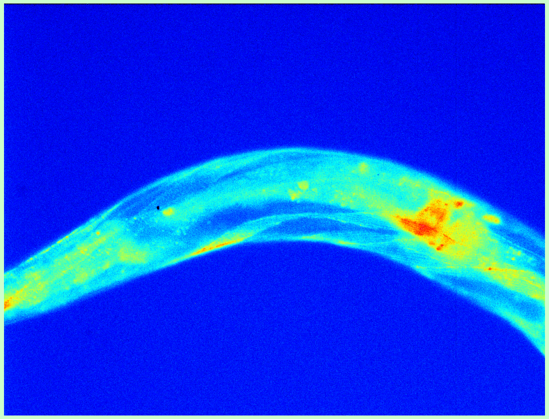
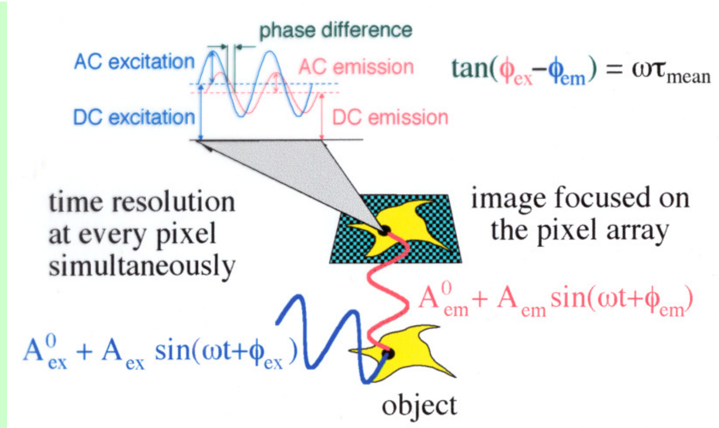
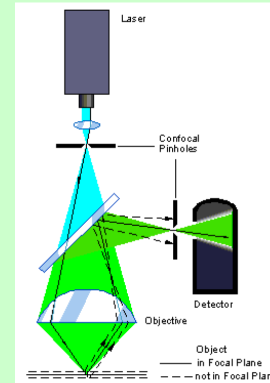
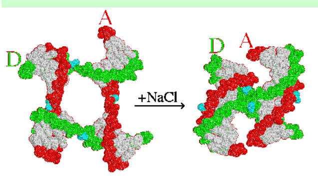


Organisms





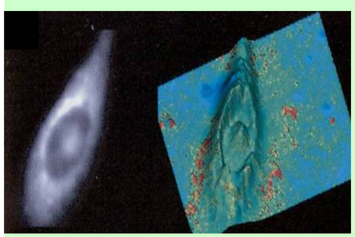
The marriage of morphology and lifetimes: Excavating the tortuous landscape of FLIM



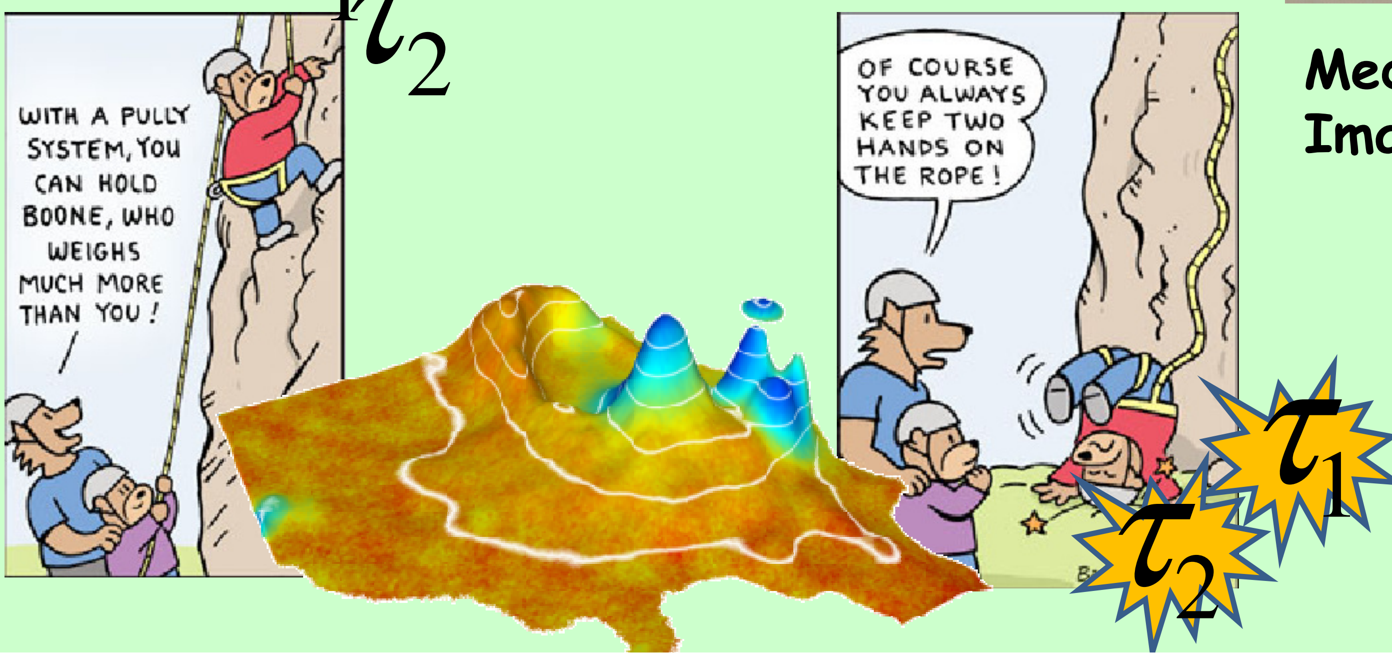
Molecules

$\tau_1\tau_2$

Medical Imaging



Cells



Measuring lifetimes has been around for a long time And The theory behind it too.

Si la loi énoncée plus haut est exacte, on doit avoir d'après la formule (2)

$$\log i = \log i_0 - at,\ \log i' = \log i_0 - at',\ \text{etc...} \qquad (3)$$

d'où

$$\frac{\log i' - \log i}{t - t'} = a,\quad \frac{\log i'' - \log i'}{t' - t''} = a, \ldots,$$

c'est-à-dire que les différences entre les logarithmes des intensités lumineuses doivent être proportionnelles aux différences des temps, et que leur rapport doit donner précisément le coefficient a.

$$\frac{di}{dt} = -ai,$$

$$(2) \quad i_t = i_0 e^{-at},$$

$$Q = \int_0^\infty i_0 e^{-at}\,dt = \frac{i_0}{a},$$

$$I = i_0 e^{-at} + y_0 e^{-bt},$$

LA LUMIÈRE

SES CAUSES ET SES EFFETS

PAR

(Alexandre)

M. EDMOND BECQUEREL

DE L'ACADÉMIE DES SCIENCES

DE L'INSTITUT DE FRANCE

PROFESSEUR AU CONSERVATOIRE IMPÉRIAL DES ARTS ET MÉTIERS, ETC., ETC.

TOME PREMIER

PARIS

LIBRAIRIE DE FIRMIN DIDOT FRÈRES, FILS ET Cie

IMPRIMEURS DE L'INSTITUT, RUE JACOB, 56

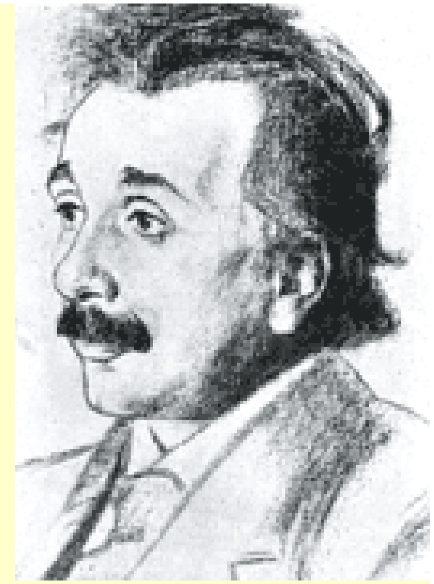
1867

$$\frac{1}{\tau} \Rightarrow$$

$$g_1 B_{12} = g_2 B_{21}$$

$$A_{21} = 16\pi^2\hbar(\omega/2\pi c)^3 B_{21}$$

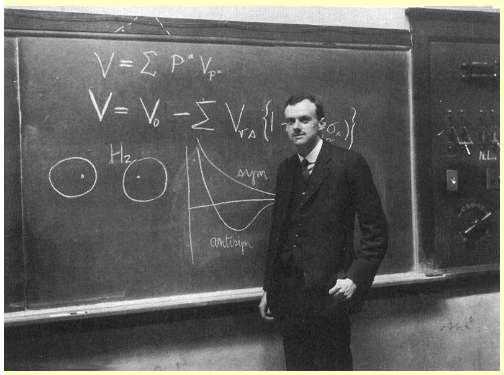
$$\frac{A_m^n}{B_m^n} = \alpha\nu^3$$



ON THE QUANTUM THEORY OF RADIATION

[1] A. Einstein. Physik Z. 18, 121 (1917)

IN a classic paper, Einstein[1] described relations connecting the rates of spontaneous emission, stimulated emission, and absorption of radiation by an atomic system in free space having two sharp energy levels.



$$\frac{1}{\tau} \Rightarrow \lambda_{if} = \frac{2\pi}{\hbar}\left|M_{if}\right|^2 \rho_f$$

Fermi's Golden Rule

Transition probability (λ_{if})

Matrix element for the interaction (M_{if})

Density of final states (ρ_f)

natural radiative lifetime

The Quantum Theory of the Emission and Absorption of Radiation P. A. M. Dirac

Proceedings of the Royal Society of London. Series A, Containing Papers of a Mathematical and Physical Character, Vol. 114, No. 767 (Mar. 1, 1927), 243-265.

The probability per unit time of a transition to a state for which each γ_k lies between γ_k' and $\gamma_k' + d\gamma_k'$ is thus (apart from the normalising factor)

$$\frac{1}{\tau} \Rightarrow \quad 2\pi \,|\, a^0 \,|^2/h \,.\, |\, v\,(W^0, \gamma' \,;\; W^0, \gamma^0)\,|^2\, J\,(W^0, \gamma')\, d\gamma_1' \,.\, d\gamma_2' \ldots d\gamma_{u-1}', \qquad (24)$$

which is proportional to the square of the matrix element associated with that transition of the perturbing energy.

How does one measure nanosecond lifetimes?

$$F(t)_{meas} = \int_0^t E(t')F_\delta(t-t')dt'$$

So called:

Time-domain:

$$F_\delta(t-t')_{meas} = \sum_i F_{\delta,i}(t-t') = \sum_i F_{0,i}\exp(-(t-t')/\tau_i)$$

Frequency domain:

Excitation repetitive pulse;e.g. $\rightarrow \quad \propto \cos(\omega t)$

$$F(t)_{meas} = \left[\sum_i F_{0,i}\tau_i + \sum_i \frac{F_{0,i}\tau_i}{1+j\omega\tau_i}e^{j\omega t}\right] = \left[\sum_i F_{0,i}\tau_i + e^{j\omega t}\sum_i \frac{F_{0,i}\tau_i}{\sqrt{1+(\omega\tau_i)^2}}e^{-j\tan^{-1}\omega\tau_i}\right]$$

$$\frac{F(t)_{meas}}{F_{meas,ss}} = 1 + \sum_i \boxed{\frac{\alpha_i}{1+j\omega\tau_i}}e^{j\omega t} = 1 + e^{j\omega t}\sum_i \boxed{\alpha_i M_i\left[\cos(\phi_{i,\omega}) + j\sin(\phi_{i,\omega})\right]}$$

$$M = \frac{1}{\sqrt{1+(\omega\tau_M)^2}} \qquad \phi = \tan^{-1}(\omega\tau_\Phi)$$

What can affect the lifetime?

Let’s see how
the old masters measured it

Die Abklingungszeiten der Fluoreszenz von Farbstofflösungen.

Von **E. Gaviola** in Berlin.

(Eingegangen am 10. Dezember 1925.)

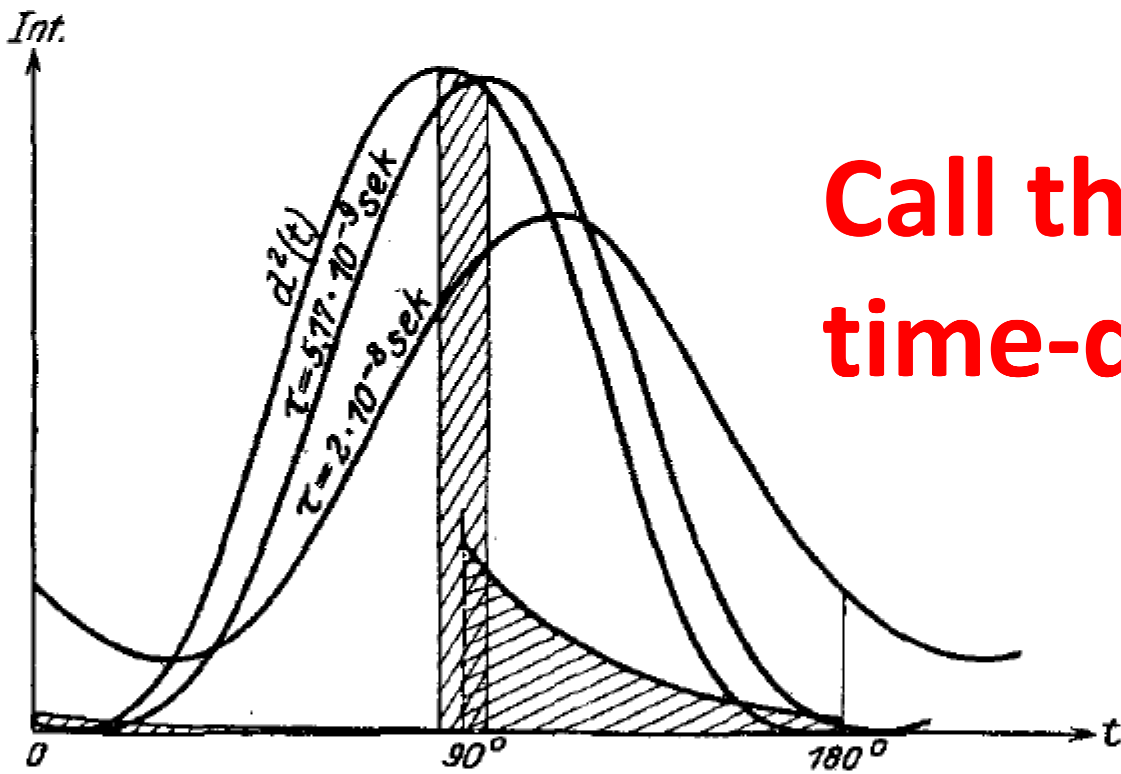


Fig. 6.

Call this the time-domain

The signal is convoluted with the excitation pulse

$$K(t^*) = \int_{-\infty}^{t^*} \frac{d^2(t)}{\tau} e^{-\frac{t^*-t}{\tau}} \, . \, dt$$

Die Abklingungszeiten der Fluoreszenz von Farbstofflösungen.

Von **E. Gaviola** in Berlin.

1925

It was difficult to measure, but look at the results!

Farbstoff	Abklingungszeiten in Wasser Sekunden	Abklingungszeiten in Glycerin Sekunden	Abklingungszeiten in Meth.-Alkohol Sekunden
Uranin	$4{,}5 \cdot 10^{-9}$	$4{,}4 \cdot 10^{-9}$	—
Fluorescein	—	—	$5{,}0 \cdot 10^{-9}$
Rhodamin B	$2{,}0 \cdot 10^{-9}$	$4{,}2 \cdot 10^{-9}$	—
Rhodulin Orange	2,7	4,3	—
Erythrosin	1,8	2,4	$2{,}6 \cdot 10^{-9}$
Tetrajodfluor . Na	1,0	2,0	2,2
Eosin 5 B	1,9	—	3,4
Uranylsulfat	—	—	1,3
Uranylsulfat in Schwefelsäure	—	—	1,9
Chinisarin in Pentan	—	—	2,9
Uranglas	—	—	> 15,0
Rubinkristall	—	—	> 15,0

Den mittleren Fehler der oben angegebenen Zahlen schätze ich zu etwa $\pm 0{,}5 \cdot 10^{-9}$ sec. Er kann unter Umständen viel kleiner sein. Die

Eine allgemeine Theorie
der zur Messung sehr kurzer Leuchtdauern dienenden
Versuchsanordnungen (Fluorometer).

Von F. Duschinsky in Berlin.

Mit 3 Abbildungen. (Eingegangen am 10. Januar 1933.)

[1]) F. Duschinsky, ZS. f. Phys. 81, 23, 1933.

"Fourier's theorem is not only one of the most beautiful results of modern analysis, but it is said to furnish an indispensable instrument in the treatment of nearly every recondite question in modern physics…
Fourier is a mathematical poem." Lord Kelvin

(March 21, 1768 – May 16, 1830)

$$f(t)=\frac{a_0}{2}+\sum_{n=1}^{\infty}[a_n\cos\frac{2n\pi t}{T}+b_n\sin\frac{2n\pi t}{T}]$$

$$a_0=\frac{2}{T}\int_0^T f(t)dt$$

$$a_n=\frac{2}{T}\int_0^T f(t)\cos(\frac{2n\pi t}{T})dt$$

$$b_n=\frac{2}{T}\int_0^T f(t)\sin(\frac{2n\pi t}{T})dt$$

Der zeitliche Intensitätsverlauf von intermittierend angeregter Resonanzstrahlung.

Von **F. Duschinsky** in Berlin.

(Eingegangen am 10. Januar 1933.)

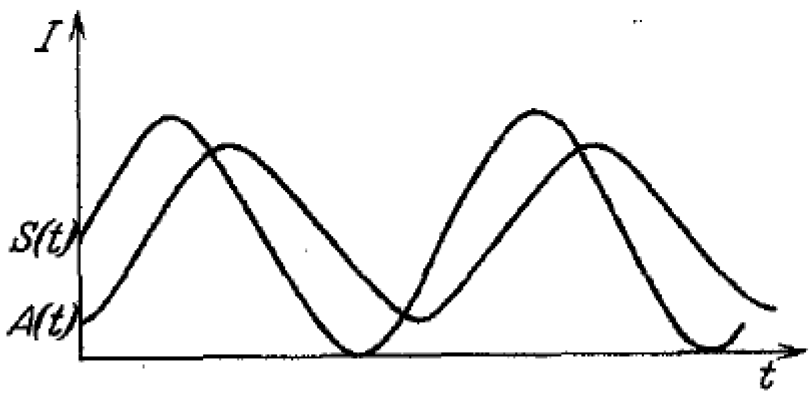


Fig. 1.

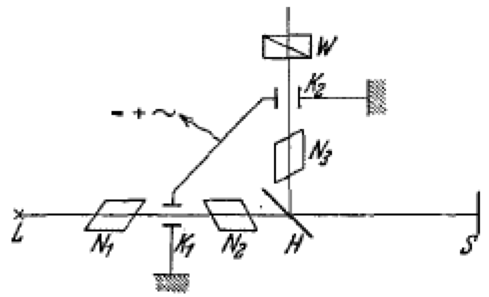


Fig. 1.

