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# Expanding the Power of Optical Microscopes to Enhance Live Cell Imaging

Announced by Harvard University and Leica Microsystems

CARS Microscopy Technology is being developed to expand the power of optical microscopes and enhance scientists' ability to image molecules in living cells and organisms. The technology was developed in the lab of Xiaoliang Sunney Xie, Ph.D., Professor of Chemistry and Chemical Biology at Harvard.

Coherent Anti-stokes Raman Scattering (CARS) microscopy allows rapid and non-perturbative imaging of biological specimens with chemical selectivity. The contrast in CARS microscopy arises from the intrinsic vibrations of molecules. Every molecule has one or more chemical bonds, the bending or stretching of which have characteristic vibrational frequencies that depend on the bond length and strength. For example, lipids, a primary component of fat, contain carbon-hydrogen bonds, which vibrate at certain distinct frequencies. CARS microscopy "tunes" into these characteristic frequencies to build chemically-selective images with extremely high sensitivity in living cells or organisms.

To image a specimen, such as tissues or cells, CARS microscopy utilizes two highly focused laser beams at different frequencies. By setting the difference between the two laser frequencies equal to the frequency of vibration of a particular chemical bond, molecules with that bond are made to vibrate coherently. This causes the sample to emit at a new frequency (called the "Anti-stokes" frequency) from the laser focus. An image is created by scanning the beams over the sample and detecting the intensity of the emitted Anti-stokes light at each position. In this way, one can map the concentration of the molecule of interest (e.g., lipid) throughout the tissue, or within a cell with 300nm lateral resolution. The method offers much higher time resolution than other vibrational imaging techniques, which allows "movies" of biological activity and chemical processes to be taken within a living cell or organism.

Harvard University

By using excitation lasers at near-infrared wavelengths, which can penetrate deep into tissue, CARS microscopy can reach a depth of nearly 0.3mm below the surface. Efforts are underway to extend CARS microscopy for not only cell biology applications, but also disease diagnostics and real-time surgical guidance.

**Why CARS microscopy?**

- Detecting the intrinsic vibrational signatures of molecules circumvents the need for fluorescent and other extrinsic labels, and permits "chemical mapping"– a visualization of the distribution of specific molecules.
- The high sensitivity of CARS allows data collection rates that are orders of

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## Expanding the Power

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magnitude faster than previous vibrational imaging techniques.

- The nonlinear nature of the CARS process assures that the signal is generated only at the laser focal point, leading to high-resolution 3D imaging of tissue and cellular structures.
- Near-infrared excitation beams allow deeper penetration in tissues than visible light, which allows noninvasive measurements with minimal photo damage.

"CARS microscopy has matured as a powerful imaging tool for biomedicine. It is complementary to magnetic resonance imaging (MRI). Although we do not have the penetration depth of MRI, we have much better spatial and time resolutions at a much lower cost," states Xie.

Harvard University's Office of Technology Development (OTD) and Leica Microsystems announced on May 15, 2007 that Harvard has licensed its CARS microscopy technology to Leica for use in the company's confocal microscopes. "This technology has far-reaching implications for helping advance important biomedical research," states Isaac T. Kohlberg, Chief Technology Development Officer, Harvard University. "Our agreement with Leica Microsystems is aligned with our strategy to partner with the best and most expert companies who, like us, are dedicated to excellence and quality."

