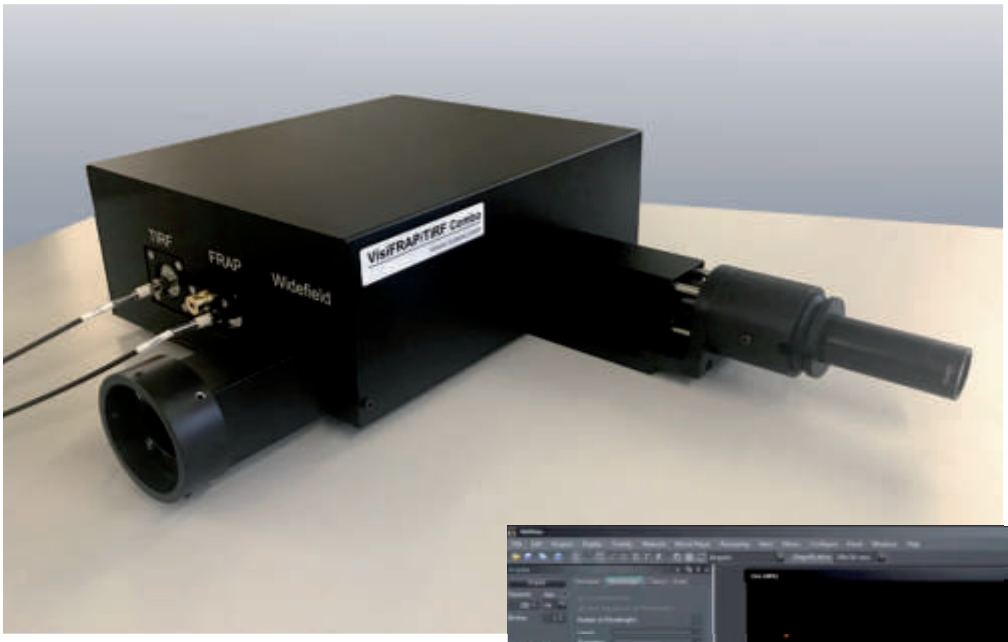
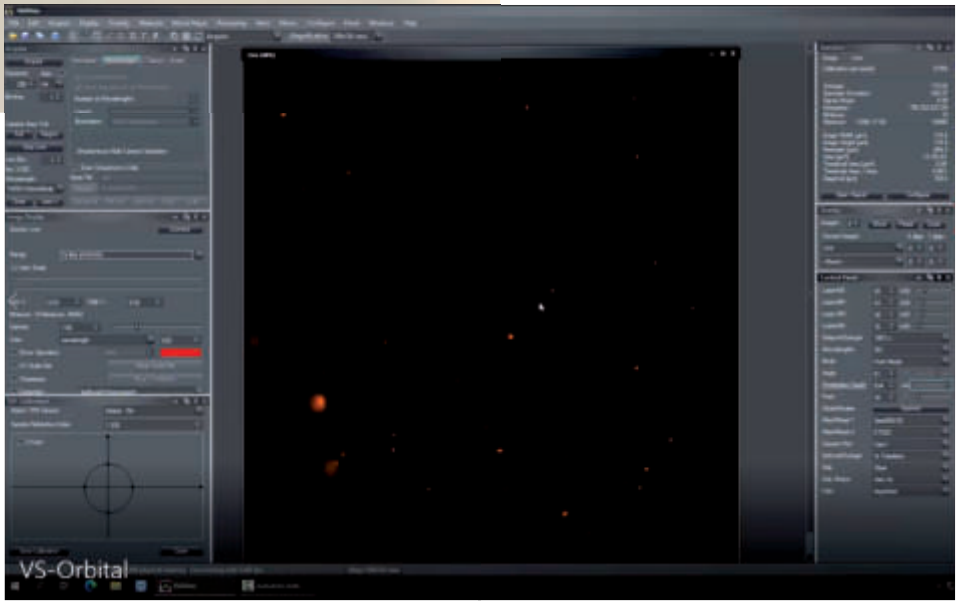
ORBITAL Ring-TIRF Technology for Total Internal Reflection Fluorescence

The Total Internal Reflection Fluorescence (TIRF) technique is the ideal method for observations of cells close the coverslip surface. By total reflection of the exciting light (typically laser) at the coverslip / medium interface, the fluorescence excitation is limited to a very thin space in the vicinity of the glass surface. The resulting fluorescence images exhibit extremely high contrast and resolution with the possibility of real-time imaging of cell membrane, actin filament behavior or single molecule tracking.

