Time-Resolved Microscopy

Modern Optics Laboratory

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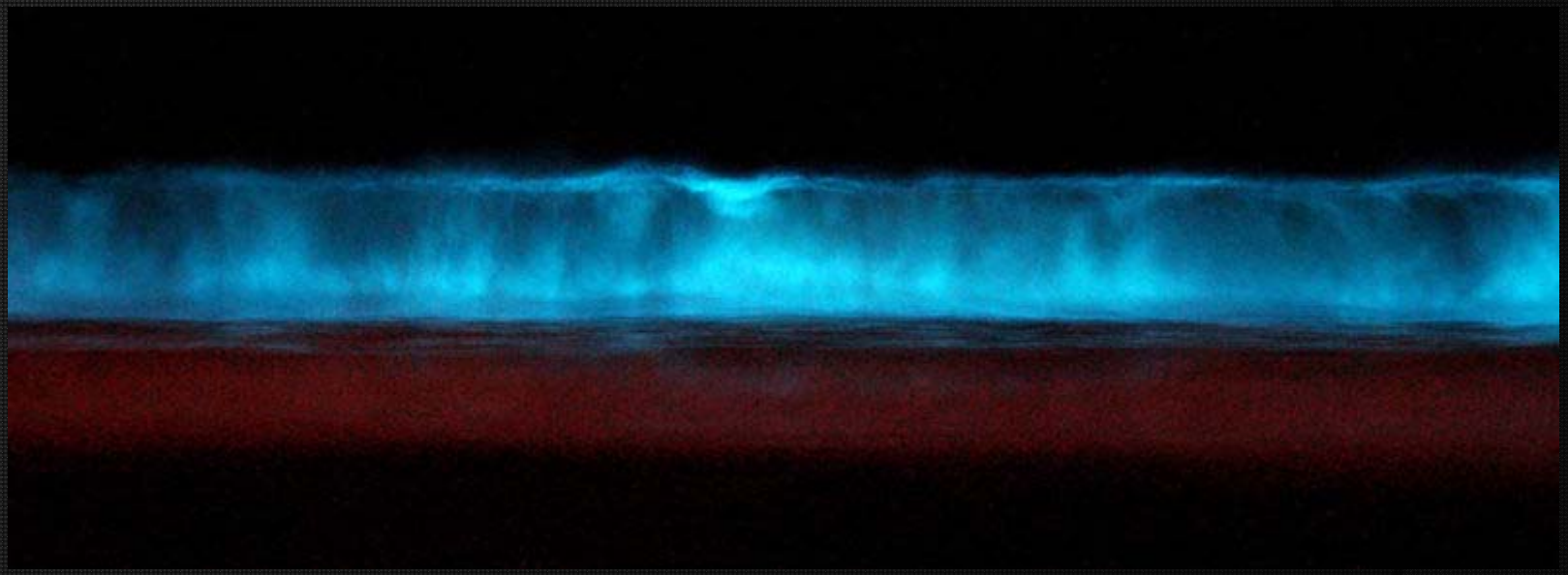


By Lauren Klabunde, 2005, Carlsbad, California

Overview

Light plays an essential role in our lives: it is an integral part of the majority of our activities. The ancient Greeks, who for ‘‘to die’’ said ‘‘to lose the light’’, were already well aware of this.

Louis de Broigle,
French physicist,
Nobel laureate

Luminescence

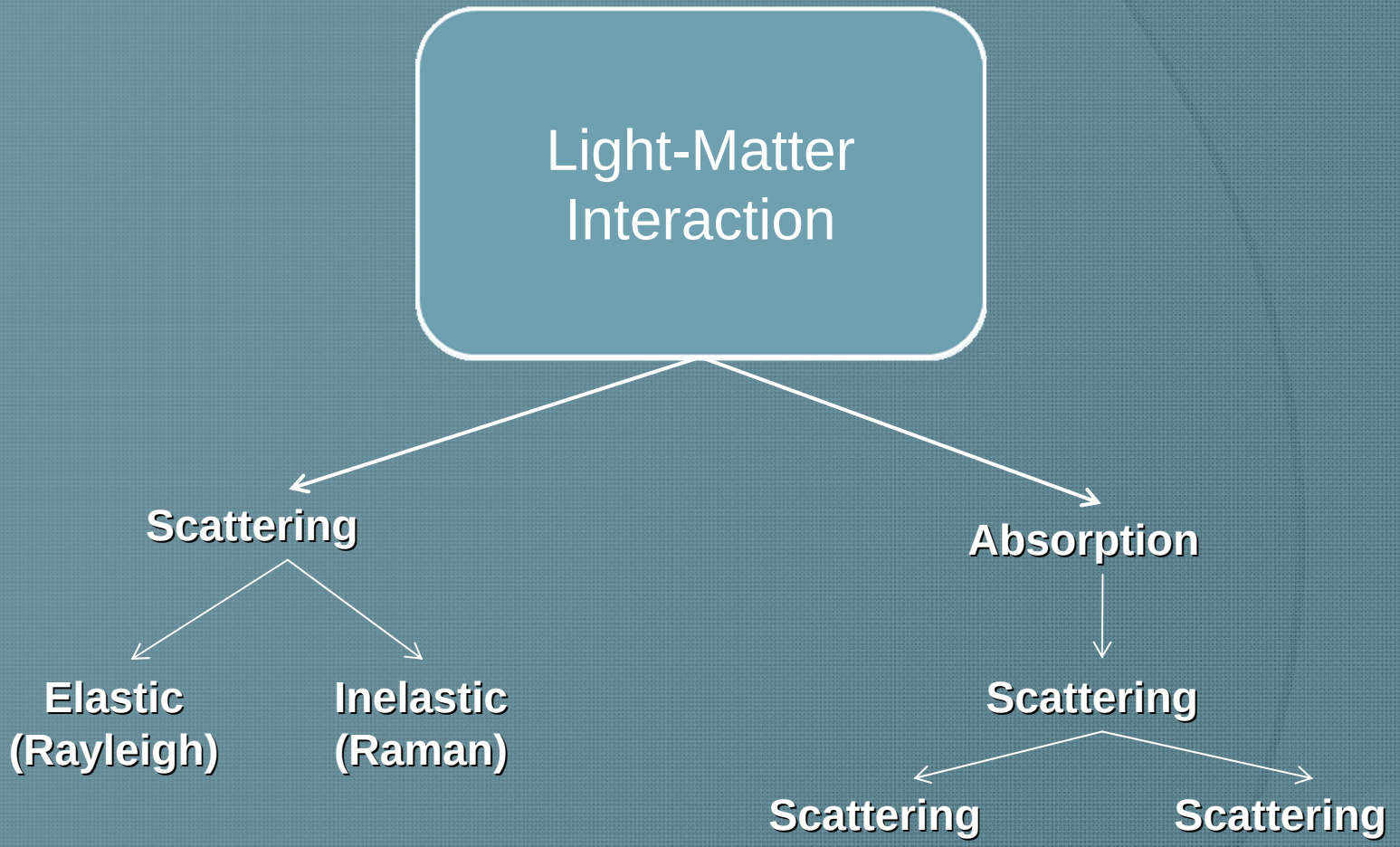
Eilhardt Wiedemann, 1888: "all those phenomena of light which are not solely conditioned by the rise in temperature".

1. ORGANIC COMPOUNDS
   - AROMATIC HYDROCARBONS
   - FLUORESCEIN
   - RHODAMINES
   - COUMARINES
   - AMINOACIDS
   - ...
2. ORGANOMETALLIC COMPOUNDS
   - RUTHENIUM COMPLEXES
   - COMPLEXES WITH LANTHANIDES
   - ...
3. INORGANIC COMPOUNDS
   - URANYL ION (UO_2^+)
   - LANTHANIDE IONS (EU^{3+}, TB^{3+}, ETC.)
   - DOPED GLASSES (ND, MN, CE, ETC.)
   - CRYSTALS (ZNS, CDS, ZNSE, ETC.)

- Photoluminescence
  - fluorescence
  - phosphorescence
- Radioluminescence
- Cathodoluminescence
- Electroluminescence
- Thermoluminescence
- Chemiluminescence
- Bioluminescence
- Triboluminescence
- Sonoluminescence

(from Latin *lumen* – light) emission of ultraviolet, visible or infrared photons from electronically excited specimen.

Overview


Light-Matter Interaction
Scattering
Absorption
Elastic (Rayleigh)
Inelastic (Raman)
Scattering
Scattering
Scattering

Ethymology

1565. First observed by Nicholas Monardes in the infusion of *Lignum Nephriticum.* Repeated by Boyle and Newton.

1842. The term introduced by Sir George Gabriel Stokes. Blue light observed from quinine similar to that seen from fluorspar.

FLUORESCENCE

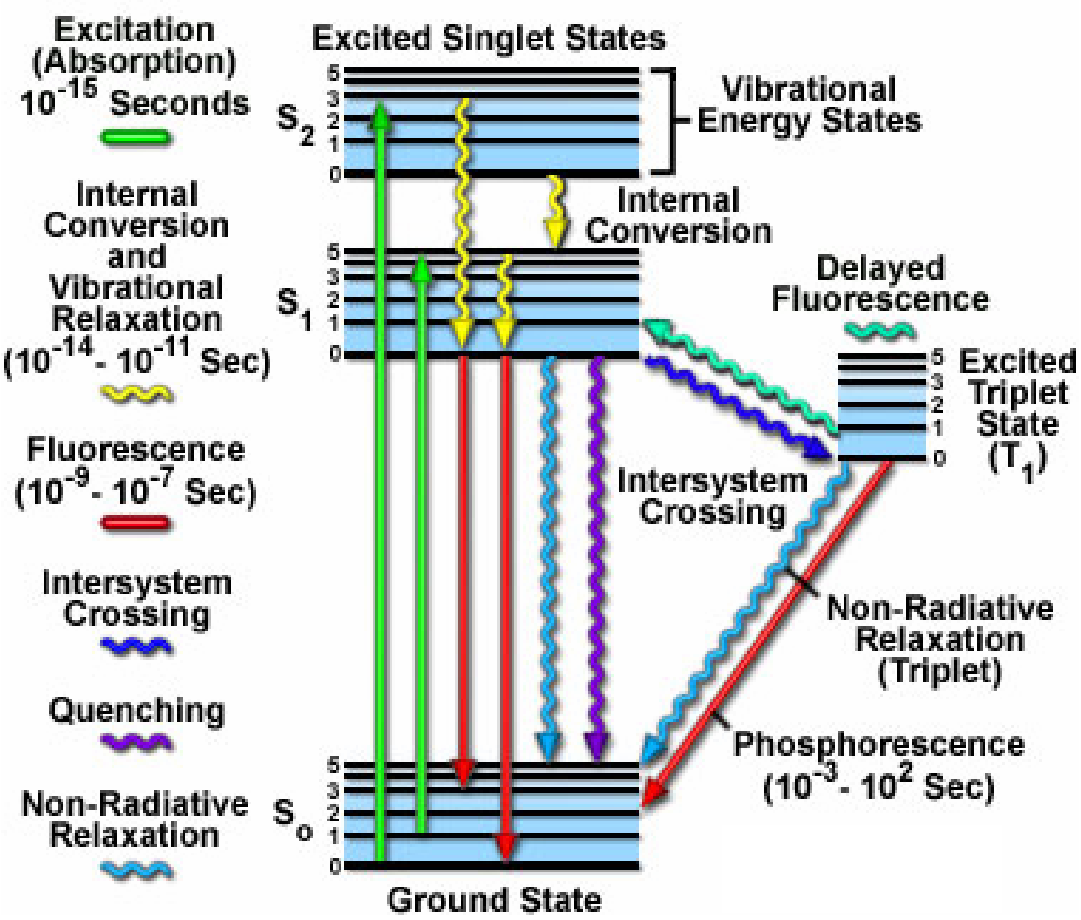
fluere – liquid
spar – crystals with low melting temperature

1608. First observed in the calcinated Bolognian Phosphor.

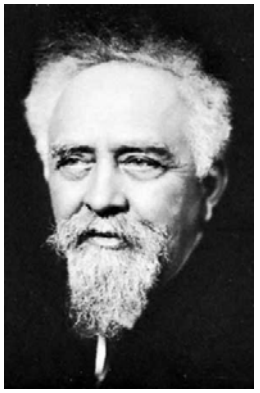
PHOSPHORESCENCE

φώς - light φόρέίν – to bear
φότός phosphor – which bears light

Perrin-Jablonski Diagram



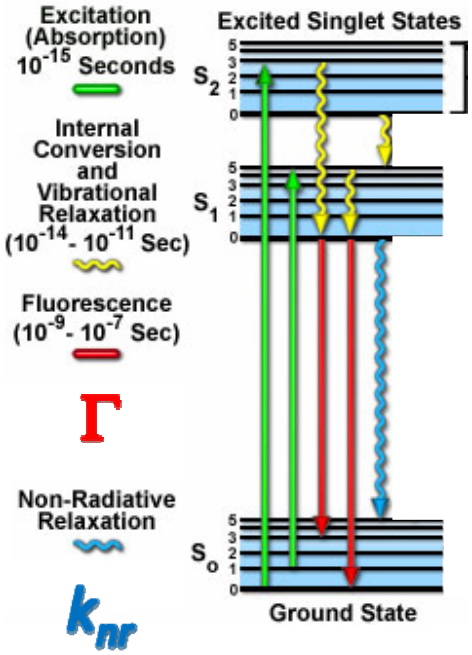
Alexander Jablonski
(1898-1980)



Jean Perrin
(1870-1942)

The diagram design is a (C) by Michael W. Davidson and The Florida State University.

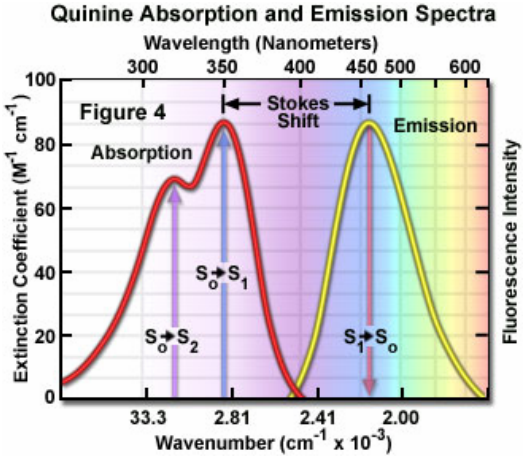
Fluorescence properties



Spectra

Stoke's shift

Mirror image rule



Fluorescence brightness

Quantum yield – number of emitted photons relative to the numbers of absorbed photons

$$Q = \frac{\Gamma}{\Gamma + k_{nr}}$$

Fluorescence lifetime

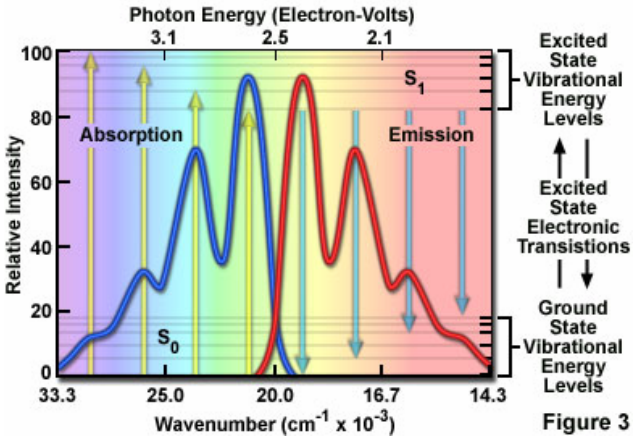
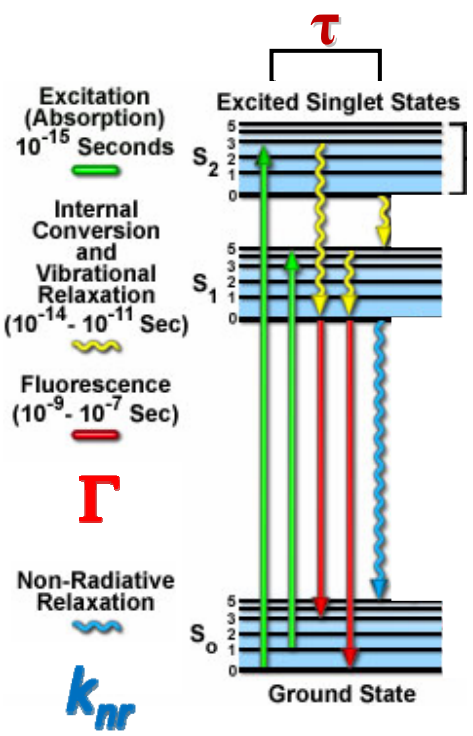


Figure 3

Fluorescence Lifetime Definition



τ

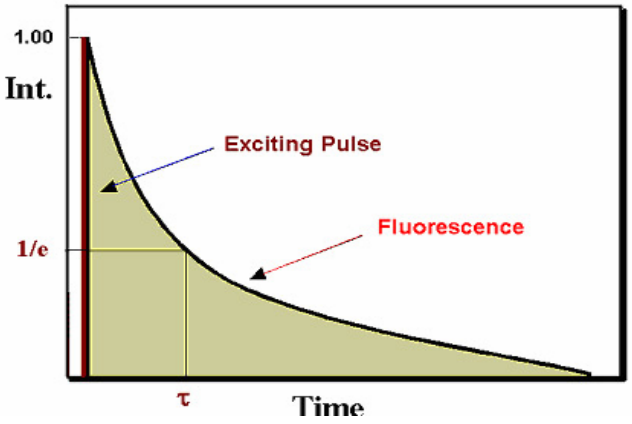
The fluorescence lifetime

[lifetime of the excited state] is defined by the average time the molecule spend in the excited state prior to return to the ground state.

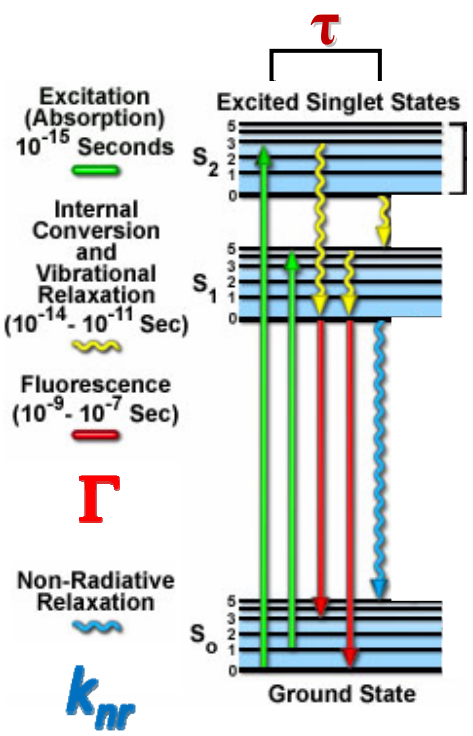
1. $\tau = \dfrac{1}{\Gamma + k_{nr}}$

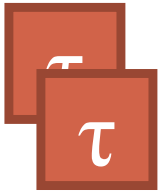
2. $n(t) = n_0 \exp(-t/\tau)$

3. $I(t) = I_0 \exp(-t/\tau)$



Multi-exponential decays





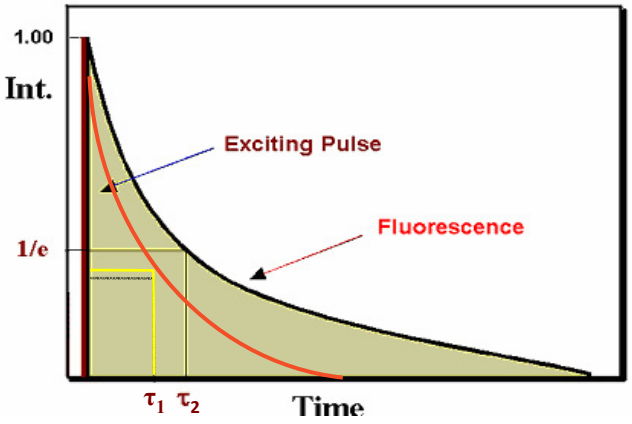
Multimple components

In case of two- and more components with different lifetime are present, the decay will become **multiexponential**.

1. $I(t) = \alpha_1 e^{-t/\tau_1} + \alpha_2 e^{-t/\tau_2}$

α – pre-exponential factor

2. $I(t) = \sum_i \alpha_i e^{-t/\tau_i}$

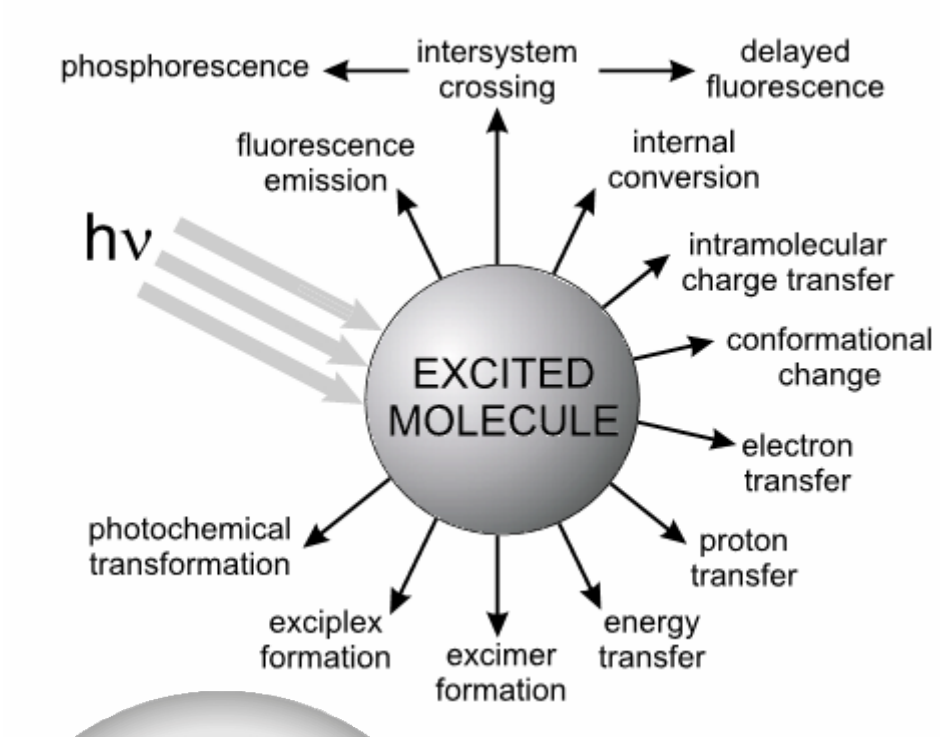


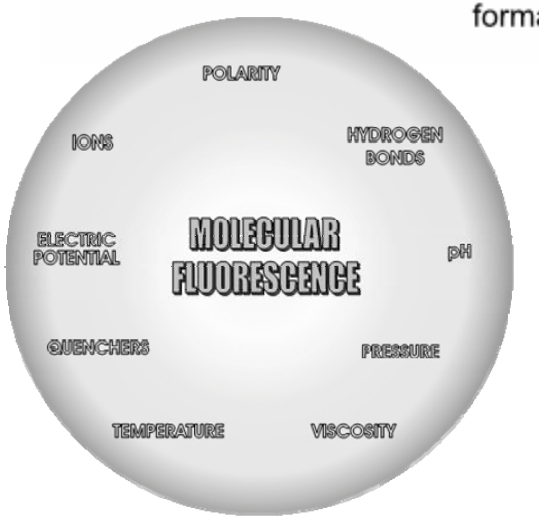
Measurement

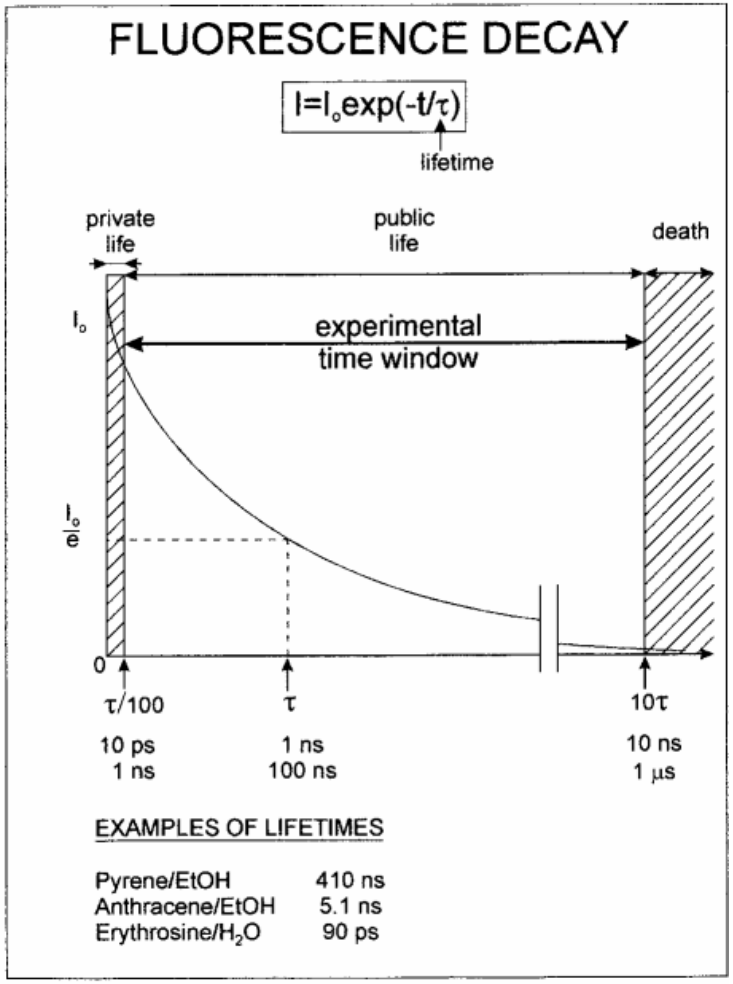
Following the excitation, the decay time is calculated from the slope of a plot of **log I(t)** versus **t**.

Time- and Frequency Domains

Lifetime basics







B. Valeur. “Molecular Fluorescence: Principles and Applications”.

TIME-CORRELATED SINGLE PHOTON COUNTING

History

1926 — First experiment of lifetime measurement by Gaviola. Phase fluorometry applied.

1984 — D. O'Connor and D. Phillips. First monography on TCSPC

TCSPC is used primarily to record fluorescence decay of dye solutions in cuvettes.

