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Institute of Materials Research and Engineering
Cancer biology

The good, the bad and the ugly

The interaction between good and malignant immune cells may promote blood cancer invasiveness and progression

Leukemia is the most common type of cancer in children. It is a particularly deadly form of cancer characterized by an abnormal increase of immature white blood cells. Subhra K. Biswas at the A*STAR Singapore Immunology Network and co-workers have now discovered a subset of immune cells called monocytes that become proinflammatory when cultured with leukemic cells. More importantly, they have reasons to believe that this ‘change of state’ might be an important driver of leukemia invasiveness and progression.

Monocytes and leukemia cells are both immune cells. The difference between them is that monocytes help protect the body against pathogens, whereas leukemia cells are cancerous.

Biswas and co-workers isolated leukemia cells from patients with leukemia, and cultured them with monocytes that they obtained from the blood of healthy subjects. The monocytes that had been exposed to the leukemic cells were found to have a higher expression level of the proinflammatory chemokine protein CXCL10.

The researchers also isolated monocytes from the bone marrow of patients with leukemia, which were found to have a higher expression level of CXCL10 in comparison to monocytes from healthy subjects. In addition, the CXCL10 protein levels in the blood were higher in leukemia patients than in normal individuals.

CXCL10 is a secreted protein, so proinflammatory monocytes that express high levels of CXCL10 because of their prior exposure to leukemia cells will release the chemokine into their cell culture medium. When the researchers collected this cell culture medium and put it in one section of a cell culture dish, leukemia cells from patients readily migrated towards the medium. Blocking CXCL10 in the medium using antibodies reduced the migration of the leukemia cells towards the proinflammatory monocyte medium.

In addition, Biswas and colleagues found that CXCL10 alone could enhance leukemia cell migration. These data indicate that proinflammatory monocytes can drive leukemia by enhancing the migratory potential of leukemia cells, which may play a key role in the spread of leukemia throughout the body. The researchers found that CXCL10 increased the expression of an enzyme in leukemia cells that enables them to chew up an extracellular net that would normally keep them in place, increasing their ability to migrate and invade. The expression of this enzyme was tightly linked to the expression of CXCL10 in patients with leukemia. The findings suggest that blockade of CXCL10 could serve as a novel therapy to treat leukemia.

Immunology

Guiding the immune response

Uric acid released from dying cells is a key driver of autoimmune diseases

When a cell dies, it secretes uric acid into its local environment to signal danger to the surrounding cells. Alessandra Mortellaro at the A*STAR Singapore Immunology Network and co-workers have discovered that uric acid interacts with other immune signals to drive the maturation of immune cells of a particular lineage that has been linked to autoimmune diseases such as multiple sclerosis and rheumatoid arthritis.

T helper 17 (Th17) cells are a subset of immune cells that secrete the proinflammatory cytokine interleukin-17 (IL-17). These cells fight foreign pathogens such as bacteria, but they also play a key role in driving many different autoimmune diseases. Determining the signaling molecules that drive Th17 maturation could lead to new therapeutic approaches for autoimmune diseases.

Previous studies have demonstrated the ability of uric acid to activate inflammation, but its role in the maturation of T cells has not been explored. When the researchers treated immature immune cells that they obtained from mouse lymph nodes with uric acid alone, the immune cells did not mature into the lineage of immune cells linked to autoimmune diseases. However, treatment with both uric acid and a second stimulus that drives the proinflammatory ‘NF-xB’ signaling pathway caused the immune cells to express Th17-related genes and secrete Th17-related cytokines, including IL-17.

Uric acid and the NF-xB signaling pathway have both been shown to induce the formation of an intracellular signaling complex called the inflammasome. Activation of the inflammasome leads to the secretion of the cytokines IL-1 and IL-18. Mortellaro and co-workers showed that uric acid and NF-xB-inducing stimuli were not able to induce Th17 maturation in mice lacking various components of the intracellular inflammasome complex. In addition, they found that IL-1 and IL-18 were required for Th17 maturation induced by uric acid and NF-xB signaling, as demonstrated by the fact that mice in which these two cytokines were absent produced less IL-17 than normal mice.

The findings suggest that tissue damage-induced release of uric acid, in combination with invasion of pathogens that activate NF-xB signaling, could lead to the skewing of immune responses along the Th17 lineage. This could then predispose individuals to autoimmune diseases. Because the inflammasome is responsible for the guidance of Th17 immune cell maturation by these stimuli, inhibiting the inflammasome could represent a strategy to block Th17 cell formation. “Our findings are important and might open up new therapeutic approaches to treat autoimmune disease,” explains Mortellaro.

Developmental biology

Switching to neurons

Researchers identify a genetic switch that controls differentiation of embryonic stem cells into neural cells

Embryonic stem cells (ESCs) hold great therapeutic promise due to their ability to maintain themselves through a process known as self-renewal and as they can potentially transform into any cell type in the body. The differentiation of ESCs into other cell types is a complex process involving the coordinated activity of multiple genes and signalling pathways; the exact mechanisms by which many of these pathways are regulated are still unclear.

In a development that promises to shed more light on these processes, a research team led by Leah Vardy at the A*STAR Institute of Medical Biology has identified a gene that is required to maintain ESCs in an undifferentiated state, and the activity of which is reduced when ESCs differentiate to progenitor nerve cells

Using microarray analytical technology, Vardy and her colleagues identified almost 200 examples of messenger RNAs (mRNAs) that are regulated at the level of protein synthesis on the differentiation of mouse ESCs into neuronal progenitor cells. Messenger RNAs are transcribed copies of gene-coding DNA sequences which are translated into proteins. Both transcription and translation can be regulated to control protein synthesis.

Further analysis revealed that one mRNA, Amd1, is regulated exclusively at the translation stage during differentiation of ESCs into neuronal progenitors. Amd1 codes for production of AMD1, an enzyme that is required for the synthesis of the polyamines spermine and spermidine, two molecules that are known to be important for growth and differentiation processes, although their precise functions in ESCs are as yet unknown. AMD1 levels are typically high in ESCs but are significantly reduced, or down-regulated, in the precursors to nerve cells.

The researchers found that regulation of Amd1 led to a decrease in production of the enzyme AMD1, with a corresponding drop in production of spermine and an altered ratio of polyamines in these cells. The team also uncovered evidence that this process is controlled by a microRNA called miR-762. In contrast, overexpression of Amd1 or addition of spermine was shown to block differentiation of ESCs into precursors to nerve cells.

Together, these results suggest that miR-762 represses Amd1 translation during differentiation of ESCs into neural progenitor cells, leading to reduced synthesis of spermine, which normally inhibits neuronal differentiation. They also demonstrate an essential role for AMD1 and the polyamines in maintaining ESC self-renewal and highlight the importance of regulation of polyamine levels for neural differentiation.

“We are now planning to further characterize the molecular targets of the polyamines in ESCs and cells undergoing directed differentiation,” says Vardy.

Cancer biology

All tumor cells are not created equal

The identification of a molecular pathway essential for the development and maintenance of tumor-initiating cells may prove invaluable to cancer treatment

Tumor-initiating cells (TICs) are cells from a cancer that can multiply and form a tumor when transplanted into an experimental animal model such as the mouse. As TICs divide and multiply to form a tumor, many of the cells lose their property of being a TIC.

Clinicians are realizing that killing these TICs is the real goal of chemotherapy; if even one is left after a course of treatment, it can regenerate a tumor. Yet the identification of which tumor cells are TICs has been a challenge. Bing Lim and co-workers at the A*STAR Genome Institute of Singapore, the Singapore Bioimaging Consortium and the Institute of Molecular and Cell Biology have now shown that a metabolic enzyme involved in synthesizing amino acids is necessary and sufficient for the formation of TICs in non-small cell lung cancer.

In normal cells, glucose is degraded to pyruvate (a process called glycolysis), which is then shunted into the mitochondria, where it proceeds to generate energy for the cell through the Krebs cycle. In the absence of oxygen — for example, in muscle cells undergoing prolonged exercise — pyruvate skips the Krebs cycle and is instead degraded into lactate in a much less energy-efficient process. This happens in cancer cells as well, even in the presence of oxygen. Some researchers suspect that this metabolic difference is a cause of cancer, while others believe that it might be just an effect. However, because the process is so energy-inefficient, the benefits it confers on cancer cells have remained unclear.

To unravel this mystery, Lim and co-workers isolated the rare TICs from primary non-small cell lung cancers at different clinical stages. They noticed that these TICs had very high levels of glycine decarboxylase (GLDC), an enzyme that degrades the amino acid glycine. Next they went on to show that active GLDC is required to make cells cancerous, and that it can do so on its own.

The researchers also found that GLDC promotes glycolysis and the accumulation of some of the nucleic acids used to build DNA and RNA, which explains why it is essential in TICs, as well as why cancer cells have an altered metabolism — the energy efficiency is not as important for them as attaining raw materials for their out-of-control growth. High levels of GLDC are correlated with high mortality in lung cancer patients, and are also found in other cancers. The finding suggests that drugs targeting GLDC, such as methotrexate, might be effective chemotherapeutic agents.

Immunology

Lose some, gain some

Cyclosporin A is a calicneurin inhibitor used for suppressing the immune system during organ transplantation and in the treatment of autoimmune diseases. It works by inhibiting a family of transcription factors called nuclear factor of activated T-cells (NFATs), which are important for T-cell development and function. However, NFATs’ effects on other cell types involved in the innate immune response had not yet been elucidated.

Jan Fric at the A*STAR Singapore Immunology Network and co-workers1 have now shown that in addition to promoting T-cell development, NFATs also suppress the maturation of myeloid cells.

Myeloid cells provide the body’s first line of immune defence, as it is their job to detect and eliminate pathogens. These findings have implications for how calicneurin inhibitors should be used clinically in the future.

As their model system, Fric and co-workers used mice that had been irradiated to remove all of their immune cells. Into these mice they injected hematopoietic stem cells, which can mature along one of two paths; they can become either myeloid cells or lymphoid cells such as T cells.

The mice got a mixture of normal control stem cells and stem cells that had been genetically engineered to express an inhibitor of NFAT signalling. Each of these cell types had different molecular tags so that the researchers could trace their distinct lineages.

Eight weeks later, the researchers found that cells with the NFAT inhibitor preferentially expanded into myeloid cells, whereas the normal control cells expanded into both myeloid and lymphoid cells in the expected ratios. There was not enough NFAT inhibitor to completely abolish T-cell development; rather, the NFAT inhibitor actively promoted development along the myeloid pathway.

These results were reconstituted in in vitro systems, whereby Fric and co-workers determined that NFAT negatively regulates myeloid expansion by suppressing the transcription of essential cell cycle genes. Inhibiting NFAT relieves this suppression and allows myeloid cells to progress more rapidly through the cell cycle, resulting in an expansion of the myeloid cell population.

“Calcineurin inhibitors are clinically used primarily to inhibit T-cell functions during transplantations or other immune disorders,” noted Fric. “Unfortunately, such a treatment can cause a higher susceptibility to infections and other side effects, due to the suppression of the immune system. Here we showed for the first time that myeloid cells can also be a target of these inhibitors. Our findings can help to better understand the drug's side effects, and eventually, long term further research can lead to therapy modifications.”

Fric thanked his A*STAR co-worker Paola Castagnoli for her support in this study. “NFAT signaling in dendritic cells has been a long-time interest of Paola Castagnoli’s group, where this research was performed,” says Fric.

Molecular biology

Preventing cell division

One of the enzymes known to regulate the cell cycle has now been shown to play a key role in mitosis

Cyclin-dependent kinase 1 (Cdk1) is a member of the Cdk family of enzymes which control the cell cycle. Philipp Kaldis at the A*STAR Institute of Molecular and Cell Biology working in collaboration with an international team of researchers has now shown that Cdk1 plays a critical role in cell division.

Moreover, the researchers discovered that by inactivating Cdk1 production, they could prevent tumor formation in mice. The new finding may open up new avenues in the development of cancer drugs and treatment.

In the mammalian genome, more than 20 different Cdks are known to exist that regulate different phases of the cycle of cell division, or mitosis.

Previous studies have shown that mice with any one of these genes knocked out remain viable, suggesting that enzymes of the Cdk family have overlapping roles. Due to technical difficulties, however, no knockout mice for Cdk1 had so far been developed.

Now, Kaldis and co-workers have not only generated Cdk1-knockout mice, but also developed adult mice in which active Cdk1 genes could be switched off by chemical means.

The researchers found in their experiment that mice lacking functional Cdk1 genes die within three and a half days of conception. Their embryos also display fewer but larger cells.

The researchers suggest the cells in very-early-stage embryos contain remnants of the products of the mother’s Cdk1 genes, which allows them to carry out limited cell division. Without Cdk1, the cells cannot divide and will instead continue to grow, suggesting that Cdk1 is critical to mitosis.

The research team studied the role of Cdk1 in liver regeneration in adult mice. They found that of all the organs in the mammalian body, the liver could tolerate the loss of Cdk1 the most as its cells only divide in adulthood during regeneration.

They made use of experimental animals whose livers were deficient in Cdk1. When they surgically removed part of the livers, the researchers found that regeneration still took place, not by producing more cells, but by increasing the size of existing cells. The researchers also studied the impact of loss of Cdk1 activity on the generation of tumors, both in cell culture and in the livers of adult mice injected with carcinogens. They discovered that without Cdk1, tumor cells could not proliferate.

“We are now able to delete Cdk1 in any tissue or tumor type we choose at any time point,” says Kaldis. “So our mouse model offers a neat method to test whether drugs targeting Cdk1 activity can be effective in treating cancers.”

Oncology

Understanding the culprit

Study using a systems biology approach reveals how the transcription factor EVI1 contributes to cancer development and tumor invasion

Since its discovery close to 25 years ago, the EVI1 gene has emerged as a major player in many different types of cancer, including leukemia and tumors of the breast, prostate and colon, among other organs. In the US, for example, there is a company called NanoOncology that was founded to develop drugs for blocking this oncogene. Yet, despite all the interest in EVI1, very few of the gene’s downstream targets are known.

Emilie Bard-Chapeau at the A*STAR Institute of Molecular and Cell Biology and co-workers have now used a systems biology approach to identify a slew of tumor-associated genes that are controlled by EVI1. The discovery could lead to new therapeutic drug strategies to combat various forms of cancer.

The EVI1 gene — short for ‘ecotropic viral integration site 1’ — encodes a zinc-finger transcription factor with two distinct DNA binding domains. When overexpressed, this oncogene leads to aggressive forms of cancer and poor patient survival. To better understand the biochemical functions of EVI1, Bard-Chapeau and co-workers searched for gene promoters and cooperating transcription factors that are actively bound by EVI1 in human ovarian cancer and chronic myeloid leukemia cell lines.

Systems biology uses a palette of analytical and computational techniques to study the complex interactions in biological systems. Using microarrays, ChIP-sequencing and immunoprecipitation assays, the researchers found that the two different zinc-finger domains of EVI1 activate unique sets of target genes, many of which are involved in cell adhesion, proliferation, colony formation and other aspects of tumor growth.

Notably, the researchers documented a strong association between EVI1 and FOS — the latter being one of the main components of the activator protein 1 (AP1) transcription factor complex that is known to drive tumorigenesis. Experiments in cell lines showed that EVI1 and FOS interact to co-regulate many hallmarks of cancer, and follow-up analyses in late-stage ovarian cancers taken from patients revealed an enrichment in expressed genes linked to both EVI1 and AP1. Taken together, the findings suggest that EVI1 expression might serve to fully elicit FOS oncogenic potential through a feed-forward regulatory loop that drives abnormal tissue changes.

“Our study has provided new mechanistic insights into the regulatory mechanism of EVI1, and revealed how EVI1 can function as a central player in many types of late-stage cancers,” says Bard-Chapeau. “Disruption of the interaction between EVI1 and FOS may be a very interesting way to prevent cancer progression.”

Cellular mechanisms that enable healthy growth can spiral out of control and give rise to cancer. For this reason, signal transduction pathways that underlie cell growth are tightly regulated, with multiple checkpoints and extensive cross-talk in between signal cascades that drive cell division and differentiation. Stephen Cohen and co-workers at the A*STAR Institute of Molecular and Cell Biology have identified a new link between growth controlling microRNAs and this cellular circuitry.

MicroRNAs (miRNAs) bind complementary nucleotide sequences on specific target messenger RNA molecules to suppress their expression of proteins. One of these miRNAs, bantam, is known to regulate cell proliferation and survival. Cohen and co-workers set out to determine how this molecule contributes to tissue development in fruit flies.

Growth control pathways are often complicated. The epidermal growth factor (EGF) signaling pathway is important in growth control and in cancer, EGF receptor (EGFR) sends signals into cells that control many aspects of cellular function, including gene expression. One of the ways EGFR signals is by reducing expression of capicua, a protein that inhibits other growth-promoting genes.

When Cohen and his team experimentally reduced capicua levels, they observed a boost in bantam expression, flagging bantam as a target of capicua-mediated inhibition. More importantly, they learned that bantam in turn feeds back to inhibit expression of capicua. The end result is a negative feedback loop that can accelerate the activation or inactivation of EGFR-mediated growth signals — for example, as bantam level rises, capicua level drops, and then bantam level rises even faster to promote cell growth.

The researchers also uncovered an additional layer of complexity in the story. They have previously found that a separate growth regulatory and cancer pathway, triggered by the protein Hippo, also modulates the level of bantam. This places bantam as a link between the Hippo and EGFR pathways; that is, a microRNA could mediate the flow of information between these pathways, so that each can influence the ‘effectiveness’ of the other.

“Regulation of bantam by both EGFR and Hippo reveals an unexpected link between these two growth-regulatory pathways,” explains Cohen. “EGFR activity can change sensitivity to Hippo activity, and vice versa.”

A better understanding of the connections between these pathways could illuminate potential triggers for tumorigenesis, although the connection between fly and human is not a straight line. “The bantam–capicua connection is not conserved in humans,” explains Cohen. “However, we have identified other new growth regulatory targets of bantam and are now studying their roles as tumor suppressors.”

**Genomics**

**Combined effects**

*New study provides mechanistic insights into how gene transcription is regulated in mammalian cells*

Mammalian cells pack their genome into a highly organized three-dimensional structure, in which the thread-like DNA is wrapped tightly around spool-like proteins. This structure, known as chromatin, plays an important role in the regulation of gene activity. An international team of researchers led by Yijun Ruan at the A*STAR Genome Institute of Singapore have now revealed a key mechanism by which the chromatin regulates gene activity.

Ruan and his co-workers developed a state-of-the-art sequencing technology called Chromatin Interaction Analysis with Paired-End-Tag (ChIA-PET), for performing the genome-wide mapping of long-range interactions between chromatin. The technology looks at different regions of regulatory DNA sequences including enhancers and promoters, which regulate gene expression by binding an enzyme called RNA polymerase II (RNAPII).

The researchers identified approximately 20 million interactions in their initial analysis. They grouped these interactions into one of three categories: interactions between promoters and nearby DNA sequences within the same gene, interactions between promoters and enhancers (which are located outside the gene on the same chromosome), and interactions between promoters on different genes.

The researchers found that interacting genes are not only expressed but also regulated in a co-ordinated manner. Genetic errors at one gene can propagate to other genes that interact with it. Ruan and his team believes that their findings can explain why some genetic diseases exert a wide variety of effects on patients.

The researchers also found that interactions between promoters could have a combinatorial effect. For example, some weak promoters could function as enhancers to regulate other promoters through interactions. Ruan and his team suggest that the classical definition of a promoter may be out of date.

The multi-gene complexes observed in this study are, in principle, similar to the ‘operons’ found in bacterial chromosomes. An operon is a cluster of genes whose functions are under the control of a single promoter.

Scientists have always tried to understand how the large number of genes in an organism is regulated and coordinated to carry out the genetic programs encoded in the genome for cellular functions in mammalian cells. It has been viewed that genes in higher organisms are individually expressed, while multiple related genes in low organisms like bacteria are arranged linearly together as operons and transcribed in single units.

“Our findings show that although genes in human genomes are located far away from each other, related genes are in fact organised through long-range chromatin interactions and higher-order chromosomal conformations,” says Ruan. “The discovery of the mechanism could open up new understanding in the genetic elements underlying human diseases.”

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Heartburn makes for an uncomfortable post-meal experience, but can also herald more serious health concerns. Indeed, gastroesophageal reflux disease (GERD) is a causative factor underlying Barrett’s metaplasia, a condition associated with changes in the epithelial cells lining the esophagus that can ultimately lead to esophageal carcinoma.

Esophageal carcinoma incidence has increased over five-fold in the Western world since 1970, but little is known about its etiology. “The fact that the five-year survival rate has not appreciably changed during this time is demoralizing and suggests that surgery, radiation and chemotherapy have not made a dent in our ability to manage this disease,” says Frank McKeon at the A*STAR Genome Institute of Singapore. However, patients may draw hope from new findings obtained by a team led by McKeon, and Wa Xian of the A*STAR Institute of Medical Biology, using a genetically-modified mouse strain that models Barrett’s metaplasia.

These animals, lacking a protein called p63, exhibited metaplasia in the epithelial cells where the esophagus meets the stomach. The team, led by McKeon and Xian, observed striking changes in gene expression in this subset of cells, with a strong correlation in the genes that were differentially expressed among p63-deficient mouse tissues and samples from human patients with Barrett’s metaplasia.

The researchers identified two marker proteins that allowed them to visualize the cells that contribute to metaplasia during embryonic development. In wild-type mice, these cells initially line the stomach, but subsequently get displaced by the expansion of a population of p63-expressing epithelial cells. In p63-deficient animals, however, these embryonic epithelial cells linger and turn metaplastic.

Importantly, wild-type mice still retain a population of these cells, dubbed ‘residual embryonic cells’ (RECs), in the stomach region adjacent to the esophagus, and analysis of human tissues revealed a similar population of RECs at the gastroesophageal junction. The researchers hypothesize that these cells represent an ‘opportunistic’ population that is normally prevented from proliferating by fully mature ‘indigenous’ epithelia. By inducing damage to this mature epithelium, GERD may thus allow RECs to proliferate unchecked (see image).

The findings suggest that Barrett’s esophagus does not arise from the typical activating mutations seen in early precursors of cancers, but rather exploits damage to the esophagus in order to expand and grow. This ‘rogue cell’ model could potentially underlie other cancers, and the researchers are now examining whether it is possible to avert esophageal carcinoma by selectively eliminating RECs. “We are identifying unique cell surface targets for targeted therapy to destroy these cells,” says Xian.

Stem cells

Promoting difference

Two proteins that regulate cell division co-operate to control neuronal differentiation

Researchers from the A*STAR Institute of Molecular and Cell Biology have identified components of the molecular mechanism that stops neural stem cells from dividing and then promotes their differentiation into different types of brain cells. During development of the nervous system, proteins called cyclin-dependent kinases (Cdks) tightly regulate the balance between the proliferation and differentiation of neural stem cells, but the exact role of each Cdk in these processes is not well understood.

To investigate the function of these proteins, the Kaldis laboratory generated mutant mice lacking the genes encoding Cdk2 and Cdk4, both of which are known to be involved in regulating cell division.

The mutant animals died of heart defects at around the fifteenth day of embryonic development, and had brain abnormalities that shed light on the role of Cdk2 and Cdk4 in neural development.

Lim and Kaldis examined the brains of the mutant animals and found that they had a larger hindbrain and enlarged cerebral ventricles compared to normal mice (see image). They also had a thinner neocortex, which lacked the usually distinct boundaries between cell layers. Closer examination revealed that the ventricular zone, which contains dividing stem cells, was unaffected, but that the neuronal layers were diminished by about 46%.

The researchers then isolated neural stem cells from the brains of the mutant and normal animals, and used antibody staining to examine the genes that they express. Cells from both types of animals expressed genes required for self-renewal, suggesting that deleting the Cdk2 and Cdk4 genes does not alter stem cell characteristics.

Although the proliferation of neural stem cells from the mutants was largely unaffected, the cells were more prone to spontaneous differentiation after an extended period in culture compared to those from normal animals. Further experiments indicated that this enhanced differentiation is largely due to alterations in cell division, so that the interval between each round of division is increased.

This may extend the time period over which chemical signals that induce differentiation can act on the cells. The absence of Cdk2 and Cdk4 may also cause an altered biochemical state that primes the stem cells to differentiate into neurons. Together, these results suggest that a high level of Cdk activity favors self-renewal of neural stem cells and discourages them from differentiating.

“We are currently analysing a particular substrate of Cdks in neural stem cells to determine the molecular mechanism of our observation,” says Kaldis.

