Dielectric and fluorescent samples imaged by scanning near-field optical microscopy in reflection

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Abstract

Dielectric fluorescent samples are imaged by scanning near-field optical microscopy in reflection. A non-metallized tapered fibre tip is used both as an emitter and a detector. Shear force feedback controls the distance between the tip and the sample and gives simultaneously a topographic image of the surface. A direct correlation with the optical image is obtained. We demonstrate that this reflection setup is suitable for dielectric samples. Images in fluorescence have been obtained on Langmuir–Blodgett films. For fluorescence detection the optical contrast is unambiguous, and consequently we estimate the detecting size of the probe at about 200 nm. Operation in reflection opens new possibilities since it allows the study of metallic, thick, opaque or dielectric sample and can be easily combined with a transmission setup. In this way, biological samples can be imaged optically simultaneously in transmission, reflection and with a shear force control.

1. Introduction

Near field optical microscopy has gone through fast developments over recent years. The technique works analogously to scanning tunnelling microscope and scanning force microscope but at variance the optical interaction is studied. In this way the optical properties of the sample a nanometre scale are obtained. The basic idea was suggested already in the beginning of the century [1]. Different schemes have been developed by several groups [2] with a lateral resolution down to 12 nm, indeed much better than obtained in classical diffraction limited microscopy.

Commonly, the near-field study of transparent samples is done with a transmission setup where an aperture obtained on a metallized tip is used as an emitter [3,4]. This technique is very promising for biological and chemical applications as all optical contrast methods

of conventional confocal microscopy can be applied on sub-wavelength scale unlimited by diffraction.

We present here images of transparent samples obtained with a reflection setup, which is more suited for reflecting, thick, opaque and absorbing samples [5–8]. Langmuir–Blodgett films have been studied in fluorescence which is excited by the light coming from the fibre tip itself. Analysing the fluorescence intensity we determine the detector size of the tip. The probe to surface distance is controlled by shear force feedback system and thus simultaneously a topographic image [9,10] is obtained.

2. Experimental setup

The experimental setup is shown in Fig. 1. For a bright field detection, a HeNe laser beam (2 mW) of wavelength $\lambda = 632.8$ nm is focused onto the face of a

Fig. 1. Reflection setup with bright field illumination and shear force detection. QD = quadrant detector, PD = photodiode, PC = personal computer.

multimode optical fibre, via a glass cover slip. Its glass face is oriented at an angle with respect to the optical axis and is overlaid with oil. Consequently the Fresnel reflection does not return to the laser source. The main part of the incoming beam is reflected by the tapered tip. The rest emerges and is reflected by the object. A fraction is reintroduced into the tip. Interference of these two reflections is detected by a photodiode (PD). A prism is used preferably to separate the incoming and reflected beams rather than a 3 dB coupler. In this way, we avoid an additional fibre to fibre connection, the fibre length is reduced and we diminish the optical signal instability due to other parameters like temperature, pressure... Moreover changing the tip is easier.

For fluorescence measurements the HeNe laser is substituted by an Ar^+ laser of wavelength $\lambda = 514 \text{ nm}$. A dichroic mirror (DM 580 Nikon, $\lambda = 560 \text{ nm}$) and a high pass filter (BA 590 Nikon, $\lambda > 590 \text{ nm}$) are used. They allow the separation of the exciting and fluorescence beams (Fig. 2). A photomultiplier (PMT Hamamatsu R5600) detects the fluorescence of the scattered light. Unlike the bright field detection which induces interference, only the fluorescence intensity coming from the sample is detected.

We have used an adiabatically pulled optical fibre as near field optical probe. In the present reflection study

the probe was not coated with metal. We chose a multimode optical fibre because it allows a better collection of the diffracted light. Its extremity is pulled with a commercial instrument (Sutter P2000) and its diameter is reduced to about 100 nm . The probe is used simultaneously as an emitter and a collector for the diffracted light.

Additionally shear force detection is used to control the tip to sample distance. The principle was presented three years ago [9,10]. A few millimetres away from the tip end the fibre is stuck onto a piezo-ceramic. The fibre is vibrated at its first resonance frequency (typically 5 kHz) with an amplitude of some tens of nanometres. Perpendicular to the fibre a laser diode is focused in front of the extremity. A quadrant detector (QD) collects the diffracted light. The difference signal is proportional to the fibre oscillation amplitude. At a few nanometres from the sample surface, forces act on the tip and the amplitude of the dither vibration decreases. Fig. 3 shows an example of the decrease of the signal where the sample surface is an Al mirror. In this case the vibration amplitude is 80 nm , and the frequency is 3.55 kHz . The physical origin of the shear force mechanism is not well understood but one remarks that the behaviour of the approach curve changes during the last 12 nm . Using a feedback system the shear force signal is kept constant. The probe follows the contour of the sample and a topographic image is realized. Because of the feedback loop, the acquisition frequency is 300 Hz .

Fig. 2. Fluorescent detection setup. PMT = Photomultiplier tube. The high pass filter blocks $\lambda_{\text{exc}} = 514 \text{ nm}$. Only $\lambda_{\text{em}} > 590 \text{ nm}$ is recorded.

Fig. 3. Shear force signal as function of the tip to sample separation. For this example the vibration amplitude is about 80 nm and the resonance frequency 3.55 kHz. Within 12 nm from the surface forces act on the tip and the vibration decreases.

The sample is stuck to a piezo-tube which allows its displacement in x and y directions. A computer is used

to generate the scan pattern and to analyze the optical and force signals.

