Fluorescence Spectroscopy

Review of Instrumentation

"Single Photon Counting is now no longer for exclusive Specialist Research"

Principles of Fluorescence

- ***What is it ?***
- ***What is it used for ?***
- ***How do we measure it ?***

Fluorescence – What it is?

– [flô res´ens]

an effect in which a substance releases electromagnetic radiation while absorbing another form of energy, but ceases to emit radiation immediately upon cessation of the input energy

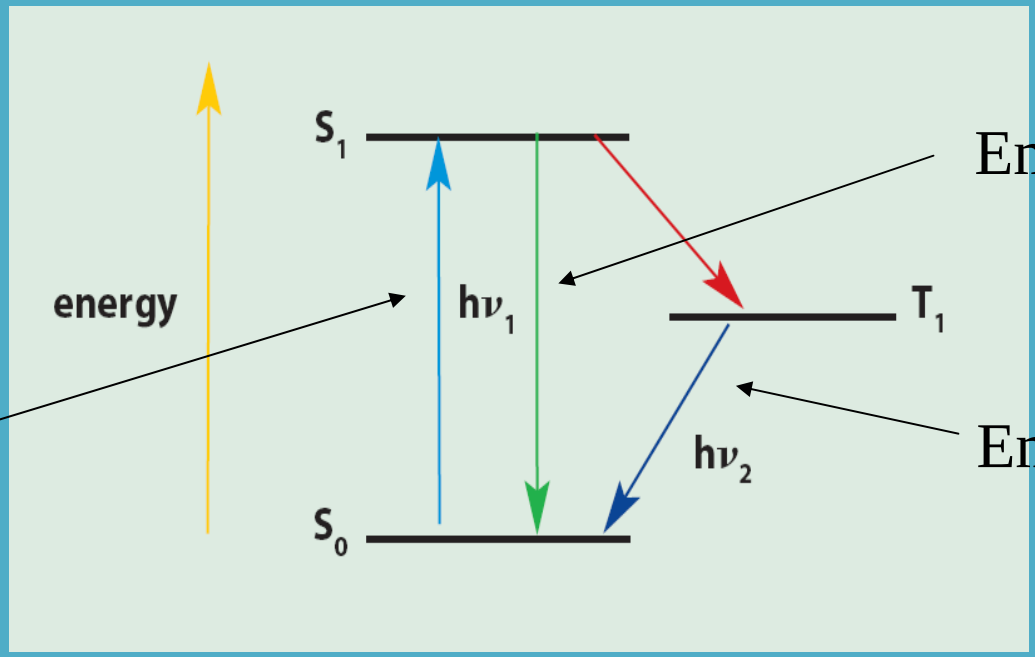
Luminescence

Luminescence is the emission of light caused by some external influence:

- Chemical reaction – chemi-luminescence
- Biochemical reaction – bio-luminescence
- Electrical discharge (recombination of ions and electrons at an electrode) – electro-luminescence
- Interaction with accelerated electrons - cathodo-luminescence
- Enhancement by the introduction of heat - thermo-luminescence
- Absorption of radiation - photoluminescence

Fluorescence & Phosphorescence

Fluorescence – the emission of light of longer wavelength by a substance as a result of the absorption of radiation of shorter wavelengths.



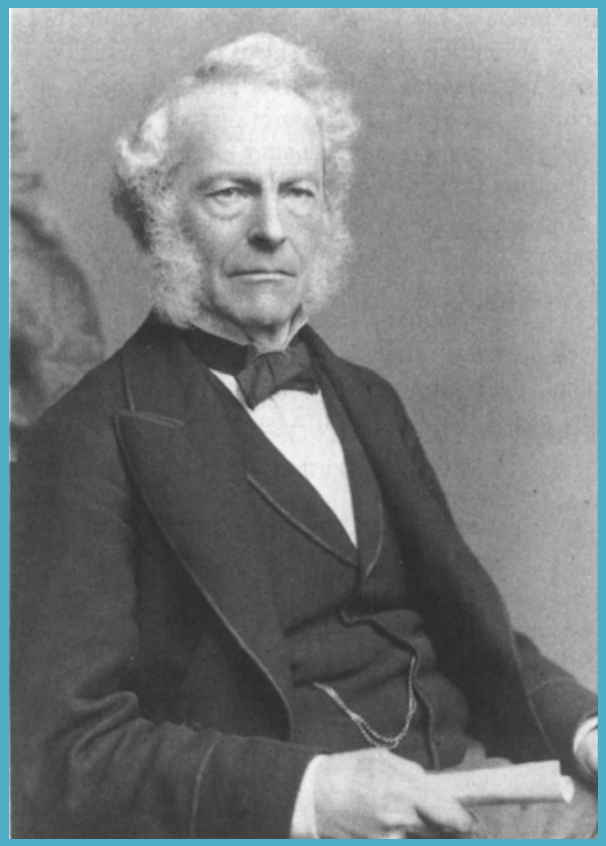
Light has wave-particle duality:

A photon is a discrete package of energy, $E = h\nu = hc/\lambda$

Fluorescence = fast decay <100ns, Phosphorescence = slower decay

Sir George Gabriel Stokes

– ***Experimental Observation - 1852***



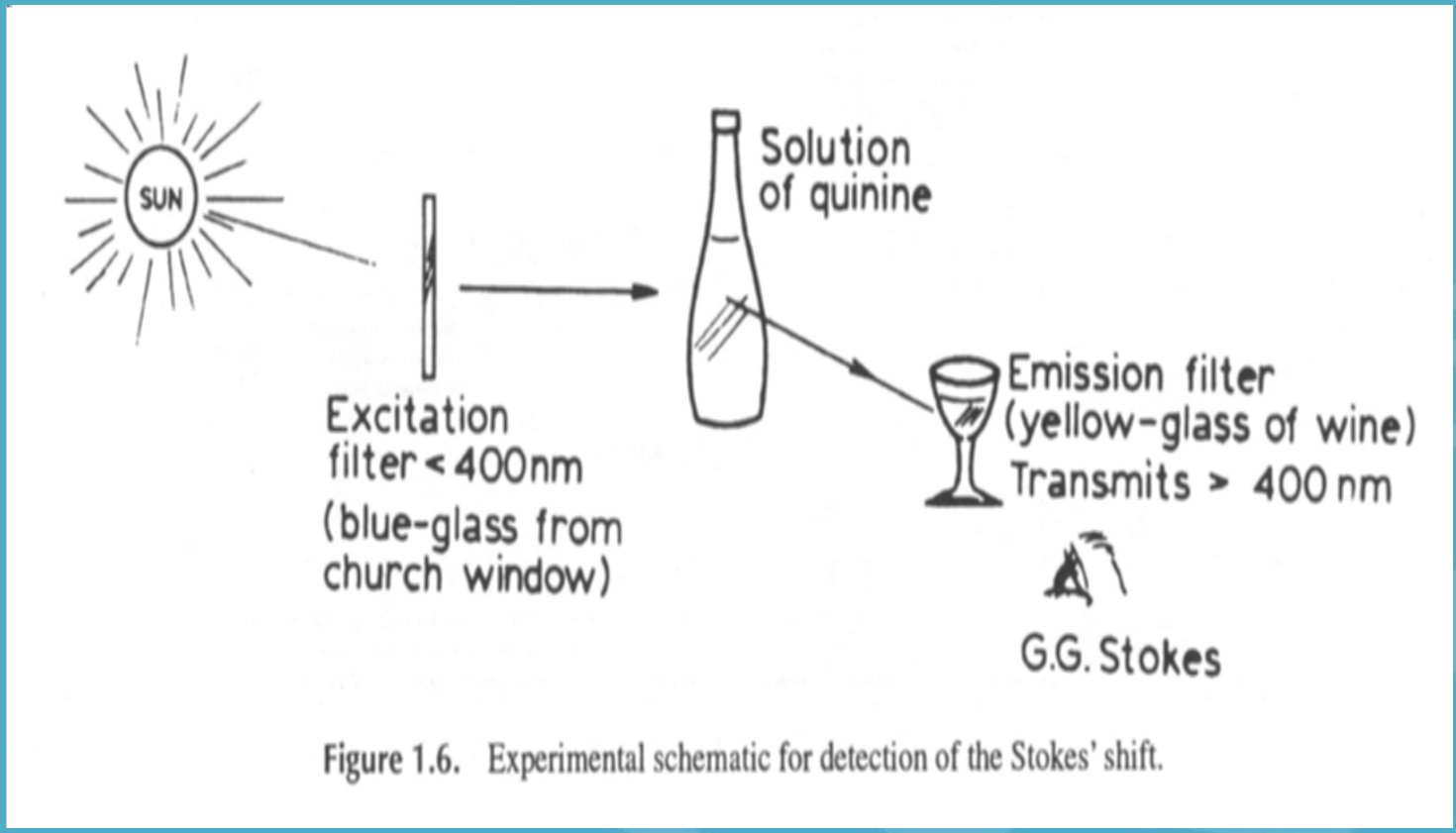


Figure 1.6. Experimental schematic for detection of the Stokes' shift.