The body’s initial response to invading bacteria or viruses is mediated by the innate immune system, wherein cells secrete signaling factors called cytokines that promote inflammation and stimulate a generalized counterattack against targets perceived as ‘foreign’. The protein Toll-like receptor 3 (TLR3), for instance, helps initiate the innate immune response against viruses.

Kong-Peng Lam at the A*STAR Bioprocessing Technology Institute and co-workers have now gained insights into how TLR3 helps ‘rally the troops’.

They showed that TLR3 recognizes viral genetic material, and subsequently undergoes activation via the enzymatic addition of phosphate chemical groups to specific amino acids — a process known as phosphorylation.

The researchers identified Bruton’s tyrosine kinase (BTK) as a TLR3-activating enzyme in this pathway. They found BTK to be a promising candidate based on its prominent role in immune function: mutations in this gene result in X-linked agammaglobulinemia (XLA), a disease characterized by failures in B cell production and function. “These patients are also very susceptible to recurrent bacterial and viral infections,” says Lam, “which suggested that BTK might be involved in innate immunity.”

To test their hypothesis, the researchers injected mice with polyribocytidylic acid, a molecule that resembles viral RNA and triggers an antiviral immune response. In normal mice, this treatment can trigger a strong inflammatory overreaction that leads to fatal septic shock. However, both BTK-deficient and TLR3-deficient mice proved resistant to septic shock, suggesting these two molecules work together in a common pathway. TLR3 activation also generates signals that stimulate production of the immunostimulatory molecule interferon β, but the absence of BTK effectively crippled this response in mice. Accordingly, BTK-deficient mice proved far less capable of clearing dengue virus from their system than wild-type animals.

Biochemical experiments clearly demonstrated that BTK was required for the phosphorylation of TLR3. Following activation, TLR3 binds to TRIF, a ‘adapter’ protein that allows it to interact with various other signaling factors. However, without BTK, TLR3 fails to undergo phosphorylation. As a result, TRIF cannot bind to downstream signaling molecules, thus stopping the signaling cascade in its tracks.

Lam now hopes to determine whether BTK represents a general component of innate immunity outside of the TLR3 pathway. In the meantime, he suggests that BTK-targeting drugs could prove a useful tool for immunomodulation. “Conceptually, BTK inhibitors could be used to dampen exaggerated immune responses when the host ‘cytokine storm’ is a curse rather than a blessing, such as in the case of SARS coronavirus infection,” says Lam.

As our primary interface with the outside world, the skin needs to be able to protect itself against infectious threats. Specialized cells known as Langerhans cells (LCs) (see image) are an essential component of this defense, helping other immune cells to distinguish friend from foe. “These cells play an important role in maintaining tolerance to cutaneous antigen, while simultaneously promoting immune responses against any invading pathogens,” explains Florent Ginhoux at the A*STAR Singapore Immunology Network.

LCs are the skin-based counterparts of dendritic cells (DCs), which reside in all tissues. However, LCs and tissue DCs are generated via distinct developmental pathways, and new research from Ginhoux and co-workers has uncovered unexpected complexity in the process by which the adult LC population arises over time.

Unlike DCs, which are generated continuously from bone marrow progenitors prior to their entry in the bloodstream and tissues, LCs are instead replenished by local precursors in the skin. In this sense, LCs are similar to microglia, immune cells residing exclusively in the brain, and Ginhoux suspected the two otherwise-similar cell types might arise from a common embryonic source. “We had previously shown that microglia derive from yolk sac progenitors,” he says. “We hypothesized that LCs could be derived from yolk sac progenitors as well.”

To test this model, the researchers used a genetic labeling strategy to trace the development of different cells in mouse embryos. Indeed, they confirmed that LCs are generated by precursors in the yolk sac, which migrate to the skin around the tenth day of mouse embryonic development, a process that requires 20 days in full. However, they were surprised to find that these yolk sac-derived cells form less than 10% of the adult LC population.

In fact, further lineage-tracing experiments revealed a later, larger wave of LC formation in the fetal liver, producing cells that migrate to the skin between the thirteenth and seventeenth day of embryonic development. “It was unexpected to find that the fetal liver contribution superseded that of the yolk sac,” says Ginhoux. He notes that although both microglia and LCs appear to emerge from common progenitors in the yolk sac, the subsequent formation of the blood-brain barrier would most likely prevent equivalent migration of liver-derived microglial precursors to the brain.

This two-wave developmental process thus appears to be specifically limited to LCs, and Ginhoux and co-workers now hope to determine what, if any, functional value is gained by this stepwise developmental process.

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A team of researchers in Singapore has determined the structure of a pair of proteins that may play an important role in tumor growth and the progression of cancer. The proteins, Vestigial (Vg) and Scalloped (Sd), normally control wing development in fruit flies, but the team found they show a remarkable structural and functional similarity to the cancer-promoting proteins called YAP and TAZ.

Led by Ajaybabu Pobbati and Wanjin Hong of the A*STAR Institute of Molecular and Cell Biology, Singapore, the research team focused on these proteins because, after binding to each other, the Vg–Sd complex binds to DNA to regulate the expression of genes that control cell proliferation. The region of Vg that binds to Sd is also present in four mammalian proteins, called Vestigial-like proteins (VGII1-4). These proteins use this region to interact with the TEAD/TEF transcription factors, the mammalian equivalents of Sd. The TEAD transcription factors also bind to YAP and TAZ. Together, they increase the expression of cell-proliferation genes that promote cancer formation.

Since little is known about the VGII proteins, the researchers used X-ray crystallography to determine the molecular structure of VGII1 bound to TEAD. Their analysis revealed that VGII1 and TEAD interact with each other in two places. The first involves structures called β-pleated sheets on VGII1 and TEAD, which bind in an antiparallel fashion, or face in opposite directions. The second consists of another structure in the VGII1 protein, called an α-helix, which sits in a groove formed by two α-helices in TEAD. The α-helices in both proteins bond to each other because of a mutual repulsion by water.

Surprisingly, Pobbati, Hong and co-workers found that both interfaces are very similar to the interfaces that mediate interactions between the TEAD and YAP proteins, despite the fact that the amino acid sequences of VGII1 and YAP bear very little resemblance to one another (see image).

Finally, the researchers investigated the function of the VGII1–TEAD4 complex, and found that it increases expression of the IGFBP5 gene, which promotes cell proliferation. The complex also promotes anchorage-independent cell proliferation, which is one of the hallmarks of cancer. Together, these findings suggest that VGII1 may play an important role in the progression of cancer, in the same way as YAP and TAZ.

“In the future, we will be using various molecular, cellular and systems biology approaches to investigate if VGII proteins have a definitive role in cancers,” says Pobbati.

Semi-invariant natural killer T (iNKT) cells wage war against infectious threats, attacking microbial cells and generating signals that enable other immune cells also to respond aggressively. iNKT cells initially undergo activation in the thymus (see image); after being ‘switched on’ via interaction with certain antigens, they undergo an initial population expansion and then migrate to peripheral immune sites where they proliferate further so they can mount an effective defense.

Some of these activating triggers are foreign in origin, such as bacterial membrane components. However, iNKT cells can also be activated by lipids produced within the thymus itself, as demonstrated in new research from a team led by Gennaro De Libero of the A*STAR Singapore Immunology Network. Previous research had indicated that such ‘self’ lipids might be an important stimulus. De Libero’s team therefore began by treating cultured mouse iNKT cells with lipids isolated from thymic cells and looking for biological signatures of activation. “We found that unusual lipids are important for thymic selection, and that these lipids are produced within unique organelles called peroxisomes,” he says.

A peroxisomal enzyme called glyceronephosphate O-acyltransferase (GNPAT) plays a central role in producing these particular lipids. Accordingly, the researchers observed that iNKT cell maturation tended to stall in mice lacking GNPAT, and these animals had considerably fewer functional iNKT cells than normal mice. Subsequent transplantation experiments demonstrated that immature iNKT cells from wild-type mice are less likely to reach full maturity when grafted into thymuses of GNPAT-deficient mice. Collectively, these experiments demonstrate that a substantial subset of developing iNKT cells is dependent on interactions with peroxisomally produced lipids in the thymus in order to undergo full activation.

Despite the team’s revelation of insightful details about the development of these important immune cells, a number of mysteries remain — for example, how mature iNKTs learn to stop targeting the antigens that switched them on in the first place. “Now that we know the stimulatory self lipids, we can address the mechanisms which reduce iNKT reactivity against them in the periphery,” explains De Libero. This would provide a means to avoid autoimmune attacks.

In parallel, he and his colleagues intend to determine whether foreign lipids also trigger immune cell maturation via a similar mechanism. “For example, T cells that recognize mycobacterial lipids are important in protecting people from tuberculosis,” he says, “and it will be important to study how these cells are selected and mature within the thymus.”

Electronics

A perfect vision

A low-temperature method could be used to ‘grow’ transparent zinc oxide films for use in displays and solar cells

The displays on flat-screen TVs and smartphones, as well as the panels on solar cells, all require materials that not only conduct electricity but are also highly transparent to visible light. One transparent electrical conductor that is typically used in the industry is indium tin oxide (ITO). Unfortunately, ITO is not only expensive but also toxic to the environment.

In a significant step forward in the field, researchers from the A*STAR Institute of Materials Research and Engineering and the A*STAR Data Storage Institute have now pioneered a low-cost methodology for the fabrication of zinc oxide thin films. “These zinc oxide thin films are highly regarded as a promising material for replacing ITO,” says Nancy Wong, a principal investigator in the research team.

Zinc oxide is a cheap and abundant material that is widely used in cosmetics such as sunscreens or baby powders. Its transparency to visible light is similar to that of ITO, but the fabrication of zinc oxide thin films on an industrial scale is considerably more challenging. In particular, to achieve the necessary electrical conductivity, small amounts of gallium need to be incorporated during growth of the films. Gallium has an additional outer electron in comparison to zinc, which is essential to achieve the necessary electrical conductivity. To date, such gallium-doped zinc oxide (GZO) films have only been realized by high-temperature processing methods.

The method developed by the A*STAR researchers involves the use of pulsed laser deposition. In this room-temperature process, an intense laser beam is used to evaporate zinc and gallium atoms. The atoms move towards a substrate that is also placed within the stainless steel chamber. They then react with oxygen gas also supplied to the growth chamber to form a zinc oxide film on the substrate. Ideal growth compositions were then found by a systematic variation of parameters such as oxygen gas pressure and substrate temperature. The best films grown achieve an optical transparency as well as electrical conductivity that match that of ITO.

Given such advantages, these GZO films could have significant commercial potential. The films may be particularly well-suited for solar panel development, as cost-reduction is a crucial factor for the solar panel industry. “The deposition can be carried out at room temperatures, which reduces the tendency to damage layers underneath, for example, in the plastic substrates applied in organic solar cells and other flexible electronic devices,” says Wang. “Entirely new applications beyond ITO could emerge this way.”

Engineered materials

Custom-made magnets

A novel approach to designing artificial materials could enable magnetic devices with a wider range of properties than those now available

The properties of a substance are largely dependent on its constituent atoms and the way that these atoms interact with each other. The finite number of atom types, however, imposes a limit on the range of properties that a conventional material can have. In contrast, a new class of engineered materials called metamaterials have no such limitation.

Metamaterials are typically composed of an array of nanostructures that can interact with electromagnetic waves in much the same way as atoms. In addition, the optical properties of these metamaterials can be tuned by altering the size and shape of nanostructures.

An international team of researchers led by Boris Luk’yanchuk at the A*STAR Data Storage Institute have now extended the properties and potential uses of metamaterials by using not one but two very different classes of nanostructures, or metamolecules.

Luk’yanchuk and the team mathematically modelled a two-dimensional array of metamolecules comprising a silicon sphere next to a partially incomplete copper ring. They studied the influence of both the sphere and the split ring on the magnetic component of an incident electromagnetic wave — a property known as magnetization.

“When the two structures were more than one micrometer apart, they both acted to increase the local magnetic field,” says Luk’yanchuk. However, they started to interact when moved closer together, and the researchers observed that the magnetization of the split ring decreases and even becomes negative for separations smaller than 0.5 micrometers.

This situation is somewhat analogous to the magnetic ordering in ‘natural’ materials. When all the atoms contribute in a positive way to a material’s magnetic properties, the material becomes a ferromagnet. However, when alternating regions of the material have opposite magnetization, the material is said to be antiferromagnetic.

“We demonstrate that our hybrid lattices of metamolecules exhibit distance-dependent magnetic interaction, opening new ways for manipulating artificial antiferromagnetism with low-loss materials,” explains Luk’yanchuk.

Although the analogy between metamaterials and magnetic materials is not a perfect one, most metamaterials are said to be ferromagnet-like. The design proposed by Luk’yanchuk and the team closely mimics antiferromagnetic ordering, and this opens an opportunity for researchers to study antiferromagnetic phenomena in metamaterials. One notable example is giant magnetoresistance, a phenomenon that is at the heart of modern electronic memories.

Luk’yanchuk affirms that a metamaterial analog would offer exciting research prospects, and comments, “We believe that our work has the potential to make a strong impact towards the development of on-chip integrated solutions for reconfigurable and optically-controlled metamaterials.”

1. Miroshnichenko, A. E., Luk’yanchuk, B., Maier, S. A. & Kivshar, Y. S. Optically induced interaction of magnetic moments in hybrid metamaterials. ACS Nano 6, 837–842 (2012).
Nanomaterials

Peeling back the sheets

Two-dimensional (2D) nanomaterials known as nanosheets have attracted a great deal of attention in recent years because of their large surface-to-volume ratio and unconventional properties. Graphene, for example, has found use in a wide range of applications in electronics as it displays both insulating and semiconducting properties.

Scientists have developed a variety of techniques for making nanosheets, but the fabrication of freestanding organic nanosheets remains a challenge. The current technology could either build small 2D fragments from small molecular units in solution or confine molecules or fragments in 2D geometries. Sometimes the 2D fragments might even aggregate into three-dimensional (3D) frameworks.

Yugen Zhang, Jackie Ying and co-workers at the A*STAR Institute of Bioengineering and Nanotechnology have now discovered an easy way of making organic nanosheets — by peeling layers off a porous polymer.

They used a porous polyisocyanurate, which contains many highly reactive, terminal isocyanate groups within its outer layers. These terminal groups can react with amine-functionalized small molecules under relatively mild conditions to give nanosheets that are subsequently isolated by filtration.

“Conventional methods for making organic nanosheets involve the deposition of thin layers of materials onto a solid support,” says Zhang. “Our method does not require the use of a support and is the first of its kind for making organic nanosheets.”

A range of amine-containing molecules can be used to make differently functionalized nanosheets — for example, amino-propanol and D-glucosamine. The latter creates blue fluorescent nanosheets that show good water solubility.

The thickness of the amorphous nanosheets is around three nanometres, and the longer the reaction time, the thinner the sheets and the smaller the dimensions of the sheets. The researchers have obtained nanosheets with thicknesses ranging from 40 to 150 nanometres.

The researchers tested how the fluorescent nanosheets perform as bioimaging materials. They found that the nanosheets had very low cytotoxicity and remained stable in water for up to four months. In addition, the fluorescent nanosheets gave off a blue light after they entered the cytoplasm of biological cells.

Furthermore, the nanosheets could act as carriers for delivering hydrophobic molecules, such as the dye molecule known as ‘Nile red’, into the cytoplasm of cells.

In the future, the researchers aim to make various nano-structured materials using this method and study the materials’ properties. “The nanosheets could also potentially be used in sensing and conducting materials,” says Zhang.

Materials science

Perfecting the defect

Simulations of defects inside copper point the way to making stronger metals

Strong metals have a tendency to be less ductile — unless the metal happens to be a peculiar form of copper known as nanotwinned copper. The crystal structure of nanotwinned copper exhibits many closely-spaced interruptions in an otherwise regular atomic array. These interruptions, despite being termed ‘defects’, actually increase the metal’s strength without reducing its ductility, making it attractive for applications such as semiconductor devices and thin film coatings. However, the relationship between the properties of these defects and those of the metals containing defects remains unclear.

Now, Zhaoxuan Wu and co-workers at the A*STAR Institute for High Performance Computing have performed a large-scale numerical simulation that sheds light on this relationship. The simulation addressed some of their previous, unexplained experimental data.

In 2009, the researchers had observed that the strength of nanotwinned copper reached a maximum when the size of the defects in its crystal structure was about 15 nanometers. When the defects were made smaller or larger, the copper’s strength decreased. This contradicted the classical model, which predicted that the metal’s strength would increase continually as the defect size was reduced.

Wu and co-workers addressed this contradiction by using a very large-scale molecular dynamics simulation to calculate how a nanotwinned copper crystal consisting of more than 60 million atoms deforms under pressure. They observed that its deformation was facilitated by three types of mobile dislocations in its crystal structure. Significantly, they found that one of these three types of dislocation, called a 60° dislocation, interacted with defects in a way that depended on the defect size.

The 60° dislocations were able to pass through small defects in a continuous manner, creating many new, highly mobile dislocations that softened the copper. On the other hand, when they encountered large defects, a three-dimensional dislocation network formed that acted as a barrier for subsequent dislocation motion, thus strengthening the copper. The simulation predicted that the critical defect size separating these two regimes of behavior occurred at 13 nanometers, very close to the experimentally measured value of 15 nanometers.

The results show that there are many different deformation mechanisms occurring in nano-structured materials like nanotwinned copper.

Understanding each of them will allow scientists to tune material properties — as Wu comments: “For example, we could introduce dislocation barriers to stop their motion, or change defect interface energies to change how they deform.” Wu adds that the next step for his research team will be to take into account the diversity in defect sizes within a single material.

Biomaterials

A new type of bone cement is unlikely to cause infection as it contains porous silica particles that slowly release antibiotics into the body

Many countries are facing the problem of an aging population, and it is estimated that the number of people aged 85 or over in the world will triple over the next fifty years. This rise in number is set to be accompanied by an increase in the prevalence of ailments that are common among the elderly, as well as the need for treatments, not least hip and knee replacements. Despite strict hygiene controls, however, it has been reported that one to three percent of patients suffer infections after hip and knee replacement surgery.

Shou-Cang Shen at the A*STAR Institute of Chemical and Engineering Sciences and co-workers have now developed a new type of bone cement that is less likely to cause problems. The bone cement slowly releases antibiotic drugs, which should dramatically reduce infection rates and the need for further interventions.

Conventional bone cements commonly use a polymer called PMMA for fixing new bone implants. They may also contain antibiotics that are released quickly into the body within one to two days. Worse still, a portion of the antibiotics may stay trapped within the bone cement, unable to be released. Shen and co-workers introduced porous silica particles that carry antibiotics inside them into the PMMA. By carefully optimising the amount of drug used, the new bone cement could release more than 70% of the antibiotics from the nanoparticles over 80 days.

The researchers found that particle size and particle number within the bone cement were critical to ensuring maximum drug release. There also had to be enough nanoparticles to ensure that they form a ‘network’ for delivering the drugs to the cement boundaries. More importantly, the particles had to be small enough so that a homogenous distribution through the cement could be achieved. If the particles were too large, this resulted in an insufficient number and they did not distribute evenly enough to create the network needed for drug diffusion to the surface.

One thing that concerns the researchers is the potential detrimental effect of the mechanical properties of the cement as a result of the incorporation of these nanoparticles. Joint replacements are load-bearing components and so their mechanical properties are critical to performance. Shen found that the bending modulus was somewhat affected though still retained 90% of its original capability. The compressive strength was completely retained despite the presence of the silica nanoparticles.

“Our next step is to make multifunctional nanoparticles for bone cements which both release drugs and are detectable to X-rays,” says Shen. “Bone cements that appear opaque to X-rays could allow for post-surgery observation and diagnosis of any problems.”

In his 1959 lecture *There’s Plenty of Room at the Bottom*, the US physicist Richard Feynman asked the question: “Why cannot we write the entire 24 volumes of the Encyclopedia Britannica on the head of a pin?” Since then, scientists have made great advances in the nascent field of nanotechnology — and among them, the reading and writing of features at the atomic scale.

Current techniques for patterning features at the atomic scale, however, have been limited by their ability to replicate colors or grayscale information. Joel Yang at the A*STAR Institute of Materials Research and Engineering and co-workers have now developed a patterning technique that produces a faithful reproduction of grayscale images with accuracy down to tens of micrometers.

Conventional micro-patterning techniques typically build on halftone printing, whereby the brightness of the image is generated by varying the density of monochromatic elements. Yang’s technique considers these elements as ‘nanoposts’ — posts of only ten nanometers in size — that are arranged in one of 17 possible patterns or ‘shades’. It then produces faithful reproductions of grayscale images using these 17 shades in hand.

As a proof of principle, the researchers replicated the patterns of a test image (pictured) onto an area of 40 square micrometers. In the densest region, the separation between individual dots was a mere 10 nanometers.

The halftone technique had been used before to create grayscale optical micrographs. However, Yang and colleagues have now pushed the approach into the realm of electron microscopy: “Our technique utilizes an electron-beam-lithography method with one of the best resolutions, allowing us to create grayscale images that are highly miniaturized,” explains Yang. “The method should be useful for creating images that can be seen under an optical microscope and may open up new avenues to adding colors to images.”

Yang and his team envisioned several uses of the miniaturized images, for example, in anti-counterfeit features to nanophotonic devices. “But above all, these are striking images,” says Yang. Indeed, one of the images — a 4,000-fold miniaturization of M. C. Escher’s mezzotint *Dewdrop* — has won last year’s Grand Prize Award of the International Conference on Electron, Ion, and Photon Beam Technology and Nanofabrication (EIPBN) conference. “Winning that award was a thrilling experience, especially as it was presented by a community of nanofabrication experts”, says Yang. “One who stares enough into the screen of a scanning electron microscope would appreciate the intrigue and aesthetic beauty of these micrographs. It is rare to see a scanning-electron-microscope image of a photo-realistic person staring back at you from the nanoworld.”

Nanomaterials

Formation in a flash

A new lithography technique enables the production of nanoscale patterns of titania for high-tech applications

Titanium dioxide, or titania, is an inorganic material commonly used as a whitening agent in food and toothpaste. It is also used as one of the main active ingredients in sunscreens. The properties that make titania useful in commercial applications — namely its whitening ability and high refractive index — are now being exploited in a wide range of technological applications.

One particular area of interest has been the application of titania in dye-sensitized solar cells — devices that can be used to convert sunlight into electricity. Such application often requires the formation of intricate surface patterns, with the key limiting factors for development being cost and speed of processing.

Now, Ramakrishnan Ganesan, Mohammad Saifullah and co-workers at the A*STAR Institute of Materials Research and Engineering have described the use of a technique called step-and-flash imprint lithography (SFIL) to produce such patterns on the nanoscale.

“The precursor method to SFIL is thermal nanoimprint lithography, which is extremely time-consuming as it requires temperature-cycling processes to form a pattern,” explains Saifullah. “A mold could be pressed into a heated (and softened) resist material or a liquid precursor could be forced into a mold and then hardened upon heating.”

Newer processes eliminate the need for heating by using irradiation with ultraviolet (UV) light to harden the polymer. Although this process may be ideal for organic polymer materials, it is more problematic when using inorganic materials such as titania as the liquid precursor materials are highly viscous and do not spread easily. As a result, the dispensing nozzle may sometimes become blocked.

The chemicals used to make titania can also be unstable in solution, so the team had to identify a mixture of components that offered a combination of stability and low viscosity.

“We found that an allyl functionalized titanium complex was stable in combination with other polymer precursors,” explains Saifullah. The final component of the mixture is a photoinitiator — which starts the polymerization process upon irradiation with UV light.

The mixture was dispensed onto the surface in the form of droplets, and the mold pressed into place to help the liquid spread. Irradiation with UV light results in hardening of the pattern, after which the mold can be removed. A final heating step burns away the organic material, leaving behind a shrunken version of the original pattern made from titania (see image). Significantly, the aspect ratio of the pattern is maintained after the heat-treatment process.

“Our current method is quite specific to titania, but after tackling this most important material, we hope to develop similar procedures for other inorganic materials,” says Saifullah.

Polymer fibers play a central role in the production of biomaterials for tissue engineering applications. Generated from self-assembling polyelectrolytes, these materials provide matrices for cells to grow and differentiate. Unfortunately, polymer fibers cannot encapsulate different cell types in a spatially defined manner for culture, thereby hindering their implementation in native tissue mimics.