ORBITAL-200
Ring-TIRF/ FRAP Technology

Orbital 200 Series
TIRF - FRAP - Widefield





ORBITAL Ring-TIRF Technology

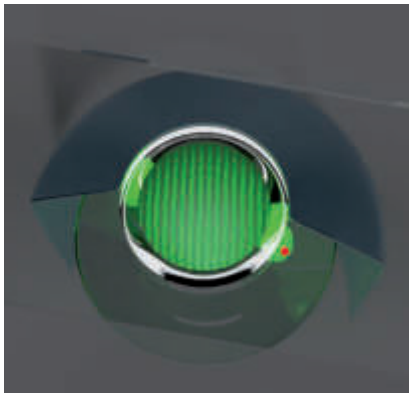
Orbital Ring-TIRF Technology

VisiTIRF-ORBITAL is a compact and powerful high speed 2D galvo driven spinning Ring-TIRF laser illumination system. It offers large and evenly illuminated field of view to enable applications such as single molecule tracking or localization super-resolution microscopy imaging. Full 360 degree positioning by free circular diameter or elliptical trajectory at the back focal plane of the high aperture TIRF objective offers illumination with minimum fringes or shading gradients.

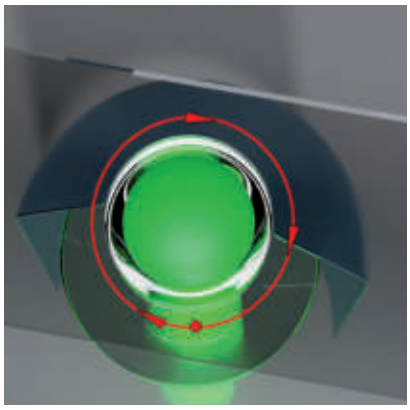
Spinning illumination for even TIRF imaging:

The fully 360 degree spinning of the laser excitation light at the back focal plane of the objective, generates an uniform imaging of samples without shadowing or artifacts. With the tratitional single point illumination an interference pattern are disturbing the quality of the image.

Objective back focal plane illumination



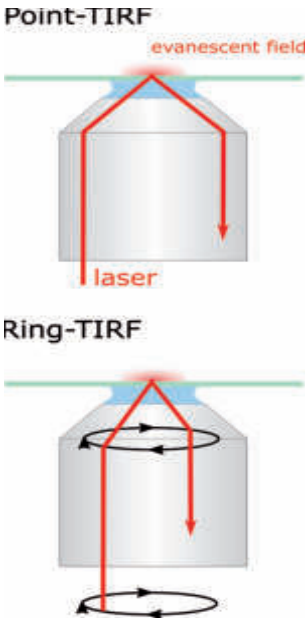
left image with point laser



right image: ring laser illumination

Modes:

- free Single Point TIRF Mode
- free RingTIRF Mode
- Hilo Mode
- Oblique illumination



Features and Benefits:

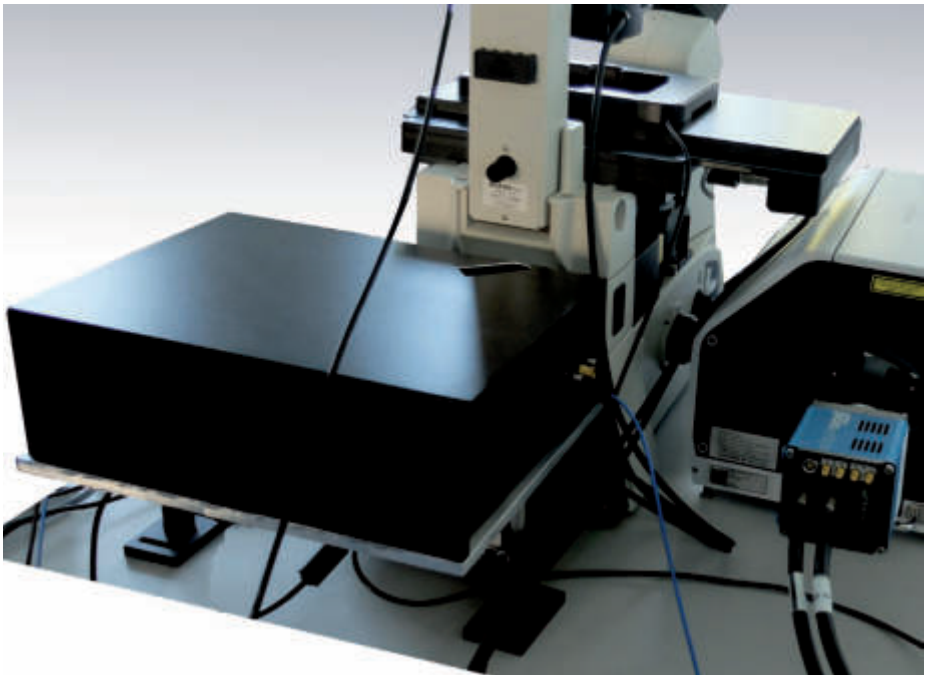
- based of 2D Galvo Scanner
- laser light focalized in the back focal plane of the objective
- even illumination without interference fringes
- fast switching of TIRF illumination angle
- Epi widefield coupling by LC liquid fiber
- fully 360-degree positioning by free circular diameter or elliptical trajectory
- TIRF angle calibration with 4 or 5 points
- calibration routines for optimum penetration depth
- TimeSharing Mode for multiple camera or DualView