Fluorescence Molecules & Spectra

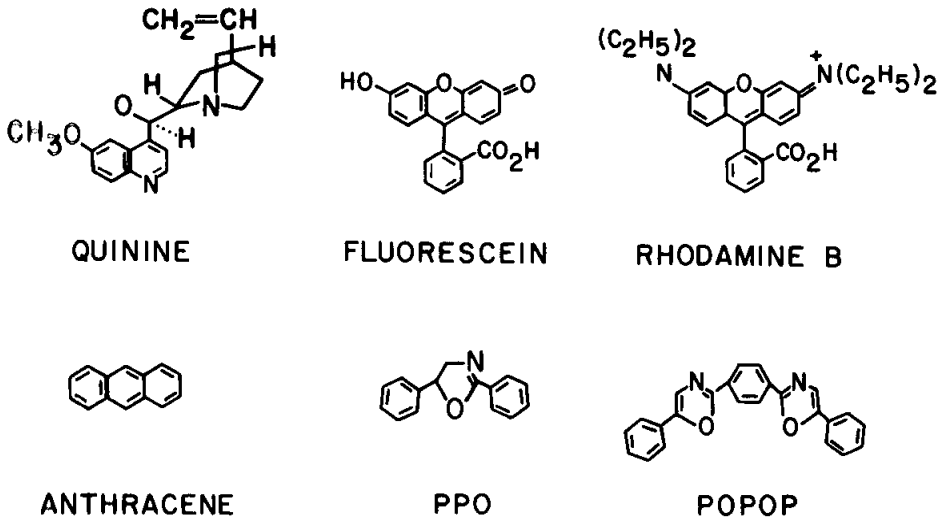
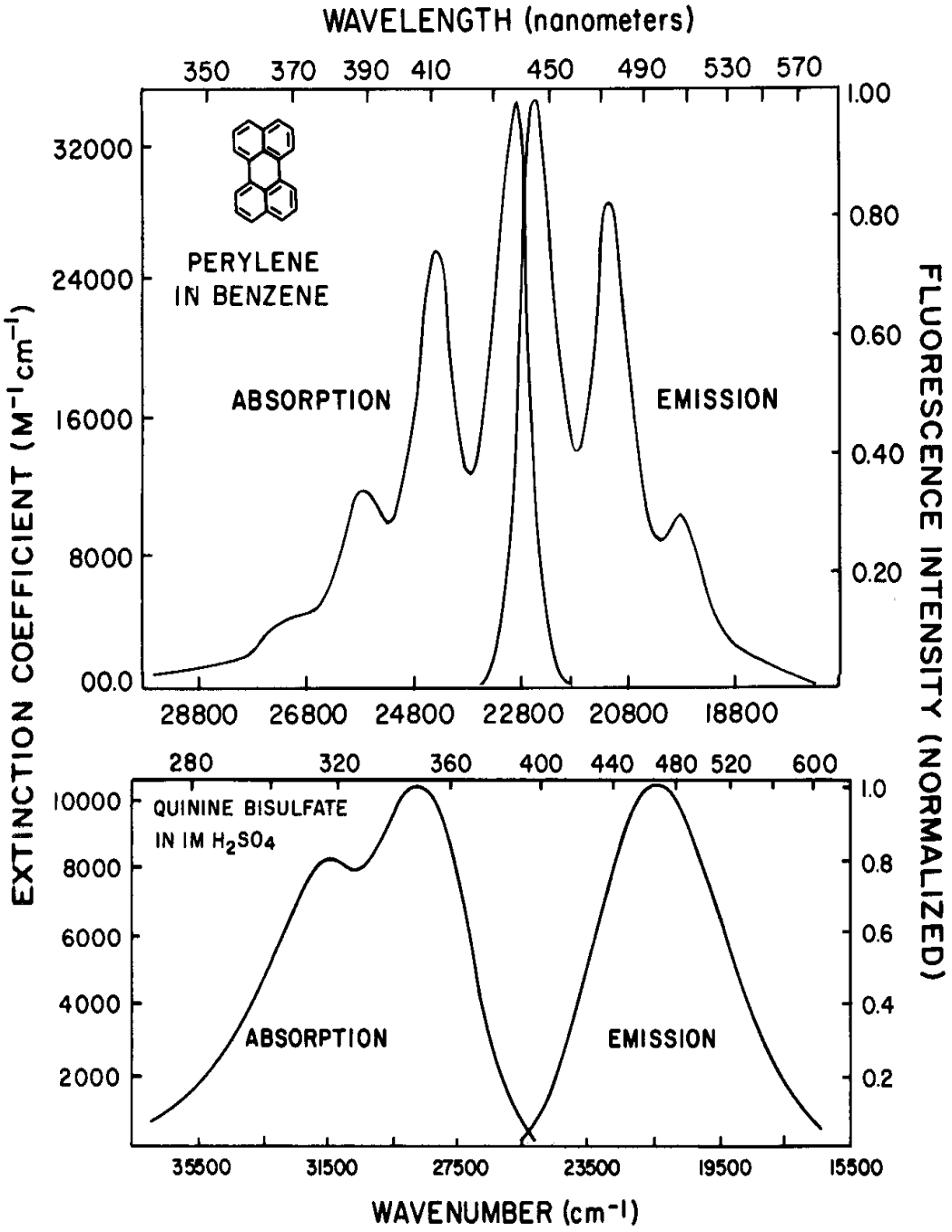
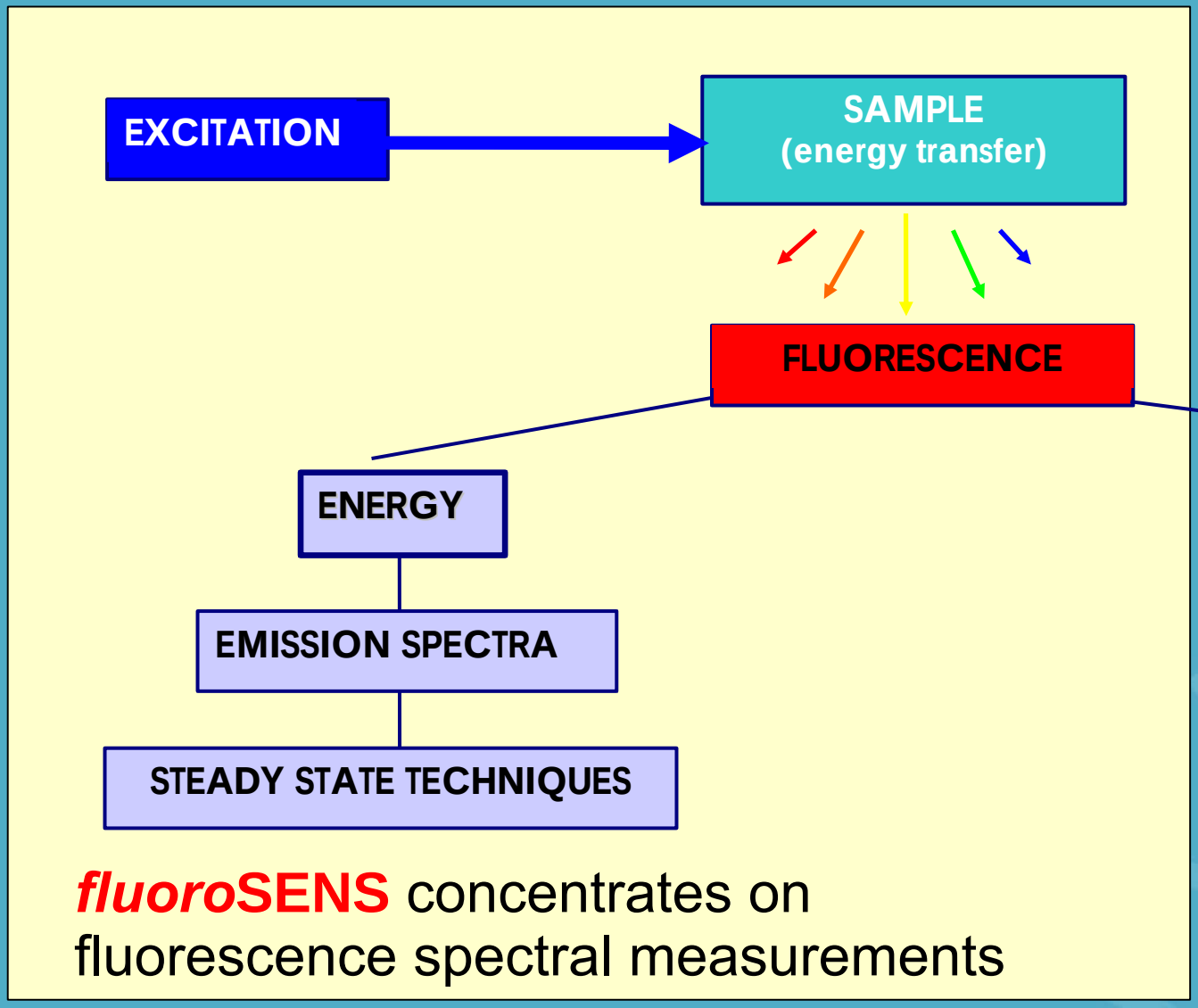


Figure 1.1. Structures of typical fluorescent substances.