Call this the frequency-domain

Every frequency component is analyzed separately

$$\left.\begin{aligned} S(t) &= X_0 + \sum_1^{\infty} X_m \cos(m\Omega t - \eta_m), \\ A(t) &= X_0 + \sum_1^{\infty} \frac{X_m}{\sqrt{1+(m\Omega\tau)^2}} \cdot \cos(m\Omega t - \eta_m - \operatorname{arctg} m\Omega\tau). \end{aligned}\right\} \quad (46')$$

Eine allgemeine Theorie der zur Messung sehr kurzer Leuchtdauern dienenden Versuchsanordnungen (Fluorometer).

Von **F. Duschinsky** in **Berlin.**

Mit 3 Abbildungen. (Eingegangen am 10. Januar 1933.)

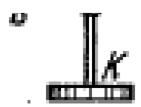
[1]) F. Duschinsky, ZS. f. Phys. **81**, 23, 1933.

$$L(t') = \int_0^{\infty} E(t' - t)\, \Phi(t)\, dt$$

Ist die Erregungsintensität $E(t)$ periodisch (mit der Frequenz ω' moduliert), so kann sie als Fourier-Reihe dargestellt werden:

$$E(t) = \sum_{\mu=0}^{\infty} E_\mu \cos(\mu\omega' t + e_\mu).$$

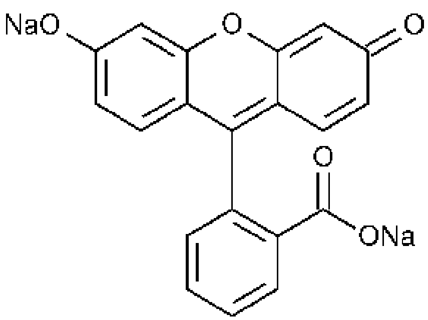
Herrn Prof. Pringsheim meinen besonderen Dank ausdrücken für alles, was ich von ihm an wissenschaftlicher Anregung erhalten habe. Ebenso bin ich Herrn Prof. Schrödinger für freundlichen Rat und wertvolle Kritik an dieser Arbeit zu großem Dank verpflichtet.



wäh ... belü ... r- ... on

Fig. 1. Schema der verbesserten Fluorometeranordnung. L Lichtquelle; N_1, N_2, N_3, N_4 Nicols, K_1, K_2 Kerr-Zellen; K Babinet-Soleil-Kompensator; S Szivessi-Platte; Z Spiegel; T Trog.

The lifetimes do
funny things
depending on
the molecular species
and
the environment



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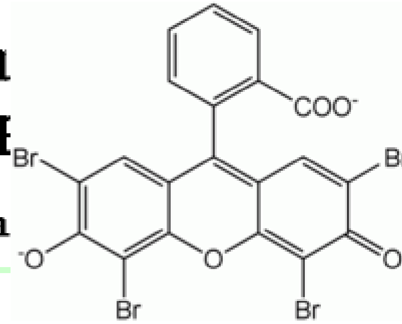
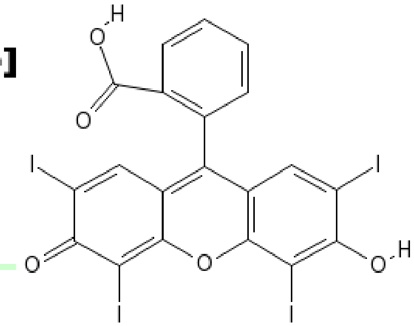


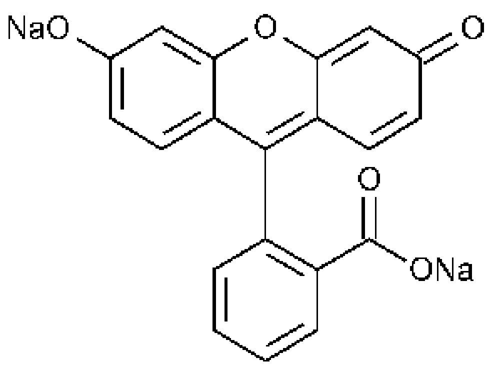


It was difficult to measure, but look at the results!

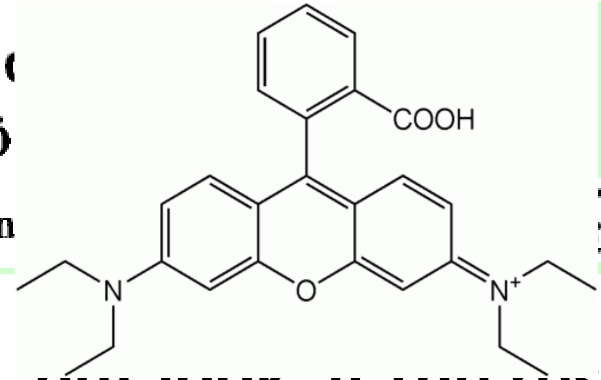
Fluorescein Eosin Y Erythroscine

Farbstoff	Abklingungszeiten in Wasser Sekunden	in Glycerin Sekunden	in Meth.-Alkohol Sekunden
Uranin	$4{,}5 \cdot 10^{-9}$	$4{,}4 \cdot 10^{-9}$	—
Fluorescein	—	—	$5{,}0 \cdot 10^{-9}$
Rhodamin B	$2{,}0 \cdot 10^{-9}$	$4{,}2 \cdot 10^{-9}$	—
Rhodulin Orange	2,7	4,3	—
Erythrosin	1,8	2,4	$2{,}6 \cdot 10^{-9}$
Tetrajodfluor. Na	1,0	2,0	2,2
Eosin 5 B	1,9	—	3,4
Uranylsulfat	—	—	1,5
Uranylsulfat in Schwefelsäure	—	—	1,9
Chinisarin in Pentan	—	—	2,9
Uranglas	—	—	> 15,0
Rubinkristall	—	—	> 15,0

Den mittleren Fehler der oben angegebenen Zahlen schätze ich zu etwa $\pm 0{,}5 \cdot 10^{-9}$ sec. Er kann unter Umständen viel kleiner sein. Die



gszeiten
rbstofflö
. Gaviola in
925



It ... measure, but look at the results!

Fluorescein

Rhodamine B

Farbstoff	Abklingungszeiten in Wasser Sekunden	Abklingungszeiten in Glycerin Sekunden	Abklingungszeiten in Meth.-Alkohol Sekunden
Uranin	4,5 . 10^{-9}	4,4 . 10^{-9}	—
Fluorescein	—	—	5,0 . 10^{-9}
Rhodamin B	2,0 . 10^{-9}	4,2 . 10^{-9}	—
Rhodulin Orange	2,7	4,3	—
Erythrosin	1,8	2,4	2,6 . 10^{-9}
Tetrajodfluor . Na	1,0	2,0	2,2
Eosin 5 B	1,9	—	3,4
Uranylsulfat	—	—	1,3
Uranylsulfat in Schwefelsäure	—	—	1,9
Chinisarin in Pentan	—	—	2,9
Uranglas	—	—	$>$ 15,0
Rubinkristall	—	—	$>$ 15,0

Den mittleren Fehler der oben angegebenen Zahlen schätze ich zu etwa $\pm$ 0,5 . 10^{-9} sec. Er kann unter Umständen viel kleiner sein. Die

**The lifetimes depend on
the dynamics of the molecules
and the dynamics
of the environment**

Die Lebensdauer der angeregten Moleküle in den wässerigen fluoreszierenden Lösungen.

Von **S. I. Wawilow** in Moskau.

Zeitschrift für Physik A Hadrons and Nuclei
Volume 53, Numbers 9-10 / September, 1929 665-674

Measuring tau using translational diffusion: dynamic quenching

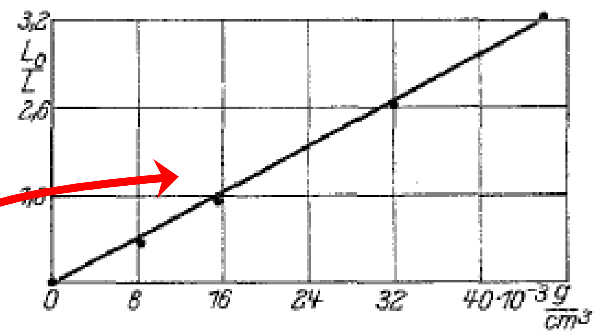
$$\bar{t} = \frac{1}{8\pi D \sigma N c},$$

$$D_1 = \frac{kT}{6\pi\sigma_1\eta}, \quad D_2 = \frac{kT}{6\pi\sigma_2\eta}$$

$$\frac{L_0}{L} = 1 + \frac{\tau N c k T}{3\eta} \cdot \alpha,$$

Tabelle 5. Rhodamin B + KJ.

c_{KJ} . . .	0	8,1 . 10^{-3} g/cm³	15,6	32	46
L_0/L . . .	1,00	1,36	1,75	2,64	3,24



anstatt σ muß $\frac{\sigma_1 + \sigma_2}{2}$

anstatt D $\left(\frac{D_1 + D_2}{2}\right)$

Auch hierbei ist (4) erfüllt, und aus der Neigung der Geraden in der Fig. 5 wird

$$\tau = 2,2 \,.\, 10^{-9} \text{ sec}$$

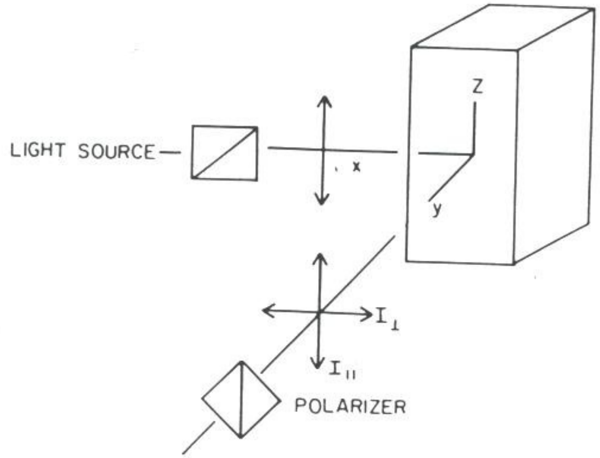
gefunden. Die direkten Messungen von Gaviola für Rhodamin im Wasser ergaben *

$$\tau = 2,0 \,.\, 10^{-9} \text{ sec.}$$

* ZS. f. Phys. **42**, 861, 1927.

Using fast rotational motions to measure the fluorescence lifetime

Francis Henri Perrin
1901-92



$$p = \frac{I_{par} - I_{perp}}{I_{par} + I_{perp}}$$

$$r = \frac{I_{par} - I_{perp}}{I_{par} + 2I_{perp}}$$

§ 3. Die Polarisation der Fluoreszenz gibt ein anderes Mittel zur Bestimmung von τ*. Nach der Formel von F. Perrin ist

$$\frac{1}{p} = \frac{1}{p_0} + \left(\frac{1}{p_0} - \frac{1}{3}\right) \tau \frac{RT}{v\eta}, \qquad (7)$$

wo p der Polarisationsgrad bei gegebenen Bedingungen ist, p_0 der Grenzwert der Polarisation bei sehr großer Zähigkeit η, v das Molekularvolumen. Nach dieser Formel berechnete F. Perrin, wie erwähnt, für Fluorescein $\tau = 4{,}5 \cdot 10^{-9}$ sec im Einklang mit den direkten Messungen von Gaviola.

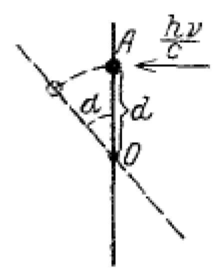


Fig. 6.

* F. Perrin, C. R. 180, 581, 1925; 182, 219, 1926; Journ. de phys. 7, 390, 1926.

But if the fluorescence is polarized then the measured "lifetime" will depend on the rotation of the molecules. Right?

Yes

Can we get the "right" lifetime?

Yes

Alexander Jablonski
(1898-1980)

Eine Theorie der zeitlichen Abklingung des Leuchtens bei polarisierter Fluoreszenz von Farbstofflösungen.

Von **A. Jabłoński** in Warschau.

Mit 2 Abbildungen. (Eingegangen am 3. April 1935.)

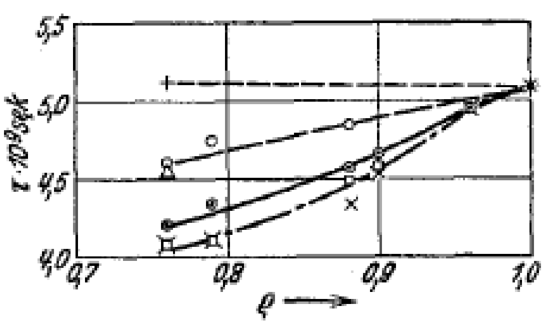
A. Jablonski, *Z. Phys.* **95**, 53 (1935).

Wir stellen also fest: *Will man direkt die Verweilzeiten der fluoreszierenden Moleküle* (in Lösungen) *bestimmen, so soll man* (falls die Erregung mit *polarisiertem* Licht erfolgt) *die Abklingung der unter dem Winkel* = 54,72° *gegen die Schwingungsrichtung des erregenden Lichtes schwingenden Komponente untersuchen.*

Enter Magic Angle

$$\eta = \int^{\infty} P(\varrho) \cdot f(\sigma - \varrho)\, d\varrho.$$

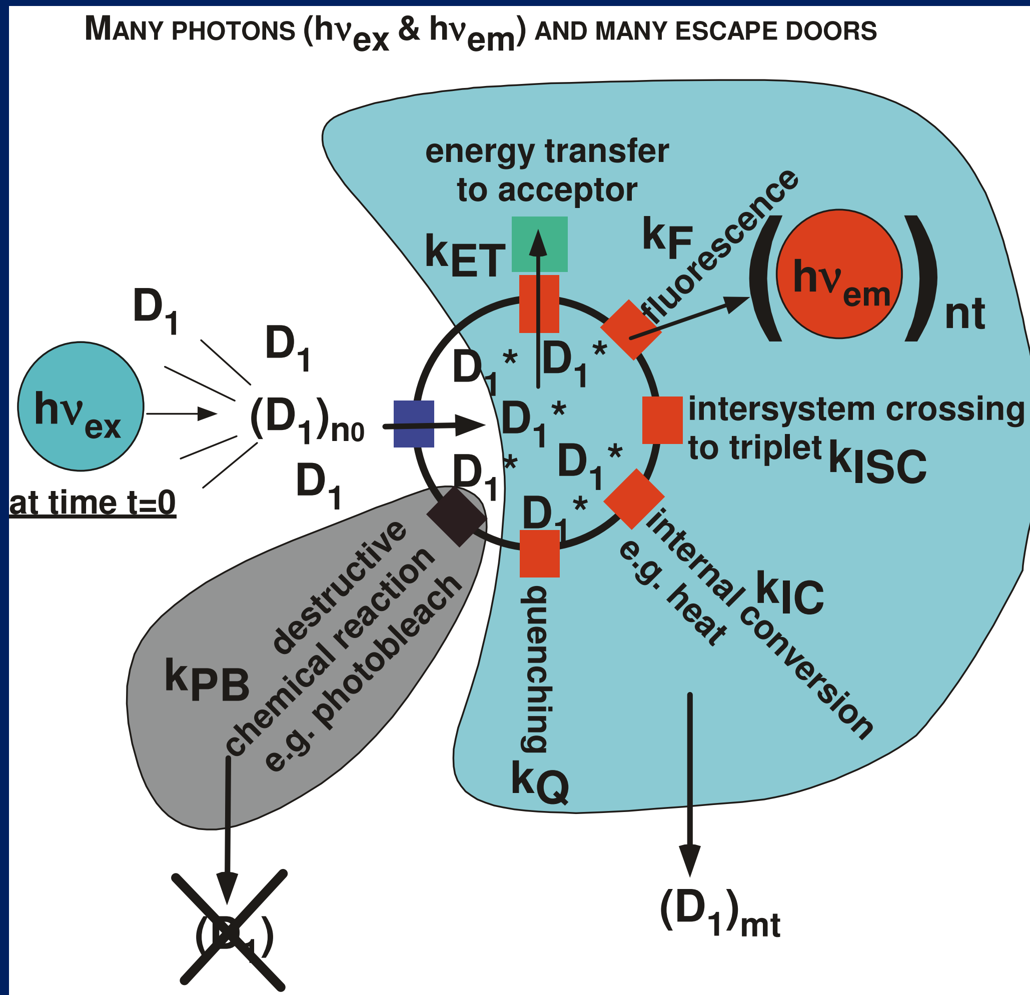
$$I_{\parallel} = \tfrac{1}{3}[A_{\parallel} + 2A_{\perp} + 2(A_{\parallel} - A_{\perp})\, e^{-\varphi t}]\, e^{-\frac{t}{\tau}},$$
$$I_{\perp} = \tfrac{1}{3}[A_{\parallel} + 2A_{\perp} - (A_{\parallel} - A_{\perp})\, e^{-\varphi t}]\, e^{-\frac{t}{\tau}}.$$



+------+ beobachtete Werte für $\bar{t}_{\perp}$,
×-----× beobachtete Werte für $\bar{t}_{\parallel}$,
⊙——⊙ berechnete Werte von $\bar{t}_{\parallel}$, $\varrho_0 = 1/3$ vorausgesetzt,
□-·-·-□ berechnete Werte von $\bar{t}_{\parallel}$, $\varrho_0 = 0,1$ vorausgesetzt,
△ beobachteter Wert für die rotationsunbeeinflußbare Komponente,
○-----○ berechnete Werte für dieselbe Komponente.

Fig. 2. Abhängigkeit der Abklingzeiten der Fluoreszenz von Fluorescein in Glycerin + Wasser-Lösungen von verschiedenem Wassergehalt von dem Depolarisationsgrade ϱ der Fluoreszenz. $\varrho = 1$ entspricht der reinen wässerigen Lösung.

