THE CNST NEWS

FALL 2018

A DISCOVERY THAT REALLY TAKES THE CAKE

INSIDE

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IN THIS ISSUE

Cover image: When electrons in a graphene sample are magnetically confined inside quantum dots, they distribute themselves in a shape resembling a wedding cake. Credit: Christopher Gutiérrez/NIST.

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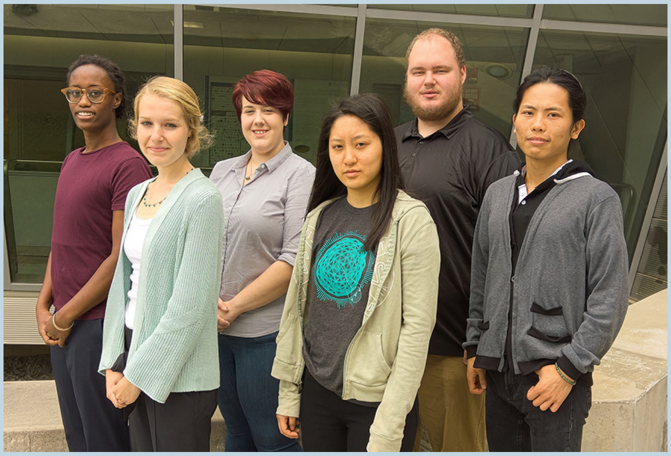


Back Row: Holland Rhodd-Lee, Devin Jessup, and Stephen Tovcimak. Front Row: Emma Rogers, Shannon Jin, and Hengming Li. Not pictured: Erik Isele and Thomas Marsh. Credit: Dill/NIST

CNST JOINS THE PHYSICAL MEASUREMENT LABORATORY

The new fiscal year heralds some significant changes. Beginning October 1, the CNST will become part of the Physical Measurement Laboratory. Merging CNST with PML will enable more effective management of programs and resources that previously spanned both organizations. Research staff from the CNST NanoLab and PML will be combined to form two new divisions within PML. The CNST NanoFab user facility will largely remain unchanged and continue to serve both NIST and external users and will retain the CNST moniker.

Left: PML acting director Carl Williams and Right: Jim Kushmerick, PML's acting deputy director. Credit: Suplee/NIST & Dill/NIST

"We welcome the CNST staff and look forward to the breadth of knowledge and experience that the staff brings with it," says PML acting director Carl Williams. "Both PML and CNST will benefit from a sharing of resources and ideas."

"I know firsthand, having been deputy director of CNST, how much the CNST personnel will contribute to PML and will continue to contribute to the overall NIST mission," says Jim Kushmerick, PML's acting deputy director.

PML's new Microsystems and Nanotechnology Division will focus on advancing nanofabrication technologies and developing integrated measurement microsystems. Staff will also collaborate with industries, universities, standards groups and other government agencies to enhance technology transfer and support U.S. industry. This division comprises six groups—photonics and plasmonics, optomechanics and microsystems, single emitters, biophysics, biomedical microtechnologies and nanoscale and nanostructure metrology.

The other new PML division, Nanoscale Device Characterization, aims to develop and advance measurements to characterize atomic- and nanoscale engineered materials and devices for innovation in computing, informatics and sensing. The division is divided into five groups—atomic scale devices, nanoscale spectroscopy, nanoscale imaging, nanoscale processes and measurements, and alternative computing.

While this will be the last issue of the *CNST News*, nanotechnology and nanoscience stories about NIST research will be an important part of the bi-monthly PML newsletter and will continue to be featured in *Tech Beats* and news releases distributed by NIST's public affairs office. To subscribe to the PML newsletter, go to the PML home page, scroll down the left-hand column, and click on the button that says "Connect with Us."

A NOVEL GRAPHENE QUANTUM DOT STRUCTURE TAKES THE CAKE

In a marriage of quantum science and solid-state physics, researchers at the National Institute of Standards and Technology (NIST) have used magnetic fields to confine groups of electrons to a series of concentric rings within graphene, a single layer of tightly packed carbon atoms.

This tiered "wedding cake," which appears in images that show the energy level structure of the electrons, experimentally confirms how electrons interact in a tightly confined space according to long-untested rules of quantum mechanics. The findings could also have practical applications in quantum computing.

Graphene is a highly promising material for new electronic devices because of its mechanical strength, its excellent ability to conduct electricity and its ultrathin, essentially two-dimensional structure. For these reasons, scientists welcome any new insights on this wonder material.

The researchers, who report their findings in the Aug. 24 issue of *Science*, began their experiment by creating quantum dots—tiny islands that act as artificial atoms—in graphene devices cooled to just a few degrees above absolute zero.

Electrons orbit quantum dots in a way that's very similar to how they orbit atoms. Like rungs on a ladder, they can only occupy specific energy levels according to the rules of quantum theory. But something special happened when the researchers applied a magnetic field, which further confined the electrons orbiting the quantum dot. When the applied field reached a strength of about 1 Tesla (some 100 times the typical strength of a small bar magnet), the electrons packed closer together and interacted more strongly.

As a result, the electrons rearranged themselves into a novel pattern: an alternating series of conducting and insulating concentric rings on the surface. When the researchers stacked images of the concentric rings recorded at different electron energy levels, the resulting picture resembled a wedding cake, with electron energy as the vertical dimension.

