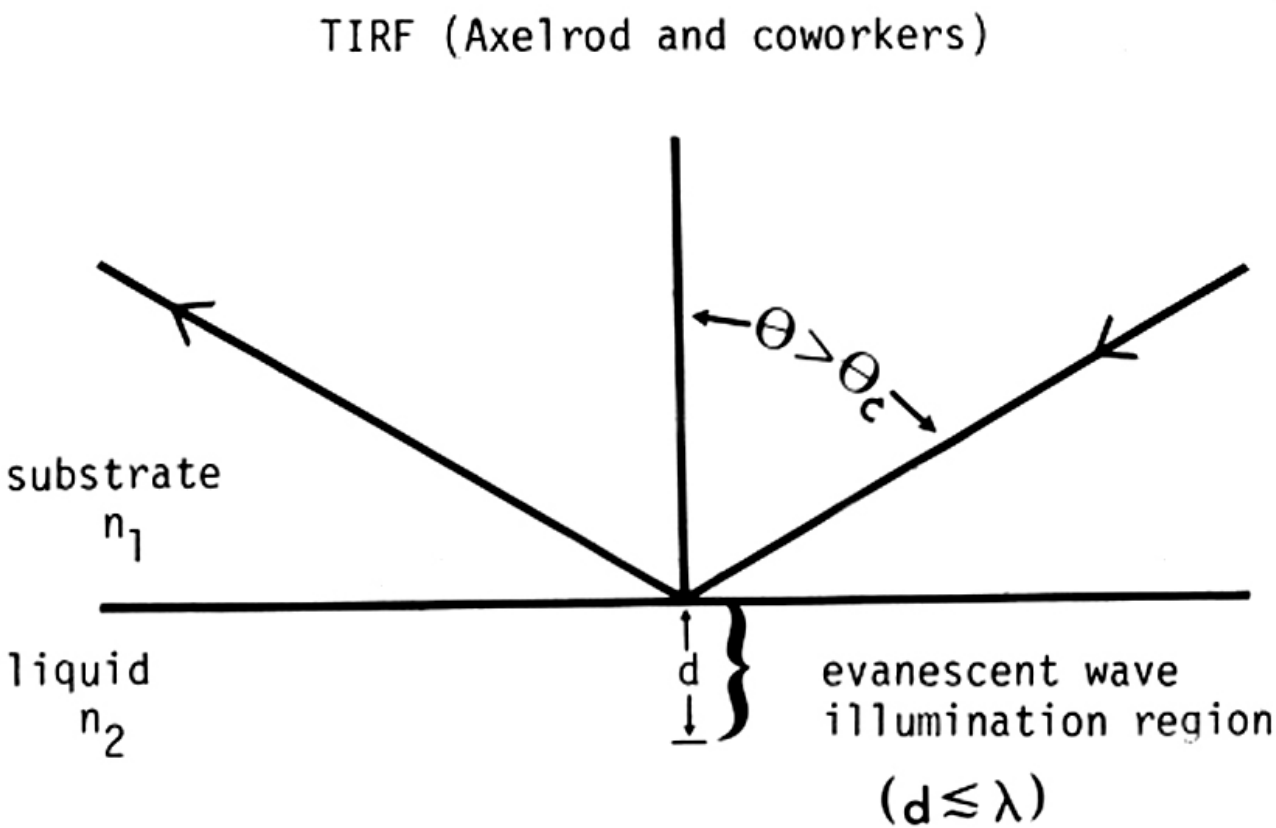
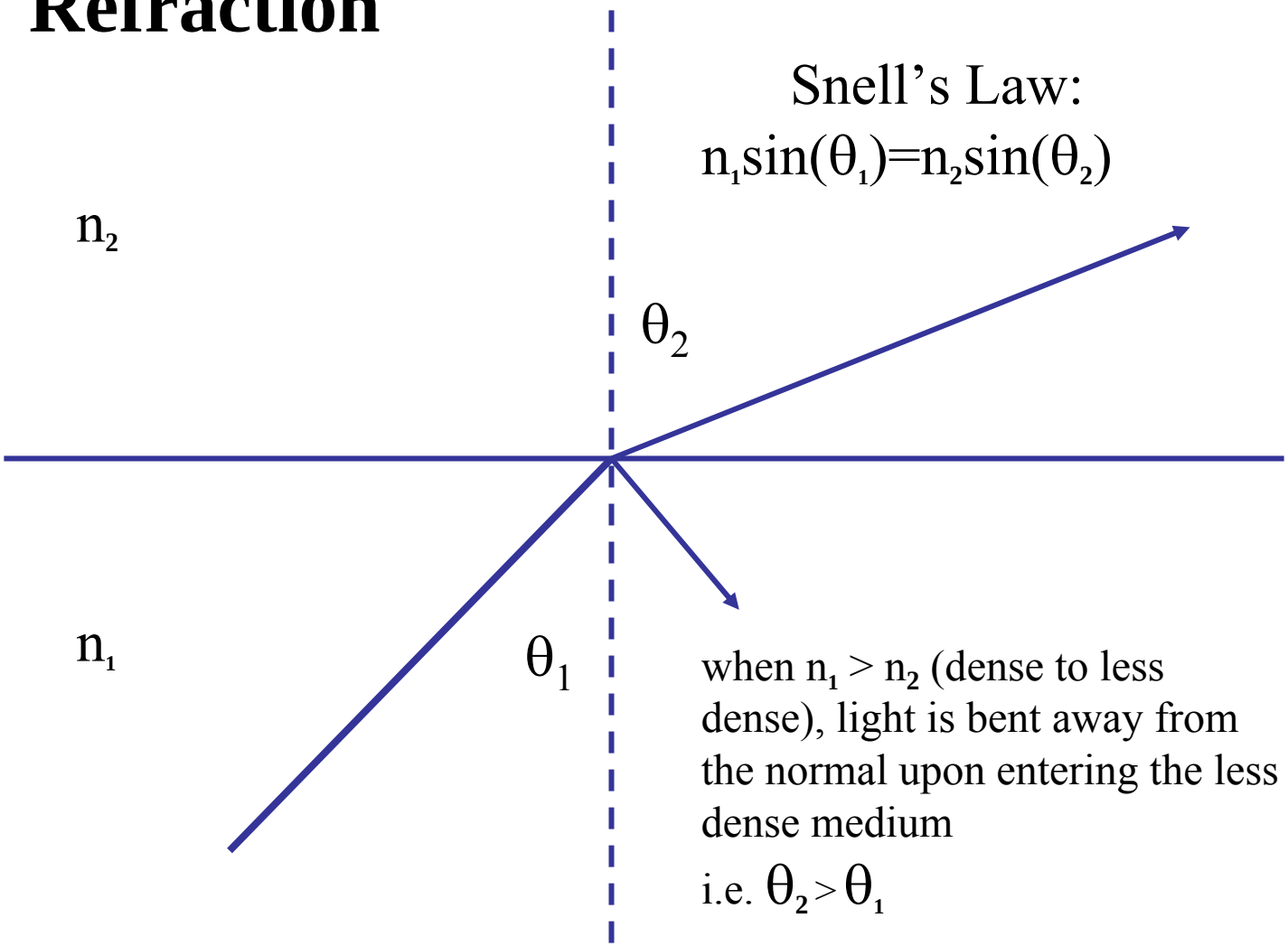
TIRF: total internal reflectance fluorescence microscopy



