HORIBA

Explore the future

Fluorescence in Biomolecular Research

HORIBA Jobin Yvon IBH Ltd

A division of HORIBA Scientific

Kulwinder Sagoo

Product Specialist

February 27, 2013

Outline

- History of IBH
- HORIBA Group
- What is Fluorescence?
- Applications relevant to Biomolecular research
- Equipment

Company Timeline

- Founders – David Birch, Bob Imhof and Tony Hallam
- Spin off company from Strathclyde University
- Role of IBH - to design fluorescence lifetime spectrometers

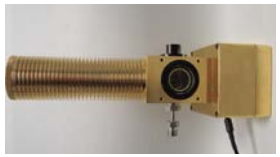
1977- IBH Consultants Ltd. formed and designs licensed

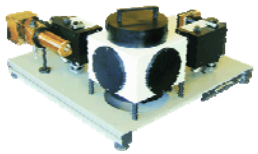
1989- IBH independent marketing & manufacturing begins

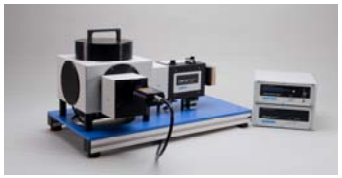
2003- IBH joins Jobin Yvon Inc.

2004- Name changed to HORIBA Jobin Yvon IBH Ltd.

2008 - HORIBA Scientific Group formed







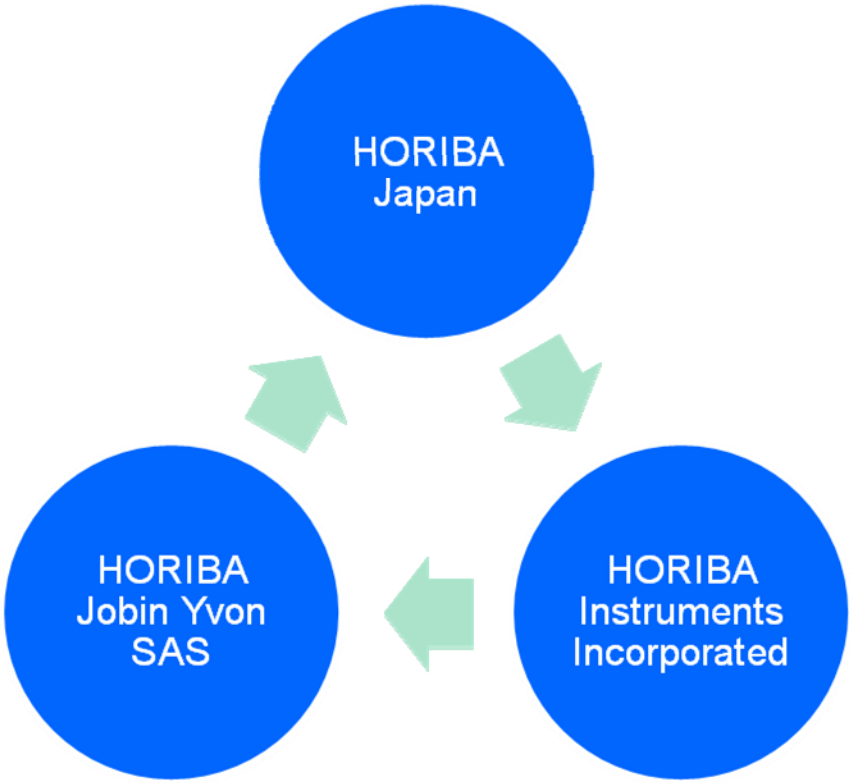
HORIBA

- $1.5 Billion sales
- Over 5000 employees
- Key business segments:
  - Automotive
  - Semiconductor systems
  - Scientific instruments
  - Environmental analyzers
  - Medical

Head Office – Kyoto, Japan

HORIBA Scientific

3 Centers of Excellence:



HOR (Kyoto, head office)

X-ray products, elemental analyzers (C,N,H,O, S), particle analyzers

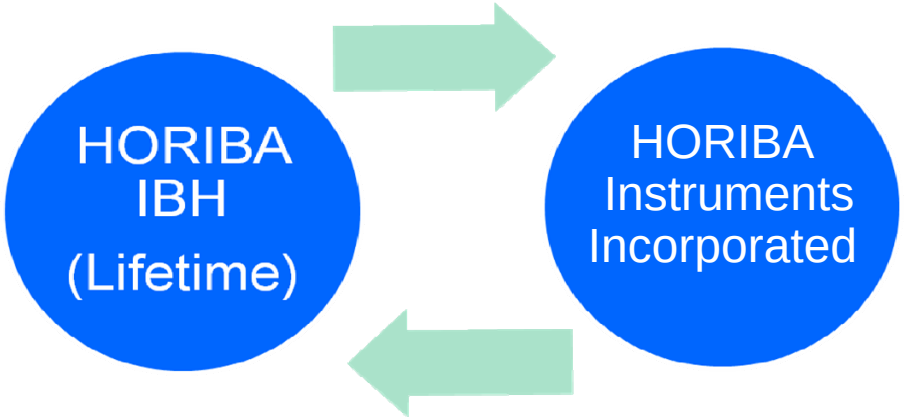
HIC (JYUS):

