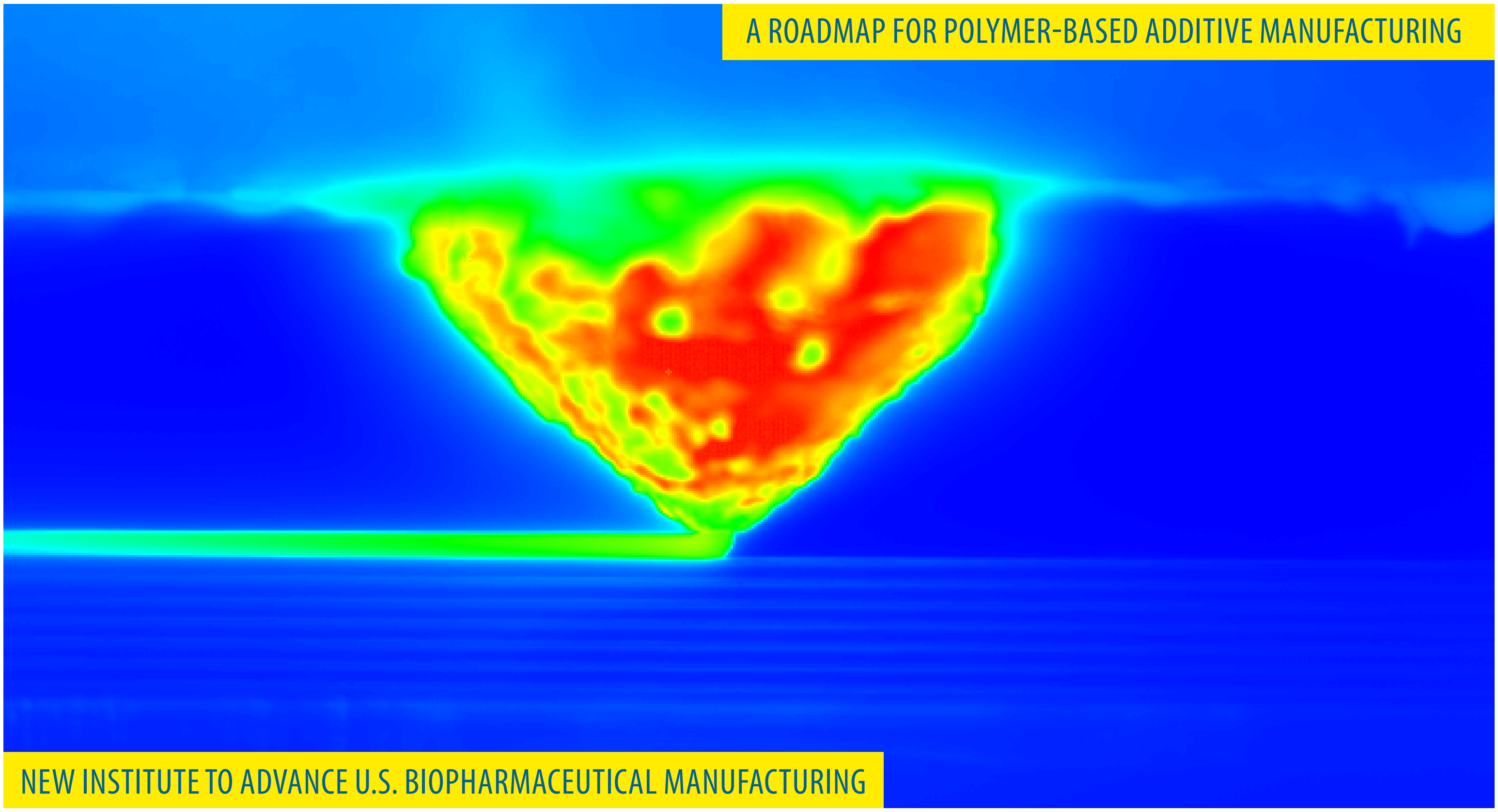
WINTER 2017

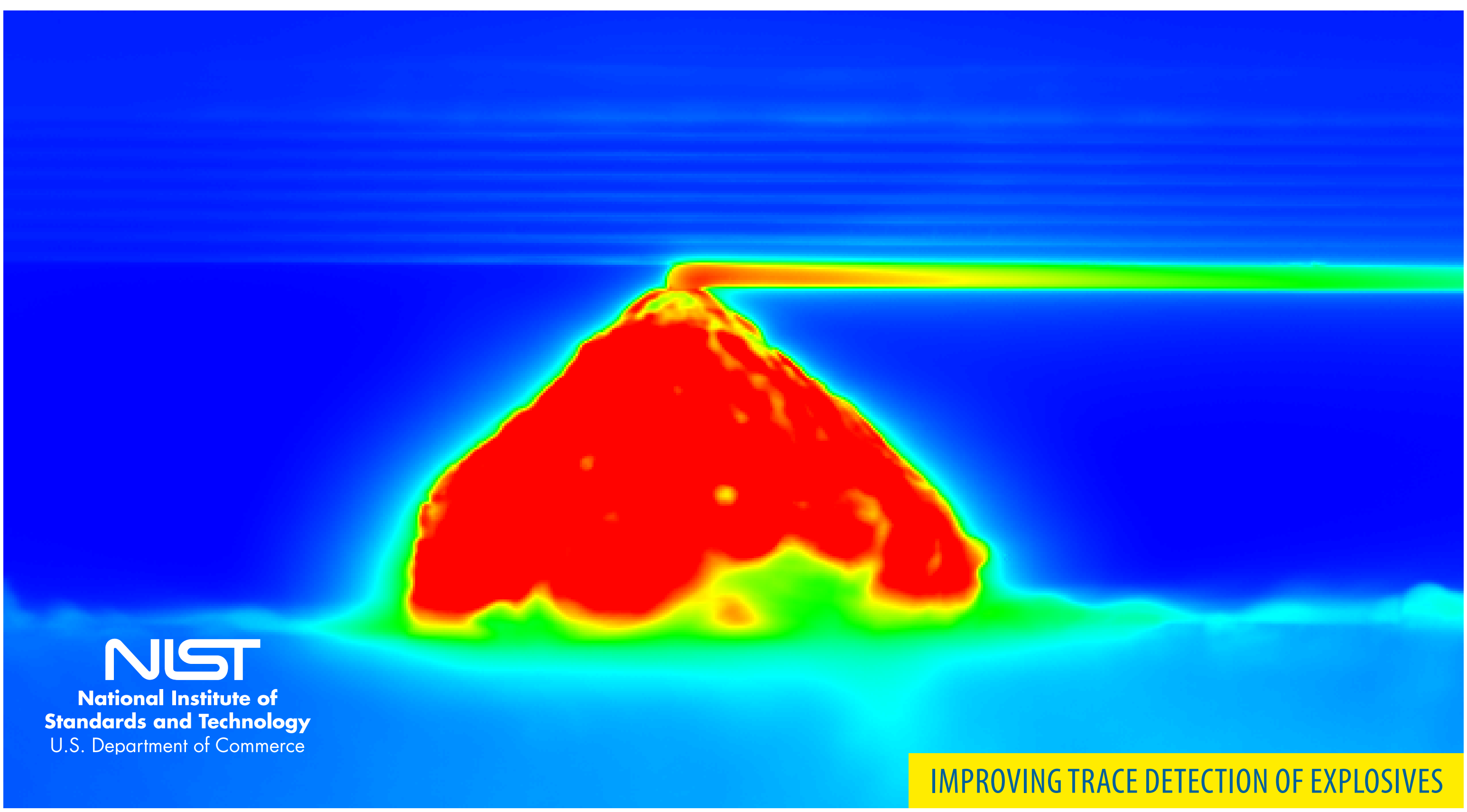
MATERIAL MATTERS

The Quarterly Magazine of NIST's Material Measurement Laboratory

A ROADMAP FOR POLYMER-BASED ADDITIVE MANUFACTURING



NEW INSTITUTE TO ADVANCE U.S. BIOPHARMACEUTICAL MANUFACTURING



IMPROVING TRACE DETECTION OF EXPLOSIVES

NIST
National Institute of Standards and Technology
U.S. Department of Commerce

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Cover Image: IR thermography measurement of materials extrusion additive manufacturing. Credit: Jon Seppala

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A MESSAGE FROM THE MML DIRECTOR

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In December 2016 at the University of Delaware, an extraordinary group of people celebrated the launch of the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), one of 14 centers in the Manufacturing USA network. Manufacturing USA centers, established with federal and matching private-sector investments, bring experts together from across entire industries to address critical technical challenges in advanced manufacturing technology areas and spur innovation.