„Orbital-200“ modulare Ring-TIRF System with 2D-FRAP Option

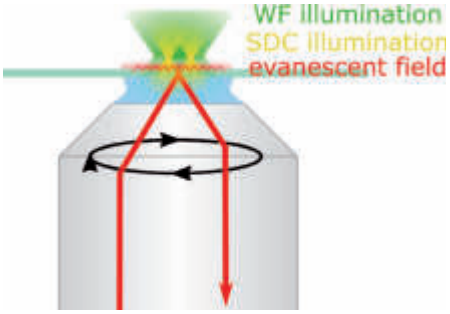
The Orbtial-200 system is based on the Orbital-100 Ring-TIRF Technology. It offers the option of 2D FRAP extension for Fluorescence Recovery, Photo-Bleaching or Photo-Activation application. A laser beam is used to perform photo bleaching or activation in user defined free selectable regions, lines or dots. The 2D-galvanometer FRAP scanner can be combined with the 2D-galvanometer TIRF scanner for comlex application.

ORBITAL-200

Ring-TIRF
FRAP
Laser
illumination



ORBITAL-200 on Nikon Ti microscope with CSU-W1-T2 Confocal



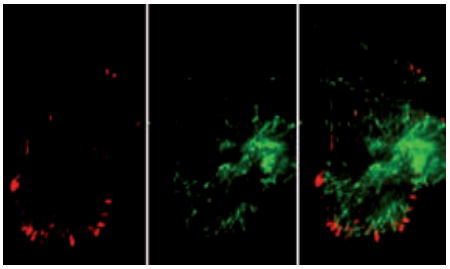
TIRF creates an evenescent wave, which is about 100nm thick above the coverslip.

Compact Design

A main factor driving the development behind this system was the lack of a compact and easy to use TIRF/FRAP combination, which often required using multi-level microscopes or complex beam combination optics. In an effort to avoid the trade-offs that come with these outdated solutions, the ORBITAL TIRF/FRAP combination became on of the most compact and versatile solution available on the market. In addition, its modular design allows us to support a wide range of microscope platforms, even legacy systems.

Widefield - FRAP – TIRF Illumination at once

Each optical input for FRAP and TIRF is coupled by a single mode optical fiber with FC-input to the Visitron laser merge system with multiple outputs. The widefield illumination input is coupled via an additional LLG-fiber typically to a LED light source.



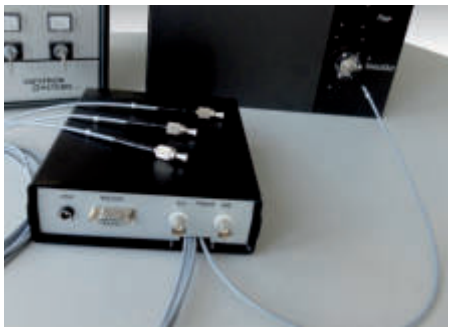
EPIchEB3gZyx labeled cells
Image courtesy of Professor Small, IMBA Vienne.

