those in an STM (120 keV compared with 2 eV), but they still induce similar changes in retinal. Liu and colleagues acquired the dynamic images of the Ret-C_{60} isomerization inside a nanotube with an electron dose of a few tens of coulombs per cm^2, which indicates that the high-energy electrons are actually more effective in inducing the *cis–trans* isomerization[5]. By confining the hybrid molecule in a nanotube they were able to restrict the motion of retinal to time scales accessible with TEM and to image the vibrational and rotational motion of the retinal. In order to reduce any type of artefact from prolonged electron-beam dosing, multiple images taken over the course of 5–20 seconds were time-averaged.

Nanotubes are convenient sample holders because a number of different types of atoms and molecules can be contained within them[6] and because they contribute a fairly small background. However, it is important to keep in mind that confining molecules inside nanotubes might also complicate the interpretation of results. For example, the molecules may interact with the inner wall of the nanotubes in a way that modifies their electronic structure. Furthermore, the structure of a molecule inside a nanotube may change as a result of strain.

The work of Liu and co-workers is the first direct observation of the dynamic behaviour of a conjugated carbon chain and it opens up the possibility of studying the role of retinal molecules in rhodopsins with atomic resolution. Moreover, as the molecules do not suffer noticeable electron-beam damage in TEM, the results suggest that it may be used to study other similar organic molecules.

The temporal resolution of this method is limited by the raster speed of TEM and is typically 0.03–0.04 seconds per frame, but it could approach 0.002 seconds per frame in a specially designed TEM. At this fairly slow imaging speed, not all molecular dynamics can be captured. However, by cooling the molecules or increasing the raster rate of TEM, intermediate states could be imaged. Combined with a laser excitation source, these improvements would allow the sort of pump–probe experiments that provide important feedback on our understanding of biochemistry.

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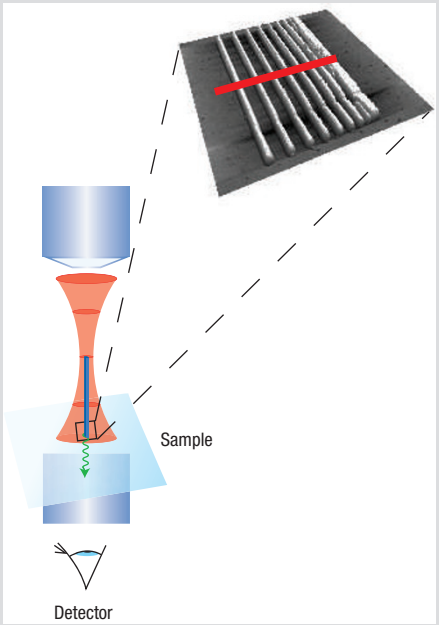
OPTICS

Nanowire lasers get in tune

A wish list of features for any new biological imaging technique would almost certainly include the ability to produce images with subwavelength resolution, a light source that can be tuned over a wide range of frequencies or wavelengths, and compatibility with physiological conditions. By combining and improving a number of existing optical technologies, Peidong Yang, Jan Liphardt and colleagues at the University of California at Berkeley and the Lawrence Berkeley National Laboratory have developed a new optical probe that just might fit the bill (*Nature* **447,** 1098–1101; 2007).

Researchers have been exploring the optical properties of nanowires made of semiconductors such as zinc oxide and gallium nitride for a number of years. When these nanowires are excited by an external light source, they can emit laser radiation — but only at certain frequencies. Being able to tune the output of nanowire lasers over a range of frequencies would significantly increase their usefulness, especially in subwavelength applications, which exploit the fact that nanowires have cross-sections that are much smaller than the wavelengths used in many imaging experiments.



The Berkeley team has now made a tunable nanowire laser from potassium niobate ($KNbO_3$) — a material that is well known for having nonlinear optical properties. When $KNbO_3$ is illuminated at two different input frequencies, it can produce light at four output frequencies, including the sum and the difference of the two input frequencies. Therefore, if a standard tunable laser provides one or both input signals, it will be possible to tune the output frequency of the nanowire laser.

Yang and co-workers perform a 'nanowire scanning microscopy' experiment to demonstrate the potential of the nanowire lasers (see image). Optical tweezers hold the nanowire (blue) in position (and also excite the lasing action in the $KNbO_3$) while a piezoelectric stage moves the sample so that it is scanned by the laser radiation (green) from the nanowire. The image is built up by measuring the amount of laser radiation that is transmitted by the sample as a function of position. The test sample (right) was a pattern of gold lines — 200 nm wide, 50 nm thick, with separation decreasing from left to right — and the line separations measured by the nanowire scanning microscope along the red line in the figure agreed with those recorded by an atomic force microscope to within ~10%.

In addition to acting as sources, the nanowires could also be operated as subwavelength photon detectors, and as they can work in sealed containers, there may also be applications in microfluidics.

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