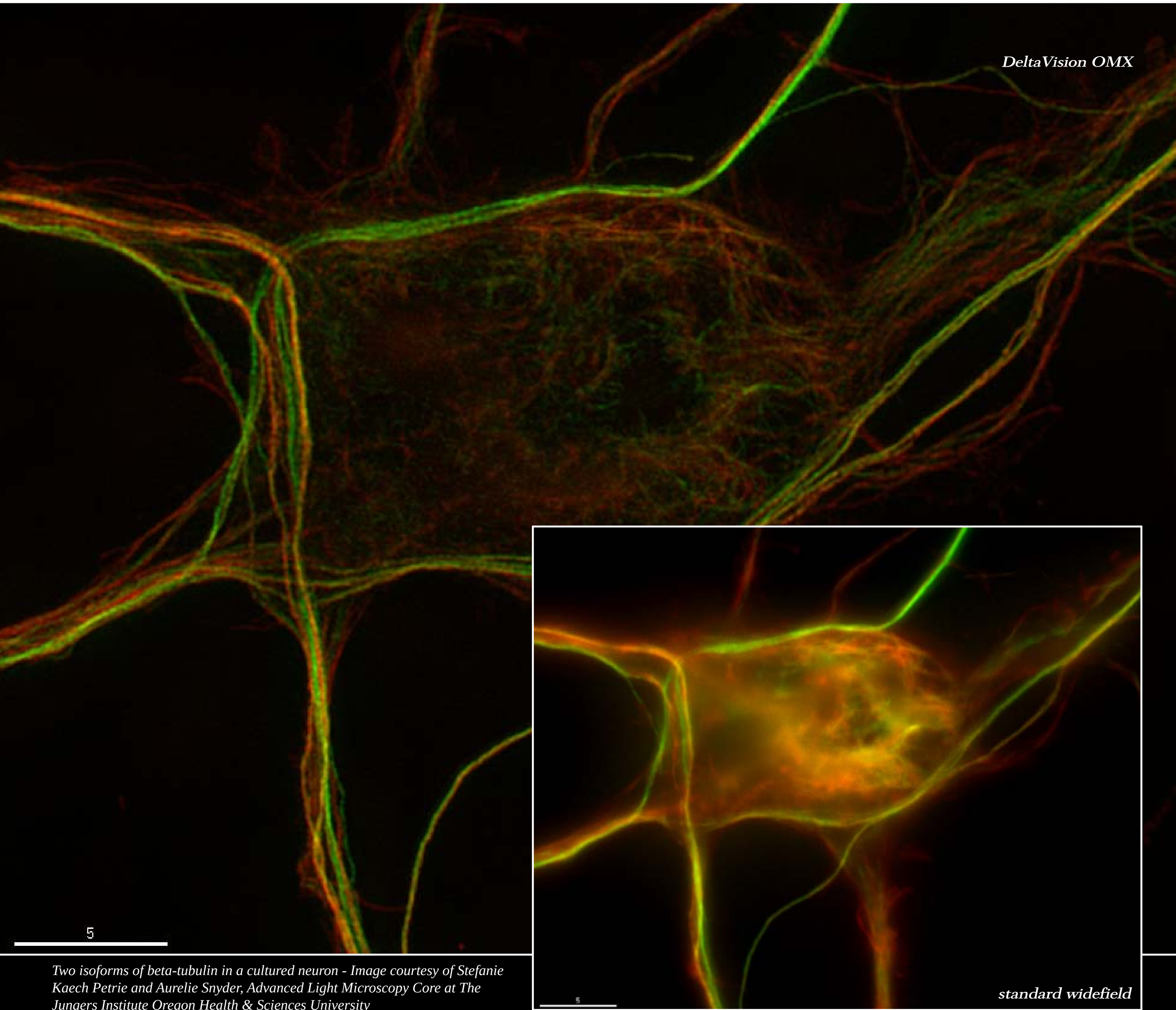
AppliedPrecision®

DeltaVision|OMX SR™

3D-SIM™ *Super-Resolution Imaging*



Two isoforms of beta-tubulin in a cultured neuron - Image courtesy of Stefanie Kaech Petrie and Aurelie Snyder, Advanced Light Microscopy Core at The Jungers Institute Oregon Health & Sciences University

What is super-resolution?

