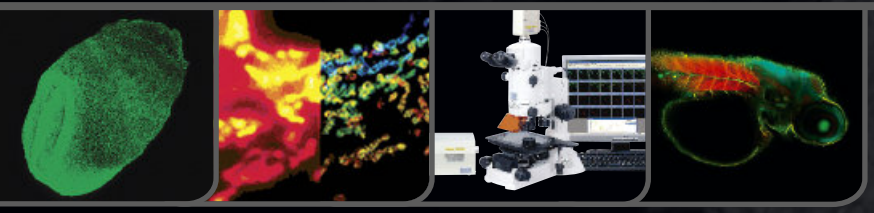
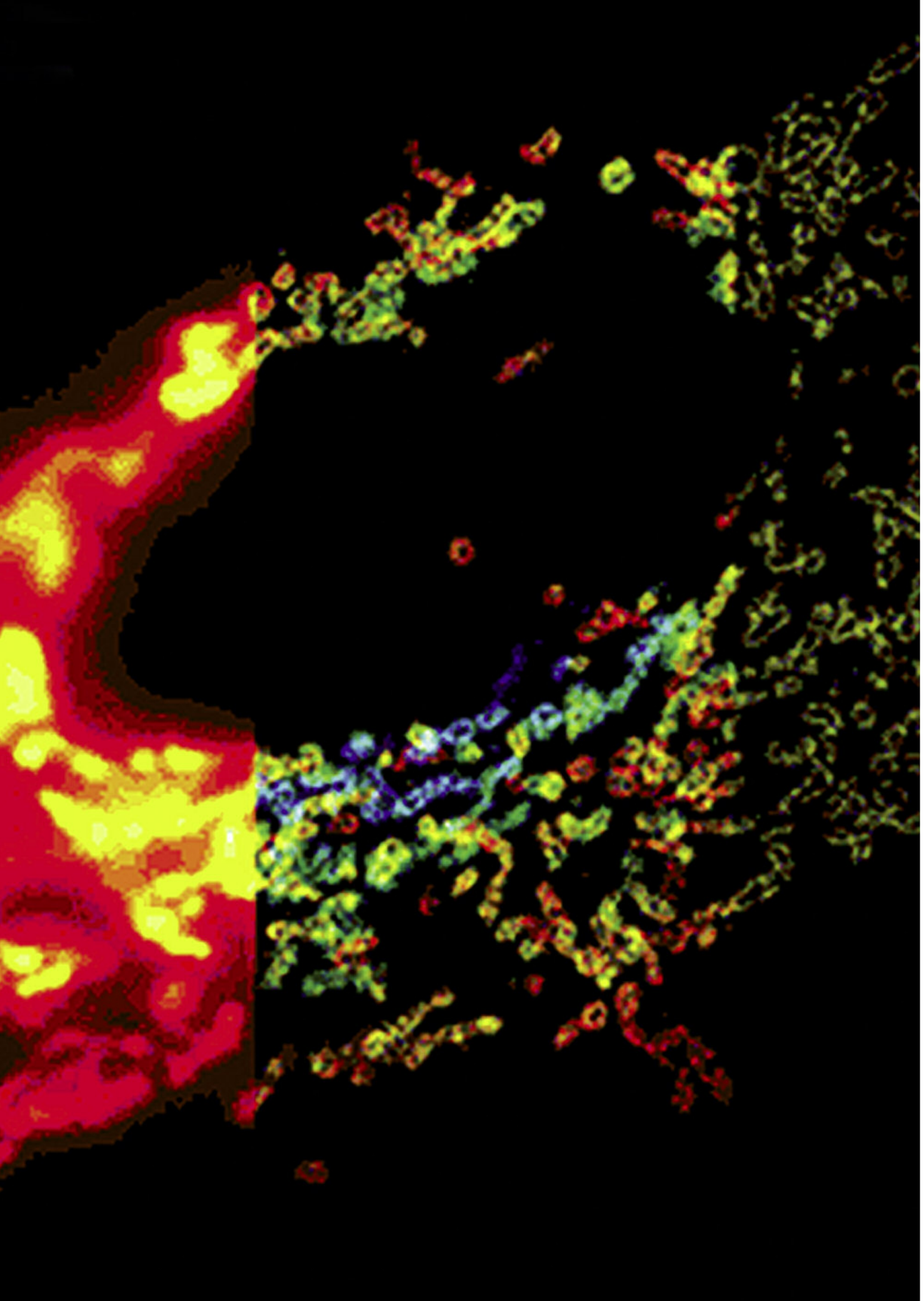
Nikon