Refraction

Snell's Law:

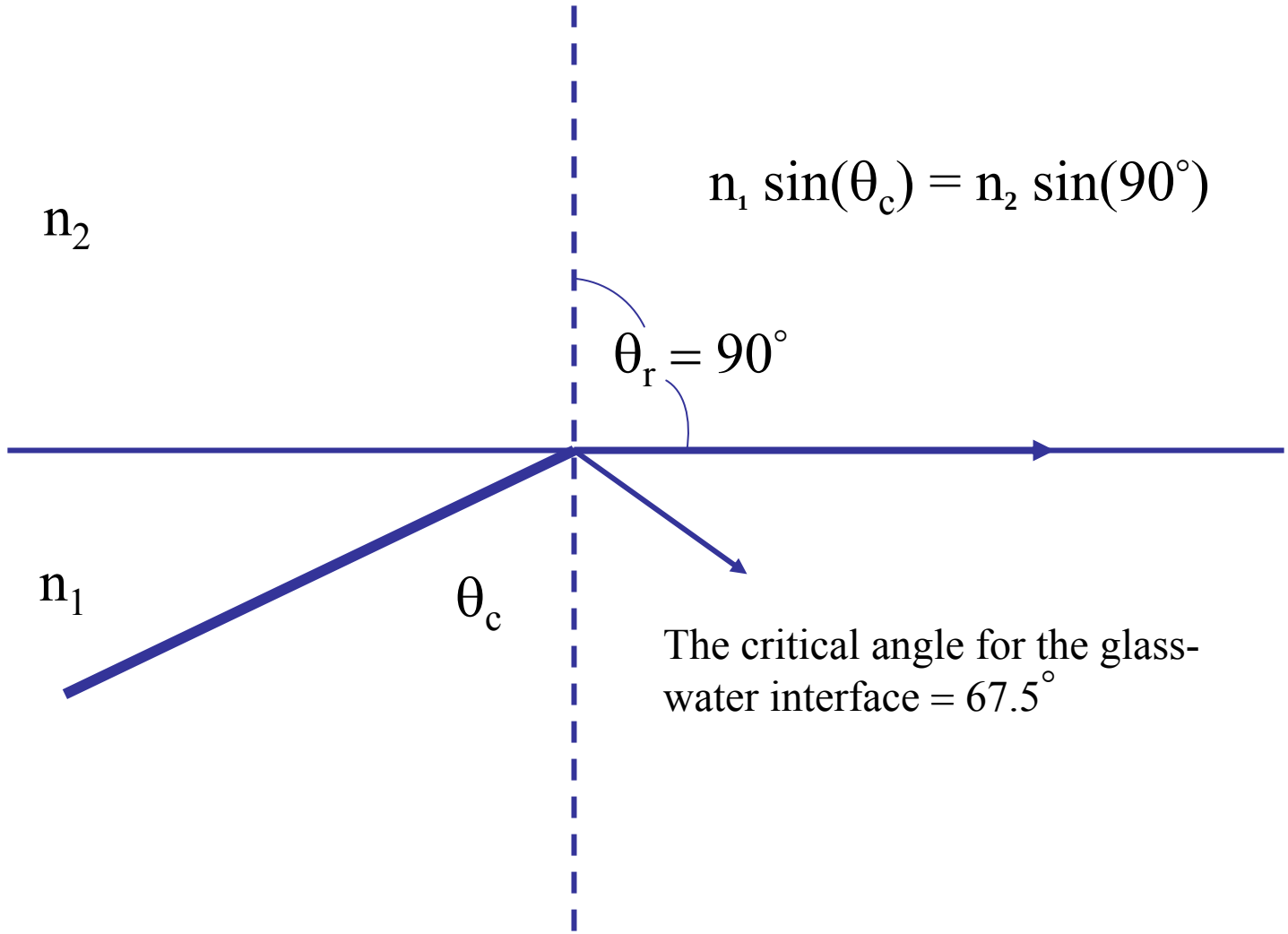
$n_1 \sin(\theta_1) = n_2 \sin(\theta_2)$