The relatively new biopharmaceutical industry has unique manufacturing challenges because its products are made by living cells in complex, changing environments. While biopharmaceuticals can be difficult to make, their success at treating cancers, autoimmune disorders, and other diseases that resist traditional therapies places them in high demand: The biopharmaceutical market, which has been led by U.S. companies, generates $200 billion in revenue worldwide and grows each year. But the U.S. faces increasing global competition. Overseas, governments are investing in infrastructure and workforce training to develop advanced manufacturing capabilities in biotech and biopharmaceuticals.

NIIMBL, the first Manufacturing USA institute funded by the Department of Commerce and the first chosen through an open competition where industry suggested the topic, will buttress U.S. leadership in biopharmaceuticals by improving the efficiency of manufacturing and making existing and new therapies available to more patients, faster. NIST's Material Measurement Lab will contribute by working with NIIMBL to develop new standards and measurement and monitoring methods for biopharmaceuticals' raw materials and production process. These efforts will yield more than medical treatments: Along with colleges and universities, NIIMBL will develop curriculum and training for new workers in the biopharmaceutical industry, which has more available jobs than employees trained to fill them, and salaries that pay more than twice the U.S. average.

What made the attendees at NIIMBL's launch so extraordinary was their commitment, whether they were newly-met collaborators or the fiercest competitors, to a common vision: an enduring infrastructure for a new, fast-growing, powerfully profitable manufacturing environment with tremendous potential to change patient care and boost regional economies.

**During the transition to a new presidential administration, Laurie Locascio, MML director, is serving as NIST's acting associate director for laboratory programs. Michael Fasolka, long-time MML deputy director, is acting director of MML*

U.S. DEPARTMENT OF COMMERCE ANNOUNCES BIOPHARMACEUTICAL MANUFACTURING INSTITUTE JOINING MANUFACTURING USA NETWORK

The U.S. Department of Commerce recently announced an award of $70 million to the new National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), the eleventh institute in the Manufacturing USA network. This is the first institute with a focus area proposed by industry and the first funded by the U.S. Department of Commerce.

NIIMBL will help to advance U.S. leadership in the biopharmaceutical industry, foster economic development, improve medical treatments, and ensure a qualified workforce by collaborating with educational institutions to develop new training programs matched to specific biopharma skill needs.

The announcement was made at the University of Delaware, which will coordinate the institute in partnership with NIST. In addition to the federal funding, the new institute is supported by an initial private investment of at least $129 million from a consortium of 150 companies, educational institutions, research centers, coordinating bodies, non-profits, and Manufacturing Extension Partnerships across the country. The consortium is establishing a new non-profit organization called USA Bio LLC to administer the cooperative agreement with NIST.

This manufacturing innovation institute was awarded under the 2014 bipartisan Revitalize American Manufacturing Innovation Act. It is the first Manufacturing USA "open topic" competition, in which industry was invited to propose institutes dedicated to any advanced manufacturing area not already addressed by another institute.

While government does not steer which new technologies get developed or how universities undertake research, the government does have a critical role to play as a catalyst and a convener. In recognition of this, the Commerce Department-funded institute was chosen from technology areas proposed by industry. Following broad dissemination of a Federal Funding Opportunity announcement, applications to establish the new institute were solicited by NIST and rigorously evaluated against written criteria by an expert panel to make the final selection.

Attendees at the December 16, 2016 NIIMBL announcement event. Photo courtesy of the University of Delaware/Evan Krape

Traditional pharmaceutical production relies on chemistry to create medical treatments. Biopharmaceutical production relies on biology—living cells produce the treatments or their components—which requires a complex manufacturing process. Biomanufacturing is used to produce many widely-used treatments for a growing number of health conditions such as cancer, autoimmune disorders, and infectious diseases—and generating billions of dollars in revenue worldwide. However, innovation is needed to allow more rapid and flexible production to meet health care demands and ensure U.S. leadership in the industry.

The institute will foster collaborative technology development to benefit the industry as a whole, reducing risk for individual companies and lowering barriers for small and medium-sized companies. Its programs will focus on advancing current manufacturing platforms as well as creating new ones for emerging products.

The institute will also seek to develop flexible, rapid manufacturing capabilities that will help to ensure that manufacturers can quickly respond to pandemics and other biological threats. Beyond its research efforts, the institute will support the development of standards that enable advances in biopharmaceutical manufacturing. Collaborating colleges and universities will work with industry to provide education and training programs, curriculum development, and certification standards that will ensure a pipeline of skilled workers.

NIIMBL joins 13 other institutes in the Manufacturing USA network, which are addressing challenges and supporting workforce development in important advanced manufacturing industries. Like all of the institutes in the network, NIIMBL will continue to seek new members and to increase its industry investments.

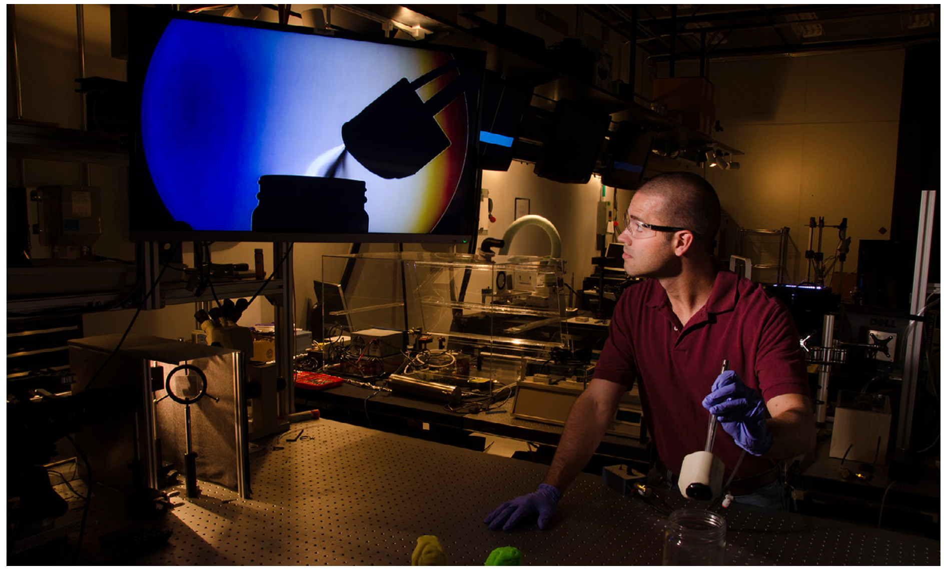
SNIFFING LIKE A DOG CAN IMPROVE TRACE DETECTION OF EXPLOSIVES

By mimicking how dogs get their whiffs, government and university researchers have demonstrated that "active sniffing" can improve by more than 10 times the performance of current technologies that rely on continuous suction to detect trace amounts of explosives and other contraband.

"The dog is an active aerodynamic sampling system that literally reaches out and grabs odorants," explained Matthew Staymates, a mechanical engineer and fluid dynamicist at NIST. "It uses fluid dynamics and entrainment to increase its aerodynamic reach to sample vapors at increasingly large distances. Applying this bio-inspired design principle could lead to significantly improved vapor samplers for detecting explosives, narcotics, pathogens—even cancer."

Following nature's lead, Staymates and colleagues from NIST, the Massachusetts Institute of Technology's Lincoln Laboratory, and the U.S. Food and Drug Administration fitted a dog-nose-inspired adapter to the front end of a commercially available explosives detector. Adding the artificial dog nose—made on a 3-D printer—to enable active sniffing improved odorant detection by up to 18 times, depending on the distance from the source.

Trace detection devices now used at points of entry and departure such as airports, seaports, and other sensitive locations typically employ passive sampling. Examples include equipment that requires swabbing hands or other surfaces and then running the sample through a chemical detector—typically an ion mobility spectrometer. Wand-like vapor detectors accommodate more sampling mobility, but unless the detector scans immediately above it, the chemical signature of a bomb-making ingredient will go unnoticed.



Matt Staymates, a mechanical engineer at NIST, uses a schlieren imaging system to visualize the flow of vapors into an explosives detection device fitted with an artificial dog nose that mimics the "active sniffing" of a dog. The artificial dog nose, which was developed by Staymates and colleagues at NIST, the Massachusetts Institute of Technology Lincoln Laboratory, and the U.S. Food and Drug Administration, can improve trace chemical detection as much as 16-fold. Copyright Robert Rathe

Aiming to uncover clues on how to improve trace detection capabilities, the researchers turned to one of nature's best chemical detectors: the dog. Through their review of previous studies, the team distilled what occurs during sniffing. Five times a second, dogs exhale to reach out, pull, and then inhale to deliver a nose full of aromas for decoding by some 300 million receptor cells.

