A Short History of Fluorescence

Beniamino Barbieri

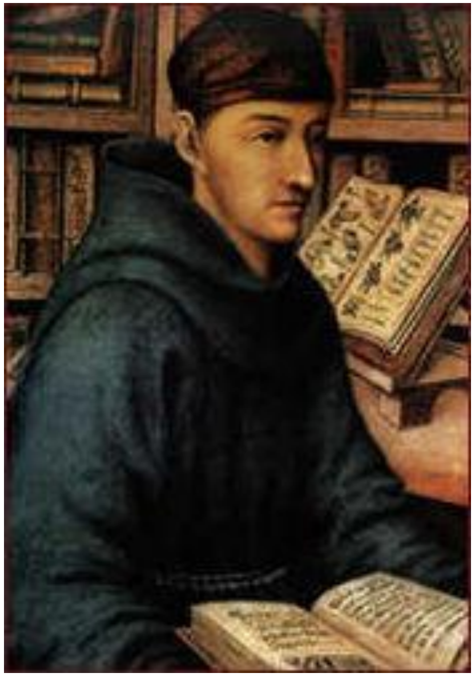
I am greatly indebted for this presentation to Professor David Jameson.

Summary

- The Discovery and Understanding of the Phenomenon
- The Discovery of Fluorescent Compounds
- Applications
- Instrumentation

Nicolás Monardes (1493-1588), a Spanish physician and botanist publishes in 1565 the *"Historia medicinal de las cosas que se traen de nuestras Indias Occidentales"* in which he describes the bluish opalescence of the water infusion from the wood of a small Mexican tree.

When made into cups and filled with water, a peculiar blue tinge was observed.



Around the same time, **Bernardino de Sahagún** (1499-1590), a Franciscan missionary, independently described the wood – called "coatli" by the Aztecs - in the Florentine Codex.

Coatli .....patli, yoan aqujxtiloni, matlatic iniayo axixpatli..
"it is a medicine, and makes the water of blue color, its juice is medicinal for the urine"

Sahagún, Florentine Codex Vol. III f. 266; CM-RAH, f. 203v.

An early Latin translation (1574) of Monardes' work by the influential Flemish botanist **Charles de L'Écluse** (1526-1609), in which the wood's name is given as *Lignum Nephriticum (kidney wood),* helped to extend awareness of its strange optical properties in Europe.

This wood was very popular in XVI - XVII Europe, because of its medicinal virtues for treating kidney ailments.

An Englishman, **John Frampton**, translated Monardes description in 1577 as ".. *white woodde which gives a blewe color*" when placed in water that was good "*for them that doeth not pisse liberally and for the pains of the Raines of the stone..*"

The German Jesuit priest **Athanasius Kircher**, among his numerous achievements, wrote a book in 1646 called *Ars Magna Lucis et Umbrae* in which he described his observation of the wood extract *Lignum nephriticum*.


ATHANASII KIRCHERI
Fuldensis Buchonii è Soc. JESU *Presbyteri;*
ARS MAGNA
LUCIS
ET
UMBRÆ,
In X. Libros digesta.
QUIBUS
Admirandæ Lucis & Umbræ in mundo, atque adeò universa natura, vires effectusque uti nova, ita varia novorum reconditiorumque speciminum exhibitione, ad varios mortalium usus, panduntur.
Editio altera priori multò auctior.
Sicuti tenebræ ejus ... ita & lumen ejus, *Psal.* 138.
AMSTELODAMI,
Apud JOANNEM JANSSONIUM à WAESBERGE.

Light passing through an aqueous infusion of this wood appeared more yellow while light reflected from the solution appeared blue.

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Robert Boyle (1664) was inspired by Monardes' report and investigated this system more fully.

He discovered that after many infusions the wood lost its power to give color to the water and concluded that there was some "essential salt" in the wood responsible for the effect.

He also discovered that addition of acid abolished the color and that addition of alkali brought it back.

In the ensuing centuries the wood was no longer used and the botanic identity of the LN was lost in a confusion of several species.

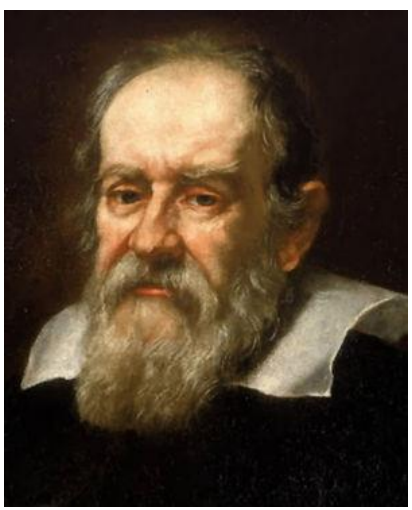
Safford, in 1915, succeeded in disentangling the botanic problem and identified the species which produced the Mexican LN as *Eynsemhardtia polystachia*.

More recently, several highly fluorescent glucosyl-hydroxichalcones were isolated from this plant.

Recent studies by **Ulises Acuña** (2009) indicate that the original blue emission observed by the Aztecs was probably due to the conversion of Coatline B, under mildly alkaline conditions, to a strongly blue-emitting compound - resembling fluorescein - with an emission maximum near 466nm and with a quantum yield near 0.8

In 1603, **Vincenzo Casciarolo**, a Bolognian shoemaker who was dreaming of producing gold, discovered that a stone, after being baked, emitted a purple-blue light in the dark.

Most likely, the stone, named lapis solaris, was barium sulfate. The discovery starts a lively debate between scientists at the time.



Galileo Galilei (1612) described the emission of light (phosphorescence) from the famous Bolognian stone, "*It must be explained how it happens that the light is conceived into the stone, and is given back after some time, as in childbirth*."

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David Brewster (1833) described that when a beam of white light passed through an alcohol solution of leaves a red beam could be observed from the side (which was of course chlorophyll fluorescence).
He considered the effect due to "dispersion".

Edmond Becquerel (1842) reports the emission of light from Calcium sulphate upon excitation in the UV.

He notes that the emission occurs at a wavelength longer than that of the incident light.

Later on (1858) builds the first phosphoroscope enabling him to measure the decay times of phosphorescence.

