Turning the light up

WE MAY NOW BE ABLE TO VIEW SINGLE CELLS WHILE THEY THEY ARE STILL WITHIN THE BODY, SAYS **S ANANTHANARAYAN**

Dr Dayong Jin and Professor Tanya Munro

Microscopy has taken great strides but it has not been kind to the specimens under view, all of which 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 used. But a group of scientists working at Macquarie University in Sydney, University of Adelaide in Australia, Shanghai Jio Tong University and Peking University in Beijing report advances in the preparation of nanocrystals that 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, Judith M 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 the 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 infra-red illumination would be gentle on the subjects of view. What is more, the surrounding material would not glow in infra-red 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 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 a 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 neighbour, 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, infra-red radiation.

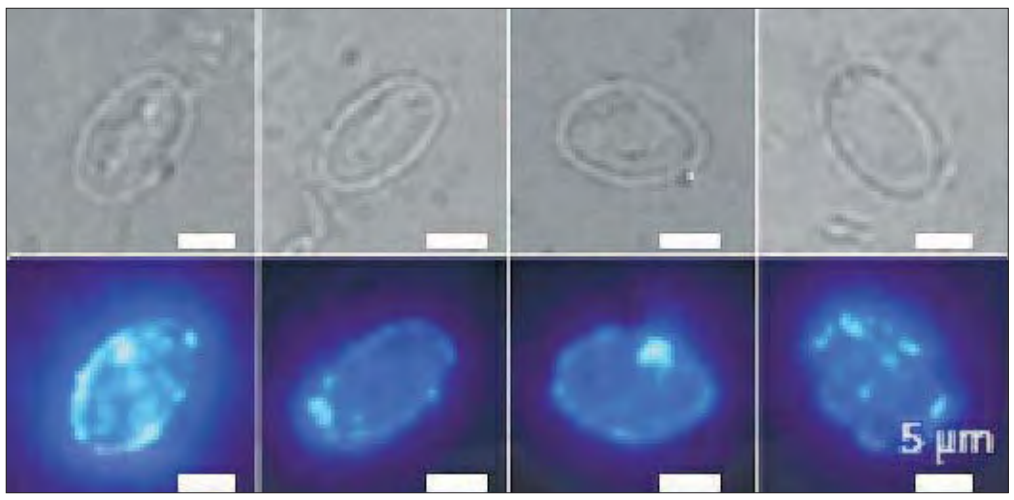
The trouble with using this effect to any good has been that the light emitted has been too faint to be of use. We now have sophisticated methods of creating nanocrystals of semiconductor materials with the correct "doping" impurities, which would make the material conduct, or act as a laser, or to select 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 a 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 centimetre, the best level of doping, has been 0.2 to 0.5 per cent.

The Aussie-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 (eight per cent) and Yb (20 per cent) 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 an optical fibre.

"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 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," she says.

"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 with the interface of experts in nanomaterials, photonics engineering and biomolecular frontiers," Jin adds.



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

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PLUS POINTS

Cosmic caterpillar

The National Aeronautics and Space Administration's Hubble telescope has spotted a light year-long cloud of interstellar gas and dust that has been dubbed a "Cosmic Caterpillar". And like its namesake, it is in the middle of a metamorphosis, with scientists eagerly watching to see what manner of beast will finally emerge.

The head of the object where the matter is most dense is a protostar, an early stage in the evolutionary life of the star, where dense spots of matter slowly attract more material through gravity to become young stars. However, astronomers are doubtful that the caterpillar will ever pull itself together and gather enough material to become a star in its own right.

Standing in the way are 65 O-type stars

The caterpillar-shaped trail of interstellar gas and dust is a protostar in an early evolutionary stage. What it might develop into is yet to be seen.

— the brightest type of stars known to scientists. These objects, seen to the right of the image, are busily blasting the caterpillar with ultraviolet radiation, smearing its matter across space and sculpting out its current shape.

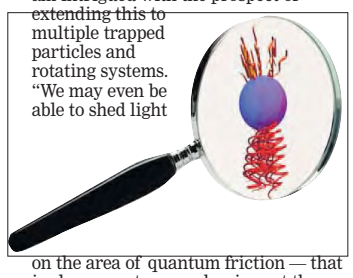
These 65 O-types are part of the Cygnus OB2 association, a cluster of over 500 stars (mostly comprised of the less-luminous B-type) with a total mass 30,000 times that of our sun.

The caterpillar — also known by its less catchy designation, IRAS 20324+4057 — might still gather material quicker than it is losing it, but scientists are unsure of the final outcome. The whole cosmic drama is taking place 4,500 light years away in the Cygnus constellation.

