Queen's University Belfast

Laser spectroscopy and Raman scattering: from excited states to biomedicine

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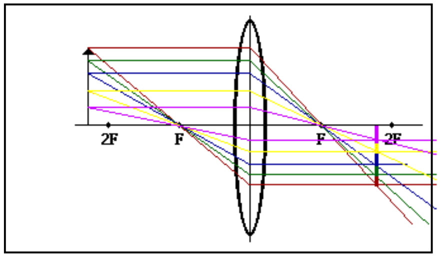
April 2005

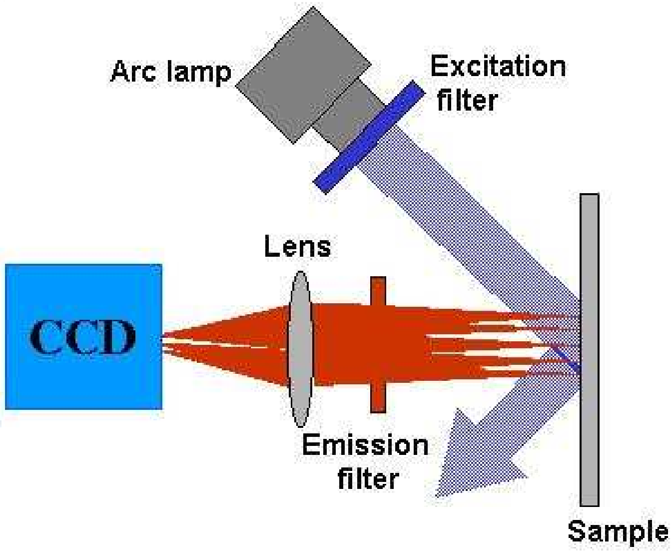
Summary

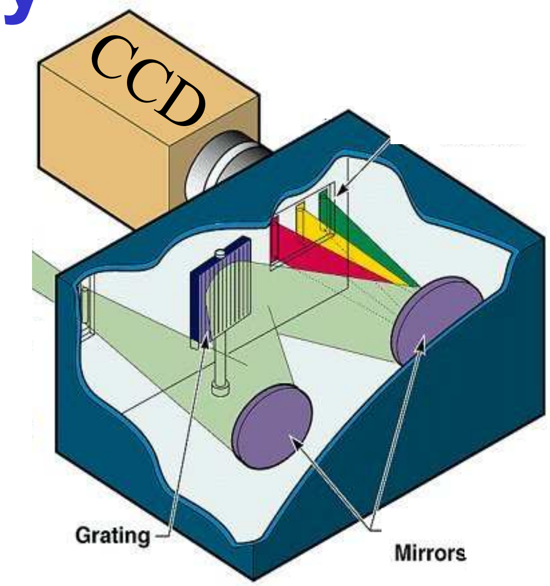
- Laser spectroscopy at QUB and Raman application overview
- Raman - strengths and challenges
- Preliminary analysis of EMCCD
  - vs conventional CCD
  - vs ICCD

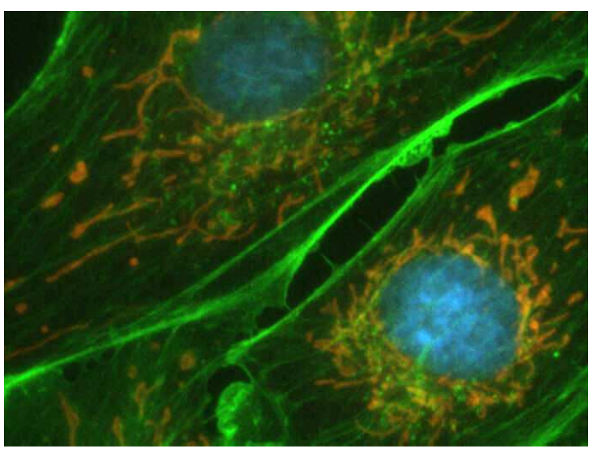
Imaging vs. Spectroscopy

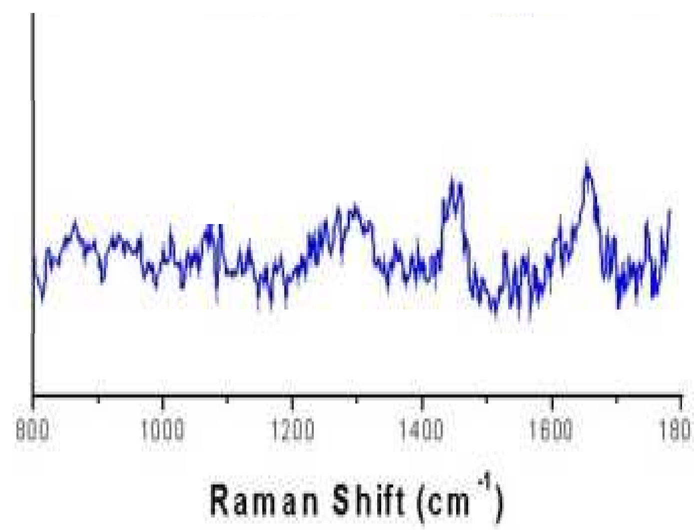


Imaging









Laser spectroscopy at QUB

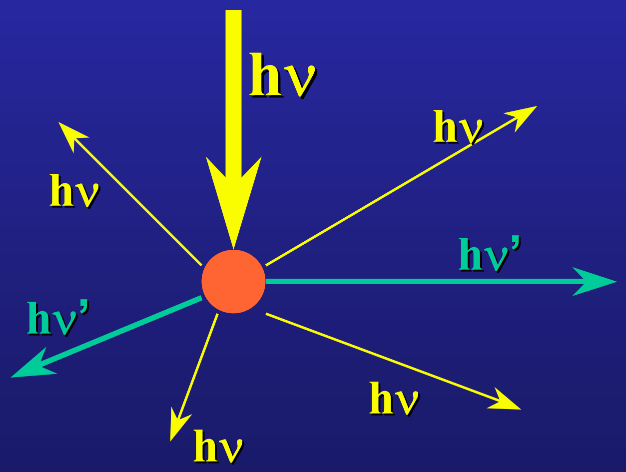
Electronic (UV-Vis and fluorescence) and Raman Spectroscopy used to probe :

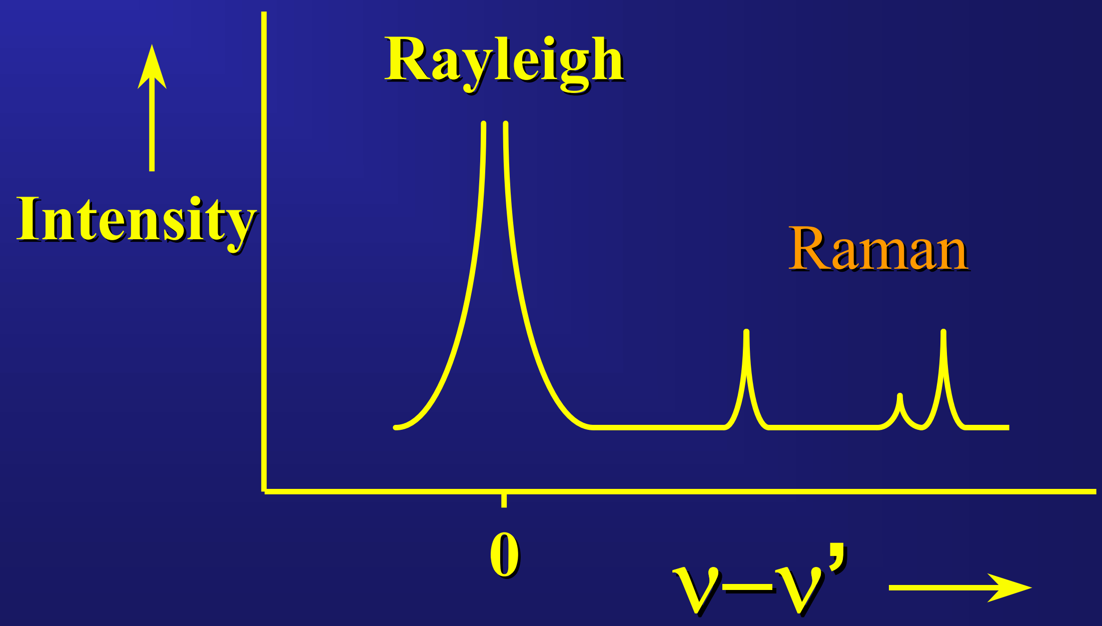
- Excited states of metal complexes – structural studies and applications

- systems of biomedical interest

The essence of the Raman experiment

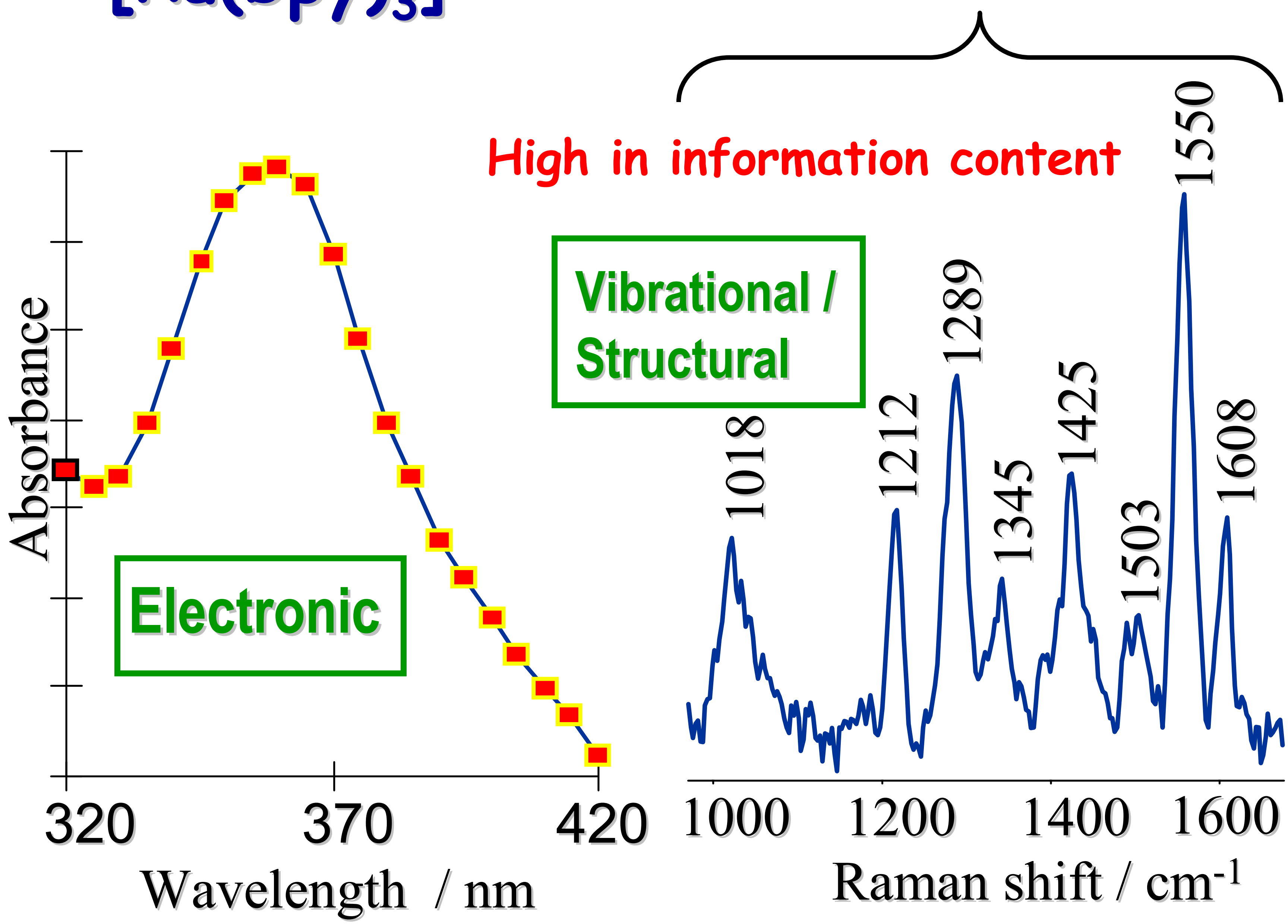
- Irradiate sample with monochromatic radiation
- Collect inelastically scattered light
- Frequency difference gives vibrational spectrum



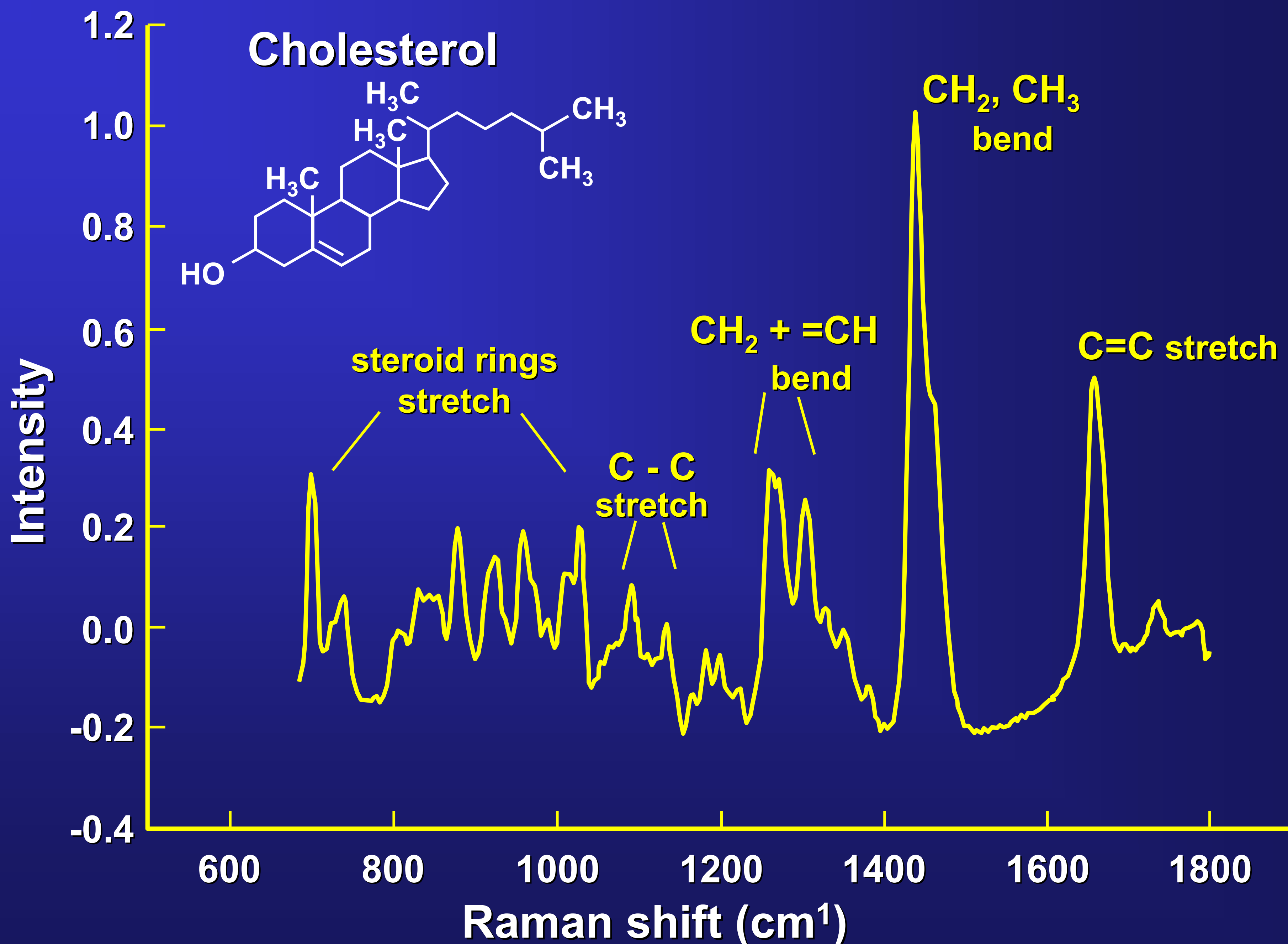


- Majority of scattered radiation will have same frequency as that of the incident radiation (***Rayleigh radiation)***.
- However, a small fraction can set molecules of sample into vibration.
- Clearly, the energy for this must come from the incident radiation
- Frequency change ≡ *the frequency of vibration of the scattering molecules*.

$[Ru(bpy)_3]^{2+}$ *



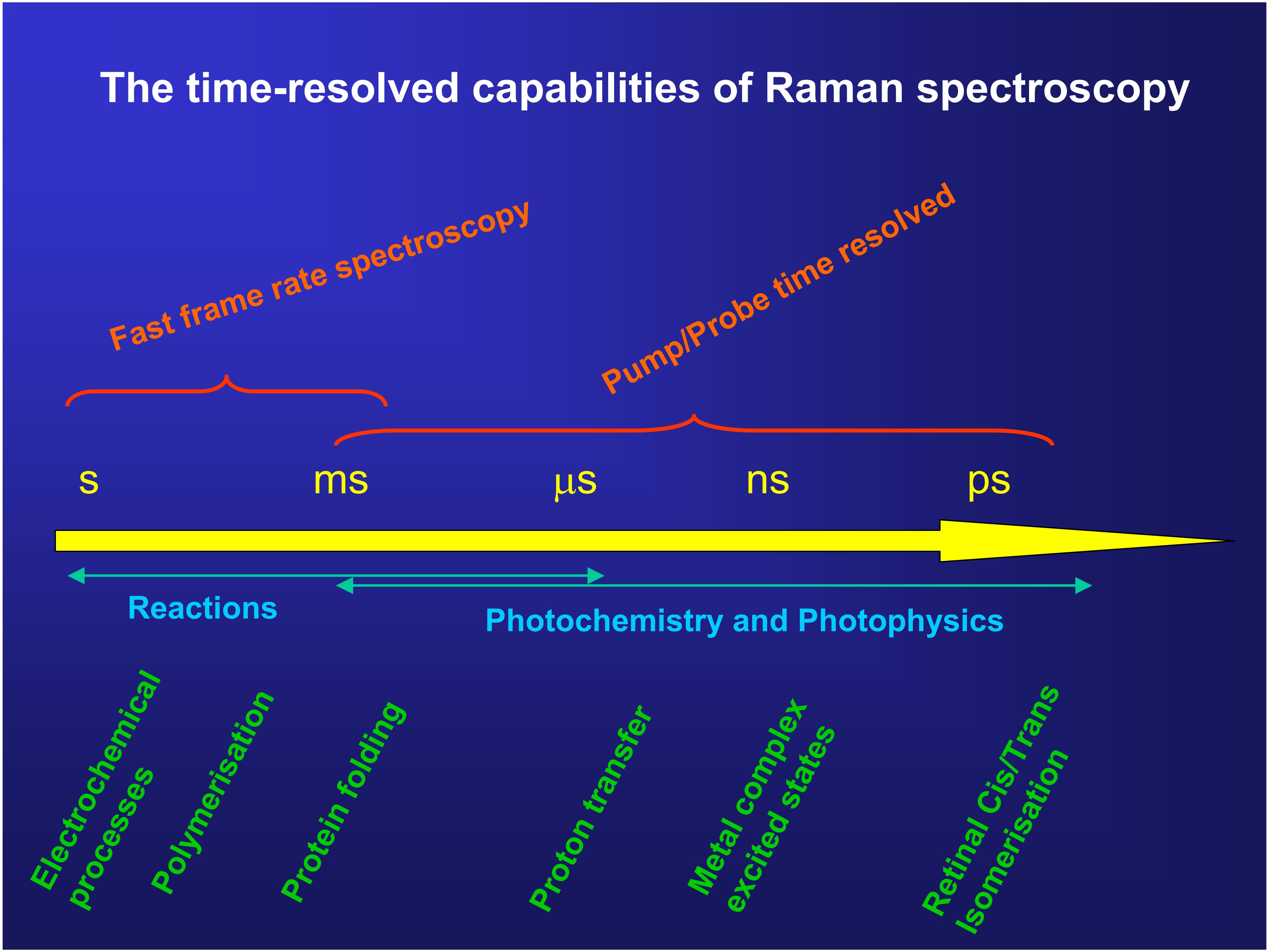
~ 7.5 nm
High in information content
Vibrational / Structural
Electronic
Absorbance
320
370
420
Wavelength / nm
1018
1212
1289
1345
1425
1503
1550
1608
1000
1200
1400
1600
Raman shift / cm-1



E.B. Hanlon *et al*, Phys. Med. Biol. 45, R1-R59, **2000**

Modern Raman spectroscopy is actually a family of techniques

- Raman scattering (non-resonant)
- Surface-enhanced scattering
- Resonance Raman scattering
- Time-resolved methods (TR^3)


