SURFACE ENHANCED VIBRATIONAL SPECTROSCOPY

IMPLEMENTATIONS IN LAB-ON-A-CHIP

Jasper Jeroen André Lozeman

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Seize the time, live now! Make now always the most precious time. Now will never come again!

Patrick Stewart as Captain Jean-Luc Picard.

Star Trek: The Next Generation - The Inner Light.

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1

Introduction

In this chapter an introduction to the multiscale catalytic energy conversion (MCEC) program and consortium will be given. The positioning of this thesis within MCEC will be discussed followed by the problem statement of the thesis. Next the overall motivation for the research performed in this thesis will be presented. Finally the thesis outline is presented, and each chapter will be described briefly.

1.1 Multiscale Catalytic Energy Conversion Research Centre

This PhD project is part of the Multiscale Catalytic Energy Conversion (MCEC) Research Centre. MCEC is a 10 year national Gravitation program, funded by the Ministry of Education, Culture and Science of the government of the Netherlands, supervised by the Netherlands Organization for Scientific Research (NWO). The grant was awarded on December 13th, 2013 with a total budget of 31.967.200 euro¹. The purpose of this Gravitation grant is to bring researchers from different disciplines together in a single consortium to create novel and excellent research in the field of catalytic energy conversion. The MCEC consortium encompasses research groups from the University of Twente (UT), University of Utrecht (UU) and the Technical University of Eindhoven (TU/e). The project is coordinated by Prof. Bert Weckhuysen (UU), with other PIs within the consortium being Prof. Albert van den Berg (UT), Prof. Detlef Lohse (UT), Prof. Rutger van Santen (TU/e), Prof. Alfons van Blaaderen (UU), and Prof. Hans Kuipers (TU/e). At present it resulted in a consortium with over 10 research groups and more than 100 scientists participating in the past or present.

As the name implies, the MCEC consortium focusses on better understanding catalytic energy conversion, both in order to get a deeper, fundamental, understanding of the processes involved and in order to design and fabricate better catalysts. This is especially relevant in our current era, where one of the major challenges of our current generation is the energy transition from fossil fuels to green alternatives. In order for the Netherlands and the European Union (EU) to reach the 2050 climate goals, more efficient, greener processes have to be developed. This has to be done for existing chemical processes, such as those using non-renewable crude oil and natural gas resources. Secondly and maybe more importantly, new chemical processes based on creating fine chemicals from renewable resources need to be developed in order to facilitate the chemical industry. Another challenge of the energy transition is the storing of green energy from, for instance wind and sun energy. These green energy forms are dependent on external factors (e.g. sun intensity, cloud coverage and wind speeds) and therefore it is difficult to regulate the supply of energy according to the demand. In order to address this problem new processes need to be developed in order to be able to store this energy in the form of chemical energy. By gaining further knowledge of existing catalytic processes associated with these chemical processes, either existing catalysts can be optimized, or new catalysts can be developed to be able to synthesize these chemicals.

The MCEC consortium, focusses on a multiscale scientific approach from the field of catalysis, chemistry, physics and engineering in order to bridge the gaps of several orders of magnitude between processes going on at the level of single atoms and molecules (nanometre length scale), catalytic particles (tens of micrometre) to the full scale reactor processes (tens of meters). To quote the official MCEC website², the final goal is: *“To develop radically improved catalytic energy conversion processes through full control over the structural complexity of catalyst materials and reactors and that are capable of efficiently converting the feedstocks of today and tomorrow.”* For more information on MCEC in general, information on individual projects and researchers and the several outreach programs, see the websites: <https://mcec-researchcenter.nl/> and <https://mcec-matters.com/homepage-one/>.

1.2 Research goal

1.2.1 Problem statement

The petroleum industry has an arsenal of analytical tools available, optimized for the analysis of crude oil and its products³. The analytical workhorse of the petroleum industry is arguably gas chromatography (GC)⁴. Since its invention in 1952 by A.T. James and A.J.P. Martin⁵, GC has been optimized and perfected for the analysis of volatile hydrocarbons. Next to GC, a typical quality control (QC) lab of a petroleum refinery has a wealth of techniques available to them, such as liquid chromatography (LC), x-ray fluorescence (XRF), inductively coupled plasma spectroscopy (ICPS), nuclear magnetic resonance (NMR) spectroscopy, infrared (IR) spectroscopy and a wide range of wet chemistry techniques³. All these techniques are, understandably, optimized for measuring either crude oil, their petroleum products, or any of the intermediate products.

Looking at the energy transition more closely, it is still unknown how much of our energy and hydrocarbon chemicals will still be obtained from traditional crude oil in 2050. One potential candidate for replacing crude oil, although falling out of favour in recent years, is biomass conversion. For more information on the discussion of biomass, the work by B. Strengers and H. Elzenga is recommended⁶. With biomass conversion the goal is to transform organic material, such as food waste, into ethanol. Compared to crude oil, food waste contains a much higher water content, and the chemical process required to transform this food waste to ethanol releases water as by-product⁷. This results in a sample stream with a high aqueous content, which is somewhat problematic for techniques such as GC, traditionally not well suited for aqueous samples. Other techniques, such as LC, could be used to measure aqueous samples or samples with a high aqueous content, but an argument can also be made to develop new analytical platforms specifically geared towards the new chemical processes expected to be involved in the energy transition.

Another aspect involved in the energy transition is the discovery of new and more efficient catalysts, to improve for instance existing catalytic processes, biomass conversion or hydrogen evolution. Although research is focused towards designing and fabricating these new catalyst⁸, a new platform capable of analysing these catalytic conversions rates on-line could make characterising these new catalysts experimentally more easy.

The BIOS lab-on-a-chip research group has a strong track record in fabricating nanostructures for surface enhanced Raman spectroscopy (SERS^{9–12}). This is a technique which combines Raman with structured, nanofabricated gold antennas in order to improve signal to noise by creating locally spots with a high electric field. Similar concepts can be applied to infrared (IR) spectroscopy to create a technique called surface enhanced infrared spectroscopy (SEIRS)¹³. Raman spectroscopy and IR spectroscopy are both vibrational techniques, capable of obtaining qualitative and quantitative information about the sample. Raman and IR spectroscopy are complementary techniques, with peaks that are strong in the Raman spectra often being weak or absent in the IR spectrum and vice versa. One main research theme in The BIOS group is micro- and nano-devices for chemical analysis. By combining SERS and SEIRS with microfluidics and micro/nanostructured devices, a new platform for investigating chemical reactions could be developed to improve e.g. limit of detection, spatial and/or temporal resolution, and throughput.

1.2.2 Goal

The goal of this thesis is as follows: *To fabricate a microfluidic device combining SERS and SEIRS for on-line reaction monitoring.* A conceptual schematic of such device was created early on in this project and can be seen in Fig. 1.1. In this figure we can identify several critical components needed to achieve the goal of this thesis. For the first component, starting at the bottom of the microfluidic chip, an attenuated total reflection (ATR) crystal is shown. This crystal can couple in IR light for IR-spectroscopy. In short, the coupled light is trapped inside the crystal as it travels through the crystal in a lateral direction by bouncing of the perpendicular surfaces due to total internal reflection. As it bounces of the surface it can interact with chemicals and substances that are in the liquid phase within a few micrometres of the crystal's surface, allowing it to be used for the sensing of said chemicals. On top of this ATR crystal, gold nanoantenna structures are shown (3 in fig. 1.1). These structures are used for surface enhanced infrared spectroscopy (SEIRS). They can be addressed by the ATR crystal with the goal of increasing the signal to noise (S/N) of the IR spectroscopy technique. The zoomed inset illustrates how a possible concept of these structures could look like. The concept shown here exists out of rectangular

Fig. 1.1: Original concept design. A microfluidic chip is shown with on the bottom an ATR device containing structures for surface enhanced infrared spectroscopy (SEIRS). These structures can be addressed by the ATR device. On the top layer of the chip structures for surface enhanced Raman spectroscopy (SERS) are shown. In the inset the figure has been rotated, since the structures are on the bottom of the top layer. The SERS structures can be addressed either from the top (back side of the structures). Or depending on the optical transparency of the ATR material, from the bottom.

gold nano-rod antennas, with a specific length, width and periodicity. On the top side of the device, a substrate with gold nanowires for surface enhanced Raman spectroscopy (SERS) is shown (4 in fig. 1.1). These SERS antennas can be either fabricated on a substrate that is transparent for the visible wavelengths (such as silicon oxide) and be addressed from the top of the device, or, in case the ATR crystal is fabricated from a material transparent for visible wavelengths (such as diamond), they can be addressed from the bottom of the device. Finally, to bring everything together, a microfluidic device (2 in fig 1.1) to introduce the sample and act as a reaction chamber, has to be designed and fabricated.

1.3 Thesis outline

In this thesis, the work done to achieve the goal as presented in this introduction is described.

Chapter 2: Theoretical introduction.

This chapter gives a basic theoretical introduction to the subject of this thesis, covering the topics as vibrational spectroscopy, microfluidics and microreactors, and nano- and micro-fabrication techniques. This introduction will cover the basics of these topics, mostly meant as an introduction for those new to one or more of these topics. At the end of each section further reading material is suggested.

Chapter 3: Spectroelectrochemistry, the future of visualizing electrode processes by hyphenating electrochemistry with spectroscopic techniques.

Here a literature review regarding the combination of spectroscopy with electrochemistry is presented. This chapter was co-written with Pascal Führer. The review covers the hyphenation of IR spectroscopy with electrochemistry and the hyphenation of Raman spectroscopy with electrochemistry, which are the sections written by the author of this thesis. The final two sections cover the hyphenation of mass-spectrometry and NMR spectrometry with electrochemistry, these two sections are written by Pascal Führer

Chapter 4: Modular microreactor with integrated reflection element for online reaction monitoring using infrared spectroscopy.

This chapter discusses the fabrication of a modular microfluidic microreactor containing microfluidic mixing structures which is combined with a silicon (Si) ATR device. Its novelty lies in the ease of fabrication, its modularity and the possibility fabricate the device outside a cleanroom. As proof of concept a Paal-Knorr reaction is performed and the results discussed.

Chapter 5: Large-area fabrication of Au nanoantennas for surface enhanced infrared spectroscopy without an adhesion layer.

In this chapter gold nanoantennas, in the form of nano-rods and nano-slits, are presented. Simulation data is shown and the fabrication is discussed. The resulting structures and proof of concept measurements are shown.

Chapter 6: Wafer-scale fabrication of high-quality tunable gold nanogap arrays for surface-enhanced Raman scattering.

This chapter, co-written with Dr. Hai Le The, covers the fabrication of gold nanogap arrays for SERS. Simulation data is shown and the method of fabrication is discussed. Proof of concept measurements have been performed and an enhancement factor is measured and calculated.

Chapter 7: Infrared waveguides and quantum cascade infrared lasers.

This is a wrap-up chapter. It covers the subject of IR waveguides and alternative IR sources, namely quantum cascade lasers (QCL). A brief summary is given towards the advantages of QCL's compared to other IR sources. It is also explained how QCLs could be more beneficial when used with waveguides compared to traditional ATR devices. Several designs for different waveguide materials are given. Simulations are discussed that have been used as basis for the waveguide designs. In addition various fabrication processes are shown.

Chapter 8: Conclusion and future outlook.

The conclusion and outlook looks back on the original goals stated in the introduction section. It discusses which goals have been achieved, and which goals need further work. additionally, a future outlooks and suggestions for further research are given.

References

- (1) Molnar, E.; Geense, C. *Where Different Worlds Meet: MCEC Overview 2014 - 2015*; 2015.
- (2) About MCEC <https://mcec-researchcenter.nl/about-mcec/> (accessed Sep 24, 2020).
- (3) Ovalles, C.; Rechsteiner, C. E. *Analytical Methods in Petroleum Upstream Applications*; CRC Press, **2015**. ISBN: 9780429170058
- (4) Beens, J.; Brinkman. The Role of Gas Chromatography in Compositional Analyses in the Petroleum Industry. *TrAC - Trends Anal. Chem.* **2000**, 19 (4), 260–275.
- (5) James, A. T.; Martin, A. J. P. Gas-Liquid Partition Chromatography: The Separation and Micro-Estimation of Volatile Fatty Acids from Formic Acid to Dodecanoic Acid. *Biochem. J.* **1952**, 50 (5), 679–690.
- (6) Strengers, B.; Elzenga, H. Availability and Applications of Sustainable Biomass. **2020**.
- (7) Zakzeski, J.; Bruijninx, P. C. A.; Jongerius, A. L.; Weckhuysen, B. M. The Catalytic Valorization of Lignin for the Production of Renewable Chemicals. *Chem. Rev.* **2010**, 110 (6), 3552–3599.
- (8) Solsona, M.; Vollenbroek, J. C.; Tregouet, C. B. M.; Nieuwelink, A. E.; Olthuis, W.; Van Den Berg, A.; Weckhuysen, B. M.; Odijk, M. Microfluidics and Catalyst Particles. *Lab Chip* **2019**, 19 (21), 3575–3601.
- (9) Le Thi Ngoc, L.; Jin, M.; Wiedemair, J.; van den Berg, A.; Carlen, E. T. Large Area Metal Nanowire Arrays with Tunable Sub-20 Nm Nanogaps. *ACS Nano* **2013**, 7 (6), 5223–5234.

- (10) Le Thi Ngoc, L.; Yuan, T.; Oonishi, N.; Van Nieuwkastele, J.; van den Berg, A.; Permentier, H.; Bischoff, R.; Carlen, E. T. Suppression of Surface-Enhanced Raman Scattering on Gold Nanostructures by Metal Adhesion Layers. *J. Phys. Chem. C* **2016**, *120* (33), 18756–18762.
- (11) Yuan, T.; Le Thi Ngoc, L.; Van Nieuwkastele, J.; Odijk, M.; van Den Berg, A.; Permentier, H.; Bischoff, R.; Carlen, E. T. In Situ Surface-Enhanced Raman Spectroelectrochemical Analysis System with a Hemin Modified Nanostructured Gold Surface. *Anal. Chem.* **2015**, *87* (5), 2588–2592.
- (12) Jin, M.; Pully, V.; Otto, C.; Van Den Berg, A.; Carlen, E. T. High-Density Periodic Arrays of Self-Aligned Subwavelength Nanopyramids for Surface-Enhanced Raman Spectroscopy. *J. Phys. Chem. C* **2010**, *114* (50), 21953–21959.
- (13) Neubrech, F.; Huck, C.; Weber, K.; Pucci, A.; Giessen, H. Surface-Enhanced Infrared Spectroscopy Using Resonant Nanoantennas. *Chem. Rev.* **2017**, *117* (7), 5110–5145.

2

Theoretical background

In this chapter a basic theoretical background for the thesis is given. This chapter is written for readers that are not familiar with one or multiple aspects of this thesis and aimed to give these readers sufficient background information regarding the work discussed in this thesis. The subjects covered are as follows: in section 2.1 the basics of vibrational spectroscopy are discussed, followed by special focus on Infrared and Raman Spectroscopy in sections 2.2 and 2.3 respectively. In section 2.4 we briefly introduce surface enhanced spectroscopy. Next, a short introduction in microfluidic chips is given in section 2.5. Finally we introduce micro and nano fabrication techniques in section 2.6.

To keep this chapter accessible, brief and understandable, some subjects might be simplified, skipped or not as adequately covered as some might like. In order to address these possible qualms and in order to provide more reading material for those interested, we will recommend further literature in most chapters.

Section 2.1 till section 2.3 cover the basics regarding vibrational spectroscopy. Most of the information stated here has been cross-referenced with the following books, unless stated otherwise: *Fundamentals of Fourier Transform Infrared Spectroscopy* by Brian C. Smith¹, *Introduction to Spectroscopy* by Pavia et al.², *IR and Raman Spectroscopy: Principles and Spectral Interpretation* by Peter J. Larkin³, *Raman Spectroscopy for Chemical Analysis* by Richard L. McCreery⁴ and *Modern Raman Spectroscopy—a Practical Approach* by Ewen Smith and Geoffrey Dent⁵.

2.1 Basics of vibrational spectroscopy

Vibrational spectroscopy is a term used to group the spectroscopic techniques of infrared (IR) spectroscopy and Raman spectroscopy. In vibrational spectroscopy, information regarding the bond vibrations of molecules can be obtained. The frequency of the vibrations is, unlike other spectroscopic techniques, often expressed in reciprocal centimetre (cm^{-1}), providing a scale that is linear with energy^{1,3}. Vibrational modes are often classified in one of six possible modes, as shown in Fig. 2.1. These modes can then be categorized as stretching and bending vibrations. In stretching vibrations, the molecules move along the internuclear axis stretching the “spring” and working against the chemical bond, while in bending vibrations only a small displacement in the angle of bond takes place^{1,3}. Since stretching of the bonds costs more energy than bending, the stretch vibrations occur at higher wavenumbers than the bending vibrations. In order to calculate the number of vibrations present in a molecule, the following equation (eq. 2.1) can be used for a linear molecule,

$$V_{lin} = 3N - 5 \quad (2.1)$$

and eq. 2.2 can be used for a non-linear molecule.

Fig. 2.1: Schematic representation of the six different vibrational modes.

$$V_{nl} = 3N - 6 \quad (2.2)$$

Where V_{lin} and V_{nl} stands for number of vibrations of a linear and number of vibrations of a nonlinear molecule respectively. N stands for the number of atoms in a molecule.

Despite both Raman and IR spectroscopy being classified as vibrational spectroscopy techniques and both providing vibrational information of a molecule, distinctive different vibrational spectra are often obtained when measuring the same sample. This has to do with the differences in how the light source interacts with the sample in the two different techniques. In IR spectroscopy, the intensity of a peak is determined by how much the dipole moment of a vibration changes compared to its normal coordinates. In Raman spectroscopy, the intensity of the peak is determined by the polarizability of the vibration. As a general rule of thumb, vibrations that have a large dipole moment often have a weak polarizability and vice versa. The change in dipole moment is easy to understand and visualize.

The CO_2 molecule will be used as an example. CO_2 is a linear molecule with 3 atoms, using eq. 2.1 this means that CO_2 has 4 possible vibrations. In Fig. 2.2 three of the four vibrations of CO_2 are shown, with the fourth vibration, the out-of-plane bending vibration, being the same as the in-plane bending vibration, only rotated 90 degrees around the horizontal axis of the molecule. The carbon molecule has a positive partial charge, the two oxygen molecules have a negative partial charge. The black arrows in this figure show the vectors of the dipole moment of the individual

Fig. 2.2: illustration of the change in dipole moment of 3 of the 4 vibrations of CO_2 . The black arrows represent the vectors of the dipole moment of the individual bonds, the blue arrow represents the vector of the total dipole moment. This figure is an illustration and the bond angles and lengths have been exaggerated. The top molecule in all three columns represents the initial state. The second and third row show the extremes of a particular vibration. The first vibration, the symmetric stretch vibration shown in the first column. The second vibrations is the asymmetric stretch, shown in the second column. The third vibration is the in-plane bending vibration shown in the third column and the fourth vibration is the out-of-plane bending vibration. The only difference between the in- and out-of-plane bending vibration is a 90° rotation of the molecule.

bonds. The dipole moment of a molecule is the sum of the dipole moments of the individual bonds. Looking at fig. 2.2 it is observable that the symmetric stretch vibration of CO_2 does not show a change in total dipole moment and therefore is not IR active. The asymmetric stretch and the bending vibrations do show a change in dipole moment and therefore are IR active, with vibrations at approximately 2400cm^{-1} and 670cm^{-1} respectively.

Polarizability describes how easily electrons can be moved by an external field. In a somewhat simplified explanation, the polarizability of a molecule changes depending on the electron density of a molecule, the length between the bonds and the strength of a bond. When again looking at fig. 2.2, it can be observed that for the symmetric stretch, in both extremes, both bond lengths change. In the first extreme, both bond lengths reduce in size, while for the second extreme, both bond lengths increase in size. This change in bond length causes a change in polarizability of the molecule, making this vibration Raman active. For the asymmetric stretch, the bond lengths also change. However for the first extreme, the bond length between the carbon atom and the left oxygen atom becomes longer, while the bond with the right oxygen atom becomes smaller. The second extreme shows the opposite, the left bond becomes smaller while the right bond becomes longer. The total polarizability of the molecule stays the same with as result that this vibration is not Raman active. For the final vibration, only the angle of the bond changes meaning that this vibration is also not Raman active.

Of course, CO_2 is a simple molecule. For more complex molecules it will be more difficult to predict the vibrational modes and which vibrations will be IR or Raman active. Intramolecular interactions can cause shifts in frequencies of vibrations, hydrogen bonds can cause peak broadening and overtones can cause mislabelling of peaks. This chapter, even though it only gives a glimpse of the theory behind vibrational spectroscopy, hopefully gives a satisfactory introduction to vibrational spectroscopy, but should not be seen as a complete guide into the subject. For further reading on the subject of vibrational spectroscopy it is recommended to read the work by Larkin, titled: IR and Raman Spectroscopy, Principles and Spectral Interpretation. Which gives a nice introduction into the field³.

2.2 Infrared spectroscopy

Infrared spectroscopy is a technique that uses infrared radiation to measure the vibrational frequency of a molecule. The infrared spectra, as can be seen in fig. 2.3 covers a wavelength range from 1.000.000 nm to 700 nm. In case of infrared spectroscopy, three different techniques can be commonly referred to. Firstly, near-infrared (NIR) spectroscopy, covering a range from 800 nm to 2.500 nm⁶ or 12.500cm^{-1} to 4.000cm^{-1} . This frequency is higher than required to match the vibrational frequency of a fundamental vibrational state⁶, but too low for most electron excitations⁷. NIR spectroscopy looks at overtones and combination modes, the so called forbidden transitions and some weak electronic transitions^{6,7}. These forbidden transitions are not allowed by the selection rules, and therefore usually have a low absorption⁷ allowing for a relative deep sample penetration. The peaks in NIR spectroscopy are usually broad, overlapping and difficult to interpret. Complex

Fig. 2.3: electromagnetic spectrum. The infrared spectrum is highlighted on the top. As a reference the visible spectrum is highlighted at the bottom. The IR spectrum can be divided in 3 different sections: The NIR region, 12.500 cm⁻¹ to 4.000 cm⁻¹, the MIR region, 4.000 cm⁻¹ to 400cm⁻¹ and the FIR region, 400 cm⁻¹ to 10 cm⁻¹. The MIR region can be further sub-divided in the group frequency region, 4.000 cm⁻¹ to 1.450 cm⁻¹ and the fingerprint region, 1450 cm⁻¹ to 400cm⁻¹.

calibration procedures are often required in order to obtain reliable quantitative or qualitative information. On the other end of the infrared spectra, from approximately 25.000 nm to 1.000.000 nm or 400 cm⁻¹ to 10 cm⁻¹, is the so called far-infrared (FIR) region³. FIR spectroscopy is mostly used in far infrared astronomy and looks at rotational vibrations.

In between the NIR and FIR region, between 2.500 to 25.000 nm or 4.000 to 400 cm⁻¹, lies the mid-infrared (MIR) region. In this MIR frequency range, the fundamental bending and stretching vibrations discussed in section 2.2.1 take place. The MIR region can be further sub-divided into the group frequency region, 4.000 cm⁻¹ to 1.450 cm⁻¹ and the fingerprint region 1.450 cm⁻¹ to 400 cm⁻¹. In this thesis, MIR spectroscopy is the only form of IR spectroscopy used. Since MIR spectroscopy is often just referred to as IR spectroscopy. When from this point onward, IR is being mentioned in this thesis, it refers to MIR, unless stated otherwise.

The most commonly used source in IR spectroscopy is a global, a silicon carbide rod heated electrically in a range from approximately 1.000°C to 1.650°C, emitting an IR spectrum. Alternative sources are synchrotron sources and IR lasers. Recently, quantum cascade lasers have been gaining attention as an alternative IR light source⁸. The detector used in IR spectroscopy is most commonly a mercury cadmium telluride (MCT) detector. This is a detector that requires cooling by liquid nitrogen to reduce noise and to properly detect the IR spectrum. Although IR spectroscopy initially was used with a monochromator to scan the frequency range, the so called dispersive IR instrument spectrometer, most modern IR instruments are the so called Fourier transform infrared (FTIR) instrument, using a Michelson interferometer. FTIR

instrumentation is faster, has a higher sensitivity and a better precision than the classic dispersive instruments. FTIR is used on a wide variety of samples, for solids, liquids and gasses, and in a wide variety of applications. Therefore several different configurations are used. Namely transmission, reflection and attenuated total reflection (ATR)-FTIR. In the next section we will give a short overview of these most commonly used IR techniques. There is no ultimate FTIR technique that is best, the use of a certain technique is usually dependent on the sample of interest. For further reading on FTIR spectroscopy, the following works are recommended: *IR and Raman Spectroscopy, Principles and Spectral Interpretation* by Peter Larkin³ and *Fundamentals of Fourier Transform Infrared Spectroscopy* by Brian C. Smith¹.

2.2.1 Transmission FTIR

In Fig. 2.4, an IR instrument in the transmission mode is shown. In transmission FTIR, the IR radiation is emitted from the source after which it is split by a beam splitter. Half of the radiation is cast on a stationary mirror while the other half is cast on a moving mirror. The light is reflected back at the beam splitter and recombined. This combination of a beam splitter, a stationary mirror and moving mirror is a so called Michelson interferometer. Because the distance travelled by the light beam hitting the moving mirror is different from the light beam hitting the stationary mirror, an interferogram is created. Certain wavelengths are blocked (by destructive interference) while others are passed through, based on the position of the mirror. After the light is re-combined it travels through the sample where absorption takes place. Next the light is redirected towards the detector where the interferogram is recorded. The detector measures the total beam intensity. Because the exact position of the moving mirror is known, Fourier transformation can be used to translate the interferogram back into a recognizable IR spectrum.

Since the source in IR spectroscopy is relatively weak in intensity and strong absorption bands are common in IR spectroscopy, the cross-section of solid and

Fig. 2.4: A simplified schematic representation of a FTIR instrument in transmission mode. Inspired by fig. 2.3 in *Introduction to Spectroscopy* by Pavia et al².

liquid samples are usually relatively small, in the order of tens of micrometres. Liquid samples can be sandwiched between two IR transparent windows, usually salts that are IR transparent such as KBr or NaCl. Alternatively liquid cells are commercially available for IR spectroscopy. Solid samples are either measured directly when thin enough, as for instance for thin polymer films, or crushed and mixed KBr. This mixture is pressed together in a press to create a pellet that can be put in the IR beam-path¹. For gaseous samples, cells exist with a path length of 5 cm up to a length of 100 meters, this path length is achieved in combination with mirrors to make multiple passes through the sample compartment¹. This long detection length can be required to measure low concentrations in air. Since symmetrical diatomic molecules (such as N₂ and O₂) are not IR active, they cause no interference in the measurement. Therefore, as long as a long enough path length can be created with little losses due to the mirrors, relative low concentrations in air can be measured.

2.2.2 Reflection FTIR

In Fig. 2.5, a FTIR instrument in reflection mode is shown. It has to be noted that several different modes of reflection FTIR exist, such as specular reflectance, diffuse reflectance Fourier transform spectroscopy (DRIFTS) and attenuated total reflection (ATR). Since ATR is quite different than specular reflectance and DRIFTS, ATR is covered as a separate topic in section 2.2.3. To define the differences between specular reflectance and DRIFT: In specular reflectance the light is reflected of a IR reflective material. Specular reflection can be used for instance to inspect the surface of reflective metals. Alternatively a thin layer of sample can be deposited on a reflective surface in what is called reflectance absorbance measurements.

In DRIFTS, the sample scatters the light in different directions due to the irregular surface of the sample. Sample types are usually powdered samples mixed with an IR transparent material such as KBr. The light reflected by the sample is then collected by a parabolic mirror and redirected back to the detector.

Fig. 2.5: A simplified schematic representation of a FTIR instrument in specular reflection mode. Inspired by fig. 2.3 in Introduction to Spectroscopy by Pavia et al².

2.2.3 ATR FTIR

In Fig. 2.6 a simplified schematic representation of an FTIR instrument in ATR mode is shown. In ATR-FTIR a crystal, which sometimes is called an ATR device or an internal reflection element (IRE), is placed in the beam path. The light is coupled into the facet of this device and due to total internal reflection, the light bounces through the ATR device. An evanescent wave is created at the interface of the crystal. Due to this evanescent wave interaction between the IR light beam and the sample on top of the crystal can take place. The evanescent wave penetrates up to several micrometres into the sample, depending on the wavelength, refractive index of the sample and the refractive index of the ATR device¹. The electric field of the evanescent wave decreases exponentially normal to the surface of the ATR device. The “path length”, as described in the Lambert beer equation, in ATR-FTIR is thus dependent on the sample and ATR-device material and the wavelength measured. Another factor in the path length is the length and height of the ATR-device, as this will influence how many times the light beam can “bounce” through the ATR device. Since the penetration depth is dependent on the wavelength, ATR spectra show different peak height ratios when comparing the same sample with reflection or transmission FTIR. Since a longer wavelength will have a bigger evanescent wave than a shorter wavelength, and thus has a longer path length¹. A slight shift in peak position compared to transmission / reflection FTIR can also be observed in ATR FTIR. This can occur due to the deeper penetration of the longer wavelengths, shifting the peak position in this direction, or due to a change of refractive index of the material when changing the wavelength. As a consequence, it also changes the penetration depth, affecting the peak shape and resulting in asymmetric peaks.

Fig. 2.6: A simplified schematic representation of an ATR-FTIR instrument. Inspired by fig. 2.3 in Introduction to Spectroscopy by Pavia et al².

2.3 Raman spectroscopy

In Raman spectroscopy, visible, UV or near-infrared light sources are used to produce vibrational spectra. When the electromagnetic wave of the Raman light source interacts with a sample several things can occur; the light can be transmitted undisturbed, it can be absorbed, it can be reflected or it can be scattered. In the case of Raman spectroscopy, the light scattering is the interaction of interest. In order to understand the process of Raman scattering, two different light-matter interactions should be discussed, namely Rayleigh scattering and Stokes scattering. As can be seen in fig. 2.7, the interaction between a photon from the Raman light source and the sample puts the molecule in a so called virtual state. Shortly after this absorption event, the molecules decays back to the ground state by emitting a photon. In the majority of the scattering events, the light emitted after the scattering has the same wavelength as the light absorbed by the molecule, so called Rayleigh scattering⁴. In some rare cases, the wavelength of the light scattered is changed in wavelength. This change in wavelength corresponds to one of the vibrational frequencies of the molecule. This can be either occur by adding vibrational energy, the so called anti-stokes scattering, or by subtracting vibrational energy, the so-called Stokes scattering⁴.

Since Stokes and anti-Stokes scattering events are rare compared to Rayleigh scattering, high powered light sources are required. Hence, Raman spectroscopy typically uses lasers in order to meet this criterion. In fig. 2.8 a schematic representation of how a typical Raman instruments works is shown. As can be seen from this somewhat simplified figure, laser light is hitting a beam splitter after which it is directed through a microscope objective onto the sample. The scattering takes

Fig. 2.7: Jablonski diagram showing the energy states of Rayleigh scattering and the Stokes scattering. Energy states are not to scale.

place and light is scattered in all directions. Of the scattered light only a small amount is scattered back into the microscope objective and of this collected light only a small percentage is the (anti) Stokes scattering of interest. This combination makes the use of lasers so important in Raman spectroscopy. However, care has to be taken of the sample when using high powered light sources. Although a higher light intensity means more scattering and a higher signal on the detector, absorption events taking place in parallel with the scattering can cause heating of the sample and in some cases even burning.

Looking back at fig. 2.8, after the scattering event takes place, the photons that reach the microscope objective pass through the beam splitter and reach a filter removing the Rayleigh scattered light. The light is redirected towards a grating which separates the light based on frequency, after which it is redirected towards a CCD detector and the vibrational spectra is obtained.

Fig. 2.8: Schematic representation of a Raman microscope. Some parts have been simplified. Partly based the figure from the work of Schmid and Dariz¹⁸.

Since no excitation state of vibrational state has to be matched in frequency. Raman spectroscopy can occur independent of the laser frequency. Therefore one might think that the choice of laser frequency is unimportant. However, this is not entirely the case. Firstly, the scattering intensity of Raman increases up to a fourth power of the light frequency⁴. Generally meaning that choosing a laser with a shorter wavelength produces more Raman scattering. However, as said before, heating of the sample might occur. This heating can be somewhat mediated by picking a laser with a frequency that is least absorbed by the sample. Additionally, especially for organic samples, fluorescence might occur, giving a broad background peak, possibly making sample peaks unrecognizable. Fluorescence in general occurs more towards the lower wavelengths (higher energy) and especially towards the UV. So in general, a laser with a shorter wavelength is preferred but for other lasers with different frequencies it should be considered if heating or fluorescence background signal is going to interfere⁴.

For further reading on the subject of Raman spectroscopy, the work of Peter Larkin discusses the principles of IR spectroscopy and Raman spectroscopy and the spectral interpretation³. The work of Ewen Smith and Geoffrey Dent is solely focused on Raman spectroscopy and goes somewhat more in depth than Larkin, covering some advanced techniques⁵. Finally the work of Richard McCreery focuses on using Raman spectroscopy for chemical analysis, covering a broad range of aspects involved⁴.

2.4 Surface Enhancement

In this thesis, two different forms of surface enhancement will be discussed, namely: Surface enhanced infrared spectroscopy (SEIRS) and surface enhanced Raman spectroscopy (SERS). In short, although there being distinct differences between SEIRS and SERS, surface enhancement can be described as a surface technique, in which the electromagnetic properties of either a nanostructured metal film or an array of nanofabricated metal structures is used to enhance the interaction between the electromagnetic radiation and the sample. By enhancing the interaction between the sample and the electromagnetic radiation, a better sample signal can be obtained resulting in, ideally, a better signal to noise (S/N) ratio. In general the quality of a substrate for surface enhancement is determined by their enhancement factor. However, in literature different ways of expressing enhancement are mentioned, hence these numbers can sometimes be misleading as its value is not always representative for practical applications.

Due to the differences in how Raman spectroscopy interacts with the sample and the different wavelengths used, SERS usually reports enhancement factors several magnitudes higher than SEIRS. Where enhancement factors of $10^1 - 10^3$ are typical values for SEIRS⁹, SERS enhancement values as high as 10^{10} are reported in literature¹⁰. For further reading about SERS, the work Eric Le Ru and Pablo Etchegoin titled: *Principles of Surface-Enhanced Raman Spectroscopy* is recommended¹⁰. For more reading on SEIRS, *Surface-Enhanced Vibrational Spectroscopy* by Ricardo Aroca¹¹ includes a chapter on the subject.

2.5 Microfluidic chips

A microfluidic chip is a small device with micrometre-sized channels etched, milled or otherwise fabricated into or on-top of it. Commonly used substrate materials used to make these microfluidic chips are: glass (SiO_2), Silicon (Si) and polydimethylsiloxane (PDMS). However, in industrial applications thermoplastic materials are also very popular, as it is easier to mass-fabricate by means of e.g. injection molding or hot-embossing. Often these microfluidic chips are combined with a (micro)reaction chamber, a sensing technique or used to study biological systems, as for example in organ-on-chip devices. There are several advantages of microfluidic devices over more conventional systems. Less chemicals have to be used, which is beneficial from both a safety and environmental perspective. Due to the small dimensions, flow is typically laminar, providing better control. Small dimensions also lead to short diffusion distances for heat and mass transfer, which is beneficial for the speed of the process under study¹². The small footprint associated with microfluidics, in principle, allows for easy parallelisation. However, the bottleneck in surrounding equipment is often limiting this parallelisation promise in practice.

Microfluidics is used in a wide variety of fields; the field of organs on chips tries to mimic human organs for personalized medicine and as an alternative for animals trails¹³. Microfluidics can also be used for the creation of monodisperse droplets for medical applications¹⁴. In chemistry microfluidics in combination with microreactors is used for screening of catalysts, finding new reactions and as devices for online reaction monitoring¹⁵. A key application of microfluidics is its use for DNA analysis and PCR, where it revolutionized the genome sequencing¹⁶. Further reading on the concepts of microfluidics can be found in the book by Patrick Tabeling titled: *Introduction to Microfluidics*¹⁷.

2.6 Micro- and nanofabrication techniques

In this section micro- and nanofabrication techniques will be briefly introduced. Unlike the previous sections covered in this chapter, where a general overview of the subject is given, this section present the subject as specific techniques as available at the University of Twente.

2.6.1 Nanofabrication: The Mesa⁺ cleanroom

One of the institutes located at the University of Twente is the Mesa⁺ institute. This institute is a leading nanotechnology research centre, with a state of the art research cleanroom as its biggest asset. The Mesa⁺ Nanolab, is arguably, one of the leading scientific cleanroom infrastructures in the world, with a working area of 1250m². The cleanroom operates at two ISO levels; the critical areas having an ISO norm of at least 5 and the walking area being at ISO level 7. In short, this means that in the critical area a maximum of 10⁵ particles with a size bigger than 0.1 μm are present in 1 m³ of air. As a comparison, a human produces on average 10.000.000.000 particles a day, depending on activity level. This level of particles per area is achieved in the cleanroom by expensive air filtration, controlled airflows, special clothing for those entering the facility and thorough training for scientist using the facility. A special

feature of the Nanolab is that certain rooms in the cleanroom are damped from vibrations by a special suspended structure, minimizing vibrations for e.g. ultra-high resolution atomic force microscopy. The highest vibration criteria (VC) achieved in the mesa⁺ cleanroom is level G, meaning that only vibrations with a maximum amplitude of 0.78 $\mu\text{m/s}$ can take place. A cleanroom is a strict necessity for fabrication nanostructures, both because of the equipment available, as well as the need for a controlled, dust-free environment.

2.6.2 Microfabrication

For microfabrication of devices and structures that do not require cleanroom fabrication, the BIOS research group has a rapid prototyping lab, a chemical lab and an assembly lab. For devices in the size-range of 100 μm to ~ 10 cm, cleanroom fabrication techniques are not required, but specialized equipment is still needed. The rapid prototyping lab is equipped with a computer numerical control (CNC) micromilling machine (Datron Neo), capable of fabricating trenches with a width down to 100 μm , positioned in height and location with few micrometre precision. The rapid protolab is also equipped with a (Formlabs Form2) 3D printer, capable of printing polymer structures down to a size of several tens of μm , depending on the resin and 3D model used. Photolithography can be performed down to ~ 1 μm in the cleanroom, while special nanolithographic techniques such as e-beam or Talbot lithography can push dimensions down to the nanoscale. Although the resolution achieved outside the cleanroom is significantly lower compared to inside, when considering the cost involved it is not always desired to perform all fabrication steps inside the cleanroom. In addition, micromilling and 3D printing techniques are much faster if multiple design iterations are required.

References

- (1) Smith, B. C. *Fundamentals of Fourier Transform Infrared Spectroscopy*; CRC Press: Cambridge, **2011**. ISBN: 9781119440550
- (2) Pavia, D. L.; Lampman, G. M.; Kriz, G. S.; Vyvyan, J. A. *Introduction to Spectroscopy*; Cengage Learning, **2008**. ISBN: 9780495114789
- (3) Peter Larkin. *IR and Raman Spectroscopy: Principles and Spectral Interpretation*; **2011**. ISBN: 9780128042090
- (4) McCreery, R. L. *Raman Spectroscopy for Chemical Analysis*; **2005**. ISBN: 9780471231875
- (5) Smith, E.; Dent, G. *Modern Raman Spectroscopy—a Practical Approach.*; **2019**; Vol. 2. ISBN: 9781119440550
- (6) Xiaobo, Z.; Jiewen, Z.; Povey, M. J. W.; Holmes, M.; Hanpin, M. Variables Selection Methods in Near-Infrared Spectroscopy. *Anal. Chim. Acta* **2010**, 667 (1–2), 14–32.
- (7) Okazaki, Y. Near-Infrared Spectroscopy—Its Versatility in Analytical. *Anal. Chem* **2012**, 28 (June), 545–562.
- (8) Weida, M. J.; Yee, B. Quantum Cascade Laser-Based Replacement for FTIR Microscopy. *Imaging, Manip. Anal. Biomol. Cells, Tissues IX* **2011**, 7902 (February 2011), 79021C.

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- (9) Ataka, K.; Heberle, J. Biochemical Applications of Surface-Enhanced Infrared Absorption Spectroscopy. *Anal. Bioanal. Chem.* **2007**, 388 (1), 47–54.
- (10) Ru, E. C. Le; Etchegoin, P. G. *Principles of Surface-Enhanced Raman Spectroscopy*; Elsevier, **2009**. ISBN 9780444527790
- (11) Aroca, R. *Surface-Enhanced Vibrational Spectroscopy*; John Wiley & Sons, Ltd: Chichester, UK, **2006**. ISBN: 9780470035641
- (12) Hartman, R. L.; Jensen, K. F. Microchemical Systems for Continuous-Flow Synthesis. *Lab Chip* **2009**, 9 (17), 2495–2507.
- (13) Huh, D.; Hamilton, G. A.; Ingber, D. E. From 3D Cell Culture to Organs-on-Chips. *Trends Cell Biol.* **2011**, 21 (12), 745–754.
- (14) Segers, T. *Monodisperse Bubbles and Droplets*. **2015**. ISBN: 9789036538992
- (15) Solsona, M.; Vollenbroek, J. C.; Tregouet, C. B. M.; Nieuwelink, A. E.; Olthuis, W.; Van Den Berg, A.; Weckhuysen, B. M.; Odijk, M. Microfluidics and Catalyst Particles. *Lab Chip* **2019**, 19 (21), 3575–3601.
- (16) Chen, T. N.; Gupta, A.; Zalavadia, M. D.; Streets, A. MCB-Seq: Microfluidic Cell Barcoding and Sequencing for High-Resolution Imaging and Sequencing of Single Cells. *Lab Chip* **2020**, 20 (21), 3899–3913.
- (17) Tabeling, P. *Introduction to Microfluidics*; **2010**. ISBN 780198568643
- (18) Schmid, T.; Dariz, P. Raman Microspectroscopic Imaging of Binder Remnants in Historical Mortars Reveals Processing Conditions. *Heritage* **2019**, 2 (2), 1662–1683.

3

Spectroelectrochemistry, the future of visualizing electrode processes by hyphenating electrochemistry with spectroscopic techniques

A review

Spectroelectrochemistry (SEC), is an combination of electrochemistry and spectroscopy. By combining these two techniques, the relevance of the data obtained is greater than what it would be when using them independently. A number of review papers have been published on this subject, mostly written for experts in the field and focused on recent advances. In this review chapter, written for both the novice in the field and the more experienced reader, the focus is not on the past but on the future. The scope is narrowed down to four techniques the authors claim to have most potential for the future, namely: infrared spectroelectrochemistry (IR-SEC), Raman spectroelectrochemistry (Raman-SEC), nuclear magnetic resonance spectroelectrochemistry (NMR-SEC) and, perhaps slightly more controversial but certainly promising, electrochemistry mass-spectrometry (EC-MS).

This work is adapted from:

J.J.A. Lozeman[†], P. Führer[†], W. Olthuis, M. Odijk. Spectroelectrochemistry, the future of visualizing electrode processes by hyphenating electrochemistry with spectroscopic techniques, *Analyst*, 2020, **145**, 2482–2509

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3.1 Combining spectroscopy and electrochemistry

3.1.1 Spectroelectrochemistry (SEC)

Spectroelectrochemistry is an established technique which hyphenates electrochemistry with spectroscopy. Electrochemistry by itself is a technique that can be used in order to determine concentrations of known compounds or to obtain information concerning reaction kinetics. However, it is less suitable for elucidating unknown reaction intermediates or products¹. By combining electrochemistry with an optical technique, more qualitative and quantitative information about the processes occurring at the electrodes can be obtained.

It is generally accepted in the SEC field that the work of Kuwana et al.² in 1964 is the first true SEC experiment. This early work on spectroelectrochemistry has resulted in a field containing a large variety of spectroscopic methods. Nowadays, a number of reviews exist concerning spectroelectrochemistry. For example the work by Dunsch from 2011, covering a wide range of multi-spectroelectrochemistry techniques³. In 2013, Oberacher et al. published a paper on mass spectrometric methods in electrochemical cells⁴. Wain and O'Connell wrote a paper in 2017 about surface-enhanced vibrational spectroelectrochemistry⁵. Also in 2017, work by Tong on nuclear magnetic resonance spectroelectrochemistry focused on the challenges and prospects was published⁶. The work by León and Mozo, published in 2018, describes in detail how to design a spectroelectrochemical cell⁷. In 2018, Zhai et al. wrote a review in which they describe the recent advances in spectroelectrochemistry⁸. Finally, in the work by Gazor-Ruiz et al. from 2019, the recent trends and challenges of spectroelectrochemistry are described⁹.

3.1.2 Visualizing the future of SEC

Most of the papers mentioned above are addressed towards experts in the fields, with a strong focus on recent advancements. This review chapter tries to add to these existing review papers, firstly by focussing on the future of spectroelectrochemistry and secondly by writing a review paper in an accessible way for newcomers to the field. To be concise, the current review is focussed on the four techniques that, in the opinion of the authors, have the biggest potential to undergo major improvements in the coming decades. The techniques covered in this review are infrared spectroelectrochemistry (IR-SEC), Raman spectroelectrochemistry (Raman-SEC), nuclear magnetic resonance spectroelectrochemistry (NMR-SEC) and, perhaps slightly more controversial but certainly promising, electrochemistry mass-spectrometry (EC-MS). These techniques will each be discussed in their own sections in the aforementioned order. The basic principle of every technique is first explained, followed by the current state of the art in the field. To conclude, every section ends with a future perspective, based on the developments in the separate fields of the SEC technique and the substantiated opinion of the authors. The authors hope that this review will inform both the newcomers as well as the experts concerning the future of SEC. At best, we aim to give an overview of how the future of SEC may look like and at worst, to initiate a scientific discussion on the subject.

3.2 Infrared spectroelectrochemistry (IR-SEC)

3.2.1 Introduction IR-SEC

3.2.1.1 IR spectroscopy

In the most basic sense of the technique, infrared (IR) spectroscopy can be described as a technique where IR radiation is absorbed by molecules. The absorption of the infrared light occurs when the frequency of the absorbed radiation is equal to the vibrational frequency of the molecule. The resulting absorption spectrum provides information about the identity of the elements and structural composition of the molecule. Only vibrational modes showing a change in dipole moment are visible in the IR spectra. As a result molecules such as N_2 cannot be detected with this technique. IR spectroscopy operates over a wide spectral window between $2.5\text{--}25\text{ }\mu\text{m}$ ($4000\text{--}400\text{ cm}^{-1}$). The most commonly used IR technique is Fourier transform infrared spectroscopy (FTIR) (Fig. 3.1).

When considering IR spectroscopy, there are some key drawbacks associated with the technique. Most importantly, IR instruments often use a silicon carbide rod (such as a Globar), heated to 1000 C or above, as a light source. Although these sources can cover a large spectral window, their power output is relatively low. This results in a relatively high detection limit compared to other analytical techniques. Another serious drawback is that water has strong absorbance bands in the IR region, which complicates measuring in aqueous solutions. In order to prevent the water bands from dominating the spectrum, Lambert-Beer law (eq. 3.1) offers a practical solution.

$$A = \varepsilon \cdot c \cdot l \quad (3.1)$$

Where A is the absorbance (arbitrary units), ε is the molar attenuation coefficient (m^2/mol), c is the concentration (mol/m^3) and l is the path length (m). In order to reduce the absorbance effect of water, the path length is the only factor that can be changed when measuring in aqueous solutions. Meaning that in practice, the path length in FTIR of aqueous samples is kept in the order of $15\text{ }\mu\text{m}$ or lower¹⁰. This shorter path length also effects the analyte. The concentrations of analytes required

Fig. 3.1: Schematic diagram by Pavia et al.³¹⁵ of an FTIR instrument. From Pavia et al. Introduction to Spectroscopy, 4E. © 2009 Brooks/Cole, a part of Cengage, Inc. Reproduced by permission. www.cengage.com/permissions

for adequate signal strengths is therefore relatively high compared to other spectroscopic techniques, forming an obvious disadvantage of IR spectroscopy.

3.2.1.2 IR-SEC spectroscopy

As explained above, the requirement of a small cell volume is seen as a disadvantage, both due to the high sample concentration needed and difficulties in sample handling with such small volumes. However, it can be seen as an advantage when combining FTIR with EC. When the cell height is smaller than the diffusion length, total electrochemical conversion is more easily achieved^{10,11}.

Due to the requirement of such a small path length, an often used cell design in IR-SEC is the so called “thin-layer” configuration. This configuration is used as follows: the sample with electrolyte is applied on an electrode and a window of IR-transparent material (such as CaF_2) is pushed against the electrode. As a result the layer of liquid analyte is sandwiched between the electrode and the transparent IR window, with a thickness of several μm . A drawback of this method is that the exact thickness of the thin-layer is not as reproducible as with a fixed cell size, making the process of taking a background reference spectrum more difficult. The thickness of the layer can be calculated by measuring the absorbance of the bulk water vibration¹², potassium ferricyanide¹³, or any other substance with a known concentration and molar attenuation coefficient and applying the Lambert-Beer law. This determination of the path length is not necessarily done using IR spectroscopy. De Lacey et al.¹⁴ used UV/Vis spectroscopy of 8 mM cytochrome c to determine the path length of their thin-layer cell. Once the path length of both the sample and the background measurement is known, a background correction can be performed.

An alternative way to make a background correction when using the thin-layer configuration is by difference spectroscopy. In difference spectroscopy, a background reference measurement is performed at one potential and then subtracted from the measurement taken at a different potential. In this way, the contribution of the bulk solution is cancelled out and a spectrum is produced showing only the changes caused by the variation of the potential. There are, however, drawbacks to this technique, as adsorption and desorption of the analyte on the electrode changes the concentration of the measured analyte¹⁵.

