APRIL 2013 – SEPTEMBER 2013

SINGAPORE

A*STAR Research

HIGHLIGHTING THE BEST OF A*STAR

FEATURES

HIGHLIGHTS

3rd NANO TODAY CONFERENCE

DECEMBER 8–11, 2013

MATRIX, BIOPOLIS, SINGAPORE

www.nanotoday2013.com

CONFERENCE TOPICS

- Synthesis and Self-Assembly of Nanocrystals, Nanoparticles and Thin Films
- Functionalization and Size-Dependent Properties of Nanocrystals, Quantum Dots and Nanowires
- Processing and Templating of Nanotubes and Nanoporous Materials
- Tailoring of Polymeric Nanoparticles, Organic-Inorganic Nanocomposites and Biohybrids
- Nanosystems for Biological and Medical Applications
- Nanosystems for Sensing, Diagnostic, Imaging, Magnetic, Electronic and Structural Applications
- Nanomaterials for Energy, Environmental, Chemical and Catalytic Applications

NANO TODAY 17.689 NEW IMPACT FACTOR

PLENARY SPEAKERS

Joseph M. DeSimone
University of North Carolina, USA

Mostafa A. El-Sayed
Georgia Institute of Technology, USA

Younan Xia
Georgia Institute of Technology, USA
2013 *Nano Today* Award Winner

INVITED SPEAKERS

- **Ritesh Agarwal**, University of Pennsylvania, USA
- **Frank Caruso**, University of Melbourne, Australia
- **Christopher J. Chang**, Howard Hughes Medical Institute and University of California, Berkeley, USA
- **Hui-Ming Cheng**, Shenyang National Laboratory for Materials Science, China
- **Yi Cui**, Stanford University, USA
- **Benoit Dubertret**, ESPCI ParisTech, France
- **Toshiaki Enoki**, Tokyo Institute of Technology, Japan
- **Harald Fuchs**, University of Münster, Germany
- **Nathan Gianneschi**, University of California San Diego, USA
- **Herbert Gleiter**, Karlsruhe Institute of Technology, Germany
- **Taeghwan Hyeon**, Seoul National University, Korea
- **Kazunori Kataoka**, The University of Tokyo, Japan
- **Ali Khademhosseini**, Harvard University, USA
- **Nicholas Kotov**, University of Michigan, USA
- **Kian Ping Loh**, National University of Singapore, Singapore
- **Jeffrey R. Long**, University of California, Berkeley, USA
- **Igor Medintz**, US Naval Research Laboratory, USA
- **Milan Mrksich**, Northwestern University, USA
- **Wolfgang Parak**, Philips-Universität Marburg, Germany
- **Nicholas A. Peppas**, The University of Texas at Austin, USA
- **Shouheng Sun**, Brown University, USA
- **Horst Weller**, University of Hamburg, Germany
- **Hong Yang**, University of Illinois, USA
- **Peidong Yang**, University of California Berkeley, USA
- **Yadong Yin**, University of California Riversides, USA
- **Shu-Hong Yu**, University of Science and Technology of China, China
- **Nanfeng Zheng**, Xiamen University, China

REGISTRATION FEES

Regular	Delegate: SGD 1,000.00 Affiliate*: SGD 900.00	Student: SGD 500.00 Student Affiliate*: SGD 450.00
1-Day Pass		SGD 400.00
Online Registration Deadline		November 25, 2013

Note: Prevailing 7% Goods and Services Tax will be charged for each registration.
**Affiliates: Staff and students from A*STAR, A*STAR research institutes, Singapore National Healthcare Group (NHG) and Singapore Health Services (SingHealth) hospitals and Singapore schools.*

CONFERENCE CHAIR

Prof. Jackie Y. Ying
Editor-in-Chief, *Nano Today*
Executive Director, Institute of Bioengineering and Nanotechnology, Singapore

Contact: nt2013chair@ibn.a-star.edu.sg

Organized by

Institute of Bioengineering and Nanotechnology
A*STAR
Innovations for Tomorrow
www.ibn.a-star.edu.sg

nanotoday
Impact Factor 17.689
www.nanotoday.com

ELSEVIER
www.elsevier.com

A*STAR Research

Contents

APRIL 2013 – SEPTEMBER 2013

FEATURES AND INNOVATIONS

RESEARCH HIGHLIGHTS

CELL BIOLOGY & IMMUNOLOGY

CHEMISTRY & MATERIALS

GENETICS & DISEASE

PHYSICAL & LIFE SCIENCE TECHNOLOGIES

ENGINEERING & NANOTECHNOLOGY

A*STAR *Research*
www.astar-research.com

*A*STAR Research* is a publication of the Agency for Science, Technology and Research (A*STAR) — Singapore's lead government agency for fostering world-class scientific research.

*A*STAR Research* is published twice a year, presenting research highlights and feature articles. All articles are first published online on the *A*STAR Research* website and available free to all readers. Register online to receive the table of contents by email as each biweekly online issue is published.

Editorial
Agency for Science, Technology and Research
1 Fusionopolis Way, #20-10 Connexis North Tower
Singapore 138632, Singapore

Editor-in-Chief: David Lane
Editorial Board: Pernille Rorth, Alfred Huan, Huck Hui Ng, Colin Stewart, Andy Hor, Keith Carpenter
Managing Editors: Kostas Repanas, Ariel Tan
Administrative Assistant: Lay Hoon Tan

ISSN 2010-0531

ON THE COVER
An artist's three-dimensional rendering of a nanopillar. Research from A*STAR may enable the production of more reliable micro- and nano-electromechanical devices. [p38]

You've read the magazine, now visit the website!

A*STAR *Research*

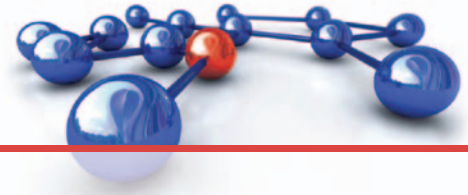
SINGAPORE

Visit the *A*STAR Research* website for the latest advanced research from the Agency for Science, Technology and Research (A*STAR).

Thanks for picking up the latest print edition of *A*STAR Research*. We hope you'll enjoy reading all about some of the latest, most cutting-edge research carried out at A*STAR.

If you'd like to know more about the world-class research work of our scientists, why not visit the *A*STAR Research* website. Updated twice a month, *A*STAR Research* brings you the very latest research from A*STAR across a broad spectrum of scientific and technology fields, from molecular biology to microelectronics, materials research to manufacturing technology. The research highlights are further enhanced by Features and Innovations articles that take a look at the people behind that work and some of the most exciting discoveries and inventions that come out of it.

*A*STAR Research* is a must-read resource for anyone who wants up-to-date coverage of the latest research and technological developments in one of Asia's most dynamic research hubs — all completely free of charge. And when you visit the website and have read the latest highlights and browsed the newest features, don't forget to register for free biweekly email alerts that bring the most recent research from A*STAR right to your inbox, so you need never miss a single edition of *A*STAR Research*.



www.astar-research.com

Adding another dimension to smartphones

A new type of plastic film developed in Singapore brings the third dimension to handheld mobile devices

The rise and rise of the smartphone and tablet markets has generated unprecedented demand for all things new in the world of mobile devices, from applications and accessories to innovative technologies designed to broaden and enrich the user experience. Now, a group of scientists in Singapore has developed an innovative, precision-engineered plastic film that can turn standard mobile phone screens into three-dimensional (3D) displays.

The new plastic film, known as EyeFly3D — devised and developed by researchers at the A*STAR Institute of Materials Research and Engineering (IMRE) and Temasek Polytechnic (TP) in Singapore — enables smartphone users to view 3D images and videos without having to wear special glasses or headgear.

With a thickness of less than 0.1 millimeters, EyeFly3D is the first innovation of its kind to offer a glasses-free 3D experience in both portrait and landscape formats. Outwardly resembling an ordinary screen protector for mobile phones, the film's uniqueness lies at the nanoscale. Using a method based on the IMRE's proprietary nanoimprinting technology, the film consists of around half a million carefully shaped lenses patterned onto its surface. This pattern, known as a lenticular lens array, gives a smoother, clearer and more transparent finish than that of previously developed 3D filters.

Conclusion of the licensing agreement between A*STAR, Temasek Polytechnic (TP) and Nanoveu Pte Ltd to enable the marketing of EyeFly3D. Standing: Andy Hor (left), executive director of the IMRE, and Raj Thampuran (right), managing director of A*STAR. Seated (left to right): Lay-Tan Siok Lie, deputy principal of TP; Alfred Chong, CEO of Nanoveu; Philip Lim, CEO of ETPL (Exploit Technologies Pte Ltd), the technology transfer arm of A*STAR.

"Our proprietary process is highly customizable and allows for rapid prototyping to suit the latest high-resolution smartphones and tablets currently on the market," says Loke Yee Chong, who currently leads the IMRE's lenticular lens array project.

Nanotech triumph

Lenticular lens technology works by presenting two different perspectives of the same image to the left and right eyes for every pixel of the image. This creates an autostereoscopic display, which enables the user to perceive 3D content without the need for extra equipment.

The innovative use of nanoimprinting to enhance existing lenticular lens technology enabled the research team to focus on design and process optimization. "One of the primary reasons for choosing nanoimprinting technology as a baseline for the development of this plastic film is the technology's capability for high-resolution patterning," Loke explains. An increasing consumer demand for higher-resolution mobile liquid-crystal displays (LCDs) has been accompanied by a dramatic reduction in pixel pitch size — presenting considerable challenges for conventional photolithographic techniques. "There has been a pertinent need to find a new micro- or nanofabrication technique for glasses-free 3D displays that is on par with the LCD-display technological advancement. Nanoimprinting technology fits the bill, with its ability to achieve very high resolution patterning, its flexibility and its manufacturing scalability through roll-to-roll."

EyeFly3D not only offers a high-quality, distortion-free viewing experience but also provides a cost-effective solution for the creation of 3D graphics and effects, as it does not require extra backlighting and thus limits battery consumption. In addition, smartphone users can easily place and peel off the reusable film themselves. With a durability similar to that of other screen protectors currently on the market, EyeFly3D can withstand the typical handling and scratches that mobile phones are subjected to.

EyeFly3D is being marketed through Nanoveu Pte Ltd, a Singapore-based nanotech start-up. The 3D technology itself will be licensed exclusively from >>>

With the newly developed plastic film, futuristic 3D capabilities are becoming a possibility for smartphones.

ETPL (Exploit Technologies Pte Ltd), the technology transfer arm of A*STAR, and TP. Originally designed for use in mobile phones, the film is expected to be affordable and readily available to consumers.

In 2009, researchers from the IMRE's nanoimprinting group and software programming specialists at TP first embarked on the development of an autostereoscopic display that exploits both groups' baseline technologies, in work aided by a National Research Foundation Translational R&D Grant and supported by A*STAR. At the time when widespread interest in 3D technology surged due to the release of films such as *Avatar* (2009), glasses-free 3D technologies were still in their relative infancy. "This posed a challenge for us as we did not yet have a reference benchmark, but at the same time, it presented an opportunity for us," says Jaslyn Law, the IMRE researcher who worked closely with colleagues from TP to come up with the patented lens array structures. "Now, 3D viewing is everywhere — from smart televisions to computers where 3D video can be seen on YouTube."

Driven by consumer demand, 3D display technologies have hit their stride and today utilize increasingly sophisticated imaging methods. Besides lenticular lenses, 3D effects can also be achieved through a device called a parallax barrier, a type of filter consisting of alternating opaque and transparent strips that give the illusion of depth. Compared with the parallax barrier, however, lenticular lenses offer the advantage of presenting brighter and sharper images.

The realization of this nanotech-enhanced lenticular lens technology is due in part to the extensive work the IMRE has performed on nanomaterials and innovative thin films. "Our nanoimprinting group at the IMRE has been working on various types of functional films, such as antireflective and antibacterial films, for a number of years," says Law. (For other recent examples of the IMRE's work on nanoimprinting, patterning and synthesis, see the article "Innovation: Fantastic plastic" and the research highlights "Nanomaterials: Copying geckos' toes" and "Nanoparticle assembly: Bridging spheres".)

Game on

In addition to bringing the initial concept of nanoimprinting technology to market, the research team has developed new applications — compatible with the Apple iOS and Android software platforms — to facilitate the transition from 2D to 3D viewing. Users will potentially be able to convert any photo or video taken on their mobile device into 3D media.

Another area in which the new technology may be applied is online security. Increasingly, banks and corporations are adopting strategies to improve web-based user authentication, and the thin-film patterning technique may open up new avenues of research into nanoencryption and electronic access systems.

There are also plans for the research team to release a software development kit, which will enable game developers to gain ground in the fast-growing handheld 3D gaming market. Video gaming is a global multi-billion-dollar industry and continues to evolve to suit the needs and preferences of mobile users.

"The success of this project is typical of what the IMRE aims to do — innovate and turn science into an exciting business opportunity," says Andy Hor, executive director of the IMRE. "I'm glad this has given us products that make life just a little bit more fun."

Making long-haul, ultrahigh bandwidth more affordable

*Researchers at A*STAR have made low-cost, ultrahigh-bandwidth telecommunication across continents a possibility through an innovative advance in silicon photonics*

Silicon photonics is an evolving technology that allows data to be transferred between computer chips by optical rays, which are able to carry larger amounts of data over shorter periods of time than conventional electrical conductors. Historically, the excessive energy consumption of the lasers used to generate the infrared beams that carry the data posed a serious challenge for the developers of silicon photonic devices. Furthermore, dissipation of heat within the silicon reduces the efficiency of lasing — thus limiting the devices' effectiveness. For these reasons, optical computers using silicon chips have relied on external lasers for their operation. In recent years, however, such problems have been overcome by new techniques that include combining silicon with indium phosphide to improve the infrared-transmission capacity of the device, boosting data relay speeds above 25 gigabits per second (Gbps). >>>

In collaboration with Japanese optical telecommunication network systems-developer Fujikura Ltd, the A*STAR Institute of Microelectronics (IME) has now pioneered the world's first 40 to 60 Gbps silicon-based optical modulators. These devices permit high-speed transfer of data over long distances in an advanced — multilevel — modulation format and greatly increase the total data communication throughput for a given optical channel. Additionally, compared to conventional non-silicon-based modulators, such as those of lithium niobate, the new modulators created by the IME and Fujikura have a much smaller energy footprint and are significantly cheaper to fabricate.

"We are proud to have jointly achieved this breakthrough with Fujikura," says Dim-Lee Kwong, professor and executive director of the IME. "This will fuel the design and development prospects of next-generation, long-haul telecommunication systems as well as truly bringing low-cost, high-performance optical communications to the masses." The researchers presented their remarkable findings at OFC/NFOEC, a global conference and exposition on optical communications and networking held in the United States, in March 2013.

Founded in 1991 as an institute for research in advanced engineering, the IME works to stimulate the microelectronics industry in Singapore by undertaking research and development in microelectronics, and supporting the needs of the industry through training and development of skilled personnel. One of the IME's goals is to encourage semiconductor companies to conduct small-scale pilots of prototypes. Under Kwong's leadership, the IME has fostered partnerships with more than 50 multinational firms ranging from major Japanese conglomerates to Forbes 500 companies. To date, the IME has successfully generated an impressive portfolio of internationally approved patents.

© iStockphoto/Thinkstock

Improved silicon-based photonic devices developed by A*STAR and Fujikura Ltd hold promise for the high-speed optical transfer of data, offering potential reductions in the cost of global telecommunication.

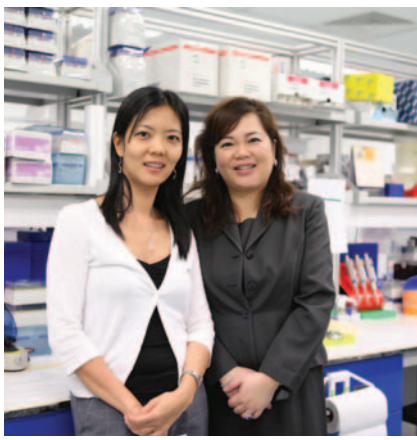
The strategic partnership between the IME and Fujikura was forged in 2006 to develop technologies for optical telecommunications. The current innovation is the latest in a line of success stories born of the relationship, allowing the IME and Fujikura to prove that reaching ultrahigh performance levels on a silicon-platform technology is now a reality. The advance is expected to provide a huge boost to the development of ultrahigh-bandwidth optical communications. Kenji Nishide, general manager of the Fujikura Optics and Electronics Laboratory, is excited about the recent breakthrough. "We have opened the door to expand silicon-based modulators in next-generation optical telecommunication networks," he says.

The IME and Fujikura are now partnering to create more prototypes of highly integrated photonic chips able to accommodate higher transmission capacities and spectral efficiencies, and working closely to refine the production cycle for the new modulators with the aim of bringing the new chips to market as early as 2015.

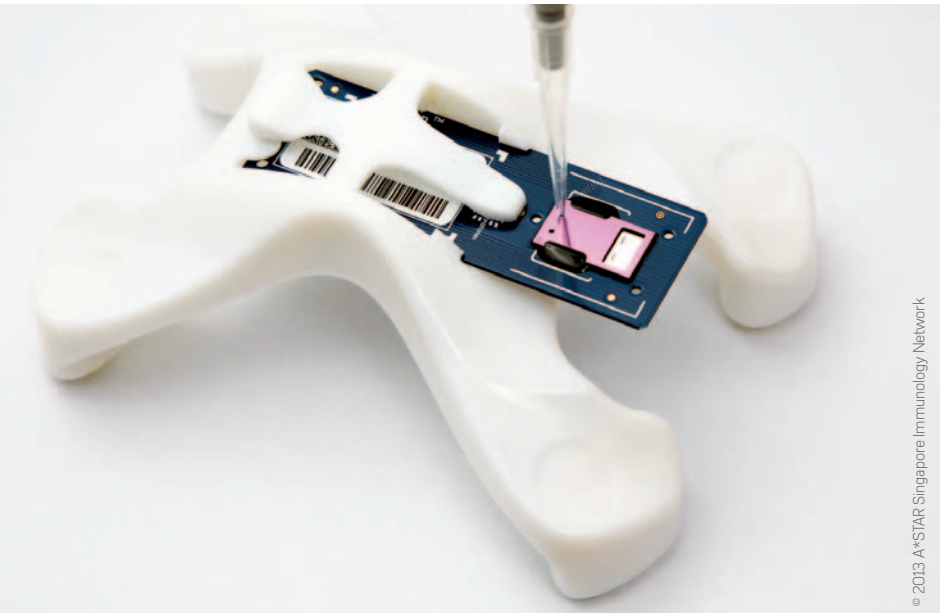
Fast and simple detection of tropical diseases

*A panel of major tropical diseases may soon be detectable from a single blood sample thanks to a biochip created at A*STAR*

While medical technology and healthcare standards have improved significantly over the past century, tropical diseases continue to pose a major threat to human health. At present, vaccines are unavailable for many major tropical infections such as dengue fever and hand, foot and mouth disease. Increased exploration of tropical rainforests, international air travel, tourism to tropical regions and human migration have also led to a rising incidence of tropical diseases. Accordingly, the rapid and accurate detection of such diseases is more >>>



Lisa Ng (left), lead virologist on the VereTrop™ project at SIgN, and Rosemary Tan (right), CEO of Veredus Laboratories Pte Ltd.



The VereChip™ biochip forms a central part of the VereTrop™ diagnostic kit, which requires only a single drop of blood to screen for 13 different major tropical diseases.

important than ever to facilitate prompt treatment and prevent potential pandemics.

In collaboration with Veredus Laboratories Pte Ltd, a leading supplier of innovative molecular diagnostic tools, a team of researchers from the A*STAR Singapore Immunology Network (SIgN) have created the VereChip™, a biochip that can identify 13 different major tropical diseases — including dengue fever, chikungunya, hand, foot and mouth disease, and malaria — from a single blood sample.

The biochip itself forms the core of the VereTrop™ diagnostic kit. Lisa Ng, the project's lead virologist at SIgN, explains that the kit's high level of automation and efficiency are the reasons why VereTrop™ is poised to revolutionize the quality of testing for tropical infectious diseases. "Tropical diseases often reflect common symptoms like fever and may not be accurately diagnosed at an early stage by doctors. This portable diagnostic kit allows accurate testing of multiple pathogenic targets from just one blood sample in a matter of hours — compared to days or even weeks for current methods," says Ng.

Together with Laurent Renia, an expert in the immunobiology of malaria at SIgN, the team successfully validated the kit with patient samples collected and tested in rural regions of the Thai–Myanmar border in northern Thailand. Due to its highly automated nature, laboratory personnel can be trained to operate the VereTrop™ kit in just one day. "This innovation opens up new possibilities for the accurate and rapid diagnosis of important infectious diseases that remain the major causes of illness in the tropics," says François Nosten, director of the Shoklo Malaria Research Unit in Thailand and clinical expert on the team. "VereTrop™'s versatility and ease of use will no doubt change the approach to diagnostics at the periphery of the health care system."

The VereTrop™ diagnostic kit is the latest in a line of success stories born of the relationship between Veredus Laboratories and A*STAR. "We have worked on several collaborative projects, including diagnostic technology for influenza and malaria, dating back to 2004," says Rosemary Tan, CEO of Veredus Laboratories. "VereTrop™ is another testament to the fruitful collaboration." The partners are now planning to register the biochip as an *in vitro* diagnostic product as early as next year.

Philip Lim, CEO of ETPL (Exploit Technologies Pte Ltd), the technology transfer arm of A*STAR, praises the success of the project and describes the novel biochip as the perfect example of a public–private partnership. "After a journey of more than three years, we are glad that such a compelling, technologically advanced product with global healthcare benefits is ready to be launched onto the market. The creation of the VereTrop™ kit is proof that local companies can work with A*STAR to achieve a competitive edge globally."

The fully automated MicroKit for rapid detection of flu viruses is one of a number of IBN's successful collaboration projects.

Partnering for better diagnostics

*A joint research center at the A*STAR Institute of Bioengineering and Nanotechnology aims to improve infectious disease diagnostics*

The A*STAR Institute of Bioengineering and Nanotechnology (IBN) has forged a strategic partnership with ARKRAY, Inc., a leader in the field of automated analysis systems, to create new detection kits for infectious diseases. ARKRAY is investing S$9.1 million to set up a base in IBN — its first Asian research center outside of its home country, Japan.

"We are delighted with the partnership. IBN certainly identifies with ARKRAY's vision of improving lives through scientific advancement and new technologies," says Jackie Y. Ying, professor and executive director at IBN.

Takeshi Matsuda, president and CEO of ARKRAY, believes that IBN is the most suitable >>>

partner for the major collaboration based on the institute's outstanding record in multidisciplinary research. "ARKRAY is excited to embark on this venture with IBN," says Matsuda. "With our new research center in Singapore, we look forward to building a strong rapport with IBN through this long-term partnership."

Founded in 2003 as the first bioengineering and nanotechnology research institute in the world, IBN has developed many notable commercialized biodevices and diagnostic technologies. These include: the MicroKit, an automated diagnostic device for rapid detection of influenza viruses; DropArray™, a miniaturized drug screening platform that allows bioassays to be performed more economically and quickly; and a second-harmonic generation microscope, which forms part of a fully automated quantification system for liver fibrosis. The institute also uses nanotechnology and biological microelectromechanical systems (bio-MEMS) to fabricate innovative miniaturized platforms for early and accurate disease detection.

Under Ying's leadership, IBN currently has over 120 active research collaborations with industrial, academic and clinical partners. Notably, its MicroKit won a Silver award at the *The Wall Street Journal* Asian Innovation Awards 2011. Another development that received recognition was the novel superbug-destroying antimicrobial agent developed by IBN and IBM in 2011, which featured as one of the *Scientific American* top ten "World Changing Ideas" that year.

IBN plays an active role in technology transfer by linking the institute and industrial partners with other global institutions. In 2009, IBN became the first biomedical research institute at A*STAR to win both A*STAR's The Outstanding Publications (TOP) Award and Patent Power Award. To date, the institute has established six spin-off companies, and it has successfully generated an impressive portfolio of over 600 active patents and patent applications, together with more than 800 publications in leading scientific journals.

ARKRAY is a leading developer and manufacturer of medical diagnostic and monitoring systems, and is particularly well known for its glycohemoglobin and urine analyzers, which help users to monitor diabetes. Founded more than 40 years ago in Kyoto, Japan, its products are currently distributed to over 80 countries. ARKRAY's new research center at IBN will house 21 researchers sharing the common goal of developing novel detection kits for infectious diseases — a project that aligns with the company's mission to contribute to the health and wellbeing of the global population.

Looking ahead, Ying shares Matsuda's enthusiasm for the new partnership, which will run for five years. "We are confident that our collaboration with ARKRAY will lead to the creation of new devices and advanced instruments for disease detection and monitoring," she says.

Innovation on the move

*The A*STAR Institute of Microelectronics and Japan's Shikino High-Tech Co., Ltd have united to develop improved technologies for motion sensing*

Motion sensing is fast becoming a 'must-have' function in consumer electronics today. For instance, motion-sensing capability is incorporated into digital cameras and camcorders, enabling image stabilization and augmentation with information such as where a shot was taken and the direction that the camera was pointing. In game consoles and smartphones, motion is used to control game play and to enable user interface functionality.

Gyro sensors, also known as gyroscopes, are the motion-sensing devices that enable such functionality by sensing changes in angular velocity. In addition to their use in consumer electronics, gyro sensors can be integrated with portable medical devices and sports equipment, allowing patients to be monitored remotely by medical staff and athletes' motion to be tracked for training purposes.

Joining efforts with a Japanese camera systems and image-processing module developer, Shikino High-Tech Co., Ltd, the A*STAR Institute of Microelectronics (IME) has signed a research agreement to pioneer an energy-efficient, high-performance application-specific integrated circuit intellectual property (ASIC IP) block for a gyro sensor to be installed in commercial handheld devices. Yuaki Osada, president of Shikino High-Tech, believes that the IME is an excellent choice of collaborative partner for the project due to the institute's proven and outstanding capabilities, particularly in the area of analog technology development.

"It is a valuable opportunity for Shikino High-Tech to work with the IME in this advanced technology project," says Osada. The agreement marks the first >>>

© iStockphoto/Thinkstock

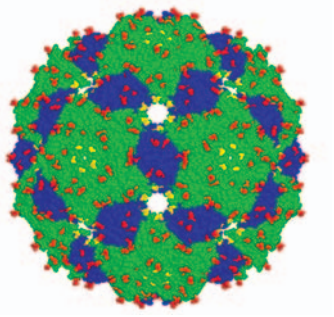
A*STAR and Shikino High-Tech Co., Ltd are teaming up to create a next-generation application-specific integrated circuit intellectual property (ASIC IP) block for use in motion-sensing technologies.

formal collaboration in Singapore for Shikino High-Tech, which boasts more than 25 years of R&D experience in Japan and an impressive record of technological inventions and patents. Osada is confident in the success of this initial research project and anticipates further exciting collaborations with the IME in the future.

The IME, founded in 1991, is a recognized leader in the development of commercial technologies and has particular strengths in the areas of microelectronics and semiconductors. Part of the institute's core mission is to provide support to industry. Its multidisciplinary approach to research, expertise in technology transfer and state-of-the-art facilities have led to the establishment of collaborations with more than 50 multinational firms and across every sector of the electronics industry.

Dim-Lee Kwong, executive director of the IME, is enthusiastic about the venture with Shikino High-Tech. Noting the IME's extensive research experience with Japanese companies, he is confident that the partnership will benefit both Shikino High-Tech and A*STAR. "This new collaboration will no doubt provide a strategic platform for the IME's researchers to leverage existing capabilities in the development of innovative gyro sensor technologies."

This new strategic partnership between the IME and Shikino High-Tech is a further testament to Singapore's position as a preferred country for Japanese companies to invest and expand in. In recent years, the number of collaborations formed between local establishments and Japanese companies has been growing steadily. According to the 2012 Singapore Business Formation Statistics Report, Japan ranks among the top investors in the Republic for that year.



Schematic representation of the Qβ virus-like particle (VLP) (blue and green) before coupling to hemagglutinin, one of the proteins present on the surface of influenza viruses that elicit an immune response in humans.

One step closer to Singapore's first influenza vaccine

*A*STAR's new vaccine, based on a virus-like particle technology created by Cytos Biotechnology AG, could strengthen Singapore's ability to tackle influenza pandemics*

Continuing population growth and urbanization are likely to increase the transmission of infections in Singapore. Higher levels of migration, travel and tourism are also intensifying the nation's susceptibility to disease transmission. Drawing on experiences from the influenza, or 'flu', outbreaks of recent years, many current research efforts have turned their focus to the development of influenza vaccines.

In late May 2009, following a global outbreak of the H1N1 influenza A virus (H1N1), the first confirmed case in Singapore was diagnosed. Despite the rigorous containment measures enforced by the government, the number of confirmed cases rose to 1,217 within 6 weeks. By June that year, the World Health Organization (WHO) declared the new strain — widely referred to as 'swine flu' — a pandemic. Three months later, when the Singapore Ministry of Health (MOH) had managed to secure a supply of vaccine from healthcare company GlaxoSmithKline, the pandemic was already in decline. Left with an excess of a vaccine that had a short expiry date, the following year the MOH decided to make their supply available to the public at no cost.

While the H1N1 pandemic was relatively mild, it did have a notable impact on the tourism and retail sectors of Singapore. If the outbreak had been more severe, like that of severe acute respiratory syndrome (SARS) in 2003, the economic and public health consequences could have been dire. These two events served to highlight the vulnerability of Singapore to infectious diseases and the nation's dependence on overseas vaccine manufacturers. Thus, to better prepare for potential future pandemics, it has become vital for Singapore to be able to quickly and locally produce large quantities of vaccine.

Singapore's very own vaccine

The A*STAR Experimental Therapeutics Centre (ETC) and Cytos Biotechnology AG, a Swiss biopharmaceutical company with a focus on vaccine development, have forged a partnership to create a vaccine that offers protection against H1N1. "This is the first time Singapore is attempting to make its own flu vaccine," says Lim Chuan Poh, chairman of A*STAR, who believes the attempt will improve Singapore's ability to respond to flu outbreaks. The choice of H1N1 as the vaccine's target strain was based on a recommendation by the WHO.

Influenza, a contagious respiratory illness caused by influenza viruses, spreads easily from person to >>>

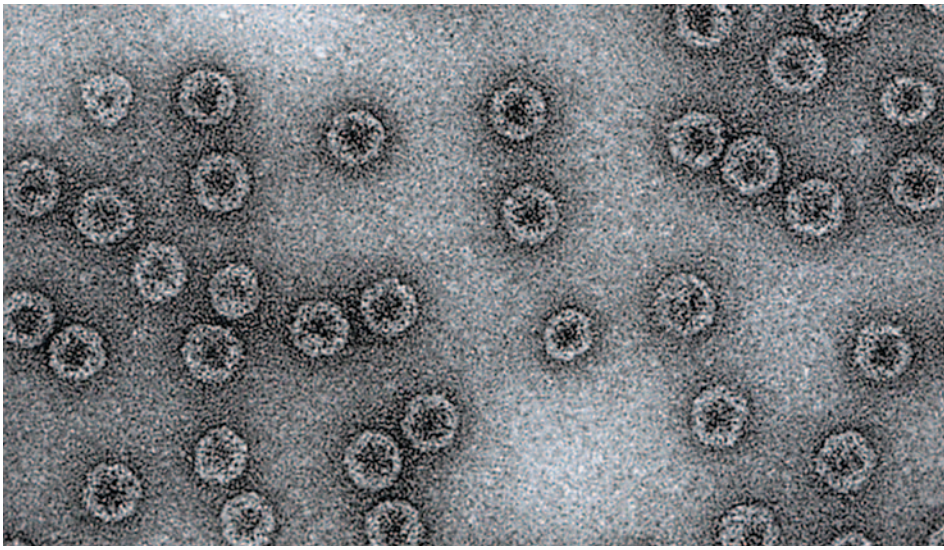
person and can affect people of any age, leading to mild to severe illness or, at times, even death. Two major proteins on the surface of influenza viruses — neuraminidase (N) and hemagglutinin (H) — are responsible for eliciting a protective antibody response in humans. Flu viruses are characterized by the types of neuraminidase and hemagglutinin that they carry, which give rise to the names of influenza subtypes, such as H1N1 and H7N9. Conventionally, chicken eggs are used to manufacture flu vaccines, but this process is time-consuming, presents limited yields and is sometimes constrained by the toxicity of certain viral strains to birds.

The new H1N1 vaccine is based on Cytos' bacteriophage Qβ virus-like particle (VLP) technology. VLPs are multi-protein structures that resemble viruses but are noninfectious due to the absence of viral genetic material. The VLP platform was chosen as the basis for the vaccine as it offers several advantages, including the easy production of influenza-subtype hemagglutinins and VLPs by recombinant techniques, which eliminates the need to work with live viruses. Furthermore, the entire process is relatively fast and economical.

Clinical trial begins

In April 2013, the first healthy volunteer was dosed with the vaccine candidate in a Phase 1 clinical trial, which will evaluate the safety and immunogenicity of the vaccine. "In the wake of the recent H7N9 bird flu outbreak, it is timely that A*STAR is taking Singapore's first H1N1 flu vaccine into clinical trial," says Lim. Christian Itin, chairman and CEO of Cytos, agrees: "We are very pleased with the fruitful collaboration, which has led to the clinical start of this novel influenza vaccine. This is an important milestone for the program and the first clinical program using Cytos' B-cell vaccine platform for a prophylactic vaccine against infectious disease."

In addition to the ETC and Cytos, several other local academic and clinical partners are participating in the research effort. The immunological aspects were tackled by researchers at the A*STAR Singapore Immunology Network (SIgN) while the ETC acted as the primary driver at the start of the clinical development program. Nonclinical efficacy experiments were performed at the DSO National Laboratories and Duke–NUS Graduate Medical School. In early 2012, 'D3', the A*STAR drug discovery and development platform, collaborated with Cytos to lead the development of the vaccine project with the aim of achieving 'proof-of-concept' in humans. At present, a clinical trial is being conducted at the SingHealth Investigational Medicine Unit and the Changi General Hospital Clinical Trials & Research Unit.



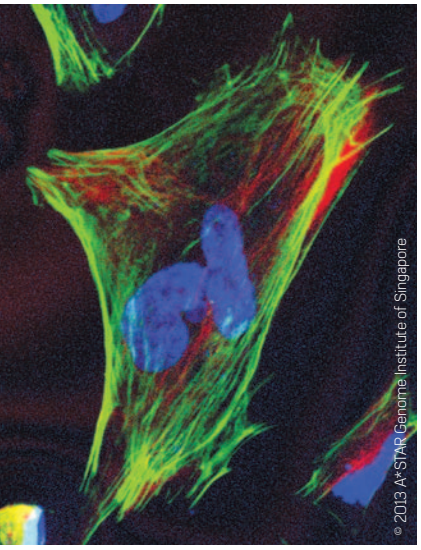
A scanning electron micrograph of Cytos' VLP.

Overall, the team aims to include 80 healthy individuals in their first clinical trial. "If this VLP-vaccine strategy proves to be effective, it can accelerate the production of vaccines against new, emerging strains of flu," says Alex Matter, professor and CEO of D3 and the ETC. "In the event of a flu epidemic, we hope to contribute to Singapore's preparedness by producing vaccine candidates for clinical trials quickly, safely and economically."

Opening new doors for vaccine production

While it is still early days for the project, the team takes pride in the progress made so far. Their efforts demonstrate that a cohesive team can be successfully formed from different institutions to work toward a common goal. In addition to overcoming great technical and clinical challenges, the team has overcome financial hurdles and secured funding for the project through grants from ETPL (Exploit Technologies Pte Ltd), the technology technology transfer arm of A*STAR, and SIgN, which together with the ETC also made substantial in-kind, as well as financial, contributions. "This experience, in our view, augurs well for the expertise, the will and the stamina of Singapore R&D to tackle ambitious projects in the biomedical arena," shares Matter.

A successful outcome for the trial will no doubt open up new opportunities to tackle emerging flu strains. "I am pleased that the collaboration with Cytos is making a meaningful contribution to Singapore's pandemic readiness, a critical aspect of our national security," says Lim. If the vaccine is approved for mass production, A*STAR subsidiaries will have the right to develop and commercialize the vaccine for Singapore and other southeast Asian countries and can earn royalties on worldwide net sales. "The potential success of this vaccine will have a significant impact not only in the region but globally as well," adds Lim.



A single cell derived from a human embryonic stem cell.

Image captured by Nani Djunaidi© 2013 A*STAR Genome Institute of Singapore

The Single-Cell Omics Centre (SCOC) provides access to sophisticated genomic and sequencing instruments.

Understanding the individual

*A*STAR's Single-Cell Omics Centre, the first research center in Asia exclusively dedicated to accelerating the understanding of how individual cells work, officially opened in April 2013*

Single-cell genomics is one of the most riveting emergent areas in contemporary life sciences research. Understanding how individual cells work could provide valuable insight into some of the fundamental biological mysteries of our time, paving the way for new diagnostics, treatments and methods of prevention for diseases such as cancer and diabetes. Early successes in the field include the elucidation of important single-cell phenomena involved in aging, the loss of sight through macular degeneration and the spread of infectious diseases. Correspondingly, the Single-Cell Omics Centre (SCOC) was established to serve as a key resource for both academia and industry in Singapore and the surrounding region, helping the research community pursue new frontiers in single-cell genomics.

The SCOC is the result of a forward-thinking collaboration between the A*STAR Genome Institute of Singapore (GIS), a prominent center for genomic discovery with the goal of using genomic sciences to improve public health and prosperity globally, and Fluidigm Corporation, an industry leader in single-cell genomic technologies and the first biochip company to be established in Singapore. The center is envisioned as a hub that brings scientists from various fields of biology together to work toward a common objective such as understanding how stem cells might be reprogrammed for therapeutic treatments. Knowledge of the processes involved would be highly beneficial for the development of new drugs and the tailoring of therapies to the needs of individual patients — so-called 'personalized medicine'.

The SCOC's official inauguration event — held in April 2013 — attracted government officials, prominent academics and industry leaders. As the first research center in Asia fully dedicated to the study of how individual cells function and behave in disease states, the SCOC aims to become a valuable resource through which researchers in the region can access integrated analytics for single-cell genomic applications. Lim Chuan Poh, chairman of A*STAR, praises the opening of the center and describes the SCOC as a perfect example of public–private partnership. "By encouraging multidisciplinary collaborations, this center will further enhance Singapore's R&D capabilities and scientific know-how. It may also lead to new, potentially life-saving applications in the biomedical sector," says Lim. Gajus Worthington, president and chief executive officer of Fluidigm, agrees: "We expect the combination of rich application diversity, ground-breaking science and the endorsement from key opinion leaders throughout Asia to make the SCOC one of the leaders of single-cell innovation in the world."

Housed in modern facilities at the GIS, and with its own dedicated laboratory space, the SCOC exploits advanced next-generation genomic equipment and sequencing technologies. The impressive set-up includes a first-of-its-kind Fluidigm C_1™ Single-Cell Auto Prep System, which automatically isolates individual cells from small quantities of tissue or larger cell populations. The SCOC also boasts two BioMark™ HD Systems — wholly manufactured in Fluidigm's factory in Singapore — that are capable of single-cell gene expression analytics and validation. Huck Hui Ng, executive director of the GIS, explains that the institute is committed to single-cell genomics as one of its new research frontiers, adding that the GIS plans to continue to build up its repertoire of new research capabilities for single-cell analyses. "Our initial collaboration with Fluidigm has borne fruit with the publication of a landmark paper by Paul Robson of the GIS," says Ng. "This larger and very important collaboration [with the SCOC] will see an even greater synergy between the technologies from GIS and Fluidigm."

In its initial phase, the SCOC will focus on single-cell analysis of lung and colon cancers in both solid and circulating tumor cells (CTCs). CTCs are cells that have been shed from a tumor and are circulating in the bloodstream. The study of CTCs is of particular importance as these cells promote the seeding and growth of additional tumors in other organs in the body — a process known as metastasis. Conventionally, samples from solid tumors are studied as an aggregate, where all cells are analyzed together in a jumbled genomic stew. At present, the SCOC is actively developing a method to suspend the cells of solid tumors in solution, and to subsequently isolate individual cells from suspension for study and sequencing using the C_1 Single-Cell Auto Prep System. The clinical implications of this research are far-reaching: the need for surgery to obtain samples from tumors may potentially be eliminated as diseases could be monitored through capture of CTCs from the blood.

One of the SCOC's anticipated follow-on projects will be the development of novel methods to >>>

compare cells that have been treated with a particular drug with those that have not. This research, planned for the near future, will offer clear insight into how individual cells react to the presence of potential therapeutic agents, providing observations that can be used as the basis from which to explore more effective treatments for diseases. "Single-cell genomics provides researchers with an opportunity for extraordinary scientific discovery. Individual cells, even from the same tissue, do not function identically. These differences can be the key to crucial biological insights, including the diagnosis and treatment of critical diseases," says Worthington, explaining his enthusiasm for the research.

The work being undertaken at the SCOC requires the analysis of a large number of cells. While in the past this process was extremely laborious and time-consuming, the SCOC's state-of-the-art instruments enable its researchers to process hundreds of cells per day in a cost-effective and efficient manner. The center is confident that its tools, methods and insightful research will quickly lead to scientific breakthroughs, and is looking forward to sharing these discoveries with the rest of the world. "Since the late 1830s we have known that the cell is the fundamental unit of life, yet it has been a challenge to comprehensively study biology at the cellular level. The technology has now arrived to do this and the local research and medical communities are abuzz with possibilities," says Robson, a GIS principal investigator and lead scientist of the center. "The SCOC aims to facilitate the research community's access to these microfluidic technologies and thus enable unparalleled insight into underlying biological mechanisms operative in health and disease," he concludes.

Huck Hui Ng (left), executive director of the A*STAR Genome Institute of Singapore (GIS), Lim Chuan Poh (center), chairman of A*STAR, and Gajus Worthington (right), president and chief executive officer of Fluidigm, at the official opening of the SCOC.

Turning unused TV frequencies into wireless broadband

The Singapore White Spaces Pilot Group takes the nation's capability in 'white space' wireless connectivity to a new level

Every day in Singapore, millions of mobile devices connect to the web wirelessly. The rapid growth of data traffic is putting a strain on current network infrastructure, prompting a need for innovative use of spectrum to increase wireless broadband capacity.

In recent years, the method of providing wireless internet access through unlicensed TV broadcasting frequencies — also known as TV white spaces (TVWS) — is gaining traction. TVWS was originally used as a buffer to minimize interference in analog broadcasts. Since the digitization of TV channels, an abundance of frequencies has been freed up. These frequencies can potentially be utilized to provide cost-effective wireless broadband.

In April 2012, the Singapore White Spaces Pilot Group (SWSPG) was formed to support Singapore's efforts to adopt TVWS for consumer and business services and applications. The founding members included A*STAR's Institute for Infocomm Research (I^2R), Microsoft Singapore, local telecommunications company StarHub, and Neul, a British mobile wireless data service provider. Five more members joined soon after, taking the number of early members up to nine.

"Our involvement in TVWS dates back to 2006 when we participated in the first IEEE TVWS >>>

From left: Paul Mitchell, senior director of Technology Policy at Microsoft; Mock Pak Lum, chief technology officer of StarHub; Tracy Hopkins, corporate vice president of Neul; and Tan Geok Leng, executive director of A*STAR's Institute for Infocomm Research.

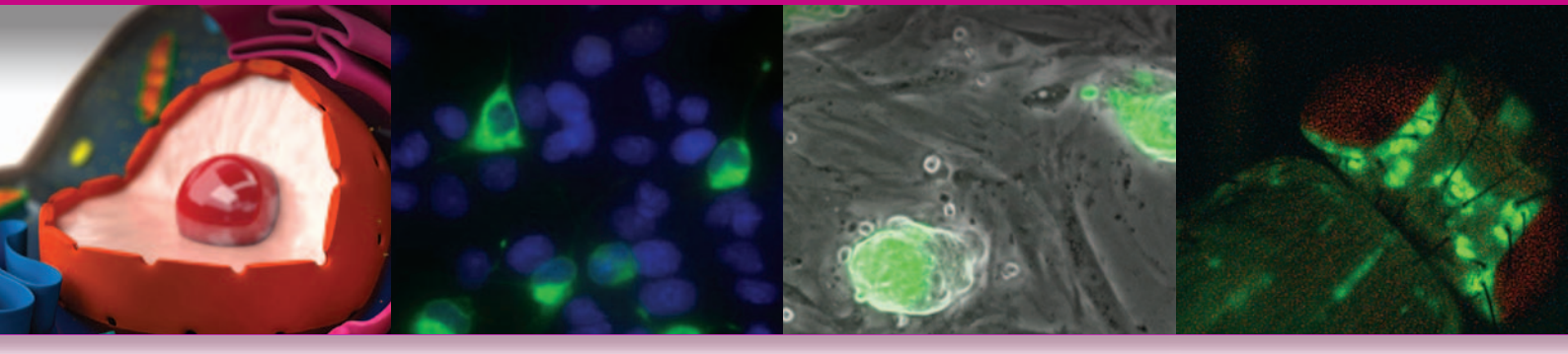
standards," comments Tan Geok Leng, executive director of the I²R. "I am proud to say that Singapore is one of the pioneers in this area of research, and continues to be a trailblazer in the global TVWS movement. The successful pilots that we have seen in Singapore have set benchmarks in showcasing the potential of TVWS technology in delivering reliable and cost-efficient wireless broadband for multiple commercial applications. With the new pilot projects, we hope to see TVWS being deployed in many other new and innovative applications."

