

# Taking turns in the spotlight

MORE USES HAVE BEEN FOUND FOR TAGGING CELLS WITH NANOPARTICLES, SAYS S ANANTHANARAYAN

Just a few months ago, the journal *Nature Nanotechnology* carried a report of getting nanoparticles that are embedded in living cells to glow strongly so that single cells could be made out. Some members of the same group of scientists, with others, now report an improvement — to get different nanoparticles to flash at different times after excitation so that they could be told apart. This is a feature that can be used in many fields — to mark and monitor different kinds of individual cells, in creating fine grained data storage or even multiplying the unique features of documents like currency notes or credit cards to discourage counterfeits.

Yiqing Lu, Jiangbo Zhao, Run Zhang, Yujia Liu, Deming Liu, Ewa M Goldys, Xusan Yang, Peng Xi, Anwar Sunna, Jie Lu, Yu Shi, Robert C Leif, Yujing Huo, Jian Shen, James A Piper, J Paul Robinson and Dayong Jin, in Australia, Beijing, Shanghai, California and Indiana, report in the journal *Nature Photonics* that they have been able to tune the time it takes for nanoparticles to decay, that is, to emit light after excitation, to vary from 25 microseconds to

662 microseconds. Differently tuned nanoparticles would then glow after their individual decay times and allow observers to tell them apart.

The technique is one way of using the same medium to carry more than one message. The idea has been refined in the field of telecommunication, where a single copper wire carries currents alternating at different frequencies. The way speech, or even data, is transmitted is by loading the signal on to a high frequency carrier wave, either over a copper wire or even a radio wave. Different streams of voice or data are loaded on different frequencies and sent out together. Receivers can then separate the different carriers, using frequency filters, and detect the different messages. The technique is called *multiplexing* and is routine in telecommunication, with huge numbers of frequencies loaded on a single optical fibre cable, for instance. And there are different kinds of multiplexing.

In the life sciences, the idea of multiplexing is used for detecting and counting members of different species at the same time. To work out the genetic features of a person's DNA, to create a tailor-made therapy for that person, for instance, each genetic feature would need to be identified in a series of scans of the DNA. But if the different features of interest could be differently labelled, the whole task could be done in a single scan. In data storage, the limitation is the number of storage elements in a given space. Now, if each element were capable of carrying more than one kind of mark, then that many different sets of data could be stored using the same number of elements. In security printing of documents, a device is to add features over and above the simple printed matter — some mark that glows in ultra violet light, for example. A genuine document can then be identified by viewing in UV light — and the more such features there are, the greater the security.

"Multiplexing typically requires a matrix of optical codes, ideally carried by nano- or micro-sized objects, each of which should be accurately identifiable at high speed and at low cost," say the authors in the paper. A useful way of multiplexing is *fluorescence*, a phenomenon where atoms in materials are excit-

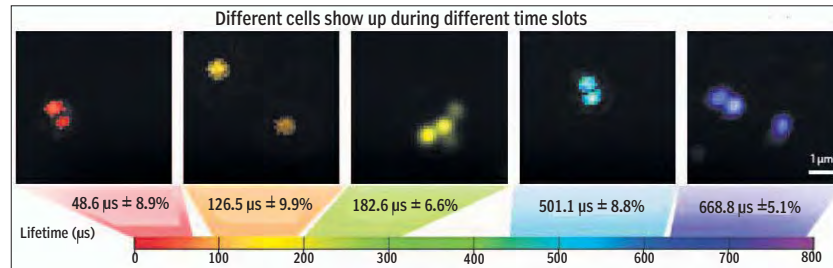
ed by absorption of light of a particular colour and then emit light, usually at a lower frequency. The best-known example is the *fluorescent lamp*, or the *tube light*, where the coating on the lamp absorbs ultra violet light and gives off nearly white light.

Using different fluorescent markers has been

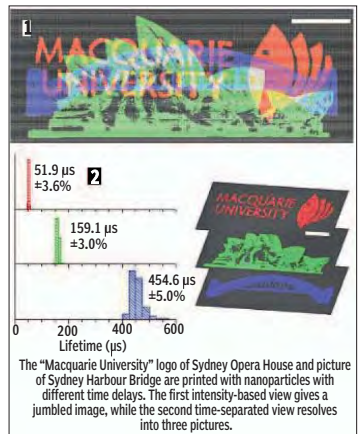
made use of.

A problem in these methods, of getting target objects to give off different unique signals, is that the glare of the original, incident radiation, obscures the faint light that has to be detected. One way out has been through the phenomenon of *upconversion*, which is where nanoparticles absorb more than one photon of the incident light and then emit at a higher, as opposed to a lower frequency. In this way, the original light can be in the infra-red but the emitted light in the visible region, which would eliminate visible glare. The work that the multinational group had last reported was to engineer the nanoparticles to give off a strong enough unconverted signal to be useful.

