Photoluminescence spectroscopy

Excited state deactivation

$A + h\nu \longrightarrow {}^{*}A$

${}^{*}A \longrightarrow B$ **photoreaction**

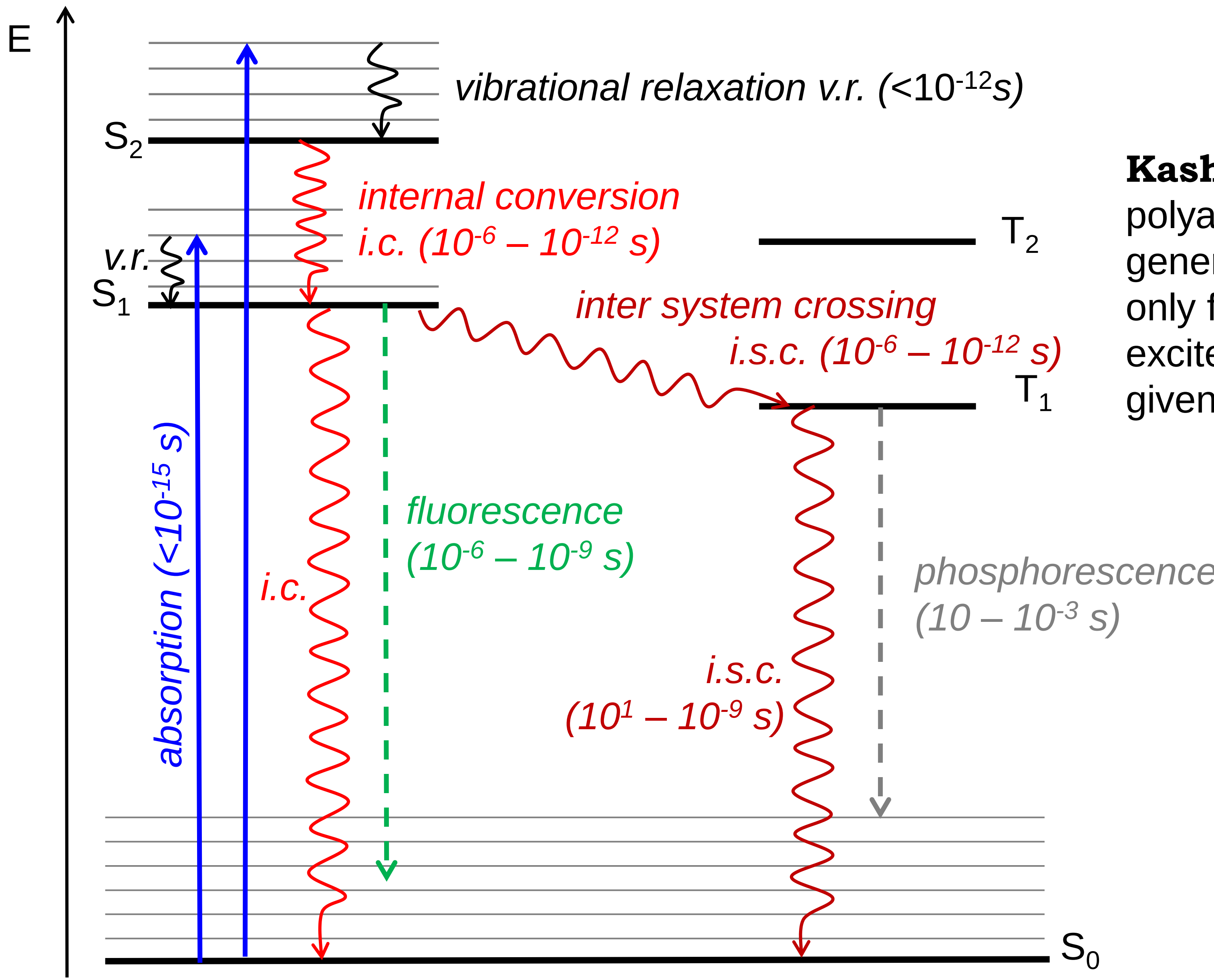
${}^{*}A \longrightarrow A + h\nu'$ **luminescence** ***(radiative deactivation)***

${}^{*}A \longrightarrow A + \text{heat}$ **degradation to heat** ***(non radiative deactivation)***

ϕ = quantum yield

$$\phi_{h\nu'} = \frac{\text{number of photons } h\nu' \text{ emitted}}{\text{number of photons } h\nu \text{ absorbed}}$$

Jablonski diagram: ***a typical organic molecule***



Kasha's rule: polyatomic molecules generally luminesce only from the lowest excited state of a given multiplicity