+ Amazingly sensitive and accurate technique with excellent time-resolution
- Slow acquisition, single dimension.

The Becker&Hickl Era.

1983 — SPC-100, the first model of TCSPC board

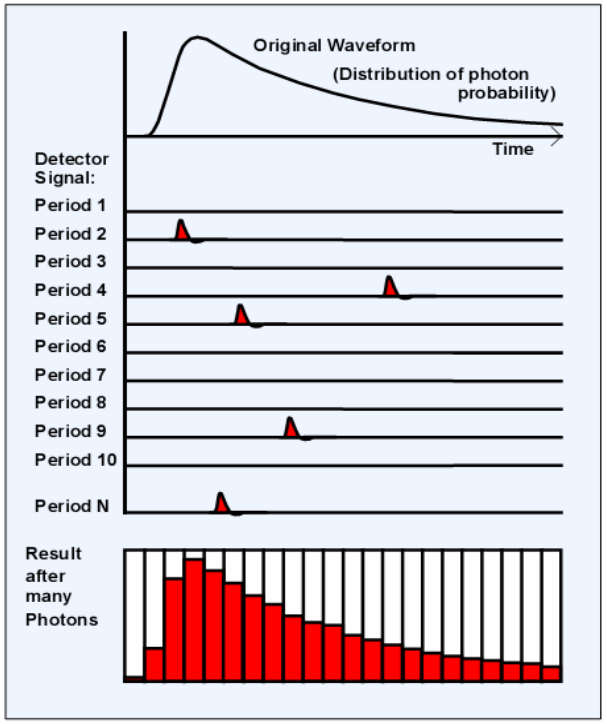
1992 — FPGA-based board introduced: the TCSPC on the rise.

1992 — Introduction of the first multidimensional system. 100 times faster than before!

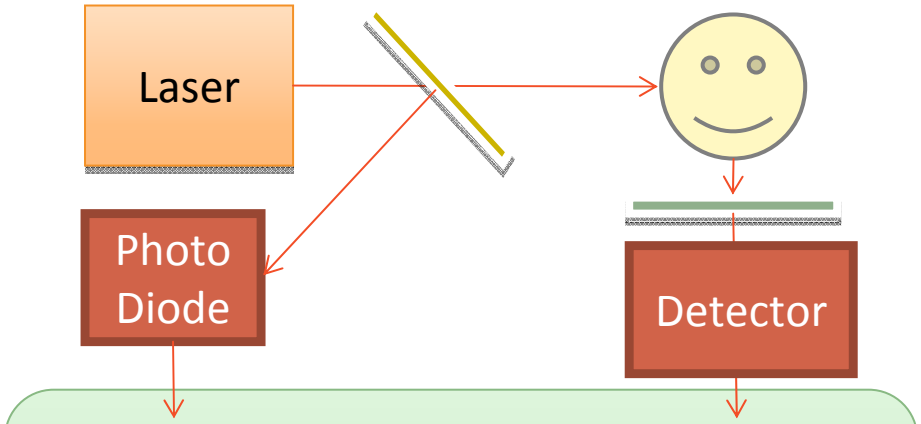
The principle

The task:
to reconstruct the fluorescence decay waveform.

Solution:
repetitive excitation and memory channels.



Building from the scratch



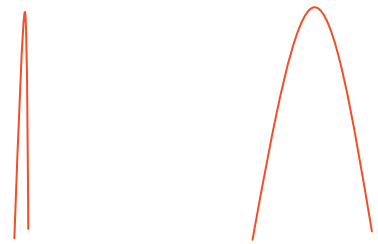
- Detect the time of photon arrival in a high-precision manner.
- Maintain low level of deviation.
- Assign each photon to the corresponding memory channel

TCSPC

The instrumentation: Light sources

The light source:

The δ-function consideration



We want

We get

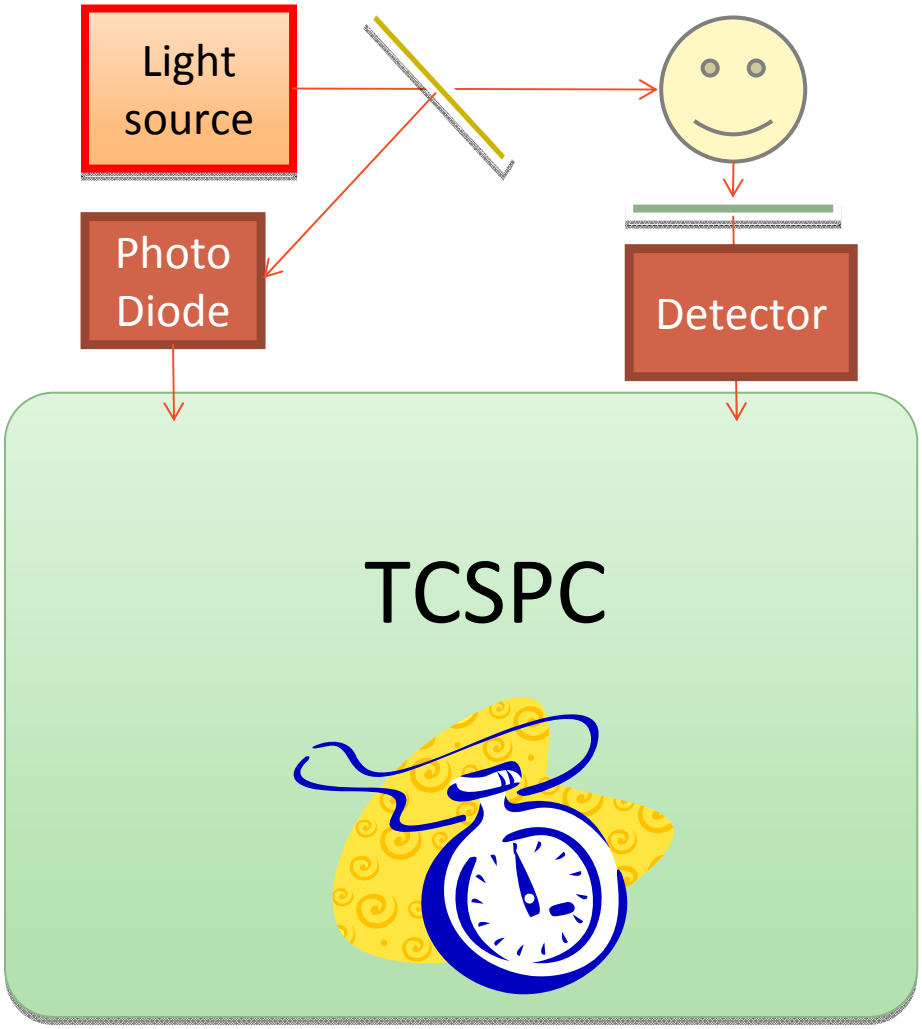
The evolution

Nanosecond flashlamp — 2ns, 50KHz

Cavity pumped dye lasers — 5ns, 80MHz

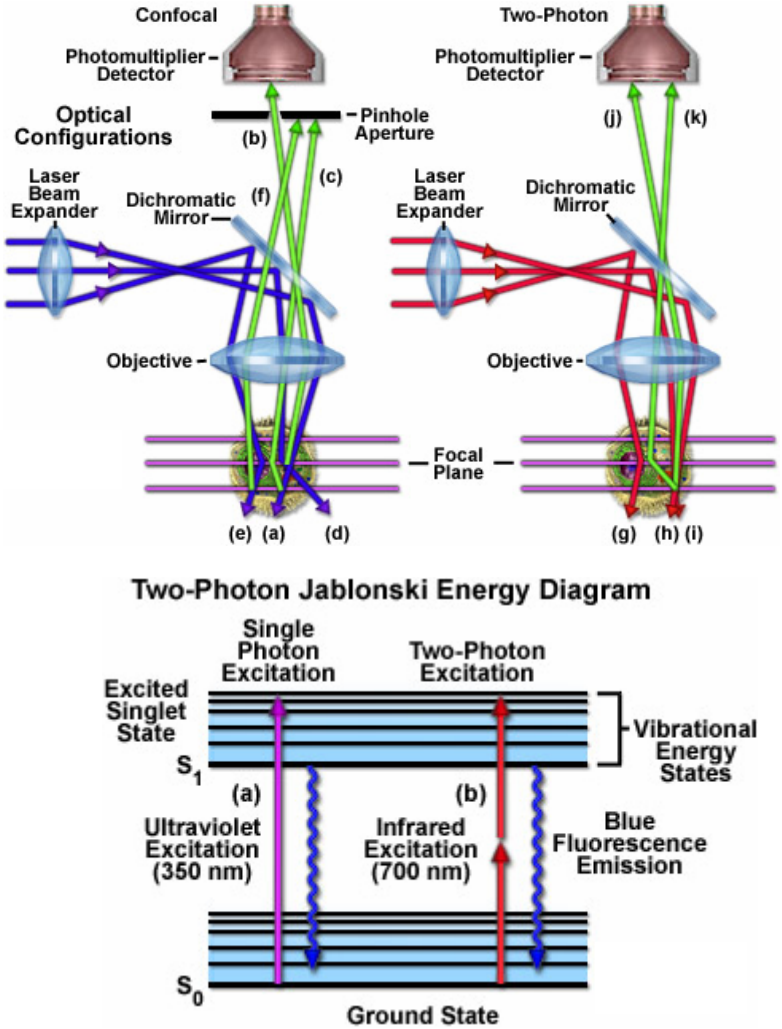
The Ti: Sapphire: the One — 100fs, 80MHz

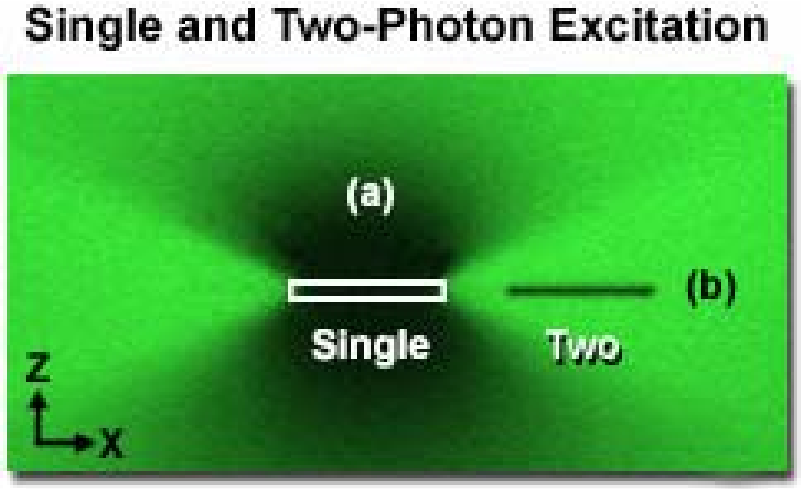
Building from the scratch



The instrumentation: The modality

The excitation:

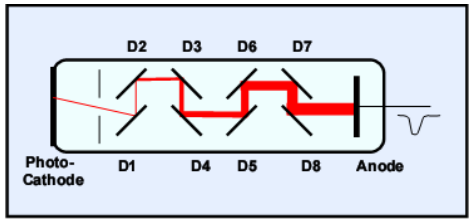




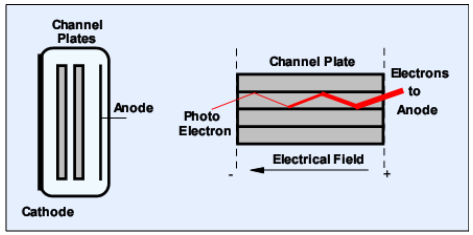
TCSPC

The instrumentation: Detectors

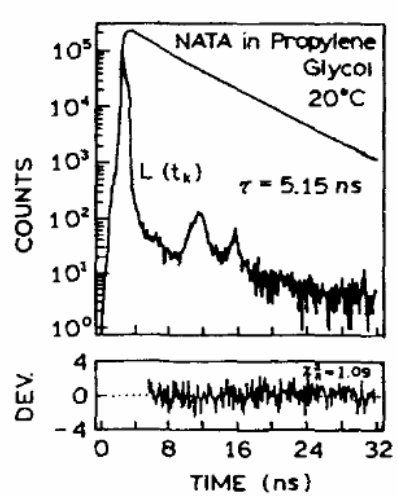
The Detector:

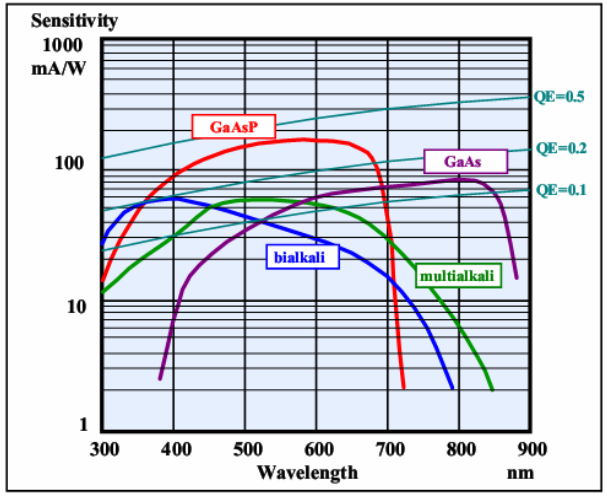


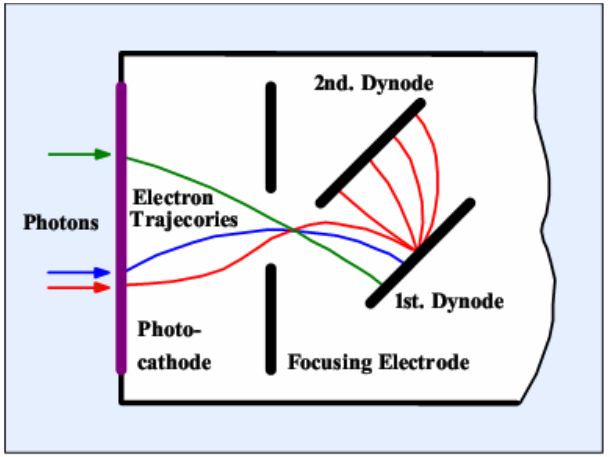
PMT
Photo
Multiplier
Tube



MCP
Multi
Channel
Plate







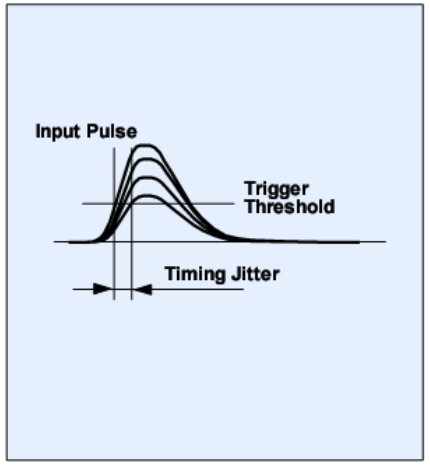
Parameters & limitations

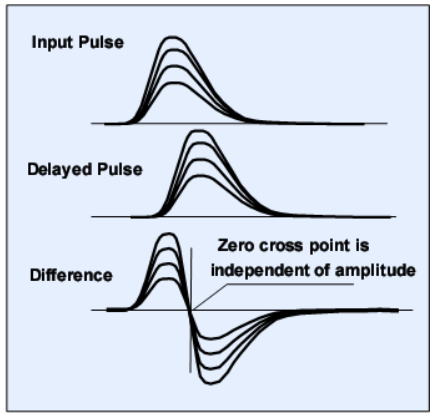
- Transit-time spread (!)
- Height distribution (jitter) (!)
- Spectral response
- Dark count rate
- Afterpulsing
- Quantum efficiency

The instrumentation: CFDs

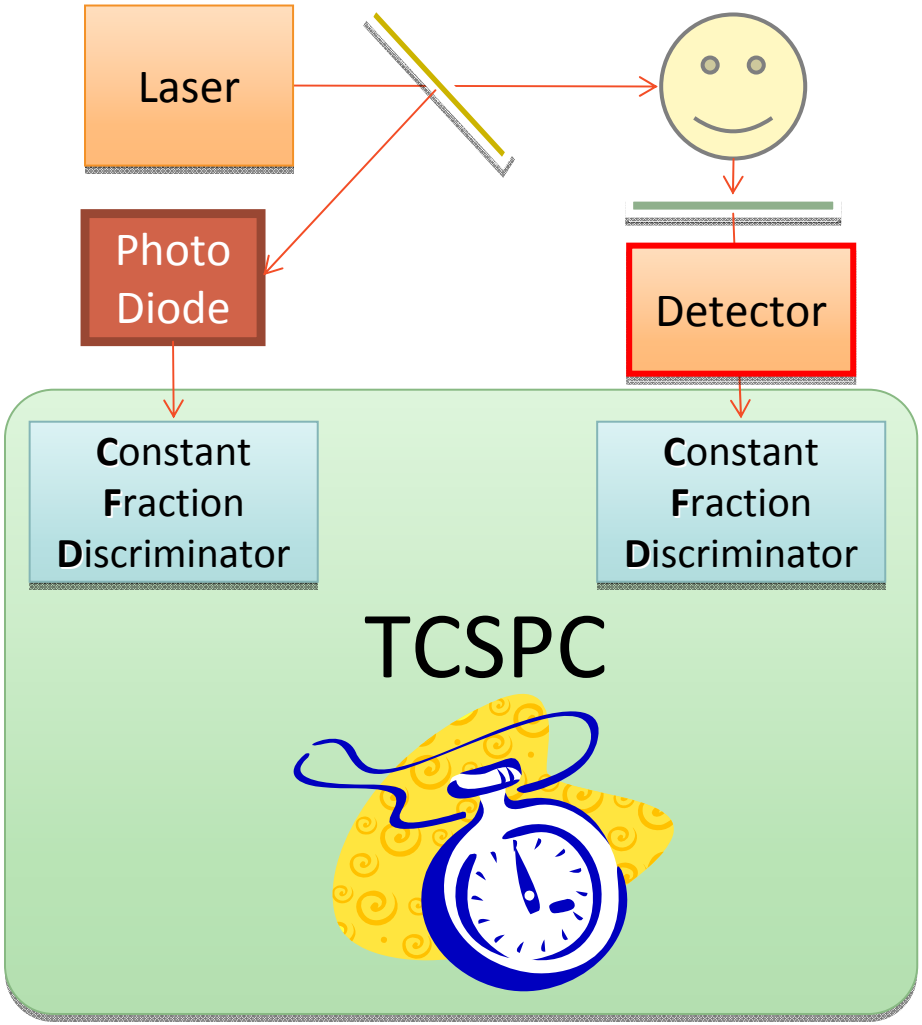
The Constant Fraction Discriminator

- Height distribution (jitter) (!)
- Dark Count





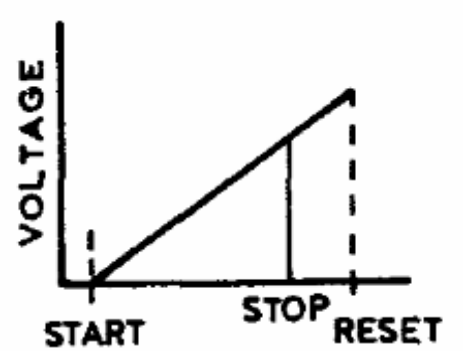
Building from the scratch

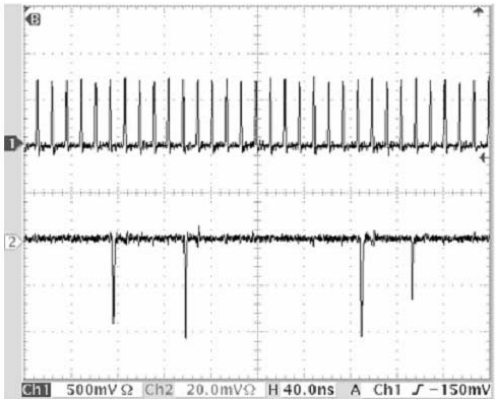


The instrumentation: TACs

The Time to Amplitude Converter

The TAC Principle





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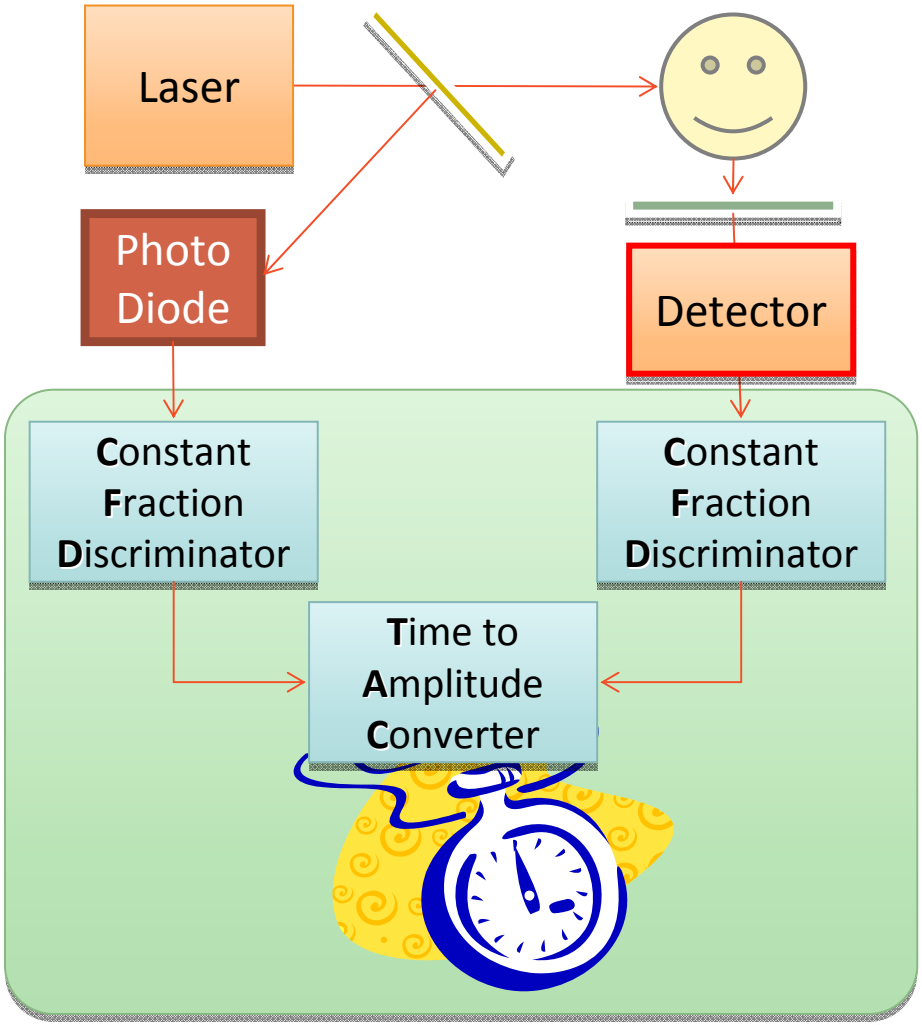
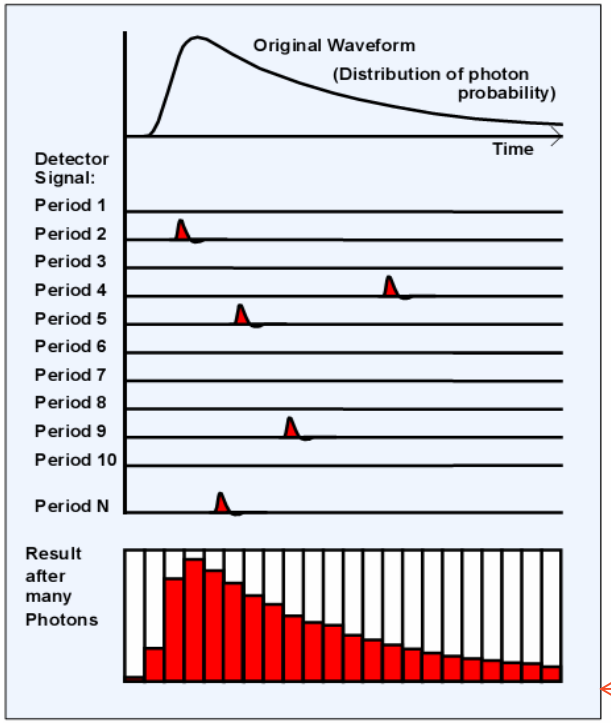


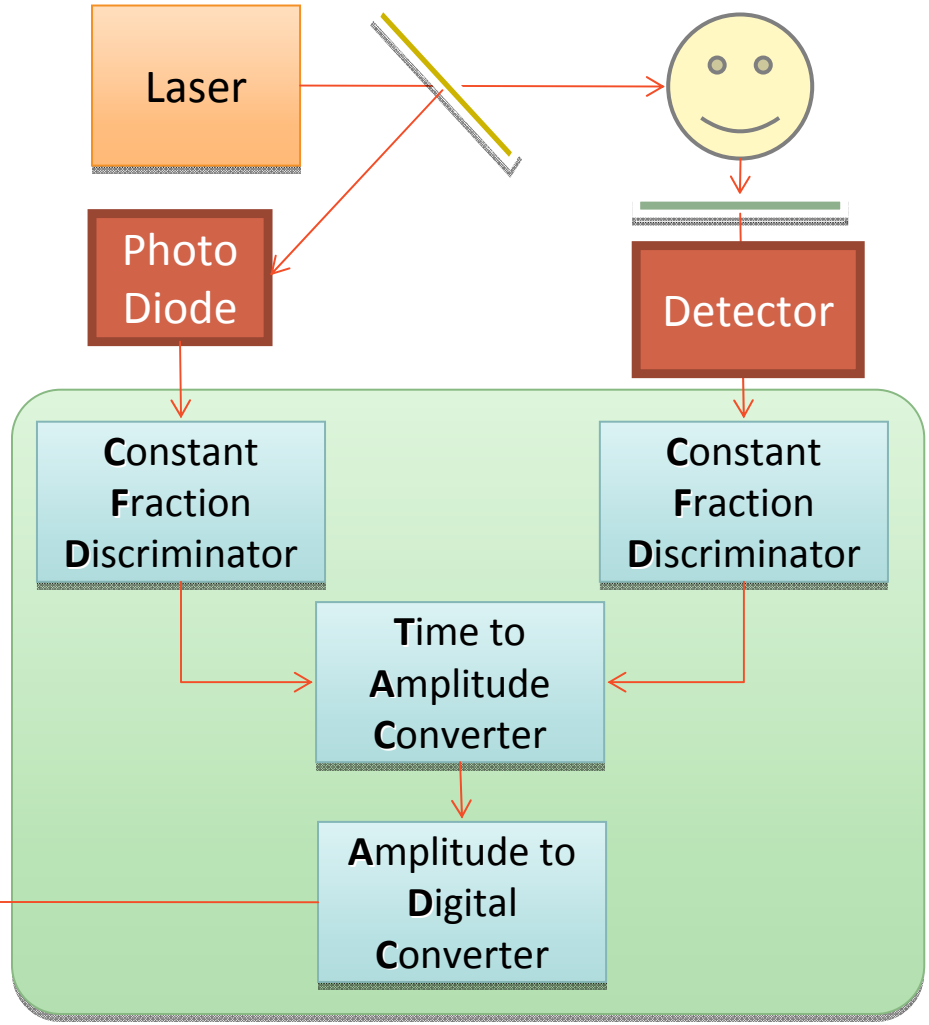
The instrumentation: ADCs

The task:
to reconstruct the fluorescence decay waveform.

