

# TIRF - Total Internal Reflection Fluorescence

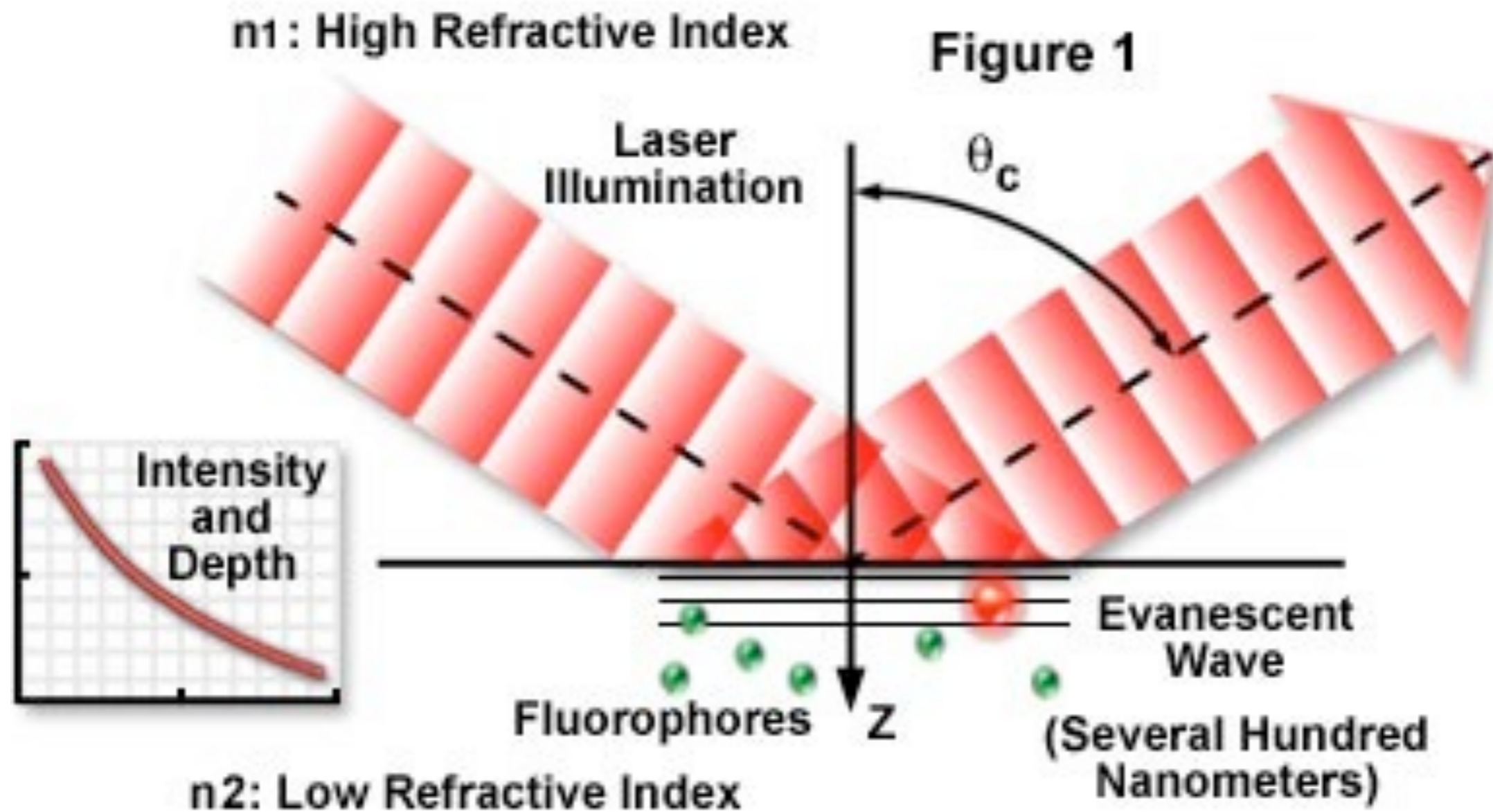
Ian Dobbie

# What is TIRF?

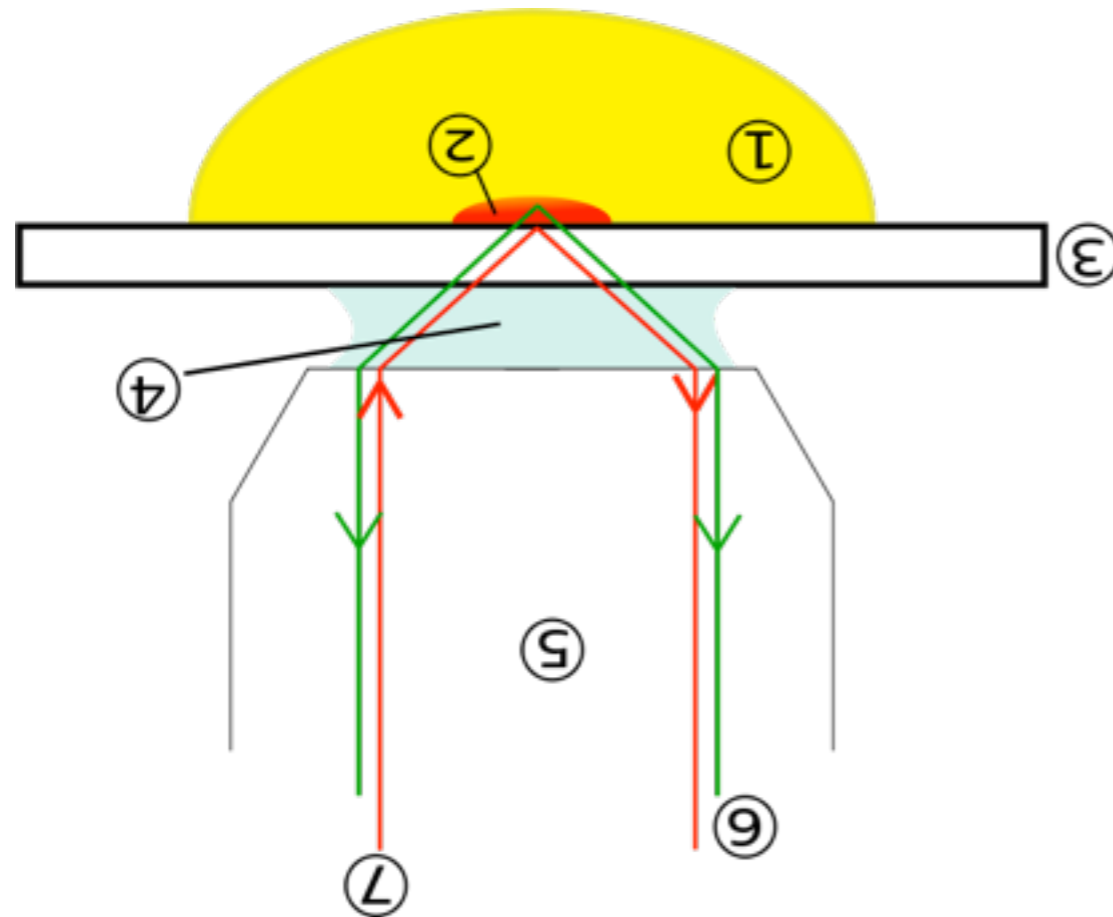
- Light striking an interface between a high refractive index material and a lower one is totally reflected above a critical angle.
- None of the illumination light enters the experimental sample.
- The field penetrates the interface, called an evanescent wave, allowing fluorescence excitation up to a depth of  $\sim 100\text{nm}$ .

