A*STAR RESEARCH

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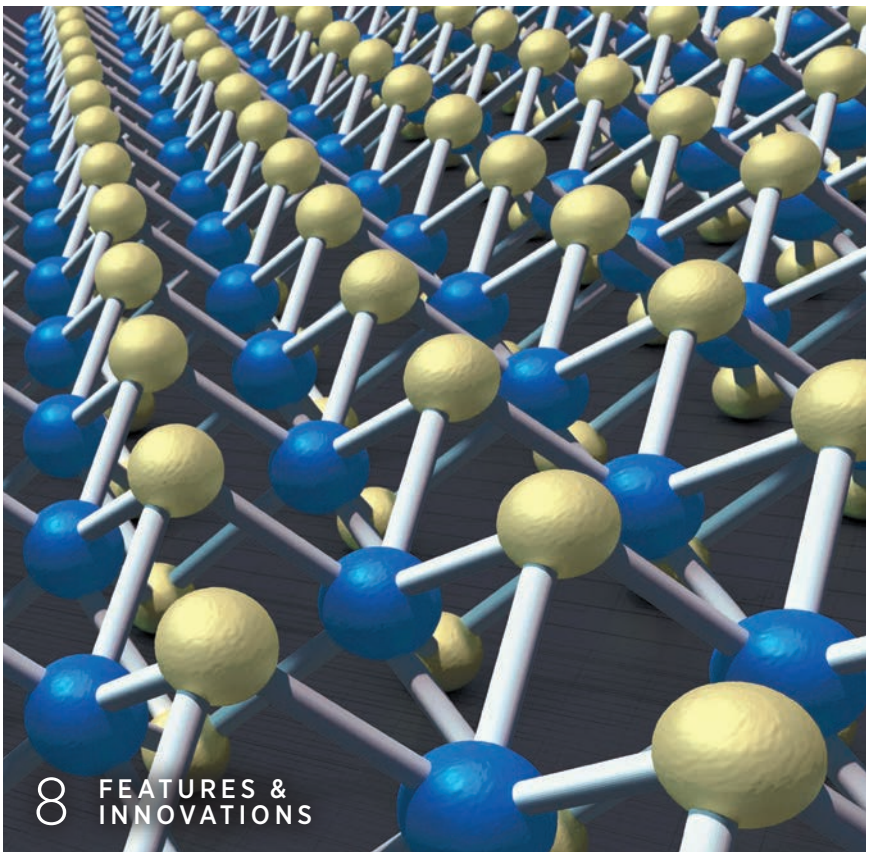

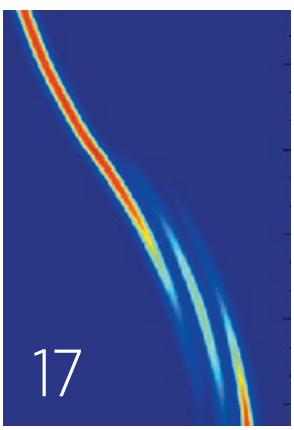

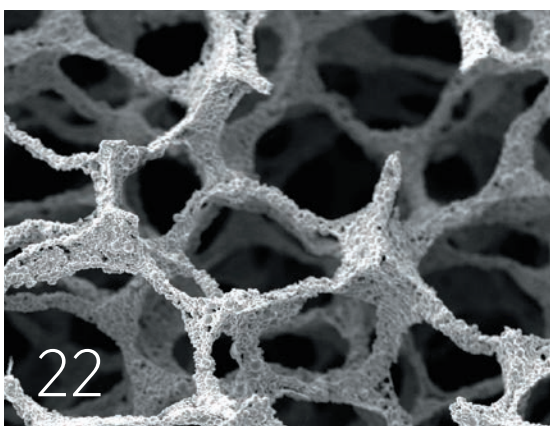

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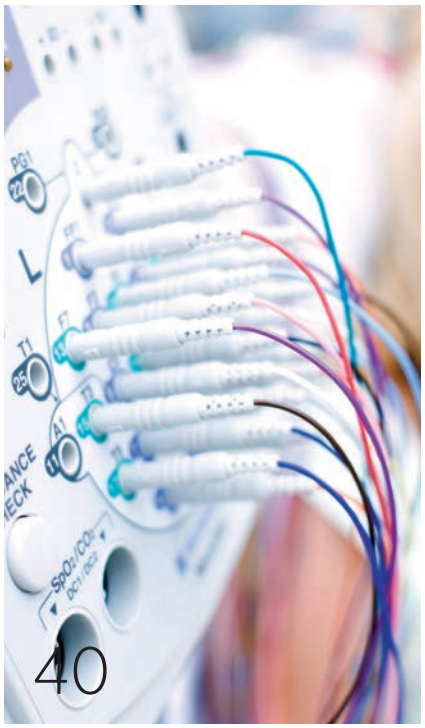

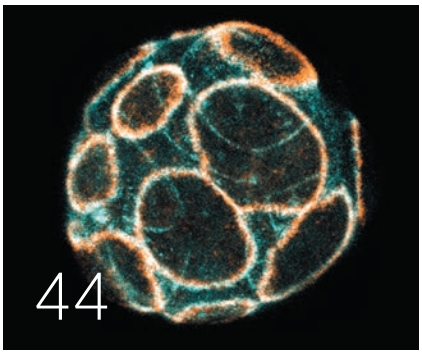

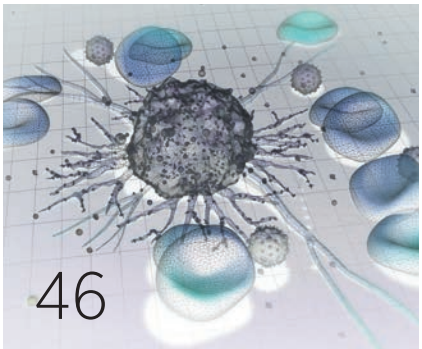

NOTES FROM THE EDITORS

The Editorial Team introduces the latest issue of *A*STAR Research*

Imagine if you never had to charge your smart watch at home again. Charging wearable technology by converting low-grade heat to electricity could soon be a possibility thanks to the thermoelectric materials discussed in our cover story, 'Putting body heat in the device game', on page 4.

Our second feature looks at the emerging field of valleytronics (page 8). Valleytronics technology, based on two-dimensional materials, such as molybdenum disulfide, could lead to smaller, less energy intensive devices.

As usual, this issue is packed with research highlights on a wide range of topics: on page 12, a team from the Institute of High Performance Computing have come up with a model that could help reduce the amount of time commuters spend waiting at the bus stop. A microfluidic 'skin on a chip' device, developed by researchers from the Singapore Institute of Manufacturing Technology and the Institute of Medical Biology, which could serve as an alternative to animal testing is discussed on page 33. And on page 49, a team from the Genome Institute of Singapore have identified more than 30 mutation hotspots for gastric cancer by harnessing the power of machine learning.

For our latest stories, please visit us at **research.a-star.edu.sg** or follow us on Twitter at: **@astar_research**. We hope you will enjoy reading the rest of this magazine.

COVER IMAGE
Could body heat be used to charge devices? [page 4]
© Rancz Andrei / Alamy Stock Photo

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PUTTING BODY HEAT IN THE DEVICE GAME

If thermoelectric materials can convert low-grade heat into electricity, we may never need to charge wearable technology at home again.

At night, most of us plug in a jumble of wires and devices as we charge our smart watches, phones and fitness trackers. It's a pile that's unlikely to get any smaller as more and more wearable tech enters our lives. Manufacturers and futurists predict that these will soon be energy self-sufficient and that we'll be free of their cords. But the question remains: how? At the moment, the only major portable power sources are solar chargers, but these have significant limitations both indoors and after dark.

Kedar Hippalgaonkar, Jianwei Xu and their co-workers at A*STAR's Institute of Materials Research and Engineering (IMRE) think they could soon use low-grade waste heat – think car exhaust or body heat – to power devices.

"An enormous amount of low-grade waste heat is being dumped into the environment," says Hippalgaonkar. Converting this heat into electricity is a big opportunity.

High-temperature thermoelectric generators are already a key source of power for space instruments. The Mars rover, Curiosity, and the interstellar space probe, Voyager 2, harness long-lasting nuclear heat. The latter has been running on this type of power for more than 40 years. "Thermoelectric power generation is not a new idea," explains Hippalgaonkar. "It's been investigated since the 1950s and there's been lots of research on new materials, but in the past most of the work focused on toxic, inorganic materials and applications with high temperatures of operation."

Hippalgaonkar observes that the proliferation of Internet of Things devices now brings with it a demand for non-toxic, portable power sources. Future body sensors and portable devices could be worn constantly if they harnessed enough body heat. "But to do that we need to develop suitable new thermoelectric materials that are efficient at lower temperatures, non-toxic and cheap to produce."

Another major opportunity is to make use of waste heat in engine exhaust from cars, aeroplanes or ships, he adds. The electricity generated could then be fed back into the vehicle, lessening its environmental footprint.

A*STAR's PHAROS project is focused on the materials that will make these thermoelectric generators possible. The five-year project started in 2016 and aims to find a material composition that is non-toxic and abundant, making it cheap, efficient, and easy to fabricate. To do this they are developing less toxic hybrid materials combining organic and inorganic elements, and they are pursuing those with potential for low temperature thermoelectric power generation.

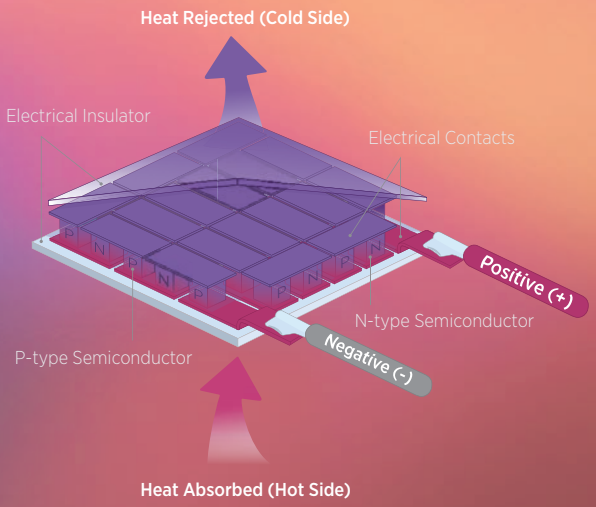
(Background) © jhillphotography/Getty Images; © traffic_analyzer/Getty Images; © Joseph Giacomin/Getty Images; (Diagram) © 2018 Nature Research

If body heat could be harnessed to power electric devices, then we could possibly one day stop worrying about our battery power.

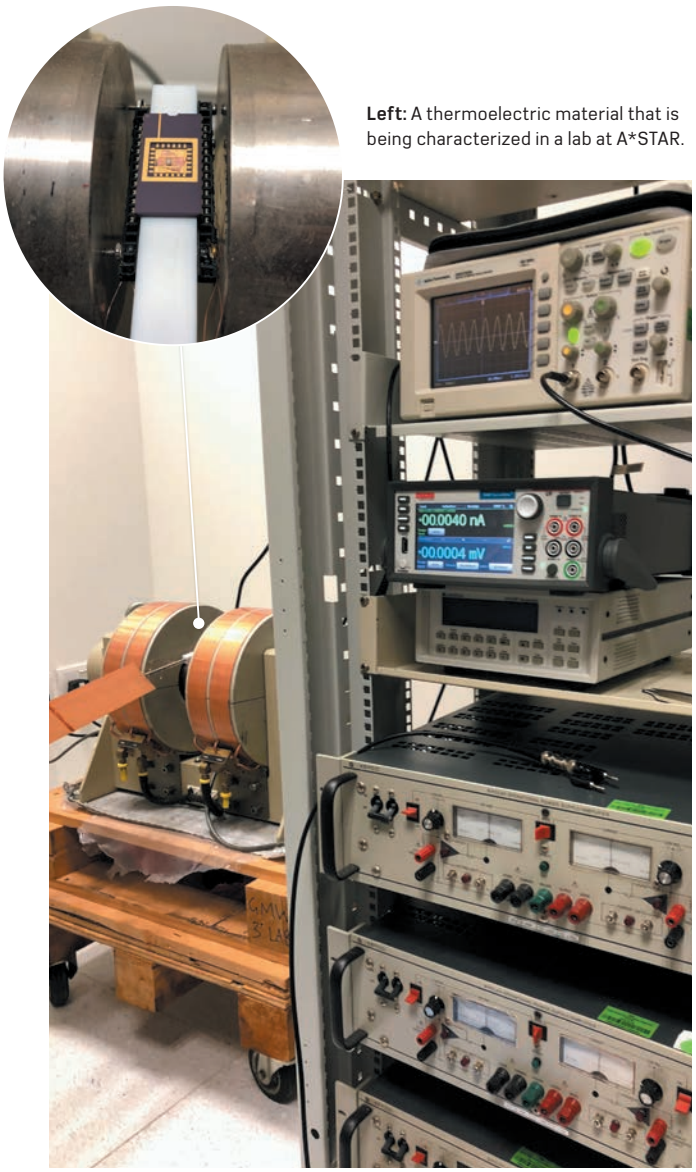
THERMOELECTRIC GENERATORS EXPLAINED

A thermoelectric generator (TEG) is a device that converts a temperature difference into a voltage, and manages the flow of electrical current around a circuit. It is a means for converting waste heat into electricity. Such devices operate due to the Seebeck effect, which was discovered by the German physicist Thomas Johann Seebeck in 1821.

A TEG is typically made by using p- and n-type doped semiconductors to create two paths that connect to metal electrodes of different temperatures, one hot, one cold. The Seebeck effect means that holes (positive electrical charge carriers) in p-type material and the electrons (negative charge carriers) in the n-type material diffuse from the hot electrode to the cold electrode, thus yielding a voltage and current flow. The process can also be operated in reverse, when it's known as the Peltier effect and the injection of an electrical current induces cooling at the material junction. Thermoelectric coolers, also known as Peltier coolers, are often used in small-scale devices to control the temperature of sensitive electronic and optoelectric devices such as laser diodes and photodetectors.



"Thermoelectrics provides you the opportunity to realize self-powered sensors"



Left: A thermoelectric material that is being characterized in a lab at A*STAR.

A sophisticated range of equipment is needed to fully characterize thermoelectric materials. For example their thermal conductivity, electrical conductivity and Seebeck coefficient all need to be measured.

The project brings together Hippalgaonkar, a solid-state physicist and an expert in the behavior of phonons, photons and electrons in nanoscale and 2D materials, and Jianwei Xu, a chemist with an extensive research background in organic materials, especially semiconducting polymers.

Turning down the heat on thermal power

To charge personal devices using thermoelectric materials, a generator harnesses the Seebeck effect, in which a temperature difference creates an electrical voltage at the junction between two different materials (often, but not exclusively p-doped and n-doped semiconductors). This voltage can be used to drive a device or charge a battery. (For more information see 'Thermoelectric generators explained' on page 5)

To date, the most well established and successful thermoelectric materials have been based on metal tellurides, including lead telluride and bismuth telluride. These are behind long-running NASA space probes. But they only work well at high temperatures, and in space an on-board nuclear isotope is used to generate this heat and to create a high temperature differential. The approach can act as a long-term, local power source, but the potential health risks of nuclear radiation mean it's not suitable for many terrestrial applications.

"There's a lack of efficient materials that operate at around room temperature and that's what we want to address with the PHAROS project," says Xu. However, it's a challenging task to identify new candidate thermoelectric materials, fabricate them and then understand what is happening to charge transfers inside them.

To date, the PHAROS team has been exploring a wide variety of conjugated semiconducting polymers (such as Polyaniline, P3HT or PEDOT:PSS) for the organic component of their hybrids, which are then combined with an inorganic component made from, say, silicon nanoparticles or 2D materials like MoS_2 and $MoSe_2$. With these, they have investigated the use of carbon nanotubes as an additive.

The team has also explored the thermoelectric potential of methylammonium lead iodide perovskites, an inorganic-organic hybrid material system that has shot to fame in recent years following its successful use in solar cells. This hybrid material rivals silicon in terms of power conversion efficiency. The big advantage of using a part-organic system is that it suits solution processing, which produces large-area, thin, flexible materials that could be cheaply ink-jet printed.

However, for a thermoelectric material to work well it ideally needs to have a large Seebeck coefficient, which is indicative of how large the voltage generated will be for a given temperature difference. And it is also important for the material to have high electrical conductivity to allow a charge to flow easily, along with low thermal conductivity to support the temperature gradient in place.

"It's very hard to achieve these attributes simultaneously," says Hippalgaonkar. "You ideally want to find a material that combines

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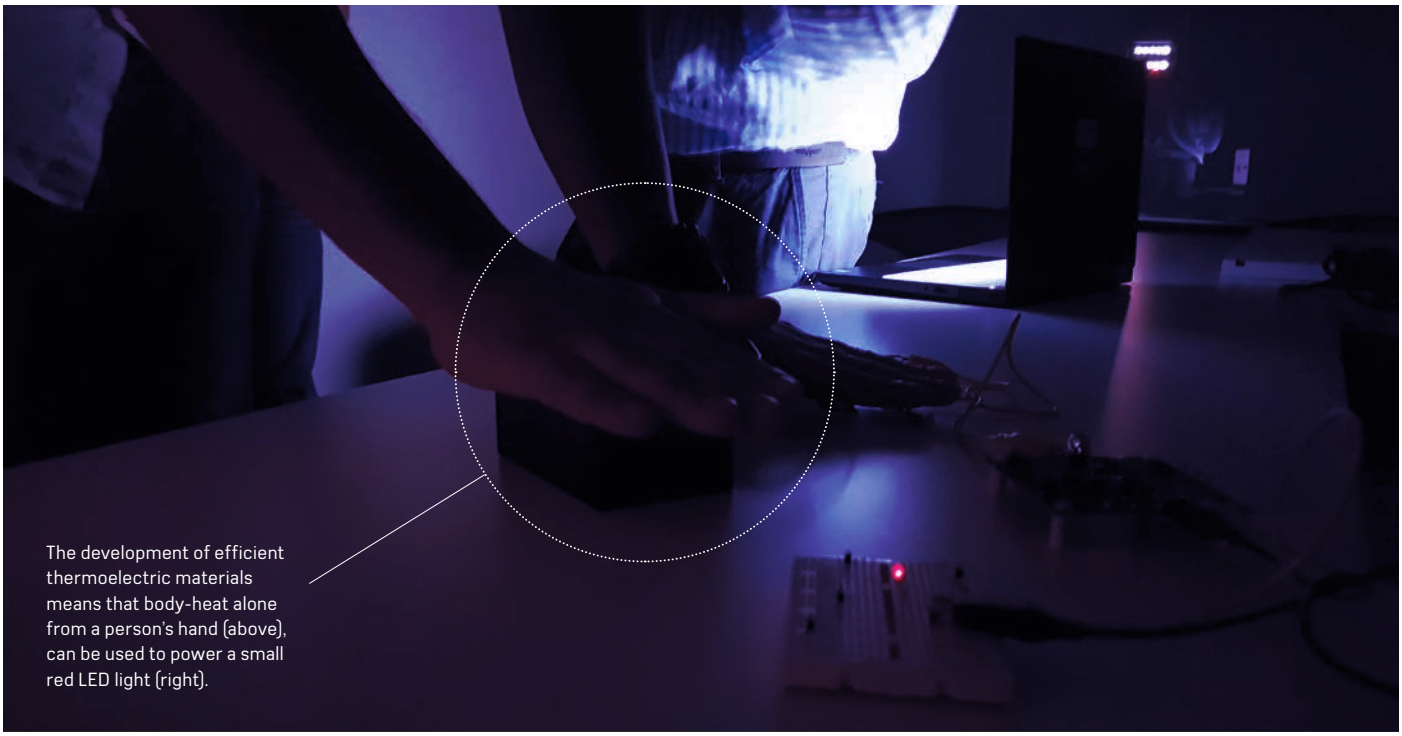

The development of efficient thermoelectric materials means that body-heat alone from a person's hand (above), can be used to power a small red LED light (right).

the low thermal conductivity of wood with the high electrical conductivity of a metal and that's not easy to do."

Materials that have a perfect score

To make comparisons between materials easier, something called the 'ZT value' was developed to take into account the Seebeck coefficient, thermal conductivity, electrical conductivity and temperature. "We really want something that has a ZT of roughly 1," says Xu, although a ZT number that high isn't necessary for a lot of uses. At present, a 1 can be achieved in bismuth telluride and lead telluride, but both materials are toxic, expensive to manufacture and rigid.

Recently, the PHAROS team has developed a safer material. They did this in a collaboration with researchers at US-based Lawrence Berkeley National Laboratory (LBNL) by optimizing a materials system that combines a carefully designed conjugated polymer with tellurium nanowires. Encouragingly, ZT values of roughly 0.1-0.2 have been achieved, as described in a recent paper.

This discovery was helped along by Shuo-Wang Yang at the Institute of High Performance Computing and his team, who helped to explain the interactions between the organic and the inorganic constituents of materials prepared by Jeff Urban's team at LBNL. With experimental and theoretical work done by Hippalgaonkar's team, the physics of how charge flows in these complex materials was detailed for the first time, laying a strong basis for future development.

"The interface between the organic and inorganic interface is very important to study," Hippalgaonkar explains. "The physics of how charge moves through such a complex landscape is very challenging to understand."

"Thermoelectric will be able to provide you the opportunity to realize self-powered sensors fastest," says Hippalgaonkar. Heart rate monitors for example have very modest power needs, on the scale of a few hundreds of microwatts. In theory, PHAROS's most recent material can achieve 10 microwatts per square centimeter. So, small-scale wearable themoelectric power is already tantalisingly close to reality, Hippalgaonkar says. And once its commercial promise starts to come into play, their work will only accelerate. ■

A material with a ZT of 1 operating at a temperature difference of 10 ˚C at room temperature generates roughly 50 microwatts of power. Enough to power a heart rate monitor.

