2012 *Microscopy Today* Innovation Awards

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

Dimension FastScan™ AFM

Bruker Nano Surfaces

Developers: Chanmin Su, Charles Meyer, Carl Masser, Ton Ruiter, Nghi Phan, and Johannes Kindt

The Dimension FastScan AFM provides at least an order of magnitude improvement in scanning speed for traditional AFM applications and two orders of magnitude greater speed for certain applications. The system is enhanced by a platform design that enables large sample scanning and algorithms that control the tip and sample interaction with higher precision using lower forces than previous commercial AFMs.

Dimension FastScan enables scientists and researchers to attain atomic resolution and measure nano-mechanical material properties *in-situ* in a fraction of the time previously required. Many advantages of AFM have been hindered by the technique's slow imaging speed as compared to other microscopy techniques, and this new product offers near-real-time results where frames per seconds are possible compared to more than 5-minutes per frame for the same image quality. Reducing the time to first explore a heterogeneous surface, find the region of interest representing the sample's morphology, and capture a publication-quality image is a clear benefit for scientists.

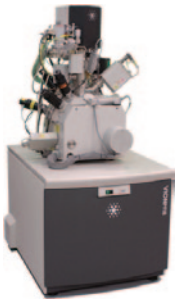
The limiting factors for the scanning speed in an AFM can be deduced and observed in the scanner, closed-loop controlling electronics, digital processing electronics, and the cantilever interacting with a sample. Because feedback loops are only as fast as their slowest component, producing a high-speed AFM that is free of image distortion, resolution degradation, and sample damage requires that the bandwidth of every component in the AFM feedback loop be improved significantly.

This high-speed AFM greatly increases productivity in capturing the large number of images needed to understand the synthesis of materials with statistically valid data quantities. The biggest advantage and most interesting application that benefits from high-speed AFM is the ability to observe dynamic processes with sufficient time resolution to study phenomena such as crystal formation, protein dynamics, or aging processes.

Vion™ Plasma Focused Ion Beam

FEI Company

Developers: Sean Kellogg, Tom Miller, and Kenny Mani



The Vion Plasma Focused Ion Beam (PFIB) from FEI provides site-specific, package-level physical analysis and reconfiguration capability for three-dimensional integrated circuits (3D ICs). Focused ion beam systems have long been used for 3D metrology, defect analysis, failure analysis, and circuit editing of structures ranging in size from tens of nanometers to a few micrometers. However, new generations of multichip integration and advanced packaging technologies use through-silicon vias (TSV) and novel chip bonding techniques to stack chips and make high-performance interconnects. These structures have linear dimensions that are at least 10× larger than the on-chip device structures to which FIB analysis has been typically applied. The resulting 1000× increase in the volume of material that must be removed during analysis (with a commensurate increased analysis time) has, until now, precluded the practical use of standard FIB instruments in these applications.

The Vion PFIB uses an inductively coupled plasma source to deliver material removal rates 20× to 50× greater than those available using conventional gallium liquid metal ion sources (LMIS). Because of its small virtual size, the LMIS is easy to focus into a small spot at low beam currents, but at beam currents above 10 nA, aberration effects severely degrade performance. The plasma source can deliver currents in excess of a microamp (>20× greater than a typical LMIS-based system) while still maintaining a well-focused beam. The Vion achieves these increases in speed without significant sacrifice in imaging resolution or low-voltage/low-current performance. The system's ability to provide site-specific cross-sectional analysis of 3D integration technologies in minutes rather than hours should accelerate advanced packaging process development and reduce time-to-market for new products.

The Vion PFIB provides a practical tool for a variety of 3D integration applications, including failure analysis of bumps, wire bonds, TSVs, and stacked dies. While the electronics industry is an obvious first use for the new Vion PFIB system, there are also important potential applications in materials science and natural resource development.