The SWSPG launched its first series of commercial pilot deployments of TVWS in September 2012. In one of the projects, the I²R is working with Power Automation Pte Ltd to develop TVWS-based infrastructure for utility metering, which could be a basis of energy grid modernization. "The benefit of using TVWS technology is tremendous," says Tracy Hopkins, vice president of Neul, "be it to support 'smart city' infrastructure, to extend connectivity into previously challenging environments or to enable ubiquitous, reliable wireless connectivity that will enhance our lives."

In June 2013, the SWSPG welcomed nine new members to expand its range of projects. The SWSPG will work with the Housing and Development Board to trial various applications for residential buildings, as well as with the Eurokars Group to cost-effectively extend its IT network. The SWSPG will also be deploying TVWS pilot projects to Singapore's new large-scale ecological park Gardens by the Bay and local island resort Sentosa to assess the viability of providing wireless internet service through TVWS. This new set of TVWS projects is the most extensive in the Asia-Pacific region to date. "The addition of these nine new members is a testament to the potential and demand for TVWS technology in Singapore," comments Mock Pak Lum, chief technology officer of StarHub.

Meanwhile, researchers at the I²R are already working on next-generation TVWS technology that will improve spectrum efficiency, scalability and quality of service. "With the momentum gained worldwide regarding the potential of TVWS, we are looking forward to more innovative use of the technologies," says Tan. "We believe TVWS is the first step to better utilize valuable frequency spectrums in order to support the exponential growth of wireless adoption."

Research Highlights

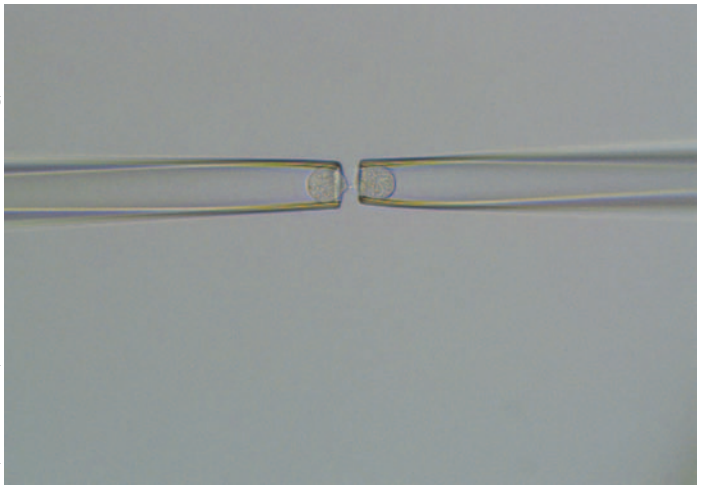


CELL BIOLOGY & IMMUNOLOGY

Development:

When fate hinges on cell–cell contact

Proper cell–cell interactions are required for the cells of early embryos to develop normally



Pulling pipettes apart to gently separate early embryonic cells.

Some 50 years have passed since scientists first proposed the so-called 'inside–outside model' of development, which holds that the inner cells of the early embryo eventually form all the definitive structures of the fetus, whereas the outer cells give rise to the placenta. Yet, the determinants of this developmental duality have remained elusive: are lineage decisions predetermined in the egg or is cell–cell contact needed to determine cell fate?

By physically separating cells in young mouse embryos, a team led by Barbara Knowles and Davor Solter from the A*STAR Institute of Medical Biology has definitively shown that extensive cell–cell interactions are required for proper lineage commitment[1].

After five rounds of cell division, a fertilized egg reaches the 32-cell stage. Chanchao Lorthongpanich, a postdoctoral fellow in the Knowles–Solter laboratory, mechanically separated cells at this and prior stages and then cultured the cells individually (see image). With her colleagues, she then measured the gene expression profiles of the separated cells. They showed that the pattern was out of sync with normal development, owing to the lack of proper cell–cell contact and the associated positional information that it confers.

"In the absence of structure and the clues provided by it, haphazard and incoherent gene expression is coupled with loss of lineage determination."

Each of the cells, known as blastomeres, failed to display gene markers characteristic of either the inner cell mass — the part of the embryo that gives rise to the fetus proper — or the nourishing trophectoderm, the precursor to the placenta. However, the researchers observed a tendency toward 'trophectoderm-like' expression consistent with cells receiving an 'outside' signal. Furthermore, when the researchers reassembled the cells, they could not organize themselves into the multiple tissue layers needed for proper development.

"In the absence of structure and the clues provided by it, haphazard and incoherent gene expression is coupled with loss of lineage determination," says Solter, who is now working to determine the exact cues by which cell–cell interactions lead to proper development. This process is reversible for a short time, but the subsequent loss of proper signals results in permanent damage to the blastomeres, according to Solter.

In addition to providing insights into the basic biology of mammalian development, the results could have important implications for human reproductive medicine. Currently, embryo screening techniques to test for genetic diseases require destroying one or two cells from the embryo at the eight-cell stage. Since the fate of blastomeres is determined by positional cues, rather than any predetermined fate, such diagnostic testing is unlikely to result in fetal malformation, Solter notes.

1. Lorthongpanich, C., Doris, T. P. Y., Limviphuvadh, V., Knowles, B. B. & Solter, D. Developmental fate and lineage commitment of singled mouse blastomeres. *Development* **139,** 3722–3731 (2012).

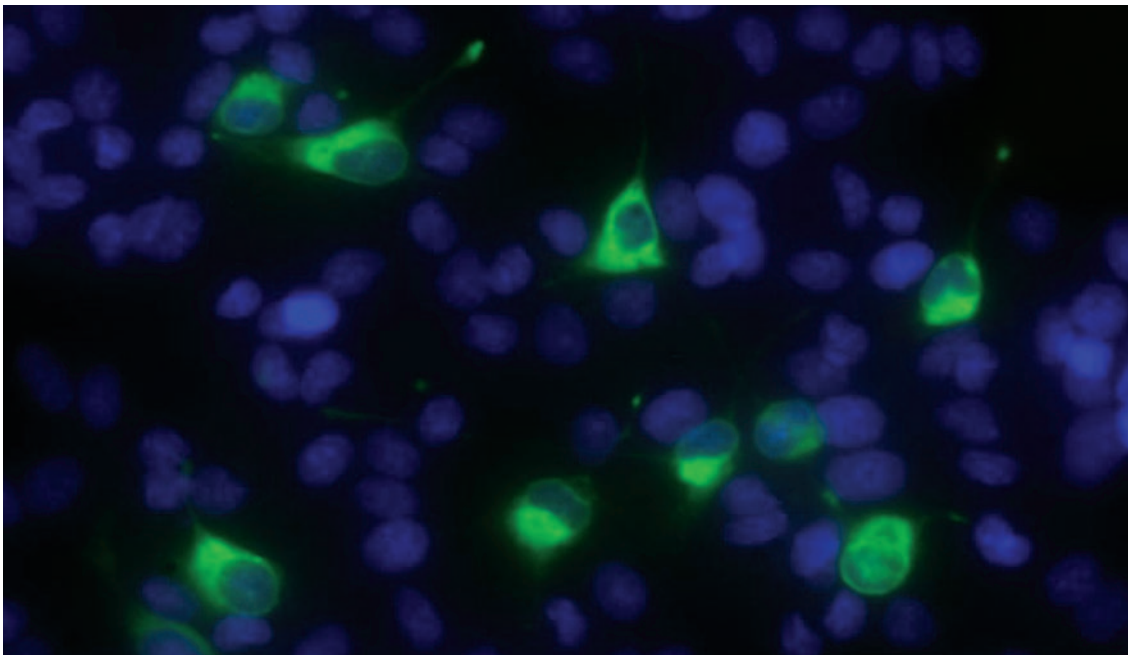
Immunology:

Bracing for a viral counterattack

Insights obtained by profiling the immune response to repeat viral infections could assist vaccine design efforts

Patients who successfully beat infection with dengue virus remain vulnerable to reinfection by other dengue variants, and these secondary infections tend to be more severe. The antibodies arising from the immune system's first encounter with the virus can play a complicated role in how these secondary infections unfold.

"Antibodies made during dengue infection can be either protective or disease enhancing," explains Katja Fink of the A*STAR Singapore Immunology Network. Fink and her team wanted to determine whether the antibodies produced very early after infection promote defense or vulnerability. To do this, her team isolated plasmablasts — immature precursors of antibody-secreting plasma cells — from two patients newly diagnosed with secondary infection[1]. After conducting assays to determine the extent to which these cells were targeting the various subtypes of dengue virus, the researchers learned that most patient plasmablasts were specifically generating antiviral antibodies (see image).



Antibodies (green) isolated from patients with a secondary dengue infection can effectively label cultured infected cells, demonstrating their strong affinity for the viral particle.

The secret of the immune system's success is its diversity, but when the body finds a threat that resembles something it has previously encountered, it specifically stimulates proliferation of cells that secrete antibodies appropriate to that threat. Fink and co-workers characterized the extent to which antibodies produced by individual plasmablasts from such patients neutralized different dengue variants in a mouse model. They found that the antibodies were generally more effective at neutralizing strains from initial infections rather than those involved in secondary infections. This is in keeping with an immunological model called 'antigenic original sin', wherein an initial encounter with a pathogen determines antibody output generated in subsequent encounters.

Importantly, the researchers found that patients with acute secondary infections were also able to successfully mount a new wave of plasmablast-mediated immune defense against the secondary strain, manifested by the generation of a collection of antibodies that effectively recognized and neutralized all four viral subtypes. More than a few very potent antibodies dominate the protective effect, according to Fink. "The immune system responds to dengue with a very diverse repertoire," she notes. Based on the timing with which the antibodies appeared, the researchers were also able to determine that they help rather than hinder the body's antiviral effort.

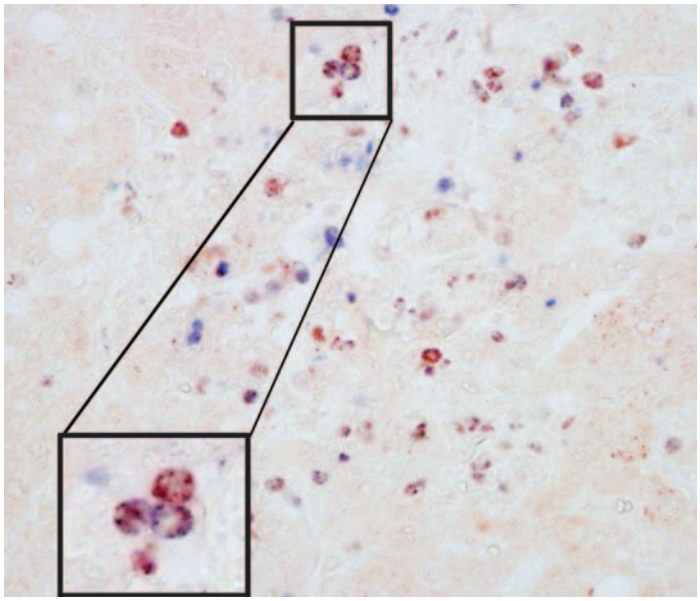
The cross-protective antibodies generated in this acute plasmablast response preferentially recognized a particular viral coat protein as a target, or 'epitope'. If validated in larger scale studies, these results could lead to better antiviral protection. "Knowledge about antibody epitopes on the virus that are naturally targeted by the human immune response could be translated into the design of vaccines," says Fink.

1. Xu, M., Hadinoto, V., Appanna, R., Joensson, K., Toh, Y. X. *et al.* Plasmablasts generated during repeated dengue infection are virus glycoprotein–specific and bind to multiple virus serotypes. *The Journal of Immunology* **189,** 5877–5885 (2012).

Cancer biology:

Awakening the body's anticancer defenses

Targeted stimulation of an immune pathway may help the body to fight back against liver cancer



The presence of natural killer cells (blue) expressing high levels of TLR3 protein (red) in HCC tumors is associated with a better prognosis and longer patient survival. Inset shows a cluster of natural killer cells with elevated TLR3.

Though it originates from the body's own cells, a tumor is as much of a hostile invader as any virus or bacterium. If the immune system is sufficiently sensitized, it can mount a counterattack just as it might fight an infection. For many patients, this response is insufficient, but researchers led by Jean-Pierre Abastado of the A*STAR Singapore Immunology Network have uncovered an immune mechanism that may help patients with hepatocellular carcinoma (HCC) keep their liver cancer at bay[1].

Certain viral infections stimulate the innate immune system via the toll-like receptor 3 (TLR3) protein, but TLR3 activation also seems to kill certain cancers. Accordingly, the researchers had previously observed improved odds of survival among Singaporean HCC patients with elevated tumor TLR3 levels[2]. Abastado and co-workers subsequently verified these initial findings in an additional cohort of patients from Hong Kong and Zurich, and demonstrated that increased survival time was associated with high TLR3 levels in both tumor cells and tumor-infiltrating immune cells.

Mouse model experiments demonstrated that TLR3 stimulation causes tumor cells to essentially 'self-destruct', while also recruiting a subset of natural killer (NK) innate immune cells to penetrate and attack the tumor. The remnants of the dead tumor cells further stimulate TLR3 signaling, accelerating the immune counterattack. "The triggering of TLR3 kick-starts a positive feedback loop that creates more cell death, and triggers more activation of TLR3 in both tumor and NK cells," explains Valerie Chew, a postdoctoral researcher in Abastado's laboratory and lead author of the research paper. In addition, TLR3 activation also summons other immune-cell subtypes; the researchers demonstrated that this immune recruitment is critical for tumor reduction in mice.

Analysis of patient HCC tumor samples revealed similar results (see image), where elevated TLR3 activity was associated with high levels of immune activity and increased tumor cell death. Why TLR3 levels are elevated in certain patients remains unclear, but Chew hypothesizes that their immune systems may be receiving a boost from certain pathogens. "It is conceivable that co-infection with viruses such as acute influenza may trigger expression of TLR3," she says, "and we believe that mounting an antiviral immune response via TLR3 actually provides protection against cancer."

As an alternative to catching influenza, HCC patients may benefit from TLR3-activating drugs such as Hiltonol, from US pharmaceutical company Oncovir. Hiltonol is in early clinical trials for the treatment of breast cancer and melanoma. Abastado's team is exploring the potential for an HCC trial of Hiltonol in collaboration with Oncovir and Han Chong Toh from the Singapore General Hospital.

1. Chew, V., Tow, C., Huang, C., Bard-Chapeau, E., Copeland, N. G. *et al.* Toll-like receptor 3 expressing tumor parenchyma and infiltrating natural killer cells in hepatocellular carcinoma patients. *Journal of the National Cancer Institute* **104,** 1796–1807 (2012).
2. Chew, V., Tow, C., Teo, M., Wong, H.L., Chan, J. *et al.* Inflammatory tumour microenvironment is associated with superior survival in hepatocellular carcinoma patients. *Journal of Hepatology* **52,** 370–379 (2010).

Cell biology:

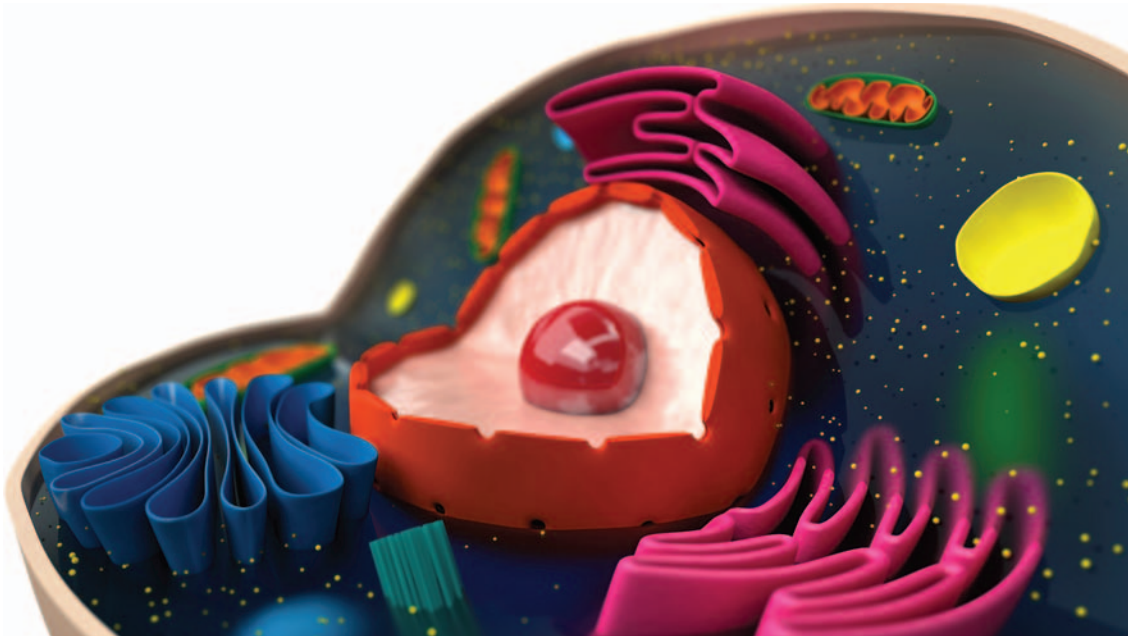
Touring the assembly line

A large-scale screen reveals how numerous signaling pathways intersect at the cell's primary protein-processing center

Many proteins undergo extensive modification after being synthesized, particularly those that are secreted or embedded in the cell membrane. This is achieved within the Golgi apparatus (see image), a cellular organelle consisting of multiple membrane-bound compartments known as cisternae. Each of these contains specific sets of protein-modifying enzymes, which sequentially modify their targets. For example, many proteins undergo glycosylation, which entails the stepwise addition of complex sugar molecules.

Golgi function depends heavily on proper organization, particularly in mammalian cells. In an ambitious study, a research team led by Frederic Bard of the A*STAR Institute of Molecular and Cell Biology has identified proteins that maintain this organelle's structure and function[1]. Many critical cellular functions are managed by signaling enzymes that either add or remove phosphate chemical groups from target proteins, known respectively as kinases and phosphatases. Bard and co-workers focused on a set of 948 proteins encompassing most of these enzymes.

The researchers used a technique called RNA interference to specifically reduce production of each protein in cultured cells, and then applied a sophisticated imaging strategy to determine the impact on different subsets of Golgi cisternae. A series of pilot experiments using treatments known to affect Golgi function enabled them to 'train' their imaging software to recognize the physiological hallmarks associated with different disruptions. In parallel, Bard and co-workers applied a targeted fluorescent labeling strategy to 'color code' the various Golgi subcompartments, allowing them to determine which of these were specifically affected in each experiment.



Many proteins undergo extensive processing and modification within the stacked compartments of the Golgi apparatus.

Using the trained imaging algorithm, the researchers identified 159 signaling factors that apparently contribute to Golgi organization and structure. Many of these were directly linked to critical Golgi functions, such as the dynamic behavior of cisternal membranes or the trafficking system that physically shuttles molecules between cisternae. Several of the targets identified specifically transmit signals in response to extracellular cues, indicating that Golgi organization may be greatly affected by the environment outside of the cell.

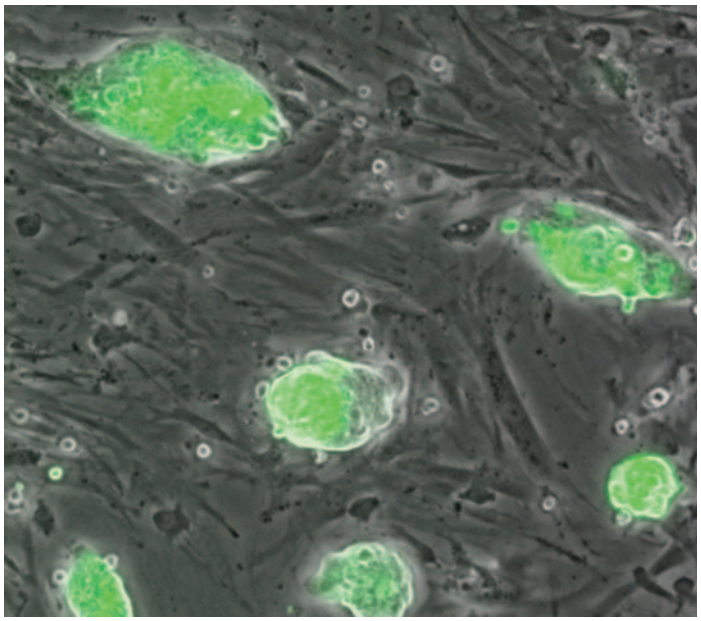
Importantly, many of these signaling factors exert a particularly strong influence on glycosylation patterns. "The sheer complexity and diversity of glyco-phenotypes arising from signaling-gene depletion were very surprising," says Bard. Given that both signaling pathways and protein glycosylation are highly prone to disruption in cancerous cells, these data suggest that the Golgi could be an important nexus for some tumorigenic processes. Bard will explore this possibility in future work. "We plan to decipher the specific cascades of glycosylation regulation that are frequently activated in tumor cells," he says.

1. Chia, J., Goh, G., Racine, V., Ng, S., Kumar, P. & Bard, F. RNAi screening reveals a large signaling network controlling the Golgi apparatus in human cells. *Molecular Systems Biology* **8**, 629 (2012).

Developmental biology:

Proteins prove the power of collaboration

Pairs of gene-regulating proteins work together to identify their proper binding sites within the genome



Fluorescently labeled stem cells express a mutated version of Sox17 that changes this protein's interaction with Oct4, enabling these cells to maintain pluripotency.

Research led by Lawrence Stanton and Prasanna Kolatkar of the A*STAR Genome Institute of Singapore has provided valuable insight into embryonic development[1]. Stanton and Kolatkar teamed up with colleagues in Singapore to determine how transcription factor proteins assemble at DNA sequences called enhancers, which help coordinate gene expression.

Multiple transcription factors often act together at a given enhancer, but biologists are unsure whether these proteins assemble sequentially at the enhancer or combine by preforming complexes that collaboratively recognize their targets.

The researchers focused on a transcription factor called Oct4, which is known to help embryonic stem cells maintain a 'pluripotent' state, from which they can give rise to any tissue in the body. However, Oct4 also helps direct the development of endodermal tissue, which forms the nervous system and other tissues. Stanton and Kolatkar suspected that Oct4's effects might be determined by the transcription factors with which it partners.

Focusing on two particular transcription factors, Sox2 and Sox17, the team identified different sets of enhancers that are selectively targeted by Sox2/Oct4 versus Sox17/Oct4. Interestingly, the binding sequences differed subtly for the two transcription factor pairs: Sox17/Oct4 preferentially bound a 'compressed' motif that was one base pair shorter than the 'canonical' motif typically bound by Sox2/Oct4. A series of experiments with mouse embryonic stem cells showed that gene enhancers associated with endoderm formation tend to contain the compressed motif and are therefore switched on by Sox17/Oct4 (see image). Conversely, Sox2/Oct4 preferentially gravitates to pluripotency-inducing enhancers containing the canonical motif. "Alternative partnering is a key mediator of this developmental switch," says Stanton.

Stanton and Kolatkar also demonstrated that Oct4 complex formation is critical in determining the DNA motif to which it binds. A mutation in the region of Sox17 that binds Oct4 proved sufficient to shift the specificity of Oct4/Sox17 so that it binds the canonical rather than the compressed motif. Furthermore, the researchers identified similar specificity-altering mutations in Sox2, and in both cases this retargeting altered the gene activation behavior of Oct4. "Single amino acid point mutations in the Sox proteins precisely switch the developmental potential of Sox2 and Sox17," Stanton says.

These findings likely represent a broader model for gene regulation and Stanton credits his multidisciplinary team of collaborators with successfully untangling the details of this process. In future work, he hopes to explore the potential of modulating gene expression by manipulating the interactions between transcription factor pairs in a more targeted fashion.

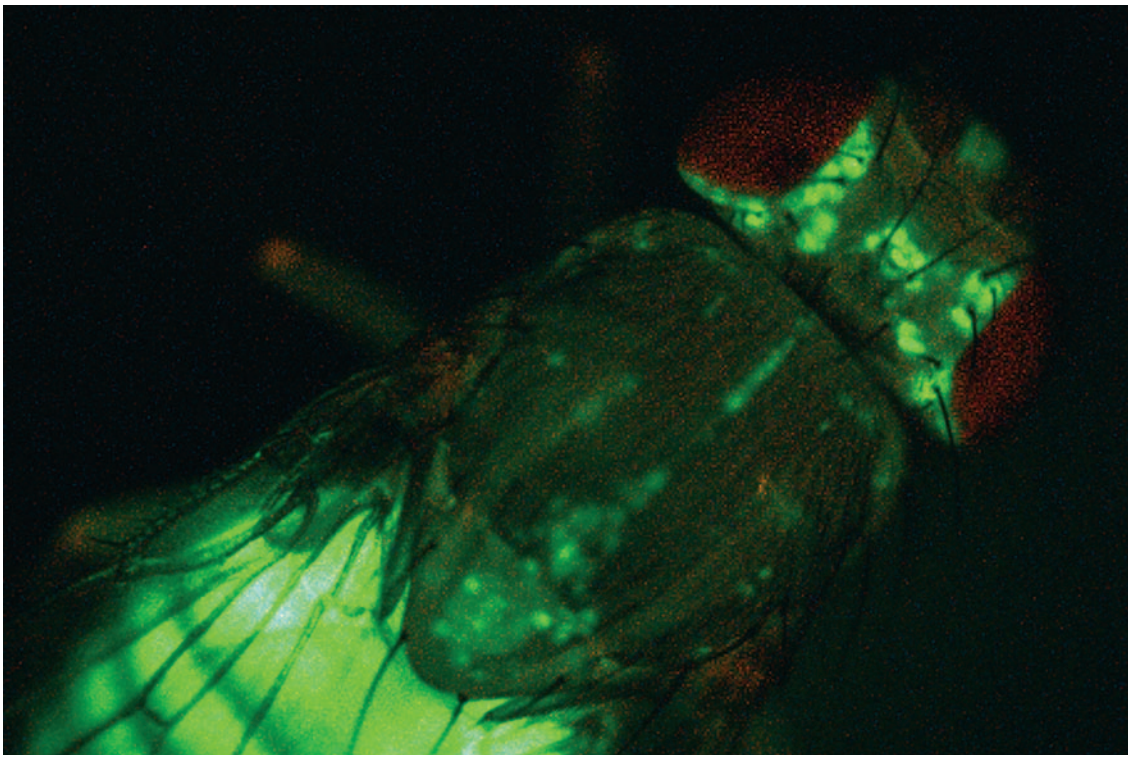
1. Aksoy, I., Jauch, R., Chen, J., Dyla, M., Divakar, U. *et al.* Oct4 switches partnering from Sox2 to Sox17 to reinterpret the enhancer code and specify endoderm. *The EMBO Journal* **32**, 938–953 (2013).

Cell biology:

Cellular damage control's link with diabetes

A cellular 'stress sensor' that also modulates metabolism could offer a therapeutic target for diabetes

An organelle called the endoplasmic reticulum (ER) helps to process newly synthesized proteins destined for delivery to the cell membrane. When the ER becomes overloaded and begins to accumulate poorly folded proteins, an 'ER stress' response ensues. ER stress tends to occur in obesity and other metabolic disorders. Now, research from Stephen Cohen and colleagues at the A*STAR Institute of Molecular and Cell Biology has revealed a potential therapeutic target linking ER stress to the onset of diabetes[1].



Experiments in a fruit fly model offer useful insights into a potentially important clinical target in human obesity and diabetes.

Cohen's team initially set out to identify components of the insulin signaling pathway. "We were screening for genes involved in tissue growth control in the fly," he says (see image). "Growth is regulated in part by the insulin pathway, so such screens can also pick up genes that function as metabolic regulators." Their screen focused on FOXO, a protein that acts as a regulator of other genes. Insulin signaling causes FOXO to segregate in the cytoplasm, so that it can no longer bind its target genes in the nucleus. The researchers therefore searched for other genes that modulate FOXO activity.

They uncovered a link between PERK, a protein that disseminates signals involved in ER stress, and FOXO. When they genetically modified fruit flies to produce excess FOXO, this protein suppressed insulin-induced growth and the flies matured with small, underdeveloped eyes. However, when the researchers also reduced PERK levels in these flies, eye development returned to normal, indicating that PERK helps amplify the effects of FOXO.

Follow-up experiments demonstrated that PERK introduces chemical modifications to FOXO that help direct this protein to the nucleus where it executes its gene regulatory functions. Cohen and colleagues demonstrated that PERK also boosts FOXO function in human cells, and found that lowering PERK activity increased these cells' sensitivity to insulin signaling. FOXO also helps to promote insulin sensitivity in cells by increasing production of the insulin receptor — which in turn inactivates FOXO. Thus, PERK contributes significantly to an important metabolic feedback loop.

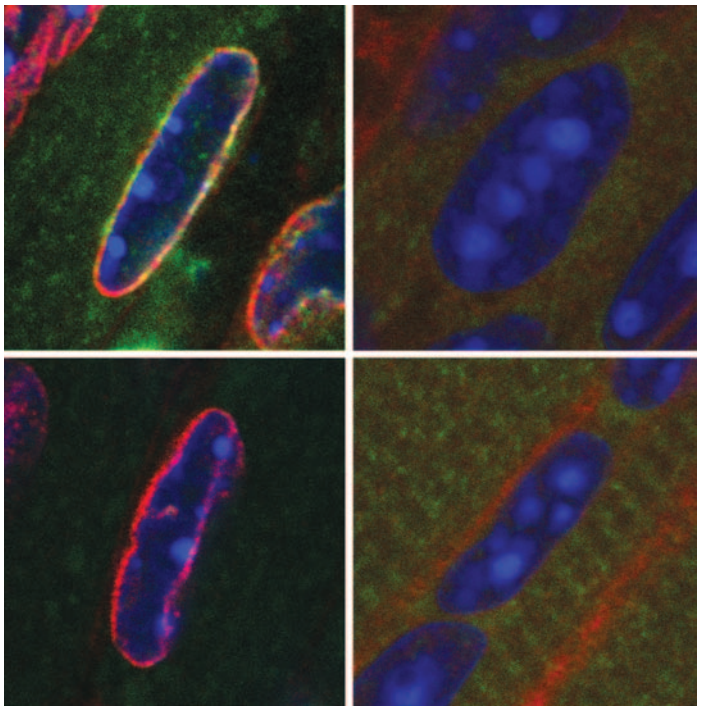
This system could be susceptible to breakdown if PERK activity were to intersect with other ER stress-induced signaling pathways. This could accelerate the onset of metabolic disease by promoting insulin resistance, a disruption of insulin signaling that is also a critical step toward onset of diabetes. "We are very interested in the potential that PERK could be used to modulate insulin responsiveness," says Cohen. "We are now exploring the biological functions of PERK to better understand its potential usefulness as a therapeutic target."

1. Zhang, W., Hietakangas, V., Wee, S., Lim, S. C., Gunaratne, J. & Cohen, S. M. ER stress potentiates insulin resistance through PERK-mediated FOXO phosphorylation. *Genes & Development* **27,** 441–449 (2013).

Cell biology:

DNA directors discovered

Two proteins that control nuclear DNA distribution also regulate development by altering gene expression



In normal muscle cells (left), heterochromatin (blue) is localized at the edges of the nucleus. In cells lacking both LBR and LamA/C (right), heterochromatin is in the center of the nucleus.

Cell biologists believe that gene expression in eukaryotic cells is partly controlled by the uneven distribution of DNA in the nucleus. Colin Stewart and Audrey Wang at the A*STAR Institute of Medical Biology, Singapore, and their international co-workers, have identified two proteins that control this distribution of DNA[1]. Their findings have important implications for disease and cellular development.

Heterochromatin is DNA that is tightly packed to prevent expression of its genes, and it is mainly tethered to the inside of the nucleus wall, also called the 'nuclear envelope'. Euchromatin is DNA that is loosely packed and ready for gene expression, and it is in the center of the nucleus.

Stewart and Wang discovered that DNA distribution depends on two proteins found in the nuclear envelope: lamin B receptor (LBR) and lamin A/C (lamA/C). They discovered that neither of these proteins is expressed in a type of photoreceptor cell that naturally has a reversed distribution of heterochromatin and euchromatin. When the researchers removed both LBR and lamA/C from normal mouse cells, they observed the same reversal, proving that the absence of these proteins is responsible for this DNA distribution.

According to Stewart, the involvement of LBR and lamA/C has important consequences. "Mutations in the lamin proteins cause a range of inherited diseases, including muscular dystrophy, enlargement of the heart, premature aging and diseases affecting skeletal development and fat production," he explains. "These [conditions] are rare, but studying them may provide insights into more pressing diseases, such as cardiovascular disease, obesity and aging."

Looking at the roles of LBR and lamA/C in more detail, Stewart and Wang also found that the proteins are expressed at different times during embryonic development; LBR is expressed initially, but it is replaced over time by lamA/C. In muscle cells, the two proteins also had opposite effects on the expression of muscle-specific genes: LBR silenced the genes, whereas lamA/C switched them on. The combination of differential expression and gene activation allows the two proteins to orchestrate cellular development.

The team's work answers some questions about the mechanisms and importance of uneven DNA distribution. Stewart notes, however, that there are now many more questions to investigate. "Is the disruption to heterochromatin a cause or consequence of diseases? What does this effect on chromatin structure mean for the stability of the genome? Does it increase susceptibility to other diseases, such as cancer?" he asks. "These are avenues that we will now pursue."

1. Solovei, I., Wang, A. S., Thanisch, K., Schmidt, C. S., Krebs, S. *et al.* LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation. *Cell* **152**, 584–598 (2013).

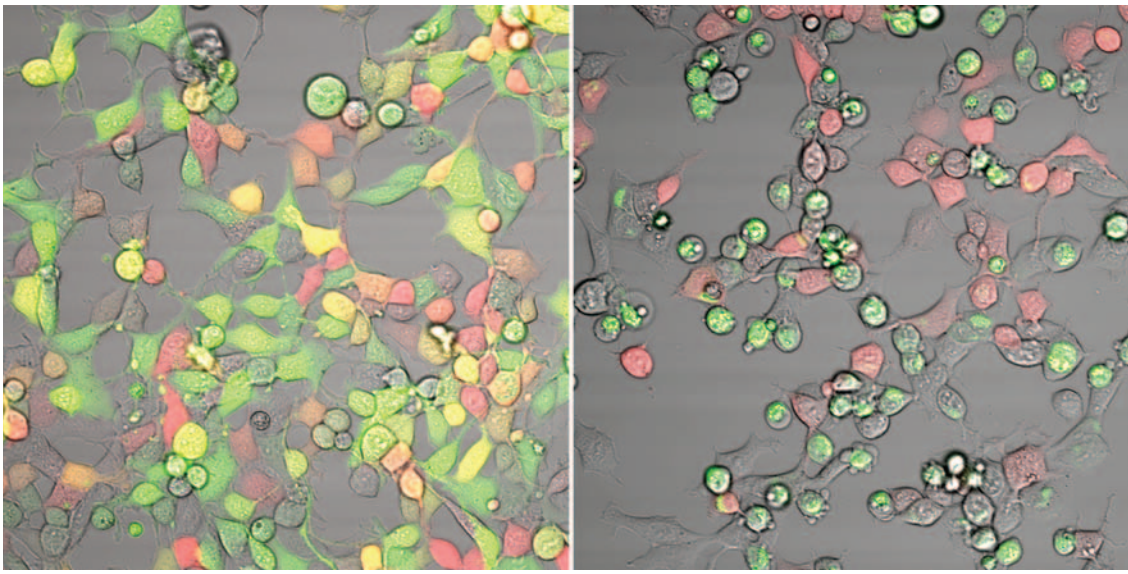
Virology:

Seeking solutions to viral migration

Revelation of antiviral properties in the protein viperin could help virologists to contain the re-emerging chikungunya virus

Although seldom fatal, persistent infection by chikungunya virus (CHIKV) afflicts patients with joint pain lasting months or even years. This insect-borne virus has received relatively little scientific attention in the 50 years since its initial description in African patients, but researchers in Singapore have now uncovered a host protein that can keep CHIKV in check[1].

"Globalization and climate warming have lent a helping hand in the resurgence of CHIKV, such that a virus originally from Africa and mosquitoes originally from Asia [could] meet in the Indian Ocean and spread to other parts of the world," explains Preston Teng, a researcher in Lisa F. P. Ng's laboratory, part of the A*STAR Singapore Immunology Network. The expanding reach of the virus motivated Ng and her co-workers to investigate how CHIKV interacts with the immune system.



Cultured cells (green) normally vulnerable to infection by chikungunya virus (red; left) acquire additional antiviral resistance after being genetically modified to produce the protein viperin (right).

"We were intrigued to find that a short, 42-amino acid fragment of viperin was sufficient to inhibit CHIKV infection and replication effectively."

Ng's team had already established that CHIKV infection triggers cellular signaling pathways mediated by the type I interferon proteins, which activate genes involved in the antiviral 'innate' immune response. As a follow-up, the team searched for specific target genes activated in immune cells collected from 24 CHIKV patients. Their analysis revealed a sharp, viral load-dependent increase in the activity of the gene encoding the protein viperin, which is involved in the defensive response to numerous viruses.

Ng and co-workers showed that forced production of viperin conferred additional protection against infection upon a human cell line normally susceptible to CHIKV (see image). Conversely, genetically modified mice lacking viperin were prone to heavier viral loads following CHIKV infection, resulting in more severe inflammatory symptoms.

Viperin thwarts different viruses by distinct mechanisms, so the researchers carved the protein into pieces to identify which segment acts against CHIKV. Unexpectedly, they found that viperin's anti-CHIKV activity resides almost entirely within a single helical segment of the protein. "We were intrigued to find that a short, 42-amino acid fragment of viperin was sufficient to inhibit CHIKV infection and replication effectively," says Teng.

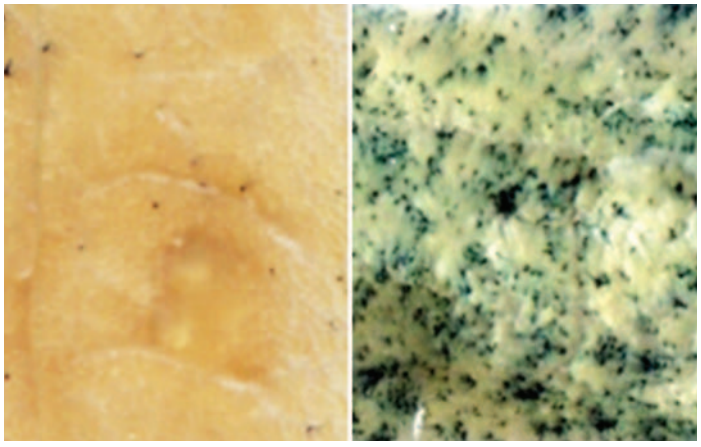
This domain helps to localize viperin to a specific cellular compartment known as the endoplasmic reticulum (ER), which contributes to the production of both host and viral proteins in infected cells. The researchers propose that viperin is capable of triggering a 'stress response' in the ER that effectively shuts down production of key viral components. This process could be exploited to bolster patient defenses against CHIKV. In future research, Ng, Teng and colleagues plan to uncover the specific antiviral mechanism of this viperin domain.

1. Teng, T.-S., Foo, S.-S., Simamarta, D., Lum, F.-M., Teo, T.-H. *et al.* Viperin restricts chikungunya virus replication and pathology. *The Journal of Clinical Investigation* **122**, 4447–4460 (2012).

Cancer biology:

In search of stem cell origins

Mapping the replacement of dead cells in the intestine uncovers a critical stem cell pool



Normally quiescent *Bmi1*-positive intestinal stem cells (ISCs) (left) undergo increased division in the event of drug-induced cell death (right). Blue staining indicates successful lineage tracing of new cells arising from ISC proliferation and maturation.

Stem cells play a critical role in replacing various cells within the intestine, but can also become drivers for colorectal cancer. The composition of these stem cell reservoirs has been debated. New research from Dmitry Bulavin of the A*STAR Institute of Molecular and Cell Biology has now clarified the organization of this tissue, yielding insights that should steer future cancer research[1].

Bulavin and co-workers had previously identified a population of '+4' cells — denoting their position within the intestinal epithelium — that appeared to contain intestinal stem cells (ISCs)[2]. They determined that activation of certain oncogenes rapidly kills these cells via a mechanism called apoptosis, a critical safeguard against cancerous growth. Accordingly, mice with a genetic mutation that increased the apoptotic response of these cells were also protected against intestinal tumors. However, subsequent findings from another team identified a different pool of putative ISCs, identifiable by their expression of the marker gene *Lgr5*.

"We want to see how apoptosis affects the development of polyps when oncogenic mutations occur in different cellular compartments in the mouse intestine."

"We could not explain the tumor-resistant phenotype of our mice using this new model," says Bulavin. "This motivated further analysis of different stem cell populations in the intestine." His team employed a technique called 'lineage tracing', which enables selective labeling of cells expressing a marker gene of interest with a fluorescent protein. Descendants of these cells become similarly labeled, allowing researchers to track the point of origin for cell populations or tissues of interest. Bulavin and co-workers then used radiation or chemical trauma to induce apoptosis, and examined the response of different cell populations to identify likely ISCs.

Their experiments revealed that a pool of +4 cells, identifiable by expression of the cellular marker gene *Bmi1*, appear to represent true ISCs. These cells are normally dormant, but sporadically replicate to replace intestinal cells lost to normal wear and tear (see image). They can also be rallied to repair more severe damage. "Massive cell death is a very powerful factor in the stimulation of these +4 ISCs," says Bulavin. "They exit quiescence and enter a proliferative state after high doses of irradiation." The previously identified pool of *Lgr5*-expressing cells in turn appears to consist of progenitor cells, which develop into ISCs only when *Bmi1*-positive ISCs are depleted.

Armed with some initial insights into how improper regulation of apoptosis affects these various cell populations, Bulavin's team is now working to understand their relevance in cancer. "We want to see how apoptosis affects the development of polyps when oncogenic mutations occur in different cellular compartments in the mouse intestine," he says.

1. Zhu, Y., Huang, Y.-F., Kek, C. & Bulavin, D. V. Apoptosis differently affects lineage tracing of Lgr5 and Bmi1 intestinal stem cell populations. *Cell Stem Cell* **12**, 298–303 (2013).
2. Demidov, O. N., Timofeev, O., Lwin, H. N. Y., Kek, C., Appella, E. & Bulavin, D. V. Wip1 phosphatase regulates p53-dependent apoptosis of stem cells and tumorigenesis in the mouse intestine. *Cell Stem Cell* **1**, 180–190 (2007).

Immunology:

Personalized vaccines edge closer

A technique for analyzing specific T-cell responses could pave the way for more rationally designed vaccines

Among different populations, a vaccine can vary in efficacy against a specific pathogen. Unraveling the discrepancy requires tools to analyze the exact nature of responses by a specific type of immune cell: the T cell. Now, a research team from Singapore and the United States has developed an approach that simultaneously identifies and characterizes T cells specific to a variety of antigens[1] — the part of the pathogen recognized by the immune system. The work could lead to more personalized and thus effective vaccine designs.

"[Our] approach can be applied to the study of any vaccine that elicits a T-cell response, allowing for an in-depth analysis of the breadth and quality of the antigen-specific T-cell response," says Evan Newell of the A*STAR Singapore Immunology Network, who led the study.

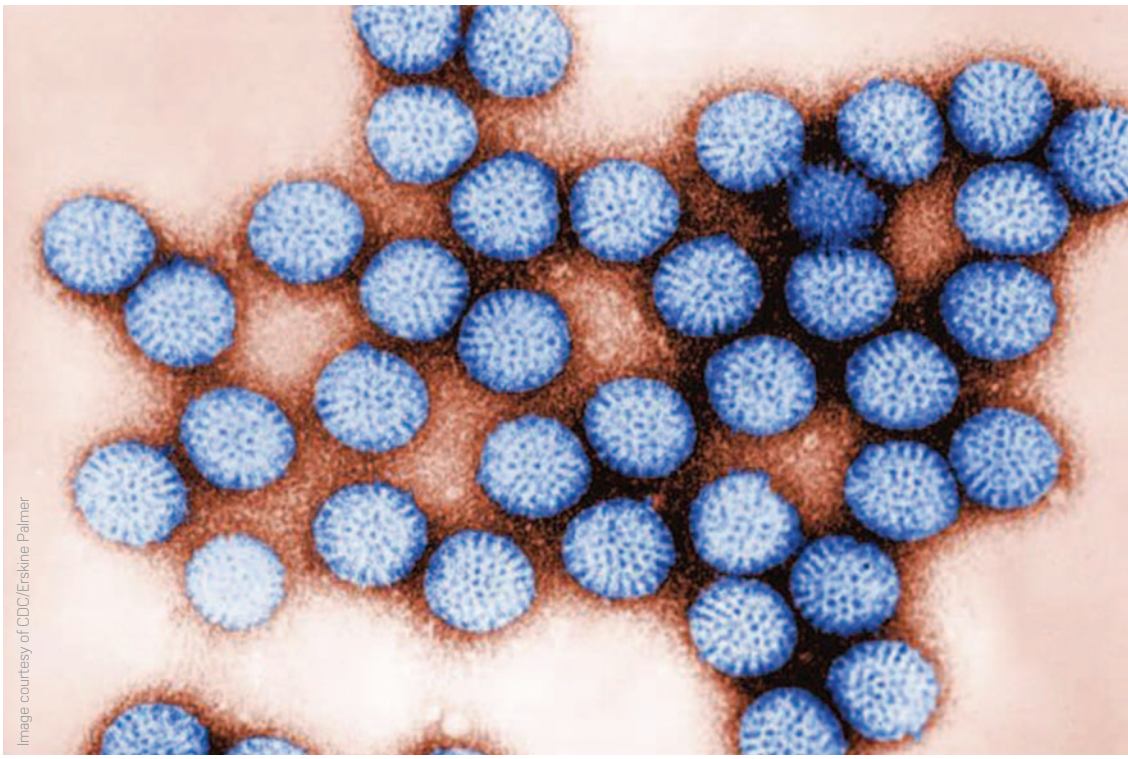


Image courtesy of CDC/Erskine Palmer

Vaccines against rotavirus (pictured) are still ineffective for vast swathes of the world's population.

