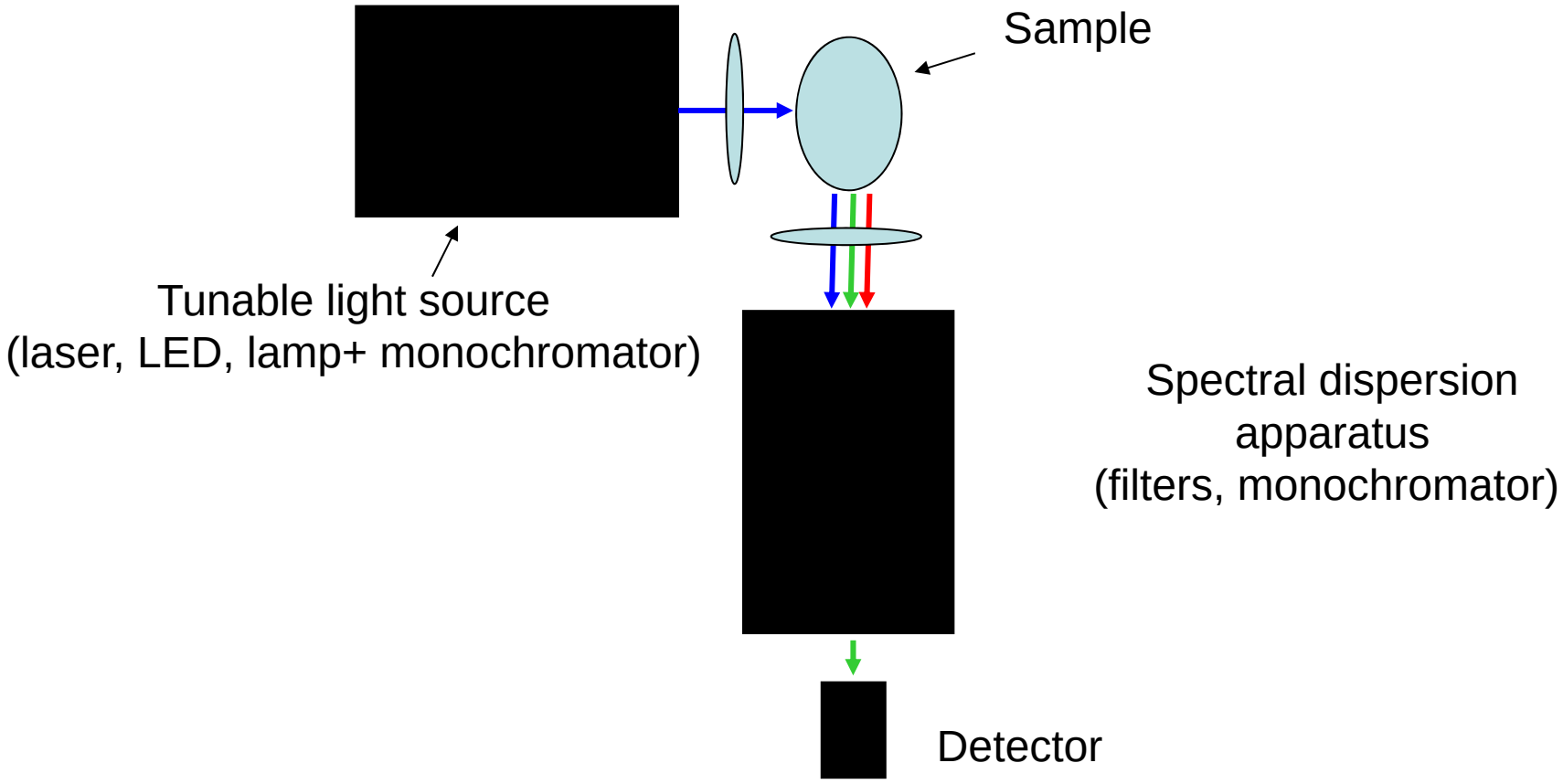
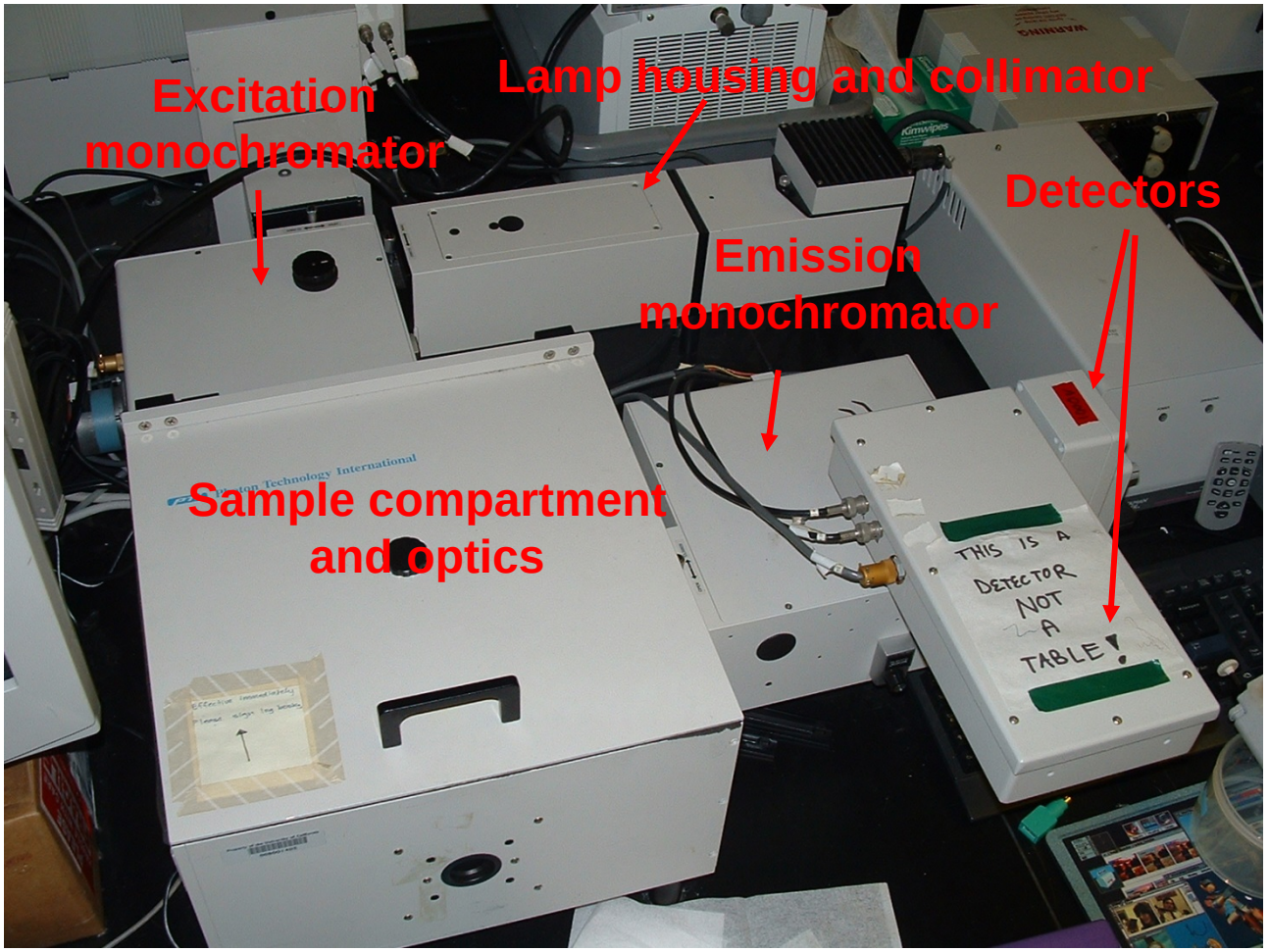
Good Practices of Fluorescence Spectroscopy

