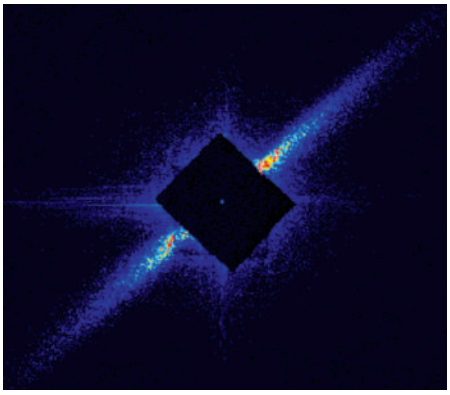
X-ray imaging for biological tissue

Proc. Natl Acad. Sci. USA **106,** 15297–15301 (2009)



Collagen has an important role in many biological tissues such as bone, tendon, teeth, skin and cartilage. Despite significant research efforts over the past five decades, the nanostructure of collagen is still not well known. Coherent X-ray diffraction imaging is a good candidate for addressing this issue, but it is limited as a result of radiation damage and the inability to invert coherent diffraction patterns from continuous objects under finite illumination from an aperture. Felisa Berenguer de la Cuesta and co-workers from University College London, UK, have got around this problem by incorporating X-ray ptychography into a coherent X-ray diffraction scheme. Ptychography is a technique that can solve the diffraction-pattern phase problem by interfering adjacent Bragg reflections coherently and thus determining their relative phase. It combines diffraction patterns from overlapping regions and often uses a raster scan of the coherent probe beam. In the scheme proposed by Berenguer de la Cuesta *et al.*, an X-ray beam of wavelength 0.14 nm was collimated down to 10 μm × 10 μm and incident on a rat tail tendon sample measuring 5 mm × 5 mm × 0.7 mm. The tendons contained micrometre-scale fibrils of collagen that were approximately 1 μm in diameter, tightly packed and well-aligned in the tissue. Owing to the array of collagen molecules within each fibril, speckle patterns at a resolution of 60–70 nm were obtained and found to be changing gradually as the beam was scanned across the tendon. Exposure time was fixed at 50 s to avoid thermal denaturation. By using a fast Fourier-transformation calculation, the team proved that these speckle patterns were associated with absorption and phase shifts in the sample. When inverted, the patterns show the disposition and orientation of the tissue.

High-resolution neuroimaging

Neuron **63,** 429–437 (2009)

Scattering, absorption and out-of-focus fluorescence impede the use of conventional confocal microscopy for deep-tissue imaging of the brain. Although a two-photon laser scanning microscope operating in the near-infrared with a high-numerical-aperture oil-immersion lens is able to perform this task, its resolution is limited. Now, Jun Ding and co-workers from Howard Hughes Medical Institute and Harvard University, USA, reveal that by exploiting stimulated emission depletion — a scheme for localizing fluorescence — the radial resolution of conventional two-photon laser scanning microscopes can be improved threefold to around 280 nm. The approach uses near-infrared photons to perform both one-photon stimulated emission depletion and two-photon excitation of a mouse brain containing the fluorophore Alexa Fluor 594. The team imaged whole-cell voltage-clamped neurons in acute brain slices of mice, and achieved greater detail than that of standard two-photon scanning laser microscopy in the structure of dendritic spines located ~100 μm below the surface of brain slices. Mismatches in the index of refraction between the immersion medium and the brain tissue were found not to deteriorate the annular illumination patterns formed at the focus spot. With efficient use of stimulated emission depletion power delivery, the researchers predict improvements in radial resolution to below 100 nm.

