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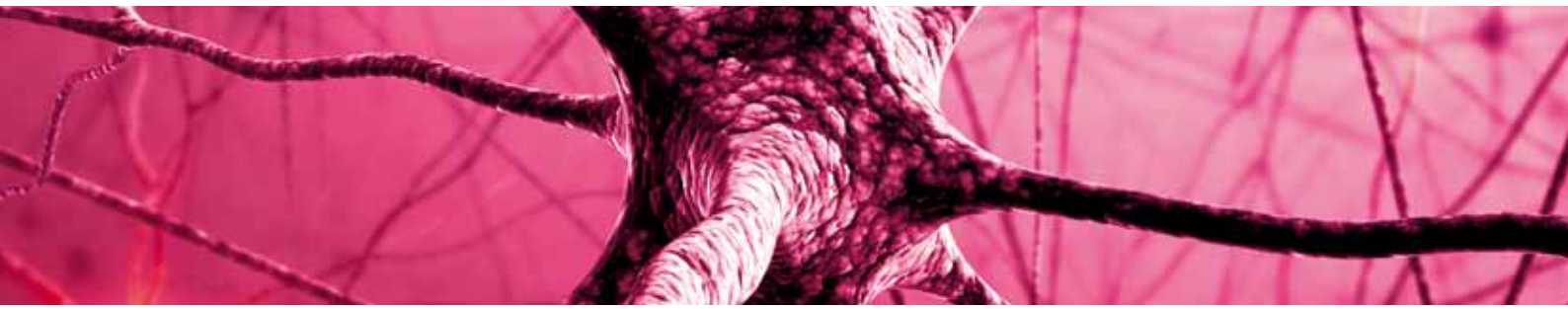

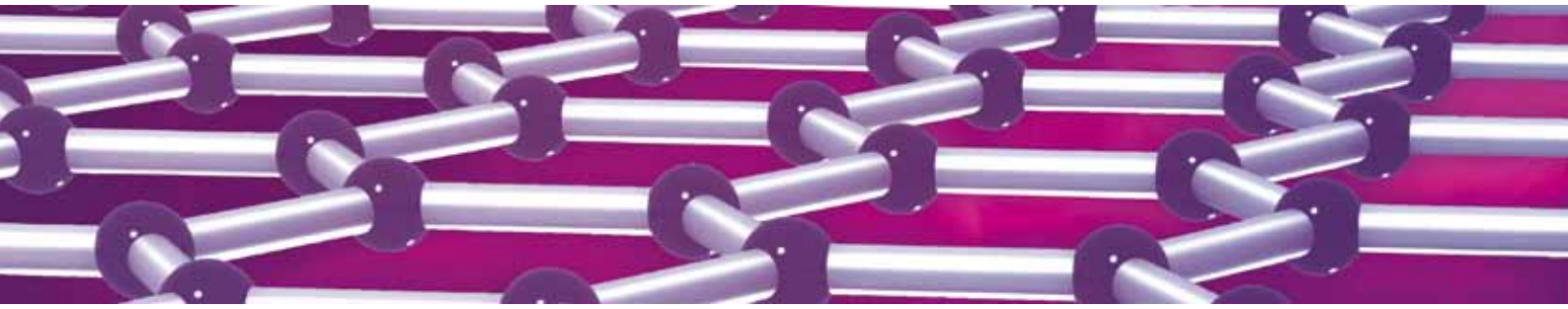

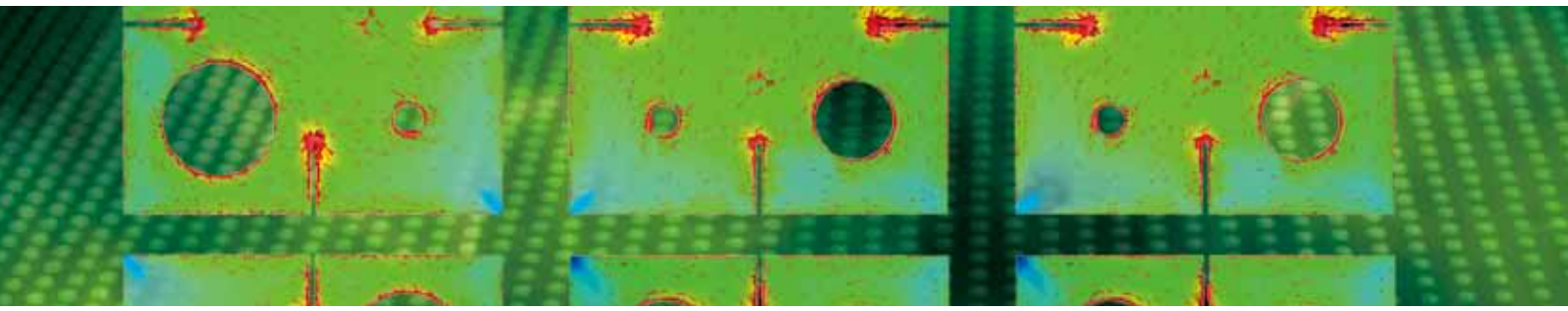

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"As we commemorate this significant milestone in A*STAR's and Singapore's history, we should note that sustained investments and efforts are what have enabled Singapore to be well positioned to seize opportunities opening up in this region."

Mr Lim Chuan Poh, Chairman,
Agency for Science, Technology and Research

1991-2011
Creating Growth
Enhancing Lives

The anniversary logo commemorates the 20th anniversary of Science and Technology planning and development in Singapore.

The emblem of "a*" riding on waves of 20 upward strokes represents the 20 research entities of A*STAR each with its unique traits and capabilities - moving forward and upward in unison. The motif symbolises A*STAR's progressive building of scientific capabilities in its 20 research entities over the past 20 years and underscores the organisation's commitment to continue to surge ahead to establish Singapore as Asia's Innovation Capital and a global R&D hub in the years to come.

The tagline, "Creating Growth. Enhancing Lives", articulates the impact and role of A*STAR in leading scientific research in Singapore for the last 20 years, creating economic growth as well as enriching the lives of Singaporeans.

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SINGAPORE
Agency for Science, Technology and Research

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A*STAR Research
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Editorial
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SINGAPORE

A*STAR *Research*

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Oncology

A double whammy

HPV18 puts women at a higher risk of developing cervical cancer because the virus converts a repressor of the cell cycle into an activator

Human papillomavirus type 16 (HPV16) and type 18 (HPV18) are the two most prevalent high-risk HPVs for causing cervical cancer—a cancer that claims the lives of more than 250,000 women around the world every year. They transform healthy keratinocytes—cells that line the cervix—into cancerous cells by disrupting the host cell cycle. In particular, they use viral genes *E6* and *E7* to encode proteins E6 and E7 that bind and inactivate tumor-suppressor proteins p53 and pRb, respectively.

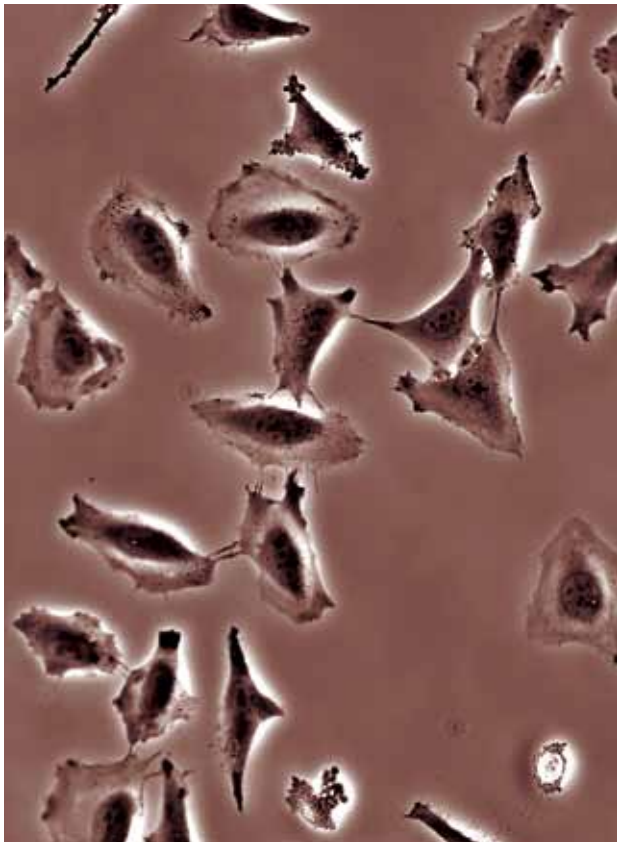
As E6 and E7 proteins of different high-risk HPVs have similar functions, however, the reason for HPV18 having a higher potential for causing cervical cancer is still unknown. Francoise Thierry at the A*STAR Institute of Medical Biology and co-workers[1] have now solved this mystery through a study of the transcriptional regulation of *E6* and *E7*.

E2F is a family of transcription factors that regulate the transcription of S-phase and mitotic genes, which are essential for cell duplication and division. There are eight members in the E2F family: E2F1–3 are activators and E2F4–8 are repressors. In normal keratinocytes, pRb binds to E2F1–3 and prevents them from activating the transcription of S-phase genes, whereas E2F5 suppresses the transcription. In HPV-infected keratinocytes, however, E7 binds to pRb and prevents it from binding to E2F1–3. As a result, E2F1–3 activate the transcription of S-phase genes, allowing the infected keratinocytes to multiply uncontrollably.

The researchers compared the HPV genomes and identified an E2F5-binding site on the E7 promoter of HPV18. They showed that in HPV18-infected keratinocytes, E2F5 binds and activates the transcription of E7. As a result, more pRb becomes inactivated and more E2F activators become available for activating S-phase genes.

In essence, HPV18 changes the role of E2F5 from being a repressor to an activator. The exclusivity of the E2F5-binding site on E7 to HPV18 is one explanation for why HPV18 has a higher potential to cause cervical cancer than other high-risk HPVs.

"Cervical cancer is a major killer of women worldwide and is caused by HPVs. Vaccines may soon reduce HPV infections but the time lag between infection and cancer is long, and many young women will die before this killer cancer virus is stopped. The aim of our research is to describe viral proteins interactions with the host cell and hence define new therapeutic targets to fight the disease," says Thierry. ■



© iStockphoto.com/Torsten Wittmann

Research into the mechanism by which human papillomaviruses cause cervical cancer (cells shown here in culture) could lead to new therapies.

1. Teissier, S., Pang, C.L. & Thierry, F. The E2F5 repressor is an activator of E6/E7 transcription and of the S-phase entry in HPV18-associated cells. *Oncogene* **29**, 5061–5070 (2010).

Stem cells

Brighten up

A fluorescent probe that selectively highlights embryonic and induced pluripotent stem cells is now available

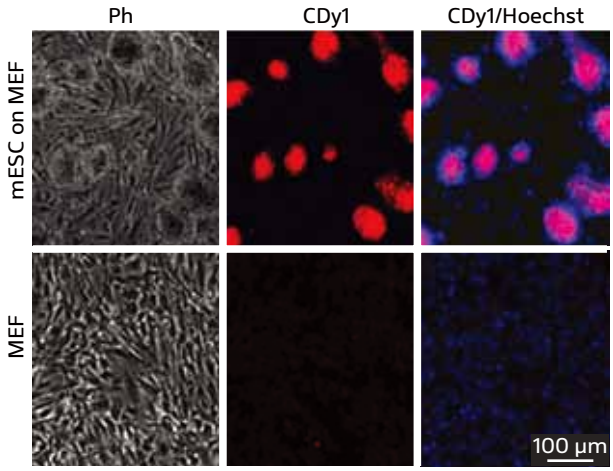
Stem cells—cells that are capable of differentiating into any type of cell in the body—have attracted widespread interest because of their potential applications in regenerative medicine and developmental biology. In the past decade, scientists have made significant progress in stem-cell research, especially in cultivation and differentiation. Yet despite these advances, there has been little change in the area of stem-cell detection.

Immunostaining is currently the most widely used method for detecting stem cells. However, the method is labour-intensive and time-consuming, and involves the use of antibodies that may affect cell physiology. Fluorescent probes—molecules that emit light upon binding specific biological targets—offer an attractive solution to problems commonly associated with immunostaining.

Young-Tae Chang at the A*STAR Singapore Bioimaging Consortium and co-workers at A*STAR's Genome Institute of Singapore and the National University of Singapore have previously synthesized several diversity-oriented fluorescence libraries (DOFLs)—collections of structurally complex and diverse fluorescent compounds—and applied them to the discovery of novel fluorescent probes for the detection of DNA, RNA and proteins. The team has now screened 280 rosamine compounds in a DOFL and identified a novel fluorescent probe that selectively highlights (stains) stem cells[1].

The researchers incubated mouse embryonic stem cells and mouse embryonic fibroblasts with each of the different rosamine compounds. They measured the fluorescence of mouse embryonic stem cells and mouse embryonic fibroblasts and selected 20 compounds that displayed the highest differences in fluorescence intensity. Flow cytometry analysis further identified a rosamine compound called CDy1 to have the highest selectivity in staining mouse embryonic stem cells.

The researchers were able to discriminate mouse embryonic stem cells from a mixed cell population using CDy1 (see image). They also found that CDy1 was effective for induced pluripotent stem cells generated from mouse embryonic fibroblasts, making it the first fluorescent probe that can stain both embryonic and induced pluripotent stem cells. Best of all, CDy1 had no effect on the physiology of stem cells, which remained capable of differentiating into other cell types after staining. The findings demonstrate the potential of CDy1 as a novel tool for detecting viable stem cells.



Microscopy images showing the selective staining of mouse embryonic stem cells (mESCs) grown on mouse embryonic fibroblasts (MEFs) using CDy1. Hoechst staining shows the nuclei of all cells. Only mESCs growing as colonies are stained by CDy1 (Ph, phase contrast image).

"Scientists previously used genetic markers to monitor the production of stem cells, but they could only do this close to the final stage of production. CDy1 allows stem cells to be detected at the early stage of production and therefore provides a means to study the early reprogramming mechanisms of stem cells," says Chang. ■

1. Im, C. N. *et al.* A fluorescent rosamine compound selectively stains pluripotent stem cells. *Angewandte Chemie International Edition* **49**, 7497–7500 (2010).

Cell biology

A new player in protein secretion

Scientists are establishing the basic molecular network of a new player involved in protein secretion and clarifying its regulation in mammalian cells

A team led by Wanjin Hong at A*STAR's Institute of Molecular and Cell Biology has identified a component of a key molecular complex involved in protein secretion by mammalian cells[1]. "Early work on protein secretion was done mainly in yeast," says Hong. "Our research is aimed at identifying molecules important for protein secretion in mammalian cells, and at understanding their functional interactions."

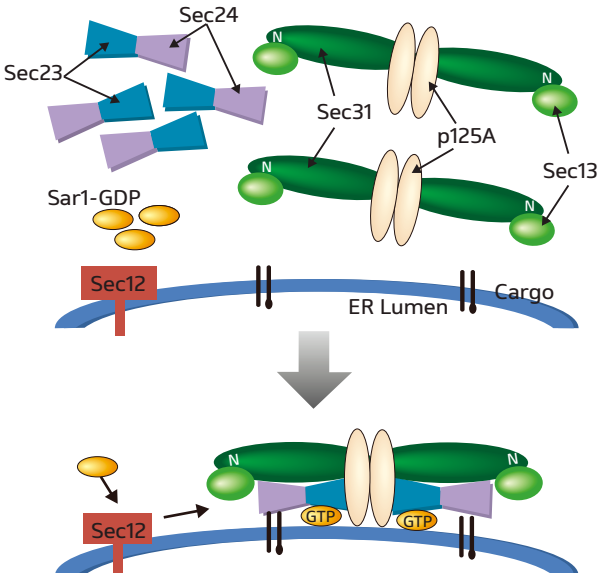
The endoplasmic reticulum (ER) is an extensive membrane network found inside the cell. It encloses an internal space called the lumen from the liquid part of the cell called cytosol. As they are synthesized, proteins targeted for secretion enter the ER lumen where protein folding takes place. They must then be carried within small membrane-enclosed sacs called vesicles to a structure known as the Golgi apparatus, before eventually being secreted from the cell.

In both yeast and mammalian cells, vesicle formation involves a multi-protein complex called coat protein II (COPII), which is assembled at specialized ER exit sites. The last COPII component to be recruited is a subcomplex containing the proteins Sec13 and Sec31 (see image). "We suspected that the Sec13/Sec31 subcomplex might be linked to molecular mechanisms that regulate vesicle formation at ER exit sites in mammalian cells," says Hong.

Using the secretion of a viral protein from rat liver cells as a model experimental system, Hong and his co-workers previously found evidence for a Sec31-interacting factor present in the cytosol. They hypothesized that this unidentified factor might be important for regulating export of the viral as well as cellular proteins from the ER lumen.

The team has now identified the factor to be p125A, a protein previously shown to interact with another COPII component known as Sec23. Their new experiments confirmed that p125A interacts directly with Sec31, and that distinct regions of p125A bind to Sec31 and Sec23.

Although p125A is enriched at ER exit sites, further experiments revealed that most of the p125A present in the cytosol is associated with the Sec13/Sec31 subcomplex, suggesting the presence of a pre-assembled complex composed of all three proteins. It was also found that the Golgi apparatus is disrupted and that protein export from the ER is affected in mammalian cells lacking p125A, supporting the view that this protein plays an important role in regulating ER export.



Schematic illustration showing the role of p125A in endoplasmic reticulum export in mammalian cells. The majority of p125A, Sec31A and Sec13 proteins probably exist in the form of a pre-assembled p125A–Sec31A–Sec13 subcomplex containing two of each of these proteins. Upon recruitment of Sar1 and the Sec23A/Sec24 subcomplex during COPII vesicle budding, the p125A–Sec31A–Sec13 subcomplex is recruited, which may open up the binding sites of p125A for Sec23A. The simultaneous interaction of p125A with both Sec31A and Sec23A on the budding vesicles may facilitate the coordination of these two COPII subcomplexes to mediate vesicle formation.

"Future work will test the hypothesis that p125A coordinates the interaction between the Sec13/Sec31 subcomplex and other COPII components such as Sec23 during their recruitment at ER exit sites," says Hong. ■

1. Ong, Y. S., Tang, B. L., Loo, L. S. & Hong, W. p125A exists as part of the mammalian Sec13/Sec31 COPII subcomplex to facilitate ER-Golgi transport. *Journal of Cell Biology* **190**, 331–345 (2010).

Cell biology

Taking wraps off mammalian Rap1

Mammalian Rap1 controls transcription through the regulation of NF-κB activity

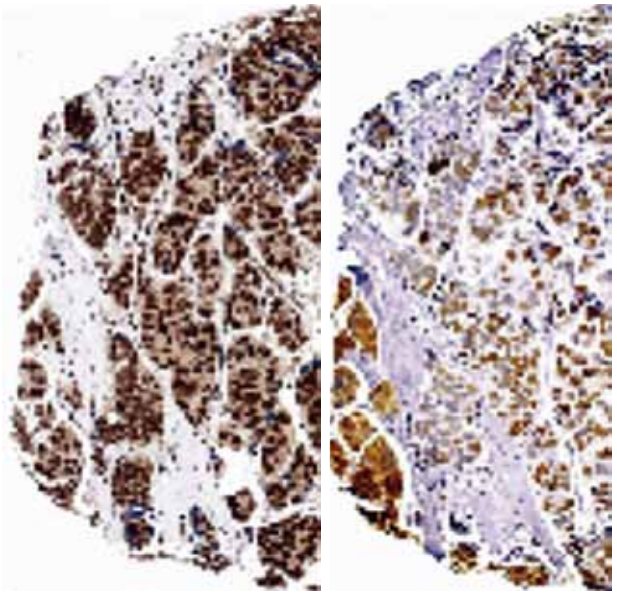
Repressor activator protein 1 (Rap1) is a protein with many roles. In budding yeasts, Rap1 is involved in regulating telomere turnover, transcription and chromatin remodeling. In mammalian cells, Rap1 plays a role in maintaining telomere length and protecting telomere ends. However, recent studies have shown that unlike yeast Rap1, mammalian Rap1 does not have a domain for binding DNA. For this reason, many scientists speculate that mammalian Rap1 may not play a role in transcription or chromatin remodeling.

Vinay Tergaonkar at the A*STAR Institute of Molecular and Cell Biology and co-workers[1] have now identified the role of mammalian Rap1 in transcription. They found that although mammalian Rap1 has lost the ability to bind DNA, the protein could control transcription by regulating the activity of the transcription factor NF-κB.

NF-κB is an important regulator of transcription and a key participant in a variety of cellular processes, including cell division and cell responses to infection. Under normal conditions, inhibitory proteins called IκBs (inhibitors of κB) bind and restrict the activity of NF-κB in the cytoplasm. When cells receive appropriate signals, special enzymes called IKKs (IκB kinases) would phosphorylate and disassociate IκB proteins to activate NF-κB. Recent studies have found that IKKs could also phosphorylate and modify the p65 subunit of NF-κB, which is needed by NF-κB to function properly as a transcription factor.

The researchers carried out a 'gain-of-function' screen to search for novel regulators of NF-κB in the human genome and identified mammalian Rap1 as a potential candidate. They found that mammalian Rap1 could bind IKKs to form complexes, and that these complexes are essential for the phosphorylation of p65 but not of IκB. They also found that mutant mice lacking Rap1 are resistant to endotoxic shock—a sign of defective NF-κB activation—and that tumor samples from breast cancer patients have high levels of Rap1 and NF-κB (see image).

Together, the results demonstrate that mammalian Rap1 is an adaptor of IKKs and a critical regulator of NF-κB. An interesting finding is that, unlike yeast Rap1, mammalian Rap1 does not control transcription directly. Instead, mammalian Rap1 controls transcription through the regulation of NF-κB activity.



Reproduced from Ref. 1. © V. Tergaonkar

Photographs showing the correlation between Rap1 (left) and NF-κB (right) in respectively stained breast cancer tissue.

"Independent findings by two other groups have also revealed extra roles played by Rap1 in mammals, showing that Rap1 in mammals can function away from the telomeres," says Tergaonkar.

NF-κB signaling has important implications in aging and cancer development. Rap1, being a regulator of NF-κB signaling, may well be a novel therapeutic target for anti-aging and anti-cancer therapy. ■

1. Teo, H. *et al*. Telomere-independent Rap1 is an IKK adaptor and regulates NF-κB-dependent gene expression. *Nature Cell Biology* **12**, 758–767 (2010).

Oncology

A very early warning

The human papillomavirus protein E2 may serve as a diagnostic marker for cervical intraepithelial neoplasia, the precursor stages of cervical cancer

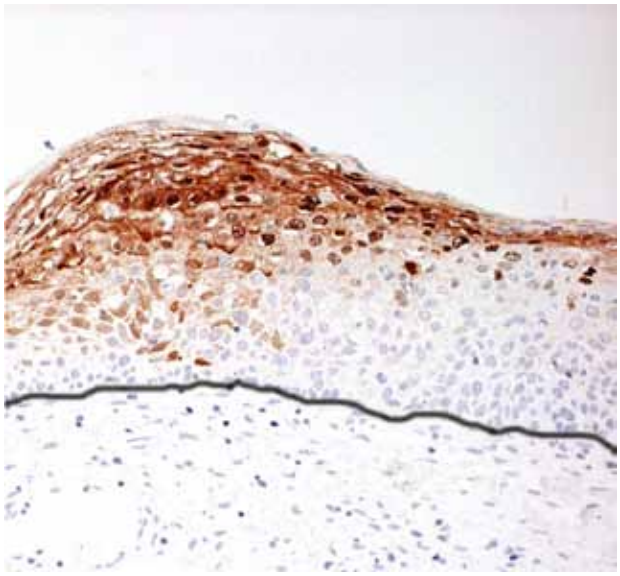
Cervical cancer is one of the most common cancers affecting women, and high-risk human papillomavirus type 16 (HPV16) and type 18 (HPV18) are responsible for causing 70% of cervical cancer cases worldwide. Cervical cancer does not develop overnight. Cells that form the surface (epithelium) of the cervix must go through a series of changes in order to become cancerous. The precancerous phase, known as cervical intraepithelial neoplasia (CIN), could take years to develop.

There are three grades of CIN: CIN1, CIN2 and CIN3. CIN1 corresponds to the earliest stage of CIN just after viral infection when the majority of epithelial cells are still normally differentiated, whereas CIN3 corresponds to the final stage of CIN prior to cancer formation when the majority of epithelial cells have become abnormally 'dedifferentiated'.

Intensive research on HPV16 and HPV18 has provided extensive knowledge on the roles of viral genes and proteins in cancer formation, but little information regarding CIN progression. Françoise Thierry at the A*STAR Institute of Medical Biology and co-workers[1] have now shown that the viral gene E2 plays a crucial role in CIN progression. They also suggested that E2—the protein encoded by the E2 gene—may serve as a diagnostic marker for CIN, which is useful for the early prevention of cervical cancer.

The researchers analyzed the gene expression of 99 cervical biopsy specimens, including 5 normal tissues, as well as 35 CIN1, 31 CIN2 and 28 CIN3 lesions. They found that viral proteins E2 and E7 were expressed at high levels in a mutually exclusive manner; CIN1 lesions expressed high levels of E2 but not of E7, whereas CIN2 and CIN3 lesions expressed high levels of E7 but not of E2 (see image). They also showed that E2 was expressed at high levels in cells forming the intermediate and upper layers of CIN1 lesions where viral DNA replication occurs, but not in cells forming the basal layers where genome integration should occur.

HPV16 and HPV18 are known to use viral proteins E6 and E7 to inactivate tumor-suppressor proteins p53 and pRb. Previous studies have suggested that the viral protein E2 is a repressor of viral gene E6 and E7 transcription and an activator of viral replication. The findings from this study are in good agreement with these assumptions.



E2 expression (stained brown) in clinical samples of precancerous HPV16-infected cervical tissue. The tissue is in an intermediate stage between CIN1 (left, higher E2 expression) and CIN2 (right, lower E2 expression).

E7 is widely used as a diagnostic marker for CIN2 and CIN3. There is currently no diagnostic marker for CIN1 or HPV infection and its progression. The researchers believe that the E2 protein, which is highly expressed in CIN1 lesions, may serve this purpose, providing the earliest warning of abnormal cervical cell growth and serving as a predictive marker for progression versus regression. ■

1. Xue, Y. *et al*. HPV16 E2 is an immediate early marker of viral infection, preceding E7 expression in precursor structures of cervical carcinoma. *Cancer Research* **70**, 5316–5325 (2010).

Proteomics

Manipulating the milieu

Cancer cells secrete proteins that help them adapt to low-oxygen environments

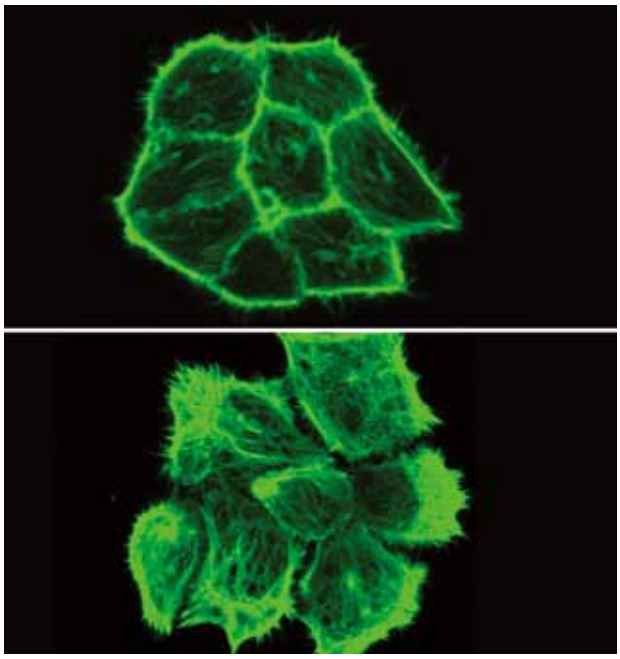
Cancer cells can grow even when oxygen and nutrient levels are low. They respond to stress either by manipulating their environment so that they can move to more hospitable locations or by inducing new vessel growth to obtain what they need from blood circulation. Sai Kiang Lim at the A*STAR Institute of Medical Biology and co-workers have now determined that proteins secreted from oxygen-deprived cancer cells may be responsible for changes in cancer cell behavior and proliferation[1].

By growing human skin cancer cells in cell culture, the researchers found that cancer cells grown in a low-oxygen environment did not stick as well to the culture dish as those grown in a normal-oxygen environment (see image), and were more prone to migrate through an artificial matrix. They reasoned that oxygen-deprived cancer cells can spread, or metastasize, to other locations in the body because of these changes.

The researchers also found that human skin cancer cells grown in a low-oxygen environment could induce the formation and outgrowth of new blood vessels from the membranes of chicken eggs. As these changes were initiated outside the cancer cells, the researchers suspected that proteins secreted by the cancer cells may be responsible for these changes.

Based on this hypothesis, Lim and her team used proteomics—the sequencing and measurement of the relative abundances of a set of proteins—to compare the differences in protein secretion between cancer cells grown in low- and normal-oxygen environments. They found an increase in the secretion level of protein-cleaving enzymes in human skin cancer cells grown in a low-oxygen environment, which they believe causes the degradation of the matrix that keeps the cells in place, permitting them to migrate and spread. They also observed that human skin cancer cells grown in a low-oxygen environment showed enhanced secretion of proteins involved in blood vessel formation.

Surprisingly, many of the secreted proteins the researchers identified are normally found within the membrane or inside cancer cells. The researchers deduced that the reason for this is because cancer cells were secreting exosomes—small vesicles that can contain proteins.



Effect of oxygen on human skin cancer cells. (Top) Cancer cells in a normal-oxygen environment. (Bottom) Cancer cells in a low-oxygen environment, showing the altered distribution of proteins (green) that affect the behavior of tumor cells.

"Cancer is a leading cause of mortality, causing one in eight deaths worldwide with 90% of these deaths attributable to metastasis. We are now focusing on understanding the mechanisms by which cancer cells make and secrete exosomes to influence their microenvironment," says Lim. "Once we find out these mechanisms, we can devise therapies to control cancer growth and spread." ■

1. Park, J. E. *et al.* Hypoxic tumor cell modulates its microenvironment to enhance angiogenic and metastatic potential by secretion of proteins and exosomes. *Molecular & Cellular Proteomics* **9**, 1085–1099 (2010).

Molecular biology

Resistance is futile

The protein ELKS plays an important role in the DNA-damage signaling pathway and blocking its function is one way to reduce drug resistance in cancer cells

Nuclear factor kappa B (NF-κB) is a family of transcription factors with important roles in eliciting cellular responses to external stimuli. NF-κB normally resides in the cytoplasm of the cell, where it remains in an inactive state bound to an inhibitor of κB (IκB). In response to stimuli, the cell activates IκB kinase (IKK) to degrade IκB, causing NF-κB to become active and move into the nucleus where it regulates gene expression and modifies cell function.

Many different stimuli can trigger IKK and NF-κB activation. The cytokine tumor necrosis factor (TNF), for example, binds TNF receptor type 1 (TNFR1) to trigger the release of TNF receptor-associated factor 2 (TRAF2), which facilitates the attachment of ubiquitin molecules to receptor interacting protein 1 (RIP1). The ubiquitin molecules form a chain that helps RIP1 recruit TGFκ-activated kinase 1 (TAK1) and IKK, providing an opportunity for TAK1 to activate IKK. This cascade of events, known as the TNF signaling pathway, is an important mechanism that keeps immune cells proliferating and stops them from dying.

Apart from cytokines, DNA-damaging agents such as infrared radiation and chemicals can also trigger IKK and NF-κB activation. Previous studies have identified several kinases involved in the DNA-damage signaling pathway, including TAK1 and ataxia telangiestasia mutated (ATM), but the cascade of events leading to IKK and NF-κB activation has been unclear.

Vinay Tergaonkar at the A*STAR Institute of Molecular and Cell Biology and co-workers[1] have now identified a protein called ELKS that acts as a molecular link between ATM and TAK1. They showed that ATM induces the 'ubiquitination' of ELKS, which promotes the formation of a TAK1–IKK signaling complex—a sequence analogous to that for TNF signaling.

"When we added DNA-damaging drugs to mouse embryonic fibroblasts derived from ELKS-deficient mice, we did not detect TAK1, IKK or NF-κB activation. However, the mouse embryonic fibroblasts had no problem activating TAK1, IKK or NF-κB in response to TNF. Our findings demonstrate that ELKS plays an important role in the DNA-damage signaling pathway," says Tergaonkar.

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There are some important implications for this finding in cancer treatment. In chemotherapy, cancer cells are known to develop resistance to drugs because of NF-κB activation. Combining chemotherapeutic drugs with NF-κB blockers can overcome chemotherapeutic drug resistance, but this may also inhibit other vital NF-κB functions, such as regulation of immune responses. Combining chemotherapeutic drugs with an ELKS blocker that blocks cellular responses to DNA damage but not to other stimuli would solve all these problems. ■

1. Wu, Z. H. *et al.* ATM and NEMO-dependent ELKS ubiquitination coordinates TAK1-mediated IKK activation in response to genotoxic stress. *Molecular Cell* **40**, 75–86 (2010).

Cancer biology

A back door to drug resistance

An investigation into how some cancers evade treatment reveals a novel pathway regulating tumor growth

Molecules of phosphate are the primary 'currency' for a variety of cellular signaling transactions, and many instructions related to cell division and other essential functions are relayed via targeted phosphorylation of specific proteins by kinase enzymes. Such signals can subsequently be switched off by protein phosphatase 2A (PP2A) enzymes, which trim away phosphate molecules.

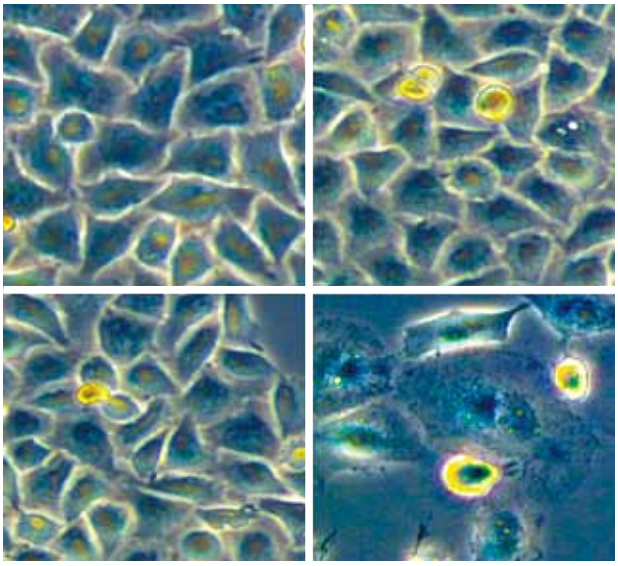
Many PP2A enzymes help keep cancer in check, and recent experiments by Qiang Yu at the A*STAR Genome Institute of Singapore and co-workers[1] have revealed a pattern of colon cancer-associated inactivation of *PPP2R2B*, a gene encoding the PP2A subunit B55β. Following up on these findings, they have now and found1 compelling evidence that B55β contributes to patient response to rapamycin, a promising cancer therapeutic.

Rapamycin targets the mTOR signaling pathway, an important engine of tumor growth, but this drug is not a universally applicable treatment option. "Clinical responses to mTOR inhibitors like rapamycin are generally unpredictable and patient drug resistance can be either intrinsic or acquired," says Yu.

PP2A enzymes also play an important role in mTOR regulation. The researchers showed that B55β expression specifically inhibits transformation and cancerous growth in cultured colorectal cancer cells. They identified several target proteins that undergo targeted dephosphorylation by B55β-containing PP2A complexes, including the well-known cancer-causing protein c-Myc. The inhibition of c-Myc appears to be a primary mechanism by which B55βconstrains cell growth, but it also represents an important link between rapamycin sensitivity and *PPP2R2B* gene expression.

Rapamycin dramatically inhibited proliferation in cancer cells that produce B55β, but when levels of the protein were reduced, the drug was largely ineffective. The researchers learned that this was because rapamycin treatment stimulates phosphorylation of c-Myc via a previously unidentified pathway, mediated by phosphoinositide-dependent kinase 1 (PDK1). This process can only be kept in check when *PPP2R2B* is being actively expressed (see image), which allows B55β to directly bind and inhibit PDK1.

According to Yu, the findings suggest a novel cancer signaling pathway that may be important in resistance to rapamycin.



Re-expression of PPP2R2B in colorectal cancer cells (right column) renders the cells more susceptible to anti-cancer drugs (lower row). Left column shows cancer cells with naturally suppressed *PPP2R2B* expression.

Therapeutics targeting PDK1 that could overcome resistance to mTOR inhibitors may be useful for other Myc-driven tumors.

Given the multi-factorial complexity of typical cellular signaling pathways, the researchers are now actively engaged in efforts to uncover other components of the PDK1–Myc cascade. "We have identified a couple of kinases in this regard, and are now in the process of developing a novel combination strategy for targeting colon cancer based on these findings," says Yu. ■

1. Tan, J. *et al*. B55β-associated PP2A complex controls PDK1-directed Myc signaling and modulates rapamycin sensitivity in colorectal cancer. *Cancer Cell* **18**, 459–471 (2010).

Molecular biology

Go or no-go?

The crystal structure of the Dom34–Hbs1 complex reveals how cells recognize and remove defective messenger RNA with stalled elongation through 'no-go' decay

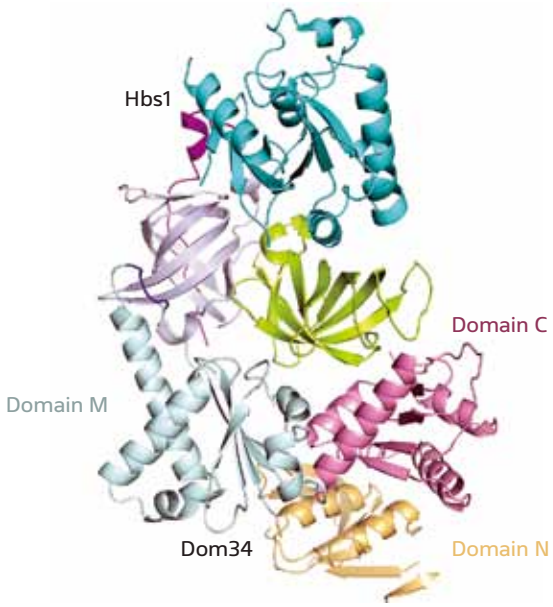
Protein synthesis is an extremely complex process. Ribosomes, which read the genetic codes of messenger RNA (mRNA) and use the information to make proteins, often encounter defective mRNA that hamper the translation process. Because of this, cells have evolved several mechanisms to detect and degrade defective mRNA. A recently identified mechanism, known as 'no-go' decay (NGD), targets mRNA with stem-loops—side branches that interrupt the translation elongation process—and is essential for maintaining the fidelity and quality of translation.

Studies on budding yeasts and fruit flies have identified Dom34 and Hbs1—the paralogs of peptide release factors eRF1 and eRF3—as two proteins required for eliminating mRNA with stem-loops through NGD. Structural analysis of yeast Dom34 has shown that the protein is similar to eRF1 in domains M and C but not in N. The relative orientation of the domains suggests that Dom34 might bind Hbs1 to form a complex (pictured) that promotes translation termination and commits the mRNA to NGD, in the same way as the eRF1–eRF3 complex promotes normal translation termination. This hypothesis, however, has never been confirmed.

