has the potential to contribute to practical applications in super-resolution imaging and thermal photovoltaics. ❐

Evgenii E. Narimanov and Alexander V. Kildishev are in the Electrical and Computer Engineering Department, and Birck Nanotechnology Center, Purdue University, West Lafayette, Indiana 47906, USA.
e-mail: evgenii@purdue.edu

References
1. Born, M. & Wolf, E. *Principles of Optics: Electromagnetic Theory of Propagation, Interference and Diffraction of Light* (Cambridge Univ. Press, 1999).
2. Jacob, Z., Alekseyev, L. V. & Narimanov, E. *Opt. Express* **14,** 8247–8256 (2006).
3. Salandrino, A. & Engheta N. *Phys. Rev. B* **74,** 075103 (2006).
4. Smolyaninov, I. I. & Narimanov, E. E. *Phys. Rev. Lett.* **105,** 067402 (2010).
5. Sun, J., Litchinitser, N. M. & Zhou, J. *ACS Photon.* **1,** 293–303 (2014).
6. Alekseyev, L. V., Podolskiy, V. A. & Narimanov, E. E. *Adv. OptoElectron.* **2012,** 267564 (2012).
7. Sun, J., Zhou, J., Li, B. & Kang, F. *Appl. Phys. Lett.* **98,** 101901 (2011).
8. Warmbier, R., Manyali, G. S. & Quandt, A. *Phys. Rev. B* **85,** 085442 (2012).
9. Romanowsky, M. B. & Capasso, F. *Phys. Rev. A* **78,** 042110 (2008).
10. Caldwell, J. D. *et al. Nature Commun.* **5,** 5221 (2014).
11. Thompson, D. W., DeVries, M. J., Tiwald, T. E. & Woollam, J. A. *Thin Solid Films* **313–314,** 341–346 (1998).
12. Schubert, M., Tiwald, T. E. & Herzinger, C. M. *Phys. Rev. B* **61,** 8187 (2000).
13. Mutschke, H., Andersen, A. C., Clément, D., Henning, Th. & Peiter, G. *Astron. Astrophys.* **345,** 187–202 (1999).
14. Esslinger, M. *et al. ACS Photon.* **1,** 1285–1289 (2014).

PHOTOACOUSTIC IMAGING

Cells make themselves heard

A photoacoustic imaging scheme that uses genetically altered cells that express an absorbing pigment can monitor *in vivo* growth of cells and tumour development.

Srirang Manohar, Aart van Apeldoorn & Wiendelt Steenbergen

The ability to visualize cell behaviour during regenerative processes or tumour growth in real-time, with high spatial resolution, is highly desirable yet considered a major challenge. If one is able to track and depict how specific cells and dynamic biological processes evolve over time *in vivo* in small animal models, a much better understanding of how diseases, or regenerative processes, occur can be gained. Now, writing in *Nature Photonics,* Jathoul *et al.*[1] report that photoacoustic imaging can provide high-quality visualization of cells that were genetically altered to produce pigment.

Fluorescent imaging of appropriately labelled cells can assist the study of cell behaviour, but has limitations as optical imaging methods fail to provide the required high-resolution interrogation at relevant tissue depths due to extensive optical scattering. Photoacoustic, or optoacoustic, imaging[2] offers an attractive alternative. It uses short pulses of light that are absorbed by the sample and, through thermoelastic expansion, converted into ultrasound pulses that can be measured using piezoelectric detectors. As ultrasound suffers low scattering and attenuation in tissue, the method can provide an image of optical absorption distribution deep within tissue.

To date, photoacoustic imaging is best known for exquisite three-dimensional visualization of vasculature with high resolution at relatively large depths. Contrast ratios as high as 100 — unmatched by any other imaging technology — are possible due to the high optical absorption of hemoglobin. Because changes in blood vessels are often associated with a variety of oncological, inflammatory and immune disorders, as well as with regenerative processes, the method is potentially highly versatile. However, in many cases, such as monitoring tumour growth in response to therapy, studying tissue regeneration in bioengineered implants, or attempting to understand the effect of cell therapies, it is also important to visualize changes in cells or groups of cells. The challenge here is that cells exhibit poor optical absorption contrast in the visible and near-infrared wavelength regimes that are typically used for such observations. Various approaches have sought to label cells with extraneously administered absorbing nanoparticles, or dyes, but these methods are not very effective due to the difficulties in precisely targeting the cells of interest. Besides, most biocompatible dyes are rather inefficient optical absorbers.

