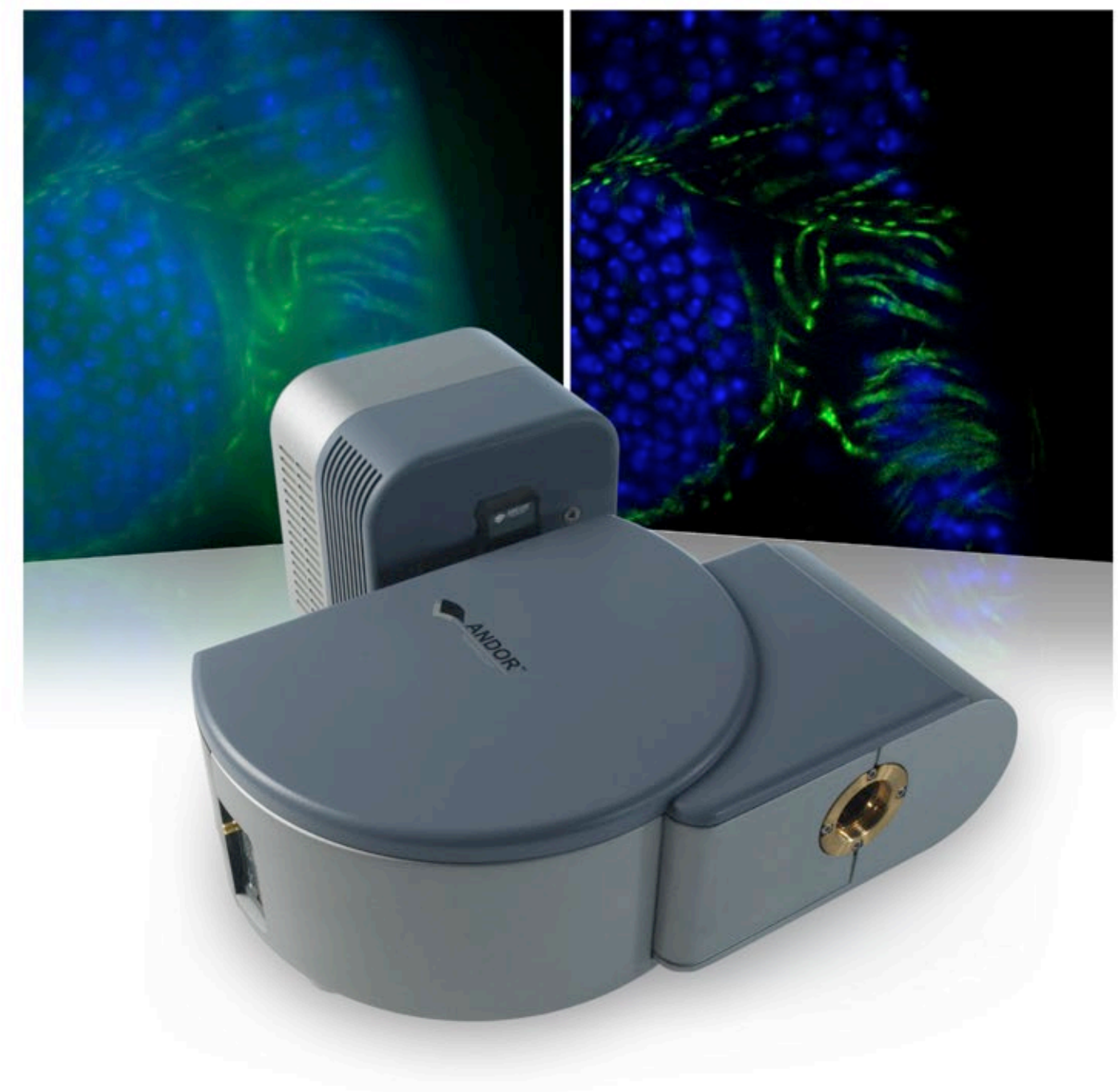
ANDOR

Revolution DSD

Simply Confocal



Revolution DSD

The personal confocal imaging unit

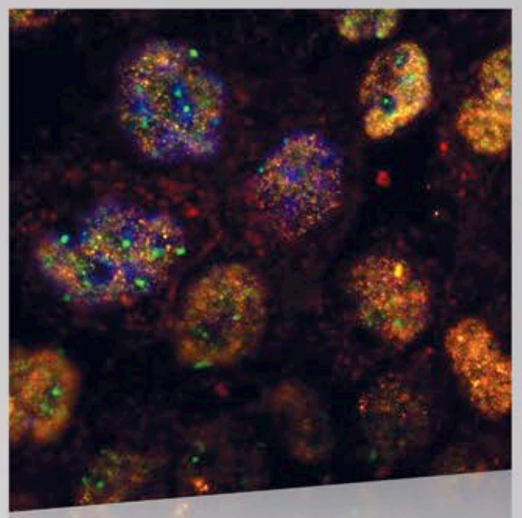
Andor's Revolution DSD is an innovative hybrid of spinning disk technology and structured illumination. This unique approach is laser-free and delivers a budget friendly confocal solution to your laboratory, offering less dependency on laser based solutions that are often restricted to core facilities. A simple device, which can even be added to an existing fluorescence microscope in your lab, the Revolution DSD will benefit your research by delivering confocal images as a routine technique in your work. Whilst laser-free, the Revolution DSD can still achieve the optical sectioning and image quality you expect of a complex laser scanning confocal system, but with low maintenance costs. Furthermore, it does not need an expert to run it!

Through our unique implementation of the confocal unit and our own motorized light source, the DSD is ideal for live cell imaging or high throughput with fixed samples. The Revolution DSD offers confocal imaging at a speed that laser scanning technology often fails to reach. Add a motorized z-drive, or use an existing one in your microscope, and capture 3D images with ease. Our iQ software includes the tools to view your 3D images, or add Imaris for 3D volume rendering and analysis.

See page 16 for technical note: Filter Exchange and Calibration

The Revolution DSD delivers high contrast, low background images with objective magnification from 10x to 100x making it suitable for large and small samples. A standard range of fluorescence filter sets are offered, but specific combinations can be requested. Through our unique implementation the filter exchange is extremely simple and so multiple combinations are possible. Being laser-free you are not restricted to fixed excitation wavelengths, so the DSD can adapt with the needs of your research and at little additional expense.

See page 18 for technical note: Optical Sectioning and Signal to Noise Ratio (SNR)



Confocal four-color image of DNA Methylated Histones.

Features	Benefits
• Pre-filtered excitation	• Lower background and improved signal:noise
• Fast excitation shutter control	• Minimize illumination, protect against photobleaching and toxicity
• Excitation filter wheel	• Up to four color fast wavelength switching in combination with DSD
• Software controlled excitation intensity	• Fine control against photobleaching and toxicity
• User friendly in-situ filter exchange	• No need to disassemble and re-align, saving considerable time
• Universal mount (DSD-*Uni*)	• DSD can be used on most microscopes • Enhances stability to avoid mis-alignment
• Optimized Disk pattern	• Optimum balance between confocality and light throughput
• Optimized algorithms and software	• Live confocal or widefield images, no post processing • Capture both widefield and confocal if required
• Andor Clara Interline CCD	• Deeper cooling for lower background • Extended red capability for red fluorophores

Key Applications

- Cellular Biochemical Imaging (e.g. ATP & pH)
- Developmental Biology
- Cell Division
- Cell Motility
- Neuroscience
- 3D Structure
- Fixed Samples
- Fluorescent proteins or dyes

The image sequence below shows selected time points from a 15 hour time-lapse zebrafish embryogenesis study with the Revolution DSD. Sox10:RFP (red) labels oligodendrocytes and Schwann cells, while Olig2:GFP (green) is expressed in oligodendrocytes and oligodendrocyte progenitor cells (OPCs). The images highlight differentiation of OPCs and also migration of oligodendrocytes which initiate neuron myelination.

This data was acquired with Andor iQ on an Olympus IX81 microscope using 20x 0.75 NA plan-apo objective. Dual channel image stacks were taken every 10 minutes for 15 hours and rendered as maximum intensity projections (MIP). Each stack comprises 25 images with 4 µm spacing in the Z dimension (96 µm thick). Frame exposure time was 500 ms for both RFP and GFP channels.

See pages 12-15 for application notes

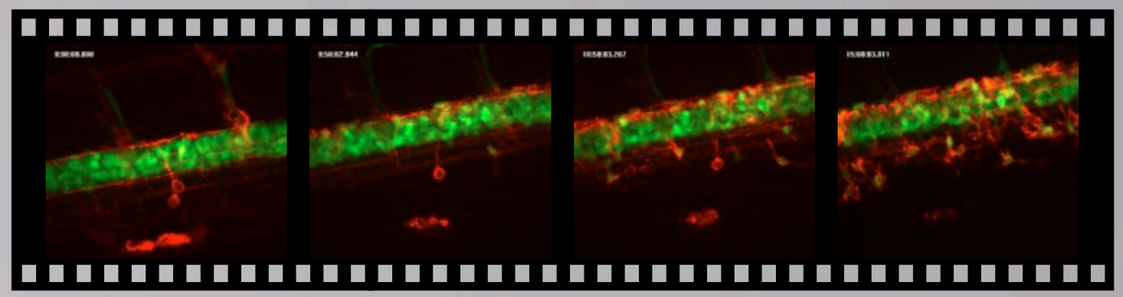


Image courtesy of Dr David Lyons and Dr Jan Soetaert, Centre for Neuroregeneration, University of Edinburgh, UK.