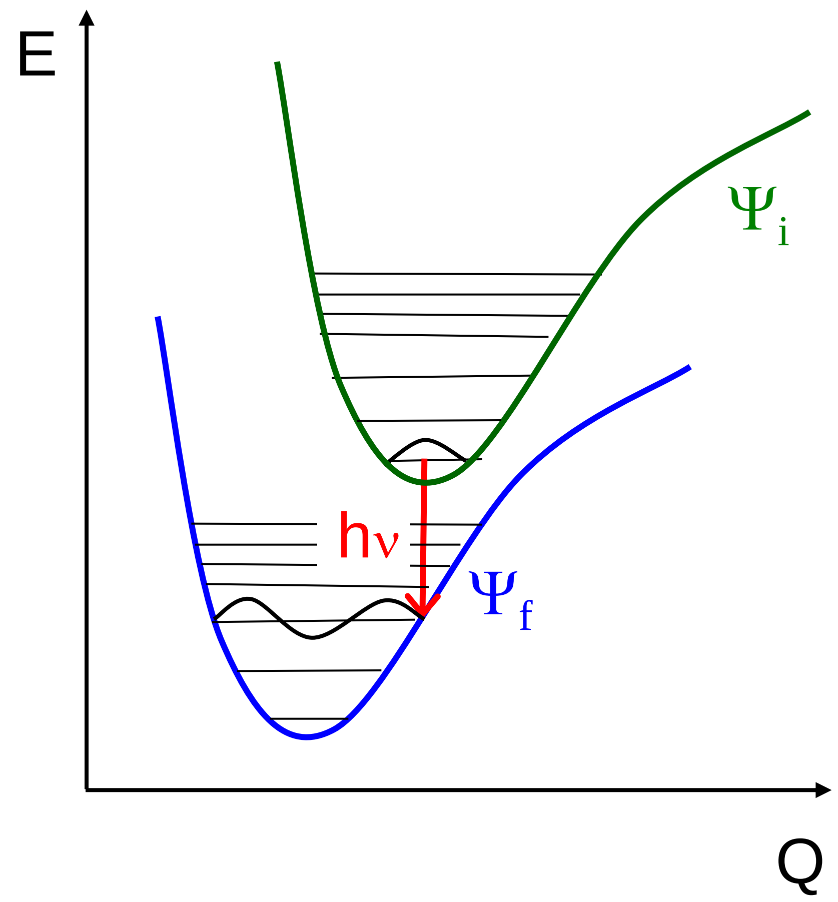
Radiative transitions

Spontaneous Emission

$$\Psi_i \rightarrow \Psi_f + h\nu$$

$$P_{em} \propto (TM)^2\ \nu^3$$

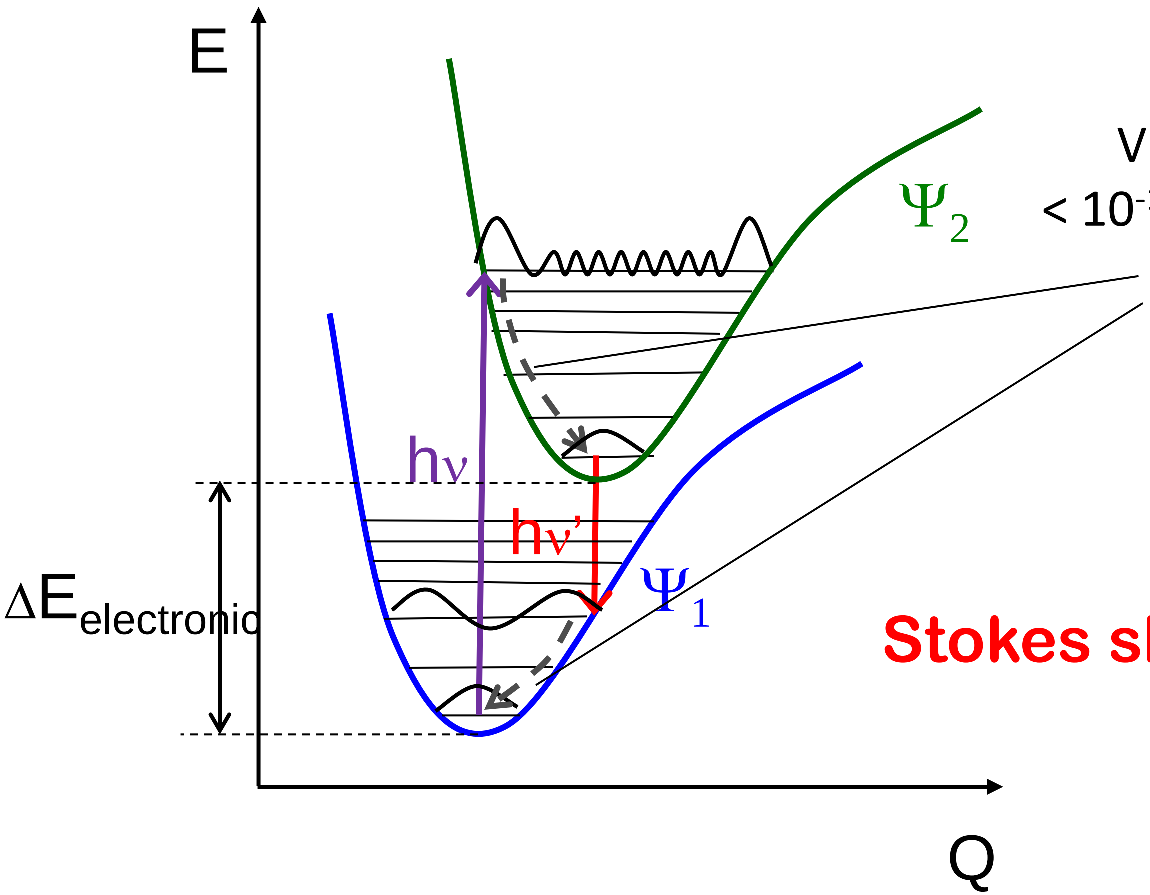
emission probability



fluorescence: spin-allowed radiative transition

phosphorescence: spin-forbidden radiative transition

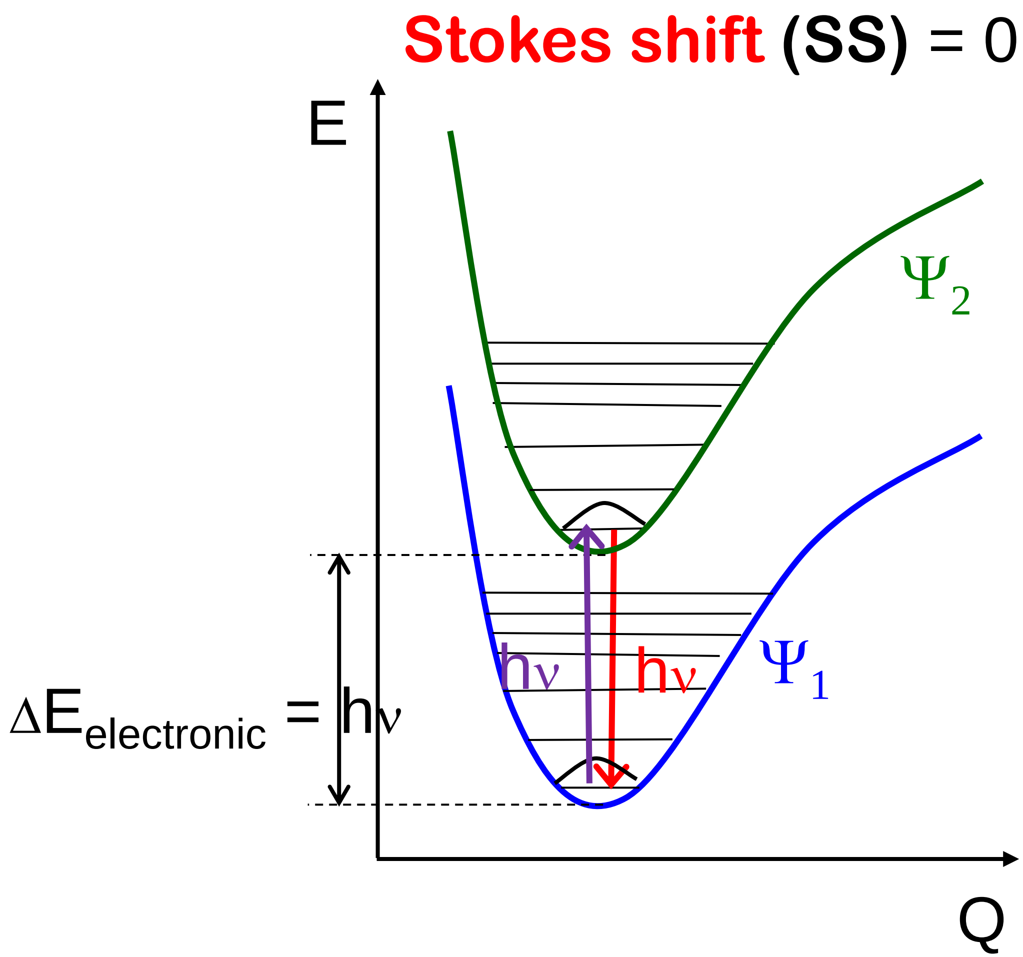
Excited state distortion



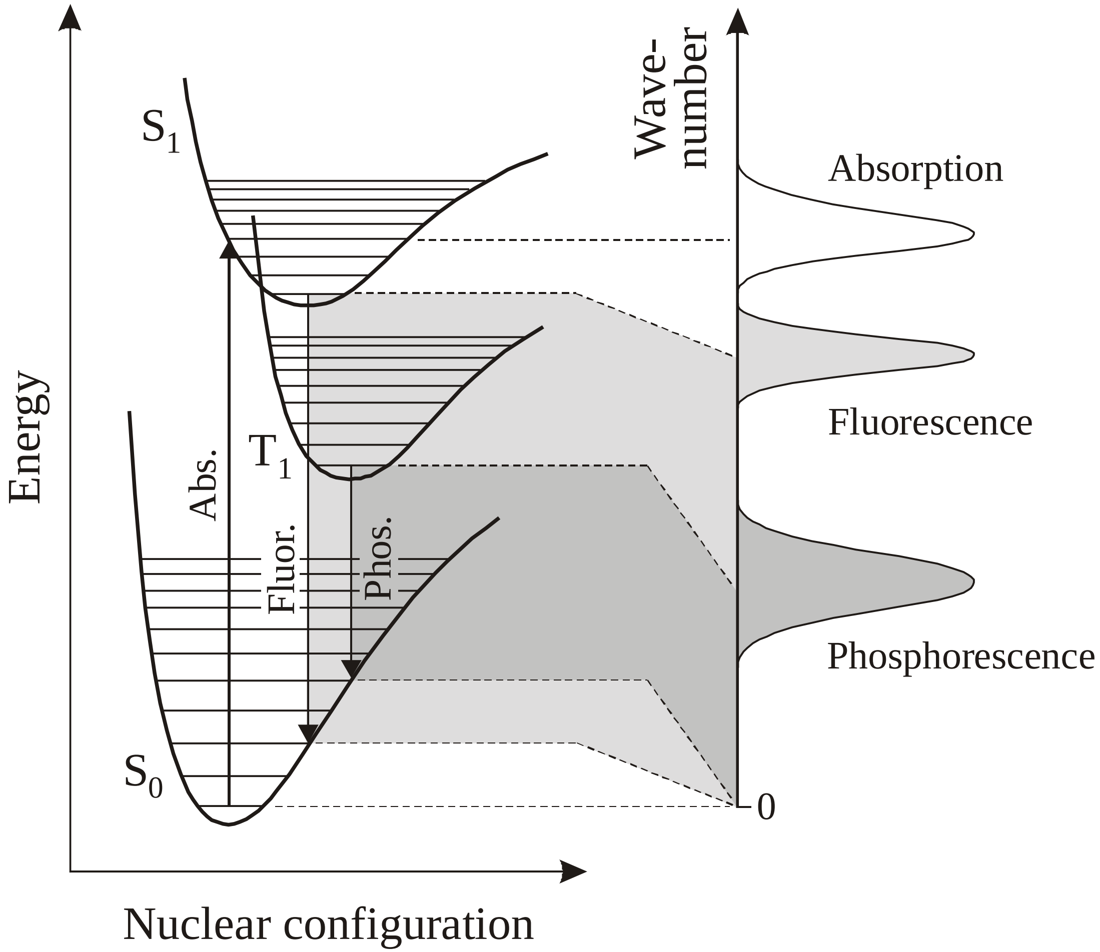
Vibrational relaxation
$< 10^{-12}$ s in condensed phase

Stokes shift (SS) = hν – hν'