For references, visit the online version of this article at:
www.research.a-star.edu.sg/feature-and-innovation/7839

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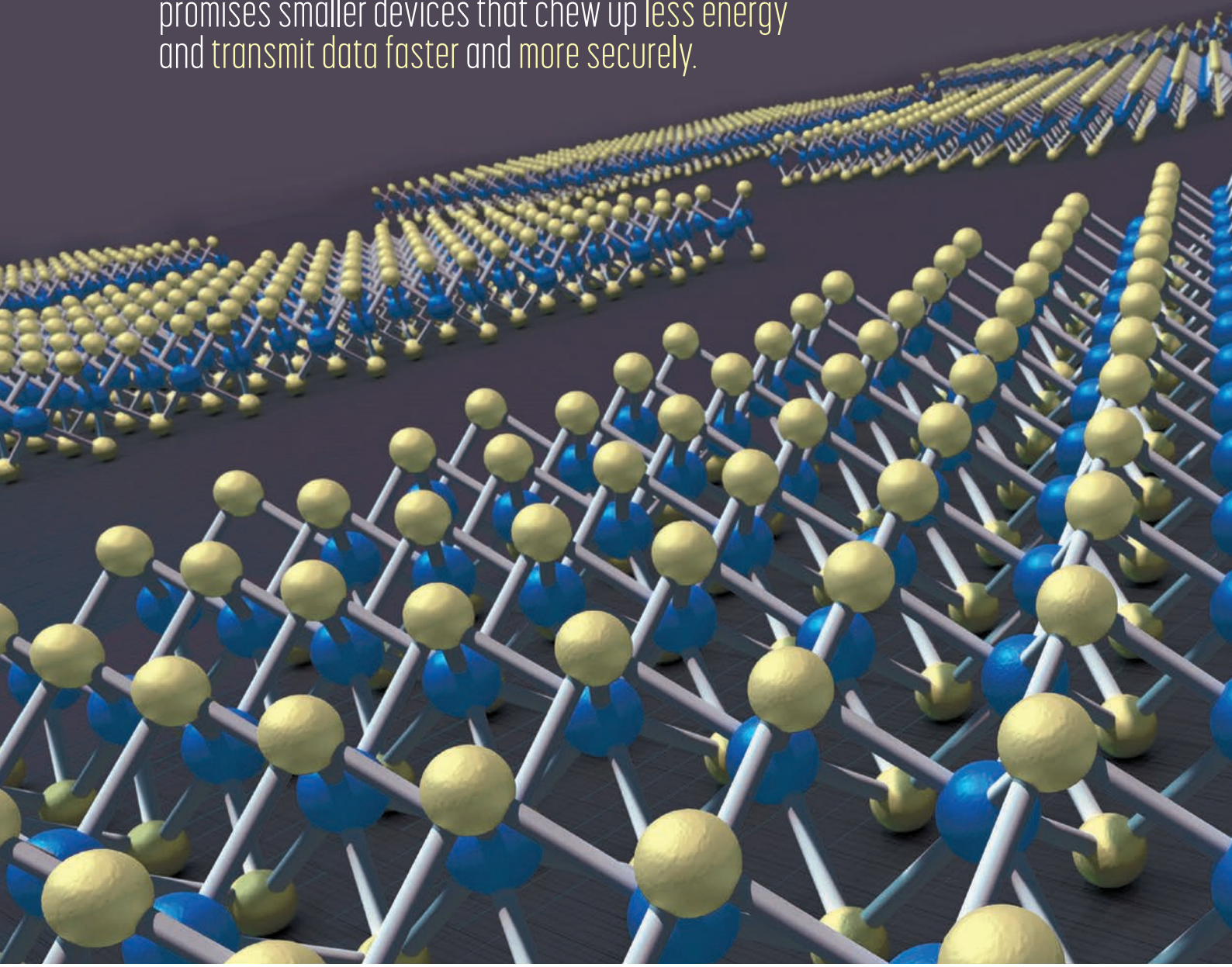
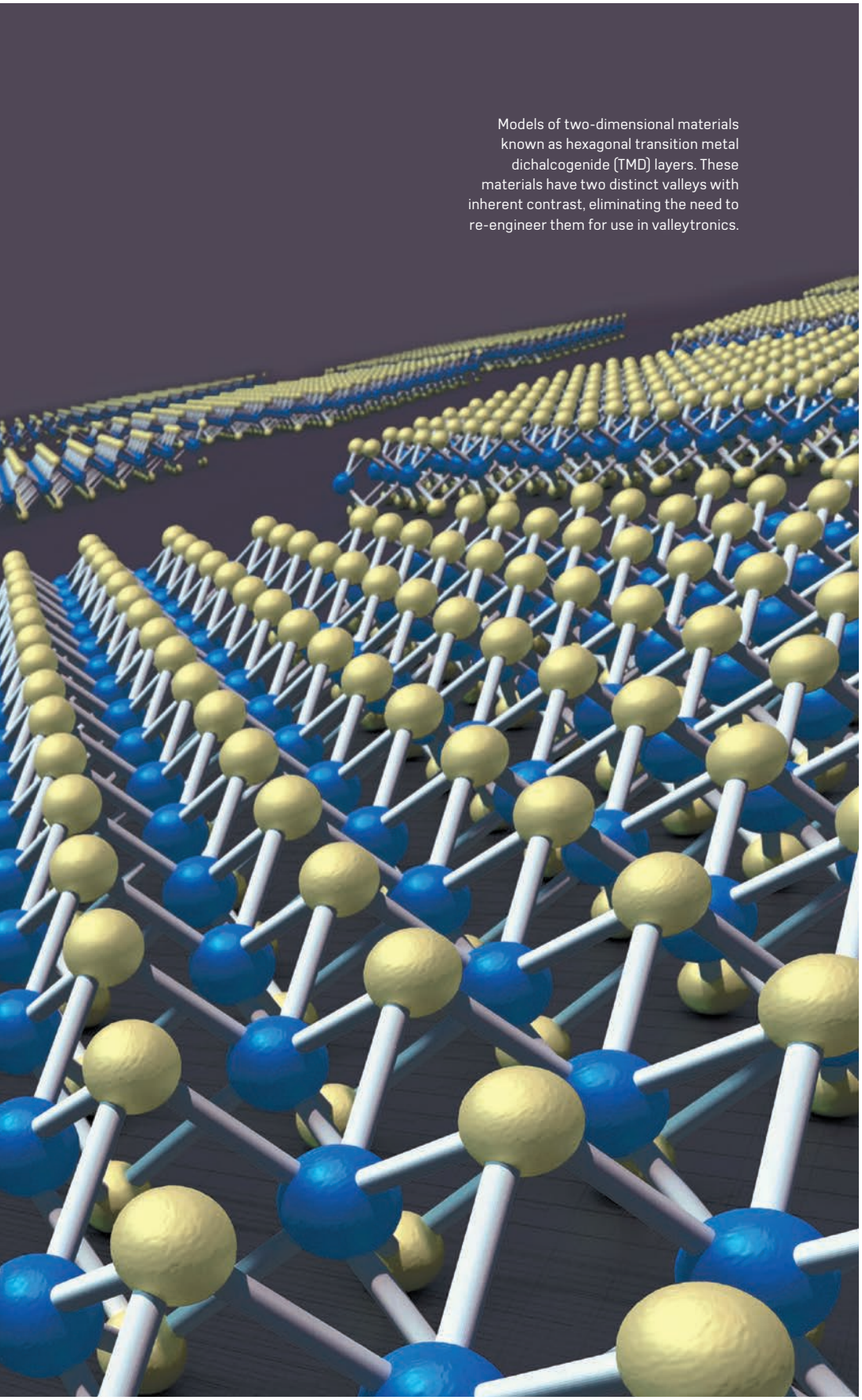
ON THE CUSP OF VALLEYTRONICS

Emerging valleytronics technology using **2D materials** promises smaller devices that chew up less energy and transmit data faster and more securely.





Models of two-dimensional materials known as hexagonal transition metal dichalcogenide (TMD) layers. These materials have two distinct valleys with inherent contrast, eliminating the need to re-engineer them for use in valleytronics.

© Robert Brook / Science Photo Library

Research into harnessing two-dimensional (2D) materials for everyday devices has had some ups and downs. However, the emerging field of valleytronics is using energy troughs to offer renewed potential.

According to Johnson Goh, a senior scientist at A*STAR's Institute of Materials Research and Engineering, valleytronics techniques could help to transmit information through 2D or very thin materials as binary digits (bits) using 'valleys' in the energy bands important to conduction and in which electrons orbit (see page 10).

The basic idea is to store information in the outermost energy bands, the conduction and valence bands, in spots where electrons dip in energy due phenomena such as local surface conditions or direction changes.

Information, says Goh, can be transmitted by controlling an electron's association with a valley, a manipulation that can be achieved using electric fields, magnetic fields and circularly polarised light in a more stable manner than the alternatives found in spintronics.

For example, in molybdenum disulfide, which is a 2D material, the presence of two inequivalent, or different, valleys mean information can be stored in a binary manner based on the valley an electron is residing in: one valley could represent a zero, while the other could represent a one. This information can then be used for computation or memory.

Goh claims that a combination of increasingly affordable 2D material production methods and the application of valleytronics could quickly act together to shrink device sizes and power consumption needs.

2D semiconductors and valleytronics

Goh argues that a combination of valleytronics and 2D or very thin materials will enable a whole host of functionalities in nanoelectronic and nanophotonic devices that can't be achieved with existing silicon-based semiconductor technology. For example, valleytronics would allow electron transport in 2D materials to be manipulated at lower energies than conventional devices.

Information is transmitted in most of today's devices using a flow of charged electrons. In addition to often requiring more electrons to communicate, this method suffers from a 'crowding' of electrons and their jostling results in scattering and some loss of electron energy as heat. In valleytronics, on the other hand, scattering losses can be suppressed because electrons in energy valleys are somewhat protected from jostling.

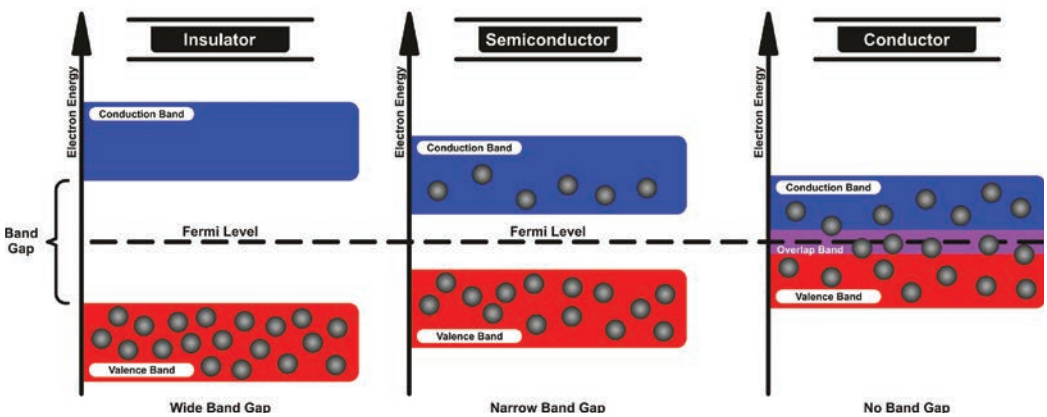
Data can also be stored more robustly in valleytronics materials than in conventional data storage systems, Goh says. "The valley is a property of the whole material, and so the valley states are destroyed only if the material is significantly modified or ceases to exist," he explains. "So instead of encoding information onto electric charges that can be lost through scattering, encoding information onto valley states should be more enduring due to the unique coupling of electron spin to valley."

Currently, Goh and other researchers at IMRE are engineering a number of new and useful 2D semiconductors for this technology by adjusting their composition to tune band gaps and thus control their conduction properties.

However, to create a binary information system using a 2D material's valley states, it's also vital to differentiate which valley a charge is associated with using 'valley contrast' — which are opposite spins hosted by valleys with opposite indices. Transition-metal dichalcogenides, such as molybdenum disulfide, have proven useful to the team as these already have two distinct valleys with inherent contrast, eliminating the need to re-engineer these materials to have this property.

Goh and his team are also trying to add to the known list of materials with this key property.

A*STAR Institute of Materials Research and Engineering scientist Johnson Goh in a laboratory that is packed with the cutting-edge equipment for investigating two-dimensional materials.



BAND THEORY OF SOLIDS (ABOVE)

Electrons in an atom's outermost conduction band determines conductivity. If an electron in the valence band below is excited enough, it can jump into the conduction band and become part of an electrical current. Valleytronics uses energy 'valleys' in these bands to encode information.

(above) © 2018 A*STAR's Institute of Materials Research and Engineering; (left) © udaix / Shutterstock

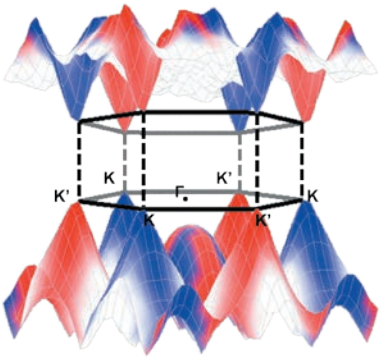
Over the past two years, in collaboration with the National University of Singapore, they have put together a suite of tools for sizing up 2D materials for their valley contrast.

Large-area 2D materials ready for the market

At the same time, Goh's colleagues are tackling one of the major obstacles to the commercialization of this technology. Finding reliable and scalable production methods for mass-scale electronics requires techniques that can form 2D materials with uniform thickness and electrical properties over areas at least as large as a four-inch wafer: the standard substrate size used in the electronics industry.

To do this, Goh turned to IMRE colleague Dongzhi Chi, who is finding ways to manufacture large-area 2D semiconductor materials using a method known as chemical vapor deposition. This technique forms materials by exposing a high-temperature substrate to gases carrying the desired atoms.

"Quantum computing will help us **showcase 2D materials' advantages** over classical electronics."



The band structure of two-dimensional materials such as tungsten disulfide has 'valleys' (left) that provide a way to encode information using an electron.

Chi and his team have already had some important success controlling the concentration spread of the chemical vapors of molybdenum disulfide during this process. By introducing a thin nickel oxide foam barrier trap to lower the chemical concentrations in the vapor, they have improved the uniformity and quality of the deposition material. "The advantage of this approach over others is ease," says Chi. "It uses chemical powders with low toxicity and minimal introduction of chemical species beyond the chemical elements in the deposited material itself, molybdenum and sulfur."

Proof of concept devices

Goh says his team is looking to demonstrate their first proof-of-concept devices by early 2019. He says these will include devices that use valleytronics to do simple things, such as switch a device on or off.

However, he adds that if valley electrons are put into superposition states they could produce a qubit — the fundamental unit for quantum computing. In fact, Goh sees the biggest future gains for valleytronics in its possible applications to "electronics such as low-power edge computing and eventually robust quantum computing."

Smaller devices mean smaller distances for information to travel and so valleytronics and quantum computing both offer advantages in data processing speeds. This has been noted by people trying to harness the spin of atoms for quantum computing. However, valleytronics may have an edge on spintronics as quantum spin is strongly linked to magnetic fields, which can introduce stability issues that aren't as problematic in valleytronics.

Because of this, Goh thinks making quantum computers that use valley states will be the key to opening up the whole 2D material field for commercialization. "Quantum computing will help us showcase 2D materials' advantages over classical electronics. If successful, companies could be more willing to invest in the infrastructure required to develop even better performing 2D materials and turn them into truly disruptive technologies." ■

For references, visit the online version of this article at: www.research.a-star.edu.sg/feature-and-innovation/7945

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PUBLIC TRANSPORT

REDUCING COMMUTERS' WAIT TIME

A computer model built on bus location and passenger payment data could reduce waiting times at bus stops

Waiting ages for a bus to arrive may soon be a thing of the past — a method for analyzing the flow of passengers on public bus networks, developed by researchers at A*STAR, could enable schedule optimization and minimize waiting times.

Global positioning systems have long enabled real-time estimations of the arrival time of buses on many of the world's public transport networks. When this automated vehicle location (AVL) data is combined with passenger information — known as automated passenger collection (APC) data and usually collected as the passenger scans their tickets — an intricate picture of human motion across a metropolitan area can be compiled. This information, in turn, can be harnessed to improve public transport.

One useful metric by which public transport systems are assessed is the average time a commuter must wait for their bus. Waiting times can be recorded manually or by video, but these methods are intrinsically very limited in scale. Instead, researchers have developed mathematical formulae that try to model average waiting times from the so-called 'headway time', the time until the next bus, based on AVL data. But this approach requires several unverified assumptions. For example, it assumes that for high frequency services, commuters arrive at the bus stop uniformly.

"We hope that our work lays the foundation for more dynamic and responsive transport operations."

Muhamad Azfar Ramli from A*STAR's Institute of High Performance Computing and collaborators from a local bus company have analysed AVL and APC data from ten bus routes in Singapore over one month. They showed that the correlation between the number of commuters boarding and the corresponding headway is poor, thus indicating that the uniform arrival assumption is incorrect in Singapore. The team then used this data to build an accurate simulation, which was able to accurately reproduce the empirical data gathered in Singapore in August 2014.

With the accuracy and efficiency of their model confirmed, the researchers were able to use the technique to propose schedules that would minimize the average commuter waiting time without necessarily imposing more buses on the network.

"We hope that our work lays the foundation for more dynamic and responsive transport operations that can be reactive not only to changes in bus locations and movements but also to commuting demand," says Ramli. At present, the analysis can only be done a day or two after data collection, but the team believes this can be done faster. "We hope that when both our estimation techniques and the available real-time data streaming technologies have improved, our methodologies can be applied in real-time and enable operators to react more accurately to dynamic changes in service quality." ■

1. Ramli, M. A., Jayaraman, V., Chee, K. H., Heong, T. K., Khoon, G. L. K. & Monterola, C. Improved estimation of commuter waiting times using headway and commuter boarding information. *Physica A* **501**, 217–226 (2018).

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A computer simulation predicts traveler waiting times at bus stops using bus location and passenger payment data.

The 'YoTube' detector helps makes AI more human-centered.

MACHINE LEARNING

IMAGE ANALYSIS TOOL HELPS PICK OUT HUMAN ACTIONS

Using deep-learning techniques to locate potential human activities in videos

When a police officer begins to raise a hand in traffic, human drivers realize that the officer is about to signal them to stop. But computers find it harder to work out people's next likely actions based on their current behavior. Now, a team of A*STAR researchers and colleagues has developed a detector that can successfully pick out where human actions will occur in videos, in almost real-time[1].

Image analysis technology will need to become better at understanding human intentions if it is to be employed in a wide range of applications, says Hongyuan Zhu, a computer scientist at A*STAR's Institute for Infocomm Research, who led the study. Driverless cars must be able to detect police officers and interpret their actions quickly and accurately, for safe driving, he explains. Autonomous systems could also be trained to identify suspicious activities such as fighting, theft, or dropping dangerous items, and alert security officers.

Computers are already extremely good at detecting objects in static images, thanks to deep learning techniques, which use artificial neural networks to process complex image information. But videos with moving objects are more challenging. "Understanding human actions in videos is a necessary step to build smarter and friendlier machines," says Zhu.

Previous methods for locating potential human actions in videos did not use deep-learning frameworks and were slow and prone to error, says Zhu. To overcome this, the team's 'YoTube' detector combines two types of neural networks in parallel: a static neural network, which has already proven to be accurate at processing still images, and a recurring neural network, typically used for processing changing data, for speech recognition. "Our method is the first to bring detection and tracking together in one deep learning pipeline," says Zhu.

The team tested YoTube on more than 3,000 videos routinely used in computer vision experiments. They report that it outperformed state-of-the-art detectors at correctly picking out potential human actions by approximately 20 per cent for videos showing general everyday activities and around 6 per cent for sports videos. The detector occasionally makes mistakes if the people in the video are small, or if there are many people in the background. Nonetheless, Zhu says, "we've demonstrated that we can detect most potential human action regions in an almost real-time manner."

1. Zhu, H., Vial, R., Lu, S., Peng, X., Fu, H., Tian, Y. & Cao, X. YoTube: Searching action proposal via recurrent and static regression networks, *IEEE Transactions on Image Processing* **27**, 2609–2622 (2018).

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OPERATIONS

THE RIPPLE EFFECTS OF SUPPLY CHAIN DISRUPTIONS

The resilience of supply chain networks to major disruptions can now be measured using a multi-factor test

By analyzing the structure of a supply chain network and the resilience of its components, A*STAR researchers have developed an analytic measure that will allow a company to determine its vulnerability to major supply chain disruptions. The measure has the potential to dramatically improve decision-making in supplier management and lower financial risk across many sectors.

Disruptions in the supply chain can have severe consequences for an unprepared venture. Think of a milk processor without a supply of milk, or a car manufacturer without a supply of engine parts. This can have immediate effects downstream in the network, as well as knock-on effects on other businesses dependent on the network for survival. When such disruption occurs on a massive scale, the economic fallout can be catastrophic and widespread. The possible scale of loss prompted Rick Goh and his team at A*STAR's Institute of High Performance Computing to start looking at ways to measure supply chain risk.

"When a man-made or natural disaster, or disruption is happening somewhere, a company may not capture the impact to its production line as the disruption may apply to its second or third tier suppliers directly, rather than to its first tier partner," says Goh. "We wanted to capture the propagation of supply chain disruption risks far beyond their immediate connection to a focal company, which may reach to the company later on but they usually realize that it is too late when it comes to them due to the loss of time across the supply chain network."

A better understanding of how disruption-caused losses propagate through a company's supply chain network could help companies improve their supplier network structure. However, this is difficult to do in practice and rarely subjected to quantitative analysis. Goh and his team wanted to develop a more reliable measure that can capture the key factors contributing to sensitivity or resilience to disruption across a multi-tier supply chain network.

"Beyond only looking at the propagation effects from one company to another, in our study, we also consider individual companies' resilience capability to overcome the disruption risks and manage the situation internally," says Goh.

By tracking the propagation of a production pause through a network of nodes using generalized mathematical models of both perfect tree and randomly constructed networks, the researchers showed that risks in a supply chain network are determined by both the resilience of companies and the structure of the supply chain network, and that mapping out and understanding these risk factors is essential to risk minimization.

"The modelling confirms that having multiple redundant suppliers, both direct and indirect, will help cushion, or even remove, any impact on one's own production, and may help prevent chained domino-effect disruptions," concludes Jesus Felix Bayta Valenzuela, first author of the study. ■

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Disruptions to supply chain networks can have significant domino-effects.

1. Valenzuela, J. F. B., Fu, X., Xiao, G. & Goh, R. S. M. A network-based impact measure for propagated losses in a supply chain network consisting of resilient components. *Complexity* **2018**, 1724125 (2018).

POWER SOURCES

SHEDDING LIGHT ON TOMORROW'S SOLAR ENERGY

Improved forecasting of sunlight could help increase solar energy generation

Analysis of numerous combined datasets can improve the accuracy of solar irradiation forecasts.

The Sun is becoming an increasingly important source of clean electricity. Accurate sunlight forecasts being developed by A*STAR researchers could greatly improve the performance of solar energy plants, making them a viable alternative to carbon-based sources of power.

A photovoltaic power plant can cover up to 50 square kilometers of the Earth's surface and can generate up to a billion Watts of electricity. This capacity depends on the amount of sunlight at that location, so the ability to predict solar irradiance is crucial for knowing how much power the plant will contribute to the grid on any particular day.

"Forecasting is a key step in integrating renewable energy into the electricity grid," says Dazhi Yang from A*STAR's Singapore Institute of Manufacturing Technology (SIMTech). "It is an emerging subject that requires a wide spectrum of cross-disciplinary knowledge, such as statistics, data science, or machine learning."

Yang, with Hao Quan from the A*STAR Experimental Power Grid Centre, and colleagues from the University of Tennessee at Chattanooga, and the National University of Singapore, has developed a numerical approach to weather prediction that efficiently combines multiple datasets to improve the accuracy of solar irradiation forecasts.

"Forecasting is a key step in integrating renewable energy into the electricity grid."

Hourly changes in the atmosphere, annual changes in the distance between Earth and the Sun, or 10-yearly changes in the Sun's internal cycles can all alter the amount of sunlight that reaches the Earth's surface. These changes occur on very different time scales, and so conventional forecasting methods model variability at different timescales separately, which makes computer processing easier. However, these methods rely on a simple addition of forecasts, with no weighting that makes more use of better forecast sub-series. Moreover, the forecasts they generate are only accurate on the timescale of the original series.

Yang and the team developed a framework that reconciles the different timescales by forming a temporal hierarchy that aggregates forecasts obtained at different timescales, such as high-frequency, hourly data and low-frequency, daily data. "Temporal reconciliation is a type of ensemble forecasting model that forecasts the next day's solar generation many times, separately, using data of different temporal granularities, hourly, two-hourly, and daily," explains Yang. "These different forecasts are then combined optimally through statistical models to produce a final forecast."

The researchers tested their numerical weather prediction method using data from 318 photovoltaic power plant sites in California over a year. Their temporal reconciliation method was shown to significantly outperform other numerical day-ahead forecasts. ■

1. Yang, D., Quan, H., Disfani, V. R. & Rodríguez-Gallegos, C. D. Reconciling solar forecasts: Temporal hierarchy. *Solar Energy* **158**, 332–346 (2017).

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PHOTONICS

LIGHT, FINELY SLICED

A triple bend structure splits light into its two polarization components with unprecedented purity

A plan to incorporate a third bent waveguide into a silicon-based light splitter led A*STAR researchers to develop a device capable of a 30-fold improvement in splitting efficiency.[1] The novel on-chip light splitter marks a major breakthrough in improving high-performance data transmission systems, as well as applications in quantum computing.

The manipulation of light in micro-scale devices is a fundamental pillar of high-speed optical circuits underpinning communications and next-generation technologies like quantum computing. Light is fast and low-power, and can be encoded to transmit data in a variety of ways. As Thomas Ang from the A*STAR Institute of High Performance Computing explains, for these manipulations to be reliable and efficient, the light needs to be as 'pure' as possible — not just tightly confined around a specific wavelength, but also of a uniform polarization.

"Light consists of a mixture of two polarization components," Ang says. "Polarization beam splitters are used to separate a mixed-polarization beam into two channels for each polarization."

When the splitting is imperfect, the two channels can contain small proportions of the opposite polarization, an effect known as crosstalk.

"Polarization beam splitters with low crosstalk are very important for high signal fidelity in high-speed optical interconnect applications," says Jun Rong Ong, Ang's partner in the study. "Current technology is limited to a crosstalk level, called the extinction ratio, of around 25 decibels, which is high enough to affect high-speed data transmission."

"We were thrilled to find that the device outperformed some of the best-in-class published results."

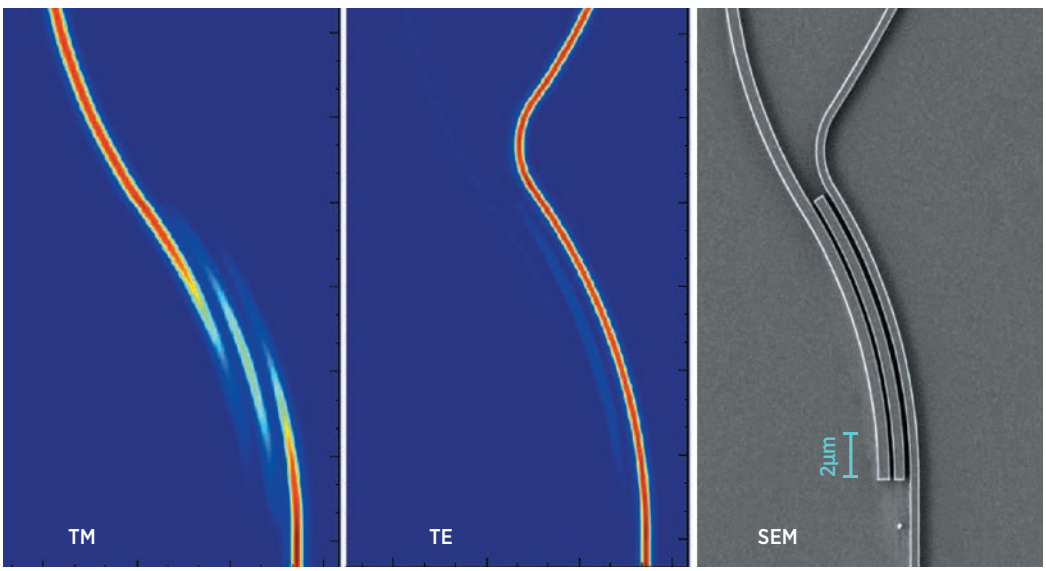
The field of polarization beam splitters is well-developed, and there have been many attempts to break this 25 decibel limit in silicon-based devices as the industry standard for fabrication. Ang, Ong and their colleagues brainstormed ways to challenge this limit, and came up with the idea of adding a third waveguide — essentially a channel or path to route light on a chip — when the status quo was no more than two.