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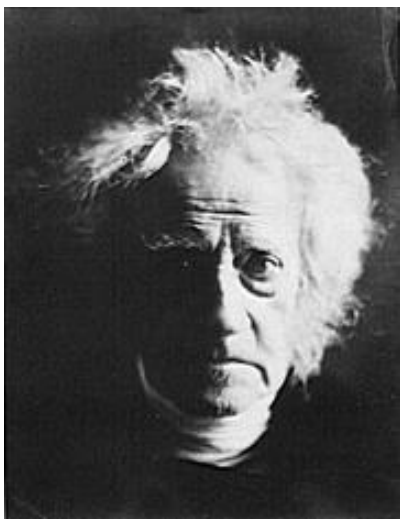
entering Quinine Sulfate



John Herschel (1845) made the first observation of fluorescence from quinine sulfate - he termed this phenomenon “epipolic dispersion”.

IV. 'Αμόρφωτα, No. I.—*On a Case of Superficial Colour presented by a homogeneous liquid internally colourless. By Sir* John Frederick William Herschel, *Bart., K.H., F.R.S., &c. &c.*

Received January 28, 1845,—Read February 13, 1845.

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XXX. *On the Change of Refrangibility of Light.* *By* G. G. Stokes, *M.A., F.R.S., Fellow of Pembroke College, and Lucasian Professor of Mathematics in the University of Cambridge.*

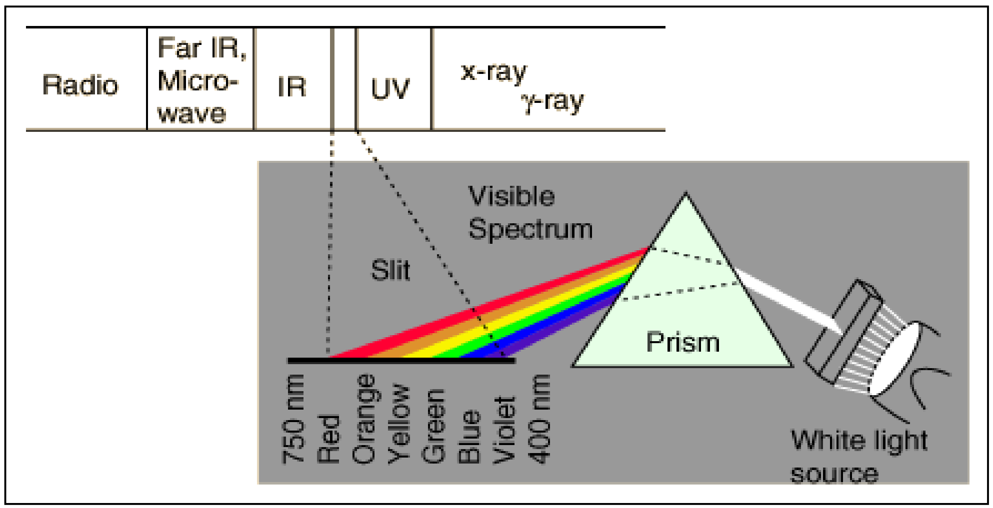
Received May 11,—Read May 27, 1852.

1. THE following researches originated in a consideration of the very remarkable phenomenon discovered by Sir John Herschel in a solution of sulphate of quinine, and described by him in two papers printed in the Philosophical Transactions for 1845, entitled 'On a Case of Superficial Colour presented by a Homogeneous Liquid internally colourless,' and 'On the Epipolic Dispersion of Light.' The solution of quinine, though it appears to be perfectly transparent and colourless, like water, when viewed by transmitted light, exhibits nevertheless in certain aspects, and under certain incidences of the light, a beautiful celestial blue colour. It appears from the experiments of Sir John Herschel that the blue colour comes only from a stratum of fluid of small but finite thickness adjacent to the surface by which the light enters.

George Gabriel Stokes (1852) published his massive treatise "*On the Change of Refrangibility of Light*" – more than 100 pages. He initially used the term "dispersive reflection" to describe the phenomenon presented by quinine sulphate.

* I confess I do not like this term. I am almost inclined to coin a word, and call the appearance *fluorescence*, from fluor-spar, as the analogous term *opalescence* is derived from the name of a mineral.

Stokes used a prism to disperse the solar spectrum and illuminate a solution of quinine. He noted that there was no effect until the solution was placed in the ultraviolet region of the spectrum.



It was certainly a curious sight to see the tube instantaneously lighted up when plunged into the invisible rays: it was literally *darkness visible*. Altogether the phenomenon had something of an unearthly appearance.

This observations led Stokes to proclaim that fluorescence is of longer wavelength than the exciting light, which led to this displacement being called the **Stokes Shift.**

He also seems to have been the first to propose, in 1864, the use of fluorescence as an analytical tool, in a lecture "*On the application of the optical properties to detection and discrimination of organic substances.”*

1905 E. Nichols and E. Merrit: first excitation spectrum of a dye

1919 O. Stern and M. Volmer: fluorescence quenching

1923 S.J. Vavilov and W.L. Levshin: fluorescence polarization of dyes

1924 S.J. Vavilov: first determination of fluorescence yield

1925 F. Perrin: theory of fluorescence polarization

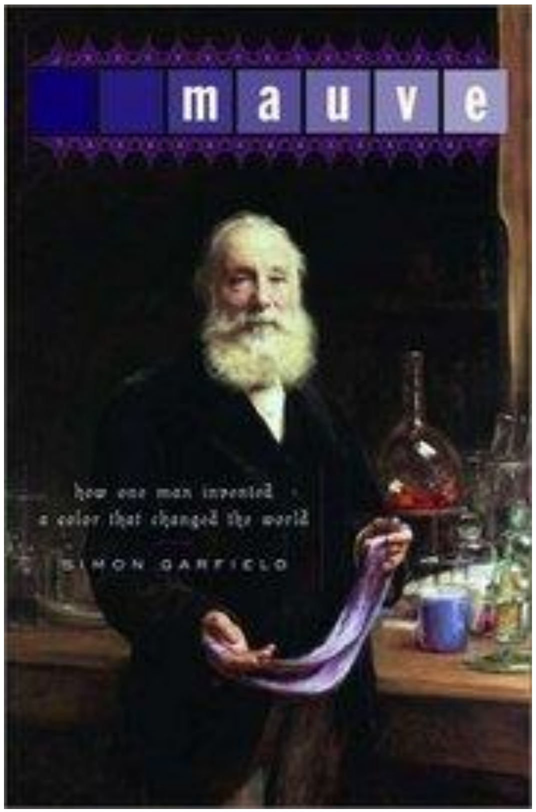
1926 E. Gaviola: first direct measurement of nanosecond lifetime

