

# Vector3

# Total Internal Reflection Fluorescence and Photomanipulation Module



Vector3 is a motorized, spinning TIRF illuminator with three key imaging modalities: TIRF, photomanipulation and widefield epifluorescence. Intelligent beam steering and optical design allow for all three imaging modes to be combined in one compact device. Vector3 offers an expansive TIRF field of view (FN20) designed for modern sCMOS cameras. A motorized scan lens corrects for sample height variation and ensures ideal TIRF illumination and photomanipulation spot size across the visible spectrum.

# Massive Field of View

Vector3 is designed to fill a field of view matched to the ever-increasing sensor size of state-of-the-art sCMOS cameras. The resulting FN20 image space allows for TIRF imaging across 150µm (100x objective) to 238µm (63x objective). Photomanipulation regions can also be drawn anywhere in the image with consistent power across all regions. The large field of view increases sample sizes and reduces overall experimental time.



# Photomanipulation

Photomanipulation is a powerful tool for studying membrane dynamics, receptor and vesicle movement, and photo conversion of specific molecules. Vector3 shares the same galvo scanners between TIRF and photomanipulation, leading to near simultaneous photobleaching and photoactivation. A custom partial reflecting dichroic allows for switching between spinning disk imaging and photomanipulation experiments in milliseconds – no filter cube swap required.









**PHOTOBLEACHING** Mammalian cells labeled with LysoBrite Red showing photobleaching ROIs (dashed circles) over time.

# Marianas Multimodal Platform

Combine Vector3 with spinning disk confocal, holography, ablation, and lightsheet systems for incredible flexibility on one system. Seamlessly control and switch imaging modes via SlideBook. LaserStack with FiberSwitcher enables millisecond switching of up to 7 lasers across modalities.





#### SLIDEBOOK ANALYSIS

SlideBook offers extensive FRAP curve analysis tools for easy graph generation and intensity quantification.

# Total Internal Reflection Fluorescence

TIRF microscopy offers a thin excitation volume of 100-150nm leading to high signal-to-noise imaging. TIRF is achieved when the excitation angle of the laser is increased past the critical angle dictated by the refractive index of glass and specimen. The resulting evanescent wave decays rapidly as it moves away from the coverslip and allows for illumination just above the glass. This thin illumination volume is ideal for samples that are difficult to resolve in widefield or even confocal imaging. The relatively low excitation power of TIRF microscopy allows for gentle, long-term imaging.



Comparison between TIRF and widefield epi-fluorescence. When in TIRF, only the bottom of the cells are visualized, leading to a large increase in signal to noise of the membrane and organelles interacting with the membrane.

Traditional TIRF systems use a fixed illumination spot to create the evanescent field. This leads to poor illumination with shadows and polarization artifacts across the field of view. Vector3 uses two fast galvo mirrors to spin the illumination spot, evening out the excitation pattern by averaging all of the positions together during a single camera exposure. The result is a clean, even field of illumination that allows for more consistent, quantitative analysis of each experiment.



#### **HILO Microscopy**

Vector3 is also designed for highly inclined and laminated optical sheet (HILO) microscopy. HILO illumination (shown in orange) is achieved just before reaching the critical angle required for TIRF evanescent wave illumination (shown in blue). HILO offers high signal-to-noise with greater penetration for imaging beyond the membrane to the nucleus and through the cell body. Combined with LaserStack and a selection of high and low-powered lasers, Vector3 is a versatile single-molecule imaging platform in both TIRF and HILO modalities.



#### Super Resolution Imaging

Vector3 is a capable super-resolution imaging system via single molecule localization microscopy (SMLM) techniques. Laser powers up to 2W ensure high laser power density across the sample in both TIRF and HILO modalities. SlideBook is compatible with established analysis pipelines for quick data processing.





TIRF image (left) and thunderSTORM reconstruction (right) of Alexa647labeled microtubules in COS7 cells. Courtesy of Dr. Chris de Graffenried, Brown University.

#### Vector2 TIRF

Vector2 TIRF is optimized for use with 512 x 512 EMCCDs (FN12) for sensitive TIRF imaging of biophysical experiments. The modular nature of Vector2 TIRF supports the documentation port on most research-grade inverted microscope frames for easy integration with existing systems.



SlideBook software supports research microscopy through the entire experimental process. By managing everything from instrument control to image processing and data analysis, SlideBook allows scientists to focus on investigation rather than instrumentation. SlideBook controls hundreds of instruments in and around the microscope from dozens of manufacturers enabling researchers to integrate their preferred components and upgrade to the latest devices once available.

