

Lab on Chip and Microfluidics

Benoît CHARLOT



l'institut
d'électronique



Part VIII.

Detection

Detection

Optical

- Fluorescence
- Absorption (UV, etc)
- Light scattering
- Refractive index
- SPR

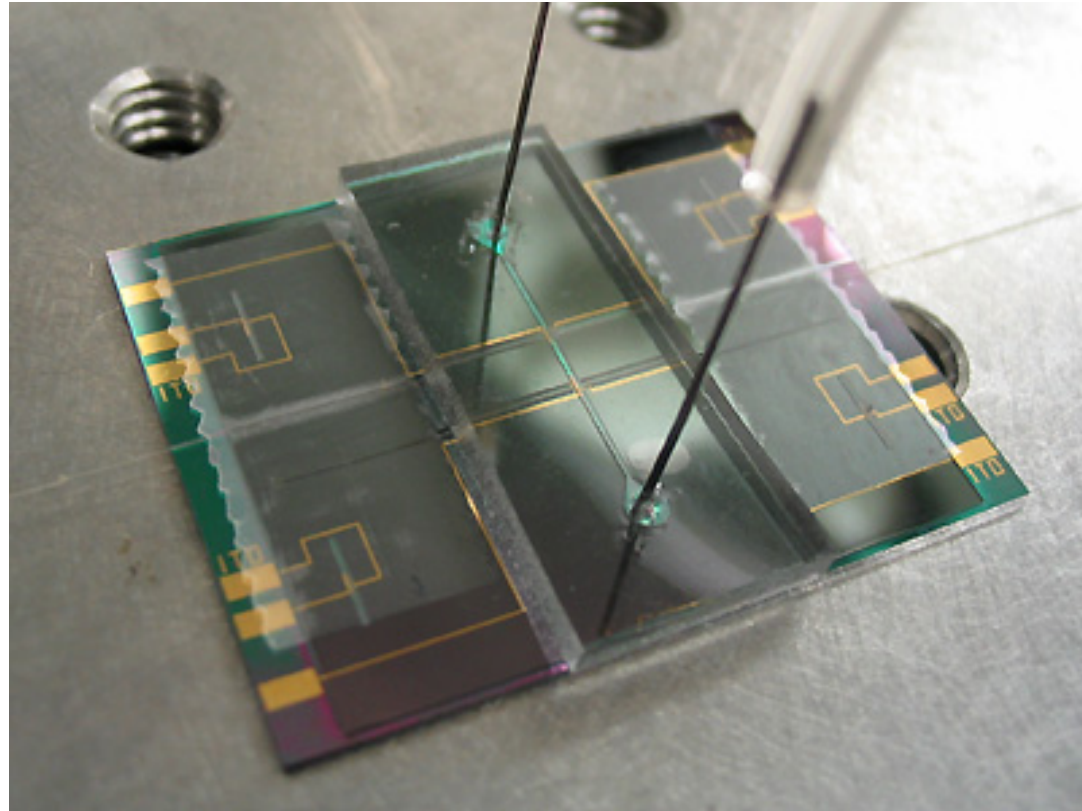
Electrochemical

- Amperometric
- Potentiometric
- Conductimetric

Mechanical

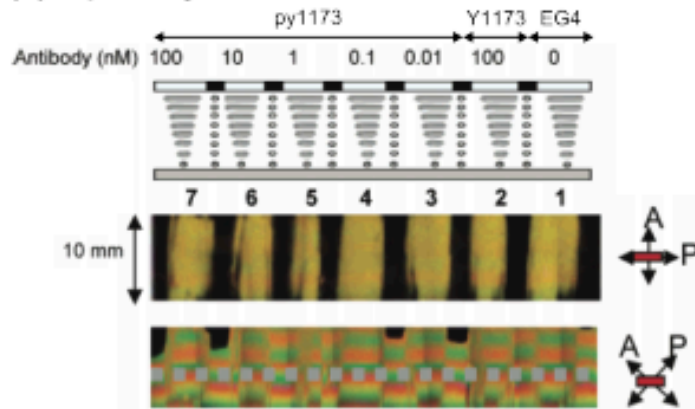
Thermal

...

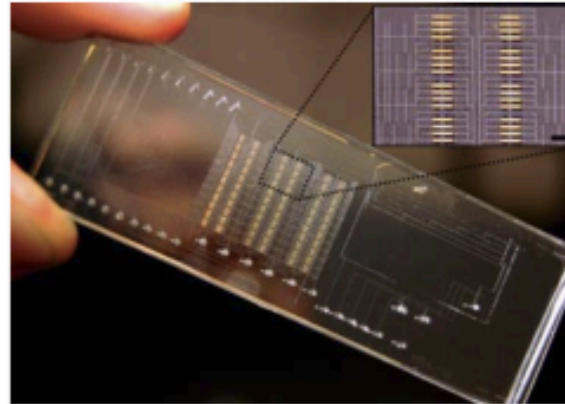


Detection

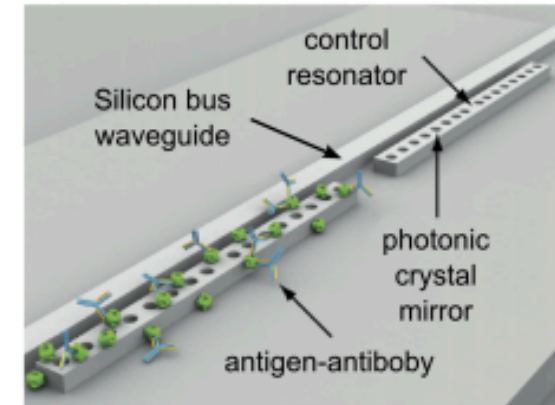
(a) liquid crystals



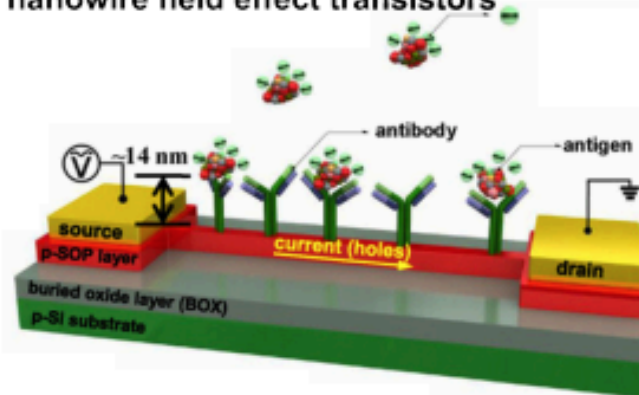
(b) surface plasmon resonance



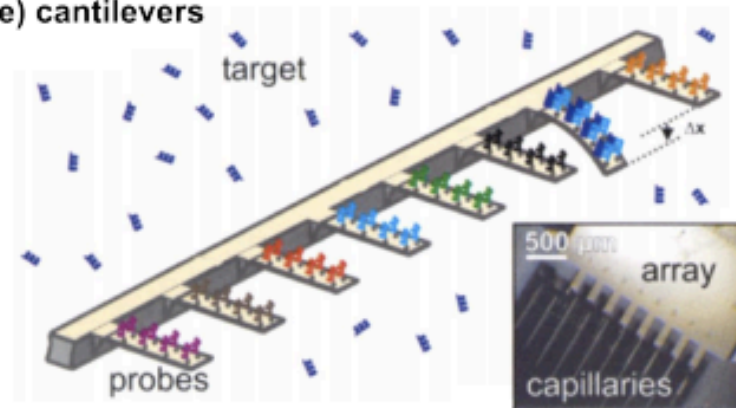
(c) resonant optical waveguides



(d) nanowire field effect transistors



(e) cantilevers



Fluorescence



Fluorescence

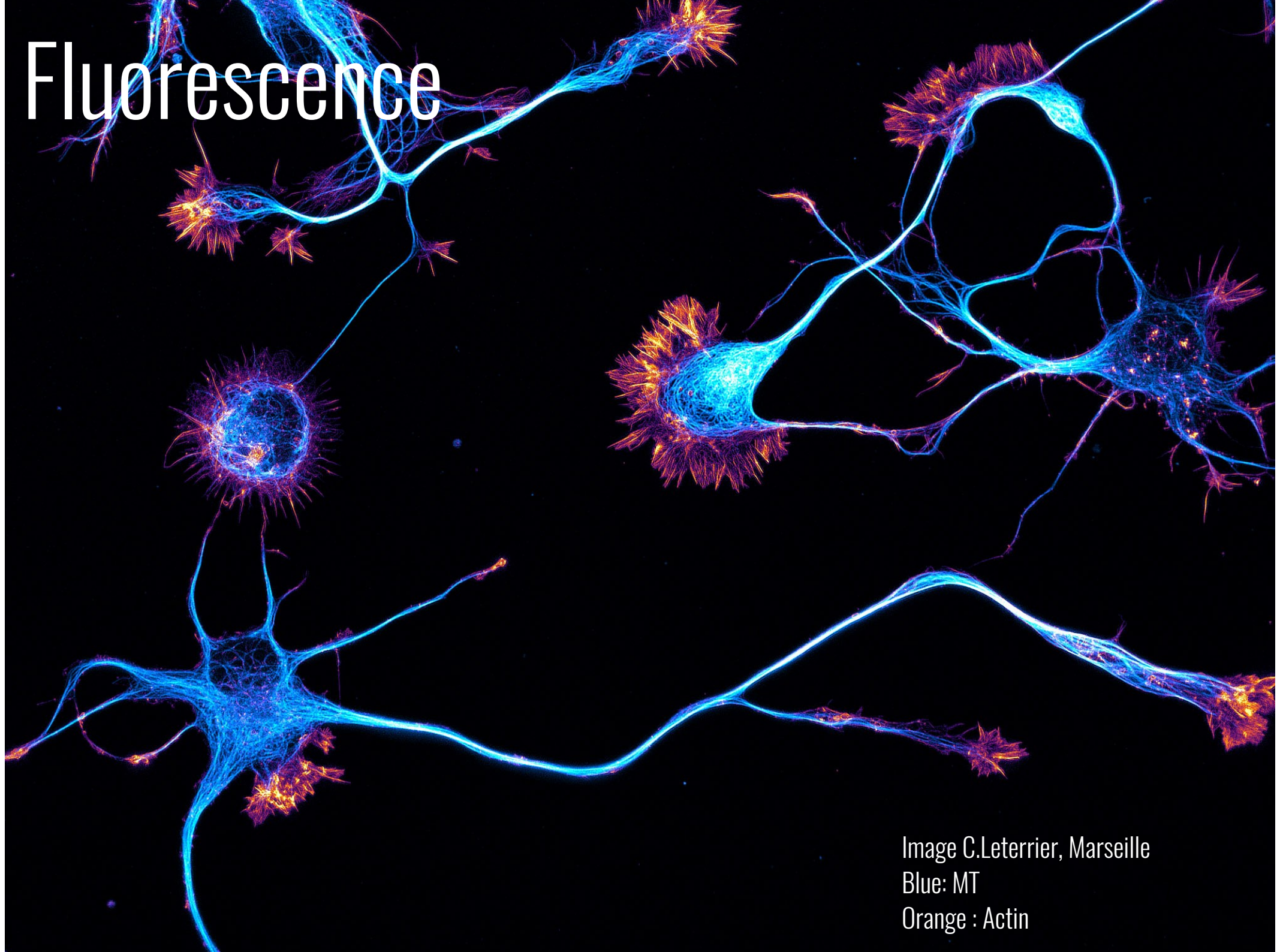


Image C.Leterrier, Marseille
Blue: MT
Orange : Actin

Fluorescence

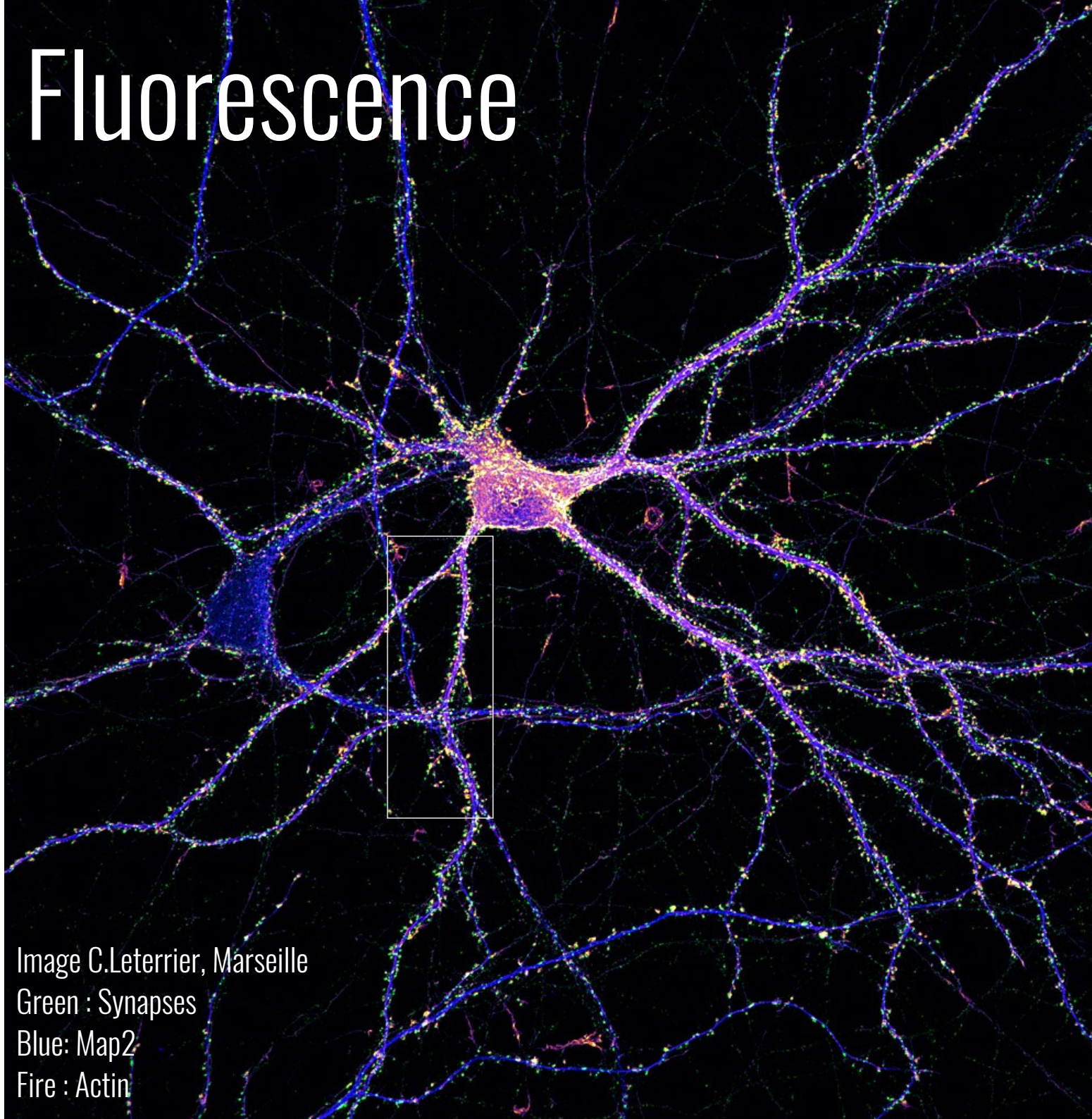
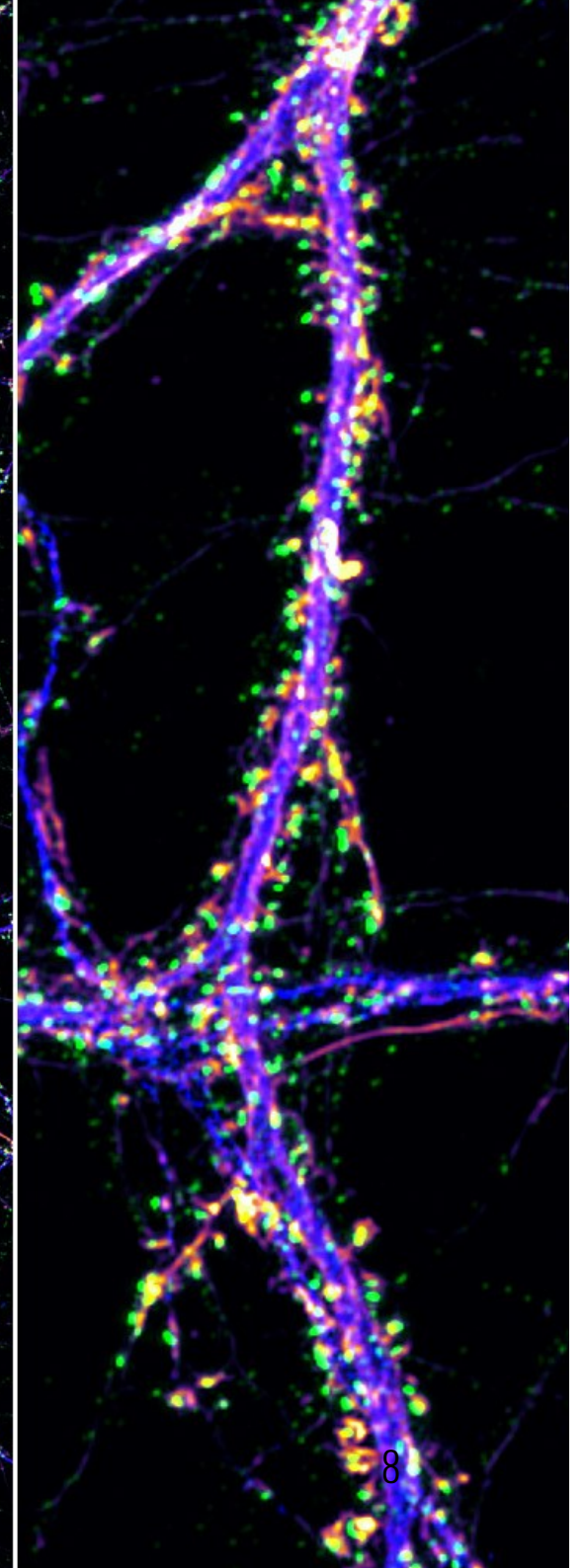


