Exploring a New Universe of Microscopy
Nikon Advanced Research Microscope Systems
Spanning the microscopy universe, from macro to super micro

Science is the pursuit of “veritas,” or truth. As scientists, we know only a tiny part of the 13 billion light years that make up our universe. The motivation to look further into space is the same desire that drives bioscientists to study the micro world. Nikon offers those in pursuit of veritas within this “microscope universe” numerous tools to help in their search.
There are innumerable areas of microscopic universe research, and the range of equipment required is extensive. Nikon meets these requirements with cutting-edge technologies and broad experience as a leading manufacturer of equipment for research into the microscopic universe. High-quality optics and manufacturing technologies enable you to get the maximum amount of information from research specimens, while NIS-Elements software works as a system brain to satisfy scientific curiosity.
Researcher’s testimonials

Maddy Parsons, Ph.D.
Group Leader (Royal Society University Research Fellow), Randall Division of Cell and Molecular Biophysics, King’s College London

1. Please tell us about your research?

We cover two main research fields; the first field focuses on adhesion receptors, such as integrins, and their roles in adhesion to the extracellular matrix, cell signaling and migration. We work with fibroblasts as a normal cell model, and also with different lines of breast carcinoma cells as a model for cancer cells. These different cell lines are known to show different levels of metastatic behavior. In cancer cells, we believe that the amount of time the receptors are in contact with the matrix, and the molecular composition of these adhesions is related to how likely the cell is to invade. We hypothesize that increased stability of adhesions results in a decreased tendency for cells to invade into the matrix. We are researching the molecules that regulate this process with an overall aim of finding novel ways to inhibit cell metastasis.

The second research field uses skin and lung epithelial cells as a model. These cells are generally not-migratory during normal homeostasis on humans, but undergo migration upon insult such as inflammation or wounding. With these cell types our focus is studying how they adhere to other cells and to the extracellular matrix, and how this is controlled by external factors. This research helps us to understand wound healing, blistering skin diseases and inflammation in lung disease.

In all of our experiments we try to mimic the in vivo environment using in vitro model systems. The cells are put into a 3D matrix, and we use different models to study different aspects of adhesion. For example, if we were looking at cancer cell invasion out of the vasculature into the extracellular matrix, we would plate cancer cells in a ‘co-culture’ on top of human endothelial cells on a 3D collagen gel to analyze invasion through this ‘barrier’. We can also change the concentration of collagen in the matrix to simulate the different mechanical stresses on the cells that are seen in vivo. In the case of epithelial cells, we can instead plate them on top of a collagen gel containing bromelin to mimic the matrix environment within the dermis. These kinds of experiments allow us to study overall effects such as migration, but also smaller details of live individual adhesions all in the same model system.

2. What applications do you use the Nikon A1Rsi and A1R-TIRF systems for?

The resonant scanner of the A1R allows imaging of short-term dynamic events in live cells over long periods, and allows fast z-stack acquisition. We use the TIRF system for studying adhesion at the cell edges, and the turnover of the adhesion receptor components. We also combine this with XYZ imaging on the A1R in a single experiment so we can follow the fate of the components as they enter the cell.

We use the spectral detector on the A1Rsi for colocalization studies. We could potentially express three fluorophores in the same cell, and want to know how these colocalize, especially during dynamic processes. The ability to spectrally image and unmix these fluorophores and remove crossstalk allows us to be confident of our colocalization results.

An example of an experiment unique to the A1R would be the experiments studying dynamic membrane protrusion events. Cells expressing fluorescent probes to label the F-actin cytoskeleton are put in a 3D collagen matrix. These cells form sub-micron protrusions that form and collapse in less than 5-10 seconds, and we believe these dynamic events contribute to cell invasion. Imaging these protrusions requires the acquisition of rapid z-stacks with the resonant scanner and piezo stage.

The protrusion dynamics (length, lifetime, etc.) of cells with and without certain proteins are studied, as well as gathering information about invasion of the cell into the matrix over a longer time period to allow us to make correlations between protrusion dynamics and the ability of the cell to invade.

To study proteins that localize to cell contacts and study the recruitment and dynamics of the cell junction we also routinely carry out TAPR analysis of adhesion receptor turnover in epithelial cells. These cell junctions are often destabilized in inflammatory lung disease and the subsequent ‘leakiness’ is associated with an increased inflammatory response. Again, these are highly dynamic, localized processes and therefore require rapid, sensitive imaging approaches to permit accurate quantification.