To overcome these limitations, Andrew Wan and Jackie Y. Ying at the A*STAR Institute of Bioengineering and Nanotechnology and co-workers have developed a method that fuses several fibers from multiple polyelectrolyte interfaces. This method creates matrices composed of well-defined, spatially patterned domains at the micrometer scale, facilitating cell co-culture within the same fiber. Ying explains that polyelectrolyte-based fibers have previously yielded three-dimensional scaffolds for cells, inspiring the team to fuse these materials to achieve co-culture.

The team’s goal is to exploit the patterning ability of their method to give structures that emulate native tissues such as the liver. “Many cell types are involved in the liver, and they are spatially patterned with respect to each other to achieve liver function,” adds Wan.

Unlike typical techniques deployed to manufacture multi-component fibers, the interfacial polyelectrolyte complexation adopted by Wan and Ying’s team is a gentle, water-based chemical process. “When two oppositely charged polyelectrolytes come into contact with each other, a complex forms at their interface,” explains Wan. “Upward drawing of this complex leads to the formation of a fiber.”

The researchers flanked a droplet of polyelectrolyte solution with two droplets of the oppositely charged polyelectrolyte, creating two interfaces from which two fibers were drawn and fused. Upon contact, the wet fibers zipped together, forming a Y-shape pattern over the droplets and producing a two-component fiber. By increasing the number of interfaces to three and four, the team obtained three- and four-component fibers. Assessments of the ability of the fibers to enable co-culture in distinct domains showed that bone-forming cells encased in the outer layers of four-component fibers exclusively propagated and accumulated in those layers. Further experiments were carried out on fibers that consisted of a central core containing endothelial cells surrounded by outer layers filled with liver cells.

The liver cells closely aggregated along the fiber without spreading to the core, where the endothelial cells had formed blood vessel-like structures.

The researchers are currently investigating ways to design better mimics of native tissue using their process. They are also planning on using the multicomponent fibers to study the influence of cellular microenvironment on cancer cell behavior.

Fused polymer-based multicomponent fibers provide well-defined domains for cell co-culture in tissue engineering

New insights into the stable magnetism of phase-change semiconductors could enable the development of ultra-high-speed data storage

Phase-change semiconductors have the ability to switch back and forth between amorphous (non-crystalline solid) and crystalline phases upon heating. As such, they are used widely in data storage and computer memory applications, for the reason that information can be written in binary form using the two distinct states.

One particular phase-change alloy currently used in rewritable disc technology is that of germanium, antimony and tellurium, or Ge$_2$Sb$_2$Te$_5$ (GST). Researchers believe that this material may prove useful for the field of spintronics, generating a way of storing data which takes advantage of the inherent angular momentum, or spin, of electrons present in the material.

Recent research indicates that the atoms in GST could naturally create a stable bond with certain metals, thereby generating a permanent and stable ferromagnetic state potentially useful for high-speed read/write storage. However, to date, researchers have been unsure exactly how GST is able to form a stable ferromagnetic state.

Now, Kewu Bai at the A*STAR Institute for High Performance Computing, together with co-workers from A*STAR’s Data Storage Institute and the Singapore University of Technology and Design, have completed an in-depth analysis of GST and its ability to maintain stable ferromagnetism when doped with iron$^1$.

“Alloying magnetic elements such as iron with semiconductors provides the materials necessary for future spintronics applications,” explains Bai. “We know very little about the processes behind ferromagnetism from doping phase-change materials with metals, because the commonly used experimental techniques, such as X-ray diffraction, transmission microscopy and X-ray absorption, are not sufficient to characterize material microstructures.”

The research team instead used first-principle calculations to determine the validity of the experiments they carried out. First-principle calculations use the inherent laws of nature — for example, bonding laws between atoms and laws for electron movements — to build up an exact picture of the chemical structures at work, rather than relying on best-fit parameters in computer models.

“We used first-principle calculations to locate the site in GST at which iron molecules preferred to bond,” explains Bai. “The mechanism that led to the observed ferromagnetism was then uncovered.”

The researchers discovered that the iron molecules preferred to bond with the antimony molecules in GST. Along certain orientations within the crystalline phase, the iron–antimony bonding becomes dominant, leading to a stable ferromagnetism in the material.

“We are still in close collaboration with the Data Storage Institute team to explore multifunctional phase-change materials further,” explains Bai. “We hope to test our criteria for other transition metals that could also cause ferromagnetism in GST.”

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Fluorescent nanoparticles loaded with organic light-emitting dyes are expected to transform live-animal imaging technologies. Compared to inorganic quantum dots, these optically stable materials are non-toxic and can easily be modified with functional groups, making them ideal when targeting specific tissues in the body. Unfortunately, traditional dyes have been known to aggregate and lose their emission intensity when incorporated in nanoparticles at high concentration. To overcome this problem, a team of researchers led by Bin Liu and Ben Zhong Tang at the A*STAR Institute of Materials Research and Engineering have now designed a family of dyes with enhanced fluorescence upon aggregation.

At the heart of the traditional dyes is a planar chromophore called triphenylamine-modified dicyanomethylene, which emits red light in dilute solutions but fluoresces weakly when aggregated. “The close vicinity of the chromophores induces fluorescence quenching due to non-radiative pathways,” says Liu.

Liu, Tang and their team reversed this phenomenon by attaching propeller-shaped tetraphenylethene pendants to each extremity of the chromophore. Contrary to planar compounds, the shape of the propellers prevents strong stacking interactions between chromophores, blocking the aggregation-caused quenching process. In addition, the physical confinement prevents these propellers from rotating freely, enabling light emission.

The team formulated the dyes using a bovine serum albumin (BSA) matrix — a biocompatible and clinically used polymer — and evaluated their performance as probes. Experimental characterization showed that the wavelength of the emission maximum of the nanoparticles remained unchanged upon encapsulation and that the intensity of the emitted light increased with the dye loading.

Live imaging of breast cancer cells revealed that the nanoparticles displayed more intense and homogeneously distributed red fluorescence in the cytoplasms (see image) than free aggregates, suggesting that BSA boosted the cellular uptake of the dyes. The team also found that the nanoparticles were optically stable in biological media and displayed good biocompatibility.

The researchers intravenously injected the nanoparticles in liver-tumor-bearing mice for in vivo imaging studies. They found that unlike free aggregates, the nanoparticles selectively accumulated in the tumor, clearly highlighting the cancerous tissue in the animals. “This demonstration underscores new research opportunities to explore similar diagnostic probes with potential clinical applications,” says Liu.

The team is currently investigating near-infrared emissive biological probes for targeted in vivo tumor imaging applications. The nanoparticles can also be utilized to understand cancer metastasis or the fate of transplanted stem cells. “These probes are promising in multimodal imaging applications through integration with magnetic resonance imaging or nuclear imaging reagents,” says Liu.

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Hollow optical fibers containing light-emitting liquids hold big promises for biological sensing applications

Processing biological samples on a small substrate the size of a computer chip is becoming a common task for biotechnology applications. Given the small working area, however, probing samples on the substrate with light can be difficult. To address this issue, Xia Yu and co-workers at the A*STAR Singapore Institute of Manufacturing Technology have now developed an optical fiber system that is able to deliver light to microfluidic chips with high efficiency.

“Our compact optical fibers are designed for use with high-throughput detection systems,” says Yu. “They are ideal for use in space-restrictive locations.”

A common way of probing biological samples is by light. In this method, the sample is excited by an external light source and the light emitted in response is detected, which provides a unique fingerprint of the substance. Conventional techniques are able to deliver light to samples and probe the response, but they are not very efficient at probing a small sample volume. A solution to this is to use optical fibers that are able to guide light to small spaces. The drawback with this technique, however, has been that it can be difficult to insert the external probe light into the optical fiber with sufficient efficiencies.

Yu and her co-workers have now circumvented this problem by using optical fibers with a hollow core (see image). The empty hollow core can be filled with liquids — in this case, with chemiluminescent solutions. The liquid is important to promote the transport of light through the core. In addition, these solutions consist of two liquids that when brought together initiate a chemical reaction that emits light. If such a solution is placed directly within the hollow core the problem of coupling light into the fiber is circumvented. This not only avoids external light sources but also promotes an established technology.

“The use of chemical luminescence is a common technique for a variety of detection assays in biology,” says Yu. “By incorporating the emission mechanism into optical fibers, we can use it as a light source for sensing applications in microfluidics systems.”

First tests for such sensing applications are already underway, although some challenges remain. For example, there might be losses in the light emitted by the fluid if the emitted light is not perfectly confined within the fiber. Such problems can be solved through improved fiber designs and an appropriate choice of materials, and applications of these fibers for microfluidic systems are promising.

Drug development

Clever crystals

Water plays a key role in the co-crystallization of active pharmaceutical ingredients

There is much more to drug development than simply identifying a potent active pharmaceutical ingredient (API). Scientists must ensure that the API can tolerate the production process, remain stable during storage and distribution, and behave appropriately inside the patient’s body after administration. One emerging technique for improving the performance of APIs with non-ideal physicochemical properties is to co-crystallize them with a second compound that modulates their behavior. Srinivasulu Aitipamula and co-workers at the A*STAR Institute of Chemical and Engineering Sciences have now developed a novel route for preparing such co-crystals.

The researchers have discovered that adding water droplets can help to form co-crystals of caffeine, a compound known to act as a central nervous system stimulant and a muscle relaxant. Caffeine is inherently unstable to humidity — a property that can be improved by forming co-crystals with biocompatible compounds such as 4-hydroxybenzoic acid (4HBA). Computer models predict that co-crystals of caffeine and 4HBA in the ratio of 1:1 should form the most stable structure. To date, however, researchers have only been able to produce 2:1 and 1:2 co-crystals.

Aitipamula and his team have now successfully formed 1:1 co-crystals of caffeine and 4HBA, in the form of a monohydrate. By grinding together a 1:1 mixture of the two components along with two drops of water, a crystal structure was formed in which each pair of crystallization partners is partly held together by a water molecule.

According to Aitipamula, the key to water’s ability to produce the 1:1 co-crystal is its capacity to both donate and accept hydrogen bonds — the intermolecular force that holds co-crystals components together. “In the case of the caffeine-4HBA co-crystal hydrate, unused hydrogen bond acceptors and donors are satisfied by forming hydrogen bonds with the water molecule,” he says. Without water, the number of hydrogen bond donors and acceptors is unbalanced, resulting in the preferential formation of the 2:1 and 1:2 crystals instead.

The process also works for other APIs, as the researchers have found. They have generated a 1:1 co-crystal hydrate of 4HBA with piracetam, a drug used to treat memory and balance problems. The results suggest that forming hydrates offers an alternative way to generate co-crystals with particular ratios of constituents, expanding the options for forming pharmaceutical materials.

The researchers are currently focused on developing new co-crystals for APIs and studying their physicochemical properties. “Our primary emphasis is to target APIs that pose problems in pre-formulation and dissolution,” Aitipamula says.

Studies reveal a new way to make superhydrophobic surfaces with better self-cleaning capabilities

Many plants and animals have textured surfaces on their body for manipulating water. Some textured surfaces are designed, for example, to improve adhesion, while others may enable the collection of water from fog in arid regions. The lotus leaf, in particular, is the most widely cited example of having a textured surface with self-cleaning properties (see image).

The surface of the lotus leaf has a hierarchical structure — comprising both micrometer and submicrometer features — that makes it difficult for water droplets to spread. As a result, water droplets form tight spheres that easily roll off the leaf, picking up dirt particles en route. Such functionality can become useful if applied to textiles or windows, and may also be used in analytical techniques for controlling fluid flow.

Linda Yongling Wu at the A*STAR Singapore Institute of Manufacturing Technology and co-workers have now developed a fast and cost-efficient way to fabricate large-scale superhydrophobic surfaces on a hard material — silica. The researchers used a laser to carve out a microstructured template that they then used to pattern a sol–gel coating. Nanoparticles were subsequently bound to the surface of the cured sol–gel surface to create a second level of hierarchy. The fabrication methodology can be adjusted to achieve different degrees of micro- and nanostructures.

In addition to the new fabrication methodology, Wu and co-workers considered various ways to optimize the water repellency of the textured surface. They found that increasing the surface roughness increases the true area of contact between the liquid and the solid, enhancing its intrinsic wetting properties. However, if the surface features are small enough, water can bridge protrusions leading to the formation of air pockets; the wettability of such a nanostructured material is then calculated as a weighted average of the wettability of the pure material and that of air. These two effects are known respectively as the Wenzel and Cassie-Baxter states.

The researchers derived an equation for calculating the surface contact angle between a water droplet and a silica surface with a certain degree of roughness. They found that there was a transition between the Wenzel and the Cassie-Baxter state, as surface structuring enters the nano dimension. The researchers found that for an optimum superhydrophobic effect, the Cassie–Baxter state must dominate the surface structure to allow a massive 83% of the surface state to be involved in air trapping with only 17% of the liquid drop surface actually in contact with the silica itself.

The researchers are hoping that their findings will generate new ideas for making innovative self-cleaning materials. “We are now developing the technology for real applications, such as easy-clean coating for solar films and structured surfaces for personal care products,” say Wu.

The discovery of graphene has brought much excitement to the nanotechnology community. Much of this excitement is due to the possibility of deriving graphene-based materials with applications in electronics, energy storage, sensing and biomedical devices. Despite the potential, however, there is a real concern that graphene-based materials may have deleterious effects on human health and the natural environment.

One particularly interesting aspect of this subject is the toxic effects of graphene-based materials on the microscopic world of bacteria. For this very reason, Jun Wei at the A*STAR Singapore Institute of Manufacturing Technology and co-workers have now compared the antibacterial activity of graphite, graphite oxide, graphene oxide and reduced graphene oxide using the model bacterium *Escherichia coli*. They showed that the two graphene-based materials kill substantially more bacteria than two graphite-based materials — with graphene oxide being the top performer.

Interestingly, graphene oxide particles had the smallest size of all the four graphene materials as measured by dynamic light scattering. Wei and co-workers believe that particles of reduced graphene oxide were larger because they aggregated both laterally and in three dimensions.

In fact, the size of the particles could well be the key to why graphene oxide is so deadly to bacteria. When the researchers studied the affected cells using scanning electron microscopy, they saw that most of the *E. coli* cells were individually wrapped by layers of graphene oxide. In contrast, *E. coli* cells were usually embedded in the larger reduced-graphene-oxide aggregates (see image). A similar cell-trapping mechanism was operational in the graphite-based materials.

So why does cell-wrapping kill more cells than cell-trapping? The researchers believe that the direct contact of cell surface with graphene causes membrane stress and irreversible damage.

Wei and co-workers also investigated chemical mechanisms by which the materials could disrupt and kill bacteria. They found that the oxidation of glutathione, an important cellular antioxidant, occurred on exposure to graphite and reduced graphene oxide. “It might be that these structures act as conducting bridges extracting electrons from glutathione molecules and releasing them into the external environment,” says Wei.

Intriguingly, while the effect of the membrane-disrupting mechanisms dies away after four hours of incubation, the oxidation mechanism shows only minor changes.

“With the knowledge obtained in this study, we envision that physicochemical properties of graphene-based materials, such as the density of functional groups, size and conductivity can be better tailored to either reduce environmental risks or increase application potential,” says Wei.

**Photonics**

**Think thin, think vibrant**

A thin liquid crystal film on gold sheets makes an ultra-compact color filter

Flat panel displays, mobile phones and many digital devices require thin, efficient and low-cost light-emitters for applications. The pixels that make up the different colors on the display are typically wired to complex electronic circuits that control their operation. Jing Hua Teng at the A*STAR Institute of Materials Research and Engineering and co-workers have now developed a display technology that requires a much simpler architecture for operation. They demonstrated that combining a thin perforated gold film with a liquid crystal layer is all that it takes to make an efficient color filter1.

“Our color filters are a lot thinner and more compact than conventional thin-film-based color filters,” says Teng. “The colors of these filters can be tuned with ease so they are very versatile in applications.”

The color selection of the devices comes from the patterned gold film. The collective motions of the electrons on the film surface — the so-called surface plasmons — absorb light at wavelengths that depend on the details of these patterns. In the present case, the patterns are narrow, nanometer-sized rings cut out of the films (see image). As the diameter of the rings changes, so does the color of the metal film. Pixels of a different color can be realized simply by patterning rings of different sizes across the same gold film.

To realize a full display, however, each of these pixels needs to be turned on and off individually. This is where liquid crystals come in.

Liquid crystals are molecules that can be switched between two different states by external stimuli, such as ultraviolet light. In their normal state the crystals let visible light pass through so that the pixel is turned on. But when ultraviolet is also present, the structure of the liquid crystal molecules will change so that it absorbs visible light (i.e. the pixel is turned off). This process can be repeated over many cycles without degrading the device itself.

Although the device works in principle, it remains a concept on the drawing board for now. This is because there are still many issues that need to be overcome, for example, the optimization of the switching speed and the contrast between ‘on’ and ‘off’ states. In future work, the researchers will need to extend their ideas so that their device can serve a larger area and produce the fundamental colors red, green and blue.

Teng and his team are quite optimistic that they will achieve this soon.

The latest advance in imaging technology helps optimize catalysts for use in onboard fuel processing

The presence of carbon monoxide (CO) impurities in hydrogen gas (H₂) can have a detrimental impact on the performance of fuel cells. Recent studies have shown that gold nanoparticles — particles less than five nanometers wide — can catalytically remove CO impurities from H₂ under mild temperature and pressure conditions. This breakthrough understanding has helped facilitate the development of fuel-cell vehicles that use ‘onboard’ fuel processing technology. Unfortunately, gold nanoparticles tend to lose their catalytic activity after a few hours of use — and scientists need to overcome this problem if gold nanoparticles are to be used.

Ziyi Zhong at the A*STAR Institute of Chemical and Engineering Sciences, Ming Lin at the A*STAR Institute of Materials Research and Engineering and co-workers have identified the subtle, atomic-scale structural transformations that can activate and de-activate gold nanoparticle catalysts, a finding that may lead to longer-lasting hydrogen fuel cells.

The researchers set out to design an improved catalyst for so-called preferential oxidation (PROX) reactions. This approach transforms CO impurities into carbon dioxide (CO₂) on a ceramic support containing metal catalysts. Previously, the team found that silica-based supports, called SBA-15, could boost CO removal by selectively absorbing the CO₂ by-product. The researchers took advantage of another SBA-15 characteristic — a mesoporous framework decorated by terminal amine groups — to engineer a novel PROX catalyst.

First, the team used amine modification to disperse a mixture of gold and copper(II) oxide (CuO) precursors evenly over the SBA-15 support. They then used heating treatment to generate gold and CuO nanoparticles on the SBA-15 support. The numerous pores in SBA-15 and the CuO particles work together to hinder agglomeration of gold nanoparticles — a major cause of catalyst de-activation.

The team then achieved a near-unprecedented chemical feat: localized structural characterization of their catalyst at atomic scale, using high-resolution transmission electron microscopy (HR-TEM) and three-dimensional electron tomography. These imaging techniques revealed that the active catalyst sites — gold or gold–copper alloy nanoparticles in the immediate vicinity of amorphous and crystalline CuO — remained stable for up to 13 hours. However, the reducing atmosphere eventually transforms CuO into copper(I) oxide and free copper; the latter of which then alloys with the gold nanoparticles and deactivates them. Fortunately, heating to >300°C reversed the alloying process and restored the catalyst’s activity.

“People working in catalysis are always curious about the ‘local structures’ of their materials,” says Zhong. “Because the Au–CuO/SBA-15 catalyst is active at room temperature, advanced characterization in our state-of-the-art facilities is possible — though it takes great patience and requires multidisciplinary collaboration.”

Inexpensive hybrid metal and oxide nanostructures prove to be a catalyst that enhance sunlight-powered hydrogen production

Nanoparticles
Two-faced materials boost hydrogen production

Hydrogen is crucial for the oil-refining industry and the production of essential chemicals such as the ammonia used in fertilizers. Since producing hydrogen is costly, scientists have long searched for alternative, energy-efficient methods to separate hydrogen atoms from abundant sources such as water.

Nanometer-scale structures consisting of cheap metal and oxide spheres were recently demonstrated as an excellent catalyst for a hydrogen-production reaction powered only by sunlight. The study was completed by Ming-Yong Han and his colleagues of the A*STAR Institute of Materials Research and Engineering, Singapore, working in collaboration with a team of researchers from Singapore and France.

Han and his team mixed 50-nanometer diameter spheres of gold into a titanium dioxide precursor such that a sphere of titanium dioxide formed on the side of each gold nanoparticle. Structures with this two-sphere arrangement are known as Janus particles, named after the two-headed god from Roman mythology. While the Janus particles were suspended in a mixture of water and isopropyl alcohol, Han and co-workers shone visible light on them and measured hydrogen production, which proceeded at a rate as fast as 2 milliliters per minute.

The researchers then used theoretical models to show that this production rate was caused by so-called plasmonics effects: that is, the electrons on the surface of the gold nanoparticle at the junction with the titanium dioxide coupled to the incoming light and formed light–matter hybrid particles called plasmon polaritons. The energy absorbed by these particles then passed into the surrounding liquid, and this drove the hydrogen-releasing chemical reaction.

“Our work provides insight into mechanisms that will be useful for the future development of high-performance photocatalysts,” says Han. Indeed, Han and his co-workers were able to improve the efficiency of the hydrogen production even further: they increased the area of the metal–oxide interface by using larger gold nanoparticles.

The Janus particles were 100 times more efficient as a catalyst for hydrogen production than bare gold nanoparticles. Moreover, they were over one-and-a-half times better than another common type of plasmonic nanoparticle, core–shell particles, in which the oxide material forms a coating around the metal nanoparticle.

“We next hope to develop a better understanding of the processes that occur at the metal–titanium-dioxide interface using a combination of experimental observations and theoretical simulations,” says Han. “This will get us closer to our ultimate goal of using solar illumination as an abundant source of renewable energy.”

Medical genetics

Finding the elements of risk

Genome-wide association studies reveal risk factors that increase the susceptibility of children to certain diseases

Children are especially vulnerable to illness, but the deck may be further stacked against them by genomic mutations that increase disease susceptibility. For example, the onset of severe dengue shock syndrome (DSS) in response to dengue virus infection and Kawasaki disease, an inflammatory condition that causes cardiovascular damage, are likely to be associated with heritable risk factors.

“Both are severe diseases occurring in young children who are otherwise healthy, and we suspect that there is a genetic predisposition,” says Chiea Chuen Khor, a research scientist with Martin Hibberd’s group at the A*STAR Genome Institute of Singapore. Their team recently collaborated on two studies that confirm this hypothesis, zooming in on genetic risk factors that yield valuable clinical insights into both conditions.

For each investigation, Khor and Hibberd joined forces with numerous other scientists to perform genome-wide association studies (GWAS). In a GWAS, researchers analyze genomic variations scattered throughout the genome in large numbers of healthy and disease-affected individuals, hoping to identify changes that show a robust statistical association with a given condition. Hibberd’s group has repeatedly demonstrated the power of such studies in the past. “These have resulted in the discovery of disease genes with unexpected functions that would never otherwise have been picked up,” he says.

Kawasaki disease has been the target of GWAS before, but Khor and Hibberd partnered with several research consortia to perform an analysis of unprecedented scale, examining nearly half a million single-nucleotide polymorphisms (SNPs) in over 11,000 individuals. Their study validated several genomic sites previously linked with disease risk, but also flagged a novel mutation in the $\text{FCGR2A}$ gene. This change appears to modulate the extent to which the immune system can respond to certain molecules, including the intravenous immunoglobulin (IVIG) that is typically used to treat Kawasaki patients. “This could explain why up to 25% of children with Kawasaki are refractory to IVIG treatment,” says Khor.

Their investigation of DSS proved equally enlightening. Based on analysis of 2,118 Vietnamese children affected by DSS versus an equivalent number of controls, they identified statistically strong disease associations for variations located near a pair of genes, $\text{PLCE1}$ and $\text{MICB}$. The latter is particularly interesting, as it encodes a protein that helps coordinate the immune antiviral response and is known to be strongly expressed in dengue-infected patients.

Khor and Hibberd will investigate the clinical ramifications of these findings more closely, but also plan in the near future to pursue higher-density searches for new risk factors using DNA sequencing-based approaches.


The human eye has a blind spot, a region where optic nerves meet and therefore has no photoreceptors for detecting and perceiving light. This blind spot, also known as the optic disc, plays a crucial part in the eye’s physiology and the diagnosis of eye diseases.

However, optic disc detection and segmentation from retinal images can become challenging due to various ocular pathologies that could degrade the image quality severely.