"We were interested in waveguides for bending and routing light, and conceptualized our new design after brainstorming within our team," says Ang. "We tested our hypothesis using computer simulations and were thrilled to find that the device outperformed some of the best-in-class published results."

Working with collaborators at the Singapore University of Technology and Design, the team then fabricated the three-waveguide device (see image below) and confirmed its vastly improved extinction ratio of 40 decibels, representing a 30-fold reduction in polarization crosstalk.

"We also expect that the device can be improved further by optimizing its design," says Ong. ■

1. Ong, J. R., Ang, T. Y. L., Sahin, E., Pawlina, B., Chen, G. F. R., Tan, D. T. H., Lim, S. T. & Png, C. E. Broadband silicon polarization beam splitter with a high extinction ratio using a triple-bent-waveguide directional coupler. *Optics Letters* **42**, 4450 (2017).



A scanning electron micrograph (right) of the three-waveguide polarization beam splitter, and the light intensity of the two polarization components (TM/TE).

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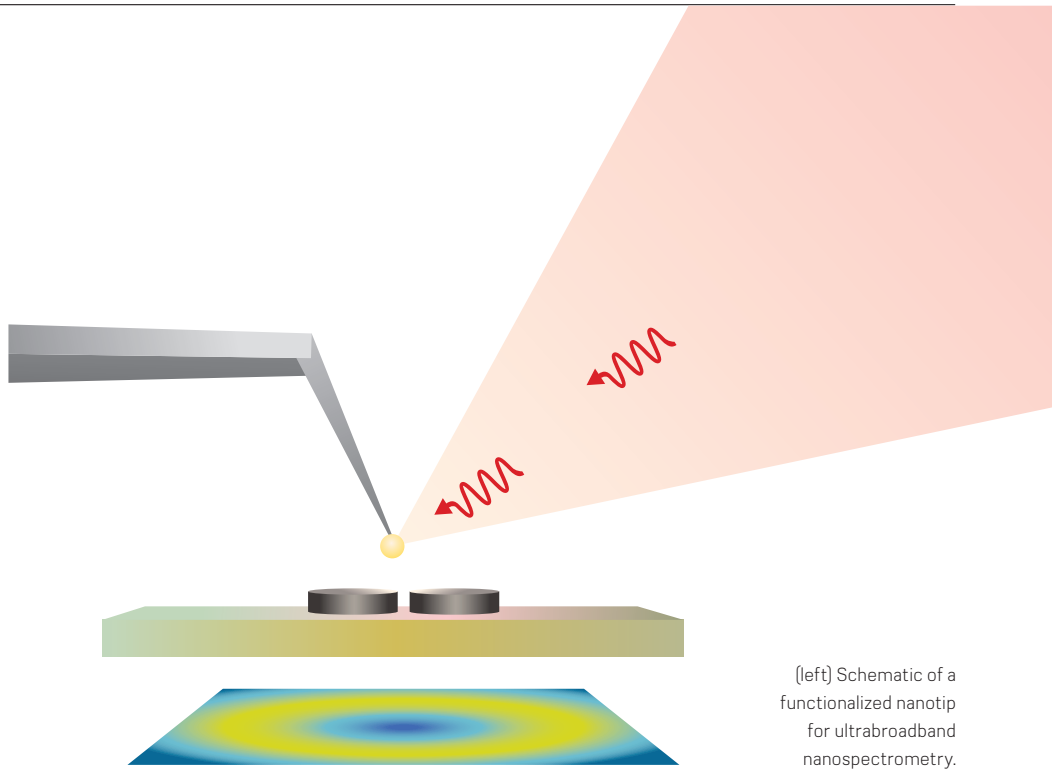
OPTICS

BRIGHTER MICROSCOPY USING BUILT-IN NANOBULBS

Hybrid nanoparticle can attach to microscope tips and light up objects too tiny to see with typical optical beams



(left) Schematic of a functionalized nanotip for ultrabroadband nanospectrometry.

By using ultrafast laser pulses to join silicon and gold atoms into a new type of nanoparticle, researchers from A*STAR and ITMO University, Russia, have created a white-light source for applications including high-resolution characterization of biomolecules[1].

The wave nature of light imposes constraints on the resolution of conventional optical microscopes, no matter how intense the source. The process of diffraction spreads out light waves at the nanoscale and produces blurry images when objects are smaller than half the source beam's wavelength.

Researchers from A*STAR's former Data Storage Insitute have recently devised a way to beat the diffraction limit. Arseniy Kuznetsov, who is currently at the A*STAR Insitute of Materials Research and Engineering, and Yefeng Yu teamed up with colleagues at Russia's ITMO University to improve near-field scanning optical microscopy (NSOM) devices. These microscopes embed optically active materials inside a sharp microscale tip. When positioned extremely close to a sample, the light-emitting tip generates evanescent waves capable of resolving items separated by just a few nanometers.

"Normally, these two materials are unmixable. But when the process happens with femtosecond laser pulses, we get a hybrid that solidifies in an unusual state."

One drawback of NSOM is that implanted light sources, such as quantum dot crystals and photoactive molecules, tend to emit in narrow regions of the electromagnetic spectrum. This makes it difficult to use these materials for emerging 'nanospectroscopy' applications that detect a sample's structural and optical features in ultra-fine detail. Broad-spectrum white light that excites many components of a specimen simultaneously would be preferable.

Kuznetsov, Yu, and their colleagues realized that silicon nanoparticles, which exhibit wide-ranging luminescence after laser excitation, might solve this white light problem if they could be made more efficient at scales suitable for NSOM tips. To realize this goal, the team deposited a thin layer of silicon on top of a gold-coated substrate, and then exposed the material to bursts of ultra-fast lasers. Spherical silicon nanoparticles containing integrated gold regions were recovered from the ablation process.

"Normally, these two materials are unmixable — melting them together won't produce a good alloy," explains Kuznetsov. "But when the process happens with femtosecond laser pulses, we get a hybrid that solidifies in an unusual state."

The researchers' experiments revealed that the gold regions of the new material produced high-energy, 'hot' electrons that transferred to silicon crystals and helped them emit ultraviolet, visible, and infrared light. Attaching the hybrid particle to the end of a microscopic tip enabled them to scan nanoscale photonic devices (as in schematic above) and map out their optical response at high speeds.

"This mixture has unique photoluminescence properties that take advantage of both components," says Kuznetsov. "We are working on ways to control their dimensions and nanocompositions." ■

1. Makarov, S. V., Sinev, I. S., Milichko, V. A., Komissarenko, F. E., Zuev, D. A. *et. al.* Nanoscale generation of white light for ultrabroadband nanospectroscopy. *Nano Letters* **18**, 535–539 (2018).

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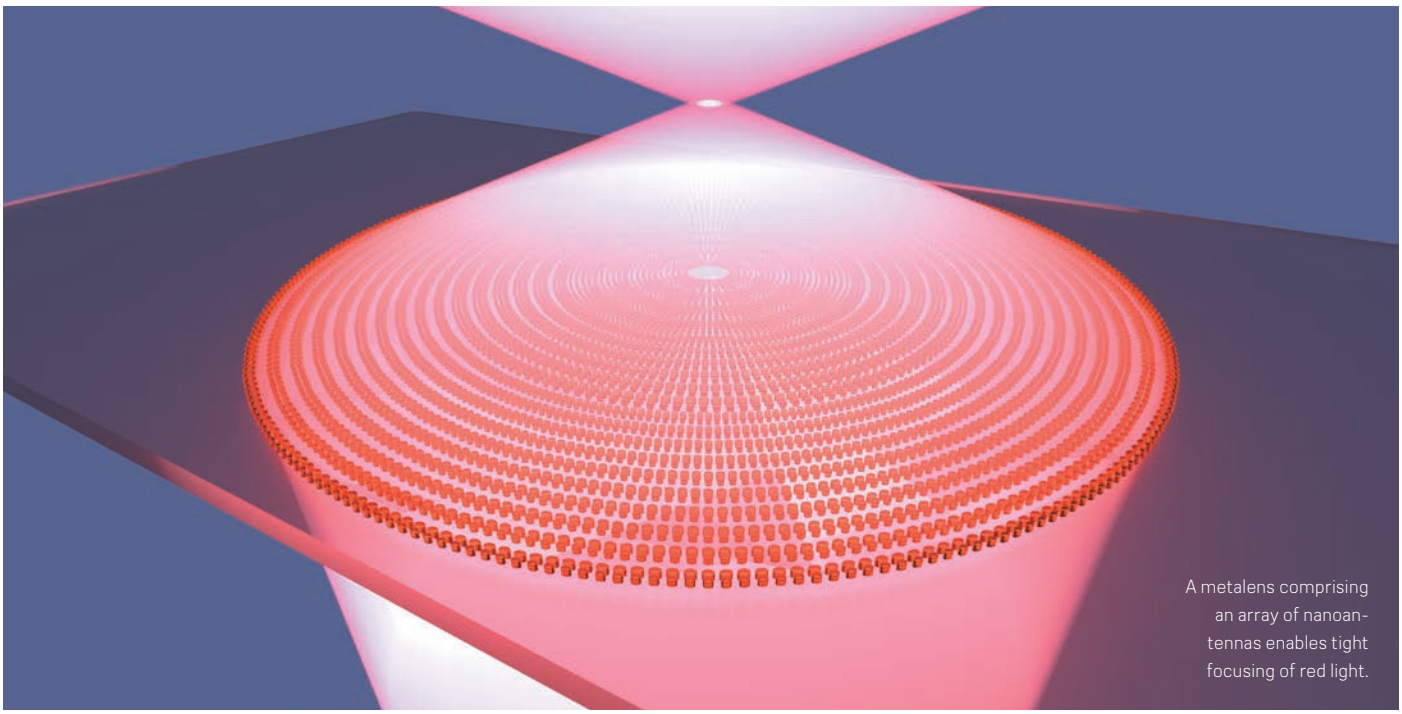

A metalens comprising an array of nanoantennas enables tight focusing of red light.

OPTICS

FLAT LENSES BRING TINY FEATURES INTO FOCUS

Flat lenses that enable high-resolution imaging are created by forming nanoscale structures on a surface

Simple flat lenses, incorporating nanoscale features, that outperform conventional bulk optics have been fabricated by scientists at A*STAR.

High-numerical-aperture optical lenses offer very high resolution and are particularly useful for low-light-intensity applications, such as single-photon optics. The numerical aperture is a measure of the conical angle of light that can be collected by the lens and, reciprocally, how tightly a beam of light can be focused. The larger the numerical aperture, the higher the resolution and the closer the lens can be to the object to be imaged. As the collection angle and, hence the amount of light collected from the object, approaches its theoretical maximum, the numerical aperture increases and gets closer to a value of one, the fundamental maximum for a lens in air.

However, high-numerical-aperture systems can be bulky, complex and expensive. And conventional glass-based optics rarely attain a numerical aperture above 0.95. Scientists from A*STAR's former Data Storage Institute have recently built a simple flat lens with a numerical aperture higher than 0.99.

"Previous attempts to make flat, high-numerical-aperture lenses ended up with devices that were very inefficient at its edges, where the light bends at very large angles," explains A*STAR scientist Ramón Paniagua-Domínguez. "We proposed a new method to overcome this limitation using nanoantennas."

The 'metalens' created by Paniagua-Domínguez and his colleagues consists of arrays of silicon discs with various diameters on a substrate, each array designed to direct the light at a specific angle. All the areas were engineered to direct light into a specific point — the focus of the lens. The area responsible for giving the lens its high-numerical-aperture comprises pairs of nanometer-scale silicon cylinders, one larger than the other, which act like antennas for visible light. Using silicon rather than a metal reduces losses and makes the concept applicable across a wide range of wavelengths.

The dimensions of the nanoantennas — the disk diameter, disk separation and the pitch of the array — can be altered to suit the metalens' wavelength range and specific application. For example, disk diameters of 190 and 150 nanometers redirect incoming red light at an angle of 82 degrees. This allowed the team to achieve a numerical aperture of 0.99 in their metalens, making it ideal for high-resolution applications.

"We are exploring more complex geometries to maximize the efficiency of the lens," says Paniagua-Domínguez. "Plus, seeking other materials to expand the range of frequencies at which the lens works." ■

1. Paniagua-Domínguez, R., Yu, Y. F., Khaidarov, E., Choi, S., Leong, V. *et al.* A metalens with a near-unity numerical aperture. *Nano Letters* **18,** 2124–2132 (2018).

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NANOPHOTONICS

A SILICON-NANOPARTICLE PHOTONIC WAVEGUIDE

A line of silicon nanoparticles can transport light efficiently at small scales

A new way to efficiently guide light at tiny scales has been demonstrated by an all-A*STAR team[1]. Their method, which involves lining up silicon nanoparticles, is promising for applications such as light-based integrated circuits, biosensors and quantum communications.

Transporting light at small scales is critical for many applications and is commonly performed using rectangular silicon waveguides — the optical circuit equivalent to wires in electronic circuits. To further shrink devices, metallic nanoparticles have been explored as an alternative, but while they are very good at confining light to small scales, they tend to leak a lot of the light.

Reuben Bakker, Arseniy Kuznetsov and their colleagues from A*STAR's former Data Storage Institute have recently come up with a more efficient method that involves a string of cylindrical silicon nanoparticles. The first nanoparticle is excited using light and then a near-field scanning optical microscope measures the light that reaches another nanoparticle further down the line (see image below). When they did this, the team found that the fall in the light intensity was low.

"This is the first experimental demonstration that shows coupled resonators can very efficiently guide light at strongly sub-wavelength dimensions and over lengths of several hundred micrometers," says Kuznetsov. "It's the first step toward a completely new approach to silicon photonics."

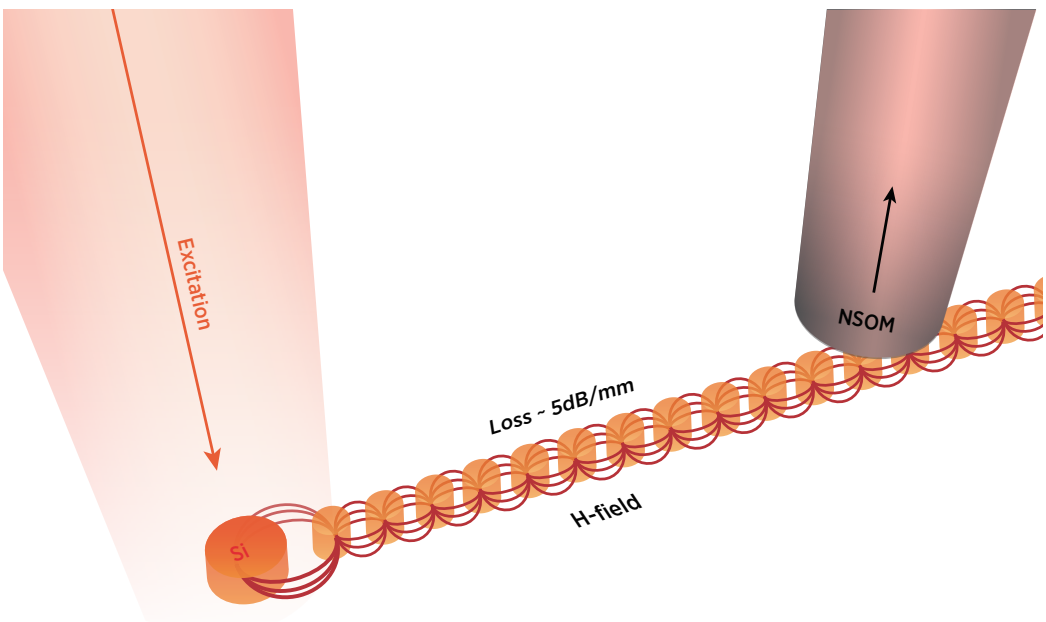
"We tweaked the geometries a little bit, but to have them perform so well after just a few iterations was quite unexpected."

The nanoparticles are not in direct contact with each other. Instead, light is transferred to the next particle through magnetic-field resonances. "Each of these particles is a resonant scatterer — so if you take one particle it will scatter light in all directions," explains Kuznetsov. "But when we line all these particles up, they work as a single waveguide without leaking light."

One big advantage of using silicon nanoparticles is that they are compatible with the fabrication processes currently used by the semiconductor industry. "You can use the same CMOS processes to do silicon photonics," says Kuznetsov. "You just change the mask and the layout and add other components without any additional complications."

Despite having modeled the system and its behavior as a waveguide before performing the measurements, the team were still amazed at how well it worked in practice. "We were surprised it worked so well," recalls Bakker. "We tweaked the geometries a little bit, but to have them perform so well after just a few iterations was quite unexpected."

The team has already demonstrated the same concept at telecommunication wavelengths. They are now working on developing various on-chip photonic components based on the concept. ■



Near-field scanning optical microscope (NSOM) measurements have shown that cylindrical silicon nanoparticles arranged in a line can transport light with low loss due to magnetic-field (H-field) resonances between them.

1. Bakker, R. M., Yu, Y. F., Paniagua-Domínguez, R., Luk'yanchuk, B. & Kuznetsov, A. I. Resonant light guiding along a chain of silicon nanoparticles. *Nano Letters* **17,** 3458–3464 (2017).

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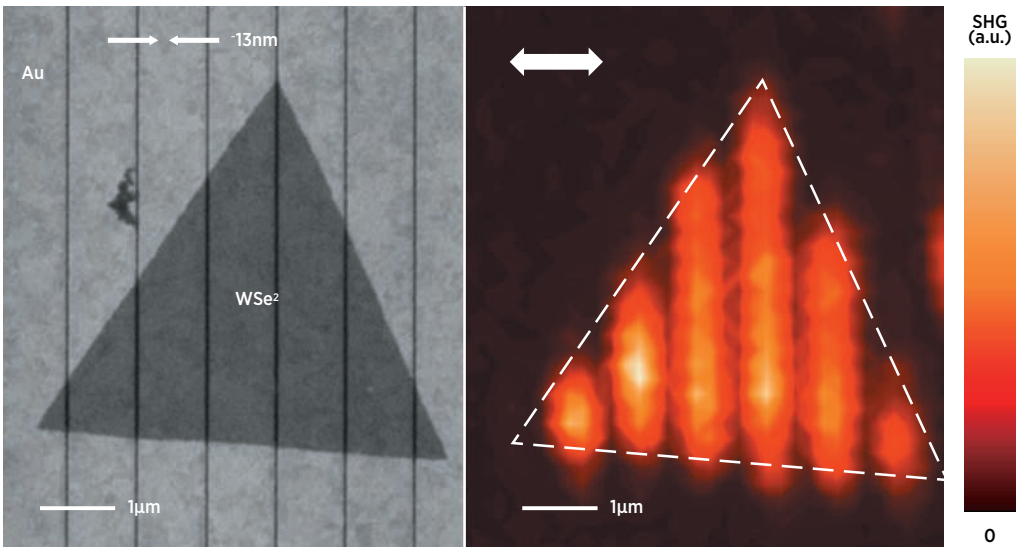
PHOTONICS

GETTING INTO THE GROOVE

Light focused into nano-grooves in a gold film bonded to an atomically thin crystal flake produces a remarkable frequency doubling effect



(Left) Scanning electron micrograph of a WeS_2 flake on a nano-groove gold film. (Right) Second harmonic generation (SHG) in monolayer WSe_2 on a nano-groove gold film.

By combining two very different light-interacting nanostructures, A*STAR researchers have demonstrated a surprisingly strong enhancement of a frequency doubling effect[1].

The functional manipulation and transformation of light in optical circuits is an exciting area of research that combines the physics of light and matter in unusual ways. At the cutting edge of this technology, researchers are exploring new structural combinations of materials that could give rise to potentially useful functions, particularly in nanoscale systems.

Joel Kwang Wei Yang, Zhaogang Dong and their colleagues at A*STAR's Institute of Materials Research and Engineering have been working on highly precise techniques for patterning thin gold films with nanoscale groove structures to enhance light interaction and produce light resonance effects known as plasmonics.

"This work was really a marvel of nanofabrication and delicate sample handling."

"We've seen from our previous studies that 10 nanometer-wide grooves in a gold film can support plasmon modes by concentrating light along the grooves," explains Yang. "We then figured that draping a two-dimensional material on to this special substrate might make for an interesting system due to confinement of light into quasi one-dimensional strips."

In collaboration with researchers from the National University of Singapore, Imperial College London and the King Abdullah University of Science and Technology in Saudi Arabia, Yang and Dong fabricated, analyzed and modelled a system consisting of the nano-grooved gold film topped with an atomically thin flake of tungsten selenide (WSe_2). The result was a remarkable 7,000-fold multiplication of an effect called second harmonic generation (SHG), by which two photons combine to produce a single photon with double the energy (or frequency).

"This work was really a marvel of nanofabrication and delicate sample handling," says Yang. "We had excellent collaborators who provided beautiful WSe_2 flakes, and a talented PhD student who has since graduated, Wang Zhuo, who worked on state-of-the-art transfer techniques to get these flakes on to our nanotrenches in the right orientation."

Notably, the sample survived both the transfer on to a separate transparent substrate and many bending cycles without degradation in SHG emission, making it a potentially resilient and versatile platform for further development.

"Gold films are flexible and retain their form well, and the nanoscale grooves produce strong optical enhancements, while monolayer WSe_2 is an efficient SHG material but interacts only weakly with light due to its atomic thickness," says Dong. "We were able to combine the advantages of both materials to achieve a flexible, ultra-compact, and efficient device for SHG, with potential applications for optical frequency doubling in nanoscale devices." ■

1. Wang, Z., Dong, Z., Zhu, H., Jin, L., Chiu, M.-H., Li, L.-J., Xu, Q.-H., Eda, G., Maier, S. A., Wee, A. T. S., Qiu, C.-W., Yang, J. K. W. Selectively plasmon-enhanced second-harmonic generation from monolayer tungsten diselenide on flexible substrates. *ACS Nano* **12**, 1859-1867 (2018).

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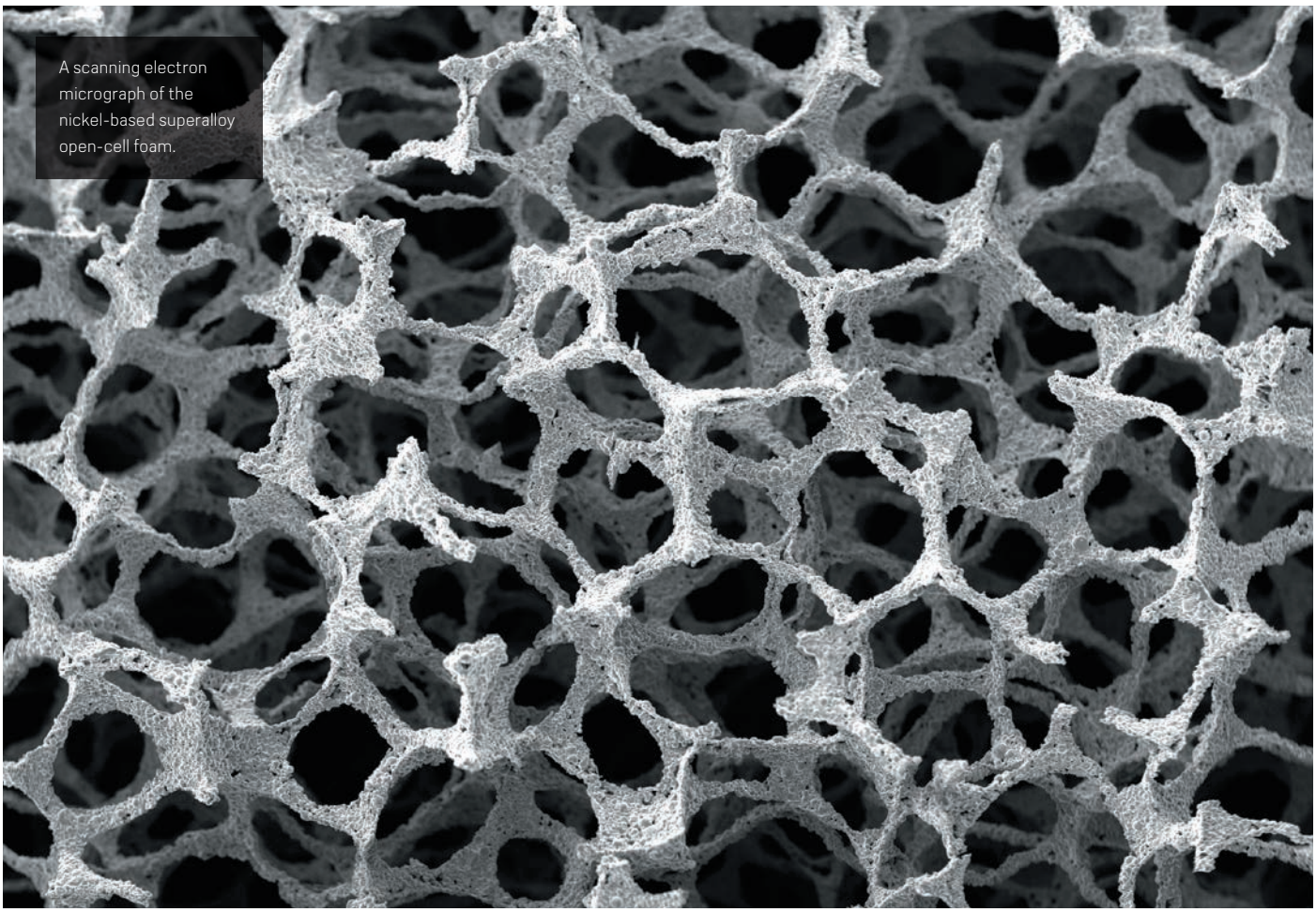

A scanning electron micrograph of the nickel-based superalloy open-cell foam.

