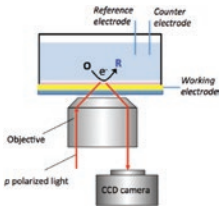
2013 *Microscopy Today* Innovation Awards

Microscopy Today congratulates the fourth annual group of Innovation Award winners. The ten innovations described below move several microscopy techniques forward: light microscopy, scanning probe microscopy, electron microscopy, ion microscopy, and hybrid microscopy-analysis methods. These innovations will make imaging and analysis more powerful, more flexible, more productive, and easier to accomplish.

Plasmonic-Based Electrochemical Microscopy

Arizona State University Center for Bioelectronics and Biosensors

Developer: Nongjian Tao



Plasmonic-based electrochemical microscopy (P-ECM) was invented to image local chemical reactions of individual nanoparticles. It determines the electrochemical current density from an optical signal generated from surface plasmon resonance rather than from electrical measurement with traditional electrochemical detection methods.

The basic principle is as follows. An electrochemical reaction taking place on an electrode is always accompanied by an electron transfer process, gaining or losing one or more electrons by the reactant molecule, which is measured as an electrochemical current in traditional electrochemical methods. The electron transfer process is also always accompanied by the conversion of chemical species between oxidized and reduced states, so one may determine the electrochemical current by monitoring the conversion of the chemical species on the surface, which is the basic principle of the P-ECM.

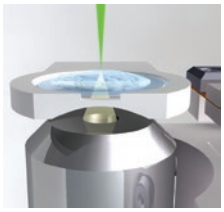
Traditional electrochemical detection measures the total electrochemical current or other related electrical quantities of an electrode, which does not provide local reaction information of the electrode. Local information, however, is important for many applications, including heterogeneous reactions on nanoscale, local activities of cells, and protein and DNA microarrays. Scanning electrochemical microscopy (SECM) overcomes this limitation and has found numerous applications. However, SECM probes local electrochemical current by scanning a microelectrode across the surface, which limits its speed from seconds to minutes and may perturb the electrochemical reactions under study. In addition, the electrical current measured by the microelectrode in SECM scales with the size of the microelectrode, making it increasingly difficult to improve the spatial resolution by shrinking the microelectrode. Instead of measuring the current with an electrode, P-ECM determines the electrochemical current density from an optical signal generated from surface plasmon resonance.

Important benefits of this new method include fast (micro-seconds) and non-invasive electrochemical current imaging and compatibility with the conventional optical microscope. Plasmonic-based electrochemical microscopy is suitable for studying heterogeneous electrochemical process and trace chemical analysis. Specific examples include high throughput screening of catalytic reactions of single nanoparticles, electroanalysis in living systems, and the study of ultra-fast electrochemical reactions.

Scanning Electron Combined Optical Microscopy

Delft University of Technology and DELMIC BV

Developers: Jacob Hoogenboom, Pieter Kruit, Christiaan Zonnevylle, and Andries Effting



Scanning Electron Combined Optical Microscopy (SECOM) integrates a fluorescence microscope (FM) with a scanning electron microscope (SEM) for fast, easy, and accurate correlative microscopy. Correlative light and electron microscopy (CLEM) is a relatively new method that combines advantages of light and electron microscopy in one experiment. Functional information obtained with a FM can be directly overlaid with structural information from the EM. However, current methods of CLEM have limitations, particularly in finding and registering the same region of interest in each type of microscope.

The SECOM platform integrates fluorescence and scanning electron microscopy in one device by equipping an SEM with an inverted FM. The SECOM system can be fitted to an SEM by replacing the specimen chamber door. The door replacement supports a motorized stage and the light optical microscope. The platform comes with an intuitive software package that is designed to easily acquire both types of information.