Image C.Leterrier, Marseille

Green : Synapses

Blue: Map2

Fire : Actin



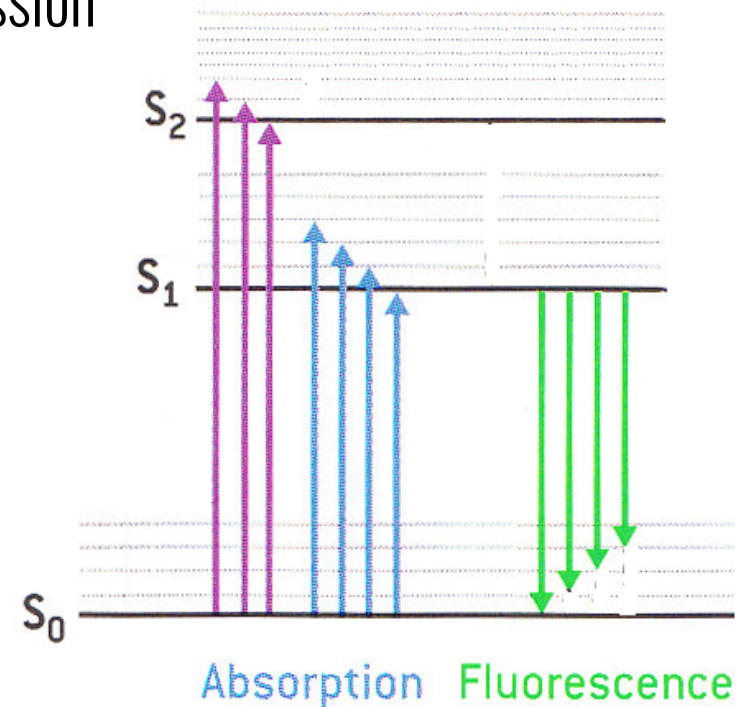
Detection Fluorescence

Fluorescence is the emission of light by a substance (Fluorophores) that has absorbed light.

Excitation : absorption of in coming light, Electron jump to higher energy level

Emission : desexcitation down to lower level, light emission

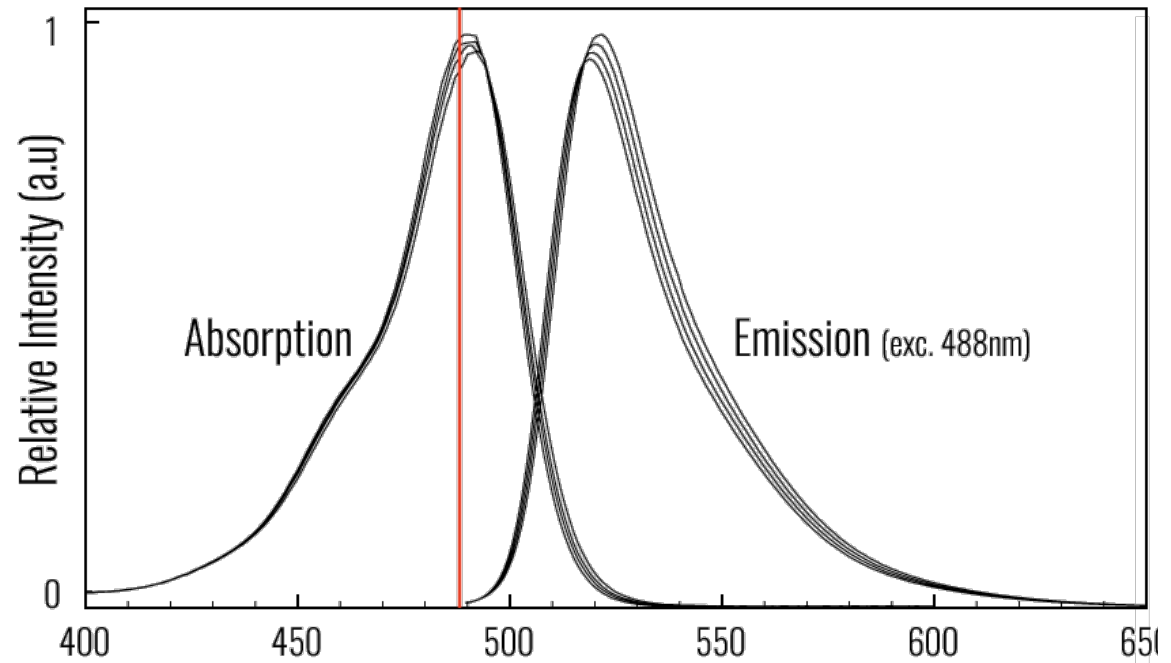
$$E = h\nu = hc/\lambda$$



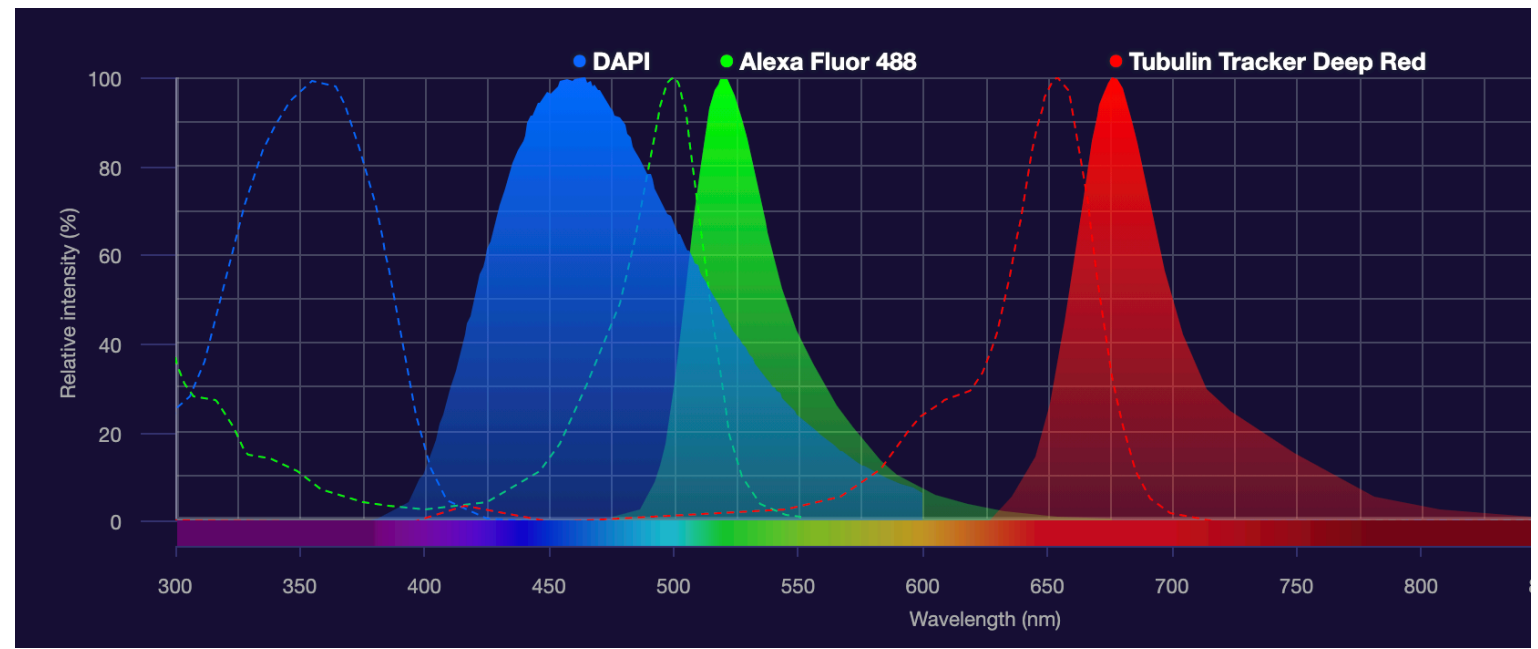
The fluorescence lifetime refers to the average time the molecule stays in its excited state before emitting a photon: 0.5 to 20 nanoseconds

Detection Fluorescence

Fluorescence spectrum ex: Fluorescein



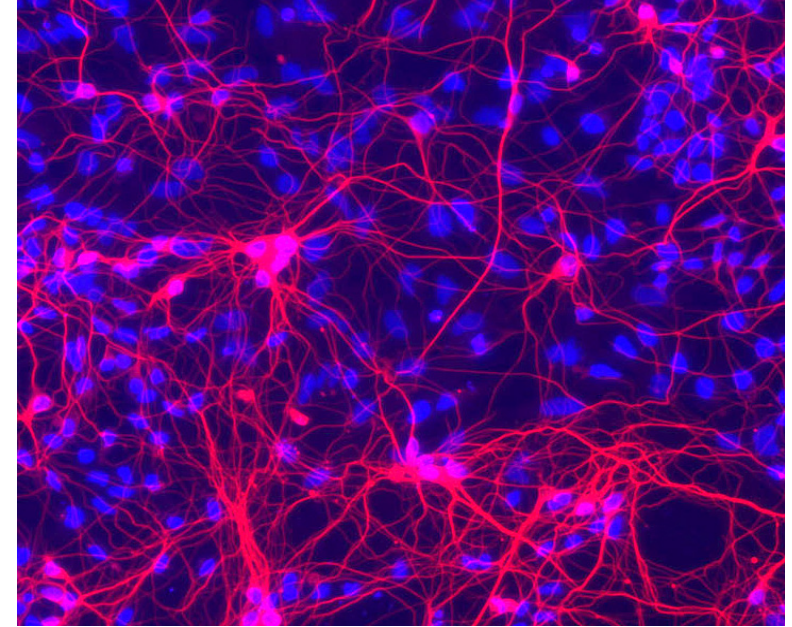
Spectraviewer



Detection Fluorescence

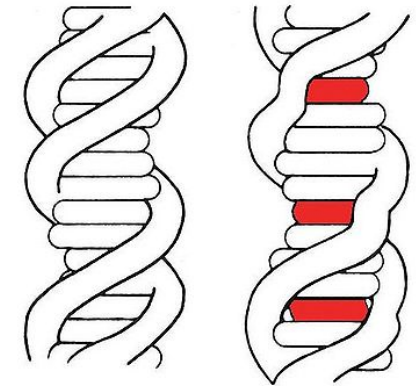
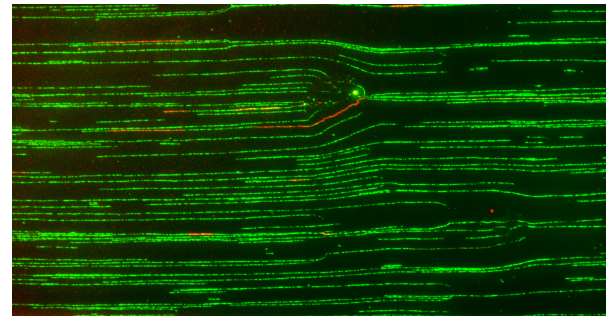
Fluorescence techniques

Staining : DAPI (4',6-diamidino-2-phenylindole)
310 /450 in inserted in DNA and links with A and T bases



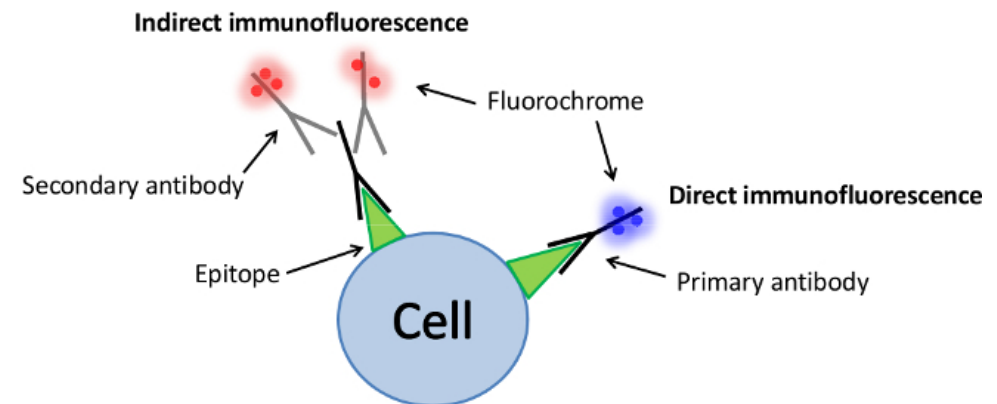
Intercalating dyes : fluorescent insert into DNA