Jathoul *et al.*[1] addressed this problem with a powerful method — they genetically

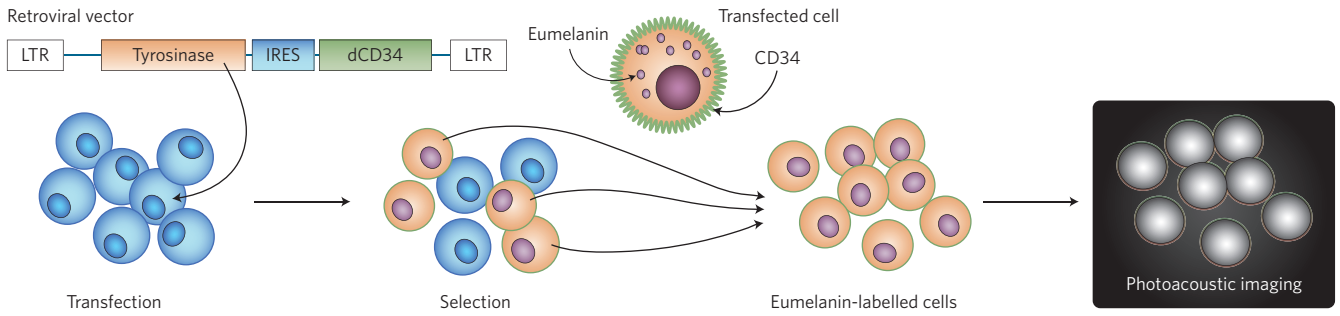

Figure 1 | Schematic showing the method for inducing photoacoustic contrast in mammalian cells in subsequent generations. The retroviral vector is incorporated into the target cells through viral transfection. The vector contains a series of important elements including 5′ and 3′ long terminal repeats (LTRs), internal ribosome entry sites (IRESs), and the coding regions for tyrosinase (orange) and dCD34 (green). After transfection, a preselection is done based on immunolabelling of a cell surface marker, CD34, which is expressed on the cell membrane. The cell surface marker can be used for the sorting of stable transfected cells, which now express both tyrosinase and CD34. The sorted cells that express tyrosinase, leading to an accumulation of eumelanin in their endosomes, are proliferated and can be used for photoacoustic imaging.

altered specific human cell lines so they express an optical absorption label for photoacoustic imaging. They used a retroviral vector to infect the cells, allowing them to express tyrosinase, the enzyme that catalyses eumelanin. When expressed in cells, the enzyme is transported to the endosome, where it is able to initiate the accumulation of eumelanin. Eumelanin — a pigment that naturally occurs in melanocytes and is responsible for the dark colour of skin and hair — has broadband optical absorption characteristics. So, through the clever stable transfection of several human cell lines, the authors were able to perform photoacoustic imaging of the spatial distribution of these cells (xenografts) in mice together with the surrounding vasculature. The imaging method allowed them to obtain exceptional resolutions, better than 100 μm at depths approaching 10 mm, in the mouse studies. The photoacoustic imager used in the study is a rather unusual set-up, as it uses an optical method of ultrasound detection based on a Fabry–Pérot etalon instead of the often used piezoelectric detectors.

Earlier work by others explored the use of transgenic expression of tyrosinase, which converts cellular tyrosine to dopaquinone. This process is the rate-limiting step in the production of melanin. Paproski *et al.*[3] developed a system in mice where tyrosinase is 'turned on' in engineered tumour cells when an inducer such as doxycycline (DOX) is administered by adding it to the animals' drinking water. Stritzker *et al.*[4] demonstrated viral-delivery of tyrosinase into cancer cells. Both works used multispectral optoacoustic imaging that allowed the tumour cells to be visualized in mouse models. However, a major drawback of these approaches is that the ability to produce melanin was restricted to the first generation of cells, a property not passed on to daughter cells, because the cells' genome was not modified.

In the present work, this problem was tackled by using a specifically engineered retroviral vector to transfect cells of interest so that they not only express tyrosinase but, importantly, also pass on the ability to produce eumelanin to subsequent generations of the cell. The authors first succeeded in stably transfecting different human cell lines using a vector containing the tyrosinase gene and CD34 (Fig. 1). By preselecting cells positive for CD34, using a combination of magnetic cell sorting and flow cytometry, they obtained purified cell populations expressing the tyrosinase gene in subsequent generations of daughter cells. On closer observation, the transfected cells were able to build up high concentrations of eumelanin, without significantly affecting their phenotype or viability. These tyrosinase-expressing cells were then injected into immunocompromised mice to study cell proliferation and vascularization using photoacoustic imaging. By using a series of different excitation wavelengths and a sophisticated photoacoustic imager, excellent visualization of the cells and the surrounding vasculature with good contrast was obtained. As the genetically-generated contrast is passed on to subsequent generations, the changes in cell proliferation could be observed over a period of 52 days in one case, and 26 days in a second case.

A high-performance photoacoustic signal detection system was crucial, as it allowed Jathoul *et al.*[1] to obtain their impressive imaging results. For the purpose of studying cell growth, the usual compromise between imaging depth and imaging resolution needed to be stretched to the limit, requiring both a high acoustic sensitivity and a good spatial resolution. The team's Fabry–Pérot-based optical ultrasound sensor made this possible.

The use of a highly focusable optical probe beam brings the benefit of a small detector size while maintaining a sensitivity of just 0.2 kPa (ref. 5). The small size offers a wide ultrasound reception angle and the use of multiple beam locations creates an array of ultrasound sensors that allow for synthetic focusing with a large numerical aperture, hence a good transverse resolution. Axial resolution is realized through a large acoustic bandwidth. Although acoustic mismatch usually creates resonances at preferred frequency ranges, hence reducing the bandwidth, mismatch was suppressed here by the properties of the applied materials, making the film-based Fabry–Pérot sensor sensitive over a range of 350 kHz (associated with objects of around 5 mm in size) to 22 MHz (objects less than 100 μm).

