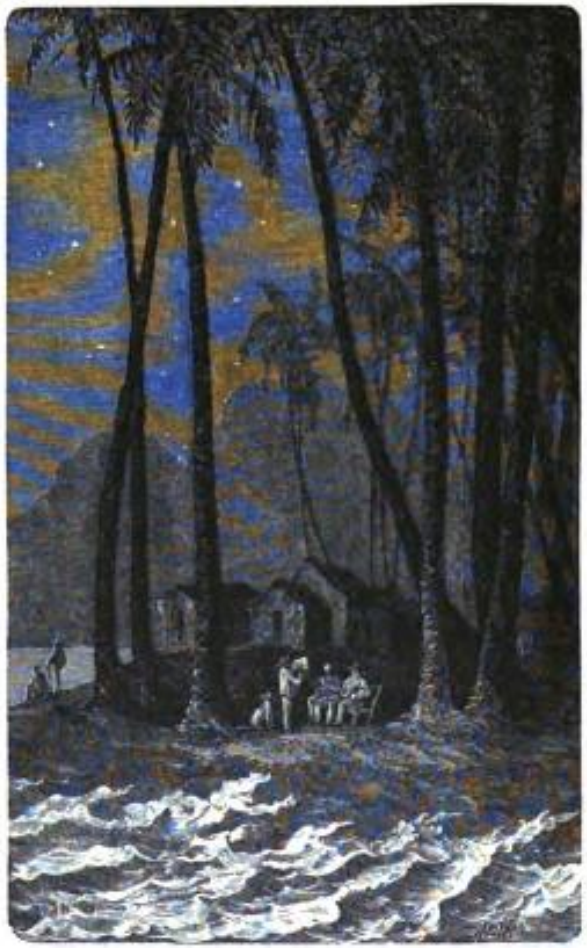
History of trials, blunders,
tribulations
and finally success
in the dark ages of
fluorescence lifetime measurements.
AND
What is a lifetime anyway?

From ancient times through
the age of alchemy and
beginnings of rational science
people were fascinated by just
the observation
of light emission.

First came bioluminescence

PLATE II.



M. DE TESSAN READING BY LIGHT OF PHOSPHORESCENT SEA.

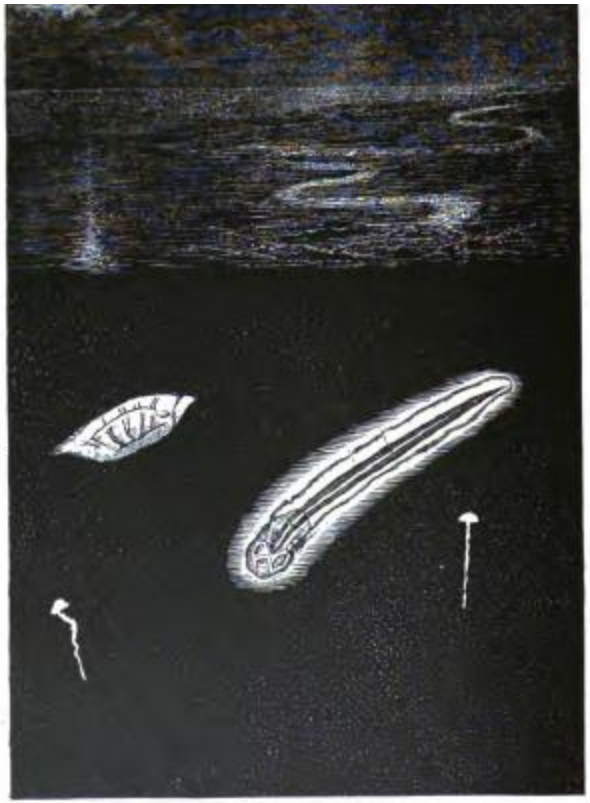
LIVING LIGHTS

A POPULAR ACCOUNT OF

PHOSPHORESCENT ANIMALS AND VEGETABLES

BY

CHARLES FREDERICK HOLDER



CHAIN OF SALPS

Salpa spinosa *Appendicularia*

PLATE XXV.

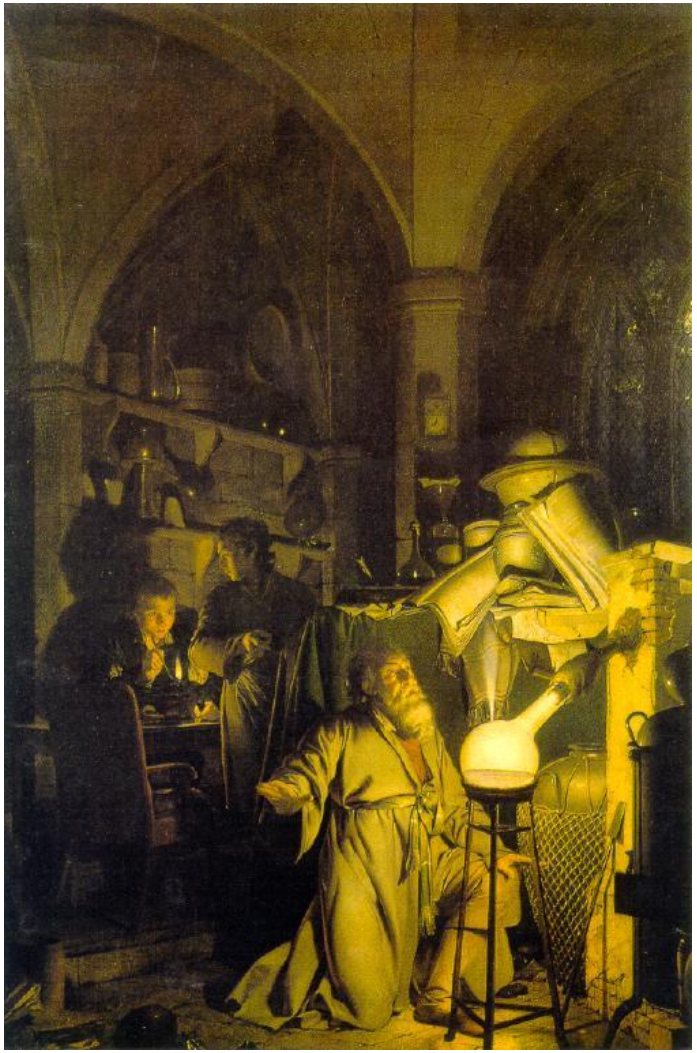
LUMINOUS WATERSPOUT.

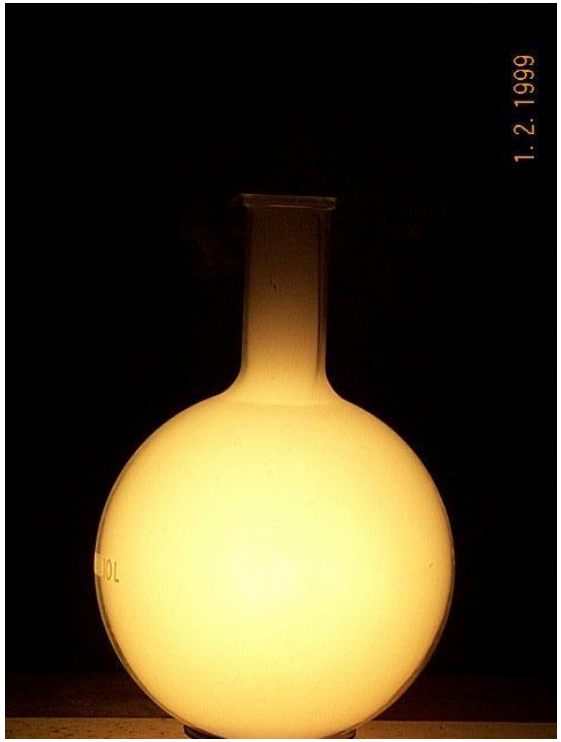
LONDON
SAMPSON LOW, MARSTON, SEARLE, AND RIVINGTON,
St. Dunstan's House
FETTER LANE, FLEET STREET, E.C.
1887

Dr. Brand in 1674-5 attempted to distil human urine and in this way discovered phosphorus.

Phosphorus (Greek "phosphoros" was the ancient name for the planet Venus) was discovered by German alchemist Hennig Brand in 1669 through a preparation from urine. Working in Hamburg, Brand attempted to distill salts by evaporating urine, and in the process produced a white material that glowed in the dark and burned brilliantly.

And there was Chemiluminescence





Misnomer: Phosphorescence of phosphorous is due to slow oxidation

Painting by Joseph Wright of Derby (18thcentury) representing the discovery of the phosphorescence of the phosphorus extracted from urine by Hennig Brand in 1669

First example of luminescence excited by light

THE PHOSPHORESCENCE

OF NATURAL OBJECTS.

INTRODUCTION.

About the latter end of the sixteenth century there lived in a narrow, winding street of the old town of Bologna, a certain cobbler, Vincenzo Cascariolo,* who devoted much of his time to alchemy. Some say that he even quitted his trade, and applied himself exclusively to chemical labours, but I am inclined to doubt the fact.

It is impossible to ascertain therefore what prominent idea, or what kind of theory reigned in the cobbler's mind on the discovery of this stone, destined to become celebrated and to immortalize his name. However, no sooner had he collected a certain number of specimens, than he hastened back to his little workshop, and began immediately to experimentize upon the mineral.

T. L. PHIPSON, PH.D., F.C.S.

1870.

It appears most probable that Cascariolo looked upon the sulphate of baryta, or heavy-spar,—for such was the object of his curiosity,—as a metallic ore, and supposed that by heating it with charcoal in a hot fire, he would be able to extract a metal —perhaps gold! His hopes in this respect were not realized, but he nevertheless succeeded in obtaining one of the most curious of substances,—a body which, to use the words of an old physicist, "absorbs the rays of the sun by day, to emit them by night."

The stone discovered by Cascariolo is now known as Barytine, or Heavy-spar (sulphate of baryta). By heating it with charcoal he had transformed it into sulphuret of barium, a substance which has the curious property of shining in the dark, after it has been exposed for some time to the rays of the sun.

Discovered in 1603

Such is the history of the discovery of the substance first known to be phosphorescent by insolation. For many years it has been sold in the streets of Bologna as a curiosity, under the name of Solar Phosphorus, or the Bologna Stone.

Fig. 1.

picked up in the secondary strata of the Monte Paterno, where he found it in lumps of considerable weight.* The German chemist, Marggraf, used to prepare solar phosphorus by powdering down the stone, and making it into thin cakes, with a mixture of flour and water, before submitting it to calcination. This "Bologna phosphorus" was the first substance known to become phosphorescent after insolation, and, consequently, it has been

1870.

T. L. PHIPSON, PH.D., F.C.S.

submitted to many and varied experiments. It is best obtained by the calcination of pulverized sulphate of baryta, made into a firm paste with common gum. It should be preserved in a bottle which closes hermetically with a glass stopper.

It will be easily understood what is meant by the term *Phosphorescence*, when we remind our readers that phosphorus, which shines so curiously in the dark, and which enters into the composition of our common lucifer matches, is the most remarkable of all phosphorescent bodies. The word "phosphorus," which signifies a substance that bears or emits a light, has frequently been applied to various other substances besides the non-metallic element termed *phosphorus* in chemistry, on account of the property these substances possess likewise of shining in the dark.

First mention of lifetimes?

"The Bologna stone, when placed in the sun attracts the rays, and retains them so long as to give light a considerable time after it is removed into the dark. "
Goethe "The Sorrows of Werter"

First quantitative measurements of luminescence lifetimes

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

PHOSPHORESCENCE PAR LA LUMIÈRE.

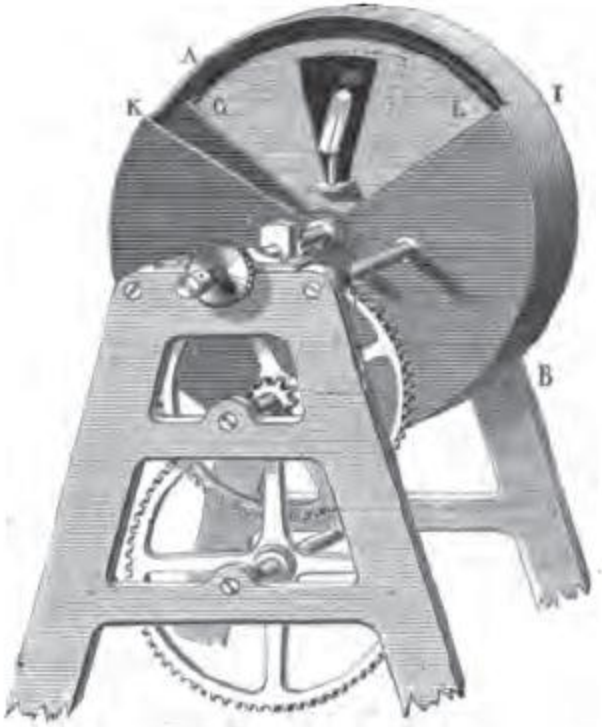


Fig. 31.

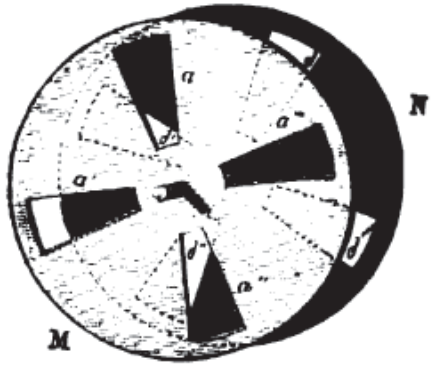
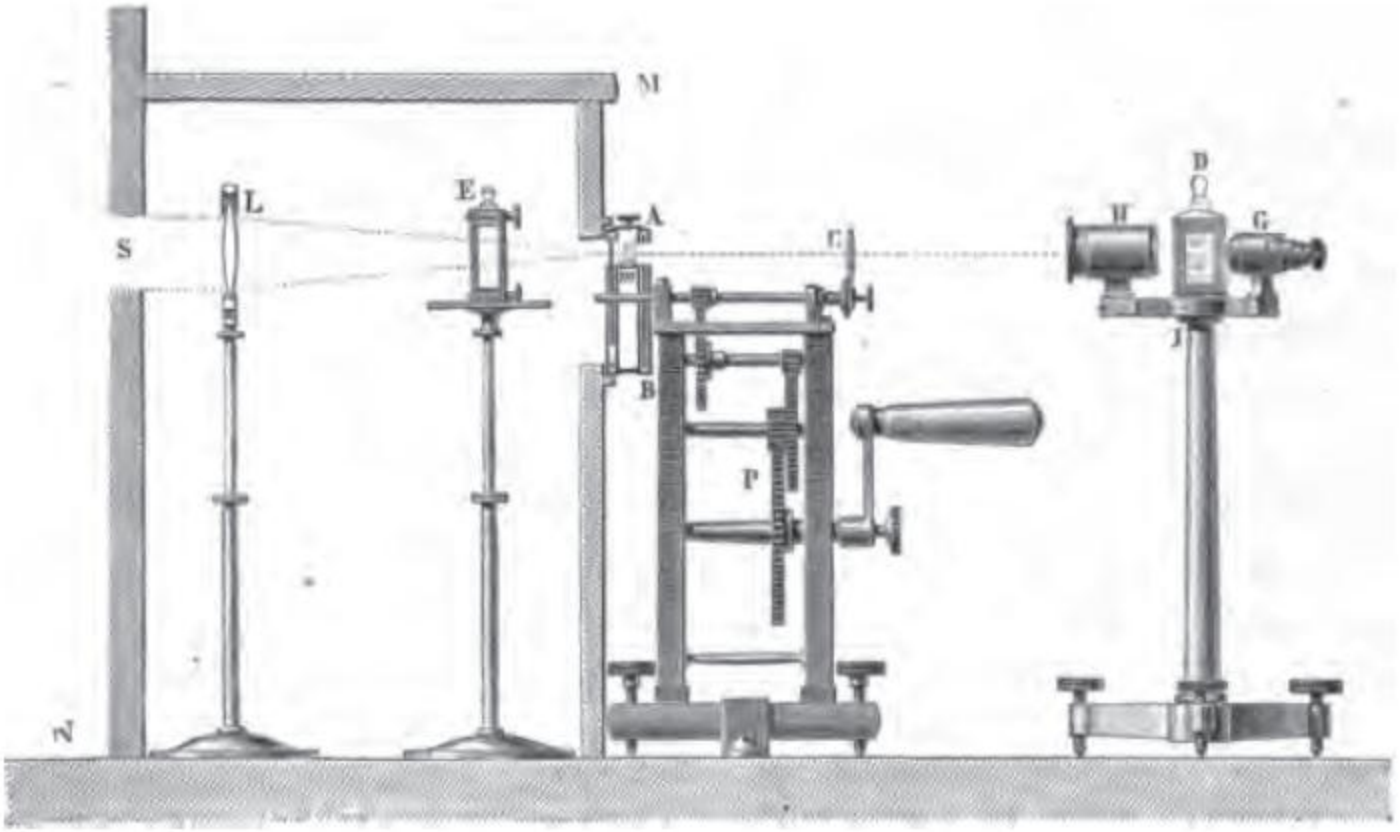
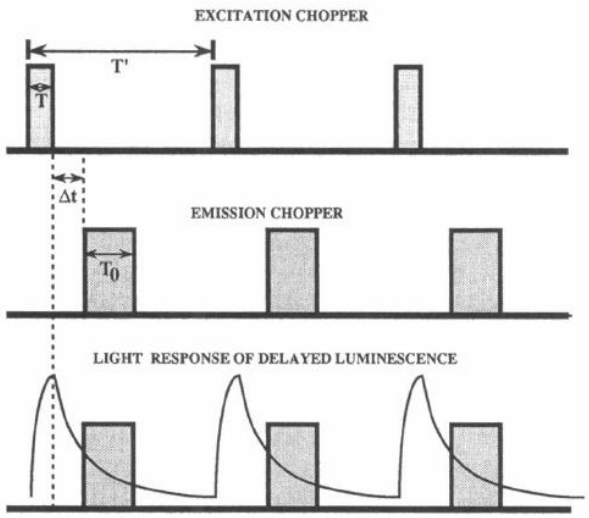


Fig. 29.

Fig. 30.



38.



Rotating mechanical chopper

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

(3) $\log i = \log i_0 - at$, $\log i' = \log i_0 - at'$, etc...

d'où

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

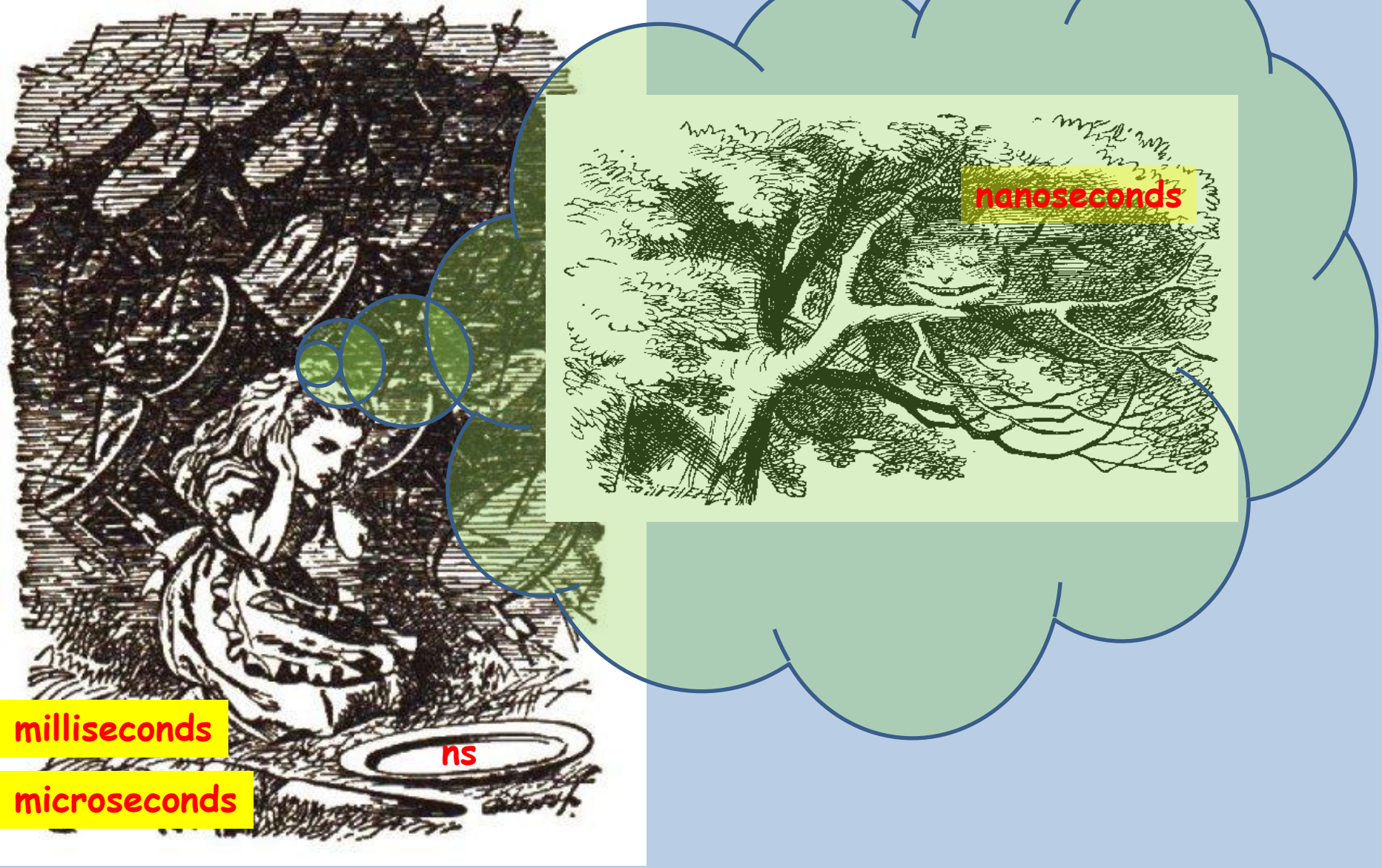
c'est-à-dire que les différences entre les logarithmes des intensités lumineuses doivent être proportionnelles aux différences des temps, et que leur rapport doit donner précisément le coefficient a.

$$\frac{di}{dt} = -ai, \qquad (2) \quad i_t = i_0 e^{-at},$$

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

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

	Valeur de a.	Valeur de t.	Valeur de $\frac{t}{a}$
Uranite naturelle (peu lumineuse).....	1,4975	»	»
Carbure d'hydrogène à teinte des sels d'urane	1,4363	0,062	0,043
Double sulfate d'urane et de potasse.....	1,3869	64,240	46,311
Double phosphate d'urane et de chaux.....	0,8206	138,750	169,083
Perchlorure d'uranium et de potassium.....	0,7682	16,950	22,064
Verre d'urane.....	0,5546	13,587	24,499
Azotate d'urane.....	0,4207	100.	237,700
Double fluorure d'uranium et de potassium..	0,3256	68,104	209,165
Verre (crown ordinaire).....	0,0436	0,184	4,220
Chaux phosphatée violette	0,0263	0,992	37,723

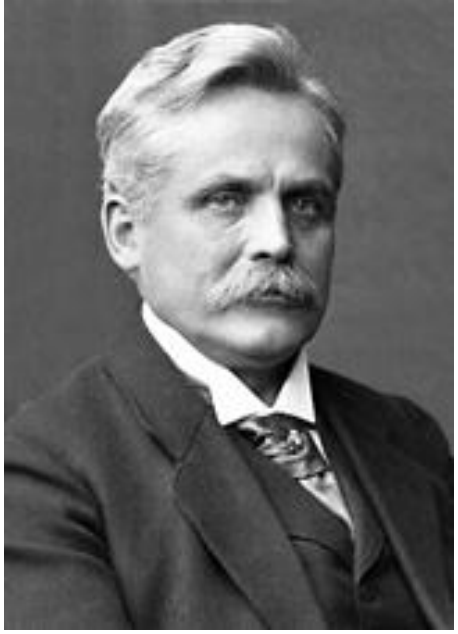


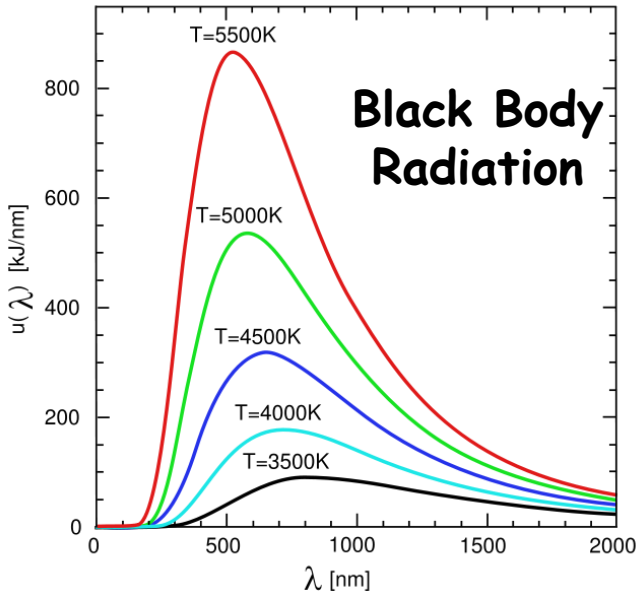
Trying to see Nanoseconds

Wien's displacement law

$$\lambda_{max} = \frac{b}{T}$$

$$f_{max} = \frac{\alpha}{h} kT \approx (5.879 \times 10^{10}\ \mathrm{Hz/K}) \cdot T$$





Wilhelm Carl Werner Otto Fritz Franz Wien

Wien made the first attempt to measure the nanosecond decay of luminescence

Wien received the 1911 Nobel Prize for his work on heat radiation.

While studying streams of ionized gas, Wien, in 1898, identified a positive particle equal in mass to the hydrogen atom. Wien, with this work, laid the foundation of mass spectroscopy. J. J. Thomson refined Wien's apparatus and conducted further experiments in 1913 then, after work by Ernest Rutherford in 1919, Wien's particle was accepted and named the proton.

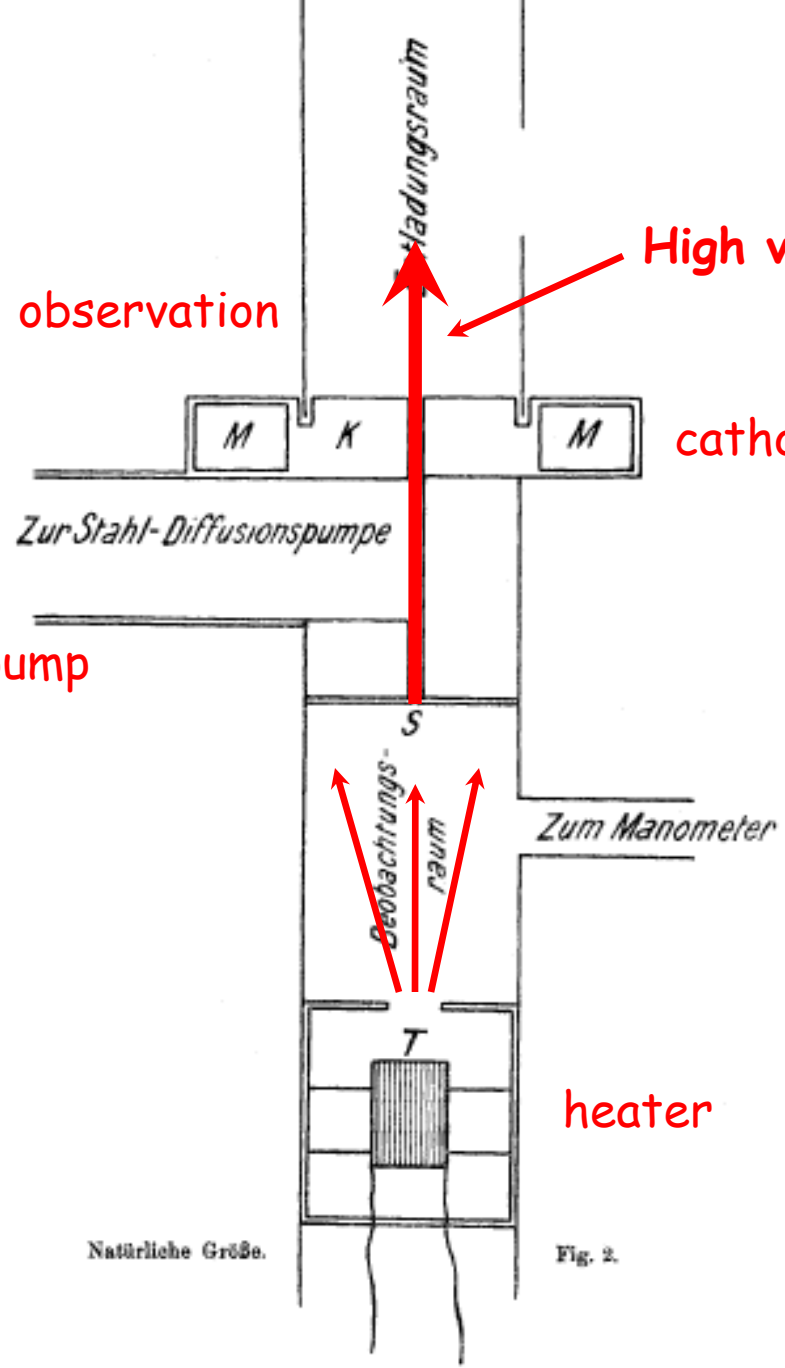


Fig. 2.

observation

High velocity atom stream

cathode

Vacuum pump

heater

Seemed to Decay exponentially

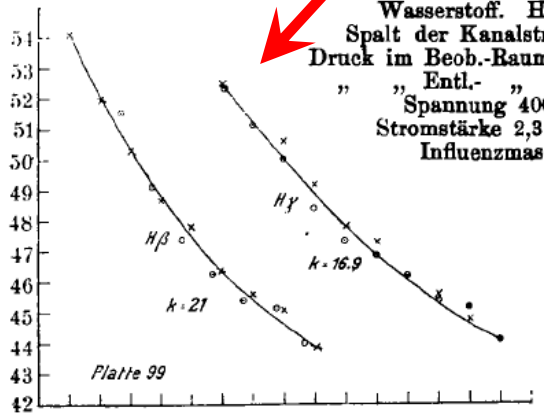


Fig. 10.

1919. № 23.

ANNALEN DER PHYSIK.

VIERTE FOLGE. BAND 60.

1. ***Über Messungen der Leuchtdauer der Atome und der Dämpfung der Spektrallinien. I;***
von W. Wien.

(Hierzu Tafel I.)

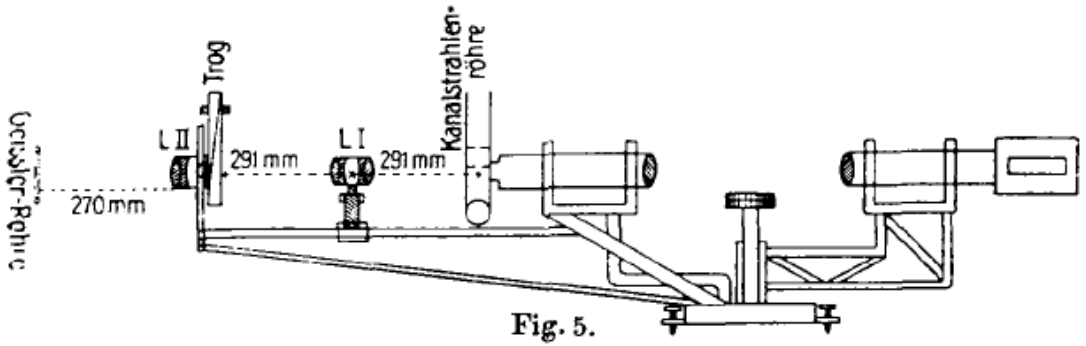


Fig. 5.