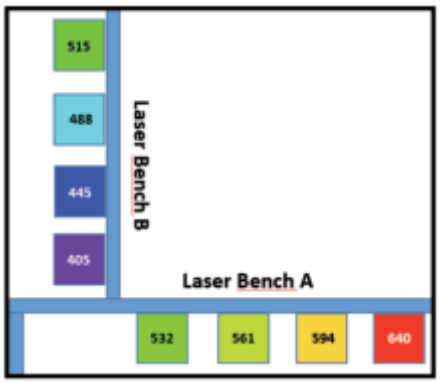
ORBITAL Technology

VS-LMS Laser Merge System

VS-LMS Flexible Multiple Laser Engine with highly stable Laser Outputs and Motorized Alignment Option

The new generation of Visitron Systems VS-LMS Laser Merge Systems is now available in a very compact design with up to 8 laser lines, three FC fiber outputs and an optional motorized alignment function. The unique design combines the beams of up to eight diode or solid state laser to a single collinear laser beam. This beam can be channeled into three different outputs for simultaneous laser application like Confocal / FRAP or TIRF.





Wavelength* (nm)	Maximum Power Rating* (mW)
405	300
445	100
488	200
515	100
532	150
561	200
594	150
640	200

Features and Benefits:

- Multi-line laser source including up to 8 lasers
- Flexible selection of diode laser modules
- All solid-state lasers for high stability and lifetime
- FC-coupling design with focus correction
- Thermally managed system
- Port Switcher with up to 3 output ports e.g. VisiScope Confocal, VisiFRAP and VisiTIRF

VS-AOM Acousto Optical Modulator - High Speed Optical Shutter

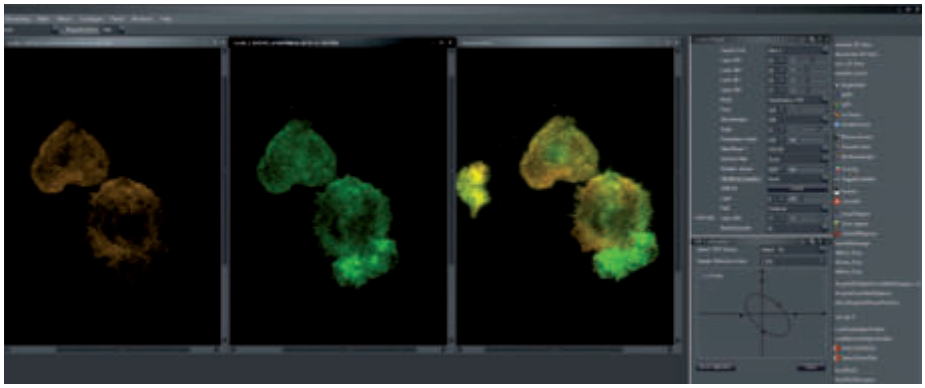
An acousto-optic-modulator (AOM) is a device which can be used for controlling the power, frequency or spatial direction of a laser beam with an electrical drive signal. It is based on the acousto-optic effect, i.e. the modification of the refractive index by the oscillating mechanical pressure of a sound wave. The AOM is used in at the VS-LMS for high speed switching and intensity control if a solid state laser is used.

VisiView Orbital Support and Features

VisiView® is a high performance imaging software for Bio-Medical applications. The software is designed as an integrated imaging software which includes comprehensive microscope control, unexpected control of peripheral device, image acquisition, analysis and documentation. Its multitasking ability supports realtime image handling and up to 6D multidimensional acquisition.

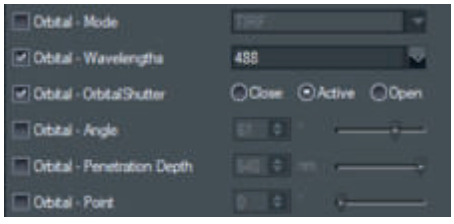
ORBITAL Technology

VisiView® Software Support



ORBITAL Setup Modes

The Center Beam Mode moves the laser to the center of the calibrated ellipse. This is a good control for your TIRF alignment and proper beam focusing. It can be used for basic widefield laser illumination.
In Widefield/HILO mode the laser spins on a calibrated ellipse but at low incident angle. This offers a more homogenous laser WF illumination than the center beam Mode, which is strongly affected by interference.
The Point Mode simulates a classical TIRF system but the angle and the direction of the laser can be freely adjusted using the angle slider and the point slider component of the ORBITAL device.
In TIRF mode the laser spins on a calibrated ellipse beyond the critical angle which is needed for total reflection. The TIRF mode removes the out-of focus blur and increases signal to noise drastically. In TIRF mode you can directly and precisely control the penetration depth of the evanescent field with a separate slider.