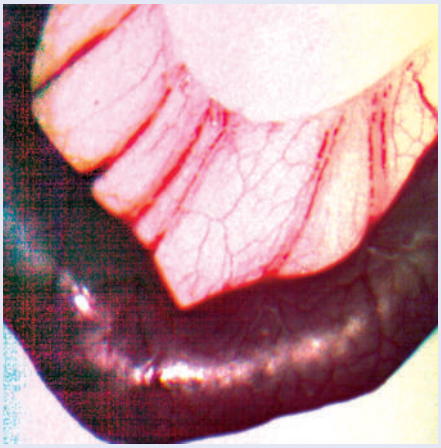
MEMS microscope images brains

Opt. Lett. **34,** 2309–2311 (2009)

Miniaturized, portable microscopes and endoscopes are desirable for biomedical and clinical imaging. The use of two-dimensional micro-electromechanical systems (MEMS) technology has allowed scientists in California and Thailand to build a two-photon microscope that is not much larger than a sugar cube. Designed and fabricated by Wibool Piyawattanametha and colleagues, the instrument is only 2.0 cm × 1.9 cm × 1.1 cm and weighs just 2.9 g. It comprises a focusing motor, a MEMS scanning mirror, a microprism and a collection of graded-index lenses. Optical fibres were used to excite the sample and collect the generated fluorescence. The microscope has a maximum field-of-view of 295 μm × 100 μm, and obtains images measuring 400 × 135 pixels. The team imaged the flow of erythrocytes in the brain

Colour endoscopy

Opt. Express **17,** 15239–15247 (2009)

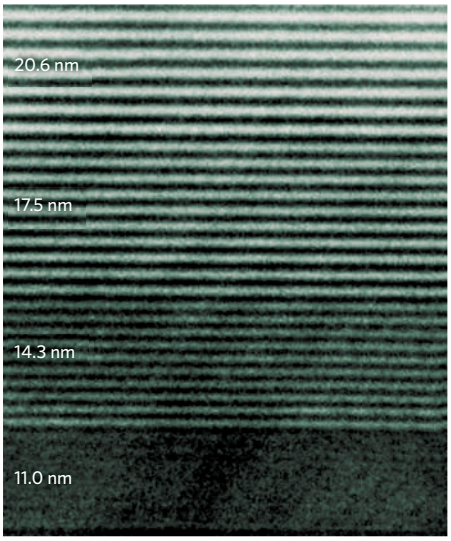


Endoscopy that uses scopes with a diameter of less than 1 mm could be a very useful tool for clinical diagnosis. The problem with current endoscopic imaging is that it is monochromatic, because its spectral bandwidth is used for the spatial encoding process. Thus, one can only view affected tissue by using a silhouette or light intensity method. The ability to observe colour changes would therefore benefit detection of the early stages of tissue abnormalities. Now, DongKyun Kang and co-workers have demonstrated a prototype of 'spectrally encoded endoscopy', which overlaps the red, green and blue spectral bands on the sample under test. A supercontinuum laser was used as the broadband light source, and this was first divided into three colours by wavelength-division optics. These three colours were then incident on a 2,400-lines-per-millimetre diffraction grating at different incident angles, resulting in a 75-nm bandwidth for each beam and thus allowing the spectral bands to overlap on the sample. A line-scan camera was used to generate two-dimensional 10-bit monochromatic images. Three samples — a colour test chart, a plastic doll and small intestine from a pig — were used to test the capability of the system. The researchers obtained clear and colourful images, reporting a resolution of around 110 μm. Although a coherent light source was used in the study, the team said that an incoherent light at lower cost can also be used, as long as sufficient light can be coupled onto the fibre.

of a live mouse and obtained transverse and axial resolutions of 1.3 μm and 10.3 μm, respectively. Applications are anticipated in physiological studies of animals or humans, and may also be useful for cellular-level diagnostics.