Solution:
repetitive excitation and memory channels.

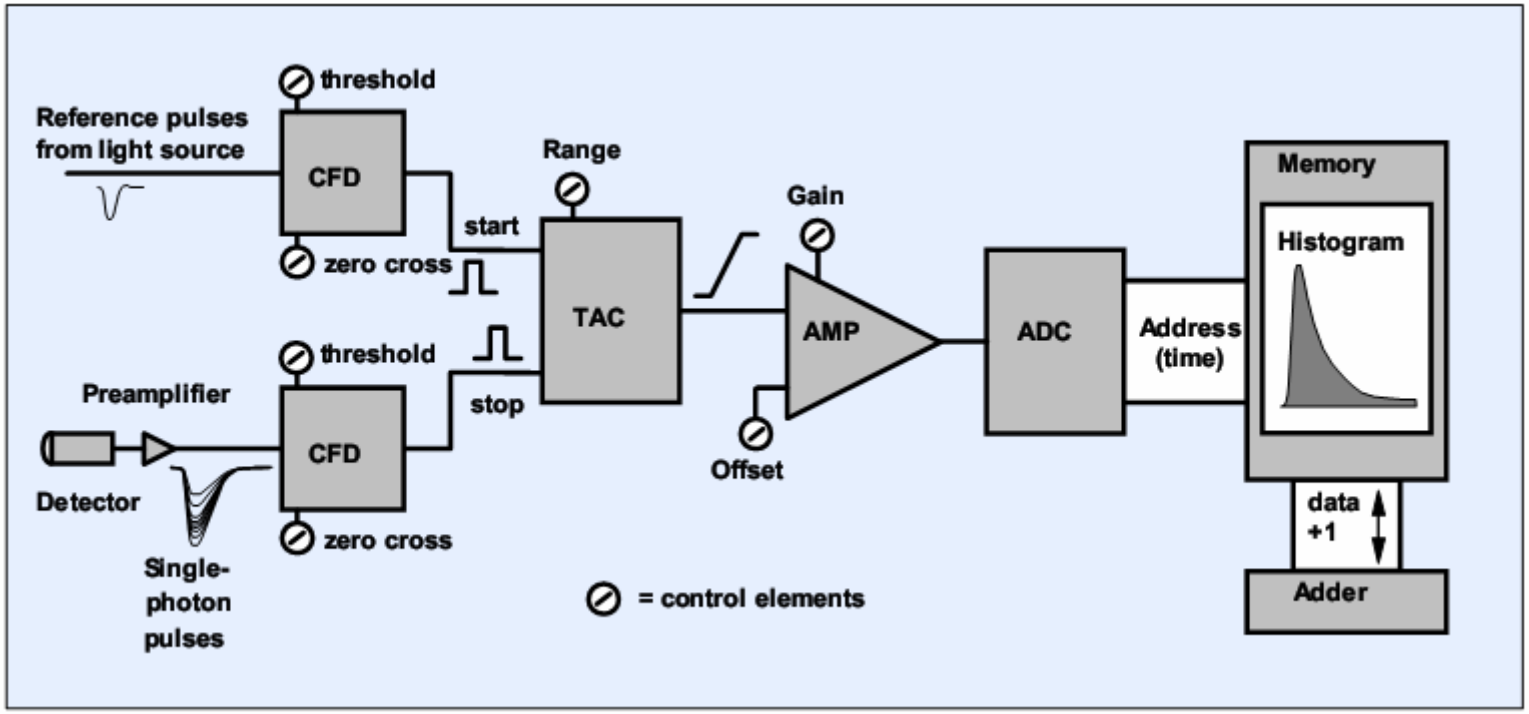


The stopwatch concept:
The measurement units: laser pulses
Arrival of the photon – start/stop



TCSPC

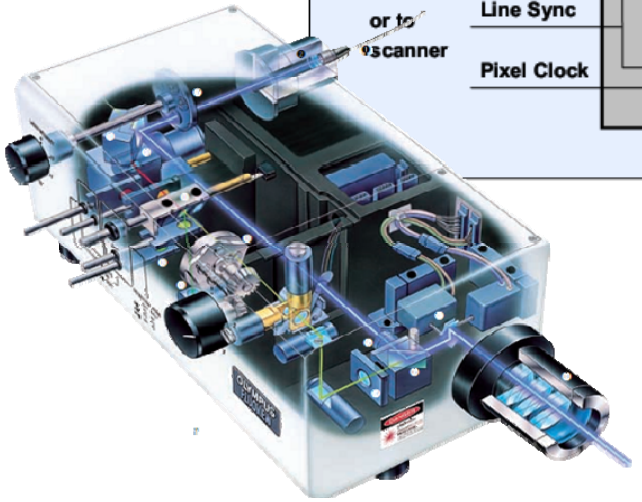
The classical scheme

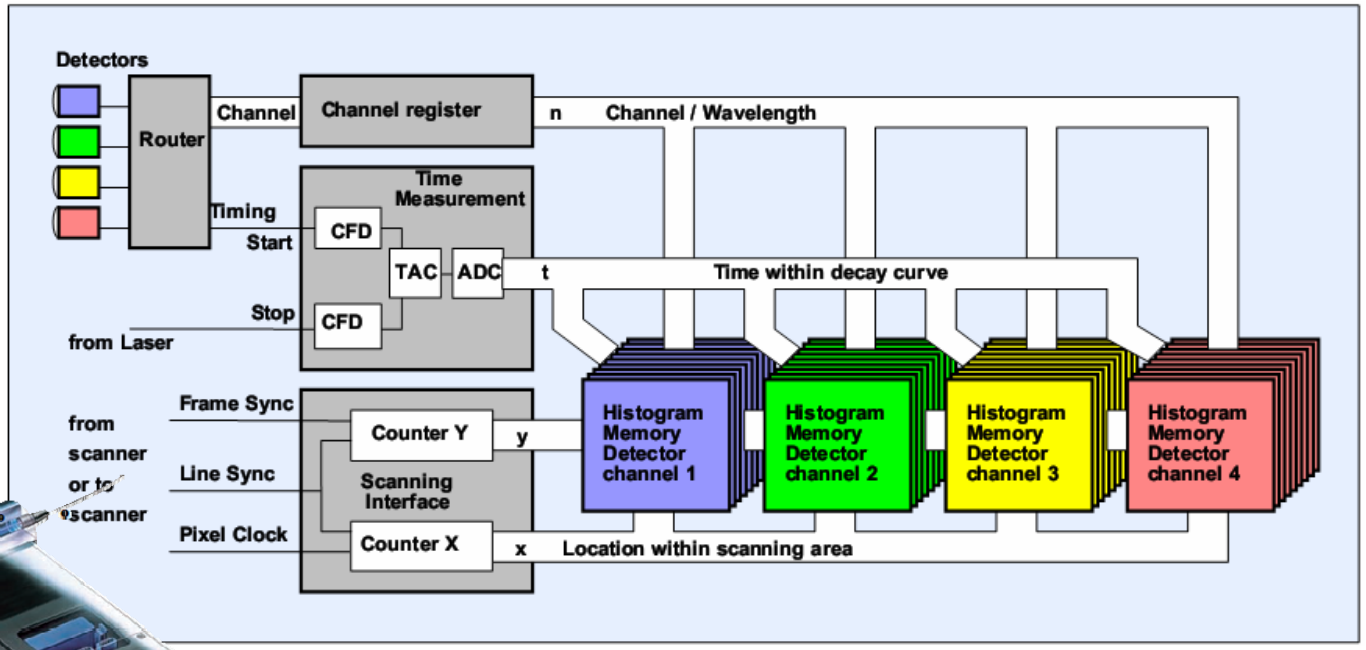


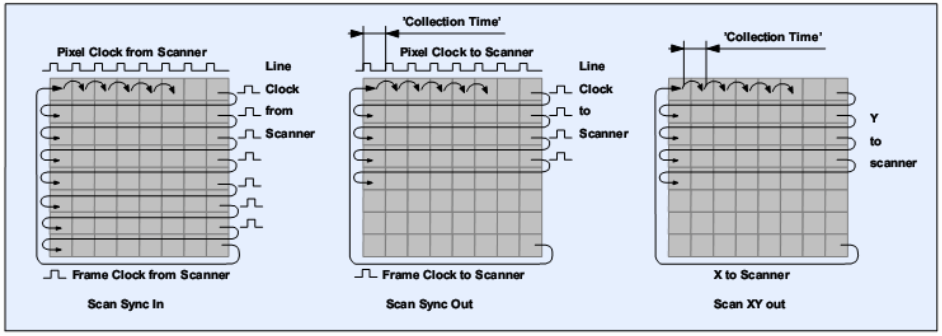
Start-stop problem and reversed mode

Introducing new dimensions: spatial

Multidimensional TCSPC:

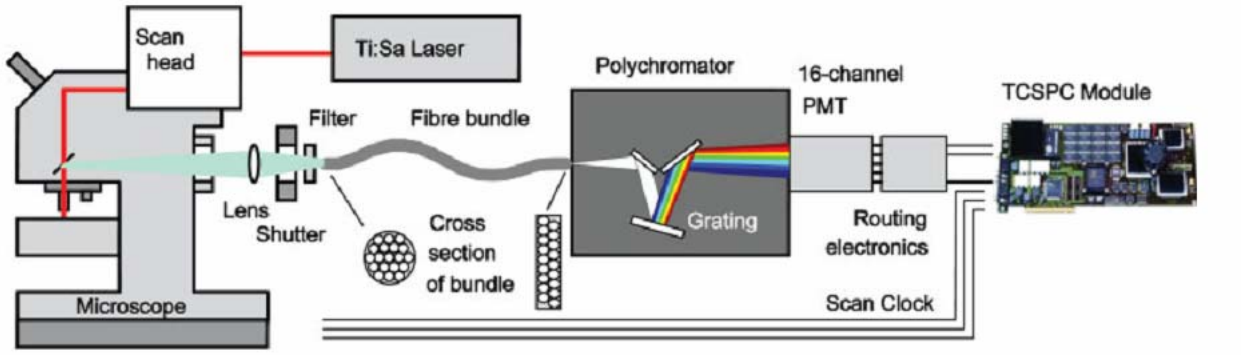


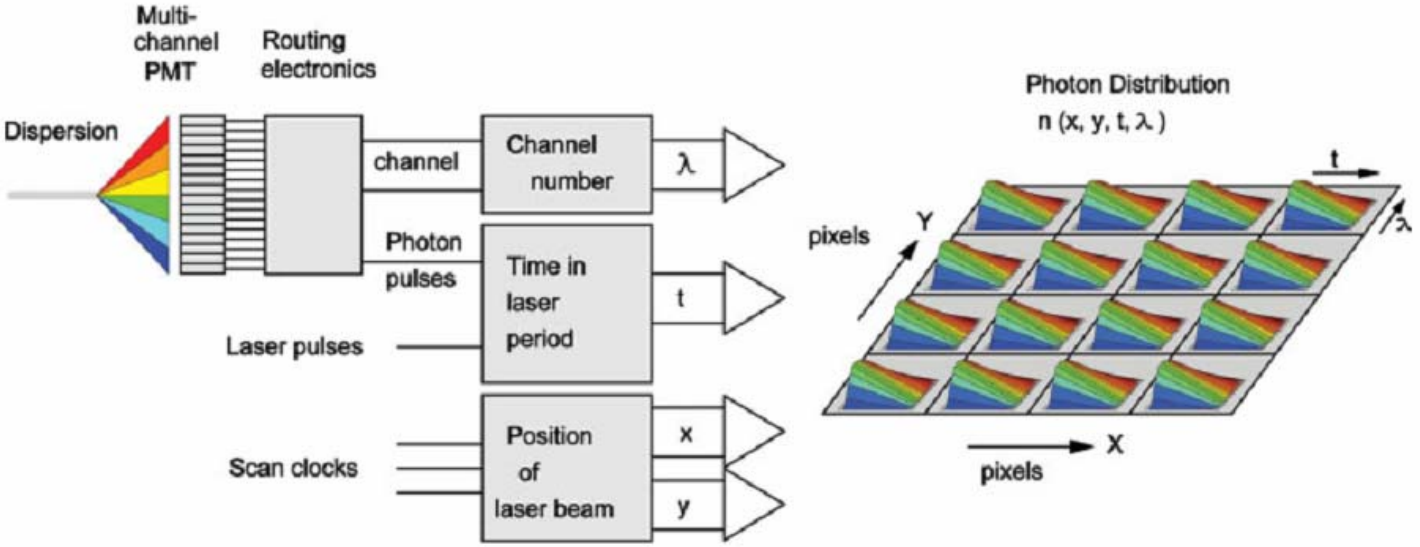




Introducing new dimensions: spectral

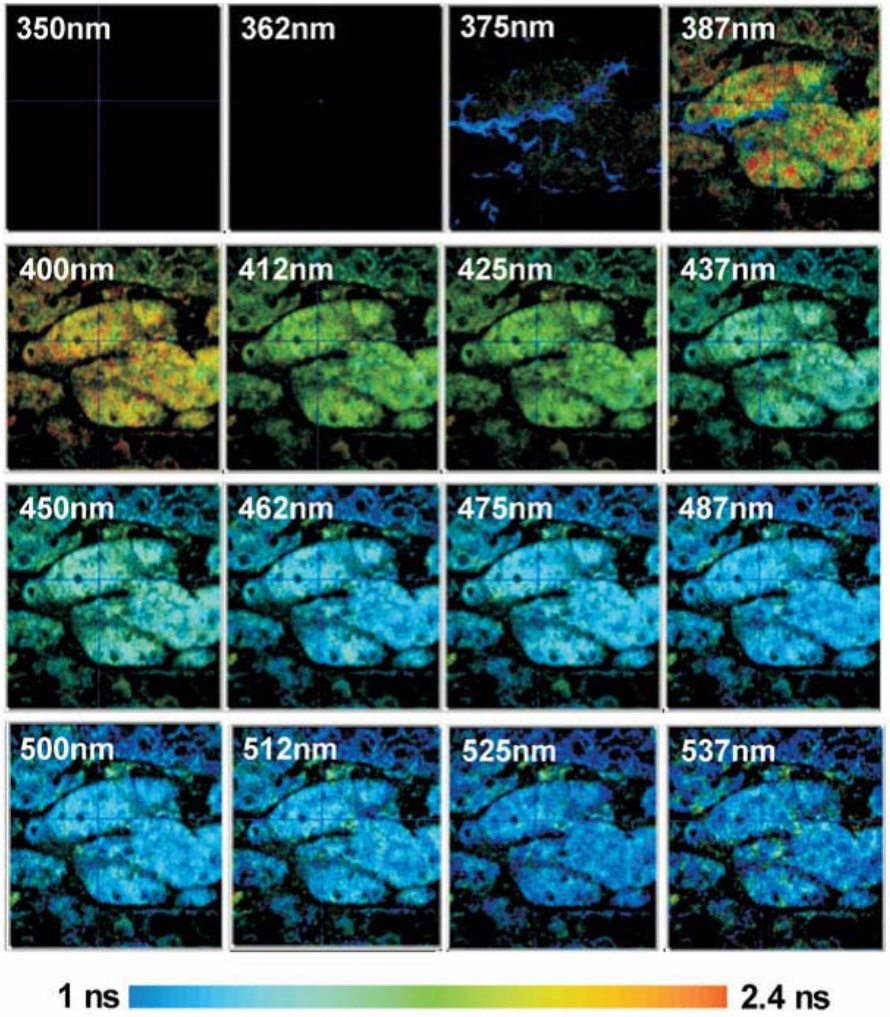
Multidimensional TCSPC:





Introducing new dimensions: spectral

Multidimensional TCSPC:



Introducing new dimensions: spectral

Multidimensional TCSPC:

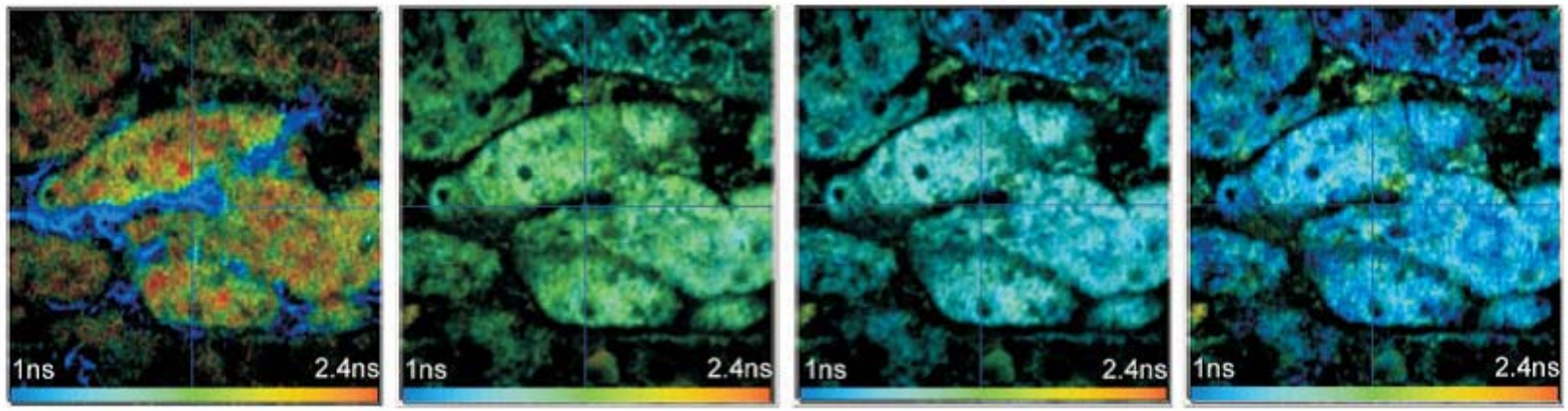
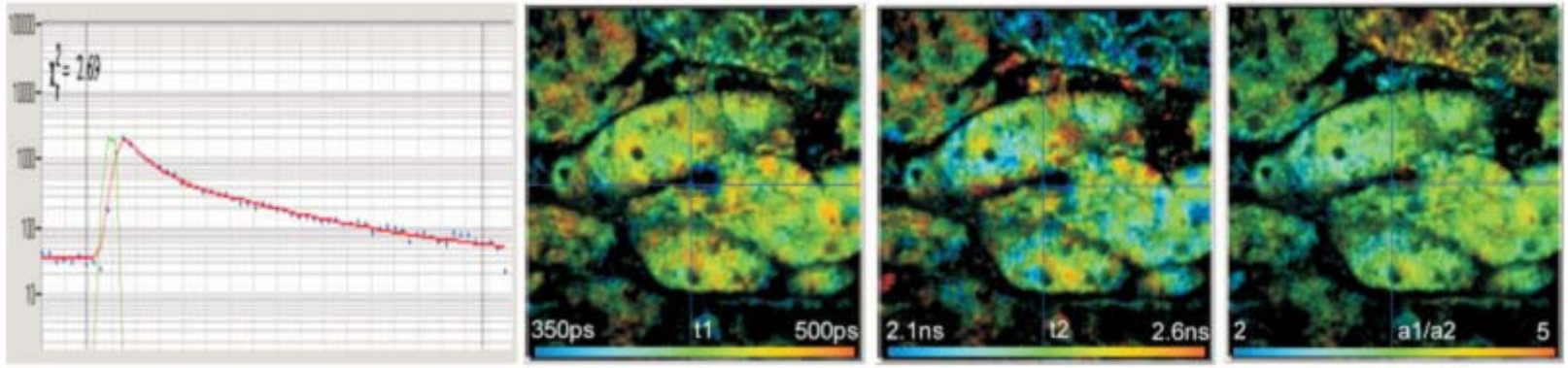
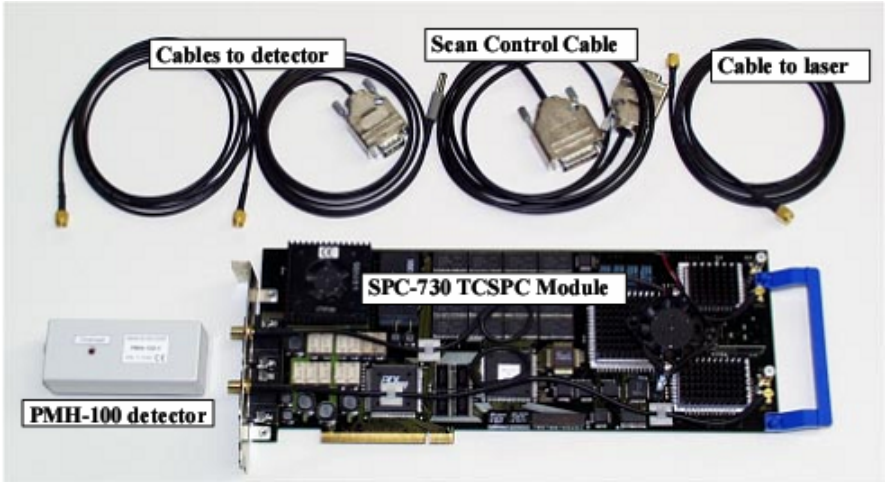


Fig. 5. Single-exponential lifetime images in the wavelength intervals from 350 to 387 nm, 400 to 437 nm, 450 to 487 nm, and 500 to 537 nm.



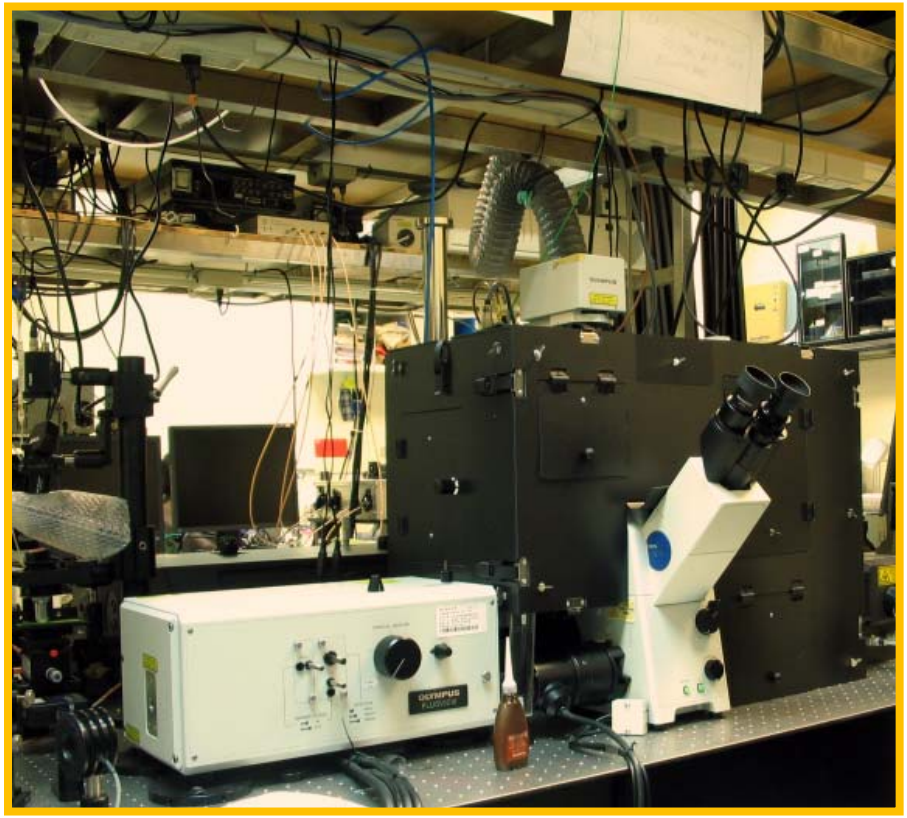
The instruments in reality

The Board

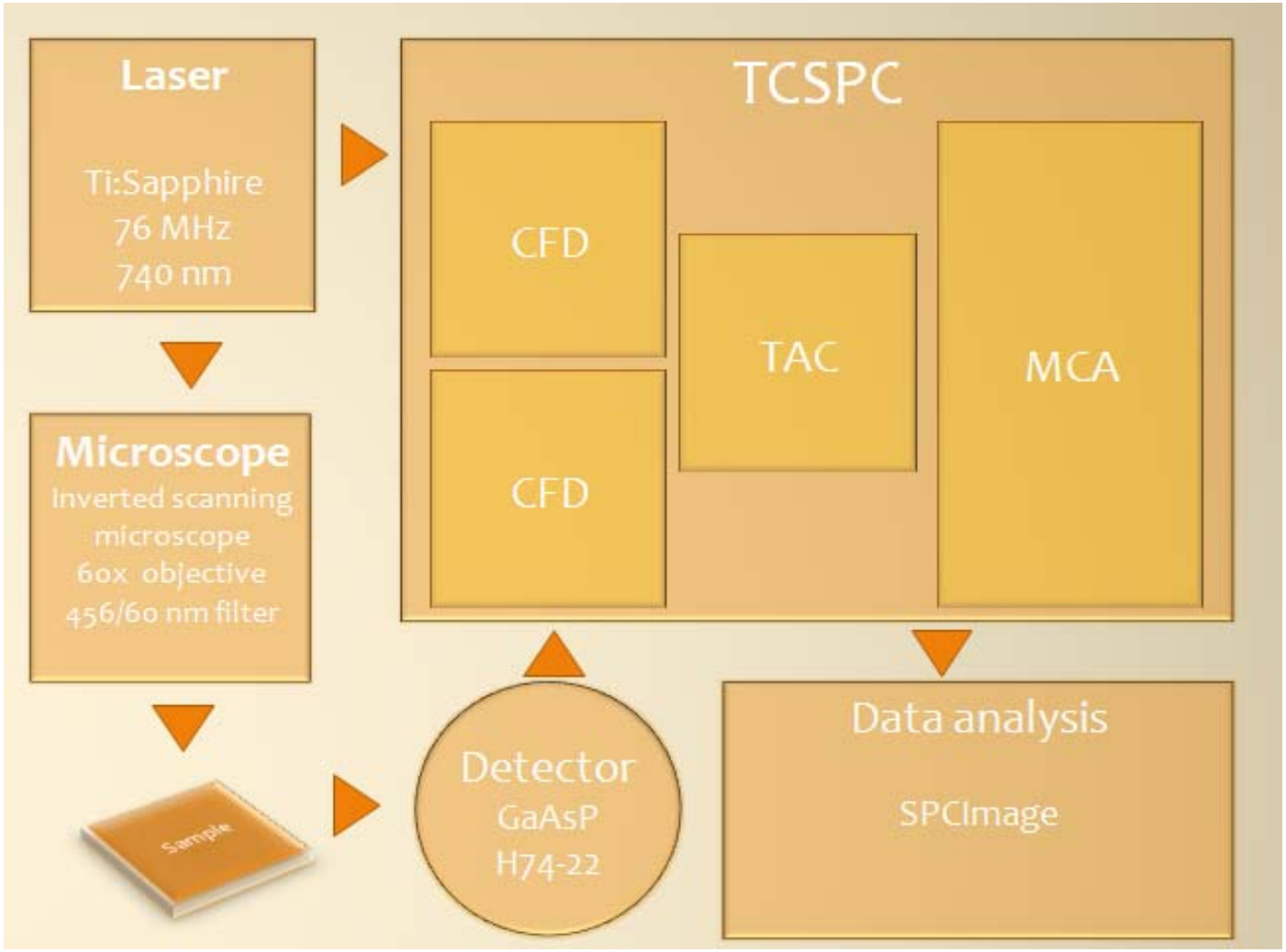


Detectors

The real lab



The setup


Laser
Ti:Sapphire
76 MHz
740 nm
Microscope
inverted scanning microscope
60x objective
456/60 nm filter
Sample
TCSPC
CFD
CFD
TAC
MCA
Detector
GaAsP
H74-22
Data analysis
SPCImage

The TCSPC characteristics

Time resolution:

Not limited by the standard Single Electron Response (SER), which limits the resolution of the analog techniques. The TCSPC is rather dependent on the Transit Time Spread (TTR), which varies in the range of 15-400 ps depending on the detector. The resolution of the board is fs per channel.