# Technology Fast Track

## Real-time, *In vivo/In situ* Small Animal Imaging

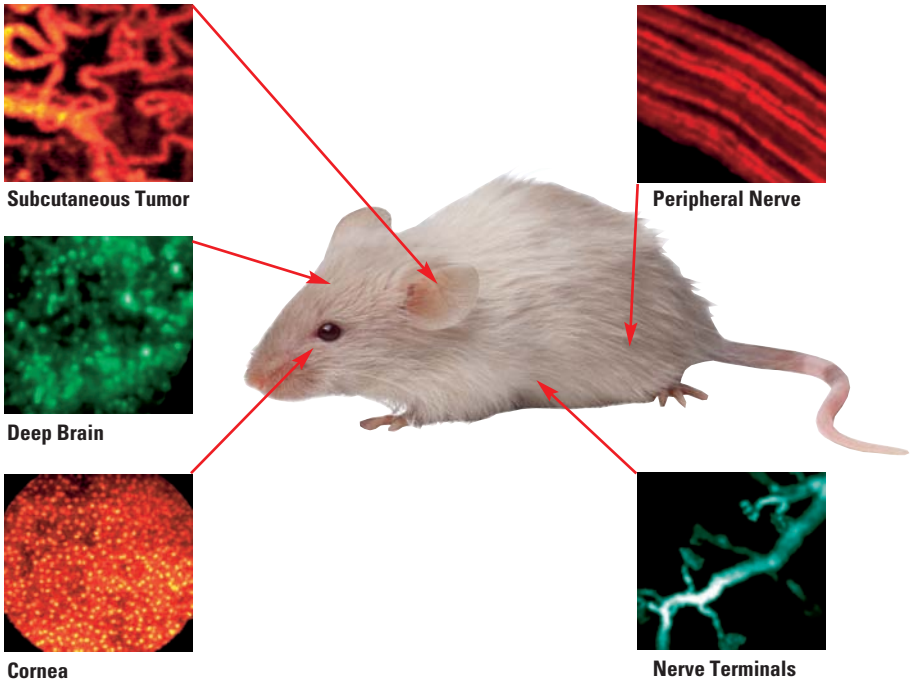
Submitted by Carolyn Custody, Mauna Kea Technologies

Sub-cellular imaging of tissue anywhere inside a living animal is now possible. From whole body imagers to intra-vital microscopy, many solutions have been developed to approach this goal. However, these approaches have always struggled with the trade-off between high resolution and low invasiveness; now that gap has been bridged.

With a fiber-optic confocal imaging system, simple probe contact with the tissue of interest generates high-speed documentation of cellular or vascular events *in vivo/in situ*. The miniature fiber-optic microprobe can be introduced with minimal invasiveness into living animals to allow convenient, repeated measurements on the same animal during time points separated by hours, days or weeks. Software quickly processes and analyzes images and allows easy movie creation.

Real-time imaging of biological events only requires the microprobe to be in contact with the fluorescent tissue. Recording of fluid dynamic movies is then only one click away. An exceptional full-field frame rate ensures high image stability as well as smooth, precise positioning of the field of view. The microprobe can be handheld, or combined to a micromanipulator or stereotaxic apparatus. From instrument setup to image acquisition and data analysis, the system is designed to be easy to use.

All of this is accomplished with the Leica FCM1000 Fiber-optic Confocal Microscope. This technology, formerly known as Cellvisio® from Mauna Kea, bridges the gap between whole animal systems and intra-vital microscopes and is the first imaging solution developed for – and fully adapted to – *in vivo* and *in situ* small animal imaging.



## A Little Can Go a Long Way . . . Fast

Impressions of the International Meeting on AFM in Biology and Medicine (AFM BioMed), Barcelona, April 19-21, 2007

By Shazia Qureshi, Amsterdam, the Netherlands

At the first AFM BioMed conference, held in April in Barcelona, Spain, world-leading life science researchers came together to share ideas and investigate how atomic force microscopy (AFM) technology can be used to solve biological bottlenecks and provide innovative solutions for healthcare. Over the three day conference, held at the CosmoCaixa Science Museum, 30 presentations and 130 posters were presented to 220 scientists. Topics covered cellular interactions, single molecular recognition, affinity, unfolding force measurements, high resolution imaging, protein membrane interactions, and more.