So, the lifetime depends on many things



lifetime of the excited state:

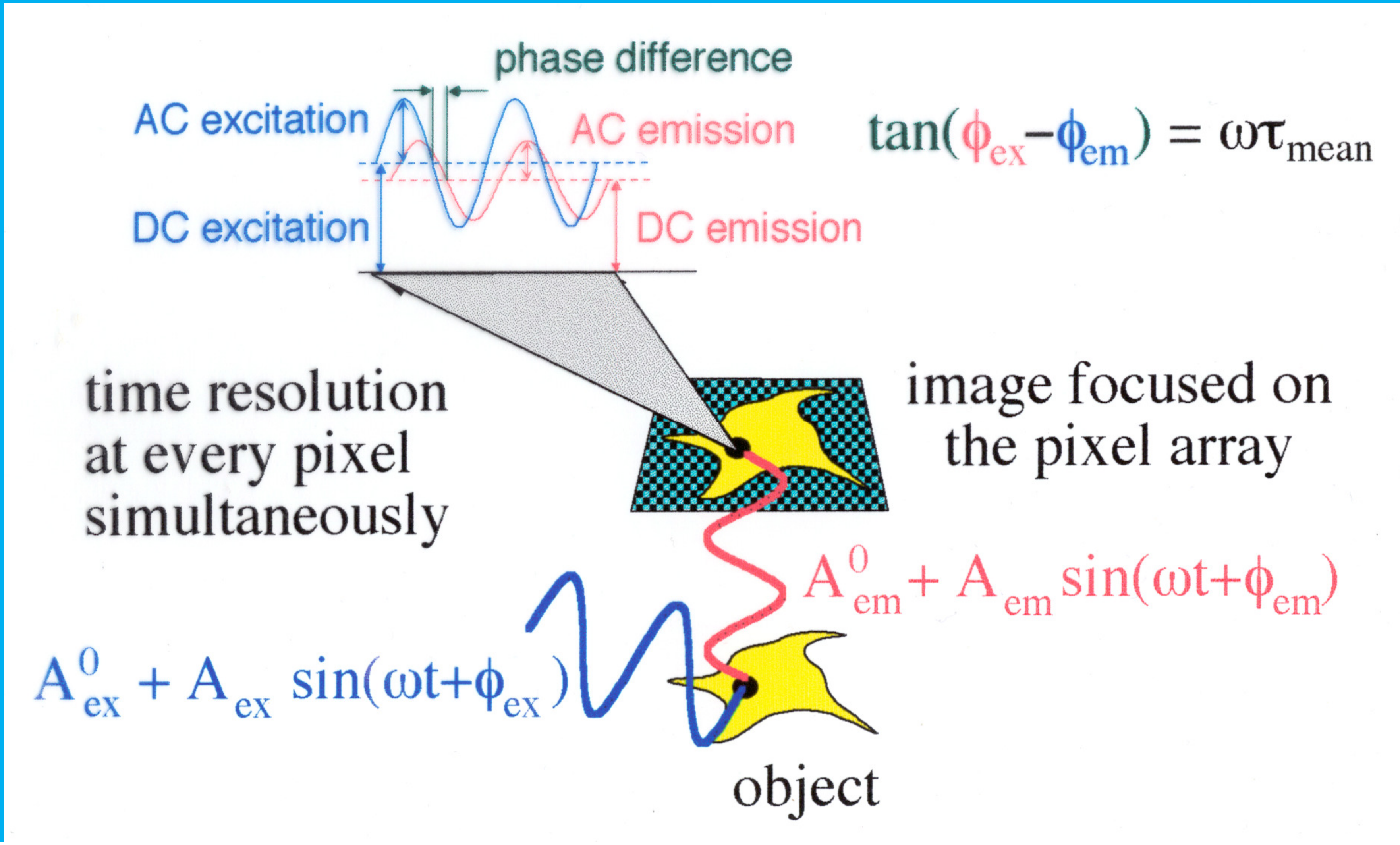
$$1/\tau = k_T + k_F + k_{ISC} + k_{IC} + k_Q + k_{PB} = \sum_j k_j$$

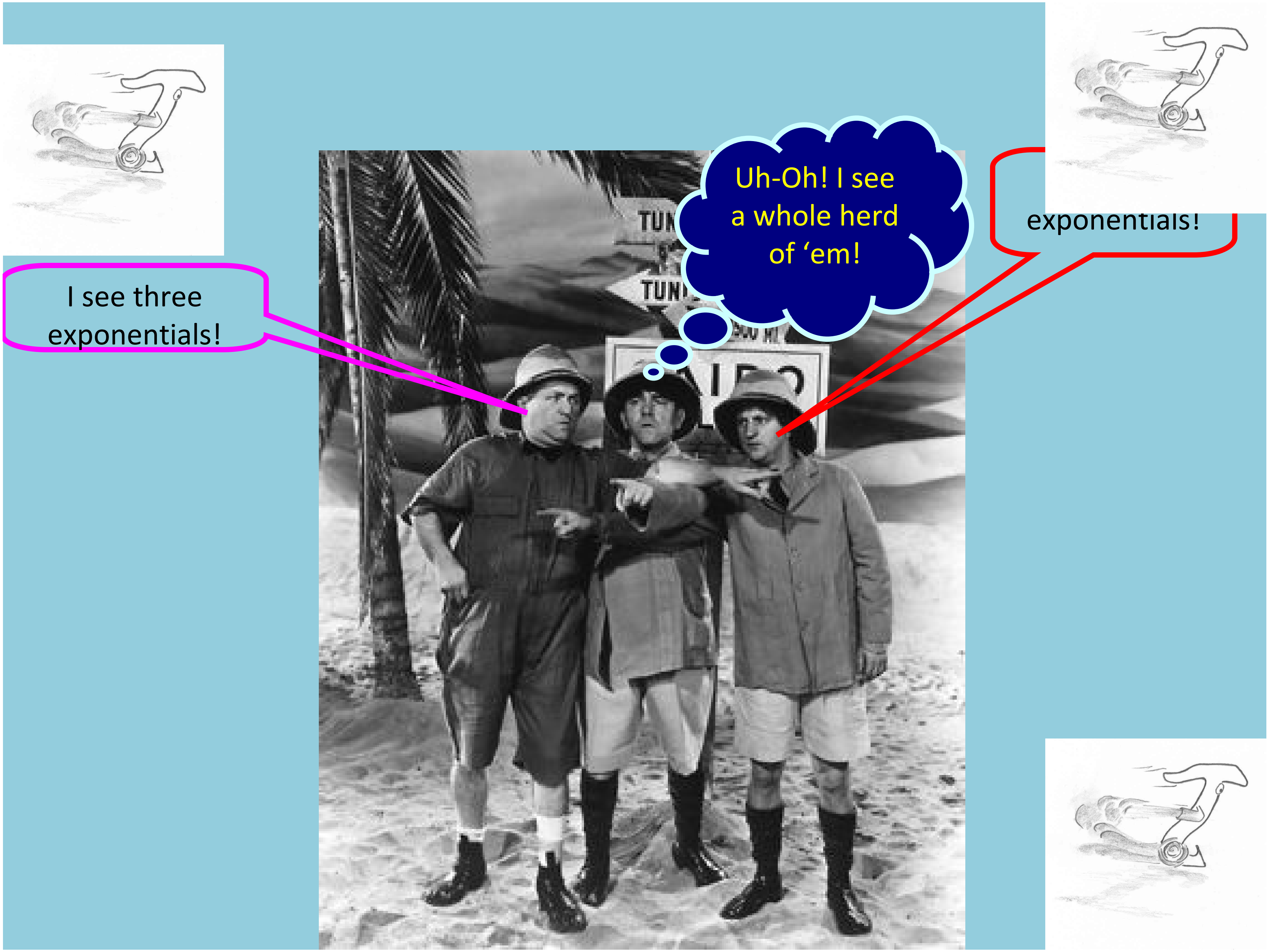
Quantum yield of the i^{th} process = $\frac{k_i}{\sum_j k_j}$

SO, now we seem all set.

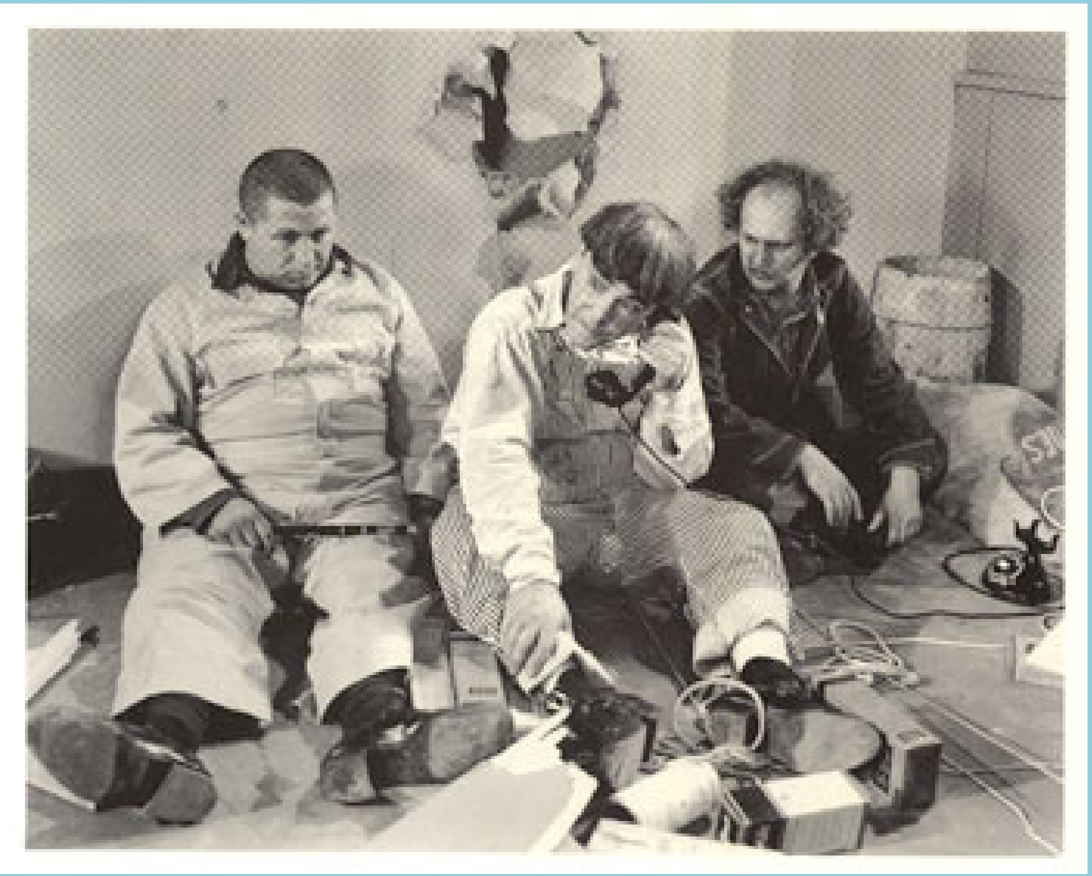
BUT…..

We want to measure fluorescence lifetimes in a fluorescence image at every location of the cell.




I see three exponentials!
Uh-Oh! I see a whole herd of ‘em!
exponentials!

What now?



Lifetimes in images are not so simple!

We need some help!

Let's start with

Model Independent Analysis

Some different ways to parameterize lifetime-resolved data

$$1/(1+j\omega t) = M_i\left[\cos\left(\phi_{i,\omega}\right) + j\sin\left(\phi_{i,\omega}\right)\right]$$

$$x = M_i\cos\left(\phi_{i,\omega}\right) \text{ and } y = M_i\sin\left(\phi_{i,\omega}\right)$$

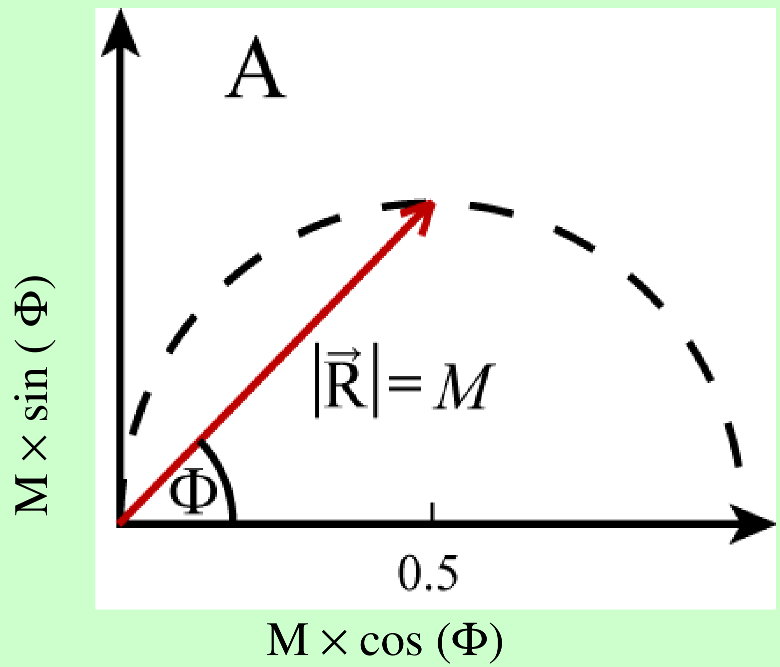
$$j = \sqrt{-1}$$

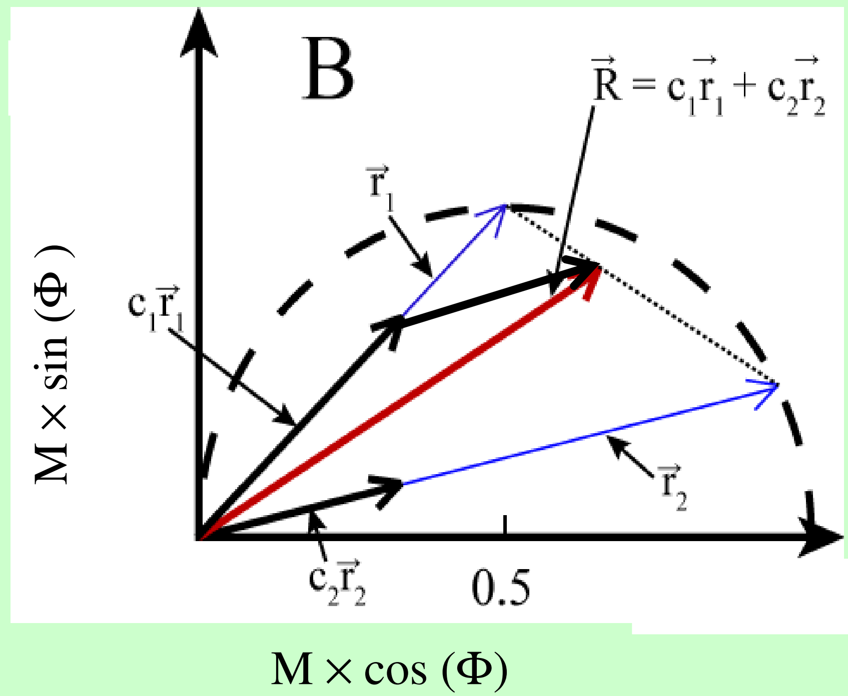
Frequency domain lifetime measurement

Data analysis with a polar plot representation

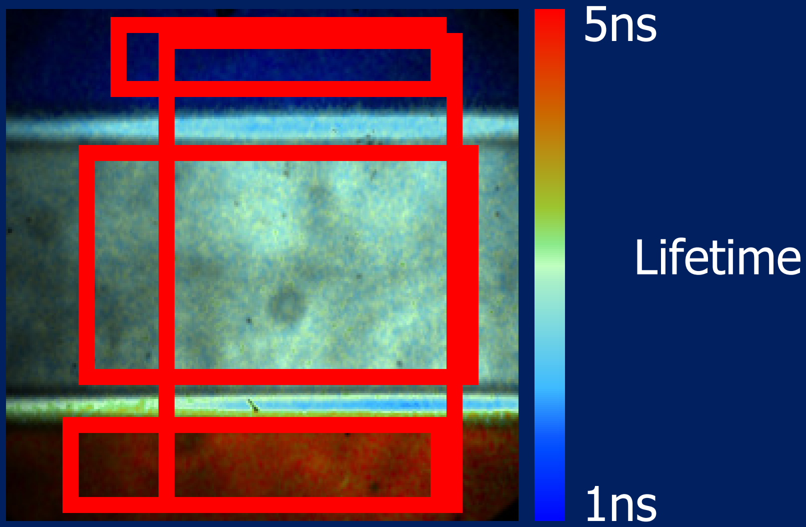
Demodulation = $$M = \frac{b/B}{a/A} = \frac{1}{\sqrt{1+(\omega\tau_M)^2}}$$

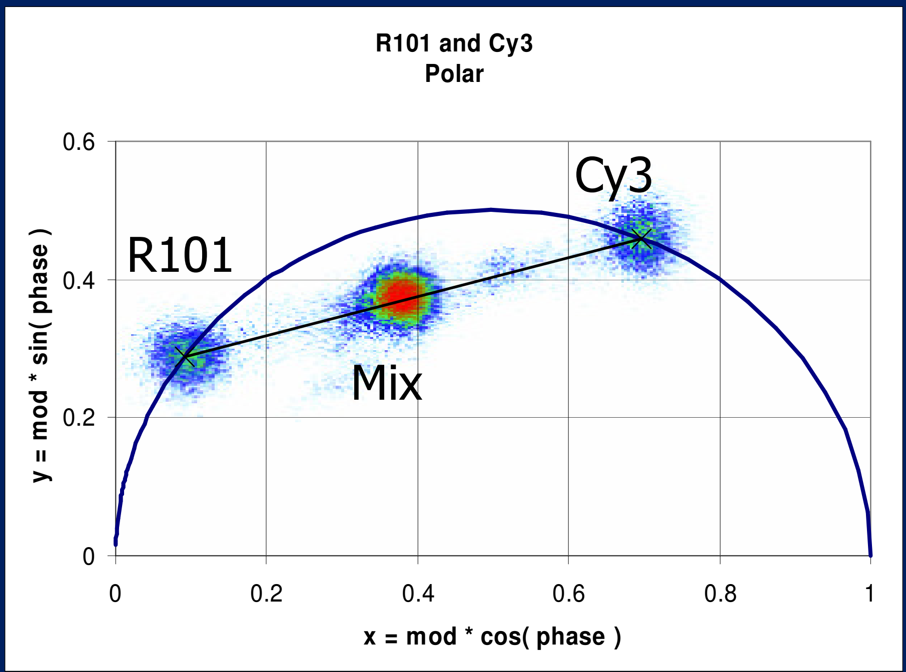
Phase shift = $$\Phi = \tan^{-1}(\omega\tau_\Phi)$$

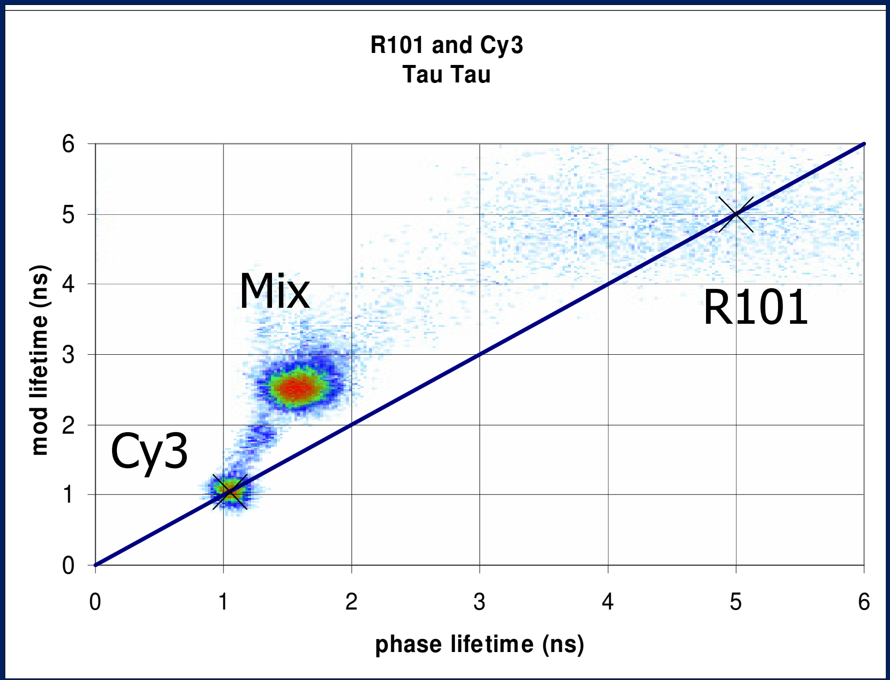




good for any signal $\propto \dfrac{1}{1+i\omega\tau}$ (for instance dielectric dispersion)