The time-resolved capabilities of Raman spectroscopy
Fast frame rate spectroscopy
Pump/Probe time resolved
s
ms
μs
ns
ps
Reactions
Photochemistry and Photophysics
Electrochemical processes
Polymerisation
Protein folding
Proton transfer
Metal complex excited states
Retinal Cis/Trans Isomerisation

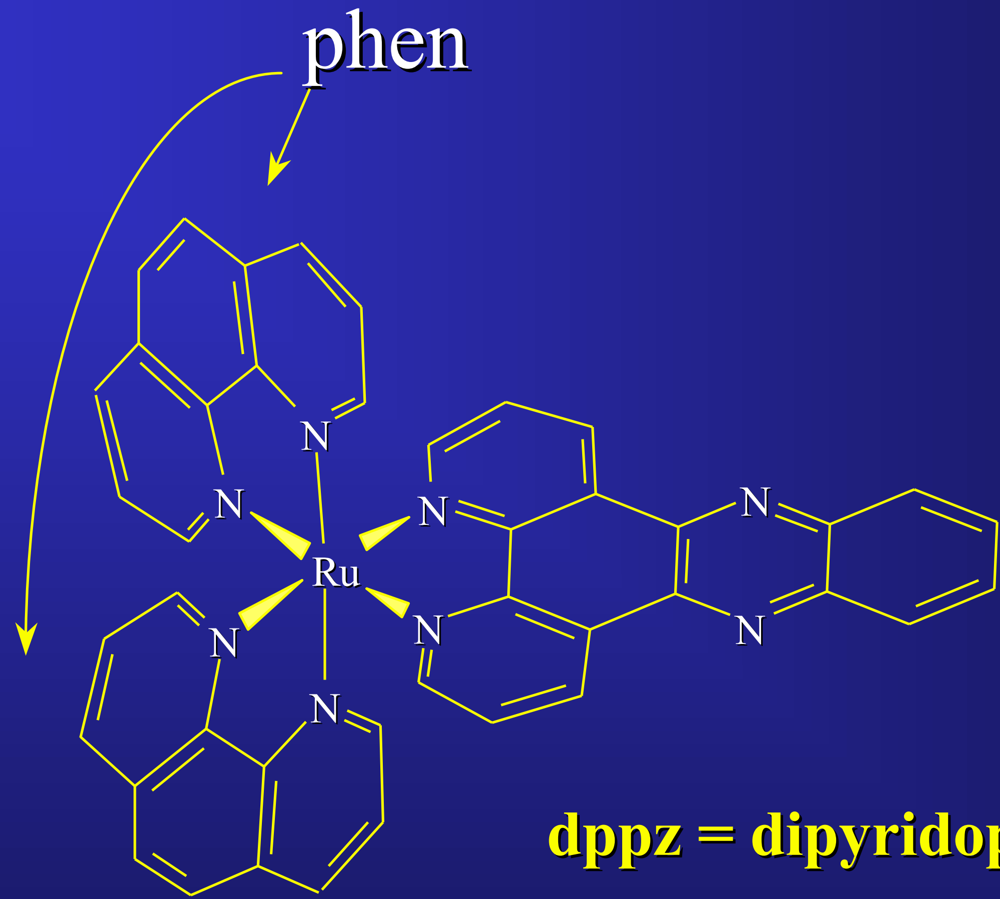
Why metal complex excited states?

Excited states underlie light-driven devices :

- light-energy conversion
- optical storage

Metal complexes offer a variety of excited state types, potentially providing the basis for a range of functions

$[Ru(phen)_2dppz]^{2+}$

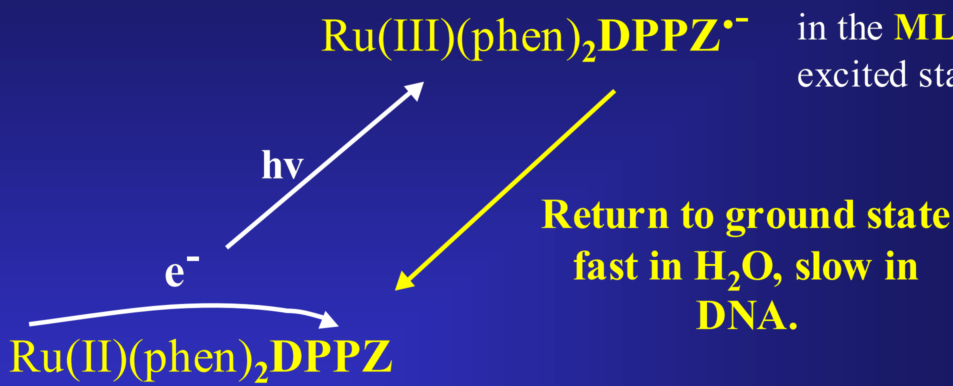


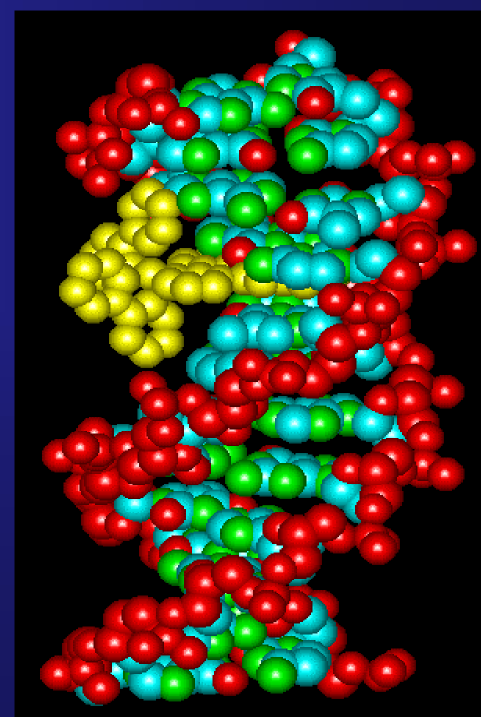
dppz = dipyridophenazine

- ns and ps TR^3 have been used to probe subtle electronic differences between states in the lowest excited state manifold.

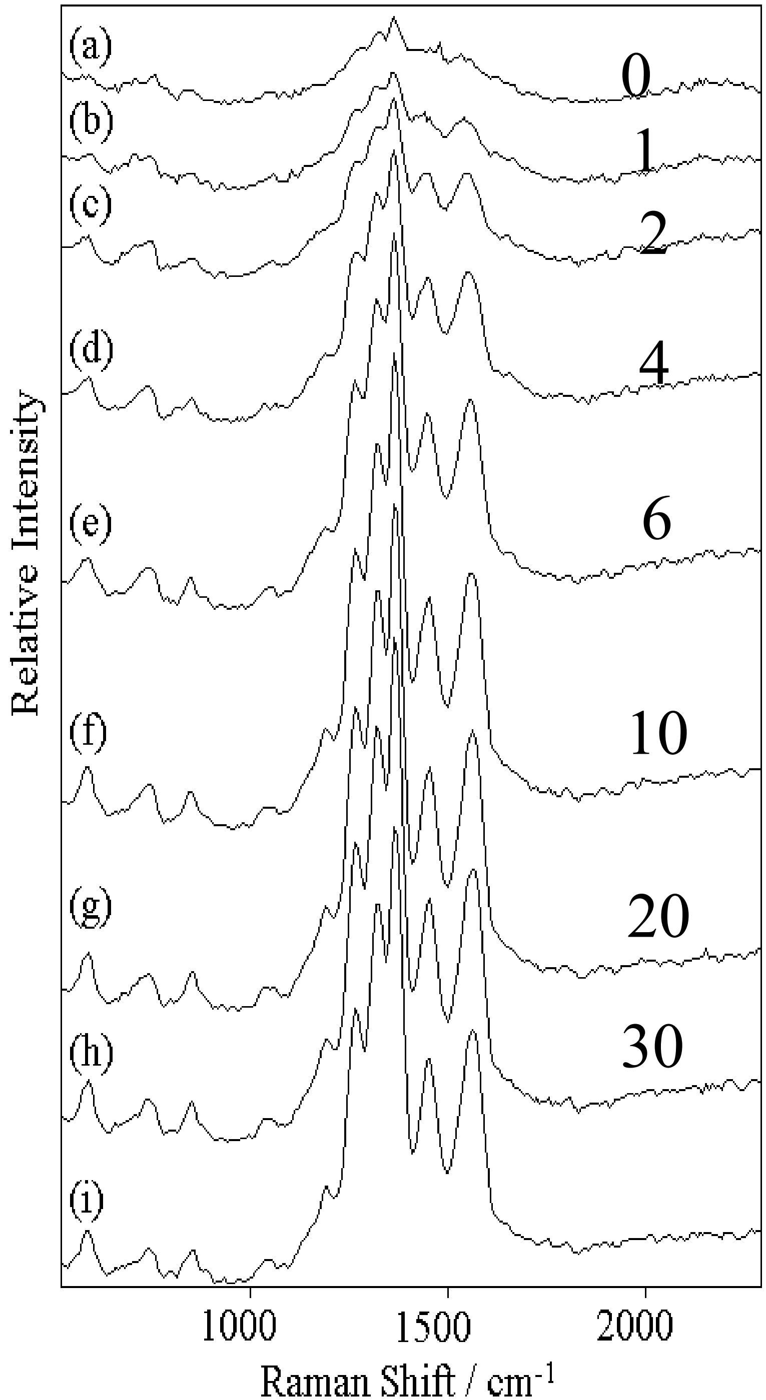
$[Ru(phen)_2dppz]^{2+}$ 'DNA light switch'

Electron localisation on the **DPPZ** ligand in the **MLCT** excited state.

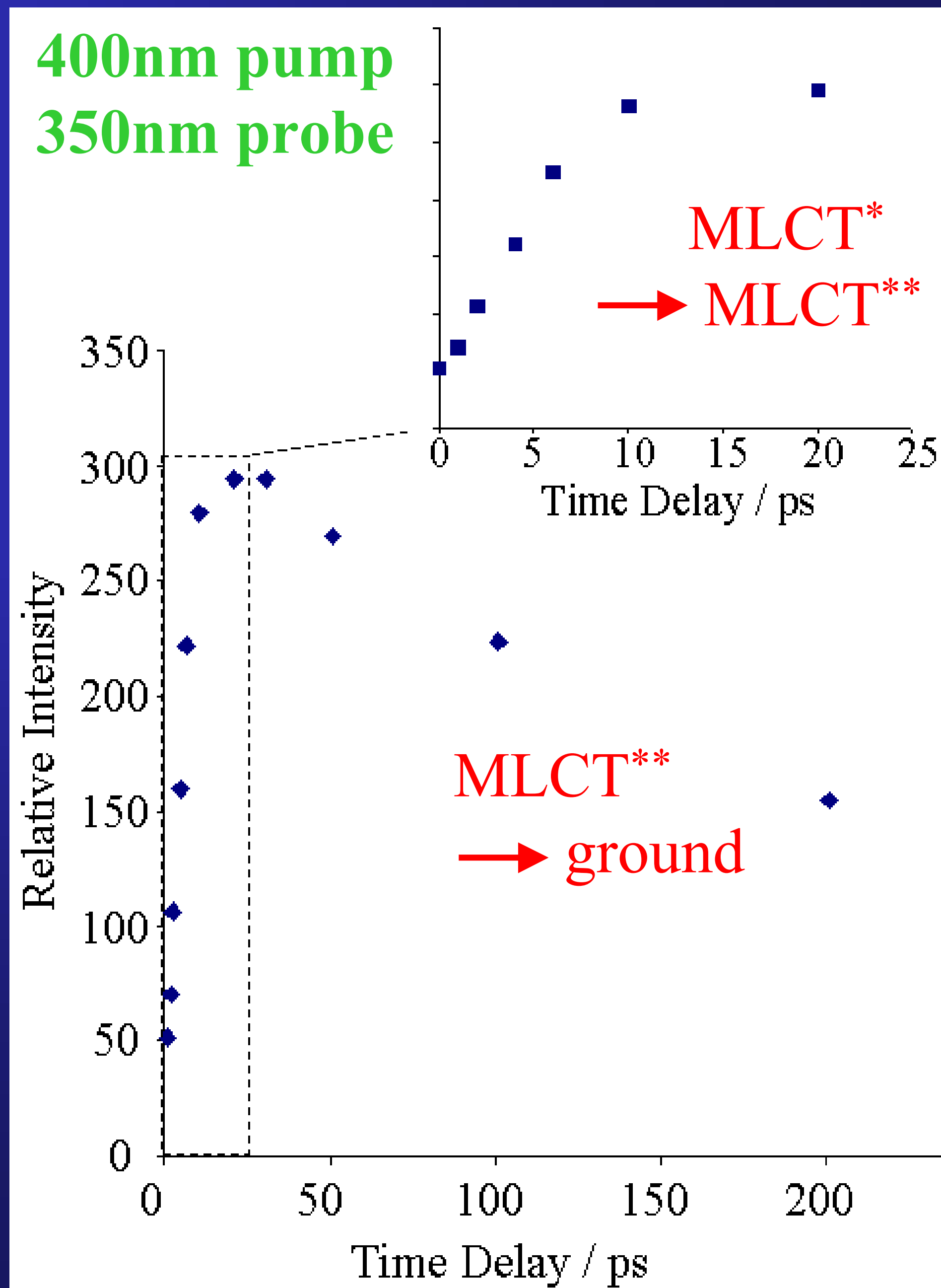




<u>**When intercalated with DNA, significant luminescent enhancement is observed.</u>



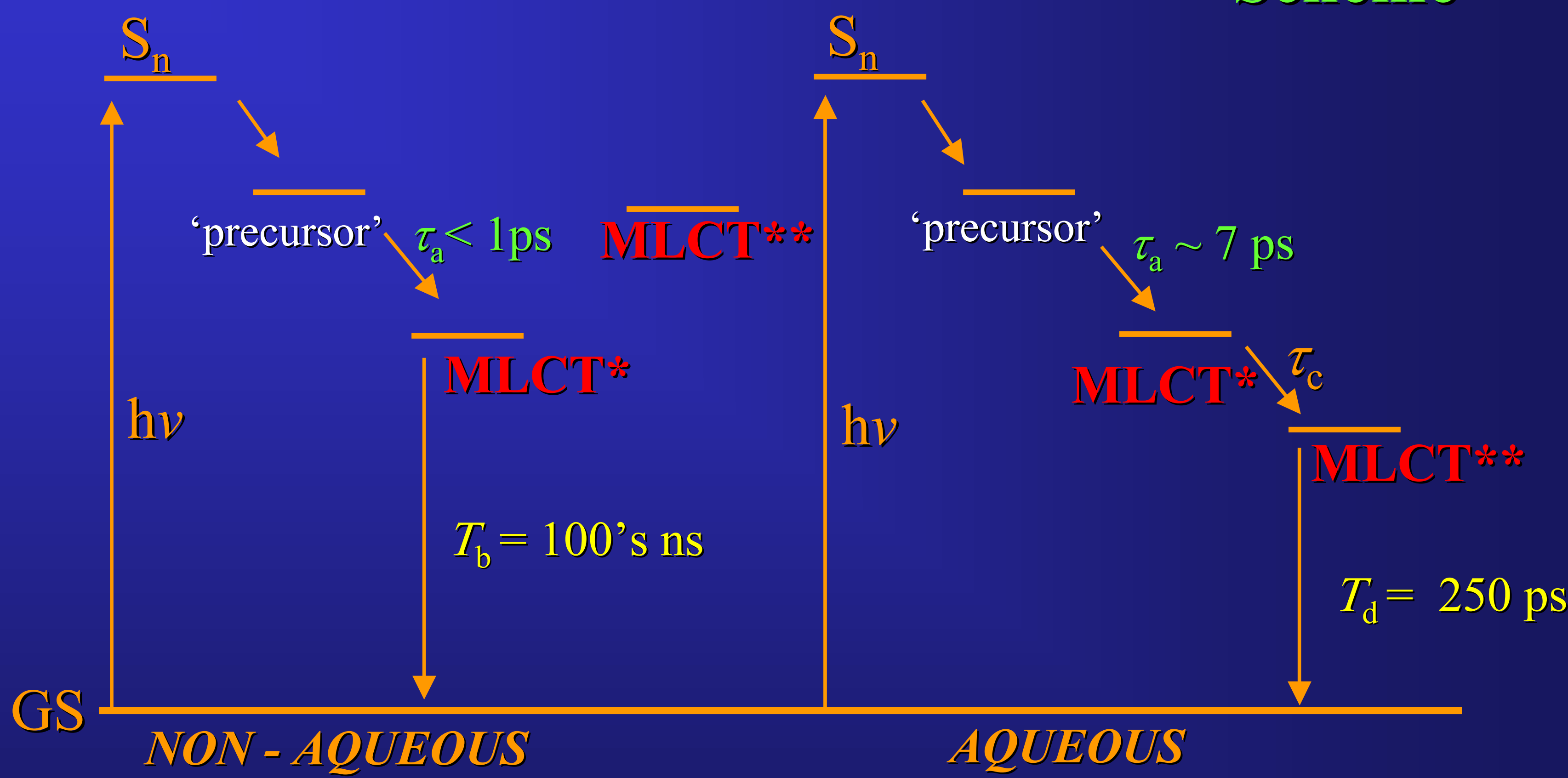
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
0
1
2
4
6
10
20
30
Relative Intensity
1000
1500
2000
Raman Shift / cm-1



400nm pump
350nm probe
MLCT*
→ MLCT**
MLCT**
→ ground
Relative Intensity
Time Delay / ps
0
50
100
150
200
250
300
350
0
5
10
15
20
25

$[Ru(phen)_2dppz]^{2+}$

Jablonski Scheme

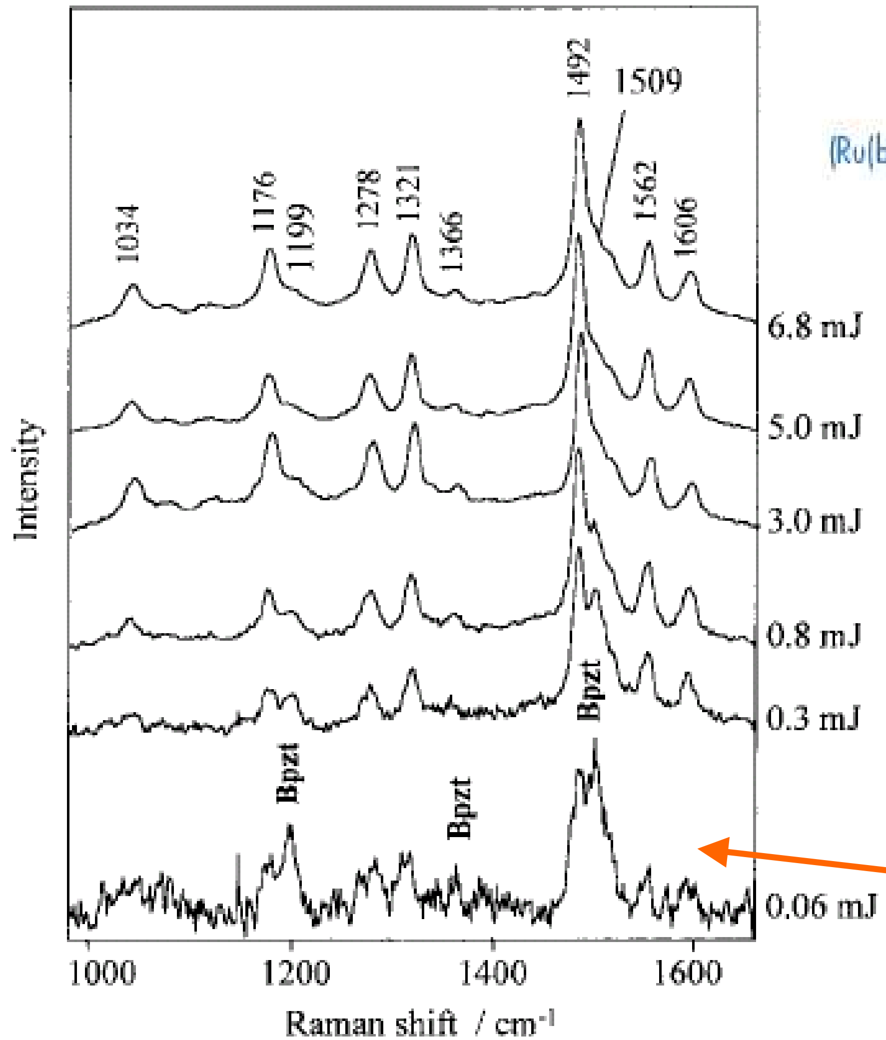


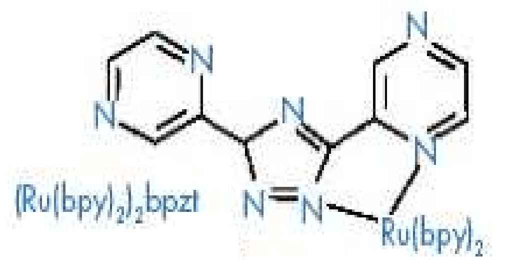
S_n - singlet state manifold accessed by initial light absorption.

