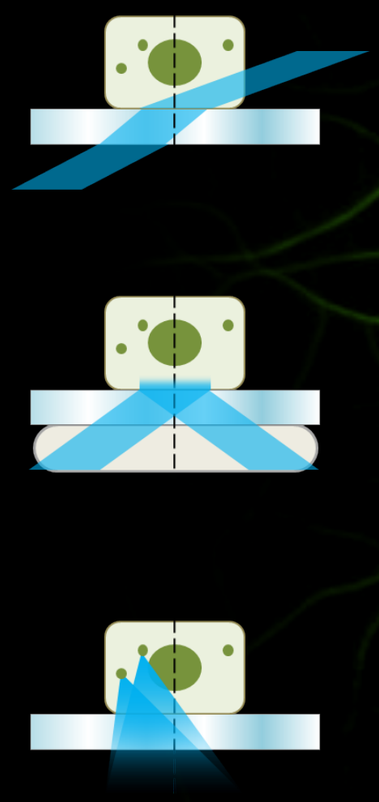


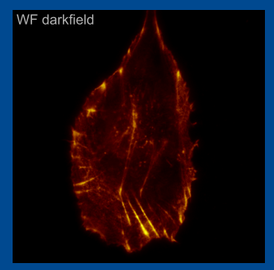
GATACA SYSTEMS

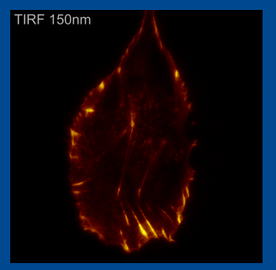
iLas²

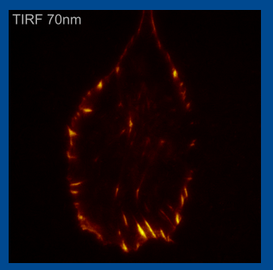
Dual Laser illuminator for TIRF microscopy and Simultaneous Targeted Laser Action