We also use FRET biosensors to measure activation of proteins in protrusions, for example RhoA-GiPaK, which controls cytoskeletal dynamics. We look at this activity in various knockdown cells. This is another type of experiment that was historically measured via biochemical methods, but using imaging allows us to measure the localized activation dynamics in much more detail.

3. What was the deciding factor for choosing this Nikon system?

One of the major factors that lead us to choose the A1R was the potential for flexibility in the system: the ability to cover all the types of experiments we wanted in a single microscope, and the possibility to add other components in the future as our research advances. Although there are other microscopes that could do parts of what we required, the A1R allows us to do everything in one system.

4. What was the performance specification that you valued most in selecting this system?

The speed of the resonant scanner was a key consideration for us, as it allows us to capture dynamic events. The ability to flip between the resonant and non-resonant galvano scanners was one of the strongest points, as there is nothing else like it on the market.

5. What do you value about this Nikon system after having used it for three years?

The Nikon A1R TIRF System is excellent and when it comes to time-lapse experiments it has changed our lives. The instrument does exactly what we wanted and has significantly increased the research possibilities available to us. We also value very highly the communication with Nikon. Their feedback and enthusiasm for science is something we have not experienced with other companies.

6. How has this system helped your research?

With the A1R systems we have been able to obtain new data that could not have been acquired or analyzed before, and this has led to new and surprising findings. Lots of assumptions have been made in the past from fixed cell studies that this work is now challenging. This has helped reinforce our position as one of the foremost labs in the world in this area of study.

Human breast carcinoma cell expressing GFP-Myc to label F-actin, plated on a thin layer of 2D collagen I labeled with Cy3 dye immobilized on a glass coverslip.

Human breast carcinoma cell expressing GFP-Myc to label F-actin, plated on a 3D collagen I gel with E-cadherin (green), CAR (red) and actin (blue). Scale bar 30 µm.

Analysis of RhoA GTPase activation in human epithelial cells using FRET biosensor. Image shows pseudocolor lookup table for RhoA FRET biosensor measured by acceptor photobleaching. Red shows high RhoA activity (20% FRET efficiency) and blue areas are low activity. Note high activity at cell-cell adhesion areas.

The Nikon T-Perfect Focus System is excellent and when it comes to time-lapse experiments it has changed our lives. The instrument does exactly what we wanted and has significantly increased the research possibilities available to us. We also value very highly the communication with Nikon. Their feedback and enthusiasm for science is something we have not experienced with other companies.
1. Please tell us about your research?

My experience is in the design, realization and utilization of optical and biophysical instrumentation, as carried out with my research team. Our main aim is to improve on existing techniques to develop new, unique technologies. Our research centers on several main themes: one is the development of higher resolution imaging solutions, especially involving fluorescence. We are interested in techniques that give X,Y resolution below 30 nm and Z resolution below 100 nm. In order to do this we need to work with systems that have an excellent signal-to-noise ratio to preserve optical quality when imaging at nanometer resolution. We recently published a paper on this reporting about our IML-SPIM setup allowing 3D super-resolution on thick biological objects (http://www.nature.com/nmeth/journal/v8/n12/full/nmeth.1744.html).

Something else we’re interested in is developing multimodal imaging systems that allow the gathering of information from multiple techniques from a single specimen. An example of this is the recent combination of an optical nanoscope and atomic force microscope built on a customized Nikon platform.

Another goal is to implement faster techniques for super-resolution imaging through both software and hardware approaches. This might be through novel combinations of existing hardware, or by improved computational analysis of data during acquisition. An example of this would be using on-the-fly data analysis during single molecule localization techniques to be able to stop acquisition once sufficient images have been acquired.

One of our biggest challenges is to be able to bring these high resolution imaging techniques to thick, highly scattering samples, tissues, organs, and ultimately in vivo. Some of the best candidates for the are methods based on multi-photon fluorescence excitation. As this is less efficient than single photon excitation it means we require systems with very high optical performance.

2. What applications do you use the Nikon systems for?

We work closely with several teams regarding applications for the newly developed systems. We have a particular focus on molecular oncology (chromatin and adhesion mechanisms), the production of insulin in beta cells, and neuroscience (neurite degeneration, Alzheimer’s disease, brain mapping, neuronal network signaling).