The SECOM has several advantages. First, the sample stage of the SECOM platform is large, 18 mm × 18 mm, meaning that all usual sample sizes for fluorescence microscopy can be accommodated. Second, a specially developed vacuum compatible immersion oil can be used to obtain the same numerical apertures (1.4) known from conventional fluorescence microscopy. Third, risk of sample damage is reduced because there is no need to transfer the sample between two microscopes. The microscopist places the sample in the sample stage once, and after that the user can easily switch between imaging with the electron microscopy and fluores-

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Microscopy Society of America Awards

Nominations are now open for the Microscopy Society of America Annual Awards. The awards process is one way in which the Microscopy Society of America recognizes the significant and diverse contributions that individuals make to our field. Deserving nominations for consideration should be submitted electronically no later than December 15th, 2013 to:

AssociationManagement@microscopy.org

The Main Society Awards Are

Distinguished Scientist Awards

These Awards recognize preeminent senior scientists from both the Biological and Physical disciplines who have a long-standing record of achievement during their career in the field of microscopy or microanalysis.

Burton Medal

The Burton Medal was initiated to honor the distinguished contributions to the field of microscopy and microanalysis of a scientist who is less than 40 years of age on January 1st of the award year. (Please note the change in the selection criterion regarding age.)

Outstanding Technologist Awards

These Awards honor technologists from both the Biological (Hildegard H. Crowley Award) and Physical Sciences (Chuck Fiori Award) who have made significant contributions such as the development of new techniques which have contributed to the advancement of microscopy and microanalysis.

Morton D. Maser Distinguished Service Award

This Award was initiated to recognize outstanding volunteer service to the Society as exemplified by Mort Maser, who served the Society for many years with great dedication. This award is made to honor an MSA member who has provided significant volunteer service to the Society over a period of years.

The Albert Crewe Award

The Albert Crewe Award was initiated to recognize the distinguished contributions to the field of microscopy and microanalysis in the physical sciences of a postdoctoral fellow of not more than 6 years' standing (since doctoral graduation).

The George Palade Award

The George Palade Award was initiated to recognize the distinguished contributions to the field of microscopy and microanalysis in the life sciences of a postdoctoral fellow of not more than 6 years' standing (since doctoral graduation).

MSA
Microscopy Society of America

Further details of the nomination process can be found on the society webpage at:
www.microscopy.org

cence microscopy. This integrated setup reduces the workflow time of correlative microscopy. Lastly, correlation between electron microscopy and light microscopy data is automatic because the electron and light beam axes are aligned to within 0.2 µm, and an overlay accuracy of 50 nm or better can be achieved.

The significance of SECOM is that CLEM methods now can be accomplished in a single instrument without transferring the sample. The integrated design and the intuitive software package of the SECOM platform makes widespread application of CLEM possible.

The applications of the SECOM platform are found mainly in the life sciences, particularly in tissue biology and thin sections. Specific applications include quickly and easily finding transfected cells, imaging of virus and bacterial infections, and characterization and analysis of bioengineered materials and tissues.

PI 87 SEM PicoIndenter™

Hysitron, Inc.

Developers: Edward Cyrankowski, Ryan Major, S.A. Syed Asif, Yuxin Feng, Derek Rasugu, and Todd Stanley

The Hysitron PI 87 SEM PicoIndenter™ provides *in-situ* nanomechanical testing with access to manipulators, ion sources, and FIB-SEM analytical detectors. The instrument is designed to perform quantitative nanomechanical testing while simultaneously imaging with the SEM or SEM/FIB. Standard test modes include nanoindentation, compression of nanoparticles or pillars, cantilever or beam bending, and tensile testing of nanowires and thin films. Furthermore, sample positioning with five degrees of freedom (X, Y, Z, rotation, and tilt) gives the user the freedom to align the sample with an ion beam for sample preparation/modification or various detectors for advanced analysis.

Using Hysitron's capacitive transducer technology, force is applied electrostatically, and displacement is measured capacitively. This design provides low thermal drift and precise positioning with < 5 nm sensitivity. A secondary x-stage enables lateral motion of the transducer for relative motion between tip and sample. The optically encoded tilt and rotation stages give the user access to a full hemisphere of motion. The PI 87 mounts easily to the SEM stage without being a permanent fixture in the microscope. The compact design of the instrument allows maximum stage tilt and minimum working distance for imaging during testing. The system connects to Hysitron's performech™ DSP-embedded controller, which allows data acquisition rates up to 39 kHz. As compared to previous *in-situ* mechanical test instruments, the PI 87 provides advanced positioning capabilities enabling a higher level of flexibility inside the SEM. Specifically, the tilt and rotation stages ensure that additional detectors (EBSD, EDS, WDS, etc.) are effortlessly within reach for advanced analysis before, during, or after the mechanical test.