In conventional microscope systems, image resolution is limited by the angle of light that can successfully traverse the light path and enter the objective lens. While objectives can be built with very high numerical apertures, a limit is reached when light can no longer cross the interfaces between different refractive indices. At this point, Brewster's angle is achieved and additional light and information (resolution) do not make it into the objective lens. This angle ultimately limits the resolution of all microscope systems. Once this limit is reached, a system is said to be diffraction limited. In recent years, new methods have been developed to surpass the diffraction limit. These methods allow precise visualization and measurement of features that are less than one-half of the size of those seen with conventional microscopy.

How is super-resolution achieved?

DeltaVision OMX SR uses 3D-SIM super-resolution technology developed by the labs of Drs. Sedat, Gustafsson, and Agard at UCSF[1]. DeltaVision OMX SR is a 3D structured illumination microscopy system that enables super resolution imaging. DeltaVision OMX SR surpasses the resolution limit by a factor of two in x, y and z, and enables imaging beyond the surface of the coverslip with multiple probes[2]. This technology will resolve features previously invisible to traditional microscopy and lets you image more of your biology, not just the biology that falls within a fraction of a micron of the basal surface of the cell. The DeltaVision OMX technology can image five, ten, fifteen, even twenty microns into cells and tissues. DeltaVision OMX SR works with conventional fluorochromes saving you valuable time and eliminating any need to genetically engineer novel or complex photoswitchable probes so that you can apply the preparation methods and fluorescent labeling reagents (antibodies and protein tags) currently used in the lab.

1. Schermellah, L et al. Science, V 320, p. 1332
2. Gustaffson, MGL et al. Biophys J., 2008 V 94, p. 4957

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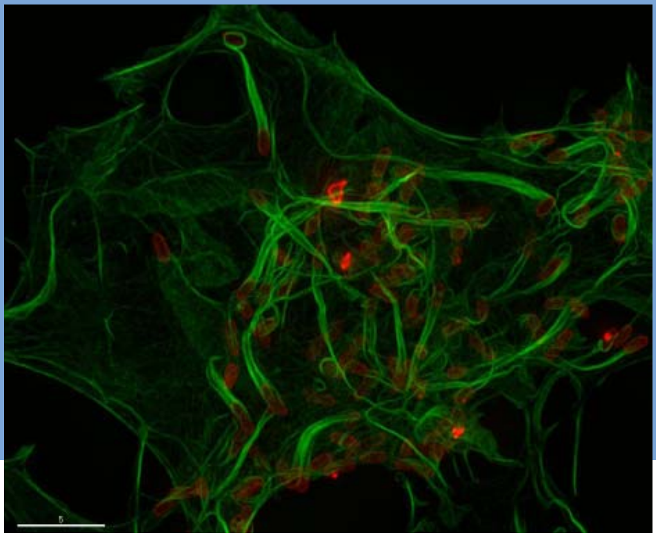
What is 3D-SIM?

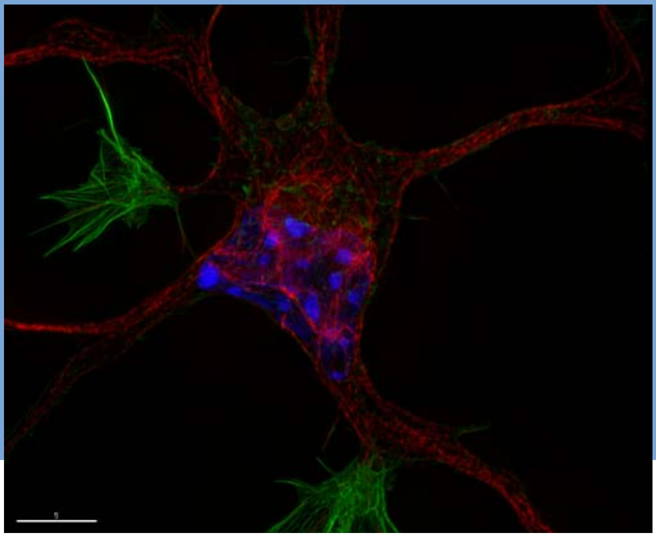
DeltaVision OMX SR extends the resolution limits of microscopy by superimposing a high-resolution sinusoidal illumination pattern onto the sample. This pattern generates moiré interference patterns with the high-resolution information contained within your sample. These moiré patterns occur at lower frequencies than the original high-resolution information in your sample. By translating and rotating this illumination pattern around the numerical aperture, twice the spatial information can be collected by the microscope effectively doubling the resolution of the microscope.