Haiwei Song and co-workers at the A*STAR Institute of Molecular and Cell Biology[1] have now shed light on the role of Dom34–Hbs1 in NGD by obtaining the crystal structure of Dom34–Hbs1 from fission yeast. They found that overall, the structure is similar to that of eRF1–eRF3, and that Dom34 and Hbs1 show similar biochemical properties to those of eRF1 and eRF3. The results suggest that Dom34–Hbs1 binds to the ribosomal A site where the complex plays a role in preventing translation elongation and promoting mRNA cleavage as an alternative event to NGD.

If Dom34–Hbs1 provides an alternative fate to NGD for ribosomes with stalled translation, it should be possible for the ribosome to undertake mRNA cleavage and NGD at strong translational pauses without Dom34–Hbs1. Consistent with this model, the researchers observed that NGD can be triggered by mRNA with a long error code that strongly blocks translation elongation, in the absence of the Dom34–Hbs1 complex.

Song and his team are now looking at how Dom34–Hbs1 functions in the context of stalled ribosomes. "Specifically, we would like to know how Dom34–Hbs1 binds to the stalled ribosome and triggers NGD, using techniques such as X-ray crystallography and electron microscopy," says Song. "We hope that these studies will reveal the molecular mechanism of NGD." ■



Crystal structure of the Dom34–Hbs1 complex

From Ref. 1. © 2010 R. Parker, H. Song

1. Chen, L. *et al*. Structure of the Dom34–Hbs1 complex and implications for no-go decay. *Nature Structural and Molecular Biology* **17**, 1233–1241 (2010).

Neurobiology

How fish avoid anxiety

Clarification of the neural basis of zebrafish avoidance response could lead to a better understanding of anxiety

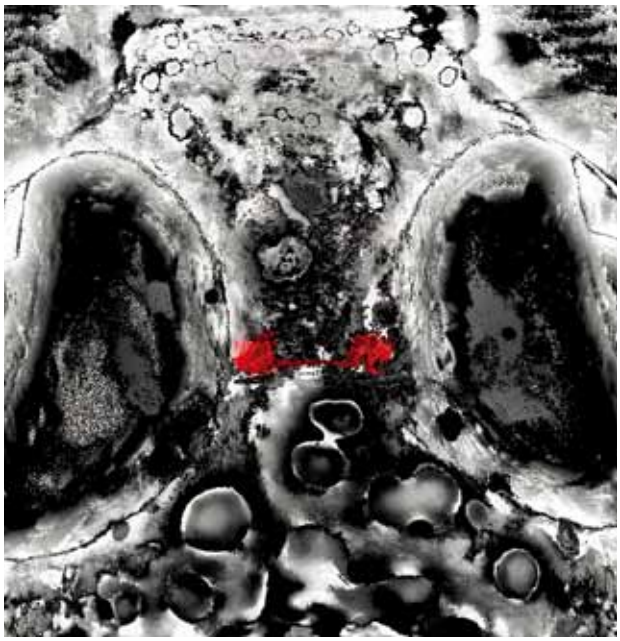
Animals quickly learn to avoid danger, but if they are first exposed to a highly stressful stimulus, they become 'helpless' and do not avoid danger even if they experience continued pain. A brain structure called the habenula has been implicated in this avoidance response, but its exact role is controversial. Suresh Jesuthasan from the A*Star Duke–NUS Neuroscience Research Partnership and co-workers[1] have conducted a study on zebrafish that clarifies the role of the habenula in avoidance response. The findings could provide a better understanding of human anxiety disorders.

The researchers placed zebrafish larvae in a rectangular box equipped with electrodes and red lights at each end. When the fish swam to one end of the box, they were shown the light and given a mild shock five seconds later. With repeated trials, the fish learned to associate the two stimuli, and to avoid the shock by moving away from that end of the box when presented with the light alone. But they failed to do so if first exposed to an electric shock from which they cannot escape.

The fish was then genetically modified to express a photoreactive protein called KillerRed in neurons projecting from the ventrolateral forebrain to the habenula (see image). The technique, known as photobleaching, enabled the researchers to selectively destroy the neurons by illuminating them with green light, which causes KillerRed to release reactive oxygen atoms that damage the cell membrane.

The researchers found that fish with damaged habenula input neurons did not display the avoidance response. They also appeared more anxious. To confirm the role of the habenula in avoidance, they then generated a new line of zebrafish expressing tetanus toxin in dorsal habenula cells. The toxin silences cells by blocking their transmission of nervous impulses. These larvae also failed to avoid the red light when it was shown to them, confirming that the learning of the avoidance response is dependent on the neural circuitry in the dorsal habenula.

The researchers suggest that the habenula could be involved in signaling stressful stimuli, with the dorsal habenula transmitting information about whether a stressful stimulus can be controlled by a specific action. And because the habenula has a similar pattern of neural connections in mammals, they further suggest that damage to the habenula could play a role in mental disorders characterized by anxiety and helplessness.



KillerRed protein (red) expressed in the zebrafish habenula

"One implication is that stimulating the habenula may reduce anxiety," says Jesuthasan. "If we know how the brain naturally switches off anxiety, we will have a different way of helping people with anxiety disorders." ■

1. Lee, A. *et al*. The habenula prevents helpless behavior in larval zebrafish. *Current Biology* **20**, 2211–2216 (2010).

Structural biology

Keeping up with the neighbors

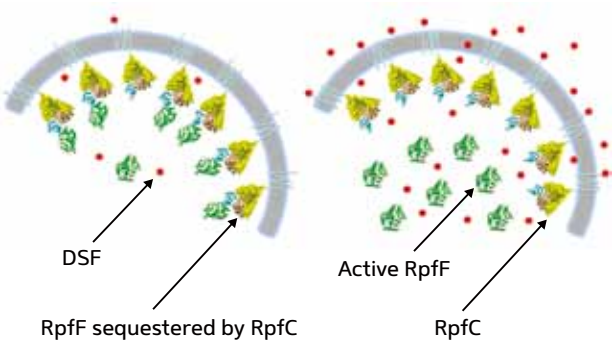
Protein structures reveal details of how some bacteria stay in touch with their community

Many bacteria are remarkably civic-minded, altering their behavior in response to changes in the density of their surrounding population. This collective organization is achieved through 'quorum-sensing' systems, which respond to molecules secreted by individual bacterial cells; once a threshold level of signaling activity is reached, bacteria respond by switching on specific quorum-responsive genes.

Several years ago, Lian-Hui Zhang and co-workers at the A*STAR Institute of Molecular and Cell Biology (IMCB) identified a novel quorum-sensing pathway, driven by the diffusible signaling factor (DSF), which appears to be employed by a number of plant and animal pathogens. Subsequent collaboration between Zhang and his IMCB colleague Haiwei Song has now revealed important details about the mechanism that enables DSF to modulate its own production and switch on genes associated with bacterial virulence[1].

This process is governed by the DSF sensor RpfC, which detects quorum signals and transmits them to the DSF-manufacturing enzyme RpfF. Song, Zhang and their co-workers began by characterizing the structural details of the RpfC–RpfF complex, revealing that RpfF belongs to a family of enzymes known as enoyl-CoA hydratases/dehydratases. They also identified a number of specific amino acids with essential roles in DSF production, most of which are positioned within a narrow pocket in the protein that most likely accommodates DSF precursor molecules.

RpfC contains a signaling domain known as REC, which binds to RpfF and thereby inhibits DSF production, and the researchers also identified key surface elements that mediate this interaction. Intriguingly, REC binding appears to lock RpfF into a configuration that leaves its catalytic pocket inaccessible, thereby physically preventing the production of DSF for as long as REC remains bound. Once DSF accumulation reaches the 'tipping point' in the external environment, however, signaling through RpfC triggers chemical modification of the REC domain, causing it to dissociate and leading to a sharp increase in DSF production and quorum signaling (see image). "Control of signal production is usually at the level of transcription," says Zhang. "The most interesting finding of this research is the structural demonstration of a novel, post-translational mechanism for quorum-sensing signal autoregulation."



When DSF levels are low (left), the interaction between RpfC and RpfF directly interferes with the production of additional DSF. As bacterial density in the environment increases (right), elevated DSF levels trigger the release of RpfF and stimulate further DSF synthesis.

As DSF signaling through RpfC also switches on the production of virulence factors that enable pathogens to get down to their dirty work, this pathway represents a potentially important therapeutic target. "Work is ongoing to identify the substrates and catalytic mechanism of RpfF," says Zhang. "The identification of key residues associated with the sensor-synthase interaction has already provided useful clues for future drug design." ■

1. Cheng, Z. *et al.* Structural basis of the sensor-synthase interaction in autoinduction of the quroum sensing signal DSF biosynthesis. *Structure* **18**, 1199–1209 (2010).

Development

Lend me your ears

A zebrafish study provides new insights into factors that influence the formation of functionally important ear stones

Cilia are hair-like sensory organelles that play essential roles in many physiological and developmental processes. Protozoa are known to use their motile cilia exclusively for locomotion in fluid. In vertebrates, however, the primary role of motile cilia is to move fluid over the cell surface. Dysfunctions in this cilia-driven fluid flow can have wide-ranging effects in the human body. For example, fields of beating cilia on the epithelial lining of the human respiratory tract drive mucus transport. In patients with the rare human genetic disorder primary ciliary dyskinesia, defects in the action of the cilia lining the respiratory tract result in failure to clear mucus from the lungs.

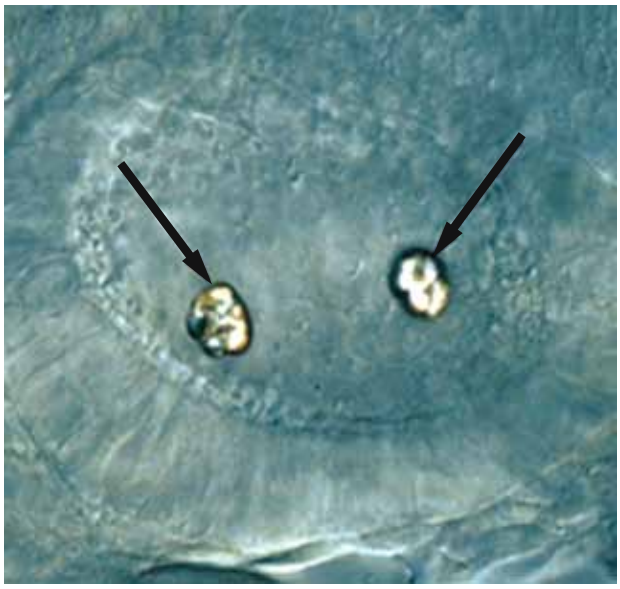
Sudipto Roy and co-workers at the A*STAR Institute of Molecular and Cell Biology[1] have now shown that the distribution of cilia in the ear is critical to the normal formation of functionally important ear stones, called otoliths, found on hair cells of the inner ear.

Motile cilia were believed to play a role in the deposition of otoliths—which have functions in hearing and balance—on specialized hair cells in the inner ear. However, the identity of the motile cilia responsible has been controversial.

To research the issue, Roy and his co-workers studied motile cilia in zebrafish. "The zebrafish is an excellent model system for studying ear development because it is easy to manipulate genetically," says Roy. Otoliths (see image), which contain proteins and calcium carbonate, crystallize from precursor particles present in the fluid-filled ear vesicle and become attached to specialized 'kinocilia' on hair cells.

The researchers used high-speed video microscopy to observe the inner ears of live embryos as well as other light and electron microscopy techniques to follow otolith formation in normal, mutant and genetically manipulated zebrafish. They showed that the ear vesicle produces many motile cilia in a spatial distribution that depends on the expression pattern of the protein Foxj1b.

Their experiments further revealed that proper otolith formation depends on the correct spatial and temporal distribution of motile cilia. "Too few or too many motile cilia result in otoliths of irregular shape and size," says Roy.



Otoliths in the ear vesicle of a zebrafish embryo

They found that kinocilia are immotile, serving as static tethers for otolith crystallization. Their evidence also suggests that kinocilia differentiation from motile cilia involves modification of the Foxj1b program by Atoh proteins. "Based on our results, it is likely that motile and immotile cilia also play important roles in the mammalian inner ear, and their dysfunction could lead to deafness," concludes Roy. ■

1. Yu. X., Lau, D., Ng, C. P. & Roy, S. D. Cilia-driven fluid flow as an epigenetic cue for otolith biomineralization on sensory hair cells of the inner ear. *Development* **138**, 487-494 (2010).

Stem cells

Essential factors

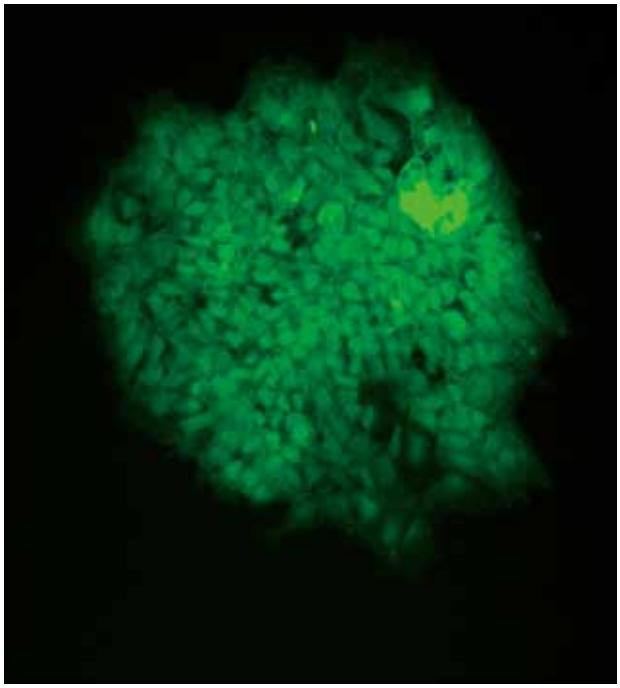
A genome-wide RNA interference screen identifies genes that govern the unique properties of human embryonic stem cells

Human embryonic stem cells (hESCs) are an important tool for research and a viable source of potential replacement cells for clinical applications. There are two properties that make hESCs unique: the ability to differentiate into any cell type, a property known as pluripotency; and the ability to go through numerous cycles of cell division without changing their undifferentiated state, a property called self-renewal. To exploit the full potential of hESCs, scientists must have a detailed understanding of the genetic network that governs these properties. For this reason, Huck-Hui Ng, Fred Bard and co-workers from the A*STAR Genome Institute of Singapore, the A*STAR Institute of Molecular and Cell Biology, Nanyang Technological University and the National University of Singapore[1] have carried out a whole-genome RNA interference screen to comb out genes that regulate these functions of self-renewal and pluripotency.

The researchers generated a stable hESC line expressing green fluorescent protein (pictured). As only undifferentiated hESCs of this line exhibit green fluorescence, the researchers could estimate the effect of a particular gene on hESC survival and proliferation by measuring the fluorescent intensity and number of hESC nuclei.

The researchers used the hESC line to screen a library of 21,121 human genes. This process identified 566 genes that had the largest effect on hESC survival and proliferation. Analyses of gene ontology and biological reactions for the 566 genes revealed a wealth of factors and pathways involved in transcription and translation. Among these were components of the transcriptional regulator PRDM14 and the chromatin remodeling complex INO80.

Previous studies have demonstrated the possibility of reprogramming human fibroblasts into pluripotent stem cells (PSCs), known as human induced PSCs, through the introduction of four transcription factors: OCT4, SOX2, KLF4 and c-MYC. The researchers assessed how the introduction of additional transcriptional factors, such as PRDM14 and NFRKB, could affect the pluripotency reprogramming process. They found that the introduction of PRDM14 and NFRKB could accelerate the reprogramming process by factors of 7 and 3.5, respectively. The results not only demonstrate the power of the whole-genome RNA interference screen, but also reveal the roles of many new factors and pathways.



From Ref. 1 © 2010 H.-H. Ng, F. Bard

Fluorescence microcopy image of human embryonic stem cells stained with green fluorescent protein

"We are extremely excited by our new findings," said Ng. "Our data have shown us the intricate network of genes and factors involved in the maintenance of the hESC state."

Further studies revealed that PRDM14 directly regulates the expression of the pluripotency gene POU5F1, and colocalizes with transcription factors OCT4, NANOG and SOX2. The findings suggest that PRDM14 is a key transcription factor required for the maintenance of hESC pluripotency. ■

1. Chia, N. Y. *et al*. A genome-wide RNAi screen reveals determinants of human embryonic stem cell identity. *Nature* **468**, 316–320 (2010).

Metabolic engineering

Hamster rules

Chinese hamster ovary and baby hamster kidney cell lines are the best choices for producing therapeutic glycoprotein that stays in the bloodstream longer

Glycosylation—the attachment of sugar chains, or glycans, to proteins—is a common chemical modification that cells use to produce functionally important glycoproteins. The glycoprotein erythropoietin (EPO) is of particular therapeutic interest as it regulates red blood cell production, which makes it useful for treating patients with blood disorders.

A glycoprotein may have one or more sialic acids attached to its N-glycans, and the extent of sialylation can have a tremendous impact on the pharmacokinetics of the glycoprotein. Highly sialylated EPO, for example, tends to stay in the bloodstream much longer and have greater efficacy, so scientists have been looking for ways to enhance EPO sialylation. One approach to achieve this is to modify the genetics of cells that produce EPO.

The conventional method for analyzing glycoprotein sialylation is costly and time-consuming. Zhiwei Song and co-workers at the A*STAR Bioprocessing Technology Institute recently adopted a technique called isoelectric focusing (IEF), which allows different molecules to be imaged separately based on differences in electrical charge, to assess the extent of EPO sialylation quickly and accurately. They have used IEF to study how CMP-sialic acid transporter, one of the best-studied nucleotide-sugar transporters, affects EPO sialylation in Chinese hamster ovary (CHO) cells[1]. They have also used IEF to show that the overexpression of GnT I—the gene that encodes the transferase enzyme GnT I—enhances EPO sialylation in a mutant CHO cell line[2]. These findings are important and will help pharmaceutical companies develop the strategies to produce highly sialylated EPO. However, the effectiveness of using other genes and other cell lines still remained unclear.

Song and his co-workers have now used IEF to perform a systematic functional analysis on 31 glycosylation-related genes in six cell lines[3]. They found that CHO and baby hamster kidney cells (pictured) were the most effective in enhancing EPO sialylation. None of the 31 genes, individually or in combination, was able to improve EPO sialylation in these two cell lines. The researchers also tested other cell lines including human embryonic kidney, monkey kidney fibroblast, mouse embryonic fibroblast and mouse myeloma cells, although none of these provided a comparable sialylation enhancement.

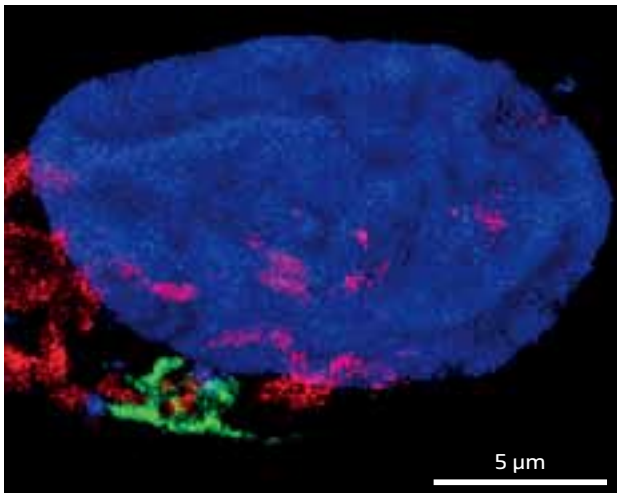


Photo: P. Zhang

Reconstructed fluorescence microscopy image of baby hamster kidney cells, showing the nucleus (blue), GnT I (red) and sialyl transferase I (green).

"Researchers in biotechnology have tried for many years to improve sialylation by expressing glycosylation-related genes," says Song. "However, each time only one or two genes have been studied. Our work represents the first systematic analysis of many genes in six commonly used mammalian cell lines, providing a general guide for engineering the glycosylation pathway in these cells." ■

1. Chan, K. F., Zhang, P. & Song, Z. Identification of essential amino acid residues in the hydrophilic loop regions of the CMP-sialic acid transporter and UDP-galactose transporter. *Glycobiology* **20**, 689–701 (2010).
2. Goh, J. S. Y. *et al.* RCA-I-resistant CHO mutant cells have dysfunctional GnT I and expression of normal GnT I in these mutants enhances sialylation of recombinant erythropoietin. *Metabolic Engineering* **12**, 360–368 (2010).
3. Zhang, P. *et al.* A functional analysis of N-glycosylation-related genes on sialylation of recombinant erythropoietin in six commonly used mammalian cell lines. *Metabolic Engineering* **12**, 526–536 (2010).

Developmental biology

Separate ways

Two guidance receptors steer the collective migration of border cells in different ways

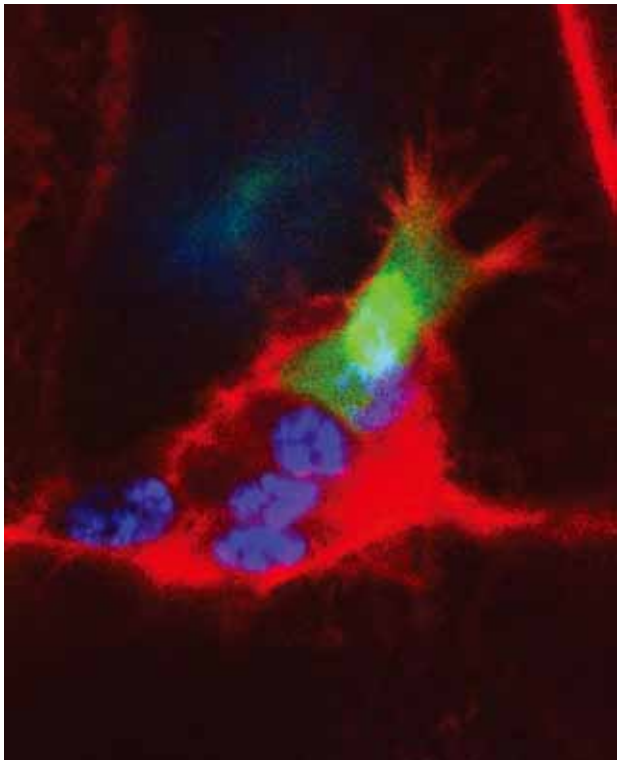
Cell migration—the orchestrated movement of cells from one location to another—is a key process in animal development. It plays important roles in tissue formation and immune response, as well as cancer metastasis. However, although scientists have performed extensive studies on the migration of individual cells, they still lack a detailed understanding of collective cell migration.

The fruit fly *Drosophila melanogaster* is one of several model organisms scientists use for studying cell migration. In the ovary of this fruit fly, a cluster of specialized border cells (pictured) detach from the epithelium and migrate to the oocyte. To reach their destination, border cells undergo collective migration in a way that allows them to squeeze between the giant nurse cells in the space between the epithelium and oocyte.

Previous studies have identified two cell-surface receptors, namely platelet-derived growth factor/vascular endothelial growth factor–related receptor (PVR) and epidermal growth factor receptor (EGFR), to be important for border cells. These receptors read guidance cues from their environment in order to direct border cells to the oocyte. Pernille Rørth and co-workers at the A*STAR Institute of Molecular and Cell Biology and the National University of Singapore[1] have now found that the two receptors change the behavior of border cells in different ways in order to steer the collective migration in the right direction.

The researchers studied the effects of PVR and EGFR on border cells using a combination of live imaging and RNA interference techniques, which reduce the expression of either PVR or EGFR in border cells. This approach allowed them to obtain quantitative information about the behavior of border cells. They found that PVR and EGFR both promote the maintenance and growth of front extensions, which in turn leads to more forward movement of the cell cluster. PVR and EGFR signaling also ensure that the extensions of cells at the front are productive and 'sticky', whereas extensions of cells at the back are not.

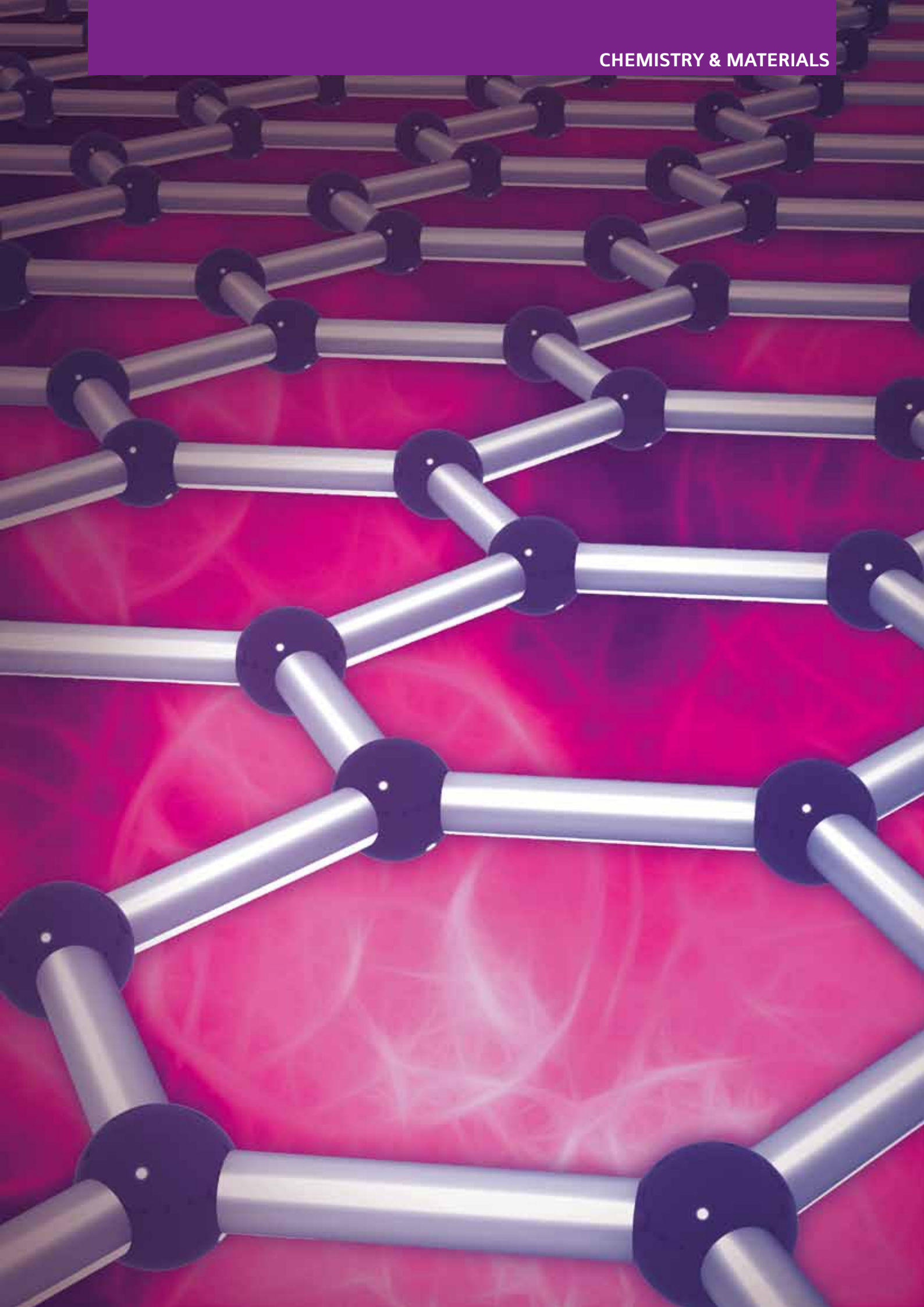
The researchers also noted functional differences between PVR and EGFR. They found that PVR induces a sliding behavior, whereas EGFR induces a tumbling behavior. In essence, PVR appears to be a better guidance receptor than EGFR.



Fluorescence microscopy image showing a cluster of border cells (blue nuclei, one cell labeled green) inside the ovary of the fruit fly *Drosophila melanogaster*

"We have previously identified the cues and receptors that are responsible for directional movement," says Rørth. "Now we have shown how they do the job—how the guidance signals change cell behavior such that cells move in the right direction." The researchers suggest that EGFR may be optimized for cell-wide or nuclear responses, whereas PVR may be optimized for more local or polarized responses. ■

1. Poukkula, M., Cliffe, A., Changede, R. & Rørth, P. Cell behaviors regulated by guidance cues in collective migration of border cells. *Journal of Cell Biology* **192**, 513–524 (2010).



Organic chemistry

Going green with copper

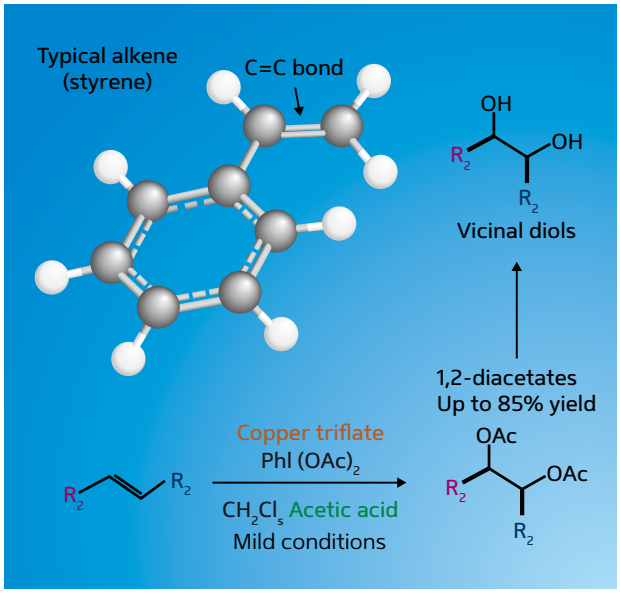
Copper salts could replace highly toxic osmium tetroxide as a catalyst for the oxidation of alkenes to diacetoxy compounds

Alkenes, also known as olefins, are a class of organic molecules having at least one double bond between adjacent carbon atoms (C=C). Carbon–carbon double bonds are valuable because they can be converted into a wide range of other functionalities of chemical and pharmaceutical interest. One such conversion is an oxidation reaction in which one of the C=C bonds is broken and two atoms of oxygen are added in its place to generate a 'vicinal diol'—a structural motif found in many naturally occurring chemical compounds and synthetic pharmaceutical drugs. The heavy metal compound osmium tetroxide is often used as a catalyst for this reaction but suffers from several drawbacks, not least its high price and notorious toxicity.

Jayasree Seayad and co-workers at the A*STAR Institute of Chemical and Engineering Sciences[1] have now devised a method for successfully converting alkenes to a diacetoxy compound—a closely related derivative of vicinal diols—using a compound of copper known as copper(II) triflate as the catalyst (see image). This copper salt is readily available, non-toxic and a fraction of the price of osmium tetroxide making the new method safe, cost-effective and environmentally benign.

In initial experiments, the researchers found that copper(II) triflate could catalyze the diacetoxylation of a common olefin known as styrene—the starting material for making polystyrene—in the presence of an oxidizing agent, $PhI(OAc)_2$, and acetic acid, under mild conditions in high chemical yield and with few side products. Further experimentation established the generality of the reaction and allowed the researchers to extend the scope of the reaction to more structurally complex olefins.

The researchers also investigated the pathway by which the reaction proceeds and proposed a mechanism in which the copper enters the reaction in a form known as copper(II), which is then oxidized to an unusual and transient form of the metal called copper(III). This then cycles between it and another form known as copper(I) to generate the observed products. Interestingly, the A*STAR team discovered that a closely related class of substrates known as homoallylic alcohols bearing an existing hydroxyl group and a longer-chain alkene can be converted in to another useful class of cyclic organic molecules.



Schematic illustration of a typical alkene (styrene) showing the C=C double bond, and the reaction scheme using copper triflate as a catalyst for the conversion of olefins to vicinal diols.

The improvements made to the alkene oxidation reaction by the use of cheap, low-toxicity copper salts and the mild conditions of the process open up the potential for its use in large-scale syntheses of vicinal diol derivatives. "We are also extending the scope of this method to the asymmetric synthesis of non-racemic diol derivatives, which will enable rapid access to the coveted chiral intermediates," says Seayad. ■

1. Seayad, J., Seayad, A. M. & Chai, C. L. L. Copper-catalyzed diacetoxylation of olefins using $PhI(OAc)_2$ as oxidant. *Organic Letters* **12**, 1412–1415 (2010).

Surface chemistry

A model of composure

Density-based methods for characterizing molecular volume reveal how liquid mixtures behave at interfaces

Estimating the surface composition of a liquid mixture may seem like an easy task at first, but the calculations are not always so straightforward. Combining two liquids together generates complex interactions that may involve breaking and remaking intermolecular bonds. Any energetic change during this process can lead to 'non-ideal' behaviors—circumstances strikingly different from theories based on pure 'ideal' liquids.

Martin Tjahjono and Marc Garland at the A*STAR Institute of Chemical and Engineering Sciences[1] have now developed a model that can accurately predict surface chemical concentrations for ideal and non-ideal binary mixtures of aqueous and organic liquids. The model is useful for a variety of applications, ranging from synthetic chemistry to heat engineering. The key to this approach is a new equation that answers a fundamental question: how best to express the physical concept of molecular volume at the surface?

Binary liquid surfaces are dynamic places that undergo constant concentration changes due to evaporation, meaning that experimental measurements of composition are not easy to perform. Instead, researchers have to use theoretical models to gauge effects like surface enrichment. However, many of these formulas are based on ideal liquid assumptions, like nearly spherical molecular shapes and the formation of finite monolayers.

Tjahjono and Garland devised a different calculation method based on a parameter called 'parachor' that mathematically connects surface tension—a direct measure of the cohesive energy present at the interface—to molecular volume, which measures size and molecular interactions. "These are two important physical properties for characterizing molecular compositions at the interface," says Tjahjono. "Parachor relates these two properties and therefore can be used to effectively describe surface composition."

While parachor models have existed for almost a century, their use has been limited due to the inexactness of describing surface molecular volume. The researchers turned to density, a factor proportional to volume, to solve this problem. In the bulk of a binary liquid, density can be measured exactly as a function of composition. Surface densities are harder to determine, but

© iStockphoto/prill

Tjahjono and Garland realized that they can be deduced mathematically in a way similar to the bulk, leading to a new surface molecular volume expression.

The modified parachor model performed flawlessly and produced theoretical surface compositions that closely matched known experimental data, no matter how non-ideally the binary liquid behaved. Using the model, the researchers resolved significant surface enrichments effects in organic hydrocarbon–water mixtures—a finding that could have implications in real-world engineering problems, notes Tjahjono. ■

1. Tjahjono, M. & Garland, M. A new modified parachor model for predicting surface compositions of binary liquid mixtures. On the importance of surface volume representation. *Journal of Colloid and Interface Science* **345**, 528–537 (2010).

Green chemistry

Going lead-free is easy

Hydrothermal synthesis offers an easier route to lead-free piezoelectric materials

Lead zirconate titanate (PZT) ceramics are high-performance piezoelectric materials that generate a voltage in response to an applied stress, and change shape in response to an applied voltage. They are an integral component of a wide range of electronic devices, including sensors, transformers and loudspeakers. Previous studies have shown that piezoelectric materials display the best piezoelectric performance when their composition is close to a transition region called the morphotropic phase boundary (MPB). In the case of PZT ceramics, the MPB state equates to a composition of 60% lead by weight. However, the production and disposal of materials with such a high lead content pose serious threats to human health and the environment.

Sodium potassium niobate (NKN) ceramics are a promising lead-free substitute for PZT, having an MPB composition of equal parts sodium and potassium. Unfortunately, the high temperatures required for conventional synthesis of NKN ceramics volatilizes the potassium atoms and can therefore affect the sodium-to-potassium ratio. These processes are also time-consuming and involve many steps, including pre-mixing, ball-milling, calcination and sintering.

Gregory Goh and Albertus D. Handoko at the A*STAR Institute of Materials Research and Engineering[1] have now demonstrated a hydrothermal process for the synthesis of NKN ceramics. The hydrothermal method is not only an environmentally friendly, low-cost technique, it is also already widely used to synthesize nanostructured materials. The researchers added niobium pentoxide to an aqueous mixture of potassium and sodium hydroxides and allowed them to react in a pressure cooker at 200 °C for 24 hours. When the reaction ended, they washed the precipitated powders with deionized water and then dried the solids in air.

X-ray diffraction studies revealed that NKN powders with close to the MPB composition could indeed be obtained hydrothermally, except that the solids were a mix of NKN and sodium niobate. The researchers found that heating the mixed phase at 800 °C for two hours gave a single-phase NKN powder with a composition practically at the MPB. The finding suggests that an atomic exchange process between the potassium and sodium atoms had occurred—similar to that which occurs during calcination in conventional processes.

Photograph of the stainless steel reactor used for the hydrothermal synthesis of NKN piezoelectric ceramics. The hydrothermal process is simple, fast and consumes much less energy than conventional methods for preparing lead-free piezoelectric materials.

"The observation that NKN solid solutions can be synthesized at compositions close to the MPB means that lead-free piezoelectric thin films can also be made by this solution technique," says Goh. "This further extends the potential of this synthesis method, as thin films are essential for miniaturized devices." ■

1. Handoko, A. D. & Goh, G. K. L. Hydrothermal synthesis of sodium potassium niobate solid solutions at 200 °C. *Green Chemistry* **12**, 680–687 (2010).

Microfluidics

A small mix-up

Instability is the key to mixing fluids with markedly different viscosities in microfluidic devices

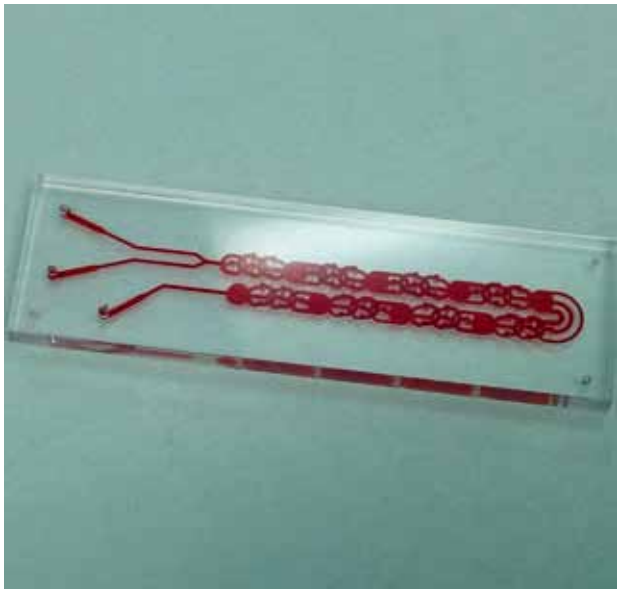
Microfluidics is the technology of designing and fabricating devices to manipulate tiny streams of fluids. In the past decade, scientists have developed a variety of microfluidic devices for use in chemical synthesis and biological analysis. These microfluidic devices often utilize an intricate network of channels and reaction chambers to mix different types of fluids at a precise ratio. Many studies assess the mixing performance of microfluidic devices using fluids with similar properties—in real-life applications, however, the properties of fluids used can vary tremendously.

Huanming Xia at the A*STAR Singapore Institute of Manufacturing Technology and co-workers[1] have now developed a microfluidic device for mixing fluids with different viscosities. The microfluidic device (pictured) features a unique network of interconnected channels and circular chambers that triggers instabilities in the fluid flow. These instabilities add up to produce a turbulent flow that dramatically improves the mixing of fluids.