μview

Meeting the growing needs of developmental biology



Solutions brochure



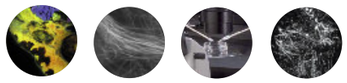
Imaging for developmental biology

Outstanding optical quality is at the heart of all Nikon's microscopic imaging systems and this, combined with state-of-the-art cell-friendly imaging technologies provides the ideal platform for microscopic imaging in developmental biology.

Nikon is one of the few microscopic imaging companies that focuses on cell care as one of the most important criteria in live cell studies. New technologies within this 'cellogy' ethos aim to achieve longer live cell studies; the capture of more continuous dynamic information; and more reliable results free from the confounding influence of cell stress resulting from phototoxicity and environmental disturbance.

In this brochure you will find information on Nikon's advanced microscopy platforms for both live cell and fixed cell imaging. These include, for example, the BioStation family of products for incubator imaging; the Eclipse Ti-E inverted microscope with Perfect Focus System (the ideal live cell imaging workstation); and the new AZ100 multizoom with C-Series confocal system for macro to micro confocal capability. You will also read about exciting new developments in super resolution imaging and hear from researchers in developmental biology who have benefitted from Nikon's imaging systems.

If you would like to find out more about Nikon's products for developmental biology, call Nikon today for information and advice suited to your specific imaging needs.

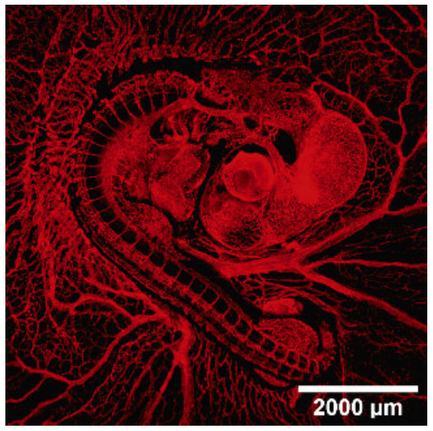


What is developmental biology?

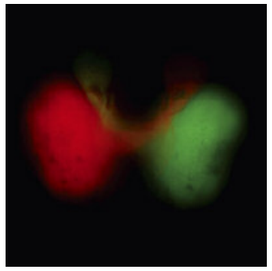
The aim of research in developmental biology is to understand the mechanisms of development, differentiation and growth in living organisms. Key areas include: the differentiation and organisation of embryonic cells; cell communication; the identification and understanding of growth factors and their effects on particular cells; the switching on and off of specific genes involved in growth processes; and the regulatory hierarchies that maintain ordered patterns of growth, differentiation and programmed cell death.

Research may involve cellular, molecular and genetic studies as well as anatomy, physiology, cell biology, and supportive disciplines such as computer modelling.

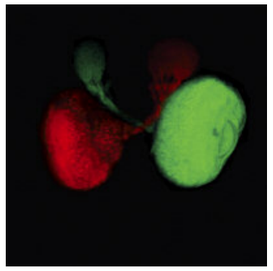
An increased understanding of developmental processes will ultimately enable intervention to control or prevent diseases such as cancer, age-related degenerative disease, congenital abnormalities and even the repair of tissues damaged by trauma or disease.



