The 14th LFD workshop on Advanced Fluorescence Imaging and Dynamics
October 21-25, 2019

A Nano-History of Fluorescence
(with some comments on the contributions of Gregorio Weber)

David Jameson
The discovery and characterization of Fluorescence

Quiz: When was the first report on “fluorescence”?

Contrary to popular belief it was not from early ancestors of Dave and Enrico
The discovery and characterization of Fluorescence

Nor was it from Enrico’s kitchen!
Nor even from the Old West!
Nicolás Monardes (1565), a Spanish physician and botanist who wrote on medicines of the New World, is usually credited as being the first to describe 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.*

Actually, **Bernardino de Sahagún**, a Franciscan missionary, independently described the wood – called “coatli” by the Aztecs.

I am indebted to Ulises Acuna for this picture and for information about these early studies.

*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”*

(7) Sahagún, B. *Matritensis Codex*; Spanish Royal Academy of History, ca. 1560–1564, f203v.
An early Latin translation (1574) 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 Mondares description 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..”
Robert Boyle (1670) 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.

I thank Katherine Reinhart from Johns Hopkins Univ. for providing a copy of the original 1670 Boyle manuscript.

Hence Boyle was the first to use fluorescence as a pH indicator!
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.
The identification of the fluorescing molecule from *Lignum Nephriticum* was finally made in 2009!
Galileo Galilei (1612) described the emission of light (phosphorescence) from the famous Bolognian stone, discovered in 1603 by Vincenzo Casciarolo, a Bolognian shoemaker. Galileo wrote: "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."

Museum of Mineralogy "L.Bombicci, University of Bologna  Pieces of Bologna Stone, barium sulphate (barite)
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).

Chlorophyll fluorescence is now very important in studies on plant health and photosynthesis and portable fluorescence instruments are routinely taken out into the field.
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).

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


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

an extremely vivid and beautiful celestial blue colour.
Enrico was so impressed by this “beautiful celestial blue color” that he recently renovated his living room to allow him to appreciate it on a daily basis.
George Gabriel Stokes (1852) published his massive treatise “On the Change of Refrangibility of Light” – more than 100 pages. In this work he initially used the term “dispersive reflection” to describe the phenomenon presented by quinine sulphate. Fortunately for all of us today, however, he then wrote:

* 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.

He wrote:

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.
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.

Fortunately for him Queen Victoria loved it! Not long afterward Perkin produced a green and a violet, and soon the canal outside his factory was turning a different color every week.

Although others – including Friedlieb Runge and Robert Rumney – had synthesized synthetic dyes, Perkins was the first to recognize the potential for commercialization and really started the synthetic dye industry.

Histologists started using the dyes to stain samples within a decade of Perkin’s discovery.
Adolph Von Baeyer (1871) a German chemist, synthesized Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 3',6'-dihydroxy.

**FLUORESCEIN!!!**

**Adolf Baeyer: Ueber eine neue Klasse von Farbstoffen.**

*(Vorgetr. vom Verf.)*


He apparently coined the name “fluorescein”, from “fluo” and “rescein,” (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".

http://www.zein-bayer.de/de/patents/olderman/antifektion/farbstein_fluorescein.g7/15/2007-8-31-55 AM
Adolph Von Beyer (1871) a German chemist, synthesized Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 3',6'-dihydroxy.

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.

10 Kilograms of fluorescein were used!

Fig. 4 The Danube at the Immendingen weir with sinkholes on the right bank and the well-stratified Oxfordian limestone behind
Adolph Von Beyer (1871) a German chemist, synthesized Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 3',6'-dihydroxy.

**FLUORESCEIN!!!**

Every year on St. Patrick’s Day, the Chicago river is dyed green with about 40 pounds of fluorescein.
The opposite of a single molecule experiment!
Adolph Von Beyer (1871) a German chemist, synthesized Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 3',6'-dihydroxy.

**FLUORESCEIN!!!**

Paul Ehrlich (1882) used uranin (the sodium salt of fluorescein) to track secretion of the aqueous humor in the eye. First *in vivo* use of fluorescence.
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*Earliest example of a Molecular Probes catalog!!!*
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R. Meyer (1897) 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.

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.


Albert Coons (1941) labeled antibodies with Fluorescein Isocyanate, thus giving birth to the field of immunofluorescence.


Riggs et al. first reported FITC, in 1958, which they synthesized to circumvent problems inherent in the isocyanate derivative, including the difficulty of its synthesis and its instability.