JAMES VINCENT/THE INDEPENDENT

At 600 million rpm

A team of researchers from the University of St Andrews claims to have created the world's fastest spinning man-made object. They were able to levitate and spin a microscopic sphere in a vacuum using only laser light. The sphere rotated at speeds of 600 million revolutions per minute before it broke apart and disappeared. In comparison, this speed of rotation is half a million times faster than the spin speed of a washing machine and more than a thousand times faster than a dentist's drill. "This is an exciting, thought-provoking experiment that pushes the boundary of our understanding of rotating bodies," said Dr Yoshihiki Arita, one of the scientists involved in the project. "I am intrigued with the prospect of extending this to multiple trapped particles and rotating systems. "We may even be able to shed light



on the area of quantum friction — that is, does quantum mechanics put the brakes on the motion or spinning particle even though we are in a near perfect vacuum with no other apparent sources of friction?"

As well as Dr Arita, the work was undertaken by Dr Michael Mazilu and Professor Kishan Dholakia of the School of Physics and Astronomy and published in the international journal *Nature Communications*.

The sphere itself was only four-millionths of a metre in diameter and was constructed from calcium carbonate. It was held in place by the "miniscule forces" of radiation produced by the laser — a phenomenon similar to balancing a beach ball on a jet of water. The team then spun the sphere by using the laser's polarisation to exert a small twist or torque. The vacuum conditions that the microscopic sphere was suspended in largely removed the drag (friction) that would have been present in a gas environment, allowing the team to achieve such a high rate of rotation.

"The rotation rate is so fast that the angular acceleration at the sphere surface is a billion times that of gravity on earth's surface – it's amazing that the centrifugal forces do not cause the sphere to disintegrate!" said Dr Mazilu.

Professor Kishan Dholakia said, "The team has performed a real breakthrough piece of work that we believe will resonate with the international community. In addition to the exciting fundamental physics aspects, this experiment will allow us to probe the nature of friction in very small systems, which has relevance to the next generation of microscopic devices. And it's always good to hold a 'world record' — even if for only a while!"

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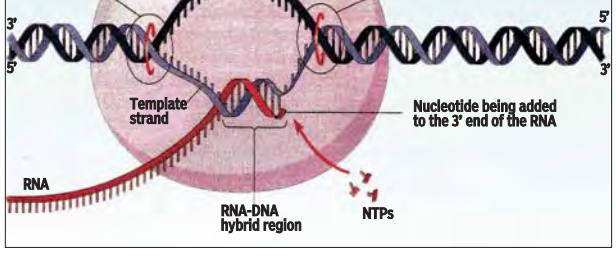
A PROOFREADING MECHANISM

TAPAN KUMAR MAITRA EXPLAINS PROKARYOTIC ELONGATION OF THE RNA CHAIN AND TERMINATION OF RNA SYNTHESIS



A close-up of the Prokaryotic Elongation Complex.

Chain elongation continues as the RNA polymerase moves along the DNA molecule, untwisting the helix bit by bit and adding one complementary nucleotide at a time to the growing RNA chain. The enzyme moves along the template DNA strand from the 3' toward the 5' end. Because complementary base pairing between the DNA template strand and the newly forming RNA chain is antiparallel, the RNA strand is elongated in the 5'→3' direction as each successive nucleotide is added to the 3' end of the growing chain. (This is the same direction in which DNA strands are synthesised during DNA replication.)

As the RNA chain grows, the most recently added nucleotides remain base-paired with the DNA template strand, forming a short RNA-DNA hybrid about eight-nine bp long. As the polymerase moves forward, the DNA ahead of the enzyme is unwound to permit the RNA-DNA hybrid to form and the DNA behind the moving enzyme is rewound into a double helix. The supercoiling that would otherwise be generated by this unwinding and rewinding is released through the action of topoisomerases, just as in DNA replication.

Like DNA polymerase, some RNA polymerases possess 3'→5' exonuclease activity that allows them to correct mistakes by removing improperly base-paired nucleotides from the 3' end of a growing RNA chain immediately after an incorrect base has been incorporated. The result is an RNA proofreading mechanism for correcting transcriptional errors that is analogous to the DNA proofreading mechanism for correcting DNA replication errors. Occasional errors in RNA synthesis are not as critical as errors in DNA replication because numerous RNA copies are transcribed from each gene and so a few inaccurate versions can be tolerated. In contrast, only one copy of each DNA molecule is made when DNA is replicated prior to cell division. Since each newly forming cell receives only one set of DNA molecules, it is crucial that the copying mechanism used in DNA replication be extremely accurate.