Fluorescence (includes IBH in Glasgow), spectroscopic components, forensics, OEM

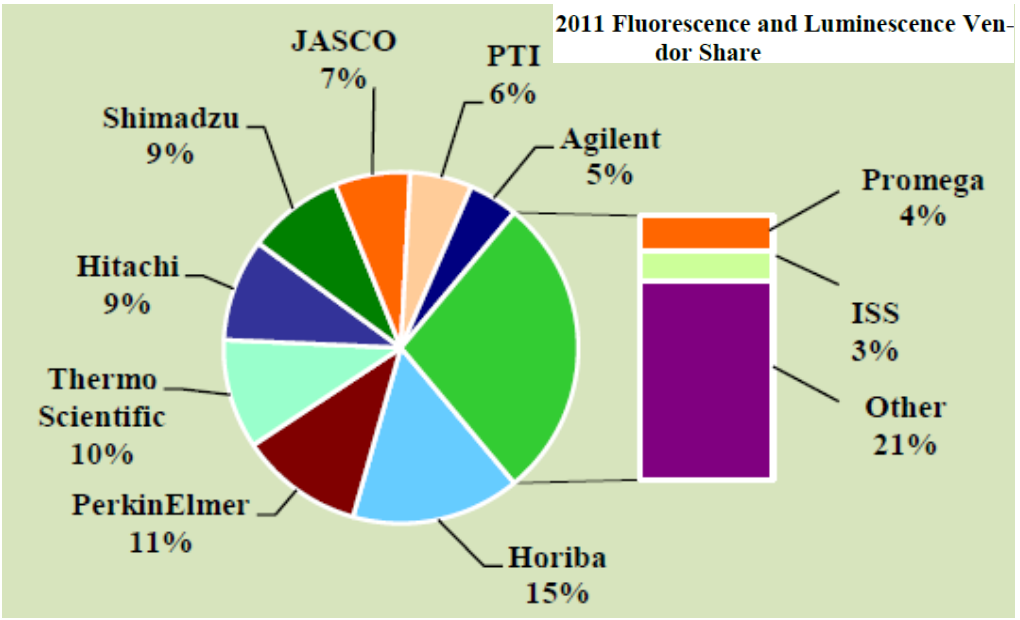
JY SAS:

Raman, elemental analyzers (ICP, GD), ellipsometry, OEM, SPRi

Fluorescence Division



2 Centers of Excellence



What is Fluorescence?

Fluorescence is a multiparameter signal

$$FL = f(I, \lambda_{exc}, \lambda_{em}, p, x, t)$$

I= intensity - measurement is quantum yield (ϕ),

λ_{exc}= excitation wavelength

–measurement of absorption spectrum,

λ_{em}= emission wavelength

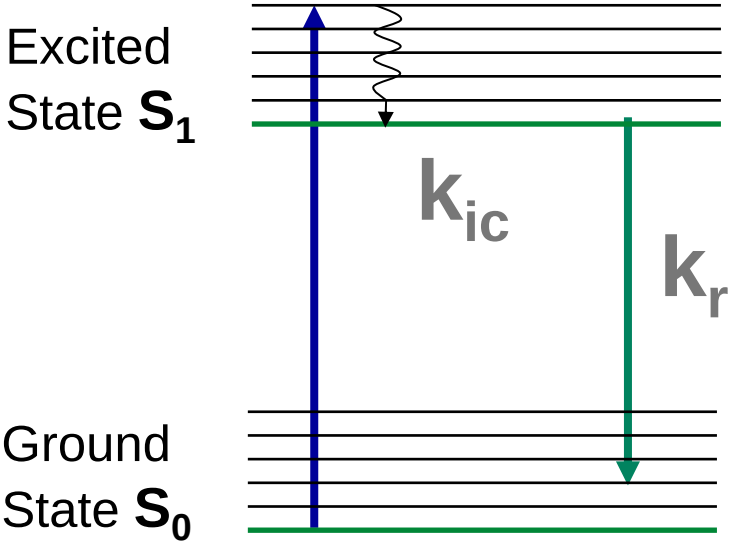
–measurement of fluorescence spectrum,

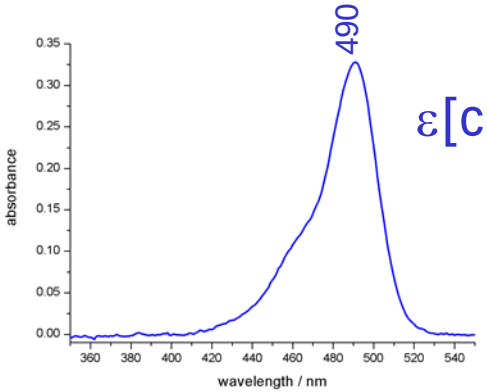
p = polarisation –measurement of anisotropy,

x= position –measurement by fluorescence microscopy,

t= time –measurement of fluorescence lifetime.