In the current paper, the group reports a way that upconverted light can provide a further dimension of multiplexing. The manner of using upconversion nanoparticles has been with crystals of sodium-yttrium fluoride



a popular way of multiplexing in detecting cells in biology, since some decades. But the method has the limitation, the authors note, of not more than about 20 colours being clearly usable, and the method calls for three to five lasers, a large number of filters and as many detectors as colours. There is, hence, the need for other means of coding biological tissue and different kinds of spectral analyses and even time delays in fluorescence are being

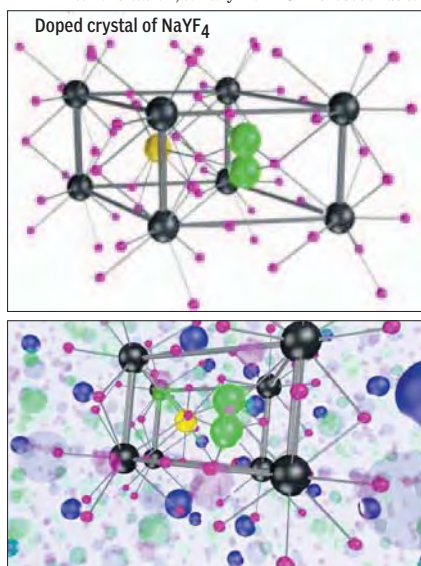


( $\text{NaYF}_4$ ) which have been doped with atoms of yttrium and thulium. Ytterbium is the *sensitiser* that absorbs a photon of light and transfers energy, without radiation, to thulium atom *emitters*. While the earlier work was to find optimum concentration of sensitiser and emitter, with strong excitation radiation, for the strongest emitted signal, the current work has been to modify the sensitiser-emitter concentrations to vary the distance between the two kinds of atoms in structure of the  $\text{NaYF}_4$  crystal, and, hence, to vary the time between excitation and emission.

The group reports nanoparticles that emit blue light with delays of a wide range, from 48 microseconds to 668 microseconds. Different kinds of cells can then be embedded with nanoparticles that have different delays, and then detected, not by the different colours emitted but by the emissions at different times after the excitation. The detection during separate time slots also serves to eliminate background light and the method has proved to be sensitive and capable of single nanoparticle detection.

The first picture shows samples of different kinds of cells detected with the help of nanoparticles that emit with different delay. The second picture is of three images embedded in the same document, and each image becoming visible according to the time window in which the document is viewed.

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## SELECTIVE SHUTDOWN

TAPAN KUMAR MAITRA EXPLAINS RNA INVOLVEMENT IN SILENCING THE EXPRESSION OF GENES CONTAINING COMPLEMENTARY BASE SEQUENCES

Regulatory proteins that bind to specific mRNAs — as is the case with the IRE-binding protein — are not the only molecules used by cells to control mRNA activity. Individual mRNAs can also be controlled by a special class of short RNA molecules that inhibit the expression of those mRNAs that contain sequences related to that of the short RNAs. Such RNA-mediated inhibition, known as RNA interference, is based on the ability of short RNAs to trigger mRNA degradation, or inhibit mRNA translation, or inhibit transcription of the gene coding for a particular mRNA.

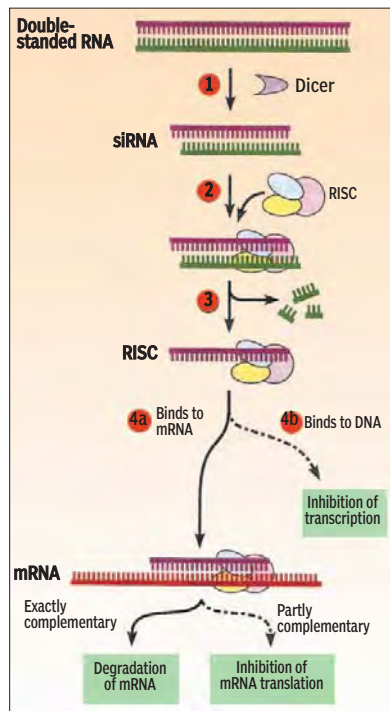
The first type of RNA interference to be discovered occurs as a response to the introduction of double-stranded RNA. For example, if plants are infected with viruses that produce double-stranded RNA as part of their life cycle, the RNA interference mechanism shuts down expression of the viral genes and thereby limits viral infection. Moreover, the effect is not limited to viral genes. If a virus is genetically engineered to contain a normal plant gene, cells infected with the virus shut down expression of their own normal copy of the same gene.