Blood vessels (red) of a 2.5 day-old chick embryo. Courtesy of Dr Yoshiko Takahashi, Molecular and Developmental Biology, Graduate School of Biological Sciences, NAIST.



Comparison of standard widefield fluorescent image (above) and AZ-C1 confocal image (below) of zebrafish eye double stained with GFP and mCherry. Courtesy of Marine Biological Laboratory, 2008 Physiology Course.



Key empowering technologies

Live cell imaging

The growth in live cell imaging technologies has allowed researchers to observe dynamic events in cells and organisms over extended periods of time to provide a window on realtime developmental processes. In contrast to fixed cell techniques, live cell imaging provides continuous information rather than having to extrapolate/interpolate from images in fixed specimens.

Genome sequencing and gene manipulation

Genome sequencing together with recombinant DNA technologies allow researchers to investigate the role of specific genes in development. Mutant organisms, gene knockouts (with permanent and inheritable genetic modifications), and gene knockdowns (with transient changes in gene expression), allow gene function, gene interactions, and gene redundancy to be determined.

Model organisms

Many model systems used in developmental biology are small, have short generation times, and are transparent in their embryonic stages, (e.g. the zebrafish: Danio rerio; the fruitfly: Drosophilia melanogaster) allowing development and the effects of gene knockdown to be visualised directly through the microscope. Conservation of genetic material, metabolic and developmental pathways between many organisms makes findings in model organisms relevant to human development.

Non-toxic fluorophores

Newer fluorescent probes, (for example, GFP family, quantum dots, photactivatable probes) allow researchers to observe and monitor specific molecules without causing damage to cells and without influencing outcomes. These probes have given rise to imaging techniques such as FRET, FLIM, FRAP, and TIRF, which allow specific molecular trafficking events to be visualised in detail. Certain probes, such as quantum dots, additionally, also enable cell lineage studies across cell generations.

DID YOU KNOW?

Nikon broke away from traditional microscope optical design in 1996 to create the revolutionary CFI60 infinity optical system. The result: long working distances, high N.A.s, and the ability to incorporate multiple imaging modules into the light path while maintaining optical quality.

DID YOU KNOW?

Nikon manufactures its own high quality, precision lenses for microscopy.

90i

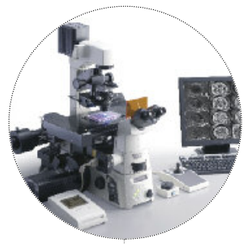
Ideal for fixed specimens

Micro Manipulators

Embryology & electrophysiology

SMZ 800, 1000 &1500

Anatomy & dissection



Eclipse Ti

Live cell imaging, confocal, multiphoton & super resolution

AZ - C Series

Macro & micro in one

BioStation Series

Ultimate care for developing organisms

The importance of environment

When imaging living cells, tissues and organisms, it is essential to maintain cells in an optimal environment, which as closely as possible mimics the in vivo environment to obtain results that reflect real life processes. Failure to control the environment, especially during long term timelapse studies may result unhealthy cells that fail to divide or exhibit abnormal morphologies confounding the interpretation of study results. A constant environment also helps to define the effects of experimental variables more accurately, and provides more consistent and reliable results.

BioStation is a combined incubator and imaging system that provides a user-specified automatically maintained environment for cells during culture and imaging. Potential cell stress from environmental changes and physical disturbance, when transferring culture vessels from incubator to microscope for imaging, is eliminated. With cell friendly phase and fluorescence imaging, Biostation IM-Q (for up to four concurrent studies) and BioStation CT (for multiuser/multistudies) are ideal for determining the effects of morphogens, performing protein localisation studies, monitoring the effects of gene knockout and knockdown etc. while minimising cell stress.

DID YOU KNOW?

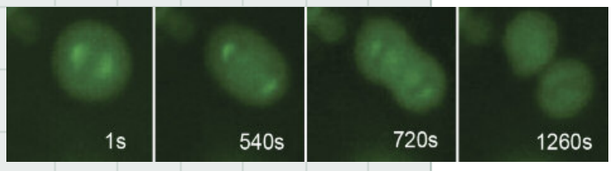
With BioStation CT you can monitor your cells via the internet or LAN – freeing you from the lab!