Fluo in PCR, TOTO, YOYO, SYBR Green



Immunofluorescence

specificity of antibodies to their antigen to target fluorescent dyes to specific biomolecule targets within a cell,

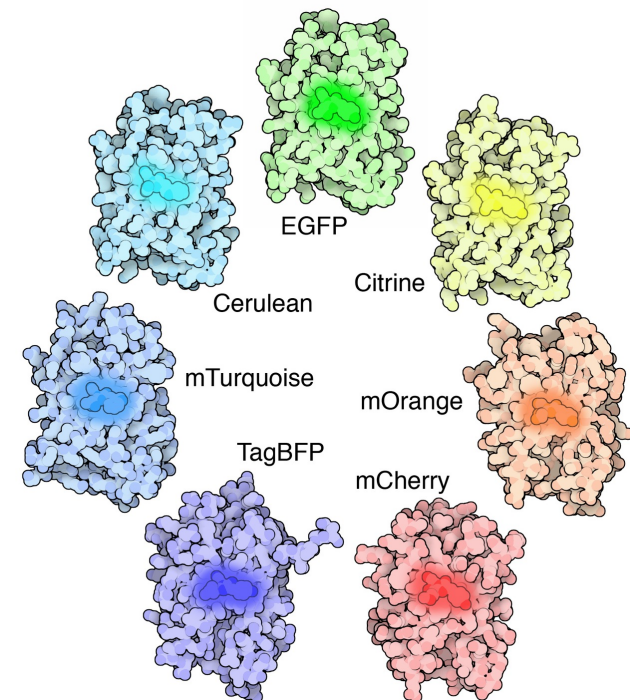
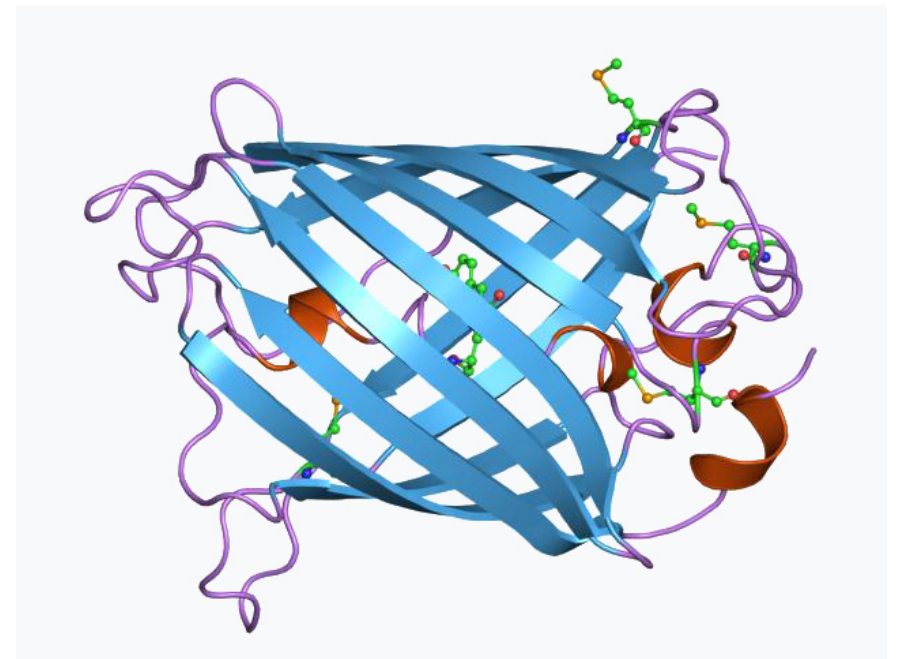


Detection Fluorescence

GFP: Green Fluorescent Protein
(26.9 kDa)
470nm/520nm

GFP gene is used as a reporter of expression.
A reporter gene is a gene that researchers attach to a regulatory sequence of another gene of interest in bacteria, cell culture, animals or plants.

Transfection : introducing **nucleic acids** into cells
(Electroporation)
Expression
Fluorescence





This frog has been engineered to express green fluorescent protein in its muscle cells.
(Image: © Jonathan Slack, University of Minnesota.)

Detection Fluorescence

FISH (Fluorescence In Situ Hybridization)

FLIM (Fluorescence Lifetime Imaging Microscopy)

FRET Fluorescence Resonance Energy Transfer

FRAP (Fluorescence Recovery After Photobleaching)

FACS (fluorescence-activated cell sorting)

Calcium imaging / GEVIS

Detection Fluorescence

Fluorescence microscopy

Epifluorescence

Confocal microscopy

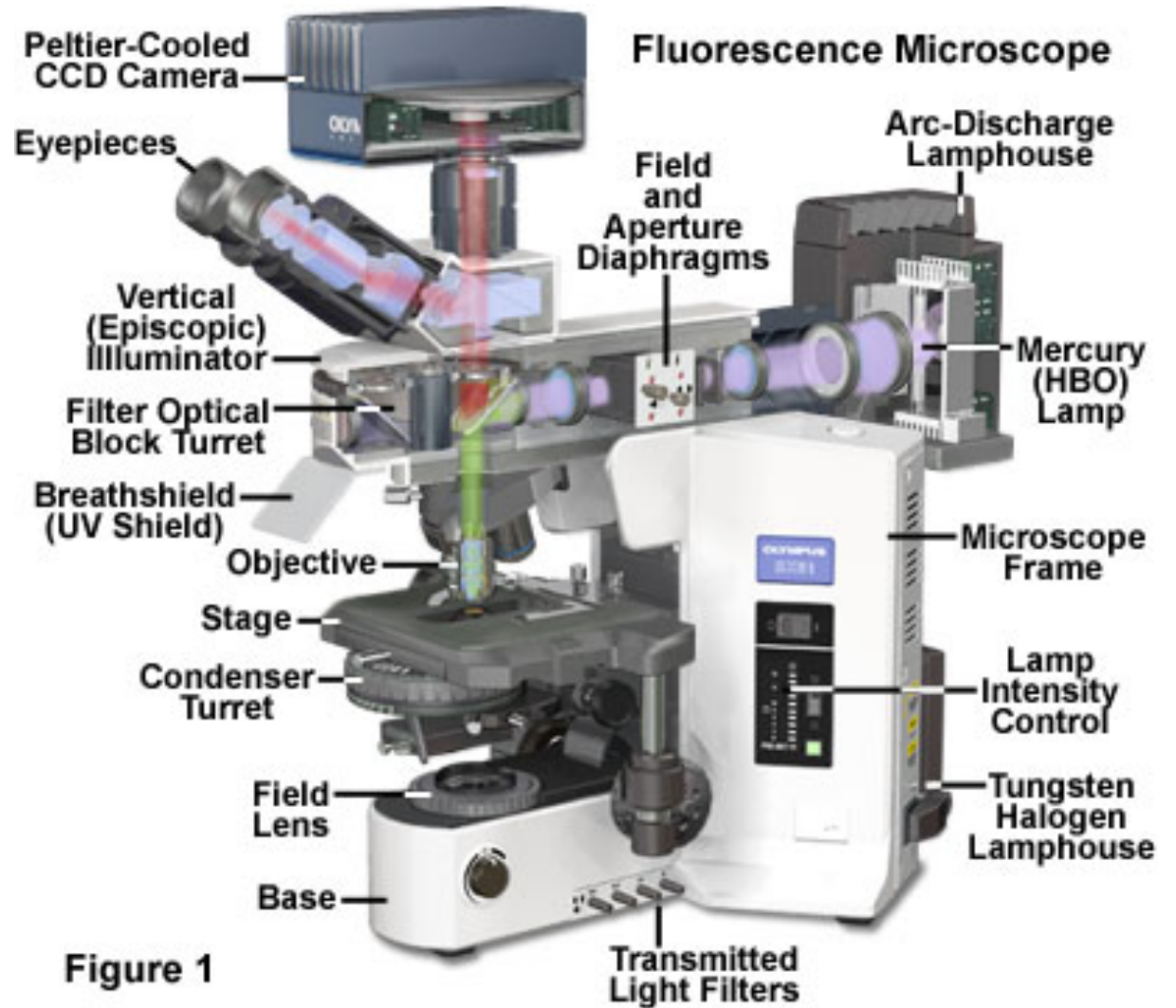
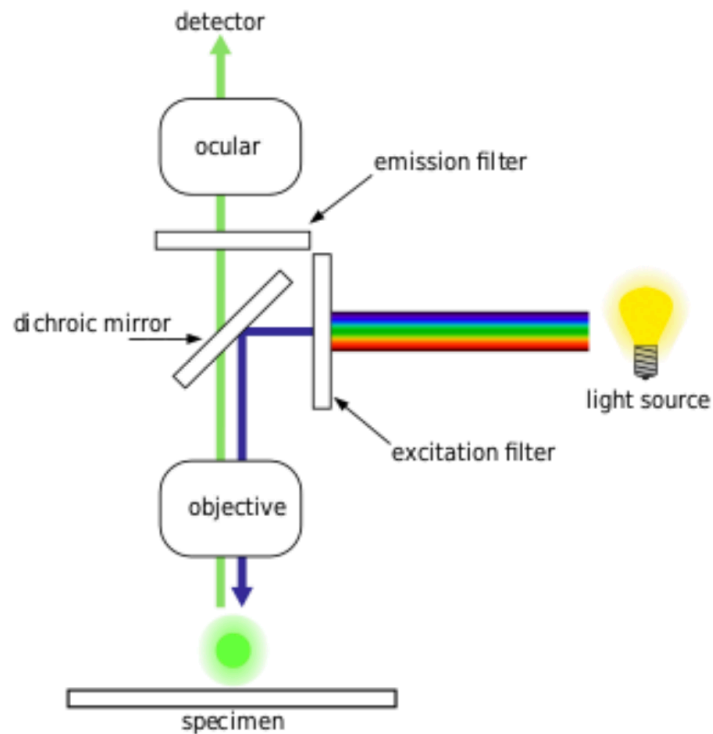
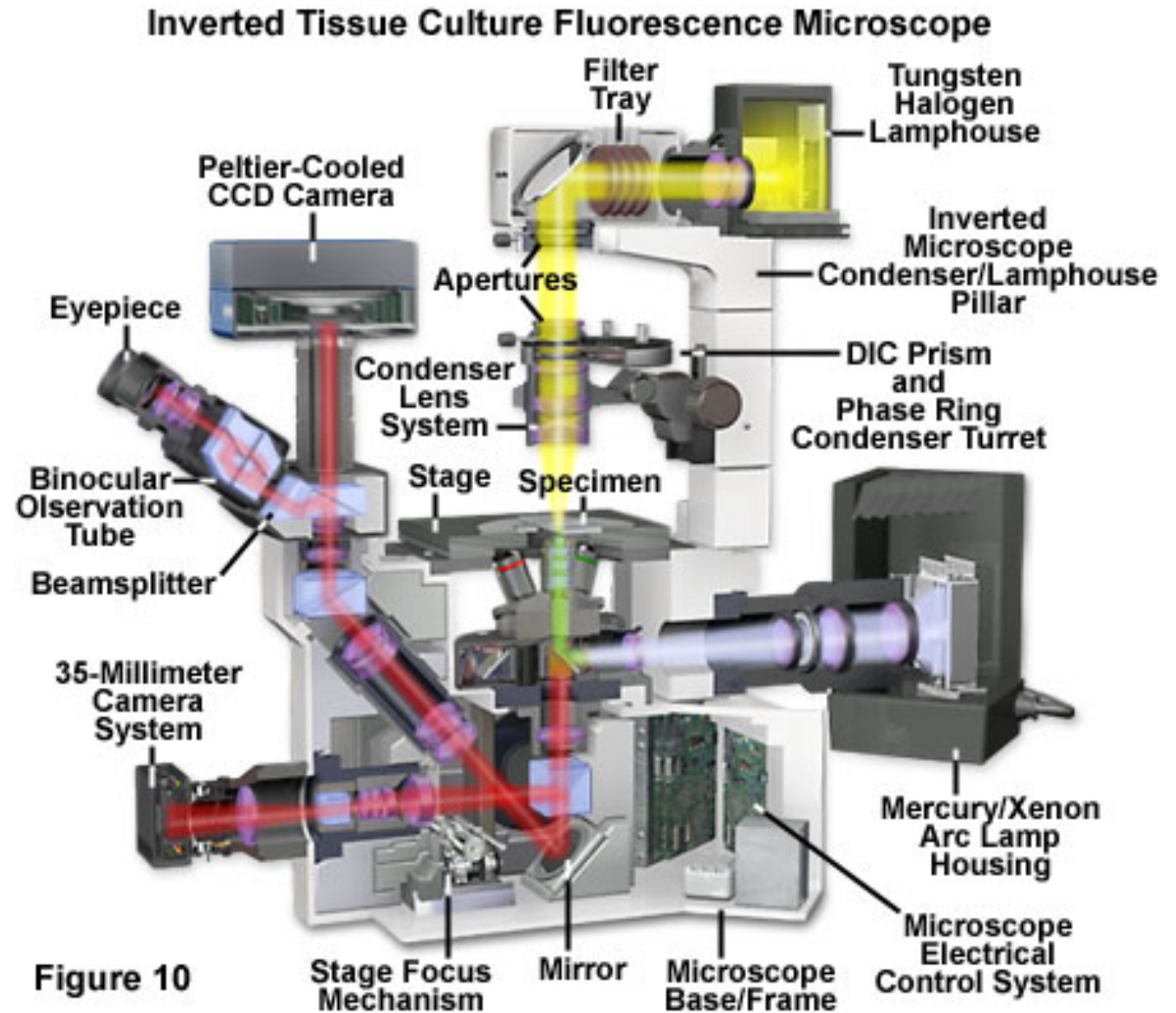


