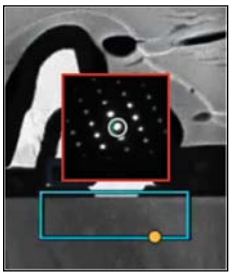
2014 *Microscopy Today* Innovation Awards

Microscopy Today congratulates the fifth group of Innovation Award winners. The ten innovations described below advance microscopy in several areas: light microscopy, scanning probe microscopy, electron microscopy, and hybrid microscopy-analysis methods. These innovations will make microscopy and microanalysis more powerful, more flexible, more productive, and easier to accomplish.

Topspin Strain Mapping

AppFive LLC and NanoMEGAS SPRL

Developers: J. K. Weiss and Amith Darbal



Topspin Strain Mapping software employs electron diffraction and electron beam precession technology to measure strain in local regions of thin specimens. The software enables the user to acquire STEM images and interactively select two areas of interest for strain measurement: (1) a reference, ideally unstrained, region and (2) an experimental, strained region. Electron diffraction patterns from the two regions are compared by the Topspin Strain Analysis software to measure strain values at every sampled position in the experimental area. Unlike other methods, which calculate strain by measuring shifts in individual diffraction spots from the patterns, Topspin Strain Analysis calculates strain by comparing the entire strained diffraction pattern to the unstrained reference pattern. This improves the accuracy and precision of the strain measurement and does not require the user to identify the spots to use for strain measurement. The addition of beam precession to the nanobeam diffraction patterns reduces the effects of specimen thickness and tilt variations, which arise from dynamical diffraction effects. This enables the robust use of automated analysis algorithms. Additionally, the increased number of high-order diffraction spots in the precession electron diffraction patterns improves the precision of the strain measurement.

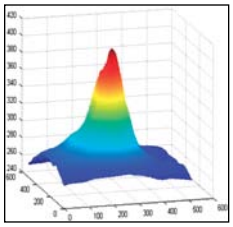
Topspin Strain Mapping is an automated solution for research and metrology applications that require strain determination and mapping, and it yields both high measurement precision (0.02% demonstrated) and high spatial resolution (nanometer-level demonstrated). Topspin Strain Mapping addresses the issue of low throughput typical of previous methods. Through the use of hardware optimized for fast diffraction pattern imaging and beam precession, a typical Topspin Strain Mapping session acquires a dataset of 200 × 200 pixels in less than seven minutes. Subsequent analysis of the dataset from such a map can produce strain maps in approximately five minutes. Additionally, there is little user input necessary for analysis, which reduces complexity and improves reproducibility.

Strain measurement is important for layered semiconducting materials where interfacial strain on electronic mobilities and properties can affect device performance. The high throughput, minimal user input, and accuracy gained by taking measurements from the entire diffraction pattern make the technique suitable for industrial applications, as well as local measurement of strain around crystal defects and precipitates that are important for fundamental applications in materials science.

VertiSense™ Scanning Thermal Microscopy Module

Applied Nanostructures, Inc. (APPNANO)

Developers: Jeremy Goeckeritz, Gary Aden, and Ami Chand



The VertiSense™ Scanning Thermal Microscopy (SThM) module from APPNANO enables commercially available atomic force microscopes (AFMs) to acquire simultaneous topographical and thermal images with nanometer resolution. At the core of the VertiSense module is an innovative thermal probe with a nano-fabricated thermocouple located at the apex of a sharp tip. The entire tip, except the thermocouple, is covered by a thermally insulating material so as to limit heat transfer to areas other than the sensor. A temperature map is generated by raster scanning the thermal probe over a sample while maintaining constant tip-surface contact via the force feedback of the AFM. When the tip is in contact with a sample having regions of different temperatures, heat transfer between the tip and the surface changes the thermocouple temperature. The thermocouple signal is processed by the VertiSense ultra-low-noise amplifier and fed to the AFM controller as an auxiliary signal to generate the temperature map. The thermocouple is capable of detecting temperature variations as small as 0.1°C and mapping samples with a lateral thermal resolution up to 20 nm—a five-fold improvement over current SThM technologies. The probe can map local temperatures up to 700°C.