Using a 3-D printer, Staymates replicated the external features of a female Labrador retriever's nose, including the shape, direction, and spacing of the nostrils. Moving air through the artificial nose at the same rate that a dog inhales and exhales allowed them to mimic the air sampling—or sniffing—of dogs.

With schlieren imaging—a technique widely used in aeronautical engineering to view the flow of air around objects—and high-speed video, the team first confirmed that their imitation nose could indeed sniff much like the real thing, a property documented in previous studies of live dogs.

With each sniff, air jets exit from both nostrils, moving downward and outward. Though it might seem counterintuitive, the air jets entrain—or draw in—vapor-laden air toward the nostrils. During inhalation, the entrained air is pulled into each nostril.

The team's first set of experiments compared the air-sampling performance of their "actively sniffing" artificial dog nose with that of trace-detection devices that rely on continuous suction. The head-to-head comparison with an inhalation system used with a real-time monitoring mass spectrometer found that sampling efficiency with the sniffing artificial dog nose was four times better 10 centimeters (3.9 inches) away from the vapor source and 18 times better at a stand-off distance of 20 centimeters (7.9 inches).

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NIST RESEARCH ENABLES ENHANCED DNA "FINGERPRINTS"

Starting January 1, 2017, DNA profiles include more data, making them even more powerful at solving cases.

Forensic labs across the country had been gearing up for a big change in the way they generate DNA profiles, the genetic fingerprints so useful in solving crimes and identifying the remains of missing persons. Forensic experts produce DNA profiles by extracting genetic material from blood or other biological evidence and analyzing sites in the DNA called markers.

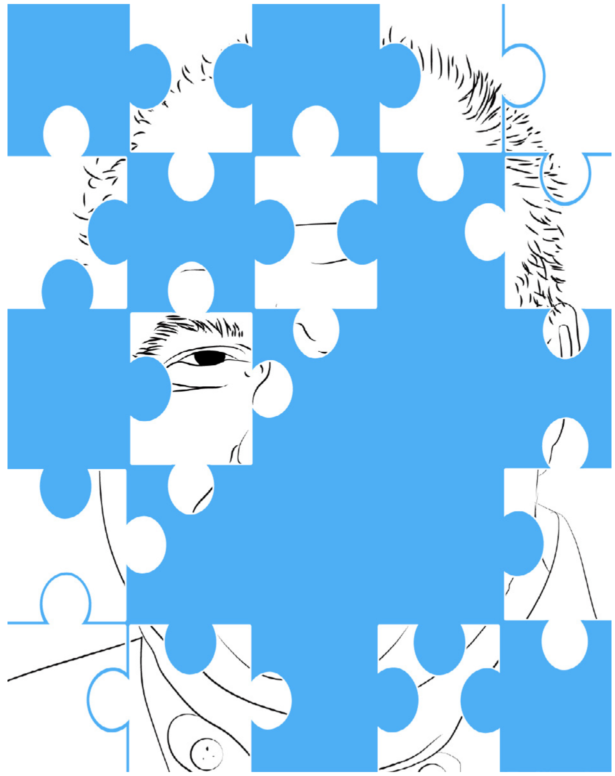
Since the FBI's National DNA Index System, or NDIS, came online in 1998, forensic labs in the United States have been generating profiles by analyzing a specific set of 13 genetic markers.

Beginning January 1, 2017, that number rose to 20, an advance made possible by close collaboration between scientists at the FBI and NIST. The additional markers will vastly increase the statistical certainty of DNA identifications and allow investigators to identify suspects that could slip through the cracks today.

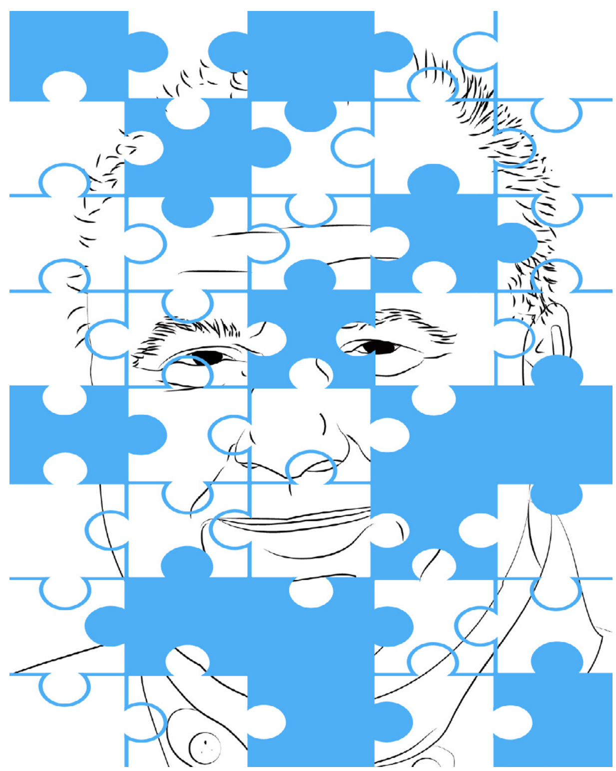
To meet the new year's deadline, all labs that submit profiles to NDIS upgraded their protocols to meet a series of quality assurance standards set by the FBI.

This upgrade was necessary in part due to the rapid growth of the system, which has expanded to include nearly 16 million profiles related to criminal investigations and 30,000 related to missing persons. NDIS now has to add more markers for the same reason a growing city might have to add a new area code. It ensures that everyone can have their own number.

In addition, this upgrade makes international DNA searches more effective by increasing the number of markers that the U.S. system has in common with those of other nations. The number of markers used in both the United States and Europe, for example, rose from eight to 15.



Until now, forensic DNA profiles in the United States have been a series of 26 numbers that can be used to identify an individual. When DNA evidence yields only a partial profile, some of the numbers will be missing. In the case illustrated here, 13 numbers, represented by puzzle pieces, are missing. There may not be sufficient information to establish an identity. Credit: Natasha Hanacek/NIST

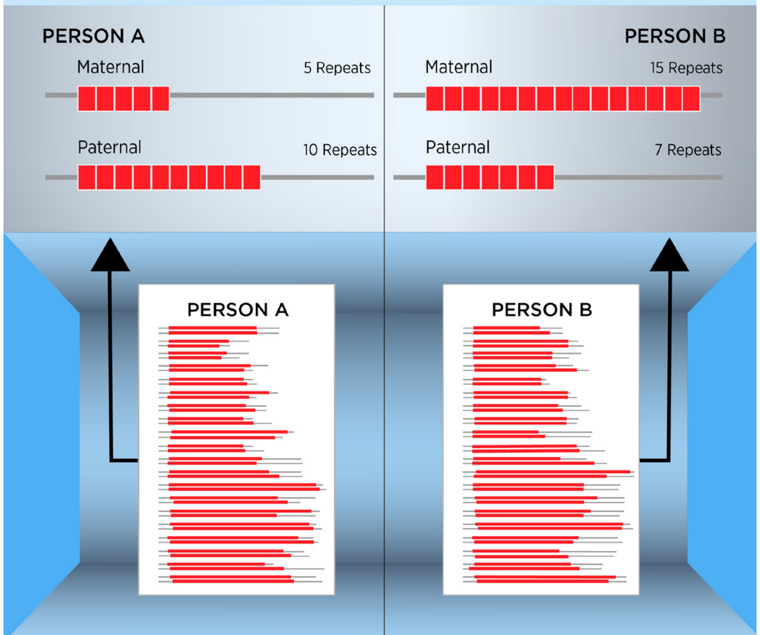


Starting January 1, 2017, forensic DNA profiles in the United States will be a series of 40 numbers. The case illustrated here also has 13 numbers missing, yet there is still sufficient information to establish an identity. These upgraded profiles are based in part on research by NIST scientists including Mike Coble, pictured above, and colleagues. Credit: Natasha Hanacek/NIST

WHAT IS A FORENSIC DNA PROFILE AND HOW DO SCIENTISTS GENERATE ONE?

A DNA profile is like a genetic fingerprint that can identify individuals and help solve crimes. To generate a DNA profile, forensic scientists extract genetic material from blood or other biological evidence and analyze specific regions in the DNA called forensic markers. Until now, DNA profiles in the United States were based on information from 13 markers. On January 1, 2017, that number will rise to 20.

We have two copies of each marker—one that we inherit from our mother and one from our father.