Figure 1

Detection Fluorescence

Inverted microscopes



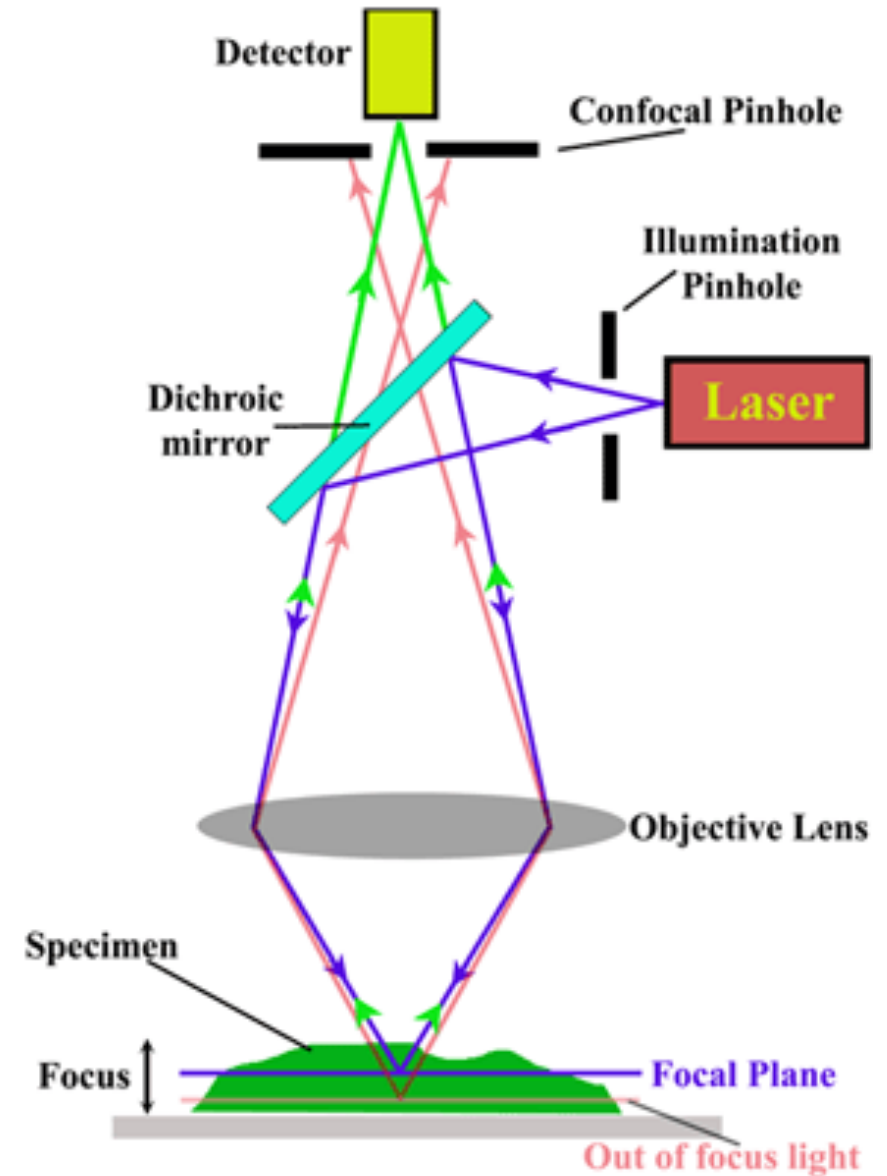
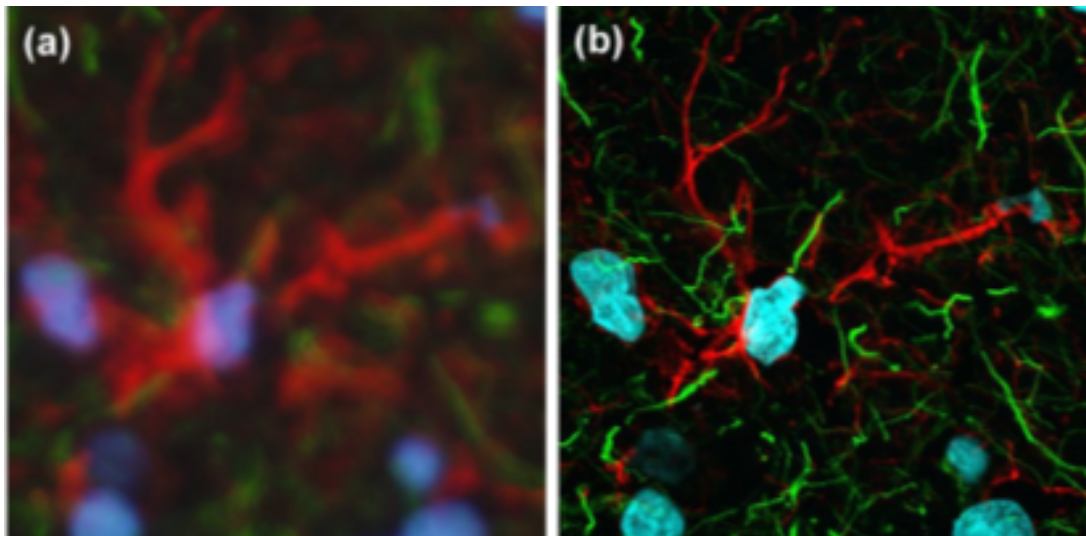
Detection Fluorescence

Confocal microscopy

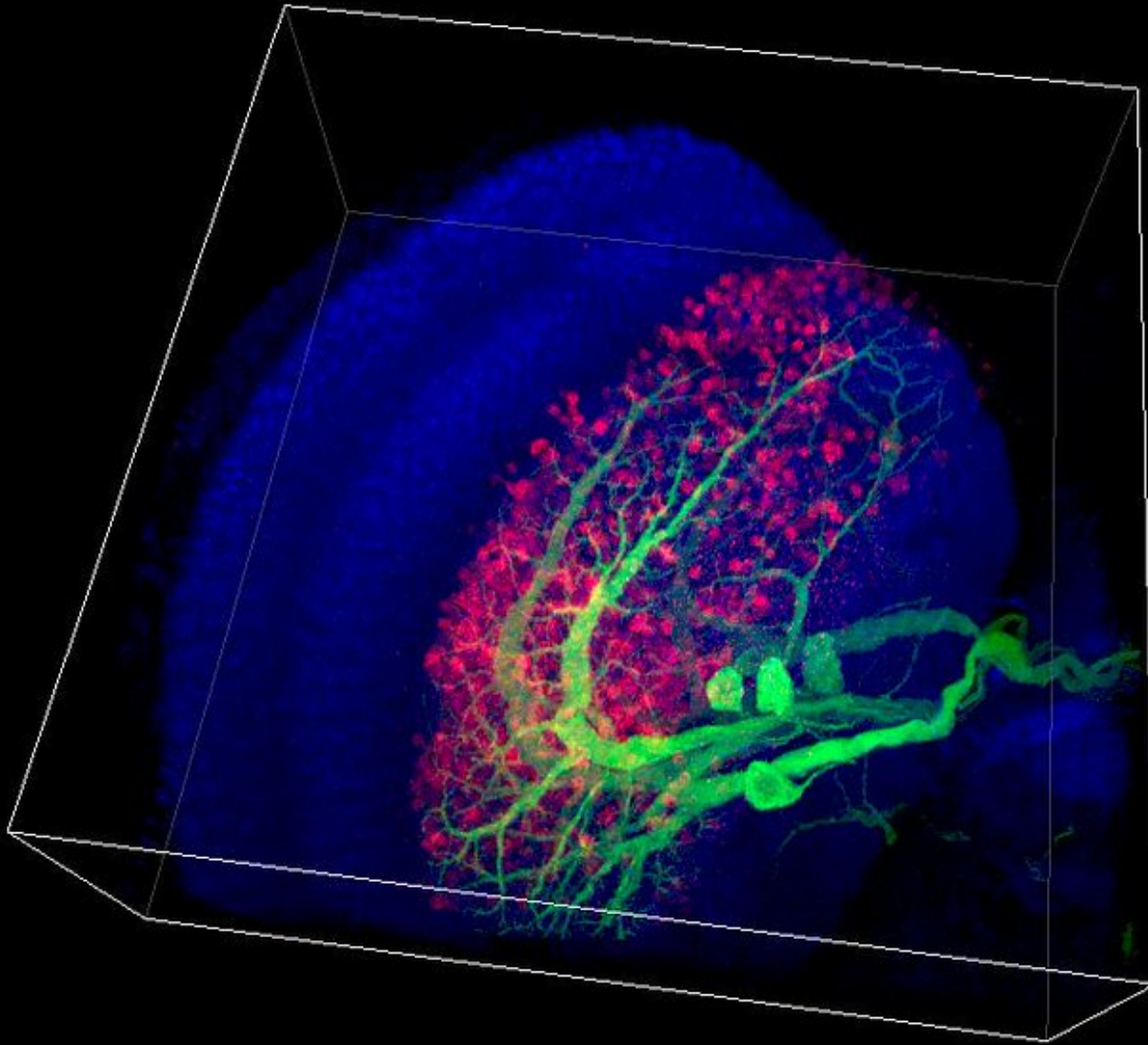
Laser scanning microscopy CLSM

use of spatial pinhole to block out-of-focus light

Z stacking of images : 3D



Detection Fluorescence



Drosophila brain, 3D confocal microscopy

Drosophila brain; triple antibody staining: Alexa 488, Alexa 568 and Alexa 633. Imaged with Zeiss LSM 800

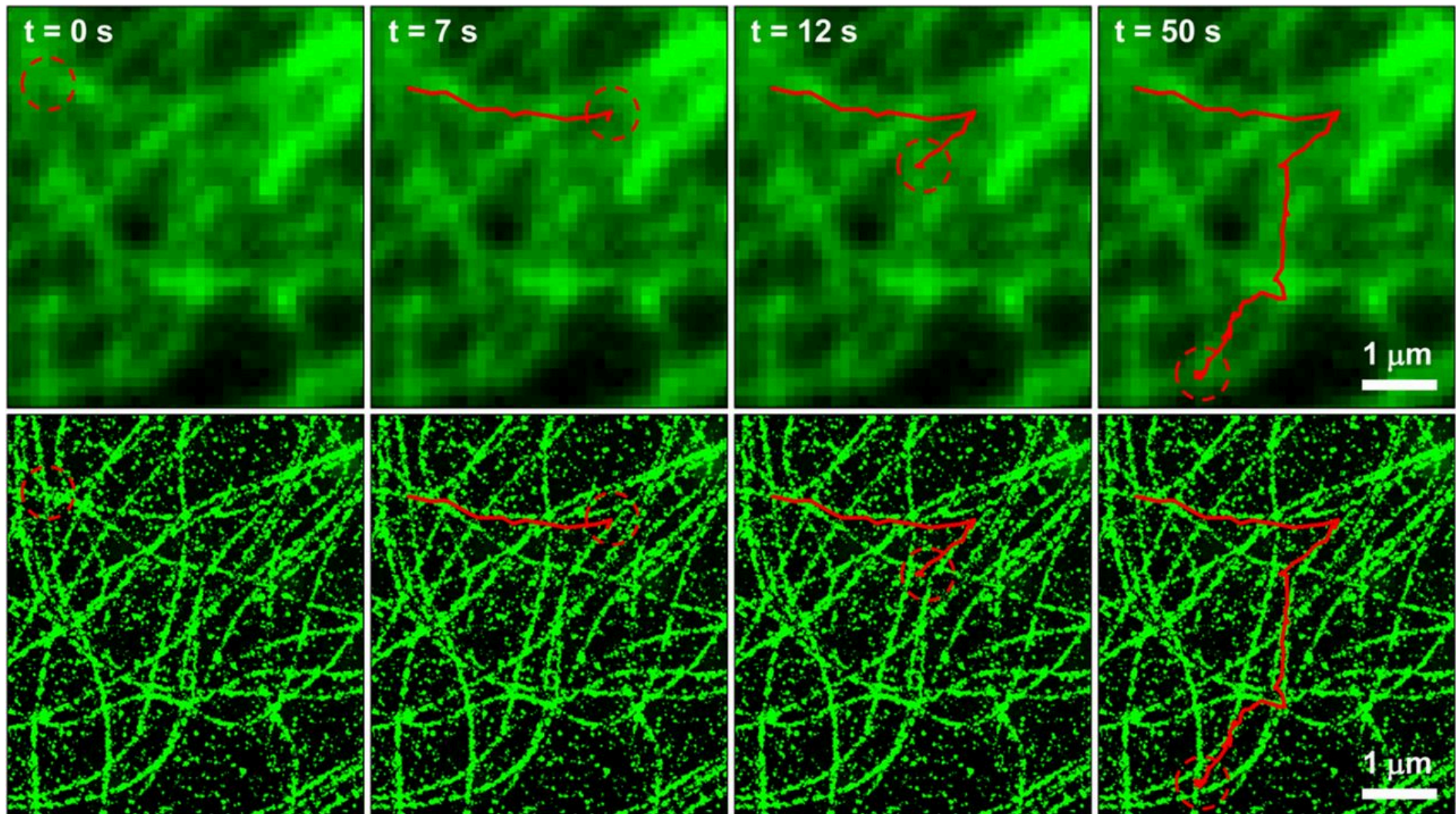
Detection Fluorescence

Confocal microscopy

**Microscopie
à fluorescence,
microscopie
confocale**

Detection Fluorescence

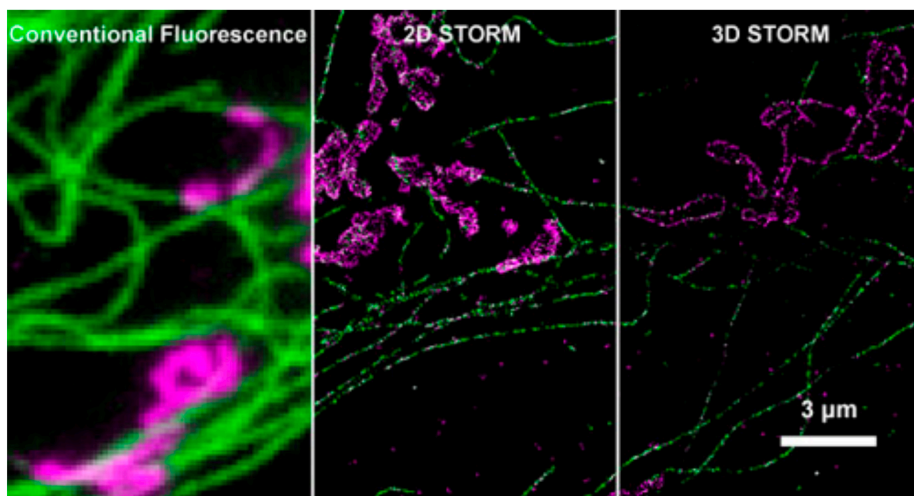
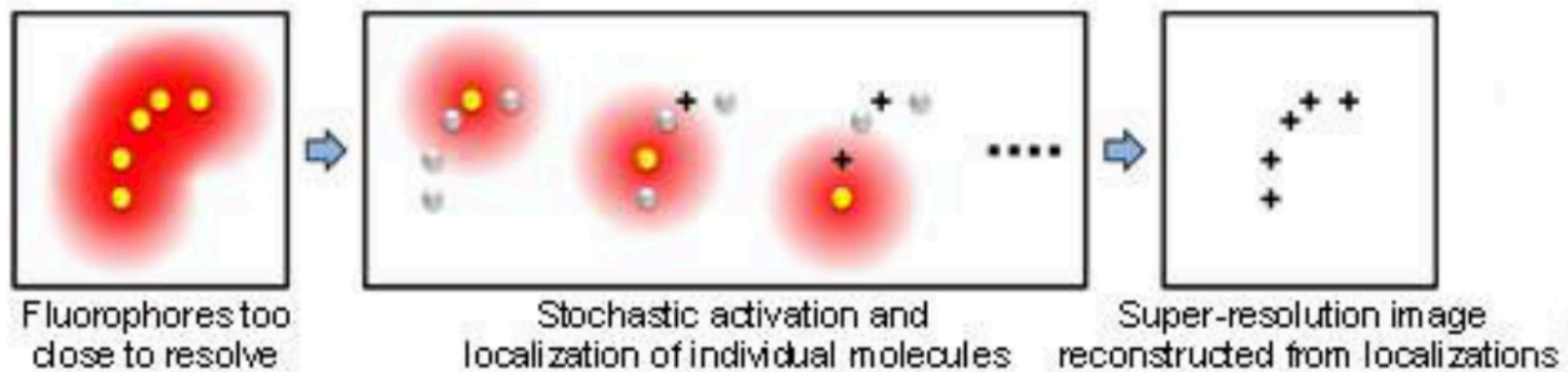
Super resolution : STORM



Detection Fluorescence

Super resolution : STORM Stochastic Optical Reconstruction Microscopy

During STORM, single fluorophores “blink” by a process of random activation from an off or dark state, to an on or emission state, quickly followed by a switch back to a dark state



Detection Electrochemical

Electrochemical sensors

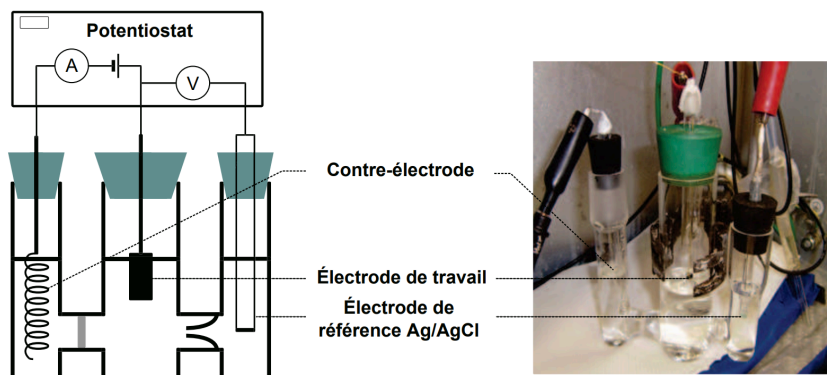
An electric potential is generated in response to a concentration change in a chemical sample

Electrochemical Cell :

Working electrode

Counter electrode

Reference electrode



$$E = E_0 + \frac{RT}{nF} \log_e \left(\frac{C_0}{C_R} \right)$$

- C_0 is the oxidant concentration
- C_R is the Reduced Product Concentration
- n is the number of electrons
- F is the Faraday constant
- T is the temperature
- R is the gas Constant
- E_0 is the electrode potential at a standard state.