General layout of a fluorimeter

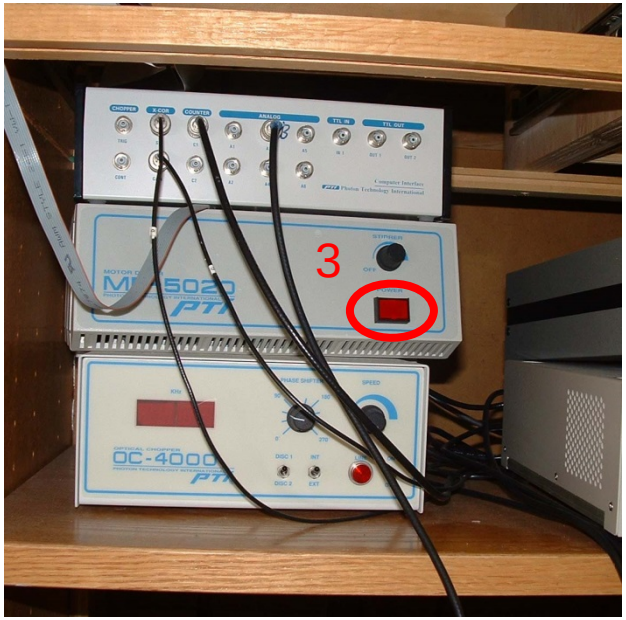


PTI Fluorimeter



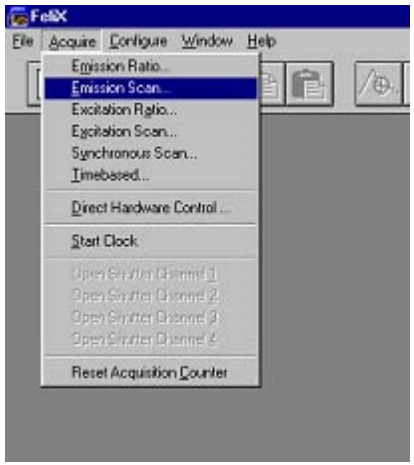
Startup procedure I

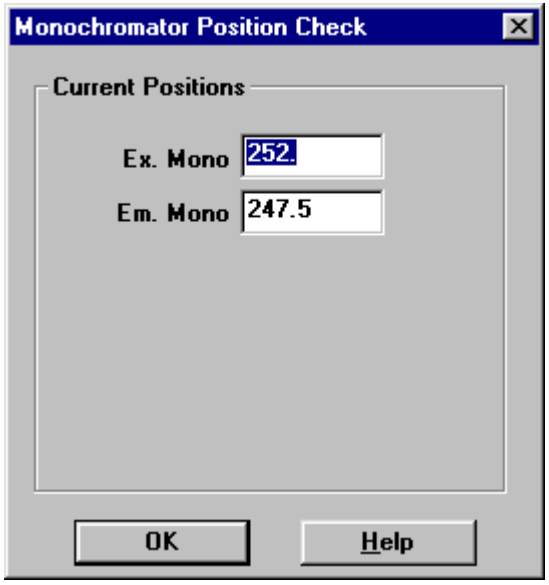
1. Turn on Xe lamp power supply.
2. Ignite Xe lamp.
3. Turn on instrument controller box
4. Start Felix software
5. Warm up the lamp. (~ 10-15 minutes)
   The lamp must be set to 72W after warmup period. Even small deviations of the lamp power reduce its life-time
5. For shutdown reverse the steps.