Fluorescence





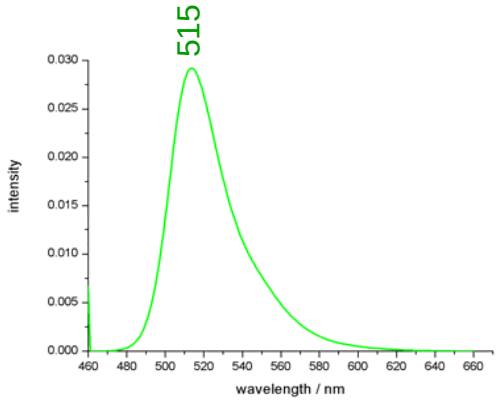
$I = I_0 10^{-\varepsilon[c]d}$

$\varepsilon[c]d$ = absorbance

ε extinction coefficient

[c] concentration

d pathlength



quantum yield ϕ

Stokes shift

Decay time τ

Radiative deactivation of the first excited singlet state

depends on molecule and environment

Fluorescence techniques

Fluorescence measurement aims to record one or more of these parameters

intensity, wavelength, time, polarisation, position (x,y,z)

Fluorescence

steady state
(average)

$$\phi = \frac{\text{emitted photons}}{\text{absorbed photons}} = k_r / (k_r + k_{nr})$$