3.2.1.3 Surface enhanced infrared absorption spectroscopy (SEIRAS)

In order to increase the signal to noise ratio (S/N) of the measurements, researchers have been using surface enhanced infrared absorption spectroscopy (SEIRAS). After the successes in the 1970's in obtaining large enhancement factors in surface enhanced Raman spectroscopy (SERS), interest started to grow to apply similar concepts in infrared spectroscopy. The first SEIRAS experiments were reported in the 1980's by Hartstein et al.¹⁶, although the term SEIRAS was only coined later^{17–19}. Since the 1990's early pioneering work in the field of SEIRAS was mostly done in the group of Osawa^{20,21}. Although not as powerful as in SERS, where local enhancement factors of up to 10^{10} have been reported^{22,23}, SEIRAS is still a valuable technique with enhancement factors of $10^1 - 10^3$ being reported²⁴. SEIRAS is performed on metallic surfaces, either in the form of roughened surfaces or arrays of nanostructures. A simplified explanation of SEIRAS is as follows: electromagnetic interactions between the IR light and the metallic structures can cause a

phenomenon called plasmon resonance. Plasmon resonance amplifies the electromagnetic field, resulting in the enhancement. This enhancement only occurs close to the surface, and is negligible at distances bigger than 10 nm. SEIRAS is a complex phenomenon and the explanation above is simplified, excluding effects such as “chemical” enhancement. Therefore, the authors of this review recommend that, for an in-depth explanation of SEIRAS, the reader reads the following literature: Osawa¹⁷, Aroca¹⁸ and Neubrech²⁵. In IR-SEC, SEIRAS is performed in combination with reflection spectroscopy and ATR spectroscopy, which will be discussed in the paragraphs below.

3.2.2 State of the art of IR-SEC

3.2.2.1 Transmission IR-SEC

In transmission IR-SEC, light emitted from the source is directed towards the sample. The sample, contained in an electrochemical cell, absorbs part of the IR radiation and the rest is transmitted through the cell towards the detector. The resulting difference between the emitted light and the detected light creates the IR spectrum. The electrochemical cell in transmission IR-SEC most frequently uses a mesh electrode configuration^{10,14,26–29}. This configuration was first reported by Murray et al.³⁰ for the use in UV/Vis-SEC and later adapted by Moss et al.¹⁰ for the use in IR-SEC.

The design by Moss et al.¹⁰ is shown in Fig. 3.2. As illustrated, a working electrode (WE) in the form of a Au mesh is sandwiched between two IR-transparent windows (CaF₂), creating a thin-layer configuration. A Teflon body keeps the cell together. The width of the cell and the WE is in the same order of magnitude as the width of the IR-light beam, allowing for the analysis of the entire cell. The design by Moss et al. has been used by several researchers over the past few years with little change in the design^{14,26–29,31}. Most changes are small, such as changing the WE material into a

Fig. 3.2: Schematic representation of the IR-SEC cell. The arrow represents the propagation of the IR-light beam (a) IR-transparent CaF₂ window mounted onto a (b) Plexiglas ring, (c) Teflon body, (d) steel surround, (e) Pt counter electrode, (f) the mesh working electrode, (g) O-ring, (h) capillary connection to the reference electrode. Reprinted from the original work by Moss et al.¹⁰, Copyright 1990, with permission from John Wiley and Sons

platinum mesh instead of a Au mesh^{28,29,31}. One notable setup that does not use the above-mentioned mesh configuration is the work done in the group of Swain^{32–34}, where boron doped diamond (BDD) deposited on undoped silicon is used as transparent electrode material. This has as benefit over the mesh configuration that light is not blocked by the electrode. Among the large number of applications found in literature, some common applications for transmission IR-SEC are investigations of proteins, such as their oxidation, reduction and folding properties^{10,14,26,27}. Another application is the study of porphyrins^{28,29,31}.

3.2.2.2 Reflection IR-SEC

Basic principles reflection IR-SEC

The basic principles for IR reflection spectroscopy are as follows: the IR light is emitted by the light source. The IR light propagates through an IR-transparent window (often CaF_2) into the electrochemical cell. Next the light beam hits the electrode, an IR reflective material, and is reflected out of the cell, as shown in Fig. 3.3. The reflected beam passes back through the same window into the detector. In Fig. 3.3, a typical schematic representation of a reflectance cell by Alwis et al.^{13,35} is shown. The WE is on top of a CaF_2 window creating a thin-layer configuration. The reference electrode (RE) is separated from the cell by a Luggin capillary and the counter electrode (CE) is looped around the WE.

Fig. 3.3: Schematic representation of a reflection setup. Republished with permission of Journal of The Electrochemical Society, original by Alwis et al.¹³; permission conveyed through Copyright Clearance Center, Inc.

Drawbacks of reflectance IR-SEC compared to transmittance IR-SEC mostly come from the somewhat more complex setup. When using a reflection setup it is necessary to introduce extra mirrors into the IR-spectrometer in order to focus the incident beam onto the electrode and focus the reflected beam onto the detector. Aligning these mirrors can be a time-consuming process, more labour intensive compared to transmission spectroscopy. The use of extra mirrors also introduces extra losses due to part of the light being absorbed instead of being reflected. An advantage of using reflection compared to transmission spectroscopy is that no light is blocked by the electrode material.

Fibre optic reflection IR-SEC

An alternative for the standard reflection setup is fibre optic reflectance spectroscopy. First developed by Shaw et al.³⁶ and used over the past decade primarily in, or in collaboration with, the group of Richter–Addo^{36–39}. The advantage of this specific configuration compared to reflection systems such as those by Alwis et al.^{13,35} is the ease of alignment of the source with the sample and electrode, allowing for smaller configuration of the setup. With the small thin-layer setup they claim better control over the applied potential than transmittance and ATR cells³⁶. This particular configuration is mostly applied to the study of porphyrins^{37–39}, although it should be noted that it can also be used for applications where reflection IR is currently used.

SNIFTIRS and PM-IRRAS

As mentioned in section 3.2.1.2, IR-SEC often operates with a thin-layer configuration. In reflection mode there are two different methods in order to perform background corrections. The first method, based on difference spectroscopy and most often reported in literature, is subtractive normalized interfacial Fourier transform infrared reflection spectroscopy (SNIFTIRS). Alternatively, polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS) is used. SNIFTIRS is an IR-SEC reflection technique first introduced in the early 1980's by Pons⁴⁰. The authors would like to note that the use of the term SNIFTIRS seems to be used inconsistently throughout literature. Several papers use the acronym SNIFTIRS^{13,35,40–42}, while other papers, seemingly using SNIFTIRS, don't make use of the term^{12,43–48}. SNIFTIRS is a technique used for a number of different applications, such as the study of the oxidation of small organic molecules (such as ethanol and ethylene glycol)^{12,43,46,47} and metal ions^{13,35,41}. SNIFTIRS is also performed in combination with SEIRAS, for example for the studies of proteins^{44,45}.

PM-IRRAS, an alternative for SNIFTIRS, was first performed by Golden et al.⁴⁹ in 1981. After this initial work, further notable improvements on the technique were done by Faguy et al.^{50,51}, the group of Corn^{52,53} and the group of Lipkowski^{54–57}. PM-IRRAS is most often used in cases where the potential modulation necessary for SNIFTIRS could damage the analyte of interest¹⁵. Additional advantages of PM-IRRAS over SNIFTIRS, as proven by Faguy et al.^{50,51}, include that PM-IRRAS is very insensitive to atmospheric water and carbon species and has an increased signal to noise ratio^{57,58}. PM-IRRAS is especially suitable for measurements of samples adsorbed on metal surfaces, such as electrodes. Explained in a simplified manner, in PM-IRRAS the polarization of the reflected light is modulated and the reflection takes place under

a grazing angle of incidence. Under s-polarization (perpendicular to the plain of incidence) there is no interaction between the light and electrode surface. However, when using p-polarization (parallel to the plain of incidence), constructive interference takes place at the electrode surface. By cycling the polarization of the light, a difference spectrum of the bulk (s-polarization) and at the electrode surface (p-polarization) can be obtained. PM-IRRAS has been reported in literature as, among others, a method for the study of self-assembled monolayers^{59,60} and lipid bilayers^{54–57}. For a more in-depth description of SNIFTIRS and PM-IRRAS the authors recommend reading the work of Guidelli¹⁵, Bard⁶¹ and Alkire et al.⁵⁷.

Electrode material and SEIRAS in reflection IR-SEC

Concerning SEIRAS in reflection IR-SEC, electrode materials used are Ni^{13,35,41}, Pt^{12,36–39,48,62}, Pd^{43,46,47} and Au^{44,45,60,63,64}. While only Liu et al. report on using the reflecting electrodes for surface enhancement^{44,45}, it is unlikely that the Au electrodes used by Hosseini et al.⁶⁰, Su et al.⁶³ and Villalba et al.⁶⁴ don't report any enhancement. For Pt, Pd and Ni, SEIRAS has been reported in the past⁶⁵, but none of the papers above mentioned FTIR-SEC papers report this effect. This might be because it is not relevant for their application or that the SEIRAS effect is negligible.

3.2.2.3 Attenuated total reflection (ATR) IR-SEC

Basic principles of ATR-IR-SEC

Attenuated total reflection infrared spectroscopy (ATR-IR) operates under the following principle: IR light is coupled into an internal reflection element (IRE), as shown in Fig. 3.4. When the light hits the sample interface at an angle higher than the critical angle (θ_{crit}), total internal reflection occurs, reflecting the light and creating an evanescence wave. This evanescence wave penetrates into the sample for several micrometres, with the electric field decaying at an exponential rate away from the surface. This has the advantage that the effective path length in ATR-IR is always in the order of several μm , and thus removes the need for a thin-layer configuration. In order to get a good estimation of the penetration depth in ATR-IR-SEC the following formula can be used⁶⁶:

$$de = \frac{\lambda}{2\pi \sqrt{\sin^2 \theta - \left(\frac{n_2}{n_1}\right)^2}} \quad (3.2)$$

In this equation de is defined as the penetration depth (m) at which the intensity of the wave is reduced to $1/e$ of its original value, or approximately 37%. Furthermore λ is the wavelength (m) used, n_1 is the refractive index of the IRE, n_2 the refractive index of the sample and θ is the angle of incidence ($^\circ$)⁶⁶. This formula can give a good estimation of the penetration depth and the effect of the IRE material on the penetration depth of the system. It can consequently be used to get an estimate of the path length through the sample.

Fig. 3.4: Internal reflection element. Reprinted and adapted from the original work by Schadle and Mizaikoff⁶⁶, Copyright 2016, with permission from SAGE Publications.

IRE material ATR-IR-SEC

Penetration depth is one reason to choose for a certain ATR material, other reasons are availability, hardness and inertness. In table 3.1, three different materials used for the IRE in ATR-IR-SEC are shown: diamond, ZnSe and Si. Diamond is more expensive than ZnSe or Si, but its large spectral window^{66,67} (2-25 μm), its chemical inertness and the high hardness are clear advantages. ZnSe is cheaper than diamond and has a similar spectral window^{66,67} (1-25 μm) but is relatively soft and can only be used between pH 5-9. Finally, and also cheaper than diamond, there is Si which is harder than ZnSe and somewhat more inert, but suffers from a reduced spectral window^{66,67} (2-7 μm). Surprisingly, Ge, a commonly used ATR material, does not seem to be used in ATR-IR-SEC. A possible reason for this is the fact that Ge is more conductive than diamond, ZnSe and Si and therefore could potentially cause interference with the simultaneous operation of the electrochemical cell.

Another variable in table 3.1 is the configuration of the beam path through the IRE. As shown in the table, a single bounce or a multi bounce configuration can be used. Reasons to choose a certain configuration include the law of Lambert-Beer (eq. 3.1), since a multi bounce configuration, where the light interacts more often with the sample, effectively has a longer path length.

Electrode material and configuration in ATR IR-SEC

Also reported in table 3.1 are the three different setups for the placement of the WE. The first configuration (denoted as I in table 3.1), is the so called optically transparent electrode. This is either made out of a thick Au mesh deposited on the IRE, similar as those reported in transmission IR-SEC (0), or an optically transparent layer deposited on top of the ATR crystal. These optically transparent electrodes, such as boron doped diamond (BDD), nitrogen doped diamond like carbon (N-DLC) or boron doped silicon (BDSi), could potentially also be used as ATR material, but this does not seem to be applied in practice. A possible reason for this could be fabrication limitations. A schematic representation of an ATR-IR-SEC design using the optically transparent electrode configuration can be seen in Fig. 3.5.

The second configuration (denoted as II in table 3.1), is similar to the first configuration in the sense that the electrode material is deposited on the top of the IRE, but instead of an optically transparent material or mesh, a thin layer of metal is

Table 3.1: Material and configuration used for IRE and electrode in ATR-IR-SEC. Setup I: Optically transparent electrode (Fig. 3.5), setup II: SEIRAS (Kretschmann configuration), setup III: Spacer setup (Fig. 3.6).

IRE	Setup	Electrode material	Configuration	Author
diamond	III	carbon nanofibres	single bounce	Dillard et al. ⁷⁴
diamond	I	boron doped diamond	single bounce	Neubauer et al. ⁷⁹
diamond	II	boron doped diamond / Au nanoparticles	single bounce	Izquierdo et al. ⁷⁸ López-Lorente et al. ³¹⁶
Si	III	carbon paper	multi bounce	Healy et al. ⁷³
Si	III	carbon paper	multi bounce	Paengnakorn et al. ⁷²
Si	II	Au / Pd	not mentioned	Yang ⁴⁶
Si	II	Au	not mentioned	Cheunquepán et al. ³¹⁷
Si	II	Au	single bounce	Dunwell et al. ³¹⁸
Si	II	Cu / Au	single bounce	Heyes et al. ⁷⁵
Si	II	Au	single bounce	Ataka et al. ²⁰
ZnSe	I or II	Sn / SnO _x	single bounce	Baruch et al. ⁷⁰
ZnSe	I	nitrogen doped diamond like carbon	multi bounce	Menegazzo et al. ⁸⁰
ZnSe	I	Au	single bounce	Pfaffeneder-Kmen et al. ⁷⁶
ZnSe	I	boron doped silicon	single bounce	Purushothaman et al. ⁸¹
ZnSe	II	Au	single bounce	Viinikanoja et al. ⁷⁷
ZnSe	II	Au	multi bounce	Zimmermann et al. ³¹⁹

deposited. This setup, known as the Kretschmann configuration^{68,69}, is a method in ATR FTIR to perform SEIRAS experiments. The thin metal layer here acts as an electrode and as a surface for SEIRAS simultaneously. This thin-layer electrode has some drawbacks: if the layer is too thick, too much IR absorbance by the material occurs, if it is too thin, holes in the continuous layer might occur. Baruch et al.⁷⁰ deposited a thin layer of Sn/SnO_x as electrode material on top of an ATR crystal. They do not mention any SEIRAS effects, but one might argue that it is likely that SEIRAS does take place. The reasoning for this is that Sn has been reported to have surface enhanced properties in the infrared region⁷¹, making it debatable whether it should be in configuration I or II. The final configuration (denoted as III in table 3.1) uses a thin porous spacer between the IRE and the electrode. As one can see in table 3.1 and in Fig. 3.6, a thin porous layer, either a Nafion membrane⁷², carbon nanoparticles⁷³ or carbon fibres⁷⁴, is placed on top of the IRE. A carbon paper electrode is placed in contact with this layer, covering the surface and functioning as a WE. This setup has as advantage that it does not need labour intensive deposition techniques.

Fig. 3.5: Schematic of an optically transparent electrode ATR IR-SEC setup, in a single bounce configuration. Reprinted with permission from Springer Nature: From the original work by Purushothaman et al.⁸¹, Copyright 2012

A large number of applications regarding ATR-IR-SEC are reported in literature. Some examples are the study of metalloproteins^{72,73}, the study of CO₂ reduction without⁷⁰ and with the combination of SEIRAS⁷⁵, the reduction of graphene oxide^{76,77}, technique development^{78–80} and practical applications such as observing lubricant degradation⁸¹.

3.2.2.4 IR-SEC microscopy

In IR microscopy, the light from an IR light source is coupled into an IR microscope. Just as with conventional microscopy, the spatial resolution is limited by the diffraction limit of the light. Since IR light has a relatively large wavelength compared to visible light, the spatial resolution is, at its lowest, in the order of about 5 μm . Nowadays, infrared microscopes with focal plane arrays are available, allowing not only IR microscopy but also imaging the sample. The combination of IR microscopy and SEC has found some limited applications by using either a conventional source or synchrotron radiation.

Conventional source

The most notable work in using IR-SEC spectroscopy with a conventional source comes from the group of Sun⁸², who developed their own IR-SEC microscope in the late 1990's. They further developed this instrument in the last two decades for a

Fig. 3.6: Schematic representation of the cell configuration using a spacer between the IRE device and the electrode. As shown, the Pt CE and calomel RE have more space than in the thin-layer configuration used for transmission and reflectance IR-SEC. In the inset one can see the IRE, the porous Nafion membrane containing the analyte and the carbon paper electrode on top. A multi-bounce configuration is used. Reprinted from the original work by - Paengnakorn et al.⁷² published by The Royal Society of Chemistry

variety of different applications regarding electrode interface reactions^{83–85}. Sun and Zhou also wrote a paper in which they further explain the use of FTIR microscopy for electrochemical applications⁸⁶.

Synchrotron source

Next to IR microscopy using conventional IR sources, IR using synchrotron radiation have also been reported. The first experiments regarding the use of synchrotron radiation with electrochemistry were reported by Melendres et al.⁸⁷ in 1995, using the frequency in the far infrared. The first experiment that coupled mid IR synchrotron radiation with electrochemistry was the work by the group of Burgess⁸⁸ in 2011, where they proved that, when using small aperture settings, synchrotron radiation has a better signal to noise ratio than conventional sources. Further notable work with synchrotron IR microscopy combined with electrochemistry was done on spatial mapping of methanol oxidation on a PtNi alloy⁸⁹, femtomole detection of ferrocyanide on ultra-microelectrodes⁹⁰ and quantitative analysis of electrochemical diffusion layers⁹¹. Other notable work regarding synchrotron radiation in combination with electrochemistry was done by Ash et al.⁹² on synchrotron based infrared microscopy to study biological redox processes under electrochemical control. In this paper they show that they can observe changes in the Flavin mononucleotide active site of a flavoenzyme with synchrotron radiation.

Although the power of synchrotron radiation is proven by the work done by researchers mentioned above, it has to be noted that the need of synchrotron radiation is a major drawback to using synchrotron based IR spectroscopy. Access to a synchrotron source is limited and will stay so in the foreseeable future.

3.2.3 Future perspective

The fields of IR and SEC are ever increasingly combined and advanced. Recently, a three-electrode electrochemical ATR cell from Pike Technologies called the Jackfish SEC Cell has appeared on the market. The J1W version of this device uses commercially available disposable IRUBIS ATR-SEIRAS devices, making IR-SEC suitable for a broader audience⁹³.

It can be postulated that further improvements in the field of IR-SEC are linked to the advancements made in the fields of IR spectroscopy and electrochemistry. These advancements will take some time to be implemented into IR-SEC, but it is logical to assume that any advancement improving the separate fields, will eventually be implemented in IR-SEC.

3.2.3.1 New Sources for IR-SEC

One of the major drawbacks of IR spectroscopy is the low intensity of the light source. High power sources are available in the form of optical parametric amplifiers (OPA) and optical parametric oscillators (OPO) but these systems are expensive and labour intensive to both operate and construct. A more affordable, user friendly and compact option is a quantum cascade laser system (QCL). Some QCL instruments such as IRSweep's Iris-f1 are complete spectrometers, making IR-laser systems more available. In Fig. 3.7, a comparison between the output of a QCL by Daylight solutions, a synchrotron and a Globar source is made by Weida and Yee⁹⁴.

Fig. 3.7: Comparison of Weida and Yee of three different IR sources of the signal through a 10 μm pinhole⁹⁴. Reprinted and adapted from the original work by Weida and Yee⁹⁴, With permission from SPIE and M. Weida.

The comparison as shown in Fig. 3.7 is made when passing the light through a 10 μm pinhole. Subsequent spot sizes of the three techniques are normalized. This comparison highlights the strength of QCLs in IR-microscopes, yet for normal spectroscopy this graph is skewed somewhat in favour of the QCL device. Nonetheless, the main message stays the same, being that a higher source power can be obtained with QCLs. Due to the higher power output of the QCLs, a shorter measurement time compared to regular IR instruments can be achieved. A commercially available QCL system from IRsweep, using optical heterodyne detection, even further improves this time resolution. This IRsweep instrument can take a spectrum with a spectral width in the order of 100 cm^{-1} within 4 μs . This allows for a more accurate understanding of the dynamic processes occurring at the electrode interface.

A drawback of QCLs is the shorter wavelength coverage compared with standard IR sources. The wavelength range of a single QCL is usually somewhere in the order of a micrometre. This can be compensated to some extent by having several lasers in a system. QCLs have already been used for several IR techniques^{95,96}, and therefore could be implemented in IR-SEC in the near future.

Fig. 3.8: Schematic representation of the evanescent wave/field with an ever decreasing IRE thickness. As one can see in (a) and (b), the smaller IRE has more reflections than the bigger IRE. When the size is small enough, as one can see in (c), a waveguide is created with a continuous evanescent field. Reprinted from the original work by Schadle and Mizaikoff⁶⁶, Copyright 2016, with permission from SAGE Publications.

3.2.3.2 From IRE to (single mode) waveguides

When having access to laser based sources the use of waveguides instead of IREs also becomes more attractive. A simplified explanation of waveguides can be found in Fig. 3.8. In Fig. 3.8a & b an IRE is shown, while Fig. 3.8c displays a single mode waveguide. The figure illustrates that reducing the size of the IRE increases the amount of bounces the light makes through the device, effectively increasing the path length. In Fig. 3.8c the thickness of the device has been reduced to such an extent that it cannot be considered an IRE anymore, but a waveguide with a continuous evanescent field along the length of the device⁶⁶. An added benefit of making the IRE smaller is that the losses due to the absorption of the IRE material are reduced, since the effective path length through the IRE decreases. While making the IRE smaller can improve the signal to noise ratio, it can also make it more difficult to couple light into the device. By using a source with a higher power and small beam size, such as the aforementioned QCLs, these losses might be mitigated. According to literature, a waveguide might lead to significantly increased sensitivity compared to an IRE device^{66,67}. Several works on infrared waveguides^{97–104} have already been published and it is therefore not unthinkable that the first IR-SEC waveguide will be published in the near future.

3.2.3.3 Novel electrode material

An interesting material for IR-SEC to be used as a (true) transparent electrode in transmission mode or as waveguide material is BDD. BDD is optically transparent for infrared light and can be used as an electrode. BDD has already been used as transparent electrode material on top of an IRE⁷⁹ and as an optical electrode in the transmission configuration^{32–34}. However, it might be possible to use the diamond

layer as IRE / waveguide and electrode simultaneously. This would make it possible to measure as close to the electrode as physically possible. A drawback of using BDD as a waveguide are the extra losses the boron doping causes at wavenumbers of 1800 cm^{-1} and higher^{32–34,105}. When the already small waveguide also functions as electrode, a device with a small footprint can be created. This way it can be easily combined with microfluidics in order to make lab-on-a-chip systems.

3.2.3.4 Microfluidics and lab-on-a-chip systems

The hyphenation of electrochemistry and microfluidics and lab-on-a-chip systems is already well established. For a review regarding this subject the authors recommend to read the paper by Odijk et al.¹⁰⁶.

Although less established than electrochemistry, the combination of IR with microfluidics is also a developing field, for example: an ATR-IR device for the study of electric field-driven processes¹⁰⁷, reaction monitoring¹⁰⁸ and the analysis of chemical reaction intermediates¹⁰⁹. A recent review written on spectroscopic microreactors for heterogeneous catalysis by Rizkin et al. also includes a section about IR¹¹⁰. The combination of IR-SEC and microfluidics is not an established field yet, although some work has been done by Führer et al.¹¹¹. It is expected that in the near future more work regarding IR-SEC is published.

3.2.3.5 Improving spatial resolution

As mentioned in section 3.2.2.4, the optical IR microscopy imaging is limited to a spatial resolution in the order of $5\text{ }\mu\text{m}$. Two different atomic force microscopy (AFM) based techniques can improve the spatial resolution of IR techniques, namely photothermal induced resonance (PTIR) and Nano-FTIR.

PTIR

PTIR is a technique that combines AFM with infrared spectroscopy. This hyphenated technique has the optical resolution of the AFM technique and the ability to obtain the chemical information usually obtained with IR. In PTIR, the probe tip of an AFM microscope is positioned close to the region of interest. Next, a tuneable IR laser is focused on the region close to the probe tip. If the sample has a vibrational frequency that corresponds to the wavelength emitted by the laser, absorption will take place. This absorption results in a thermal expansion of the sample, which then will be measured by the AFM cantilever^{112,113}. The resulting spatial resolutions for commercially available instruments are in the order of 20 nm , at least two orders of magnitude lower compared to optical microscopy^{112,113}. To our knowledge, this technique has not yet been combined with SEC. One possible explanation could be that SEC introduces too much heat in the sample, which could interfere with the AFM measurement.

Nano-FTIR

In Nano-FTIR, FTIR and scattering-type scanning near-field optical microscopy (s-SNOM) are combined. IR light from a conventional IR laser source or a synchrotron source is focused on a conductive AFM probe tip. This probe tip acts as an antenna for the IR light and focuses and enhances it. The near field interaction between the probe tip and the sample influences how the light is being scattered. Recording the scattered light results in a spectrum of the sample. While imaging the surface, a map

with special resolution as small as 10 nm can be obtained^{112,114,115}. One application of Nano-FTIR combined with SEC was reported by Lu et al.¹¹⁴ where they study the molecular structure of graphene-liquid interfaces, allowing new possibilities for the characterization of graphene-electrolyte interfaces.

3.2.3.6 Advanced SEIRAS structures

Finally, SEIRAS could be improved by replacing rough metal films with nanofabricated structured SEIRAS arrays or slits. Nanofabricated plasmonic nanoantennas have shown to be promising for the use in SEIRAS¹¹⁶. These devices have been reported to have an improved SEIRAS effect, although they usually only operate at a small wavelength range. This however would make them a suitable to be used with QCLs, as they can be tuned to the same wavelength as the relatively narrow-band QCL laser.

3.3 Raman spectroelectrochemistry (Raman-SEC)

3.3.1 Introduction Raman-SEC

3.3.1.1 Raman Spectroscopy

Just like IR spectroscopy, Raman spectroscopy is a technique used to get information from the vibrations of molecule. In short, Raman spectroscopy operates based on the Raman scattering effect. A light source, often a laser with a wavelength in the near infrared, visible or near ultraviolet, is directed at the sample. When the light interacts with the sample, photon scattering takes place. Most of the light is elastically scattered from the sample, also known as Rayleigh scattering, and has the same wavelength as the incoming light. A small percentage of the light is inelastically scattered, also known as Raman scattering, resulting in a shift in wavelength corresponding to the frequency of a molecular vibration. The shift can be to higher wavelengths (Stokes shift) or lower wavelengths (anti-Stokes shift).

Raman is often considered to be complementary to IR spectroscopy. The reason for this is that, in order to be visible in Raman spectroscopy, the vibration has to experience a change in polarizability. In other words, a change in how easy it is to distort the electron cloud around the molecule. As mentioned in the previous section, IR active molecules require a change in dipole moment in order to be visible in IR spectroscopy. Often vibrations that are polarizable do not have a dipole moment and vice versa, making the techniques complementary.

A typical setup used in Raman spectroscopy is shown Fig. 3.9, as reported by Schmid and Dariz¹¹⁷. It consists of a light source, most often a laser, focused on the sample using a microscope objective. The scattered light is guided back into the instrument and a filter removes the Rayleigh scattered light. The Raman scattered light is guided onto the grating of a monochromator and eventually reaches the detector, where it records the resulting spectrum.

3.3.1.2 Surface enhanced Raman Spectroscopy (SERS)

Fleischmann et al.¹¹⁸ made the first observation of SERS in 1974. Since then the work by the group of Van Duyne^{119,120}, the group of Moskovits¹²¹⁻¹²³, Otto¹¹⁹ and Schlücker¹²⁴ further pioneered the field of SERS. In a simplified explanation, SERS is a

Fig. 3.9: Schematic representation of a Raman setup. Reprinted from the original work by Schmid and Dariz¹¹⁷, Copyright 2019, with permission from Creative Commons.

technique that is used to enhance a Raman signal, similar to SEIRAS. This is primarily done through the electromagnetic interaction of the incident Raman light beam with the metal surfaces^{18,23}. This results in a phenomenon called plasmon resonance which amplifies the laser field²³. This phenomenon only occurs very close to the surface of the metal substrate. Typically, the analyte is required to be relatively close to the substrate, not further away than 10 nm^{18,23}. The substrate, most often Ag or Au, should contain microstructures, either periodically nanofabricated or randomly orientated as encountered in roughened surfaces, to increase the intensity of the enhancement.

A second factor for enhancement that should be discussed is the “chemical” enhancement. Unlike the well-defined electrochemical enhancement, the term “chemical” enhancement describes more of a collection of different phenomena¹²⁵. Simplified, the chemical enhancement mostly describes phenomena due to the charge transfers between the analyte molecule and the metal substrate and other metal substrate / analyte molecule interactions. The chemical enhancement is significantly lower than the electrochemical enhancement, and much harder to theoretically explain and predict¹²⁴.

In practice, distinctions can be made between three different kinds of SERS substrate. The first are randomly orientated enhancement surfaces, such as roughened (electrode) surfaces and randomly orientated nanoparticles deposited or grown on a surface, which are relatively easy to fabricate. Second are the so called

structured arrays, which can exist of a self-assembled array nanoparticles deposited on a substrate or nanofabricated arrays. These structured arrays can obtain higher enhancement factors than the roughened surfaces but are more difficult to fabricate. Finally, in order to improve measuring in liquid samples, metallic particles can be used in order to create SERS active colloidal solutions²³. This last form, using colloidal particles, cannot be used in SERS-SEC, since it cannot function as or be in contact with the WE easily. With the right combination of SERS substrate material and configuration of the structures, enhancement factors of up to 10^{10} can be achieved, depending on the used definition of SERS enhancement²³.

One important note on working with nanoparticles is the need for proper cleaning of the particles in order to maximize the SERS effect. The recent work of Montiel et al. describes some of the methods that can be used for the cleaning of these particles¹²⁶.

SERS is a complex field with persistent misconceptions¹²². Therefore a complete review on the subject is beyond the scope of this study. The authors of this review recommend that the reader reads the work cited for a more in-depth explanation of SERS^{23,65,118–124,127}.

3.3.1.3 Raman-SEC

In Raman-SEC, a Raman instrument, often a Raman confocal microscope, is coupled to an electrochemical cell. Compared to FTIR SEC, a Raman setup is somewhat more versatile in the cell design. Since Raman is a scattering technique, it is not limited to transparent electrodes as is the case with transmission IR-SEC. Measurements usually take place at the electrode surface, so the laser is most often used in a configuration where it is focused on the electrode. Even compared to reflection and ATR IR-SEC, the setup of the cell can be considered more user friendly. The optical window of the cell can be made out of standard materials since a visible light source can be used. The thin-layer configuration is not necessary, as water does not have strong absorptions in typical wavelengths used for Raman spectroscopy. Moreover, confocal lenses are often used. Once access to a Raman instrument is available, combining it with SEC is therefore relatively straightforward.

3.3.1.4 SERS-SEC

As written above, the first report of the observation of the SERS phenomenon is considered to be made by Fleischmann et al.¹¹⁸. In this experiment, SERS was observed by studying pyridine on a Ag electrode. This experiment highlights the good synergy between SEC and Raman. The metallic electrodes used for the electrochemistry are naturally suited to perform SERS measurements, and adapting an electrode to be more SERS active usually does not negatively interfere with the electrode behaviour. For this reason, Raman-SEC measurements will experience the SERS effect to some extent, which can be either negligible or indeed the selling point of a certain configuration.

3.3.2 State of the art of Raman-SEC

3.3.2.1 Cell configuration and electrodes

There is a large variety of different materials for Raman electrodes. Reports range from standard metallic electrodes such as Au electrodes, to cells made by 3D printing¹²⁸, screen-printed electrodes^{129–136} and even electrodes on fabric¹³⁷.

In the literature, several different cell configurations are described. Most commonly used is the stationary electrode configuration. Other configurations include the rotating cell, the linearly moving cell or the flow through cell. Two important reasons to choose a certain cell are the ease of use and the balance of fabrication versus potential photo-degradation. Photo-degradation of samples can be a severe problem in Raman spectroscopy. Constant illumination of a substrate can heat up the sample and eventually cause burning or evaporation of the sample or matrix. In the next section, we will discuss the different cells, their advantages and disadvantages, and how they deal with photo degradation.

Stationary electrode

The stationary electrode, as shown in Fig. 3.10 is the most commonly used configuration. Since the laser is stationary with respect to the electrode, alternatives should be considered in order to reduce damage due to the laser beam. Depending on how susceptible the sample is to photo degradation, one should limit the laser power and the exposure time of the sample.

Linearly moving electrodes and cells

In order to reduce photo degradation, several setups have been designed to limit the time the Raman laser remains on a single spot on the substrate. One such a device operates by moving the entire cell and its electrode as can be seen in

Fig. 3.11. The cell moves together with the electrode in a linear path with respect to the Raman light source, reducing the time for which a single spot on the electrode

Fig. 3.10: Example of a stationary electrochemical cell used for Raman experiments. Reprinted from the original work by Fleischmann et al.¹¹⁸, Copyright 1974, with permission from Elsevier.

Fig. 3.11: Schematic of a moving electrode cell. Ne is the Neon lamp and D is the rotating disk rotating around the axis represented by the black dot. Reprinted from the original work by Niaura et al.¹³⁸, Copyright 1997, with permission from John Wiley and Sons.

is exposed to the Raman source. The linear moving electrode was first developed by Niaura et al.¹³⁸ and is mostly used by Mažeikienė et al. to study polyaniline^{139–144}, dyes^{145–148} and hexacyanoferrates^{149,150}.

Rotating electrodes and cells

Next to the moving electrode system, a rotating Raman system has been first developed by Kiefer and Bernstein¹⁵¹ in 1971. One of the first rotating Raman-SEC setups was reported by Yamada et al.¹⁵² in 1978, as shown in Fig. 3.12. An advantage from an electrochemical point of view for both the rotating electrode and the moving electrode is that there are no mass transport limitations due to the added convection. Some use of the rotating electrode has been reported in the 70's and 80's^{152–156}, but, despite the potential of the rotating electrode, little mention of its use is reported in recent literature. An alternative to the rotating electrode configuration is the rotating cell configuration, in which instead of the electrode the entire cell is being rotated. This device was first used by Kavan et al.¹⁵⁷ in 2009. The WE is positioned at the centre of the motor axis while the sample rotates. Despite the potential for this setup, it is only mentioned once more in literature, for Fullerene C₆₀ in an aqueous medium¹⁵⁸.

Flow through cell

Another way to reduce the chance of photo-degradation is a flow through cell, which is easy to use for non-viscous liquids, but not applicable for solid samples. Most commonly, a flow through cell either operates by having a constant flow of fresh liquid flowing over the WE^{77,159} or by continuous mixing of the electrolyte solution¹⁶⁰.

Alternative designs

A special configuration that is worth mentioning is the setup by Ibañez et al.¹⁶¹, using a 4-electrode system in order to study the charge transfer at a polarisable liquid/liquid interface. Another interesting design is the Raman spectroscopy of a carbon super capacitor by Bohomme et al.¹⁶²

Fig. 3.12: Rotating Raman-SEC device. Reprinted from the original work by Yamada et al.¹⁵², Copyright 1978, with permission from Elsevier.

3.3.2.2 Application Raman-SEC

Materials

Raman-SEC is used in a multitude of research fields and for different applications. An important research field for Raman-SEC is material science to investigate novel materials. One example is the research done on carbon nanotubes. Most of this extensive research comes from the groups of Dunsch and Kavan^{163,164,173,174,165–172}, although some work has been performed elsewhere^{175–180}. A related field is the investigation of graphene^{77,160,181–187}. Another popular application of Raman-SEC within material science is to get more insight on conductive polymers, studied by the group of Malinauskas^{139–144,188} and others^{189,190,199,191–198}. A number of applications are reported on the investigation of dyes^{145–148,200,201}, small organic molecules that can form monolayers on the electrode material^{202–206} and protein / cell studies^{138,207–209}.

Sensing applications and point of care

Research aimed towards real world applications is also present in Raman-SEC, although underrepresented and almost solely performed by the group of Brosseau. Some interesting applications include a portable SERS-SEC device for the detection of melanine in milk¹³⁴, the detection of DNA biomarkers in synthetic urine¹³⁵, the detection of 6-thiouric acid in synthetic urine²¹⁰, the quantitative detection of uric acid in synthetic urine¹³⁶ and a point of care device based on SERS-SEC on a fabric substrate¹³⁷.

3.3.3 Future perspective

Raman-SEC is already a more mature technique than for example IR-SEC. This does not mean, however, that the technique has stopped to progress. The authors of this review are of opinion that most future development will take place on the

SERS substrates and the development of new applications for Raman-SEC, especially towards real world applications.

3.3.3.1 Nanofabricated SERS structures

Further developments towards structured nano arrays for SERS applications are expected. As is shown in Fig. 3.13 by Yuan et al.²¹¹, highly structured nano arrays consisting of thin Au lines can be used as SERS substrate, resulting in high enhancement factors while still functioning as electrode. New lithographic techniques make the wafer-scale fabrication of such substrates easier²¹², while novel methods to improve the adhesion of these Au structures on silicon²¹³ have also been developed in recent years.

Compared to, for instance, Au islands and randomly distributed particles, these nanostructured arrays can achieve a higher reliability, since the enhancement is higher and equal over the entire surface. This makes these structures more suitable for quantitative measurements compared to the randomly organized substrates.

3.3.3.2 Electrochemical-surface oxidation enhanced Raman scattering (EC-SOERS)

EC-SOERS is a relatively new technique that was first introduced by Perales-Rondon et al.^{214,215}. According to Perales-Rondon et al., EC-SOERS cannot be easily explained using the classical models of SERS^{214,215}. In EC-SOERS, enhancement is only observed when the Ag WE is undergoing anodic oxidation. They postulate that during this process nanostructures are formed that are favourable for SERS enhancement. Garoz-Ruiz claims that this technique might be an alternative for molecules that are usually not very sensitive in SERS. Several questions need to be answered to determine the potential of this SOERS techniques for the future, such as elucidating the enhancement mechanism and determining suitable applications.

Fig. 3.13: In-situ SERS-SEC system by Yuan et al.²¹¹ (a) Schematic representation of the Raman SEC analysis system, (b) SEC-cell composed of a SEC-chip directly bonded to a small-volume microfluidic sample chamber with an optical interface to a microscope objective, (c) SEC-cell consisting of a Pt CE and nanostructured Au WE patterned on the Si SEC-chip and an external Ag/AgCl RE, and (d) representative SEM image of the Nanostructures Au WE. Reprinted with permission from Yuan et al.²¹¹. Copyright 2015 American Chemical Society.

3.3.3.3 Improving time and spatial resolution

Time resolved Raman spectroscopy

Most commonly, Raman-SEC is performed using the so-called steady state method. In the steady state method, the electrode potential is set to a desired value and given time to stabilize^{216,217}. After this stabilization period, a Raman spectrum is acquired. This steady state method has the benefit of being highly reproducible in obtaining data. However, information is lost when operating in this steady state configuration, such as information on reactive intermediates. Several experiments have been done in the 1990's regarding time resolved Raman spectroscopy^{218–223}. Van den Beld et al. have recently used an in-house developed graphene spectroelectrochemical cell to study the adsorption behaviour of simple redox couples in a more dynamic fashion, i.e. acquiring real-time Raman spectra while recording cyclic voltammograms at a scan rate of 25 mV/s¹⁸⁷. Also recently, Zong et al.²¹⁷ published on a transient SERS-SEC instrument that can acquire data at millisecond time resolution without the need to repetitively cycle the data acquisition. Further improvements in the field of time-resolved Raman-SEC might lead to a better understanding of reaction intermediates at the electrode surface.

Spatial resolution

One method in Raman spectroscopy that can be used in order to improve the spatial resolution is tip-enhanced Raman spectroscopy (TERS). First proposed by Wessel²²⁴ in 1985 and first independently developed by Stöckle et al.²²⁵, Anderson²²⁶, Hayazawa et al.²²⁷, and Pettinger²²⁸ in 2000. In short, a metal or metal-coated tip is illuminated with a laser. The metal tip acts as a substrate for surface enhancement and can therefore be used for sensing. In general, TERS achieves an enhancement factor of about 10^3 to 10^6 , and has a spatial resolution between 10-80 nm²²⁹, smaller than the diffraction limit of light. The combination of SEC and TERS is a relatively new field, first introduced in 2015 by Korouski et al.²³⁰ and Zheng et al.²³¹ We expect that TERS-SEC will find more interest in the future.

3.3.3.4 Real world applications

As shown in paragraph 3.2.2, most of the research done with Raman-SEC is on fundamental research for material sciences. Recently, a paper was published on compact Raman-SEC for a portable point-of-care device¹³⁴, as well as a paper on a low-cost portable device for the study of carbon nanotubes¹⁷⁹. These advancements might make Raman-SEC more accessible, possibly enabling a break-through towards more real-world applications.

3.3.3.5 Shell-isolated-enhanced-Raman-spectroscopy particles (SHINERS) and catalysis

Another new development related to SERS are so called SHINERS. SHINERS, developed by the group of Tian²³², are promising SERS particles coated with an inert layer for the use of catalyst monitoring. This both protects the typically Au nanoparticles from the high temperature and pressure conditions required for catalysis and at the same time prevents the Au to act as a catalyst, while the particles still exhibit the SERS effect²³². The group of Tian²³³ also performed notable work on combining SHINERS with electrochemistry. In the work Li et al.²³⁴ they combine SHINERS with electrochemical methods (EC-SHINERS) in order to monitor the pyridine absorption on single crystal Au surfaces *in-situ*. The structured nanowires

mentioned before (Fig. 3.13) could also be made into SHINERS-like structures by coating them with Si. Although these SHINERS-like nanowires would no longer be suitable for use as electrodes, they could find applications in the field of e.g. elucidating photoelectrocatalytic reaction mechanisms.

3.4 Nuclear magnetic resonance spectroelectrochemistry (NMR-SEC)

3.4.1 Introduction NMR

Nuclear magnetic resonance (NMR) spectroscopy is a technique which is often used to obtain structural data about analytes. It typically operates within radio frequencies in the range of 60 to 1000 MHz (wavelengths of ca. 5 to 0.3 m). These low-energy waves can interact with nuclei that possess a magnetic spin, for example the isotopes ^1H and ^{13}C . For NMR spectroscopy, the different spin states of the nuclei get separated with a powerful magnetic field. The surrounding atoms and functional groups in a molecule influence how strongly the outside magnetic field affects the target nucleus locally. As a consequence, NMR spectroscopy is able to obtain comprehensive structural information of the molecule.

The sample can be manipulated and analysed with radio frequency radiation generated with a transmitter inside the spectrometer and the NMR probe around the sample. Nowadays, there are many different pulse patterns being used for sample excitation under varying experimental conditions.

For more detailed information, the authors refer the reader to a recent review paper by Colnago et al.²³⁵ and the references therein, explaining the fundamentals and challenges of NMR spectroscopy.

3.4.2 State of the art of NMR-SEC

NMR spectroscopy is a versatile tool to identify molecules non-destructively and accurately. It is most commonly used for the analysis of organic compounds, although there are also many applications which involve metals or metalorganic compounds. This versatility makes NMR spectroscopy a good coupling partner to EC, which can e.g. struggle to attribute signals correctly in the presence of side reactions. By combining the two techniques, however, mechanistic and kinetic information can be obtained *in-situ* accurately and in a much more flexible way than e.g. with UV/Vis or IR spectroscopy, which can be limited by the functional groups present in the analyte.

However, because of the strong dependency of NMR measurements on the electromagnetic environment of a nucleus and the involvement of a strong outside magnetic field, NMR-SEC is not trivial. Combining EC with NMR leads to interference between the electrical and the magnetic fields, often caused by the electrode configuration, which can worsen the signal to noise ratio and signal resolutions in general. Additionally, instrumental failure upon simultaneous use of both systems has been reported, making real-time *in-situ* measurements challenging²³⁶. Pioneering work on NMR-SEC was performed by Wieckowski and associates. Many of their reported approaches were based on transferring the reaction products from an EC-cell into an NMR tube and then analysing them with an NMR spectrometer in

an offline manner^{237,238,247,239–246}. Other works included dedicated SEC-NMR cells^{248,249} or placing an EC-cell into an NMR spectrometer²⁵⁰.

A simple way to avoid possible interference between the two methods is to use an online technique which separates the electrochemical and the NMR-spectroscopic steps. However, this leads to much longer response times than is necessary to observe electrochemical intermediates and short-lived reaction products and is difficult to automate. To our knowledge, online EC-NMR methods are rarely being reported.

In this review chapter, we instead focus on the liquid sample *in-situ* EC-NMR methods reported in recent years and briefly mention their respective applications. For information on NMR applications in battery research, Hu et al. have recently published a review paper²⁵¹.

3.4.2.1 Three-electrode cells as NMR tube insert

Because of the small confines imposed by standard NMR spectrometers, most of the recently reported *in-situ* NMR-SEC approaches for liquid samples rely on integrating a homemade EC cell into a standard NMR test tube. An integral part of this kind of setup is the use of chokes to reduce the encountered interference between the NMR spectrometer and the potentiostat, which was specifically characterized in some recent publications^{252,253}.

Carbon fibre electrodes

Recent publications using carbon fibre WEs are based on a design originally published by Klod et al. in 2009²⁵⁴. The design is shown in Fig. 3.14 and incorporated three electrodes. The WE and CE both consisted of carbon fibre filaments in sealed

Fig. 3.14: Schematic of a three-electrode NMR-SEC cell with carbon fibre WE and CE, partially placed in the NMR detection area. Reprinted with permission from Klod et al.²⁵⁴ Copyright 2009 American Chemical Society.

glass capillaries and the pseudo-RE was a PTFE-covered Ag wire with a AgCl tip. Only the active region of the WE was placed in the NMR detection area. The motivation to use carbon fibres was the higher possible reaction rate to obtain enough electrolysis product for ^1H and ^{13}C NMR measurements in lower amounts of time. Recent applications of this cell included the analysis of *p*-benzoquinone^{254,255}, 2-diphenylaminothiophene based compounds²⁵⁶ and a perfluoroalkylated fullerene²⁵⁷. In the last case, ^{19}F NMR spectroscopy was performed as opposed to the more common ^1H and ^{13}C experiments. The measurements in all of the applications just mentioned were performed over large timeframes of up to 17 hours of electrolysis with periodic NMR spectroscopy sampling. Nunes et al. used a very similar configuration with the RE placed above the NMR detection area in a 10 mm NMR tube for the reduction of 9-chloroanthracene²³⁶. They used a steady-state free precession (SSFP) pulse sequence to obtain a strong enough signal for ^{13}C experiments in the span of 11 minutes while halting the 150 minute long electrolysis process during the measurement. Huang et al. used a different variation of the three-electrode design in which the WE carbon fibres just above the NMR detection area were loaded with a commercial electrocatalyst and a Pt gauze served as CE²⁵⁸. They reported successful ^{13}C measurements of the electrocatalytic oxidation of ^{13}C -labeled ethanol by averaging 128 scans.

In 2013, Bussy et al. reported a slightly different three-electrode configuration in which carbon microfibres were again used as WE and CE²⁵⁹. The RE was replaced with a Pd wire. In this iteration, shown in Fig. 3.15, the electrodes were interfaced with the glass capillaries slightly differently and placed in different positions. The CE was still placed beneath the NMR detection area while the WE and the pseudo-RE were

Fig. 3.15: Schematic of a three-electrode NMR-SEC cell with carbon microfibre WE and CE placed above and below the NMR detection area. Reprinted by permission from Springer Nature: from the original work by Bussy et al.²⁵⁹, Copyright 2012.

placed above it. Using this design, the oxidation of phenacetin was monitored periodically over the span of 6 hours²⁵⁹. Another application of this NMR-SEC cell was reported by Boisseau et al. when they performed ultrafast 2D COSY experiments on 9-chloroanthracene²⁶⁰. Periodic NMR samples were taken every 3 minutes in a chronoamperometric experiment over a timeframe of 80 minutes.

While the previously described cell designs focused on placing the WE close to or in the NMR detection area Gomes et al. reported an NMR-SEC cell with three-electrode setup where all electrodes were deliberately placed above the NMR detection area. They used a carbon fibre WE, a platinized Pt CE, and a Ag/AgCl pseudo-RE²⁶¹. By moving the electrodes outside of the detection area, Gomes et al. investigated the influence of the interference between the magnetic and the electric fields on mass transport within the cell. They reported that, by exploiting the resulting magnetoelectrolysis effect^{262–264}, which causes the solution to be stirred by a magnetohydrodynamic force, the electrochemical reaction rate for the electrochemical reduction of *p*-benzoquinone was greatly increased. The reaction was monitored via ¹H NMR spectroscopy.

Thin film electrodes

While carbon microfibre approaches could aim to improve the electrochemical conversion inside the NMR-SEC cell, thin films with large surface areas hold the advantage of being near transparent to radio frequency radiation if they are thinner than their respective skin depth²⁶⁵. This makes it possible to move the WE and possibly CE further into the NMR detection area to better monitor the electrochemical reaction close to the electrodes. One of the older approaches to introduce electrodes into an NMR tube based on a metal film was published by Zhang and Zwanziger in 2011²⁵³. They used a thin Au film deposited on a 3 mm glass tube with small bored holes for ion exchange that served as WE and extended into the NMR detection area. A pseudo-RE was positioned inside of the WE glass tube and a curved platinized Pt-foil CE was placed between the NMR tube wall and the WE. With this metal film-based setup, Zhang and Zwanziger investigated the spectroelectrochemistry of *p*-benzoquinone, caffeic acid and 9-chloroanthracene in mechanistic approaches and with sampling times of up to two minutes. Cao et al. reported a similar NMR-SEC cell in which a Au coated glass tube sealed with a Nafion membrane to allow ion-exchange was used²⁶⁶. Additionally, a Pt wire CE was placed inside the WE tube and a Pt foil pseudo-RE was placed between the NMR tube wall and the WE tube. Only the WE reached into the NMR detection area. They performed real-time ¹H NMR-SEC *in-situ* measurements on quinone.