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

Shimomura, Johnson and Saiga (1962) 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 victoreia*, not produced by bacteria.
“The jellyfish *Aequorea* and its light-emitting organs”

70 mgs of purified GFP were obtained. The 30,000 jellyfish weighed about 1.5 tons

The outer ring of the jellyfish had to be isolated. Initially scissors were used but then a “ring-cutting” machine was built.
Polarizers have been in use for a very long time - the Vikings used a “sunstone” (thought to have been composed either of the mineral cordierite or iceland spar – calcite – both of which are naturally polarizing materials) to observe the location of the sun on foggy or overcast days. Since scattered sunlight is highly polarized compared to light coming along the direction to the sun, the distribution of the sky’s brightness could be observed through the sunstone and hence the sun’s position could be localized and, if the time of day were known, the compass directions.
In 1808, Malus observed sunlight reflected from the windows of the Luxemburg Palace in Paris through an Iceland spar (Calcite) crystal that he rotated.

Malus discovered that the intensity of the reflected light varied as he rotated the crystal and coined the term “polarized” to describe this property of light.

He published his findings in 1809: “Sur une propriété de la lumière réfléchie par les corps diaphanes” (Bull. Soc. Philomat. I:16)

Malus also derived an expression for calculating the transmission of light as a function of the angle ($\theta$) between two polarizers. This equation (Malus’ Law) is now written as: $I_\theta = I_0 (\cos^2 \theta)$
Sir David Brewster (1781-1868)

David Brewster studied the relationship between refractive index and angle of incidence on the polarization of the reflected light

III. *On the law of the partial polarization of light by reflexion. By David Brewster, LL.D. F.R.S. L. & E.*

Read February 4, 1830.

He discovered that for normal glass and visible light, an incidence angle of ~56 degrees resulted in total reflection of one plane of polarization – this angle is now known as *Brewster’s Angle*

\[ \theta_B = \tan^{-1} \left( \frac{n_2}{n_1} \right) \]

This discovery allowed Brewster to construct a polarizer composed of a “pile of plates”
William Nicol (1770-1851)

In 1828, Nicol joined two crystals of Iceland spar, cut at an angle of 68°, using Canada balsam.

Edwin Herbert Land (1909-1991)

In 1929 Edwin Land patented the sheet polarizer (the J-sheet), consisting of crystals of iodoquinine sulfate embedded in nitrocellulose film followed by alignment of the crystals by stretching which led to dichroism. In 1938 he invented the H-sheet which was comprised of polyvinyl alcohol sheets with embedded iodine.

Other important calcite polarizers developed around this time include: Glan-Foucault; Glan-Thompson; Glan-Taylor; Wollaston; Rochon

But the Henry Ford of polarizers was…..
A very important paper connecting polarization and energy transfer between identical fluorophores was carried out by Gaviola and Pringsheim in 1924.

Über den Einfluß der Konzentration auf die Polarisationscharakteristik der Fluoreszenz von Farbstofflösungen.

Von E. Gaviola und Peter Pringsheim in Berlin.
Mit zwei Abbildungen. (Eingegangen am 24. März 1924.)

Tabelle 2. Uranin in ganz wasserfreiem Glycerin.

<table>
<thead>
<tr>
<th>C</th>
<th>p</th>
<th>C</th>
<th>p</th>
<th>C</th>
<th>p</th>
<th>C</th>
<th>p</th>
</tr>
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<tr>
<td>1/4</td>
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<td>1</td>
<td>6,5</td>
<td>1/256</td>
<td>15</td>
<td>1/2048</td>
<td>39,2</td>
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<tr>
<td>1/8</td>
<td>6</td>
<td>1/32</td>
<td>8,1</td>
<td>1/512</td>
<td>19,5</td>
<td>1/4100</td>
<td>43,5</td>
</tr>
<tr>
<td>1/16</td>
<td>3,2</td>
<td>1/128</td>
<td>11,1</td>
<td>1/1024</td>
<td>30,7</td>
<td>etwa 1/20000</td>
<td>45</td>
</tr>
</tbody>
</table>

(note: uranin is the sodium salt of fluorescein)
We now come to Francis Perrin, son of the famous physicist Jean Perrin.

For an excellent discussion of the scientific contributions of the Perrins see the article by Mario Berbaran-Santos in “New Trends in Fluorescence Spectroscopy” 2001, Eds B. Valuer and J-C. Brochon.
In 1925 - 1926, Francis Perrin published several important papers describing a quantitative theory of fluorescence polarization including what is now considered his classic paper containing most of the essential information that we use to this day.

