ORBITAL Ring-TIRF Technology for Total Internal Reflection Fluorescence

The Visitron Systems Orbital family technology is designed to implement the ring-Total Internal Reflection Fluorescence microscopy (TIRF) technology via a circular or elliptical beam rotation at the back focal plane (BFP) of the TIRF objective. This technique is superior to the typical Point-TIRF approach as it offers an illumination with minimal interference fringes and shadowing effects. However, the typical Point-TIRF and Ring-TIRF technologies generally use Gaussian beams to achieve optimal focusing of the excitation light at the sample plane. The profile of the gaussian beam, however, has a drawback on the excitation uniformity and intensity profile.

Preliminary 08-2024

Technical Note:

ORBITAL Ring-TIRF Technology

ORBITAL-110 IsoTIRF

Ring-TIRF combined with VS-Homogenizer Technology

The new Visitron VS-Orbital-110 IsoTIRF tackles these limitations by providing a flat intensity profile whilst maintaining an optimal focusing of the laser beam at the sample plane. This new design was used to enhance the uniformity of laser illumination for the Ring-TIRF technology offering an even evanescent field illumination of large areas and allowing for high-sensitivity imaging of living cells, without the need for mathematical shading correction.

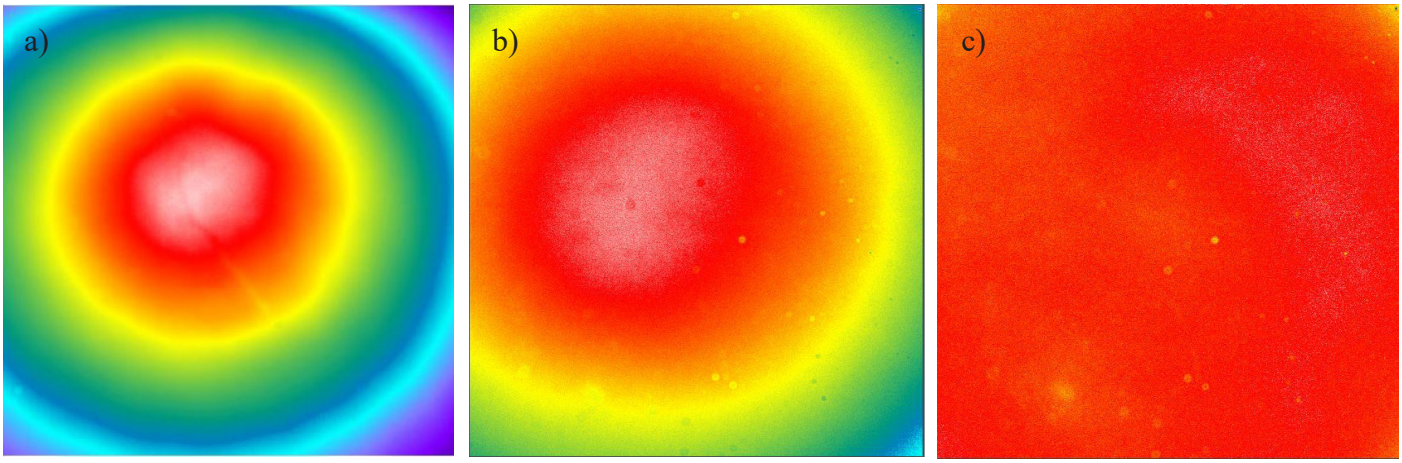


Figure 1: We offering two different input optics either left side a) with higher laser throughput b) with better uniformitiy. The used sample was a text marker which is tested via 488 nm laser excitation and the roll-off values defining the homogenity. Used camera was sCMOS Prime BSI with 13.3mm x 13.3mm, 18.8mm diagonal.

a) about 80%; b) about 40% and c) with new ISO homogenizer optics about 5%.

ORBITAL Ring-TIRF Technology

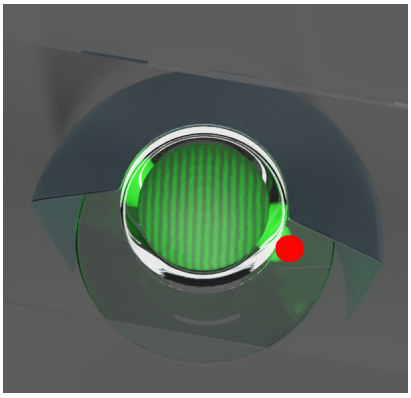
ORBITAL Ring-TIRF Technology

The VisiTIRF-ORBITAL is a compact and powerful high speed 2D galvo driven spinning Ring-TIRF laser illumination system. It offers a large and evenly illuminated field of view to enable applications such as single molecule tracking or SMLM - Single Molecule Localisation Microscopy for super-resolution 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 minimal fringes or shading gradients.