Microscopy TODAY

Innovation Awards

Congratulations to the winners of the 2012 awards:

- Bruker Nano Surfaces
- FEI Company
- Gatan Inc.
- University of California at San Francisco
- Leica Microsystems
- Lumen Dynamics
- Olympus America
- Pacific Northwest National Laboratory
- Hummingbird Scientific
- Rebellion Photonics
- Technical University of Denmark
- Tsinghua University
- Institute of Metals of the Chinese Academy of Sciences
- Johns Hopkins University
- Tousimis Research Corporation

Next entry deadline: March 15, 2013

Application forms at www.microscopy-today.com

K2 Summit Direct Detection Camera

Gatan, Inc.
Lawrence Berkeley National Laboratory
University of California at San Francisco

Developers: Sander Gubbens, Paul Mooney, Peter Denes, and David Agard

The K2 Summit Direct Detection Camera is a new type of camera for transmission electron microscopy. The product contains a direct detection transmission CMOS detector, which gives the highest resolution images of any electron-imaging sensor available today. The camera runs in a mode of constantly collecting images at 400 frames per second. The camera then, through a high-bandwidth link, passes the complete frames to dedicated high-throughput hardware designed for the express purpose of processing these images in real time. By collecting and processing full-frame images so quickly the detector can identify and record individual electron events (counting) as they reach the sensor. By counting every single electron event, the camera can eliminate the background noise typically seen in devices that simply read out the charge deposited by an electron striking a piece of silicon. By removing this source of noise, the camera can offer higher image quality and sensitivity than previously available in an electron-imaging device.

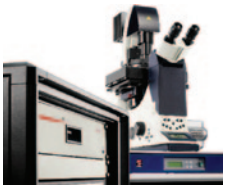
This device is also capable of operating in a Super-Resolution mode. In this mode, the sensor is able to localize the electron event with sub-pixel accuracy, effectively doubling the number of pixels available for imaging (from 3840 × 3712 to 7680 × 7424). Again the processing is all done on full frames in real time as the images are collected. The camera is also capable of reading out a sub-region of the detector. The high throughput processing chain can then capture bursts of images at greater than 1000 frames per second. In addition, the camera employs a pixel design giving radiation hardness 10× greater than any other direct-detection sensor available (pixel lifetime of >5 billion electrons).

The biggest initial application for this type of camera will be in the field of low-dose biological imaging for structural biology. This type of work is not limited by microscope performance but by the signal-to-noise ratio that can be achieved before the imaging damages the specimen. Another important application for this technology is to study dynamic systems (*in-situ* microscopy).

Leica SR GSD Super-Resolution Microscope System

Leica Microsystems CMS GmbH

Developers: Marcus Dyba and Jonas Foelling of Leica Microsystems, Germany, based on an invention by Prof. Dr. Stefan W. Hell, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany



The Leica SR GSD is a fluorescence imaging system enabling life scientists to observe cells and individual molecules with about 10-fold higher resolution power compared to standard light microscopes. The highlights of its performance are: resolution down to ~20 nm, drift suppression thanks to the SuMo Stage (Suppressed Motion Stage), and effective localization thanks to powerful laser sources and sensitive detection.

With the super-resolution microscopy method Ground State Depletion followed by Individual Molecule return (GSDIM), developed by Prof. S. Hell at the Max-Planck-Institute in Göttingen, exclusively licensed by Leica Microsystems, it is possible to overcome the diffraction limit. Typically in fluorescence imaging individual fluorescent molecules light up upon excitation but all 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: controlled laser light turns them into a dark state. From time to time, individual molecules spontaneously return to the fluorescent state, while their neighbors remain non-illuminating. In this way, the signals of individual molecules can be acquired sequentially using a highly sensitive camera system. Other improvements in the SR GSD system that enhance super-resolution microscopy are drift compensation, live super-resolution image display, and high-power lasers that allow the use of standard fluorochromes.

The Leica SR GSD is a multi-modal system for fluorescence imaging in widefield, total internal reflection fluorescence (TIRF), and super-resolution microscopy modes. The system is capable of resolving details as small as 20 nanometers and thus builds a bridge between light microscopy and electron microscopy. The system provides detailed insights into cellular structures that cannot be seen with diffraction-limited light microscopy. It should find application in understanding the structure of fluorescently labeled specimens in neurobiology, cell biology, virology, microbiology, and physiology.