The new method involves a combination of two technologies. First, complexes of candidate peptide antigens and major histocompatibility complex (MHC) molecules, which bind antigens for presentation to T cells, are each tagged with a unique combination of heavy metal markers. These so-called 'tetramers' are then incubated with patient samples containing T cells, which attach to any complexes with peptides that match those recognized by receptors on the T-cell surface. Next, a high-throughput, multiplexing technique called mass cytometry is used to analyze the coupled T cells at single-cell resolution. "This yields an unprecedented amount of information from each sample," says Newell.

As a proof of principle, Newell and his co-workers analyzed blood and intestinal tissue from healthy volunteers for T cells that recognize rotavirus (see image). "Rotavirus infection is a major problem in the developing world, partially because vaccines do not work well there," explains Newell. The gut pathogen causes severe diarrhea in young children and kills more than 500,000 people worldwide each year.

The researchers stained the T cells from the healthy individuals with a series of 77 candidate rotavirus epitopes. Each epitope is a slightly different site on a rotavirus protein — the antigen — that is recognized by the immune system. From this larger collection, they identified and validated six rotavirus epitopes and simultaneously analyzed the T cells specific for each of them.

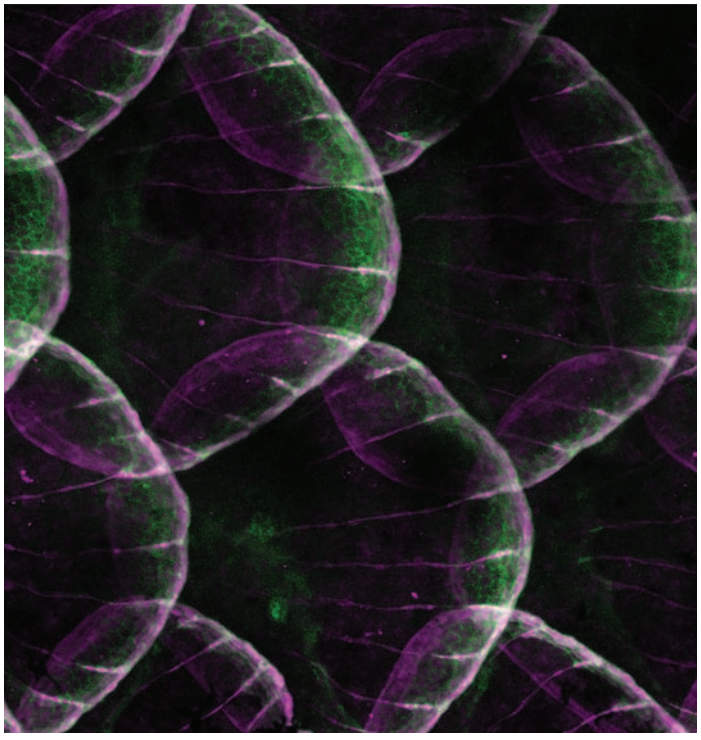
"We were surprised to find that T cells targeting different parts of the rotavirus had dramatically different characteristics; and, only one set of these T cells were found to be present in the intestine of normal donors," says Newell. "These analyses should allow faster identification of correlates for vaccine efficacy that can be used to speed the testing of new vaccines," he adds.

1. Newell, E. W., Sigal, N., Nair, N., Kidd, B. A., Greenberg, H. B. & Davis, M. M. Combinatorial tetramer staining and mass cytometry analysis facilitate T-cell epitope mapping and characterization. *Nature Biotechnology* **31,** 623–629 (2013).

Development:

Assumption about fish skeletons proves false

Evidence that disproves a long-standing assumption about fish development gives insight into the evolution of skeletons



Magenta-colored fluorescent labeling of cells in the scales of a 30-day-old fish shows that they originate in the mesoderm of the embryo. The green labeling indicates bone precursor cells.

A Singapore-based research team has used fluorescent labeling of embryonic cell populations to pinpoint the origin of scales and fins in modern-day fish. These tissues are evolutionary relics of the first skeletons and were widely assumed to originate from an embryonic cell population known as the 'trunk neural crest'. Now, research led by Tom Carney of the A*STAR Institute of Molecular and Cell Biology has shown that scales and fins actually develop from a cell population called the mesoderm[1,2].

In both mammals and fish, two embryonic cell populations contribute to the formation of the skeleton — the cranial neural crest generates most of the skull and jaw bones, and the mesoderm generates the remainder of the skeleton. In mammals, trunk neural crest cells, a third population, do not contribute to skeletal development, but previous cell-labeling studies suggested that they did in ancient vertebrates and still do in some modern animals. Despite inaccurate cell labeling in these studies, the widely held assumption remained. However, no-one had directly demonstrated the role of these trunk neural crest cells.

Carney and his team labeled embryonic cell populations with fluorescent molecules to determine which populations contribute to the fin skeleton and scales in modern fish. New cells originating from these populations also expressed the label, allowing the team to see which cells in the body developed from each population.

"We found that fish scales and fin rays derive entirely from the mesoderm, with no [trunk] neural crest contribution," explains Carney. "This [finding] indicates that the mesoderm can generate this ancient type of bone and suggests that when skeletons first evolved, they developed from the mesoderm."

The team's discovery is contrary to the previous assumption about the role of the trunk neural crest in skeleton development. They therefore investigated further using genetic manipulation and more cell labeling.

"We went on to show that the trunk neural crest in fish does not generate any skeletal-type cells," says Carney. "This suggests that the contribution of cranial neural crest cells to the skeleton may have evolved independently in the head and has never been a general feature of the whole neural crest."

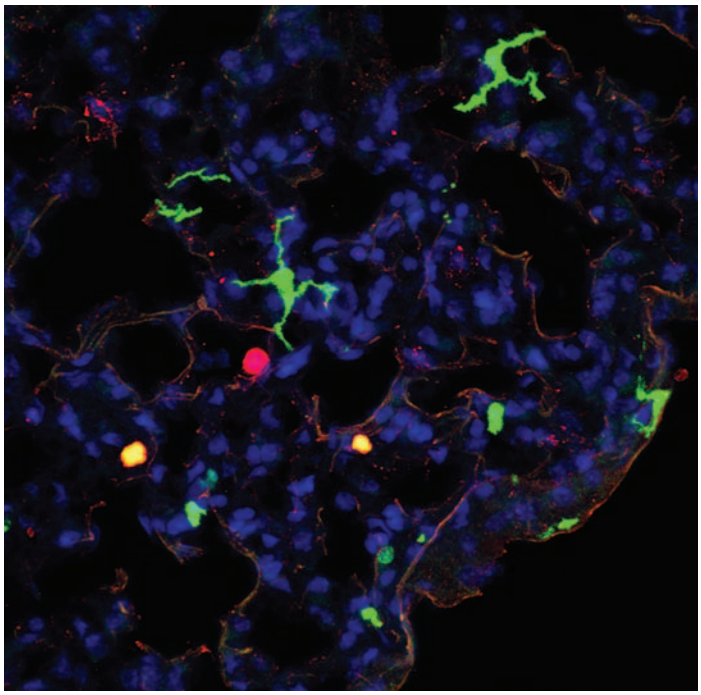
The results are likely to change our understanding of skeleton evolution. Carney says that: "Many of the important 'innovations' of the skeleton can now be considered as 'inventions' of the mesoderm, with the neural crest only playing a specific role in generating the skeleton of the head."

1. Lee, R. T. H., Thiery, J. P. & Carney, T. J. Dermal fin rays and scales derive from mesoderm, not neural crest. *Current Biology* **23,** R336–R337 (2013).
2. Lee, R. T. H., Knapik, E. W., Thiery, J. P. & Carney, T. J. An exclusively mesodermal origin of fin mesenchyme demonstrates that zebrafish trunk neural crest does not generate ectomesenchyme. *Development* **140,** 2923–2932 (2013).

Immunology:

White blood cells show their stripes

A newly discovered subset of white blood cells in mice and humans could improve treatments for infections and autoimmune diseases



Discovery of the CD11b$^+$ subset (green) of murine dendritic cells and its direct human counterpart (not shown) could lead to improved therapeutics.

For the human immune system to work effectively, the body must be able to distinguish invading pathogens, such as fungi and bacteria, from its own healthy tissue. A group of white blood cells known as dendritic cells (DCs) has a critical role in this task: DCs recognize pathogens, then activate and regulate the immune system accordingly. Immunologists therefore believe that DCs could be harnessed for new therapies against fungal and bacterial infections, as well as autoimmune diseases such as multiple sclerosis.

Florent Ginhoux and co-workers at the A*STAR Singapore Immunology Network, together with researchers in the United Kingdom, United States and Japan, have identified a subset of DCs that exists in mouse and human mucosal tissues[1]. The murine subset is called CD11b$^+$ and the human equivalent is called CD1c$^+$. The existence of similar cell populations in the two species will assist in translating the results of murine experiments to human biology and also further the development of clinical therapies.

Contamination by other cell types in previous studies limited the true understanding of the function of CD11b$^+$ cells, Ginhoux notes. "In this study, we found new markers to specifically identify CD11b$^+$ DCs."

Parts of the body that are exposed to the external environment, such as the lungs and gut, contain DCs (see image). They act as messengers, presenting fragments of pathogens to other white blood cells called CD4 T-helper cells that trigger appropriate immune responses. One particular T-helper, Th17, in concert with DCs, specializes in activating the protective response to fungal or bacterial infections.

"We found that CD11b$^+$ DCs secrete a specific cytokine protein named interleukin-23 (IL-23). This protein induces and governs Th17 cells that secrete a second cytokine — IL-17 — a very potent inflammatory mediator against fungi in the lungs of mice as well as of humans," explains Ginhoux.

During infections that fight and clear pathogens, IL-17 can be very powerful. If cytokine IL-23 secretion is unregulated, however, it induces exacerbated Th17 responses because of an excess of IL-17 release. This regulation failure has been linked to the development of psoriasis, Crohn's disease and multiple sclerosis. Controlling the activity of DCs that regulate IL-23 and subsequent Th17 cell responses could therefore prove useful therapeutically.

"There are two major applications for this research," says Ginhoux. Firstly, a vaccine strategy targeting CD11b$^+$ DCs to trigger a potent IL-17-dependent immune response could prevent fungal and bacterial infections. Secondly, selectively inhibiting CD11b$^+$ DCs may lead to better control of IL-17-dependent autoinflammatory disorders.

1. Schlitzer, A., McGovern, N., Teo, P., Zelante, T., Atarashi, K. *et al.* IRF4 transcription factor-dependent CD11b$^+$ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* **38**, 970–983 (2013).

Malaria:

Parasites inflict collateral damage in the brain

Brain-damaging complications of malaria arise from the immune response to parasite antigens absorbed by blood vessels

Most deaths caused by the malarial parasite *Plasmodium falciparum* result from the onset of cerebral malaria. This severe neurological condition arises when parasites accumulate within the brain vasculature. Numerous studies over the years, using a mouse model of experimental cerebral malaria (ECM), have also revealed that host immune cells play a critical part.

Previous research from Laurent Rénia at the A*STAR Singapore Immunology Network had highlighted the prominent contribution of cells known as cytotoxic T lymphocytes (CTLs). His team has now uncovered the mechanism by which these cells promote ECM[1]. CTLs are normally responsible for destroying cancerous or infected cells, but the researchers suspected that ECM may result from parasite-targeting CTLs that also attack and damage blood vessels in the brain.

Rénia and co-workers developed tools for detecting these CTLs and their target cells. They then determined that mice infected with *Plasmodium berghei* ANKA (PbA), an ECM-causing parasite subtype, specifically elicit CTLs targeted against a particular polypeptide chunk from a parasite-derived protein. Mice infected with PbA began producing CTLs that recognize this polypeptide within five days, and these cells migrated to the brain shortly afterward. The researchers also examined three *Plasmodium* parasite strains that do not trigger ECM and were surprised to find that these elicited a similar CTL response.

© iStockphoto/Thinkstock

Without prompt medical attention, cerebral malaria can manifest as early as 10 days after being bitten by a mosquito infected with *Plasmodium falciparum*.

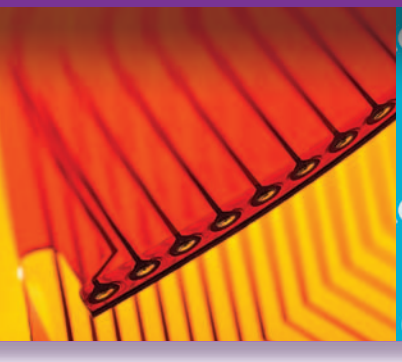
A closer examination of blood vessels from the brains of infected mice revealed the missing piece of the puzzle. Red blood cells infected by ECM-causing parasites exhibit a tendency to accumulate within these vessels, while those from non-ECM-causing parasites do not. Rénia and co-workers determined that when this accumulation occurs, the endothelial cells that line these blood vessels absorb and then display CTL-targeted parasite proteins via a mechanism termed 'cross-presentation'.

These cross-presenting cells subsequently become targets for CTL-mediated destruction, creating leaks that give malarial parasites access to the brain. Importantly, prompt treatment with antimalarial drugs can rapidly clear these parasites from the blood vessels, thereby preventing the onset of ECM.

Rénia hypothesizes that ECM specifically arises from parasite species possessing some innate characteristic that makes infected cells 'stickier'. "We were surprised that this subtle difference in parasite biology of sequestration versus non-sequestration leads to such huge differences in pathology," he says. Despite there being other known examples of endothelial cross-presentation, the mechanism remains poorly understood. Rénia is keen to uncover how *Plasmodium* deflects the immune response. "This is an interesting biological question, because endothelial cells are not infected by these parasites," he says.

1. Howland, S. W., Poh, C. M., Gun, S. Y., Claser, C., Malleret, B. *et al.* Brain microvessel cross-presentation is a hallmark of experimental cerebral malaria. *EMBO Molecular Medicine* **5**, 984–999 (2013).

Research Highlights

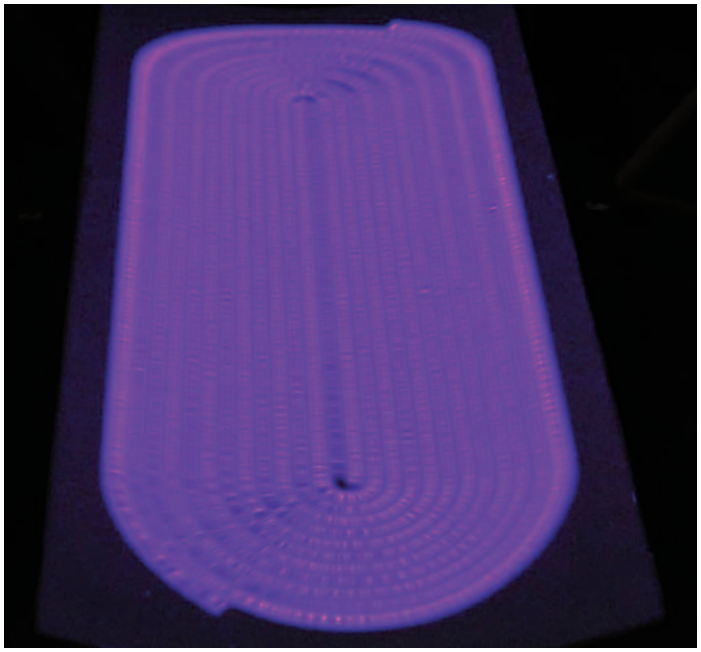



CHEMISTRY & MATERIALS

Manufacturing:

Plasma treatments on a roll

A revolutionary atmospheric-pressure plasma boosts adhesion of polymer films for roll-to-roll solar-cell production



A new 'diffuse coplanar surface barrier discharge' (DCSBD) plasma source (pictured) that rapidly cleans flat polymer sheets in open-air conditions holds great promise for roll-to-roll manufacturing.

Mass manufacture of photovoltaic materials is often achieved inexpensively by screen printing organic solar cells onto plastic sheets. The polymer known as poly(ethylene terephthalate), or PET, is a key part of the technology. Well known as the inexpensive plastic used to make soda bottles, PET has garnered increasing use as an optoelectronic substrate because of its strength and flexibility. But printing conductive solar-cell coatings onto PET is a challenge: it has a non-reactive surface and is frequently contaminated with static electric charges, which makes adhesion to other materials difficult.

Linda Wu from the A*STAR Singapore Institute of Manufacturing Technology and co-workers have now devised an innovative plasma treatment to 'activate' PET surfaces for improved bonding during roll-to-roll processing[1]. The team's experiments with 'diffuse coplanar surface barrier discharge' (DCSBD) technology show that large-area PET sheets can be microscopically abraded and chemically modified to increase surface adhesion nearly instantaneously, thanks to plasma ions generated under open-air conditions.

Plasma treatments can quickly clean the surfaces of PET and other plastics[2] without affecting their underlying properties or appearance. Normally, this technology requires clean rooms and vacuum chambers to turn noble gases into polymer-scrubbing plasma ions.

The DCSBD technique, on the other hand, operates at atmospheric pressure and generates its plasma from ordinary air molecules. It achieves this through an inventive system of parallel, strip-like electrodes embedded inside an alumina ceramic plate. Applying a high-frequency, high-voltage electric field to these strips produces a thin and very uniform plasma field from ambient gases close to the ceramic plate (see image). The planar arrangement of this device makes it simple to treat only the top of the substrate using DCSBD in roll-to-roll lines.

When the researchers treated a PET substrate with a DCSBD plasma source, they saw immediate changes to the polymer surface: single-second plasma exposure times were sufficient to transform it from a water-repellent to a water-attractive surface. These modifications occurred uniformly over the entire PET substrate and provided improved adhesion power that lasted for more than 300 hours. X-ray and atomic force microscopy revealed that the short plasma bursts increased the proportion of surface polar groups and significantly enhanced microscale roughness.

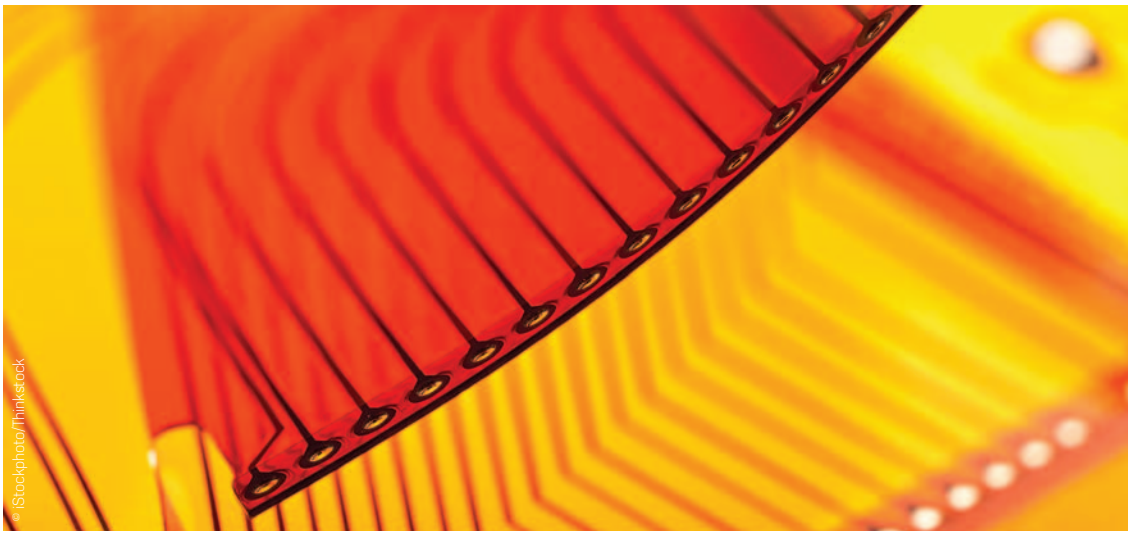
Wu notes that the DCSBD technology is safe to touch, easy to operate, and can be deployed in humid and dusty industrial environments. The team is currently investigating if the high power densities present in these atmospheric plasmas can be exploited for future nanomaterial deposition applications.

1. Homola, T., Matoušek, J., Hergelová, B., Kormunda, M., Wu, L. Y. L. & Černak, M. Activation of poly(ethylene terephthalate) surfaces by atmospheric pressure plasma. *Polymer Degradation and Stability* **97,** 2249–2254 (2012).
2. Homola, T., Matoušek, J., Hergelová, B., Kormunda, M., Wu, L. Y. L. & Černak, M. Activation of poly(methyl methacrylate) surfaces by atmospheric pressure plasma. *Polymer Degradation and Stability* **97,** 886–892 (2012).

Organic electronics:

A faster way to move electrons

A low-cost molecule boosts the stability and amplification characteristics of solution-based polymer semiconductors



Research at the A*STAR Institute of Materials Research and Engineering is reducing the barriers hindering the development of electronic circuits made from flexible polymers.

Replacing traditional rigid silicon wafers with semiconductors made from flexible polymers would herald an age of advanced, 'wearable' electronics. Switching to these semiconductors, known as organic field-effect transistors (OFETs), would also reduce manufacturing costs significantly. However, most plastic materials have trouble moving electrons and their polar opposites — positively charged empty 'holes' inside semiconductor lattices — with sufficient speed for electronic amplification.

Prashant Sonar and co-workers from the A*STAR Institute of Materials Research and Engineering in Singapore have now developed a polymer for solution-based OFET processing that has inherently high carrier mobility and extraordinary air stability[1]. Unlike silicon, polymers are difficult to pack into crystalline structures containing regular pathways for charge carriers. The team's polymer, however, has specifically designed hydrogen bond interactions that create ordered networks for transporting electrons and holes.

Most polymers used in OFETs have a 'donor–acceptor' arrangement of conjugated molecules to enhance the mobility of charge carriers. Using special catalysts, chemists can link together small units of electron-rich and electron-poor aromatic molecules to form an alternating chain of 'block' copolymers. Sonar and co-workers investigated whether fluorenone — an inexpensive and chemically stable molecule with three fused aromatic rings and a central carbonyl unit — could act as a new type of acceptor block for OFET polymers.

The researchers anticipated that the unusual polarity of fluorenone's carbonyl unit might help it stick to aromatic hydrogen atoms and improve solid-state packing. To test this concept, they made a copolymer consisting of fluorenone and an aromatic donor known as diketopyrrolopyrrole (DPP), a compound designed to be compatible with large-scale solution processing. The resulting block copolymer had exceptional thermal stability: it melted only at external temperatures over 300 °C.

When Sonar and co-workers used a technique called spin-coating to convert the fluorenone–DPP copolymer into an OFET device, they observed impressive amplification characteristics and one of the highest hole mobilities ever recorded for solution-processed transistors. Their tests also showed that this material retained its valuable electronic attributes without decomposing in air — a problem that plagued earlier generations of OFETs. Optical measurements revealed the basis of this high stability: the fluorenone units make electrons in the copolymer's highest energy states less accessible and therefore less susceptible to air-based impurities.

"Fluorenone is a commercially available, cheap starting material, which has never been studied for OFET use before," says Sonar. The team is now investigating how to utilize it as a novel building block for high-performance organic electronic applications by carefully 'engineering' chemical improvements onto its molecular framework.

1. Sonar, P., Ha, T.-J. & Dodabalapur, A. A fluorenone based low band gap solution processable copolymer for air stable and high mobility organic field effect transistors. *Chemical Communications* **49**, 1588–1590 (2013).

Thin poles standing in water barely affect waves rolling past them. Similarly, nanostructured devices typically do not interact with light waves.

Plasmonics:

A flexible bridge between two worlds

A novel material shows its credentials to facilitate the integration of photonic and electronic components in practical devices

Many devices used in everyday life — whether they be televisions, mobile phones or barcode scanners — are based on the manipulation of electric currents and light. At the micro- and nanoscales, however, it is typically challenging to integrate electronic components with photonic components. At these small dimensions, the wavelengths of light become long relative to the size of the device. Consequently, the light waves are barely detectable by the device, just as passing waves simply roll past thin poles in a water body (see image).

Better integration of photonic and electronic components in nanoscale devices may now become possible, thanks to work by Khuong Phuong Ong and Hong-Son Chu from the A*STAR Institute of High Performance Computing and their co-workers in Singapore and the United States[1]. From computer simulations, they have identified that the compound $BiFeO_3$ has the potential to be used to efficiently couple light to electrical charges through light-induced electron oscillations known as plasmons. The researchers propose that this coupling could be activated, controlled and switched off, on demand, by applying an electrical field to an active plasmonic device based on this material. If such a device were realized on a very small footprint, it would give scientists a versatile tool for connecting components that manipulate light or electric currents.

"The fact that, in theory, the properties of $BiFeO_3$ [could] be [so readily controlled] by applying an electric field makes it a promising material for high-performance plasmonic devices," explains Ong. He says that they expected such favorable properties after they had calculated the behavior of the material. But when they studied the behavior of the proposed $BiFeO_3$-based device, they found that it could outperform devices based on $BaTiO_3$, which is one of the best materials currently used for such applications.

Like $BaTiO_3$, $BiFeO_3$ can be fabricated relatively easily and cheaply. The new material is therefore a particularly promising candidate for device applications. Ong, Chu and their collaborators will now explore that potential. "We will design $BiFeO_3$ nanostructures optimized for applications such as optical devices for data communication, sensing and solar-energy conversion," says Ong.

According to Ong and Chu, an important step on the path to producing practical devices will be assessing the compatibility of $BiFeO_3$-based structures with standard technologies, which typically use materials known as metal-oxide semiconductors. This future work will involve collaborations with experimental groups at the A*STAR Institute of Materials Research and Engineering, and the National University of Singapore.

1. Chu, S. H., Singh, D. J., Wang, J., Li, E.-P. & Ong, K. P. High optical performance and practicality of active plasmonic devices based on rhombohedral $BiFeO_3$. *Laser & Photonics Reviews* **6**, 684–689 (2012).

Materials:

Two ways to tame a radical

Trapping free electrons with polycyclic aromatic molecules creates materials with enhanced optical, electronic and magnetic properties

Molecules containing multiple aromatic rings may find increasing use as 'smart' materials, thanks to a new strategy to stabilize free electrons in these chemical frameworks.

Replacing traditional semiconductors with flexible and lightweight organic components has the potential to realize significant cost savings for manufacturers. Recently, a promising class of organic materials known as open-shell polycyclic aromatic hydrocarbons (PAHs) has gained researchers' attention (see image). These molecules consist of interlocked, benzene-like rings and contain unpaired electrons, or 'free-radical' centers. Interactions between the radical centers and aromatic electrons make these compounds extremely responsive to light- and electron-based stimuli. Unfortunately, these same radical electrons can quickly degrade PAH chemical structures, rendering them unusable.

Jishan Wu from the A*STAR Institute of Materials Research and Engineering in Singapore and an international team of co-workers have now devised a new stabilization strategy that promises to make open-shell PAHs even more practical[1]. Through clever modification of a prototypical compound known as Chichibabin's hydrocarbon, the team has produced two types of PAHs that retain active radical centers for unprecedented amounts of time.

Chichibabin's hydrocarbon has a sextet of aromatic rings that thermodynamically stabilize radical centers. However, it also has a strong chemical affinity for oxygen atoms and tends to polymerize in their presence. To resolve this issue, Wu and co-workers used a process known as benzannulation to add four additional aromatic benzene rings to the PAH framework. They anticipated this design could enhance thermodynamic stability and block kinetic polymerization interactions.

When the researchers chemically excited the tetrabenzo-Chichibabin's hydrocarbon to an open-shell system, they saw that the radical centers remained active for an unusually long time — two full days — before returning to the low-energy ground state. Using a combination of high-resolution spectroscopy and theoretical calculations, the team discovered the radical's benzene rings were oriented at right angles to one another, while the ground-state compound had a relatively flat, butterfly-like ring layout. The large energy barrier between these two geometries kept the radical active. "This opens the possibility of accessing each form of the PAH molecule and understanding its physical properties," says Wu.

The researchers also modified the tetrabenzo-Chichibabin's hydrocarbon with aromatic fluorenyl rings that have well-known radical stabilizing effects. In fact, the stabilizing capacity of this compound proved so strong that the open-shell radical became the lowest-energy state, and the molecule remained stable for months under ambient air and light conditions.

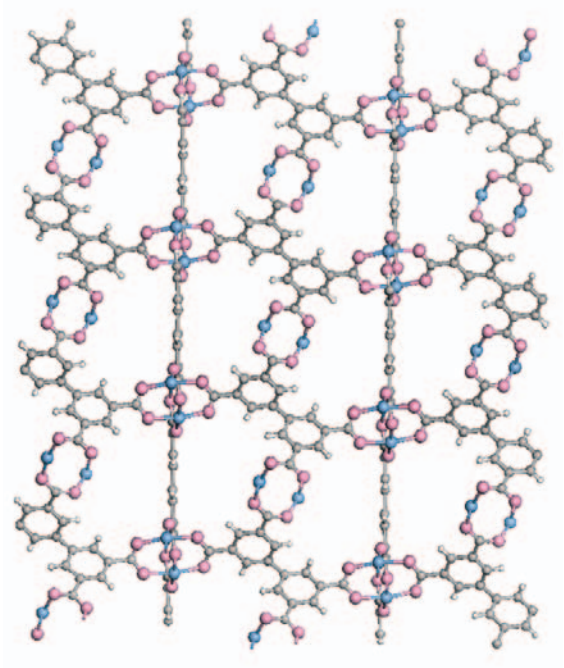
Experiments revealed these new open-shell PAHs to have valuable properties including enhanced two-photon absorption, a strong magnetic response and multiple redox states. Wu notes that these findings may lead to the development of better photodynamic therapies and magnetic imaging techniques in the future.

1. Zeng, Z., Sung, Y. M., Bao, N., Tan, D., Lee, R. *et al.* Stable tetrabenzo-Chichibabin's hydrocarbons: Tunable ground state and unusual transition between their closed-shell and open-shell resonance forms. *Journal of the American Chemical Society* **134,** 14513–14525 (2012).

Materials:

Magnetic mystery solved

Defects in metal–organic frameworks induce low-temperature ferromagnetism and could yield novel materials for industry



The metal–organic framework (MOF) is built from clusters containing copper (blue), oxygen (pink) and carbon (black), joined by carbon-based linkers.

Highly porous materials known as metal–organic frameworks (MOFs) are showing promise as catalysts and drug-delivery vehicles. Some scientific sleuthing by A*STAR researchers could now help industry to exploit the magnetic properties of MOFs for applications such as biomedical sensors.

The structure of MOFs resembles atomic scaffolding: clusters of atoms containing metal ions are linked together in a three-dimensional lattice by carbon-based aromatic molecules (see image). One particular MOF — known as HKUST-1 — has attracted attention because it was unexpectedly found to be ferromagnetic, albeit at the low temperature of −268.45 °C or less[1].

Each metal cluster in the material contains a pair of copper ions held together by four carboxylate groups, which contain carbon and oxygen atoms. Each metal ion carries an unpaired electron, which acts like a tiny bar magnet. The magnetic fields of the two unpaired electrons in a cluster would normally oppose each other — the 'north' of one electron lining up with the 'south' of its neighbor — negating any overall magnetism. Even if one copper cluster became magnetic, it would have to align with many other clusters throughout the material to produce ferromagnetism. Yet the organic linker molecules hold the clusters too far apart for the clusters to influence each other directly.

An international team led by Shuo-Wang Yang of the A*STAR Institute of High Performance Computing in Singapore and Lei Shen of the National University of Singapore has now solved the mysterious origin of the ferromagnetism[2]. By modeling the behaviors of ions and electrons in a series of MOFs, the team showed that if a copper ion is absent from a cluster, its carboxylate group will carry an unpaired, magnetic electron instead. Its magnetic field affects itinerant electrons in the MOF's organic linkers, which in turn affect any unpaired electrons in the next copper cluster. If the magnetic message reaches enough clusters, the material as a whole becomes ferromagnetic.

The researchers made a range of MOFs containing the twin copper motif, and found that around 0.57% of the metal ions were indeed missing from the structure — enough to generate ferromagnetism at low temperatures, they calculated. Such copper vacancies are "inevitable, especially for large organic–metal complex systems such as MOFs," the researchers note.

Yang and co-workers also predicted that a MOF containing a non-aromatic organic linker blocks the magnetic coupling between two adjacent clusters, and confirmed by experiments that it was not ferromagnetic. The researchers now hope to create new ferromagnetic materials by designing MOFs with deliberate metal-ion vacancies.

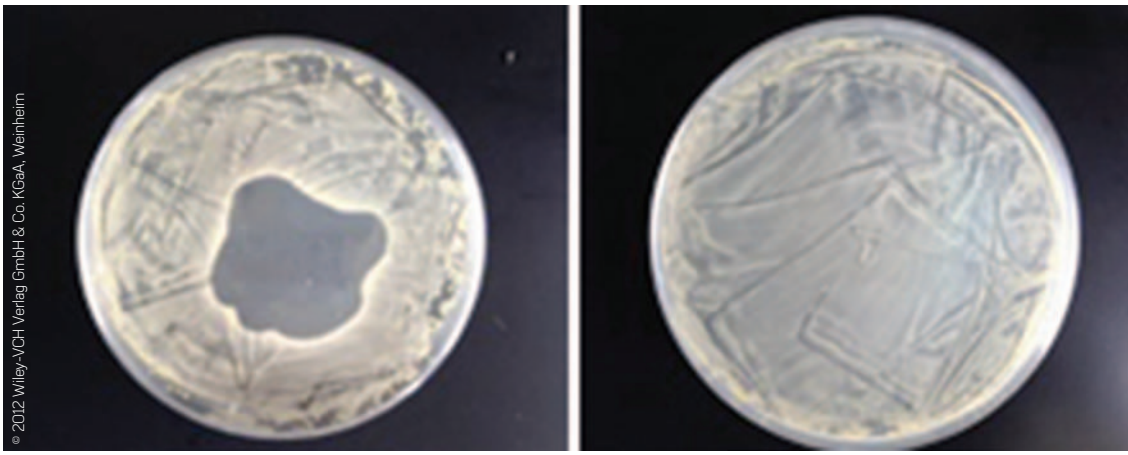
1. Chui, S. S., Lo, S. M., Charmant, J. P., Orpen, A. G. & Williams, I. D. A chemically functionalizable nanoporous material. *Science* **283,** 1148–1150 (1999).
2. Shen, L., Yang, S.-W., Xiang, S., Liu, T., Zhao, B. *et al.* Origin of long-range ferromagnetic ordering in metal–organic frameworks with antiferromagnetic dimeric-Cu(II) building units. *Journal of the American Chemical Society* **134,** 17286–17290 (2012).

Antimicrobial materials:

Pathogen-proof surfaces for better health

A hydrogel with potent antibacterial activity promises to protect hospital patients from difficult-to-treat infections

Coating medical supplies with an antimicrobial material is one approach that bioengineers are using to combat the increasing spread of multidrug-resistant bacteria. Multidrug-resistant *Staphylococcus aureus* (MRSA) and related pathogens, for example, can lengthen hospital stays and even cause death. A research team at the A*STAR Institute of Bioengineering and Nanotechnology in Singapore has now developed a highly effective antimicrobial coating based on polymers[1]. The coating can be applied to medical equipment, such as catheters, explains Yi-Yan Yang, who led the research.



An antibacterial hydrogel coated onto the center of a Petri dish (left) prevents bacterial growth, whereas an untreated Petri dish (right) is completely covered with bacteria.

The gel coating was highly effective at killing a range of multidrug-resistant bacteria and fungi and preventing pathogens from growing.

Yang's coating was inspired by a well-known family of antimicrobial materials called cationic polymers. On contact, these materials kill microbes by attaching to, infiltrating and ultimately rupturing their cell walls. When these polymers are modified to form a coating, however, their antimicrobial activity is usually compromised. They also tend to accumulate a layer of dead microorganisms on their surface. "This can trigger an immune response and inflammation in the patient, and may also block the antimicrobial function of the coating," Yang explains.

To overcome these limitations, Yang and her team developed their polymer-based hydrogel coating to have antifouling as well as potent antimicrobial properties. They made the coating by combining a 'block' of poly(ethylene glycol) (PEG) — which is known for its fouling resistance — with a polycarbonate. They then made the polycarbonate block functional by adding two components: cationic groups to capture passing pathogens; and water-repellent hydrophobic units to puncture their lipid-rich cell membranes and kill the cell.

Yang and her team showed that their gel coating was highly effective at killing a range of multidrug-resistant bacteria and fungi and preventing pathogens from growing on surfaces (see image). A simple rinse with a buffer solution was sufficient to remove the dead cells, confirming the coating's antifouling capabilities. The team also confirmed that the coating is harmless to red blood cells and does not irritate the skin.

Furthermore, the researchers showed that the hydrogel could be added to the surface of a standard hospital catheter, preventing microbial growth. As the coating can be formed under mild, physiological conditions, the hydrogels can also be used as a wound dressing, Yang notes. "For example, hydrogel dressings could form after spraying the gel precursor solution onto wounds," she says.

According to Yang, the research team's next step will be to investigate wound healing using these gels in animal studies. "At the same time, we will also seek industry partners to help commercialize these hydrogels, especially for medical device coating applications," she says.

1. Liu, S. Q., Yang, C., Huang, Y., Ding, X., Li, Y. *et al.* Antimicrobial and antifouling hydrogels formed in situ from polycarbonate and poly(ethylene glycol) via Michael addition. *Advanced Materials* **24,** 6484–6489 (2012).

Data storage:

Making the switch

Magnetic materials that change their properties when heated could pack more data onto hard drives

A three-layer sandwich of magnetic materials could help to pack more data onto hard drives.

A 'sandwich' of three iron alloy layers could help to create computer hard drives that can store more data than ever before. Tiejun Zhou and co-workers at the A*STAR Data Storage Institute in Singapore expect that their development, based on a new technology called heat-assisted magnetic recording (HAMR), could boost the capacity of disks[1].

Conventional hard drives contain a tiny electromagnet — a write head — that hovers over a spinning disk coated with a ferromagnetic material. The electromagnet induces the magnetic field within small regions of the disk to point either up or down, encoding one bit of data.

Heat can jumble these magnetic bits and destroy the data. The latest disks use materials with a very large coercivity — a measure of how difficult they are to demagnetize. However, write heads must exert even greater magnetic fields to encode data in such materials. The balance between bit size, coercivity and the electromagnet's strength ultimately puts an upper limit on disk density of about 1 terabit per square inch.

In HAMR systems, each recording region is briefly heated above its Curie temperature, a point when magnetic coercivity drops significantly and a much smaller field can write the bit. Once the region cools, the coercivity rises and the bit locks into place.

Zhou's team found a way to reduce both the writing temperature and the switching field in HAMR systems. The upper iron–platinum layer of the sandwich stores data bits; the lower iron–cobalt layer helps to channel the write head's magnetic field, enabling data writing; and the middle iron–rhodium layer acts as a switch between the two. The middle layer is antiferromagnetic at room temperature so blocks any magnetic coupling between the other layers. At about 350 kelvin, however, it becomes ferromagnetic, allowing the layers to couple.

Iron–platinum normally has a Curie temperature of about 750 kelvin, but that plummets when coupled to the iron–cobalt layer. Data can therefore be written to the iron–platinum layer once the iron–rhodium layer becomes ferromagnetic, at about 350 kelvin.

Coupling also reduces the coercivity of the iron–platinum layer, so a write head would need only to generate one-third of the usual magnetic field to encode a bit. "Theoretically, the bit can occupy a space as small as 100 square nanometers," says Zhou. The team now plans to reduce the size of the nanocrystals in each data region of the iron–platinum layer, while maintaining its high coercivity.

1. Zhou, T. J., Cher, K., Hu, J. F., Yuan, Z. M. & Liu, B. The concept and fabrication of exchange switchable trilayer of FePt/FeRh/FeCo with reduced switching field. *Journal of Applied Physics* **111**, 07C116 (2012).

Alternative energy:

A cooler way to clean hydrogen

Converting bioethanol into hydrogen for fuel cells becomes significantly simpler with innovative metal catalysts

A process known as ethanol steam reforming is creating opportunities for fuel cell researchers, thanks to the recent rise of the bioethanol industry. This technique generates hydrogen gas (H_2) directly within fuel cell systems onboard vehicles by decomposing bioethanol in the presence of special catalysts — an approach that could use current gasoline delivery infrastructures to power alternative energy transportation. Currently, ethanol steam reforming suffers from a major obstacle: its multiple reaction pathways can produce toxic carbon monoxide (CO) by-products that ruin fuel cell membranes.

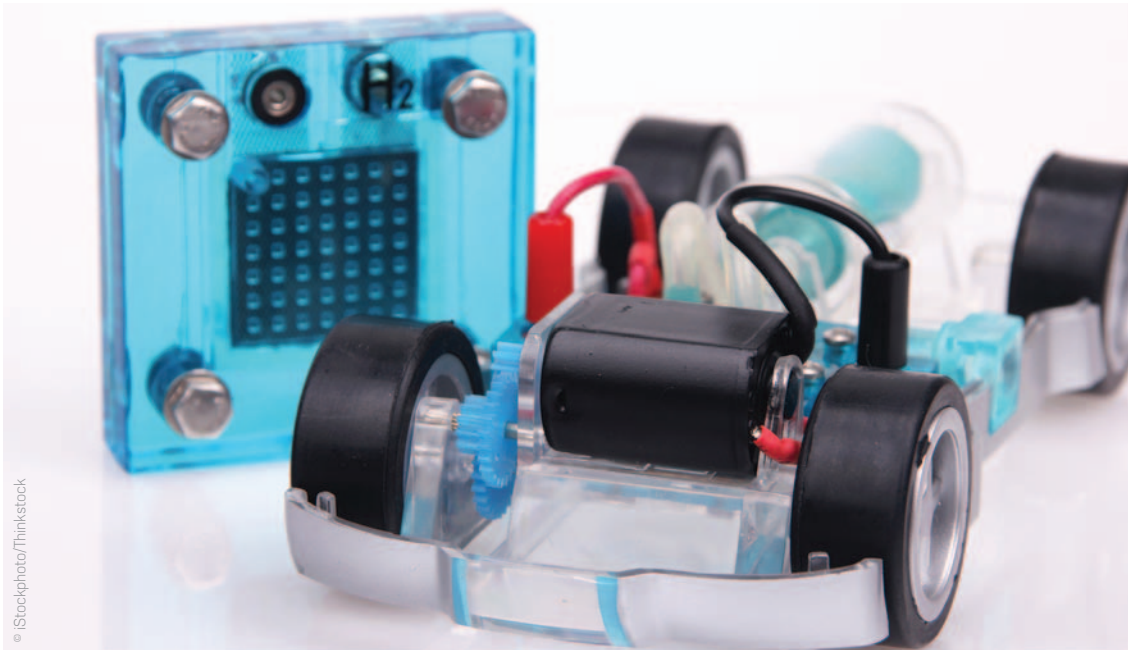
Lin Huang, Jianyi Lin and co-workers from the A*STAR Institute of Chemical and Engineering Sciences in Singapore have now prepared a novel metal catalyst that can eradicate CO emissions from ethanol-derived H_2 at temperatures 50 °C lower than previous catalysts[1].

Low-temperature ethanol steam reforming boosts the safety and efficiency of fuel processing onboard vehicles, but requires a careful choice of catalysts. Rhodium (Rh), a relatively scarce transition metal, has gained attention among chemists because it targets ethanol's carbon–carbon bond — the most difficult part of the alcohol to decompose. However, Rh catalysts tend to generate CO and methane by-products when steam reforming conditions fall below 350 °C.

Huang, Lin and co-workers investigated whether they could resolve Rh's shortcomings with cobalt (Co), a less expensive transition metal that has high selectivity toward H_2 production at low temperatures. They explored whether Co could be combined with Rh on a nanostructured oxide surface to produce a dual-component catalyst. While making a mixed catalyst is relatively straightforward, finding one that maximizes the benefits of both metals for efficient steam reforming is not as easy. Therefore, the team investigated how different metallic precursors could achieve an ideal interaction between Rh and Co atoms on the supporting surface.

Their experiments revealed that catalysts consisting of Rh and Co, prepared from metal carbonyl precursors, gave high yields of extraordinarily clean H_2 with no CO emissions at temperatures as low as 300 °C. According to Huang, these findings indicate that atomic interactions between the metals favor a particular pathway, known as the water–gas shift, which converts CO and water into H_2 and carbon dioxide. However, mixed catalysts made from metal nitrate precursors failed to yield CO-free H_2, presumably because of poor atomic interactions.

The team now faces two challenges: uncovering the mechanistic reasons why supported Rh–Co dual-component catalysts are so effective; and, reducing the build-up of carbonaceous coke deposits that adversely affect catalytic activity and stability during ethanol steam reforming.