Inside each marker is a section of genetic code that repeats itself, like a single word typed over and over. The number of repeats at each marker varies from person to person, and the chances that two people who are not closely related have the same number of repeats at both copies of all 20 markers is infinitesimal: less than one in a billion billion.

If you lined up the number of repeats for all the markers, you'd have something like a very long Social Security number that can be used to identify a person. That is a DNA profile.

PERSON A	PERSON B
17, 17	11, 14
18, 23	17, 17
10, 10	10, 14
15, 16	15, 19
11, 12	12, 13
11, 11	10, 10
13, 14	10, 13
15, 16	13, 13
18, 22	19, 24
10, 11	10, 13
12, 15	13, 16
13, 14	16, 16
28, 32	32, 32
15, 15	15, 17
10, 10	10, 11
21, 23	20, 23
5, 10	7, 15
8, 9	6, 9
8, 8	8, 11
18, 19	17, 18

DESIGN BY NATASHA HANACEK/NIST

The new markers also help solve a problem that often comes up in cases where the DNA has started to break down. In those cases, forensic analysts can't always get a read on all 13 markers, and they end up with a partial profile.

"If you've got a case where seven markers drop out, the statistics may be too weak to establish an identity," said Mike Coble, a research geneticist at NIST. When that happens, a perpetrator might escape the notice of investigators and remain free to commit more crimes. "But if you start with 20 markers, seven can drop out and you'll still have what's considered a full profile today," Coble said.

Of the seven new NDIS markers, three were first identified by Coble and his colleagues at NIST. And those three markers were chosen because they are particularly useful in cases where the DNA has started to break down.

A Fragile Molecule

A marker is a stretch of DNA that occurs at a specific location within a person's total set of genes, just as a building occurs at a specific address within a city. And like many buildings, forensic markers aren't very interesting inside.

"We've intentionally chosen markers that don't tell you anything about how a person looks or behaves," said Doug Hares, the biologist and FBI official who manages NDIS. "And they have no predictive value in terms of medical conditions."

But forensic markers do contain a section of genetic code that repeats itself, like a single word typed over and over. The number of repeats at each marker varies from person to person, and the chances that two people that are not closely related have the same number of repeats at all 13 markers is less than one in a trillion.

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If you lined up the number of repeats for all the markers, you'd have something like a very long social security number that can be used to identify people, and that's what a DNA profile is. Because we have two copies of each marker—one inherited from our mother and one from our father—a DNA profile based on 13 markers is 26 numbers long. Since the NDIS upgrade on January 1, 2017, a DNA profile is now a series of 40 numbers.

To get those numbers, forensic analysts don't need to read the genetic code inside the markers; they just measure how long the markers are and from that deduce the number of repeats. But DNA is a wisp of a molecule, and under certain conditions, the bonds that hold it together are easily broken.

Heat, sunlight, and bacteria can all damage DNA. If a blood stain on a piece of clothing has been exposed to the elements, if evidence doesn't turn up until years after a crime, or if evidence is stored improperly, the DNA can begin to break down. If a break occurs within a marker, scientists can't measure how long it is, and they get a partial profile.

But some markers withstand damage better than others.

Size Matters

Markers vary in length, with certain ones always being relatively short, and others always longer. When DNA is damaged, it starts breaking apart, like a ribbon cut to pieces. When that happens, the shorter markers tend to remain intact just because they're small pieces already.

But only a few of the markers in the original set of 13 are particularly short, so in 2004, Coble and his colleagues at NIST set out to find a few new ones.

"We were looking for markers that were short and that showed a lot of variability," Coble said. "If half the population has the same number of repeats, it won't be very good for telling people apart."

They started with a list of more than 1,000 candidate markers, most of which were unknown when the original set of 13 markers was chosen a decade earlier. After running thousands of tests, including tests using artificially degraded DNA, they found 27 "mini-markers" that worked.

Of those 27, three are among the seven new NDIS markers. The other four new markers aren't minis, but they have exceptionally high variability.

Collaborative Science

Much of the research that yielded the mini-markers grew out of the effort to identify the victims of the 9/11 terror attacks. That effort was complicated by the fact that fires smoldered for months in the rubble at Ground Zero and, because heat damages DNA, the remains recovered there often yielded partial profiles. To help resolve those profiles, NIST scientist John Butler worked with colleagues at the New York City Medical Examiner's Office to develop new methods for working with badly degraded DNA—methods that made it possible to identify remains that otherwise would have never been returned to the victims' families.

That research was the precursor to the search for mini-markers at NIST. More recently, NIST worked closely with the FBI to validate the new 20-marker profiling kits that forensic labs use to generate DNA profiles. The agencies coordinated a series of trials in which crime labs used the kits against test DNA with known profiles to ensure that the results were accurate and that kits from different manufacturers produced equivalent results.

The FBI announced the upgrade after the trials ended, giving crime labs two years to put the new kits into production and to pass a series of quality assurance tests. According to the FBI's Hares, many labs have been using the new kits for almost a year.

For crime victims seeking justice, for defendants seeking a fair trial, and for families looking for missing loved ones, a lot depends on the accuracy and reliability of DNA profiles. The transition to 20-markers hasn't been quick, but it has allowed time for testing and validation.

"DNA profiles can be incredibly powerful clues for solving cases," said Hares. In the last year alone, NDIS has aided more than 40,000 investigations. "We've been working for years to make sure that this transition goes smoothly."

UNIVERSE'S CONSTANTS NOW KNOWN WITH SUFFICIENT CERTAINTY TO COMPLETELY REDEFINE THE INTERNATIONAL SYSTEM OF UNITS

Fundamental constants are physical quantities that are universal in nature. For example, the speed of light in vacuum and the charge of a single electron are the same everywhere in the universe. That is why scientists would like to use invariant quantities of nature to define the seven base measurement units of the International System of Units (SI), or the modern metric system, rather than to rely on measurements of physical artifacts.

According to a recent evaluation and update of the values of the fundamental constants by researchers at NIST, the uncertainties in measurements of the constants have now been reduced to such exceedingly low levels that all of the SI units can now be linked to them.

This new and redefined SI will benefit science, technology, industry, and commerce by helping to ensure the long-term stability of these base units and the entire international measurement system.

The latest update (http://dx.doi.org/10.1063/1.4954402) of the values of the fundamental constants was authored by NIST's Peter Mohr, David Newell, and Barry Taylor, who lead the international Task Group on Fundamental Constants of the Committee on Data for Science and Technology (CODATA). This task group updates the values every four years. The new quantities represent the latest comprehensive adjustment of values of the constants. In the summer of 2017, the task group will perform a special update to produce the final values for four fundamental constants to be adopted in the fall of 2018 by an international body known as the General Conference on Weights and Measures (Conférence Générale des Poids et Mesures, or CGPM).

The seven base units in the SI are the meter, kilogram, second, ampere (a measure of electric current), kelvin (a measure of temperature), mole (a measure of the amount of a substance), and candela (a measure of luminous intensity). The goal of the new SI is to define all of these units completely in terms of fundamental constants with exact values. Some constants, such as the speed of light, are currently defined in this way, as exact quantities.

A nearly perfect 1-kg silicon sphere reflecting its connection with a 1-kg prototype platinum-iridium alloy cylinder. Credit: PTB

Examples of fundamental constants range from the magnitude of the elementary charge of a single electron or proton to the extraordinary number of particles in one mole of a substance, described by the Avogadro constant. Another example is the Planck constant, a quantity at the heart of quantum physics that will be used to redefine the kilogram as an invariant property of nature instead of a standard platinum-iridium cylinder.

The evaluation and update reduce the uncertainties in both the Planck and Avogadro constants by almost four times compared to the previous evaluation, to just 12 parts per billion. These uncertainties decreased by reconciling measurements in different "watt-balance" devices around the world and new highly accurate X-ray measurements of a softball-sized sphere of silicon that is a nearly perfect crystal and is made almost entirely of the same isotope of silicon (99.9995 percent silicon-28). The update reduces the relative uncertainty by almost two times, to 0.6 parts per million, for the Boltzmann constant, which can be used to determine the amount of energy in a gas at a certain temperature.

"The reduced uncertainties in these four fundamental physical constants are very significant," said NIST chemist Donald Burgess, co-editor of the *Journal of Physical and Chemical Reference Data* (JPCRD). "These now ultra-small uncertainties in the constants will allow the CGPM to revise the International System of Units so that the seven base units will be exactly defined in terms of fundamental constants. In turn, many equations that describe the laws of nature—such as the relationship between energy and temperature as expressed through Boltzmann's constant—will now be exact and not depend on measurement units that have inherent uncertainties because of the way that they are currently defined."

