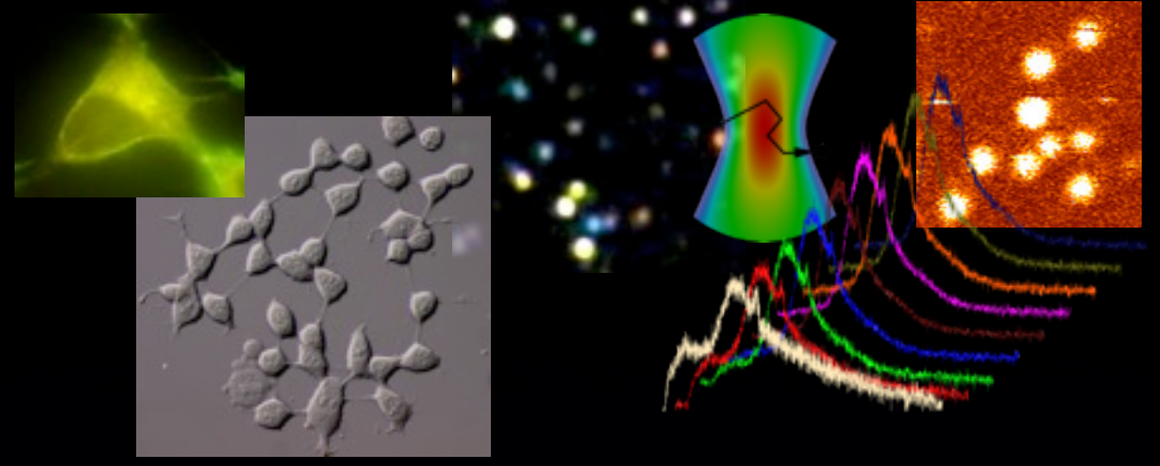


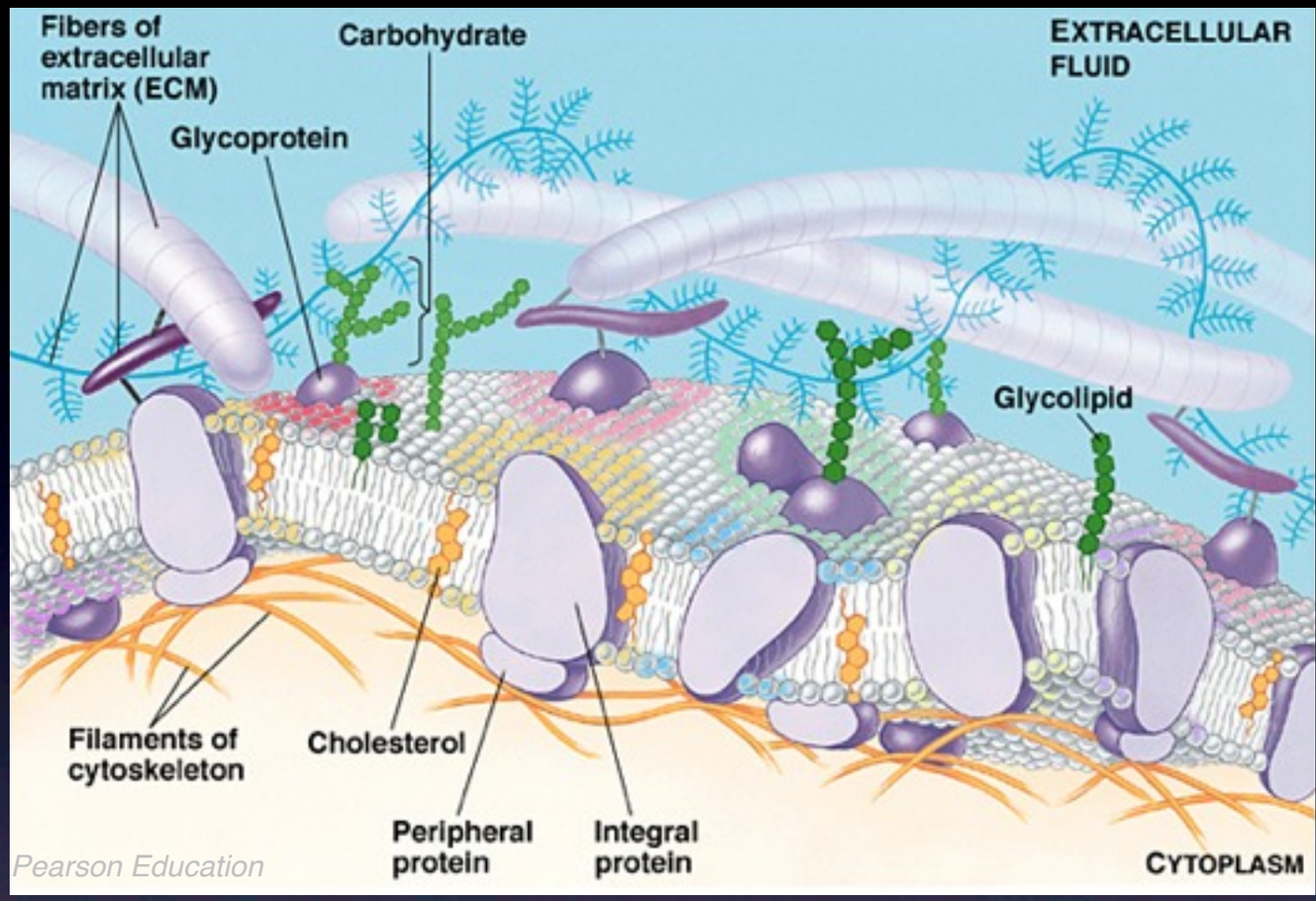
Imagerie et spectroscopie de fluorescence par excitation non radiative

comment s'affranchir de la limite de diffraction

Rodolphe Jaffiol,
Cyrille Vézy, Marcelina Cardoso Dos Santos

LNIO, UTT, Troyes
NanoBioPhotonics group **http://lnio.utt.fr**

Motivations :