3. Results and discussions

Fig. 4 shows a $2.8 \times 1.4 \mu\text{m}^2$ area of a latex sphere multilayer (Molecular Probes, Eugene, OR, USA). The pixel size is 28 nm. Two kinds of fluorescent spheres with a size of 282 nm are mixed. Only one type absorbs at 551 nm and fluoresces around 636 nm. Corresponding wavelengths for the other type are respectively 650 nm and 700 nm. Fig. 4a shows the filtered shear force image, in which we recognize the “hexagonal” structure of the packing. Fig. 4b represents the corresponding bright field image in reflection. Although the contours of each sphere are resolved, several appear with mutually different contrast.

Fig. 4. Shear force (a) and optical bright field (b) maps of a latex sphere multilayer. Image size is $2.8 \times 1.4 \mu\text{m}^2$. The pixel size is 28 nm.

According to the shear force image, this behaviour is not due to a shift of the optical image. Additional to topographic effects the optical contrast can have two origins: First, the sample is composed of two kinds of spheres. Considering the exciting wavelength, absorption can reduce drastically the reflected light level and introduce an optical contrast. Second, the tip is not metallized, and will accept light over its entire apex. Multiple interferences can thus be measured. The resulting phase, coming from this contribution can be different for each sphere and gives rise to a change of contrast level.

In the same way, an indium-tin-oxide (Baltracon, Balzers) film has been studied. It is composed of small crystallites of about 50–300 nm in size. The transmission coefficient is about 85%. Fig. 5a shows the shear force image. The scan area is $3 \times 3 \mu\text{m}^2$ with 200×200 pixels. Structures with 100 nm in size are resolved. However this type of sample is difficult to image with a vibrating tip. The dither amplitude is in the same order of magnitude as the size of the structures to be imaged. The topographic variations are so extensive that the probe cannot detect the whole contour of the surface. Corresponding optical image is shown in Fig. 5b. The crystallites appear light and the background black. We note that this contrast is inverted compared to that obtained with a combined PSTM-AFM [11]. In this case the measurements are done in transmission and because of their refractive index the grains appear dark. Although the two systems use different tips (pulled fibre and cantilever) and illumination modes (reflection and transmission), the results on the ITO sample agree well.

Fluorescence technique is a contrast method used in conventional microscopy and is of a great interest in biology and chemistry. In the near field reflection mode it allows to decorrelate the optical image from the topographic contrast as it appears in bright field too.

Diethylene glycol diamine pentacosadiyonic amide (DPDA) sample polymerized by UV radiation have been studied. The film have been fabricated by Langmuir–Blodgett techniques on glass slide [12]. It is suited as a test sample for near field optical microscopy because of its strong fluorescence (green excitation–red emission) and the weak thickness (6 nm) of the monolayer.

Topographic and fluorescence maps are shown in Fig. 6. The scan area is $12 \times 12 \mu\text{m}^2$ for 300 pixels.

Fig. 5. Shear force (a) and optical bright field (b) images ($3 \times 3 \mu\text{m}^2$, 15 nm pixel size) of an indium-tin-oxide film (Baltracon, Balzers).

We note on the topographic image (a) that the film is composed of several domains with a thickness of few nanometres created during the film formation. However, the shear force contrast is inverted with respect to the topography, and the film seems to be lower than the glass part. This behaviour, not totally explained, could be due to adhesion forces that the tip detects in the

Fig. 6. $12 \times 12 \mu\text{m}^2$ (40 nm pixel size) images of a DPDA film. On the shear force image (a) the contrast is inverted in respect with the topography. (b) is the fluorescence corresponding image where we recognize the film domains.

vicinity of this kind of inhomogeneous sample. Fig. 6b is the corresponding fluorescence map. The black parts are the areas where no film covers the glass. The fluorescence domains appear clearly but because of the multimode fibre we cannot study the polarisation of

this signal. We did several measurements on the same area without notable photobleaching.

In order to investigate the detector size of the probe, we plotted in Fig. 7 a fluorescence contour line. We define the resolution like the full width at half maxi-

Fig. 7. Fluorescence contour of a line in the optical image 6b. The passage between fluorescence and non fluorescence parts is done within 150 to 400 nm. We estimate the detector size at about 200 nm.

imum of the signal jump between fluorescent part (above) and glass part (lower). We note that this jump can be done within 150 to 400 nm, and we estimate an average value of about 200 nm.

4. Conclusions

We have presented images of dielectric and fluorescent sample obtained with a scanning near-field optical microscope in reflection. We described near field optical setup in reflection, with shear force control which is a means to control the tip to sample distance. A force image is also realized, and can be compared to the optical image. The main advantage of this configuration compared to other reflection setup is that the optical fibre extremity collects the optical field that it generates.

We show that the contrast mechanism in bright field images is rather complicated and correlated to the sample topography. This effect could be attenuated by using different mechanisms like polarisation study [13] for several wavelengths.

In order to analyze true optical contrast, we imaged Langmuir-Blodgett films where we distinguish fluorescence and non fluorescence areas. This last result allows an estimation of the detector size at about 200 nm. Possibly lateral resolution could be improved using a

metal coated fibre. However, the issue of a bare fibre or a metal coated fibre for optimum operation in reflection is not clear yet and will be subject of further research.

This results are promising especially for biological sample studies. We can imagine a setup combining reflection and transmission detection. Results could be compared to each other for a better understanding of surface optical properties.

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