An established technology

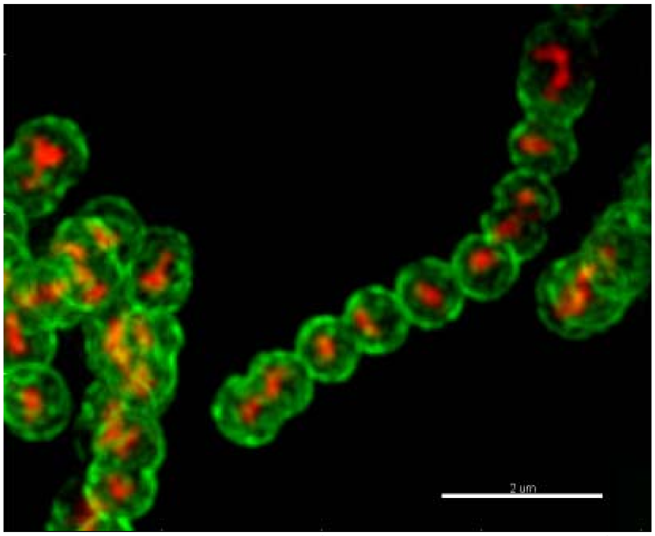
DeltaVision OMX systems have been in place around the world since 2008. As scientists make groundbreaking discoveries using 3D-SIM, the demand for the technology continues to grow. DeltaVision OMX super-resolution systems are in use at a number of institutions around the world including: The University of California, Davis, The Oxford University, The Samuel Lunenfeld Research Institute, The Georgia Institute of Technology, The University of Dundee, The University of Illinois, Cold Spring Harbor Laboratories, Ludwig Maximilian University of Munich, University of Technology Sydney, Massachusetts Institute of Technology and University of Cambridge.



R. parkeri infected Cos7 cells - Image courtesy of Matt Welch, University of California at Berkeley



Hippocampal neurons - Image courtesy of Erik Dent, University of Wisconsin Madison



Streptococcus cells - Image courtesy of Cynthia Whitchurch, University of Technology

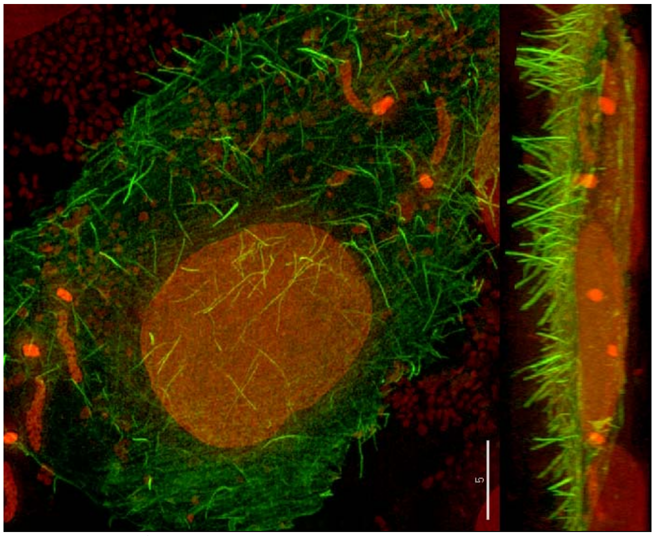


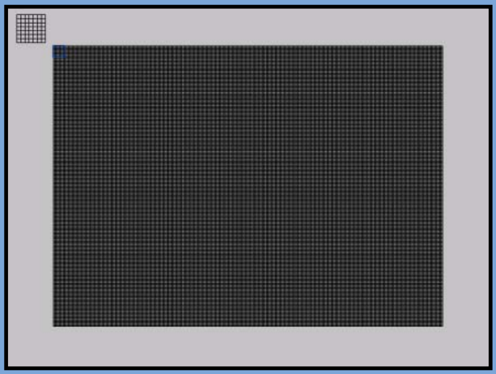
Image courtesy of Renato Mortara, Universidade Federal de São Paulo (UNIFESP), Sao Paulo, Brazil

How does 3D-SIM work?