Zero-distorsion

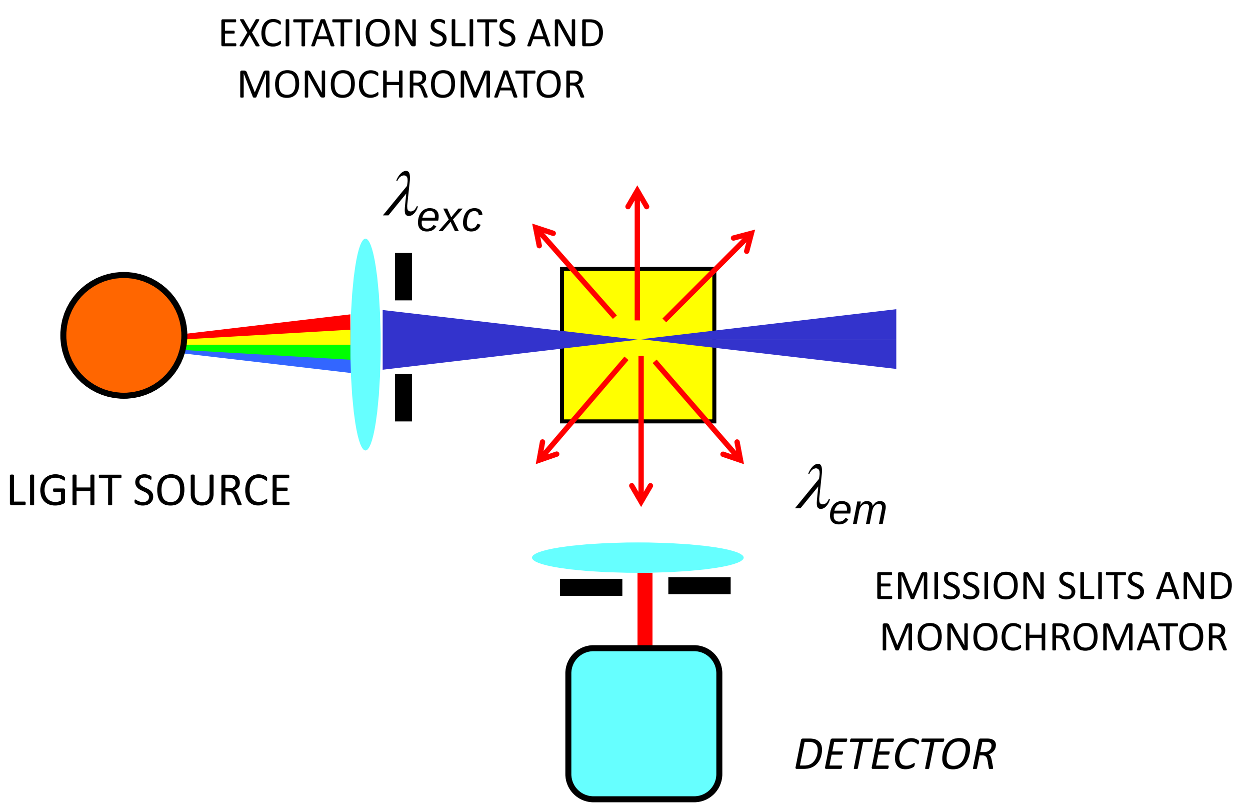


Absorption, fluorescence and phosphorescence bands



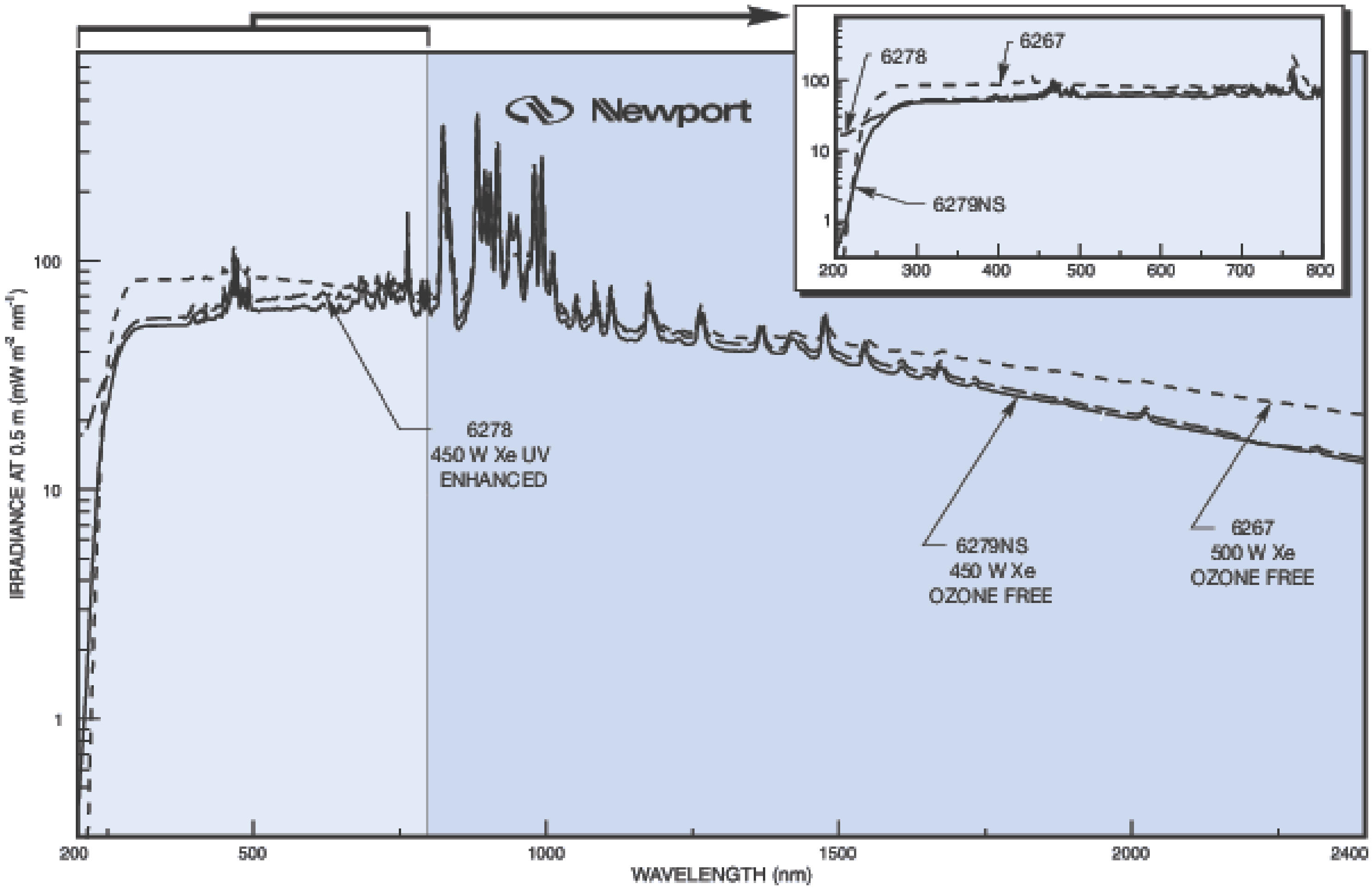
SPECTROFLUORIMETER

(Luminescence spectra)

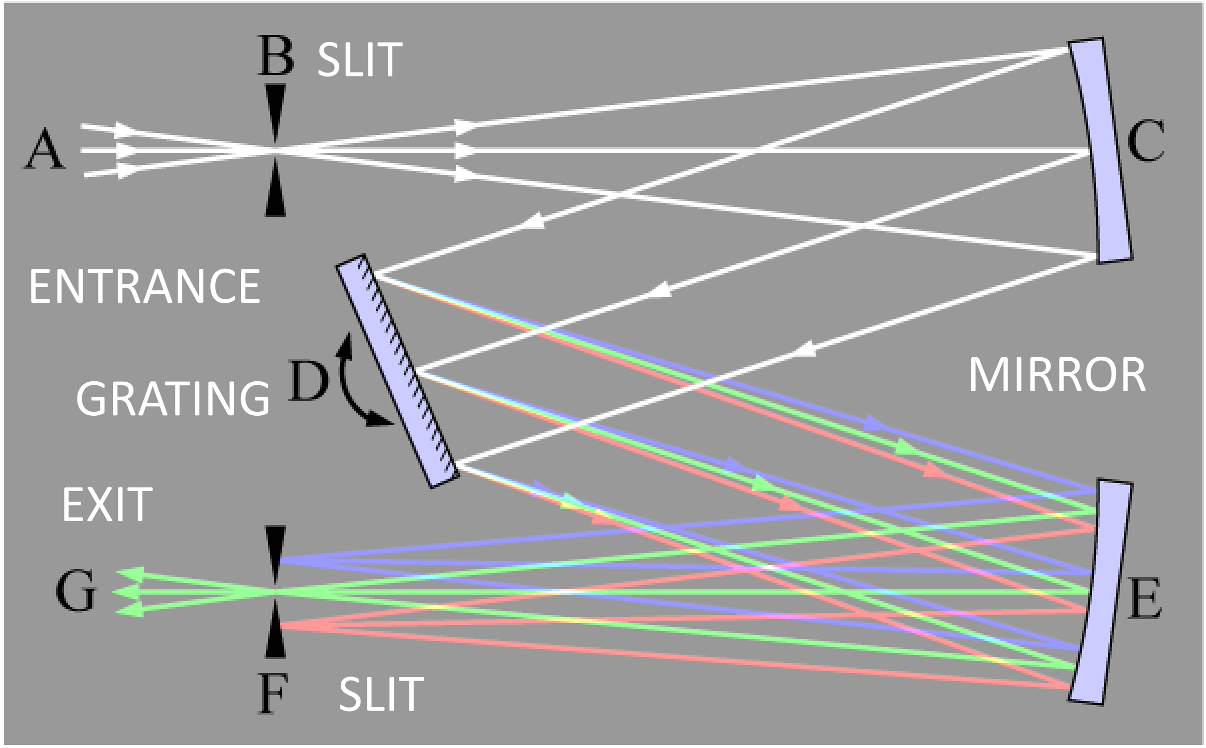


LIGHT SOURCE

Xe lamp (450W)



Monochromator

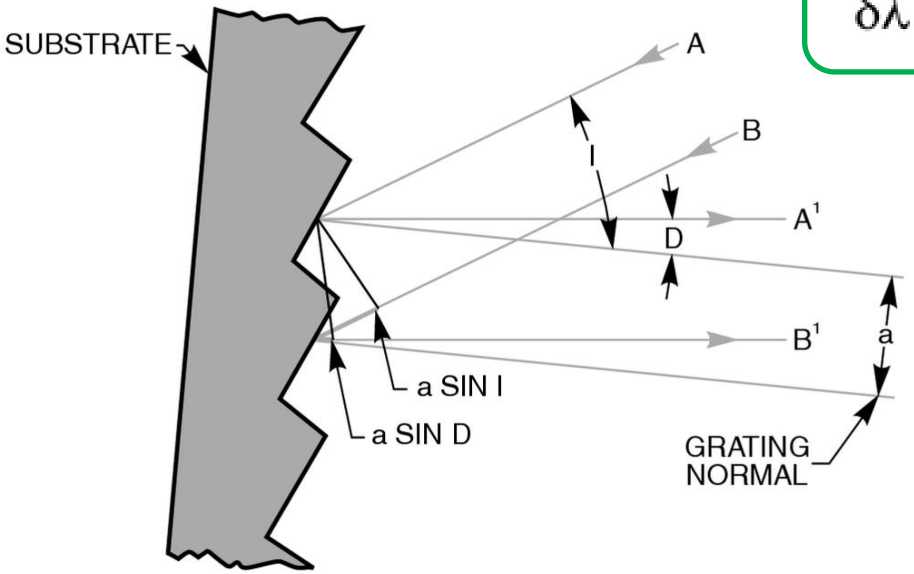


Grating: constructive interference

$$a(\sin I + \sin D) = m\lambda$$

dispersion

$$\frac{\delta D}{\delta \lambda} = \frac{m}{a \times \cos D}$$



a size of the reflecting element (groove)

m integer number indicating the order of interference

Grating: interferenza costruttiva

Alternative equation

$$\boxed{m\lambda = 2\,a\cos\phi\,\sin\theta}$$

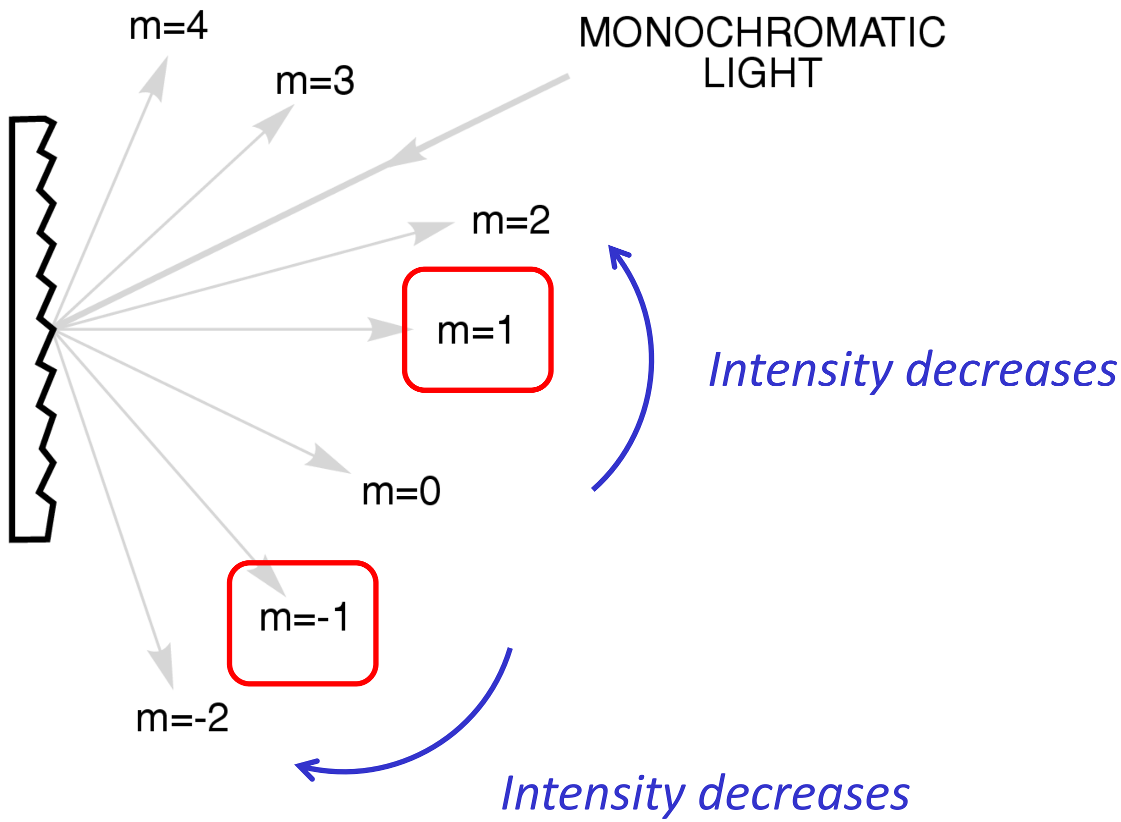
ϕ = Half the included angle between the incident ray and the diffracted ray at the grating