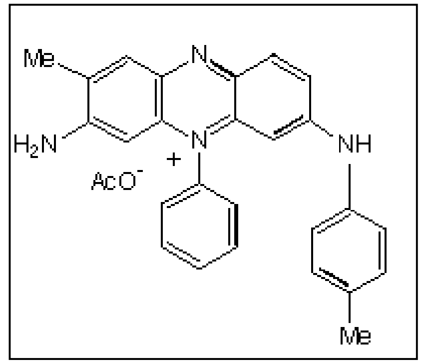
1935 A. Jablonski: diagram

1948 T. Förster: QM theory of dipole-dipole interaction

The Discovery Of Fluorescent Compounds

William Henry Perkin





In 1856, at the age of 18, William Henry Perkin set out with idea of making *quinine* by oxidizing *allytoluidine* –instead he accidentally produced the synthetic dye, *mauve,* a derivative of coal tar with an aniline base.

Adolph Von Baeyer (1871) a German chemist, synthesized Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 3',6'-dihydroxy.

He apparently coined the name “fluorescein”, from “fluo” and resorcin, (resorcinol) which he reacted with phthalic anhydride.

In 1905 he was awarded the Nobel Prize in Chemistry "in recognition of his services in the advancement of organic chemistry and the chemical industry, through his work on organic dyes and hydroaromatic compounds".

More compounds

1874 H. Caro eosin

1880 C. Liebermann polycyclic aromatic hydrocarbons

1882 Paul Erlich used uranin (the sodium salt of fluorescein) to track secretion of the aqueous humor in the eye. First *in vivo* use of fluorescence.

1887 K. Noack published a book listing 660 compounds arranged according to the color of their fluorescence.

1897 R. Meyer used the term "fluorophore" to describe chemical groups which tended to be associated with fluorescence; this word was analogous to "chromophore" which was first used in 1876 by O.N. Witt to describe groups associated with color.

Applications and Instrumentation

In 1864 G.G. Stokes proposes the use of fluorescence as an analytical tool, in a lecture "*On the application of the optical properties to detection and discrimination of organic substances.”*

In 1867 F. Goppelsröder introduced the term form "Fluoreszenzanalyse“ and performed the first fluorimetric analysis in history: the determination of Al(III) by the fluorescence of its morin chelate.

One of the first uses of fluorescein was in 1877 in a major ground-water tracing experiment in southern Germany.

The results of this experiment showed that the River Danube and Rhine are connected by underground streams.

Fluorescein was placed in the Danube and about 60 hours later it appeared in an affluent of the Rhine.

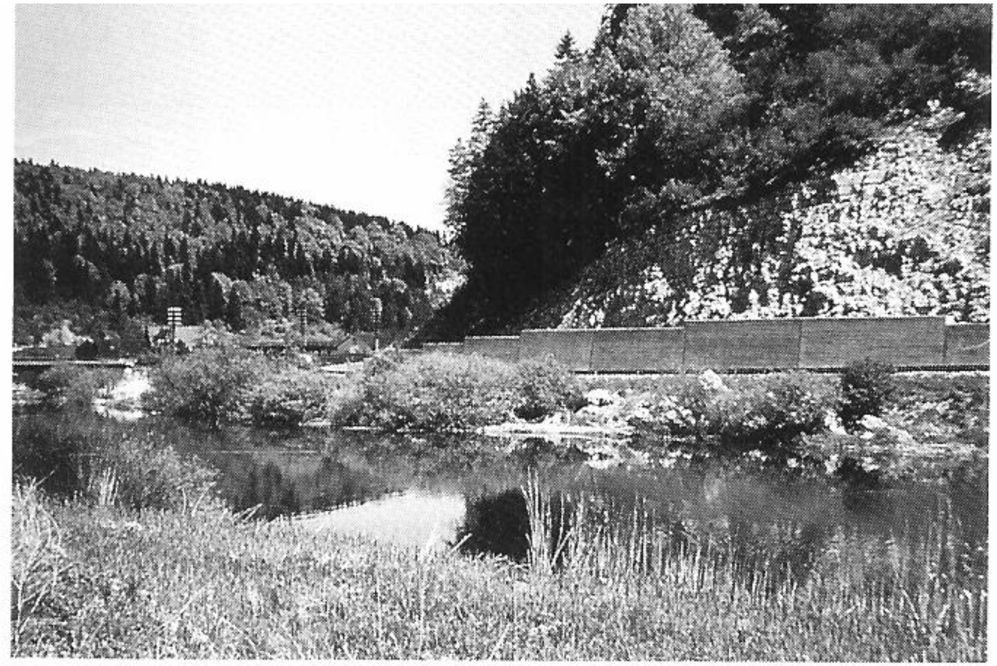


Fig. 4 The Danube at the Immendingen weir with sinkholes on the right bank and the well-stratified Oxfordian limestone behind

10 Kilograms of fluorescein were used!

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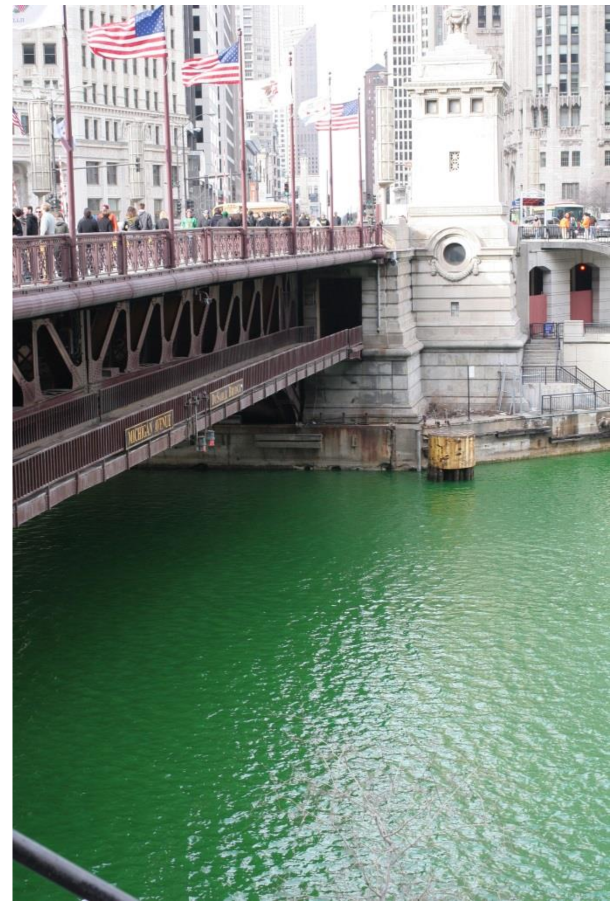
Two miracles appear that day, the river turns a perfect shade of green something that many other cities have tried but have not been successful at doing, and the second miracle by starting with the color orange giving the impression that river will be orange only to convert the river to that true Irish green. We believe that is where the leprechaun comes in.