Fluorescence nano-imaging

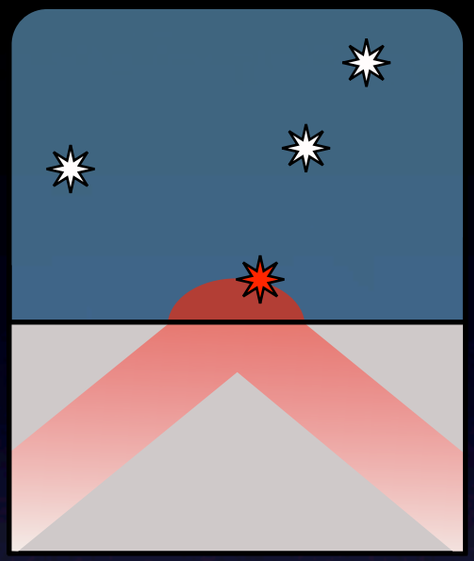
nanoscale spectroscopy

nano-FCS

FCS : Fluorescence Correlation Spectroscopy

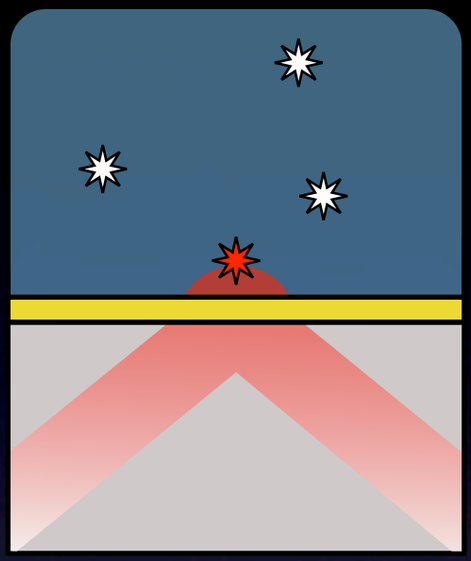
State of the art

TIRFM



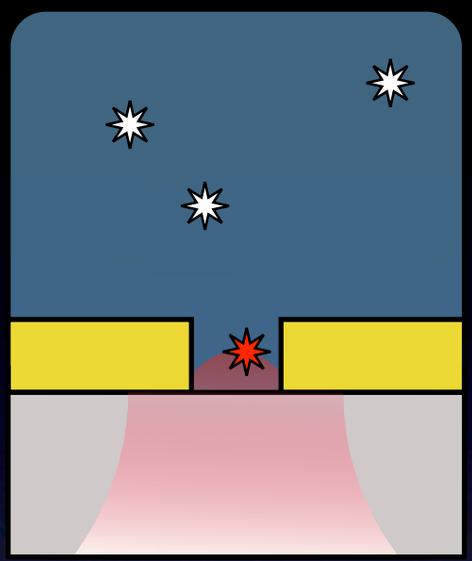
Opt Lett 29 (2004) 569

Surface plasmon



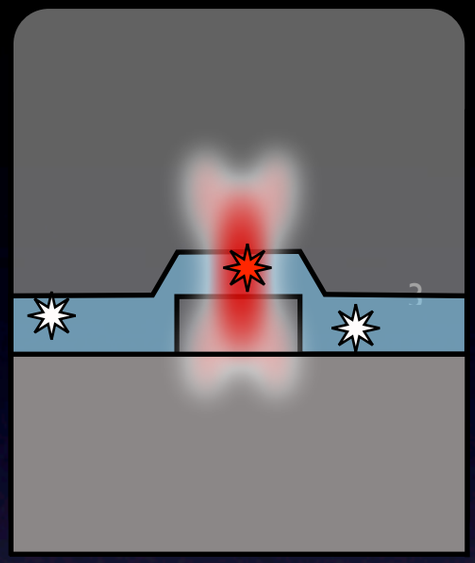
Opt Exp 14 (2006) 7878

nano-hole



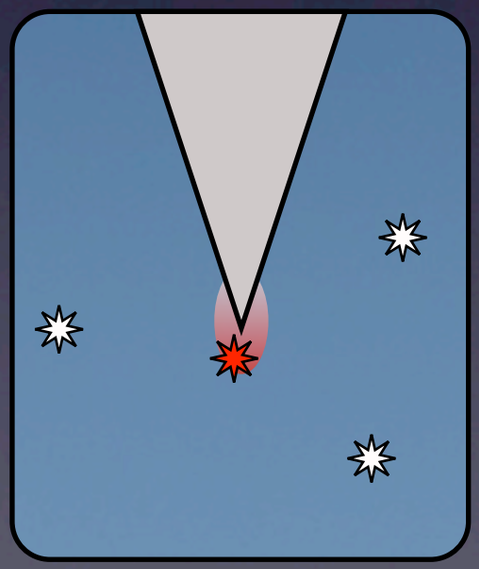
Science 299 (2005) 682
PRL 95 (2005) 117101

sub-µm fluidic system



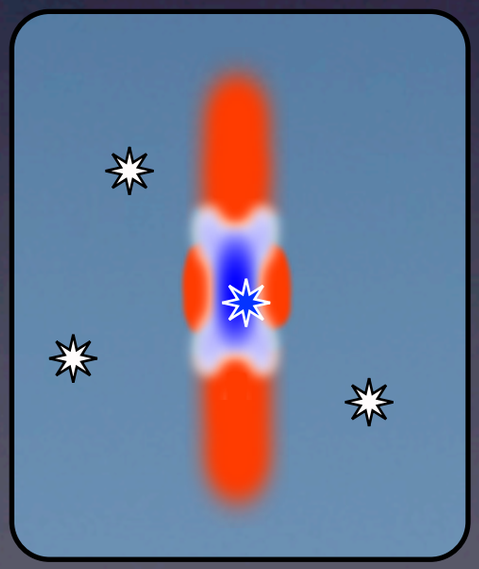
Anal Chem 76 (2004) 1618

SNOM-NFOM



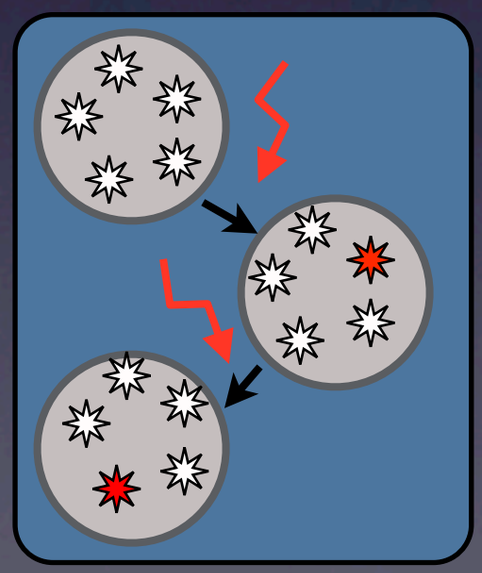
APL 93 (2008) 163904

STED



PRL 94 (2005) 178104

STORM / PALM



Nat. Methods 3 (2006) 793

objectives

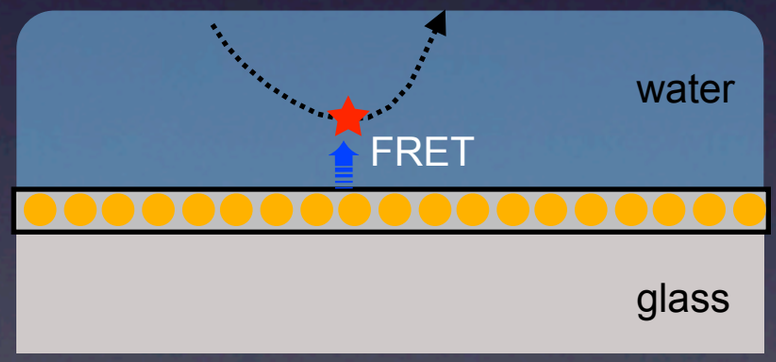
The goal : local illumination with conventional microscope ?

→ The technique:

- based on a new design of the substrate
- compatible with conventional techniques of observation in biology such as: phase contrast, DIC...

→ The method is based on the “activation” of the glass substrate:

thin film of Quantum dots (Qdots) in PMMA (thickness ≈10nm)

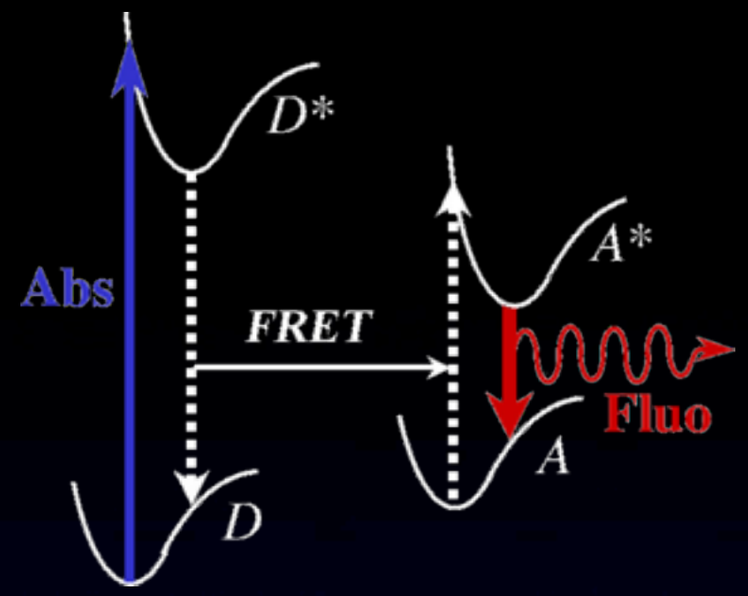


→ The concept:

The illumination of the sample will not be done directly by the laser, but through a Förster non-radiative energy transfer (FRET) from the QDots to dyes

