The findings on nanotubes described by Bonner and co-workers are, therefore, fully consistent with the movement of a proportion of any deposited particle through the pleura. However, the fact that no carbon black was seen in the pleural tissues suggests that, in contrast to carbon nanotubes, these compact particles are small enough to pass rapidly through the pleura, into the pleural space fluid and out again through the pores in the chest wall. By contrast, the profusion of nanotubes visible in and around the pleura suggests that the movement of the nanotube aggregates is impeded, probably as a consequence of their fibrous or irregular shape. The relative bulk of the nanotubes may slow down their movement and cause them to lodge in the sensitive pleural tissue where they may then exert pathogenic effects over time (Fig. 1).

As more research comes to light we can look forward to further illumination of the behaviour of carbon nanotubes in the pleura, including their retention time and the role of factors such as fibre length or aggregate size. Along with actual measurements of nanotubes in the air of the workplace, such studies should culminate in rational risk assessment and safe management for those handling carbon nanotubes. ❐

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BIOIMAGING

Second window for *in vivo* imaging

Enhanced fluorescence from carbon nanotubes and advances in near-infrared cameras have opened up a new wavelength window for small animal imaging.

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Near-infrared light (700–2,500 nm) can penetrate biological tissues such as skin and blood more efficiently than visible light because these tissues scatter and absorb less light at longer wavelengths. Simply hold your hand in sunlight and your fingers will glow red owing to the preferential transmission of red and near-infrared light. At wavelengths longer than 950 nm, however, this effect diminishes owing to increased absorption by water and lipids. Nonetheless, a clear window exists at wavelengths between 650 nm and 950 nm for optical imaging of live animals[1,2] (Fig. 1). In practice, however, this window is not optimal because tissue autofluorescence produces substantial background noise and the tissue penetration depth is limited to between 1 and 2 cm (ref. 3).

In 2003, simulations and modelling studies[4] of optical imaging in turbid media such as tissue or blood suggested that it would be possible to improve signal-to-noise ratios by over 100-fold by using quantum dot fluorophores that emit light at 1,320 nm (instead of 850 nm). However, the lack of biocompatible fluorescent probes in this second near-infrared window between 1,000 and 1,350 nm has prevented the use of this highly sensitive spectral range for *in vivo* imaging.

On page 773 of this issue, Hongjie Dai and colleagues from Stanford University and Soochow University in China report a way to generate biocompatible fluorescent single-walled carbon nanotubes that emit between 950 and 1,400 nm (ref. 5). These bright

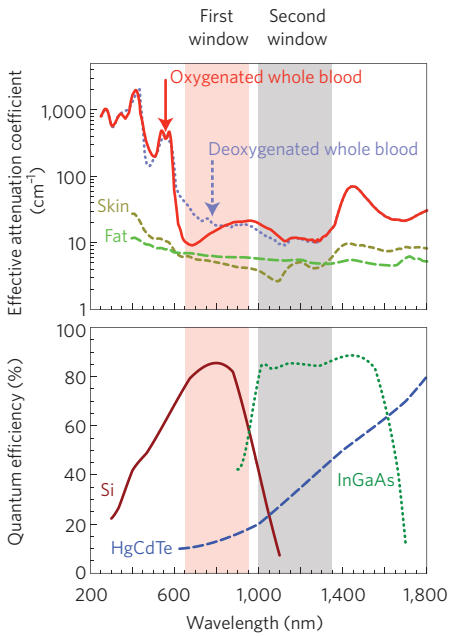

Figure 1 | Optical windows in biological tissues. Top: These plots of effective attenuation coefficient (on a log scale) versus wavelength show that absorption and scattering from oxygenated blood, deoxygenated blood, skin and fatty tissue is lowest in either the first (pink shaded area) or second (grey) near-infrared window[9,10]. Bottom: Sensitivity curves for typical cameras based on silicon (Si), indium gallium arsenide (InGaAs) or mercury cadmium telluride (HgCdTe) sensors. Si and InGaAs cameras are sensitive within the first and second near-infrared windows, respectively, whereas HgCdTe is most sensitive at longer wavelengths.

nanotubes allowed deep and highly sensitive *in vivo* imaging of blood vessels immediately beneath and through the deep layers of skin.

Single-walled carbon nanotubes are grown on solid substrates, so they must be detached, debundled and coated with a suitable hydrophilic layer before they can be used as soluble fluorophores. Dai and co-workers observed that the frequently used sonochemical method for coating the tubes with a layer of biocompatible phospholipids shortens the tubes and creates defects that quench the fluorescence. However, they found that the tubes remained fluorescent and intact if they were first coated with sodium cholate (a biological lipid) before replacing the cholate with the phospholipid-polyethylene glycol coating in a second step. This two-step process generated nanotubes that had a biocompatible surface coating and emitted stable and strongly enhanced fluorescence at near-infrared wavelengths.

When the exchanged nanotubes were injected into the bloodstream of normal and tumour-bearing mice, a detailed map of blood vessels (including the smaller ones inside the tumour) fluoresced brightly through the skin without any interference from background autofluorescence. Furthermore, a 15-times lower dose of nanotubes was needed to achieve such detailed signals when compared with nanotubes prepared using the direct sonochemical method. This work shows the advantages of fluorescence imaging in the second near-infrared window and has opened up new imaging possibilities. However, for

these nanotubes to be useful contrast agents, a complete study of their biodistribution and pharmacokinetics is necessary.

Early mice studies suggest efficient excretion of biocompatible carbon nanotubes[6], but the long-term fate of nanotubes that remain in the body is still largely unknown. Furthermore, the non-biodegradable nature of these particles and their needle-like structure is a concern because tissue damage and chronic toxicity have been observed in lungs following inhalation[7]. Nevertheless, the developments by the Stanford–Soochow team should help to resolve these controversies in the near future through sensitive and specific near-infrared fluorescence imaging.

Other promising contrast agents for *in vivo* optical imaging between 1,000 and 1,350 nm include semiconductor quantum dots and plasmonic nanoparticles such as gold nanorods. Unlike carbon nanotubes, the size and shape of these agents can be finely adjusted to modulate pharmacokinetics and biodistribution, and they also offer unmatched tunability in their optical properties. However, near-infrared quantum dots contain highly toxic semiconductor compounds (such as PbS, PbSe, InAs or HgTe) so their use for *in vivo* applications has been limited.

Optical imaging between 1,000 and 1,350 nm has also been impeded by the lack of sensitive and low-cost CCD (charge-coupled device) cameras. Silicon, the material most commonly used in CCD cameras, is not sensitive at wavelengths longer than 1,000 nm. Instead, near-infrared cameras use semiconductor alloys with narrower bandgaps such as InGaAs and HgCdTe. InGaAs cameras have a high quantum efficiency in the second near-infrared window, whereas HgCdTe cameras are available in higher-resolution arrays, but with a lower overall quantum efficiency. In general, these types of sensors are becoming more affordable with adequate sensitivity and resolution (0.1–0.3 megapixels) for most *in vivo* imaging applications.

Along with new near-infrared bioluminescence probes[8], the second near-infrared window offers a tremendous new opportunity for sensitive *in vivo* fluorescence imaging of small animals. However, translation to clinical applications will require researchers to demonstrate that these nanoprobes are biocompatible and that they can out-perform other more-established shorter-wavelength probes such as indocyanine. Nevertheless, the window of opportunity is large and remains to be explored. ❐

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