The mechanism that allows a double-stranded RNA to silence the expression of specific genes is illustrated. First, a ribonuclease known as Dicer cleaves the double-stranded RNA into short fragments about 21-22 base pairs in length. The resulting double-stranded fragments, called siRNAs (small interfering RNAs), are then combined with a group of proteins to form a complex known as RISC (RNA-induced silencing complex). After being incorporated into a RISC, one of the two strands of the siRNA is degraded. The remaining single-stranded RNA then binds the RISC via complementary base pairing to a target mRNA molecule.

If pairing between the siRNA and the mRNA is a perfect match (or very close), the mRNA is degraded by Slicer, a ribonuclease component of the RISC that cleaves the mRNA in the middle of the complementary site. If the match between the siRNA and mRNA is imperfect, translation of the mRNA may be inhibited without the mRNA being degraded. And, in some cases, the RISC may enter the nucleus and be guided by its siRNA to complementary nuclear DNA sequences. After associating with these gene sequences, the RISC silences their expression by stimulating DNA methylation and/or recruiting an enzyme that adds methyl groups to histones, thereby triggering the formation of a transcriptionally inactive, condensed form of chromatin (heterochromatin).

RNA interference may have originally evolved to protect cells from viruses that utilise double-stranded RNA. However, it also turns out to be a powerful laboratory tool that allows scientists to selectively shut down any gene they wish to study. Since complete genome sequences are now available for a variety of organisms, the function of each individual gene can be systematically explored by using RNA interference to turn it off.

Researchers simply synthesise (or purchase) short siRNAs that are complementary to sequences present in the genes they wish to silence. Introducing these synthetic siRNAs into cells allows individual genes to be turned off one at a time. To illustrate the extraordinary power of



When a cell encounters double-stranded RNA (1), the enzyme Dicer cleaves the double-stranded RNA into siRNAs about 21-22 base pairs in length. The resulting siRNA (2) is combined with RISC proteins and one of the two RNA strands (3) is degraded. The remaining siRNA strand then binds the RISC via complementary base pairing to a target mRNA molecule (4a) in the cytoplasm or to a target DNA sequence (4b) in the nucleus, thereby silencing gene expression at either the translational or transcriptional level. The most common situation (indicated by the solid arrows) is an exact complementary match between the siRNA and a corresponding mRNA, which triggers mRNA degradation by Slicer, an enzyme component of the RISC.

this approach, synthetic siRNAs have already been used to individually turn off almost all of the 19,000 genes in the worm *C. elegans*.

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## Galileo's puzzle solved

A VISUAL ILLUSION THAT MAKES VENUS LOOK BIGGER THAN JUPITER HAS FINALLY BEEN EXPLAINED AFTER 400 YEARS, WRITES STEVE CONNOR

Scientists have finally come up with an explanation for a visual illusion that was first identified in the 16th century by Galileo Galilei who noticed how large the planet Venus appeared to the naked eye when compared to Jupiter — which is quite the reverse when seen through a telescope.

Venus is nearer to earth than Jupiter and therefore appears brighter in the night sky; however this alone cannot account for its larger-than-life appearance. There must be another reason to do with the way the eye perceives light compared to the optical reality of a telescope, scientists said.

Viewed directly with the naked eye, Venus appears to have a "radiant crown" which makes it look eight to 10 times bigger than Jupiter even though Jupiter is four times larger when seen from earth.

Galileo was the first to realise this radiant crown was something to do with human perception, or, as he described it, an "impediment of our eyes" which the telescope eliminated, but he put it down to some kind of optical interference to the light from the planets as the light entered the human eye.

However, scientists have now shown that the effect is caused by the way the light-sensitive cells at the back of the eye respond to images of different intensity set against a dark background. Venus appears larger because its brighter-than-Jupiter image is much exaggerated by the visual centres of the brain to create a bigger radi-

ant crown than Jupiter's, according to the study published in the journal *Proceedings of the National Academy of Sciences*.

"They believe the effect influences the way we see everything because the human retina and brain are finely tuned to respond to the contrast between light objects against a dark background. This made them appear larger than light objects of the same size set against a light background," said Jose-Manuel Alonso of the State University of New York College of Optometry.

"Galileo was the first to say that our eye was distorting reality. He could see that Venus appeared to be much larger than Jupiter when seen with the naked eye and that the opposite was true when he looked through his telescope," Dr Alonso said.

Galileo said that the effect was some kind of size illusion created by the eyes. "Either because their light is refracted in the moisture that cover the pupil, or because it is reflected from the edges of the eyelids and these reflected rays are diffused over the pupil, or for some other reasons," Galileo wrote.