GILDEN pλotonics

Fluorescence Spectroscopy



***fluoro*SENS** concentrates on fluorescence spectral measurements

Jabłoński Diagram - 1935

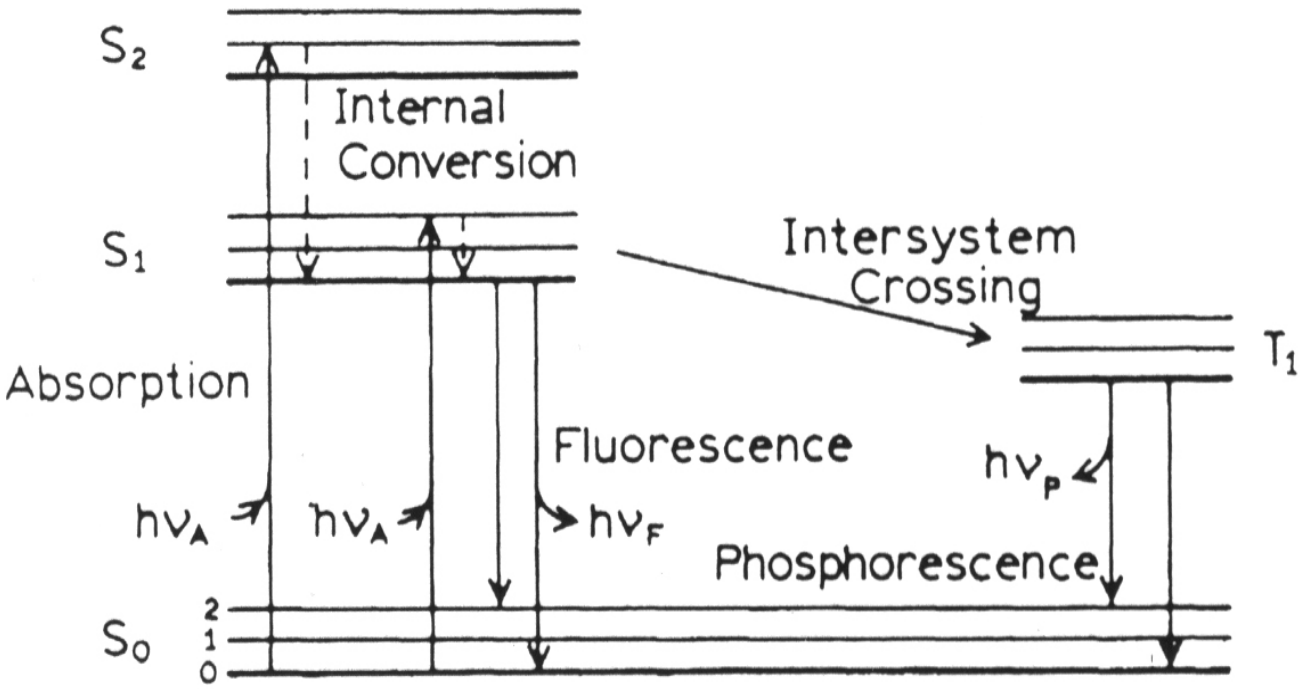


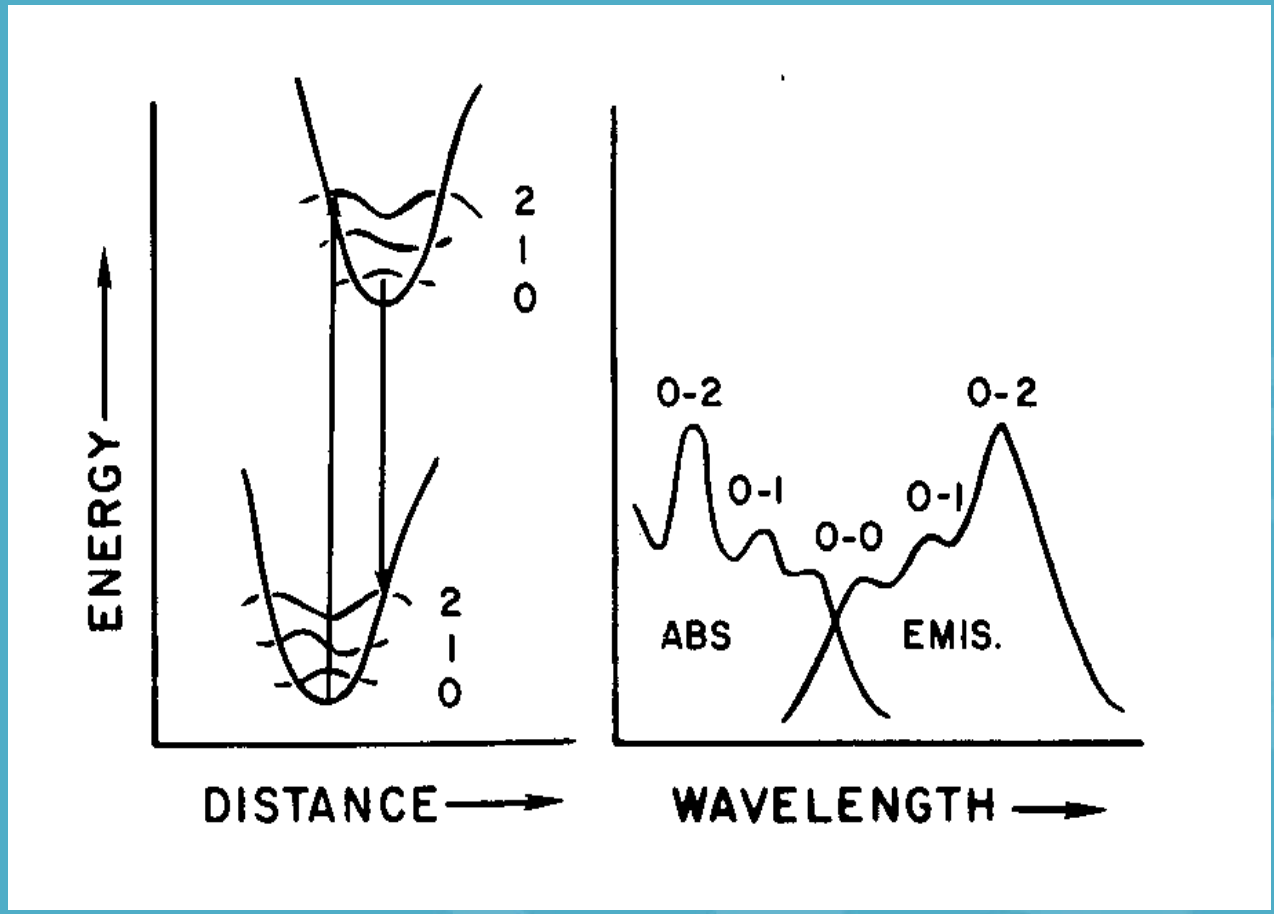
Figure 1.5. One form of a Jabłoński diagram.

Prof. Alexander Jabłoński

Frank-Condon Principle & Mirror Image Rule

Generally,

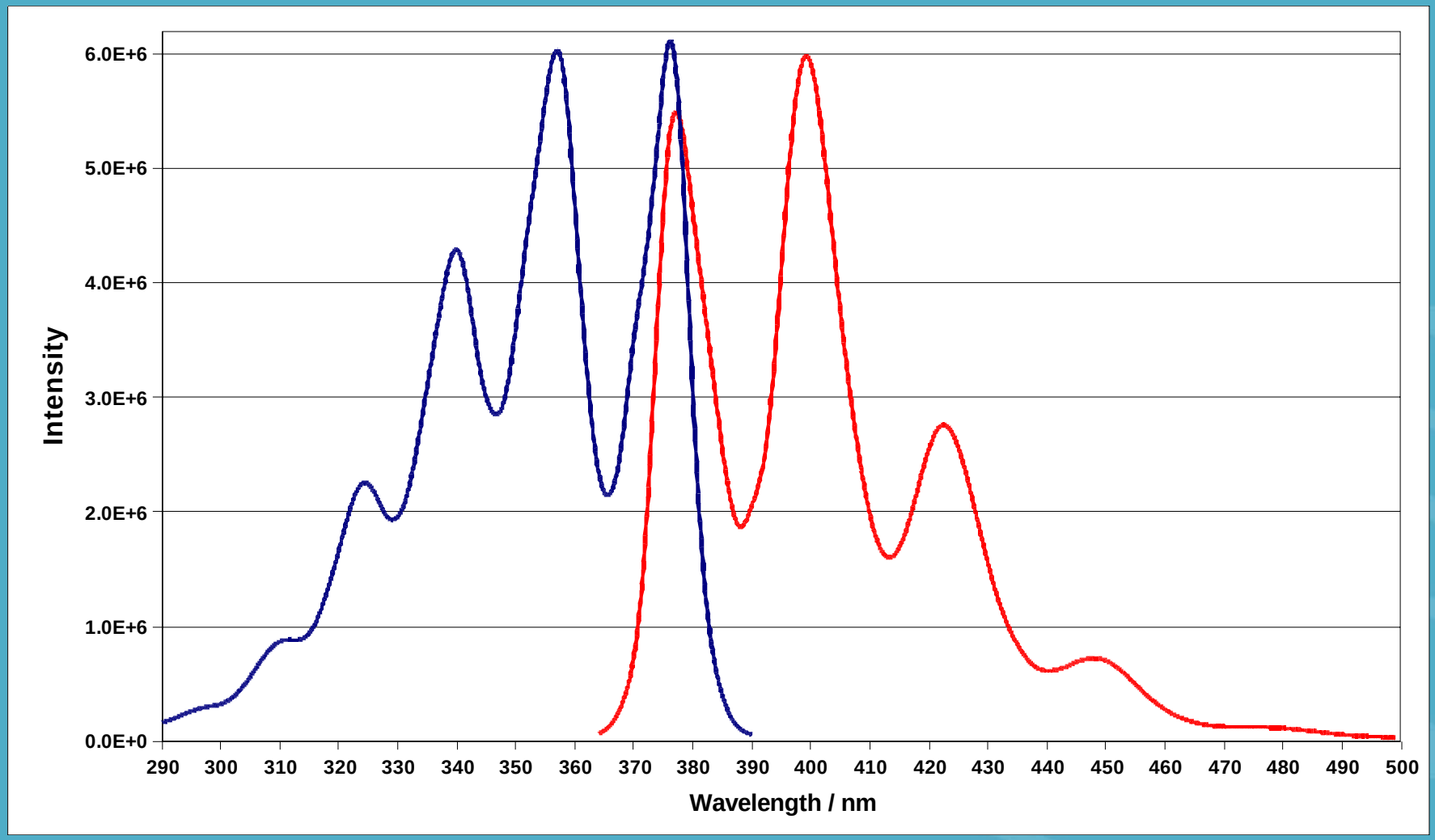
the fluorescence spectrum appears as a mirror image of the absorption spectrum, specifically the absorption spectrum representing the S_0 to S_1 transition.