DSD System Configuration

The building blocks of our DSD system allow you to configure everything from an upgrade of your existing epi-fluorescence microscope to a fully configured live specimen imaging workstation. Andor's goal is to provide support in meeting your needs. Please contact us with special requests.

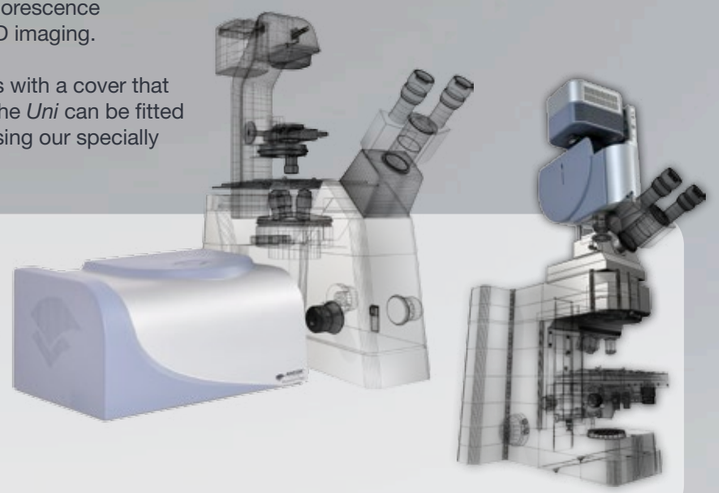
DSD Baseline Configuration Includes:

- Integrated high resolution cooled CCD camera
- DSD 600 differential spinning disk unit operating at 600rpm for confocal imaging
- Andor AMH Metal Halide motorized light source
- Choice of interchangeable filters
- iQ image acquisition and control workstation

DSD Core and Uni

The DSD 600 attaches to research-grade fluorescence microscopes and enables sectioned and 3-D imaging.

The *Core* is offered on inverted microscopes with a cover that provides rationalized cable management. The *Uni* can be fitted to both inverted and upright microscopes using our specially designed universal mount.

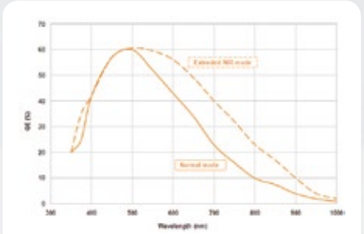


Features

- Unique design for easy filter exchange
- Full spectrum, laser-free
- A range of interchangeable filter turrets
- Excellent confocality
- Real-time control and viewing
- Active background rejection
- High throughput
- Highly cost effective
- Suitable for live and fixed specimens

Clara Interline CCD

Andor's expertise in scientific camera performance optimization has been harnessed to deliver the highest sensitivity interline CCD on the market. Based around the popular ICX285 sensor from Sony, the Clara is ideally suited to high-resolution cell microscopy.

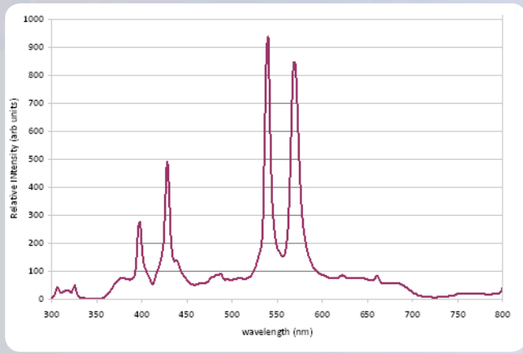


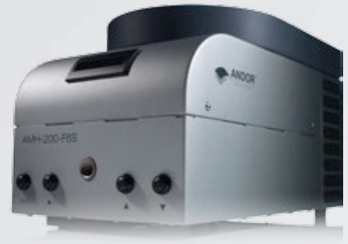
Features

- Ideal for high-resolution cell microscopy
- 1.3 megapixel interline CCD; 6.45 µm pixels
- Very low readout noise (< 3e-)
- Deep thermoelectric vacuum cooling down to -55°C
- Broad Vis-NIR wavelength coverage with enhanced NIR mode
- Up to 20 MHz readout speed

Pre-filtered Metal Halide Light Source (AMH-200 series)

The AMH-200 is a motorized 200 Watt DC stabilized metal halide light source with 2200 hours lamp life. This motorized white light source, with its long-life easy exchange lamp, is ideal for heavy routine use or live cell imaging. The unique combination of the DSD and AMH means that we can offer up to four wavelengths at any one time instead of just three, and with faster switching and shuttering.





Features

- Wavelength range 380 - 700 nm
- ~60 ms filter wheel switching time
- Motorized intensity/shutter, 0% - 100%, 1% resolution, 40 ms Open/Close
- Optical coupling, UV/Vis liquid light guide, NA 0.5, length 2 m (optional 3 m)
- Light source, 200 W stabilized metal halide lamp
- Lamp lifetime typically 2200 hours
- USB interface

Specification sheets
andor.com/microscopy

Software

iQ

Multi-dimensional Imaging Software

Andor iQ is our flagship live cell imaging software, designed with flexibility and power in mind. iQ (image and Quantify) occupies a central role in our Revolution product range and provides optimized control of Andor's Clara CCD camera. iQ also provides support for a wide range of automation hardware, making it a powerful and flexible core for live-cell imaging systems.

Features

- Powerful control and acquisition software platform
- Accessible dashboard interface and wizard structure
- Multidimensional at its core – from fast time-lapse to 5D/6D imaging
- ImageDisk Virtual Memory System for huge data sets
- High performance graphics with OpenGL
- Read the ImageDisk directly with Imaris 7.1 and later

MetaMorph NX

Though we offer our own integrated software, we also recognise that many laboratories around the world use MetaMorph as their imaging software package. Consequently Molecular Devices have included support for the DSD in the newest software platform.

The next generation of MetaMorph Software streamlines the workflow for all tasks and provides an entirely new user-focussed interface. With one-click access to features, integrated hardware setup, and synchronized, unobstructed views of your data, you can become an imaging expert in minutes.

Features

- "Ribbon" interface for convenient access to commonly used options
- Selectable context specific acquisition modes
- Easy installation and configuring of microscope devices
- Multi-threading for software interaction during acquisition
- Live data review and analysis
- Settings recall function from previous experiments
- Improved the speed of acquisitions

Imaris

Visualize, Measure, Understand

Imaris delivers all the necessary functionality for visualization, segmentation and interpretation of multidimensional datasets. By combining speed, precision, and intuitive ease-of-use, Imaris provides a complete set of features for handling multi-channel image sets of any size up to 50 gigabytes.

Imaris will read, visualize, and analyze images acquired from almost any confocal or widefield microscope. Imaris provides important communication with iQ and will read the iQ ImageDisk directly, avoiding the save and open cycle required for third party data. Imaris has been designed for life science applications where data processing is important. Its workflow approach has been designed to remove the burden of selecting and managing multiple imaging tools to increase time spent on research.

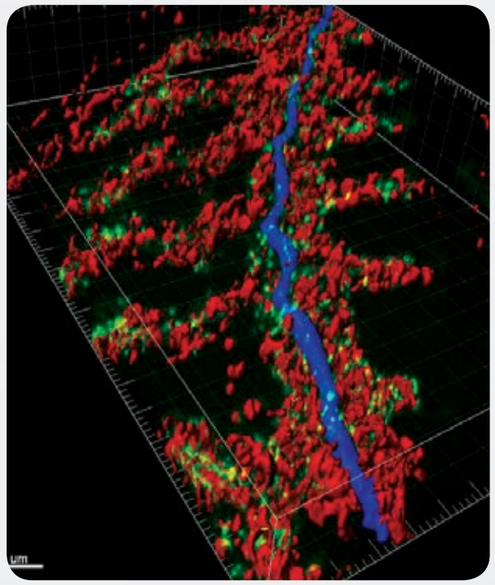
Features

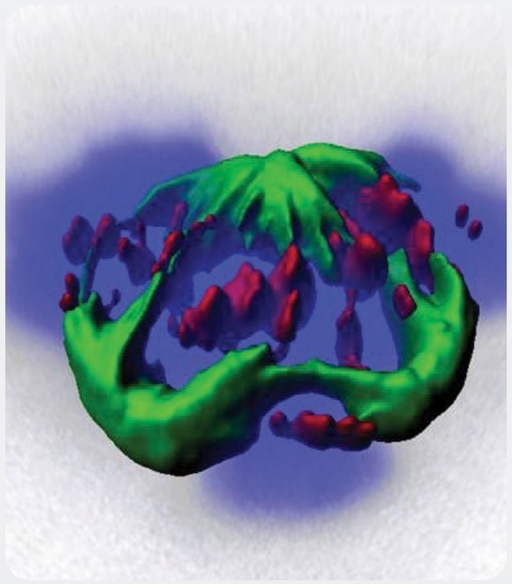
- Advanced volume rendering including Maximum Intensity Projection (MIP), blend projection, and real-time shadow rendering
- Surfaces, segmentation and interactive iso-surfaces, region growing, and semi automatic surface generation
- Spots, segmentation, and interaction – identify and interact in 3D with hundreds of objects
- Smart handling of huge images (> 50 GB)
- Multithreading and advanced computer graphics – high-resolution multiple light sources and 3D holographic rendering

ImarisCell

ImarisCell is an Imaris module specifically designed for the analysis of 2D, 3D, and 4D images of cells and their components. ImarisCell enables researchers to qualitatively and quantitatively examine micro relationships that exist between cells and within cells using the plasma membrane to segment.