In more recent publications, Sorte et al. constructed an NMR-SEC cell with an interdigitated Au WE and CE (IGEs) in the NMR detection area and a Ag/AgCl pseudo-RE above it^{267,268}. The design is shown schematically and as a photograph fig. 3.16 The IGEs were printed on polyimide films and could be brought into close proximity inside a 5 mm NMR tube to promote better electrochemical performance. Sorte et al. successfully used this setup for *in-situ* NMR-SEC measurements of the electrochemistry of ferrocene (¹H)²⁶⁸ and of the oxidation of methanol and ethanol (¹³C)^{267,268}.

Fig. 3.16: Schematic and photograph of an NMR-SEC cell with interdigitated WE and CE placed in the NMR detection area. Reprinted from the original work by Sorte et al.²⁶⁸, Copyright 2016, with permission from Elsevier

Different modified tin oxide glass based coatings were explored by Zhang et al., Wang et al. and Cao et al.^{269–272} Zhang et al. created a nano-polyaniline film (PAn) via electro-polymerization on an indium tin oxide (ITO) electrode²⁷². The schematic and a photograph are shown in Fig. 3.17. This PAn WE was then assembled with a Pt wire CE and a Ag pseudo-RE inside of a 5 mm NMR tube where only the WE extended into the NMR detection area. Using this simple NMR-SEC, they conducted real-time ^1H NMR-SEC experiments with hydroquinone. In 2019, Wang et al. used the same cell

Fig. 3.17: Schematic and photograph of an ITO/PAn WE based three-electrode NMR-SEC cell. Reprinted from the original work by Zhang et al.²⁷², Copyright 2018, with permission from Elsevier.

design to characterize the electrocatalytic performance of composite catalysts consisting of varying combinations of Pt, carbon, MoS₂ and graphene nanosheets electrodeposited on ITO-based WEs^{269,270}. They investigated the oxidation of ethanol with real time ¹H NMR-SEC measurements, among other techniques.

Lastly, Cao et al. used a slightly different substrate with a similar cell design²⁷¹. They employed two fluorinated tin oxide glass (FTO) slices positioned back to back to create a two sided WE and used a Pd wire as pseudo-RE. After synthesizing Pt nanocrystals on the WE surface, they characterized the electrocatalytic performance of the WE to oxidize short-chain alcohols with real-time ¹H NMR-SEC measurements.

Metal coil electrodes

A compromise between good NMR-SEC performance and a simple fabrication process was reported by da Silva et al. in 2019²⁷³. They focused on developing a clutter-free NMR-SEC cell with simple materials and provided an instructional video on how to assemble such a cell. The design schematic is shown in Fig. 3.18 and comprises two Pt wire coils around a glass capillary as WE and CE, as well as a Ag wire inside the capillary as pseudo-RE. All components were located above the NMR detection area. Da Silva et al. reported using the magnetoelectrolysis effect to stir the solution in a real-time ¹H NMR-SEC experiment on the oxidation of ascorbic acid, finding a doubled reaction rate in comparison to pure EC experiments.

3.4.2.2. Sealed pouch cell

Aside from the previously described approaches that focused on using standard NMR tubes as carriers, there have also been advances for different electrochemical reaction monitoring liquid *in-situ* cells. One of those was reported in 2019 by Richter et al.²⁷⁴ who assembled electrocatalyst-coated carbon paper electrodes separated by a glass fibre film into a sealed plastic pouch cell, based on a pouch design for capacitor characterization by Wang et al.²⁷⁵ The design schematic as well as photographs inside the solid state NMR spectrometer are shown in Fig. 3.19. With this cell and a solid state instrument, Richter et al. quantitatively monitored the oxidation of ethanol in real-time ¹³C NMR-SEC *in-situ* experiments with a time-resolution in the order of 10 minutes and during a total electrolysis time of 100 hours. The cell had to be punctured due to the evolution of CO₂ as reaction product.

Fig. 3.18: Schematic of a coil-based three-electrode NMR-SEC cell. Reprinted from the original work by da Silva et al.²⁷³, Copyright 2019, with permission from Elsevier

Fig. 3.19. (A) schematic of a plastic pouch NMR-SEC cell and (B and C) photographs thereof. Reprinted from the original work by Richter et al.²⁷⁴, Copyright 2019, with permission from Creative Commons

3.4.3 Future perspective

The integration of the EC cell into a standard NMR tube is a simple and affordable approach. Since it is always done manually, however, it may be difficult to reproduce a cell exactly, even if an instructional video is provided²⁷³. Creating a standardized electrochemical cell insert for NMR tubes could make NMR-SEC more accessible and allow for more comparable research between different research groups and experimental setups. On the other hand, creating more specialized equipment might lead to instrumental improvements in further avoiding negative interference between the two systems and making NMR-SEC methods more robust for real applications. An example of the influence of the instrumentation has very recently been given by Gomes et al. in a review about the application of low magnetic field strengths for NMR-SEC²⁷⁶.

Especially ^{13}C NMR-SEC currently suffers from poor time-resolution. Underlying causes are low electrochemical reaction rates and the low natural occurrence of the isotope, often leading to both very long electrolysis times of tens of hours and long measurement times of tens of minutes to achieve spectra of good enough quality.

There are general NMR spectroscopy advancements such as extra sample preparation steps or specific pulse sequences that can help to improve the sensitivity and reduce the timeframe of experiments. Specific pulse techniques like e.g. SSFP are already being investigated for applications in NMR-SEC²³⁶, but, to our knowledge, sample preparation techniques are still not being investigated. One example is dynamic nuclear polarization (DNP)^{6,236,251}, where a solidified sample can be polarized much more strongly in the solid state and then get quickly dissolved and analysed while retaining this hyperpolarization, leading to improved signal intensities.

A different approach to increase the reaction yield and reduce the time necessary for ^{13}C measurements could rely on the previously introduced NMR-SEC exclusive magnetoelectrolysis effect^{261–264}. Many cell designs rely on diffusion to move the reaction products into the NMR detection area. By investigating the exact influence of cell and electrode geometries on the stirring induced by the magnetoelectrolysis effect and optimizing them, the sensitivity limitations imposed by electrochemical reaction rates and product diffusion could be greatly alleviated. One approach to do this in the three-electrode cell type could for example focus on micromachining electrodes. By designing them in such a way that they induce defined non-uniformities in the magnetic field, the magnetoelectrolysis effect could be further investigated on a more fundamental level. Additionally, the possibilities of high-surface area electrodes to improve the conversion speed should be further investigated, since the larger currents can lead to more product but also to complications in the NMR-SEC setups.

To conclude, there are many promising areas for NMR-SEC developments to take place in the near future as it still is very much a developing technique with great potential.

3.5 Mass Spectroelectrochemistry (EC-MS)

3.5.1 Introduction EC-MS

Mass spectrometry (MS) is a powerful and versatile technique to identify and quantify various kinds of analytes almost universally. The general MS process consists of the ionization of the analyte followed by the mass selection of the ions for their mass to charge ratio m/z and finally, the detection of the ions in a universal ion detector. There are many ionization and mass selector techniques with their respective advantages and drawbacks, varying for example in the ionization success for different kinds of molecules and the mass resolution that is achievable. Additionally, tandem mass spectrometry is the intentional fragmentation of the ions, for example via collision with an inert gas. The fragments can then be mass selected and detected or fragmented again for more complex experiments. More detailed information about mass spectrometry can be found in the literature^{277,278}.

While MS is not generally regarded as a spectroscopic method²⁷⁹, its universal capabilities to generate qualitative as well as quantitative data make it an excellent method to pair with EC. However, three precautions need to be kept in mind: Firstly, the analyte needs to be successfully ionized. Secondly, MS requires a high-vacuum environment for the mass selector. And thirdly, MS generally uses a destructive detection method, making the analyte unavailable for follow-up procedures. Because of these precautions, *in-situ* MS measurements are not possible in the same way as with, for example, the previously described IR spectroscopy. This also means that almost no surface-related data can be obtained. However, MS is an excellent method to quantify and identify reaction products and intermediates in online approaches. The qualitative information about reaction products and their structure gained complements the electrochemical information that can be obtained, especially for more complex reactions. In the following sections, we will present

often used as well as novel and innovative experimental setups used for EC-MS measurements and discuss future prospects. We focus on coupling mechanisms for liquid samples and not on the electrochemical and mass spectrometric instrumentation.

3.5.2 State of the art of EC-MS

Liquid EC-MS can only be realized as an online method. Since the lifetime of intermediates and unstable products is limited by definition, the method needs to have a low response time between these two steps to be able to detect them. In the following sections, we present a selection of recently reported online EC-MS methods to achieve low response times and successful intermediate detection. Methods with coupled chromatography steps will be excluded since they strictly raise the response time between product generation and detection. In research questions, where no intermediates need to be detected, online EC inductively coupled plasma (ICP) MS can be used, since it destroys species information. In 2019, Kasian et al.²⁸⁰ reviewed EC-ICP-MS as a technique for catalyst research in an online configuration with reported response times 30-40 s^{281,282}.

3.5.2.1 Coupled electrospray ionization (ESI) techniques

A useful ionization technique for liquid samples is electrospray ionization (ESI). For this technique, the analyte solution is introduced into a capillary-like device which is electrically connected to the MS inlet interface. By applying a high potential of several kV, a Taylor cone can be created that emits charged droplets towards the MS inlet. These charged droplets disintegrate into smaller and smaller droplets to finally form ionized molecules²⁸³. The high potential necessary for this process can be dangerous. Therefore, special care has to be taken to decouple the EC reaction from the ionization. In combined systems, the ESI potential can be applied to the EC system as a floating potential²⁸⁴.

Commercial thin-layer EC-MS flow cells

As has been mentioned previously^{10,11}, thin-layer cells and microfluidic devices can hold several advantages over bigger cell geometries for better electrochemical performance. Over the last decade, there have been many applications of commercial thin-layer flow cells for EC-MS, often but not always coupled to liquid chromatography (LC). In a review from 2014, Faber et al. covered the principal designs and flow charts of these setups²⁸⁵, focusing on applications in metabolism studies. In a more recent publication, Zabel and Weber reported the use of a thin-layer flow cell setup with a response time of ~25 s for the study of sulphuric amino acid and glutathione oxidation²⁸⁶. As using thin-layer flow cells is an established and commercialized approach, the authors do not go into further detail here.

Microfluidic EC-MS flow cells

As described previously, microfluidic EC methods can hold many advantages over methods with larger reactors. Since EC-MS methods are online techniques, a microfluidic EC cell generally does not need to be specifically designed for coupling to MS. In 2012, Odijk et al.²⁸⁷ reported an EC chip coupled to a microchip-ESI, which was previously developed by Qiao et al.²⁸⁸. They investigated the oxidation of

mitoxantrone as well as the influence of frit channels on the EC performance of the chip. As a special precaution to separate the electrical circuits, a grounded transfer tubing with high resistance was used to transfer the EC products to the microchip-ESI. Using the same chip design as Odijk et al., Van den Brink et al. integrated a commercially available ESI emitter to couple the chip to an MS system²⁸⁹ (Fig. 3.20). Van den Brink had less issues with circuit separation, as the ESI needle itself was grounded while the orifice of the MS was set to high voltage. Using this latter setup, response times of ~4.5s were achieved.

EC-MS droplet in front of MS-interface

A coupling method with a simple configuration was employed by Yu et al. in 2018²⁹⁰, as shown in Fig. 3.21. They used a droplet-approach in which a microscopy glass cover slide served as a carrier. This setup was developed from a previously reported pure MS approach with a glass carrier from Jiang et al.²⁹¹. The glass carrier was operated with a sample volume of 80 μL forming a droplet in one corner. In that corner, there was also a three-electrode setup consisting of a Pt WE and CE as well as a Ag/AgCl RE. In addition to the electrochemical reaction, the WE and CE were also used to form the electrospray together with the MS inlet. The authors do not specify a response time between the reaction and subsequent detection, but demonstrate real-time behaviour of the setup system to obtain mechanistic EC reaction data. As a droplet-based approach, this setup is very small and simple to use. A disadvantage is the non-continuity of the experiment. The droplet is gradually desaturated of the analyte and saturated with reaction products over the course of the experiment which limits the versatility of this method. Additionally, the electrospray removes more and more liquid, slightly changing the entire system over time. The exact positioning and amount of the liquid in the droplet is also difficult to exactly

Fig. 3.20: Photographs of a microfluidic EC cell integrated with a commercially available ESI needle in an EC-MS setup. Reprinted with permission from Van den Brink et al.²⁸⁹ Copyright 2015 American Chemical Society.

Fig. 3.21: Schematic of the droplet-based EC-MS approach. Reprinted with permission from Yu et al.²⁹⁰ Copyright 2018 American Chemical Society.

reproduce. As a consequence, this setup is well suited for qualitative mechanistic studies but not for quantitative investigations.

Thin liquid layer double-barrel capillary

Qiu et al.²⁹² and later Guo et al.²⁹³ reported a homemade capillary device where the EC reaction takes place inside a thin liquid layer formed just outside the tip of a hydrophilic double-barrel capillary. One of the two barrels is capped with previously *in-situ* generated carbon while the other barrel contains the analyte solution as well as a AgCl/Ag wire. Primary ions for the formation of an electrospray are generated with a piezoelectric pistol at the beginning of the capillary.

By positioning the EC reaction at the tip outside of the capillary at a distance of 2 mm to the MS inlet, the response time is reduced greatly. While the authors don't give an exact value, they succeeded in detecting a diamine intermediate formed by the oxidation of uric acid with a reported half-life of about 23 ms²⁹⁴ which makes this method an excellent online EC-MS technique with a very low response time. Later, Guo et al. applied this technique to detect intermediates in organometallic Ru- and

Fig. 3.22. Schematic of the thin-layer double-barrel capillary setup. Reprinted from the original work by Qiu et al.²⁹². Copyright 2016, with permission from Creative Commons

Ir-based chemiluminescence experiments. Qiu et al.²⁹² have created a powerful online EC-MS method with a small footprint, as shown in Fig. 3.22. Their setup needs careful preparation to form the carbon electrode, but shows an excellent response time. The ionization process is unusual, however, and probably the most variable part between different experiments. In addition to this drawback, there is no RE, further complicating the use of this setup. With careful calibration, however, this method can serve as a great tool to investigate short-lived reaction products and intermediates.

3.5.2.2 Differential electrochemical mass spectrometry (DEMS)

A different approach to ionize quickly transport reaction products is the differential electrochemical mass spectrometry (DEMS) method, where volatile compounds inside a liquid evaporate through a membrane or porous frit into the MS. This technique is inherently restricted by the vapor pressure of the analyte and was first mentioned as a dedicated technique by Wolter and Heitbaum in 1984^{295,296}. The main component of DEMS is a non-wetting porous membrane that allows volatile compounds to evaporate into a two-step vacuum as a function of their formation rate, hence the name “differential”²⁹⁷.

Thin-layer DEMS cells

Modern DEMS systems, as described by Abd-El-Latif et al.²⁹⁷, are generally built in a thin-layer configuration, where an electrode is placed on one side and a porous membrane on the other side. The electrochemical reaction products have to cross from the electrode to the other side of the cell to pass the membrane, causing response times of up to 2 s.

Fig. 3.23: DEMS cell; A: exploded sideview; B: top view. Image republished and modified with permission of Journal of The Electrochemical Society, from the original work by Jusys²⁹⁸. Copyright 1999, permission conveyed through Copyright Clearance Center, Inc.

One example of a thin-layer cell still in use at present with modifications was published by Jusys et al. in 1999, and is depicted in Fig. 3.23²⁹⁸. It was originally designed for a combination of DEMS and an electrochemical quartz crystal microbalance (EQCM) in two separate chambers. The membrane used in this instance is a porous Teflon membrane, placed on top of a stainless steel frit that leads to an MS inlet. In 2007, Heinen et al. slightly changed the cell to include a Pt thin-film layer on a Si prism instead of an EQCM²⁹⁹ to allow a combined EC-IR-MS method. These two designs have been used in multiple recent publications with applications ranging from CO oxidation and desorption on nanocrystals³⁰⁰, ethylene glycol oxidation^{301,302}, methanol oxidation³⁰³ to the oxygen evolution reaction³⁰⁴. The reported response time of the setups lies in the range of 1-2 s^{298,301}.

A simpler one chamber thin-layer cell was reported by Trimarco et al. in 2018³⁰⁵. Here, they used a previously reported microfabricated Si membrane with supporting SiO₂ pillars and an integrated sampling chamber to separate the liquid phase from the gaseous phase³⁰⁶. The thin-layer cell had a simple one chamber geometry with a WE directly above the membrane chip, allowing for low response times. A CE and RE were positioned in slanted side channels. The membrane chip itself was mounted onto a vacuum system to enable DEMS, shown schematically in Fig. 3.24. Using this setup, Roy et al. investigated the oxidation evolution reaction catalysed by nanoparticle catalysts³⁰⁷. Additionally, Winiwarter et al. investigated the mechanisms of partial propene oxidation³⁰⁸.

Microfluidic DEMS

In a similar fashion to the ESI method, a microfluidic approach for DEMS has recently been reported. In 2015, Trimarco et al. reported a “sniffer chip” assembly that uses the previously mentioned microfabricated membrane³⁰⁶. The

Fig. 3.24: Membrane DEMS setup for thin-layer cell; a) membrane chip photograph; b) scanning electron microscope image of the membrane chip; c) schematic of the membrane chip; d) schematic of the setup. Image reprinted and modified with permission from Trimarco et al.³⁰⁵. Copyright 2018 American Chemical Society.

Fig. 3.25: Membrane fabrication scheme. Reprinted from the original work by Trimarco et al.³⁰⁶, with the permission of AIP Publishing.

microfabrication process is illustrated in Fig. 3.25. The Si and SiO₂ based membrane was incorporated into a microfluidic chip with integrated electrodes a small total internal volume of ca. 31 nL and a low response time about 0.5 s. Trimarco et al. made proof of principle measurements of hydrogen and oxygen evolution reactions in an aqueous solvent. An envisioned application is to use the chip as a dip-in probe that immediately saturates with the gasses in the solution to provide a quick qualitative readout of the sample.

3.5.2.3 Microfluidic EC-SIMS (secondary ion mass spectrometry)

A different ionization approach based on a microfluidic chip was recently reported by Liu et al.³⁰⁹, Wang et al.³¹⁰ and Yu et al.³¹¹ They were using microfluidic EC cells with a silicon nitride (SiN) membrane on the backside of a thin Au WE, as shown in Fig. 3.26. In SIMS, a primary ion beam is used to ionize the analyte molecules as secondary ions. In this device, the primary ion beam was used to drill a hole through the SiN membrane and the WE, exposing the liquid to the vacuum of the mass spectrometer in a ~2 μm wide spot. For sequential mechanistic measurements, new holes could be drilled to capture the current state close to the electrode in the instant the hole is created. All of the publications cited above focused on imaging the electrode/electrolyte interface, by using KI^{309,311} or ascorbic acid³¹⁰ as analytes with

Fig. 3.26: Schematic of an EC-SIMS cell. Reprinted from the original work by Liu et al.³⁰⁹ - Published by The Royal Society of Chemistry.

a response time of under 100 ms and imaging integration times of 1-2 minutes. Wang et al. also succeeded in detecting ascorbic acid oxidation intermediates.

3.5.3 Future perspective

We have shown several established and innovative approaches to couple electrochemical devices to mass spectrometers. Table 3.2 shows the different techniques and semi-quantitative statements about their reported response times, advantages and restrictions. Compared to other spectroelectrochemical methods, however, EC-MS is not being researched to the same degree. As stated previously, the most important part in coupling these techniques is the ionization technique.

The already established microfluidic ESI methods described previously mostly use commercially available nano-ESI needles. A simple way to improve on the methods could lie in the monolithic fabrication of the device. By integrating the needle into the chip design, it could be simpler to setup, avoiding damage to the nano-ESI needle and possible leaks. More importantly, it reduces dead volume. Additionally, the influence of the needle geometry on the ionization success could be investigated more systematically. This has been demonstrated in a relatively straightforward manner by the group of Girault^{312,313}, using a single carbon paste electrode. To the best of our knowledge it has not been demonstrated with a three-electrode

Table 3.2: Overview of the described EC-MS techniques and their special characteristics.

Ionization method / technique	Response time	Advantages	Drawbacks	References
ESI, thin-layer	~25 s	standardized, exchangeable electrode	long transfer times, special care in gasket handling	286
ESI, microfluidic	~4.5 s	optimized design, very low analyte amounts	limited flexibility, difficult fabrication	287,289
ESI, droplet	fast enough to detect intermediates	very small footprint, simple fabrication	static experiments, poor reproducibility	290
ESI, double-barrel capillary	≤23 ms	extremely fast, small footprint	complicated fabrication and operation	292,293
DEMS, thin-layer	1-2 s	simple operation, measures formation rate	long transfer times, restricted to volatile analytes	298–305,307,308
DEMS, microfluidic	~0.5 s	simple and fast operation, very small footprint	restricted to volatile analytes, difficult fabrication	306
SIMS, microfluidic	<100 ms	very fast and sensitive, can probe electrode/electrolyte interfaces	small probed area, destroys the WE	309–311

microchip yet. Additionally, there have been ESI setups where the capillary itself served as an electrochemical reactor in the past^{284,314}. This approach promises the lowest possible response times and simplest setups and should be investigated further even in present times.

The microfluidic possibilities of using EC-DEMS chips to monitor EC reactions are still only emerging. A spin-off company, SpectroInlets, has created a dedicated system to use their membrane chip technology. It is to be expected that microfluidic DEMS will be a topic of future research. Lastly, as a general note, there are limitations to the use of ESI and DEMS. While DEMS is strongly restricted to volatile analytes, ESI can struggle with the ionization of certain classes of compounds and ion suppression effects²⁷⁷. It is therefore also important to investigate other already established ionization methods as a coupling partner to EC. We presented an example that employed SIMS to obtain impressive information about electrode / electrolyte interfaces and could also take short snapshots of intermediates. There are, however, more established and likely also suitable techniques like e.g. atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), atmospheric pressure plasma jet (APPJ) or ICP ionization, the latter having been already mentioned in section 3.5.2 for methods where species information is not important. These methods mostly use external gasses to nebulize and/or ionize the analytes and thus require additional instrumentation outside the EC cell. They can, however, offer better ionization efficiencies or better versatility than other ionization methods that require less instrumentation because they use different mechanisms to create the ions necessary for MS.

3.6 Concluding remarks

3.6.1 IR-SEC

We expect that the advent of QCL lasers will change the future of IR-SEC, solving the inherent problems of low intensity light sources. The QCL lasers will make laser IR spectroscopy more accessible, allowing for faster analysis times. The higher source power will alleviate the need for using the thin-layer configurations and at the same time push the field of ATR IR-SEC towards the use of waveguides. This, together with advancements in nanofabrication will create the possibility of constructing complete IR-SEC lab on chip systems.

By making use of IR-transparent conductive waveguides, both the waveguide and the electrode can be combined into one structure. The footprint of the device could be made small enough to be installed as sensing systems in organs on chip, as wearable sensors or in miniaturized high throughput screening platforms.

3.6.2 Raman-SEC

Unlike IR-SEC Raman-SEC is already a mature technique. However, especially the field of SERS-SEC can be further developed by combining it with new advancements in the field of nanofabrication. These advancements will possibly lead to developments where SERS enhancement structures can give more reliable and

uniform enhancements and at the same time increase the area of the enhancement structures and simultaneously decrease the cost.

At the moment Raman-SEC is mostly used for a large number of applications in material sciences, but as of yet does not have a large number of real world applications, some notable exceptions are for instance the work by the group of Brosseau^{134–137}. When the advancements of SERS-SEC as highlighted above take place, the technique will likely become more attractive for real world applications.

3.6.3 NMR-SEC

NMR spectroscopy is a very versatile technique that still has much potential to grow and mature. As a consequence, NMR-SEC is also still in its infancy and will benefit from general NMR improvements. There are, however, certain NMR-SEC specific recommendations that can be made.

Because of the unique restrictions caused by the interference between NMR and EC systems, dedicated NMR-SEC instruments should be investigated. Also, standardizing or making the often used three-electrode cell type commercially available would be beneficial to make research from different groups both more comparable and reliable.

NMR-SEC can already be used for real-time ^1H measurements, but especially real-time ^{13}C measuring methods still need to be improved. Research approaches could be based on sample preparation techniques or the exploitation of NMR-SEC specific effects like magnetoelectrolysis to reduce the time needed for an NMR-SEC experiment.

3.6.4 EC-MS

Although research towards EC-MS is not being performed to the same degree as the SEC techniques covered in this review, clear paths for future advancements can be discussed. Any improvements on EC-MS regarding the response time between reactions at the electrodes and the ionization of the sample would make the technique more attractive. Directly combining the ionization mechanism, e.g. ESI, with a microfluidic system via new microfabrication techniques could result in monolithic devices with very short response times. Additional ionization techniques that are used for MS but could also be of interest for EC-MS are SIMS, APCI, APPI, APPJ and ICP, as partly already mentioned previously. These other ionization techniques could help to increase the ionization efficiency of certain analytes while also mostly not suffering from the same coupling problems of EC with MS as is the case with ESI. Some of them have their own application restrictions, however, making a careful selection all the more important.

3.6.5 Hyphenation

Hyphenation of the (already hyphenated) SEC techniques, also known as multi-spectroelectrochemistry, is quite common in literature. In 2011 Dunsch wrote a review paper on this very topic³.

As the field of SEC advances, so will the number of hyphenation of different techniques. Already in 2011 Dunsch predicted a greater variety in triple multi-

spectroelectrochemistry. In order to get more information from a system, we expect that more and more techniques will be combined into multi-spectroelectrochemistry in the future.

References

- (1) Kaim, W.; Fiedler, J. Spectroelectrochemistry: The Best of Two Worlds. *Chem. Soc. Rev.* **2009**, *38* (12), 3373–3382.
- (2) Kuwana, T.; Darlington, R. K.; Leedy, D. W. Electrochemical Studies Using Conducting Glass Indicator Electrodes. *Anal. Chem.* **1964**, *36* (10), 2023–2025.
- (3) Dunsch, L. Recent Advances in in Situ Multi-Spectroelectrochemistry. *J. Solid State Electrochem.* **2011**, *15* (7–8), 1631–1646.
- (4) Oberacher, H.; Pitterl, F.; Erb, R.; Plattner, S. Mass Spectrometric Methods for Monitoring Redox Processes in Electrochemical Cells. *Mass Spectrom. Rev.* **2015**, *34* (1), 64–92.
- (5) Wain, A. J.; O'connell, M. A. Advances in Surface-Enhanced Vibrational Spectroscopy at Electrochemical Interfaces. *Adv. Phys. X* **2017**, *2* (1), 188–209.
- (6) Tong, Y. Y. J. In Situ Electrochemical Nuclear Magnetic Resonance Spectroscopy for Electrocatalysis: Challenges and Prospects. *Curr. Opin. Electrochem.* **2017**, *4* (1), 60–68.
- (7) León, L.; Mozo, J. D. Designing Spectroelectrochemical Cells: A Review. *TrAC Trends Anal. Chem.* **2018**, *102*, 147–169.
- (8) Zhai, Y.; Zhu, Z.; Zhou, S.; Zhu, C.; Dong, S. Recent Advances in Spectroelectrochemistry. *Nanoscale* **2018**, *10* (7), 3089–3111.
- (9) Garoz-Ruiz, J.; Perales-Rondon, J. V.; Heras, A.; Colina, A. Spectroelectrochemical Sensing: Current Trends and Challenges. *Electroanalysis* **2019**, 1254–1278.
- (10) Moss, D.; Nabedryk, E.; Breton, J.; Mantele, W. Redox-Linked Conformational Changes in Proteins Detected by a Combination of Infrared Spectroscopy and Protein Electrochemistry. Evaluation of the Technique with Cytochrome C. *Eur. J. Biochem.* **1990**, *187* (3), 565–572.
- (11) van den Brink, F. T. G. G.; Olthuis, W.; van den Berg, A.; Odijk, M. Miniaturization of Electrochemical Cells for Mass Spectrometry. *TrAC Trends Anal. Chem.* **2015**, *70*, 40–49.
- (12) Christensen, P. A.; Jones, S. W. M. An in Situ FTIR Study of Ethanol Oxidation at Polycrystalline Platinum in 0.1 M KOH at 25 and 50 °C. *J. Phys. Chem. C* **2014**, *118* (51), 29760–29769.
- (13) Alwis, L. K. H. K.; Mucalo, M. R.; Ingham, B. Anodically Polarized Nickel Electrodes in DMSO or DMF Solutions of Pseudohalide Ions: IR Spectroelectrochemical Studies. *J. Electrochem. Soc.* **2013**, *160* (11), 803–812.
- (14) De Lacey, A. L.; Hatchikian, E. C.; Volbeda, A.; Frey, M.; Fontecilla-Camps, J. C.; Fernandez, V. M. Infrared-Spectroelectrochemical Characterization of the [NiFe] Hydrogenase of *Desulfovibrio Gigas*. *J. Am. Chem. Soc.* **1997**, *119* (31), 7181–7189.
- (15) Guidelli, R. *Bioelectrochemistry of Biomembranes and Biomimetic Membranes*; 2016.
- (16) Hartstein, A.; Kirtley, J. R.; Tsang, J. C. Enhancement of the Infrared Absorption from Molecular Monolayers with Thin Metal Overlayers. *Phys. Rev. Lett.* **1980**, *45* (3),

- 201–204.
- (17) Osawa, M. Surface-Enhanced Infrared Absorption. In *Near-Field Optics and Surface Plasmon Polaritons*; Kawata, S., Ed.; Springer Berlin Heidelberg: Berlin, Heidelberg, 2001; pp 163–187.
- (18) Aroca, R. *Surface-Enhanced Vibrational Spectroscopy*; John Wiley & Sons, Ltd: Chichester, UK, 2006.
- (19) Hu, Y.; López-Lorente, Á. I.; Mizaikoff, B. Graphene-Based Surface Enhanced Vibrational Spectroscopy: Recent Developments, Challenges, and Applications. *ACS Photonics* **2019**, *6* (9), 2182–2197.
- (20) Ataka, K. I.; Yotsuyanagi, T.; Osawa, M. Potential-Dependent Reorientation of Water Molecules at an Electrode/Electrolyte Interface Studied by Surface-Enhanced Infrared Absorption Spectroscopy. *J. Phys. Chem.* **1996**, *100* (25), 10664–10672.
- (21) Osawa, M.; Ataka, K. I.; Yoshii, K.; Yotsuyanagi, T. Surface-Enhanced Infrared ATR Spectroscopy for in Situ Studies of Electrode/Electrolyte Interfaces. *J. Electron Spectros. Relat. Phenomena* **1993**, *64–65* (C), 371–379.
- (22) Im, H.; Bantz, K. C.; Lindquist, N. C.; Haynes, C. L.; Oh, S. H. Vertically Oriented Sub-10-Nm Plasmonic Nanogap Arrays. *Nano Lett.* **2010**, *10* (6), 2231–2236.
- (23) Ru, E. C. Le; Etchegoin, P. G. *Principles of Surface-Enhanced Raman Spectroscopy*; Elsevier, 2009.
- (24) Ataka, K.; Heberle, J. Biochemical Applications of Surface-Enhanced Infrared Absorption Spectroscopy. *Anal. Bioanal. Chem.* **2007**, *388* (1), 47–54.
- (25) Neubrech, F.; Huck, C.; Weber, K.; Pucci, A.; Giessen, H. Surface-Enhanced Infrared Spectroscopy Using Resonant Nanoantennas. *Chem. Rev.* **2017**, *117* (7), 5110–5145.
- (26) Di Bari, C.; Mano, N.; Shleev, S.; Pita, M.; L. De Lacey, A. Halides Inhibition of Multicopper Oxidases Studied by FTIR Spectroelectrochemistry Using Azide as an Active Infrared Probe. *J. Biol. Inorg. Chem.* **2017**, *22* (8), 1179–1186.
- (27) Kato, Y.; Noguchi, T. Long-Range Interaction between the Mn₄CaO₅ Cluster and the Non-Heme Iron Center in Photosystem II as Revealed by FTIR Spectroelectrochemistry. *Biochemistry* **2014**, *53* (30), 4914–4923.
- (28) Tutunea, F.; Ryan, M. D. Visible and Infrared Spectroelectrochemistry of Cobalt Porphinones and Porphinediones. *J. Electroanal. Chem.* **2012**, *670*, 16–22.
- (29) Tutunea, F.; Atifi, A.; Ryan, M. D. Visible and Infrared Spectroelectrochemistry of Zinc and Manganese Porphinones: Metal vs. Porphyrin Reduction. *J. Electroanal. Chem.* **2015**, *744*, 17–24.
- (30) Murray, R. W.; Heineman, W. R.; O'Dom, G. W. Optically Transparent Electrodes. **1967**, *39* (13), 1666–1668.
- (31) Wei, Z.; Ryan, M. D. Infrared Spectroelectrochemical Reduction of Iron Porphyrin Complexes. *Inorg. Chem.* **2010**, *49* (15), 6948–6954.
- (32) Dai, Y.; Proshlyakov, D. A.; Zak, J. K.; Swain, G. M. Optically Transparent Diamond Electrode for Use in IR Transmission Spectroelectrochemical Measurements. *Anal. Chem.* **2007**, *79* (19), 7526–7533.
- (33) Zak, J. K.; Butler, J. E.; Swain, G. M. Diamond Optically Transparent Electrodes: Demonstration of Concept with Ferri/Ferrocyanide and Methyl Viologen. *Anal. Chem.* **2001**, *73* (5), 908–914.
- (34) Stotter, J.; Zak, J.; Behler, Z.; Show, Y.; Swain, G. M. Optical and Electrochemical Properties of Optically Transparent, Boron-Doped Diamond Thin Films Deposited on

- Quartz. *Anal. Chem.* **2002**, 74 (23), 5924–5930.
- (35) Alwis, K. H. K. L.; Mucalo, M. R.; Lane, J. R. A Fundamental in Situ IR Spectroelectrochemical Study of the Electrical Polarization of Nickel, Copper and Gold Electrodes in the Presence of the Unstable Tellurocyanate Ion in DMSO and DMF Solutions. *RSC Adv.* **2015**, 5 (40), 31815–31825.
 - (36) Shaw, M. J.; Henson, R. L.; Houk, S. E.; Westhoff, J. W.; Jones, M. W.; Richter-Addo, G. B. Fiber-Optic Infrared Reflectance Spectroelectrochemistry: Isomerization of a Manganese Pyranyl Complex. *J. Electroanal. Chem.* **2002**, 534 (1), 47–53.
 - (37) Xu, N.; Lilly, J.; Powell, D. R.; Richter-Addo, G. B. Synthesis, Characterization, and Infrared Reflectance Spectroelectrochemistry of Organoruthenium Nitrosyl Porphyrins. *Organometallics* **2012**, 31 (3), 827–834.
 - (38) El-Attar, M. A.; Xu, N.; Awasabisah, D.; Powell, D. R.; Richter-Addo, G. B. Cyclic Voltammetric and Fiber-Optic Infrared Spectroelectrochemical Studies of Six-Coordinate (Por)Ru(NO)Cl Compounds (Por = Porphyrinato Dianion). *Polyhedron* **2012**, 40 (1), 105–109.
 - (39) Awasabisah, D.; Xu, N.; Gautam, K. P. S.; Powell, D. R.; Shaw, M. J.; Richter-Addo, G. B. Preparation, Characterization, Electrochemistry, and Infrared Spectro-electrochemistry of Ruthenium -Nitrosyl Porphyrins Containing η^1 -O-Bonded Axial Carboxylates. *Eur. J. Inorg. Chem.* **2016**, 2016 (4), 509–518.
 - (40) Pons, S. THE USE OF FOURIER TRANSFORM INFRARED SPECTROSCOPY FOR IN SITU RECORDING OF SPECIES IN THE ELECTRODE-ELECTROLYTE SOLUTION INTERPHASE. *J. Electroanal. Chem.* **1983**, 150, 495–504.
 - (41) Aurbach, D.; Chusid, O. In Situ FTIR Spectroelectrochemical Studies of Surface Films Formed on Li and Nonactive Electrodes at Low Potentials in Li Salt Solutions Containing CO₂. *J. Electrochem. Soc.* **1993**, 140 (11), L155–L157.
 - (42) Mucalo, M. R.; Li, Q. In Situ Infrared Spectroelectrochemical Studies of the Corrosion of a Nickel Electrode as a Function of Applied Potential in Cyanate, Thiocyanate, and Selenocyanate Solutions. *J. Colloid Interface Sci.* **2004**, 269 (2), 370–380.
 - (43) Fang, X.; Wang, L.; Shen, P. K.; Cui, G.; Bianchini, C. An in Situ Fourier Transform Infrared Spectroelectrochemical Study on Ethanol Electrooxidation on Pd in Alkaline Solution. *J. Power Sources* **2010**, 195 (5), 1375–1378.
 - (44) Liu, L.; Zeng, L.; Wu, L.; Jiang, X. Revealing the Effect of Protein Weak Adsorption to Nanoparticles on the Interaction between the Desorbed Protein and Its Binding Partner by Surface-Enhanced Infrared Spectroelectrochemistry. *Anal. Chem.* **2017**, 89 (5), 2724–2730.
 - (45) Liu, L.; Zeng, L.; Wu, L.; Jiang, X. Label-Free Surface-Enhanced Infrared Spectroelectrochemistry Studies the Interaction of Cytochrome c with Cardiolipin-Containing Membranes. *J. Phys. Chem. C* **2015**, 119 (8), 3990–3999.
 - (46) Yang, Y. Y.; Ren, J.; Zhang, H. X.; Zhou, Z. Y.; Sun, S. G.; Cai, W. Bin. Infrared Spectroelectrochemical Study of Dissociation and Oxidation of Methanol at a Palladium Electrode in Alkaline Solution. *Langmuir* **2013**, 29 (5), 1709–1716.
 - (47) Wang, L.; Meng, H.; Shen, P. K.; Bianchini, C.; Vizza, F.; Wei, Z. In Situ FTIR Spectroelectrochemical Study on the Mechanism of Ethylene Glycol Electrocatalytic Oxidation at a Pd Electrode. *Phys. Chem. Chem. Phys.* **2011**, 13 (7), 2667–2673.
 - (48) Zhang, Q.; Song, W. L.; Showkot Hossain, A. M.; Liu, Z. Di; Hu, G. J.; Tian, Y. P.; Wu, J. Y.; Jin, B. K.; Zhou, H. P.; Yang, J. X.; et al. Synthesis, Crystal Structure,

- Electrochemistry and in Situ FTIR Spectroelectrochemistry of a Bisferrocene Pyrazole Derivative. *Dalt. Trans.* **2011**, 40 (14), 3510–3516.
- (49) Golden, W. G.; Dunn, D. S.; Overend, J. A Method for Measuring Infrared Reflection-Absorption Spectra of Molecules Adsorbed on Low-Area Surfaces at Monolayer and Submonolayer Concentrations. *J. Catal.* **1981**, 71 (2), 395–404.
 - (50) Faguy, P. W.; Richmond, W. N. Real-Time Polarization Modulation Infrared Spectroscopy Applied to the Study of Water and Hydroxide Ions at Electrode Surfaces. *J. Electroanal. Chem.* **1996**, 410 (1), 109–113.
 - (51) Faguy, P. W.; Richmond, W. N.; Jackson, R. S.; Weibel, S. C.; Ball, G.; Payer, J. H. Real-Time Polarization Modulation in Situ Infrared Spectroscopy Applied to the Study of Atmospheric Corrosion. *Appl. Spectrosc.* **1998**, 52 (4), 557–564.
 - (52) Green, M. J.; Barner, B. J.; Corn, R. M. Real-Time Sampling Electronics for Double Modulation Experiments with Fourier Transform Infrared Spectrometers. *Rev. Sci. Instrum.* **1991**, 62 (6), 1426–1430.
 - (53) Barner, B. J.; Green, M. J.; Sáez, E. I.; Corn, R. M. Polarization Modulation Fourier Transform Infrared Reflectance Measurements of Thin Films and Monolayers at Metal Surfaces Utilizing Real-Time Sampling Electronics. *Anal. Chem.* **1991**, 63 (1), 55–60.
 - (54) Zamlynny, V.; Zawisza, I.; Lipkowski, J. PM FTIRAS Studies of Potential-Controlled Transformations of a Monolayer and a Bilayer of 4-Pentadecylpyridine, a Model Surfactant, Adsorbed on a Au(111) Electrode Surface. *Langmuir* **2003**, 19 (1), 132–145.
 - (55) Zawisza, I.; Bin, X.; Lipkowski, J. Potential-Driven Structural Changes in Langmuir-Blodgett DMPC Bilayers Determined by in Situ Spectroelectrochemical PM IRRAS. *Langmuir* **2007**, 23 (9), 5180–5194.
 - (56) Garcia-Araez, N.; Brosseau, C. L.; Rodriguez, P.; Lipkowski, J. Layer-by-Layer PMIRRAS Characterization of DMPC Bilayers Deposited on a Au(111) Electrode Surface. *Langmuir* **2006**, 22 (25), 10365–10371.
 - (57) Alkire, R. C.; Kolb, D. M.; Lipkowski, J.; Ross, P. N. *Diffraction and Spectroscopic Methods in Electrochemistry*; Advances in Electrochemical Sciences and Engineering; Wiley, 2009.
 - (58) Monyoncho, E. A.; Zamlynny, V.; Woo, T. K.; Baranova, E. A. The Utility of Polarization Modulation Infrared Reflection Absorption Spectroscopy (PM-IRRAS) in Surface and: In Situ Studies: New Data Processing and Presentation Approach. *Analyst* **2018**, 143 (11), 2563–2573.
 - (59) Wang, T.; Bai, J.; Jiang, X.; Nienhaus, G. U. Cellular Uptake of Nanoparticles by Membrane Penetration: A Study Combining Confocal Microscopy with FTIR Spectroelectrochemistry. *ACS Nano* **2012**, 6 (2), 1251–1259.
 - (60) Hosseini, P.; Wittstock, G.; Brand, I. Infrared Spectroelectrochemical Analysis of Potential Dependent Changes in Cobalt Hexacyanoferrate and Copper Hexacyanoferrate Films on Gold Electrodes. *J. Electroanal. Chem.* **2018**, 812 (October 2017), 199–206.
 - (61) Bard, A. J. *Electroanalytical Chemistry: A Series of Advances*; Electroanalytical Chemistry: A Series of Advances; Taylor & Francis, 1986.
 - (62) Neophytides, G.; Quaroni, L.; Büchi, F. N.; Orfanidi, A.; Neophytides, S. G.; Schmidt, T. J. A New In-Situ Spectroelectrochemical Setup for FTIR Measurements in

- Operating High Temperature Polymer Electrolyte Fuel Cells. *Electrochem. commun.* **2013**, *34*, 200–203.
- (63) Su, P.; Prabhakaran, V.; Johnson, G. E.; Laskin, J. In Situ Infrared Spectroelectrochemistry for Understanding Structural Transformations of Precisely Defined Ions at Electrochemical Interfaces. *Anal. Chem.* **2018**, *90* (18), 10935–10942.
- (64) Villalba, M.; De Leo, L. P. M.; Calvo, E. J. PM-IRRA Spectroelectrochemistry of Hexacyanoferrate Films in Layer-by-Layer Polyelectrolyte Multilayers. *ChemElectroChem* **2014**, *1* (1), 195–199.
- (65) Aroca, R.; Ross, D.; Domingo, C. Surface-Enhanced Infrared Spectroscopy. *Appl. Spectrosc.* **2004**, *58*, 324A–338A.
- (66) Schadle, T.; Mizaikoff, B. Mid-Infrared Waveguides: A Perspective. *Appl. Spectrosc.* **2016**, *70* (10), 1625–1638.
- (67) Sieger, M.; Mizaikoff, B. Toward On-Chip Mid-Infrared Sensors. *Anal. Chem.* **2016**, *88* (11), 5562–5573.
- (68) Hatta, A.; Suzuki, Y.; Wadayama, T.; Suëtaka, W. Kretschmann ATRIR Spectroscopy of Investigating Metal/Liquid(or Solid) Interfaces. *Appl. Surf. Sci.* **1991**, *48–49* (C), 222–226.
- (69) Kretschmann, E. The Determination of the Optical Constants of Metals by Excitation of Surface Plasmons. *Zeitschrift für Phys.* **1971**, *241* (4), 313–324.
- (70) Baruch, M. F.; Pander, J. E.; White, J. L.; Bocarsly, A. B. Mechanistic Insights into the Reduction of CO₂ on Tin Electrodes Using in Situ ATR-IR Spectroscopy. *ACS Catal.* **2015**, *5* (5), 3148–3156.
- (71) Aroca, R.; Price, B. A New Surface for Surface-Enhanced Infrared Spectroscopy: Tin Island Films. *J. Phys. Chem. B* **1997**, *101* (33), 6537–6540.
- (72) Paengnakorn, P.; Ash, P. A.; Shaw, S.; Danyal, K.; Chen, T.; Dean, D. R.; Seefeldt, L. C.; Vincent, K. A. Infrared Spectroscopy of the Nitrogenase MoFe Protein under Electrochemical Control: Potential-Triggered CO Binding. *Chem. Sci.* **2017**, *8* (2), 1500–1505.
- (73) Healy, A. J.; Ash, P. A.; Lenz, O.; Vincent, K. A. Attenuated Total Reflectance Infrared Spectroelectrochemistry at a Carbon Particle Electrode; Unmediated Redox Control of a [NiFe]-Hydrogenase Solution. *Phys. Chem. Chem. Phys.* **2013**, *15* (19), 7055–7059.
- (74) Dillard, C.; Singh, A.; Kalra, V. Polysulfide Speciation and Electrolyte Interactions in Lithium-Sulfur Batteries with in Situ Infrared Spectroelectrochemistry. *J. Phys. Chem. C* **2018**, *122* (32), 18195–18203.
- (75) Heyes, J.; Dunwell, M.; Xu, B. CO₂ Reduction on Cu at Low Overpotentials with Surface-Enhanced in Situ Spectroscopy. *J. Phys. Chem. C* **2016**, *120* (31), 17334–17341.
- (76) Pfaffeneder-Kmen, M.; Casas, I. F.; Naghilou, A.; Trettenhahn, G.; Kautek, W. A Multivariate Curve Resolution Evaluation of an In-Situ ATR-FTIR Spectroscopy Investigation of the Electrochemical Reduction of Graphene Oxide. *Electrochim. Acta* **2017**, *255*, 160–167.
- (77) Viinikanoja, A.; Kauppila, J.; Damlin, P.; Suominen, M.; Kvarnström, C. In Situ FTIR and Raman Spectroelectrochemical Characterization of Graphene Oxide upon Electrochemical Reduction in Organic Solvents. *Phys. Chem. Chem. Phys.* **2015**, *17*

- (18), 12115–12123.
- (78) Izquierdo, J.; Mizaikoff, B.; Kranz, C. Surface-Enhanced Infrared Spectroscopy on Boron-Doped Diamond Modified with Gold Nanoparticles for Spectroelectrochemical Analysis. *Phys. Status Solidi Appl. Mater. Sci.* **2016**, *213* (8), 2056–2062.
- (79) Neubauer, D.; Scharpf, J.; Pasquarelli, A.; Mizaikoff, B.; Kranz, C. Combined in Situ Atomic Force Microscopy and Infrared Attenuated Total Reflection Spectroelectrochemistry. *Analyst* **2013**, *138* (22), 6746–6752.
- (80) Menegazzo, N.; Kahn, M.; Berghauser, R.; Waldhauser, W.; Mizaikoff, B. Nitrogen-Doped Diamond-like Carbon as Optically Transparent Electrode for Infrared Attenuated Total Reflection Spectroelectrochemistry. *Analyst* **2011**, *136* (9), 1831–1839.
- (81) Purushothaman, B. K.; Pelsozy, M.; Morrison, P. W.; Lvovich, V. F.; Martin, H. B. In Situ Infrared Attenuated Total Reflectance Spectroelectrochemical Study of Lubricant Degradation. *J. Appl. Electrochem.* **2012**, *42* (2), 111–120.
- (82) Sun, S.; Hong, S.; Chen, S.; Lu, G.; Dai, H.; Xiao, X. In Situ Scanning FTIR Microscopy and IR Imaging of Pt Electrode Surface towards CO Adsorption. *Sci. China, Ser. B Chem.* **1999**, *42* (3), 261–267.
- (83) Li, J. T.; Chen, S. R.; Ke, F. S.; Wei, G. Z.; Huang, L.; Sun, S. G. In Situ Microscope FTIR Spectroscopic Studies of Interfacial Reactions of Sn-Co Alloy Film Anode of Lithium Ion Battery. *J. Electroanal. Chem.* **2010**, *649* (1–2), 171–176.
- (84) Li, J. T.; Chen, Q. S.; Sun, S. G. In Situ Microscope FTIR Studies of Methanol Adsorption and Oxidation on an Individually Addressable Array of Nanostructured Pt Microelectrodes. *Electrochim. Acta* **2007**, *52* (18), 5725–5732.
- (85) Gong, H.; Sun, S. G.; Chen, Y. J.; Chen, S. P. In Situ Microscope FTIRS Studies of CO Adsorption on an Individually Addressable Array of Nanostructured Pt Microelectrodes - An Approach of Combinatorial Analysis of Anomalous IR Properties. *J. Phys. Chem. B* **2004**, *108* (31), 11575–11584.
- (86) Sun, S.-G.; Zhou, Z.-Y. Chapter 5 - In-Situ Microscope FTIR Reflection Spectroscopy and Its Applications in Electrochemical Adsorption and Electrocatalysis on Nanostructured Surfaces. In *In-situ Spectroscopic Studies of Adsorption at the Electrode and Electrocatalysis*; Sun, S.-G., Christensen, P. A., Wieckowski, A., Eds.; Elsevier Science B.V.: Amsterdam, 2007; pp 139–178.
- (87) Melendres, C. A.; Beden, B.; Bowmaker, G. A. Far Infrared Spectroscopy of a Platinum Electrode “in-Situ” Using a Synchrotron Source. *J. Electroanal. Chem.* **1995**, *383* (1–2), 191–193.
- (88) Rosendahl, S. M.; Borondics, F.; May, T. E.; Pedersen, T. M.; Burgess, I. J. Synchrotron Infrared Radiation for Electrochemical External Reflection Spectroscopy: A Case Study Using Ferrocyanide. *Anal. Chem.* **2011**, *83* (10), 3632–3639.
- (89) Tu, K.; Lardner, M. J.; Morhart, T. A.; Rosendahl, S. M.; Creighton, S.; Burgess, I. J. Spatial Mapping of Methanol Oxidation Activity on a Monolithic Variable-Composition PtNi Alloy Using Synchrotron Infrared Microspectroscopy. *J. Phys. Chem. C* **2016**, *120* (41), 23640–23647.
- (90) Rosendahl, S. M.; Borondics, F.; May, T. E.; Burgess, I. J. Step-Scan IR Spectroelectrochemistry with Ultramicroelectrodes: Nonsurface Enhanced