Shijian Lu at the A*STAR Institute for Infocomm Research has now provided a solution to this long-standing problem by developing a computer algorithm that is able to detect the optic disc from retinal images with unprecedented precision and accuracy.1

The variations in optic disc appearance for different eyes have made it difficult for computer algorithms to pinpoint disc centre and boundary with sufficient accuracy for medical diagnostics. Often, diseases or other features in the eye such as blood vessels make assignments difficult.

The basis on which algorithms identify the optic disc is usually through its brighter appearance compared to surrounding areas. Through such an analysis, a region can be identified in which the optic disc is most likely to be.

The algorithm developed by Shijian Lu now takes the information on the probable locations for the disc and refines it by taking a step further — assuming that the optic disc is usually round. The circular transformation method developed by Lu looks for maximum variations in brightness along radial lines spreading out from the region of the probable location of the optic disc. By passing through several filters, the researchers could identify the disc boundary, and consequently the disc center.

In tests on standardized retina photographs, the algorithm was able to identify the optic disc with 98.8% detection accuracy. The placement error of the disc center was only six pixels.

Moreover, the sampling speed of the photos was only five seconds. This can be enhanced even further by at least a factor of ten as the software was written on a non-optimized software package.

Such accuracy and sub-second speeds make this method promising for clinical use. “This is a breakthrough for automatic computer aided diagnosis of ocular diseases, because few state-of-the-art techniques can handle the optic disc segmentation for severely degraded pathological retinal images,” says Lu.

Clinical trials under more difficult circumstances than the standardized photographs will follow. If successful, this new method could greatly improve the detection of eye diseases. ■

Immunology

Vaccine hope for virile virus

Exploiting the early immune response in Chikungunya fever promises to provide protection

Chikungunya fever is a viral disease that has re-emerged to cause epidemics in the Pacific region within the last decade. It is caused by the Chikungunya virus (CHIKV), which is transmitted by mosquitoes and causes symptoms including fever, rash and joint pain. It can be incapacitating, with some patients developing severe chronic symptoms, and it is sometimes fatal. The main current control measure is to prevent exposure to mosquitoes; a vaccine would reduce the threat of CHIKV.

Lisa Ng of the A*STAR Singapore Immunology Network and co-workers1 have now provided insight into the natural immune response that may help in developing a vaccine. Ng’s group showed previously that the initial immune response to CHIKV is spearheaded by a specific class of antibody that disables the virus when bound to it. Their latest research reveals a way to exploit this clinically.

Working with clinicians at the Tan Tock Seng Hospital, Ng and her team took blood samples from CHIKV-infected patients and tested them to see if they contained any antibodies that recognize proteins from the surface of the virus. They found that at early stages of recovery, patients’ blood contained large amounts of an antibody that targets a protein known as E2, which projects from the surface of CHIKV (see image). The same antibody was found in different groups of patients, showing that it is a reliable indicator of early infection.

The team confirmed that this antibody neutralizes CHIKV by adding blood samples to virus which was then used to infect susceptible human cells. If the blood samples contained the antibody, infection rates were reduced, whereas removing the antibody from the samples beforehand left infection rates high.

Having identified that the antibody recognizes E2, the researchers then tested its ability to recognize fragments of the protein. This allowed identification of the epitope, or the exact site on the protein, that the antibody binds to, which they called E2EP3.

When they vaccinated mice with a protein fragment equivalent to this epitope, the mice produced the same antibody in response. On subsequent infection with CHIKV, the vaccinated mice also showed milder symptoms, making the epitope a promising basis for a future vaccine in humans.

“[This study is] highly relevant for the rational design of CHIKV vaccines and for the development of diagnostics for optimal clinical management of patients,” says Ng. “It may also inspire similar studies with other arthritic arboviruses that in many parts of the world cause severe morbidity with extensive incapacitation.”

Immunology

For one and for all

The infection of one dengue virus subtype can lead to the production of antibodies that confer protection against other dengue virus subtypes

Dengue fever is a mosquito-borne illness caused by the dengue virus that is endemic in Singapore and other tropical regions of the world. Over half of Singaporeans harbor antibodies that confer protection against the dengue virus, but how soon after infection these antibodies are produced in the body, and how broadly protective they are against the different strains, or subtypes, of the dengue virus, is unclear. An international team of researchers led by Katja Fink at the A*STAR Singapore Immunology Network have now observed an unexpectedly early and high production of antibodies in the human body after both primary and secondary infection of the dengue virus.

Dengue virus infection can cause a variety of symptoms, ranging from fever, pain or rash to hemorrhage — even death. Severe symptoms are seen more frequently in patients who have been infected with the dengue virus more than once. These so-called secondary infections are therefore more dangerous than primary infections.

Fink and co-workers took blood samples from individuals who were presented to the clinic with fever and screened the samples for dengue viruses, as well as antibodies against dengue viruses. Based on the test results, they classified the patients into one of three groups: normal (non-dengue) fever, primary dengue infection, and secondary dengue infection.

The researchers found that blood samples from patients with dengue infection contained a much higher number of antibody-producing immune cells than in those from patients with normal fever. When they exposed cells in culture to antibodies produced by these immune cells, they found the cells were protected against not just one but four major dengue virus subtypes. Antibodies from patients with secondary infections seemed to neutralize infections by all four dengue strains even better than those from patients with primary infections, suggesting that their immune response to re-exposure to the virus is stronger than the response of patients who are exposed to the virus for the first time.

“Our findings can explain why dengue patients are protected against all four dengue strains for several weeks after infection with one strain,” says Fink. “However, the enormous numbers of activated cells also create inflammation in the body, which can contribute to the symptoms observed in dengue patients.” As patients with secondary infections have stronger, more improperly regulated immune responses than those with primary infections, the findings could explain why secondary infection is often more severe than primary infection.

Variations in immune genes associated with increased susceptibility to common kidney disease

IgA nephropathy (IgAN) is the most common condition affecting the glomeruli, or small blood vessels in the kidney. It is characterized primarily by the deposition of IgA antibodies in the glomeruli, which leads to inflammation and scarring of the blood vessels. The disease is more prevalent in Asian than in Western countries, and although genetic and environmental factors play a role in its development, very little is known about the genetic risk factors involved.

A large team of researchers led by Jianjun Liu at the A*STAR Genome Institute of Singapore have identified a number of genetic variants that are associated with increased risk of a common kidney disease called IgAN in Chinese individuals of Han descent.

Liu and his co-workers performed a genome-wide association study comparing the genomic data of nearly 1,500 Han Chinese individuals with IgAN with those of approximately 2,700 healthy controls. In the first phase of the study, they analyzed almost 450,000 common single nucleotide polymorphisms (SNPs), or sequence variations at individual positions in the DNA sequence. This confirmed that a number of known genetics variants are associated with increased susceptibility to IgAN.

The researchers also identified several more previously unknown genetic variants. After confirming these initial findings, they analyzed these genetic variants in another 2,700 individuals with IgAN and about 3,500 controls.

Some of the newly identified SNPs lie within the region of the genome containing the major histocompatibility complex (MHC) genes, which encode proteins that are critical for proper function of the immune system. Other SNPs were found in the genes encoding tumour necrosis factor, a signaling molecule that is important for the development of B cells of the immune system, and α-defensins, a group of molecules that have antibiotic properties and are involved in the inflammatory response to infection. They also provide migratory cues for immune cells and induce them to release small signaling molecules called cytokines.

The findings show that variations in genes involved in immunity and inflammation can influence susceptibility to IgAN and the development of the disease.

“These novel SNPs have not been studied in non-Chinese populations yet, so we don’t know whether they will show the similar association in other populations,” says Liu. “The SNPs only explain a small proportion of genetic risk for IgAN and many additional genetic risk variants need to be discovered. We are collaborating with other groups on a meta-analysis of IgAN where independent GWAS datasets will be combined to discover new variants.”

A protein thwarts developmental abnormalities by preventing removal of critical chemical marks from embryonic DNA

When a mammalian egg gets fertilized, it essentially undergoes a genomic ‘reset’ that transforms it into an embryonic cell capable of developing into the full spectrum of adult tissues. Daniel Messerschmidt and co-workers at the A*STAR Institute of Medical Biology have now identified the protein TRIM28 as a key player in this reprogramming process.

Genetic activity is not exclusively determined by sequences of nucleotides, but also depends on ‘epigenetic marks’ — chemical modifications, such as the addition of methyl groups to DNA, that can dramatically affect gene expression. After fertilization, many parental genes are stripped of their methyl groups (a process called demethylation), but certain maternally- or paternally-inherited copies of specific genomic loci are ‘imprinted’ and retain their original methylation pattern.

TRIM28 normally contributes to DNA methylation, which makes it seem a likely contributor to establish imprinting patterns, but Messerschmidt and his team have determined that this is not the case. “To our surprise, TRIM28 is not required to establish imprints, as we might have predicted, but instead helps maintain these features after fertilization,” says Messerschmidt.

In the earliest stages of development, gene expression depends entirely on proteins synthesized from maternal messenger RNA (mRNA) within the oocyte. Therefore, the researchers investigated the early function of this gene with embryos generated by using sperm from normal male mice to fertilize oocytes that lack the Trim28 gene. Messerschmidt and co-workers observed a staggering variety of defects in the resulting embryos (see image), none of which successfully developed to term.

Closer analysis revealed unexpected demethylation of numerous loci that are normally imprinted, indicating that TRIM28 typically insulates these regions against epigenetic modification. Some loci were more commonly affected than others, but overall the effects were highly heterogeneous among littermates.

“We were surprised by the extreme variability,” says Messerschmidt, “as all the embryos were genetically identical and should therefore display the same defects.” Interestingly, subsequent expression of the intact paternal Trim28 gene was insufficient to rescue these embryos later in development, suggesting that damage arising from early abnormalities accumulates over the course of development.

“Once a defect has occurred and imprinting is lost at a locus, it cannot be recovered, impacting on later embryonic stages,” says Messerschmidt. By exploring how TRIM28 protects its target genes and attempting to identify other, non-imprinting-related functions for this protein, Messerschmidt and his team hope to gain insights that might ultimately prove valuable in predicting — or possibly even preventing — human birth defects.

A simple new method of extracting viral RNA from blood samples allows quick, on-the-spot identification of dengue fever in patients

Dengue fever is a disease passed to humans by mosquitoes. Millions of people every year are infected worldwide, and around 4,000–5,000 of these cases will suffer severe complications or death. Dengue fever most commonly affects young people between the ages of 15 and 24.

Currently, doctors identify dengue fever by clinical observations followed by a series of laboratory tests of blood and urine samples. These tests can take seven to ten days to complete, and require highly skilled staff and specialist equipment. Due to the complexity of the process, there is also a chance of cross-contamination during the procedure.

For these reasons, researchers are keen to develop quicker, more accurate ways of identifying viruses such as dengue fever. Siti Mohamed Rafei and co-workers at A*STAR’s Institute of Microelectronics, together with scientists from Veredus Laboratories in Singapore and the National University of Singapore, have designed and built a new self-contained microsystem that can ascertain the presence of dengue fever in blood samples within 30 minutes. Crucially, the new cartridge can be operated by non-skilled staff.

The microsystem works by extracting viral RNA from patients’ blood samples. Using a silicon-based viral extraction chip and a cartridge containing reservoirs pre-filled with the different reagents required to extract viral RNA, the microsystem is fully self-contained.

In conventional virus detection systems, the chance of cross-contamination is high because the extraction process requires extensive manual pipetting of reagents. In the newly designed system, the silicon chip is embedded in a polymeric cartridge that allows the user to preload all necessary reagents, making it fully self-contained and disposable. This added feature is extremely useful for testing infectious disease that might be highly virulent or contagious.

The cartridge is placed inside a handheld computer device with a touch screen. Pressing the start button operates a pre-determined series of plungers, which release the reagents into the silicon chip containing the blood sample. The reagents allow for the extraction of viral RNA and virus identification readout within 30 minutes.

The sequence of plungers and their speed are fully computer-controlled, thus the cartridge is configurable, user-friendly and does not require specialist knowledge to operate. In addition, the cartridge is adaptable to multiple biochemical protocols, not just to the viral RNA for dengue fever as described here. In future, the researchers hope to identify many infectious diseases with this technology.

Transcription factor proteins essentially act as genetic on/off switches, binding specific stretches of DNA that enable them to stimulate or repress the activity of neighboring genes. However, it remains a challenge to authoritatively define the DNA sequences individual factors prefer.

Work from Prasanna Kolatkar’s team at the A*STAR Genome Institute of Singapore now offers valuable insights into this process. The human genome encodes 20 members of the Sox transcription factor family, which play a key role in many developmental processes, and Kolatkar and colleagues are exploring how these proteins recognize their respective targets.

Previous experiments have defined a core TTGT binding sequence for Sox proteins, but other determinants are clearly involved. The researchers began by comparing the structures of the complexes formed by the DNA-binding ‘high mobility group’ (HMG) domains of either Sox4 or Sox17 with a specific target DNA sequence. Both proteins formed highly similar complexes in these experiments, although there were subtle differences in the Sox4 HMG-DNA interaction that prompted closer investigation.

In follow-up experiments, the researchers assessed the binding efficiency of the Sox4 HMG against a broad variety of DNA sequences. To their surprise, they learned that although this domain preferentially binds to a specific ‘primary motif’, it can also interact strongly with various ‘secondary motifs’ (see image). All of these motifs contain the TTGT core, but with variations in the flanking sequences. Significantly, Kolatkar’s team noted a ‘positional interdependence’ effect within these flanking sequences, such that Sox4 HMG required the presence of specific nucleotide pairs to bind effectively — for example, favoring CT or AA over other combinations.

The researchers determined that the subtle differences they had observed in the Sox4 binding complex actually represented the amino acids that enabled Sox4 to sense and discriminate between its primary and secondary binding sites. “The same protein can recognize two separate DNA motifs for binding with different affinities using slight changes at the interaction surface,” says Kolatkar.

These findings suggest that transcription factors in general may have greater flexibility with regard to their site preferences than was previously recognized, and Kolatkar’s team is now delving more deeply into the interaction behavior of the rest of the Sox family. If this model is confirmed, it could represent an important system for control of gene expression; for example, certain genes containing ‘secondary’ regulatory motifs may only get switched on when transcription factor levels are especially high. “This could help us to rationalize the mechanisms underlying genomic regulatory network data,” says Kolatkar.

Chikungunya virus has caused epidemics in Africa, Asia and recently Europe. It is transmitted to humans by *Aedes* (see image), a genus of mosquitoes that also transmit dengue fever. Symptoms of a Chikungunya viral infection include acute fever followed by joint pain that can last for days, weeks, or even years. The disease may be fatal for newborns and the elderly; what’s worse is that there are no vaccines or treatments currently available for the disease.

Lucile Warter at the A*STAR’s Singapore Immunology Network and co-workers have now identified two regions of the virus recognized by human antibodies that can neutralize Chikungunya virus. The researchers have also shown the first proof that the virus is capable of direct cell-to-cell transmission. The findings could help explain how the virus avoids being bound by extracellular neutralizing antibodies and increases the efficiency of infection.

To discover the antibody-binding sites, researchers incubated the virus with previously identified antibodies and then isolated resistant variants. Sequencing and structural analysis revealed that the resistant variants had mutations in several regions, suggesting that these regions were the antibody binding sites. The mutations were in the viral fusion loop ‘groove,’ and the envelope E2 domain B. The latter domain has been shown to be important in neutralizing antibody recognition of related RNA viruses.

Results of analyses of the resistant strains, including rapid *in vivo* spread compared with wild-type virus, led the researchers to suspect that Chikungunya virus might be capable of direct cell-to-cell transmission. Warter and co-workers confirmed this hypothesis by culturing infected cells with uninfected cells in medium containing neutralizing antibodies. Many new cells became infected, but no viral particles were detectable outside the cells.

Microscopic examination of infected cells also showed increased concentrations of viral particles at points of cell-cell contact. Direct cell-to-cell transfer offers the advantage of spreading a large number of viral particles directly into another cell where they can begin reproducing, without the risks of traversing the extracellular space. This provides obvious advantages over the canonical mode of viral spread, broadcasting viral particles inside an organism.

“Chikungunya is the first alphavirus known to be capable of cell-to-cell transmission,” says Warter. “Additional studies will be necessary to identify molecular mechanisms associated with this route of transmission, as well as to investigate whether this kind of transmission occurs *in vivo*.”

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Tyrosine kinase inhibitors (TKIs) kill cancerous cells by inducing programmed cell death. They are of enormous therapeutic benefit to patients with chronic myeloid leukemia (CML) and certain types of lung cancer, but their effectiveness may vary from individual to individual. Previous studies have estimated that one in five patients finds TKIs to be ineffective. An international team of researchers including Yijun Ruan and Axel Hillmer at the A*STAR Genome Institute of Singapore, Sin Tiong Ong and King Pan Ng at the Duke-National University of Singapore Graduate Medical School, Charles Chuah at the Department of Haematology, Singapore General Hospital and Darren Wan-Teck Lim at the National Cancer Centre has now identified a common genetic variation linked with resistance to TKIs.

Ng, Hillmer and their colleagues sequenced and compared the genomes of five CML patients, three of whom were resistant to treatment with TKIs, focusing on several candidate genes that are known to be involved in the cell death signaling pathway. The researchers found that what the three TKI-resistant patients all had in common was a 2,903 base pair deletion in the non-coding region of the BIM gene, which encodes a member of the BCL2 family of cell death genes.

They then screened the genomes of more than 2,500 healthy individuals, and found that this deletion is a common variation that occurs in approximately one eighth of East Asian individuals but is not found in Africans or Europeans. Further experiments revealed that the deletion alters processing of the messenger RNA that is transcribed from the BIM gene. As a result of this, the coding sequence of the cell death activation domain is preferentially removed, so that the BIM proteins synthesized from the transcript are faulty.

Finally, the researchers showed that the deletion in the BIM gene is a useful biomarker that predicts which patients are at risk for developing TKI resistance. They examined the responses of 203 East Asian cancer patients to the drug imatinib, and found that CML patients with the deletion were more likely to be resistant to it.

In lung cancer patients, the deletion was associated with the duration of the drug response, and predicted a shorter period of survival without disease progression. Those with the deletion had progression-free periods of about 6.5 months, on average, compared to an average of nearly 12 months in those without it.

“Asian CML patients could be screened for the presence of the deletion to determine the ones who have a higher chance of being resistant to TKI treatment,” says Hillmer. “It will be interesting to investigate the frequency of the deletion polymorphism in other populations and to translate the findings in the clinical practice.”

Cancer genomics

An integrative approach to liver cancer

New research sheds light on the molecular mechanisms by which a virus contributes to cancer

Hepatocellular carcinoma (HCC) is the third leading cause of cancer deaths worldwide and is associated with exposure to hepatitis B virus (HBV). Patients carrying the virus have a 100-fold greater risk of developing HCC, but exactly why was unclear until now. Wing Kin Sung at the A*STAR Genome Institute of Singapore and the National University of Singapore, John Luk at the A*STAR Institute of Molecular and Cell Biology and the National University of Singapore and co-workers have now identified genetic mechanisms by which a virus contributes to this common form of cancer.

To investigate, the researchers obtained samples of liver tumors and adjacent non-cancerous tissues from 88 Chinese HCC patients, and used advanced DNA sequencing technology to analyze their genomes for HBV integration sites. They identified 399 sites at which HBV was integrated into the genome, and found that they were randomly distributed across the whole genome, but that most were clustered within a small number of 'hotspots'. The vast majority of the integration sites (344 out of 399; more than 86%) were found only in the samples obtained from liver tumors.

The researchers analyzed breakpoints in the HBV genome — sites at which the circular genome of the virus breaks before being integrated into the genome of the host cell. They found that about 40% of breakpoints occur within a restricted region where three critical genetic elements are located.

This region, approximately 400 base pairs in length, contains the enhancer, a short regulatory sequence that binds proteins and enhances expression of the viral genes; the X gene, which plays critical roles in infection and replication; and the core gene, which encodes a protein envelope for the viral DNA. The high number of breakpoints in the region may facilitate HBV insertion into the host genome, which in turn may promote cancer formation by interrupting the coding sequences of tumor suppressor genes.

The researchers also examined the prevalence of HBV insertions in DNA obtained from HCC patients. More than 92% of the patients in the sample had HBV integrated into their genomes, and the majority of these were found only in DNA from the tumors.

Non-cancerous tissues were also found to contain integrated viral genomes, but DNA isolated from the tumors tended to have more HBV integration sites. Thus, HBV integration patterns differ between cancerous and non-cancerous tissues, and there is a complex relationship between HBV integration and cancer development.

Sung, Luk and co-workers are now in the process of finding mutations in HCC for drug discovery and novel therapies.

Molecular biology

Genetic disease linked to protein build-up

Findings may lead to new approach to treat developmental problems associated with cell nuclear membranes

Mutations of the gene Lmna previously thought to be directly responsible for a group of laminopathies — serious developmental conditions including premature ageing and a form of muscular dystrophy — in fact cause them by allowing a critical protein to accumulate. An international collaborative group of researchers including Ya-Hui Chi and co-workers at the A*STAR Institute of Medical Biology have discovered in mice that reducing levels of the protein, Sun1, resulted in decreased severity of the diseases and longer life spans. This breakthrough finding may eventually lead to changes of the treatment strategy for developmental conditions.

The inner membrane of the cell nucleus is strengthened by a meshwork of protein filaments known collectively as the nuclear lamina. In mammals, the Lmna gene encodes two of the proteins that form the lamina filaments. Mice with two copies of dysfunctional Lmna genes model human Emery-Dreifuss muscular dystrophy (EDMD), and mice with genes incorporating a mutation that deletes 40 amino acids from the Lmna gene show features of the premature ageing syndrome Hutchinson-Gilford progeria (HGPS). All these mice have misshapen cellular nuclei, degenerative tissues and organs, and short lives.

Recent research has shown that, as well as keeping the membrane in shape, the nuclear lamina is involved in activating genes, repairing DNA and organizing the nucleus. In order to investigate these roles, the researchers generated EDMD and HGPS model mice with genes encoding dysfunctional Sun1, a protein involved in linking the nuclear lamina and the cytoskeleton within the cell. To their surprise, these mice showed milder developmental defects and lived longer.

In fact, cells from EDMD and HGPS model mice display an excessive accumulation of Sun1. The researchers found the same to be true of human cells taken from those afflicted by HGPS. Their developmental problems were alleviated by lowering the level of Sun1. Further work suggested that the accumulation of Sun1 was the result not of increased production of the protein, but reduced degradation.

“Collectively the findings implicate Sun1 build-up as the common event of the disorders,” says Chi. “We suspect that clinical trials and therapies that target the protein products of dysfunctional genes without resolving the Sun1 accumulation are ineffective or useless against HGPS. In fact, our experimental evidence shows that reduced metabolic turnover of Sun1 is a major cause of HGPS.”

Chi and co-workers now want to investigate what factors interact with Sun1 for it to accumulate, and also if there are any other proteins responsible for HGPS.

Cancer biology

On the trail of a mysterious killer

A bonanza of genomic sequence data gives researchers valuable new insights into a poorly understood cancer

Stomach cancer doesn’t get the same publicity as lung or breast cancer, but it is a health threat to be taken very seriously. “Gastric cancer is the second leading cause of worldwide cancer mortality, with an annual death rate of over 700,000 individuals,” explains Patrick Tan of the A*STAR Genome Institute of Singapore. He notes that this disease is especially prevalent in Asia; gastric cancer is the fifth most common cancer amongst Singaporean men.

Remarkably little is known about the biological triggers of gastric tumor formation. Tan recently led a large international team of researchers that identified genetic risk factors for this particular cancer. They performed a massive dragnet screen for mutations, sequencing 18,000 genes in 15 different tumors and comparing them against equivalent sequences from adjacent, noncancerous tissue.

The results proved illuminating. For example, although half of all gastric cancer cases are associated with infection by the bacterium Helicobacter pylori, there were no obvious differences in mutational profiles from H. pylori-positive and -negative tumors. However, Tan notes that this may also be a result of limited sample size. In general, the researchers encountered striking diversity across their samples, but also uncovered patterns upon closer examination. “Although most individual genes were only mutated in a small proportion of samples — usually less than 10% — many of the genetic abnormalities represented different components of the same functional pathway,” says Tan.

Many mutations observed by the team affect cellular adhesion pathways, which can influence tumor progression and metastasis. One gene in this pathway, FAT4, caught the researchers’ attention; laboratory experiments confirmed that disruption of this gene confers tumorigenic properties on cells. Tan and co-workers subsequently identified FAT4 mutations in genomic data from various other cancers as well. They also identified another previously unknown tumor suppressor gene, ARID1A; importantly, this gene acts in a cancer-associated signaling pathway targeted by existing drugs, suggesting that it may provide a clinically useful indicator for planning patient treatment.