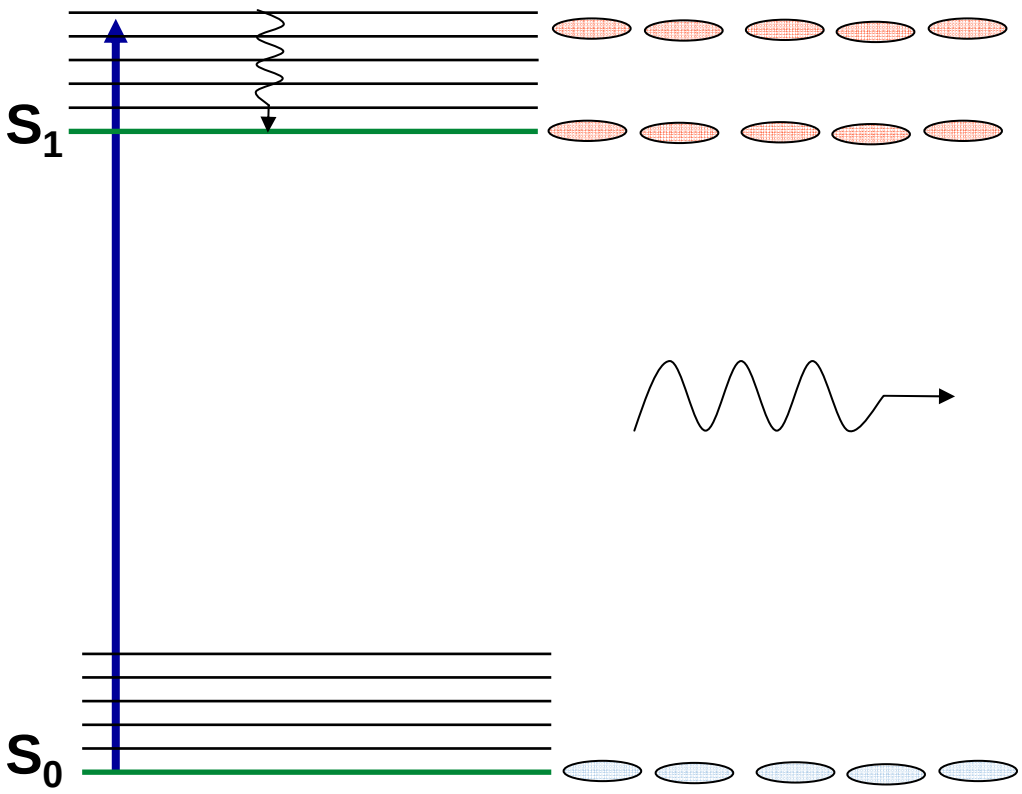
time-resolved

τ- fluorescence lifetime

$= 1 / (k_r + k_{nr})$

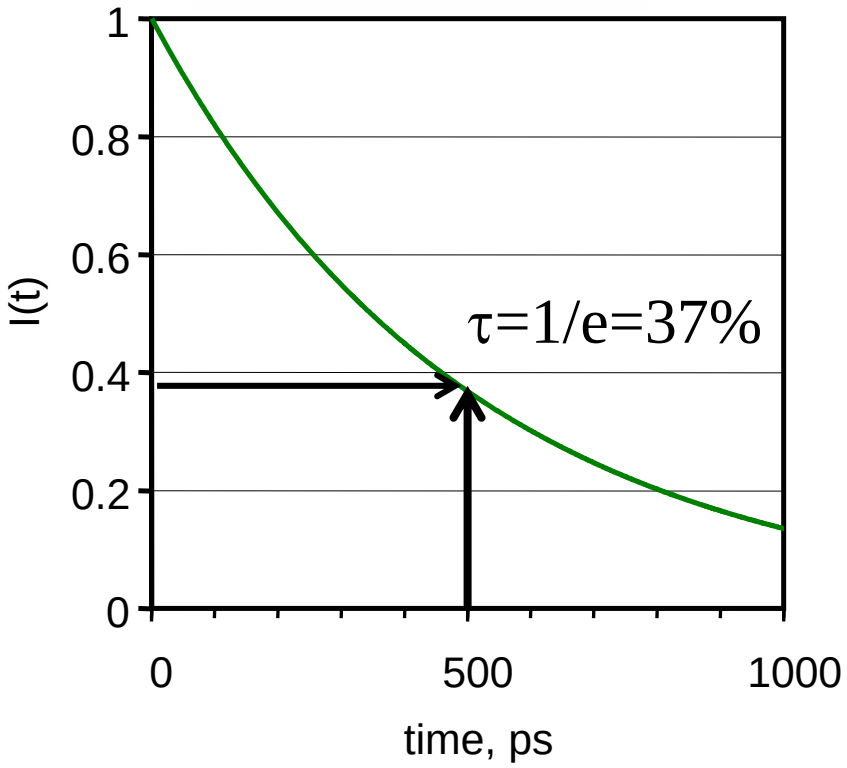
Absolute measurement

The fluorescence lifetime



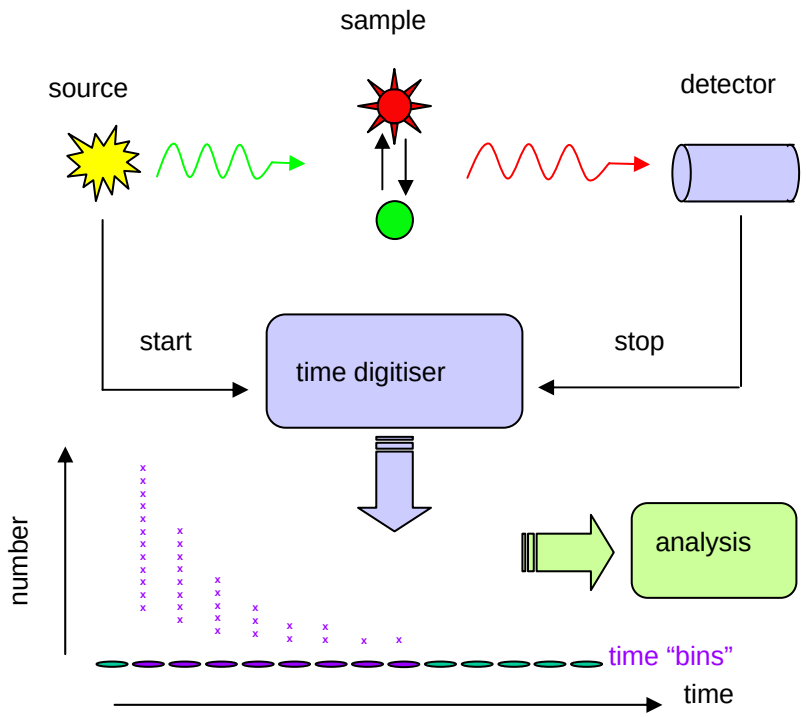
molecular ensemble

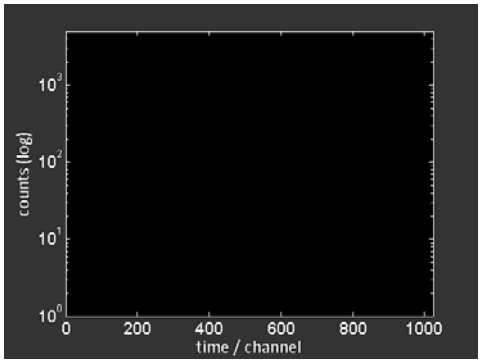
$$I(t) = I_0 \exp(-t/\tau)$$



$$\tau = \frac{1}{k_r + k_{nr}}$$

Practical set up





www.picocomponents.com

Applications

- Examples
  - Fast acquisition of short lifetimes
  - FRET (Foerster Resonance Energy Transfer)
  - Stern-Volmer quenching
  - Lanthanide luminescence
  - Time-resolved anisotropy
  - Protein Fluorescence
  - Solar cell analysis
  - Singlet oxygen measurements
  - Materials research
  - Photophysical research

Why measure lifetimes?