Researchers can map organelles and other cellular components within individual cells, even to the level of multiple populations of vesicles. With features such as tracking, and modules like MeasurementPro, it is possible to then examine the relationships between those components.





BITPLANE
explore the next dimension

Accessories

APZ-X00 Piezo Z-Stage

Specifically designed for researchers utilizing deconvolution and 3D imaging, the APZ-X00 offers 100 µm, 200 µm, 250 µm, and 500 µm travel models. The APZ-X00 provides rapid and precise movement of the specimen container. The 250 and 500 µm versions can accept a micro-plate insert for multi-well scanning.

Features

- 100 µm, 200 µm, 250 µm and 500 µm travel range
- Accuracy/Linearity of 0.5% of travel
- Stage Control via Analog (0 - 10 VDC), USB and RS232
- Settling time of 10 - 20 ms
- Inserts for slide, Petri dishes and microtitre plates
- Output-Position Signal 0.0 - 10.0V

Motorized XY & Z Control

Features

- Open and closed loop stages
- Typical travel >100 x 75 mm, with 0.02 µm resolution
- 30 mm/sec travel speed
- Perform multi-field scans for 6D imaging
- Create 4D mosaics using iQ software
- Repeatability 0.2 to 0.3 µm rms

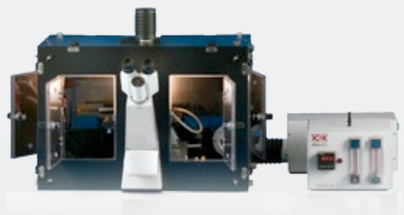
Piezo Objective Control

Features

- PI PIFOC P721 - 100 µm travel
- PI PIFOC P725 - 400 µm travel
- Setting time can be tuned to < 10 ms
- 1.25 nm resolution
- Analogue or digital control
- Oil and water objectives

Stage Incubator

The CO_2 Microscope Stage Incubator (MSI) is a very compact solution to create a suitable environment for cell cultures right on the microscope stage, allowing prolonged observations of living cells. In the MSI cells are proven to proliferate as well as they do in a regular bench-top incubator. Humidifying and pre-heating options prevent medium evaporation and avoid condensation.

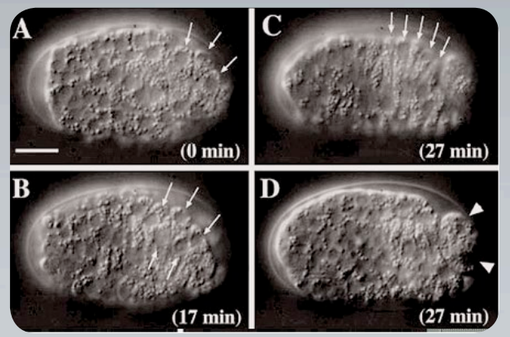


Features

- Available for Piezo inserts
- Electric, Water, and Cryo options
- Temperature range: 3°C above Tamb to 50 °C.
- Cryo with WJ: heating and cooling between 10 to 50 °C
- Temperature regulation - ±0.3°C (Water) and ±0.1°C (Water and Cryo)
- CO_2 range - 0-100%

Photoactivation and Ablation

MicroPoint Laser Illumination and Ablation



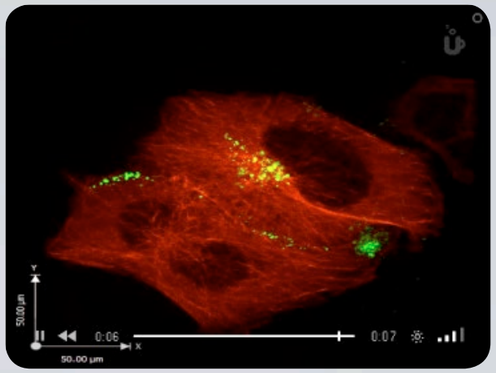
Micropoint Laser ablation of muscle precursor cells in C. elegans

MicroPoint is a pulsed laser delivery system with bio and industrial imaging applications. It is available in manual and galvo-steered versions and delivers 337 nm pulses from a Nitrogen pulsed laser (~3ns, 70 µJ) via optical fiber to a dye cell resonator. Dye cells are available to convert the UV pulses to more than 20 user-exchangable wavelengths ranging from 365 to 656 nm. Custom-designed achromatic microscope adapters ensure that near diffraction-limited pulses are delivered to the specimen. MicroPoint is an excellent photo-stimulation tool providing ablation, uncaging, activation, and bleaching capabilities, adapted by tuning wavelength and energy.

Features

- Simultaneous laser delivery and image acquisition
- Multiple regions of interest with diffraction-limited performance
- Ablation, uncaging, activation and bleaching
- 365-656 nm - adapt to specific targets
- User control of ablation and illumination plane provided by z-axis telescope
- Incremental control of energy provided by manual or motorized variable attenuator slide
- Low maintenance with safety features and fiber optic delivery

Mosaic Digital Illumination



Simultaneous photo-activation in discrete regions of GAG-GFP (green) for particle tracking.

The Mosaic digital illumination system utilizes digital mirror device (DMD) technology to control the illumination field of a fluorescence microscope. Using laser or arc lamp sources, Mosaic achieves real time and near-diffraction limited resolution. Mosaic can simultaneously and precisely excite multiple regions of interest with complex geometries (parallel multi-region illumination) and allow simultaneous imaging. Mosaic is unique, yet flexible, operating over wavelengths ranging from 380-800 nm (365 nm on special request) including uncaging, photo-conversion, activation, and bleaching.

Its simple optical design is readily integrated with complex optical microscopes to realize diffraction limited imaging with minimal loss over a broad spectral range. Unlike traditional galvo-scanning systems where pixels are addressed sequentially, Mosaic provides truly parallel illumination of multiple complex ROIs.

Features

- Unlimited flexibility in shape, complexity, and opacity of illumination mask
- Simultaneous illumination of multiple regions of interest
- Applications include channelrhodopsin, glutamate uncaging and photo-activation
- Precise illumination of areas of interest that protects target specimen and fluorophore
- No scanning - truly parallel illumination and imaging
- Longest lifetime and lowest maintenance with rugged semiconductor device (DMD MTBF > 650,000 hours DMD lifetime > 100,000 hours

Principle of Operation

Andor's Revolution DSD utilizes a unique spinning disk implementation. The patented optical design of the DSD has a structured illumination pattern (SIP) built into the spinning disk to modulate the illumination field. In detection the DSD uses this unique double-sided disk design (Figure 1) to exploit both transmitted (T) and reflected (R) fluorescence emission images to differentiate between in-focus and out-of-focus information. Computer subtraction of these images (T-R) creates a confocal result and provides the name "differential spinning disk"[1].

Structured illumination has been used previously in a number of instruments, but has never been combined with a spinning disk implementation. The DSD provides a unique blend of speed and image quality; images can be acquired at up to 100 fps. The fact that the SIP is generated by a spinning disk means SIP artefacts, which are a well known problem with other approaches[2], are no longer an issue.

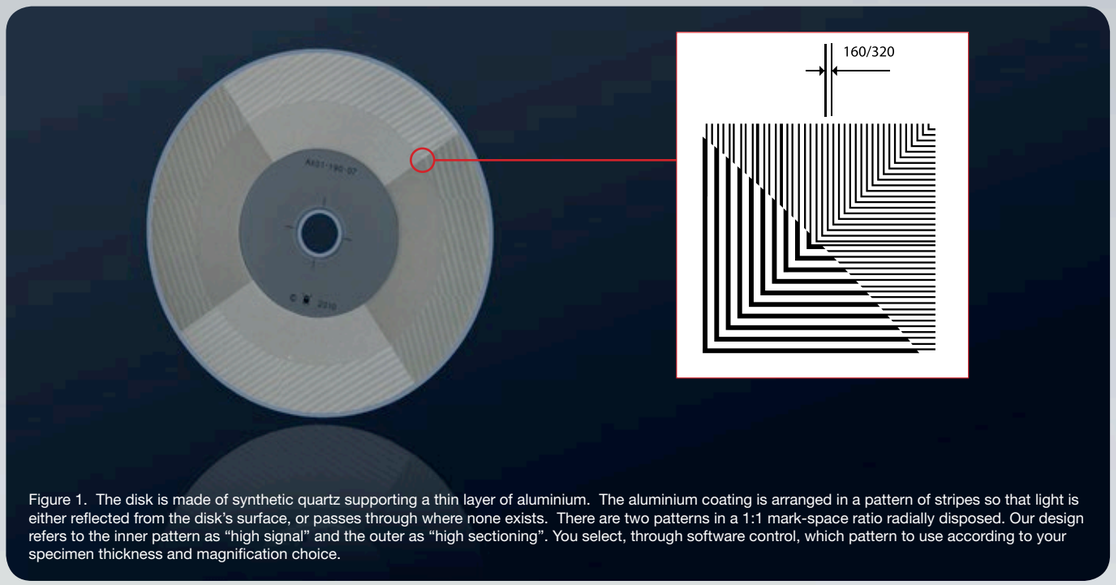


Figure 1. The disk is made of synthetic quartz supporting a thin layer of aluminium. The aluminium coating is arranged in a pattern of stripes so that light is either reflected from the disk's surface, or passes through where none exists. There are two patterns in a 1:1 mark-space ratio radially disposed. Our design refers to the inner pattern as "high signal" and the outer as "high sectioning". You select, through software control, which pattern to use according to your specimen thickness and magnification choice.