θ = Grating angle relative to the zero order position

$$\boxed{I = \theta + \phi \text{ and } D = \theta - \phi}$$

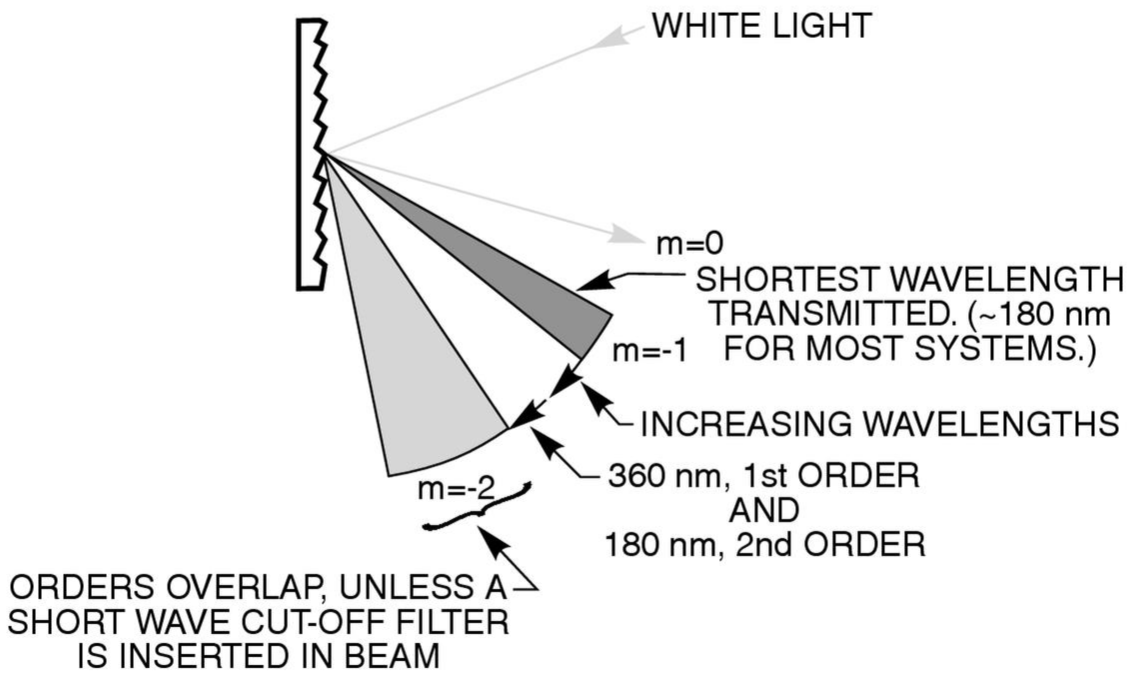
Grating: orders

$$a(\sin I + \sin D) = m\lambda$$

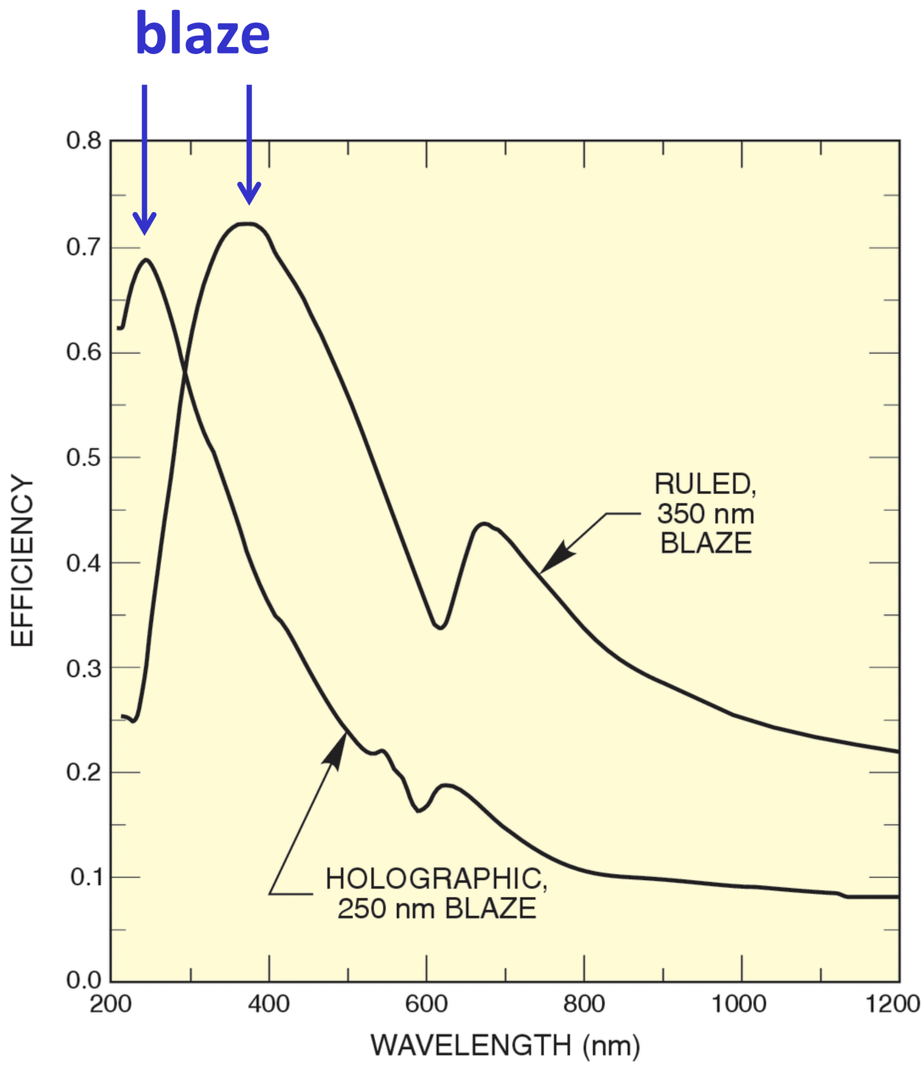


Grating: le armoniche

$$a(\sin I + \sin D) = m\lambda$$



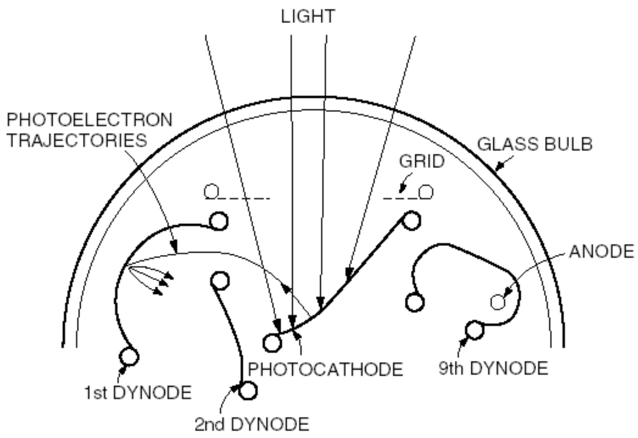
Grating: spectral efficency

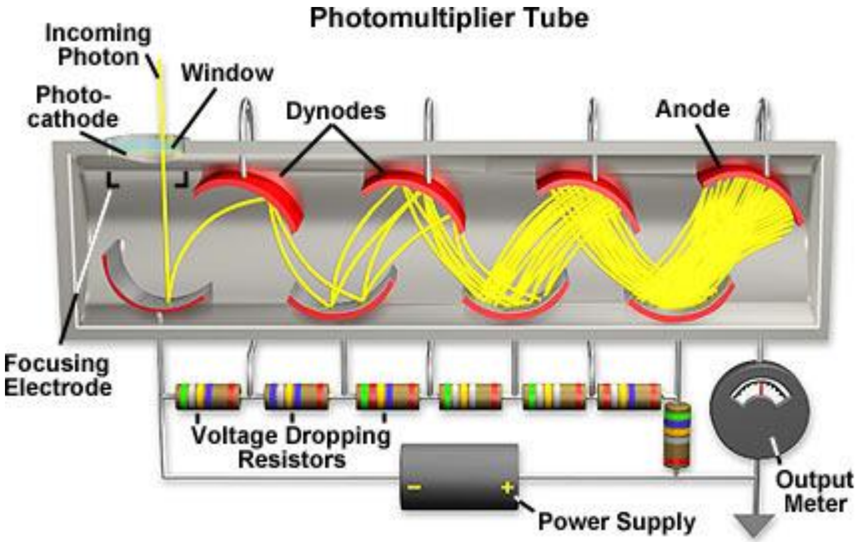


Detector: PhotoMultiplier Tube (PMT)