- Reveal information about kinetic rates, dynamics and structure of fluorophore
- Single molecule sensitivity
- Independent of sample intensity
  - Absolute measurement
  - Concentration independent (within limits)
- Sensitive to local environment
  - pH, local charged group, quenchers
  - Temperature, polarity, viscosity
- Extra Specificity
  - Discriminate against unwanted fluorescence and excitation
- Other information
  - Additional dimension to fluorescence data map
  - Increases specificity of the measurement

Why measure lifetime in BioScience?

- Lifetime time scale of <1ns to 10ns is comparable with many types of molecular motion (physiological and chemical processes)
  - allows probing of the environment surrounding the fluorophore

    nm distances (10^{-9}m)

    ns timescale (10^{-9}s)
- Also, very sensitive, works in-situ, & non-destructive
- Fluorophores can be intrinsic or extrinsic

Examples of Fluorescence in Biochemistry & Medicine

- Fluorescence Microscopy
  - Tissues & cells - label anitibodies with fluorophore and monitor interaction with antigen
  - FLIM (Fluorescence lifetime imaging microscopy) – bio-molecular interactions resulted in changes in fluorescence lifetime
- FRET – to monitor ie. protein interactions and for biosensors
- Biotechnology – develop biosensors for detection of glucose monitoring
- DNA – sequence sorting by labelling specific terminal bases with a specific fluorophore (chain termination method)

Questions related to Biomolecular research

How large is the biomolecule of interest?

Has my protein denatured?

Are you exploring new sensitisers for photodynamic therapy?

What proportion of my sample is bound?

Is the biomolecule thermally stable?

Has a protein-protein interaction occurred?

Has the drug bound to the active region of the biomolecule?

Has the molecular microenvironment changed?

How mobile is my biomolecule after a binding interaction has occurred?

Has immobilising my biomolecule affected conformation and function??

Is the active site of the biomolecule accessible for binding ?

Has there been a conformational change?

How can fluorescence lifetimes help?

STRUCTURE/ CONFORMATION

Monitor:
- Changes in conformation because of unfolding, denaturing or binding
- Inter vs Intra molecular distances
- Photosensitisation

Using techniques such as:
- Fluorescence lifetime
- TRES
- FRET
- TR anisotropy
- Phosphorescence lifetime

SIZE / MOBILITY

Monitor:
- Viscosity
- Rotational diffusion
- Binding
- Restricted mobility

Using techniques such as:
- Fluorescence lifetime
- FRET
- TR Anisotropy

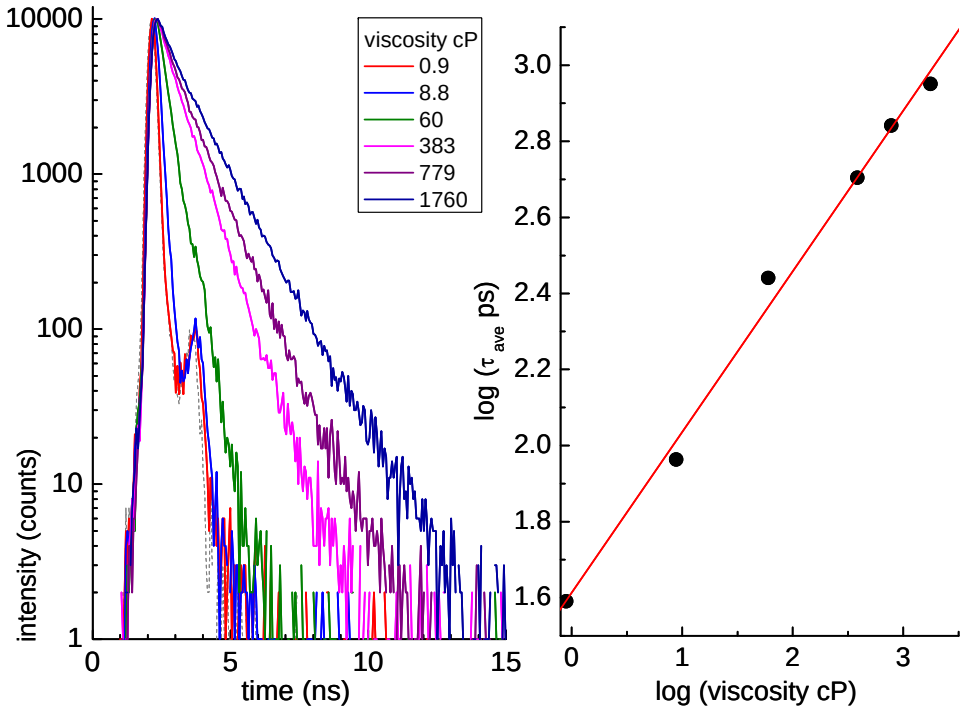
FUNCTION

Monitor:
- Binding of proteins, ligands, drugs, etc.
- Bound vs unbound
- Change in rate of rotation of species upon binding