The researchers evaluated the performance of their microfluidic device in mixing glycerol and sodium hydroxide (NaOH), fluids with a 680-fold difference in viscosity. They identified changes in the fluid interface as glycerol and NaOH move along the microfluidic device.

By adding a pH indicator to the glycerol that turns red upon mixing with NaOH, the researchers found that when glycerol and NaOH met up initially, the interface between the two fluids was relatively smooth, meaning that there was little mixing. However, when the fluids entered the interconnected channels, the fluid interface became notably unstable, indicating strong mixing.

The interconnected channels had parts splitting larger stream of fluids into smaller streams, and parts merging smaller streams of fluids into larger streams. The splitting-and-merging process forced the fluid interface to break up and recombine in a random and chaotic manner. Furthermore, when the fluids entered the circular chambers, the sudden change in flow direction and speed forced the fluid interface to expand and become more unstable.



Photograph of a microfluidic device for mixing fluids with large viscosity contrast.

"A large viscosity contrast usually retards the mixing of fluids. However, it can be turned into a positive factor as viscous flow instabilities can be produced with specially designed configurations. The current design may be integrated into a microfluidic system to meet the mixing requirements for various applications," says Xia.

The microfluidic device could mix glycerol and NaOH even at low flow rates. Best of all, the microfluidic device does not require energy to operate and has the benefit of low fabrication cost. The researchers believe that the new microfluidic device will find use in many applications, such as the formulation of polymers, cosmetics, food and beverages. ■

1. Xia, H. M., Wang, Z. P., Koh, Y. X. & May, K. T. A microfluidic mixer with self-excited 'turbulent' fluid motion for wide viscosity ratio applications. *Lab on a Chip* **10**, 1712–1716 (2010).

Metamaterials

Designed for a response

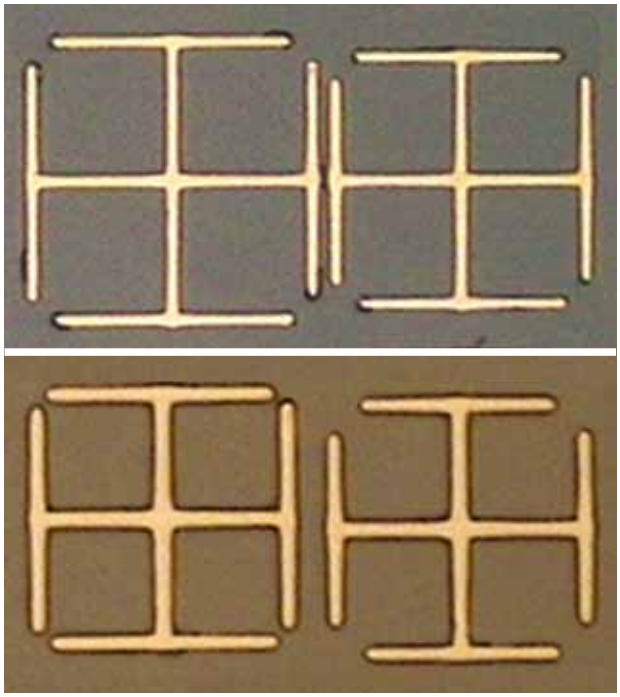
Hybrid designs could greatly enhance the resonant response of terahertz metamaterials

Terahertz radiation—a type of electromagnetic radiation that falls between microwave and infrared—can pass through a variety of non-conducting materials, such as clothing, wood and plastic. Terahertz radiation is therefore ideal for medical imaging and security screening, and is perfect for communication and astronomy because of its ability to penetrate through fog and cloud. However, terahertz-band technology has a fundamental weakness: detecting terahertz signals is almost impossible using conventional materials because such materials do not generate the desired electromagnetic response. For this reason, many scientists have turned to artificial materials for answers.

Metamaterials are artificially designed materials with extraordinary electromagnetic properties. They are designed and fabricated using periodic microstructures in such a way as to interact with and manipulate electromagnetic radiation. Most scientists involved in metamaterials research are focusing on using the technology for making invisibility cloaks and perfect lenses, but there is much more that metamaterials can do. Some studies have demonstrated the possibility of achieving an electromagnetic response to terahertz waves in metamaterials by altering the dimensions of the scattering elements, called split ring resonators (SRRs). However, so far such studies have only achieved this type of response for radiowave, microwave, infrared and visible wavelengths—not for terahertz radiation.

Researchers at the A*STAR Data Storage Institute and the National University of Singapore[1] have now enhanced the electromagnetic response of metamaterials in the terahertz regime by arraying SRRs of various sizes in the same metamaterial (see image). To fabricate the metamaterials, the researchers patterned silicon substrates by photolithography to form planar arrays of SRRs, and then applied a lift-off process to obtain the metamaterial in its usable form. Using terahertz time-domain spectroscopy to measure the electromagnetic responses of metamaterials with various SRR patterns, they found that the resonant response of 'hybrid' metamaterials consisting of SRRs of various sizes was up to 364 times that of metamaterials made up of uniform SRRs.

The transmission spectra of the hybrid metamaterials showed broad resonance peaks, demonstrating that the material responds to terahertz radiation in a way that could be used for signal detection. The researchers found that the characteristics of the resonant peaks were dependent not only on the sizes of the SRRs, but also on the separation between them: resonant peaks became broader as SRRs were brought closer to each other. Computer simulations showed that there was strong mutual coupling between adjacent SRRs.



Two examples of hybrid metamaterials consisting of a planar array of split ring resonators of various sizes.

The researchers could tune the resonant response by varying the SRR design—a novel approach to realizing tunable and sensitive metamaterials for detecting terahertz radiation. ■

1. Lim, C. S. *et al*. Hybrid metamaterial design and fabrication for terahertz resonance response enhancement. *Optic Express* **18**, 12421–12429 (2010).

Biomaterials

Lasers make the cut

Photolithography using a two-photon laser makes it possible to produce precise microstructured scaffolds for tissue engineering

The repair of organs, such as human kidney and liver, hinges on the development of three-dimensional (3D) tissue scaffolds with well-defined microstructures. Andrew Wan, Jackie Y. Ying and co-workers at the A*STAR Institute of Bioengineering and Nanotechnology have now developed a photolithography method that can be used to fabricate microstructured 3D tissue materials with high precision[1].

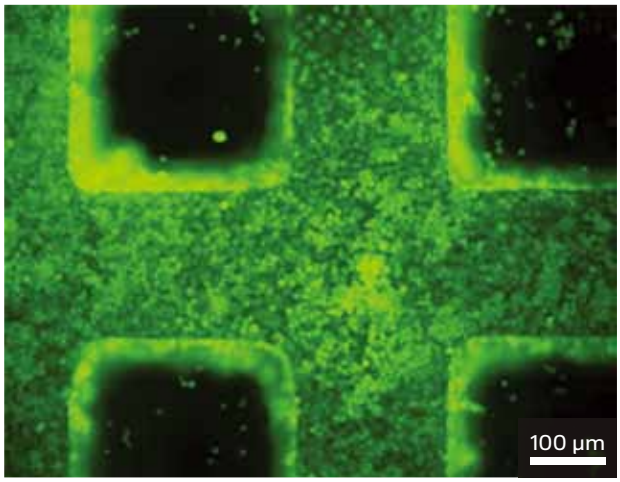
"Fine microstructures are important as they allow us to better define the interactions between cells, which in turn leads to better scaffold function," says Wan.

Typical approaches for the fabrication of scaffolds involve physical processes that lead to poorly defined and heterogeneous pore geometry. One way to resolve this issue is to apply layer-by-layer methods, such as soft lithography, to form layered structures with microscopic internal patterns. However, the resulting materials lack the fine structure desired for advanced tissue engineering.

Wan and his team developed a two-photon laser scanning photolithography technique to produce scaffolds with finer microstructures than can be achieved using previous approaches. The researchers adapted design and manufacturing software for the creation of microscopic objects to the conversion of a drawing of a scaffold into digital information. That information is then translated into an actual scaffold using the laser scanning process. The two-photon laser excites 'crosslinkable' molecules in the polymer to form a dense polymer network that replicates the computerized design. The scaffold can then be obtained by washing away the unreacted molecules using an organic solvent.

The researchers successfully used their approach to fabricate a small cube composed of microscopic pores in just two hours. The scaffold reproduced the original design with high resolution and fidelity, and was also highly transparent and easily observed by fluorescence microscopy—a boon for monitoring cell seeding.

The researchers evaluated the performance of the porous cube for liver tissue engineering and discovered that primary liver cells, or hepatocytes, cultured on the scaffold exhibited good cell attachment, viability and cell–cell interactions (see image). Furthermore, unlike cells seeded on flat polymer surfaces, these hepatocytes produced more of the liver-specific compounds albumin and urea.



A fluorescence microscopy image of hepatocytes growing on a microstructured three-dimensional scaffold fabricated by two-photon laser scanning photolithography.

As complex organs such as the liver consist of more than one cell type, the researchers are currently looking into ways of employing microstructured scaffolds to produce patterned co-cultures of cells. "These 3D spatially defined co-cultures would allow us to better reproduce the function of the tissue or organ that we would like to engineer," says Wan. The researchers add that this technique would be particularly useful for studying cell–drug interactions and developing effective therapies. ■

1. Hsieh, T. M., Ng, C. W. B., Narayanan, K., Wan, A. C. A. & Ying, J. Y. Three-dimensional microstructured tissue scaffolds fabricated by two-photon laser scanning photolithography. *Biomaterials* **31**, 7648–7652 (2010).

Environmental science

Pick your plastic bag

A life cycle assessment study sheds light on the environmental benefits and drawbacks of using bioplastic bags

Plastic bags have become an integral part of our daily lives, used for everything from grocery shopping to product packaging. The massive production of plastic bags, however, consumes vast quantities of non-renewable resources, including crude oil and natural gas. Replacing conventional plastics with bioplastics—plastics derived from renewable biomass such as vegetable starch—is one way to reduce our reliance on fossil fuels, but the environmental benefits and drawbacks of taking such a step have been unclear. Hsien Hui Khoo at the A*STAR Institute of Chemical and Engineering Sciences and co-workers[1] have now conducted a life cycle assessment (LCA) to investigate whether the use of bioplastic bags is indeed good for the environment.

LCA is a technique used for analyzing the environmental impacts associated with all stages of a manufacturing process, and involves compiling an inventory of the energy and resources consumed and emissions and waste generated in the production of a particular product. The researchers used LCA in their study to compare the benefits of using polyhydroxyalkanoate (PHA) bags imported from the USA with those of using polyethylene bags produced locally in Singapore.

Polyethylene is currently the most widely used material for making plastic bags. The production of polyethylene bags requires the extraction and refining of fossil fuels, the conversion of fossil fuels into polyethylene, and the extrusion of polyethylene into plastic bags. The researchers estimate that 1.22 kg of crude oil, 0.4 kilograms of natural gas and 48 megajoules of energy are needed to make 1 kilogram of polyethylene bags.

PHA, on the other hand, is a bioplastic made from cornstarch. The production of PHA bags involves corn cultivation, harvesting, wet milling and fermentation. The researchers estimate that 4.86 kilogram of corn and 81 megajoules of energy are needed to produce 1 kilogram of PHA bags. To their surprise, Khoo and his team found that the energy consumed in the production of PHA bags is 69% higher than that for polyethylene bags.

Although corn growing can help offset carbon emissions via photosynthesis, the researchers found that PHA bag production requires higher energy input during production compared to polyethylene bag production. Therefore, PHA bags can be considered to be environmentally friendly only if the entire production process is performed using renewable energy.

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The researchers caution that before bio-based materials can be regarded as sustainable alternatives to conventional plastics, a few challenges have to be overcome. "The main issue lies in reducing the energy demands of the conversion of biomass into plastic-like properties," says Khoo. ■

1. Khoo, H. H., Tan, B. H. T. & Chng, K. W. L. Environmental impacts of conventional plastic and bio-based carrier bags. *International Journal of Life Cycle Assessment* **15**, 284–293 (2010).

Data storage

Explore new dimensions

An advanced channel model may help push the capacity limits of future magnetic recording media

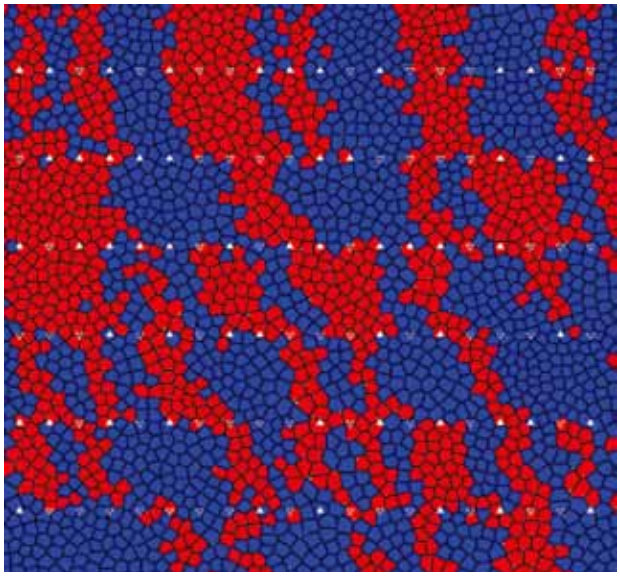
Two-dimensional magnetic recording (TDMR) is a data storage architecture that has recently been proposed as a way to boost the magnetic bit density in hard disk drives. Kheong Sann Chan at the A*STAR Data Storage Institute and co-workers[1] have developed and tested a channel model that allows realistic and computationally efficient simulation of the recording process in TDMR systems, taking into account the magnetic media's magnetization dynamics.

It has become increasingly difficult to raise data storage capacity and performance by downscaling the magnetic grains that comprise magnetic recording media because at such small scales the magnetization orientation that encodes the data bits become thermally unstable. Increasing the magnetic bit density also results in a lower signal-to-noise ratio and inter-track interference—the inability of read/write heads to differentiate between individual tracks on the revolving disk drive platters.

The TDMR architecture aims to circumvent these issues by employing a track-overlapping writing method called shingled writing as well as more powerful but complex two-dimensional (2D) detection and error-correction coding schemes. The main challenges for TDMR involve determining the capacity limits of a suitable 2D magnetic recording channel and developing encoding and decoding algorithms and detectors that can approach those limits while at the same time taking account of the recording medium's microscopic magnetic behavior.

Chan and his co-workers developed a grain flipping probability (GFP) model that can dramatically shorten the time required to simulate the microscale behavior of TDMR systems from days to just seconds. "The model's main purpose is to reproduce the most realistic waveforms possible through micromagnetic simulations, at speeds that simulations cannot match," says Chan.

Micromagnetic simulations solve differential equations numerically to determine the magnetization dynamics of magnetic grains in the presence of a time-varying magnetic field, such as that from a write head. According to Chan, although micromagnetic simulations are the most realistic way to model the behavior of granular magnetic media, such methods are too time-consuming for use in channel simulations as they require millions, or even billions, of data bits to be simulated.



Magnetization profiles calculated using the GFP model for a fine-grained magnetic medium. Red and blue represent opposite polarities of magnetization, and white arrows indicate the polarity of the written bits.

The GFP model requires an initially computationally intensive characterization phase, but subsequently provides fast and accurate 2D readback waveforms that include the effects captured from micromagnetic simulations and statistical effects derived from the granularity of the recording medium itself (see image). The researchers have demonstrated the versatility of their model by implementing and testing three read channel detectors based on the GFP TDMR approach. ■

1. Chan, K. S. *et al.* Channel models and detectors for two-dimensional magnetic recording. *IEEE Transactions on Magnetics* **46**, 804–811 (2010).

Polymer chemistry

Radical predictions

Computer-aided molecular design can help scientists find more efficient catalysts for polymerization reactions

Free radicals may have a bad reputation for causing ozone depletion and premature aging, but they are in fact extremely useful for making novel materials, particularly polymers. Skilled chemists can link up a wide range of free radicals into long-chain polymers using a technique called atom transfer radical polymerization (ATRP). Using different metal catalysts, one can precisely control the rates of polymerization and termination and therefore the architecture and functionality of the polymer.

Fabio di Lena and Christina Chai at the A*STAR Institute of Chemical and Engineering Sciences[1] have now performed the first-ever theoretical modeling of copper-catalyzed ATRP to explain quantitatively how radical polymerization rates are influenced by molecular structures and properties—laying out a critical roadmap for the production of next-generation polymer materials.

The success of an ATRP reaction depends on how well the metal catalyst generates and deactivates organic radicals by intermittently stealing or giving up electrons. If radical production is too fast, the polymerization stops, while sluggish activation or deactivation makes it hard to produce high-quality polymers. Unfortunately, fine-tuning ATRP rates is tricky because researchers must simultaneously optimize many diverse factors such as catalyst and radical geometries, solvents and reaction conditions.

To solve this problem, di Lena and Chai turned to computer-aided molecular design, a technique widely employed in the pharmaceutical drug discovery. They first performed theoretical calculations to extract hundreds of numerical parameters or 'molecular descriptors' corresponding to specific structural and chemical properties for a series of ATRP copper catalysts and organic radicals. They then conducted sophisticated statistical analyses on the data to reveal subsets of principal descriptors that had the most influence over polymerization rates.

Next, the team combined their chemical intuition with stringent testing to further narrow the list of descriptors. Finally, biology-inspired artificial intelligence techniques called genetic function algorithms were used to produce mathematical models that relate ATRP rates to algebraic combinations of descriptors like energy levels, molecular volumes and bond lengths. According to di Lena, these models are striking because they agree with the generally accepted mechanistic picture of ATRP and can provide unprecedented predictive insights.

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"This method should facilitate the design of new ATRP catalysts by screening, in a virtual way, hundreds of metal complexes at time," says di Lena. "Labs will only need to prepare the most promising candidates, saving time and money." Di Lena is also confident that the method will become a powerful tool for developing polymers with tailored properties and functions. ■

1. Di Lena, F. & Chai, C. L. L. Quantitative structure–reactivity modeling of copper-catalyzed atom transfer radical polymerization. *Polymer Chemistry* **1**, 922–930 (2010).

Green chemistry

The heat is on

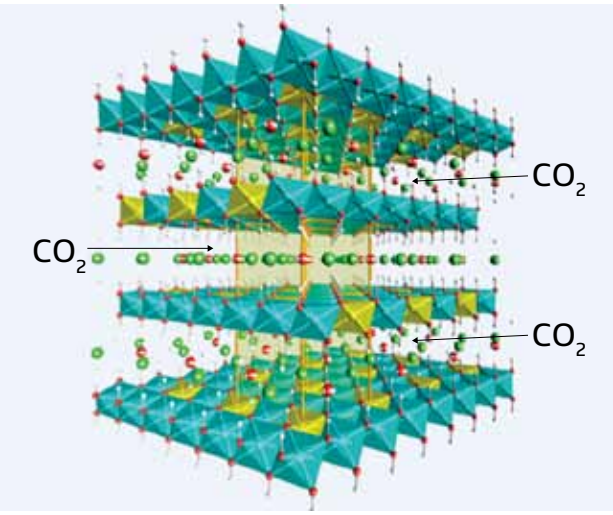
New findings reveal how layered metallic hydroxide crystals can trap carbon dioxide gas at elevated temperatures

'Scrubbing' carbon dioxide (CO_2) from industrial exhaust gases is one of the critical steps needed to reduce CO_2 emissions. It remains a major challenge for researchers, however, to find materials that can reliably soak up CO_2 under the extreme conditions common to real-world industrial processes. A study by Jizhong Luo and co-workers from the A*STAR Institute of Chemical and Engineering Sciences in Singapore[1] now promises to help mitigate CO_2 emissions by uncovering never-before-seen structural details of high-temperature sorption materials called layered double hydroxides (LDHs).

Composed of positively charged sheets of metal oxides interspersed with relatively open spaces holding anions and water molecules (see image), LDHs have large, active surfaces that can react with CO_2 and transform the gas into solid carbonate ions. Recently, scientists have used LDHs as part of an innovative technology called the sorption-enhanced water-gas shift that combines high-temperature hydrocarbon processing with CO_2 removal in a single step. However, when LDHs reach their adsorption limits, they must be regenerated by heating to temperatures high enough to induce an internal structural transformation—a process known as calcination that can eventually destabilize the metal oxide layers.

Luo and his co-workers set out to understand the high-temperature performance of these adsorbents by adjusting the chemical composition of a typical magnesium–aluminum LDH. The researchers replaced the triply charged aluminum cations with iron, gallium and manganese cations and systematically observed how these substitutions affected structure, adsorption and thermal stability. Their results revealed, for the first time, the role such metal species play in LDH-based CO_2 fixation.

Surprisingly, the researchers found that the new cations influenced the physical properties of the LDH more than its chemical behavior. "Generally, people may think that differences in chemical composition between LDHs will lead to different CO_2 adsorption sites, and therefore different carbon capture capacities," notes Luo. "However, our research demonstrates that the temperature-dependent structural evolution of LDHs is a much more important parameter." Luo and his co-workers showed that



Layered double hydroxide crystals can store CO_2 gas molecules between sheets of metal cations (blue octahedra) at high temperatures, paving the way for improved 'scrubbing' of emissions.

distinct calcination temperatures for each LDH compound, as well as a unique quasi-amorphous phase, are key to maximizing CO_2 adsorption levels.

The empirical ground-rules laid out by this study should help researchers select even better candidates for industrial CO_2 scrubbers. "High-temperature CO_2 adsorbents are a hot topic right now in carbon capture and sequestration," Luo says. "In the future, we plan to use combinations of triply charged metal cations to better tune the CO_2 capturing performance of LDHs." ■

1. Wang, Q. *et al.* The effect of trivalent cations on the performance of Mg-M-CO_3 layered double hydroxides for high-temperature CO_2 capture. *ChemSusChem* **3**, 965–973 (2010).

Polymer chemistry

Gel control

The gelation behavior of a biodegradable hydrogel can be controlled precisely by adjustments to pH

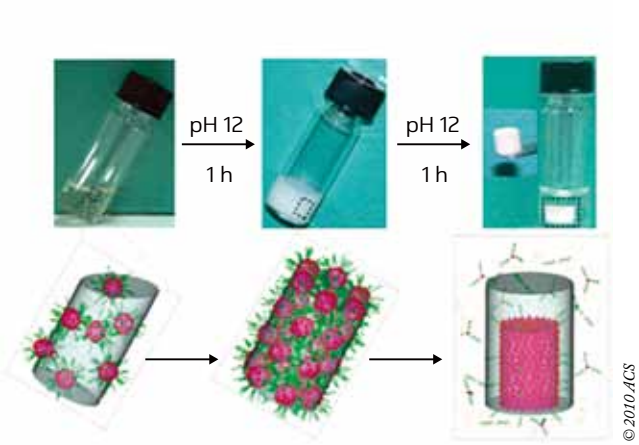
Hydrogels are a unique class of cross-linked polymer that can hold a large amount of water. They are the material of choice for making sensors, wound dressings, contact lenses and a myriad of products with important engineering and biomedical applications. Scientists have developed a diverse range of hydrogels with tunable properties that can be activated or terminated through light, pH or temperature changes. However, there is one critical property they have yet to gain control: gelation—the process that converts the liquid 'sol' phase to the gel phase.

Ye Liu and co-workers at the A*STAR Institute of Materials Research and Engineering[1] have now developed a hydrogel with a gelation process that can be activated, terminated and reinitiated in a precise manner through pH changes. The hydrogel-forming material was synthesized using a one-pot, two-step polymerization approach. The researchers mixed aminoethyl piperazine (AEPZ) with *N,N'*-bis(acryloyl)cystamine (BAC) at a molar ratio of 1:2 to yield terminal vinyl groups, and then introduced polyethylene glycol (PEG) to the mixture to give a solution of a branched polymer called poly(BAC2-AEPZ1)-PEG, or BAP.

Under highly alkaline conditions (pH 12), the BAP solution formed a watery hydrogel that became more viscous over time. Aging the hydrogel for 24 hours or longer caused the BAP to fall out of solution and change into a compact solid hydrogel (see image). The researchers were able to interrupt this gelation process by lowering the pH of the solution, and reinitiate the process by increasing the pH back to 12. The aged compact hydrogel has good stability and is able to maintain its shape in solution for over half a year.

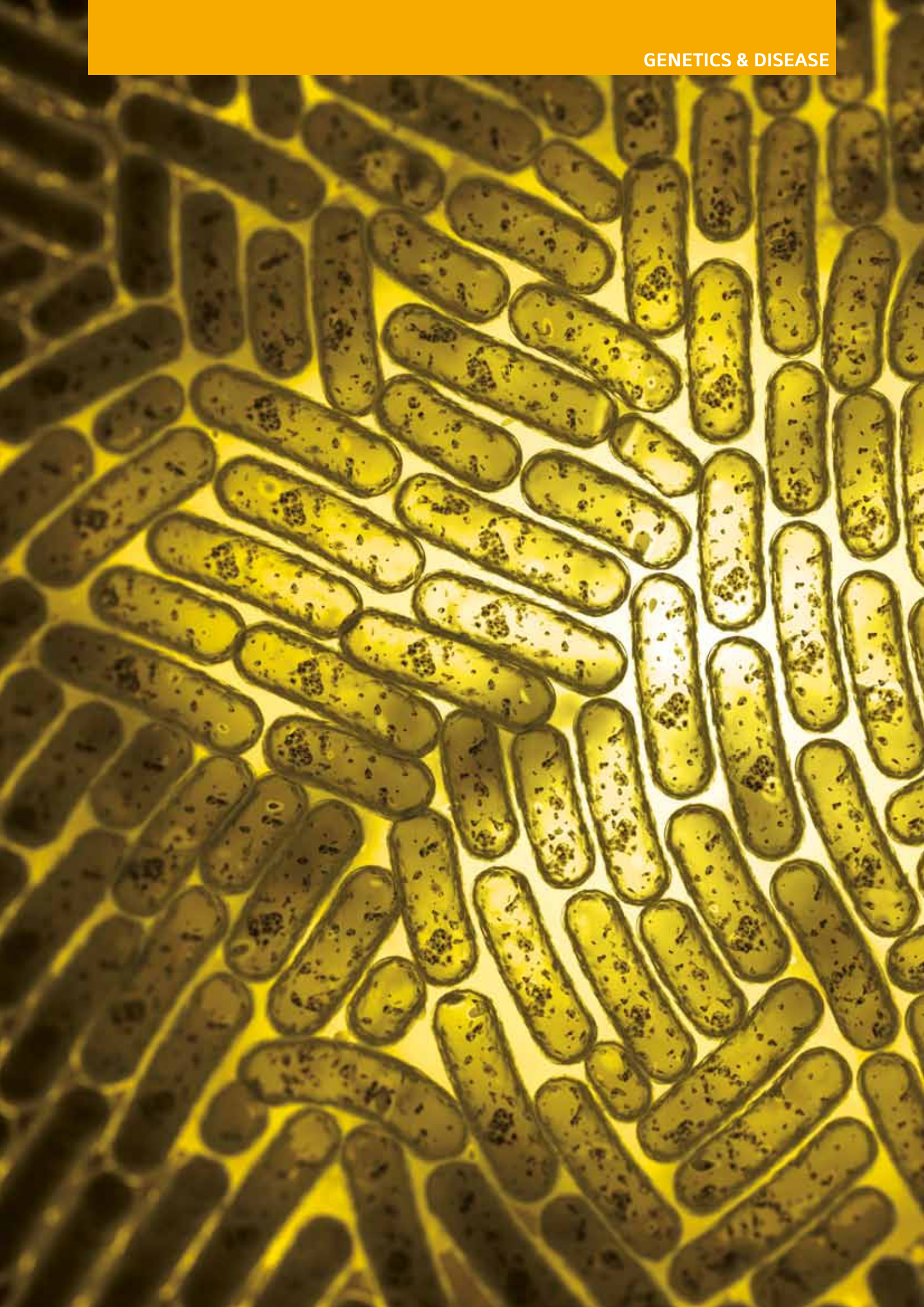
Using nuclear magnetic resonance spectroscopy to analyze the BAP molecules, the researchers found that at high pH, large numbers of BAP molecules aggregate together to form micelle-like core–shell structures. This process is accompanied by thiol–disulfide exchange between BAP molecules, which results in the formation of a stable hydrogel with chemical cross-linkages. As this reaction no longer proceeds below pH 7, the gelation can be controlled easily by changing the pH.

The hydrogel is biocompatible, biodegradable and could easily be made into various shapes and sizes. The researchers believe their approach could be used for the design, synthesis and self-assembly of hydrogels with novel properties. "It is difficult to control the degree of cross-linkages using other approaches once the polymerization has started," says Liu. "To the best of my knowledge, we are the first to have this control in a hydrogel system." ■



Photographs and schematic illustrations of the hydrogel as it changes from the sol form (left) at low pH to a gel form (center) at pH 12 and eventually a compact solid hydrogel (right) on further aging.

1. Wu, D. C., Loh, X. J., Wu, Y. L., Lay, C. L. & Liu, Y. 'Living' controlled *in situ* gelling systems: thiol-disulfide exchange method toward tailor-made biodegradable hydrogels. *Journal of American Chemical Society* **132**, 15140–15143 (2010).



Medical genetics

Susceptibility loci for meningococcal disease

A genome-wide association study identifies genetic factors that increase the risk of meningococcal disease

Meningococcal disease is a life-threatening and debilitating illness that affects people of all ages, but infants and children under five years of age are most at risk. It often begins with flu-like symptoms, such as fever, headache and stiff neck, but can quickly progress into meningitis—inflammation in the lining of the brain and spinal cord—and even septicemia (blood poisoning) and death.

Meningococcal disease is caused by the bacterium *Neisseria meningitides* (see image). Four serogroups of *N. meningitides,* namely serogroups B, C, Y and W135, are responsible for most of the cases of meningococcal disease in the developed world, while serogroup A strains cause devastating epidemics in sub-Saharan Africa. Currently, vaccines are available for all serogroups except for serogroup B, which remains a health threat in many developed countries.

Family studies have shown that host genetic factors are important in determining the susceptibility and clinical outcomes of diseases, but scientists have yet to find the genetic factors that contribute to a higher risk of meningococcal disease. Sonia Davila at the A*STAR Genome Institute of Singapore and co-workers[1] have now conducted a genome-wide association study in Europe and identified two genetic variants—or single-nucleotide polymorphisms (SNPs)—that increase the risk of developing meningococcal disease.

The researchers recruited 475 people with meningococcal disease and 4,703 people without from the UK for initial screening. They sampled over half a million common SNPs and identified an initial set of 79 of interest in connection with meningococcal disease.

To remove false positives, the researchers further performed two replication studies that helped pinpoint two SNPs, rs1065489 on the gene *CFH* and rs426736 on *CFHR3*, that are highly associated with susceptibility to meningococcal disease.

CFH is an important regulator of the alternative complement pathway, an innate component of the immune system's natural defense against pathogens, and *N. meningitides* is known to evade the host immune system by binding itself to CFH. The discovery of the two SNPs suggests that genetic variations on the CFH-related pathway may increase susceptibility to meningococcal disease. The finding could have implications for both vaccine development and potential new therapies.

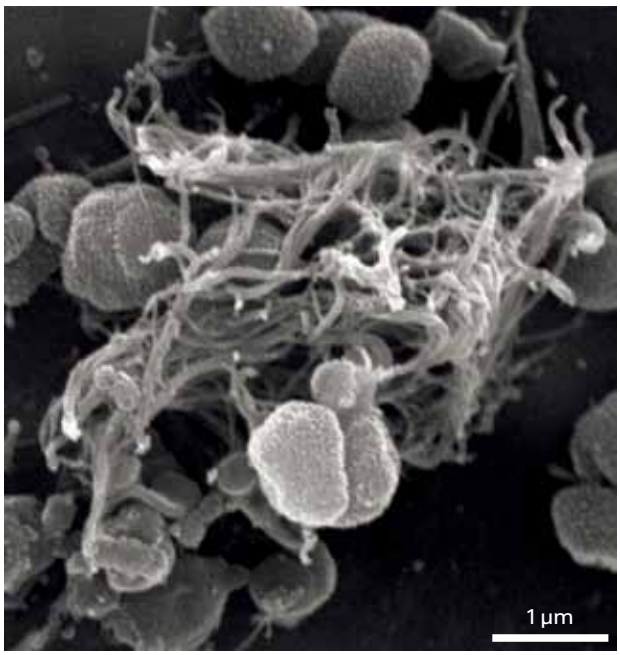


Image: Ariann Hey, Imperial College London, UK

Scanning electron microscopy image of the interaction between host cells and *N. meningitides* 48 hours after infection.

"Genome-wide association studies allow us to interrogate the whole genome in an unbiased fashion and identify genes, as well as genetic variants, involved in human diseases. With advances in the development of next-generation sequencing technologies, we should be able to sequence the complete human genome at a reasonable financial cost in the very near future," says Davila. ■

1. Davila, S. *et al.* Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. *Nature Genetics* **42**, 772–776 (2010).

Metabolism

Another way to keep thin?

A highly truncated receptor protein variant retains the ability to modulate metabolic activity in mice

The brain relies heavily on signals transmitted by fat cells in order to recognize when it is time to stop eating and start exercising. These signals, which take the form of leptin molecules, are detected by a family of specialized leptin receptors in the hypothalamus, called OB-R. Malfunctions in the signaling process are known to cause obesity in both animal models and humans.

The body produces several alternative versions of OB-R, some of which appear to be incapable of initiating the signal cascades normally induced by leptin molecules. The most enigmatic of these receptor variants is OB-Re, which completely lacks a membrane anchor and is released directly into the bloodstream. Weiping Han at the A*STAR Singapore Bioimaging Consortium, Cai Li at the University of Texas Southwestern Medical Center in the USA and co-workers[1] have now teamed up in an effort to uncover the functions of OB-Re.

Normally, such a study would be performed by creating a mouse strain in which the receptor gene is disrupted. However, since OB-Re and other OB-Rs are encoded by the same stretch of DNA, the researchers instead created transgenic animals that produced artificially elevated levels of OB-Re. Surprisingly, the results they obtained were the exact opposite of what they initially predicted.

"As the soluble form shares the same leptin-binding sites with the long-form receptor, we thought that the soluble form would function as a negative regulator in leptin signaling by binding to leptin and thus reducing free leptin availability," says Han. "However, we were completely wrong." After 16 weeks, OB-Re-overexpressing mice weighed less than their normal counterparts (see image), and magnetic resonance imaging revealed a significant reduction in the proportion of body fat in these animals. The transgenic animals also displayed notable changes in metabolic activity, consuming less food but more oxygen. These mice also had higher levels of circulating leptin, with the majority of hormone in complex with receptor molecules.

The researchers have developed several models that might explain these unexpected findings. One possibility is that this soluble receptor form prevents the clearance of leptin from the bloodstream, thereby prolonging its activity. However, Han and Li suggest that the receptor could also be modulating energy metabolism via some yet-unknown leptin-independent mechanism. "We are following up on this question," says Han. "If the soluble leptin receptor has an independent function on its own, it may be quite interesting to pursue targeting it for the treatment of obesity."

A transgenic mouse with over-expression of OB-Re (right) has lower body weight and body fat content than its wild-type littermate (left).

1. Lou, P.-H. *et al.* Reduced body weight and increased energy expenditure in transgenic mice over-expressing soluble leptin receptor. *PLoS ONE* **5**, e11669 (2010).

Immunology

Infection turned protection

Cells that fight herpes viruses also help us fend off other viruses

Viral infections can kill, but a recent study shows that they can also save us. A group led by researchers from the A*STAR Singapore Institute for Clinical Sciences[1] have provided evidence that herpes virus infections in humans can induce an immune response that can contribute to protection against other unrelated pathogens.

Herpes virus infections are very common in humans, affecting more than 90% of the population. They normally cause persistent infection, remaining in the body harmlessly for many years, but are also associated with the development of cancers such as Burkitt's lymphoma.

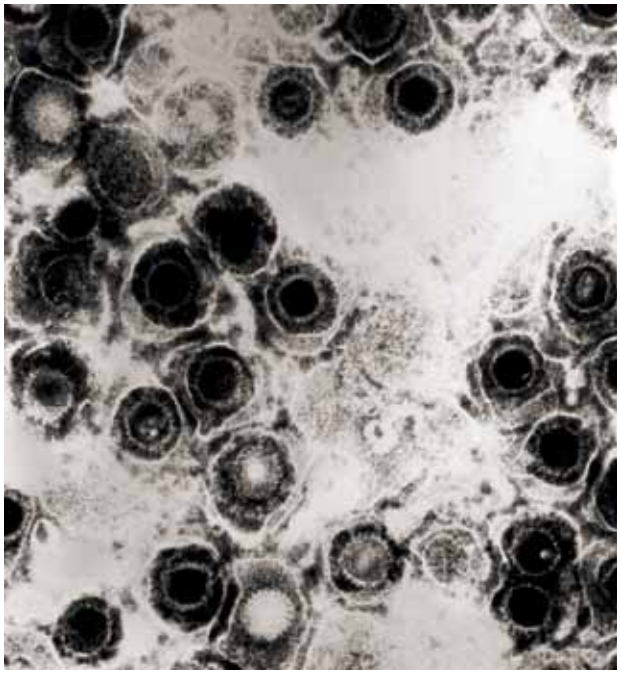
The researchers evaluated CD8 T cells in 50 patients, all of whom carried herpes viruses, such as Epstein Barr virus (EBV) and human cytomegalovirus (HCMV), and who were also infected with other viruses.

T cells are the immune system's first line of defense. When activated by infectious agents, they kill infected cells and retain a memory of the microbe so that they can respond to future infections. Herpes viruses are known to alter the composition of the body's T-cell repertoire, so that up to 20% of T cells are specific to them.

The researchers found that patients with acute hepatitis B virus infection had a greatly expanded T-cell population, containing activated T cells specific not only to HBV, but also to EBV and HCMV. Similar findings were observed in the T-cell populations of patients infected with dengue fever, influenza and adenovirus.

They then went on to investigate how herpes virus-specific T cells might be activated by other infections. They purified T cells from the healthy volunteers carrying EBV and HCMV, then incubated them with signaling molecules called cytokines—known to be produced in response to infection—to mimic the T-cell activation observed in patients with viral infections.

In all of the samples, one particular cytokine called interleukin-15 was found to activate herpes virus-specific T cells. These results suggest that interleukin-15 produced during new viral infections can contribute to the activation of herpes virus-specific T cells.



Herpes simplex virus 1 (shown here under transmission electron microscopy) causes cold sores and is very common in humans.

These findings show that activation of herpes virus-specific T cells is a common feature of the human immune response to infection by other viruses. As well as contributing to the immune response to current infections, this mechanism may also prevent the reactivation of persistent herpes viruses. The researchers believe that the ability of persistent viruses to leave a functional imprint on T cells has deeper consequences that require further elucidation.

1. Sandalova, E. *et al.*, Contribution of herpesvirus specific CD8 T cells to anti-viral T cell response in humans. *PLoS Pathogens* **6**, e1001051 (2010).

Medical genetics

Many a little makes a mickle

Genetic variations related to estrogen production have a cumulative effect on breast and endometrial cancer risk

For women, estrogen is a good thing when everything is working in balance. The hormone promotes the development of female sexual characteristics, such as breasts, stimulates the thickening of the endometrium (the inner lining of the uterus) and regulates many other aspects of the body, such as menstruation and bone growth. However, many studies have shown that when a woman is exposed to too much estrogen, her risk of developing breast and endometrial cancer increases.