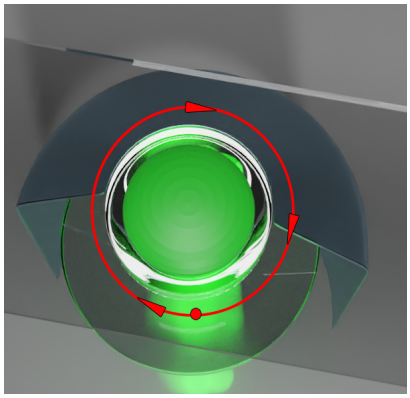
Spinning illumination for even TIRF imaging:

The 360 degree spinning of the laser excitation light at the back focal plane of the objective allows for an uniform imaging of samples without shadowing or artifacts. With the traditional single point illumination an interference pattern is often disturbing the quality of the image.

focal plane illumination



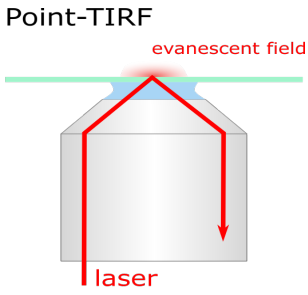
left image with point laser illumination and interference pattern

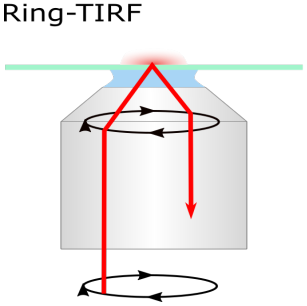


right image with ring laser illumination without interference pattern

Modes:

- free Single Point TIRF Mode
- free RingTIRF Mode
- HILO Mode
- oblique illumination





Features and Benefits:

- based on 2D galvo scanner
- laser light focused in the back focal plane of the objective
- uniform illumination without interference fringes
- fast switching of TIRF illumination angle
- Epi widefield coupling by Liquid Light Guide
- fully 360-degree positioning by free circular diameter or elliptical trajectory
- TIRF angle calibration with 4 or 5 points
- calibration routines for penetration depth control and equalization
- TimeSharing Mode for multiple camera or SplitView operation

ORBITAL-110 IsoTIRF Upgradeable

The simplest and most compact Orbital VS-Orbital-110 Iso-TIRF system is the upgraded Orbital-100 that comprises the ring-TIRF technology alongside an additional light pathway with an LED or white illumination source for epifluorescence / wide-field. The standard TIRF illumination sources are single-mode laser diodes (for the visible spectrum, i.e. 400-700 nm) coupled into a polarization maintaining fiber. This technology, however, can be implemented to the different Orbital family systems like the Orbital 200, 500 and 600.

ORBITAL-110

Ring-TIRF Laser Illumination

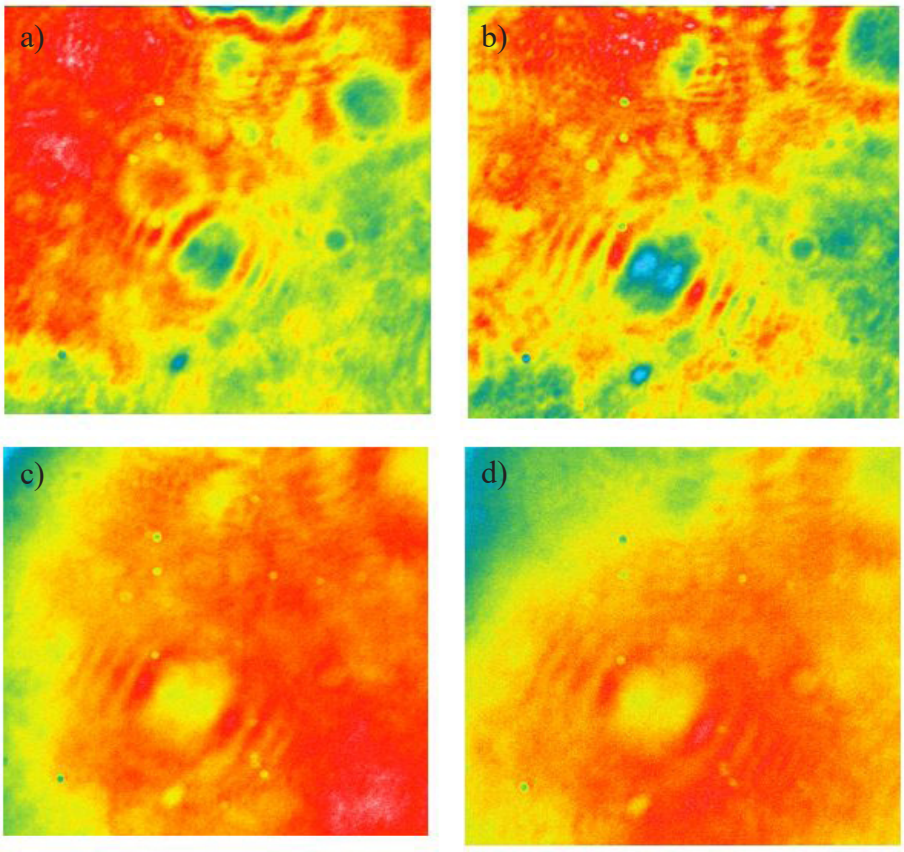
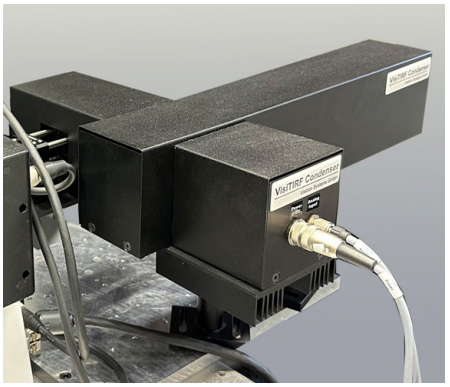
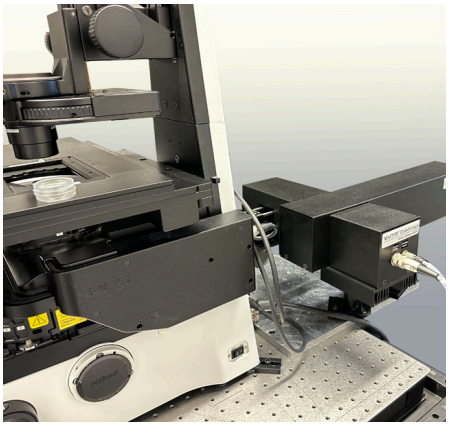