Hamamatsu R928

Figure 1: Electro Optical Structure

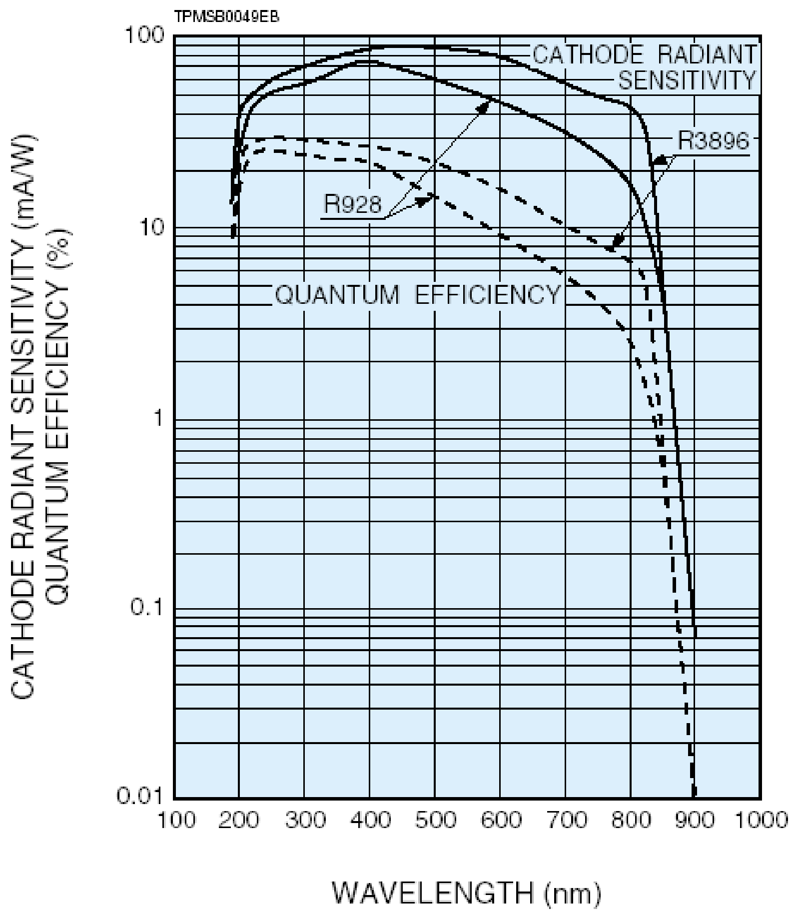




PhotoMultiplier Tube (PMT)

Spectral efficiency

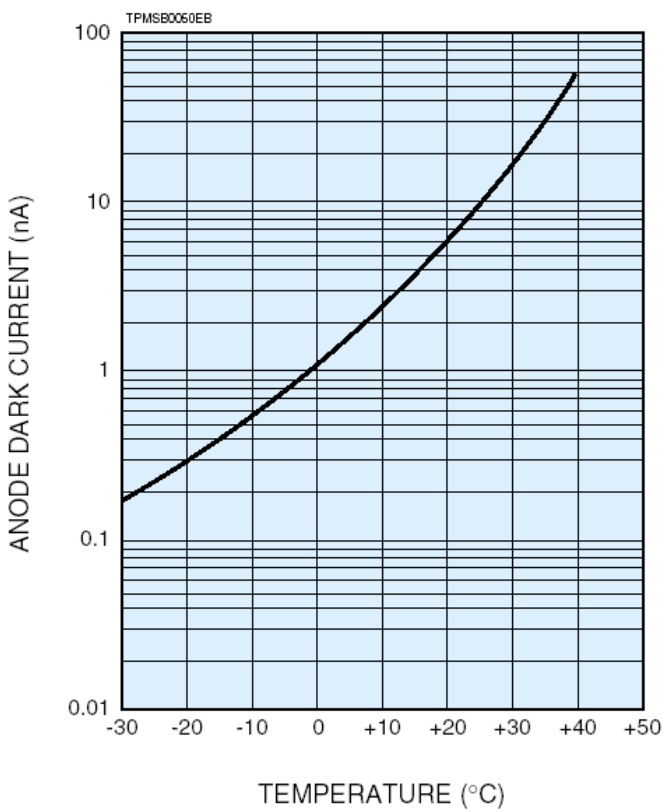
Figure 2: Typical Spectral Response



PhotoMultiplier Tube (PMT)

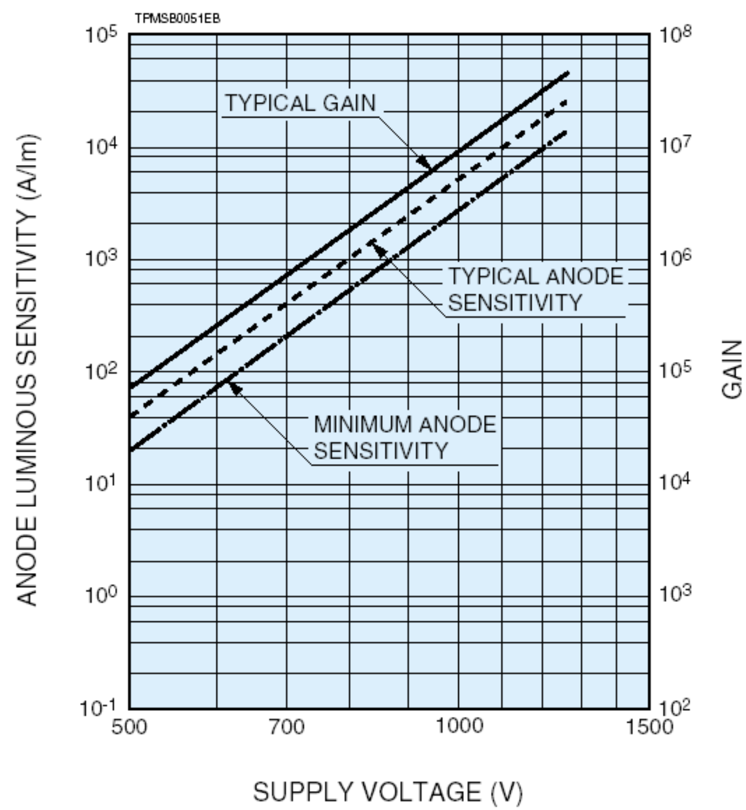
Dark current/Temperature

Figure 3: Typical Temperature Characteristics of Dark Current (at 1000 V, after 30 min storage in darkness)



Signal/Voltage

Figure 4: Anode Luminous Sensitivity and Gain Characteristics



Modular fluorimeter (FL920 Edinburgh)

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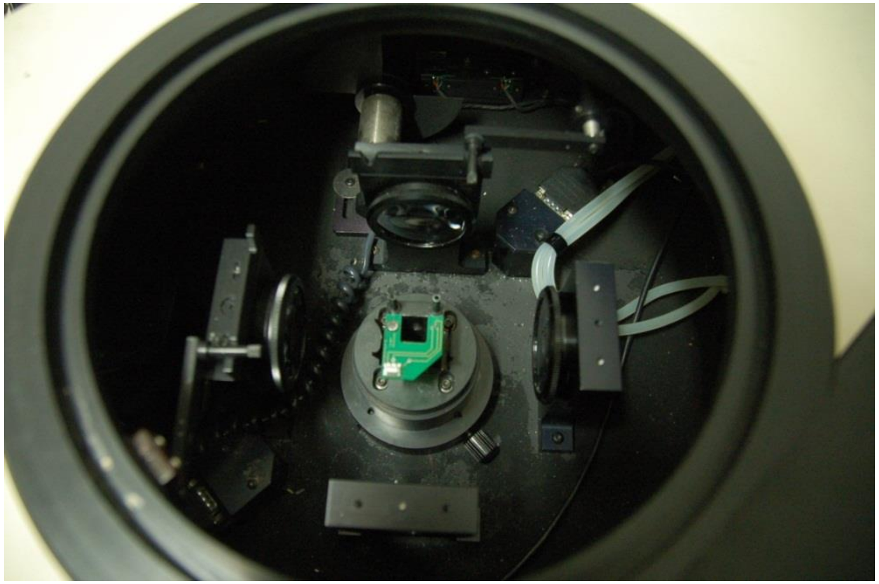

Compact fluorimeter (Perkin-Elmer LS 55)

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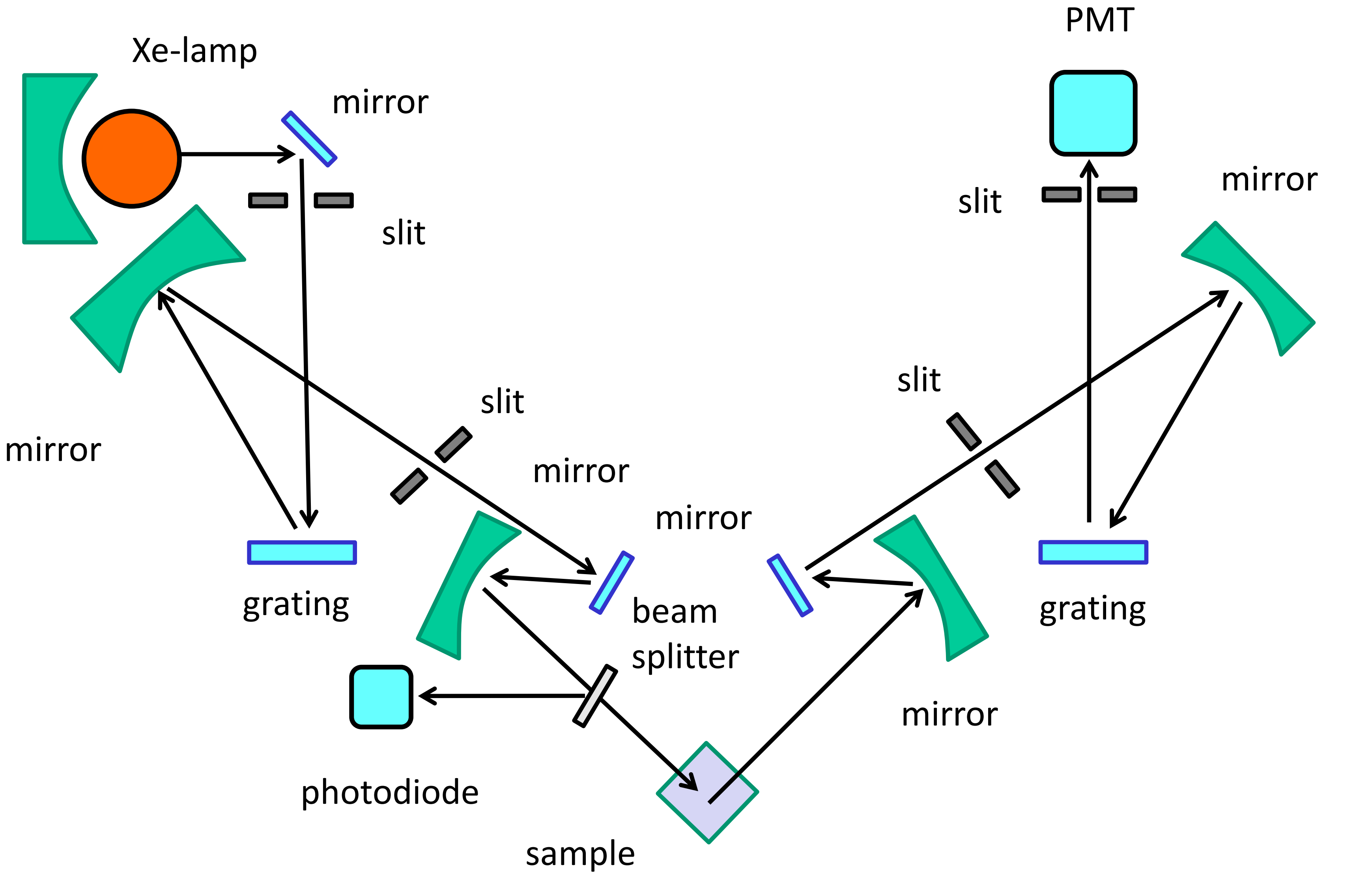


Parameters to be set (general)

Lamp Mode: fluorescence / phosphorescence

Built-in filters: Selection of the excitation / emission filters present inside the instrument

PMT voltage: from 650 to 900 volts.

