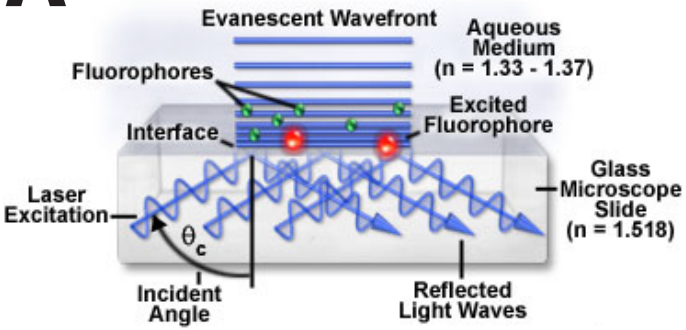
TIRF

Total Internal Reflection Fluorescence microscopy or Evanescent Wave Microscopy

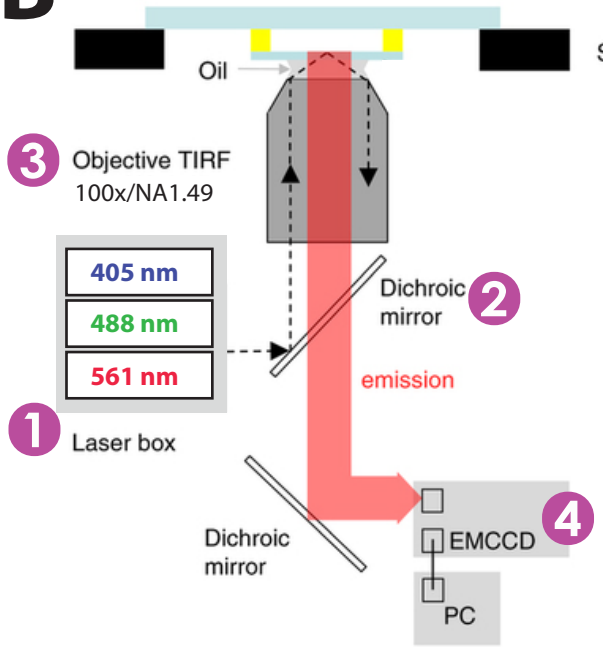
- **Live imaging and observations of fixed samples**
  - Green and Red fluorescence
- **Temperature, Humidity, CO2 control**
- **Observations of biological structures/ proteins near the plasma membrane**

Bleaching	★★☆☆	*low*
Resolution	★★☆☆	*medium*
Speed acq.	★★★☆	*good*
Multicolor	★★★☆	*3*
Depth (z)	★★★★	*<100 nm*

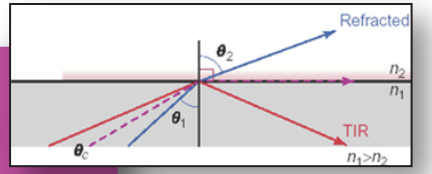
A



B



Some key TIRF formulas



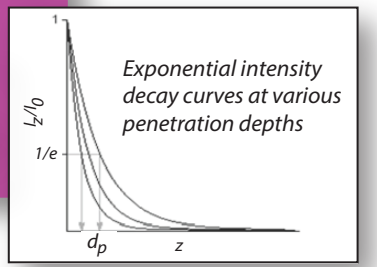
Snell's law:
n1.sin θ1=n2.sin θ2
(θ: angle of incidence, n: refractive index)

Critical angle:
at the critical angle θc, θ2=90°; sin90°=1;
n1.sinθc=n2
$\theta c = \sin^{-1}(n2/n1)$
If n1=1.515 and n2= 1.36, $\theta c = \sin^{-1}(1.36/1.515)=63.85°$

Evanescent field:
$I_z = I_0 \exp^{-z/d_p}$

$$d_p = \frac{\lambda}{4\pi\sqrt{n_1^2 \sin^2\theta_1 - n_2^2}}$$

I: intensity
z: distance
λ: wavelength
d_p: penetration of depth



Exponential intensity decay curves at various penetration depths

Lasers 1
405 nm 50 mW
488 nm 50 mW
561 nm 25 mW

Mounted filters 2
TIRF Quad Filterset 405/488/561/640
TIRF FITC : ex 488/10 DM502 em 530/43
TIRF TRITC : ex 561/10 DM580 em 605/50

Objectives 3
10x 0.3 Plan Fluor DIC C / N1 WD 16mm
40x 1.3 Oil Plan Fluor DIC H WD 0.2mm
63x 1.4 Oil Plan Apo DIC H WD 0.21mm
100x 1.49 Oil CFI Apochromat TIRF

Detector 4
Camera Andor iXon+
(Model : DU-897E-CS0-#BV-500)
Pixels : 512 x 512 (16um x 16um)
56 fps full frame
Cooling -80°C

Microscope 5
Nikon Eclipse Ti with PFS

Software 6
- Windows 7
- NIS elements

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