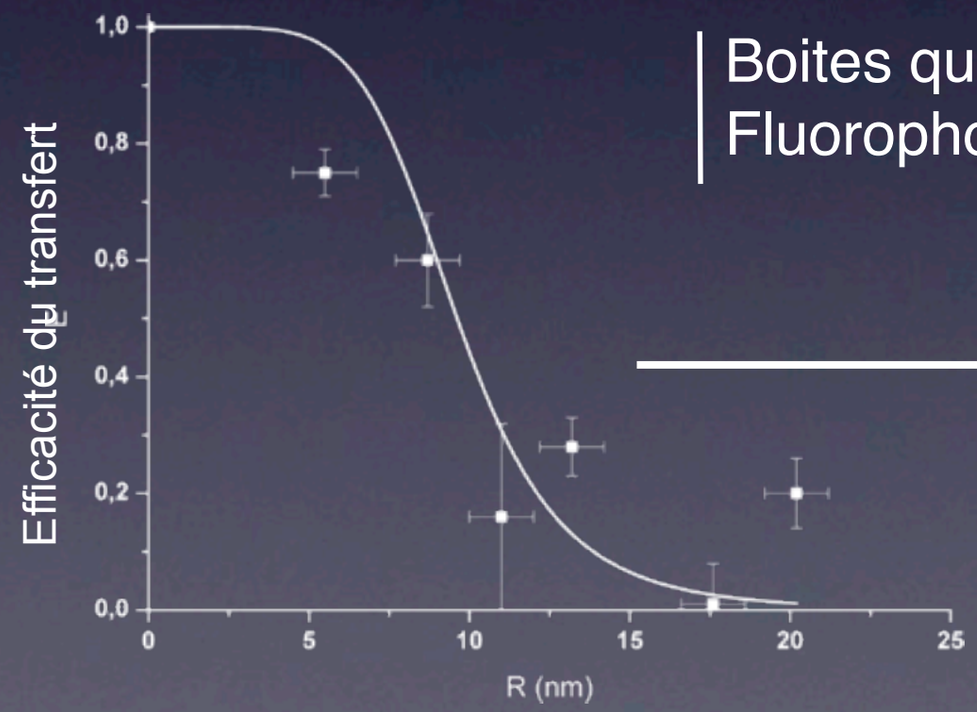
FRET

Taux de transfert : $$k_{Förster} = \frac{1}{\tau_D}\left(\frac{R_0}{R}\right)^6 \propto \frac{\Phi_D}{\tau_D}\frac{I_R}{R^6}$$



⟶ Dépendence en R^{-6} → forte sélectivité spatiale

R_0 : distance de Förster (efficacité de transfert de 50%)



Boites quantiques : FortOrange (Evident Technologies)
Fluorophore : Alexa647 (Invitrogen)

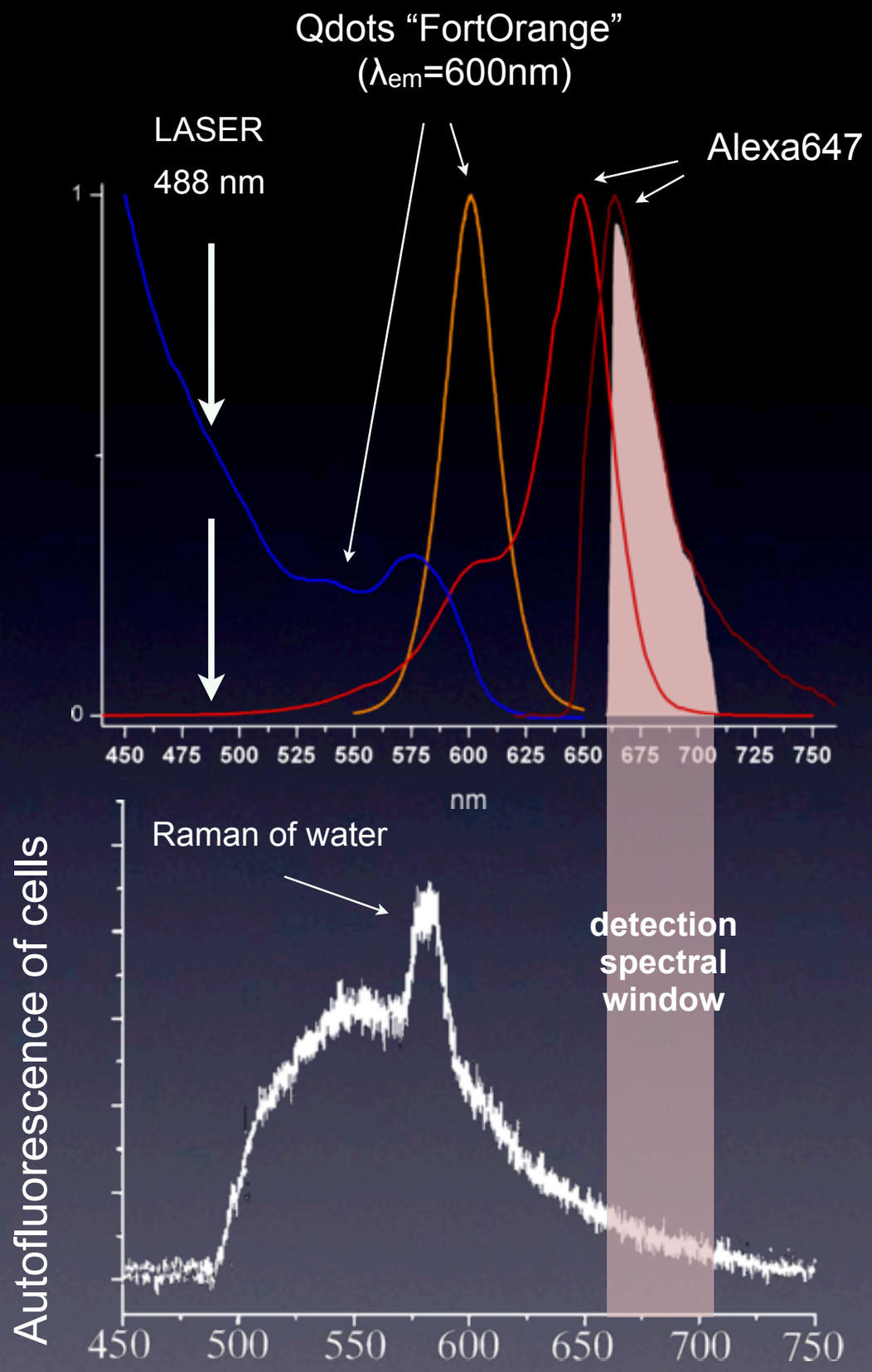
⟶ $R_0^{exp} = 9.6 \pm 0.4$ nm

($R_0^{th} \approx 9.4$ nm)

Qdots advantages

- high photo-stability in regards to photobleaching
- important stoke shift

- definitively eliminates the autofluorescence of cells and Raman scattering of water...
- avoids the direct excitation of the acceptor (Alexa647)

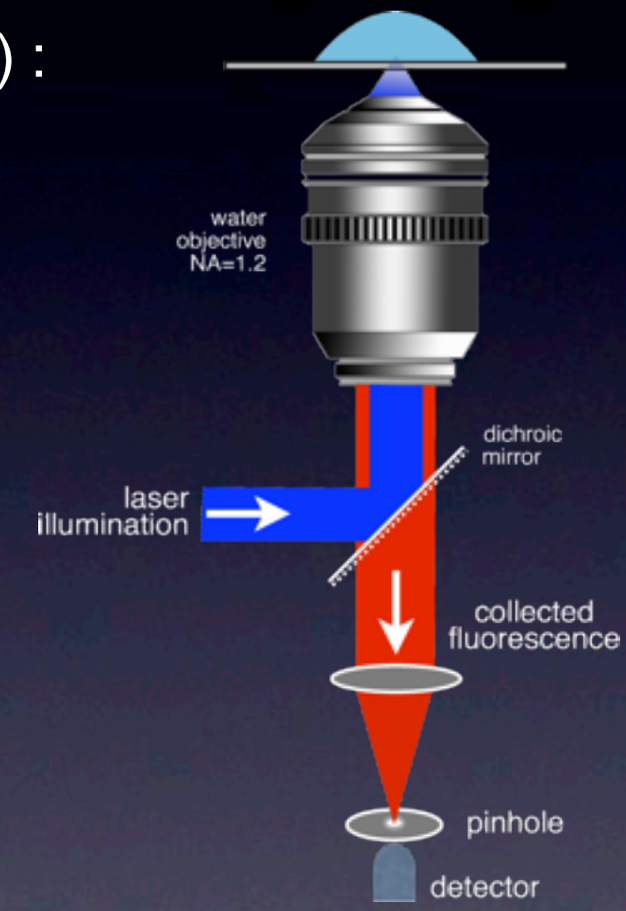


FCS in nano-volume: *motivations*

➠ in Biology

➠ Limitations of “conventional FCS” (based on confocal setup) :