3. What was the deciding factor for choosing Nikon systems?

One of the main factors was Nikon’s reputation and experience in confocal laser scanning microscopy, which started with the PCM2000. Nikon systems are very efficient in terms of signal-to-noise ratio. When working at the nanoscale this becomes a critical factor.

Most importantly, the assistance given by Nikon is superb, with well qualified personnel and fast responses.

4. What was the performance specification that you valued most in selecting these systems?

The modularity and flexibility of the systems was very attractive as the basis for a platform for development. Nikon software is flexible and user friendly—Nikon has created some of the best software I’ve ever used. The Perfect Focus System is also very robust and useful for our time lapse systems.

We are excited to explore the growing possibilities of intelligent microscope systems, such as CLEM (Controlled Light Exposure Microscopy).

5. What do you value about this Nikon system after having worked with it?

We have at our disposal a new-generation confocal system, which has been overbooked throughout the last year, and it has been up and running 100 percent of the time.

6. How has this system helped your research?

The systems have allowed us to make affordable measurements with high reproducibility. The systems are really high-value work horses for nanobiotechnology and neuroscience labs.
Paul Ronald Selvin, Ph.D.  Professor, Department of Physics, University of Illinois at Urbana-Champaign

1. Please tell us about your relationship with Nikon.
Nikon understands the uncertainty and sensitivity of my field. I’ve worked hand in glove with the company to create a flexible system, perfectly tailored to my laboratory needs.

I have worked closely with Nikon engineers to build an imaging system that fully supports my research goals. For example, side by side with Nikon engineers and product managers, I hawked the stage of the company’s Ti microscope so it moves in incredible plus/minus 10 nanometer increments—improving upon the former range of plus/minus 30 nanometers and also reducing vibrations. I also collaborated with the company to better control the temperature of the system (to prevent drift), using different lasers to enable viewing of different fluorophores, and viewing work with a very high numerical aperture objective.

I have come to depend on Nikon to work with me through many iterations of change to my systems to get them just right. The support I received from the company was nothing short of fantastic. If I had known then what I know now, every single one of my microscopes would be Nikon.

2. Please tell us about the development of FIONA.
It’s a partnership that really blossomed when I became the 2010 Nikon Fellow and worked closely with the company to develop a specific system for my work at the Marine Biological Laboratory. Nikon allowed me to take my custom microscope back to the lab and shortly thereafter we received a grant that allowed us to purchase it. Our team continued to innovate new ways to produce clear images of the tiny specimens we study. We invented FIONA, or Fluorescence Imaging with One Nanometer Accuracy. As the name implies, FIONA allows researchers to determine the position of a fluorophore to about a nanometre in the x-y plane, anywhere from a millisecond or longer, depending on its brightness.

FIONA coupled with creative dye selection and manipulation allows researchers to study cytoplasmic molecular motors, both in vitro and in vivo. Our team has captured the movements of these motors and found that they move by “walking,” one “foot” in front of the other with a stride ranging from 16 to 74 nanometers.

3. Please tell us about your other research.
Our team has also developed a series of super-resolution techniques by separating the distance between dyes to achieve a better diffraction-limit of about 250 nm. By relying on successive photobleaching of the dyes—either permanently, or transiently—we have demonstrated that it is possible to get down to about 8 nm between two dyes, and approximately 50 nm measuring up to 20 dyes. We now use the system to study neuronal synapses and measure distances on DNA and RNA.

4. What do you think of Nikon and Nikon products?
When you’re in the business of single molecules, good accuracy and resolution is key. I count on our Nikon instrumentation to allow us to see cells in a consistent, reliable fashion. The customization, in which I collaborated with Nikon, has fine-tuned our equipment to create the ideal conditions for viewing these tiny samples. However, what I value most is the support I receive from Nikon. I have called up or e-mailed them so many times with various questions or challenges, and I always get a totally professional response in a rapid time-scale. That makes all the difference.