CASE STUDY: INHERITED MICROCEPHALY & BRAIN DEVELOPMENT

The inherited disease microcephaly (MCPH) is characterised by a small, but architecturally normal brain. Apart from mental retardation, there are generally no other apparent developmental abnormalities suggesting that MCPH genes may have a specific role in neurogenesis. Mutation in the ASPM gene at the MCPH5 locus is the most common cause of MCPH. Around 100 mutations have been identified but there are few genotype/phenotype correlations. "We are trying to find out more about the function of ASPM and the effect of ASPM mutations on the developing brain," explains Dr Jacquelyn Bond of the Section of Ophthalmology and Neuroscience, Leeds Institute for Molecular Medicine.

Using BioStation IM, Dr Bond is planning a series of rapid 4-hour live cell studies using C-terminal constructs to look at protein localisation and mitotic redistribution in the cell. "In pilot studies we could easily see the GFP-tagged protein moving to the spindle pole – it was beautiful! We are also planning to use confocal imaging, especially FRET and FRAP, for protein localisation, protein interaction and trafficking studies using our Nikon inverted microscope configured for TIRF and four-laser confocal imaging. By inserting a GFP- or RFP-linked ASPM fragment construct into cells, we can photobleach the spindle pole and determine, by the reappearance of fluorescence, whether further ASPM-GFP fusion proteins migrate into the area, indicating a transitory interaction with the spindle.

"There is still a great deal to find out about MCPH," Dr Bond comments. "We have affected families that cannot be mapped to known loci indicating that there are likely to be further



MCPH genes. We also need to find out more about factors that regulate the transcription and activity of MCPH genes. Besides being of benefit to families affected by MCPH, research contributes to the understanding of brain development in general, which may have an impact on other neurodevelopmental diseases."

Kind to living cells

The light used to image living specimens can cause cell damage. Care is, therefore, required to minimise light-induced cell stress especially in longer term studies and, especially, when using confocal techniques. A number of technologies have been designed to give researchers more control over excitation light, thereby delivering only enough excitation to achieve a signal, and to capture as much fluorescence light as possible so that less excitation light can be reduced.

AOM (acousto-optical modulator) and AOTF (acousto-optical tunable filter) modules allow researchers to control laser wavelength and intensity resulting in precise targeting of light.

CLEM (Controlled Light Exposure Microscopy) automatically delivers only enough light to cells to create an image. CLEM reduces light exposure to allow imaging for up to six times longer without noticeable cell damage or deterioration in image quality (Hoebe et al 2007).

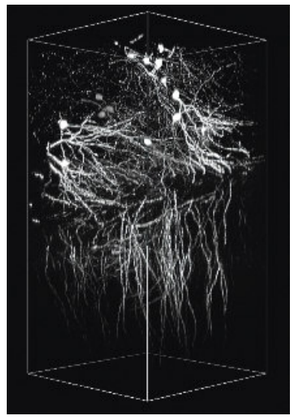
Hybrid confocal scanning: Switching between non-resonant and resonant scanners allows users to tailor sensitivity and scan speed to each individual application. Use of both scanners also enables simultaneous photo-activation and imaging.

Spectral imaging: With only one scan required to capture all spectral data in a sample, image acquisition time is reduced. Simultaneous excitation by up to four lasers is possible with fast spectral unmixing (512 x 512 pixel, 32-channel unmixed in less than 1 second).