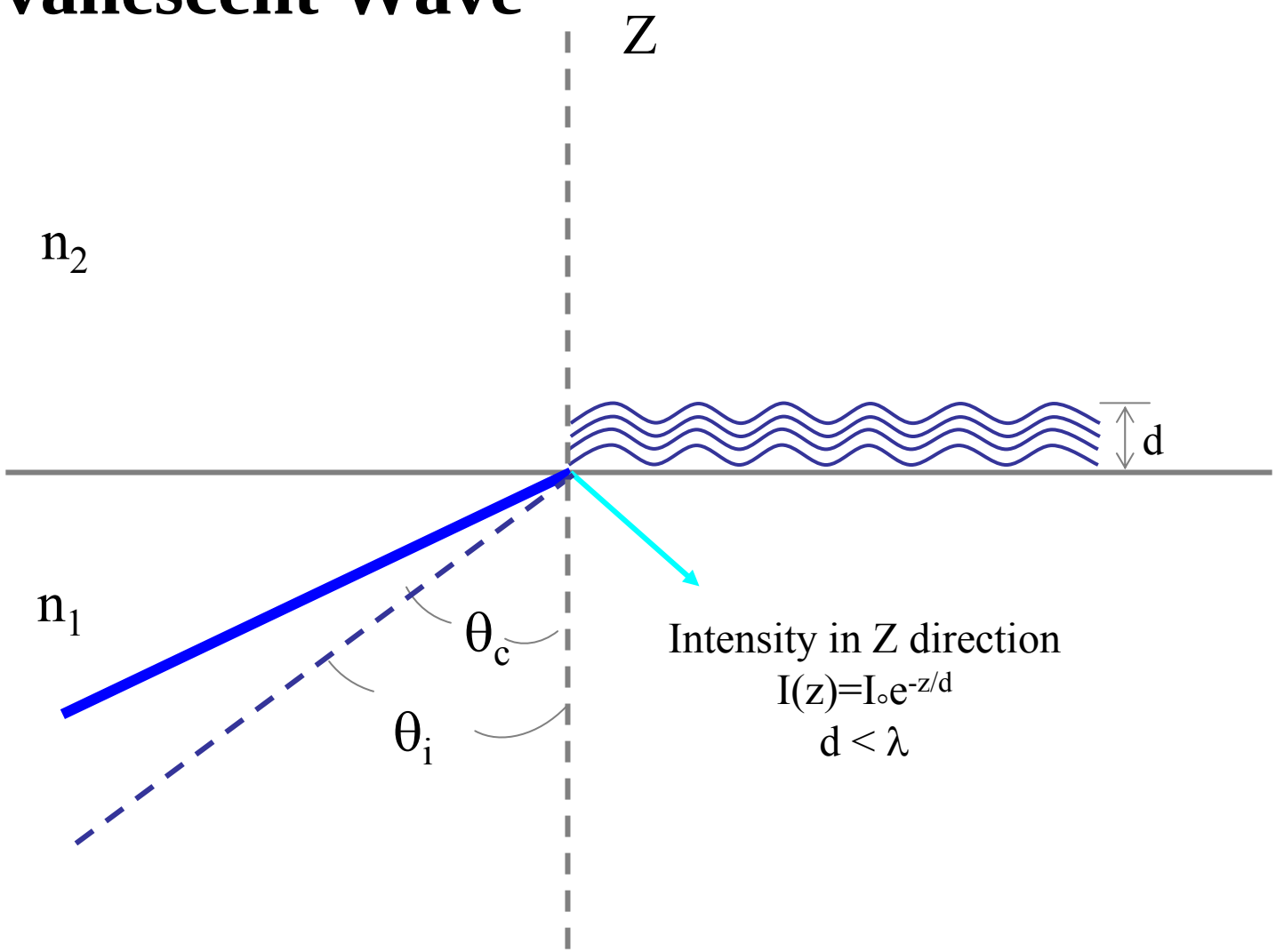
when $n_1 > n_2$ (dense to less dense), light is bent away from the normal upon entering the less dense medium