The optical path of the DSD is illustrated in Figure 2. The white excitation beam, depicted in green having passed through the excitation filter, is reflected off a dichroic mirror then passes on through the disk, and in doing so generates the structured illumination pattern that is projected into the specimen.

The emitted light from the specimen will ultimately follow two independent paths and so is represented by orange and yellow for purposes of clarity. The light from the sample contains both confocal (C, in-focus) and widefield (WF, out of focus) information. Following the orange/yellow emission path, the emitted light reaches the disk at which point the light that is transmitted (T, yellow) through the disk is now comprised of the confocal signal and about half of the widefield, whilst the reflected (R, orange) is made up of half widefield with no confocal element.

The resulting split light paths, transmitted and reflected, run independently and with the aid of a prism are projected side-by-side onto the CCD detector (Clara camera). Equation 1 depicts the signals of the transmitted and reflected light paths:

$$T = 0.5\,WF + C; \quad R = 0.5\,WF - C \quad \text{(equation 1)}$$

A confocal image is created by subtracting the two images from each other; a widefield image is created by adding the two images together as in equation 2. The process of creating the confocal image actively rejects the background out of focus light, known as active imaging, and enhances the confocal signal by a factor of ≈ 2.

$$2C = T - R; \quad WF = T + R \quad \text{(equation 2)}$$

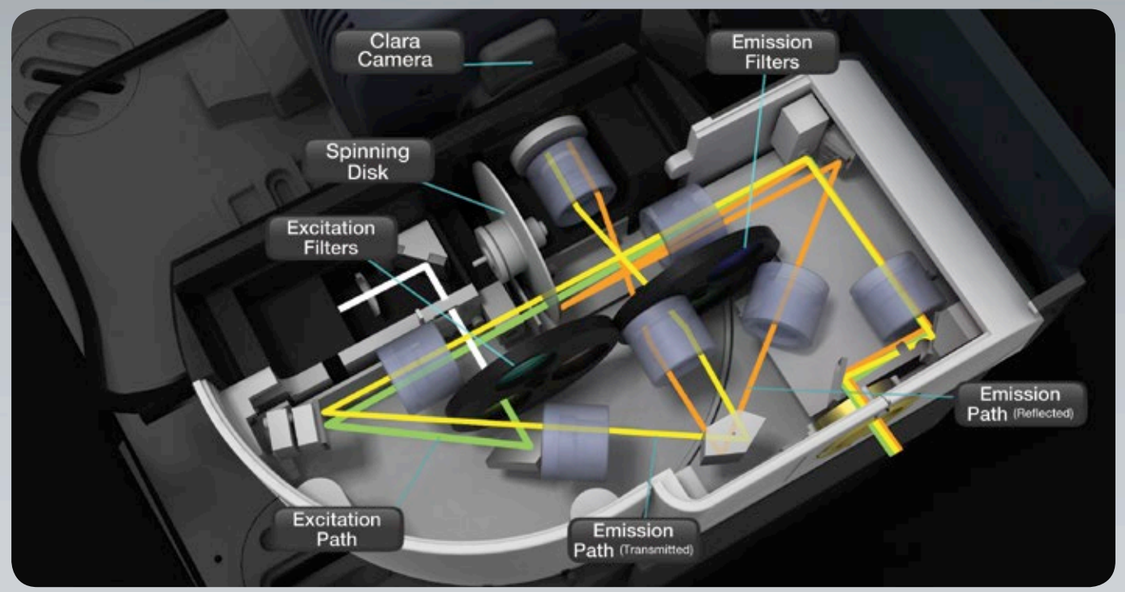


Figure 2. This schematic of the optical path shows the excitation beam in green, while the emission fluorescence beams are shown in orange and yellow to illustrate the different optical paths of the reflected and transmitted signals, respectively. The two signals are combined at the prism and projected onto two halves of the camera.

Figure 3. shows the basic principle of how the raw images are processed in order to achieve the final confocal image. It is worth noting that the Revolution DSD algorithms involve more than just the simple equations previously, but also include essential image registration to avoid mis-alignment artefacts, and the ability to adjust the confocal black level through changing the weighting of the subtraction. All of this can be performed in real-time with a live image, with no post capture processing required.

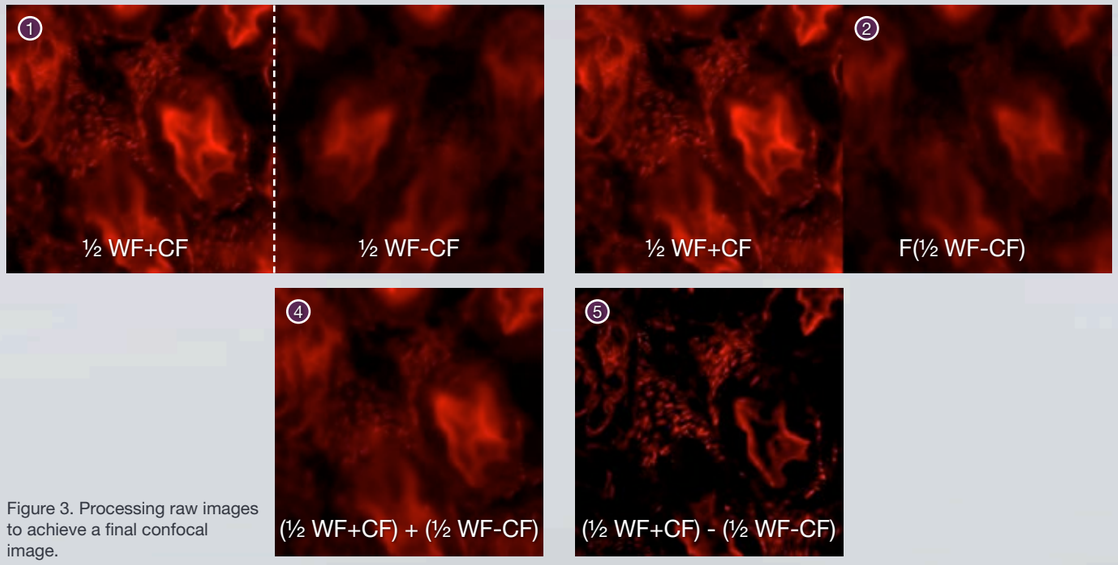


Figure 3. Processing raw images to achieve a final confocal image.

1. Split image about axis of symmetry
2. Flip ½W-C about horizontal axis
3. Register images in real time (>20 Hz)
4. Weighted addition gives widefield image
5. Weighted subtraction gives confocal image
6. Repeat process for each label

Application Note

Real-Time Observation of Nanotube Disassembly

Prof. Dr. Ben L. Feringa and Dr. Wesley R. Browne from the University of Groningen, the Netherlands, are using synthetic chemistry to create new light-responsive nanoscale structures that could one day find use in applications such as smart materials and drug delivery. The photoreactivity of the structures being developed places the Andor Revolution DSD confocal microscope as a key asset in studying the dynamic properties of the materials in real time.

Scientists in the field of nanotechnology strive to create smart nano and microscale structures, incorporating switch and motor units that mimic Nature's fascinating mechanical cellular systems. In the research groups of Feringa and Browne, molecular motors and switches are built into new smart materials through self-assembly and give these materials dynamic properties that respond to external stimuli. Recently the Groningen team created micron-long nanotubes that are tens of nanometers in diameter using a functional amphiphilic building block. The amphiphile is highly fluorescent in the blue region, but at the same time undergoes rapid photochemical conversion to a green fluorescent structure that cannot form the nanotubes. Hence with light the nanotubes can be forced to disassemble (Figure 1).

Dr. Browne says that their work builds on that of other research groups, in particular that of Takuzo Aida's group at the University of Tokyo, which has developed several beautiful examples of nanotube-forming molecular systems. However, by building a reactive element into their amphiphile, the Groningen team's new nanotube systems exhibit unprecedented multifunctionality. "Not only can they be studied by fluorescence microscopy, but we can use light to control the stability and structure of the tubes, ultimately allowing us to trigger the tubes' disintegration in a controlled manner to form new types of structures," he says.

Following the UV-triggered disassembly of the nanotubes in real time at room temperature was singularly the biggest challenge faced by the team because conventional point-scanning, PMT-based confocal systems could provide images but could not provide the time resolution needed. The rapid switching between excitation/emission wavelengths allowed for by the DSD together with the speed of image acquisition offered by the Clara CCD camera was critical.