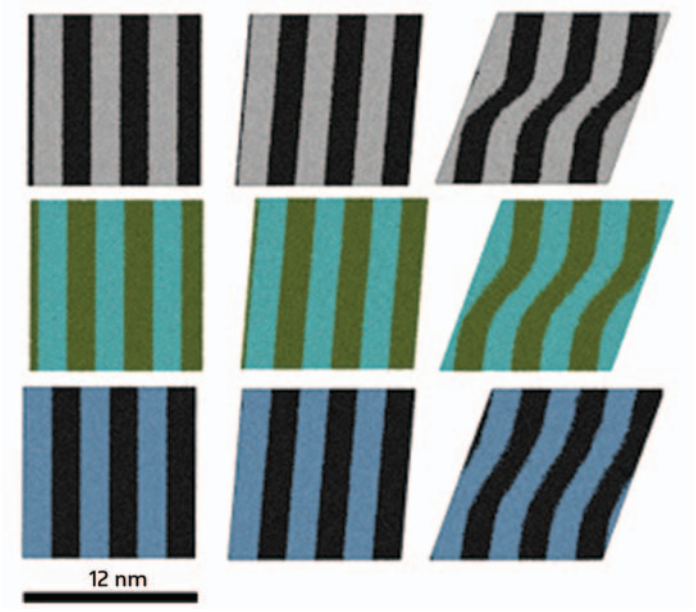
A new dual-component rhodium–cobalt (Rh–Co) catalyst can convert bioethanol into hydrogen fuel at low temperatures without carbon monoxide emissions.

1. Huang, L., Choong, C., Chen, L., Wang, Z., Zhong, Z., Campos-Cuerva, C. & Lin, J. Monometallic carbonyl-derived CeO_2-supported Rh and Co bicomponent catalysts for CO-free, high-yield H_2 generation from low-temperature ethanol steam reforming. *ChemCatChem* **5,** 220–234 (2013).

Metallic glass:

How nanoscale islands react under strain

High-level simulations reveal that plastic deformation in super-resilient alloys is governed by atomic zones with characteristic lengths



Snapshots of atomic movements captured during simulations of iron–phosphorous (top), magnesium–aluminum (middle) and copper–zirconium (bottom) metallic glasses undergoing increasing mechanical strain (left to right).

Quick-cooling molten atoms give metal alloys a glassy, or random, atomic structure that generates higher elasticity and better wear- and corrosion-resistance than their crystalline alloy counterparts. However, these 'metallic glasses' also suffer from brittleness that makes them shatter. Findings from Yong Wei Zhang of the A*STAR Institute of High Performance Computing in Singapore and co-workers may now make it easier to use metallic glass in practical engineering applications[1]. They have discovered that a fundamental relationship between material plasticity and atomic 'islands', known as 'shear transition zones' (STZs), enables precise measurement and prediction of fracturing in these materials.

When an external force strains a metallic glass, most of its atoms respond elastically and try to return to their original positions. Researchers believe that shattering occurs when STZs appear and begin to deform irreversibly. If present in high enough numbers, the STZs will generate shear bands that propagate through a cascade-like process and make the glass fracture.

Despite their importance, defining the extent of STZs remains a point of controversy among researchers. Zhang and co-workers used atomic calculations to explore the development of STZs within three metallic glasses — iron–phosphorous, magnesium–aluminum and copper–zirconium. They selected these materials because of their increasingly different 'Poisson's ratios', a mechanical constant that describes how a material 'pinches in', like a rubber band, when pulled lengthwise. Zhang and co-workers suspected that this ratio could be related to STZ formation.

They first simulated mechanical strain in each of the three types of metallic glass (see image) and observed whether the atomic movements were plastic or irreversible. Then, they correlated the plastic movements with a mathematical function based on interatomic distances. Intriguingly, they discovered that they could extract a constant 'characteristic length' parameter to measure the size of STZ islands that developed during deformation.

Zhang explains that the nanoscale lengths of STZs are reminiscent of 'defects' seen in crystalline metals, and their positive correlation with the Poisson's ratio of a material can help predict fracture problems. Their calculations showed that bigger STZ islands had more resistance to pinching in, and shattered only when relatively large shear bands formed.

By connecting basic materials physics to atomic deformation zones, the team hopes to lay the groundwork for a new generation of metallic glasses with greater resistance to brittle fracture. "Understanding the connection between Poisson's ratio, STZ size and fracture toughness is very important for the development of metallic glasses with good mechanical properties," says Zhang.

1. Murali, P., Zhang, Y. W. & Gao, H. J. On the characteristic length scales associated with plastic deformation in metallic glasses. *Applied Physics Letters* **100**, 201901 (2012).

Carbon capture:

Making use of minerals

Ammonium salts could provide a viable way of removing carbon dioxide from the atmosphere via carbon mineralization

Removing excess carbon dioxide (CO_2) from the atmosphere may be essential to curb severe climate change. Possible, but expensive, methods include burying the gas underground between rock layers or 'scrubbing' the CO_2 in power station cooling towers before it is released. James Highfield at A*STAR's Institute of Chemical and Engineering Sciences, together with co-workers at the National Junior College of Singapore and Åbo Akademi University in Finland, has now described a cheaper and more permanent solution that would prevent the CO_2 escaping back into the atmosphere[1,2].

"Magnesites are commodities in their own right as smoke- and fire-retardants, and have potential for heavy-metal ion sequestration."

Their work focused on using carbon mineralization, a process that involves a reaction between CO_2 and minerals, such as magnesium silicates, to form solid carbonates. Mineralization occurs naturally between the atmosphere and rocks, and the carbonates remain geologically stable for millions of years. Crucially, plentiful raw materials would be available to conduct this type of CO_2 removal on a vast scale.

Natural carbon mineralization is very slow, so scientists are working to accelerate the process in an energy-efficient and carbon-neutral way. Using ammonium salts and magnesium-silicate-rich serpentine rocks, Highfield and co-workers induced rapid carbon mineralization. They also found that milling the solids could convert serpentine directly into stable carbonate.

To accelerate the extraction of magnesium (as soluble sulfate) from serpentine, the researchers used ammonium sulfate. This reaction generates by-products such as iron oxide that may be useful for the steel industry. They trapped the leftover ammonia in water, and recycled this by-product in an aqueous wash with the magnesium solution to produce a mineral form of magnesium hydroxide called brucite. Finally, the researchers carbonated the brucite in a pressurized reactor. The heat generated by this exothermic process was recycled to help power the initial magnesium extraction.

A key aim throughout the processing was to recycle as much ammonium sulfate as possible. The final products, magnesites (magnesium carbonates), could also be useful. "Magnesites are commodities in their own right as smoke- and fire-retardants, and have potential for heavy-metal ion sequestration," the team notes.

Highfield and co-workers discovered that the yield of recycled ammonium sulfate drops considerably at temperatures of 400–450 °C, although reactions at these temperatures produce the most brucite. They suggest that this may be rectified by either increasing the humidity during the process or performing the reaction at a lower temperature to extract an alternative mineral to brucite.

"By virtue of their rich chemistry with magnesium, ammonium salts are likely to become ubiquitous in the field of CO_2 mineralization," the team says.

© iStockphoto/Thinkstock

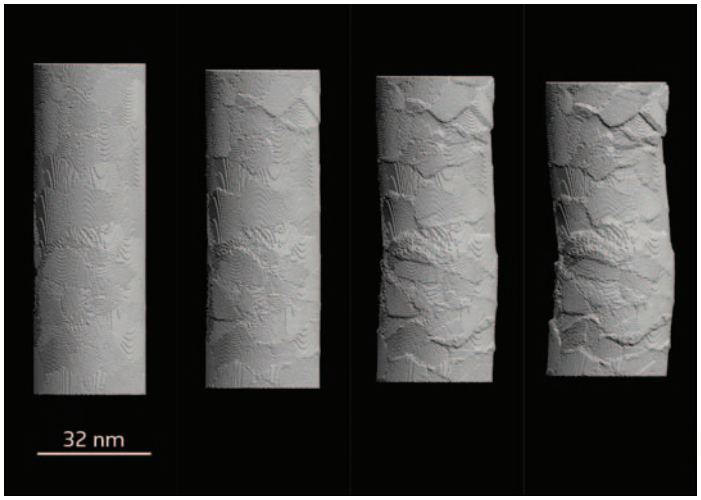
Rapid mineral carbonization of CO_2, using recyclable ammonium sulfates to extract magnesium from serpentine rock, produces magnesites and iron oxides, which have uses in industry.

1. Highfield, J., Lim, H.-Q., Fagerlund, J. & Zevenhoven, R. Activation of serpentine for CO_2 mineralization by flux extraction of soluble magnesium salts using ammonium sulfate. *RSC Advances* **2**, 6535–6541 (2012).
2. Highfield, J., Lim, H.-Q., Fagerlund., J. & Zevenhoven, R. Mechanochemical processing of serpentine with ammonium salts under ambient conditions for CO_2 mineralization. *RSC Advances* **2**, 6542–6548 (2012).

Nanomaterials:

Sized-up for strength

Experiments and numerical simulations show that miniaturized ultra-small platinum cylinders weaken when their constituents are reduced in number



Simulated deformation of a platinum nanopillar under increasing levels of compression from left to right.

Miniaturizing microscopic metallic objects while enhancing their strength is critical to developing high-performance devices that integrate transistor-like electronics with mechanical components. When these objects consist of small crystals, or grains, such as polycrystalline nanopillars, their mechanical behavior is difficult to predict because the grains vary in size and orientation. Researchers from the California Institute of Technology in the United States and the A*STAR Institute of High Performance Computing (IHPC), Singapore, have now determined how miniaturization and intrinsic granular structure impact the deformation of ultra-small platinum cylinders[1].

The team used a combined experimental and computational approach to overcome the knowledge gap hindering the production of reliable micro- and nano-electromechanical devices. Team member Zhaoxuan Wu from the IHPC explains that this approach allowed them to reduce the size of the experimental samples to tens of nanometers. It also allowed them to perform large-scale atomic simulations on comparable nanostructures, which provided a means to directly link structure and mechanical properties. "This is rarely achievable in such studies," he notes.

The researchers first generated a template by depositing a polymer film on a gold-coated silicon surface and perforating it with nano- to micrometer-sized cylindrical holes. Next, they synthesized the metal nanostructures in these holes from a platinum precursor solution. Dissolving the template then produced nanopillars that displayed well-defined grains of similar sizes and grain boundaries, or interfaces.

Compression experiments on the nanostructures showed that the thinnest nanopillars remained almost cylindrical under low pressure but weakened dramatically, and bent irreversibly, under high pressure. In contrast, wider nanopillars exhibited a smoother deformation and delayed failure. This 'smaller is weaker' trend is contrary to the fate observed for metallic single crystals: they become stronger with smaller diameters. Wu and co-workers also found that reducing the number of grains across a nanopillar's diameter weakened the structure.

In agreement with their experimental results, the researchers' numerical simulations revealed that the compressed nanopillars gradually underwent reversible and subsequent irreversible deformation (see image). Moreover, the simulations indicated the origin within the nanostructures of the irreversible deformation and dislocation motions. The nanopillars contain a high density of grain boundaries that promote the formation of dislocations. These dislocations, through which a specific type of deformation develops, propagate across an entire grain or from one grain to another inside the cores. Close to the nanopillar surface, the grains easily slide against each other to create atom-sized steps, reducing material strength.

"We are further examining the effects of microstructural flaws and oxidations on the mechanical behavior of nanomaterials," says Wu.

1. Gu, X. W., Loynachan, C. N., Wu, Z., Zhang, Y.-W., Srolovitz, D. J. & Greer, J. R. Size-dependent deformation of nanocrystalline Pt nanopillars. *Nano Letters* **12**, 6385–6392 (2012).

Magnetic materials:

Forging ahead with a back-to-basics approach

Atomic-level simulations hint at how to control the magnetic properties of layered materials for data storage applications

Scientists have recently started to explore the possibility of using an intrinsic property of the electron known as spin for processing and storing information. Magnetic fields can influence the dynamics of electron spin, so harnessing this potential relies on precision engineering of crystalline storage materials. Chee Kwan Gan and co-workers at the A*STAR Institute of High Performance Computing and the A*STAR Data Storage Institute in Singapore have used theoretical calculations to show how the magnetic characteristics of specific materials can be controlled at the atomic level[1]. Their results could lead to novel magnetic recording devices.

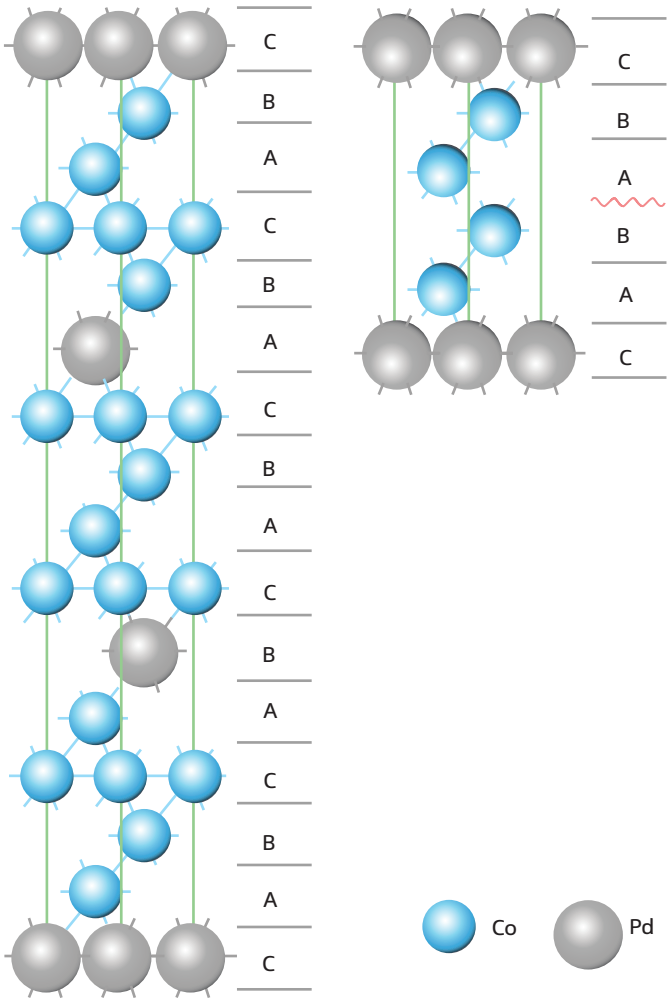
"Nature sometimes makes 'mistakes'. It is important to understand these defects and subsequently use them to control the material's physical properties."

One promising route to such spintronic devices is to design structures consisting of alternating layers of different magnetic atoms. The strength of the magnetic influence is stronger in the direction of the multilayer stack than it is parallel to the planes of the atoms. This so-called perpendicular magnetic anisotropy is useful for spintronic memory devices because it allows a greater storage density than a conventional electronic device.

The properties of these structures, however, are highly sensitive to the precise arrangement of the crystal. Just one misplaced layer of atoms — a stacking fault — can noticeably alter device performance (see image). Previous studies usually ignored these special defects, "but nature sometimes makes 'mistakes'," explains Gan. "It is important to understand these defects and subsequently use them to control the material's physical properties."

Gan and his team went back to basics to better understand how atom-level imperfections affect the properties of these multilayers. They used a powerful mathematical approach known as density functional theory. This approach uses only fundamental equations from quantum mechanics to model the behavior of electrons in these structures, without requiring any prior assumptions.

The researchers modeled a material consisting of alternating layers of cobalt and palladium atoms. Multilayers of these atoms have previously exhibited a large perpendicular magnetic anisotropy when the cobalt layers are less than 0.8 nanometers thick. Gan and co-workers then assessed how stacking faults and the ratio of cobalt to palladium atoms affected this anisotropy. Their results showed that a stacking fault could enhance the magnetic anisotropy in structures with a relatively thick cobalt layer. They also found that the anisotropy increased almost linearly with increasing cobalt content.



The atoms in a crystalline material known as Co_4Pd_1 are arranged into stacked layers labeled A, B and C (left). A single 'mistake' in this arrangement (right) can affect the material's properties.

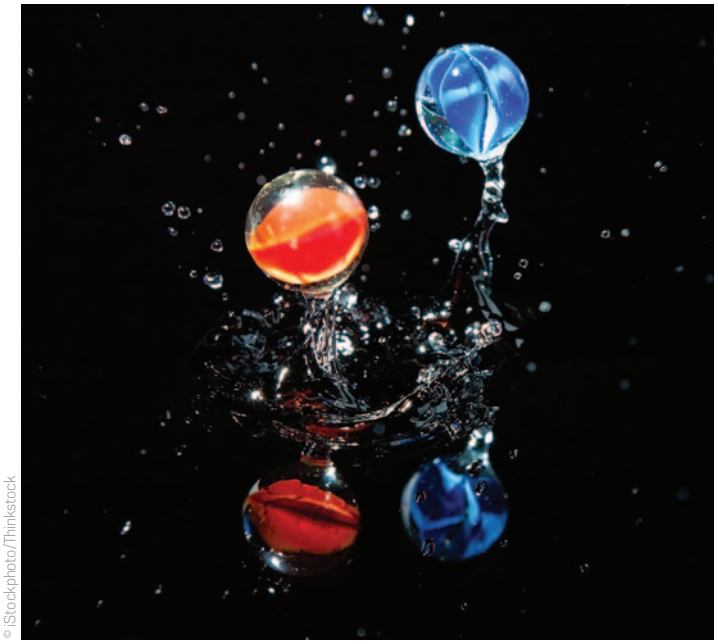
High magnetic anisotropy materials have potential for use in the next generation of ultrafast and high-capacity magnetic random-access memory, Gan explains. The improved understanding of these materials from this research will guide the way to realizing such devices.

1. Wu, G., Khoo, K. H., Jhon, M. H., Meng, H., Lua, S. Y. H. *et al.* First-principles calculations of the magnetic anisotropic constants of Co–Pd multilayers: Effect of stacking faults. *Europhysics Letters* **99**, 17001 (2012).

Smart materials:

Fused liquid marbles show their strength

A superglue polymerization strategy that fortifies encapsulated 'liquid marble' water droplets also strengthens their market potential



Confining water droplets inside metal–organic liquid marbles could make cosmetic and personal care products easier to apply.

'Liquid marbles' are a peculiar new substance made by rolling water droplets into powders incapable of dissolving in water. The resulting micro- and nanoscale-particles act like soft solids and can speed along surfaces without leaving water marks. Such non-stick, hydrophobic behavior has potential application in drug delivery and microfluidic technology. However, liquid marbles suffer from erratic structures prone to collapse. Jia Min Chin, Jianwei Xu and co-workers from A*STAR's Institute of Materials Research and Engineering, and Institute of Bioengineering and Nanotechnology, have now developed a scheme to stabilize liquid marbles quickly and safely using vapors from ordinary superglue[1].

Many powders used to make liquid marbles are based on metal–organic frameworks (MOFs), a type of crystal in which metal ions are interspersed with rigid organic molecules. Chin, Xu and co-workers investigated whether MOFs known as NH_2-MIL-53(Al), a combination of aluminum atoms and aminophenyl compounds, could grow directly on the surfaces of alumina microparticles. This approach, the team theorized, might provide extra structural control over liquid marble stability. After confirming MOF growth with X-ray measurements, the team modified the microparticles with either hydrocarbon or fluorocarbon chains, converting them into 'superhydrophobic' powders. Then, they produced alumina-supported liquid marbles by adding micro-sized water droplets.

The researchers found that their new liquid marbles had greater stability than usual, thanks to its reactive amino groups and high surface roughness. Yet, they sought to further boost its resilience. When they spotted small gaps between the MOF–alumina microparticles with scanning electron microscopy, they inferred that certain gas molecules might enter these pores and create a cross-linked network through a process called air–liquid interfacial polymerization.

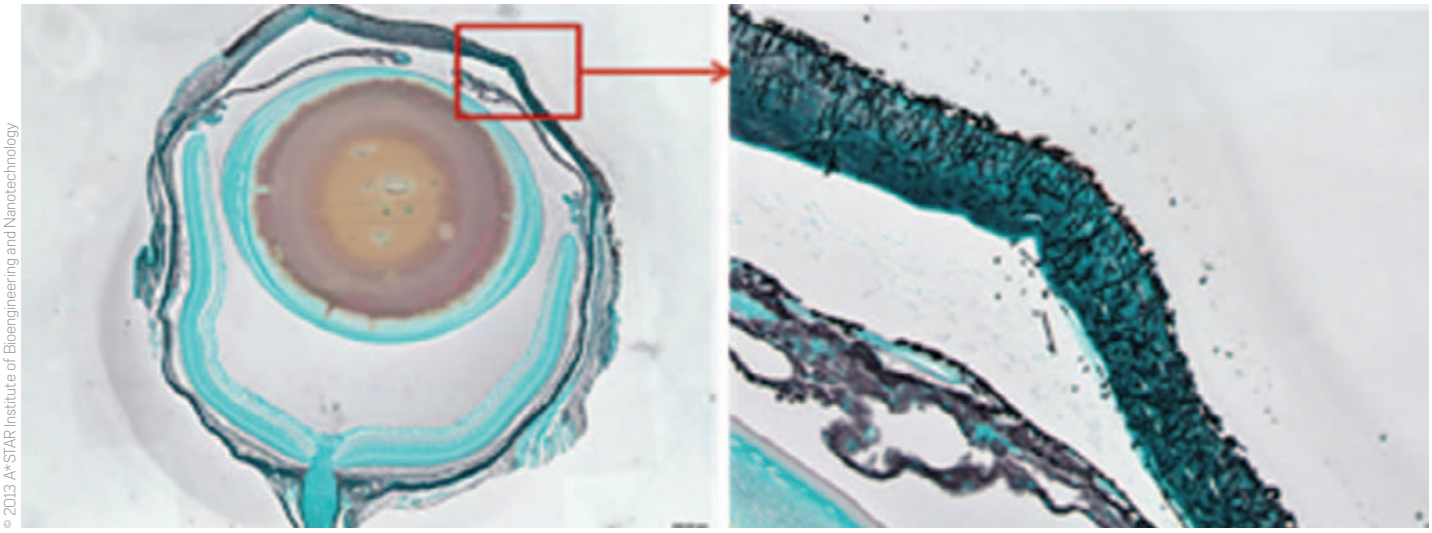
Forensic scientists often use superglue vapors to uncover fingerprints at crime scenes; the trace water in finger smudges reacts rapidly with adhesive fumes and generates visible polymer structures. Taking a cue from this method, the team exposed their MOF–alumina liquid marble to superglue vapors in a Petri dish and saw a rigid polymer casing form within a few minutes. Chin notes that this procedure requires no heat, UV radiation, or chemical initiators — an unprecedented finding for liquid marble encapsulation. "Furthermore, the only solvent required was water, qualifying this as a 'green' reaction," she adds.

The liquid marble retained its unique non-wetting behavior on surfaces, even with the protective polymer coating. These stabilizing attributes promise big dividends in areas such as gas purification and personal care products: two patents have already been filed this year in efforts to commercialize this technology.

1. Chin, J. M., Reithofer, M. R., Tan, T. T. Y., Menon, A. G., Chen, E. Y. *et al.* Supergluing MOF liquid marbles. *Chemical Communications* **49,** 493–495 (2013).

Research Highlights

GENETICS & DISEASE



Microscope images of a mouse eye (left) show that the polymeric imidazolium compound PIM-45 protects the cornea by reducing fungal invasion into the cornea (right; fungi are stained black).

Biomaterials:

A robust cure for a fungal eye infection

Stable, inexpensive and easy-to-prepare active ingredients for topical treatments effectively clear a fungal eye infection

Pathogenic microbes that become encased within a protective and adhesive polymeric coating, forming a biofilm, are among the most difficult forms of infections to treat. Fungal keratitis, for example, is a common form of eye infection caused by fungi that can form a biofilm on the patient's cornea, which if left untreated may lead to blindness. A novel pair of antifungal compounds that clear these biofilms more effectively than existing treatments has now been developed by researchers led by Yugen Zhang and Jackie Ying at the A*STAR Institute of Bioengineering and Nanotechnology, Singapore[1].

Zhang, Ying and co-workers developed their compounds from a family of antimicrobial materials called amphiphilic polymers. These materials incorporate both polar and non-polar subunits, a characteristic that is crucial to their function: the polar group helps to anchor the polymer to the microbe's charged surface, which allows the non-polar tail to then penetrate and rupture the microbe's lipid membrane. The researchers' compounds incorporated a polar unit called an imidazolium group. Interestingly, previously developed imidazolium-based amphiphilic structures simply featured a long-chain non-polar tail. However, in a modification to the usual design, Zhang and Ying's team developed short-chain amphiphilic materials consisting of repeating polar and non-polar subunits.

The researchers showed that the amphiphilic components of these materials — named IBN-1 and PIM-45 — make them particularly effective for treating biofilms. In tests against biofilm-protected fungal cells grown on the surface of a contact lens, the team showed that IBN-1 and PIM-45 were more effective than fluconazole and amphotericin B, the drugs currently used to treat these infections. "The amphiphilic structure and its high solubility in water allow our short-chain polymers to better penetrate the biofilms," Ying explains. Using mice, the team then showed that their compounds could curtail fungal growth in the eye itself (see image).

As well as proving more efficacious than current treatments, the compounds also offer several practical advantages. Amphotericin B and fluconazole are fragile structures, requiring careful protection from heat and light. "Once a package of Amphotericin B is opened, it can be used for one to two days only," says Zhang. "In comparison, our compounds can be stored in water or a buffer solution at room temperature for at least six months." In addition, IBN-1 and PIM-45 are easy and inexpensive to prepare, he adds.

The team is currently working with industry to commercialize the polymer technology, according to Ying. "We will also explore other applications of these materials," she says.

1. Liu, L., Wu, H., Riduan, S. N., Ying, J. Y. & Zhang, Y. Short imidazolium chains effectively clear fungal biofilm in keratitis treatment. *Biomaterials* **34,** 1018–1023 (2013).

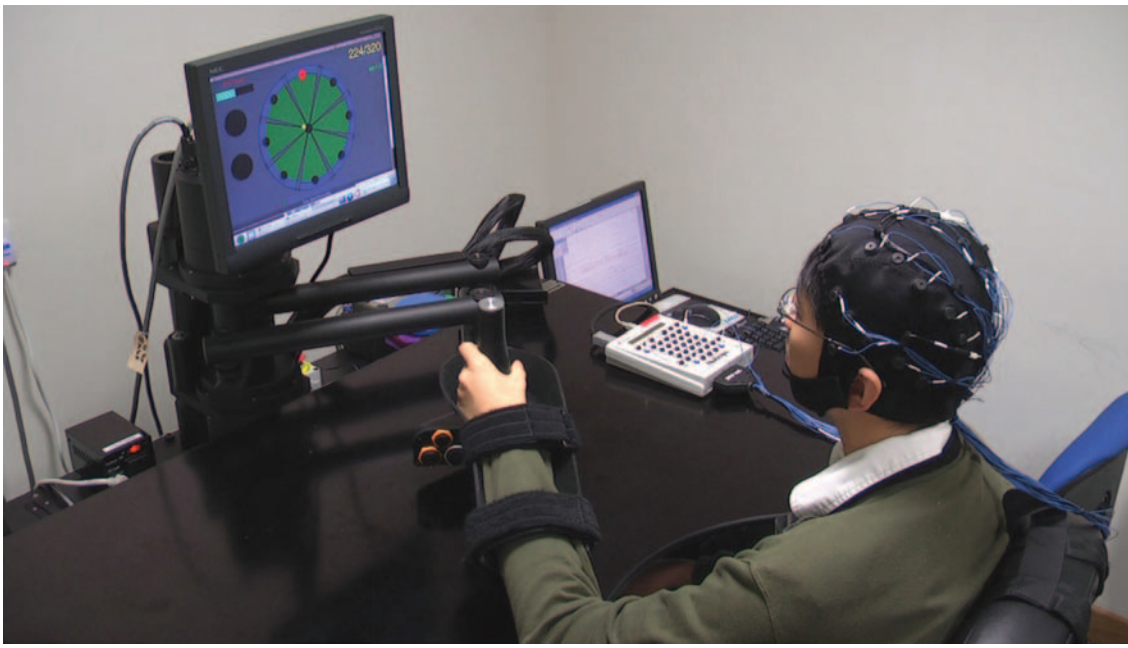
Neuroscience:

Predicting stroke recovery

By monitoring brain connections in their resting state, researchers show that rehabilitation based on brain–computer interfaces could be superior to robot-assisted programs

Changes in the pattern of connections in the resting brain predict the extent to which stroke patients will recover following rehabilitation, according to new research led by Cuntai Guan of the A*STAR Institute for Infocomm Research, Singapore, and Karen Chua of the Tan Tock Sen Hospital, Singapore, in collaboration with Bálint Várkuti of the University of Tübingen, Germany[1].

Strokes are caused by blockage of, or damage to, blood vessels in the brain. They are a leading cause of death, often result in speech deficits and paralysis on one side of the body, and frequently cause brain damage and disability. Rehabilitation, however, can help to partially restore the motor deficits.



A user operating the brain–computer interface stroke rehabilitation system.

Guan and his co-workers studied nine individuals who had recently suffered their first stroke. The team trained the participants for one month using either a robot-assisted rehabilitation program or a brain–machine interface (BCI). Via scalp electrodes, the BCI reads brain waves associated with movement planning, and then translates them into commands that move a robotic arm (see image).

The researchers used functional magnetic resonance imaging to examine connections in the participants' brains. They also used a standardized clinical scale to assess their upper-limb movements, both before and after rehabilitation. Specifically, they examined long-range connections within the so-called default mode network, a set of brain regions that become active when external stimuli are ignored and the mind is allowed to wander instead.

Guan and co-workers found that patients rehabilitated with the BCI recovered better than those who received robot-assisted rehabilitation. This was associated with increased connectivity between certain components of the default mode network — especially the anterior cingulate cortex, the inferior parietal lobule, and the supplementary motor cortex. Furthermore, these connectivity changes accurately predicted the extent to which the stroke patients would recover.

A stroke often disrupts long-range connections within the brain, but neuroscientists now widely believe that the brain can build or strengthen alternative pathways to compensate for the damage, leading to some functional recovery. The enhanced connectivity in the brain's resting state observed by the team could therefore be an aftereffect of these processes, and may reflect increased cooperation between the regions involved, which compensates for the damage caused by the stroke.

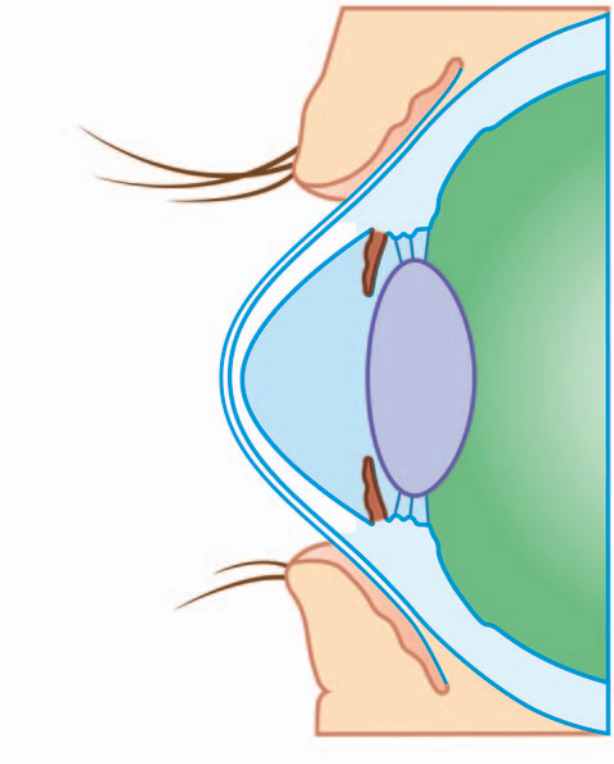
"Stroke rehab is a complex and effortful process," says Guan, "and in terms of saving therapists' time, there is currently a lack of efficient and productive approaches." The team's BCI stroke rehabilitation system can supplement other approaches. "We are now working with industry to further develop [the system] and make it accessible to patients in hospitals, rehab centers, and eventually the home."

1. Várkuti, B., Guan, C., Pan, Y., Phua, K. S., Ang, K. K. *et al.* Resting state changes in functional connectivity correlate with movement recovery for BCI and robot-assisted upper-extremity training after stroke. *Neurorehabilitation and Neural Repair* **27,** 53–62 (2013).

Eye disease:

Genes with a blinding influence

Identification of specific genetic variants associated with common eye disorders could improve treatment and prevention



Keratoconus causes distortion of the cornea and can lead to blindness. Thin corneas are an indication of a risk of developing the disease.

The eye is covered by a clear and protective layer called the cornea, and abnormal thickness of the cornea can result in eye disease. An international research team including Chiea Chuen Khor of the A*STAR Genome Institute of Singapore has pinned down 27 genetic variations that are strongly associated with a heritable trait known as central corneal thickness (CCT)[1]. Some of these variations are also directly linked to eye diseases, so the findings may lead to better prevention and treatment.

Extreme thinning of the cornea is associated with rare eye disorders, whereas milder thinning is linked to more common problems. These include primary open angle glaucoma (POAG), which is the second leading cause of blindness worldwide, and a progressive eye disease called keratoconus, which affects 1 in 2,000 people and causes distortion of the cornea and visual impairment (see image).

In several previous studies, researchers investigated the genetic basis for CCT by comparing the corneal thickness of individuals with their genetic fingerprints. This revealed 11 genetic variations linked to CCT, but with varying levels of certainty; the new study solidified these findings and uncovered further associated genetic variations.

Khor and co-workers achieved this by collecting and re-analyzing the data from 13 previous studies as a whole. They identified a total of 27 genetic variations that are strongly linked to CCT; 16 of these had previously eluded detection. Furthermore, the researchers found that six of these variations indicated a risk of developing keratoconus, with one of the six also linked to POAG.

The new study identified a higher number of genetic variations associated with eye disease than previous ones because it included data from over 20,000 individuals. "[Our study] is three to four times the size of previous studies, and it combines data from Asians and Europeans for the first time," explains Khor. "As such, the results are correspondingly rich in detail."

The team's findings not only provide greater insight into the genetic basis for corneal thinning, but also demonstrate that associated genetic variations increase the risk of common eye diseases. According to Khor, this could open up new avenues for dealing with these diseases.

"We now know the biological targets that are relevant for disease to occur, and some of these gene targets may be amenable to drug modifications," he says. "For prevention, individuals [who carry] multiple risk variants, and who are deemed to be at very high risk of disease, can be screened earlier to intervene before blindness occurs."

1. Lu, Y., Vitart, V., Burdon, K. P., Khor, C. C., Bykhovskaya, Y. *et al.* Genome-wide association analyses identify multiple loci associated with central corneal thickness and keratoconus. *Nature Genetics* **45,** 155–163 (2013).

Genomics:

Sweet success with citrus

The sweet orange's parents and mechanism for producing vitamin C are revealed in its draft genome sequence

The sweet orange, *Citrus sinensis*, has long dominated fruit production worldwide. Yet attempts to study this fruit's genetics and improve its desirable traits have proved difficult because it reproduces asexually and seedlings are nearly identical to the mother plant. Plant biologists had even failed to determine with certainty which fruits had been crossed to produce the sweet orange, over 2,000 years ago in China. An international research team, including members from the A*STAR Genome Institute of Singapore (GIS), has now broken the deadlock by sequencing the genome of the sweet orange[1]. The team has also revealed the fruit's parentage: pummelo, which is similar to grapefruit, and mandarin, a small and easy-peeling orange.

Xiaoan Ruan of the GIS along with Qiang Xu and Ling-Ling Chen of Huazhong Agricultural University, China, and their co-workers compared the orange's genome with those of pummelo, *Citrus grandis*, and mandarin, *Citrus reticulata*, using simple sequence repeat and single-nucleotide polymorphism markers — two types of short and highly variable DNA sequence data.

One-quarter of the sweet orange's markers matched pummelo, and three-quarters matched mandarin. The researchers also knew that the sweet orange's chloroplast — the organelle that performs photosynthesis — originated in pummelo, indicating that this fruit was the maternal parent. Plants inherit DNA only from their 'fathers', whereas they inherit DNA, chloroplasts and mitochondria from their 'mothers'. Ruan and his co-workers therefore inferred that the original breeders first crossed a female pummelo with a male mandarin, and then crossed the resulting hybrid with a male mandarin, resulting in the asexual sweet orange.

"The findings provide new tools and approaches for future plant breeding using genetic modification or engineering for high-yield vitamin C production."

The research team also mined the sequence data to uncover the genetic underpinnings of one of orange's most important traits: production of vitamin C, a powerful antioxidant essential for connective tissue building and wound-healing. They searched for genes similar to *GalUR*, which produces a key enzyme in the vitamin C production pathway and found 18 copies. Other vitamin-C-rich crops, such as papaya and apple, contain between 13 and 17 copies only. From studies of when and where genes are expressed during development, the team observed that the *GalUR* genes are highly expressed in orange fruits. "*GalUR* may be the most important contributor to vitamin C accumulation in orange fruit," says Ruan.

Availability of the sweet orange genome will facilitate the study of many other important traits, including disease resistance, flavor, sugar content and fruit color, the team notes. "The findings provide new tools and approaches for future plant breeding using genetic modification or engineering for high-yield vitamin C production," says Ruan.

© iStockphoto/Thinkstock

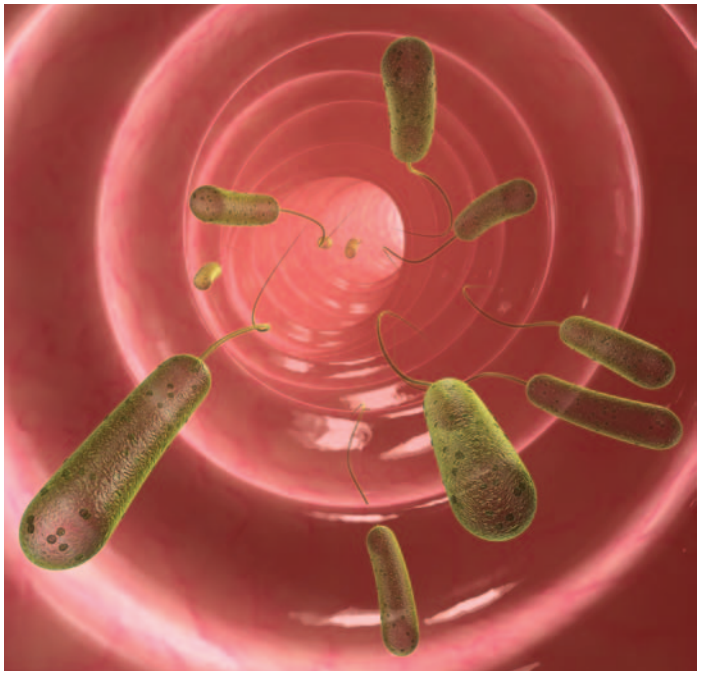
The sweet orange is the product of a cross between pummelo and mandarin, and then a re-cross of the resulting hybrid with mandarin.

1. Xu, Q., Chen, L.-L., Ruan, X., Chen, D., Zhu, A. *et al.* The draft genome of sweet orange (*Citrus sinensis*). *Nature Genetics* **45,** 59–66 (2013).

Gene sequencing:

Sensitive software detects diversity

A new computer program readily identifies rare mutations harbored within diverse populations of cancer cells and microorganisms



LoFreq detects single-nucleotide variants of *E. coli* bacteria with greater sensitivity than competing programs.

A tumor is not a uniform mass of identical cells. However, teasing apart genetic heterogeneity within a biopsied tumor can be difficult. Researchers often fail to tell the difference between a rare variant in a DNA dataset or a small error because of imprecision in existing high-throughput sequencing technologies.

Now, a new computer program developed at A*STAR could help. Thanks to open-source software called LoFreq — so-called because it can detect mutations at extremely low frequencies — researchers can reliably pick out rare subpopulations of cells from heterogeneous populations of cancer cells, microorganisms and other biological samples[1].

"This is key to a wide range of scientific investigations, from understanding how pathogens evolve and escape the immune system, to uncovering the processes through which cancers grow and spread," says Niranjan Nagarajan, a senior scientist at the A*STAR Genome Institute of Singapore, who helped to develop the program.

Nagarajan and his co-workers wrote the algorithm that forms the foundations of LoFreq. Their aim was for the software not only to adapt to sequencing biases, but also to detect single DNA differences with frequencies below the specific level of noise introduced by sequencing errors. The researchers first tested the program against existing computer programs for analyzing large DNA datasets using simulated sequences from dengue virus. They then validated the approach using real genomic libraries from samples of *Escherichia coli* bacteria, human gastric cancer biopsies, and dengue viruses collected before and after antiviral drug treatment — an exposure that often leads to the evolution of drug resistance in some subpopulations of virus.

"Previous attempts to describe this evolution have had to wait for the selection process to near completion," Nagarajan says. "In this new work, we have greatly increased the sensitivity of detecting these mutations and thus can catch their evolution in 'real time', observing how this process develops."

LoFreq proved itself to have near-perfect specificity for rare variants, with significantly improved sensitivity compared to existing methods, regardless of the high-throughput sequencing platform. The method also pinpointed a handful of low-frequency polymorphisms in whole-genome readouts from individual gastric cancer patients, and flagged mutational hotspots in dengue samples from a clinical drug trial.

"Almost anybody who is interested in studying evolutionary processes at a higher resolution, ranging from researchers who study how viruses and bacteria evolve and become more pathogenic, to cancer scientists looking at the evolution of a tumor," could benefit from LoFreq, Nagarajan says. The software is freely available at http://sourceforge.net/projects/lofreq.

1. Wilm, A., Aw, P. P. K., Bertrand, D., Yeo, G. H. T., Ong, S. H. *et al.* LoFreq: a sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. *Nucleic Acids Research* **40**, 11189–11201 (2012).

Cancer biology:

The importance of thorough editing

Insights into an abnormally edited RNA molecule may yield new weapons against a hard-to-kill cancer

Diagnosis of the brain cancer glioblastoma multiforme (GBM) is particularly bad news for patients due to limited available medical options and poor outcomes. Even treatments that can eliminate other malignancies, such as chemotherapy and surgery, buy only limited time for GBM patients.

"The disease usually recurs due to extensive invasion of tumor cells into the normal brain tissue and therapeutic resistance," explains Shu Wang of the A*STAR Institute of Bioengineering and Nanotechnology in Singapore. "This cancer's highly lethal nature results in a median survival time of around a year for patients with advanced GBM." As scientists hunt for effective treatments, research from Wang and colleagues could offer a useful strategy for fighting this deadly disease[1].

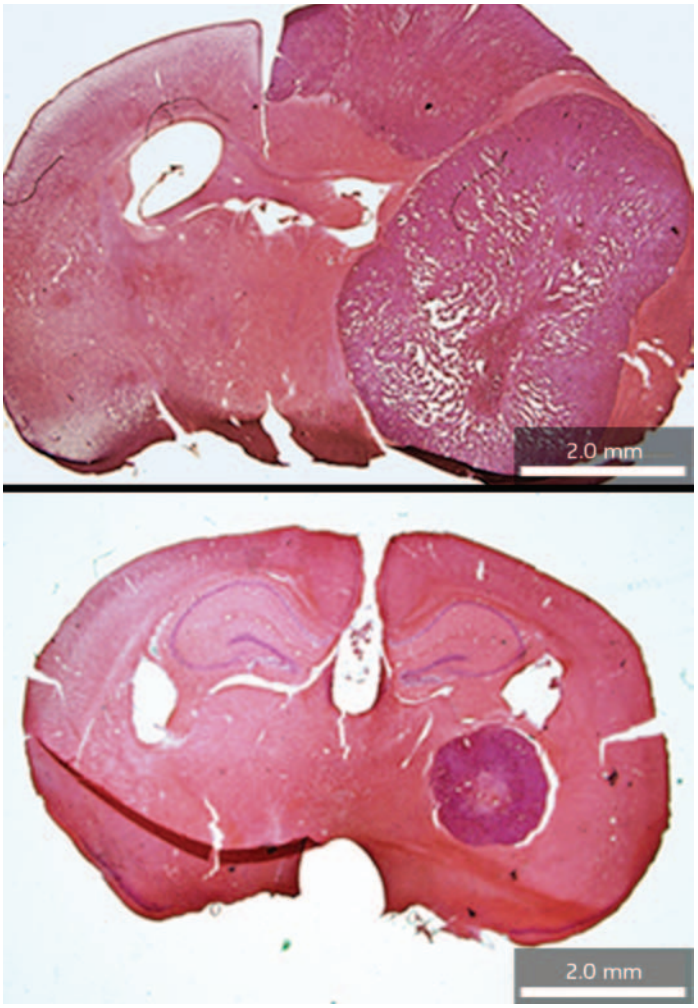
"Introduction of a single base difference by adenosine-to-inosine 'mutation' in miR-376a* affects the selection of its target genes and redirects its function from inhibiting to promoting glioma cell invasion."

Rather than producing protein-coding messenger RNAs (mRNAs), some genes encode short microRNAs that regulate other genes by binding target sequences on the mRNAs that they produce. Some microRNAs undergo further enzymatic 'editing', in which certain adenine nucleotides are changed to inosine, and this 'A-to-I' modification can markedly alter a microRNA's target preference. Since previous studies have indicated a link between abnormal mRNA editing and brain cancer, Wang and co-workers investigated a group of mRNA-encoding genes known as the miR-376 cluster.