Internal Pulse Generator for the X-Cite® XLED1

Lumen Dynamics Group Inc.

Developers: Thomas B. Porter and Stewart A. Clark

The Internal Pulse Generator (IPG) provides precise time control of individual LEDs within the X-Cite XLED1 Fluorescence Illuminator down to the microsecond timescale, allowing the pulsing of single LEDs at user-defined intervals from 10 µs to 18 hours. The LEDs can be independently switched on and off for a defined time period and coordinated with a sync out signal that can be referenced

to the LED switching with a positive, negative, or zero time offset. For example, before the LED is turned on, a negative offset allows a signal to be sent out to peripheral equipment. The LEDs can be synchronized to operate: (a) together from a tandem pair or from the maximum number of LEDs in the system, (b) with a combination of internally synchronized and external arbitrary pulse sequence(s), or (c) with all LEDs run independently. The IPG will normally be configured to operate in a free-running mode that will repeat the pulse sequencing at the end of the specified cycle. When operating solely from the IPG, the LEDs will always be phase-aligned with respect to each other. Finally, with IPG the XLED1 can serve not only as a premium light source, but as a central triggering device as well, synchronizing peripherals such as cameras and stages with LED on/off events.

Because the IPG is within the XLED1, the researcher benefits from the intrinsic characteristics of LEDs for fluorescence excitation: long lifetime, spectral specificity, reduced photobleaching and phototoxicity, and a full spectrum of wavelength options. Furthermore, without this innovation, the flexibility described above in controlling the light source would only be possible by introducing an external pulse generator: an extra piece of hardware costing thousands of dollars and requiring additional effort to integrate into the imaging system.

Fast pulsing allows applications such as optogenetics or calcium imaging. Rapidly timed switching between LEDs facilitates imaging of live cell events, and some researchers believe that fast pulsing may extend the imaging time for live cells.

SCALEVIEW Microscope Objectives

Olympus America Inc.

Developers: Katsuyuki Abe, Masahiro Sakakura, Takashi Kasahara, and Takeshi Uehara

These two light microscope objectives, when used with a specific clearing reagent, provide a significant increase in imaging depth into cleared tissue. There are two SCALEVIEW optics: (a) a 25×, NA 1.0 objective, designed to image 4 mm deep into contiguous tissue and (b) a 25×, NA 0.9 objective, designed to image up to 8 mm deep. Both objectives are designed to boost the capabilities of multiphoton and confocal microscopies and allow scientists to look deeper than they ever could before. In the mouse brain, for instance, neurons and blood vessels far beyond the white matter, as deep as 4 mm beneath the surface to the hippocampus, have been clearly viewed and recorded using the 4 mm objective. With the 8mm objective, structures as deep as the thalamus and hypothalamus have been observed.

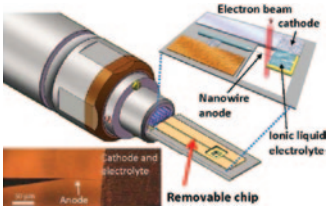
Without a clearing agent, light scattering within tissue prevents imaging deep into the mouse brain. Previous clearing agents, however, tended to quench the fluorescence signals from labeled cells. A clearing reagent to overcome this problem was invented by Atsushi Miyawaki and his team at the RIKEN Brain Science Institute in Japan (Hiroshi Hama, Hiroshi Kurokawa, Hiroyuki Kawano, Ryoko Ando, Tomomi Shimogori, Hisayori Noda, Kiyoko Fukami, and Asako Sakauo-Sawano), and their results were published online in the August edition of *Nature Neuroscience* (Hama et al., Aug 30, 2011). The clearing reagent is commercially available as SCALEVIEW-A2 by Olympus. The new objectives work well because they were specifically designed and engineered to match precisely the 1.38 refractive index of the clearing reagent. With this system, bright fluorescent protein signals can be retained and imaged in a cleared sample.

Beyond the mouse brain, applications of these objectives may extend to human brains as well. In addition to the brain, the new objectives are appropriate for viewing entire organs and embryos to better understand the mechanisms of development.