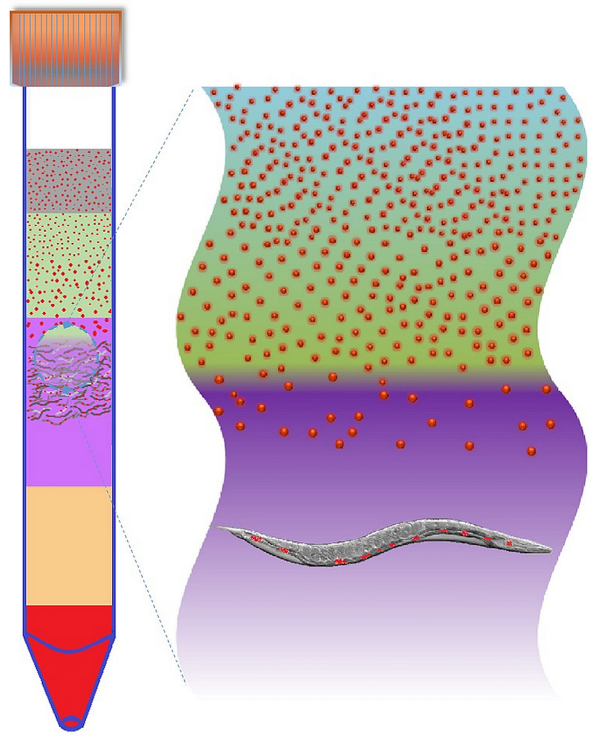
This update of the fundamental physical constants is appearing in both the JPCRD (published by the American Institute of Physics) and the *Reviews of Modern Physics* (published by the American Physical Society). The JPCRD came into existence as a consequence of an act of Congress, the Standard Reference Data Act of 1968 (Public Law 90-396), which gave NIST the primary responsibility in the federal government to make critically evaluated scientific and technical reference data available to scientists, engineers, and the general public.

NIST UPDATES 'SWEET' 1950S SEPARATION METHOD TO CLEAN NANOPARTICLES FROM ORGANISMS

Sometimes old-school methods provide the best ways of studying cutting-edge tech and its effects on the modern world.

Giving a 65-year-old laboratory technique a new role, researchers at NIST have performed the cleanest separation to date of synthetic nanoparticles from a living organism. The new NIST method is expected to significantly improve experiments looking at the potential environmental and health impacts of these manufactured entities. It will allow scientists to more accurately count how many nanoparticles have actually been ingested by organisms exposed to them.

A paper describing the new method appears in the current issue of the journal *ACS Nano*.



Graphic displaying the NIST-designed sucrose density gradient used to separate excess engineered nanoparticles (ENPs) in solution from roundworms and any ENPs they consumed. Loose or externally attached ENPs are trapped in the uppermost salt layer and lower density sucrose layers. Worms with ingested ENPs move downward to settle in the higher density sucrose layers. Credit: M. Johnson/NIST

The common roundworm *Caenorhabditis elegans* has been used in recent years as a living model for laboratory studies of how biological and chemical compounds may affect multicellular organisms. These compounds include engineered nanoparticles (ENPs), bits of material between 1 and 100 nanometers (billionths of a meter, or about 1/10,000 the diameter of a red blood cell). Previous research has often focused on quantifying the amount and size of engineered nanoparticles ingested by *C. elegans*. Measuring the nanoparticles that actually make it into an organism is considered a more relevant indicator of potential toxicity than just the amount of ENPs to which the worms are exposed.

Traditional methods for counting ingested ENPs have produced questionable results. Currently, researchers expose *C. elegans* to metal ENPs such as silver or gold in solution, then rinse the excess particles away with water followed by centrifugation and freeze-drying. A portion of the "cleaned" sample produced is then typically examined by a technique that determines the amount of metal present, known as inductively coupled plasma mass spectrometry (ICP-MS). It often yields ENP counts in the tens of thousands per worm; however, those numbers always seem too high to NIST researchers working with *C. elegans*.

"Since ICP-MS will detect all of the nanoparticles associated with the worms, both those ingested and those that remain attached externally, we suspect that the latter is what makes the 'ENPs' per-worm counts so high," said NIST analytical chemist Monique Johnson, the lead author on the *ACS Nano* paper. "Since we only wanted to quantify the ingested ENPs, a more robust and reliable separation method was needed."

Luckily, the solution to the problem was already in the lab.

In the course of culturing *C. elegans* for ENP-exposure experiments, Johnson and her colleagues had used sucrose density gradient centrifugation, a decades-old and established system for cleanly separating cellular components, to isolate the worms from debris and bacteria. "We wondered if the same process would allow us to perform an organism-from-ENP separation as well, so I designed a study to find out," Johnson said.

In their experiment, the NIST researchers first exposed separate samples of *C. elegans* to low and high concentrations of two sizes of gold nanospheres, 30 and 60 nanometers in diameter. The researchers put each of the samples into a centrifuge and removed the supernatant (liquid portion), leaving the worms and ENPs in the remaining pellets. These were centrifuged twice in a salt solution (rather than just water as in previous separation methods), and then centrifuged again, but this time, through a uniquely designed sucrose density gradient.

"From top to bottom, our gradient consisted of a salt solution layer to trap excess ENPs and three increasingly dense layers of sucrose [20, 40, and 50 percent] to isolate the *C. elegans*," Johnson explained. "We followed up the gradient with three water rinses and with centrifugations to ensure that only worms with ingested ENPs, and not the sucrose separation medium with any excess ENPs, would make it into the final pellet."

Analyzing the range of masses in the ultrapurified samples indicated gold levels more in line with what the researchers expected would be found as ingested ENPs. Experimental validation of the NIST separation method's success came when the worms were examined in detail under a scanning electron microscope (SEM).

"For me, the eureka moment was when I first saw gold ENPs in the cross section images taken from the *C. elegans* samples that had been processed through the sucrose density gradient," Johnson said. "I had been dreaming about finding ENPs in the worm's digestive tract and now they were really there!"

The high-resolution SEM images also provided visual evidence that only ingested ENPs were counted. "No ENPs were attached to the cuticle, the exoskeleton of *C. elegans*, in any of the sucrose density gradient samples," Johnson said. "When we examined worms from our control experiments [processed using the traditional no-gradient, water-rinse-only separation method], there were a number of nanospheres found attached to the cuticle.

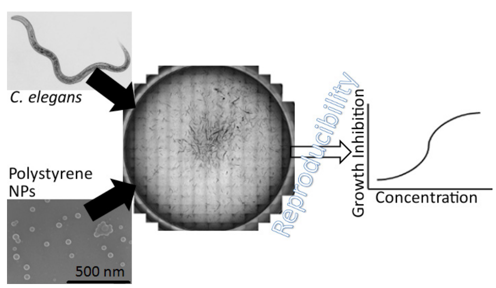
Now that it has been successfully demonstrated, the NIST researchers plan to refine and further validate their system for evaluating the uptake of ENPs by *C. elegans*. "Hopefully, our method will become a useful and valuable tool for reducing the measurement variability and sampling bias that can plague environmental nanotoxicology studies," Johnson said.

M.E. Johnson, S.K. Hanna, A.R. Montoro Bustos, C.M. Sims, L.C.C. Elliott, A. Lingayat, A.C. Johnston, B. Nikoobakht, J.T. Elliott, R.D. Holbrook, K.C.K. Scott, K.E. Murphy, E.J. Petersen, L.L. Yu and B.C. Nelson, Separation, sizing, and quantitation of engineered nanoparticles in an organism model using inductively coupled plasma mass spectrometry and image analysis, *ACS Nano*, January 24, 2017, http://dx.doi.org/10.1021/acsnano.6b06582

NIST RESEARCHERS USE A STANDARDIZED *C. ELEGANS* TOXICITY TEST TO ASSESS NANOMATERIAL TOXICITY

Toxicity assays are an important component in identifying potential health or environmental hazards associated with chemicals. The development of robust, standardized toxicity assays to understand the potential risks of chemicals in water is critical to making scientifically informed decisions pertaining to chemical management and usage. However, new classes of chemicals, such as nanomaterials, behave differently from their larger counterparts and dissolved organic and inorganic chemicals, and thus pose challenges for traditional toxicity assays.

Researchers from NIST have tested the feasibility of standardized *Caenorhabditis elegans* (*C. elegans*) toxicity assay (ISO 10872) for assessing the toxicity of nanomaterials suspended in water. *C. elegans* is a nematode, which are possibly the most abundant multicellular organisms on the planet. Because of this ubiquity, nematodes are useful in toxicity assays that evaluate the potential effects of pollutants on the environment.



