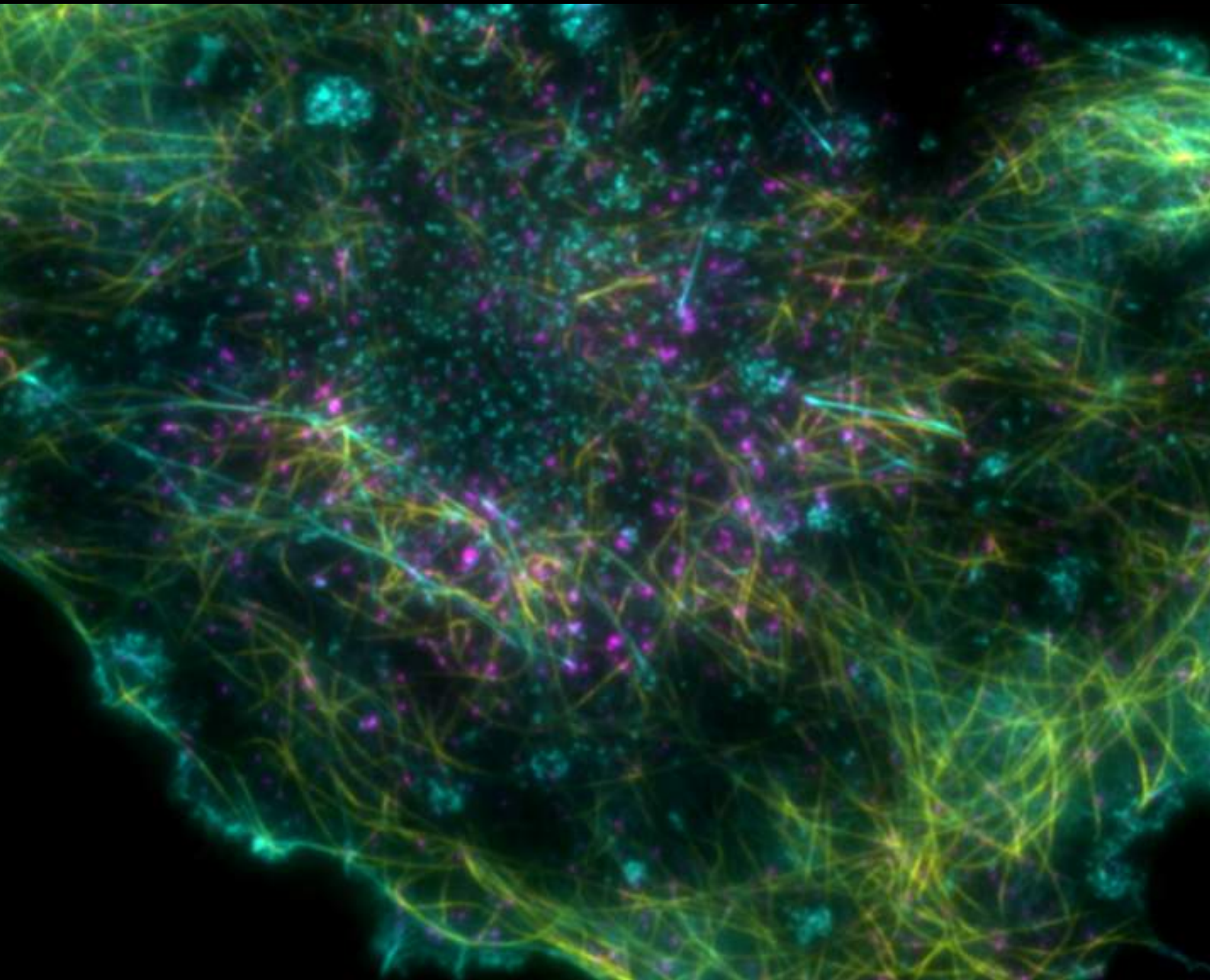




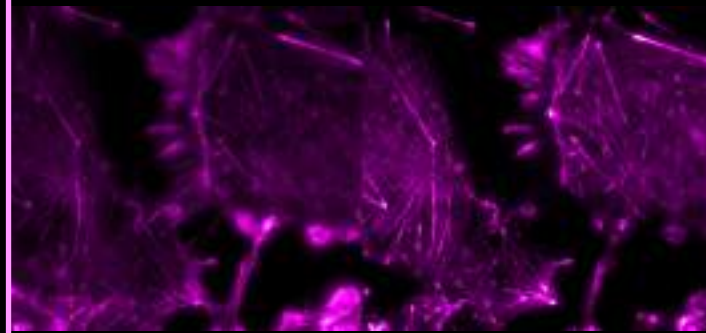
Abbelight TIRF

For automatic, multicolor
and optically perfect
TIRF imaging



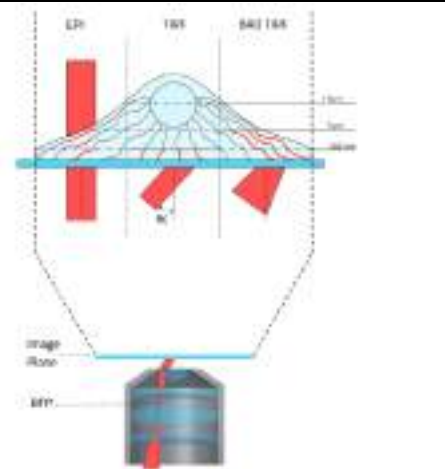
The study of membranes and events close to the coverslip is very much answered in biology research such as **cell adhesion, endocytosis, exocytosis or osmosis**.

Total Internal Reflection Fluorescence microscopy (TIRF) (Axelrod, 2013) is the most suitable imaging method as it is offering an advantageous optical sectioning (up to a few hundred nanometers after the coverslip). Nevertheless, in practice, TIRF microscope remains quite difficult to preset for non-expert users. It requires perfect optical conjugation of the microscope and very precise positioning of the beam in the Back Focal Plane (BFP) of the objective (Mattheyses et al., 2010), resulting in experiments that are not reproducible from day to day.



EPI illumination no optical sectioning EPI illumination nanoscale sectioning

Sectioning and BFP focus



Features and advantages

- **Adaptable** technology to any inverted microscope
- **The largest uniform and speckle-free field of view** on the market thanks to Abbelight ASTER illumination strategy