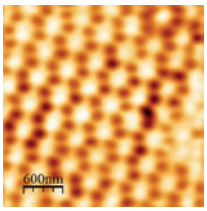
The mechanical properties of small volumes have been found to vary significantly from those of the bulk for a wide range of materials. Pairing high sensitivity of nanomechanical testing with high-resolution SEM has created a new tool for exploring the material deformation mechanisms that may occur during a nano-scale mechanical test.

Materials applications include characterization of fracture onset and measurement of the effects of crystal grain orientation, grain size, and grain structure on mechanical properties through pre- and post-test EBSD mapping. Microelectronics applications include nanomechanical failure analysis related to mismatches of thermal expansion and temperature cycling in highly non-uniform stress situations.

3TB4000 Triple Beam NSOM/AFM & SEM/FIB Microscope™

Nanonics Imaging Ltd.

Developers: Aaron Lewis, Anatoly Komissar, and Andrey Ignatov



The 3TB4000 enables triple beams of light, electron, and ion beams to co-exist simultaneously but unobtrusively with the full complement of other SPM functionalities. This is the first triple-beam instrument with the ability to integrate atomic force microscopy (AFM) and near-field scanning optical microscopy (NSOM) with SEM and FIB technology to provide nanoscale tomographic characterization.

The innovation in this instrument is an open architecture that provides access to the SEM/FIB beams without any obstruction or interference to the injectors, detectors, or beam lines. Open access refers both to the geometry of the instrument and the geometry of a NanoToolKit™ of probes developed for this system. The SPM probe tip developed for this instrument is exposed to the electron and ion beam and sits at the eucentric point, where the position of the three beams on the sample remain the same with sample tilt. The instrument allows any size sample permitted in the SEM/FIB stage to be scanned with the SEM over a large XY range, a range that is not normally accessible to rapid probe placement in SPM. The system brings functional tomographic imaging protocols, not available without SPM and NSOM, within the SEM/FIB environment, enabling true 3D functional characterization of materials with nanoscale resolution.

Applications of the present device include high spatial resolution cathodoluminescence for which the SEM beam in the triple beam instrument can excite carriers with extreme precision. The exposed tip of the NSOM probe can approach within 100 nm of the electron beam and profile nanometrically the emission as a function of distance from the electron beam. In addition, such nano-optical profiling can be correlated with the AFM topography because the NSOM probe is also an AFM

probe. Another application is the detection of fluorescently labeled biological tissue allowing tomographic nano-optical analysis because an NSOM beam, unlike all other optical beams, is confined in all three directions. Additional probe tips provide electrical, thermal, and gas delivery. Furthermore, plasmonic devices also can be investigated with such a combination. While the electron beam stimulates the plasmons to give off light, the FIB provides an online trimming tool so that plasmonic devices can be studied *in situ* with nano-optical resolution.

Nano-FTIR

Neaspec GmbH

Developers: F. Huth, R. Hillenbrand, and F. Keilmann

Nanoscale chemical identification and mapping of organic materials is now possible with nano-FTIR. Conventional infrared spectroscopy is non-invasive and highly sensitive to molecular bonds, but its spatial resolution is limited by diffraction to about half the incident infrared wavelength (several μm). The new technique of nano-FTIR combines the nanoscale spatial resolution of near-field microscopy with the analytical power of Fourier transform infrared (FTIR) spectroscopy. Nano-FTIR allows fast and reliable chemical identification of organic and inorganic material with about 1,000 times better spatial resolution (20 nm) when compared to the standard FTIR method. Because nano-FTIR is based on atomic force microscopy (AFM), it only requires standard AFM sample preparation.