Counting efficiency:

$$SNR = \sqrt{N}$$

Near-ideal efficiency

Sensitivity:

$$S = \frac{(Rd * N/T)^{1/2}}{Q}$$

Rd – dark count rate (300 s^{-1}),
N- number of time channels (256)
T – measurement time (100s)
Q – quantum efficiency (0.1)

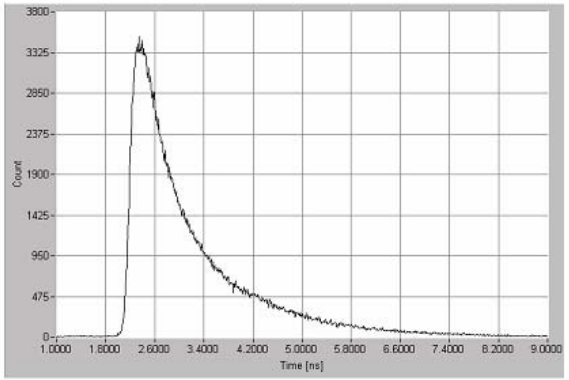
S=280 photons/s

Count rate:

Pile-up problem and insignificance for the TCSPC. Possible rates: $5x10^6$/s

Acquisition time:

More than 300.000 in 100 ms.



Summary

1. The **TCSPC** systems were sensitive and precise from the beginning, however, limitation in the light source repetition rate and slow electronics made the acquisition times enormously big.

2. The basic principle of the TCSPC is based on the repetitive excitation, collection of the photons, placing them in the corresponding memory channel and further building of the decay curve.

3. Upon the introduction with the high-repetition rate lasers with ultra-short pulses (Ti:Sapphire) the main limitation factor of the system is the detector with the transit time spread around 300ps, and time uncertainity introduced by afterpulses, dark noise, amplitude jitter and color sensitivity.

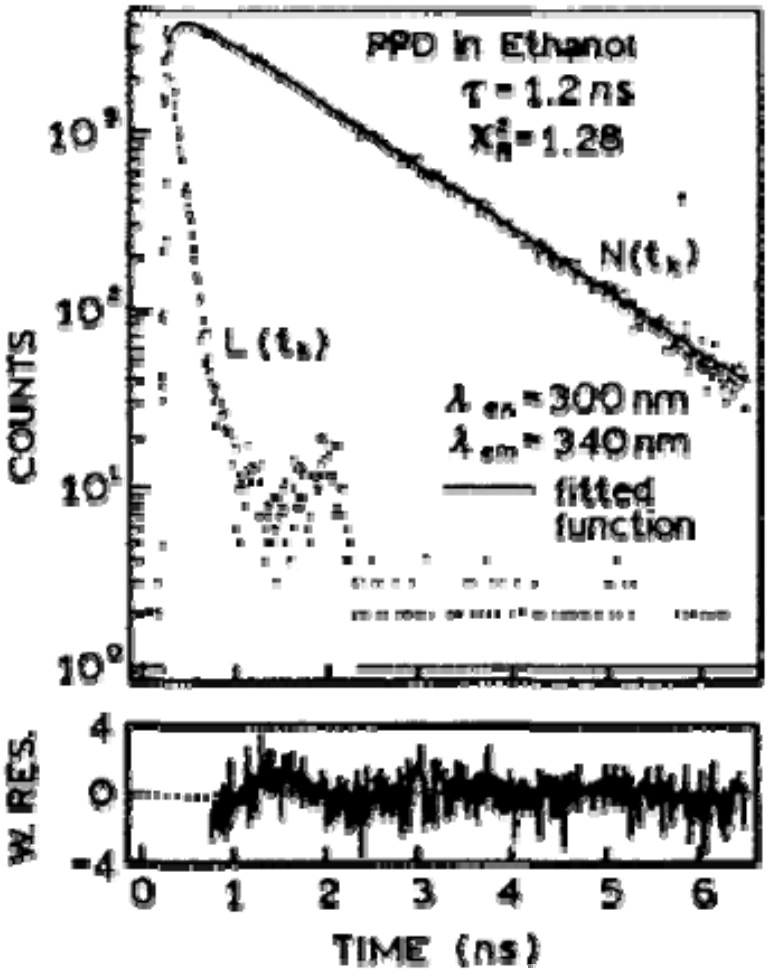
4. The limitations of the PMTs are addressed by the electronics of TCSPC with the noise and jitter filltered and corrected by the CFDs, high-precision working TAC and ADCs.

5. Currently additional modalities are introduced to the market: multidimensional TCSPC allows to correlate lifetime with other parameters, such as spatial coordinates (scanning) and spectrum-resolved.

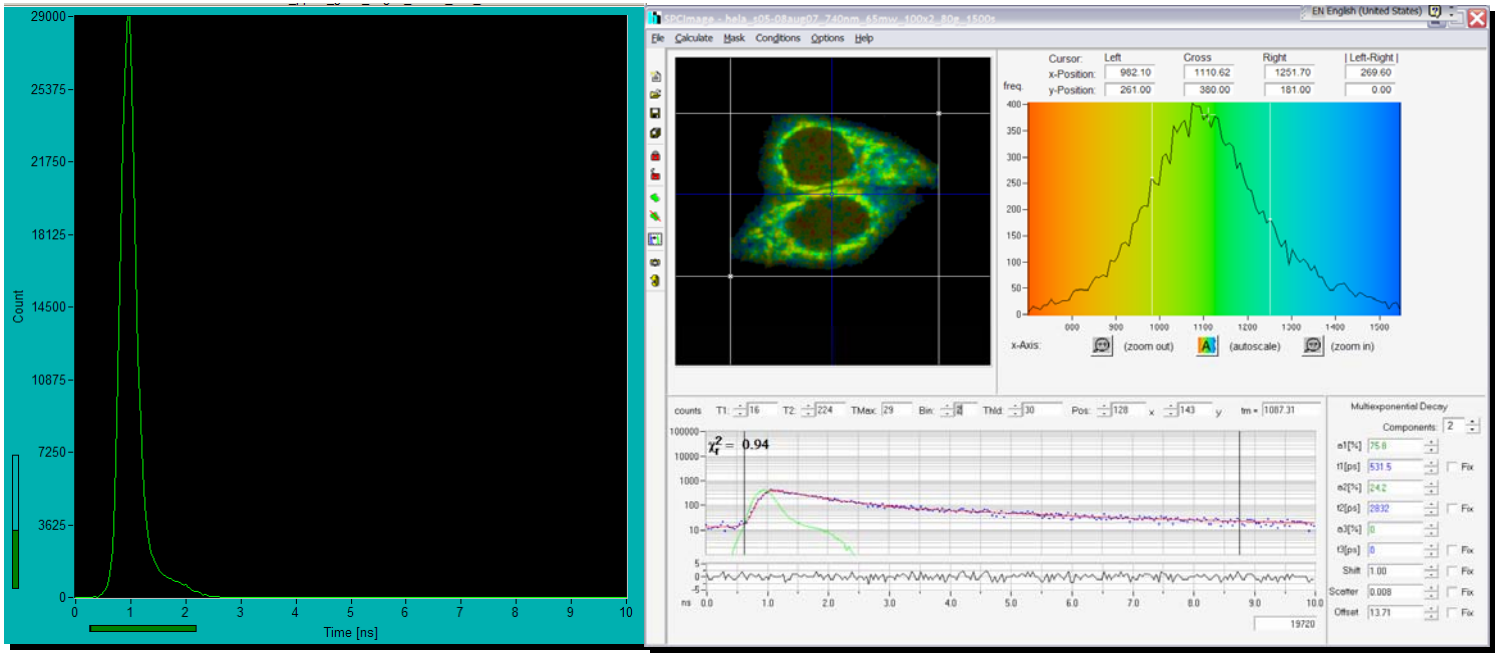
DATA ANALYSIS

Got the data. What next?

- Development of the decay model.
- Calculation of the Instrument Response Function.
- Convolution of the model with IRF
- Fitting of the resulting model with the data.



The Instrument Response Function

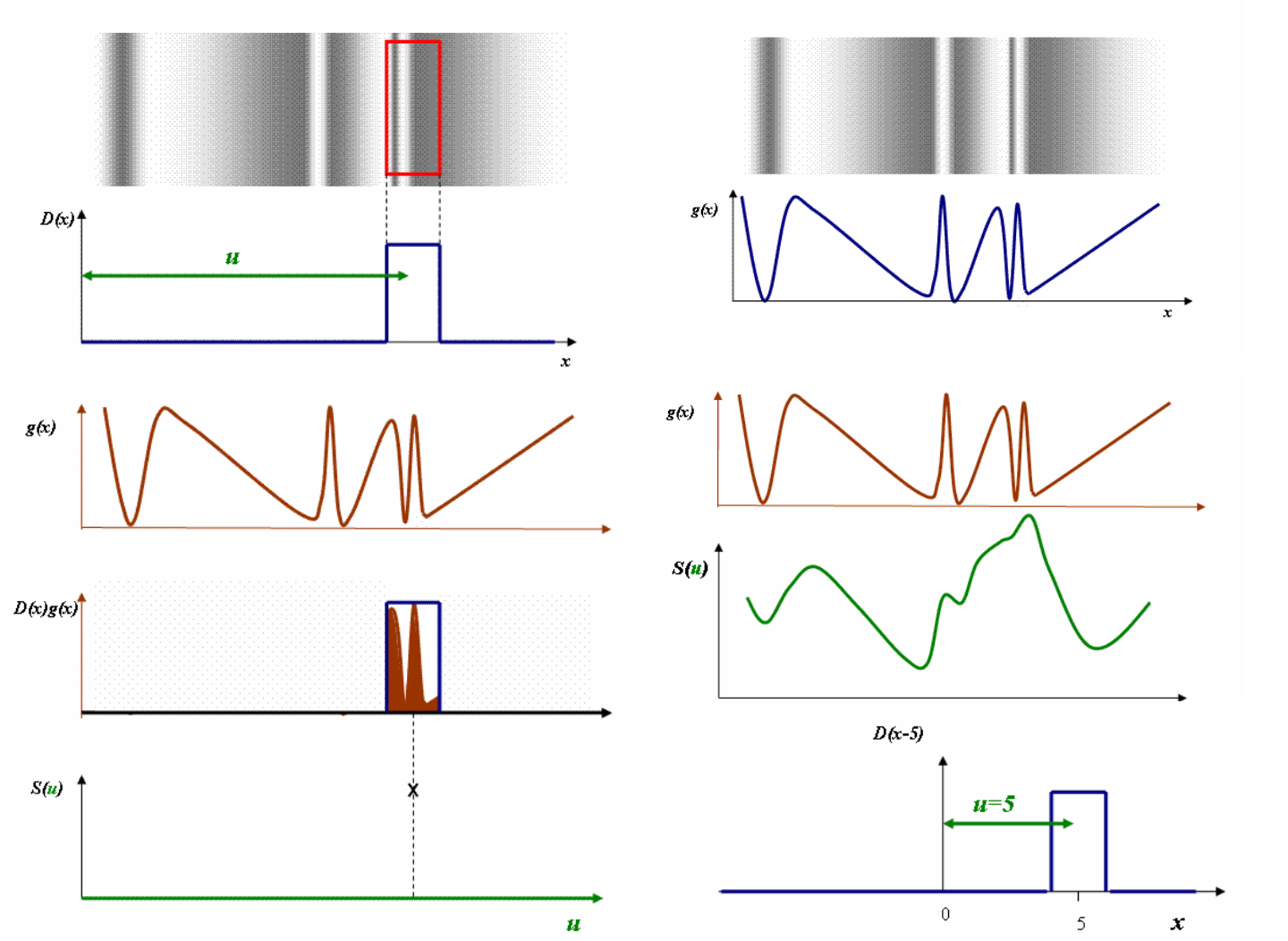


Measurement:

-Scattering for single-photon excitation

- Second Harmonic Generation for 2-photon excitation.

The Instrument Response Function



$$f(x) = g(x) \oplus h(x)$$

$$S(u) = \int g(x)D(x-u)dx$$

Deriving the model function

$$I_0 + \sum_{i=1}^{n} a_i\left(\exp\left(-t/\tau_i\right)\right)$$

Data set for analysis

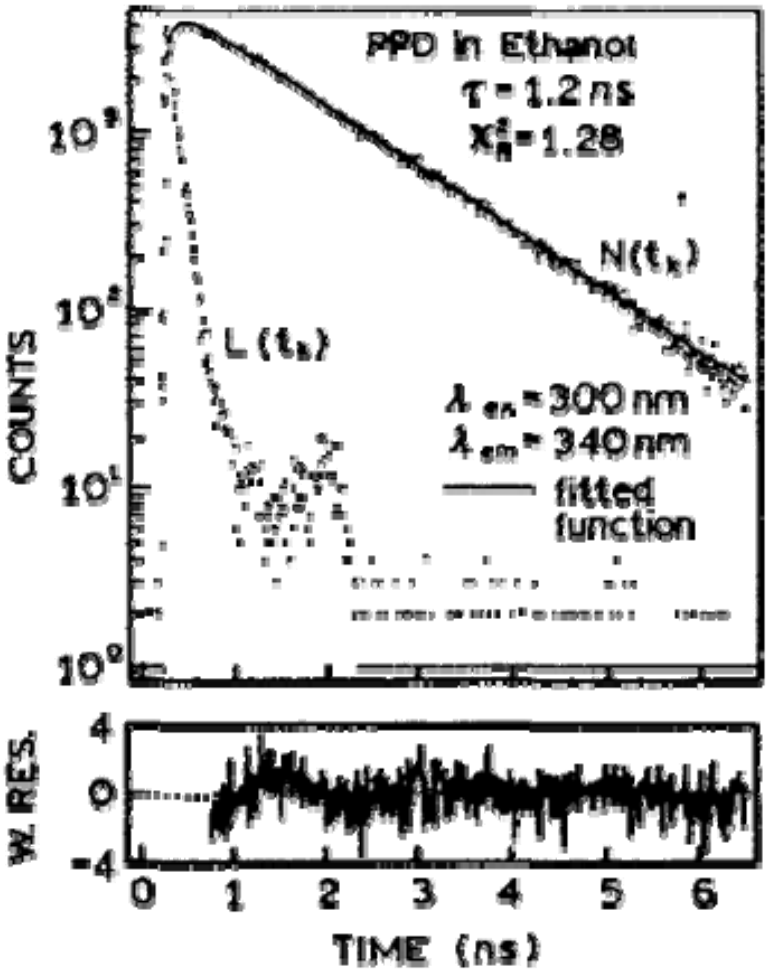
Instrument response (IRF)

+ N(t_k) Measured photons distribution over **t**

+ N*c*(t_k) Calculated decay

+ Residuals

$$I(t) = \int_{-\infty}^{\infty} L(t_k)\left\{I_0 + \sum_{i=1}^{n} a_i\left(\exp\left(-t/\tau_i\right)\right)\right\}dt$$



Convolution improves the temporal resolution

Fitting the data

Standard deviation

$$\sigma_k = [N(t_k)]^{1/2}$$

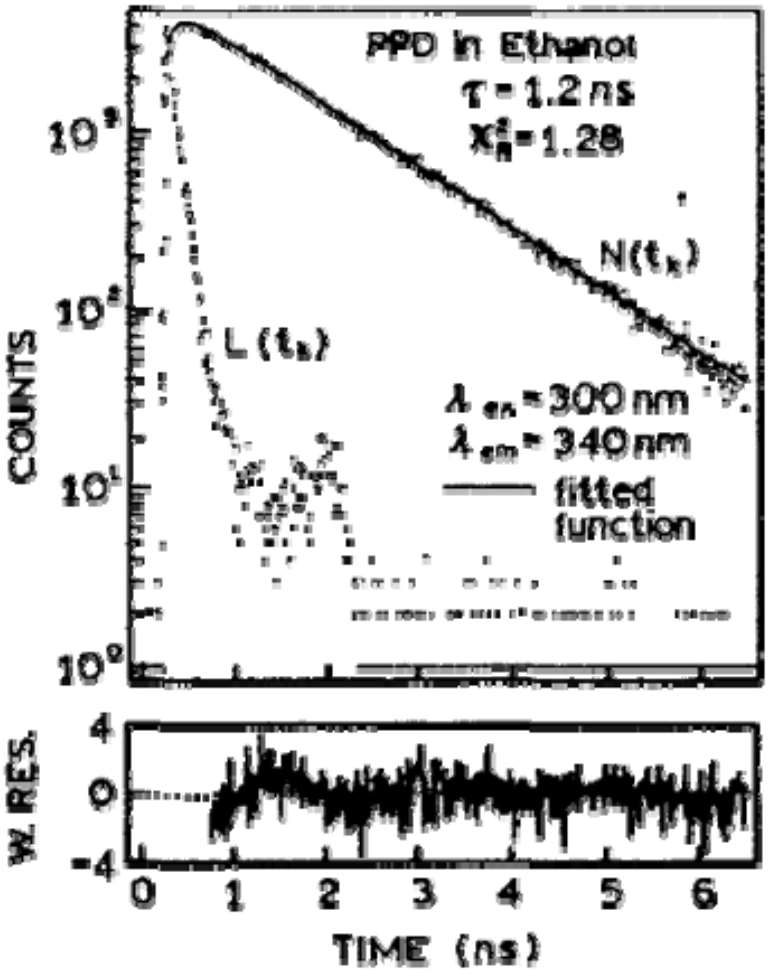
Goodness-of-fit parameter calculation

$$\chi^2 = \sum_{k=1}^{n} \frac{[N(t_k) - N_c(t_k)]^2}{N(t_k)}$$

Reduced goodness-of-fit

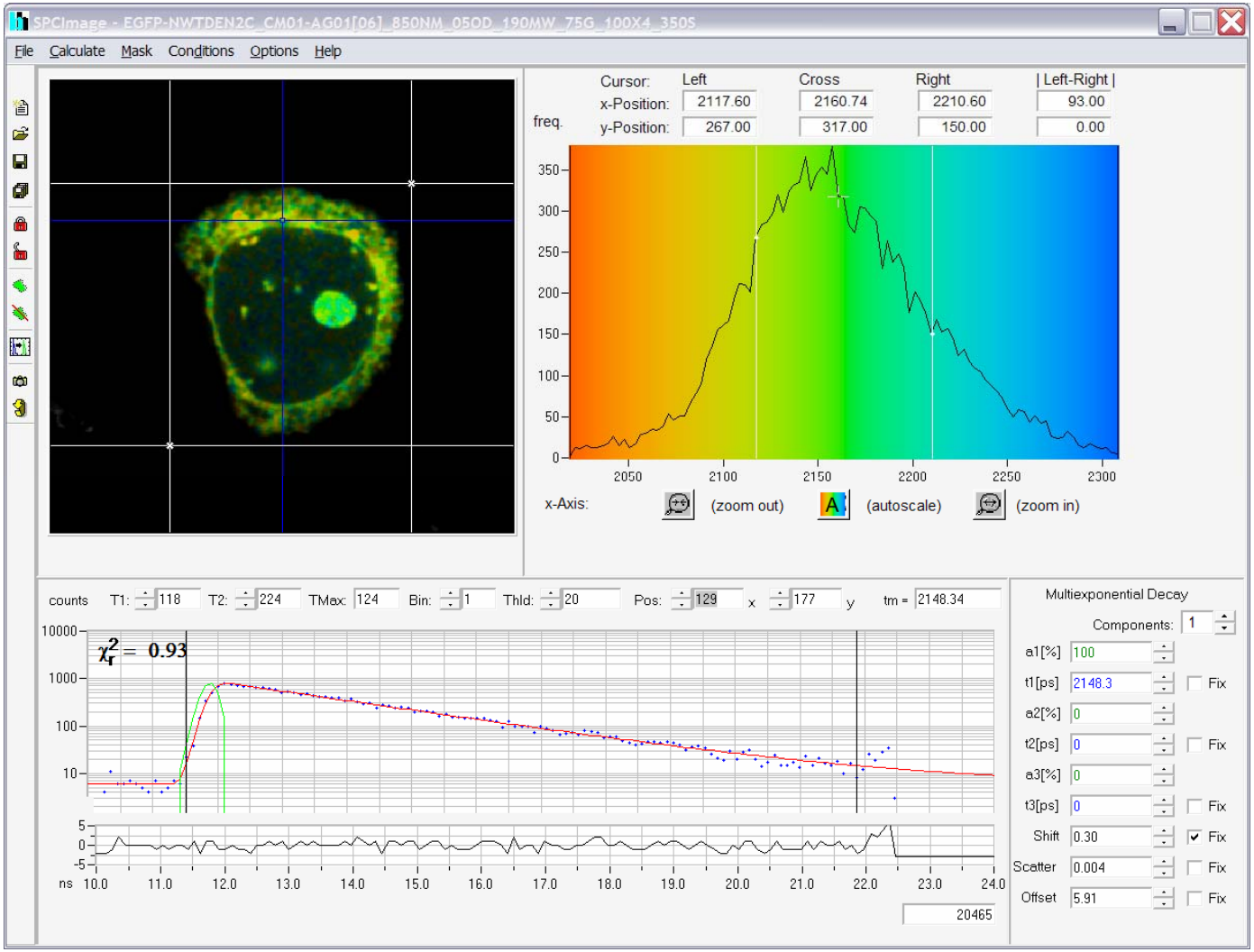
$$\chi_R^2 = \frac{\chi^2}{n - p}$$

Where
n- number of time channels,
p – number of parameters



Levenberg-Marquardt Algorithm.

Color-Coding



Summary

1. The data obtained is convolved by the Instrument Response Function. To restore the original values and increase the temporal resolution a separately measured Instrument Response Function is convolved with the model function.

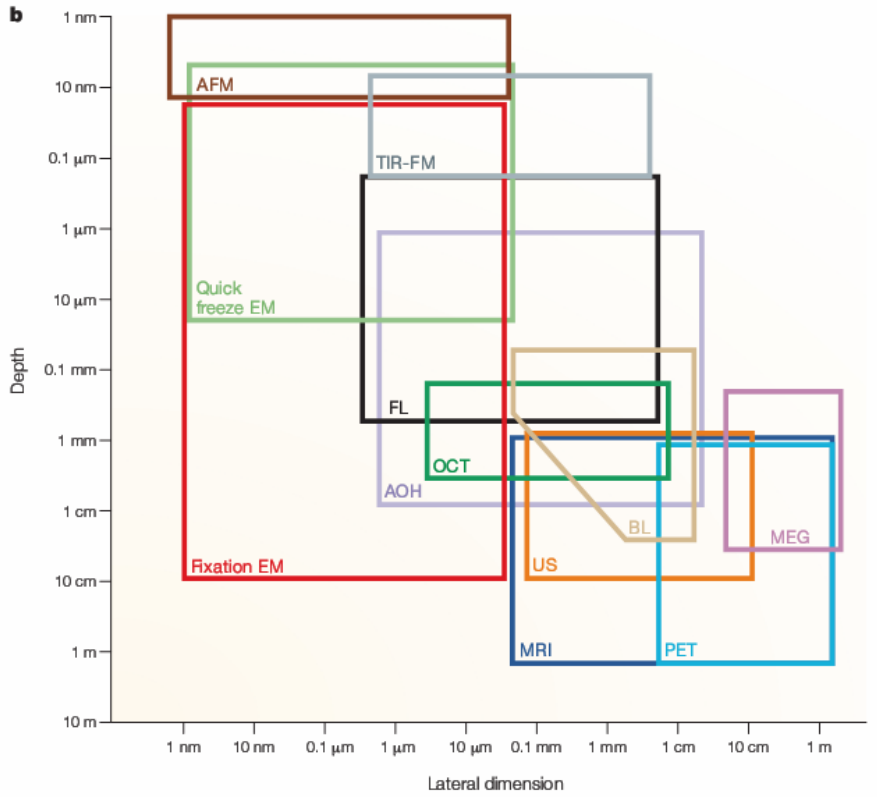
2. Upon convolution the temporal resolution increases almost x10.