In their ongoing analysis of the gastric cancer genomic landscape, Tan and his co-workers will now investigate major structural alterations — including chunks of chromosome that have been duplicated, deleted or flipped around — as well as changes in how chromosomal DNA becomes chemically modified. Collectively, these data may eventually provide a handy atlas for oncologists. “We hope to apply these technologies to gastric cancer patients treated in clinical trials, to identify accurate molecular predictors of disease relapse and treatment response,” says Tan.

Photonics

Getting a fair compensation

**Compensation doping can improve the efficiency of silicon optical modulators**

Silicon is widely used in electronics devices, such as computer chips and solar cells. It is also becoming the material of choice for making photonic devices that lie at the heart of communications, including light-emitting diodes, photodetectors and optical modulators.

One of the drawbacks of silicon photonic devices is ‘insertion loss’ — the loss of optical signals when these devices are integrated into the optical network. Silicon optical modulators, for example, may have comparable switching speed and modulation efficiency as optical modulators made of other materials such as lithium niobate, but their insertion loss can be on the double. Improving the optical performance of silicon modulators is highly desirable as these devices are compatible with complementary metal–oxide–semiconductor (CMOS) technology that is widely used in today’s electronic devices.

Xiaoguang Tu and co-workers at the A*STAR Institute of Microelectronics have now demonstrated how to improve silicon modulators by using an appropriate way of doping the silicon with electrons and holes\(^1\).

Doping can provide active modulators with extra electrons and holes. The process normally involves implanting acceptor and donor impurities in the main component of the modulator — the silicon waveguide. Unfortunately, the extra carriers produce a reduction in efficiency due to their light absorption. The loss efficiency of silicon modulators is typically 20% worse than that of lithium niobate modulators. There are several possible routes to minimize the loss efficiency, but they all tend to degrade the devices in one way or another.

Tu and his team overcame the problem using an approach called compensation doping (see image). In this approach, the central area of the silicon waveguide is highly doped as usual, so that the electrons and holes remain on opposite sides of the central plane. Moving away from the center, however, the doping is reduced so that the total number of carriers and the light they absorb is compensated.

The researchers monitored several characteristics of the devices while varying the profile of the non-compensated region on the cross-section of the modulator. They found that in the best-case scenario, the loss efficiency of silicon modulators was comparable to that of lithium niobate modulators without affecting the modulation efficiency or the shifting speed.

“With these improvements, silicon modulators may become a main competitor of lithium niobate modulators currently on the market,” says Tu. “These modulators may also be the perfect candidate for future integrated photonics and electronics circuits.” Tu and his team are now working towards improving the performance of silicon modulators further by exploring new structure designs and doping profiles.

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Biophysics

Order in chaos

A combination of random protein movements and the elasticity inside muscles helps to maintain a steady force during skeletal muscle contraction

The process of skeletal muscle contraction is based around protein filaments sliding inside sarcomeres — the structural units of muscle fiber. Inside each sarcomere is a set of filament motors, which appear in different densities in different areas. Scientists previously thought that the motor force would change according to the filament load in the muscle, but recent studies have shown that the motor force actually maintains a constant level during the muscle contraction. Despite such breakthroughs, however, it remains unclear exactly how this constant force is maintained in an otherwise chaotic system.

Bin Chen of the A*STAR Institute of High Performance Computing and Huajian Gao at Brown University, US, have now built a model to illustrate the process of skeletal muscle contraction and show how a constant force can be sustained by the protein motors.

The two key proteins in muscle contraction are actin and myosin. Myosin drives the system, forming a thick filament made up of numerous motors which ‘grab’ onto, bind to and slide past the thinner actin filaments during contraction. This ‘grabbing’ and sliding motion has been shown to be fairly chaotic in nature, with attachment and release happening at random.

When the weight of an object exerts a load on the filaments — for example, when you try to lift something up — the muscles must contract, requiring the protein motors to generate a force opposite to the load.

Chen and Gao have created a new skeletal muscle fiber model to demonstrate how contraction forces work.

“Our model is designed for the sarcomere,” Chen explains. “We consider the thin filament as an elastic rod under a filament force, which is driven by multiple stochastic myosin motors that convert the chemical energy of adenosine-5’-triphosphate (ATP) hydrolysis into stored elastic energy and then function like swinging arms.”

The results show that the unique way in which the myosin motors randomly attach and release from actin, coupled with the elastic properties of the motors, generate a consistent force across the whole sarcomere. When there is a higher filament load, more myosin motors are attached to the actin, but the overall motor force remains constant. “This regulation mechanism may exist in various biological processes and dramatically induces order within a chaotic system,” explains Chen. “Our modeling framework can also be further adapted to study the behaviors of other actomyosin complex structures, which is part of our plan for future work in this area.”

Hard drives

A bit of progress

Information in most computer memories is stored in the form of ‘bits’ represented by the polarization of tiny magnets on the surface of memory devices such as the computer’s hard drive. The capacities of these devices have increased exponentially over the last 30 years, a feat made possible by progressively reducing the area taken up by the magnets storing the information. In modern machines, these magnets are so small that reducing their size any further risks creating unstable data, due to random flipping of the direction of polarization of the magnets at higher densities. Now, Mojtaba Ranjbar and colleagues at the A*STAR Data Storage Institute have honed a key technology, called bit-patterned media, to overcome this problem and allow data to be stored at previously unattainable densities.

Bit-patterned media technology replaces the continuous magnetic film traditionally used in hard drives with an array of small, patterned magnetic dots (see image), each of which stores a bit of data. By carefully designing the size and shape of these dots, data can be stored at very high densities without the instability that would be encountered if a continuous film were used.

Using bit-patterned media, however, is not without its own difficulties, chief among which is a problem known as ‘switching field distribution’, whereby the magnetic field required to write or erase data in each dot differs slightly and by an unknown amount. As a result, the magnetic field applied by a hard drive write head may be too small, or too large, resulting in data errors.

Previous work by other researchers sought to minimize the switching field distribution problem by covering all of the magnetic dots with a continuous magnetic film placed on top of the dots, which alters the magnetic interactions between individual dots. The approach called ‘capped bit-patterned media’ traditionally requires different magnetic materials for the dots and film, introducing additional fabrication complexity.

Ranjbar and co-workers used the same material for the film and dots, and positioned the dots above the film rather than below it. This approach allowed a particularly simple fabrication process, in which dots were etched in a controlled fashion, leaving a continuous, unetched film underneath and obviating the need for a separate deposition step to introduce a new magnetic material. The researchers found that this simplified process successfully reduced switching field distribution, and also lowered the field strengths necessary for writing data.

Ranjbar comments, “Combined with the ease of fabrication, this technology should prove useful in bit-patterned media for next-generation hard disk drives.”

A modified approach to fabrication of magnetic memory elements may lead to a new generation of stable, ultra-high-capacity hard drives

Developmental biology

Fishy fingers

Mutant zebrafish provide a model for an emerging class of human diseases

A team of researchers led by Phillip Ingham of the A*STAR Institute of Molecular and Cell Biology has created mutant zebrafish that highlight the unusual mechanism underlying how cells respond to a key molecular signal controlling vertebrate development.

The talpid3 gene was originally discovered through a mutation in chickens that causes a characteristic limb deformity, called polydactyly, as well as craniofacial defects. These abnormalities arise through disruption of the primary cilium, a finger-like structure that protrudes from the cell surface and plays a crucial role in the cellular response to the Hedgehog signal.

Animals with mutations in the Hedgehog gene are also characterized by severe malformations of the limbs, brain and face. Related defects are found amongst the broad range of abnormalities associated with ciliopathies, an emerging class of human genetic disease that share dysfunction of the primary cilium as their underlying cause.

To analyze talpid3 function in a system more amenable to genetic manipulation, Ingham and his colleagues identified the zebrafish version of the gene and fused it to the gene encoding green fluorescent protein. This allowed them to visualize the Talpid3 protein in live zebrafish embryos, revealing that it is localized to a structure called the centriole, which is critical for development and positioning of the primary cilium.

They then mutated the gene using a DNA cutting enzyme called an endonuclease that they genetically engineered to target specific DNA sequences found only in the talpid3 gene. Surprisingly, the resulting mutant fish developed more or less normally, apart from abnormal kidneys, but died about one month after hatching.

Ingham reasoned that the talpid3 gene product supplied to the egg by the mother might suffice to support normal embryonic development. To test this, the team transplanted mutant egg progenitors into normal ‘surrogate’ females — as predicted, embryos derived from the mutant eggs failed to make primary cilia, disrupting the cellular response to Hedgehog and causing the same abnormalities associated with the chicken mutant.

These findings underline the crucial role of the primary cilium in Hedgehog signaling in all vertebrates. They provide a new model for investigating the interaction between Talpid3 and other proteins implicated in ciliopathies, as well as the role of the primary cilium in the cellular response to Hedgehog.

“It is becoming clear that components of the Hedgehog signaling pathway interact at the primary cilium, but exactly why is unclear,” says Ingham. “We are now generating mutations in other genes that affect primary cilia and in genes encoding Hedgehog pathway components. This will give insights into human ciliopathies.”

Robotics

Gesturing for control

New intelligent algorithms could help robots to quickly recognize and respond to human gestures

Many works of science fiction have imagined robots that could interact directly with people to provide entertainment, services or even health care. Robotics is now at a stage where some of these ideas can be realized, but it remains difficult to make robots easy to operate.

One option is to train robots to recognize and respond to human gestures. In practice, however, this is difficult because a simple gesture such as waving a hand may appear very different between different people. Designers must develop intelligent computer algorithms that can be ‘trained’ to identify general patterns of motion and relate them correctly to individual commands.

Now, Rui Yan and co-workers at the A*STAR Institute for Infocomm Research in Singapore have adapted a cognitive memory model called a localist attractor network (LAN) to develop a new system that recognize gestures quickly and accurately, and requires very little training1.

“Since many social robots will be operated by non-expert users, it is essential for them to be equipped with natural interfaces for interaction with humans,” says Yan. “Gestures are an obvious, natural means of human communication. Our LAN gesture recognition system only requires a small amount of training data, and avoids tedious training processes.”

Yan and co-workers tested their software by integrating it with ShapeTape, a special jacket that uses fiber optics and inertial sensors to monitor the bending and twisting of hands and arms. They programmed the ShapeTape to provide data 80 times per second on the three-dimensional orientation of shoulders, elbows and wrists, and applied velocity thresholds to detect when gestures were starting.

In tests, five different users wore the ShapeTape jacket and used it to control a virtual robot through simple arm motions that represented commands such as forward, backwards, faster or slower.

The researchers found that 99.15% of gestures were correctly translated by their system. It is also easy to add new commands, by demonstrating a new control gesture just a few times.

The next step in improving the gesture recognition system is to allow humans to control robots without the need to wear any special devices. Yan and co-workers are tackling this problem by replacing the ShapeTape jacket with motion-sensitive cameras.

“Currently we are building a new gesture recognition system by incorporating our method with a Microsoft Kinect camera,” says Yan. “We will implement the proposed system on an autonomous robot to test its usability in the context of a realistic service task, such as cleaning!”

Photonics

Beam me up

‘Tractor beams’ of light that pull objects towards them are no longer science fiction

Tractor beams are a well-known concept in science fiction. These rays of light are often shown pulling objects towards an observer, seemingly violating the laws of physics, and of course, such beams have yet to be realized in the real world. Haifeng Wang at the A*STAR Data Storage Institute and co-workers have now demonstrated how a tractor beam can in fact be realized on a small scale.

“Our work demonstrates a tractor beam based only on a single laser to pull or push an object of interest toward the light source,” says Wang.

Based on pioneering work by Albert Einstein and Max Planck more than a hundred years ago, it is known that light carries momentum that pushes objects away. In addition, the intensity that varies across a laser beam can be used to push objects sideways, and for example can be used to move cells in biotechnology applications. Pulling an object towards an observer, however, has so far proven to be elusive.

In 2011, researchers theoretically demonstrated a mechanism where light movement can be controlled using two opposing light beams — though technically, this differs from the idea behind a tractor beam.

Wang and co-workers have now studied the properties of lasers with a particular type of distribution of light intensity across the beam, or so-called Bessel beams. Usually, if a laser beam hits a small particle in its path, the light is scattered backwards, which in turn pushes the particle forward.

What Wang and co-workers have now shown theoretically for Bessel beams is that for particles that are sufficiently small, the light scatters off the particle in a forward direction, meaning that the particle itself is pulled backwards towards the observer. In other words, the behavior of the particle is the direct opposite of the usual scenario. The size of the tractor beam force depends on parameters such as the electrical and magnetic properties of the particles.

Although the forces are not very large, such tractor beams do have real applications, says Wang. “These beams are not very likely to pull a human or a car, as this would require a huge laser intensity that may damage the object,” says Wang. “However, they could manipulate biological cells because the force needed for these doesn’t have to be large.”

Such applications are the driving force for future experimental demonstrations of such pulling effects. The technology could, for example, be used to gauge the tensile strength of cells, which would be useful to investigate whether cells have been infected. “For instance, the malaria-infected blood cell is more rigid, and this technology would be an easy-to-use tool to measure this,” adds Wang.

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Microfluidics

Creating chaos

A new microfluidic device can operate as a mixer or a valve, improving the efficiency of micro-scale laboratory apparatus

A quiet revolution is taking place in the fields of biology and chemistry. Microfluidic devices, which allow fluid manipulation in micro-scale channels, are slowly but surely finding their place on the lab bench. These tools are increasingly taking the place of the usual macro scale glassware and offer a number of benefits including faster processing, less reagents, less waste and greater reaction control. However, at these small scales, fluids tend to flow in parallel layers which do not interact — a phenomenon known as laminar flow — meaning that mixing of reagents becomes difficult. However, recent work by Huanming Xia and colleagues from the Singapore Institute of Manufacturing Technology based at A*STAR introduced a new microfluidic device which changes laminar fluid flow into an oscillating flow, which substantially enhances the efficiency of mixing.

The A*STAR team used the natural elasticity of a thin, flat silicone membrane freely supported on a circular stepped cavity separating two chambers through which liquid flows perpendicular to the membrane. When fluid is pumped through the chamber, the membrane deflects, becoming convex downstream of the flow, although the flow remained laminar and stable. Further deflection of the membrane occurs until the elasticity and lift forces of the silicone makes the membrane bounce back and the process then repeats, leading the generation of an oscillating fluid flow. The device can also work as a valve; at higher pressures, the membrane completely blocks the forward flow whilst reverse flow forces the membrane to the ceiling of the upper chamber, completely blocking fluid transfer.

The researchers also demonstrated the mixing behavior of their device in a Y-shaped fluidic element in which a membrane oscillator was incorporated into one channel. When fluid was pumped into the other chamber, the presence of the membrane prevented mixing of the two liquid streams. Subsequent introduction of a second liquid at low pressure allowed the fluid streams to meet at the intersection point of the Y-shaped channel, although at this point flow was laminar and no mixing occurred. However, increasing the pressure from the oscillator-containing channel led to the generation of oscillatory behavior with the result that the two fluid flows mixed chaotically.

The researchers are also working on an improved oscillator design employing a thin metal spring foil in place of the silicon rubber diaphragm. Such measures are intended to improve still further the mixing performance of the system and lead to more durable membrane mixing systems.

A novel live-cell imaging technique offers new opportunities to understanding immune responses in the skin

Biologists often use a technique called multi-photon imaging to examine live cells. The technique is unique in that it uses multiple photons of high wavelengths to stimulate fluorescent labels, causing them to emit light. It is superior to more conventional fluorescence imaging techniques, such as confocal microscopy, as it has a higher spatial resolution and enables greater depth of penetration into tissues.

Lai Guan Ng at the A*STAR Singapore Immunology Network and an international team of co-workers have extended the capability of multi-photon imaging further so that it can now be used to directly visualize immune responses in skin.

The skin is known to have two layers: the dermis and the epidermis. The epidermis is predominantly avascular, containing specialized skin cells called keratinocytes, whereas the underlying dermis contains highly vascularized lymphatic vessels.

Conventional fluorescence microscopy only allows imaging into the epidermis and limited structures of the dermis below. The multi-photon imaging technique developed by Ng and his team offers simultaneous imaging of multiple cellular and structural components through the epidermis and into the dermis (see image).

The researchers provide a step-by-step guide to preparing a live mouse ear skin model which can be used to probe skin response to localized injury or disease over several hours. They placed a glass slide over the ear, which acts as a window through which to observe the tissue without surgery. The mouse ear is a good site for imaging because it requires minimal pre-treatment and is easily accessible.

The researchers could take images more quickly — for example, on a per-second basis — by adjusting various experimental parameters, thereby enabling them to study fast-moving cells such as rolling leukocytes.

The researchers recommend albino mice to prevent any artifacts arising from photodamage to the skin. They provide a comprehensive checklist for preparation of the ear for imaging to ensure that the integrity of the blood vessels is not being compromised. They also describe a protocol to induce local laser injury, the biological response of which can then be studied. The protocol has a troubleshooting section that can be used to resolve problems that others may encounter when they come to repeat the experiment.

So far, the researchers have used their approach to study a variety of skin conditions, for example, parasitic infection, T-cell lymphoma migration and neutrophil response to sterile injury amongst others.

“We envision that this approach will not only continue to unravel new knowledge relating to the skin immune system, but also gradually become a standard approach for assessing drug delivery as well as percutaneous and intradermal vaccine applications in preclinical studies,” says Ng.

An innovative computer program brings color to grayscale images

Creating a high-quality realistic color image from a grayscale picture can be challenging. Conventional methods typically require the user’s input, either by using a scribbling tool to color the image manually or by using a color transfer. Both options can result in poor colorization quality limited by the user’s degree of skill or the range of reference images available.

Alex Yong-Sang Chia at the A*STAR’s Institute for Infocomm Research and co-workers have now developed a computer program that utilizes the vast amount of imagery available on the internet to find suitable color matches for grayscale images. The program searches hundreds of thousands of online color images, cross-referencing their key features and objects in the foreground with those of grayscale pictures.

“We have developed a method that takes advantage of the plentiful supply of internet data to colorize gray photos,” Chia explains. “The user segments the image into separate major foreground objects and adds semantic labels naming these objects in the gray photo. Our program then scans the internet using these inputs for suitable object color matches.”

Given the vast amount of visual data available online, not all of the chosen images are useful. Once the initial color images have been found, the program then filters them to find the most realistic and suitable matches for the grayscale object inputs.

“Our method automatically detects and segments salient objects from an internet photo,” explains Chia. “It then exploits shape and appearance information of these objects to compute its relevance to the original grayscale image data.”

The grayscale image is then automatically colored using the information collected from internet-based images (pictured). Plausible colorization of images is vitally important, however, as the human eye can quickly distinguish between real and ‘false’ coloring. To this end, the user has the final say over the choice of colors. “The program generates several image colorizations and the user can pick the one that fits best from a graphical user interface,” explains Chia.

To demonstrate the capability of the program, Chia and his team showed a group of people their colored grayscale images alongside real color pictures, asking them to identify which ones had been colored artificially. “Our colored images were classed as ‘real’ up to 65% of the time,” says Chia. “Overall the colorization results are visually pleasing and perceptually meaningful to users.”

The researchers hope to expand the range of applications using this technology in the future. They envision that the technology may one day become so powerful that it could be used to generate realistic animations.

The preclinical animal study of drugs can be a costly and lengthy process. However, owing to some basic similarities with humans and their short development time, the zebrafish has emerged as a useful model for drug screening and disease profiling. For these experiments, zebrafish embryos are usually contained in the wells of a ‘multi-well plate’ — however, controlling the medium in which they are submerged and the addition of other chemicals, as well as imaging of the tissues and organs inside the zebrafish, are not straightforward using this setup.

Hanry Yu at the A*STAR Institute of Bioengineering and Nanotechnology and co-workers have devised a new and efficient microfluidic device for the growth, live imaging and monitoring of tissues and organs of zebrafish. The researchers show how the multichannel platform, which they call ‘fish and chips’, can detect abnormalities in the tail morphology and eye of the zebrafish, in the presence of valproic acid — a drug known to cause birth defects if taken by the mother during pregnancy.

The fish-and-chips platform created by Yu and co-workers has three sections (see image): eight fish tanks that can each hold one zebrafish; a gradient generator that controls the administration of drugs and chemicals to the tanks; and eight outlet channels for the removal of waste products. Zebrafish have been monitored in microfluidic setups in the past, but the new platform allows the diagonal flow of solutions. As a result, the embryos remain within a consistent flow of growth medium and drugs. Yu and co-workers are able to monitor developmental changes under the influence of different concentrations of drug molecules because of this gradient method.

Another advantage is the 1.4-millimeter diameter of the individual tanks — a size that sufficiently restricts the movement of the zebrafish to allow fluorescence imaging of the fish without the need for complex manipulation of the zebrafish with needles and anaesthesia.

Using imaging methods, Yu and co-workers are able to see various tissues and organs of the zebrafish including the brain, eye, ear, olfactory bulbs, melanophores, notochord, epidermis, trunk and the distinct chambers of the heart. These detailed imaging possibilities, together with the ability to monitor long-term development of the zebrafish embryo from eight to 92 hours post-fertilization, make the fish-and-chips platform an attractive tool in drug discovery.

“Toxicity is a major cause of drug failures in clinical trials and our novel fish-and-chips device can be used as the first step in drug screening during the preclinical phase to complement existing animal models and improve toxicity testing. Our next step will involve investigating cardiotoxicity and hepatotoxicity on the chip,” says Yu.

Hydrogen gas (H\textsubscript{2}) is an ideal energy carrier for fuel cells, but finding sustainable ways to produce large quantities of hydrogen continues to be a technological challenge. Jia Zhang at the A*STAR Institute of High Performance Computing and co-workers\textsuperscript{1} have now used sophisticated calculations to uncover a critical chemical mechanism that may make catalytic transformation of safe, renewable liquid ethanol into hydrogen fuel easier than ever before.

Currently, steam reforming is the popular method for producing hydrogen gas from ethanol. In this technique, ethanol is injected into a hot, steam-filled chamber containing a catalyst such as rhodium. The catalyst promotes the dissociation of ethanol molecules into smaller molecules, such as carbon monoxide and H\textsubscript{2}. Although chemists have had good success in using steam reforming to ‘crack’ ethanol, they have had difficulties in improving the efficiency of the catalyst because of the many diverse and complex chemical reactions at play in the system.

According to Zhang, catalysts need to selectively crack the carbon–carbon bonds of surface-adsorbed ethanol to be viable for steam reforming. Recent experimental efforts have shown that ‘stepped’ catalyst surfaces — tiny staircase–like defects present in a normally flat rhodium surface — are unusually active at both carbon-hydrogen and carbon–carbon bond cleaving. One problem, however, is that the actual mechanism of ethanol decomposition on stepped surfaces is still unclear.

The research team overcame this challenge by using high-powered computer simulations to work out which ethanol decomposition pathways are most probable on a particular stepped rhodium surface known as rhodium \textsuperscript{(211)}. Exhaustive calculations using density functional theory (DFT) methods revealed that there were two ways of breaking ethanol down into H\textsubscript{2}, and both shared a common intermediate species with the chemical formula CH\textsubscript{3}COH.

Crucially, the team found that this CH\textsubscript{3}COH intermediate exists only on stepped rhodium surfaces. While flat catalyst surfaces fracture ethanol through an oxametallacycle intermediate, the step–type defects preferentially absorb the alcohol and then activate the decomposition cycle by sequentially removing hydrogen atoms from the intermediate. The researchers note that the surface-sensitivity of ethanol steam reforming is an important finding because step-defects are extremely common on state-of-the-art nanoscale rhodium catalysts.

“Steam reforming is a very complicated chemical process, and our current DFT study on ethanol decomposition mechanism is just the tip of the iceberg — many factors such as temperature, concentration, substrate influence, and water effects can influence the results,” says Zhang. “However, this work is an important first step for establishing theoretical rules to guide development of new, high-performance catalyst materials.”

The detection of small quantities of molecules is important for a myriad of applications, ranging from gas sensing to biomedical diagnostics. The majority of these applications require the sensors to be cheap and disposable, yet sensitive enough to detect molecules down to the single-molecule level. Ping Bai and co-workers at the A*STAR Institute of High Performance Computing and the Institute of Materials Research and Engineering have now studied the properties of thin metallic films with holes in them that are particularly promising for molecular sensing.