Paul Ronald Selvin, Ph.D.  Professor, Department of Physics, University of Illinois at Urbana-Champaign

Bo Huang, Ph.D.  Assistant Professor, Department of Pharmaceutical Chemistry, Department of Biochemistry and Biophysics, University of California, San Francisco

1. Please tell us about your involvement with N-STORM.
I was part of the team that developed the Stochastic Optical Reconstruction Microscopy (STORM) technology, and I have been working closely with Nikon engineers in building up the N-STORM hardware and software. With Nikon N-STORM, we can see things never before imagined. I am very happy to see our lab work turning into a commercial product, which allows many more research groups to have access to this tool in their pursuits to understand the fundamental mechanisms of the biological world.

2. Please tell us about the possibilities of N-STORM.
They are endless. With a spatial resolution that is more than an order of magnitude better than conventional fluorescence microscopy, STORM enables the observation of subcellular and even sub-organellar structures with unprecedented detail. For many decades, scientists had to rely on indirect methods to investigate biological systems at these scales, leaving many questions unanswered simply because we could not “see.” Now STORM makes it possible to address them by direct visualization. This is an opportunity for every one of us to see profound changes in how we understand the inner workings of life.

Besides the much improved resolution, N-STORM has two very important features: 3D imaging, which is often required to resolve intracellular structures, and multicolor imaging, which is essential for studying biomolecular interactions. In addition, it can utilize fluorescent probes identical to how biologists labeled specimens before. These features make N-STORM not only a powerful but also a versatile tool that will find applications in numerous fields of biological and biomedical sciences.

3. Please tell us about Nikon and Nikon products.
As a lab focused on both the development of new microscopy techniques and the application of these techniques, we found Nikon instruments are a good balance between user-friendliness and customizability. N-STORM offers the robustness and image quality for biologists in our group to perform their everyday experiments. The same microscope also provides the flexibility in design for our physicists to add in or modify components, enabling it to perform new functionalities that have not been previously conceived.

4. Please tell us about your future research.
In addition to pushing the super-resolution microscopy technology further into live cell observation and molecular-scale resolution, we have had our eye on the application of super-resolution microscopy in cell biology and neurobiology. We are using STORM in combination with Structured Illumination Microscopy (SIM), benefiting from the higher resolution of STORM as well as the fact that SIM puts fewer constraints on the sample. We have set out to study the architecture of subcellular structures such as the centrosome, as well as molecular mechanisms controlling the development of neurons in the central nervous system. In the end, we hope our work will set an example of how super-resolution microscopy can bridge our structural knowledge of macromolecules and their functionalities in the cellular context. There are an infinite number of biological questions that need to be answered. We are pleased that technology like STORM will help scientists begin to answer them.
Atsushi Miyawaki, M.D., Ph.D.  
Senior Team Leader, Laboratory for Cell Function Dynamics, RIKEN Brain Science Institute

1. Please tell us about the development of light imaging and Scale

I have long believed that there is much information to be obtained from the intermediate stage between a biochemical approach such as cell component analysis using electrophoresis and optical imaging of living specimens—for example, from research into fixed specimens. In particular, fluorescent protein technology and gene induction technologies have enabled the selective labeling of nerve cells regardless of depth; however, there is as yet little technology for visualizing the neural networks running from the surface to the depths of the brain together. The Scale reagent we have developed can make biological tissue transparent like jelly. This has enabled deep imaging without damage to the specimen.

Dr. Hiroki Hama focused attention on the fact that the PVDF membrane used for transferring proteins in western blotting becomes transparent in high-concentration urea solution. In addition, we were already aware that most fluorescent proteins were urea-resistant, though high-concentration urea solution is generally used for protein denaturation. The impetus for the Scale project was the belief that tissue clearing technology could probably be developed based on urea. Initially, it took quite a long time for the entire brain of a mouse to become transparent when soaked in our early reagents. However, our capacity for patience seems to have paid off. The Scale technology was improved and finally the Scale A2 reagent was completed. We now know that making a cut in the mouse forebrain at E15.5. The mouse was fixed by PFA at P10 and subjected to treatment with ScaleA2 solution for tissue clearing.

2. What part did the AZ-C1 macro confocal microscope system play?

A wide range of applications are possible when used in combination with Scale technology.

For example, if neurons fire, the transcription of immediate-early genes such as c-fos and Arc is enhanced. Therefore, neuronal firing can be monitored with the fluorescence signal of a reporter gene of fluorescent protein such as Venus located downstream of a promoter that controls c-fos and Arc expression. Quantifying this signal by capturing a number of slices of the brain and reconstructing three-dimensional images entailed tremendous effort. Using the AZ-C1, however, we proved that it is possible to simplify the quantification by projecting three-dimensional information of the brain, made transparent with Scale, to construct a two-dimensional image.