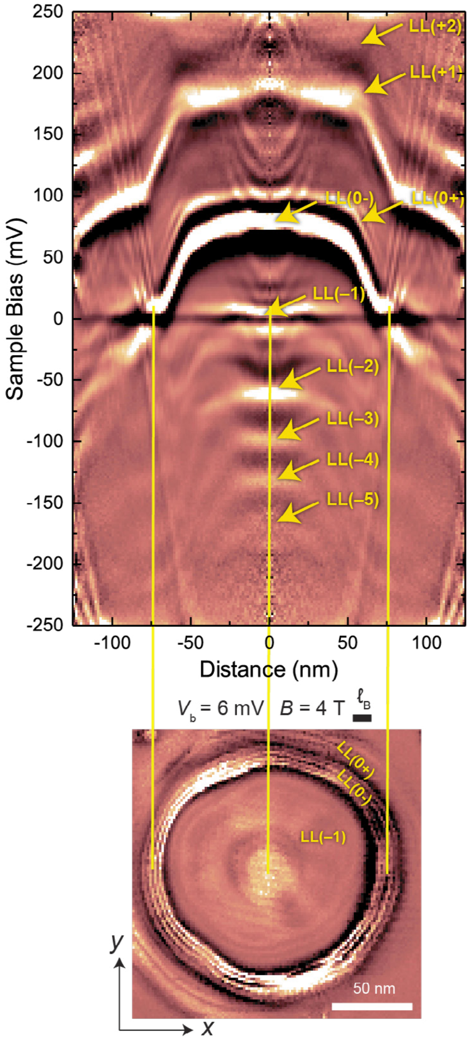
A scanning tunneling microscope, which images surfaces with atomic-scale resolution by recording the flow of electrons between different regions of the sample and the ultrasharp tip of the microscope's stylus, revealed the structure.

"This is a textbook example of a problem—determining what the combined effect of spatial and magnetic confinement of electrons looks like—that you solve on paper when you're first exposed to quantum mechanics, but that no one's actually seen before," said NIST scientist and co-author Joseph Stroscio. "The key is that graphene is a truly two-dimensional material with an exposed sea of electrons at the surface," he added. "In previous experiments using other materials, quantum dots were buried at material interfaces so no one had been able to look inside them and see how the energy levels change when a magnetic field was applied."

Graphene quantum dots have been proposed as fundamental components of some quantum computers.

"Since we see this behavior begin at moderate fields of just about 1 Tesla, it means that these electron-electron interactions will have to be carefully accounted for when considering certain types of graphene quantum dots for quantum computation," said study co-author Christopher Gutiérrez, now at the University of British Columbia in Vancouver, who performed the experimental work at NIST with co-authors Fereshte Ghahari and Daniel Walkup of NIST and the University of Maryland.

This achievement also opens possibilities for graphene to act as what the researchers call a "relativistic quantum simulator." The theory of relativity describes how objects behave when moving at or close to light speed. And electrons in graphene possess an unusual property—they move as if they are massless, like particles of light. Although electrons in graphene actually travel far slower than the speed of light, their light-like massless behavior has earned them the moniker of "relativistic" matter. The new study opens the door to creating a table-top experiment to study strongly confined relativistic matter.



Scanning tunneling spectroscopy image shows that magnetically confined electrons are arranged in a wedding cake-like structure of energy levels (top panel), which creates a series of insulating and conducting rings within graphene (bottom panel). Credit: NIST

Collaborators on this work included researchers from the Massachusetts Institute of Technology, Harvard University, the University of Maryland NanoCenter and the National Institute for Material Science in Ibaraki, Japan.

The measurements suggest that scientists may soon find even more exotic structures produced by the interactions of electrons confined to solid-state materials at low temperatures.

Christopher Gutiérrez, Daniel Walkup, Fereshte Ghahari, Cyprian Lewandowski, Joaquin F. Rodriguez-Nieva, Kenji Watanabe, Takashi Taniguchi, Leonid S. Levitov, Nikolai B. Zhitenev and Joseph A. Stroscio. Interaction Driven Quantum Hall Wedding cake-like Structures in Graphene Quantum Dots. *Science*. Published August 24, 2018. DOI: 10.1126/science.aar2014