1919. № 23.

ANNALEN DER PHYSIK.
VIERTE FOLGE. BAND 60.

1. ***Über Messungen der Leuchtdauer der Atome und der Dämpfung der Spektrallinien. I;***
von W. Wien.

(Hierzu Tafel I.)

By knowing the velocity of the gas molecules he could calculate the lifetime

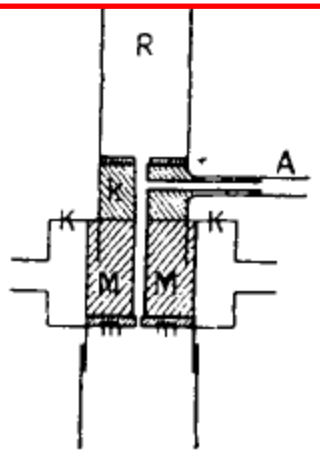


Fig. 1.

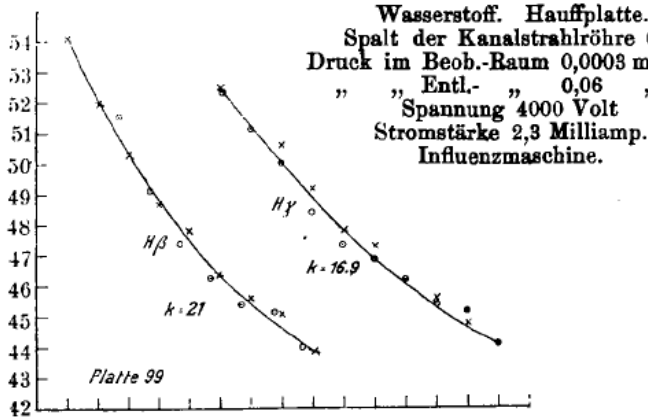


Fig. 10.

Nach Knudsen[1]) geht durch einen Kanal von der Länge l, der Breite a und der Tiefe L die Gasmasse

$$Q = \frac{4}{3}\sqrt{\frac{2}{\pi}}\sqrt{\frac{N m}{p_1}}\,\frac{a^2 b}{L}\,(p_1 - p_2)$$

in der Zeiteinheit, wo $p_1 - p_2$ die Druckdifferenz ist.

Ferner ist

$$p = \frac{N m v^2}{3}.$$

Hier ist v die mittlere Geschwindigkeit, aus der Summe der Quadrate berechnet. Dabei ist

$$v = \sqrt{\frac{3\pi}{8}}\,u,$$

also

$$p = N m \frac{\pi}{8} u^2.$$

Setzen wie nun $p_2 = 0$, $p_1 = p$, so ist

$$Q = \frac{4}{3}\sqrt{\frac{2}{\pi}}\sqrt{\frac{\pi}{8}}\,\frac{a^2 b}{L}\,N m u.$$

1919. № 23.

ANNALEN DER PHYSIK.

VIERTE FOLGE. BAND 60.

1. ***Über Messungen der Leuchtdauer der Atome und der Dämpfung der Spektrallinien. I;***

von W. Wien

(Hierzu Tafel I.)

$1/\tau$ *measured*

This is close to the theoretical relaxation time for the model of an oscillating electron to decay

aus Tabelle II

für	H_α	$2a = 6{,}68 \cdot 10^7\ \mathrm{sec}^{-1}$
,,	H_β	$2a = 6{,}06 \cdot 10^7\ \mathrm{sec}^{-1}$
,,	H_γ	$2a = 6{,}62 \cdot 10^7\ \mathrm{sec}^{-1}$.

Aus den Beobachtungen bei 2000 Volt ergibt sich

für	H_β	$2a = 6{,}38 \cdot 10^7\ \mathrm{sec}^{-1}$.

Wenn man mit dieser Zahl den Wert vergleicht, der sich aus der Elektronentheorie für ein schwingendes Elektron ergibt nach der Formel

$$2a = \frac{8\pi^2 e^2}{3mc\lambda^2}$$

(e Ladung, m Masse des Elektrons, λ Wellenlänge, c Lichtgeschwindigkeit), so ergibt sich für H_α ($\lambda = 656{,}3\ \mu\mu$)

$$2a = 5{,}35 \cdot 10^7\ \mathrm{sec}^{-1}, \quad = 1/\tau$$

Theoretical for a Oscillating electron

$1/\tau$ *theoretical*

Wie bereits erwähnt, ergibt die Theorie eines um eine Gleichgewichtslage schwingenden Elektrons für die Dämpfungskonstante den Wert

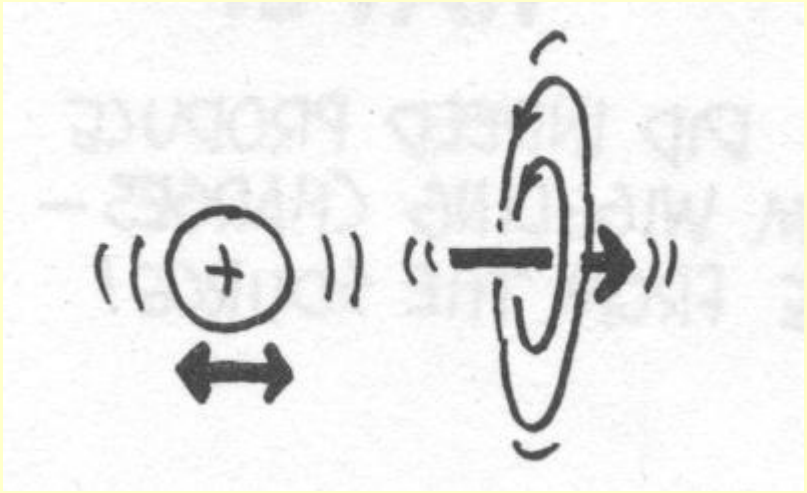
(I) $$2a = \frac{8\pi^2 e^2 \nu^2}{3mc^3},$$

wenn $\nu = \frac{c}{\lambda}$, e Ladung, m Masse des Elektrons bezeichnen.

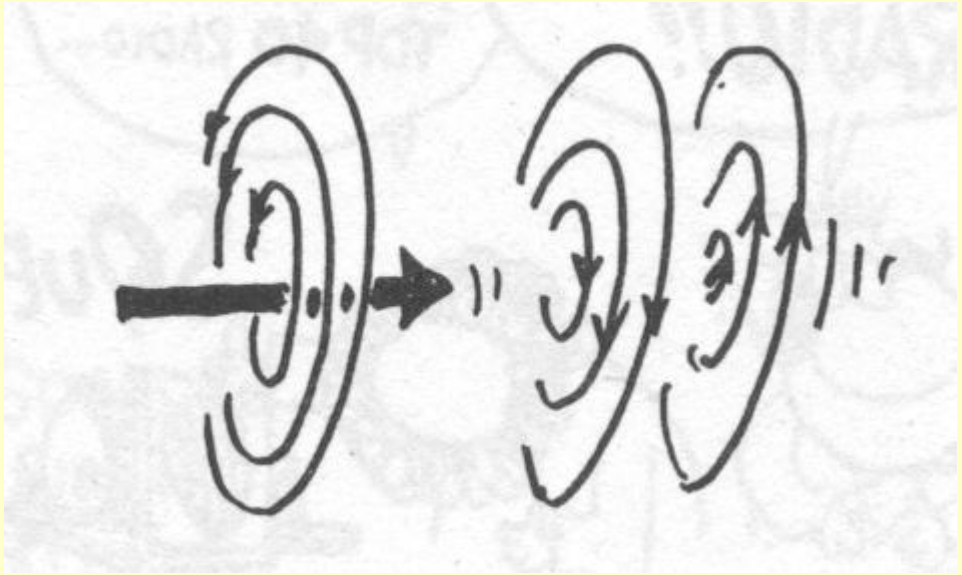
An accelerating (oscillating) charge emits radiation

Maxwell found this out; but never saw it proved

$$\nabla \times E = -\partial B/\partial t$$



$$\nabla \times B = \mu_0 \varepsilon_0 \, \partial E/\partial t$$



Free decay of oscillating real dipole
Emits radiative energy

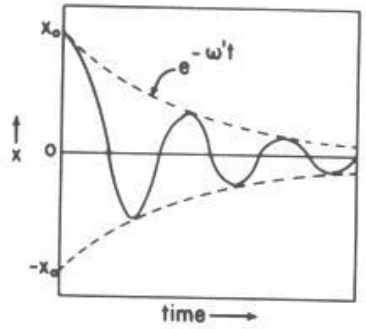


FIG. 15-4. Damped oscillations of an elastically bound electron. In the classical atom the damping would be much less rapid than is shown here.

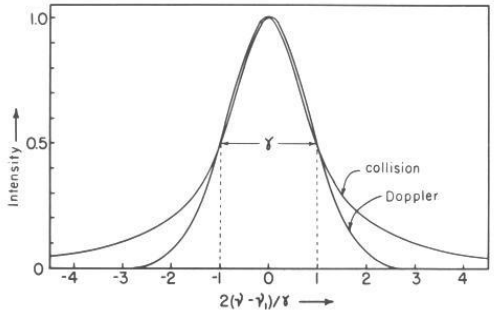


FIG. 15-7. Line shapes due to collision and radiation broadening and to Doppler broadening. Both curves have the same half-width, γ.

$$\Rightarrow \frac{\gamma/2\pi}{(\nu-\nu_1)^2+\left(\gamma/2\right)^2}$$

Spectral distribution
Natural linewidth

$$m_e\frac{d^2x}{dt^2}+(\mu)\frac{dx}{dt}+kx=0$$

Radiation damping constant

Emission from a damped electric harmonic oscillator (radiation damping)

$$\frac{1}{\tau}=\frac{\mu}{m}=\frac{2}{3}\frac{e^2\omega^2}{mc^3}$$

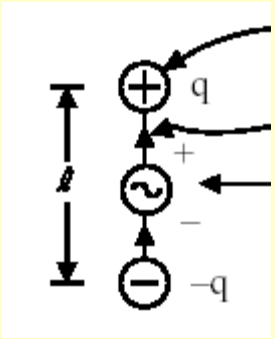
Actually gives the right fluorescence lifetime and spectral line width

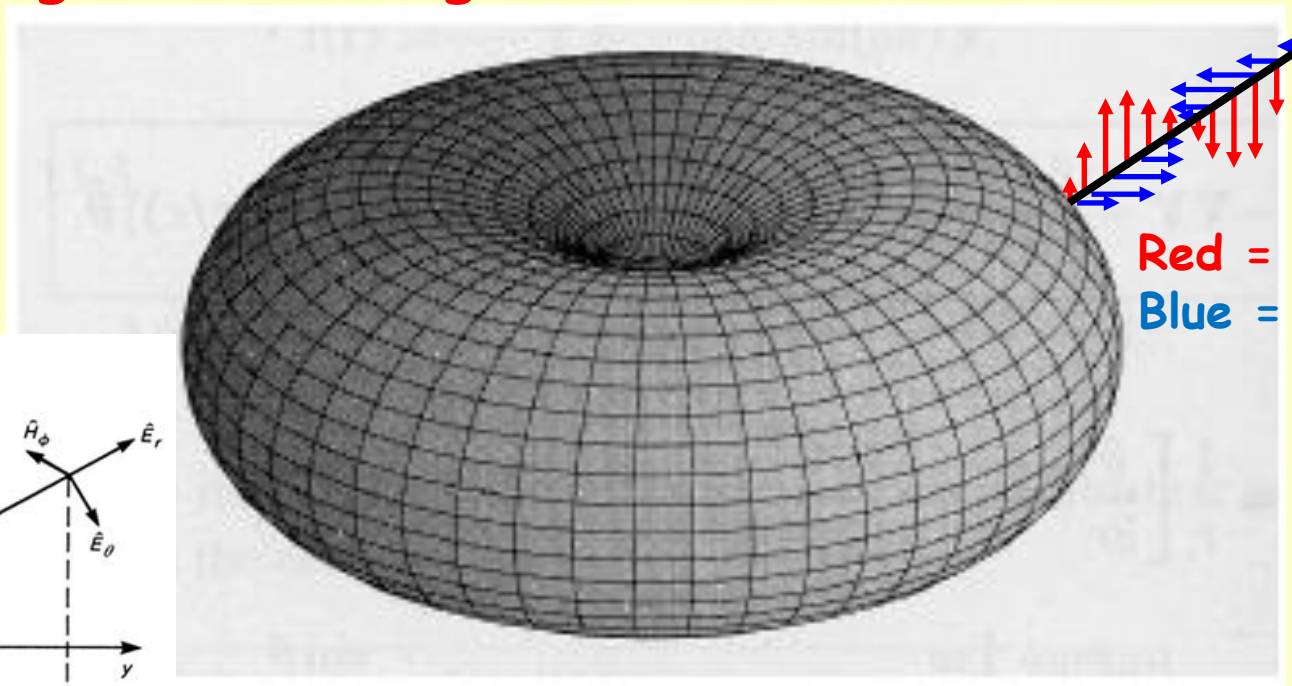
$$m_e\frac{d^2x}{dt^2}+(\eta+\mu)\frac{dx}{dt}+kx=e\left|\vec{E}_0\right|\sin\omega t$$

For absorption
Add forcing function

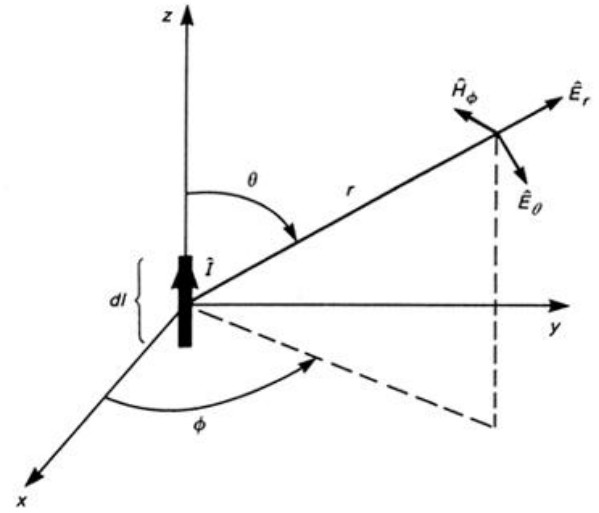
Hertz Dipole Radiation

This emission doughnut is valid for
light scattering and fluorescence emission





Red = E-field
Blue = B-field

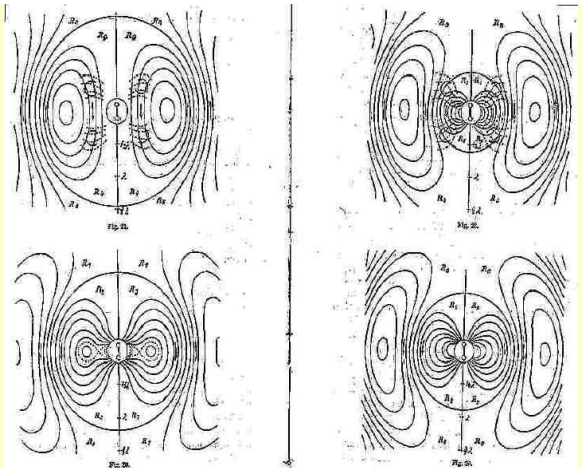


$$E_r = 60\beta^2 I\, dz \left[\frac{1}{(\beta r)^2} - \frac{j}{(\beta r)^3}\right] \cos\theta\, e^{-j\beta r}$$

$$E_\theta = j30\beta^2 I\, dz \left[\frac{1}{\beta r} - \frac{j}{(\beta r)^2} - \frac{1}{(\beta r)^3}\right] \sin\theta\, e^{-j\beta r}$$

$$H_\phi = j\frac{\beta^2}{4\pi} I\, dz \left[\frac{1}{\beta r} - \frac{j}{(\beta r)^2}\right] \sin\theta\, e^{-j\beta r}$$

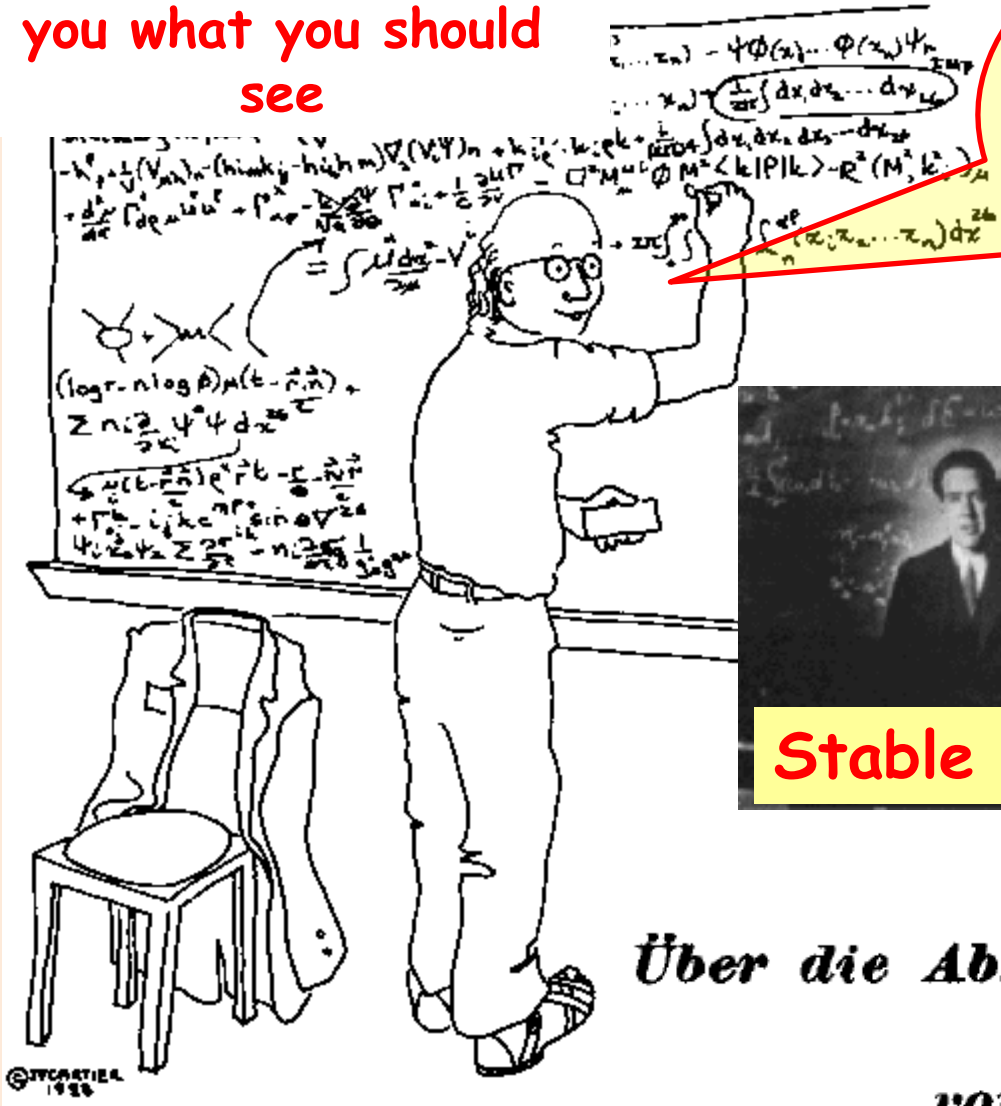
$$E_\phi = H_r = H_\theta = 0$$



Everything seemed fine

And then…..

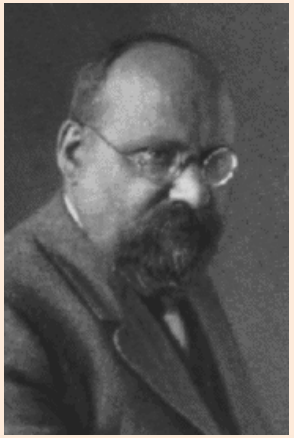
In come the theoreticians to tell you what you should see



"At this point we notice that this equation is beautifully simplified if we assume that space–time has 92 dimensions."

Hey look, it is simple if you just know the basic theory! Photons don't just start popping out right away – there is a delay time. Let me show you how it goes!

Stable orbits!



Gustav Mie (1868 - 1957)

Über die Abklingung der Lichtemission eines Atoms;

von Gustav Mie.

Annalen der Physik (1921) Vol 371, Issue 20 Pages, 229–292

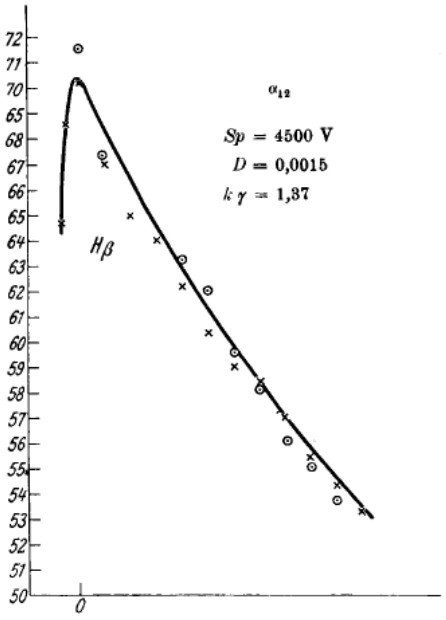


Fig. 3.

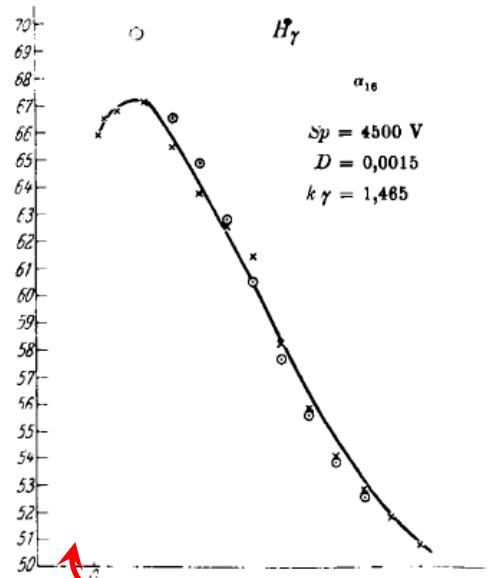


Fig. 4.

1921. № 20.

ANNALEN DER PHYSIK.

VIERTE FOLGE. BAND 66.

1. *Über Messungen der Leuchtdauer der Atome und die Dämpfung der Spektrallinien. II;*

von W. Wien.

Die Miesche Theorie ergibt, daß die aus dem Spalt in das hohe Vakuum austretenden Wasserstoffatome nicht sogleich mit der vollen Stärke zu leuchten beginnen. Erst nach einer gewissen Zeit erreichen sie die volle Intensität des Leuchtens und klingen dann allmählich ab. Hieraus geht hervor, daß die Abklingungskurve nicht in ihrem ganzen Verlauf durch eine Exponentialfunktion dargestellt werden kann, daß sie vielmehr zunächst von einer solchen abweichen muß.

für die Konstante der Exponentialfunktion bezogen auf 1 cm der Wegstrecke der Kanalstrahlen. Die Messung der Dopplerdigkeit der Kanalstrahlen $v = 3{,}17 \cdot 10^7$ cm/sec. Hieraus ergibt sich die Abklingungskonstante

$$2\alpha = k\gamma v = 4{,}35 \cdot 10^7 \text{ sec}^{-1}$$

also etwas kleiner als in meiner ersten Mitteilung und auch etwas kleiner als der aus der Elektronentheorie für H_α sich ergebende Wert

$$2\alpha = 5{,}35 \cdot 10^7 \text{ sec}^{-1}.$$

Wien then introduced the "dark time" based on the theory of Mie

Before we get carried away,
let's be fair to Professor Mie

As we will see, he was actually on the right track,
for the wrong reason

Über die Abklingung der Lichtemission eines Atoms;

von Gustav Mie.

$$N_1 = N \cdot e^{-\gamma \cdot \frac{x_1}{v}},$$

$\gamma =$ rate of the initial decay from a stable orbit to a state that was unstable and could fluoresce.

$$dI = N_1 \cdot \gamma \cdot \frac{dx_1}{v} \cdot f\left(\frac{x - x_1}{v}\right)$$

$$= \frac{N \cdot \gamma}{v} \cdot e^{-\frac{\gamma \cdot x_1}{v}} \cdot f\left(\frac{x - x_1}{v}\right) \cdot dx_1 .$$

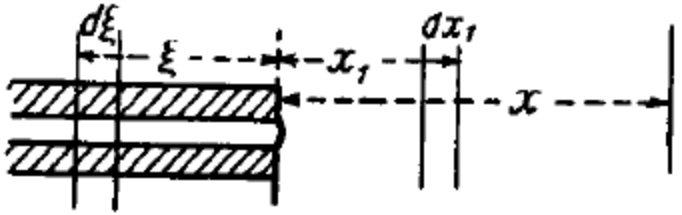


Fig. 3.

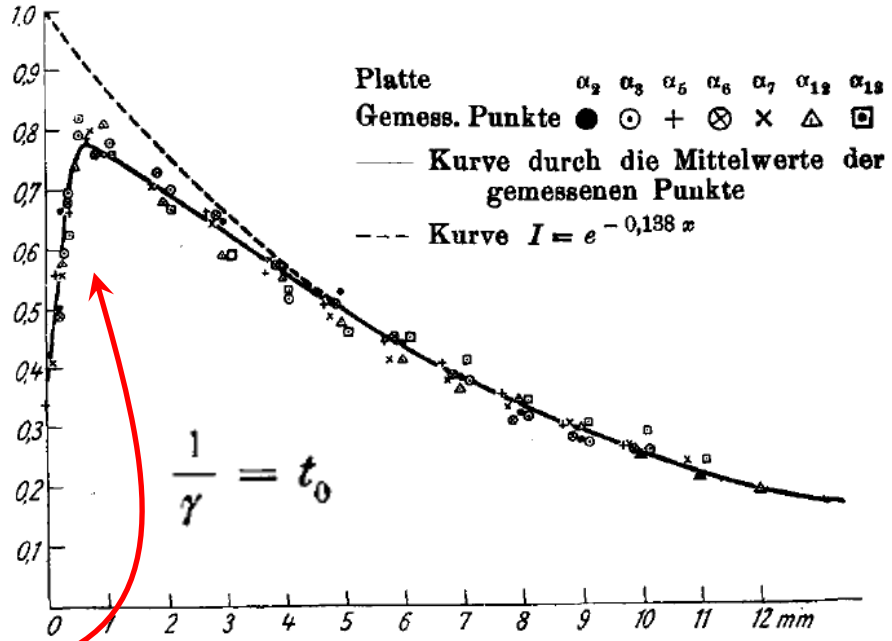


Fig. 6. H_β. 4500 Volt.

$$\int_0^\infty e^{-\gamma \cdot \tau} \cdot f(t - \tau) \cdot d\tau$$

This convolution effect does happen in energy transfer and the triplet state excitation

Leider ist uns bisher noch nicht bekannt, wie lange es durchschnittlich dauert, bis ein Atom, nachdem es erregt worden ist, zu leuchten beginnt, oder, mit anderen Worten, wie lange Zeit ein Elektron auf einer höherquantigen Bahn durchschnittlich verweilt, ehe es beginnt, unter Ausstrahlung von Lichtwellen auf eine Bahn von niedrigerer Quantenzahl überzugehen.

"**Men, it has been well said, think in herds; it will be seen that they go mad in herds, while they only recover their senses slowly, and one by one.**", *MEMOIRS OF EXTRAORDINARY POPULAR DELUSIONS, BY CHARLES MACKAY 1856*

Off we are to see the "dark times"

There was a flurry of 15-16 papers reporting on the "dark time"

We only consider two

PHYSICAL OPTICS

1914

BY

ROBERT W. WOOD, LL.D.

PROFESSOR OF EXPERIMENTAL PHYSICS IN THE JOHNS HOPKINS UNIVERSITY

Front row left to right: R. W. Wood, Max Planck, Albert Einstein

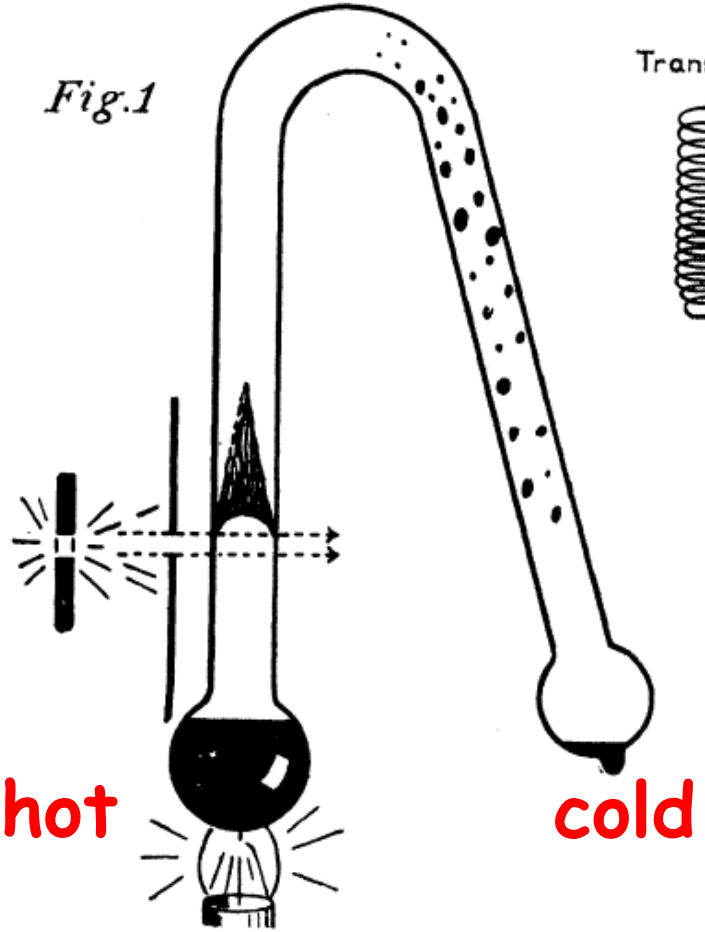
R. W. Wood, Professor of Optics at John Hopkins University delighted the scientific world in the first half of the 20th century with his showmanship at scientific lectures, exceeding the modern antics of Richard Feynman. His fame encompassed his ability to debunk frauds.

The Time Interval between Absorption and Emission of Light in Fluorescence.

By R. W. Wood, For. Mem. R.S., Johns Hopkins University, Baltimore.

(Received June 12, 1921.)

Fig. 1



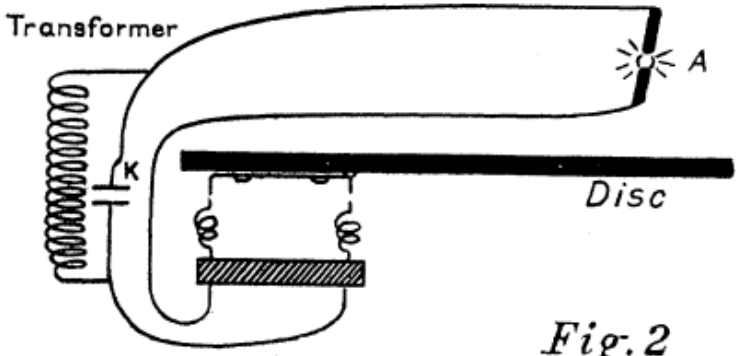


Fig. 2

The Synchrono-Phosphoroscope.

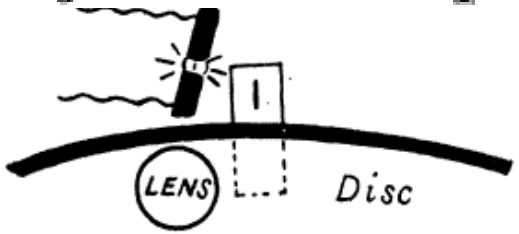


Fig. 3

"Double, double toil and trouble; Fire burn, and cauldron bubble." - (Act IV, Scene I).