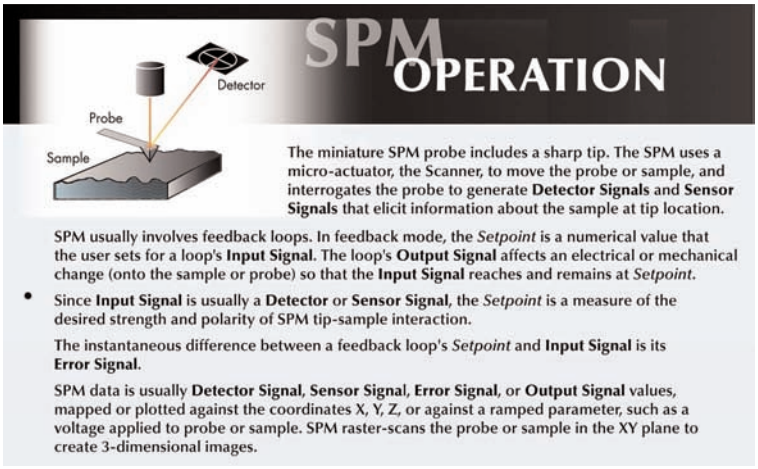



**Figure 1**

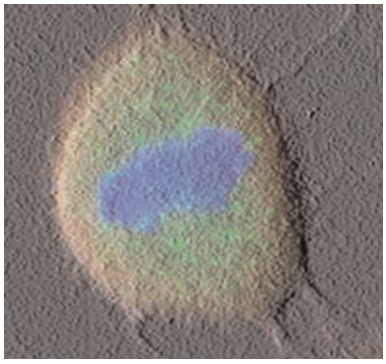
**What is AFM?**

AFM has only been around since 1986, but it has been an important tool in the nanotechnology revolution in materials sciences and physics, enabling the rapid advancements seen in the data storage and semiconductor industries. The expectation is that life science and medicine will be next. At the newly opened London Centre for Nanotechnology, nanotechnology is considered to be a "disruptive" technology for healthcare research[1], meaning that it will cause a massive change in both the way biomedical research is performed and in the potential applications that will result.

AFM is a type of scanning probe microscope, which, unlike optical and electron microscopes, does not use electromagnetic radiation (light or electrons) to image an object, but instead "feels" the object and creates an image by measuring the force between the probe and the object (*See Figure 1*). The first type of scanning probe microscope was the scanning tunneling microscope, built by Gerd Binnig and Heinrich Rohrer in 1981. It won them the Nobel Prize in physics 5 years later (interestingly, they shared the prize that year with Ernst Ruska, who built the first working electron microscope 50 years earlier). Binnig[2] was also instrumental in inventing the AFM in 1986. In announcing the Nobel prize for Binnig and Rohrer, the Nobel committee aptly compared this new non-optical, quantum-mechanical imaging principle to the Braille method of reading. "In Braille, it is the reader's fingers that detect the impressed characters, but a much more detailed picture of the topography of a surface can be obtained if the surface is traversed by a fine stylus, the vertical movement of which is recorded. What determines the amount of detail in the image – the resolution – is the sharpness of the stylus and how well it can follow the structure of the surface."[3] This was, in other words, a whole new way of seeing.

**AFM in combination with optical microscopy**

On display at the AFM BioMed conference was one example of a combined-feature system: an integration of Veeco's BioScope™ II AFM with one of Leica's DMI inverted optical microscopes. Leica and Veeco share a common philosophy that the integrated system should be easy to operate. Their ultimate aim is to enable researchers to concentrate on their scientific work and on the sample; the instrument itself should be a tool, not something that takes up time with complex settings and adjustments.



AFM overlayed with optical microscope Hela cells

Dr. Elizabeth Adams uses a combination of optical and atomic force microscopy in her research at the Delaware Biotechnology Institute, University of Delaware. "AFM is a really powerful technique, as it is able to complement data generated from a range of different methods, which helps us learn a more complete story, not just from one angle," she says. "We're interested in looking at immunological cells and how they respond to different stimuli. For example, by using a fluorescent probe with the confocal microscope, it's possible to locate specific molecules. By combining the AFM, we can then relate

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## The Origin of Optic Production

By Claudia Moch, Leica Corporate Communications

The first era of significant glass production began in Egypt in approximately 1370 BC. For a long time, Alexandria was regarded as the glass trading center of the Mediterranean. It was not until the tenth century AD that the art of glass making reached central Europe, where many glassworks were established in the forests of Bohemia and Bavaria.