In addition to temperature mapping, the design of the thermal probe's cantilever, tip shape, and materials allows the VertiSense system to perform thermal conductivity contrast mapping. In conductivity mapping mode (CMM), the system takes advantage of heat from the AFM laser. When the AFM laser is positioned directly above the thermocouple, it heats the

Microscopy Innovation Awards

Congratulations to the winners of the 2014 *Microscopy Today* Innovation Awards:

- Abberior Instruments
- AppFive
- Applied Nanostructures
- Asylum Research, an Oxford Instruments company
- CoolLED
- FEI
- Leica Microsystems
- Max Planck Institute for Biophysical Chemistry
- NanoMEGAS
- Stanford University
- University of Missouri
- Yale University

Entry deadline: **March 15, 2015**

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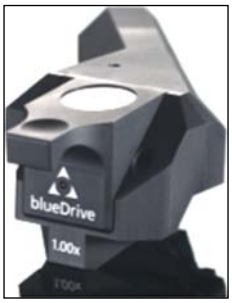
thermocouple 10–20°C above room temperature. Thus, as the tip is brought in contact with a sample, heat flows from the tip to the sample depending on the conductance of the sample. As a result, areas of different thermal conductivity are recorded through a change in the thermocouple temperature.

The VeriSense thermal probe allows, for the first time, the user to acquire thermal images in multiple modes of operation, such as contact mode, non-contact mode, tapping mode, and other mixed modes. Applications include the investigation of heat-assisted magnetic recording phenomena, measurement of hot-spots in electronics to determine potential failure points, assessment of thermoelectric and photovoltaic devices, and high-resolution thermal microscopy of both hard and soft biostructures.

blueDrive™ Photothermal Excitation for AFM

Asylum Research, an Oxford Instruments company

Developers: Aleks Labuda, Deron Walters, and Jason Cleveland



Though originally limited to topographic imaging, modern atomic force microscopes (AFMs) can now measure mechanical, electrical, electromechanical, and magnetic properties. They can image samples in gas or liquid environments and under the influence of temperature, magnetic and electric fields, and mechanical strain. Despite these many capabilities, a single mode of AFM operation, known as AC mode or Tapping Mode, is the basis for the majority of these measurements.

Tapping Mode oscillates the AFM cantilever as it raster scans the sample such that the tip intermittently touches the surface. Most AFMs use piezoacoustic excitation to produce the cantilever oscillation. In blueDrive™, a new excitation method, known as photothermal excitation, is used. A blue laser is introduced into the AFM optical path in addition to the laser used for sensing the cantilever motion. This blue laser is focused on the base of the cantilever, and its power is modulated at the desired drive frequency. This causes rapid heating and cooling of the cantilever in a localized area. This creates mechanical stresses, amplified by the bimetallic effect for metal-coated cantilevers, that result in cantilever oscillation. The critical difference between blueDrive and piezoacoustic excitation is that blueDrive excites the cantilever directly and does not excite any other mechanical resonances in the AFM. blueDrive produces cantilever responses that are indistinguishable from the measured thermal resonances. No other resonances appear in the frequency response because blueDrive only excites the cantilever. The oscillation amplitude is stable over time, typically drifting by less than 1% over many hours. Almost any cantilever type is compatible, and the benefits of blueDrive apply to imaging in both air and liquids. blueDrive is only available for Asylum Research Cypher™ AFMs.

The scientists at Asylum Research did not invent photothermal excitation itself. Similar technology was developed earlier by groups performing specialized low-amplitude, frequency-modulated AFM. The innovations at Asylum were, first, to recognize that photothermal excitation would be useful for the more common amplitude-modulated Tapping Mode AFM imaging and for related nanomechanical imaging modes, and, second, to develop blueDrive photothermal excitation as a commercial product that is simple and productive to use.

Applications include imaging of proteins, membranes, and cells in liquid; capturing dynamic events like thermal transitions in polymers; and quantitatively measuring viscoelastic properties.

pE-4000 Universal LED Fluorescence Illumination System

CoolLED, Ltd.

Developer: CoolLED, Ltd.

The pE-4000 is a light microscope illumination system built of powerful light emitting diode (LED) wavelength modules that can match the filters of any microscope. The system provides full spectral coverage using a patent-pending "wavelength-grouping" concept that reduces the number of optical and electrical components required. The result is a compact, affordable system that does not compromise performance. LED wavelengths are divided into spectral groups that can simply be considered as violet, blue, green, and red channels. The user can select LED wavelength channels that match the fluorophores being used. The LEDs from each channel will then move into the optical path.

Until now, it has been impractical to offer illumination across the spectrum for all fluorescent stains and corresponding filter sets in one LED system. The number of discreet excitation wavelengths within even the most advanced unit has been limited. Users have been forced to compromise when selecting a light source: choosing between reduced performance (for ease-of-use) or complicated operation (for functionality). The pE-4000 incorporates 16 selectable LED wavelengths in a single system.