By Dan O'Leary, courtesy of www.ChicagoStPatsParade.com

[Although fluorescein was used for several years, recently a vegetable-based dye was substituted].



St. Patrick's Day 2014, Chicago

(courtesy of Darcy Lear)

Inorganic

1929 Nichols and Slattery report the first observation of the intense fluorescence of uranium in a NaF matrix.

1930 Hieger found that the fluorescence spectrum (measured photographically) of 1,2-benzanthracene resembled the spectra of some cancer-producing coal tars.

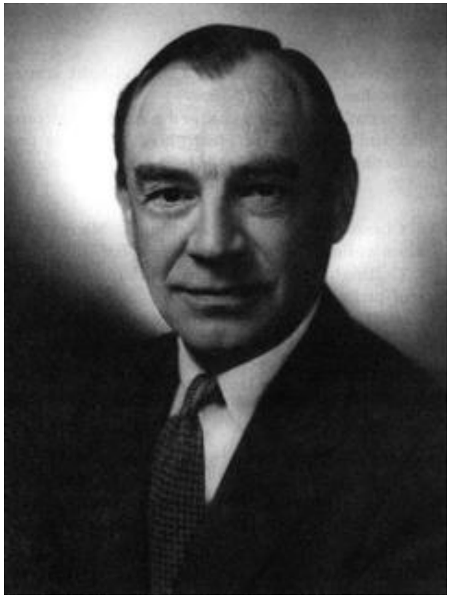
1933 Cook and his associates isolated a few grams of 3,4-benzypyrene from two tons of coal tar and demonstrated that its fluorescence spectrum was identical to that of the active tars.

Drugs and Clinical

1941 Albert Coons labeled antibodies with FITC, thus giving birth to the field of immunofluorescence

1943 Brodie and Udenfriend introduce a simple method for the determination of quinine, and its dextro-rotary stereoisomer quinidine, in plasma.

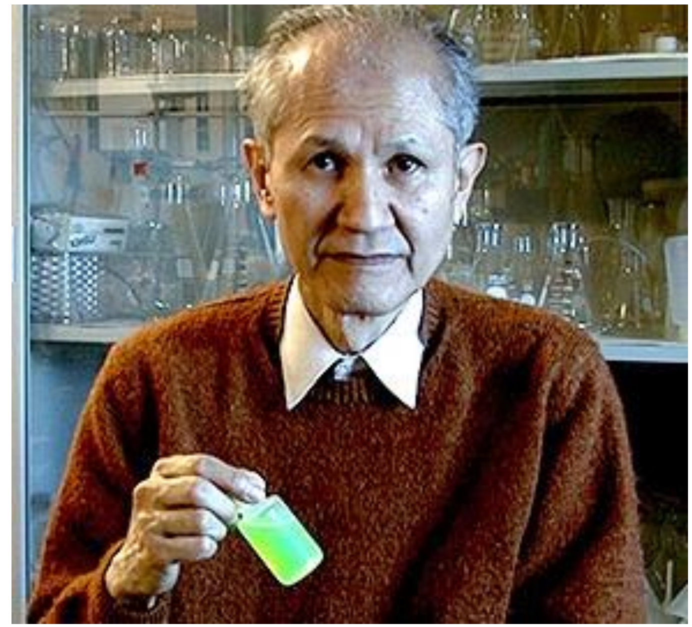
1948 Saltzman introduces fluorimetric methods for salicylates in blood.



Inorganic

1952 Gregorio Weber
synthesized dansyl chloride for attachment to proteins and used polarization to study protein hydrodynamics - these studies initiated the field of quantitative biological fluorescence.

1962 Shimomura, Johnson and Saiga discovered Green Fluorescent Protein in the *Aequorea victoria* jellyfish



Osamu Shimomura in the lab in the basement of his home. He is holding a sample of GFP isolated from Aequorea victorea, not produced by bacteria.

The Instrumentation

During World War II, the United States government issued a desperate call to scientists and doctors: find a treatment for malaria! Since Japan had taken over most of the world's supply of quinine-the best known treatment-Allied forces in the Pacific Theater needed a new drug, and fast.

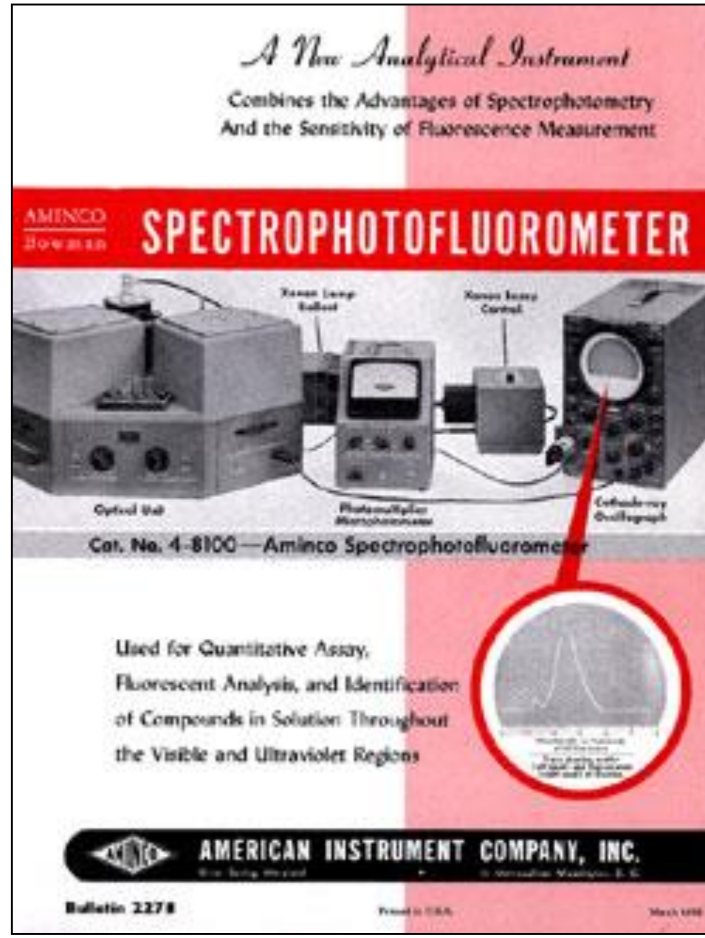
With an instrument called a fluorometer, Brodie and Udenfriend could measure how much of the drug was in a patient's plasma sample.