The Time Interval between Absorption and Emission of Light in Fluorescence.

By R. W. Wood, For. Mem. R.S., Johns Hopkins University, Baltimore.

As the temperature of the "condenser" end of the tube rises, the velocity of the vapour becomes less, the luminosity draws down towards the illuminated region, and presently appears in the form of a beautiful green flame, concave on the under side as shown in Plate 5, fig. 1. The flame form is due obviously to the high velocity of the vapour along the axis of the tube, and the low velocity close to the wall. Absolutely no sign of luminosity is seen in the region traversed by the exciting beam, except a trace on the inner wall of the tube where the velocity of the vapour is very low. At the centre of the tube the dark region extends 2 or 3 mm. above the beam. The form of

Fig. 4

Fig. 5

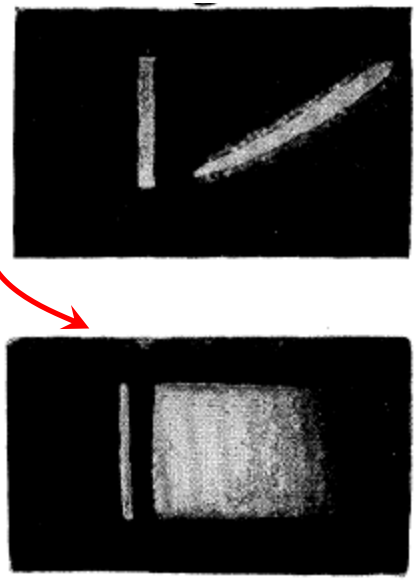


Fig. 7

"Curiouser and curiouser!"

tube. The phosphoroscope now showed a beautifully displaced band, as shown on Plate 5, fig. 7. The time interval in this case was about the same as in the other experiment, 1/15,000 second.

The Time Interval between Absorption and Emission of Light in Fluorescence.

By R. W. Wood, For. Mem. R.S., Johns Hopkins University, Baltimore.

(Received June 12, 1921.)

Source: *Proceedings of the Royal Society of London. Series A, Containing Papers of a Mathematical and Physical Character*, Vol. 99, No. 700, (Aug. 2, 1921), pp. 362-371

In the case of the fluorescence, or rather phosphorescence, of mercury vapour, I have succeeded in measuring the time interval, the vapour remaining non-luminous during the process of the absorption of light, and bursting into luminosity about 1/15000th of a second later. This, I believe, is the first case ever observed of a photo-luminescent body remaining dark during the period of excitation.

"Delays have dangerous ends". - (Act III, Scene II).

But then comes
a suggestion for improvement
in instrumentation
from long ago
from across the channel

ON THE MEASUREMENT OF CERTAIN VERY SHORT INTERVALS OF TIME.

Lord Raleigh

[*Nature*, Vol. LXIX, pp. 560, 561, 1904.]

In order to obtain a measure of the double refraction, which is rapidly variable in time, somewhat special arrangements are necessary. At the receiving end the light, after emergence from the trough containing the bisulphide of carbon, falls first upon a double image prism, of somewhat feeble separating power, so held that one of the images is extinguished when the leyden is out of action. The other image would be of full brightness, but this, in its turn, is quenched by an analysing nicol. When there is double refraction to be observed, the nicol is slightly rotated until the two images are of equal brightness. This equality occurs in two positions, and the angle between them may be taken as a measure of the effect. A full discussion is given in the paper referred to.

Known as the Kerr Cell

The problem thus presented has been very skilfully treated by MM. Abraham and Lemoine (*Ann. de Chimie*, t. XX, p. 264, 1900).

ON THE MEASUREMENT OF CERTAIN VERY SHORT INTERVALS OF TIME.

Lord Raleigh

[*Nature*, Vol. LXIX, pp. 560, 561, 1904.]

Raleigh also suggested how to use the Kerr cell to measure fast fluorescence lifetimes

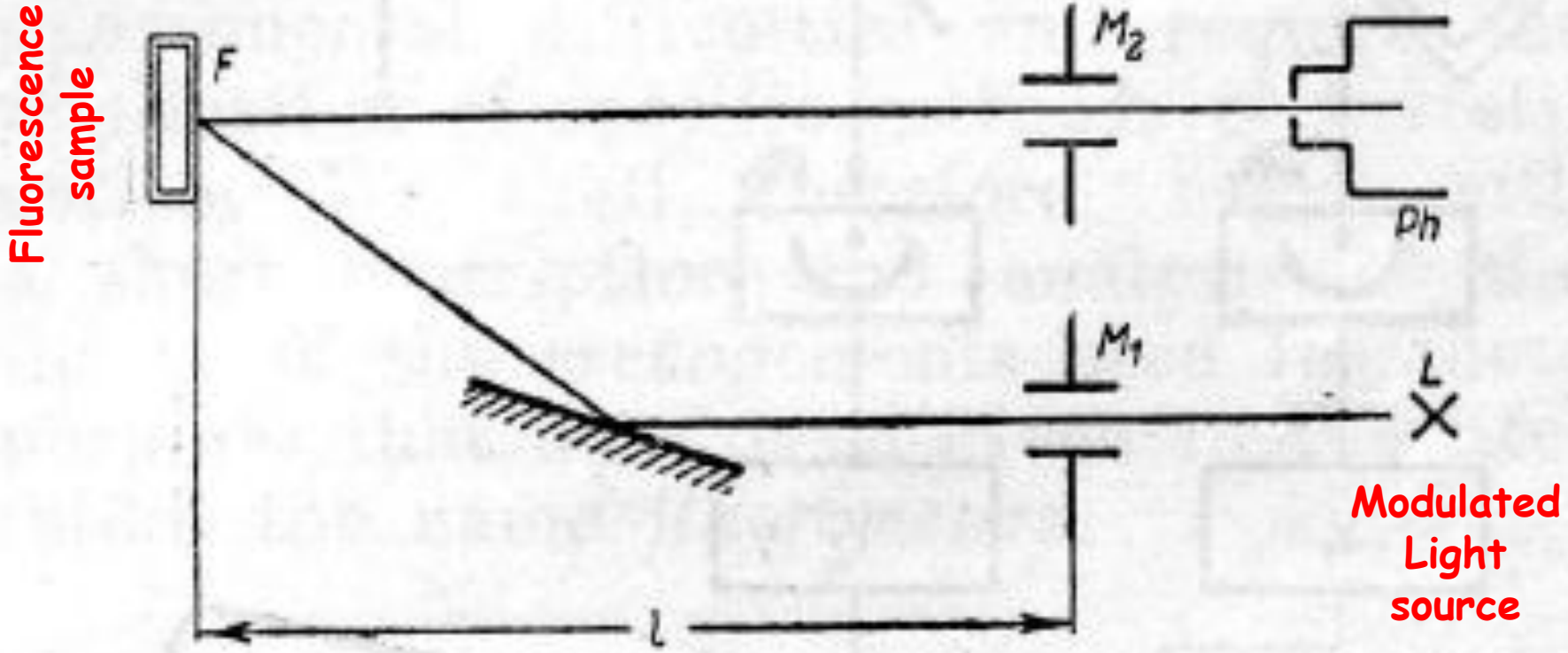


Fig. 2. Fundamental scheme of fluorometer with variable length of optical path

THE DETERMINATION OF THE TIME BETWEEN EXCITATION AND EMISSION FOR CERTAIN FLUORESCENT SOLIDS [3]) Phys. Rev. **22**, 566, 1923.

By Philip F. Gottling

First use of the Kerr cell

To measure lifetimes

IT HAS been shown recently[1] that some fluorescent substances remain dark during a definite period of excitation, in other words, that the exciting energy was imprisoned for a short but definite and measurable interval of time within the fluorescent substance.

[1] R. W. Wood, Proc. Roy. Soc. A. **99**, 362, 1921.

Gottling believes Mie, Wien and Wood concerning the delay (dark time)

Again: "This above all: to thine own self be true". - (Act I, Scene III).

Let's see how he does his experiments.
He wanted to measure faster than Wood.
So he uses a Kerr cell for shorter pulses.

Apparatus to measure the speed of light

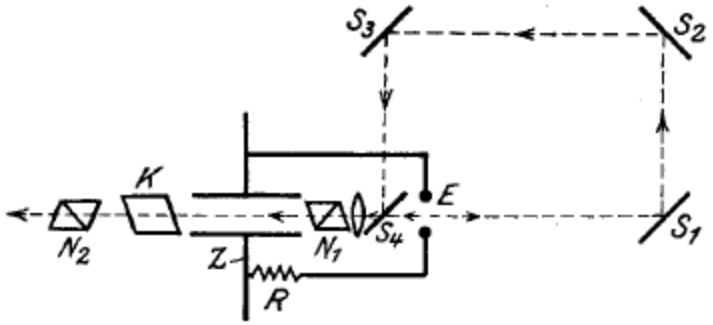


Fig. 1. Anordnung von Abraham und Lemoine.

[2] Abraham and Lemoine, Compt. Rend., p. 206, 1899
[3] Rayleigh, Scientific Papers, Vol. V, p. 190

Gottling's Result: the same "dark times"

15-16 "dark time papers followed

"A friend should bear his friend's infirmities."

Julius Caesar 4.3.85, Cassius to Brutus

And from the popes of
times on high,
came down to us
the word:
from darkness
"Let there be light"!
Bursting into luminosity!

The solution

Gaviola was one of the most outstanding scientists produced by Argentina in all of its history. The encyclopaedic *Notable Twentieth Century Scientists (McMurray,* 1998) places Gaviola in this category.

Ramón Enrique Gaviola was born in the city of Mendoza on August 31, 1900. In 1917, he was a student in *La Plata University*, when his professor, Richard Gans, advised him that if he really wanted to 'learn physics' he had to do it in Germany. Following the suggestion, Gaviola studied physics in the *Georg August Universität*, Göttingen, from 1922 to 1923, and in the *Friedrich Wilhelms Universität*, Berlín, from 1923 to 1926. The list of his professors is impressive: James Franck, David Hillbert, Richard Courant, Max Born, Richard Pohl, Hans Reichenbach, Max Plank, Max von Laue, Edler von Mises, Peter Pringsheim, Wolfgang Köhler, Albert Einstein, Walter Nernst and Lise Meitner. His Ph.D. thesis (1926) was co-directed by Walter Nernst and Max von Laue.

The eight papers (five of them before his graduation) on fluorescence and polarisation published by Gaviola in *Zeitschrift für Physik* and in *Annalen der Physik* are the basis of the scientific field that has relevance in today's biology and biochemistry: Fluorescence Spectrometry. Gaviola constructed the first-phase—fluorometer in the 1920s and measured with great precision the lifetime of the excited state of fluoresceine.

Die Abklingungszeiten der Fluoreszenz von Farbstofflösungen;
von Enrique Gaviola

Abklings Zeit Fluor Gavioloa Annalen der Physik 1926
Volume 386, Issue 23 (p 681-710)

Suggestion from Lord Raleigh

Kerr Cell modulation

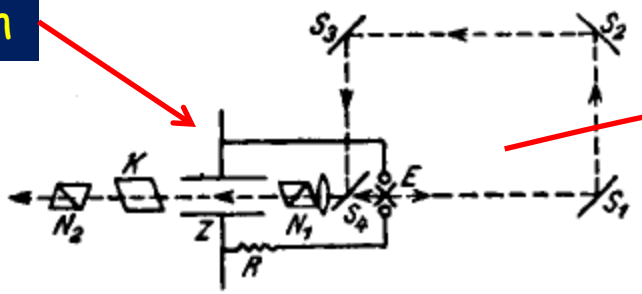


Fig. 1

Light Pulse

Abraham und Lemoine[1]) 1) Compt. rend. **129.** S. 206. 1899.

$$\mathfrak{E} = \mathfrak{E}_0 \sin\left(2\pi\frac{t}{T}\right)$$

Die hindurchgelassene Lichtintensität ist also

$$d^2 = \sin^2(\pi B l \mathfrak{E}^2).$$

Im Laufe einer Periode geht die Intensität

(4) $$D^2 = \int_0^{\pi} d^2\,dt = \int_0^{\pi} \sin^2\left(p\sin^2\left(2\pi\frac{t}{T}\right)\right)dt$$

hindurch.

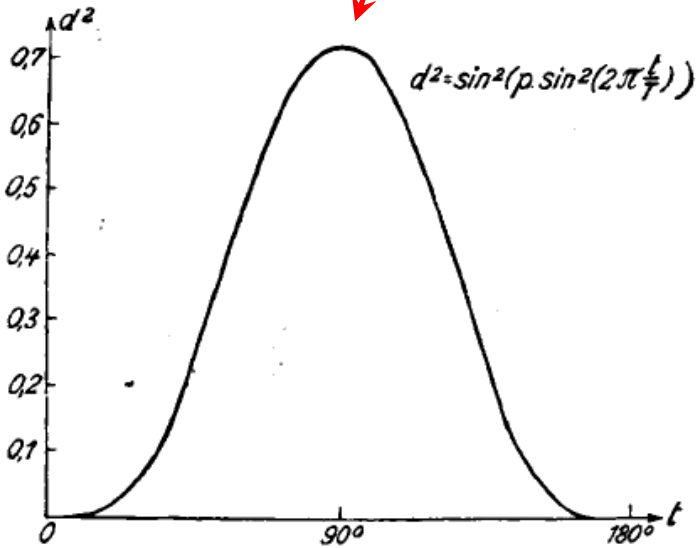


Fig. 3

Wir machen nun zunächst die Annahme, daß diese Lichtmenge in Form einer abnehmenden Exponentialfunktion

$$E = C e^{-\frac{t-t^*}{\tau}} \qquad t \geqq t^* \tag{12}$$

remittiert wird, wo τ die mittlere Abklingungszeit ist und C aus der Bedingung

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

sich zu

$$C = \frac{d^2(t^*)\,dt}{\tau} \quad ^1) \tag{13}$$

bestimmt.

Assumption:
an exponential decay.
See below!

Von dem zur Zeit t $(t \leq t^*)$ absorbierten Lichte wird zur Zeit t^* nur noch $\frac{d^2(t^*)}{\tau} e^{-\frac{t^*-t}{\tau}}$ emittiert. Die gesamte zur Zeit t^* emittierte Intensität erhält man, wenn man den vorigen Ausdruck über t von $-\infty$ bis t^* integriert. Nennen wir diese Intensität $K(t^*)$, so ist

$$K(t^*) = \int_{-\infty}^{t^*} \frac{d^2(t)}{\tau} e^{-\frac{t^*-t}{\tau}}\,dt. \tag{14}$$

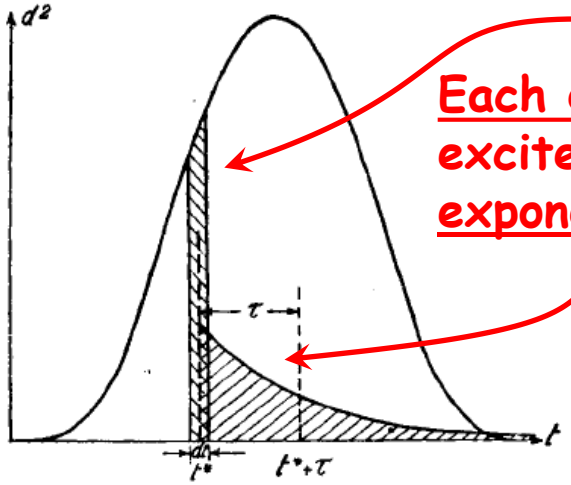


Fig. 13

Each delta function portion of the light pulse excites a number of molecules that decay as an exponential following the instant of their excitation

Die Abklingungszeiten der Fluoreszenz von Farbstofflösungen;
von Enrique Gaviola

Die Abklingungszeiten der Fluoreszenz von Farbstofflösungen.

Von **E. Gaviola** in Berlin.

(Eingegangen am 10. Dezember 1925.)

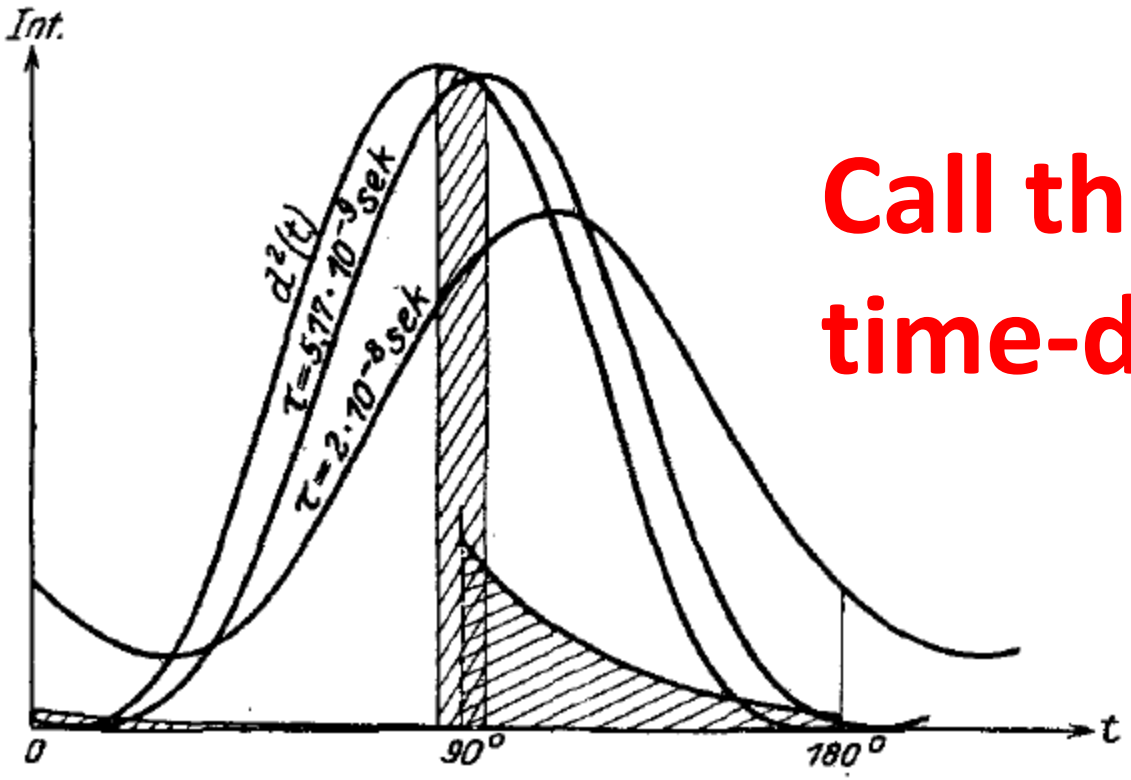


Fig. 6.

Call this the time-domain

The signal is convoluted with the excitation pulse

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

No Dark times!

And Gaviola showed that lifetimes depend on the molecular species (according to Einstein's & Schroedinger's theories)
And the dependence on the environment

But it was difficult to measure (involved a deconvolution)

Die Abklingungszeiten der Fluoreszenz von Farbstofflösungen.

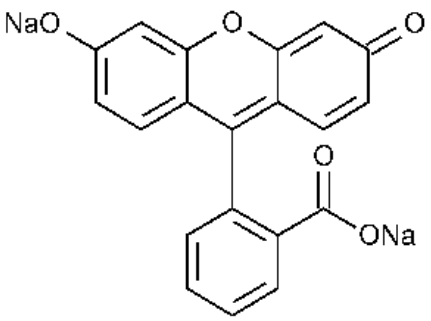
Von **E. Gaviola** in Berlin.

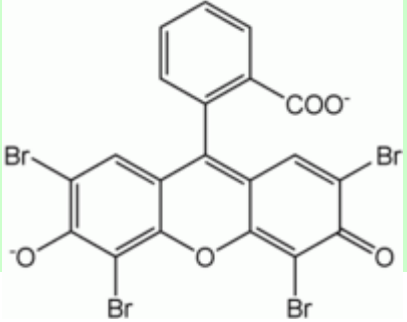
1925

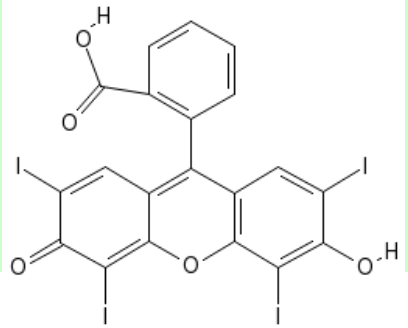
It was difficult to measure, but look at the results!

Farbstoff	Abklingungszeiten in Wasser Sekunden	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	—	—	1,3
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





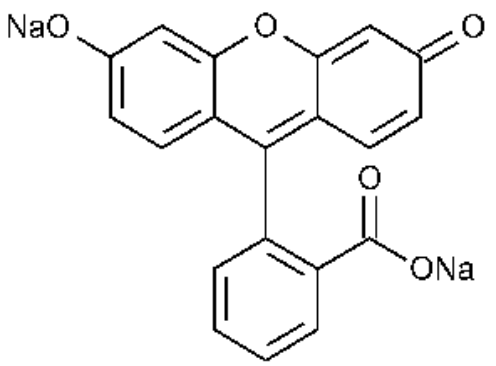


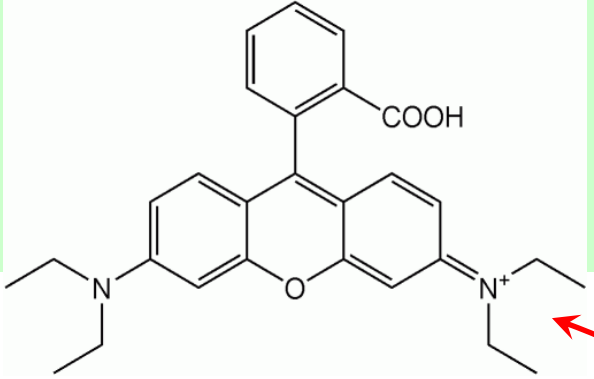
fluorescein Eosin Y Erythroscine

Intersystem crossing

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	—	—	1,3
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





Fluorescein

Rhodamine B

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	—
			$2{,}6 \cdot 10^{-9}$
			2,2
			3,4
			1,3
			1,9
			2,9
			> 15,0
Rubinkristall	—	—	> 15,0

Twisted Internal Charge Transfer
Rotation of amine groups
are Viscosity dependent

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

But wait…
What about Wood's early results?

ON TIME-LAGS IN FLUORESCENCE AND IN THE KERR AND FARADAY EFFECTS

By E. Gaviola

JUNE, 1929 PHYSICAL REVIEW VOLUME 33

The Origin of the Idea of Time-Lags[123]

In the classical theory there is no possibility of time-lags in fluorescence: If a classical oscillator is excited at a given moment, it will begin to emit radiation (if capable of doing so) at the very time of excitation and the intensity of the radiation will decrease exponentially with time, because of the damping of the oscillator.

The Bohr atom, with its stationary states in which the electrons could remain for some time without radiating, gave rise to the possibility of conceiving the existence of "dark-times." In fact, the statement that the excited stationary states had a measurable mean life was often misinterpreted in the sense that *most* atoms, if not all, would remain in the excited state during the said mean life and then fall to the normal level emitting radiation.

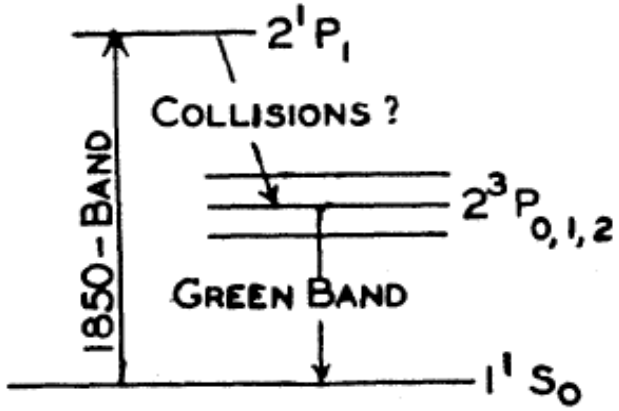


Fig. 2.

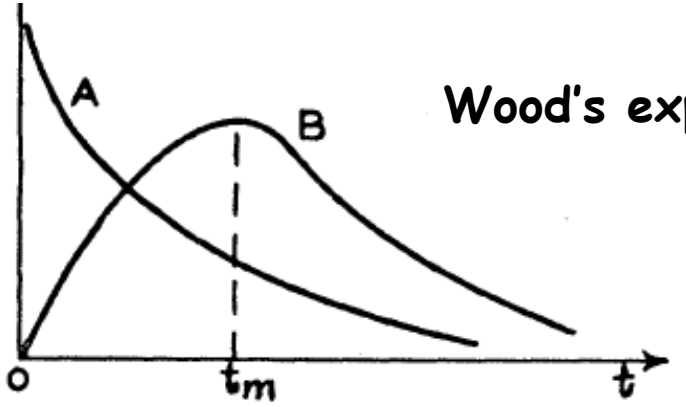


Fig. 3.

Wood's experiment

mercury vapor

reach B a maximum at a time

$$t_m = [\tau_1\tau_2/(\tau_1-\tau_2)]\cdot ln\,\tau_1/\tau_2$$

$$N_B = N_A{}^0(e^{-t/\tau_1} - e^{-t/\tau_2})\,[\tau_2/(\tau_1-\tau_2)].$$

During 1928, Gaviola carried out the first experimental work on spontaneous atomic emission of radiation, theoretically described by Albert Einstein in 1917. Gaviola observed the spontaneous emission lines from a mercury discharge at 435,8 nm and 404,6 nm from common 2^3S_1 upper level down to the 2^3P_1 and 2^3P_0 lower levels, under widely varying conditions of pressure and with various added buffer gasses' (Siegman, 1986).

"An experimental test of Schroedinger's theory", Nature 122, 722 (1928)

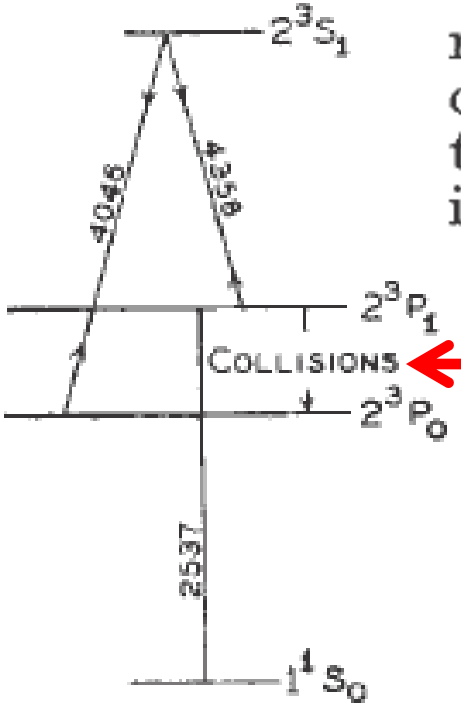


FIG. 1.

This proves conclusively that in our case the ratio of the intensities of the lines in emission does not depend on the populations of the lower levels, in contradiction with the common interpretation of Schrödinger's theory.

The relative population of the two lower levels 2^3P_1 and 2^3P_0 can be changed several hundred times by introducing a few millimetres of nitrogen or water vapour into the tube containing the mercury vapour.

NOTE!

The experimental part of this investigation was done in Prof. R. W. Wood's laboratory in the Johns Hopkins University.

E. GAVIOLA.
Department of Terrestrial Magnetism,
Carnegie Institution of Washington,

What about Wien's early results?

Ueber die Abklingung der Balmerserie
Annalen d Physik Port 1928 392, 20 581-589

von Johannes Port

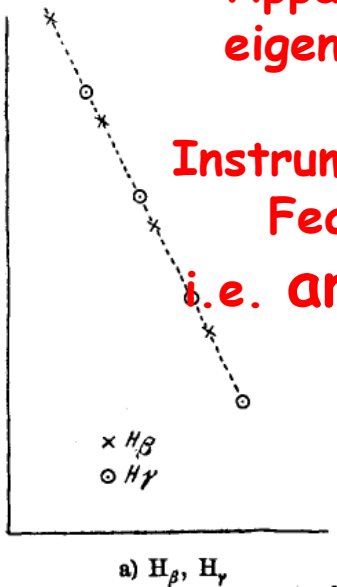
"Hydrogen follows a pure exponential decay without any deviation..."

Die Abklingung von Wasserstoffkanalstrahlen folgt einem reinen Exponentialgesetz ohne jegliche Abweichung in der Nähe des Spaltes. Weder die von W. Wien gefundene Abweichung noch die von J. Stark gefundene Abklingung eines Stoßleuchtens sind Atomeigenschaften.

$$e^{-2\alpha t} = e^{-2\alpha \frac{x}{v}}.$$

Das Abklingleuchten der Wasserstoffkanalstrahlen findet nach einem reinen Exponentialgesetz statt. Die bisher gefundene Abweichung ist keine Atom-, sondern eine Apparatureigenschaft. Sie ist darauf zurückzuführen, daß das Metall des Kanalstrahlspaltes dauernd Gas abgibt, so daß sich eine Gaswolke von höherem Druck in unmittelbarer Nähe des Spaltes bildet, die zu neuer Anregung des Leuchtens führt. Diese Gasabgabe verschwindet erst nach längerem Betrieb mit stärkeren Strömen.

Apparatur-eigenschaft = Instrumentation Feature i.e. artifact

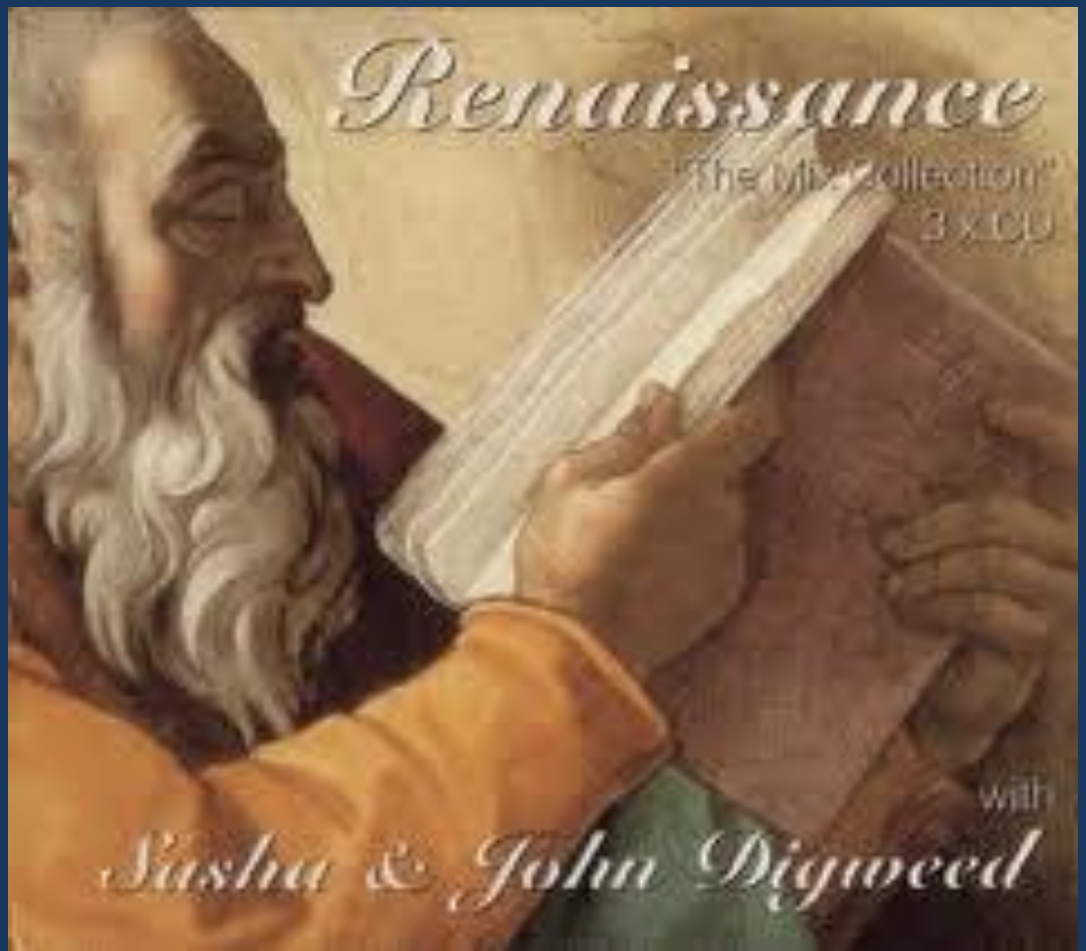


a) H_β, H_γ

NOTE!