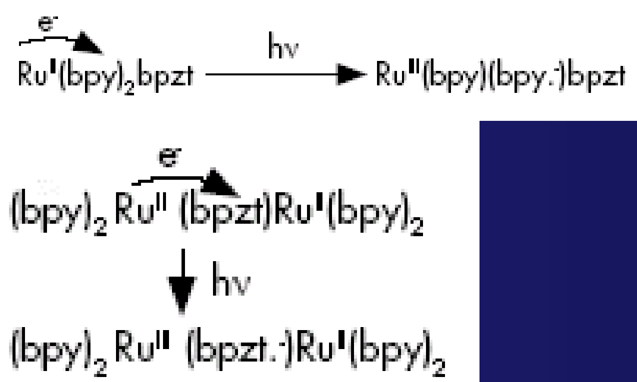
τ_a varies with solvent environment.

C.Coates *et al* J Phys Chem B, <u>105</u>, 12653, **2001**

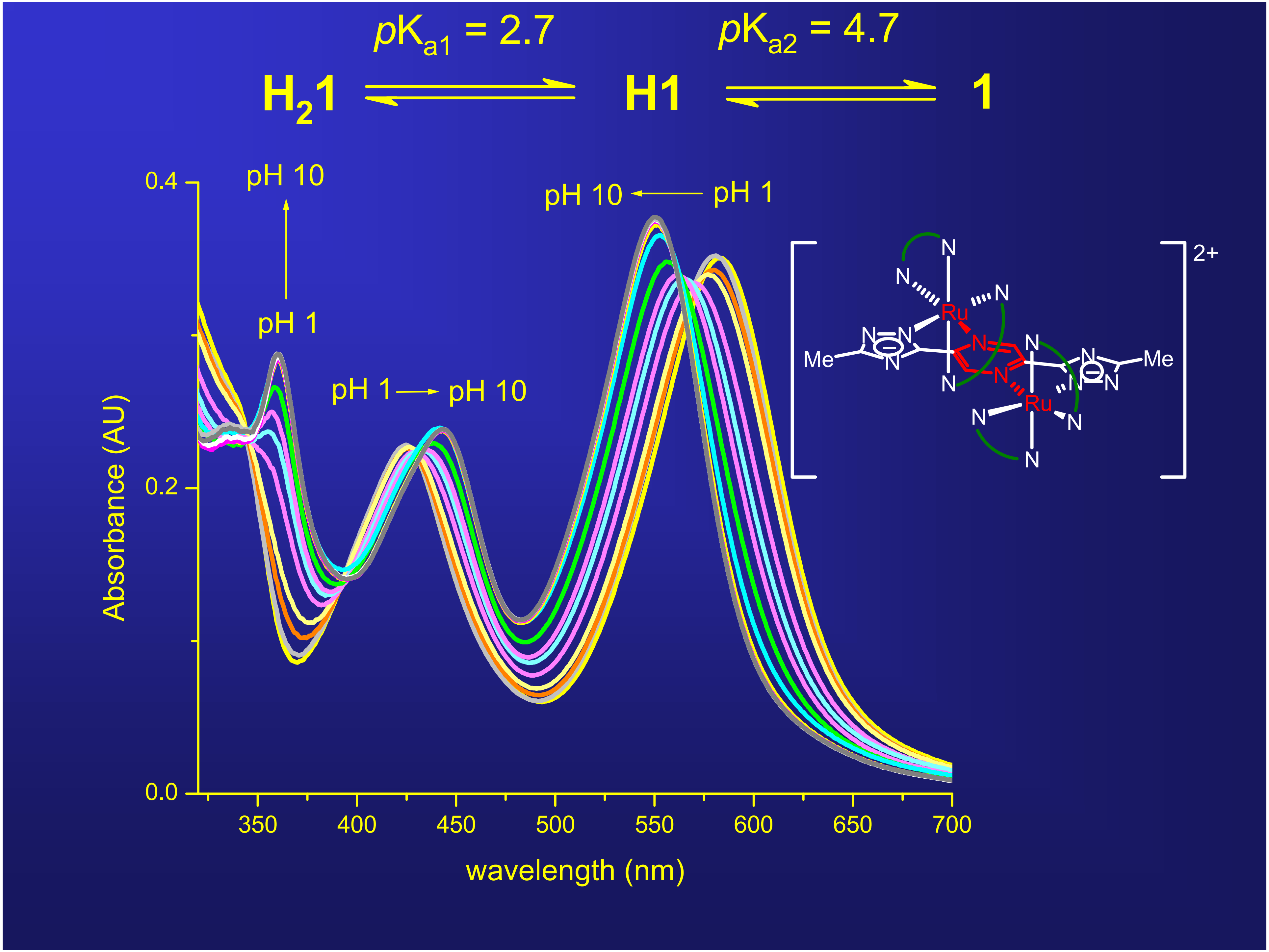
Power-Resolved Resonance Raman





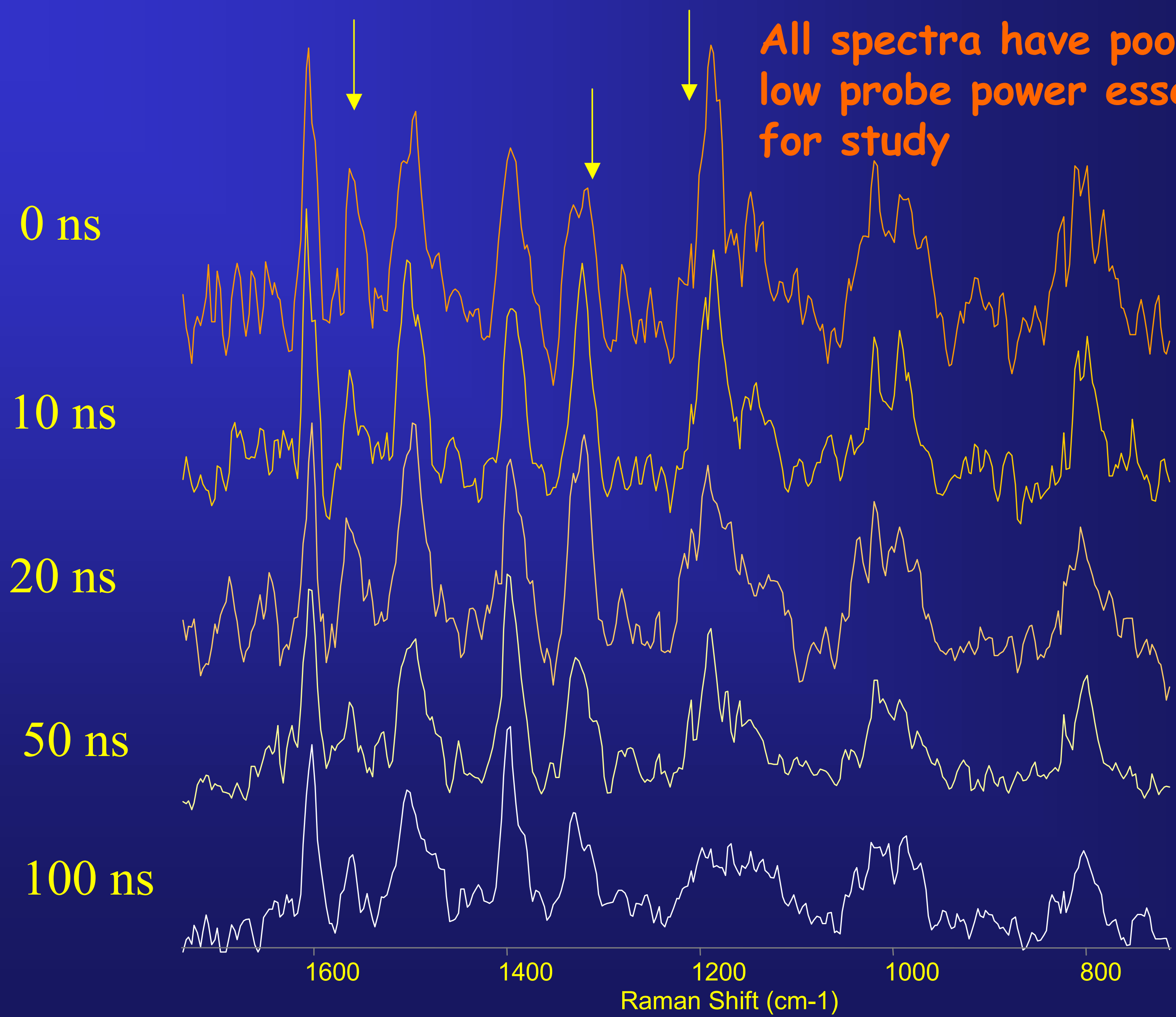


@ low power,
S/N is an issue



pKa1 = 2.7
pKa2 = 4.7
H21
H1
1
0.4
0.2
0.0
Absorbance (AU)
pH 10
pH 1
pH 1 → pH 10
pH 10 ← pH 1
350
400
450
500
550
600
650
700
wavelength (nm)
2+
Ru
N
Me

TR³ spectra of **1** in H_2O: 532 nm pump, 355 nm probe



All spectra have poor S/N – low probe power essential for study

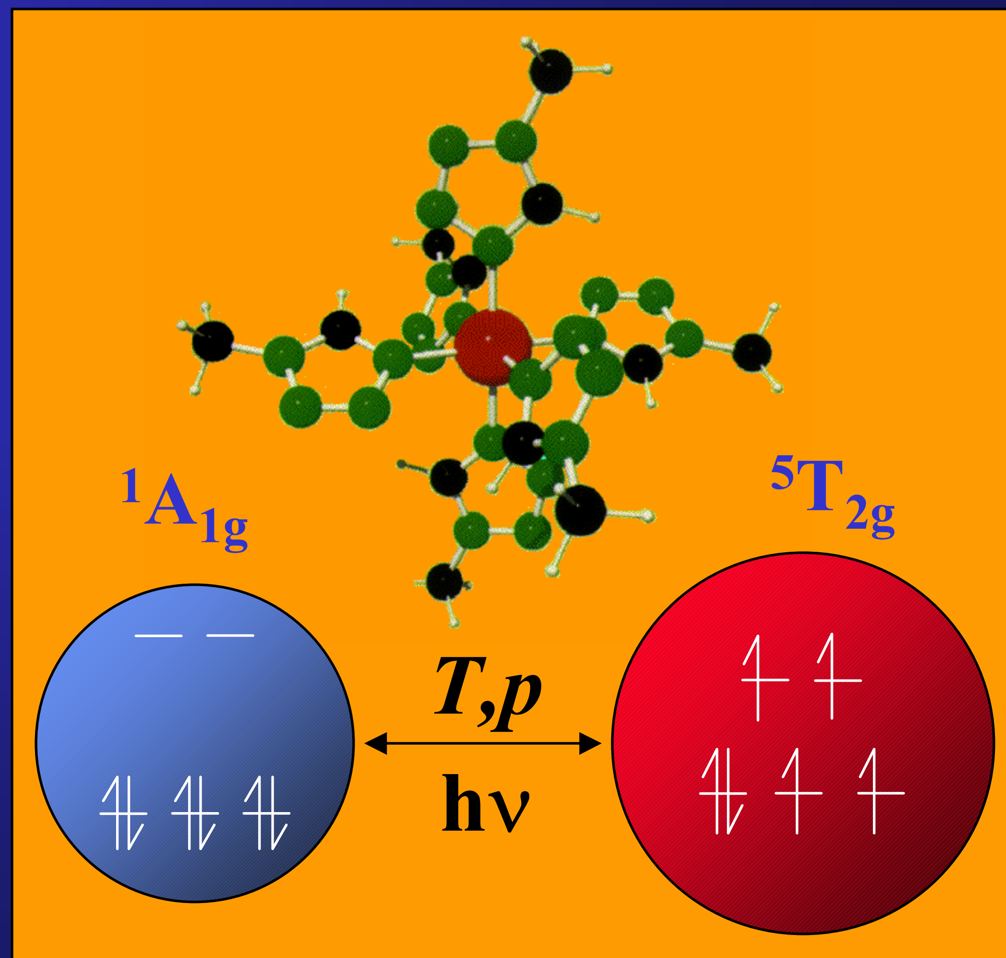
Metal complexes as devices

Spin Crossover

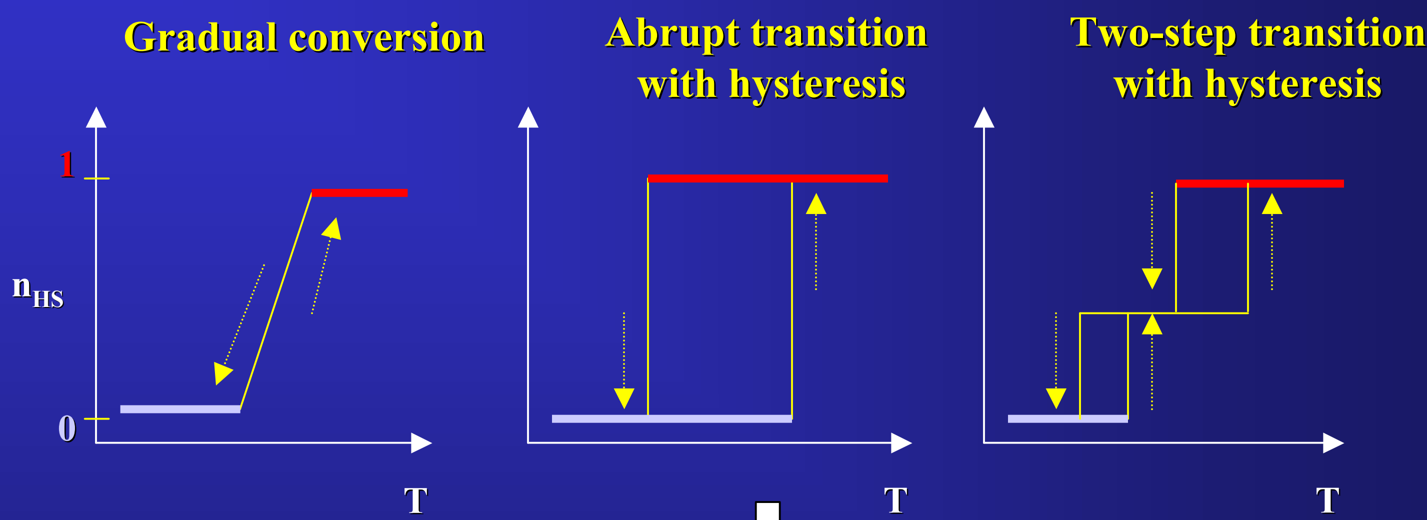
The magnetic and optical properties of some transition metal complexes can be altered by varying the temperature
(*thermal spin crossover*) :

This *spin crossover* can also be brought about by light and by pressure.

Light-induced changes in colour, magnetism, volume in spin-crossover materials form the basis for light-driven devices



Spin-crossover in the solid state: types of transitions



Possible applications in memory and display devices and as molecular switches

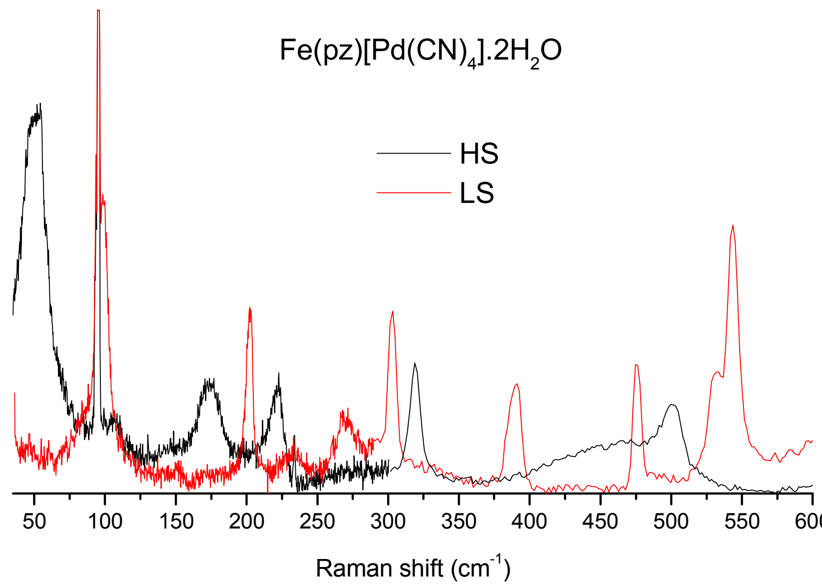
O. Kahn et al. Science, 279, p. 44, 1998

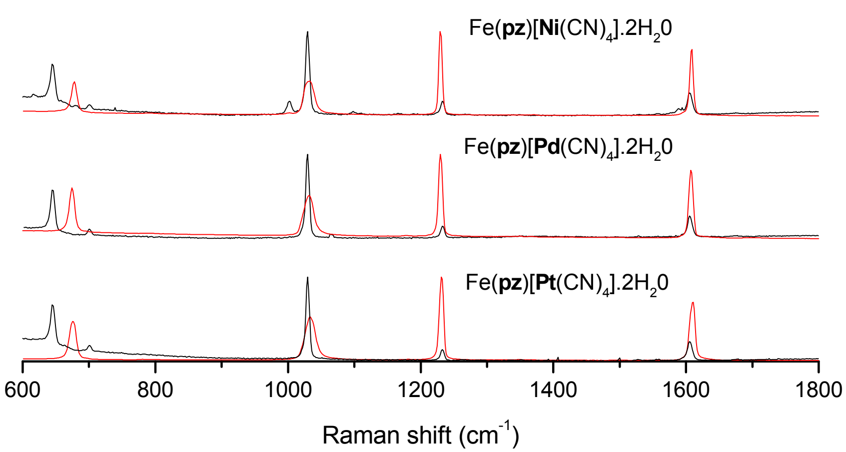
Cooperative phenomena, such as encountered in the solid state are key to such applications

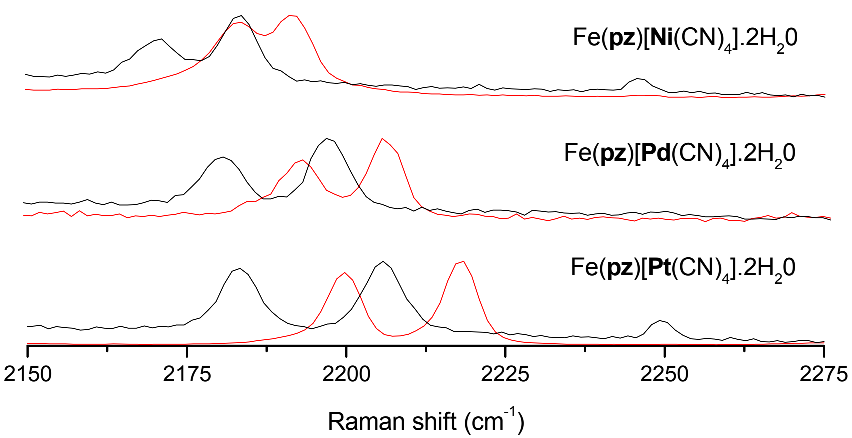
Raman spectra display useful marker bands for the high- and low-spin species

RAMAN SPECTRA : distinct spectral regions

- Low-frequency modes from 2D polymeric sheet:
- CN-stretches
- Pyrazine (pyridine) internal modes
  (straightforward assignments)







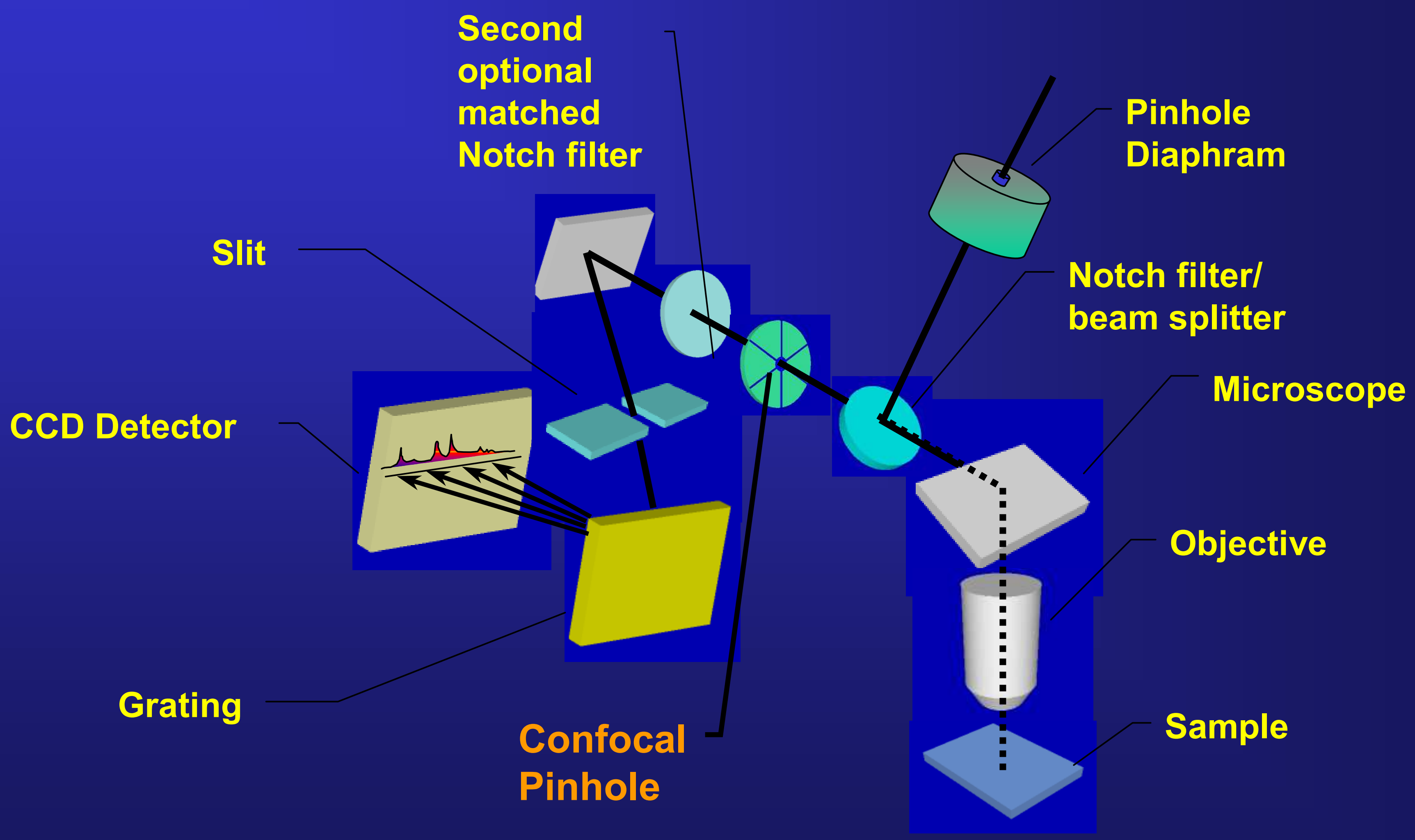
• Raman is an informative technique for probing spin-crossover complexes, but there can be some problems in applying it :

Since the spin equilibrium can be perturbed by light and temperature, acquisition of the Raman spectrum may simultaneously perturb the equilibrium…….