3. After the convolution the fitting procedure is applied to let the model function follow the measured decay.

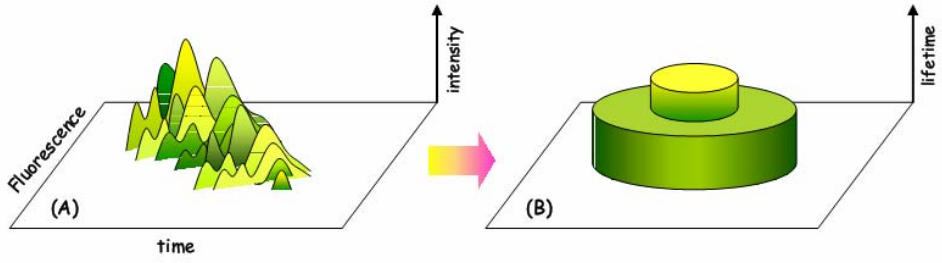
THE APPLICABILITY OF FLIM

FLIM and the others

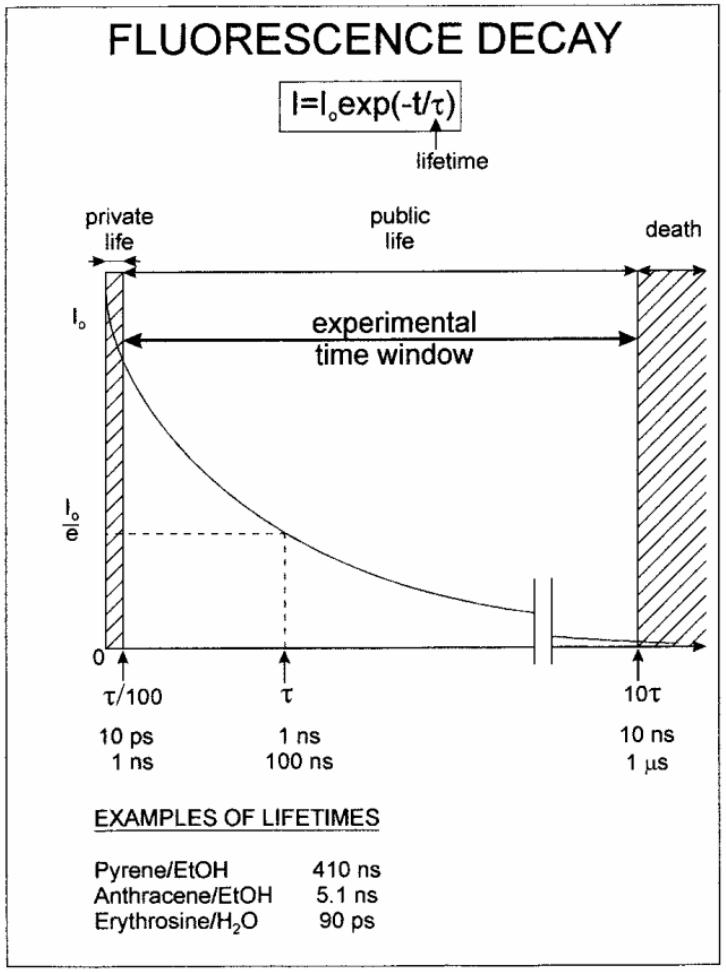
- Atomic Force Microscopy
  - Limited penetration depth
  -Ability to perform the live objects imaging
- Electron Microscopy
  -Good penetration depth
  - Pre-processing required
- Fluorescence Microscopy
  -Dependence on the excitation and emission wavelengths
  - Dependence on the intensity of the excitation
  - Dependence on the sample concentration



R. Tsien, "Imagining Imaging Future", Nature 2003.



Experimental time window



The 150fs resolution by the current generation lasers

Picoseconds resolution by the detectors

Confocal/2-photon microscopy: excitation volume of 0.1fL

Spatial resolution: 200-300 nm.

FLIM probing / Quenching

a. Close proximity: Static
In the close distance. Intramolecular reactions.

c. Collisional: Dynamic quenching
In solutions. Intermolecular reactions.

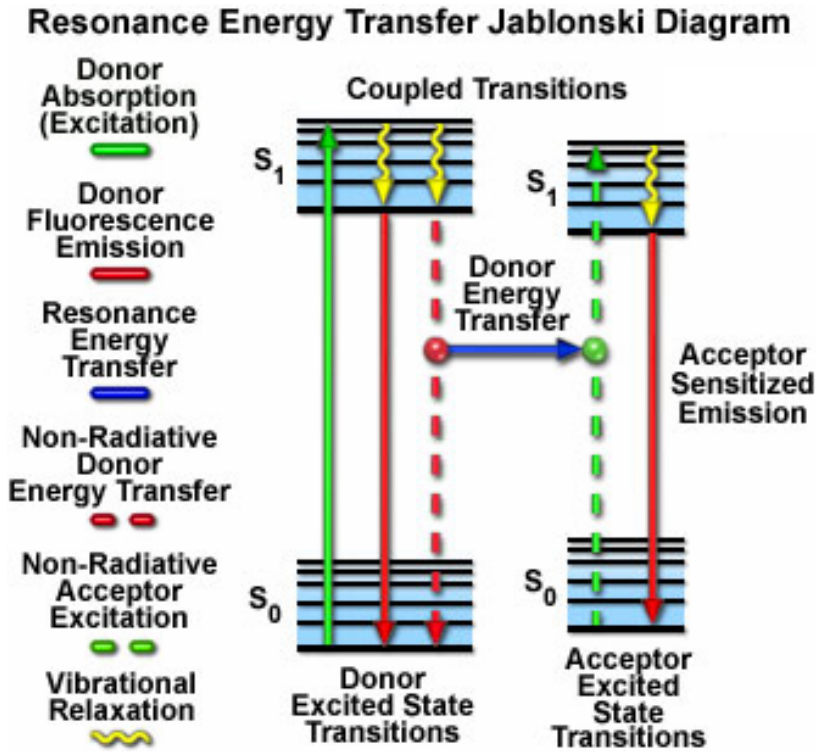
b. Distant: Non-radiate transfer of energy
At the distance of 2-10 nm. Non-radiative energy transfer. Intermolecular

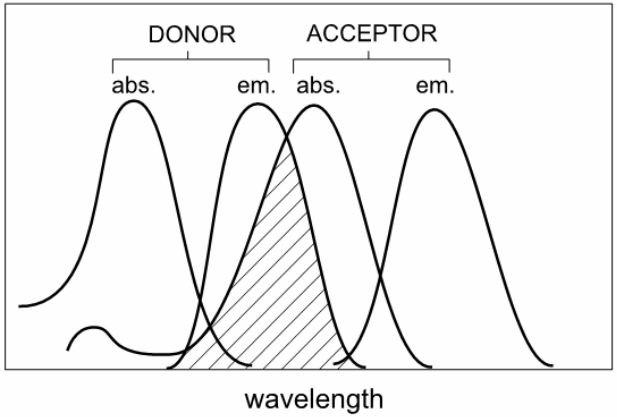
Conditions for FRET to happen

1.Spectral overlap between donor emission and acceptor excitation.

2.Relative orientation of the donor and acceptor transition dipoles.

3.Necessary level of the donor quantum yield.





FLIM probing / Quenching

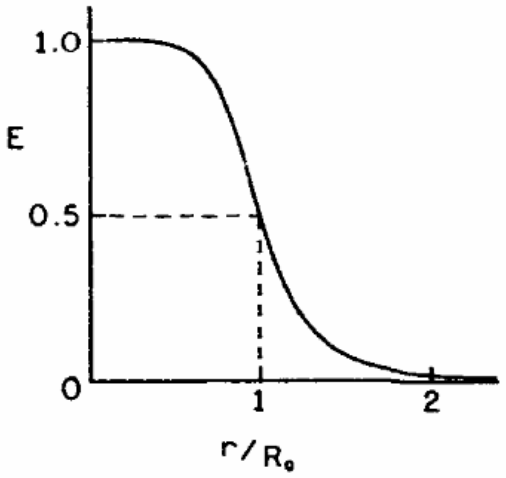
Rate calculation

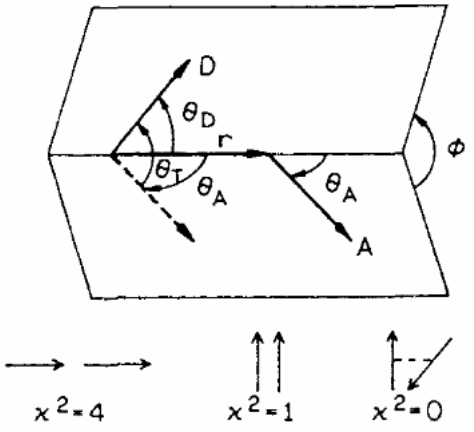
$$k_T = \frac{Q_D \kappa^2}{\tau_D r^6}\left(\frac{9000 \ln 10}{128 \pi N n^4}\right)\int_0^\infty F_D(\lambda)\varepsilon(\lambda)\lambda^4 d\lambda$$

$\boldsymbol{\tau_D}$ – is the lifetime of the **D** w/o **A**
$\boldsymbol{Q_D}$ - quantum efficiency of the donor,
$\boldsymbol{\kappa}$ – orientation factor, $\boldsymbol{r}$ – distance between molecules, $\boldsymbol{F_D}$ – normalized fluorescence intensity, $\boldsymbol{\varepsilon}$ - extinction coefficient

$$k_T = \frac{1}{\tau}\left(\frac{R_0}{r}\right)^6$$ **R_0 - Forster distance**

$$E = \frac{R_0^6}{R_0^6 + r^6} = \frac{1}{1 + (r/R_0)^6}$$

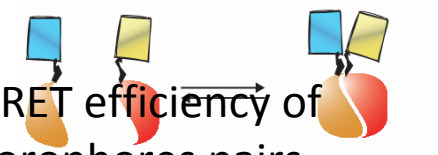




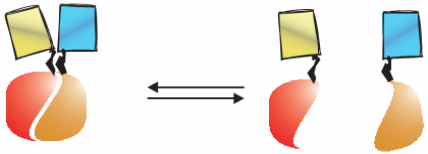
The FLIM-FRET saga

Interactions

Protein-protein interactions
Dimers formations
Contraction of cells
Detection of clustered distribution
Genotyping (via PCR)
Location-specific activity of molecules
DNA-protein interactions
Molecules
Relative or

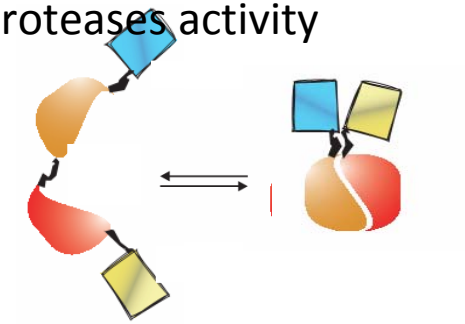


Disruption



Conformations

- FRET efficiency of fluorophores pairs
- Caspases activity
- Detection of apoptosis
- Proteases activity



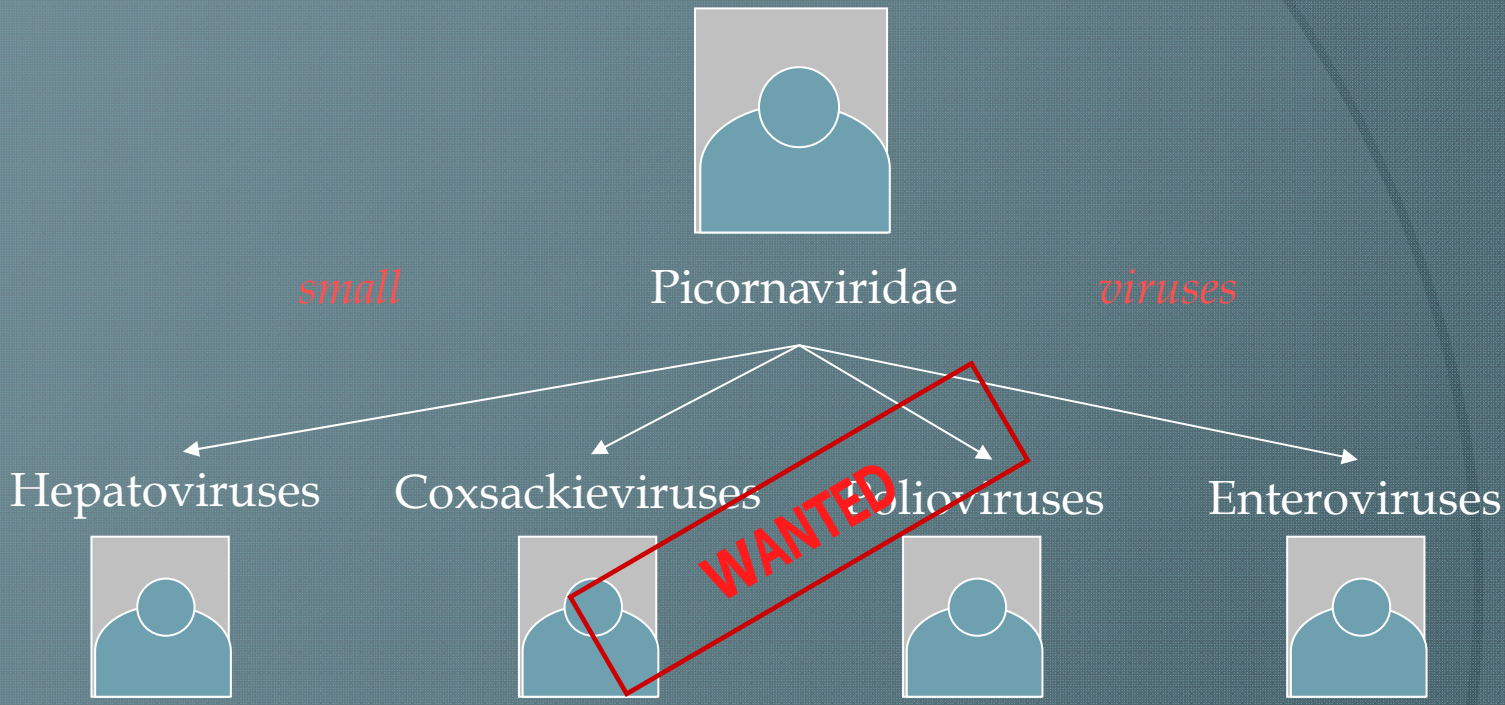
Summary

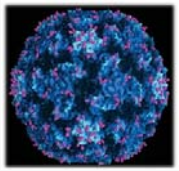
1. The applicability of FLIM is explained with the time scale of the average lifetime comparable with the dynamical processes in the environment.

2. Combination of FLIM with RET provides with the indirect “Spectroscopic Ruler”.

3. The FLIM/RET combination provides with a rich toolkit, whereas the imagination is the limit.

The subject: Enterovirus





Single-stranded RNA of positive sense:

Can act as an mRNA in infected cells

- Hand-foot-and-mouth disease
- Cold
- Meningitis
- Hepatitis
- Myocarditis

and much much more…

Enterovirus: Dossier

Pathology:

Enteroviruses are associated with:

- Hand-foot-and-mouth disease **HFM**
- Encephalitis
- Aseptic meningitis
- Pulmonary oedema
- Poliomyelitis-like paralysis

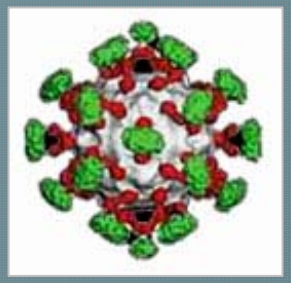
Taiwan, 1998
the biggest outbreak,
1,5 million infected
78 dead

Singapore, 2007
700 infected within one record week

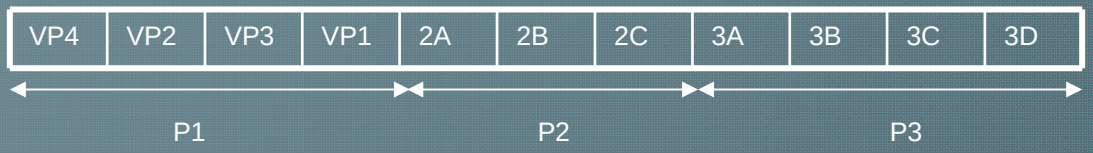
- Australia
- Hungary
- the United States
- Brazil
- Bulgaria
- Malaysia

Diagnostics
Asymptomatic
Serious neurological outcomes.
Enterovirus 71 – the most common non-polio enterovirus associated with poliomyelitis-like paralysis.

The Infection



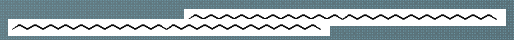
Single-stranded positive charged RNA

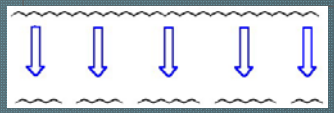


Viral infection → Translation into a single polyprotein → 2A → Maturation cleavage

The 2A protease ($2A^{pro}$)

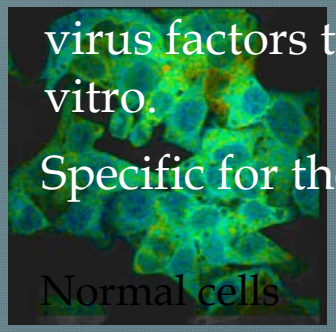
Chemotrypsin-like cysteine protease



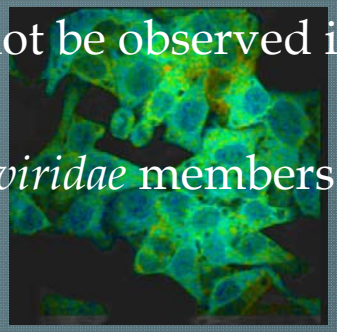


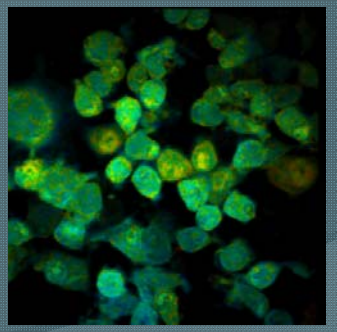
Can be modified by a number of host and virus factors that can not be observed in vitro.

Specific for the *Picornaviridae* members

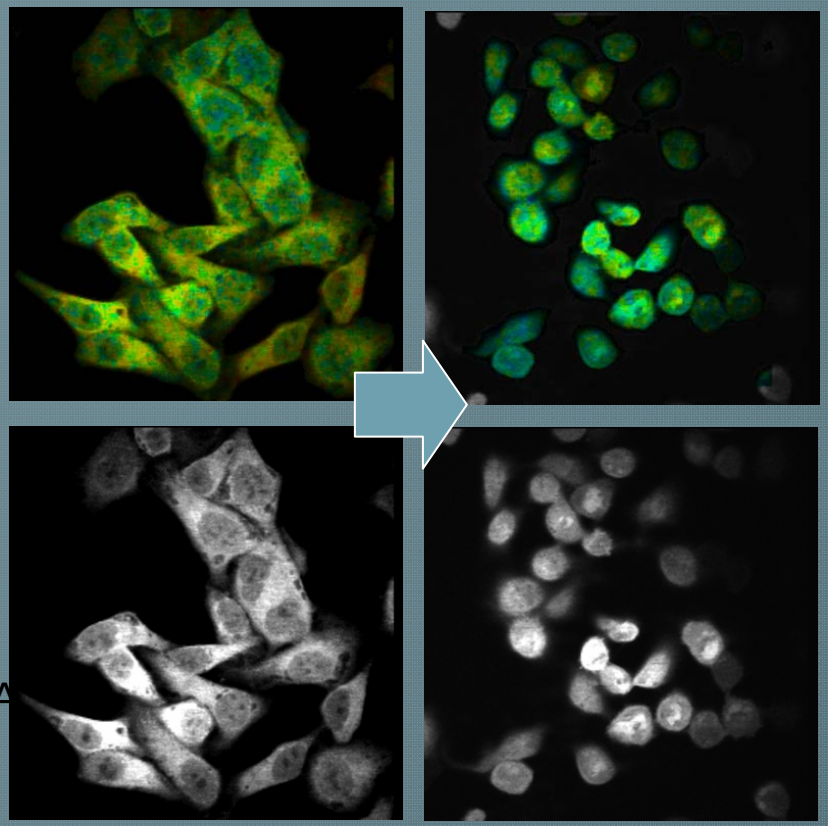


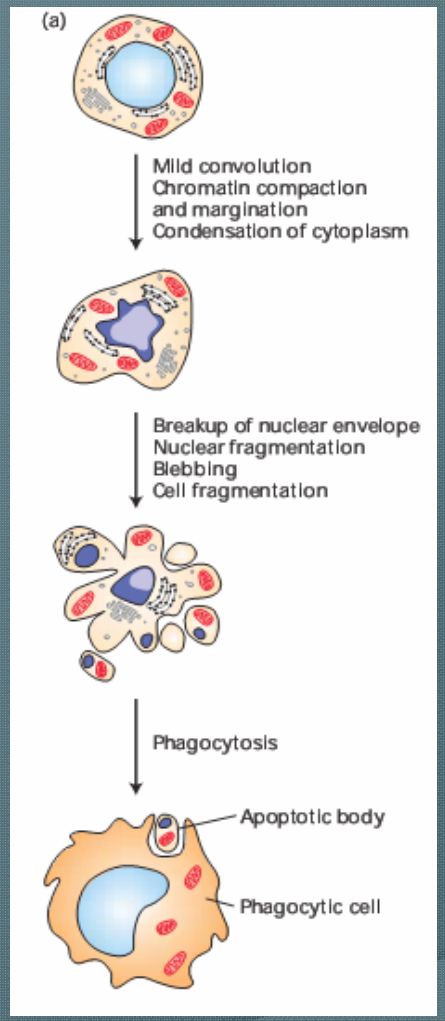
Normal cells





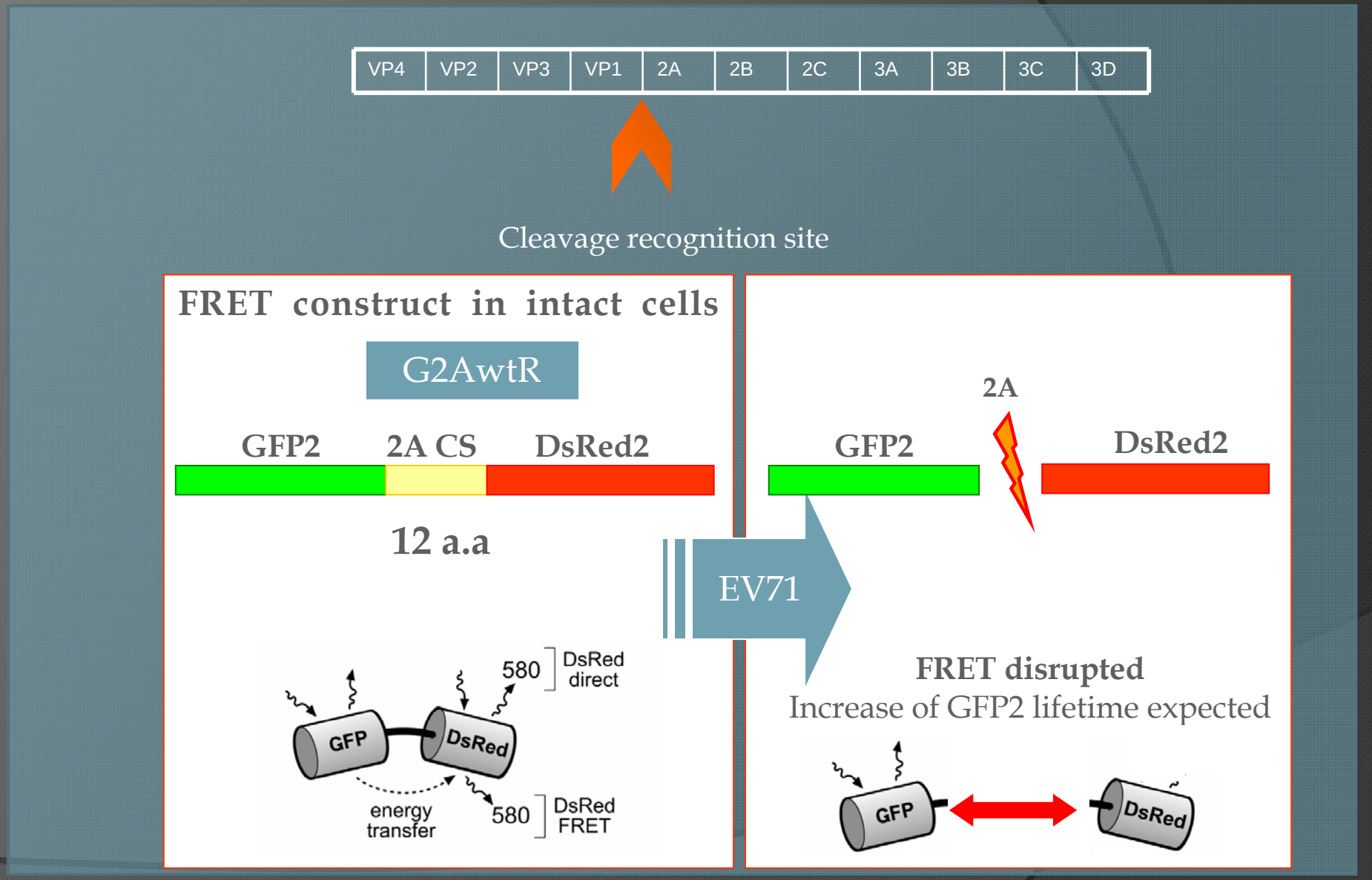
The Infection





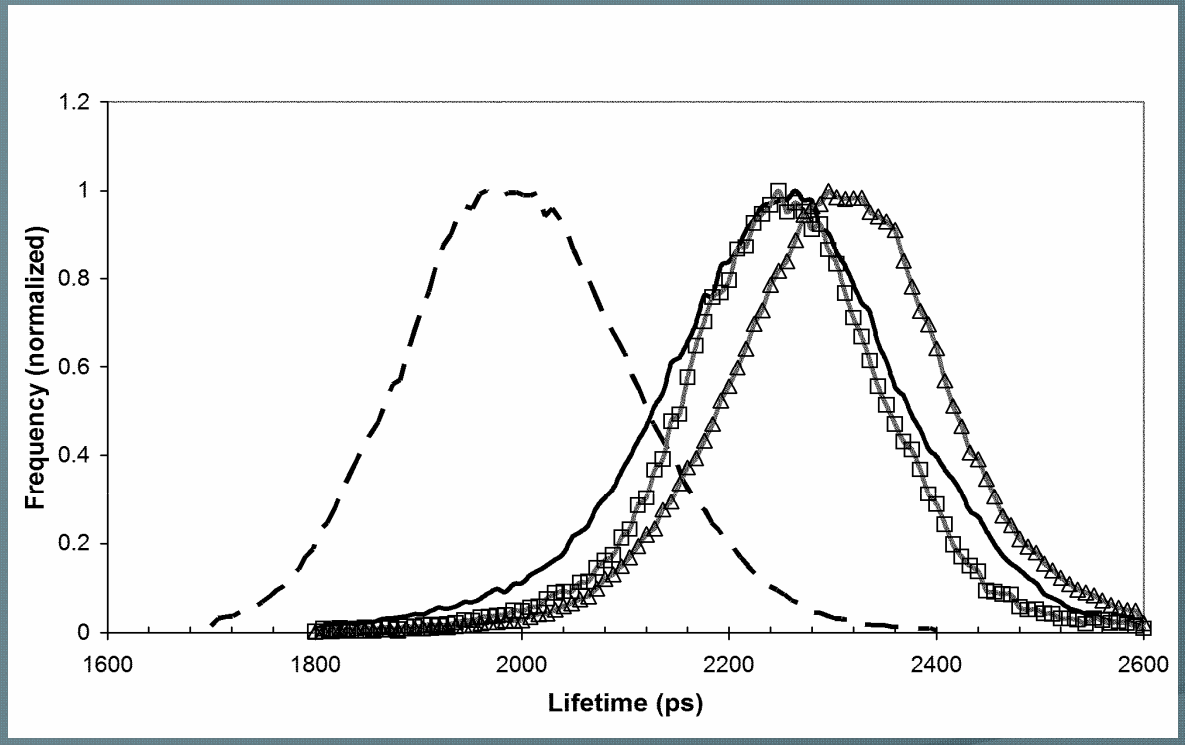
The key event: activation of the caspases – death executioners.