1956

Aminco-Bowman (Silver Spring, MD)

Farrand Optical Co. (Walhalla, NY)

Cost: over $8,000 – which is about $130,000 in 2023 !



Fluorometers

(courtesy of ISS)

(courtesy of Agilent)

Fluorescence Microscopy

Otto Heimstaedt and Heinrich Lehmann (1911-1913) developed the first fluorescence microscopes as an outgrowth of the UV microscope (1901-1904).
The instrument was used to investigate the autofluorescence of bacteria, protozoa, plant and animal tissues, and bioorganic substances such as albumin, elastin, and keratin.

Stanislav Von Prowazek (1914) employed the fluorescence microscope to study dye binding to living cells.

A. Policard and A. Paillot (1928) introduce vertical illumination

Evgenii M. Brumberg and T.N. Krylova (1953) introduce dichroic mirror

Johan Sebastian Ploem (1965) introduces the cube housing the dichroic and filter still in use today.

1969 The Leitz Ploemopak includes 4 cubes that can be interchanged

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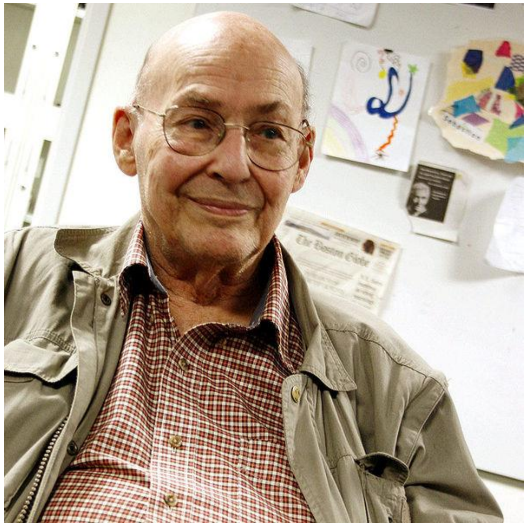
Confocal Microscopy

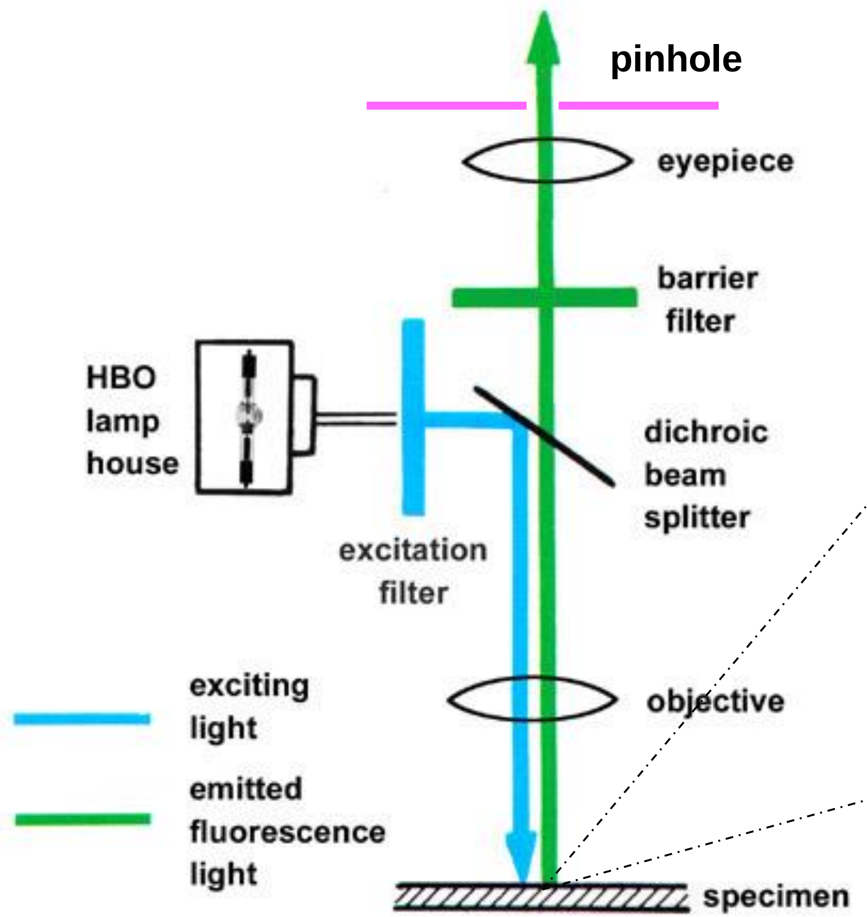
1957 Marvin Minsky files a patent

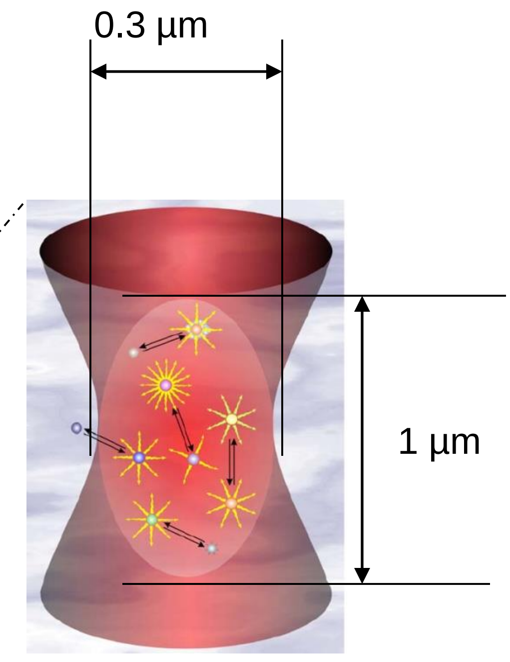
1978 Thomas and Christoph Cremer introduce laser scanning