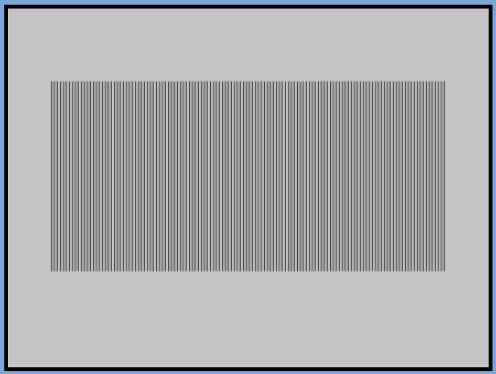
The resolution of the microscope is limited by the amount of spatial information that can pass sucessfully through the optics. This is represented by the fine grid pattern (panel 1). If we mix that high-resolution information with a known signal that we can resolve (panel 2), we generate a new pattern, the moiré pattern (panel 3). Here the pattern that we see is the difference between the two patterns and can easily be represented without high-resolution methods.

Likewise, in 3D-SIM, a three-dimensional illumination pattern is superimposed on the sample. This pattern generates a new pattern (moiré pattern) that contains both illumination and sample data. By carefully reconstructing the sample data from the moiré pattern, the DeltaVision OMX system creates a super-resolution three-dimensional image of the original sample.

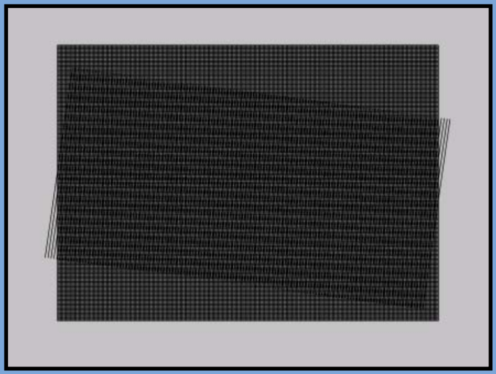
Because the illumination pattern is three-dimensional, the image that is created contains both lateral (2D) and the axial (3D) data. This is limited only by how deep into the sample the illumination pattern can be maintained before light scattering attenuates the illumination pattern (usually tens of microns into the sample). This illumination pattern can be efficiently generated with multiple excitation wavelengths allowing use of multiple fluorochromes in the same sample. 3D-SIM is the only super resolution method that can use multiple probes in the same sample (currently up to 4) and that can image well beyond the sample substrate.