…….unless the photon flux in the Raman excitation beam can be kept sufficiently low.

Raman spectroscopy and microscopy – ***a natural coming together***

Horiba JY Raman Microscope - Schematic



Some applications of Raman microscopy

- Raman studies of lung brush samples
- Mapping of pharmaceutical samples
- Studies at single cell level
- Raman mapping of retinal tissue

Raman mapping of retinal tissue

Retinitis Pigmentosa (RP) is a group of eye diseases which cause progressive vision loss due to death of the light-sensitive cells in the retina at the back of the eye.

:

In the present work the retina of an animal model which possesses a human RP mutation, is being investigated by Raman microscopy to evaluate the potential of this technique for analysis of the multi-layered retina.

Two strands to the study:

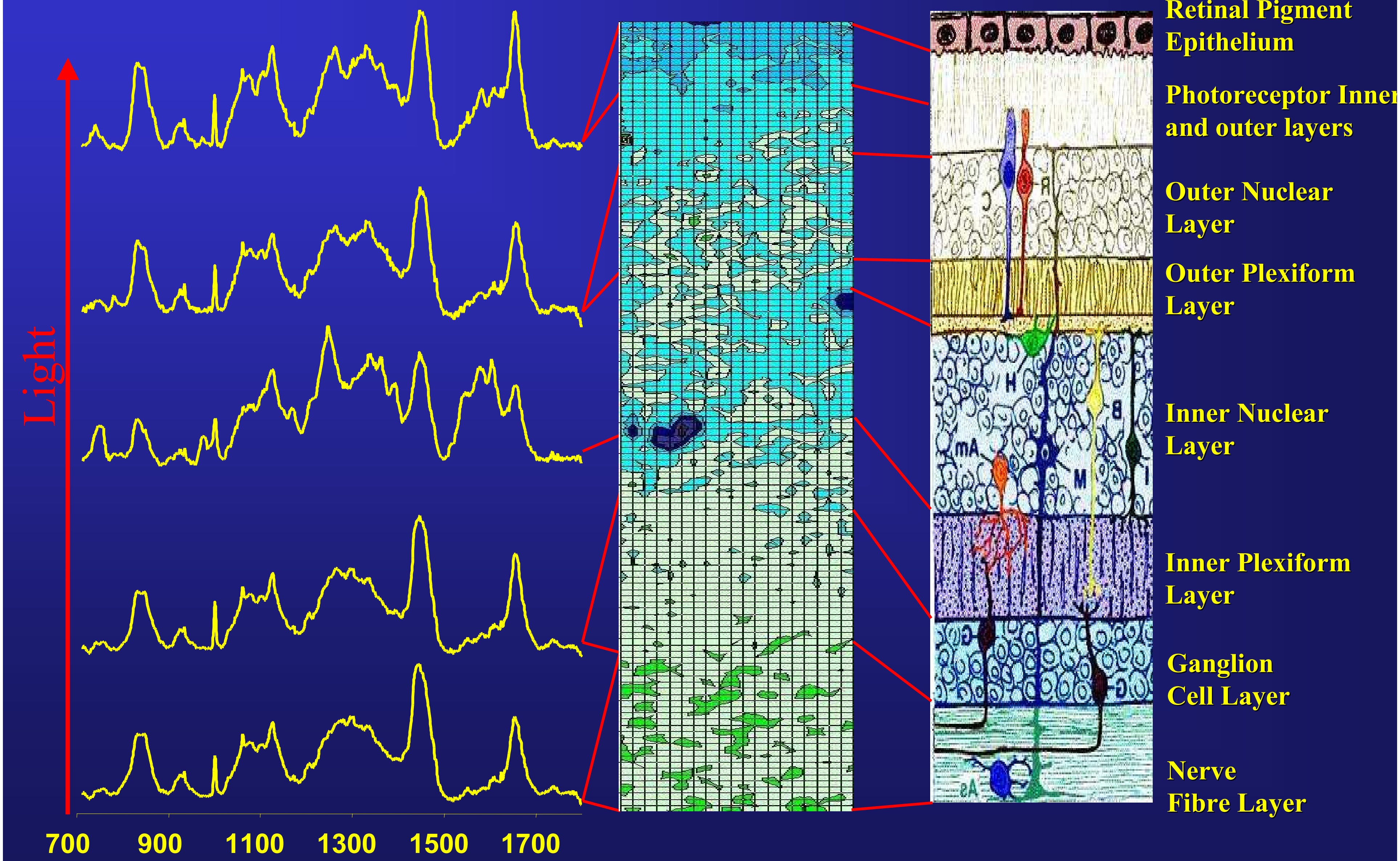
Mapping the Raman spectral variations across a section of retina, spanning the photoreceptor region through to the ganglion cell layer.

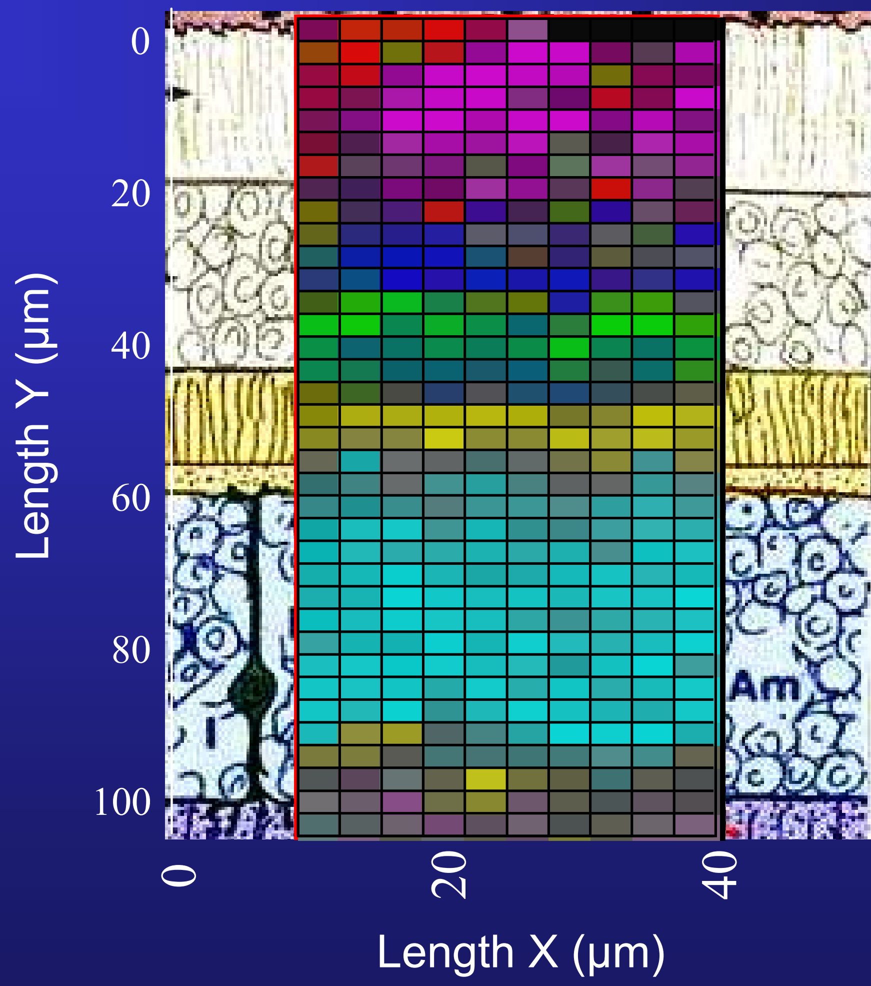
:

Mapping the Raman signal variation in the photoreceptor outer region for normal retina and transgenic pig model RP

Attention is focussed on the photoreceptor region, since this is where the pathology of RP presents itself.

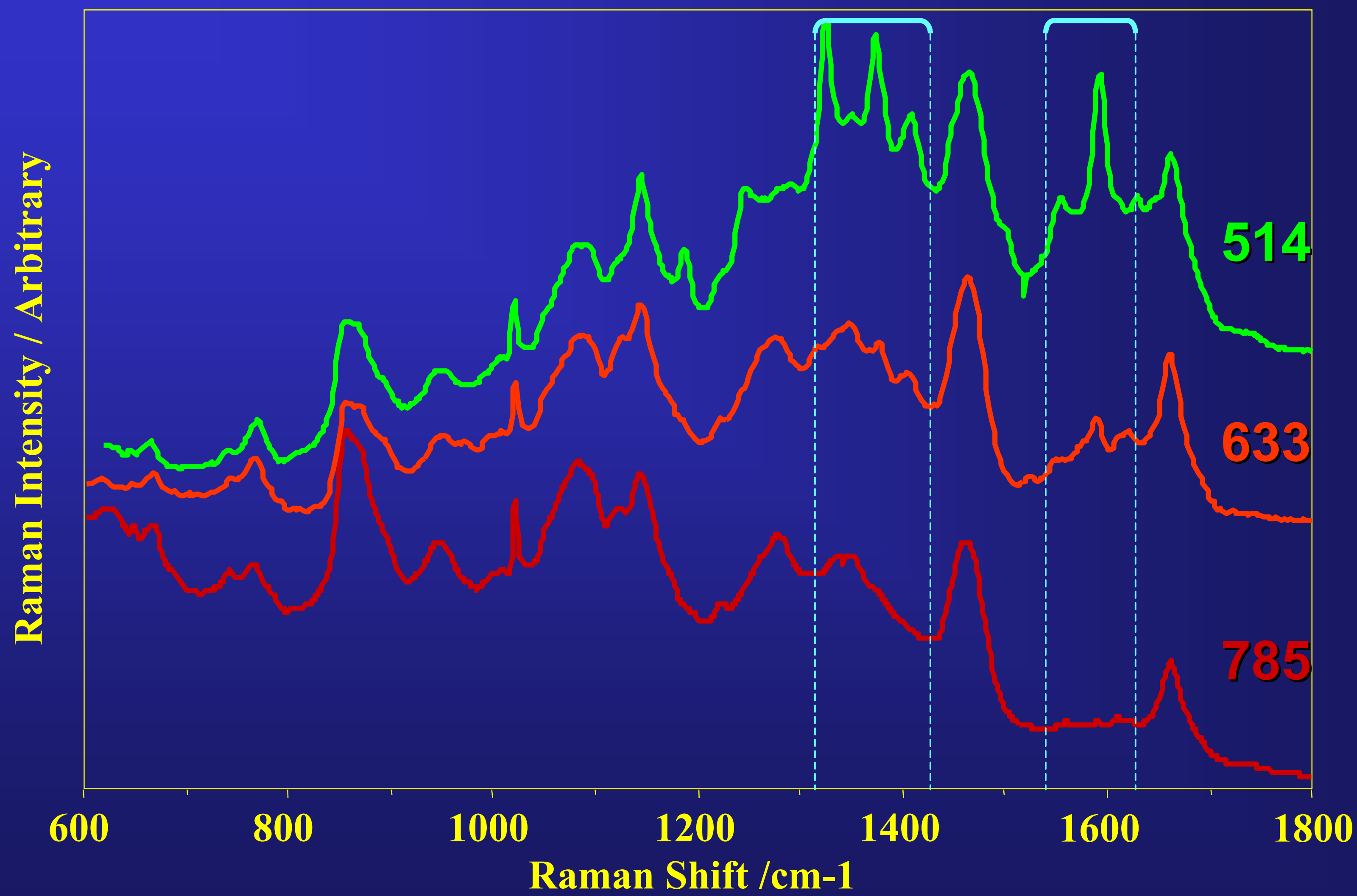
Comparison of schematic retina section with a 1μm spacing Raman map of a section of retina





0
20
40
60
80
100
Length Y (µm)
0
20
40
Length X (µm)
Am

Raman spectra of the photoreceptor inner segment at three wavelengths



Raman spectroscopy is widely applicable as a 'chemical vision' technique:

- for both ground and excited states of molecules
- in solution, solid phase or on surfaces
- within the broad field of biological research/diagnosis

  e.g.**Raman microscopy has the potential to probe the pathology of RP and biochemical composition/processes in the retinal layers.**

Selected examples of work from other laboratories.

Protein folding

A. Smith Group, University of Leeds

UVRR and ultra-rapid mixing

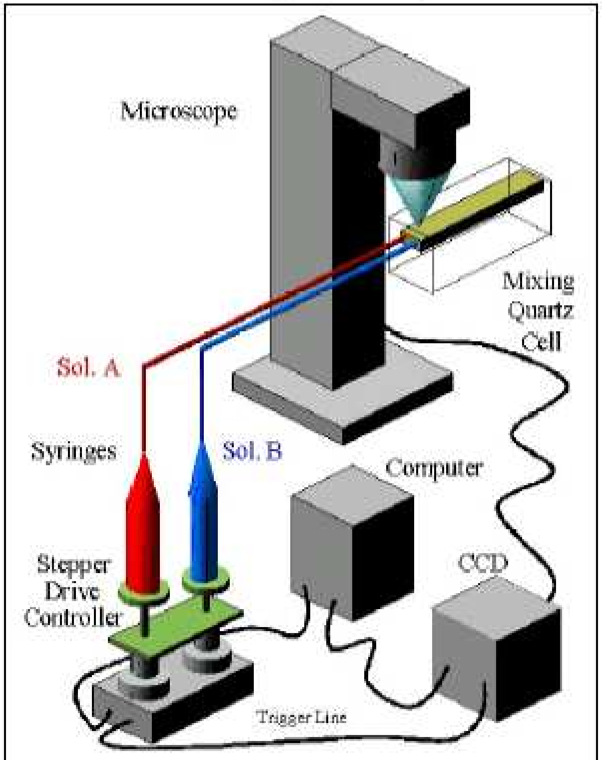


Fig. 1 The experimental setup.

- Detailed **structural** (e.g. conformational changes) and kinetic information can be probed by **Raman**

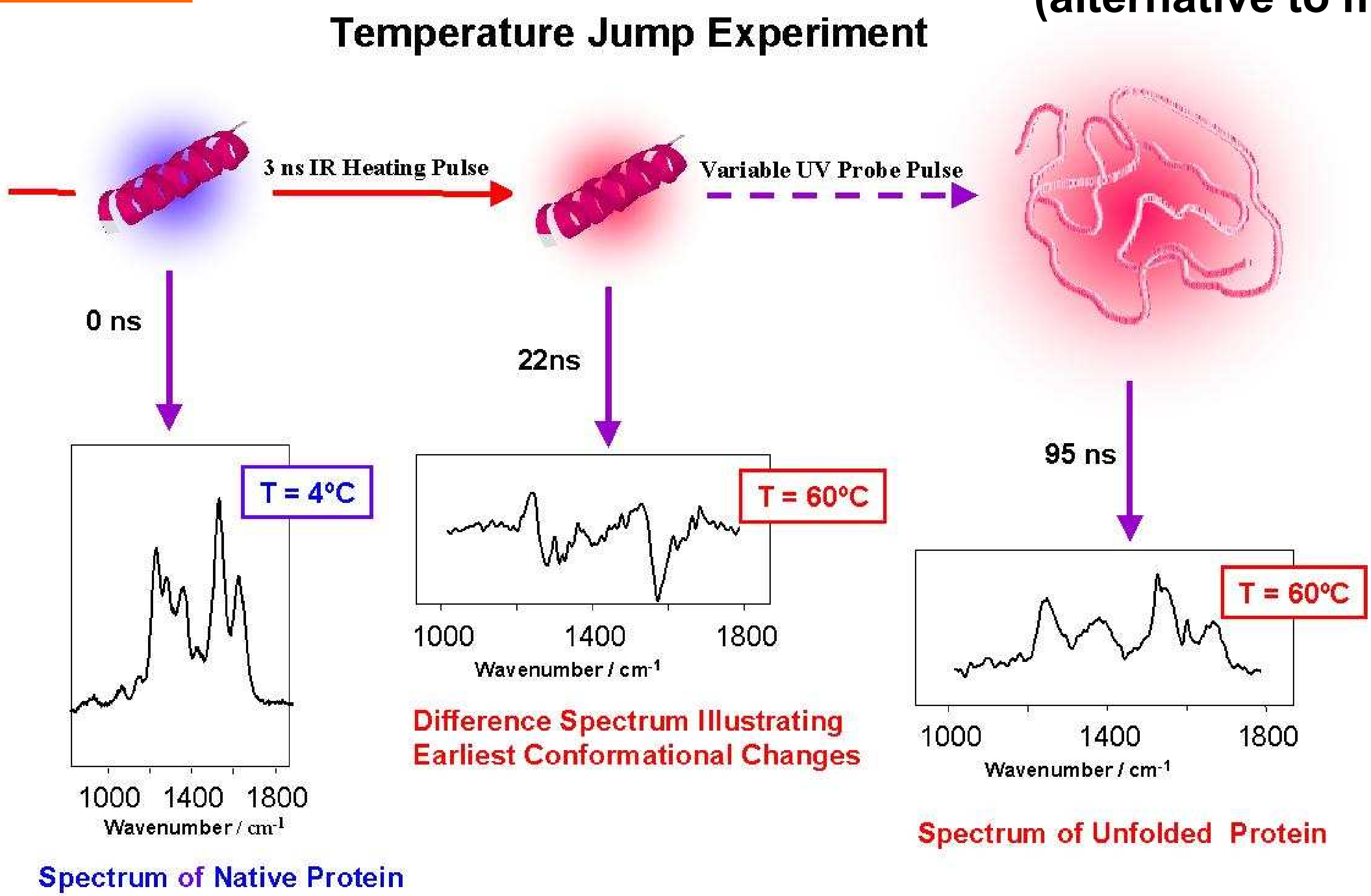
- Special efforts needed to minimise mixing times. Now possible to have this as short as 150 μs

- Would benefit from fast spectral acquisition on the **ms** (or less) timescale without being restricted by read noise *(EMCCD)*.

S.A. Asher Group Univ. of Pittsburgh

The temperature jump initiates unfolding ('denaturation')
UVRR spectra obtained at intervals from **ten to several hundred ns** following the heat-producing pump pulse and monitor the time scale on which re-folding occurs.