- Detection of near Femtomole Quantities Using Synchrotron Radiation. *Anal. Chem.* **2013**, *85* (18), 8722–8727.
- (91) Lardner, M. J.; Tu, K.; Barlow, B. C.; Rosendahl, S. M.; Borondics, F.; Burgess, I. J. Quantitative Analysis of Electrochemical Diffusion Layers Using Synchrotron Infrared Radiation. *J. Electroanal. Chem.* **2017**, *800*, 184–189.
 - (92) Ash, P. A.; Reeve, H. A.; Quinson, J.; Hidalgo, R.; Zhu, T.; McPherson, I. J.; Chung, M. W.; Healy, A. J.; Nayak, S.; Lonsdale, T. H.; et al. Synchrotron-Based Infrared Microanalysis of Biological Redox Processes under Electrochemical Control. *Anal. Chem.* **2016**, *88* (13), 6666–6671.
 - (93) Sigrist, J. A.; Lins, E. S.; Morhart, T. A.; Briggs, J. L.; Burgess, I. J. Optimization of a Commercial Variable Angle Accessory for Entry Level Users of Electrochemical Attenuated Total Reflection Surface-Enhanced Infrared Absorption Spectroscopy (ATR-SEIRAS). *Appl. Spectrosc.* **2019**, *73* (12), 1394–1402.
 - (94) Weida, M. J.; Yee, B. Quantum Cascade Laser-Based Replacement for FTIR Microscopy. *Imaging, Manip. Anal. Biomol. Cells, Tissues IX* **2011**, 7902 (February 2011), 79021C.
 - (95) Ramirez, J. M.; Vakarin, V.; Gilles, C.; Frigerio, J.; Ballabio, A.; Chaisakul, P.; Roux, X. Le; Alonso-Ramos, C.; Maisons, G.; Vivien, L.; et al. Low-Loss Ge-Rich Si₀₂Ge₀₈ Waveguides for Mid-Infrared Photonics. *Opt. Lett.* **2017**, *42* (1), 105.
 - (96) Wägli, P.; Chang, Y. C.; Homsy, A.; Hvozdar, L.; Herzig, H. P.; De Rooij, N. F. Microfluidic Droplet-Based Liquid-Liquid Extraction and on-Chip IR Spectroscopy Detection of Cocaine in Human Saliva. *Anal. Chem.* **2013**, *85* (15), 7558–7565.
 - (97) Lo, P. G.-Q.; Bettiol, A. A.; Lim, A. E.-J.; Ang, K.-W.; Younis, U. Propagation Loss Improvement in Ge-on-SOI Mid-Infrared Waveguides Using Rapid Thermal Annealing. *IEEE Photonics Technol. Lett.* **2016**, *28* (21), 2447–2450.
 - (98) Gawith, C. B. E.; Khokhar, A. Z.; Littlejohns, C.; Murugan, G. S.; Penades, J. S.; Mittal, V.; Nedeljkovic, M.; Wilkinson, J. S.; Mashanovich, G. Z.; Carpenter, L. G. Germanium-on-Silicon Waveguides Operating at Mid-Infrared Wavelengths up to 85 Mm. *Opt. Express* **2017**, *25* (22), 27431.
 - (99) Singh, N.; Casas-Bedoya, A.; Hudson, D. D.; Read, A.; Mägi, E.; Eggleton, B. J. Mid-IR Absorption Sensing of Heavy Water Using a Silicon-on-Sapphire Waveguide. *Opt. Lett.* **2016**, *41* (24), 5776.
 - (100) Mittal, V.; Wilkinson, J. S.; Murugan, G. S. High-Contrast GeTe₄ Waveguides for Mid-Infrared Biomedical Sensing Applications; Broquin, J. E., Nunzi Conti, G., Eds.; International Society for Optics and Photonics, 2014; p 89881A.
 - (101) Cheng, Z.; Chen, X.; Wong, C. Y.; Xu, K.; Tsang, H. K. Mid-Infrared Suspended Membrane Waveguide and Ring Resonator on Silicon-on-Insulator. *IEEE Photonics J.* **2012**, *4* (5), 1510–1519.
 - (102) Chang, Y. C.; Wägli, P.; Paeder, V.; Homsy, A.; Hvozdar, L.; Van Der Wal, P.; Di Francesco, J.; De Rooij, N. F.; Peter Herzig, H. Cocaine Detection by a Mid-Infrared Waveguide Integrated with a Microfluidic Chip. *Lab Chip* **2012**, *12* (17), 3020–3023.
 - (103) Malmström, M.; Karlsson, M.; Forsberg, P.; Cai, Y.; Nikolajeff, F.; Laurell, F. Waveguides in Polycrystalline Diamond for Mid-IR Sensing. *Opt. Mater. Express* **2016**, *6* (4), 1286.
 - (104) Osman, A.; Khokhar, A. Z.; Nedeljkovic, M.; Wu, Y.; Mashanovich, G. Z.; Debnath, K.; Qu, Z.; Soler Penades, J. Suspended Low-Loss Germanium Waveguides for the

- Longwave Infrared. *Opt. Lett.* **2018**, *43* (24), 5997.
- (105) TZENG, Y. Diamond Transmissive Windows. *Diam. Film. Technol.* **1991**, *1*, 31.
- (106) Odijk, M.; van den Berg, A. Nanoscale Electrochemical Sensing and Processing in Microreactors. *Annu. Rev. Anal. Chem.* **2018**, *11* (1), 421–440.
- (107) Susarrey-Arce, A.; Tiggelaar, R. M.; Morassutto, M.; Geerlings, J.; Sanders, R. G. P.; Geerdink, B.; Schlautmann, S.; Lefferts, L.; van Houselt, A.; Gardeniers, J. G. E. A New ATR-IR Microreactor to Study Electric Field-Driven Processes. *Sensors Actuators B Chem.* **2015**, *220*, 13–21.
- (108) Herzig-Marx, R.; Queeney, K. T.; Jackman, R. J.; Schmidt, M. A.; Jensen, K. F. Infrared Spectroscopy for Chemically Specific Sensing in Silicon-Based Microreactors. *Anal. Chem.* **2004**, *76* (21), 6476–6483.
- (109) Karabudak, E.; Mojet, B. L.; Schlautmann, S.; Mul, G.; Gardeniers, H. J. G. E. Attenuated Total Reflection-Infrared Nanofluidic Chip with 71 nL Detection Volume for *in Situ* Spectroscopic Analysis of Chemical Reaction Intermediates. *Anal. Chem.* **2012**, *84* (7), 3132–3137.
- (110) Rizkin, B. A.; Popovic, F. G.; Hartman, R. L. Review Article: Spectroscopic Microreactors for Heterogeneous Catalysis. *J. Vac. Sci. Technol. A* **2019**, *37* (5), 050801.
- (111) Führer, P.; Lozeman, J. J. A. J. A.; de Boer, H. L. H. L.; Bomer, J. G. J. G.; Olthuis, W.; Odijk, M. SPECTROELECTROCHEMICAL DETECTION OF P-BENZOQUINONE AND HYDROQUINONE IN AN ELECTROCHEMICAL MICROREACTOR WITH AN INTEGRATED ATR-IR IRE. *22nd Int. Conf. Miniaturized Syst. Chem. Life Sci. μ TAS 2018* **2018**, *4*, 2127–2130.
- (112) Gross, E. Challenges and Opportunities in IR Nanospectroscopy Measurements of Energy Materials. *Nano Res.* **2019**, *12* (9), 2200–2210.
- (113) Dazzi, A.; Prater, C. B. AFM-IR: Technology and Applications in Nanoscale Infrared Spectroscopy and Chemical Imaging. *Chem. Rev.* **2017**, *117* (7), 5146–5173.
- (114) Lu, Y. H.; Larson, J. M.; Baskin, A.; Zhao, X.; Ashby, P. D.; Prendergast, D.; Bechtel, H. A.; Kostecki, R.; Salmeron, M. Infrared Nanospectroscopy at the Graphene-Electrolyte Interface. *Nano Lett.* **2019**, *19* (8), 5388–5393.
- (115) Huth, F.; Govyadinov, A.; Amarie, S.; Nuansing, W.; Keilmann, F.; Hillenbrand, R. Nano-FTIR Absorption Spectroscopy of Molecular Fingerprints at 20 nm Spatial Resolution. *Nano Lett.* **2012**, *12* (8), 3973–3978.
- (116) Adato, R.; Aksu, S.; Altug, H. Engineering Mid-Infrared Nanoantennas for Surface Enhanced Infrared Absorption Spectroscopy. *Biochem. Pharmacol.* **2015**, *18* (8), 436–446.
- (117) Schmid, T.; Dariz, P. Raman Microspectroscopic Imaging of Binder Remnants in Historical Mortars Reveals Processing Conditions. *Heritage* **2019**, *2* (2), 1662–1683.
- (118) Fleischmann, M.; Hendra, P. J.; McQuillan, A. J. Raman Spectra of Pyridine Adsorbed at a Silver Electrode. *Chem. Phys. Lett.* **1974**, *26* (2), 163–166.
- (119) Jeanmaire, D. L.; Duyne, R. P. VAN. Surface Raman Spectroelectrochemistry Part1. Heterocyclic. *J. Electroanal. Chem.* **1977**, *84*, 1.
- (120) Stiles, P. L.; Dieringer, J. A.; Shah, N. C.; Van Duyne, R. P. Surface-Enhanced Raman Spectroscopy SERS: Surface-Enhanced Raman Spectroscopy Raman Scattering: Inelastic Scattering of a Photon from a Molecule in Which the Frequency Change Precisely Matches the Difference in Vibrational Energy Levels. *Annu. Rev. Anal.*

- Chem* **2008**, *1*, 601–626.
- (121) Moskovits, M. Surface-Enhanced Spectroscopy. *Rev. Mod. Phys.* **1985**, *57* (3), 783–826.
 - (122) Moskovits, M. Persistent Misconceptions Regarding SERS. *Phys. Chem. Chem. Phys.* **2013**, *15* (15), 5301–5311.
 - (123) Moskovits, M. Surface-Enhanced Raman Spectroscopy: A Brief Retrospective. *J. Raman Spectrosc.* **2005**, *36* (6–7), 485–496.
 - (124) Schlücker, S. Surface-Enhanced Raman Spectroscopy: Concepts and Chemical Applications. *Angew. Chemie - Int. Ed.* **2014**, *53* (19), 4756–4795.
 - (125) Kim, J.; Jang, Y.; Kim, N. J.; Kim, H.; Yi, G. C.; Shin, Y.; Kim, M. H.; Yoon, S. Study of Chemical Enhancement Mechanism in Non-Plasmonic Surface Enhanced Raman Spectroscopy (SERS). *Front. Chem.* **2019**, *7* (August), 1–7.
 - (126) Montiel, M. A.; Vidal-Iglesias, F. J.; Montiel, V.; Solla-Gullón, J. Electrocatalysis on Shape-Controlled Metal Nanoparticles: Progress in Surface Cleaning Methodologies. *Curr. Opin. Electrochem.* **2017**, *1* (1), 34–39.
 - (127) Otto, A. Surface-Enhanced Raman Scattering of Adsorbates. *J. Raman Spectrosc.* **1991**, *22* (12), 743–752.
 - (128) dos Santos, M. F.; Katic, V.; dos Santos, P. L.; Pires, B. M.; Formiga, A. L. B.; Bonacin, J. A. 3D-Printed Low-Cost Spectroelectrochemical Cell for In Situ Raman Measurements. *Anal. Chem.* **2019**, *91* (16), 10386–10389.
 - (129) Martín-Yerga, D.; Pérez-Junquera, A.; Hernández-Santos, D.; Fanjul-Bolado, P. In Situ Activation of Thick-Film Disposable Copper Electrodes for Sensitive Detection of Malachite Green Using Electrochemical Surface-Enhanced Raman Scattering (EC-SERS). *Electroanalysis* **2018**, *30* (6), 1095–1099.
 - (130) Clarke, O. J. R.; St Marie, G. J. H.; Brosseau, C. L. Evaluation of an Electrodeposited Bimetallic Cu/Ag Nanostructured Screen Printed Electrode for Electrochemical Surface-Enhanced Raman Spectroscopy (EC-SERS) Investigations. *J. Electrochem. Soc.* **2017**, *164* (5), B3091–B3095.
 - (131) Hernández, C. N.; Martín-Yerga, D.; González-García, M. B.; Hernández-Santos, D.; Fanjul-Bolado, P. Evaluation of Electrochemical, UV/VIS and Raman Spectroelectrochemical Detection of Naratriptan with Screen-Printed Electrodes. *Talanta* **2018**, *178* (August 2017), 85–88.
 - (132) Martín-Yerga, D.; Pérez-Junquera, A.; González-García, M. B.; Perales-Rondon, J. V.; Heras, A.; Colina, A.; Hernández-Santos, D.; Fanjul-Bolado, P. Quantitative Raman Spectroelectrochemistry Using Silver Screen-Printed Electrodes. *Electrochim. Acta* **2018**, *264*, 183–190.
 - (133) Lynk, T. P.; Clarke, O. J. R.; Kesavan, N.; Brosseau, C. L. Development of a Sustainable Plasmon-Enhanced Spectroelectrochemical Sensor Using Avocado Pit (Persea Americana) Extract. *Sensors Actuators, B Chem.* **2018**, *257*, 270–277.
 - (134) Robinson, A. M.; Harroun, S. G.; Bergman, J.; Brosseau, C. L. Portable Electrochemical Surface-Enhanced Raman Spectroscopy System for Routine Spectroelectrochemical Analysis. *Anal. Chem.* **2012**, *84* (3), 1760–1764.
 - (135) Karaballi, R. A.; Nel, A.; Krishnan, S.; Blackburn, J.; Brosseau, C. L. Development of an Electrochemical Surface-Enhanced Raman Spectroscopy (EC-SERS) Aptasensor for Direct Detection of DNA Hybridization. *Phys. Chem. Chem. Phys.* **2015**, *17* (33), 21356–21363.

- (136) Zhao, L.; Blackburn, J.; Brosseau, C. L. Quantitative Detection of Uric Acid by Electrochemical-Surface Enhanced Raman Spectroscopy Using a Multilayered Au/Ag Substrate. *Anal. Chem.* **2015**, *87* (1), 441–447.
- (137) Bindesri, S. D.; Alhatab, D. S.; Brosseau, C. L. Development of an Electrochemical Surface-Enhanced Raman Spectroscopy (EC-SERS) Fabric-Based Plasmonic Sensor for Point-of-Care Diagnostics. *Analyst* **2018**, *143* (17), 4128–4135.
- (138) Niaura, G.; Gaigalas, A. K.; Vilker, V. L. Moving Spectroelectrochemical Cell for Surface Raman Spectroscopy. *J. Raman Spectrosc.* **1997**, *28* (12), 1009–1011.
- (139) Mažeikienė, R.; Niaura, G.; Malinauskas, A. In Situ Time-Resolved Raman Spectroelectrochemical Study of Aniline Polymerization at Platinum and Gold Electrodes. *Chemija* **2018**, *29* (2), 81–88.
- (140) Mažeikienė, R.; Niaura, G.; Malinauskas, A. A Comparative Raman Spectroelectrochemical Study of Selected Polyaniline Derivatives in a PH-Neutral Solution. *Synth. Met.* **2010**, *160* (9–10), 1060–1064.
- (141) Mažeikienė, R.; Niaura, G.; Malinauskas, A. A Comparative Multiwavelength Raman Spectroelectrochemical Study of Polyaniline: A Review. *J. Solid State Electrochem.* **2019**, *23* (6), 1631–1640.
- (142) Mažeikienė, R.; Niaura, G.; Malinauskas, A. Red and NIR Laser Line Excited Raman Spectroscopy of Polyaniline in Electrochemical System. *Synth. Met.* **2019**, *248* (September 2018), 35–44.
- (143) Mažeikienė, R.; Niaura, G.; Malinauskas, A. Raman Spectroelectrochemical Study of Polyaniline at UV, Blue, and Green Laser Line Excitation in Solutions of Different PH. *Synth. Met.* **2018**, *243* (March), 97–106.
- (144) Mažeikienė, R.; Niaura, G.; Malinauskas, A. Study of Redox and Protonation Processes of Polyaniline by the Differential Multiwavelength Raman Spectroelectrochemistry. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2019**, *221*, 117147.
- (145) Mažeikienė, R.; Niaura, G.; Eicher-Lorka, O.; Malinauskas, A. Raman Spectroelectrochemical Study of Meldola Blue, Adsorbed and Electropolymerized at a Gold Electrode. *J. Colloid Interface Sci.* **2011**, *357* (1), 189–197.
- (146) Mažeikienė, R.; Niaura, G.; Malinauskas, A. Electrocatalytic Reduction of Hydrogen Peroxide at Prussian Blue Modified Electrode: An in Situ Raman Spectroelectrochemical Study. *J. Electroanal. Chem.* **2011**, *660* (1), 140–146.
- (147) Mažeikienė, R.; Niaura, G.; Malinauskas, A. Raman Spectroelectrochemical Study of Electrodeposited Prussian Blue Layer: Dependence of Spectral Intensity on Underlying Electrode Roughness. *Chemija* **2017**, *28* (1), 28–32.
- (148) Mažeikienė, R.; Niaura, G.; Malinauskas, A. Raman Spectroelectrochemical Study of Electrode Reactions of Hydroquinone at Electrodes Modified with Nile Blue and Other Azine Type Redox Mediators. *J. Solid State Electrochem.* **2019**, 2307–2316.
- (149) Mažeikienė, R.; Niaura, G.; Malinauskas, A. SERS Spectroelectrochemical Study of Electrode Processes at Copper Hexacyanoferrate Modified Electrode. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **2017**, *181*, 200–207.
- (150) Mažeikienė, R.; Niaura, G.; Malinauskas, A. Electrochemical Redox Processes at Cobalt Hexacyanoferrate Modified Electrodes: An in Situ Raman Spectroelectrochemical Study. *J. Electroanal. Chem.* **2014**, *719*, 60–71.
- (151) W. Kiefer; H. J. Bernstein. Rotating Raman Sample Technique for Colored Crystal

- Powders. Resonance Raman Effect in Solid KMnO₄. *Appl. Spectrosc.* **1971**, 25 (6), 609–613.
- (152) Yamada, H.; Amamiya, T.; Tsubomura, H. Resonance Raman Spectra of Rose Bengal Adsorbed on a ZnO Electrode. *Chem. Phys. Lett.* **1978**, 56 (3), 591–594.
- (153) Hildebrandt, P.; Macor, K. A.; Czernuszewicz, R. S. Novel Cylindrical Rotating Electrode for Anaerobic Surface-Enhanced Raman Spectroscopy. *J. Raman Spectrosc.* **1988**, 19 (1), 65–69.
- (154) McQuillan, A. J.; Hester, R. E. Raman Spectral Studies at Rotating Pyrolytic Graphite Electrodes. *J. Raman Spectrosc.* **1984**, 15 (1), 15–19.
- (155) Pemberton, J. E.; Buck, R. P. A Rotating Disk Cell for Raman Spectroelectrochemistry: Investigation of an Electrode Surface Species and an Electrogenerated Solution Species. *Appl. Spectrosc.* **1981**, 35 (6), 571–576.
- (156) Pemberton, J. E.; Buck, R. P. Dithizone Adsorption at Metal Electrodes. *J. Electroanal. Chem. Interfacial Electrochem.* **1982**, 136 (1), 201–208.
- (157) Kavan, L.; Janda, P.; Krause, M.; Ziegls, F.; Dunsch, L. Rotating Cell for in Situ Raman Spectroelectrochemical Studies of Photosensitive Redox Systems. *Anal. Chem.* **2009**, 81 (5), 2017–2021.
- (158) Pospíšil, L.; Gál, M.; Hromádová, M.; Bulíková, J.; Kolivka, V.; Cvaka, J.; Nováková, K.; Kavan, L.; Zukalová, M.; Dunsch, L. Search for the Form of Fullerene C₆₀ in Aqueous Medium. *Phys. Chem. Chem. Phys.* **2010**, 12 (42), 14095–14101.
- (159) Damlin, P.; Kvarnström, C.; Petr, A.; Ek, P.; Dunsch, L.; Ivaska, A. In Situ Resonant Raman and ESR Spectroelectrochemical Study of Electrochemically Synthesized Poly(p-Phenylenevinylene). *J. Solid State Electrochem.* **2002**, 6 (5), 291–301.
- (160) McGlashen, M. L.; Blackwood, M. E.; Spiro, T. G. Resonance Raman Spectroelectrochemistry of the C₆₀ Radical Anion. *J. Am. Chem. Soc.* **1993**, 115 (5), 2074–2075.
- (161) Ibañez, D.; Plana, D.; Heras, A.; Fermín, D. J.; Colina, A. Monitoring Charge Transfer at Polarizable Liquid/Liquid Interfaces Employing Time-Resolved Raman Spectroelectrochemistry. *Electrochem. commun.* **2015**, 54, 14–17.
- (162) Bonhomme, F.; Lassègues, J. C.; Servant, L. Raman Spectroelectrochemistry of a Carbon Supercapacitor. *J. Electrochem. Soc.* **2001**, 148 (11), 450–458.
- (163) Kavan, L.; Dunsch, L. Ionic Liquid for in Situ Vis/NIR and Raman Spectroelectrochemistry: Doping of Carbon Nanostructures. *ChemPhysChem* **2003**, 4 (9), 944–950.
- (164) Kalbáč, M.; Kavan, L.; Gorantla, S.; Gemming, T.; Dunsch, L. Sexithiophene Encapsulated in a Single-Walled Carbon Nanotube: An in Situ Raman Spectroelectrochemical Study of a Peapod Structure. *Chem. - A Eur. J.* **2010**, 16 (38), 11753–11759.
- (165) Kalbáč, M.; Vales, V.; Kavan, L.; Dunsch, L. Doping of C₇₀ Fullerene Peapods with Lithium Vapor: Raman Spectroscopic and Raman Spectroelectrochemical Studies. *Nanotechnology* **2014**, 25 (48), 485706.
- (166) Kalbac, M.; Kavan, L.; Dunsch, L. In Situ Raman Spectroelectrochemistry of SWCNT Bundles: Development of the Tangential Mode during Electrochemical Charging in Different Electrolyte Solutions. *Diam. Relat. Mater.* **2009**, 18 (5–8), 972–974.
- (167) Kavan, L.; Raptá, P.; Dunsch, L. In Situ Raman and Vis-NIR Spectroelectrochemistry at Single-Walled Carbon Nanotubes. *Chem. Phys. Lett.* **2000**, 328 (4–6), 363–368.

- (168) Tarábek, J.; Kavan, L.; Dunsch, L.; Kalbac, M. Chemical States of Electrochemically Doped Single Wall Carbon Nanotubes as Probed by Raman Spectroelectrochemistry and Ex Situ X-Ray Photoelectron Spectroscopy. *J. Phys. Chem. C* **2008**, *112* (36), 13856–13861.
- (169) Kavan, L.; Frank, O.; Green, A. A.; Hersam, M. C.; Koltai, J.; Zólyomi, V.; Kürti, J.; Dunsch, L. In Situ Raman Spectroelectrochemistry of Single-Walled Carbon Nanotubes: Investigation of Materials Enriched with (6,5) Tubes. *J. Phys. Chem. C* **2008**, *112* (36), 14179–14187.
- (170) Kavan, L.; Dunsch, L.; Kataura, H. In Situ Vis-NIR and Raman Spectroelectrochemistry at Fullerene Peapods. *Chem. Phys. Lett.* **2002**, *361* (1–2), 79–85.
- (171) Kalbáč, M.; Kavan, L.; Dunsch, L. In Situ Raman Spectroelectrochemistry as a Tool for the Differentiation of Inner Tubes of Double-Wall Carbon Nanotubes and Thin Single-Wall Carbon Nanotubes. *Anal. Chem.* **2007**, *79* (23), 9074–9081.
- (172) Kalbáč, M.; Kavan, L.; Zukalová, M.; Dunsch, L. In-Situ Vis-near-Infrared and Raman Spectroelectrochemistry of Double-Walled Carbon Nanotubes. *Adv. Funct. Mater.* **2005**, *15* (3), 418–426.
- (173) Kalbac, M.; Kavan, L.; Zukalová, M.; Dunsch, L. Influence of an Extended Fullerene Cage: Study of Chemical and Electrochemical Doping of C70 Peapods by in Situ Raman Spectroelectrochemistry. *J. Phys. Chem. C* **2007**, *111* (3), 1079–1085.
- (174) Kalbac, M.; Hsieh, Y. P.; Farhat, H.; Kavan, L.; Hofmann, M.; Kong, J.; Dresselhaus, M. S. Defects in Individual Semiconducting Single Wall Carbon Nanotubes: Raman Spectroscopic and in Situ Raman Spectroelectrochemical Study. *Nano Lett.* **2010**, *10* (11), 4619–4626.
- (175) Koh, C. S. L.; Lee, H. K.; Phan-Quang, G. C.; Han, X.; Lee, M. R.; Yang, Z.; Ling, X. Y. SERS- and Electrochemically Active 3D Plasmonic Liquid Marbles for Molecular-Level Spectroelectrochemical Investigation of Microliter Reactions. *Angew. Chemie - Int. Ed.* **2017**, *56* (30), 8813–8817.
- (176) Zhang, L.; Liao, V.; Yu, Z. Raman Spectroelectrochemistry of a Single-Wall Carbon Nanotube Bundle. *Carbon N. Y.* **2010**, *48* (9), 2582–2589.
- (177) Ibañez, D.; Romero, E. C.; Heras, A.; Colina, A. Dynamic Raman Spectroelectrochemistry of Single Walled Carbon Nanotubes Modified Electrodes Using a Langmuir-Schaefer Method. *Electrochim. Acta* **2014**, *129*, 171–176.
- (178) Tominaga, M.; Watanabe, N.; Yatsugi, Y. In Situ Raman Spectroelectrochemical Study of Potential-Induced Molecular Encapsulation of β -Carotene inside Single-Walled Carbon Nanotubes. *J. Electroanal. Chem.* **2017**, *800*, 156–161.
- (179) Colina, A.; Ruiz, V.; Heras, A.; Ochoteco, E.; Kauppinen, E.; López-Palacios, J. Low Resolution Raman Spectroelectrochemistry of Single Walled Carbon Nanotube Electrodes. *Electrochim. Acta* **2011**, *56* (3), 1294–1299.
- (180) Salvatierra, R. V.; Moura, L. G.; Oliveira, M. M.; Pimenta, M. A.; Zarbin, A. J. G. Resonant Raman Spectroscopy and Spectroelectrochemistry Characterization of Carbon Nanotubes/Polyaniline Thin Film Obtained through Interfacial Polymerization. *J. Raman Spectrosc.* **2012**, *43* (8), 1094–1100.
- (181) Kalbac, M.; Farhat, H.; Kong, J.; Janda, P.; Kavan, L.; Dresselhaus, M. S. Raman Spectroscopy and in Situ Raman Spectroelectrochemistry of Bilayer 12C/13C Graphene. *Nano Lett.* **2011**, *11* (5), 1957–1963.
- (182) Frank, O.; Dresselhaus, M. S.; Kalbac, M. Raman Spectroscopy and in Situ Raman

- Spectroelectrochemistry of Isotopically Engineered Graphene Systems. *Acc. Chem. Res.* **2015**, *48* (1), 111–118.
- (183) Binder, J.; Urban, J. M.; Stepniewski, R.; Strupinski, W.; Wysmolek, A. In Situ Raman Spectroscopy of the Graphene/Water Interface of a Solution-Gated Field-Effect Transistor: Electron–Phonon Coupling and Spectroelectrochemistry. *Nanotechnology* **2016**, *27* (4), 045704.
- (184) Bousa, M.; Frank, O.; Kavan, L. Progressive in Situ Reduction of Graphene Oxide Studied by Raman Spectroelectrochemistry: Implications for a Spontaneous Activation of LiFePO₄ (Olivine). *Electroanalysis* **2014**, *26* (1), 57–61.
- (185) Bouša, M.; Frank, O.; Jirka, I.; Kavan, L. In Situ Raman Spectroelectrochemistry of Graphene Oxide. *Phys. Status Solidi Basic Res.* **2013**, *250* (12), 2662–2667.
- (186) Gupta, S.; Carrizosa, S. B.; Jasinski, J.; Dimakis, N. Charge Transfer Dynamical Processes at Graphene-Transition Metal Oxides/Electrolyte Interface for Energy Storage: Insights from in-Situ Raman Spectroelectrochemistry. *AIP Adv.* **2018**, *8* (6), 065225.
- (187) Van Den Beld, W. T. E.; Odijk, M.; Vervuurt, R. H. J.; Weber, J. W.; Bol, A. A.; Van Den Berg, A.; Eijkel, J. C. T. In-Situ Raman Spectroscopy to Elucidate the Influence of Adsorption in Graphene Electrochemistry. *Sci. Rep.* **2017**, *7* (October 2016), 1–8.
- (188) Mažeikienė, R.; Niaura, G.; Malinauskas, A. Raman Spectroelectrochemical Study of Electrode Processes at Hybrid Polyaniline - Copper Hexacyanoferrate Modified Electrode. *J. Electroanal. Chem.* **2018**, *808* (September 2017), 228–235.
- (189) Gribkova, O. L.; Iakobson, O. D.; Nekrasov, A. A.; Cabanova, V. A.; Tverskoy, V. A.; Tameev, A. R.; Vannikov, A. V. Ultraviolet-Visible-Near Infrared and Raman Spectroelectrochemistry of Poly(3,4-Ethylenedioxythiophene) Complexes with Sulfonated Polyelectrolytes. The Role of Inter- and Intra-Molecular Interactions in Polyelectrolyte. *Electrochim. Acta* **2016**, *222*, 409–420.
- (190) Zanfrognini, B.; Colina, A.; Heras, A.; Zanardi, C.; Seeber, R.; López-Palacios, J. A UV-Visible/Raman Spectroelectrochemical Study of the Stability of Poly(3,4-Ethylenedioxythiophene) Films. *Polym. Degrad. Stab.* **2011**, *96* (12), 2112–2119.
- (191) Bilal, S.; Shah, A. U. H. A.; Holze, R. Raman Spectroelectrochemical Studies of Copolymers of O-Phenylenediamine and o-Toluidine. *Vib. Spectrosc.* **2010**, *53* (2), 279–284.
- (192) Shreepathi, S.; Holze, R. Spectroelectrochemistry and Preresonance Raman Spectroscopy of Polyaniline-Dodecylbenzenesulfonic Acid Colloidal Dispersions. *Langmuir* **2006**, *22* (11), 5196–5204.
- (193) Nekrasov, A. A.; Gribkova, O. L.; Iakobson, O. D.; Ardabievskii, I. N.; Ivanov, V. F.; Vannikov, A. V. Raman Spectroelectrochemical Study of Electrodeposited Polyaniline Doped with Polymeric Sulfonic Acids of Different Structures. *Chem. Pap.* **2017**, *71* (2), 449–458.
- (194) Morávková, Z.; Dmitrieva, E. Structural Changes in Polyaniline near the Middle Oxidation Peak Studied by in Situ Raman Spectroelectrochemistry. *J. Raman Spectrosc.* **2017**, *48* (9), 1229–1234.
- (195) Morávková, Z.; Dmitrieva, E. The First Products of Aniline Oxidation – SERS Spectroelectrochemistry. *ChemistrySelect* **2019**, *4* (30), 8847–8854.
- (196) Grzeszczuk, M.; Kepas, A.; Kvarnstrom, C.; Ivaska, A. Effects of Small Octahedral Mono, Di, and Trivalent Hexafluoroanions on Electronic and Molecular Structures

- of Polypyrrole Monitored by in Situ UV-Vis-NIR and Resonance Raman Spectroelectrochemical Measurements. *Synth. Met.* **2010**, *160* (7–8), 636–642.
- (197) Crowley, K.; Cassidy, J. In Situ Resonance Raman Spectroelectrochemistry of Polypyrrole Doped with Dodecylbenzenesulfonate. *J. Electroanal. Chem.* **2003**, *547* (1), 75–82.
- (198) Ferreira, L. M. C.; Grasseschi, D.; Santos, M. S. F.; Martins, P. R.; Gutz, I. G. R.; Ferreira, A. M. C.; Araki, K.; Toma, H. E.; Angnes, L. Unveiling the Structure of Polytetraruthenated Nickel Porphyrin by Raman Spectroelectrochemistry. *Langmuir* **2015**, *31* (14), 4351–4360.
- (199) Blacha-Grzechnik, A.; Piwowar, K.; Zassowski, P.; Motyka, R.; Zak, J. In-Situ Raman Spectroelectrochemical Studies on Thionine Layer Electrochemically Grafted to the Gold Surface. *Electrochim. Acta* **2017**, *245*, 902–911.
- (200) Wilson, A. J.; Willets, K. A. Unforeseen Distance-Dependent SERS Spectroelectrochemistry from Surface-Tethered Nile Blue: The Role of Molecular Orientation. *Analyst* **2016**, *141* (17), 5144–5151.
- (201) Xu, C.; Geng, H.; Bennett, R.; Clayton, D. A.; Pan, S. Ti@TiO₂ Nanowire Electrode with Polydisperse Gold Nanoparticles for Electrogenenerated Chemiluminescence and Surface Enhanced Raman Spectroelectrochemistry. *J. Phys. Chem. C* **2013**, *117* (4), 1849–1856.
- (202) Song, P.; Guo, X.; Pan, Y.; Wen, Y.; Zhang, Z.; Yang, H. SERS and in Situ SERS Spectroelectrochemical Investigations of Serotonin Monolayers at a Silver Electrode. *J. Electroanal. Chem.* **2013**, *688*, 384–391.
- (203) Shegai, T.; Vaskevich, A.; Rubinstein, I.; Haran, G. Raman Spectroelectrochemistry of Molecules within Individual Electromagnetic Hot Spots. *J. Am. Chem. Soc.* **2009**, *131* (40), 14392–14398.
- (204) Itoh, T.; McCreery, R. L. In Situ Raman Spectroelectrochemistry of Electron Transfer between Glassy Carbon and a Chemisorbed Nitroazobenzene Monolayer. *J. Am. Chem. Soc.* **2002**, *124* (36), 10894–10902.
- (205) Paxton, W. F.; Kleinman, S. L.; Basuray, A. N.; Stoddart, J. F.; Van Duyne, R. P. Surface-Enhanced Raman Spectroelectrochemistry of TTF-Modified Self-Assembled Monolayers. *J. Phys. Chem. Lett.* **2011**, *2* (10), 1145–1149.
- (206) Pomfret, M. B.; Pietron, J. J.; Owruksy, J. C. Measurement of Benzenethiol Adsorption to Nanostructured Pt, Pd, and PtPd Films Using Raman Spectroelectrochemistry. *Langmuir* **2010**, *26* (9), 6809–6817.
- (207) Karaballi, R. A.; Merchant, S.; Power, S. R.; Brosseau, C. L. Electrochemical Surface-Enhanced Raman Spectroscopy (EC-SERS) Study of the Interaction between Protein Aggregates and Biomimetic Membranes. *Phys. Chem. Chem. Phys.* **2018**, *20* (6), 4513–4526.
- (208) McLeod, K. E. R.; Lynk, T. P.; Sit, C. S.; Brosseau, C. L. On the Origin of Electrochemical Surface-Enhanced Raman Spectroscopy (EC-SERS) Signals for Bacterial Samples: The Importance of Filtered Control Studies in the Development of New Bacterial Screening Platforms. *Anal. Methods* **2019**, *11* (7), 924–929.
- (209) Ibañez, D.; Santidrian, A.; Heras, A.; Kalbáč, M.; Colina, A. Study of Adenine and Guanine Oxidation Mechanism by Surface-Enhanced Raman Spectroelectrochemistry. *J. Phys. Chem. C* **2015**, *119* (15), 8191–8198.
- (210) Greene, B. H. C.; Alhatab, D. S.; Pye, C. C.; Brosseau, C. L. Electrochemical-Surface

- Enhanced Raman Spectroscopic (EC-SERS) Study of 6-Thiouric Acid: A Metabolite of the Chemotherapy Drug Azathioprine. *J. Phys. Chem. C* **2017**, *121* (14), 8084–8090.
- (211) Yuan, T.; Le Thi Ngoc, L.; Van Nieuwkastele, J.; Odijk, M.; van Den Berg, A.; Permentier, H.; Bischoff, R.; Carlen, E. T. In Situ Surface-Enhanced Raman Spectroelectrochemical Analysis System with a Hemin Modified Nanostructured Gold Surface. *Anal. Chem.* **2015**, *87* (5), 2588–2592.
- (212) Le-The, H.; Lozeman, J. J. A. J. A.; Lafuente, M.; Muñoz, P.; Bommer, J. G. J. G.; Duy-Tong, H.; Berenschot, E.; Van Den Berg, A.; Tas, N. R. N. R.; Odijk, M.; et al. Wafer-Scale Fabrication of High-Quality Tunable Gold Nanogap Arrays for Surface-Enhanced Raman Scattering. *Nanoscale* **2019**, *11* (25), 12152–12160.
- (213) Le-The, H.; Tiggelaar, R. M.; Berenschot, E.; van den Berg, A.; Tas, N.; Eijkel, J. C. T. Postdeposition UV-Ozone Treatment: An Enabling Technique to Enhance the Direct Adhesion of Gold Thin Films to Oxidized Silicon. *ACS Nano* **2019**, *13*, 6782–6789.
- (214) Perales-Rondon, J. V.; Hernandez, S.; Heras, A.; Colina, A. Effect of Chloride and PH on the Electrochemical Surface Oxidation Enhanced Raman Scattering. *Appl. Surf. Sci.* **2019**, *473* (October 2018), 366–372.
- (215) Perales-Rondon, J. V.; Hernandez, S.; Martin-Yerga, D.; Fanjul-Bolado, P.; Heras, A.; Colina, A. Electrochemical Surface Oxidation Enhanced Raman Scattering. *Electrochim. Acta* **2018**, *282*, 377–383.
- (216) Bard, A. J.; Stratmann, M. Encyclopedia of Electrochemistry. Wiley-VCH: Weinheim 2007.
- (217) Zong, C.; Chen, C. J.; Zhang, M.; Wu, D. Y.; Ren, B. Transient Electrochemical Surface-Enhanced Raman Spectroscopy: A Millisecond Time-Resolved Study of an Electrochemical Redox Process. *J. Am. Chem. Soc.* **2015**, *137* (36), 11768–11774.
- (218) Shi, C.; Zhang, W.; Birke, R. L.; Lombardi, J. R. SERS Investigation of the Adsorption and Electroreduction of 4-Cyanopyridine on a Silver Electrode. *J. Electroanal. Chem.* **1997**, *423* (1–2), 67–81.
- (219) Murgida, D. H.; Hildebrandt, P. Proton-Coupled Electron Transfer of Cytochrome C. *J. Am. Chem. Soc.* **2001**, *123* (17), 4062–4068.
- (220) Murgida, D. H.; Hildebrandt, P. Active-Site Structure and Dynamics of Cytochrome c Immobilized on Self-Assembled Monolayers-A Time-Resolved Surface Enhanced Resonance Raman Spectroscopic Study This Work Was Supported by the Deutsche Forschungsgemeinschaft, the Volkswagenstiftung, and Th. *Angew. Chem. Int. Ed. Engl.* **2001**, *40* (4), 728–731.
- (221) Shi, C.; Zhang, W.; Lombardi, J. R.; Birke, R. L. Nanosecond Time Scale Kinetics in the Flavin Mononucleotide on an Electrode Surface Using Time-Resolved Surface-Enhanced Raman Spectroscopy. *J. Phys. Chem.* **1992**, *96* (25), 10093–10096.
- (222) Zhang, W.; Vivoni, A.; Lombardi, J. R.; Birke, R. L. Time-Resolved SERS Study of Direct Photochemical Charge Transfer between FMN and a Ag Electrode. *J. Phys. Chem.* **1995**, *99* (34), 12846–12857.
- (223) Shi, C.; Zhang, W.; Birke, R. L.; Lombardi, J. R. Detection of Short-Lived Intermediates in Electrochemical Reactions Using Time-Resolved Surface-Enhanced Raman Spectroscopy. *J. Phys. Chem.* **1990**, *94* (12), 4766–4769.
- (224) Wessel, J. Surface-Enhanced Optical Microscopy. **1985**, *2* (9), 1538–1541.
- (225) Stöckle, R. M.; Suh, Y. D.; Deckert, V.; Zenobi, R. Nanoscale Chemical Analysis by Tip-Enhanced Raman Spectroscopy. *Chem. Phys. Lett.* **2000**, *318* (1–3), 131–136.

- (226) Anderson, M. S. Locally Enhanced Raman Spectroscopy with an Atomic Force Microscope. *Appl. Phys. Lett.* **2000**, *76* (21), 3130–3132.
- (227) Hayazawa, N.; Inouye, Y.; Sekkat, Z.; Kawata, S. Metallized Tip Amplification of Near-Field Raman Scattering. *Opt. Commun.* **2000**, *183* (1), 333–336.
- (228) PETTINGER, B.; PICARDI, G.; SCHUSTER, R.; ERTL, G. Surface Enhanced Raman Spectroscopy: Towards Single Molecule Spectroscopy. *Electrochemistry* **2000**, *68* (12), 942–949.
- (229) Shao, F.; Zenobi, R. Tip-Enhanced Raman Spectroscopy: Principles, Practice, and Applications to Nanospectroscopic Imaging of 2D Materials. *Anal. Bioanal. Chem.* **2019**, *411* (1), 37–61.
- (230) Kurouski, D.; Mattei, M.; Van Duyne, R. P. Probing Redox Reactions at the Nanoscale with Electrochemical Tip-Enhanced Raman Spectroscopy. *Nano Lett.* **2015**, *15* (12), 7956–7962.
- (231) Zeng, Z. C.; Huang, S. C.; Wu, D. Y.; Meng, L. Y.; Li, M. H.; Huang, T. X.; Zhong, J. H.; Wang, X.; Yang, Z. L.; Ren, B. Electrochemical Tip-Enhanced Raman Spectroscopy. *J. Am. Chem. Soc.* **2015**, *137* (37), 11928–11931.
- (232) Li, J. F.; Huang, Y. F.; Ding, Y.; Yang, Z. L.; Li, S. B.; Zhou, X. S.; Fan, F. R.; Zhang, W.; Zhou, Z. Y.; Wu, D. Y.; et al. Shell-Isolated Nanoparticle-Enhanced Raman Spectroscopy. *Nature* **2010**, *464* (7287), 392–395.
- (233) Sharma, B.; Frontiera, R. R.; Henry, A.-I.; Ringe, E.; Van Duyne, R. P. SERS: Materials, Applications, and the Future. *Mater. Today* **2012**, *15* (1–2), 16–25.
- (234) Li, J. F.; Zhang, Y. J.; Rudnev, A. V.; Anema, J. R.; Li, S. B.; Hong, W. J.; Rajapandian, P.; Lipkowski, J.; Wandlowski, T.; Tian, Z. Q. Electrochemical Shell-Isolated Nanoparticle-Enhanced Raman Spectroscopy: Correlating Structural Information and Adsorption Processes of Pyridine at the Au(Hkl) Single Crystal/Solution Interface. *J. Am. Chem. Soc.* **2015**, *137* (6), 2400–2408.
- (235) Colnago, L. A.; Andrade, F. D.; Souza, A. A.; Azeredo, R. B. V.; Lima, A. A.; Cerioni, L. M.; Osán, T. M.; Pusiol, D. J. Why Is Inline NMR Rarely Used as Industrial Sensor? Challenges and Opportunities. *Chem. Eng. Technol.* **2014**, *37* (2), 191–203.
- (236) Nunes, L. M. S.; Moraes, T. B.; Barbosa, L. L.; Mazo, L. H.; Colnago, L. A. Monitoring Electrochemical Reactions in Situ Using Steady-State Free Precession ¹³C NMR Spectroscopy. *Anal. Chim. Acta* **2014**, *850*, 1–5.
- (237) Kobayashi, T.; Babu, P. K.; Chung, J. H.; Oldfield, E.; Wieckowski, A. Coverage Dependence of CO Surface Diffusion on Pt Nanoparticles: An EC-NMR Study. *J. Phys. Chem. C* **2007**, *111* (19), 7078–7083.
- (238) Babu, P. K.; Chung, J. H.; Oldfield, E.; Wieckowski, A. CO Surface Diffusion on Platinum Fuel Cell Catalysts by Electrochemical NMR. *Electrochim. Acta* **2008**, *53* (23), 6672–6679.
- (239) Day, J. B.; Vuissoz, P. A.; Oldfield, E.; Wieckowski, A.; Ansermet, J. P. Nuclear Magnetic Resonance Spectroscopic Study of the Electrochemical Oxidation Product of Methanol on Platinum Black. *J. Am. Chem. Soc.* **1996**, *118* (51), 13046–13050.
- (240) Babu, P. K.; Kim, H. S.; Oldfield, E.; Wieckowski, A. Electronic Alterations Caused by Ruthenium in Pt-Ru Alloy Nanoparticles as Revealed by Electrochemical NMR. *J. Phys. Chem. B* **2003**, *107* (31), 7595–7600.
- (241) Tong, Y. Y.; Hee, S. K.; Babu, P. K.; Waszczuk, P.; Wieckowski, A.; Oldfield, E. An NMR Investigation of CO Tolerance in a Pt/Ru Fuel Cell Catalyst. *J. Am. Chem. Soc.* **2002**,

- 124 (3), 468–473.
- (242) Tong, Y. Y.; Rice, C.; Wieckowski, A.; Oldfield, E. A Detailed NMR-Based Model for CO on Pt Catalysts in an Electrochemical Environment: Shifts, Relaxation, Back-Bonding, and the Fermi-Level Local Density of States. *J. Am. Chem. Soc.* **2000**, *122* (6), 1123–1129.
- (243) Tong, Y. Y.; Rice, C.; Wieckowski, A.; Oldfield, E. 195 Pt NMR of Platinum Electrocatalysts: Friedel-Heine Invariance and Correlations between Platinum Knight Shifts, Healing Length, and Adsorbate Electronegativity. *J. Am. Chem. Soc.* **2000**, *122* (48), 11921–11924.
- (244) Tong, Y.; Rice, C.; Godbout, N.; Wieckowski, A.; Oldfield, E. Correlation between the Knight Shift of Chemisorbed CO and the Fermi Level Local Density of States at Clean Platinum Catalyst Surfaces. *J. Am. Chem. Soc.* **1999**, *121* (13), 2996–3003.
- (245) Rice, C.; Tong, Y.; Oldfield, E.; Wieckowski, A. Cyclic Voltammetry and 195Pt Nuclear Magnetic Resonance Characterization of Graphite-Supported Commercial Fuel Cell Grade Platinum Electrocatalysts. *Electrochim. Acta* **1998**, *43* (19–20), 2825–2830.
- (246) Yano, H.; Inukai, J.; Uchida, H.; Watanabe, M.; Babu, P. K.; Kobayashi, T.; Chung, J. H.; Oldfield, E.; Wieckowski, A. Particle-Size Effect of Nanoscale Platinum Catalysts in Oxygen Reduction Reaction: An Electrochemical and 195Pt EC-NMR Study. *Phys. Chem. Chem. Phys.* **2006**, *8* (42), 4932–4939.
- (247) Babu, P. K.; Kim, H. S.; Chung, J. H.; Oldfield, E.; Wieckowski, A. Bonding and Motional Aspects of CO Adsorbed on the Surface of Pt Nanoparticles Decorated with Pd. *J. Phys. Chem. B* **2004**, *108* (52), 20228–20232.
- (248) Tong, Y. Y.; Wieckowski, A.; Oldfield, E. NMR of Electrocatalysts. *J. Phys. Chem. B* **2002**, *106* (10), 2434–2446.
- (249) Vuissoz, P. A.; Ansermet, J. P. H.; Wieckowski, A. Probing by NMR the Effect of Surface Charges on the Chemisorption Bond. *Phys. Rev. Lett.* **1999**, *83* (12), 2457–2460.
- (250) Slezak, P. J.; Wieckowski, A. Electrode Potential Dependent NMR Spectra of Surface ¹³CO on Polycrystalline Platinum. *J. Electroanal. Chem.* **1992**, *339* (1–2), 401–410.
- (251) Hu, J. Z.; Jaegers, N. R.; Hu, M. Y.; Mueller, K. T. In Situ and Ex Situ NMR for Battery Research. *J. Phys. Condens. Matter* **2018**, *30* (46), 463001.
- (252) Webster, R. D. In Situ Electrochemical-NMR Spectroscopy. Reduction of Aromatic Halides. *Anal. Chem.* **2004**, *76* (6), 1603–1610.
- (253) Zhang, X.; Zwanziger, J. W. Design and Applications of an in Situ Electrochemical NMR Cell. *J. Magn. Reson.* **2011**, *208* (1), 136–147.
- (254) Klod, S.; Ziegls, F.; Dunsch, L. In Situ NMR Spectroelectrochemistry of Higher Sensitivity by Large Scale Electrodes. *Anal. Chem.* **2009**, *81* (24), 10262–10267.
- (255) Klod, S.; Dunsch, L. A Combination of in Situ ESR and in Situ NMR Spectroelectrochemistry for Mechanistic Studies of Electrode Reactions: The Case of p-Benzoquinone. *Magn. Reson. Chem.* **2011**, *49* (11), 725–729.
- (256) Rapta, P.; Haubner, K.; Machata, P.; Lukeš, V.; Rosenkranz, M.; Schiemenz, S.; Klod, S.; Kivelä, H.; Kvarnström, C.; Hartmann, H.; et al. Charged States in Diphenylamino Endcapped Thiophenes with a 1,4-Phenylene Core: In Situ Electron Spin Resonance/Ultraviolet-Visible-near Infrared and Nuclear Magnetic Resonance Spectroelectrochemistry and Quantum Chemical Study. *Electrochim. Acta* **2013**, *110*, 670–680.

