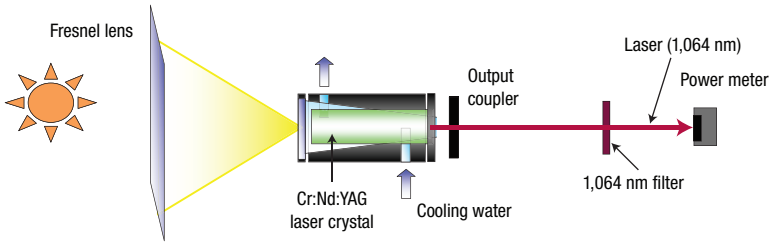

Figure 2 A schematic of the solar-powered laser design. A high-performance 1.3-m^2 Fresnel lens collects the light and a highly efficient, broad-absorption-band chromium-doped Nd:YAG ceramic laser gain medium enables most of the Sun's wide spectrum to be captured.

Before the team considers using the lasers for magnesium oxide treatment it is first optimizing its laser design and performing trials to see how the lasers perform in the field. It has recently installed three such solar-pumped lasers at a test bed near Chitose airport in Hokkaido, north Japan, and is monitoring their operation.

Yabe holds the opinion that once optimized his solar-pumped lasers could also compete with conventional Nd:YAG lasers and have wider applications than energy generation. He hopes to have a product on the market within the next 12 months. "A commercial 1-kW continuous-wave YAG laser sells for around ¥25 million [$200,000] in Japan, our solar-pumped lasers should cost an order of magnitude less."

Although solar-pumped lasers could potentially become important in the future it is important not to forget that they do have some obvious limitations — the main one being that they can't operate at night or during poor weather. However, according to Yabe, there is sufficient sunlight in Japan for them to run for up to 2–4 hours per day.

References

1. Yabe, T. *et al. Appl. Phys. Lett.* **90,** 261120 (2007).
2. Yabe, T. *et al. Appl. Phys. Lett.* **89,** 261107 (2006).
3. Landoa, M. *et al. Opt. Commun.* **222,** 371–381 (2003).
4. Ikesue, A., Kamata, K. & Yoshida, K. *J. Am. Ceram. Soc.* **78,** 2545–2547 (1995).
5. Krupin, V., Kagan, Y. & Yogev, A. *Proc. SPIE* **2016,** 50–60 (1993).

BIOPHOTONICS

Unravelling animal anatomy

An elegant approach to imaging the interior organs of small animals with improved visibility and accuracy looks set to help drug development and disease research.

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Imagine being able to accurately image all the major organs inside an animal and their relative positions by simply injecting the animal with a small amount of chemically inert fluorescent dye, illuminating it and analysing the fluorescence that is generated. This apparently improbable feat has now been accomplished by Elizabeth Hillman and Anna Moore on a mouse, as reported on page 526 of this issue[1].

In essence, the method — dynamic fluorescence molecular imaging (DFMI) — enables researchers to accurately determine the exact co-ordinates of the origin of the emitted fluorescence deep within the body, without resorting to additional computed tomography (CT) or magnetic resonance imaging (MRI) scans (Fig. 1). The speed and simplicity of DFMI combined with its use of low-cost instruments will surely endear the approach to many end-users.

Biophotonics imaging (BPI) has intrigued scientists for centuries and remains the basis of many diagnostic techniques in modern medicine. At the microscopic level, the wide use of photonics in biology and medicine stems from its high sensitivity, which enables the detection of minute amounts of light-emitting materials in living organisms.

Indeed, recent advances in high-resolution diffuse optical tomography (DOT) systems and algorithms now make it possible to resolve the uptake of molecular probes in target tissues that are within a few millimetres of the excitation light source. At such shallow depths (about 1 mm) BPI has a resolution superior to other imaging methods but unfortunately the resolution reduces sharply as the depth increases.