NIST researchers assessed a standard *C. elegans* toxicity assay to determine if test modifications were needed for nanomaterials. They found that commonly used methods to maintain particle stability impact nematode growth and, therefore, alter assay results.

The NIST group conducted a cause-and-effect analysis of the *C. elegans* assay to identify protocol steps that were expected to contribute the most to the variability of the assay results. This information was used to design and conduct a sensitivity analysis, in which certain parameters were altered, especially those that might be modified for nanomaterials testing, to determine how they impacted the assay results. Complete population sampling of the nematode cultures under experimental treatment was achieved with state-of-the-art automated large-field-of-view microscopy imaging. They found that altering the quantity of food (*Escherichia coli*) provided to the nematodes during the assay drastically changed results. Both the chemical control compound and a positively charged polystyrene nanoparticle were more toxic with lower food concentrations. Additionally, they found that shaking the plates during the assay, which could help to keep insoluble nanomaterials suspended, inhibited growth and reproduction of the nematodes, thus impacting assay results.

Overall, these results highlight the need to assess the robustness of standardized aquatic toxicity assays for use with nanomaterials and to understand the potential interactions that may lead to unforeseen problems in these tests.

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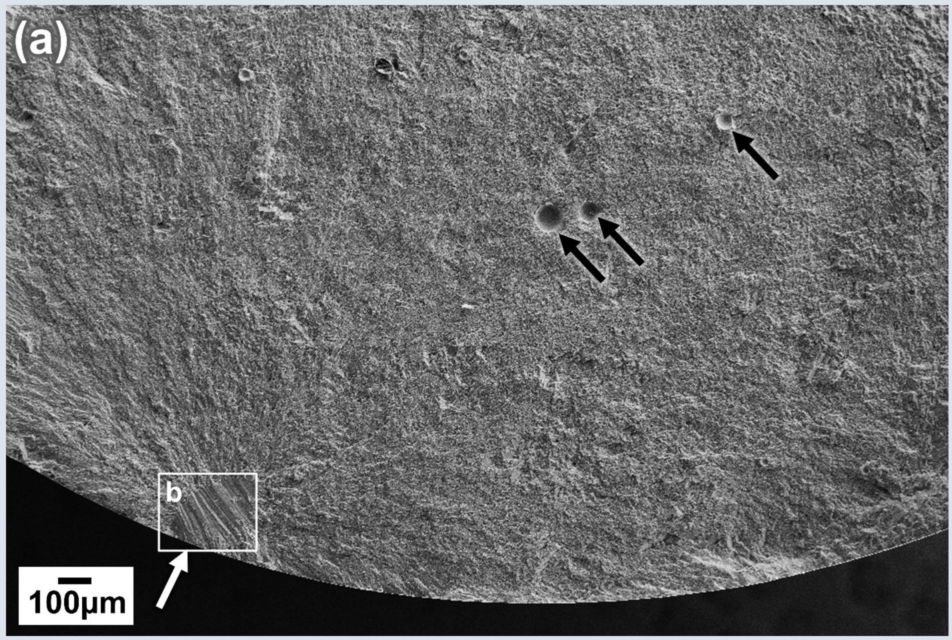
NIST RELEASES ROADMAP FOR POLYMER-BASED ADDITIVE MANUFACTURING

Additive manufacturing is a high-priority technology growth area for U.S. manufacturers. Innovative additive manufacturing processes that fabricate parts layer-by-layer directly from a 3-D digital model have great potential for producing high-value, complex, individually customized parts. Companies are beginning to use additive manufacturing as a tool for reducing time to market, improving product quality, and reducing the cost to manufacture products. Metal-based additive manufacturing parts are already in use in a number of applications, including automotive engines, aircraft assemblies, power tools, and manufacturing tools.

In support of the development of polymer-based additive manufacturing, NIST has released the *Measurement Science Roadmap for Polymer-Based Additive Manufacturing* (https://doi.org/10.6028/NIST.Additive manufacturingS.100-5), a guide that identifies future desired capabilities, challenges, and priority R&D topics in polymer-based additive manufacturing. The report is the result of the "Roadmap Workshop on Measurement Science for Polymer-Based Additive Manufacturing," held June 9-10, 2016 at the NIST campus in Gaithersburg, Maryland. The workshop brought together nearly 100 additive manufacturing experts from industry, government, national laboratories, and academia to identify measurement science challenges and associated R&D needs for polymer-based additive manufacturing systems. The workshop was sponsored by the National Science Foundation, Division of Civil, Mechanical and Manufacturing Innovation and NIST's Material Measurement Laboratory. Additive manufacturing is an important research priority for NIST and a key component of MML's five-year strategic plan (https://mmlstrategy.nist.gov/).

By identifying high priority goals and challenges in polymer-based additive manufacturing, the report can serve as a roadmap for R&D, standards development, and other future efforts. It includes detailed analyses of the complexities surrounding material characterization, process modeling, *in situ* measurement, performance, and other cross-cutting challenges for polymer-based additive manufacturing. As such, the report can help guide public and private decision-makers interested in furthering the capabilities of polymer-based additive manufacturing, and accelerating its more widespread use, and contribute to a robust national research agenda for polymer-based additive manufacturing.



Fracture surface of AM (electron beam melting) titanium alloy (Ti-6Al-4V) high-cycle fatigue fracture surface showing crack initiation at internal lack-of-fusion defect (white arrow).[4]

WORKSHOP ON MECHANICAL BEHAVIOR OF ADDITIVE MANUFACTURING COMPONENTS

The worldwide market for additive manufacturing products and services is projected to exceed $6.5 billion by 2019[1]. Despite this prediction, there are no metal additive manufacturing parts currently being used in any fatigue or fracture critical applications[2,3]. As a result, ASTM and NIST held a workshop May 4-5, 2016, during the ASTM Committee Week in San Antonio, TX to determine and prioritize the research, standards, and data needs required to overcome the barriers to the acceptance of additive manufacturing parts for these important applications. This meeting brought together over 150 representatives from industry, academia, and government (research and regulatory) agencies who presented, discussed, evaluated, and prioritized research and standardization needs to enable the use of additive manufacturing parts in fatigue and fracture critical applications. The report of this workshop, Findings from the NIST/ASTM Workshop on Mechanical Behavior of Additive Manufacturing Components (https://doi.org/10.6028/NIST.AMS.100-4), is available for download. This report should help guide the planning of research and standards development to enable the use of additive manufacturing parts while avoiding fatigue and fracture failures.

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OUTREACH AND PARTNERING

MML AND PARTNERS PROPOSE DEVELOPMENT OF REFERENCE MATERIAL STANDARDS FOR CIRCULATING TUMOR DNA

On December 15, 2016, the leader of NIST's Bioassay Methods group Kenneth Cole served as a panelist at the Foundation for the National Institutes of Health (FNIH) Cancer Biomarker Steering Committee Meeting in Bethesda, MD. He and scientific leaders from Jansen, National Cancer Institute, Harvard Medical School, and AstraZeneca presented a proposed project on development of reference material standards for circulating tumor DNA.

Blood based biomarkers (liquid biopsies) are key for realizing precision medicine. Genetic material such as ctDNA, circulating tumor RNA, circulating tumor microRNA, fragments of tumor cells (exosomes), and fully-intact circulating tumor cells can be obtained non-invasively from the peripheral blood and used to identify actionable mutations for targeting therapy, or resistance if targeted therapy fails to achieve a response. However, many technologies becoming available to enrich, isolate, expand, and interpret ctDNA present multiple challenges to safely and rapidly reducing the results to practice.

The proposed project is to develop suitable control material that aids interpretation of results for drug development, clinical research, therapeutic decision-making, and regulatory applications. If approved, this project will be implemented and funded by the FNIH and NIST will play a key role in developing and validating the ctDNA reference material standards.