Gataca systems
21/23 rue Aristide Briand 92170 Vanves, France
Tel: +33 141906740

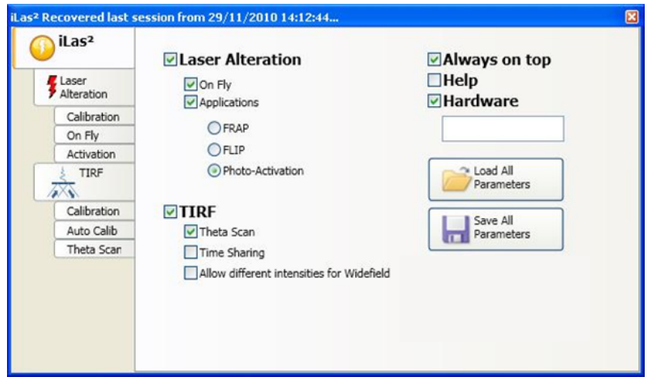
www.gataca-systems.com



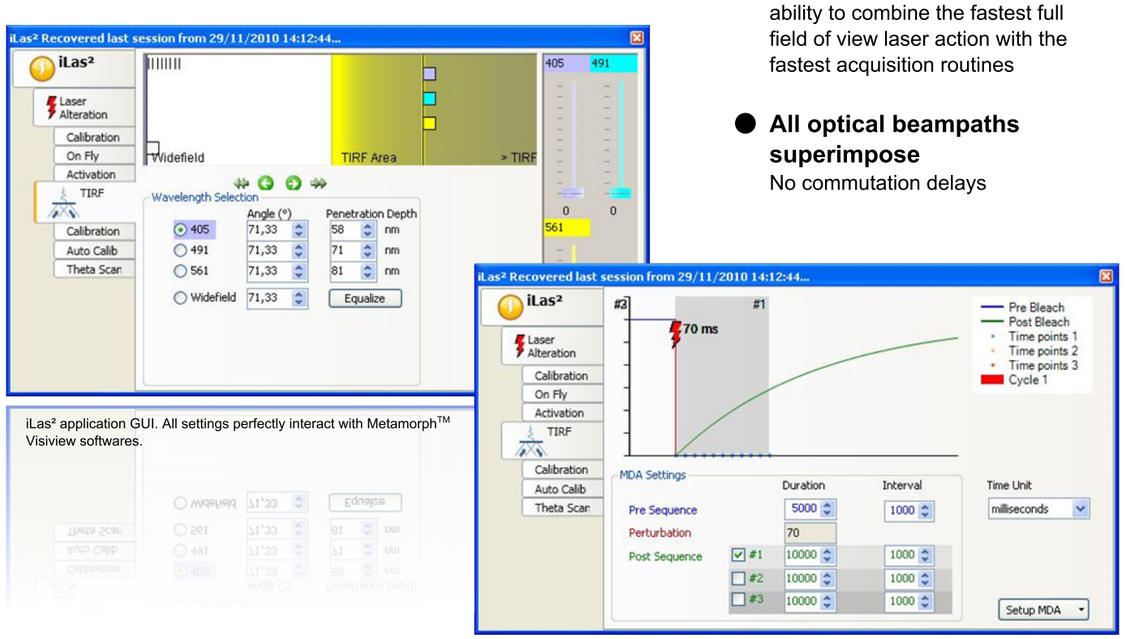




The **iLas²** system is a unique **multi-application device** that offers **complete control** over any other laser illumination. It provides researchers the ability to **manage and modify** the position and focalization of laser light in **real time**.



iLas² platform set up menu. Choose from a large range of applications that can be combined to carry out simple to very complex experiments. iLas² is known for its ability to develop imaging platforms that meet the criteria for a multiple user model.



iLas² application GUI. All settings perfectly interact with Metamorph™ Visiview softwares.

- **Uniform illumination TIRF**
  with multiwavelength controls and penetration depths
- **Calibrated angle and penetration depth**
  take into account the wavelength
- **Uniform wide-field illumination**
  laser illumination with limited background signal : Dark field fluorescence
- **Close to coverslip optical sectioning**
  Oblique illumination
- **FRAP/Photoactivation**
  iLas is known for providing the ability to combine the fastest full field of view laser action with the fastest acquisition routines
- **All optical beampaths superimpose**
  No commutation delays

Optical

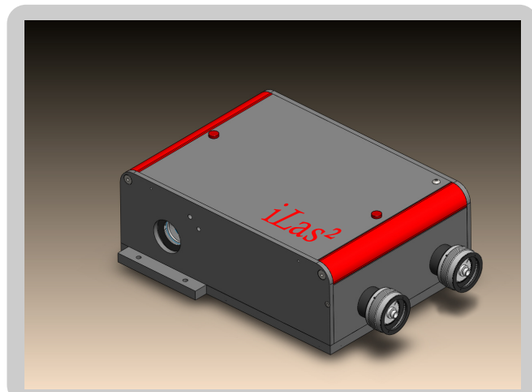
Wavelength range	375-650nm
Connectivity	Illumination port (see compatibly list below)
Fiber connection	Singlemode PM FC/APC
Objectives	TIRF objectives
Laser class	3B

TIRF Control

Intensity modulation	0-100% (200 steps)
Response time	< 1 ms
External trigger	yes
Angular resolution	0.3°

Compatibility

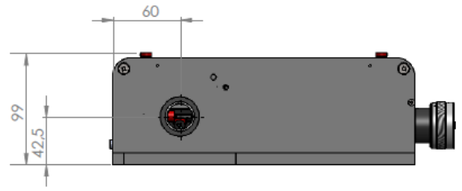
LEICA	DMI serie
NIKON	TI serie
OLYMPUS	IX1 / IX3 series
ZEISS	Axio Observer / Axiovert 200