"In this case the 'switching' between the blue tube-forming fluorescence and green tube-disrupting fluorescence is such that we need to be able to rapidly switch between excitation and emission wavelengths to capture the entire image simultaneously," Dr. Browne says. "This is not practical with PMT-based systems that use scanning to produce an image and require much higher total light intensities."

The researchers used the DSD Revolution confocal microscope to monitor a cross-section of a nanotube during irradiation. When exposed to ultraviolet light, they could see the blue fluorescence decreasing and the green fluorescence increasing in intensity. As green fluorescence increased, they observed matching structural changes within the tube until it eventually disassembled. "Furthermore with the DSD we are able to collect a widefield and confocal image with the one system," says Dr. Browne. In widefield mode the DSD can use external excitation sources so it is not restricted to the excitation wavelengths provided for by the interchangeable filter sets.

The researchers also used the DSD microscope to follow controlled disassembly of the nanotube/vesicle system, which could be accomplished by varying the intensity or wavelength of light (Figure 2.). They found, for example, that irradiation at 365 nm using a UV lamp held above the sample instead of 390 nm light from the DSD's filtered superbright white light source allowed them to slow the disassembly process. "The precise software control of the illumination intensity that is possible with the DSD and the flexibility and speed of switching between excitation wavelengths was central to discovering the functionality of the nanotubes," Dr. Browne says.

For Prof. Feringa's and Dr. Browne's research teams it was important that the DSD could be connected to the side port of any microscope. The researchers use it on a set-up that can also perform widefield imaging and Raman spectroscopy. "The DSD Revolution Confocal system is an ideal workhorse instrument, and a number of projects make use of it," Dr. Browne says. "The key benefit is that at a relatively low cost we have access to a powerful microscopy system that allows optical, widefield, and confocal fluorescence, and together with a Shamrock303 spectrograph and a spectroscopy camera on the second port of the microscope we are able to obtain emission spectra of the fluorophores and carry out Raman microspectroscopy at the flick of a switch on the one sample without any changes in sample position."

In addition, the DSD Revolution confocal microscope uses a bright white light source, which eliminates the expense and safety precautions of working with lasers. "In the future we can easily change the system to a different excitation emission combination - something that would be prohibitively expensive with lasers," Dr. Browne says.

Research Paper: Light–induced disassembly of self-assembled vesicle-capped nanotubes observed in real time, Nature Nanotechnology (2011), doi:10.1038/nnano.2011.120.

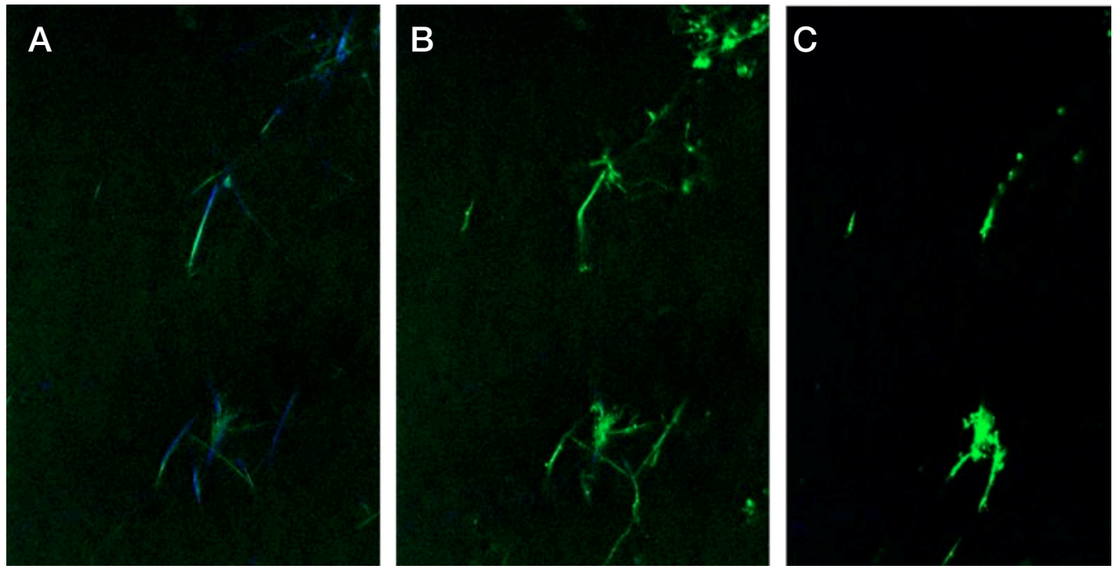


Figure 1. Amphiphilic building blocks. The amphiphile is highly fluorescent in the blue region, excited at 406nm, but at the same time undergoes rapid photochemical conversion at this wavelength to a green fluorescent structure that cannot form the nanotubes. Image A shows mature nanotubes that are already undergoing conversion to green fluorescence owing to the violet excitation of the blue signal. In B, 12 seconds later the majority of tubes have converted to green and by 48 seconds (C) they are disassembling.

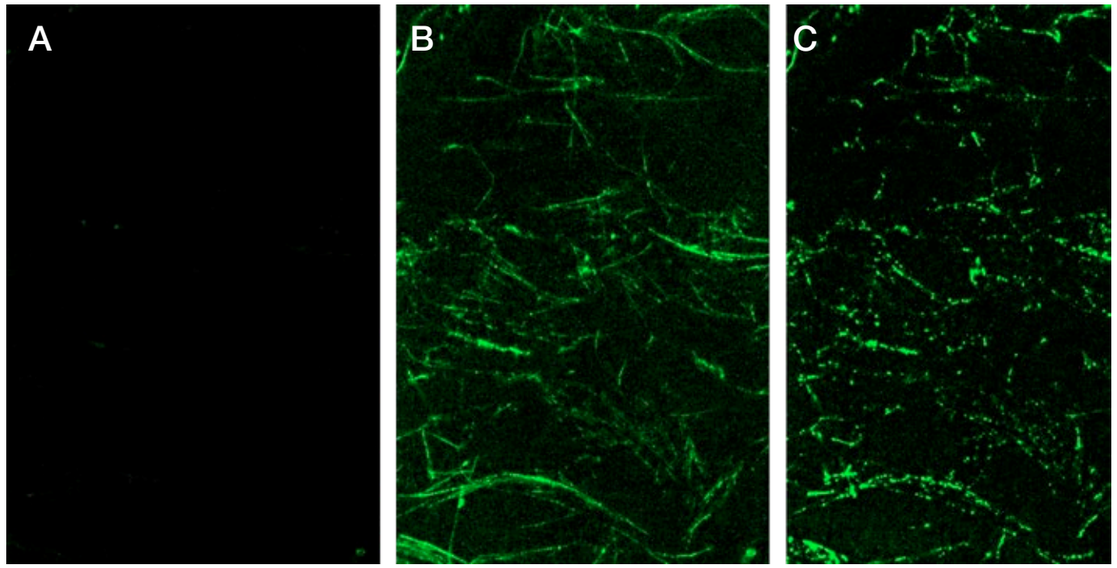


Figure 2. The amphiphile conversion can be regulated with more control using lower intensity and shorter wavelengths. Here the nanotubes are only imaged for green emission (excitation at 494nm) so avoiding excitation at 406nm, whilst shorter wavelength excitation was controlled using an external UV light source. A, shows no evidence of nanotubes undergoing conversion as seen by the lack of green signal. Significant conversion is only observed 41 seconds later in B, with disassembly evident as long as 1 minute and 13 seconds later in C.

Application Note

Imaging Intracellular pH in Living Cells

Intracellular pH is important in many functions that take place in a cell. For example, pH affects protein structure and the function of lysosomes, mitochondria, and other organelles. Changes in energy metabolism also often correlate with pH changes, and so scientists would like to monitor ATP and pH in a cell simultaneously.

The Andor Revolution DSD confocal microscope is helping Dr. Gary Yellen's lab at Harvard Medical School develop genetically encoded fluorescent sensors for monitoring metabolism of single cells. "We are interested in sub-cellular compartmentalization of metabolites, and the optical sectioning afforded by the DSD allows us to assess the distribution of our sensors when they are genetically targeted to different cellular locations (e.g., plasma membrane, mitochondria, etc; Figures 1a and 1b)," he says.

Dr. Yellen's research team recently developed a genetically encoded pH sensor that could be used with GFP-based ATP sensors (Figure 1.) This type of probe was needed to monitor ATP and pH changes in a cell and to correct for the pH sensitivity of ATP sensors. They used the red fluorescent protein mKeima as a basis to engineer a genetically encoded ratiometric pH sensor they named pHRed. The sensor has a fluorescence emission peak at 610 nm and dual excitation peaks at 440 and 585 nm that allow ratiometric imaging.

Postdoctoral research fellow Mathew Tantama imaged energy-dependent changes in cytosolic and mitochondrial pH with the Revolution DSD, demonstrating pHRed's capability to monitor intracellular pH in living cells. "The Andor Revolution DSD offered us a practical, cost-effective solution for optical sectioning with the flexibility of a white light source," Dr. Yellen says. The microscope's broadband prefiltered light source and user-selectable filters gave the researchers access to a wide variety of excitation wavelengths. "During the process of sensor development, color requirements often change, making the fixed wavelengths of laser-based systems constraining or cost-prohibitive for an individual lab," he adds. The unique optical architecture of the Revolution DSD enables capture of both conventional and confocal fluorescence images simultaneously. This feature provides further flexibility for the researchers who can choose between the sensitivity of widefield and the resolution of confocal imaging according to conditions and experimental requirements.