Many exceptions occur mainly caused by the molecule changing its shape after excitation

Example of Mirror Rule

Anthracene in Cyclohexane, 10^{-5}M, degassed



Exceptions to Mirror Rule

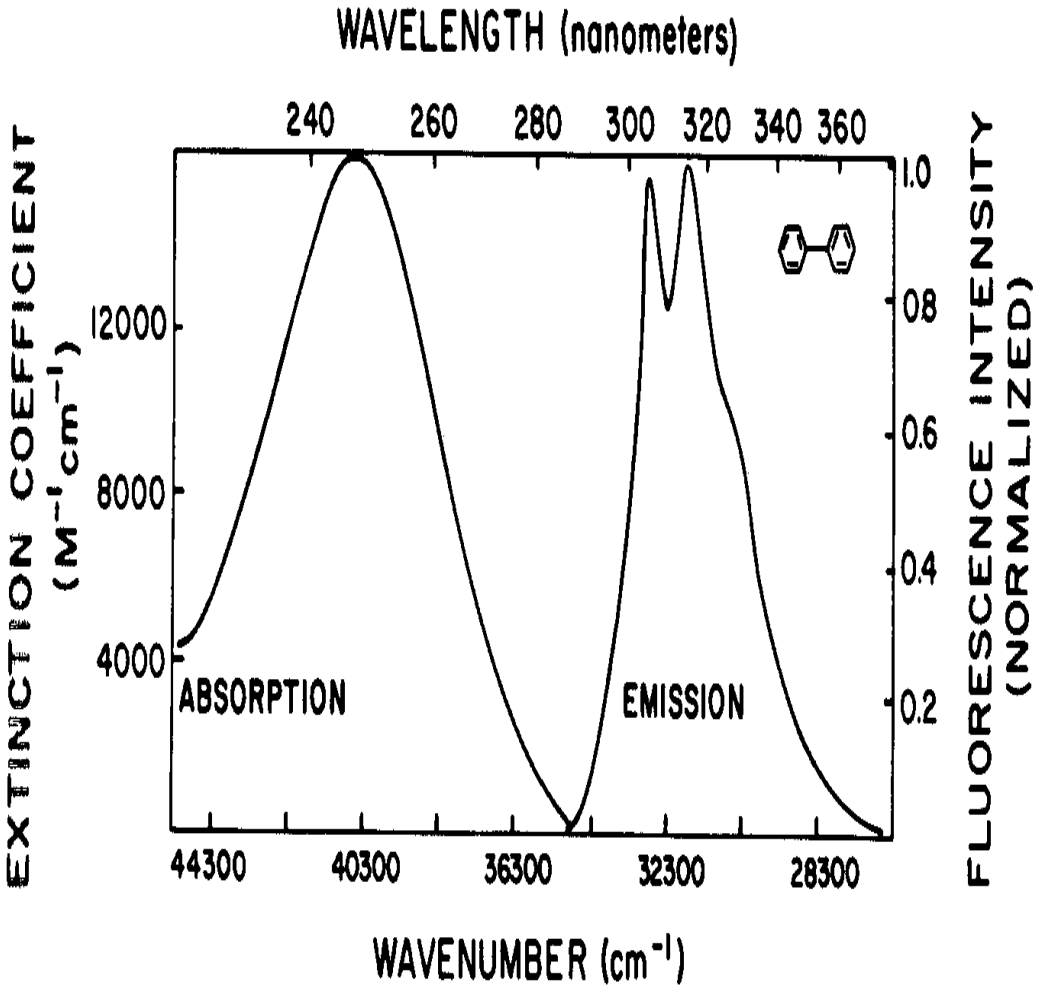


Figure 1.6. Absorption and emission spectra of biphenyl. (From Ref. 2.)

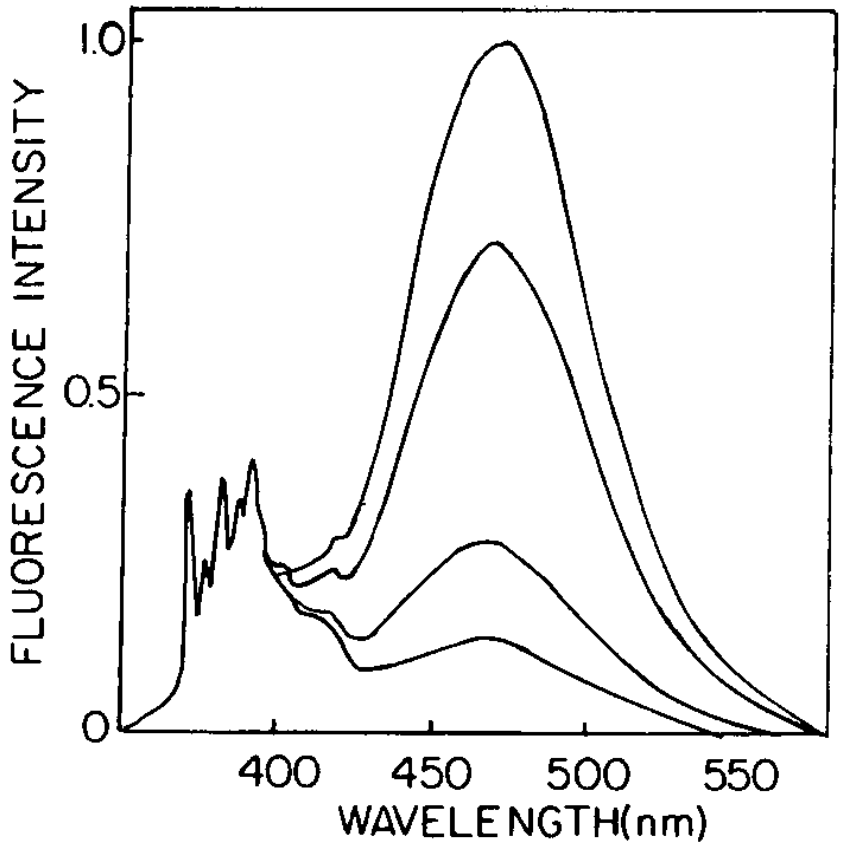


Figure 1.7. Emission spectra of pyrene and its excimer. The relative intensity of the excimer peak (470 nm) decreases as the total concentration of pyrene is decreased from 6×10^{-3} M (top) to 0.9×10^{-4} M (bottom). (From Ref. 6.)

Luminescence Parameters

- **Fixed parameters**
  - Φ quantum efficiency
  - I fluorescence intensity
  - P fluorescence polarisation
  - r fluorescence anisotropy

- **Dependence on 1 parameter**
  - I(λ) spectra - excitation, emission, synchronous
  - I(c) titration
  - I(T) temperature
  - r (λ) spectrally resolved anisotropy
  - I(t) time-resolved fluorescence measurements

- **Dependence on 2 parameters**
  - I (λ, t) time-resolved emission spectra
  - I (λ_{exc}, λ_{em}) excitation-emission spectral maps
  - I (λ, T) temperature resolved spectra
  - r (λ, t) time and spectrally resolved anisotropy

Luminescence Parameters

Luminescence emitted by a sample can be characterised by five parameters:

- ***excitation wavelength*** ***-fluoroSENS***
- ***emission wavelength*** ***-fluoroSENS***
- ***emission intensity*** ***-fluoroSENS***
- ***polarisation*** ***-fluoroSENS***
- ***decay-time***

Measurement of steady-state excitation and emission spectra are widely used to identify many chemical, physical and biological processes.

Importance of Luminescence

- The measurement of Luminescence / Fluorescence guarantees an increase in sensitivity 10 to 100 times over conventional absorption spectroscopy.

- nanomolar to picomolar samples can be analysed

- **Single photon counting sensitivity** gives added sensitivity guaranteeing an increase in sensitivity of 1,000 to 10,000 times over conventional absorption spectroscopy

- femtomolar samples can be analysed with ease!