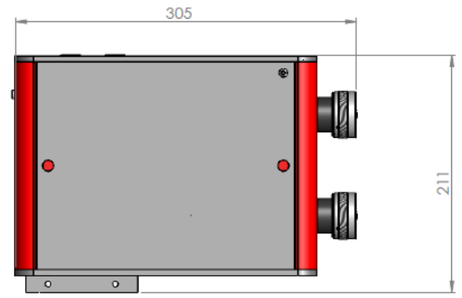


FRAP/PA Control

Intensity modulation	0-100% (200 steps)
Scanning speed	20 000 Hz
External mudulation	yes
Response time	< 1 ms

Dimensions





For more informations on iLas²:
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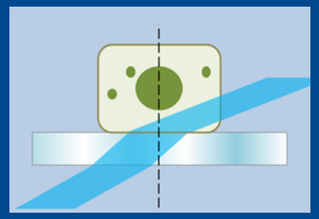


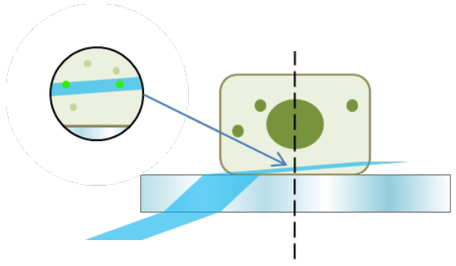
Applications

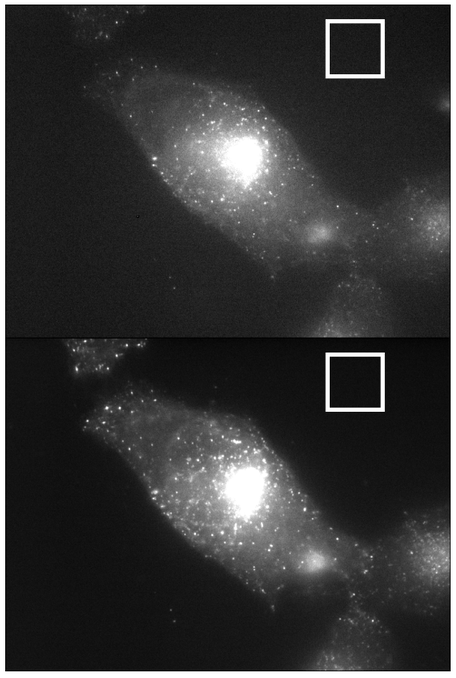
The iLas² uses its fast galvo driven TIRF to improve image quality and to enable demanding acquisition protocols.

Dark Field illumination / Oblique illumination sectioning

- **Lower backgroung**
- **Lower illumination needed**
- **Close to coverslip optical sectioning**
- **No need for wide field light source**

In addition to other capabilities, iLas² enables users to conduct wide-field acquisition taking advantage of a tilted illumination to lower background blur that results from out-of focus planes and to enhance the excitation illumination (Dark field laser illumination). As a result, users maintain image quality and achieve less excitation power with less observational bleaching or faster acquisition rates. The oblique illumination sectioning is the extension of the dark field laser illumination. For high incident angles but smaller than the critical angle, starting the TIRF domain, the angle of the excitation beam going through the sample is so high that the illuminated thickness is very thin (around 2µm).





Wide-field images of living M10 cells, expressing YFP-Langerine (B.Cinquin & J.Salamero, Institut Curie, Paris). Left image has been illuminated with perpendicular laser illumination. Right image has been illuminated with 50° tilted illumination using the same power and acquisition settings. Background went down from 157 to 76 gray levels (white square region).

Single molecule (ie PALM, STORM, ...)