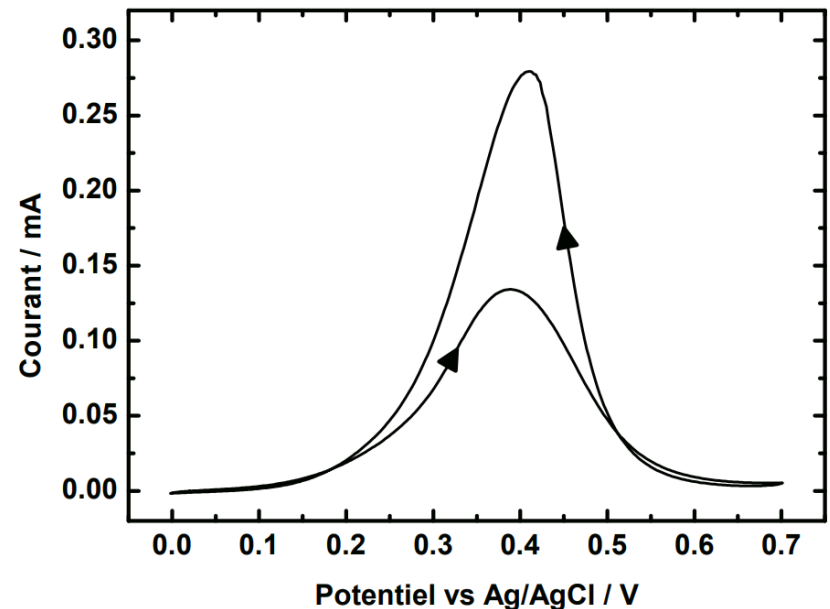
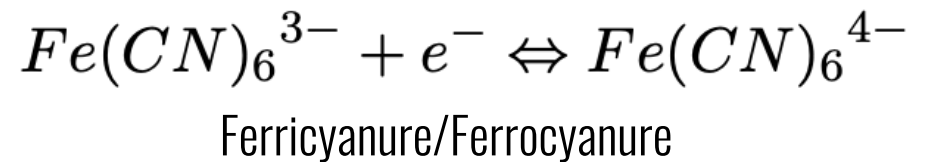
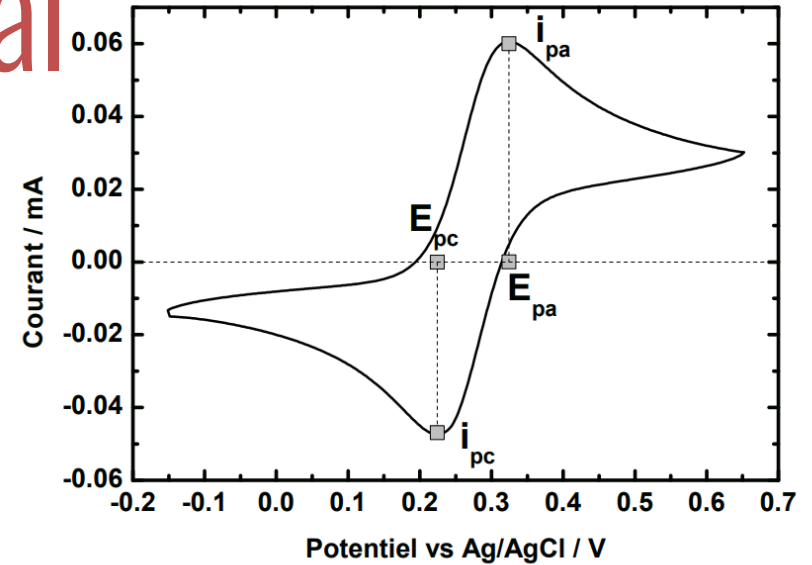
Detection Electrochemical

Cyclic Voltammetry

the working electrode potential is ramped linearly versus time.

The potential is measured between the working electrode and the reference electrode

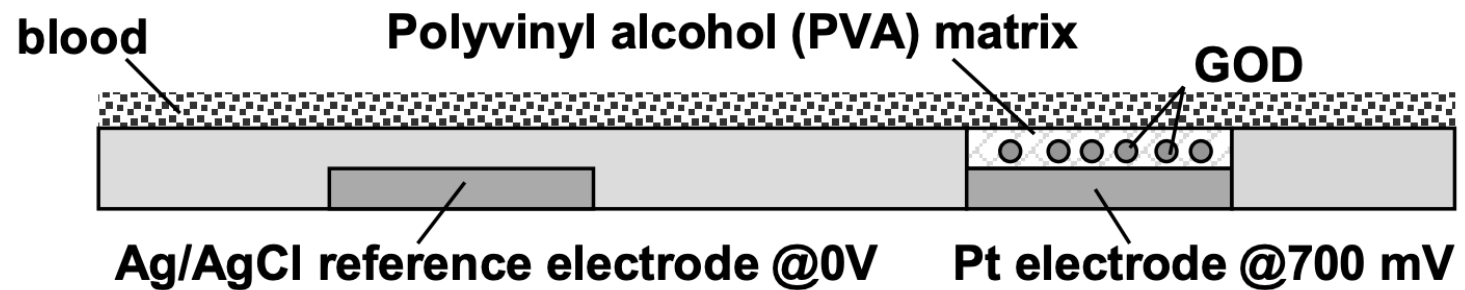
The analyte has to be redox active within the potential window to be scanned.



Oxydation du glucose avec une électrode d'or

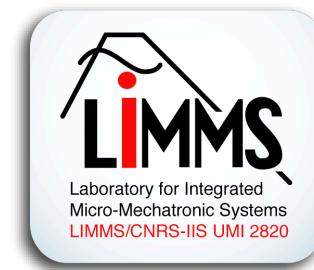
Detection Electrochemical

Glucose detection in blood



- Reactions at the Pt electrode:
 - Glucose oxidized in the presence of oxygen and GOD:
 - » $\text{Glucose} + \text{O}_2 \rightarrow \text{gluconolactone} + \text{H}_2\text{O}_2$
 - Hydrogen peroxide is oxidized and cause the current flow:
 - » $\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^-$

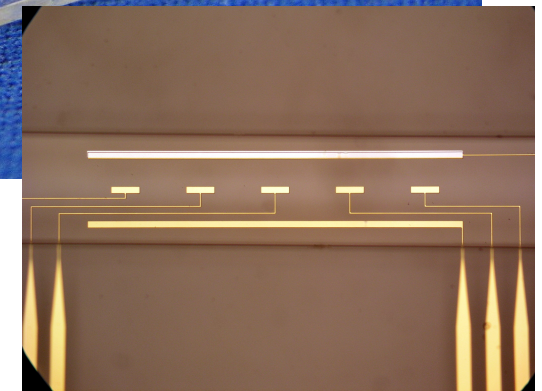
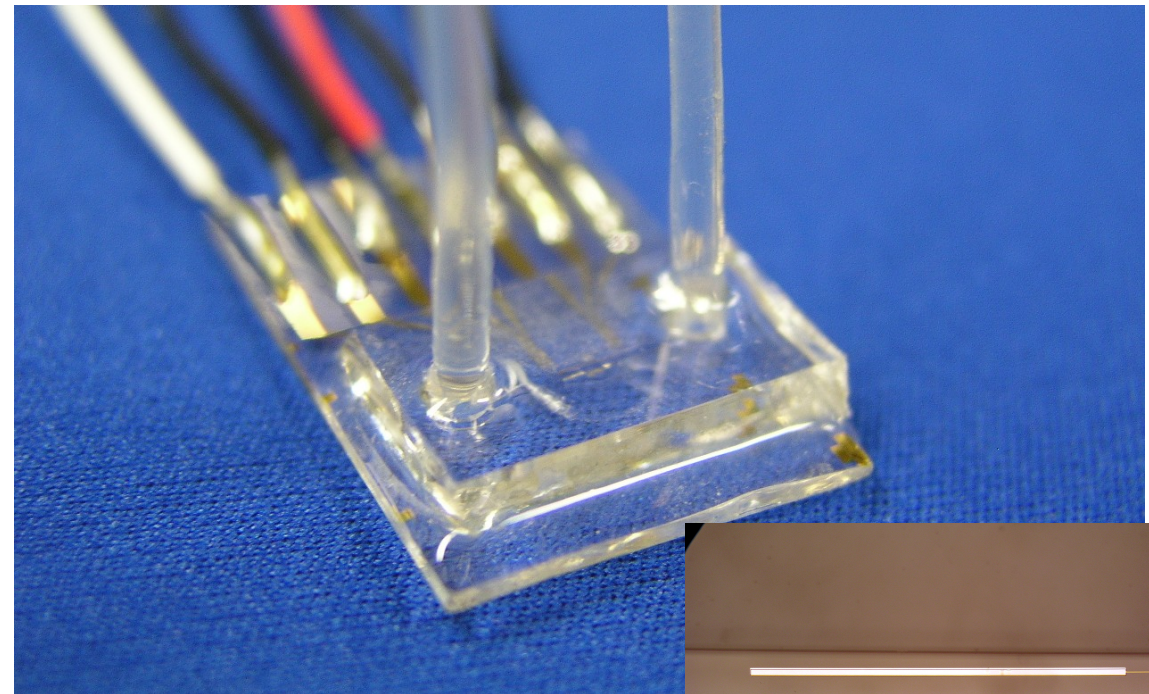
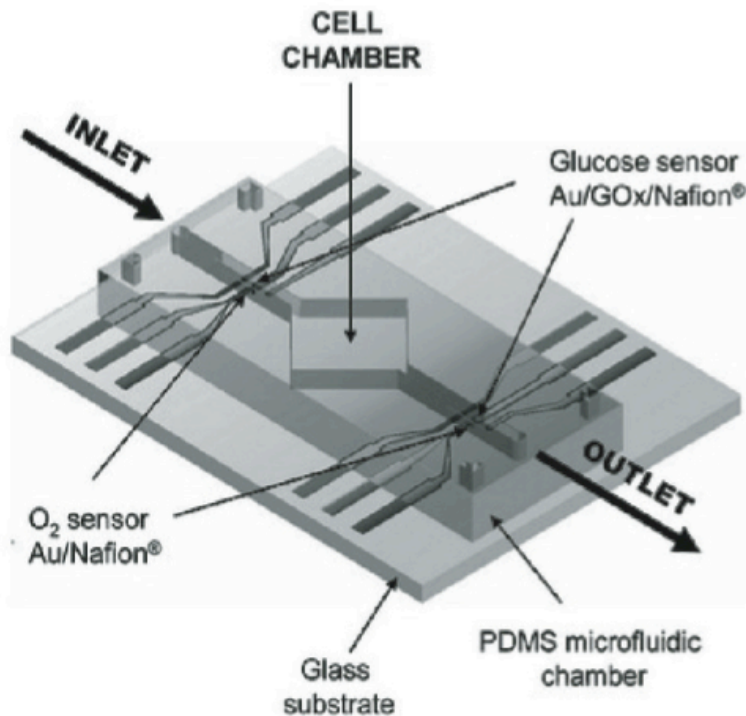
Detection Electrochemical



Integrated microfluidic device for sensing dynamic response of cells and tissues

Nazare Pereira Rodrigues, Teruo FUJII

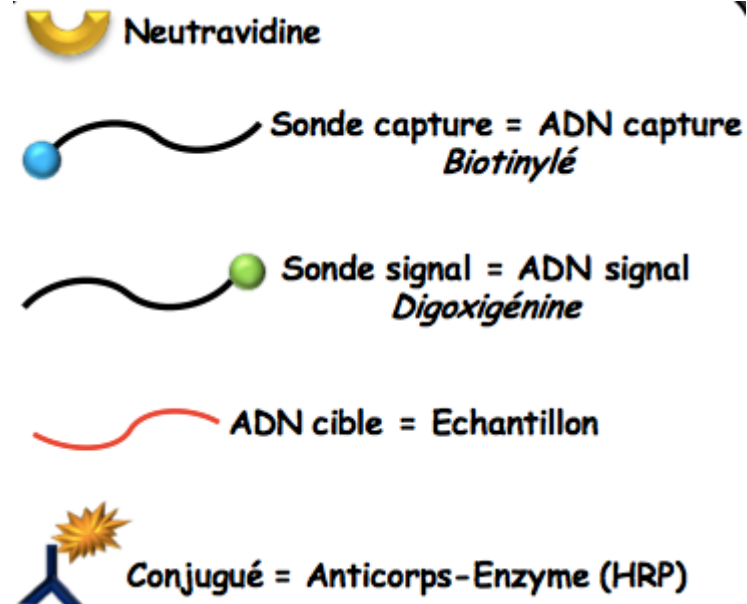
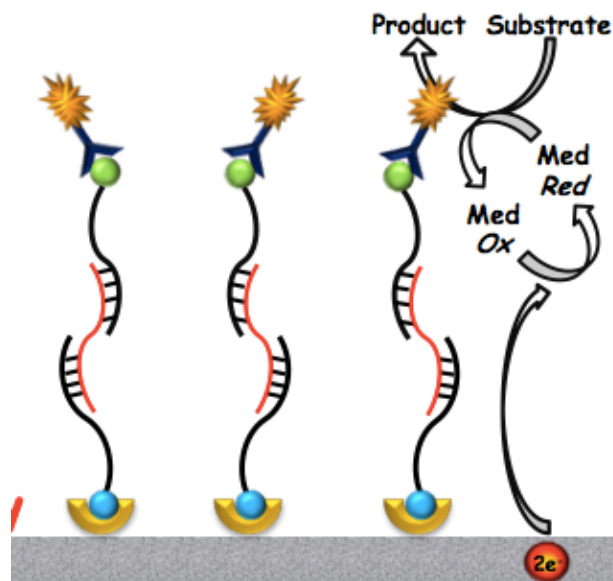
- Monitoring of essential nutrients such as dissolved oxygen and glucose during cell culture
- Specific membranes in way to achieve a selective and sensitive detection