(alternative to mixing)



J. Am. Chem. Soc. **2001,** *123,* 2388−2392

Transient UV Raman Spectroscopy Finds No Crossing Barrier between the Peptide α-Helix and Fully Random Coil Conformation

Igor K. Lednev, Anton S. Karnoup, Mark C. Sparrow, and Sanford A. Asher*

Contribution from the Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Note S/N limitation

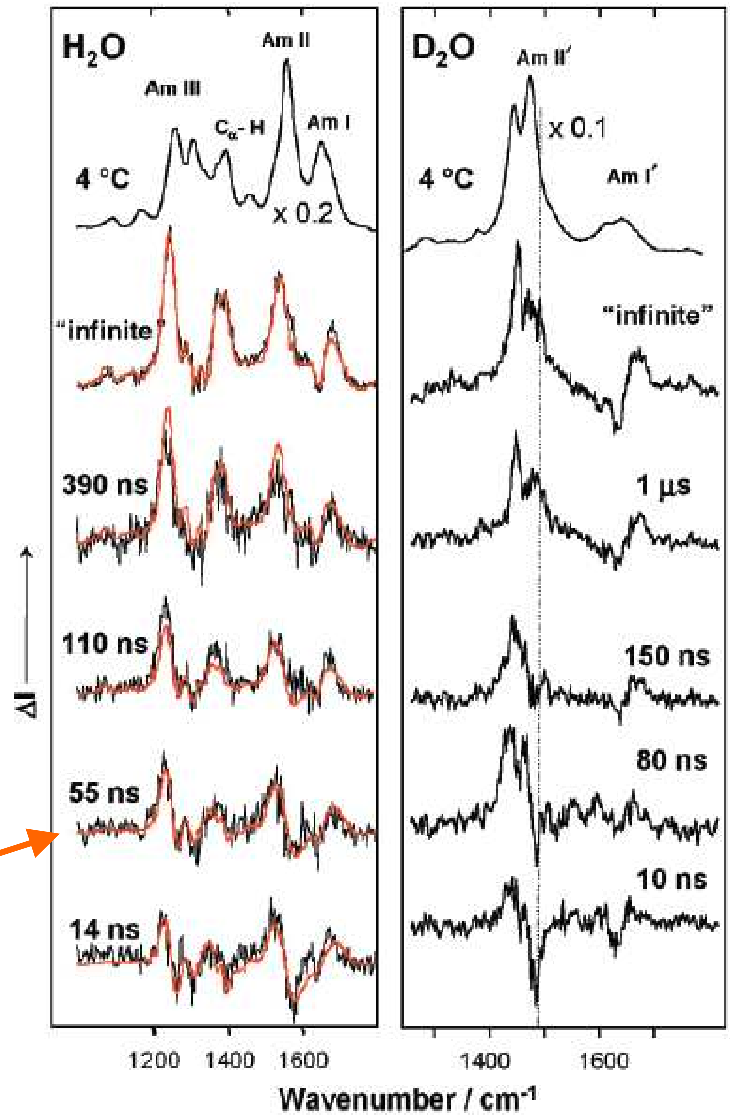


Figure 3. AP UVRS spectra (top curves) measured in H_2O (D_2O) solution at 4 °C and transient difference UVRS of AP in H_2O (D_2O) solution initially at 4 °C measured at different delay times after a *T*-jump of ~31 (~22) °C. The steady state difference UVRS spectrum between 35 (26) and 4 °C is the transient difference spectrum for an infinite delay time. Red curves: Transient spectra modeled with eq 1 by using pure α-helix and random coil basis spectra and their measured temperature dependence with no free parameters.

Low-Light Detection

• ***Many Raman approaches require detection of low photon fluxes.***

This may come about through a combination of factors:

- Raman is a weak phenomenon.
- Trace analysis / low concentration
- Short exposure times for fast processes or fast mapping
- Confocal configurations
- Attenuated excitation power - to reduce photo-induced effects or ensure low excited state population.

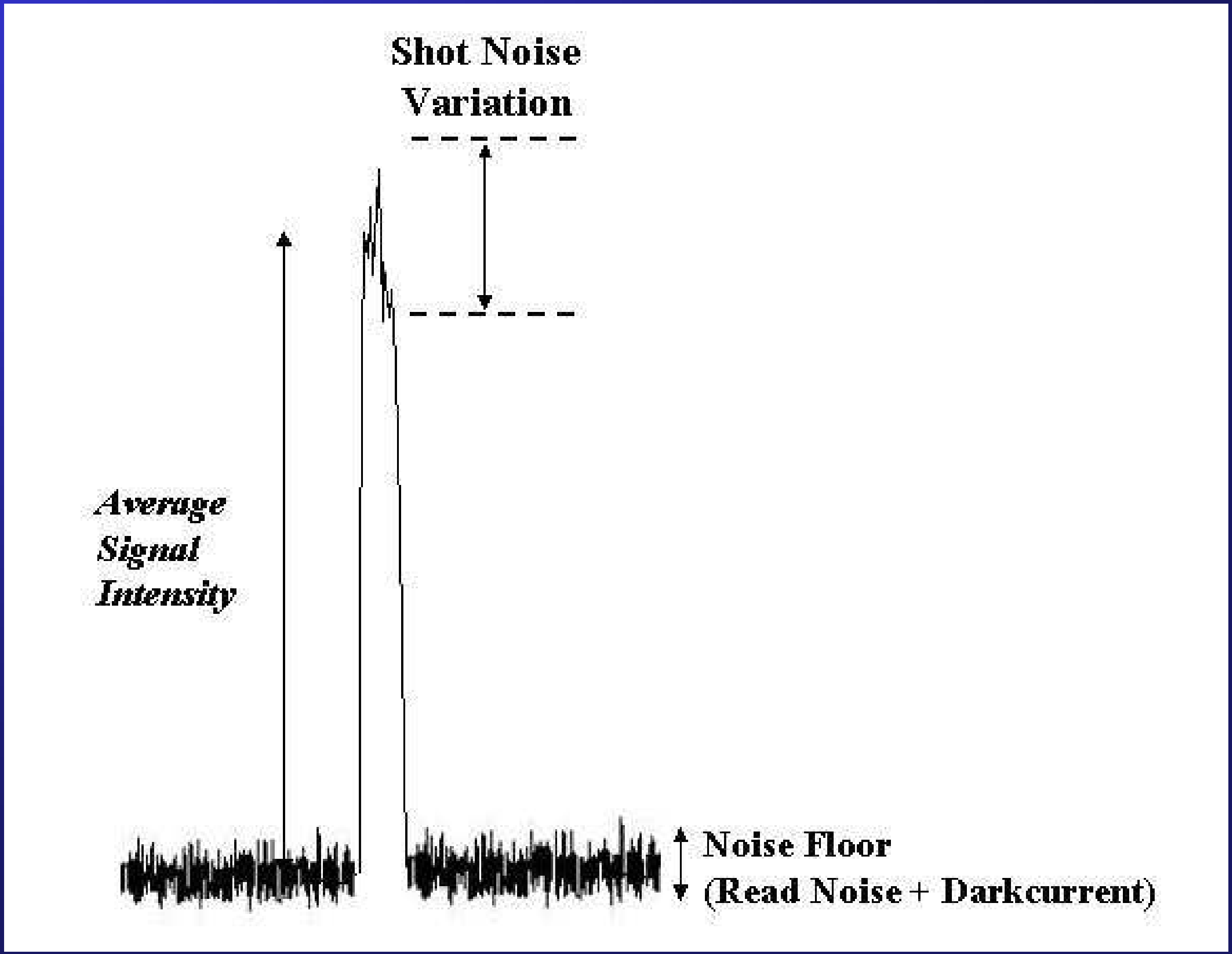
Instrument Sensitivity

Two Fundamental parameters determine sensitivity:

1. **Noise – Defines the Detection Limit to overcome. Lower Noise = Less photons needed for detection.**

2. **Quantum Efficiency – QE is a measure of the detector's ability to capture photons. Higher QE means less photons are required to overcome the Noise.**

Illustration of Principal Noise Sources



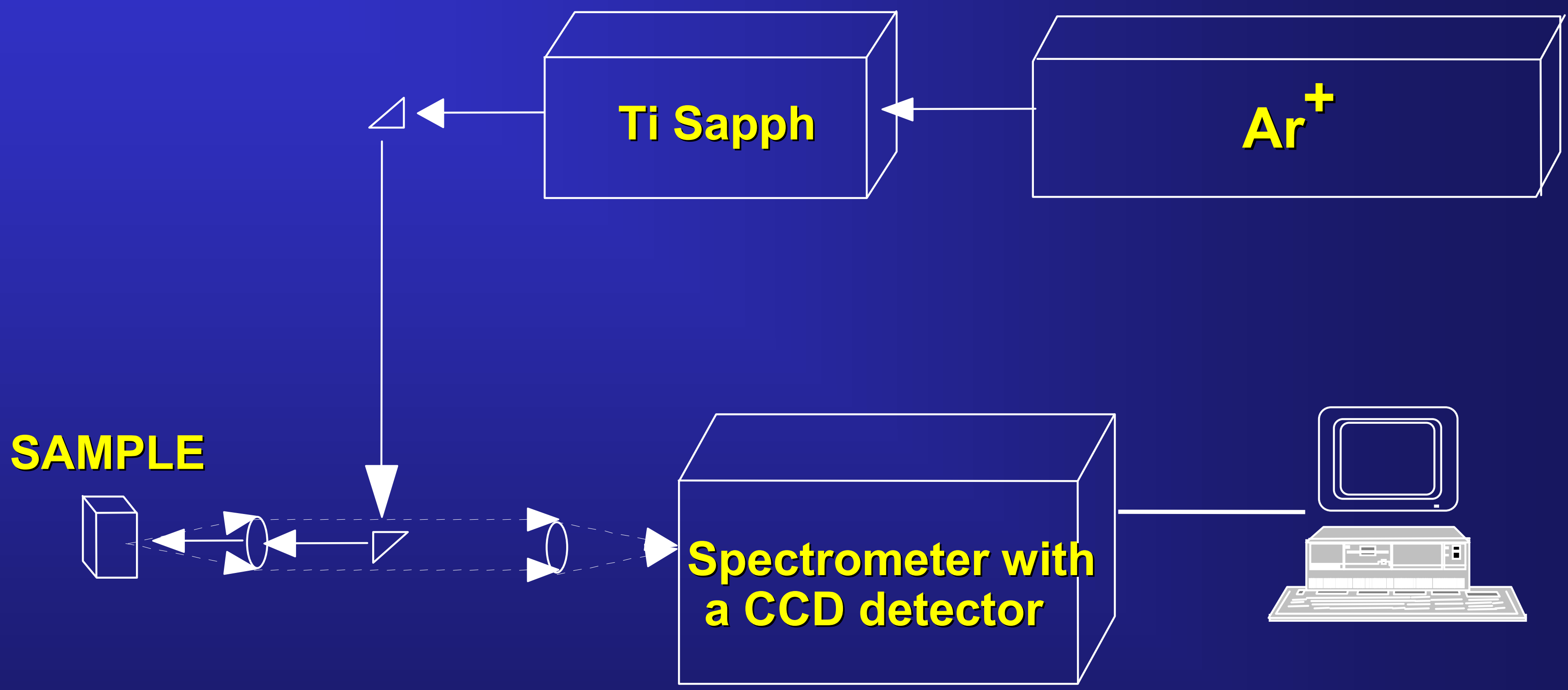
We will now show some preliminary evaluation of new Newton EMCCD camera……

- ✓ Effect of EM gain in overcoming noise floor
- ✓ Ability to reduce laser excitation power
- ✓ Ability to reduce exposure time
- ✓ Ability to vastly extend dynamic range through accumulation of multiple spectra without summing noise floors.
- ✓ Comparison to ICCD performance (QE difference on shot noise)

newton^{EM} DU971N

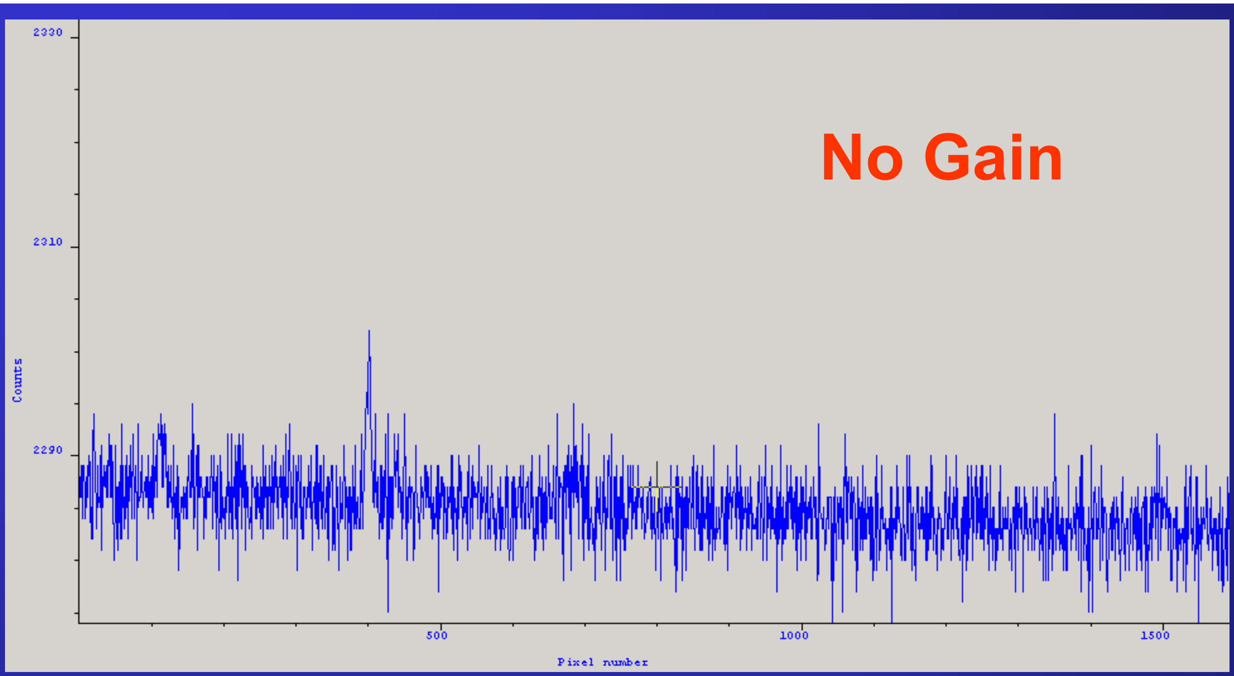
- **1600x400 pixels (also available as 1600x200)**
- **3MHz readout**
- **Back-illuminated**
- **16 bit A/D**
- **-75 ^{0}C air cooled (-100 ^{0}C water cooled)**
- **USB2**

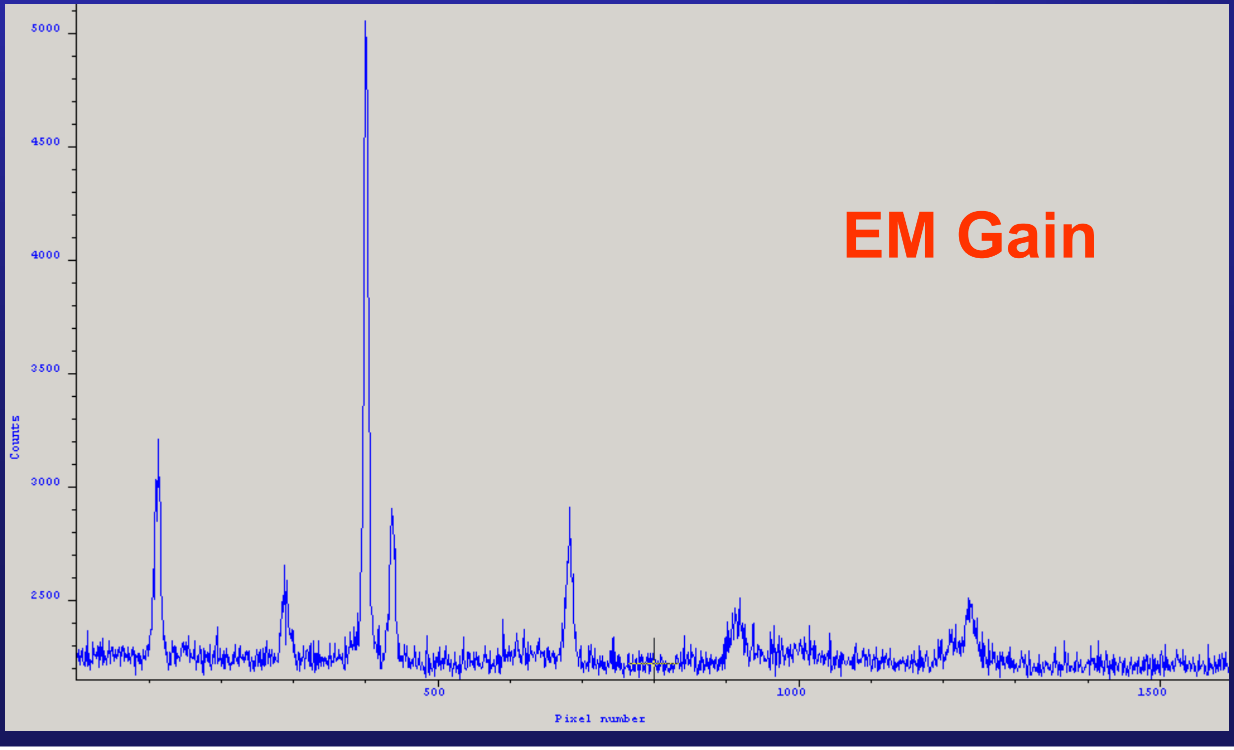
Some recent exploratory studies in QUB Raman Lab