i.e. $\theta_2 > \theta_1$

Total internal reflection and the critical angle



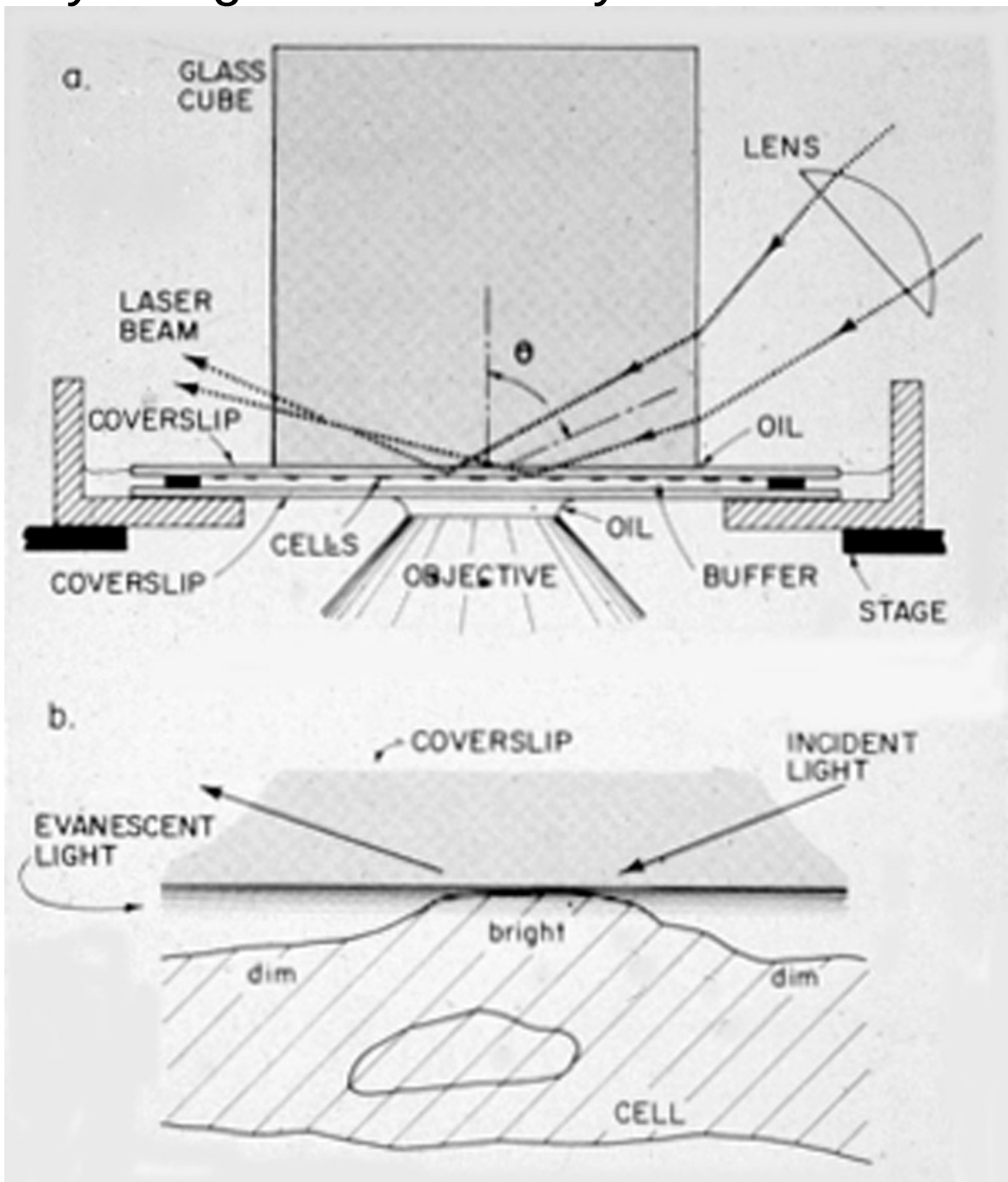
Evanescent Wave



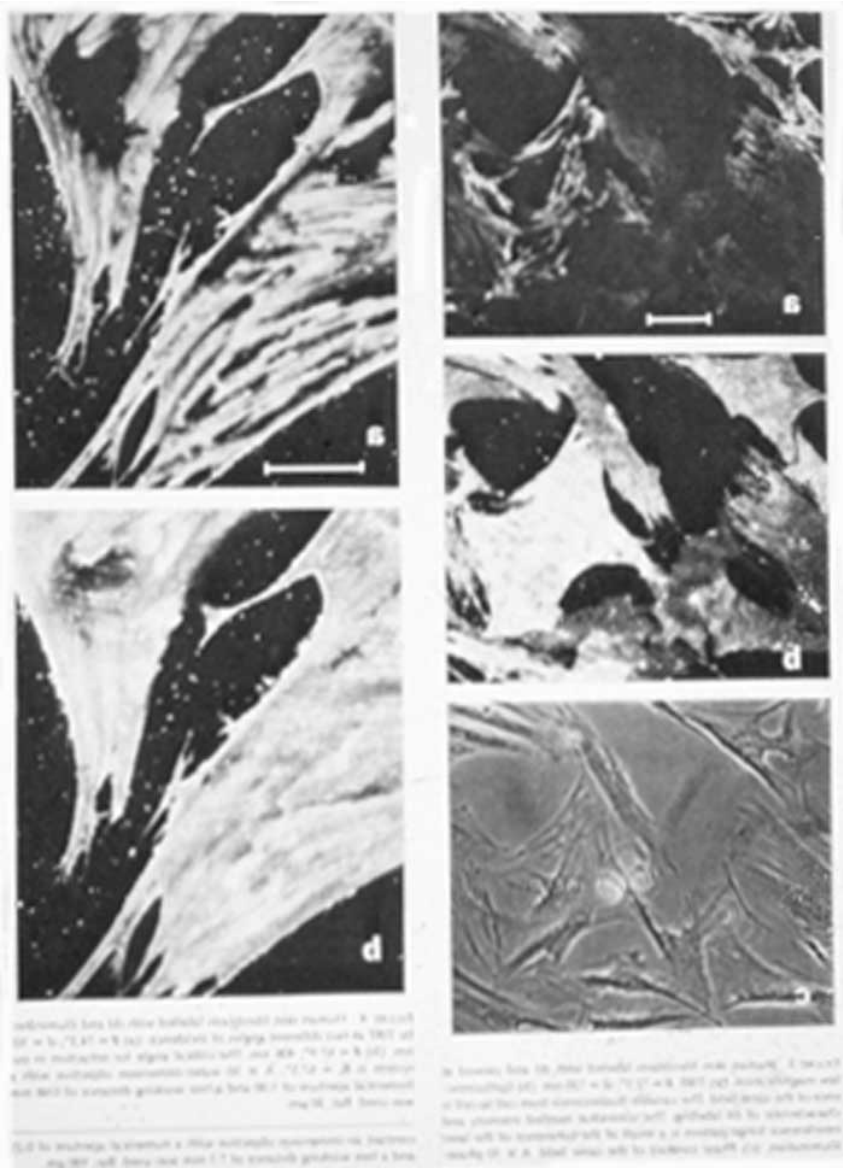
Depth of evanescent wave (d) depends on angle of incidence, θ_i: it is largest close to the critical angle, θ_c

$$d = \frac{1}{\sqrt{\frac{\sin^2 \theta_i}{\sin^2 \theta_c} - 1}} \times \frac{\lambda}{4\pi n}$$