Ideal Fluorimeter

- ***Extremely Sensitive, and no noise signals***
- ***Measures Excitation and Emission Spectra*** ***i.e. The photon flux emitted at each wavelength***
- ***Not Sensitive to interferent signals e.g.***

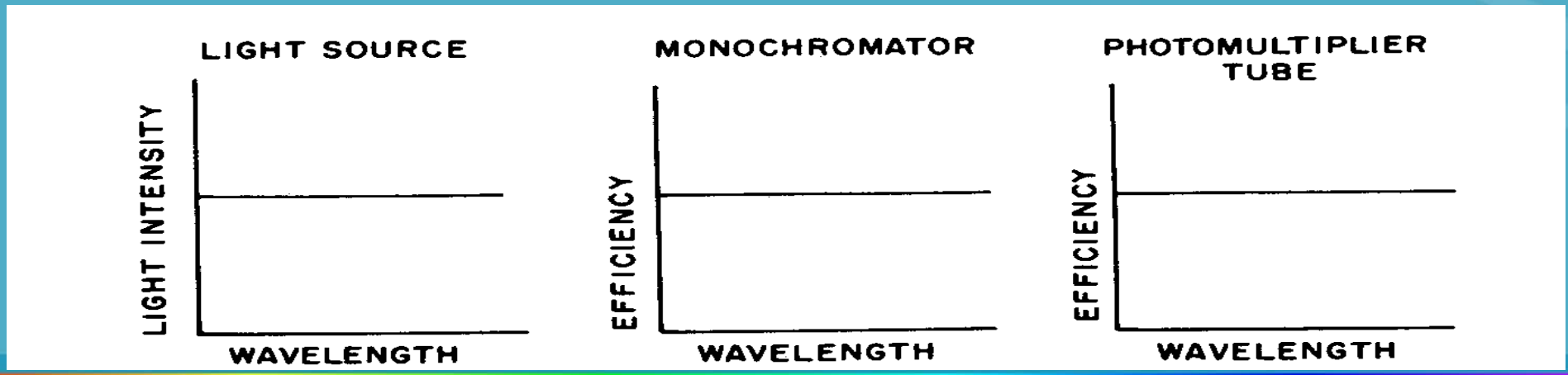
  Raman, Rayleigh Scattering, straylight,

  Fluorescence from solvents, etc.
- ***Measures the "True Spectra"***

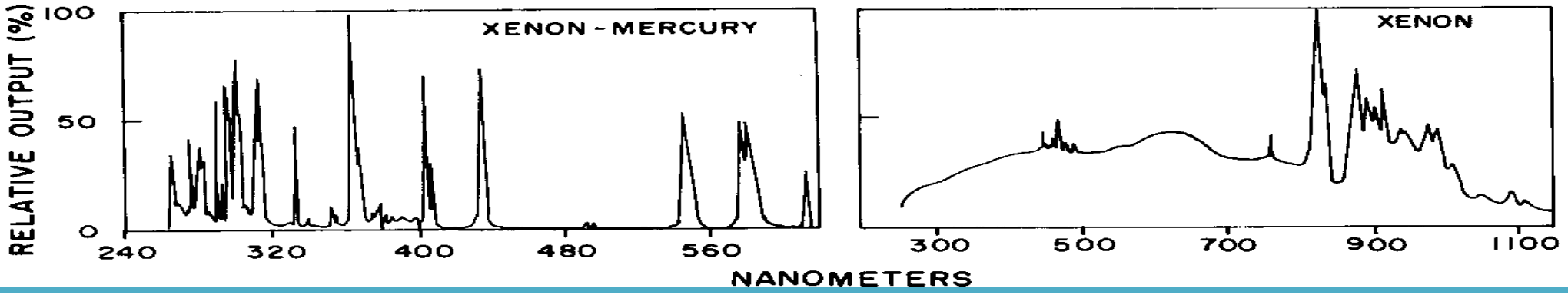
  Corrected for the non-uniform spectral output of light sources and wavelength dependent efficiency of monochromators and detectors.

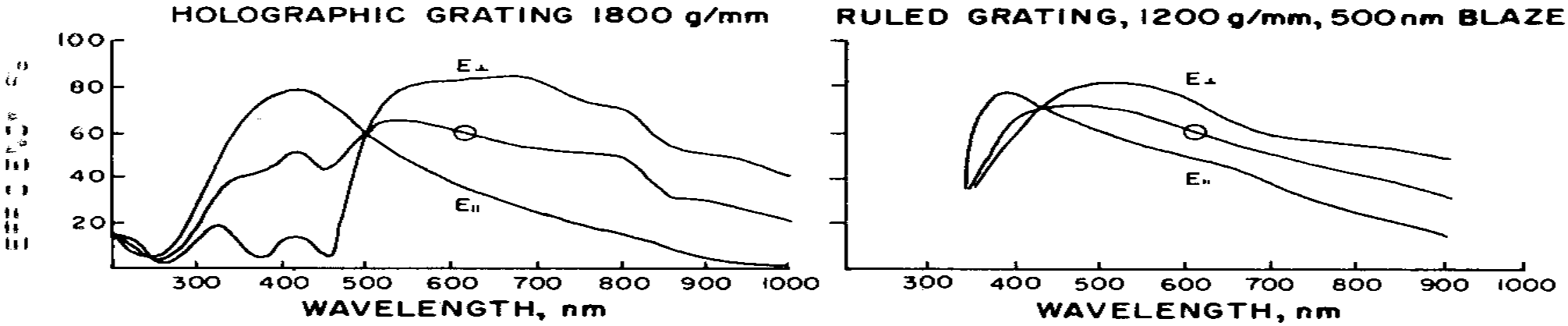
Ideal Fluorimeter

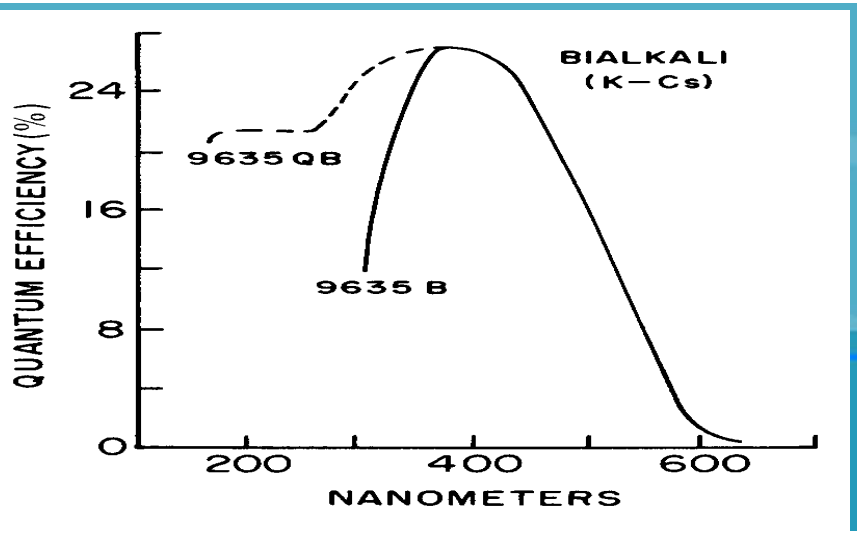
- ***The light source must yield a constant photon output at all wavelengths***
- ***The monochromator must pass photons of all wavelengths with equal efficiency***
- ***The monochromator must be independent of polarisation***
- ***The detectors must detect photons of all wavelengths with equal efficiency***



Distortions in Excitation & Emission Spectra







Introducing: *fluoro*SENS

Versatile Bench-top Single Photon Counting Fluorimeter

- High Technical Performance
- Low Cost

***fluoroSENS** is a fully integrated, computer controlled,*

***SINGLE PHOTON COUNTING** fluorimeter with comprehensive standard features and optional accessories.*

The fluoroSENS truly smashes the established price / performance ratio for a high sensitivity fluorimeter

Major Benefits of *fluoro*SENS

- **Ultimate Sensitivity** - measure down to femto-molar concentrations.
- **Spectral Range** – SPC detectors can cover VUV to NIR, i.e. 110nm to 1.7μm.
- **Exceptionally High Dynamic Range** - weak and strong fluorescence peaks can be viewed simultaneously.
- **High Spectral Resolution** - fine line structure can be resolved.

NO photo-bleaching !!

*fluoro*SENS: key features

- ***Single Photon Counting*** **Sensitivity & Dynamic Range**
- ***High Resolution*** **Exc & Em Monochromators**
- ***High Aperture & High Throughput*** **Optical Design – collects & transfers more signal to detector. → more signal**
- ***Reference Detector*** **for** ***Corrected Excitation Spectra***
- ***Corrected Emission Spectra***
- ***Unique Transmission Detector*** **for checking sample stability**

*fluoro*SENS: key features

- ***Integrated, high stability design***
- ***Xenon Lamp optical stability < 0.1%***
- ***Pre-aligned and calibrated system***
- ***Integrated filter changer - standard !***
- ***α-BBO Glan-Thompson motorised Polarisers***
- ***Choice of Blue or Red Sensitive PMT, NIR detector, InGaAs photodiode, etc.***
- ***Choice of unique digital signal processing module for enhanced s/n performance***