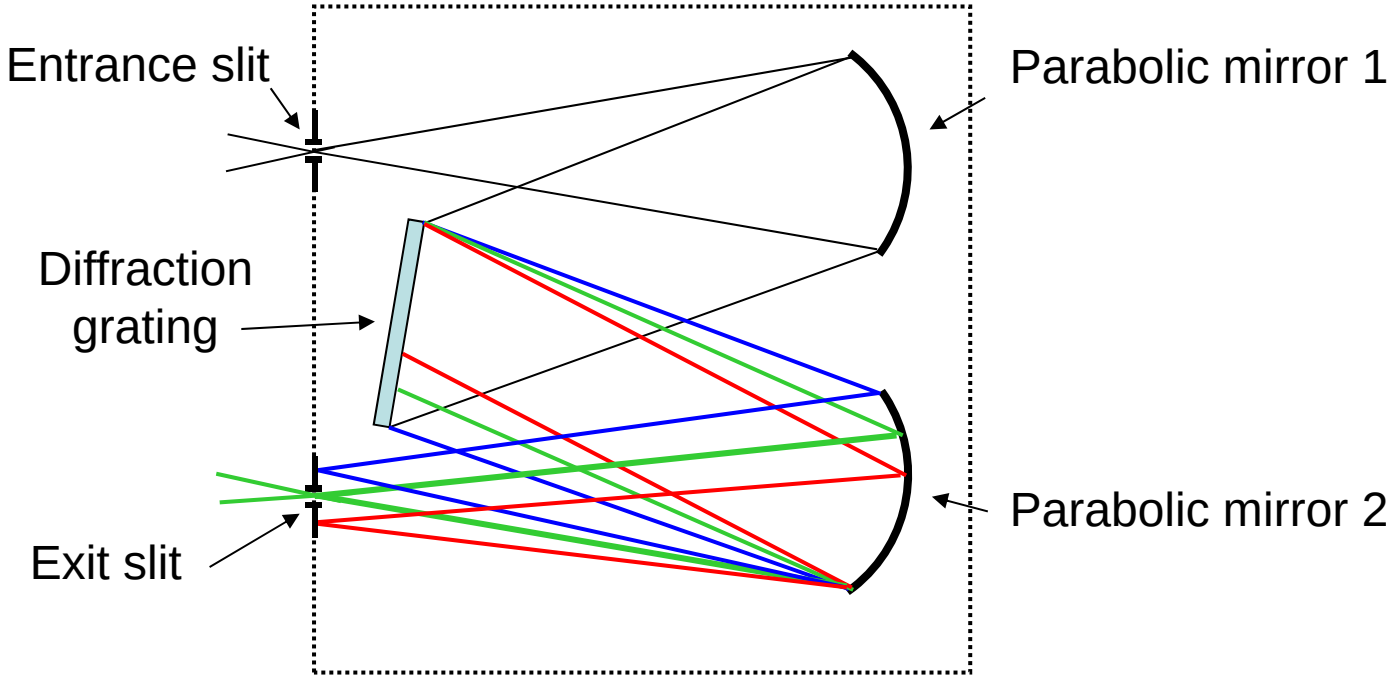
Startup procedure 2

1. Select the type of experiment
2. Input wavelength from monochromators counters into the program





Cherny-Turner Monochromator



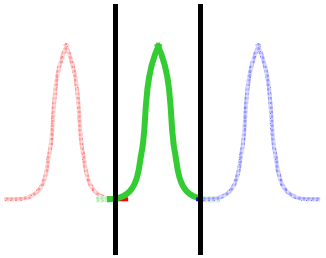
$$d(\sin\theta_m(\lambda) - \sin\theta_i) = m\lambda$$

- θ_i – angle of incidence, θ_m – angle of diffraction, d – grating pitch, m – an integer number, λ – wavelength of light.
- Tuning of the output wavelength is done by rotation of the grating (θ_i)
- Imaging system

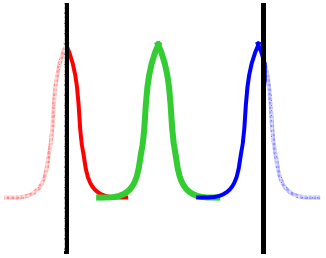
Do we need slits? I

Exit slit

- Exit slit determines spectral resolution of the instrument
- Resolution is determined by the product of the monochromator linear dispersion (nm/mm) and the slit width
- Monochromator resolution depends on the grating pitch and the (focal) length of the monochromator
- For PTI fluorimeter with 1200 LPM grating dispersion is 4 nm/mm.
- 1 turn of the slit micrometer = 0.5 mm slit opening = 2 nm.
- **180º = 1 nm (closes clockwise)**
- **Completely open slit = 25 nm**
- **Use it wisely!!!**