Multimodal Electrochemical Probe for *in-situ* TEM

Pacific Northwest National Laboratory
Hummingbird Scientific

Developers: Chongmin Wang, Wu Xu, Jiguang Zhang, Jun Liu, S. Thevuthasan, Donald R. Baer, Norman Salmon, and Daan Hein Alsem



This multimodal electrochemical probe is an electrical-biasing transmission electron microscope (TEM) sample holder that enables implementation of versatile electrochemical experiments for *in-situ* TEM. The probe consists of two main components: the body of the TEM holder with electrical connector and a removable chip. The body of the holder features a slide-in point connector, which enables connection of up to eight wires to an electrochemical testing control unit (such as a potentiostat). An electrochemical device may be loaded onto the removable chip, and the chip connects to the body through a spring-loaded sliding mechanism.

The innovative feature of this probe is the design of the removable chip that is machined out of "cheap" printed electronic board and can be modified to allow attachment of a miniaturized electrochemical device. Because of the relatively large size of the chip and the ease of machining different configurations, a wide range of electrochemical devices may be implanted onto the chip. One typical example is the fabrication of a lithium ion nanobattery on the chip that allows *in-situ* TEM observation of the structural evolution of an electrode during dynamic charging and discharging of the battery. In this nanobattery application, $LiCoO_2$ coated on aluminum foil

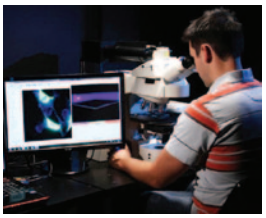
is used as the cathode, an ionic liquid is used as the electrolyte, and a single nanowire dipped into the electrolyte is used as the anode. Because of the low vapor pressure of the ionic liquid, the electrolyte can be directly loaded into the TEM column without sealing. The special sliding electrical connector allows connection between the holder and chip to be made in a few seconds.

This probe is a platform for studying some fundamental physical and chemical processes in electrochemical devices such as lithiation-induced structural evolution of electrodes, charge and ion transport, corrosion kinetics, electrolysis and electroplating, and nucleation and growth of nanoparticles from a solution.

ARROW Hyperspectral Imaging Video Camera

Rebellion Photonics

Developer: Robert Kester



The ARROW is a real-time hyperspectral imaging video camera that allows microscope users to simultaneously detect, track, and monitor processes and interactions within a biological cell. It offers real-time operation with two orders of magnitude higher sensitivity than existing spectral imagers. The ARROW replaces an ordinary digital camera with a system that collects spectrally resolved images. Unlike other spectral imaging systems that scan images one wavelength at a time, or scan a laser spot across the image one pixel at a time, the ARROW behaves like an ordinary camera in that the data are collected in a snapshot rather than in a scanning format. This eliminates scanning artifacts when imaging dynamic objects, provides improved light collection, and acquires images at much higher speeds.

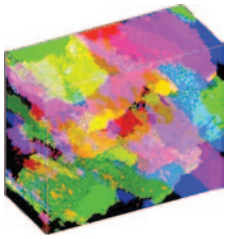
The ARROW's operation is based on a compact array of hundreds of line-imaging spectrometers facilitated through the use of a custom-fabricated optical element, known as an image mapping mirror, coupled to an array of prisms and imaging lenses. The image mapping mirror is a set of thin mirror facets, each with separate tilt angles, designed to encode the full 3D (x-y-λ) data onto a 2-D detector array (x-y) without the use of moving parts or filters. Three recent technological advances made this device possible. First, was the steady decrease in cost for large-format detector arrays, together with the decrease in pixel size. The second advance involved the manufacturing tools for making precision array optical elements. Third was the increased computing power available in desktop computers, enabling algorithms that can readily display and analyze the large datasets produced by these instruments.

Because the ARROW images all at once, instead of scanning, it offers a wide variety of new possibilities for researchers including the following: real-time tracking of the chemicals within cells, imaging over 10 fluorescent dyes simultaneously (instead of the normal 3), removing background light (such as autofluorescence) without losing information, and real-time spectral unmixing for tracking dynamic processes.