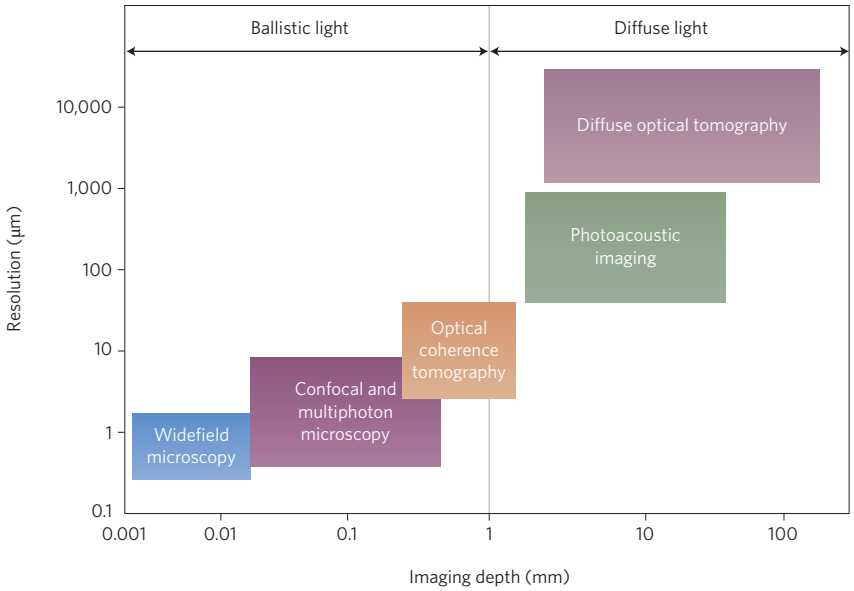

Figure 2 | Methods for three-dimensional optical imaging of biological tissues compared by their performance in terms of resolution versus imaging depth. Imaging methods relying on ballistic (unscattered) light are limited to depths below 1 mm. Imaging methods based on diffuse light ordinarily lead to a strong deterioration of the resolution to depth ratio. This limitation is overcome in photoacoustic imaging by exploiting the high transparency of biological tissues to ultrasound.

Figure 2 compares the photoacoustic imaging set-up used by Jathoul *et al.*[1] with other optical modalities for biomedical imaging. The approximately constant ratio of imaging depth to imaging resolution for optical modalities that work with ballistic light (microscopies and optical coherence tomography) is now extended into the regime of diffuse, multiply scattered light.

The use of genetically modified cells expressing a label that allows visualization by photoacoustics enables the functional imaging of cells *in vivo*. By developing different retroviral vectors in which a gene of interest is co-expressed with tyrosinase, the behaviour of cells under the influence of any co-expressed gene over time can be studied. The method employed by Jathoul *et al.*[1] enables easy *in vivo* photoacoustic imaging of otherwise invisible cells, which can not only be used to investigate various aspects of tumour development, metastases and therapeutic responses, but can also potentially be used to study tissue regenerative processes in the future. However, the potential for clinical application, for

example for tracking cell fate in cell-based therapies, is complicated by the need for genetic alteration, which is controversial.

From the point of view of imaging technology, although the photoacoustic imager used by Jathoul *et al.*[1] demonstrates an impressive performance, it comes with a price: the stepwise scanning and the point-by-point tuning of the laser wavelength for optimum sensitivity strongly limit the speed of operation, with scanning times of 6–8 minutes. The biological process of interest in this study is obviously much slower, however the long scanning time will inhibit the imaging of fast transients such as blood oxygenation in the brain, as well as the imaging of blood flow using contrast agents[6] or photoacoustic Doppler shifts[7], and it may compromise image quality in the case of background motion, for example, caused by breathing. Further developments of this approach into a multichannel version present a new challenge for the photonics community. ❐

Srirang Manohar, Aart van Apeldoorn and Wiendelt Steenbergen are at the MIRA Institute of Biomedical Technology and Technical Medicine, University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands.
e-mail: w.steenbergen@utwente.nl

References

1. Jathoul, A. P. *et al. Nature Photon.* **9,** 239–246 (2015).
2. Wang, L. V. (ed.) *Photoacoustic Imaging and Spectroscopy* (CRC Press, 2009).
3. Paproski, R. J., Heinmiller, A., Wachowicz, K. & Zemp, R. J. *Sci. Rep.* **4,** 5329 (2014).
4. Stritzker, J. *et al. Proc. Natl Acad. Sci. USA* **110,** 3316–3320 (2013).
5. Zhang, E., Laufer, J. & Beard, P. *Appl. Opt.* **47,** 561–577 (2008).
6. Razansky, D., Buehler, A. & Ntziachristos, V. *Nature Protoc.* **6,** 1121–1129 (2011).
7. Fang, H., Maslov, K. & Wang. L. V. *Phys. Rev. Lett.* **99,** 184501 (2007).