In a difficult process, potash, obtained from burning trees, was melted together with silica sand and lime to make a greenish-colored glass. The word 'potash' comes from the English words pot and ash, referring to its discovery in wood ash. Potash has been used since antiquity to make glass and soap. The glass produced in this way was extremely expensive. Until well into the Middle Ages, glass was generally the privilege of town dwellers, where it was used for church windows, jewelry, and medicine bottles.

**Development of optical glass**

In 1884, physicist and optician Ernst Abbe appealed to glass company founder Otto Schott to dedicate his career to the further development of glass on a scientific basis. Ever since then, the Mainz, Germany company known today as SCHOTT AG has improved and redeveloped its optical glass types and melting techniques. Under the umbrella of the "Optics for Devices" division, the manufacture of special optical glass continues to this day.

As a result of tougher environmental regulations implemented a few years ago, most of the optical glass produced and marketed by SCHOTT AG is now free of lead and arsenic. This is designated as N glass. Today, industry classifies glass into three categories: normal glass, colored glass, and technical glass. Technical glass is used in the chemical industry and also for making optics.

"At Leica Microsystems we use 200 different types of optical glass," says Claus Gunkel, head of the Leica Optic Center. "No two glass types are the same. The refractive index, color dispersion, and chemical composition give each optical glass type its own individual character." This is a key factor to consider when designing microscope objectives.

**Delivered in blocks**

Glass deliveries from SCHOTT AG arrive at Leica's Wetzlar, Germany factory in rough blocks. The price per kilogram varies and can be as high as 1000 euros for certain glass types. Due to its great homogeneity, block glass is ideal for making microlenses.

Frank Weiler, of the MRP Optical Glass Company, measures the weight of a glass block that has just arrived from SCHOTT AG.

The entire block of glass is first clamped in a boring machine and one or more glass columns are cut out with a diamond-charged hollow drill. Depending on the required diameter of the final lens, the glass cylinder is then trimmed to within 30 microns of its specified raw diameter, from which the lens blanks are later sliced off.

**Machines have their limits**

From this point onward in the manufacturing process, the skill and intuition of a precision optician is crucial. The lens radius is ground to an accuracy of 1 micron. To avoid stress in the optical glass, lenses are not tightly clamped for grinding and polishing, but are rather fixed on exactly dimensioned substrates with special thermoplastic cement – the hand of a craftsman is needed. "Machines reach their limits when polishing the lenses, as the shapes of the radii must be machined to a precision of 25 nanometers," says Martin Henche, head of surface working in the Leica Optic Center. The threshold value he refers to is equivalent to a few thousandths of the thickness of a human hair. "This is an interactive process between a precision optician and the polishing machine," explains Henche.

Leica's meticulous optical quality standards demand considerable experience and care from precision opticians. The fact that lenses are ground and polished by hand is the best guarantee of precision. And the quality of the spheres is checked with special interferometers to meet the highest specifications.

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# Open Forum

## The Beginnings of Live Cell Microscopy

By Terry Sharrer, Editor. Originally published in *The Scientist*

When Leonard Hayflick began his cell culture work at the Wistar Institute in the 1950's, the field was facing a nagging problem. Culture flasks were so big that microscope objective lenses could not come reasonably close to the subject. Hayflick told his Leitz sales representative about the problem, and the sales rep returned with an inverted chemist's microscope that was popular among crystallographers. With slight modification, this microscope became a workhorse for cell culture work.