3D-OMiTEM

Technical University of Denmark
Tsinghua University
Institute of Metal Research, Chinese Academy of Sciences
Johns Hopkins University

Developers: Haihua Liu, Søren Schmidt, Henning Friis Poulsen, Andy Godfrey, Zhiquan Liu, John A. Sharon, and Xiaoxu Huang



The 3D-OMiTEM produces 3D orientation maps from electron-transparent specimens that are substantially thicker than the average grain size. Existing 3D electron backscatter diffraction (3D-EBSD) techniques require destructive serial sectioning of the sample as part of the data collection process and have a resolution of only 20–50 nm. The 3D-OMiTEM is the first technique that can allow non-destructive 3D orientation mapping with a resolution down to 1 nm. The technique can be installed on any computer-controlled TEM where a large sample tilt-angle range is available.

Data collection is based on automated conical scanning dark-field imaging, achieved using scripting codes for automatic operation of the microscope with respect to the beam tilts. At each sample tilt, the beam tilt angle is varied to cover up to 10 diffraction rings, and for each diffraction ring images are acquired over equiangular steps of the azimuthal angle in the range 0–360°. To obtain 3D information, this procedure is repeated over a wide range of sample tilt angles (up to ±60°), in steps typically of 1° or 2°, so that in total more than 100,000 images are collected and stored in the memory of a dedicated computer. A software package is used for orientation determination and 3D reconstruction. The discrete set of rotations that best brings a set of theoretical diffraction vectors onto a subset of the measured diffraction vectors determines the crystallographic orientation of each voxel in the investigated region of the sample. A volume formed by adjacent voxels of the same orientation is identified as a grain.

This new method has a wide range of potential applications in materials science, solid-state physics, and nanotechnologies. The generation of 3D orientation maps for nanocrystalline materials provides information on structural parameters such as the position, size, morphology, and orientation of each crystallite or particle. One example in materials science is to use the technique for 3D studies of deformation mechanisms (dislocation activity, crystal rotation, grain boundary sliding) in nanocrystalline materials.

Tousimis touch screen 931 with "Stasis Software"

Tousimis

Developers: Chris Tousimis, Hyun Park, Andrew Mitz, and Yianni Tousimis

The Tousimis touch screen 931 with the patent pending "Stasis Software" was conceived out of the need to meet current demands for critical-point drying (CPD) processing that lie outside the traditional biological and materials sample types. Today many laboratories are seeking alternative CPD methods to dry much more difficult sample types than in the past, including various gel types, metal-organic frameworks, nanoparticles, graphene, and others. By employing the new "Stasis Software" platform in the 931, these difficult samples may be processed.

Traditional critical point dryers have not been able to automatically handle thicker matrix samples or molecular level sample types because of difficulty in removing the embedded intermediary fluid within the sample. The "Stasis Software" option provided with the 931 enables the user to customize the length of time and the number of cycles that the sample will sit in a stasis environment. "Stasis Software" allows the 931 to achieve optimal fluid viscosity and density while the sample goes through repeated cycles and periods of process dormancy during the processing run. This dormancy allows the intermediary liquid to leave the sample and the liquid CO_2 to completely penetrate the sample. Complete penetration by liquid CO_2 is essential for proper critical point drying because any region of the sample with residual intermediary fluid will not go through the critical point at the critical temperature and pressure for CO_2. This will cause structural collapse and insufficient drying.

The Tousimis touch screen 931 is able to CPD process samples via three operational modes: (a) Default Auto-Sequence, the pre-programmed default mode that has preset values that work best for the majority of CPD samples. (b) Manual Operational Mode, which allows the user to operate the system in a push-button mode using the touch screen as a controller. (c) Custom Auto-Sequence Mode, which permits individual user recipes to be entered and stored for later use. This mode also includes the "Stasis Software."

Applications for the Tousimis touch screen 931 include a variety of nanomaterials. The instrument also should be able to process yet-to-be-discovered difficult samples.

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