THROUGH A PRISM BRIGHTLY: A NEW WAY TO MEASURE THE LIGHT-WARPING PROPERTIES OF HYPERBOLIC METAMATERIALS

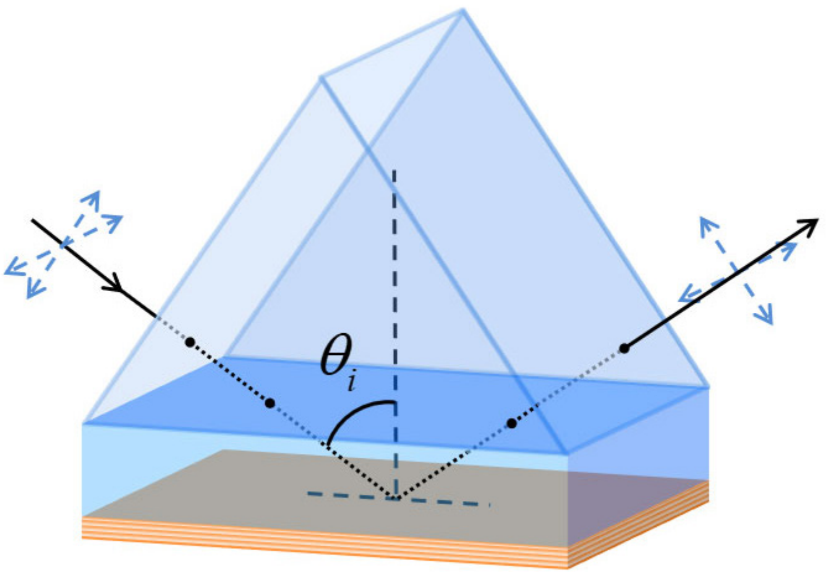


Illustration of incident light traveling through a glass prism before entering and after exiting the hyperbolic metamaterial, which is composed of stacked metal and transparent oxide layers. By sending incident light through the glass prism, the researchers minimize the mismatch between the wavelengths of light in air and in the hyperbolic metamaterial. This enables the light to penetrate farther into the metamaterial and more accurately assess its electromagnetic properties. Credit: NIST

Manipulating light in a variety of ways—shrinking its wavelength and allowing it to travel freely in one direction while stopping it cold in another—hyperbolic metamaterials have wide application in optical communications and as nanoparticle sensors. But some of the same optical properties that make these metamaterials so appealing make them frustratingly difficult to evaluate.

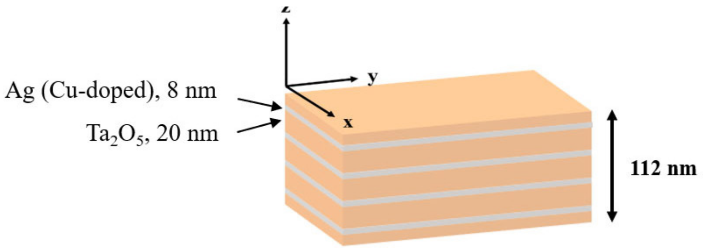
For example, the mismatch between the wavelength of incident light, traveling through air, and the much shorter wavelength inside these metamaterials typically prevents the incident light from penetrating very far. That property can be used to create a nanoparticle sensor but poses a problem for measuring just how well a hyperbolic metamaterial performs its light-warping feats, characterized by an electrical property known as permittivity. If light can't probe deeply into a hyperbolic metamaterial, it can't accurately assess the permittivity.

CNST researchers have now developed a new measurement method that circumvents this difficulty. Using an off-the-shelf glass prism to enhance the interaction of incident light with hyperbolic metamaterials, a team led by Cheng Zhang of the CNST and the University of Maryland's NanoCenter and Henri Lezec of NIST has devised a simple and much more accurate way to determine the permittivity.

Zhang, Lezec and their colleagues, who include researchers from the J.A. Woollam Co. in Lincoln, Nebraska, and the University of Michigan in Ann Arbor, described their findings in a recent issue of *ACS Photonics*.

The glass prism serves two functions. Light traveling through glass has a wavelength intermediate in size between that of the incident light and the light inside a hyperbolic metamaterial. By sending light into the glass prism before it enters the hyperbolic metamaterial, the researchers minimize the mismatch in wavelength, enabling the light to penetrate farther into the material. In addition, the shape of the prism directs the light to strike the hyperbolic metamaterial at the optimum angle to probe the material.

Because the technique uses an off-the-shelf prism and does not require any modification of the hyperbolic metamaterial, it promises to serve as a reliable and easy to adopt method to characterize a broad class of highly anisotropic materials—structures whose optical properties depend on the angle at which light strikes the surface. As Zhang and his colleagues fabricate more complex versions of such materials, using nanoengineered layers of different compounds, such measurements will take on added importance.



Cross-sectional view of the hyperbolic metamaterial examined in the new study shows layers of copper-doped silver and tantalum pentoxide. Credit: NIST

Cheng Zhang, Nina Hong, Chengang Ji, Wenqi Zhu, Xi Chen, Amit Agrawal, Zhong Zhang, Tom E. Tiwald, Stefan Schoeche, James N. Hilfiker, L. Jay Guo, and Henri J. Lezec. Robust Extraction of Hyperbolic Metamaterial Permittivity Using Total Internal Reflection Ellipsometry. *ACS Photonics*, Publication Date (Web): March 13, 2018. DOI: 10.1021/acsphotonics.8b00086