Exploiting higher-order diffraction

Phys. Rev. Lett. **103,** 110801 (2009)



Fresnel zone-plate microscopy relies on flat plates made of concentric rings (zones) of alternating transparent/opaque material to focus light by diffraction and constructive interference, rather than through conventional refractive lenses. The approach is particularly useful for investigating structures that have features that are beyond the resolution of conventional imaging or that are buried underneath a material opaque to visible wavelengths. Unfortunately, the resolution of zone-plate microscopy is limited by the width of the outermost zone, which is typically ~25 nm. Pushing the resolution limit down to around 10 nm, therefore, becomes a significant nanofabrication challenge. Stefan Rehbein and co-workers from Germany have overcome this problem by taking advantage of the fact that the numerical aperture of a Fresnel zone-plate is directly proportional to its diffraction order. In other words, third-order imaging, in theory, results in a threefold increase in resolution. Using the third-order diffraction from a zone-plate objective with 25-nm feature size, a real-space image resolution of 12.5 nm was achieved. Although there are trade-offs in efficiency and a decrease in focal length as higher diffraction orders are used, further improvements of the scheme may pave the way towards sub-10-nm soft-X-ray imaging.

Imaging magnetic vortices

Opt. Express **17,** 16160–16165 (2009)

Using the human eye to 'see' magnetism — particularly that of type II superconductors — would be a great benefit for researchers striving to understand the basics of magnetism, as well as for potential applications in data storage and spintronics. By harnessing the magneto-optical Kerr effect, researchers from Technion–Israel Institute of Technology have shown that it is possible to image individual vortices carrying quanta of magnetic flux at the surface of a type II superconductor. Daniel Golubchik and co-workers evaporated a 40-nm-thick film of europium selenide on a 200-nm-thick niobium superconducting film. They then illuminated the sample with 548-nm light from a 100 W mercury lamp, and imaged the reflected light using a CCD camera with a field of view of 110 μm × 160 μm. They report imaging with a spatial resolution of 0.8 μm, which is limited only by optical diffraction and is comparable to those of non-optical techniques such as electron microscopy or magnetic-force microscopy. The benefit of this optical approach is that it allows much faster measurements over significantly larger areas — imaging from one to several thousand vortices was demonstrated.

Golden promise

Nature Nanotech. **4,** 688–694 (2009)

Gold-plated carbon nanotubes (CNTs) can function as high-contrast agents for photoacoustic and photothermal imaging. However, owing to the low absorption of CNTs at near-infrared wavelengths and concerns over their toxicity, they are generally not used for *in vivo* applications. Now, Jin-Woo Kim and co-workers in the USA and Russia report how gold-plated CNTs can enhance near-infrared contrast by a factor of approximately 100 when imaging lymphatic vessels in mice with an 850-nm laser. The benefit of the gold-plating is that it allows a much lower concentration (pico- and femtomolar) of CNTs to be used for high-resolution, deep-tissue imaging. Although further investigation is needed, Kim *et al.* say that their preliminary data suggests that the gold-plated CNTs have minimal cytotoxicity. The team is now investigating the potential of these coated CNTs for the molecular detection and eradication of metastasis in sentinel lymph nodes, and for the real-time tracking of gold-plated CNTs in ear and skin samples.

Nonlinear nanosource

Nano Lett. doi:10.1021/nl901986g (2009)

Stefano Palomba and Lukas Novotny from the University of Rochester, USA, have demonstrated high-resolution near-field imaging and spectroscopy using a nonlinear nano-optical light source. Four-wave mixing at the junction of a metallic dimmer (consisting of two gold nanoparticles) was exploited as a highly localized, coherent and frequency-tunable source for high-resolution fluorescence imaging. To prepare the dimmer, the tip of an optical fibre was pushed onto a nanoparticle and the resulting combination was then pushed onto a second nanoparticle. The pulses focused onto the dimmer were provided by a Ti:Sapphire laser with a duration of ~200 fs, a repetition rate of ~76 MHz and wavelength tunable in the range of 740–821 nm. The laser also pumped an optical parametric oscillator providing wavelengths of 1078–1170 nm. By using a delay line between the two excitation pulses, the four-wave mixing at the dimmer could be turned on or off, because the nonlinear mixing process only occurs if the pulses are overlapping. The image was created by raster scanning the sample underneath the dimmer and detecting fluorescence, and the excitation efficiency was maximized by overlapping the four-wave mixing frequency with an absorption peak of the sample. The imaging is almost background-free because the sample does not by itself produce light at the four-wave mixing frequency.