The 19th century German physicist Hermann von Helmholtz came nearer to the truth when he said the "irradiation illusion", as he called it, was caused by our sensation of the object and not by the optics of the eye.

"The latest research demonstrates that the sensation Helmholtz used to explain the irradiation illusion is a 'non-linear' response of the visual system when objects are presented on dark backgrounds," Dr Alonso explained.

The edges of a light object appeared blurred and this was effectively magnified by the brain so that the entire object appeared bigger than it should. Venus, being nearer to earth, was brighter than Jupiter and so it appeared bigger against the dark background of the night sky, he said.



Venus (left) and Jupiter (right) seen above a crescent moon.

THE INDEPENDENT

### PLUS POINTS

#### Changing paradigm

Carbon storage is different at different spots in the same soil. The unique ability to sequester carbon. By doing so, it lowers the amount of carbon released in



Carbon storage is different at different spots in the same soil.

the atmosphere and plays a significant role in the global carbon cycle. Though the concentration of carbon dioxide in the atmosphere has increased over

the years, the rates of carbon sequestration have remained unchanged. Recent scientific developments indicate a shift in our understanding of how sequestration happens in nature, making previous estimates of soil's carbon absorption capacity questionable. A paper published in *Nature Communications* by scientists from the Technische Universität München, Freising-Weihenstephan, Germany, has shown that sequestration of carbon does not happen uniformly across all types of soils. Instead, there is preferential absorption at certain hotspots in the same soil.

For the study, researchers used soil samples similar to natural top soil and mixed them with litter having labelled carbon and nitrogen isotopes. Carbon and nitrogen were labelled in the litter to distinguish the new sequestration from the existing one. The incubation continued for 42 days, after which the soil was divided into fractions based on particle size and density. Samples were then analysed using ultrasensitive nanoscale secondary iron mass spectrometry technique (Nano-SIMS). This allowed them to get the elemental distribution of the samples at very high resolution.

Less than 19 per cent of the soil showed evidence of new sequestration. The labels showed that the new sequestration had happened only in organomineral clusters with rough surfaces. In the soil, some mineral particles appeared as individual particles with mostly plain surfaces, whereas others were aggregated in clusters of several small particles. This clustering caused rough surfaces.

There are no clear answers as to why such a preference occurs. Commenting on the study, S Kundu, principal scientist, division of environmental soil science, Indian Institute of Soil Science, Bhopal, says, "The type of soil taken for this investigation contained 18.5 per cent clay and 18.4 per cent silt and was dominated by chlorite/illite type of minerals. I am sure a distinctly different picture will emerge if the soil were of the vertisol type, containing 40-60 per cent clay, dominated by smectite/vermiculite type of minerals. More research is needed to interpret the result of this investigation in the context of carbon/nitrogen sequestration in soils of diverse physical, chemical and biological properties."

MANUPRIYA/CSE-DOWN TO EARTH FEATURE SERVICE

#### Locust control

Locust swarms destroy crops and threaten the livelihood of millions. The problem is more severe in Asia and Africa because these insects, which belong to grasshopper family *Acrididae*, breed easily in warm, moist conditions,



According to a new study, the microsporidian gut parasite, *Paranosema locustae*, can be used to check the swarming behaviour of migratory locusts and control locust plagues. Researchers found that the parasite causes hindgut acidity and controls the locust's immune response, which suppresses growth of the hindgut bacteria.

These bacteria are behind the production of the aggregation pheromone (chemicals released in the locust's faecal pellets that encourage swarming behaviour in other locusts). Reduction in hindgut bacteria and the consequent reduction in the aggregation pheromone cause a drop in serotonin and dopamine levels — the neurotransmitters that initiate and maintain swarm behaviour.

The experiments were conducted by exposing uninfected locusts to glass chambers containing faecal volatile chemicals from both healthy locusts and others infected with *Paranosema*. The locusts aggregated more and displayed a higher antennal response to uninfected faeces compared to infected faeces.

The research was led by Wangpeng Shi from the department of entomology, China Agricultural University, Beijing, and published in the 14 January issue of *Proceedings of the National Academy of Sciences*.

Carlos E Lange, research scientist working at the Centre for Parasitological Studies and Vectors in Buenos Aires, Argentina, notes that the parasite has already been introduced to affect outbreaks of locusts in parts of Argentina and China with positive results.

But Gregory A Sword, professor, department of entomology, Texas A&M University, says, "The role of aggregation pheromone in mediating the initial attraction, subsequent phase change and swarm formation is considerably overstated in this article."

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