To keep estrogen at optimum levels, the body has devised a network of enzymes—encoded by different genes—for regulating estrogen metabolism. Many scientists believe that genetic variations or single nucleotide polymorphisms (SNPs) on certain genes involved in estrogen metabolism may increase breast and endometrial cancer risk. Despite numerous studies, however, scientists have yet to find a metabolic gene or SNP that has a major impact on the development of these hormone-related cancers.

Jianjun Liu at the A*STAR Genome Institute of Singapore and co-workers suspect that breast and endometrial cancer risk may be influenced not by a single metabolic gene or SNP but by 'weak' SNPs on several metabolic genes. They have performed a candidate gene-based association study of the metabolic genes in European populations and identified multiple SNPs that work together to increase breast and endometrial cancer risk[1].

The researchers invited 1,596 breast cancer patients, 719 endometrial cancer patients and 1,730 healthy individuals from Sweden to participate in an initial 'discovery' screening. They interrogated 239 SNPs on 35 metabolic genes using traditional methods, but could not identify any significant association with breast or endometrial cancer. However, when they took the cumulative effect of SNPs into consideration, they found a strong association with SNPs on genes involved in androgen–estrogen conversion.

To confirm their results, the researchers invited another 2,245 breast cancer patients and 1,287 healthy individuals from Finland to participate in a second 'validation' screening. They interrogated 120 SNPs on genes involved in androgen–estrogen conversion and found a strong association as before. They also found through

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statistical analysis that *CYP19A1* and *UGT2B4* are two major metabolic genes associated with breast and endometrial cancer. Interestingly, CYP19A1 is a gene responsible for encoding aromatase, an enzyme responsible for catalyzing the final step in androgen–estrogen conversion—an aromatase inhibitor is currently used for treating hormone-related breast cancer.

The findings demonstrate that seemingly insignificant SNPs, when added up, can have a cumulative effect that can promote certain hormone-related cancers. The knowledge gained through the study could help in the development of better measures for the prevention and treatment of breast and endometrial cancers. ■

1. Low, Y. L. *et al.* Multi-variant pathway association analysis reveals the importance of genetic determinants of estrogen metabolism in breast and endometrial cancer susceptibility. *PLoS Genetics* **6**, e1001012 (2010).

Immunology

The origin of microglia

A large part of the development of brain immune cells takes place at the embryonic stage

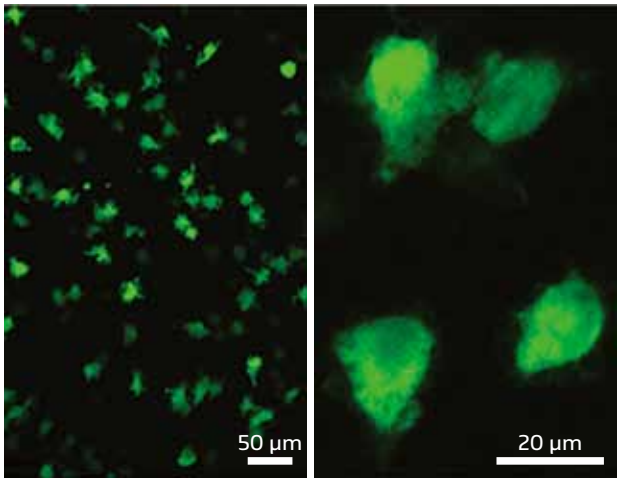
Microglia are macrophages that reside in the central nervous system (CNS), constantly scavenging the brain for damaged neurons, plaque and foreign antigens. The CNS needs the right amount of microglial activity to maintain its wellbeing—too much or too little microglial activity could contribute to the development of neurodegenerative disorders such as Alzheimer's and Parkinson's disease.

Because microglia are so important to CNS health, scientists believe it might be possible to treat neurological disorders through the manipulation of microglia activity. In order to achieve this, however, it is important to understand microglial origins and regulation. A recent study by Florent Ginhoux and co-workers at the A*STAR Singapore Immunology Network[1] has brought us closer to this goal.

Previous studies concerning the origins of microglia had drawn inconsistent conclusions. Ginhoux and his co-workers used innovative approaches to pinpoint where, when and how microglia arise in the CNS. Recent studies had found that microglial progenitors—the precursors of microglia—were already present in the developing brain at birth, but the researchers wanted to find out if these cells were definitive progenitors that will persist in the adult brain and how they came about during the development of the embryo.

To answer this, they used mice that express fluorescent proteins in immune cells and studied embryos of different ages to see when fluorescent cells could first be seen in the brain (see image). At embryonic day 9.5 of the 20 days of mouse development, they detected fluorescent cells not only in the developing brain but also in the yolk sac, which is known to give rise to the primitive macrophages present in early development. To test whether these yolk-sac primitive macrophages were the precursors of microglia, they used a type of mouse that can be made to irreversibly express fluorescence only within these yolk sac macrophages and their progeny. In this model, fluorescence was detected in adult microglial cells, showing unequivocally that microglia come from these yolk-sac progenitors.

These results are especially interesting because they show that microglia, as well as having specialized functions in the CNS, also have a unique origin. They are seeded into the brains of embryos by precursors from the embryonic yolk sac before the precursors of most types of macrophages have even developed.



Fluorescent protein-tagged microglia in the brain of embryonic mice

"Our work reveals the unique origin of microglial cells in the immune system and that there are differentiation processes that only occur during early embryonic life. These results may lead to the development of new strategies for the treatment of various brain disorders," says Ginhoux.

1. Ginhoux, F. *et al.* Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* **330**, 841–845 (2010).

Molecular biology

Taking full control of diabetes

The maintenance of insulin secretion by itself is not enough for the prevention and treatment of diabetes

Type 2 diabetes is a metabolic disorder characterized by high blood glucose levels. Two hormones, secreted by the pancreas, are responsible for regulating the level of blood glucose in the human body. These hormones are insulin, which is released in response to high blood glucose to stimulate glucose uptake in the liver and muscle and so lower blood glucose levels, and glucagon, which is secreted at low blood glucose levels to increase glucose production from the liver and so restore normal blood glucose levels. It is commonly accepted that type 2 diabetes is the result of reduced insulin secretion from the pancreas and impaired insulin sensitivity in peripheral metabolic organs. Several studies have reported an association between elevated glucagon secretion and type 2 diabetes, but this is generally interpreted as a consequence, rather than a cause, of diabetes.

Weiping Han and co-workers at the A*STAR Singapore Bioimaging Consortium[1] now have evidence that dysregulated glucagon secretion, or the lack of inhibition of glucagon secretion in the presence of high blood glucose levels, plays an important role in the development of type 2 diabetes. The researchers studied what would happen when they fed a high-fat diet to normal mice and to genetically modified 'knockout' mice with impaired glucose tolerance, reduced insulin secretion and almost no glucagon secretion. A high-fat diet is known to reduce insulin sensitivity in mice. As the knockout mice had impaired insulin secretion, it was predicted that they would develop high blood glucose levels sooner than the control mice after high-fat feeding.

The biggest surprise was that the control mice had developed high blood glucose, whereas the knockout mice had not. This finding is extremely important because it shows that reduced insulin secretion and sensitivity may not be sufficient to cause high blood glucose; dysregulated glucagon secretion is also needed.

Glucagon and insulin are synthesized by alpha and beta cells, which reside in islets of endocrine cells in the pancreas. Most studies have been devoted to the study of beta cells because of the traditional view that insulin deficiency is the cause of type 2 diabetes, and insulin maintenance has conventionally been the method of choice for controlling diabetes.

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"Our findings demonstrate that diabetes is a bi-hormonal disease. A better understanding of alpha cells and glucagon secretion regulation may help uncover new therapeutic targets for the treatment of type 2 diabetes," says Han. "In addition to promoting glucose-dependent insulin secretion and peripheral insulin sensitivity, maintenance of glucagon secretion control might help prevent and treat type 2 diabetes." ■

1. Gustavsson, N. *et al.* Delayed onset of hyperglycaemia in a mouse model with impaired glucagon secretion demonstrates that dysregulated glucagon secretion promotes hyperglycaemia and type 2 diabetes. *Diabetologia* **54**, 415–422 (2011).

Infectious disease

Desktop diagnosis

An innovative system for detecting and identifying the viruses responsible for infectious disease should facilitate decentralized screening of suspect cases

In 2009, there was an outbreak of a highly contagious influenza strain called H1N1. According to the World Health Organization, there were 8,480 officially reported cases and 72 deaths across 39 countries within a month of the first case surfacing. Although H1N1 ultimately caused relatively few deaths, the event shows that highly virulent influenza strains could spread rapidly around the world and underscores the importance of identifying infected patients quickly.

Conventional influenza diagnoses are often conducted at centralized clinical diagnosis laboratories. The procedures are complex and the diagnosis, even when performed by skilled operators, can take several hours. Mo-Huang Li and Jackie Y. Ying at the A*STAR Institute of Bioengineering and Nanotechnology and co-workers[1] have now developed a fully automated portable desktop system for rapidly diagnosing infectious diseases and successfully applied it for the diagnosis of influenza.

The desktop system has several advantages over existing methods. It is fully automated and does not need to be assisted by highly skilled operators. This makes it potentially suitable for use at immigration checkpoints, train stations, airports and outpatient clinics, rather than having to depend on centralized clinical establishments. Its all-in-one design should also make it cheap to manufacture, and reduces the risks of hardware contamination and accidental viral exposure.

The researchers have shown experimentally that their system can efficiently detect influenza viruses in samples that contain as few as 100 viral particles per milliliter. It does so by applying the real-time polymerase chain reaction (RT-PCR) process to amplify the viral RNA before molecular analysis.

All the operator has to do is to take a swab from the patient, add a solution, and inject it with a syringe needle into a disposable self-contained cartridge (pictured) holding all of the necessary RT-PCR reactants. The entire preparation and diagnostic process is thereafter fully automated and takes just 2.5 hours to complete. The sealed cartridge containing all of the waste products can then be safely disposed of.

The researchers have successfully used their automated system to type and subtype seasonal H1N1 influenza viruses, and showed that the system has comparable sensitivity to that of conventional diagnostic methods. In principle, the system could be used to diagnose other infectious diseases caused by viruses that have many subtypes leading to similar patient symptoms.

Photograph of the self-contained cartridge that houses the necessary reagents and fully automates the essential molecular diagnosis protocols without manual intervention or contamination risk.

"We hope that the use of our portable device will aid the decentralized diagnosis of infectious diseases and alleviate the burden on healthcare personnel in the diagnosis of an overwhelming number of suspect cases," says Li. ■

1. Xu, G. *et al.* A self-contained all-in-one cartridge for sample preparation and real-time PCR in rapid influenza diagnosis. *Lab on a Chip* **10**, 3103–3111 (2010).

Molecular biology

Solving an age-old problem

Genetically modified mice could help scientists find a cure for Hutchinson–Gilford progeria syndrome

Hutchinson–Gilford progeria syndrome (HGPS) is a rare genetic disease that affects one in every 4–8 million newborns. Children with HGPS appear normal at birth, but develop symptoms of aging, including growth retardation, skin wrinkles, hair loss and fragile bones, within the first 12 months. There is no known cure for HGPS, and few children with HGPS ever reach the age of 13—90% of them die from complications of atherosclerosis, such as heart attack or stroke.

Genetic studies have shown that HGPS is caused by mutations in the gene LMNA. In 2003, Colin Stewart and co-workers at the A*STAR Institute of Medical Biology and the National Cancer Institute in the USA reported an *LMNA*-mutated mouse line that exhibits a variety of HGPS symptoms. Seven years later, the same group has used the mouse line to reveal the molecular etiology and pathology of HGPS[1].

Fibroblasts are cells responsible for synthesizing the extracellular matrix (ECM) of connective tissues and collagen in skin cells. The researchers compared fibroblasts obtained from *LMNA*-mutated mice at embryonic day 13 with those obtained from *LMNA*-mutated mice at postnatal day 14–21. They observed proliferation arrest and cell death in postnatal, but not embryonic, fibroblasts.

The researchers also compared the gene expression and growth of *LMNA*-mutated mice with those of normal mice. They found that *LMNA*-mutated mice had reduced ECM and cell adhesion gene expression, as well as abnormal skeletal and vascular development. The findings suggest that mutations in *LMNA* cause problems in postnatal development by altering ECM and cell adhesion gene expression, which in turn, affect skeletal and vascular maintenance.

A question raised by these findings is how mutations in *LMNA*, a gene responsible for encoding nuclear envelope proteins, can have such large effects on the cell membrane and ECM. The researchers therefore analyzed all the signaling pathways that might potentially be affected and found that mutations in *LMNA* could inhibit the canonical Wnt signaling pathway by reducing nuclear localization and transcriptional activity of Lef1.

Composite image showing the skull of a normal mouse (left) and a mouse with progeria (right). Background image is a section from the pulmonary artery of a progeric mouse, showing extensive apoptosis (brown staining) in the vascular smooth muscle.

Defects in canonical Wnt signaling could cause problems in ECM synthesis. The finding suggests that HGPS is a disease of the ECM and connective tissue and that there may be therapeutic targets in the canonical Wnt signaling pathway for HGPS.

"Our findings may also provide insights into how arthrosclerosis develops, as a consequence of vascular smooth muscle defects, during aging," says Stewart. ■

1. Hernandez, L. *et al*. Functional coupling between the extracellular matrix and nuclear lamina by Wnt signaling in progeria. *Developmental Cell* **19**, 413–425 (2010).

Infectious disease

Rapid resistance

A strain of H1N1 influenza virus that rapidly develops resistance to the antiviral drug oseltamivir has emerged

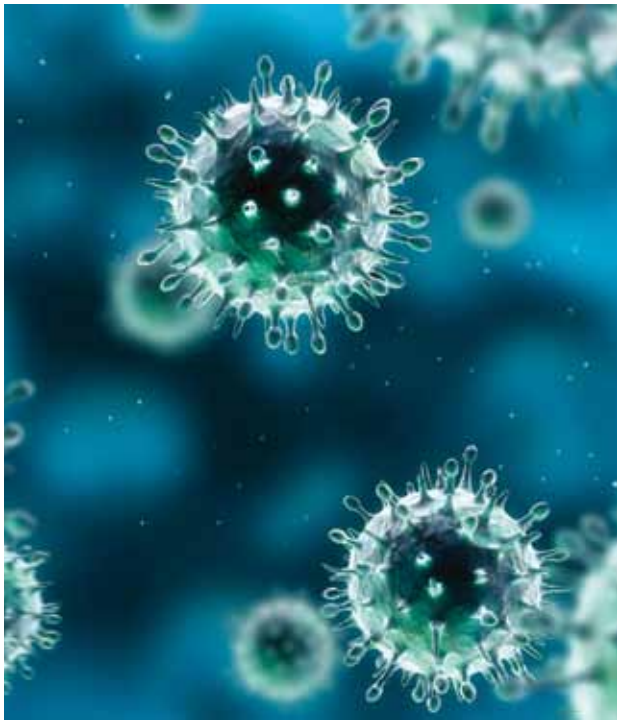
Influenza viruses can become resistant to antiviral drugs by undergoing mutations, and a drug-resistant virus combined with a pandemic could kill millions of people. Masafumi Inoue at the A*STAR Experimental Therapeutics Centre, Timothy Barkham from Tan Tock Seng Hospital in Singapore and co-workers[1] have identified a strain of the influenza A virus subtype H1N1 that developed resistance to the drug oseltamivir more quickly than any case reported previously.

Oseltamivir is an antiviral drug commonly used to fight severe influenza infections. It prevents the spread of viruses by blocking the activity of neuraminidase, a protein found on the surface of influenza viruses that allows them to break away from infected cells. The researchers investigated an H1N1 influenza virus strain that had undergone mutation and substituted the amino acid histidine at position 275 with another amino acid. They found that the mutant was resistant to the effects of oseltamivir and continued to replicate despite treatment.

"The actual mutation occurs not because of the drug but by chance," explains Inoue. "However, the drug kills off all viruses except those that are resistant, thereby enriching the population of resistant viruses."

The researchers sequenced respiratory samples from a female patient in Singapore who came down with flu symptoms soon after a trip to Hawaii. They treated her with oseltamivir on the fourth day of her illness, at the height of her fever. All of the pandemic H1N1 influenza virus particles found in the patient's samples before and 14 hours after antiviral treatment contained the wild-type neuraminidase protein sequence, suggesting that the virus would respond to the antiviral drug. However, 38 hours after antiviral treatment had begun, 24% of the H1N1 influenza virus particles in her respiratory sample were the mutant virus. At 45 hours after treatment, this proportion had risen to 52%. As the patient's wild-type and mutant viruses were otherwise quite similar, the results suggest that the mutant viruses developed within the patient from the wild-type virus that she had initially been infected with.

Oseltamivir treatment in this case was initiated at the height of the patient's fever when H1N1 influenza viral replication—and therefore the possibility for replication mistakes—was likely at its peak. The researchers suggest that this may account for the rapid emergence of the mutant virus in this patient. "If a patient fails to respond to oseltamivir, it would be reasonable to suspect resistance, prompting the use of alternative antiviral drugs," says Inoue. "The findings also remind us that a drug-resistant virus could emerge literally overnight."



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1. Inoue, M. *et al.* Emergence of oseltamivir-resistant pandemic (H1N1) 2009 virus within 48 hours. *Emerging Infectious Diseases* **16**, 1633–1636 (2010).

Immunology

Cells on patrol

A subset of human immune cells monitors blood vessels for viral infection

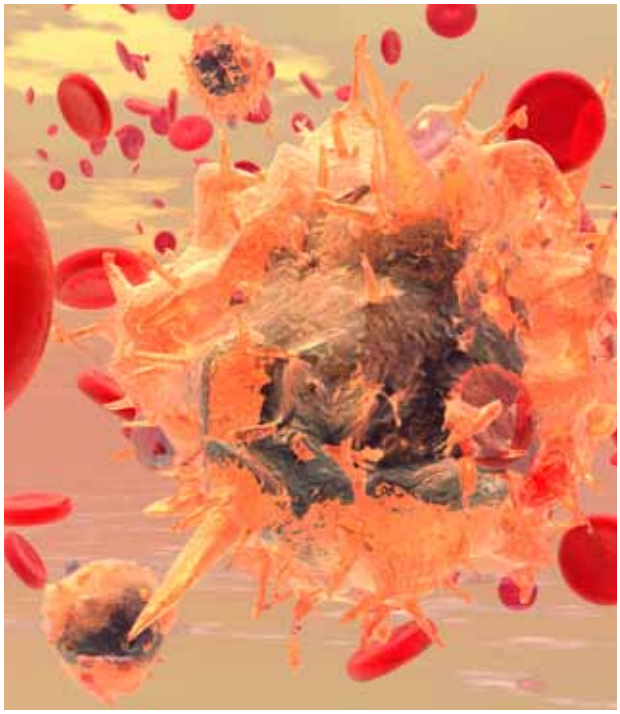
Monocytes are a type of white blood cell that play an important role in the immune system by fighting invading pathogens. Subhra K. Biswas at the A*STAR Singapore Immunology Network, Fréderic Geissmann of King's College London and co-workers[1] have now identified a subset of human monocytes that are involved in the surveillance of tissues that have been injured or infected by viruses.

Mice have two subsets of monocytes for different purposes: one subset secretes proinflammatory cytokines in response to pathogens, while the other subset patrols blood vessels to sense tissue damage. However, scientists were unsure if similar monocyte subsets with corresponding functions exist in humans.

The researchers compared the gene expression of the two subsets of mouse monocytes with that of human monocytes. They found that the expression profile of the proinflammatory subset of mouse monocytes was similar to that for human monocytes of subset $CD14^+$, which express the cell surface protein CD14. The profile of the mouse monocyte subset involved in patrolling tissues, on the other hand, was similar to that for human monocytes expressing the cell surface protein CD16 along with very low levels of CD14, representing a newly resolved subset of monocytes that the authors call $CD14^{dim}CD16^+$.

When the research team injected human $CD14^{dim}CD16^+$ cells into mice, they found that they attached to and patrolled the blood vessels, while the $CD14^+$ cells did not. $CD14^+$ cells were quite efficient at engulfing foreign particles, while $CD14^{dim}CD16^+$ cells were not. These functional differences between the human monocyte subsets were similar to the differences in function between the mouse monocyte subsets.

Exposing $CD14^+$ cells to bacterial cues led to the production of inflammatory cytokines, while $CD14^{dim}CD16^+$ cells were poor responders. However, treatment of the $CD14^{dim}CD16^+$ cells with herpes or measles virus did lead to cytokine secretion. Biochemical and computational modeling revealed that viral activation induced different signaling pathways in the two human monocyte subsets, which may explain why they have divergent cytokine responses to viral exposure. The researchers also observed $CD16^+$ cells in blood vessels in the kidneys of patients with lupus, indicating that this monocyte subset may modulate or induce such autoimmune diseases.



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The similarity between mouse and human monocyte subsets suggests that identifying the role of mouse monocytes in mouse models of disease would be likely to parallel the role of human monocytes in the corresponding human disease. "Identification of human monocyte subsets with distinct functions in inflammation and infection may open future options in the development of more specific strategies to target human diseases," says Biswas.

1. Cros, J. *et al.* Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity* **33**, 375–386 (2010).

Molecular biology

Go back in time

Stem-cell technology can help reveal the pathology of a wide range of genetic diseases, including progeria

Hutchinson–Gilford progeria syndrome (HGPS) is a rare genetic disease that affects one in every 4–8 million newborns. Children with HGPS appear normal at birth, but eventually develop many symptoms associated with aging, such as skin wrinkles, growth retardation, osteoporosis (loss of bone mass) and atherosclerosis (hardening of blood vessels), within their first year of life. There is no known cure for HGPS, and few children with HGPS have ever reached adulthood as 90% of them die from complications, such as heart attack or stroke, at an early age.

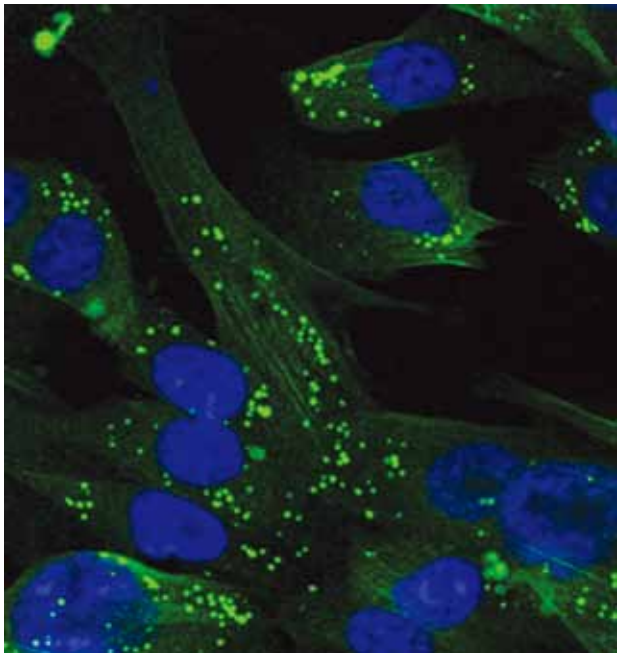
Although HGPS seems to accelerate the aging process, remarkably the disease does not cause neural degeneration, diabetes, cancer or cataracts. It appears that HGPS mainly affects cells that make up the bone, cartilage, collagen, subcutaneous fat and blood vessels. Alan Colman and Colin Stewart at the A*STAR Institute of Molecular Biology and co-workers[1] have now begun to understand the basis of the observed pathology through stem-cell technology.

The researchers obtained skin cells, known as fibroblasts, from two patients with HGPS. They transformed the fibroblasts into stem cells by injecting them with the standard 'Yamanaka's cocktail' formula comprising transcription factors OCT4, SOX2, KLF4, and CMYC.

Stem cells derived in this way are called induced pluripotent stem cells (iPSCs) and have the ability to differentiate into almost any type of cell. The researchers used the iPSCs derived from HGPS patients to generate a variety of cell types, including neural progenitor cells, endothelial cells, fibroblasts, vascular smooth muscle cells (VSMCs) (pictured) and mesenchymal stem cells (MSCs).

Previous studies have shown that HGPS is caused by mutations in the gene *LMNA*. The mutated gene produces the mutant protein progerin, but exactly how progerin causes HGPS is unclear.

The researchers analyzed all their generated cell types and found that progerin levels were highest in MSCs, VSMCs and fibroblasts, and lowest in neural progenitors. Progerin-expressing MSCs and VSMCs had more DNA damage and nuclear abnormalities, and were more sensitive to stress and low-oxygen environment than normal MSCs and VSMCs.



Vascular smooth muscle cells derived from iPSCs generated from a progeria patient's skin cells. Green spots (concentrations of the protein calponin) are not seen in cells from healthy patients.

MSCs are stem cells that can differentiate into bone cells, cartilage cells and fat cells, whereas VSMCs are cells that surround the larger blood vessels. The results therefore suggest that progerin may cause fragile bones, saggy skin and cardiovascular diseases partly by exerting toxic effects on those cell types that accumulate the most progerin and partly by depleting the number of stem cells available for cell replacement. The findings will help scientists develop new strategies to combat HGPS. ■

1. Zhang, J, *et al*. A human iPSC model of Hutchinson Gilford Progeria reveals vascular smooth muscle and mesenchymal stem cell defects. *Cell Stem Cell* **8**, 31–45 (2010).

Immunology

A new weapon against chikungunya

Antibodies derived from an individual who had previously been exposed to the chikungunya virus raise hope of a new therapy

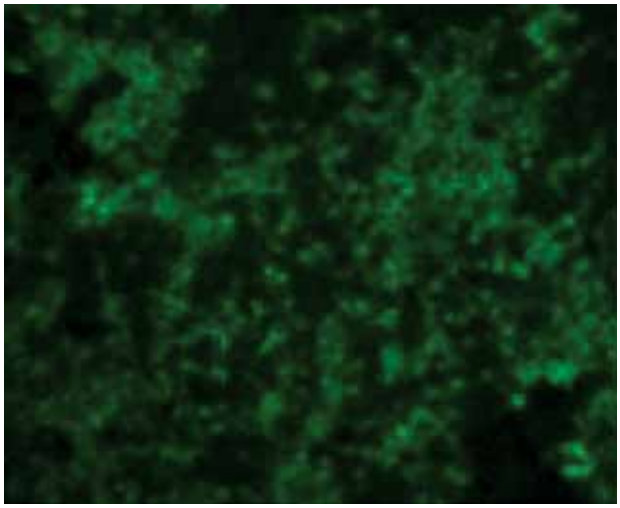
Chikungunya fever, caused by the chikungunya virus, is a mosquito-borne, infectious disease endemic to Africa and Southeast Asia. The symptoms of chikungunya fever range from acute high fever to rheumatoid arthritis (chronic joint pain). There is currently no vaccine or treatment for chikungunya fever, and the disease could be fatal to individuals with a weak immune system. Lucile Warter at A*STAR Singapore Immunology Network and co-workers[1] have now derived from an individual who had previously been infected but subsequently recovered from chikungunya fever two powerful antibodies that could neutralize a variety of chikungunya virus strains.

The researchers isolated antibody-synthesizing cells from the individual, and then characterized the antibodies that these cells produced. In cell culture experiments, they found two antibodies that were able to prevent the chikungunya virus from entering into the host cells and inhibit the virus from spreading from one infected cell to an uninfected one. "These antibodies may be useful not only for protecting human beings from being infected with the chikungunya virus, but also for treating individuals who are infected with the virus," explains Warter.

The antibodies efficiently blocked the infectivity of different chikungunya virus strains obtained from Singapore, Africa and Indonesia. The result shows that the antibodies can target a broad range of chikungunya virus strains and should therefore be useful for the eventual development of a treatment for the chikungunya virus.

The chikungunya virus belongs to a group of viruses called alphaviruses. Although the antibodies could also neutralize a closely related alphavirus called o'nyong-nyong virus, another mosquito-borne infectious disease with symptoms common to chikungunya fever, tests showed they were not effective against other alphaviruses. The antibodies bound to proteins on the chikungunya virus envelope, which is on the outer surface of the virus. The differences between the envelope proteins of the alphaviruses may explain the variable efficacies of the antibodies in fighting infection by the different alphaviruses tested.

"The identification of these chikungunya protein components recognized by human neutralizing antibodies provides insight into the way that the chikungunya virus interacts with its human host," says Warter. While further experiments are needed to determine whether the antibodies can also combat the chikungunya virus infection in vivo in animal models, the isolation and characterization of these antibodies constitute a first step in drug development to fight this fast-spreading virus. In addition, the new findings provide important insights that could help dissect the mechanisms underlying chikungunya virus-associated chronic arthritis. ■



Infected cells stained with anti-chikungunya virus plasma

1. Warter, L. *et al.* Chikungunya virus envelope-specific human monoclonal antibodies with broad neutralization potency. *Journal of Immunology* **186**, 3258–3264 (2011).

Hepatology

A boost in defense

Gene therapy restores adaptive immunity in patients with chronic hepatitis B and directs T cells to tumor cells in patients with hepatitis B virus-related hepatocellular carcinoma

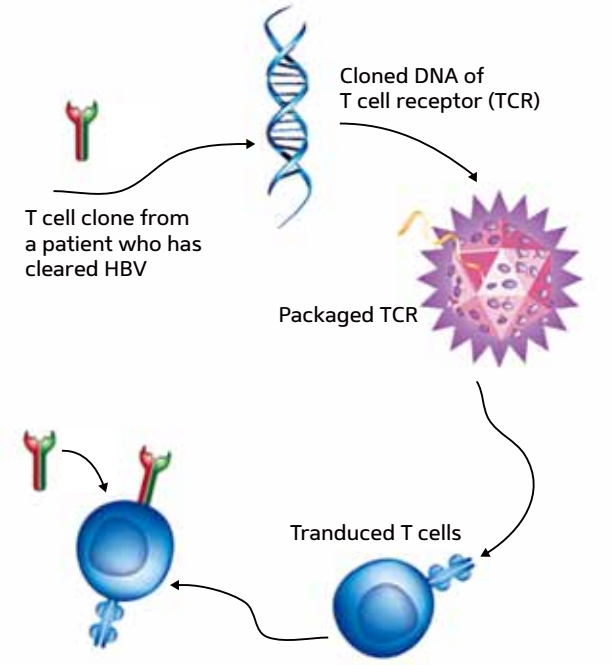
Hepatitis B virus (HBV) is the culprit behind hepatitis B, a type of liver infection that affects people all over the world, particularly those in China, Southeast Asia and Africa. An HBV infection might either be acute (brief) or chronic (long-lasting). Acute HBV infection may cause a variety of symptoms, including yellow skin and eyes, nausea and abdominal pain, but rarely death. Chronic HBV infection is more dangerous and can lead to severe consequences, such as cirrhosis and hepatocellular carcinoma (HCC), that are often fatal.

Patients with acute HBV infection usually do not require treatment because their body is equipped with T cells, a special type of immune cells that is capable of eliminating various virally infected and tumor cells. Unfortunately, T cells that specifically target HBV are either deleted or dysfunctional in patients with chronic HBV infection.

Current treatments, such as antiviral therapy, can reduce the number of HBVs in the body and clear the virus from the bloodstream, but are unable to eliminate HBV completely from the liver or set up immunity against HBV. As a result, patients with chronic HBV infection must pay for expensive treatments and remain at risk of HCC for the rest of their life. Antonio Bertoletti at the A*STAR Singapore Institute for Clinical Sciences and co-workers[1] have now investigated the possibility of using gene therapy to restore the immune response of T cells towards HBV.

The researchers inserted HBV-specific T cell receptor (TCR) genes into T cells obtained from patients with and without chronic HBV infection (see image). They used immunostaining to demonstrate that T cells from both groups of patients expressed the same amount of TCRs on the cell surface after gene transfer. They also used intracellular staining to show that the T cells could produce interferon, tumor necrosis factor and interleukin—cytokines for inhibiting viral replication, tumor growth and microbial infection.

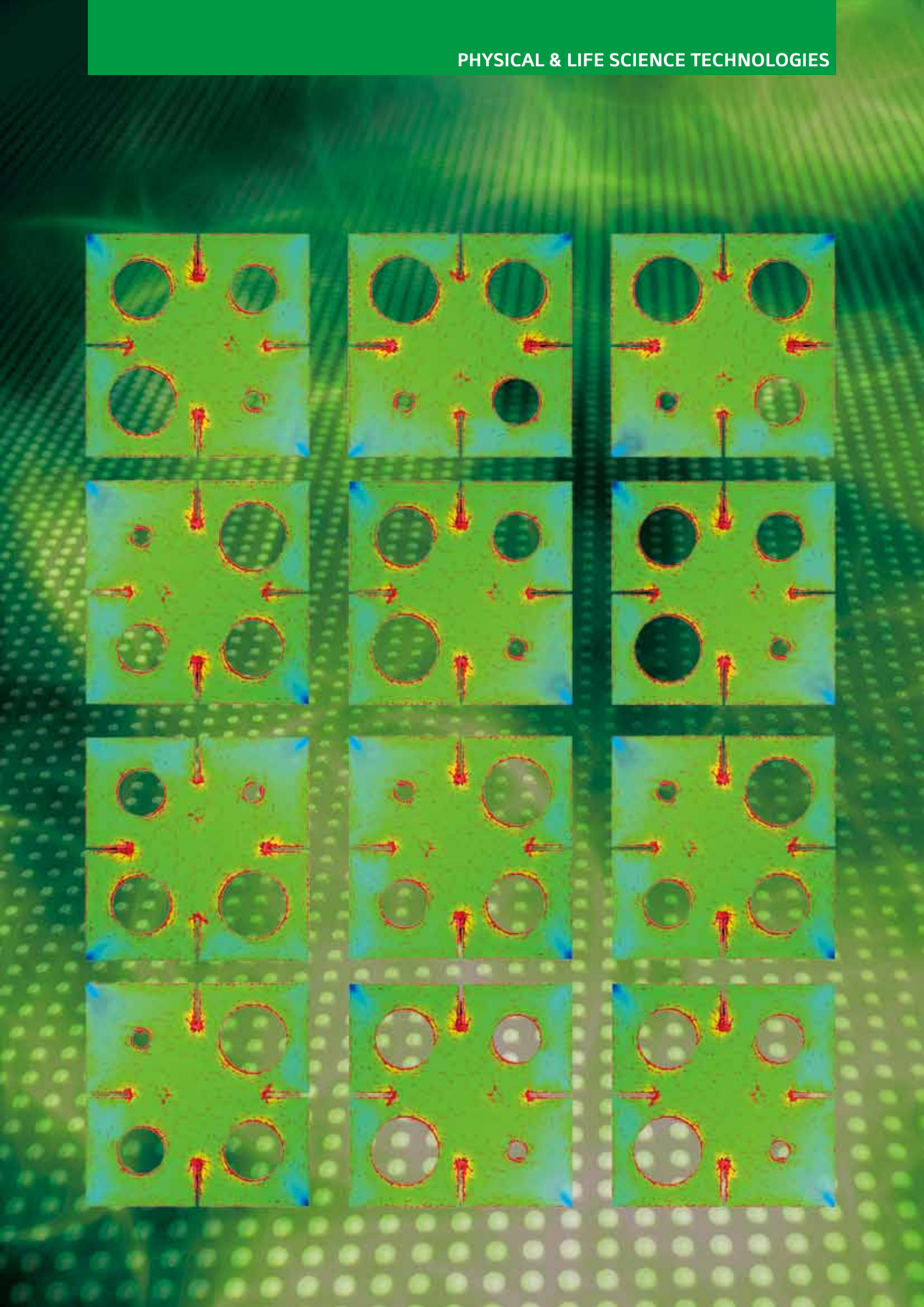
The researchers tested the genetically modified T cells on various cell lines and found that the T cells could destroy HBV-infected liver cells and recognize HCC cells expressing HBV antigen. They also tested the genetically modified T cells on mice and showed that the T cells were effective in reducing tumor growth.



The process of TCR gene transfer inserts gene materials into T cells to restore their immunity

"Our findings suggest that HBV-specific TCR gene transfer could potentially be used in HBV-HCC patients to target tumors and the underlying infection," says Bertoletti. If true, the new gene therapy could help save millions of lives and generate important revenue.

1. Gehring, A. J. *et al.* Engineering virus-specific T cells that target HBV infected hepatocytes and hepatocellular carcinoma cell lines. *Journal of Hepatology* DOI: 10.1016/j.jhep.2010.10.025



Bioprocess engineering

Healing a broken heart

A new bioprocessing system enables large-scale production of heart muscle cells from stem cells

Heart attack is a leading cause of death in the developed world, and although stem cell therapies offer hope for treatment, they have so far met with limited success. In preclinical studies, human embryonic stem cells (hESCs) grafted into the damaged hearts of mice led only to partial repair, because the cells do not efficiently differentiate into functional heart cells.

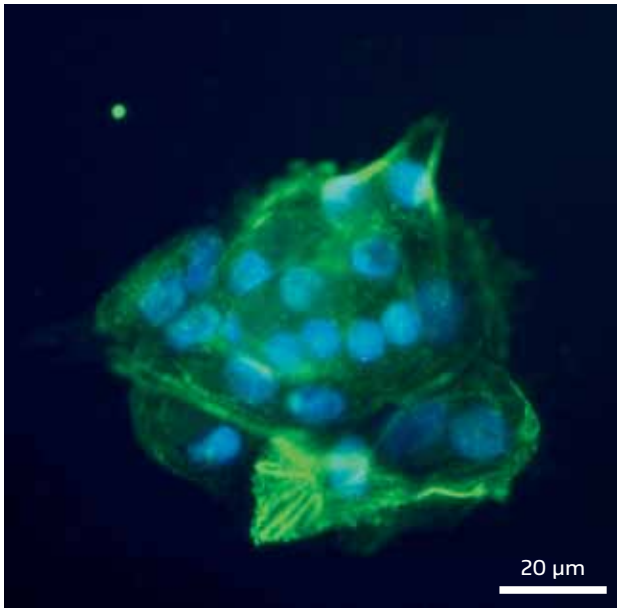
Steve Oh at the A*STAR Bioprocessing Technology Institute and co-workers[1] have now developed a new method for producing heart muscle cells (cardiomyocytes) from hESCs that could eventually lead to an effective cell transplantation therapy for heart failure. The technique involves growing hESCs in 'microcarrier' cultures containing microscopic beads to which the cells can adhere, and inducing them to differentiate into cardiomyocytes by treating them with a compound that inhibits an enzyme called MAPK.

The conventional cell culture method involves growing hESCs in two-dimensional dishes and using expensive growth factors to induce cardiomyocyte differentiation. Under these conditions, the cells aggregate into large clusters called embryoid bodies, and only a small proportion of these differentiate into functional cardiomyocytes.

By contrast, hESCs grown in microcarrier cultures form small aggregates of controlled size that do not clump together (see image), leading to a higher surface-to-volume ratio. As a result, the team achieved a three-fold increase in the number of cells that grew and successfully differentiated. The researchers also showed that the microcarrier culture system can be used for screening drugs and evaluating how toxic they are to cardiomyocytes.

Significantly, the system can be scaled up to produce huge numbers of cardiomyocytes in suspension culture. It is estimated that 1–2 billion of these cells are needed to regenerate the tissue damaged in a heart attack, and according to the researchers' calculations, a 90-liter bioreactor containing microcarrier cultures could produce the 20 billion cells needed for clinical trials.

"The major advantage of our method is the ability to grow cells without growth factors in suspension cultures," says Oh. "This allows for large-volume scale-up in controlled bioreactors, instead of production in hundreds of stacks of disposable plastic trays, which is much more labor-intensive and has batch-to-batch variation."