Good

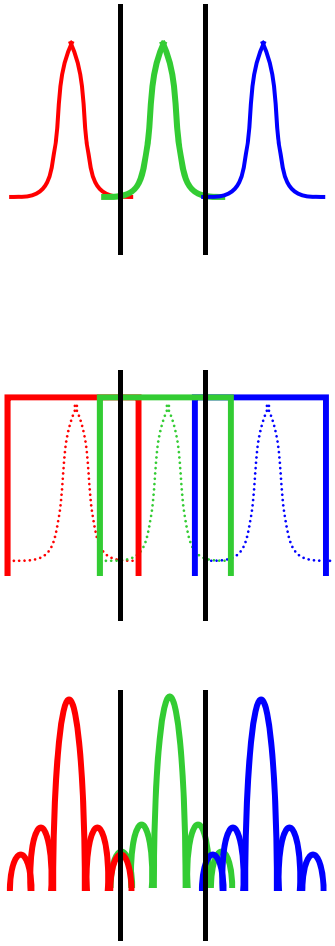


Bad

Why do we need slits? II

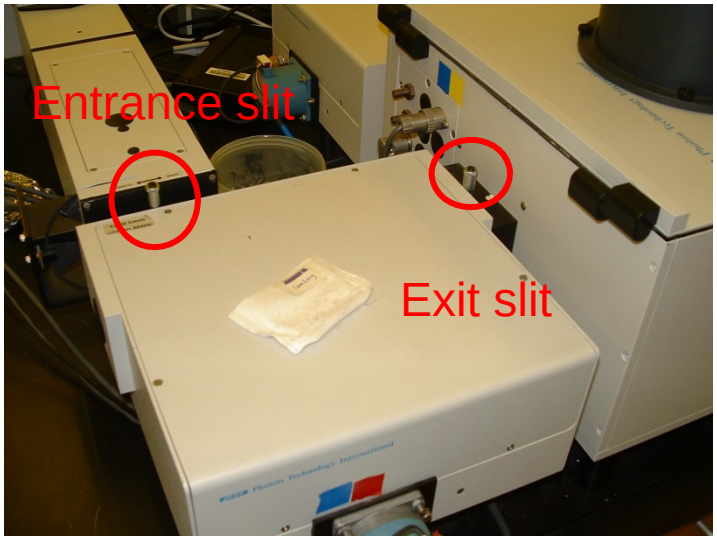
Entrance slit

1. Determines the amount light getting into the monochromator (and onto the detector!)
2. Reduces amount of the stray light
3. Affects spectral resolution
   - Too wide – reduces resolution due to imaging effects
   - Too narrow – diffraction effects reduce resolution
   - Optimal setting – normal slit width (~ 25 μ for PTI machine), rarely used.

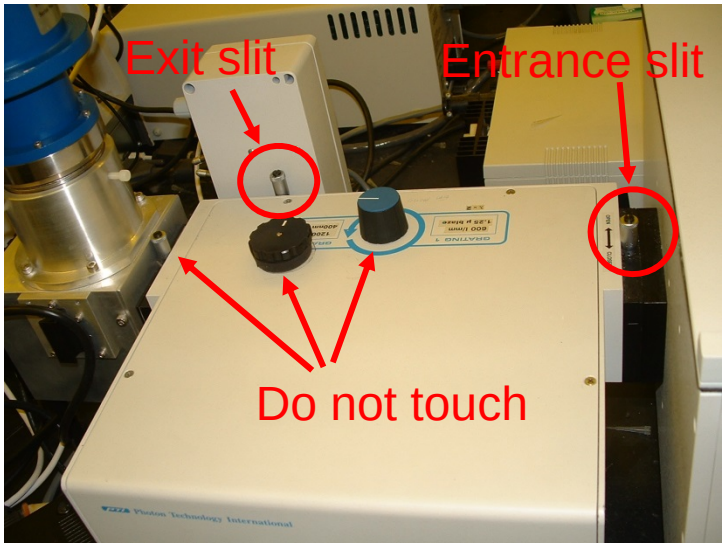


Practical Recipe for Setting Slits

1. Use the narrowest possible slit.
2. Set entrance and exit slits to the same width. (Monochromator magnification is equal to 1!).
3. If the spectral resolution is not that important (most of organic materials have broad emission and absorption bands), set the slits widths using fluorescence signal magnitude as a reference (discussed later).