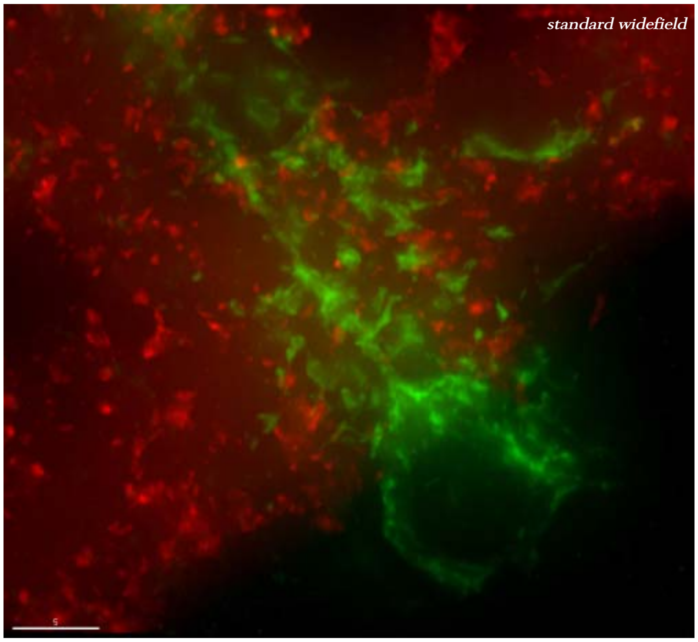
panel 1

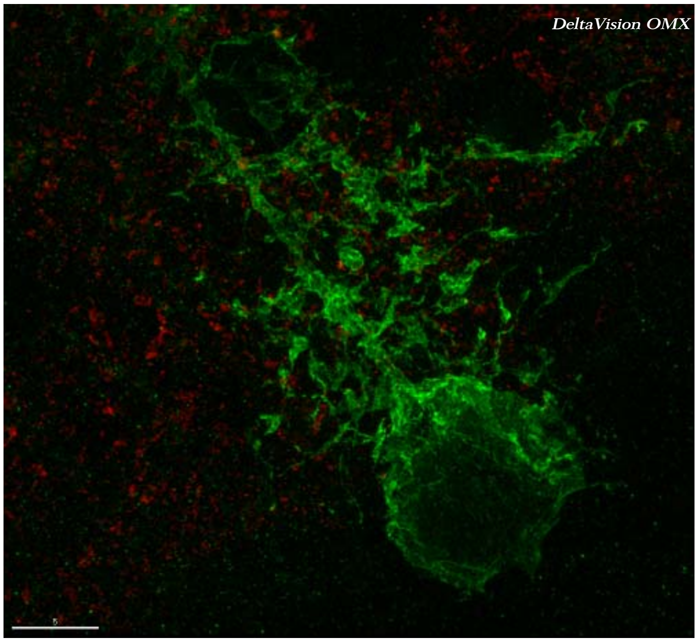


panel 2



panel 3





Glial cell - Image courtesy of Ed Ruthazer, McGill University

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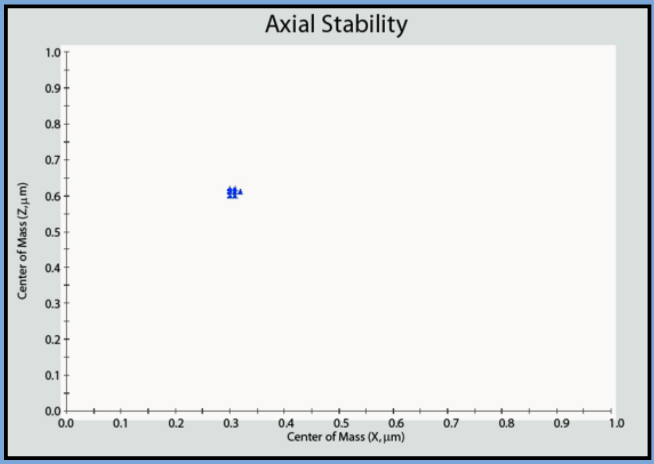
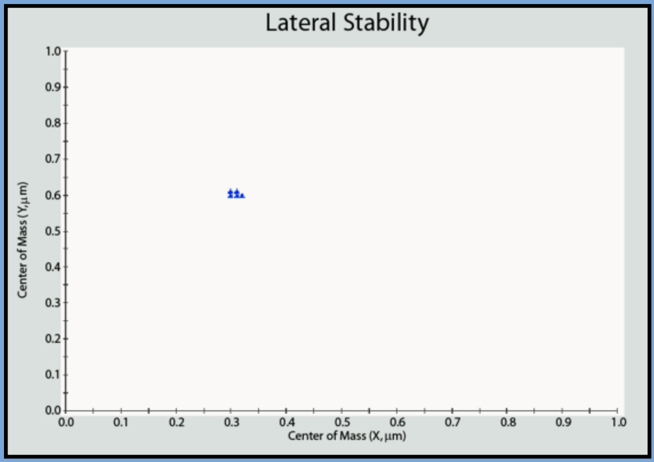
What does it take?