ADVANCED MATERIALS

SUPPRESSING SOUND IN EXTREME PLACES

Tailor-made metallic foams could soak up sound in high pressure, high temperature environments such as jet engines

Superalloy foams could make ship and aircraft engines much quieter, new research suggests[1].

The engine and exhaust system of a jet is the aircraft's main source of noise, but jet engines are far too hot for materials typically used for sound deadening such as polymer foams. One possibility for reducing aircraft engine noise, is to marry regular sound insulation with extremely heat-resistant metal superalloys like those already used for the jet's turbine blades.

Regular polymer foams can be used as a template from which to create heat-resistant sound-suppressing superalloy metallic foams, show Wei Zhai and Xu Song from the Singapore Institute of Manufacturing Technology at A*STAR, and their colleagues. The team developed a technique in which they coated a slurry of the nickel-based superalloy on to a polymer foam, then burnt off the polymer to leave behind an open-cell metallic foam with the same structure as the original polymer[2]. Inexpensive polyurethane household cleaning sponges make good foam templates, Zhai says.

Teaming up with Xiang Yu and Fangsen Cui from A*STAR's Institute of High Performance Computing, the researchers developed a predictive model for optimizing the metallic foam's pore structure for a given task. First, they produced a range

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of metallic foam structures by growing them on polymer templates with a range of porosities and pore cell sizes. The researchers then tested the acoustic properties of these metallic foams, to measure how foams with different pore structures soaked up sound. This data formed the basis of the predictive model.

The team found that, generally speaking, sound absorption is better when the pores are smaller. The smaller the pores, the longer and more difficult the sound wave's path will be through the material, and the more time the material has to damp sound energy by converting it to heat — a phenomenon known as the thermo-viscous effect.

The next step is to better control the template replication process, so that a tunable gradient of pore sizes can be formed within a single foam block. "The sound absorption and mechanical performance of such gradient metallic foams will be further improved," says Zhai.

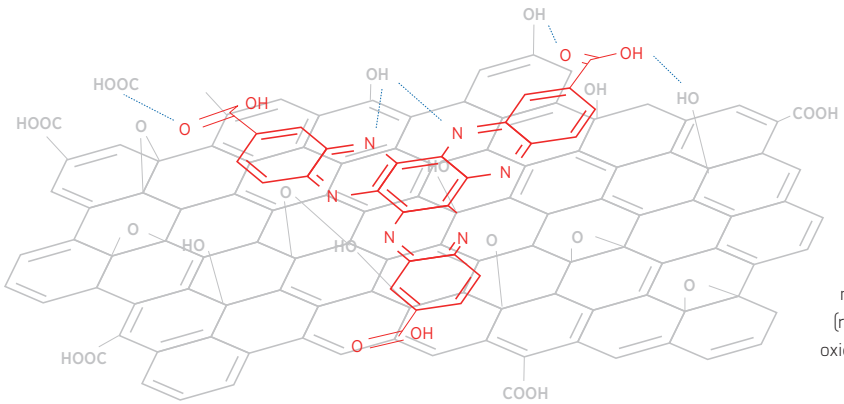
The metallic foams' exceptional properties in addition to sound absorption — low weight, noncombustible, and structural strength — has great potential for aircraft and ship engine and exhaust components. "The improved foam could be used as the core material of a sandwich-structured composite material, which possess both mechanical strength and acoustic damping performance in one component," says Zhai. ■

1. Zhai, W., Yu, X., Song, X., Ang, L. Y. N. Cui, F. *et al.* Microstructure-based experimental and numerical investigations on the sound absorption property of open-cell metallic foams manufactured by a template replication technique. *Materials and Design* **137**, 108 (2018).

MATERIALS

BATTERIES POISED FOR ORGANIC REVOLUTION

Cathode material made from organic molecules enhances the green credentials of rechargeable batteries



Hydrogen bonds (blue) strongly attach the redox active organic molecule HATNTA (red) to a graphene oxide support (grey).

Incorporating organic materials into lithium ion batteries could lower their cost and make them more environmentally friendly, A*STAR researchers have found. The team has developed an organic battery cathode, the positive electrode, which has significantly improved electrochemical performance compared to previous organic cathode materials. Crucially, the new material is also robust, remaining stable over thousands of battery charge/discharge cycles.

The cathode is a critical component in Li-ion batteries. An electron-deficient, rigid organic molecule called hexaazatrinaphthalene (HATN) was previously investigated as an organic cathode material for lithium ion batteries. However, its promising initial performance declined rapidly during use, because the molecule began to dissolve into the battery's liquid electrolyte.

Yugen Zhang and his colleagues from the A*STAR Institute of Bioengineering and Nanotechnology have developed a new cathode material, in which HATN was combined with graphene oxide in a bid to prevent the organic material from dissolving.

In graphene oxide, a single-atom thick sheet of carbon atoms is partly covered by a layer of oxygen atoms. "Graphene oxide has excellent electronic conductivity, and surface oxygen functionality that may form hydrogen-bonding interactions with HATN," Zhang says. He explains that this made graphene oxide a promising candidate for forming a HATN-graphene oxide nanocomposite.

The nanocomposite's performance exceeded expectations. The materials combined to form core-shell nanorods in which the HATN was coated with graphene oxide. "Graphene oxide and HATN formed a very nice composite structure, which solved the dissolution issue of HATN in electrolyte and gave the cathode very good cycling stability," Zhang says. A lithium ion battery using this material as its cathode retained 80 per cent of its capacity after 2,000 charge/discharge cycles.

The team saw even better performance when they combined graphene oxide with a HATN derivate called hexaazatrinaphthalene tricarboxylic acid (HATNTA). A battery made from this material retained 86 per cent of its capacity after 2,000 charge/discharge cycles. The improved performance is probably due to the polar carboxylic acid groups on the HATNTA molecule, which attached the molecule even more strongly to the graphene oxide.

The team is continuing to develop new materials to improve the performance of organic cathodes, Zhang says. Aside from investigating alternatives to graphene oxide, the team also is working on HATN-based porous polymers for use as organic cathode materials, which should enhance the flow of ions during battery charge and discharge. ■

1. Wang, J., Tee, K., Lee, Y., Riduan, S. N. and Zhang, Y. Hexaazatriphenylene derivatives/GO composites as organic cathodes for lithium ion batteries. *Journal of Materials Chemistry A* **6**, 2752-2757 (2018).

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CHEMISTRY

TUNING INTO THE POTENTIAL OF PHOSPHORENE

Better understanding of the wetting characteristics of phosphorene could pave the way for new applications in biological engineering

Commerical applications for the use of phsphorene in the biological sciences could result from the work of A*STAR researchers. They have developed a technique for investigating the wetting behavior of water on phosphorene — the single layer form of black phosphorus.

Phosphorene, unlike other commonly used 2D materials, such as graphene and molybdenum disulfide, possesses structural anisotropy, meaning it exhibits different physical properties along axes in different directions. This property could allow phosphorene with adjustable wettability to be fabricated for use in the biological sciences. Yet until now, little was known about the wetting behavior of this material.

To realize the potential of phosphorene, however, requires a thorough understanding of how it interacts with biomolecules and fluids. This drove Chen Shuai, and colleagues from the A*STAR Institute of High Performance Computing to develop a technique for investigating the wetting characteristics of water droplets on phosphorene.

"The contact angle of a water droplet on phosphorene is important for biological applications."

The researchers investigated the contact angle, a measure of the relative strength of the interaction between the phosphorene and the water droplets, which determines its wettability characteristics. Many properties of phosphorene, such as electronic band gap and atomic/molecular adsorption, are layer-dependent, so they also considered the wetting behavior on multilayer phosphorene.

To do this they first used molecular dynamics simulations to observe the effects of different droplet sizes and the number of phosphorene layers on the contact angle.

As phosphorene has strong structural anisotropy, they also explored the diffusion behavior of water droplets on phosphorene — both with and without defects — for their effect on contact angle.

"The contact angle of a water droplet on phosphorene is important for biological applications of phosphorene," explains Chen. "Because it is an intrinsic property, we investigated the effect of water droplet size, number of phosphorene layers, and defect distribution on the contact angle of both pristine and defective phosphorene."

"We found that the contact angle decreased when the number of phosphorene layers increased from one to three, but then converged to a constant value when the number of layers was larger than three," says Chen. "The results for defective phosphorene demonstrate that the contact angle along different directions increased with increasing defect concentration."

The work demonstrates that the wetting property of phosphorene is tunable with the number of layers and the defect distribution, which are critical for manipulating the water wetting and protein adsorption on phosphorene-based devices for use in biological and nanofluidic applications.

"Based on these results from our research, we now intend to explore the interaction of phosphorene with biomolecules in a water environment," says Chen. ■

1. Chen, S., Cheng, Y., Zhang, G, Pei, Q. & Zhang, Y-W. Anisotropic wetting characteristics of water droplets on phosphorene: Roles of layer and defect engineering. *Journal of Physical Chemistry C*, **122**, 4622 – 4627 (2018).

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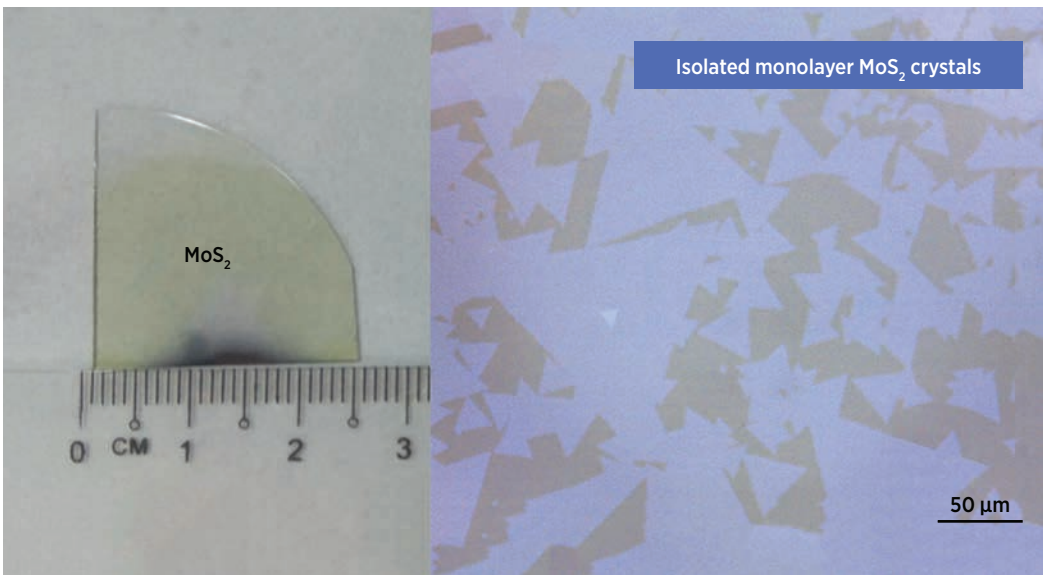
A*STAR researchers have developed a technique for investigating the wetting behavior of water on phosphorene.

MATERIALS

NO BARRIER TO APPLICATIONS FOR A REMARKABLE 2D MATERIAL

An innovative technique for growing large, uniform sheets of single-layer molybdenum disulfide could lead to new flexible electronic and optoelectronic devices



Two-dimensional monolayer MoS_2 was grown on sapphire (left). The optical microscope image on the right shows isolated monolayer MoS_2 crystals at the periphery of the film.

Mass production of large, uniform sheets of single-layer molybdenum disulfide, MoS_2, is difficult, which limits its commercial application. A*STAR researchers have modified an existing manufacturing technique to enable the use of MoS_2 in a range of technologies from photodevices to flexible, transparent sensors.[1]

The two-dimensional material has attracted considerable attention because of its extraordinary physical, electronic and optoelectronic properties, including flexibility, transparency and semiconducting characteristics. But fabricating large-scale, defect-free single layers of MoS_2 is challenging.

"The advantage of this approach is the ease of implementation as well as a reduction in contamination."

Dongzhi Chi and his team from the A*STAR Institute of Materials Research and Engineering, in collaboration with colleagues from the National University of Singapore and the Indian Institute of Science Education and Research, have modified a current technique, known as chemical vapor deposition (CVD), to produce uniform, centimeter-sized sheets of MoS_2 crystals with large grain sizes.

"The physical properties of MoS_2 vary greatly with its thickness," explains Chi. "To maintain its remarkable electronic and physical properties we need a method that can uniformly deposit MoS_2 films over a large area with high crystallinity."

Although CVD is an effective technique for fabricating large-area, uniform sheets of MoS_2 of varying thickness on different substrates, and significant progress has been made in improving the quality of MoS_2 monolayers produced by the technique, little attention has been paid to controlling the chemical vapors using physical barriers during the growth of MoS_2 crystals.

By introducing a nickel oxide (NiO) barrier, the researchers were able to control the concentration and distribution of chemical vapors during the growth of MoS_2 crystals. Because NiO reacts with molybdenum trioxide (MoO_3), one of the chemical reactants used in the growth process, it traps and lowers the MoO_3 concentration, allowing the uniform deposition of monolayers of MoS_2 over a large area.

"The advantage of this approach is the ease of implementation as well as a reduction in contamination, and it allows for control of the chemical exposure during the growth process," says Chi.

The work has led to further advances in the fabrication of uniform and large-area MoS_2 monolayers, and could also be applied to other two-dimensional materials.

"We are now looking to scale up our fabrication process for producing even larger sheets, which could pave the way for next-generation optoelectronic and sensor technologies," says Chi. ■

1. Lim, Y-F., Priyadarshi, K., Bussolotti, F., Gogoi, P. K., Cui, X. *et al.* Modification of vapor phase concentrations in MoS_2 growth using a NiO foam barrier. *ACS Nano* **12**, 1339 – 1349 (2018).

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CATALYSIS

STEPPING UP WATER SPLITTING

Cheap and accessible cobalt-based catalysts could fast-track the industrial-scale production of hydrogen from water

A low-cost and simple alternative to expensive platinum-based catalysts that generate hydrogen from water could soon be available, thanks to A*STAR researchers.

A team from A*STAR's Institute of Materials Research and Engineering has developed a one-step approach to synthesize cobalt phosphides that exhibit good electrochemical activity in the hydrogen evolution reaction (HER). "Unlike the expensive and scarce platinum-based catalysts, these phosphorus-functionalized cobalt catalysts are particularly promising because of their long-term stability and suitability to large scale industrial implementation," explains Yun Zong, who led the team with Zhaolin Liu.

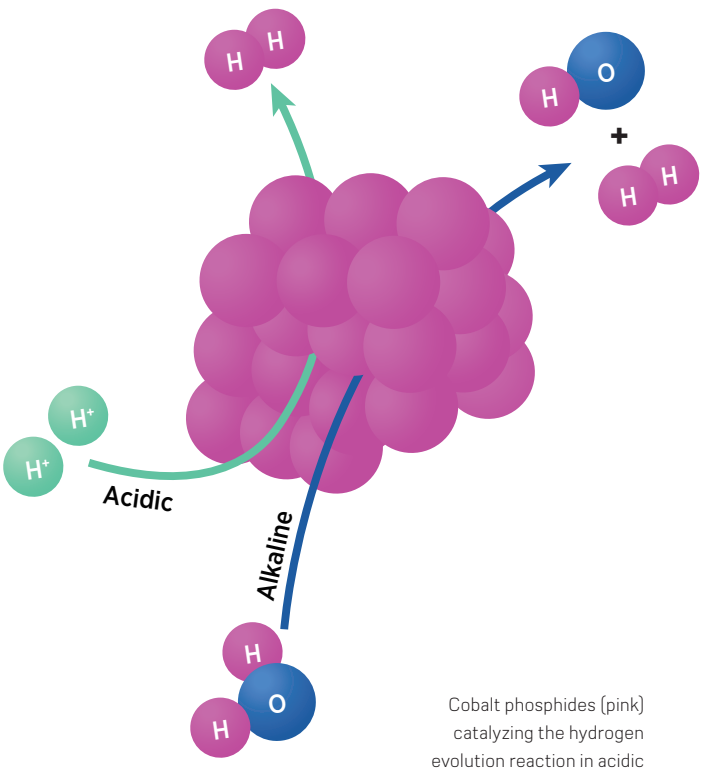
Hydrogen is an energy-dense, clean and renewable fuel source that plays a key role in many industrial processes, such as the production of ammonia as well as the processing and refining of fossil fuels. Yet, it is predominantly produced through the steam reforming of natural gas, a high-temperature extraction from hydrocarbons that emits carbon dioxide in large quantities.

Water electrolysis and light-driven water splitting, which isolate hydrogen from water, have emerged as attractive environmentally-friendly alternatives to this process. These electrochemical approaches depend on the slow formation of oxygen at the anode concomitant with the hydrogen evolution reaction at the cathode. However, while hydrogen evolution has proven easier under acidic conditions, electrocatalysts developed to speed up the anodic reaction have shown higher activity in alkaline media. In response to this, the team has devised nanostructured cobalt phosphides that are able to facilitate the hydrogen evolution reaction regardless of the pH of the aqueous media[1].

The researchers have concocted a facile and scalable synthesis that yields catalysts in one step. In this one-step synthesis, a phosphorus-containing compound reacts directly with commercially available cobalt salts when heated to 330 degrees Celsius. This unusual gas–solid 'phosphidation' produces a mixture of cobalt-containing compounds, including mono- and bimetallic phosphides, oxides, and trace metal with a small amount of gaseous phosphine.

"Each of these cobaltous species is expected to benefit the overall HER activity," says Zong, noting that most gas–solid phosphidation reactions only give the monometallic derivative in low yields. He adds that conventional syntheses require multiple steps involving the production of a cobalt precursor and the subsequent phosphide formation.

In addition to integrating their cobalt-base catalysts into a water splitting system, the team is currently working on screening other transition metal salts and optimizing the synthetic procedures. "These protocols may be extended to one-step phosphidation of other transition-metal salts," says Zong, adding that insight into the direct phosphidation of cobalt salts provides valuable input for the future large-scale synthesis of transition metal phosphides.



Cobalt phosphides (pink) catalyzing the hydrogen evolution reaction in acidic and alkaline media.

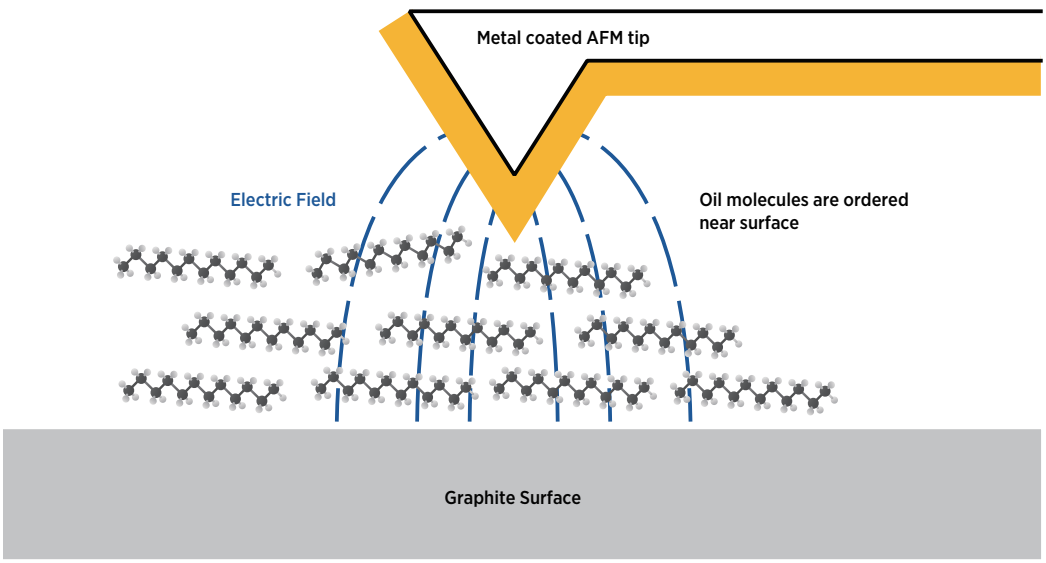
Adapted with permission from Ref 1. Copyright (2018) American Chemical Society.

1. Sumboja, A., An, T., Goh, H. Y., Lübke, M., Howard, D. P. *et al.* One-step facile synthesis of cobalt phosphides for hydrogen evolution reaction catalyst in acidic and alkaline medium. *ACS Applied Materials and Interfaces* **10**, 15673–15680 (2018).

CHEMISTRY

ANOTHER STEP CLOSER TO TUNABLE LIQUIDS

By investigating the influence of electric fields on liquids, scientists could develop a new technique for controlling their friction and adhesive properties



The sharp, metal-coated tip of an atomic force microscope (AFM) can apply very high electric fields across liquid molecules (in this case an oil) right next to a surface.

Researchers at A*STAR have developed a technique for investigating the effect of electric fields on the properties of oil-based lubricants, which could lead to new applications in nanofluidics and nanotribology. Using electrical fields to modify the properties of liquids in contact with a surface can be used in several applications, such as electrophoresis, where an electric current can separate molecules by size.[1]

When a liquid is confined between closely spaced surfaces, it may form ordered layers, leading to changes in the liquid's viscosity and molecular structure. Understanding the mechanical properties of these ordered layers is important for the development of nanotechnology devices and lubricants.

"This [work] represents another step closer to controllable lubrication and/or liquid flow on surfaces."

This led Sean O'Shea and Eugene Soh from the A*STAR Institute of Materials Research and Engineering to develop a technique for investigating whether the mechanical properties of liquids, such as flow or surface adhesion, can be tuned by applying external electric fields.

"These are the type of engineering questions to be addressed in order to develop 'smart' surfaces for applications that require electrically controllable adhesion, lubrication or flow," says O'Shea. "However, we first need to investigate the presence of any significant electrically induced effects."

Studies so far have primarily used water or ionic liquids because their polar nature means they are significantly influenced by electric fields. However, the use of ionic liquids is expensive, so the researchers used the more conventional lubricants undecanol and tetradecane, which consist of long-chain hydrocarbons that provide thick ordered layers.

With a strong electric field between the tip of an atomic force microscope (AFM) and a graphite substrate submerged in the liquids, the researchers were able to produce highly ordered layers of hydrocarbons along the surface of the graphite. A feature of these ordered layers is that they give rise to oscillatory forces that can be measured by the AFM.

Although oscillatory forces were observed when no electric field was applied — indicating ordered layers in the liquid close to the surface — these forces appeared far less frequently when a strong electric field was applied across undecanol, and slightly less frequently in tetradecane.

But when the liquids were boiled at 140 degrees Celsius to remove the small amounts of water present in the oils, the oscillatory forces remained present even at high electric field strengths.

"Our work suggests that in addition to changes in molecule orientation, another mechanism, which arises from the presence of trace amounts of water, must be considered when electric fields are applied," says O'Shea. "This represents another step closer to controllable lubrication and/or liquid flow on surfaces." ■

1. Soh, E. J. H.& O'Shea, S. J. Effect of electric field and trace water on confined undecanol and tetradecane. *Journal of Physical Chemistry C* **122**, 3326 – 3333 (2018).

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MACHINE LEARNING

PREDICTING DIFFUSION DATA

A high-throughput computer model predicts diffusion data for the transport of light elements within solids

The diffusion of low-mass elements into metals has been efficiently modeled by A*STAR researchers using a machine learning approach[1].