#### NVIDIA CUDA GPU Acceleration

GPU acceleration of computationally-intensive operations such as deconvolution

#### User-Selectable App Appearance

Select a color scheme from dozens of options Switch on-the-fly from dark to light themes

#### SlideBook Open File Format

Directory-based open file format for big data and high performance computing applications

#### Volume Rendering

3D and 4D volume view visualization tools support a userspecified bounding box and a storyboard interface where multiple perspectives can be assembled into a single movie



### Capabilities

### Capture

Control hundreds of devices including microscopes, stages, lasers, wheels, piezos, scanners, shutters and much more.

# Scripting

Macro scripting for capture and analysis enhances the flexibility and power available to users.

### 🕢 Vie

Visualize data through any numbers of portals, from single images to z-stacks, time lapse, color channels and 4D views.

### Analyze

Analyze images and extract statistical data via a wide variety of algorithms while maintaining original data integrity.

### Communicate

Present and export data easily as 16-bit TIFFs, 3D movies, graphs or spreadsheets. Data is directly portable to MATLAB and Excel and adheres to Open Microscopy Environment (OME) standards.

# 📣 MATLAB

Through hierarchical and conditional capture, user-supplied MATLAB programs can control experimental workflows.

# 📢 Aivia

Aivia is an innovative and complete 2D-to-5D image visualization, analysis and interpretation platform with artificial intelligence-guided image analysis.

### System Capture Consoles

Consoles are a single easy-to-use window featuring all frequent controls and status displays

### Multiwell and Montage

Streamlined multiwell interface Montaging with a variety of methods

### 3D Capture Status

Volumetric projection during 4D capture supported across all instruments



### Partners

## Microvolution

Microvolution® software delivers nearly instantaneous deconvolution by combining intelligent software programming with the power of a GPU.

# Dell

The latest high-power computer workstations control all microscope hardware and enable high-speed processing, segmentation and volume rendering of terabyte (TB) datasets.

# Application Data



CELL DYNAMICS Lifeact GFP in mammalian cells.





CELL MORPHOLOGY Courtesy of Dr. Chris Bakal and Oliver Inge at the Institute of Cancer Research in London.





# Application Data





Paxillin-GFP and Actin-Mars expressed in mammalian cells migrating across the coverslip.



**CYTOSKELETAL DYNAMICS** GFP-cax expressed in RPE-1 cells.



#### IN VITRO MICROTUBULES

Alexa Fluor 488 labeled microtubules on Alexa Fluor 647 labeled "seeds." Courtesy of Dr. Margot Quinlan, University of California Los Angeles.



VESICLE TRAFFICKING Mammalian cells labled with Lysobrite Red imaged in TIRF (white) and widefield (magenta).





GROWING MICROTUBULES EB3 GFP expressed in mammalian cells.



#### **EXOCYTOSIS** Mammalian cells labled with Lysobrite Red.

# Support and Maintenance

A variety of software and equipment support levels help keep systems running well for years. A Software Support Agreement allows labs to run the latest version of SlideBook with new acquisition and analysis features. It includes direct access to 3i staff via email, phone and video chat. A System Maintenance Agreement adds an annual preventative maintenance visit, 3i service visits and 3i coordination of any repairs, although repair and replacement parts are not included. A System Extended Warranty adds full coverage for repairs and replacement parts. Additionally, 3i application scientists may provide in-person and webinar-based application training.

	Software Maintenance	System Maintenance	System Warranty
Phone, Email and Video Chat Support		Ĵ	o
SlideBook Software Releases	Radio:		Gadico.
Service Visits and Annual PM Visit			
Repairs Coordinated by 3i		×	×
Application Training   In-Person or Online			
Full Warranty Coverage of all System Hardware			

#### **BUILT BY SCIENTISTS FOR SCIENTISTS**

3i designs and manufactures technologies for living cell, live cell, and intravital fluorescence microscopy including superresolution, computer-generated holography, spinning disk confocal, multi-photon and lightsheet. SlideBook software manages everything from instrument control to image capture, processing and data analysis. 3i was established in 1995 by a group of cell biologists, neuroscientists, and computer scientists to provide advanced multi-dimensional microscopy platforms that are intuitive to use, modular in design, and meet the evolving needs of investigators in the biological research community.





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