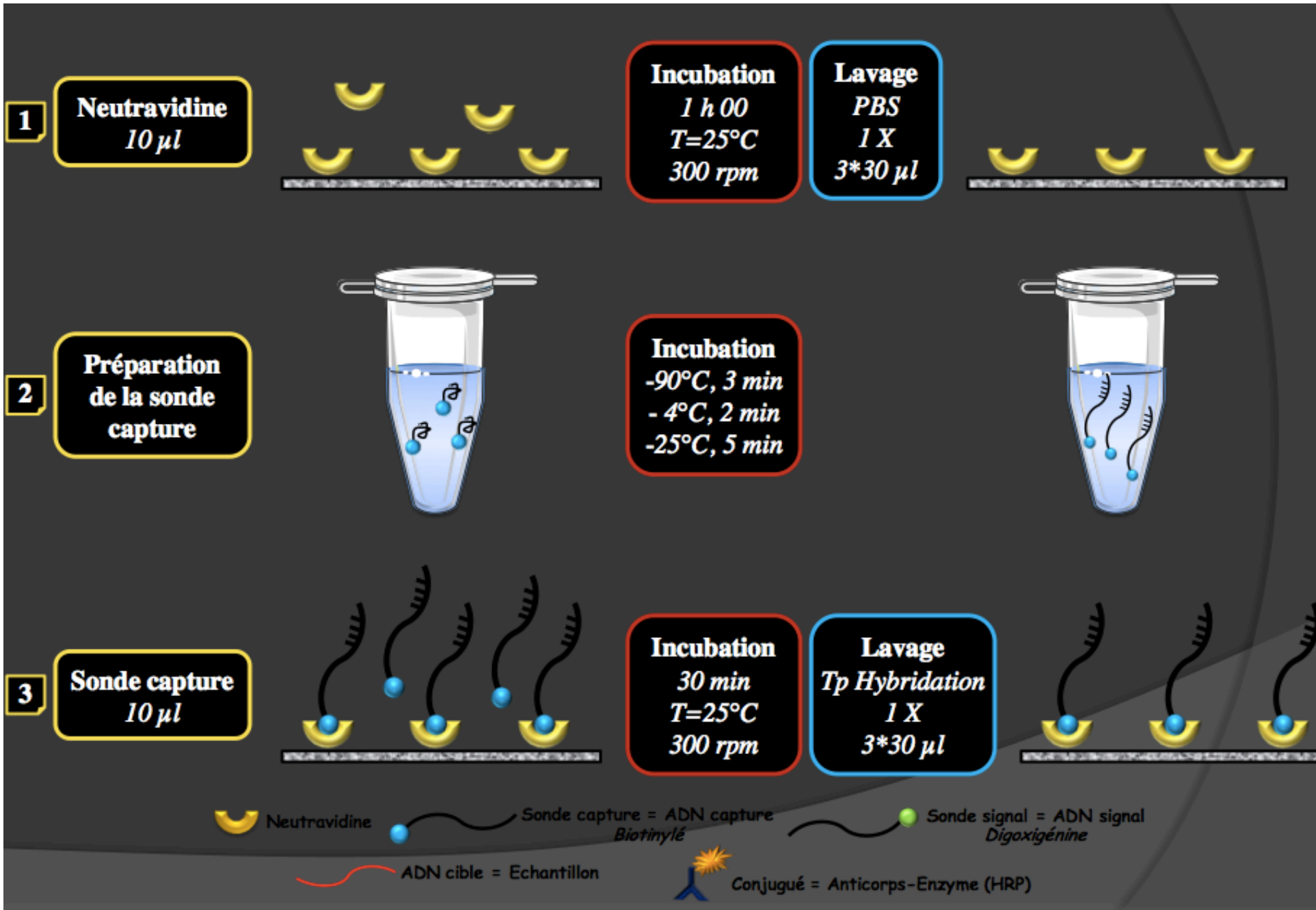
Detection Electrochimical

DNA genosensor

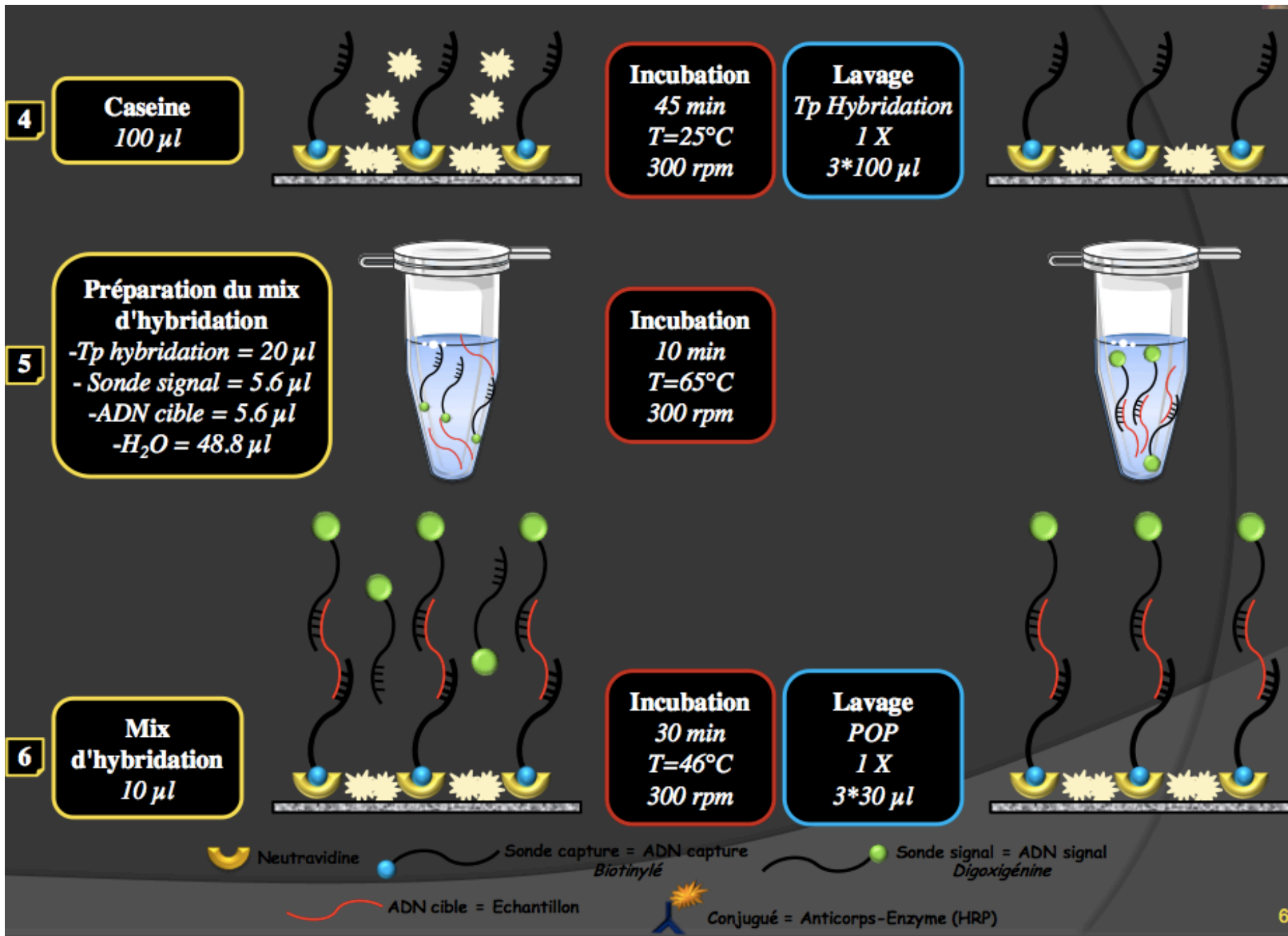
Détection d'ADN cible par électrochimie



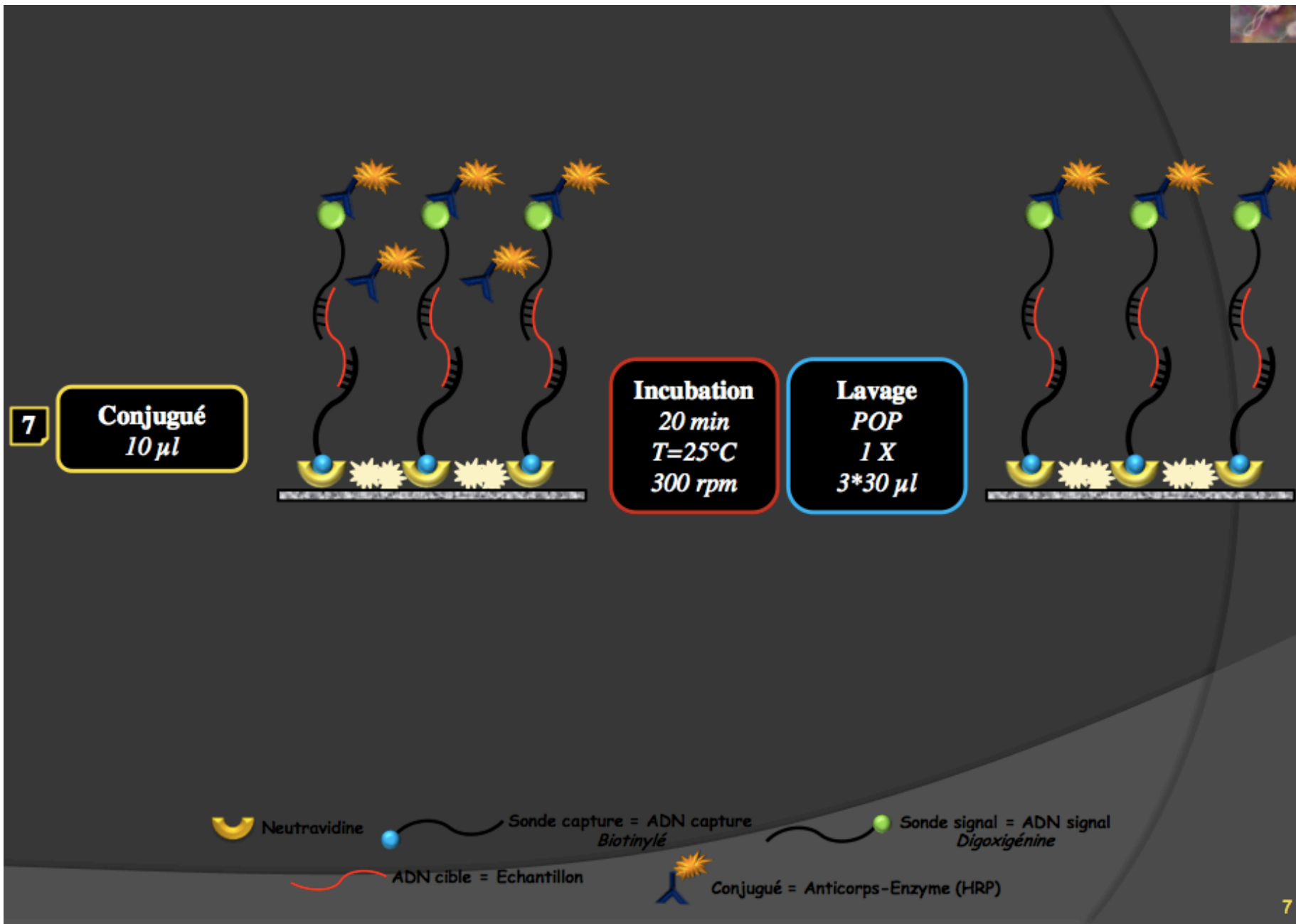
Detection Electrochimique



Detection Electrochimique



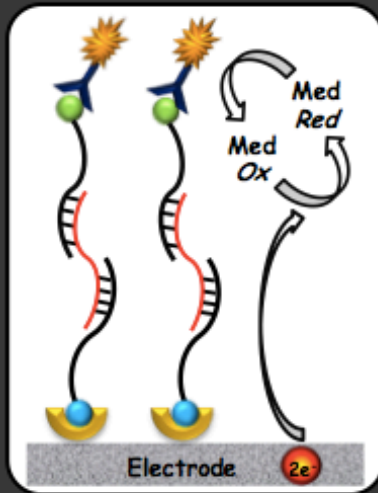
Detection Electrochimique



Detection Electrochimical

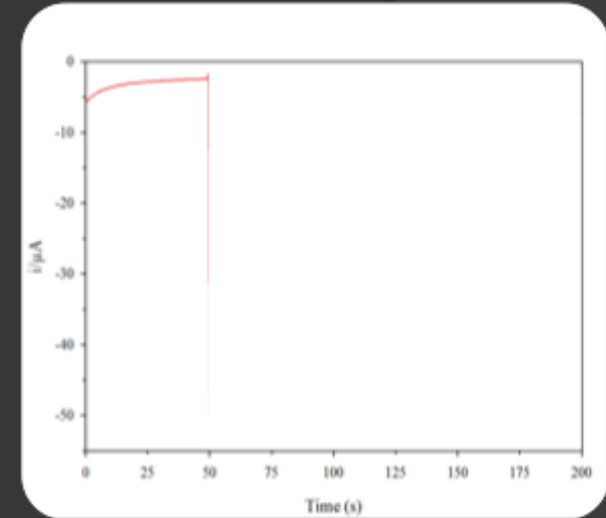
8

Tampon acétate
MPMS= Médiateur
90 μ l



9

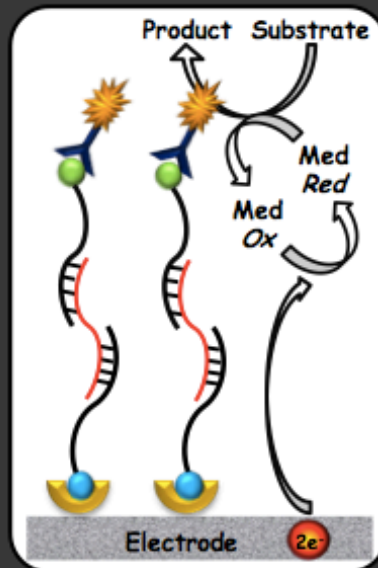
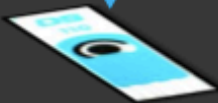
Enregistrement
Ampérométrie
-0,2 V vs Ag/AgCl
80 s