Solid-state diffusion, in which atoms migrate through the lattice of a host material, underpins a variety of important processes that range from undesirable (corrosion) to useful (metal-joining processes). In one mechanism called 'interstitial diffusion', light elements, such as nitrogen, move through lattices made up of much bigger atoms, such as metals, by squeezing between them. Yingzhi Zeng and colleagues at the A*STAR Institute of High Performance Computing have now developed a rapid predictive model for this phenomenon.

"Typical examples of interstitial diffusion include surface hardening of steel through carburization or nitridation, and the diffusion of oxygen in titanium for the design of implant and aerospace alloys" Zeng says. This process is important to understand, but particularly difficult to probe experimentally. The challenge stems from the heavy-duty specialized equipment that is often required, and because as Zeng explains, "most experimental techniques rely on surface measurements, and so are inherently limited to a few nanometers under the surface."

Computational studies can circumvent these technical difficulties; first-principle methods have been shown to reliably predict diffusion transport rates, but they are time-consuming. Yingzhi Zeng and co-workers have dramatically sped up calculations of diffusion activation energies — the energy required for a light element to move through its host lattice — through machine learning.

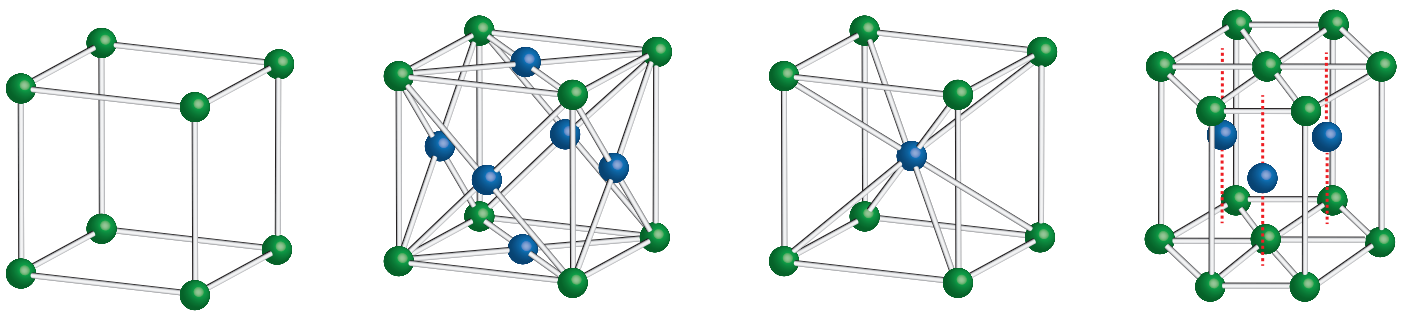
"Our predicted results have offered large amounts of reliable data — 554 new sets of diffusion data."

They first 'trained' a model on a set of existing data, consisting of experimental activation energies complemented with first-principle calculations. The dataset was selected for consistency: for example only high temperatures and small solute concentrations were considered. 94 systems were used, each consisting of one solute (boron, carbon, oxygen or nitrogen) diffusing through a metal host adopting one of three most widespread lattice arrangements: body center cubic (bcc), face-centered cubic (fcc) or hexagonal close packed (hcp).

The accuracy of the model was verified by using it to predict known activation energies, and comparing the calculated results with the experimental values. It was then used to calculate activation energies for systems for which no experimental data have been reported. "Our predicted results have offered large amounts of reliable data — 554 new sets of diffusion data covering almost all the metals in the periodic table with the three common crystal structures of bcc, fcc, and hcp — for the conditions that are most commonly used in experiments," Zeng says.

The immediate aim of the study is two-fold: to go on to predict transport rates in materials, and to gain insight into the factors driving the diffusion process. But the team won't stop there. "We are planning to develop a mobility database for materials microstructure simulation," Zeng says. ■

1. Zeng, Y., Li, Q., Bai, K. Prediction of interstitial diffusion activation energies of nitrogen, oxygen, boron and carbon in bcc, fcc, and hcp metals using machine learning. *Computational Materials Science* **144**, 232–247 (2018).



Unit cells in crystal lattices. (left to right) Simple cubic (sc), cubic face-centered (fcc), cubic body-centered (bcc) and hexagonal (hcp)

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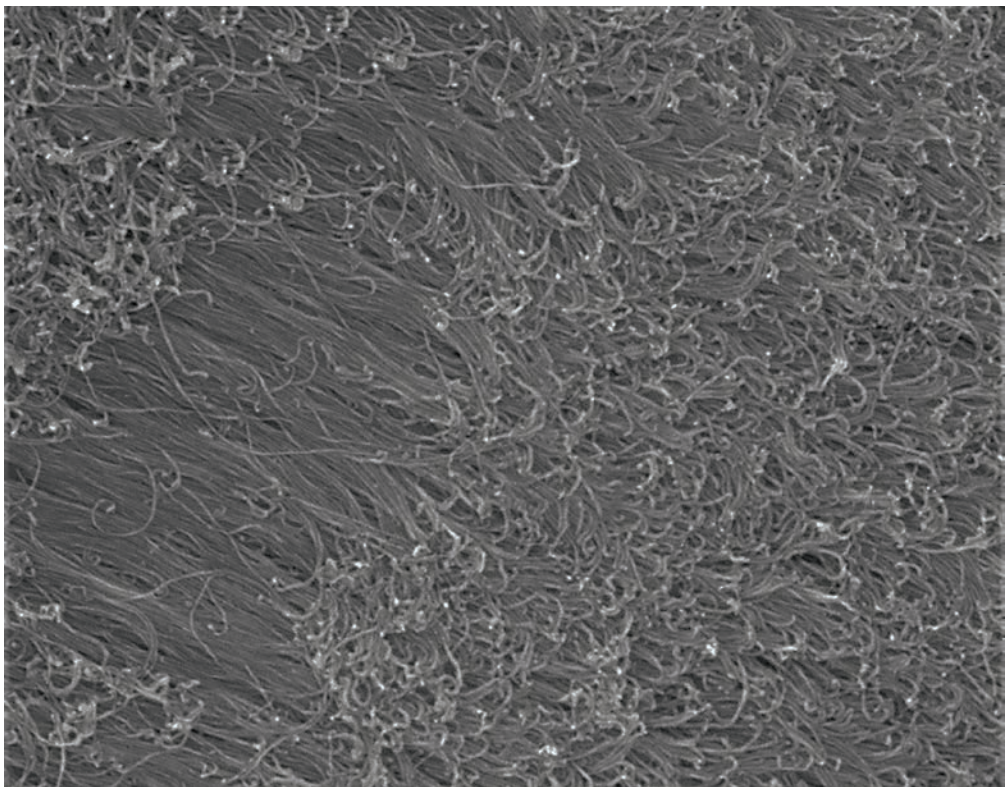
CHEMISTRY

WIRED FOR GROWTH

Thiol molecules drive gold atoms to form a forest of nanowires with useful properties



A forest of gold nanowires grown using 4-mercaptobenzoic acid.

Adjustments to sulfur-containing molecules have enabled researchers to precisely control the growth of gold nanowires, which are potentially useful in various applications including biosensors and catalysis[1].

Ligand molecules are used to prevent nanostructures from growing too large, or forming unwanted shapes. Suzhu Yu of the A*STAR Singapore Institute of Manufacturing Technology and colleagues had previously found that sulfur-containing molecules called thiols, which bind to gold, could be used to grow very thin gold nanowires. Now they have investigated how exactly these thiols do their job, and shown that different types of thiols can fine-tune the shape and size of the nanowires.

The researchers attached gold particles a few nanometers wide to a wafer of silicon, and then dipped this assembly into a solution containing a gold compound, a thiol ligand, and a reducing agent that generated gold atoms. When they used a ligand called 4-mercaptobenzoic acid (4-MBA), the nanoparticles sprouted a forest of gold nanowires that were 6 nanometers across and about 1 micrometer long in 15 minutes.

"This active surface growth mechanism is fundamentally different from other gold nanowire growth strategies."

The ligand binds strongly to any exposed part of the gold nanowire, and interactions between ligand molecules keep them densely packed on the wire's surface. This prevents gold atoms in solution from sticking to the sides of the wire, so that they only join the growing wire at the junction between the wire and the wafer. Consequently, the gold nanowire grows like hair sprouting from a follicle, rather than forming a sphere. "This active surface growth mechanism is fundamentally different from other gold nanowire growth strategies," says Yu.

Changing the position of the chemical groups in the ligand also had a dramatic effect on nanowire growth. In 4-MBA, the thiol group is on the opposite side of the molecule to a carboxylic acid group. If these groups are side by side, as in 2-MBA, the carboxylic acid interferes with the packing of the ligands around the nanowire, allowing gold atoms to sneak through and form short, lumpy nanostructures. A mixture of 4-MBA and 3-MBA ligands allowed some gold atoms to stick to the sides of the nanostructure as it grew, creating a tapered nanowire. Another ligand, 2-naphthalenethiol, made the gold nanowire extremely water repellent — a potentially useful property in functional surfaces.

The team hopes to use this approach to make other gold nanostructures, and explore how they can be used as high efficiency electrodes in flexible sensors, or as catalysts to turn carbon dioxide into useful products. ■

1. Wang, Y., He, J., Yu, S. & Chen, H. Effect of thiolated ligands in Au nanowire synthesis. *Small* **13**, 1702121 (2017).

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CONCRETE

A HARD LOOK AT POLYMERS IN CEMENT MIX

Computer simulations have zoomed in on the role of superplasticizers in concrete performance

Modelling how superplasticizers can reduce water proportions in cement mixtures which help develop more efficient superplasticizers, as well as enhance concrete performance, shows the first comprehensive study conducted by A*STAR[1].

Superplasticizers are polymers that act as dispersants in cement mixtures. They hinder the aggregation of the cement particles, allowing dramatic reductions in the volume of water in the mixture without impacting on its flow and workability. While some water is needed to enable cement to harden to concrete through hydration, reducing the proportion of water in cement mixtures results in stronger products.

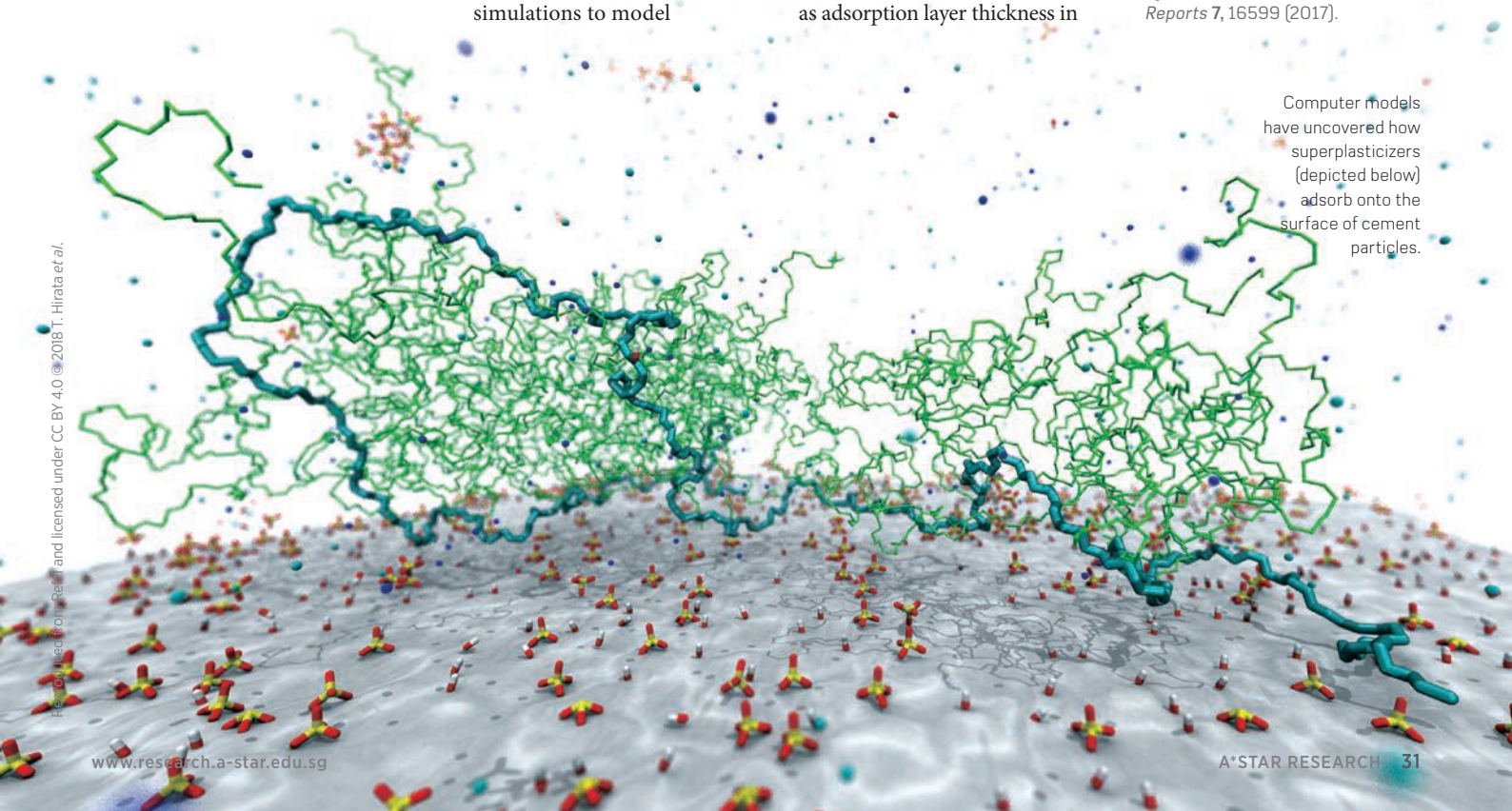
In collaboration with the global chemical company, Nippon Shokubai, Jianwei Zheng at the A*STAR Institute of High Performance Computing and colleagues used molecular dynamics simulations to model the adsorption of three novel superplasticizers – polycarboxylate ethers (PCEs) – onto the surface of magnesium oxide particles in a cement mixture.

PCE-based superplasticizers have negatively-charged carboxylic acid groups in their polymer backbone that adsorb electrostatically to particles in cement such as magnesium oxide. The long polyethylene glycol groups then act as spacers, preventing the cement particles from clumping. A number of earlier, smaller modeling studies suggested that the thickness of this adsorbed polymer layer directly correlates with the amount of dispersion seen. Zheng's team is the first to carry out a comprehensive modeling study to determine how the shape of the polymer influences layer construction and depth.

"We describe the correlation of molecular structures of PCE-type superplasticizers with polymer conformation as well as adsorption layer thickness in cement pore solution," explains Zheng. The team found that the thickness of the layer depends on how the polymers initially orientate themselves against the particle surface. Those that start perpendicular to the surface gradually form a tail with a loop on their end. These polymers eventually form the desired thicker layer. By contrast, those that start out parallel to the surface grow into a tail and result in a thinner layer.

The team plan to conduct further simulations with different polymeric structures to see if the layer depth can be increased further. "More efficient superplasticizers may be designed in near future," says Zheng. "The effect of superplasticizers on cement hydration will be under consideration in future models." ■

1. Hirata, T., Ye, J., Branicio, P., Zheng, J., Lange, A. *et al.* Adsorbed conformations of PCE superplasticizers in cement pore solution unraveled by molecular dynamics simulations *Scientific Reports* **7**, 16599 (2017).



Computer models have uncovered how superplasticizers (depicted below) adsorb onto the surface of cement particles.

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A new mathematical method should minimize the loss of flavor molecules into plastic food packaging.

POLYMER CHEMISTRY

FORMULA FOR FLAVOR

Model predicts plastic food packaging's propensity for absorbing aroma molecules from their contents

Your favorite foods and beverages could soon taste even better, thanks to new calculations developed at A*STAR. One significant source of flavor loss for food and beverages is from aroma molecules that are absorbed into the plastic packaging materials used to store the products. Researchers have now developed a method to quickly predict aroma molecule absorption by different packaging polymers[1].

Experimentally measuring the loss of flavor molecules and other organic compounds into their polymer packaging is difficult and slow, explains Jianwei Zheng from the A*STAR Institute of High Performance Computing. "The concentration of organic compounds such as flavor molecules in a beverage is very dilute," he says. And the absorption process from the beverage into the polymer can take a few months to reach equilibrium, he adds.

Zheng and his colleagues teamed up with researchers from the Coca-Cola Company in Atlanta, USA, to develop a faster method. The team developed a mathematical calculation to rapidly predict the extent to which the packaging would absorb organic molecules from the beverage products they contain.

The team adapted a well-known model of polymer properties, first developed in the 1940s, called Flory-Higgins theory. This mathematical model was originally developed to describe the mixing behavior of a polymer with a solvent.

To adapt the model so that it could describe the mixing behavior of polymers with organic compounds, such as aroma molecules, the team combined Flory-Higgins theory with group contribution methods (GCM). "Group contribution methods assume the properties of a substance are the sum of contributions from all constituent chemical groups," Zheng explains. So the properties of the molecule CH_3-CH_2-CH_2-COOH, for example, can be calculated by summing the properties of one CH_3 group, two CH_2 groups and one COOH group. "The data of each group is usually estimated from experimental data," Zheng says.

In the case of food packaging, the calculation essentially predicts the extent to which a given aroma molecule will dissolve into a given polymer by summing the mixing of each chemical component of the polymer with each chemical component of the aroma molecule.

The team tested its approach using aroma molecules such as limonene and eugenol, and polymers such as PET and PVC. "The calculation is very fast, and the results were consistent with available experimental data," Zheng says.

The researchers are now refining and expanding their approach, "fine-tuning group contribution parameters and building up the database for more groups," he adds. ■

1. Hong, B., Zhang, L., Zheng, J., Sullivan, M. B., You, X. *et al.* Fast estimation of sorption of organic compounds in polymeric packaging materials. *Food Packaging and Shelf Life* **16**, 97–102 (2018).

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BIOMATERIALS

RECONSTRUCTING SKIN ON A CHIP

A microfluidic organ-on-a-chip device combines skin culture and testing capabilities to offer a substitute to animal testing

Microfluidics could fulfill a growing need for alternatives to animal testing for the development of pharmaceuticals and cosmetics. A multidisciplinary team, led by Zhiping Wang from the A*STAR Singapore Institute of Manufacturing Technology, and Paul Bigliardi from the A*STAR Institute of Medical Biology, have produced a scalable credit-card sized device that simultaneously facilitates skin cell culture and testing[1].

State-of-the-art alternatives to animal testing rely on reconstructed skin. However, these three-dimensional tissue models are typically generated from static cell cultures on a collagen matrix that readily shrinks. "When collagen contracts, we don't know whether compounds under investigation are going through the skin or through gaps between the device and the skin during permeation tests," explains Gopu Sriram, one of the lead authors.

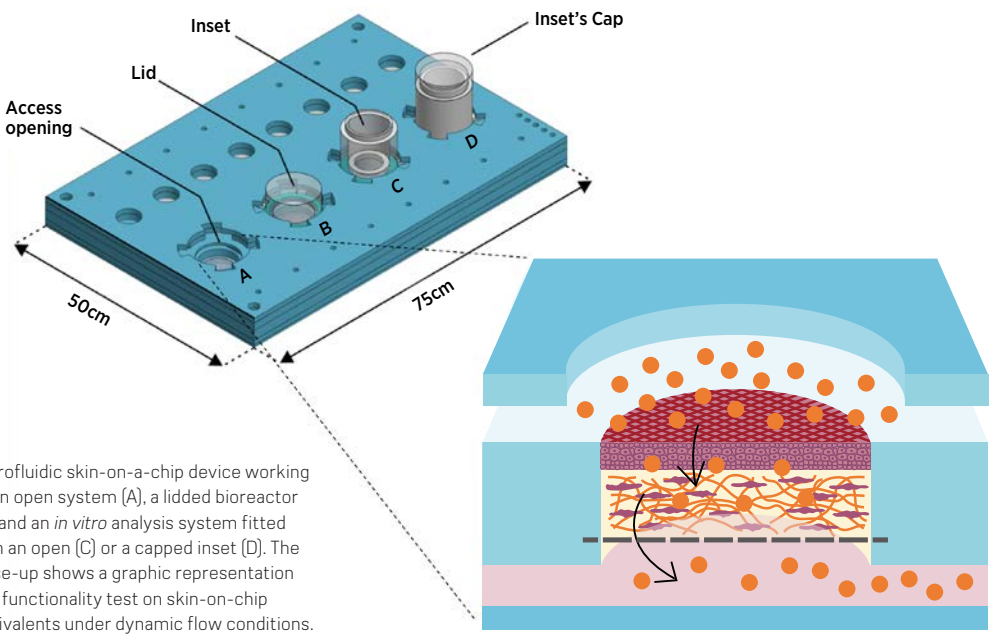
To address these problems, the researchers developed a method to grow skin on a matrix using the protein fibrin, preventing skin contraction. The skin is grown directly in the microfluidic device where the tests are conducted, without further manipulation or transfer.

"Compared to conventional skin reconstruction, the skin-on-chip platform offers better skin morphology and performance, in terms of barrier function."

Skin cultured in the microfluidic device exhibited enhanced maturation of the epidermis, the top protective layer of the skin. This translated to a nearly two-fold increase in epidermis thickness compared to standard skin equivalents. "This enhanced epidermis correlated with lower chemical permeability than in conventional systems," says Yuri Dancik, another lead author. "Compared to conventional skin reconstruction, the skin-on-chip platform offers better skin morphology and performance, in terms of barrier function," adds Wang. It can also facilitate downstream assays using commercially available skin equivalents or natural skin.

According to Massimo Alberti, another lead author, these enhancements stem from the use of microfluidics. Under static conditions, nutrients and medium passively diffuse through the skin. By contrast, in the microfluidic chip, a continuous flow generates pressure that pushes the culture medium through the matrix and may act as a "stressor for the cells and the extracellular matrix, which may also activate some mechanically-triggered signaling pathways," he says. This stimulation also promotes the formation of a superior basement membrane, a "Velcro-like protein layer that anchors the epidermis to the connective tissue called dermis," says Sriram.

In addition to automating their system, the researchers are currently working to improve their model to better mimic natural human skin. They plan to increase the complexity of their model by adding immune cells and enhancing its barrier function. They are also optimising the microfluidic device by simulating blood flow dynamics and implementing additional microenvironment controls "to promote conditions that will bring the system closer to human skin," says Alberti. ■



Microfluidic skin-on-a-chip device working as an open system (A), a lidded bioreactor (B), and an *in vitro* analysis system fitted with an open (C) or a capped inset (D). The close-up shows a graphic representation of a functionality test on skin-on-chip equivalents under dynamic flow conditions.

1. Sriram, G., Alberti, M., Dancik, Y., Wu, B., Wu, R. *et al.* Full-thickness human skin-on-chip with enhanced epidermal morphogenesis and barrier function. *Materials Today* **21**, 326–340 (2018).

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INFECTIOUS DISEASES

DOUBLING DOWN ON INFECTION

A new understanding of the way chikungunya virus protects mice against malaria could lead to improved patient care

Chikungunya virus infection may reduce the severity of malaria, according to a discovery by A*STAR scientists, which could lead to the development of new malaria treatments.

Spread by different mosquito species in many tropical countries, the two infections commonly occur in tandem, recent studies have found. Cases of chikungunya virus have risen significantly in the last decade and co-infection with malaria is frequently misdiagnosed as malaria only.

As diagnosis methods improve, a better understanding of the effects of co-infection will help treatment options. Previous research by Laurent Rénia of the A*STAR Singapore Immunology Network (SIgN) has shown that 70-80 per cent of mice infected with the parasite *Plasmodium berghei*, widely used by scientists as a model of human malaria, die within six to 12 days.

In a new study, a team led by Rénia and Lisa Ng, also of SIgN, showed similar results for mice infected with *P. berghei*, both four days before and after being also infected with chikungunya virus. However, among another group simultaneously infected with both, close to 75 per cent survived for at least 26 days.

"Targeting this same pathway to prevent or reduce CD8⁺ T cell migration to the brain could offer a new way to treat malaria."

The researchers found that mice infected with both pathogens had lower levels of *P. berghei* in their brains and less damage to the blood brain barrier than those infected only with the malaria-causing parasite.

CD8⁺ T cells, a type of white blood cell, play important roles in the body's defenses against pathogens including malaria, using various methods to attack and kill infected or malignant cells. The team showed that levels of CD8⁺ T cells were lower in the brains of co-infected mice than in those of mice with malaria only, while remaining similar in the spleen.

Rénia and Ng went on to discover the complex chain of molecular events behind the protective effects of co-infection. Namely CD4⁺ T cells, which regulate immune responses, secrete a protein called interferon gamma, causing changes in levels of proteins involved in cell signaling and trafficking. This, in turn, reduces migration of CD8⁺ T cells to the brain, which, in the case of infection with malaria alone, can cause severe neurological damage.

This improved understanding of the mechanisms underlying the protective effect of chikungunya virus on malaria in mouse models could provide the basis for human therapies. "Targeting this same pathway to prevent or reduce CD8⁺ T cell migration to the brain could offer a new way to treat malaria," says Ng. ■

1. Teo, T-H., Howland, S. W., Claser, C., Ng, L., Rénia L. *et al.* Co-infection with Chikungunya virus alters trafficking of pathogenic CD8⁺ T cells into the brain and prevents Plasmodium-induced neuropathology. *EMBO Molecular Medicine* **10**, 121–138 (2017).