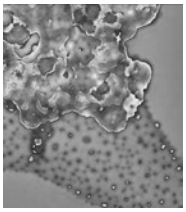
The nano-FTIR method breaks the diffraction limit in FTIR by combining apertureless near-field microscopy, also known as scattering-type scanning near-field optical microscopy (s-SNOM), with Fourier transform infrared spectroscopy. In operation, a focused broadband infrared beam illuminates a standard metal-coated AFM probing tip. The metallic tip acts as an antenna, concentrating the incident light at the tip. In this way an infrared nanofocus is created at the tip apex, the size of which is only determined by the size of the tip apex. For near-field optical microscopy and nano-FTIR, this means that sharper probes will produce both better field confinement and higher infrared field strength. The light backscattered from the oscillating metallic tip is analyzed with an asymmetric Fourier transform spectrometer based on a Michelson interferometer. To separate the weak near-field signals from the dominant background contributions, the detector signal is demodulated at a higher harmonic of the tip vibration frequency. Translation of the reference mirror with a piezo stage yields an interferogram of the demodulated signal, which can be transformed to a complex-valued near-field spectrum carrying information about the local dielectric function, refractive index, and local absorption by the sample.

Applications of this technology include high spatial resolution chemical identification and mapping for organic and inorganic semiconductor technology, photovoltaics, polymer science, and life sciences. The s-SNOM technology of nano-FTIR also allows mapping of the near fields of nanophotonic and plasmonic structures.

Phase Focus Virtual Lens®

Phase Focus Ltd. and Gatan, Inc.

Developers: John Rodenburg, Andrew Maiden, Martin Humphry, Bernd Kraus, and Michael Sarahan



The Phase Focus Virtual Lens® is a digital replacement for a microscope's conventional image-forming optics. A specimen is illuminated by a patch of illumination referred to as the "probe," which is typically much larger than the desired resolution. Because the method automatically computes the probe's phase and amplitude distribution, deleterious effects of non-uniformities in the illumination can be eliminated. The probe is shifted relative to a number of approximately known overlapping positions on the specimen. At each position, the transmitted or reflected diffraction pattern is recorded on a standard two-dimensional array detector. The Phase Focus Virtual Lens® phase retrieval algorithm processes the diffraction patterns to create an image pair from the specimen: a modulus image and a phase image. The simple hardware requirements comprise a computer to implement the digital image formation process, an coherent illumination source (for example, a diode laser for visible light applications or a field emission gun for electron applications), a conventional 2D detector (for example, a CCD camera), and a means of shifting the illumination laterally with respect to the specimen.

The Phase Focus Virtual Lens®-enabled electron microscope operates at electron wavelengths without the need of magnification optics. A Phase Virtual Virtual Lens® transmission electron microscopy capability was integrated into a SEM of standard specification and operating at 30 keV. A resolution improvement of a factor of five over that available from the host instrument's conventional electron optics was obtained, revealing atomic-scale structure.

The Phase Focus Virtual Lens® overcomes the shortcomings of (and even need for) conventional focusing optics such as glass and quartz lenses at visible and ultraviolet wavelengths, nanofabricated gold zone plates at X-ray wavelengths, and electromagnetic lens optics at electron wavelengths. It therefore eliminates the limitations and aberrations of conventional lens-based instruments. A 3D version enables separation of thin tomographic "slices" through a thick specimen. Moreover, the method simultaneously provides quantitative phase images, which can be used to characterize specimen thickness profiles, or electric and magnetic field phenomena.

The Phase Focus Virtual Lens®-enabled electron microscope is expected to support the imaging of specimens at sub-atomic resolutions, surpassing that available from today's state-of-the-art transmission electron microscopes. In addition, the simultaneously produced phase information is expected to enable applications in semiconductor analysis, stain-free cell imaging, and magnetic field mapping.

Poseidon 500

Protochips, Inc.

Developers: John Damiano, David Nackashi, and Dan Gardiner

The Poseidon 500 is an *in-situ* transmission electron microscope (TEM) electrochemistry sample holder with electrolyte flow and a selection of electrode configurations and materials. Typical electrochemistry tools are large and cannot provide real-time visualization of nanoscale processes occurring during reactions; whereas, TEMs usually cannot have liquids for electrochemical experiments in the specimen chamber under vacuum conditions.