Für die Anregung zu dieser Arbeit und das fördernde Interesse während ihrer Ausführung danke ich Hrn. Geh. Rat Professor Dr. W. Wien herzlichst.

That was
the end of the dark times
and the end of the dark ages



Enter Fourier
For analysis

"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)

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

$$a_0 = \frac{2}{T}\int_0^T f(t)dt \qquad a_n = \frac{2}{T}\int_0^T f(t)\cos(\frac{2n\pi t}{T})dt \qquad b_n = \frac{2}{T}\int_0^T f(t)\sin(\frac{2n\pi t}{T})dt$$

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.

Der zeitliche Intensitätsverlauf von intermittierend angeregter Resonanzstrahlung.

Von **F. Duschinsky** in Berlin.

(Eingegangen am 10. Januar 1933.)

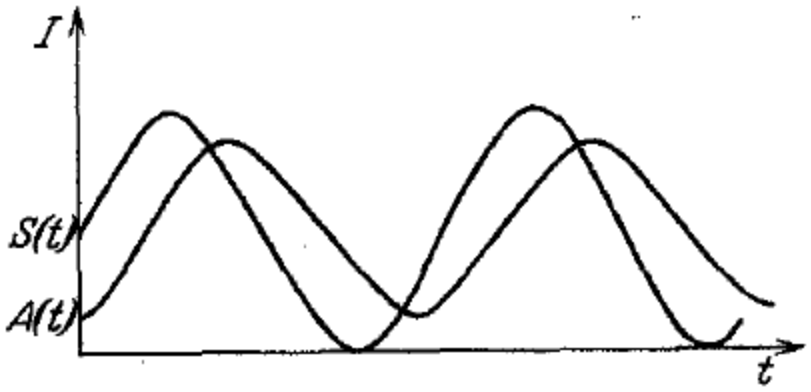


Fig. 1.

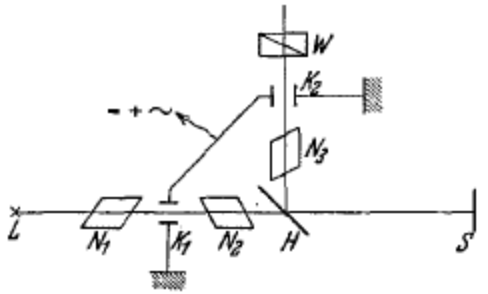


Fig. 1.

Every frequency component is analyzed separately

Call this the frequency-domain

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

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.

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

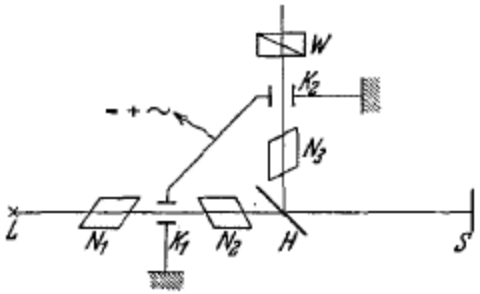
Ist die Erregungsintensität $E(t)$ periodisch (mit der Frequenz ω' moduliert), so kann sie als FOURIER-Reihe dargestellt werden:

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

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.

Fig. 1. Schema der verbesserten Fluorometeranordnung. *L* Lichtquelle; *m* ... *N*1; *N*2, *N*3, *N*4 Nicols; *F*1, *F*2 Keil-Zellen; *K* Babinet-Soleil-Kompensator; *S* Szivessi-Platte: *Z* Spiegel; *T* Trog.

For the Gaviola fluorimeter, the theory demonstrates the following possibility: One is free to choose any Fourier component of the excitation and acquisition signal, without having to know the actual waveform of the repetitive pulse.



$$K_1 - H - S - H - K_2$$

(t_l ist die Zeit des schädlichen Lichtweges).

Reihe nach mit „Sende- oder Erregungsfunktion", $S(t)$, „Ausstrahlungsfunktion", $A(t)$ und „Empfangsfunktion", $E(t)$, bezeichnet werden sollen.

$$\overline{S(t - t_l - \vartheta) \cdot E(t)} = \overline{A(t - t_l) \cdot E(t)}.$$

Wir nehmen nun an, daß die Funktionen $S(t)$, $E(t)$ und $A(t)$ in Form von Fourierreihen gegeben sind:

$$\left.\begin{aligned} S(t) &= S_0 + \sum (S_m \cos m x + s_m \sin m x), \\ E(t) &= E_0 + \sum (E_n \cos n x + e_n \sin n x), \\ A(t) &= A_0 + \sum [A_m \cos (m x - \gamma_m) + a_m \sin (m x - \gamma_m)]. \end{aligned}\right\} \quad (11')$$

$$\left.\begin{aligned} \Omega t &\equiv x, \\ \Omega \vartheta &\equiv \sigma, \\ \Omega \tau &\equiv \varrho, \\ \Omega t_l &\equiv l \end{aligned}\right\}$$

Die analoge Darstellung von

$$g = \overline{A(t - t_l) \cdot E(t)}$$

ist jetzt sehr einfach, denn die Funktion $A(t)$ spielt hier genau dieselbe Rolle wie $S(t - \vartheta)$ in

$$f = \overline{S(t - t_l - \vartheta) \cdot E(t)}.$$

$$f = S_0 E_0 + \sum_{m=1}^{\infty} (U_m \cos m \sigma + u_m \sin m \sigma).$$

$$g = S_0 E_0 + \sum_{m=1} \left(U_m \cdot \frac{1}{1 + m^2 \varrho^2} + u_m \cdot \frac{m \varrho}{1 + m^2 \varrho^2} \right). \quad (16)$$

$$\boxed{\sum_{m=1}^{\infty} (U_m \cos m \sigma + u_m \sin m \sigma) = \sum_{m=1}^{\infty} \frac{U_m + u_m \cdot m \varrho}{1 + m^2 \varrho^2}.} \quad (17)$$

Every frequency component is analyzed Separately

It is easy to analyze with Fourier techniques

ON THE LAW OF DECAY OF LUMINESCENCE OF COMPLEX MOLECULES

By L. A. TUMERMAN

(Received July 9, 1940)

JOURNAL of PHYSICS Vol. IV, No. 1—2 1941 151-166

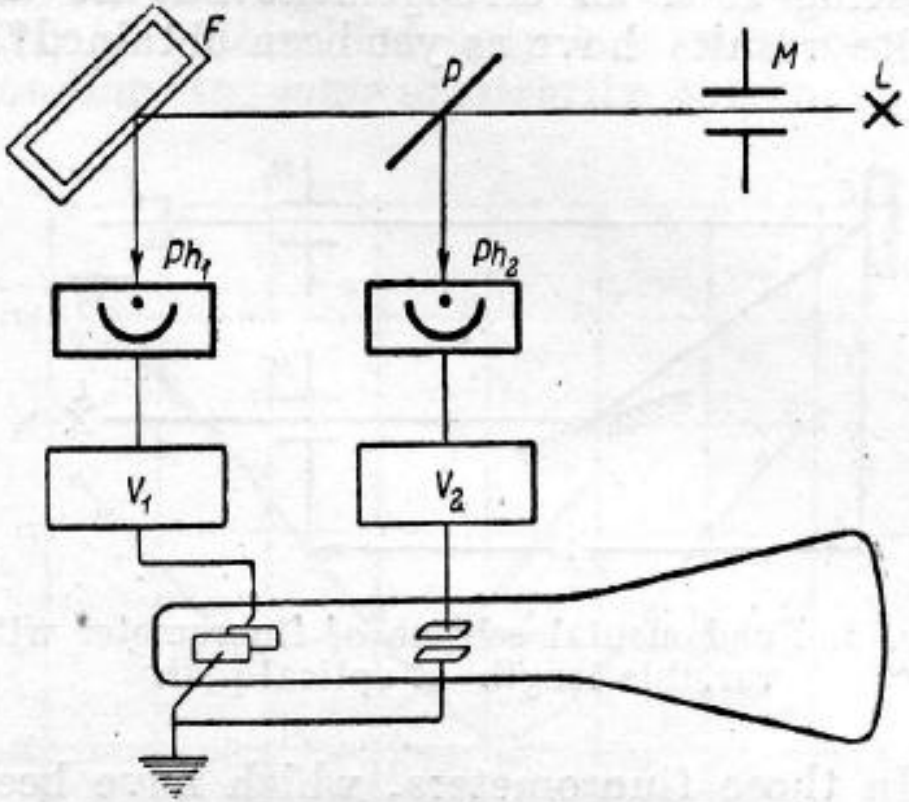


Fig. 1. Fundamental scheme of fluorometer based on a measurement of the phase displacement between the functions $S(t)$ and $A(t)$

$$A(t) = S_0 + \frac{S_1}{\sqrt{1+\omega^2\tau^2}} \cos(\omega t - \varphi), \qquad (6)$$

where

$$\operatorname{tg}\varphi = \omega\tau. \qquad (7)$$

The photocurrents of both elements, amplified by the arrangements V_1 and V_2 are passed to the plates of an oscillograph and by the Lissajous figures or in some other manner the phase displacement between them is determined.

The Determination of the Fluorescence Lifetimes of Dissolved Substances by a Phase Shift Method

E. A. Bailey, Jr., and G. K. Rollefson
Department of Chemistry and Chemical Engineering, University of California, Berkeley, California
(Received December 23, 1952)

$$dI/dt = -k_1 I,$$

$$dI/dt = -k_1 I + k_2 J(t),$$

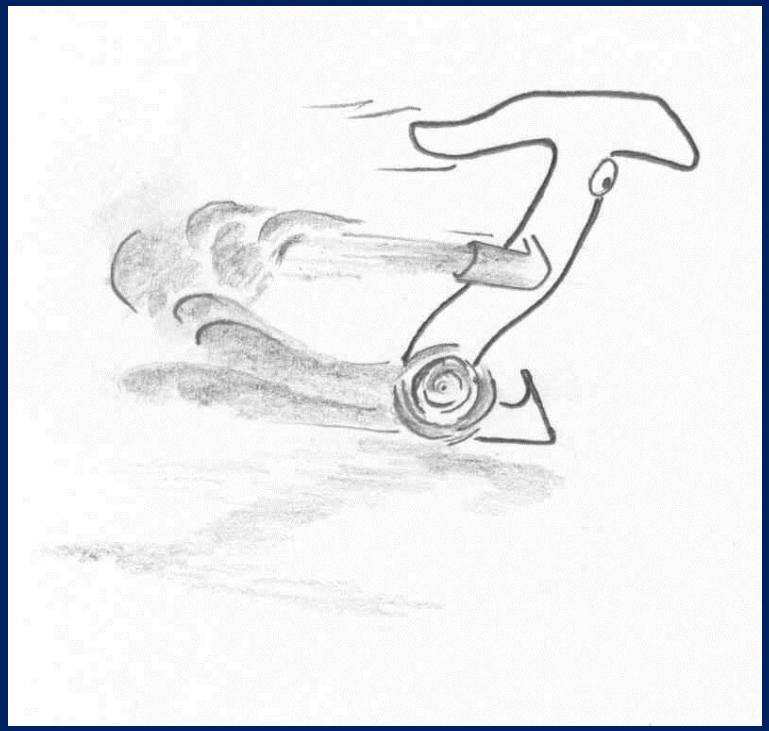
$$J(t) = a_0/2 + \sum (a_n \cos n\omega t + b_n \sin n\omega t).$$

$$I = \frac{a_0 k_2}{2k_1} + k_2 \sum \frac{a_n \cos(n\omega t - \phi_n)}{(k_1^2 + n^2\omega^2)^{\frac{1}{2}}} + k_2 \sum \frac{b_n \sin(n\omega t - \phi_n)}{(k_1^2 + n^2\omega^2)^{\frac{1}{2}}} + Ce^{-k_1 t}. \quad (4)$$

$$\sin\phi_n = n\omega/(k_1^2 + n^2\omega^2)^{\frac{1}{2}},$$
$$\cos\phi_n = k_1/(k_1^2 + n^2\omega^2)^{\frac{1}{2}},$$

$$\tan\phi_n = n\omega/k_1 = n\omega\tau.$$

And from then on Lifetimes have lived happily ever more



PHOSPHORIC PHENOMENON

PHOSPHORESCENCE

OR, THE

EMISSION OF LIGHT

BY

MINERALS, PLANTS, AND ANIMALS.

BY

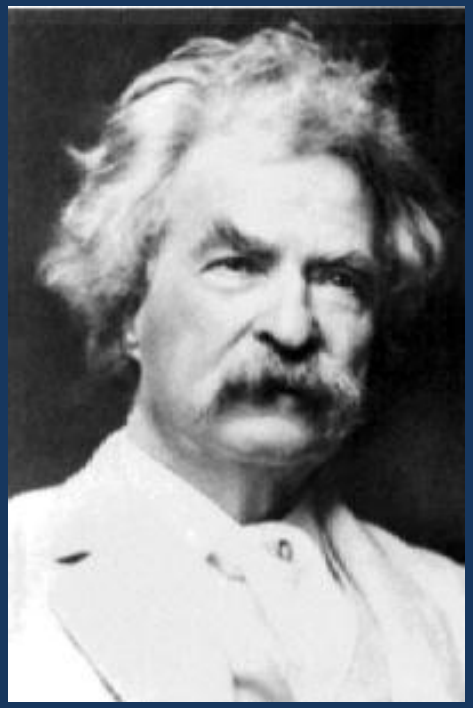
T. L. PHIPSON, PH.D., F.C.S.,

MEMBER OF THE CHEMICAL SOCIETY OF PARIS, ETC.

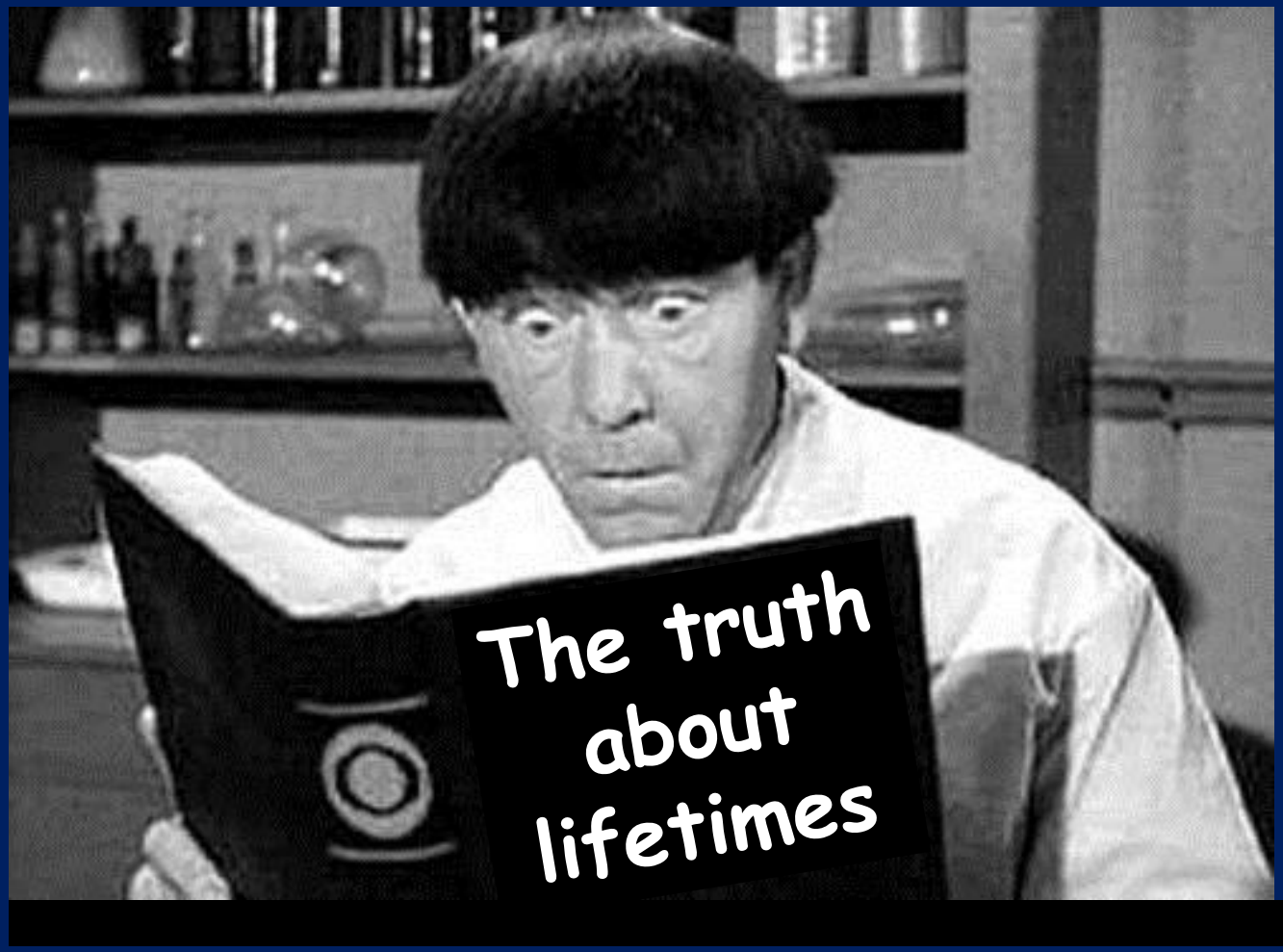
LONDON:

L. REEVE & CO., 5, HENRIETTA STREET, COVENT GARDEN.

1870.

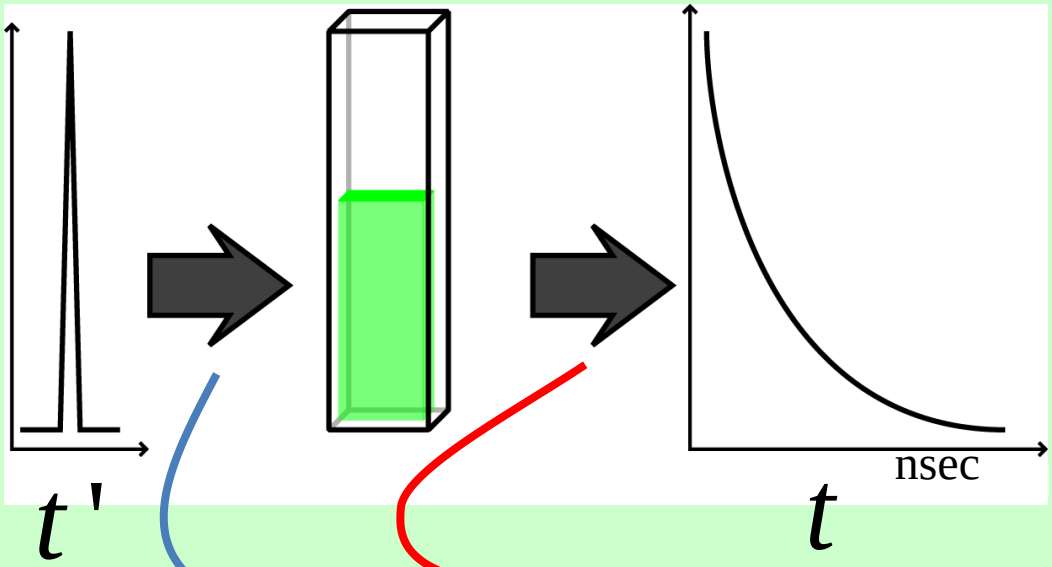


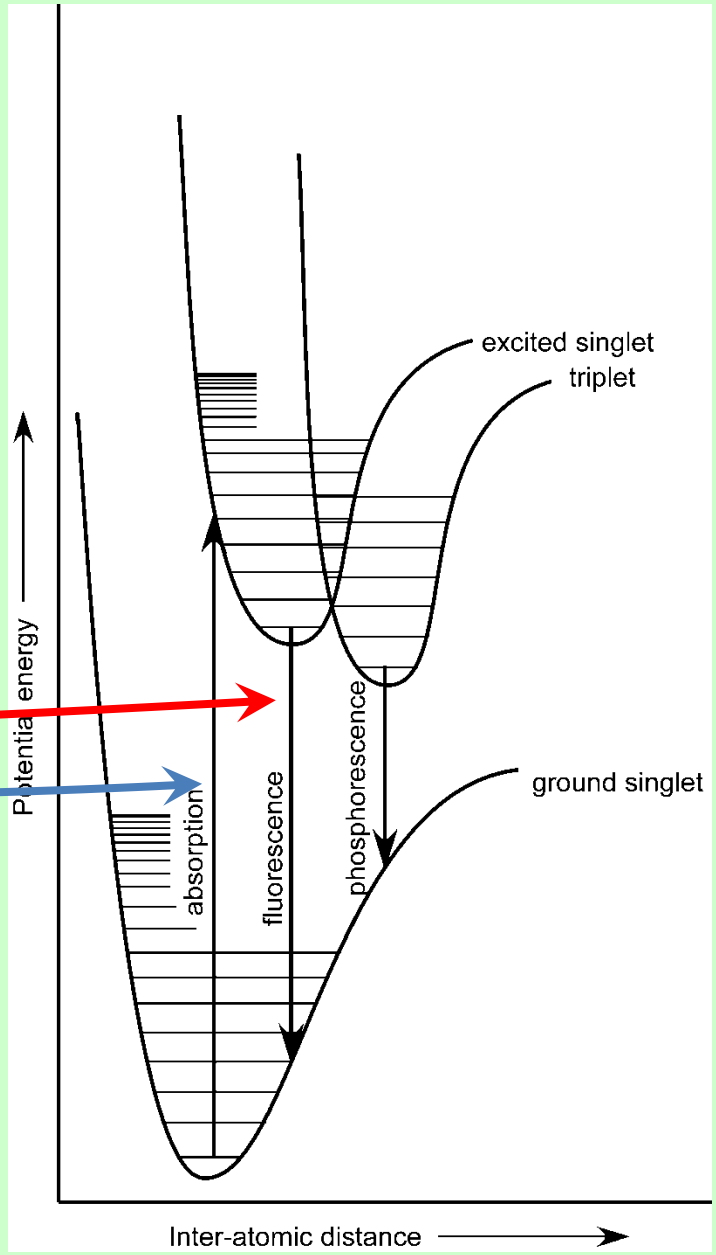
"You cannot depend on your eyes when your imagination is out of focus."
Mark Twain



What are lifetimes really?

"What is a lifetime?"





$$F_0 \exp(-(t - t')/\tau)$$

God does not roll dice

God does not role dice

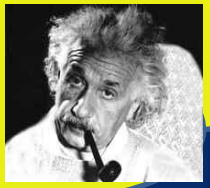
God does not role dice

God does not roll dice

.....

.....

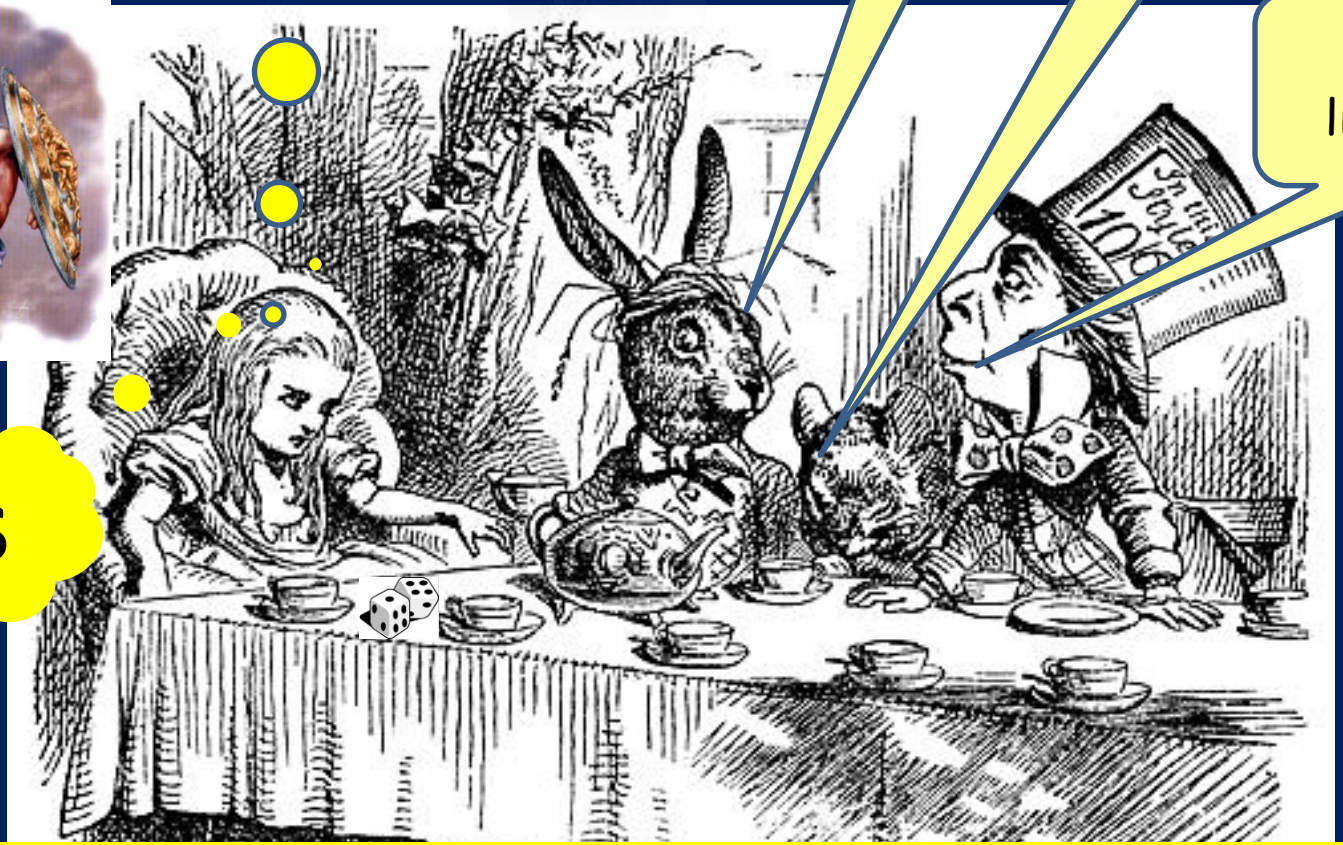
.....



Now we come to a time

When we have to decide

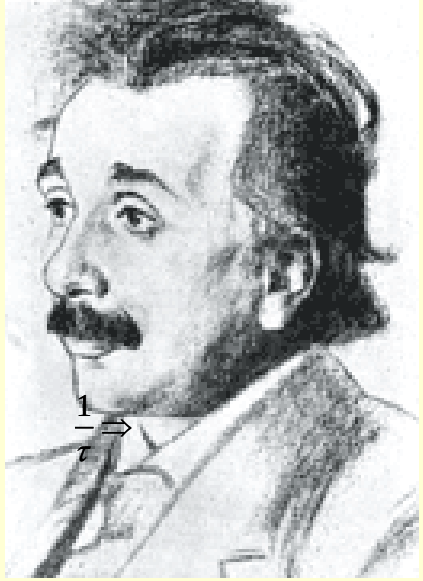
What a lifetime is



OPPS

"If it was so, it might be; and if it were so, it would be; but as it isn't, it ain't. That's logic."

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



$$\varrho = \frac{A_m^n/B_m^n}{\exp\left[(\varepsilon_m - \varepsilon_n)/kT\right] - 1}$$

$$p_n \exp\left(-\varepsilon_n/kT\right) B_n^m \varrho = p_m \exp\left(-\varepsilon_m/kT\right)(B_m^n \varrho + A_m^n)$$

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

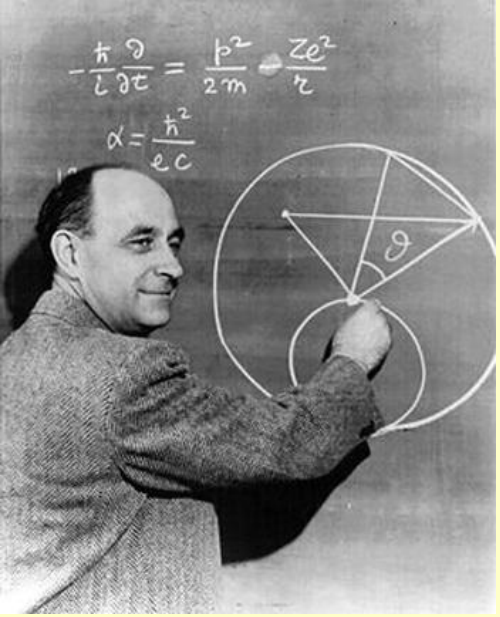
$$\frac{1}{\tau} \Rightarrow$$

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

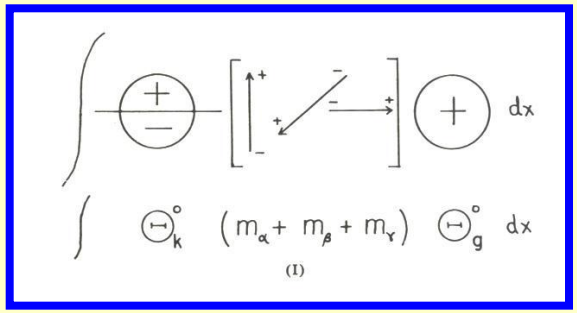
ON THE QUANTUM THEORY OF RADIATION

[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.

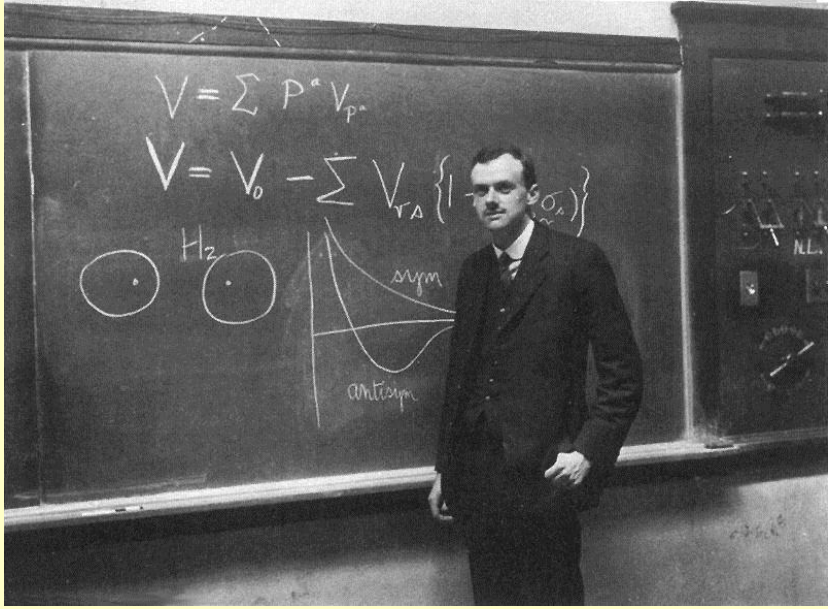


Transition moment



Transition Dipole

m=er



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

Fermi's Golden Rule

Transition probability — *Matrix element for the interaction* — *Density of final states*

OPPS - Dirac's Golden Rule

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 "*attribution of Enrico Fermi's golden rule to Pauli is also miscast; it was Dirac who developed time-dependent perturbation theory, including this formula, to calculate radiative transitions with his other great invention, the quantized radiation field. More than 20 years later, Fermi, in his Chicago lectures, called the formula a golden rule, and many physicists, with their habitual disregard for history, have ever since attributed it to Fermi*"
KURT GOTTFRIED (*kg13@cornell.edu*) , *Cornell University , Ithaca, New York*

If the dynamics is non-coherent with the light oscillations
And if the end molecular states are distributed statistically
(no perfectly two-state transitions)
we can use normal statistics.