- (257) Zalibera, M.; Machata, P.; Clikeman, T. T.; Rosenkranz, M.; Strauss, S. H.; Boltalina, O. V.; Popov, A. A. 19F NMR-, ESR-, and Vis-NIR-Spectroelectrochemical Study of the Unconventional Reduction Behaviour of a Perfluoroalkylated Fullerene: Dimerization of the C70(CF3)10- Radical Anion. *Analyst* **2015**, *140* (21), 7209–7216.
- (258) Huang, L.; Sorte, E. G.; Sun, S. G.; Tong, Y. Y. J. A Straightforward Implementation of in Situ Solution Electrochemical ¹³C NMR Spectroscopy for Studying Reactions on Commercial Electrocatalysts: Ethanol Oxidation. *Chem. Commun.* **2015**, *51* (38), 8086–8088.
- (259) Bussy, U.; Giraudeau, P.; Silvestre, V.; Jaunet-Lahary, T.; Ferchaud-Roucher, V.; Krempf, M.; Akoka, S.; Tea, I.; Boujtita, M. In Situ NMR Spectroelectrochemistry for the Structure Elucidation of Unstable Intermediate Metabolites. *Anal. Bioanal. Chem.* **2013**, *405* (17), 5817–5824.
- (260) Boisseau, R.; Bussy, U.; Giraudeau, P.; Boujtita, M. In Situ Ultrafast 2D NMR Spectroelectrochemistry for Real-Time Monitoring of Redox Reactions. *Anal. Chem.* **2015**, *87* (1), 372–375.
- (261) Ferreira Gomes, B.; Ferreira da Silva, P.; Silva Lobo, C. M.; da Silva Santos, M.; Colnago, L. A. Strong Magnetoelectrolysis Effect during Electrochemical Reaction Monitored in Situ by High-Resolution NMR Spectroscopy. *Anal. Chim. Acta* **2017**, *983*, 91–95.
- (262) Kelly, E. J. Magnetic Field Effects on Electrochemical Reactions Occurring at Metal/Flowing-Electrolyte Interfaces. *J. Electrochem. Soc.* **1977**, *124* (7), 987–994.
- (263) Gomes, B. F.; Nunes, L. M. S.; Lobo, C. M. S.; Cabeça, L. F.; Colnago, L. A. In Situ Study of the Magnetoelectrolysis Phenomenon during Copper Electrodeposition Using Time Domain NMR Relaxometry. *Anal. Chem.* **2014**, *86* (19), 9391–9393.
- (264) Monzon, L. M. A.; Coey, J. M. D. Magnetic Fields in Electrochemistry: The Lorentz Force. A Mini-Review. *Electrochem. commun.* **2014**, *42*, 38–41.
- (265) Mincey, D. W.; Popovich, M. J.; Faustino, P. J.; Hurst, M. M.; Caruso, J. A. Monitoring of Electrochemical Reactions by Nuclear Magnetic Resonance Spectrometry. *Anal. Chem.* **1990**, *62* (11), 1197–1200.
- (266) Cao, S. H.; Ni, Z. R.; Huang, L.; Sun, H. J.; Tang, B.; Lin, L. J.; Huang, Y. Q.; Zhou, Z. Y.; Sun, S. G.; Chen, Z. In Situ Monitoring Potential-Dependent Electrochemical Process by Liquid NMR Spectroelectrochemical Determination: A Proof-of-Concept Study. *Anal. Chem.* **2017**, *89* (7), 3810–3813.
- (267) Sorte, E. G.; Jilani, S.; Tong, Y. Y. J. Methanol and Ethanol Electrooxidation on PtRu and PtNiCu as Studied by High-Resolution In Situ Electrochemical NMR Spectroscopy with Interdigitated Electrodes. *Electrocatalysis* **2017**, *8* (2), 95–102.
- (268) Sorte, E. G.; Tong, Y. J. Interdigitated Metal Electrodes for High-Resolution in Situ Electrochemical NMR. *J. Electroanal. Chem.* **2016**, *769*, 1–4.
- (269) Wang, J.; Cao, X.; Fang, L.; You, X.; Wong, K.; Cao, S.; Xiao, C.; Cai, S.; Huang, Y.; Zhang, X.; et al. MoS₂ Nanoflower Supported Pt Nanoparticle as an Efficient Electrocatalyst for Ethanol Oxidation Reaction. *Int. J. Hydrogen Energy* **2019**, *44* (31), 16411–16423.
- (270) Wang, J.; You, X.; Xiao, C.; Zhang, X.; Cai, S.; Jiang, W.; Guo, S.; Cao, S.; Chen, Z. Small-Sized Pt Nanoparticles Supported on Hybrid Structures of MoS₂ Nanoflowers/Graphene Nanosheets: Highly Active Composite Catalyst toward Efficient Ethanol Oxidation Reaction Studied by in Situ Electrochemical NMR

- Spectroscopy. *Appl. Catal. B Environ.* **2019**, 259 (March), 118060.
- (271) Cao, S. H.; Liu, S.; Sun, H. J.; Huang, L.; Ni, Z. R.; Jiang, W. L.; Zhan, M.; Zhou, Z. Y.; Sun, S. G.; Chen, Z. Versatile, Robust, and Facile Approach for in Situ Monitoring Electrocatalytic Processes through Liquid Electrochemical NMR Spectroscopy. *Anal. Chem.* **2019**, 91 (3), 1686–1691.
- (272) Zhang, X. P.; Jiang, W. L.; Cao, S. H.; Sun, H. J.; You, X. Q.; Cai, S. H.; Wang, J. L.; Zhao, C. Sen; Wang, X.; Chen, Z.; et al. NMR Spectroelectrochemistry in Studies of Hydroquinone Oxidation by Polyaniline Thin Films. *Electrochim. Acta* **2018**, 273, 300–306.
- (273) Silva, P. F. da; Gomes, B. F.; Lobo, C. M. S.; Queiroz Júnior, L. H. K.; Danieli, E.; Carmo, M.; Blümich, B.; Colnago, L. A. Electrochemical NMR Spectroscopy: Electrode Construction and Magnetic Sample Stirring. *Microchem. J.* **2019**, 146 (November 2018), 658–663.
- (274) Richter, J. B.; Eßbach, C.; Senkovska, I.; Kaskel, S.; Brunner, E. Quantitative: In Situ ¹³C NMR Studies of the Electro-Catalytic Oxidation of Ethanol. *Chem. Commun.* **2019**, 55 (43), 6042–6045.
- (275) Wang, H.; Köster, T. K. J.; Trease, N. M.; Ségalini, J.; Taberna, P. L.; Simon, P.; Gogotsi, Y.; Grey, C. P. Real-Time NMR Studies of Electrochemical Double-Layer Capacitors. *J. Am. Chem. Soc.* **2011**, 133 (48), 19270–19273.
- (276) Gomes, B.; Lobo, C.; Colnago, L. Monitoring Electrochemical Reactions in Situ with Low Field NMR: A Mini-Review. *Appl. Sci.* **2019**, 9 (3), 498.
- (277) Bruins, A. P. An Overview of Electrochemistry Combined with Mass Spectrometry. *TrAC - Trends Anal. Chem.* **2015**, 70, 14–19.
- (278) Dass, C. *Fundamentals of Contemporary Mass Spectrometry*; Wiley Series on Mass Spectrometry; Wiley, 2007.
- (279) Plieth, W.; Wilson, G. S.; Gutiérrez De La Fe, C. Spectroelectrochemistry: A Survey of in Situ Spectroscopic Techniques (Technical Report). *Pure Appl. Chem.* **1998**, 70 (7), 1395–1414.
- (280) Kasian, O.; Geiger, S.; Mayrhofer, K. J. J.; Cherevko, S. Electrochemical On-Line ICP-MS in Electrocatalysis Research. *Chem. Rec.* **2019**, 19 (10), 2130–2142.
- (281) Klemm, S. O.; Topalov, A. A.; Laska, C. A.; Mayrhofer, K. J. J. Coupling of a High Throughput Microelectrochemical Cell with Online Multielemental Trace Analysis by ICP-MS. *Electrochem. commun.* **2011**, 13 (12), 1533–1535.
- (282) Klemm, S. O.; Karschin, A.; Schuppert, A. K.; Topalov, A. A.; Katsounaros, I.; Mayrhofer, K. J. J. Corrigendum to “Time and Potential Resolved Dissolution Analysis of Rhodium Using a Microelectrochemical Flow Cell Coupled to an ICP-MS” [Journal of Electroanalytical Chemistry 677–680 (2012) 50–55] (S1572665712001865) (10.1016/j.jelechem.2012.05.006). *J. Electroanal. Chem.* **2013**, 693, 127.
- (283) Yuill, E. M.; Baker, L. A. Electrochemical Aspects of Mass Spectrometry: Atmospheric Pressure Ionization and Ambient Ionization for Bioanalysis. *ChemElectroChem* **2017**, 4 (4), 806–821.
- (284) Xu, X.; Lu, W.; Cole, R. B. On-Line Probe for Fast Electrochemistry / Electrospray Mass Spectrometry . Investigation of Polycyclic Aromatic Hydrocarbons. **1996**, 2700 (12), 4244–4253.
- (285) Faber, H.; Vogel, M.; Karst, U. Electrochemistry/Mass Spectrometry as a Tool in

- Metabolism Studies-A Review. *Anal. Chim. Acta* **2014**, 834 (1), 9–21.
- (286) Zabel, R.; Weber, G. Comparative Study of the Oxidation Behavior of Sulfur-Containing Amino Acids and Glutathione by Electrochemistry-Mass Spectrometry in the Presence and Absence of Cisplatin. *Anal. Bioanal. Chem.* **2016**, 408 (4), 1237–1247.
- (287) Odijk, M.; Olthuis, W.; van den Berg, a; Qiao, L.; Girault, H. Improved Conversion Rates in Drug Screening Applications Using Miniaturized Electrochemical Cells with Frit Channels. *Anal. Chem.* **2012**, 84 (21), 9176–9183.
- (288) Qiao, L.; Lu, Y.; Liu, B.; Girault, H. H. Copper-Catalyzed Tyrosine Nitration. *J. Am. Chem. Soc.* **2011**, 133 (49), 19823–19831.
- (289) van den Brink, F. T. G.; Büter, L.; Odijk, M.; Olthuis, W.; Karst, U.; van den Berg, A. Mass Spectrometric Detection of Short-Lived Drug Metabolites Generated in an Electrochemical Microfluidic Chip. *Anal. Chem.* **2015**, 87 (3), 1527–1535.
- (290) Yu, K.; Zhang, H.; He, J.; Zare, R. N.; Wang, Y.; Li, L.; Li, N.; Zhang, D.; Jiang, J. In Situ Mass Spectrometric Screening and Studying of the Fleeting Chain Propagation of Aniline. *Anal. Chem.* **2018**, 90 (12), 7154–7157.
- (291) Jiang, J.; Zhang, H.; Li, M.; Dulay, M. T.; Ingram, A. J.; Li, N.; You, H.; Zare, R. N. Droplet Spray Ionization from a Glass Microscope Slide: Real-Time Monitoring of Ethylene Polymerization. *Anal. Chem.* **2015**, 87 (16), 8057–8062.
- (292) Qiu, R.; Zhang, X.; Luo, H.; Shao, Y. Mass Spectrometric Snapshots for Electrochemical Reactions. *Chem. Sci.* **2016**, 7 (11), 6684–6688.
- (293) Guo, W.; Ding, H.; Gu, C.; Liu, Y.; Jiang, X.; Su, B.; Shao, Y. Potential-Resolved Multicolor Electrochemiluminescence for Multiplex Immunoassay in a Single Sample. *J. Am. Chem. Soc.* **2018**, 140 (46), 15904–15915.
- (294) Owens, J. L.; Marsh, H. A.; Dryhurst, G. Electrochemical Oxidation of Uric Acid and Xanthine. An Investigation by Cyclic Voltammetry, Double Potential Step Chronoamperometry and Thin-Layer Spectroelectrochemistry. *J. Electroanal. Chem.* **1978**, 91 (2), 231–247.
- (295) Wolter, O.; Heitbaum, J. Principles of Differential Electrochemical Mass Spectroscopy (Dems). *Ext. Abstr. Meet. - Int. Soc. Electrochem.* **1984**, 6, 454–456.
- (296) Wolter, O.; Heitbaum, J. Adsorption of Co on a Porous Pt-Electrode in Sulfuric Acid Studied By Dems. *Berichte der Bunsengesellschaft/Physical Chem. Chem. Phys.* **1984**, 88 (1), 6–10.
- (297) Abd-El-Latif, A. A.; Bondue, C. J.; Ernst, S.; Hegemann, M.; Kaul, J. K.; Khodayari, M.; Mostafa, E.; Stefanova, A.; Baltruschat, H. Insights into Electrochemical Reactions by Differential Electrochemical Mass Spectrometry. *TrAC - Trends Anal. Chem.* **2015**, 70, 4–13.
- (298) Jusys, Z.; Massong, H.; Baltruschat, H. New Approach for Simultaneous DEMS and EQCM: Electro-Oxidation of Adsorbed CO on Pt and Pt-Ru. *J. Electrochem. Soc.* **1999**, 146 (3), 1093–1098.
- (299) Heinen, M.; Chen, Y. X.; Jusys, Z.; Behm, R. J. In Situ ATR-FTIRS Coupled with on-Line DEMS under Controlled Mass Transport Conditions—A Novel Tool for Electrocatalytic Reaction Studies. *Electrochim. Acta* **2007**, 52 (18), 5634–5643.
- (300) Brimaud, S.; Jusys, Z.; Jürgen Behm, R. Shape-Selected Nanocrystals for in Situ Spectro-Electrochemistry Studies on Structurally Well Defined Surfaces under Controlled Electrolyte Transport: A Combined in Situ ATR-FTIR/Online DEMS

- Investigation of CO Electrooxidation on Pt. *Beilstein J. Nanotechnol.* **2014**, *5* (1), 735–746.
- (301) Schnaidt, J.; Heinen, M.; Jusys, Z.; Behm, R. J. Electro-Oxidation of Ethylene Glycol on a Pt-Film Electrode Studied by Combined in Situ Infrared Spectroscopy and Online Mass Spectrometry. *J. Phys. Chem. C* **2012**, *116* (4), 2872–2883.
- (302) Schnaidt, J.; Heinen, M.; Jusys, Z.; Behm, R. J. Mechanistic Aspects of the Electro-Oxidation of Ethylene Glycol on a Pt-Film Electrode: A Combined in Situ IR Spectroscopy and Online Mass Spectrometry Study of Kinetic Isotope Effects. *Catal. Today* **2013**, *202* (1), 154–162.
- (303) Reichert, R.; Schnaidt, J.; Jusys, Z.; Behm, R. J. The Influence of Reactive Side Products in Electrocatalytic Reactions: Methanol Oxidation as Case Study. *ChemPhysChem* **2013**, *14* (16), 3678–3681.
- (304) Gorlin, M.; De Araujo, J. F.; Schmies, H.; Bernsmeier, D.; Dresch, S.; Gliech, M.; Jusys, Z.; Chernev, P.; Kraehnert, R.; Dau, H.; et al. Tracking Catalyst Redox States and Reaction Dynamics in Ni-Fe Oxyhydroxide Oxygen Evolution Reaction Electrocatalysts: The Role of Catalyst Support and Electrolyte pH. *J. Am. Chem. Soc.* **2017**, *139* (5), 2070–2082.
- (305) Trimarco, D. B.; Scott, S. B.; Thilsted, A. H.; Pan, J. Y.; Pedersen, T.; Hansen, O.; Chorkendorff, I.; Vesborg, P. C. K. Enabling Real-Time Detection of Electrochemical Desorption Phenomena with Sub-Monolayer Sensitivity. *Electrochim. Acta* **2018**, *268*, 520–530.
- (306) Trimarco, D. B.; Pedersen, T.; Hansen, O.; Chorkendorff, I.; Vesborg, P. C. K. Fast and Sensitive Method for Detecting Volatile Species in Liquids. *Rev. Sci. Instrum.* **2015**, *86* (7), 075006.
- (307) Roy, C.; Sebok, B.; Scott, S. B.; Fiordaliso, E. M.; Sørensen, J. E.; Bodin, A.; Trimarco, D. B.; Damsgaard, C. D.; Vesborg, P. C. K.; Hansen, O.; et al. Impact of Nanoparticle Size and Lattice Oxygen on Water Oxidation on NiFeOxHy. *Nat. Catal.* **2018**, *1* (11), 820–829.
- (308) Winiwarter, A.; Silvioli, L.; Scott, S. B.; Enemark-Rasmussen, K.; Sariç, M.; Trimarco, D. B.; Vesborg, P. C. K.; Moses, P. G.; Stephens, I. E. L.; Seger, B.; et al. Towards an Atomistic Understanding of Electrocatalytic Partial Hydrocarbon Oxidation: Propene on Palladium. *Energy Environ. Sci.* **2019**, *12* (3), 1055–1067.
- (309) Liu, B.; Yu, X. Y.; Zhu, Z.; Hua, X.; Yang, L.; Wang, Z. In Situ Chemical Probing of the Electrode-Electrolyte Interface by ToF-SIMS. *Lab Chip* **2014**, *14* (5), 855–859.
- (310) Wang, Z.; Zhang, Y.; Liu, B.; Wu, K.; Thevuthasan, S.; Baer, D. R.; Zhu, Z.; Yu, X. Y.; Wang, F. In Situ Mass Spectrometric Monitoring of the Dynamic Electrochemical Process at the Electrode-Electrolyte Interface: A SIMS Approach. *Anal. Chem.* **2017**, *89* (1), 960–965.
- (311) Yu, J.; Zhou, Y.; Hua, X.; Liu, S.; Zhu, Z.; Yu, X. Y. Capturing the Transient Species at the Electrode-Electrolyte Interface by: In Situ Dynamic Molecular Imaging. *Chem. Commun.* **2016**, *52* (73), 10952–10955.
- (312) Rohner, T. C.; Rossier, J. S.; Girault, H. H. Polymer Microspray with an Integrated Thick-Film Microelectrode. *Anal. Chem.* **2001**, *73* (22), 5353–5357.
- (313) Rohner, T. C.; Rossier, J. S.; Girault, H. H. On-Line Electrochemical Tagging of Cysteines in Proteins during Nanospray. *Electrochem. commun.* **2002**, *4* (9), 695–700.

- (314) Van Berkel, G. J.; Quirke, J. M. E.; Tigani, R. A.; Dilley, A. S.; Covey, T. R. Derivatization for Electrospray Ionization Mass Spectrometry. 3. Electrochemically Ionizable Derivatives. *Anal. Chem.* **1998**, *70* (8), 1544–1554.
- (315) Pavia, D. L.; Lampman, G. M.; Kriz, G. S.; Vyvyan, J. A. *Introduction to Spectroscopy*; Cengage Learning, 2008.
- (316) López-Lorente, Á. I.; Izquierdo, J.; Kranz, C.; Mizaikoff, B. Boron-Doped Diamond Modified with Gold Nanoparticles for the Characterization of Bovine Serum Albumin Protein. *Vib. Spectrosc.* **2017**, *91*, 147–156.
- (317) Cheuquepán, W.; Pérez, J. M.; Orts, J. M.; Rodes, A. Spectroelectrochemical and DFT Study of Thiourea Adsorption on Gold Electrodes in Acid Media. *J. Phys. Chem. C* **2014**, *118* (33), 19070–19084.
- (318) Dunwell, M.; Yang, X.; Yan, Y.; Xu, B. Potential Routes and Mitigation Strategies for Contamination in Interfacial Specific Infrared Spectroelectrochemical Studies. *J. Phys. Chem. C* **2018**, *122* (43), 24658–24664.
- (319) Zimmermann, A.; Künzelmann, U.; Dunsch, L. Initial States in the Electropolymerization of Aniline and P-Aminodiphenylamine as Studied by in Situ FT-IR and UV-Vis Spectroelectrochemistry. *Synth. Met.* **1998**, *93* (1), 17–25.

4

Modular microreactor with integrated reflection element for online reaction monitoring using infrared spectroscopy

We report on the fabrication of an internal reflection element (IRE) combined with a modular polymer microfluidic chip that can be used for attenuated total reflection (ATR) infrared spectroscopy. The IRE is fabricated from a silicon wafer. Two different polymers are used for the fabrication of the two types of modular microfluidic chips, namely polydimethylsiloxane (PDMS) and cyclic olefin copolymer (COC). The microfluidic chip is modular in the sense that several layers of mixing channels, using the herringbone mixer principle, and reactions chambers, can be stacked to facilitate the study of the desired reaction. A model Paal-Knorr reaction is carried out to prove that the chip works as intended. Furthermore, we highlight the strength of IR spectroscopy as a tool for reaction monitoring by identifying the peaks and showing the different reaction orders at the different steps of the Paal-Knorr reaction. The reduction of the aldehyde groups indicates a (pseudo) first order reaction whereas the vibrational modes associated with the ring formation indicate a zero order reaction. This zero order reaction can be explained with literature, where it is suggested that water acts as a catalyst during the dehydration step, which is the final step in the pyrrole ring formation.

This work is adapted from:

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4.1 Introduction

Online reaction monitoring in microfluidics has the potential to make reaction synthesis cheaper, safer, faster and more environmentally friendly^{1,2}. In a review by Jensen et al. it is stated that for microreactors to be applicable to more than laboratory applications, sensors must be integrated into the systems. Now, almost 20 years later, only a limited amount of papers have been published regarding online reaction monitoring in microfluidic microreactors, most notably the work by or in association with the groups of H. Gardeniers³⁻⁷, K. Jensen^{2,8} and several others^{9,10,19-22,11-18}. These techniques for direct online measurement operate using different types of sensors, such as mass-spectrometry¹⁴, fluorescence and UV/Vis spectroscopy^{10,15,18,19,21}, electrochemistry^{4,16,17} and IR/NIR spectroscopy^{3,5,7,9,11-13,20,22}. These reported works are often geared towards a specific application and do not expand to broader fields. In our group, we have obtained ample expertise with (modularly designed) microreactor systems²³⁻²⁶. Here, we propose a modular microreactor system, making it possible for the microreactor to be adapted to a wide variety of reactions of interest. This is accomplished by changing the number of layers and the polymer material of the reactor chamber. By combining these microreactors with a silicon (Si) internal reflection element (IRE), online attenuated total reflection (ATR) Fourier transform infrared (FTIR) spectroscopy can be performed on-chip. The goal of the authors is to provide a versatile microreactor design enabling online reaction monitoring.

ATR-FTIR is already used in an industrial setting as reaction monitoring technique. Industrial scale reactions can be monitored with ATR fibre optics probes, closely monitoring the progress of the reaction. In a research setting, (ATR)-FTIR, often in combination with MS and NMR, is frequently used to confirm that the expected product is formed by performing measurements ex-situ²⁷. In-situ reaction monitoring in a research setting is traditionally performed on the macroscale, e.g. in large reaction vessels. A major drawback is that mass transport can be slow, for instance between the bulk and the surface of a catalyst. By performing reactions in a microfluidic chip, better control over mass transport, concentrations and temperature can be obtained, and less chemicals can be used¹. A microfluidic chip can screen reaction conditions, such as ratios of chemicals and concentrations, at higher throughput than classical batch reactions¹. Additionally, lesser amount of chemicals can be used, which is beneficial for economic, environmental and safety reasons.

In literature, hyphenated ATR-FTIR with microfluidic reaction chambers^{3,5,7,12,13} is described. Although these microfluidic devices are well designed, they might be difficult to replicate outside the research group, due to the highly specific and somewhat complex reaction chamber designs. Our simplified fabrication method for this ATR-FTIR chip is easily replicable, compared to the devices mentioned in existing literature. The simplicity and modularity of the chip makes it applicable for a wide variety of reactions. The goal of this work is to show that with limited fabrication techniques, a relatively cheap, modular chip for online reaction monitoring in a microfluidic device equipped with (passive) mixing structures and a reaction chamber can be achieved. To test the functionality of our chip, we performed a Paal-Knorr

reaction on-chip. We highlight the strength of IR as a tool for reaction monitoring by identifying the peaks and determining the reaction orders.

4.2 Materials and methods

Here we describe the fabrication of an internal reflection element (IRE) for ATR-FTIR spectroscopy, combined with a modular microfluidic reaction chamber and mixing channels. The final conceptual design of the modular device can be seen in Fig. 4.1.

4.2.1 Fabrication of the silicon IRE

The fabrication of an IRE for ATR-FTIR was achieved by etching facets into a silicon (Si) wafer. The fabrication of the IRE was based on a simplified version of the workflow reported by Herzig-Marx et al²⁸, a schematic representation of the fabrication can be seen in the form of a process flow in Fig. 4.2. In step 1: a 300 nm layer of low-stress silicon rich nitride (SiRN) (Tempress systems, dichlorosilane 77.5 sccm ammonia 20 sccm nitrogen 250 sccm 820-850-870°C 20 Pa) was deposited by low-pressure chemical vapour deposition on a 525 μm double polished Si wafer. In step 2 photolithography was used to create a layer of Olin Oir 907-17 positive photoresist which acted as a mask for selective SiRN etching. Unlike the previously reported method²⁸, where test etching pits were created to align the $\langle 100 \rangle$ crystalline plane, our fabrication method aligned the flat of the wafer with the mask using the micro positioning stage of the mask aligner (EVG6200 NT). This way, the same alignment was achieved, while reducing the fabrication steps. After the pattern

Fig. 4.1. Two different configurations of the IRE combined with the microfluidic mixer and reaction chamber layers. Several tubings can be connected to the inlets of the microfluidic layer in order to introduce the reactants. a): IRE with a single layer of a combined mixing and reaction chamber. Several tubings, two inlets for introducing the reactants (A and B) and one outlet for the product (C), are connected to the device. Highlighted with numbers are: (1) mixing channels. (2) reaction chamber. (3) polymer microfluidic chip. (4) Si IRE. Chips with the single microfluidic layer which can be used for simple reactions, such as a Paal-Knorr reaction (reaction mechanism shown in Fig. 4.5) b) Si-ATR device with a three microfluidic layers, which can be used for reactions with more than two reactants. Several tubings are connected to the device, four inlets for introducing the reactants (A, B, D and F) and one outlet for the final product (C). Holes are punched or drilled in two layers allowing the transport of liquid between different layers (C and E)

Fig. 4.2: Process flow for Si-IRE. Step 1-4: creating SiRN mask for selective KOH etching of Si. Step 5 selective KOH etching of Si to create the facets. Step 6: Dicing of the wafer to obtain the Si-IRE.)

had been developed, the SiRN was selectively etched as shown in step 3 (PlasmaTherm, 790-series, trifluoromethane 100sccm oxygen 9 sccm, 5.3 Pa) by use of directional reactive ion etching followed by stripping of the photoresist in step 4. In step 5, the wafer was immersed in a 25wt.% potassium hydroxide (KOH) solution at 70°C for 7.5 h, which etched the Si wafer until a thin layer of 50 μm Si was left, which created the ATR facets with the dimensions of l: 4.15 mm w: 10 mm. Finally the remaining SiRN layer was stripped in a 50% hydrofluoric acid solution and the devices were diced (DISCO, DAD3220) from the wafer in step 6, which created the ATR chips with the dimensions l: 30 mm w: 15 mm h: 0.525 mm. Although the devices were made using the MESA⁺ Nanolab cleanroom facilities, the dimensions of the facets were in the order of several millimetres, therefore the use of a cleanroom is not strictly necessary, enabling the fabrication of cheap Si ATR devices.

4.2.2 Microfluidic chip design

Two polymers have been used to fabricate the modular microfluidic chips. These are: polydimethylsiloxane (PDMS) and cyclic olefin copolymer (COC). These different polymers required different fabrication and bonding techniques, both with their own advantages and disadvantages. An added benefit to using different polymers is their different chemical inertness²⁹, allowing the user to change polymers in order to facilitate the requirements of a desired reaction. As shown in Fig. 4.1, the chip consisted of a mixing channel (Fig. 4.1a, 1) followed by a reaction chamber (Fig. 4.1a, 2), where the reaction took place. The reaction chamber was shaped as an elongated hexagon, which prevented the formation of air bubbles during filling and prevented the formation of stagnation zones in the corners. The mixing channel was required since the flow was operated in the laminar regime. The mixer ensured that reactants were introduced as a homogeneously mixed solution into the reaction chamber. The mixing channels were of the herringbone design, which was first reported by Stroock et al.³⁰. This mixer created a chaotic flow by adding a transverse flow component. In the PDMS design, the herringbone structures were recessed into PDMS. For the COC design the herringbone structures were protruded into the channel.

Table 4.1: Fabrication specifications of the two different polymer chips

Material	Cleanroom	Channel width (μm)	Channel length (mm)	Chemical inertness
PDMS	Yes	200	70	Low
COC	No	600	215	High

Several different variations of the mixing and reaction chamber were designed and fabricated (see Fig. A1 and Fig. A2). These variations could be combined by stacking up to three layers on top of each other, accommodating different reaction requirements. The chips had been designed such that the walls cover the sensing area of the ATR device as little as possible, preventing losses due to absorption of the polymer of the microfluidic chip.

4.2.3 Microfluidic chip fabrication

The PDMS devices were made in a cleanroom facility by use of lithography, containing structures with dimensions in the order of 10 μm . The COC chip was made with a different technique, namely by micromilling, which eliminated the need for a cleanroom facility. This resulted in a lower resolution device, which mostly affected the width of the mixing channels and the size of the herringbone structures. This micromilling technique could also be used for similar polymers such as poly(methyl 2-methylpropenoate), poly(oxymethylene), poly(tetrafluoroethylene), polystyrene and polycarbonate. A comparison of the PDMS and COC chip design and material properties is given in Table 4.1.

4.2.3.1 PDMS chip

The process flow for the fabrication PDMS chip can be seen in Fig. 4.3. The PDMS chip was fabricated by soft lithography, starting with a layer of SU-8 (MicroChem NANOTM SU-8 100, spin speed: 4000 RPM) followed by a pre-bake (subsequent heating steps: 50°C 10 min, 65°C 30 min, 95°C 35 min) and lithography, producing a mould which defined the negative of the channels/reaction chamber without mixing structures. After exposure, a post exposure bake was performed (subsequent heating steps: 50°C 10 min, 65°C 10 min, 75°C 35 min). Before resist development, a second SU-8 layer was applied on top of the first layer (MicroChem NANOTM SU-8 25, spin speed: 1000 RPM) followed by a pre-bake (subsequent heating steps: 50°C 10 min, 65°C 30 min, 95°C 35 min) which defined the herringbone mixing structures. After exposure, a post exposure bake was performed, (subsequent heating steps: 50°C 10 min, 65°C 10 min, 75°C 35 min) followed by a development step (Propylene glycol methyl ether acetate, RER600, ARCH Chemicals) and a hard bake (subsequent heating steps: 50°C 10 min, 65°C 10 min, 100°C 10 min, 120°C 120 min). The resulting SU-8 mould contained channels and a reaction chamber with a height of 85 μm . The mixing structures on top of the channels added an additional height of 13.6 μm . This fabricated mould could be reused to make a number of PDMS devices. The PDMS was mixed with a cross-linker (Sylgard 184 kit, containing polymer and cross-linker), degassed, (20.000 Pa for 45 min) and casted on the SU-8 structures, followed by curing in an oven at 60°C for 3 h. Lastly, the PDMS replica was peeled off the structures followed by punching holes in the in- and outlets with a biopsy puncher

Fig. 4.3: Process flow fabrication PDMS microfluidic chip. Step 1: SU-8 negative mould for reaction chamber and channels. Step 2-3: SU-8 negative mould for mixing channels. Step 4-5: Casting the PDMS chip on the SU-8 mould. Step 6: Bonding the PDMS chip on the Si-IRE.

($\varnothing 1$ mm). Next, the PDMS was bonded to the Si-IRE or to a second PDMS chip by an activation of the Si or PDMS surface by an oxygen plasma treatment (Cute plasma system, Femto science).

4.2.3.2 COC chip

In Fig. 4.4 the process flow of the fabrication for the COC chip is illustrated. The COC chip was fabricated by micromilling (DATRON Neo) a square piece of COC polymer (DENZ Bio-Medical GmbH) with the thickness of 1.0 mm. The designs (Solidworks) for part A and B (see Fig. A2) were loaded simultaneously in the milling machine after which the program controls the mill (smallest tool used for the most delicate features in this design: $\varnothing 0.2$ mm at 15.000 RPM and a feed rate of 75 mm/min. larger tool for features such as the reaction chamber: a $\varnothing 1.0$ mm at 20.000 RPM and a feed rate of 200 mm/min). Subsequently the solvent bonding procedure of the COC chip for parts A and B was conducted. The COC chip components are exposed to hexane vapour for 8 minutes in a closed petri-dish. Next, the parts were pressed together by hand, while being aligned using alignment pins (see Fig. A3). To complete the bonding, the parts were further pressed together using a hot press (Auto C-PL, Carver, Inc.) operated at 65°C and a force of 1.1 kN/cm² for 20 min. For bonding COC to the Si IRE unit, NOA-81 (Norland Products Inc.) adhesive was used. A droplet of NOA-81 is deposited on a glass microscopy slide and spin coated at 1500 RPM for 60 s to coat the glass slide with a thin uniform layer of glue. Next, the COC piece was pressed onto the coated glass slide, transferring a thin layer of NOA-81 adhesive. The ATR-device and the COC chip are aligned in the homemade alignment holder, pressure was applied by hand while the device is exposed to UV light (365 nm, 10 s, 400 W/cm²).

Fig. 4.4: Process flow fabrication COC microfluidic chip. Step 1-2: Micromilling different layers of the COC chip. Step 3: Solvent bonding of two separate COC chips. Step 4: Bonding the COC chip on the Si-IRE by using NOA-81 adhesive.

4.2.4 Measurement setup

The chip was mounted in an in-house constructed IR beam aligner. This aligner was used to couple the IR light from the Bruker Vertex 70 into the ATR device and consecutively couple the outgoing beam back into the IR spectrometer. Using a neMESYS syringe pump (CETONI GmbH) fitted with two 5mL syringes (Hamilton Model 1005-LTN), reagents are introduced into the microfluidic chip. In Fig. A4 images of the setup are shown.

4.2.5 Reaction monitoring and data processing

A Paal-Knorr reaction has been performed on-chip, where the reagents hexane-2,5-dione (HD) (Sigma-Aldrich) and butan-1-amine (BA) (Sigma-Aldrich) created the reaction products 1-butyl-2,5-dimethyl-1H-pyrrole (BDP) and water. The reaction mechanism as described in literature^{31–34}, is shown in Fig. 4.5. As proof of concept, we use the PDMS chip to perform this reaction. Before the reactants are introduced to the chip, a background scan is made of an empty chip (64 scans, 1 cm^{-1}). Using a syringe pump, the chip was filled with a 1:1 mixture of HD (neat) and BA (neat), resulting in a final concentration of 4.3 mol/l HD and 5.1 mol/l BA. The time required to fill the chip was 1 min. As soon as the chip is filled, the flow is halted and FTIR spectra are recorded (64 scans, 1 cm^{-1} resolution) every 3.5 minutes for a period of 21 minutes.

Data processing was performed using SpectraGryph. Firstly, the spectra are normalized on the 2930 cm^{-1} CH_2 asymmetric C-H stretch vibration. The spectra were further treated by with a second order derivative filter^{35,36}, enhancing the resolution of the spectra and reducing the baseline offset^{35,36}. The resulting second order derivative spectra were then integrated and plotted versus time. The resulting graph is used to determine the reaction order.

Fig. 4.5: Reaction mechanism for the Paal-Knorr reaction of hexane-2,5-dione and butan-1-amine to 1-butyl-2,5-dimethyl-1H-pyrrole and water side product, as described in the literature^{31–34}. Pathway A describes the hemiaminal cyclization step and pathway B describes the enamine cyclization step. The different intermediate products are highlighted with the roman numbers I-V and the final product, BDP, with the roman number VI

4.3 Results and discussion

4.3.1 Fabrication result IRE

In Fig. 4.6a, a fabricated Si ATR device is shown while in Fig. 4.6b, a microscope image of one of the facets from the same device is included. As can be seen in this figure, the etched section of the Si surface looks grainy as a result of the KOH treatment. The protected part of the Si device and the facet itself, which are the areas that interact with the light beam, look smooth. From this optical study of the Si surface, we conclude that the deposited SiRN layer is thick and homogenous enough to protect the Si surface from this KOH etching. In the same microscope image, the etching depth can be calculated. Based on the preferential etching of the $\langle 100 \rangle$ crystalline plane into Si, an etching angle of 54.7° is created resulting in a calculated depth of 0.472 mm. Taking into account the etching rate of KOH of $1 \mu\text{m}/\text{min}$ and an etching time of 7.5 hours, this closely matches the expected etching depth of 0.475 mm.

Fig. 4.6: a) Si ATR-device with the etched facets. b) microscope image of one of the facets, etching depth confirmed based on the width and angle of the facets.

4.3.2 Fabrication result microfluidic chip

In Fig. 4.7, two images of two different chips are shown. Fig. 4.7a demonstrates a single PDMS microfluidic chip bonded to a Si-IRE. This chip is a single-layer device, where a mixing channel (length 70 mm) and a reaction chamber are integrated in one PDMS layer. The PDMS bonding procedure has a high success rate. In 6 devices fabricated, none showed any leakage before or after the reaction. Failure is only observed when using too high flow rates ($>1 \mu\text{l/s}$), causing the tubing to disconnect from the chip. Fig. 4.7b shows a double-layered device of COC bonded to a Si-IRE. This device consists of a single COC reaction chamber in the bottom layer and a single mixing channel in the top layer (l: 215 mm). Bonding the several COC layers has a high success rate, rarely showing any leakage or separation. The bonding of the COC to the Si-IRE is more finicky. In most of the cases, the bonding using NOA-81 shows some signs of leakage. This is corrected by using a disposable glass pipet to introduce a small amount of NOA-81 to the leakage area. It is then observed that the NOA-81 gets pulled into the opening between the Si-IRE and COC chip, probably by capillary forces. The UV exposure is performed again to cure the NOA-81 and a visual check is performed to determine whether any glue entered the reaction chamber. After this fix, about 75% of the COC-IRE devices show no sign of leakage.

In Fig. 4.8a and Fig. 4.8b, microscope images of a PDMS and COC device are shown respectively. As explained in section 4.2.2, the width of the COC channels is significantly larger than that of the PDMS chip. The herringbone structures are clearly

Fig. 4.7: a) Single layer PDMS chip on Si-IRE. b) double layer COC chip on Si-IRE

Fig. 4.8: Mixing channels characterized. a) mixing channels PDMS chip. Width channel: 200 μm , length channel: 70mm, depth channel: 85 μm , length herringbone 275 μm , width herringbone 35 μm , depth herringbone 14 μm . b) mixing channels COC chip. Width channel: 600 μm , length channel: 215 mm, depth channel: 300 μm length herringbone 850 μm , width herringbone 280 μm , depth herringbone 50 μm . c) PDMS chip with food dye. Flow rate of the two different dyes: 0.1 $\mu\text{l/s}$ for a total flow rate of 0.2 $\mu\text{l/s}$. As can be observed, total mixing is achieved in the second mixing channel. d) COC chip with food dye. Flow rate of the two different dyes: 0.1 $\mu\text{l/s}$ for a total flow rate of 0.2 $\mu\text{l/s}$. as can be observed, total mixing is achieved in the ninth mixing channel.

visible and no significant defects can be observed in both the structures and the channels. However, the COC chip clearly has some milling artefacts on the edges of the channels, even after cleaning. In Fig. 4.8 (c and d), a macroscopic image of the PDMS and COC chips is shown respectively. The chips are filled with aqueous solutions containing food dyes in order to determine the mixing performance. Independent of the polymer used and the resulting differences in the width of the channels and grooves of the herringbone, total mixing occurs before the food dyes enter the detection chamber. For the PDMS chips this occurs observed in the second channel, for the COC in the ninth channel. (for RGB colour analysis see Fig. A5 and A6) . Therefore it can be concluded that the length of the PDMS channel is, as designed, much longer than the minimum required mixing length. This will guarantee that liquids for the Paal-Knorr reaction, where HD (0.00143 $\text{pa}\cdot\text{s}$ at 25°) is about 1.5 times more viscous than water (0.00089 $\text{pa}\cdot\text{s}$ at 25°), will still be properly mixed.

4.3.3 Measurement results

In Fig. 4.9a, the spectra of the Paal-Knorr reaction are shown at 3.5 minute interval. In Fig. 4.9b, the same spectra are shown, zoomed in on the 1800-900 cm^{-1} region. The most distinct peaks are highlighted with roman numerals. In Fig. 4.9a, peaks I-IV are highlighted. Table 4.2 contains the peak assignment. Peak I is a combination of the NH_2 stretch vibration of BA and the OH stretch of H_2O . The NH_2 stretch vibration, with its distinct double peaks (characteristic of a primary amine) is somewhat broadened, possibly due to hydrogen bridging with the ketone groups of

Fig. 4.9: IR-spectra of the Paal-Knorr reaction. Spectra have been normalized to the CH₃ stretch vibration. a) 6 spectra at 3.5 minute interval. b) same spectra as in a) extending from 1900 to 900 cm⁻¹. c-h) enlarged regions of specific peaks of the same spectra. Direction of the arrow indicates if the peaks increase or decrease over time. Line plots of spectra c till h are shown in A7.

HD. Despite the conversion of the primary amine to a tertiary amine, an increase in the peak intensity over time can be observed. This is caused by the formation of H₂O, which has a peak at the same wavelength, and gives two molecules for every single molecule of primary amine being converted. Peak II (CH₂ and CH₃ stretch), peak III (CH₂ stretch) and peak IV (CH₂ stretch of a CH₂-N) show, as expected, little change in peak area. Looking at Fig. 4.9c, peak V at ~1720 cm⁻¹ is highlighted. This peak is the most distinct peak and is largely separated from the other peaks in the spectra. It has a high peak intensity and originates from the C=O stretch vibration of HD. In Fig. 4.9d, two peaks are highlighted. Peak VI is the total sum of several peaks. Starting from the left, a sharp peak at 1660 cm⁻¹ can be observed decreasing over time, possibly NH₂ deformation vibration of BA. Going towards the right, a broad peak increases over time; this can be attributed to the O-H deformation of H₂O. Peak VII, which also increases over time, is the ring skeletal vibration of BDP. In Fig. 4.9e, the fingerprint region starts and more peak convolution can be observed. Most peaks in this figure are caused by the different C-H₂ and C-H₃ deformation stretch vibrations. Fig. 4.9f shows the decrease of peak VIII, representing the C-H₂ and C-H₃ deformations adjacent to a C=O stretch vibration. The peak in Fig. 4.9g, numbered IX, is increasing over time and is probably caused by the C-N deformation of a tertiary amine. Either BDP or the intermediate imine component (fig. 4.5 intermediate III.) could be responsible for this peak. However, if this peak is caused by the imine intermediate, a shift over time would be expected as the imine is converted to the enamine (fig.

Table 4.2: Peak assignment.

Peak in fig. 4.9.	HD (cm ⁻¹)	BA (cm ⁻¹)	BDP (cm ⁻¹)	Water (cm ⁻¹)	Unknown (cm ⁻¹)	Peak
I		3370 ³⁷⁻³⁹				NH ₂ stretch
I				3350		O-H stretch
I		3300 ³⁷⁻³⁹				NH ₂ stretch
	3000 ³⁹⁻⁴²					CH ₃ stretch
II	2960 ³⁹⁻⁴²	2960 ³⁷⁻³⁹	2960 ^{39,43}			CH ₃ stretch
III	2930 ³⁹⁻⁴²	2930 ³⁷⁻³⁹	2930 ^{39,43}			CH ₃ stretch
IV		2875 ³⁷⁻³⁹	2875 ^{39,43}			CH ₂ -N stretch
V	1715 ³⁹⁻⁴²					C=O stretch
VI					1660	Unknown
				1645		O-H deformation
		1600 ³⁷⁻³⁹				NH ₂ deformation
VII			1520 ^{39,43}			Ring skeletal vibration
		1460 ³⁷⁻³⁹	1460 ^{39,43}			C-H deformation
	1410 ³⁹⁻⁴²					C-H deformation
			1410 ^{39,43}			C-H deformation
		1380 ³⁷⁻³⁹				C-H deformation
VIII	1360 ³⁹⁻⁴²					C-H deformation
IX			1300 ^{39,43}			C-N Deformation
	1260 ³⁹⁻⁴²					Skeletal vibrations
	1230 ³⁹⁻⁴²					Skeletal vibrations
	1200 ³⁹⁻⁴²					Skeletal vibrations
X	1160 ³⁹⁻⁴²					Skeletal vibrations

3.5. IV) and consequently into the final BDP product. For this reason its highly likely that this peak corresponds to the C-N deformation of BDP. Fig. 4.9h shows peak X, decreasing over time. This peak is assigned to the skeletal vibration of HD.

In Fig. 4.10(a-c), the second order derivative of 3 peaks are shown. As one can see in these plots, negligible changes in peak intensity can be observed between the 17.5min and 21.0min spectra, suggesting that the reaction has come to completion.

Fig. 4.10: Second order derivative of 3 peaks. a) C=O stretch vibration. b) ring skeletal vibration. c) C-N deformation vibration. d) plotted integrated area of the second order derivative C=O stretch vibration against time. e) plotted integrated area of the second order derivative ring skeletal vibration against time. f) plotted integrated area of the second order derivative C-N deformation vibration.

In Fig. 4.10(d-f) plots of the area of this derivative versus time have been shown. For these plots, the points at 21.0 min have been left out. The peaks plotted are those most separated in the spectrum and where the vibrational frequency can be assigned to a single molecule. In Fig. 4.10a, the second order derivative spectra of the C=O stretch vibration peak of HD is shown at different measurement times. The area of these peaks are plotted against time in Fig. 4.10d, showing an exponential decay of the C=O vibration peak area. This suggests that the intermediate products formed by

removal of the C=O bond (Fig. 4.5 intermediates I and either II or V depending on the pathway) are formed at an exponential rate. This also suggests that this process is a first or pseudo first order reaction. When looking at the ring skeletal vibration of BDP (Fig. 4.10b) and its increase over time (Fig. 4.10e), a linear line can be plotted. Looking at Fig. 4.5, the ring skeletal is formed at the final step to form the end product, suggesting that side intermediate product V is converted into BDP at an linear rate. It also suggest that this process is a zero order reaction. Looking at the C-N deformation peak (Fig. 4.10c) and the plotted increase over time (Fig. 4.10f), a linear fit can also be made. This suggest that the tertiary amine is formed at an linear rate, indicating that this process is also a zero order reaction. The data in fig. 4.10d suggest that the first reaction step in the pyrrole formation by Paal-Knorr is a first order reaction. Since the chemicals used in the current experiment are used in excess, this could also mean that this is pseudo first order reaction. The zero order reaction of the formation of the C-N bond and the BDP ring is more peculiar and suggest that this process is limited by reactant molecules.

This might be explained by Mothana and Boyd³³, which states that water is used as a catalyst during the reaction. Catalysis by water takes place primarily during the hydroxyl removal from the diol intermediate and the dehydration step, which occurs for the final two steps in the pyrrole ring formation for pathway A (fig. 4.5). Since the reaction is performed with low concentration of water present, zeroth order reaction behaviour is to be expected if pathway A is indeed the pathway of the reaction. The result of these IR measurements seem to be in line with the conclusions made by Mothana and Boyd³³ that water is used as a catalyst, although more research is required to confirm this.

4.4 Conclusion

We successfully fabricated a modular microfluidic device, which can adapted to the number of steps of the reaction of interest. We show a single layer PDMS chip and a double layer COC chip to highlight the modularity of the device. We prove that the mixer structures function as designed by using a food dye, demonstrating that the mixing channels are overdesigned and therefore suitable for liquids that are more viscous. The microfluidic chip is combined with a Si-IRE that can be used for ATR-FTIR spectroscopy. A model Paal-Knorr reaction has successfully been performed on-chip. We show the strength of IR by highlighting and assigning the most important vibrational frequencies and determining the reaction orders of different steps of the reaction. Finally, the data obtained corresponds to that described in literature³³, suggesting that water is used as a catalyst during the dehydration step, which is the final step in the pyrrole ring formation.