Figure 2: Evaluation measurement of the VS-Orbiatl-110 ISOTirf technology by testing the homogeneity after using different excitation wavelengths. The sample used was a text marker which was tested via 405 nm (a), 488 nm (b), 561 nm (c), and 640 nm (d) laser excitations, respectively. The roll-off values defining the homogeneity after the diverse excitation wavelengths are 11%, 6%, 11%, and 15% for a), b), c), and d), respectively. Used camera was sCMOS Prime BSI with 13.3mm x 13.3mm, 18.8mm diagonal



new compact ORBITAL-110 design



Applications:

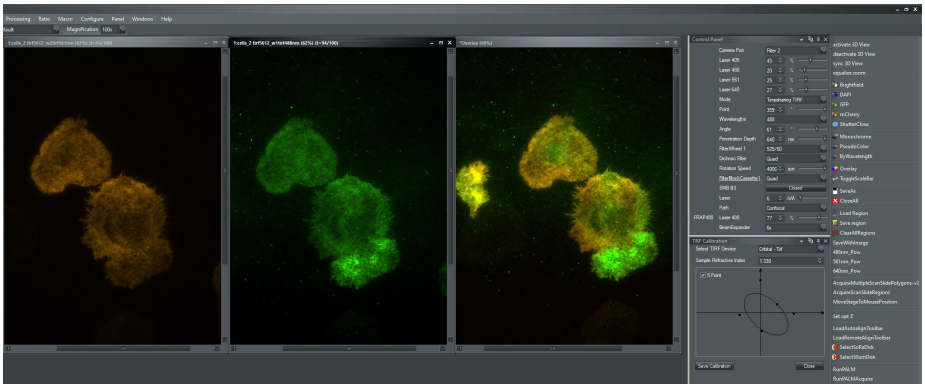
- single molecule localisation & tracking
- kinetic studies of single molecule interactions
- kinetic studies of protein
- super-resolution techniques e.g. PALM or STORM

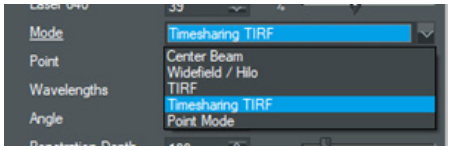
ORBITAL Technology

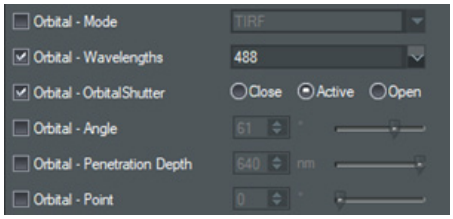
VisiView® Software Support

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, control of a vast number of peripheral devices, image acquisition and analysis. Its multitasking ability supports realtime image handling and up to 6D multidimensional acquisition. The ORBITAL setup, calibration and control is seamlessly integrated into the VisiView capture mode. In the TIRF illumination configuration, the user can setup five different TIRF modes. Which are Center Beam, Widefield/HILO, RingTIRF, Timesharing TIRF and Point TIRF.







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.

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