**J. de Physique 1926**

\[
P = P_0 \left( \frac{1}{1 + \left(1 - \frac{1}{3} P_0 \right) \frac{RT}{V_\eta} \tau} \right)
\]

Polarization remained largely in the province of the physicists for almost two decades, until Gregorio Weber began his thesis work with the famous enzymologist Malcolm Dixon in Cambridge in the mid-1940’s.

Weber’s subsequent theoretical and experimental work – which extended Perrin’s earlier contributions and also developed what became modern instrumentation - brought fluorescence polarization to the attention of the biochemical community, and so ushered in a new scientific discipline – quantitative biological fluorescence.
The Time Interval between Absorption and Emission of Light in Fluorescence.


(Received June 12, 1921.)

Some experiments were then made at the University of Wisconsin, in collaboration with Prof. C. E. Mendenhall, during my visit to Madison in December. We used a high pressure, six-cylinder pump, and obtained a jet velocity of about 200 metres per second, with a fine glass nozzle about 0.2 mm. in diameter. More recently, Prof. Mendenhall has increased the velocity to 230 metres per second, and, by blackening one side of the jet tube, leaving a small clear space for the entrance of the sunlight, has assured himself that there is no displacement as great as 0.1 mm. (observing the fluorescent patch with a short-focus lens). This means that the duration of the fluorescence is less than 1/2,300,000 second.
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Anthracene

< 0.1 mm
The Time Interval between Absorption and Emission of Light in Fluorescence.


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i.e. < 435ns

anthracene
Microfluidic Space-Domain Time-Resolved Emission Spectroscopy of Terbium(III) and Europium(III) Chelates with Pyridine-2,6-Dicarboxylate

Vicente Nuñez,† Srigokul Upadhyayula,‡,‡† Brent Millar,‡,† Jillian M. Larsen,† Ali Hadian,† Sanghoon Shin,† Prashanthi Vandrangi,† Sharad Gupta,† Hong Xu,† Adam P. Lin,† Georgi Y. Georgiev,†,‡,§ and Valentine I. Vullev*†,‡,§
Ein Fluorometer.
Apparat zur Messung von Fluoreszenzabklingungszeiten.

Von E. Gaviola in Berlin.
Mit 9 Abbildungen. (Eingegangen am 24. März 1927.)

Fig. 1. Original apparatus of Gaviola¹ for the measurement of fluorescence lifetimes, described in text. B, Source of exciting light; T, cuvette containing the fluorescent solution; S, mirror.
Gaviola was one of the most outstanding scientists produced by Argentina in all of its history.

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.
<table>
<thead>
<tr>
<th>Farbstoff</th>
<th>Abklingungszeiten</th>
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<tbody>
<tr>
<td></td>
<td>in Wasser Sekunden</td>
</tr>
<tr>
<td>Uranin</td>
<td>$4,5 \times 10^{-9}$</td>
</tr>
<tr>
<td>Fluorescein</td>
<td>—</td>
</tr>
<tr>
<td>Rhodamin B</td>
<td>$2,0 \times 10^{-9}$</td>
</tr>
<tr>
<td>Rhodulin Orange</td>
<td>$2,7$</td>
</tr>
<tr>
<td>Erythrosin</td>
<td>$1,8$</td>
</tr>
<tr>
<td>Tetrajodfluor. Na</td>
<td>$1,0$</td>
</tr>
<tr>
<td>Eosin 5 B</td>
<td>$1,9$</td>
</tr>
<tr>
<td>Uranylsulfat</td>
<td>—</td>
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<tr>
<td>Uranylsulfat in Schwefelsäure</td>
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<tr>
<td>Chinisarin in Pentan</td>
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</tr>
<tr>
<td>Uranglas</td>
<td>—</td>
</tr>
<tr>
<td>Rubinkristall</td>
<td>—</td>
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</table>
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

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."

\[ \tan \Delta \phi = \omega \tau. \]

Fig. 5. The transmitted light signal, fluorescent light signal, and the tracer signal (sweep speed: 0.034 \( \mu \)sec/cm).

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

Slide from Bob Clegg
Milestones in the Theory of Resonance Energy Transfer

1922  G. Cario and J. Franck demonstrate that excitation of a mixture of mercury and thallium atomic vapors with 254nm (the mercury resonance line) also displayed thallium (sensitized) emission at 535nm.