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

Fermi's Golden Rule

Transition probability

Matrix element for the interaction

Density of final states

This is a Transition Dipole

$$\rho(\omega)\,d\omega = \left(\frac{\omega}{c}\right)^2 \frac{d\omega/c}{\pi^2} = \frac{\omega^2 d\omega}{\pi^2 c^3}$$

This is the <u>density of light modes in the cavity having frequencies between</u> ω and $\omega + d\omega$

Nowadays we can vary the natural radiative lifetime of fluorescence!

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

Fermi's Golden Rule

Transition probability

Matrix element for the interaction

Density of final states

$$\left(\tau\right)_{\substack{\text{natural}\\ \text{radiative}\\ \text{lifetime}}} = \frac{1}{\lambda_{if}}$$

$$M_{if} = e\left\langle f\left|\vec{r}\right|i\right\rangle = e\int \psi_f r \psi_i d\vec{r}$$

This QM rate expression is true of any incoherent kinetic process where the system has equilibrated to a quasi-steady-state. May not be true if time < 10^{-12} seconds for fluorescence.

But … In Spontaneous Emission there is no perturbation in the QM description. So, how does this work?

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

Fermi's Golden Rule

Transition probability

Matrix element for the interaction

Density of final states

Short answer (Dirac and QED): Light fields consist of quantized "modes" , and each mode of a light field is filled with (a probability of) a certain number of photons of energy hν. If there are no photons then each mode still has an energy = 1/2 hν. This **zero point field** oscillates in occupation, causing a perturbation to the excited state (see the density of final states above). This, together with radiation damping causes the spontaneous emission, and the basic theory for the Fermi Golden rule can still be applied. The density of modes is important for enhanced fluorescence techniques (e.g. metal enhanced fluorescence).

P. W. Milonni, Semiclassical and quantum.electrodynamical approaches in nonrelativistic radiation theory, PHYSICS REPORTS (Section C of Physics Letters) 25, No. 1(1976)1—81.

P. W. Milonni, Field quantization and radiative processes in dispersive dielectric media, JOURNAL OF MODERN OPTICS, 1995, VOL. 42, NO. 10, 1991-2004

$$e^{-t/\tau}$$

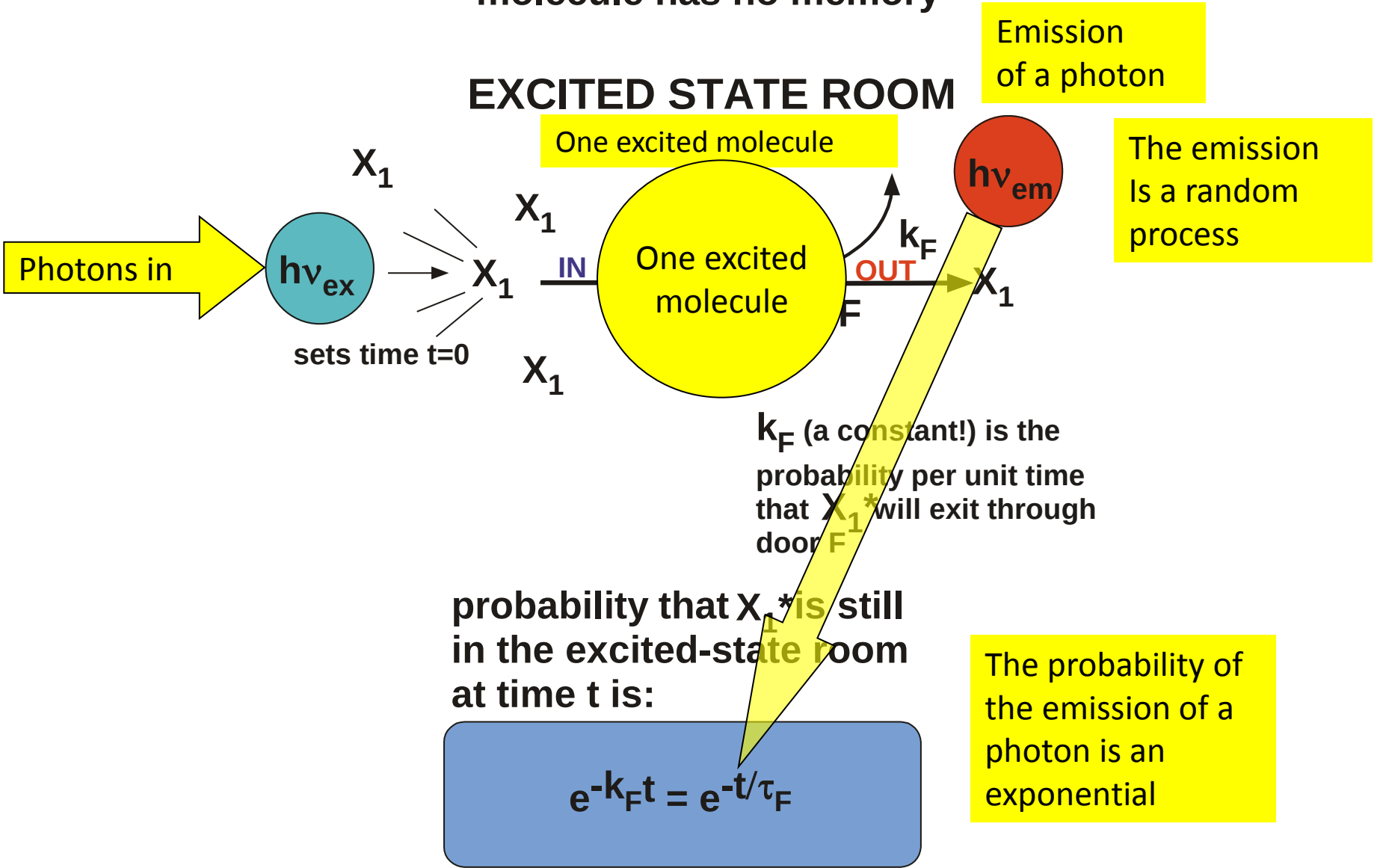
What is hidden in a lifetime?

An easy way
to interpret just about everything
from fluorescence measurements

MOLECULE IN THE EXCITED STATE

- *some statistics* -

Simple case: one door in, and one door out, one molecule occupation, molecule has no memory



Proof: that $e^{-k_F T}$ is the probability that X* is in the room at time t=T.

1. k_F is the probability *per unit time* that X*, which is already in the excited state at any time "t", will leave the excited state through door "F" (fluorescence).
   1.1 $k_F\Delta t$ is the *approximate* probability that X*, which is already in the excited state at time "t=0", will leave the excited state through door "F", after the "short" time, Δt.
2. $(1-k_F)$ is the probability *per unit time* that X*, which is already in the excited state at any time "t", will remain in the excited state.
   2.1 $(1-k_F\Delta t)$ is the *approximate* probability that X*, which is already in the excited state at time "t=0", will remain in the excited state for the "short" time Δt.
      2.1.1 another Δt time step: $(1-k_F\Delta t)(1-k_F\Delta t)$ is the *approximate* probability that X*, which is already in the excited state at time "t=0", will remain in the excited state for the time $2\Delta t$.
      ... and so on for $3\Delta t$, etc.
3. Consider the total time from t=0 to t=T. Divide up this time interval into "n" time points, so that $\Delta t = T/n$. The more time intervals, the smaller Δt.
   3.1 $(1-k_F\Delta t)^n = (1-k_F T/n)^n$ is the *approximate* probability that X*, which is already in the excited state at time "t=0", will remain in the excited state for the longer time $T = n\Delta t = n(T/n)$.
   3.2 Take the limit as n->infinity. This Too many is the *definition of an exponential*.

Too many details

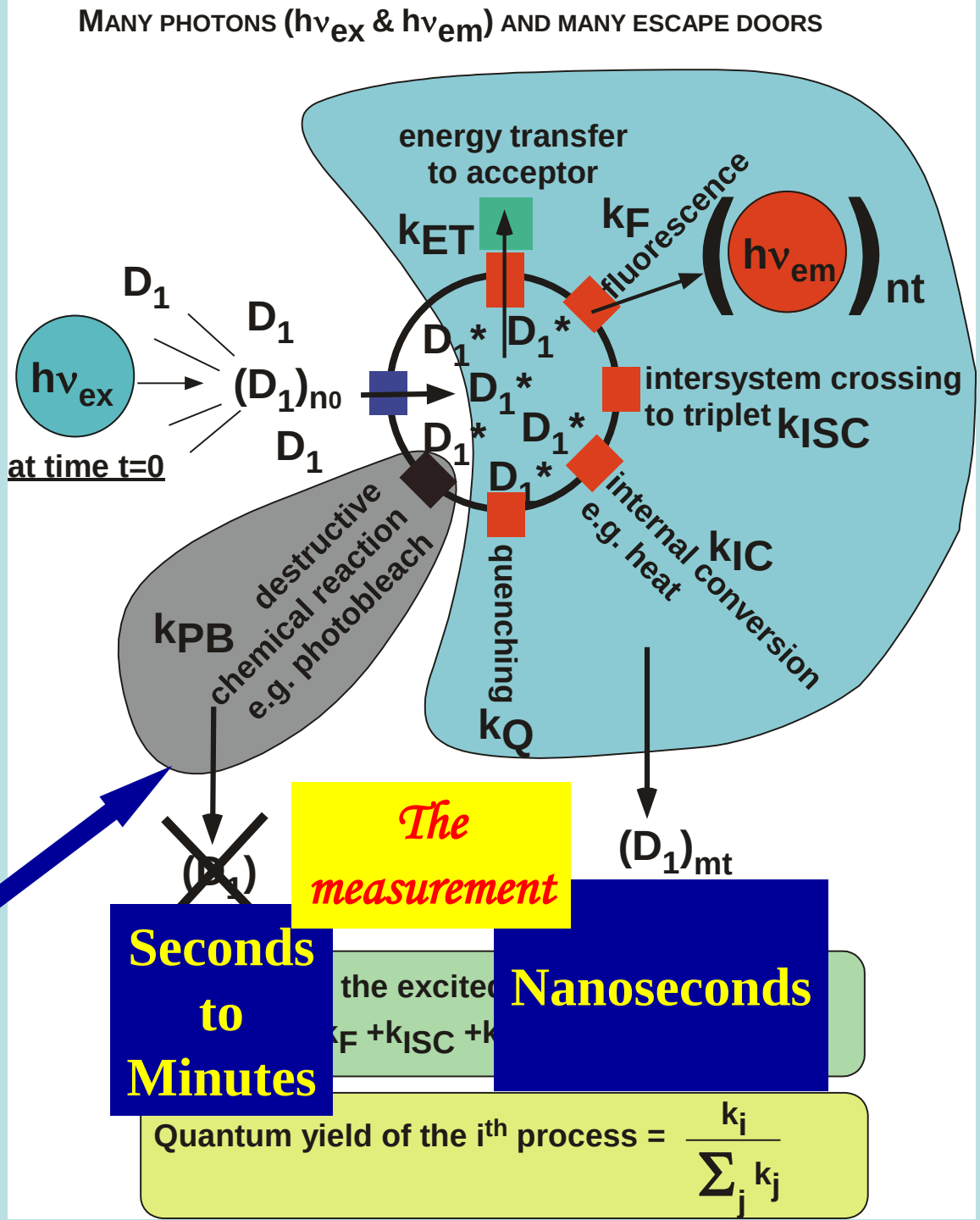
The exact probability that X* remains in the excited state until t=T is:

$$exp(-k_F t)$$

QED

The rate of leaving any of the doors can be used to measure the rate of leaving any OTHER door

In particular the photolysis, in the second to minute scale, can be used to measure the nanosecond scale exit rates



The probability /time for going through the fluorescence door is always the same!

The total rate of leaving the excited state is GREATER.

QuantumYield of fluorescence =

$$\frac{\text{total number of photons emitted}}{\text{total number of molecules originally excited}}$$

$$= \frac{k_F}{(\sum_{i \neq F} k_i + k_F)}$$

We can measure the efficiency of energy transfer from JUST the fluorescence lifetimes

Quantum Yield of energy transfer =

$$\frac{\text{total number of energy quanta transferred}}{\text{total number of molecules originally excited}}$$

$$= \frac{k_{Transfer}}{(k_{Transfer} + k_F)} \quad \left(1/\tau_{+transfer}\right) = \left(1/\tau_{-transfer}\right) \quad Y = 1 - \left(\frac{\tau_{+transfer}}{\tau_{-transfer}}\right)$$

The **quantum yield of energy transfer** is the fraction of excited molecules that transfer a quantum of energy to the acceptor (Y)

Determining rate of process "p" by measuring the rate of process "m"

Rate of deactivation ($1/\tau$) and Q.Y. of process "m" in the *presence* of *all* paths of deactivation (measuring process "m"):

$$\frac{1}{\tau_m} = \sum_j k_j \; ; \; (Q.Y.)_m = \frac{k_m}{\sum_j k_j}$$

Simple – This is the reason we measure fluorescence!

Rate of deactivation (1/t) in the *absence* of path "p" of deactivation:

$$\left(\frac{1}{\tau_m}\right)_{\neq p} = \sum_{j \neq p} k_j ; \; (Q.Y.)_{m;\neq p} = \frac{k_m}{\sum_{j \neq p} k_j}$$

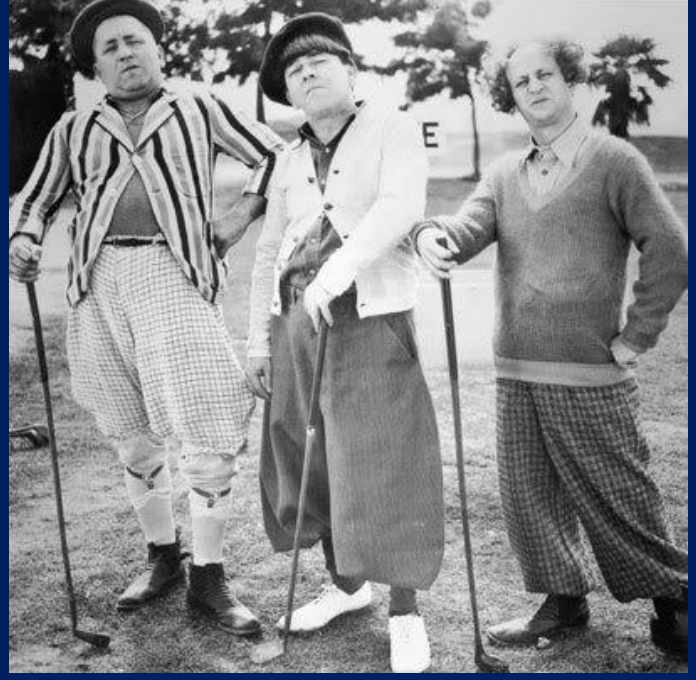
Combine the two rates and quantum yield measurements

$$\frac{1}{\tau_m} - \left(\frac{1}{\tau_m}\right)_{\neq p} = k_p \; ;$$

"p" can be FRET

$$\frac{(Q.Y.)_m^{-1} - (Q.Y.)_{m;\neq p}^{-1}}{(Q.Y.)_m^{-1}} = \frac{k_p}{\sum_j k_j} = (Q.Y.)_p$$

So, we can determine "p" by measuring "m"



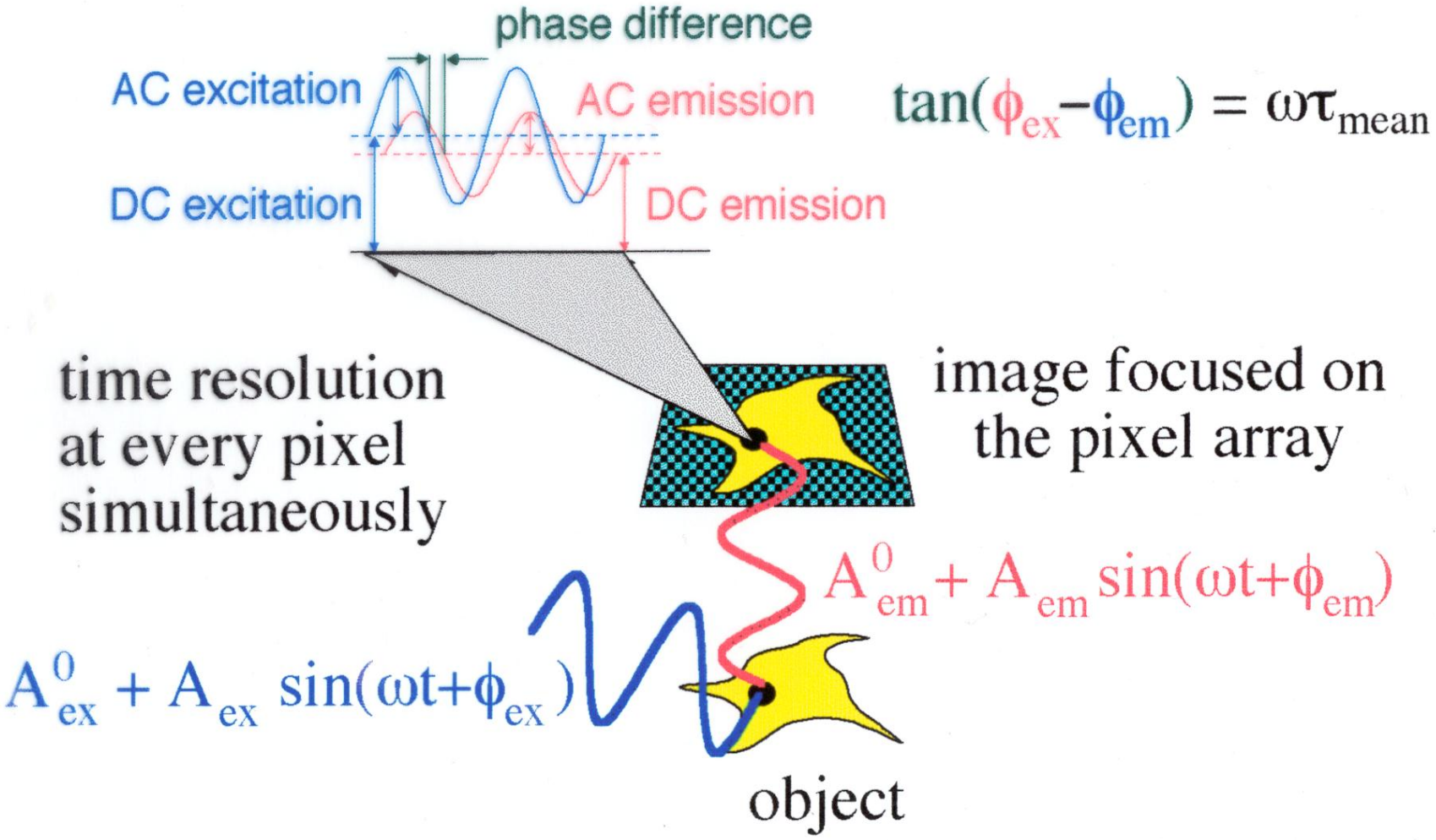
$$e^{-t/\tau_{measured}}$$

$$\frac{1}{\tau_{measured}} = k_{fluorescence} + \sum_{i \neq fluorescence} k_i$$

End of section 1

Fluorescence Lifetime Imaging (FLI)
&
Some useful information
for your lab sessions

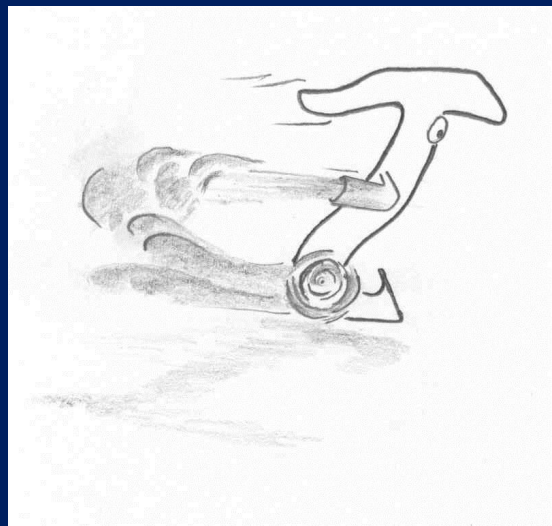
We want to measure fluorescence lifetimes <u>in a fluorescence image</u> at <u>every location in the cell</u>.



FLIM

Measuring
Nanosecond fluorescence lifetimes
at many pixels in an image
used to be difficult

We look at
The early pioneers



THE REVIEW OF SCIENTIFIC INSTRUMENTS VOLUME 30, NUMBER 6 JUNE, 1959

Microscope Phase Fluorometer for Determining the Fluorescence Lifetimes of Fluorochromes

BENJAMIN D. VENETTA
Department of Anatomy, Western Reserve University School of Medicine, Cleveland 6, Ohio

1959

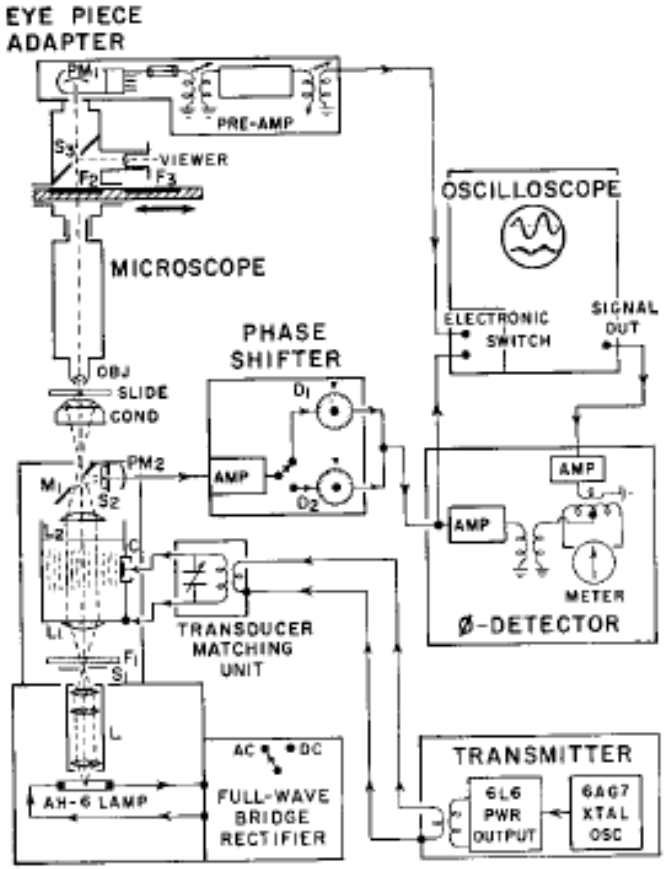


FIG. 1. Block diagram of the microscope phase fluorometer.

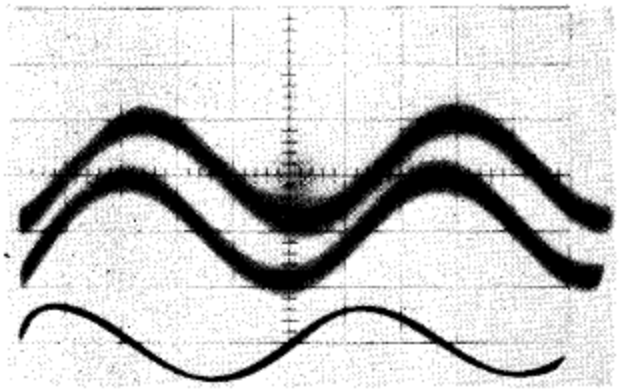


FIG. 5. The transmitted light signal, fluorescent light signal, and the tracer signal (sweep speed: 0.034 μsec/cm).

$$\tan\Delta\phi = \omega\tau.$$

The instrument was capable of dissecting the image into areas of interest, and can therefore be classified as an imaging fluorescence lifetime instrument. Lifetime measurements were carried out on "fluorphores bound to the **nuclei of tumor cells, as well as autofluorescence of biological tissue samples**."

MEASUREMENT OF FLUORESCENCE DECAY TIME IN LIVING CELLS

CH. N. LOESER, ELLEN CLARK, MARJORIE MAHER and H. TARKMEEL

University of Connecticut Health Center, Department of Anatomy, Farmington, Conn. 06032, USA

Experimental Cell Research 72 (1972) 480–484

1972

"Ascites tumor cells, liver cells, fibroblasts, bacteria, and cell fractions, after incubation with a fluorochrome and appropriate washing, can be suspended in a cuvette (or in the case of single cells, placed on a microscope slide) and the fluorescent decay time can be read out digitally in nanoseconds. The instrument is most accurate where actual decay values are > 2 ns! "

Table 1. *Intracellular fluorescence decay times of ANS, TNS, BP, and 2-AN*

Medium[a]	Cell type	Decay time (nsec)
0.3×10^{-4} M ANS	Ascites	7.8 ± 0.2[b]
0.3×10^{-4} M TNS[c]	Ascites	8.8 ± 0.1
16 % saturated BP	Ascites	15.2 ± 0.1
0.3×10^{-4} M 2-AN	Ascites	16.3 ± 0.1
0.3×10^{-4} M ANS	*Bacterium megaterium*	10.3 ± 0.3

[a] BP was made up as a saturated solution in propylene glycol and diluted with saline. The other compounds were made up in Krebs-Ringer, pH 7.3 ± 0.1.
[b] Standard error.
[c] Limited solubility in aqueous solution.

1976-
1979

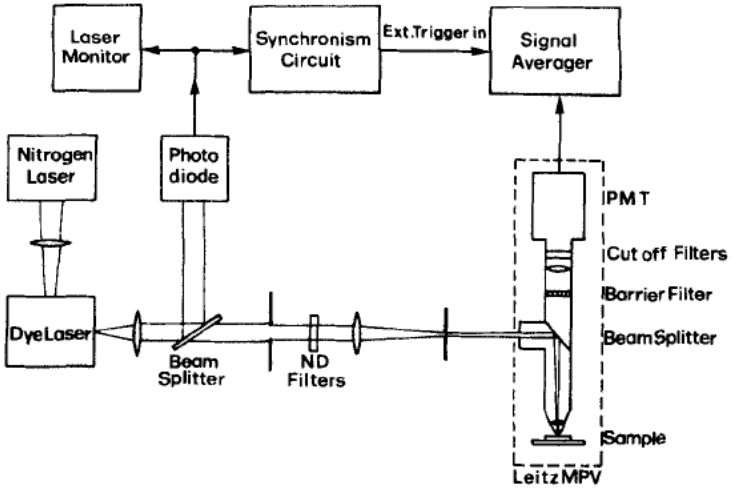
FLUORESCENCE OF COMPLEXES OF QUINACRINE MUSTARD WITH DNA. I. INFLUENCE OF THE DNA BASE COMPOSITION ON THE DECAY TIME IN BACTERIA

G. BOTTIROLI,* G. PRENNA,*† A. ANDREONI,‡ C. A. SACCHI‡ and O. SVELTO‡

*Centro di Studio per l'Istochimica del C.N.R., Istituto di Anatomia Comparata dell'Università, Pavia, Italy and ‡Centro de Studio per l'Elettronica Quantistica e la Strumentazione Elettronica del C.N.R., Istituto di Fisica del Politecnico, Milano, Italy

Photochemistry and Photobiology, Vol. 29, pp. 23 28. 1979.

$$\tau = \frac{\int_0^\infty t n(t)\,dt}{\int_0^\infty n(t)\,dt} = \tau_D\left(1 - \frac{\sqrt{\pi}}{2}\frac{[Y]}{[Y]_0}\right),$$



The fluorescence of several bacterial DNAs stained with quinacrine mustard have been investigated using a laser microfluorometer with **a spatial resolution of ~0.3 *micro*-m and a temporal resolution of ~0.3 ns** connected to a digital signal averager.

We explain this result on the basis of an energy transfer mechanism between dye molecules intercalating AT:AT sequences (donors) and dye molecules bound to either GC:GC or GC:AT sequences (acceptors).

Andreoni, **A.**, Sacchi, C.A., Svelto, O., Longoni, A., Bottiroli, G., and Prenna, G., in ***Proceedings of the Third European Elecrro-Optics Conference***, H.A. Elion, Editor, SPIE, Washington, 258-270, (1976).

Fluorescence Decay Analysis in Solution and in a Microscope of DNA and Chromosomes Stained with Quinacrine

DONNA J. ARNDT-JOVIN, SAMUEL A. LATT, GEORGE STRIKER AND THOMAS M. JOVIN

THE JOURNAL OF HISTOCHEMISTRY AND CYTOCHEMISTRY

Vol. 27, No. 1, pp. 87–95, 1979

Fluorescence Lifetimes of Quinacrine Bound to DNA and Poly[d(A-T)] (Three-Component Analysis)[a]

DNA or polymer[b]	(A-T)[c]	(A-T)[d]	rte[e]	⟨τ⟩ (nsec)	τ_1 (nsec)	fce %	τ_2 (nsec)	fce %	τ_3 (nsec)	fce %	$\langle r_w^2 \rangle$	⟨c⟩
Poly[d(A-T)][f]	1	1	1	18	1.1	6	7.9	33	26	60	2.71	1040
Clostridium acidurici	0.7	0.24	.2	14	3.0	29	11	36	27	34	1.20	566
Proteus mirabilis	0.6	0.14	.15	14	3.3	29	13	45	30	25	2.34	2326
Bacillus subtilis	0.55	0.098	.12	12	2.6	35	11	39	27	26	1.26	437

1979

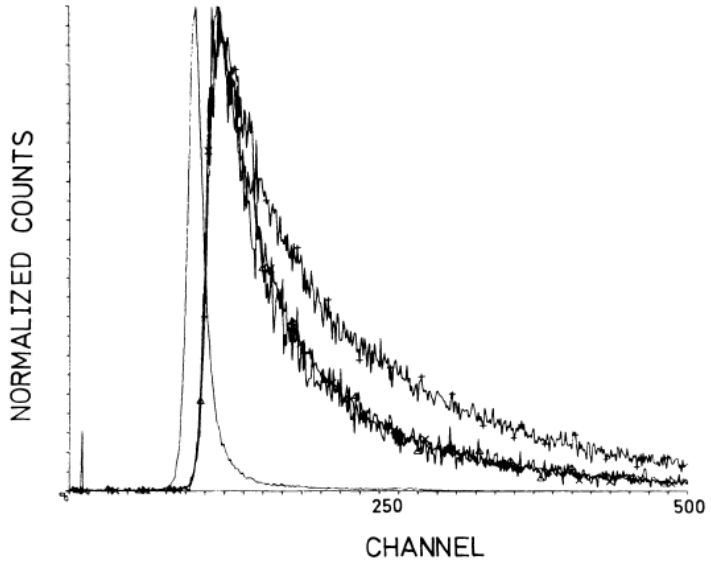


FIG. 4. Fluorescence decay curves for quinacrine bound to cytologic samples taken by microfluorometry. Decay curves were taken on the microscope as described in Materials and Methods and data were analyzed is in Table III, 0.127 nsec/channel. Solid line, flash lamp; open triangle, nuclei from a normal human XY male; ×, nuclei from *Drosophila virilis*; +, nuclei from *Samoae leonensis*.

The new laboratory based FLIM instruments were first reported about 1989

What changed later in the 1980s?