Metallic thin films with nanometer-sized holes in them are known to transmit light of particular wavelengths very efficiently. The efficiency arises from surface plasmon polaritons (SPPs) — the collective movements of electrons on the metal surface — which are able to focus light into tiny spots much smaller than the wavelength of light used (see image).

These SPPs can be used to detect the molecules through the fluorescence of tracer molecules attached to them. This fluorescence is also strongly enhanced by the SPP and can easily be detected by a microscope even for small quantities of molecules. “The whole setup is ultra-compact to support a point-of-care sensing system,” explains Bai.

Bai and his colleagues studied two sensing arrangements. In the first arrangement, light is directed at a film with nanoholes at an oblique angle from the same side as the sample. In the second arrangement, the film is illuminated from the back so that light is travelling through the holes first. The researchers found that each scheme has its own advantages.

In the ‘reflection’ scheme, the SPP effect is stronger as the light is directly aimed at the sample and does not have to cross the metal film. However, a thicker film is needed so that the light does not pass through. In the ‘transmission’ scheme, the intensity of the light emitted by the molecules is weaker, but the advantage there is that filters and other sensors can possibly be included with the metal film, and the film thickness can be much thinner.

“There is therefore no clear advantage for either sensing modes of such films,” says Bai. “One thing that is clear from the study, however, is the clear benefits of using metal films with nanoholes as a molecular sensing platform,” says Bai.

“This is merely a snapshot of our whole project. Ultimately, our sensing technology will be utilized in hospitals and test centers, for example, in prostate cancer screening, or even used at home just like glucose test kits,” adds Bai.

Data storage

Memory that does it all

Using the correct annealing temperature is key to making fast, non-volatile computer memory

Computers often do not run as fast as they should because they are constantly transferring information between two kinds of memory: a fast, volatile memory connected to the CPU, and a slow, non-volatile memory that remembers data even when switched off. A universal memory that is fast, power-efficient and non-volatile would allow new designs that avoid this bottleneck. Hao Meng and co-workers at the A*STAR Data Storage Institute have now shed new light on how to manufacture such a memory.

The researchers explored a special class of universal memory called spin-transfer torque magnetic random access memory (MRAM). A spin-transfer torque MRAM typically comprises two magnetic films that are separated by an insulating layer. The resistance between the two films is low if the magnetization direction in each film is parallel, and high if it is anti-parallel. Information is stored in the relative magnetization between the two films, and read out by measuring resistance. The magnetization directions can be switched by applying spin torque to the films’ magnetic domains (using a spin polarized electric current).

High-temperature annealing is a key step in the manufacture of an MRAM cell. Annealing alters the crystal structure of the cell materials, which in turn changes the degree of magnetization and how the cell functions. In particular, the greater change in resistance between parallel and anti-parallel magnetizations, the better the memory will function. Previous studies have shown that this resistance change increases as the annealing temperature increases, but drops if the annealing temperature rises too much.

Meng and co-workers extended this analysis to other critical MRAM characteristics. They focused on a cell made with CoFeB magnetic films, which has a natural magnetization direction outside of the plane of the film. They found that the annealing temperature that yielded maximum resistance variation exceeded the temperature necessary for maximum thermal stability. This is critical information for design engineers, who must balance these two metrics against each other.

Meng and co-workers also found that the minimum current density necessary to change the film magnetization increased with annealing temperature. A lower current is desirable for practical cell operation. The current density could be lowered by reducing the thickness of the magnetic films. However, lower thicknesses also produced an undesirable reduction in resistance variation. By explicitly demonstrating the trade-offs necessary in the design of spin torque MRAMs, the data is expected to help engineers design the next generation of these promising devices.

Optomechanics

Swift light switching at the microscale

Faster signal storage and optical processing in nanomachined devices edge closer to realization

A system that has only two possible stable states, such as a light switch, is called bistable by scientists and engineers. Bistability in microscale devices could pave the way to compact optical switching and memory elements. In the bistable systems found so far, however, switching between states takes too long to make the approach practical. Now, thanks to the recent observation of bistability in an array of micrometer-sized rings, fast microscale optical switches in novel photonic devices are a step closer to development.

Yefeng Yu of the A*STAR Data Storage Institute and his co-workers in Singapore and France observed this bistability in a device consisting of two 60-micrometer-wide silicon rings into which they could feed laser light of wavelengths specific to the particular ring geometry they used. One segment of each ring hung above a gap, and these free-hanging arcs deformed slightly as light flowed through the ring. The deformation of the rings, in turn, changed their optical properties. As a result of this interplay between optical and mechanical forces, the researchers observed stable behavior at two wavelengths of the light; not at one, as expected. By changing the wavelength of the incoming light, Yu and co-workers could conveniently switch between these two states.

“To our knowledge, this is the first time that optical bistability has been induced by optical forces acting on mechanical motion,” explains Yu. “Similar phenomena are usually produced by thermal effects.” Relying on heating mechanisms, however, means that the typical times required to switch between the two stable states are relatively long, on the order of milliseconds. Using optical effects gave Yu and his co-workers a much faster means to control the switching process. “The switching time in our system is currently at the microsecond level,” says Yu. “But there is some space for reducing this time through design optimization.”

This thousand-fold acceleration should assist practical applications. The two stable states of the system, for example, can be used to encode information in terms of ‘zeros’ and ‘ones’, as it is in digital computers. But instead of using electrons to process information, the two states of Yu and his co-workers’ optomechanical device should allow the representation of information.

“We envisage using our new system to implement optical logic gates for data processing,” Yu says. But there may be many more possible uses for these devices. “Applications we want to explore include tunable lasers, biosensor and optomechanical memories.”

Nanomaterials

Surrounding effects

Metals nanoparticles could play a key role in next-generation light detectors, optical circuits, and cancer therapies. For these future technologies to be realized, it is important to understand what happens when nanoparticles are caused to undergo vibrations, and the consequent scattering of light that can occur due to oscillations, or surface plasmons, in their free electron cloud. However, little is known about exactly how these vibrations are affected by the nanoparticle’s immediate surroundings — in particular, how the environment affects the dissipation of energy from a nanoparticle when it vibrates.

Sudhiranjan Tripathy at the A*STAR Institute of Materials Research and Engineering and co-workers, collaborating with Arnaud Arbouet and colleagues from the National Center of Scientific Research (CNRS) in France, have now analyzed the effect of different environments on individual gold nanoparticles, their acoustic vibrations and associated energy dissipation.

The researchers examined individual nanorings made of gold using transient absorption spectroscopy, which involves exciting the sample with a pulse of laser light before measuring the absorbance of light at various wavelengths. They measured both the vibration period and damping time — the rate at which the nanoring loses its energy to its surroundings.

“When a metallic system is downsized to nanometric dimensions, its vibration modes can become very different in comparison to its bulk form,” explains Tripathy. “For example, the damping of the acoustic vibrations is strongly affected by the elastic properties of the environment and the interface between the nanoparticle and its environment.”

Previous spectroscopy studies have experimented with large groups of nanoparticles, but the collective approach has its limits because nanoparticles of different sizes may have different vibration periods. The researchers overcame the problem by working with individual nanorings, but the workaround did have its own difficulties.

The first challenge was the nanofabrication of perfectly controlled and characterized nano-objects. Secondly, there was the issue of detecting and monitoring the acoustic vibrations of one single metal nano-object. This meant that the researchers had to measure relative changes on the order of one in ten million. The researchers studied individual nanorings that were surrounded by either air or glycerol, and focused on how the different environments affected the damping time of the vibrations. This provided valuable insight into how energy dissipated from the nanorings to their environment. Most tellingly, the damping times were significantly shorter in the highly viscose glycerol.

“Our work opens up exciting perspectives including the use of metal nanoparticles as mass sensors, or as nanosized probes of the elastic properties of their local environments,” says Tripathy.

Nanoengineering

Targeting cancer stem cells

Patented droplet microarray technology allows medical researchers to come to grips with rare cells

A miniaturized microarray technology patented by the A*STAR Institute of Bioengineering and Nanotechnology (IBN) can be used to measure the resistance of cancer stem cells (CSCs) to chemotherapeutic drugs. Jackie Y. Ying and IBN co-workers, who invented the DropArray™ technology (pictured), say recent studies show it can be used for developing more effective cancer drug screening, as well as saving time, cost and the amount of material needed for analysis.

Cancer stem cells are a sub-group of tumor cells that are particularly resistant to chemotherapy and are drivers of metastasis, such as the spread of cancer via the blood stream. But they are so scarce that it is difficult to study their drug responses with standard laboratory methods.

Using DropArray™, however, the IBN researchers were able to investigate drug resistance in CSCs with high-content screening methods — whereby fluorescent tags are attached to compounds which identify cells of interest, then sensed automatically using microscopes. These methods, which typically employ 96-well or 384-well plates, demand at least 5,000 or 2,500 fluorescing cells respectively per well for detection. This is more than the number of CSCs that would typically be present.

Instead of wells in conventional microplates, DropArray™ uses plates coated with water-repellent material except for an array of two-millimeter-diameter spots that hold samples in the form of droplets.

The key to the DropArray™ technology is a layer of proprietary oil with which the plate is covered to prevent evaporation and cross contamination between the droplets. This enables the entire plate to be rinsed easily and precisely in a specially built automated apparatus. Using DropArray™ only 500 fluorescing cells are needed for detection.

The researchers studied the drug response of CSCs from liver, breast and colon tumors and, using DropArray™, compared them to typical tumor cells with respect to the impact of a range of doses of drugs used in the treatment of these cancers. In the liver tumor cells, for instance, they looked at sensitivity to doxorubicin which stimulates apoptosis or cell suicide. By testing for a protein synthesized during apoptosis, they found normal tumor cells were far more sensitive to the drug than CSCs. The results for the other tumors were similar. The researchers also showed in mice that the material containing CSCs was much more effective at generating new tumors.

“We are now able to apply DropArray™ to develop novel drug screening assays using rare CSCs and facilitate cancer therapy research,” says Ying.

Nanoparticle synthesis

Joined at the hip

Hybrid ‘Janus’ nanoparticles made from gold and titania have high catalytic activity and extraordinary durability

As recently as twenty-five years ago, chemists considered gold to be one of the most inert metallic elements, until the discovery that nanoscale-sized dispersions of gold had high catalytic activity forced a re-think of old principles. Researchers soon found that gold nanoparticles could promote many industrially important reactions, such as the removal of harmful carbon monoxide gas from emission streams. Whilst the benefits of nanoscale gold are well-attested, preparing the material in a durable and reusable form remains a significant challenge that limits its uptake by manufacturers.

Work by the teams of Ming-Yong Han of the Institute of Materials Research and Engineering and Yong-Wei Zhang from the Institute of High Performance Computing both at A*STAR has revealed that the stability of gold nanoparticle catalysts can be enhanced by coating them with protective titania (TiO$_2$) layers. Conceived by co-author Zhi Wei Seh, an A*STAR National Science Scholar, this new technique produces so-called Janus nanostructures that retain nearly all the catalytic activity of bare gold nanoparticles without suffering from irreversible aggregation that diminishes the reactivity of the latter.

Named after the twin-faced Roman god of beginnings and transitions, Janus nanostructures join two or more equal-sized components together through very small junctions — an arrangement that maximizes the active surface area of each substance. The beneficial effects of pairing gold nanoparticles with titania is well known, but until the work by A*STAR researchers, a detailed understanding of the mechanism by which these two species fuse together had proved elusive.

Han and co-workers used an unconventional chelating compound called titanium diisopropoxide bis(acetylacetonate) to nucleate the growth of TiO$_2$ onto gold at extremely slow rates. By carefully controlling the addition of this reagent to rod- and spherical-shaped gold nanoparticles, the researchers observed three distinct nanostructures (see image): a Janus geometry; a partially encapsulating ‘eccentric’ geometry; and a ‘concentric’ core-shell arrangement.

Catalytic experiments revealed that the reactivity and durability of gold-titania Janus structures have unique advantages over other nanoparticles. Due to the exposed nature of their gold surfaces, the former catalyze the reduction of the molecule 4-nitro phenol at much faster rates than eccentric and concentric nanoparticles whose gold surfaces are more confined. Furthermore, the protective TiO$_2$ coating of the hybrid catalysts allowed them to be reused repeatedly with little loss of activity. In contrast, bare gold nanoparticles agglomerated into un-reactive clumps after just five usage cycles.

Further theoretical investigations by the team revealed that the formation of Janus nanostructures as the energetically stable species is promoted by the addition of smaller volumes of the titania precursor — a finding that may help the researchers generate other metal–oxide hybrids for catalytic applications in the near future.

Nanomaterials

Making a bluer light

The light that a luminescent particle emits is usually less energetic than the light that it absorbs. Some applications require the emitted light to be more energetic, but this so-called upconversion process has been observed in only a small handful of materials. Xiaogang Liu at the A*STAR Institute of Materials Research and Engineering and co-workers have now succeeded in expanding the list of upconversion materials, easing the path to new applications.

Traditional upconversion particles are distinguished by their evenly-spaced or ‘ladder-like’ energy levels which their internal electrons can take on. The even spacings allow an electron to be promoted up in energy many times consecutively, by absorbing many photons of the same color. When an electron that has been promoted to a high energy finally relaxes back to the lowest-energy state, it emits a photon which is more energetic than the photons that excited it to begin with.

Nanoparticles doped with elements from the lanthanide group of the periodic table are capable of upconversion, and are useful for biological imaging because their high-energy emission can be clearly distinguished from background noise. However, only three elements from the lanthanide series are efficient at upconversion: erbium, thulium, and holmium. This list is so short because of the simultaneous requirements that an upconversion particle exhibit a ladder-like electronic energy structure and also efficient emission.

Liu and colleagues solved this problem by using different lanthanides to perform different stages of the upconversion process. Sensitizer elements absorb incident light and transfer the absorbed energy to nearby accumulators, whose electrons rise to high energy levels. Then, the energy stored in accumulators transfers by hopping through many migrators, until an activator is reached. Finally, the activator releases a high-energy photon.

By assigning different elements to each of these four functions, the researchers were able to ease the requirements on any individual element.

In addition, unwanted interactions among different elements were avoided by separating them spatially inside a single spherical nanoparticle that has sensitizers and accumulators in the core, activators in the shell and migrators in both the core and the shell. This design allowed Liu and his team to observe a spectrum of colors from the upconverted emission of europium, terbium, dysprosium and samarium. The same approach may also allow other elements to emit efficiently.

“Our results may lead to advances in ultrasensitive biodetection,” says Liu, “and should inspire more researchers to work in this field.”

Optical materials

Holey gold

Imaging nanoporous metals with beams of electrons provides deep insights into the unusual optical properties of these materials

Gold is usually thought of as being a shiny metal — however, in its porous form, gold actually appears dull and black. The surfaces of nanoporous gold are rough and the metal loses its shine. Michel Bosman at the A*STAR Institute of Materials Research and Engineering and co-workers have now experimentally demonstrated that the dullness is a consequence of the way incoming light couples to the electrons on the gold surface.

A beam of light hitting metal can cause all of the electrons at the surface to oscillate in unison. If the light is within an appropriate narrow band of wavelengths, it gets absorbed by the surface and creates half-matter hybrid particles known as surface plasmon polaritons (SPPs). Bosman and his team showed that the narrow-band absorption of many SPPs across a surface can combine to give the broadband high-absorption characteristics of nanoporous materials. “Our measurements show that these materials are not black at all when looked at up close; they are actually very colorful,” explains Bosman. “They only appear black to us because we look at them from far away, where over a large area all the different colors have been absorbed.”

These effects caused by the SPPs occur at the sub-micron level. For this reason, conventional optical imaging methods do not offer the resolution necessary to view SPPs directly. In response, the team used imaging techniques based on electron beams. By firing electrons at the surface and measuring the energy that they lose during their interaction with the material, Bosman and his team were able to calculate the energy required to create a SPP, and from this they could infer the wavelength of light that it would absorb.

The researchers scanned their electron beam across both gold and silver films, which enabled them to generate a two-dimensional map showing both the wavelength of light absorbed at a particular point as well as the local surface geometry (see image). The varying shape and size of the nanopores gave rise to SPPs that absorb light at a wide range of wavelengths.

The concept could lead to improved power conversion efficiency in photovoltaic devices. “These results show that it is possible to design the color of a gold or silver film,” says Bosman. “It will, for example, be possible to more efficiently absorb the energy of sunlight, by tuning the light absorption of the gold or silver to that of the solar spectrum.”

Certain metallic nanostructures are known to exhibit a distinctly asymmetric spectral feature. This characteristic feature, known as a Fano resonance, has attracted a considerable amount of attention due to its potential in sensing applications.

Fano resonance is caused by the interference of two eigen-modes (modes of electron excitations), so its shape and wavelength are sensitive to slight variations in the environment. A small change in the refractive index, for example, could lead to a big change in the Fano resonance.

So far, most of the metallic structures used to generate Fano resonances have been made of gold. The wavelength of such Fano resonances is typically in the infrared region, which is not ideal for practical sensing applications. Jing Bo Zhang and co-workers at the A*STAR Data Storage Institute have now proposed a silver dual-disk ring nanostructure for generating Fano resonance in the visible range.

The nanostructure comprises a dual-disk ring consisting of two silver disks, measuring tens of nanometers wide, placed inside a silver ring. The researchers calculated the optical modes of the structures using the finite-difference time-domain (FDTD) method. They found that the coupling between one of the dual-disk eigenmodes and one of the ring eigenmodes produces a Fano resonance just below 700 nanometers in wavelength, well within the visible spectrum.

The shape and wavelength of the Fano resonance can be finely tuned by varying the geometric parameters that define the dual-disk ring structure. The key capability of a biomolecule sensor is its reaction to a change in the surroundings. The calculations showed that by increasing the refractive index of the environment, the Fano resonance is strongly red-shifted. This is to simulate a case in which a thin coat of a dielectric material, such as a layer of specific biomolecules, is assumed to cover the nanostructure.

The calculations were promising but had to be verified experimentally. The researchers used electron beam lithography and corresponding nanoprocessing techniques to fabricate silver dual-disk rings on quartz and indeed observed Fano resonance in the visible light range.

Observation of the Fano resonance and its sensitivity to environmental changes in the visible range is an important result for sensing applications. The researchers aim to improve the design of the nanostructure further. “We have already determined and fabricated the optimum geometry of dual-disk ring structures for biosensing,” says Zhang. “Next we are going to functionalize the surface of the structure chemically to examine and improve the sensing power experimentally.”

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Microelectronics

Two at a time

New design reduces the areal footprint of nanowire transistors by a factor of two

Semiconductor chip makers first began the production of three-dimensional (3D) transistors in 2011. Engineers can pack more 3D transistors onto a single chip because they are much more compact than traditional transistors.

For future generations of semiconductor chips, however, there is a need to shrink these 3D transistors further and the use of vertical nanowires in the transistor design is one of the promising approaches. Moreover, the area taken up by a nanowire-based transistor is typically half that of a planar transistor — or even less if considering more complicated components, like inverters. Xiang Li at the A*STAR Institute of Microelectronics and co-workers have now integrated two transistors onto a single vertical silicon nanowire, pushing the areal density limit of nanowire transistors even further.

The researchers used wrap-around gates, or ‘gate-all-around’ gates, in the making of their device. These gates consist of a vertical cylinder, at the center of which lies the nanowire. They are much better at controlling the transistor current than traditional planar gates. Li and co-workers decreased the area required for a gate-all-around nanowire transistor by a factor of two by constructing two transistors out of a single vertical nanowire. Their design involves two wrap-around gates, one above the other, separated by a thin dielectric layer to isolate them electrically (see image).

Unlike other independent double-gate transistor designs, such as those employing a vertical fin-like channel, changing the gate voltage applied to one transistor does not change the threshold (or turn-on) voltage of the other. This means that either of the gates can modulate the nanowire current independently.

As a result, Li and co-workers were able to construct a simple logic device using just one nanowire. For a nanowire doped with negative carriers, current was able to flow when both gate voltages were high, but current stopped when either gate voltage was low.

This device therefore functioned as an ‘AND’ digital gate, but used only half the area it otherwise would require. The stacked gate arrangement may also be useful for enabling an emerging type of transistor, called a tunnel field effect transistor (TFET). Because TFETs rely on the tunneling of electrons across a barrier rather than the thermal activation of electrons, they turn on very quickly and consume very little power.

Li says the tunnel junction required for a TFET could be formed between the two gates of the dual-gate nanowire geometry, allowing a particularly compact implementation. The dual-gate design could also be used for other technologies, such as non-volatile memory.

Photonics

The smaller the better

Waveguides that combine metallic and semiconductor structures can be made more compact

Increasing the areal density at which electronic components can be integrated onto a computer chip has always been key to the revolution of technological applications. However, achieving the same feat in the world of optics has been proven difficult as light waves cannot be compressed to sizes below their wavelength by conventional semiconductor-based optical waveguides.

Metallic structures, in theory, are able to provide such functionality through so-called plasmonic effects. In practice, however, the large optical losses have hampered the implementation of such schemes. Combining the benefits of conventional optics with plasmonics, Shiyang Zhu and co-workers at the A*STAR Institute of Microelectronics have now demonstrated how structures made of semiconductor and metals represent a more viable approach to effectively miniaturize optical circuits.

Plasmonic effects are based on motions of electrons at the surface of metals that act like an antenna on incoming light. They can be very effective to squeeze light into small volumes, although transport losses when guiding light along such small volumes are much higher than for conventional semiconductor waveguides.

Zhu and colleagues observed waveguides based on semiconductor silicon. First, ridges are etched out of silicon chip to form the basis for the waveguide architecture. The surface of the silicon is then oxidized to provide electrical insulation of the silicon before it is covered in a thin copper layer (see image).

This architecture has the benefit of very efficiently squeezing light into the waveguide via the surrounding copper layer, but it travels mostly along the core made of silicon and not the metal. Silicon is transparent for light at telecommunications frequencies and thus shows low losses. “These waveguide structures are not only compatible with the fabrication processes of silicon computer chips,” says Zhu. “More importantly, the use of silicon and silicon oxide and related semiconductors enables further possibilities to potentially achieve other effects, such as light amplification, and control over the plasmon properties.”

Having previously shown that such waveguides are able to guide light efficiently, the researchers have now demonstrated a number of complex photonic structures, including the splitting of light beams at multiple junctions, the propagation of light across multiple kinks and steps, resonator structures that show light interference effects and many more.

“This is only a first step towards the varied and complex effects possible with these structures,” says Zhu. “The next step is to demonstrate some of the active functionality, especially to combine waveguides with ultracompact plasmonic light modulators based on related designs for complete functional nanoplasmonic circuits.”

Data storage

Keeping things in place

*Fluid dynamics simulations aim to better predict how air circulating in a hard disk drive perturbs the vibrating read/write head*

Engineers rely on sophisticated simulation software to understand how air and other fluids flow over objects like an airplane wing or a golf ball. The software typically maps the air and object to a three-dimensional array (mesh) of small cells and iteratively calculates the forces in each cell over a series of time steps. Such simulations would be useful for predicting the effects of air flow on moving parts of a spinning hard disk drive — a critical step in the testing of new designs. Unfortunately, parts moving at high frequencies are notoriously difficult to simulate because they are shifted by a few nanometers only.

Ningyu Liu and co-workers at the A*STAR Data Storage Institute have now made an important step towards solving this problem. They developed a program that can accurately simulate the force of air acting on the actuator assembly (the arm that suspends the read/write head over the spinning magnetic disk in a hard disk drive) and determine the amplitude of the assembly’s vibration.

The program is unique in that it uses fluid dynamics to describe the interaction between the read/write arm and the air surrounding it.

In a hard disk drive (see image), the read/write head moves quickly back and forth over the magnetic disk, which rotates at up to tens of thousands of revolutions per minute. In high-density hard disk drives, however, the distance between the read/write head and the disk (the “flying height”) may only be a few nanometers — or even less for compact, high-storage disks of the future.

“Variation in the amplitude of vibration can have a critical impact on the performance of hard disk drives, even if the difference is on the sub-nanometer scale,” says Liu.

To improve fluid dynamics simulations and better predict how air, which is sealed inside the hard disk drive and driven into circulation by the rotating disks, affects the vibration of the arm suspending the read/write head, Liu and his co-workers described the space right around the arm as a continuous vibrating surface, or boundary, instead of breaking it into many cells. They tested their simulations by comparing them to measurements of arm vibrations in a home-built simplified hard disk drive.