Early design circa 1980 by Dan Axelrod



74.3 deg;
d=105nm



72.5 deg; d=120nm

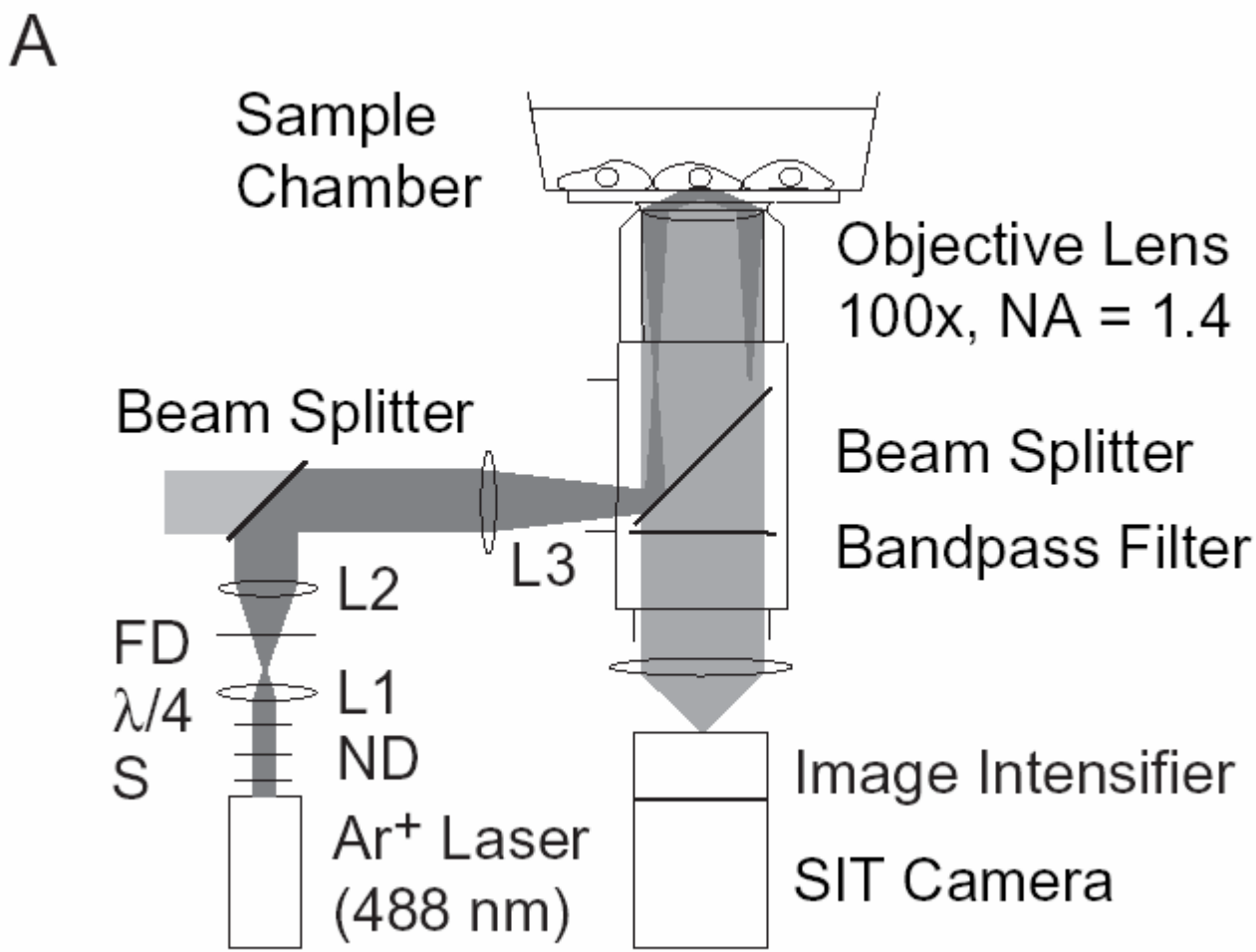
epi

74.3 deg; d=405nm

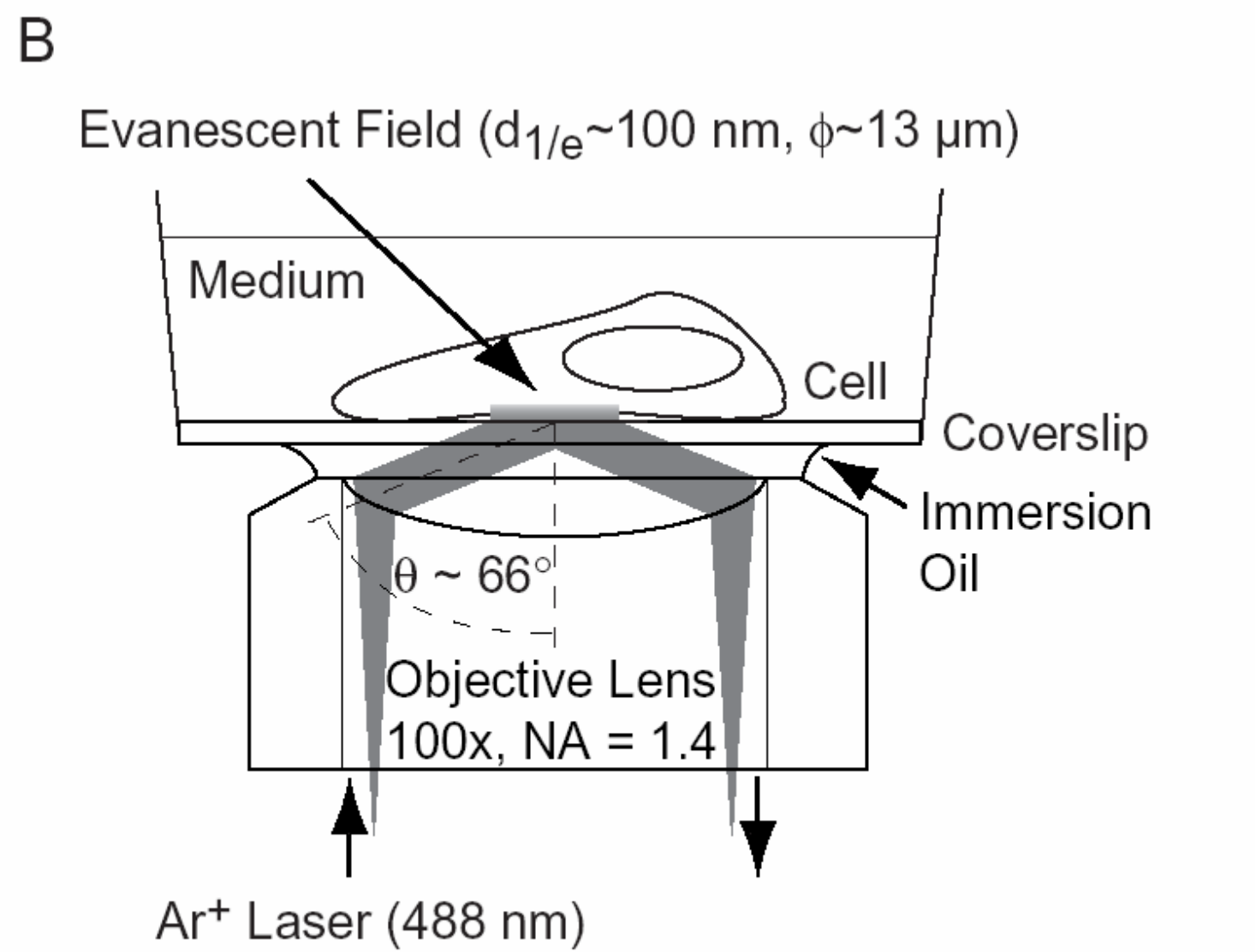