The researchers transfected the pHRed sensor and the mVenus-based Perceval ATP/ADP sensor into live cells. For intensity ratio imaging they imaged the cells on an inverted microscope equipped with a Revolution DSD system controlled by Andor iQ software. Ratio imaging of the two sensors used excitation filters 445/20 nm, 482/18 nm, or 578/16 nm filters. The DSD unit contained a 59022bs dichroic, 492 nm short pass, 490 nm short pass, and 590 nm short pass filters. Emission light was passed through 525/39 nm or 629/56 nm filters. They typically acquired images every 10 seconds with 2x2 binning and 50 to 400 ms exposure times using a 20X/0.75NA objective. The speed performance of the Revolution DSD allowed imaging of several wavelengths at multiple XY positions while maintaining an adequate temporal resolution.

After capturing the images, they subtracted background and bleed-through of Perceval fluorescence into the pHRed channel before calculating ratios. At the shared wavelength of 445 nm there was little bleedthrough during excitation. The researchers calculated pixel-by-pixel ratios, identified regions of interest around cells, and calculated averaged measurements using thresholding with ImageJ software. They found that pHRed's intensity ratio responds with an apparent pKa of 6.6 and a greater than 10-fold dynamic range.

"This is the first ratiometric sensor based on a single red fluorescent protein that can be used in multiple imaging modalities," Dr. Yellen says. "There are several other genetically-encoded pH sensors that utilize the blue to yellow color range or that provide intensity-based readouts only. Our red-colored pH sensor can be used in conjunction with other genetically-encoded sensors with the possibility of minimal color channel crosstalk."

Research Paper: Imaging Intracellular pH in Live Cells with a Genetically Encoded Red Fluorescent Protein Sensor, Journal of the American Chemical Society, 2011, 133 (26), pp 10034–10037, DOI: 10.1021/ja202902d.

Additional related publication: Imaging Cytosolic NADH-NAD+ Redox State with a Genetically Encoded Fluorescent Biosensor, Yin Pun Hung, John G. Albeck, Mathew Tantama, Gary Yellen, Cell Metabolism - 5 October 2011 (Vol. 14, Issue 4, pp. 545-554) http://dx.doi.org/10.1016/j.cmet.2011.08.012

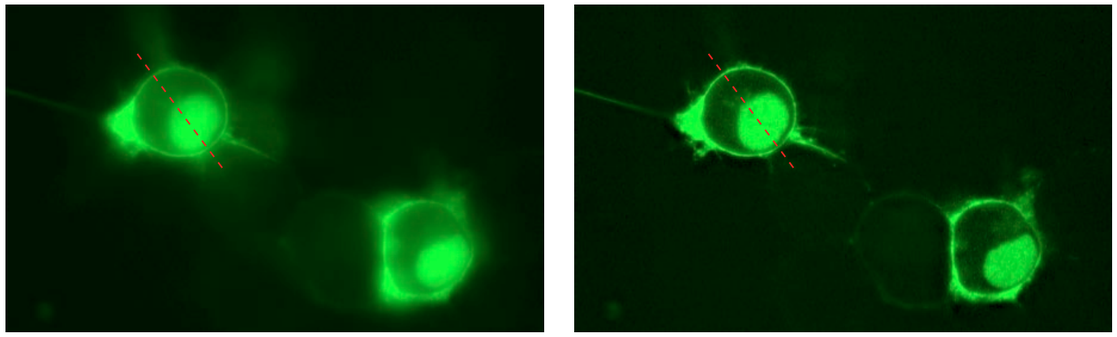


Figure 1a. The researchers transfected Neuro-2A cells with a single fusion construct of pHRed-Perceval targeted to the plasma membrane and the nucleus. The Andor Revolution DSD was used to collect both widefield and confocal signals at a single z-plane through the center of the cells using a 40X 0.95NA air objective. The widefield image is on the left, and the confocal image is seen on the right. Only the green Perceval emission is represented in the image.

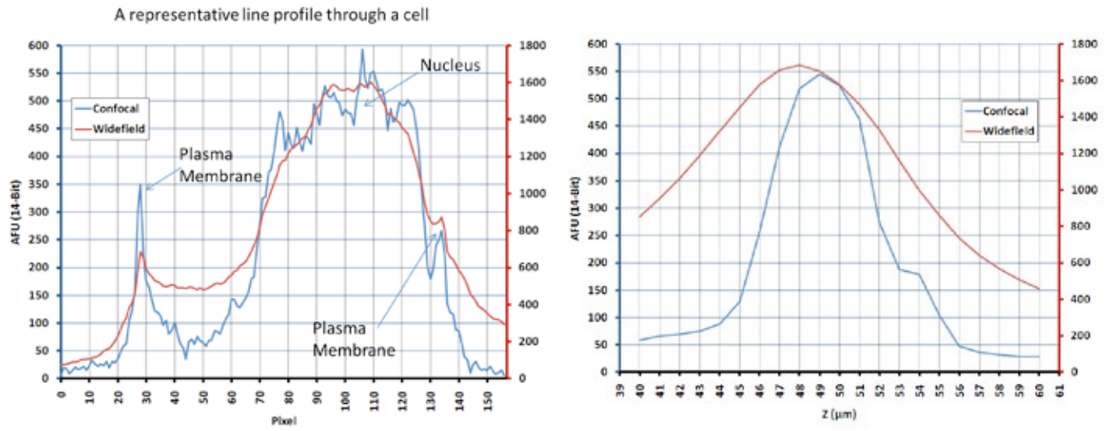


Figure 1b. The widefield and confocal signals were simultaneously acquired with the Andor DSD system, and data are shown for a representative line profile through a cell (left) and a representative axial intensity profiled through the nucleus (right). Subcellular compartments such as the plasma membrane and nucleus are better defined in the confocal image compared to widefield, allowing more spatially accurate measurements. The widefield mode of the DSD is useful when spatial variation is minimal but more sensitivity is required.

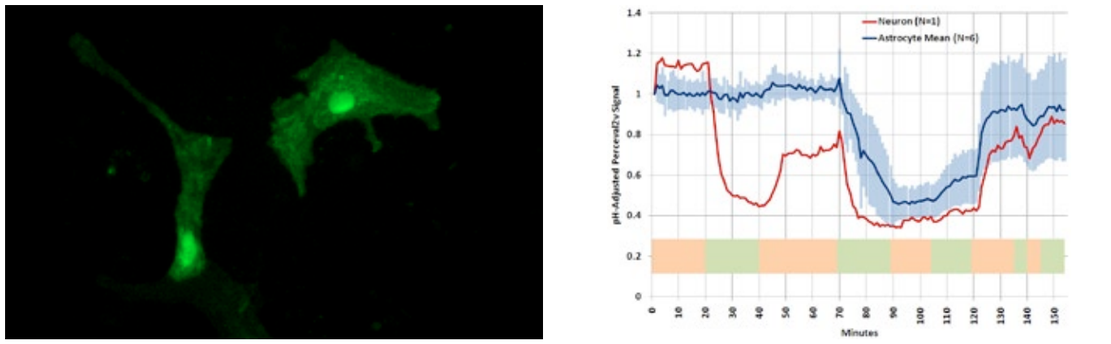


Figure 2. Mouse primary astrocytes (pictured above) as well as neurons were transfected with a single fusion construct of pHRed-Perceval targeted to the plasma membrane and nucleus. Data acquired with the DSD confocal microscope (graph above) demonstrates that the sensors report neuronal response to glutamate challenge (t = 20 to 40 min) with a decrease in ATP/ADP but astrocytes did not. All cells responded to lowering of extracellular glucose (t = 70 to 120 min). In this experiment, images in 4 separate color channels at 4 stage positions were taken at each time point with only the green Perceval emission being represented in the image.

Technical Note

Filter Exchange and Calibration

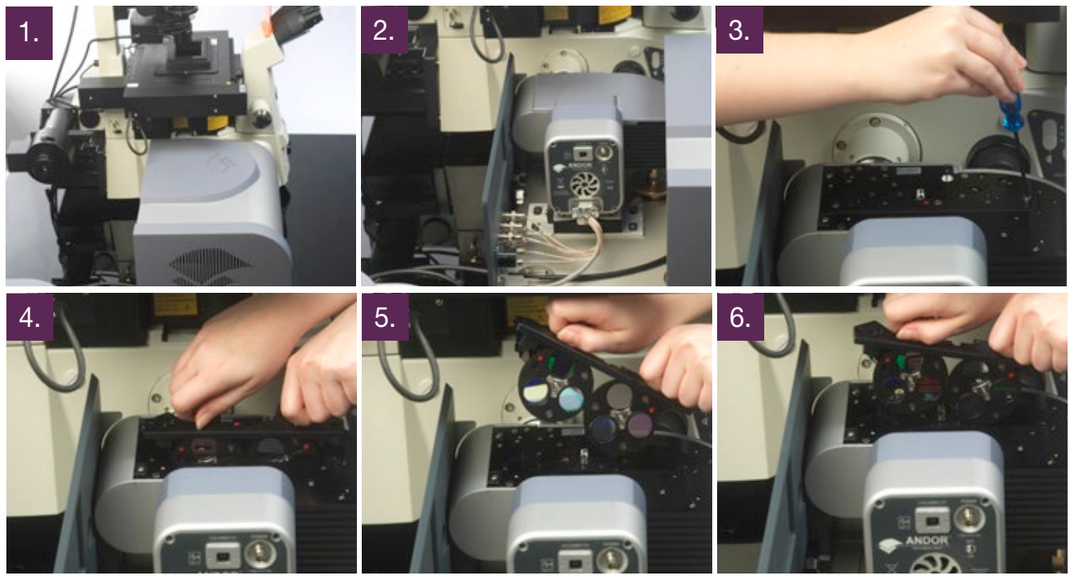
Rapid Filter Turret Exchange
Andor's unique design of the Revolution system allows the DSD to remain fixed to the microscope and the camera fixed to the DSD during filter turret switching. This removes any requirement to re-align the optical and mechanical assembly. The filter turrets, shown right, contain the excitation, dichroic, and emission filters for the DSD.