The cause of this unwanted drop in performance is that many types of tissue are strong absorbers or scatterers of light. As light impinges on tissue, light-absorbing molecules, such as proteins and skin pigments, rapidly attenuate it as it propagates, a factor that is defined in terms of the absorption coefficient. Penetration can be increased by using near-infrared (NIR) light in the wavelength region of 650–900 nm, where absorption of photons by constituents of the body is low. Unfortunately, cellular organelles scatter light intensely (including in the NIR region), a phenomenon resulting in the diffusion of light. Together, light scattering and absorption phenomena are responsible for the poor optical quality and resolution of deep-tissue imaging.

Algorithms and tomography systems have been developed to address this problem by modelling the propagation of photons in a heterogeneous medium. Mathematically, photon transport in tissue is modelled either by transport theory or other analytical descriptions. Typically, these theories assume that absorbing and scattering materials are uniformly distributed in tissue. However, this assumption is far from reality because different tissues interact with photons differently. In addition, fluorescent materials used in molecular optical imaging are known to perfuse on different timescales in different organs. What Hillman and Moore have done for the first time is to use this time-dependent correlation analysis of the fluorescence to perform accurate

deep-tissue imaging and map the internal organs of an animal.

The technique's arrival is timely. Recent developments in molecular medicine have been impressive and now require the development of imaging techniques to help exploit their full potential. For example, the availability of transgenic mice has enabled the development of a variety of small-animal models of human diseases. Central to the success of these models is the application of non-invasive BPI in drug development, detection of diseases and the monitoring of response to treatment[2,3].

A variety of high-resolution imaging methods such as DOT systems have been used to image molecularly targeted tissues such as tumours[4,5]. Although these approaches essentially provide high-resolution images of molecular targets in small tissue volumes, anatomical landmarks needed to localize the position of the fluorescent materials are typically obtained by using a complementary imaging method such as CT or MRI. The limitation is that obtaining accurate co-registration with these complex and expensive imaging methods is difficult and time consuming.

The beauty of the innovative all-optical approach of Hillman and Moore is that it obtains both molecular and anatomical information without the need for secondary imaging methods. Based on the well-established concept of perfusion imaging, Hillman and Moore use differential dynamics of inert optical imaging agents in tissues to delineate different organs in small animals.

The DFMI system consists of two glass mirrors angled at 45° relative to the mouse to provide simultaneous imaging of three orthogonal views. By using a CCD camera to image the dynamics of optical contrast agents injected through tail veins, the authors were able to extract anatomical information of various internal organs. Image processing was accomplished by principal component analysis (PCA) or a non-negative least-squares-fitting routine.

Although quantitative parameters were not determined — which is important in molecular imaging, striking images of internal mouse organs were obtained. It also appears that the DFMI method revealed additional anatomical features — small and large intestines — that are not readily visible in CT-generated scans of mouse anatomy. The technique also poses an additional bonus — it takes only 20 seconds to complete the imaging process.

The NIR fluorescent dye indocyanine green is approved for use in humans and provides a suitable inert dynamic contrast agent because it perfuses rapidly throughout all the organs of the body and quickly clears from the blood, adding to the flexibility and appeal of the technique. In addition to using the dynamic contrast imaging agent to delineate internal organs, the DFMI approach can exploit the differences in the transient retention of the contrast agent between diseased and surrounding normal tissues to detect diseased tissue, as reported by other investigators[6,7]. Furthermore, the ability to obtain instant images without the need to reduce background fluorescence provides a unique opportunity for real-time image acquisition.

Although other researchers have discriminated between tissue types based on dynamic optical methods[6–8], this study represents the first imaging demonstration of PCA and correlation analysis of an image series to map internal organs of small animals using dynamic contrast-enhancement agents. What is lacking at this stage is a specific application demonstrating the uses of the method in a realistic disease model. In the current form, the name DFMI might be considered inappropriate because its use in molecular as opposed to anatomical imaging has not yet been demonstrated.

When two dyes are used — one for molecular and another for anatomical imaging, it is possible that the accumulation of a target-specific molecular probe in specific tissues and organs will alter the dynamics or preclude the uptake of the added dynamic contrast agent in those regions, thereby potentially limiting the use of this technology for studies spanning long periods of time. Ideally, the use of a single imaging agent to delineate internal organs and localize target tissue would rapidly elevate the DFMI technology reported here to a preferred method for molecular optical imaging of small animals.