The Poseidon 500 system also integrates microfluidics so the electrolyte can be refreshed, the concentration changed, or a new type of electrolyte introduced without taking the holder out of the microscope. The resulting reaction dynamics can be quantitatively analyzed and correlated with effects imaged in the TEM at the same time that spectra are acquired with the potentiostat/galvanostat. The key intellectual property in the system is the semiconductor-based technology called Environmental Chips (E-chips™), which provides both active and passive sample supports. The active E-chip, to which the sample is affixed, has three electrical leads that act as reference, working, and counter electrodes. The passive E-chip has spacers with well-defined heights that set the liquid layer thickness. Samples can be biased and measured with low-current signals during analysis.

The Poseidon 500 *in-situ* electrochemistry system allows users, for the first time, to image and analyze dynamic nanoscale reactions in their real-world native environment. At some level, all reactions occur at the nanoscale on the surface. This new system overcomes previous technological limitations in electrochemical experiments by combining the E-chip technology with ultra-low noise electronics and novel liquid flow capabilities in a platform that fits nearly all modern TEMs.

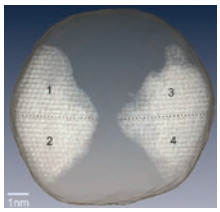
Applications of this device include real-time imaging of electrochemical processes involved in the development of improved materials and processes for batteries, fuel cells, and catalysts, as well as for more traditional processes like electroplating and corrosion prevention. Nanoscale processes must be understood to advance each of these fields. For example, many new battery systems rely on nanomaterials for operation, and detailed investigations of individual nanostructures and their interactions under various cycling conditions are of interest.

Atomic Resolution Electron Tomography

Jianwei Miao, University of California, Los Angeles

Developer: Jianwei Miao

This general electron tomography method achieves atomic scale resolution without the use of *a priori* information. By combining a novel projection alignment and tomographic reconstruction method with a scanning transmission electron microscope (STEM), this system has determined the structure of an Au nanoparticle at 2.4 Å resolution in three dimensions. In this experiment, a tilt series of 69 projections from a ~10 nm Au nanoparticle was acquired using annular dark-field (ADF) imaging in a STEM.



The tilt series was aligned with the center-of-mass method at atomic level precision. The reconstruction of the aligned tilt series was conducted with an equally sloped tomography (EST) algorithm. The EST algorithm iterated back and forth between real and reciprocal space through the use of the pseudopolar fast Fourier transform. Using the iterative EST algorithm, a preliminary 3D reconstruction was obtained after 500 iterations. A tight 3D support (that is, close to the true boundary of the particle) was determined by convolving the reconstruction with a Gaussian window and selecting a suitable cut-off. Using the tight support, another 500 iterations were performed to obtain a final reconstruction. The 3D surface morphology and internal lattice structure revealed by this method are consistent with a distorted icosahedral multiply twinned particle.

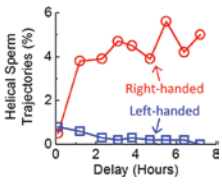
Electron tomography with atomic resolution, however, has not been demonstrated previously for several reasons. First, aligning the projections of a tomographic tilt series to a common axis with atomic scale precision is technically challenging. Second, radiation damage limits the number of projections that can be acquired from a single object. Finally, specimens cannot usually be tilted beyond ±79°, preventing acquisition of data from the "missing wedge." Now these limitations can be overcome or alleviated by applying the present novel approach.

Transmission electron microscopy (TEM) can routinely resolve atoms in the 2D projection of a 3D object. Scanning probe microscopy and surface electron diffraction techniques can determine the surface structure at the atomic level. The method described here represents a general technique to determine the 3D local structure of materials at atomic-scale resolution. It is expected this general method will find application in materials science, nanoscience, solid-state physics, and chemistry.