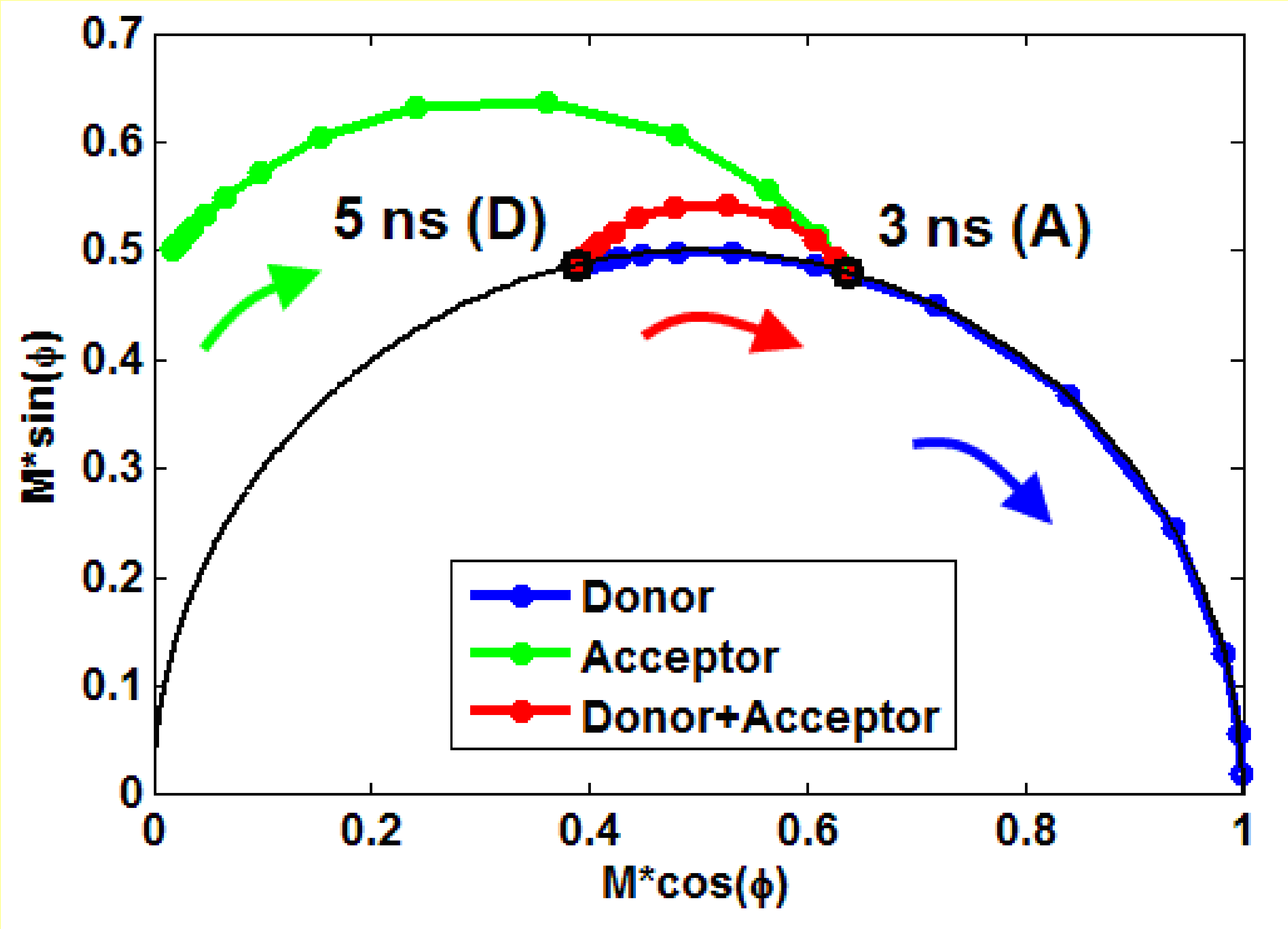

5ns
Lifetime
1ns


R101 and Cy3
Polar
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
y = mod * sin(phase)
x = mod * cos(phase)
R101
Mix
Cy3


R101 and Cy3
Tau Tau
6
5
4
3
2
1
0
0
1
2
3
4
5
6
mod lifetime (ns)
phase lifetime (ns)
Mix
R101
Cy3

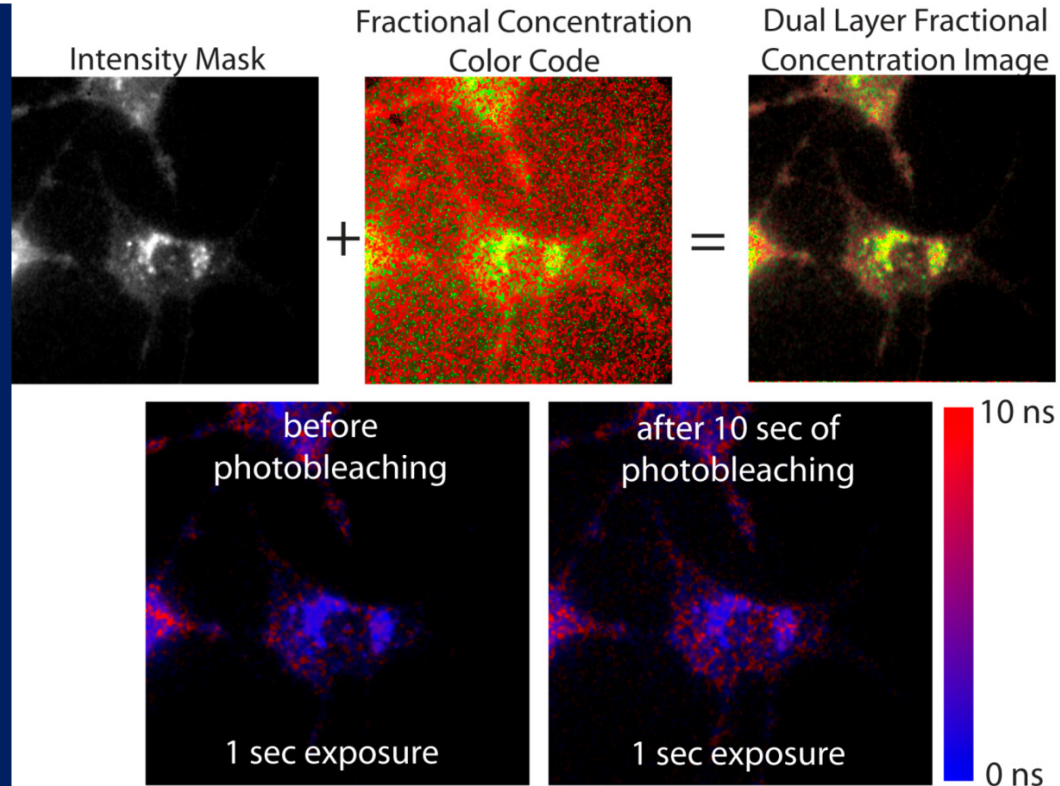
Observing the fluorescence of:

Product species of an excited state reaction
Product and directly excited species
Directly excited species

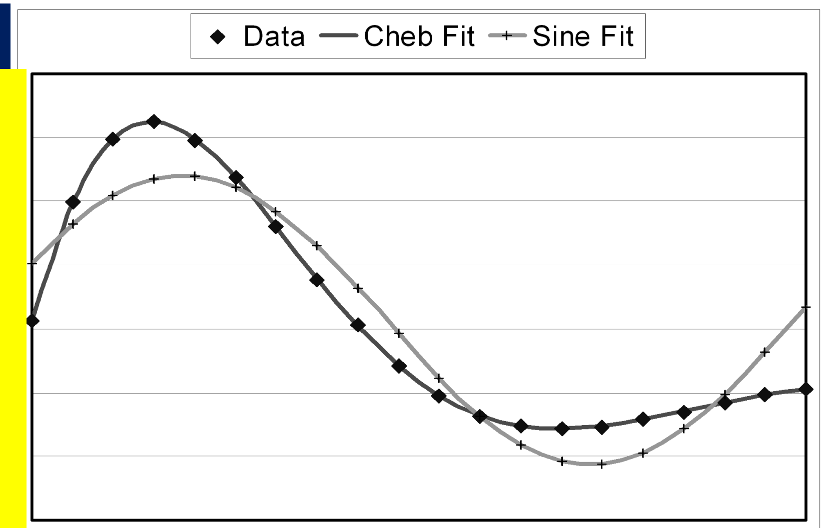


Of course we have to be sure that we get rid of artifacts

Chebyshev fits – very fast single pass not interative



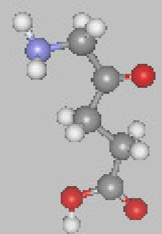
Correction for photobleaching



Malachowski, G.C., Clegg, R.M., and **Redford, G.I.,** "Analytic solutions to modelling exponential and harmonic functions using Chebyshev polynomials: fitting frequency-domain lifetime images with photobleaching," Journal of Microscopy 228(3), 282-295 (2007)

Photodiagnostics and phototherapy

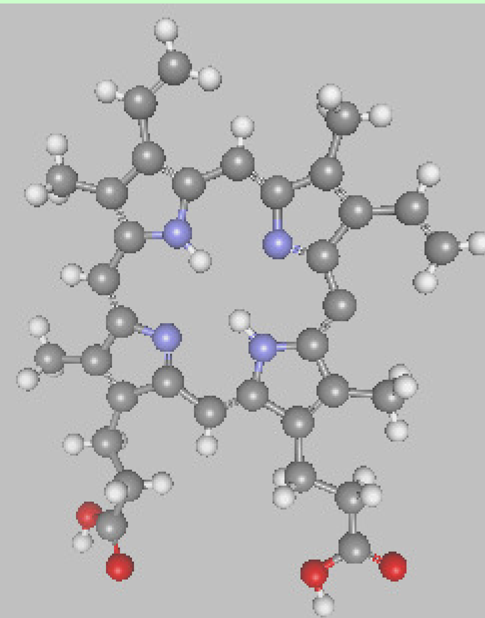
ALA

8 x 

δ-aminolevulinic-acid

Normal cellular

heme synthesis



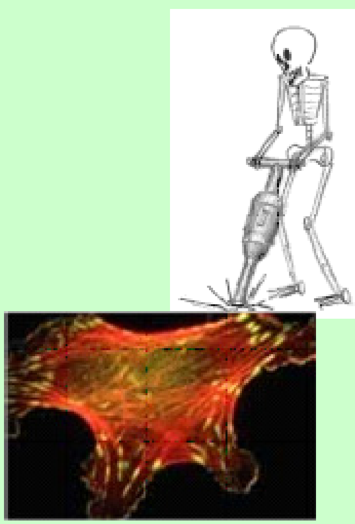
PpIX-protoporphyrin IX

Fluorescence

or

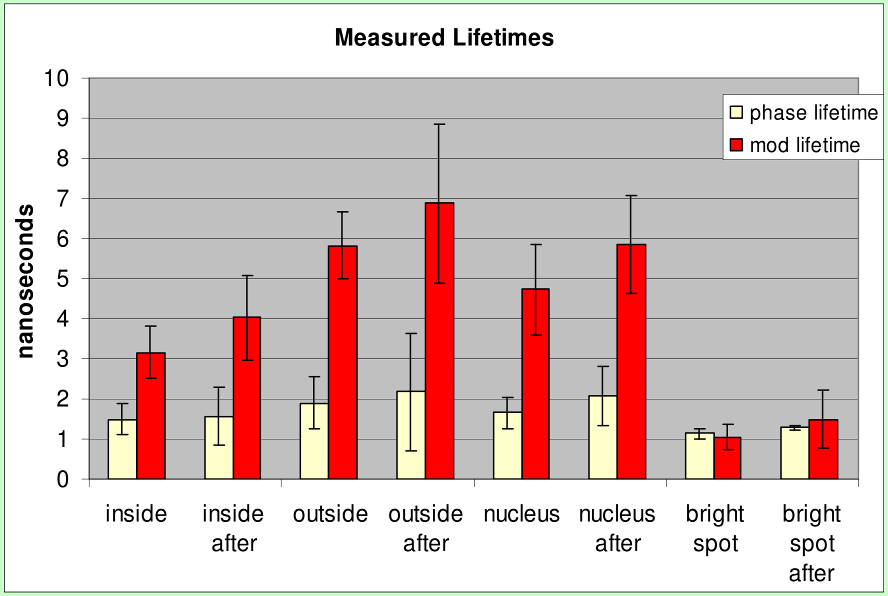
hν

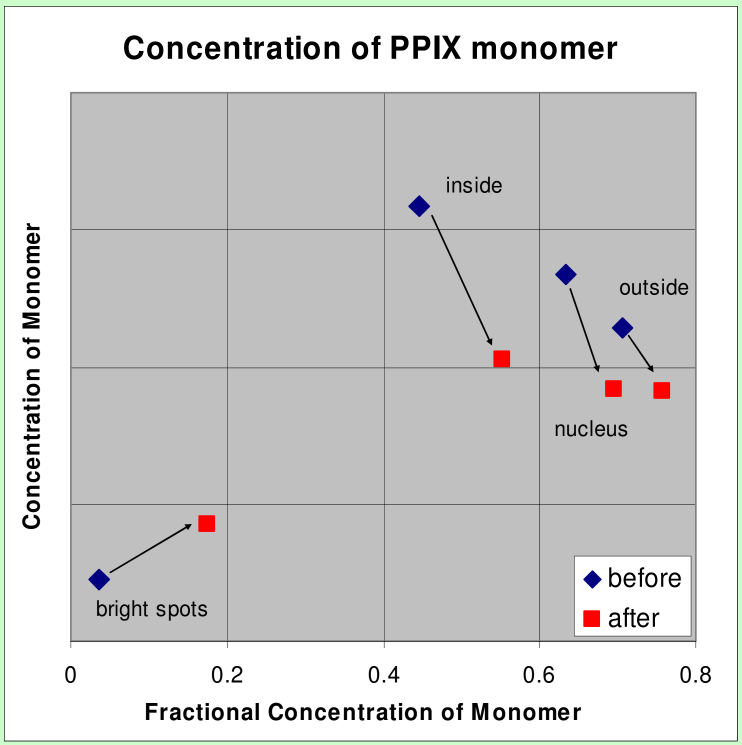
$^1O_2$



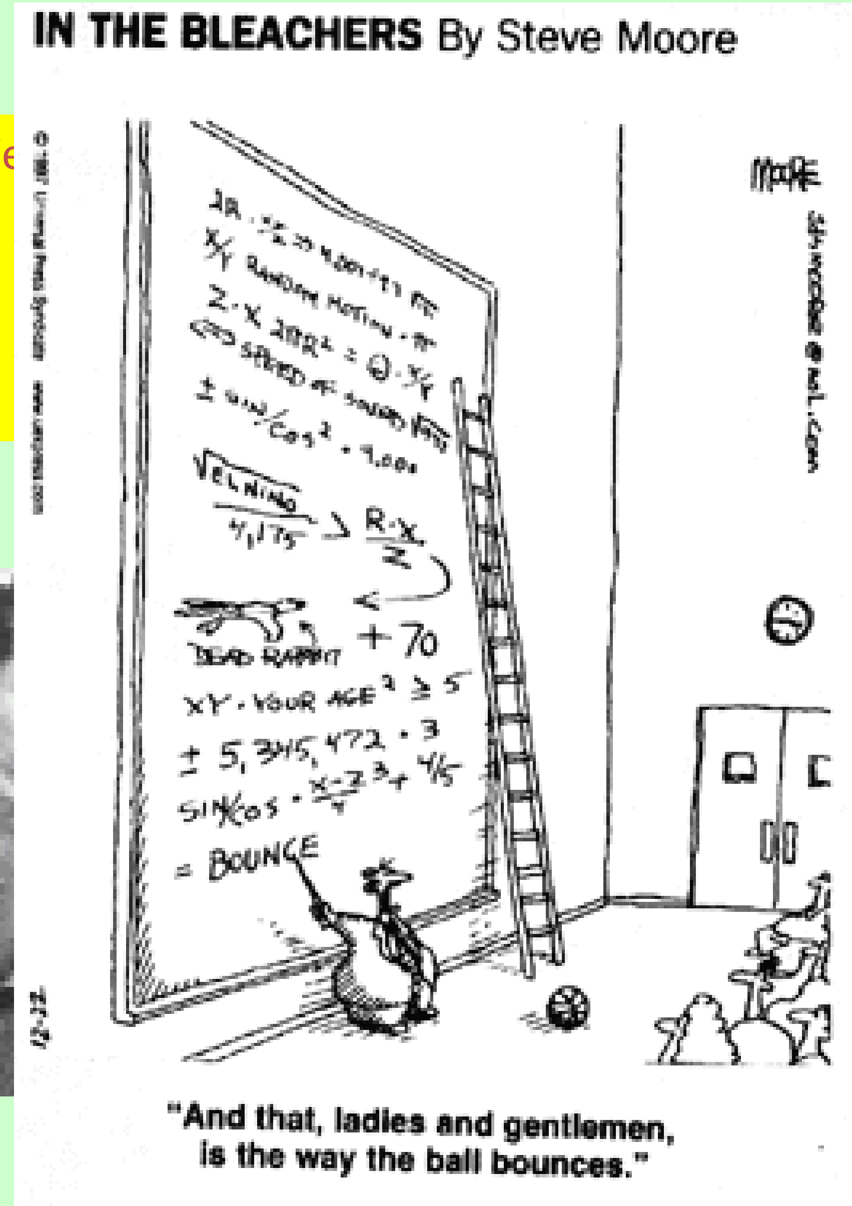
The monomer of PpIX forms ROS and is used for phototherapy