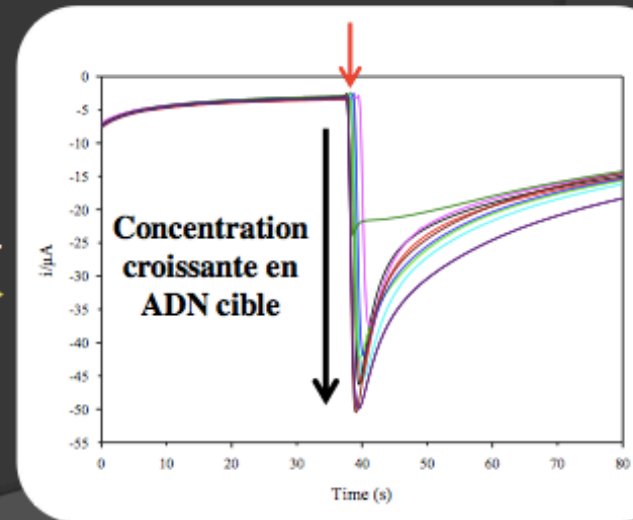
10

Peroxyde d'hydrogène
(H₂O₂) = Substrat
10 μ l

Enregistrement



Enregistrement



Neutravidin



Capture probe
Biotin



Signal probe



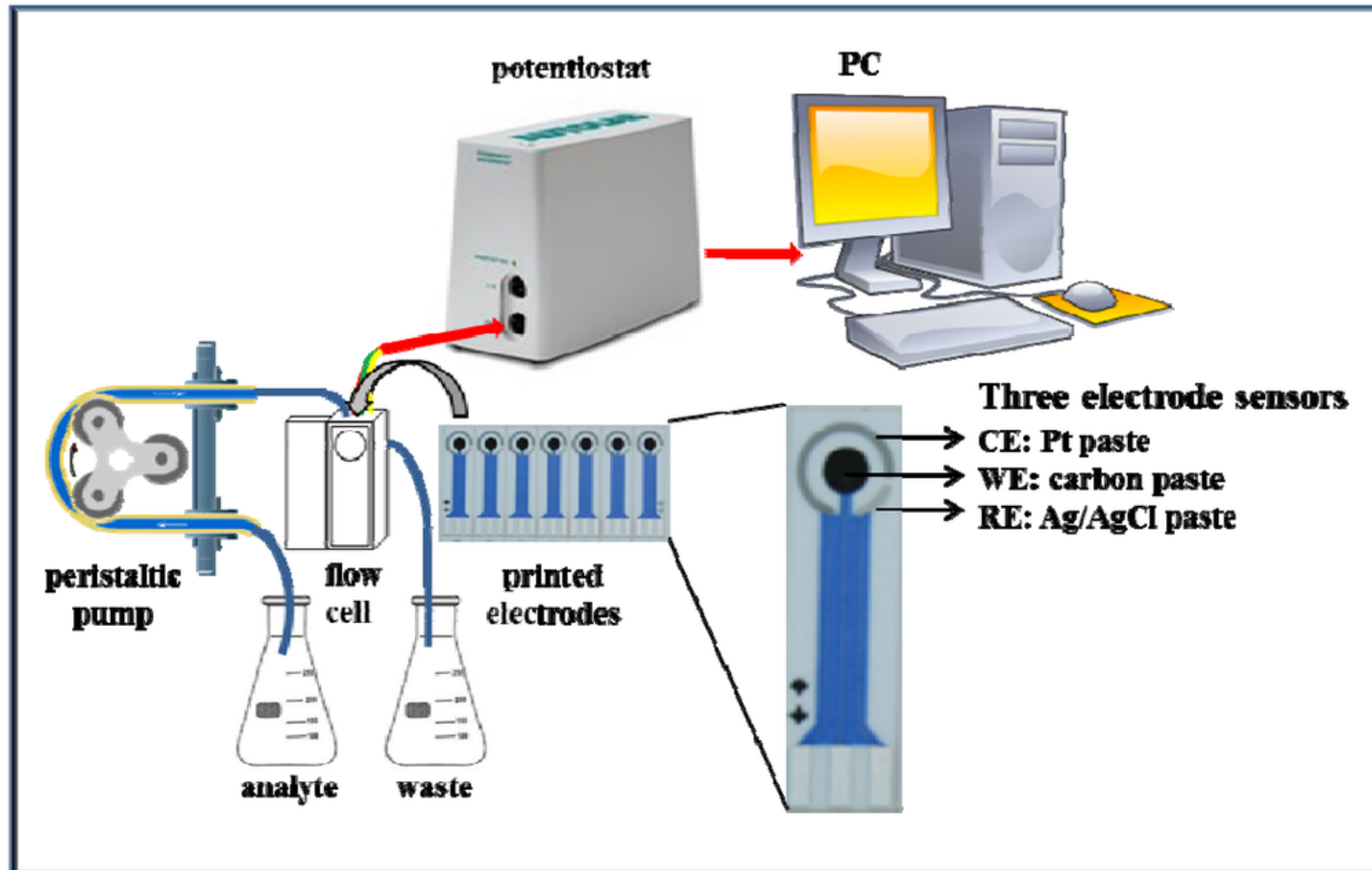
DNA target



Antibody-Enzyme (HRP)

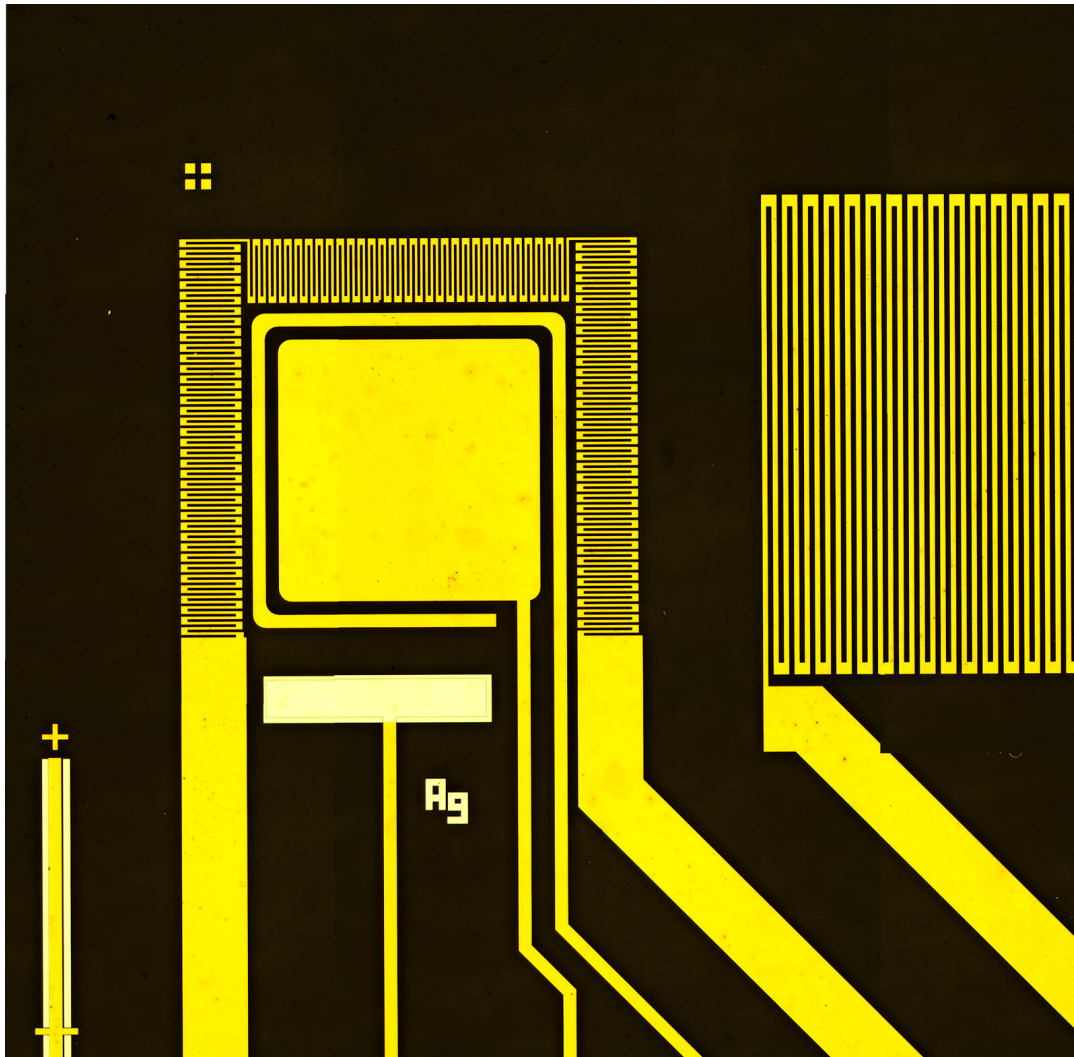


Detection Electrochemical

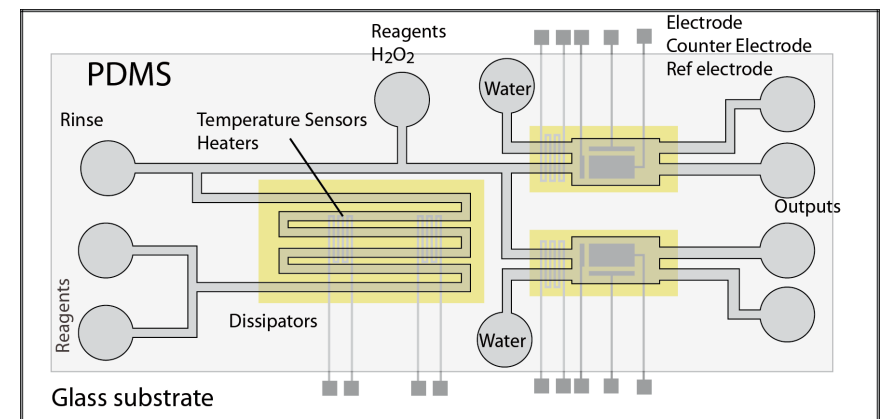
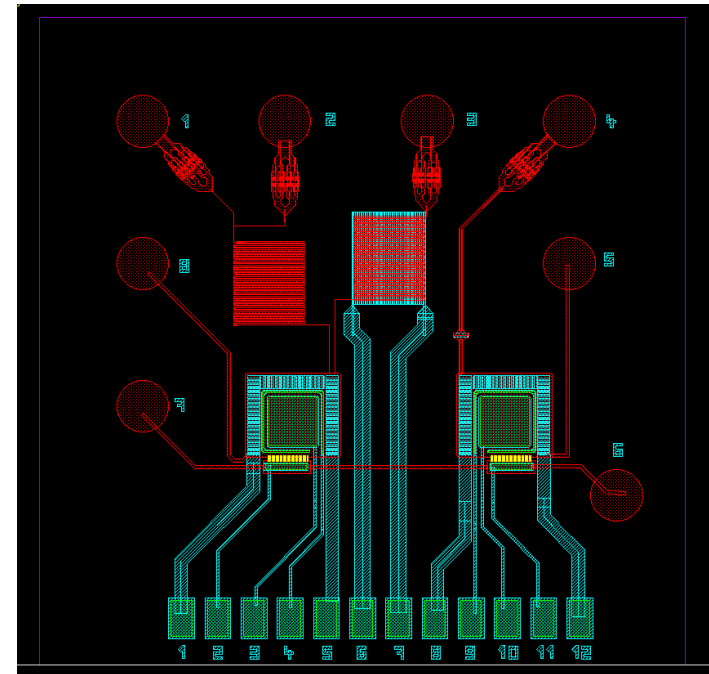


Detection Electrochemical

Intégration en microfluidique



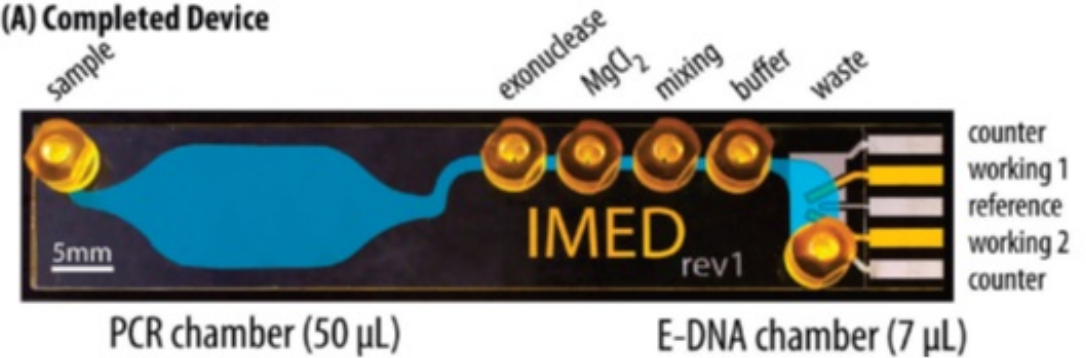
B.Charlot, IES, J.Baudart LOMIC, L.Bathelmebs, Images



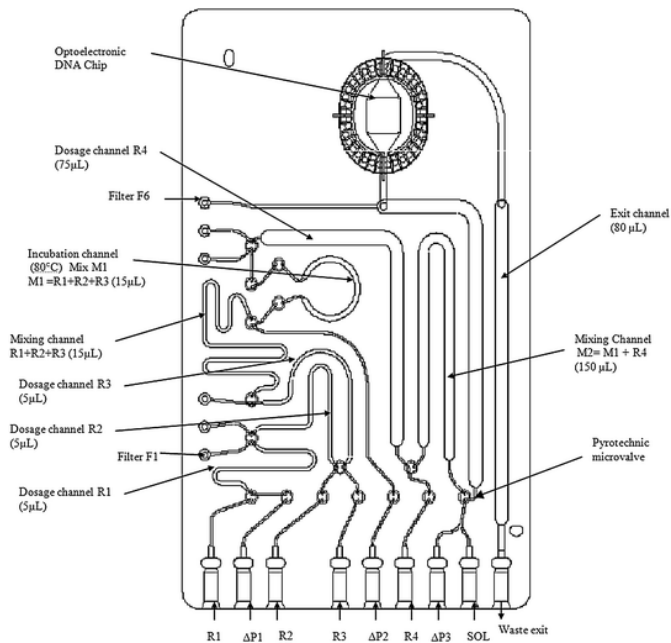
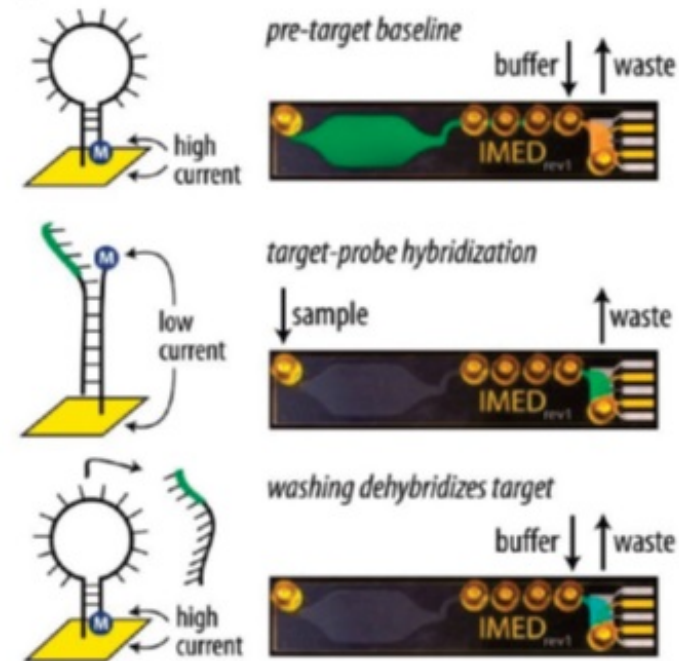
Detection Electrochemical