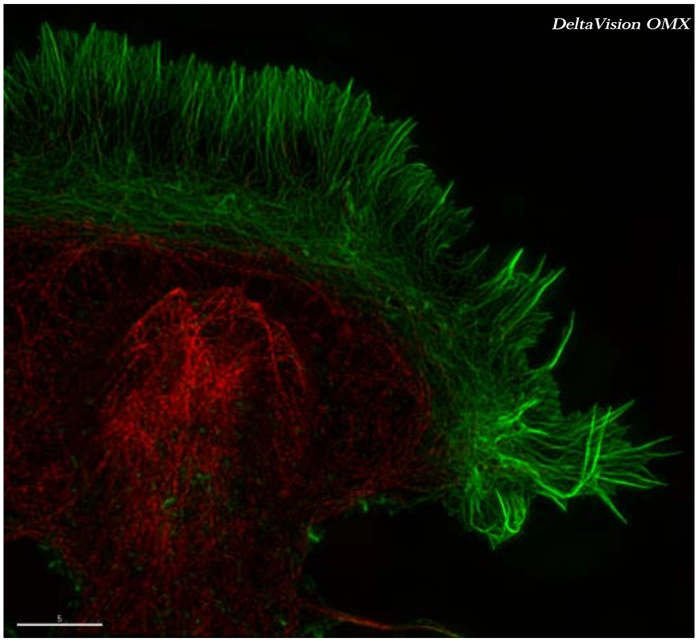
This level of spatial resolution plus axial and lateral stage stability can only be achieved with careful engineering and validation. Beginning with meticulous system design, each component is selected to deliver the highest performance possible.

- API certified highest quality PSF objective lens
- Custom high-performance reverse light-path beam splitters
- Custom detectors
- Custom laser designs
- Proprietary motion control
- Proprietary timing control
- Proprietary light scrambling methods
- Proprietary light structure generation
- Fully optimized microscopy chamber
- Fully integrated laser table
- Integrated electronics
- Ergonomic workstation design





The unique stage design of the DeltaVision OMX renders the best stability and repeatability available (lateral and axial stability images



Hippocampal neurons - Image courtesy of Eric Dent, University of Wisconsin Madison

DeltaVision OMX Specifications *(subject to change without notice)*

General Information

- Three-dimensional Imaging and Analysis System
- High spatial resolution beyond the diffraction limit
  - ~100 nm lateral (depending on wavelength and optics)
  - ~200 nm axial (depending on wavelengths and optics)
- Up to four-channels per data set
- Super resolution beyond the cover slip using 3D-SIM Structured Illumination Microscopy (per technology inventors Sedat, Agard and Gustafsson at UCSF)
- Custom Applied Precision transilluminator with ultra-white LED
- Laser fluorescence illumination
- Image acquisition software
- Full suite of image processing and analysis tools
- Proprietary image reconstruction software
- 3-D Modeling and analysis
- Toolkit, calibration slides, alignment optics and immersion oil kits

Imaging Components

- Custom optics assembly with novel optical light path
- High precision, repeatable X, Y, Z stage
  - Absolute accuracy: +/- 0.02%
  - Repeatability: +/- 10 nm (Z), +/- 150 nm (X, Y)
  - Step resolution: 5 nm (Z), 10 nm (X, Y)
  - Maximum travel: 25 mm (X, Y, and Z)
- Novel Z-axis closed-loop piezo crystal drive
- Closed loop diffraction grating motion and rotary stage
- Fixed optics light path
- Integrated proprietary FPGA timing architecture
- Integrated motion control system
- Integrated camera control architecture
- Standard filter set (DAPI, Alexa488, Alexa594, Alexa642)
- 405 nm and 488 nm high power lasers
- High-speed excitation shutter for each laser (2 ms)
- Neutral Density filter wheel for each laser
- Novel widefield scrambling technology
- API certified Olympus Plan-Apo 60X, 1.42 NA PSF “A” quality objective lens for optimal 3D imaging performance
- Custom monochrome 20 MHz camera with Sony ICX285 ER progressive scan CCD