The Assay Construct

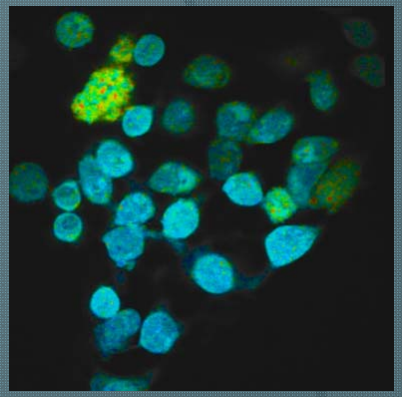


Results

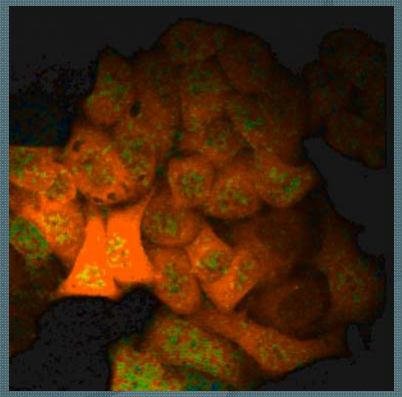
G2AwtR/Mock
G2Awt / Infected
GFP / Mock
GFP / Infected



Infected



Mock



2.5 ns

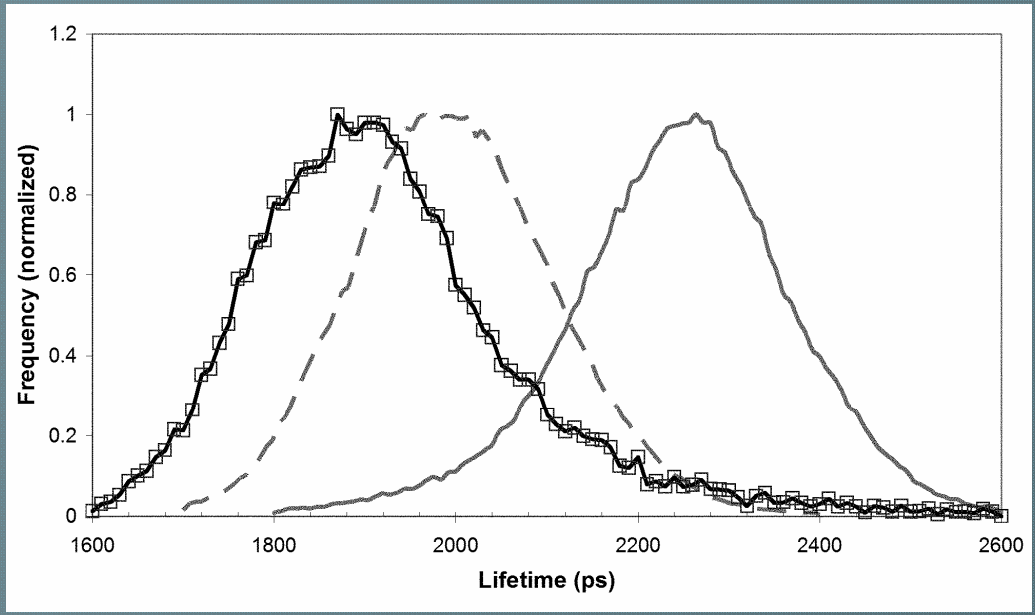
2 ns

Controls :: Apoptosis effect

G2AwtR/Mock

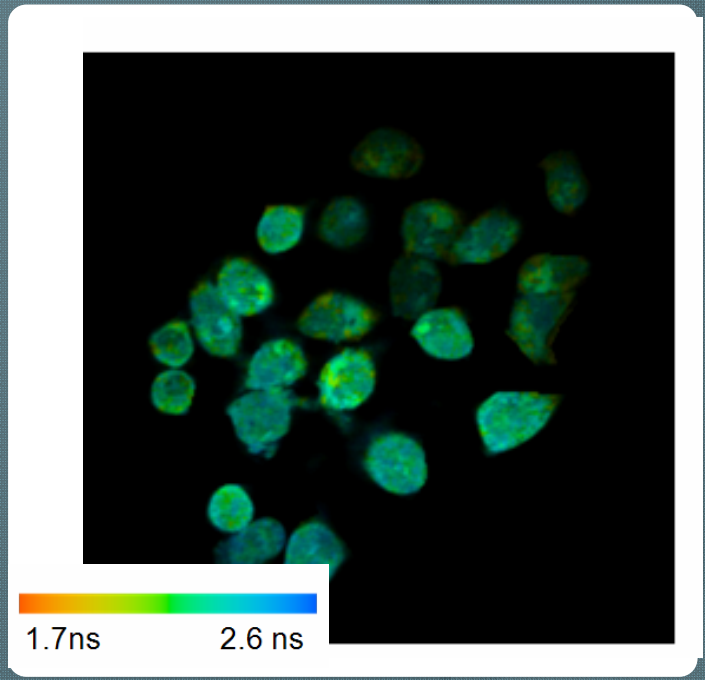
G2AwtR / Infected

G2AwtR :: Treated with cisplatin



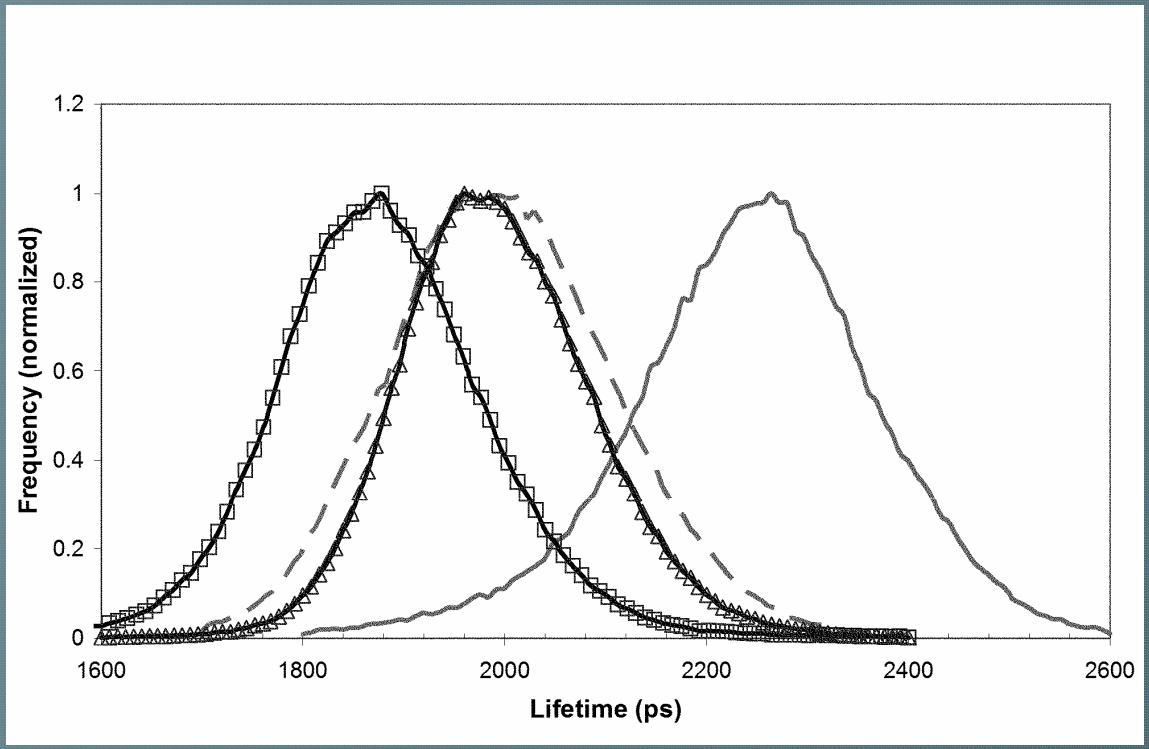
G2AwtR lifetime histogram measured upon the initiation of apoptosis in cells by cisplatin

2A causes apoptosis:



Controls :: Disruption specificity

G2AwtR/Mock
G2AwtR / Infected
G2AmutR / Mock
G2AmutR / Infected



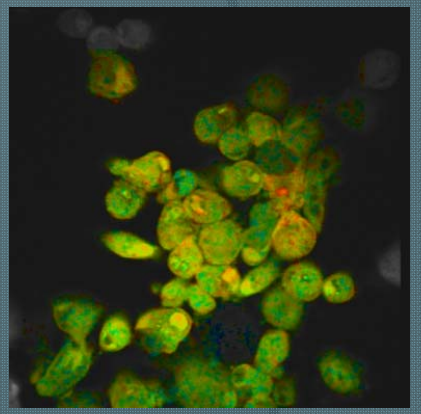
Averaged lifetime histograms of the mutant construct G2AmutR in mock and EV71 infected HeLa cells.

Specificity of the disruption:

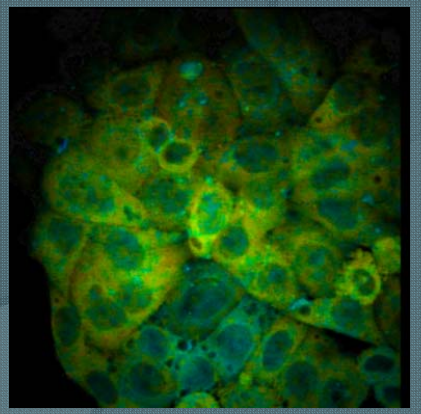
Mutant controls

1.6ns 2.4ns

Infected

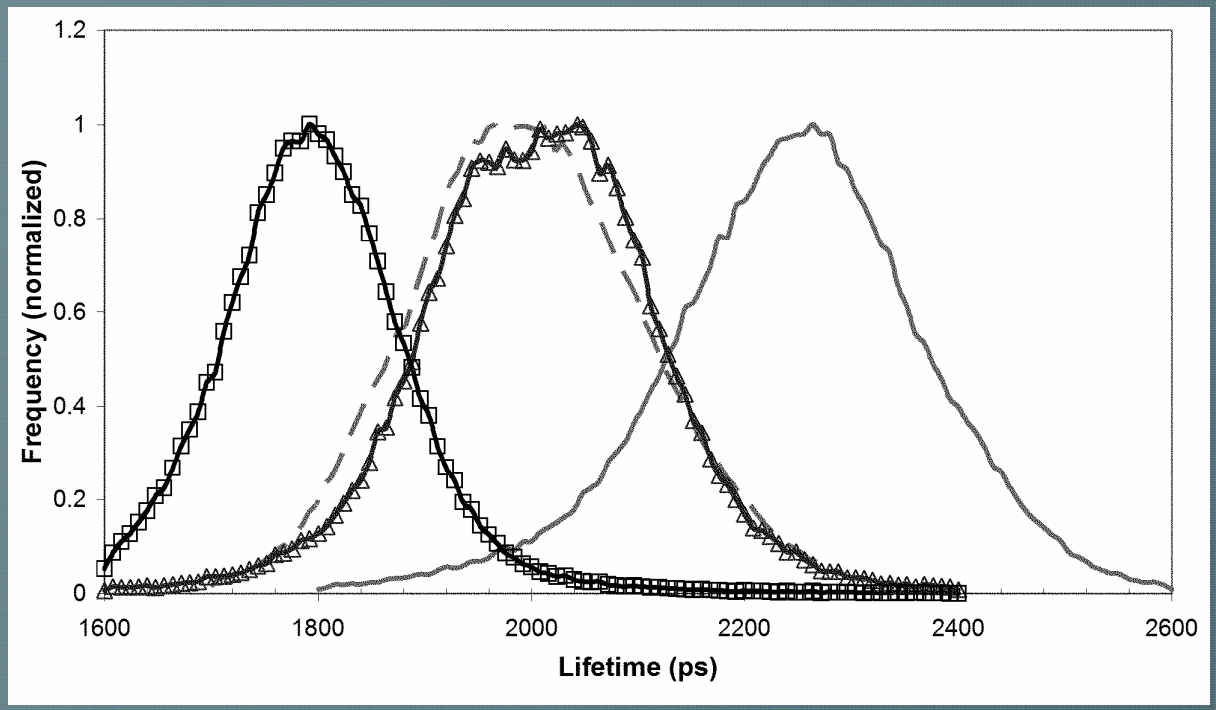


Mock



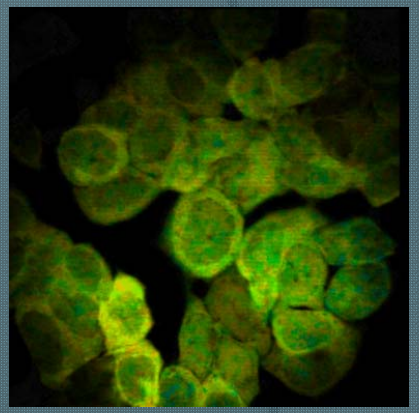
Controls :: Virus specificity

G2AwtR/Mock
G2AwtR / Infected
G2AmutR / Mock
G2AmutR / Infected

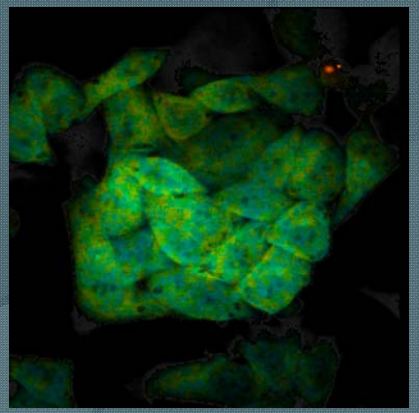


1.6ns 2.4ns

Infected

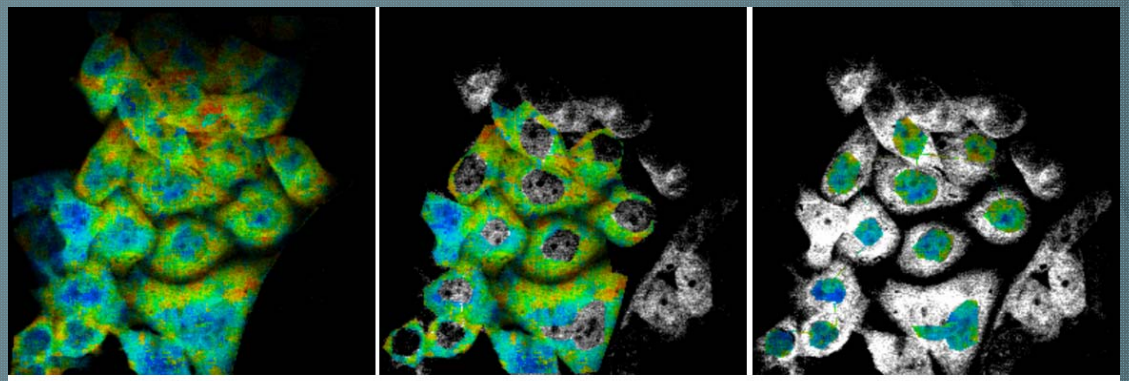


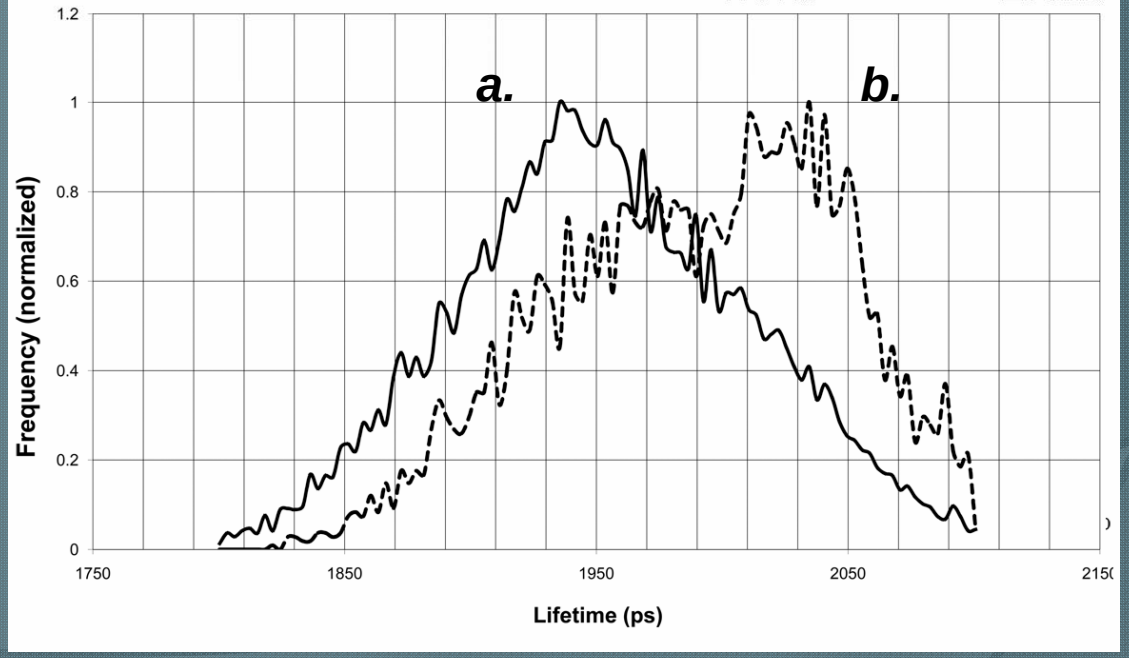
Mock



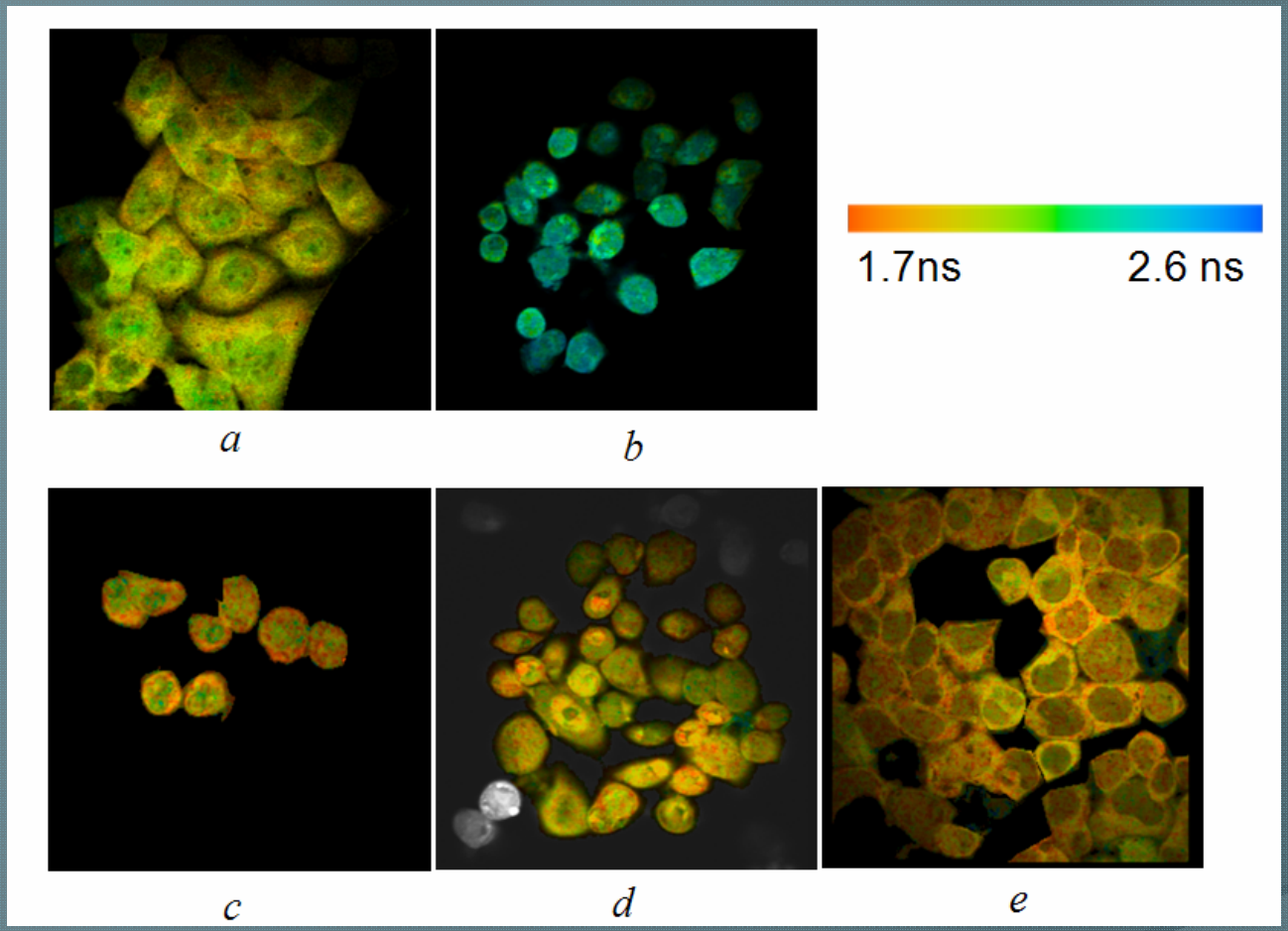
Averaged lifetime histograms for G2AwtR in mock and HSV-infected HeLa cells.