Liu and his colleagues showed that simulations that do not include the vibrating boundary underestimate or overestimate the amplitude of the vibrating arm by as much as 40%. They are now in the process of performing similar simulations on smaller and thinner disk drives, which, according to Liu, “require a huge computer resource.”

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The understanding of oxidation and corrosion processes is essential for a wide range of applications, particularly those related to the nuclear industry. Zhi Gen Yu at the A*STAR Institute of High Performance Computing and co-workers have now performed calculations to study how nitrogen degrades zirconium — a material widely used for cladding fuel rods in nuclear reactors — and found that nitrogen atoms entering zirconia (the oxidized form of zirconium) do not simply replace oxygen atoms. Instead, the researchers showed that nitrogen atoms combine with oxygen atoms to form nitrosyl (NO) radicals, which bind the zirconium lattice. They believe that this mechanism promotes the corrosion of zirconia in nuclear reactors.

The nuclear disaster in Fukushima last year is a recent and drastic example that illustrates the importance of studying corrosion processes in zirconium, with the goal of developing methods to prevent deteriorating processes. “Following the accident in Fukushima there were reports that due to the high temperatures and the presence of steam, oxidation of the zirconium cladding — designed to protect the nuclear-fuel rods — produced hydrogen, which only exacerbated the heat problem,” explains Yu.

An important property of zirconium is that, when exposed to air, it naturally forms a thin layer of zirconia, which acts as a barrier against further oxidation and corrosion. The stability of zirconia is normally very high. At elevated temperatures (as present when a reactor core overheats), however, the stability decreases substantially and the zirconia layer loses its protective function — just when it is most needed.

Scientists have yet to grasp the mechanism underlying the corrosion of zirconium. However, they know that one of the factors that influence the corrosion process is nitrogen impurities. To better understand the role of nitrogen in corrosion when it enters zirconia, Yu and co-workers have calculated the probability of every chemical processes that may happen in zirconia as nitrogen molecules intrude. They found that among all possible basic structures associated with nitrogen, the most likely species to form is NO molecules, which then occupy the sites where single oxygen atoms originally resided.

“We expect that for every two nitrogen atoms introduced, three oxygen atoms in the lattice are removed,” says Yu. “Our results suggest that two of the removed oxygen atoms combine with nitrogen to form NO defects, whereas the remaining oxygen atoms escape, leaving behind vacancies. Such vacancies could provide paths for oxygen diffusion, which promotes the rate of corrosion.”

The finding that nitrogen can combine with oxygen in zirconia to form NO molecules may lead to safer materials for nuclear reactors.

Transistors made from graphene nanoribbons make efficient magnetic field sensors

Graphene — a single layer of carbon atoms packed in a hexagonal lattice — has a number of appealing properties owing to its two-dimensional geometry. It has, for one thing, good electrical conductivity that is of interest to high-speed electronic applications. Seng Ghee Tan at the A*STAR Data Storage Institute and co-workers at the National University of Singapore have now shown that graphene has additional applications in magnetic data storage. They have developed a method to measure magnetic fields by detecting changes in the electrical resistance of graphene. “The findings could open up new avenues in the development of miniaturized magnetic field sensors,” says Tan.

Electrons move inside graphene almost without any hindrance from the atoms of the two-dimensional carbon sheet. This good transport property is of interest to the development of magnetic field sensors because the change in charge transport in the presence of a magnetic field can lead to a measurable change in electrical resistance. Unfortunately, in previous devices thermal excitations of the electrons at room temperature have dominated over this magnetoresistance effect and so far have hindered the use of graphene for this purpose.

To address this problem, Tan and co-workers used a transistor device made from graphene nanoribbons (see image). Unlike conventional graphene sheets, the geometric restriction of the nanoribbons leads to a gap in the electronic states (bandgap) of the ribbons, which makes them semiconducting similar to silicon.

The nanoribbon transistor modifies the bandgap in a way that prevents the flow of electrical charges through the device (high resistance). A magnetic field, however, causes the bandgap of the nanoribbons to close, so that electrical charges now can travel freely across the device (low resistance). Overall, the researchers were able to change the electrical resistance by more than a factor of a thousand by varying the magnetic field from zero to five teslas. In addition, the electronic bandgap in the off state was sufficiently large so thermal excitations of the electrons were minimal.

“We could suppress the noise considerably because of the energy barrier of the device,” says Tan. “As a result, we have a better chance to deliver a high magnetoresistance signal even at room temperature.”

For commercial applications, however, further research may be required, as the fabrication of the devices remains challenging. The width of the graphene nanoribbons is only 5 nanometers, which is smaller than the feature size of present commercial transistor structures. Nevertheless, the impressive device performance achieved in the laboratory clearly demonstrates the potential of graphene also for magnetic applications.

Mechanical failure of short nanowires is characterized by smooth, ductile deformations, while long nanowires fail catastrophically

Most materials will break when a force is applied to an imperfection in their structure — such as a notch or dislocation. The behavior of these imperfections, and the resulting breakage, differ markedly between small structures, such as nanowires, and larger, bulk materials. However, scientists lacked complete understanding of the precise mechanics of nanowire breakages, owing in part to inconsistent behavior in experiments. These inconsistencies are now resolved thanks to numerical simulations by Zhaoxuan Wu and his co-workers at the A*STAR Institute for High Performance Computing, Singapore, and collaborators in the USA.

The researchers focused on metal nanowires with a so-called ‘face-centered cubic crystal structure’ because they exhibit two different failure modes. Previous experiments by other groups showed that these nanowires can break as the result of a ductile process, in which a narrow neck is formed smoothly and continuously before failure. Other experiments showed that the failure was caused by a brittle fracture, which happened suddenly. To complicate matters further, atom-scale simulations of these experiments predicted that only ductile necking should be occurring.

Wu and co-workers approached the problem by searching for a set of nanowire parameters that they could use to predict the type of failure. They used molecular dynamics software to simulate a series of cylindrical copper nanowires with a diameter of 20 nanometers and lengths ranging between 188 nanometers and 1,503 nanometers. They ‘cut’ a notch of 0.5 nanometers into the nanowire surface, which served as an initial deformation, and then applied tensile stress along the nanowire’s long axis.

These simulations predicted that long nanowires were brittle and would fail abruptly, while short nanowires less than 1,500 nanometers in length would ductile and would exhibit a smooth deformation before failure. In other words, says Wu, they “fail gracefully”. Previous nanowire simulations failed to identify these two regimes because the nanowire lengths considered were too short. The difference in behavior results from the fact that, for a given strain, long nanowires store a greater quantity of elastic energy than shorter wires.

This insight allowed Wu and co-workers to derive a simple expression for the length at which nanowires switch between failure modes. Both this expression, and the full simulation results, matched experimental data well. The results, says Wu, resolve an outstanding scientific issue, and provide a basic engineering principle for the design of nanoscale mechanical systems. Whether the model applies to nanowires with very small diameters, where classical plasticity effects begin to be lost, remains to be tested.

Biomaterials

Building up stem cell production

A three-dimensional fiber scaffold promotes large-scale stem cell proliferation and differentiation to levels suitable for tissue transplants

Thanks to the ability of pluripotent stem cells to self-renew and differentiate into a wide variety of specialized cell types, they are expected to revolutionize the treatment of illnesses such as type I diabetes and Parkinson’s disease. Before this becomes a reality, however, scientists must develop culture systems to mass-produce these cells. To overcome the limitations of previous single-layer-substrate systems, a research team in Singapore has developed three-dimensional scaffolds that stimulate stem cell proliferation and differentiation under defined chemical conditions. Importantly, the system can be scaled up. The scaffolds consist of microscopic fibers obtained by weaving together polymer strands bearing opposite charges.

Hongfang Lu and Andrew Wan from the A*STAR Institute of Bioengineering and Nanotechnology led the research. Wan notes that the fiber-based scaffold not only avoids the need to consume large quantities of key growth factors, but it would also shield the cells from the shear stresses generated in large-scale bioreactors.

To manufacture the scaffold, the researchers opted for a positively charged biopolymer called chitin, which they extracted from crab shell, and a negatively charged polymer called sodium alginate. After depositing one droplet of each of these water-soluble polymers onto a sterile substrate, they brought the droplet interfaces into contact using forceps; this formed a chitin–alginate complex. Held together by intermolecular electrostatic interactions, the complex extended into a continuous fiber. The team reeled the fiber onto a holder to complete the three-dimensional system.

By suspending the stem cells in the alginate solution, Lu, Wan and co-workers incorporated the cells into the scaffold during fiber formation, resulting in a network of uniformly distributed cells (see image). Preliminary tests showed that when the researchers destroyed the scaffold with enzymes, they could recover a high number of the cells.

Lu explains that their system provided a ‘micro-environment’ in which cells could grow in aggregates. When sub-cultured over many generations, the encapsulated stem cells remained pluripotent and did not undergo any genetic mutations. Moreover, the cells displayed excellent viability when frozen in the fiber for storage; in addition, they could either self-renew or differentiate, depending on the media available to them. “The small dimensions of the fibers are useful because they allow nutrients and growth factors to efficiently diffuse towards the cells within the scaffold,” she adds.

The team is now planning to exploit their approach to produce transplantable tissue for cell-based therapy. “Our system allows us to generate large numbers of cells for tissue-engineering applications,” says Wan.

Novel coding technique patented by A*STAR researchers

A pioneering error correction technique developed at the A*STAR Data Storage Institute holds promise for the development of next-generation computers

Over the past decade, tablet computers and smartphones have taken the world by storm, in no small part due to the way in which they can be switched on almost instantly. The race has been on to develop computers that can similarly be up and running in a matter of moments. Such advances are currently hindered due to the fact that computers need to boot up, as silicon memory chips cannot hold information if the power is turned off. In order to retain information even if the power is turned off, the memory needs to be non-volatile, as is the type of memory commonly found in memory sticks. However, existing memory technologies are expensive, difficult to scale up and often cannot keep up with the demands of current desktop computers. A key contender for future non-volatile memories is the so-called spin-torque transfer magnetic random access memory (STT-MRAM).

Breakthrough in coding and design

Although STT-MRAM devices have been the subject of intense research in the past few years, key hurdles remain. Focusing in particular on the technological aspects of STT-MRAM, Dr. Cai Kui and her non-volatile memory (NVM) Coding Team from the A*STAR Data Storage Institute have now patented an algorithm for correcting errors arising when information is stored and read out incorrectly. Their novel approach significantly enhances the error tolerance of STT-MRAM devices. “This is a breakthrough work that will help provide bigger tolerances and ease the engineering challenges in STT-MRAM material and process development,” says Pan telis Alexopoulos, Executive Director of the Data Storage Institute.

Essentially, STT-MRAM devices encode information through the relative magnetic field orientation of two thin films. The magnetic fields of the two films are oriented parallel or antiparallel to each other. The relative orientation of the fields can be switched because one of the films is a hard magnet whose orientation stays fixed, while the other one is a soft magnet that can be easily realigned. This realignment can be achieved, for example, by an electrical current in which the magnetic property of the electrons — their ‘spin’ — is polarized in the desired orientation by passing through an appropriate oxide layer in the device. The read-out of the stored information is straightforward as the electrical resistance of a current passing through the device depends on the relative orientation of the two layers. If the magnetic layers are aligned in the same direction, the electrical resistance is smaller than it would be the other way round.

The absolute value of this electrical resistance is more difficult to control and depends on many parameters such as device size or film thickness. Despite on-going efforts to minimize such influences, these parameters inevitably vary from device to device, and as a consequence, the electrical resistance is never really precise. This poses a problem during the conversion of this analogue information into the digital signals used in computing — what should be a digital ‘0’ signal might be sensed as a ‘1’.

This jeopardizes the usability of memory chips containing billion of these devices, explains Cai: “At present, there is a considerable amount of effort devoted to the device design, material improvement, and process development for STT-MRAM. However, at the same time, there is little work done in the area of coding and signal processing to correct STT-MRAM cell errors.” Efforts so far have been limited to conventional error correction procedures that could correct only a very limited number of errors in STT-MRAM devices. In these so-called error correction codes (ECCs), redundancy is built into the encoded information — the data is converted into more bits than needed. As there is a well-defined relationship between the redundant bits and the input data, it is possible to perform an error correction that enables the retrieval of the stored information even in the presence of errors and noise. However, in these earlier schemes the information is considered strictly as digital ‘0s and ‘1s only during the decoding, which means that this hard decoding error correction capability is limited.

For this reason, Cai and colleagues developed a new design of the memory sensing and detection architecture that is based on soft decision decoding. The soft decision decoding goes beyond the strict limitation of ‘0’ and ‘1’ of the bits and also considers the probability of each detected bit being a ‘0’ or ‘1’. The use of such additional information leads to significantly fewer decoding errors than the hard decision decoding that does not take such probabilities into account.

Improving performance across the board

An important component of the new design is the soft-output channel detector, which measures the probabilities of the bits
read out being set as ’0’ and ’1’, and feeds this information into the soft decision decoding process of the particular STT-MRAM error correction code utilized here — the so-called low-density parity-check (LDPC) codes.

The improved design also includes a new quantization scheme for STT-MRAM. This is the process that converts the analogue signal into the digital signal. To ensure a high-quality conversion, the analogue information is best encoded into a large number of quantization bits, which greatly increases computational efforts. However, the enhanced error correction procedure means that fewer quantization bits can be used. This not only simplifies the management of such devices but also maximizes the number of information bits that can be stored in a STT-MRAM cell.

Remarkably, Cai and her colleagues have successfully shown that the new scheme achieves a 20% increase in the tolerance towards variations in electrical resistance of the devices. Such relaxed demands greatly ease the manufacturing process of the devices and also will be important when it comes to further reducing STT-MRAM device sizes, as Alexopoulos comments: “DSI’s design of LDPC coding with soft decision decoding for STT-MRAM has a better error correction capability, paving the way for the industry to reduce the cell feature size of future STT-MRAM devices.”

The new error correction approach developed by Cai may well keep STT-MRAM in the running when it comes to replacing flash drives in computers, and lengthy computer boot-up times could soon be a thing of the past. One of the next challenges for the non-volatile memory coding team will be to focus on further scaling of STT-MRAM. “We will further investigate how ECCs with soft decoding can help to improve the various performance of STT-MRAM, and eventually contribute to the scaling of STT-MRAM,” says Cai. “We will also design different ECCs for STT-MRAM with different applications.”

The successful demonstration of a technique capable of producing color images with a record-breaking resolution of 100,000 dots per inch (dpi) could guide the printing industry into uncharted territory. The resolution provided by the new method, which is based on the tailored scattering of light by nanostructures, surpasses commercial techniques by more than a factor of ten and reaches the fundamental limit set by the laws of optics.

Currently leading technologies for color printing, such as the inkjet and laserjet methods, deposit tiny dots of dye on a surface. The size of these dots is typically in the micrometer range, which means that the highest attainable resolution for these techniques is below 10,000 dpi. Approaches that yield finer dots have been demonstrated in research laboratories, but these techniques are so far limited to producing monochrome images and are not scalable. The method now developed at the A*STAR Institute of Materials Research and Engineering (IMRE), with the participation of the Institute of High Performance Computing (IHPC), provides a fundamentally different approach that is free of the technological limitations of colorant-based methods. “We use tiny metal nanostructures to create color,” explains Joel Yang, the lead researcher on this project. “This is somewhat similar to staining glass, where different colors are produced by using different metals. We use only one metal, however, which we deposit in a thin, uniform layer on tiny posts of varying diameter.”

The posts are only a few tens of nanometers in size, and Yang and his co-workers have now shown that, depending on the disks’ diameter and density, different colors can be generated through a mechanism known as plasmon resonance. To demonstrate the power of their technique, Yang’s team has created a 50 micrometer × 50 micrometer photorealistic full-color image with pixels at 250 nanometer pitch (see above image). This resolution is at the so-called optical diffraction limit, which is the fundamental resolution limit of any optical imaging system.

Fine prospects for high-resolution printing

A method for printing color images of ultimate resolution has been developed at the A*STAR Institute of Materials Research and Engineering

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This new method now pushes the door for commercial uses wide open. “At the current stage, we can think already of a number of applications, including branding and brand protection for products meant for viewing under a microscope — bio-assays or microscope calibration kits, for instance — or the production of anti-counterfeit features,” says Yang. “Following further development, there should be the potential for applications such as high-density optical data storage or coating all sorts of surfaces with colors that won’t fade.”

Importantly, with a view to commercial applications, the new technique can be scaled up. The creation of the nanostructures that make up the image may be quite involved. Once a master template is made, however, pattern-replication methods such as nano-imprint or photolithography can be used to mass-produce micro-images. Furthermore, technologies for imprinting large areas are available, too.

Indeed, first steps from laboratory to market have already been taken: One Singapore provisional patent is filed and another is pending. Furthermore, the team is working with Exploit Technologies Pte Ltd (ETPL), A*STAR’s technology-transfer arm, to assess the interest of companies for collaborations, and to explore opportunities for licensing the technology.


Strengthening stem cell research

Remarkable strides are being made in stem cell research at the A*STAR Institute of Bioengineering and Nanotechnology, leading to the development of novel therapeutic and diagnostic strategies

As the world’s first bioengineering and nanotechnology institute, the A*STAR Institute of Bioengineering and Nanotechnology (IBN) has gained international prominence for its exceptional multidisciplinary approaches to biomedical research. Not limiting its scope to materials science and nanotechnologies, the Institute is also surging ahead on developments based on stem cell research.

In recent weeks, IBN has released breakthrough news on two fronts — a promising discovery for breast cancer therapy and a novel approach to drug screening, both of which demonstrate the power of harnessing stem cell-based research.

Targeting tumors

In a landmark finding that may impact future courses of cancer treatment, a group of IBN researchers at the Institute’s Drug and Gene Delivery Research Group has shown that neural stem cells (NSCs) have an innate ability to target tumors outside the central nervous system. Based on a series of mouse experiments, the IBN study is the first of its kind to demonstrate that NSCs derived from human induced pluripotent stem (iPS) cells could target cancerous tissues other than those in the brain, such as breast tumors and cancerous cells in the lung, stomach and bone.

“We have generated a new type of cells with tumor-homing property from freshly-produced human pluripotent stem cells. Using our in-house baculoviral transduction technology, we can engineer the cells into cancer gene therapy delivery vehicles,” explains Shu Wang, IBN Group Leader.

What makes the IBN group’s approach particularly outstanding is that using iPS cells to derive NSCs would enable an efficient and reliable method of standardizing and producing cell therapy products in large quantities. As the iPS cells are developed from the patient’s own cells, the new
Stem cell research may continue to give rise to novel medical breakthroughs. In a move that may greatly facilitate cancer therapy research, a research team led by Jackie Y. Ying, Executive Director of IBN, has developed a biochip that is capable of analyzing the effect of drugs on cancer stem cells (CSCs), a rare form of cancer cells that are typically more resistant to chemotherapeutic drugs than other cells in a tumor.

The miniaturized technology, which has been patented and named the ‘DropArray™’, stands apart from other biochips developed to date in several respects: “In traditional biological assays, microplates — a flat plate with multiple wells in which samples are placed — are commonly used. Each well requires the presence of at least 2,500 or 5,000 cells, depending on the type of plate used, for viable analysis,” explains Ying. “By comparison, IBN’s Droplet Array is a flat, rectangular glass plate on which a series of spots, each two millimeters in diameter, are arranged.”

Sample ‘droplets’ can then be pipetted onto these two-millimeter-diameter spots, after which the Droplet Array is coated with a layer of proprietary oil to prevent evaporation and cross-contamination between the droplets during the rinsing process. “Being one-fifth the size of a well in a standard microplate, each spot on IBN’s Droplet Array requires only 500 cells for screening,” says Ying. “This massive reduction in sample volume not only saves money, but is also particularly advantageous for studying limited samples containing scarce cells, such as cancer stem cells.”

The Droplet Array could also potentially reduce industry reliance on animal experiments, as in vitro results may complement or replace those achieved through animal studies. “This is timely in light of the European Commission (EC) ban on testing cosmetic products and ingredients on animals and the EC ban on marketing cosmetic products that contain ingredients that have been tested on animals,” says Ying.

So far, the Droplet Array has been used to examine drug responses of CSCs extracted from breast, liver and colon cancer cells. “It was found that chemotherapeutic drugs such as doxorubicin, which usually induce cell death in liver cancer cells, demonstrated poor efficacy in liver CSCs,” says Ying. “The CSCs from the breast and colon tumors also showed much greater ability to survive the effects of anti-cancer drugs. By providing a viable platform to investigate the effect of anti-cancer drugs on CSCs, the Droplet Array technology could boost the development of more effective cancer drugs.”

Commenting on some of the challenges faced by the research group during the development of the novel biochip, Ying notes, “The main challenge was to overcome the issues of sample scarcity and purity while deriving cancer stem cells for biological studies. We found the answer by using the Droplet Array technology to study drug.
resistance in cancer stem cells. Our novel platform can maximize the use of scarce and precious cells like cancer stem cells in drug screening.”

**Success through collaboration**

The type of collaborative work that led to the discovery of human neural stem cells with tumor targeting ability and the development of the Droplet Array demonstrates the effectiveness of interdisciplinary approaches to problem-solving, which have clearly become the order of the day at IBN.

Ying comments, “IBN advocates a multidisciplinary approach to solving exciting and challenging biomedical problems. The teams at IBN are project-based rather than discipline-based, and we encourage our researchers, who come from diverse backgrounds and specializations in science, engineering and medicine, to interact and collaborate with each other, because better and more comprehensive solutions emerge when different fields of expertise come together.”

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**A future vision for media**

**A*STAR’s Snap2Tell image recognition technology is at the forefront of new media applications for mobile phone and iPad users**

New technologies to support the rapidly evolving world of media and communications are big business. Creating successful image recognition software is a key area of research, since multimedia relies heavily on the power of imagery and customers are demanding more information more rapidly.

Teaching computers to ‘see’ and recognize images accurately is difficult. The key is to train computers to recognize and group together images that have the same or similar numerical pixel data. The sheer amount and complexity of image data available means that advanced image recognition is still beyond current technologies.

An image recognition system called Snap2Tell, developed by Yiqun Li and co-workers at the A*STAR Institute for Infocomm Research is now being integrated into a new mobile phone app for Malaysian newspaper The Star as a new function called iSnap. This could have many potential applications for media use in future.

**How Snap2Tell works**

“The initial objective of the Snap2Tell technology is to simplify the process of getting information on the move,” explains Li. “By capturing an image using the phone camera, relevant information on the topic at hand is displayed to the user immediately. Taking a picture is much easier than typing a sentence on the small key pad on the phone.”

The Snap2Tell software works by recognizing image information present in a pre-prepared database of Snap2Tell readable images. Snap2Tell matches the image to the database, and then accesses further information which has been pre-associated with that image.

**Enhancing newspapers with iSnap**

Snap2Tell is now being applied to mobile augmented reality applications, in the form of iSnap. iSnap is free to use for mobile phone and iPad users as soon they have downloaded the Star Mobile app. The user simply points the phone camera at an iSnap marked article or advert, and a set of icons will appear on top of the articles on the phone screen. The icons represent different additional information relevant to the article. When an icon is touched, iSnap will automatically take the reader to further information through a variety of multimedia, such as websites, social network forums, or video clips.

In The Star newspaper, certain articles and advertisements are now printed with an iSnap logo next to them. iSnap is different to QR codes, currently used widely in advertising print media such as billboard posters. Essentially barcode squares that phone users can scan, QR codes direct the user’s phone to a company’s product website. With iSnap, the code is not needed — the software recognizes images related to the product.

In addition, iSnap brings up a whole array of different multimedia information via icons on the touch-screen — not just one website. This means that the user has a choice of which media form they would like to view, rather than scrolling through websites to find a video, audio file, or written commentary, to give but a few examples. The user also has the choice to save their
favorite information to their phone, should they wish.

Advantages and disadvantages
Media and advertising companies are delighted with the possibilities that the Snap2Tell technology brings. Paper advertisements can potentially come to life through mixed media — for example viewing the latest TV adverts or reading the specifications and reviews for the latest model of a car. News articles can be accompanied by film footage, interviews and associated story links and commentaries, and even billboard posters could carry the iSnap logo in future and lead the ‘snap-per’ to film trailers or the latest news on a certain product.

Furthermore, a more targeted audience could also be reached in future, as Li explains: “The information can also be stored in the user’s phone if the user is interested in the product being advertised. We have developed a mobile app called Snap2Remind for bringing up more information on printed advertisement pictures. The app also provides location-based reminders to alert users when they pass by the shops where the advertised products are being sold.”