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References

- (1) Jensen, K. F. Microreaction Engineering — Is Small Better? *Chem. Eng. Sci.* **2001**, *56* (2), 293–303.
- (2) Moore, J. S.; Jensen, K. F. Automated Multitrajectory Method for Reaction Optimization in a Microfluidic System Using Online IR Analysis. *Org. Process Res. Dev.* **2012**, *16* (8), 1409–1415.
- (3) Susarrey-Arce, A.; Tiggelaar, R. M.; Sanders, R. G. P.; Geerdink, B.; Lefferts, L.; Gardeniers, J. G. E.; van Houselt, A. Temperature Dependence of the 1727 cm^{-1} Interstitial Oxygen Absorption Band Studied by Attenuated Total Internal Reflection Infrared Spectroscopy in a Newly Developed Microreactor. *J. Phys. Chem. C* **2013**, *117* (42), 21936–21942.
- (4) van Leeuwen, M.; Krommenhoek, E. E.; Heijnen, J. J.; Gardeniers, H.; van der Wielen, L. A. M.; van Gulik, W. M. Aerobic Batch Cultivation in Micro Bioreactor with Integrated Electrochemical Sensor Array. *Biotechnol. Prog.* **2010**, *26* (1), 293–300.
- (5) Karabudak, E.; Mojet, B. L.; Schlautmann, S.; Mul, G.; Gardeniers, H. J. G. E. Attenuated Total Reflection-Infrared Nanofluidic Chip with 71 nL Detection Volume for *in Situ* Spectroscopic Analysis of Chemical Reaction Intermediates. *Anal. Chem.* **2012**, *84* (7), 3132–3137.
- (6) Susarrey-Arce, A.; Tiggelaar, R. M.; Gardeniers, J. G. E.; Van Houselt, A.; Lefferts, L. CO Adsorption on Pt Nanoparticles in Low E-Fields Studied by ATR-IR Spectroscopy in a Microreactor. *J. Phys. Chem. C* **2015**, *119* (44), 24887–24894.
- (7) Susarrey-Arce, A.; Tiggelaar, R. M.; Morassutto, M.; Geerlings, J.; Sanders, R. G. P.; Geerdink, B.; Schlautmann, S.; Lefferts, L.; van Houselt, A.; Gardeniers, J. G. E. A New ATR-IR Microreactor to Study Electric Field-Driven Processes. *Sensors Actuators B Chem.* **2015**, *220*, 13–21.
- (8) Moore, J. S.; Jensen, K. F. “Batch” Kinetics in Flow: Online IR Analysis and Continuous Control. *Angew. Chemie* **2014**, *126* (2), 480–483.
- (9) Sagmeister, P.; Williams, J. D.; Hone, C. A.; Kappe, C. O. Laboratory of the Future: A Modular Flow Platform with Multiple Integrated PAT Tools for Multistep Reactions. *React. Chem. Eng.* **2019**, *4* (9), 1571–1578.
- (10) Abu, E. A.; Bryan, S. A.; Seliskar, C. J.; Heineman, W. R. Assessing a Spectroelectrochemical Sensor’s Performance for Detecting $[\text{Ru}(\text{Bpy})_3]^{2+}$ in Natural and Treated Water. *Electroanalysis* **2012**, *24* (7), 1517–1523.
- (11) Wilkin, O. M.; Maitlis, P. M.; Haynes, A.; Turner, M. L. Mid-IR Spectroscopy for Rapid on-Line Analysis in Heterogeneous Catalyst Testing. *Catal. Today* **2003**, *81* (3), 309–317.
- (12) Sriram, K. K.; Nayak, S.; Pengel, S.; Chou, C. F.; Erbe, A. 10 nm Deep, Sub-Nanoliter Fluidic Nanochannels on Germanium for Attenuated Total Reflection Infrared (ATR-IR) Spectroscopy. *Analyst* **2017**, *142* (2), 273–278.
- (13) Aguirre, A.; Kler, P. A.; Berli, C. L. A.; Collins, S. E. Design and Operational Limits of an ATR-FTIR Spectroscopic Microreactor for Investigating Reactions at Liquid-Solid Interface. *Chem. Eng. J.* **2014**, *243*, 197–206.
- (14) Hu, X.; Dong, Y.; He, Q.; Chen, H.; Zhu, Z. Fabrication of a Polystyrene Microfluidic Chip Coupled to Electrospray Ionization Mass Spectrometry for Protein Analysis. *J. Chromatogr. B* **2015**, *990*, 96–103.
- (15) Li, Y.; Lin, B.; Ge, L.; Guo, H.; Chen, X.; Lu, M. Real-Time Spectroscopic Monitoring of

- Photocatalytic Activity Promoted by Graphene in a Microfluidic Reactor. *Nat. Publ. Gr.* **2016**, No. June, 1–9.
- (16) Urban, S.; Weltin, A.; Flamm, H.; Kieninger, J.; Deschner, B. J.; Kraut, M.; Dittmeyer, R.; Urban, G. A. Sensors and Actuators B : Chemical Electrochemical Multisensor System for Monitoring Hydrogen Peroxide , Hydrogen and Oxygen in Direct Synthesis Microreactors. *Sensors Actuators B. Chem.* **2018**, 273 (February), 973–982.
 - (17) Lin, Y.; Zhang, Z.; Zhao, L.; Wang, X.; Yu, P.; Su, L.; Mao, L. A Non-Oxidative Electrochemical Approach to Online Measurements of Dopamine Release through Laccase-Catalyzed Oxidation and Intramolecular Cyclization of Dopamine. *Biosens. Bioelectron.* **2010**, 25 (6), 1350–1355.
 - (18) Chen, L.; West, J.; Auroux, P.; Manz, A.; Day, P. J. R. Ultrasensitive PCR and Real-Time Detection from Human Genomic Samples Using a Bidirectional Flow Microreactor. *Anal. Chem.* **2007**, 79 (23), 9185–9190.
 - (19) Slyadnev, M. N.; Lavrova, M. V.; Erkin, M. A.; Kazakov, V. A.; Ganeev, A. A. Development of a Multireactor Microfluidic System for the Determination of DNA Using Real-Time Polymerase Chain Reaction. *J. Anal. Chem.* **2008**, 63 (2), 192–198.
 - (20) Martínez Arias, E. L.; Fazzio Martins, P.; Jardini Munhoz, A. L.; Gutierrez-Rivera, L.; Maciel Filho, R. Continuous Synthesis and in Situ Monitoring of Biodiesel Production in Different Microfluidic Devices. *Ind. Eng. Chem. Res.* **2012**, 51 (33), 10755–10767.
 - (21) Richardson, J. N.; Aguilar, Z.; Kaval, N.; Andria, S. E.; Shtoyko, T.; Seliskar, C. J.; Heineman, W. R. Optical and Electrochemical Evaluation of Colloidal Au Nanoparticle-ITO Hybrid Optically Transparent Electrodes and Their Application to Attenuated Total Reflectance Spectroelectrochemistry. *Electrochim. Acta* **2003**, 48 (28), 4291–4299.
 - (22) Richard, R.; Dubreuil, B.; Thiebaud-Roux, S.; Prat, L. On-Line Monitoring of the Transesterification Reaction Carried out in Microreactors Using near Infrared Spectroscopy. *Fuel* **2013**, 104, 318–325.
 - (23) Odijk, M.; Baumann, A.; Lohmann, W.; Van Den Brink, F. T. G.; Olthuis, W.; Karst, U.; Van Den Berg, A. A Microfluidic Chip for Electrochemical Conversions in Drug Metabolism Studies. *Lab Chip* **2009**, 9 (12), 1687–1693.
 - (24) van den Brink, F. T. G. G.; Olthuis, W.; van den Berg, A.; Odijk, M. Miniaturization of Electrochemical Cells for Mass Spectrometry. *TrAC Trends Anal. Chem.* **2015**, 70, 40–49.
 - (25) Geitenbeek, R. G.; Vollenbroek, J. C.; Weijgertze, H. M. H.; Tregouet, C. B. M.; Nieuwelink, A. E.; Kennedy, C. L.; Weckhuysen, B. M.; Lohse, D.; Van Blaaderen, A.; Van Den Berg, A.; et al. Luminescence Thermometry for: In Situ Temperature Measurements in Microfluidic Devices. *Lab Chip* **2019**, 19 (7), 1236–1246.
 - (26) van den Brink, F. T. G.; Wigger, T.; Ma, L.; Odijk, M.; Olthuis, W.; Karst, U.; van den Berg, A. Oxidation and Adduct Formation of Xenobiotics in a Microfluidic Electrochemical Cell with Boron Doped Diamond Electrodes and an Integrated Passive Gradient Rotation Mixer. *Lab Chip* **2016**, 16 (20), 3990–4001.
 - (27) Bruice, P. Y. *Organic Chemistry*; Prentice Hall, 2011.
 - (28) Herzig-Marx, R.; Queeney, K. T.; Jackman, R. J.; Schmidt, M. A.; Jensen, K. F. Infrared Spectroscopy for Chemically Specific Sensing in Silicon-Based Microreactors. *Anal. Chem.* **2004**, 76 (21), 6476–6483.
 - (29) Wypych, G. *Handbook of Polymers*; Elsevier Science, 2016.

- (30) Stroock, A. D.; Dertinger, S. K. W.; Ajdari, A.; Mezić, I.; Stone, H. A.; Whitesides, G. M. Chaotic Mixer for Microchannels. *Science* (80-.). **2002**, 295 (5555), 647–651.
- (31) Amarnath, V.; Anthony, D. C.; Amarnath, K.; Valentine, W. M.; Wetterau, L. A.; Graham, D. G. Intermediates in the Paal-Knorr Synthesis of Pyrroles. *J. Org. Chem.* **1991**, 56 (24), 6924–6931.
- (32) Amarnath, V.; Amarnath, K.; Valentine, W. M.; Eng, M. A.; Graham, D. G. Intermediates in the Paal-Knorr Synthesis of Pyrroles. 4-Oxoaldehydes. *Chem. Res. Toxicol.* **1995**, 8 (2), 234–238.
- (33) Mothana, B.; Boyd, R. J. A Density Functional Theory Study of the Mechanism of the Paal-Knorr Pyrrole Synthesis. *J. Mol. Struct. THEOCHEM* **2007**, 811 (1–3), 97–107.
- (34) Abbat, S.; Dhaked, D.; Arfeen, M.; Bharatam, P. V. Mechanism of the Paal-Knorr Reaction: The Importance of Water Mediated Hemialcohol Pathway. *RSC Adv.* **2015**, 5 (107), 88353–88366.
- (35) Lasch, P. Spectral Pre-Processing for Biomedical Vibrational Spectroscopy and Microspectroscopic Imaging. *Chemom. Intell. Lab. Syst.* **2012**, 117, 100–114.
- (36) Smith, B. C. *Fundamentals of Fourier Transform Infrared Spectroscopy*; Intergovernmental Panel on Climate Change, Ed.; CRC Press: Cambridge, 2011.
- (37) Teixeira-Dias, J. J. C.; de Carvalho, L. A. E. B.; da Costa, A. M. A.; Lampreia, I. M. S.; Barbosa, E. F. G. Conformational Studies by Raman Spectroscopy and Statistical Analysis of Gauche Interactions in N-Butylamine. *Spectrochim. Acta Part A Mol. Spectrosc.* **1986**, 42 (5), 589–597.
- (38) Kipkemboi, P. K.; Kiprono, P. C.; Sanga, J. J. Vibrational Spectra of T-Butyl Alcohol, t-Butylamine and t-Butyl Alcohol + t-Butylamine Binary Liquid Mixtures. *Bull. Chem. Soc. Ethiop.* **2003**, 17 (2), 211–218.
- (39) Pretsch, E.; Bühlmann, P.; Badertscher, M. *Structure Determination of Organic Compounds : Tables of Spectral Data.*; Springer, 2009.
- (40) Hudson, R. L.; Gerakines, P. A.; Ferrante, R. F. IR Spectra and Properties of Solid Acetone, an Interstellar and Cometary Molecule. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **2018**, 193, 33–39.
- (41) Tensmeyer, L. G.; Hoffmann, R. W.; Brindley, G. W. Infrared Studies of Some Complexes between Ketones and Calcium Montmorillonite. Clay-Organic Studies. Part III. *J. Phys. Chem.* **1960**, 64 (11), 1655–1662.
- (42) Dellepiane, G.; Overend, J. Vibrational Spectra and Assignment of Acetone, Aαα Acetone-D3 and Acetone-D6. *Spectrochim. Acta* **1966**, 22 (4), 593–614.
- (43) Akbaşlar, D.; Demirkol, O.; Giray, S. Paal-Knorr Pyrrole Synthesis in Water. *Synth. Commun.* **2014**, 44 (9), 1323–1332.

5

Gold nanoantennas for surface enhanced infrared spectroscopy

Background: This chapter contains agglomerated work regarding surface enhanced infrared spectroscopy performed over a period of 4 years. Most results discussed in this chapter are obtained towards the end of the PhD with the help of the K. Srivastava. Earlier fabrication work has been done with H. Le-The. At the start of the PhD, literature research and some initial fabrication work has been performed by the author of this thesis, which helped in setting the course of this chapter. Because the results have been obtained over a period of 4 years, using different techniques, this chapter will cover a number of different results.

Abstract: This work reports on the fabrication of large-area Au nanoantennas on a Si substrate for surface-enhanced-infrared-absorption-spectroscopy. Two different kinds of nanoantennas are fabricated, namely nano-rods and nano-slits. Fabrication is achieved by E-beam lithography. The need for an adhesion layer is eliminated using a UV-ozone pre-treatment. To our knowledge, this is the first time this technique is used to fabricate Au nanoantennas on Si, while at the same time obtaining a decent adhesion. Next to using the standard positive resist for EBL lithography, we also propose a workflow using a negative photoresist to make the nano-rod antennas, potentially speeding up the process by skipping the lift off procedure. Although still requiring optimization, our first fabrication attempt show promising results. In order to get the optimal enhancement for a given wavelength, we used FDTD simulations to simulate the structure length and pitch. After the simulations, the structures were fabricated and a comparison between the simulated results and fabricated structures was made.

This work is partially adapted from:

J. J. A. Lozeman[†], K. Srivastava[†], H. Le-The, A. van den Berg, M. Odijk, Large-area fabrication of Au nanoantennas for surface enhanced infrared spectroscopy without an adhesion layer, *in Enhanced Spectroscopies and Nanoimaging 2020, Proceedings of SPIE*. 1146818

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5.1 Surface enhanced infrared spectroscopy

Infrared (IR) spectroscopy is a powerful analytical technique used in a wide variety of fields such as the pharmaceutical industry, petroleum industry, feed and food industry, and forensic sciences. With IR spectroscopy, information about the molecular vibrations of a sample can be obtained, which can be translated into the molecular structure of the analyte in question, making IR spectroscopy a strong qualitative technique. Since IR spectroscopy is a spectroscopic technique, the Lambert-Beer law can be applied, allowing the user to obtain not only qualitative, but quantitative information as well. IR spectroscopy is a versatile tool, with operational modes including transmission and reflection spectroscopy, as well as attenuated total reflection (ATR). These different operational modes make it possible for IR spectroscopy to be used on solid, liquid and gaseous samples, giving the technique a wide field of potential applications. Hyphenating IR with separation techniques and secondary detection techniques is also not uncommon, examples are liquid chromatography IR, gas chromatography IR, size-exclusion chromatography IR, thermogravimetric analysis IR and atomic force microscopy IR, enabling even more applications such as the measurements of complex samples. This wide applicability, general ease of use of the instrumentation and the wealth of both quantitative and qualitative information it provides, makes IR spectroscopy a popular technique used in a large number of laboratories around the world.

One major drawback of IR spectroscopy is the low source power of the instrument. This can cause a low signal to noise (S/N), which limits the user to samples with small cross-sections and requires the instrumentation to operate with expensive, liquid nitrogen cooled detectors to be able to properly detect the lower IR wavelengths. In recent years, two different approaches to increase the S/N have been gaining attention. The first is to increase source power, for instance by using lasers. The development of QCL lasers has made high power mid-IR laser more accessible. And increasing the peak power of the light source by several orders of magnitude compared to Global sources¹. The second approach, and the focus of this chapter, is by using nanofabricated metallic structures to act as antennas for surface enhanced infrared spectroscopy (SEIRS).

SEIRS, first observed by Hartstein *et al.*² in the 1980's and further pioneered by the group of Osawa in the 1990's^{3,4}, is a technique often compared with surface enhanced Raman spectroscopy (SERS), although there are some distinct differences. These differences are most notable when comparing the enhancement factors of the two techniques, for SERS⁵⁻⁷ enhancement factors of up to 10^{10} have been reported, while SEIRS enhancement factors^{8,9} are more modest, in the range of 10^{1-5} . SEIRS substrates can be roughly categorized in two different groups, namely substrates consisting of roughened metallic films, or resonantly tuned nanoantennas. The resonantly tuned nanoantennas, with fine-tuned nanostructured parameters such as the shape, length, width, height and periodicity can provide a higher sensitivity than the roughened metallic films⁹. However, fabrication of these nanoantennas is quite labour-intensive, requiring cleanroom processing and high-resolution nanoscale lithography. The enhancement factor is directly related to the quality and reproducibility of the Au-nanoantennas and for practical applications, large-area fabrication is preferred. Moreover, adhesion of the nanoantennas to Si/SiO₂

substrates is poor and metal adhesion layers like Ti/Cr can affect the plasmonic resonance of the nanoantennas¹⁰⁻¹².

In SEIRS, the size and shape of the antenna are critical for determining the enhancement strength and resonance wavelength. As stated by Neubrech et al.¹³, the resonances in SEIRS, which occur along the long axis of the nanoantennas, can be considered as a standing wave, comparable with that of radiofrequency antennas¹³. The main difference when comparing to radiofrequency antennas, is that in SEIRS the electromagnetic radiation does penetrate into the antenna material. This penetration of the radiation into the antenna material causes a compressed effective wavelength compared to the wavelength of the external radiation field¹³. Neubrech et al. proposes a formula that can be used to determine the resonance wavelength in a half-wave dipole antenna for SEIRS, as followed¹³:

$$\lambda = \frac{2L}{m} n a_1 + a_2 \quad (5.1)$$

In this equation (5.1) L is the length of the antenna along the long axis of the antenna and m is the mode number, and n is the refractive index of the medium surrounding the antenna. a_1 is a parameter which depends on the antenna geometry and material and a_2 is a constant, accounting for the phase difference that is associated with the reflection occurring at the end of the antenna¹³. From this formula, we can determine that the resonance wavelength scales linearly with the antenna length and the refractive index of the medium surrounding the antenna. Which makes this formula an ideal tool for designing nanoantennas for the mid-infrared. It allows researchers to make a prediction for the resonance wavelength of antennas with different lengths, based on the experimental results of a small number of antenna lengths, as long as the other parameters stay the same.

Another aspect of SEIRS that should be discussed, is the resonance peak and its interaction with the sample. In a IR spectrum of an IR-nanoantenna, a broad absorbance peak can be observed, corresponding to the resonance wavelength¹³. When a sample molecule, with a molecular vibration in the wavelength range of the resonance wavelength, gets in close proximity of an IR-nanoantenna, dips and / or asymmetric peak shapes can be observed, depending on the match between the narrow molecular vibration and the broad plasmonic vibration¹³. These dips and / or asymmetric peak shapes often have a so called fano-profile, changing shape and strength depending on the match between the resonance frequency and the vibrational frequency. There are several methods of explaining these shapes, using quantum mechanics, the classical theory approach, as well as a numerical approach that can be used to address the fano-form of these peaks. In one approach, the shape of the peak can be explained as the results of constructive and destructive interference, originating from two excitation pathways¹³. One excitation pathway being the a broad spectral line caused by the plasmonic excitation of the nanoantennas and the second one being the narrow line caused by the molecular vibration¹³. The vibrational mode from the molecule constructively and destructively interferes with the electromagnetic field from the broad, plasmonic resonance,

resulting in the asymmetric shape of the measured vibrational signal. The different line shapes are dependent on the phase of the electromagnetic interaction¹³. When there is a perfect alignment between the plasmonic resonance and the molecular vibration, a so called anti-absorption peak can be observed in the spectrum¹³. The more the plasmonic resonance is detuned from the molecular vibration, the more the intensity of the anti-absorption peak decreases and the more asymmetric the shape of the peak gets. For a more in-depth explanation, and different approaches to address the fano-form, the reader is suggested to read the work *Surface-Enhanced Infrared Spectroscopy Using Resonant Nanoantennas* by Neubrech et al.¹³, which does not only give a good explanation of the theory behind the fano-shape, but also gives a deeper insight in the theory behind SEIRS.

In this work, we present two different nanoantenna configurations, nano-rods and nano-slits, designed based on FDTD simulations. The design of these structures have been reported before in literature, nano-rod antennas have been reported, by amongst others, Huck *et al.*^{14,15} and Adato *et al.*^{16,17}, while nano-slit antennas were first reported by Huck *et al.*¹⁸. The novelty of this work comes with the fabrication of these antennas. Our fabrication technique does not rely on the use of a metallic adhesion layer, but uses the previously reported UV-Ozone treatment¹⁹, simplifying the procedure and potentially improving the enhancement factor. Additionally, we propose a technique to fabricate the Au nano-rods with the use of negative photoresist E-beam lithography (EBL), speeding up the fabrication process by skipping the lift off procedure. As far as the authors are aware, this is the first time that SEIRS nanoantennas have been fabricated with such a process.

5.2 Experimental section

5.2.1 Simulations

Simulations were performed prior to fabrication in order to determine the structure's optimal shape and array configuration. The simulations were performed by FDTD simulation (Lumerical 2020a Launcher 1.1.2305). Two different nanoantenna designs were simulated, namely nano-rods and nano-slits (fig. 5.1). The nano-slits designs are the inverse of the nano-rod designs. Several parameters have been varied in order to find the optimal conditions. The parameters varied were: the length (l) of the structures, the pitch between the structures in the x direction (P_x) and the pitch between the structures in the y direction (P_y). The simulations where

Fig. 5.1: Schematic representation of top view of the nano-antennas. (a) Au nano-rods in a periodic pattern on Si substrate. (b) Au nano-slits in a periodic pattern on a Si substrate.

performed with the following boundary conditions: perfect matched layer (standard) for isolated antennas and periodic boundary conditions in the x and y direction and perfect matched layer in the z direction for the periodic antenna arrays.

5.2.2 Fabrication

Fabrication of the nano-structures was performed in the cleanroom at the MESA⁺ Institute for Nanotechnology. Three different fabrication processes will be discussed in the next section: the fabrication of the nano-slits and nano-rods using a positive resist and the fabrication of nano-rods using a negative resist.

5.2.2.1 Nano-slits

In Fig. 5.2, the process flow for the fabrication of the nano-slits is shown. On a Si wafer (one-sided polished (OSP), P-type <100>, 525 μm thick) a thin Au layer of 10 nm was sputtered on the wafer (in-house build sputtering system, pressure: 6.6×10^{-3} mbar power: 200 W, deposition rate 41.5 nm/min). Next, the wafer was treated with UV-Ozone for 5 min. (UVP PR-100 UV-Ozone photoreactor) following the procedure described by H. Le-The *et al.*¹⁹ Subsequently, another 40 nm of Au was sputtered to achieve the final structure height of 50 nm. A 255 nm coating of PMMA (NANOtm 950 PMMA Series Resists in chlorobenzene, Organic Polymer Solution. Spin rate: 2500 rpm for 60 s) was spin-coated on the wafer followed by a bake step (180°C for 120 s). Next, the PMMA was patterned using EBL (Raith EBPg 5150, Raith nanofabrication. Dose: 1400 $\mu\text{C}/\text{m}^2$, beam current: 5 nA), which resulted in a total writing time of 45 min. Following the lithography step, the resist was subsequently developed in a solution of 1:3 MIBK:IPA for 90 s. The excess Au is etched away with ion beam etching (Oxford i300 RIBE, current: 50 mA, voltage: 300 V, accelerator voltage: 500 V). Finally, the remaining resist was stripped away by 99% nitric acid followed by O₂ plasma treatment (Tepla 300, 500 sccm O₂ 500 sccm N₂, 800 W, 10 min).

5.2.2.2 Nano-rods (positive resist)

In Fig. 5.3, the process flow describing the fabrication of the nano-rods using positive resist is shown. A silicon wafer (OSP, P-type <100>, 525 μm thick) was used as a substrate. A 255 nm coating of PMMA (NANOtm 950 PMMA Series Resists in

Fig. 5.2: Fabrication process flow Au nano-slits using positive photoresist.

Fig. 5.3: Fabrication process flow for the nano-rods using positive photoresist.

Chlorobenzene, Organic Polymer Solution. Spin rate: 2500 rpm for 60 s) was spin-coated on the wafer followed by a bake step (180°C for 120 s). Next, the PMMA was patterned using EBL (Raith EBPG 5150, Raith nanofabrication. Dose: 1400 $\mu\text{C}/\text{m}^2$, beam current: 5 nA), which resulted in a total writing time of 45 min. After the lithography procedure, the resist was developed in a solution of 1:3 MIBK:IPA for 90 s. Following the successful development, 10 nm of Au was sputtered on the wafer (in-house build sputtering system pressure: 6.6×10^{-3} mbar power: 200 W, deposition rate 41.5 nm/min). Next, the wafer was treated with UV-Ozone for 5 min. (UVP PR-100 UV-Ozone photoreactor). Subsequently another 40nm of Au was sputtered to achieve the final structure height of 50 nm. Finally, the wafer was submerged in a 99% nitric acid till lift off was completed.

5.2.2.3 Nano-rods (negative resist)

Fig. 5.4 describes the process flow of the fabrication of the nano-rods using negative resist. On a silicon wafer (one-sided polished (OSP), P-type <100>, 525 μm thick) a thin Au layer of 10 nm was sputtered onto the wafer (In-house build sputtering system pressure: 6.6×10^{-3} mbar power: 200 W, deposition rate 41.5

Fig. 5.4: Fabrication process flow for the nano-rods using negative photoresist.

nm/min). Next, the wafer was treated with UV-Ozone for 5 min. (UVP PR-100 UV-Ozone photoreactor). In order to complete the Au-layer, another 40 nm of Au was sputtered to achieve the final structure height of 50 nm. A 400 nm coating of AR-n7520 (Allresist GmbH. Spin rate: 4000 rpm for 60 s) was spin-coated on the wafer followed by a bake step (85°C for 60 s). Next, the negative resist was patterned using EBL (Raith EBPG 5150, Raith nanofabrication. Dose: 1900 $\mu\text{C}/\text{m}^2$, beam current: 5 nA). Subsequently, the resist was developed in a solution of AR-300:47 (Allresist GmbH) for 90 s. The excess Au is etched away with ion beam etching (Oxford i300 RIBE, current: 50 mA, voltage: 300 V, accelerator voltage: 500 V). Finally, the remaining resist was stripped away by 99% nitric acid followed by O₂ plasma treatment (Tepla 300, 500 sccm O₂ 500 sccm N₂, 800 W, 20 min).

5.2.3 Measurements

Measurement of a wafer containing the nanoantennas of the nano-rod design have been performed using a Bruker vertex 70v. The large area nanoantennas were aligned with the IR-beam using an in-house build aligner, containing polarization filter. Measurements were performed in transmission mode. For the nanoantennas of the nano-slit design a PerkinElmer Spotlight 400 FT-IR imaging system has been used. The nano-slits where measured in the reflection mode.

Fig. 5.5: Simulation results showing normalized electric field density for the Au nano-antennas. Dimensions a and b: l: 1500 nm, w: 50 nm, h: 50 nm. Dimensions c and d: l: 1100 nm, w: 230 nm, h: 50 nm. (a) Nano-rod structure excited with parallel polarized light. (b) Nano-slit structure excited with parallel polarized light. (c) Nano-rod structure excited with parallel polarized light. (d) Nano-slit structure excited with parallel polarized light.

5.3 Results and discussion

5.3.1 Simulations results

In Fig. 5.5 the simulation results of a nano-rod and nano-slit are shown. Two different dimensions have been simulated. (a-b) Show antennas with the dimensions of l : 1500 nm, w : 50 nm, h : 50 nm, while (c-d) shown antennas with the dimensions of: l : 1100 nm, w : 230 nm, h : 50 nm. If the structures are excited with polarized light (E_{parallel} for the nano-rods and $E_{\text{perpendicular}}$ for the nano-slits) resonant accumulation of charges occurs, acting as hot spots for SEIRS. The accumulations of the charges occurs at the edges of the nano-rods while in the nano-slits the accumulation appears in the centre of the slits. These results correspond with literature, as shown by Huck *et al.*¹⁸. Also visible in fig. 5.5 is the effect of the width on the distribution of the intensity of the hotspots.

Fig. 5.6: Optimizing nano-rod antennas to make comparisons with literature. Dimensions of the antennas are: w : 50nm. h : 50nm. Substrate: CaF.
(a) Varying the length of the antennas will change the resonant frequency. (b) comparison of the results in fig. 5.6a with literature¹³. (c-d) By varying the pitch between antennas (pitch in y direction: P_y , pitch in x direction: P_x) with a given length, a maximum intensity can be determined. Shown are antennas with a length of 1500 nm. (c) Plotted are antennas with a P_x of 4 μm , while varying the P_y . A maximum enhancement is obtained at a P_y of 3 μm . (d) Plotted are antennas with a P_y of 3 μm , while varying the P_x . A maximum enhancement intensity is found at a P_x of 4.2 μm , resulting in an optimal enhancement for antennas with these particular dimension when using a periodicity of P_x of 4.2 μm and a P_y of 3 μm .

Shown in, Fig. 5.6a, the length of an antenna is critical in order to determine the frequency at which the resonance takes place. If the length of an antenna is increased, the wavelength where resonance occurs also increases. These results show high agreement with existing literature¹⁴. Next, the periodicity between the antennas was investigated by varying the distance between the antennas in the P_y direction and P_x direction. Periodicity is measured as the distance from the centre of one antenna to the centre of its neighbouring antenna. A change in enhancement can be observed due to the so called far-field coupling of the antennas²⁰. In fig. 5.6b, one example of the optimal periodicity between antennas with a size of l: 1500 nm w: 50 nm h: 50 nm is studied. First, the periodicity for P_x is set to 4 μm , while the P_y is varied, the optimal signal is obtained at an P_y of 3 μm . Next, P_y is set to 3 μm while P_x is varied, resulting in an optimal value at 4.2 μm . These results are wavelength dependent, but nicely show the dependence of the enhancement factor on the periodicity of the structures, as also shown by Bagheri et al.²⁰. In Fig. 5.7a the difference in resonance frequency between nano-rod and nano-slit nanoantennas is shown. Unlike the antennas in fig. 5.6, these antennas are simulated on a Si substrate and have a width of 230 nm, more in line with the experimental results. The resonance frequency between the nano-slits and nano-rods are almost identical, corresponding with literature¹⁸. In fig. 5.7b a comparison between nano-slits on a pure Si wafer, nano-slits on a Si wafer with 10 nm layer of SiO_2 and a Si wafer with 125 nm of SiO_2 is made. The enhancement peak is shifted to a higher wavenumber if the thickness of the SiO_2 layer is increased.

5.3.2 Fabrication results

5.3.2.1 Positive resist fabrication

In Fig. 5.8, the fabrication results of some of the fabricated nanoantennas are shown. Fig. 8a and 8b show the nano-slits and nano-rods fabricated with positive photoresist, respectively. The quality of the antennas look as expected, the slits and rods have straight walls and sharp corners and show little defects. The dimensions

Fig. 5.7: antennas with w: 230 nm and h: 50 nm. (a) comparison between nano-rod antennas and nano-slit antennas on a Si substrate with the exact same dimensions. (b) comparison between nano-slit antennas with in grey on a Si substrate, in blue on a Si substrate with 10 nm SiO_2 and in green on a Si substrate with 125 nm SiO_2 .

Fig. 5.8: Fabrication results of nanoantennas fabricated with the positive resist procedure (a) nano-slits with designed dimensions l: 2500 nm w: 70 nm measured dimension: l: 2410 nm w: 90 nm. (b) nano-rods with designed dimensions l: 2500 nm w: 230 nm measured dimension: l: 2540 nm w: 180 nm.

indicate some variations compared to the designs, which is most significant for the width of the structures. For instance, the width of the slits shown in fig. 8a is measured to be 90 nm, while being designed to be 70 nm. Additionally, the width for the rods in Fig. 5.8b is measured to be 180 nm, while being designed to be 230 nm. This is probably caused by the beam size during the EBL process and could be improved by optimizing the procedure. Fig. B1 contains additional SEM images of different variations of nano-slits.

5.3.2.2 Negative resist fabrication

More peculiar are the nano-rods fabricated by the negative photoresist procedure. In Fig. 5.9a and 5.9b, the first results of the negative resist nano-rods are shown. These Au nano-rods show deviation from the design, most notably it shows sharp spiky features on the Au rods and wide variety in the width of the structures. These spiky features are possibly a result of the negative resists cross linking and can be improved by optimizing the EBL procedure. Although roughness is usually

Fig.

5.9: Fabrication results of nano-rod antennas fabricated with the negative resist procedure (a) nano-rods with designed dimensions l: 1000 nm w: 70 nm measured dimension: l: 1000 nm w: 130-250 nm. (b) zoomed image of the nano-rods.

attributed to be a beneficial contribution to surface enhancement, roughness in this size is probably detrimental for the enhancement.

5.3.3 Measurements results

5.3.3.1 Nano-slits

In Table B1 a table is shown containing the dimensions and configurations of the fabricated nano-slits. In Fig. B2 the spectra of 3 sets of nano-slits are shown. Although the spectra are noisy, clear peaks can be seen in these spectra. In fig. 5.10a the resonance frequency of the experimental results of nano-slit arrays on a Si substrate are compared with simulation results. A general trend between the simulation and experimental results can be observed, although there is a clear difference between the two that increases as antenna length decreases. The same phenomenon can be observed in fig. 5.10B where the same comparison is made but this time with a 10nm thick layer of SiO₂ between the antenna array and the Si substrate. In this plot two sets of experimental results are shown, measured on different days and with different sample batches, showing the reproducibility of the fabrication process. An overlap of the simulated spectra with the experimental spectra can be seen in Fig. B2d. Although these results do show correlation between simulated and fabrication results, it is clear that either optimization of the simulation or the fabrication process is required. One possible reason for the offset between the simulation and experimental results are defects that occur during the fabrication. Shown in Fig. B1, some debris can be observed in the corners of the nano-slit antennas. Possibly from remaining resist or redeposited Si, or Au from the ion beam etching process. It is not unthinkable that these debris have an effect on the resonance frequency and this effect might increase as the antenna length decreases.

5.3.3.2 Nano-rods

In fig 5.11, the simulation and experimental results of an array of gold nano-rods with the dimensions of l: 1100 nm, w: 230 nm h: 50 nm P_x: 2600 nm P_y: 2400 nm, fabricated using the positive resist procedure, are shown. As can be seen in this

Fig. 5.10: Resonance frequency of simulations compared with experimental results. Structures with dimensions: h: 50 nm, w: 230 nm. (a) nano-slits on a Si substrate. (b) nano-slits on a Si substrate with 10 nm of SiO₂.

Fig. 5.11: Nano-rod antenna with the dimensions of l: 1100 nm, w: 230 nm h: 50 nm Px: 2600 nm Py: 2400 nm. (a) simulation results (b) experimental results.

figure, the absorption peak of the measured structures (1360cm^{-1}) is at a comparable frequency as the simulated results (1367cm^{-1}). A small dip in the signal at 1250 cm^{-1} can be seen in the experimental results. This dip is probably caused by contaminants present on the wafer. It should be noted that this dip has a fano shape, characteristic for the coupling of plasmonic and vibrational excitations²¹.

5.4 Conclusion and Outlook

We show that the fabrication technique to improve the Au adhesion to Si by using UV-ozone treatment works for the fabrication of Au nano-rod and nano-slit antennas. This UV-ozone treatment removes the need for a metallic adhesion layers for the fabrication of Au nanostructures on Si substrates, which not only simplifies the fabrication process, but the absence of a metallic adhesion layer can also improve the enhancement factor. We show the measurement result of a number of different nano-slit arrays and compare them with the simulation results. We show that there is a correlation between the simulation and experimental results, although an offset between the two is observed, which increases for decreasing antenna length. It is also shown that the resonance frequency between two different batches of nano-slit antennas, fabricated on different days is comparable, suggesting high repeatability of the fabrication technique. Although only one set of nano-rod arrays has been measured, the result of this measurement corresponds with the results of the simulations.

For future work, the experiments performed for the nano-slit arrays should be repeated for the nano-rod arrays to prove that the tunability is also valid for the nano-rod antenna arrays. Furthermore, in order to more properly be able to use the simulations as a tool for predicting the resonance frequency of novel structures of in future, a better understanding of the mismatch between the experimental results and simulated results must be obtained. After addressing the aforementioned points an indication of the enhancement factor should be obtained in order to be able to make a comparison with literature.

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References

- (1) Weida, M. J.; Yee, B. Quantum Cascade Laser-Based Replacement for FTIR Microscopy. *Imaging, Manip. Anal. Biomol. Cells, Tissues IX* **2011**, 7902 (February 2011), 79021C.
- (2) Hartstein, A.; Kirtley, J. R.; Tsang, J. C. Enhancement of the Infrared Absorption from Molecular Monolayers with Thin Metal Overlayers. *Phys. Rev. Lett.* **1980**, 45 (3), 201–204.
- (3) Osawa, M.; Ataka, K. ichi; Yoshii, K.; Yotsuyanagi, T. Surface-Enhanced Infrared ATR Spectroscopy for in Situ Studies of Electrode/Electrolyte Interfaces. *J. Electron Spectros. Relat. Phenomena* **1993**, 64–65 (C), 371–379.
- (4) Ataka, K. I.; Yotsuyanagi, T.; Osawa, M. Potential-Dependent Reorientation of Water Molecules at an Electrode/Electrolyte Interface Studied by Surface-Enhanced Infrared Absorption Spectroscopy. *J. Phys. Chem.* **1996**, 100 (25), 10664–10672.
- (5) Le-The, H.; Lozeman, J. J. A. A. J. J. A.; Lafuente, M.; Muñoz, P.; Bommer, J. G. J. G. J. G.; Duy-Tong, H.; Berenschot, E.; van den Berg, A.; Tas, N. R. N. R.; Odijk, M.; et al. Wafer-Scale Fabrication of High-Quality Tunable Gold Nanogap Arrays for Surface-Enhanced Raman Scattering. *Nanoscale* **2019**, 11 (25), 12152–12160.
- (6) Lozeman, J. J. A.; Führer, P.; Olthuis, W.; Odijk, M. Spectroelectrochemistry, the Future of Visualizing Electrode Processes by Hyphenating Electrochemistry with Spectroscopic Techniques. *Analyst* **2020**, 145 (7), 2482–2509.
- (7) Ru, E. C. Le; Etchegoin, P. G. *Principles of Surface-Enhanced Raman Spectroscopy*; Elsevier, 2009.
- (8) Ataka, K.; Heberle, J. Biochemical Applications of Surface-Enhanced Infrared Absorption Spectroscopy. *Anal. Bioanal. Chem.* **2007**, 388 (1), 47–54.
- (9) Kühner, L.; Hentschel, M.; Zschieschang, U.; Klauk, H.; Vogt, J.; Huck, C.; Giessen, H.; Neubrech, F. Nanoantenna-Enhanced Infrared Spectroscopic Chemical Imaging. *ACS Sensors* **2017**, 2 (5), 655–662.
- (10) Debu, D. T.; Ghosh, P. K.; French, D.; Herzog, J. B. Surface Plasmon Damping Effects Due to Ti Adhesion Layer in Individual Gold Nanodisks. *Opt. Mater. Express* **2017**, 7 (1), 73.
- (11) Jiao, X.; Goeckeritz, J.; Blair, S.; Oldham, M. Localization of Near-Field Resonances in Bowtie Antennae: Influence of Adhesion Layers. *Plasmonics* **2009**, 4 (1), 37–50.
- (12) Huang, J. S.; Callegari, V.; Geisler, P.; Brüning, C.; Kern, J.; Prangsma, J. C.; Wu, X.; Feichtner, T.; Ziegler, J.; Weinmann, P.; et al. Atomically Flat Single-Crystalline Gold Nanostructures for Plasmonic Nanocircuitry. *Nat. Commun.* **2010**, 1 (9), 1–8.

- (13) Neubrech, F.; Huck, C.; Weber, K.; Pucci, A.; Giessen, H. Surface-Enhanced Infrared Spectroscopy Using Resonant Nanoantennas. *Chem. Rev.* **2017**, *117* (7), 5110–5145.
- (14) Huck, C.; Toma, A.; Neubrech, F.; Chirumamilla, M.; Vogt, J.; De Angelis, F.; Pucci, A. Gold Nanoantennas on a Pedestal for Plasmonic Enhancement in the Infrared. *ACS Photonics* **2015**, *2* (4), 497–505.
- (15) Huck, C.; Neubrech, F.; Vogt, J.; Toma, A.; Gerbert, D.; Katzmann, J.; Härtling, T.; Pucci, A. Surface-Enhanced Infrared Spectroscopy Using Nanometer-Sized Gaps. *ACS Nano* **2014**, *8* (5), 4908–4914.
- (16) Adato, R.; Aksu, S.; Altug, H. Engineering Mid-Infrared Nanoantennas for Surface Enhanced Infrared Absorption Spectroscopy. *Biochem. Pharmacol.* **2015**, *18* (8), 436–446.
- (17) Adato, R.; Yanik, A. A.; Amsden, J. J.; Kaplan, D. L.; Omenetto, F. G.; Hong, M. K.; Erramilli, S.; Altug, H. Ultra-Sensitive Vibrational Spectroscopy of Protein Monolayers with Plasmonic Nanoantenna Arrays. *Proc. Natl. Acad. Sci.* **2009**, *106* (46), 19227–19232.
- (18) Huck, C.; Vogt, J.; Sendner, M.; Hengstler, D.; Neubrech, F.; Pucci, A. Plasmonic Enhancement of Infrared Vibrational Signals: Nanoslits versus Nanorods. *ACS Photonics* **2015**, *2* (10), 1489–1497.
- (19) Le-The, H.; Tiggelaar, R. M.; Berenschot, E.; van den Berg, A.; Tas, N.; Eijkel, J. C. T. Postdeposition UV-Ozone Treatment: An Enabling Technique to Enhance the Direct Adhesion of Gold Thin Films to Oxidized Silicon. *ACS Nano* **2019**, *13*, 6782–6789.
- (20) Bagheri, S.; Weber, K.; Gissibl, T.; Weiss, T.; Neubrech, F.; Giessen, H. Fabrication of Square-Centimeter Plasmonic Nanoantenna Arrays by Femtosecond Direct Laser Writing Lithography/ Effects of Collective Excitations on SEIRA Enhancement. *ACS Photonics* **2015**, *2* (6), 779–786.
- (21) Bagheri, S.; Giessen, H.; Neubrech, F. Large-Area Antenna-Assisted SEIRA Substrates by Laser Interference Lithography. *Adv. Opt. Mater.* **2014**, *2* (11), 1050–1056.

6

Wafer-scale fabrication of high-quality tunable gold nanogap arrays for surface-enhanced Raman scattering

In this chapter we report a robust and high-yield fabrication method for wafer-scale patterning of high-quality arrays of dense gold nanogaps, combining displacement Talbot lithography based shrink-etching with dry etching, wet etching, and thin film deposition techniques. By using the self-sharpening of <111>-oriented silicon crystal planes during the wet etching process, silicon structures with extremely smooth nanogaps are obtained. Subsequent conformal deposition of a silicon nitride layer and a gold layer results in dense arrays of narrow gold nanogaps. Using this method, we successfully fabricate high-quality Au nanogaps down to 10 nm over full wafer areas. Moreover, the gap spacing can be tuned by changing the thickness of deposited Au layers. Since the roughness of the template is minimized by the crystallographic etching of silicon, the roughness of the gold nanogaps depends almost exclusively on the roughness of the sputtered gold layers. Additionally, our fabricated Au nanogaps show a significant enhancement of surface enhanced Raman scattering (SERS) signals of benzenethiol molecules chemisorbed on the structure surface, at an average enhancement factor up to 1.5×10^6 .

This work is adapted from:

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6.1 SERS substrate fabrication techniques

Nanometer-sized metallic nanogaps made of gold (Au) or silver (Ag) have found many applications including biomedical and chemical sensing,¹ surface-enhanced Raman spectroscopy²⁻⁷ and nonlinear optics⁸ due to their remarkable electrochemical and optical properties. For the surface enhanced Raman spectroscopy, the intensity of the Raman scattering signal is boosted due to the modified emission of the scattering signal and the improved excitation of the studied molecules caused by the near-fields generated in metallic nanogap regions.⁹ To significantly enhance signals, the gap spacing needs to be much smaller than the incident wavelength.¹⁰ As a result, a strongly confined electromagnetic field is generated in the nanogap region by localized plasmons.¹¹ Using metallic nanogap structures, local enhancement factors up to 10^9 have been achieved, thus enabling the detection of a single molecule.^{2,3} Many methods have been reported to fabricate metallic nanogap structures, such as electron beam lithography (EBL),⁵ EBL-based angular deposition,⁴ electrochemical deposition,¹² electromigration¹³ break junction,^{14,15} and atomic layer deposition (ALD).^{2,7} EBL-based methods offer a high feasibility in designing the size, position, shape and orientation of the metal nanogaps. However, their feature of serial patterning limits their use for high-yield and low-cost fabrication of large-area metal nanogap arrays. Moreover, the quality of the metal nanogaps strongly depends on the sidewall roughness of the patterned resist structures, which is mostly limited by the properties of the resist.^{4,5} Other indirect methods, such as electrochemical deposition, electromigration, and break junction, can produce very narrow metal nanogaps down to 5 nm.¹²⁻¹⁵ However, using these methods it is difficult to design a reproducible fabrication process, and thus they are unsuitable for mass production at low-cost. ALD has recently come to be considered as an effective method to pattern well-defined metal nanogaps as narrow as 3 nm.^{2,7} However, the fabrication process involves many complicated steps, which makes it time consuming. Additionally, the metal nanogaps patterned by the ALD technique cannot be rapidly reproduced, whereas other template-based methods allow the metal nanogaps to be reused by replacing the metal layer sputtered over the template surface. Therefore, there is a need for a robust fabrication technique for wafer-scale patterning of dense metal nanogap arrays at high throughput but low-cost. Recently, a compelling technique for patterning periodic nanostructures has been reported by Solak et al., termed displacement Talbot lithography (DTL).¹⁶ Using this technique with a monochromatic UV beam (365 nm wavelength) allows high-yield patterning of highly ordered photoresist (PR) nanostructures, i.e. lines, holes or dots, with dimensions down to approximately 100 nm, over full wafer areas. However, patterning PR nanostructures smaller than 50 nm using this technique still requires the use of a deep-UV laser, i.e. an ArF laser at 193 nm wavelength. To overcome this limitation, we developed a robust technique for shrink-etching the patterned PR nanostructures using a mixture of O_2/N_2 plasma.¹⁷ As a result, bottom antireflection coating (BARC) nanostructures with dimensions down to sub-30 nm could be reliably fabricated from the initially patterned PR nanostructures.

In this paper, we report and demonstrate a robust and high-yield method for wafer-scale patterning of dense arrays of tunable Au nanogaps, combining the DTL-

based shrink-etching technique with dry etching, wet etching, and thin film deposition techniques. Silicon (Si) structures consisting of extremely smooth nanogaps were fabricated by combining the dry etching process with the self-sharpening of <111>-oriented Si crystal planes during the wet etching process. Such etching combination has been reported and used to fabricate various Si-based structures.^{18–23} Subsequent conformal deposition of a silicon nitride layer and a gold layer resulted in dense arrays of high-quality gold nanogaps over full wafer areas. The gap spacing can be tuned down to 10 nm at high uniformity by changing the Au layer thickness. Moreover, we showed the potential of our fabricated Au nanogaps for surface-enhanced spectroscopy by measuring SERS signals of benzenethiol (BT) molecules chemisorbed on the structure surface.

6.2 Experimental section

6.2.1 Patterning periodic 50 nm BARC nanolines

A BARC layer of 187 ± 2 nm (AZ BARLi II 200, MicroChem Corp., Japan), and a PR layer of 200 ± 2 nm (PFI88 photoresist diluted 1 : 1 with propylene glycol methyl ether acetate (PGMEA), Sumitomo Chemical Co., Ltd, Japan) were spincoated on the surface of an oxidized (~ 30 nm thick SiO_2) 4-inch Si wafers (525 μm thick, P-type, <100>, single side polished, Okmetic, Finland), followed by baking at 185 °C for 1 min and 90 °C for 1 min, respectively (Fig. 6.1a). DTL (PhableR 100C, Eulitha, Switzerland) was utilized to pattern PR nanolines of approximately 110 nm in width (250 nm pitch) at an exposure dose of 1 mW cm^{-2} for 75 s, using a wafer-scale phase shift mask of 250 nm grating period purchased from Eulitha (Fig. 6.1b). After the exposure, the oxidized Si wafer was post-exposure baked on a hotplate at 110 °C for 1 min, developed in the OPD4262 developer for 1 min, and rinsed with DI water to complete the fabrication of PR nanoline arrays (Fig. 6.1c). It is worth mentioning that the patterned PR nanolines were aligned to the [110] crystal orientation of the Si wafers – PR nanolines were patterned perpendicularly to the primary flat of the Si wafers. Subsequently, these PR nanolines were shrink-etched into ~ 50 nm BARC nanolines, using a two-step reactive plasma etching process, i.e. 50/50 sccm O_2/N_2 plasma for 1 min and 45 s, and 50 sccm N_2 plasma for 2 min (Fig. 6.1d). These plasma etching processes were conducted using a wafer-scale parallel plate reactive ion etching

Fig. 6.1: Fabrication process of high-quality tunable Au nanogap arrays.

system (in-house built TEske system, NanoLab Cleanroom) at 13 mTorr, and 25 W.¹⁷ Patterning periodic Cr nanolines using lift-off process

A thin Cr layer of approximately 6 nm was deposited over the surface of the patterned wafer using a sputtering system (in-house built T'COathy system, NanoLab Cleanroom) (Fig. 6.1e). The sputtering process was conducted at 200 W, and a pressure of 6.6×10^{-3} mbar adjusted by using an argon (Ar) flow. Subsequently, a lift-off process was performed in a 99% HNO₃ solution, followed by sonication in DI water for 10 min. As a result, periodic Cr nanolines of approximately 200 nm in width (250 nm pitch) were fabricated on the surface of the oxidized Si wafer (Fig. 6.1f). These Cr nanolines were then used as a hard mask for further processing of both the SiO₂ masking layer and the bulk Si substrate.

6.2.2 Patterning high-quality SiN nanogaps

Reactive plasma etching was used to directionally etch the SiO₂ masking layer and the bulk Si substrate, which resulted in periodic Si nanotrenches of ~50 nm in width and ~150 nm in depth (Fig. 6.1g–h). This plasma etching was also conducted in the TEske system at wafer-level, 25 sccm fluoroform (CHF₃), 5 sccm O₂, 10 mTorr, and 25 W for 13 min. Thereafter, the Cr mask was removed completely in a Cr etchant solution (Cr etch 200, MicroChemicals GmbH, Germany), leaving the SiO₂ nanolines as a masking layer (Fig. 6.1i). After rinsing with DI water and drying with N₂, the patterned wafer was cleaned in the O₂ plasma (TePla 300, PAV TePla AG, Germany) at 500 W for 20 min. Subsequently, the fabricated Si nanotrenches were etched in a 25% KOH solution at 75 °C for 15 s (Fig. 6.1k), cleaned using the standard RCA-2 cleaning process, and immersed in a 50% HF solution for 10 min to completely remove the SiO₂ masking layer, thus leaving only the Si structures as a template for the deposition of a low stress silicon nitride (SiN) layer. Prior to the deposition of the SiN layer, the wafer was cleaned in a 99% HNO₃ solution for 10 min, in a 69% HNO₃ solution at 95 °C for 10 min, in DI water using a quick dump rinser, and spin-dried. A thin SiN layer of approximately 35 nm was deposited over the Si template by using low-pressure chemical vapor deposition (LPCVD) in a high temperature tube furnace (Tempress Systems, Inc., The Netherlands) at 850 °C for 40 min (Fig. 6.1l). The thickness of the deposited SiN layer was measured using an ellipsometry system (M-2000UI, J.A. Woollam Co., United States) at an angle of 75°.