Changing the Filter Turret - Step by Step
The images below show the actions required to change the filter turret. Remove the existing filter turret by lifting the lid and removing 3 screws (1-5). Insert new set (6) and repeat steps 1 to 5 in reverse. To guide you through the process, a Filter Change Wizard in Andor iQ, provides step by step instructions.

Multiband Blocking Filter Insert
In addition to the turret, each DSD filter set has a multi-band emission filter, which is inserted into the tube connecting the DSD to the Clara CCD camera.

The ability to exchange multiband emission filters without removing the camera from the system allows for more rapid system configuration and is unique to the Revolution DSD.

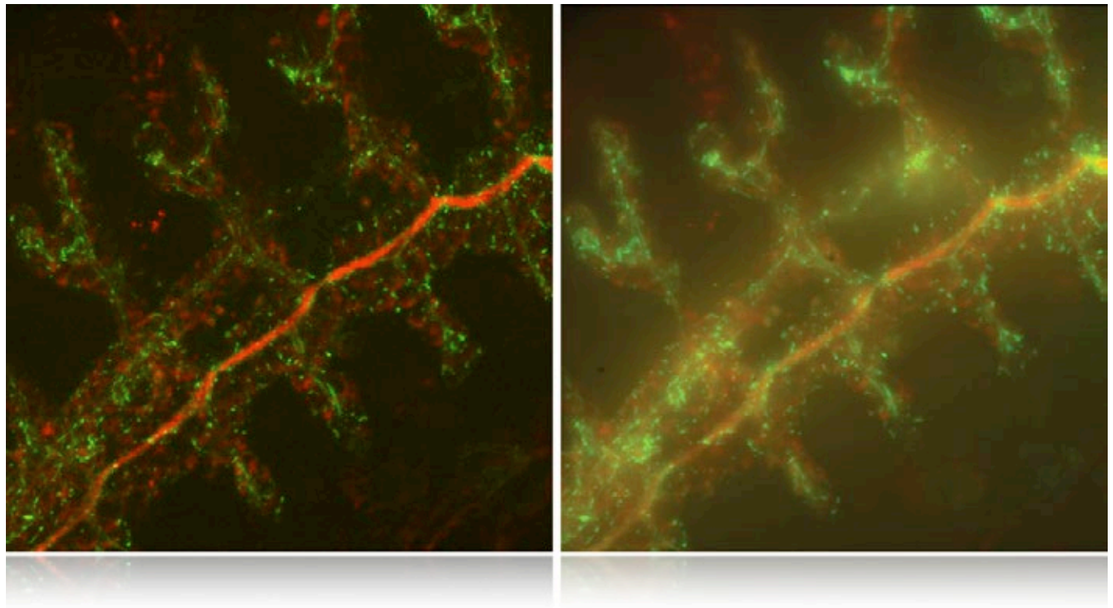
One-click Calibration
The last step in changing the DSD filter set is to run a calibration in Andor iQ. This can be done with a single mouse click and no user intervention is required to complete the process, which takes just one minute.



Lines	Turret Name	Excitation & Emission Filter Pairs Centre/FWHM	Pre Filters	Fluorophores
2	GFP/RFP	457/50 & 525/50, 556/20 & 617/73	457/50, 556/20	GFP, RFP
2	CFP/YFP	427/10 & 475/50, 500/24 & 542/50	427/10, 500/24	CFP, YFP, CFP-YFP
2	Cy3/Cy5	534/36 & 577/24, 635/31 & 690/50	534/36, 635/31	Cy3, Alexa 555, Cy5, Alexa 647
3	D/F/T	406/15 & 457/50, 494/20 & 536/40, 575/25 & 628/40	406/15, 494/20, 575/25	DAPI, FITC, Texas Red
4	D/F/T/CY5	387/11 & 440/40, 485/20 & 521/21, 560/25 & 607/34, 650/13 & 700/45	387/11, 485/20, 560/25, 650/13	DAPI, FITC, TRITC, Cy5
	Custom	On Special Request		

Source Pre-filters - Significant Benefits
Revolution DSD supports light sources with fast wavelength and intensity switching, giving three distinct advantages.

- DSD offers four or more wavelengths in contrast to a standard filter turret
- DSD wavelength switching times are much reduced
- Instrumental background is reduced because only desired source wavelengths reach the DSD

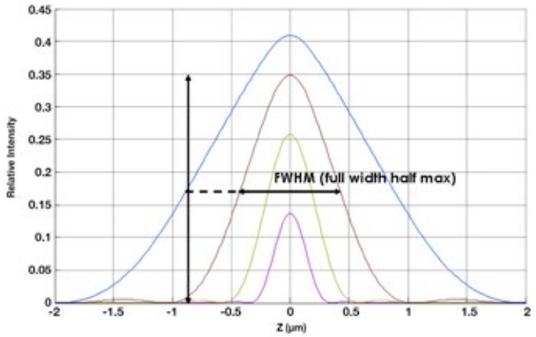


Developing airways in the Drosophila embryo. A luminal protein is shown in red and a secreted marker is shown in intracellular green puncta. Vasilios Tsarouhas and Christos Samakovlis, Wenner-Gren institute, Stockholm University, Sweden.

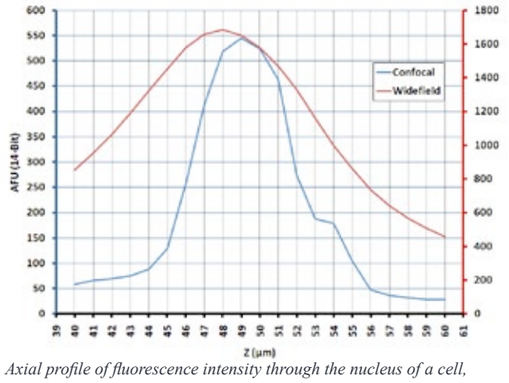
Technical Note

Optical Sectioning and Signal to Noise Ratio (SNR)

Spinning at 6000 rpm, the disk produces a dynamic SIP that is projected into the specimen focal plane where it is in sharp focus. At increasing defocus the SIP loses contrast and eventually becomes impossible to differentiate from the general widefield or out of focus fluorescence. The manner in which the confocal signal, C, changes with focus position, z, is known as the axial response function. A convenient way to describe the axial response function is by its full-width-half-maximum or FWHM as illustrated in graph ***A*** below.



A. *Shows the theoretical axial response function (ARF) of the DSD system with different SIP pitches from 40-320 µm and showing the full width half maximum or FWHM. All data computed for 60X/1.4 oil immersion lens.*



B. *Axial profile of fluorescence intensity through the nucleus of a cell, showing the increased axial resolution from the confocal mode of the DSD compared to widefield. Image captured with 40x dry 0.95 NA objective.*

Depending on the specimen and the goals of the study, we can trade signal to noise for axial resolution, magnification, or specimen exposure time and illumination level. This series of trade-offs becomes most complex for live cell specimens, where specimen health, photo-bleaching, and toxicity can be critically important. Photo-bleaching leads to loss of signal over time and toxicity in live specimens.

	Confocal Sectioning (µm)	
Mag/NA	High Sectioning	High Signal
10/0.4	15	28
20/0.5	6.4	12.2
20/0.7	4.6	8.8
20/0.9	3.6	7
40/0.7	2.5	4.4
40/1.0	1.7	3.2
40/1.3	1.35	2.45
60/1.2	1	1.8
60/1.4	0.85	1.55
100/1.4	0.55	1

Table 1. The theoretical FWHM ARF data for the Revolution DSD are listed above. This will help to explain how we estimate signal to noise ratio and also how we can trade signal for optical sectioning and choose the DSD SIP best suited to particular types of specimen.

In this respect the DSD compares well with laser scanning confocal since photo-bleaching is a nonlinear effect in which high illumination power has the greatest effect. Figure 1. shows measured Mito-Tracker photo-bleaching with living MDCK cells imaged by the DSD.