↳ $V_{eff} \approx 0.2 - 1\ \mu m^3$



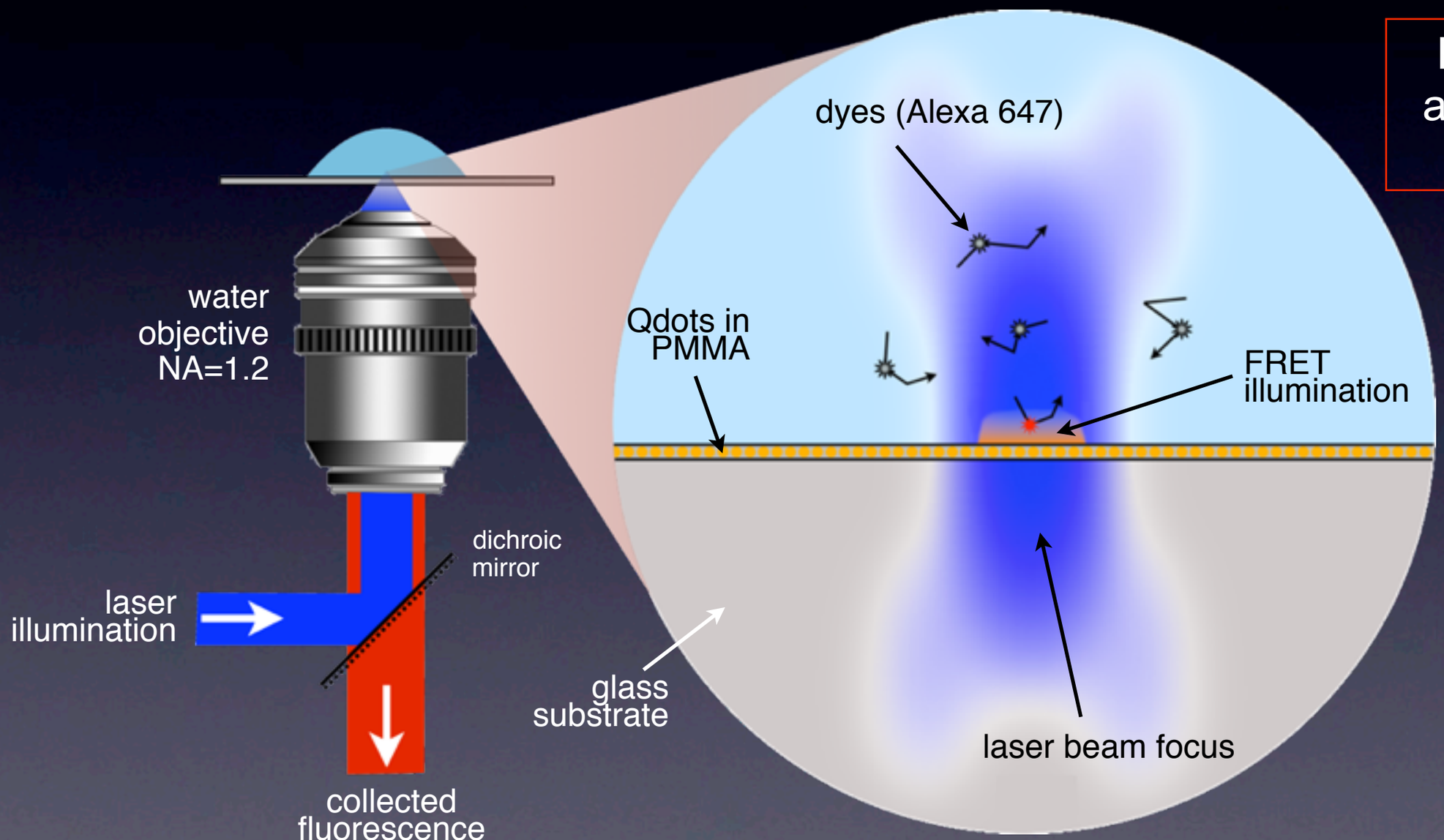
⟶ *Concentration working range : sub-nM to ≈ 50nM*

⟶ *Use of ultra pure solvents (↘ background)*
ultra purified water, PBS for in vivo observation...

➠ Motivations: extend the concentration working range to µM or higher

FCS in a confined volume

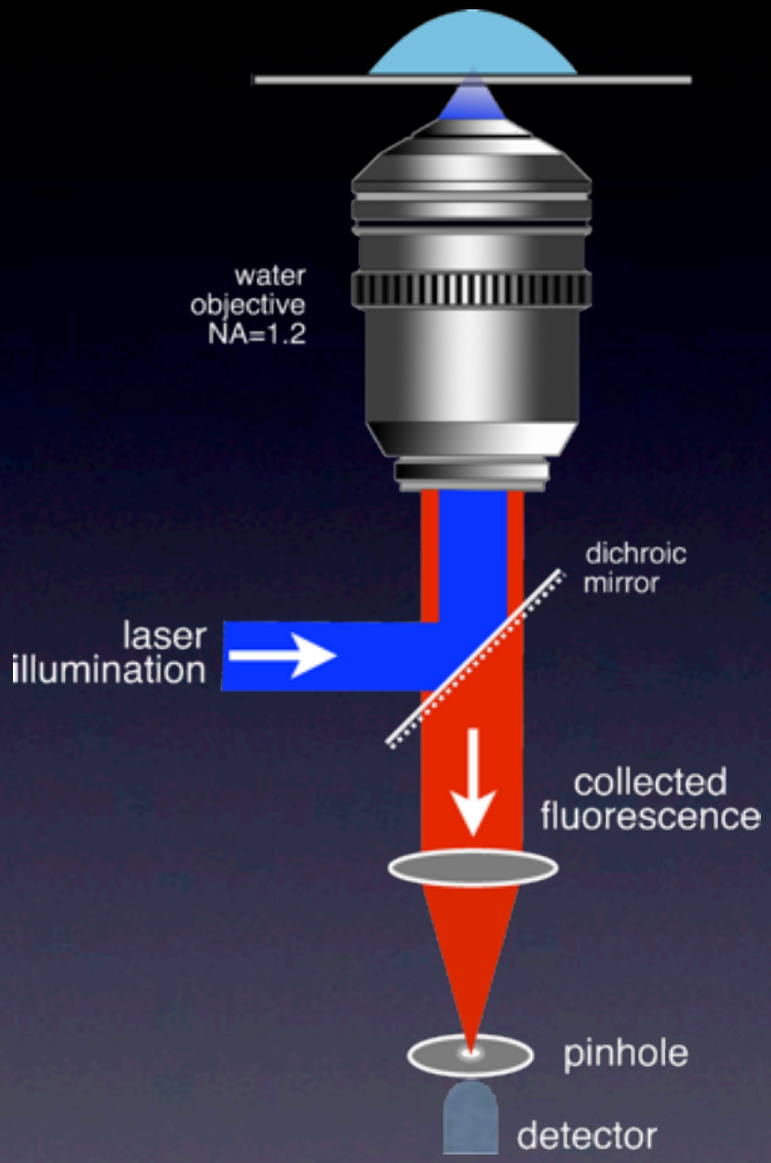
The excitation of Alexa647 will not be done directly by a focused laser beam, but through a FRET process

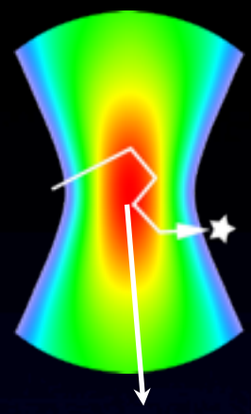


FRET only occurs at the vicinity of the interface

depth ≈10 nm

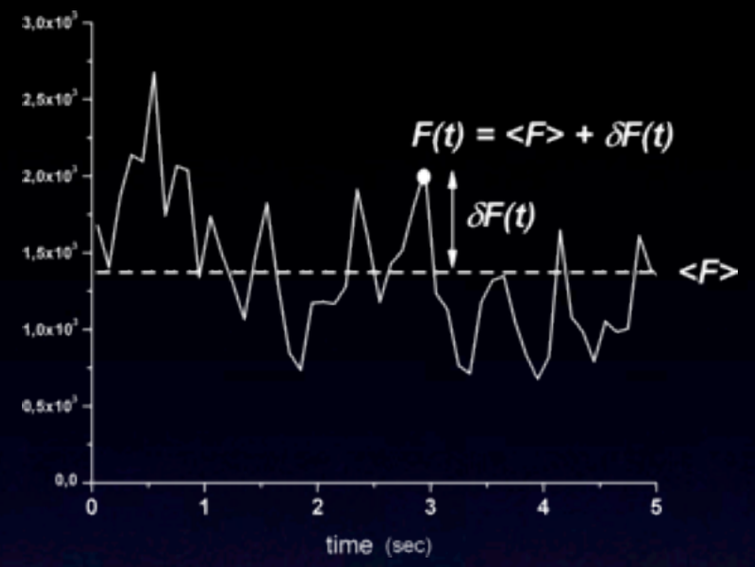
FCS: fluorescence Correlation Spectroscopy