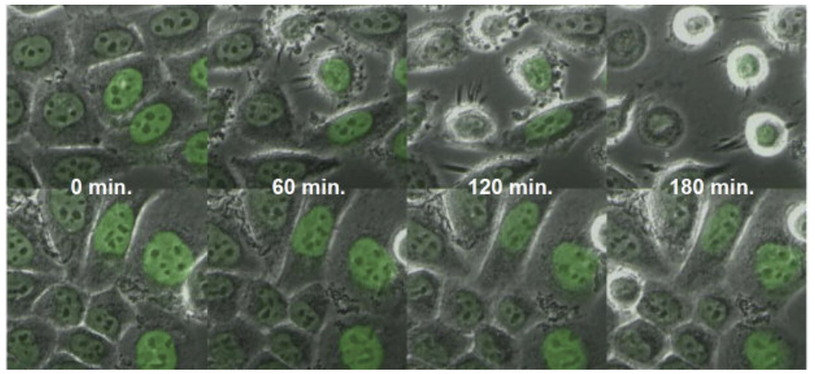
Multiphoton: Ideal for imaging deeper into tissues, multiphoton uses more cell-friendly longer wavelength light for excitation. Excitation also takes place only in the plane of focus, reducing phototoxicity and photobleaching.

Increased fluorescence efficiency: New optical technologies in the A1R confocal system increase fluorescence transmittance by 30% and increase signal-to-noise ratios. The result is increased image brightness, reduced need for further scans and reduced light exposure to cells.

Hoebe et al (2007). Nat. Biotechnol;25(2):249-53.



Fixed neuronal cells of mouse brain expressing eGFP captured using NDD and CFI Apo LWD 40x WI Lambda S objective. Courtesy of Dr. Satoru Kondo, Department of Cellular Neurobiology, University of Tokyo.

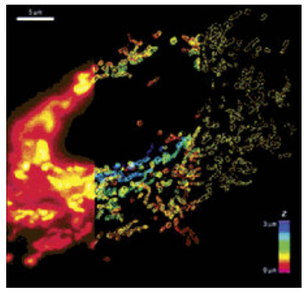


Time-lapse acquisition of HeLa cells expressing GFP tagged histone-2B. The transmitted light and fluorescence images were simultaneously acquired in the absence (A) or presence (B) of CLEM.

Super resolution fluorescence microscopy

Nikon's N-SIM* (Structured Illumination Microscopy) super resolution microscope, ideal for 2-D and 3-D imaging of both fixed and live cells, is based on the Eclipse Ti inverted research level microscope with CFI Apo TIRF 100x oil objective lens. With the TIRF-SIM system, higher resolution TIRF observations are possible to give more detailed structural information near the cell membrane. It provides fast imaging capability (0.6 sec/frame) enabling the capture of rapid cellular events. N-SIM is able to resolve cell structures, such as actin fibres (110-120nm) close to the cell membrane, which would be indistinguishable by conventional light microscopy (Gustaffson 2000).

N-STORM**, also based on the Eclipse Ti, provides multi-spectral 2-D and 3-D nanoscale imaging of cellular structures and, in principle, imaging at the molecular scale in fixed specimens. STORM (stochastic optical reconstruction microscopy) technology constructs a fluorescence image from the highly accurate localisation of fluorescent molecules in a sample using photoswitchable fluorophore pairs. This method has allowed 3-D imaging of the entire mitochondrial network and the spatial relationship between mitochondria and microtubules in mammalian cells (Huang et al 2008). Several photoswitchable fluorophore pairs have been characterised (Bates et al 2007) enabling versatile and powerful, high contrast imaging. 3-D imaging can be achieved with an optical switchover device eliminating the need for time consuming serial sectioning.



Comparison of conventional and STORM images of mitochondria in a mammalian cell. The mitochondrial outer membrane protein Tom20 was labelled. Left panel: Conventional image. Middle panel: 3D STORM image, the z-dimension information is colour-coded according to the colour scale bar (bottom right). Right panel: xy cross-section of the STORM image. Image courtesy of Zhuang Research Group, Dept of Chemistry and Chemical Biology, Harvard University, Cambridge, MA.