6.2.3 Patterning high-quality tunable Au nanogaps

Au layers at different thicknesses were deposited over the SiN coated Si templates by using the T'COathy sputtering system at 200 W, 6.6×10^{-3} mbar.²⁴ By adjusting the sputtering time, Au nanogaps of different gap spacings down to ~10 nm were fabricated over full wafer areas (Fig. 6.1m). It is worth mentioning that all the deposited Au layers were treated with UV-ozone (PR-100 UV-ozone Photoreactor, UVP Inc., United States) for 5 min. High-resolution scanning electron microscopy (HR-SEM) images were taken by using a HR-SEM system (FEI Sirion microscope, United States) at a 5 kV acceleration voltage and a spot size of 3. The surface roughness of the deposited Au layers was determined from atomic-force microscopy (AFM) images (scan field: 500 × 500 nm²), recorded in contact modes using an AFM system (Dimension Icon, Bruker Corp., United States) in air.

6.2.4 Finite-difference time-domain simulations

The plasmonic behavior of the Au nanogaps was simulated by using finite-difference time-domain (FDTD) method by means of a commercial software package (Lumerical Solutions Inc., FDTD solutions). The dimensions of the Au nanogap structures were determined from the HR-SEM images. Given the translational symmetry of the structures, two-dimensional FDTD simulations were performed. Conformal mesh (mesh accuracy 4) and mesh refinement were used to define the structure with a minimum mesh size of 0.1 nm. Antisymmetric boundary conditions were used in both boundaries in the x-axis to simulate an infinitely periodic array of Au nanogaps, thus reducing the computational time. Perfectly matched layer (PML) boundary conditions with a steep angle profile were selected along the y-direction, which, ideally, absorb all incident light without creating any back reflections. A plane wave source propagating in the y-direction with an electric field parallel to the x-direction was employed to excite the surface plasmons. Convergence test was made to determine the optimum distance between the SERS structure and the PML boundary conditions in the y-axis (2 μm). The values of the dispersion relations for the different materials used (Au and Si) were extracted from the literature.²⁵ To represent the dispersion relation of the Si_3N_4 material, a Cauchy model²⁶ with coefficients of $B = 1.9832$, $C = 0.0096$, and $D = 6 \times 10^{-4}$ extracted from the characterization of a Si_3N_4 layer by ellipsometry, was used. The auto shutoff intensity was fixed at 1×10^{-6} , and an excitation wavelength of 785 nm was used in the simulations.

6.2.5 Reflection measurements

Reflection measurements were performed on different Au nanogap samples featuring different gap spacings, i.e. ~ 30 nm, ~ 20 nm, and ~ 10 nm, in order to determine the reflection minimum of the different gap spacings. These reflection spectra were used to select the proper excitation wavelength for the FDTD simulations and the SERS measurements. The reflection measurements were conducted by using a microscope (Leica DM6000 FS microscope fitted with an Ocean Optics HR4000 detector, Germany) with a bright light source (Leica type 11307072060, Germany). A 100 $\times$ magnification, and a numerical aperture of 0.9 was used for the measurements. A continuous gold substrate was used as a background for the reflection measurement.

6.2.6 Surface-enhanced Raman scattering measurements

For the Raman measurements, a confocal Raman microscope (WITec Alpha300R, Germany) fitted with an UHTS300 spectrometer was used. The laser wavelength was chosen based on the result of the reflection measurements. Two different laser sources were used for the Raman measurements, i.e. a laser with an excitation wavelength of 785 nm (Topica Photonics XTRA 785 nm single frequency diode laser), and a laser with a wavelength of 532 nm (WITec 532 nm laser). The laser was focused on the substrate using a lens with a 100 $\times$ magnification, and a numerical aperture of 0.9. The Raman microscope was operated in a backscatter configuration. The elastically backscattered excitation beam was filtered from the spectrum using an edge filter. Polarization of the light beam with respect to the sample was controlled by rotating the sample using a rotation plate for rough alignment and a half-wave

dish for precise alignment. The integration time was 100 ms, and the power applied was 20 mW. It has to be noted that the measured signals were stable at this applied power. For the samples, a $5 \times 5 \mu\text{m}^2$ map of 2500 measurements was created, in which for every point in the map the enhancement factor was calculated without performing the averaging. For the reference BT sample, a total of nine measurements were performed and averaged before being used to calculate the enhancement factor. In this paper, BT molecule was chosen as a reference molecule as it is generally accepted as a reference molecule to determine SERS enhancement factors in literature.^{27–29} Moreover, it can form a well-defined monolayer over the structure surface, and has strong Raman peaks. A monolayer of BT molecules was deposited on the Au nanowires by immersing the Au nanogaps in a 10 mM solution of BT molecules in ethanol for 15 hours. Before immersing completely in the solution, the Au nanogaps were slowly immersed perpendicularly to the solution surface so that the solution could enter all Au nanogaps by capillary flow. Subsequently, the Au nanogaps were rinsed with ethanol, and dried with N_2 gas. After the functionalization of the Au nanogaps, SERS Raman spectra were measured. In addition, a neat BT solution in a glass vial was also measured using the same power and same integration time as for the SERS measurements, which was then used to determine the enhancement factor.

6.2.7 Determination of the enhancement factor

A large variety of methods have been reported to determine the enhancement factor of a SERS substrate.³⁰ However, each of them has its own advantages and disadvantages, and the reported enhancement factors vary widely depending on the method used. In this paper, we used a method reported and used by Pilot et al.²⁹ Le Ru et al.³⁰ and Lin et al.³¹ In this method, the enhancement factor is calculated by comparing the normalized results of a SERS structure, functionalized with a reference molecule (BT), against the normalized results of the neat solution of the same reference molecule. The enhancement factor is approximated from the following equation:²⁹

$$EF = \frac{C^{\text{Raman}} \cdot I^{\text{SERS}}}{C^{\text{SERS}} \cdot I^{\text{Raman}}} \times \eta^N, \quad (6.1)$$

Where C^{Raman} is the number of the BT molecules per unit of volume in the neat BT solution. The value of C^{Raman} can easily be calculated by dividing the density of the liquid by the molecular weight, and subsequently multiplying it by the Avogadro's number. This results in a C^{Raman} of 5.86×10^{21} molecules per cm^3 . C^{SERS} is the surface density of the BT molecules forming a monolayer on the surface of the Au nanogaps. The surface density of the BT monolayer on a flat gold surface has been reported in literature,^{32–34} and is in the order of 3.3×10^{14} molecules per cm^2 . Using this value and the estimated surface of the Au nanogaps (Fig. C16), the surface densities of the BT monolayer on the surface of our fabricated Au nanogaps can be calculated, which are 7.67×10^{14} molecules per cm^2 , 8.22×10^{14} molecules per cm^2 , and 6.48×10^{14} molecules per cm^2 for the Au nanogaps of ~ 30 nm, ~ 20 nm, and ~ 10 nm, respectively. I^{RAMAN} is the integrated intensity of the peak at 1070 cm^{-1} in the neat BT solution, and I^{SERS} is the integrated intensity of the same peak of the SERS sample.^{35–37} η^N ($7.231 \times 10^{-4} \text{ cm}$) the integrated area of the normalized collection efficiency of

the instrument. The normalized collection efficiency was obtained by taking several Raman spectra of a Si wafer at various positions along the z-axis. For each spectrum, the area of the Si peak at 520 cm^{-1} was then obtained, and then normalized against the area measured at the focal point. The result obtained was a bell-shaped curve with the offset from the focal point on the y-axis and the normalized area on the x-axis (Fig. C17).

6.3 Results and discussion

6.3.1 Patterning periodic 50 nm BARC nanolines

Fig. 6.2a shows the HR-SEM images of periodic PR nanolines patterned on an oxidized Si wafer applying DTL. The top-view image shows well-fabricated PR nanolines (250 nm pitch) with a high uniformity in terms of the line width. The width of the PR nanolines was approximately 114 nm with a relatively small variation (standard deviation) of 2 nm. For the uniformity measurement of the fabricated PR nanolines, four areas were selected over a full wafer area (Fig. C1–C2). The cross-sectional image shows a highly vertical PR sidewall, thus indicating a robust fabrication process achieved by applying the DTL technique. Fig. 6.2b shows the HR-SEM images of BARC nanolines shrink-etched using a mixture O_2/N_2 plasma, starting from the patterned PR nanolines. It is remarkable that the BARC nanolines were found to be still uniform over a full wafer area (Fig. C3), and that their sidewalls remained highly vertical after the shrink-etching process, as shown in the cross-sectional image. These periodic BARC nanolines of $50 \pm 3\text{ nm}$ in width were subsequently used as a template to pattern Cr nanolines by using a lift-off process.

Fig. 6.2: Top-view (scale bar: 1 μm) and cross-sectional (scale bar: 500 nm) HR-SEM images of (a) periodic PR nanolines, and (b) $\sim 50\text{ nm}$ BARC nanolines shrink-etched from the patterned PR nanolines.

6.3.2 Patterning periodic Cr nanolines using lift-off

Fig. 6.3a shows a top-view HR-SEM image of periodic Cr nanolines (~ 6 nm thickness) patterned on the surface of an oxidized Si wafer. A high width uniformity of the fabricated Cr nanolines was obtained over a full wafer area (Fig. C4), with an average width of approximately 50 nm. It is worth mentioning that the lift-off process was conducted in a 99% nitric acid (HNO_3) solution as BARC cannot be dissolved in acetone. Moreover, the patterned wafer needs to be immersed immediately into DI water for sonication after the immersion in the HNO_3 solution as drying the wafer before the sonication could result in the permanent sticking of unwanted Cr residues on its surface (Fig. 6.3b). Such Cr residues are attributed to the collapse of a Cr layer deposited on the sidewall of the BARC nanolines during the sputtering process. In addition, deposition of a thick Cr layer of approximately 12 nm also made it difficult to obtain the complete lift-off of Cr (Fig. 6.3c). We attribute this to the fact that the Cr layer completely covered the BARC nanolines, thus preventing the diffusion of HNO_3 to completely dissolve the BARC.

6.3.3 Patterning high-quality Si nanogaps

Fig. 6.4a shows the HR-SEM images of Si nanotrenches after directionally dry etching the bulk Si in a parallel plate reactive ion etching system (in-house built TEske system, NanoLab Cleanroom). From the cross-sectional image, a well-defined pattern of Si nanotrenches that have relatively vertical sidewalls is seen, indicating an anisotropic etching process. Moreover, the top-view image shows no damage to the area that is protected by the Cr layer after the etching process. This indicates that a continuous Cr layer was formed at the thickness of approximately 6 nm, and that this Cr layer was sufficient to withstand the etching of the SiO_2 masking layer and the bulk Si substrate for 13 min (~ 150 nm deep in Si). It is clear from the cross-sectional image that the top edge of the SiO_2 masking layer was slightly damaged. We attribute this damage to the faceting formation at these SiO_2 edge regions during the dry etching process. However, such damage was not found to affect the formation of Si structures after the wet etching in a potassium hydroxide (KOH) solution (Fig. 6.4b). A high uniformity of the etched profile into the sidewall of the Si nanotrenches was obtained, in which the etching virtually ceased at $\langle 111 \rangle$ -oriented Si crystal planes (etching rate of 12.5 nm min^{-1}). It is worth noting that over-etching of the wafer in

Fig. 6.3: (a) Top-view HR-SEM image of periodic Cr nanolines with ~ 50 nm spacing fabricated by using a lift-off process. (b) Unwanted Cr residues caused by drying the wafer before the sonication in DI water. (c) A thick deposited Cr layer (~ 12 nm thick) resulted in an undesired structures after the lift-off process. Scale bar represents 1 μm .

Fig. 6.4: Top-view (scale bar: 1 μm) and cross-sectional (scale bar: 500 nm) HR-SEM images of the fabricated (a) Si nanotrenches after dry etching, (b) Si structure after wet etching in the KOH solution, and (c) after wet etching of the SiO_2 masking layer.

the KOH solution could lead to the formation of undesirable v-grooves in the bulk Si substrate (Fig. C5). After wet etching of the SiO_2 masking layer, a dense array of extremely smooth Si nanogaps of approximately 120 nm (250 nm pitch) was obtained (Fig. 6.4c). Since the roughness effects of the patterned Cr nanolines and SiO_2 masking layer on the quality of the Si nanogaps are eliminated by the subsequent crystallographic etching of Si, using this Si template for further patterning of Au nanogaps significantly improves their quality. Fig. 6.5 shows the HR-SEM images of a fabricated Si template after coating with a SiN layer. The top-view image shows well-defined SiN nanolines with high uniformity in width (~ 200 nm) and periodicity (~ 250 nm) (Fig. C6). It can be observed from the cross-sectional image that the SiN layer (~ 35 nm thick) was conformally deposited over the Si template, resulting in very smooth and uniform SiN nanogaps of approximately 50 nm (Fig. 6.5b).

6.3.4 Patterning high-quality tunable Au nanogaps

Fig. 6.6a shows the HR-SEM images of a dense array of high-quality Au nanogaps fabricated by sputtering a Au layer over the SiN-coated Si template. A high uniformity in gap spacing of the fabricated Au nanogaps was obtained over a full wafer area (Fig.

Fig. 6.5: (a) Top-view (scale bar: 500 nm) and (b) cross-sectional (scale bar: 200 nm) HR-SEM images of a fabricated Si template coated with a SiN layer.

Fig. 6.6: (a) Top-view (scale bar: 1 μm) and cross-sectional (scale bar: 200 nm) HR-SEM images of a fabricated array of Au nanogaps. (b) A close-up HR-SEM image (scale bar: 200 nm) shows well-defined Au nanogaps with a gap spacing of approximately 10 nm. The gap spacing was tuned from (c) ~30 nm to (d) ~20 nm, and (e) ~10 nm by increasing the thickness of the deposited Au layers from ~67 nm to ~90 nm, and ~113 nm, respectively. Scale bars represent 200 nm.

C7). It is worth noting that the top-view HR-SEM images of the Au nanogaps fabricated from different batches showed a high reproducibility of the fabrication process (Fig. C8). It is clearly shown in the cross-sectional image that the sputtered Au layer completely covered the structure surface. It is worth mentioning that no metallic adhesion layer was used to improve the adhesion of the sputtering Au layer with the SiN surface. Therefore, the adverse effects caused by metallic adhesion layers on the optical and electrical properties of pure Au were eliminated.^{38,39} Fig. 6.6b shows a closeup top-view HR-SEM image of Au nanogaps after the sputtering of a Au layer approximately 113 nm thick. Well-defined Au nanogaps of approximately 10 nm were fabricated at the periodicity of 250 nm. Since the roughness of the template was minimized by the crystallographic etching of Si, the roughness of the Au nanogaps depended almost exclusively on the roughness of the sputtered Au layers. Decreasing the thickness of the Au layer was found to decrease its roughness (Fig. C9–C11),⁴⁰ though the Au gap spacing became wider (Fig. 6.6c–e). This can be improved by depositing a thicker SiN layer than the currently used SiN layer of ~35 nm, thus narrowing down the SiN nanogaps. Then, a thinner gold layer is required in order to obtain a similar Au gap spacing. In our fabrication process, only the photoresist nanolines are serially patterned (approximately 10 min per wafer), whereas the other processes can be performed in batch. All together, the process may offer a unique combination of high geometrical control and a good potential for low-cost production. Furthermore, it is worth mentioning that our SiN coated Si templates can be reused by replacing the Au layer with another fresh layer, without damaging the template material or altering its dimensions.

6.3.5 Finite-difference time-domain simulations

Fig. 6.7 shows the finite-difference time-domain (FDTD) simulation results of the local field intensity enhancement factor at Au nanogaps with different gap spacings at the excitation wavelength of 785 nm. The color scale represents the normalized amplitude of the enhanced electric field $|E|$ with respect to the amplitude of the incoming electric field $|E_0|$. As can be seen in Fig. 6.7, the reduction of the gap spacing resulted in an increase in the enhanced electric field at the Au nanogap region, which can be explained from the increasing surface plasmon resonance (SPR) strength when decreasing the gap spacing^{41,42} Similar results have also been reported for different structures when reducing the gap spacing.^{42,43} In addition, Fig. 6.7c clearly shows that there are two major regions of the enhanced electric field generated within the ~ 10 nm Au nanogaps. Awang et al. reported a similar observation when simulating the electric field distribution within the gap of the Au nanograting pairs supported on the SF10 glass substrate,⁴³ which they attributed to the corner effect of the structure.

6.3.6 Surface-enhanced Raman scattering measurements

Fig. 6.8a shows the reflectance spectra measured on various Au nanogap samples containing different gap spacings. A red shift and formation of a second peak at ~ 525 nm were observed in the spectra when reducing the gap spacing. The appearance of such second peak at lower wavelengths has been reported in the literature, both for two nanoparticle systems⁴⁴ and nanowires.⁵ Romero et al. attributed the formation of these peaks to the strong interactions of single particle multipoles.⁴⁵ However, it has to be noted that the explanation of Romero et al. was based on the two nanosphere system. Fig. 6.8b shows the SERS signal of the pure BT used as the reference. Fig. 6.8c shows the SERS signals measured on various Au nanogap samples containing different gap spacings of ~ 30 nm, ~ 20 nm, and ~ 10 nm, after coating a monolayer of BT molecules. As can be seen at the 1070 cm^{-1} peak in Fig. 6.8d, there

Fig. 6.7: FDTD-simulated distribution of the local field intensity enhancement factor ($|E|^2/|E_0|^2$) at the wavelength of 785 nm for Au nanogaps of (a) ~ 30 nm, (b) ~ 20 nm, and (c) ~ 10 nm. The yellow dashed lines indicates the SiN boundary.

Fig. 6.8: (a) Reflectance spectra. SERS signals (b) of pure BT, and (c) measured on Au nanogap arrays of ~30 nm, ~20 nm, and ~10 nm gap spacing, with (d) a close-up image. (e) Calculated enhancement factors of the spectra obtained from the measurements over a $5 \times 5 \mu\text{m}^2$ area for the ~10 nm Au nanogaps, and (f) the corresponding histogram.

is a slight increase in the measured signal with decreasing gap spacing from ~30 nm to ~10 nm. It might be surprising that the enhancement in the electric field strength in the simulated gap region (see Fig. 6.7), which is about 10-fold from the ~20 nm to ~10 nm gaps, causes only a relatively small increase in the measured signal. However, this can be explained by the fact that the fraction of the scattered signal

that reaches the objectives ($NA = 0.9$, $\alpha_{\text{adm}} \approx 64^\circ$) decreases with an increasingly narrow gap and consequently lower escape angle. It must furthermore be noted that the BT monolayer forms only on the sample surface after drying (white lines in Fig. 6.7), so that the high field enhancement in the entire gap in the ~ 10 nm Au nanogaps does not contribute to the measured SERS signal (Fig. C12–C13). It is thus expected that the ~ 10 nm Au nanogaps are especially useful for measuring in solution. It is worth mentioning that all the Au nanogaps were slowly immersed perpendicularly to the BT solution surface before immersing completely in the solution. We expect that the solution could wet the entire Au nanogaps by the capillary flow. This procedure was followed because immersing the Au nanogaps into the BT solution, parallel to the solution surface, resulted in a significant drop in the signal intensity, especially for the ~ 10 nm Au nanogaps (Fig. C14). We attribute this to a lack of penetration of the solution with analyte molecules to the region of the nanogaps, caused by the hydrophobic nature of Au. Therefore, in that case, although there is a strongly enhanced electric field at this region (see Fig. 6.7), a lower SERS signal is generated, leading to a significant drop in the totally obtained signal. This would imply that the method of sample introduction is an important factor in the present SERS structures. To investigate the homogeneity of the enhancement over the structure surface, a map of $5 \times 5 \mu\text{m}^2$ containing 2500 excitation points (50 lines containing 50 points per line) was constructed, and the enhancement factor was calculated using eqn (1). As shown in Fig. 6.8e, for the Au nanogaps of ~ 10 nm gap spacing, a relatively high uniformity in the enhanced factor was obtained (Fig. 6.8f). For the structures with gap spacings of ~ 30 nm and ~ 20 nm, the maps of the enhancement factor are shown in Fig. C15. The average enhancement factors calculated by averaging 2500 spectra of each map and their relative standard deviation were $1.1 \times 10^6 \pm 19.7\%$, $1.1 \times 10^6 \pm 22.7\%$, and $1.5 \times 10^6 \pm 31.7\%$ for the Au nanogaps of ~ 30 nm, ~ 20 nm, and ~ 10 nm, respectively (Fig. C15). These obtained enhancement factors are comparable to those reported in the literature^{35–37} at similar gap spacings. These enhancement factors are expected to be enhanced by further narrowing down the gap spacing. This can be done by conformal deposition of a thicker SiN layer than the currently used SiN layer of ~ 35 nm before sputtering the gold layer. In addition, it is noteworthy that for the second, smaller peak at around 525 nm in the reflectance spectra, no signal was observed when excited by a 532 nm laser (Fig. C18).

6.4 Conclusions

In summary, we report a robust method for wafer-scale patterning dense arrays of high-quality Au nanogaps, combining DTL-based shrink-etching with dry etching, wet etching, and thin film deposition techniques. Using this fabrication method, SiN-coated Si templates with extremely smooth SiN nanogaps were fabricated over full wafer areas, which were then used as a template to pattern high-quality Au nanogaps by sputtering a Au layer over the structure surface. The quality of the Au nanogaps was significantly enhanced by minimizing the roughness of the template used for the Au deposition. By varying the thickness of deposited Au layers, we successfully demonstrated the fabrication of high-quality tunable Au nanogaps down to ~ 10 nm gap spacing over full wafer areas. Our fabricated Au nanogaps showed a significant enhancement of SERS signals (up to 1.5×10^6) of BT molecules chemisorbed on the

nanogap surface compared to those obtained when using a pure BT solution. Moreover, using the FDTD simulation allowed us to predict the location of the hot-spot regions. As a result, a tailored sample introduction procedure was introduced for our fabricated Au nanogap arrays in order to obtain good SERS signals, especially for the ~ 10 nm Au nanogaps. In addition, the simulations indicated that the ~ 10 nm and ~ 20 nm Au nanogap arrays are expected to give higher enhanced signals when measuring in solution as the enhanced electric field in between the gap is then fully employed. With its simple operation, our fabrication method is suitable for the high-yield and low-cost patterning of high quality tunable Au nanogaps that can be used in many applications, including biomedical and chemical sensing,^{3,4} and surface-enhanced spectroscopy.^{6,46}

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References

- (1) Chen, X.; Guo, Z.; Yang, G. M.; Li, J.; Li, M. Q.; Liu, J. H.; Huang, X. J. Electrical Nanogap Devices for Biosensing. *Mater. Today* **2010**, *13* (11), 28–41.
- (2) Im, H.; Bantz, K. C.; Lindquist, N. C.; Haynes, C. L.; Oh, S. H. Vertically Oriented Sub-10-Nm Plasmonic Nanogap Arrays. *Nano Lett.* **2010**, *10* (6), 2231–2236.
- (3) Lim, D. K.; Jeon, K. S.; Kim, H. M.; Nam, J. M.; Suh, Y. D. Nanogap-Engineerable Raman-Active Nanodumbbells for Single-Molecule Detection. *Nat. Mater.* **2010**, *9* (1), 60–67.
- (4) Siegfried, T.; Ekinici, Y.; Solak, H. H.; Martin, O. J. F.; Sigg, H. Fabrication of Sub-10 Nm Gap Arrays over Large Areas for Plasmonic Sensors. *Appl. Phys. Lett.* **2011**, *99* (26), 99–101.
- (5) Le Thi Ngoc, L.; Jin, M.; Wiedemair, J.; van den Berg, A.; Carlen, E. T. Large Area Metal Nanowire Arrays with Tunable Sub-20 Nm Nanogaps. *ACS Nano* **2013**, *7* (6), 5223–5234.
- (6) Fu, Q.; Zhan, Z.; Dou, J.; Zheng, X.; Xu, R.; Wu, M.; Lei, Y. Highly Reproducible and Sensitive SERS Substrates with Ag Inter-Nanoparticle Gaps of 5 Nm Fabricated by Ultrathin Aluminum Mask Technique. *ACS Appl. Mater. Interfaces* **2015**, *7* (24), 13322–13328.
- (7) Cai, H.; Wu, Y.; Dai, Y.; Pan, N.; Tian, Y.; Luo, Y.; Wang, X. Wafer Scale Fabrication of Highly Dense and Uniform Array of Sub-5 Nm Nanogaps for Surface Enhanced Raman Scattering Substrates. *Opt. Express* **2016**, *24* (18), 20808.
- (8) Kim, S.; Jin, J.; Kim, Y. J.; Park, I. Y.; Kim, Y.; Kim, S. W. High-Harmonic Generation by Resonant Plasmon Field Enhancement. *Nature* **2008**, *453* (7196), 757–760.
- (9) Le Ru, E. C.; Etchegoin, P. G. SERS Enhancement Factors and Related Topics. In *Principles of Surface-Enhanced Raman Spectroscopy*; Elsevier, 2009; pp 185–264.
- (10) Zuloaga, J.; Prodan, E.; Nordlander, P. Quantum Description of the Plasmon Resonances of a Nanoparticle Dimer. *Nano Lett.* **2009**, *9* (2), 887–891.
- (11) Schuller, J. A.; Barnard, E. S.; Cai, W.; Jun, Y. C.; White, J. S.; Brongersma, M. L.

- Plasmonics for Extreme Light Concentration and Manipulation. *Nat. Mater.* **2010**, *9* (3), 193–204.
- (12) Wu, Y.; Hong, W.; Akiyama, T.; Gautsch, S.; Kolivoska, V.; Wandlowski, T.; De Rooij, N. F. Batch Fabrication of Gold-Gold Nanogaps by E-Beam Lithography and Electrochemical Deposition. *Nanotechnology* **2013**, *24* (23).
 - (13) Johnston, D. E.; Strachan, D. R.; Johnson, A. T. C. Parallel Fabrication of Nanogap Electrodes. *Nano Lett.* **2007**, *7* (9), 2774–2777.
 - (14) Suga, H.; Sumiya, T.; Furuta, S.; Ueki, R.; Miyazawa, Y.; Nishijima, T.; Fujita, J. I.; Tsukagoshi, K.; Shimizu, T.; Naitoh, Y. Single-Crystalline Nanogap Electrodes: Enhancing the Nanowire-Breakdown Process with a Gaseous Environment. *ACS Appl. Mater. Interfaces* **2012**, *4* (10), 5542–5546.
 - (15) Perrin, M. L.; Verzijl, C. J. O.; Martin, C. A.; Shaikh, A. J.; Eelkema, R.; Van Esch, J. H.; Van Ruitenbeek, J. M.; Thijssen, J. M.; Van Der Zant, H. S. J.; Dulić, D. Large Tunable Image-Charge Effects in Single-Molecule Junctions. *Nat. Nanotechnol.* **2013**, *8* (4), 282–287.
 - (16) Solak, H. H.; Dais, C.; Clube, F. Displacement Talbot Lithography: A New Method for High-Resolution Patterning of Large Areas. *Opt. Express* **2011**, *19* (11), 10686.
 - (17) Le-The, H.; Berenschot, E.; Tiggelaar, R. M.; Tas, N. R.; van den Berg, A.; Eijkel, J. C. T. Shrinkage Control of Photoresist for Large-Area Fabrication of Sub-30 Nm Periodic Nanocolumns. *Adv. Mater. Technol.* **2017**, *2* (3), 1600238.
 - (18) Hashiguchi, G.; Sakamoto, H.; Kanazawa, S.; Mimura, H. Fabrication and Emission Characteristics of New Wedge-Shaped Silicon Emitters. *Appl. Surf. Sci.* **1994**, *76–77* (C), 41–46.
 - (19) Yuki, K.; Hirai, Y.; Morimoto, K.; Inoue, K.; Niwa, M.; Yasui, J. Fabrication of Novel Si Double-Barrier Structures and Their Characteristics. *Jpn. J. Appl. Phys.* **1995**, *34* (Part 1, No. 2B), 860–863.
 - (20) Berenschot, J. W.; Oosterbroek, R. E.; Lammerink, T. S. J.; Elwenspoek, M. C. Micromachining of {111} Plates in 〈001〉 Oriented Silicon. *J. Micromechanics Microengineering* **1998**, *8* (2), 104–106.
 - (21) De Boer, M. J.; Tjerkstra, R. W.; Berenschot, J. W.; Jansen, H. V.; Burger, G. J.; Gardeniers, J. G. E.; Elwenspoek, M.; Van Den Berg, A. Micromachining of Buried Micro Channels in Silicon. *J. Microelectromechanical Syst.* **2000**, *9* (1), 94–103.
 - (22) Lee, M.; Jeon, Y.; Moon, T.; Kim, S. Top-down Fabrication of Fully CMOS-Compatible Silicon Nanowire Arrays and Their Integration into CMOS Inverters on Plastic. *ACS Nano* **2011**, *5* (4), 2629–2636.
 - (23) Wilbers, J. G. E.; Berenschot, J. W.; Tiggelaar, R. M.; Dogan, T.; Sugimura, K.; Van Der Wiel, W. G.; Gardeniers, J. G. E.; Tas, N. R. 3D-Fabrication of Tunable and High-Density Arrays of Crystalline Silicon Nanostructures. *J. Micromechanics Microengineering* **2018**, *28* (4).
 - (24) Le-The, H.; Berenschot, E.; Tiggelaar, R. M.; Tas, N. R.; van den Berg, A.; Eijkel, J. C. T. Large-Scale Fabrication of Highly Ordered Sub-20 Nm Noble Metal Nanoparticles on Silica Substrates without Metallic Adhesion Layers. *Microsystems Nanoeng.* **2018**, *4* (1), 4.
 - (25) Palik, E. D.; Ghosh, G. Handbook of Optical Constants of Solids. Academic Press: San Diego 1998.
 - (26) Jenkins, F. A.; White, H. E. *Fundamentals of Optics*; Mc Graw Hill, 1981.

- (27) Le Thi Ngoc, L.; Yuan, T.; Oonishi, N.; Van Nieuwkastele, J.; van den Berg, A.; Permentier, H.; Bischoff, R.; Carlen, E. T. Suppression of Surface-Enhanced Raman Scattering on Gold Nanostructures by Metal Adhesion Layers. *J. Phys. Chem. C* **2016**, *120* (33), 18756–18762.
- (28) Michieli, N.; Pilot, R.; Russo, V.; Scian, C.; Todescato, F.; Signorini, R.; Agnoli, S.; Cesca, T.; Bozio, R.; Mattei, G. Oxidation Effects on the SERS Response of Silver Nanoprism Arrays. *RSC Adv.* **2017**, *7* (1), 369–378.
- (29) Pilot, R.; Bozio, R. Validation of SERS Enhancement Factor Measurements. *J. Raman Spectrosc.* **2018**, *49* (3), 462–471.
- (30) Le Ru, E. C.; Blackie, E.; Meyer, M.; Etchegoin, P. G.; E. C. Le Ru, *; E. Blackie; M. Meyer, and; Etchegoin†, P. G. No Title. *J. Phys. Chem. C* **2007**, *111* (37), 13794–13803.
- (31) Lin, X.-M.; Cui, Y.; Xu, Y.-H.; Ren, B.; Tian, Z.-Q. Surface-Enhanced Raman Spectroscopy: Substrate-Related Issues. *Anal. Bioanal. Chem.* **2009**, *394* (7), 1729–1745.
- (32) Wan, L. J.; Terashima, M.; Noda, H.; Osawa, M. Molecular Orientation and Ordered Structure of Benzenethiol Adsorbed on Gold(111). *J. Phys. Chem. B* **2000**, *104* (15), 3563–3569.
- (33) Käfer, D.; Bashir, A.; Witte, G. Interplay of Anchoring and Ordering in Aromatic Self-Assembled Monolayers. *J. Phys. Chem. C* **2007**, *111* (28), 10546–10551.
- (34) Tatara, S.; Kuzumoto, Y.; Kitamura, M. Surface Properties of Substituted-Benzenethiol Monolayers on Gold and Silver: Work Function, Wettability, and Surface Tension. *Jpn. J. Appl. Phys.* **2016**, *55* (3).
- (35) Gopinath, A.; Boriskina, S. V.; Reinhard, B. M.; Dal Negro, L. Deterministic Aperiodic Arrays of Metal Nanoparticles for Surface-Enhanced Raman Scattering (SERS). *Opt. Express* **2009**, *17* (5), 3741.
- (36) Yang, L.; Yan, B.; Premasiri, W. R.; Ziegler, L. D.; Negro, L. D.; Reinhard, B. M. Engineering Nanoparticle Cluster Arrays for Bacterial Biosensing: The Role of the Building Block in Multiscale SERS Substrates. *Adv. Funct. Mater.* **2010**, *20* (16), 2619–2628.
- (37) Reinhard, B. M.; Negro, L. D. Engineered SERS Substrates Employing Nanoparticle Cluster Arrays with Multiscale Signal Enhancement. 9013689, 2015.
- (38) Habteyes, T. G.; Dhuey, S.; Wood, E.; Gargas, D.; Cabrini, S.; Schuck, P. J.; Alivisatos, A. P.; Leone, S. R. Metallic Adhesion Layer Induced Plasmon Damping and Molecular Linker as a Nondamping Alternative. *ACS Nano* **2012**, *6* (6), 5702–5709.
- (39) Lahiri, B.; Dylewicz, R.; De La Rue, R. M.; Johnson, N. P. Impact of Titanium Adhesion Layers on the Response of Arrays of Metallic Split-Ring Resonators (SRRs). *Opt. Express* **2010**, *18* (11), 11202–11208.
- (40) Melo, L. L.; Vaz, A. R.; Salvadori, M. C.; Cattani, M. Grain Sizes and Surface Roughness in Platinum and Gold Thin Films. *J. Metastable Nanocrystalline Mater.* **2004**, *20–21*, 623–628.
- (41) Stiles, P. L.; Dieringer, J. A.; Shah, N. C.; Van Duyne, R. P. Surface-Enhanced Raman Spectroscopy SERS: Surface-Enhanced Raman Spectroscopy Raman Scattering: Inelastic Scattering of a Photon from a Molecule in Which the Frequency Change Precisely Matches the Difference in Vibrational Energy Levels. *Annu. Rev. Anal. Chem* **2008**, *1*, 601–626.

- (42) Yang, J. L.; Li, R. P.; Han, J. H.; Huang, M. J. FDTD Simulation Study of Size/Gap and Substrate-Dependent SERS Activity Study of Au@SiO₂ Nanoparticles. *Chinese Phys. B* **2016**, 25 (8).
- (43) Awang, R. A.; El-Gohary, S. H.; Kim, N.-H.; Byun, K. M. Enhancement of Field–Analyte Interaction at Metallic Nanogap Arrays for Sensitive Localized Surface Plasmon Resonance Detection. *Appl. Opt.* **2012**, 51 (31), 7437.
- (44) Tolga Atay; Jung-Hoon Song, and; Nurmikko*, A. V. Strongly Interacting Plasmon Nanoparticle Pairs: From Dipole–Dipole Interaction to Conductively Coupled Regime. **2004**.
- (45) Romero, I.; Aizpurua, J.; Bryant, G. W.; García De Abajo, F. J. Plasmons in Nearly Touching Metallic Nanoparticles: Singular Response in the Limit of Touching Dimers. *Opt. Express* **2006**, 14 (21), 9988.
- (46) Xia, L.; Yang, Z.; Yin, S.; Guo, W.; Li, S.; Xie, W.; Huang, D.; Deng, Q.; Shi, H.; Cui, H.; et al. Surface Enhanced Raman Scattering Substrate with Metallic Nanogap Array Fabricated by Etching the Assembled Polystyrene Spheres Array. *Opt. Express* **2013**, 21 (9), 11349–11355.

7

Towards single mode Infrared Waveguides as alternative for attenuated total reflection devices

This chapter discusses two waveguide designs for mid-infrared spectroscopy. Based on Lumerical mode solution simulations and literature we design two different waveguides based on germanium (Ge). The first waveguide is consists of Ge on silicon (Si) and the second design is a free-hanging Ge waveguide. We discuss a cleanroom process flow that can be used to fabricate the design. Due to the ensuing Covid-19 pandemic lockdown which occurred in 2020, the fabrication process could not be executed during the current PhD project.

This chapter contains work performed by:

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7.1 Waveguides for infrared spectroscopy

Attenuated total reflection (ATR) Fourier transform infrared spectroscopy (FT-IR) is a surface sensitive spectroscopic technique. In Fig. 7.1a a schematic representation of an internal reflection element (IRE) for an ATR device is shown, inspired by the work from Schadle et al.¹. Under the right conditions, light can be coupled into an ATR device. When the refractive index (n) of the material at the interface (e.g. air or sample) is smaller than the refractive index of the ATR device and the angle of incidence is bigger than the critical angle, total internal reflection takes place². At the interface where the total internal reflection takes place an evanescent wave is formed interacting with the sample. This evanescent wave penetrates up to several micrometre into the sample, depending on the refractive index of the ATR device, refractive index of the sample and the wavelength used². For ATR the path length (i.e. the optical path length in the lambert-beer equation) is a function of the number of complete bounces the light undergoes in the ATR device and the penetration depth. As can be seen when comparing fig. 7.1a with fig. 7.1b, when reducing the height of a ATR device while keeping the other dimensions the same, the amount of bounces, and therefore the path length in the sample, increases. Depending on the situation, this can be either beneficial or detrimental for a measurement. If the molar attenuation coefficient of the analyte is high or the source power is low, a shorter path length is preferred compared to a case where the a sample has a low molar attenuation coefficient or a high source power.

Fig. 7.1: A simplified schematic of **(a)** microfluidic chip on a ATR device **(b)** same chip with the an ATR device reduced in height **(c)** same chip on a waveguide. Figure inspired by the work of Schadle et al.¹

The standard FT-IR light source consists of a so called globar, a silicon carbide rod that emits light in a broad wavelength range when heated. The biggest drawback of this globar is the relatively low power output, especially when focusing on small spot sizes. In Fig. 7.2, Weida et al.³ makes a comparison between globar sources, synchrotron sources and quantum cascade laser (QCL) sources, cast through a 10 μm pinhole. Although this comparison is somewhat unfavourable for the globar due to the relative large spot size generated by the globar, it does show the strength of the QCLs in this comparison. Since in this chapter, focussing the light on a few micrometre spot to couple light into a waveguide, the comparison is surely relevant for our work. One can see that a globar has a maximum output of 1×10^{-9} Watts into 2 cm^{-1} bandwidth. This is significantly lower than the 2×10^{-6} Watts into 2 cm^{-1} for the synchrotron and the 0.5 Watts into 2 cm^{-1} for the QCL. This high source power allows for the possibility to use a longer path length compared to the classical globar sources. Again looking at Fig. 7.1, it is shown that if the size reduces far enough, no bounces are occurring anymore and a continuous evanescent wave is formed¹. Describing the behaviour as a series of bounces does not adequately cover the physics involved at this point. Different models based on wave analysis are required to describe these waveguides¹. If the height of these waveguides is reduced sufficiently, so-called single mode waveguides can be created⁴, tuned to a specific wavelength range. Although these single mode waveguides reduce the spectral range that can be addressed, they might be ideal to combine with structures that also are tuned at specific wavelengths such as the surface enhanced infrared spectroscopy (SEIRS) as discussed in chapter 5. By combining the advancement in QCL lasers with single mode mid-IR waveguides and nano-fabricated SEIRS arrays, tuned to a specific wavelength range, a new level of sensitivity for IR-spectroscopy might be achieved.

Fig. 7.2: Comparison of Weida and Yee of three different IR sources of the signal through a 10 μm pinhole³. Reprinted and adapted from the original work by Weida and Yee³, With permission from SPIE and M. Weida.

7.2 Design criteria

In theory, the size and refractive index of the waveguide determines to which wavelength the waveguide is tuned⁵. In order to be applicable for a large amount of applications, a waveguide material that is transparent in a large spectral range is preferred. Another important aspect of the waveguide is the cladding, since part of the wave will interact with the cladding material. For this reason, the cladding material should also be IR transparent. When picking the cladding material it has to be taken in consideration that the refractive index of the cladding should be lower than the refractive index of the waveguide itself. Some additional design criteria are: chemical inertness and not toxic.

To design a mid-IR waveguide to fit to these criteria preliminary research was performed to find the right material that could function as waveguide in combination with a suitable cladding material. The summarized results of this preliminary research are shown in table 7.1. The refractive indices mentioned in this table have been looked up with the help of a database website⁶, with the citation mentioning the original source. Based on this table ZnSe and BaF₂, CaF₂ and KBr immediately are eliminated. ZnSe has only a small operable pH window and is toxic, making it not ideal to work with. BaF₂ is soluble in water and toxic. CaF₂ and KBr are soluble in water to some extent, which is not ideal when measuring in aqueous solutions. Based on the spectral window available of the remaining materials, Ge, diamond and GaAS

Table 7.1: Mid-IR transparent materials.

Material	n at 10 μm	Spectral range (μm)	Aqueous solutions	Note	Ref.
CaF ₂	1.30	1 till 8	Slightly soluble in water		10,11
BaF ₂	1.40	1 till 11	Soluble in water	Harmful if swallowed or inhaled	10–12
KBr	1.53	1 till 40	Soluble in water		11,13
ZnSe	2.40	2 till 18	Operable between pH 5-9	Toxic if swallowed or inhaled, very toxic for aquatic life	2,4,14–16
Si	3.41	2 till 8	Soluble at pH > 12		2,4,14,17
Si ₃ N ₄	1.62	2 till 8	Insoluble		18
Al ₂ O ₃	1.10	2 till 7	Slightly soluble in alkaline		4,11,15
Ge	4.00	2 till 15	Insoluble		2,4,14,17
Diamond	2.37	0.3 till >20	Insoluble		2,4,14,19
GaAS	3.27	1 till 15	Unknown	Toxic if swallowed or inhaled, may cause cancer	4,20,21

are among the most promising ones. GaAs is somewhat toxic and therefore is less ideal. Which leaves Ge and diamond as a suitable candidate. The waveguide material should have a higher refractive index than the cladding, hence Ge seems to be more promising with its refractive index of 4.00 against the refractive index of 2.37 of diamond. Additionally, Ge might be easier as a fabrication material in cleanroom processes due to the many similarities between Ge and Si.

For cladding material several choices remain. From a theoretical point of view, a Ge waveguide on diamond might be ideal, due to the difference in refractive index and the wide spectral range. However, diamond is an expensive material and not a very common cleanroom material, especially not as a substrate. Which leaves Si, Si_3N_4 and Al_2O_3 as a suitable cladding material. Si and Si_3N_4 are commonly used cleanroom material and would make a practical candidate. Another candidate that would make a good cladding and has not been discussed yet is air. Air has low refractive index and mostly contains diatomic molecules such as O_2 and N_2 , that don't absorb IR radiation. Based on this two waveguide designs have been developed, namely: a Ge waveguide on Si cladding, inspired partly by Gallacher et al.⁷ and freestanding germanium waveguide, partly inspired by the work of Zhou et al.⁸ and Penadés et al.^{5,9}.

7.3 Experimental

In this section, the simulation parameters are discussed.

7.3.1 Simulations

Preliminary simulations have been performed by the author in order to determine the optimal configuration for the dimension for the waveguide. It has to be noted that the author is not well versed in performing Lumerical simulations and therefore the simulations are used more as a guideline than a definite template in order to design the waveguide.

Simulations have been performed using Lumerical mode solutions software (Lumerical 2020a Launcher 1.1.2305). Settings of the simulations are as followed: a

Fig. 7.3: Example of a simulation using Lumerical mode solutions. Shown is a germanium waveguide with a width of $4\ \mu\text{m}$ and a height of $2\ \mu\text{m}$, coupled with a $10.5\ \mu\text{m}$ wavelength light source. The colour bar represents the E-field.

2D simulation in a finite difference eigenmode solver (FDE) with a x-dimension of 50 μm and a y-dimension of 50 μm . The boundary conditions in the x and y directions are metal. The maximum mesh size is 0.05 μm . A wavelength sweep is performed from 9 μm (1111 cm^{-1}) to 14 μm (715 cm^{-1}) in 23 steps. The width of the waveguide is varied between 2 and 5 μm with steps of 1 μm and the height is varied from 2 till 3 μm with a step of 1 μm .

7.4 Discussion and results

7.4.1 Simulation results

Typical cross-section of a simulated waveguide can be seen in fig. 7.3. The bottom layer represents the silicon substrate and the square in the middle the Ge waveguide. The colour gradient in the centre represents the electric field. In table 7.2 the results of the simulations are shown. The results of the simulations tracked the mode of the waveguide, till the size of the waveguide was reduced to such an extent that the mode could not fit in the waveguide anymore. This was used as a cut-off value and as an indication of the maximum wavelength that can be coupled into the a waveguide with a certain dimension. next to width and height of the waveguide the bend radius was varied. As can be seen in this table, the bigger the radius the larger the wavelength being able to be contained in waveguide.

Table 7.2: simulation results of the plotted E-fields with lumerical mode solutions. E-fields plotted in a Ge waveguide using the dimensions stated in table. Wavelength varied from 9 μm to 14 μm .

width (μm)	height (μm)	bend radius (μm)	max wavelength (μm)
2	2	x	<9
2	3	x	9.9
3	2	x	9.9
3	3	x	12.6
4	2	x	11.7
4	3	x	13.3
5	2	x	>14
5	3	x	>14
5	2	200	<9
5	2	400	12
5	2	600	12.9
5	2	800	12.9
5	2	1000	13.2

7.4.2 Design

The simulation results, combined with the previously mentioned literature^{5,7-9} are used to make several waveguide designs. Three different widths for the waveguide are used, namely $3\text{ }\mu\text{m}$, $5\text{ }\mu\text{m}$ and $7\text{ }\mu\text{m}$. additionally 3 different radius and waveguide lengths are used, these being a length of 20 mm and a radius of 12.73 mm, a length of 10 mm and a radius of 6.36 mm and a length of 5 mm with a radius of 3.18 mm. This results in a total of 9 design fabrication for the first fabrication round. 3 of the 9 Clewin designs can be seen in fig. 7.4.

7.4.3 Proposed fabrication process

Two different fabrication processes have been developed while using the same mask. In fig. 7.5 the fabrication process for the Ge on Si waveguide is shown while in fig. 7.6 the fabrication process for the free-hanging Ge waveguide is shown. As can be seen in these figures, the fabrication of the Ge on Si waveguide is less complicated

Fig. 7.4: 3 different Clewin designs for the waveguide. (a) waveguide with a width of $5\text{ }\mu\text{m}$ and a length of 20 mm and a radius of 12.73 mm. (b) waveguide with a width of $5\text{ }\mu\text{m}$ and a length of 10 mm and a radius of 6.36 mm. (c) waveguide with a width of $5\text{ }\mu\text{m}$ and a length of 5 mm and a radius of 3.18 mm.

Fig. 7.5: Fabrication of the Ge on Si waveguide. Step 1: spin coat positive photoresist on the Ge on Si wafer. Step 2: perform lithography. Step 3: cryogenic etching using SF_6 / O_2 . Step 4: strip resist.

to fabricate compared to the free-hanging Ge waveguide. This is because the free-hanging waveguide requires anodic bonding of a Ge wafer to a Si wafer containing a thin layer of SiO_2 . Additionally the thickness of the Ge wafer needs to be etched down to a suitable thickness in the order of several μm . Finally, the free-hanging Ge waveguide requires an extra etching step to remove the SiO_2 under the Ge waveguide in order for the device to be free-hanging.

Fig. 7.6: Fabrication of the free-hanging Ge waveguide. Step 1: anodic bonding of the Ge wafer to the Si wafer with a thin layer of SiO_2 . Step 2: Reduce the thickness of the Ge wafer by etching with H_2O_2 at 50°C . Step 3: spin coat positive photoresist on the Ge wafer. Step 4: perform lithography. Step 5: cryogenic etching using SF_6 / O_2 . Step 6: Remove a layer of SiO_2 by BHF etching. Step 7: strip resist.

7.5 Conclusion and outlook

Two different waveguide designs have been developed. A Ge on Si waveguide, with arguably a relatively easy fabrication procedure. And a free-hanging Ge waveguide, which with its air “cladding” can be used over a wider spectral range but has a more complicated fabrication process. The design of these waveguides have been made with the help of Lumerical Mode solutions and literature. Due to the Covid-19 lock-down the fabrication could not be performed during the course of this project. Currently the co-author of this chapter is in the initial phase of starting the cleanroom fabrication.

References:

- (1) Schadle, T.; Mizaikoff, B. Mid-Infrared Waveguides: A Perspective. *Appl. Spectrosc.* **2016**, *70* (10), 1625–1638.
- (2) Peter Larkin. *IR and Raman Spectroscopy: Principles and Spectral Interpretation*; 2011.
- (3) Weida, M. J.; Yee, B. Quantum Cascade Laser-Based Replacement for FTIR Microscopy. *Imaging, Manip. Anal. Biomol. Cells, Tissues IX* **2011**, 7902 (February 2011), 79021C.
- (4) Sieger, M.; Mizaikoff, B. Toward On-Chip Mid-Infrared Sensors. *Anal. Chem.* **2016**, *88* (11), 5562–5573.
- (5) Penadés, J. S.; Sánchez-Postigo, A.; Nedeljkovic, M.; Ortega-Moñux, A.; Wangüemert-Pérez, J. G.; Xu, Y.; Halir, R.; Qu, Z.; Khokhar, A. Z.; Osman, A.; et al. Suspended Silicon Waveguides for Long-Wave Infrared Wavelengths. *Opt. Lett.* **2018**, *43* (4), 795.
- (6) <https://refractiveindex.info/>. date accessed 30-10-2020
- (7) Gallacher, K.; Griškevičiūtė, U.; Millar, R. W.; Paul, D. J.; Sorel, M.; Ortolani, M.; Baldassarre, L. Low Loss Ge-on-Si Waveguides Operating in the 8–14 Mm Atmospheric Transmission Window. *Opt. Express* **2018**, *26* (20), 25667.
- (8) Zhou, W.; Cheng, Z.; Wu, X.; Zhu, B.; Sun, X.; Tsang, H. K. Fully Suspended Slot Waveguides for High Refractive Index Sensitivity. *Opt. Lett.* **2017**, *42* (7), 1245.
- (9) Penades, J. S.; Ortega-Moñux, A.; Nedeljkovic, M.; Wangüemert-Pérez, J. G.; Halir, R.; Khokhar, A. Z.; Alonso-Ramos, C.; Qu, Z.; Molina-Fernández, I.; Cheben, P.; et al. Suspended Silicon Mid-Infrared Waveguide Devices with Subwavelength Grating Metamaterial Cladding. *Opt. Express* **2016**, *24* (20), 22908.
- (10) Li, H. H. Refractive Index of Alkaline Earth Halides and Its Wavelength and Temperature Derivatives. *J. Phys. Chem. Ref. Data* **1980**, *9*, 161–289.
- (11) Lide, D. R. *CRC Handbook of Chemistry and Physics*; 2003; Vol. 84.
- (12) Safety Data Sheet: Barium Fluoride. *ThermoFischer Scientific*. date accessed 30-10-2020
- (13) Li, H. H. Refractive Index of Alkali Halides and Its Wavelength and Temperature Derivatives. *J. Phys. Chem. Ref. Data* **1976**, *5* (329–528).