ORBITAL Synchronisation

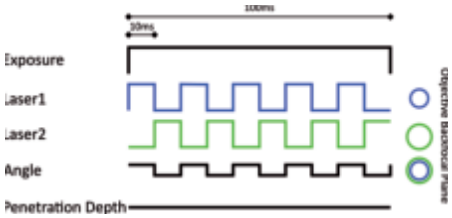
If using an ORBITAL, not only the elliptical laser deflection is externally controlled by the ORBITAL controller, but also the laser shuttering, which allows precise synchronisation of laser and angle. Further the stable image quality is assured by synchronizing the laser rotation speed with camera exposure. If exposure times are too low, the system switches to arc scanning instead of scanning full ellipses.

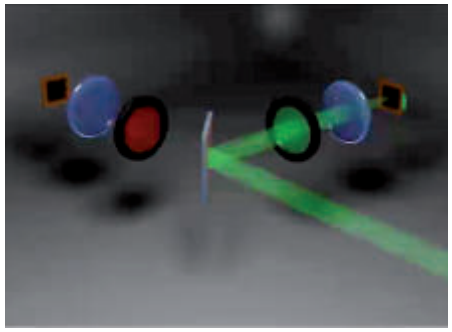
ORBITAL Technology

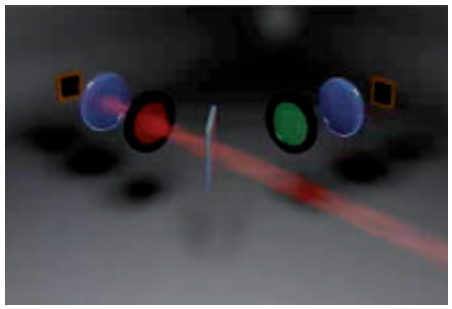
VisiView® Software Support

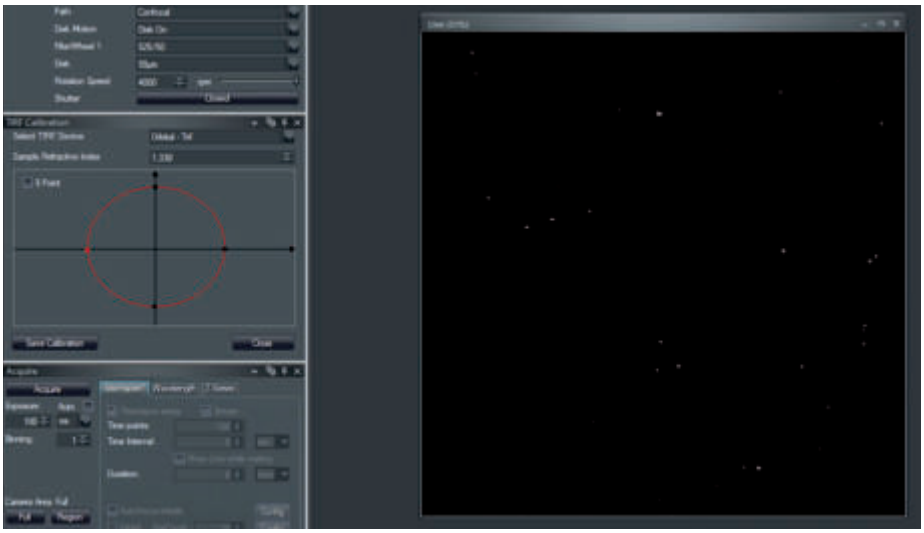
VisiView ORBITAL Support and Features

ORBITAL calibration:
By default the calibration is specific for the magnification and illumination you have chosen and will be switched automatically. For the internal calculations, it is required that a magnification with a suitable objective is selected. Make sure you have a TIRF objective and that the magnification is configured with the appropriate refractive index and numerical aperture.









VisiView ORBITAL TIRF angle calibration by 4 or 5 points
with 200nm TetraSpec beads

ORBITAL Timesharing Mode

Usually simultaneous multi-wavelength TIRF imaging suffers from a variation of the penetration depth which is like optical refraction wavelength dependent. This means in order to create equal penetration depths distinct angles are required. But this is not possible if the lasers are switched on at the same time.
The Time-Sharing Mode circumvents this limitation and makes simultaneous multi-wavelength TIRF imaging at equal penetration depth possible.
If set to Time-Sharing Mode the ORBITAL will switch and change the active laser and the incident angle after each elliptical sweep. The time for each elliptical sweep is basically calculated by:

A÷floor A/10ms where as A=(Exposure time)/(number of Wavelengths)

Thus the ellipses for all wavelengths are done exactly with same exposure time and at complete trajectories but different incident angles. The required angles will be calculated from the selected wavelengths automatically.

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