** SIM technology licensed from the University of California, USA*
*** STORM technology licensed from Harvard University, USA*

Bates M et al (2007).Science. 2007;317(5845):1749-53.
Gustafsson MG. (2000) J Microsc;198(Pt 2):82-7.
Huang B et a. l(2008). Nat Methods;5(12):1047-52.

CASE STUDY: A MODEL FOR DEVELOPMENT OF THE THALAMUS

Understanding how the human brain develops has great significance in understanding, and possibly treating, neurological disorders and CNS injury. At the MRC Centre for Developmental Neurobiology, King's College, London, Dr Steffen Scholpp, working with Professor Andrew Lumsden, is interested in the early development of the thalamus. "Located in the centre of the brain, the thalamus is the 'gateway to the cortex'," Dr Scholpp explains, "and is the most important processing centre in the brain." Key questions are how does the thalamus form? What regulates its development? And how does it acquire its distinct functions?"

The MRC-funded group is studying embryonic development in the zebrafish (Danio rerio). The MRC zebrafish group use a C-Series confocal microscope equipped with an acousto optic modulator (AOM). "It is a compact system that meets our needs, is very easy to use and does not require a dedicated technician. The C-Series is also modular making it easy to add further imaging equipment as required."

The MRC team carry out timelapse studies over periods of 15-30 hours and employ a variety of fluorescence contrast techniques to study brain development in wild-type and mutant zebrafish strains in vivo. Optical sectioning with dedicated software enables 3-dimensional digital reconstruction of the specimen for greater insight into spatial dynamics. "With the help of model organisms such as the zebrafish, molecular biology tools, and imaging equipment such as the C-Series confocals, we hope to unravel more complexities of the developing brain," Dr Scholpp concludes. "Work across many model organisms indicates that many developmental principles and processes are common to all species. Work in the zebrafish may, therefore help to reveal the secrets of brain development in humans – the most complex brain of all."

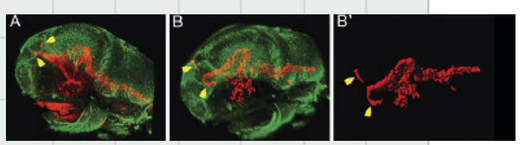


Fig. 1: Images, after deconvolution and 3-D reconstruction, show entire zebrafish at 2-days old.

Microscopes for developmental biology

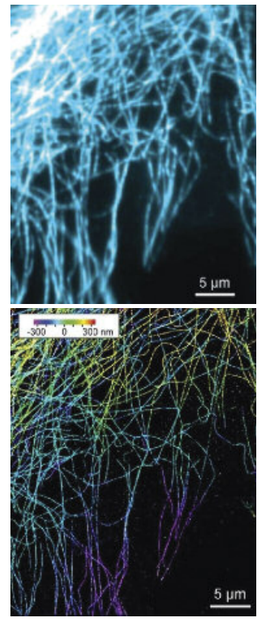
Whatever the imaging technique, a quality optical system delivering high resolution, clear and bright images is essential for maximising visual information.

Inverted microscopes: From the Eclipse TS100 series to the advanced Eclipse Ti system, Nikon's inverted microscopes offer exceptional optical quality and vibration-free design. The modular design of the Ti accommodates many different imaging techniques to create a versatile live cell imaging workstation. Built-in Perfect Focus System (PFS) ensures in-focus images during extended timelapse studies.

Upright microscope: The Eclipse i-series upright microscopes deliver superb images over the entire magnification range, ideal for fixed specimen observations.

Confocal systems: From the all-new state-of-the art A-series, with high resolution galvanometer scanner and high speed resonant scanner, to the modular entry level C-series, Nikon has a confocal to suit virtually every research need.

Multizoom microscope: With magnifications from 5x to 400x, the AZ100 multizoom combines stereo zoom and compound microscopy to offer macro and micro imaging in one instrument. In combination with the C-series confocal system, the AZ100 C-series offers micro-to-macro confocal capability.