A cluster of cardiomyocytes stained with an antibody that binds the cytoskeletal protein alpha actinin (green). Nuclei are stained blue.

Furthermore, the serum-free media with MAPK inhibitor is a well-defined system that allows for good manufacturing practice for cell therapies, and there is room to optimize the cardiomyocyte production process. "The method can be improved by supplementing the culture medium with nutrients," adds Oh, "and integrating hESC expansion with differentiation on the microcarriers to achieve even higher yields of cardiomyocytes." ■

1. Lecina, M., Ting, S., Choo, A., Reuveny, S. & Oh, S. Scalable platform for hESC differentiation to cardiomyocytes in suspended microcarrier cultures. *Tissue Engineering Part C: Methods* **16**, 1609–1619 (2010).

Biosensors

Morpholino offers you more

Morpholino-based biosensors are more stable, more specific and more sensitive than conventional biosensors for DNA detection

Molecular biologists and geneticists often use DNA microarrays to detect DNA, measure gene expression levels and detect the single-nucleotide polymorphisms that are responsible for various genetic diseases. A DNA microarray has tens of thousands of biosensors on its surface; each biosensor contains a short DNA fragment, known as a probe, for recognizing DNA targets. Ideally, the DNA biosensor should have high sensitivity (the ability to detect very low concentrations of targets), high specificity (the ability to distinguish the difference between similar targets) and high stability (the ability to withstand wear and tear over many cycles).

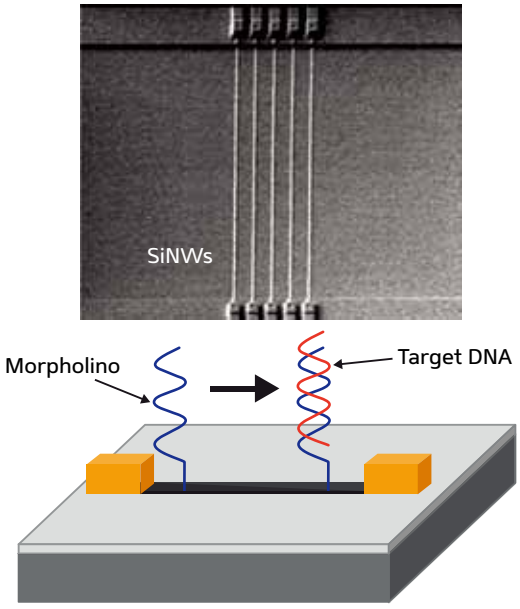
In recent years, scientists have taken an interest in silicon nanowire (SiNW) biosensors as an option to improve microarray performance. SiNW biosensors are more sensitive than conventional biosensors because probes are functionalized onto the silicon surface, which has a large surface-to-volume ratio. However, SiNW biosensors rely on an electric field for DNA detection, so using negatively charged DNA fragments as probes may interfere with measurement.

Guo Jun Zhang and co-workers at the A*STAR Institute of Microelectronics[1] have now overcome this problem by using 'morpholinos' as probes. Morpholinos, first discovered in 1985, are synthetic molecules that structurally resemble naturally occurring DNA molecules. Each morpholino molecule comprises a nucleic acid base, a morpholine ring and a non-ionic phosphorodiamidate linkage. More importantly, Morpholinos have many properties that are superior to DNA, including higher stability and higher specificity.

The researchers fabricated SiNW sensors on a 'silicon-on-wafer' substrate (see image). They then treated the SiNW device with aminopropyltriethoxysilane, glutaraldehyde and morpholino. X-ray photoelectron spectroscopy showed that the morpholino molecules were chemically bonded to the silicon surface through glutaraldehyde molecules, and fluorescence microscopy revealed that the morpholino molecules had been hybridized to the DNA target molecule.

The researchers tested the performance of their morpholino-based SiNW device and found that it was extremely sensitive, specific and stable. The sensors responded immediately after the addition of a sample containing DNA targets to the device, even when the concentration of the targets was in the femtomolar range. Adding a sample containing structurally similar non-targets to the device produced no effect. After 20 cycles of hybridization and denaturation, the fluorescence intensity of the morpholino-modified silicon surface only decreased by about 13%.

Although the Morpholino-based SiNW biosensors are still at an early stage in development, they are expected to have broad application in future DNA diagnostics. ■



Scanning electron microscopy image (top) of a cluster of parallel SiNWs, and a schematic illustration (bottom) of the Morpholino-based sensing mechanism for a single SiNW.

1. Zhang, G. J., Luo, Z. H. H., Huang, M. J., Tay, G. K. I. & Lim, E. J. A. Morpholino-functionalized silicon nanowire biosensor for sequence-specific label-free detection of DNA. *Biosensors and Bioelectronics* **25**, 2447–2453 (2010)

Microfluidics

A thousand captures at a time

A new breed of microfluidic devices can accelerate drug discovery by making it possible to assess the effects of thousands of drug candidates on ion channels simultaneously

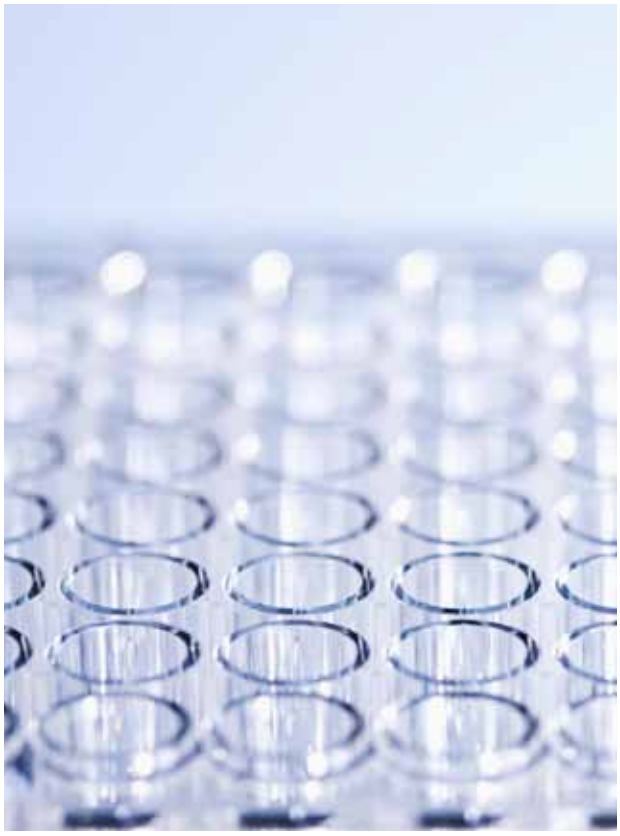
Patch clamping is a widely used laboratory technique to study ion channels in living cells. It is conventionally performed by pressing the open tip of a glass pipette against the membrane of a cell, applying gentle suction to establish a tight seal and then rupturing the cell membrane to measure the flow of ions across the entire cell. This approach works well for small-scale studies, but is very time-consuming for large-scale studies, such as in the evaluation of the effects of a massive library of drug candidates on thousands of cells.

Microfluidics technology makes it possible to miniaturize and automate various laboratory techniques, including patch clamping, which can be implemented by fabricating a microscopic aperture to capture the cell and penetrate the cell membrane. Levent Yobas and co-workers at the A*STAR Institute of Microelectronics previously developed a specialized method to form glass capillaries with well-rounded tips that form a tight seal with the cell of interest in a silicon-based microfluidic chip. The researchers have now combined this technology with a micromolding process for efficient fabrication[1].

The microfluidic chip consists of a silicon microchip substrate and a PDMS (elastic polymer) capping layer inscribed with tiny wells that serve as reservoirs for drugs and other liquids. The researchers used a standard 1,536-well plate as a cast to shape the capping layer, and a silicon substrate containing 1,536 inlets with associated microfluidic networks and glass capillaries. The researchers aligned and bonded the capping layer and the microchip together, ensuring that the wells of the capping layer were aligned with the inlets of the silicon substrate.

For now, the microfluidic device stands as a proof of concept. A single unit (4×4 well array) has been tested with rat basophilic leukemia cells where 12 individual cells were captured by applying suction to the integrated glass capillaries. The remaining 4 wells were used to flow the cell culture and other solutions. In theory, the researchers can scale up the device to capture thousands of cells at a time. The technology will speed up the drug discovery process by providing scientists with a huge array for studying ion channels.

"The use of cheap materials, such as silicon and PDMS, to produce a high-throughput array could dramatically reduce the cost of drug discovery while providing ease of use," says Tushar Bansal, who is currently leading the project. Bansal is currently teaming with other researchers in the field to study multiple drug reactions on insulin cells using the new microfluidic array. ■



Microfluidics technology has helped turn a standard 1,536-well microplate into a high-throughput platform for patch clamping analyses.

1. Tang, K. C., Reboud, J., Kwok, Y. L., Peng, S. L. & Yobas L. Lateral patch-clamping in a standard 1536-well microplate format. *Lab on a chip* **10**, 1044–1050 (2010).

Sensors

A portable device for virus detection

A device that runs on batteries and fits in the palm of your hand can detect viruses in just 35 minutes

Recent infectious disease outbreaks, including the avian flu in 1997, the severe acute respiratory syndrome (SARS) in 2002–2003 and the H1N1 flu in 2009, are reminders of how vulnerable humans are to viruses. Our experience with pandemics has taught us that the early detection of infected individuals is crucial to preventing the spread of such contagions. A team led by Pavel Neuzil, a principal research scientist previously of the A*STAR Institute of Bioengineering and Nanotechnology (IBN), has developed a technology for the rapid detection of viruses that has now been adapted as a portable device for rapid virus detection in the field[1]. The technology could help save millions of lives, particularly in developing countries and remote areas.

Real-time (RT) reverse-transcription polymerase chain reaction (PCR) is currently the most sensitive and reliable technique for detecting viruses. The technology involves making complementary DNA copies of viral RNA, multiplying the number of DNA copies and staining them with a fluorescent dye. The subtle differences in fluorescence intensity reflect the presence and quantity of viral RNA.

Conventional PCR devices are large, heavy and expensive. The two key components that make up the bulk of an RT-PCR device are the thermal cycler and the fluorescence detection system. The thermal cycler, consisting of a heater and sensor, controls the temperature of the sample in a cyclic manner; the fluorescence detection system consists of a metal halide light source or blue laser to excite the fluorescence and a photomultiplier tube to detect the excited light. The researchers miniaturized their PCR device by replacing the conventional aluminum block with a silicon-based micromachined 'lab-on-a-chip', the conventional light source with a light-emitting diode, and the photomultiplier tube with a photodiode.

Tests confirmed that the portable RT-PCR device (pictured) could detect H5N1 viruses in as little as 35 minutes. Not only is the device reliable and fast, it is also cheap to manufacture—some 50 times cheaper than competing devices—and uses little power, making it particularly suitable for field and point-of-care applications. Its cost-effectiveness may even make it attractive as an educational tool.

Low-cost, portable virus detection could help prevent the spread of pandemic disease.

"The detection device is already ready to use in its current form and is suitable for monitoring avian influenza outbreaks, especially in rural areas of developing countries," says Juergen Pipper, a member of the research team and a senior research scientist previously with the IBN. "We can also adjust the system so that it can detect other infectious diseases, including SARS." ■

1. Neuzil, P. *et al.* Rapid detection of viral RNA by a pocket-size real-time PCR system. *Lab on a chip* **10**, 2632–2634 (2010).

Biomedical diagnosis

Automated eye test

The development of an automated system for analyzing images of the retina will facilitate computer-aided diagnosis of eye diseases

Optical coherence tomography (OCT) is an imaging technique that is being increasingly used to diagnose progressive eye conditions such as glaucoma. Shijian Lu and computer scientists at the A*STAR Institute for Infocomm Research have now teamed up with ophthalmologists to develop an automated method for analyzing OCT images[1].

OCT uses a light source with particular properties to generate cross-sectional images of translucent or opaque materials such as biological tissue. It does this by capturing light reflected by internal structures or discontinuities between tissue layers.

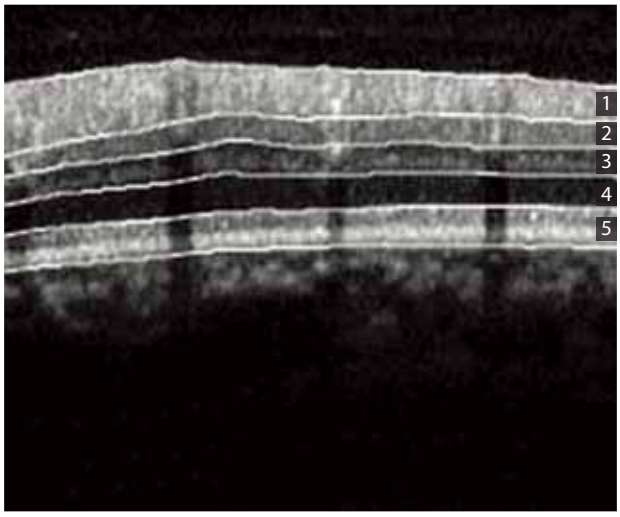
Glaucoma involves the loss of nerve cells from within the retinal nerve fiber layer (RNFL). This feature of the disease can be detected by measuring the RNFL thickness as well as that of other retinal layers from OCT images.

"Accurate and reliable automated analysis of OCT images should greatly increase the efficiency of diagnostic screening," says Lu. Their method implements advanced spectral-domain OCT, which offers faster scanning, improved signal sensitivity and increased image definition. However, the automated segmentation of different anatomical layers within the retina from OCT images remains a challenging task.

Lu and his co-workers developed a system that automatically segments images generated by spectral-domain OCT into five retinal layers, including the RNFL (see image). "Our system is specifically designed for computer-aided diagnosis of glaucoma and other eye diseases, and has features not found in previously proposed methods for analyzing OCT images," says Lu.

The new system first detects retinal blood vessels and then uses these to cut the image into multiple vessel and non-vessel sections. Based on the known retinal anatomy, it then detects boundaries within the non-vessel sections and classifies them into different retinal layers. Lastly, the retinal layer boundaries of the vessel sections are determined by interpolation, resulting in a complete picture of retinal boundary positions. Having determined the boundary positions, the thickness of the RNFL can be measured and quantified for glaucoma diagnosis and the monitoring of disease progression.

To test the performance of their proposed OCT segmentation technique, the researchers used it to analyze spectral-domain OCT images from four healthy subjects at the University Eye Center of the Chinese University of Hong Kong. They showed that that the technique accurately segments an OCT image into five layers, allowing the efficient assessment of RNFL thickness.



Automatic segmentation of a spectral-domain OCT retinal image into five layers. The glaucoma can be diagnosed based on the thickness variation of the nerve fiber layer. 1, retinal nerve fiber layer; 2, ganglion cell layer; 3, inner plexiform/nuclear layer; 4, outer plexiform/nuclear layer; 5, photoreceptor layer.

"We now intend to test the technique using larger-scale datasets and to introduce further improvements that are needed to allow reliable segmentation of layers much thinner than the RNFL," says Lu. ■

1. Lu, S. *et al.* Automated layer segmentation of optical coherence tomography images. *IEEE Transactions on Biomedical Engineering* **57**, 2605–2608 (2010).

Biomaterials

Close to the bone

Porous structures made from a copolymer of polyhydroxybutyrate and polyethylene glycol are ideal scaffolds for bone regeneration

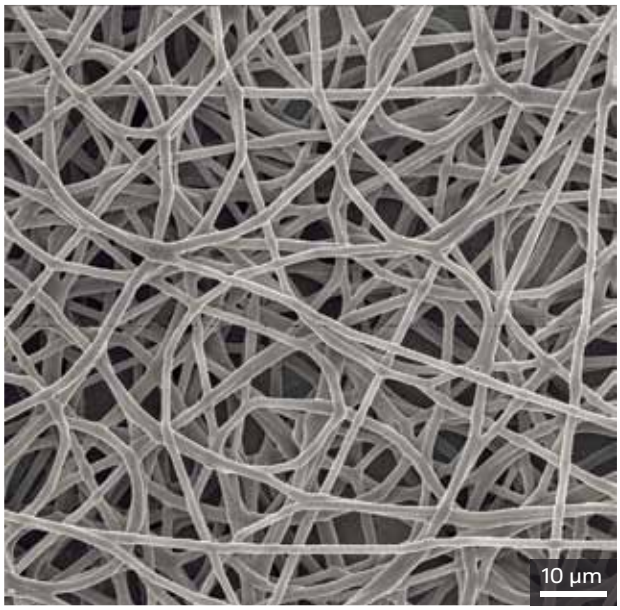
Osteoporosis, or bone deterioration, is a public health problem that affects one in three women and one in 12 men over the age of 50. Patients with osteoporosis have fragile bones and are at risk of hip, wrist and rib fractures. Tissue engineering, which uses stem cells, growth factors and scaffolds—porous structures for supporting cell proliferation, differentiation and tissue formation—to generate new body tissues, is an attractive method for repairing fragile or fractured bones. However, the technology is still at its infancy and there remain a number of hurdles to overcome.

In tissue engineering, the use of scaffolds that mimic natural extracellular matrixes can greatly enhance the quality and success of tissue formation. Li Xu at the A*STAR Institute of Materials Research and Engineering and co-workers[1] have now developed a series of copolymers for fabrication of scaffolds that mimic the mechanical properties, surface chemistry and porosity of the extracellular matrix of human bone. The scaffolds are biodegradable and absorb water and calcium minerals well, making them ideal for bone regeneration.

Polyhydroxybutyrate (PHB) is a biocompatible, biodegradable and readily available polymer produced by bacteria. However, PHB is rarely used as a scaffold material because it is brittle and hydrophobic. Xu and his co-workers made PHB ductile and hydrophilic by incorporating segments of polyethylene glycol (PEG) into the backbone of PHB. They then used an electrically driven technique called electrospinning to weave PHB–PEG copolymer fibers into porous structures (pictured).

Tensile measurements revealed that the developed copolymers could be strained up to 20 times its original length before failure. Laboratory tests showed that the flexible and porous copolymer scaffolds could absorb water much better than PHB scaffolds and retain their structural integrity throughout cell culture work.

When small pieces of the porous structures were immersed into a pool of 'simulated body fluid' containing various salts, including sodium chloride, calcium chloride and disodium hydrogen phosphate, the researchers found that the porous structures could soak up the salts and undergo mineralization without further surface modification—facilitating the formation of an extracellular matrix much akin to human bone.



Scanning electron microscopy image of a porous copolymer scaffold

"Scaffolds made using the new PHB–PEG copolymer may become useful for bone generation, and hence improve the quality of life of osteoporosis patients," says Xu. In addition, the co-polymers are biocompatible and biodegradable, and should therefore cause little transplant rejection in patients. He and his co-workers believe their technique may also be extended to other types of tissues. ■

1. Liu, K. L. *et al*. Designing poly[(R)-3-hydroxybutyrate]-based polyurethane block copolymers for electrospun nanofiber scaffolds with improved mechanical properties and enhanced mineralization capability. *Journal of Physical Chemistry B* **114**, 7489–7498 (2010).

Biomaterials

The right gel for the right cell

It is possible to control the differentiation of stem cells by changing the stiffness of a hydrogel

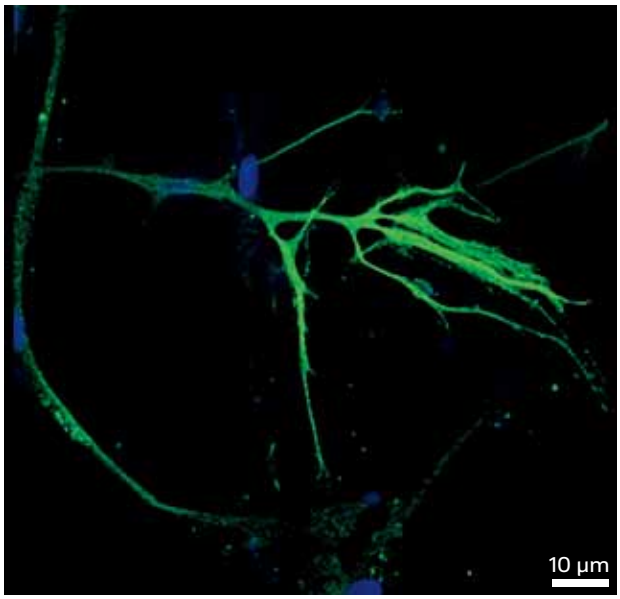
Recent advances in tissue engineering have given scientists the ability to produce a variety of cell types from stem cells using artificial growth media. Some scientists, for example, have grown neurons, muscle and bone cells on collagen-coated polyacrylamide gels from human mesenchymal stem cells. The technique holds the promise of growing functional artificial tissue for repairing or replacing damaged human tissue.

Recent experiments have suggested that the stiffness of the hydrogel used as a growth medium may play an important role in the growth and development of stem cells. Motoichi Kurisawa and co-workers at the A*STAR Institute of Bioengineering and Nanotechnology[1] have now developed a biodegradable hydrogel with tunable stiffness for culturing human mesenchymal stem cells. The researchers studied the effects of hydrogel stiffness on stem cell properties and found that it is possible to control stem cell differentiation—the end product or cell type produced from stem cells—by changing the hydrogel stiffness.

The biodegradable hydrogel was made by adding hydrogen peroxide and horseradish peroxidase to a gelatin–hydroxyphenylpropionic acid (HPA) conjugate. The stiffness and gelation rate of the gelatin–HPA hydrogel could be independently controlled by varying the amount of hydrogen peroxide and horseradish peroxidase used, respectively. This allowed the researchers to alter the mechanical strength of the gelatin–HPA hydrogels without changing the polymer precursor solution or affecting its gelation rate over a wide range of stiffness.

Kurisawa and his colleagues cultured human mesenchymal stem cells on two different hydrogels: one as soft as brain tissue (stiffness of 600 pascals) and one as stiff as muscle (12,800 pascals). They found that hydrogel stiffness strongly affected the attachment, focal adhesion, migration and proliferation of human mesenchymal stem cells. On the stiffer gel, the human mesenchymal stem cells had a larger spreading area, more organized cytoskeletons, more stable focal adhesion, faster migration and a higher proliferation rate.

The researchers also found that hydrogel stiffness strongly affected the differentiation of human mesenchymal stem cells. On the softer gel, the stem cells expressed the protein markers of neurons (see image), whereas on the stiffer gel, the stem cells expressed the protein markers of muscle cells.



Immunofluorescence image of neuronal protein markers expressed in human mesenchymal stem cells differentiated in gelatin-HPA hydrogels.

The findings show that stem cell properties are affected not only by biochemical parameters, but also by physical parameters. A good understanding of how these physical parameters influence stem cell properties is crucial for tissue engineering applications. The hydrogel is also biodegradable, meaning that it could be injected into human wounds to repair damaged tissue. ■

1. Wang, L. S., Boulaire, J., Chan, P. P. Y., Chung, J. E. & Kurisawa, M. The role of stiffness of gelatin–hydroxyphenylpropionic acid hydrogels formed by enzyme-mediated crosslinking on the differentiation of human mesenchymal stem cell. *Biomaterials* **31**, 8608–8616 (2010).

Biomaterials

Finding the right support

Scaffolds that are able to support stem-cell proliferation and differentiation in culture may not have the same effects in the human body

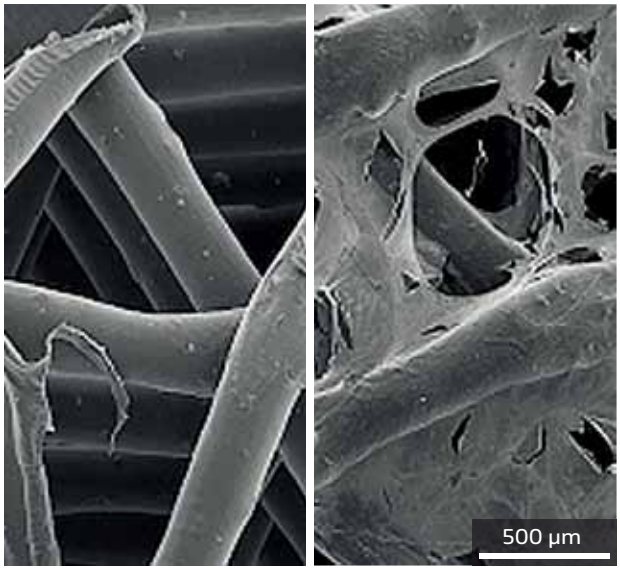
Stem cells have attracted much media attention because of their potential in regenerative medicine. For example, many studies have demonstrated the possibility of using mesenchymal stem cells (MSCs)—stem cells derived from bone marrow—to grow skin, muscle, bone and connective tissues in culture. The general approach involves the use of porous biomaterials, or scaffolds, to support the proliferation and differentiation of MSCs. However, Simon Cool at the A*STAR Institute of Medical Biology and National University of Singapore and co-workers have now discovered that some scaffolds may perform differently in therapeutic applications than they do in experiments[1].

"Human MSCs are being trialed for the treatment of trauma as well as a plethora of acute and chronic diseases," says Cool. "With the amount of investment and clinical activities, there is a growing expectation that physicians will be using MSCs on patients within the next five years. For this reason, scientists are urgently searching for scaffolds that promote the vigorous growth of MSCs in the human body."

Previous studies have used polycaprolactone and tricalcium phosphate (PCL-TCP) scaffolds (pictured) for growing bone tissue. The composite material is highly porous and biodegradable, yet sturdy enough to withstand pressure in the human body. The researchers investigated the regenerative capacity of human MSCs seeded in PCL-TCP scaffolds and compared the difference in culture and in rats with broken femurs.

MSCs flourished on the PCL-TCP matrix, and after several weeks these cells had colonized the scaffold and began to exhibit clear signs of developing into bone tissue, including the expression of differentiation-specific genes and the accumulation of calcium deposits. This process could be further accelerated by cultivating cells with chemicals that specifically stimulate bone-cell maturation.

For their implantation experiments, the researchers specifically chose a sample of donor cells that exhibited the most orthodox MSC-like behavior. However, after three weeks, only three of the six recipient animals displayed regeneration at the implantation site, and the other rats showed no evidence of new femoral bone growth. Moreover, the new bone tissue only covered half of the area that needed treatment.



Scanning electron microscopy images showing the bare scaffold (left) and the growth of human MSCs on the scaffold after two weeks (right).

The findings demonstrate that sometimes *in vitro* data are not sufficient to predict the growth of stem cells in scaffolds *in vivo*. "When we started out, we expected to find strong correlation between how MSCs performed in culture and their ability to heal tissue when transplanted," says Cool. "Instead, our research highlighted the need to develop better benchmarking standards prior to transplantation." ■

1. Rai, B. *et al.* Differences between *in vitro* viability and differentiation and *in vivo* bone-forming efficacy of human mesenchymal stem cells cultured on PCL-TCP scaffolds. *Biomaterials* **31**, 7960–7970 (2010).

Forensics

A call for evidence

Forensic scientists have developed an automated system to extract volatile data from mobile phones

Many people today rely heavily on instant messaging services such as AIM, Windows Live Messenger and Google Talk for communications, and an increasing number of users are accessing these online chat services from their mobile phones. For forensic investigators, such conversations may provide valuable evidence, but retrieving the instant messages from mobile phones remains a great challenge.

Vrizlynn Thing and co-workers at the A*STAR Institute for Infocomm Research[1] have now developed an automated system to extract volatile application data such as incoming and outgoing instant messages from mobile phones running on Google's Android mobile operating system. The forensic system and methodology, in theory, could extend to other mobile operating systems.

Previous experimental groups have used state-of-the-art forensic systems to extract call logs, SMS messages, contacts, emails and images from mobile phones, but attempts to retrieve instant messages have met with no success. The reason for the difficulty is that unlike computers, mobile phones tend to store application data in volatile memory, which is overwritten whenever the user types or sends a new message.

Thing and her co-workers have developed a memory acquisition tool called Memgrab and a memory dump analyzer called MDA for collecting and analyzing volatile information on the Android platform. The Memgrab tool connects to an Android phone and retrieves a bit-by-bit copy of the volatile memory, while the MDA tool decodes and extracts useful information from the retrieved data.

The researchers conducted an experiment to examine the performance of Memgrab and MDA in automatically retrieving and analyzing data during a chat session. They used the Android phone to send 15 messages to a computer and receive 15 messages from the computer in return. They found that, depending on the typing speed and waiting time, the acquisition rate for incoming messages could vary from 75.6% to 100%. However, in all of their tests, their acquisition rate for outgoing messages was consistently 100%.

Based on their statistics, the researchers are confident that their system is capable of capturing close to 100% of instant messages in real-life situations. "Digital forensics is a very important area and technology is advancing at an exponential rate. However, without a more sophisticated mobile device forensics tool, potentially important evidence could be lost forever," says Thing. "To the best of our knowledge, our study represents the first work in the modeling and analysis of dynamic evidence on a mobile phone." The researchers are now applying the methodology and porting the system to other mobile operating systems. ■

1. Thing, V. L. L., Ng, K. Y. & Chang, E. C. Live memory forensics of mobile phones. *Digital Investigation* **7**, S74–S82 (2010).

Modeling

Triumph in the sky

A tool to assess the performance of air cargo supply chain operations provides insights into the effects of industry reforms

The cargo industry has undergone a major transformation over the past 20 years, with air freight now preferred for a wide range of products. In 2006, the air cargo industry was responsible for distributing 35% of all international trade, and the Asia-Pacific region accounted for almost half of all air deliveries.

The spectacular performance of the air cargo industry has fueled not only the development of regional logistics industries but also local economic growth. And as the air cargo industry continues to grow, the necessity for economic, industrial and airport operations reforms becomes inevitable. Xue-Ming Yuan at the A*STAR Singapore Institute of Manufacturing Technology and co-workers at the National University of Singapore[1] have now developed a diagnostic tool called the air-cargo supply-chain operations reference (ACSCOR) model to evaluate the impact of these reforms on airport performance.

The ACSCOR model is quite similar to the SCOR model, the standard tool most industries use for assessing supply chain management practices. However, whereas the SCOR model evaluates performance on three levels, namely customer interactions, product transactions and market interactions, the ACSCOR model covers four levels: the airport, airfreight sector, logistic industry and economy.

The researchers demonstrated the usefulness of the ACSCOR model by applying it to case studies of Hong Kong Chak Lap Kok International Airport and Singapore Changi International Airport—two of the world's busiest air cargo hubs. Using air traffic, capacity and cost data for 2002–2007, the ACSCOR model indicated that air cargo traffic is significantly influenced by the airport's operational efficiency, logistics support and economic environment. They also found that in order for the air cargo industry to remain competitive, the air freight sector must integrate with other supporting sectors in the logistics industry to form a seamless network for cargo transport.

In the case of Hong Kong, cost control was found to be important as it can have negative effects on air cargo traffic. In the case of Singapore, the ACSCOR model finds that enhanced utilization of physical facilities at the airside or landside may be beneficial. "The ACSCOR model shows that Singapore

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Changi International Airport has allowed Singapore's economy to benefit from higher returns in terms of the spillover effect to the overall air cargo supply chain," says Yuan.

The findings demonstrate the usefulness of ACSCOR as a diagnostic tool for providing decision makers with an in-depth insight into the impact of various factors on air cargo traffic. ■

1. Yuan, X. M., Low, J. M. W. & Tang, L. C. Roles of the airport and logistics services on the economic outcomes of an air cargo supply chain. *International Journal of Production Economics* **127**, 215–225 (2010).

Bioimaging

Core targets

A tiny silicon–oxygen-based polyhedron enters cellular nuclei to light them up selectively

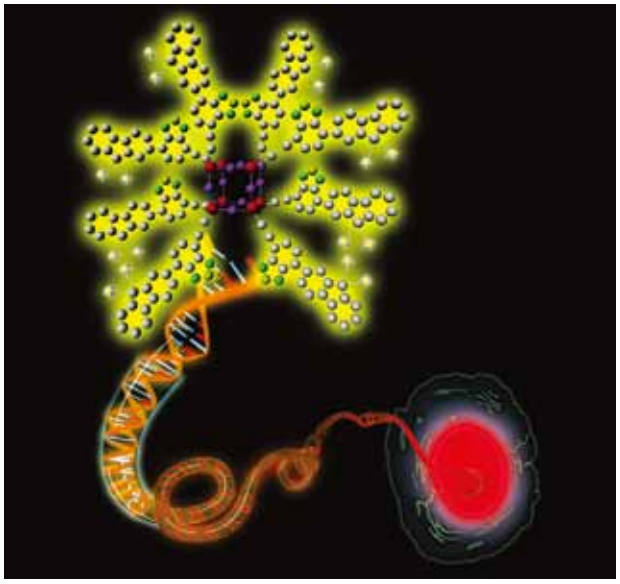
Nuclei are complex, well-defined organelles carrying genetic information that is critical to the cell. Visualizing these organelles through fluorescence imaging techniques promises to reveal the mechanisms that govern genetic information and provide ways to predict and treat genetic diseases. Working closely with Xinhai Zhang at the A*STAR Institute of Materials Research and Engineering, a research team led by Bin Liu at the National University of Singapore has now developed a method to create ultrasmall, highly selective fluorescent nanoprobes for a cellular nucleus imaging technique known as two-photon excited fluorescence (TPEF) microscopy[1].

Researchers have proposed a number of fluorescent substances to illuminate nuclei within cells. However, light-induced phenomena, such as cellular autofluorescence and severe photodamage, tend to degrade the performance of these probes. In the TPEF technique, each nanoprobe produces a fluorescent signal by absorbing not one but two low-energy photons of near-infrared light. This two-photon process significantly reduces the effects of photodamage and cellular autofluorescence while enhancing resolution, making TPEF advantageous over traditional one-photon fluorescence microscopy. "TPEF imaging is more powerful than one-photon imaging, in particular for *in vivo* and tissue imaging where strong biological autofluorescence exists," say Zhang.

Instead of a traditional step-by-step synthesis, the researchers adopted a 'bottom-up' approach to synthesize the nanoprobes for their TPEF scheme. These nanoprobes consist of tiny inorganic silicon–oxygen cages surrounded by short positively charged polymer chains. The team obtained cages and chains separately before joining them together, and the synthesis lends itself well to producing TPEF nanoprobes with various light-emission colors and bio-recognition capabilities.

The small, rigid cages facilitate the incorporation of the probes into cellular nuclei, while the positively charged and light-sensitive chains contribute to water-solubility and optical properties. According to Liu, these features combine to ultimately produce TPEF-suitable light-up probes (see image).

The team discovered that the fluorescence of the probes became substantially more intense upon exposure to nucleic acids, such as double-strand DNA and RNA. This is because the positively charged probes bind tightly to the negatively charged nucleic acids through attractive electrostatic interactions, increasing the micro-environmental hydrophobicity of the probes and their fluorescence. Furthermore, the probes selectively stained the nuclei of breast cancer and healthy cells with low toxicity.



Schematic illustration showing a positively charged nanoprobe (upper left) binding to a negatively charged double-strand DNA molecule (center), resulting in enhanced fluorescence that allows the visualization of a cellular nucleus (bottom right).

The researchers are currently expanding their probe collection to include other intracellular target applications. They are also further optimizing the TPEF performance of the probes. "These nanoprobes can open up new ways of interrogating biological systems in a high-contrast and safe fashion," say Zhang. ■

1. Pu, K.-Y., Li, K., Zhang, X. & Liu, B. Conjugated oligoelectrolyte harnessed polyhedral oligomeric silsesquioxane as light-up hybrid nanodot for two-photon fluorescence imaging of cellular nucleus. *Advanced Materials* **22**, 4186–4189 (2010).

Optics

Mastering bandwidth

Researchers have developed a tunable, low-cost laser device that could help advance fiber-optic communications

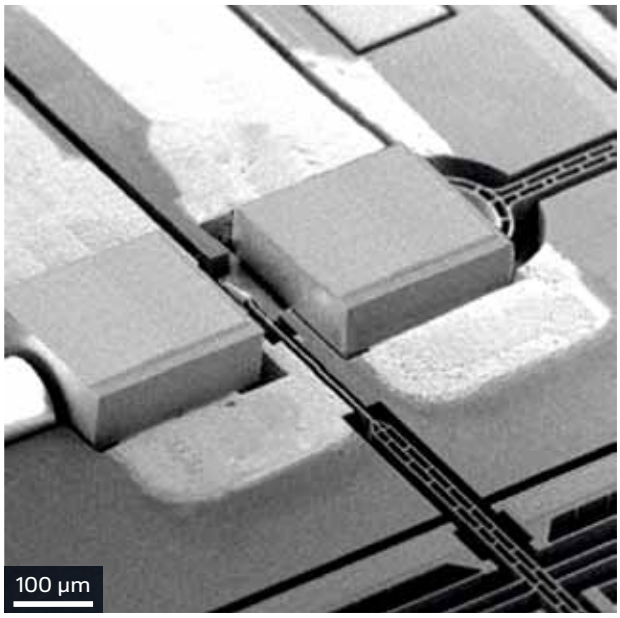
Transmitting information as pulses of light through fiber-optic cables is the fastest and highest-bandwidth communications technology that exists today. Yet even this technology is being pressed to carry ever-greater quantities of information. One way to overcome this problem is to transmit light of different wavelengths simultaneously—an approach known as wavelength division multiplexing. However, the technique requires the use of tunable lasers, which are relatively expensive to produce. Hong Cai at the A*STAR Institute of Microelectronics and collaborators from Nanyang Technological University and Hong Polytechnic University[1] have now developed a low-cost and tunable laser device made specifically for this purpose.

The new laser is constructed using microelectromechanical systems (MEMS) technology to achieve wavelength tunability. By moving a tiny mirror, the laser switches between different operating modes, each of which produces a different wavelength. This tuning capability is built into a 'master' laser, which injects laser light into a secondary 'slave' laser. The slave laser increases the power of the emitted light, suppresses unwanted wavelengths, and allows for the encoding of information by modulating the light intensity. The two-part configuration surpasses the performance of conventional tunable lasers, without increasing bulk or cost.

Cai and her co-workers first calculated how the power and wavelength of the master laser would affect the behavior of the slave laser. They found that the synchronization, or locking, between the two lasers can only be obtained for a certain range of powers and wavelengths, and that the slave output is always shifted to a longer wavelength than the master output.

The researchers then built and experimentally characterized the performance of their lasers. When integrated into a silicon chip (pictured), the laser device is only 3 millimeters long, 3 millimeters wide and 0.8 millimeters thick, and outputs light at wavelengths tunable over a range of 12 nanometers. Most importantly, compared with conventional single-laser devices, the new tunable laser has a narrower spectral distribution, lower noise and higher overall power.

Fabrication of the laser device using MEMS technology means that it can be made in large batches at low cost per device. The small size of the laser device also allows it to be more easily integrated into larger electronic and optical systems, including bandwidth-hungry communication networks. However, there remains considerable development work before this goal can be reached. "Our next steps will be to undergo a detailed physical study of laser operation, allowing us to optimize its performance and then to prototype it to meet the real needs of industry," says Cai. ■



A scanning electron microscopy image of the master and slave lasers integrated onto a silicon substrate to form a miniature tunable laser.

1. Cai, H. *et al.* Discretely tunable micromachined injection-locked lasers. *Journal of Micromechanics and Microengineering* **20**, 085018 (2010).