One product of this cluster, miR-376a*, typically undergoes A-to-I editing in healthy brain tissue. By analyzing primary tumor tissue as well as glioma-derived cell lines, the researchers determined that many GBM cells instead tend to carry the unedited form of miR-376a*. Importantly, a comparative analysis of patient tumors indicated that excessive levels of unedited miR-376a* are associated with considerably greater tumor volume (see image).

Follow-up studies by the team supported this connection. Relatively noninvasive glioma-derived cells became far more aggressive in culture when forced to express RNAs that mimic unedited miR-376a*; when transplanted into mice, these cells formed large, irregular tumors that soon killed their hosts. By comparison, cells expressing edited miR-376a* were comparatively quiescent, with limited tumor growth. Wang and co-workers also determined that unedited miR-376a* alters expression of a distinct subset of genes relative to its edited counterpart, some of which appear to contribute directly to its harmful effects.

"Introduction of a single base difference by adenosine-to-inosine 'mutation' in miR-376a* affects the selection of its target genes and redirects its function from inhibiting to promoting glioma cell invasion," says Wang. He adds that these results also highlight forced expression of edited miR-376a* as a possible GBM treatment strategy. He and his team are now exploring different delivery mechanisms for such a therapeutic intervention.



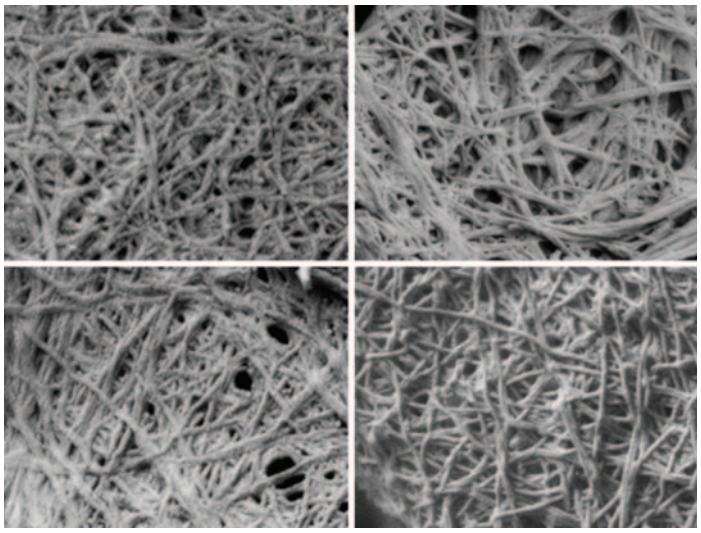
Unedited miR-376a* promotes aggressive tumor growth (dark red) in a mouse brain tumor model (top), whereas tumor growth (dark red) modulated by the edited form of the same microRNA, differing by a single base, is limited and noninvasive (bottom).

1. Choudhury, Y., Tay, F. C., Lam, D. H., Sandanaraj, E., Tang, C., Ang, B.-T. & Wang, S. Attenuated adenosine-to-inosine editing of microRNA-376a* promotes invasiveness of glioblastoma cells. *The Journal of Clinical Investigation* **122**, 4059–4076 (2012).

Amyloid formation:

Designer proteins light the way forward

Insight into the mechanism of protein aggregation provides a model system that could lead to treatments for several associated diseases



Electron microscopy images reveal that amyloids formed by the designed peptide LD6 (top left) have a similar structure to naturally occurring amyloids in diabetes type 2 (top right), Alzheimer's disease (bottom left) and thyroid cancer (bottom right).

The assembly of abnormal proteins into aggregates called amyloids is a characteristic of several diseases, including Alzheimer's, Parkinson's, diabetes type 2 and thyroid cancer. Exactly how amyloids form is unknown, but work performed at A*STAR has shown that the mechanism can be accurately recreated with specifically designed protein fragments[1].

The research team included Charlotte Hauser and Anupama Lakshmanan at the A*STAR Institute of Bioengineering and Nanotechnology (IBN), Daniel Cheong at the A*STAR Institute of High Performance Computing and international collaborators.

The study was based on previous work in which the IBN team designed peptides, or short protein fragments, that self-assemble in water to produce amyloids. In their recent study, the researchers used experimental techniques and computer modeling to compare the structural properties of aggregates formed by two of these peptides — LIVAGD (LD_6) and IVD (ID_3) — with those of peptide fragments from naturally occurring amyloid proteins (see image).

"We found that our rationally designed peptides exhibit a similar self-assembly mechanism to several amyloid-forming peptide sequences implicated in disease," says Hauser. "This provides a fresh perspective on the process of amyloid formation."

The team also aimed to clarify the role of the amino acid phenylalanine, which is often found within regions of proteins that are important for amyloid formation. Consequently, scientists thought that phenylalanine played a crucial role in the mechanism. The team's findings, however, challenge this idea: the peptides that the researchers designed contained no phenylalanine or similar amino acids, yet still formed amyloids.

Analysis of another naturally occurring peptide, KLVFFAE (KE_7), reinforced this finding. KE_7 is a fragment of amyloid-β, a protein involved in Alzheimer's disease. Scientists believed that two adjacent phenylalanines in its structure were crucial for amyloid formation, but Hauser and co-workers discovered that KE_7 forms aggregates in a different way to the amyloid-forming peptides.

"This suggests that phenylalanine is not as essential for amyloid formation as previously postulated," explains Hauser. "It shows that there might be other core sequences that are more important."

Hauser notes that the new study forms the basis for tackling amyloid fibril formation in disease. "The fundamental mechanism of amyloid formation is believed to be common across all amyloid-related diseases, so drugs could be developed to effectively treat multiple diseases," she explains. Insufficient knowledge of protein self-assembly has hampered the search for a way to prevent or cure amyloid formation. "Our findings put forth a simplified model to study this hallmark of several degenerative disorders and design therapeutics for its control and prevention."

1. Lakshmanan, A., Cheong, D. W., Accardo, A., Fabrizio, E. D., Riekel, C. & Hauser, C. A. E. Aliphatic peptides show similar self-assembly to amyloid core sequences, challenging the importance of aromatic interactions in amyloidosis. *Proceedings of the National Academy of Sciences USA* **110,** 519–524 (2013).

Wound healing:

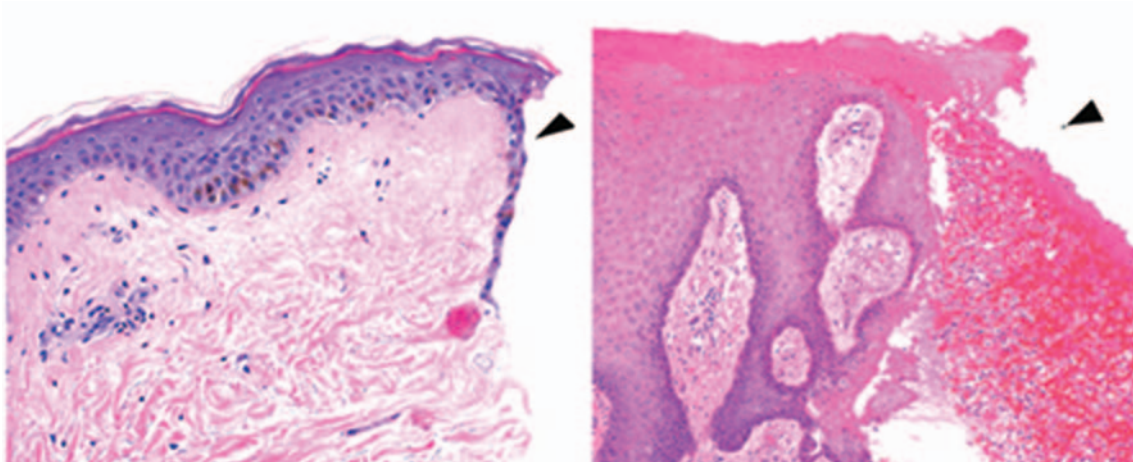
‘See-saw’ switch sends cells on the march

An unusual switching mechanism that determines a single gene’s RNA output directly controls wound healing

Many genes are transcribed into messenger RNA (mRNA) molecules that provide instructions for protein synthesis. Other genes encode regulatory RNAs known as ‘microRNAs’, which can block protein translation by binding to specific sequences on target mRNAs. Now, researchers led by Prabha Sampath of the A*STAR Institute of Medical Biology have identified a gene that uses an unusual ‘see-saw’ mechanism to regulate wound healing by switching between production of mRNA and microRNA[1].

Sampath and colleagues studied healing with cultured skin cells called keratinocytes, searching for microRNAs that affect the migration of these cells to close newly inflicted wounds. The team focused on miR-198, a microRNA normally produced at high levels but suppressed shortly after injury. Interestingly, miR-198 is derived from the same gene that encodes the FSTL1 protein. The researchers subsequently determined that FSTL1 protein levels rise at the same time as miR-198 levels fall in damaged keratinocyte cultures.

Closer analysis revealed that the microRNA is actually a direct by-product of the mRNA encoding FSTL1, indicating that cells switch between production of FSTL1 and the microRNA, which is ‘edited’ from the mRNA. By experimentally reducing keratinocyte production of FSTL1 without affecting miR-198, the researchers inhibited wound healing and identified several healing-associated genes. Forced miR-198 expression had a similar effect on keratinocytes, and inhibited this same set of genes.



Tissue sections from a normally healing wound (left) and a chronic non-healing diabetic ulcer (right). Keratinocytes (blue) fail to migrate to the wound edge (arrowhead) in the diabetic tissue because of abnormalities in the FSTL1/miR-198 switching mechanism.

“We plan to use ‘anti-miR-198’ molecules to eliminate anti-migratory miR-198.”

To determine whether these experimental results hold true for human healing, Sampath and co-workers examined patients with chronic non-healing ulcers (see image), a common consequence of diabetes. “Foot ulcers occur in about 15% of diabetic patients, and in 84% of cases they lead to amputation,” says Sampath. The team consistently identified high levels of miR-198 and low levels of FSTL1 in cells near the wound edge, indicating an apparently malfunctioning ‘switch’.

In many cases, these diabetic ulcers also exhibit defects in a cell signaling pathway triggered by the transforming growth factor-β (TGF-β) protein. Sampath and co-workers revealed a direct link between TGF-β activity and FSTL1 production. They showed that TGF-β facilitates expression of the FSTL1 protein and blocks the expression of a protein that promotes miR-198 processing; without TGF-β signaling, miR-198 production prevails and wound healing is blocked.

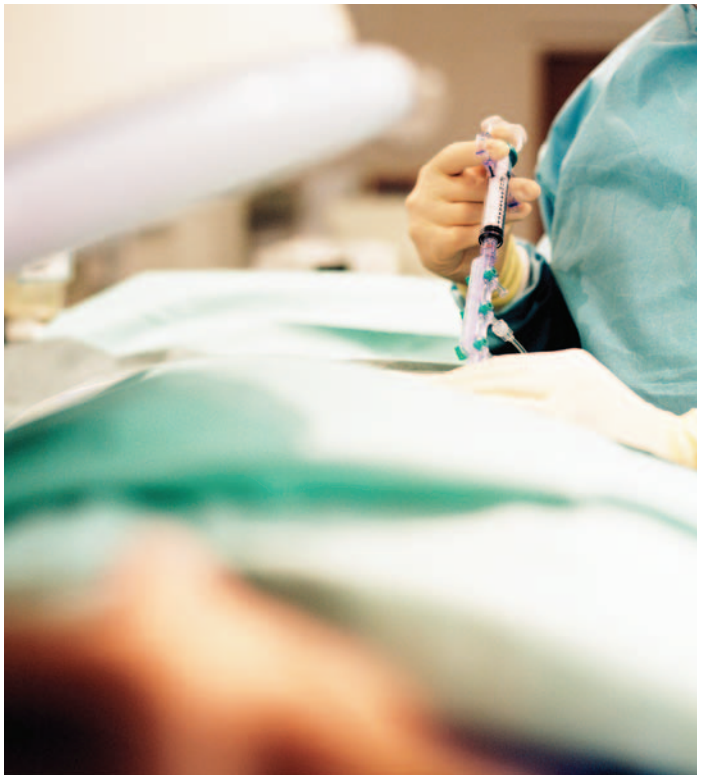
Previous attempts to treat chronic diabetic ulcers with TGF-β have failed. Having uncovered this TGF-β-modulated FSTL1 switch, however, Sampath now sees a promising way forward for this and other conditions with impaired healing. “We plan to use ‘anti-miR-198’ molecules to eliminate anti-migratory miR-198,” she says. “Coupled with pro-migratory FSTL1 peptides, this may result in effective wound healing.”

1. Sundaram, G. M., Common, J. E. A., Gopal, F. E., Srikanta, S., Lakshman, K. *et al.* ‘See-saw’ expression of microRNA-198 and FSTL1 from a single transcript in wound healing. *Nature* **495**, 103–106 (2013).

Cancer biology:

A biomarker for myeloma

A protein called FAIM could help doctors to parse which cancer patients will respond to multiple myeloma therapy



Discovery of a biomarker for multiple myeloma may improve treatments for newly diagnosed and relapsed patients.

A number of drugs exist that can extend the lifespan of people with multiple myeloma (MM), but none of these medicines are curative. Thus, medical researchers continue to search for targets for new drug therapies as well as new ways to predict which particular patients will be most responsive to existing treatment options.

A protein called FAIM — newly implicated in MM by a team of A*STAR scientists — could help to achieve both of these goals[1]. "As FAIM is important to keep MM cells alive, it could be a useful and novel target for the development of novel therapeutic interventions," says Lam Kong Peng from the A*STAR Bioprocessing Technology Institute in Singapore, who led the work. "Concurrently, the significant association of elevated FAIM expression with poorer survival outcomes of MM patients suggests that FAIM can also be used as a poor-risk marker for selection of patients for more targeted therapy."

FAIM, short for 'Fas apoptosis inhibitory molecule', is a protein activated by IRF4, a key driver of the blood cell differentiation that gives rise to MM. Although scientists had previously implicated FAIM expression in the survival of pancreatic tumor cells, the protein's role in other types of cancer was unknown.

Lam's group charted the role of FAIM in MM. Through a series of cell-based studies, they demonstrated that the protein is overexpressed by known myeloma growth factors such as IGF1. FAIM then acts through a signaling axis involving Akt, an important signaling protein, and IRF4. By genetically disrupting FAIM or its signaling partners, the researchers could induce MM cell death in the laboratory. They now hope to achieve the same effect in the body by making a safe and effective drug. "Our next step will be to design and screen for specific inhibitors to inhibit the expression or function of FAIM protein," Lam says.

In addition, the researchers studied clinical specimens, showing that people with symptomatic MM had higher FAIM levels than either healthy individuals or patients with premalignant conditions. They also analyzed biopsies taken from two clinical cohorts: one included people newly diagnosed with MM and treated quickly and aggressively with both high-dose chemotherapy and stem cell transplantation; the other included a set of relapsed patients and those treated in clinical trials with a proteasome inhibitor drug called bortezomib. In both cases, Lam and co-workers found that FAIM expression correlated with worse overall survival rates, demonstrating the prognostic value of the biomarker.

1. Huo, J., Xu, S., Lin, B., Chng, W.-J. & Lam, K.-P. Fas apoptosis inhibitory molecule is upregulated by IGF-1 signaling and modulates Akt activation and IRF4 expression in multiple myeloma. *Leukemia* **27,** 1165–1171 (2013).

Genomics:

A rare view of gene regulation

A new technique allows biologists to profile patterns of gene regulation in clinical specimens and other rare cell populations

Mapping all of the chemical, or epigenetic, changes to chromosomes that affect which genes are turned on or off — and thus determine the fate of genomically identical cells in the body — usually requires a large amount of starting cellular material. This technical limitation has impeded the analysis of gene regulation in many rare cell types and in small clinical biopsy samples.

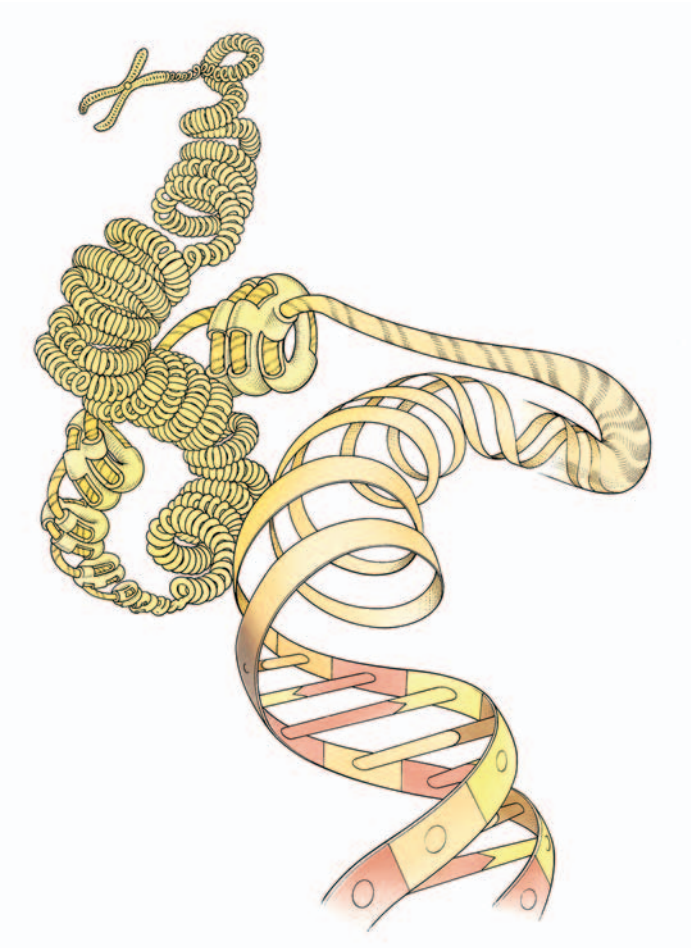
"We developed a technique for epigenetic analysis of very small numbers of cells, and used it to identify gene regulatory elements in the genome of germ cells in the mouse embryo."

Now, a team of biologists led by A*STAR scientists has developed a protocol for characterizing these changes that requires up to 100 times fewer cells than previously needed[1]. As a proof of principle, the researchers used the approach to chart which genes are activated or repressed in mouse reproductive cells that eventually give rise to eggs or sperm.

"We developed a technique for epigenetic analysis of very small numbers of cells, and used it to identify gene regulatory elements in the genome of germ cells in the mouse embryo," says one of the team leaders, Shyam Prabhakar from A*STAR's Genome Institute of Singapore (GIS).

The team, which is co-led by the GIS's Huck Hui Ng, started with a standard technique for epigenetic analysis known as ChIP-seq. This method combines a way of investigating the interactions between proteins and DNA called 'chromatin immunoprecipitation', or ChIP, with high-throughput DNA sequencing. ChIP-seq can reveal modifications to chromosome structures that affect gene expression (see image). Typically, ChIP-seq requires millions of cells to work properly. Prabhakar and Ng's team, however, adapted the technique so that it could work with just 10,000 cells. This involved miniaturizing various aspects of the protocol so that it worked reliably with smaller volumes.

Using the small-scale ChIP-seq method on germ cells taken from embryonic mice, the researchers revealed many previously unknown epigenetic features needed for proper maintenance and development of these early precursors of eggs and sperm. They include changes to promoters and enhancer elements that control how germ-cell specific genes are activated, as well as repressive marks on some genes that are needed only later in development. Interestingly, the analysis also showed that genetic elements called retrotransposons and genes involved in activating immune responses are both silenced, preserving genomic integrity and preventing cell death in these important reproductive cells.



© Dorling Kindersley RF/Thinkstock

Epigenetic changes to chromosome structure influence whether or not certain genes are active.

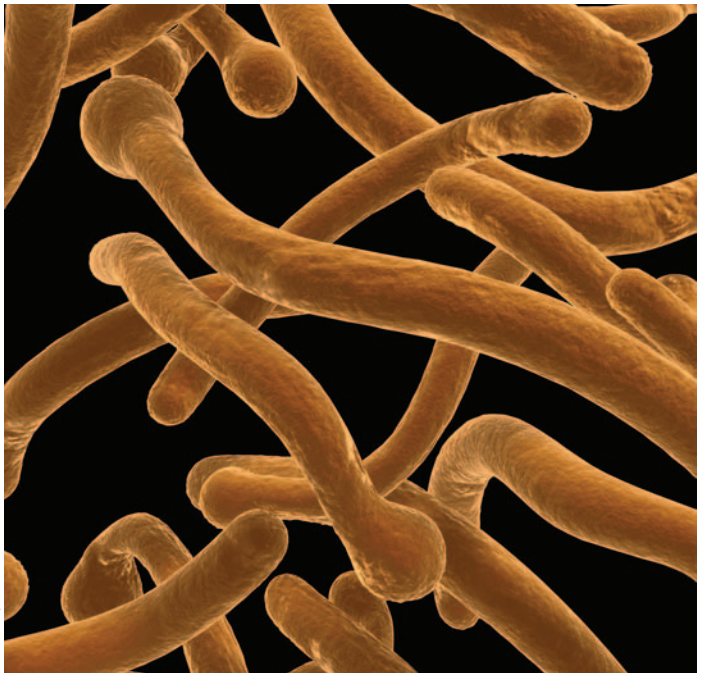
Next, Prabhakar, Ng and co-workers plan to apply the method to study rare cell populations in humans, including biopsies taken from cancer patients. "This technology now enables us to study other cell types of limited quantity," Ng says.

1. Ng, J.-H., Kumar, V., Muratani, M., Kraus, P., Yeo, J.-C. *et al.* In vivo epigenomic profiling of germ cells reveals germ cell molecular signatures. *Developmental Cell* **24,** 324–333 (2013).

Fungal biology:

Finding yeast's better half

The discovery of strains of Candida albicans *that contain only one set of chromosomes will facilitate identification of therapeutic targets*



Through a process known as concerted chromosome loss, *Candida albicans* cells (pictured) containing two sets of chromosomes can form mating-competent and viable cells that contain a single set of chromosomes.

Scientists long believed that the fungal pathogen *Candida albicans* was incapable of producing haploid cells — which contain only one copy of each chromosome, analagous to eggs and sperm — for mating. Mixing of genes in sexual reproduction helps generate the diversity that is the raw material for evolution, and *C. albicans*' inability to reproduce sexually appeared to give it a disadvantage. An international research team, including Yue Wang at the A*STAR Institute of Molecular and Cell Biology in Singapore, has now found viable haploid strains of *C. albicans*. The finding illuminates *C. albicans*' evolution and pathogenicity[1].

The team's discovery of the haploids was serendipitous. Team members in Ching-Hua Su's laboratory at the Taipei Medical University, Taiwan, studying *C. albicans* strains growing in media containing an antifungal drug, noticed a strain with half the usual amount of genetic material. Further analysis revealed that the strain lacked one of each of its eight chromosomes, indicating that it was in fact haploid.

Work in Wang's laboratory at A*STAR and in Judith Berman's laboratory at the University of Minnesota in the United States, revealed many strains of both *in vitro* and *in vivo* haploids. Not only could the haploids form all of the same developmental stages as their diploid counterparts, they formed cells that could mate.

The haploid cells were less fit than diploid cells: they grew slowly and were avirulent in mice. They were also ephemeral, as after a short time in culture they duplicated their own genetic material to become diploid. These so-called auto-diploids were also less virulent than wild-type cells. When two haploids mated, however, they produced diploid cells with a mixed genetic background and increased virulence and growth.

"Generation of haploid cells through random loss of chromosomes followed by mating between haploid cells of opposite sex generates genetic variation, which is important for adaptation and evolution of this fungus," explains Wang.

The haploids will serve as an invaluable research tool in genetic and drug-discovery studies. Studying gene function in a diploid organism requires deleting both copies of a gene, which is technically very difficult, Wang notes. Researchers will now be able to delete genes in a single step.

Wang's team painstakingly screened thousands of isolates to identify several stable haploid strains. From these, they constructed a set of strains that will make genetic manipulation rapid and effective. "Preliminary screens of a small number of mutants have already identified several new genes important for virulence, promising more discoveries in the near future," says Wang.

1. Hickman, M. A., Zeng, G., Forche, A., Hirakawa, M. P., Abbey, D. *et al.* The 'obligate diploid' *Candida albicans* forms mating-competent haploids. *Nature* **494,** 55–59 (2013).

Cancer biology:

Charting a tumor's genomic roots

Whole-genome sequencing gives researchers a deeper understanding of factors contributing to the onset and progression of gastric cancer

By combining the skills and knowledge of a large number of cancer and genomics experts, the Singapore Gastric Cancer Consortium has successfully generated genome sequences for tumor samples from two gastric cancer patients[1]. The effort was coordinated by Niranjan Nagarajan, Yijun Ruan and Patrick Tan from the A*STAR Genome Institute of Singapore.

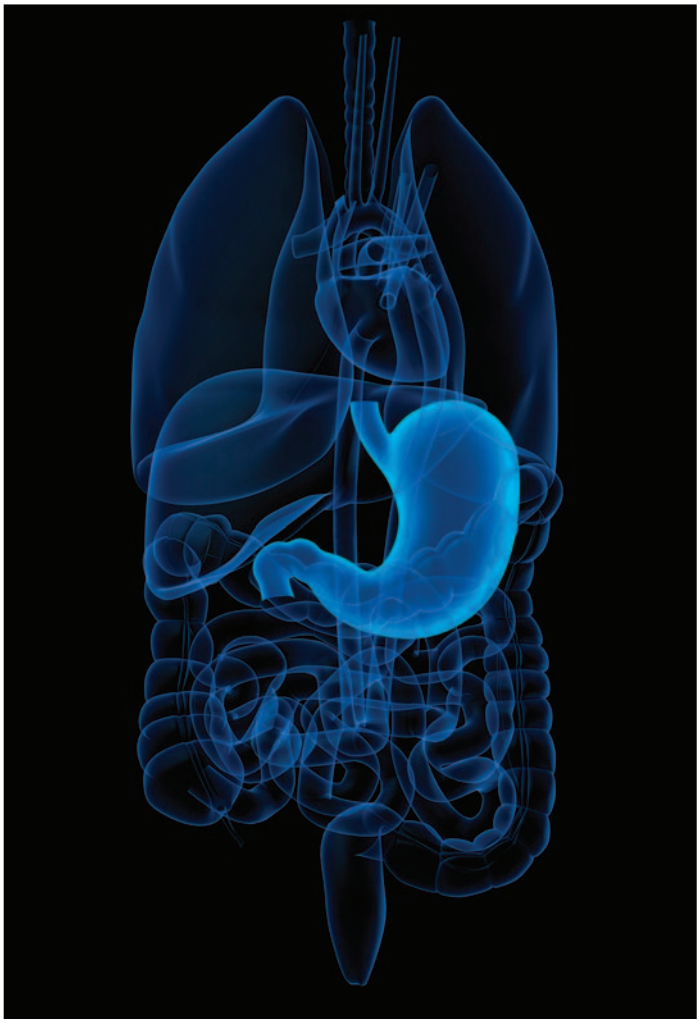
Modern DNA sequencing instruments can deliver a high-quality human genome sequence within a week for less than US$10,000. This technology offers a promising tool for revealing the specific genomic disruptions that underlie poorly understood cancers such as gastric cancer, which remains extremely challenging to diagnose and treat. Unfortunately, the repetitive sequences and large-scale chromosomal rearrangements observed in cancer genomes make it difficult to accurately assemble relatively short DNA sequence 'reads' into a complete sequence.

"We were able to recover the pathogen genome associated with this cancer from out of massive amounts of sequencing data."

Nagarajan credits their success to a combination of sophisticated analytical tools. "We had deep-sequencing data from two complimentary approaches as well as a new assembly approach available to us," he says, "and these provided an ideal test-bed for carrying out this study."

The researchers began by generating genome segments dubbed 'contigs' based on overlap between short sequencing reads. Then they used a long-range mapping technique called DNA paired-end tag analysis to accurately assemble these into even larger contigs that span a considerable portion of the genome. The resulting coverage allowed them to conduct a detailed census of small-scale sequence alterations as well as larger-scale chromosomal rearrangements. Their work revealed more than five times as many changes than could be detected with more limited conventional sequencing strategies.

Gastric cancer is unusual in that it can be triggered by infection with the bacterium *Helicobacter pylori*. Nagarajan and co-workers' approach allowed them to identify differences between *H. pylori*- and non-*H. pylori*-associated cancer cases. "We were able to recover the pathogen genome associated with this cancer from out of massive amounts of sequencing data," says Nagarajan. "We also found a mutational signature that can be linked to infection and is likely to have had a disproportionate impact in tumorigenesis." The researchers were further able to define mutational characteristics that might contribute to specific categories of cancer-related genomic damage, such as unnatural expansion of repetitive sequences or sizeable insertions and deletions.



© iStockphoto/Thinkstock

Gastric cancer, caused by infection with the bacterium *Helicobacter pylori*, is second only to lung cancer as the leading cause of death.

This work demonstrates the valuable clinical information that tumor genome sequencing can provide. Nagarajan and co-workers are now developing improved computational tools that could make better sense of the avalanche of genomic data that can be generated from a single tumor.

1. Nagarajan, N., Bertrand, D., Hillmer, A. M., Zang, Z. J., Yao, F. *et al.* Whole-genome reconstruction and mutational signatures in gastric cancer. *Genome Biology* **13**, R115 (2012).

Cancer biology:

Targeting tumors with 'stapled' peptides

Designer peptides containing chemically stabilized helices emerge as a potent way to activate anti-tumor proteins inside cells

Cancer biologists consider many cancer-related proteins as 'undruggable' because they resist treatments from traditional drugs. David Lane and co-workers at the A*STAR p53 Laboratory, Bioinformatics Institute and Experimental Therapeutics Centre are therefore developing a new generation of therapeutics that can reach such proteins and activate their innate cancer-fighting abilities. The team's latest work reveals that 'stapled' peptides — chemically stabilized helices of amino acids — can activate the tumor-suppressing protein p53 inside cells by disrupting interactions with Mdm2, its regulator protein[1].

"Peptides isolated from phage display experiments are highly specific and potent against the individual protein they are selected against."

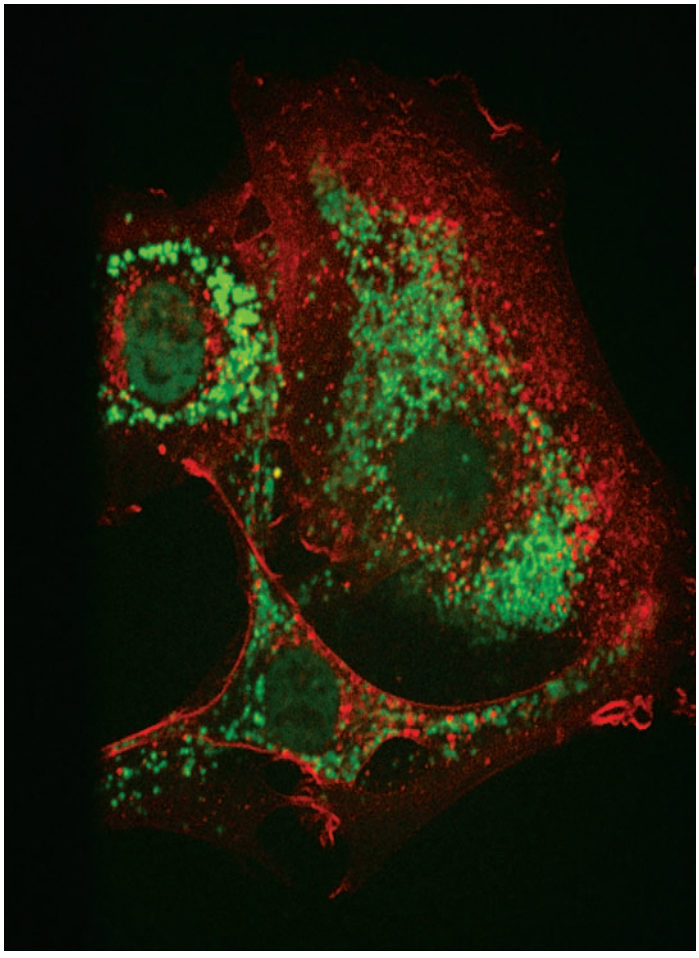
Chemical biologists have realized that interfering with the crucial p53:Mdm2 interaction is a viable cancer treatment strategy; however, it is no easy task to find drug compounds that permeate cells and survive to reach their targets. Peptide α-helices that adhere tightly to Mdm2's p53 binding domain make attractive drug candidates because of their low toxicity and site-specific potency. Unfortunately, native peptide helices tend to unravel and decompose inside cells.

To remedy this, Lane and co-workers explored the concept of holding peptide α-helices in place by connecting parts of them with a hydrocarbon chain. First, they studied potential peptides using phage display, a technology that enables precise expression and rapid screening of peptide chains on the surfaces of virus-like particles.

By combining computer modeling with *in vitro* testing, the team generated stapled peptides known as 'sMTIDEs' with extraordinary affinity for binding p53. "Peptides isolated from phage display experiments are highly specific and potent against the individual protein they are selected against," says Christopher Brown, the study's lead author.

The researchers observed some surprising results when they compared the biological activity of their sMTIDE peptides to SAH-8, a stapled peptide derived from wild-type p53, and Nutlin, a small molecule that affects p53 production. The sMTIDEs could activate the p53 genes inside cell reporter assays quickly and at low concentrations. Their stapled peptide reached unprecedented levels of p53 activation and proved to have few toxic side-effects (see image). Furthermore, by making a single amino acid substitution on the sMTIDE chain, the researchers could activate or extinguish this activity without affecting *in vitro* binding.

Brown believes that these findings could help resolve a controversy brewing among scientists over the intracellular activity of stapled peptides.



A microscopy image of a stapled peptide (fluorescently labeled green) being incorporated into a live murine T22 cell array. The peptide will activate the p53 tumor suppressor protein. Cell membrane is labeled in red.

Peptides isolated from wild-type proteins, he notes, may show off-target binding because of evolved interactions with other proteins. The sMTIDEs, on the other hand, "prove the claims that peptides can enter cells. This has re-invigorated interest in the field and gives peptide researchers a set of validated and robust reagents," he says.

1. Brown, C. J., Quah, S. T., Jong, J., Goh, A. M., Chiam, P. C. *et. al.* Stapled peptides with improved potency and specificity that activate p53. *ACS Chemical Biology* **8**, 506–512 (2013).

Obesity:

How inflammation influences appetite

Insensitivity to a hormone that balances food intake with energy expenditure explains the contradictory effects of different inflammatory conditions on appetite

Sustained low-grade inflammation and an above-average appetite are commonly found in obese individuals. Therefore, it seems counterintuitive that the acute inflammation associated with many illnesses normally suppresses appetite. A team led by Weiping Han of the Singapore Bioimaging Consortium at A*STAR has used mice to elucidate the molecular mechanisms that explain the different effects of chronic and acute inflammation on appetite[1]. The study also helps to explain why obesity compromises appetite-suppression mechanisms.

"We believe that the lower than normal rates of *Pomc* transcription caused by the combination of both of these effects accounts — at least, in part — for why obese individuals have a larger-than-normal appetite despite chronic inflammation."

The team's insights center around the different effects of acute inflammation and chronic obesity-related inflammation on the transcription of a gene expressed in neurons of the hypothalamus of the brain. A neurohormone called leptin controls transcription of the pro-opiomelanocortin (*Pomc*) gene, which suppresses appetite. Leptin is normally produced by fat cells at levels that ensure food intake matches energy expenditure. However, obese individuals often become insensitive to leptin, leading to a larger-than-normal appetite.

Previous studies by other groups and Han showed that in well-fed animals of normal body weight, leptin inhibited appetite by causing a protein called STAT3 to migrate to the nucleus of POMC neurons. The nuclear STAT3 suppressed appetite by sustaining normal levels of the *Pomc* gene's transcription.

In their recent study, again in well-fed animals of normal body weight, Han and co-workers found that acute inflammation — such as that caused by a viral infection — suppressed this translocation of STAT3, but more than compensated for the slight reduction in *Pomc* transcription by causing a protein called RELA to migrate to the nucleus. The nuclear RELA elevated the rate of *Pomc* transcription to above the normal level. This is consistent with the loss of appetite associated with most illnesses.

The researchers also found that in obese mice, methylation — a DNA modification that usually silences gene expression — prevented the nuclear RELA from binding to the *Pomc* promoter. RELA also blocked STAT3 from entering the nucleus.

"We believe that the lower than normal rates of *Pomc* transcription caused by the combination of both of these effects accounts — at least, in part — for why obese individuals have a larger-than-normal appetite despite chronic inflammation," explains Han. He also notes that the role of RELA in sequestering STAT3 from *Pomc* promoter sequences provides a much-needed explanation of how chronic inflammation contributes to leptin resistance.

"We're hopeful that these insights into how different causes of inflammation interfere with leptin signaling might help to identify more effective and safer drugs to curb the appetite of obese individuals or better still, prevent leptin resistance," says Han.

© iStockphoto/Thinkstock

Managing weight gain could become easier thanks to new insights that explain how acute inflammation, such as that caused by influenza, suppresses appetite but chronic inflammation increases it.

1. Shi, X., Wang, X., Li, Q., Su, M., Chew, E. *et al.* Nuclear factor κB (NF-κB) suppresses food intake and energy expenditure in mice by directly activating the *Pomc* promoter. *Diabetologia* **56,** 925–936 (2013).

Cancer:

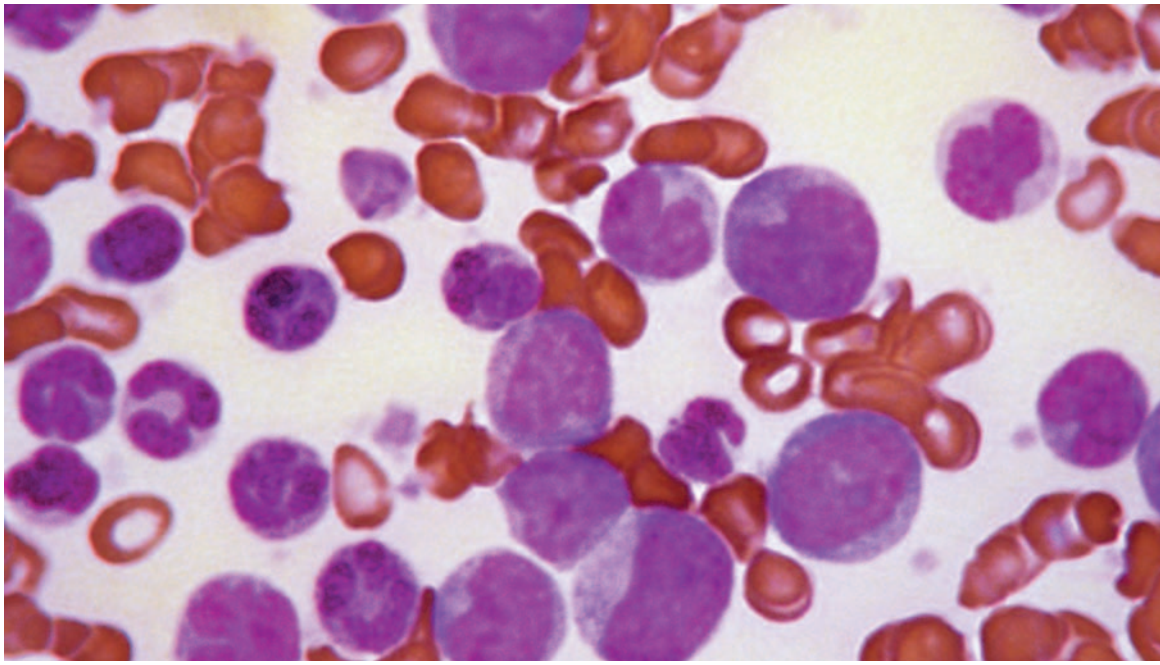
Leukemia 'blasted' through new drug target

By inhibiting an enzyme called MNK, researchers have found a way to halt the growth of blast crisis phase CML

Once chronic myeloid leukemia (CML) has progressed to its most advanced stage, it is a tough cancer to treat. Drugs that help control earlier stages of the disease no longer work, leaving patients who develop so-called 'blast crisis CML' with few effective treatment options. Encouragingly, a team of scientists in Singapore has discovered a promising way to kill the cells that are responsible for this type of aggressive leukemia. They demonstrated in mice that compounds targeted at a specific enzyme prevent blast crisis cells from behaving like cancer stem cells[1].

"The identification of a 'druggable' target in the terminal stage of CML is the first concrete step toward finding a life-extending treatment for this deadly disease," say Sharon Lim and Ong Sin Tiong, the authors of the study from the Duke–National University of Singapore (Duke–NUS) Graduate Medical School.

The finding was the result of a long-standing research collaboration between Duke–NUS and the A*STAR Experimental Therapeutics Centre. The research team started by characterizing blast crisis-stage leukemia stem cells (see image). These cells self-renew owing to elevated levels of the cancer-causing protein β-catenin. This property would make β-catenin a good drug target; however, this protein is also required for normal blood cell development. Lim, Ong and co-workers therefore searched for a protein that is involved in activating β-catenin, but in cancerous cells only.



A smear of blood cells showing chronic myeloid leukemia cells in the stage known as 'blast crisis' (magenta).

Image courtesy of CDC/Stacy Howard

The researchers found their target in an enzyme called MAP kinase interacting serine/threonine kinase, or MNK. In leukemia cells, they showed that MNK adds a chemical tag to another protein called eIF4E. This modification in turn prompts eIF4E to trigger β-catenin production. This 'MNK-eIF4E axis' proved to be the ideal drug target because the pathway is not involved in normal β-catenin expression in healthy cells.

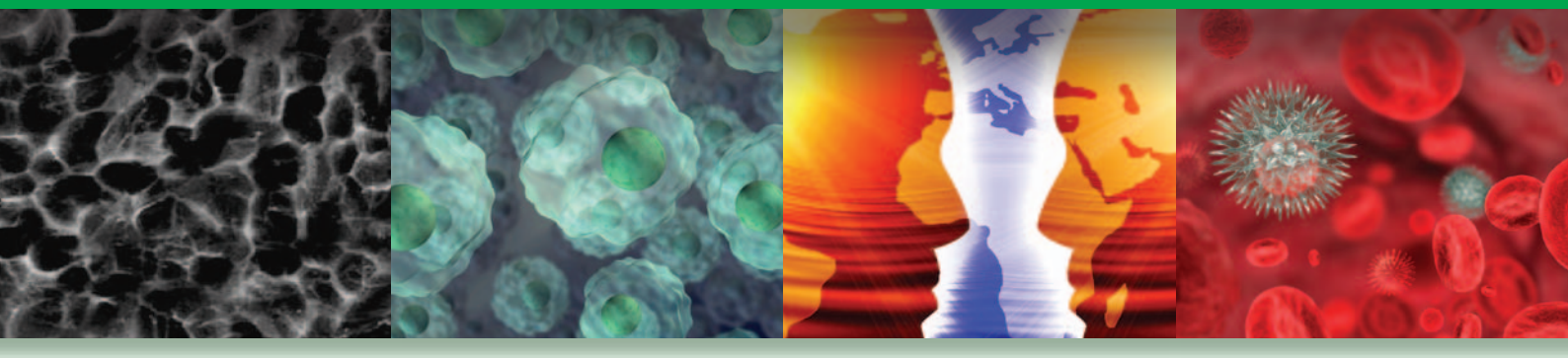
Lim, Ong and co-workers next treated leukemia and healthy cells with a small-molecule drug that blocks MNK function. They also gave the compound to mice implanted with human blast crisis cells. They found that drug treatment reduced the cancer burden by preventing the leukemia stem cells from self-renewing. Importantly, the treatment did not affect normal stem cell function.

"This [finding] will represent a critical step toward finding the best and safest MNK inhibitors to test in patients," say A*STAR scientists Jeffrey Hill and Kassoum Nacro. The collaborative team is currently working on inhibitors of MNK and other selected kinases to treat blast crisis CML.

Funding for the study was provided by the Duke–NUS Signature Research Program funded by A*STAR; the Ministry of Health (Singapore); and the National Research Foundation Singapore Clinician Scientist Award (CSA), awarded to Professor Ong by the National Medical Research Council (NMRC).

1. Lim, S., Saw, T.-Y., Zhang, M., Janes, M. R., Nacro, K. *et al.* Targeting of the MNK-eIF4E axis in blast crisis chronic myeloid leukemia inhibits leukemia stem cell function. *Proceedings of the National Academy of Sciences USA* **110**, E2298–E2307 (2013)..

Research Highlights



PHYSICAL & LIFE SCIENCE TECHNOLOGIES

Bioinformatics:

Analysis of sequence data falls into line

Formal mathematics underpins a new approach that standardizes the analysis of genome information

Researchers in Singapore have developed and tested mathematical tools, or algorithms, that are more accurate and robust than those currently used in analyzing high-throughput genetic sequencing data[1]. The algorithms can determine the location and activity of specific nucleic acid sequences in a broad range of high-throughput techniques that detect gene–protein interactions. The research group, led by Shyam Prabhakar of the A*STAR Genome Institute of Singapore, also showed they could use the algorithms to generate meaningful results from degraded tissue and tissue constructed from several different cell types.

Two new mathematically based algorithms for analyzing genomic data are not only more accurate and robust than their predecessors but also allow integration of data from different sources.

The rapid expansion in the application of high-throughput sequencing was possible because of a parallel growth in bioinformatics techniques to analyze the huge amount of data that the technique generated. High-throughput sequencing began as a technology for rapidly sequencing whole genomes; now it can detect gene activity, DNA methylation, microRNA binding and interactions between genes, transcription factors and regulatory elements. Each of these different sequencing techniques spawned its own specialized analytical methods, many of them based on heuristics — practical strategies that work but may require optimization.