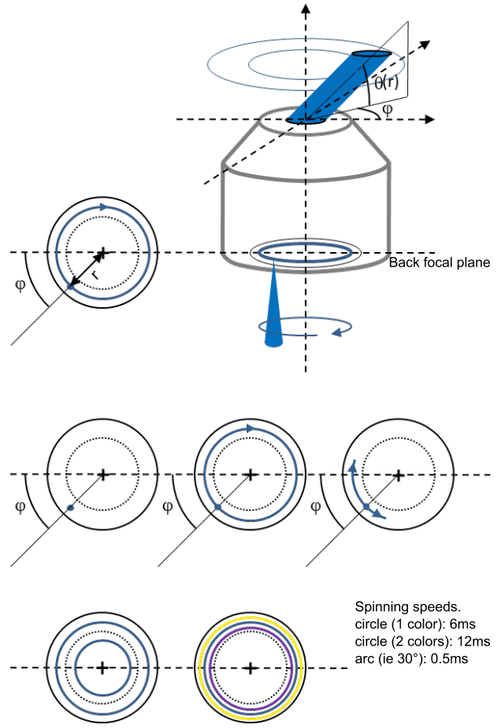
- **TIRF or WF capabilities**
- **Lower background for better event detection**
- **Remove artifacts due to field non uniformity**

Single molecule detection and tracking are very demanding techniques. Both require high performance imaging capabilities and premium optical quality at the excitation and at the emission. iLas² provides the ability to produce wide-field laser illumination (either wide-field, oblique or TIRF) while it significantly improves the illumination uniformity. Thus, the probalitity to excite and to detect are not modulated by random fringe patterns and artifacts are avoided on high resolution reconstructed images.

- **Uniform illumination TIRF**
  When a laser beam is focussed at the back of an objective and spins to describe a circle, each point of that circle creates a parallel beam which has the same incidence angle onto the coverslip. Thus in TIRF and for a given wavelength, the evanescent wave resulting from each spot has the same penetration depth. However, interference induced patterns depend on the azimuth of the beam. Being able to spin the Laser beam very rapidly during the exposure time of the camera will blur uniformities such as fringes or ring patterns.

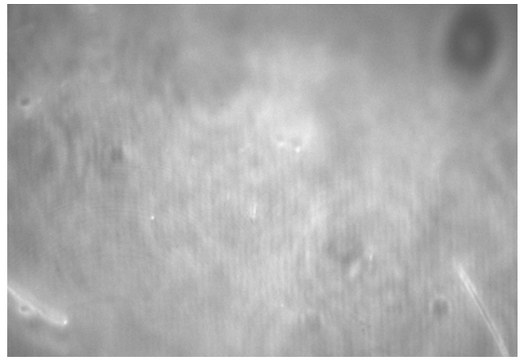
- **Ultra fast incidence angle / TIRF penetration motorization**
  The iLas² galvonometer based motorization enables changing the TIRF penetration depth in less than a millisecond, making it compatible with "overlap" streaming acquisition. Even complex multicolor Widefield/TIRF experiments can be carried out.

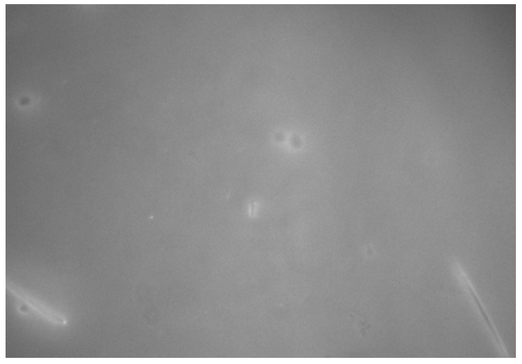
- **Multiple wavelengths / Wavelength correction**
  The fast motorization can be used to correct the penetration depth for its wavelength dependency. Advanced acquisition functions are also available to image simultaneously several channels even at different penetration depths.

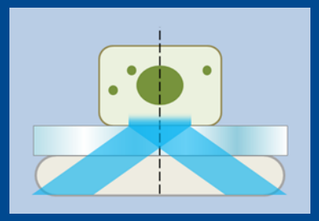


Spinning speeds.
circle (1 color): 6ms
circle (2 colors): 12ms
arc (ie 30°): 0.5ms

Images of a fluorescent layer made with TIRF illumination. In stationary mode (left), diffraction rings and fringes of various frequencies can be observed. In "spinning" mode those modulations disappear.