By increasing the voltage sensitivity increases

If the voltage is too high, the system goes off the scale (saturation)

Parameters to be set (scanning)

mode: emission, excitation

λ excitation / emission: Depends on the properties of fluorophore

Spectral range: λ_{max}, λ_{min} in nm

based on the properties of fluorophore

Scan speed: in nm /min

Increasing the speed the resolution of the spectrum worsens
Increase the noise
For the same spectral range the acquisition time decreases

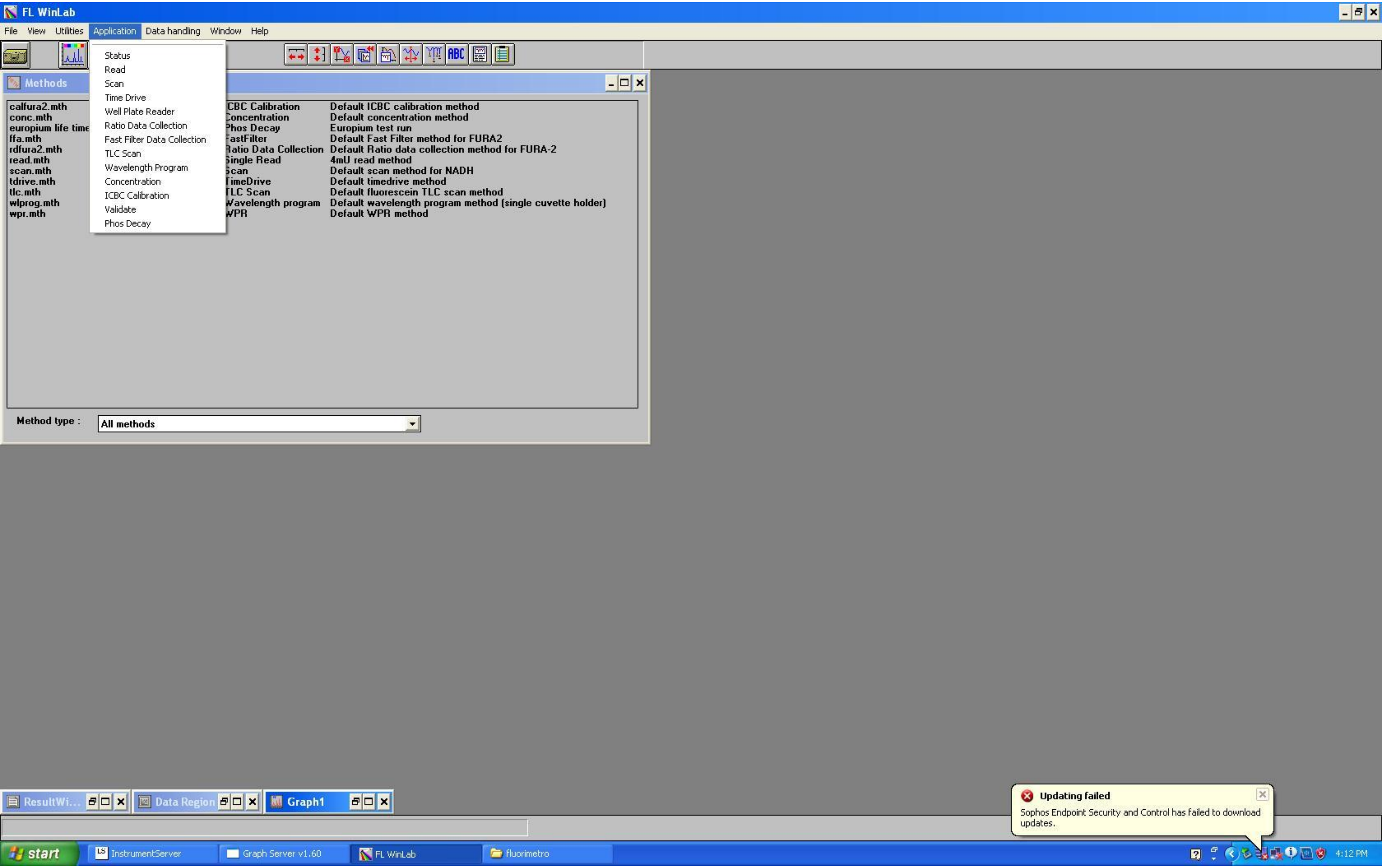
Parameters to be set (scanning)

Excitation slit: in nm

It is expressed as a passband
Increasing the slit the resolution of the spectrum of excitation decreases
By increasing the slit the measured signal does not change (in case of photodiode correction) but decreases the noise

Emission slit: in nm

It is expressed as a passband
Increasing the slit the resolution of the emission spectrum decreases
By increasing the slit the signal increases


FL WinLab
File View Utilities Application Data handling Window Help
Status
Read
Scan
Time Drive
Well Plate Reader
Ratio Data Collection
Fast Filter Data Collection
TLC Scan
Wavelength Program
Concentration
ICBC Calibration
Validate
Phos Decay
Methods
calfura2.mth
conc.mth
europium life time
ffa.mth
rdfura2.mth
read.mth
scan.mth
tdrive.mth
tlc.mth
wlprog.mth
wpr.mth
Default ICBC calibration method
Default concentration method
Europium test run
Default Fast Filter method for FURA2
Default Ratio data collection method for FURA-2
4mU read method
Default scan method for NADH
Default timedrive method
Default fluorescein TLC scan method
Default wavelength program method (single cuvette holder)
Default WPR method
Method type :
All methods
ResultWi...
Data Region
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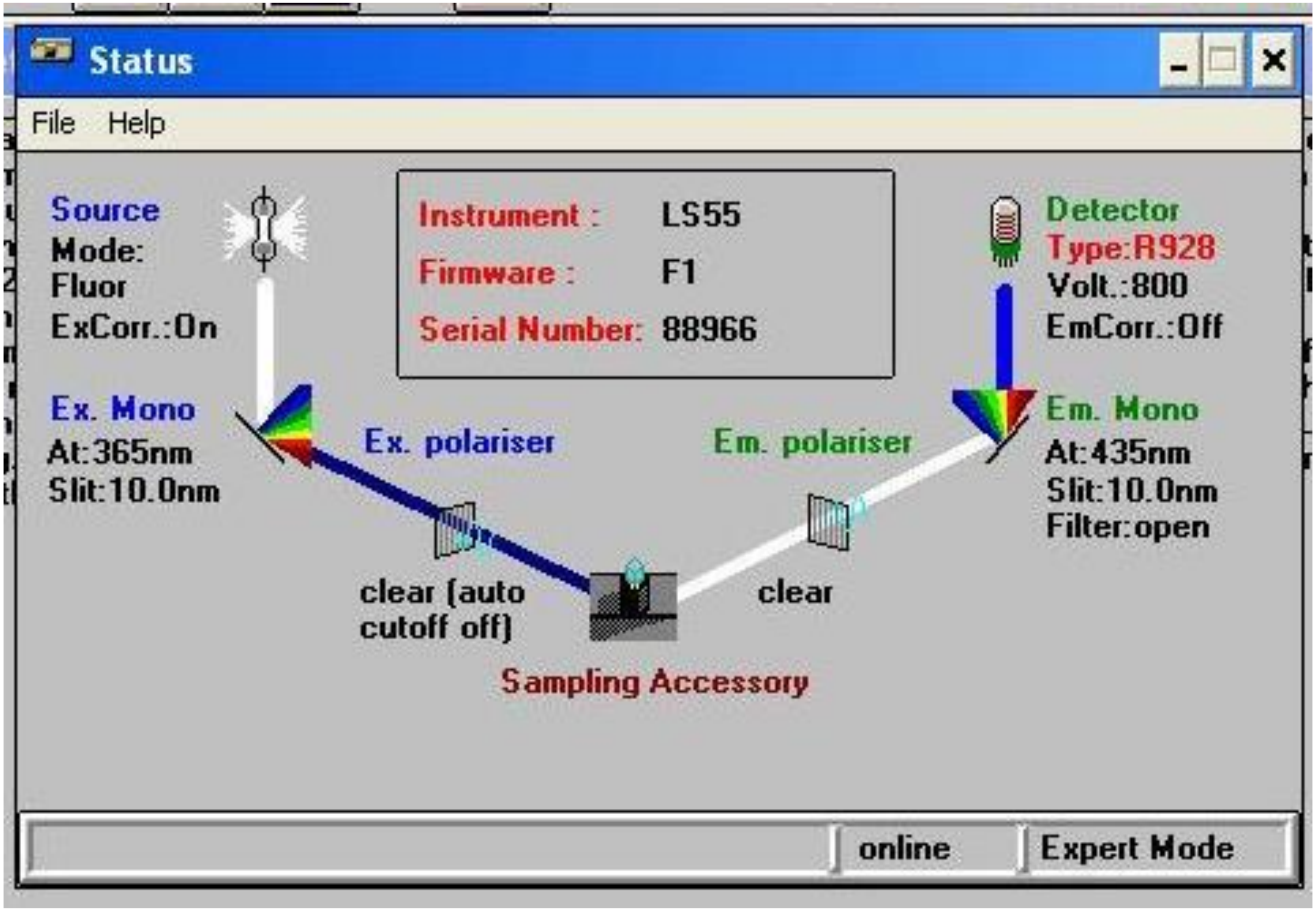
Sophos Endpoint Security and Control has failed to download updates.
start
InstrumentServer
Graph Server v1.60
FL WinLab
fluorimetro
4:12 PM