Experimental set-up for ground state Raman spectroscopy

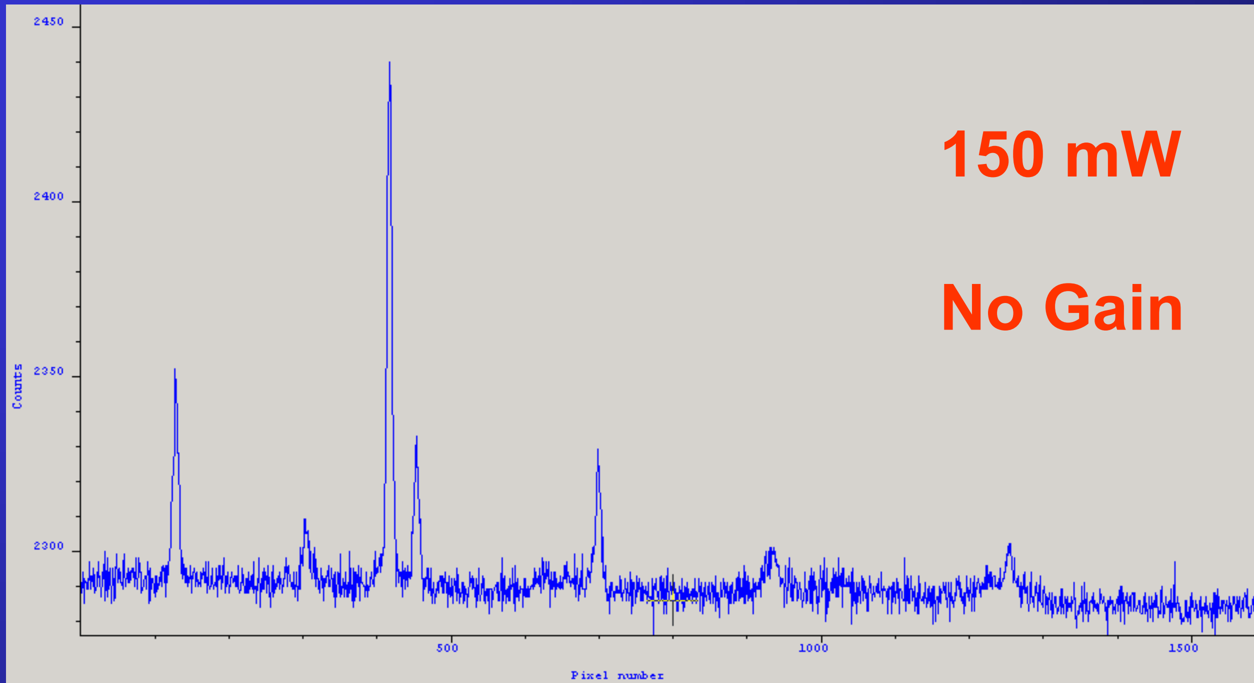
Effect of EM Gain

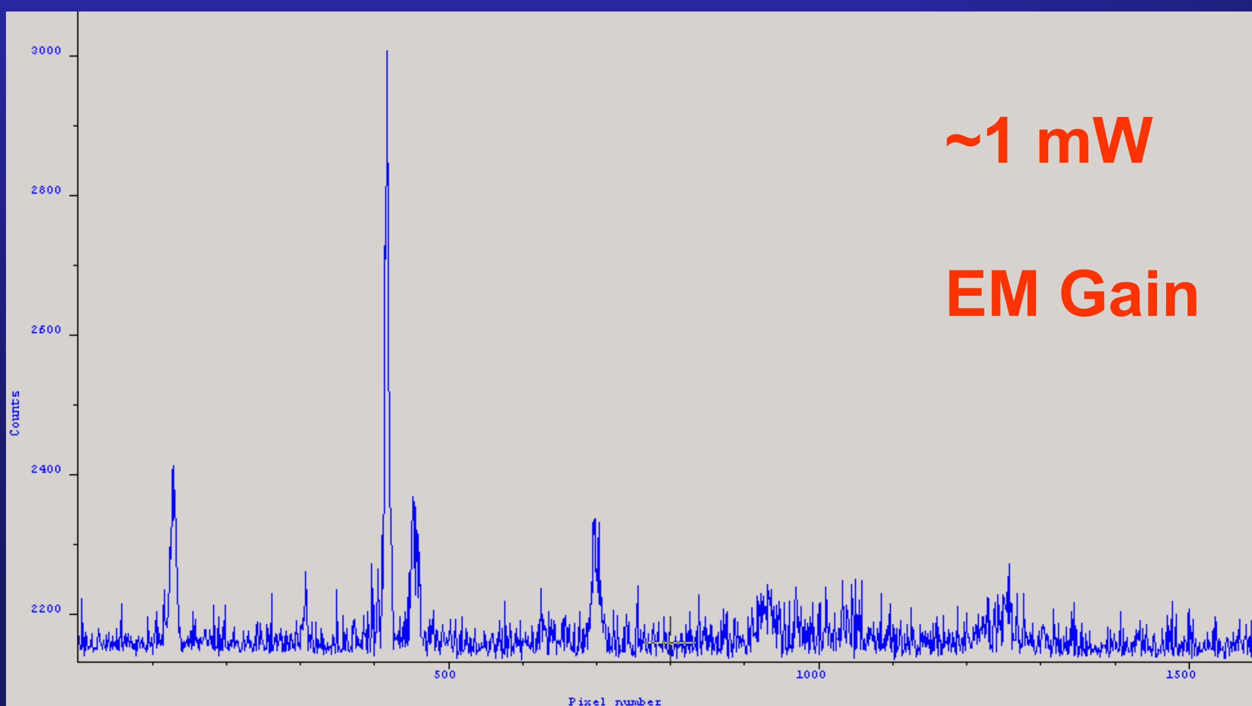




Acetonitrile/Toluene Mixture

20 μs exposure



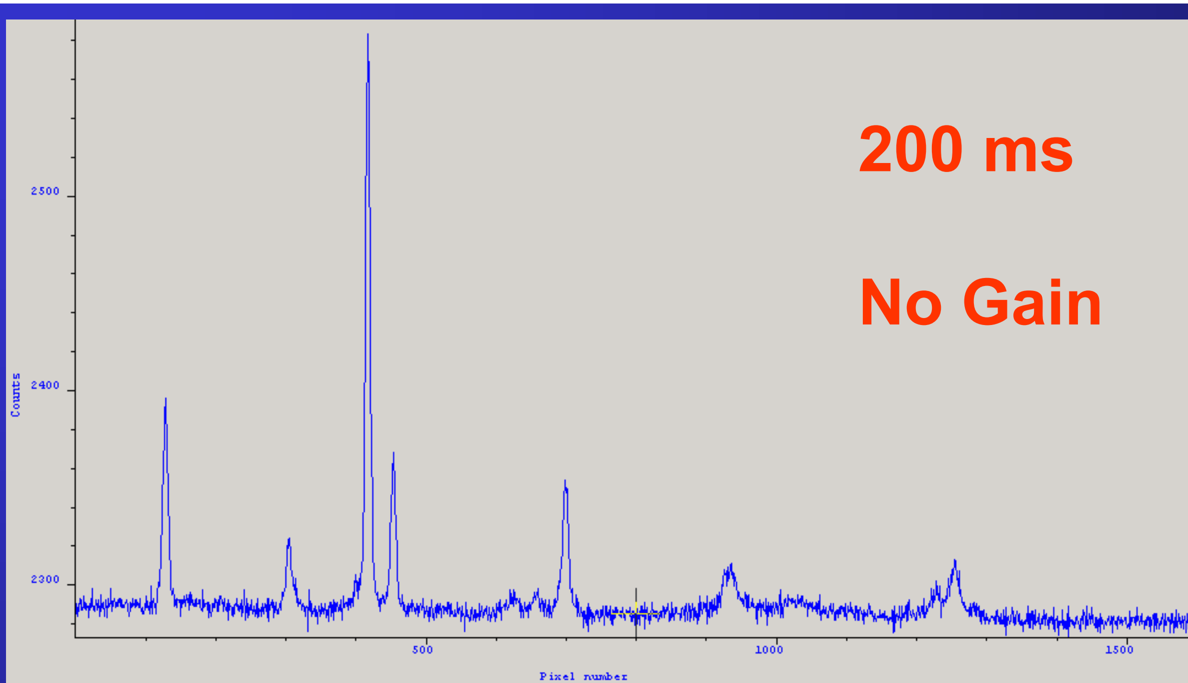


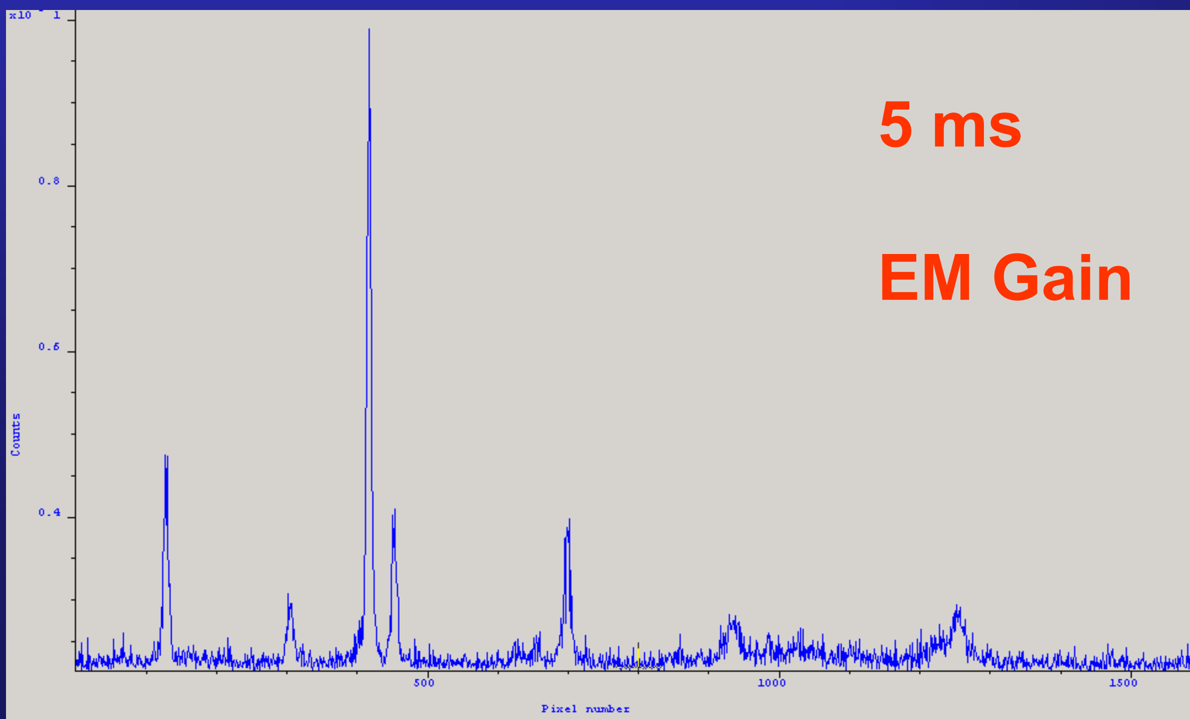
Ability to reduce laser power

0.1 s exposure

Implications:

- Preserve photolabile samples
- Minimise sample heating
- Power-resolved studies
- TR^3
- Safety



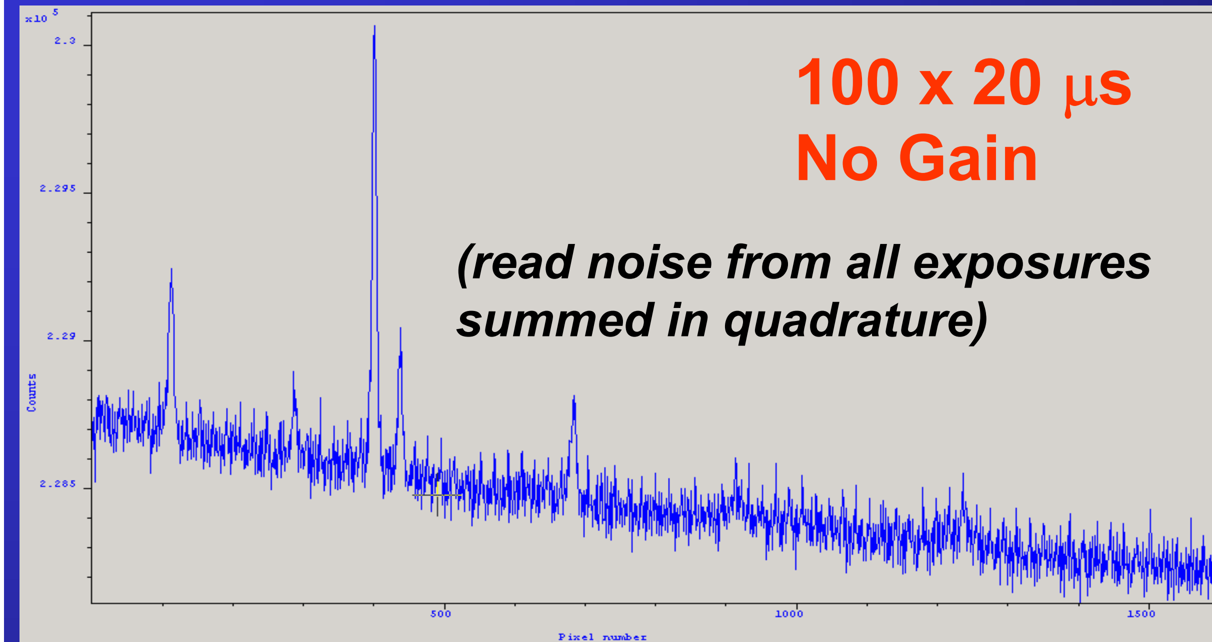


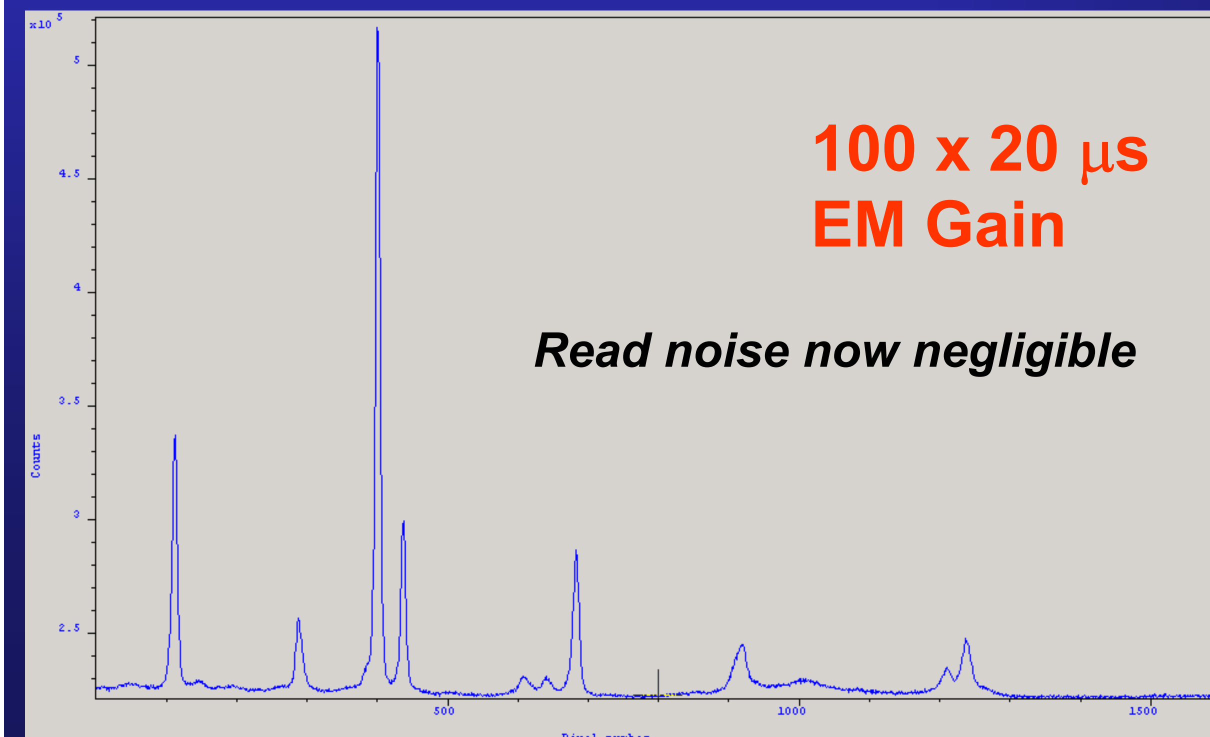
Ability to use shorter exposures / faster frame rates

150 mW laser

Implications:
- Follow fast processes
- Faster data acquisition
- Preserve photolabile samples
- Minimise sample heating

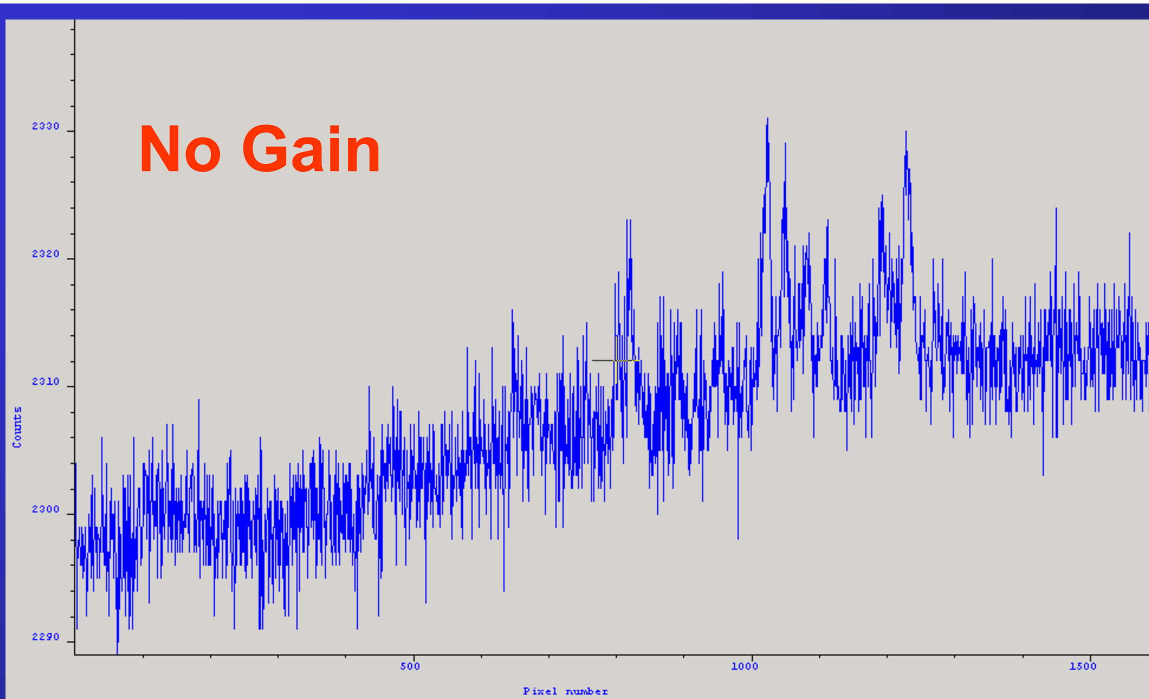
Effect of EM Gain on ‘accumulated’ spectra

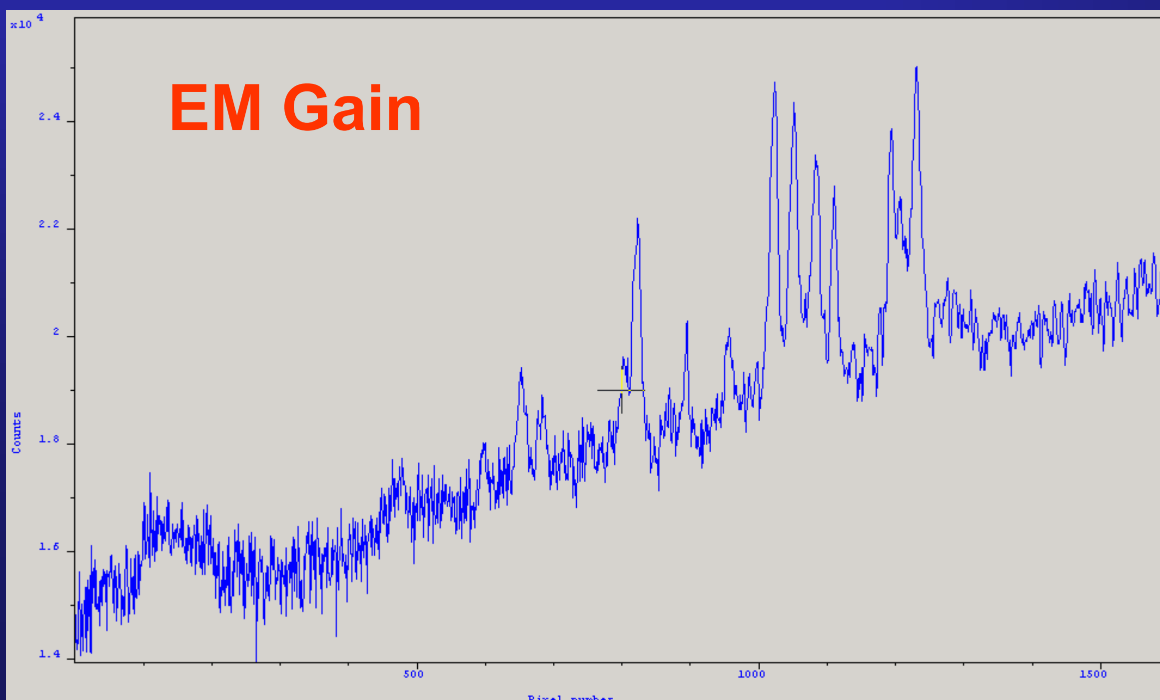




Implications:

- Significantly extend dynamic range, enabling trace analysis alongside intense features.
- e.g. look at both sides of an equilibrium, weighted very much to one side.