Biosensors

Hormonal attractions

Ultrasmall silicon wires could detect subtle changes in estrogen receptor-binding DNA sequences that are implicated in breast cancer

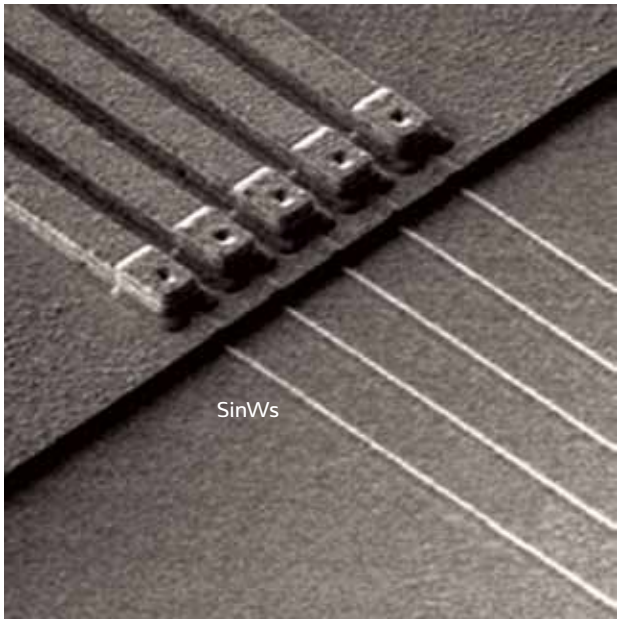
Estrogen receptor (ER) proteins play a major role in controlling the transcription of genetic information from DNA to messenger RNA in cells. Understanding how ER proteins interact with specific DNA regulatory sequences may shed new light on important physiological processes in the body, such as cell growth and differentiation, as well as the development and progression of breast cancer. Guo-Jun Zhang at the A*STAR Institute of Microelectronics and co-workers[1] have now developed a detector that uses silicon nanowires (SiNWs) to evaluate these interactions.

The magnitude of the transcriptional activity that arises from the ER–DNA binding varies from one gene to another. Some genes are highly affected while others are only marginally changed. Zhang and his co-workers therefore investigated how slight variations in nucleotide composition affect the binding affinity between ER and DNA. By combining this new information with existing experimental data on gene expression, the researchers could predict transcriptional outcome following ER–DNA binding and gain new insight into ER signaling.

Most imaging techniques developed for the study of interactions between ER proteins and DNA targets are time-consuming and require the use of fluorescent labels. A number of label-free methods exist, but they lack the sensitivity needed to distinguish subtle changes in ER–DNA binding. The new system created by Zhang's team is both label-free and highly sensitive.

The researchers prepared their ER-based sensor by modifying a nanostructured biosensing platform previously used to detect cardiac biomarkers and the dengue virus. They generated SiNW arrays on a silica substrate (pictured) through optical lithography and covered the silicon surfaces with functional organosilane and organic molecules, which allowed them to immobilize the ER proteins on the nanowires. Next, a well-shaped sample holder, constructed of insulating material, was pasted around the SiNW area.

After exposing the ER-functionalized nanowires with the target DNA, the team measured the change in resistance induced by ER–DNA complex formation to assess the binding affinity. Upon binding to ERs, DNA strands increased the overall increase in resistance of the SiNWs by adding negative charges.



Scanning electron microscopy image of a sensor formed from five individual SiNWs

The researchers discovered that the sensor could detect ultralow levels of ER-bound DNA and discriminate ER-specific from mutant DNA sequences. Moreover, the DNA easily detached from the ER-functionalized nanowires upon contact with a detergent, enabling the regeneration of the sensor.

"The SiNW array biosensor platform is now helping us in the multiplexed characterization of protein–DNA interactions," says Zhang.

1. Zhang, G.-J. *et al.* Highly sensitive and reversible silicon nanowire biosensor to study nuclear hormone receptor protein and response element DNA interactions. *Biosensors and Bioelectronics* **26**, 365–370 (2010).

Quantum physics

A degree of entanglement

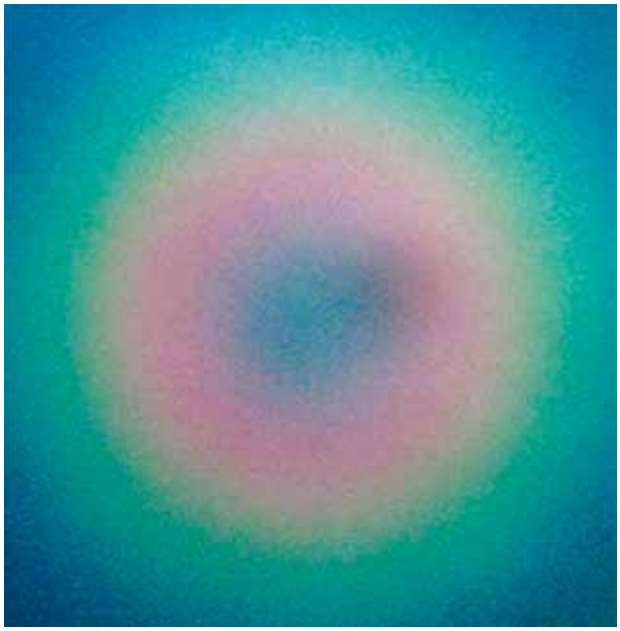
The invisible quantum link between pairs of photons can be tailored for specific applications

Entanglement is one of the most bizarre implications of quantum mechanics. It acts like an invisible link between two distant quantum bodies so that whatever happens to one instantaneously affects the other. Experimentally, this phenomenon has already been verified and studied using light. A relatively simple technique known as spontaneous parametric down conversion (SPDC) can produce photons that are connected in this unusual way, and the method is now used in many laboratories throughout the world. Dmitry Kalashnikov and Leonid Krivitsky at the A*STAR Data Storage Institute[1] have now shown that small differences in the properties of these two photons can affect the degree of entanglement, and that the effect can be put to good use.

When a material with nonlinear optical properties absorbs an incoming photon, it emits two new photons of lower energies. Although these two photons travel in different directions, they remain entangled, meaning that the polarization of one photon is linked to the polarization of the other. "In reality," explains Kalashnikov, "the angle of emission and the energy of each photon can vary within a small band called the SPDC linewidth." This linewidth makes it difficult to observe the polarization entanglement of the photon pair.

Kalashnikov and Krivitsky have now thoroughly investigated how the quantum state of photons emitted at different angles and energies within the linewidth are connected. They shot a continuous beam of laser light at a bulk crystal of beta-barium borate, and detected the photon pairs produced (see image) using two single-photon counters. One counter scanned and measured light at different emission angles, while the other counter detected light after it had passed through a spectrometer that selected photons with a specific energy. This experiment was repeated for various choices of emission angle and energy to get a full map of the polarization state of the photon pairs using a procedure referred to as quantum tomography.

"We found that the generated polarization-entangled states are highly specific to the choice of energy and emission angle," says Kalashnikov. "Thus, it is possible to obtain a wide range of polarization-entangled states using just a single nonlinear crystal. The generated states can be efficiently tailored to fit specific applications by placing a specially designed sample of quartz just after the beta-barium borate."



Light from a nonlinear crystal is emitted over a range of emission angles (as indicated by the concentric circles) and energies (as indicated by the different colors) with various levels of quantum entanglement.

Based on their results, the researchers hope to investigate highly entangled photon states further with the aim of implementing them in quantum information science. ■

1. Kalashnikov, D. A. & Krivitsky, L. A. Spectrally resolved quantum tomography of polarization-entangled states. *New Journal of Physics* **12**, 093040 (2010).

Complex systems

Beat the traffic

A complex network analysis reveals additional information about Singapore's public transportation system

Analyzing a complex network, such as a national transport system, by traditional topological mapping can only tell you so much about the way the network functions. To provide a much richer picture, one needs to analyze the network's dynamics—the flow of passengers over time.

Singapore is the third most densely populated country in the world and its people rely heavily on the public transport system for commuting. The system comprises a Rapid Transit System (RTS) with 93 stations and a wider bus network with 4,000 stations, servicing between them approximately 4.5 million passenger trips each day. To obtain a deeper understanding of how the network functions, Tianyou Zhang at the A*STAR Institute of High Performance Computing and co-workers[1] have carried out a weighted complex network analysis that in combination with traditional topological mapping analysis has revealed additional information about Singapore's RTS.

"The dynamic properties of the Singapore public transportation network differ significantly from the static properties indicated by the topological analysis," says Zhang. "The weighted complex network analysis reveals much more about the parts of the network that are under strain."

For example, the analysis highlighted hub nodes within the RTS network that experience disproportionately high passenger traffic. However, that pattern shifts over time, the analysis showed. In order to study passenger flows within the network, the team used one week's worth of passenger data, spanning seven days of January 2008. The dynamic analysis revealed key differences between travel at the weekend and during the working week. Although fewer trips were made at the weekend, they were to more distinct locations.

Zhang says that more passenger data would reveal further details about changes to the flows within the system over time. "It would be interesting to conduct a larger-scale study in which fluctuations across weeks or even years could be analyzed," he says. "Moreover, if more fine-scale data could be obtained, perhaps on an hourly basis, we could derive and compare the in and out statistics separately for the networks." This could reveal, for example, more about the different travel routes taken in a single day, he says.

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However, the analysis developed by Zhang and his co-workers doesn't just apply to complex transport networks. "We would like to extend our analyses to other classes of networks," says Zhang. "Such networks could vary from natural systems such as gene networks within organisms, to the interconnectivity of one of the most complex man-made networks—the internet." ■

1. Soh, H. *et al*. Weighted complex network analysis of travel routes on the Singapore public transportation system. *Physica A* **389**, 5852–5863 (2010).

Green chemistry

Carbon capture with a copper twist

Recycling of carbon dioxide emissions gets significantly easier thanks to a novel copper–organocatalyst complex

Schemes for storing carbon dioxide (CO_2) emissions underground often make the headlines, but chemically 'fixing' this molecule onto the frameworks of other compounds is a potentially more lucrative proposition. Transforming CO_2 into useful products like polymers and pharmaceuticals could simultaneously reduce greenhouse gases and boost manufacturers' profits. Unfortunately, the expensive metal catalysts and hot temperatures typically needed to break CO_2 apart and rearrange the bonds render this technology too pricy for most applications.

Yugen Zhang and Dingyi Yu at the A*STAR Institute of Bioengineering and Nanotechnology[1] have now discovered a way to make CO_2 fixation more economical than ever before. The researchers developed a mixed copper–organocatalyst system that can convert almost any molecule bearing terminal alkyne groups—outward-facing carbon–carbon triple bonds—into carboxylic acids via CO_2 insertion at room temperature. Alkynyl carboxylic acids have extensive applications in synthetic chemistry, so the discovery could have wide-reaching impact.

Adding CO_2 onto a hydrocarbon is an energy-intensive process that generally requires the creation of a new carbon–carbon bond. Consequently, chemists have been hunting for metal catalysts that can lower these energy barriers to cost-effective levels. Recent studies have identified copper complexes as promising catalysts because they could activate a variety of substrates by bonding to the carbon atom and then incorporating CO_2 in between the carbon and its neighboring metal atoms. However, copper-catalyzed additions of CO_2 to terminal alkynes have had limited success so far.

Zhang and Yu discovered that previous experiments were simply too hot to stabilize the important copper–alkyne intermediates. By mixing a copper catalyst containing a basic ligand with alkyne molecules and CO_2 at room temperature, instead of the usual 100 °C, the researchers obtained several types of alkynyl carboxylic acids in excellent yields. "We could achieve broad tolerance for different substrates because we use mild reaction conditions—no strong base or acid, no heating, no oxidant or reductant," says Zhang.

However, alkynes containing deactivating 'electron-withdrawing' substituents remained stubbornly inert with copper catalysts. To resolve this, the researchers synthesized N-heterocyclic carbenes (NHCs)—molecules with proven CO_2-activating behavior—into a large, robust polymer. When used as a copper catalyst ligand, the unique structure of poly-NHC enabled it to surround the metal and enhance the chances of CO_2 conversion, boosting yields from 2% to 70% for a typical electron-withdrawing alkyne. Furthermore, the solid structure of the poly-NHC–copper catalyst makes it compatible with industrial systems, a technological advantage that Yu and Zhang are currently investigating. ■

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1. Yu, D. & Zhang, Y. Copper- and copper–N-heterocyclic carbene-catalyzed C–H activating carboxylation of terminal alkynes with CO_2 at ambient conditions. *Proceedings of the Natural Academy of Sciences* **107**, 20184–20189 (2010).

Antennas

Running circles around it

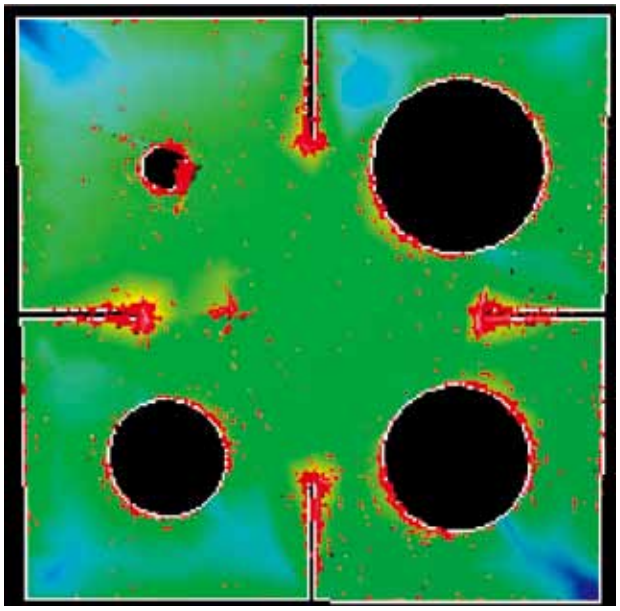
An antenna design relying on circular holes provides strong performance for radiofrequency identification

Radiofrequency identification (RFID) is a technology that grants users the ability to track the origin, destination and characteristics of any RFID-tagged product in a warehouse simply by using an RFID reader. The ideal handheld RFID reader should be small, but the limit of miniaturization for such devices is often restricted by the size of the reader's antenna, which must be able to receive signals in the ultrahigh-frequency (UHF) range of 840 to 960 megahertz. Nasimuddin and co-workers at the A*STAR Institute for Infocomm Research[1] have now demonstrated a new, compact antenna design with superior sensitivity for UHF signals.

The antenna consists of one to four circular holes cut out of a square patch (see image). By giving each of the holes a different radius, Nasimuddin and his co-workers made the antenna extremely sensitive to circularly polarized radiation. This allowed the antenna to operate without maintaining any particular orientation relative to a signal source, in this case the RFID tag. In addition to the variously sized circular holes, the antenna has four long, uniform and symmetrically arranged slits that can be customized to achieve the desired operating frequency range without affecting the antenna's polarization sensitivity.

The final, constructed antenna was designed to operate at 900 megahertz; it had circular holes of between 6 and 12 millimeters in radius, a side length of 90 millimeters and thickness of about 5 millimeters, making the antenna smaller than other antenna designs suitable for similar frequencies, including designs that rely on truncated corners. Part of the reason for the reduced size is the long, meandering path taken by the current flowing across the antenna's surface, which brings the operating frequency closer to the UHF range without requiring a physical increase in antenna size.

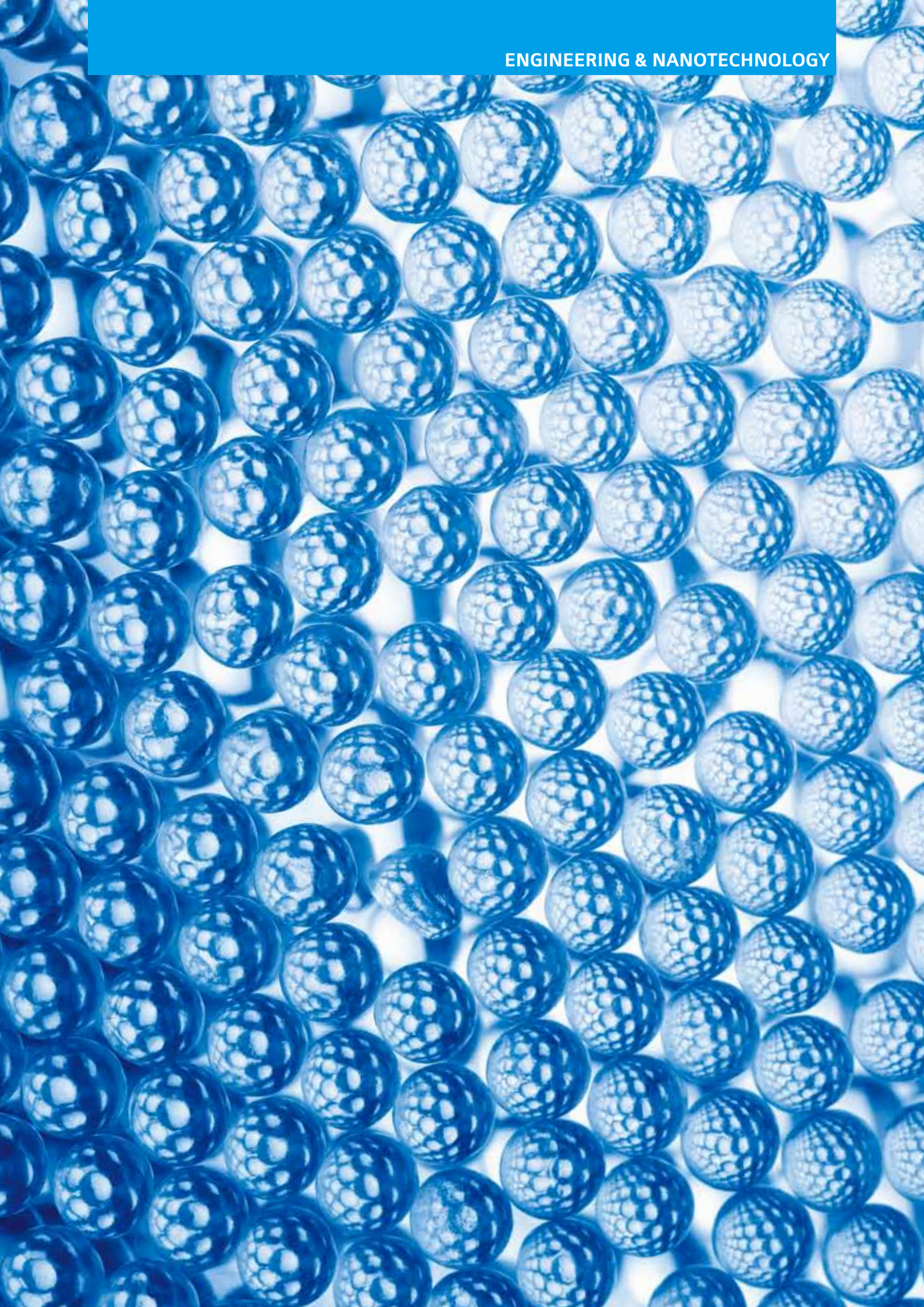
The antenna was able to sustain reading ranges of up to 96 centimeters, depending on the dielectric constant of the substrate that was used. It was also able to detect circularly polarized radiation over a wide range of incoming angles with an angular width of 100°. In addition, by varying the slit widths, the research team was able to tune the operating frequency over a range of tens of megahertz.



A color map showing the current distribution around an asymmetrically holed and symmetrically slotted antenna for UHF applications.

The combination of these strong performance characteristics and the antenna's tunability and small size make the asymmetrical design particularly versatile and attractive. "Our proposed antenna design may become useful for portable and handheld wireless devices, such as handheld RFID readers, medical implant device readers and small portable wireless devices," says Nasimuddin. ■

1. Nasimuddin, Chen, Z. N. & Qing, X. Asymmetric-circular shaped slotted microstrip antennas for circular polarization and RFID applications. *IEEE Transactions on Antennas and Propagation* **58**, 3821–3828 (2010).



Applied physics

A head-to-head comparison

Hard disk drives could cram ten terabits of data per square inch of area using a new read head design

To keep up with market demand, the technology roadmap has the areal density of hard disk drives (HDDs) reaching ten terabits per square inch (Tb/in^2) by around 2015. Increasing the areal density to such levels requires the magnetic domains for storing data to be made extremely small, with an equally small but sensitive read head for reading the data.

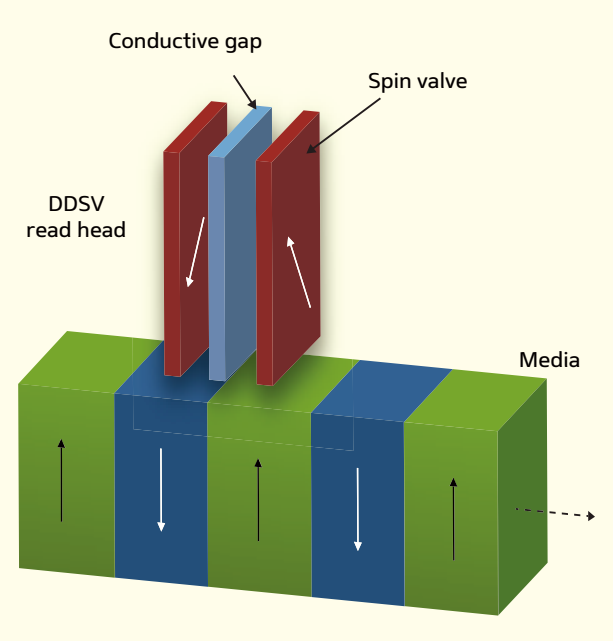
Many manufacturers have used 'spin valves' for reading data at high densities. The read heads in such systems consist of multilayered sensors that exploit the giant magnetoresistance (GMR) effect to detect extremely weak magnetic fields. When the spin valve passes over a magnetic domain, the magnetization of one of the sensor layers (the 'free' layer) rotates, generating a dramatic change in electrical resistance, or GMR, that can easily be measured.

Unfortunately, the magnetization in the sensor's reference or 'pinned' layer is prone to noise from adjacent magnetic domains. Manufacturers have improved the magnetic stability of these read head by replacing the pinned layer with a synthetic antiferromagnetic (SAF) structure and embedding the spin valve sensor between two magnetic shields. However, the SAF structure occupies a large portion of the spin valve's total thickness and manufacturers have difficulty reducing this thickness below 20 nanometers.

Gu Chang Han and co-workers at the A*STAR Data Storage Institute[1] have now explored the feasibility of using differential dual spin valves (DDSV) in ultrahigh-density (>10 Tb/in^2) HDDs. A DDSV, essentially two spin valves separated by a conductive gap layer (see image), only responds to a localized field transition and therefore requires no magnetic shield to filter out noise.

The researchers fabricated two DDSVs for comparison. One DDSV, called DDSV-A, comprised a simple spin valve, a conductive gap layer and a spin valve with an SAF structure. Another DDSV, called DDSV-B, comprised a spin valve with an SAF structure, a conductive gap layer and a spin valve with a double SAF structure.

The researchers measured the electrical resistance of both DDSVs as a function of magnetic field and found DDSV-B to have a higher pinning field with higher magnetic stability. As no magnetic shield is required for filtering out noise, the reading resolution of the DDSV is limited only by the thickness of the gap layer and free layers—typically just several nanometers. "The DDSV allows higher reading resolution, which enables more data to be crammed into HDDs," says Han. ■



Simplified schematic illustration of a read head based on a differential dual spin valve (DDSV).

1. Han, G. C., Qiu, J. J., Wang, C. C., Ko, V. & Gao, Z. B. A differential dual spin valve with high pinning stability. *Applied Physics Letters* **96**, 212506 (2010)..

Laser patterning

Below the surface

New laser engraving technology can be used to make markings inside a block of stainless steel

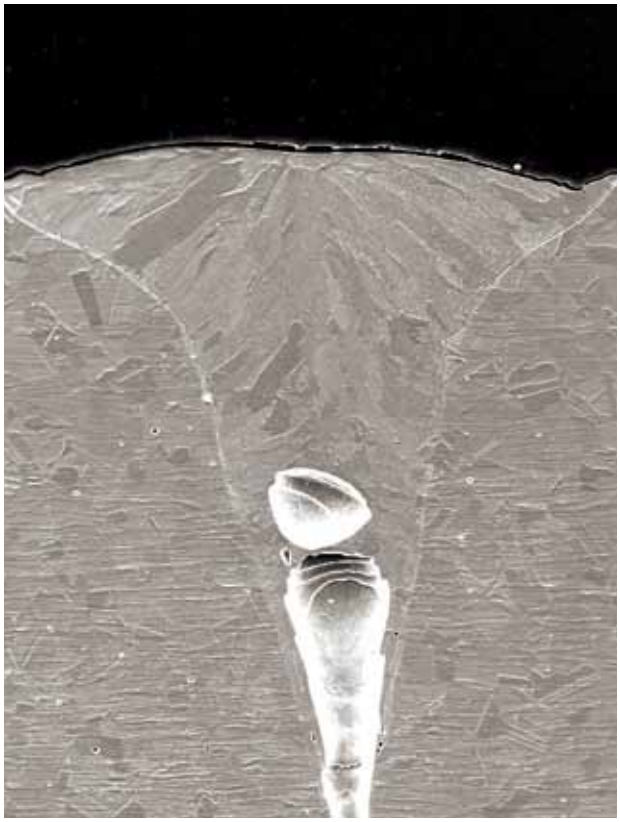
Sub-surface laser engraving has been used for many years to burn images, patterns or words inside solid materials, typically glass. When a computer system is used to drive the movements of the laser head, the engraving process itself becomes extremely precise and fast. And because the process does not disturb the surface of the material, the images, patterns or words inside the material will never fade or deteriorate.

For a long time, sub-surface laser engraving has been used exclusively for producing markings inside transparent materials. Zhongli Li at A*STAR's Singapore Institute of Manufacturing Technology and co-workers[1] have now demonstrated the possibility of laser engraving inside metals. Using a 'Nd-YAG' laser, the team was able to create a series of small holes, or microcavities, below the surface of a block of stainless steel (see image). After surface polishing, the laser markings become completely invisible.

The new technology is useful for fabricating hidden markings that are difficult to remove and could therefore be used to make security watermarks in the automotive, aerospace and military industries. The markings themselves can be revealed using non-destructive techniques such as X-ray imaging.

An additional use of the technology is to create 'closed' porous metals surface. The desired density and distribution of pores, which determine the electrical resistivity, acoustic and electromagnetic properties of the patterned block, can be produced simply by programming the laser beam traces. The flexibility of patterning these closed porous metal surface could even open new avenues of research.

The researchers found that the working principle of sub-surface laser engraving in metals is very different from that in glass. When the laser pulse enters a slab of glass, the intense heat of the laser pulse causes changes to both the microstructure and the refractive index of the glass. It is these defects, or 'cloud points', that appear opaque under light. In contrast, the laser pulse is not able to penetrate a block of stainless steel and is instead absorbed at the surface. The intense heat generated by the absorption of the laser pulse causes the metal to melt, forming a microcavity when the laser point is moved and the molten material solidifies.



Cross-sectional scanning electron microscopy image of a microcavity formed in a block of stainless stell by laser engraving.

The depth of the microcavity can be controlled by varying the peak power of the laser.

The researchers also demonstrated their technique using other metals, including mild steels and titanium alloy. "It will be interesting to see if the laser engraving technique is applicable to other materials. We plan to investigate this issue further," says Li. ■

1. Li, Z. L. *et al.* Direct patterning in sub-surface of stainless steel using laser pulses. *Optics Express* **18**, 15990–15997 (2010).

Wireless networks

Sharing in privacy

Information theory shows the best way to share wireless spectrum securely

Spectrum scarcity and transmission security are two of the largest challenges facing modern wireless communications. One solution to spectrum scarcity is to establish a 'cognitive radio' network that allows licensed 'primary' users, such as mobile carriers and television broadcasting companies, to share the idle spectrum with unlicensed 'secondary' users, such as private users. This approach ensures that no wireless spectrum goes to waste.

Information theory is an extremely useful tool for studying the transmission rates of different communication channels in a given network. It is also a powerful tool for the study of security issues because it can calculate the capacities of legitimate and illegitimate (eavesdropping) communication channels. Ying-Chang Liang at the A*STAR Institute for Infocomm Research and co-workers at Nanyang Technological University[1] have now used information theory to calculate the maximum rate at which information can be securely transmitted between primary and secondary users. Their findings demonstrate the most secure way to operate a cognitive radio network.

The researchers began by mathematically formulating the problem of finding the 'secrecy capacity'—the maximum secure rate of transmission of a secondary transmitter broadcasting to a secondary receiver over spectrum that is shared with a primary user, while at the same time being spied on by an eavesdropper. This is a complex optimization problem that needs to account for transmission power and interference strengths. By developing a novel set of mathematical transformations, Liang and his co-workers were able to reduce this problem to a sequence of simpler problems that are performed routinely on unsecured cognitive radio networks.

As a result, the team was able to prove that beamforming is the optimal transmission strategy for a secure cognitive radio channel. Beamforming involves the use of multiple transmission antennas, each carrying a scaled version of the transmitted signal, to shape the transmission beam, and is used in unsecured cognitive radio networks to avoid interference with the primary user. Liang and his co-workers have shown that beamforming is also the best way to maximize the secrecy capacity of a secure cognitive radio network.

The researchers also introduce three beamforming approaches that produce sub-optimal secrecy capacities, but that have the benefit of reduced calculational complexity. Their study of cognitive radio security can be applied broadly, says Liang. "These results are relevant both for existing cellular wireless networks and for new technologies like femtocells, which broadcast cellular signals to indoor users over spectrum that is shared with outdoor users." ■

© iStockphoto/fotoVoyager

1. Pei, Y., Liang, Y.-C., Zhang, L., Teh, K. C. & Li, K. H. Secure communication over MISO cognitive radio channels. *IEEE Transactions on Wireless Communications* **9**, 1494–1502 (2010).

Photonics

Optimizing the design of a compact coupler

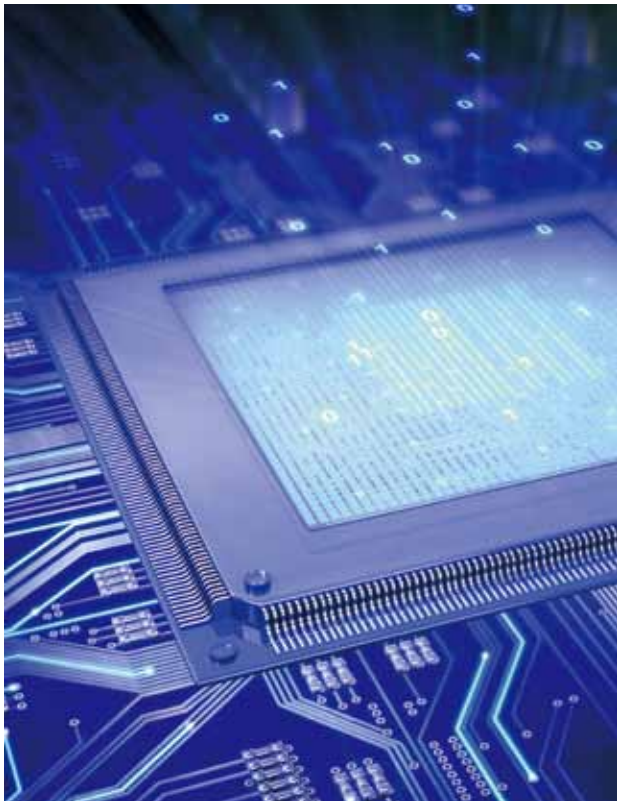
An optimization program that combines the use of two parallel algorithms has found an ultra-compact design for an optical coupler

Integrated circuits—the tiny silicon chips running inside computers, mobile phones and cameras—represent one of the most important innovations of the twentieth century. The development of integrated circuits has not only reduced the size and power consumption of various electronic devices, but also created a multi-billion-dollar industry. Scientists would like to replicate the success of integrated circuits in high-performance computing and telecommunications with photonic integrated circuits, but shrinking the size of various photonic components to fit on a tiny chip is not easy.

There are many basic components in a photonic integrated circuit. One is the multimode interference (MMI) coupler, a device that is responsible for splitting and coupling optical signals utilizing the interference of propagating light. It is possible to use, for example, a 2×2 MMI coupler—a coupler with two optical inputs and two optical outputs—for coupling waves in a laser. Most MMI couplers designed to date have a device length of over 100 micrometers, making them too large for use in high-density photonic integrated circuits.

Qian Wang at the A*STAR Data Storage Institute and co-workers[1] have come up with a powerful and generic optimization program that they have applied to design an ultra-compact MMI coupler. The optimization program combines the use of two algorithms: finite-difference time-domain (FDTD) calculation, a popular computation method for modeling the electrodynamics in photonic devices, and particle swarm optimization (PSO), an intelligent optimization scheme that searches iteratively for the best solution to a problem. Simply combining FDTD and PSO, however, is not efficient and results in impractical computation times. The optimization program parallelizes the FDTD and PSO algorithms to cut down the amount of time needed to find the best solution. Running the optimization program on 12 parallel processors, for example, is almost ten times faster than running the same program on a single processor.

The researchers tested the performance of their optimization program in finding the most compact design for 2×2 MMI couplers with coupling ratios of 50:50 and 90:10. The numerical results showed that in both cases, it is possible to shrink the MMI coupler down to about ten micrometers on an indium phosphide chip with low excess loss. They verified their results by comparison with those obtained from a full simulation using a parallel three-dimensional FDTD-only method. They found good agreement between both sets of results. The findings demonstrate the potential of the optimization program in designing compact components in photonics integrated circuits. ■



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1. Wang, Q. & Ho, S.-T. Ultracompact multimode interference coupler designed by parallel particle swarm optimization with parallel finite-difference time-domain. *Journal of Lightwave Technology* **28,** 1298–1304 (2010).

Plasmonics

Smooth operator

Ultrasmooth silver surfaces promise invisibility cloaks, ultrahigh-resolution lenses and other enhanced photonic devices

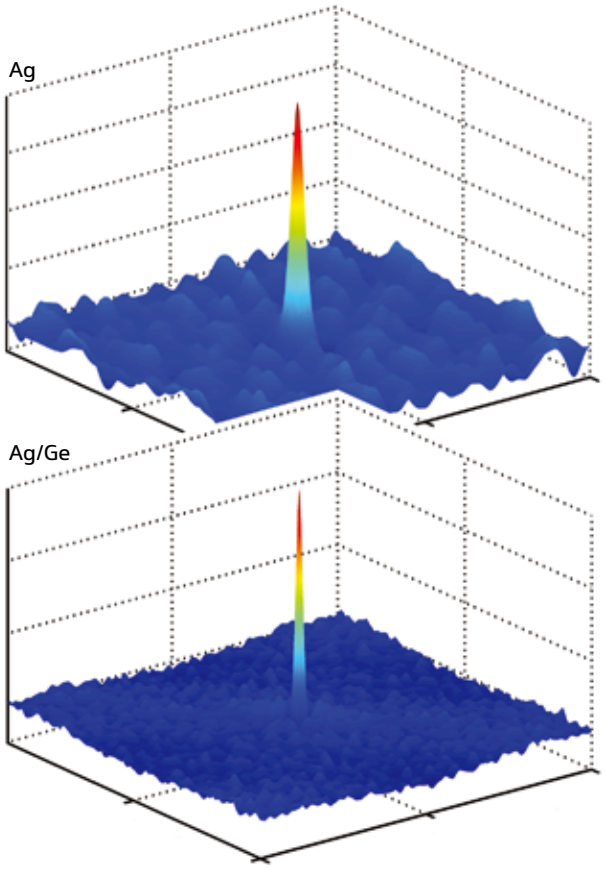
Surface plasmon resonance—the coherent excitation of free electrons on the surface of metals—is crucial to the operation of extremely small photonic devices, which have applications ranging from optoelectronic circuits and molecular sensors to the 'metamaterials' that could one day be used to make invisibility cloaks. To minimize scattering losses in metals, the metal surfaces need to be extremely smooth. Jinghua Teng at the A*STAR Institute of Materials Research and Engineering and co-workers have now developed an effective method to fabricate smooth thin films of silver with enhanced plasmonic properties[1].

As surface plasmon resonances are confined to surfaces, it is possible to miniaturize plasmonic devices to sizes that go far below the wavelength of light used to excite the electrons, but at such dimensions, losses become an obstacle to performance. "One of the grand challenges for plasmonics and metamaterials application is the minimization of loss," says Teng. "Applications of plasmonics in real-world devices will to a great extent depend on our ability to fabricate structures with low optical losses."

These losses originate from absorption as well as the scattering of light due to small imperfections and roughness on the surface of a metal. Therefore, it is paramount to use metal films with ultrasmooth surfaces. At the same time, the fabrication technique used for preparing the metal films needs to be scalable for industrial applications—and evaporative deposition techniques are currently the best option.

The researchers have now optimized the evaporation technique for depositing thin silver films on silicon and quartz substrates. They first deposited a seed layer of nickel or germanium on the substrate, then deposited the silver. The seed layer leads to a smoother surface, and the researchers found the silver–germanium film to be the smoothest (see image). However, because of optical losses in germanium, the silver–nickel films showed superior plasmonic properties. "Overall, the silver–nickel structure is the best because of its low losses and enhanced plasmonic properties," says Teng, "It will be an essential material for future low-loss plasmonic device applications."

The researchers are now searching for ways to enhance the plasmonic and metamaterial devices. "We are now trying to apply this silver–nickel film in applications like superlenses to achieve higher resolution in optical imaging. It can also be implemented in many other plasmonic devices and applications, such as metamaterials and plasmonic circuits," says Teng. ■



Correlation plots showing the smoothness of thin silver films prepared on bare silicon (left) and on a germanium seed layer (right). The narrower and shorter the center peak, the smoother the film.

1. Liu, H. *et al.* Enhanced surface plasmon resonance on a smooth silver film with a seed growth layer. *ACS Nano* **4**, 3139–3146 (2010).

Bio-detection

Building bridges with DNA

A highly specific electronic sensor array detects messenger RNA below femtomolar concentrations

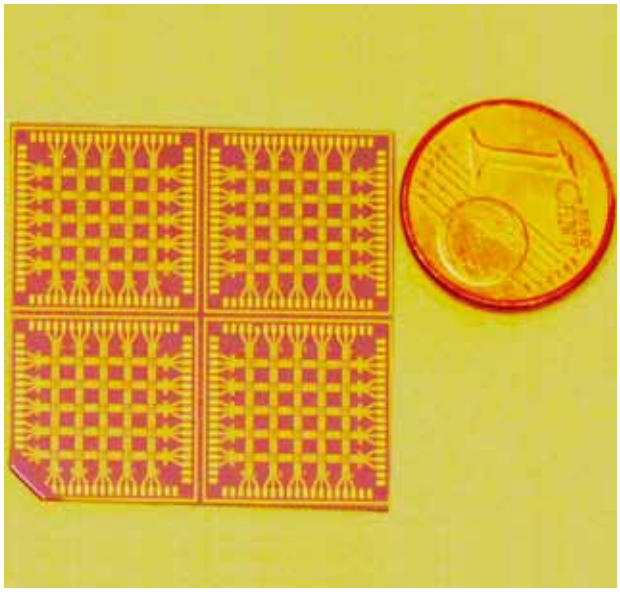
Electrical sensor arrays are useful tools for rapid and early diagnosis of cancer and other genetic diseases. Zhiqiang Gao and co-workers at the A*STAR Institute of Bioengineering and Nanotechnology have developed an array that can sense messenger RNA (mRNA) for quantitative gene expression profiling[1].

Conventional gene expression profiling methods rely on the amplification of target DNA through polymerase chain reaction (PCR) technologies and the detection of fluorescent labels. However, these methods are expensive to run and have low throughput. The researchers designed a high-sensitivity PCR-free device that directly quantifies gene expression using a two-step hybridization of the target mRNA. "Our goal is to develop a highly portable molecular diagnostic system," says Gao, who led the research group.