Prabhakar and his colleagues recognized that almost all sequencing analyses are concerned with solving two major classes of problems, long studied in the fields of signal processing — signal detection and signal strength estimation. Standard mathematical techniques already existed for solving such problems. The researchers therefore adapted these techniques to sequencing analyses. They reasoned that the formal mathematical basis underlying the techniques would allow them to be optimized or tuned. They also realized that the same approaches could be used across a broad range of applications, thus enabling data integration.

The researchers developed two algorithms: DFilter for detecting and locating the binding of regulatory proteins to the genome; and EFilter for estimating gene activity through levels of messenger RNA, the genetic material used as a template for building proteins. Across several sequencing technologies, the researchers benchmarked both algorithms against existing analytical methods. They found that DFilter and EFilter outperformed the more specialized algorithms. The new algorithms also facilitated the analysis and comparison of multiple and diverse datasets.

Prabhakar and co-workers also used their new algorithms to analyze data from complex, heterogeneous tissue in the embryonic mouse forebrain. They searched for functioning transcription factors and gained useful insights, despite the fact that individual transcription factors could not be assigned to specific cell types.

"We intend to make DFilter and EFilter widely available," says Prabhakar, "perhaps via cloud genomics providers, if all goes according to plan."

1. Kumar, V., Muratani, M., Rayan, N. A., Kraus, P., Lufkin, T. *et al.* Uniform, optimal signal processing of mapped deep-sequencing data. *Nature Biotechnology* **31,** 615–622 (2013).

Cell membranes:

Synthetics save time and cut costs

An approach that directly inserts proteins into polymer-based cell membranes improves drug-screening platforms

Screening for critical drug targets known as G-protein-coupled receptors (GPCRs) is now possible without the need to extract these proteins from their native cells. Extraction requires the use of stabilizing lipids, which damage the structural integrity and functionality of GPCRs. The cell-free approach was developed by Madhavan Nallani from the A*STAR Institute of Materials Research and Engineering, Singapore, along with co-workers in Singapore, Germany and Austria[1].

Nallani and co-workers focused on GPCRs because they are ideal drug targets that pass through cell membranes. These proteins are involved in cell communication, cell adhesion and signal transduction, as well as major illnesses, including hypertension and diabetes.

"This cell-free expression provides an easy way to produce proteins directly from their DNA."

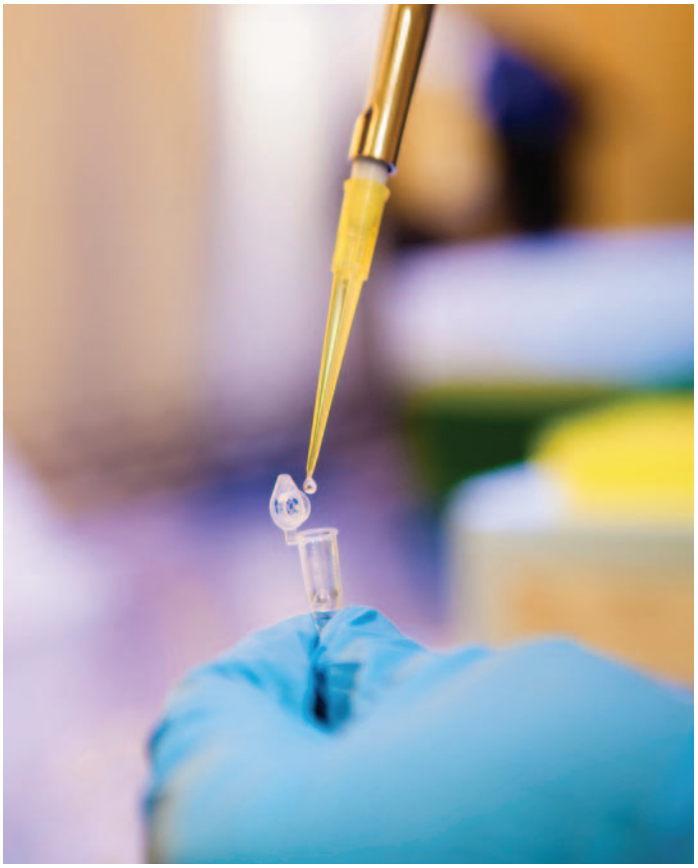
The team's approach produced GPCRs directly in artificial cell-like pockets called polymersomes. "This [approach] circumvents the tedious protein isolation process and the use of lipids or detergents as stabilizing agents," says Nallani.

As a proof of concept, the researchers synthesized the GPCR dopamine receptor D2 *in vitro* in the presence of polymersomes and the DNA that encodes for the protein. "This cell-free expression provides an easy way to produce proteins directly from their DNA," notes Nallani. The proteins generated by transcription and translation became incorporated into the polymer-based membranes via spontaneous self-assembly.

Characterization using fluorescently labeled antibodies that are receptor-specific showed that the self-assembled product displayed stronger fluorescence than the unmodified polymersomes, confirming the insertion of the receptor. This characterization also indicated that the membrane-incorporated portion of the receptor was properly oriented in the polymersomes.

Nallani's team further investigated the structure of the polymersome-inserted receptor using a fluorescently tagged dopamine. "Dopamine will bind only if the receptor is correctly folded and oriented," explains Nallani. After incubation, the GPCR-modified polymersomes showed higher fluorescence than the negative controls, indicating that the receptor maintained its structural integrity upon insertion into the polymersome.

The researchers assessed the potential use of the GPCR-modified polymersomes for drug screening and biosensing by synthesizing the receptor in the presence of polymersomes, which they patterned onto glass surfaces. Incubation of the resulting material with fluorescently labeled dopamine illuminated the patterns — proof of a successful GPCR addition. Exposure of these fluorescent patterns to increasing concentrations of unlabeled dopamine caused the fluorescence to decrease, showing that dopamine displaced the fluorescent ligand.



© iStockphoto/Thinkstock

Proteins for drug screening can now be isolated using a cell-free approach developed at A*STAR.

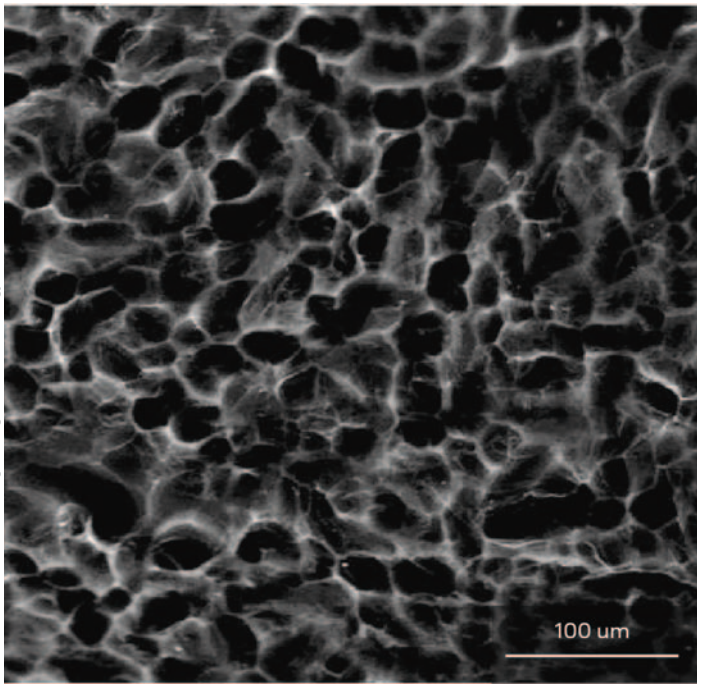
"We are developing this technology through a commercialization project from ETPL (Exploit Technologies Pte Ltd), A*STAR's technology transfer arm, and focusing on small-molecule and antibody screening," says Nallani. His team is planning to form a start-up company with this approach within the next few months.

1. May, S., Andreasson-Ochsner, M., Fu, Z., Low, Y. X., Tan, D. *et al.* In vitro expressed GPCR inserted in polymersome membranes for ligand-binding studies. *Angewandte Chemie International Edition* **52,** 749–753 (2013).

Bioengineering:

Greater anticancer potency with less risk

When injected as a hydrogel rather than in solution, an anticancer protein treats liver tumors more effectively



A scanning electron microscope image of the injectable hydrogel containing interferon-α2a.

Proteins and other therapeutic compounds injected directly into the blood stream tend to be broken down rapidly by the immune system. Now, researchers in Singapore have demonstrated in mice that the anticancer protein, interferon-α2a (IFN-α2a), can be delivered more effectively to liver tumors when incorporated into an injectable hydrogel[1].

Motoichi Kurisawa and co-workers at the A*STAR Institute of Bioengineering and Nanotechnology say their work indicates that hydrogels can protect therapeutic proteins from attack while releasing them in a controlled manner. As a result, more of the anticancer agent accumulates in tumors, and patients are at lower risk of side effects. "Our hydrogel system can also be developed to incorporate other potent drugs, proteins or small molecule drugs to achieve controlled release of therapeutics for various diseases," Kurisawa says.

Hydrogels first attracted the interest of medical researchers because the large amount of water they contain provides an environment that prevents proteins from denaturing or losing their shape. The injectable hydrogel developed by Kurisawa and co-workers is composed of a substance known as a hyaluronic acid–tyramine conjugate. The linkage reaction to form the gel is catalyzed by hydrogen peroxide, the level of which controls the stiffness of the gel, and horseradish peroxidase, which can tune the rate of gelation.

Kurisawa and co-workers previously demonstrated that proteins such as lysozyme and α-amylase could be incorporated and released by the hydrogel[2]. Their more recent *in vitro* work with IFN-α2a showed that the gel remained unaffected after incorporating the protein and that the protein was released via diffusion. Importantly, the IFN-α2a released from the gel could inhibit the proliferation of liver tumor cells and induce apoptosis, or 'cell suicide'.

In live mice, the injected hydrogel delivered up to three times as much IFN-α2a to liver tumor sites compared to direct injection in solution. The hydrogel treatment was also more effective: the average size of the tumors in mice injected with the hydrogel reduced significantly, whereas those in mice injected with IFN-α2a in solution remained the same. Cells in the tumors of hydrogel-treated mice also showed a decrease in proliferation and an increase in apoptosis. In addition, the hydrogel-delivered IFN-α2a inhibited the development of nutritive blood vessels in these mice.

"We are now planning to develop another hydrogel system to enhance the half-life of therapeutic proteins in the body even more," Kurisawa says. "And we are modifying our current hydrogel system to allow hepatitis treatment through the sustained release of interferon."

1. Xu, K., Lee, F., Gao, S. J., Chung, J. E., Yano, H. & Kurisawa, M. Injectable hyaluronic acid-tyramine hydrogels incorporating interferon-α2a for liver cancer therapy. *Journal of Controlled Release* **166,** 203–210 (2013).
2. Lee, F., Chung, J. E. & Kurisawa, M. An injectable hyaluronic acid–tyramine hydrogel system for protein delivery. *Journal of Controlled Release* **134,** 186–193 (2009).

Bio-imaging:

Probing for deeper diagnostics

Multi-armed polymers with dual fluorescent and magnetic imaging capabilities boost the resolution of cancer detection tools

Molecular probes that selectively latch onto tumor cells and emit imaging signals can detect cancer without invasive procedures. These tools, however, have specific deficiencies. Fluorescent probes that image individual molecules have poor depth penetration into cells. The alternative, magnetic resonance imaging (MRI) probes, resolves cells in three dimensions but with low resolution. Bin Liu at the A*STAR Institute of Materials Research and Engineering, Singapore, and co-workers have now solved this problem with a biocompatible polymer that combines MRI and fluorescence imaging in a single molecular probe[1].

A new complex that combines fluorescent imaging probes with high-contrast MRI chelating agents promises to make noninvasive cancer detection even more accurate.

According to Liu, designing a probe with joint imaging capabilities is challenging because fluorescent and MRI-active materials display different biological behaviors. Substances that emit fluorescent light are often lethal to cells at low concentrations. In contrast, to produce sufficient imaging signals, MRI probes require substantial injections of substances called chelated gadolinium (Gd(III)) agents.

Liu and her team devised a strategy to overcome the dissimilar dosage requirements with polymers known as 'hyperbranched' polyglycerols (HPGs). These materials have a tree-like structure of repeating molecular units that radiate from a core. HPGs also have a promising biomedical track record because of their water solubility and low cytotoxicity. Liu and co-workers envisaged using HPGs to encapsulate fluorescent organic molecules as their core. Then, they reasoned, high densities of Gd(III) agents could attach to the numerous hydroxyl attachment points present on the HPG surfaces.

After synthesizing a fluorescent molecule consisting of fused aromatic rings, the researchers attached eight of them to a rigid polysilicate cage, known as polyhedral oligomeric silsesquioxane. With the stable core in place, they initiated growth and outward branching of the HPG into a spherical protective shell — a tricky procedure, notes Liu, as it required carefully controlling the reagents and polymerization conditions. The new nanospherical probe converted over 50% of light photons into fluorescent emissions, a remarkably high quantum yield arising from the water-repellent nature of the dense HPG shell.

Next, the team attached Gd(III) agents to the probe's exterior and tested its dual detection capabilities inside MCF-7 breast cancer cells. Both MRI and fluorescence imaging revealed that the nanoprobe was well integrated into cell structures with no obvious changes to cell viability. The probe demonstrated high photostability when exposed to laser light — a key attribute for fluorescence imaging — and had promising magnetic properties that compared favorably with commercial MRI probes. "Combining both imaging techniques in one probe simultaneously boosts resolution and penetration depth," says Liu. "The different signals can also validate each other to improve detection accuracy."

1. Liu, J., Li, K., Geng, J., Zhou, L., Chandrasekharan, P., Yang, C.-T. & Liu, B. Single molecular hyperbranched nanoprobes for fluorescence and magnetic resonance dual modal imaging. *Polymer Chemistry* **4**, 1517–1524 (2013).

By writing data on adjacent overlapping tracks, just as the shingles overlap on this roof, the shingled magnetic recording approach promises to increase hard drive capacities.

Data storage:

Shingled tracks stack up

Simply changing the pattern by which data is recorded may lead to increased hard drive capacities

Modern hard drive technology is reaching its limits. Engineers have increased data-storage capacities by reducing the widths of the narrow tracks of magnetic material that record data inside a hard drive. Narrowing these tracks has required a concordant reduction in the size of the magnetic write head — the device used to create them. However, it is physically difficult to reduce the size of write heads any further. Kim Keng Teo and co-workers at the A*STAR Data Storage Institute, Singapore, and the Niigata Institute of Technology, Japan, have recently performed an analysis that highlights the promise of an alternative approach, which may sidestep this problem completely[1].

In a conventional hard drive, a write head stores data by applying a magnetic field to a series of parallel, non-overlapping tracks. Halving the width of the track effectively doubles the data-storage capacity, but also requires the size of the write head to be halved. The head therefore produces less magnetic field than is needed to enable stable data storage. This is because the small magnetic grains that are characteristic of modern hard drive media need to be thermally stable at room temperature.

"A relatively small difference in the way that writing occurs calls for a completely new approach to head design."

Shingled magnetic recording represents a step toward solving this problem as it allows for narrower track widths without smaller write heads. Rather than writing to non-overlapping tracks, the approach overlaps tracks just as shingles on a roof overlap (see image). Tracks are written in a so-called 'raster' pattern, with new data written only to one side of the last-written track.

Teo and co-workers analyzed the scaling behavior of this approach by using both numerical analysis and experimental verification. Their results showed that the size of the data track is not limited by the size of the write head, as in conventional hard drives. Instead, the track size is limited by the size of the magnetic read head, and by the 'erase bandwidth', which represents the portion of the track edge that is affected by adjacent tracks.

"This is a paradigm shift for the industry," says Teo. "A relatively small difference in the way that writing occurs calls for a completely new approach to head design." Teo expects the shingled approach to be a useful stop-gap measure prior to the arrival of more advanced, next-generation technologies in the next decade or so that will apply more radical modifications to the hard drive such as the use of heat to assist the write head.

1. Teo, K. K., Elidrissi, M. R., Chan, K. S. & Kanai, Y. Analysis and design of shingled magnetic recording systems. *Journal of Applied Physics* **111**, 07B716 (2012).

Imaging:

A brighter future for cell tracking

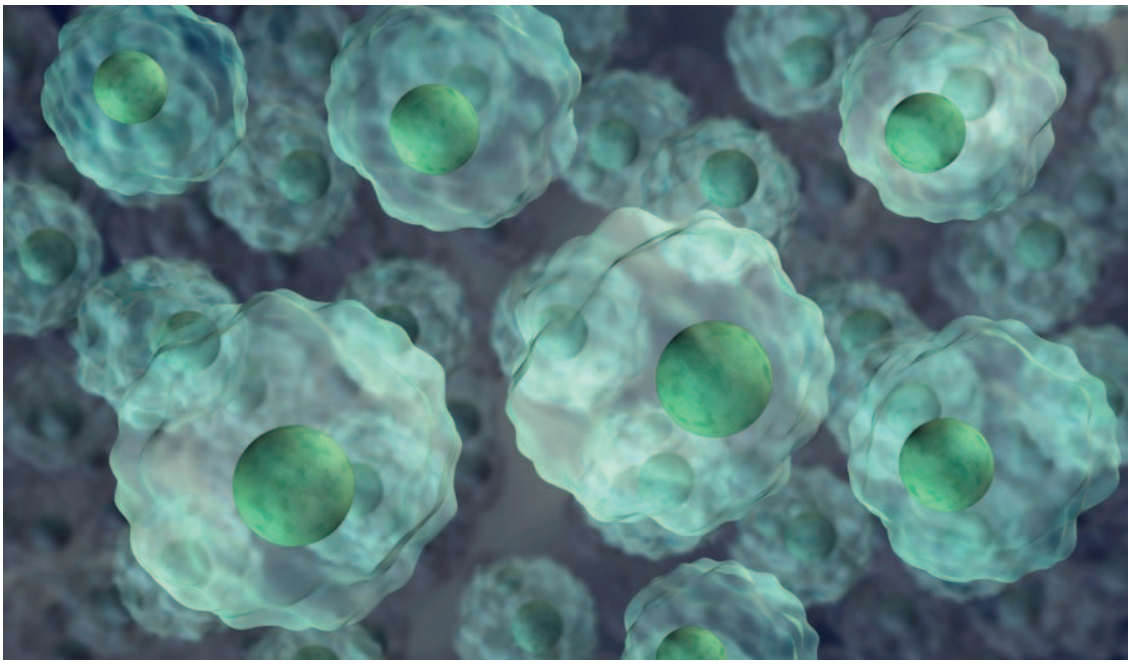
Fluorescent organic nanoparticles operating as cell tracers outperform existing methods for long-term tracking of living cells

A research team in Asia has developed a method for tracking, or 'tracing', cells that overcomes the limitations of existing methods. The team's fluorescent organic tracers will provide researchers with a noninvasive tool to continually track biological processes for long periods. Applications for the tracers include following carcinogenesis or the progress of interventions such as stem cell therapies.

Bin Liu and Ben Zhong Tang of the A*STAR Institute of Materials Research and Engineering in Singapore and their co-workers developed probes composed of a small number of molecules that aggregate[1]. The aggregation means that the probes have more detectable fluorescence and less leakage than that provided by single-molecule probes. Importantly, rather than 'blink', the team's tracers show steady fluorescence, and do not contain heavy metal ions that can be toxic for living systems.

Compared with their existing inorganic counterparts, the team's carbon-based tracers show greater chemical stability and improved biocompatibility with cell biochemistry. They are also more resistant to bleaching by light and do not interfere with normal biochemical processes. Furthermore, the fluorescent signals emitted by the probes do not overlap with the signal naturally emitted by cells.

The tracers developed by Liu, Tang and their colleagues are examples of 'quantum dots', as they are composed of a small number of molecules with optical characteristics that rely on quantum-mechanical effects. Technically, they are referred to as aggregation-induced emission dots (AIE dots) as they become photostable and highly efficient fluorescent emitters when their component molecules aggregate.



Tracking specific cells in normal development and in disease has now become easier through the use of organic fluorescent quantum dots, which display significant advantages over existing methods.

The assembly of the AIE dots began with the synthesis of organic molecules, specifically 2,3-bis(4-(phenyl(4-(1,2,2-triphenylvinyl) phenyl)amino)phenyl)fumaronitrile (TPETPAFN), which the researchers then encapsulated in an insoluble lipid-based matrix. Next, the researchers attached small peptide molecules derived from the human immunodeficiency virus (HIV) to exploit the ability of these peptides to promote efficient uptake of AIE dots into living cells.

"Our AIE dots could track isolated human breast cancer cells *in vitro* for 10 to 12 generations and glioma tumor cells *in vivo* in mice for 21 days," says Liu. "They outperform existing commercial inorganic quantum dots, and open a new avenue in the development of advanced fluorescent probes for following biological processes such as carcinogenesis, stem cell transplantation and other cell-based therapies."

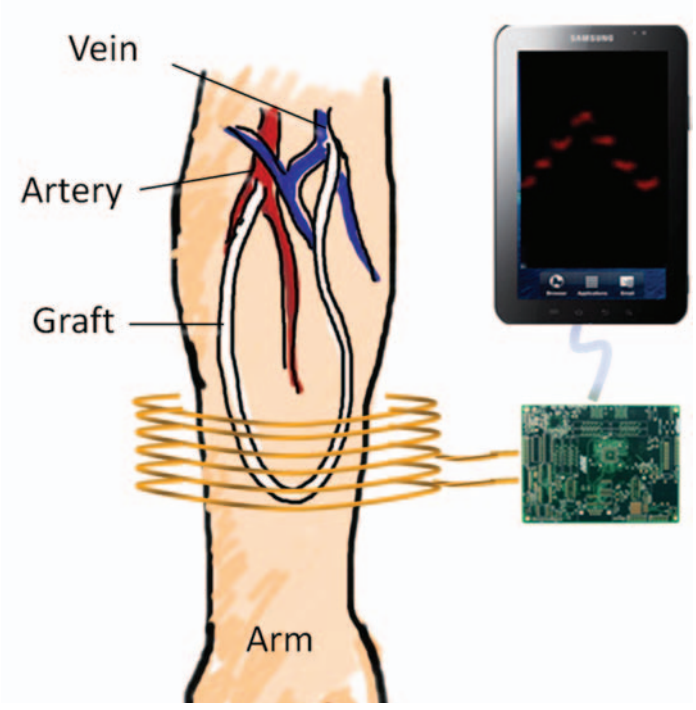
Future work by Liu, Tang and co-workers will aim to broaden the application of the organic tracers for their use in conjunction with magnetic resonance and nuclear imaging techniques.

1. Li, K., Qin, W., Ding, D., Tomczak, N., Geng, J. *et al.* Photostable fluorescent organic dots with aggregation-induced emission (AIE dots) for noninvasive long-term cell tracing. *Scientific Reports* **3**, 1150 (2013).

Biomedical engineering:

No batteries required

Microscale medical sensors inserted under the skin can be powered wirelessly by an external handheld receiver



A handheld reader (top right) wirelessly powers and interrogates a tiny blood-pressure sensor embedded inside a prosthetic graft, inserted in this case as a conduit for haemodialysis in a patient with kidney failure.

Implantable electronic devices potentially offer a rapid and accurate way for doctors to monitor patients with particular medical conditions. Yet powering such devices remains a fundamental challenge: batteries are bulky and eventually need recharging or replacing. Jia Hao Cheong at the A*STAR Institute of Microelectronics, Singapore, and his co-workers are developing an alternative approach that eliminates the need for a battery[1]. Their miniature devices are based on wireless power-transfer technology.

The research team has developed a microscale electronic sensor to monitor blood flow through artificial blood vessels. Surgeons use these prosthetic grafts to bypass diseased or clogged blood vessels in patients experiencing restricted blood supply, for example. Over time, however, the graft can also become blocked. To avoid complete failure, blood flow through the graft must be monitored regularly, but existing techniques are slow and costly.

These limitations prompted the researchers to develop a bench-top prototype of a device that could be incorporated inside a graft to monitor blood flow. The implant is powered by a handheld external reader, which uses inductive coupling to wirelessly transfer energy, a technology similar to that found in the latest wireless-charging mobile phones. The team developed an application-specific, integrated circuit for the implant designed for low power use (see image).

The incoming energy powers circuits in the device that control sensors based on silicon nanowires. This material is piezoresistive: as blood flows over the sensor the associated mechanical stresses induce a measurable increase in electrical resistance, proportional to the flow pressure.

Key to the success of the device is its ability to work with a very limited power supply. Most of the incoming energy is absorbed by skin and tissue before it can reach the implant, which may be inserted up to 50 millimeters deep.

"Our flow sensor system achieves an ultralow power consumption of 12.6 microwatts," Cheong says. For example, the sensor transmits its data to the handheld reader passively, by back-scattering some of the incoming energy. "We have tested our system with 50-millimeter-thick tissue between the external coil and implantable coil, and it successfully extracted the pressure data from the implantable device," he adds.

Cheong and his co-workers' tests showed that the prototype sensor was also highly pressure sensitive, providing pressure readings with a resolution of 0.17 pounds per square inch (1,172 pascals). "The next step of the project is to integrate the system and embed it inside a graft for [an experimental] animal," Cheong says.

1. Cheong, J. H., Ng, S. S. Y., Liu, X., Xue, R.-F., Lim, H. J. *et al.* An inductively powered implantable blood flow sensor microsystem for vascular grafts. *IEEE Transactions on Biomedical Engineering* **59**, 2466–2475 (2012).

Optics:

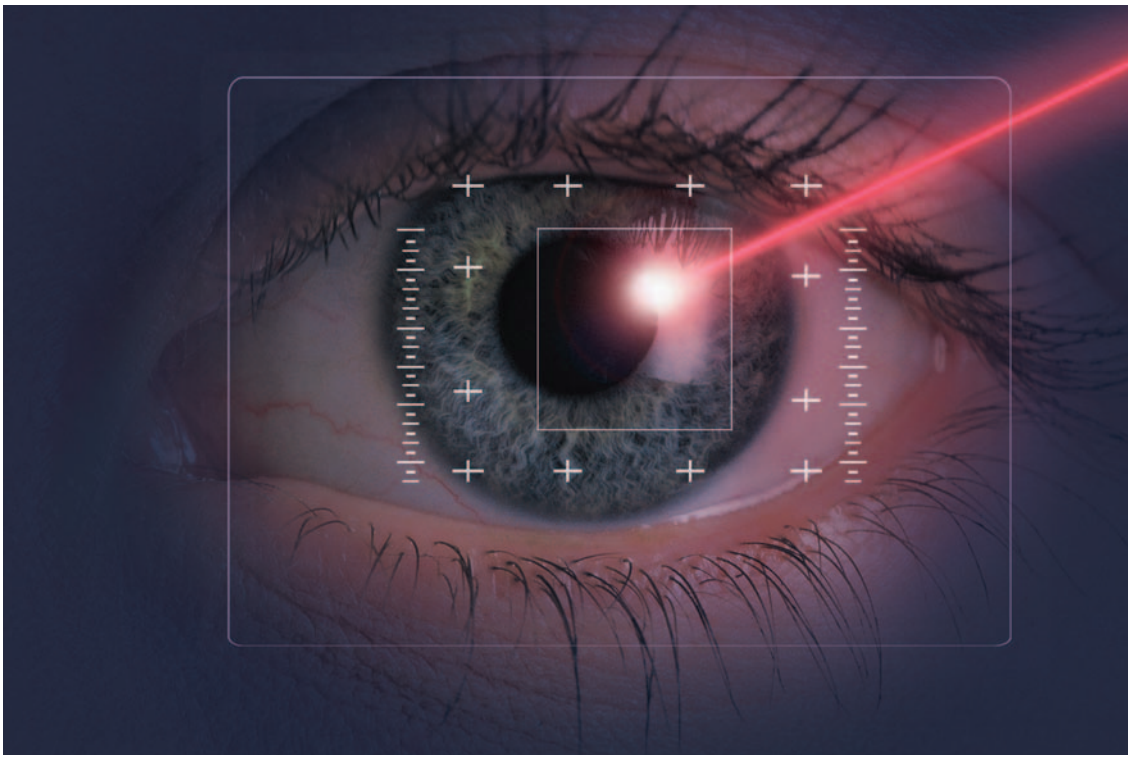
Statistics light the way

Revelation of how photoreceptive cells in the eye distinguish between different light sources could pave the way for a novel class of optical devices

Millions of years of evolution have molded our eyes into highly sensitive optical detectors, surpassing even many man-made devices. Now, Leonid Krivitsky and his co-workers at the A*STAR Data Storage Institute and the A*STAR Institute of Medical Biology, Singapore, have shown that the photoreceptor cells found in the retina are even sensitive to the statistical properties of light[1]. This ability could be harnessed in 'bioquantum' interfaces, a novel class of optical devices that use biological systems to detect the quantum nature of light.

Light comprises discrete bundles of energy known as photons. A 40-watt light bulb, for example, creates more than 10^{19} (a one followed by 19 zeros) visible photons every second. Nevertheless, attenuated sources that generate light pulses containing just a few photons are also useful. In such ultralow-intensity light pulses, the statistical distribution of photons emitted in a single pulse depends on the light source.

Warm light sources such as light-bulb filaments generate photons in bunches. Lasers, in contrast, create photons randomly — each is emitted independently of the next. Krivitsky and his co-workers experimentally demonstrated that rod photoreceptor cells in the eye can distinguish between pulses of light from either a laser or a thermal light based only on these differing distributions. "Showing that such cells can assess photon statistics provides hope for accessing the quantum properties of light using biodetectors," says Krivitsky.



Pulses of low-level laser light trigger electrical responses of cells from the retina, which demonstrate these cells' sensitivity to photon statistics.

Krivitsky and his team trapped a photoreceptor cell from a frog on the end of a suction pipette. Then they fired green-light laser pulses at the cell through an optical fiber. The same device could also imitate a thermal light source when they placed a rotating disk of ground glass and an aperture into the beam path.

They observed that rhodopsin molecules in the cell absorbed the incoming photons, which generated an ion current. The researchers amplified and measured this current as the average number of photons in each light pulse increased. They noticed a much sharper increase in detected current for the laser light than the pseudothermal pulses. This is because, while the average photon number is the same, an individual pseudothermal pulse was more likely to have a low number of photons. The photon distribution of the laser pulses, on the other hand, was much narrower.

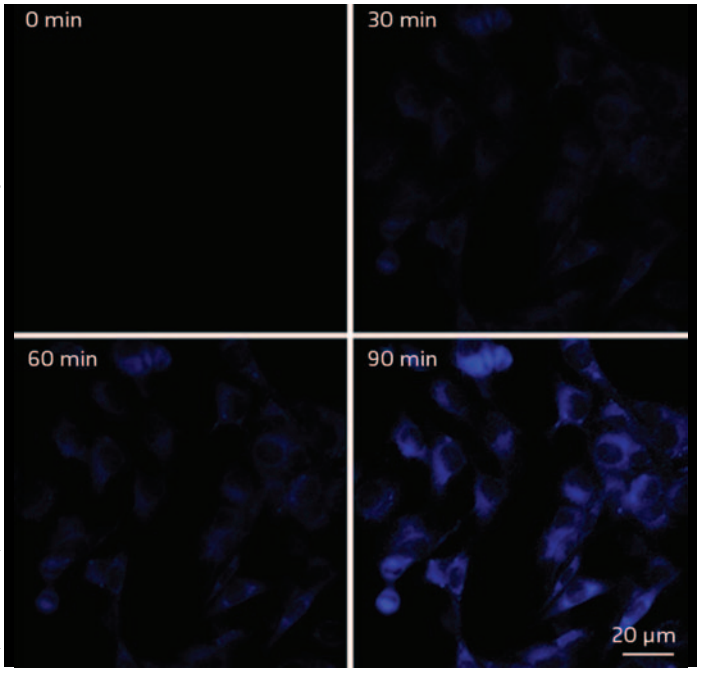
The two types of photon emitters investigated in these experiments are examples of 'classical' light sources. "The next step is to investigate quantum light, such as pulses with a fixed number of photons," notes Krivitsky.

1. Sim, N., Cheng, M. F., Bessarab, D., Jones, C. M. & Krivitsky, L. A. Measurement of photon statistics with live photoreceptor cells. *Physical Review Letters* **109,** 113601 (2012).

Imaging:

Cells marked for death

A fluorescent indicator could help scientists identify useful drugs that modulate the process of cell death



Treatment with the drug staurosporine triggers onset of apoptosis in cultured cells, resulting in increased blue fluorescent signal over time.

Apoptosis is a programmed death mechanism that eliminates unwanted or injured cells from the body. Defects in apoptotic regulation can lead to serious physiological problems such as tissue damage or uncontrolled cancerous growth. Apoptosis is therefore a prominent target for drug development.

A molecular probe devised by Bin Liu and Ben Tang of the A*STAR Institute of Materials Research and Engineering, Singapore, could accelerate drug discovery by giving scientists a clear view of apoptotic onset in living cells[1]. Recent years have seen an explosion in the use of fluorescent molecules to track biological processes, but existing probes have proven inadequate for monitoring cell death. "Commercial apoptosis probes do not have good cell permeability, and their background signal is also quite high," explains Liu.

As an alternative, the researchers employed a mechanism called 'aggregation-induced emission', initially developed in Tang's laboratory[2]. Their probe contains a molecule called tetraphenylethene (TPE), which normally gathers into insoluble fluorescent clumps in aqueous media. This accumulation can be prevented by attaching a short polypeptide sequence that renders the probe water-soluble.

In the presence of an enzyme known as caspase-3, however, this polypeptide is clipped off. "Caspase-3 exists in all cells in an inactive form, but the enzyme becomes activated upon induction of apoptosis," says Liu. Dying cells therefore produce a fluorescent signal as TPE is released and begins to aggregate. The researchers initially demonstrated the effectiveness of their probe in solutions containing different concentrations of caspase-3, and showed that their molecule is a highly responsive sensor. Compounds that inhibit caspase-3-blocked fluorescent signaling confirm that the probe is exclusively activated by this enzyme.

Liu and Tang achieved similar success in cultured cells, and pretreatment with their probe allowed them to directly monitor the onset of apoptosis over the course of 90 minutes (see image). "We observed very low background and strong, time-dependent signaling," says Liu, "which opens new opportunities to continuously monitor biological processes." To test the molecule's usefulness for drug screening, the researchers observed cultured cells after treatment with a selection of apoptosis-inducing drugs. Based on the rate of increase in fluorescence, they were able to compare the relative efficacy of each drug, demonstrating the probe's promise as a valuable clinical tool.

The researchers have now begun optimizing the probe for use *in vivo*, which should allow scientists to conduct more meaningful drug testing in live animals. The team will determine whether the probe can be used for continuous monitoring of drugs taken either orally or injected.

1. Shi, H., Kwok, R. T. K., Liu, J., Xing, B., Tang, B. Z. & Liu, B. Real-time monitoring of cell apoptosis and drug screening using fluorescent light-up probe with aggregation-induced emission characteristics. *Journal of the American Chemical Society* **134**, 17972–17981 (2012).
2. Luo, J., Xie, Z., Lam, J. W. Y., Cheng, L., Chen, H. *et al.* Aggregation-induced emission of 1-methyl-1,2,3,4,5-pentaphenylsilole. *Chemical Communications*, 1740–1741 (2001).

Data storage:

Measuring the downside of downsizing

Sensitive measurements of lubricant transfer in hard disk drives will aid the design of more stable and compact components

Understanding how much lubricant is transferred from the rotating disk to the slider is crucial for designing better hard disk drives.

To keep pace with the rapidly growing consumer demand for data storage, hardware engineers are striving to cram as much electronic information into as small a space as possible. Jinmin Zhao, Mingsheng Zhang and co-workers at the A*STAR Data Storage Institute, Singapore, have now devised a technique to assess the impact of making these devices more compact[1]. Insights resulting from this work will guide the future design of stable disk drives.

The primary components of a hard disk drive are a rotating disk coated with a thin film of magnetic material and a magnetic head on a moving arm, also called a slider (see image). The slider includes magnetic read/write elements that can encode a single bit of binary information by altering the properties of the thin film at a small spot on the surface. A smaller spot enables a higher density of data storage.

Current technology is rapidly approaching one trillion bits per square inch, but this requires the separation between the head and disk to be less than 2 nanometers. This narrow requirement, however, creates its own problems. Lubricant used on the surface of the disk to protect it from corrosion can attach to the slider, which adversely affects the reliability of the hard disk drive. "We have carried out a systematic and quantitative study on how the variation of slider optical properties affects the accuracy of the measured lubricant thickness on the slider surface," says Zhang.

Zhao, Zhang and their co-workers analyzed a lubricant-coated slider using a technique known as spectroscopic ellipsometry. Measuring the intensity of light reflected from a sample slider provided a highly accurate estimate of the thickness of the lubricant film. Ellipsometry is a fast and non-destructive technique that, unlike some of the alternative approaches, does not require ultrahigh vacuum conditions. This technique, however, does require accurate knowledge of the optical properties of the slider. A typical slider is made of aluminum oxide and grains of titanium carbide of many different shapes and sizes; thus, its optical properties vary from position to position.

Zhao and the team's study demonstrated that the uncertainty in lubricant thickness is approximately proportional to the uncertainty in the slider's optical constants, and it becomes particularly pronounced for thicknesses below 2 nanometers.

"This lubricant transfer will be more serious in future heat-assisted magnetic recording," explains Zhang. "The next step in this research will focus on how to reduce the lubricant transfer, especially in this type of device."

1. Zhao, J. M., Zhang, M. S., Yang, M. C. & Ji, R. Ellipsometric measurement accuracy of ultrathin lubricant thickness on magnetic head slider. *Microsystem Technologies* **18,** 1283–1288 (2012).

Investigating how individuals really 'view' one another is now possible using a model developed at the A*STAR Institute of High Performance Computing.

Social cognition:

Exploring implicit racial biases computationally

A numerical simulation based on individual preferences provides insights into social attitudes that are unattainable through physical experiments

A Singaporean computer scientist and his American colleague have created a computer simulation of how humans perform on a widely employed test of implicit or unconscious social attitudes, particularly racial bias[1].

The success of their model opens the way to undertaking experiments virtually, which cannot be conducted in real human subjects, and exploring the factors underlying the formation of such social biases. "This will help us to better account for people's behavior as a function of their attitudes and beliefs, and possibly provide some insight into consumers' tastes, preferences and purchasing habits," says Boon-Kiat Quek of the A*STAR Institute of High Performance Computing, who carried out the study with Andrew Ortony of Northwestern University in the United States.

The Implicit Association Test (IAT), first published in 1998, is designed to test a person's internal association between concepts and objects, such as whether insects or flowers are deemed pleasant or unpleasant. The computer-based test requires participants to sort concepts or objects flashed on the screen into one of two categories by pressing a left- or right-hand key. The system records the time taken to make each choice.

The IAT is now widely employed in many different forms in studies of social psychology, and has been used as evidence for the existence of society-wide unconscious or implicit social preferences, such as young over old. Another widely publicized result is that almost all white people show an apparent bias for whites over blacks, whereas only about half of the black individuals tested prefer blacks over whites.

Quek and Ortony, however, could see nothing in the IAT that would allow them to decide whether or not those attitudes were strictly positive or negative, or if — for instance — people had positive attitudes toward both whites and blacks, but preferred one more than the other. They also found no indication of the absolute strength of the bias. To explore these issues, they developed a computational model representing the steps involved in processing the IAT in humans.

Using their relatively simple model, Quek and Ortony were able to replicate human performance on the IAT. They argue that such models could be useful in providing data on unconscious assumptions that could not be obtained otherwise, because the memories and brains of human subjects cannot be taken apart and manipulated.

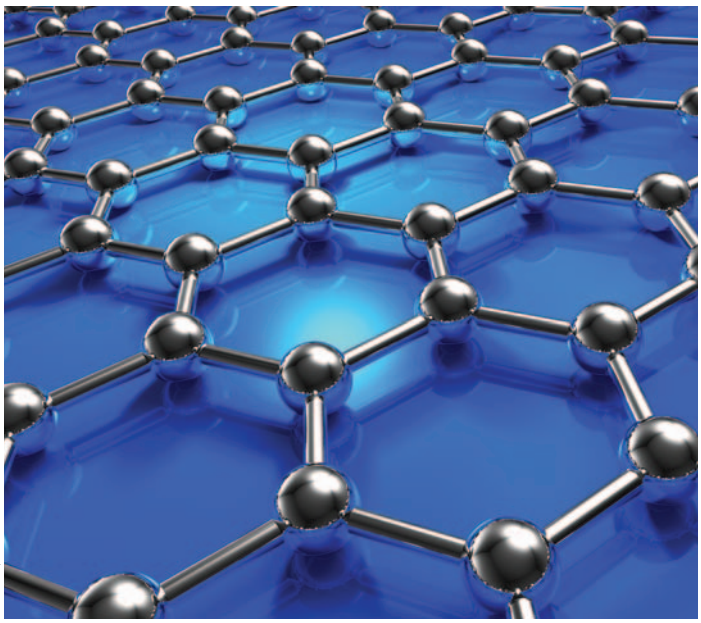
"There are many possible extensions of this work," says Quek. "For instance, performing virtual experiments to understand how people acquire the implicit attitudes that they possess."

1. Quek, B.-K. & Ortony, A. Assessing implicit attitudes: What can be learned from simulations? *Social Cognition* **30**, 610–630 (2012).

Electronics:

Graphene makes a magnetic switch

Tiny nanoribbons of carbon could be used to make a magnetic field sensor for novel electronic devices



Graphene — a thin sheet of carbon atoms — could be used to make a magnetic field sensor.

Researchers in Singapore have designed an electronic switch that responds to changes in a magnetic field[1]. The device relies on graphene, a strong and flexible electricity-conducting layer of carbon atoms arranged in a honeycomb pattern.

Seng Ghee Tan of the A*STAR Data Storage Institute, along with colleagues at the National University of Singapore, used theoretical models to predict the properties of their proposed device, known as a magnetic field-effect transistor.

The transistor is based on two nanoribbons of graphene, each just a few tens of nanometers wide, which are joined end to end. The atoms along the edges of these nanoribbons are arranged in an 'armchair' configuration — a pattern that resembles the indented battlements of castle walls. If these edges were in a zigzag pattern, however, the material would have different electrical properties.

One of the nanoribbons in the team's transistor acts as a metallic conductor that allows electrons to flow freely; the other, slightly wider, nanoribbon is a semiconductor. Under normal conditions, electrons cannot travel from one nanoribbon to the other because their quantum wavefunctions — the probability of where electrons are found within the materials — do not overlap.

A magnetic field, however, warps the distribution of electrons, changing their wavefunctions until they overlap and allowing current to flow from one nanoribbon to the other. Using an external field to change the electrical resistance of a conductor in this way is known as a magnetoresistance effect.

The team calculated how electrons would travel in the nanoribbons under the influence of a 10-tesla magnetic field — the rough equivalent of that produced by a large superconducting magnet — at a range of different temperatures.

Tan and colleagues found that larger magnetic fields allowed more current to flow, and the effect was more pronounced at lower temperatures. At 150 kelvin, for example, the magnetic field induced a very large magnetoresistance effect and current flowed freely. At room temperature, the effect declined slightly but still allowed a considerable current. At 300 kelvin, the magnetoresistance effect was approximately half as strong.

The researchers also discovered that as the voltage across the nanoribbons increased, the electrons had enough energy to force their way through the switch and the magnetoresistance effect declined.

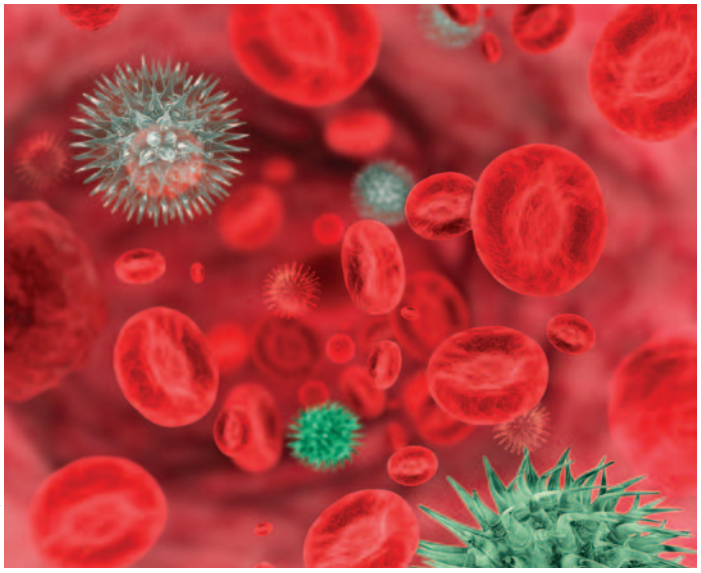
Other researchers recently produced graphene nanoribbons with atomically precise edges, similar to those in the proposed design. Tan and his colleagues suggest that if similar manufacturing techniques were used to build their device, its properties could come close to matching their theoretical predictions.

1. Kumar, S. B., Jalil, M. B. A. & Tan, S. G. High magnetoresistance in graphene nanoribbon heterojunction. *Applied Physics Letters* **101,** 183111 (2012).

Biopurification:

Convection trumps diffusion

Breakthrough technology quickly separates large proteins and viruses from their surroundings, which improves purification of vaccines and protein therapuetics



Viruses can be captured efficiently and with high precision on chromatographic surfaces using a technique developed at the A*STAR Bioprocessing Technology Institute.

