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Turning the light up

We may now be able to view single cells while they are still within the body, says S.Ananthanarayanan.

Microscopy has taken great strides, but it has not been kind to the specimens under view. The specimens need to be sliced, stained, dried or otherwise prepared and placed under the objective lens, in a device thousands of times larger, and often they are destroyed by the radiation that is used.

But a group of scientists working at the Macquarie University, Sydney, the University of Adelaide, in Australia and the Shanghai Jio Tong University, Shanghai and the Peking University, Beijng, China report advances in preparation of nanocrystals, which could help single cells light themselves into view at the end of a glass fibre, which could be inserted into the body, to see that cell right where it is. Jiangbo Zhao, Dayong Jin, Erik P. Schartner, Yiqing Lu, Yujia Liu, Andrei V. Zvyagin, Lixin Zhang, JudithM. Dawes, Peng Xi, James A. Piper, Ewa M. Goldys and Tanya M. Monro report in the journal, ***Nature Nanotechnology***, that they have found that using stronger lighting than previously attempted allows use of nanoparticles that glow much more brightly to help single cells to be spotted and traced.

One challenge in normal microscopy is that we not only need to see the faint light that comes from the object we want to study, but also to keep out the glare from neighbouring objects. While using light of higher frequencies, like blue or violet, could help build more sensitive microscopes, this kind of light is more energetic and could also damage the delicate cells that it shines upon.

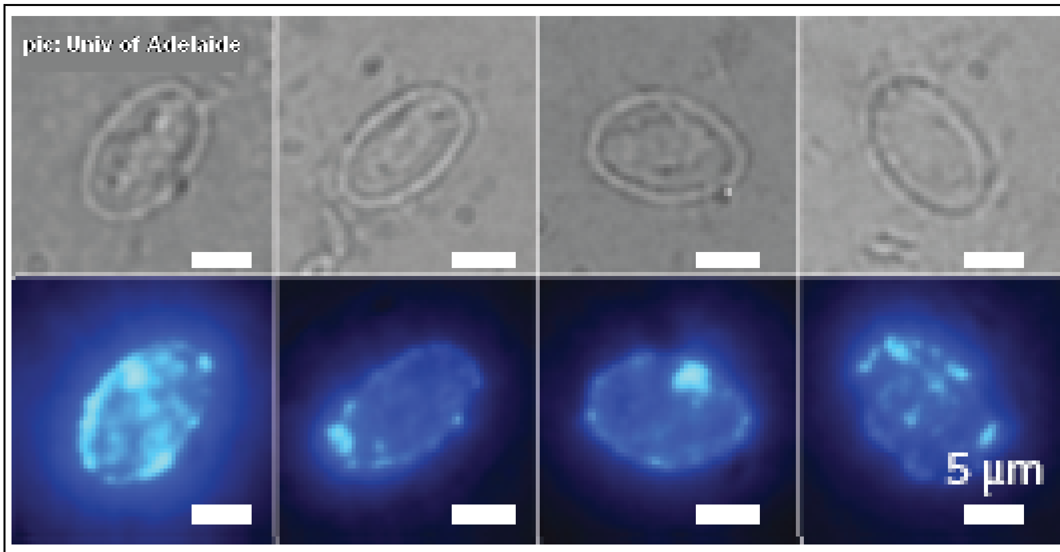
One way out has been the idea of introducing into the cell, minute, artificial, nanoparticles, which would glow in visible colours when the cells and surroundings are bathed in low energy, infra-red light. The glow would be readily captured in the microscope and the IR illumination would be gentle on the subjects of view. What is more, the surrounding material would not glow in IR light, and there would be no glare.

This effect, of giving off red, green or blue light when bathed in the infra-red, looks like the opposite of fluorescence, where we get of a lower frequency than used to start with. The effect, which is called ***upconversion***, is quite different and involves semiconductor materials and laser light. In floursecence and the laser, an atom absorbs a photon of light and then emits, after some time, the same photon, or a less energetic one. But in upconversion, an atom that is in an excited state, having absorbed a photon, gets into a yet higher energy state by absorbing more energy. Now, when it de-excites, it gives off a high energy photon of shorter wavelength.

The elements whose atoms allow this effect fall in a group where the penultimate electron shell of the atoms are in the process of getting filled. These, ***inner shell*** electrons have energy states to or from which they can move with transfer of energy either by radiation or by passing the energy on to another atom in the crystal lattice. This kind of transfer of energy to a neighbor, without radiation, is actually a problem in designing lasers, because in lasers, all de-excitation should be emission, stimulated by a photon emitted by another atom. But where it happens, it could become useful, like in generating visible light under illumination by low energy, IR radiation.

The trouble with using this effect to any good has been that the light emitted has been too faint to be of use. We now have sophisticated methods of creating nanocrystals of semiconductor materials with the correct ‘doping’ impurities, which would make the material conduct, or act as a laser, or to select upconversion colours. But, there has been no success in increasing the emission of light by upconversion. Typically, doping atoms like ytterbium (Yb^{3+}) are the agents that absorb infra-red radiation and transfer the energy, without radiation, to atoms of erbium (Er^{3+}), or thulium (tm^{3+}) or holmium (Ho^{3+}). But optimising the geometry of the nanocrystals, for brighter emission, has always fallen short of overcoming an intrinsic roadblock, which arises from clustering of dopant ions. If the density of the dopants is increased beyond an optimum level, an effect known as concentration quenching sets in and the brightness of emission decreases. At weak excitation radiation, of less than 100 watts per square cm, the best level of doping has been 0.2 to 0.5 %.

The Aussi-Chinese group experimented with much stronger excitation radiation, an area that has not been explored so far, and found that a different balance sets in between the radiation, the dopants and the upconverting atoms, which overcomes concentration quenching. As a result, under strong illumination, a nanocrystal doped with high levels of Tm -8% and Yb-20% could make use of easier access to radiation and non-radiative energy transfer and could give off very bright emission, an increase of a factor of 70. A single nanocrystal could then be remotely detected, using a optical fibre.



Cells of Giardia Lamblia, a small intestine parasite, labelled with upconverting nanocrystals and viewed in tranmission(upper panel) and luniniscence(lower)

"Up until now, measuring a single nanoparticle would have required placing it inside a very bulky and expensive microscope," says Professor Tanya Monro, Director of the University of

Adelaide's Institute for Photonics and Advanced Sensing (IPAS) and ARC Australian Laureate Fellow. "For the first time, we've been able to detect a single nanoparticle at one end of an optical fibre from the other end. That opens up all sorts of possibilities in sensing."

"Using optical fibres we can get to many places such as inside the living human brain, next to a developing embryo, or within an artery – locations that are inaccessible to conventional measurement tools. This advance ultimately paves the way to breakthroughs in medical treatment. For example, measuring a cell's reaction in real time to a cancer drug means doctors could tell at the time treatment is being delivered whether or not a person is responding to the therapy," says Prof Monro.

"Material scientists have faced a huge challenge in increasing the brightness of nanocrystals," says Dr. Jin, ARC Fellow at Macquarie University's Advanced Cytometry Laboratories. "Using these optical fibres, however, we have been given unprecedented insight into the light emissions. "The trans-disciplinary research from multiple institutions has paved the way for this innovative discovery," says Jin, "with the interface of experts in nanomaterials, photonics engineering, and biomolecular frontiers,." Dr Jin adds.