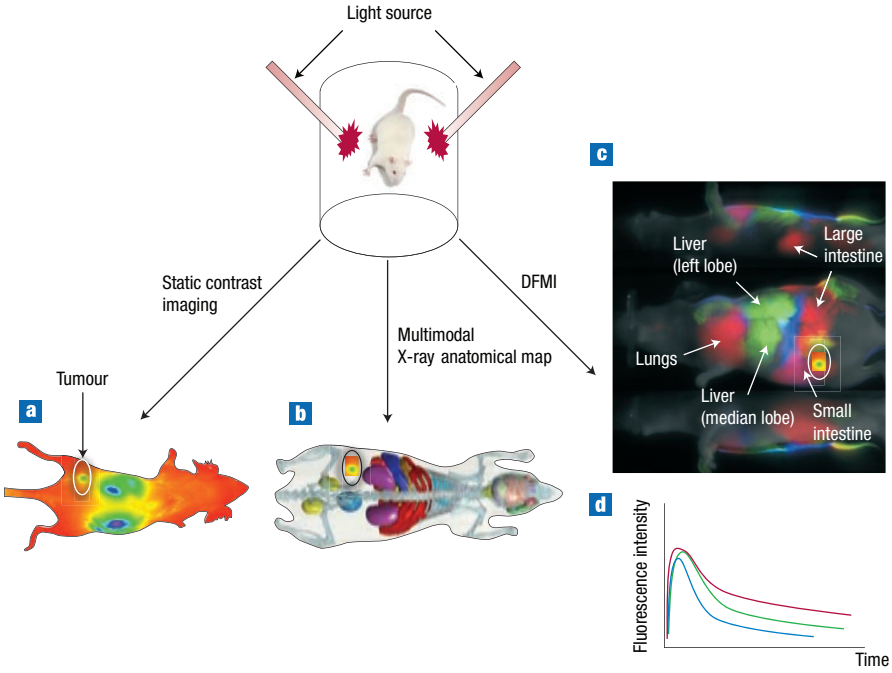

Figure 1 Tumour imaging. **a**, Static imaging, **b**, multimodal co-localization imaging and, **c**, all-optical DFMI. Dynamic fluorescence molecular imaging positions the tumour close to the small intestine, an organ that is not visible in the multimodal image. The image of the tumour from the static image was manually inserted at the corresponding anatomical regions in images **b** and **c** to illustrate co-localization of the molecularly targeted tumour with anatomy. Part **b** was adapted with permission from refs 1, 9. Copyright (2007) IOP. **d**, Perfusion rates of the fluorescent dye in different parts of the body. The colours correspond to the colours of the regions indicated in part **c**.

References

1. Hillman, E. M. C. & Moore, A. L. *Nature Photon.* **1,** 526–530 (2007).
2. Weissleder, R., Tung, C.-H., Mahmood, U. & Bogdanov, A. Jr *Nature Biotechnol.* **17,** 375–378 (1999).
3. Achilefu, S., Dorshow, R. B., Bugaj, J. E. & Rajagopalan, R. *Invest. Radiol.* **35,** 479–485 (2000).
4. Ntziachristos, V., Ripoll, J., Wang, L. V. & Weissleder, R. *Nature Biotechnol.* **23,** 313–320 (2005).
5. Patwardhan, S. V., Bloch, S. R., Achilefu, S. & Culver, J. P. *Opt. Express* **13,** 2564–2577 (2005).
6. Cuccia, D. J. *et al. Appl. Opt.* **42,** 2940–2950 (2003).
7. Gurfinkel, M., Ke, S., Wang, W., Li, C. & Sevick-Muraca, E. M. *J. Biomed. Opt.* **10,** 034019 (2005).
8. Barbour, R. L., Graber, H. L., Pei, Y., Zhong, S. & Schmitz, C. H. *J. Opt. Soc. Am. A* **18,** 3018–3036 (2001).
9. Dogdas, B., Stout, D., Chatziioannou, A. F. & Leahy, M. *Phys. Med. Biol.* **52,** 577–587 (2007).