NANOPARTICLE MANUFACTURING: LOOKING AT THE BIG PICTURE

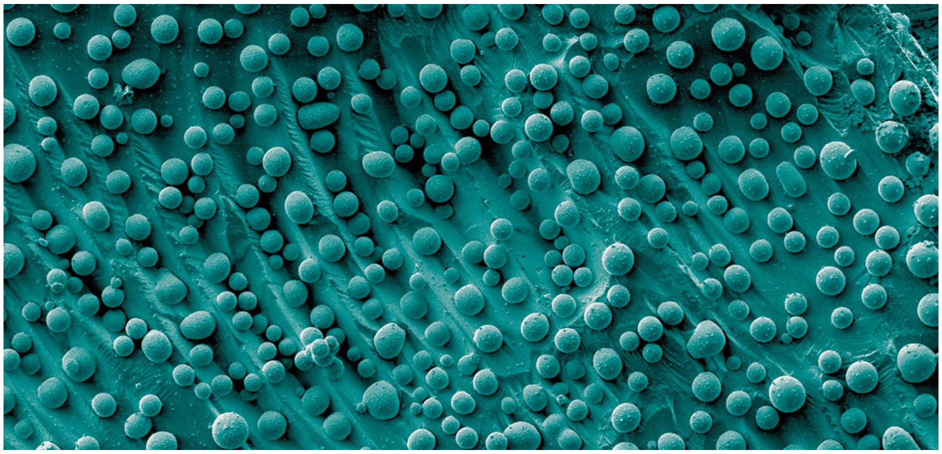
Nanoparticle manufacturing, the production of material units less than 100 nanometers in size (100,000 times smaller than a marble), is proving the adage that "good things come in small packages." Today's engineered nanoparticles are integral components of everything from the quantum dot nanocrystals coloring the brilliant displays of state-of-the-art televisions to the miniscule bits of silver helping bandages protect against infection. However, commercial ventures seeking to profit from these tiny building blocks face quality control issues that, if unaddressed, can reduce efficiency, increase production costs and limit commercial impact of the products that incorporate them.

To help overcome these obstacles, the National Institute of Standards and Technology (NIST) and the nonprofit World Technology Evaluation Center (WTEC) advocate that nanoparticle researchers, manufacturers and administrators "connect the dots" by considering their shared challenges broadly and tackling them collectively rather than individually. This includes transferring knowledge across disciplines, coordinating actions between organizations and sharing resources to facilitate solutions.

The recommendations are presented in a new paper in the journal *ACS Applied Nano Materials*.

"We looked at the big picture of nanoparticle manufacturing to identify problems that are common for different materials, processes and applications," said NIST physical scientist Samuel Stavis, lead author of the paper. "Solving these problems could advance the entire enterprise."

The new paper provides a framework to better understand these issues. It is the culmination of a study initiated by a workshop organized by NIST that focused on the fundamental challenge of reducing or mitigating heterogeneity, the inadvertent variations in nanoparticle size, shape and other characteristics that occur during their manufacture.



Electron micrograph showing gallium arsenide nanoparticles of varying shapes and sizes. Such heterogeneity can increase costs and limit profits when making nanoparticles into products. A new NIST study recommends that researchers, manufacturers and administrators work together to solve this, and other common problems, in nanoparticle manufacturing. Credit: A. Demotiere, E. Shevchenko/Argonne National Laboratory

"Heterogeneity can have significant consequences in nanoparticle manufacturing," said NIST chemical engineer and co-author Jeffrey Fagan.

In their paper, the authors noted that the most profitable innovations in nanoparticle manufacturing minimize heterogeneity during the early stages of the operation, reducing the need for subsequent processing. This decreases waste, simplifies characterization and improves the integration of nanoparticles into products, all of which save money.

The authors illustrated the point by comparing the production of gold nanoparticles and carbon nanotubes. For gold, they stated, the initial synthesis costs can be high, but the similarity of the nanoparticles produced requires less purification and characterization. Therefore, they can be made into a variety of products, such as sensors, at relatively low costs.

In contrast, the more heterogeneous carbon nanotubes are less expensive to synthesize but require more processing to yield those with desired properties. The added costs during manufacturing currently make high purity nanotubes only practical for high-value applications such as digital logic devices.

"Although these nanoparticles and their end products are very different, the stakeholders in their manufacture can learn much from each other's best practices," said NIST materials scientist and co-author J. Alexander Liddle. "By sharing knowledge, they might be able to improve both seemingly disparate operations."

Finding ways like this to connect the dots, the authors said, is critically important for new ventures seeking to transfer nanoparticle technologies from laboratory to market.

"Nanoparticle manufacturing can become so costly that funding expires before the end product can be commercialized," said WTEC nanotechnology consultant and co-author Michael Stopa. "In our paper, we outlined several opportunities for improving the odds that new ventures will survive their journeys through this technology transfer 'valley of death.'"

Finally, the authors considered how manufacturing challenges and innovations are affecting the ever-growing number of applications for nanoparticles, including those in the areas of electronics, energy, health care and materials.

S.M. Stavis, J.A. Fagan, M. Stopa and J.A. Liddle. Nanoparticle manufacturing—heterogeneity through processes to products. *ACS Applied Nano Materials*, August 2018. DOI: 10.1021/acsanm.8b01239

NIST PUTS THE OPTICAL MICROSCOPE UNDER THE MICROSCOPE TO ACHIEVE ATOMIC ACCURACY

Over the last two decades, scientists have discovered that the optical microscope can be used to detect, track and image objects much smaller than their traditional limit—about half the wavelength of visible light, or a few hundred nanometers.

That pioneering research, which won the 2014 Nobel Prize in Chemistry, has enabled researchers to track proteins in fertilized eggs, visualize how molecules form electrical connections between nerve cells in the brain, and study the nanoscale motion of miniature motors.

Now, research developments at the National Institute of Standards and Technology (NIST) enable the microscopes to measure these nanometer-scale details with a new level of accuracy.

“We put the optical microscope under a microscope to achieve accuracy near the atomic scale,” said NIST’s Samuel Stavis, who served as the project leader for these efforts.