1924 E. Gaviola and P. Pringsham observed that an increase in the concentration of fluorescein in viscous solvent was accompanied by a progressive depolarization of the emission.

More than 50 years ago, the German scientist Förster discovered that close proximity of two chromophores changes their spectral properties in predictable ways (Förster, 1948a).
Milestones in the Theory of Resonance Energy Transfer

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1924 E. Gaviola and P. Pringsham observed that an increase in the concentration of fluorescein in viscous solvent was accompanied by a progressive depolarization of the emission.

1925  J. Perrin proposed the mechanism of resonance energy transfer

1928 H. Kallmann and F. London developed the quantum theory of resonance energy transfer between various atoms in the gas phase. The dipole-dipole interaction and the parameter $R_0$ are used for the first time

1932  F. Perrin published a quantum mechanical theory of energy transfer between molecules of the same specie in solution. Qualitative discussion of the effect of the spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor

1946-1949 T. Förster develop the first complete quantitative theory of molecular resonance energy transfer

More than 50 years ago, the German scientist Förster discovered that close proximity of two chromophores changes their spectral properties in predictable ways (Förster, 1948a).
Theodor Förster (1910-1974)

The 1st Theodor Förster International Lecture series at the University of Cambridge was inaugurated at the Li Ka Shing Centre for Cancer Research, on the 12th of November 2007 by Professor Enrico Gratton.
The first commercial spectrofluorimeters with monochromators for both excitation and emission were inspired by the work of Bowman at the NIH and were produced by Aminco-Bowman and Farrand. These early instruments allowed biologists to use fluorescence to develop clinically relevant assays for a wide variety of biological molecules.
1956

Cost: over $8,000
– which is about $100,000 in 2017 dollars
Fluorescence in the 20th Century

Most of the basic principles of fluorescence were developed during the 1920's and 1930's.

- Excited state lifetime (Gaviola)
- Quantum yield (Wavilov)
- Polarization of fluorescence (Weigert, F. Perrin)
- Fluorescence resonance energy transfer (J. and F. Perrin; T. Förster)

Until the second half of the 20th century, however, the use of fluorescence in biology and biochemistry was, descriptive in nature and primarily limited to a role in the isolation, purification and quantification of fluorescent substances such as riboflavin and porphyrins. True “quantitative” biological fluorescence began with the pioneering work of Gregorio Weber.
The Seminal Contributions of Gregorio Weber to Modern Fluorescence Spectroscopy

During the last few decades, fluorescence spectroscopy has evolved from a narrow, highly specialized technique into an important discipline widely utilized in the biological, chemical and physical sciences.

As in all scientific disciplines, the development of modern fluorescence spectroscopy has benefited from the contributions of many individuals from many countries.

However, one individual, Gregorio Weber, can be singled out for his outstanding and far-reaching contributions to this field.
Biographical Sketch of Gregorio Weber

1916  Born in Buenos Aires, Argentina (July 4)

Photo courtesy of Francisco Barrantes
Biographical Sketch of Gregorio Weber

1916  Born in Buenos Aires, Argentina (July 4)

1943  M.D. degree from the University of Buenos Aires (teaching assistant with Bernardo Houssay)

Bernardo Houssay was awarded the 1947 Nobel Prize in Physiology and Medicine for his discovery of the role of pituitary hormones in the regulation of glucose in the blood. He was also the first Argentine and Latin American to be awarded with a Nobel Prize in some field of the Sciences.

Photo courtesy of Tom Jovin
Biographical Sketch of Gregorio Weber

1916  Born in Buenos Aires, Argentina (July 4)

1943  M.D. degree from the University of Buenos Aires  
(teaching assistant with Bernardo Houssay)

1943/47  Attended Cambridge University supported by a  
British Council Fellowship. 
Thesis Advisor - Malcolm Dixon

1947  Awarded Ph.D. - Thesis title “Fluorescence of  
Riboflavin, Diaphorase and Related Substances”

1948 /52  Independent investigator at the Sir William Dunn  
Institute of Biochemistry at Cambridge University

1953  Joined Biochemistry Department of Sheffield  
University

1962  Joined the Biochemistry Division of the Chemistry  
Department at the University of Illinois  
at Urbana-Champaign
At the University of Buenos Aires, Bernardo Houssay suggested that his young protégé apply for a prestigious British Council Fellowship to support Ph.D. studies at Cambridge University, UK.