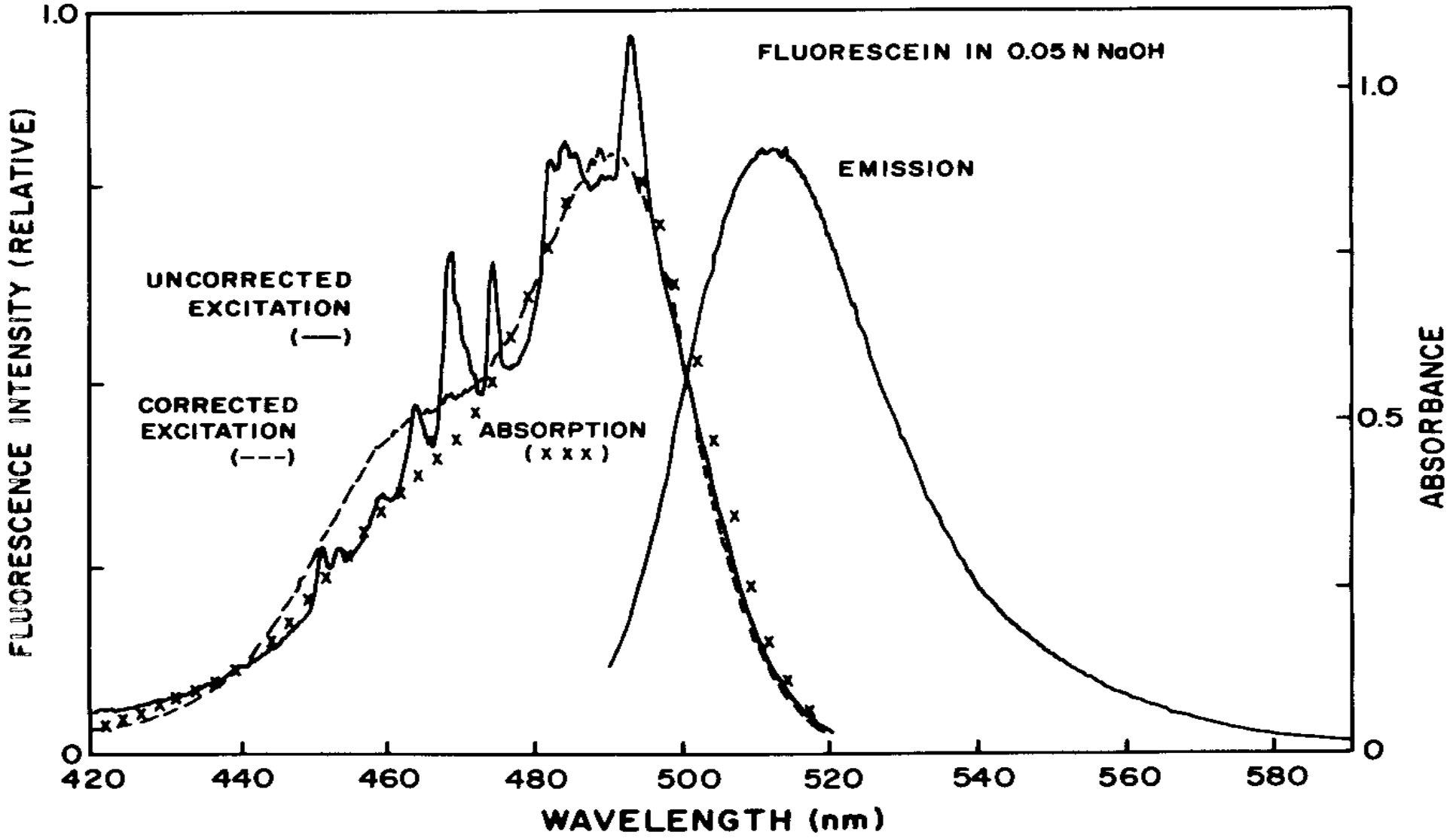
*fluoro*SENS: key features

- ***Flexible sample chamber with full side access for accessories – wide range of accessories***
- ***Comprehensive Fluorescence Spectra software for data collection, analysis and presentation***
- ***Method Files: recall complete experimental parameters***
- ***Compact Desktop Size – 0.6m (D) x 0.83m (W)***
- ***USB 2.0 interface (no interface cards for PC)***
- ***Power supply – 90 to 260V ac, 50/60 Hz***

Reference Detector

- ***STANDARD in every fluoroSENS instrument***
- ***Fluorescence Signal depends on Excitation Intensity Level***
- ***Large Area Silicon Photodiode***
- ***Detector Calibrated to give "true" correction of excitation intensity***
- ***Simultaneous Acquisition during Excitation Scans***
- ***Provides on-line, real time, corrected Spectra for best measurement performance – unique at this price level***

Example of Corrected Exc. Spectrum



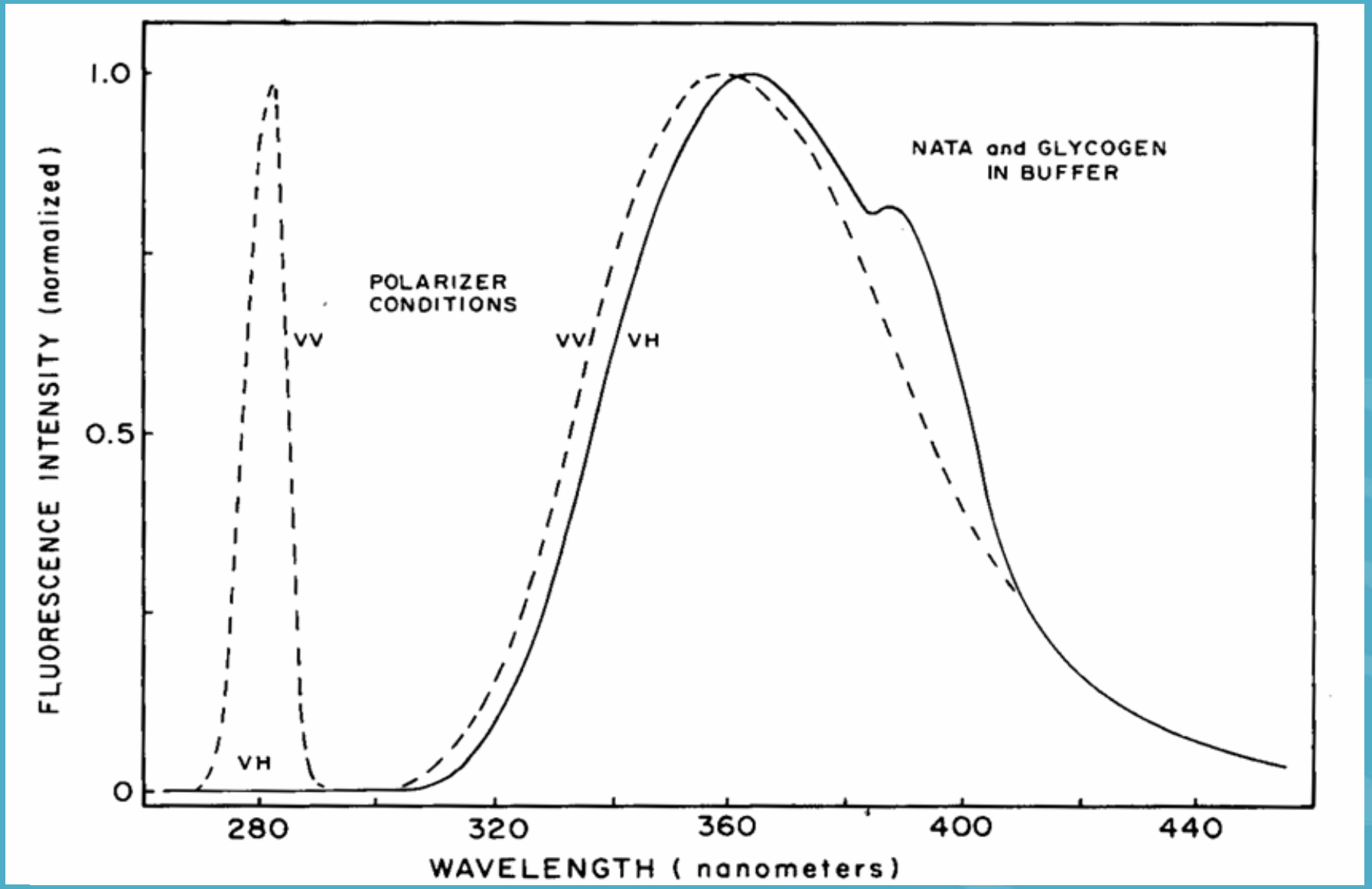
Corrected Emission Spectra

- ***STANDARD in every fluoroSENS instrument***

- ***Factory determined correction function using National Standards Accredited tungsten calibration lamp***

- ***Guarantees that one measures the "true" emission spectrum from the sample***

Example of un-Corrected Spectra



Automated Filter Changer

- ***Unique, standard component in fluoroSENS***

- ***Auto insertion of order sorting filter as measurement proceeds.***

- ***Up to 5 filters selectable***

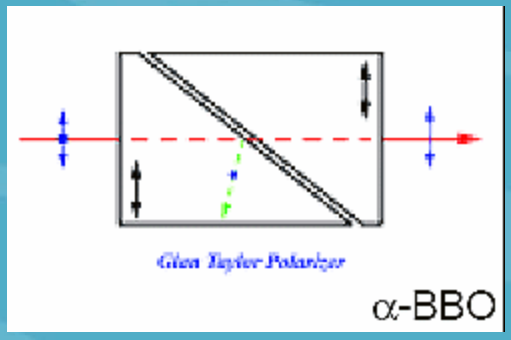
- ***Filter selection automatically included in the spectral correction functions***

- ***Reduces stray and scattered light***

- ***Does not necessarily remove Raman scattered signal from solvents, depends on filter used***

Anisotropy / Polarisation option

- ***Key measurement in Life Sciences, Drug Interaction Studies, Photo-Chemistry, Polymer studies, etc.***
- ***Steady-state anisotropy obtained from polarised excitation and emission spectra.***
- ***Allows study of molecular motion***
- ***α-BBO Glan-Thompson Polarising Prisms***
- ***Ideal for Spectral Range 200nm to 3500nm***
- ***Superb Extinction Coefficient 5 x 10^{-6} guarantees exceptional polarisation purity***
- ***Motorised Positioning into beam***
- ***Motorised Angular Selection***
- ***0º , 90º, Magic Angle, free selection***



Applications of Anisotropy

- ***Micro-viscosities of Cell Membranes***
- ***Rotational Diffusion of Proteins***
- ***Associated Reactions between Biological Molecules***
- ***Denaturisation of DNA***
- ***Segmental Mobility of Biopolymer-bound fluorophore or Antibody molecules***
- ***Lipid Bilayers labeled with Diphenylhexatriene (DPH)***

Transmission Detector option

- ***Unique to fluoroSENS***

- ***Allows direct monitoring of the optical density of a sample – ideal for life science customers***

- ***Real-time, on-line check of sample photo-bleaching, cell death, sample settling, etc.***