Researchers looking to isolate individual proteins from complex environments usually turn to chromatography, a technique where mobile solutions of biomolecules flow through columns packed with solid, porous particles. Separation occurs when attractive chemical forces cause the molecules to adsorb onto the solid while contaminants pass through. Despite major progress, however, chromatographic purification of viruses and other large biomolecules remains challenging: their spatial heft makes it hard for them to diffuse through columns in a reasonable amount of time.

Pete Gagnon and co-workers at the A*STAR Bioprocessing Technology Institute in Singapore have discovered a new chromatography approach that can boost the capacity and resolution of large-scale biological purifications[1]. Instead of relying on chemical attraction, the team's 'steric exclusion chromatography' (SXC) technique exploits the physical distribution of biomolecules and a dissolved polymer to drive adsorption at a chromatography surface — a strategy that generates extremely fast binding kinetics and virus purification efficiencies thousands of times greater than current techniques.

No two compounds dissolved in a solution can occupy the same space. In addition, random movements and collisions create narrow zones adjacent to surfaces where smaller dissolved molecules are statistically absent. As these zones create excess free energy, materials in the solution spontaneously rearrange themselves to reduce the excess.

Gagnon and his team exploited this effect by dissolving proteins into polyethylene glycol (PEG), creating PEG-free zones around the biomolecules and an inert chromatography surface. When the biomolecules randomly encounter the surface, their PEG-deficient zones fuse together to reduce the system's free energy and they become stabilized on the solid support. Because larger biological species are more affected by this phenomenon, they tend to associate with the chromatography surface, whereas smaller compounds are swept through the column and eliminated.

By performing the separation in special monolithic columns that transport dissolved materials through convection, not diffusion, the researchers were able to purify viruses with unprecedented efficiency. They achieved binding capacities of 10 trillion virus particles per milliliter of monolith, despite the passage time through the column being only 6 seconds. Some 99.8% of *E. coli* proteins and 93% of DNA contaminants were removed. Virus recovery was 90%, and critically, the viruses retained full biological activity.

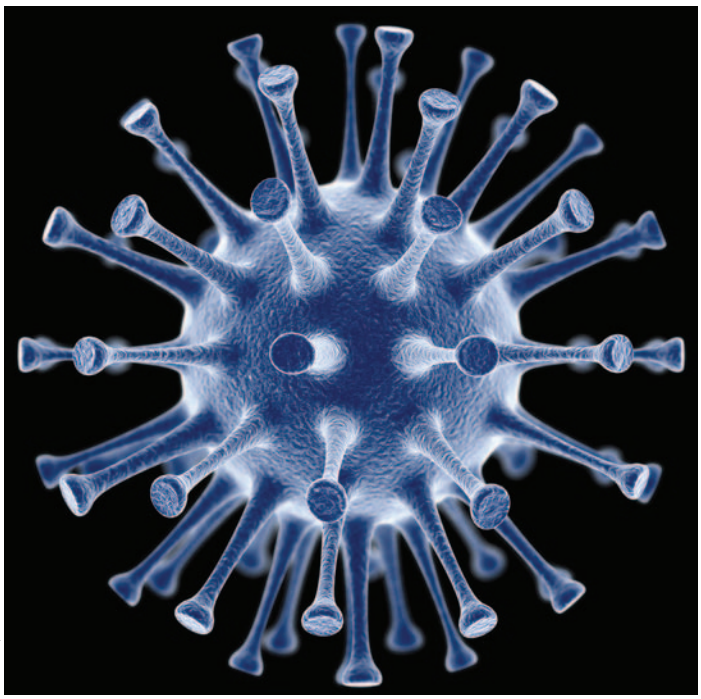
Gagnon notes that this unexpected discovery drastically improves upon the sluggishness and low efficiency problems currently associated with size-based chromatography methods. "Steric exclusion chromatography provides process developers with a rapid, high-precision tool needed to support effective and economical industrial purification."

1. Lee, J., Gan, H. T., Latiff, S. M. A., Chuah, C., Lee, W. Y. *et al.* Principles and applications of steric exclusion chromatography. *Journal of Chromatography A* **1270**, 162–170 (2012).

Pathology:

All sorted on a single microchip

A microchip that can identify human pathogens in a single test could revolutionize the diagnosis of infections



© iStockphoto/Thinkstock

PathChip detects all clinically relevant viruses and bacteria that infect humans, including the human influenza virus.

Quick diagnosis of an infection is critical to providing early treatment; but, currently, multiple tests can be required to identify the pathogen responsible. Now, Christopher Wong at the A*STAR Genome Institute of Singapore and an international research team have demonstrated that a recently developed alternative called PathChip can improve diagnoses[1].

Owing to the lack of a single standard test for detecting all illness-causing pathogens, accurate diagnosis of an infection can be a trial-and-error process. "Doctors order tests to confirm their diagnosis," explains Wong. "If they suspect their patient has dengue fever, they order a dengue test. Often, the result is negative, so they need to order more tests."

PathChip is designed to provide accurate diagnosis of respiratory tract infections in just one test. This tool consists of a chip covered in molecules that can recognize genetic material from 70,000 different pathogens. By observing which genetic material from a patient sample binds to PathChip, scientists can immediately identify the pathogens present.

"In this study, we wanted to determine the performance of PathChip relative to existing methods that are approved by the US Federal Food and Drug Administration (FDA)."

The team extracted genetic material from samples collected from 290 children with respiratory tract infections. Then, they used three methods to identify the pathogens present. Two of these, manufactured by EraGen and Luminex, are FDA-approved methods currently in clinical use. The third was PathChip.

PathChip not only matched the performance of existing tests, but, owing to its wider coverage, it also detected pathogens that the other tests missed entirely. "PathChip made a diagnosis in 20 per cent more patient samples than the approved methods," Wong explains.

Wong says that PathChip can also cope with the constant evolution of pathogens that changes their genetic make-up and produces new strains. "Even with genetic changes, we remain able to detect viruses," he says. "For example, the recent Middle East respiratory syndrome coronavirus, and the H7N9 bird flu virus in China, emerged after we manufactured PathChip. However, we could still detect these viruses without modification. With the other methods, new tests have to be designed."

According to Wong, this proven performance means PathChip has the potential to revolutionize diagnosis in hospitals. "Doctors can order a single test and treat their patients based on the results. This could avoid unnecessary diagnostic tests, shorten hospital stays and reduce antibiotic use."

As the next step toward this, Wong says that he wants to take PathChip into clinical trials.

1. Simões, E. A. F., Patel, C., Sung, W.-K., Lee, C. W. H., Loh, K. H. *et al.* Pathogen chip for respiratory tract infections. *Journal of Clinical Microbiology* **51**, 945–953 (2013).

Data storage:

Better hard drives ready for lift-off

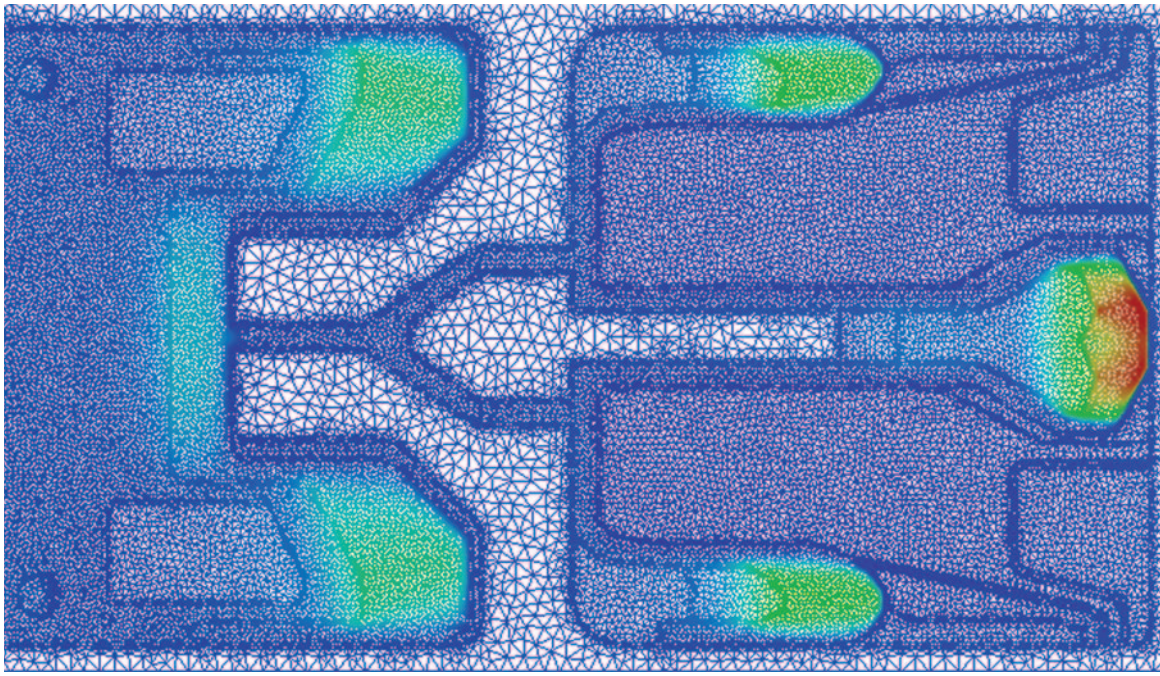
An improved software algorithm enables more efficient modeling and development of computer hard drives of the future

Operating a hard disk drive is as complex as keeping a superfast car on the road. Read/write heads within the hard disk must process a huge amount of data at high speed. Controlling the motion of the slider housing these heads is crucial: if the slider crashes, it could destroy the hard disk.

Researchers at the A*STAR Data Storage Institute (DSI) in Singapore have now developed a computational algorithm for studying the properties of the slider in a hard drive that is faster than existing algorithms[1]. Instead of taking days to finish, dynamic simulations using the new algorithm take only an hour, notes Wei Hua from the research team. "It greatly improves our simulation and research abilities," he adds.

A read head typically moves across the disk surface of a hard drive at more than 7,000 revolutions per minute. The flying height of this fast-moving head is as low as 2 nanometers from the surface of the disk, some 50,000 times less than the width of a human hair. Controlling this motion is not easy, notes Hua. "The slider housing the read/write head flies on the fast-rotating hard drive disk, owing to a very thin layer of air. This air bearing pushes the slider upward, while a suspension bearing pushes the slider down toward the disk."

Thermal effects control the distance of the head to the surface when it is being pushed down. To understand these effects, and other factors that control disk and head movements at high speed, fine-grained computer simulations are necessary.



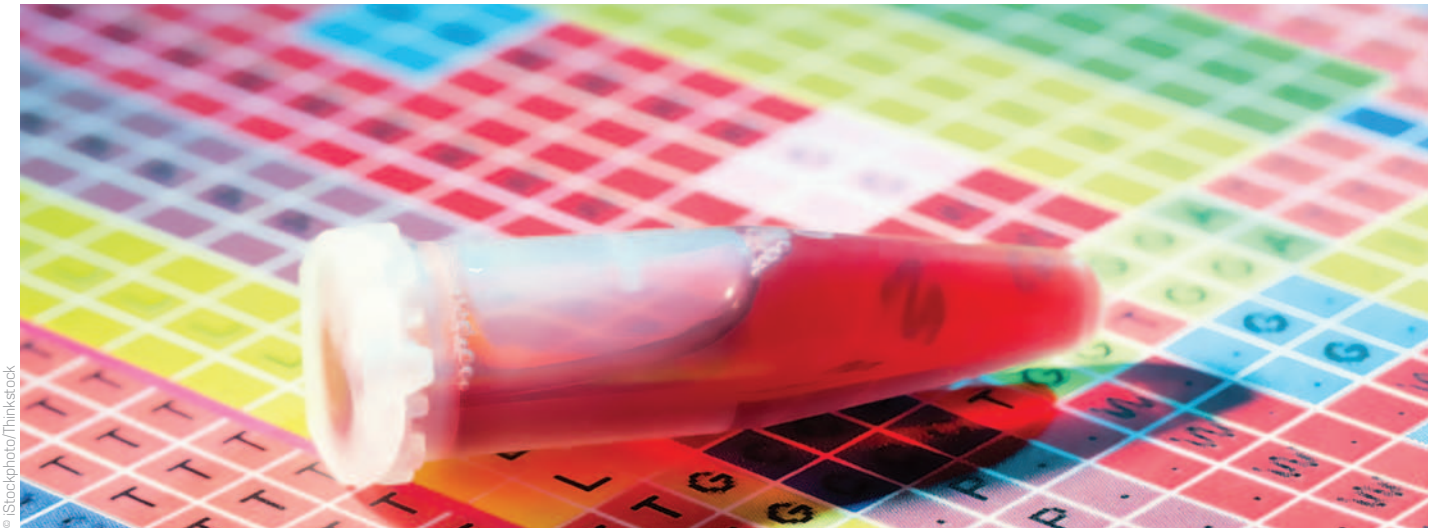
The triangular mesh used to model the properties of a hard disk read/write head. The colors represent the pressure profile of the head; red indicates areas of high pressure.

> Instead of taking days to finish, dynamic simulations using the new alorithm take only an hour.

Hua and co-workers expanded the DSI's ABSolution air bearing simulation software for faster and more precise modeling. Instead of dividing the hard drive slider into a structured rectangular mesh typically used to aid calculations, the researchers used an unstructured triangular mesh that accurately captures the geometry of the read/write head (see image). Moreover, the algorithm better implements the dynamic effects that occur in drive heads, meaning that overall the code works faster and more efficiently.

This modeling software should prove useful in the future development of drive heads, Hua notes. Modeling the interaction between the slider and the rest of the drive is also important. "Influences such as those from the air suspension and disk effects are now being considered," he explains. Hua and co-workers will use the improved algorithm to model slider properties that were almost impossible to simulate using the previous versions.

1. Hua, W., Yu, S., Zhou, W. & Myo, K. S. A fast implicit algorithm for time-dependent dynamic simulations of air bearing sliders. *Journal of Tribology* **134,** 031901 (2012).



Microelectronics:

Automating cancer detection

A sensor technology developed at A*STAR may soon replace time-consuming and expensive methods for detecting DNA methylation as a marker of cancer.

*A sensor developed at A*STAR can detect bladder cancer cells and track tumor progression*

Microelectronic engineers in Singapore have developed and tested sensor technology that can detect and measure a chemical signature of bladder cancer[1]. The light-based sensor could eventually be used for the early diagnosis and subsequent tracking of the progression and treatment of many different tumors, according to Yong Shin at the A*STAR Institute of Microelectronics, who led the research. After further testing of the technology, Shin and co-workers are planning to develop a lab-on-a-chip device incorporating the sensor that can process fluid samples within about five minutes.

Genes that suppress tumors can be deactivated by the attachment of a methyl group to a specific DNA sequence — cytosine next to guanine — in their promoter region. The methyl group prevents the gene from being used as a template for protein synthesis and reduces the capacity of the cell to control its own proliferation.

Several well-established chemical methods exist for detecting such DNA methylation, but they are expensive, time-consuming and dependent on laboratory expertise. Shin and co-workers therefore investigated direct physical methods as an alternative. They focused particularly on silicon micro-ring resonators that amplify light at specific resonant frequencies. The resonators developed by the researchers are very sensitive detectors of a shift in light frequency, including the shift that occurs when a methyl group is attached or detached to DNA.

Shin and co-workers tested the capacity of silicon micro-ring resonators to discriminate between methylated and unmethylated forms of genes known to trigger cancer in bladder cells. They fashioned separate DNA probes to capture one or other form when they passed a solution of the genes, amplified by the polymerase chain reaction, over a silicon chip to which the probes were attached. The resonators clearly distinguished between the forms within five minutes. Moreover, the method allowed the team to quantify the density of methylation, which means the technique should be able to track changes in patterns of methylation.

"Our sensors could be widely useful for DNA methylation detection specifically and rapidly in the field," says Shin.

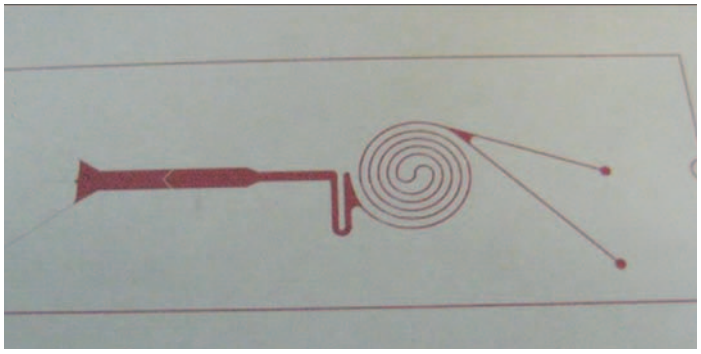
He also notes that the team has published several research papers on using silicon micro-ring resonators. "Among the techniques we have published is a novel technique that can be integrated with the methylation-specific sensor to amplify the methylated DNA from low amounts of DNA," he explains. "So, we are now trying to make a single microfluidic-based chip system that integrates several techniques, such as DNA extraction, conversion, amplification and detection."

1. Shin, Y., Perera, A. P., Kee, J. S., Song, J., Fang, Q. *et al.* Label-free methylation specific sensor based on silicon microring resonators for detection and quantification of DNA methylation biomarkers in bladder cancer. *Sensors and Actuators B: Chemical* **177,** 404–411 (2013).

Microelectronics:

Taking the heat off microfluidic chips

Replacing a high-temperature processing technique with an infrared treatment allows the manufacture of tiny devices without damaging the polymer components



Researchers have developed a cooler way to create microfluidic devices with channels of just 10 micrometers in width.

Microfluidic devices are allowing microelectronic engineers to shrink laboratories to the size of a computer chip. By ferrying reagents through a series of microscopic channels and reservoirs carved into a flat plate, researchers can develop new chemical reactions or monitor the cellular effects of drugs on a much smaller scale, potentially saving time and money.

Some of these microfluidic devices even have electrical components that act as heaters or sensors, for example. But researchers have struggled to develop a rapid, low-cost method for creating the detailed metal patterns that make up these circuits.

Conventional techniques tend to require high-temperature processing, which can damage the transparent polymers typically used to build microfluidic devices, such as polycarbonate (PC) or poly(methyl methacrylate) (PMMA). Despite this drawback, the polymers are preferred over more robust alternatives because they "have very good optical properties, which most microfluidic devices require, and they are viable for plastic injection molding, which enables high-volume production," explains Zhaohong Huang of the A*STAR Singapore Institute of Manufacturing Technology.

"[Polymers] have very good optical properties, which most microfluidic devices require, and they are viable for plastic injection molding, which enables high-volume production."

Huang and his co-workers developed an alternative process that avoids exposing the polymers to high temperatures, and used it to build complex metal-patterned microfluidic devices (see image)[1]. They first covered sheets of PC or PMMA with thin layers of chromium, copper and nickel, and added a coating of a light-sensitive material called a photoresist. At this stage, the 'sandwich' would normally be baked at around 100 °C to remove any residual solvents after the coating process. But these temperatures would soften and warp the polymer, potentially cracking or loosening the metal layer.

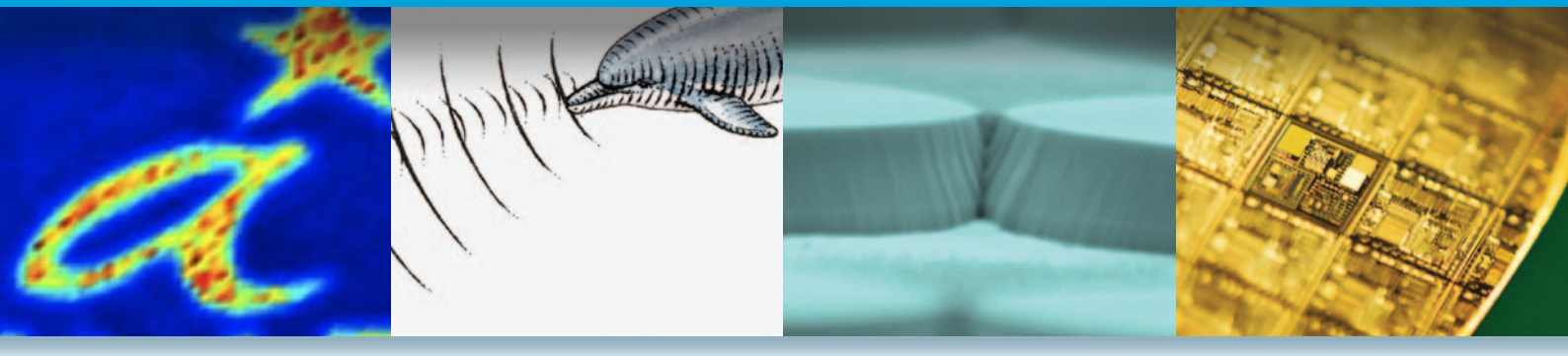
Instead, Huang's team used infrared heating elements to eliminate the solvents. The metal layer acted as a protective barrier, reflecting more than 95% of any infrared radiation that hit it, meaning that the radiation warmed the photoresist layer but not the polymer beneath.

The researchers then used standard photolithography processes to create the microfluidic device. They placed a patterned mask over the sandwich and shone ultraviolet light to erode some areas of the photoresist; then, they etched away the exposed areas of metal beneath using a wash of chemicals. Stripping off any remaining photoresist left a clean metal pattern, which had features as small as 10 micrometers in width.

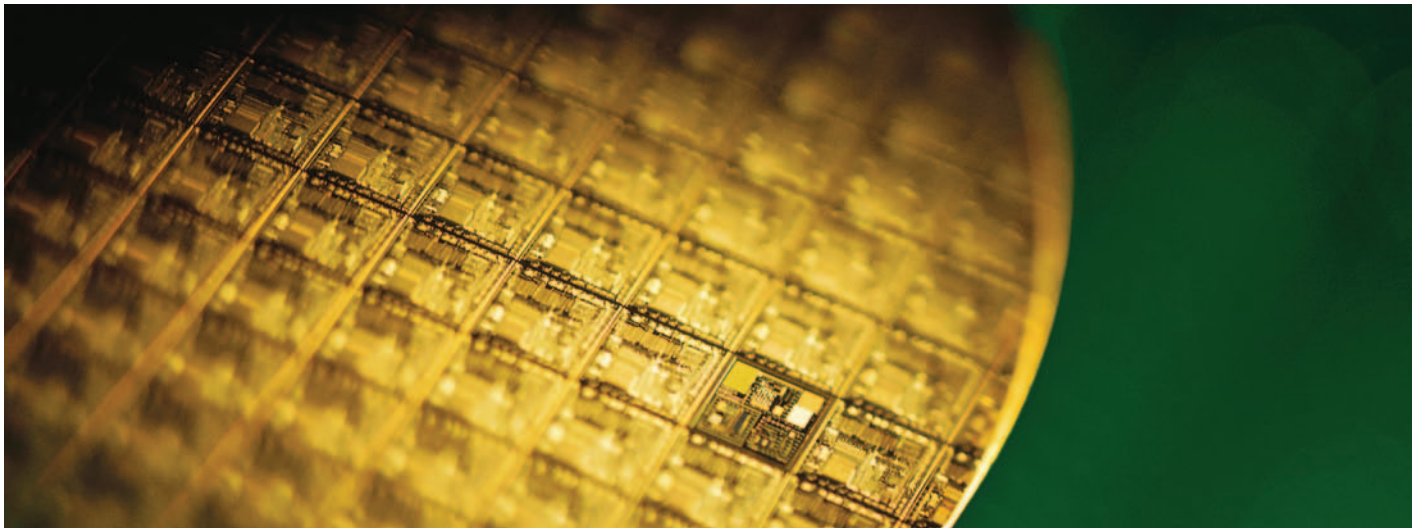
"If the surface finish is gold, our method can cut costs by more than 90%," says Huang. His team is now refining the process, and creating patterns of different metals with catalytic properties, which could speed up chemical reactions inside microfluidic devices.

1. Huang, Z. H., Lim, B. C. & Wang, Z. F. Process development for high precision metal patterning on low glass transition polymer substrates. *Microelectronic Engineering* **98**, 528–531 (2012).

Research Highlights



ENGINEERING & NANOTECHNOLOGY



Stacking ever-more components on computer chips is aggravating crowding. A computer model developed at A*STAR now allows researchers to predict how a silicon wafer deforms as a consequence.

Computer chips:
Building upward safely

A computer model provides important clues for the production of tightly packed electronic components

Greater numbers of ever-smaller components are required to fit on computer chips to meet the ongoing demands of miniaturizing electronic devices. Consequently, computer chips are becoming increasingly crowded. Designers of electronic architectures have therefore followed the lead of urban planners and started to build upward. In so-called 'three-dimensional (3D) packages', for example, several flat, two-dimensional chips can be stacked on top of each other using vertical joints.

Controlling the properties of these complex structures is no easy task, as many factors come into play during production. FaXing Che and Hongyu Li and co-workers from the A*STAR Institute of Microelectronics, Singapore, have now developed a powerful modeling method that allows large-scale simulations — and optimization — of the fabrication process[1], which provides welcome assistance to designers.

Among the challenges of producing tightly packed computer chips is the need to prevent warpage of the underlying silicon wafer as electronics components are stacked on it (see image). Warpage leads to a number of unwanted effects. "Strong warpage can cause wafer breakage, it makes tight packing more difficult and some processing machines cannot handle high-warpage wafers," explains Li. The degree of warpage depends on many design and process parameters, and optimizing the procedure experimentally is time-consuming and costly.

Using their computer model, Che and Li studied a wide range of parameters that influence the warpage of an 8-inch diameter silicon wafer. They focused, in particular, on how a silicon substrate responds to the deposition of layers of copper — through which electrical currents eventually flow. "This is the first time that a model has been able to predict warpage [at] the level of the entire wafer," says Li. Moreover, the stress on the wafer can be determined accurately. The calculated values agreed well with experimental data. Importantly, with the computer simulations, the researchers could explore regimes that cannot be easily studied experimentally, such as how the depth of the connections between layers influences wafer warpage.

The next goal is to simulate even larger wafers with variable connection sizes, explains Li. "Today, there are two industry standards for 3D packaging applications, 8-inch and 12-inch wafers, but the latter are becoming increasingly important," she says. The team's model is applicable to these larger wafers, too, but it requires optimization. Currently, Che, Li and their co-workers are collecting warpage and stress data for 12-inch wafers. They will use these data for developing their model further, according to Li.

1. Che, F., Li, H. Y., Zhang, X. W., Gao, S. & Teo, K. H. Development of wafer-level warpage and stress modeling methodology and its application in process optimization for TSV wafers. *IEEE Transactions on Components, Packaging and Manufacturing Technology* **2**, 944–955 (2012).

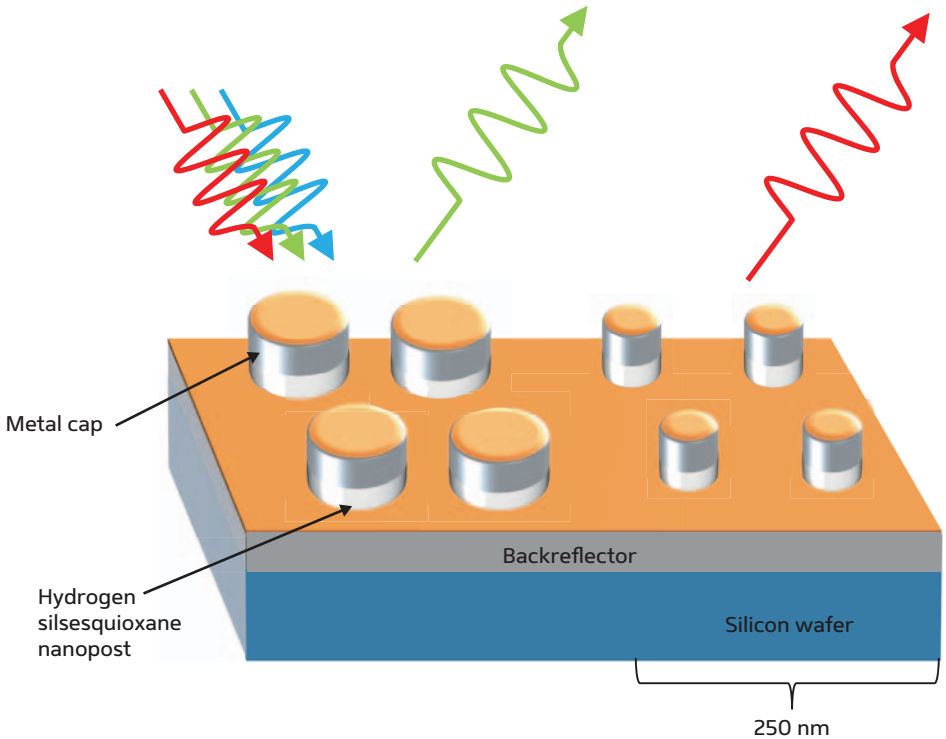
Nanotechnology:

Color printing reaches new highs

Color printing at the highest resolution possible is enabled by the use of arrays of metal-coated nanostructures

Commercial laser printers typically produce pin-sharp images with spots of ink about 20 micrometers apart, resulting in a resolution of 1,200 dots per inch (dpi). By shrinking the separation to just 250 nanometers — roughly 100 times smaller — a research team at A*STAR can now print images at an incredible 100,000 dpi, the highest possible resolution for a color image[1]. These images could be used as minuscule anti-counterfeit tags or to encode high-density data.

To print the image, the team coated a silicon wafer with insulating hydrogen silsesquioxane and then removed part of that layer to leave behind a series of upright posts of about 95 nanometers high. They capped these nanoposts with layers of chromium, silver and gold (1, 15 and 5 nanometers thick, respectively), and also coated the wafer with metal to act as a backreflector.



Variation in post size and spacing in the metal array alters which incoming wavelength of light (red, green or blue) is reflected back.

Each color pixel in the image contained four posts at most, arranged in a square. The researchers were able to produce a rainbow of colors simply by varying the spacing and diameter of the posts to between 50 nanometers and 140 nanometers.

When light hits the thin metal layer that caps the posts, it sends ripples — known as plasmons — running through the electrons in the metal. The size of the post determines which wavelengths of light are absorbed, and which are reflected (see image).

The plasmons in the metal caps also cause electrons in the backreflector to oscillate. "This coupling channels energy from the disks into the backreflector plane, thus creating strong absorption that results in certain colors being subtracted from the visible spectrum," says Joel Yang, who led the team of researchers at the A*STAR Institute of Materials Research and Engineering and the A*STAR Institute of High Performance Computing.

Printing images in this way makes them potentially more durable than those created with conventional dyes. In addition, color images cannot be any more detailed: two adjacent dots blur into one if they are closer than half the wavelength of the light reflecting from them. Since the wavelength of visible light ranges about 380–780 nanometers, the nanoposts are as close as is physically possible to produce a reasonable range of colors.

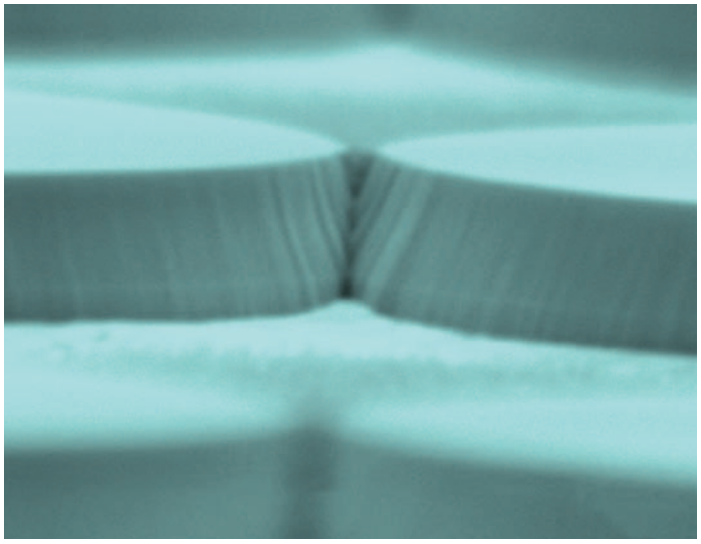
Although the process takes several hours, Yang suggests that a template for the nanoposts could rapidly stamp many copies of the image. "We are also exploring novel methods to control the polarization of light with these nanostructures and approaches to improve the color purity of the pixels," he adds.

1. Kumar, K., Duan, H., Hegde, R. S., Koh, S. C. W., Wei, J. N. & Yang, J. K. W. Printing colour at the optical diffraction limit. *Nature Nanotechnology* **7**, 557–561 (2012).

Semiconductors:

Touching moments with a radiant outcome

Microstructures made of adjoining semiconductor disks could lead to powerful nanoscale sensors



Terahertz radiation is greatly enhanced in the tiny V-shaped gap, just a fraction of a micrometer wide, between pairs of touching semiconductor disks.

Many users of microwave ovens have had the frightening experience of leaving a fork, crumpled piece of aluminum foil or some other pointy metal item inside the cooking chamber. The sharp metal object acts as an antenna for the oven's microwave radiation, causing strong local heating or sparking. Jing Hua Teng from the A*STAR Institute of Materials Research and Engineering (IMRE) and colleagues in Singapore and the UK have now observed a similar antenna effect, involving a different sort of electromagnetic radiation — known as terahertz (THz) radiation — in a microfabricated semiconductor structure[1]. Their discovery could find application in areas ranging from biosensing to airport security scanners.

Teng and his co-workers developed tiny semiconductor structures made of the chemical elements indium and antimony. From this material, they produced disks of 20 micrometers in diameter, which they arranged such that pairs just touched. The gap between contiguous disks was merely tens to hundreds of nanometers wide (see image). When the researchers exposed the structures to THz radiation, they found that the radiation intensity in the gap was enhanced by more than a hundred times.

> "Building a device such as ours for visible light is much more challenging, as it would involve even smaller structures."

Confining and enhancing THz radiation is significant for two reasons, according to Teng. First, electromagnetic waves in the THz range can be used in a broad range of applications, for example, to study the structure of large biomolecules. As this sort of radiation can penetrate textiles but is less energetic than X-rays — or microwaves — it is also well suited for use in body scanners at airports. The second reason as to why the new results are important is more fundamental. "We have produced this particular touching-disk structure to test, in the THz regime, intriguing theoretical predictions made for optical radiation," explains Teng. "Building a device such as ours for visible light is much more challenging, as it would involve even smaller structures."

The now-verified theoretical predictions came from collaborators at Imperial College London in the UK. "For the present work, the IMRE is in charge of the materials growth and the structure fabrication, while Imperial College contributes structure design and characterization," says Teng. The A*STAR researchers are now focused on practical applications: they will further explore the unique properties of their semiconductor materials and try to develop devices for THz technology. The group has already succeeded in tuning the THz response of their structure[2], meaning that they can conveniently adjust the frequency response of their device for different applications.

1. Hanham, S. M., Fernández-Domínguez, A. I., Teng, J. H., Ang, S. S., Lim, K. P. *et al.* Broadband terahertz plasmonic response of touching InSb disks. *Advanced Materials* **24**, OP226–OP230 (2012).
2. Deng, L., Teng, J. H., Liu, H., Wu, Q. Y., Tang, J. *et al.* Direct optical tuning of the terahertz plasmonic response of InSb subwavelength gratings. *Advanced Optical Materials* **1**, 128–132 (2013).

Device physics:

Simulating electronic smog

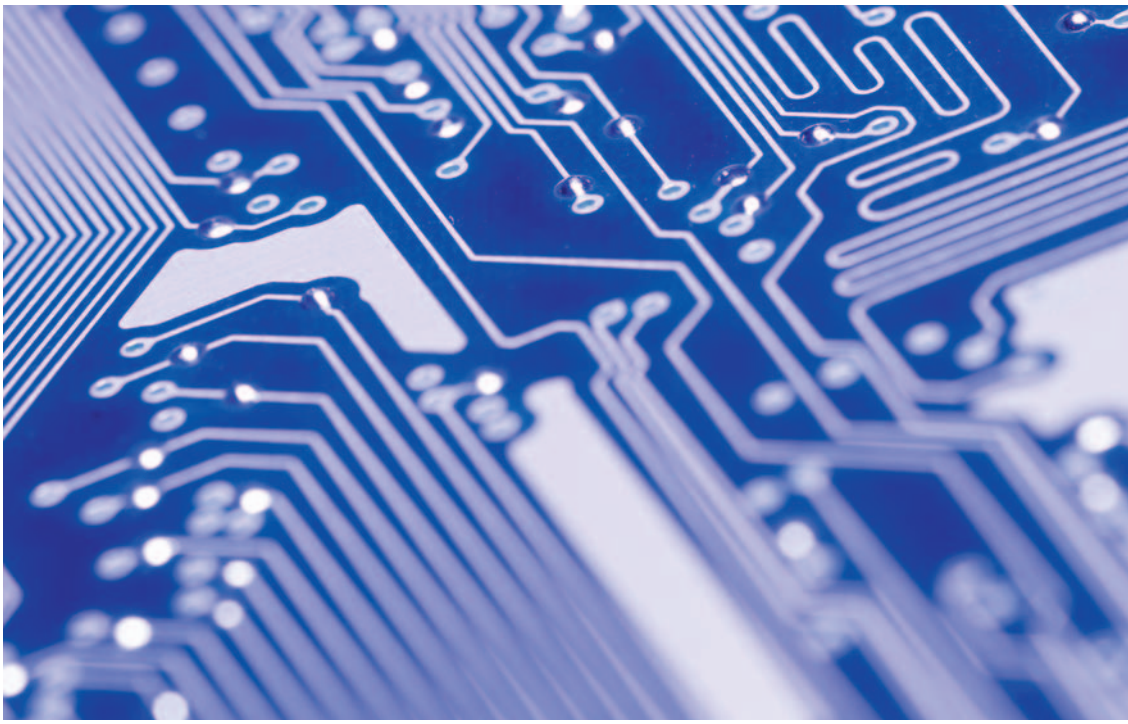
A mathematical model that predicts the electromagnetic radiation produced by circuit boards could help to improve designs and lower costs

A research team from A*STAR and Samsung Electronics has developed a fast and accurate way to estimate the electromagnetic emissions from printed circuit boards that could help designers to ensure that devices meet regulatory standards[1].

Circuits that carry rapidly changing electrical currents can generate unwanted electromagnetic waves, wasting energy, causing interference with other electrical equipment and potentially posing health risks to users. To ensure that such emissions are within acceptable limits, electronic products such as mobile phones and laptops must undergo tests for this 'electronic smog' before they can be marketed.

Those tests have traditionally been done in large rooms designed to capture all the electromagnetic waves emitted from the device, explains Wei-Jiang Zhao of A*STAR's Institute of High Performance Computing, Singapore, who led the study. An alternative to this costly process involves scanning the electromagnetic field very close to the device's circuit boards (the near field), and then calculating the resulting radiation at a distance (the far field). But those calculations can take powerful computers many hours to complete.

The mathematical model developed by Zhao and co-workers translates near-field measurements into an accurate estimate of far-field radiation in less than 10 minutes on a standard desktop computer. "Our simulation technique could help to shorten the product design cycle, save laboratory space and reduce product development cost," says Zhao.



An A*STAR research team's mathematical model can estimate the electromagnetic emissions from a printed circuit board.

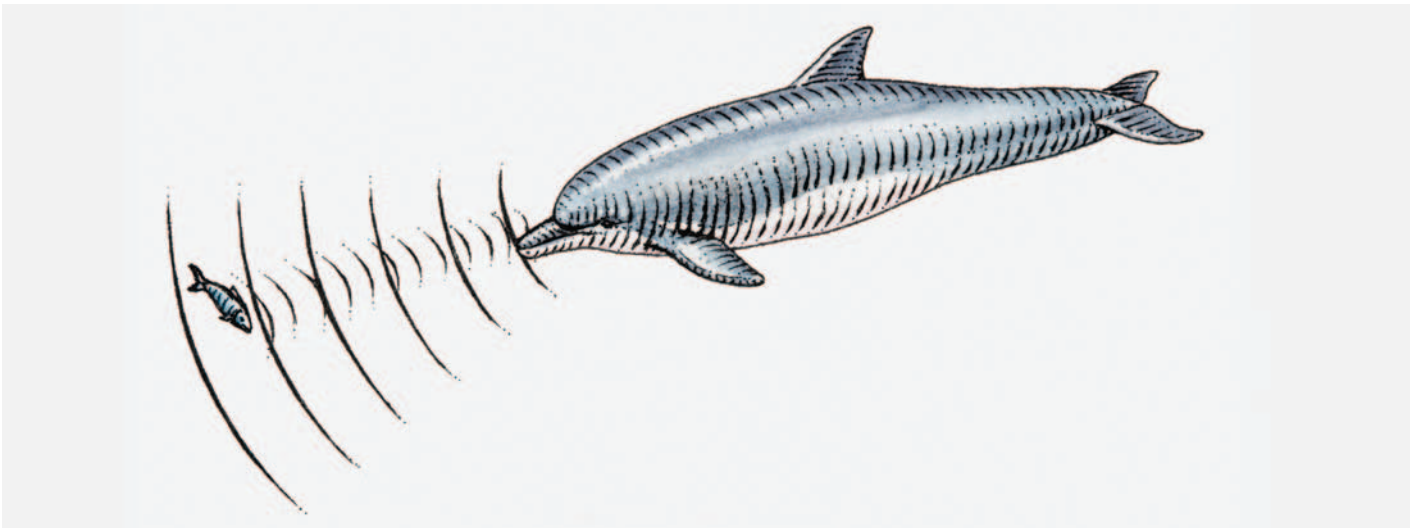
© iStockphoto/Thinkstock

The researchers' model mathematically mimics the readings from a scan of the near-field above a printed circuit board. Their simulation relies on a series of virtual magnetic dipoles — effectively tiny, imaginary bar magnets — that collectively replicate the variations in the measured magnetic field.

The simulation runs iteratively, each time altering the magnetic dipoles so that they fit the data better. This process of 'differential evolution' eventually produces a solution that is a sufficiently close match to the circuit-board's near field. The researchers then use those magnetic dipoles to simplify their calculation of the far-field radiation produced by the device.

The researchers tested their model using simulated near-field data from a thin, L-shaped metal strip laid on a small circuit board. The data contained 1,273 sample points, each 10 millimeters above the board. The model could approximate this magnetic field using just a few virtual magnetic dipoles. The match improved as they added more dipoles, until they reached very good agreement at nine dipoles — adding a tenth did not significantly improve the match. The team is now working to refine the system to make it suitable for use by the electronics industry.

1. Zhao, W.-J., Wang, B.-F., Liu, E.-X., Park, H. B., Park, H. H. *et al.* An effective and efficient approach for radiated emission prediction based on amplitude-only near-field measurements. *IEEE Transactions on Electromagnetic Compatibility* **54,** 1186–1189 (2012).



Dolphins use echolocation to locate prey and navigate. Researchers have harnessed the same principle to determine the inclination of millimeter-sized ultrasonic sensors.

Micromachining:

Inclinations sounded out

A novel type of tilt sensor may extend the capabilities of ultrasonic devices already used in a range of applications

Echolocation is a powerful technique that uses sound or ultrasound waves to locate objects and surfaces. Ships and submarines, for example, use it to avoid collisions, and dolphins and microbats use it to locate prey (see image). Hongbin Yu and co-workers from the A*STAR Institute of Microelectronics, Singapore, have now used echolocation to measure the inclination of millimeter-sized ultrasonic sensors[1]. In this new setting, their technique should extend the capabilities of devices that already use ultrasonic components, whether for locating defects in materials, visualizing anatomical structures or determining range.

Yu and his co-workers built on the success that so-called 'capacitive micromachined ultrasonic transducers' (CMUTs) have achieved over the past decade in generating and detecting ultrasound signals. These devices are fabricated using silicon micromachining technology, so the components are very compact and can be conveniently integrated with standard electronics components, which are also based on silicon.

"Our main goal was to explore a new application of the CMUT device," says Yu. Consequently, the researchers harnessed these ultrasonic components for measuring tilt angles. They used three micromachined CMUTs — two senders and a common receiver — each measuring less than a tenth of a millimeter across. To test this array, they immersed it in a bath filled with oil. As they tilted the device, the oil surface stayed level — in the same manner that the water surface in a tilted glass would remain horizontal. However, the distances between the surface and the sensors at the bottom changed such that one sensor became closer to the surface than the other.

By measuring how long it took the ultrasound waves to travel from each of the senders to the receiver, via the oil surface where the waves were reflected, Yu and his co-workers could accurately determine the distances between the sensors and the surface. They could then calculate the tilt angle that the CMUT array had relative to the oil surface.

As many devices already contain ultrasonic components, the new sensor should be useful in a number of applications, according to Yu. "As one example, in an automotive robotic arm equipped with ultrasound transducers for fault detection, a tilt-sensing function should help improve the arm-control accuracy without greatly increasing the complexity of the device," he explains.

Other areas where tilt-angle measurements are important include level determination for instrumentation and motion-state monitoring. With the team's innovation, such functionality may now be added to ultrasonic medical-imaging and non-destructive materials-testing devices.

1. Yu, H., Guo, B., Haridas, K., Lin, T.-H., Cheong, J. H. *et al.* Capacitive micromachined ultrasonic transducer based tilt sensing. *Applied Physics Letters* **101,** 153502 (2012).