1989 John White, WB Amos and M Fordham improve the LSM

1999 Stephen Hell demonstrates STED



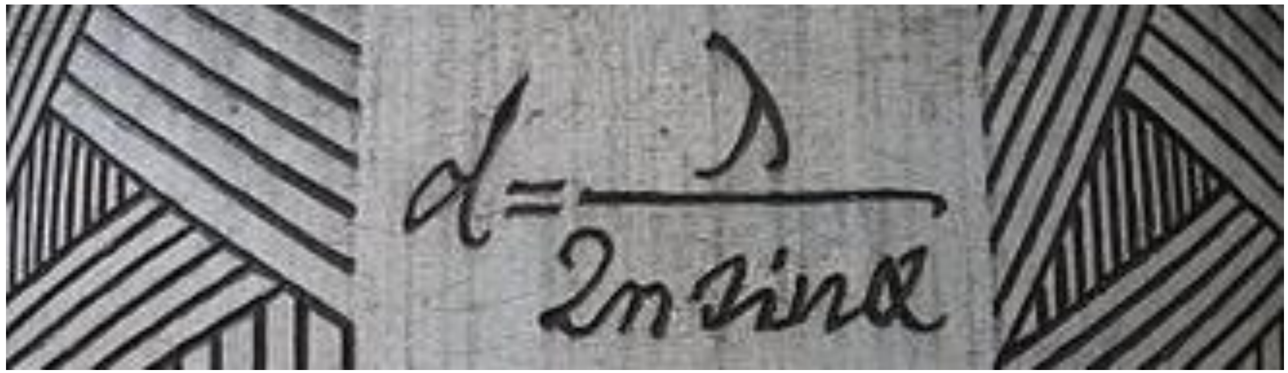




Volume: 10^{-15} L
Size (µm): 0.3 x 1
No. molecules: ≈ 1

Ernst Abbe

1873 - Resolution



Stefan Hell

1999 - STimulated Emission Depletion (STED):

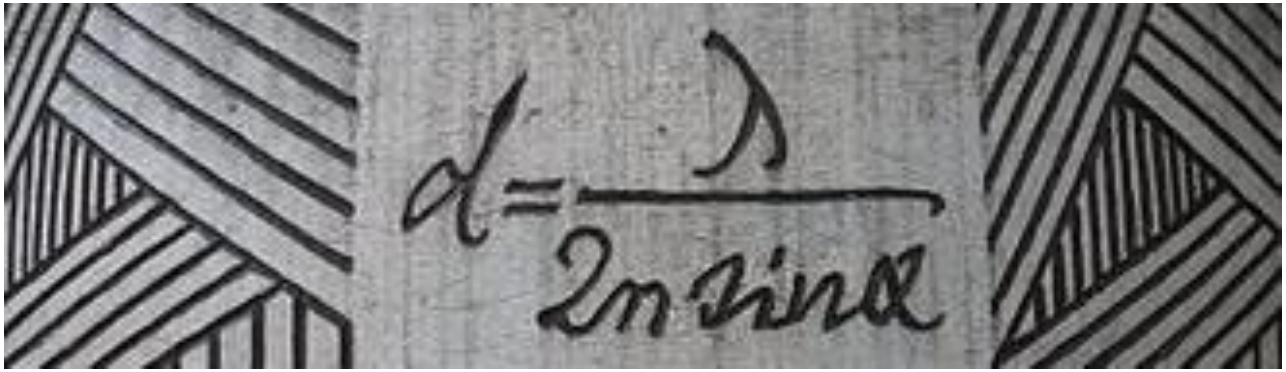
STED microscopy generates a sub-diffraction volume by engineering a donut-shaped PSF of the STED beam relative to the Gaussian-shaped PSF of the excitation beam. The depletion PSF has a zero-intensity spot at the center of the excitation PSF and strong intensity at the periphery of the excitation PSF.

$$d = \lambda / \left(2n\, sin\alpha \sqrt{1 + (I_{STED}/I_{Sat})}\right)$$

Both lasers are pulsed in pSTED.

The Pulsed STED (pSTED) microscopy

Ernst Abbe



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<u>STimulated Emission Depletion (STED):</u>