phase

Axelrod, J. Cell Biol. 1981

TIRF excitation using the objective



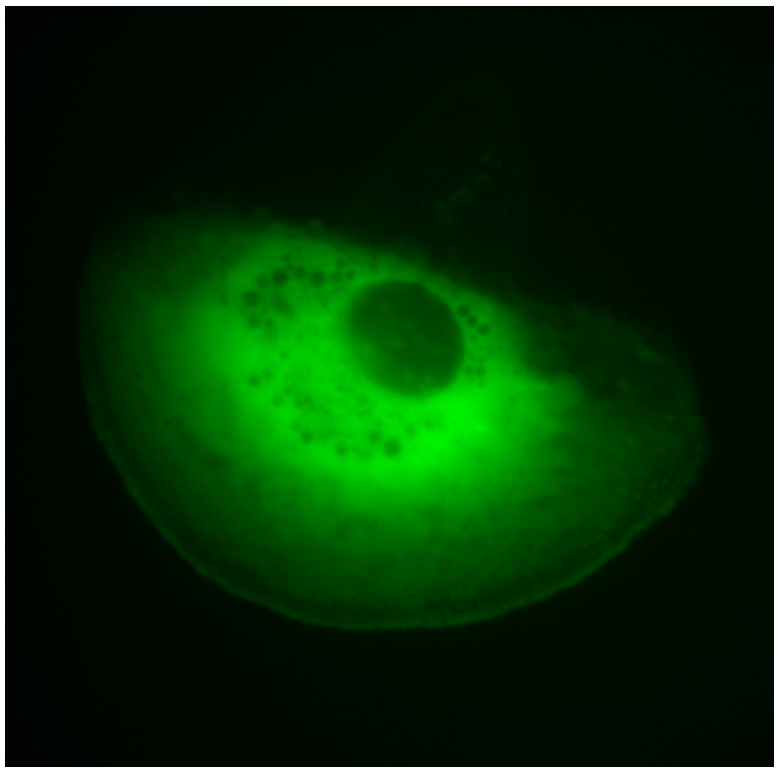
Iino and Kusumi, J. Fluorescence (2001)