Travel to England in 1943 was an adventure - Weber's voyage took 44 days in a convoy.
At Cambridge, Weber entered St. John’s College where he met Malcolm Dixon, the well-known enzymologist, and talked with him about applying techniques of Physical Chemistry to the study of proteins.

At that time, Dixon was already acknowledged as one of the world’s preeminent physical biochemists and the leading authority on enzymes. He had recorded the first absorption spectrum of cytochrome c.
At Cambridge, Weber entered St. John’s College where he met Malcolm Dixon, the well-known enzymologist, and talked with him about applying techniques of Physical Chemistry to the study of proteins.

Dixon suggested that Weber consider applying fluorescence techniques to the study of the naturally fluorescent flavin and flavoprotein systems.

At that time, Weber knew little about fluorescence but soon learned that there were a number of low molecular weight flavin compounds, such as riboflavin and FAD, that differed greatly in fluorescence intensity. A few flavoproteins had been purified but only one of them showed fluorescence comparable to the free prosthetic group.

Gregorio Weber was thus given the task of “sorting out” this area.
Microviscosity
versus
Macroviscosity

The final chapter of Gregorio Weber’s thesis is devoted to the application of polarization measurements to determine the viscosity of gels.
the low microscopic viscosity necessary for the rapid diffusion of metabolites within it.

Note on the determination of protoplasm viscosity.

The polarization method appears very convenient for the determination of the viscosity of protoplasm.

Both the microscopic and macroscopic viscosity of the protoplasm are of importance; the first in relation to diffusion processes and the second in relation to the organization of the cell. The fluorescent method would allow the determination of the microscopic viscosity only.
This prescient observation anticipated the work he would publish 24 years later which first delineated the application of fluorescence probes to studies of the physical state of lipid systems.
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The use of fluorescence probes in model and biological membrane systems has become an extremely important and wide-spread technique which, in recent years, has been extended to fluorescence microscopy.
Designing fluorescence probes - The beginning...

From 1948 to 1952 Weber carried out independent investigations at the Sir William Dunn Institute of Biochemistry at Cambridge. He began to delve more deeply into the theory of fluorescence polarization and also to develop methods which would allow him to study proteins which did not contain an intrinsic fluorophore.

He invested considerable time and effort in synthesizing a fluorescent probe which could be covalently attached to proteins and which possessed absorption and emission characteristics appropriate for the instrumentation available in post-war England.

The result of two years of effort was dimethylaminonaphthalene sulfonyl chloride or dansyl chloride - a probe which is still utilized today.
From 1948 to 1952 Weber carried out independent investigations at the Sir William Dunn Institute of Biochemistry at Cambridge. He began to delve more deeply into the effects of metals on enzymes and also to develop methods which would allow him to study proteins which did not contain intrinsic fluorophores.

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The result of two years of effort was dimethylaminonaphthalene sulfonyl chloride or dansyl chloride - a probe which is still utilized today.
Designing fluorescence probes - continued...

Bis-ANS

LAURDAN  \( R = -(CH_2)_{10}CH_3\)
PRODAN   \( R = CH_2CH_3\)
DANCA    \( R \)
The 1952 papers

With Dansyl chloride and with new instrumentation Weber began to investigate several protein systems, publishing his theory and experimental results in two classic papers published in Biochemical Journal in 1952.

“Polarization of the fluorescence of macromolecules. I. Theory and experimental method.”

“Polarization of the fluorescence of macromolecules. II. Fluorescent conjugates of ovalbumin and bovine serum albumin.”

The theory paper - which interestingly contains an acknowledgment to F. Perrin for his suggestions - includes an extension of Perrin’s theory of depolarization to the case of ellipsoidal molecules carrying randomly oriented oscillators of absorption and emission.
Polarization theory continued...

In subsequent years, Weber continued to advance the theory of fluorescence polarization.