Hayflick, now professor of anatomy at the University of California, San Francisco, used the microscope for three major career achievements: the discovery of Mycoplasma pneumoniae,[1] the determination that normal human diploid cells undergo a limited number of doublings,[2] and the development of a vaccine-producing cell strain, "WI-38."[3]

By isolating Mycoplasma, Hayflick advanced the process of sorting out bacterial and viral pathogens for pneumonia and influenza, which together had been the leading cause of death in the United States before 1950.


The microscope was a low power affair, originally intended as a laboratory inverted microscope for the observation of chemical reactions in Erlenmeyer flasks, thus the name Chemist's Microscope. The microscope enjoyed a much wider audience for use as a workhorse inverted microscope to observe cell growth in tissue culture vessels. A binocular head is attached to the inverted nosepiece through a straight body tube, and light passes through a series of prisms and mirrors. Focus is achieved with a rack and pinion mechanism that moves the stage upward and downward. A chrome pillar contains the lamp house and condenser assembly that illuminates specimens from above. (*by Jan Hinsch, Leica Microsystems*)

Hayflick and colleague Paul Moorhead showed that normal embryonic human diploid cells become quiescent and eventually die after about fifty population doublings. This observation overturned Alexis Carrel's half century-old assertion that any cell could be kept alive indefinitely in vitro. The so-called Hayflick limit first defined aging at the cellular level and led future investigators to the telomere and telomerase.

WI-38, from an elected abortus in Sweden, became the preferred producer strain for virtually all human virus vaccines. The primary WI-38 culture held potential for yielding twenty million metric tons of material in its fifty population doublings. At least one-sixth of humanity has had artificial immunizations grown in WI-38.

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## The Objective

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this to topography, which makes it possible to see how these structures are interrelated and how they change over time. That's why we are moving toward combining these techniques."

Dr. Adams presented research at the conference that looked at one particular ligand-receptor interaction that occurs during the innate immune response to fungal pathogens. Using AFM and confocal laser scanning microscopy, she was able to follow the binding of glucan (a polysaccharide found in fungal cell walls) to Dectin-expressing cells (Dectin is a glucan receptor found on some types of leukocytes), and show that this binding was selective and specific. What does this mean? Dr. Adams says, " We've looked at these cells highly expressing a protein that's important to an innate immune response, so that we can begin to understand why the innate immune response doesn't seem to be able to protect certain people from catastrophic diseases."

**In summary**

The first AFM BioMed conference showed just how powerful a tool AFM is for life science researchers wanting nanoscale imaging and manipulation to study cells, molecules, and forces. There was more to hear and learn than this article can cover. But it was clear from the conference that the pace of development is fast, and several instrument innovations are coming from the researcher community themselves as they address AFM's limitations in order to answer new scientific questions that arise. That promises even more exciting technology for the 2nd AFM BioMed conference, planned for 2008 in the U.S.

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*Editor's Note: Veeco Instruments Inc. a leading provider of instrumentation to the nanoscience community, and Leica Microsystems, a leading designer and manufacturer of optical microscopy imaging systems, have finalized a product collaboration to drive research in biological and nano-medicine communities. The initial phase of the collaboration focuses on the integration of Veeco's BioScope™ II AFM with Leica's DMI series of inverted microscopes. The alliance will yield high-resolution images for cell biology, enabling researchers to uncover positive ways to impact human disease and treatment. Veeco and Leica Microsystems debuted their collaboration at the AFM BioMed conference in Barcelona, Spain, April 19-21, 2007.*

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# Upcoming Events

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- Microscopy & Microanalysis, Ft. Lauderdale, FL
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- Health and Colony Management of Laboratory Fish
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**http://www.mdibl.org/courses/fishhealth07.shtml**

**Marine Biological Laboratory**

- Optical Microscopy & Imaging in the Biological Sciences
  (**October 9-18, 2007**)

**http://www.mbl.edu/education/courses/summer/index.html**

**Cold Spring Harbor Laboratory**

- Immunocytochemistry, *In Situ*, Hybridization, and
  Live Cell Imaging (**October 18-31, 2007**)

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