



Total Internal Reflection Fluorescence (TIRF)

Introduction to the basic of Total Internal Reflection

The optical phenomenon of total internal reflection (TIR) can be observed in everyday life. The century old handcraft of gemstone cutting is a typical example. Keeping the light within a diamond and creating as many reflections as possible before it exits, produces the so called “fire” of a gemstone. The light undergoes multiple internal reflections before it gets refracted. The same principle is used in modern fiber optic data translation.

The goal of using total internal reflection fluorescence (TIRF) spectroscopy in biological applications is to study events close to the interface of two different media. A simple example would be a droplet of water on a glass plate. There is no refraction if you follow a beam along the optical axis ($\alpha_1=0^\circ$) of such a system. Increasing the angle of the incoming beam ($\beta_2=30^\circ$) would lead to refraction away from the optical axis. The reason for this is that the refractive index of glass is bigger than the one of water. If you further increase the angle of the incoming beam above the so called “critical angle”, total reflection occurs ($\gamma_3 > \alpha_{critical}$). The critical angle is defined by the density (the refractive index η) of the two media forming the interface. The relationship between the angle of the incoming and the refracted beam is given by the law of refraction:

$$\eta_1 \cdot \sin \alpha_1 = \eta_2 \cdot \sin \alpha_2$$

$$\Leftrightarrow \frac{\sin \alpha_1}{\sin \alpha_2} = \frac{\eta_1}{\eta_2} \equiv \eta_{21}$$

Assuming that the beam travels from the optically denser medium into the less dense medium, the critical angle is calculated as follows:

$$\alpha_{critical} = \arcsin \eta_{21}$$

For the interface of our example formed by water and glass, the refractive indices are $\eta_{water} = 1,333$ and $\eta_{quartz} = 1,544$, respectively. Hence the critical angle for this system is $59,7^\circ$.



What happens at the water/glass interface if the incoming beam hits it above the critical angle?

The term “total internal reflection” (TIR) is a little misleading in this case. However, the not so frequently used expression “attenuated total reflection” (ATR) describes the phenomenon a bit better. Looking at the interface and especially into the medium with the lower index of refraction (water), a standing (evanescence) wave is established whose intensity decreases exponentially with distance. In other words, part of the wave (intensity) penetrates into the surrounding medium. The penetration depth depends on the angle, the wavelength, and the index of refraction of the two media involved. Coming back to the example given above the penetration depth slightly above, the critical angle (60°) equals roughly 1.5 times the wavelength used.

How is TIRF technically realized in the TILL Photonics setup?

Changing the example described above to a more biological one - by using a cell surrounded by media instead of a water droplet and a cover slip instead of simple glass – shows the possibility to investigate biological applications. As shown in the previous section, one has to control the angle of the incident light. In our setup we use a laser as an excitation source. The laser is focused into the back focal plane of the objective. Assuming the laser is focused into the center of the back focal plane, the light exits the objective along the optical axis of the system. Moving the focus away from the center towards the edge of the back focal plane leads to the fact that the light exits the objective under a certain angle. The further you move the focus away from the center, the bigger the angle of the exiting light gets. To be able to reach the critical angle necessary for total internal reflection, you need a least an objective with an NA of > 1.39 for a glass/water interface. Using a standard 1.4 NA objective makes it nearly impossible to adjust a TIRF system. Therefore objectives with extremely high NA have been developed. The standard TIRF objective has an NA of 1.45. The highest NA objective commercially available is the 1.65 from Olympus. The NA of the objective limits the achievable angle of the excitation light, and therefore also limits the minimal penetration depth!

TILL TIRF Solutions

Currently we support our own fully motorized microscope - the IMIC, the Nikon TE2000, the Zeiss Axiovert 200/100 and the Olympus IX50/70/71. Our TIRF setup is fully integrated into our Imaging System. Apart from the equipment listed below you need the TILL Imaging System, and as an option, a controller for the AOTF. You can also buy “stand alone” TIRF components meaning you have to integrate them into your existing setup. If you already have a laser, please talk to us about its specifications. We are happy to assist you in integrating our TIRF equipment into your existing setup.



TIRF with single line laser

TIRF Equipment needed (“stand alone”): TILL-TIRF condenser, TILL-TIRF shutter, laser fiber, TIRF objective, filter set, adjustment kit, single line laser

Optional: adapter for mounting the laser fiber onto the laser

To upgrade this to a turnkey TILL Imaging TIRF system you need the complete Imaging system plus the equipment listed above.

TIRF with multi line laser

TIRF Equipment needed (“stand alone”): TILL-TIRF condenser, TILL-TIRF shutter, laser fiber, TIRF objective, filter sets, adjustment kit, multi line laser

Optional: adapter for mounting the laser fiber onto the laser, acousto-optic tunable filter (AOTF)

To upgrade this to a turnkey TILL Imaging TIRF system you need the complete Imaging system, and the TILL AOTF control board plus the equipment listed above.

TIRF with multiple different lasers

This product will be released soon. Our Laser Combiner solution is going to be a modular system. The idea is to be as flexible as possible by giving you the opportunity to select between a huge variety of different lasers and therefore laser lines. Please contact us directly for getting more information about this product .

Applications

- Tracking of secretory granules in intact cells
- Selective visualization of cell/substrate contact regions
- Protein translocation
- Selective detection of membrane proteins, e.g. ion channels
- Visualization of single molecules near the surface
- Force measurements on single molecular contacts
- Long term fluorescence observation of living cells