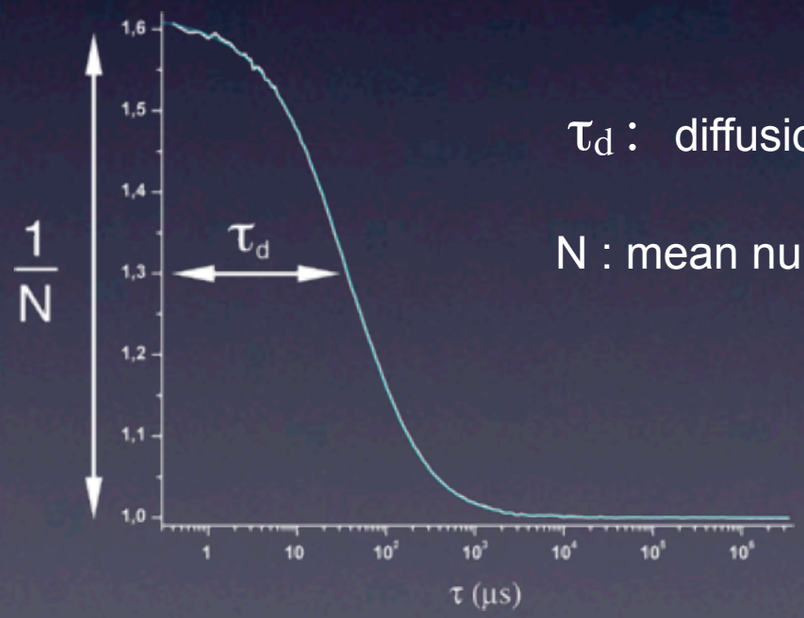
Fluorescence

$\overrightarrow{F(t)}$



Confocal volume

Typical autocorrelation curve G(τ) :



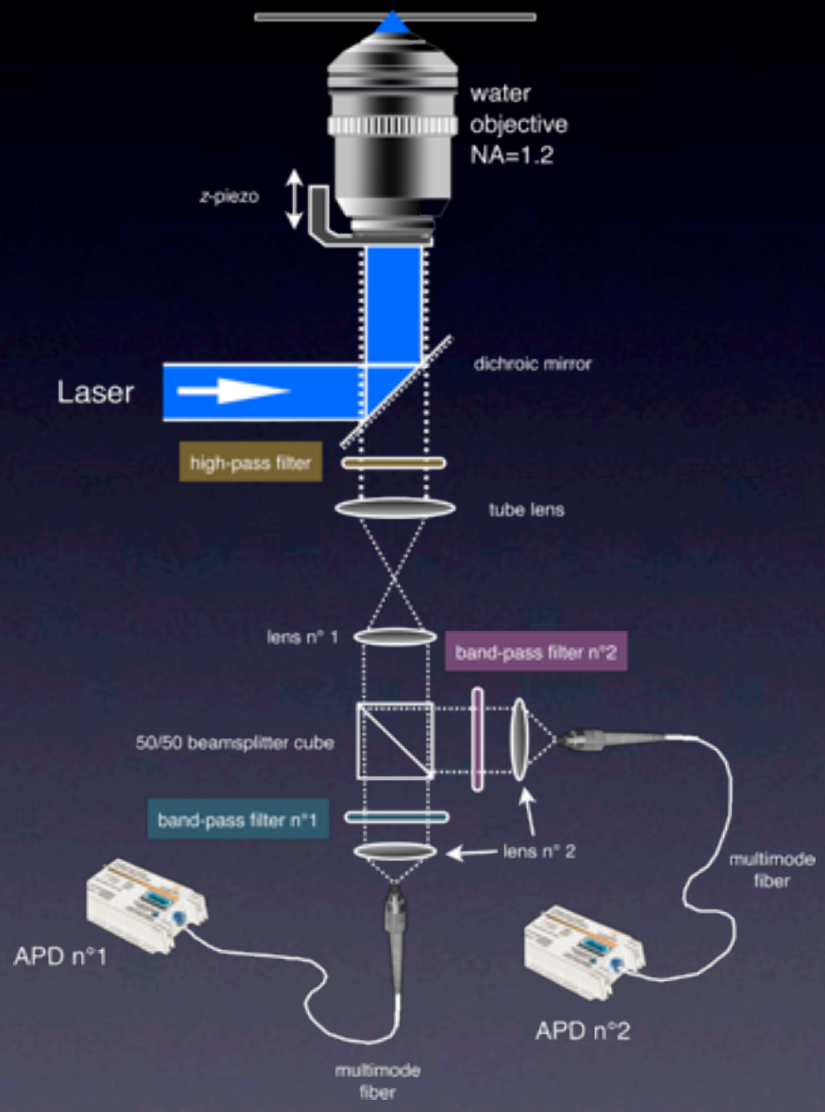
τ_d : diffusion time

N : mean number of molecules

FCS : autocorrelation of the collected fluorescence signal F(t) emitted from dye molecules diffusing through the confocal volume

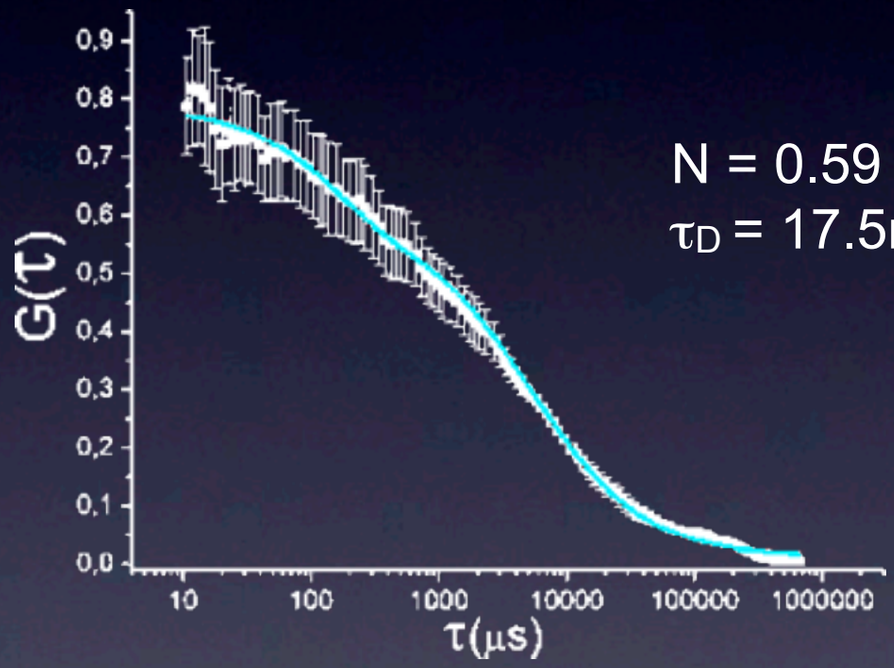
FCS in a confined volume

The confocal microscope:



Experimental ACF @ 405nm with Alexa647 in water:

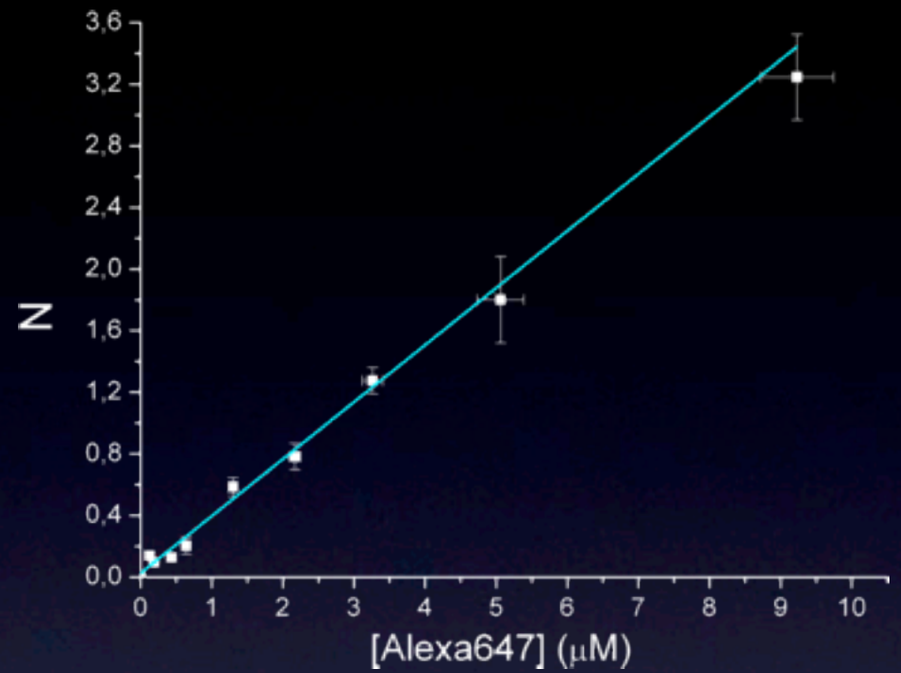
$C_{alexa=}$ 1 µM



N = 0.59
τ_D = 17.5ms

Laser power @ 405nm : ≈100 nW

FCS at high concentrations



$$N = V_{eff} \cdot C$$

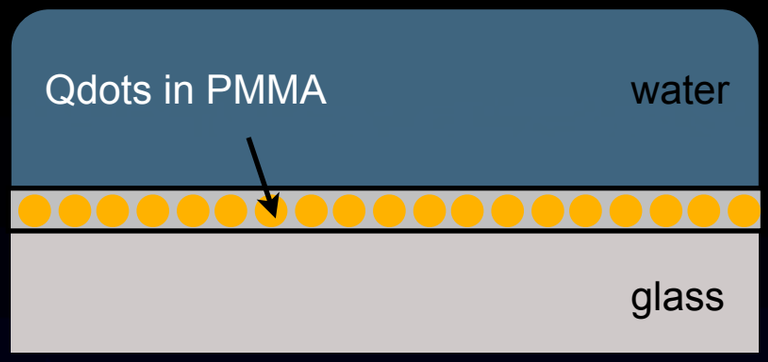
→ The effective observation volume @ 405nm:
V_{eff} = **0.62±0,03** aL (attolitre)

↳ ≈ 700 times smaller than confocal one (@ 632.8nm)

(@ 488 nm: V_{eff} = **2.15±0,05** aL)

strong reduction of V_{eff}:
allows a concentration working range in FCS up to 10 µM !

Application to cell adhesion



- PMMA totally biocompatible for cell culture
- PMMA is hydrophobic → perfect for cell adhesion

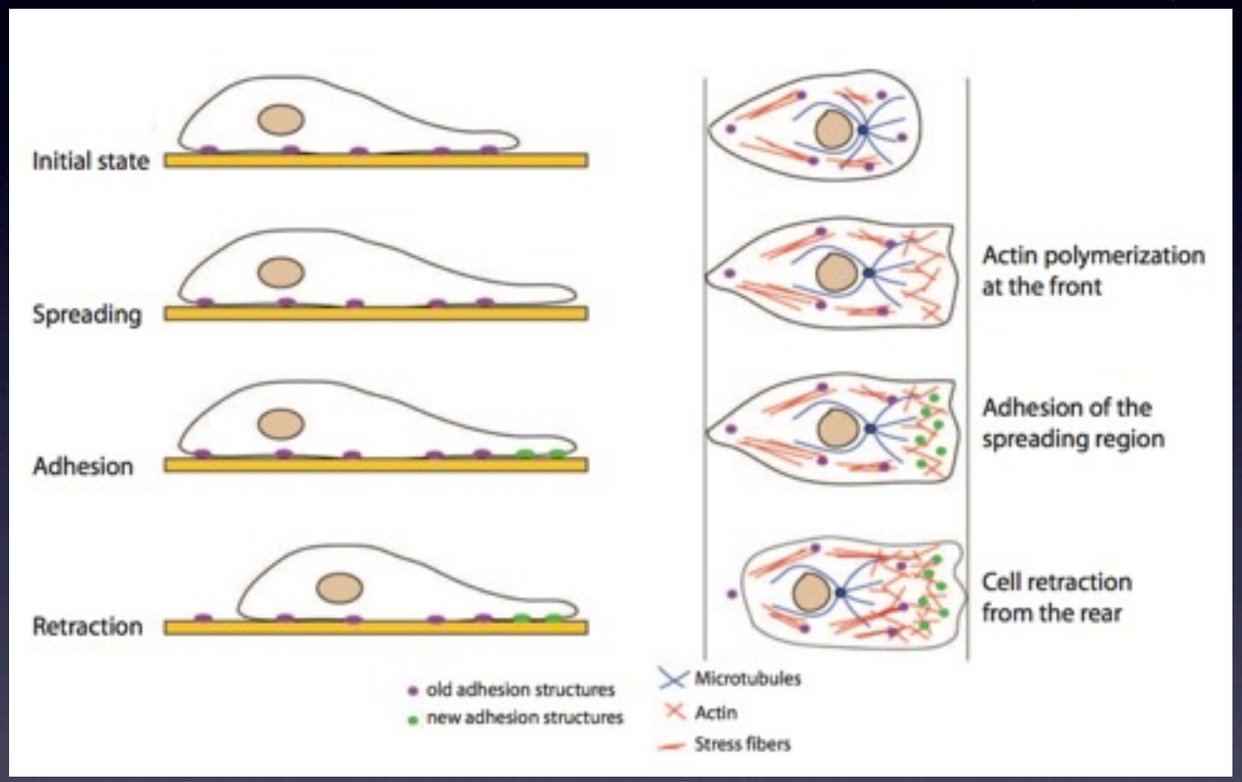
Cell spreading on a surface is associated with important functional changes relevant to survival, proliferation, migration processes... of cells.

The specific junction between cell and surface takes place at the plasma membrane level.

→ the functional units of adhesive contact include multiprotein complexes (integrins, FAKs...) linked to actin filaments.

cell migration

Thèse L. Golé (LPCML)