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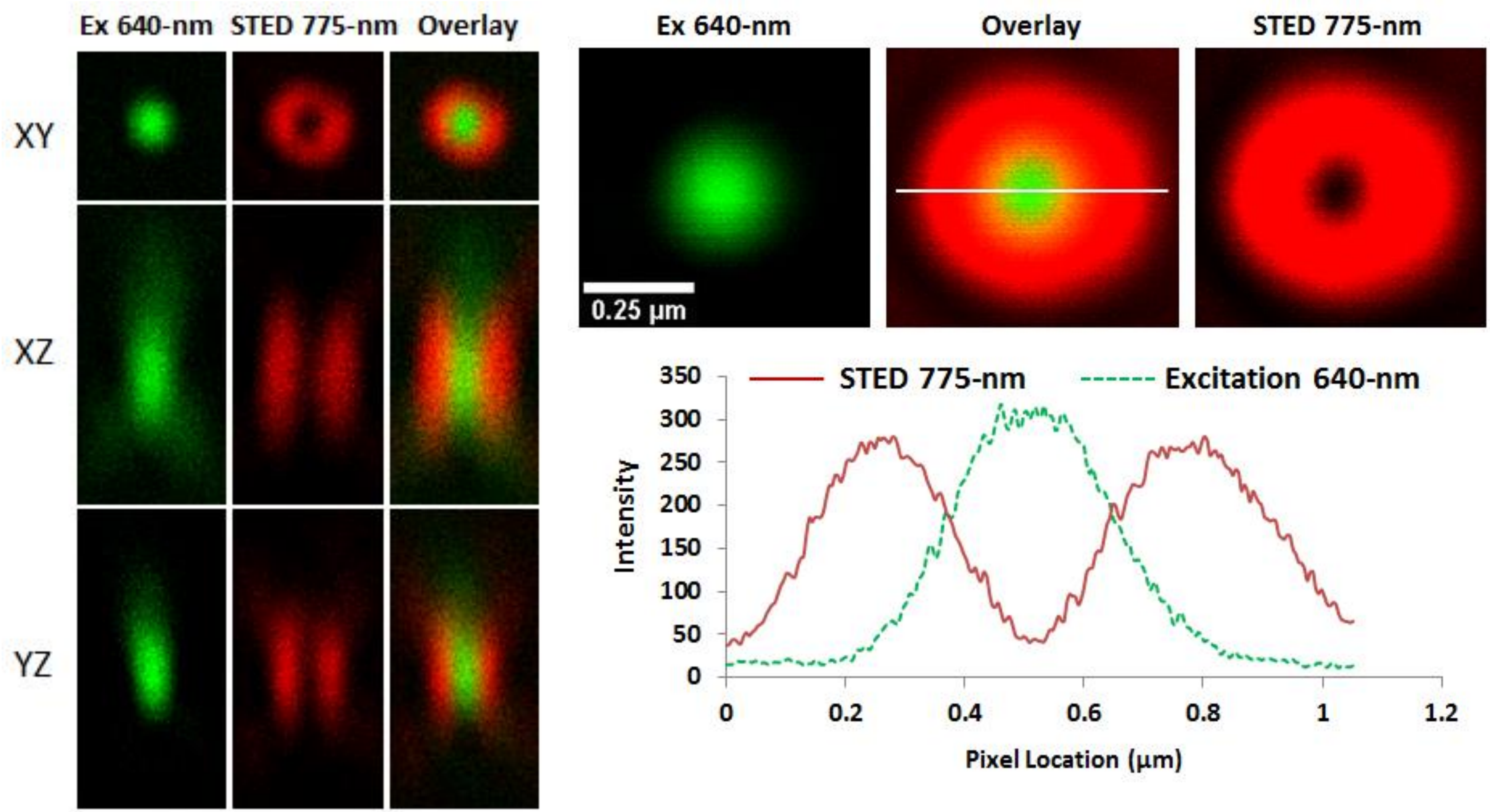
$$\mathbf{d} = \lambda / \left(2n\, sin\alpha \sqrt{1 + (I_{STED}/I_{Sat})}\right)$$

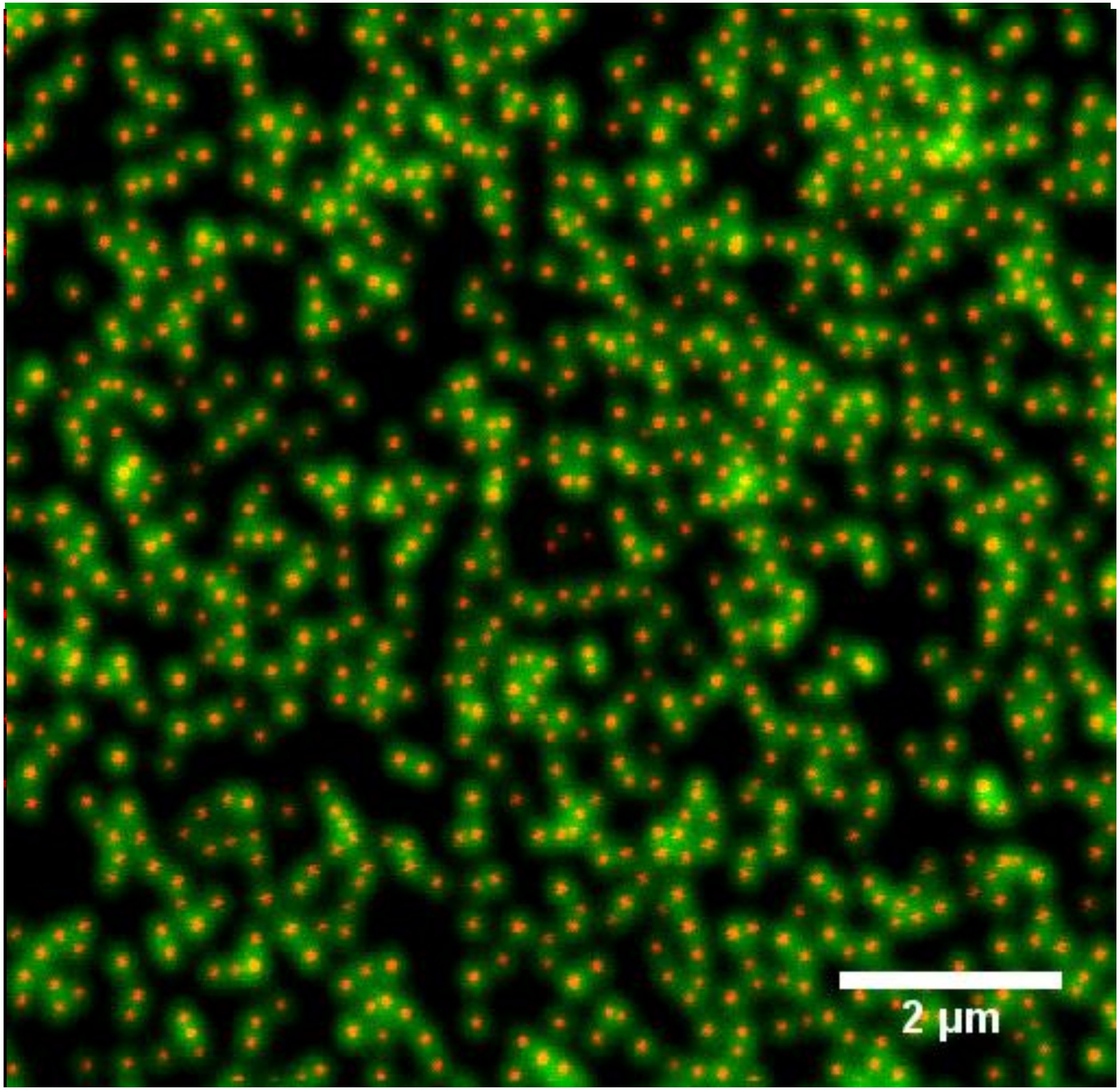
Both lasers are pulsed in pSTED.