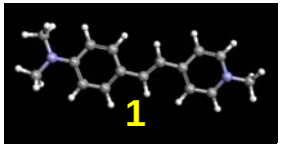
Using techniques such as:
- Fluorescence lifetime
- TR anisotropy
- Fluorescence quenching

Measurement Example

Monitoring viscosity changes



Mitochrondrial probe



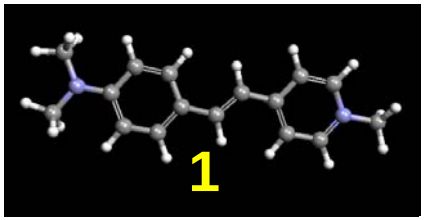
Stillbenoid molecule (DASPMI)– molecular rotor sensitive to changes in local nanoscale viscosity

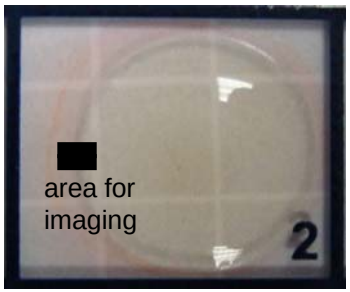
Viscosity dependent intermolecular re-arrangement that affects lifetime

G. Hungerford et al. **2009**. *Monitoring sol to gel transitions via fluorescence lifetime determination using viscosity sensitive fluorescent probes.* J. Phys. Chem. B. 113, 12067-12074.

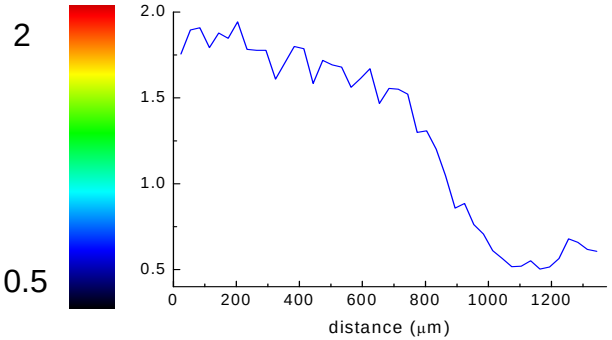
Viscosity Study- Cont'd

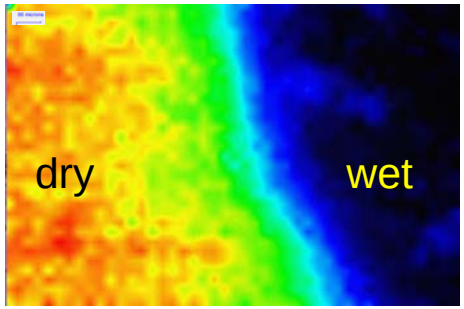
- Polysaccharides
  - Repeating units of monomers linked by glycosidic bonds
  - Important role in animal and plant nutrition and structure
  - Gellan gum is used in food industry, drug delivery and tissue engineering