Images exhibit superior contrast because background is avoided by selecting only wavelengths matched to the filters in the microscope. The user can choose from the 16 selectable wavelengths in the pE-4000 to match any commercially available single-band or multi-band filter set. The pE-4000 can be operated in either of two modes: as a simple white light source or as an advanced system with full control over functionality. Remote control under imaging software is simple to configure.

Standard functions include optical feedback, analog control, and a simple function generator. This makes the system versatile and ideally suited for use in the growing areas of optogenetics and electrophysiology. Matching to a microscope takes only a few moments, and the result is an optimized illumination source that will minimize the risk of sample bleaching and enhance image contrast. This system is suitable for any application requiring stable, controllable, and repeatable illumination. Advanced techniques in life sciences will benefit, particularly live cell imaging and Förster resonance energy transfer (FRET).

Automated Metrology Workflow: ExSolve™ and Metrios™

FEI

Developer: FEI

This workflow addresses the needs of customers requiring automated, high-throughput sampling for advanced semiconductor manufacturing metrology. The ExSolve™ wafer TEM prep (WTP) system is an automated sample preparation system that can prepare site-specific, 20 nm thick lamellae from whole wafers of up to 300 mm in diameter. Until now, most sample preparation for TEM analysis used slow, manual processes that break the wafer into multiple hard-to-track and hard-to-handle pieces. ExSolve fully automates lamella creation from whole wafers and provides sample tracking, eliminating most of the manual sample handling and data tracking overhead. The ExSolve includes front opening universal pod (FOUP) handling and is designed to be located in the fab near the manufacturing line. ExSolve is part of a fast, complete workflow that includes TEMLink™ and the Metrios™ TEM.

The Metrios system is the first TEM dedicated to providing the fast, precise measurements that semiconductor manufacturers need to develop and control their wafer fabrication processes. Extensive automation of basic TEM operation and measurement procedures minimizes requirements for specialized operator training. Its advanced automated metrology delivers greater precision than manual methods and provides customers with higher throughput and lower cost-per-sample than other TEMs.

Preparation of the extremely thin samples required for TEM analysis has always been a bottleneck because of the artistry required by the technician. This new, automated sample preparation workflow will prepare site-specific thin sections from full wafers in a fraction of the time. The process is more reliable, and the resulting samples are more uniform and repeatable in thickness and uniformity. When imaged downstream in the Metrios TEM, this preparation results in more reliable process control information.

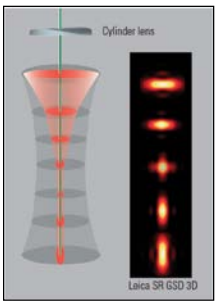
Specifically designed workflows improve pathfinding and technology development, yield ramp and process verification, and high-volume production control. The ExSolve WTP workflow and automated Metrios TEM address the needs of customers who require automated, high-throughput sampling at advanced technology nodes. These are companies working at the bleeding edge of semiconductor technology, such as those developing three-dimensional and multi-gate devices, at the 20 nm node and smaller.

Super-Resolution 3D Localization Microscopy

Leica Microsystems CMS GmbH

Developer: Leica Microsystems CMS GmbH

The Leica SR GSD 3D is a new fluorescence imaging system enabling life scientists to examine cells and individual



molecules with nearly 10-fold higher resolution than with standard light microscopy—in all three dimensions. The system achieves this by using a super-resolution microscopy technique known as GSDIM (ground state depletion followed by individual molecule return) to overcome the diffraction limit. Typically in fluorescence imaging, individual fluorescent molecules light upon excitation, but they activate at the same time. This makes distinguishing individual molecules impossible if they are closer together than the diffraction limit (typically half of the wavelength of the light used). With GSDIM, almost all of the fluorescent molecules in the specimen are switched off for most of the time: high-power laser illumination is used to convert them into a dark state. Spontaneously, individual molecules return to the fluorescent state, while their neighbors remain inactive. In this way the signals from individual molecules can be acquired sequentially using a high-speed EMCCD camera, and the positions of the detected molecules in the specimen can be calculated. Three-dimensional information is gathered via a deliberately induced image aberration (astigmatism) using a cylindrical lens. Finally, a super-resolution image is created from the location of many thousands of molecules. Even small closely spaced cellular components below the diffraction barrier can be resolved using the technique.

Using GSDIM technology, it is possible to control the emission of fluorochromes and localize the position of single molecules down to a lateral precision of 20 nm. Building upon this, the Leica SR GSD offers 3D super-resolution imaging, reaching an axial precision of 50 nm. The system provides true-to-detail imaging of the spatial arrangement of proteins and other biomolecules in cells. One of the strengths of GSDIM is that it uses conventional fluorescence markers routinely used in bioimaging.