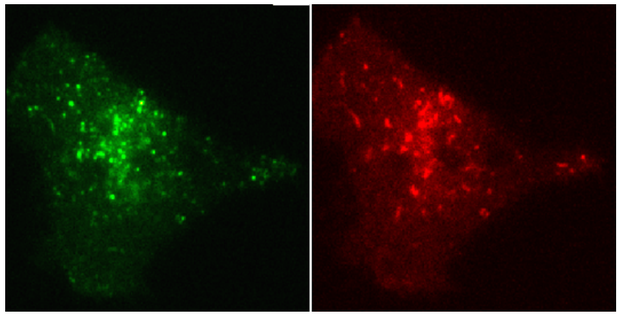




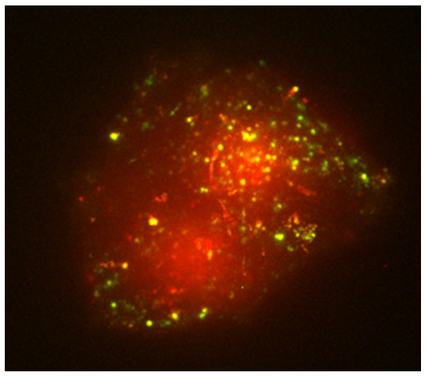
Total Internal Reflection Fluorescence

- **Spinning TIRF and fast angle motorization**
- **Simultaneous multi-wavelength TIRF with penetration depth adaptation**
- **Unmatched illumination uniformity**

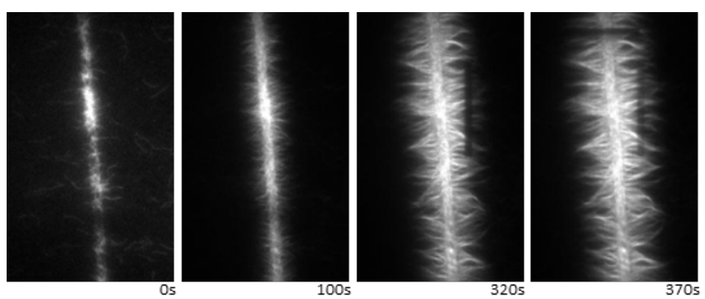
Total Internal Reflection Fluorescence (TIRF) microscopy is the ideal technique for observation close to the coverslip surface as it provides the highest axial resolution possible (between 60 to 300nm depending on the angle of incidence). This technique covers a large field of applications such as single molecule tracking, imaging secretion processes, interaction of cell membrane with matrix components or actin filament behavior.



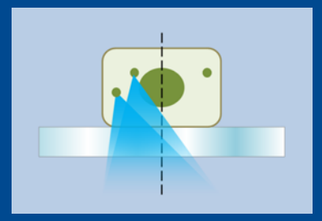
Double transfected M10 stable cell line (Langerine-YFP in green; mCherry-Rab11A in red). Images were acquired at 10fps, 100ms exp in stream mode using an image splitter (dualview,dv2) to get simultaneous detection of the two fluorescences in TIRF.
Image taken with PICT-IBiSA team @ Mifobio 2010, fr.



Single transfected M10 stable cells (mCherry-Rab11A) in Ultra Fast TIRF/ WF.
Images were acquired at 10fps/100ms (for 2 minutes), streaming both time and penetration depths (TIRF/wide-field). Here is shown the overlay of Maximum Intensity projections for TIRF illumination (green; 600 frames), while red color represents wide-field illumination (600 frames). Our Ultra fast dual imaging modality allows to rely plasma membrane appearance of single vesicles (TIRF) with their movements within the cell body (note “trajectories” in red that end up in yellow when entering the evanescent field). Image taken with B. Cinquin and J. Salamero @ Institut Curie, Paris.



In-vitro actin polymerization. The actin filament growth starts from a longitudinal micro-pattern functionalized with an activator of nucleation. Images were acquired at 1 frame every 10s in TIRF
illumination. TIRF is necessary in order to remove the high background of actin monomers in solution. FRAP experiments have been realized to investigate the filaments polarity and growth mechanism from the imposed nucleation geometry. Image courtesy of L.Blanchoin, iRTSV/LPCV, CEA Grenoble.