- ***Large Area Silicon Photodiode***

Software

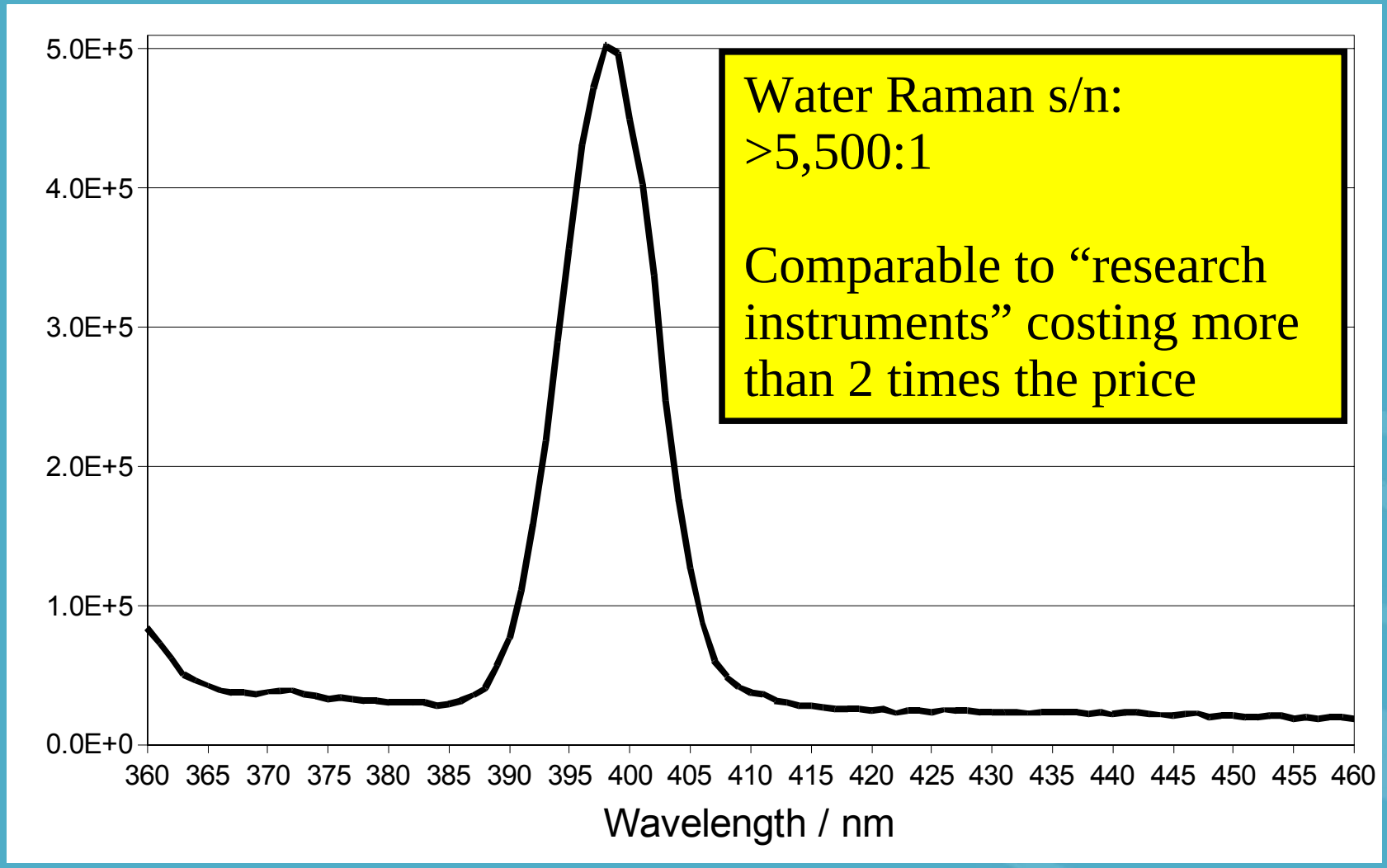
- ***Ex, Em, Sync Scans***
- ***Ex-Em, Sync Mapping***
- ***Anisotropy***
- ***Corrected Exc and Emission Spectra***
- ***Flexible 2D, 3D, and Contour charting***
- ***Flexible Analysis features***
- ***Spectral Arithmetic (+, -, x, /)***
- ***Normalisation***
- ***$1^{st.}$, $2^{nd.}$, $3^{rd.}$ Spectral Derivatives***
- ***Integration***
- ***Anisotropy***
- ***Spectral Correction (post acquisition)***

Sample Chamber Accessories

- ***Single Cell Holder - standard***
- ***Water Thermostattable Cell Holder with Magnetic Stirrer***
- ***Automated Four Position Cell Holder***
- ***XYZ(or R) Sample Positioner***
- ***Front Face Cell Holder with Rotation***
- ***Stopped Flow Cell***
- ***Optical Fibre input and output for remote fluorescence measurements***
- ***EPR Dewar***
- ***Cryostats (Oxford Instruments N_2 and He)***
- ***Plate Reader (needs optical fibre accessory)***