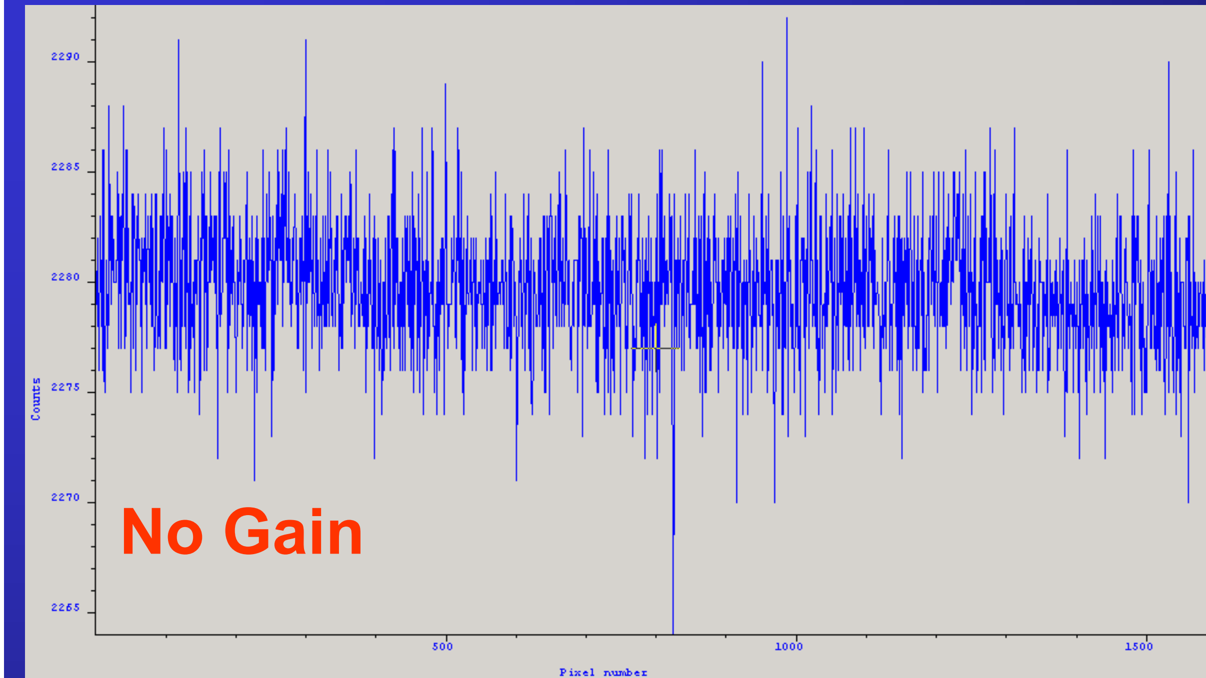


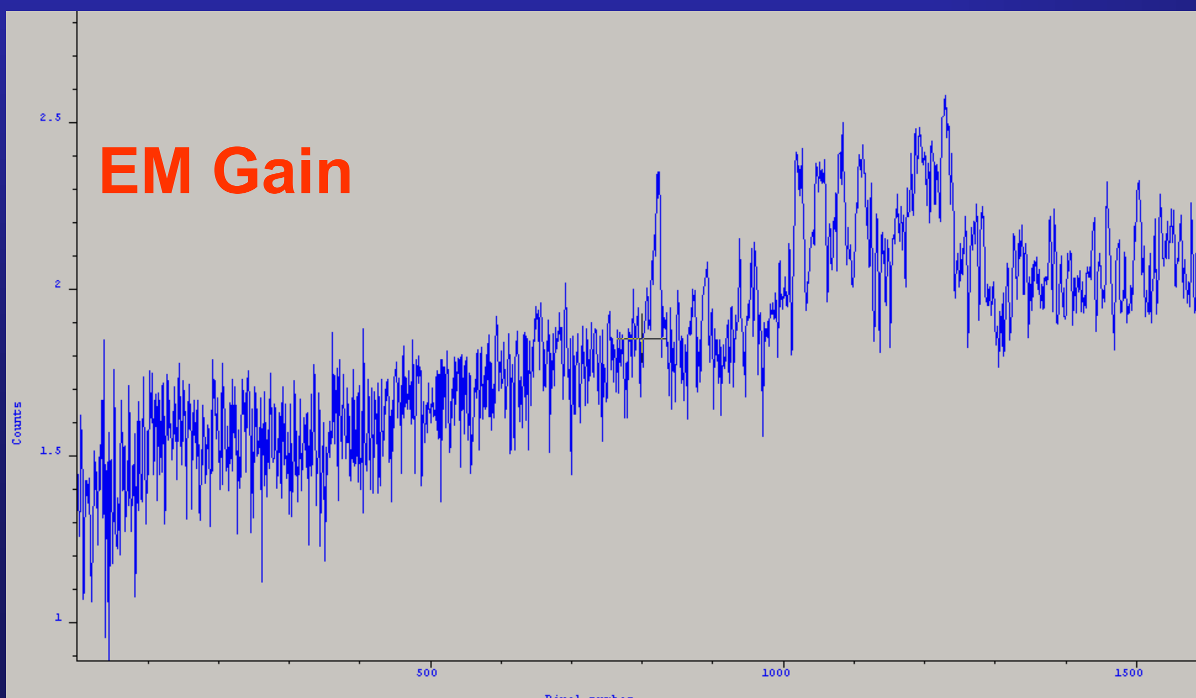


EM amplification of weak signal from dilute $[Ru(phen)_2dppz]^{2+}$

N.B. also amplifying some fluorescence Background – remaining shot noise detection limit.

- ✓ **S/N is sufficient to determine relative intensities of prominent marker bands.**
- ✓ **1s exposure time – perfect for photolabile complexes ………… …..(and busy schedules)**

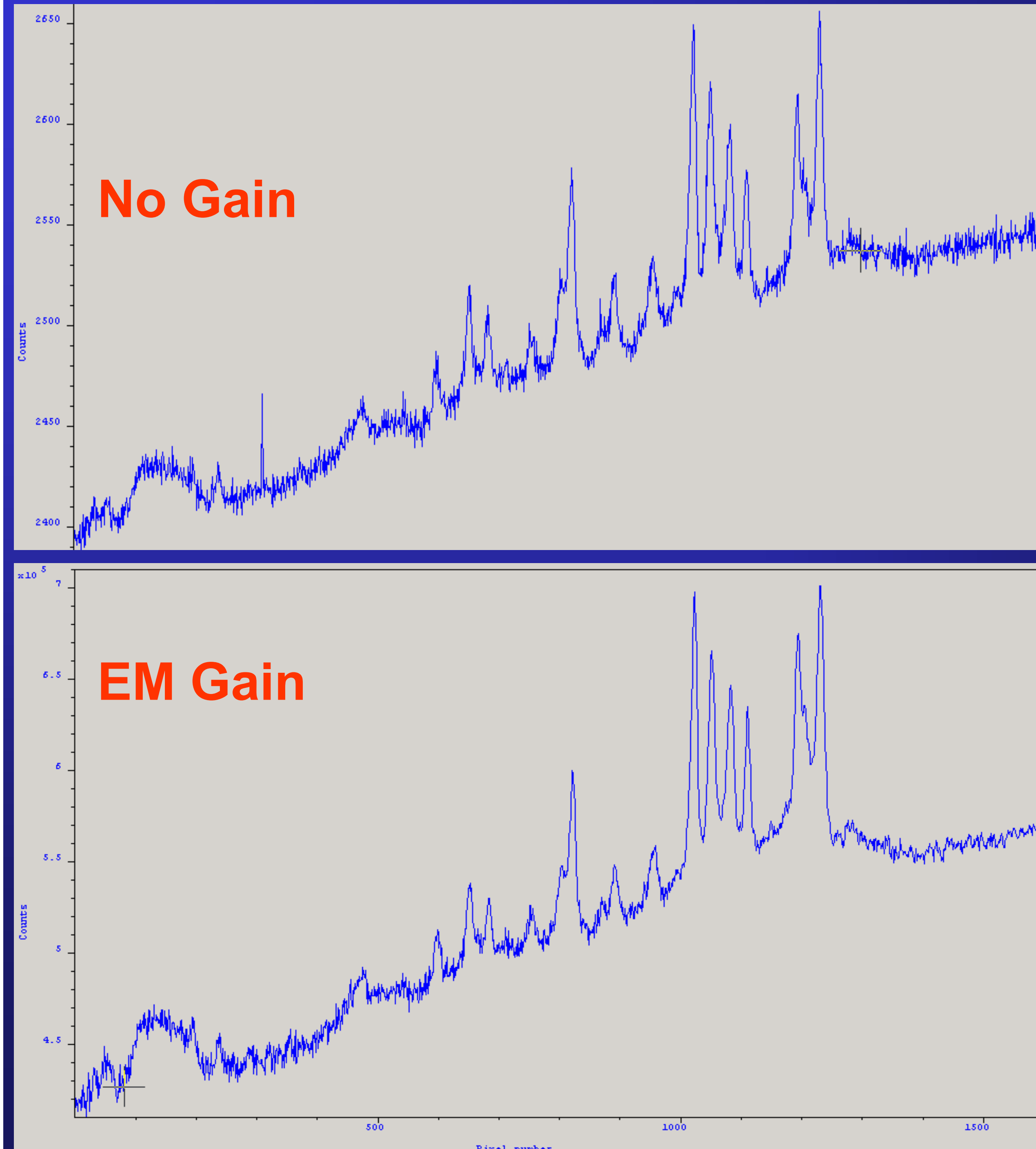




EM amplification of <u>even weaker</u> signal from dilute $[Ru(phen)_2dppz]^{2+}$

At the detection limit!

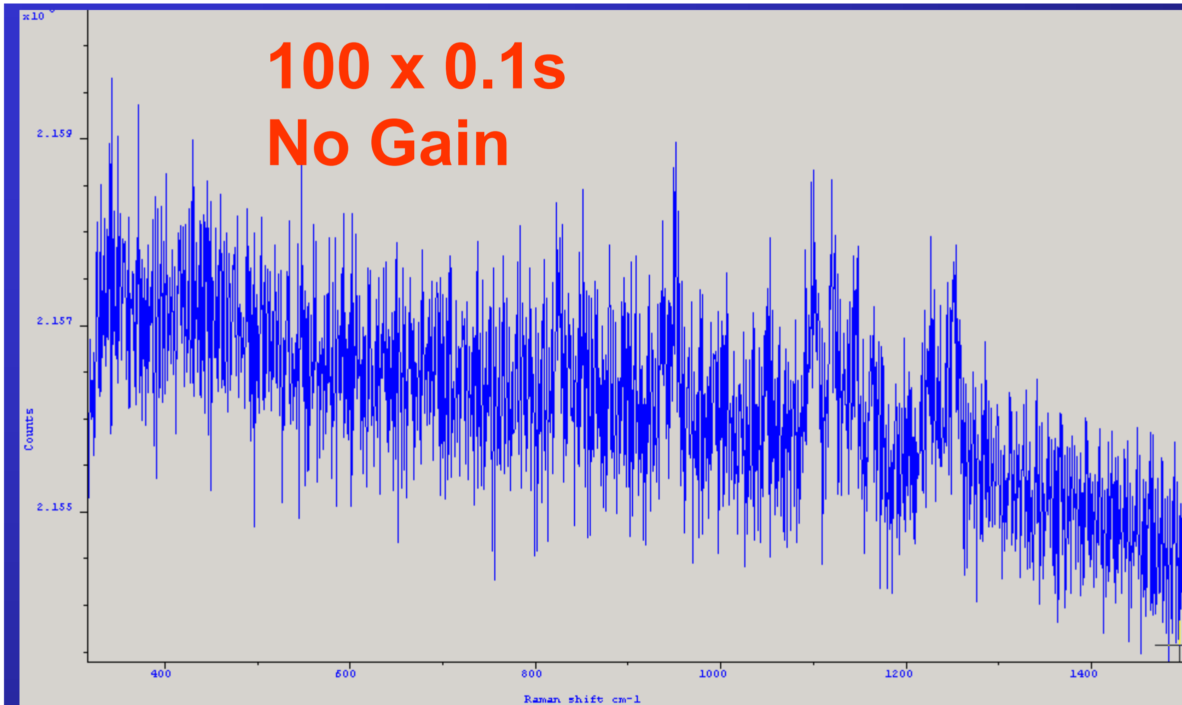
Difference between seeing something identifiable………… or nothing at all!

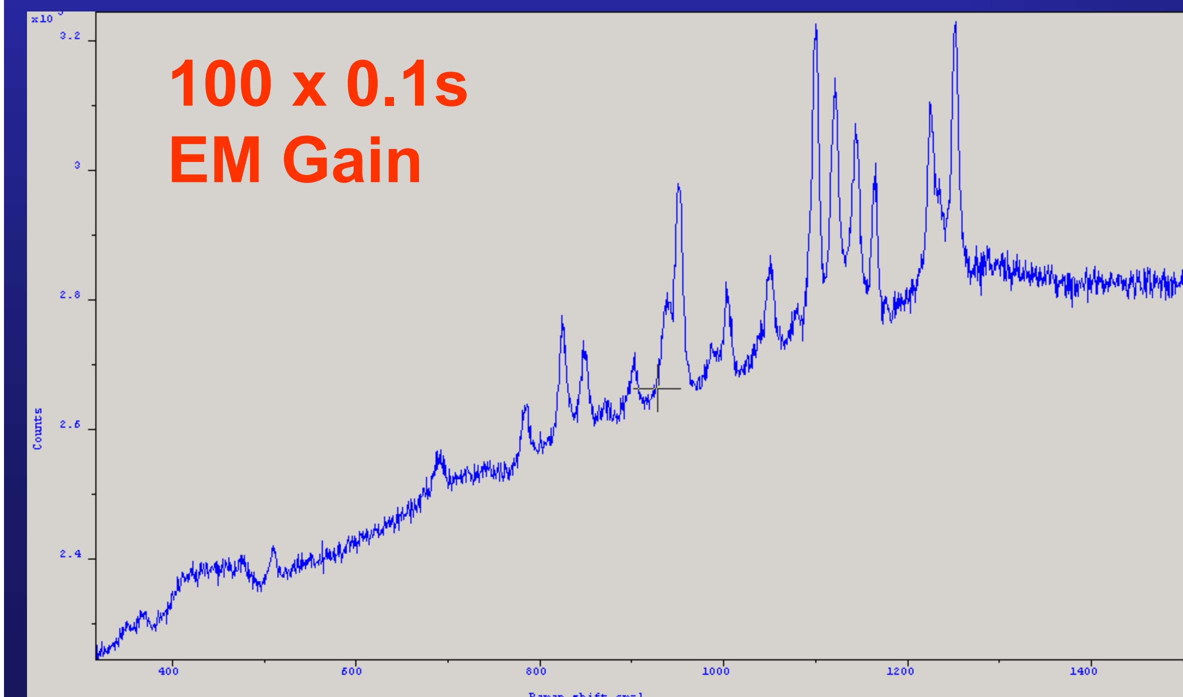


EM amplification of stronger signal from $[Ru(phen)_2dppz]^{2+}$

With stronger signals/longer exposures, still makes a difference……..

but more subtle.





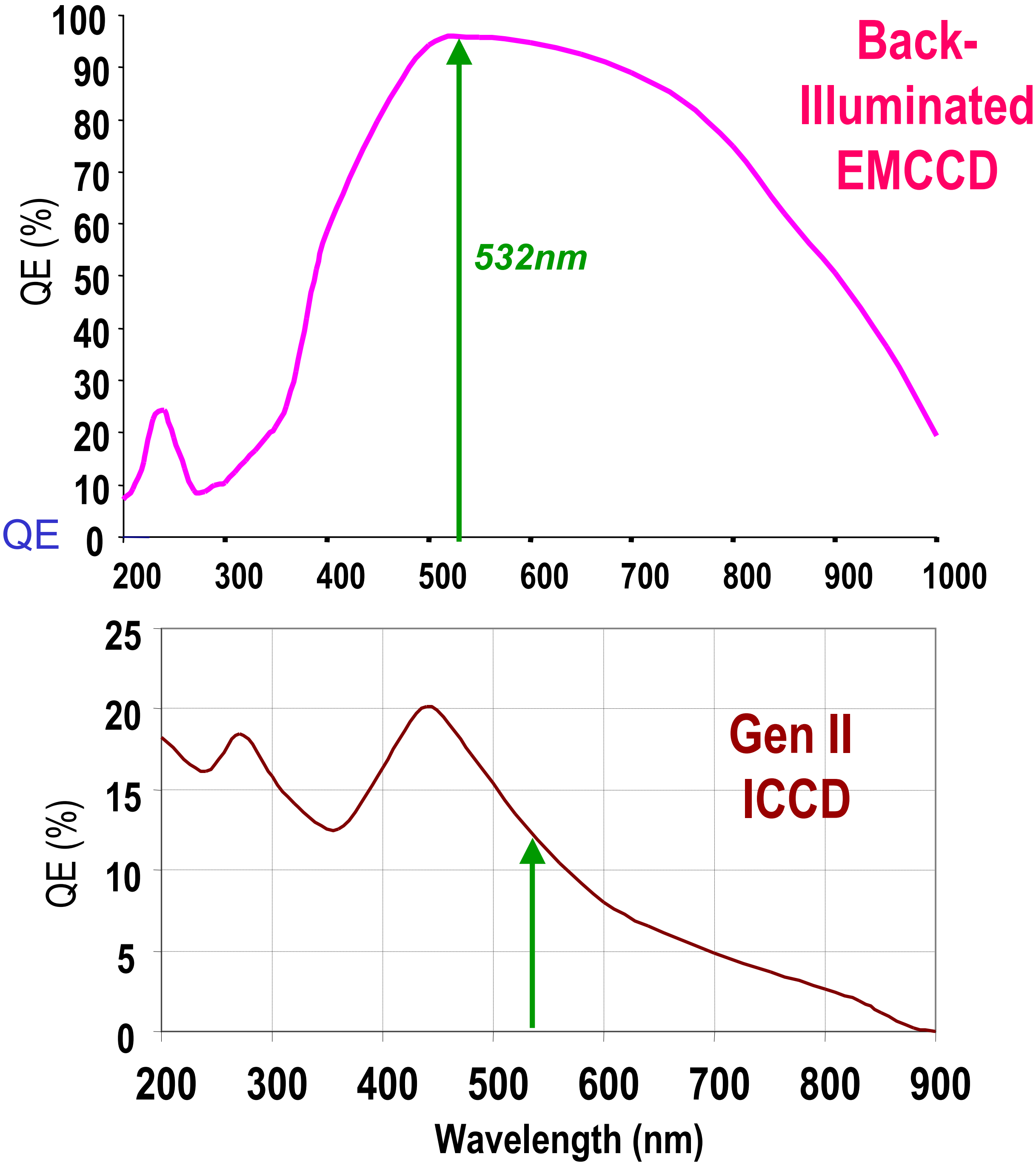
Effect of EM Gain of 'accumulated' spectra $[Ru(phen)_2dppz]^{2+}$

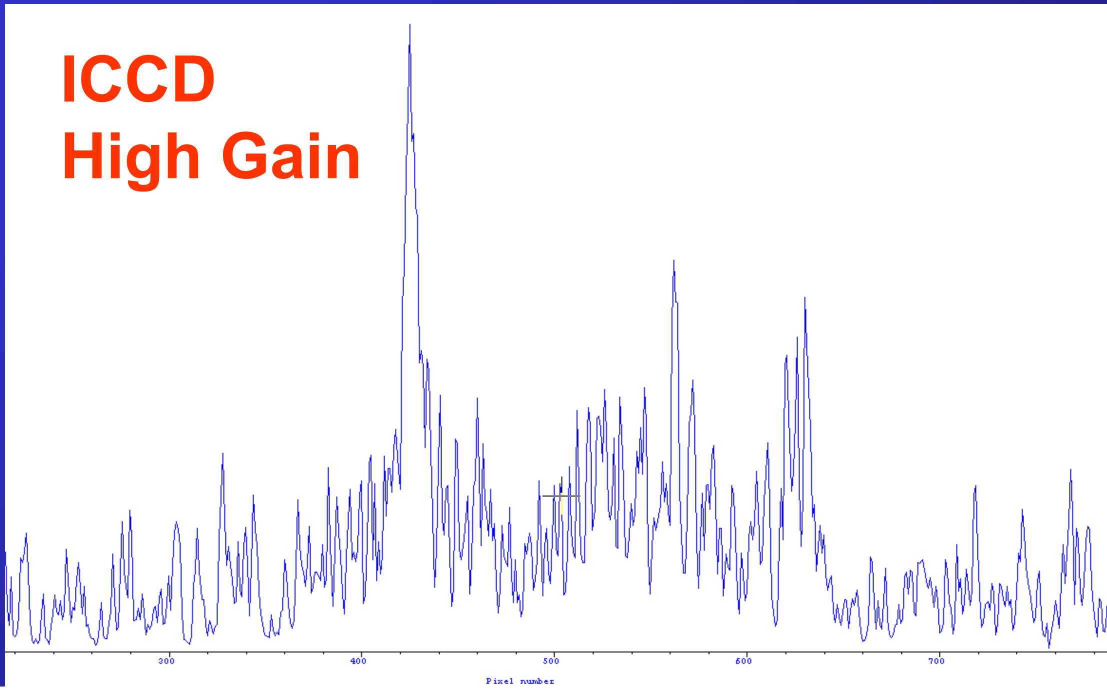
Again, if accumulations were required to extend dynamic range, EM can make a dramatic difference to weak sources.