- (14) Smith, B. C. *Fundamentals of Fourier Transform Infrared Spectroscopy*; Intergovernmental Panel on Climate Change, Ed.; CRC Press: Cambridge, 2011.
- (15) Querry, M. Optical Constants of Minerals and Other Materials from the Millimeter to the Ultraviolet. *Contract. Rep.* **1987**, 71.
- (16) Safety Data Sheet: Zinc Selenide. *ThermoFischer Scientific*. date accessed 30-10-2020
- (17) Li, H. H. Refractive Index of Silicon and Germanium and Its Wavelength and Temperature Derivatives. *J. Phys. Chem. Ref. Data* **1993**, 9, 561–658.
- (18) Kischkat, J.; Peters, S.; Gruska, B.; Semtsiv, M.; Chashnikova, M.; Klinkmüller, M.; Fedosenko, O.; Machulik, S.; Aleksandrova, A.; Monastyrskiy, G.; et al. Mid-Infrared Optical Properties of Thin Films of Aluminum Oxide, Titanium Dioxide, Silicon Dioxide, Aluminum Nitride, and Silicon Nitride. *Appl. Opt.* **2012**, 51 (28), 6789.
- (19) Dore, P.; Nucara, A.; Cannavò, D.; De Marzi, G.; Calvani, P.; Marcelli, A.; Sussmann, R. S.; Whitehead, A. J.; Dodge, C. N.; Krehan, A. J.; et al. Infrared Properties of Chemical-Vapor Deposition Polycrystalline Diamond Windows. *Appl. Opt.* **1998**, 37 (24), 5731.
- (20) Skauli, T.; Kuo, P. S.; Vodopyanov, K. L.; Pinguet, T. J.; Levi, O.; Eyres, L. A.; Harris, J. S.; Fejer, M. M.; Gerard, B.; Becouarn, L.; et al. Improved Dispersion Relations for GaAs and Applications to Nonlinear Optics. *J. Appl. Phys.* **2003**, 94 (10), 6447–6455.
- (21) Safety Data Sheet: Gallium Arsenide. *ThermoFischer Scientific*. date accessed 30-10-2020

8

Conclusion and outlook

This chapter provides a conclusion on the research goal stated in the Introduction chapter of the thesis and gives an outlook for future work of some of the components mentioned.

The goal of this thesis, as stated in the **Introduction** is as follows: *To fabricate a microfluidic device combining SERS and SEIRS for on-line reaction monitoring.* In the Introduction section of this thesis we explained the goal with the use of Fig. 8.1. In this section we will conclude the thesis by using the same figure as a reference. As discussed, four components are critical for this thesis:

The first is the attenuated total reflection (ATR) crystal and the second component is the microfluidic device. In **Chapter 4** we discussed a successfully fabricated modular microfluidic device, which can be adapted to the reaction of interest. The microfluidic chip is combined with a silicon internal reflection element (Si-IRE) that can be used for attenuated total reflection (ATR)-FTIR spectroscopy. A model Paal-Knorr reaction is successfully performed on-chip, proving the functionality of the device. We show the strength of IR by highlighting and assigning the most important vibrational frequencies and determine reaction orders. We conclude that this chapter successfully combines the two components mentioned above.

Fig. 8.1: Original concept design. A microfluidic chip is shown with on the bottom an ATR device containing structures for surface enhanced infrared spectroscopy (SEIRS). These structures can be addressed by the ATR device. On the top layer of the chip structures for surface enhanced Raman spectroscopy (SERS) are shown. In the inset the figure has been rotated, since the structures are on the bottom of the top layer. The SERS structures can be addressed either from the top (back side of the structures). Or depending on the optical transparency of the ATR material, from the bottom.

Component 3 (Fig. 8.1) is the array of structures for surface enhanced infrared spectroscopy (SEIRS). In **Chapter 5** we cover the fabrication process for nanoantennas, in a nano-rod and nano-slit configuration, without the need for an adhesion layer. We show the simulation results and compare them with fabrication results, showing reasonable correlation. We also proof the tunability of the nano-slits, allowing for the tuning of the resonance frequency with vibrations of interest. For future work on this component, an indication of the enhancement factor should be obtained in order to be able to make a comparison with literature and make a claim regarding its enhancement capability. Furthermore the structures should be included in a microfluidic chip, such as the one mentioned in Chapter 4, in order to show its functionality as an online measurement technique. Due to the ease of fabrication of the ATR device, and the fact that the structures are already fabricated on a silicon substrate, little complications are expected in combining the SEIRS structures with a Si-IRE device.

The final component, (4 in Fig. 8.1) is the inclusion of structures for surface enhanced Raman spectroscopy (SERS). In **Chapter 6** we report a robust method for wafer-scale patterning dense arrays of high-quality Au nanogaps for SERS. We quantify the enhancement of the Au by chemisorbing benzenethiol (BT) molecules on the nanogap surface. We compared these results with those obtained when using a pure BT solution and found a maximum enhancement factor of 1.5×10^6 . Although the strength of the structures are adequately described, they are not yet integrated in a microfluidic chip as shown in Fig. 8.1. This can be achieved by redesigning the process flow so that the silicon substrate can be replaced with silicate. This way the SERS structures can be addressed from the back-side as shown in the figure. It should be quite easy to replace the substrate with silicate, this will change the shape of the cavity under the enhancement structure. Although this will probably have little effect on the enhancement itself, this can only be confirmed by re-determining the enhancement factor.

Chapter 7 introduces the concept of waveguides for mid-infrared spectroscopy. Although not initially a goal of this thesis, waveguides show a promising alternative for ATR devices such as those mentioned in chapter 4, especially in combination with a mid-IR QCL laser. Although the fabrication of these waveguides is delayed due to the global Covid-19 pandemic, we do show promising simulation results and two different process flows for two different waveguide designs. For future work these waveguides should be fabricated using the aforementioned fabrication procedures. Next, a comparison of signal to noise between the results obtained with the waveguide versus more traditional ATR devices should be performed in order to be able to say something about its promise as a platform for IR spectroscopy.

Appendices

Appendix A: page 148

Supplementary information for chapter 4: Modular microreactor with integrated reflection element for online reaction monitoring using infrared spectroscopy

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Supplementary information for chapter 6: Wafer-scale fabrication of high-quality tunable gold nanogap arrays for surface-enhanced Raman scattering

A

Supplementary information for chapter 4:

*Modular microreactor with integrated reflection element
for online reaction monitoring using infrared
spectroscopy*

Fig. A1: Four different PDMS chip designs. (a) mixing channel followed by reaction chamber. (b) long mixing channel. (c) reaction chamber (d) mixing channel followed by a long channel without mixing channels.

Fig. A2: The two different parts for the COC chip. A large reaction chamber and a channel containing mixing structures

Fig. A3: Alignment of the two different COC parts with alignment pins. After aligning and bonding of the two parts the pins are removed from microfluidic chip.

Fig. A4: Aligner used to align the fabricated chip with the Bruker Vertex 70 FTIR instrument. a) zoomed out view of the setup I: Bruker Vertex 70 FTIR instrument. II: alignment tool III: Nemesys syringe pump. b) zoomed in view on the aligner tool. Several micro stages are visible to move the chip in X, Y, Z, direction and adjust the pitch and roll of the chip. Adjustments can also be made to the in X, Y, Z, direction of the mirrors as well as the roll axis of the mirror. IV: mirror for coupling the light into the IRE device. V: mirror for coupling the light exiting the chip into the FTIR instrument. c) zoom in on the chip holder. VI: resistors that can be used to heat the chip, not used for the current study. d) zoom in on the chip. VII: the microfluidic chip. VIII: the two inlets connected to the chip. IX: the outlet connecting the chip with the waste container.

Fig. A5: Colour analysis of the dyes in the COC chip. Each point averaged over 50 point in the y directions, indicated by the white dotted line . Both visually and based on the RGB values, separation can be observed till channel 6. Based on the RGB value, separation might still be present in channel 7 and 8. But it is clear that total mixing has occurred by the time liquid reaches channel 9.

Fig. A6: Colour analysis of the dyes in the PDMS chip. Each point averaged over 50 point in the y directions, indicated by the white dotted line . Both visually and based on the RGB values, separation can be observed till channel 2. Based on the RGB value and visual observation total mixing has occurred by the time liquid reaches channel 3.

Fig. A7: Zoomed in line spectra of the Paal-Knorr reaction

B

Supplementary information for chapter 5:

Au nanoantennas for surface enhanced infrared spectroscopy

Fig. B1: Additional SEM images of the nano-slit arrays. In all images significant debris can be observed on and around the nano-slits. (a-c): slit arrays with a dimension of l: 1300 nm h: 50 nm w: 230 nm. (d-f): slit arrays with a dimension of l: 700 nm h: 50 nm w: 230 nm.

LENGTH (nm)	WIDTH (nm)	HEIGHT (nm)	GAP (x) (nm)	GAP (y) (nm)	No SiO ₂ - Simulation (cm- 1)	No SiO ₂ - Experimental (cm- 1)	10nm SiO ₂ - Simulation (cm- 1)	10nm SiO ₂ - Experimental 23-09-2020 (cm- 1)	10nm SiO ₂ - Experimental 10-Oct-2020 (cm- 1)	125nm SiO ₂ - Simulation (cm- 1)
500	230	50	1100	1800	2049	2880	2089	3796	3200	2570
700	230	50	1100	2000	1720	2102	1895	2685	2660	2272
900	230	50	1300	2200	1560	1660	1734	2080	2073	2024
1100	230	50	1500	2400	1298	1355	1564	1772	1740	1836
1300	230	50	1700	2600	1136	1100	1309	1545	1550	1675
1500	230	50	1900	2800	1038	out of range	1167	1370	1410	1552

Table B1: Dimensions nano-slit arrays and their resonance frequencies.

Fig. B2. Reflection spectra obtained from several nano-slit antenna arrays. spectra have been smoothed and baseline corrected. (a-b) spectra of antennas on 10 nm SiO_2 clear interference from SiO_2 can be seen at $\sim 1200 \text{ cm}^{-1}$. (c) spectra of antennas on Si wafer. (d) comparison of the results shown in b with simulation results.

C

Supplementary information for chapter 6:

Wafer-scale fabrication of high-quality tunable gold nanogap arrays for surface-enhanced Raman scattering

Fig. C1: A wafer-scale array of PR nanolines patterned on an oxidized Si wafer. Four areas indicated by white squares were selected for the uniformity measurement of the fabricated PR nanolines.

Fig. C2: Top-view HR-SEM images of the fabricated PR nanolines at the four selected areas (see Fig. C1). Scale bars: 1 μm

Fig. C3: Top-view HR-SEM images of the fabricated BARC nanolines at the four selected areas (see Fig. C1). Scale bars: 1 μm .

Fig. C4: Top-view HR-SEM images of the fabricated Cr nanolines at the four selected areas (see Fig. C1). Scale bars: 1 μm .

Fig. C5: Cross-sectional HR-SEM image of undesirable v-grooves in bulk Si substrate caused by over etching in the KOH solution. Scale bar represents 100 nm.

Fig. C6: Top-view HR-SEM images of the fabricated SiN nanogaps at the four selected areas (see Fig. C1). Scale bars: 1 μm .

Fig. C7: Top-view HR-SEM images of the fabricated Au nanogaps at the four selected areas (see Fig. C1). Scale bars : 1 μm

Fig. C8: Top-view HR-SEM images of the Au nanogaps fabricated from three different batches. Scale bars: 1 μm .

Fig. C9: AFM images (scan field 500×500 nm²) of Au layers deposited on the surface of oxidized Si wafers at different thicknesses.

Fig. C10: Top-view HR-SEM image (scale bar 2 μ m) of a dense array of sub-20 nm Au nanogaps with a close-up image (scale bar: 200 nm), fabricated by sputtering a Au layer of approximately 90 nm thick over the SiN coated Si template.

Fig. C11: Top-view and cross-sectional HR-SEM images of the fabricated Au nanogaps with different gap spacings of (a) ~30 nm, (b) ~20 nm, and (c) ~10 nm. Scale bars represent 500 nm

Supporting information-C1 Finite-difference time-domain simulations

We estimated the normalized SERS intensity from the local electromagnetic enhancement generated in our Au nanogaps by analyzing the near-field distributions at 785 nm (excitation) and 852 nm (BT Raman shift at ~999 cm⁻¹) wavelengths (see Fig. C12). The normalized SERS intensity – SERS intensity enhancement – was calculated as follows:¹

$$\text{Normalized SERS intensity} = \frac{|E|_{785\text{nm}}^2 \cdot |E|_{852\text{nm}}^2}{|E_0|_{785\text{nm}}^2 \cdot |E_0|_{852\text{nm}}^2} \quad (\text{C.1})$$

where $|E|$ is the locally enhanced electric field and $|E_0|$ is the incident electric field calculated for 785 nm, and 852 nm wavelengths.

Due to the exponential decay of the enhanced near field, only the molecules adsorbed on the surface of the SERS structures can show the increased Raman scattering intensity. Therefore, we calculated the expected normalized SERS intensity from only a monolayer of BT covered completely the surface of our Au nanogaps (white lines in Fig. C12). The thickness of a BT monolayer coated on a Au surface was estimated using Materials Studio software (Accelrys Inc., United States), which is approximately 0.8 nm. The SERS-enhanced intensities integrated over the thickness of the considered BT monolayer were 4.1×10^6 , 8.2×10^6 , and 2.3×10^7 for Au nanogaps of ~30 nm, ~20 nm, and ~10 nm, respectively (Fig. C12). This indicated that although there was a considerable increase in the enhanced electric field in the simulated gap region, only a relatively small increase in the calculated SERS intensities due to the presence of a BT monolayer.

Despite of the fact that the simulations proved to be useful for understanding the origin of the behavior differences of our Au nanogaps of different gap spacings – the simulations follow the expected trend from the experimental measurements – no

absolute comparison between the calculated SERS intensity and the measured SERS intensity is intended. Because our calculated SERS intensity includes only the contribution from the local field enhancement, without considering the chemical enhancement. Moreover, the simulations were performed using a two-dimensional model, which was simplified from the three-dimensional structure of the fabricated Au nanogaps.

Fig. C12: FDTD-simulated distribution of the local field intensity enhancement factor ($|E|^2/|E_0|^2$) for Au nanogaps of a) ~30 nm, b) ~20 nm, and c) ~10 nm at excitation (785 nm) and Raman (852 nm or 999cm⁻¹) wavelengths. The yellow dashed lines indicate the SiN boundary, whereas the white lines on the Au surface indicate the BT monolayer.

Fig. C13: FDTD-simulated distribution of the SERS intensity ($|E|^4$) for Au nanogaps of a) ~ 30 nm, b) ~ 20 nm, and c) ~ 10 nm. The yellow dashed lines indicate the SiN boundary, whereas the white lines on the Au surface indicate the BT monolayer.

Fig. C14: SERS signals measured on Au nanogaps of ~ 30 nm, ~ 20 nm, and ~ 10 nm gap spacing. All the Au nanogaps were immediately immersed into the BT solution, parallel to the solution surface.

Fig. C15: Calculated enhancement factors and the corresponding histogram of the obtained spectra over a $5 \times 5 \mu\text{m}^2$ area for Au nanogaps of a) ~ 30 nm and b) ~ 20 nm. C14: SERS signals measured on Au nanogaps of ~ 30 nm, ~ 20 nm, and ~ 10 nm gap spacing. All the Au nanogaps were immediately immersed into the BT solution, parallel to the solution surface.

Fig. C16: a) Simulation of the allowed angle range of our fabricated Au nanogaps. b) Estimation of the reachable surface of the Au nanogaps (red lines). It is worth noting that in case of ~10 nm gaps the scattering signal from the cavity under the gap may not reach to the detector. Therefore, the surface of this cavity did not consider in the totally estimated surface. Scale bars represent 100 nm.

Fig. C17: Normalized collection efficiency.

Fig. C18: SERS signal of ~ 10 nm Au nanogap array with an excitation laser of 525 nm. High fluorescence background is visible, but no signal of BT is observed (2500 scan average).

References:

- (1) Ru, E. C. Le; Etchegoin, P. G. *Principles of Surface-Enhanced Raman Spectroscopy*; Elsevier, 2009.

Scientific output

Peer-reviewed journal articles

H. Le-The[†], **J.J.A. Lozeman[†]**, M. Lafuente, P. Muñoz, J.G. Bomer, H. Duy-Tong, E. Berenschot, A. Van Den Berg, N. R. Tas, M. Odijk, J. C. T. Eijkel, Wafer-scale fabrication of high-quality tunable gold nanogap arrays for surface-enhanced Raman scattering. *Nanoscale*, 2019, **11**, 12152–12160.

J.J.A. Lozeman[†], P. Führer[†], W. Olthuis, M. Odijk, Spectroelectrochemistry, the future of visualizing electrode processes by hyphenating electrochemistry with spectroscopic techniques, *Analyst*, 2020, **145**, 2482–2509.

J.J.A. Lozeman, K. Srivastava, H. Le-The, A. van den Berg, M. Odijk, Large-area fabrication of Au nanoantennas for surface enhanced infrared spectroscopy without an adhesion layer, in *Enhanced Spectroscopies and Nanoimaging 2020*, edited by Prabhat Verma, Yung Doug Suh, Proceedings of SPIE, 2020, **11468**, 1146818.

J.J.A. Lozeman, T. Elsbecker, S. Bohnenn, H. de Boer, M. Krakkers, G. Mul, A. van den Berg, and M. Odijk, Modular microreactor with integrated reflection element for online reaction monitoring using infrared spectroscopy, *Lab on a Chip*, 2020, **20**, 4166-4174

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Conference contributions (main presenter)

J.J.A. Lozeman, J.C. Vollenbroek, J.G. Bomer, H.L. de Boer, A. van den Berg, M Odijk, Modular ATR FT-IR microreactor chip for optimizing reaction conditions. *In MicroTAS: 22nd International Conference on Miniaturized Systems for Chemistry and Life Sciences* (poster presentation). 11-15 November, 2018. Kaohsiung, Taiwan.

J.J.A. Lozeman, K. Srivastava, H. Le-The, A. van den Berg, M. Odijk, "Large-area fabrication of Au nanoantennas for surface enhanced infrared spectroscopy without an adhesion layer. *SPIE Nanoscience + Engineering* (poster / digital presentation). 24-28 August. Digitally, San Diego, United States of America.

Conference contributions (co-author)

P. Führer, **J.J.A. Lozeman**, H.L. de Boer, J.G. Bomer, W. Olthuis, M. Odijk, Spectroelectrochemical detection of p-benzoquinone and hydroquinone in an electrochemical microreactor with an integrated ATR-IR IRE. *In MicroTAS: 22nd International Conference on Miniaturized Systems for Chemistry and Life Sciences* (poster presentation). 11-15 November, 2018. Kaohsiung, Taiwan.

H. Le The, **J.J.A. Lozeman**, J.G. Bomer, H.D. Tong, E. Berenschot, A van den Berg, J.C.T. Eijkel, Wafer-scale fabrication of high-quality sub-10 nm gold nanogaps. *In MicroTAS: 22nd International Conference on Miniaturized Systems for Chemistry and Life Sciences* (poster presentation). 11-15 November, 2018. Kaohsiung, Taiwan.

C.B.M. Tregouet, C.L. Kennedy, R. Kotni, S. Kölling, J. G. Bomer, **J.J.A. Lozeman**, D. Lohse, A. van den Berg, A. van Blaaderen, M. Odijk. Massively-parallelized production of femtoliter droplets and its application to self-assembled nanoparticle clusters for novel metamaterials. *In MicroTAS: 23rd International Conference on Miniaturized Systems for Chemistry and Life Sciences* (poster presentation). 27-31 October 2019. Basel, Switzerland.

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Contributions

All the work in this thesis is, to some degree, a collaborative effort. In this section I will clearly outline per chapter the individual contribution of each person that worked on the chapter.

Chapter 1: Introduction

Chapter 1 is written by Jasper Lozeman. Final edits were performed by Mathieu Odijk and Albert van den Berg.

Chapter 2: Theoretical Background

Chapter 2 is written by Jasper Lozeman. Final edits were performed by Mathieu Odijk.

Chapter 3: Spectroelectrochemistry, the future of visualizing electrode processes by hyphenating electrochemistry with spectroscopic techniques

Chapter 3 was a collaborative process by Jasper Lozeman and Pascal Führer. Jasper Lozeman was responsible for section 3.1 till 3.3. Pascal Führer was responsible for section 3.4 and 3.5. Section 3.6 was written by both Jasper Lozeman and Pascal Führer. Final edits were performed by Mathieu Odijk and Wouter.

Chapter 4: Modular microreactor with integrated reflection element for online reaction monitoring using infrared spectroscopy

Jasper Lozeman designed the chips, came up with the concepts and wrote the chapter. Jasper Lozeman, Tobias Elsbecker and Sylvie Bohnenn have fabricated the

chips and performed the experiments. Herein Jasper Lozeman mostly focussed on the ATR fabrication and the PDMS microfluidic chip fabrication. Tobias Elsbecker mostly focused on the COC microfluidic chip fabrication, bonding of the COC to the Si-ATR and the experimental design. Both Jasper Lozeman and Sylvie Bohnenn performed the experiments. Hans de Boer designed and fabricated the aligner, used to align the chip with the Bruker Vertex V70. Max Krakkers designed the herringbone mixers. Final edits were done by Guido Mul, Albert van den Berg and Mathieu Odijk.

Chapter 5: Au nanoantennas for surface enhanced infrared spectroscopy

Jasper Lozeman and Mathieu Odijk came up with the original designs and concepts. Ketki Srivastava performed the Numerical simulation and fabricated the nano-slit and the negative resist nano-rod antennas. Hai Le-The fabricated the positive resist nano-rod antennas. Experiments have been performed by Jasper Lozeman and Ketki Srivastava. Experiments have been performed in the BIOS lab at the university of Twente, at the PCS group at the university of Twente and at the ICC group at the university of Utrecht. Jasper Lozeman was responsible for writing the chapter. Figures and graphs have been made by both Jasper Lozeman and Ketki Srivastava. Albert van den Berg and Mathieu Odijk were responsible for some final edits.

Chapter 6: Wafer-scale fabrication of high-quality tunable gold nanogap arrays for surface-enhanced Raman scattering

Hai Le the, Jasper Lozeman, Albert van den Berg, Niels Tas, Mathieu Odijk and Jan Eijkel worked on the initial concept. Hai Le The created the antenna designs, performed the fabrication and wrote the sections regarding to the fabrication, the introduction and the conclusion. Jasper Lozeman performed the enhancement measurements and wrote the sections regarding the measurement results and surface enhancement. Marta Lafuente and Pablo Muñoz performed the simulations. Johan Bommer, Hien Duy-Tong and Erwin Berenschot contributed to the fabrication. Final edits were performed by Albert van den Berg, Niels Tas, Mathieu Odijk and Jan Eijkel.

Chapter7: Towards single mode infrared waveguides as alternative for attenuated total reflection devices

Chapter 7 is written by Jasper Lozeman. Initial conceptualization was done by Jasper Lozeman and Mathieu Odijk. The simulation are performed by Jasper Lozeman. The mask design and process flow has been designed by Rob Haverkate. Final edits were done by Mathieu Odijk.

Chapter 8: Conclusion and Outlook

Chapter 8 is written by Jasper Lozeman. Final edits were done by Mathieu Odijk.

Summary

The goal of this thesis, stated in **Chapter 1**, is to develop a lab-on-a-chip device for online reaction monitoring using surface enhanced vibrational spectroscopy. Vibrational spectroscopy covers techniques of infrared (IR) spectroscopy and Raman spectroscopy. Both techniques provide vibrational information, although in fundamentally different ways.

In **Chapter 2** a theoretical background is presented discussing the basic techniques covered in this thesis. This theoretical chapter is followed by a literature study in **Chapter 3** regarding spectroelectrochemistry. Although spectroelectrochemistry is not a major subject in this study, the two sections discussing IR spectroscopy and Raman spectroscopy provide further insight into these techniques.

In **Chapter 4**, a modular, microfluidic microreactor is presented. This microreactor has an integrated internal reflection element for attenuated total reflection (ATR)-IR spectroscopy. A Paal-Knorr reaction is performed as proof-of-concept. To highlight the strength of IR spectroscopy as a tool for reaction monitoring, the peaks are identified and different reaction orders of various steps of the Paal-Knorr reaction are calculated.

Chapter 5 and **Chapter 6** cover surface enhanced infrared spectroscopy (SEIRS) and surface enhanced Raman spectroscopy (SERS), respectively. In **Chapter 5** we discuss several different fabrication techniques to fabricate nano-rod and nano-slit nanoantennas. Furthermore we discuss FDTD simulations that can be used in order to predict the wavelength these antennas are tuned to and which configuration gives the strongest enhancement. Finally we present some very initial measurement results. **Chapter 6** discusses a high-yield fabrication method for wafer-scale patterning of high-quality arrays of dense gold nanogaps, using displacement Talbot lithography. Additionally, we prove that the Au nanogaps show a significant enhancement of signal of benzenethiol molecules chemisorbed on the structure surface, with an average enhancement factor up to 1.5×10^6 .

In **Chapter 7** we discuss the promise of mid-IR waveguides and propose a fabrication process how these waveguides could be fabricated. The dimensions used to design these waveguides are based on FDE simulations and literature.

In summary, all of the components as stated in the goal above have been addressed, although full integration into a single device has not been achieved. How this can be accomplished is discussed in **Chapter 8** and no major obstacles are expected.

Samenvatting

Het doel van dit proefschrift, als beschreven in **Hoofdstuk 1**, is om een lab-on-a-chip-apparaat te ontwikkelen om online reacties te volgen met behulp van “*surface-enhanced*” (oppervlakte versterkte) vibratiespectroscopie. Vibratiespectroscopie omvat de technieken infrarood (IR) spectroscopie en Raman-spectroscopie. Beide technieken verschaffen informatie over moleculaire vibraties, zij het op fundamenteel verschillende manieren.

In **Hoofdstuk 2** wordt een theoretische achtergrond gepresenteerd waarin de basistechnieken die in dit proefschrift aan bod komen worden besproken. Dit theoretische hoofdstuk wordt gevolgd door een literatuurstudie in **Hoofdstuk 3** over spectro-elektrochemie. Hoewel spectro-elektrochemie geen hoofdonderwerp is in dit proefschrift, geven de twee secties met betrekking tot IR-spectroscopie en Raman-spectroscopie verder inzicht in de besproken technieken.

In **Hoofdstuk 4** wordt een modulaire, microfluïdische microreactor gepresenteerd. Deze microreactor heeft een geïntegreerd intern reflectie-element voor attenuated-total-reflectie IR-spectroscopie. Als voorbeeld is er een Paal-Knorr-reactie uitgevoerd. Om de kracht van IR-spectroscopie als techniek om reacties te volgen te benadrukken, werden de pieken geïdentificeerd en werden verschillende reactieorders bij de verschillende stappen van de Paal-Knorr-reactie berekend.

Hoofdstuk 5 en **Hoofdstuk 6** behandelen respectievelijk oppervlakte-versterkte infraroodspectroscopie (SEIRS) en oppervlakte-versterkte Raman-spectroscopie (SERS). In Hoofdstuk 5 bespreken we verschillende fabricagetechnieken om nano-rod en nano-slit antennes te fabriceren op nano-schaal. Verder bespreken we FDTD-simulaties die kunnen worden gebruikt om de golflengte te voorspellen waarop deze antennes zijn afgestemd en welke configuratie de beste versterking geeft. Ten slotte presenteren we enkele preliminaire meetresultaten. **Hoofdstuk 6** behandelt een fabricagemethode met hoge opbrengst voor het maken van hoogwaardige arrays van dicht op elkaar staande gouden nanogaps, met behulp van Talbot-lithografie. Bovendien bewijzen we dat de Au-nanogaps een significante verbetering vertonen van het signaal van benzeenethiol-moleculen die zijn gechemisorbeerd op het structuuroppervlak, met een gemiddelde versterkingsfactor van 1.5×10^6 .

In **Hoofdstuk 7** bespreken we de potentie van mid-IR golfgeleiders en stellen we een fabricageproces voor. De afmetingen die worden gebruikt om deze golfgeleiders te ontwerpen, zijn gebaseerd op FDE-simulaties en literatuur.

Samengevat worden alle in het doel genoemde componenten zijn behandeld, hoewel de combinatie tot één apparaat niet volledig is bereikt. Hoe dit alsnog kan worden gerealiseerd, wordt besproken in **Hoofdstuk 8**, waarbij geen grote obstakels worden verwacht

Words of Gratitude

In this section of the thesis I am allowed to take my time and thank all who have made it possible for me to complete this thesis. Whereas it is expected of me to write the thesis in a formal style, write in English, and use scientific language and terms, in this section of the thesis I'm allowed to write in a more informal style. This, I will wholeheartedly embrace in an attempt to convey my thanks and emotion properly to those who worked so hard to support, assist, teach and motivate me to complete this thesis. Furthermore, I might add a story or anecdote about a person, so that when I read this 30 years from now, I can reminisce about the good time I had at BIOS. After re-reading this section, I also feel it's my obligation to tell you that this section can be experienced as cringy, and might be better enjoyed with a drink.

The professors of BIOS

I would like to start by thanking the professors, who are the beating heart of BIOS.

Firstly, I would like to thank **Albert van den Berg**, one of my promotors and the head of the research group. I would describe Albert as a large fire; bright, present, and sometimes slightly scary. Albert is always passionate about research and always gave good and useful feedback. However, sometimes the fire that is Albert is burning a bit too bright, especially when adding too much fuel. I can clearly remember organizing an archery tag teambuilding contest with Cristina and Vasilis. With Albert on one team and Mathieu on the other, too much fuel was on the fire and Albert burst into a blazing firestorm. When the two approached in the middle of the battlefield, Mathieu's knee met Alberts face, resulting in quite an injury. Despite the fact that the fire can be unpredictable sometimes, in the end he keeps everyone warm and fed, and he creates a place for people to gather around. For that I will always be thankful to Albert.

If Albert is the fire of BIOS, then **Jan Eijkel** is the (microfluidic) water. Jan is in general a calm professor. Like a gentle stream he nourishes the mind, be it in a technical meeting or in a philosophical discussion during a coffee break. He gently carries you downstream to a point he tries to convey, and if you have a good counter argument, the river always branches, willing to take a different path. Sometimes, when, for instance, concerning a certain U.S. president, the calm river can turn into a raging torrent, but it won't be long before the stream calms down again. I had the pleasure of working with Jan and Hai on the paper: *Wafer-scale fabrication of high-quality tunable gold nanogap arrays for surface-enhanced Raman scattering*. I learned a lot in the few meetings we had, where you were very educative. Jan is a fun professor to spar with and a fountain of wisdom.

Since it seems that I have developed a theme for the elements I would describe **Wouter Olthuis** as the earth (pun intended). Wouter is without a doubt a master of electrical engineering, and as an electrical engineer it is always a good idea to have a connection to earth. I worked together with Pascal and Wouter on our paper *Spectroelectrochemistry, the future of visualizing electrode processes by hyphenating electrochemistry with spectroscopic techniques*, and the feedback we got from Wouter was always clear, precise and detailed. Wouter always had answers to our questions or at least knew in which direction he had to point us. Wouter shared my passion for Star Trek and I always enjoyed our coffee break discussion on this subject and other (sci-fi) series.

If I follow the Chinese elements, then I would describe **Loes Segerink** as metal. Like metal, she is pretty cool, joining in a lot of BIOS events such as the BATA and being a big fan of hardstyle music. Although I have never had the opportunity to work together with Loes, I do remember a certain night during a conference in Taiwan where, after a drink, I learned some cool dance moves from Loes.

In the last months of my PhD **Tim Segers** joined the BIOS group. Due to the Corona crisis I have not had the chance to speak to him yet, but I wish him the best and hope he can become a strong element of BIOS.

Finally, we end up with the last professor, my promotor and direct supervisor. He is undoubtedly the most important professor for my project. Staying in theme I could associate **Mathieu Odijk** either with air if comparing him with the Greek elements, or wood if comparing with the Chinese elements. I would say he is both. He always stood strong like a tree, a stationary point in the landscape, where I could go to when I needed help or when I had questions. And when I did not need his help, he was still there, like a gentle breeze, not pushing but gently blowing me in the right direction. Mathieu's supervising style is really calm and honest which really worked well with me. There are a lot of things during my PhD I would have done differently in hindsight and a lot of things I would still like to do. I hope I lived up to your expectations and I will forever be grateful for the opportunity you gave me four years ago. Because you did not only give me the best job I could wish for, in a research group I doubt one would find anywhere else, you also introduced me to the place where I met Esther and where I would make friends that will stay with me for a lifetime, and for that I will always be grateful.

The Committee

I would like to thank **Bert Weckhuysen** for being my co-promotor. Bert is the leader of the MCEC communities and a great professor and organizer. The few meetings I have had with Bert have been very valuable.

A large number of measurements have been performed in the labs of the PCS group headed by **Guido Mul**. A lot of my work would not have been possible or would have taken longer without access to the PCS labs and for this I'm very thankful to Guido. Additionally, I have had a number of informative and pleasant discussions with Guido and one of his PhD students, Mozghan. Finally I want to thank Guido for being on my committee and taking the effort to read my thesis.

Sonia García Blanco is a professor in the Optical Sciences group and we have had some discussions during the initial phase of my PhD, which were really helpful. Sonia and one of her PhD students, Pablo helped me a lot in using the simulation software Lumerical, for which I'm really thankful. Sonia is always extremely busy so I want to thank her for taking the time in reading my thesis and for being on my committee.

I would describe **Han Gardeniers** as the nicest professor on campus. He is always extremely helpful and the few lectures of him that I have seen during the MCEC schools were always very interesting and informative. A lot of work Han did in the past formed a basis for the current thesis and I learned a lot of the literature he produced. It is a pleasure to have Han on my committee and I hope he enjoyed reading my thesis. Thank you Han!

I would like to thank **Wesley Browne** for being on my committee on such a short notice. Thanks for taking you for being on the committee and for taking the time and interest in reading my thesis.

I would like to especially thank **Ian Burgess**. Ian provided a lot suggestions to improve the thesis for which I'm really thankful. Due to the time difference Ian will have to wake up extremely early in the morning and be sharp enough to judge a PhD defence. Thank you for all your effort!

BIOS Staff

The most important person in BIOS is undoubtedly **Hermine Knol**, without her the group would be in constant chaos. She makes sure everyone is in time for meetings and appointments. She also makes sure that the PhD's and professors are aware of all the rules involved in doing a PhD. It must be difficult for her to keep everyone in line but somehow she manages. Thank you, Hermine for all your efforts.

Hans de Boer is an expert in designing and fabricating a wide variation of mechanical instruments. In this project, he was key in designing and fabricating the aligner used to align the ATR chip with the IR spectrometer. Hans does an amazing job in translating vague and uncertain ideas from PhD's and professors into an actual device which always exceeds our expectations. Thank you for all your help Hans and I wish you the best with your retirement.

If anyone in BIOS has a problem in the cleanroom, they go to **Johan Bomer**. Johan is a silent, stoic man. He is always there to help or answer questions and does not shows that he is always extremely busy. If I learned anything during my time at BIOS, it is that everybody always has some problem in the cleanroom, and Johan is always there to help. For this I would really and wholeheartedly say, thank you Johan!

Paul ter Braak might be one of the coolest staff members around BIOS. I enjoyed our coffee break discussions about Star Wars and other topics. I also appreciate all the discussions we had regarding the chemical lab. It always made me feel better that there was someone at BIOS that was willing to listen and discuss lab safety. I wish you the best in the future and good luck with your plant experiments!

Lisette Knippenborg and I have not had a chance to work together. Lisette is mostly responsible for the lab downstairs and does a lot of "*DNA stuff*", which is far outside of my comfort zone. Most of my conversations with Lisette are based around

the coffee corner, from which I have to conclude that she is a fun colleague to have around. I wish you, Nora and Jim the best in the future.

Jan van Nieuwekastele is a former staff member that helped me a lot during the initial stage of the PhD with the microscopes and visible spectrometer. Somewhere in my second year he decided to pursue new career opportunities, but I still learned a lot from him, thank you Jan!

Eddy de Weerd carries a lot of the educational tasks of BIOS. Eddy also knows where to get the best cakes in Enschede and his birthday was always a treat. Thank you for all you do for BIOS, Eddy!

The newest member of the staff is **Pieter Huchshorn**. Due to the Corona crisis I have not had to change to work with you yet. You have a challenging but fun task ahead as a technician at BIOS and I wish you the best!

Although not technically BIOS staff, I would also like to thank the **cleaning staff**. On those early mornings you were usually one of the first to greet me, which was always appreciated. I'm sure you have seen some horrifying things, cleaning at a university. I would like to thank you all for the great job you all did keeping our offices and hallways clean.

Collaborators and students

Firstly I would like to thank **Ketki Srivastava**, a former master student of mine, a current BIOS PhD student, and one of my paranymphs. Ketki is the typical story of the student that became the master. Ketki grasped the theory extremely fast and also in the cleanroom she proved to be an excellent student. One of my biggest fears when starting my PhD is that all the result would be put in a book, the book would be put on a shelf, and it would never again see the light of day. So I would not only like to thank Ketki for all the work she has done in the past, which is really amazing work, I would also like to thank her for continuing with large parts of the works mentioned in this thesis. So, thank you Ketki and I hope you will enjoy your PhD as much as I have.

Hai Le The is a former BIOS PhD student and was the cleanroom wizard at BIOS. He helped me a lot during my PhD with cleanroom processes, which was much appreciated. We worked together on the paper: *Wafer-scale fabrication of high-quality tunable gold nanogap arrays for surface-enhanced Raman scattering*, which was a pleasure to work on. Hai has now started a new job at ASML and with this BIOS has lost a great asset. Hai, thanks for everything and I wish you, Hoa and baby Elena the best in this new chapter of your life.

I have had the pleasure to have **Pascal Führer** as an office mate. Pascal is always helpful and never afraid to give critique, which always improved my work. Pascal also always gave interesting facts about whatever is on his mind, which ranges from informative to funny, depending on the situation. People say that Germans don't have any humour, but Pascal has proven that stereotype wrong. I had the pleasure of working together with Pascal on the paper: *Spectroelectrochemistry, the future of visualizing electrode processes by hyphenating electrochemistry*. Which I think was a great success. Thank you for everything Pascal!

I had the honour of supervising **Rob Haverkate** during his master project. Rob was the most self-reliant student I have ever seen and I'm afraid I was not much use to him. Rob is an extremely stoic and strong person that never complains. He is one of the most hardworking persons I know and I'm sure he can do anything he sets his mind to. Thank you for everything Rob and best of luck in the future!

Tobias Elsbecker and **Silvie Bohnenn**, thanks for all the work you two did during your bachelor project on the ATR device. I hope you learned a lot and that the project was enjoyable.

Pablo Muñoz Galindo helped me a lot during the first few years of my PhD. Sadly the help was mostly one sided since his knowledge in optical science far outweighed my own. During the end of Pablo's PhD he became more busy and sadly I never got the chance to really thank him for all his help. So, I would like to say, thank you Pablo for all your help.

Some initial collaboration effort attempts were made with **Mozhgan Moradzaman**. Although this collaboration in the end did not bear any fruits, I learned a lot from Mozhgan and I would like to thank her for this.

Historical figures

There are some people from my past that I would like to thank. These people appeared in a critical point in my life and steered me in the direction I ended up today. I might not have realized at the time how important they were and I don't think I ever thanked them properly.

I would like to start by thanking **Ad van de Haar**, Chemistry teacher at Groevenbeek college. My time at Groevenbeek was a difficult one, and going to school was never enjoyable for me. That is, till I got chemistry from Ad. Ad made chemistry and learning fun. He was always in for a joke and his way of teaching really motivated me as a student. If it wasn't for Ad, I would never have known that learning could be fun and I would have never developed a love for chemistry. For this, I am really grateful, thank you Ad!

After Groevenbeek I went to the ROC Midden Nederland for my education as laboratory technician. During my time here I was taught by a lot of great teachers. However, one teacher stood out, which was **Peter de Vries**. During these teenage years I could be quite demanding. Where most teachers were nice, considerate and easy going, Peter was more honest and direct on how the world was. His directness and Dutch "nuchterheid" were refreshing and helped me to better understand the world we live in. I learned a lot from all my teachers, but Peter was the only one that went the extra step to teach his students life skills. Thank you Peter.

Of all the internships I performed during my education, the people at **Labora** where the only ones that also invested time in developing me as a person, they pushed me to better myself. I'm sure that at times I could be quite tiresome, but it was really appreciated. Thank you for the fun and interesting times at Labora!

During my bachelor, at the Hogeschool van Utrecht, I really started to enjoy learning. For which I want to thank great teachers like **Gert Legemaat**, **Aloys Pouwels** and **Els Willems**. I also clearly remember the teacher **Rob Niekel**, who towards the

end of my bachelor recommended me against doing a master, since I would never be able to finish it. I would really like to thank him for this opinion, since it served as a great motivation to prove him wrong.

During my master I had a teacher called **Henk Lingeman**. Henk was overseeing my master program and he taught me much. He especially showed me how peculiar people working in academia can be. This prepared me for some of the people I met during my PhD. Thank you Henk!

Co-workers

There are a lot of co-workers, fellow PhD's and post-docs that I would like to thank for the fun times at BIOS. Firstly I would like to thank, in a random order, the PhD's and post-docs that finished before me: **Marinke van der Helm, Burcu Gumuscu, Rik Rurup, Marzena Krzek, Maria Perez Zabalza, Felix Loser, Julia Mikhal, Laura Folkertsma-Hendriks, Martijn Tibbe, Floris van den Brink, Miguel Solsona, Juan Wang and Anke Vollertsen**. Thank you all for your help, the fun coffee breaks and adventures during the workweek(s) and showing me the way in how to do a PhD.

I want to take my time to thank **Hugo Albers**. I remember fondly how he introduced me and Esther to the food festival in Enschede. This happened when I just started my PhD and it was a lot of fun. Hugo's fondness for cooking was very infectious to Esther and as a result, I enjoyed a lot of great meals. Hugo, Josh and me enjoyed a short trip in Taiwan which was a lot of fun and where we had some great adventures. Thanks Hugo!

I would like to thank **Corentin Tregouet**, he started his post-doc around the same time as I started my PhD and he is in general a fun person to be around. We had some great fun in the MCEC meetings and was always in for some BIOS event or another. His positive work attitude was infectious and his attempts at learning Dutch were both amusing and impressive. He did some amazing work and I wish him all the best in his academic career.

I especially would like to thank **Anne Leferink** for being such a fun person. Anne is very open and tells things how she sees it. Her honesty is refreshing and always comes with a dash of humour sprinkled in. She is a great teacher and without a doubt motivates a lot of students during their studies. AST is lucky to have Anne as a researcher / teacher. Thanks Anne!

Furthermore I would like to thank my current BIOS co-workers, again in a random order: **Alessandro Carlino, Luca Carnevale, Koen Jorissen, Ruben Kolkman, Edo de Kruiff, Stella Kruit, Lanhui Li, Heleen Middelkamp, José Rivera Arbelaez, Cristina Tiflidis, Eiko Westerbeek, Mariia Zakharova, Dodo Wasserberg and Rajendra Shukla**. You guys make BIOS great!

In **Nienke van Dongen** I found an ally regarding the situation of the chemical lab, which was a welcome change. Nienke is a fun person to be around and although she likes cats, she is a good person in general. The coffee breaks reached new heights of funny-ness when Nienke, Josh, Jeroen, Jorien, Vasilis, Paul and Esther were participating, often resulting in me crying of laughter and straining muscles in my face I did not know I had. Keep on being funny Nienke!

Alessia Broccoli took over my difficult task as moderator of the BIOS Thursday afternoon talk (previously known as Monday morning talks). This moderator task (official title being Iron Fist) is more time consuming than most people think, especially in these Corona times where everything is digital. Good luck with your PhD and the BIOS talks, Alessia. Don't let anyone forget the power of the Iron Fist!

Together with **Tanja Hamacher, Douwe de Bruijn**, Josh and Jeroen we organized the BIOS workweek to Denmark and Sweden. This was a lot of fun to organize and I think it was well received due to our group effort. I fondly remember the amazing talk organized by Tanja about the history of Denmark and Douwe's administrative skills, making sure we keep to our budget. You are both amazing scientist and I wish you well with the continuation of your PhD's

A special thanks should go out to my office mates, who were locked up with me in our office without any choice in the matter. It must have been a difficult time but (for most of you) it's finally over. Pascal, thanks again for being such an amazing office mate and Esther, I will come around to thanking you later. This thanks section I would like to reserve for **Elsbeth Bossink**. Elsbeth is probably the most considerate office mate one could think of. She is possibly the most polite, well-mannered and respectful person around BIOS (the bar is low, but still a nice achievement). She is a workaholic that always plans to take days off but never does. She is always ready to help people and probably never says no to anything. But behind this polite, well-mannered woman, hides a roller-coaster nut. I remember well, during our trip to Walibi Holland, she had to go on every rollercoaster, several times. Where the most hardened colleagues were tired and sick, barely holding themselves together, Elsbeth was still laughing and having fun going through loops and corkscrews. Thank you for the fun times Elsbeth and although I know that you don't need it, good luck with the rest of you PhD!

Finally, I would like to thank all my **MCEC colleagues**. We had some fun times at the MCEC schools and trips to the universities involved in the project. I learned a lot and achieved things I could not achieve without the program. I would like to especially thank those at the MCEC office. I would like to thank **Emke Molnar, Nina Versteeg, Anne-Eva Nieuwelink, Christine Geense, Daan van Arcken** and those working behind the scenes making everything possible. Thanks for all your effort!

Family and Friends

Josh Loessberg-Zahl is a crazy – and liberal – American which probably says enough about him. But through the years I found out Josh is more than that. Josh is a kind colleague, that is always ready to help someone. Josh is a great teacher and scientist, but an even greater friend. I enjoyed our after work jogs and adventures we had. Josh, I'm sorry for all the America jokes I made in the past and I apologize for those I will make in the future. Thanks for putting up with me and thanks for being a great friend!

Jorien Berendsen is the most serious and clearheaded person in our Enschede friend group. Jorien is a great person to be around and always helpful in giving housebuilding advice. Although she might not want to admit it, she is great fun and

a funny person. Thank you Jorien for putting up with us crazy people and their stupid hobbies.

Jeroen Vollenbroek is a great friend and one of my paranymphs. We went on a trip to Japan together which was great fun and I remember fondly. Jeroen is an expert in alcohol and is impossible to outdrink. He always has either a cup of coffee in his hand or a bottle of beer, but somehow functions as a human being. Jeroen is a great scientist and electrical engineer and an amazing colleague. I'm sure that after my PhD we still have a lot of adventures. Thank you for all the fun times and jokes and thanks for being a great friend!

Vasilis Papadimitriou is a great colleague and a good friend. When there was a new movie to be seen, Vasilis, Jeroen Esther and I were always ready to go to the movies to see it. Vasilis is also a great cook and I remember fondly how we enjoyed his Greek sausages during a summer barbecue. Thanks for all the fun times Vaaaaaaaaaaaaasilis!

Stefan Dekker is a great colleague and friend, who is the most helpful person I know. When Esther and I bought our house, he helped a great deal in renovating and even motivated us on projects we were not sure if we would be capable of finishing. He also often joined for the movies and is a great friend to have around.

One of my oldest friends is **Jeroen Nanninga**. Although nowadays we don't always have as much contact as in the past, Jeroen is still a valuable friend that I appreciate having around. Jeroen, thanks for all the fun times!

I want to thank my master friends, **Simone Mooibroek, Eliane van Dam, Sander van Doorn, Carol de Ram en Teun de Gelder**. Thanks for all the fun during and after our master. The master would probably be much more difficult and much less fun without you guys around. Although we have not been able to hang out during Corona, I hope we can all meet again soon!

I would like to thank Esther's parents, **Francisca Srioetami** and **Denny Tanumihardja**. Thank you for accepting me and being so extremely welcoming when we visited Indonesia. I hope we can visit you again soon, so I can thank you in person.

I would also like to thank Esther's sister and brother-in-law, **Gabriela** and **Andrew Eaton**. You guys are great fun and amazing people to be around. Esther is always visibly happy when she calls with Gaby which then always makes me happy. I hope we can all meet again soon!

Ik wil graag mijn familie bedanken. **Jaap en Fieke, Rosalien, Sjaak, Nick en Maik van den Hoek. Evert en Niesje van den Hoek, Diana en Anthony de Zwart** en de kids, **Corina van den Hoek en Klaas Jan** en de kids en **Brenda Jansen van den Hoek en Anton** en de kids. Hoewel we nooit echt erg close zijn geweest, zijn jullie wel familie en waardeer ik de moeite die iedereen neemt om in ieder geval een keer per jaar samen te zijn. Hoewel het vorig jaar niet is gelukt door Corona, (of laten we dat maar de schuld geven) hoop ik dat we dit jaar weer de mogelijkheid hebben om elkaar te zien.

Janny en Theo van Oossanen zijn de tante en oom die vaak langs komen. Hoewel ik vaak grapjes maak over Janny, waardeer ik haar enorm als een tante. Geen

feestdag is compleet zonder Janny en Theo en ik heb jullie ook enorm gemist deze kerst. Hopelijk kunnen we elkaar snel weer zien!

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The final person I want to thank is the most important person on this list and more important to me than anything. **Esther Tanumihardja**, words can't describe how thankful I am to you. You give my live colour and meaning. Thank you for everything Esther.

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