This second paper (1972) is a re-examination of previous treatments by several groups (including Weber) and presents the generally accepted equation for the time-dependence of fluorescence polarization owing to rotational diffusion of fluorophores attached to rigid macromolecules.

\[ r(t) = \sum_{i=1}^{3} c_i \exp(-t/\tau_i) + [(F + G)/4] \exp(-|6D - 2\Delta|t) + [(F - G)/4] \exp(-|6D + 2\Delta|t), \]

where

- \( r(t) \) is the polarization anisotropy \( 2 \) at time \( t \).
- \( D = (D_1 + D_2 + D_3)/3 \), the mean rotational diffusion constant.
- \( \Delta = (D_1^2 + D_2^2 + D_3^2 - D_1D_2 - D_1D_3 - D_2D_3)/\tau_i \).
- \( c_i = a_1a_2a_3; (ijk) = (123), (231), \) or \( (312) \).
- \( \alpha_1, \alpha_2, \alpha_3 \) are the direction cosines of the absorbing dipole with respect to the principal rotation axes.
- \( \tau_1, \tau_2, \tau_3 \) are the corresponding direction cosines of the emitting dipole.
- \( F = \sum_{i=3}^{3} \alpha_i^2 \).
- \( G = \sum_{i=1}^{3} D_i(a_i^2\alpha_i^2 + \alpha_i^2\alpha_j^2 + \alpha_i^2\alpha_k^2) - D_i \neq j \neq k \neq i \).
In 1953, Hans Krebs recruited Weber for the new Biochemistry Department at Sheffield University.

Krebs received the Nobel Prize in 1953 for his elucidation of the metabolic reactions which produce energy in cells – the Tricarboxylic Acid or Krebs Cycle.

During his years at Sheffield Weber continued to lay the foundations of modern fluorescence spectroscopy developing both fluorescence theory and instrumentation.
Professor Sir Hans Krebs Nobel Prize:
Celebration dinner in the Old Staff Club (Oct 27, 1953)
Gregorio Weber’s pioneering contributions during these early years included his report, with Laurence (in 1954) of the fluorescence properties of anilino-naphthalene sulfonate (ANS).

Fluorescent Indicators of Adsorption in Aqueous Solution and on the Solid Phase. By G. Weber and D. J. R. Laurence. (Department of Biochemistry, University of Sheffield and Postgraduate Medical School, London, W. 12)

It is interesting to note that even today, 60 years after that first report, ANS is still being used in protein studies, quite often as an indicator of the “molten globular” state.
Intrinsic Protein Fluorescence

During his years at Sheffield, Weber and his postdoctoral fellow, John Teale, began their studies on the fluorescence of the aromatic amino acids and proteins.

In the late 1950’s and early 1960’s, Weber and Teale published a series of important papers including the first description of the excitation and fluorescence spectra of the aromatic amino acids - tryptophan, tyrosine and phenylalanine.

Figure 7 from this paper has been reproduced many times in review articles and books.
Ultraviolet Fluorescence of the Aromatic Amino Acids

By F. W. J. TEALE AND G. WEBER.
Department of Biochemistry, The University, Sheffield 16

(Received 25 June 1956)

Fig. 4. Excitation spectrum of phenylalanine fluorescence in water. Abscissa: wavelength (mμ). Ordinate: molecular extinction coefficient. The continuous line is the optical density spectrum; the dots are the values of log Ω in equation (5).

Fig. 5. Excitation spectrum of tyrosine fluorescence in water. Co-ordinates are as in Fig. 1.

Fig. 6. Excitation spectrum of tryptophan fluorescence in water. Co-ordinates are as in Fig. 4.

Fig. 7. Fluorescence spectra of the aromatic amino acids in water. Abscissa: wavelength (mμ). Ordinate: relative number of quanta.
Phase Fluorometry

During the 1950’s, Gregorio Weber started to think about constructing a fluorescence lifetime instrument. Influenced perhaps by the work of fellow Argentinean Enrique Gaviola, Weber worked on designing a phase fluorometer. At that time Birks and others had also built several types of phase fluorometers.

At the University of Illinois in the mid-1960’s Weber, together with his graduate student Richard Spencer, constructed a highly versatile phase and modulation fluorometer utilizing the principle of cross-correlation (Annals New York Acad. Sci. 158, 361).

The cross-correlation approach proved to be the key to modern phase fluorometry and is still used universally today.

MEASUREMENTS OF SUBNANOSECOND FLUORESCENCE LIFETIMES WITH A CROSS-CORRELATION PHASE FLUOROMETER

Richard D. Spencer and Gregorio Weber
When Enrico Gratton joined Weber’s laboratory as a postdoctoral fellow from 1975-1976, he worked, at the suggestion of Weber, on the development of a phase and modulation fluorometer with continuously variable light modulation frequencies.