The Leica SR GSD 3D is a multi-modal microscopy system for fluorescence imaging in widefield, TIRF, and super-resolution. It can be used for determining the structure of labeled specimens in neurobiology, cell biology, virology, microbiology, and physiology.

Ultraparallel RESOLFT Superresolution Microscopy

Max Planck Institute for Biophysical Chemistry and Abberior Instruments GmbH

Developers: Stefan W. Hell and Andriy Chmyrov

RESOLFT microscopy is a method of lens-based (far-field) fluorescence microscopy that provides spatial resolution below the diffraction limit. STED, RESOLFT, and other techniques overcame the diffraction barrier because they can separate adjacent features by prompting their molecules to

different states. The fact that the separation is accomplished by the states rather than by the process of focusing made a fundamental difference: the resolution is no longer limited by the "imperfection" of focusing, that is, by the wavelength of light.

In its most basic implementation, RESOLFT microscopy resembles stimulated emission depletion (STED) microscopy, employing a doughnut-shaped beam for switching-off molecular fluorescence but leaving a point with vanishing intensity. This prevents fluorescence from everywhere except at the vanished intensity point, which is then scanned across the sample. Importantly, RESOLFT microscopy uses a different molecular mechanism for "off-switching"— it uses reversibly switchable fluorescent proteins (rsFPs) that are toggled between "fluorescent" and "non-fluorescent" long-lived states. The RESOLFT process usually consists of three steps: switching on all rsFPs within an area or volume, switching-off all rsFPs except those located at the intensity minimum, and reading out the fluorescence signal that is produced by the fluorophores at the minimum left in the on-state. The benefit of using long-lived fluorophore states is that the required intensity is 100–10,000 times lower than in STED microscopy, making it highly suitable for imaging living cells.

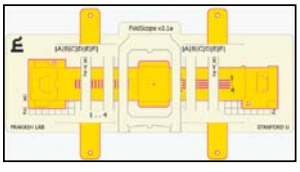
The innovation in the new method converts RESOLFT microscopy from a single-spot method into a highly parallelized multiple spot method that cuts down the recording time for large areas. It was demonstrated that 100,000 minima, acting like donuts, can be created and read out simultaneously with a camera-based detection system. Using the maximum possible density of donuts and the current characteristic switching times for rsFPS, the described method becomes the fastest implementation of RESOLFT imaging that is possible today. Recording times for a large field of view (for example, 100 µm×100 µm) have been cut down to 1–2 seconds per frame with this new parallelized RESOLFT method.

The parallel RESOLFT method is highly suited to the imaging of living targets labeled with rsFPs at quasi-video rate speed with super-microscopy resolution. The immediate applications are in cell biology and neurophysiology.

Foldscope: an Origami-based Print-and-fold Microscope

Manu Prakash of Stanford University

Developers: Manu Prakash, James Cybulski, and James Clements



Foldscope is an ultra-low-cost origami-based approach to large-scale manufacturing of light optical microscopes, specifically brightfield, darkfield, and fluorescence microscopes. Merging principles of optical design with origami enables high-volume fabrication of microscopes from 2D media. Flexure mechanisms created via folding enable a flat compact design. Structural loops in folded paper provide kinematic constraints as a means for passive self-alignment. This rugged instrument can survive harsh field conditions while providing a diversity of imaging capabilities expected in cost-effective, portable microscopes for science and education.

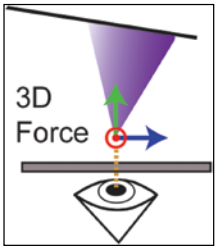
This print-and-fold light microscope can be assembled from a flat sheet of paper. Although it costs less than a dollar in parts, it can provide sub-micron resolution (800 nm). It weighs less than two nickels (8.8 g), is small enough to fit in a pocket, requires no external power, and can survive being dropped from a three-story building or being stepped on by a person. The Foldscope can be assembled from a flat sheet of paper in under 10 minutes. Its scalable design is inherently application-specific, rather than general-purpose, gearing toward particular applications in global health, field-based citizen science, and K–12 science education. Using this platform, there are potential innovations for various imaging modalities (brightfield, darkfield, fluorescence, lens-array) and such scalable manufacturing strategies as capillary encapsulation lens mounting, carrier tape lens mounting, self-alignment of micro-optics by folding, and the paper microscope slide.