DASPMI entrapped in Gellan Gum analysed using FLIM

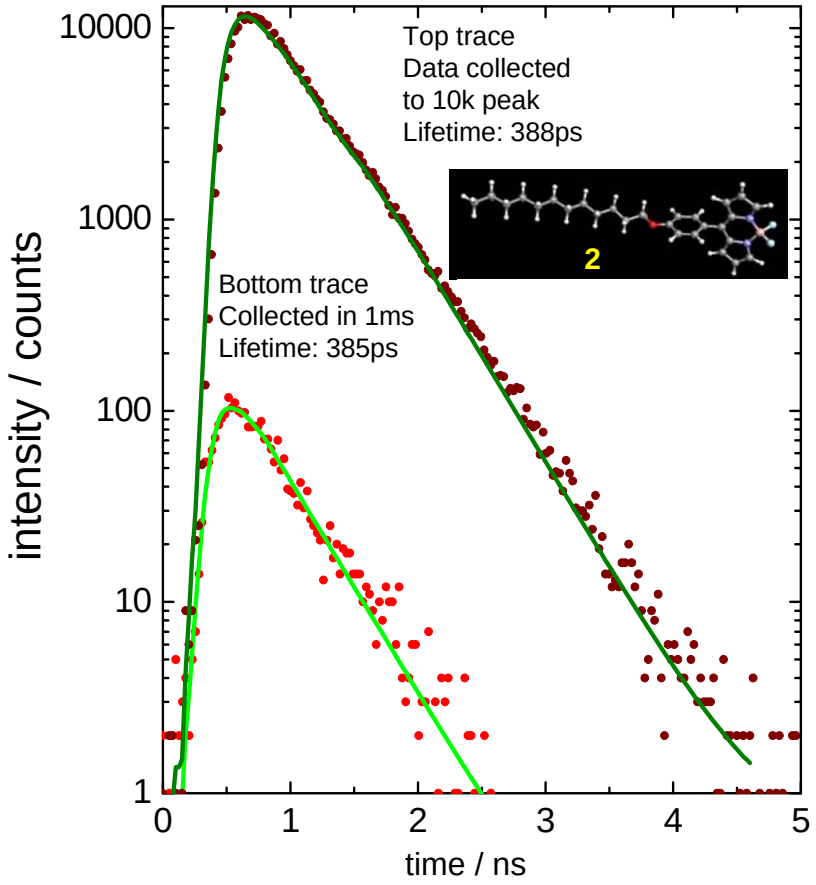




Viscosity changed ~600x moving from dry to wet front

G. Hungerford et al. **2012**. *In-situ formation of silver nanostructures within a polysaccharide film and its application as a potential biocompatible fluorescence sensing medium*. Soft Matter. 8, 653-659.

Measurement Example



Picosecond lifetimes

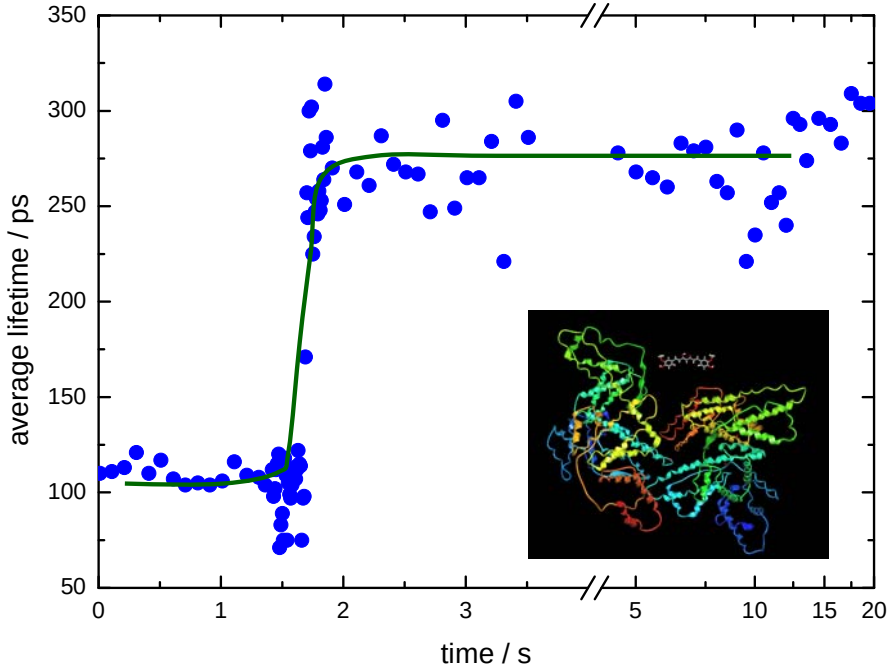
Fast Acquisition

Example of fast data acquisition with DeltaDiode excitation with 100MHz repetition rate and low deadtime (<10ns) timing electronics (Bodipy Derivative)

D. McLoskey et al. **2011**. *Fast time-correlated single-photon counting fluorescence lifetime acquisition using a 100 MHz semiconductor excitation source*. Meas. Sci. Technol. 22, 067001.

Measurement Example- Binding Study

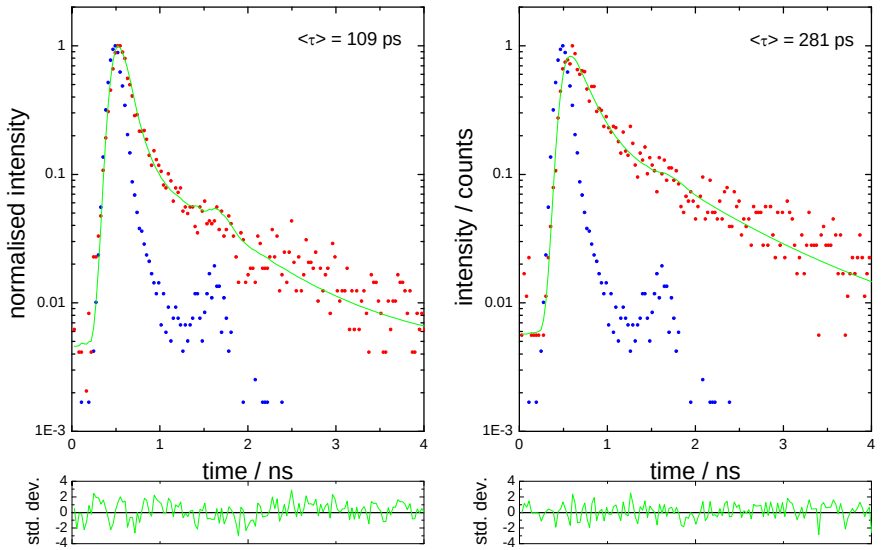
HSA binding to curcuminoid



- Protein binding important to understand interactions with variety of target molecules
- Phenolic compounds provide anti-oxidant activity with potential health benefits