At the heart of this device is a pair of vertically aligned gold microelectrodes, separated by an insulating 'nanogap' and deposited onto a silicon chip using standard photolithography. After anchoring polythymine probes complementary to the mRNA polyadenine tails on the top microelectrodes, they attached target-specific 'capture probes' on the bottom microelectrodes. Hybridization of the target mRNA extremities with the immobilized probes bridged the nanogap. Finally, the team coated the resulting mRNA–DNA complex with silver to generate a conducting wire between the electrodes for signal detection.

The researchers then designed an array with capture probes specific to an mRNA called GAPDH. They found that the device (pictured) selectively bound to target strands present in total RNA, which also contains other RNA molecules, even at extremely low concentrations. The strength of the detected signal increased in proportion to the GAPDH concentration. The researchers also observed a current when they covered the top electrodes with GAPDH-specific capture probes, indicating that a few target strands, which are longer than the nanogap, could reach the opposite electrode.

The sensitivity and detection limit of the array increased with the length of the genes and surpassed the performance of the best direct mRNA expression profiling methods. The team also demonstrated that the device readily distinguished mismatched mRNA sequences—proof of its specificity—and achieved the same level of performance as quantitative PCR methods for the analysis of real-life samples, particularly for normal and breast cancer tissues.



Photograph of the prototype electronic sensor array for ultra-sensitive and specific mRNA detection.

"The vertical nanogap structure and two-step–two-probe hybridization eliminate much of the background noise," says Gao. "The metallization of DNA generates an excellent signal."

The ultrahigh signal-to-noise ratio and minimal sample preparation requirements enhance the potential adaptability of this array to miniaturized analytical platforms. The team is currently optimizing the sensor and developing new prototypes for commercialization. ■

1. Chen, X., Roy, S., Peng, Y. & Gao, Z. Electrical sensor array for polymerase chain reaction-free messenger RNA expression profiling. *Analytical Chemistry* **82**, 5958–5964 (2010).

Photonics

The full spectrum

The successful growth of high-quality indium nitride thin films makes it possible to produce nitride-based light-emitting diodes with a full visible emission spectrum

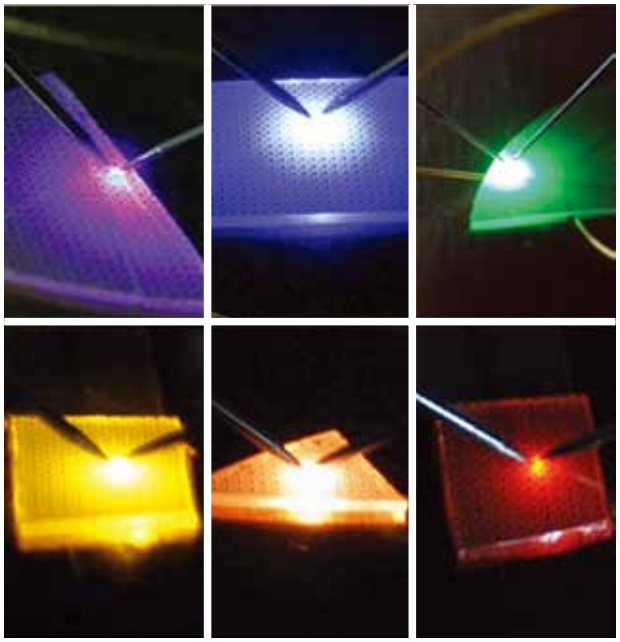
White light-emitting diodes (LEDs) are increasingly finding applications from indoor lighting to flat panel displays. Their success is founded on advances in the growth of semiconductor compounds based on nitrogen, in particularly the blue light emitters aluminum nitride (AlN) and gallium nitride (GaN), which are the key compounds used in white LEDs. Another nitride semiconductor, indium nitride (InN) is also of interest for its red and infrared light emission. Together, all three compounds could cover the entire visible spectrum, from red to violet. Unfortunately, however, the growth of InN thin films with sufficient quality has been difficult. Wei Liu at A*STAR's Institute of Materials Research and Engineering and co-workers have now developed a growth technique and layer design that overcomes previous fabrication problems with InN LEDs[1].

One of the problems in growing high-quality InN thin films is the development of electrical charge on the surface of samples during growth. All LEDs contain a p–n junction comprising two neighboring regions—one with a surplus of negative charge and one with a surplus of positive charge. The surface charge compensates for charge at the p–n junction, and also leads to defects that slow down the electrons as they move through the InN film. Both effects are detrimental to the operation of LEDs.

The researchers have now discovered a solution to this problem. "We have demonstrated that a cap layer is able to overcome this problem," says Liu. The problem with charge accumulation is limited to InN, so a very thin cap layer made from another nitride semiconductor such as SiN, GaN or ZnN is sufficient to prevent the development of surplus electrical charge.

The impact of the cap layer on the sample quality is significant. An example is the transport of electrons. With the cap layer, the electrons move more than 100 times faster through the InN, promising significant performance enhancements for LEDs and other electronic devices.

The realization of efficient InN LEDs will make it possible to achieve complete coverage of the visible spectrum using nitride-based semiconductors. "This means that group-III nitrides can completely replace gallium arsenide and indium phosphide as materials for yellow, red and infrared LEDs," says Liu. The advantage is that LEDs of all colors can be grown on the same chip in a single growth process. In addition, according to Liu this has considerable environmental benefits. "Gallium arsenide and indium phosphide are toxic materials and the precursors used for their growth are also toxic. GaN, AlN and InN, on the other hand, are environmentally friendly materials." ■



Examples of LEDs based on various semiconductor compounds

1. Liu, W., Tan, R. J. N., Soh, C. B. & Chua, S. J. The effects of cap layers on electrical properties of indium nitride films. *Applied Physics Letters* **97**, 042110 (2010).

Biosensors

Under the wire

A device based on silicon nanowires can detect dengue viruses in less than 30 minutes

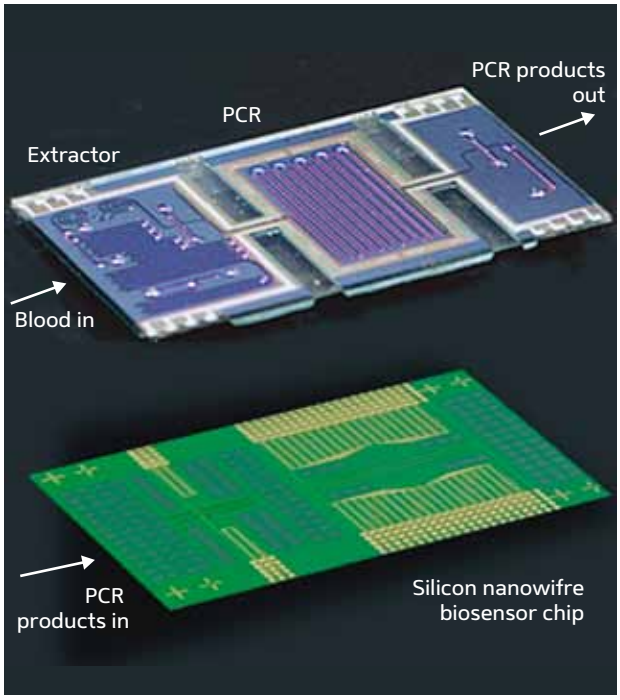
Dengue is an infection caused by one of four types of dengue viruses. Humans usually catch dengue from mosquitoes in tropical areas, particularly in Southeast Asia. The symptoms of dengue are very similar to those of influenza: infected individuals commonly experience headaches, rash and pain in muscles and joints. Some, however, may develop severe complications such as dengue hemorrhagic fever and dengue shock syndrome, which in extreme cases can be deadly.

The incidence of dengue around the world has grown dramatically in recent years. The World Health Organization estimates that 50 million people catch dengue every year, and that 2.5 billion people are exposed the virus. Early detection of dengue viruses could help doctors implement timely clinical treatment and better manage infected patients, but the results of current diagnostic tests conducted in hospital laboratories can take up to several days to obtain.

Guo-Jun Zhang at the A*STAR Institute of Microelectronics and co-workers[1] have developed a silicon nanowire-based biosensor that can detect the 'reverse transcription polymerase chain reaction' product of dengue type 2 (DEN-2) viruses in less than 30 minutes. The device utilizes silicon nanowires affixed with peptide nucleic acid (PNA) probes to recognize complementary DNA fragments of DEN-2.

PNA is an artificially synthesized polymer similar to DNA but without phosphate groups, which allows it to bind DNA better than DNA itself due to the lack of electrostatic repulsion. In a typical test using the new device, researchers prepare complementary DNA fragments of the dengue viral RNA and then 'amplify' the number of fragments. The binding of complementary DNA fragments to the PNA probe is then detected by a change in charge and resistance of the silicon nanowire to which the PNA probe had been bound.

As a proof of concept, the researchers demonstrated that the device (pictured) could be used to detect a complementary DNA fragment of DEN-2 that is 69 base pairs long. The detection time was less than 30 minutes, even when the concentration of DNA fragments was below ten femtomolars. They also demonstrated that the device was highly specific—only complementary DNA fragments of DEN-2 were recognized by the device in mixed samples.



Photograph of the diagnostic device comprised of an extractor, a polymerase chain reaction (PCR) unit, and a silicon nanowire biosensor chip for the detection of dengue viruses.

"Our silicon nanowire-based device will significantly reduce the reliance on labor-intensive and time-consuming laboratory tests requiring trained laboratory staff," says Zhang.

The results highlight the potential of silicon nanowire-based devices in the diagnosis of dengue viruses. The researchers plan to integrate their device into handheld diagnostic systems. ■

1. Zhang, G. J. *et al.* Silicon nanowire biosensor for highly sensitive and rapid detection of Dengue virus. *Sensors and Actuators B: Chemical* **146**, 138–144 (2010).

Printed circuits

Keep the noise down

An efficient model reveals how the distribution of power causes electrical noise in printed circuits

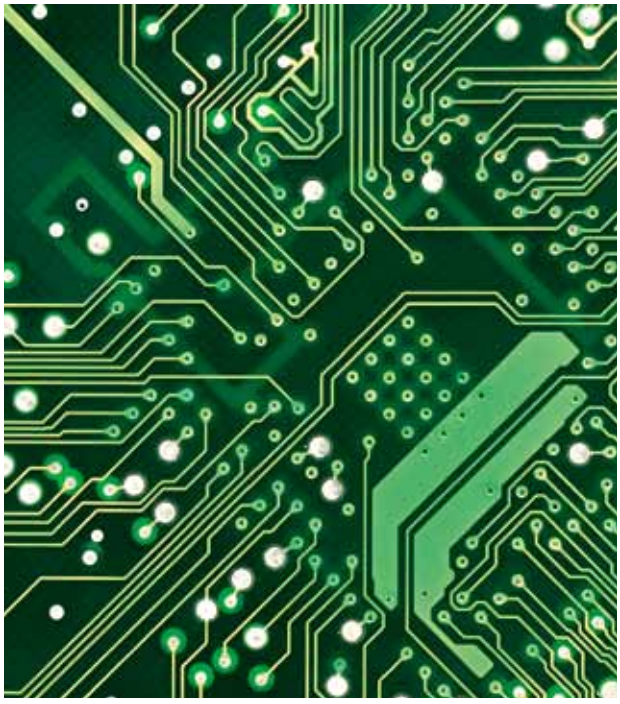
The major source of electronic signal noise in printed circuit boards is not, as one might expect, active electronic components like transistors or diodes, but rather the network of wires responsible for powering these active components.

Although numerical models for printed circuit power distribution networks exist, they are limited in the geometries that they can handle, and are often computationally intensive. This makes it challenging for engineers to design circuits in a way that minimizes noise. Xing-Chang Wei and Er-Ping Li from the A*STAR Institute of High Performance Computing[1] have now developed a more efficient way of calculating the electrical signal noise caused by this power distribution network.

The power distribution network in a multilayered printed circuit consists of multiple patterned planes of wires, called signal traces, and power and ground planes. Holes, or 'vias', through the multilayered board connect the signal traces to the ground and power planes, and also to other planar layers patterned with circuits. Current fluctuations flowing through this complex network of signal traces and the compensating currents flowing in the ground and power planes can create crosstalk between different areas of the circuit, resulting in noise that interferes with the processing of real data. This noise is particularly problematic in high-frequency and high-power circuits.

Wei and Li had previously calculated this noise by modelling the ground planes, signal traces and vias with equivalent circuit elements with well-understood behavior. These elements included distributed resistances, capacitors, inductors and one-dimensional transmission lines. This modeling approach could only be applied, however, to networks with a single pair of ground and power planes.

In the present work, the researchers extended this technique to handle multilayered power distribution networks by constructing equivalent circuits for different portions of the network, one at a time. These circuit models were then cascaded together to describe the entire system. The researchers also took into account interactions between vias by including additional capacitors and inductors. However, when the horizontal distance between vias was greater than three times the vertical distance between the relevant ground and power planes, these interactions could be ignored.



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The model was compared against a commercially available simulator, called HFSS, that described the printed circuit using a three-dimensional finite element mesh, and was found to produce accurate results in about one tenth of the computation time. The researchers achieved this substantial boost in computational efficiency by decomposing the three-dimensional problem into a series of simpler two-, one- and zero-dimensional problems. ■

1. Wei, X.-C. & Li, E.-P. Integral-equation equivalent-circuit method for modeling of noise coupling in multilayered power distribution networks. *IEEE Transactions on Microwave Theory and Techniques* **58**, 559–565 (2010).

Microtechnology

An alignment assignment

Photonics and microelectromechanical systems fabricated separately on different wafers can now be aligned precisely by finishing with a single processing step

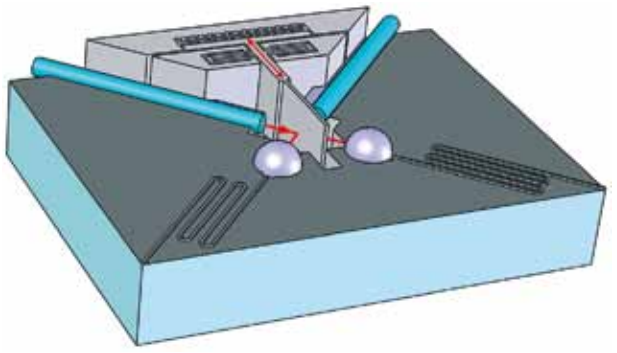
Microelectromechanical systems (MEMS), which consist of tiny moving parts driven by electrical signals, have found ready applications in optical communication systems. They are attractive in part because they can be integrated with other electrical and optical components to create a multifunctional device in a single package, which reduces fabrication costs and allows for greater performance. However, this integration requires precise alignment of the constituent parts in order to avoid signal loss.

One approach to achieve accurate alignment is to manufacture both the optical MEMS components and any other electronic or photonic components on the same silicon wafer. Optical MEMS devices, however, are often ten times thicker than other optical components. This means that different fabrication techniques are needed for the different components, making alignment difficult.

Another approach is to fabricate MEMS and electrical components on two separate wafers that are then bonded together. Achieving good alignment in this scheme is made difficult, however, by the coarse bonding processes that are available. Qingxin Zhang and co-workers at the A*STAR Institute of Microelectronics[1] have now refined the two-wafer approach by combining the final fabrication step for each component into a single process.

The research team aligned an optical MEMS structure with a silicon photonic structure (see image). The two wafers bearing the respective components were processed independently in the first step: the MEMS structure was fabricated on a bulk silicon wafer and the photonic structure on a silicon-on-insulator wafer. The wafers were then bonded together using benzocyclobutene—a commonly used bonding agent for MEMS—at 250 °C, and the two structures were completed simultaneously using a single step of deep reactive ion etching.

The use of a single fabrication step to complete the final integrated device allowed Zhang and his co-workers to meet strict alignment specifications, achieving a misalignment of less than one micrometer laterally and less than half a micrometer vertically. They also used their strategy to construct and characterize a functioning optical switch in which a MEMS mirror is displaced by a driving voltage to connect and disconnect an optical pathway. The signal loss between a source optical fiber and the silicon waveguide in the device was just 2.4 decibels, which is well within acceptable limits.



A schematic view of the MEMS actuators integrated with silicon photonics on a silicon-on-insulator substrate. Red arrows indicate the direction of light or mechanical motion.

The new approach allows scientists to merge photonic and MEMS components fabricated on two different wafers into a single device. Future work will focus on optimizing the MEMS design and fabrication process, and demonstrating reconfigurability. ■

1. Zhang, Q. *et al*. A two-wafer approach for integration of optical MEMS and photonics on silicon substrate. *IEEE Photonics Technology Letters* **22**, 269–271 (2010).

Photonics

A light choice

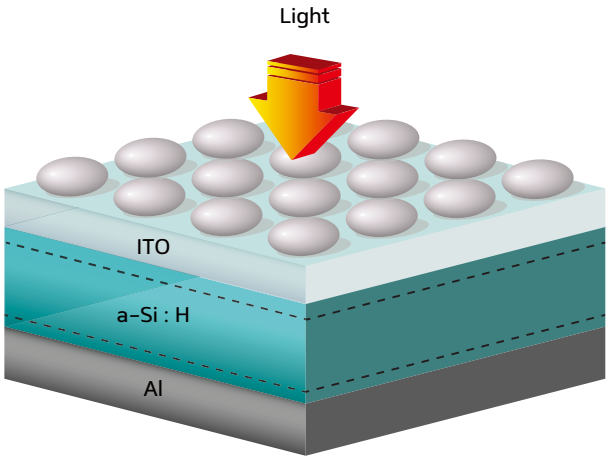
Solar cells can be made thinner and lighter with the help of aluminum particles

Solar cells are a key technology in the drive toward cleaner energy production. Unfortunately, solar technology is not yet economically competitive and the cost of solar cells needs to be brought down. One way to overcome this problem is to reduce the amount of expensive semiconductor material used, but thin-film solar cells tend to have lower performance compared with conventional solar cells. Yuriy Akimov and Wee Shing Koh at the A*STAR Institute of High Performance Computing[1,2] have now improved the light conversion efficiency of thin-film solar cells by depositing aluminum particles on the cell surface.

Metallic nanoparticles can direct light better into the solar cell and prevent light from escaping. In conventional 'thick-film' solar cells, the nanoparticles would have little effect because all the light is absorbed by the film due to its thickness. For thin films, however, the nanoparticles can make a big difference (see image). Their scattering increases the duration the light stays in the film, bringing the total absorption of light up to a level comparable with that for conventional solar cells. "The strategy allows us to reduce the production costs of solar cells by several times and makes photovoltaics more competitive with respect to other forms of power generation," says Akimov.

The researchers modeled the light absorption efficiency of solar cells for various nanoparticle materials and sizes. In particular, they compared the properties of silver versus aluminum nanoparticles. In most studies on the subject, silver particles have been preferred. These have optical resonances in the visible part of the spectrum that are even better at focusing the light into the solar cell. Unfortunately, there is a tradeoff: the optical resonances also cause the absorption of light by the nanoparticles, which means the solar cell is less efficient.

In the case of silver, this resonance is right in the key part of the solar spectrum, so that light absorption is considerable. But not so for aluminum nanoparticles, where these resonances are outside the important part of the solar spectrum. Furthermore, the aluminum particles handle oxidation well and their properties change little with variations in shape and size. And more importantly, their scattering properties are robust in comparison with silver nanoparticles. "We found that nanoparticles made of aluminum perform better than those made of other metals in enhancing light trapping in thin-film solar cells," says Akimov. "We believe aluminum particles can help make thin-film solar cells commercially viable." ■



Schematic illustration of a silicon solar cell (a-Si:H) sandwiched between aluminum (Al) and transparent indium tin oxide (ITO) electrical contacts. Aluminum nanoparticles on the top (gray) enhance the absorption of light.

1. Akimov, Y. A. & Koh, W. S. Design of plasmonic nanoparticles for efficient subwavelength light trapping in thin-film solar cells. *Plasmonics* **6**, 155–161 (2010).
2. Akimov, Y. A. & Koh, W. S. Resonant and nonresonant plasmonic nanoparticle enhancement for thin-film silicon solar cells. *Nanotechnology* **21**, 235201 (2010).

Nanomaterials

Peapod power

A new type of composite material with peapod structures can help improve the performance of lithium-ion batteries

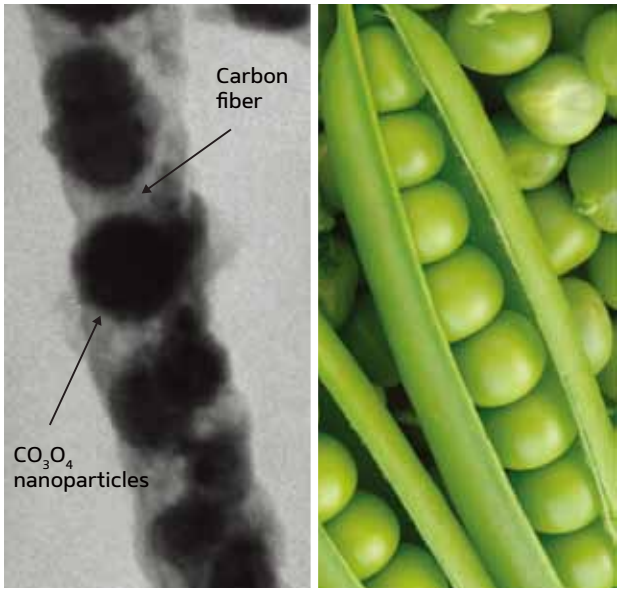
Lithium-ion batteries are used to power a wide range of electronic devices, including computers, cameras, digital audio players and calculators. Tremendous effort has been devoted to the development of lithium-ion batteries, especially in improving the efficiency and integrity of the battery electrodes. This is because during the discharging and charging processes, lithium ions are repeatedly incorporated into and extracted from the electrodes by alloy formation or chemical conversion. These recurring events are known to cause the progressive degradation of the electrodes, irreversibly damaging battery performance.

Yu Wang at the A*STAR Institute of Chemical and Engineering Sciences and co-workers[1] have now demonstrated an elegant strategy to reduce the degradation problem and increase the capacity retention of lithium-ion batteries over many charge–discharge cycles. The strategy involves the use of a composite material with a peapod structure comprising cobalt oxide (Co_3O_4) nanoparticles embedded in carbon fibers (see image).

Cobalt oxide is a promising material for anodes in lithium-ion batteries because its capacity for holding ions is higher than that of conventional electrode materials, such as tin. In addition, Co_3O_4 can be easily converted to $LiCoO_2$, which is the material currently used in commercial cathodes. The researchers made the peapod structures by heating cobalt carbonate hydroxide nanobelts coated with layers of polymerized glucose in an inert atmosphere at 700 °C and then in air at 250 °C. Electrodes built using the peapod composite had enhanced lithium storage and capacity retention—delivering 91% of the total possible capacity after 50 charge–discharge cycles.

"The Co_3O_4 nanoparticles act as active materials to store lithium ions and the hollow carbon fibers protect and prevent the Co_3O_4 nanoparticles from aggregating and collapsing," says Wang. The carbon fibers also play the role of conducting electrons from the nanoparticles.

According to Wang, aside from the promising application in lithium-ion batteries, the fabrication of the peapod composite is an achievement in itself, as it is the first time that such isolated magnetic nanoparticles embedded in hollow fibers have been produced. Scanning electron microscopy revealed that the peapod composite exhibits a uniform morphology, with pod lengths of up to several micrometers and pod diameters of as small as 50 nanometers. The researchers believe that their method could be extended to generate encapsulated nanoparticles using a wide range of materials with applications beyond lithium-ion batteries, for example, in gene engineering, catalysis, gas sensing and the manufacture of capacitors and magnets. ■



Left: © 2010 ACS. Right: © iStockphoto.com/pixbook

Cobalt oxide nanoparticles embedded in carbon fibers (left) to form peapod-like structures improve the lifetime of electrodes in lithium-ion batteries.

1. Wang, Y. *et al*. Designed functional systems from peapod-like Co@carbon to Co_3O_4@carbon nanocomposites. *ACS Nano* **4**, 4753–4761 (2010).

Data storage

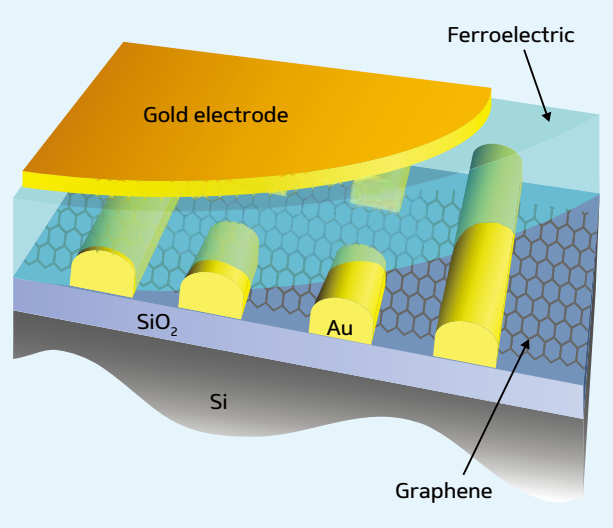
Carbon–ferroelectric memory

Nonvolatile memory based on ferroelectric–graphene field-effect transistors is now a step closer to reality

A fundamental component of a field-effect transistor (FET) is the gate dielectric, which determines the number of charge carriers—electrons or electron vacancies—that can be injected into the active channel of the device. Graphene has recently become the focus of attention as a viable, high-performance replacement for silicon in FETs, and in recent studies on graphene-based FETs, scientists have investigated the use of thin films of a ferroelectric material for the gate dielectric. Such films offer several interesting advantages for use in graphene-based FETs: their strong electrical polarization makes it possible to introduce a much higher density of carriers than can be achieved using standard dielectrics, and they have remnant electric polarization—a property that could allow graphene–ferroelectric FETs to be used for nonvolatile memory by storing a certain level of carrier density in the absence of an electrical field.

Two collaborating teams from the A*STAR Institute of Materials Research and Engineering and the National University of Singapore, led by Kui Yao and Barbaros Özylmaz, respectively, previously demonstrated a basic graphene–ferroelectric memory device in which the polarization in the ferroelectric film was controlled by the electrical bias applied to the gate terminal. In that structure, a thin ferroelectric film was deposited on top of a graphene layer, where it injects charge carriers and thus modulates the resistance of the graphene. Unfortunately, however, the two distinct resistance states that could be read as an information bit could only be realized by polarizing and depolarizing the ferroelectric film, which presented problems due to the instability of the depolarization state.

Now, the two teams have collaborated to fabricate an improved device[1] that includes an additional silicon dioxide (SiO_2) dielectric gate below the graphene layer (see image). The SiO_2 gate, a long-standing component in traditional FETs, effectively provides a reference point from which to measure the effect of ferroelectric gating. By monitoring the resistance of the device as a function of the voltages applied to the top and bottom gates, the researchers developed a quantitative understanding of the performance and switching behavior of graphene–ferroelectric FETs. For use as a nonvolatile memory device, the SiO_2 dielectric gate also simplifies bit writing by providing an additional background source of charge carriers, allowing the ferroelectric polarization to be switched between two stable states corresponding to two opposite polarization orientations.



Schematic illustration of an improved graphene–ferroelectric FET with SiO_2 basal layer.

The new device developed by the research team achieved impressive practical results, capable of symmetrical bit writing with a resistance ratio between the two resistance states of over 500% and reproducible nonvolatile switching over 100,000 cycles. ■

1. Zheng, Y. *et al.* Graphene field effect transistors with ferroelectric gating. *Physical Review Letters* **105**, 166602 (2010).

Photonics

Better optical fiber networks

A new architecture for optical fiber networks promises more cost-efficient fiber-optic networks for the consumer market

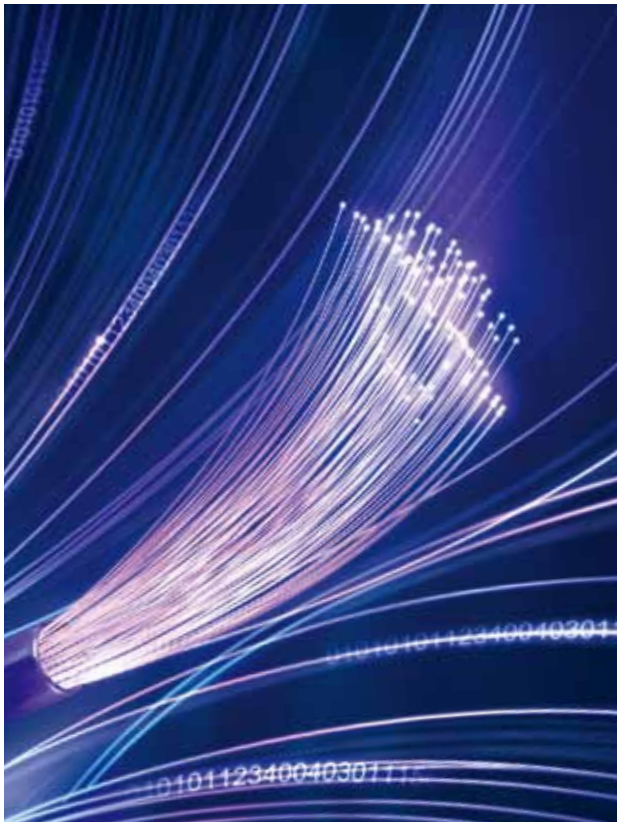
The household demand for increased internet bandwidth has grown tremendously because of the popularity of data-intensive internet activities such as movie streaming. Conventional copper telephone lines struggle to meet this demand, and modern optical fiber networks connecting the homes of consumers to the network backbone are becoming necessary. Jing Zhang and co-workers at the A*STAR Institute of Microelectronics have now demonstrated a network scheme that considerably reduces the cost of fiber-optic installations and could make them more attractive for consumer use[1].

A key component of any optical fiber network is the laser that transmits information down the fiber. Unlike the silicon-based electronic circuits that control the data flow through the network, these lasers are made from semiconductor materials other than silicon, which is a poor light-emitter. This makes integrating lasers with silicon electronic circuits cumbersome and expensive, and so reducing the number of lasers in the network could substantially lower the cost of connecting users to the internet.

One widely adopted scheme for reducing the number of expensive lasers in the network is to transmit data to multiple homes at once using a single laser, with a transmission protocol ensuring that the correct data packet is sent to the correct user. Yet although this configuration reduces the number of lasers considerably, each connected household still needs a laser to send data back the other way.

The network architecture proposed by Zhang and his co-workers eliminates the laser at the consumer end. Instead, they propose using two strands of optical fiber: one to transmit data to the consumer as usual and another to send a continuous laser beam to all linked consumers. An integrated silicon chip at the consumer end picks up the incoming continuous laser beam, encodes it with the signal intended for back transmission, and then redirects this laser beam back to the internet provider. "Fiber is cheaper than lasers, particularly as it can be used for more than 20 years once it is installed," says Zhang.

In their experiment, the researchers also demonstrated the practical viability of this scheme for the operation of commercial fiber-optic networks. They fabricated an integrated silicon circuit for this task and have already achieved successful operation at speeds of up to 10 gigabits per second. "Given the cost benefits, these transceiver devices may significantly accelerate the deployment of optical fiber networks," says Zhang. "Our work has attracted serious commercial interest for collaboration on the development of silicon photonic transceivers." ■



© iStockimage/Andrey Voladin

1. Zhang, J., Liow, T.-Y., Lo, G.-Q. & Kwong, D.-L. 10Gbps monolithic silicon FTTH transceiver without laser diode for a new PON configuration. *Optics Express* **18**, 5135–5141 (2010).

Laser physics

Getting silicon into order

Short pulses of laser light can crystallize amorphous silicon and create a nanostructured surface texture ideal for solar-cell applications

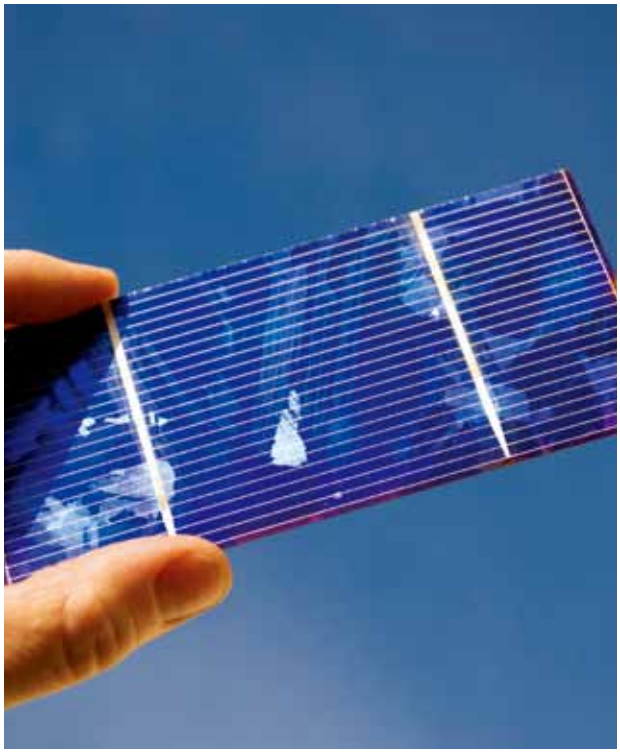
The importance of silicon for almost every element in modern-day electronic devices and computers is due largely to its crystalline atomic structure. Crystalline silicon, however, is much more expensive to produce than its non-crystalline or amorphous form, which has limited the cost reduction achievable in devices such as silicon-based solar cells. Xincai Wang at the A*STAR Singapore Institute of Manufacturing Technology and co-workers[1] have now shown that ultrafast pulses of light can be used to crystallize amorphous silicon and to texture its surface.

The useful electronic properties of silicon stem from the way the atoms are arranged into a regular and repeating lattice. Amorphous silicon, on the other hand, does not display this long-range order: nearby atoms are bonded in the same way but the structure is not homogenous through the whole material. While lacking the extraordinarily useful properties of its crystalline relative, amorphous silicon does have some advantages. Thin films of amorphous silicon can be grown at low temperatures and therefore at much lower cost. In fact, amorphous silicon is used widely in the field of photovoltaics for the conversion of sunlight into electrical power. But modifying the material could improve both electrical efficiency and optical sensitivity.

The researchers used laser radiation to achieve this level of material control. They focused pulses of light just 150 femtoseconds in duration into a spot 30 micrometers in diameter. This spot was then scanned across an 80-nanometer-thick layer of amorphous silicon deposited on a glass substrate.

The first noticeable effect was a change in color: the treated area was darker than the untreated region. Closer inspection using an electron microscope revealed that the laser created 'nano-spikes' in the silicon. This texturing is useful for photovoltaic applications because it reduces light reflection from the surface and thereby increases absorption: an effect the researchers confirmed directly.

Raman spectroscopy—a powerful technique for analyzing atomic structure—was then used to compare treated and untreated samples. The Raman spectrum for the untreated region had two peaks characteristic of an amorphous structure. But that of the treated sample displayed a third sharper peak indicative of a crystalline atomic structure. This state change was likely caused by the excitation of electrons at the surface of the silicon by the laser pulses, which weakened the interatomic bonds in a way fundamentally different from simple thermal melting.



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"Our process has potential applications in the fabrication of high-efficiency thin-film silicon for solar cells, as well as thin-film transistors and other novel optoelectronic devices," says Wang. ■

1. Wang, X.C. *et al*. Femtosecond laser induced surface nanostructuring and simultaneous crystallization of amorphous thin silicon film. *Optics Express* **18**, 19379–19385 (2010).

Microfabrication

The light approach

The patterning of templates using ultraviolet light is a promising new method for assembling transparent microwire networks

Materials that conduct electricity but which are also transparent to light are important for electronic displays, cameras and solar cells. The industry's standard material for these applications is indium tin oxide (ITO), but the spiraling cost and limited supply of indium has prompted a search for alternatives.

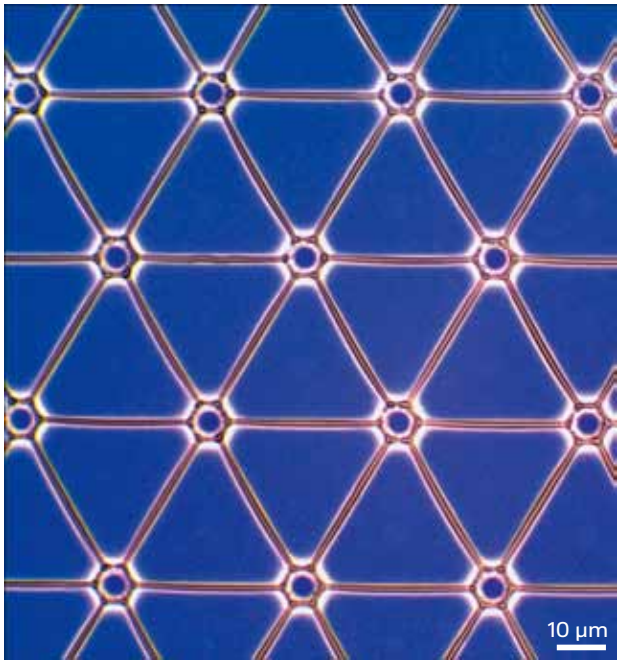
One promising approach is to build mesh-like networks of ultrathin conducting metal wires that light can pass through. Ivan Vakarelski at the A*STAR Institute of Chemical and Engineering Sciences and Xiaosong Tang and Sean O'Shea at the A*STAR Institute of Materials Research and Engineering[1] have now refined the process of making these tiny meshes so that it is feasible for large-scale manufacturing.

The secret to fabricating such intricate microstructures is to encourage metal nanoparticles to assemble themselves from a liquid suspension. This requires a predefined template to guide the self-assembly—in the same way that coffee granules assemble into a ring under a cup as spilt liquid evaporates.

A few years ago, Vakarelski and his co-workers demonstrated the possibility of using latex microparticles as a template for such a mesh using a solution containing gold nanoparticles. "As the solvent evaporated, a liquid bridge network developed around the latex particles, leaving behind a network of microwires formed by the self-assembly of the gold particles," explains O'Shea. "This is an easy approach for research purposes, but is difficult to control on a manufacturing scale."

To tackle this problem, the researchers turned to the technique of photolithography, which involves using ultraviolet light to draw patterns in a photoresist film. The exposed and hardened parts of the photoresist then act as a precise template for self-assembly of gold nanoparticles. "It is difficult, however," says Vakarelski, "to produce spheres replicating the latex particle template using photolithography. We tried several alternative structures and found that arched structures work just as well."

Using photolithography to produce a template of arch-like structures and the same solution of gold nanoparticles, the researchers prepared a high-quality gold microwire mesh (pictured) with conductance and transparency comparable to those of high-grade ITO. "An added advantage of the arched structures is that, unlike latex microparticles, we are not restricted to a hexagonal network topology," says O'Shea. Indeed, the researchers successfully produced networks of rectangles, hexagons and triangles. "Using this technique we plan to explore special functional networks using other types of particles, including semiconducting particles, magnetic particles, carbon nanotubes, DNA and proteins," says Vakarelski. ■



Optical microscopy image of a fully connected hexagonal network of microwires prepared by the self-assembly of gold nanoparticles on a photoresist template.

1. Tang, X., O'Shea, S. J. & Vakarelski, I. U. Photoresist templates for wafer-scale defect-free evaporative lithography. *Advanced Materials* **22**, 5150–5153 (2010).