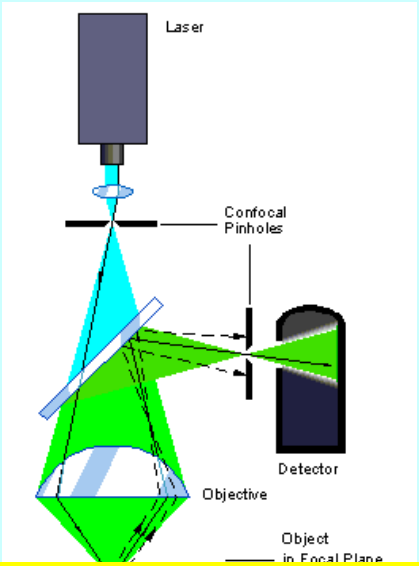
Light sources, detectors (Intensifiers, CCDs), computers, etc.

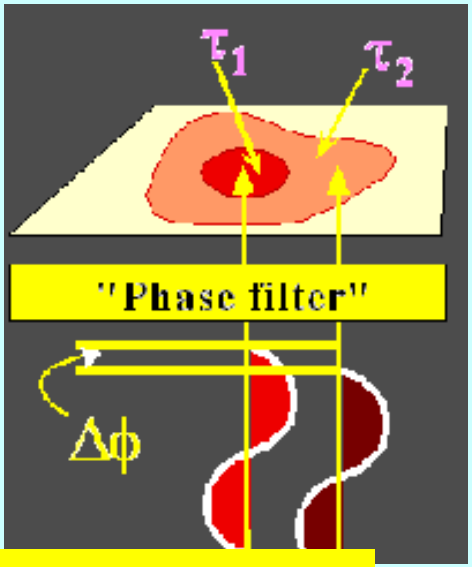
Parts became available commercially; major progress in microscopes

Commercial packages for image analysis and date handling and display

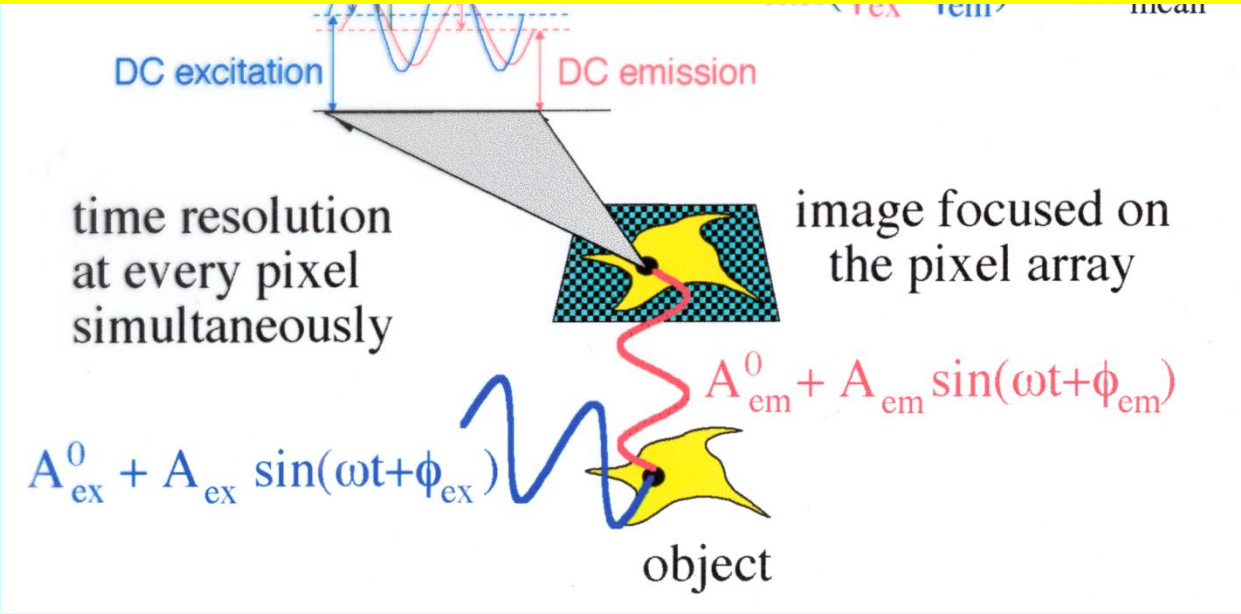
Interest grew in the biology community for quantitative imaging

2 WAYS
TO DO IT
2-hν scanning
&
full-field
FLI





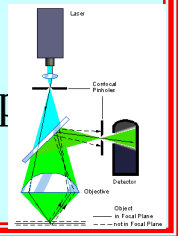
How does one we do it?



Fluorescence lifetime-resolved imaging microscopy (FLI)

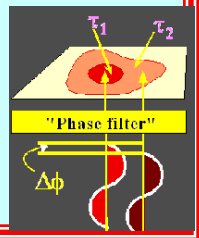
Scanning 2-hν FLI

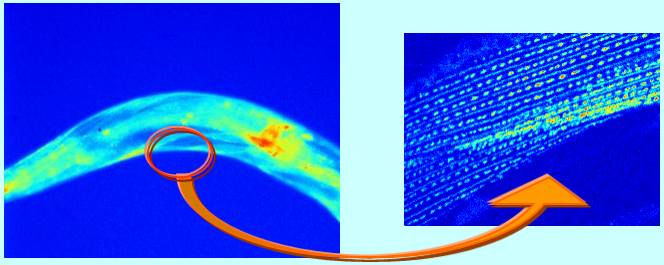
- Spatial confinement of excitation-diffraction limited focussing
  0.3 m x 1 m ($h\nu_{ex}$=700 nm, NA=1.3)
- confocal effect
- Little or no photodamage outside of 2-hν region
- Depth of penetration
- 3-D images possible
- UV-excitation (localized)
- PM detection - multifrequencies - Fourier spectrum
- Detection straight forward
- Photoactivation of caged comp

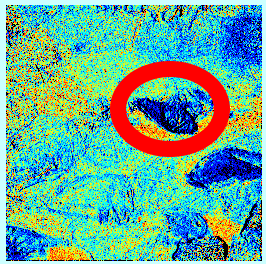


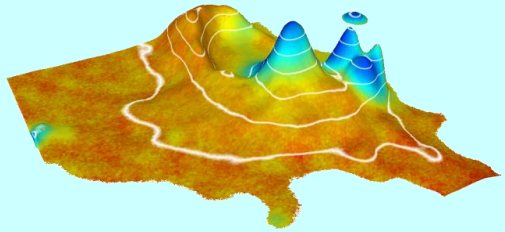
Full-field FLI

- Simultaneous pixel measurement
- Attach to any microscope
- Simplicity of optical construction & operation
- FLIE (endoscopy)
- Real-time applications
- CCD data acquisition (long integration times possible without unreasonable total measurement time)
- Phosphorescence (DLIM)
- 3-D possible with image deconvolution; spinning disk
- Rapid time resolution for kinetics in millisecond range.



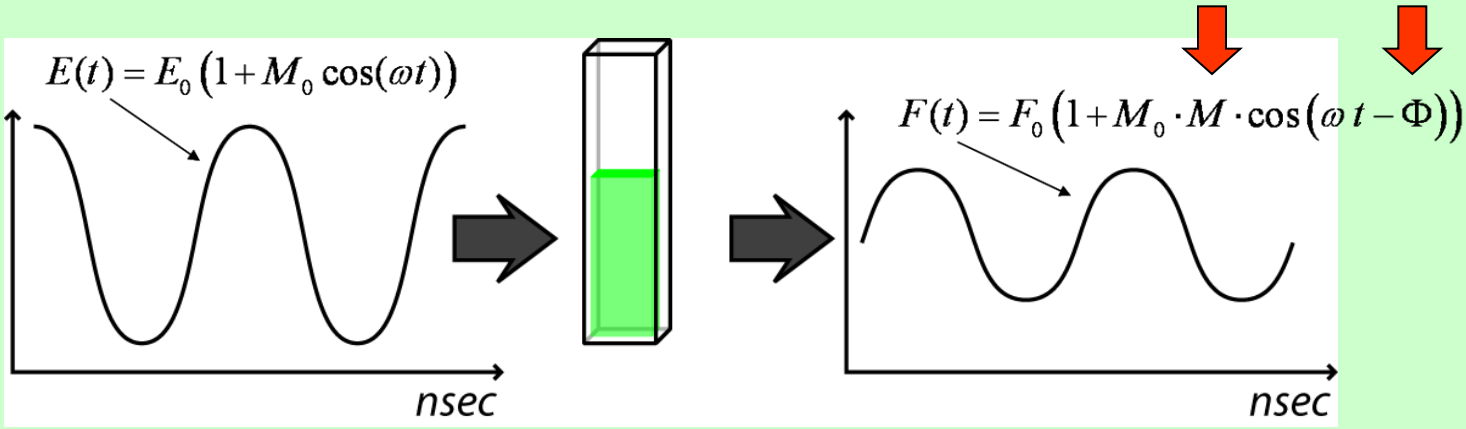






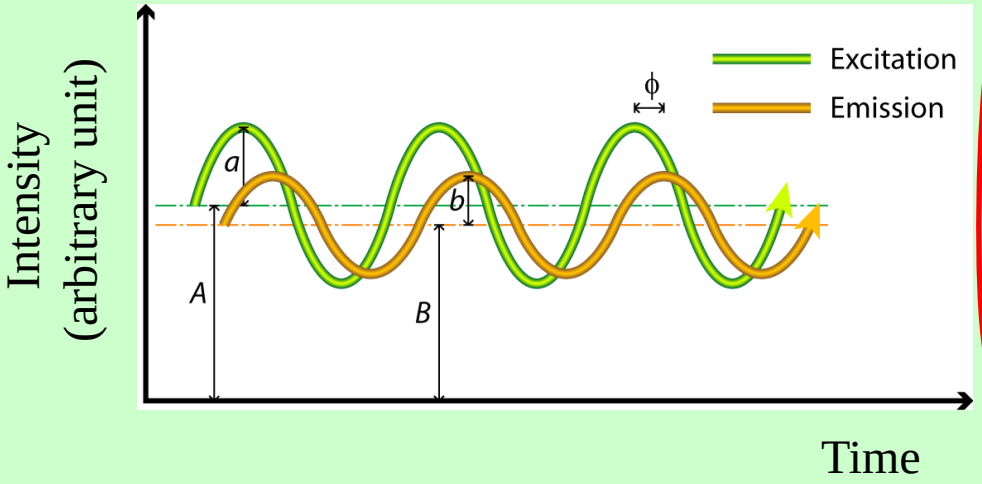
We will discuss only
full field imaging
and
the frequency domain

Frequency domain lifetime measurement



The modulation and phase are measured quantities

For single lifetime component

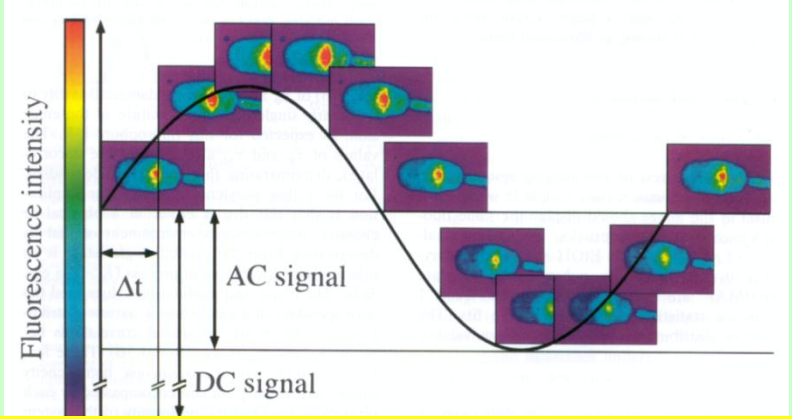


$$M = \frac{b/B}{a/A} = \frac{1}{\sqrt{1+(\omega\tau)^2}}$$

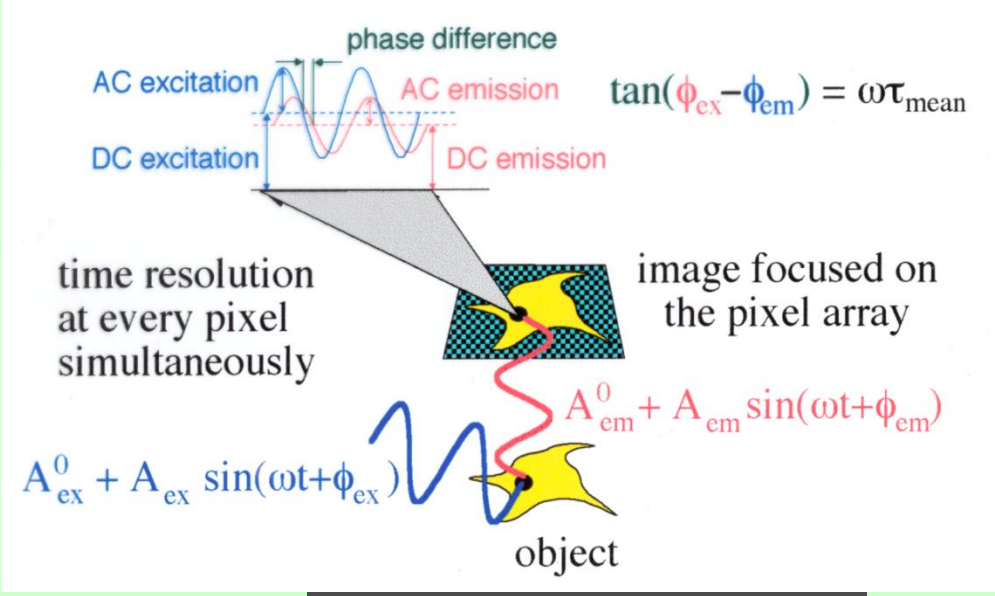
$$\phi = \tan^{-1}\left(\omega\tau_\phi\right)$$

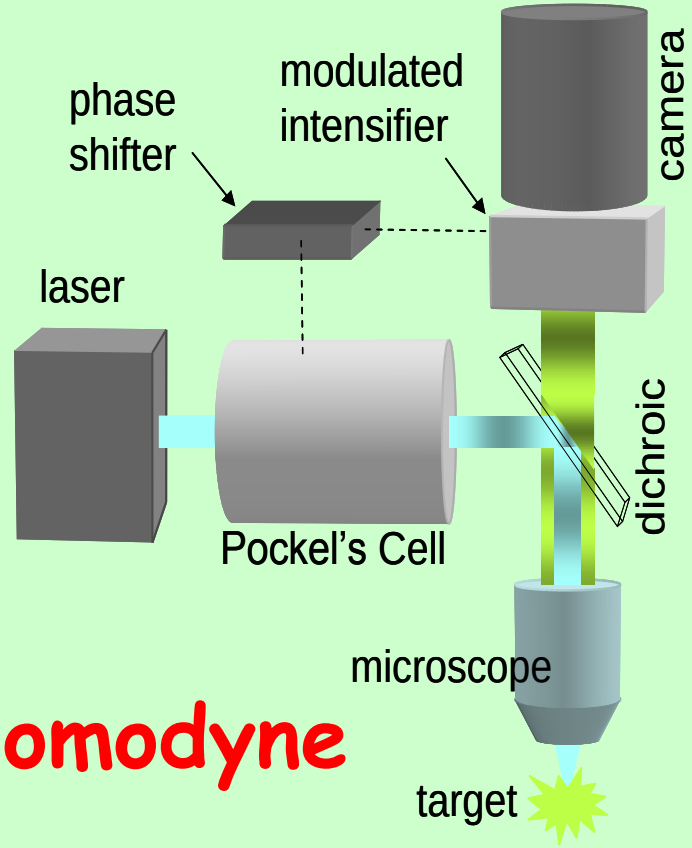
Remember this function

$$\frac{1}{1+i\omega\tau} = Me^{-i\tan^{-1}(\omega\tau)}$$



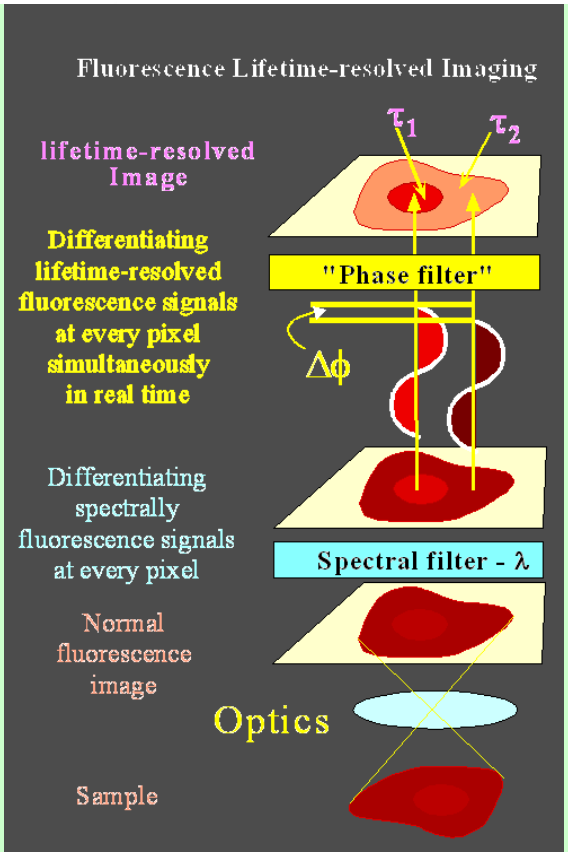
Boar sperm labeled with a lifetime dye molecule; Note the variation of the fluorescence intensity over the period of the excitation modulation.





Homodyne

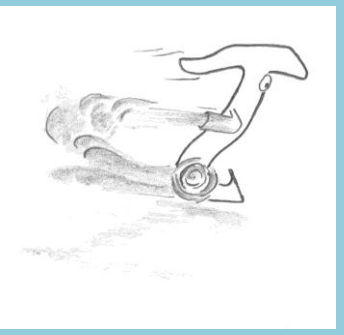
FULL FIELD FLIM



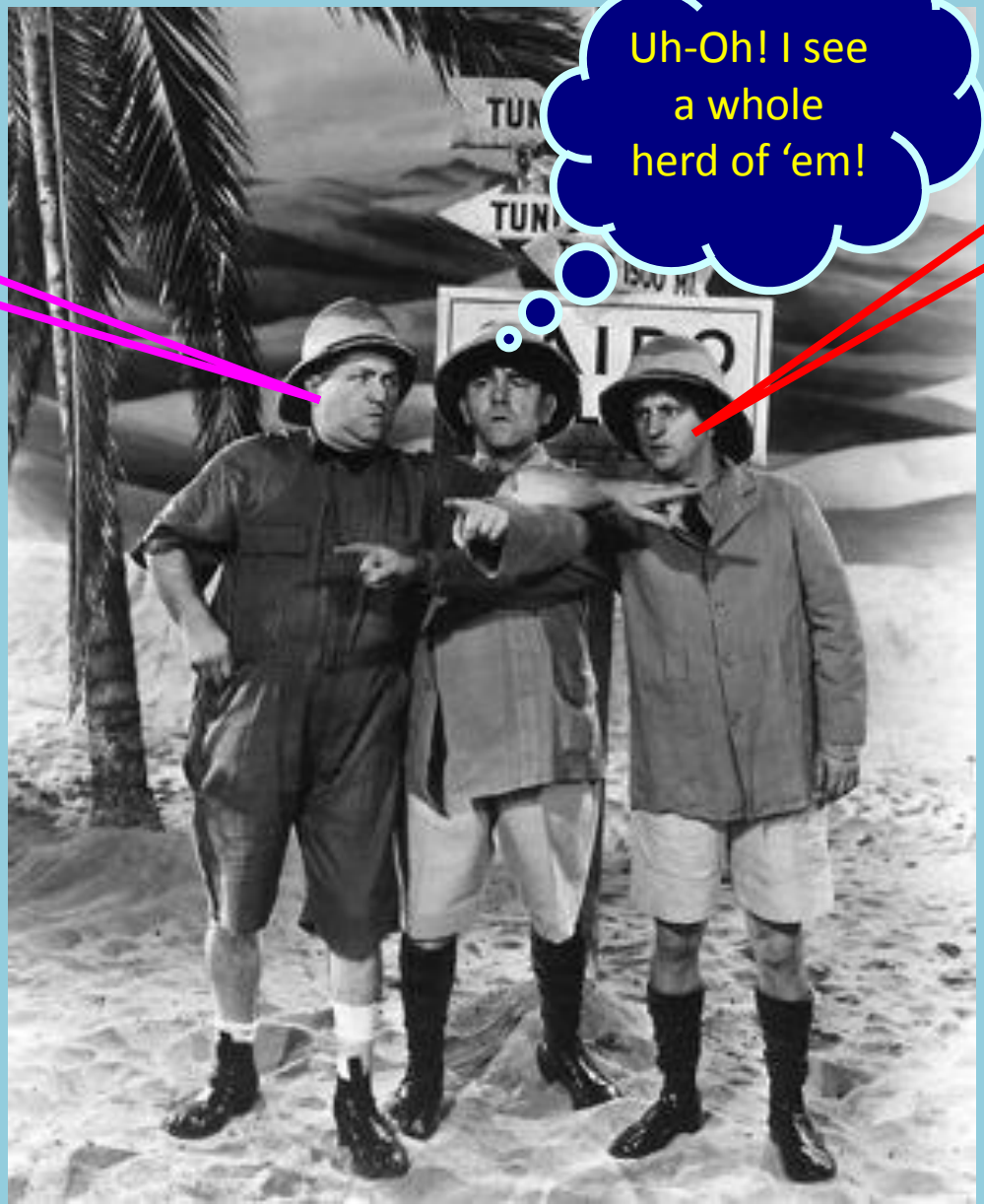
Frequency domain is convenient for acquiring data fast

SO, now we seem all set.

BUT how about the analysis?.



I see three exponentials!



exponentials!

Usually there are several lifetime components
- In an image we have 10^5 – 10^6 pixels-

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

Fluorescence response

Time-domain (notorious non-orthogonality of exponentials)

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

What now?

Lifetimes in images are not so simple!

We need some help!

APRIL, 1941 JOURNAL OF CHEMICAL PHYSICS VOLUME 9

Dispersion and Absorption in Dielectrics

I. Alternating Current Characteristics*

KENNETH S. COLE, *Department of Physiology, Columbia University, New York, New York*

Complex dielectric constant → $\epsilon^* = \epsilon' - i\epsilon''$ → $z = x + iy = |z|e^{i\theta}$

ROBERT H. COLE, *Research Laboratory of Physics, Harvard University, Cambridge, Massachusetts*

(Received February 4, 1941)

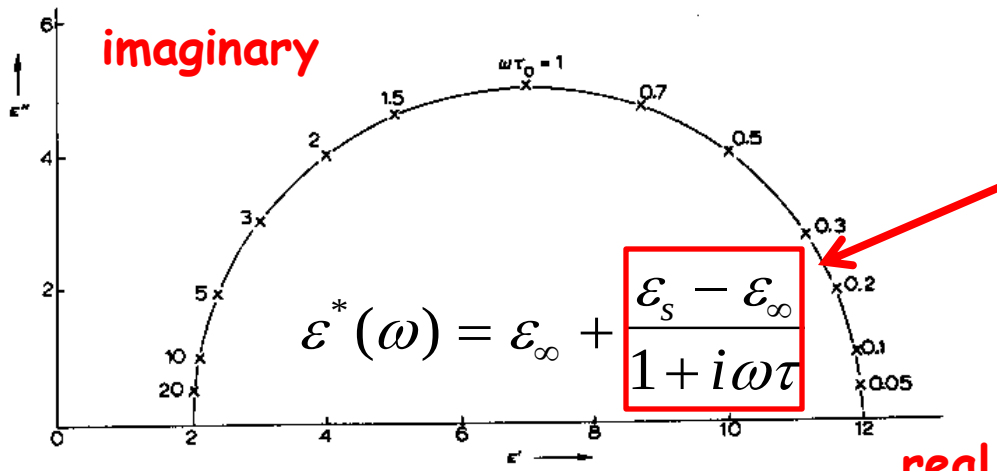


Fig. 48
Cole–Cole plot for a single relaxation time (calculated).

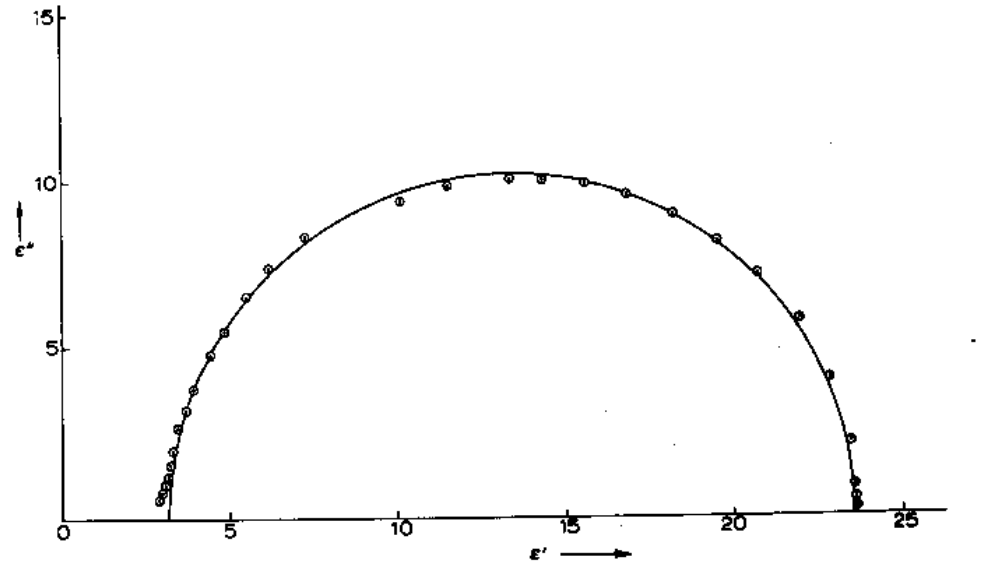


Fig. 49
Cole–Cole plot for heptanol-2 at −61.5°C.

$$\propto \frac{1}{1+i\omega\tau}$$

Cole-Cole plot for dielectric dispersion with a single relaxation time

For the case of a single relaxation time the points $(\varepsilon', \varepsilon'')$ lie on a **semicircle** with center on the ε' axis and intersecting this axis at $\varepsilon' = \varepsilon_s$ and $\varepsilon' = \varepsilon_\infty$.

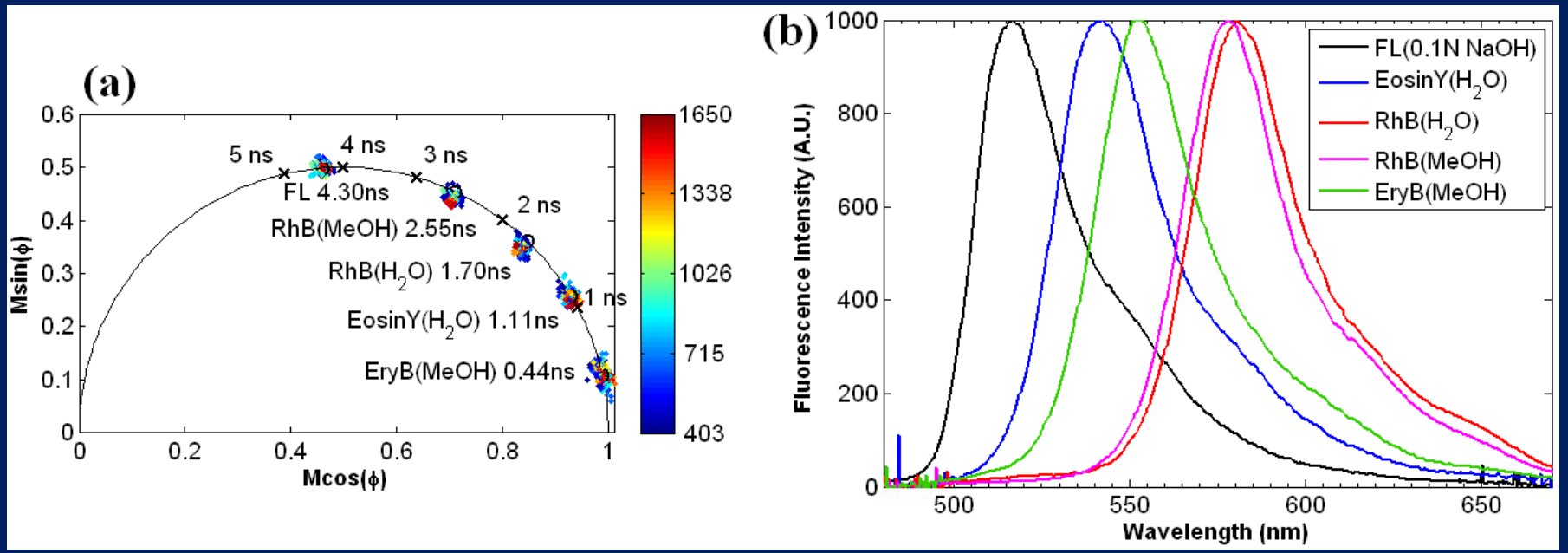
Model Independent Analysis Some different ways to parameterize lifetime-resolved data

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

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

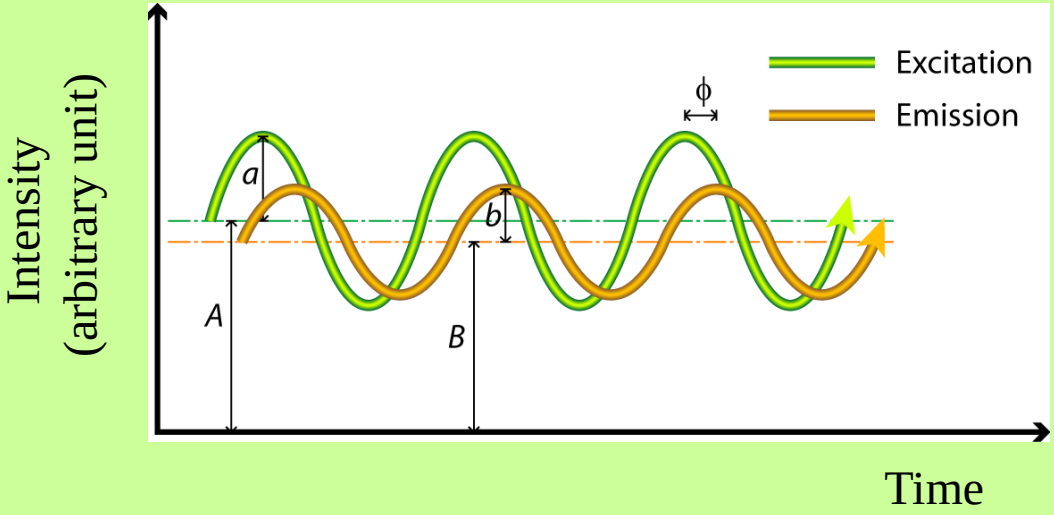
$$j = \sqrt{-1}$$

Near single fluorescence lifetimes belong on the semicircle throughout the emission spectrum



We call this a "polar plot"

Fine and good for a single lifetime component



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

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

But

What about multiple lifetimes?