Enrico returned to Urbana in 1978 as an Assistant Professor in the Physics Department. By this time he had finished the first true multifrequency phase and modulation instrument, utilizing a Pockels cell as the light modulator, thus completing Weber’s vision.
Weber’s work with phase and modulation fluorometry also led indirectly to the development of the Phasor approach to lifetime imaging by Enrico Gratton
Awards

American Academy of Arts and Sciences - 1968
1st National Lecturer of the Biophysical Society - 1969
Guggenheim Foundation Fellow - 1970
Corresponding Member of the National Academy of Exact Sciences of Argentina - 1971
National Academy of Sciences (U.S.) - 1975
Rumford Premium of American Academy of Arts and Science - 1979
ISCO Award for excellence in Biochemical Instrumentation - 1983
First recipient of Repligen Award for the Chemistry of Biological Processes: Awarded by the American Chemical Society -1986
First recipient of the International Jablonski Award for Fluorescence - 1996
Established in 1839, the Rumford Premium is one of the oldest scientific prizes in the US. and recognizes contributions to the fields of heat and light, broadly interpreted. The endowment was created by a bequest to the Academy from Benjamin Thompson, Count Rumford, in 1796.

Previous winners include:

Repligen Corporation Award in Chemistry of Biological Processes

The Repligen Award

The Repligen Award for Chemistry of Biological Processes was established in 1985 and consists of a silver medal and honorarium. Its purpose is to acknowledge and encourage outstanding contributions to the understanding of the chemistry of biological processes, with particular emphasis on structure, function, and mechanism. The Award is administered by the Division of Biological Chemistry of the American Chemical Society.

1986 Gregorio Weber 2001 Rowena G. Matthews
1987 Thomas C. Bruice 2002 C. Dale Poulter
1989 Stephen J. Benkovic 2004 JoAnne Stubbe
1990 Harold A. Scheraga 2005 David E. Cane
1992 Frank H. Westheimer 2007 Michael Marletta
1993 Jeremy R. Knowles 2008 Hung-Wen (Ben) Liu
1994 Judith P. Klinman 2009 Frank Rauschel
1995 W. Wallace Cleland 2010 Ronald T. Raines
1996 William P. Jencks
1997 James A. Spudich
1998 David S. Eisenberg
1999 Christopher T. Walsh
2000 Perry A. Frey
International Symposia Honoring Gregorio Weber

Fluorescence Symposia

Bocca de Magra – 1986
International Symposia Honoring Gregorio Weber

Fluorescence Symposia

Bocca de Magra – 1986
Frascati - 1991
Maui - 1995
Maui - 1999
Kauai - 2002
Kauai - 2005
Kauai - 2008
Kauai - 2011
Kauai - 2014
Búzios - 2017
11th International Weber Symposium

Búzios, Brazil

2021

???
These Weber meetings are known for their total focus on rigorous science!

(Enrico demonstrating fluctuations)
and at the last meeting in Buzios Brazil Enrico gave a hands-on course on diffusion and particle tracking
But that’s another story...............
Gregorio Weber

In tribute to the outstanding contributions of Gregorio Weber (1916-1997) to the field of fluorescence, the Laboratory for Fluorescence Dynamics (LFD) organizes and sponsors the

- International Weber Symposium on Innovative Fluorescence Methodologies in Biochemistry & Medicine
- Gregorio Weber International Prize in Biological Fluorescence

See also:

- List of Publications by Gregorio Weber, maintained by LFD.
- Gregorio Weber Award for Excellence in Fluorescence Theory and Applications, sponsored by ISS, Inc.
- Gregorio Weber Homepage, maintained by David Jameson.
- Tributes to Gregorio Weber, collected by David Lloyd.


Excerpt from the article published by David M. Jameson in *Biophysical Journal* 75(1), 419-421, 1998.

"Early on the morning of July 18, 1997, at home and surrounded by family and friends, Gregorio Weber died of leukemia at the age of 81. His death ended a remarkable scientific career, which began in Buenos Aires, took form in England at Cambridge and Sheffield, and flourished at the University of Illinois at Urbana-Champaign.