Conclusion

Many factors must be considered when choosing an instrument for scientific research. DSD-based systems will not replace point scanners or laser spinning disks in all conditions, but they do represent a viable and cost-effective alternative in many applications, ranging from analysis of fixed tissue to live-cell imaging. DSD can be tuned to a broad range of fluorophores, operate at high and low magnification, and acquire high-resolution images at frame rates in the order of 1 to 10 Hz. At 2x2 binning and with regions of interest this frame rate can exceed 25 Hz, with a limit of 100Hz defined by the disk rotation speed.

Revolution DSD delivers a new price-performance level in the confocal microscopy market. Its flexibility, imaging performance and low maintenance requirements will find favour in a wide range of research applications and environments.

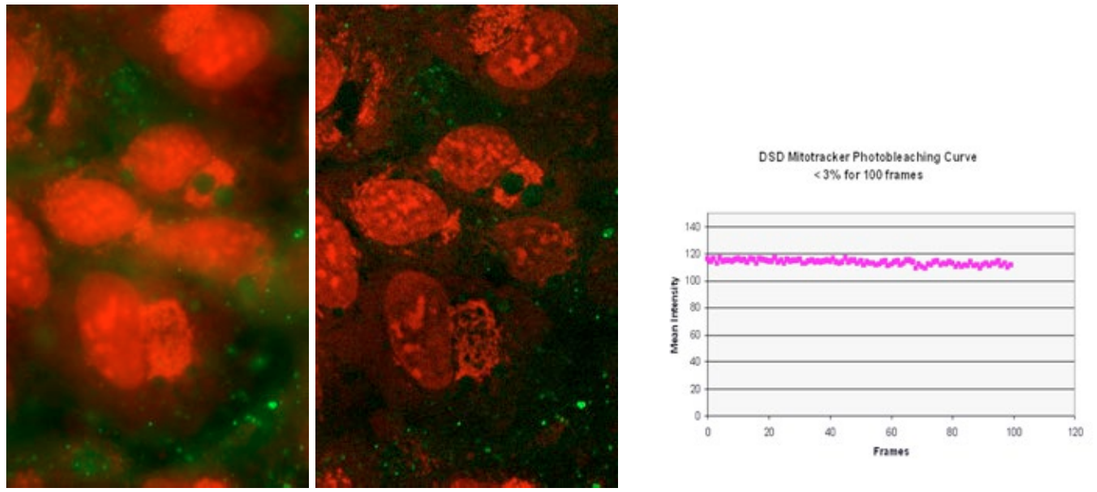
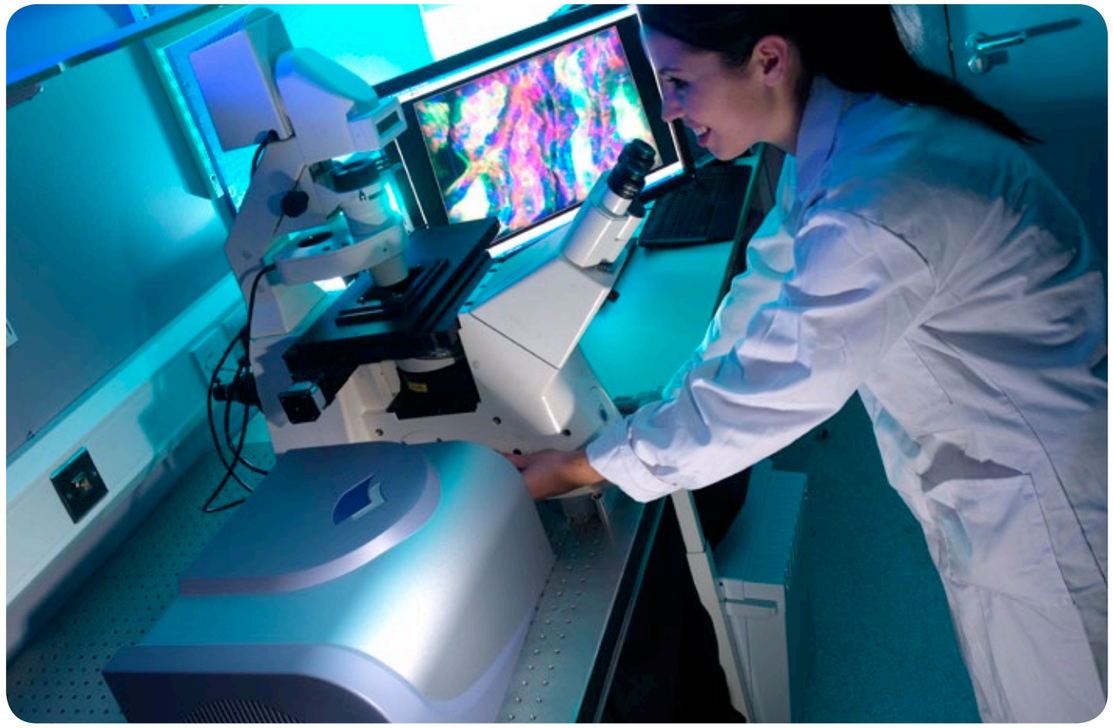


Figure 1. A two-color image series of living MDCK cells acquired with Revolution DSD. Mito-tracker label shown in red and lysotracker in green. 200 images were acquired over a period of approximately 3 minutes of imaging and analyzed for bleaching as shown in the intensity graph on the right. Mito-tracker is known to bleach rapidly, but as this data shows, DSD shows low levels of photo-bleaching with <3% recorded over the 200 frame series (100 per channel).



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1. R. Juskaitis et al (October 1996). Efficient real-time confocal microscopy with white light sources. Nature, pp. 804-806.

2. L. Schaefer et al (Oct 2004) "Structured illumination microscopy: artefact analysis and reduction utilizing a parameter optimization approach" Journal of Microscopy Volume 216 Issue 2, Pages 165 - 174 Published Online: 28 Oct 2004

3. V. Poher et al (August 2008). Improved sectioning in a slit scanning confocal microscope. Opt Lett, pp. 1813-1815.

Andor Customer Support

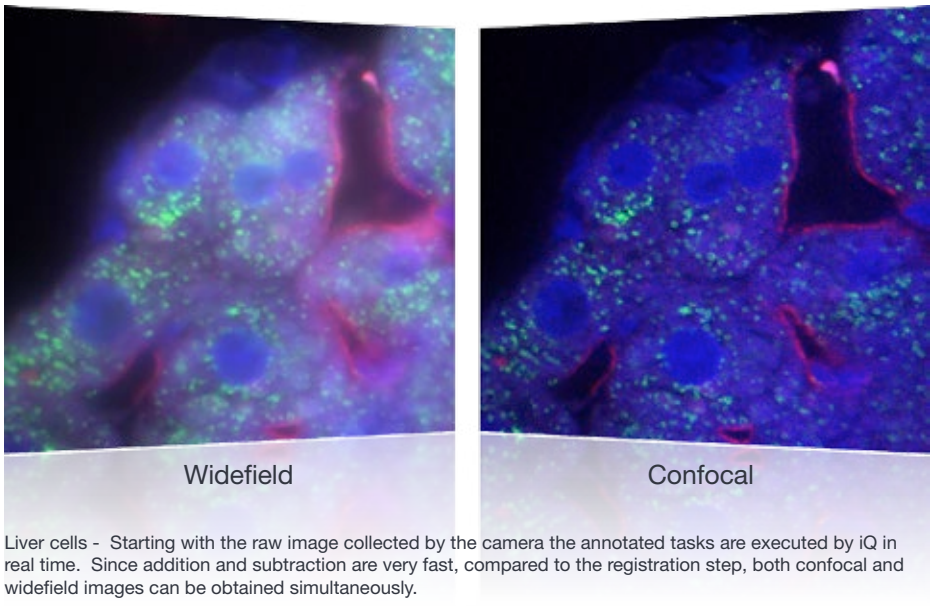
Andor products are regularly used in critical applications and we can provide a variety of customer support services to maximise the return on your investment and ensure that your product continues to operate at its optimum performance.

Andor has customer support teams located across North America, Asia and Europe, allowing us to provide local technical assistance and advice. Requests for support can be made at any time by contacting our technical support team at andor.com/support.

Andor offers a variety of support under the following format:

- On-site product specialists can assist you with the installation and commissioning of your chosen product
- Training services can be provided on-site or remotely via the Internet
- A testing service to confirm the integrity and optimise the performance of existing equipment in the field is also available on request.

A range of extended warranty packages are available for Andor products giving you the flexibility to choose one appropriate for your needs. These warranties allow you to obtain additional levels of service and include both on-site and remote support options, and may be purchased on a multi-year basis allowing users to fix their support costs over the operating lifecycle of the products.



Liver cells - Starting with the raw image collected by the camera the annotated tasks are executed by iQ in real time. Since addition and subtraction are very fast, compared to the registration step, both confocal and widefield images can be obtained simultaneously.

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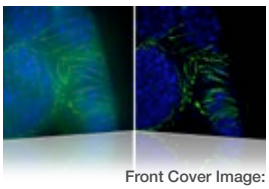

Front Cover Image:

Ovariole of Drosophila melanogater showing germarium and early stage egg chambers. Green: F-actin stained with Alexa Fluor 488 phalloidin. Blue: DNA stained with DAPI.

Courtesy of Dr. Eurico Sá, Molecular Genetics group, IBMC, Porto, Portugal