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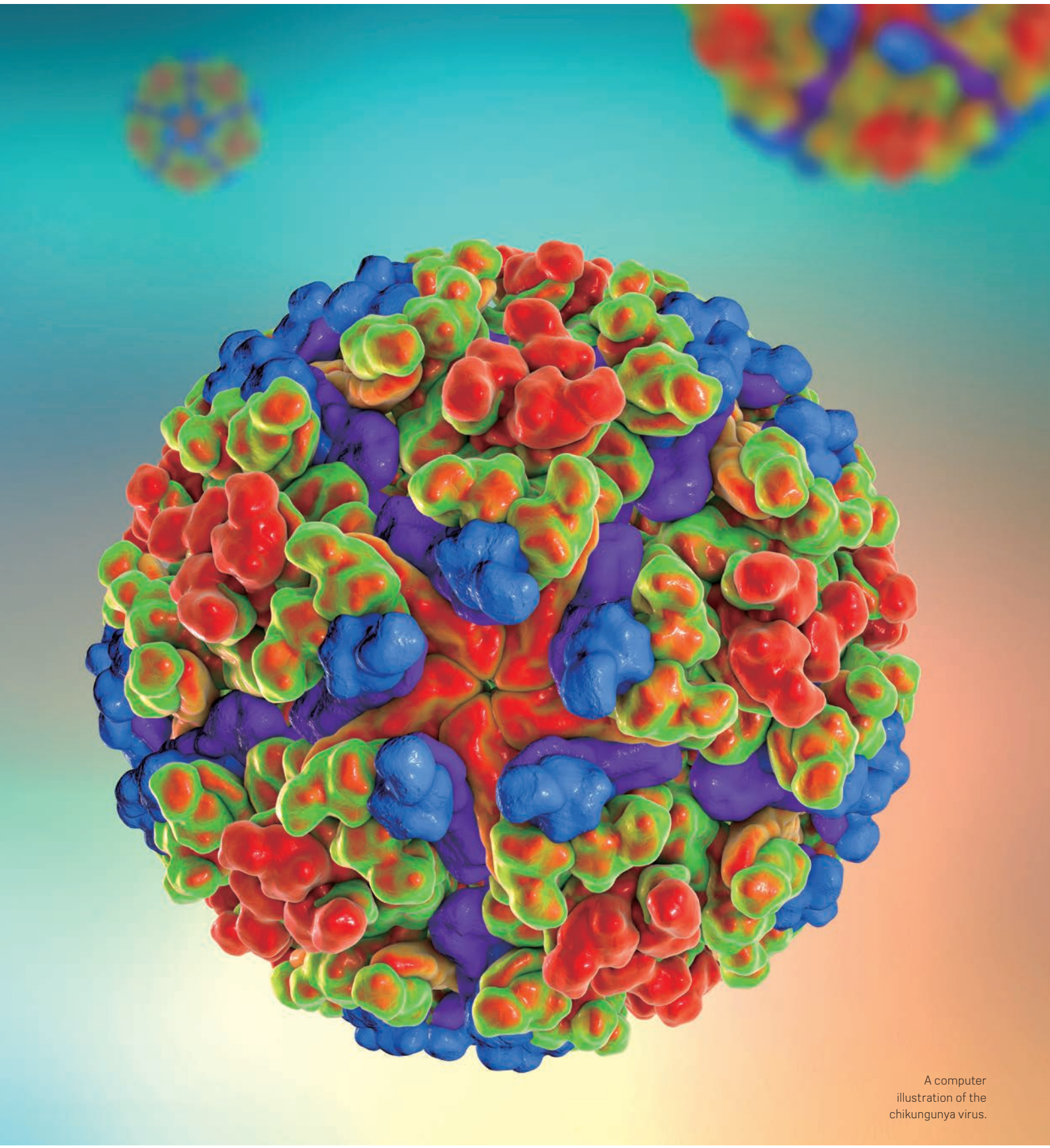
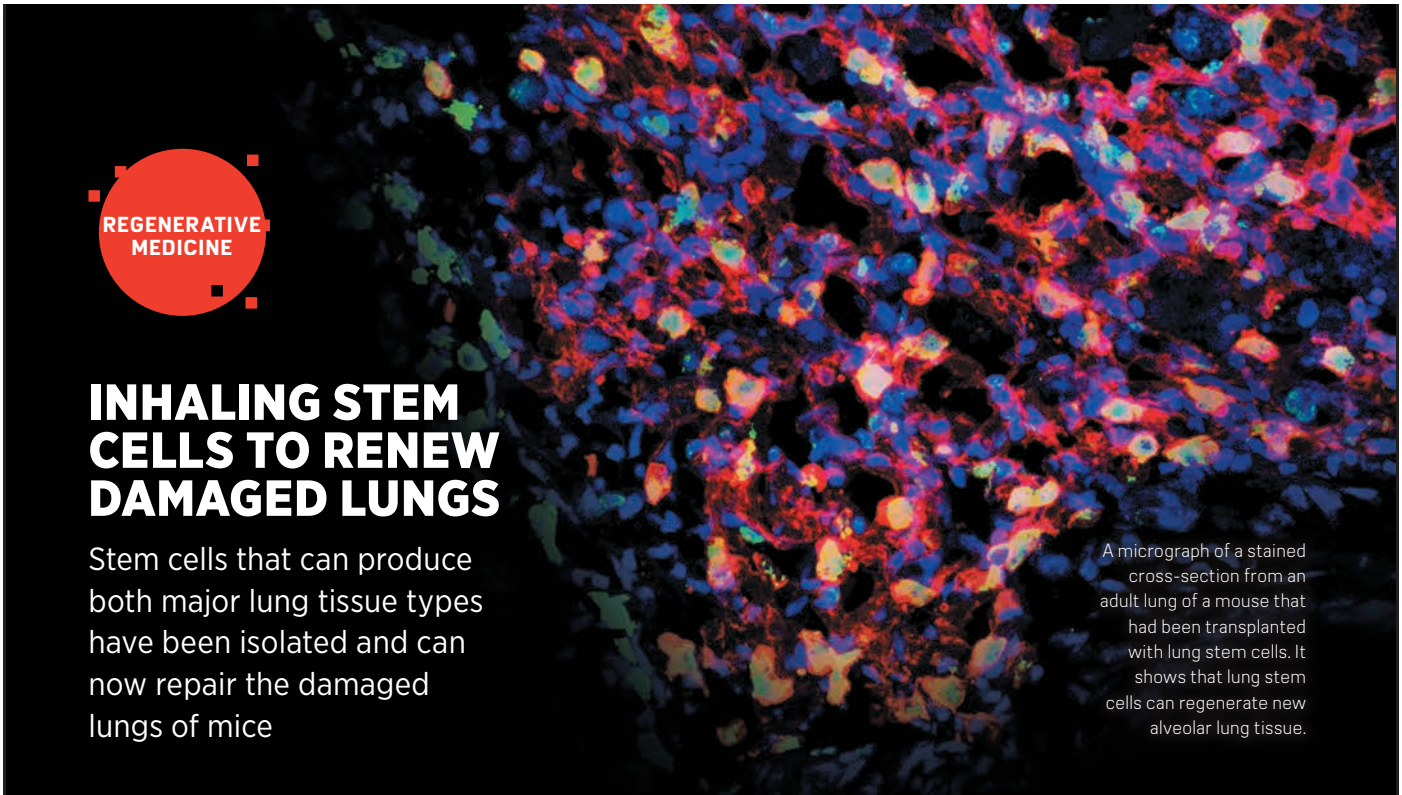


A computer illustration of the chikungunya virus.



REGENERATIVE MEDICINE

INHALING STEM CELLS TO RENEW DAMAGED LUNGS

Stem cells that can produce both major lung tissue types have been isolated and can now repair the damaged lungs of mice

A micrograph of a stained cross-section from an adult lung of a mouse that had been transplanted with lung stem cells. It shows that lung stem cells can regenerate new alveolar lung tissue.

By isolating transient stem cells that can grow the two main types of lung tissue, an international team of researchers have partially restored the damaged lungs of mice, bringing hope for the possibility of human lung repair[1].

Our lungs are susceptible to a range of diseases. Currently, the only treatment for damaged lungs is a transplant, for which demand greatly outstrips supply.

The lungs are thus a prime target for the emerging regenerative medicine field, but "there is currently a dearth of regenerative therapies for lung diseases," notes Kyle Loh from the Stanford University School of Medicine in the USA, who jointly directed the work.

This gap in medical science exists because adult stem cells' ability to mature into cells from different tissues decreases as they age. Scientists had previously only been able to isolate stem cells that produced either — but not both — of the two major lung tissue types: one is found in the airways that transport air, and the other makes up the alveoli, where carbon dioxide in the blood is exchanged with inhaled air.

Now, Bing Lim of the A*STAR Genome Institute of Singapore and colleagues have shown that a single population of stem cells derived from mouse embryos can produce both tissue types. They also demonstrated that the stem cells can be used to repair damaged lungs in mice.

Lung stem cells extracted in an early lung development stage were grown in the laboratory. The researchers generated 100 billion billion (i.e. 10^{20} or about 10 million times the number of cells in the body of an adult human) new lung stem cells over six months.

They then tested whether these stem cells could regenerate lung tissue after damage to the lungs.

To achieve this, the team had to find a safe and easy way to deliver the cells. They did this by getting the mice to inhale a droplet of liquid containing the lung stem cells.

"On injection into the toxin-injured lungs of laboratory mice, the lung stem cells could regenerate new airway and alveolar lung tissue," says Loh. "The critical advance of the paper was that *both* cell types were regenerated," he notes.

"In the future, we hope that lung stem cell transplantation can be used to regenerate new lung tissue [in humans] *in vivo*," he adds, but notes that they first have to test the safety and efficacy in animal models of end-stage lung disease.

The team also plans to extend their lab work to human cells to see whether they can identify equivalent stem cells that can regenerate both kinds of lung tissue. ■

1. Nichane, M., Javed, A., Sivakamasundari, V., Ganesan, M., Ang, L. T. *et al.* Isolation and 3D expansion of multipotent Sox9+ mouse lung progenitors. *Nature Methods* **14**, 1205–1212 (2017).

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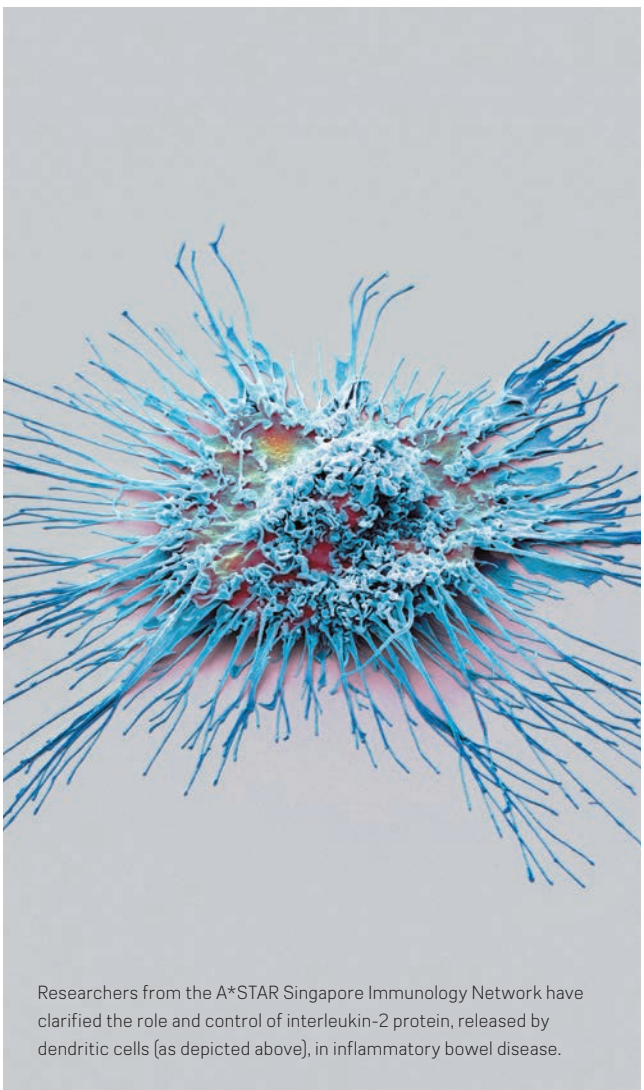
INFLAMMATORY BOWEL DISEASE

FINDING THE CELLS AND THE SIGNALS

Clarifying the role of a protein released by immune 'dendritic' cells could yield better treatments



Researchers from the A*STAR Singapore Immunology Network have clarified the role and control of interleukin-2 protein, released by dendritic cells (as depicted above), in inflammatory bowel disease.

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Possibilities for new treatments to combat Inflammatory Bowel Disease (IBD), which afflicts millions of people worldwide, may come about from the insights of A*STAR researchers. IBD arises when the immune system mounts an overly aggressive response against microorganisms, dietary substances, or cells of the gut. A*STAR researchers have identified specific cells and molecular signals that maintain appropriate immune activity, and that may be disrupted when IBD develops.

The human intestine carries a complex mixture of microorganisms: some benefit the digestive system and general health, while others can cause serious illness. The cells of the immune system need to balance responding effectively to attack harmful infections in the gut with tolerating harmless microorganisms and other gut contents. If the immune response is over-aggressive, it causes the intestinal inflammation that leads to the symptoms of chronic IBD.

"We discovered that interleukin-2 protein secreted by specialized immune cells, called dendritic cells, in the intestinal lining, keep inflammation in check," says Alessandra Mortellaro of the A*STAR Singapore Immunology Network. She explains that interleukin-2 released by dendritic cells achieves this effect by suppressing the activity of other immune system cells called inflammatory T-cells. In a further demonstration of the sophisticated immune control network, the dendritic cell interleukin-2 also promotes the generation of other specialized T cells that suppress the immune response.

Interleukin-2 is released by many types of cells in the body, including dendritic and T-cells. To clarify the specific role of interleukin-2 released by dendritic cells, the A*STAR team sought a way to separate the effects of different sources of the protein. "To achieve this, we generated mice that did not specifically produce interleukin-2 from dendritic cells," says Mortellaro. These mice suffered severe and spontaneous intestinal inflammation.

"[Finding that interleukin-2 performs distinct and specific functions] was a real breakthrough moment."

The researchers also discovered that the mice had a different form of inflammation than mice in which interleukin-2 production was knocked out from both dendritic cells and T-cells. Finding that the dendritic cell interleukin-2 performs distinct and specific functions "was a real breakthrough moment," explains Mortellaro. The processes in mice are assumed to be similar to those in humans.

By unraveling molecular details of the signaling systems to reveal the specific and previously unknown role of interleukin-2 from dendritic cells, the researchers hope to prompt new research into possibilities for treatment. One option could be finding ways to specifically boost interleukin-2 production by dendritic cells. The researchers also want to understand if dendritic cell interleukin-2 regulation is involved in other inflammatory and autoimmune diseases affecting tissues such as the skin. ■

1. Mencarelli, A., Khameneh, H. J., Fric, J., Vacca, M., Daker, S. E. *et al.* Calcineurin-mediated IL-2 production by CD11c^{high}MHCII^{+} myeloid cells is crucial for intestinal immune homeostasis. *Nature Communications* **9**, 1102 (2018).

GENETICS

DNA TEST REVEALS GENETIC RISK FACTORS FOR ECZEMA

Assay combines microfluidics and next-generation sequencing to reveal more mutations than existing targeted gene tests

A gene test developed by A*STAR researchers reveals mutations that predispose people to atopic dermatitis, an inflammatory skin disorder.

A new diagnostic test developed at A*STAR is helping researchers around the world identify gene mutations that put people at risk of severe eczema and other chronic skin diseases[1].

The skin's outer layer forms a protective barrier against the external environment, thanks to a gene called *FLG*. People lacking a functional copy of this gene, which encodes a thread-like structural protein known as filaggrin or 'filament aggregating protein', often develop skin deficiencies that can trigger a severe form of eczema known as atopic dermatitis — an inflammatory skin condition that causes persistent rashes that are red and itchy. Mutations in *FLG* can also predispose people to asthma and other autoimmune diseases.

Physicians currently have several gene tests that can help them identify patients with suspected *FLG*-associated health problems. But these tests generally target only a handful of common mutations found primarily in Caucasian populations from Northern Europe, which limits their usefulness in Asia and other regions.

Now, John Common and his colleagues from the A*STAR Institute of Medical Biology have created a more globally applicable test that combines microfluidics and next-generation sequencing technologies to decode the entire span of the *FLG* gene.

As a proof of concept, they used the assay on blood samples from a large cohort of Singaporean patients of Chinese, Malay and Indian descent. The team identified 51 different disease-causing mutations, most of which would be missed by existing screening methods.

Knowing whether someone has inherited a mutant copy of *FLG* is important clinical information, notes Common. "If a mutation is present in a disease-susceptible newborn, then early intervention with standard moisturizers could help to prevent or delay the onset of atopic dermatitis," he says. "This would improve the quality of life for not just the child but also the family, and prevent the need for more-expensive topical and systemic immunotherapies downstream."

Since publishing a description of the assay last year, Common has made the test available to the global research community, which has rapidly adopted it. In the United States, for example, a team of dermatologists used the technique to identify population-specific mutations in African-American children with severe atopic dermatitis. And in the United Kingdom, the assay helped researchers find *FLG* mutations in Bangladeshi families with the skin condition.

According to Common, it is too early for the assay to be used routinely in clinical practice — more-rigorous quality controls and standard operating procedures need to be established. However, he believes their assay will ultimately supplant targeted tests that reveal only a predefined set of mutations — both in Asia and around the world. ■

1. Wong, X. F. C. C., Denil, S. L. I. J., Foo, J. N., Chen, H., Tay, A. S. L. *et al.* Array-based sequencing of filaggrin gene for comprehensive detection of disease-associated variants. *The Journal of Allergy and Clinical Immunology* **141**, 814–816 (2018).

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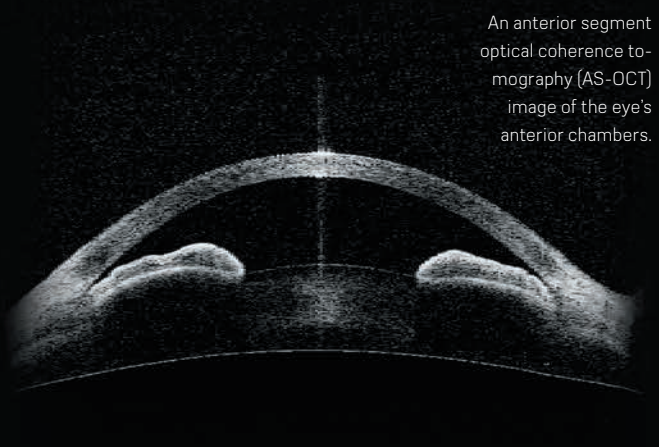
GLAUCOMA

A BIRD'S EYE VIEW FOR IMPROVED DIAGNOSIS

An algorithm paves the way for accurate, rapid diagnosis of closed-angle glaucoma



An anterior segment optical coherence tomography (AS-OCT) image of the eye's anterior chambers.

An effective method of screening patients for closed-angle glaucoma has been developed by A*STAR researchers.

In closed-angle glaucoma, the optic nerve is damaged because the aqueous fluid in the eye does not drain properly. This is because the gap between the cornea and the iris, known as the anterior chamber angle, where fluid normally flows through the eye, has narrowed significantly or become blocked. The resulting build-up of pressure within the eye, which can happen rapidly, can cause irreversible blindness.

Current diagnosis of closed-angle glaucoma involves analyzing 'anterior segment optical coherence tomography' (AS-OCT) images, which are cross-sectional pictures of the eye's anterior chambers. However, manual comparison of AS-OCT images, particularly those taken using different systems, takes considerable time and expertise.

"We wanted to create a fully-automated, rapid method for screening AS-OCT images that could ascertain whether closed-angle glaucoma is present," explains Huazhu Fu at A*STAR's Institute for Infocomm Research, who led the project alongside Mani Baskaran at Singapore Eye Research Institute, with scientists from China and the UK. "Our algorithm compares each individual image with a library of exemplar images, before producing a set of clinical parameters from each image that clinicians can use to validate diagnosis."

Fu's team created a reference dataset of labelled images taken by different AS-OCT machines of several thousand individual eyes, both with and without clinically-diagnosed glaucoma. Their algorithm then uses the dataset to analyze newly-uploaded images.

"Although the eye structure shown in every AS-OCT image is different, local regions within each eye are similar. When our algorithm receives a new eye image, it searches the annotated dataset for 'best-fit' images that are the closest match in terms of eye structure and anterior chamber angle, before transferring the labels from these selected library images to the new image," Fu explains.

"This will significantly improve screening for patients in clinics or community settings and provide support for clinicians."

The algorithm then applies a smoothing technique to refine the structural labeling and ensure it is accurate for each unique eye shape. This allows very precise clinical measurements of the anterior chamber angle and other diagnostic features.

During tests, the algorithm was able to segment and measure one AS-OCT image in ten seconds, providing a potentially invaluable tool for clinicians.

"Our algorithm accurately interprets single or multiple AS-OCT scans, is fully automated and does not depend on specialist training," says Baskaran. "This will significantly improve screening for patients in clinics or community settings and provide support for clinicians."

The team are expanding their trials to larger datasets and aim to incorporate machine-learning techniques into the algorithm to further improve accuracy and performance. ■

1. Fu, H., Xu, Y., Lin, S., Zhang, X., Wong, D. W. K., *et al.* Structure segmentation and quantification for angle-closure glaucoma assessment in anterior segment OCT. *IEEE Transactions on Medical Imaging* **36,** 1930–1938 (2017).

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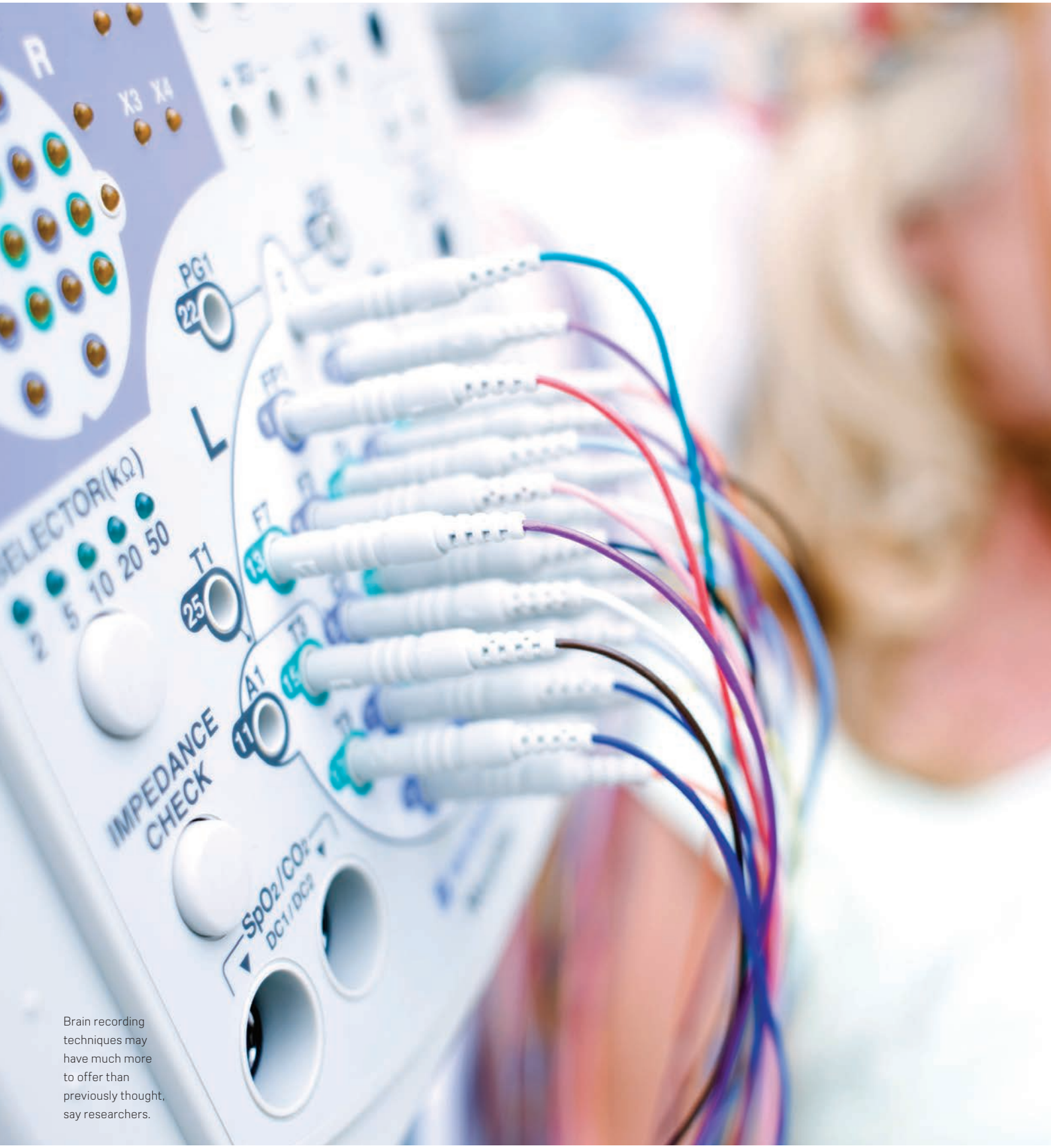

Brain recording techniques may have much more to offer than previously thought, say researchers.