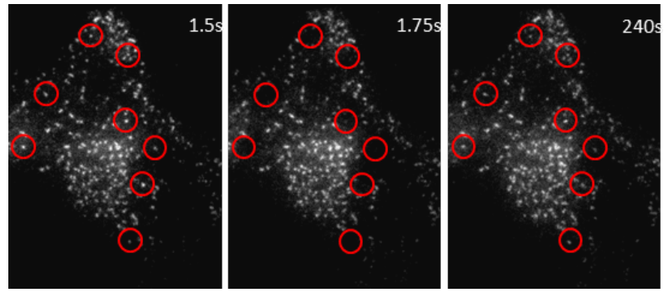


FRAP / Photo-activation

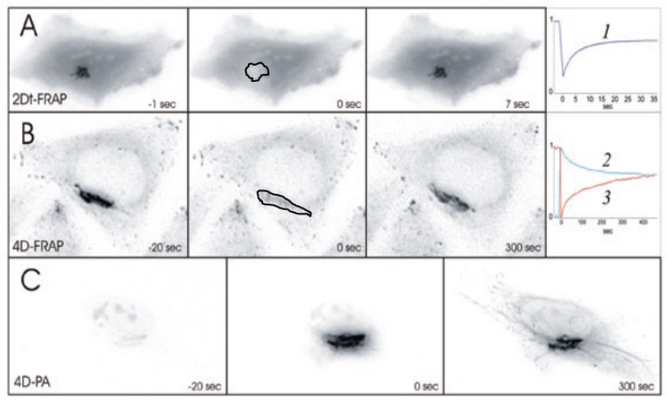
- **Galvo-based / Vectorial mode at 20kHz**
- **On-the-fly photoperturbation**
- **Auto-calibration algorithm**
- **Fast Multi ROI/Point targeting**

Localized laser action techniques such as Fluorescence Recovery After Photobleaching (FRAP, FLIP), photoactivation, uncaging, photo-ablation are very powerful tools to photo-manipulate tissues or to analyze intracellular dynamics of proteins and other macromolecular complexes. For example, FRAP permits perturbation of the steady state fluorescence distribution by bleaching fluorescence in selected regions. After the bleaching step, researchers can observe and analyze how the fluorescence distribution returns to the same or a different steady state, giving appraisal on the spatiotemporal half life of the molecule of interest within one particular site of a living sample. Photo-activation or photo-conversion make use of photo-convertible probes, allowing morphological “pulse and chase” experiments.

The iLas² system provides an easy-to-use interface to manage the lasers, set-up ROIs and plan the experiment. In order to simplify the acquisition process and enhance steering speed, iLas² is driven by its own electronic. Vectorial scanning and live action mode provide the ability to measure the fastest phenomena. The user can bleach fast-moving structures and analyze their recovery as they continue to move with the help of a tracking algorithm.



HeLA cells expressing (mRFP-LCa clathrin lightchain). Images were acquired in TIRF (100ms exp).The clathrin accumulates at the plasma membrane intoclathrin-coated pits. Several single-point ROIs werebleached at once to enables multiple quantifications.A 2 step post-bleach sequence was acquired inorder to compromize good precision on t1/2 and lowobservational photo-bleaching (4fps followed by0.25fps). Image taken by G. Montagnac @InstitutCurie, Paris.



2D+t and 3D+t FRAP/PA wide-field sequences of acquisitions and their associated redistribution curves. In all case, the whole Golgiapparatus (volume of interest) has been submitted to laser illumination . A) GFP-dymeclin (2D+t, curve 1). B) GFP-Rab6A (3D+t,curve 3) and C) PA-GFP-Rab6A (3D+t, curve 2). All recovery curves show the average intensity over time in the Golgi apparatusvolume. Figure taken from G.I.T Imaging & Microscopy (Gueudry, C. et al. , 24-26, 3/2006)