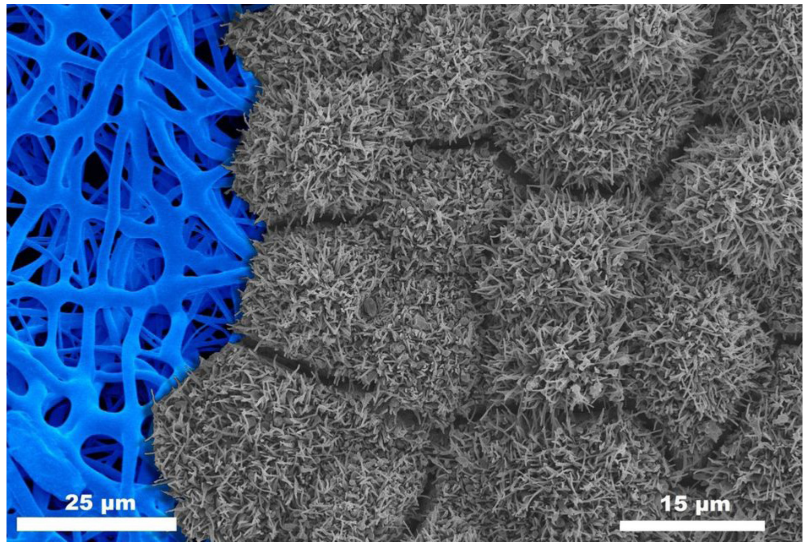
MML COLLABORATES ON MARINE ENVIRONMENTAL SCIENCE

(L to R) Daniela Tizabi, Amanda Maggio, Mary Bedner, and Elena Legrand

NIST, the Hollings Marine Laboratory (HML) in Charleston, SC, and the University of Maryland Institute of Marine and Environmental Technology (IMET) in Baltimore, MD, have been collaborating to promote scientific understanding and technology in the complex, multi-disciplinary field of marine environmental science. While both HML and IMET have similar missions to sustain, protect, and restore coastal ecosystems, each organization has unique facilities and skillsets that complement each other in the partnership and enhance the scientific capabilities available to scientists at both organizations. To stimulate scientific interchange in areas of mutual interest, three new postdoctoral scientists have recently joined IMET and HML. Amanda Maggio is working with Rusty Day (NIST-HML) and Colleen Burge (IMET) to study coral samples collected over a wide geographic range, assessing both the bacterial communities as well as the correlation of boron isotope ratios with ocean pH measurements as a potential indicator of ocean acidification. Elena Legrand is working with Tracey Schock and Ashley Boggs (NIST-HML) and Sook Chung (IMET) to understand viral diseases and hormonal signaling of blue crabs to support potential aquaculture activities and restocking programs. Kehau Hagiwara is working with Dan Bearden (NIST-HML) and Frank Robb (IMET and the Institute for Bioscience and Biotechnology Research (IBBR)) to determine metabolomics fingerprints of extremophile bacteria from ocean vents and other extreme environments, to identify compatible solutes (or *hypersolutes*) that may be able to be used for cryopreservation or to reduce oxidative stress. In addition to the three postdocs, a new graduate fellow, Daniela Tizabi, has joined NIST in Gaithersburg and IMET. Daniela completed a SURF internship this past summer with Trina Formolo (NIST-IBBR) working on the characterization of the new monoclonal antibody standard NIST is developing to support the biopharmaceutical industry, in preparation for her graduate studies to identify potential antibiotic compounds derived from sea sponge symbionts. Lastly, Mary Bedner (NIST, Gaithersburg), is currently on detail to IMET and has been facilitating the partnership, coordinating the graduate research fellowship, as well as establishing collaborative research with multiple IMET faculty related to water sustainability including measurement science to promote recirculating aquaculture technology and the prediction and identification of harmful algal blooms.

OUTREACH AND PARTNERING

NEW PUBLIC-PRIVATE PARTNERSHIP TO DEVELOP STANDARDS FOR REGENERATIVE MEDICINE



Scanning electron micrograph of retinal pigment epithelium (RPE) cultured on a nanofiber scaffold. RPE and nanofibers were imaged separately and combined. There are two scale bars since RPE and nanofibers were imaged at different magnifications. Nanofibers were pseudocolored and shadowing was added to the RPE.

NIST has partnered with the Standards Coordinating Body for Gene, Cell and Regenerative Medicines and Cell-based Drug Discovery (SCB) to develop industry-wide standard methods and protocols for characterizing and manufacturing these cutting-edge therapies, with an aim of accelerating their use as mainstream treatments for a variety of human diseases and injuries.

The field of regenerative medicine manipulates genes, cells, and tissues to repair or replace diseased, damaged, or missing organs, skin, bone, and other cells and tissues. Regenerative medicine has the potential to alleviate the shortage of donor organs and restore appearance and full function to patients who have experienced severe burns or physical trauma.

Because of the complexity of regenerative medicine treatments, they have been slow to transition from the laboratory to the clinic. The traditional measurements of efficacy, potency, purity, and quality that work with traditional pharmaceuticals aren't always sufficient for regenerative medicine treatments.

To address these issues, NIST and the SCB, a non-profit founded by the Alliance for Regenerative Medicine, announced on September 19, 2016 that they had signed a Memorandum of Understanding, forming a partnership to explore the regenerative medicine industry's needs and develop standards and other products to increase confidence in measurements of gene- and cell-based therapies and manufacturing processes.

SNIFFING LIKE A DOG CAN IMPROVE TRACE DETECTION OF EXPLOSIVES

On the basis of those results, the team chose to outfit a commercially available vapor detector with a bio-inspired 3D-printed inlet that would enable it to sniff like a dog, rather than to inhale only in 10-second intervals, the device's normal mode of operation. The switch resulted in an improvement in odorant detection by a factor of 16 at a stand-off distance of 4 centimeters (1.6 inches).

"Their incredible air-sampling efficiency is one reason why the dog is such an amazing chemical sampler," Staymates said. "It's just a piece of the puzzle. There's lots more to be learned and to emulate as we work to improve the sensitivity, accuracy, and speed of trace-detection technology."

M. Staymates, W. MacCrehan, J. Staymates, R. Kunz, T. Mendum, T-H. Ong, G. Geurtsen, G. Gillen and B.A. Craven, Biomimetic Sniffing Improves the Detection Performance of a 3D Printed Nose of a Dog and a Commercial Trace Vapor Detector, *Scientific Reports*, December 1, 2016, http://dx.doi.org/10.1038/srep36876

AWARDS

PLANT ELECTED AAAS FELLOW

Anne Plant, Chief of MML's Biosystems and Biomaterials Division, has been elected to the rank of fellow of the American Association for the Advancement of Science (AAAS) by the AAAS Council. Plant was recognized for "distinguished contributions to the field of quantitative biology through leadership in promoting reliability in research and applications, particularly in biomolecular materials and cellular measurements." Each year, the AAAS Council elects members whose "efforts on behalf of the advancement of science or its applications are scientifically or socially distinguished." In October 2016, the AAAS Council elected 391 members as fellows of the association, in recognition of their contributions to innovation, education, and scientific leadership.

ZOOK RECEIVES PECASE

On January 9, 2017, MML's Justin Zook received the Presidential Early Career Award for Scientists and Engineers (PECASE). The award represents the highest honor bestowed by the U.S. Government for excellence in early scientific research careers. Zook is a scientist in MML's Joint Initiative for Metrology in Biology (JIMB) and co-leader of the Genome in a Bottle Consortium, which is developing reference materials, data, and methods for human genome sequencing. These standards help answer the question "So, you've sequenced my genome. How well did you do?" by providing benchmark samples to assess measurement performance for the rapidly advancing array of genome-sequencing technologies that are being developed for the health care market. Justin is creating methods to compare and integrate whole-genome DNA sequencing data from multiple technologies and sequencing runs, using machine learning to arbitrate sequence calls, yielding highly confident results that can act as our best estimate of "truth." The NIST data and reference materials are being used to refine and optimize new sequencing technologies and as the basis for regulatory oversight of clinical sequencing assays. Justin's newest work is helping to bring previously inaccessible variant types and genomic regions into reach.

LOCASCIO JOINS ASTM INTERNATIONAL BOARD OF DIRECTORS, RECEIVES ACS LEADERSHIP IN CHEMICAL MANAGEMENT AWARD

NIST Acting Associate Director for Laboratory Programs Laurie Locascio will serve a three-year term on the ASTM International board of directors. Locascio has served as director of MML since 2012, and prior to that, as division chief of the NIST Biochemical Science Division. Her technical expertise lies in bioengineering and her research portfolio includes the development and applications of microfluidic systems for biomedical research.

Locascio has also been awarded the Earle B. Barnes Award for Leadership in Chemical Management, sponsored by the Dow Chemical Co. Foundation, and given by the American Chemical Society. The award recognizes outstanding leadership and creativity in promoting the sciences of chemistry and chemical engineering in research management at NIST, and will be presented April 4, 2017.

AWARDS

FLEISHER AWARDED KAMINOW PRIZE BY THE OPTICAL SOCIETY OF AMERICA

Adam J. Fleisher of MML's Gas Sensing Metrology Group has been awarded the Ivan P. Kaminow Young Professional Prize by the Optical Society of America (OSA). This prize is given to one recipient per year and includes funding to attend the OSA *Winter Leadership Meeting*, February 6-9 in Washington, DC and any OSA-managed meeting in 2017.