Elongation of the growing RNA chain proceeds until RNA polymerase copies a special sequence, called a termination signal, which triggers the end of transcription. In bacteria, two classes of termination signals can be distinguished that differ in whether or not they require the participation of a protein called rho (r) factor. RNA molecules terminated without the aid of the rho factor contain a short GC-rich sequence followed by several U residues near their 3' end. Since GC base pairs are held together by three hydrogen bonds, whereas AU base pairs are joined by only two hydrogen bonds, this configuration promotes termination in the following way: first, the GC region contains sequences that are complementary to each other, causing the RNA to spontaneously fold into a hairpin loop that tends to pull the RNA molecule away from the DNA; and then the weaker bonds between the sequence of U residues and the DNA template are broken, releasing the newly formed RNA molecule.

In contrast, RNA molecules that do not form a GC-rich hairpin loop require participation of the rho factor for termination. Genes coding for such RNAs were first discovered in experiments in which purified DNA obtained from bacteriophage λ was transcribed with purified RNA polymerase. Some genes were found to be transcribed into RNA molecules that are longer than the RNAs produced in living cells, suggesting that transcription was not terminating properly. This problem could be corrected by adding rho factor, which binds to specific termination sequences 50-90 bases long located near the 3' end of newly forming RNA molecules. The rho factor acts as an ATP-dependent unwinding enzyme, moving along the newly forming RNA molecule toward its 3' end and unwinding it from the DNA template as it proceeds.

Whether termination is rho-dependent or depends on the formation of a hairpin loop, it results in the release of the completed RNA molecule and of the core RNA polymerase. The core polymerase can then bind sigma factor again and reinitiate RNA synthesis at another promoter.

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Who's afraid of tiny holes?

IF YOU HAVE AN AVERSION TO ANYTHING THAT MAKES YOUR SKIN CRAWL, YOU MAY HAVE TRYPOPHOBIA, WRITES **ROGER DOBSON**

If the photo of the seedhead of a lotus flower makes your skin crawl, you may well have a phobia about holes. Researchers have discovered that a substantial minority of Britons suffer from trypophobia, the largely unstudied fear of clusters of tiny holes. The sight of these small, irregularly or asymmetrically placed holes can make people sick, itch, shake and even cry, according to the results of the first academic study into the phenomenon.

Although the phobia is thought to be a relic of an evolutionary survival mechanism that associates such patterns with dangerous animals, the researchers noted that some people have an aversion to obviously innocuous holes — such as those formed by soap bubbles or found in aerated chocolate, like Aero bars.

"It is quite extraordinary that images of something as innocuous as the bubbles in a bar of chocolate can bring about this level of aversion," said psychologists Dr Geoff Cole and Professor Arnold Wilkins of Essex University, whose interest was sparked when a colleague reported having the phobia.

"We have found that significant numbers of people are affected, and that others, who would not be classed as having a phobia, have a dislike of those same images. That supports our theory that it is an evolutionary defence mechanism. For many people, trypophobia affects their everyday lives and can be quite debilitating." In the study, researchers carried out a number of experiments with different images to estimate the prevalence of trypophobia. They exposed 300 men and women aged 18 to 55 years to an image of the seedhead of the lotus flower. It was chosen because it was found to be the most often reported trigger for the phobia.

Results show that 18 per cent of the women and 11 per cent of the men had an aversion to the image; they found it uncomfortable or repulsive. The researchers also examined images of potentially dangerous animals and showed that they, too, could be trypophobic. The blue-ringed octopus, box jellyfish, the Brazilian wandering spider, the death stalker scorpion, inland Taipan snake, king cobra, marbled cone snail, poison dart frog, puffer fish and the stone fish all possess a pattern or body shape similar to that in the trypophobic images.

The researchers suggest that during evolution, specific patterns became a rapid identifying feature for danger, in the form of poisonous animals. While many people feel uncomfortable looking at these images, those with a phobia have an exaggerated response. "We argue that trypophobia arises because the images and objects share a simple visual property with potentially dangerous objects," the researchers said. "We have found that images responsible for a previously undescribed but relatively common form of visual phobia possess a property characteristic of images that are generally uncomfortable to view. Importantly, we have also found that images of animals well known to be dangerous also possess this visual property.

"We therefore suggest that trypophobia arises because the inducing stimuli share a core spectral feature with such organisms. This feature does not reach conscious awareness, but it induces aversion because of the survival value of such aversion."

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Aero bars can trigger trypophobic responses