But sometimes that complicates things



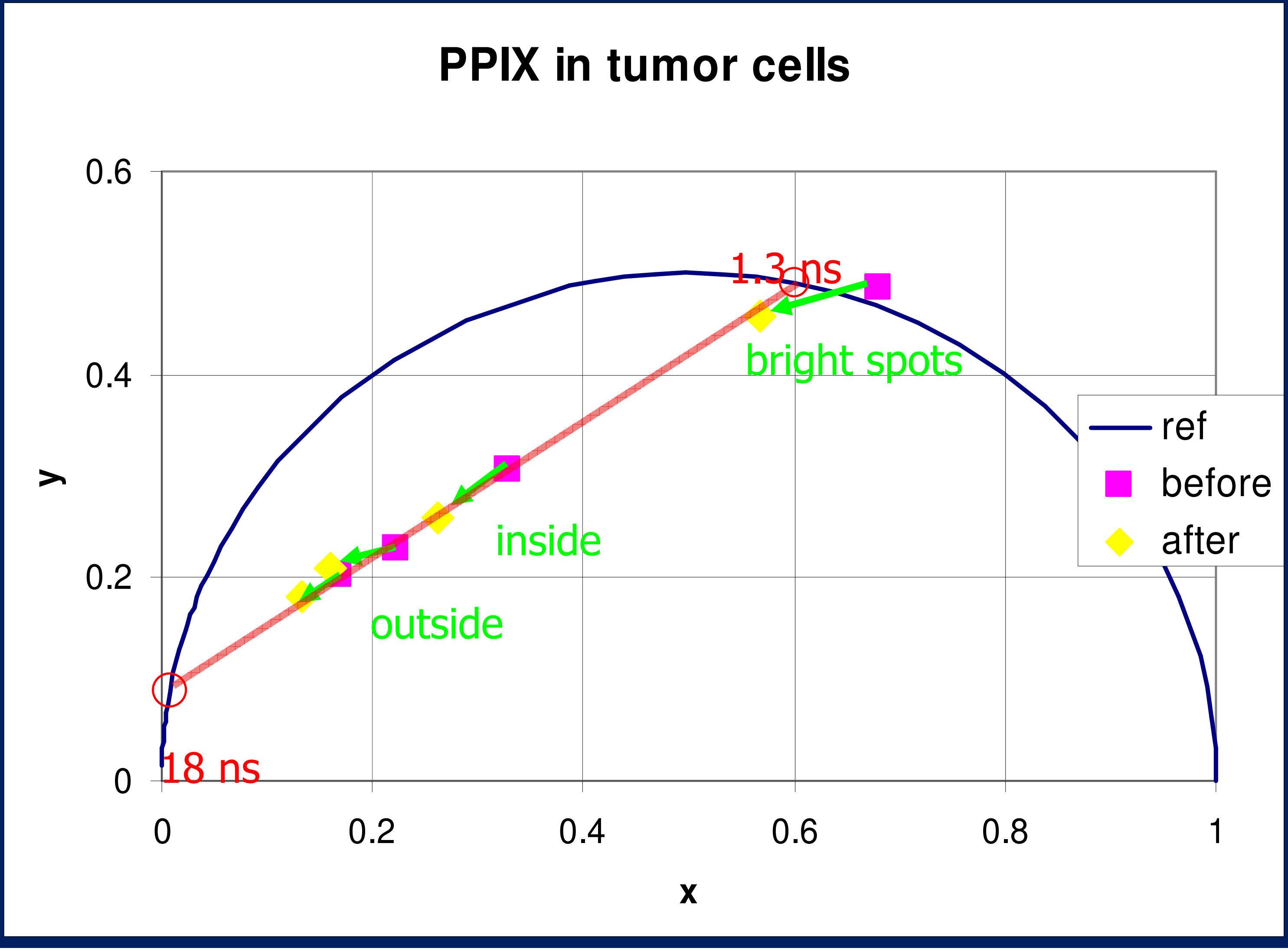


We

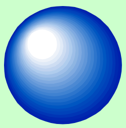
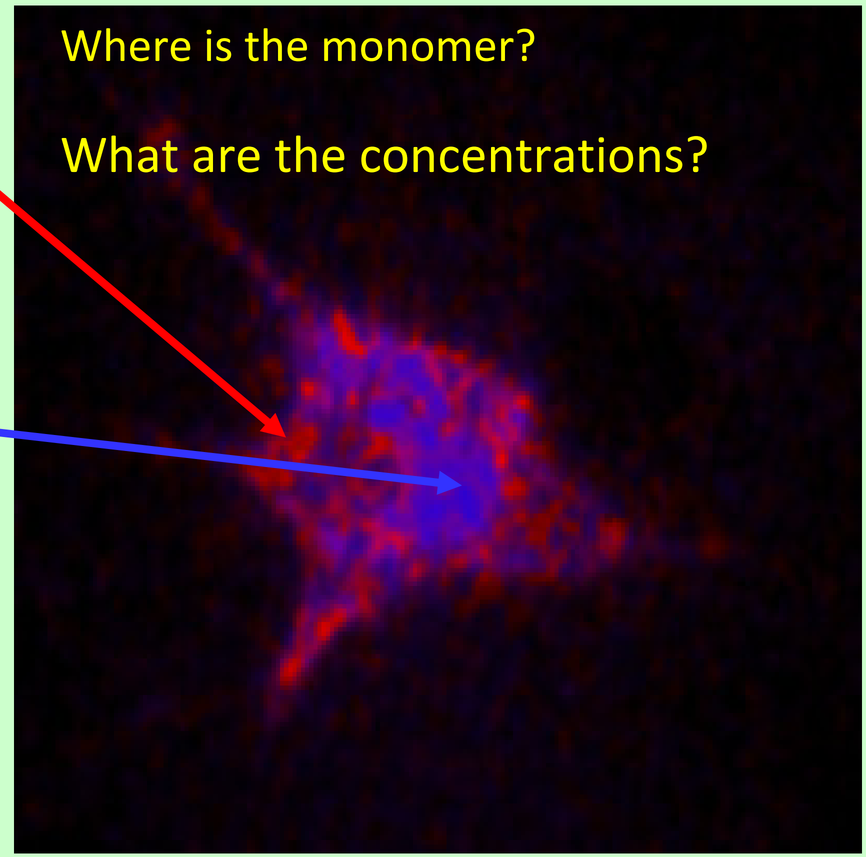
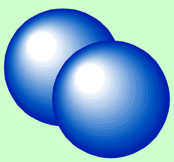
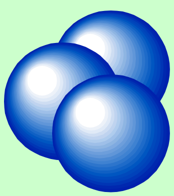


Polar Plot analysis

Fluorescence intensity and dynamic response change upon illumination

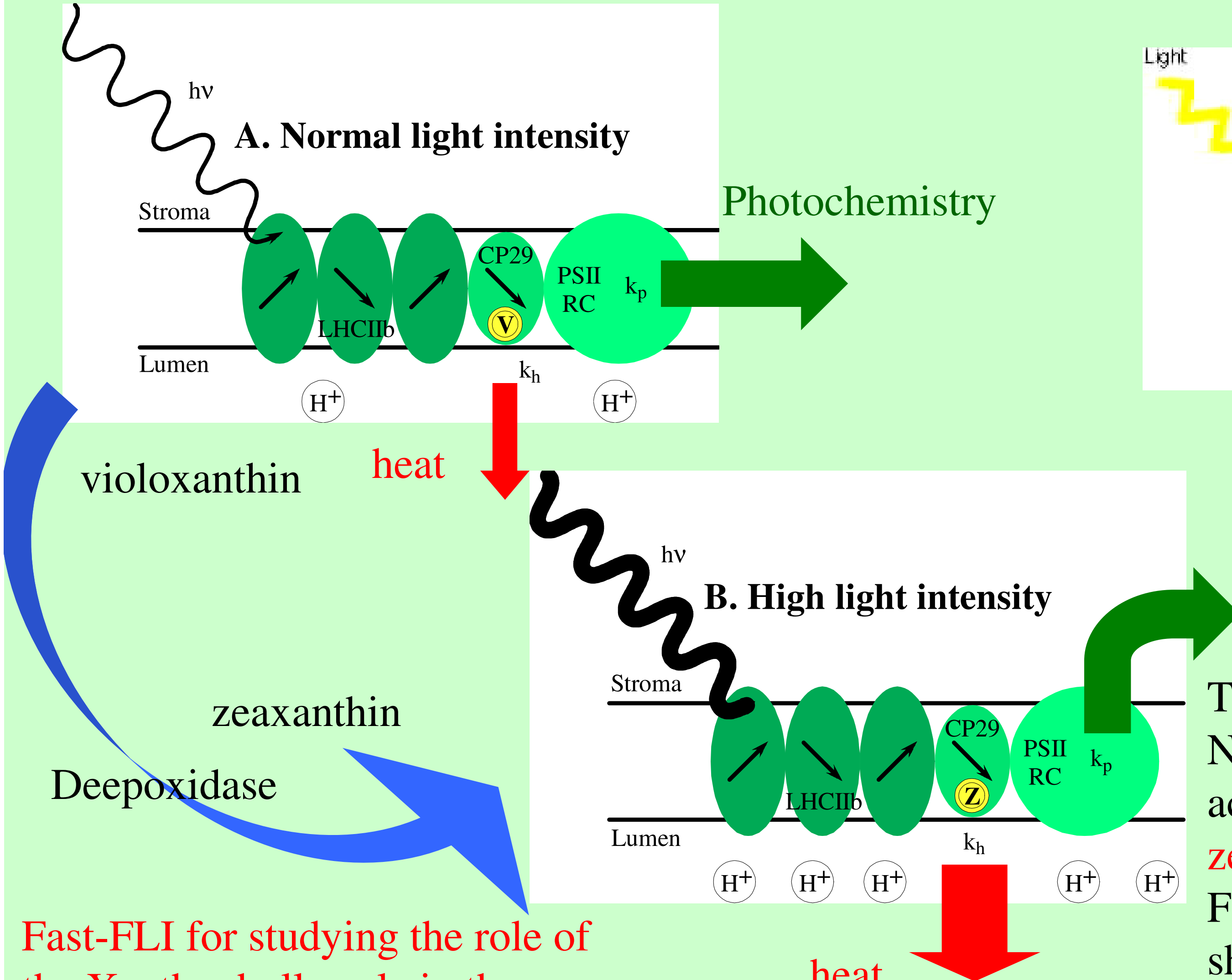


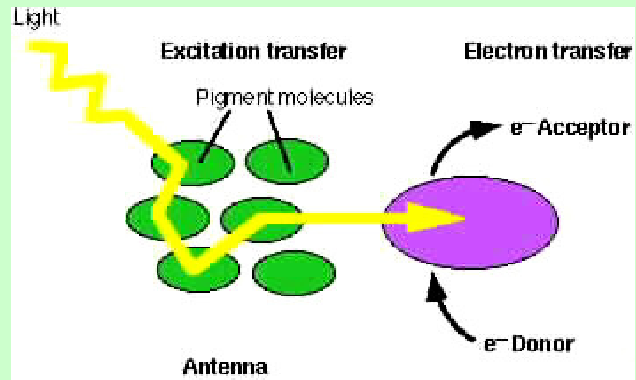
Protoporphyrin IX

		Lifetime	Location
	Monomer	17-18 ns	
	Dimer	~2 ns	
	Higher Multimers	<2 ns	

Monomers and Multimers ≈ same spectra

Real-Time Fluorescence Lifetime-Resolved Images of individual cells of Wild Type and NPQ mutants of *Chlamydomonas reinhardtii*



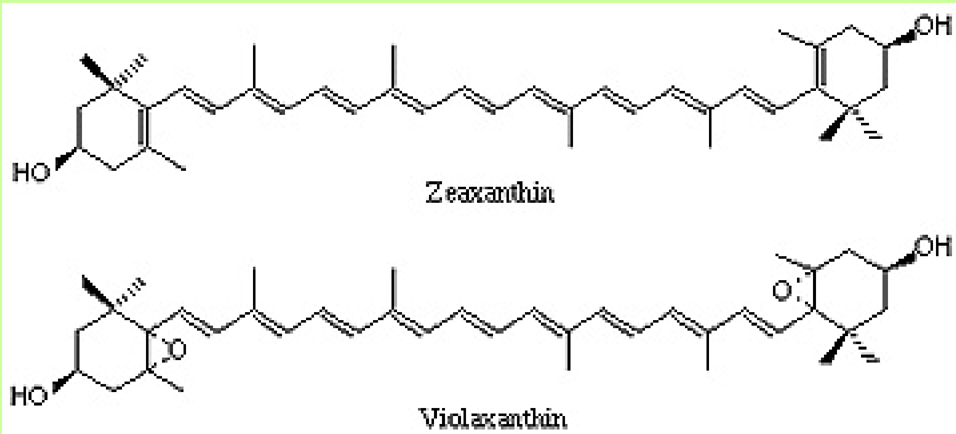


Fast-FLI for studying the role of the Xanthophyll cycle in the non-photochemical quenching process in single cells.

The mutants (NPQ1 and NPQ2) used in this work, accumulate violoxanthin and zeaxanthin, respectively. Fluorescence transient studies showed strong quenching for NPQ2 in comparison to the WT and NPQ1.

NPQ mutants of the green alga *Chlamydomonas reinhardtii*

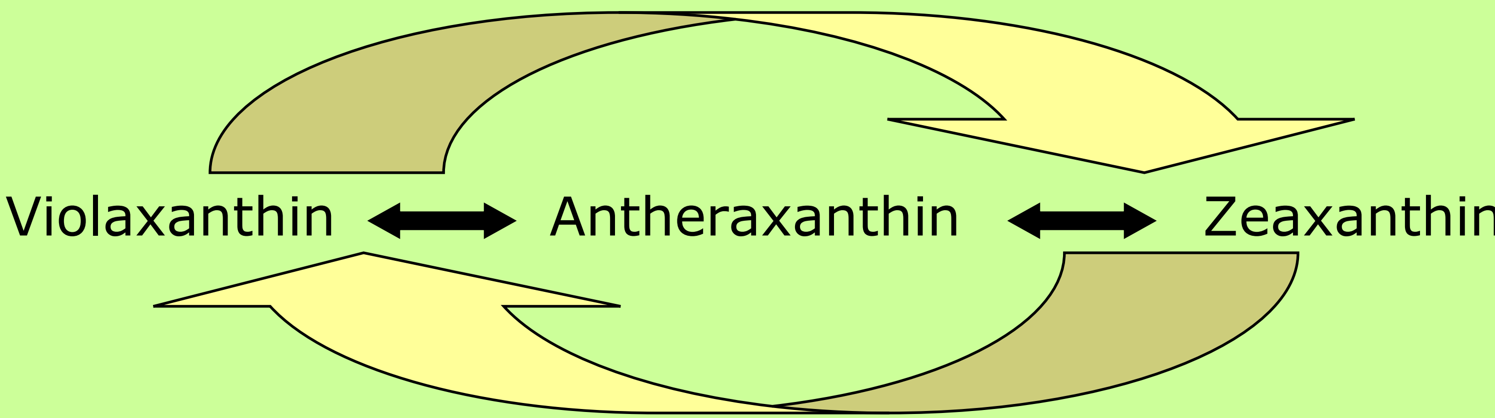
Mutants from Krishna K. Niyogi



The Xanthophyll cycle

Deepoxidase

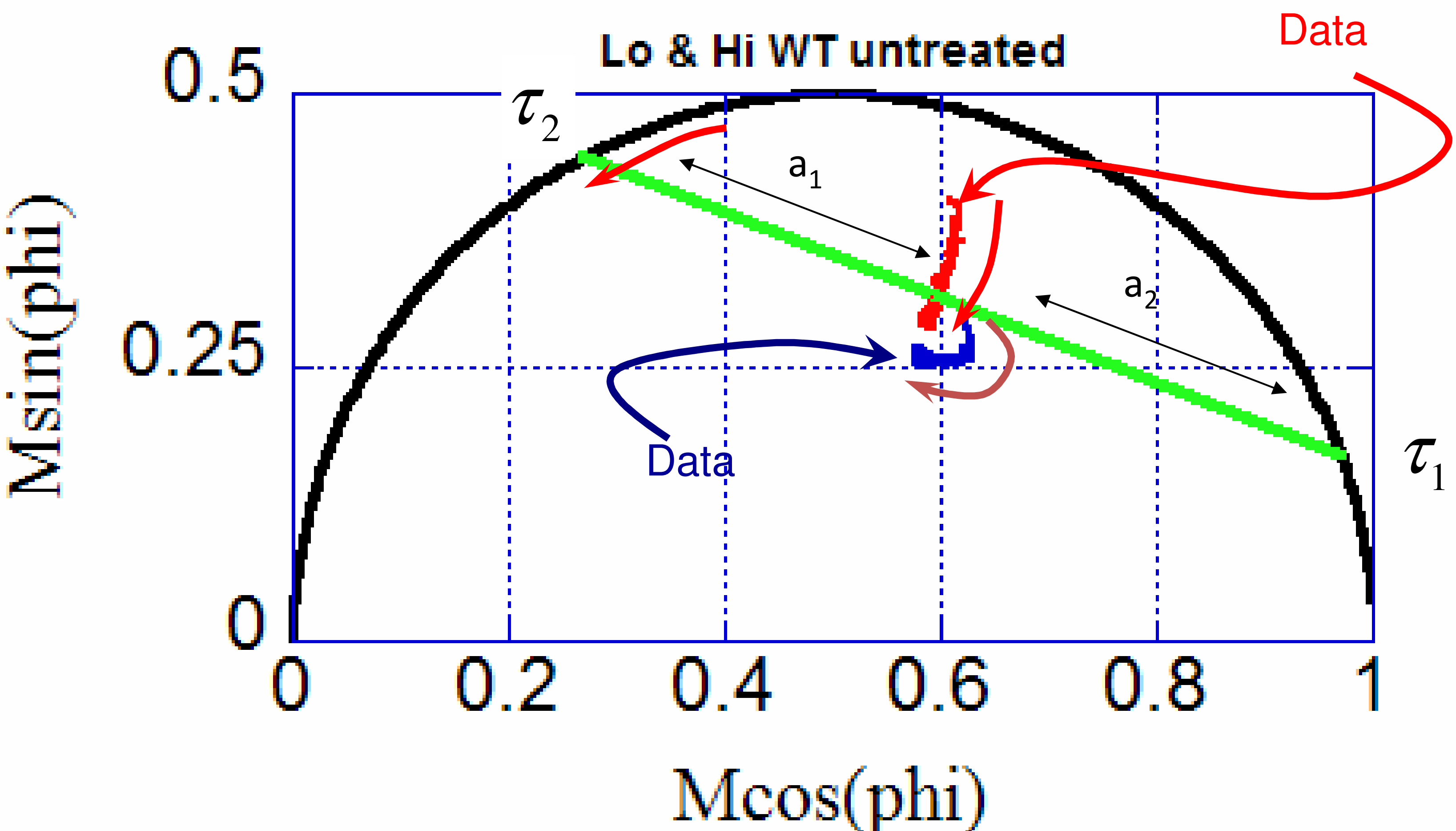
Mutant NPQ1: Deepoxidase



Epoxidase

Mutant NPQ2: Epoxidase

Lifetime lever – long time biochemical changes in the cells

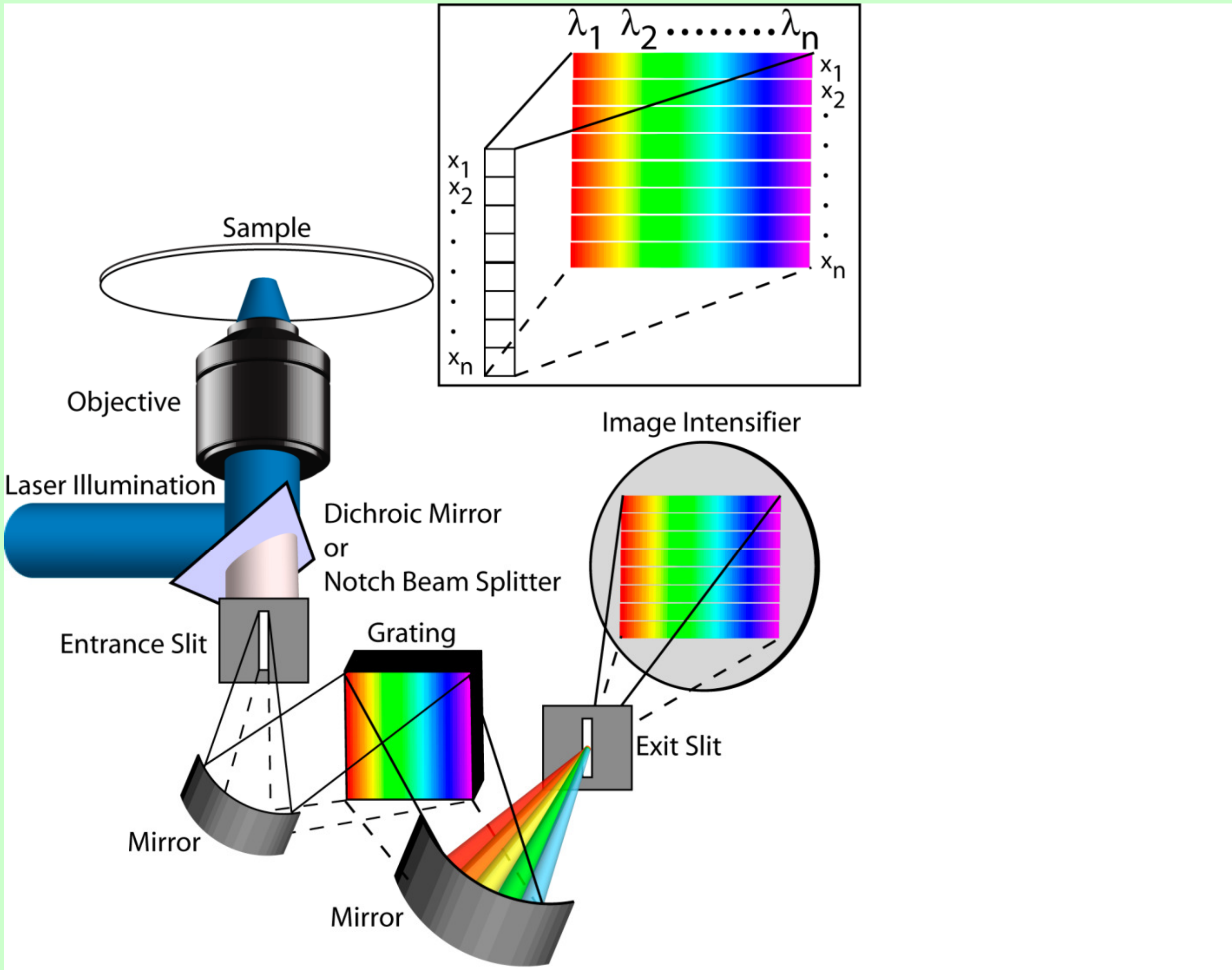


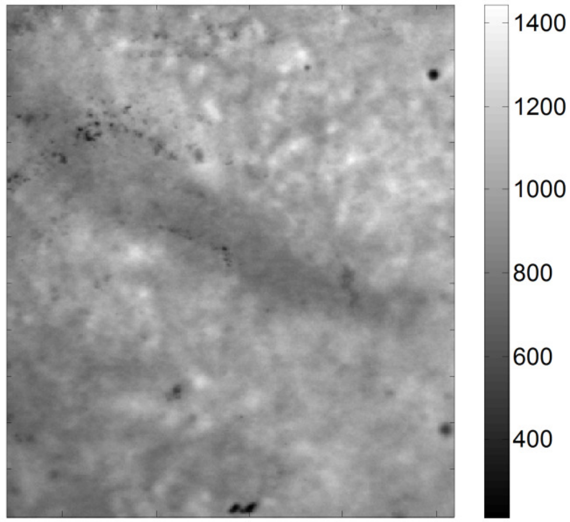
$$Signal \neq a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2}$$