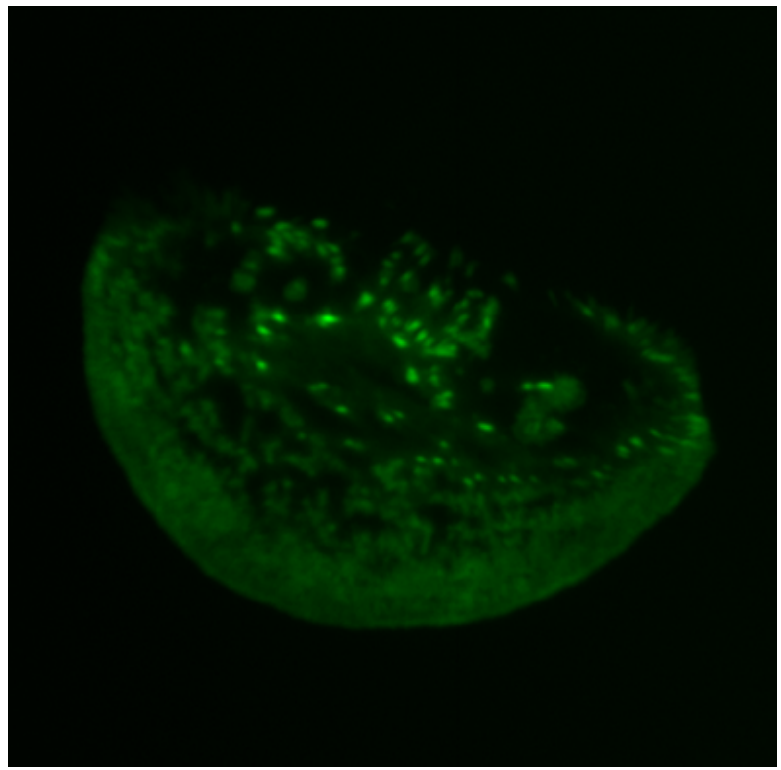
Iino and Kusumi, J. Fluorescence (2001)

Expression of EGFP-paxillin Mutant in MDA-MB-231 Human Breast Cancer Cells Olympus 60x, 1.45 NA

