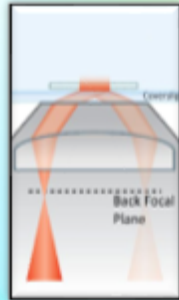


Tips & Tricks : TIRF HILO

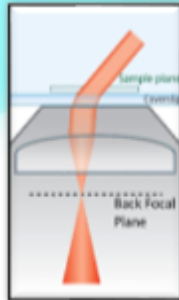
Our new NikonTi2 inverted microscope has been designed for TIRF HILO and FRET experiment. It is equipped with a chamber that maintains CO₂, humidity, and temperature, ensuring optimal conditions for live-cell imaging. This system is compatible with various sample holders, including 35 mm dishes, slides, and multiwell plates. Illumination is provided by an 8-channel LED system (365, 400, 435, 470, 500, 550, 580, 635, 740 nm) and very powerful lasers (100mW) at 405, 488, 561 and 640 nm for TIRF, HILO and Single Particules Tracking (SPT). Image acquisition is optimized with a Photometrics Kinetix camera (sCMOS, 96% quantum efficiency), ensuring exceptional sensitivity and resolution. This microscope has three lens: a 20X/0.8 dry, a CFI Plan Apo Lambda D 60X/1.42 Oil, and an Aplanachromat TIRF 100X/1.49 Oil.

How do TIRF & HILO work ?

TIRF relies on the principle of total internal reflection. When a laser beam passes through a high-refractive-index medium (e.g., glass) and reaches an interface with a lower-index medium (e.g., a cell), it reflects entirely if the critical angle is exceeded. This generates an evanescent wave that penetrates only 100-200 nm into the lower-index medium, selectively exciting fluorophores near the membrane.

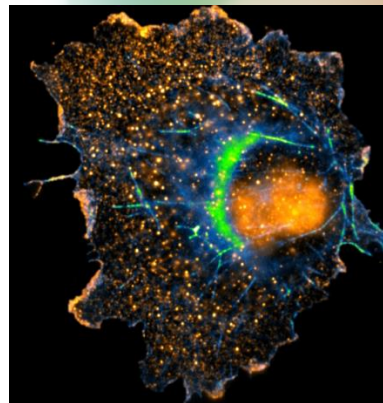


HILO modifies the TIRF approach by steeply tilting the laser beam without reaching the critical angle. This creates a thin light sheet that penetrates deeper into the sample while minimizing out-of-focus fluorescence.



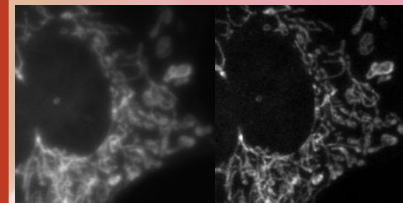
Why Should you use TIRF ?

TIRF can be used to study dynamic processes at the membrane with high spatial and temporal resolution. It enables the visualization of receptor dynamics, endocytosis, lateral movement, exocytosis, focal adhesions, and migration. Since it selectively illuminates structures within 100-200 nm of the coverglass, it reduces background fluorescence and phototoxicity, making it ideal for long-term live-cell imaging.

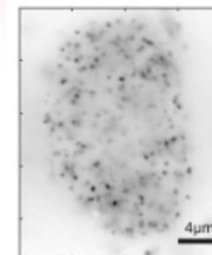


Why Should you use HILO ?

By using an inclined illumination angle, HILO selectively excites fluorophores in a thin optical section, reducing background fluorescence while penetrating deeper into the cell compared to TIRF. This makes it ideal for tracking the movement of individual molecules and vesicles in the cytoplasm or nuclei with high spatiotemporal resolution.

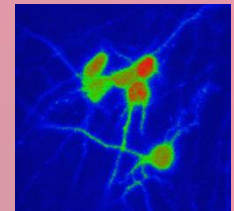


Single molecules



What is FRET ?

Förster Resonance Energy Transfer (FRET) is a technique that relies on the non-radiative transfer of energy between two fluorophores (a donor and an acceptor) when they are within 1-10 nm of each other. This distance sensitivity makes FRET ideal for detecting protein-protein interactions in real time.



Reminder: Our Stellaris system allows precise fluorescence lifetime imaging (FLIM), providing the same information with greater accuracy, as the measurement is not based on intensity ratios.



ALWAYS KEEP IN MIND :

You are the only one responsible for your data

Never use the platform's computers as a back-up for your data !