Two lifetime pools of Chlorophyll Molecules

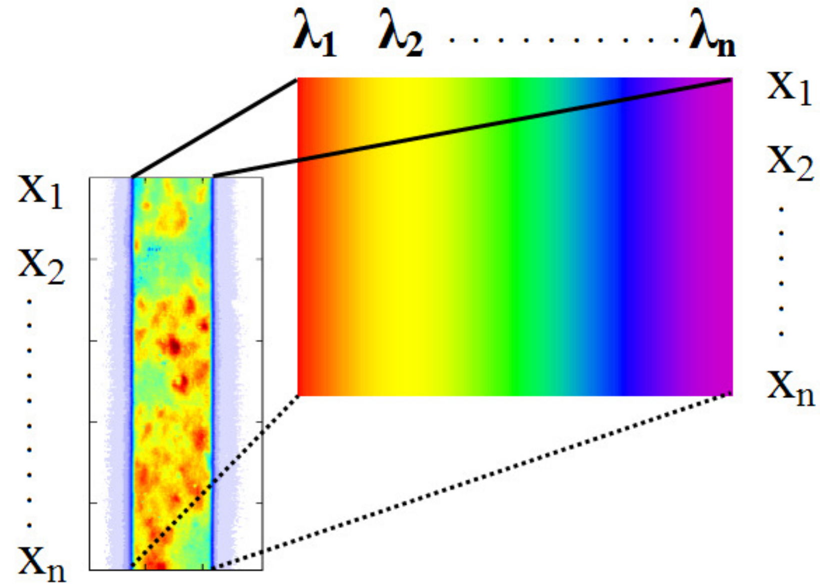
Holub, O., Seufferheld, M.J., Gohlke, C., Govindjee, Heiss, G.J., and Clegg, R.M., "Fluorescence lifetime imaging microscopy of Chlamydomonas reinhardtii: non-

Spectral FLIM

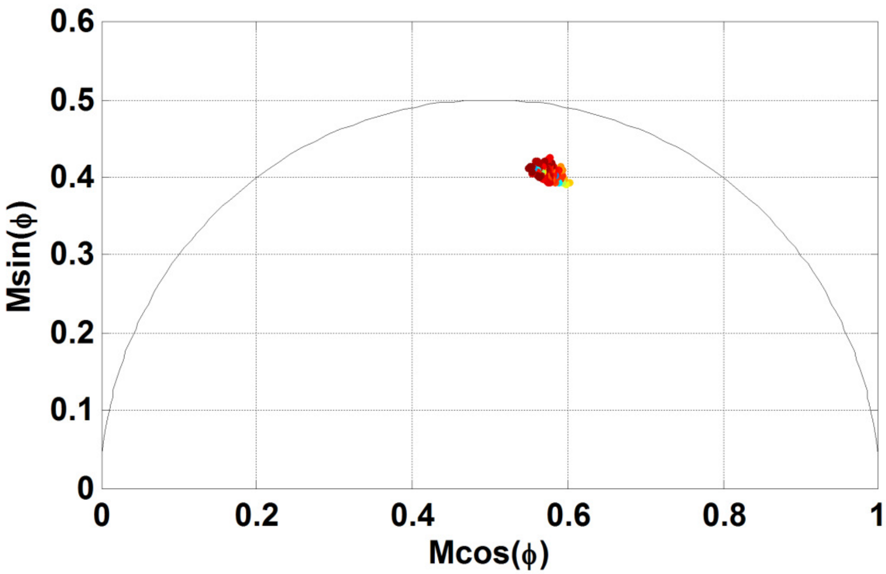


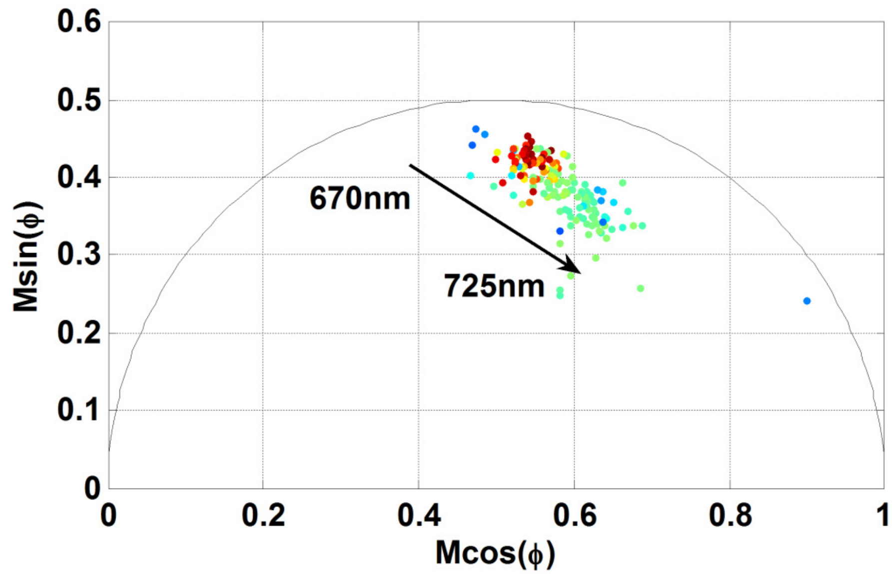


Avocado Leaves



Different wavelengths – different lifetimes





Combining morphology + lifetime resolution
Localized spatial frequencies

$$w(x,y,a)=\iint g\left(\frac{x-x'}{a},\frac{y-y'}{a}\right)f(x',y')\,dx'dy'$$

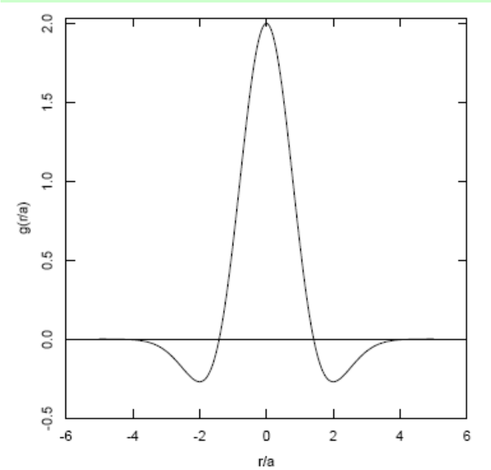


FIG. 1.—Mexican hat-generating wavelet $g(r/a)$

Wavelets

+

Denoising

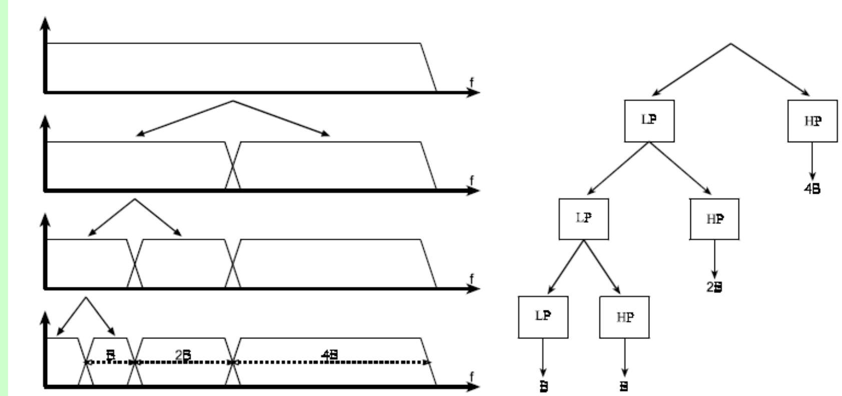
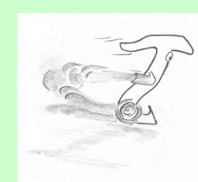
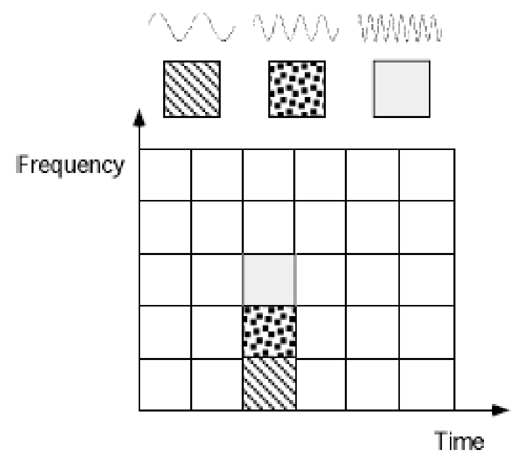


Figure 4
Splitting the signal spectrum with an iterated filter bank.

Chasing lifetimes in the morphology and noise



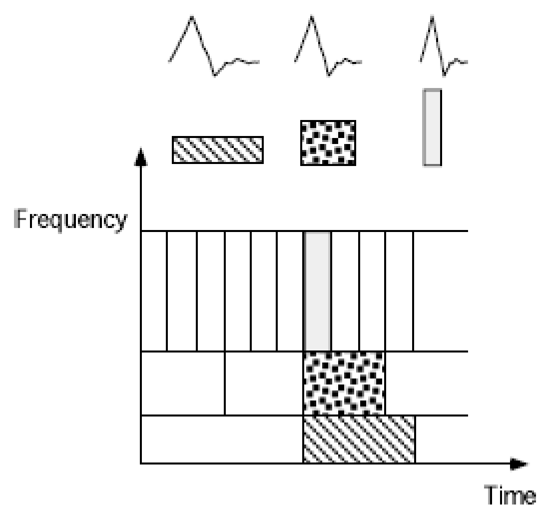
$$a_0 + \sum_{k=1}^{\infty} (a_k \cos kx + b_k \sin kx)$$

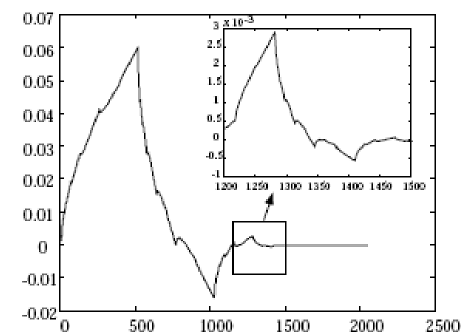


Fourier basis functions, time-frequency tiles, and coverage of the time-frequency plane.

$$\Phi_{(s,l)}(x) = 2^{-\frac{s}{2}} \Phi\left(2^{-s}x - l\right)$$

$$W(x) = \sum_{k=-1}^{N-2} (-1)^k c_{k+1} \Phi(2x + k)$$





Daubechies mother wavelet

Daubechies wavelet basis functions, time-frequency tiles, and coverage of the time-frequency

An Introduction to Wavelets

Amara Graps

WHAT DO SOME WAVELETS LOOK LIKE?

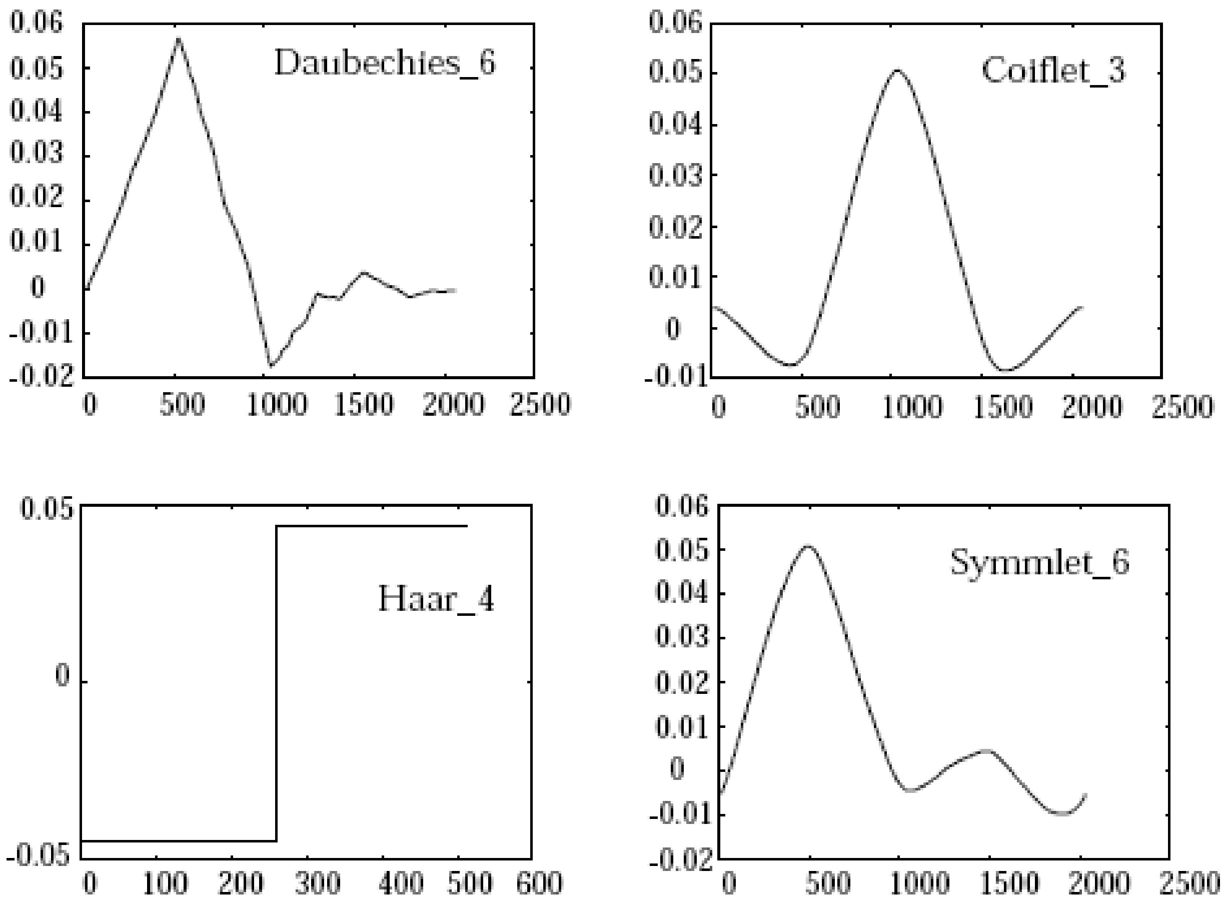
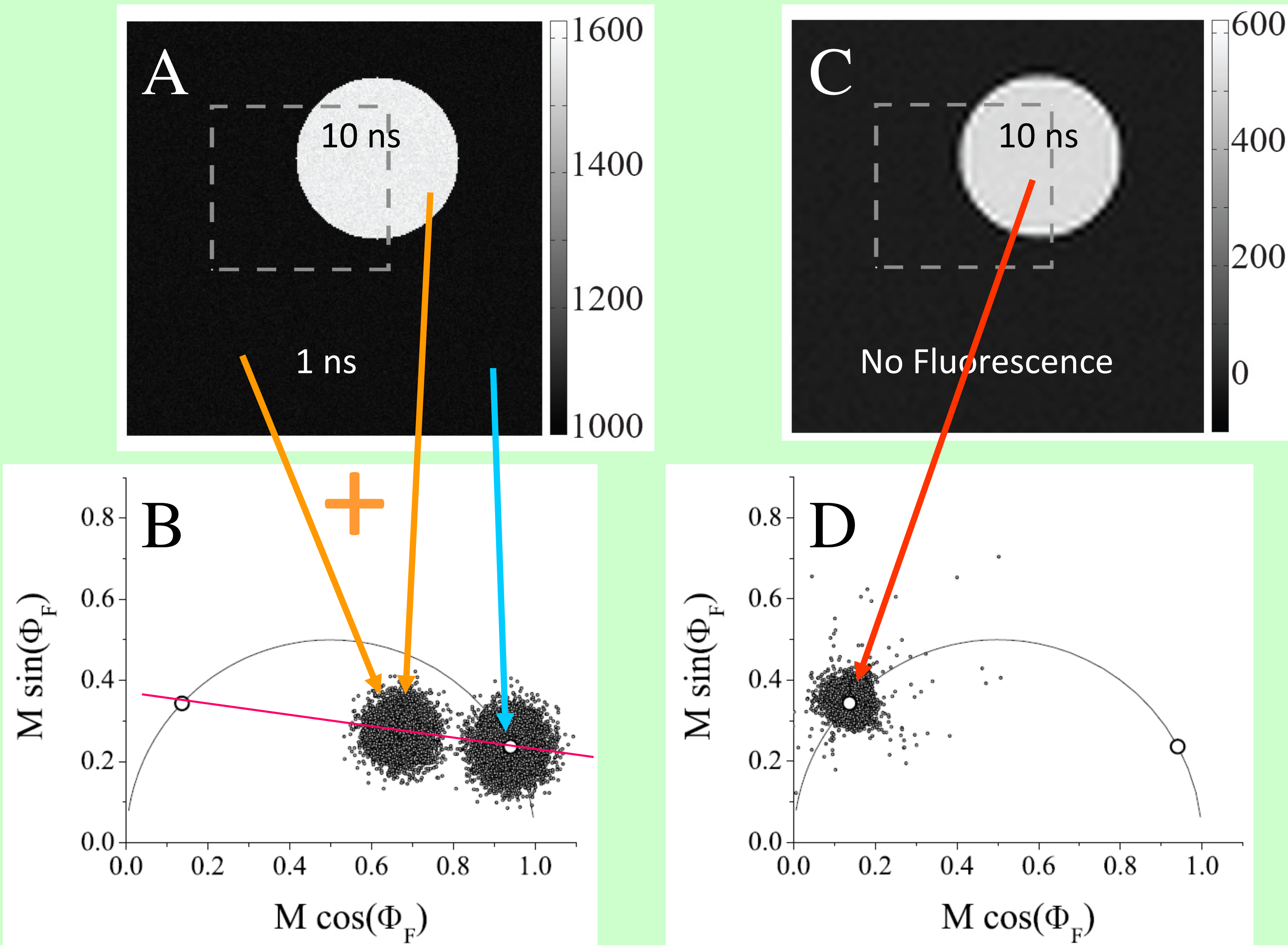
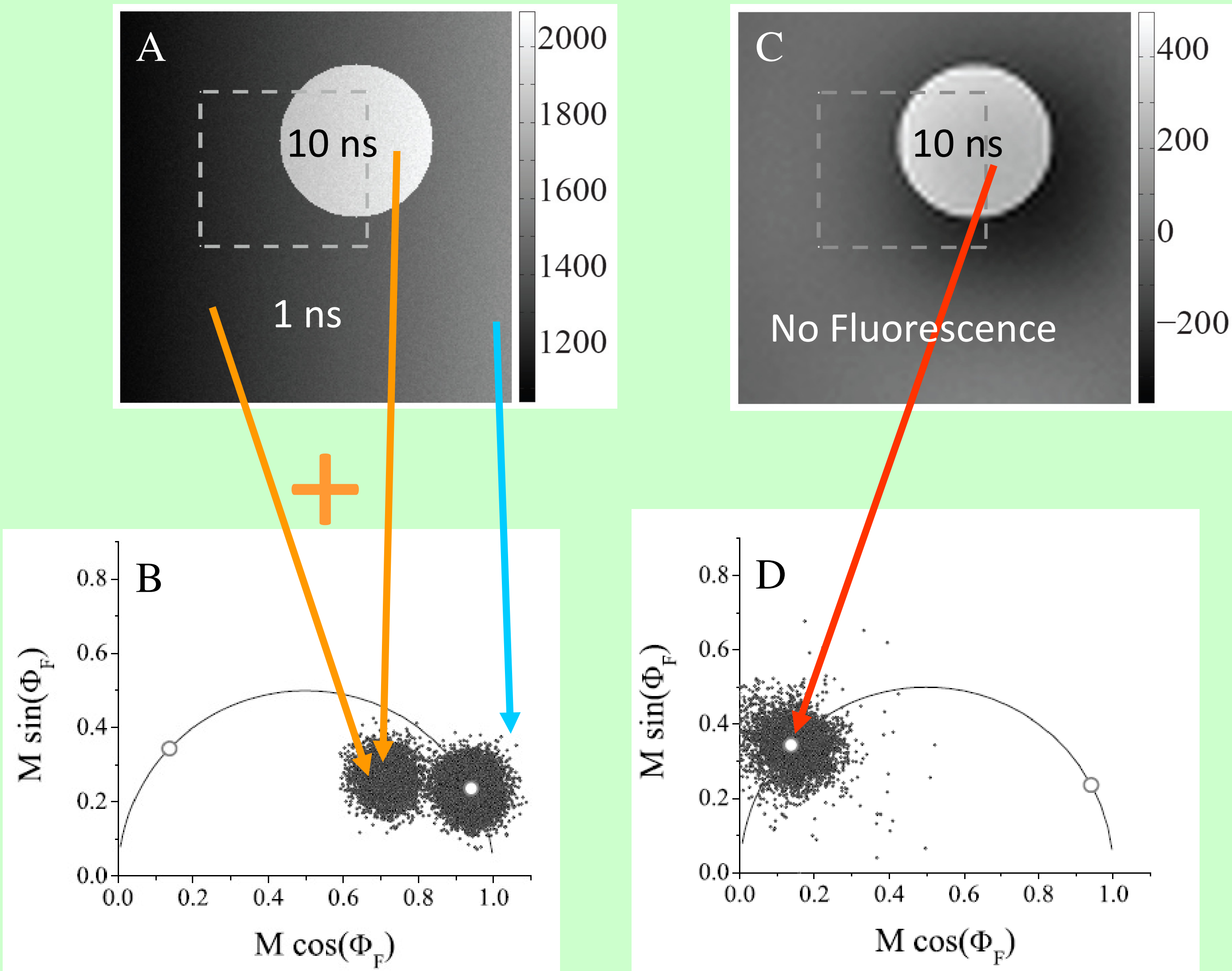