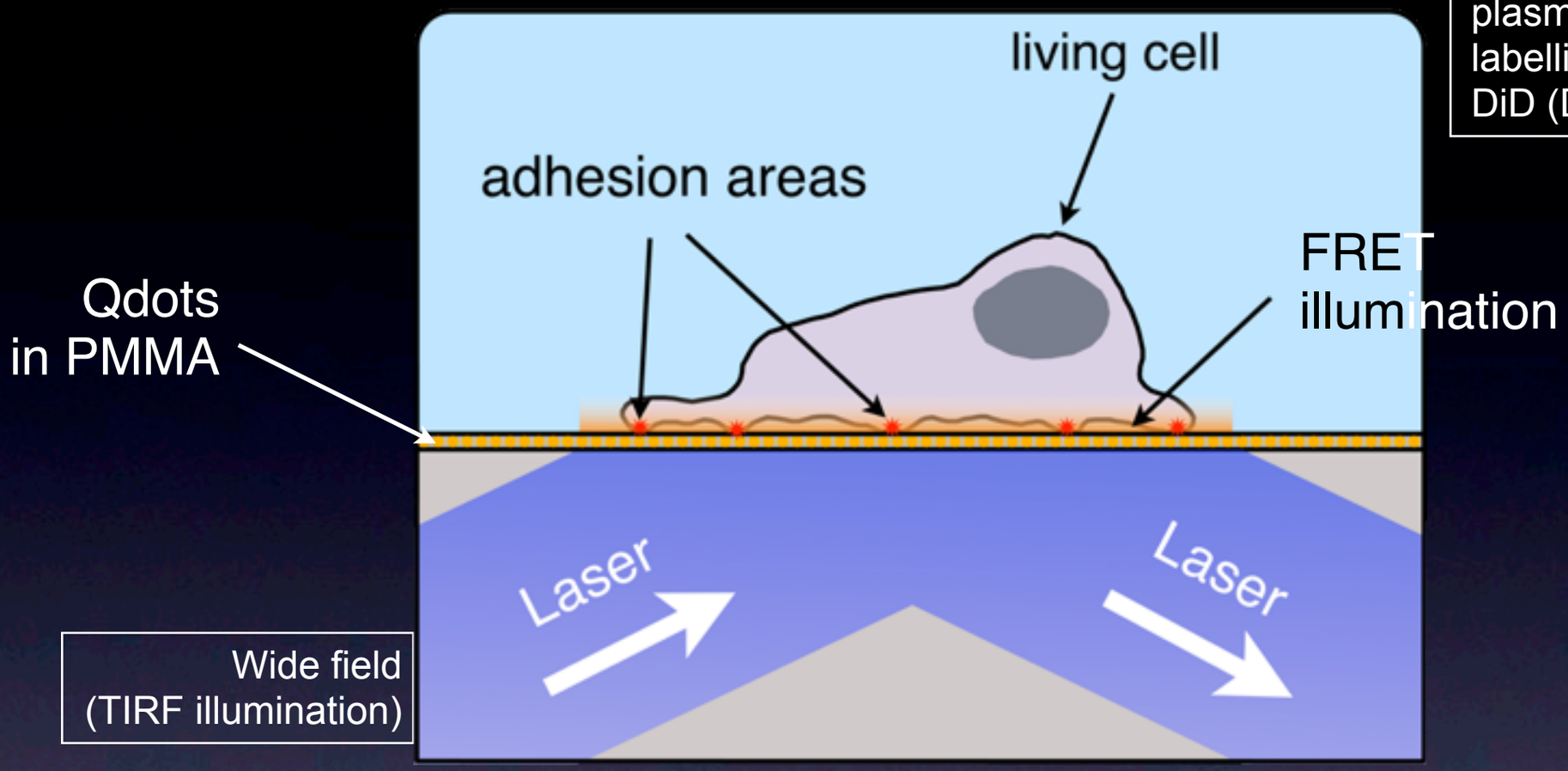


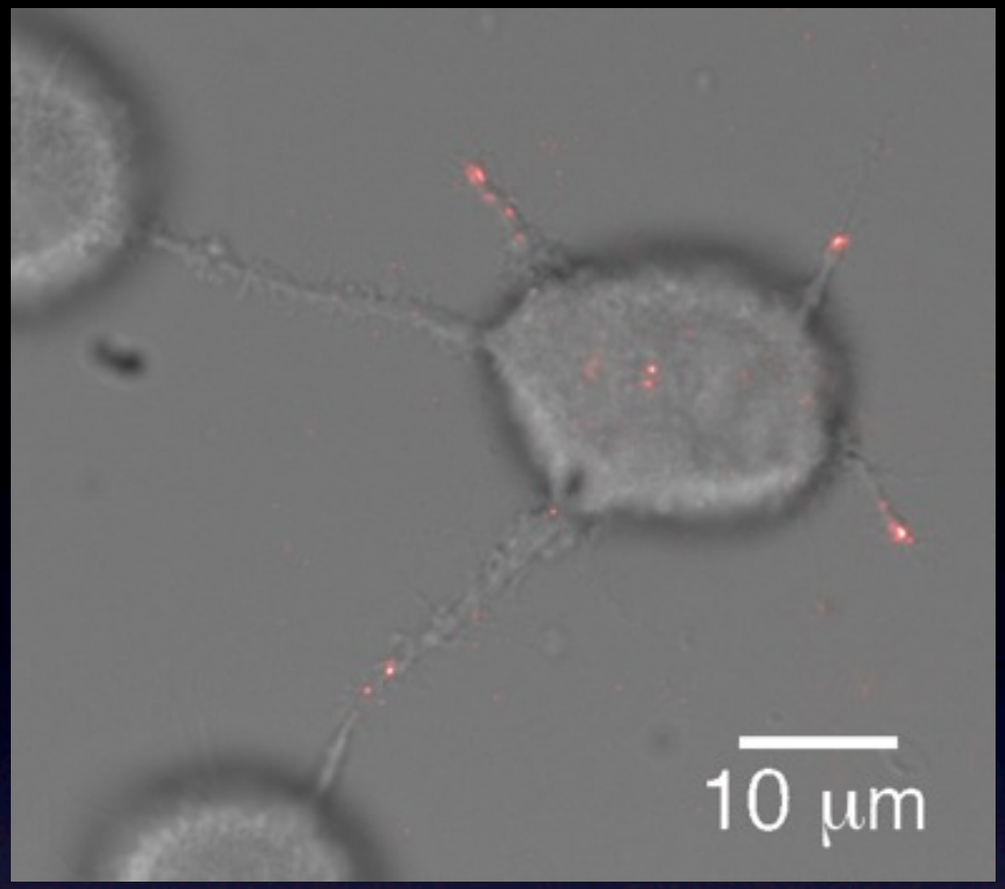
movie :

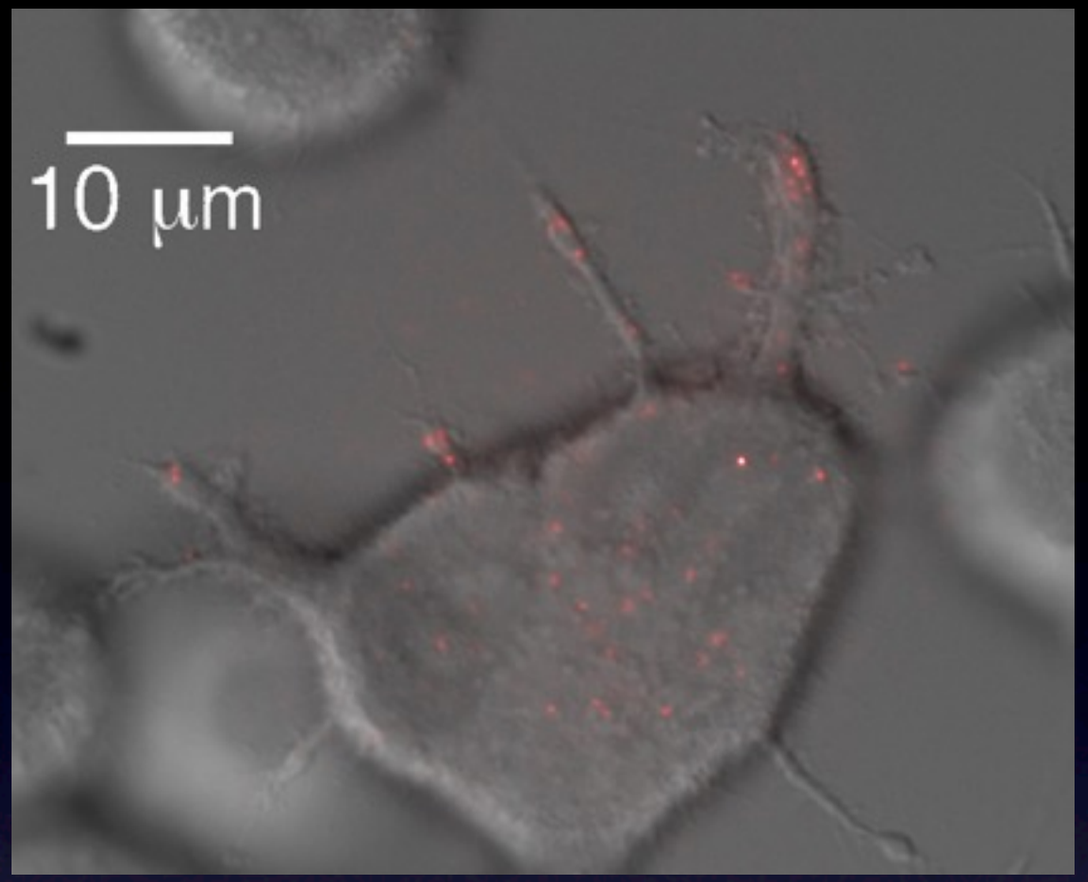
MDCKII cells @37°C
DIC (x60, 0N=1.45)
146 c 109 μm^2