The imaging depth limit with single-photon excitation is generally only around 0.2 mm due to the influence of scattered light; while it is 0.8 mm with two-photon excitation. As Scale technology minimizes light scattering in the sample, it is more effective when used with a single-photon excitation microscope. It may also be beneficial for the AZ-C1.

For example, if the YFP gene is administered to the cerebral cortex of the right hemisphere of the brain of an embryonic mouse, it is observed that axons project into the left side of the cerebral cortex across the corpus callosum during nervous system development (see the image below). The AZ-C1 imaging system is effective in this kind of research, in which wide-area images must be obtained.

1x objective lens (AZ-Plan Apo, NA 0.1, W.D. 35 mm) 2x objective lens (AZ-Plan Fluar, NA 0.2, W.D. 45 mm)

3. Future demand for microscopes and imaging

It would be ideal if there were a microscopic imaging system that could freely zoom in and out.

I want to see our fluorescent-imaging technology and Scale technology make a major contribution to human pathological diagnosis. I am interested in the establishment of the technology for use in finding lesions in large tissue mass. I believe that Scale technology will contribute to medical science, as well as to academic disciplines such as developmental biology. I also expect AZ-C1 to make a contribution.

4. On Nikon

Professor Roger Tsien (2008 Nobel laureate for chemistry), who has conducted numerous joint research and development projects with Nikon, was my adviser. Therefore, I have a long-standing relationship with Nikon. I hope that the company will develop products that challenge researchers.
1. What kind of research are you engaged in?

I have long been interested in the mechanism by which secreted proteins are efficiently produced. To study this, I have conducted a series of studies into the dynamics of inner membrane proteins and the dynamics of cargo protein molecules themselves. After my biochemistry research, I started fluorescence imaging, and then went on to use single molecule techniques, such as FCS (fluorescence correlation spectroscopy) and single molecule tracking. Because I want to understand what makes “living” cells, I have a boundless interest in changes in cell structure and in the dynamic behavior of multiple molecules.

2. Why were you interested in the N-SIM super resolution microscope?

Initially, I anticipated that I would be able to observe the microstructures of endomembranes that cannot be recorded with a confocal microscope. When I used it in practice, I discovered that this was not the only thing I could now do—and when I used it in combination with an A1Rsi confocal microscope system, it felt as if a whole new world had been opened up.

3. How did you find the N-SIM to use in practice?

It was extremely user-friendly and easier to use than I had imagined. First, the A1Rsi is highly extensible and operations, including external inputs, are extremely simple. This makes it easy to use—even for an amateur of optics like me. We use FLIM information obtained with the TCSPC unit for analyzing the FRET efficiency. Although there are sometimes difficulties with the acceptor-bleaching method, with FLIM, results are obtained quickly with few artifacts appearing, making it useful for FRET measurements when, for example, studying structural changes in molecules. Photon static information, including FLIM and TCSPC/PCH information, from living cells obtained with the TCSPC unit supplements detailed structural information obtained with the N-SIM. Nikon’s 60x water-immersion objective lens is the default lens for the TCSPC unit. Nikon’s engineers have been giving thorough consideration to my requests. Instead of responding to my wishes by saying “it can’t be done,” they are properly considering how they can bring them to fruition. Nikon products are also outstanding in terms of system extensibility. It seems so difficult to attach third-party peripheral devices to other manufacturer’s products, particularly in Japan.

4. How have you been using the A1Rsi and the N-SIM in combination with one another?

I mainly use the N-SIM for observing cell structures as still images. On the other hand, the A1Rsi can perform ultra-high-speed image acquisition and allows FRAP and photo stimulation. Unless both devices are used, the information we can obtain is limited. The combination of N-SIM and A1Rsi allows us to acquire high-resolution images of specific structures. This is because A1Rsi highlights and activates only regions labeled with photoactivatable proteins such as PA-GFP. The ability to highlight only specific structures represents an extremely powerful capability.

5. What are the advantages of connecting analysis units such as the TCSPC unit to the A1Rsi confocal microscope system?

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6. What developments would you like to see for N-SIM in the future?