Status
File Help
Source
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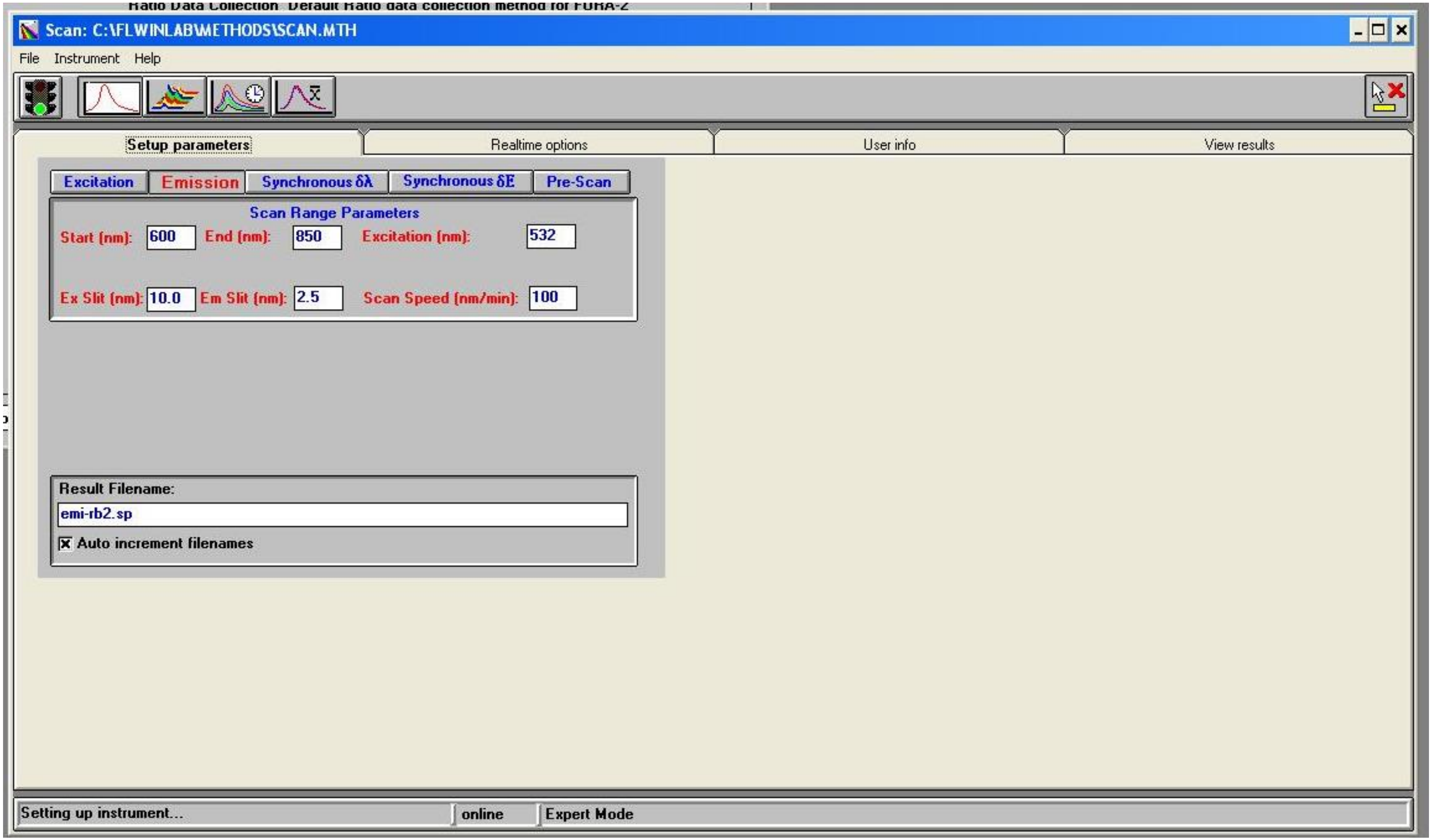
ExCorr.:On
Instrument : LS55
Firmware : F1
Serial Number: 88966
Detector
Type:R928
Volt.:800
EmCorr.:Off
Ex. Mono
At:365nm
Slit:10.0nm
Ex. polariser
Em. polariser
Em. Mono
At:435nm
Slit:10.0nm
Filter:open
clear (auto
cutoff off)
clear
Sampling Accessory
online
Expert Mode



Ratio Data Collection Default Ratio data collection method for FURA-2
Scan: C:\FLWINLAB\METHODS\SCAN.MTH
File
Instrument
Help
Setup parameters
Realtime options
User info
View results
Excitation
Emission
Synchronous δλ
Synchronous δE
Pre-Scan
Scan Range Parameters
Start (nm): 600
End (nm): 850
Excitation (nm): 532
Ex Slit (nm): 10.0
Em Slit (nm): 2.5
Scan Speed (nm/min): 100
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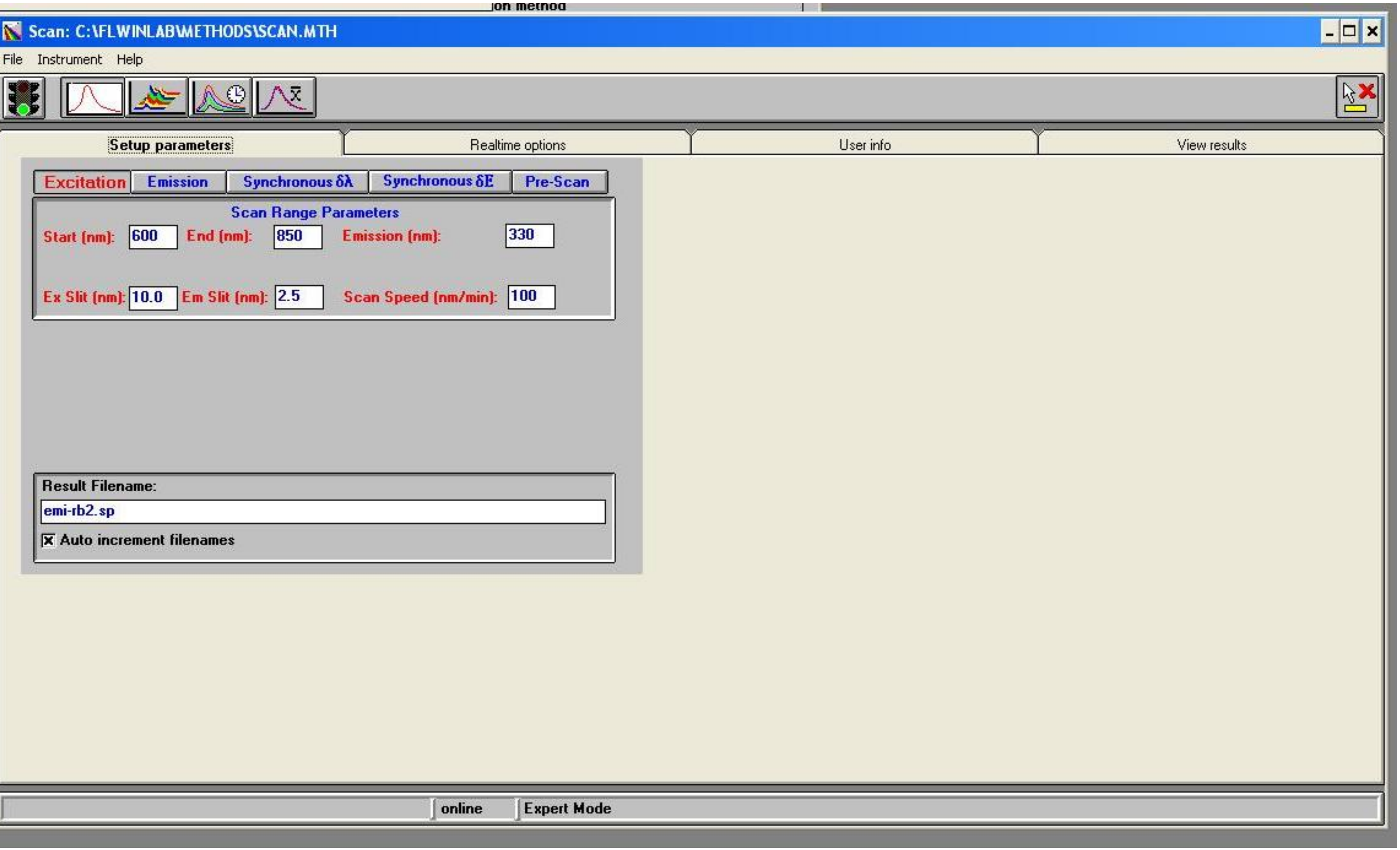
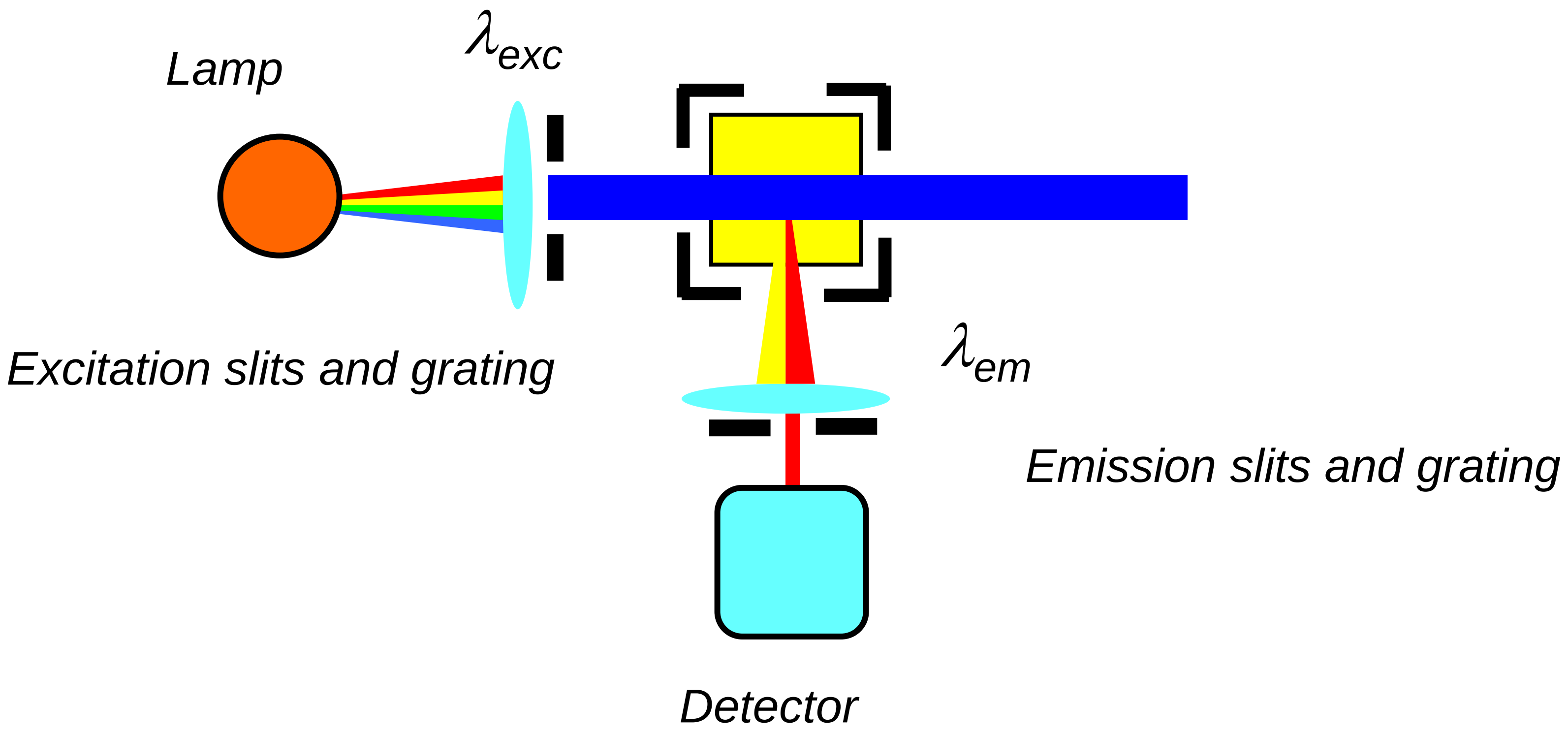
Auto increment filenames
Setting up instrument...
online
Expert Mode