Gregorio Weber's research career, spanning more than half a century, was characterized by an unbroken chain of highly original and important contributions to fluorescence spectroscopy and protein chemistry. Born in Buenos Aires, Argentina, in 1916, Weber completed his M.D. degree at the University of Buenos Aires in 1942. While attending medical school, he worked as a teaching assistant for Bernardo Houssay, who was to receive the Nobel Prize for Physiology and Medicine in 1947. Houssay nominated Gregorio Weber for a British Council Fellowship, which would support his graduate studies at Cambridge University. Travel to England during the war years was an adventure, and his voyage took 44 days in a convoy that endured occasional U-boat attacks. At Cambridge, Malcolm Dixon, the well-known enzymologist, became his thesis advisor and suggested that the young Argentinean investigate the fluorescence of flavins and flavoproteins. Weber soon learned that, during the 1920s and 1930s, fluorescence had already greatly impressed the physicists and to some extent the biologist, but had not drawn much attention from the chemists. For example, a fellow Argentinean, the physicist Gaviola, had already constructed a phase fluorometer in the 1920s and had measured the excited state lifetime of fluorescein with good precision. Weber soon came upon the writings of Francis Perrin (the son of Jean Perrin, who had worked on the translational diffusion of macroscopic particles), on the depolarization of fluorescence by Brownian rotation and on energy transfer. Perrin's beautifully crafted theories and clarity of thought and expression inspired Weber to apply these methods to biochemistry."

Read more on the Gregorio Weber Homepage, maintained by David M Jameson.
Perspectives on Fluorescence
A Tribute to Gregorio Weber

A Fluorescent Lifetime: Reminiscing About Gregorio Weber: Jameson, David M.

Gregorio Weber’s Roots in Argentina: Barrantes, Francisco

The Labyrinthine World of Gregorio Weber: Jovin, Thomas

Measurements of Fluorescence Decay Time by the Frequency Domain Method: Gratton, Enrico

Personal Recollections of Gregorio Weber, My Postdoc Advisor, and the Important Consequences for My Own Academic Career: Visser, Antonie
http://thejamesonlab.wordpress.com

Gregorio Weber Related links

Tribute to Gregorio Weber site
Weber Prize (PhD thesis award)
Gregorio Weber Award
(senior investigator award)
http://www.iss.com/events/weber.html
Tributes to Gregorio Weber (1916-1997)

Gregorio Weber's distinguished career and many contributions to our understanding of fundamental processes of biophysics and biochemistry have been extensively documented. On this web page are personal tributes to an exceptional scientist by some of those who knew and worked with him in Buenos Aires, Cambridge, Sheffield and Urbana.

- Gregorio Weber in Cambridge by Brian Hartley
- The First Floor by Brian Hartley
- Memories by Hal Dixon
- Reminiscences by Fred Sanger
- Friendship Renewed in Sheffield in 1952-53 by Quentin Gibson
- Gregorio Weber: Some Recollections by Pauline Harrison
- Appreciation by Theo Hofmann
- Recollections of Gregorio by Maurice Kaye
- Gregorio by Stanly Ainsworth
- “Stay in Sheffield” Gregorio’s Sage Advise by Graham Palmer
- Gregorio as Teacher by Peter J. Large
- Golden Days of Biophysics at Sheffield by David Lloyd
- Memories of the Biochemistry Department Sheffield 1961 and in particular of Gregorio Weber by Charles Williams
- My Best of Times: with Gregorio in Sheffield and Urbana (1954-1964) by Lorna B. Young
- Weber Memoir by Woody Hastings
- A Roman Connection by Maurizio Brunori
- My Mentor at Urbana, Rome, Corvallis and Beyond by Sonia Anderson
- Fond Memories from Argentinian Interactions by Leonardo Erijman and Elizabeth Jares-Erijman
- Two Memories in Parallel by George and Tamara Mitchell
- A Superb Interaction by David Laker
- An Appreciation by Carola Eisenberg
- Short Snippets
I do not feel able to comment on Gregorio’s published scientific work as it was in a rather different field from my own interests, but I do believe that his contribution to science was considerably more than has appeared in print. During the time that we were both working in the Cambridge Biochemical Laboratory he would frequently come over to my bench to see what I was doing, discuss my work and make useful suggestions. I found this stimulating and often helpful for my work. Gregorio had a considerably wider knowledge of science than I did, and was a wonderful person.

It was of course not possible to evaluate the effect of these meetings on my work, but I do feel that my publications and those of other members of the lab owe much to Gregorio’s influence. It is always stimulating to know that someone is interested in your work especially if they can make helpful suggestions.
Final observations...

You know David, when I was much younger an older colleague said to me “Gregorio, when you pass the age of 60 you will begin to notice that your students have more ideas than you and better ideas than you”

Huh?

Gee, really Professor?

...I have not found this to be the case...

How about you Enrico?

And David?

In their dreams!