# Total internal Reflection TIRF

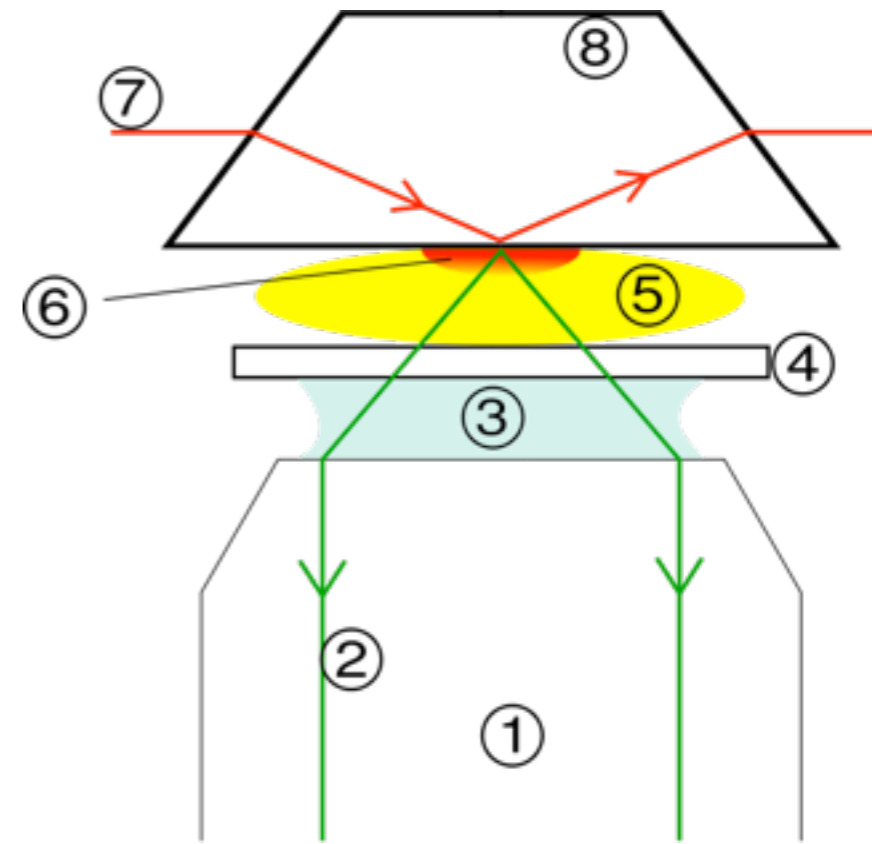
## Total Internal Reflection Fluorescence Microscopy



# Two common TIRF setups



Objective based TIRF



Prism based TIRF

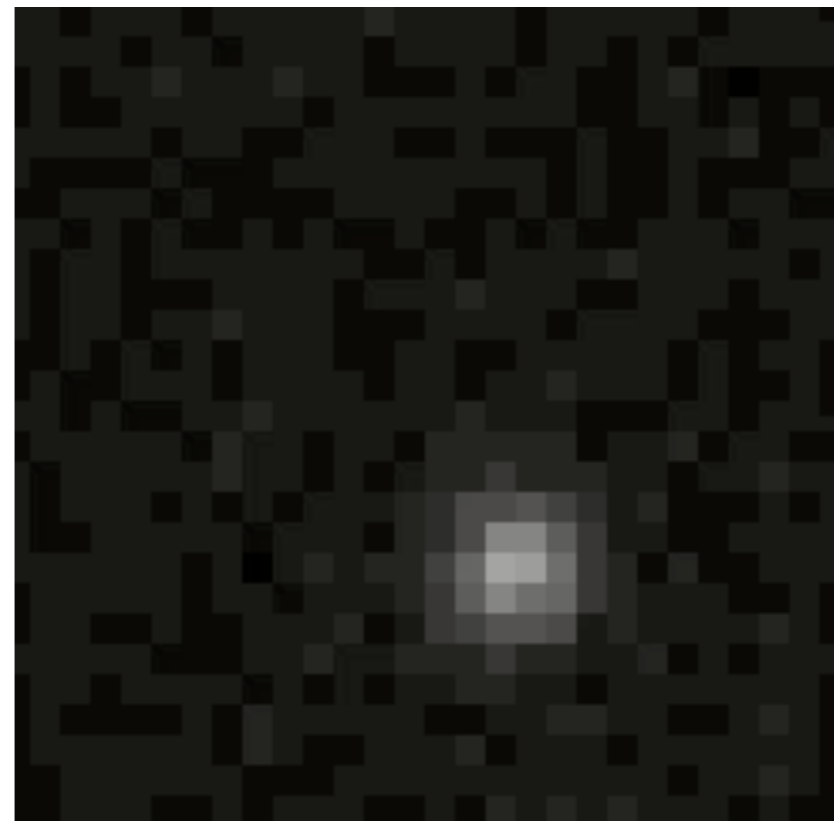
# Uses of TIRF

Two advantages of TIRF:

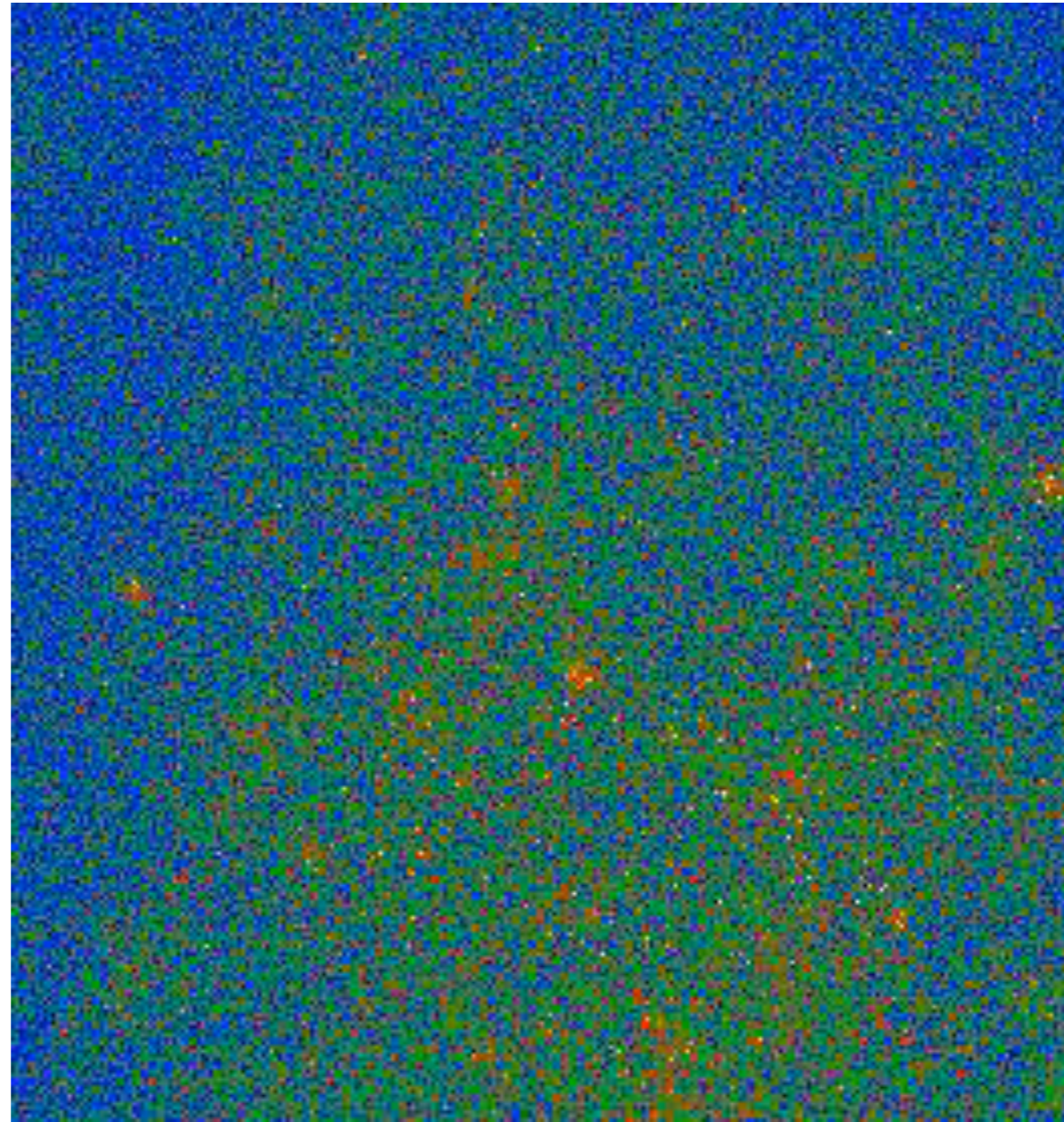
- 1) Extremely low background.
- 2) Localisation to glass surface.

TIRF has often been used to visualise single fluorescent molecules or localization specific structures such as cell surface receptor binding or a single motor molecule.

A single myosin V molecule crawling across an actin filament



# Example TIRF data.



Single GFP molecules diffusing in solution