Because optical microscopes have not traditionally been used to study the nanometer scale, they typically lack the calibration—comparison to a standard to check that a result is correct—necessary to obtain information that is accurate at that scale. A microscope may be precise, consistently indicating the same position for a single molecule or nanoparticle, but at the same time it can be highly inaccurate. The location of the object identified by the microscope to within a billionth of a meter may, in fact, be millionths of a meter off due to unaccounted-for errors. “Precision without accuracy can be very misleading,” said Jon Geist, a NIST co-author of the study.

To address the problem, NIST has developed a new calibration process that closely examines and corrects these imaging errors.

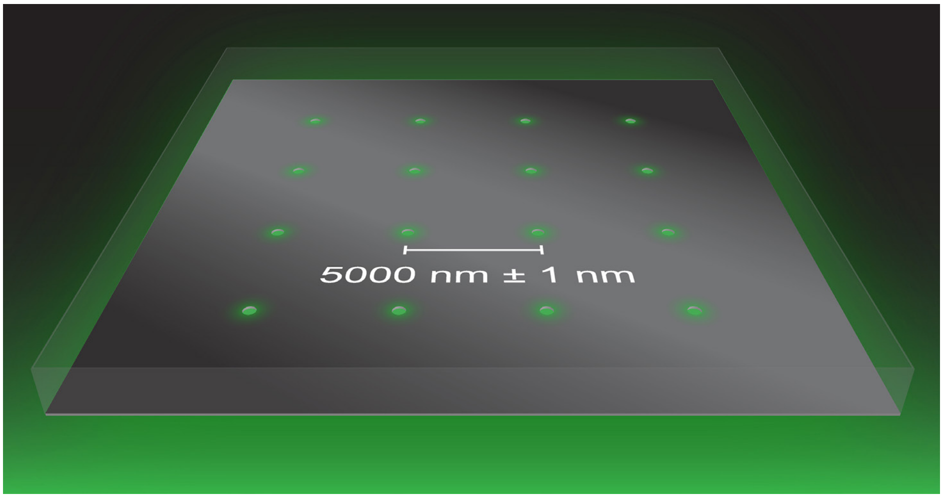


Illustration shows an array of apertures with a spacing of 5000 nanometers (nm) ± 1 nm. The apertures pass light through a metal film on a glass slide. Imaging the aperture array with an optical microscope results in apparent errors in the spacing between apertures. Knowledge of the true spacing allows correction of these imaging errors. This calibration process enables accurate measurements of position across a large image. Credit: NIST

The process uses reference materials—objects with characteristics that are well-known and stable—that have the potential for mass production and widespread distribution to individual laboratories.

This is important because optical microscopes are common laboratory instruments that can easily magnify different samples, ranging from delicate biological specimens to electrical and mechanical devices. As well, optical microscopes are becoming increasingly capable and economical as they incorporate scientific versions of the lights and cameras in smartphones.

The NIST team relied on nanometer-scale fabrication processes to develop the reference material. The researchers used electron beam lithography and ion milling to form an array of pinhole apertures through a thin film of platinum on a glass slide. The process enabled the team to space the apertures 5,000 nanometers apart, to within an accuracy of about 1 nanometer. In this way, the researchers built a measure of accuracy into the aperture positions.

Shining light through the array of apertures creates an array of points for imaging. But because all microscope lenses have imperfections, errors inevitably occur during imaging that change the apparent positions of the points, making the spacing between the apertures appear to be larger or smaller than the actual spacing engineered by the team. Knowledge of the true spacing allows correction of the imaging errors and calibration of the microscope for measurements of position with high accuracy across a wide field of view.

Even a small error can lead to a large problem. Consider, for example, a microscope having an actual magnification of 103 times when the expected magnification, as specified by the manufacturer, is 100 times. The resulting error of 3 percent adds up over large distances across a microscope image. Because of lens imperfections, a subtler problem also occurs—the microscope magnification changes across the image, causing image distortion. To solve this problem, the NIST team designed aperture

continued on page 9

Craig R. Copeland, Jon Geist, Craig D. McGray, Vladimir A. Aksyuk, J. Alexander Liddle, B. Robert Ilic and Samuel M. Stavis. Subnanometer localization accuracy in widefield optical microscopy.
Light: Science & Applications. Accepted article posted online 16 May 2018. DOI: 10.1038/s41377-018-0031-z

HELPING THE MICROCHIP INDUSTRY GO *(VERY LOW)* WITH THE FLOW

A new NIST analysis reveals a heated source of potentially expensive errors.

Process Engineer Richard Kasica of NIST's Center for Nanoscale Science and Technology holds a wafer of the type typically used in the plasma-enhanced chemical vapor deposition chamber at center. Credit: NIST

A new study by scientists at the National Institute of Standards and Technology (NIST) has uncovered a source of error in an industry-standard calibration method that could lead microchip manufacturers to lose a million dollars or more in a single fabrication run. The problem is expected to become progressively more acute as chipmakers move to pack ever more features into ever smaller space.

The error affects a process called chemical vapor deposition (CVD), which occurs inside a vacuum chamber as an ultra-rarefied gas flows across a silicon wafer to form a solid film. CVD is widely used to fabricate many kinds of high-performance microchips containing as many as several billion transistors each. It creates complex 3D structures by building up successive layers—sometimes as thin as a few billionths of a meter—of different materials. A complementary process called plasma etching uses small flows of exotic gases to produce tiny features on the surface of semiconducting materials.