Controls :: location specificity



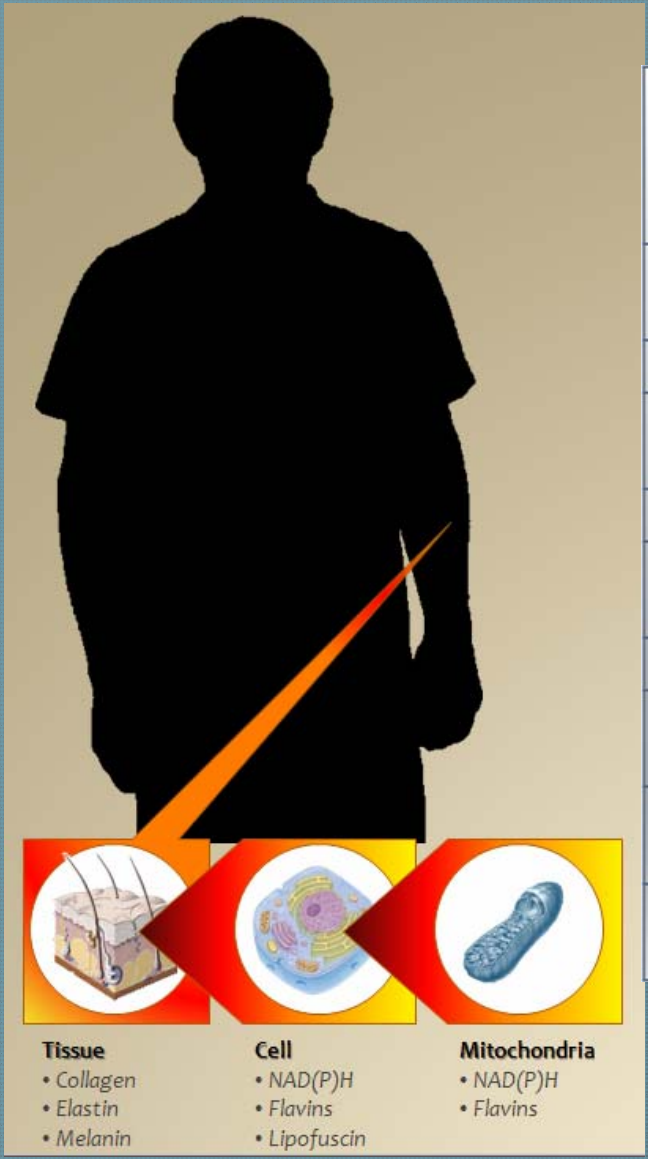


Summary



a. Wild type, mock
b. Wild type, infected
c. Wild type, cisplatin
d. Mutant, infected
e. Wild type, HSV

Overview



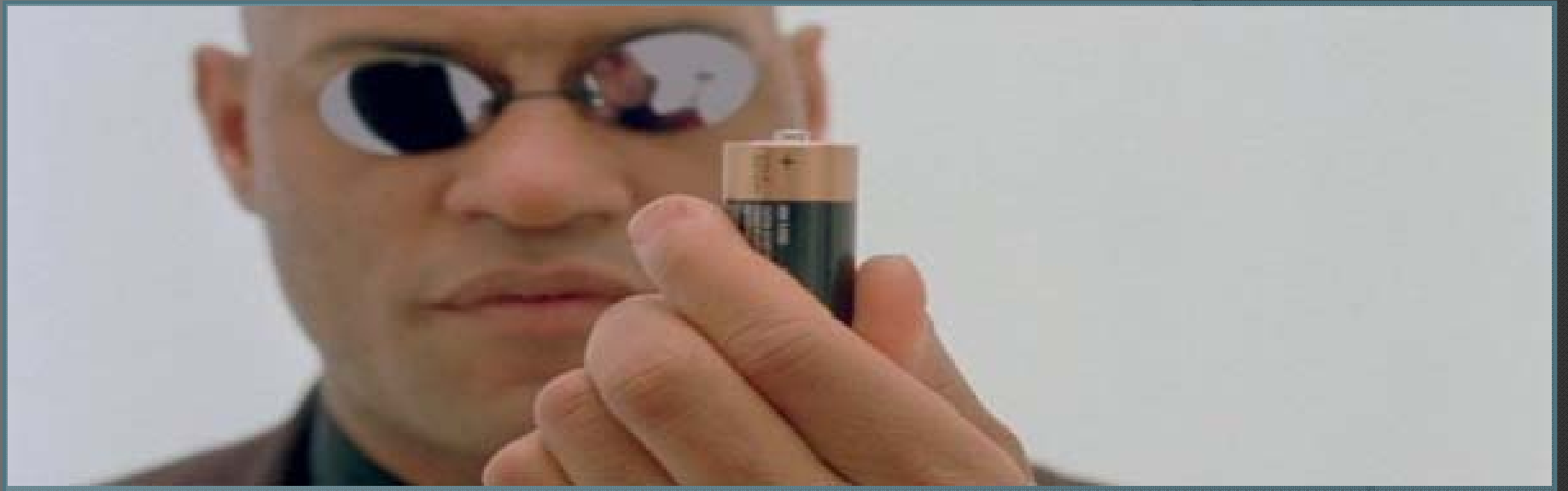
Fluorophore	Main exc. peaks (nm)	Main emission peaks (nm)	Fluorescence lifetime (ns)
Tryptophan	275	350	2.8, 1.5
Collagen	340, 270, 285	295, 395, 310	9.9, 5.0, 0.8
Elastin	460, 360, 425, 260	520, 410, 490, 410	6.7, 1.4, 7.8, 2.6, 0.5
NADH	350	460	0.6, 0.2, 2.5, 6
FAD, Flavins	450	535	~4
FMN	440	520	4.7
β-carotene		520	9.6, 2.0, 0.3
Porphyrins	400	610, 675	9.6, 2.0, 0.3
Lipofuscin	340-395	540, 430-460	-

The autofluorescence

- Tryptophan

Fluorophore	Main exc. peaks (nm)	Main emission peaks (nm)	Fluorescence lifetime (ns)
Tryptophan	275	350	2.8, 1.5
Collagen	340, 270, 285	295, 395, 310	9.9, 5.0, 0.8
Elastin	460, 360, 425, 260	520, 410, 490, 410	6.7, 1.4, 7.8, 2.6, 0.5
NADH	350	460	0.6, 0.2, 2.5, 6
FAD, Flavins	450	535	~4
FMN	440	520	4.7
β-carotene		520	9.6, 2.0, 0.3
Porphyrins	400	610, 675	9.6, 2.0, 0.3
Lipofuscin	340-395	540, 430-460	-

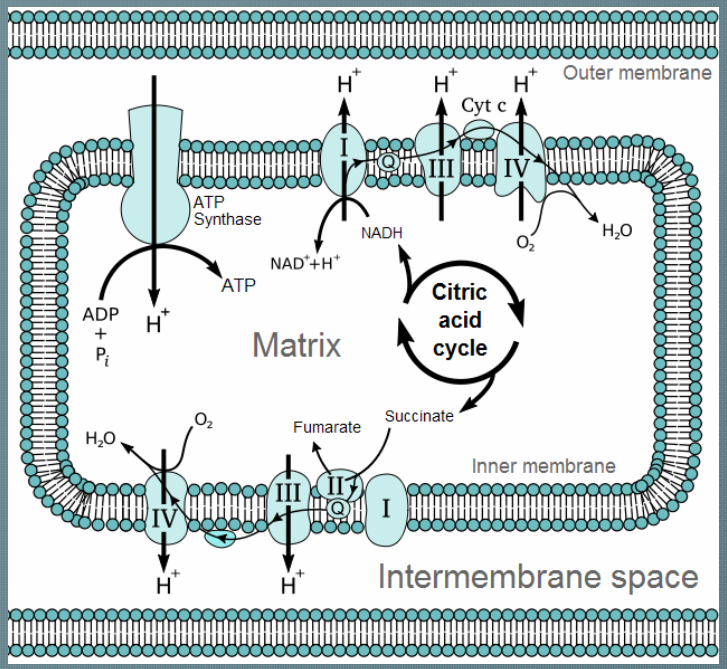
We are the batteries



ATP (Adenosine triphosphate) is the main energy source in cells, enabling all the synthesis and other endergonic reactions, as well as increasing the rate of exergonic.

NADH (Reduced nicotinamide adenine diphosphate) is a part of the oxidative phosphorylation pathway, which is the most efficient way to produce ATP in aerobic cells.

The NADH faces



The NADH, involved in the first step of the oxidative phosphorilation, serves as an electron donor, creating the intermembrane potential.

The potential is used by ATP synthase to phosphorylate ADP and AMP.

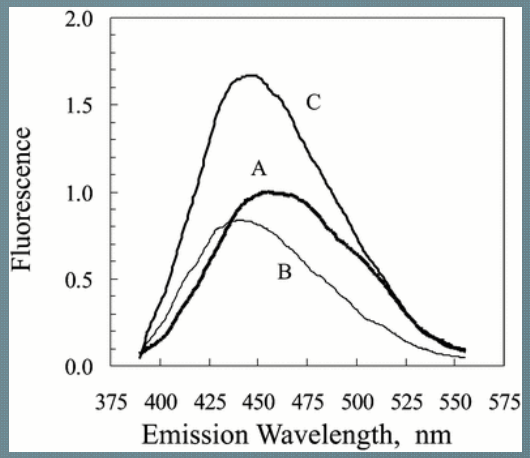
2

When involved in the ATP production, the molecules bind to enzymes (bound pool, mainly located in mitochondria).

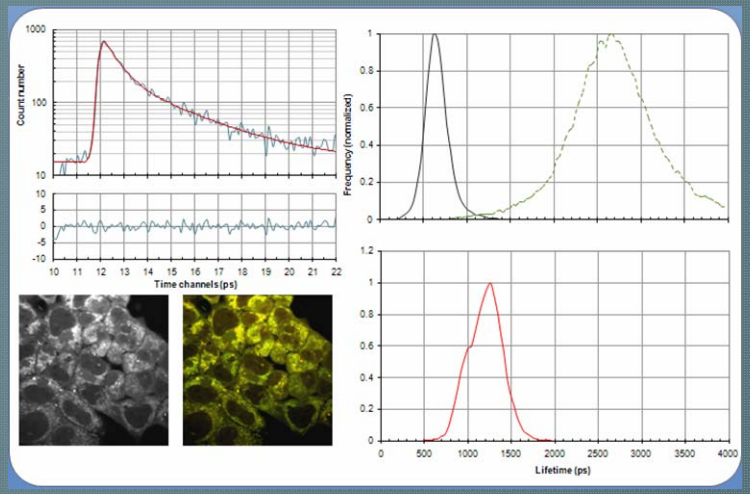
Otherwise, distributed as single molecules in cytosole (free pool).

THE RATIO BETWEEN TWO POOLS ILLUSTRATE THE METABOLICAL ACTIVITY OF THE CELLS.

The lifetime advantage



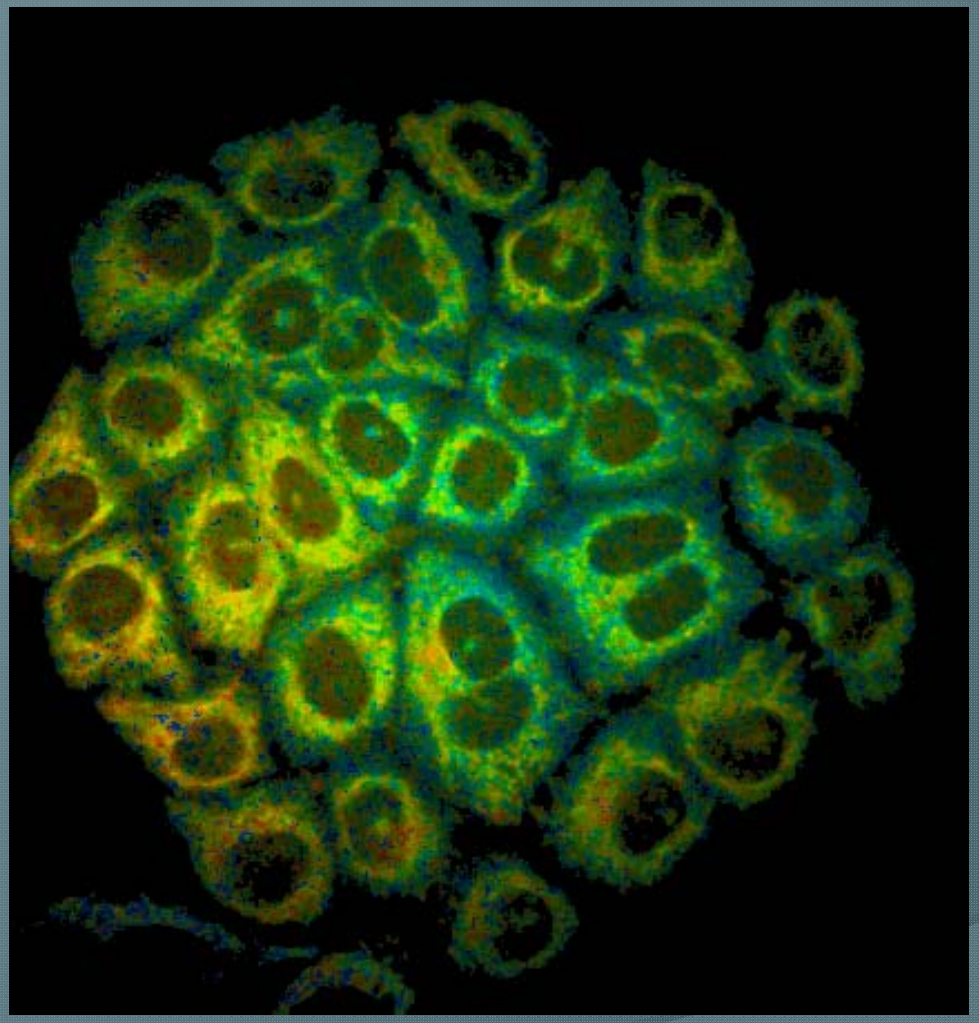
The spectra:
Difference between the peaks for free (A) and bound forms (B, C) are just 10-20 nm, whereas the spectrum width ~150 nm wide.



The lifetime:

Difference between the peaks for free and bound forms are well spearated: 400 ps vs ~2800 ps.

The lifetime advantage



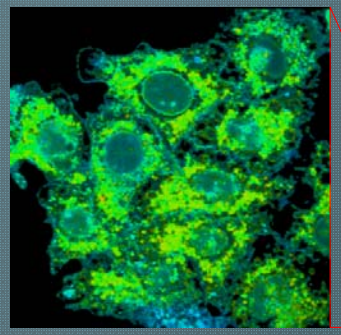
MOL autofluorescence profile

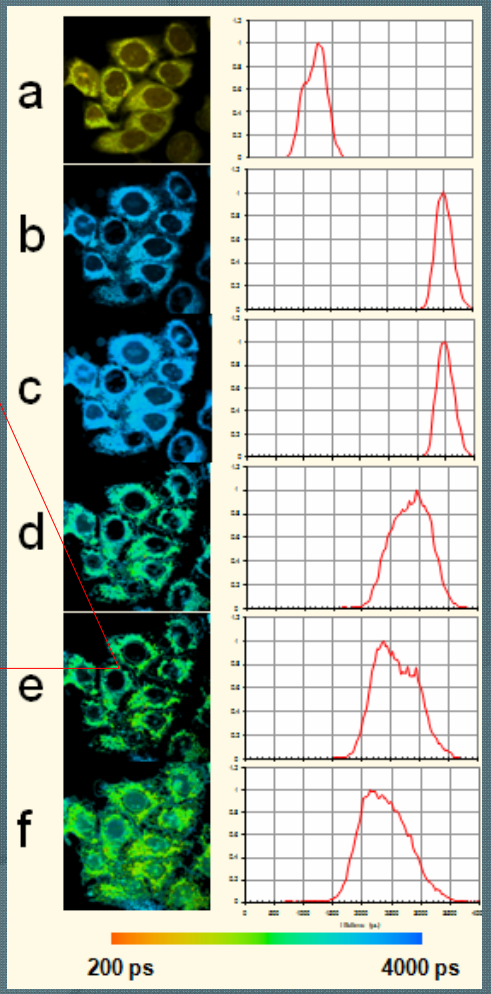
The dynamics of the NADH fluorescence in response to apoptosis inducer Staurosporine STS.

HWWang[1], V. Ghukasyan[1], CTChen[2], HWGuo[1], JSYu[1], YHWei[2], and FJKao[1]
Institute of Biophotonics Engineering, Institute of Biochemistry and Molecular Biology, National Yang-Ming University

Completed

Lifetime observation (τ_m) in HeLa cells at 15 (a), 30(b), 45 (c), 60(d), 75 (e) minutes and 10 hours (f) after treatment with apoptosis inducer staurosporine (STS). Magnified image shows characteristic to apoptosis morphological changes.





MOL autofluorescence profile

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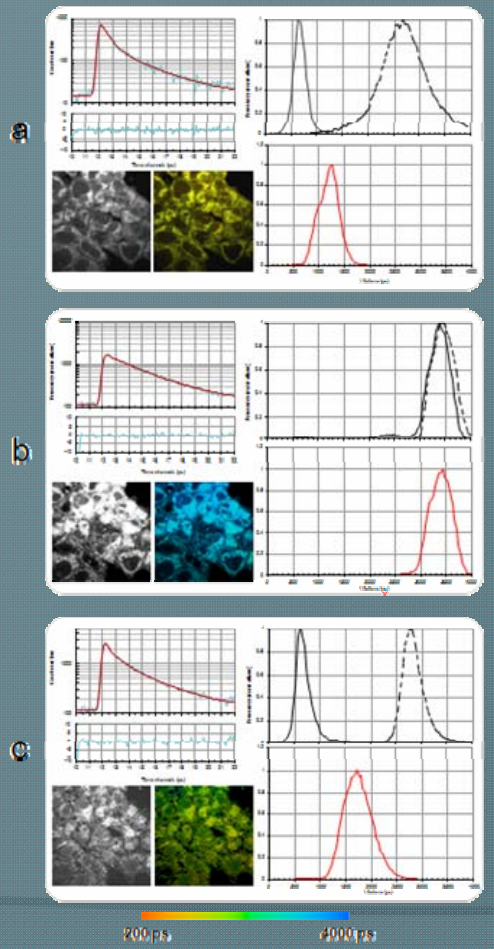


2-exponential lifetime observation in 143B cells at 15 (a), 30 (b) and 45 (c) minutes upon STS treatment.

τ_1 and τ_2 (black) as well as $\tau_{av.}$ (red) are shown along with the fluorescence decays.

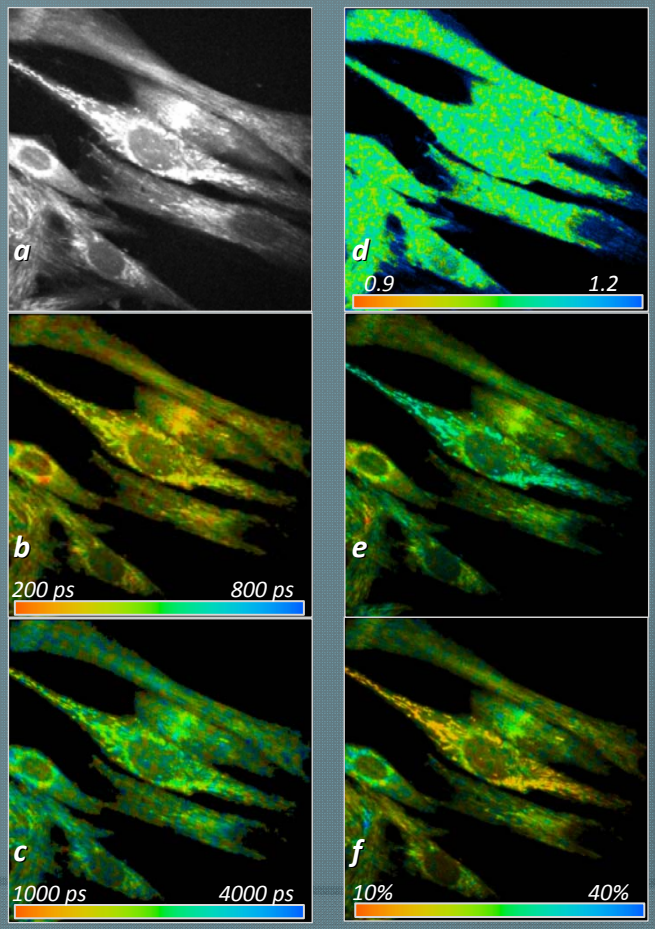
Completed

MOL autofluorescence profile

The influence of aging on the NADH fluorescence lifetime

V. Ghukasyan[1], CTChen[2], YHWei[2], and FJKao[1]
Institute of Biophotonics Engineering,
National Yang-Ming University

In progress



Goal
Study dependence of fibroblast donor's age and NADH lifetime. Expected difference should reflect changes in metabolism observed earlier.

Representative FLIM data for fibroblasts.
Images: *a).* fluorescence intensity image, *b).* color-coded image of the τ1 lifetime distribution, *c).* color-coded image of the τ2 lifetime distribution, *d).* χ2 distribution, *e.* distribution of the pre-exponential factor a1, *f).* distribution of the pre-exponential factor a2.

MOL autofluorescence profile

The influence of aging on the NADH fluorescence lifetime

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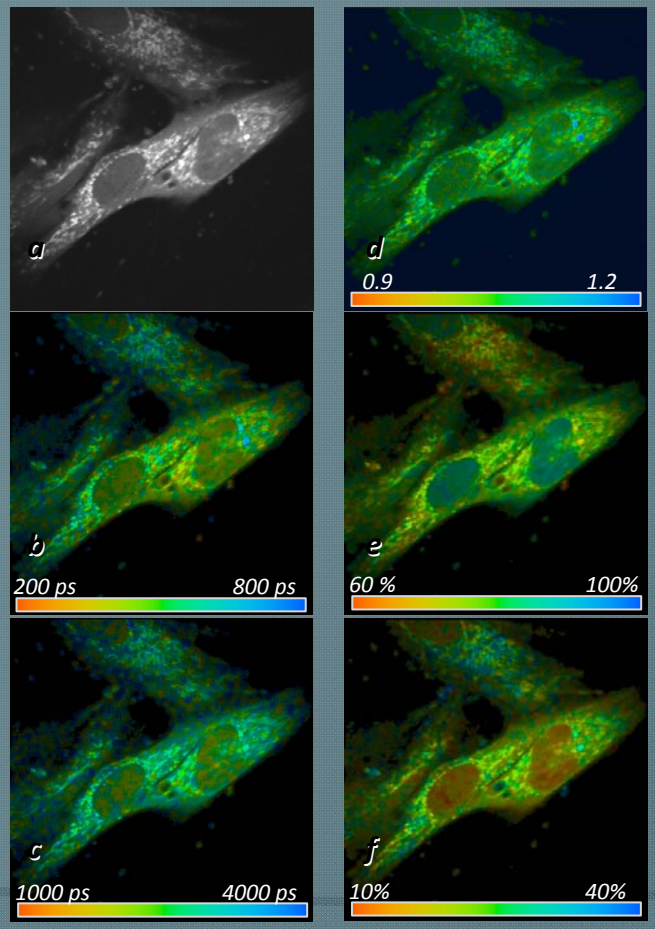
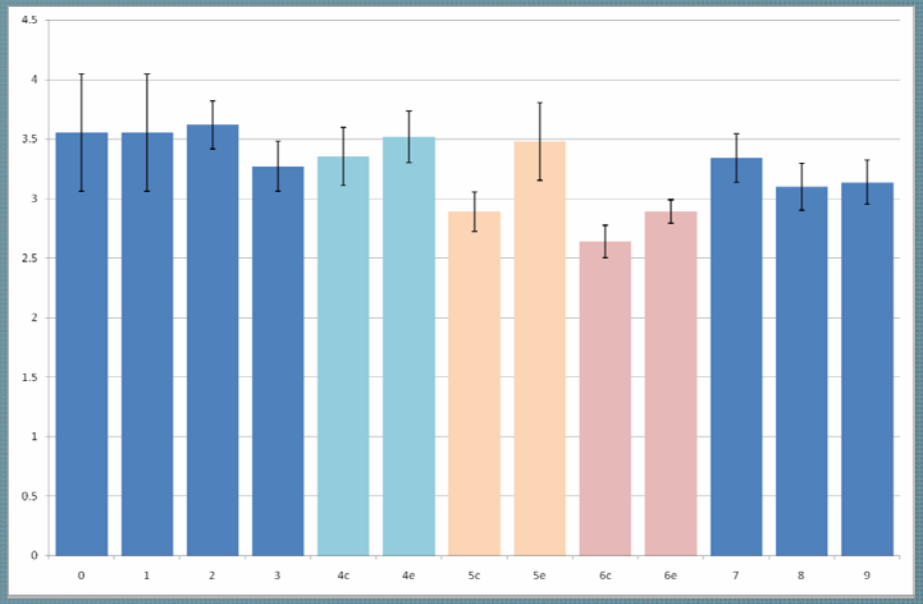
Institute of Biochemistry, National Yang-Ming University

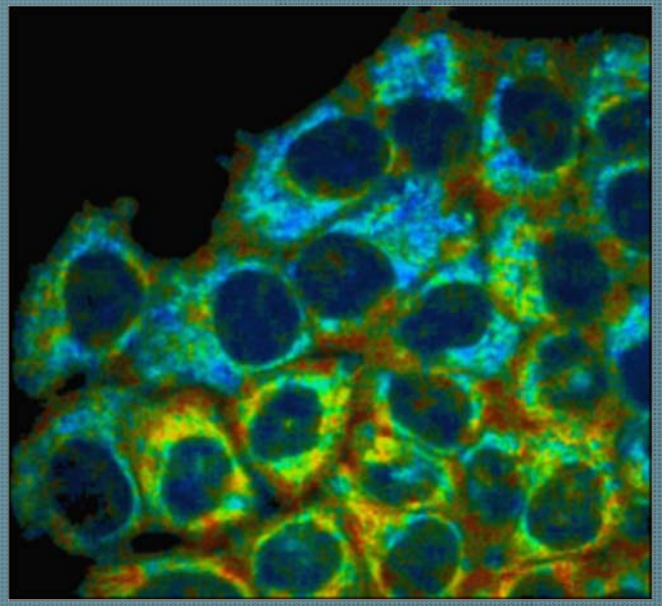
In progress

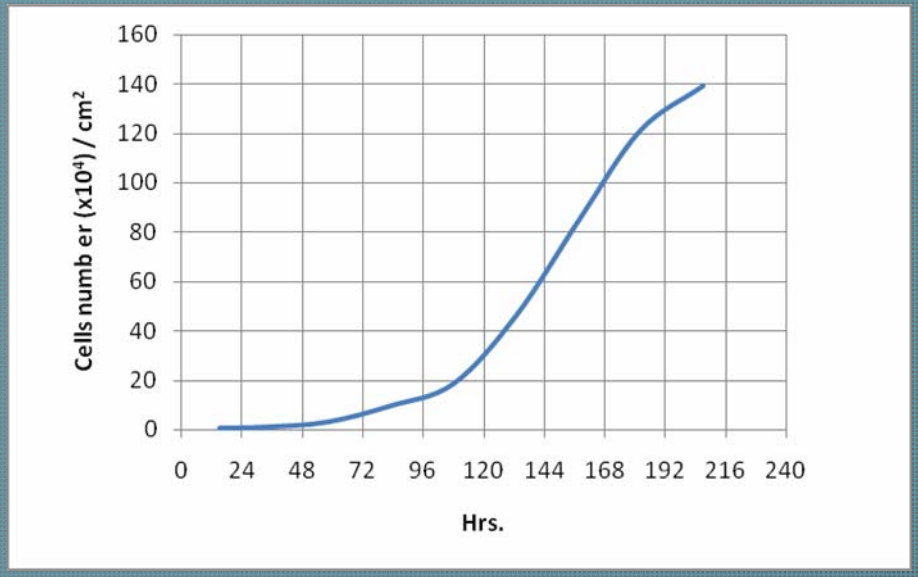


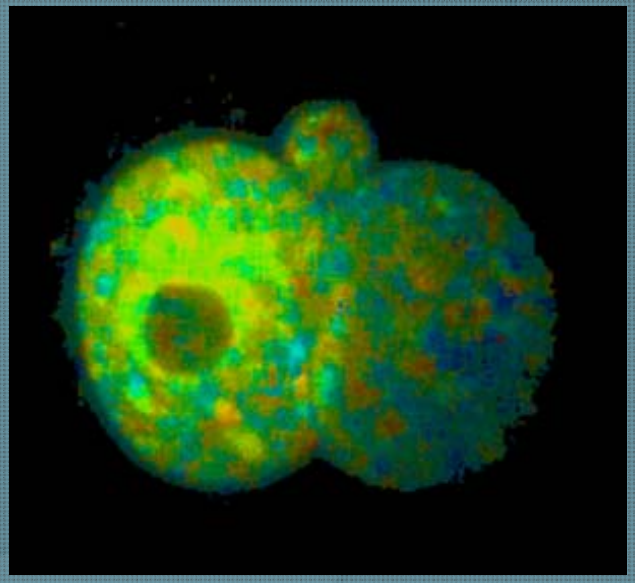
Representative FLIM data for fibroblasts from donor C (13 yrs old). Images: *a).* fluorescence intensity image, *b).* color-coded image of the τ1 lifetime distribution, *c).* color-coded image of the τ2 lifetime distribution, *d).* χ2 distribution, *e.* distribution of the pre-exponential factor a1, *f).* distribution of the pre-exponential factor a2.