Comparison of conventional light microscopy (top) and N-STORM imaging (above) of microtubules. Image courtesy of Dr Yasushi Okada, Cell Biology, Medical Department of Graduate School, University of Tokyo, Japan.

Stereozoom microscopes: The SMZ series offers zoom ratios from 6.3: 1 to 15:1 with exceptional optical quality and vibration resistant, ergonomic design.

BioStation: Eliminating the need to remove specimens from the incubator for imaging, BioStation IM-Q is ideal for timelapse studies involving up to four parallel experiments using the Hi-Q4 dish, while BioStation CT is a multiuser system accommodating 30 vessels which are transferred robotically between the microscope stage and vessel rack.

Super resolution microscopes: Nikon has two super resolution microscopes: N-SIM* with lateral resolution twice that of conventional light microscopes, and N-STORM**, with a lateral resolution of ~20nm and axial resolution of ~50nm enabling the visualisation of cellular features indistinguishable by conventional light microscopy.

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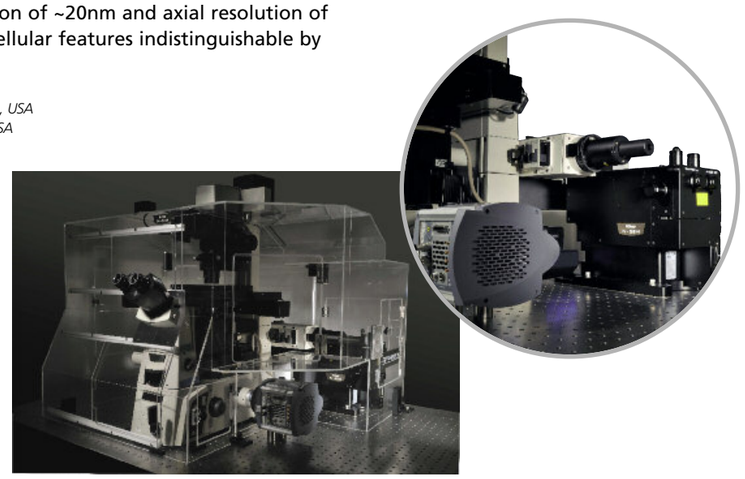
*** STORM technology licensed from Harvard University, USA*



Supporting developmental biology

As well its comprehensive range of microscopes, Nikon also supplies specialist objectives, digital imaging equipment and image analysis software to enhance imaging capabilities in developmental biology.

Digital imaging

Nikon's digital cameras can be incorporated into developmental biology imaging systems to enable immediate image capture, archiving and immediate image sharing via a network. The DS-Vi1 model offers high frame rates and increased sensitivity in a 2 million pixel 1/2 inch format CCD, featuring multiple live capture modes, picture sizes and digital transfer rates, with video display rates of up to 27 frames per second possible.

NIS-Elements

Nikon's intuitive NIS-Elements software simplifies workflow and speeds up image acquisition times while providing features such as image stitching, object counting and volume views. It is a powerful image management tool, which integrates cameras, components and peripherals with image archiving, analysis and visualisation tools.