The exact amount of vapor injected into the chamber is critically important to these processes and is regulated by a device called a mass flow controller (MFC). MFCs must be highly accurate to ensure that the deposited layers have the required dimensions. The potential impact is large because chips with incorrect layer depths must be discarded.

"Flow inaccuracies cause nonuniformities in critical features in wafers, directly causing yield reduction," said Mohamed Saleem, Chief Technology Officer at Brooks Instrument, a U.S. company that manufactures MFCs among other precision measurement devices. "Factoring in the cost of running cleanrooms, the loss on a batch of wafers scrapped due to flow irregularities can run around $500,000 to $1,000,000. Add to that cost the process tool downtime required for troubleshooting, and it becomes prohibitively expensive."

Modern nanofabrication facilities cost several billion dollars each, and it is generally not cost-effective for a company to engage in constant fine tuning.

MFCs are typically calibrated using the "rate of rise" (RoR) method, which makes a series of pressure and temperature measurements over time as gas fills a collection tank through the MFC.

"Concerns about the accuracy of that technique came to our attention recently when a major manufacturer of chip-fabrication equipment found that they were getting inconsistent results for flow rate from their own instruments when they were calibrated on different RoR systems," said John Wright of NIST's Fluid Metrology Group, whose members conducted the error analysis.

Wright was particularly interested because for many years he had seen that RoR readings didn't agree with results obtained with NIST's "gold standard" pressure/volume/temperature/time system. He and colleagues developed a mathematical model of the RoR process and conducted detailed experiments. The conclusion: Conventional RoR produces errors because it does not correct for higher temperatures caused by the heat of compression.

"Very simply stated, gas that's running through the pipe into the collection tank has kinetic energy, but eventually stops moving," Wright said. "Where does that energy go? It turns into heat. And that is not easily accounted for: it is difficult to measure the temperature of stationary gas."

Wright and colleagues found that RoR readings can be off by as much as 1 percent, and perhaps considerably more. That might not seem like a lot, but current low-end flow rates for CVD in the semiconductor industry are in the range of one standard cubic

HELPING THE MICROCHIP INDUSTRY GO *(VERY LOW)* WITH THE FLOW (CONT'D.)

centimeter (1 sccm)—about the volume of a sugar cube—per minute. And they will soon shrink by a factor of 10.

Precise flow measurement is a particularly serious concern for manufacturing processes that use etching of deposited layers to form trench-like features. In that case, the MFC is often open for no more than a few seconds.

"A tiny amount of variation in the flow rate has a profound effect on the etch rate and critical dimensions of the structures" in very large-scale integrated circuits, said Iqbal Shareef of Lam Research, a company headquartered in California that provides precision fabrication equipment to microchip manufacturers.

"So, we are extremely concerned about flow rates being accurate and consistent from chamber to chamber and wafer to wafer," Shareef said. "Our industry is already headed toward 0.2 or 0.3 sccm flow rates, and it will be down to 0.1 sccm very soon."

"We are talking about wafer uniformity today on the nanometer and even subnanometer scale," Shareef said.

That's *very* small. But it's what the complexity of three-dimensional chip manufacturing increasingly demands. Not so long ago, "a 3D integrated circuit used to have four layers of metals," said William White, Director of Advanced Technology at HORIBA Instruments Incorporated, a global firm that provides analytical and measurement systems. "Now companies are regularly going to 32 layers and sometimes to 64. Just this year I heard about 128." And some of those chips have as many as 3,000 process steps.

Many companies are already re-examining their practices in light of the NIST publication, which provides needed theoretical explanations for the source of RoR flow measurement errors. The theory guides designers of RoR collection tanks and demonstrates easy-to-apply correction methods. RoR theory shows that different temperature errors will occur for the different gases used in CVD processes. The NIST publication also provides a model uncertainty analysis that others can use to know what level of agreement to expect between MFCs calibrated on different RoR systems.

"NIST serves as a reliable reference for knowledge and measurement where industry can assess agreement of their systems," Wright said. "As manufacturers' measurement needs push to ever lower flows, so will NIST calibration standards.

"Each 300 mm wafer costs about $400, and contains 281 dies," Brooks' Saleem said. "Each die in today's high-end integrated circuits consists of about three to four billion transistors. Each wafer goes through 1-2 months of processing that includes multiple runs of separate individual processes," including chemical vapor deposition, etch, lithography and ion implantation. All those use expensive chemicals and gases.

NIST PUTS THE OPTICAL MICROSCOPE UNDER THE MICROSCOPE TO ACHIEVE ATOMIC ACCURACY (CONT'D. FROM PAGE 7)

arrays and calibration processes that worked across large fields of view.

The aperture arrays, which would enable individual researchers to perform calibrations in their own laboratories, could improve by a factor of 10,000 the ability of optical microscopes to accurately locate the position of single molecules and nanoparticles.

Stavis and his colleagues, including first author Craig Copeland of NIST and the Maryland NanoCenter at the University of Maryland, reported their findings in a recently posted article in *Light: Science & Applications*.

"We have identified and solved an underappreciated problem," said Copeland.