Lensfree On-Chip Microscopy

Aydogan Ozcan, University of California, Los Angeles

Developer: Aydogan Ozcan



The lensfree on-chip imaging technique can track the 3D trajectories of more than 1,500 individual human sperms within an observation volume of ~8–17 mm^3 with sub-micron accuracy. This computational imaging platform relies on holographic lensfree shadows of sperms that are simultaneously acquired at two different wavelengths, emanating from two partially coherent sources that are placed

at 45 degrees with respect to each other. This multi-angle and multi-color illumination scheme permits dynamic tracking of the 3D motion of human sperms across a field-of-view greater than 17 mm^2 and a depth-of-field of ~0.5–1 mm with sub-micron positioning accuracy. The large statistical sample provided by this lensfree imaging platform revealed that only about 5% of motile human sperms swim along well-defined helices and that this percentage can be significantly suppressed under seminal plasma. Furthermore, among these observed helical paths, a significant majority (~90%) preferred right-handed helices over left-handed ones, with a helix radius of ~0.5–3 μm, a helical rotation speed of ~3–20 rotations/sec, and a linear speed of ~20–100 μm/sec. This high-throughput 3D imaging platform could in general be quite valuable for observing the statistical swimming patterns of various other microorganisms, leading to new insights in their 3D motion and the underlying biophysics.

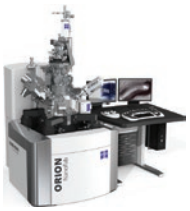
Based on this new lensfree computational imaging platform, it is possible to visualize, for the first time, the helical trajectories of human sperms, an observation that could not be reported before this innovation, mostly because the tight 3D radii of such helices, as well as the rapid rotation speed of human sperm, make it rather challenging to image using the limited sample volume and depth-of-field of conventional microscopy techniques.

These results shed new light on 3D swimming patterns of human sperms, revealing several important observations that have so far been hidden due to limited capabilities of existing optical imaging platforms. This multi-angle and multi-color illumination scheme permits us to computationally track the 3D motion of human sperms across a field-of-view of >17 mm^2 and depth-of-field of ~0.5–1 mm with sub-micron 3D positioning accuracy, which is not possible using other imaging or tracking platforms.

ORION NanoFab

Carl Zeiss Microscopy LLC

Developer: Carl Zeiss Microscopy



The ORION NanoFab is a nanofabrication tool that integrates helium, neon, and gallium focused ion beams on a single platform. The helium and neon ion beams are based on the gas field ion source (GFIS) technology. The same instrument also provides a traditional gallium focused ion beam (FIB). Thus, ORION NanoFab is a truly multi-ion beam platform that allows users to choose from three ion beams (helium, neon, and gallium) depending on the application at hand.

In the ORION NanoFab, helium or neon gas is ionized by a large electric field on an atomically sharp tip to generate the helium or neon ion beam. The beam is then focused and used for nanofabrication or imaging of the sample. Because of the small spot size (< 0.5 nm for helium and < 1.9 nm for neon) and the interaction dynamics of light ions with the sample, sub-10 nm sized structures can be routinely fabricated via ion beam milling, ion beam lithography, or by use of a gas injection system. Neon ions are heavier than helium ions, which makes structuring processes considerably faster; whereas, the helium ions allow imaging of the sample at a very high resolution.

ORION NanoFab also features an optional state-of-the-art gallium FIB. There are traditional applications for which gallium FIB is better suited, for example, massive removal of material and prototyping of structures that are not affected by gallium implantation (smallest feature size is about 30 nm). However, for emerging applications where sub-10 nm feature size is critical or gallium implantation is a problem, users can switch to neon or helium ion beams in ORION NanoFab. When the nanofabrication is complete, samples can be imaged in high resolution in the same machine without removing the sample. Thus, ORION NanoFab is a versatile machine that enables new solutions for the challenges faced in nanofabrication.

ORION NanoFab allows researchers and companies to prototype devices in fewer steps and without contamination by using neon and helium ion beams. Results have been demonstrated for the following applications: nanopores for bioanalysis applications, graphene nanoribbon patterning, imaging of shale and coal for oil and gas recovery, ion beam lithography, plasmonic devices, and semiconductor circuit edit tasks.

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