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NEUROIMAGING

STATISTICAL MACHINE LEARNING REVEALS DEEP BRAIN ACTIVITY

Scientists demonstrate non-invasive brain recordings can 'see through' the cerebral cortex and characterize activity in deeper structures

Many neurophysiological processes, such as memory, sensory perception and emotion, as well as diseases including Alzheimer's, depression and autism, are mediated by brain regions located deep beneath the cerebral cortex. Techniques to non-invasively image millisecond-scale activity in these deep brain regions are limited. Now, an international team including A*STAR researchers has shown that magnetoencephalography (MEG) and electroencephalography (EEG) can be used to characterize fast timescale activity in these deep brain structures.

In a recent advance[1], A*STAR's Pavitra Krishnaswamy, in a research team spanning the United States, Sweden, and Finland, developed a statistical machine learning approach to resolve deep brain activity with high temporal and spatial resolution. The researchers used simulated test cases and experimental MEG/EEG recordings from healthy volunteers to demonstrate that their approach accurately maps out this deep brain activity amidst concurrent activity in cortical structures.

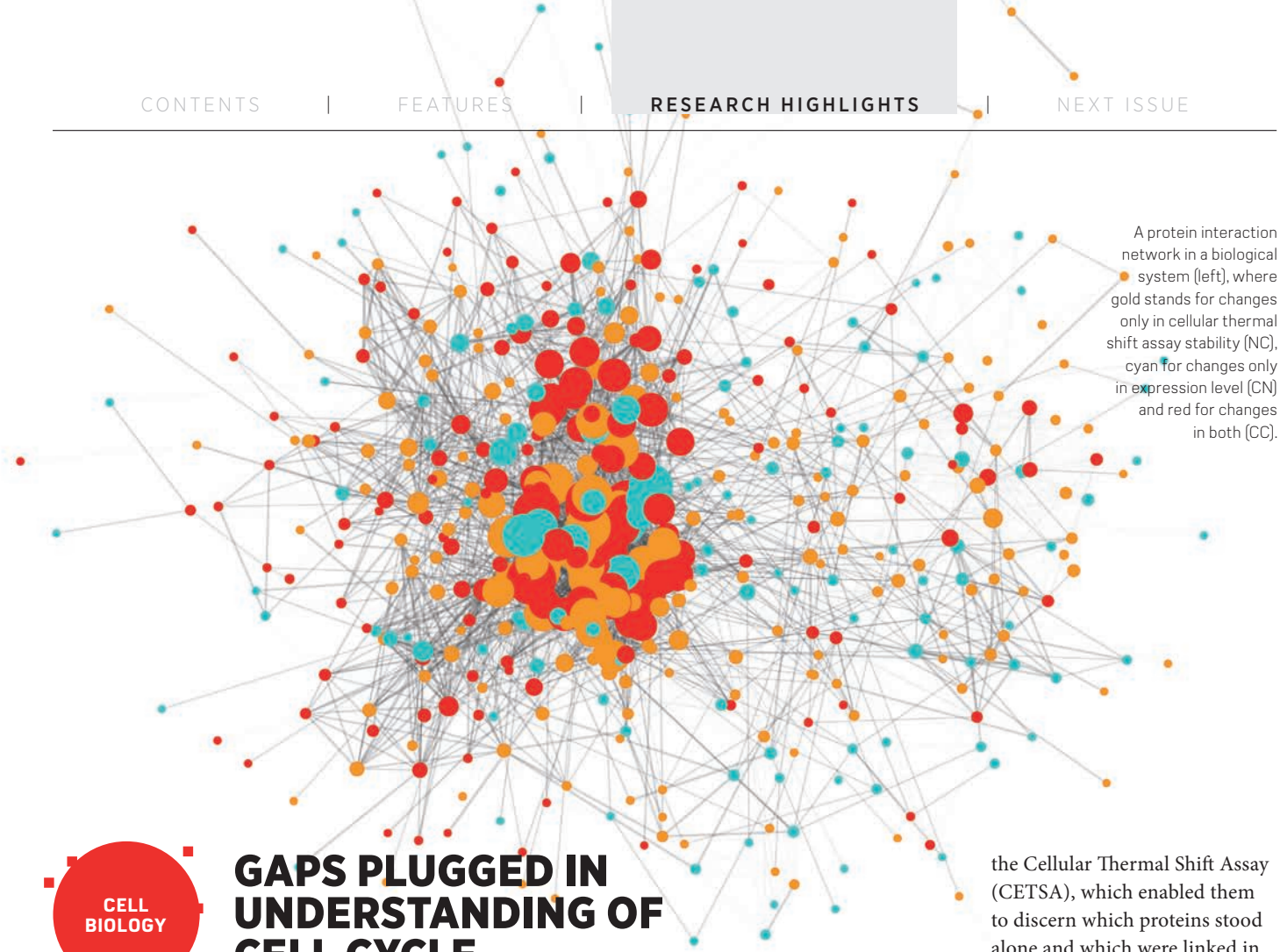
Deep brain activity typically generates weak MEG/EEG signals that are easily drowned out by louder signals arising from cortical activity. Therefore, characterizing the deep brain sources becomes akin to 'picking out needles in a haystack'. Rather than solely relying on how 'loud' the brainwaves are, the team leveraged the fact that deep brain activity generates distinct spatial patterns across multiple MEG/EEG sensors positioned over the head.

"If all of the cerebral cortex were active at the same instant, the cortical signal would "completely dwarf" that of the deep brain."

The concept of sparsity — referring to how limited subsets of neurons across the brain 'fire' in sequential and coordinated patterns — also underpins the team's research. If all of the cerebral cortex were active at the same instant, says Krishnaswamy, the cortical signal would "completely dwarf" that of the deep brain. However, when only a limited number of cortical regions are simultaneously active, it is possible to train an algorithm to see through into the deep brain. "When just a limited portion of the cortex is active, even though it appears louder than ongoing deep brain activity, it is possible to transform the data into a space where the deeper signals also have a distinct 'voice'."

Some of Krishnaswamy's collaborators will now look to validate the approach for possible neuroscience and clinical applications. She will investigate ways to further develop such statistical machine learning approaches for adjacent applications in medical image analysis where the goals are to resolve low signal-to-noise features, enhance reconstruction quality and ultimately reduce diagnostic error. ■

1. Krishnaswamy, P., Obregon-Henao, G., Ahveninen J., Khan, S., Babadi, B., Iglesias, J. E., Hämäläinen, M. S. & Purdon, P. L. Sparsity enables estimation of both subcortical and cortical activity from MEG and EEG. *Proceedings of the National Academy of Sciences USA* **114**, E10465—E10474 (2017).



A protein interaction network in a biological system (left), where gold stands for changes only in cellular thermal shift assay stability (NC), cyan for changes only in expression level (CN) and red for changes in both (CC).

CELL BIOLOGY

GAPS PLUGGED IN UNDERSTANDING OF CELL CYCLE

Similarities between the two gap phases of the cell cycle indicate a default biochemical program in living cells

As a cell replicates, it pauses twice in the process, as if to gather its strength. Scientists long thought the two 'gap' phases were under different regulatory control circuits, but a new study from A*STAR overturns this idea.

Pär Nordlund from the A*STAR Institute of Molecular and Cell Biology and colleagues found that the protein complexes formed during the gap 1 (G1) and gap 2 (G2) phases of the cell cycle are remarkably similar — suggesting that the cell is hardwired for a default biochemical mode of operation when it's not actively replicating genetic material or dividing itself.

"This observation of similar biochemical programs in G1 and G2 cells is new, exciting, and unexpected," Nordlund says.

Cells in our bodies multiply through a four-stage process: cells first increase their mass and prepare for DNA replication during G1; they then copy DNA during the synthesis stage; next, they check the fidelity of duplicated DNA and assemble the materials needed for division during G2; and finally they align replicated chromosomes and divide during mitosis.

Transition from each stage is a tightly regulated event, requiring the assembly and disassembly of various protein complexes to execute many different functions — including to provide molecular checkpoints on cell-cycle progression.

Researchers had previously shown that the expression levels of some of these proteins, and their corresponding RNA molecules, rise and fall at certain points of the cell cycle. However, those analyses overlooked the dynamic interactions between proteins and their binding partners that underpin how the cell moves through the phases of its cycle.

Nordlund and his colleagues profiled the dynamics of interaction states between all the proteins found in human blood cells during the transition of the cell cycle's four stages. They used a technique previously developed by Nordlund's team — the Cellular Thermal Shift Assay (CETSA), which enabled them to discern which proteins stood alone and which were linked in protein complexes.

They identified more than 750 proteins that formed complexes or broke them apart at some point in the cell cycle. However, most of these altered protein interaction states occurred during the synthesis and mitosis stages of the cell cycle — not during the gap phases.

Differences between G1 and G2 were comparatively minor, despite the unique roles played by gap stages in readying the cell for the next phase of the cycle. "This implies a well-working constant program that the cell reverts to," says Nordlund. "It extends our basic understanding of the biochemical programs in one of the fundamental processes in living cells." ■

1. Dai, L., Zhao, T., Bisteau, X., Sun, W., Prabhu, N. *et al.* Modulation of protein-interaction states through the cell cycle. *Cell* **173,** 1481–1494. e13 (2018).

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IMMUNOLOGY

BUILDING IMMUNITY

Insights into the development of neutrophils could lead to therapeutic options for inflammatory diseases and cancers

Steps in the development of first-responder immune cells have been revealed for the first time by A*STAR researchers. These insights help to illustrate how the functions of these cells might be harnessed to treat disease.

The immune cells studied were neutrophils, which regulate responses to infection, disease and injury. These cells survive in the blood for only a short time, so must be continually replenished from cells developing in the bone marrow to avoid a life-threatening condition called neutropenia. However, the process of their development is poorly understood.

"Neutrophils are considered the first line of defence of our immune system," explains Lai Guan Ng who led the study and is from the A*STAR Singapore Immunology Network. "Better understanding their development may lead to better treatment of neutrophil-mediated inflammatory diseases or cancer."

Neutrophils are known to originate from granulocyte–macrophage progenitor cells, but it has been unclear what steps these cells go through to produce functionally mature neutrophils. Ng and colleagues aimed to address this by analyzing the properties of developing cells in the bone marrow and identifying neutrophils through their expression of specific surface protein markers.

The researchers identified a population of proliferative cells that were committed to developing into neutrophils, which they called neutrophil precursors, or preNeus. These could develop into immature neutrophils and mature neutrophils, with each of these three cell populations having a distinct gene expression profile.

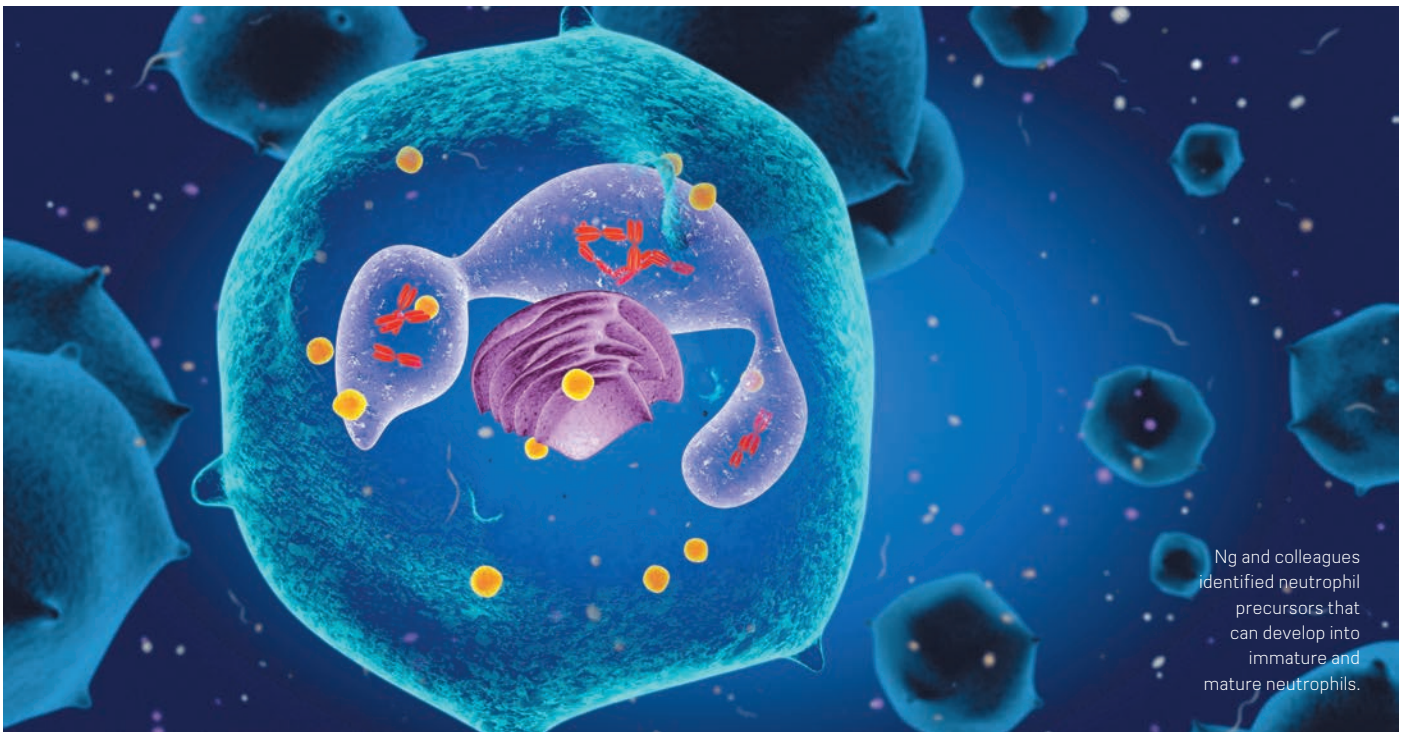
Given the importance of neutrophils to the immune response, Ng and colleagues studied how the neutrophil populations are affected in diseases. They showed that the numbers of preNeus in bone marrow increased in response to sepsis and in an animal model of pancreatic cancer. Additionally, in the cancer model, higher numbers of circulating immature neutrophils were found to be associated with larger tumors.

"We believe that our study not only provides a fundamental framework for neutrophil subset characterization, but also serves as a basis for therapeutic interventions for neutrophil-related diseases," says Ng.

One such intervention relates to patients who receive bone marrow transplantation for blood cancers. In preparation for transplantation, neutrophils are eradicated and subsequent replacement of these cells takes some time, leaving patients vulnerable to serious infection.

"Given the proliferative and unipotent nature of the preNeus," says Ng, "we will explore the potential of these cells to rapidly reconstitute the neutrophil pool when transferred during transplantation." ■

1. Evrard, M., Kwok, I. W. H., Chong, S. Z., Teng, K. W. W., Becht, E. *et al.* Developmental analysis of bone marrow neutrophils reveals populations specialized in expansion, trafficking and effector functions. *Immunity* **48**, 364–379 (2018).



Ng and colleagues identified neutrophil precursors that can develop into immature and mature neutrophils.

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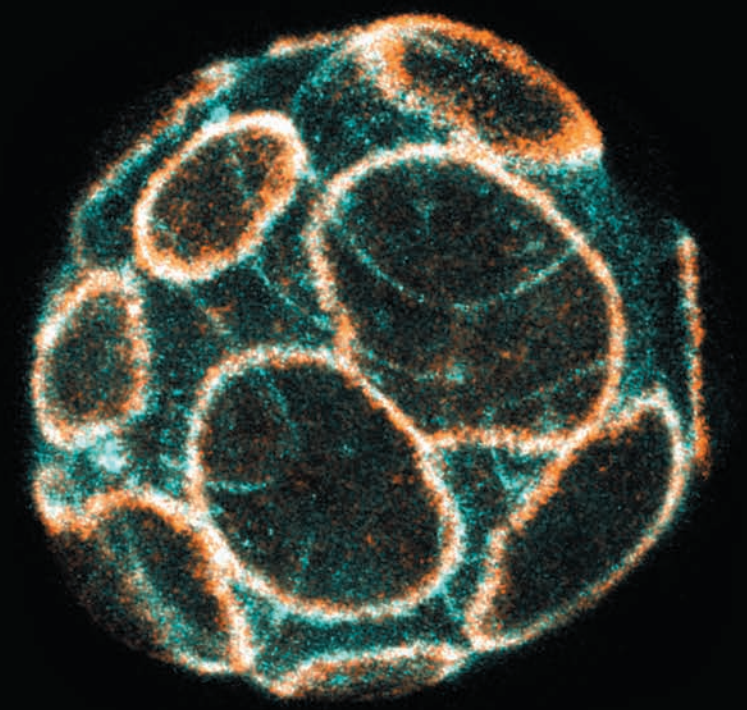
DEVELOPMENT

HOW THE EARLY EMBRYO IS LIKE A SOCCER BALL

Rings of filamentous protein zip together to seal the embryo, creating watertight junctions between adjacent cells



The early embryo becomes a watertight cluster of cells, creating junctions between them that function like the stitches between panels of a soccer ball.

The process, discovered by A*STAR scientists, centers on a protein called actin, which fashions a thread-like network inside every cell of the body. In the early mouse embryo, actin additionally forms a ring-like structure on the outer surface of cells.

Similar actin rings, found in certain cells of the adult body, typically contract inward to aid in wound closure and cell division. However, the team found that in a crucial stage of early development, as the embryo matures from a slack 16-cell aggregate to a tight 32-cell ball, the actin rings expand *outward* toward the borders of neighboring cells, where they interlock and recruit other proteins to seal the embryo into its cohesive spherical form.

This helps to make the outermost cells watertight, an essential step in the proper development of the early embryo. Errors in this process "may lead to implantation failure, fetal defects and miscarriages," says Jennifer Zenker, postdoctoral scientist at the A*STAR Institute of Molecular and Cell Biology and the first author of the new study.

"An advanced knowledge of the fundamental cellular and molecular mechanism controlling early development could further improve the application of assisted reproductive technologies, stem cell research and birth rates," she adds.

"This allows us to follow the real-time development of the mouse embryo in 3D at the frequency of minutes."

Zenker works in the laboratory of developmental biologist Nicolas Plachta, who co-led the study together with Maté Biro, a biophysicist at the University of New South Wales in Australia.

The researchers used high-speed imaging techniques to observe the dynamics of the actin rings on the surface of 2.5-day-old mouse embryos. They labeled the actin filaments with fluorescent markers and watched as the expanded rings recruited other factors at the cell boundaries that stabilize the stretched loops.

The resulting tension then promoted the zipping of adjacent rings at cell junctions, thereby sealing the embryo from outside fluids. "This shows the importance of the crosstalk between the actin network

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Actin rings expand outwards to help create watertight junctions between adjacent cells in the early embryo.

and other types of filaments of the internal skeleton for its formation, organization and turnover," Zenker says.

According to Zenker, research teams had previously failed to see this dynamic process because they looked only at fixed specimens at a single time point. "Our lab uses advanced imaging technologies to study the living mouse embryo," she says. "This allows us to follow the real-time development of the mouse embryo in 3D at the frequency of minutes." ■

1. Zenker, J., White, M. D., Gasnier, M., Alvarez, Y. D., Lim, H. Y. G.*et al.* Expanding actin rings zipper the mouse embryo for blastocyst formation. *Cell* **173**, 776–791. e17 (2018).

IMMUNOLOGY

TRACING THE ORIGIN OF MAST CELLS

Mast cells originate from two distinct sites during embryonic development

Mast cells, a type of white blood cell found mainly in tissues (as opposed to the bloodstream) arise from a membrane that surrounds a mouse embryo as well as the embryo itself, a Singapore-French team, has shown.

In the developing embryo, blood cells, both red and white, arise from cells in special regions known as hemogenic endothelia. There are two key regions: one in the embryo itself, within the aorta-gonado-mesonephros, or AGM — a region that eventually develops into the dorsal aorta, the testis or ovary; and one in a membrane surrounding the embryo, called the yolk sac. The team, led by Marc Bajénoff from the Centre d'Immunologie de Marseille Luminy (CIML), France, in collaboration with Florent Ginhoux's group at A*STAR's Singapore Immunology Network, wanted to understand the fate of cells derived from the two different regions.

"First we established a way to specifically label cells from the two different hemogenic endothelia so we could follow their progeny," said Ginhoux.

They did this by developing a new genetic marker specific for all hemogenic endothelium cells, which was invisible until 'switched on' by a dose of the drug tamoxifen. From that point onwards all marked cells fluoresced, making them and their progeny visible.

"Mast cells are important players in innate immunity — especially in the skin."

As the yolk sac hemogenic endothelium was present on day seven of embryonic development, but gone by day 10.5, the team could tag cells deriving from a specific source by appropriately timing the tamoxifen dose.

Examining the embryos as they developed, they were surprised to find that many tagged cells in the embryonic skin were mast cells that had originated from cells in the yolk sac.

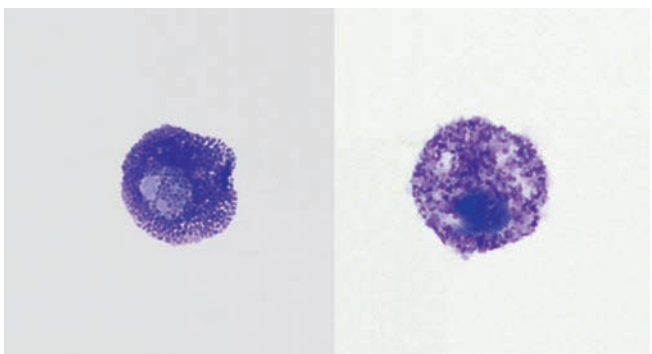
However, by the time of birth, almost all the skin mast cells had been replaced by new cells derived from the AGM.

"Mast cells are important players in innate immunity — especially in the skin. In the adult they are early sentinels, and help neutralize toxins such as bee and snake venom. They are also activated by Immunoglobulin E antibodies and involved in allergy," said Ginhoux.

The mast cells from the two origins had distinct characteristics and differences in gene expression, suggesting they might have varied functions.

"The skin of the developing embryo is surrounded by amniotic fluid — it's a very different environment from adult skin — so the mast cells probably have different functions in the embryo," said Ginhoux.

Finally, they showed that mast cells in adult mouse skin are not replenished from the bone marrow, contrary to established thought. How the number of mast cells is maintained in adult skin remains a mystery. ■



© Dr. Rasha Msallam, SIgN

A peritoneal cavity mast cell (left) and a skin mast cell (right).

1. Gentek, R., Ghigo, C., Hoeffel, G., Bulle, M. J., Msallam, R. *et al.* Hemogenic endothelial fate mapping reveals dual developmental origin of mast cells. *Immunity* **48**, 1160–1171.e5 (2018).

DRUG DESIGN

LEADING THE CHARGE AGAINST TUMORS

By using nanosized charged polymer balls to attack tumors directly rather than carry drugs, researchers have found a way to overcome drug resistance

A new approach to fighting cancer using large, electrically charged molecules to physically disrupt tumor cells has been demonstrated by A*STAR researchers[1]. Tumors cannot develop resistance to the treatment and it prevents cancer from spreading.

Doctors have a vast array of drugs at their disposal for attacking tumors. But while these drugs can often be very effective initially, tumors frequently become resistant to them, causing the cancer to bounce back. Furthermore, cancer cells can migrate to other organs and seed the formation of secondary tumors.

These problems are being increasingly untenable. "More and more anticancer drugs are being given to each patient to overcome drug resistance and prevent the spread of cancer," says Yi Yan Yang of the A*STAR Institute of Bioengineering and Nanotechnology. Yang and her co-workers have devised a method that overcomes both problems.

They used micelles — nanoscale balls made up of long polymer molecules. Micelles are often used to encapsulate anticancer drugs and ferry them to tumors, where they release their payload. But the micelles developed by Yang's team do not transport drugs; rather they are themselves the weapon that physically destroys the tumor.

"Our micelles are much faster; they killed cancer cells within 30 minutes."

The polymers have one positively charged end. These ends, which form the cores of the micelles, attach to the negatively charged membranes of cancer cells and physically disrupt them. As the outer membrane surfaces of healthy cells are electrically neutral, the micelles do not affect them. In addition, since the blood vessels near tumor tissues are leaky, the micelles preferentially accumulate at tumors, and hence target tumor tissues.