Hybrid nanostructures:

Getting to the core

Insights into self-assembled, multicomponent nanostructures on nanowires provide an innovative fabrication approach for high-performance devices

Material scientists expect the new multifunctional properties of hybrid nanostructures will transform the development of high-performance devices, including batteries, high-sensitivity sensors and solar cells. These self-assembling nanostructures are typically generated by depositing ultrasmall objects with different properties on the surfaces of tiny semiconducting wires. However, the factors that govern their formation remain elusive, making these structures difficult to control and design.

To fill this gap, Bharathi Srinivasan and co-workers from the A*STAR Institute of High Performance Computing have developed a computational approach that sheds light on the self-assembly of these nanostructures on multi-sided, or polygonal, nanowires. They first identified how different nanostructure patterns grow on nanowires by conducting energy calculations in a theoretical analysis before analyzing these patterns by performing numerical simulations[1].

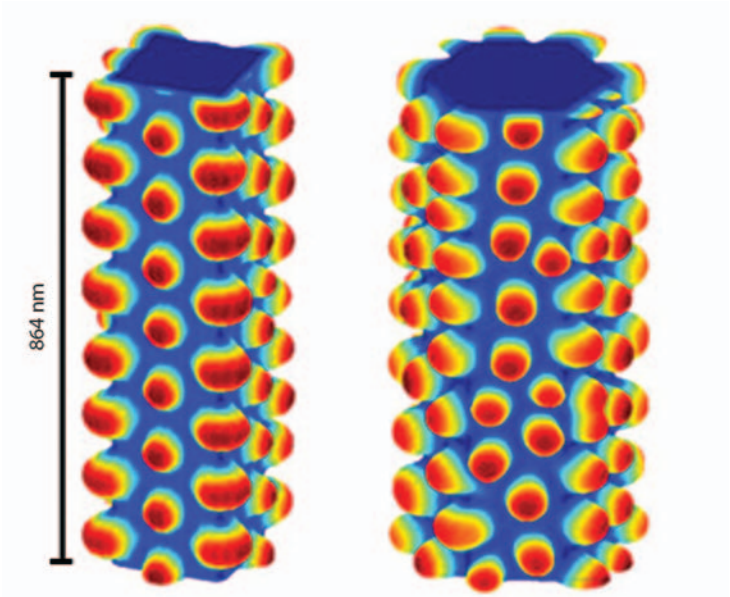
Srinivasan's team designed two- and three-dimensional (2D and 3D) models of nanowires with a square, hexagonal or octagonal core surrounded by various shell configurations. Analysis of the energy profiles of these configurations showed that the researchers could control shell morphology by changing the core size. The theoretical analysis also revealed the transitions between these different configurations — a valuable insight into the self-assembly mechanism.

For the numerical simulation, the researchers constructed a 'phase-field' model, which mathematically defined the phase transitions of the shell material. This allowed them to simulate the self-assembly process of the nanostructures on the nanowires after depositing the 'seed' in the form of 'quantum dots', which are miniature semiconductors. The equations used in the simulation describe both the thermodynamics and the kinetics of self-assembly, Srinivasan notes.

Both the 2D and 3D simulations showed that the deposited shells underwent morphological transformations that mirrored the energy calculations. At the initial deposition stage — the lowest size range — the shells consisted of perfect cylinders in the 2D model, and they formed ultrasmall rings, or 'nanorings', stacked along the vertical direction of the nanowire, in the 3D model.

As the core expanded, the 2D models indicated that the shells could break into smaller wires. For the intermediate-sized cores, each wire sat on the sides of the core. For the largest-sized cores, they sat on the corners. In the 3D simulations, the nanorings divided into quantum dots that materialized into columns on the nanowire facets and migrated toward the ridges upon further growth (see image). Simulations of heat treatment yielded the same configurations as those during growth.

"Our future work [will be] to understand the growth of different hybrid nanostructures, including quantum dots on shells, nanorings and other quantum dots," says Srinivasan.



Simulations showing the potential growth of quantum dots on the ridges and facets of nanowires with square (left) and hexagonal cores (right).

1. Lu, L.-X., Bharathi M. S. & Zhang, Y.-W. Self-assembly of ordered epitaxial nanostructures on polygonal nanowires. *Nano Letters* **13**, 538–542 (2013).

Land use in cities can now be modeled by computer software that allows researchers to predict details such as energy use on individual plots of land.

Urban planning:

City dynamics yield to computer modeling

A new computer model of city dynamics could pave the way to planning sustainable urban areas

The sustainability of cities is a challenge facing planners across the globe. The numerous complex and wide-ranging interactions between energy consumption, water use, transportation and population dynamics make cities intrinsically complicated systems to study.

Christopher Monterola and co-workers at A*STAR's Institute of High Performance Computing, Singapore, have created a computer modeling system capable of characterizing land-use patterns in different cities[1]. This software provides planners with the ability to define the features of a particular city as well as compare and contrast these features with those of other cities.

A city is a complex system, and complex systems evolve as a result of highly interacting units driven by a simple mechanism, Monterola notes. "Understanding the underlying simplicity in the growth of cities will allow us to model the emergence of city dynamics more accurately and, more importantly, learn to shape a city's growth based on our desired outcomes."

The team worked with high-resolution image data for Singapore and eight North American cities. They painstakingly categorized land use into business, residential or industrial sectors, pixel by pixel, for each city. To analyze the dispersion and aggregation of land-use types across the urban space, the computer model used two parameters — 'spatial entropy', which describes how a given sector is spread across space, and an 'index of dissimilarity', which measures the relative mixing of sectors.

"The lower the entropy number, the more densely clustered a given sector is," explains Monterola. "In the cities studied, industrial areas were generally clustered and distinct from residential and business zones. There is 'safety in numbers', but only if the resources required by [a] specific sector are not compromised."

The index of dissimilarity helped to define the efficiency of different urban factors, especially transportation and energy consumption. In follow-up work, the team successfully modeled the emergence of land use in cities, the surface temperatures for individual plots of land and even accurately estimated ridership — how many people are using public transport at any one time.

"The good visual and statistical resemblance of our simulations to actual cities hints at the robustness of this work so far," says Monterola. "We will add more details, including schools, churches and so on, with the aim of capturing the day-to-day routines of people in a city." Monterola believes that this groundwork will yield predictive models of different urban activities, resulting from easily measured parameters that will be useful as guides for planners.

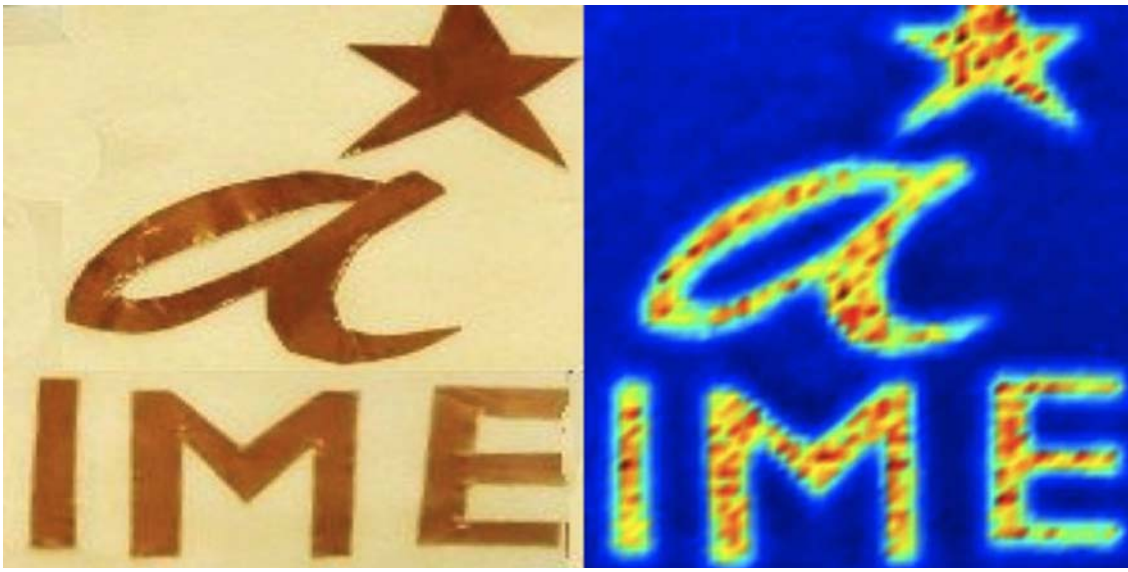
1. Decraene, J., Monterola, C., Lee, G. K. K. & Hung, T. G. G. A quantitative procedure for the spatial characterization of urban land use. *International Journal of Modern Physics C* **24**, 1250092 (2013).

Terahertz technology:

Seeing more with less

Single-chip integration of the components needed for sending and receiving terahertz radiation should help applications in imaging and communication

Terahertz technology is an emerging field that promises to improve a host of useful applications, ranging from passenger scanning at airports to huge digital data transfers. Terahertz radiation sits between the frequency bands of microwaves and infrared radiation, and it can easily penetrate many materials, including biological tissue. The energy carried by terahertz radiation is low enough to pose no risk to the subject or object under investigation.



Terahertz radiation can penetrate materials such as a paper envelope and reveal the contents (left) in an accurate image (right).

"In a commercial terahertz transmitter–receiver unit, the central module alone measures typically around 190 by 80 by 65 millimeters, which is roughly 1 million cubic millimeters."

Before terahertz technology can take off on a large scale, however, developers need new kinds of devices that can send and receive radiation in this frequency range. Worldwide, electronic engineers are developing such devices. Now, Sanming Hu and co-workers from the A*STAR Institute of Microelectronics (IME), Singapore, have designed novel circuits and antennas for terahertz radiation and efficiently integrated these components into a transmitter–receiver unit on a single chip[1]. Measuring just a few millimeters across, this area is substantially smaller than the size of current commercial devices. As such, it represents an important step toward the development of practical terahertz technologies.

Hu and his co-workers based their terahertz design on a fabrication technology known as BiCMOS, which enables full integration of devices on a single chip of only a few cubic millimeters in size. "Currently, commercial products for terahertz technologies use discrete modules that are assembled into a device," explains Hu. These module-based devices tend to be considerably more bulky than fully integrated systems.

"In a commercial terahertz transmitter–receiver unit, the central module alone measures typically around 190 by 80 by 65 millimeters, which is roughly 1 million cubic millimeters," says Hu. The novel design of Hu's team unites the essential components of a terahertz device in a smaller two-dimensional area of just a few millimeters along each side. According to Hu and his co-workers, this compact device paves the way toward the mass production of a fully integrated terahertz system.

As the next step, the team will use the IME's cutting-edge technologies to build more complex structures composed of several two-dimensional layers, which will be based on their new designs. Although the team is not pursuing any specific applications, their devices potentially open up a wide range of possibilities. These include wireless short-range transfers of datasets — the content of a Blu-ray disc could be sent in as little as a few seconds, for example — high-resolution biosensing, risk-free screening of patients and passengers and see-through-envelope imaging (see image).

1. Hu, S., Xiong, Y.-Z., Zhang, B., Wang, L., Lim, T.-G. *et al.* A SiGe BiCMOS transmitter/receiver chipset with on-chip SIW antennas for terahertz applications. *IEEE Journal of Solid-State Circuits* **47**, 2654–2664 (2012).

Adding gold particles of 40 nanometers in diameter to the surface of a gold-coated prism boosts the sensitivity of chemical sensors.

Sensors:

Going for gold

Specifically sized gold nanoparticle spheres increase the sensitivity of a light-based chemical detector

A sensor that relies on reflected light to analyze biomedical and chemical samples now has greater sensitivity, thanks to a carpet of gold nanoparticles. Xia Yu of the A*STAR Singapore Institute of Manufacturing Technology, along with her students and colleagues, has determined the ideal size of nanoparticle to improve surface plasmon resonance (SPR) sensors[1].

SPR sensors contain a prism with one face covered in a thin film of gold. As laser light shines through the prism, it mostly reflects off the gold into a detector. However, if the light hits the gold at a particular angle, some of it couples with electrons in the metal to produce electromagnetic waves called surface plasmon polaritons. Stronger coupling leads to less light being reflected toward the detector.

When a liquid sample flows across the gold film, it changes the refractive index in that region and slightly alters the angle at which the light arrives at the metal. This hampers the formation of polaritons meaning that more of the light is reflected toward the detector. Varying the angle of the laser beam and monitoring the intensity of the reflected light reveals the composition of samples flowing over the metal surface.

Other researchers have shown that gold nanoparticles can enhance the sensor's responsiveness. Incoming light sparks localized plasmon resonances around the nanoparticles that couple to the sensor surface, which causes larger changes in the intensity of the reflected light. This makes the device more sensitive to the light's angle of arrival and therefore able to detect lower concentrations of the chemicals being tested.

Yu's team calculated the optical responses of four different gold nanoparticles — ranging in diameter from 40 to 80 nanometers — determining that they would be most effective when held about 5 nanometers above the gold surface. The researchers then mounted the different nanoparticles onto gold films using a sulfur-containing molecule called dithiothreitol, which provided the optimum 5-nanometer gap.

The team's calculations had suggested that the electric field of the surface plasmon polaritons would be hundreds of times greater when 40-nanometer particles were added to the surface. "The stronger the electric field, the more sensitive the sensors," says Yu. Tests using different concentrations of glycerin and formamide solutions confirmed that the 40-nanometer particles did offer the greatest increase in sensitivity. "The detection limit is at least three orders of magnitude higher than current commercial SPR sensors," says Yu.

Yu now hopes to apply this discovery to ultrasensitive sensors that can detect traces of cancer biomarkers.

1. Zeng, S., Yu, X., Law, W.-C., Zhang, Y., Hu, R. *et al.* Size dependence of Au NP-enhanced surface plasmon resonance based on differential phase measurement. *Sensors and Actuators B: Chemical* **176,** 1128–1133 (2013).

Cybersecurity:

Plugging smart grid weaknesses

As well as company privacy, security frameworks for smart grids must protect the privacy of individual consumers

Power companies are increasingly upgrading to smart grids — national or state-based intelligent computer systems that collect information from consumers and suppliers in order to automatically improve the grid's efficiency and reliability. The National Institute of Standards and Technology in the United States has produced a set of cybersecurity guidelines, called NISTIR 7628, for smart grid programmers across the globe. However, Aldar Chan and Jianying Zhou at the A*STAR Institute for Infocomm Research in Singapore point out that, although the guidelines are comprehensive, they lack standardized instructions for scenarios that may arise with new technologies such as electric vehicles. Chan and Zhou have also identified two key weaknesses within NISTIR 7628[1].

New protocols are needed in the smart grid security framework to protect the privacy of individuals charging electric vehicles.

When people plug in and charge electric vehicles, the security risks bridge the 'cyberworld' and the real world. "If there is no binding of identities between the cyber and physical domains, how can we be sure the information provided by the smart grid accurately reflects what is happening in the real world?" asks Chan. "We have little knowledge about cross-domain vulnerabilities, not to mention security mechanisms to withstand coordinated cyber–physical attacks."

Chan and Zhou examined the NISTIR 7628 framework using the scenario of a person charging an electric vehicle on a smart power grid. This framework is designed to provide a very secure system because as well as requiring a user login to pay for electricity, the car itself also needs device authentication when plugged in. In this way, a car reported as stolen would be barred from charging. Nevertheless, there may be ways of altering plug-in systems that would allow stolen vehicles to charge.

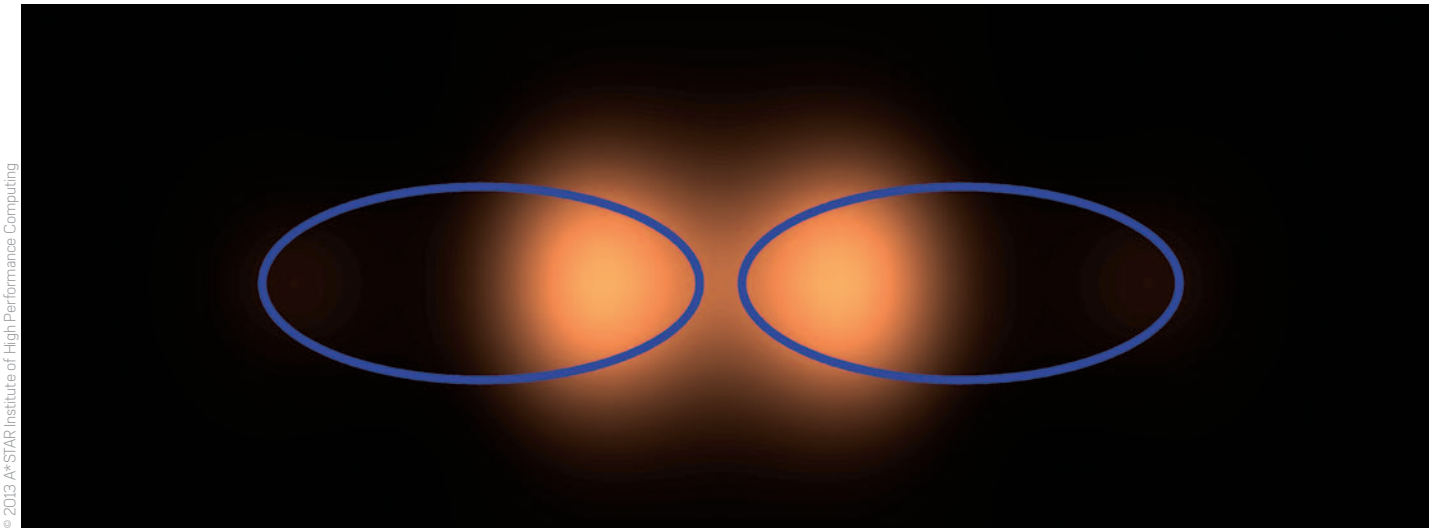
"NISTIR 7628 seems to separate cybersecurity from physical security without proper guidelines on how the two should be blended under this scenario," explains Chan. "These gaps could mean the system is open to a coordinated cyber–physical attack."

Chan and Zhou also examined the data that the smart grid system would hold. These include personal and banking details, and the physical location of the vehicle and how long it had been there — the perfect combination for criminals to exploit.

"NISTIR 7628 takes a utility company-centric perspective here," explains Chan. "Although there is caution about consumer privacy issues involving smart meters, little attention is paid to driver privacy."

Chan and Zhou are keen to improve the NISTIR 7628 framework: "We are developing a cyber–physical authentication protocol to strengthen login security, and a protocol to balance accountability and privacy regarding the location data the smart grid can hold on individuals."

1. Chan, A. C. & Zhou, J. On smart grid cybersecurity standardization: Issues of designing with NISTIR 7628. *IEEE Communications Magazine* **51**, 58–65 (2013).



An optical antenna made of silver, represented by the two blue ellipses, can concentrate red light at a subwavelength scale.

Optics:

Nanotechnology's benefits brought into focus

Mathematical modeling confirms that a nanometer-scale device that can concentrate light into a tiny spot improves optical sensing

Conventional lenses, made of shaped glass, are limited in how precisely they can redirect beams of incoming light and make them meet at a point. Now, a team led by Zhengtong Liu at the A*STAR Institute of High Performance Computing, Singapore, has proposed a novel approach to 'superlens' systems that can surpass this classical limit of focusing light[1].

The team used numerical modeling to develop the design. Concentrating radiation into a smaller volume in this way enhances the interaction between light and matter, and thus the concept could prove useful in highly sensitive sensors of the future.

Light is a type of wave. Unlike the rise and fall in seawater at a beach, however, a light wave consists of oscillating electric and magnetic fields. The wavelength — the distance a wave travels in one oscillation cycle — imposes a limit on the minimum size to which light can be focused. However, this limit does not apply over small distances that are comparable to the wavelength, which is known as the near-field regime.

The researchers designed a silver nanostructure embedded in glass. Their device combined two separate elements. One component was a nanoantenna — similar to the radio-frequency antennas used to detect television-carrying signals, but reduced in size to match the wavelength of optical radiation. The other component was a superlens made of a thin slab of silver. The purpose of the superlens was to move the light detected by the nanoantenna into an imaging plane. "Using nanoantennas to concentrate light is not a new idea," says Liu. "But by adding a superlens to translate the concentrated spot of light, we can overcome limitations imposed by the optical properties of the material."

"Our concept is targeted at biomedical and chemical sensing applications."

Liu and co-workers mathematically modeled the optical response of this device to an incoming beam of red light. They then altered the dimensions of the structure to maximize the enhancement in electric field. In this way, they were able to show that a 20-nanometer-thick superlens, separated by 34 nanometers from an antenna made of two silver ellipses, could increase the electric field of light by a factor of 250 (see image).

Confining light into these super intense 'hotspots' could prove a boon for optical detection systems. "Our concept is targeted at biomedical and chemical sensing applications," explains Liu. "The next step is to seek collaboration opportunities to actually make the sensor and test it in the field."

1. Liu, Z., Li, E., Shalaev, V. M. & Kildishev, A. V. Near field enhancement in silver nanoantenna-superlens systems. *Applied Physics Letters* **101,** 021109 (2012).

Data storage:

Maintaining privacy on the cloud

A data-sharing scheme utilizing an encryption manager shows the way toward low-cost, flexible and secure cloud storage services

Wider adoption of cloud storage services by organizations has been hindered by security and privacy issues. A consequence of storing data on the cloud is that, by its very nature, the storage infrastructure is not owned by the same organization that owns the data. In addition, the data of one user is stored along with that of many others. Traditional schemes for ensuring security can only protect data privacy by sacrificing convenient operations such as searching and sharing.

Now, Shu Qin Ren and his colleague Khin Mi Mi Aung at the A*STAR Data Storage Institute in Singapore have devised a scheme that would not only allow organizations to store data on the cloud without loss of privacy but also permit searching and sharing of the data[1]. The system is able to preserve the benefit of the cloud's specialized low-cost storage infrastructure while overcoming its current privacy and flexibility limitations. "The scheme may potentially push forward the wider adoption of cloud storage usage for organizations," says Ren.

A data-sharing scheme involving an encryption key manager could make storage on the cloud secure as well as searchable.

The solution proposed by the researchers involves a central 'key manager', who specifically manages data authentication and access authorization. In their scheme, data stored on the cloud is encrypted by its owner and hence is indecipherable to anyone else — including the cloud storage provider. A secret key required to unlock the encryption is generated and kept by the owner, who also determines an access policy for other users. This policy is implemented by the key manager, who generates a second access key, which is then passed back to the owner. Next, the owner wraps the original encryption key in this second layer of protection. The key manager is then able to pass on the second 'public' key to authorized third parties to allow them to access the data.

Under traditional privacy schemes, the owner manages both the encryption of and access to their data. Sharing with a third party typically involves retrieval and decryption of the data by the owner and therefore some loss of privacy. Under Ren and Aung's scheme — entitled 'Privacy Preserved Data Sharing' — the third party only deals with the key manager and, after authorization, receives the public key without interacting with the data's owner, thus allowing privacy to be maintained.

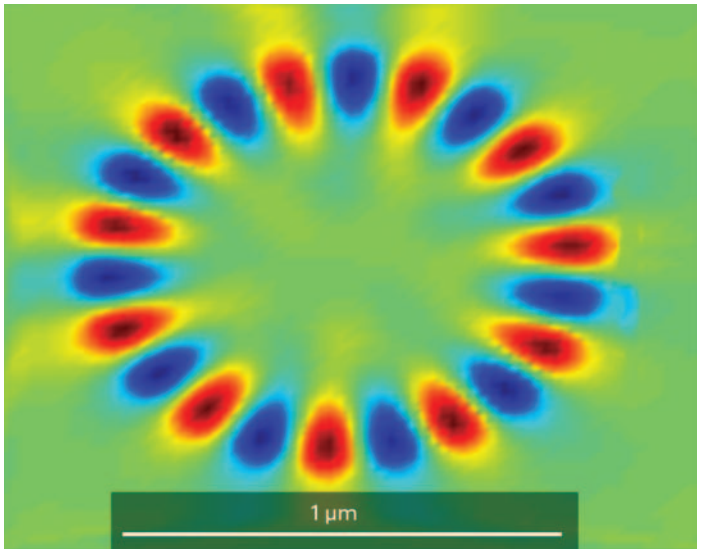
"The research team is now building a secure data searching and sharing prototype to test on structured data such as in databases," says Ren. "The next step is to support unstructured data."

1. Ren, S. Q. & Aung, K. M. M. PPDS: Privacy Preserved Data Sharing scheme for cloud storage. *International Journal of Advancements in Computing Technology* **4**, 493–499 (2012).

Optics:

A step in time saves two

A technique that reduces the time to simulate the operation of active optical devices aids the design of nanoscale lasers



An efficient FDTD simulation can quickly calculate the electric and magnetic field patterns inside a nanocavity laser.

Tiny optical components — the heart of modern communications systems — might one day increase the operational speed of computers. When designing these components, optical engineers rely on mathematical simulations to predict the performance and efficiency of potential devices. Now, Qian Wang at the A*STAR Data Storage Institute and co-workers have developed a neat mathematical trick that more than doubles the speed of this usually slow computation[1]. Their method also enables more accurate modeling of increasingly complicated structures.

In the mid-nineteenth century, the physicist James Maxwell established a set of equations that describe the flow of light. The oscillating electric and magnetic fields of an optical pulse react to the optical properties of the medium through which it is travelling. "Combining Maxwell's equations with equations that describe light–matter interactions can provide a powerful simulation platform for optoelectronic devices," explains Wang. "However, running the computations is usually time-consuming."

"Currently we are applying our approach to design integrated nanolasers as a next-generation on-chip light source for various applications."

Finite-difference time-domain (FDTD) simulations are a well-established method for modeling the flow of light in optical devices. This technique models a device as a grid of points and then calculates the electric and magnetic fields at each position using both Maxwell's equations and knowledge of the fields at neighboring points. Similarly, calculating the time evolution of light using Maxwell's equations is simplified by considering discrete temporal steps. Smaller spatial and temporal steps yield more accurate results but at the expense of a longer calculation time.

Electron density in a semiconductor is a key determiner of a material's optical properties. This density varies at a slower rate than the electric and magnetic fields of the optical pulse. Wang and his colleagues therefore eliminated calculation of this material property at every time step to shorten the calculation.

The researchers proved the usefulness of their approach by modeling a semiconductor laser, consisting of a cylindrical cavity 2 micrometers in diameter that traps light at its edges (see image). The trapped light supplies the optical feedback required for lasing. They simulated the operation of this device using an FDTD spatial grid with a 20-nanometer resolution and 0.033 femtosecond time steps. The calculated field pattern in the cavity was the same whether the active optical properties of the semiconductor were calculated at every time increment or once every 100 steps. Yet, this simplification reduced the computation time by a factor of 2.2.

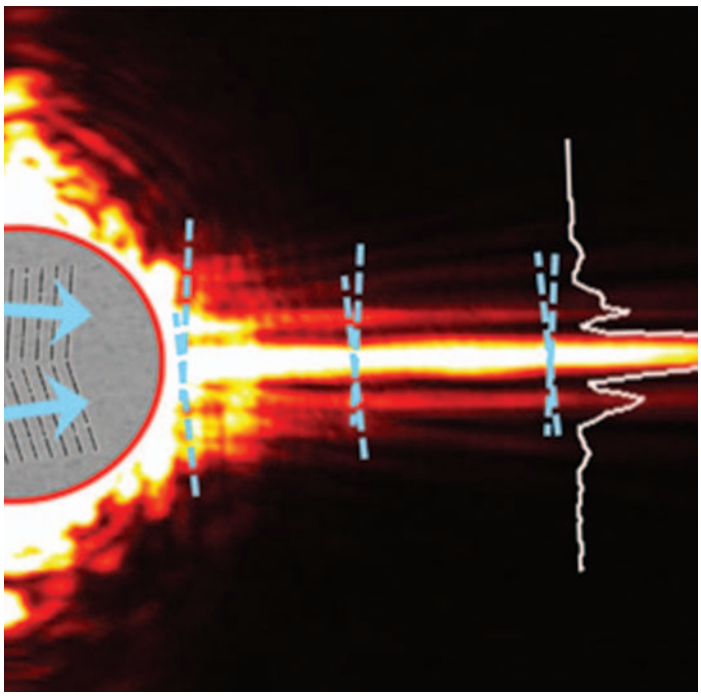
"Currently we are applying our approach to design integrated nanolasers as a next-generation on-chip light source for various applications," says Wang.

1. Ravi, K., Wang, Q. & Ho, S.-T. Efficient FDTD simulation of active photonic devices with multiple temporal resolutions. *IEEE Photonics Technology Letters* **24,** 584–586 (2012).

Plasmonics:

A wave without diffraction

Optical computing could benefit from the recent development of a novel electromagnetic wave



When laser light hits grooves in a thin film of gold (left), it generates surface plasmon polariton waves (cyan dashed lines) that converge and interfere to create a nondiffracting beam (orange).

An unusual wave that does not spread out as it travels could become a key component in speedy computer chips that use beams of light to carry and process data. Jiao Lin, a physicist at the A*STAR Singapore Institute of Manufacturing Technology, helped to develop the electromagnetic wave, which can travel some 80 micrometers in a straight line without diffracting[1].

The wave is formed when light hits the surface of a metal, creating ripples in the sea of electrons there. Under certain conditions, the ripples — known as surface plasmons — can couple with the incoming light to create electromagnetic waves that stick tightly to the metal surface as they travel. Known as surface plasmon polaritons, these waves have a shorter wavelength than the light, which makes them more attractive as data carriers.

Although light can zip around a computer much faster than electrons, optical components tend to be much larger than those in conventional circuits — their size is dictated by the wavelength of the light they handle. Using surface plasmon polaritons offers the best of both worlds, explains Lin, because the signals can travel at the speed of light along metal waveguides that are as compact as conventional circuits. Unfortunately, surface plasmon polaritons diffract as they travel over the metal, which erodes the quality of the signals they carry. Previous attempts to prevent this diffraction were moderately successful, but caused the polaritons to veer off course.

The wave developed by Lin and co-workers is a previously unknown solution to Maxwell's equations, which describe how electromagnetic fields behave. Once the team had formulated a mathematical description of this wave, known as a localized cosine-Gauss beam, Lin helped to turn it into a reality. The team carved two sets of tiny grooves, each roughly 10 micrometers long, into a thin layer of gold stuck to a glass backplate. They slightly angled the grooves to make a chevron pattern (see image).

Shining near-infrared laser light at the grooves generated two surface plasmon polaritons that soon converged and interfered constructively with each other. This resulted in a tightly focused beam that skimmed across the gold without diffracting, covering a much greater distance than previous efforts had achieved. The team tracked the narrow beam as it traveled over the surface using a near-field scanning optical microscope.

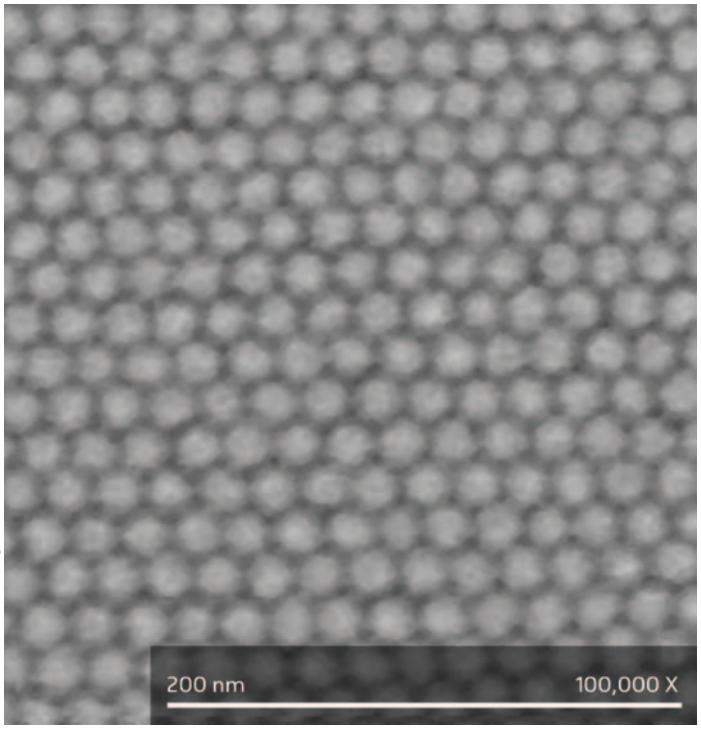
Lin says that as well as helping to create faster and more energy-efficient computers, the beams could also be used in the laboratory to trap and manipulate nanoparticles.

1. Lin, J., Dellinger, J., Genevet, P., Cluzel, B., de Fornel, F. & Capasso, F. Cosine-Gauss plasmon beam: A localized long-range nondiffracting surface wave. *Physical Review Letters* **109**, 093904 (2012).

Data storage:

Synchronized at the write time

Numerical simulations show how to avoid imperfections in the next generation of high-density data storage



A scanning electron microscope image of a bit-patterned recording medium, with ordered arrays of magnetic islands.

The rise of the Internet and the move from paper to digital information has driven a need for large-volume electronic data storage. Maria Yu Lin and her co-workers at the A*STAR Data Storage Institute, Singapore, have now established some important design principles to consider when developing bit-patterned media recording (BPMR)[1,2] — a potential high-density magnetic recording system of the future.

Conventional hard disk drives store a single data bit in a continuous magnetic medium consisting of many 'grains'. However, the number (approximately 10–15) and the size of these grains (about 6–10 nanometers) naturally limits the maximum density at which digital information can be stored. The BPMR technique offers much higher storage capacity because it records the data in a regular array of single-grain magnetic islands (see image), which can be much smaller than multiple grain bits in continuous media, according to Lin.

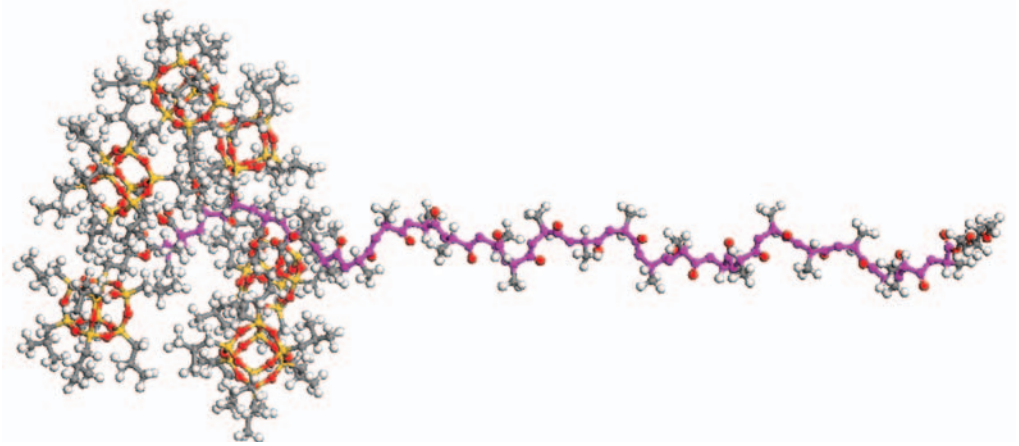
"Multiple grains must be used per data bit in continuous-media storage," she explains. "Ideally, bit-patterned media [will] achieve one grain per bit because the magnetic cells are patterned in isolated and ordered arrays known as 'islands'."

A number of practical hurdles, however, are preventing the use of BPMR in computer hard disks. One problem is that the islands are separated by non-magnetic spaces — only some 25–65% of the surface is magnetic. The data can be passed from the writing 'head' only when it is aligned with an island on the spinning disk. Therefore, the writing process must be synchronized with the position of the magnetic islands. However, manufacturing defects, variations in disk spinning speed and vibrations can all cause temporal misalignment, which in turn causes writing errors.

Adding information to the disk that tells the writing head its exact position is one way to correctly time the writing process. This includes synchronization sectors and error correction information; however, this information reduces the capacity of the disk for data storage. Lin and co-workers used computer simulations to theoretically analyze the optimum number of the synchronization sectors. They also analyzed how the additional information would relate to variations in disk spin speed. They investigated the system with read/write spindle motors suffering from high, medium, low and zero speed variation.

"The analysis indicates that the total additional information needed for synchronization and error correction for a motor with a medium rotation variation is 11.75%," says Lin. "Compared to the potential gain in terms of data density that this technology enables, such a total overhead is acceptable."

1. Lin, M. Y., Chan, K. S., Chua, M., Zhang, S., Kui, C. & Elidrissi, M. R. Modeling for write synchronization in bit patterned media recording. *Journal of Applied Physics* **111**, 07B918 (2012).
2. Lin, M. Y., Elidrissi, M. R., Chan, K. S., Eason, K., Chua, M. *et al.* Channel characterization and performance evaluation of bit-patterned media. *IEEE Transactions on Magnetics* **49**, 723–729 (2013).



Polylactic acid (PLA)-based organic–inorganic polymers (above) self-assemble into nanoparticle spheres with the potential for drug delivery. During polymerization, PLA (magenta) forms one of two mirror-image structures.

Nanoparticles:

Polymer knots with silicon hearts

Biocompatible complexes for drug delivery applications get a structural boost from nanoscale silicon cages

Protein-based drugs show promising activity against many hard-to-treat targets. Getting these biomolecules past the body's numerous defenses, however, requires innovative technology such as drug-delivering nanoparticles. Polylactic acid (PLA) is a potential candidate because it is nontoxic, biodegradable, and spontaneously assembles into tiny structures under the right conditions. Chaobin He from the A*STAR Institute of Materials Research and Engineering in Singapore and co-workers have developed a robust method to synthesize PLA nanoparticles using copolymer technology and a rigid 'nanocage' made from silicon[1].

During polymerization, PLA forms into one of two mirror-image compounds, known as L-type or D-type (see image). When chemists mix L- and D-type PLA chains together, their complementary shapes interlock through a process known as stereocomplexation. Recently, chemists have found that constructing PLA chains containing discrete 'blocks' of L- and D-compounds brings unprecedented control over nanoparticle formation — allowing them to produce distinct shapes.

Although stereocomplexation improves the mechanical attributes of PLA nanoparticles, many of these compounds aggregate undesirably after a few days in water. He and his team investigated whether they could retain the nanoparticles' shape using silsequioxane, a stiff and small framework of silicon–oxygen atoms that has a strong record of boosting polymer strength at the molecular level.

After connecting silsequioxane to individual L- and D-type PLA chains, the researchers used a process called atom transfer radical polymerization to generate organic–inorganic hybrid co-polymers with well-defined PLA and silsequioxane segments. When they mixed two block copolymers with complementary L- and D-PLA segments into polar organic solvents that hold slight electrical charges, the chains self-assembled into nanoscale spheres. Because copolymers without matching L- and D-segments remained in solution under the same conditions, the team deduced that stereocomplexation is the primary force driving nanoparticle formation.

Experiments revealed that the silicon nanocages significantly improved PLA nanoparticle stability: even after a month in diluted aqueous solution, these hybrid compounds retained their unique shapes. Furthermore, the team found that incorporating longer silsequioxane units into the PLA chains caused the nanoparticles to assemble into smaller spheres. According to He, this suggests that the inorganic constituent can influence the probability of stereocomplexation — findings that open opportunities to precisely tune nanoparticle size and shape.

He and co-workers anticipate that their nanoparticles might enhance the properties of PLA plastics used for medical implants by acting as novel 'filler' substances. He explains that the tiny compounds should enhance interfacial adhesion inside large sheets of PLA, thereby augmenting its ductility and toughness.

1. Tan, B. H., Hussain, H., Leong, Y. W., Lin, T. T., Tjiu, W. W. & He, C. Tuning self-assembly of hybrid PLA-P(MA-POSS) block copolymers in solution *via* stereocomplexation. *Polymer Chemistry* **4**, 1250–1259 (2013).

Fluid dynamics:

Resolving shockwaves more accurately

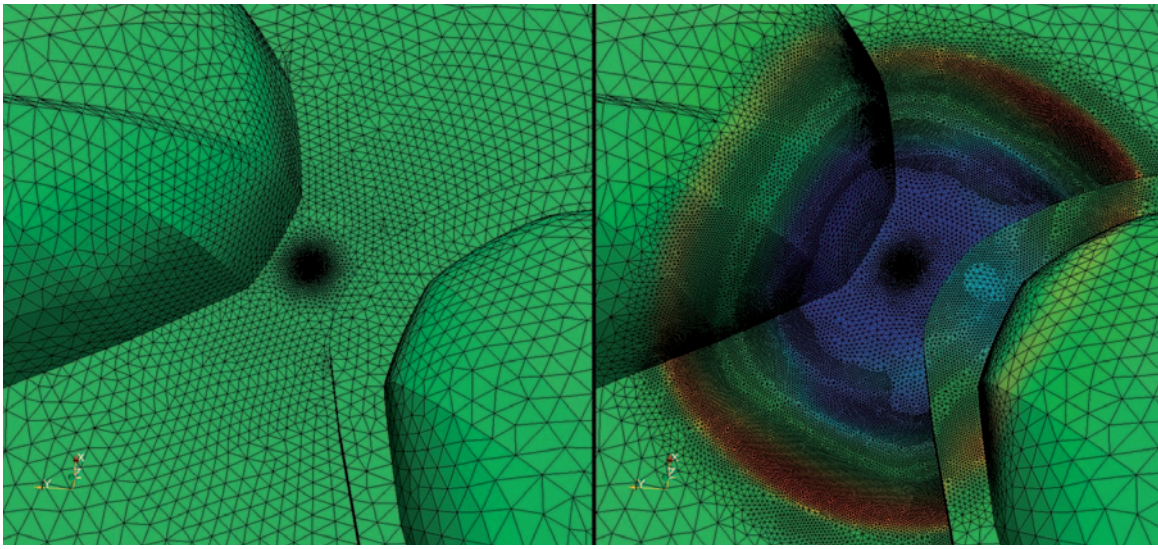
A new computational scheme enables more stable simulations of shockwaves in fluids and may be scalable for large engineering designs

Vinh-Tan Nguyen and co-workers at the A*STAR Institute of High Performance Computing in Singapore have developed a more robust and efficient way to simulate shockwaves under various flow scenarios[1]. Previous techniques for shockwave simulation are specific to particular flow problems, whereas this new method is applicable to shockwaves in any high-speed flow scenario, for example in aerodynamics or explosions.

A shockwave is generated when a discontinuous change in fluid properties follows an abrupt increase in the pressure, temperature and density of the flow. "Strong and unsteady shockwaves can produce oscillations, which affect the stability of numerical solutions in the three-dimensional (3D) computational domain," explains Nguyen. "The main aim of our technique is to resolve the front of a shockwave while preserving the overall accuracy of the simulation."

In computational fluid dynamics, flows can be simulated with different levels of accuracy — a low-order approximation is based on a hypothesis, whereas a high-order approximation is one that is closest to reality, or the 'finest-tuned' approximation. Simulation accuracy is maintained by using as high-order approximation as possible, as well as by altering the resolution of the 3D computational mesh — a grid of interconnected data points that covers the spatial area of the flow.

"Simulating flows using high-order approximations triggers oscillations, which cause miscalculations at the front of shockwaves where the flow is discontinuous," explains Nguyen. "It therefore becomes counterproductive to have high-order approximations in place right across shock regions."



Simulations of shockwaves in fluids as they initiate (left) and propagate (right), using a specially tuned computational mesh.

"With precise detection through the shockwave sensor we can apply the right capturing scheme to treat each shockwave."

To overcome this problem, Nguyen and his team placed a shockwave sensor within the flow to identify high-gradient shockwave fronts as they appeared. They then applied shock-capturing schemes to resolve the fronts by reducing the approximation order in those specific regions.

Finally, the researchers increased the spatial resolution of the computational mesh in the localized shock areas to compensate for the lower-order approximations (see image). The 3D mesh is also programmed to rebuild itself following contact with a shockwave.

"With precise detection through the shockwave sensor we can apply the right capturing scheme to treat each shockwave, regardless of its strength," explains Nguyen. "Our mesh adaptation procedure then simultaneously refines the mesh in shockwave regions and coarsens it in areas of least change, reducing computational costs significantly."

In addition to its potential application in aerodynamics and blast analysis, the researchers believe that this scheme may be useful for simulating the interface between air and water, with huge potential for marine and offshore applications.

1. Nguyen, V.-T., Nguyen, H. H., Price, M. A. & Tan, J. K. Shock capturing schemes with local mesh adaptation for high speed compressible flows on three dimensional unstructured grids. *Computers & Fluids* **70**, 126–135 (2012).

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.

A*STAR currently oversees the following research institutes, consortia and centers and supports extramural research with universities, hospital research centers, and other local and international partners.

A*STAR Computational Resource Centre (A*CRC)
Bioinformatics Institute (BII)
Bioprocessing Technology Institute (BTI)
Data Storage Institute (DSI)
Experimental Power Grid Centre (EPGC)
Experimental Therapeutics Centre (ETC)
Genome Institute of Singapore (GIS)
Institute for Infocomm Research (I^2R)
Institute of Bioengineering and Nanotechnology (IBN)
Institute of Chemical & Engineering Sciences (ICES)
Institute of High Performance Computing (IHPC)
Institute of Materials Research and Engineering (IMRE)
Institute of Medical Biology (IMB)
Institute of Microelectronics (IME)
Institute of Molecular and Cell Biology (IMCB)
National Metrology Centre (NMC)
SERC Nanofabrication and Characterisation Facility (SNFC)
Singapore Bioimaging Consortium (SBIC)
Singapore Cancer Syndicate (SCS)
Singapore Consortium of Cohort Studies (SCCS)
Singapore Immunology Network (SIgN)
Singapore Institute for Clinical Sciences (SICS)
Singapore Institute of Manufacturing Technology (SIMTech)
Singapore Stem Cell Consortium (SSCC)
The Chemical Synthesis Laboratory (CSL)


SINGAPORE