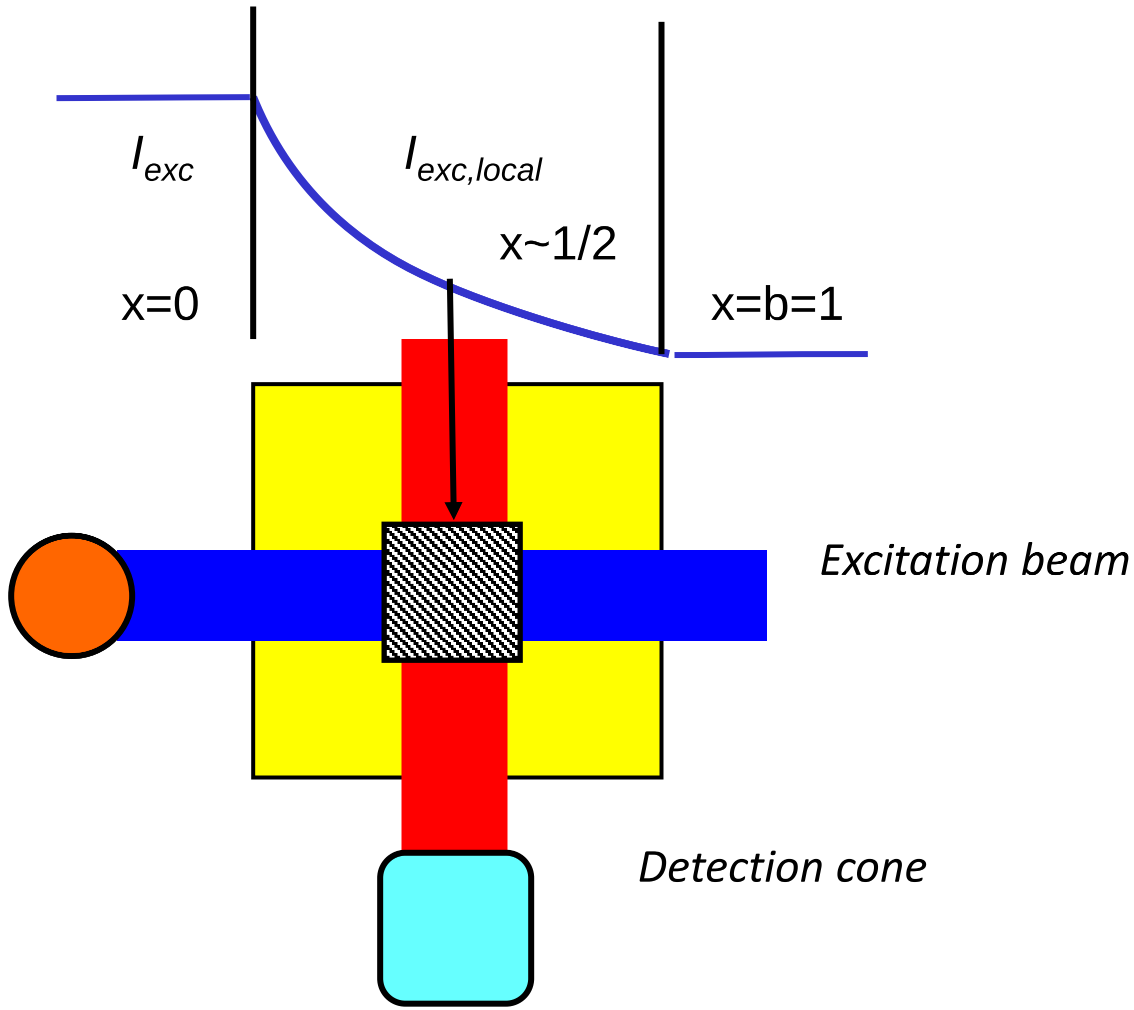
on method
Scan: C:\FLWINLAB\METHODS\SCAN.MTH
File
Instrument
Help
Setup parameters
Realtime options
User info
View results
Excitation
Emission
Synchronous δλ
Synchronous δE
Pre-Scan
Scan Range Parameters
Start (nm): 600
End (nm): 850
Emission (nm): 330
Ex Slit (nm): 10.0
Em Slit (nm): 2.5
Scan Speed (nm/min): 100
Result Filename:
emi-rb2.sp
Auto increment filenames
online
Expert Mode

Dependency of the intensity on concentration

Mask are used In ordr to minimize scattering from the cuvette corners.
Emission is detected at 90° with respect to excitation focussing on the center of the cuvette.



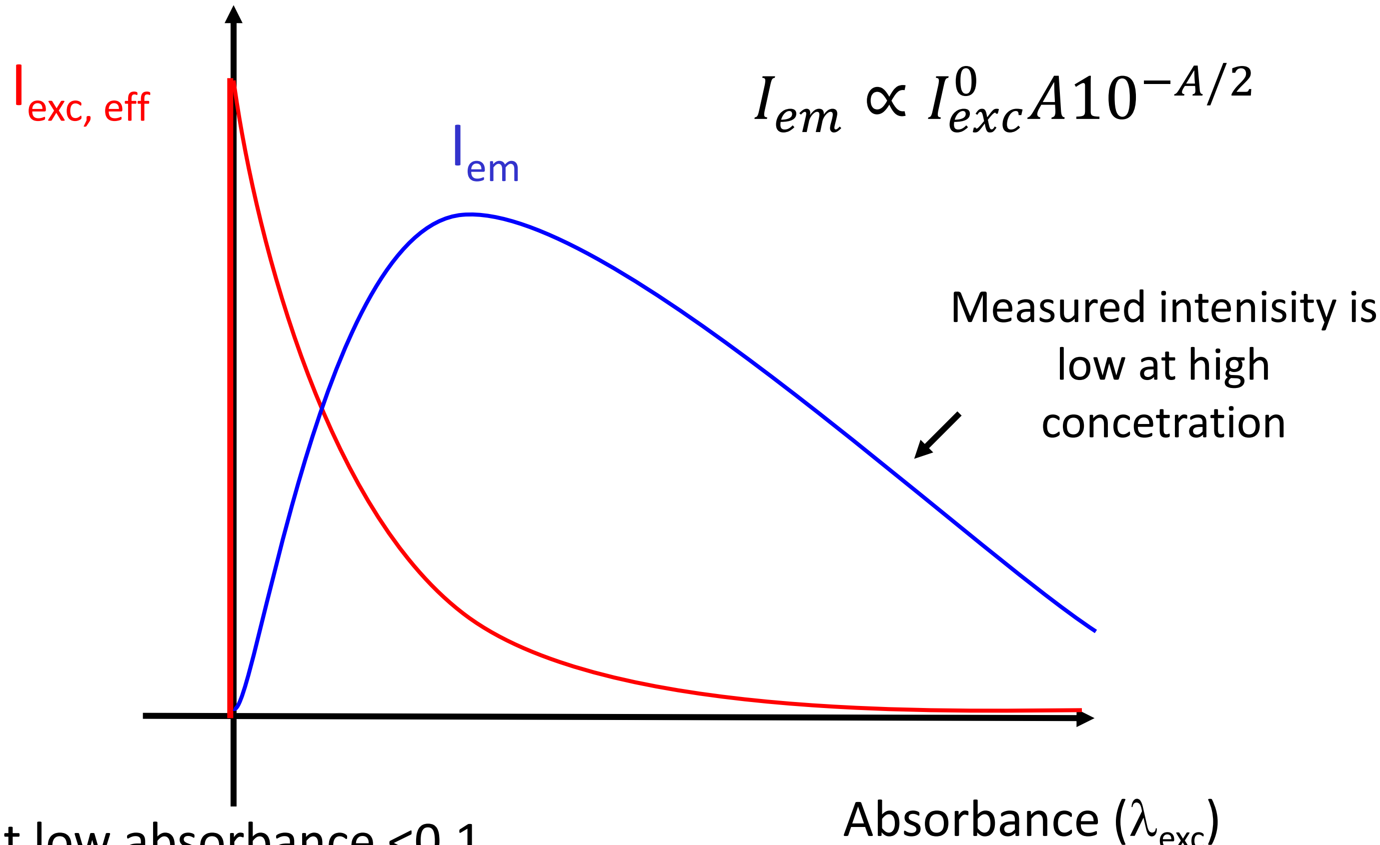
Dependency of the intensity on concentration



$$I_{exc}(x) = I_{exc}^{0} 10^{-Ax}$$

$$d[A^*] \propto -dI_{exc}(x) \propto I_{exc}^{0} A 10^{-Ax}$$

Dependency of the intensity on concentration



At low absorbance <0.1

$$I_{em} \propto A$$

Correction of fluorescence will be treated in detatil by Prof. Silvi