Fig. 4. Several different families of wavelets. The number next to the wavelet name represents the number of vanishing moments (A stringent mathematical definition related to the number of wavelet coefficients) for the subclass of wavelet. These figures were generated using WaveLab.

combination of wavelet analysis and FLIM with simulated data
Background constant
The wavelet analysis has completely removed the background contribution



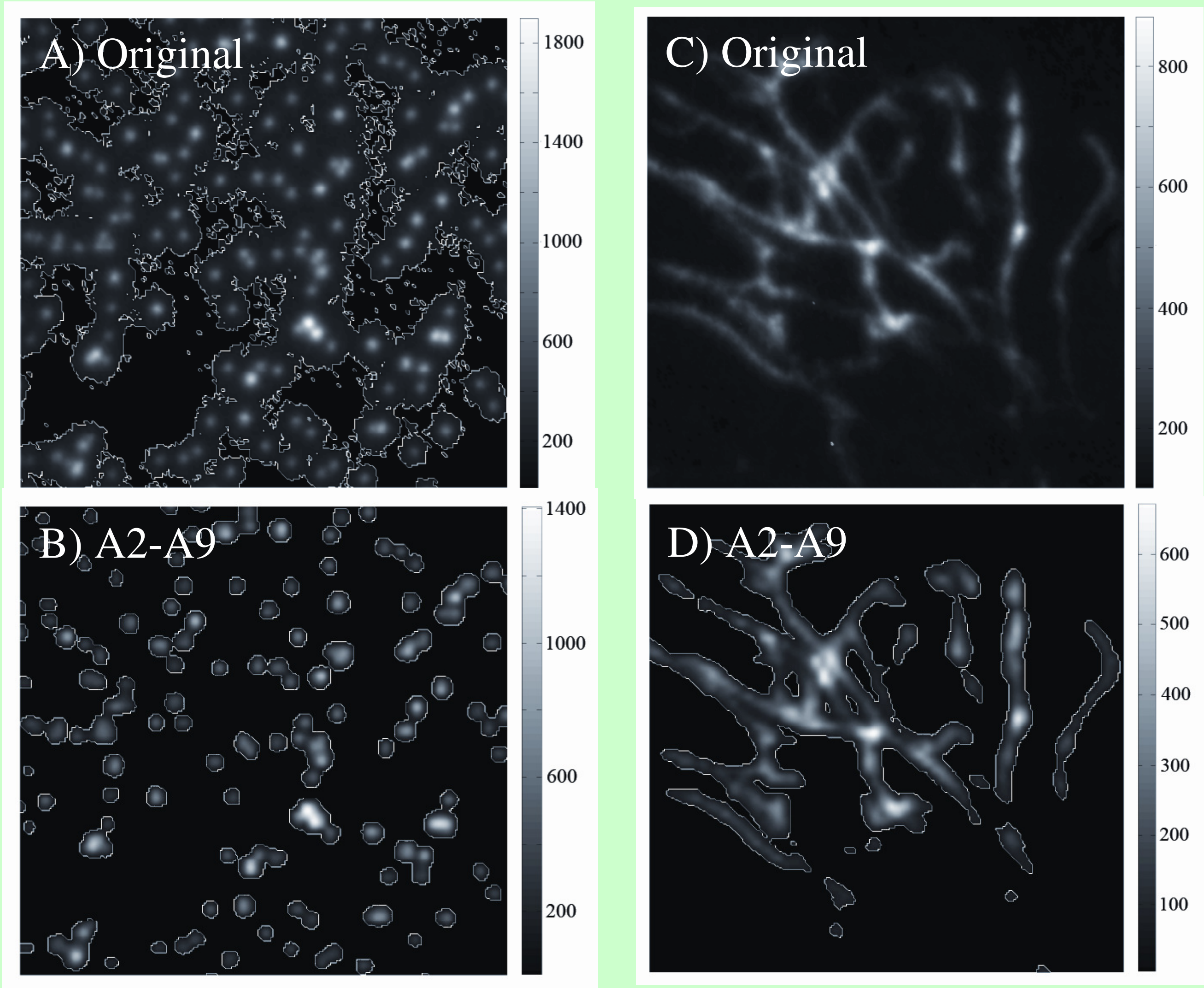
Combination of wavelet analysis and FLIM with simulated data
The background in this simulated data is increasing in amplitude with a constant gradient from left to right.



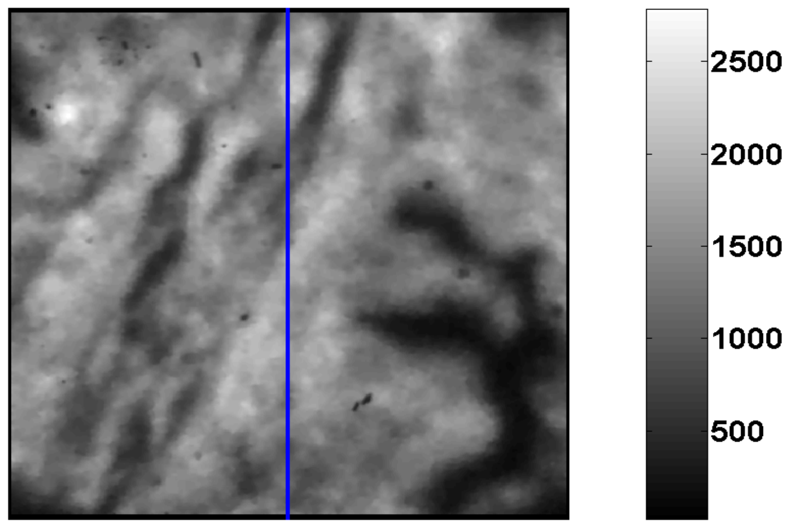
Finding morphology using wavelets

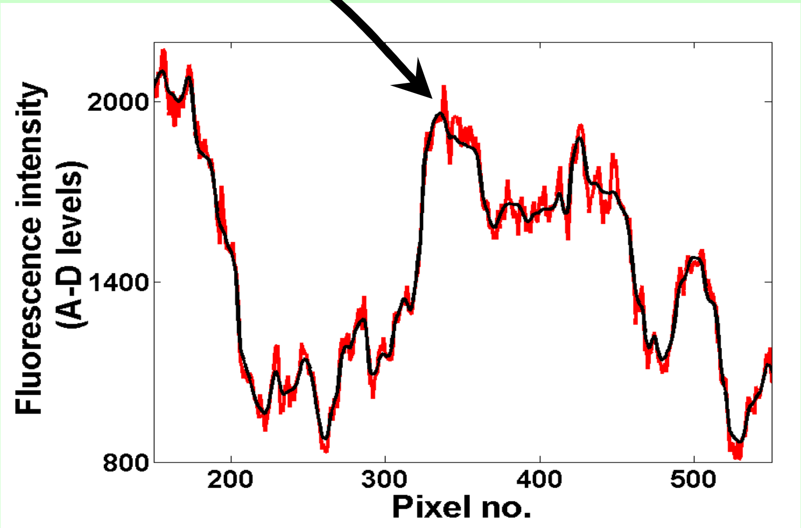
Background subtraction using wavelet on the **fluorescent beads** image (A and B) and on the **dendrites in a *Drosophila melanogastor* larva** expressing membrane-tagged GFP (C and D). The original images (A and C) and the edited image analyzed with wavelet (B and D) are compared.

final images are reconstructed by the 'wrcoef2' function from the difference in the approximation data level 2 (containing both high and low spatial frequency components) and level 9 (containing mostly low spatial frequency component)



Variance stabilized Gaussian Denoising





Red – raw data; Black – denoised

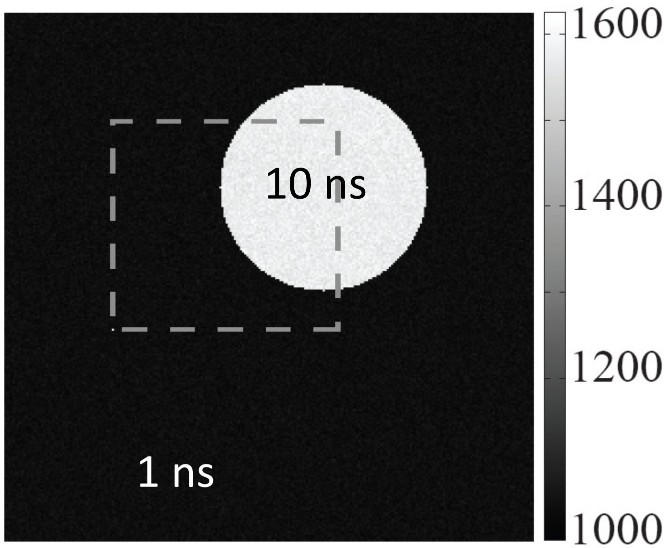
Line profile from an image of prostate tissue

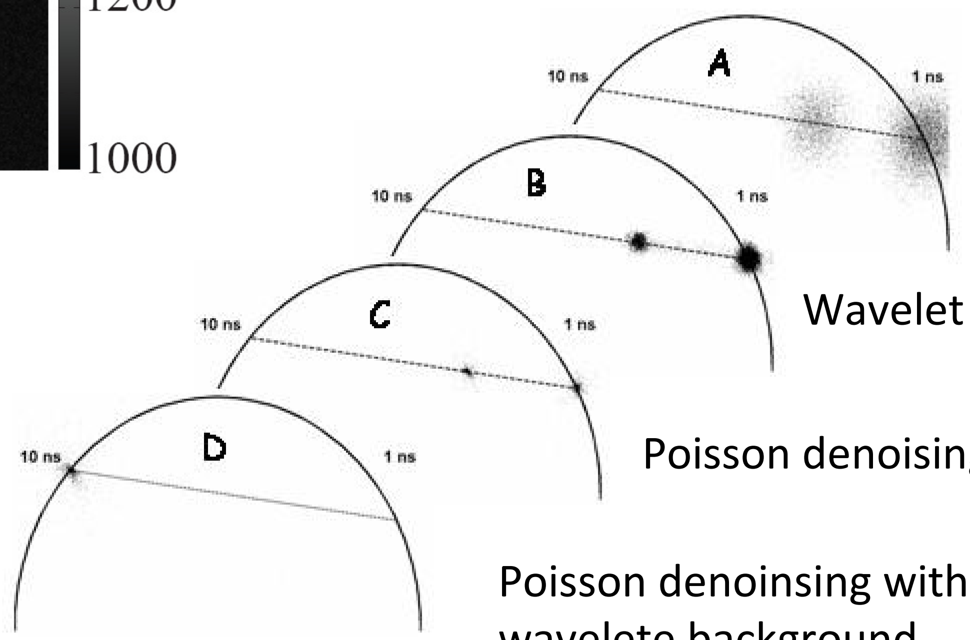
Spatial frequency cuts (intervals) can be selected