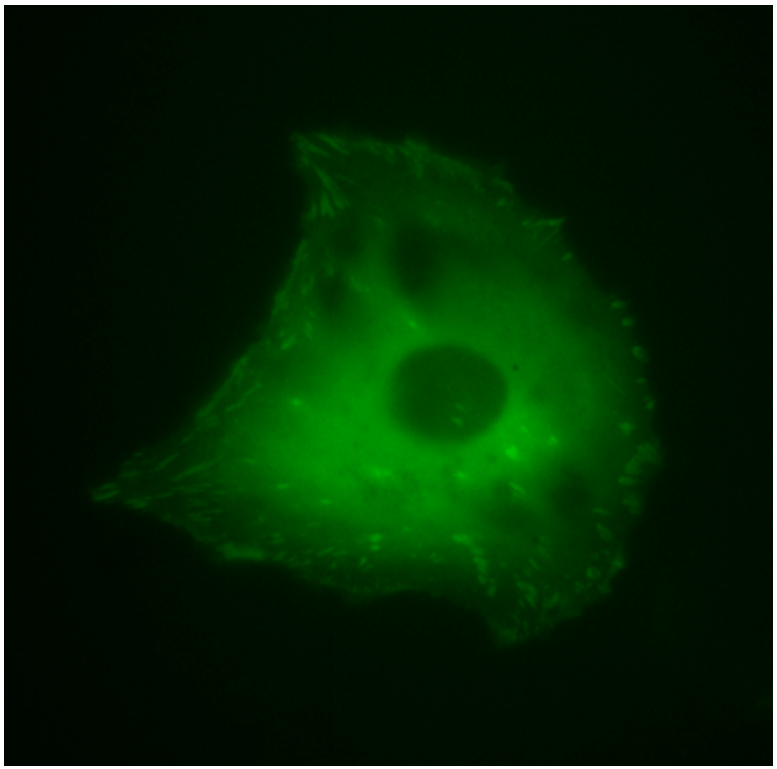
EPI
EGFP



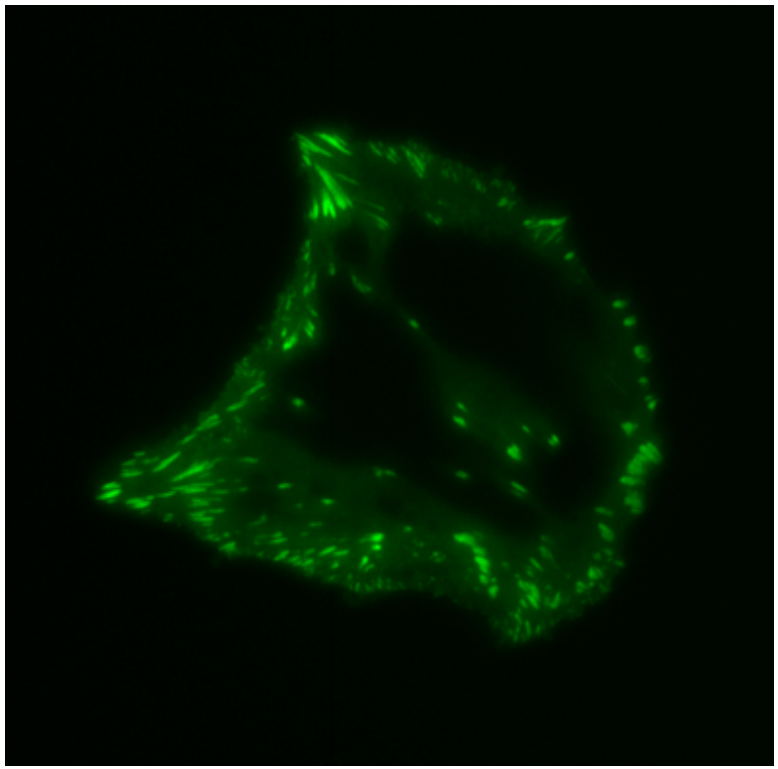
TIRF



EPI

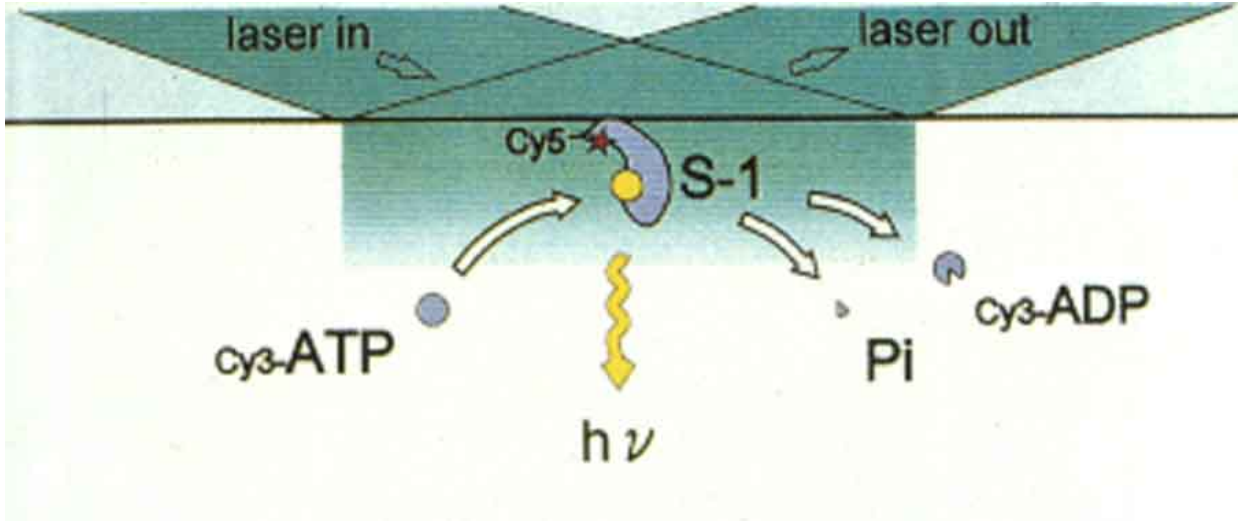


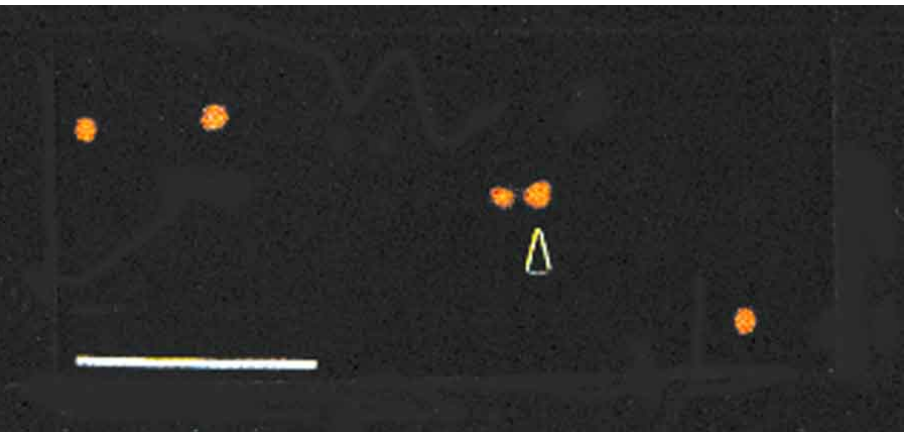
TIRF

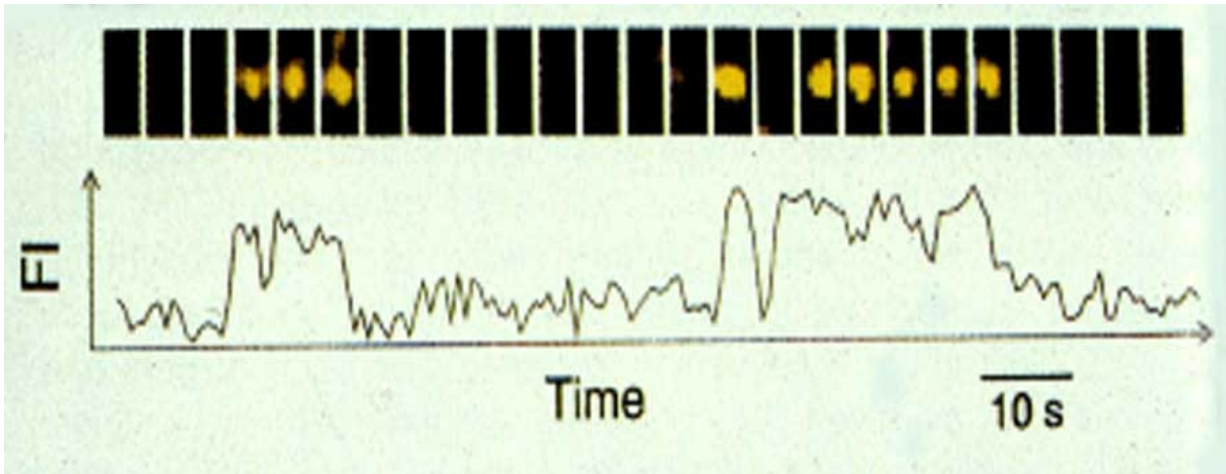


Single molecule studies using TIRF

Yanagida et al Nature 374: 555 (1995)


laser in
laser out
Cy5
S-1
Cy3-ATP
Cy3-ADP
Pi
h ν




FI
Time
10 s