Foldscope has implications for both science education and global health. Many children around the world have never used a microscope, even in developed countries like the United States. A universal program providing "a microscope for every child" could foster interest in science at an early age. Disease-specific Foldscope designs are an important vision for future development. For example, brightfield images are possible of *Giardia lamblia*, *Leishmania donovani, Trypanosoma cruzi* (Chagas parasite), *Escherichia coli, Bacillus cereus*, *Schistosoma haematobium,* and *Dirofilaria immitis.* In the future, darkfield and fluorescence Foldscopes will be adapted for diagnostics. Sensitivity and specificity will be measured for various disease-specific Foldscopes as clinical validations against existing diagnostic standards.

Direct Three-Dimensional Atomic Force Microscopy

Gavin M. King and Krishna P. Sigdel of the University of Missouri–Columbia

Developers: Gavin M. King and Krishna P. Sigdel



In conventional atomic force microscopy (AFM), the tip-sample interaction force vector is not directly accessible. However, with direct 3D AFM, light from a focused laser scatters off an AFM tip apex to rapidly and precisely measure the tapping tip trajectory in three-dimensional space. These data yield three-dimensional cantilever spring constants, effective masses, and the tip-sample interaction force components via Newton's second law. In one instance, significant lateral forces

representing 49% and 13% of the normal force (about 150 pN) were observed in common tapping mode conditions as a silicon tip intermittently contacted a glass substrate in aqueous solution. As a consequence, the direction of the force vector tilted considerably more than expected. When addressing the surface of a lipid bilayer, the behavior of the force components differed significantly from that observed on glass. This is attributed to the lateral mobility of the lipid membrane coupled with its elastic properties. Direct access to interaction components Fx, Fy, and Fz provides a more complete view of tip dynamics that underlie force microscope operation and can form the foundation for improved 3D AFM.

Existing 3D AFM techniques require recording thousands of frequency shift curves at different lateral locations, followed by off-line integration (to yield energy) and lateral differentiation (to yield lateral force). That procedure is inherently slow and is largely restricted to studies of static samples.

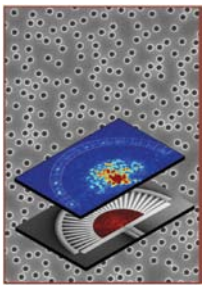
The direct 3D AFM is expected to permit new research in many areas of nanoscience. The instrument has already provided the first measurements of the 3D trajectory of a tapping tip as it interacts with a surface, the first determination of the 3D spring constants of an AFM tip, and the first direct determination of the 3D tip-sample force vector in AFM.

The direct 3D AFM opens new avenues in multi-dimensional AFM in perturbative operating conditions such as mapping the 3D trajectory of flexible and disordered protein motifs in physiological buffer solution. In addition to biophysics, the method is applicable to other fields that use AFM routinely such as nanotechnology, materials science, and chemistry.

Chip-Scale Random Spectrometer

Hui Cao and Brandon Redding of Yale University

Developers: Hui Cao and Brandon Redding



The operation of this compact, general-purpose on-chip spectrometer is based on multiple-scattering in disordered nanostructures. Traditional spectrometers rely on a grating or prism to disperse light into different wavelengths, and the spectral resolution scales with the optical path length from the grating to the detectors. By using a disordered structure as the dispersive element, the random spectrometer overcomes this trade-off by increasing the optical path length through multiple scattering. Optical scattering in random media has been studied for years because of its prevalence in natural systems such as biological tissue and the atmosphere. But historically the goal has been to mitigate the effects of scattering. In this work, disorder was intentionally introduced into the device and shows that optical scattering can be used to improve device performance. In this case, the long path length through a scattering medium enables high spectral resolution in a small footprint.

The proof-of-principle device was fabricated on a silicon-on-insulator wafer, and the disordered structure consisted of cylinders etched in the silicon. The spectrometer operates by measuring the seemingly random intensity pattern produced by light diffusing through the scattering structure. Because the scattering structure is fixed, the same input wavelength will always produce the same intensity pattern, whereas different wavelengths produce distinct patterns. These intensity patterns can be used as fingerprints to identify the input spectrum. To overcome the low-transmission usually associated with a disordered structure, a photonic crystal boundary was introduced to confine the light within the semi-circular scattering structure until it reaches the detectors. Out-of-plane scattering loss was reduced by introducing structural correlations to the positions of the scattering elements. The result is a 25 μm radius random spectrometer that provides 0.75 nm spectral resolution with a 25 nm bandwidth at $\lambda = 1500$ nm.

The on-chip random spectrometer could enable a host of new spectroscopy applications which were previously impractical because of the large size and cost of existing spectrometers, including new applications in field spectroscopy, lab-on-a-chip, and hyperspectral imaging. Moreover, the extremely compact size of the random spectrometer provides the possibility of integration with other photonic components. This could enable lab-on-a-chip systems with enhanced sensitivity and new functionality.

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