Edges not smoothed

Rebecca Willett and Robert Nowak

Polar Plots





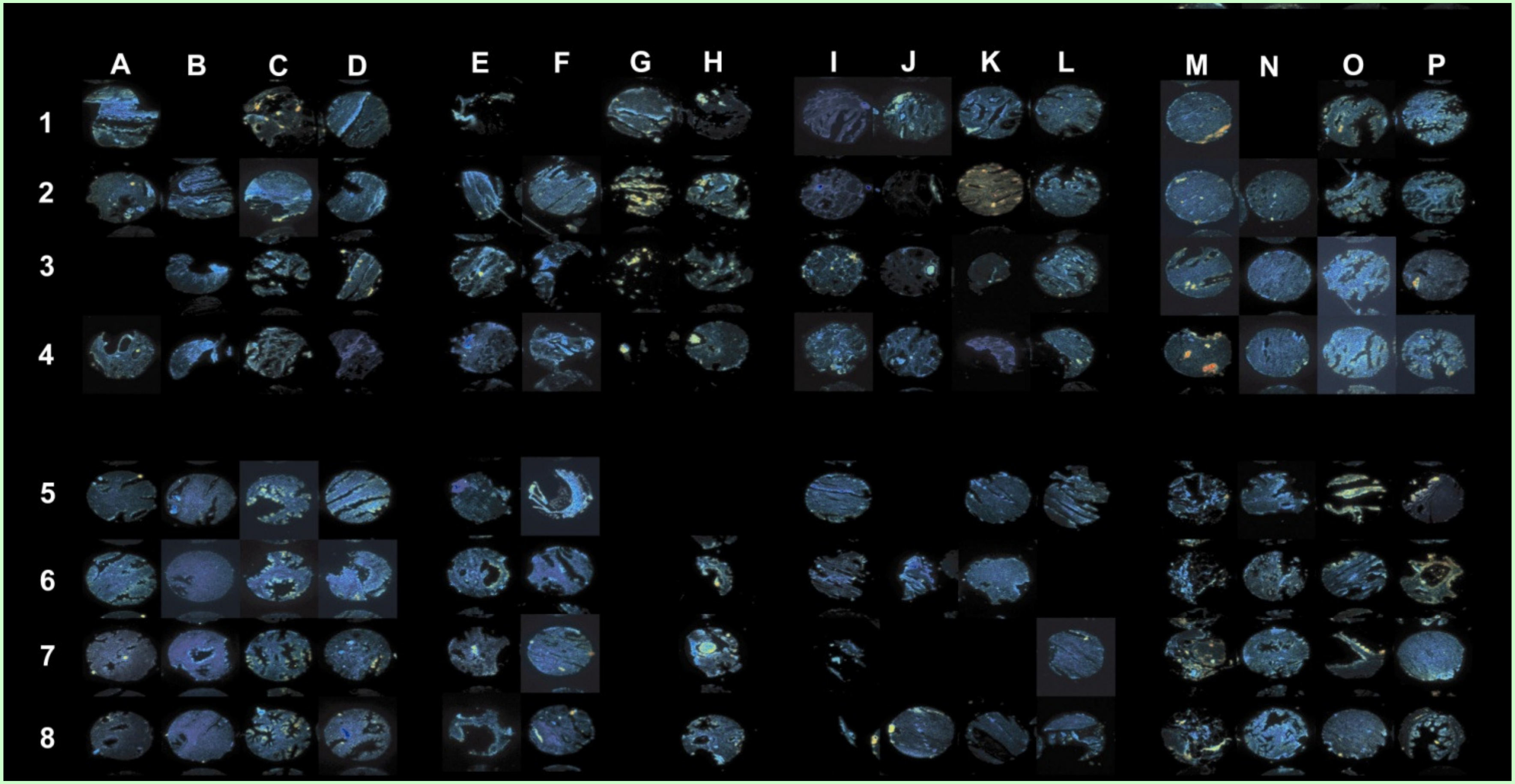
Raw data

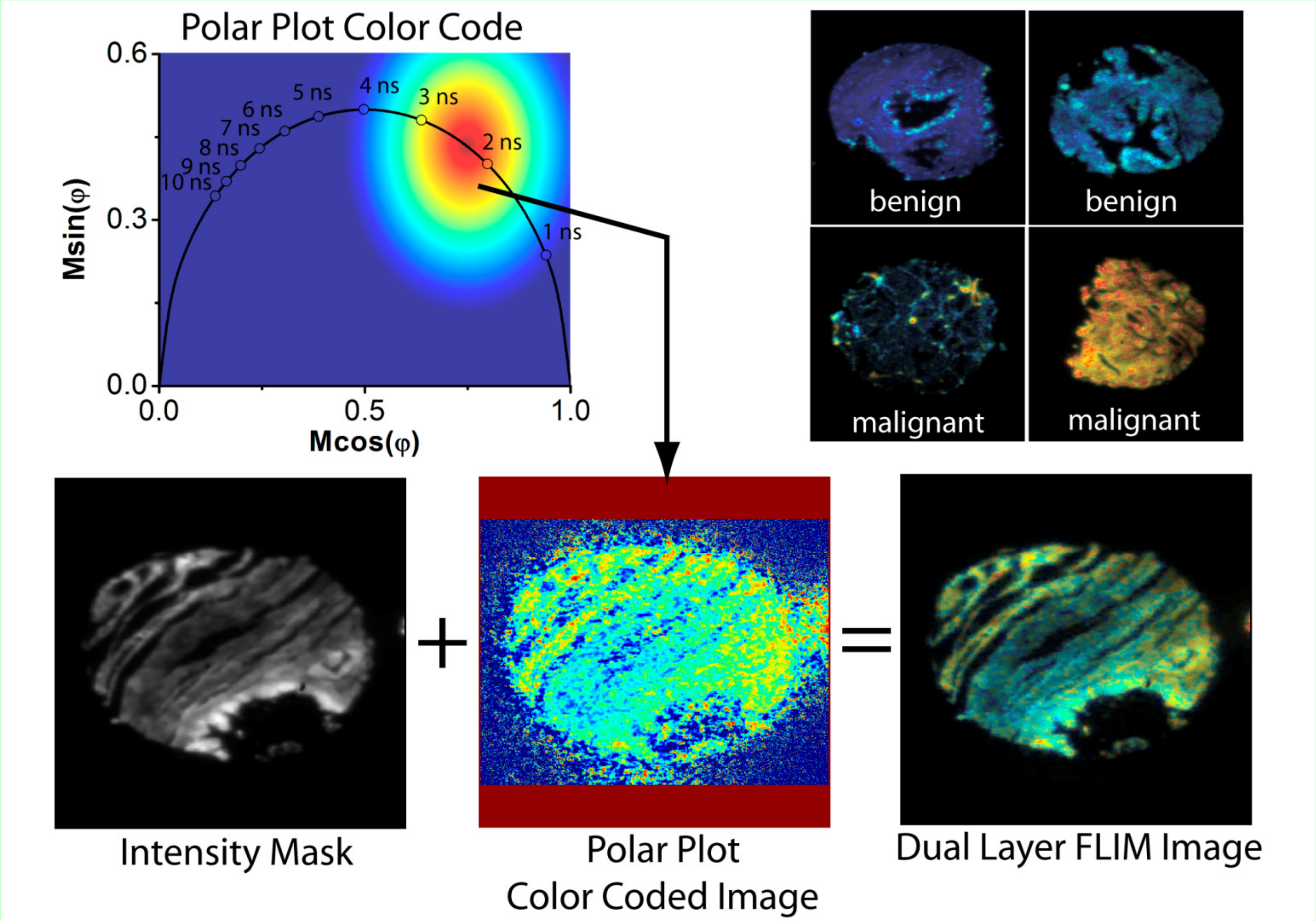
Wavelet smoothing

Poisson denoising

Poisson denoinsing with wavelete background subtraction

FLIM image of a prostate tissue biopsy microarray

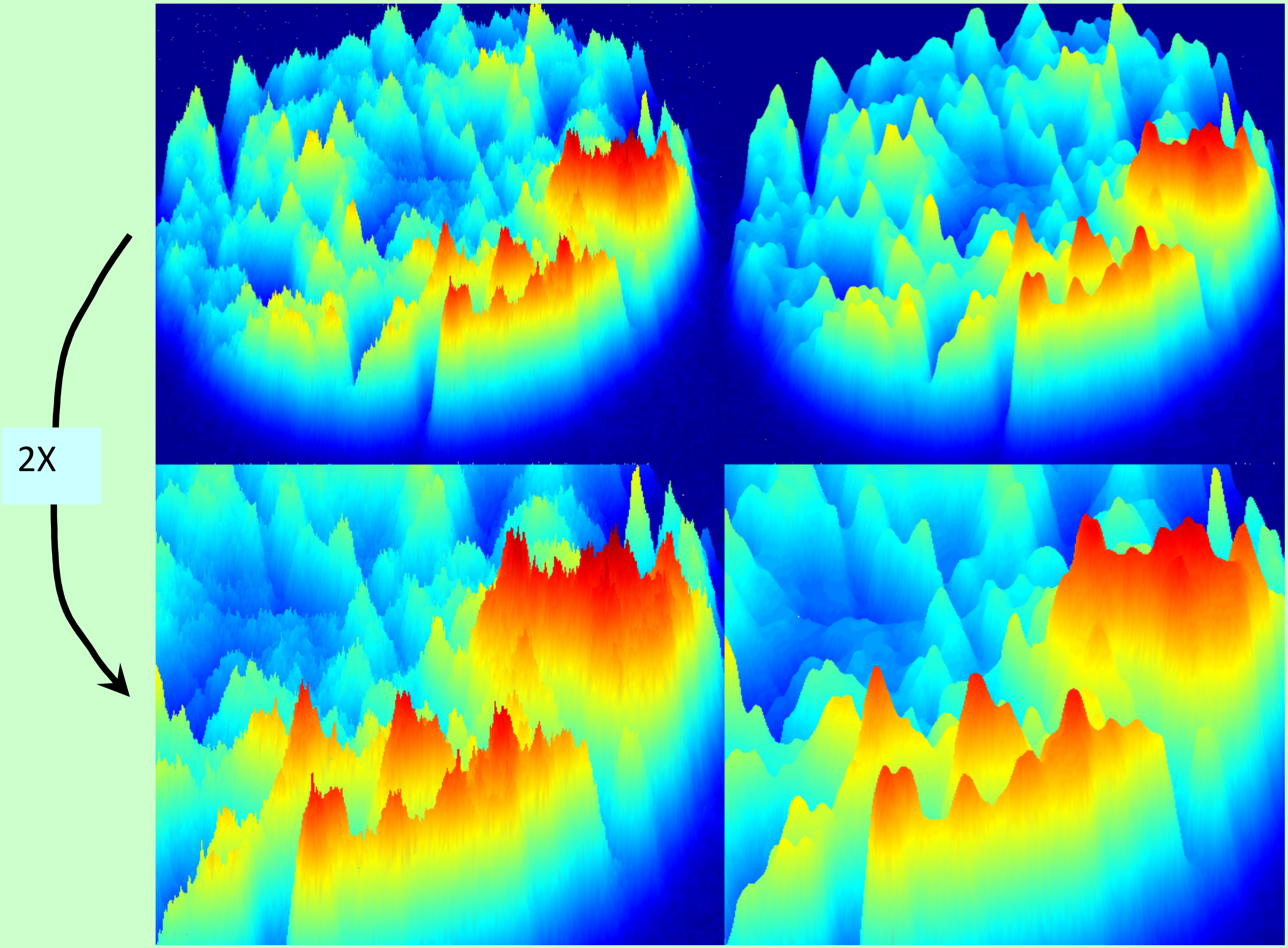


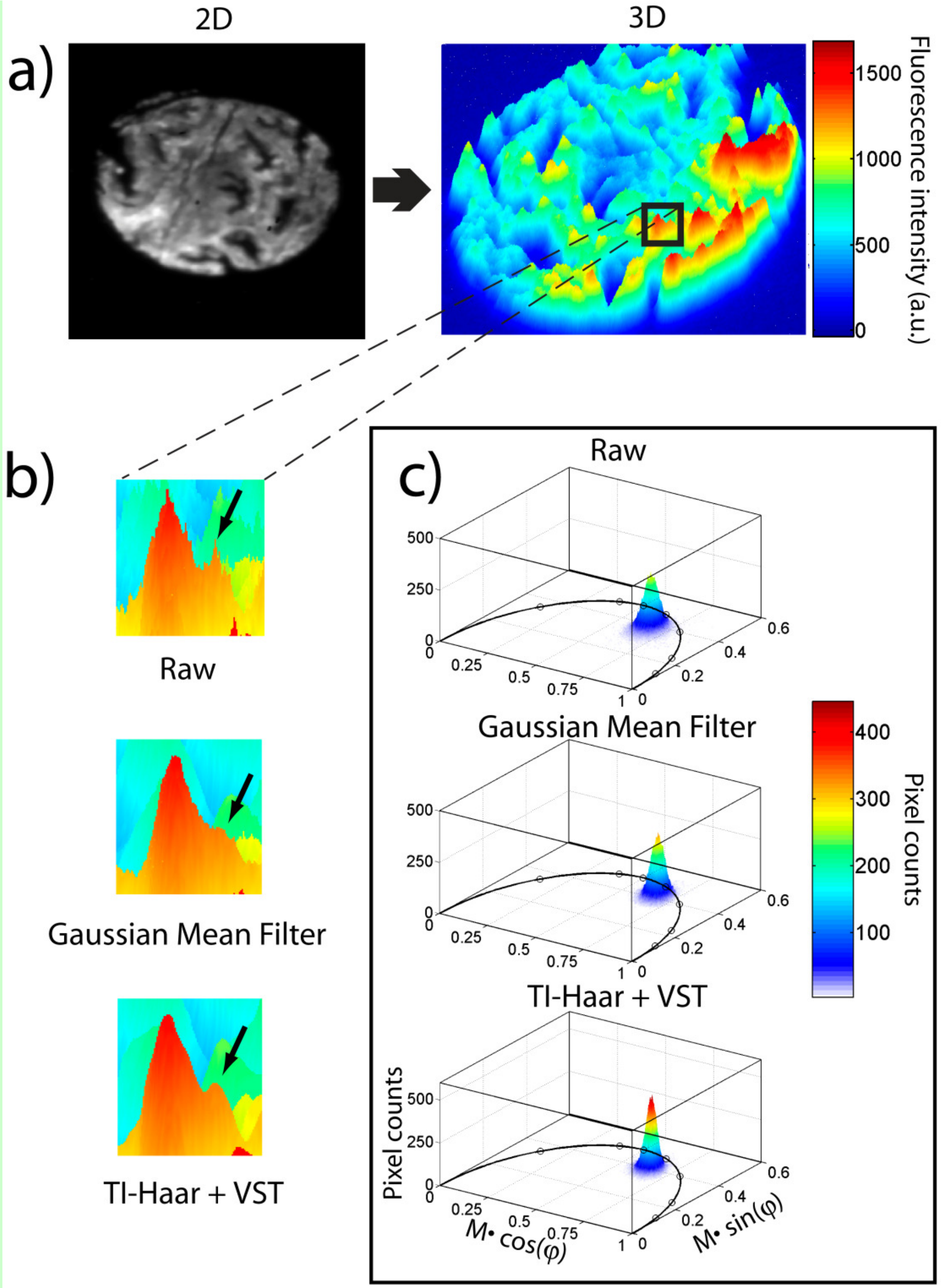


Combining morphological features and FLIM signals (wavelets)
and
Using denoising to assist in the overall analysis

Left: raw fluorescence intensity images of a prostate tissue core

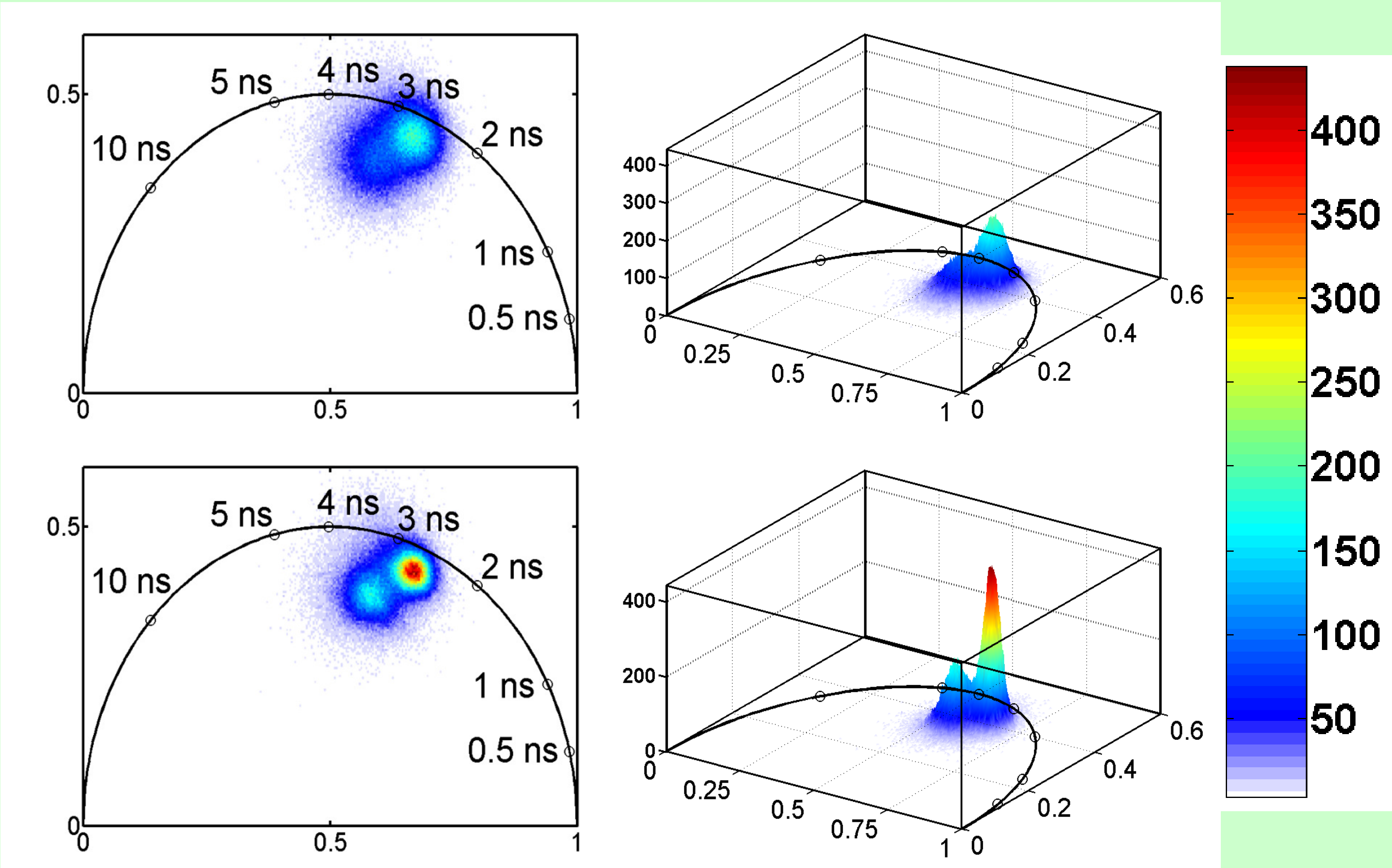
Right: denoised images

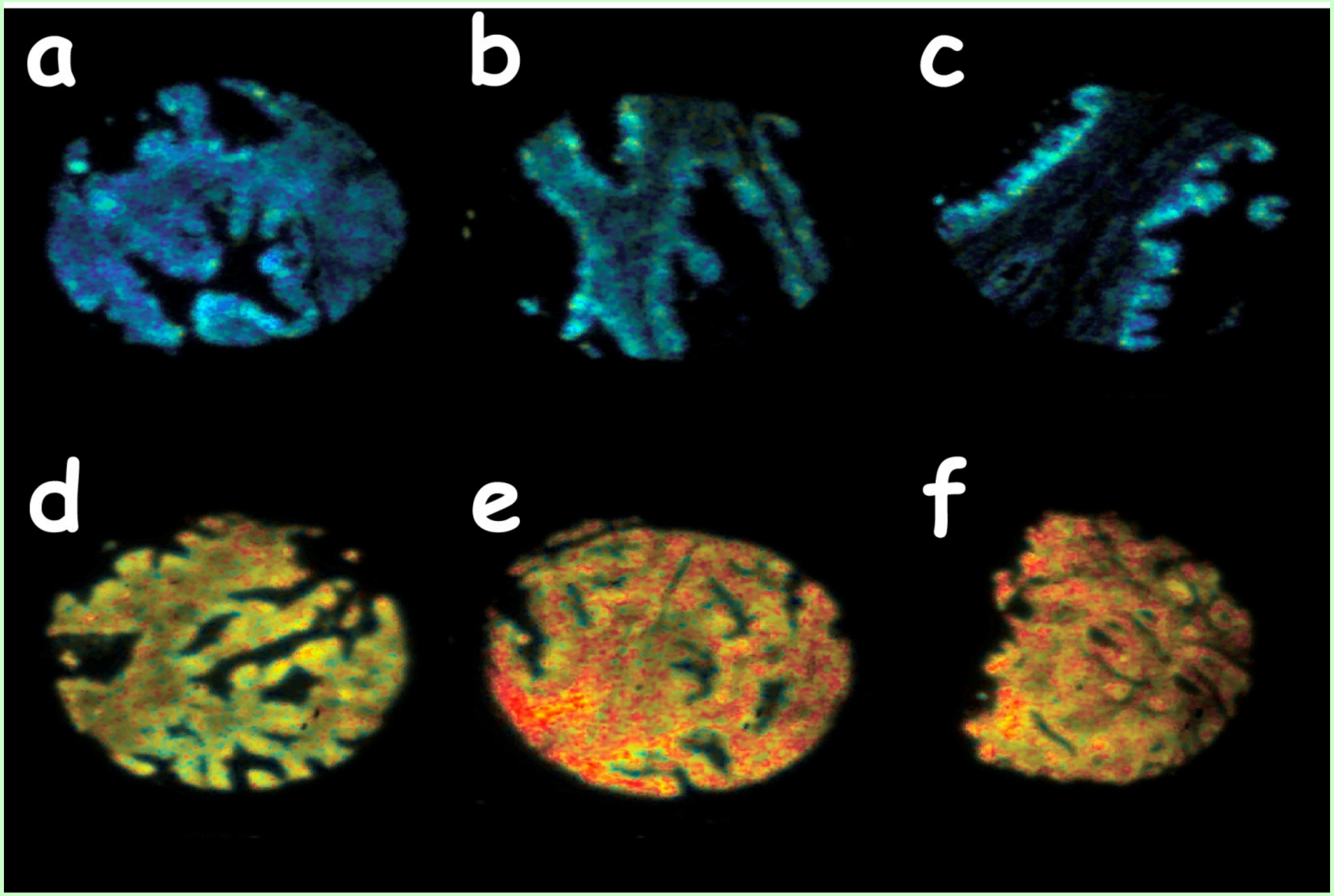



a)
2D
3D
Fluorescence intensity (a.u.)
1500
1000
500
0
b)
Raw
Gaussian Mean Filter
TI-Haar + VST
c)
Raw
Gaussian Mean Filter
TI-Haar + VST
500
250
0
0
0.25
0.5
0.75
1
0.2
0.4
0.6
Pixel counts
400
300
200
100
M• cos(φ)
M• sin(φ)

Polar plot histograms

of entire FLIM images of a benign and a malignant prostate tissue core
Top: **before** denoising
Bottom: **after** denoising





Dual layer FLIM images (intensity image used to mask color coded lifetime image) - Color indicates the fluorescence lifetime distribution of each pixel. Blue indicates normal fluorescence lifetimes while red indicates a significant shift in fluorescence lifetimes from benign tissue. **a-c)** Benign tissue cores. **d)** Low-grade cancer. **e & f)** High-grade cancer. Note that the lifetime distributions can be complex (the fluorescence lifetimes reflect multiple species – i.e., free and enzyme-bound species). The color coding represents an overall shift in the relative amounts of each species and therefore accomplishes representation of complex data in an easily visible fashion.

SO…..

With FLIM
We need all the
help we can get.
Morphology helps!

Full Field FLI

Peter Schneider

Oliver Holub

Christoph Gohlke

Glen Redford

(polar plot ALA–PPIX)

+

Spinning disk, wavelets and denoising

Chittaton Buranachi, Bryan Spring, Rohit Bhargava

(dendritesALA–PPIX, prostate FLIM, redox sensor)

Yi-Chun Chen (Polar Plot, spectral FLIM, photosynthesis)

Photosynthesis:

Govindjee

Oliver Holub

Christoph Gohlke

Gregor Heiss

Shizue Matsubara

[redacted]