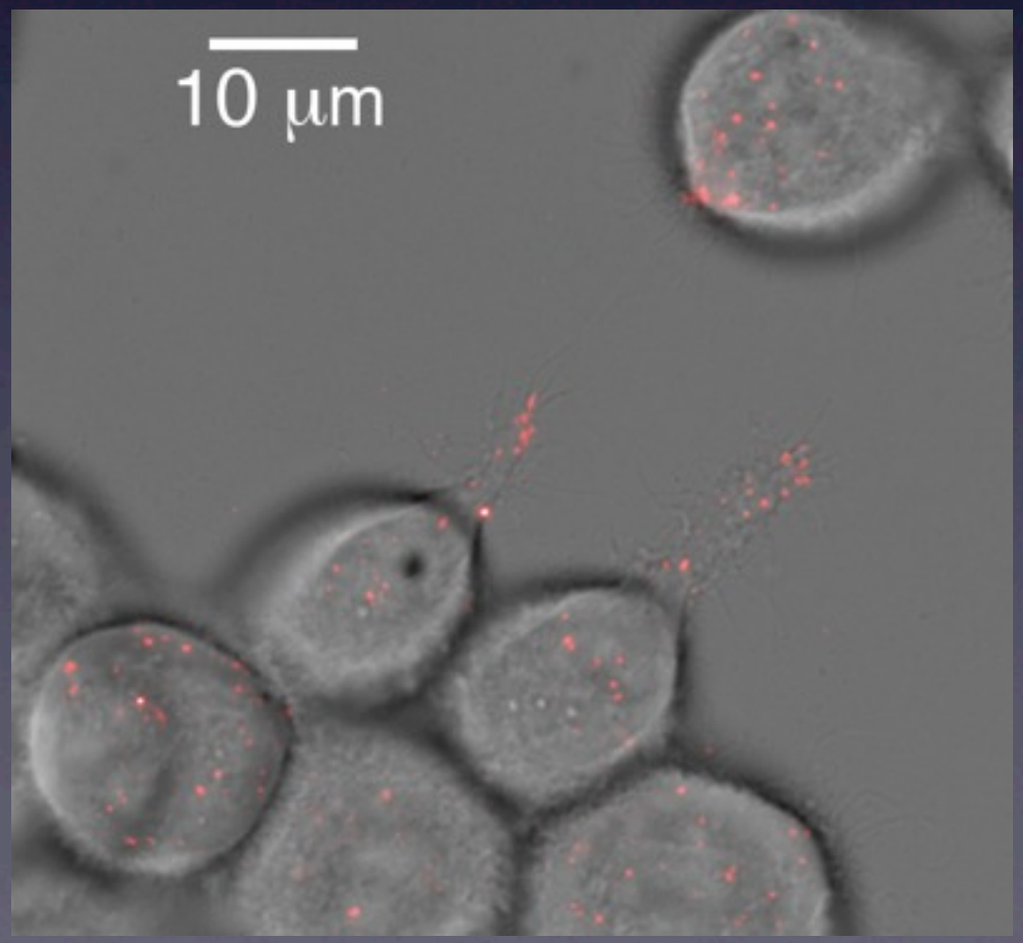
Cell adhesion

plasma membrane labelling with DiD ($DiIC_{18}$ (5) oil)









Images of adhesion areas

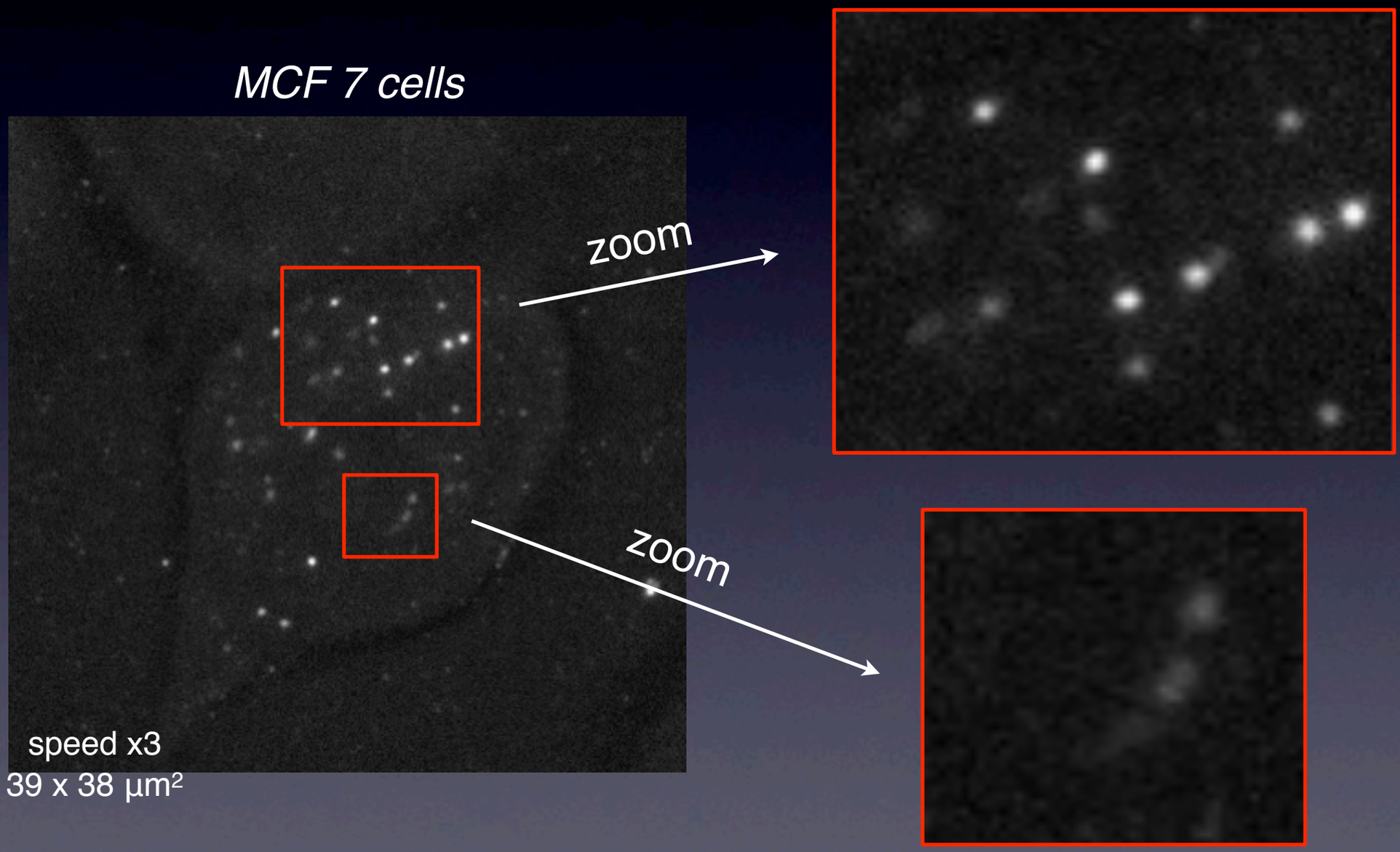
(*MCF-7 human breast cancer cells*)

in red scale: FRET illumination image

in grey scale: DIC picture

Cell adhesion

→ towards a dynamic view of cell adhesion:



Conclusions

- We propose a new scheme for local illumination, based on non-radiative energy transfer

  → strong reduction of the excitation depth (≈10 nm)

- This new illumination scheme was employed to observe cell adhesion

P. Winckler, R. Jaffiol, J. Plain & P. Royer,
Nonradiative Excitation Fluorescence: Probing Volumes Down to the Attoliter Range,
J. Phys Chem. Letters
vol 1 (2010) p 2451-2454

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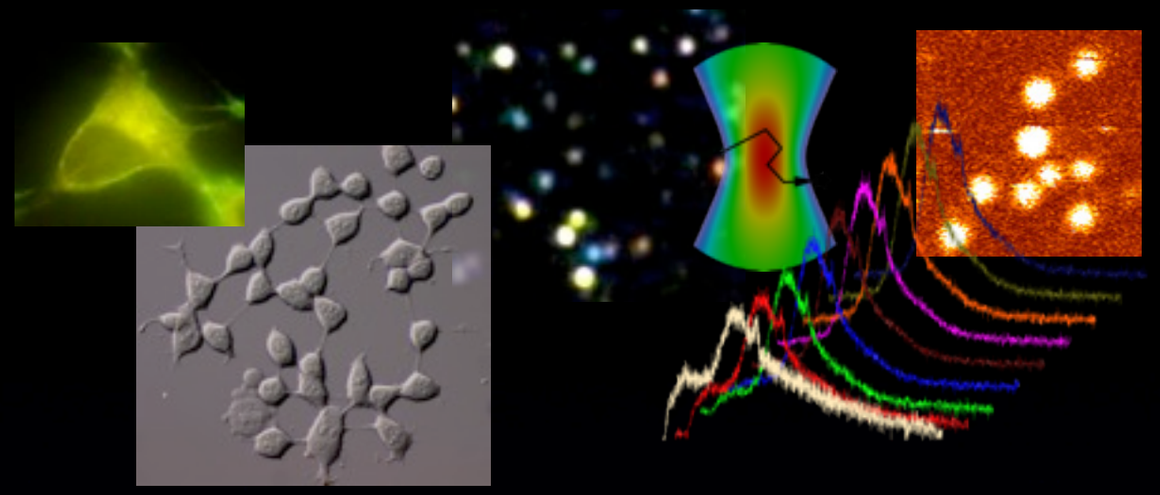

Funding :

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Agence Nationale de la Recherche ANR

Aube Conseil Général

CONTRE LE CANCER LA LIGUE

Thank you for your attention !