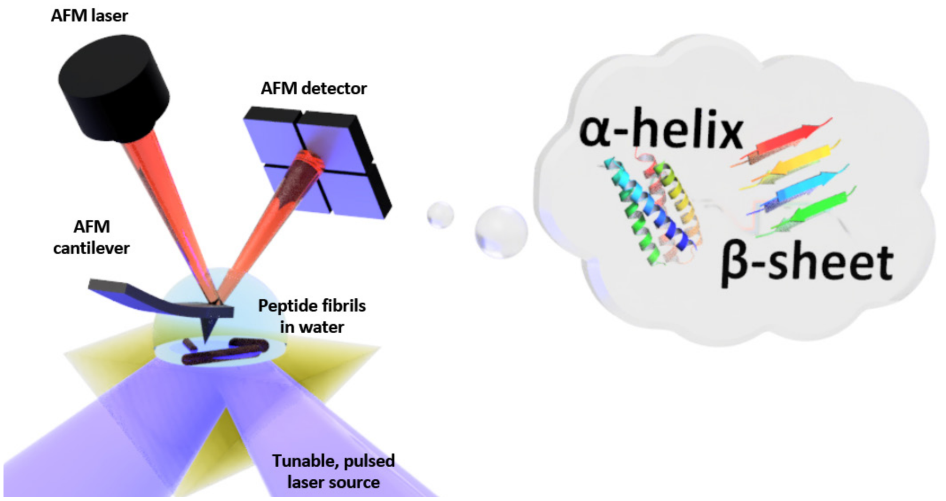
Having calibrated their optical microscope using the arrays, the team reversed the process, using their microscope to identify imperfections in the prototype arrays from the nanofabrication process. "We tested the limits of nanofabrication to control the aperture spacing," noted co-author Rob Ilic, manager of NIST's NanoFab. The ease and speed of optical microscopy could facilitate quality control of aperture arrays in a production process.

Finally, the team exploited the inherent stability of the aperture arrays to evaluate whether fluorescent nanoparticles, often used as fixed points of reference in optical microscopy, actually remained fixed to a particular point or if they moved around. The researchers found that while unintentional motions of their optical microscope made views of the nanoparticles blurry, using the aperture array showed that the nanoparticles were not actually moving at atomic scales.

THE SHAPE IN WATER: NANOMETER-SCALE MEASUREMENTS OF THE

NIST-developed method could provide new insights into Alzheimer's and other diseases.

Tinkering with a method they helped develop over the last few years, scientists have measured at the nanometer scale the characteristic patterns of folds that give proteins their three-dimensional shape in water. Developed by researchers at the National Institute of Standards and Technology (NIST) and their colleagues, this technique will help scientists gain insights about the behavior of biomolecules in watery environments similar to those in cells. These insights, in turn, could increase our understanding of major diseases, including Alzheimer's, that are related to "mistakes" in protein folding.



Schematic of the setup for photo-thermal induced resonance (PTIR), which includes an infrared laser source and atomic force microscope (AFM) cantilever with a sharp tip that touches the sample and vibrates in response to the sample's light-induced expansion. PTIR can determine the folding pattern (called for example α-helix, β-sheet) of peptides (amino acid chains) in water with nanometer-scale resolution. Credit: NIST

Life as we know it couldn't survive if proteins didn't fold into precise patterns leading to helices, sheets and other shapes that give proteins their three-dimensional structure. The precise shapes of proteins enable them to carry oxygen, fend off harmful bacteria and perform other essential tasks in the body. Proteins that fold improperly cannot function and sometimes generate toxic fragments, such as those associated with neurodegenerative disorders.

To understand the intricacies of folding, scientists need to study the detailed arrangement of chains of amino acids that are shorter and simpler than proteins—called peptides—and how they fold, assemble and rotate to create a variety of shapes, or conformations. Biologists prefer to examine proteins and peptides immersed in water because that environment closely approximates the conditions inside living cells.

Previously established techniques for determining the conformation of proteins, such as infrared spectroscopy, lack the fine spatial resolution to study the tiny and diverse assemblies of properly folded and misfolded proteins. In addition, these techniques don't work well in an aqueous environment because water strongly absorbs infrared light, confounding the analysis. Water had also posed severe challenges for a pioneering technique, known as photo-thermal induced resonance (PTIR), that recently enabled researchers to examine peptide structure and conformation in air with nanoscale resolution.