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

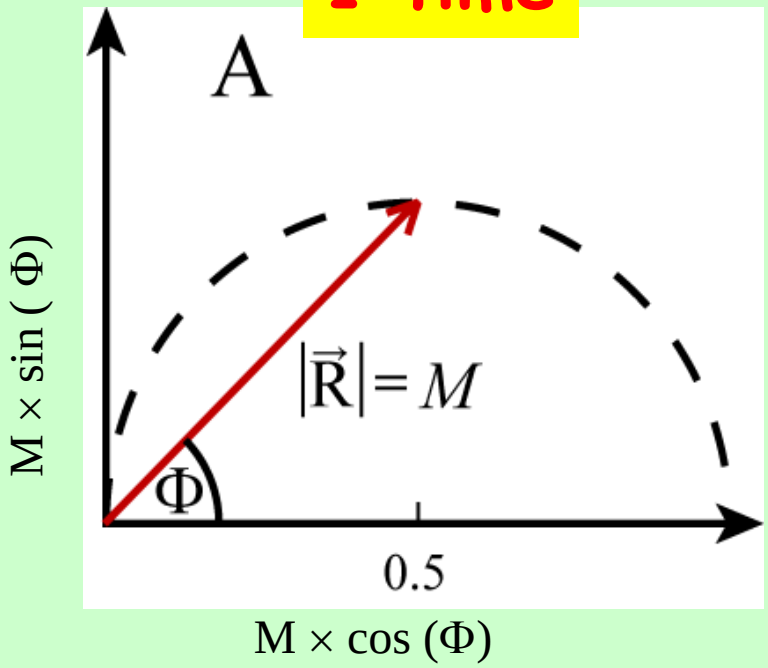
Frequency domain lifetime measurement

Data analysis with a **polar plot** representation

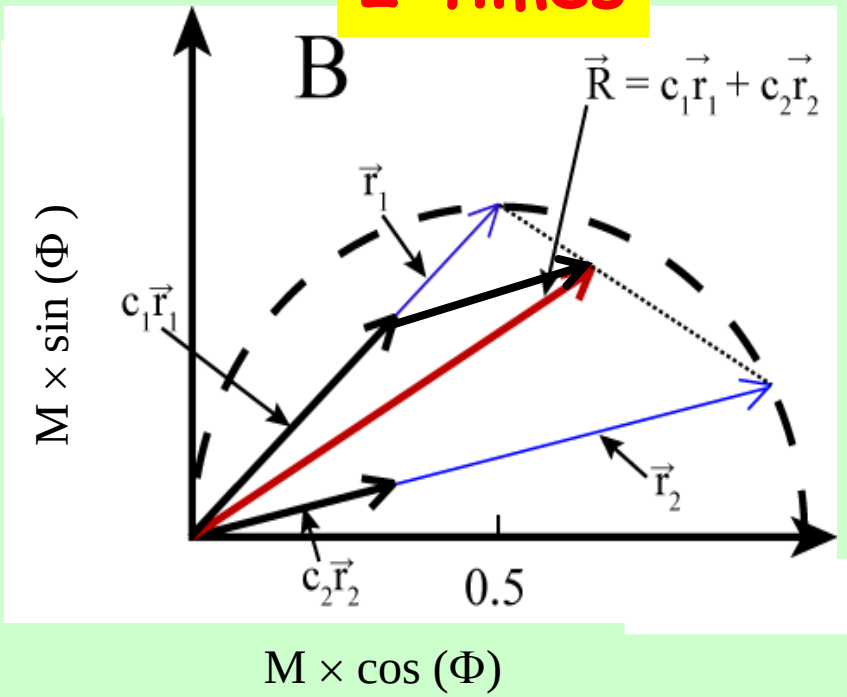
Demodulation = $M = \frac{b/B}{a/A} = \frac{1}{\sqrt{1+(\omega\tau_M)^2}}$

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

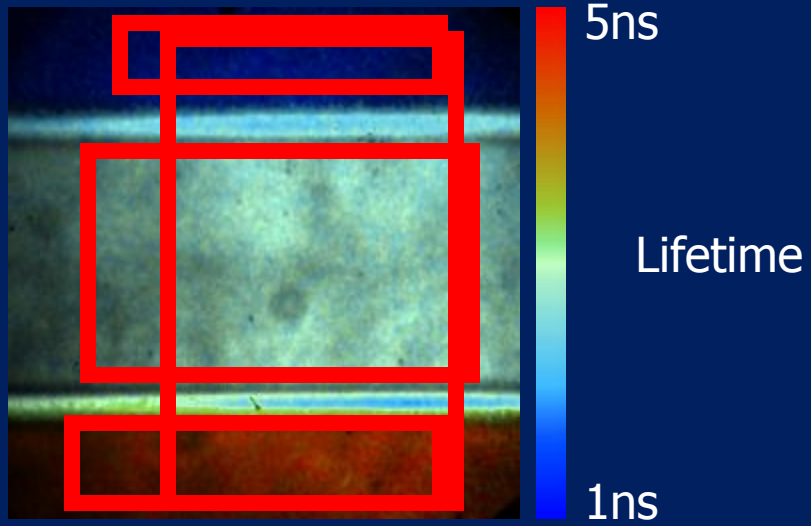
1 time



2 times



good for any signal $\propto \sum_j \frac{1}{1+i\omega\tau_j}$ (for instance dielectric dispersion)

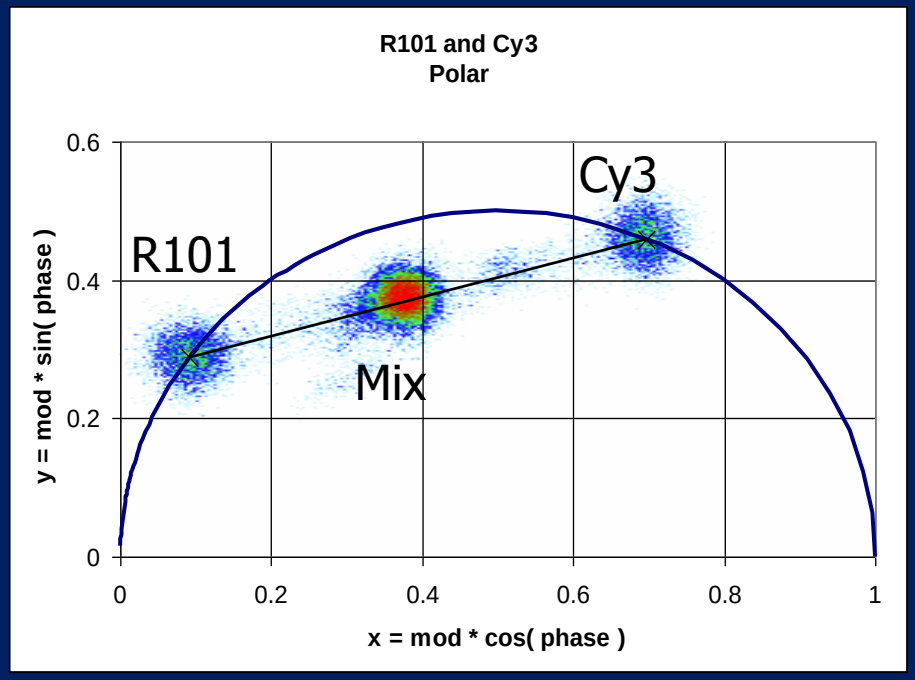


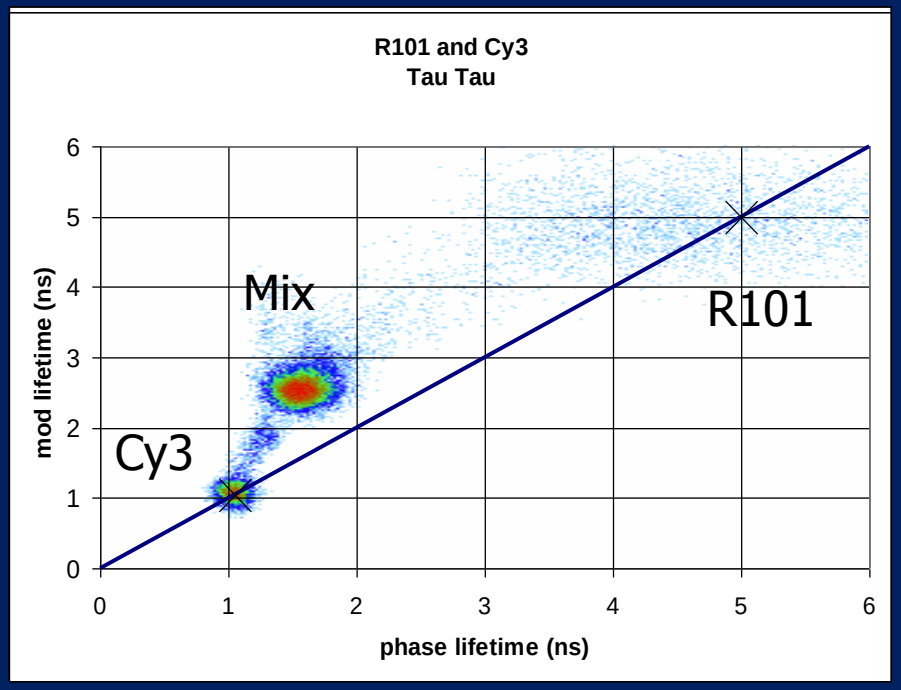
Model free data projection
No fitting

M & φ Measured parameters

$$M = 1/\sqrt{1+(\omega\tau)^2} \qquad \varphi = tan^{-1}(\omega\tau)$$

$$x = M \cdot cos(\varphi) \qquad y = M \cdot sin(\varphi)$$



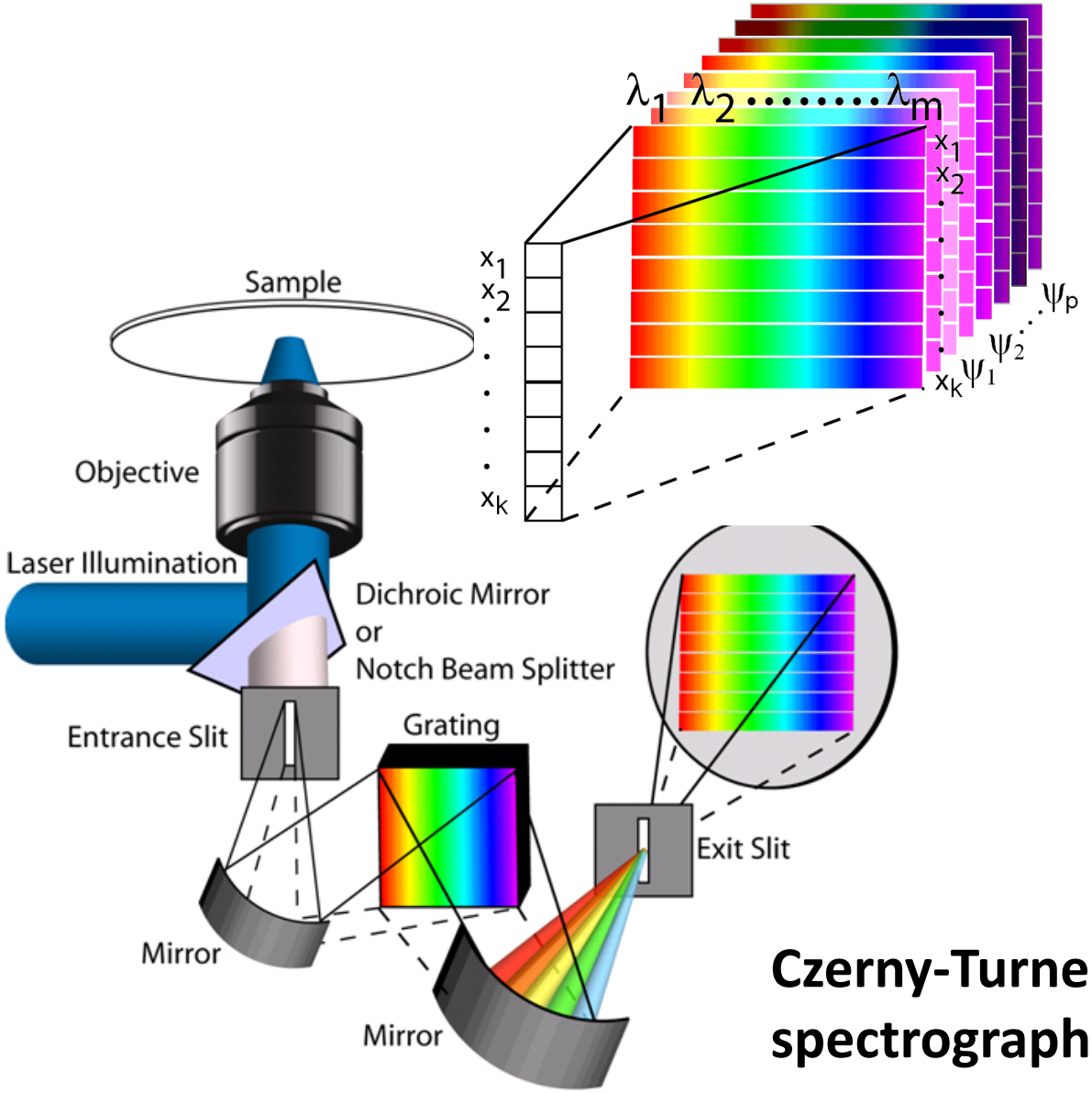


One more thing:

Spectral FLIM

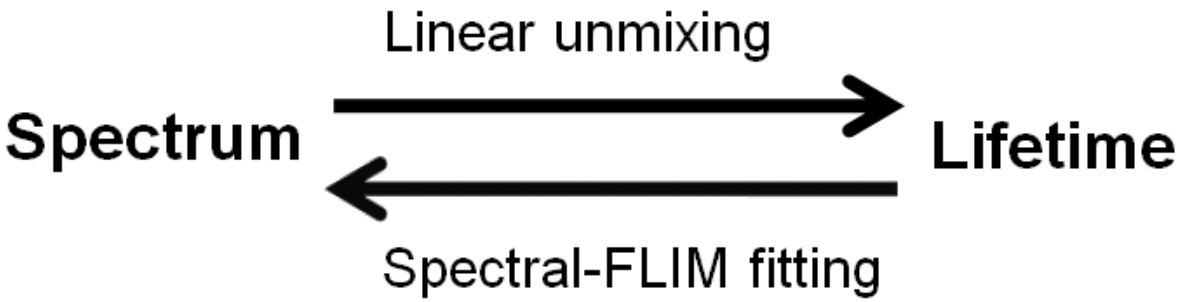
spectral dispersion
of fluorescence emission
is environmentally sensitive

Spectral-FLIM



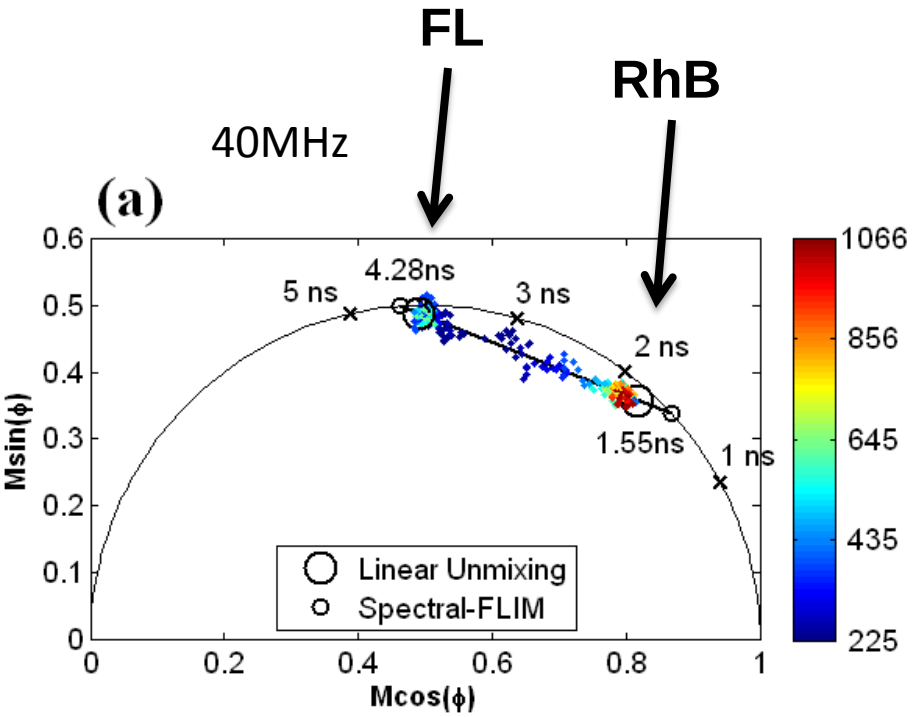
Czerny-Turner spectrograph

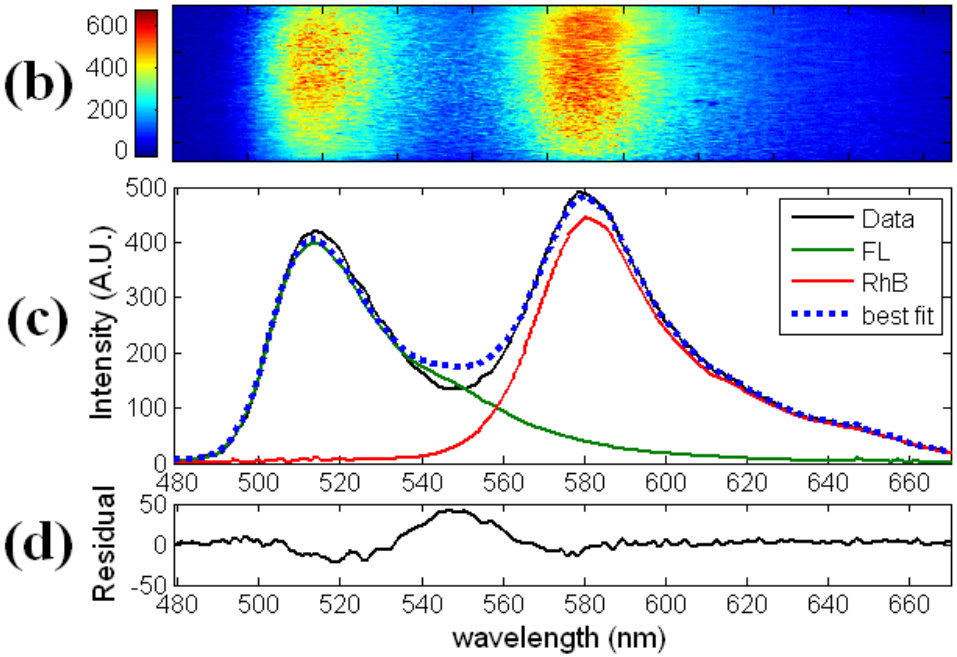
Spectral-FLIM + polar plot



(b) The Spectral-FLIM data at one of the phase shift on the ICCD

(c) The spectrum calculated from (b) and the linear unmixing results.



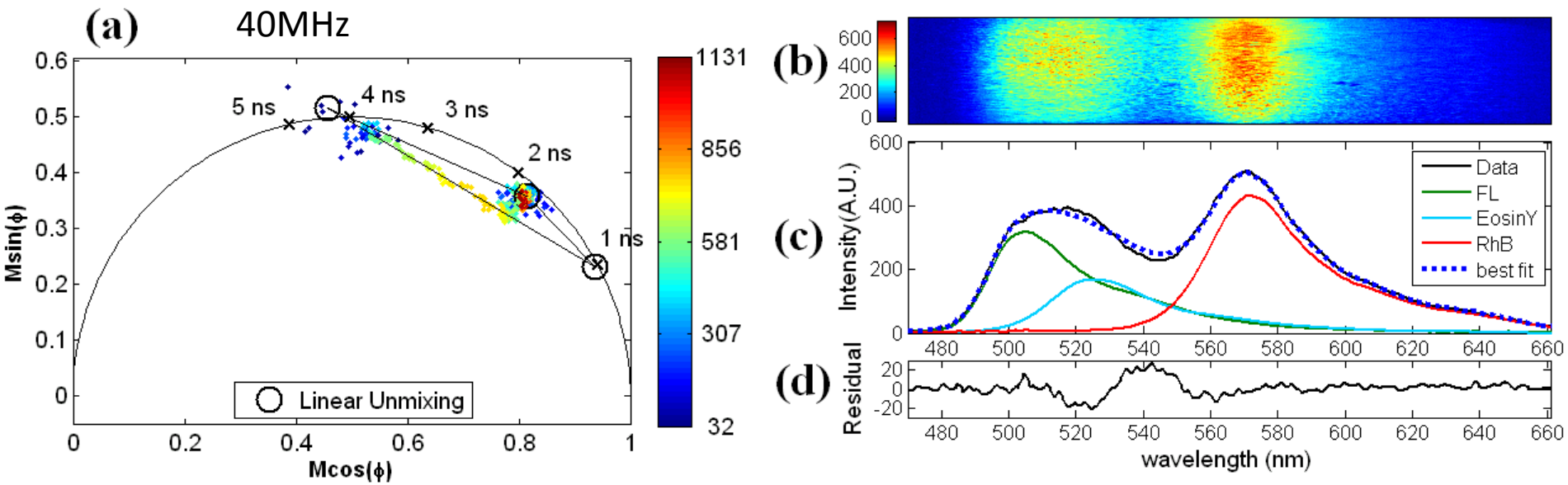


Spectral-FLIM + polar plot

Separate multiple (3) lifetimes.

(b) The Spectral-FLIM data at one phase shift on the intensifier

(c) The spectrum calculated from (b) and the linear unmixing results.



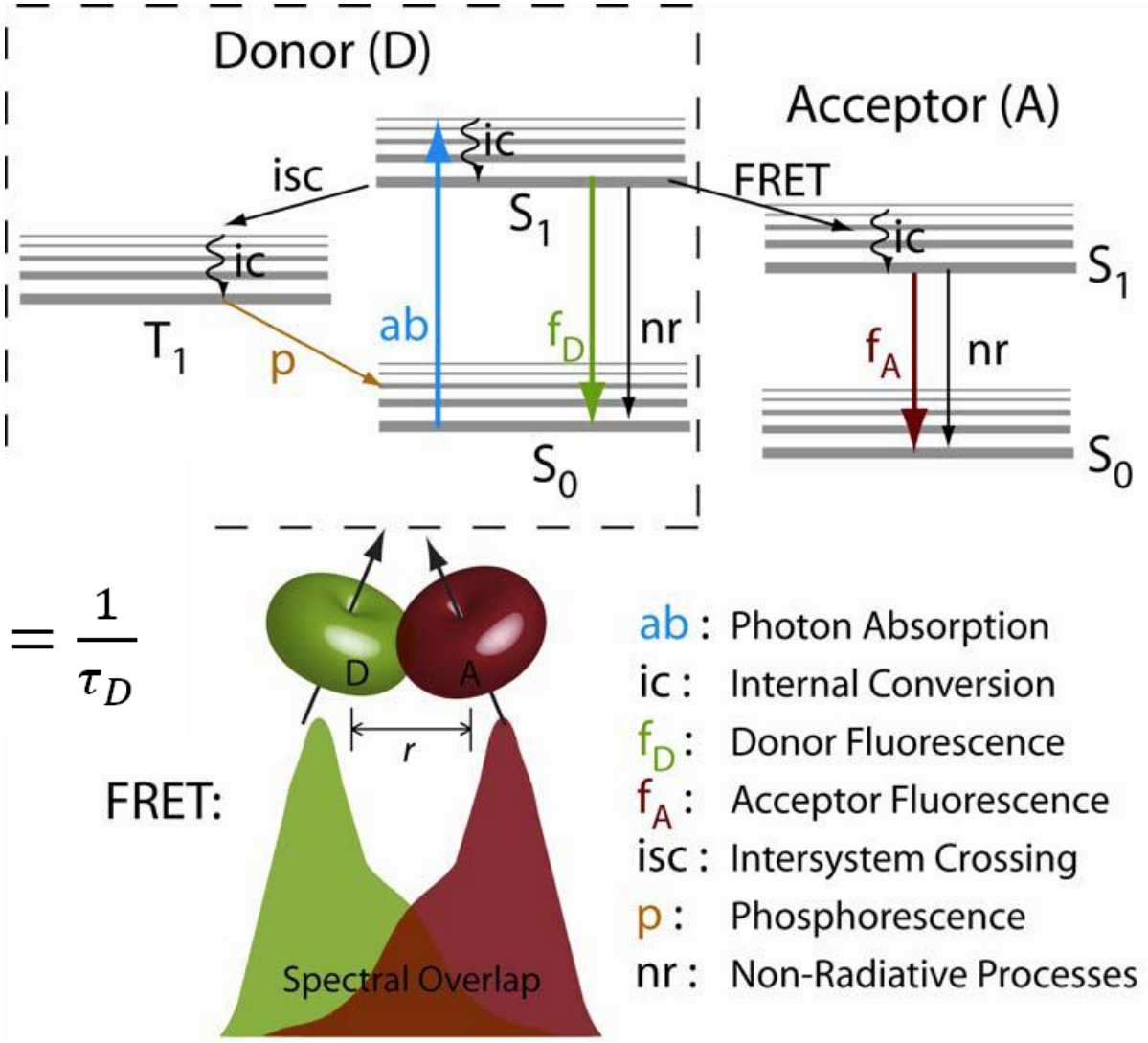
Förster Resonance Energy Transfer

Molecular ruler :
< 10 nm

$$k_{et} = \frac{1}{\tau_{D,0}}\left(\frac{R_0}{r}\right)^6$$

$$k_D = k_{D,0} + k_{et}$$

$$= \frac{1}{\tau_{D,0}}\left[1 + \left(\frac{R_0}{r}\right)^6\right] = \frac{1}{\tau_D}$$

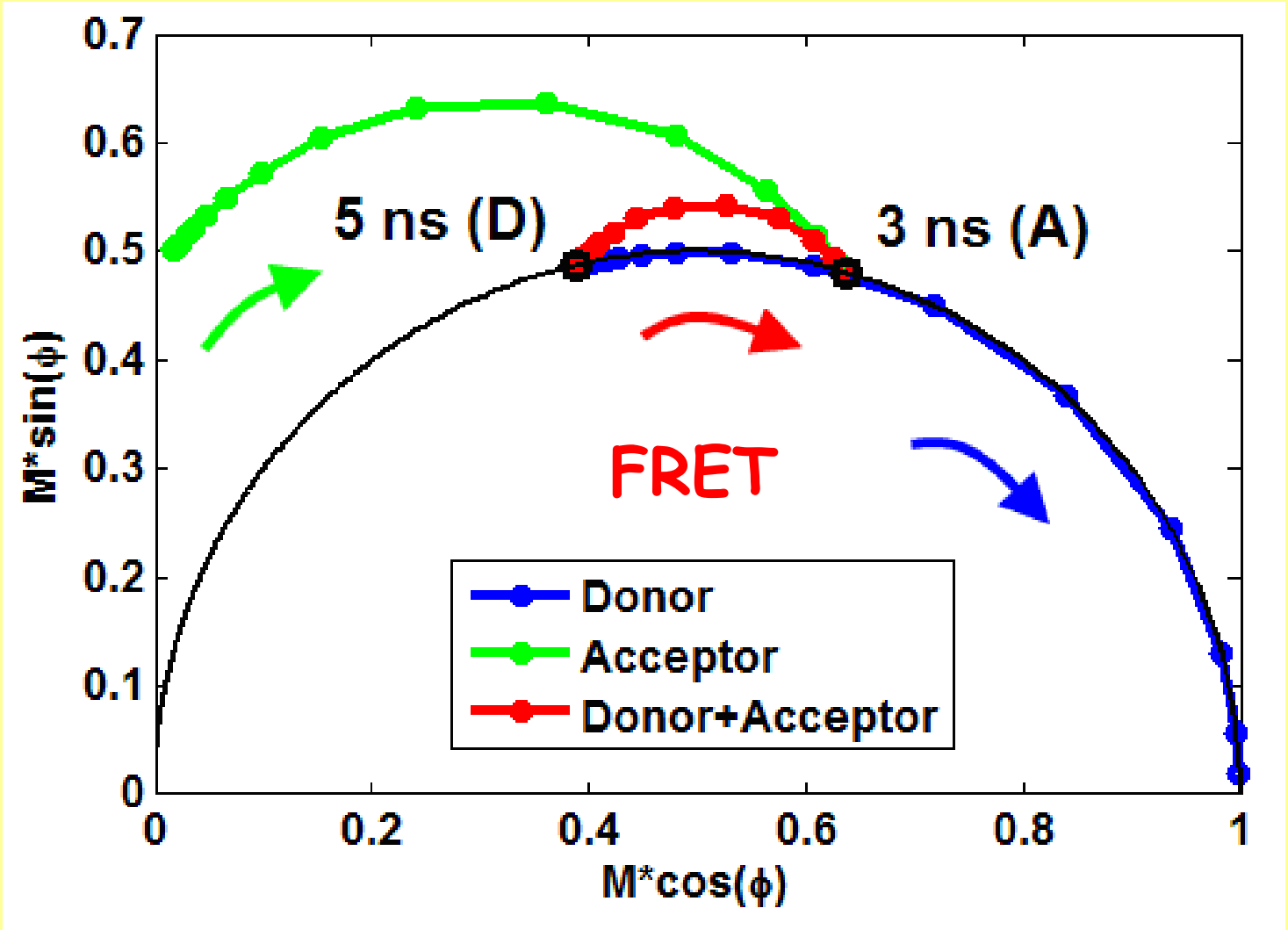


Observing the fluorescence of:

Product species of an excited state reaction

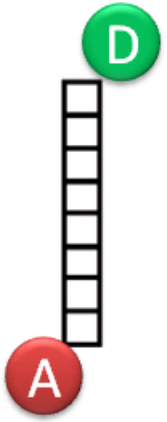
Product and directly excited species

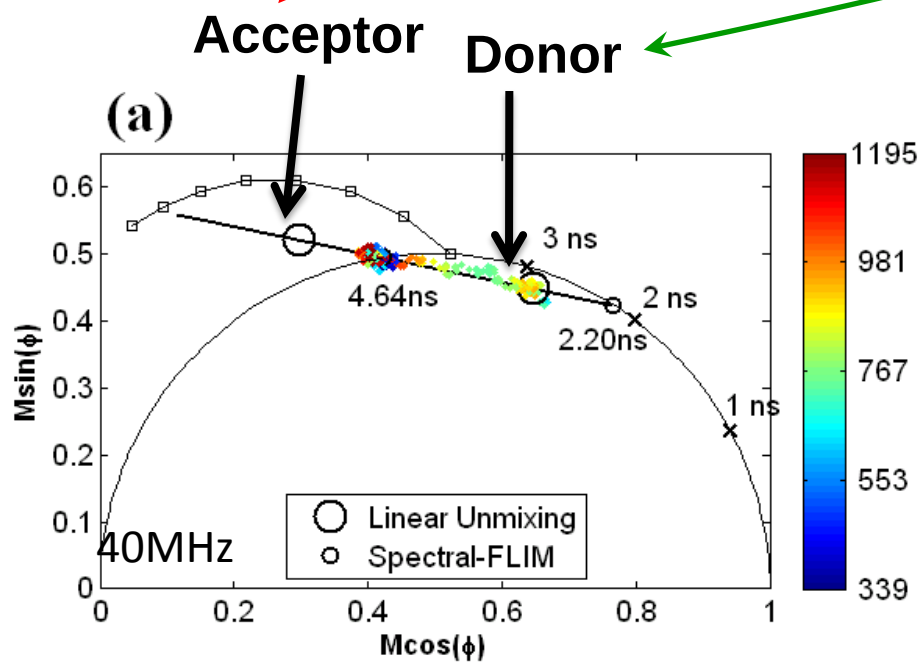
Directly excited species

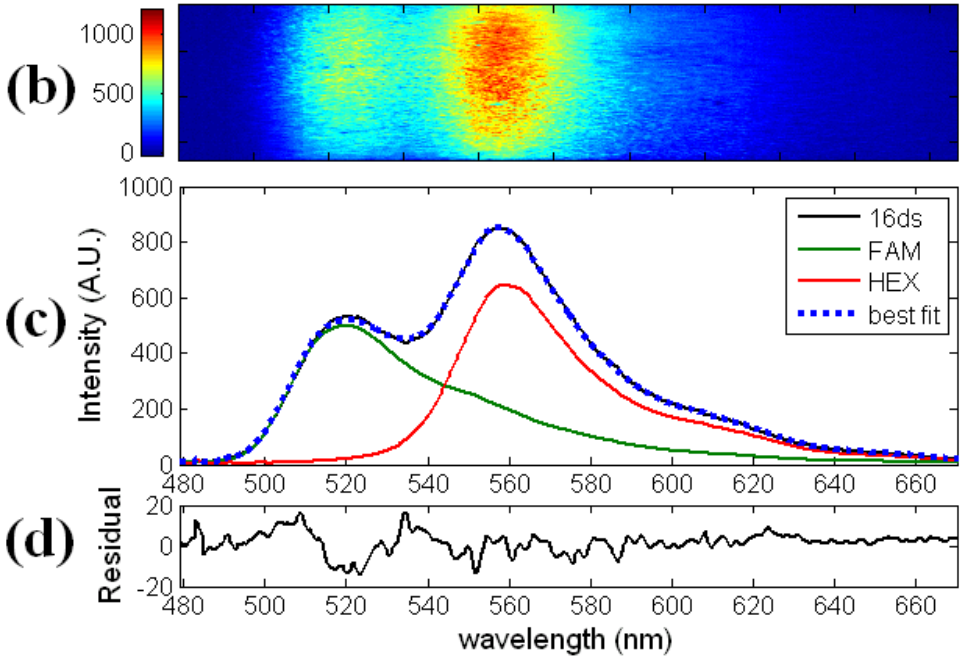


Spectral-FLIM/FRET data

- Unmix donor and acceptor spectra.
- Fourier analysis of separate D & A images
- Advantage : FRET efficiency calculated more accurately