- **Automatic, quantitative and reproducible TIRF** to define the optimal position of the TIRF angles and get reliable data over time, whatever your sample is
- **Chromatic aberration-free multicolor TIRF imaging** which allows you to image several structures on your samples simultaneously
- **Multi-dimensional acquisition** with multiple scenarios, having options designed to best meet the needs of TIRF applications

Abbelight's TIRF technology

This technology is a hardware and software solution that provides an **optically perfect TIRF imaging**. **Adaptable on any combination of microscopes, objectives, XYZ stages and accessories**, our new product guarantees the **automatically calibrated and reproducible positionned TIRF angles**. Coupled with a detection optical module, it offers an optimal and simultaneous multicolor imaging with the benefits of **ASTER** technology. This technology provides an **ultra-widefield and speckle-free illumination** of biological structures close to the coverslip such as membranes, focal adhesions, and much more.

Adaptable

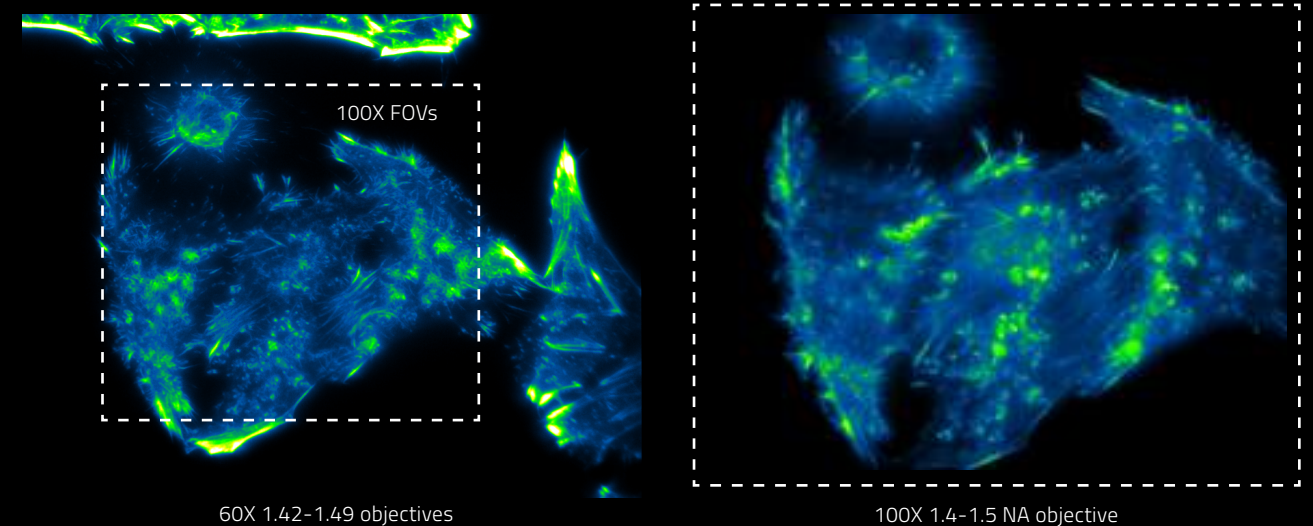
The optical system installed in Abbelight SAFe excitation device is designed to obtain **the optimal TIRF angle position** and get the **perfect TIRF illumination** compatible with any combination of:

Microscopes

Objectives

XYZ stages

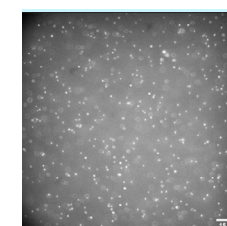
Accessories



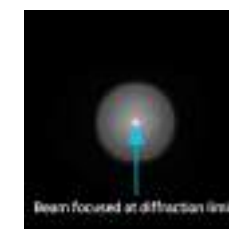
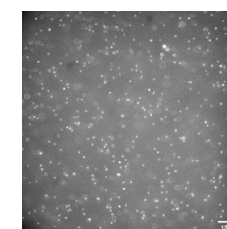
EPI Illumination

IMAGE PLANE

BACK FOCAL PLANE



Beam NOT perfectly focused on BFP
No difference in EPI



Beam perfectly focused on BFP.
No difference in EPI

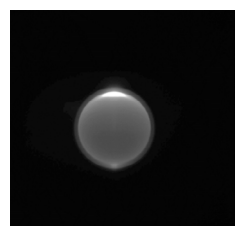
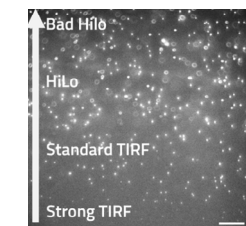
Without Abbelight SAFe technology

With Abbelight SAFe technology

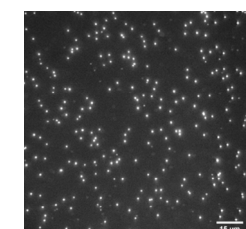
TIRF Illumination

IMAGE PLANE

BACK FOCAL PLANE



Beam NOT perfectly focused on BFP.
TIRF not available



Beam perfectly focused on BFP.
Perfect TIRF

Very large and uniform FOV

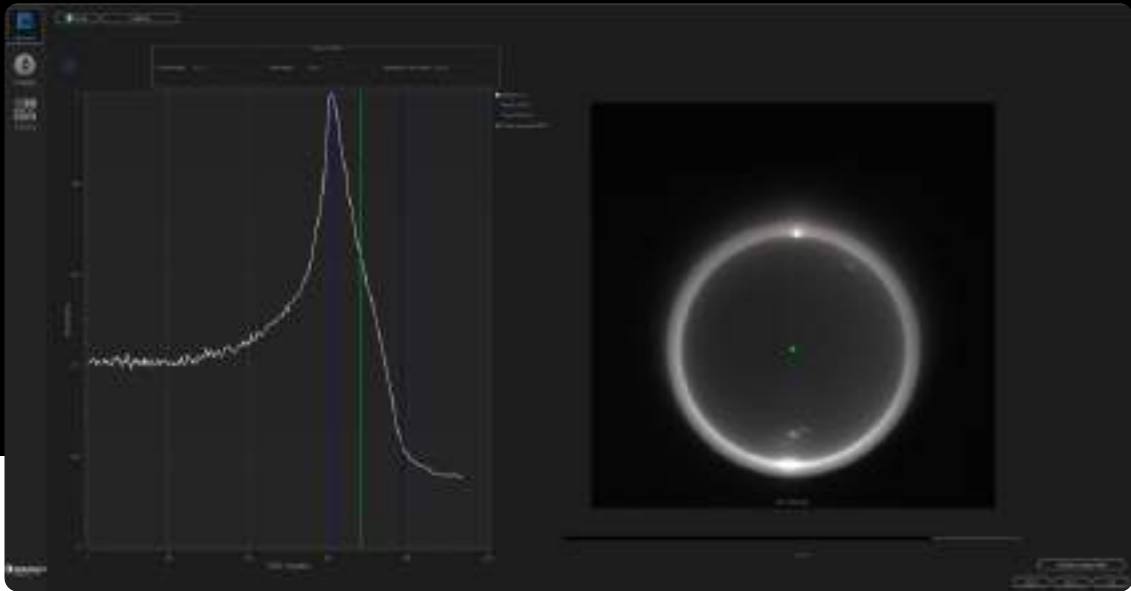
Abbelight patented ASTER method offers **homogeneous TIRF illumination** of the sample over the **largest FOV** on the market.

| | Simultaneous 2C | Simultaneous 4C |
|------|---------------------------|---------------------------|
| 60X | 250 x 250 μm^2 | 170 x 250 μm^2 |
| 100X | 150 x 150 μm^2 | 100 x 100 μm^2 |

Blue F-Actin-AF488 - Green Tub-AF568 - Red Clathrin-AF647

Automatic, quantitative and reproducible

Our technology combined with a **software application** and a calibration sample allows automatic definition of the TIRF angles with **<1.2% error**, leading to reliable data over time.