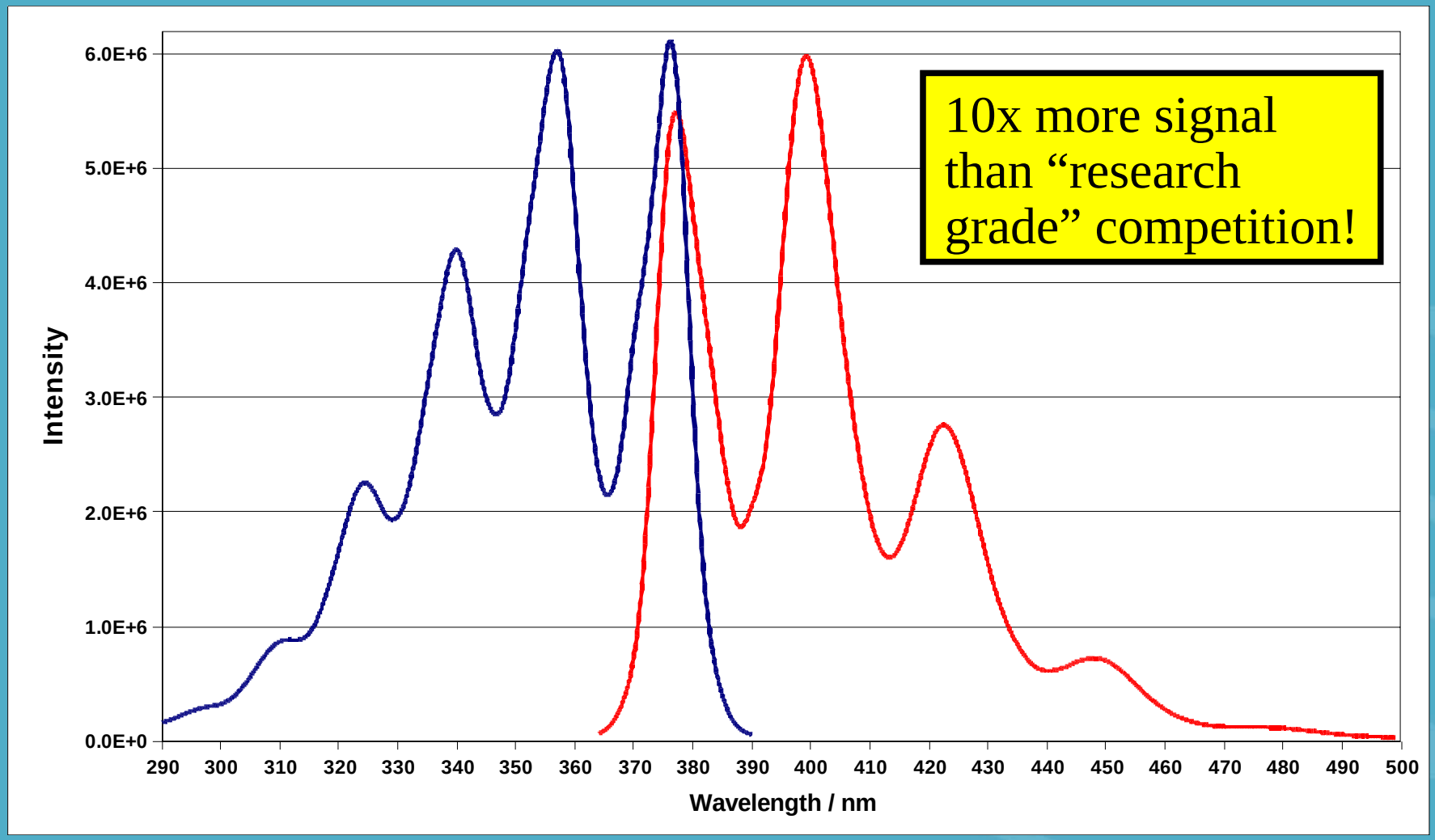
Performance

Sensitivity: Water Raman signal-to-noise



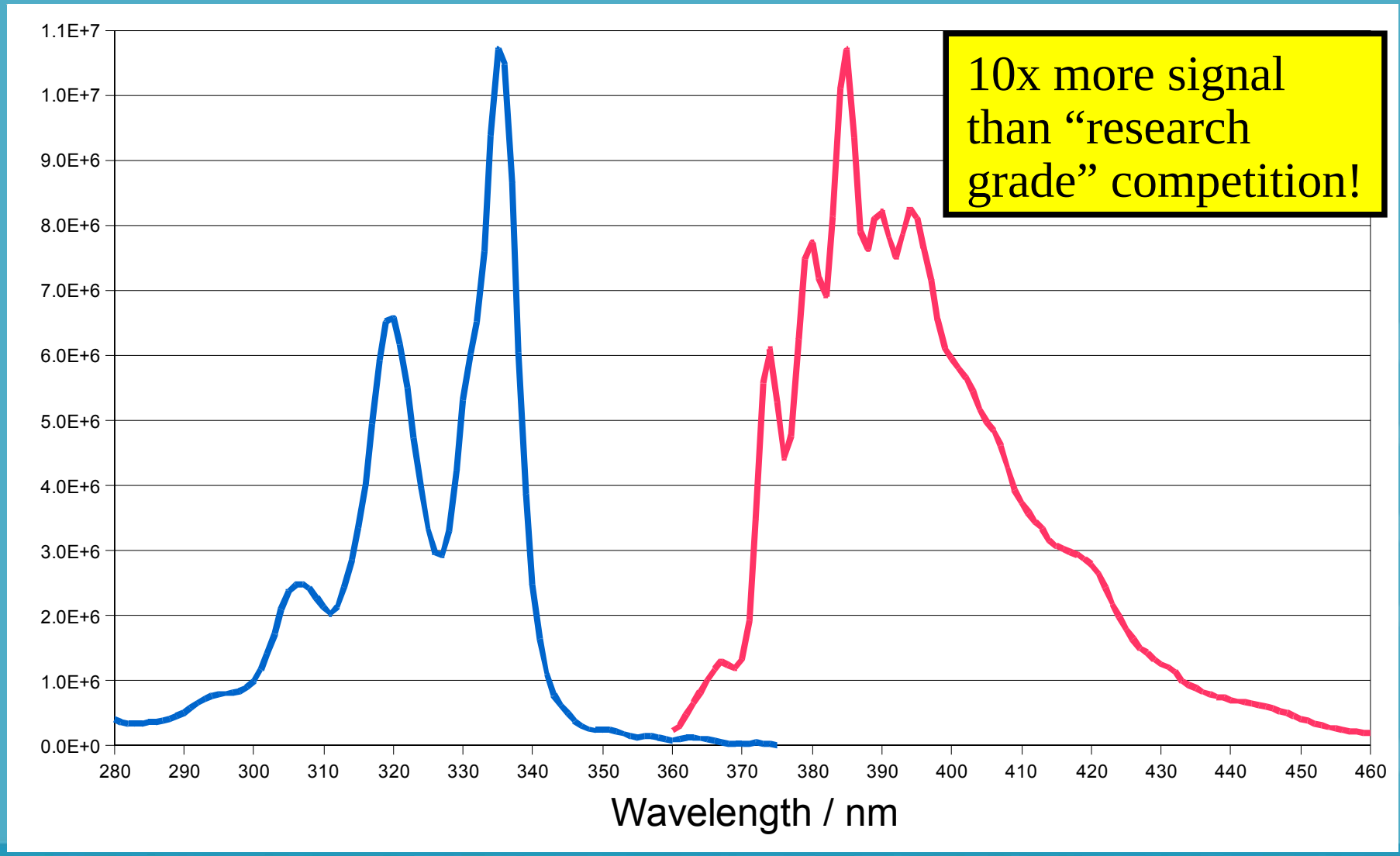
Performance

Anthracene in Cyclohexane, 10^{-5}M, degassed



Performance

Pyrene in Cyclohexane, 10^{-5}M, degassed



*fluoro*SENS Summary

- ***Single Photon Counting Sensitivity*** **- *First time in an instrument in this price bracket***
- ***Superb Signal-to-Noise Ratio and Sensitivity***
- ***Superb spectral resolution and minimum straylight***
- ***Standard features*** **include** ***Reference Detector and Corrected Emission channel***
- ***Comprehensive software for spectral measurements, analysis and presentation***
- ***Extremely good price / performance ratio*** **– *technical performance of "research instrument" for the price of a "standard routine" instrument***

Application Fields

Pharmaceuticals and Medicine
- Immuno assay methods
- Advanced research
- Routine Analysis
- Drug Interaction studies
- DNA, proteins, nucleic acid, virus, etc.

Industry
- Quality Control
- Plastics, polymers,
- Optical brightners
- Phosphor coatings
- Cosmetics, sunscreens,
- Health care

Environmental
- Monitor trace quantities of materials organics, inorganics, mutagenic materials, carcinogens, etc.
- Geological analysis, e.g. Oil and Oil in rock.
- Synchronous scanning for quantitative PAH analysis.
- Exc-Em mapping for complete fluorescence fingerprint

Food Science & Agriculture
- Assessment of shelf life, & packaging
- Bacterial growth
- Pesticide analysis
- Packaging -polymers & plasticisers

Application Fields

Analytical Chemistry
- Measurement of extremely low analyte concentrations
- Identification and detection of single molecules
- Analysis of complex mixtures of fluorescent substances

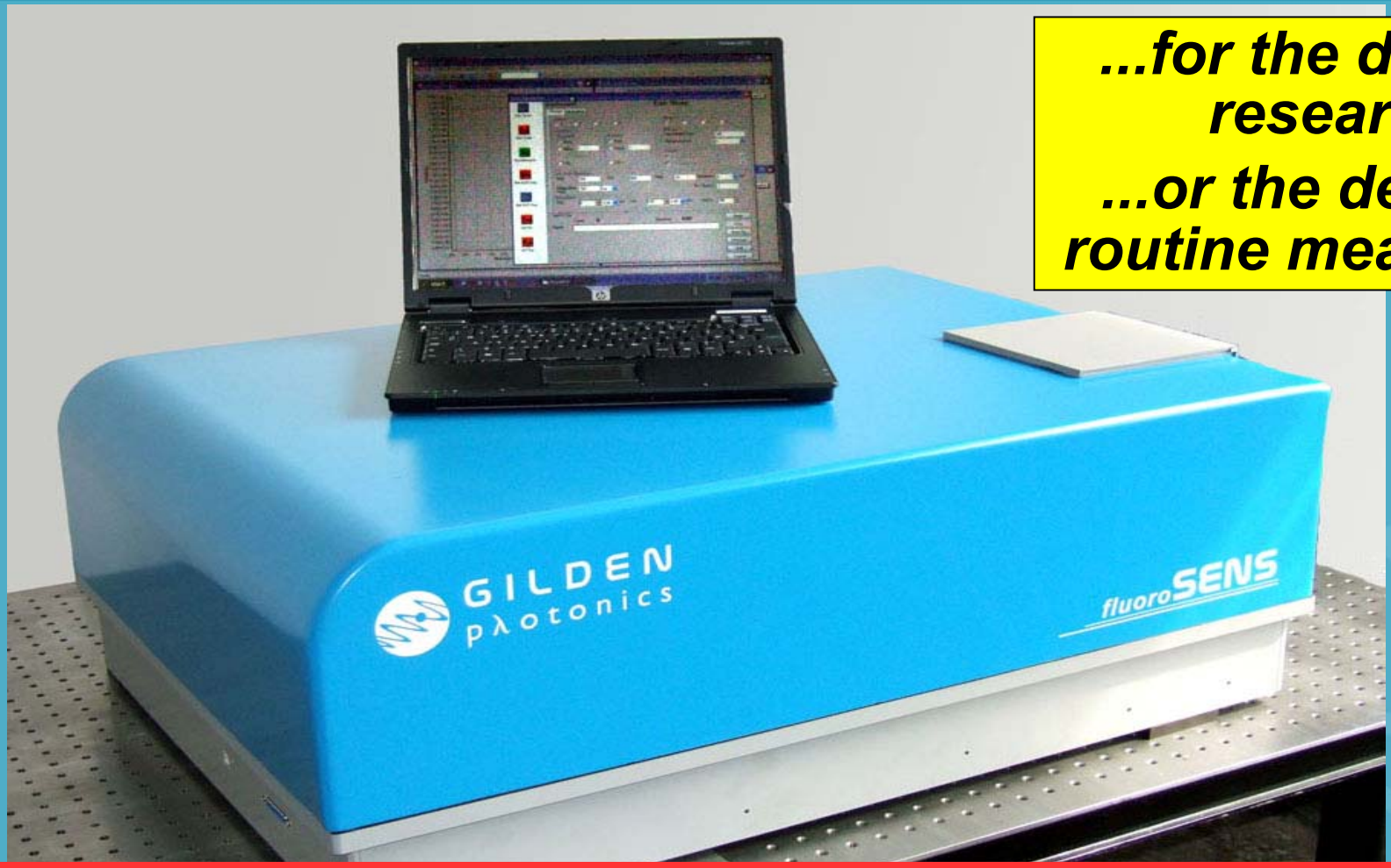
Pharmacology
- Monitoring of drug interaction with biological systems
- Anaesthesiology research
- Quality control
- High throughput screening

Biochemistry and Medicine Photochemistry
- Drug monitoring in photodynamic therapy
- Investigation of protein structure and folding
- Investigation of protein-antibody interactions
- Determination of donor-accepter distances
- Investigation of permeability and structure of membranes
- Enzyme research in proteins and membranes
- Investigation of dynamics and structure of nucleic acids
- DNA-sequencing and sizing

Photophysics and
- Characterisation of excited states dynamics of molecules
- Electron and proton transfer
- Intra-molecular relaxations
- Michelle structure and reaction in molecules
- Excimer and exciplex formation
- Polymer structure and dynamics
- Solvent-solute interactions
- Study of monolayers and surfaces

*fluoro*SENS

a new star is born...



...for the demanding researcher...

...or the demands of routine measurements

Choose the fluoroSENS and impact your research without devastating your budget !

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application and technical support.

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