Objectives

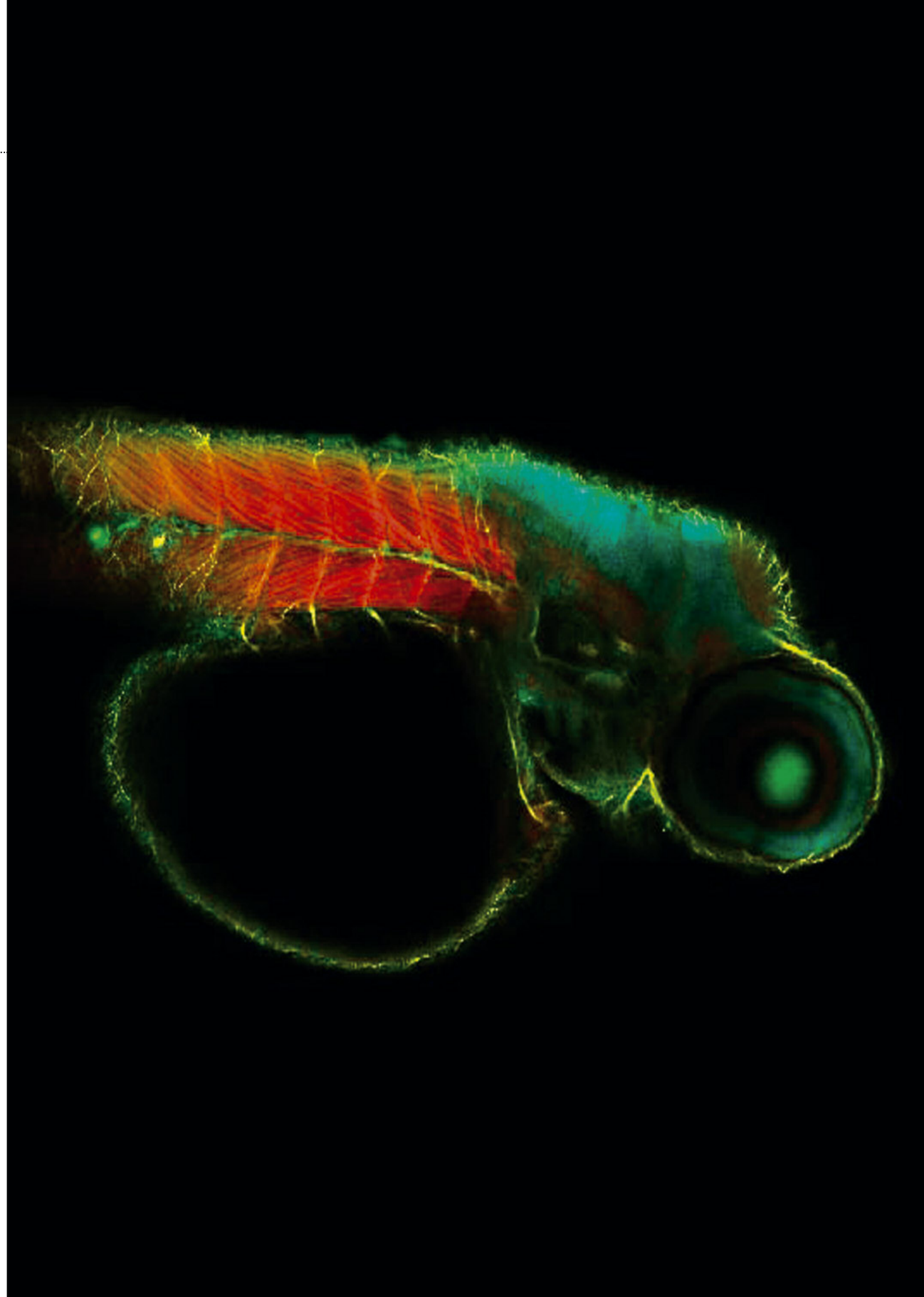
Nikon manufactures its own glass, ensuring quality in the entire research, development and production process. Nikon's CFI60 optical system provides both long working distances as well as high N.A.s. Unique nano crystal coat technology, used in all new Lambda S (λS) series objectives, virtually eliminates internal lens element reflections to result is higher light transmission, high contrast image acquisition, faster image capture at lower excitation levels, and reduced photobleaching and damage to live cells. These objectives are ideal for near infrared applications, such as multiphoton imaging and laser tweezers, spectral imaging and multiprobe studies, which require high transmission rates across a broad range of wavelengths.

Environmental enclosures

From heated stages to full environmentally controlled microscope enclosures, Nikon supplies Okolab and Solent Scientific products for environmental control.

BioStation

BioStation is the ultimate solution for live cell imaging of developmental processes. Specimens are maintained and imaged in situ in an optimal controlled environment to dramatically reduced unwanted experimental variables and risk of contamination.



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