Kinetic TCSPC

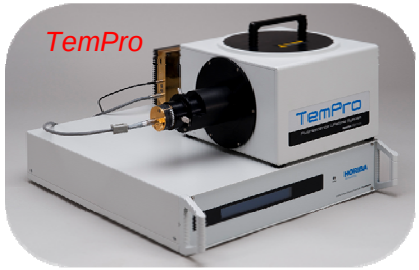
- 10 000 decays, 10ms/decay
- <τ> vs macrotime
- <τ> increases upon binding
- total volume - 5µl



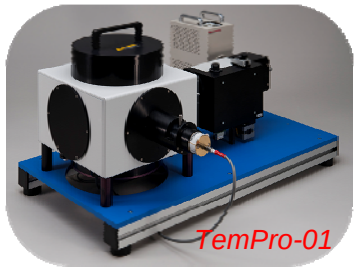
Fluorescence Lifetime systems

dedicated systems

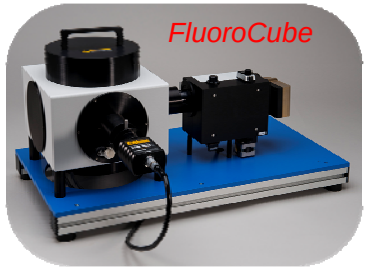
TemPro



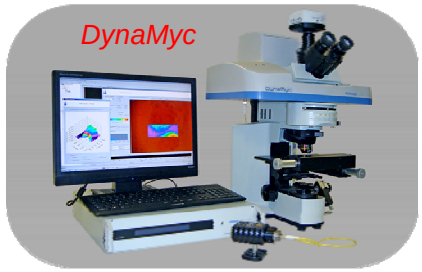
TemPro-01



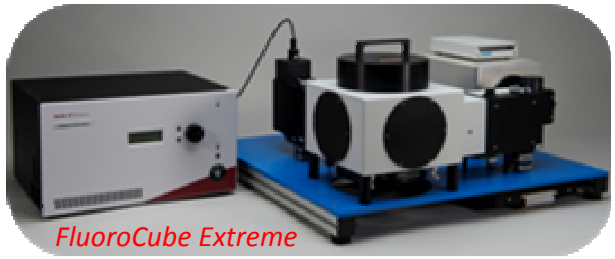
FluoroCube



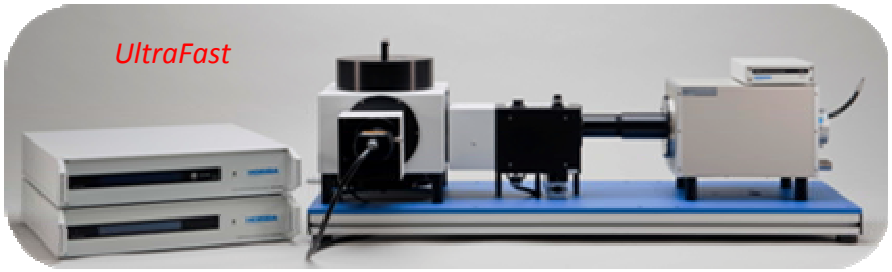
DynaMyc



FluoroCube Extreme



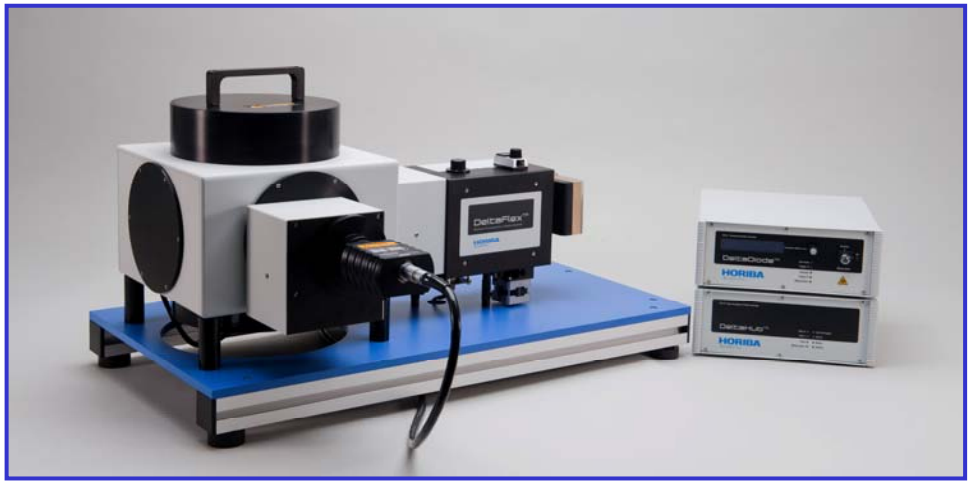
UltraFast



Our New Delta Series

- DeltaPro

- DeltaFlex



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FluoroFest

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Thank you