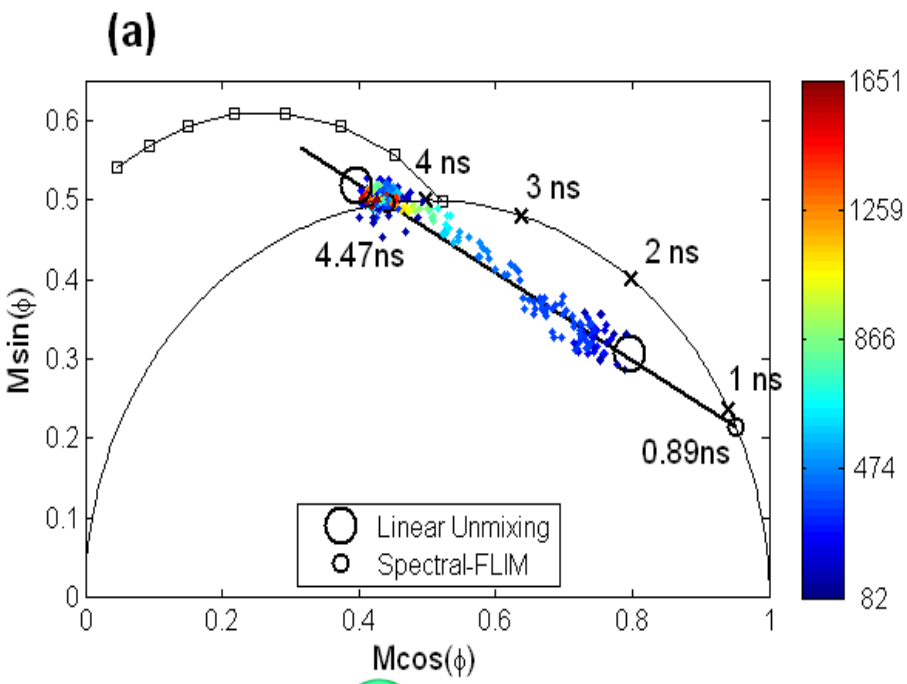


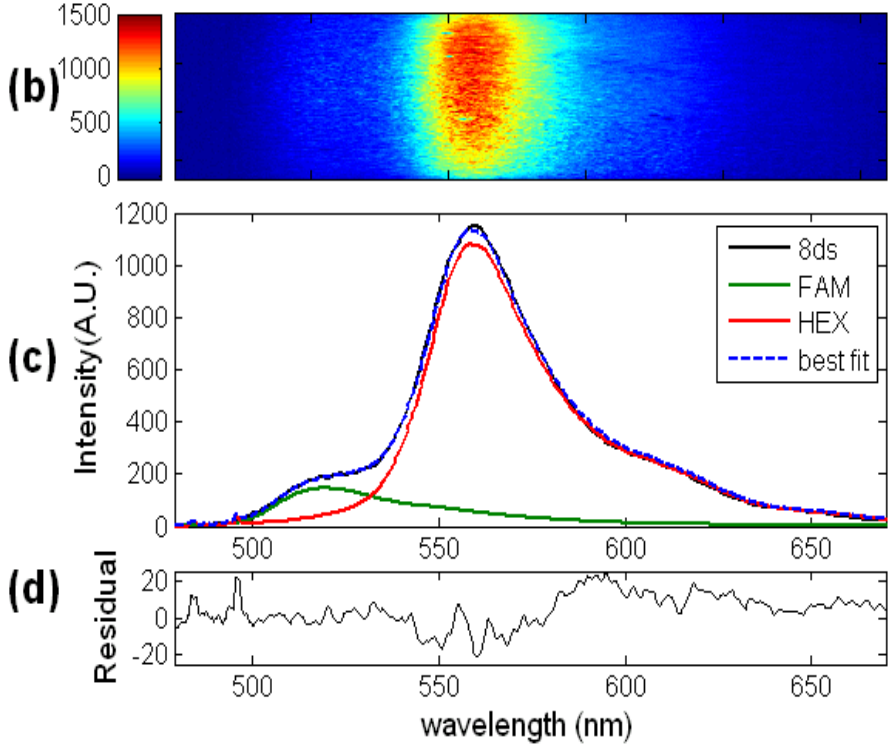


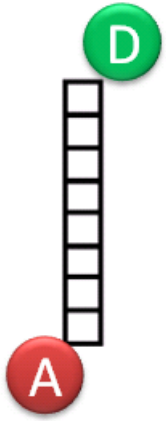


Spectral-FLIM/FRET data

Hex - 8mer - FAM





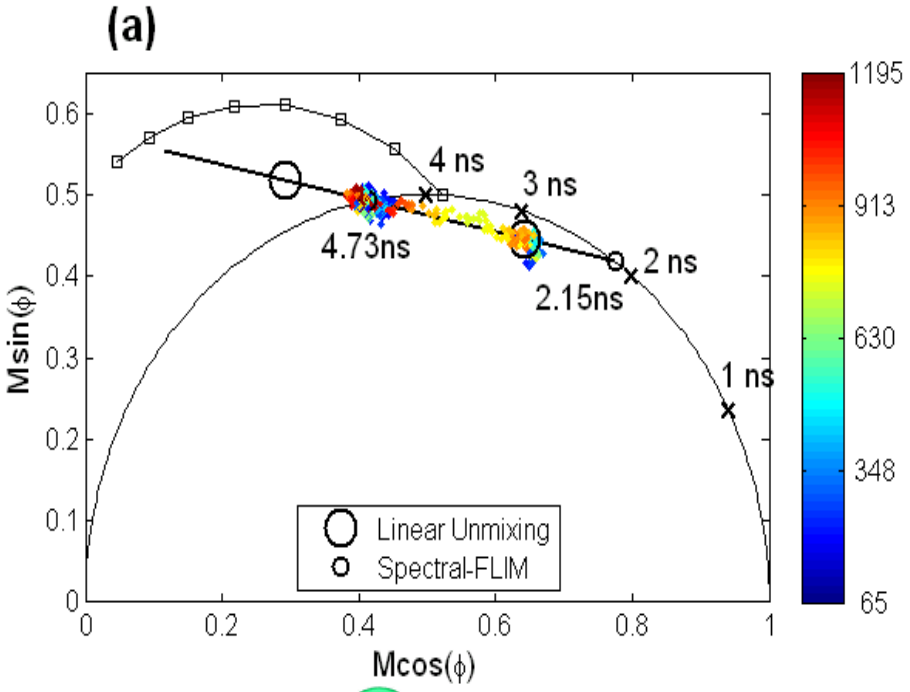


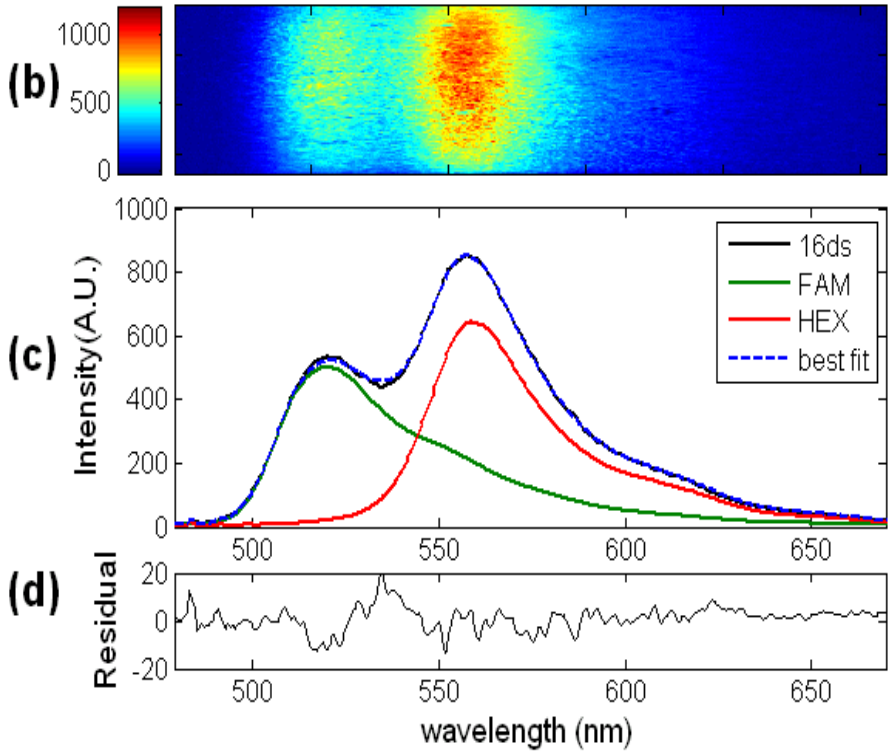
8mer

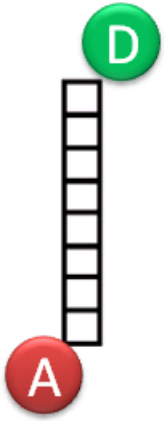
E=0.77

Spectral-FLIM/FRET data

Hex - 16mer - FAM





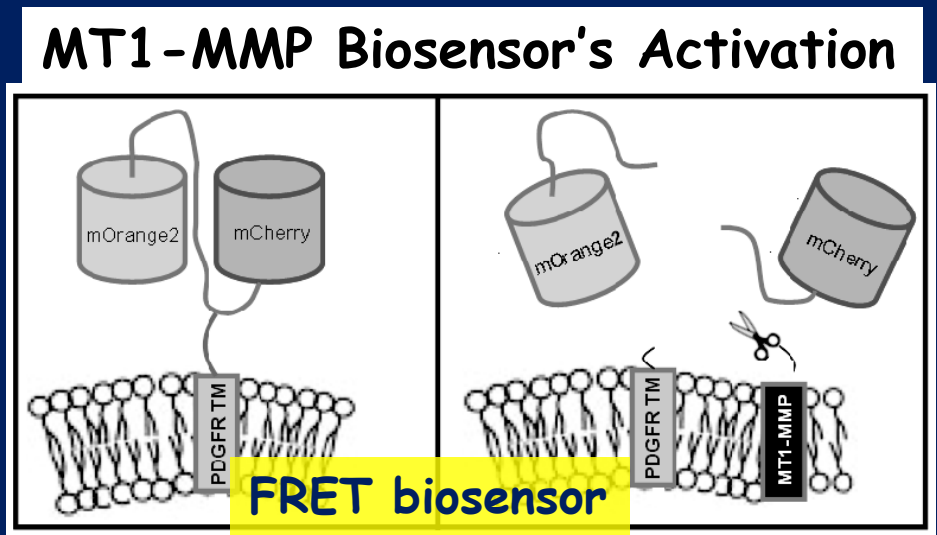


16mer

E=0.44

Membrane Type 1 Matrix Metalloproteinase (MT1-MMP)

- destruction of ECM (extracellular matrix) proteins
- transmission of signaling cascades to facilitate invasion during metastatic events
- understand the spatiotemporal activation patterns of MT1-MMP
- HT1080 cells singly transfected with the MT1-MMP biosensor.
- HT1080 cells endogenously produce MT1-MMP which cleaves the biosensor.

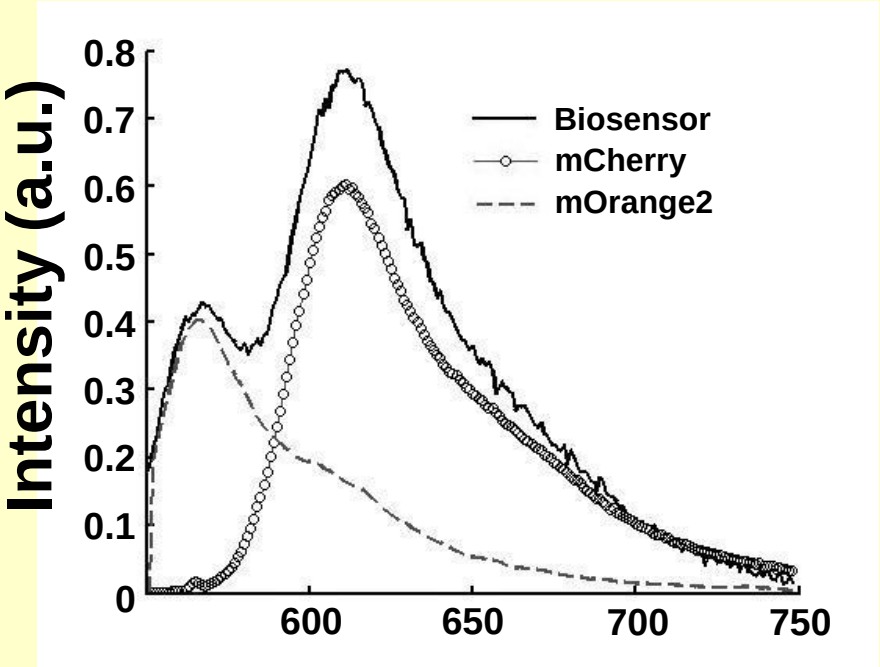


FLIM and Phase Suppression

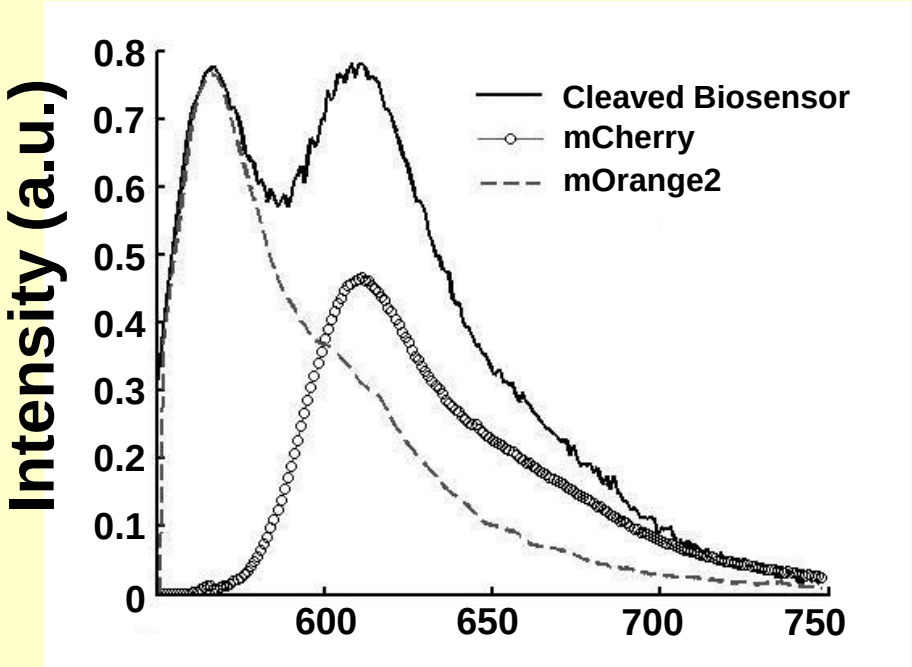
- Avoid controls
- Very rapid
- Determine concentrations
- Increase sensitivity
- Avoid steady state fluorescence artifacts

mOrange2/mCherry MT1-MMP Biosensor Emission Spectra with 532nm Excitation

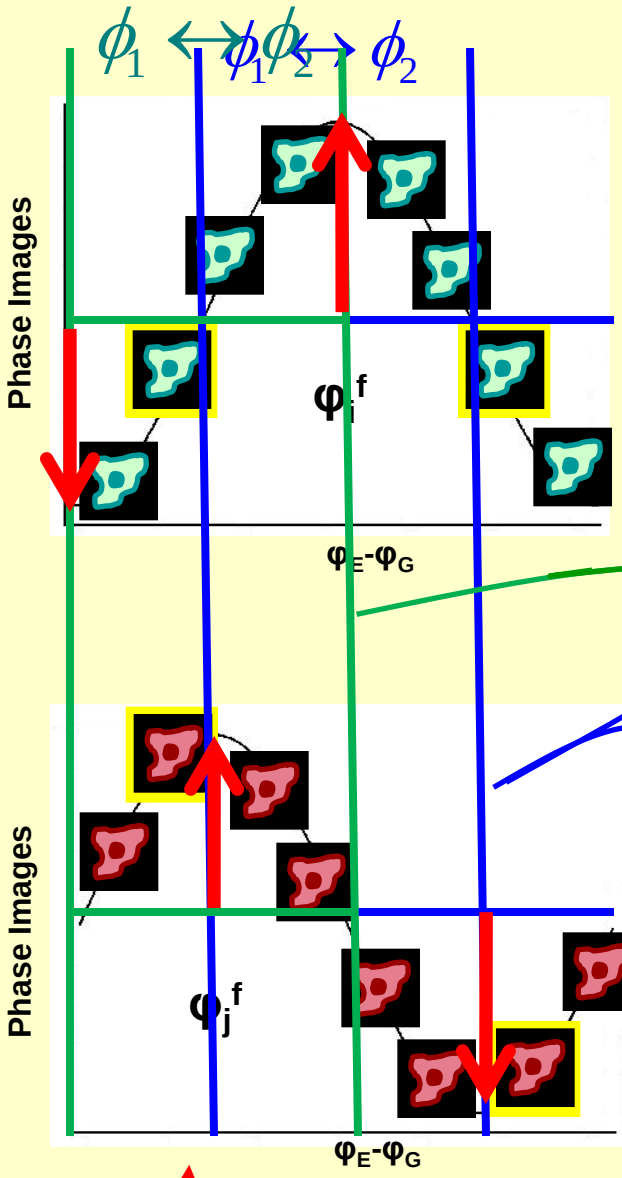
Intact Biosensor



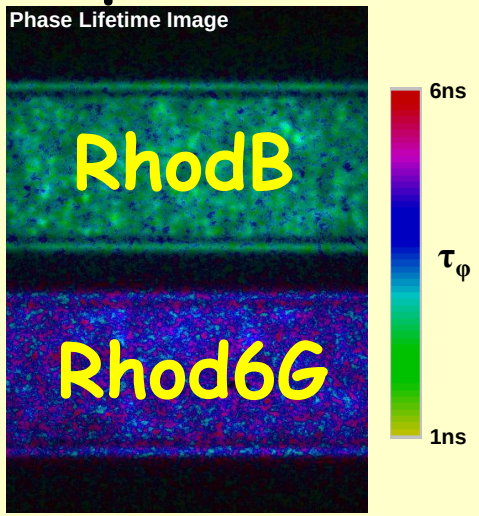
Cleaved Biosensor

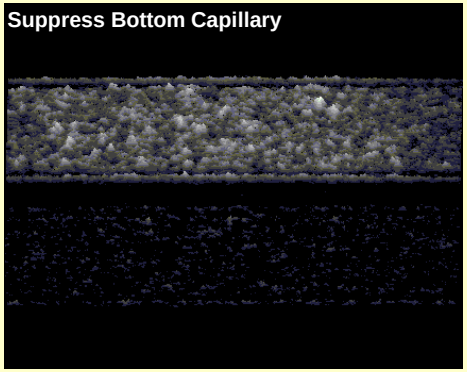


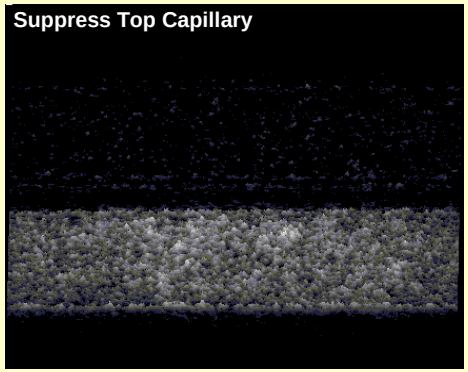
Phase Suppression (how it works)



Capillaries



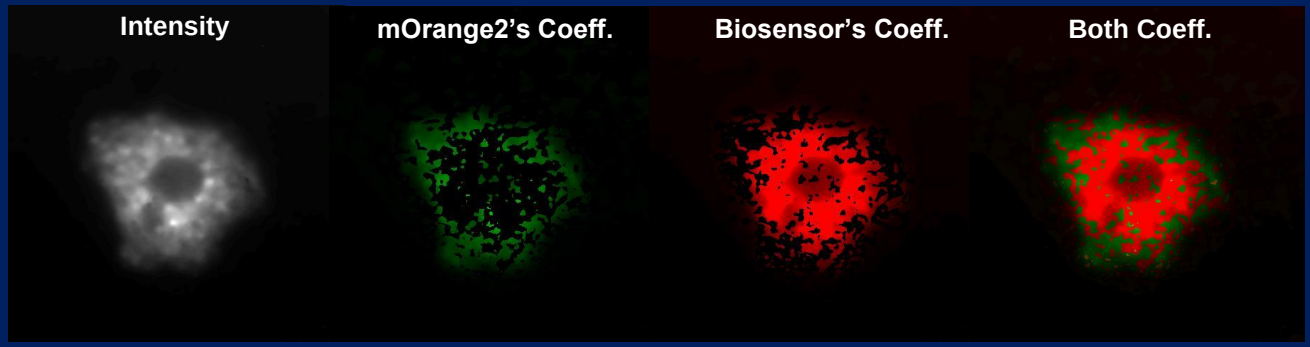




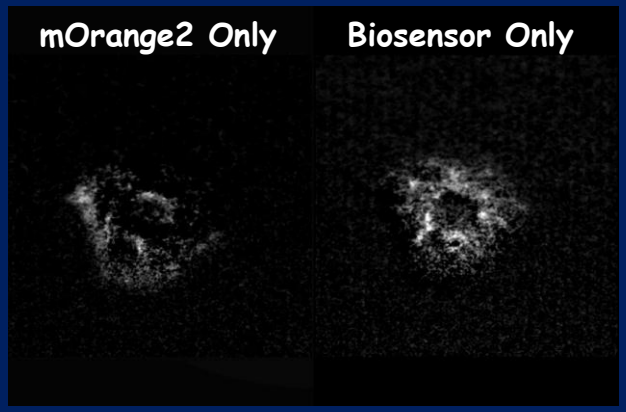
Homodyne measurements at selected phases

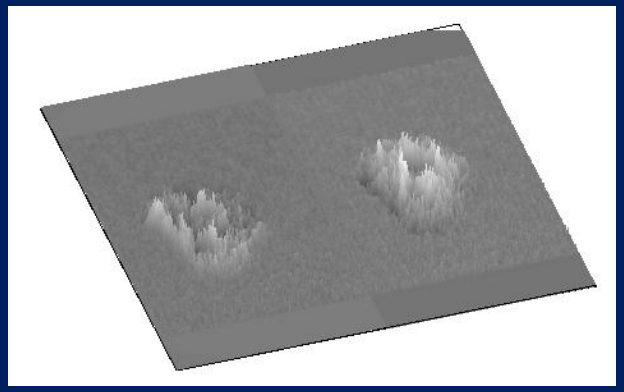
Phase Suppression of HT1080 cell (produces MT1-MMP) transfected with MT1-MMP biosensor

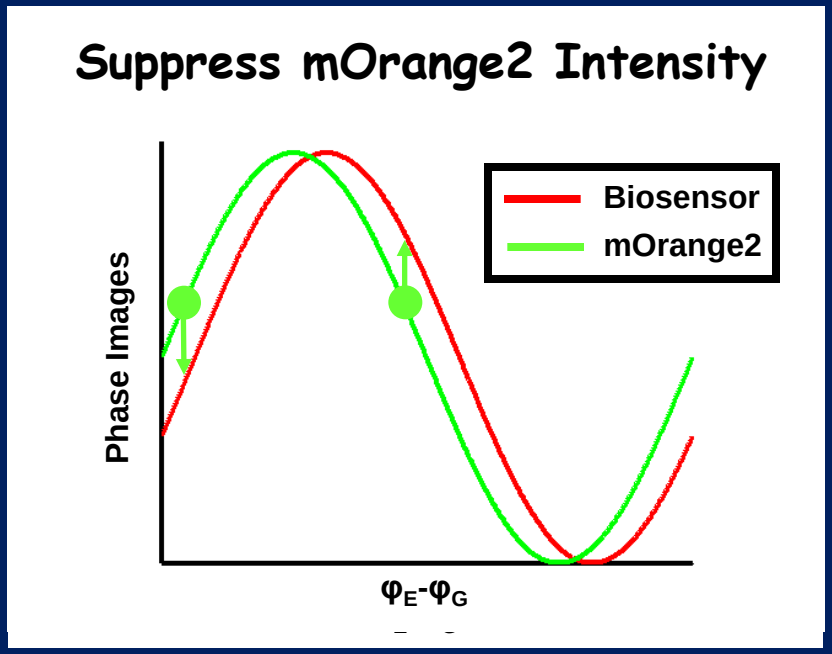
Results from Polar Plot



Results from Phase Suppression

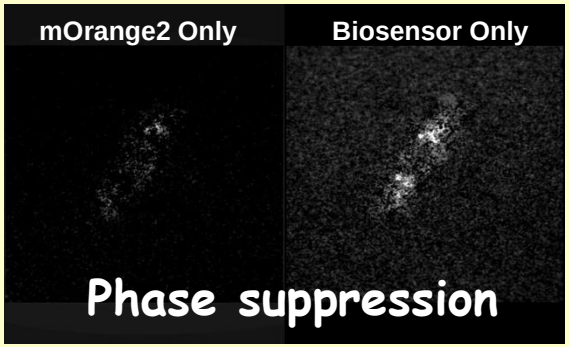


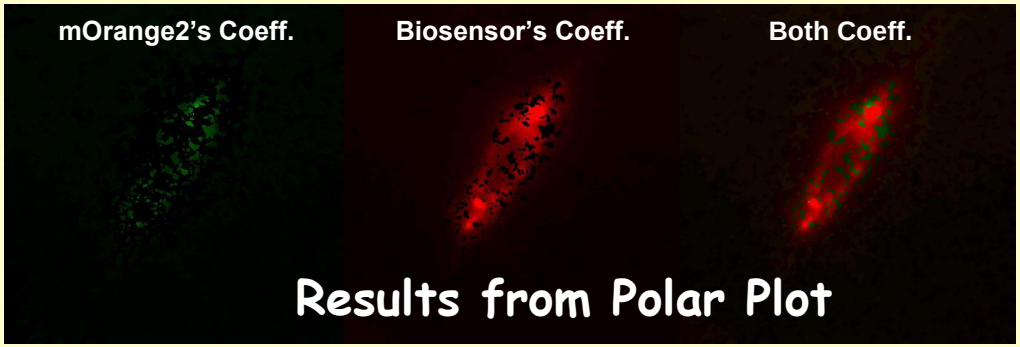


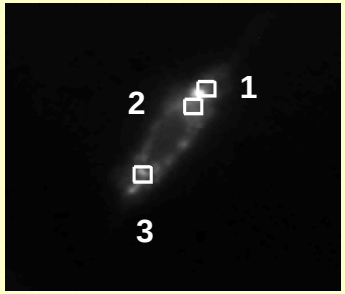


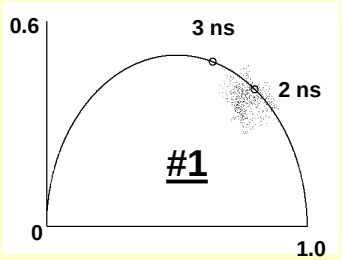
HT1080 cells transfected with MT1-MMP biosensor. Treated with the MMP inhibitor GM6001

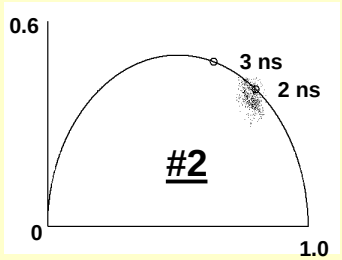
Before Wash

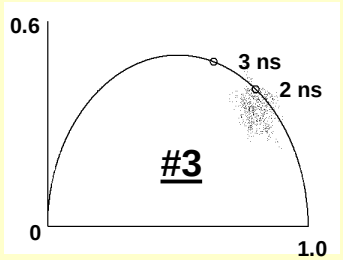




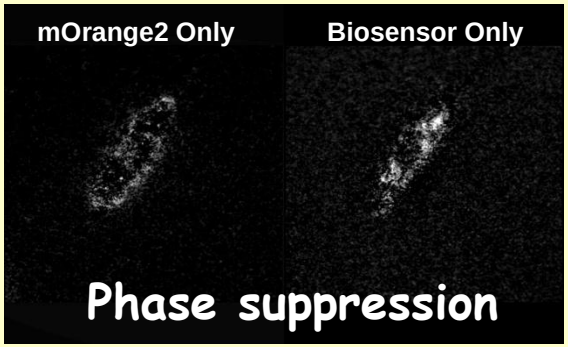


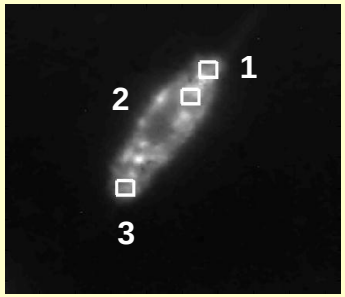


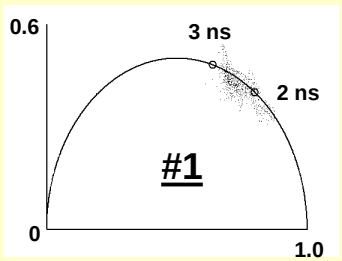


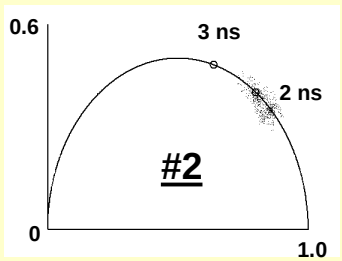


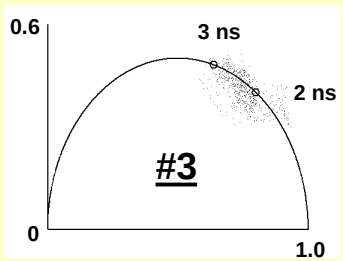
After Wash











Photosynthesis photograph taken with a leaf as film

Nature's CCD

Roger P. Hangarter & Howard Gest

Full Field FLI :

Glen Redford (UIUC)

Bryan Spring (UIUC)

Chittanon (Keng) Buranachai (UIUC)

Yi-Chun Chen (UIUC)

John Eichorst (UIUC)

Kevin Teng (UIUC)

Photosynthesis:

Govindjee (UIUC)

Shizue Matsubara, Rosanna Caliandro (Forschungszentrum, Jülich)

MT1-MMP biosensor

Peter Wang

Biopsies

Rohit Bahrgava (UIUC)