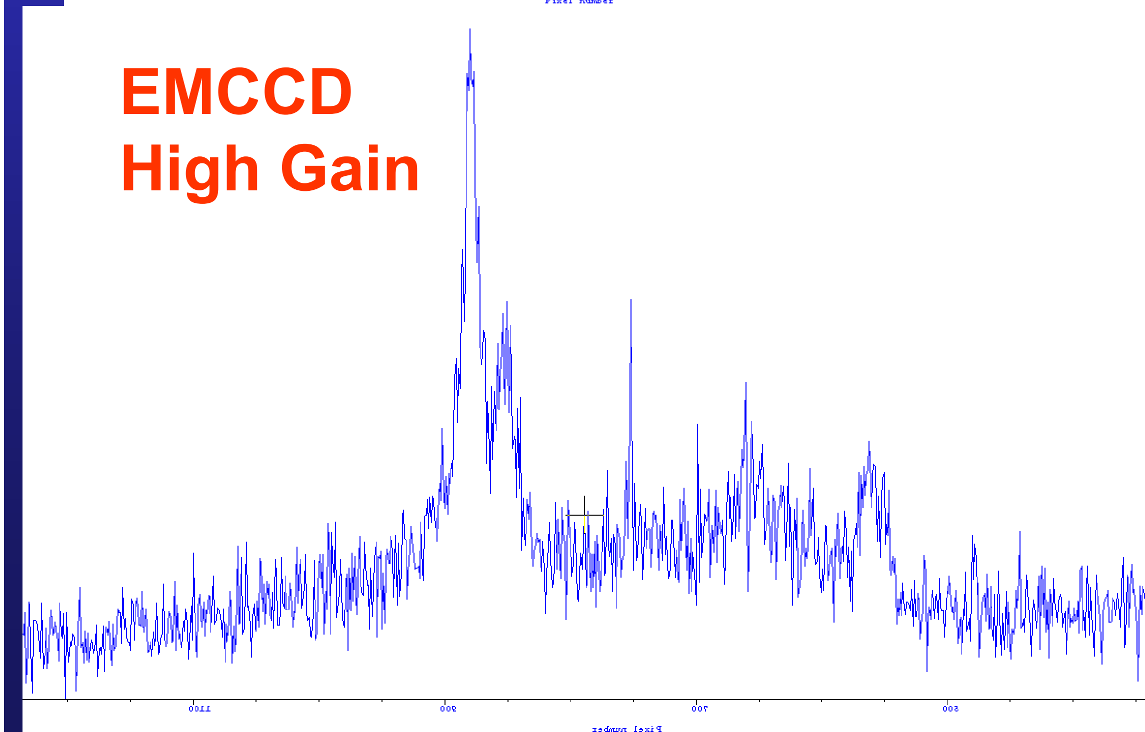
e.g. weak solutions in solvent

EMCCD vs ICCD

- Each single photon sensitive
- Marked difference in QE
- Expect enhanced Signal to Shot Noise for EMCCD





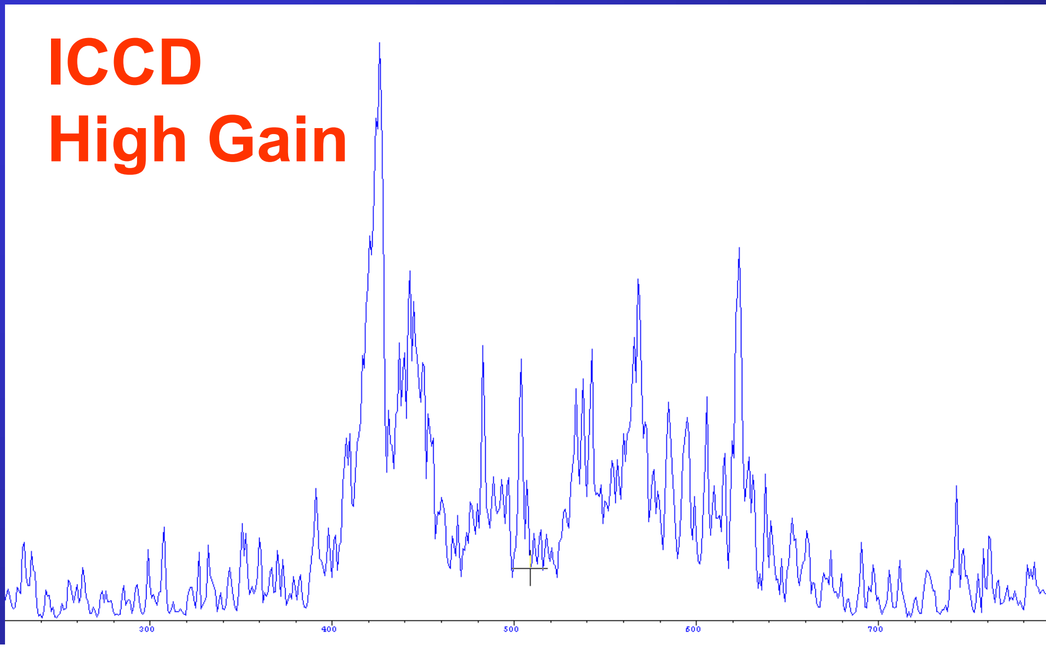


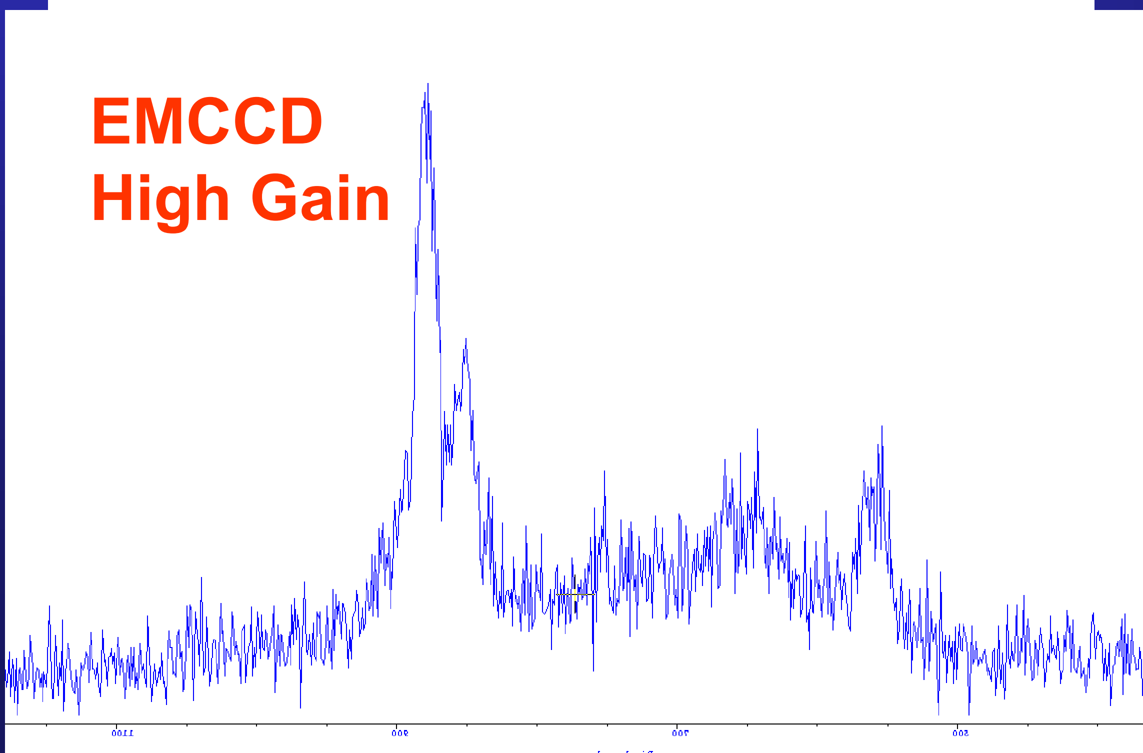
Comparison of ICCD vs EMCCD - Acetonitrile/ Toluene mixture

$\lambda_{ex.}$ = 532nm

Exposure time = 1s

Power = 1mJ/pulse



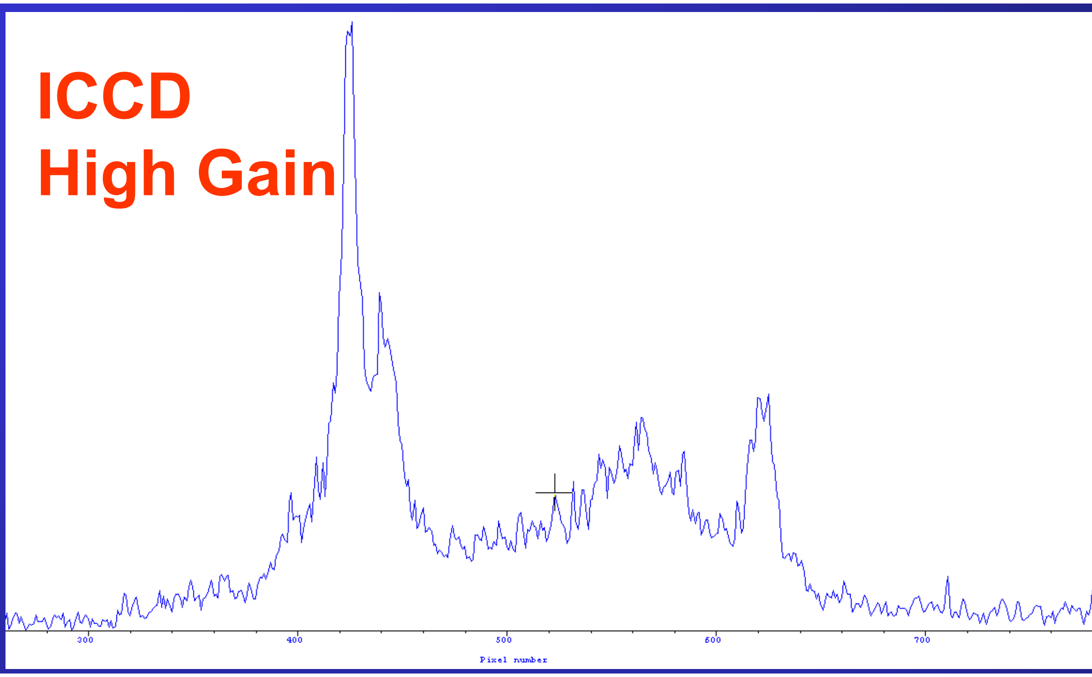


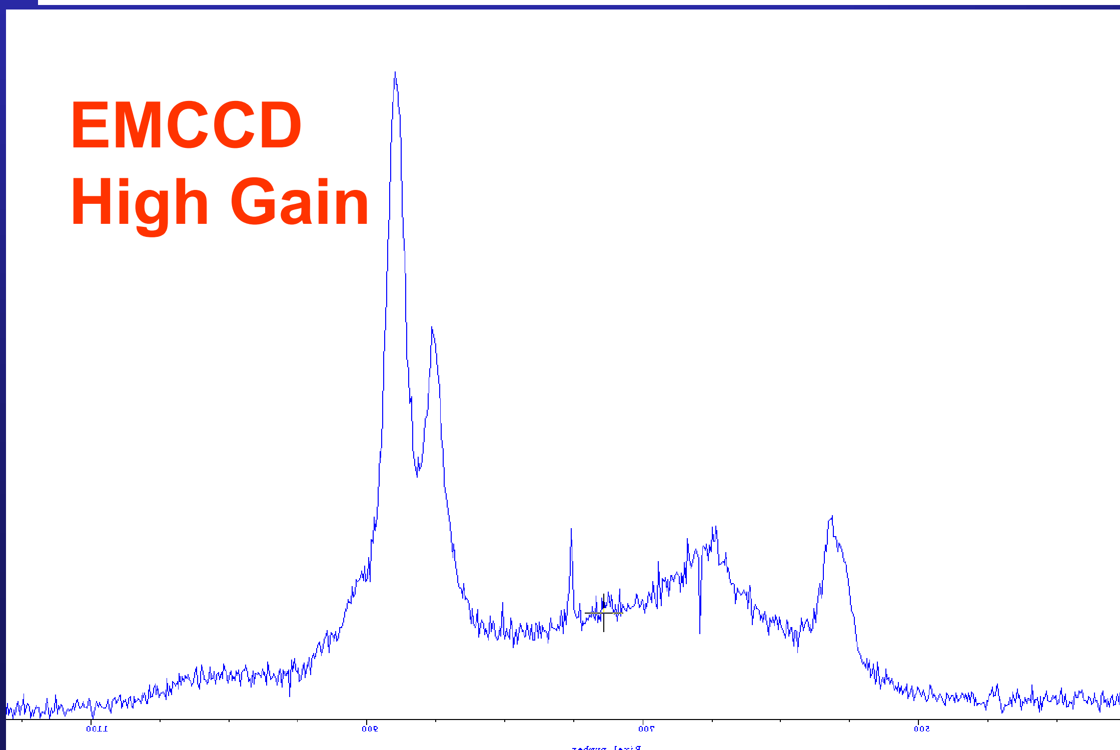
Comparison of ICCD vs EMCCD - Acetonitrile/ Toluene mixture

$\lambda_{ex.}$= 532nm

Exposure time = 1s

Power = 2mJ/pulse



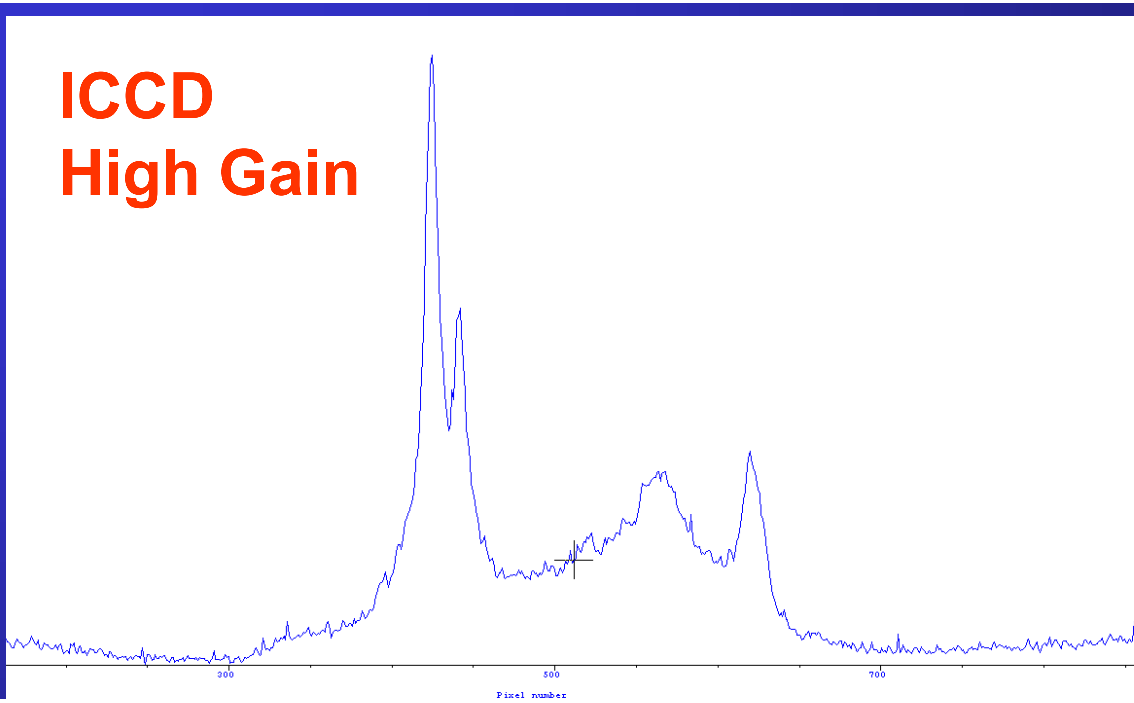


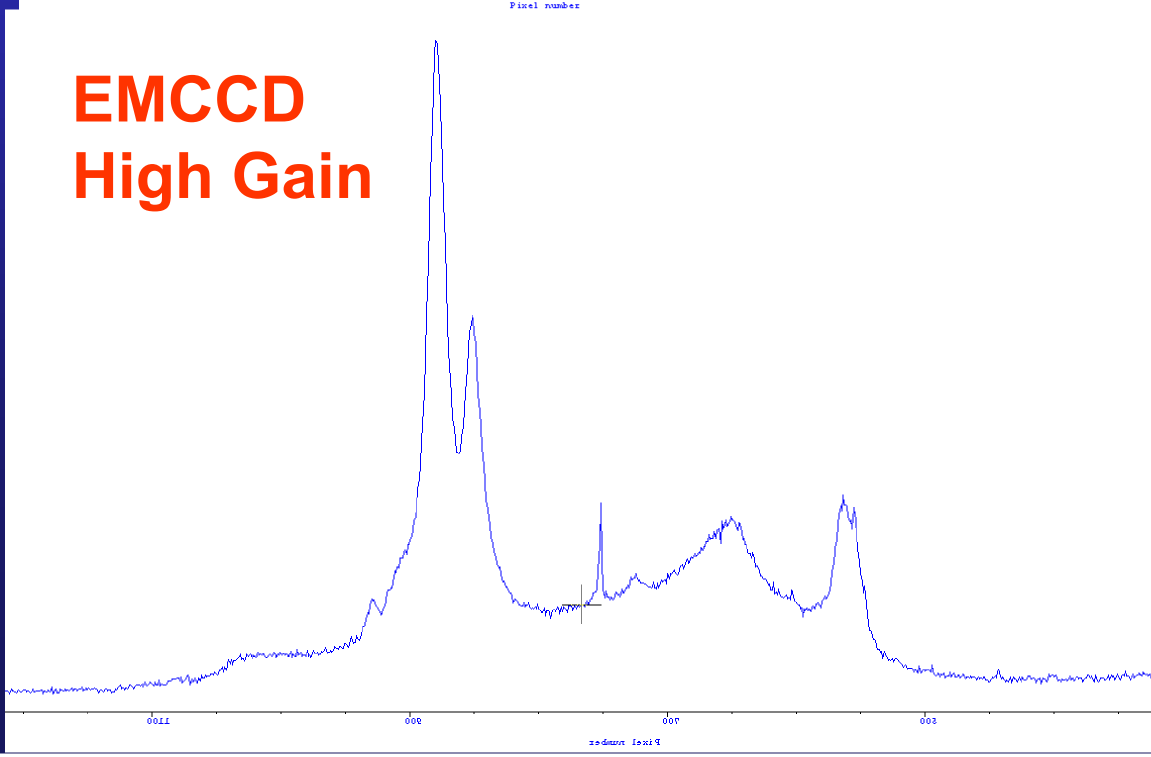
Comparison of ICCD vs EMCCD - Acetonitrile/ Toluene mixture

$\lambda_{ex.}$ = 532nm

Exposure time = <u>10s</u>

Power = 2mJ/pulse



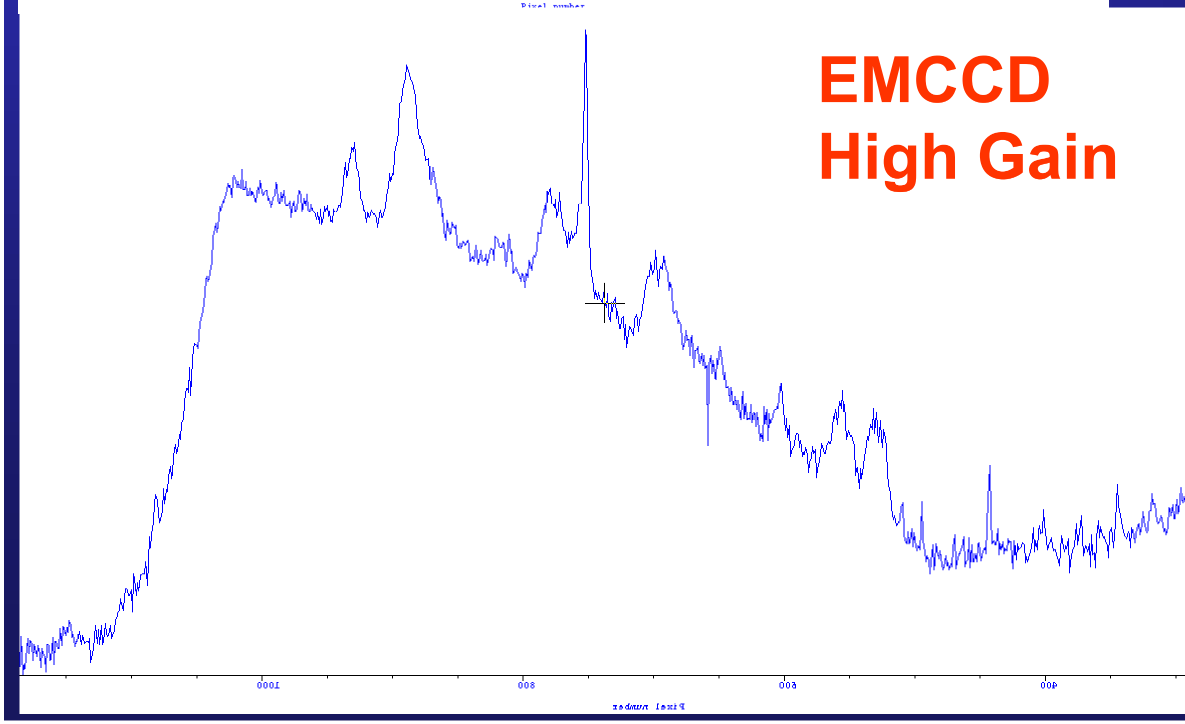


Comparison of ICCD vs EMCCD – Acetonitrile/ Toluene mixture

$\lambda_{ex.}$ = 532nm

Exposure time = 100s

Power = 2mJ/pulse



Comparison of ICCD vs EMCCD $[Ru(phen)_2dppz]^{2+}$

$\lambda_{ex.}$ = 532nm

Exposure time = <u>100s</u>

Power = 2mJ/pulse

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