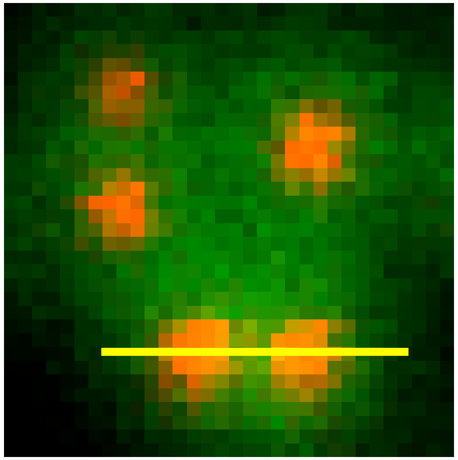
The spatial alignment

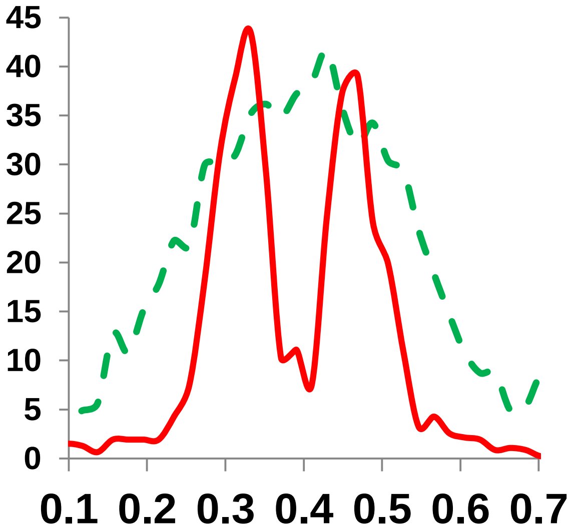
Measure the XY/XZ/YZ intensity profiles of the laser beam reflected from a 80-nm gold nanoparticle (i.e. the point spread function of each laser)



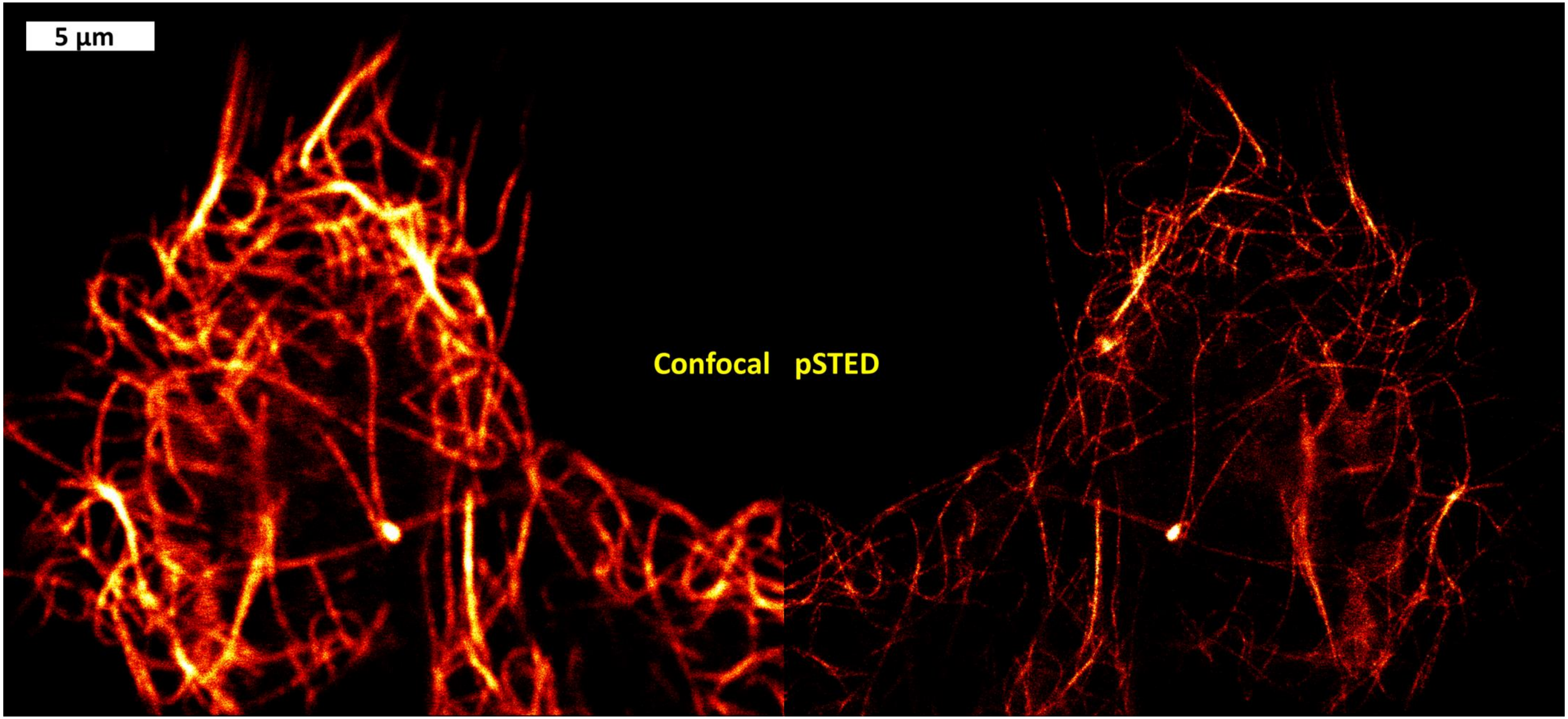
Confocal vs. pSTED, 60-nm fluorescent beads







Confocal vs. STED, Microtubule-SiR in Live HeLa cells



Microtubule labeled by SiR in live HeLa cells
Paul R. Selvin's lab, Physics, University of Illinois – Urbana and Champaign

Single Molecule and Confocal Microscopes

(courtesy of ISS)

(courtesy of Carl Zeiss)

Other Applications/Instruments

Flow Cytometry

(courtesy of Sony-ICyt)

Genome Sequencing

(courtesy of Pacific Biosciences)

Microwell Plate Readers

(courtesy of Molecular Devices)

Immunoassay Chemistry Analyzer

(courtesy of Abbott Diagnostics)

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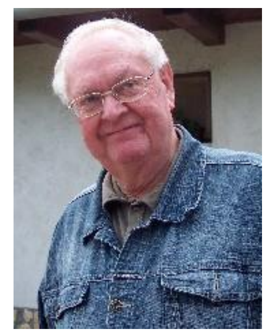




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Thank you.