For advertising and media companies, Snap2Tell opens up a wide range of possibilities in terms of consumers accessing further information without page or print limitations. Companies can also monitor the popularity of different articles and products by looking at the access rates for each individual item.

There are, however, some limitations of the technology as it currently stands. iSnap requires a phone with a touch-screen and access to the internet. In addition, media companies’ servers need to be big enough to host and produce multimedia on a large scale. They also need to develop a database of Snap2Tell readable images, by uploading each image as it is needed in printed form. This could eliminate some smaller local companies from participating.

Future plans for Snap2Tell technology
Li is quick to point out that, as in the West, newspapers may not last in Asia. However, she remains optimistic about the future of iSnap and Snap2Tell: “Even if there are no printed newspapers in the future, there may still be other paper media such as posters, signboards, paintings, drawings, CD covers, and book covers, for example.”

Snap2Tell image recognition is not limited to printed materials; it can be applied to physical objects or scenes as well.

A*STAR researchers are now working to produce more complex versions of the Snap2Tell technology and expanding it into applications for 3D physical objects or scenes. Pointing the phone camera to a real building could bring up a similar touch-screen experience, providing the mobile users with information about the building and its location. This could mean that sight-seeing for tourists could take on a new twist, without the need for carrying guidebooks.

“We are planning to work on more complicated cases of augmented reality on mobile phones,” says Li. “The iSnap may not be just an overlay of icons on top of printed media. It may include 3D object rendering on top of physical objects or scenes in future.”

Fantastic plastic

Drawing their inspiration from nature, A*STAR researchers develop a brand new type of anti-reflective plastic

Nature has long been a source of inspiration for both scientists and artists alike. Some of the most ingeniously designed products and gadgets familiar to millions of people worldwide owe their origin to seemingly simple forms and patterns found in plants and wildlife. The ability to adapt these natural forms to develop ever more innovative products and processes has given rise to the field of biomimetics — literally meaning ‘imitation of life’.

One of the most notable examples of biomimetic design to date is Velcro, famously inspired by the tiny, adhesive hairs found on the underside of geckos’ feet. Insects, too, have provided intriguing clues for the development of many new technologies ranging from self-cooling systems inspired by termite mounds to cicada wing-inspired nanosensors. Now, a team of researchers based at the A*STAR Institute of Materials Research
and Engineering (IMRE) working in collaboration with industry partners have succeeded in developing a new type of high-quality, anti-reflective plastic inspired by another unlikely source: the eyes of a moth.

The eyes have it
Moths are renowned for their ability to see well in the dark. Moth eyes are coated with a special anti-reflective layer that lends them the unusual distinction of having one of nature’s least reflective surfaces. Composed of a hexagonal array of conical nanostructures, the anti-reflective layer enables moths to maximize light capture and minimize reflection, thereby reducing the chances of being spotted by predators, even in settings that appear pitch-dark to the human eye.

“Our group has been working on bio-inspired surfaces for a number of years,” explains Low Hong Yee, senior scientist and team leader of the project at the IMRE. “The anti-reflection properties found on some insect eyes are rather well-known and it was natural that we looked into the moth-eye structures. Mimicking moth-eye nanostructures have been attempted by others — however, our unique approach is in the combination of micro- and nanostructures in a hierarchical arrangement. These structures are even closer to mimicking the insect eye.”

Using a high-precision method known as nanoimprinting, the IMRE team were able to ‘reconstruct’ the moth-eye nanostructures and utilize direct patterning techniques to reduce surface glare. Nanoimprinting is a technique closely associated with the semiconductor and data storage industries — however, the method is becoming more and more widely used in other domains as a means to fine-tune the physical and optical properties of many different kinds of components, for example, in the optics and biomedical industries.

One of the main advantages of nanoimprinting is that materials can be manipulated in terms of their physical as opposed to their chemical properties. New plastics can therefore be developed without the need to use harmful chemicals. Indeed, this type of nanoimprinting is viewed as a way of moving towards cost-effective, environmentally sustainable manufacturing practices.

Anti-reflective plastics currently on the market typically exhibit a reflectivity of around 1% of visible light. In contrast, the new plastic developed at IMRE reflects less than 0.2% of visible light — attaining a five-fold increase in anti-reflective power. The new plastic maintains a reflectivity of less than 0.7% even at angles of up to 45 degrees. Combined with the reduced amount of glare, the new plastic may find a host of applications in the development of new and improved TV displays, windows and organic solar cells.

“We are also developing complementary research that allows the technology to be easily ramped up to an industrial scale,” says Low. Several companies are now in the process of licensing the anti-reflective nanostructure technology from Exploit Technologies Pte Ltd, the technology transfer arm of A*STAR.

Partnering with industry
The new plastic is notable for being the first successful outcome of the IMRE-led Industrial Consortium On Nanoimprint (ICON), a multi-agency effort backed by Singapore’s leading trade and industry development bodies, including the Economic Development Board, International Enterprise Singapore and SPRING Singapore. By encouraging companies to adopt versatile, industry-ready nanoimprinting technology, ICON is dedicated to building collaborative pre-competitive research and development projects.

By focusing on strengthening academia-industry partnerships, ICON is raising the bar for innovative research in Singapore. “The ultra-low reflection as required by our industrial partner was a challenge for the team,” says Low. “We proposed a new design rule that has not been reported by others, both theoretically and experimentally. We had a sound hypothesis and we had earlier developed a unique hierarchical nanoimprint process; these two starting points helped us to focus and develop our designs experimentally.”

As a testament to the potential of the technology, many industry experts have also added their voices in extolling the work of the consortium. Wilson Kim Woo Yong, director of global marketing at Young Chang Chemical Co., Ltd., comments, “The outstanding results from this consortium work will benefit our company’s expansion into new markets such as in the touch-screen panel and solar business sectors.”

Tatsuo Shirahama, president of Innox Co. Ltd., states, “We have been very impressed with the developed technology and with the excellent team of researchers working on the anti-reflective structures.”

“The results from the consortium work are key in the decision making for our future strategic planning,” says Yuji Akatsu from NTT Advanced Technology (NTT-AT).

“This is an exciting innovation — mimicking nature through the nanoimprint technology to solve real-world problems,” comments Andy Hor, IMRE’s executive director. “I am very pleased that the collaboration with industry has helped move this R&D from the laboratory to application in the industry. The development of the new plastic is a testament to the strength of Singapore’s advanced R&D capabilities, the benefits of nanoimprint technology and the confidence that companies place on our technologies.”

With regard to future directions for her research group, Low hints that there may be more nature-inspired innovations in store: “Soon, we will be releasing the results of a second consortium project, which is also a biomimicry project,” she says. “It is a project that will aim to develop anti-microbial plastic, taking clues from marine species.”
Bright future for new LED manufacturing process

An award-winning method that improves the efficiency of high-powered lighting has been developed by A*STAR’s Singapore Institute of Manufacturing Technology

A new technique for making brighter, longer-lasting LEDs (Light Emitting Diodes) has taken the first leap from research laboratory towards the three-billion-US-dollar global market in high-powered lighting. The new manufacturing system, called liquid forging, dramatically improves the way tiny electronic devices keep cool and looks set to revolutionize production of next generation LEDs.

Many electronic parts need heat sinks to prevent burnout. Effective cooling of high-powered LEDs for homes, offices and streetlights is a serious engineering challenge for a global market expanding 10% annually. If heat is allowed to build, it can damage parts causing them to dim and lose efficiency. The award-winning liquid-forging method developed by A*STAR’s Singapore Institute of Manufacturing Technology (SIMTech) provides a solution.

“Liquid forging is a hybrid between forging and casting,” says Chua Beng Wah, the lead researcher on the SIMTech project. “It is especially useful if you need to manufacture lighter components with intricate features like heat sinks using wrought aluminum alloys.”

The process provides a significant additional benefit for thermal engineers: the thermal conductivity of liquid-forged products beats conventional techniques such as casting by a factor of two. “The method is ideally suited to heat sink design,” adds Beng Wah.

In April this year, A*STAR’s technology transfer arm, Exploit Technologies Pte Ltd (ETPL), licensed the patented technology to a leading LED thermal management firm. The agreement allows the firm to build lightweight, high-performance LED heat sinks using the liquid-forging process.

Liquid forging was developed by a SIMTech team led by John Yong. In 2008, Yong’s team won Singapore’s highest honor for exceptional research, the National Technology Award, for their discovery.

The process is highly scalable allowing complex parts — using composite materials such as copper and aluminum — to be created in a single step. This development means heat sinks and light fixtures can be formed as one piece significantly minimizing assembly costs. The system also allows more elaborate designs like complex arrays of pins and fins that increase surface area for improved heat dissipation. Furthermore, the final product requires less machining, partly because the process uses raw materials more efficiently. The resultant heat sink can be anodized, improving thermal performance by an additional 10–15%.

But liquid forging is not restricted to cooling LEDs. ETPL’s Chief Executive Officer Philip Lim explains, “Liquid forging is a low-cost system with the potential to compete with traditional manufacturing processes in the biomedical, aerospace and automotive industries. Amongst other things, this technique could be used to make alloy wheel trims, electronic casings or pistons.”

With products predicted to be on the shelves as early as 2013, the future for this new technology seems bright.
Forging new ties

A*STAR and a UK-based biotech company join forces to develop leading-edge diagnostic devices based on silicon nanowire technology

Rapid advances in DNA sequencing technologies and emerging diagnostic tools in recent years have led to the proliferation of biotech companies keen to bring new and innovative ideas to market. More and more biotech firms are turning to Singapore to drive innovations forward, drawing on the country’s wealth of expertise in enabling biomedical research and development to flourish. A*STAR takes a leading role in developing biomedical engineering applications and enabling partnership enterprises to be technologically competitive.

A new collaboration between the A*STAR Institute of Microelectronics (IME) and QuantuMDx Group (QMDx), a UK-based biotech company established in 2008, aims to develop innovative and affordable medical devices based on some of the latest advances in bioelectronics. The collaboration brings together the expertise of the IME in developing silicon-based nanowire technologies and QMDx’s innovative designs for a suite of novel diagnostic and DNA sequencing technologies.

“This is an important strategic collaboration for the IME with a key industry partner, validating the potential of silicon microelectronics for next-generation molecular diagnostics,” says Abdur Rub Abdur Rahman, Head of the Bioelectronics department at the IME.

One of the key goals of the new partnership will be to develop and commercialize QMDx’s handheld DNA sequencing nanowire biosensor—a device that may greatly aid medical practitioners to perform advanced diagnostic tests in a wide variety of settings, with the twin advantages of being affordable and portable. Designed by QMDx’s chief scientific officer Jonathan O’Halloran, the nanowire biosensor—currently in prototype—would enable rapid and accurate genomic sequencing at the point of need. “Nanowire biosensors fulfill an important requirement and milestone in the roadmap of biomedicine—namely, highly sensitive and multiplexed multi-analyte sensing to enable next-generation point-of-care diagnostics,” says Rahman.

The IME’s advanced silicon nanowire technology is well-suited to the design requirements of a handheld diagnostic device. As Rahman notes, “IME’s highly sensitive silicon nanowire technology is manufactured using a CMOS-compatible top down approach. This allows for scaling the sensing area and multiplexing as demanded by the application. For point-of-care applications, it is desired that the handheld instrument be as minimalist as possible. This can be achieved with CMOS-addressable nanowires with built-in sensing circuits using our approach.”

There are two basic approaches to manufacturing nanowires: top down and bottom up. “In the bottom up approach, wires are manufactured en masse, but they have to be assembled individually between contacts, which is a cumbersome and non-mass producible process,” explains Rahman. “In the top down approach, nanowires are precision-located, meaning that they can be created on desired locations. This method is mass-manufacurable, reproducible and convenient; hence our approach is advantageous.”

The capabilities of nanowire technology are being studied on many different levels at the IME, with projects ranging from applications in nanoscale pressure sensors to the development of ‘seamless’ memory devices.

The IME has been actively engaged in nanowire development for diagnostics for approximately three years. With regard to some of the future research directions for his Bioelectronics team, Rahman comments, “Our current work is focused on improving the reliability of our platform for clinical deployment. Future developments include smart packaging and fluidic interfacing schemes, and a highly multiplexed CMOS nanowire platform with built-in sensing circuitry.”

Dim-Lee Kwong, Executive Director of the IME, who presided over the signing of the research collaboration agreement, comments, “The collaboration with QMDx to deliver a technology breakthrough clearly demonstrates the potential of the IME’s cross-disciplinary expertise and capabilities in the bioelectronics industry. We look forward to working with our partner towards the commercialization of their first nanowire biosensor.”

Jonathan O’Halloran, Chief Scientific Officer at QuantuMDx (third from left), Dim-Lee Kwong, Executive Director of the IME (fourth from right), Abdur Rub Abdur Rahman, Head of the Bioelectronics department at the IME (third from right) and colleagues at the signing of the research collaboration agreement on 23 February 2012

Silicon nanowire array (top left), microchip with silicon nanowire arrays (top right), and the prototype of packaged Silicon nanowire biosensor from IME (bottom). Images (top left and bottom): From the 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences 3–7 October 2010, Groningen, The Netherlands
In an ambitious and far-sighted move, A*STAR Singapore and the University of Illinois at Urbana-Champaign, USA, opened the Advanced Digital Sciences Center (ADSC) in Singapore in 2009. The Center is the culmination of many years of collaboration between the two institutions. A*STAR granted S$75 million to open ADSC — the first affiliated center outside the USA for the University of Illinois — with the aim of leading the race in computing research and promoting signature technology projects in the digital age.

“At ADSC, we want to transform the way society uses and interacts with information technology,” explains Marianne Winslett, director of ADSC. “Our two research programs, interactive digital media and the smart grid, are University of Illinois specialties and are important areas for Singapore, which would like to grow those sectors of its expanding knowledge-based economy.”

The researchers based at ADSC are involved in developing virtual reality interfaces which will allow seamless interaction between humans and the digital world. “We are working on realistic immersive telepresence using just ordinary cameras, low-cost microphones, personal PCs or laptops, and the public internet,” describes Winslett.

“Our smart grid projects are about understanding, controlling, and securing the power grid. For example, finding low-cost, non-intrusive ways to get real-time information about how consumers are using power at home and at work means we can help avoid black-outs and power surges during periods of high demand.”

Changing scenes

A new piece of virtual reality software developed by Jiangbo Lu and colleagues at ADSC has recently been licensed through Exploit Technologies, the technology transfer arm of A*STAR. Lu’s team have developed a way of allowing online chat users a choice of different video backgrounds which can be altered during real-time chat.

“In our research, we like to see whether one’s ordinary laptop can be turned into something magical,” explains Lu. “Our new program — CuteChat — allows the chat background behind the user to be changed easily at will, without any special equipment needed.”

The CuteChat algorithm works by ‘cutting out’ the foreground objects (namely, the person) in a video feed and placing them into a different backdrop.

“A key challenge for us was how to make sure the vision algorithm was intelligent enough to reliably delineate the foreground objects from live video feed,” explains Lu. “Also we had to stay within the limits of real-time processing speed, keeping it affordable on an ordinary laptop.”

Through these technologies, CuteChat can heighten online privacy by allowing users to transport their online self to any chosen location, thus concealing where they really are. In addition, slides can be posted behind the user as they work through a presentation. With the addition of a depth camera, CuteChat employs gesture recognition to allow the user to page through presentation slides or online photo albums with a wave of the arm.

Image recognition in medical science

ADSC has another group of researchers dedicated to the field of image recognition, teaching computers to ‘see’. In a second project licensed by Exploit Technologies, Gang Wang and co-workers have developed a novel way of using image technology to recognise medical pills and capsules. This helps to verify that the correct drugs are being administered to patients.

“The project is inspired by the fact that there can be errors in hospital packaging, and different companies produce the same drugs in different forms,” explains Wang. “It is very hard for humans to manually
input information such as exact size, shape, and color, which some online pill verification programs require.”

“The main challenge was the variation of pill appearance due to changes in lighting, perspectives, rotation, scale, and color,” describes Wang. “We overcame these difficulties by developing advanced image features in the software to represent pills of all varieties.”

Wang hopes to continue developing the software to work with the different computer systems in hospitals and may develop a mobile phone application for use by the general public at home.

A unique PhD program
Perhaps one of the highlights of the collaboration between A*STAR and the University of Illinois over the years has been the development of their unique PhD program. The two institutions allow Singaporean students to study for a PhD split between them — two years spent in the USA followed by two years spent at an appropriate A*STAR research institute in Singapore. The result is a PhD from the University of Illinois for Singaporean students, with their high levels of skills and research returning to their home country.

This popular PhD program has expanded with the opening of ADSC, allowing A*STAR-funded top students from Singapore onto the AUIP program, splitting their time between Urbana-Champaign and ADSC.

Xinqi Chu is a current PhD student on the AUIP program studying computer vision and machine learning.

“The University of Illinois has a set of eminent researchers and an excellent facility in which to work,” describes Chu. “The support provided by the AUIP program is good enough for me to concentrate on my work and worry less about living expenses. I also have access to other funding initiatives that might not be available elsewhere.”

Further, international students are now able to apply for scholarships through the Singapore International Graduate Award (SINGA) PhD program, allowing them to work towards a PhD from the National University of Singapore or Nanyang Technological University whilst conducting research at ADSC. Shin Hwei Tan, originally from Malaysia, is one such international student due to start her PhD in the fall:

“I obtained my BS and MS degrees in Computer Science from the University of Illinois,” Tan explains. “The SINGA program provides a good platform for students from around the world to communicate with leading researchers and professors from top Singaporean universities.”

The bright future for ADSC
Due to the importance of digital technology in today’s world, ADSC is certain to go from strength to strength in terms of developing commercial projects from their research in future.

“The gap between basic research and commercial potential is narrow for interactive digital media,” explains Marianne Winslett.

“We have a number of results in the pipeline that we expect to head toward commercialization in the near future, in telepresence, video analytics, web mining, and energy usage monitoring.”

Fighting cancer cells by stealth

Through a combination of two powerful methodologies, researchers at the A*STAR Institute of Bioengineering and Nanotechnology drive forward promising developments for gene therapy

The way in which cancer can spread silently and unnoticed in the body — with symptoms in some cases remaining latent for months, years, or even decades — is often noted as its most deadly feature. Researchers around the world have been devising ever more sophisticated strategies to fight cancer — including ‘stealth’ techniques designed to outwit the body’s immune system so as to deliver therapeutic drugs, genes, proteins and viruses to carefully targeted disease sites. Such approaches may help to turn the tables, enabling researchers to one day realize the ultimate goal of silencing the silent killer.

In a move that reflects the strong, collaborative nature of research at the A*STAR Institute of Bioengineering and Nanotechnology (IBN), a multidisciplinary team of IBN researchers led by Shu Wang, Jackie Y. Ying and Andrew Wan have demonstrated an ingenious stealth-attack method that may help to lead to the more effective elimination of cancer cells1.

The new method involves coating therapeutic viruses with specially fabricated microfibers composed of peptides and DNA. The microfibers crucially help to ‘trick’ the body into bringing down its natural defenses. So far, experiments on mice with brain tumors have shown that the microfiber-coated therapeutic viruses can lead to delayed tumor growth and prolonged survival time.
A boost for biomedical research

The new work conducted by Wang and colleagues notably brings together two biomedical innovations developed at IBN. The first is a unique microfiber fabrication technique developed by Andrew Wan and co-workers in 2006. The technique is distinguished by the incorporation of silica into polyelectrolyte-complex fibers, making them highly flexible and suitable as tissue engineering scaffolds.

The second innovation, in turn a result of IBN’s extensive experience in investigating engineered insect viruses to treat cancer and neurological disorders, is the successful delivery of therapeutic genes to human embryonic stem cells using a baculoviral vector, achieved by Shu Wang and co-workers in 2006. This study demonstrated the possibility of effective and safe genetic manipulation methods in human embryonic stem cells — an important goal in furthering applications of stem cells in developmental biology and regenerative medicine. Shu Wang has contributed to numerous cancer research studies at IBN, including the successful treatment of mouse brain tumors using genetically engineered insect viruses.

The new work builds on the two IBN-developed innovations in order to bring gene therapy — the targeted replacement of disease-causing mutated genes with functional genes — one step closer to clinical reality. To date, the development of synthetic tissue fibers has been limited by the considerable challenges of constructing fibers made up of more than a single type of biomolecular material, such as peptide, protein, RNA or DNA. The team of IBN researchers was able to circumvent this problem by using a water-based procedure that enables the combination of positively-charged peptides and negatively-charged plasmid DNA. The biomolecules effectively combine to form the microfibers and, using the same water-based process, baculoviral vectors can be added to the DNA solution so as to coat therapeutic viruses with the microfibers.

“Experimental work in the area of virus encapsulation thus far has employed polymers and lipids,” explains Wang. “There are certain limitations associated with the usage of these synthetic materials. For example, polyesters based on lactic acid and glycolic acid (PLGA) generate acidic degradation products that cause transient tissue inflammation and reduce cell proliferation, and cationic lipids trigger allergic-type immune reactions.”

The new method with improved biocompatibility therefore represents a powerful approach to virus-based gene therapies: “For the very first time, we have shown that two biomolecules, namely peptides and DNA, can interact with each other to form structured fibers in a test tube,” says Wang. “Since these biomolecules are readily metabolized in the human body to naturally occurring molecules and have no adverse toxicity, they hold strong biomedical potential for the delivery of therapeutic drugs, genes, proteins and viruses to combat cancer.”

Prospects for gene therapy

Gene therapy is set to become one of the most important medical treatment strategies of the twenty-first century that may help to lead the fight against many currently incurable diseases such as cancer, AIDS, Parkinson’s and Alzheimer’s diseases, cystic fibrosis, multiple sclerosis, and diabetes. Advancements that spur progress in effective gene delivery are therefore of key interest to medicinal chemists, pharmaceutical companies, and all those involved in drug design, development and delivery.

“The favorable in vivo application of the virus-containing bio-macromolecule microfibers makes them attractive candidates for localized gene therapy in an environment with complementary components,” says Wang. “Fiber encapsulation may also be valuable in minimizing the effects of adaptive immune responses directed against repeatedly injected gene therapy viral vectors by shielding the encapsulated viruses against neutralizing antibodies. Thus, the results from this study open new avenues for the development of biomolecular material-based drug delivery systems.”

Since its establishment in 2003 as the world’s first institute to specialize in bioengineering and nanotechnology, IBN has been steadily building on its many achievements in drug and gene delivery, microfabrication technologies, and cell and tissue engineering. Situated in the heart of Biopolis — one of Singapore’s premier research hubs dedicated to advancing biomedical sciences through a wide variety of public and private partnerships — and with over 150 scientists, engineers and medical specialists currently based at the Institute, IBN is uniquely geared towards playing an active role in transferring innovative developments from the laboratory bench to industry.

Commenting on the remarkable achievements borne out of collaborative efforts at the Institute and the hotly-pursued research area of regenerative medicine, IBN executive director Jackie Y. Ying says, “This innovative application of microfibers with viral vectors is an exciting development for gene therapy that was made possible through multidisciplinary collaboration between biologists, chemists and materials scientists at IBN. Our fibrous materials are also of great interest as biocompatible tissue engineering scaffolds for applications in regenerative medicine.”

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

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

A*STAR currently oversees the following research institutes, consortia and centers and supports extramural research with universities, hospital research centers, and other local and international partners.

A*STAR Computational Resource Centre (A*CRC)
Bioinformatics Institute (BII)
Bioprocessing Technology Institute (BTI)
Data Storage Institute (DSI)
Experimental Power Grid Centre (EPGC)
Experimental Therapeutics Centre (ETC)
Genome Institute of Singapore (GIS)
Institute for Infocomm Research (I2R)
Institute of Bioengineering and Nanotechnology (IBN)
Institute of Chemical & Engineering Sciences (ICES)
Institute of High Performance Computing (IHPC)
Institute of Materials Research and Engineering (IMRE)
Institute of Medical Biology (IMB)
Institute of Microelectronics (IME)
Institute of Molecular and Cell Biology (IMCB)
National Metrology Centre (NMC)
SERC Nanofabrication and Characterisation Facility (SNFC)
Singapore Bioimaging Consortium (SBIC)
Singapore Cancer Syndicate (SCS)
Singapore Consortium of Cohort Studies (SCCS)
Singapore Immunology Network (SIgN)
Singapore Institute for Clinical Sciences (SICS)
Singapore Institute of Manufacturing Technology (SIMTech)
Singapore Stem Cell Consortium (SSCC)
The Chemical Synthesis Laboratory (CSL)