Intégration en microfluidique

(A) Completed Device



(F) E-DNA Detection

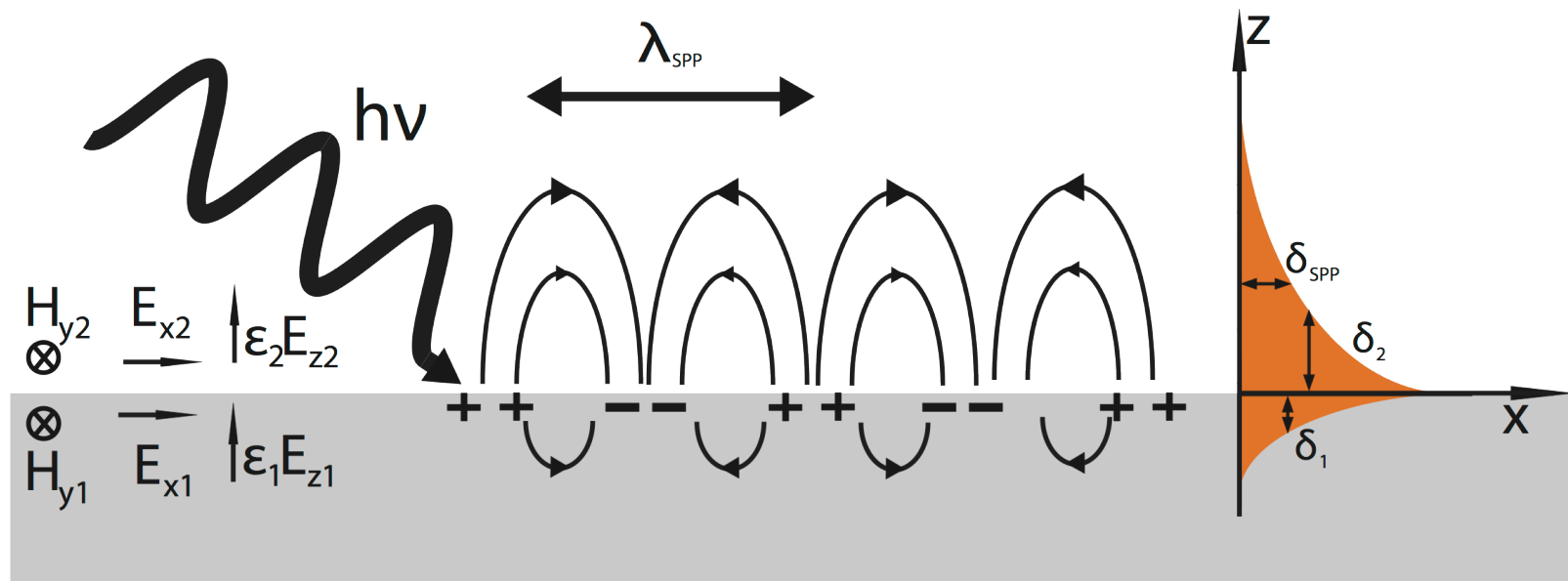


Detection Surface plasmon resonance

A plasmon is a quantum of plasma oscillation = quasiparticle.

Surface plasmon (SP) is an evanescent surface electromagnetic wave caused by collective and coherent free electron oscillation at a metal-dielectric interface

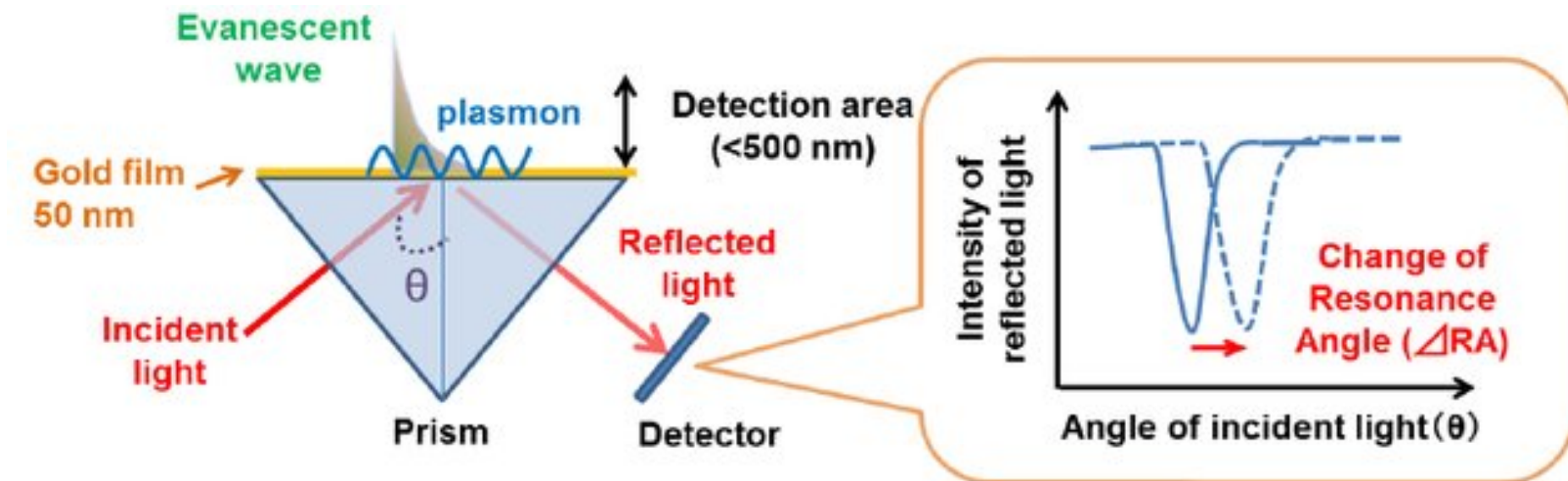
Plasmons can couple with a photon to create another quasiparticle called a plasmon polariton.



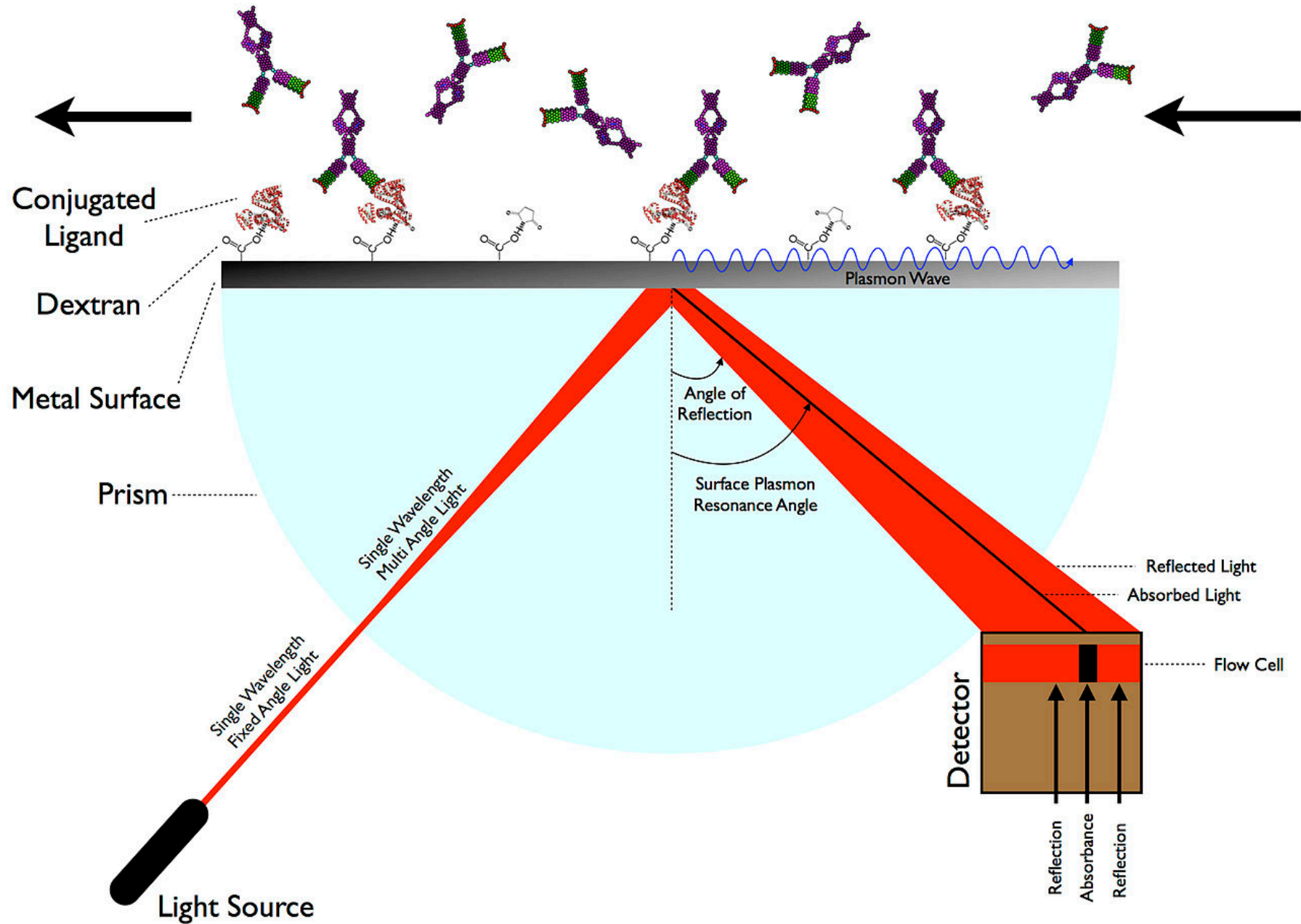
Detection Surface plasmon resonance

Surface plasmon resonance (SPR) including propagating (PSPR) and localized (LSPR) are observed in the continuous metal film and isolated metal nanostructure.

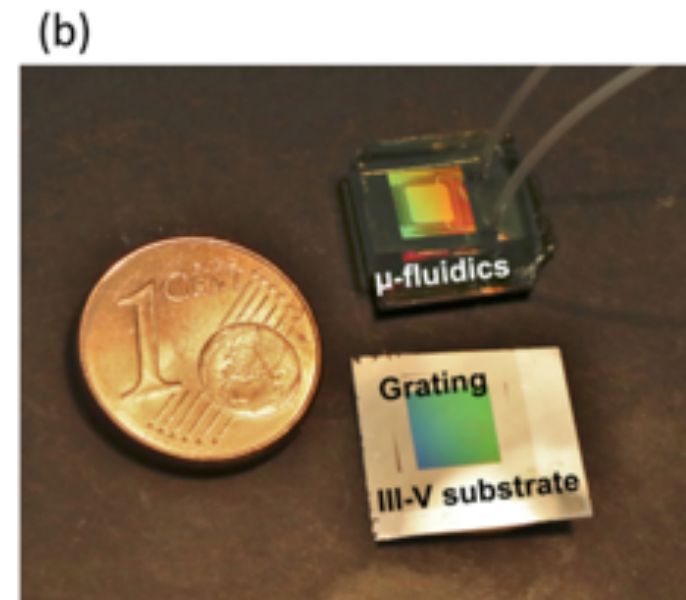
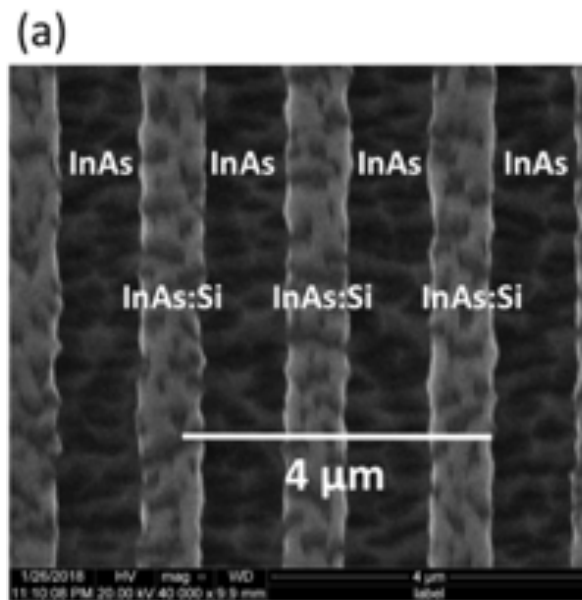
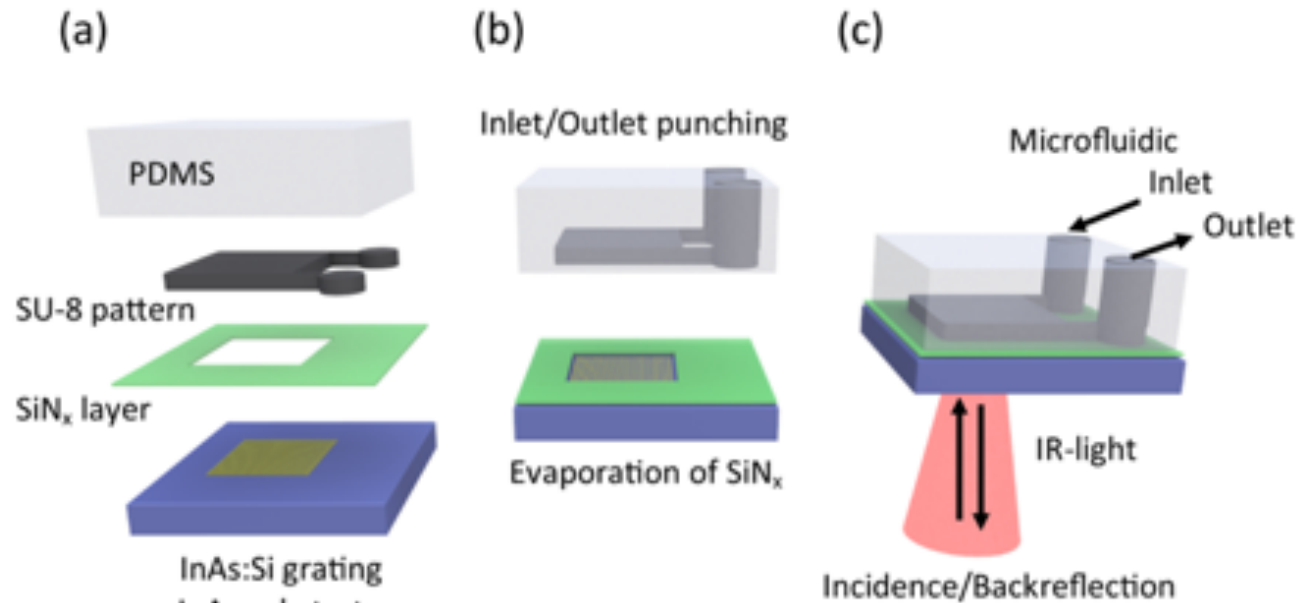
The SP waves are excited by incident light and is confined to the metal-dielectric interface with an extension of only ~ 200 nm from the interface.



Detection Surface plasmon resonance



Detection Surface plasmon resonance



Microfluidic surface-enhanced infrared spectroscopy with semiconductor plasmonics for the fingerprint region

Mario Bomers,^a Benoît Charlot,^a Franziska Barho,^a Antoine Chauvel,^a Aude Mezy,^b Laurent Cerutti,^a Fernando Gonzalez-Posada^a and Thierry Taliercio*