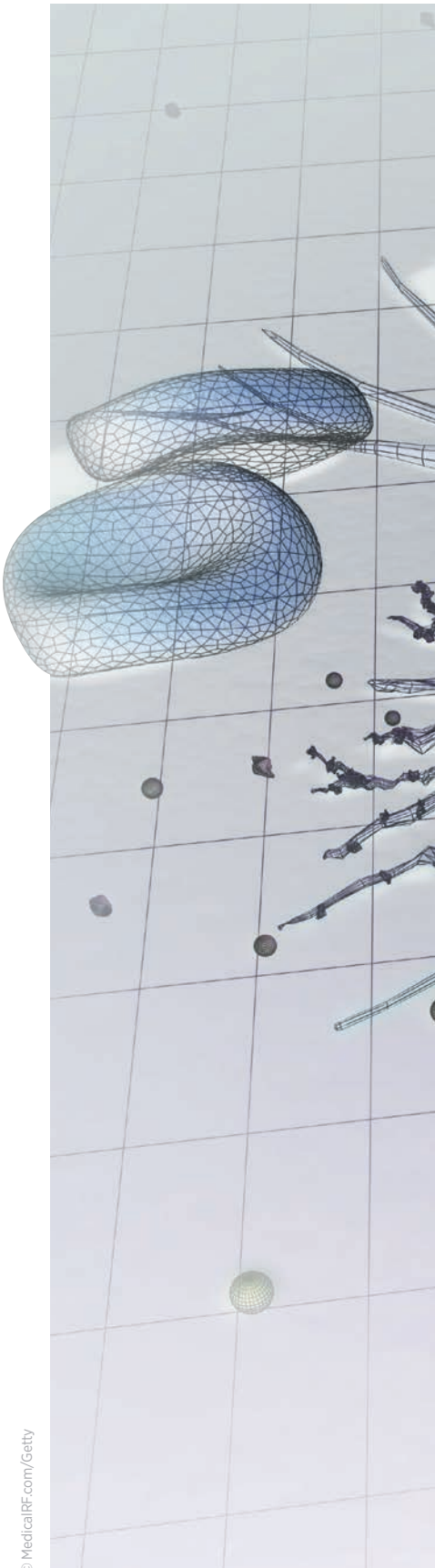
The researchers demonstrated the effectiveness of their method by using the micelles to destroy human liver cancer cells in mice.

Because this is a physical mechanism, tumor cells cannot develop resistance to it. Furthermore, it is effective against cancer stem cells, which are widely believed to be the cells responsible for spreading cancer to other parts of the body.

An additional advantage is the speed at which the micelles attacked cancer cells. "The anticancer drug doxorubicin takes several hours to kill cancer cells," says Yang. "Our micelles are much faster; they killed cancer cells within 30 minutes."

The micelles could be combined with other treatments. "In the future, we will investigate using the micelles with small-molecule anticancer drugs," says Yang. "We may be able to reverse drug resistance." ■

1. Park, N. H., Cheng, W., Lai, F., Yang, C., de Sessions, P. F. *et al.* Addressing drug resistance in cancer with macromolecular chemotherapeutic agents. *Journal of the American Chemical Society* **140**, 4244–4252 (2018).



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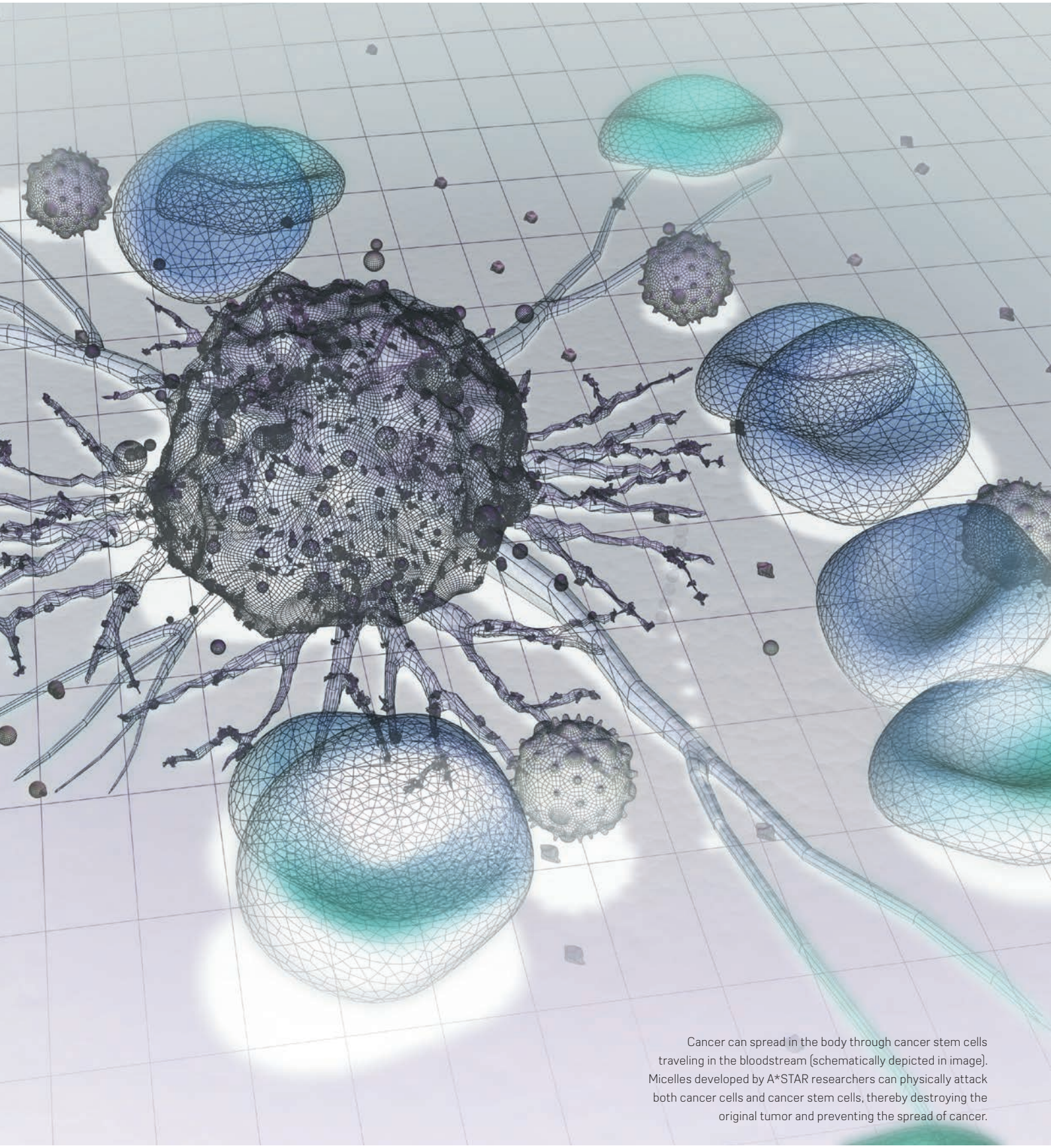
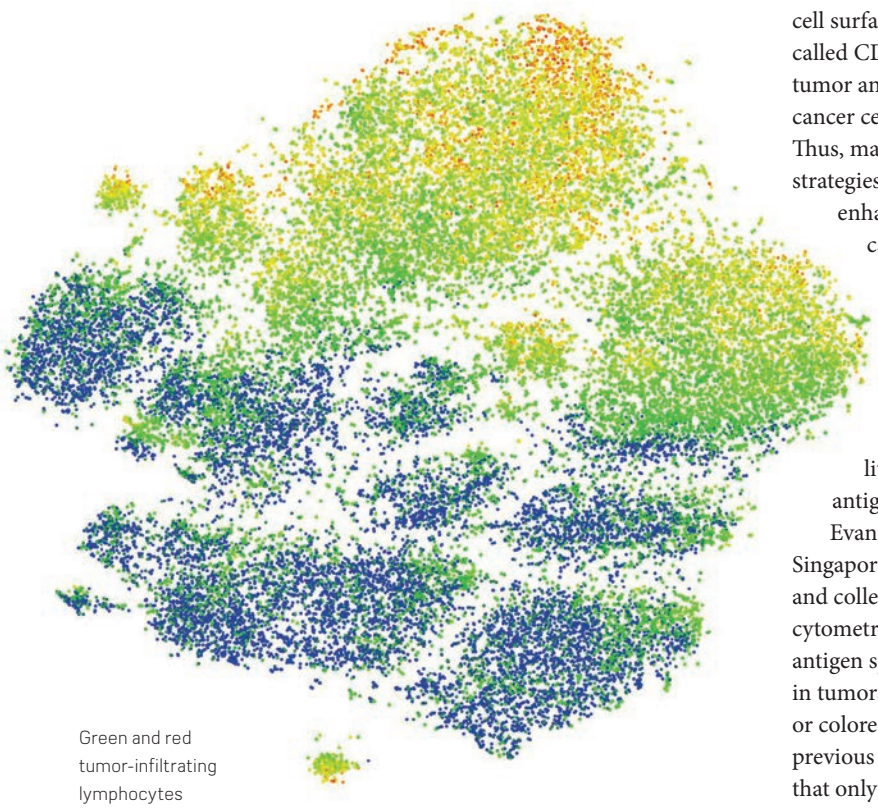


Cancer can spread in the body through cancer stem cells traveling in the bloodstream (schematically depicted in image). Micelles developed by A*STAR researchers can physically attack both cancer cells and cancer stem cells, thereby destroying the original tumor and preventing the spread of cancer.

CANCER IMMUNOTHERAPY

AN UNEXPECTED INFILTRATOR

Virus-targeting white blood cells in tumors offer intriguing insights into responsiveness to immunotherapy



Green and red tumor-infiltrating lymphocytes expressing CD39 in human lung cancer.

Bystander cells present in human lung and colorectal tumors could indicate how well a patient will respond to immunotherapy, an A*STAR-led study finds.

Directing patients' immune responses against cancer cells shows great promise for the treatment of tumors that are unresponsive to conventional therapies. Unfortunately, such approaches do not work in the majority of patients. Efforts to improve their efficacy are centered on combination approaches and predictive biomarkers of response and resistance, as well as furthering understanding of the antigen specificity of tumor-infiltrating immune cells.

The presence of white blood cells in tumors, known as tumor-infiltrating lymphocytes (TILs), is associated with better results in treating most types of cancer. TILs that express their cell surface glycoprotein CD8, called $CD8^+$ TILs, can find tumor antigens on the surface of cancer cells and destroy them. Thus, many immunotherapeutic strategies aim to stimulate, enhance and maintain the cancer-killing ability of these cells. However, $CD8^+$ TILs are a highly diverse population of cells, not only across patients but also within individual tumors, and little is known about the antigens that activate them.

Evan Newell of the A*STAR Singapore Immunology Network, and colleagues, used mass cytometry to investigate the antigen specificity of $CD8^+$ TILs in tumors from patients with lung or colorectal cancer. In line with previous reports, they showed that only a small proportion of the $CD8^+$ TIL population targets mutation-associated cancer antigens. However, they found a significant number of $CD8^+$ TILs that are specific for antigens unrelated to tumors.

"We were surprised by how often we were able to detect $CD8^+$ TILs specific for cancer unrelated antigens infiltrating human tumors. These cells are specific for other common viral infections so shouldn't necessarily be crawling into tumors," says Newell.

$CD8^+$ TILs targeting virus-derived antigens were found in nine of 24 lung cancer patients and 21 of 42 colorectal cancer patients, and unlike tumor-specific $CD8^+$TILs, they did not express CD39, a molecule associated with chronic immune cell stimulation, on their surface.

Although the role of these bystander cells is still to be identified, the results suggest that CD39 could be a useful marker for tumor-specific $CD8^+$ TILs and of patients' responsiveness to $CD8^+$ TIL-directed cancer immunotherapies. As Newell explains, "Very low frequencies of CD39 expressing $CD8^+$ TIL in patients with lung cancer associated with epidermal growth factor receptor mutations correlate with poor response rates to immunotherapy." This could have important implications for the treatment of these tumors, which are particularly prevalent in East Asian patients.

In addition to testing whether CD39 could be a useful biomarker, Newell and colleagues are working on other cancer types and TILs, such as $CD4^+$ T cells, to establish whether similar principles apply. ■

1. Simoni, Y., Becht, E., Fehlings, M., Loh, C.H., Koo, S. L. *et al.* Bystander $CD8^+$ T cells are abundant and phenotypically distinct in human tumour infiltrates. *Nature* **557,** 575–579 (2018).

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CANCER GENOMICS

TRACKING DOWN SUSPECTED CANCER-INDUCING MUTATIONS USING MACHINE LEARNING

Machine learning helps locate dozens of mutations statistically linked to gastric cancer in non-coding DNA regions

By using machine learning, A*STAR researchers have identified 34 hotspots in non-coding DNA that are strongly statistically linked with gastric cancer.

By harnessing the power of machine learning, A*STAR researchers have identified more than 30 mutation hotspots for gastric cancer in regions of DNA that do not code for proteins[1]. This information could be used to diagnose gastric cancer and monitor the effectiveness of treatments.

The molecular machinery inside cells uses instructions encoded in DNA to make proteins for a wide range of functions. When the code becomes corrupted through mutations, the resulting proteins may not perform as expected, and in the worst-case scenario, a cell can become cancerous. Thus, many studies have investigated links between mutations in protein-coding DNA and various cancers.

But DNA that encodes for proteins makes up a meagre 2 per cent of the human genome; the remaining 98 per cent is known as non-coding DNA and its relationship with cancer is largely unstudied.

Now, by using machine learning to analyze the whole genomes of tumors from 212 gastric cancer patients, Anders Skanderup at the A*STAR Genome Institute of Singapore and colleagues have identified 34 hotspots in non-coding DNA that are strongly statistically correlated with gastric cancer.

The team chose to study gastric cancer because there was both a clinical need and a research opportunity. "Gastric cancer is one of the top cancer killers in the world, and there's a strong gastric cancer research community in Singapore," says Skanderup. "So it made a lot of sense for us to study it."

The team's method had two steps. Machine learning was used to first identify all the mutations in each genome of each patient's tumor. It then looked for patterns across all these patients' tumor genomes. "We were looking for regions with an unexpectedly high rate of mutations across the patients, which would indicate that something suspicious was going on in those regions," explains Skanderup.

Of the 34 hotspots, 11 were sites at which CTCF — a protein that controls the expression of genes by determining whether they are copied into RNA — binds. "We discovered that these hotspots had an unexpectedly high rate of mutations, which we couldn't explain in terms of random chance," says Skanderup. "When we probed further, what immediately jumped out at us was that 11 of them overlapped with CTCF-binding sites. That was so striking because we wouldn't have expected any such sites among the 34 hotspots." He notes that other studies had also found links between mutations and CTCF-binding sites, which suggests that these sites may play an important role in gastric cancer.

The team now intends to explore the relationship between non-coding mutations and how well patients respond to treatments. ■

1. Guo, Y. A., Chang, M. M., Huang, W., Ooi, W. F., Xing, M. *et al.* Mutation hotspots at CTCF binding sites coupled to chromosomal instability in gastrointestinal cancers. *Nature Communications* **9**, 1520 (2018).

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GENETICS

IDENTIFYING STOMACH CANCER RISK

Genetic and epigenetic testing of samples from people with cellular changes to the stomach lining could help predict their risk of developing cancer

People with a potentially precancerous condition are more at risk for developing gastric cancer if their stomach lining samples show specific chromosomal alterations. Genetic testing of these samples can also improve detection of a cancer-causing bacterial infection, according to research published by Singaporean scientists.

"Patients with intestinal metaplasia exhibiting telomere shortening and chromosomal instability are significantly more likely to progress to gastric cancer."

Intestinal metaplasia is a condition, usually without symptoms, in which the cells lining the stomach change to resemble intestinal cells. It occurs in an estimated one quarter of the world's population, and has the potential to develop into gastric cancer. It is typically diagnosed through routine endoscopic examinations of the gastrointestinal tract, but these tests are expensive and invasive, so surveillance endoscopies are only cost-effective in countries where gastric cancer rates are high.

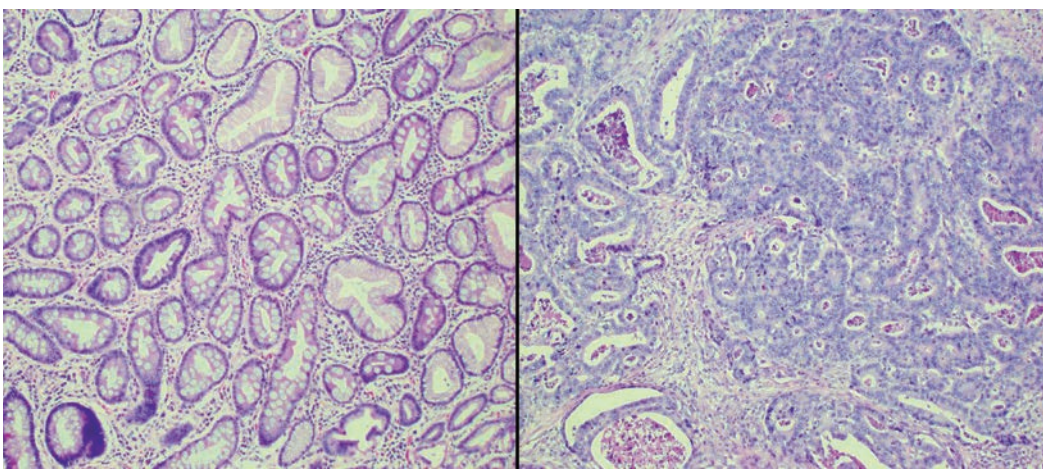
Patrick Tan of A*STAR's Biomedical Research Council, and colleagues, investigated the genetic and epigenetic changes that occurred over a period in people with intestinal metaplasia. They studied stomach tissue samples, showing different stages of the condition, from almost 150 cancer-free people involved in the Gastric Cancer Epidemiology Program, led by Khay Guan Yeoh of the National University Hospital Singapore, which conducted surveillance endoscopies in almost 3,000 Singaporeans over several years.

"Our data suggest that patients with intestinal metaplasia exhibiting telomere shortening and chromosomal instability are significantly more likely to progress to gastric cancer," says Tan. Telomeres are structures that protect the ends of chromosomes from deterioration. Intestinal metaplasias showing normal genetic patterns were more likely to revert back to healthy gastric tissue.

The team also found that genetic sequencing of gastric tissue could identify infection by *Helicobacter pylori* bacteria more accurately than microscopic examinations. *H. pylori* infection of the stomach is common and is usually asymptomatic, but it can lead to stomach ulcers and is a risk factor for the development of intestinal metaplasia and gastric cancer in some people. Treating it early with antibiotics is important and can delay progression of intestinal metaplasia to gastric cancer.

Microscopic detection of *H. pylori* infection is challenging in samples with concurrent intestinal metaplasia, explains Tan, so genetic sequencing could prove useful in these patients. Besides detecting the bacterium's genetic material, sequencing can also provide information about its virulence and resistance to antibiotics, influencing treatment decisions and clinical outcomes, Tan says.

"It is essential to validate our findings in additional cohorts of intestinal metaplasia patients, preferably those that represent independent populations from different countries," says Yeoh. "We are now working with several international groups to perform this validation study."



Genetic sequencing of gastric samples from people with intestinal metaplasia (left) could help identify those who are more at-risk of developing gastric cancer (right).

1. Huang, K. K., Ramnarayanan, K., Zhu, F., Srivastava, S., Xu, C. *et al.* Genomic and epigenomic profiling of high-risk intestinal metaplasia reveals molecular determinants of progression to gastric cancer. *Cancer Cell* **33**, 137–150.e5 (2018).

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NANOMEDICINE

GREEN TEA FOR DRUG DELIVERY

A component of green tea can help deliver drugs to tumors in the body

A component of green tea known as epigallocatechin gallate (EGCG) facilitates efficient transportation of anticancer drugs to tumors.

Wrapping an anticancer drug in a nanoscale case made of a polymer and a component of green tea is an extremely effective way to deliver high doses of the drug to tumors, an A*STAR team has shown[1]. They demonstrated the system's potential by using it to inhibit the growth of liver tumors in mice.

The molecules of some polymers are water attracting (hydrophilic) on one end and water repellent (hydrophobic) on the other. When placed in water, such polymers form nanoscale balls known as micelles with their hydrophilic ends facing outward. These micelles make excellent vehicles for transporting drugs to a desired site in the body. That is because an insoluble drug can be stashed in the hydrophobic core of the micelle, preventing it from harming other tissues until it is released at the tumor, while the hydrophilic outer surface enables the micelle to travel in the bloodstream. Micelles can be designed to target tumors through various mechanisms.

Although polymeric micelles have been well studied, in clinical trials they have generally failed to live up to expectations. Their poor performance was down to their low drug-loading capacity — often they can carry only about 10 per cent of their total weight — and their instability, which causes them to break up and offload their cargo before reaching a target, damaging healthy tissue.

Now, Motoichi Kurisawa at the A*STAR Institute of Bioengineering and Nanotechnology, and colleagues, have shown that micelles with hydrophilic tips made of the polymer poly(ethylene glycol), commonly known as PEG, and hydrophobic tails of epigallocatechin gallate (EGCG) — a component of green tea — overcome both these problems. The researchers found that the micelles had a remarkably high drug-loading capacity of over 80 per cent for the anticancer drug doxorubicin. They were also stable in the bloodstream.

"Epigallocatechin gallate (EGCG) can interact with different types of drugs, including antibodies, proteins, small drugs and even nucleic acid."

"We didn't expect such a high loading capacity," says Kurisawa. "Usually micelles become unstable as you increase their drug-loading capacity, and yet our micellar nanocomplexes are soluble in water. That came as a surprise both to us and to some of the reviewers of the paper, who asked us to check our work. But we confirmed the results."

The results also demonstrated the versatility of the micelles. "In a previous study, we utilized the protein antibody drug; this time, we used a small drug," says Kurisawa. "This is the advantage over existing systems: EGCG can interact with different types of drugs, including antibodies, proteins, small drugs and even nucleic acid. However, specific polymer drug carriers have to be designed to encapsulate specific drugs in conventional techniques."

In addition to being useful for transporting anti-cancer drugs, EGCG is also known to be a powerful anti-oxidant with anti-cancer properties. The team is now exploring this synergism. ■

1. Liang, K., Chung, J. E., Gao, S. J., Yongvongsoontorn, N. & Kurisawa, M. Highly augmented drug loading and stability of micellar nanocomplexes composed of doxorubicin and poly(ethylene glycol)–green tea catechin conjugate for cancer therapy. *Advanced Materials* **30**, 1706963 (2018).

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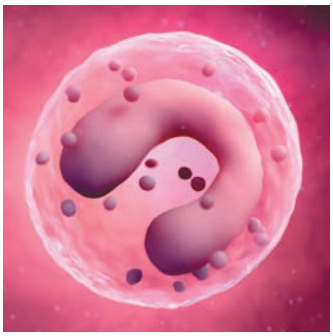
NEXT ISSUE

Here's a sneak peek of the material covered in the next issue of *A*STAR Research*

NEUROSCIENCE

BRAIN REGION CONTROLS HUNGER IN MICE

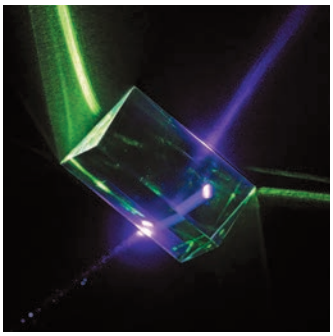
A previously unsuspected region in the brain has been implicated in controlling feeding in mice



IMMUNOLOGY

AGING WHITE BLOOD CELLS MAY BOOST INFLAMMATION

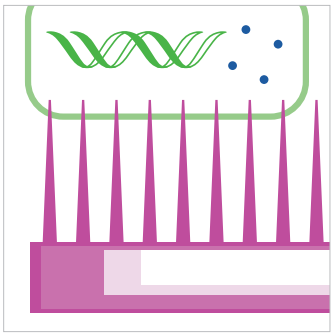
A class of white blood cells becomes more inflammatory as they get older



SENSING

WAVELENGTH SHIFT COULD LEAD TO CHEAP INFRARED SCANS

Using a crystal to link visible light to infrared opens a window on infrared sensing



ANTIMICROBIALS

THE KILLING TOUCH FOR BACTERIA

Tiny zinc oxide-based spikes that mimic natural antibacterial coatings puncture and damage microbial cell walls via oxidation

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X-ray sources used in medicine and other industries have remained virtually unchanged for over a century. Leveraging the unique properties of novel 2D materials, Dr. Wong Liang Jie and a team of collaborators have conceived a method to generate intense, continuously tunable X-rays on a microchip scale. The laser beam-like quality of the X-ray output also allows for more precise pinpointing of medical and dental X-rays, enabling lower dosages and leading to safer, more efficient and less costly X-ray sources in the future.

Dr. Wong Liang Jie

Scientist, Singapore Institute of Manufacturing Technology
PhD and Postdoctoral Fellow, Massachusetts Institute of Technology

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