The cell culture growth

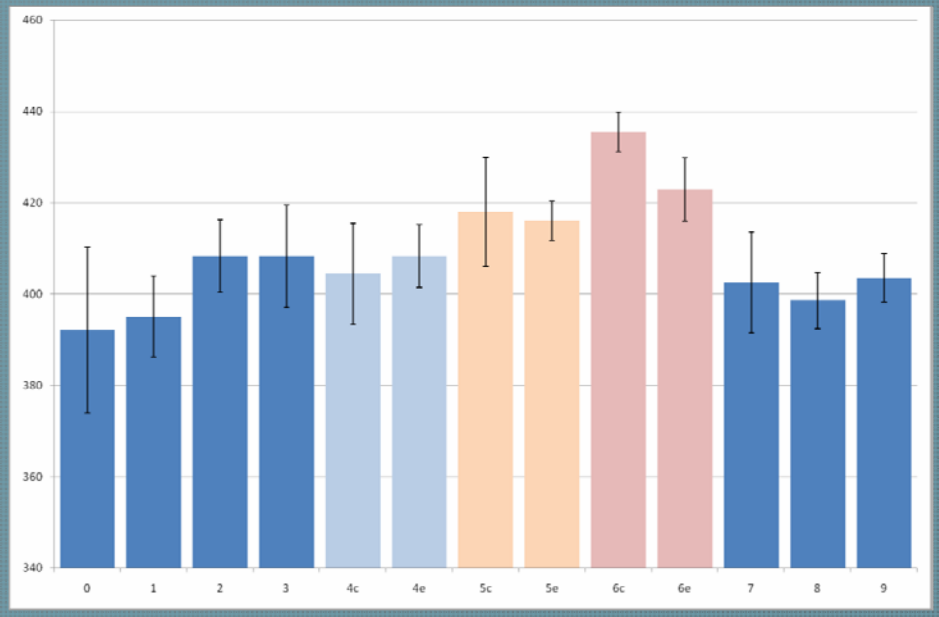








Overview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360
340
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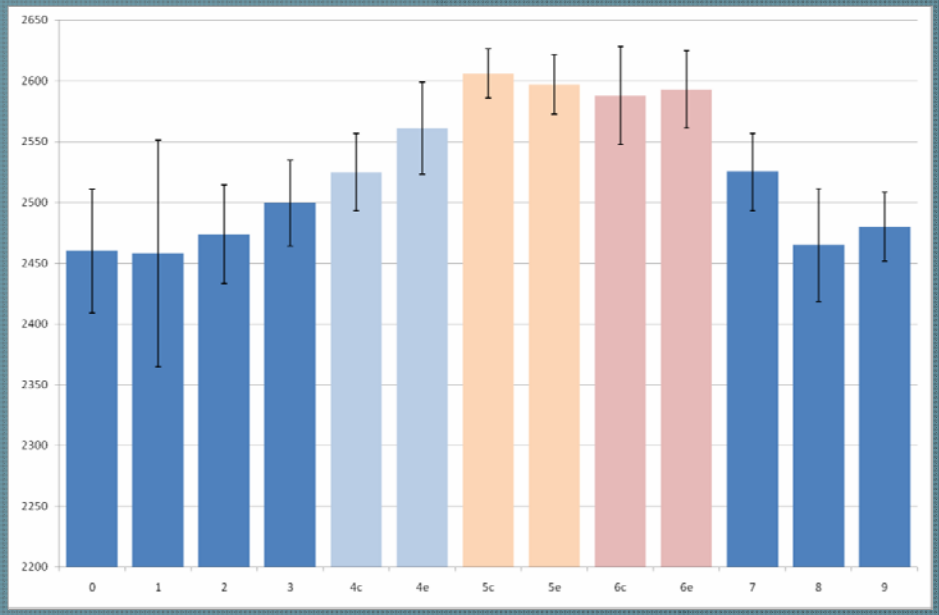
2
3
4c
4e
5c
5e
6c
6e
7
8
9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2600
2550
2500
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2400
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2
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5c
5e
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6e
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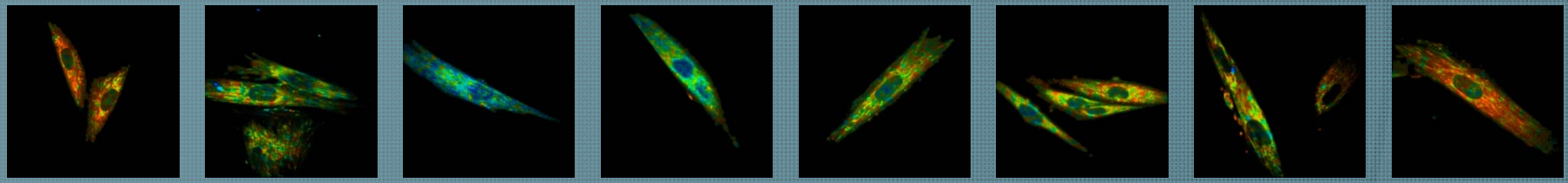
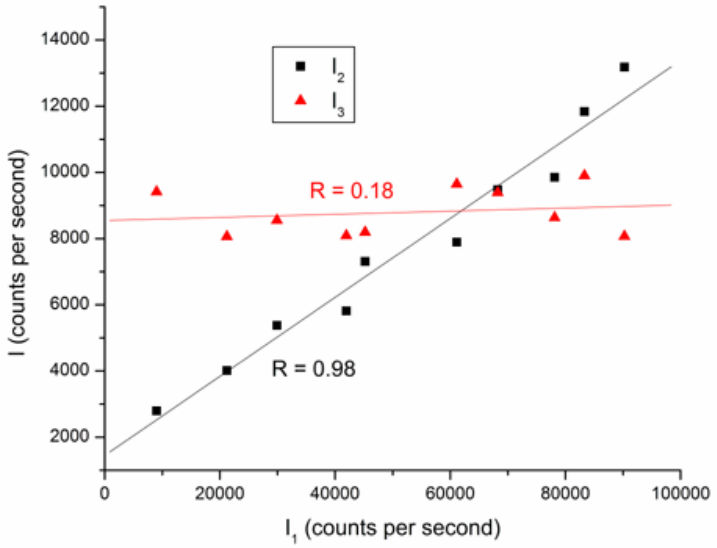
8
9



Fluorescence Correlation Spectroscopy

Finding the pattern: correlation

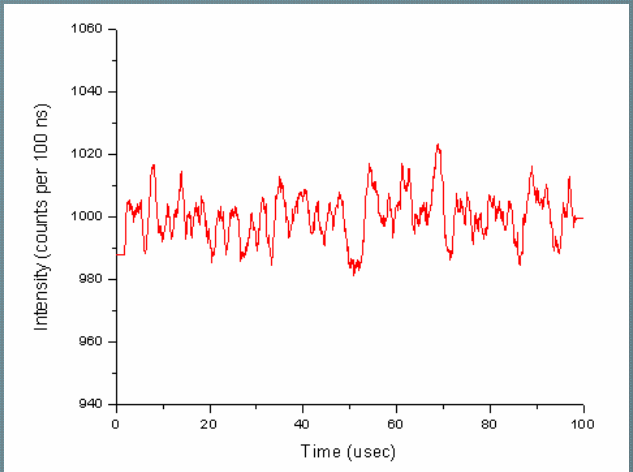
I_1 (Counts per Second)	I_2 (Counts per Second)	I_3 (Counts per Second)
9012	2794	9412
21228	4011	8064
29903	5377	8554
41948	5815	8092
45223	7307	8194
61131	7893	9647
68245	9475	9390
78116	9852	8634
83310	11837	9900
90254	13183	8069



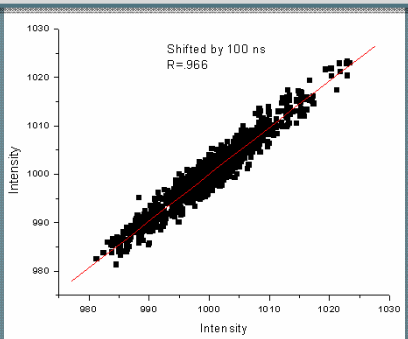
$$R = \sqrt{\frac{\sum_{i=1}^{M} (I_1 - < I_1 >)(I_2 - < I_2 >)}{(M-1)(SD_1 * SD_2)}}$$

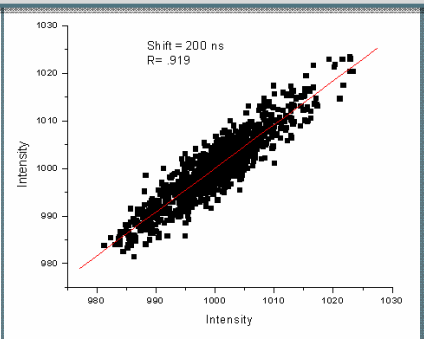
http://fcsxpert.com/classroom/theory/fcs_correlation.html

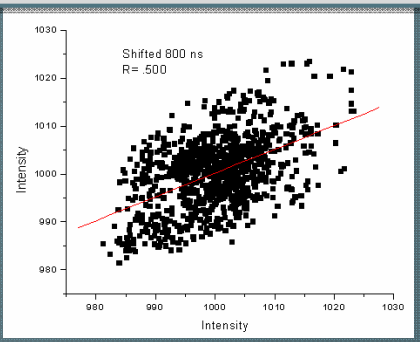
Finding the pattern: AUTOcorrelation

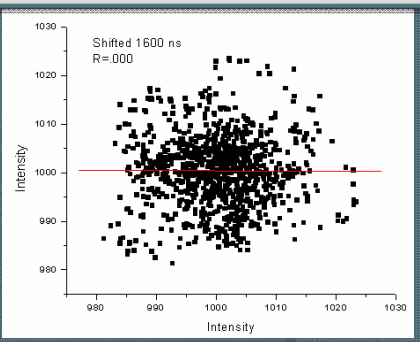


Shift in time - correlation







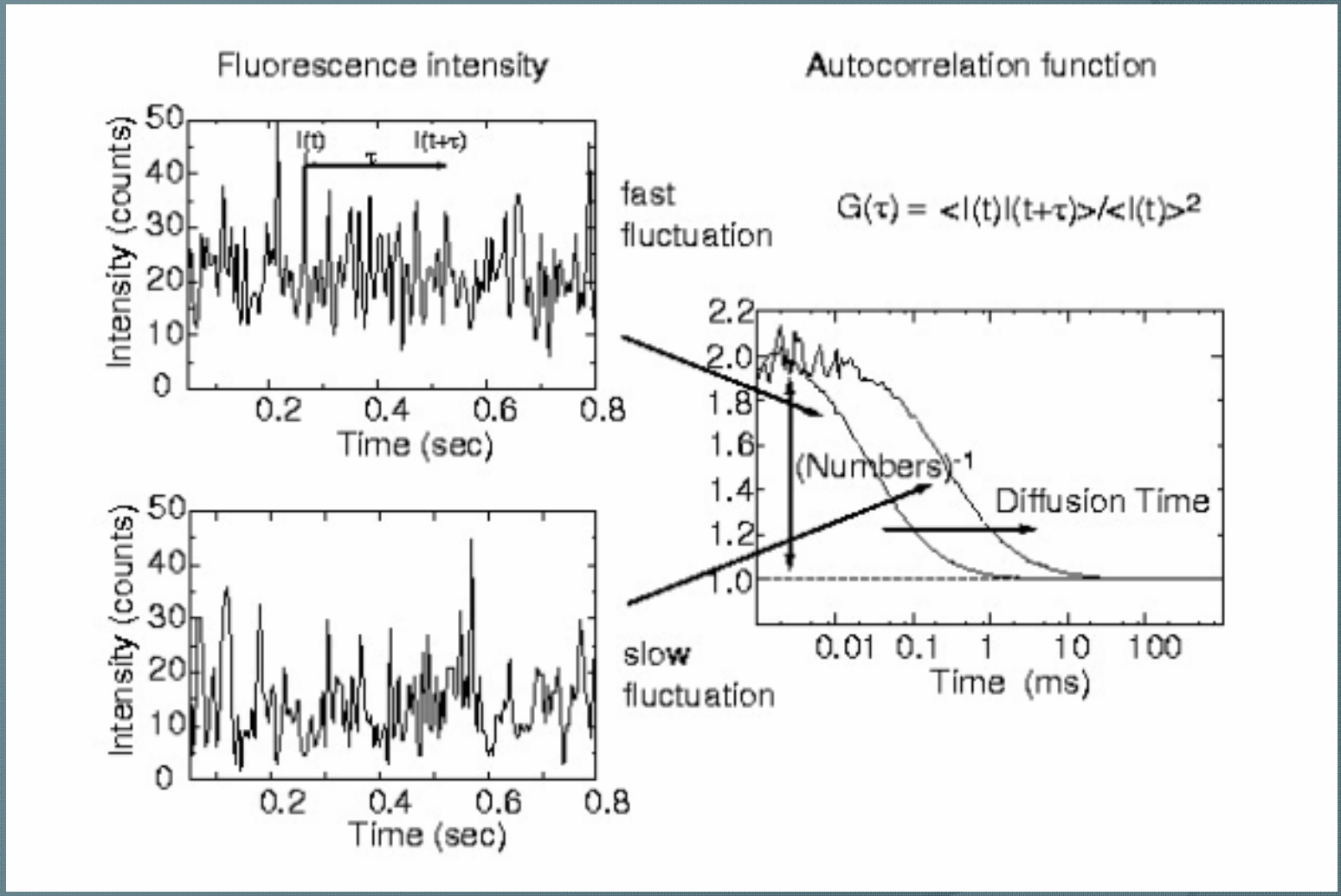


$$R = \sqrt{\frac{\sum_{i=1}^{M} (I_1 - < I_1 >)(I_2 - < I_2 >)}{(M-1)(SD_1 * SD_2)}}$$

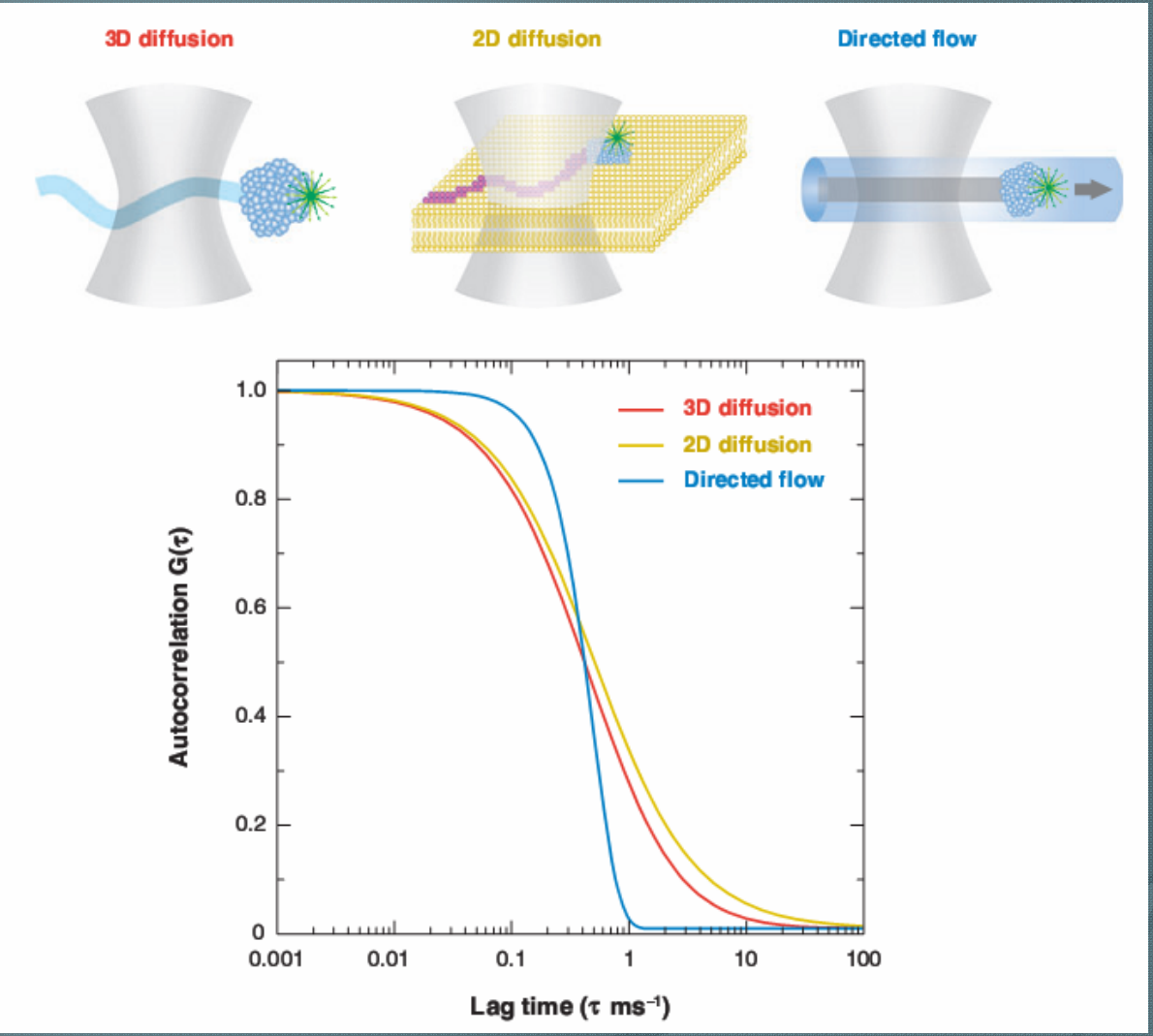
$$G(\Delta t) = \frac{\sum_{i=0}^{M} I\ (i * \tau)(I((i+m)\tau))}{< I >^2 (M-1)}$$

http://fcsxpert.com/classroom/theory/fcs_correlation.html

Finding the pattern: the FCS curve

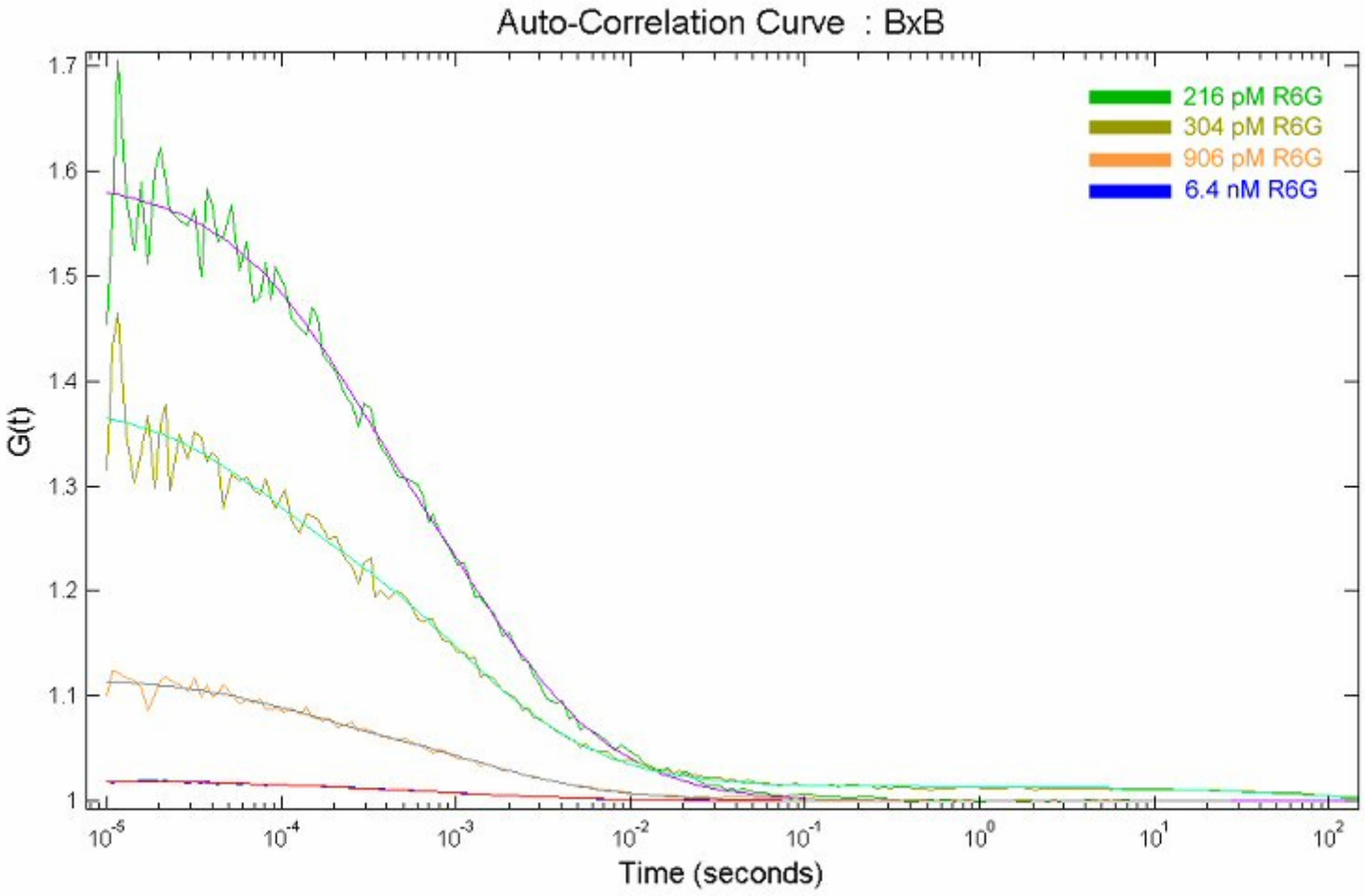


The diffusion

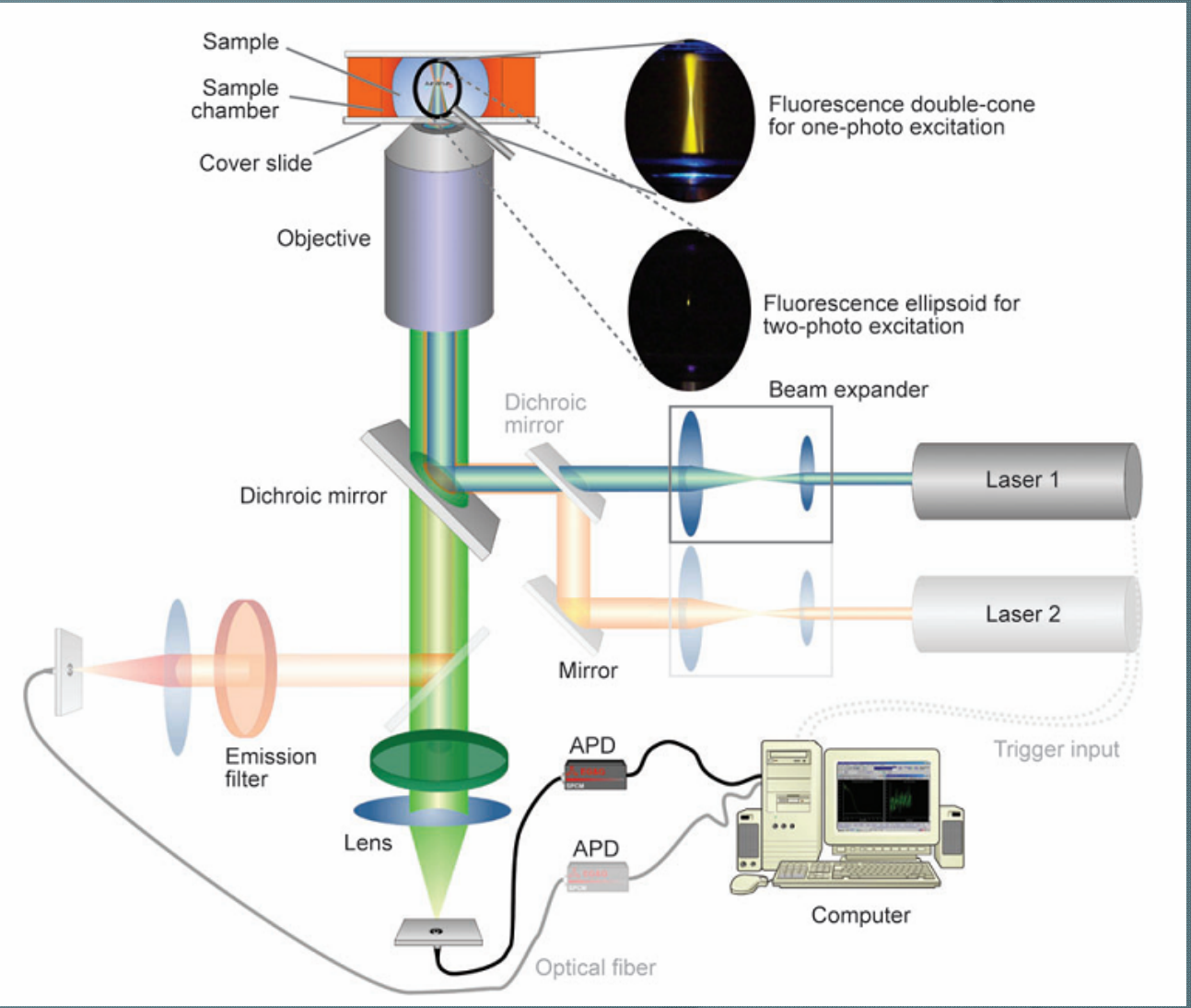


E. Hauswein, P. Schwille, Annu. Rev. Biophys. Biomol. Struct. 36:151-169, 2007

The mechanism



Instrumentation



E. Hauswein, P. Schwille, Annu. Rev. Biophys. Biomol. Struct. 36:151-169, 2007

Applications

DNA structure and interactions
Dynamics of nucleic acids
Transport of nucleosome core particles
Rates of Mg2+ and Na+ dependent conformational changes in a single RNA molecule
Proteins interactions and assembly
Thermodynamic analysis of DNA-protein interaction
Conformational dynamics and folding
Protein incorporation in viral membranes
Particles trackingG
Gene delivery vesicles dynamics,
…

SIZE

MOBILITY

CONCENTRATION

SM INTERACTIONS

CONFORMATIONS

PARTICLES TRACKING

DIFFUSION