Firstly, faster image acquisition and simultaneous acquisition of two colors. Even without two cameras, if it could offer dual-view display with just a single camera, this would be quite effective—even with only 256 pixels. Users can probably be divided into those who want to view a larger area, and those who want to see faster change. Secondly, improvements in illumination pattern contrast and a better CCD camera with a lower dark current may help improve reconstruction of dark (noisy) samples and should lead to wider use of the N-SIM in cell biology.

In addition, I feel that successful application of the N-SIM depends on proper imaging techniques and knowledge. I hope that Nikon is involved in promoting optical imaging education.

7. Please describe your impressions of Nikon.

Nikon’s engineers have been giving thorough consideration to my requests. Instead of responding to my wishes by saying “it can’t be done,” they are properly considering how they can bring them to fruition. Nikon products are also outstanding in terms of system extensibility. It seems so difficult to attach third-party peripheral devices to other manufacturer’s products, particularly in Japan.

Dr. Wada (right)

Three-dimensional image of phagosomes taken with a super resolution microscope

(DM6000B-A1Rsi+TCSPC cells observed using a super-resolution microscope Nikon’s N-SIM-based Illumination Microscopy System). Phagosomes (highlighted with green fluorescence), which incorporate beads wrapped in Venus-SNAP-23 (which are marked with a *), are visible in the cells. After Phagocytosis, the addition of an A1Rsi-labeled antibody that recognizes the IgG used in opsonization allows confirmation that there are beads outside the cells (highlighted with red fluorescence).
NIS-Elements imaging software is an ideal tool to have when you peer into a microscopy universe and discover something remarkable. As a single software platform, it allows everything from integrated control of a wide range of devices and analysis of acquired images, to output of results as information. NIS-Elements provides the support you need to achieve your research goals.

Get Vision
Integrating hardware devices with your ideas…

Give Information
…into a software system designed for building experiments and analyzing images for data output.

*“Turning vision into information” is the mission statement of Nikon Corporation’s Instruments Company.*
All you need is optics

The Nikon process: From raw material to final product

Nikon was established in 1917 under the name of Nippon Kogaku Kogyo, or Japan Optical Industries. As Nikon has grown, optics has been and continues to be the company’s core technology.

Materials technology

Nikon conducts the entire lens manufacturing process, beginning from melting glass materials, on-site. The company has amassed a wealth of experience over its 90-year history.

Strict testing is the key to the highest quality, best performance and maximum reliability. As the world’s finest lens manufacturer, rigid testing criteria is unmatched in the industry.

Nikon’s Perfect Focus System was chosen as one of The Scientist magazine’s Top Innovations of 2008. The PFS is a real-time focus correction mechanism that keeps a specimen in focus by correcting focus drift caused by mechanical effects and thermal change over extended periods. It tracks optical offset and corrects it by sampling every five milliseconds with infrared light, enabling acquisition of more reliable data during time-lapse imaging of live cells. The panel of judges, including David Piston, Simon Watkins, Klaus Hahn and Steven Wiley, has collectively published more than 700 academic articles. They chose Nikon’s PFS as a product most likely to impact the life science market.

The Nikon N-SIM super resolution microscope was chosen as one of the best 10 microscopy innovations in the 2011 awards sponsored by Microscopy Today magazine. The N-SIM was developed with “Structured Illumination Microscopy” technology licensed from the University of California, San Francisco (UCSF). It offers nearly double the resolution of conventional optical microscopes and achieves super resolution of up to 85 nm in multiple colors. It can also capture super resolution images at a temporal resolution of 0.6 sec/frame, allowing study of dynamic interactions in living cells.

Measurement and inspection technology

Strict testing is the key to the highest quality, best performance and maximum reliability. As the world’s finest lens manufacturer, rigid testing criteria is unmatched in the industry.

Perfect Focus System

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Original lenses and coating design methods developed by Nikon have been continuously improved. Nikon is passionate about the challenges set by innovative design.

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Nikon imaging centers—world leading microscopy facilities—provide researchers access to the latest in cutting-edge imaging and microscopy equipment. They also serve as learning centers by offering training courses on basic and advanced light microscopy techniques.

http://www.nikonimagingcenters.com/

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*Products: Hardware and its technical information (including software)

Monitor images are simulated.

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TO ENSURE CORRECT USAGE, READ THE CORRESPONDING MANUALS CAREFULLY BEFORE USING YOUR EQUIPMENT.