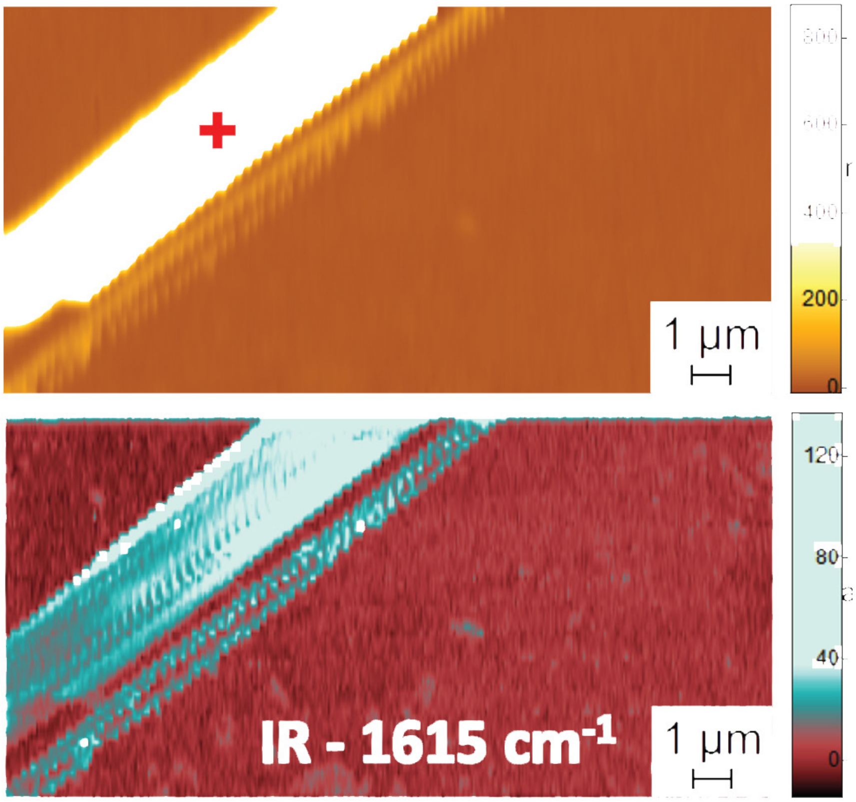
NIST researchers and their colleagues have now demonstrated that PTIR can be adapted to obtain conformational structure at the nanoscale *in water* using two chemically similar peptides known as diphenylalanine and Boc-diphenylalanine. Diphenylalanine is related to beta-amyloid, a sticky, larger peptide linked to Alzheimer's disease.

"PTIR is a powerful technique that had already shown promise for the study of biological systems, but the possibility to use this with samples in a liquid environment will greatly improve its use in this area," said Georg Ramer of NIST and the University of Maryland in College Park.

Ramer and NIST researcher Andrea Centrone, along with their colleagues at the University of Cambridge in England, described their work in an article recently posted online at *ACS Nano*.

G. Ramer, F.S. Ruggeri, A. Levin, T.P. J. Knowles and A. Centrone. Determination of Polypeptide Conformation with Nanoscale Resolution in Water. *ACS Nano*. Published online June 22, 2018.
DOI: 10.1021/acsnano.8b01425

FOLDED STRUCTURE OF BIOMOLECULES IN LIQUIDS



Atomic force microscope image showing topography (top) and PTIR absorption image indicating composition and conformation (bottom) of a diphenylalanine peptide fibril in water. Additional data, recording the spectrum of infrared radiation absorbed by the peptide fibrils, provides information on their folding pattern. The PTIR spectrum indicated, for example, that diphenylalanine assumes a pure anti-parallel β-sheet conformation. Credit: NIST

PTIR determines the chemical composition of materials with nanoscale resolution by combining an atomic force microscope (AFM) with light from an infrared laser that operates over a range of wavelengths. The characteristic wavelengths of infrared light that are absorbed by the sample are akin to a molecular fingerprint, revealing its chemical composition. At each site on the sample where infrared is absorbed, the material heats up, causing it to rapidly, but ever so slightly, expand. The expansion is detected, with the sharp tip of the AFM protruding from a cantilever, which oscillates like a diving board each time the sample expands. The more light that is absorbed by the sample, the greater its expansion and the larger the strength, or amplitude, of the oscillations.

As good as PTIR is, using the method in a water environment is problematic. Water strongly absorbs infrared light, producing an absorption signal that can interfere with efforts to discern the sample's chemical structure. In addition, the drag force exerted by water is much stronger than in air and it typically weakens the PTIR signal, as it strongly damps the oscillations of the AFM's cantilever.

To limit water's absorption of infrared light, the team placed a prism between the laser and the sample. The prism served to confine the infrared light to the sample's surface, minimizing the amount that could leak out and interact with the water. To address the damping problem, the team used a laser that could operate at frequencies up to 2,000 kilohertz. That enabled the researchers to match the frequency of the laser pulses to one of the higher frequencies at which the cantilever oscillates. Like pushing a child on a swing at just the right interval, the frequency matching enhanced the amplitude of the cantilever's oscillations, partially offsetting the damping due to water.

To demonstrate the accuracy of their method, the team compared PTIR measurements of diphenylalanine and other peptide samples in two environments: water and air. (The peptides folded similarly in both mediums, making it easier to perform the comparison.) Remarkably, the scientists achieved similar spatial resolution and contrast in water and air, demonstrating for the first time that measurements in a water environment can be performed accurately, revealing the precise conformation of peptides with nanoscale resolution.

"This finding is important to biologists who want to understand protein structure and folding in environments as close as possible to those in cells," said Centrone.

CNST Center for Nanoscale Science & Technology

SUPPORTING THE DEVELOPMENT OF NANOTECHNOLOGY FROM DISCOVERY TO PRODUCTION

CENTER FOR NANOSCALE SCIENCE AND TECHNOLOGY

The CNST is a national user facility purposely designed to accelerate innovation in nanotechnology-based commerce. Its mission is to operate a national, shared resource for nanoscale fabrication and measurement and develop innovative nanoscale measurement and fabrication capabilities to support researchers from industry, academia, NIST and other government agencies in advancing nanoscale technology from discovery to production. The Center, located in the Advanced Measurement Laboratory Complex on NIST's Gaithersburg, MD campus, disseminates new nanoscale measurement methods by incorporating them into facility operations, collaborating and partnering with others and providing international leadership in nanotechnology.

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