Photonics

A more sensitive device

Electron resonances could greatly enhance the response of the photodetectors critical to the operation of optical chips

Optical chips are the latest innovation in silicon technology with the potential to revolutionize telecommunications. Their operation relies on several key components, including light-emitting devices, waveguides and photodetectors. Engineers are looking for ways to miniaturize these components without sacrificing the data-processing speed of the integrated optical chips. Patrick Guo-Qiang Lo and co-workers at the A*STAR Institute of Microelectronics[1] have now fabricated a highly sensitive photodetector by exploiting the enhancement effects of electron resonances that occur at metal contacts.

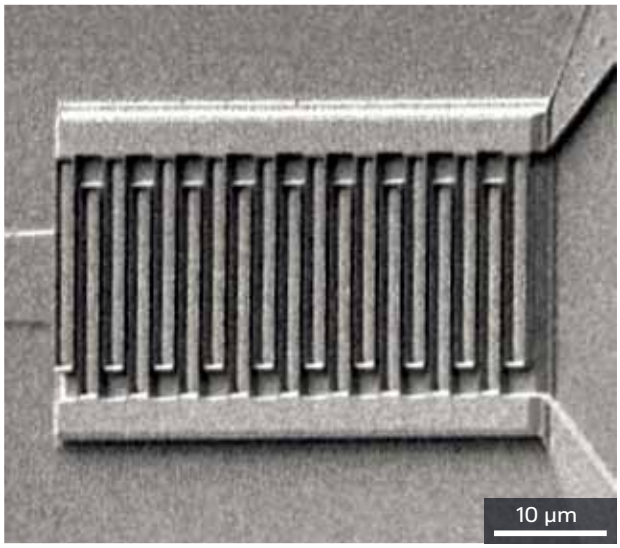
Surface plasmon polaritons—the collective movements of electrons at the surface of metals—are known to enhance and focus electromagnetic waves in their vicinity. The plasmon effect has been studied extensively for its ability to enhance the performance of optical devices, but in this study the researchers applied the phenomenon to improved the sensitivity, and hence speed, of semiconductor detectors.

Photodetectors on a silicon chip are generally designed to pick up light arriving through silicon waveguides. The light travelling through the silicon waveguides is detected by germanium, another semiconductor, which is grown directly on top of the silicon structure. However, the sensitivity of the germanium detector needs to be enhanced considerably in order to increase the speed and reduce the footprint of the photodetector further.

Plasmonic resonances can easily enhance the sensitivity of this light detection. The researchers introduced plasmons by adding thin aluminum contacts on top of the device (pictured). The plasmonic effects in the metal films channel considerably more light from the silicon waveguide into the photodetector, with important implications for device performance. "The enhanced photodetection enables the use of smaller devices, which in turn means that the device speed can be increased considerably," explains Lo.

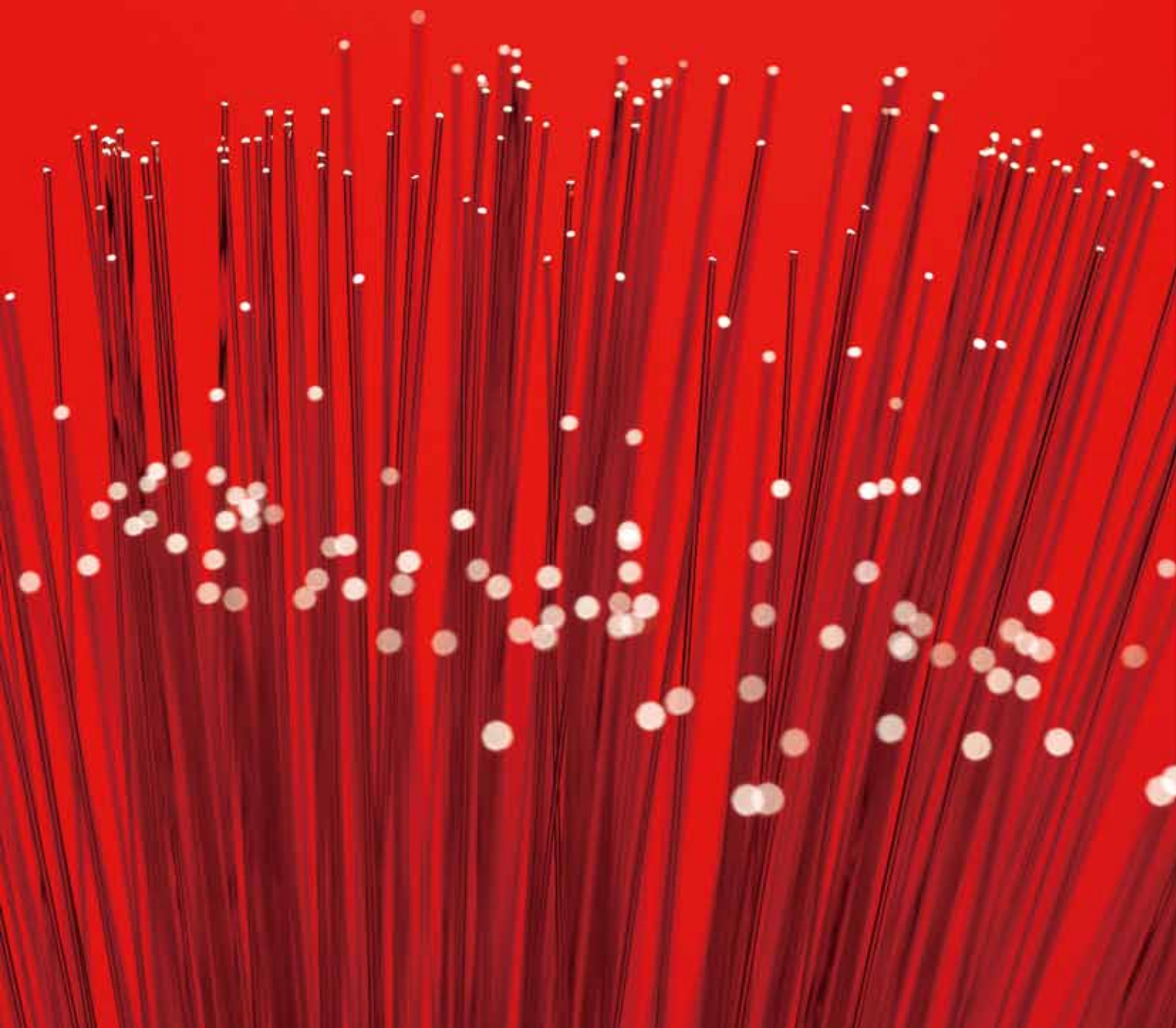
The researchers demonstrate detection speeds of 37.6 picoseconds or faster, corresponding to a data transmission speed of 11.4 gigahertz—several orders of magnitude faster than that achievable by current broadband connections.

At the same time, these speeds still lag behind the full potential of these detectors. One of the reasons, says Lo, is loss that arises from the plasmonic resonances, which absorb some of the light and therefore reduce the amount of light that arrives at the detector. "The response of the detector is lower than what we expected from our design," says Lo. "Enhancing the plasmonic properties of the detector, for example through the design of different geometries, could alleviate such problems and enable a further miniaturization of photodetectors on silicon chips." ■



Scanning electron microscopy image of the germanium–silicon-based photodetector with metal contacts to induce plasmonic light enhancement

1. Ren, F.-F. *et al*. Surface plasmon enhanced responsivity in a waveguided germanium metal-semiconductor-metal photodetector. *Applied Physics Letters* **97**, 091102 (2010).



The landscape of stem-cell research

Stem-cell research is diversifying more than ever in Singapore.

When the first human embryonic stem (ES) cell line was established in 1998, people had high expectations that regenerative medicine would soon become a reality. With the ability to differentiate into any type of cell, ES cells could help restore lost or damaged organs, even neurons in the brain. Amidst much-publicized ethical concerns surrounding the use of ES cells early in the new century, scientists in 2006 discovered a means to reprogram adult cells into 'all-purpose' stem cells known as induced pluripotent stem (iPS) cells. At the time, iPS cells looked like the silver bullet that would obviate the ethical concerns that had previously blocked stem-cell research and lead to a cascade of therapeutic applications.

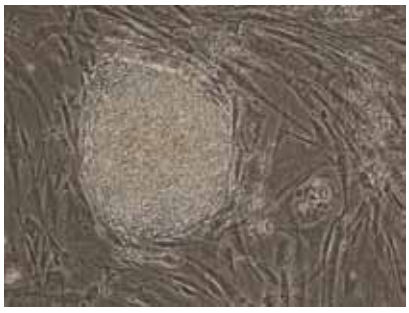
Almost five years later, it is now recognized that there is still much to be proved before stem-cell therapies can be brought to the clinic. "The initial enthusiasm has passed," says Davor Solter, professor and senior principal investigator of the Mammalian Development group at the A*STAR Institute of Medical Biology (IMB). "Our expectations for stem cells now have more reasonable proportions."

In Singapore, a country known for its strong commitment to stem-cell science, research is diversifying more than ever. About 20 groups at various A*STAR institutions are working with a variety of objectives, from establishing ES cell lines to developing bioprocess technologies and utilizing iPS cells to study disease. A*STAR has pledged its continuing support to stem-cell research through relatively liberal regulations and ample public grants. In the past five years, A*STAR has allocated S$110 million to its Singapore Stem Cell Consortium.

Modeling disease

Much attention and hope have been invested in iPS cells. The potential of iPS cells as a substitute for ES cells fascinates many well-established stem-cell researchers, and competition has been heating up to create safer and more useful iPS cell lines. "An iPS cell is very similar to an ES cell, but easier to make. We've found iPS cells to be very useful for modeling certain diseases," says Alan Colman, principal investigator of the Stem Cell Disease Models laboratory at the IMB.

Colman is known for his contribution to the 'birth' of Dolly, the world's first mammal to be cloned from an adult somatic cell. Colman's contribution to the project occurred in 1996 when he was a research director of PPL Therapeutics in the UK. At that time, his team collaborated with scientists at the neighboring Roslin Institute to further evaluate the somatic cell nuclear transfer (SCNT) technology, which involves the removal of the nucleus from an oocyte (an immature egg cell) and its substitution (via injection) by a single nucleus taken from a somatic cell. With a few chemical tweaks, the new cell begins dividing like a fertilized egg. In the case of Dolly, the donor nucleus was taken from a sheep mammary cell. The arrival of "Dolly" transformed contemporary views of the stability of the genome in somatic cells since it has been previously concluded that a complete resetting of the genetic program in any somatic cell was not possible.



Induced pluripotent stem cells are used by Alan Colman to model a rare aging disease, Hutchinson–Gilford progeria syndrome.

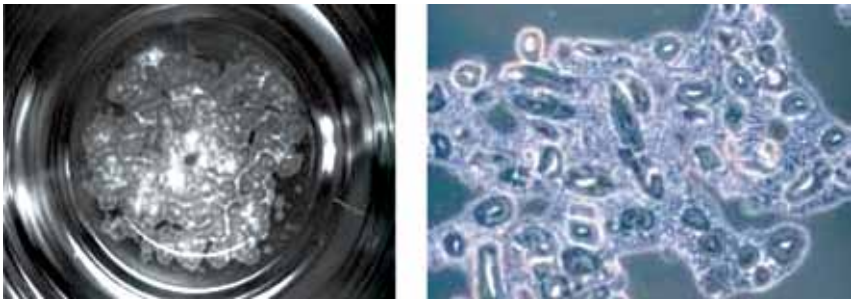
In 2002, Colman joined ES Cell International, a Singapore-based company that specializes in developing human ES cell lines. Five years later, he moved to A*STAR to take advantage of iPS cells to look into aging mechanisms. He has abandoned nuclear-transfer technology and now uses ES cells as experimental controls only.

Currently, most researchers at Colman's laboratory are devoted to developing iPS cells to model several human genetic diseases including Hutchinson–Gilford progeria syndrome, a premature aging disease. The disease is caused by a single mutation of the 'nuclear lamin A' gene. The mutation leads to the production of a mutant protein called progerin, which affects the integrity of nuclear envelope and triggers the acceleration of aging. Colman explains that while this mutant protein is produced in most cells, only certain cells suffer and accelerate aging. "We are trying to understand why, in this disease, only certain cells are affected. A patient's central nervous system remains fine, but they develop cardiovascular problems."

His team's approach is to take fibroblast skin cells from a patient and reprogram them using retroviruses carrying four transcription factors. This results in the production of many iPS cells, which can then be used to study the pathology of the disease in the early stages of development. Colman says that aspects of this particular disease resemble normal aging, so "studying progeria could lead to a better understanding of normal human aging."

New technologies

Although iPS cells have taken up much of the limelight over the past few years, the majority of papers published by A*STAR researchers still involve the use of ES cells. Many scientists working on both types of

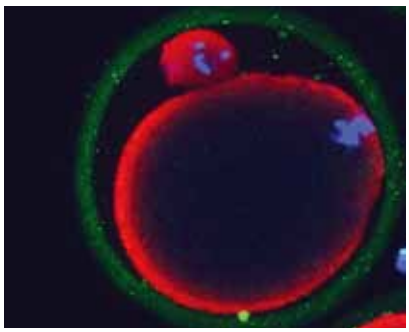


Comparison of 2D colony on surfaces (left) with 3D cultures on cylindrical microcarriers (right) for the growth of human ES cells. 3D-cultured cells, developed by Steve Oh, develop to 2–4 times higher densities than 2D colonies.

cells share a common challenge: how to make a variety of desired cell types efficiently. Researchers have been struggling to produce a sufficient number of usable cells for possible therapeutic applications. An equally important issue is the safety of the stem cells, as past research has shown that ES and iPS cells can switch spontaneously into cancer cells.

Steve Oh and Andre Choo at the A*STAR Bioprocessing Technology Institute are striving to address solutions to some of these problems[1,2]. Oh, a principal scientist in the Stem Cell Group, has invented a method to attach human ES cells to rod-shaped, cellulose microcarriers and grow them as a three-dimensional cluster in suspension cultures. The conventional method is to culture human ES cells on two-dimensional surfaces in a Petri dish, but this approach has limitations in terms of the yield of cells. Oh's approach allows long-term, serial growth in suspension cultures and achieves 2–4 times higher cell densities than using conventional cultures.

For their next step, Oh and his colleagues have triggered the aggregated cells to differentiate into cardiomyocytes (heart muscle cells) in serum-free culture conditions. But the major problem is that a small number of stem cells in these clusters of mature cells remain undifferentiated, which can develop into tumors known as teratomas. Choo, senior scientist in the Stem Cell Group, has developed several antibodies that bind selectively to undifferentiated ES cells and kill them. "Then we can collect pure populations of cardiomyocytes," says Choo. He is aiming to improve the accuracy of the method to make it possible to guide antibodies to the remaining undifferentiated populations of cells. "We could then target cancer stem cells, or another disease indication in the future," Choo adds.



An immunofluorescence image of an oocyte. Davor Solter has been studying the myth of the oocyte-to-embryo transition, and takes a cautionary stance on human ES cells and human iPS cells.

These techniques are promising but there are still a number of problems to be solved. For example, Oh says ES cells tend to differentiate spontaneously in suspension cultures, so he needs to develop another method to prevent this from happening. "Transferring the cells to larger volumes of culture is a major challenge," he says. Work is ongoing to scale the process to controlled bioreactors of several liters.

Back to basics

Despite the rapid progress in stem-cell research over the past few years, Solter has taken a step back from stem cells to examine how an oocyte transforms into an embryo in such a short period of time. "Development starts not from fertilization but goes way back to the processes involved in making oocytes. Sperm cells only contribute DNA and maybe some other things," he says. His laboratory, which he runs in collaboration with Barbara Knowles, a professor and principal investigator, is focusing on determining the molecules and processes that are involved in the activation of embryonic genomes in oocytes.

Although there are some cases of treatment using adult stem cells, such as for serious burns, Solter raises serious questions regarding the potential therapeutic application of ES cells. "For example, if pancreatic stem cells are applied by injection into blood vessels, they may not know what to do. How that is going to work is totally unclear," he says. He also has doubts about the eventual clinical use of iPS cells, suggesting instead that iPS cells could be bypassed with yet another technology. Solter says researchers may soon be able to differentiate fibroblasts directly into organs or neurons, in which case iPS cell technology would become another transient development in the saga of stem-cell therapy.

Not everybody agrees with that opinion, however. "I personally don't think direct trans-differentiation will be easier in general," argues Colman. "That approach will be difficult to use if large numbers of cells are needed, since often the differentiated cells cannot divide well. It may remain preferable to grow up large numbers of iPS cells and then differentiate these into large numbers of the desired cell type." Colman says convincing other scientists that their cell types behave in the same manner as cells in patients is as difficult a challenge as making a variety of adult-like differentiated cell types.

Beyond all the debate in stem-cell research, researchers universally agree that a better understanding of the underlying developmental biology is essential and could lead to remarkable and exciting outcomes in the future. "At the present time, we do not know how to exactly apply differentiated derivatives of ES cells for cell and tissue replacement," Solter says. "In many cases these cells would have to precisely integrate in the right place in the right organ and it is not clear how to achieve that." ■

1. Leung, H. W, Chen, A., Choo, A. B. H., Reuveny, S. & Oh, S. K. W. Agitation can induce differentiation of pluripotent stem cells in microcarrier cultures. *Tissue Engineering Part C: Methods* **17**, 165–172 (2011).
2. Oh, S. K. W. *et al.* Long term microcarrier suspension cultures of human embryonic stem cells. *Stem Cell Research* **2**, 219–230 (2009).

Robots with a human touch

'Social' robots with human-like mannerisms allow for more natural interactions with human users.

In 2005, when Martin Saerbeck was studying computer science at Bielefeld University in Germany, he programmed a service robot called BIRON. Mounted with a pan-tilt camera on top, BIRON was able to follow a human pointing gesture and focus on the object pointed at. On one occasion, however, BIRON's camera lost track of Saerbeck's hand and the robot appeared to be sleeping or to have lost interest. Without thinking, Saerbeck waved his hand in front of BIRON and said "Wake up!"

"But then, I thought, 'What am I doing?'" says Saerbeck. "I didn't program waving detection so I should have known it wouldn't move, but it was natural for me to talk to the robot in that way." The experience is a demonstration of how people are naturally inclined to use normal human social skills when interacting with technology.

Inspired by such experiences, Saerbeck went on to develop a series of programs and architectures that enable robots to mimic human-to-human communication in a natural and readily understandable manner. "I don't want to think too much about how to interact with the device or how to control it," he says. Saerbeck, now a research scientist at the A*STAR Institute of High Performance Computing, is interested in developing robots that can be programmed to express words or reactions in response to a dialog with a human instead of simply responding to a few preset keywords. The goal is to enable the user to understand the state that the current state of the robot in a natural way.

In recent years, 'social' robots—cleaning robots, nursing-care robots, robot pets and the like—have started to penetrate into people's everyday lives. Saerbeck and other robotics researchers are now scrambling to develop more sophisticated robotic capabilities that can reduce the 'strangeness' of robot interaction. "When robots come to live in a human space, we need to take care of many more things than for manufacturing robots installed on the factory floor," says Haizhou Li, head of the Human Language Technology Department at the A*STAR Institute for Infocomm Research. "Everything from design to the cognitive process needs to be considered."

Martin Saerbeck is developing a tutoring application using the iCAT research platform.

Mimicking human interaction

Li leads the ASORO program, which was launched in 2008 as A*STAR's first multidisciplinary team for robot development covering robotic engineering, navigation and mechatronics, computer vision and speech recognition. The program's 35 members have developed seven robots, including a robot butler and a robot home assistant. Their flagship robot is OLIVIA, a robot receptionist who also acts as a research platform for evaluating various technologies related to social robotics.

Li unveiled the latest version of OLIVIA—'OLIVIA 2.1'—at the RoboCup 2010 competition in Singapore. In her robotic receptionist mode, OLIVIA welcomes guests as a robotic receptionist and responds to a few key phrases such as "Can you introduce yourself?" She is also able to track human faces and eyes, and detect the lip motions of speakers. Eight head-mounted microphones enable OLIVIA to accurately locate the source of human speech and turn to the speaker. Intriguingly, OLIVIA even performs certain gestures learned from human demonstrators. Li is now discussing collaborations with Saerbeck to upgrade the OLIVIA platform.

Haizhou Li (right) introduces the capabilities of OLIVIA—Singapore's first social robot.

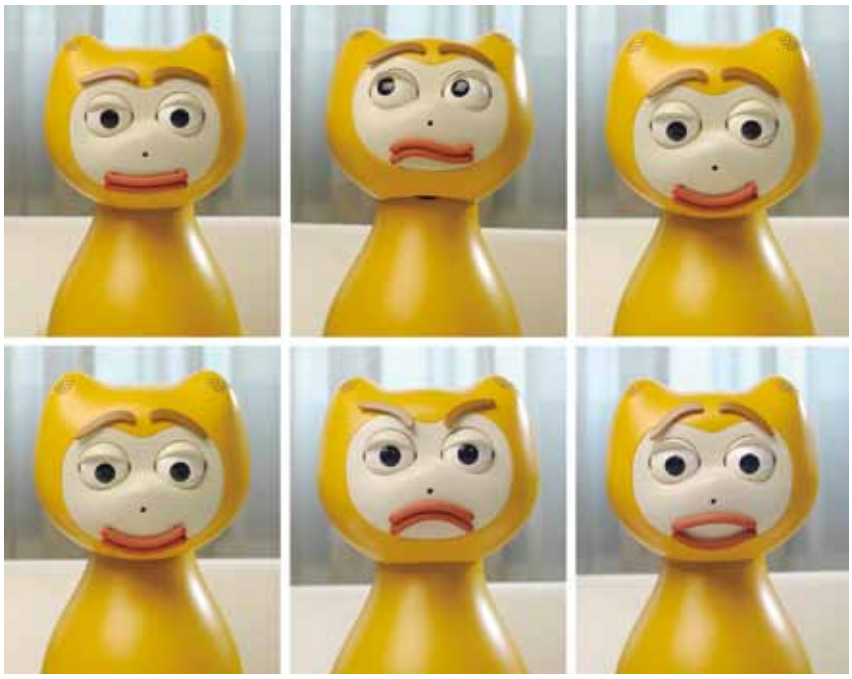


Photo: Philips Research

The interactive iCAT robot for language teaching

One of the core premises of enhanced human–robot interaction is the concept of 'believability', says Saerbeck. A robot is controlled by a highly sophisticated and technical architecture of programs, sensors and actuators, and without detailed attention to the robot's 'animation', the robot can appear mechanical and alien.

For example, if a vacuum cleaning robot were to bump into an object and say "Ouch," people would understand that it is simulating that it was hurt. However, static animations are not sufficient to give the impression of a life-like character. If the robot continuously bumped and repeated the same reaction, the animation would no longer be convincing. "What we are investigating now is how we can take context into account, and how behaviors can develop over time," says Saerbeck.

The research is guided by psychology, social science and linguistics to create models for appropriate actions using animation frameworks. He is also working on more sophisticated programming so that robots can autonomously cope with a wider range of situations instead of resorting to conventional programming of prescribed sequences of actions that humans are expected to perform.

Help from a robotutor

Bringing all these technological elements together, Saerbeck is currently developing a robotic tutor that assists vocabulary-learning tasks for school children. The project dates back to the time when he worked at Philips Research in the Netherlands. In one experiment, his team divided 16 children aged 10–11 years into two groups, and varied the degree of social interaction with a cat-shaped robot named iCAT. All of the children had good language skills and were given the same artificial language to study. The result was that children in the class with a more socially responsive robot scored significantly higher than the children that interacted with a robot in the style of current learning programs. The children with the social iCAT also showed significantly higher intrinsic and task motivation.

At A*STAR, Saerbeck is still using iCAT as a prototype platform. His team is now planning to build a completely new desktop-based static robot tailored for tutoring applications and equipped with specialized hardware for teaching. The design has yet to be fixed, but the researchers are evaluating various technologies including touch screens, flash cards, projectors and gesture-based interfaces.

Haizhou Li with OLIVIA

Meanwhile, Li is also upgrading OLIVIA to enable her to learn from speakers and to understand naturally spoken queries. Ultimately, he plans OLIVIA to be able to deliver information or take actions such as making a taxi booking and shaking hands. However, Li admits that there are many technological gaps that need to be filled before 'OLIVIA 3.0' becomes a reality.

OLIVIA's current technologies are already at a sufficiently advanced stage of development to attract commercial interest—aspects of OLIVIA have been applied in a commercial surveillance system. But, according to Li, there remains much scope for improvement, for example, in the accuracy of visual and speech understanding and real-time compliant control.

It's all about context

One of the biggest challenges is to improve robotic 'attention', says Li. "In human-to-human interaction, we share a natural concept of communication—we know when the conversation starts and ends, and when we can start talking in a group. We are now trying to facilitate this kind of ability in a robot." Li's team is developing new algorithms and cognitive processes that could enable a robot to engage in conversation with both visual and auditory attention, accompanied by natural body language.

As the context of communications is also influenced by the robot design, OLIVIA 3 is being designed in a completely different style from the version demonstrated at RoboCup 2010. The design idea is based on market research garnering public opinion about how people wanted a robotic receptionist to look. As opinions varied with age, ethics and social status, OLIVIA 3's final appearance is still under discussion. "We also have to consider cultural contexts when we build robots—our aim is to develop social robots that people can interact with comfortably," says Li. ■

The digital side of biology

The rapid development of computational tools is shedding light on new genetic and molecular studies, but it is also creating headaches for the scientists involved in this research.

Revolutions in science come in waves. One of the epoch-making events in modern biology came in 1995 when J. Craig Venter, an American biologist, decoded the whole genome of the Haemohilus influenzae bacterium using a 'shotgun' sequencing technique that involved the computational assembly of data.

At that time, many molecular biologists, including Wayne Mitchell who is now with the A*STAR Experimental Therapeutic Centre (ETC), had experienced years of frustration seeing their work in gene cloning beaten to the finish line time and again by rival laboratories. Inspired by Venter's demonstration of how computers can be used to vastly accelerate biological research, Mitchell decided to self-train himself to acquire the computational skills needed to perform this type of analysis. At first, his new direction was not entirely welcomed by his colleagues, who raised their eyebrows and asked why he would waste his time with such an endeavor. "I think they couldn't get out of an old paradigm," recalls Mitchell.

Fifteen years have passed since then, and Mitchell appears to have rightly captured the tide of the times as the computerization of biological research gains momentum. He has become one of the global pioneers who are armed with both biological and computational expertise. As the founding leader of the Informatics Group at the ETC, Mitchell took the initiative to build up information technology platforms such as electronic content management systems and new networks to transmit large amounts of data at faster speeds. On the research side, Mitchell and his colleagues are utilizing computerized processes—from the robotic screening of several thousands of chemical compounds with high-throughput computing machines, to the computer modeling of chemical structures—in a bid to develop therapeutic drug candidates for cancer and other diseases[1].

"Modern biology is all about automated machines churning out huge amounts of data, which then have to be managed, stored, analyzed and visualized," says Mitchell. "None of these procedures amount to rocket science, but if you don't do it, there is actually no point to conducting the experiment in the first place."

Multiple data analysis

The advent of the information technology era has completely changed biological research. Network speed, storage capacity and computer clusters have seen continual improvements, and the development of new algorithms and knowledge management protocols has led to the introduction of novel computer-based methods such as sequence alignment, machine learning techniques for pattern identification and ontologies for formal data classification. Together these platforms have made possible what are now widely used experimental technologies, including sequencers, microarrays, detectors for single-nucleotide polymorphisms and mass spectrometers. Large databases of sequencing results have also become publicly available, spurring informaticians to design tools that make the data readily available to experimental scientists. "Digital computing is the servant of non-digital, brain-based computing," Mitchell says.

Biologists' interest in research aided by cutting-edge computational tools, a field known as computational biology, has taken hold across A*STAR's institutes. At the A*STAR Genome Institute of Singapore (GIS), almost all research involves computing, and one third of its investigators are either computer scientists or biologists equipped with strong computer skills. Now that sequencing has become routine, "there is more demand for computer researchers to analyze the massive quantities of data," says Neil Clarke, deputy director of the GIS and a molecular biologist as well as a self-trained bioinformatician.

In a recent study from the GIS, Clarke's team investigated one of the most

Wayne Mitchell

GIS researchers Neil Clarke (top left), Ken Sung (top right), Niranjan Nagarajan (bottom right) and Anbupalam Thalamuthu (bottom left)

important DNA regulatory systems—how transcriptional factors and nucleosomes compete with each other to obtain a position to bind to DNA[2]. The researchers examined multiple publicly available, high-resolution datasets of genome-wide nucleosome position and performed computer analyses to compare positions in vivo and in vitro. They found that the key to this analysis is to know not the precise nucleosome location but rather its broader regional occupancy.

Ken Sung, a principal investigator and computer scientist at the GIS, and co-workers have recently designed a microarray to identify DNA mutations of the H1N1 influenza virus with high accuracy. In combination with software called EvoSTAR, the microarray chip can sequence the virus directly from blood samples without amplifying the virus beforehand. The kit has not only improved the efficiency of testing compared with other chip-based methods, but also reduced research costs by a factor of ten. It enables researchers to perform large-scale biosurveillance studies to track the changes in influenza during a pandemic, which can help prevent the spread of the virus.

Elsewhere in the GIS, large-scale sequencing has brought great benefits to the emerging field of 'metagenomics', in which researchers directly study microbial samples from nature, bypassing a difficult culturing step in the lab.

Niranjan Nagarajan, a principal investigator and computer scientist, is developing novel tools to accurately analyze metagenomic datasets for both marker gene sequencing and whole-genome sequencing. "New technologies are able to deeply profile these communities, but what researchers get is very fragmented information. They want to use the data to reconstruct a more comprehensive picture of a sample," says Nagarajan.

Finding out the correlation between variations and phenotypes for multi-marker studies is another computationally intensive field. "It is a highly complex, high-dimensional challenge to handle millions of variables and construct optimal mathematical models. We spend a lot of time building into a model analyses or predictions that reduce the complexity of the problem," says Anbupalam Thalamuthu, a principal investigator and statistician at the GIS. Thalamuthu is developing a model for the genetic analysis of polymorphisms of diseases including various infectious diseases, breast cancer and dengue.

Modeling a virus

At the A*STAR Institute for Infocomm Research, Victor Tong, an assistant department head, is developing three-dimensional (3D) models of immune cells and viruses to see which location of a virus is likely to activate strong immune responses. In the past, researchers used traditional wet lab procedures to analyze thousands or even tens of thousands of different combinations of peptide sequences. But such an approach was not cost-effective and it took considerable time to pinpoint specific regions in pathogen proteins that can trigger effective immune responses. "This way, computational analysis can help accelerate the research," says Tong, a biochemist and computer scientist. "When an antigenic peptide triggers an immune response, there must be a 3D fit between the peptide and the receptor binding site of the host immune cell. Hence, the use of 3D models is important for such studies," he adds.

Tong's recent studies include the analysis of chikungunya virus, a re-emerging tropical disease that causes flu-like symptoms in Singapore and elsewhere around the world. His team has built a model and analyzed how the pathogen mutates across different times and geographical locations. They have found that the virus has accumulated different mutations within a specific region of a structural protein. Tong hopes his work on modeling the virus will lead to the design of new types of vaccines that contain precise fragments of the virus triggering immune responses. Using current technologies, vaccines contain randomly selected fragments of the virus or the entire attenuated dead virus.

Visualizing data

Although computers can produce mountains of interesting data, the data remain useless unless biologists are able to extract meaning from the data set. This is where they need the aid of computer scientists. "Biologists are interested in finding out what kinds of shapes cells are taking in reaction to treatments. Our work is to visualize the results of data analysis in a way that is understandable for biologists," says William Tjhi, a research engineer at the

A*STAR Institute of High Performance Computing (IHPC).

In collaboration with Frederic Bard, a biologist at the A*STAR Institute of Molecular and Cell Biology, Tjhi is developing a methodology to transform millions of digitized cell images into numerical values or matrices. He then performs an analysis to find areas in which one coherent pattern can be separated from another. "Based on this cluster analysis, biologists make their own interpretation," he says. Being able to develop an initial visual understanding of the data helps biologists plan detailed experiments. But in the past, the reliability of visual approaches was variable because biologists needed to categorize cells manually either under the microscope or by utilizing a tool called a classifier. Such methods are heavily dependent on human intervention in determining the initial definitions of interesting patterns. Tjhi is trying to reduce the level of human intervention in the data-creation process so that researchers can minimize subjectivity.

To expand the cluster analysis approach for full-scale operation, Tjhi is collaborating with Bard and Rick Goh, a senior research engineer at the IHPC, on a project to tackle the 'millions of cells problem'. So far, Tjhi's software is capable of performing analyses on 50,000 cells, but the team is trying to beef up the capability to a few million cells. Goh is currently assessing the kinds of high-performance computing techniques and tools that are needed for such a platform, which could include hybrid architectures such as multicore systems, accelerators and graphics processing units.

Victor Tong

The computer trap

The new engineering solutions that have revolutionized biological research have revealed previously unimagined aspects of biology and refuted numerous biological myths. However, such technological development has leaped so far ahead of the information infrastructure that supports it that data are now being accumulated much faster than can be digested or synthesized. As Mitchell points out, the informatics aspect of the computational support system is becoming a bottleneck.

One of the issues faced by many researchers in this area is a shortage of data storage and memory capacity. High-throughput machines churn out terabytes of data every day, and many biologists would regard it unthinkable to delete any of the data in order to conserve storage capacity. "Our terabytes of storage can run out so easily," says Goh. The large volumes of data are also affecting data mobility. "On our servers, we can move terabytes of data in less than a few hours. But moving data from one storage system to another can take days, or even weeks—and that is the time needed even before analysis starts," Goh adds. One potential technique, instead of moving data, is to move the computing code and process the data on its original system, thus eliminating the time for data transfer.

The rapid generational change in experimental technologies is also a constant headache. "We spend a lot of time thinking about what applications and new technologies to work on, and how we can analyze the data acquired using them," Clarke says.

Moreover, as researchers are sharing more and more data among the community, standardization is becoming another imminent issue requiring serious discussion. "Data could be inconsistent and not interpretable using other databases. There is no quality control for bioinformatics," Tong points out.

Building a bridge

Aside from hardware issues, perhaps the biggest challenge for computational biology is the dialogue between computer scientists and biologists, many researchers say. Whenever building a model for analysis or predictions, or visualizing matrix data, computer scientists may not always be able to understand what it is biologists want to know. "If I just take data given by biologists and put them into my cluster analysis, the results would be poor," says Tjhi. "I need to understand how the data are generated by their experiments. I need to take into consideration this fact when incorporating data into the analysis, and then the results generally become better." Tjhi admits that understanding such different disciplines is far from an easy task, but there is no short cut, he says. "We have to communicate more with the biologists. More time needs to be invested in this kind of project in order for people to understand each other."

William Tjhi (left) and Rick Goh (right)

Many researchers involved in computational biology are hoping to employ a person who can understand the languages of both sides of this research. Mitchell, whose role is to talk to both biologists and computer scientists, agrees that the biggest issue is communication. But he is more optimistic about the future. "Younger scientists and undergraduate students feel more comfortable with computer-based methods because computers are already a natural extension of their daily lives. For the next generation, the computational–experimental dialogue will naturally become routine. I'll need to find another job." ■

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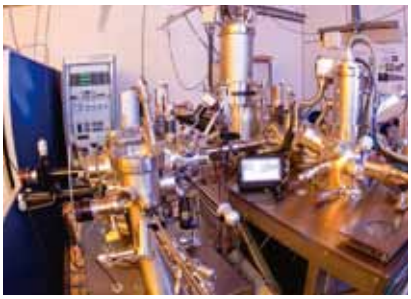
2. Goh, W. S., Orlov, Y., Li, J. & Clarke, N. D. Blurring of high-resolution data shows that the effect of intrinsic nucleosome occupancy on transcription factor binding is mostly regional, not local. *PLoS Computational Biology* **6**, e1000649 (2010).

The next big thing

*A new international project involving A*STAR researchers could set the stage for atomic-scale molecular computing.*



An ultrahigh-vacuum atomic fabrication system pioneered by researchers at the IMRE will be used in the AtMol project to construct molecular chips.

As makers of computer microchips continue their consumer-driven pursuit of higher processor power and lower energy consumption, they are rapidly approaching the physical limits of miniaturization. The nanometer-scale transistors that comprise the latest microprocessor's logic circuits simply cannot be made any smaller, leaving industry with little choice but to increase the number of transistors on each chip and operate the processor at higher frequency—both of which lead to increased power consumption and heat generation.

Scientists and engineers around the world are searching for new architectures that could supersede conventional silicon-based processors as the basis for the next generation of computers. Although quantum and optical computers have received much attention, devising an industrially viable platform for implementing such computing systems remains a distant target. Molecule-based computing, on the other hand, despite having a shorter history of development, is much closer to implementation compared to other possible computing technologies and could emerge as a promising next-generation technology if a commercially useful fabrication platform can be developed.

Christian Joachim from the National Center for Scientific Research (CNRS) in France and a visiting international professor at A*STAR's Institute for Materials Research and Engineering (IMRE) is leading a collaboration between ten European Union partners and the IMRE in an ambitious four-year project starting in 2011 to develop a complete fabrication process for single-molecule-based computing. The Atomic Scale and Single Molecule Logic Gate Technologies project, dubbed AtMol, is

Christian Joachim

funded to the tune of close to 10 million Euros and brings together some of the most prestigious institutes in Europe. "IMRE's unique 'atom tech' research group is a key partner in AtMol because we have the technological know-how and the only atomic-scale interconnection machine in the world," says Joachim. Two more of these cryogenic ultrahigh-vacuum fabrication instruments are under construction as part of the AtMol project.

The project has coalesced out of technology developed by Joachim's team at the IMRE and CNRS for constructing molecular machines, and follows recent demonstrations of a process that allows single molecules to be deposited with atomic precision in arrangements that could provide computing capabilities. "We are developing a process to package a molecular chip in a way that separates the molecular logic circuits from the backside interconnects and allows the molecular circuit to be brought out from vacuum without destroying it," says Joachim. "Using A*STAR's patented back interconnect process, we can preserve the atomic precision of the top part of the chip."

The development of a packaging process that effectively separates the nanoscale molecular circuits from the microscale connections that interface with conventional silicon-based circuitry is a critical step forward in demonstrating the potential and viability of molecular processing technology. "A molecular chip derives its processing power from a set of well-connected molecules, where each molecule acts like a conventional logic gate, or even better, a small processor, and the interconnections among molecules are formed by wires just one atom wide," says Joachim. "We are aiming at 0.01-nanometer precision, placing each molecule optimally on the substrate. This would not be possible without our atomic fabrication technology and encapsulation process."

Releasing the restrictions of microscale processing will also allow the AtMol team to investigate new ways to achieve logic functions. "In AtMol, we are optimally aiming to do away with transistors all together, instead using atomic-scale quantum effects to achieve gate logic functions." Such an approach would allow miniaturization far beyond that achievable in conventional transistor-based nanocircuits, and according to Joachim, could mean that the circuit complexity could be raised without increasing the lateral dimensions of the circuit. "We already have the technology to connect a single molecule to four nanoscale electrodes with atomic precision. I am looking forward to seeing how far we can push our molecular chip technology by the end of this four-year project." ■

Agency for Science, Technology and Research
SINGAPORE

The Agency for Science, Technology and Research (A*STAR) is Singapore's lead government agency dedicated to fostering world-class scientific research and talent for a vibrant knowledge-based economy.

A*STAR actively nurtures public-sector research and development in biomedical sciences, physical sciences and engineering, and spurs growth in Singapore's key economic clusters by providing human, intellectual and industrial capital to our partners in industry and the healthcare sector.

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