During the past year, Fleisher was heavily involved in several high-profile OSA activities, including the planning and organization of an OSA Incubator Meeting on Precision Measurements in Air Quality & Turbulence, as well as participation in the CLEO Science & Innovations Active Optical Sensing sub-committee and the Fourier Transform Spectroscopy organizing committee. He also reviewed numerous papers for premier OSA journals and conferences, and volunteered as a judge at local science fairs. Fleisher and his colleagues have also recently published papers in *Optics Letters and Optics Express*, and given contributed and/or invited talks at CLEO, FiO, FTS, and LACSEA conferences. His research involves the development of advanced optical sensing techniques using optical frequency combs, quantum-cascade lasers, and high-finesse optical resonators.

PRITCHETT RECEIVES PROFESSIONAL AWARD FROM NOBCCHE

MML Research Chemist Jeanita Pritchett has been selected as the recipient of the 2016 Henry C. McBay Outstanding Teacher Award from the National Organization for the Professional Advancement of Black Chemists and Chemical Engineers (NOBCChE). Henry McBay was a renowned chemist and educator who taught in the Atlanta University System (Morehouse College, Spelman College, and Atlanta University) for over 41 years and one of the seven founders of NOBCChE. In honor of his legacy, NOBCChE established the Henry C. McBay Outstanding Teacher Award to recognize STEM educators for demonstrating outstanding contributions to the education and mentoring of young scientists and engineers. Pritchett was nominated by the Chemistry Department at Montgomery College and was selected based on her passion for STEM and commitment to the educational development of others. The award was presented at the 43rd NOBCChE annual awards ceremony and reception on November 11, 2016.

OBRZUT RECEIVES IEC 1906 AWARD

The United States National Committee of the International Electrochemical Commission (IEC) recently presented MML Materials Research Engineer Jan Obrzut with the "1906 Award" for developing graphene standards. Obrzut was recognized for his "essential contributions to support the development of graphene standardization in IEC/TC 113." As the project leader of IEC/TC 62607-6-4, Obrzut and his team developed the first Technical Specification on graphene in the 626A7-6-x series, which will be published in 2016.

Created in 2004 by the IEC Executive Committee, the 1906 Award commemorates the IEC's year of foundation and honors IEC experts around the world whose work is fundamental to the IEC. The Award also recognizes exceptional and recent achievement - a project or other specific contribution - related to the activities of the IEC and which contributes in a significant way to advancing the work of the commission.

PHILLIPS SELECTED AS FELLOW OF AOAC INTERNATIONAL

MML Research Chemist Melissa Phillips has been selected to be a Fellow of AOAC International. Phillips was selected for this honor in recognition of her dedication, commitment, and meritorious service to the analytical community through the AOAC. She has served on numerous stakeholder panels, working groups, and expert review panels (ERPs). Most recently she served as co-chair of the Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) Proficiency Testing Task Force. Last year, the SPIFAN ERP received the AOAC Expert Review Panel of the Year Award. Phillips is a member of the AOAC Technical Division on Reference Materials (TDRM) and currently serves as the TDRM newsletter editor. She is currently a member of the editorial board for the Journal of AOAC International, and served as a special guest editor for an special issue featuring reference materials. Phillips also serves as a member of the AOAC Official Methods Board and is a member of the Technical Programming Council. AOAC International is an independent, third party, not-for-profit association and voluntary consensus standards developing organization founded in 1884.

PETERSEN AWARDED THE SUSTAINABLE NANOTECHNOLOGY ORGANIZATION'S 2016 EMERGING INVESTIGATOR AWARD

MML scientist Elijah Petersen recently received the Sustainable Nanotechnology Organization Emerging Investigator award at the 2016 Sustainable Nanotechnology Organization conference. The award was announced by the Royal Society of Chemistry journal *Environmental Science: Nano*. Petersen was selected for his pioneering research contributions and his commitment and leadership to the wider sustainable nanotechnology community.

NIST STANDARD STORY

NIST scientists have thoroughly measured and characterized more than 1,300 physical products, NIST Standard Reference Materials®, to help people in industry, academia, and government agencies calibrate instruments, verify their test methods, and develop new measurement methods. NIST reference materials, for example, help manufacturers make interoperable parts in far-flung facilities, medical labs check the accuracy of cholesterol and other clinical tests, and scientists monitor environmental threats.

NO HUMANS WERE HARMED

WHAT

Standard Reference Material® 2668 Toxic Elements in Frozen Urine, and 3668 Mercury, Perchlorate, and Iodide in Frozen Human Urine

Together, these two SRMs provide values for more than one dozen environmental contaminants that are harmful to human health, plus a few nutritional elements that public health authorities monitor.

Each SRM contains 10 vials, each with 1.5 milliliters of frozen human urine. Five vials in each SRM contain concentrations of the elements of interest that represent the 50th percentile of the U.S. population (that is, 50% of us have concentrations of, say, mercury in our urine below the levels in the SRM.) Five vials contain concentrations of the elements of interest that represent the 95th percentile. Most test labs will see samples that fall within this range, since it covers so much of the population.

WHY

SRMs 2668 and 3668 help the Centers for Disease Control and Prevention (CDC; which collaborated on their development) and other labs monitor people's exposure to toxins in the environment.

Each SRM provides multiple vials so that customers can run multiple quality control checks, comparing their results to NIST's. If the test labs results aren't with the range specified by NIST, customers know to look for problems with their test processes or equipment.

NIST has supported the nation's public health system with reference materials for toxins in urine since the early 1980s, but those were freeze-dried samples that had to be reconstituted with water, introducing the possibility of errors. In clinical chemistry, a best practice is to match your reference material's characteristics to the properties of the kind of samples you usually see in your lab. Since urine tests in clinical labs are conducted on liquid samples, NIST developed liquid SRMs to more closely imitate real-world conditions.

(L to R) Gulchekhra Shakirova of the CDC and NIST's Savelas Rabb and John Molloy package SRMs 2668 and 3668 at the CDC. Credit: Jeffrey M. Jarrett, CDC

WHO

Since the early 1960s, the CDC has conducted surveys on the health of the population. A new survey—questionnaires and medical and dental exams of 5,000 people in 15 counties—begins every other year. The CDC's findings help to shape public policy and new health programs and services. For example, information from the health survey about the nutritional status of Americans informed a program to fortify bread, flour, cereal and other grain products with folate, critical to the correct formation of the spine in developing embryos. After folate was introduced into products, The CDC's health survey saw a 33% reduction in the number of women of childbearing age with low levels of the nutrient. The survey's findings on the population's levels of lead prompted the removal of lead from gasoline and helps the CDC pinpoint areas where lead contamination (such as from old paint) is still an issue.

The survey continues to monitor the population for lead and other environmental contaminants, and is now monitoring for some emerging contaminants like perchlorate, a component of rocket fuel and some fertilizers. Perchlorate in the environment concerns public health experts because it can interfere with the thyroid gland's absorption of iodine, essential for production of thyroid hormone. The Environmental Protection Agency is considering regulating perchlorate in drinking water. Recognizing the increasing interest in perchlorate, the NIST team developed SRM 3668, the world's first reference material for the substance.

HOW

No humans were harmed in the making of this product: The CDC, with permission from a board that examines the ethics and safety of studies involving people, collected some 34 liters of urine from, no doubt, a lot of volunteers. For each SRM, the urine was homogenized, divided into two portions, and spiked to represent the concentrations of the elements of interest in the 50th and 95th percentiles of the U.S. population. The SRMs are stored and shipped frozen and thawed at room temperature before use.

Learn more: https://www-s.nist.gov/srmors/view_detail.cfm?srm=2668

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MATERIAL MEASUREMENT LABORATORY

The Material Measurement Laboratory supports the NIST mission by serving as the national reference laboratory for measurements of matter, providing broad support for chemical, biological, and materials sciences. Our fundamental and applied measurement science research expands possibilities for determining the composition, structure, and properties of manufactured, biological, and environmental materials, and the processes that create them. In addition, MML drives the development and dissemination of tools—including measurement protocols, certified reference materials, critically evaluated data, and best practice guides—that help assure quality measurements of matter. Our research and measurement services support progress in areas of national importance including advanced materials, energy, environment, food safety and nutrition, forensic science, health care, manufacturing, physical infrastructure, and safety and security. MML also coordinates the NIST-wide Standard Reference Materials® (SRM) and Standard Reference Data programs.

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