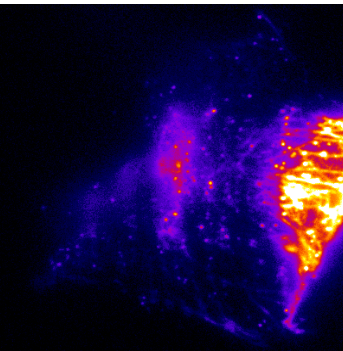
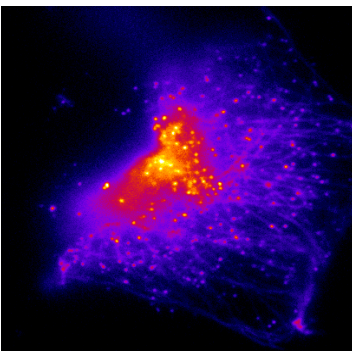
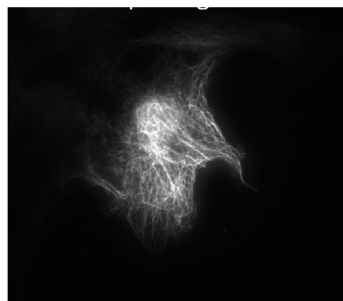
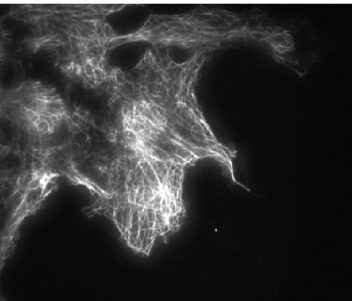


Visualization of the results of calibration: The algorithm uses the two planes (BFP/Image plane) to calculate the **critical angle**, **maximum angle** and an **optimized TIRF**

Abbelight's ASTER illumination

ASTER TIRF illumination, co-developed with Sandrine L  v  que-Fort and published in Nature Communication (A. Mau et al., 2021), **outperforms standard TIRF strategy** because **it breaks classical interference fringes** like the Ring/Azimuthal TIRF method.

ASTER TIRF illumination also surpasses Ring/Azimuthal method because it is offering a **>95% uniform excitation over the largest FOV**, while Ring/Azimuthal TIRF is strongly limited by the gaussian shape of the laser irradiance.

| STANDARD TIRF | ASTER TIRF |
|---|---|
|  |  |
| Interference fringes issues | No fringes offering reliable image |
| RING/AZIMUTAL TIRF | ASTER TIRF |
|  |  |
| Gaussian illumination leading to non uniform excitation | > 95% excitation uniformity over the whole FOV |

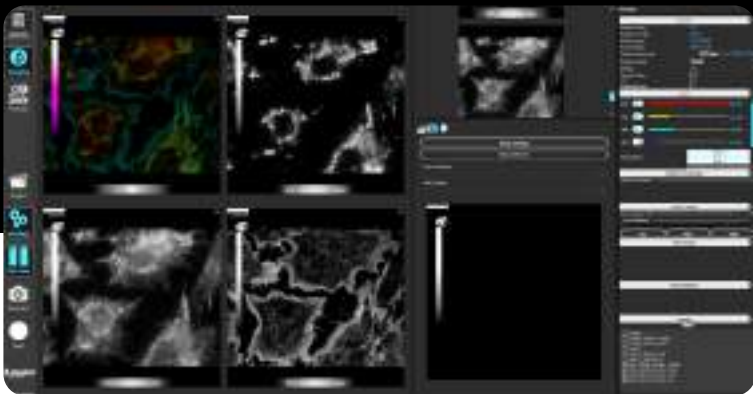
Abbelight TIRF

- **Software application integrated into Abbelight™ SAFe NEO** aids in calculation and backup of TIRF angles based on image processing algorithm, that exploits image plane intensity as well as distribution and segmentation of your objective's BFP
- **Calibration sample** based on nanometric beads embedded into a highly controlled and long life-time gel with refractive index of water. The calibration sample is supplied with our Support Program Abbelight Care 1
- **Ultra stable TIRF stage** based on a closed-loop long range piezo stage offering nanoscale repositionning of the beam at BFP



Reliable Multicolor imaging

A robust optical design and software architecture leads to highly stable, multicolor imaging without chromatic aberration, optical artefact or misalignment over time.