Optional Components

- Fluorescent protein filter set (CFP, YFP, EGFP, mCherry, DIC)
- 514 nm, 592.5 nm, 642 nm Lasers
- Custom monochrome 10 MHz camera with E2V back-thinned, frame-transfer, CCD camera upgrade package available

Image Processing and Analysis Software

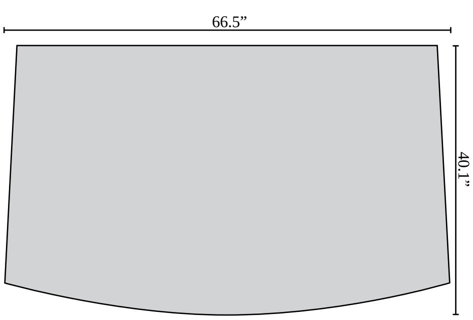
- 4-D Image Window (X, Y, Z, Channel)
- Multi-processor enabled software tools
- Multi-threaded image processing
  - SI Reconstruction
  - Volume rendering
- System-level processing queueing
  - SI Reconstruction
  - Volume rendering
  - Quick projections
  - TIFF file conversion
  - Movie conversion (AVI and Quicktime®)
- Orthogonal Viewer
- Arbitrary line profiles
- Co-localization measurements (2 methods)
- Data extraction tools
- Interactive volume rendering
- Fully network compatible
- Image output formats (mpg, tiff, psd, mov)
- 3D modeling from 2D image data volume
- Per channel image analysis:
  - Image rotation on X, Y or Z axis
  - Volume View
  - 2D and 3D measurements
  - Histogram with non-destructive min/max intensity adjustments
  - Numerical data inspection from region of interest with vexport
  - 3D data graph from region of interest

Ergonomic Design

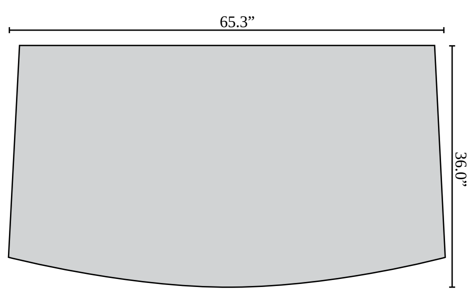
- Optics Module
  - Light tight enclosure
  - Vibration isolation (mechanical and acoustic)
  - Class 100 HEPA filtering with positive pressure
  - Integrated lighting
  - 3 ft x 4 ft x 6ft (90 cm x 120 cm x 180 cm)
- Laser Module
  - Laser safety interlocks
  - Rigid optical design
  - 2.5 ft x 4 ft x 4 ft (105 cm x 120 cm x 120 cm)
  - Integrated electronics rack
- Ergonomically designed workstation desk
  - 3 ft x 4ft x 2.5 ft (90 cm x 120 cm x 105 cm)
- Maximum system weight = 1500 lbs (680 kg)

Electrical/Environmental Specifications

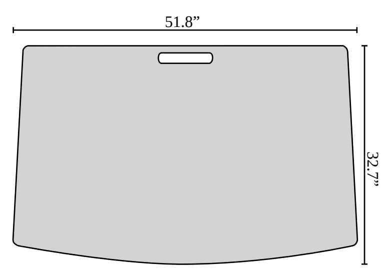
- Minimum Size: 8 ft x 16 ft x 8 ft high
- Dry, clean air or nitrogen lines:
  - 100 psi regulated for clean gun, de-ionized
  - 100 psi regulated for OMX table
- Room temperature maintained at 72° F (+/- 2° F over 4 hour period). System heat load ~ 5 kW.
- System power: 200-240 V ± 10%, 50 Hz ± 5%, 20 Amperes, single outlet located within 6 feet of laser/electronics cabinet
  - Additional power: 120 V± 10%, 60 Hz ± 5% or 200-240 V ± 10%, 50 Hz ± 5%
  - Installation category 2 (office power)
  - Total power ~3-5 kW
  - An Uninterruptible Power Source or power conditioner supplied by user is recommended



Optics Module Cabinet



Laser Module Cabinet



Workstation Table

Applied Precision, Inc
1040 12th Ave NW
Issaquah, WA 98027
Tel: 425.557.1000
www.appliedprecision.com