Multi-view imaging page of Abbelight NEO Safe software suite.

Abbelight’s multicolor strategy

Optics inside the illumination device are designed to **reduce drastically chromatic aberration** for the range **488-640 nm**, thus offering **TIRF excitation over a wide spectrum**.

Special **custom dichroic CUBES** have been designed to **remove ghost images** and also to **reduce crosstalk between channels**, thus offering up to **4 colors simultaneous imaging**.

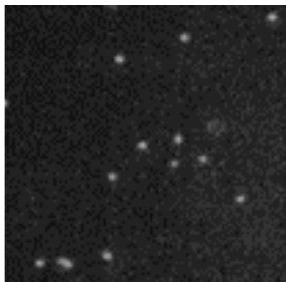
Highly stable optomechanical components holding dichroic cubes have been carefully chosen to **simplify the alignment of the 4 colors images**. A **software digital alignment tool** has also been developed to offer < 0,7 pixel precision of alignment.

STANDARD DICHOIC



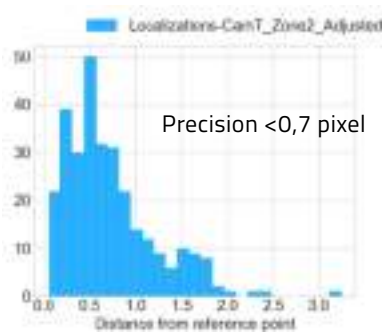
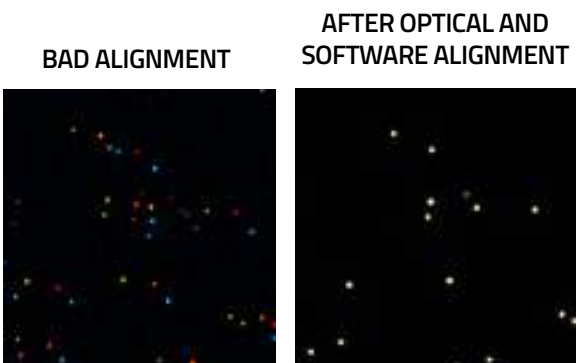
Ghost images

ABELLIGHT CUBE



No ghost images leads to reliable data

30 x 30 mm³ dichroic cube designed to reduce aberration and crosstalk.



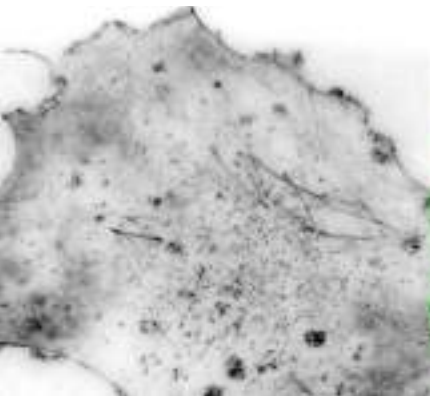
Bead position differences in pixels

Multi-dimensional acquisition for TIRF and multicolor imaging

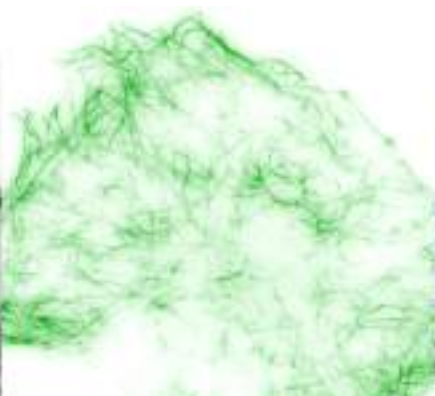
Multiple acquisition scenarios with adjustable parameters developed to best meet the needs of TIRF applications



Time lapse multicolor imaging



Variable angle TIRF multicolor



Multi-position multicolor imaging



Abbelight SAFe modular platform* for microscopy and super-resolution



- For a large Field Of View, Widefield and automatic TIRF
- For automatic, multicolor and optically perfect TIRF imaging
- For 2D sequential multicolor SMLM imaging, TIRF and widefield imaging
- For a complete solution for multiple microscopic modalities: WF, 2D and 3D SMLM and TIRF

MICROSCOPY

Widefield

TIRF

SAFe M45

SAFe M90

SAFe MN 180

SAFe MN 360

NANOSCOPY

SMLM 2D

SMLM ultimate 3D

*compatible with all inverted microscopes





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