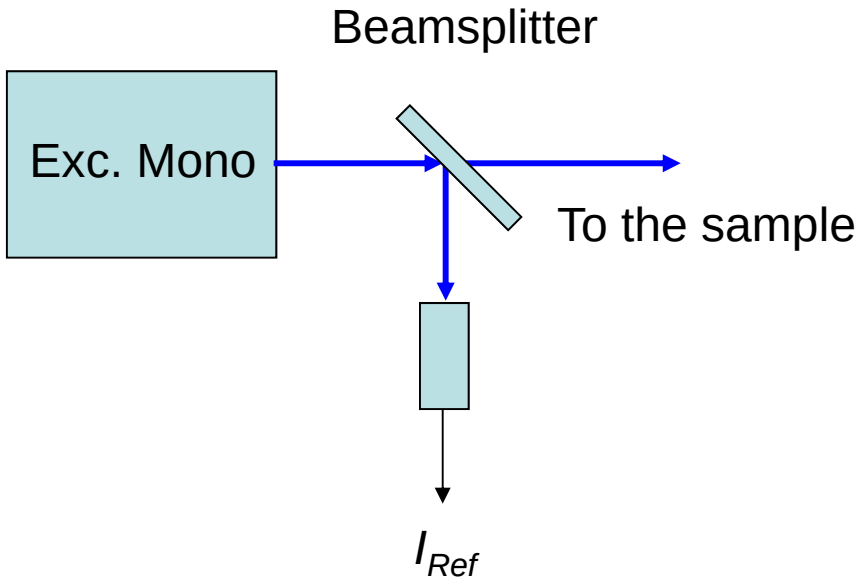


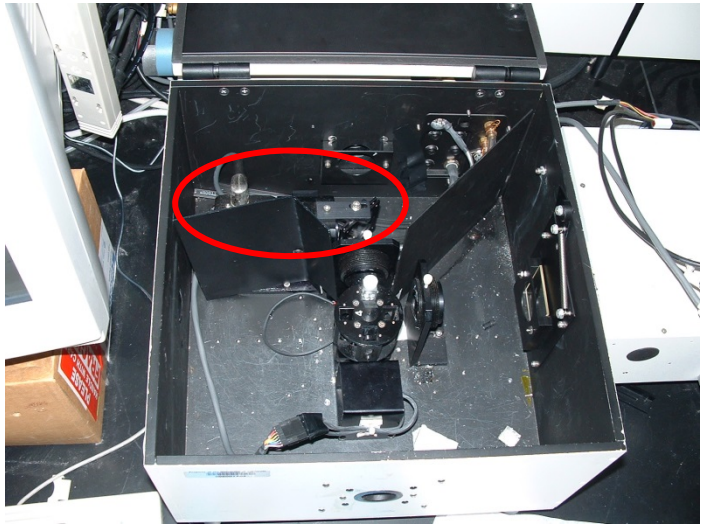
Excitation mono



Emission mono

Setting up Excitation Mono



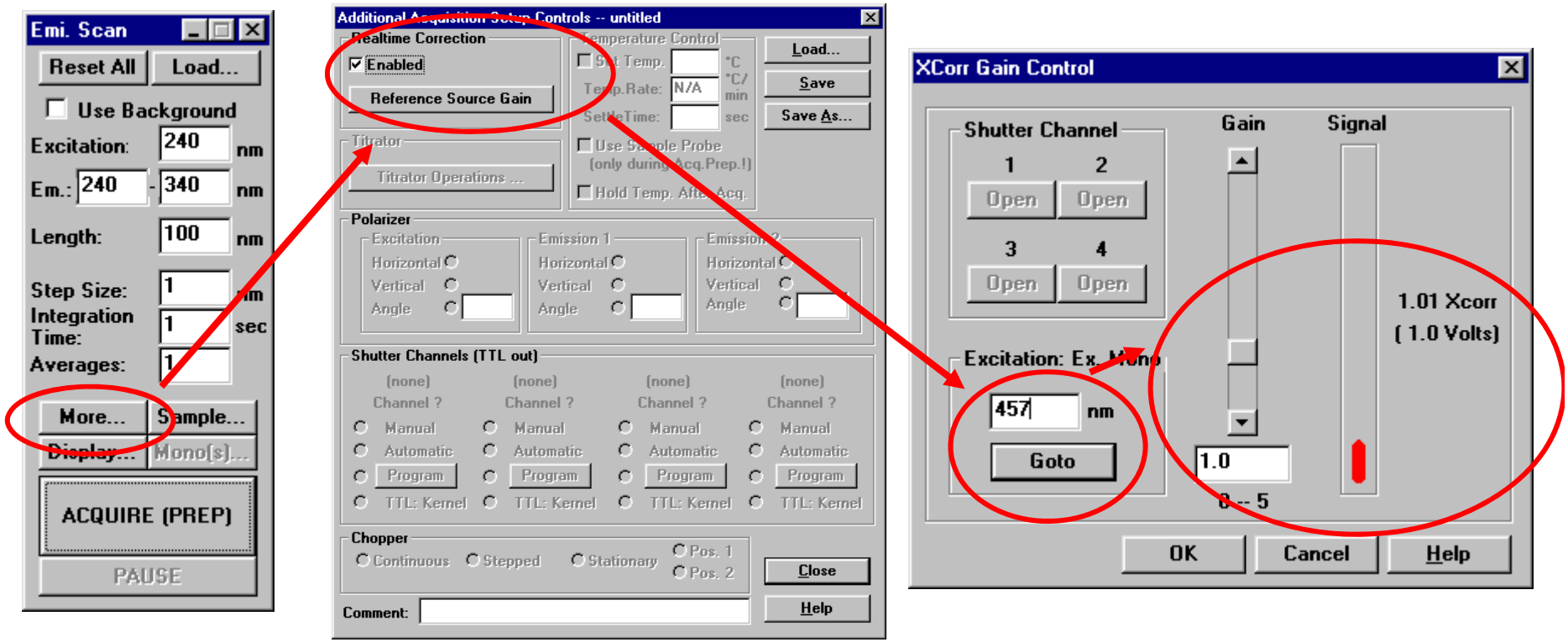


$$I_{PL} \sim I_{Exc} / I_{Ref} = I_{Exc} / (AI_{Exc}) = const$$

Source reference allows to compensate
for the lamp intensity fluctuations and differences
of the excitation light intensity at different wavelengths

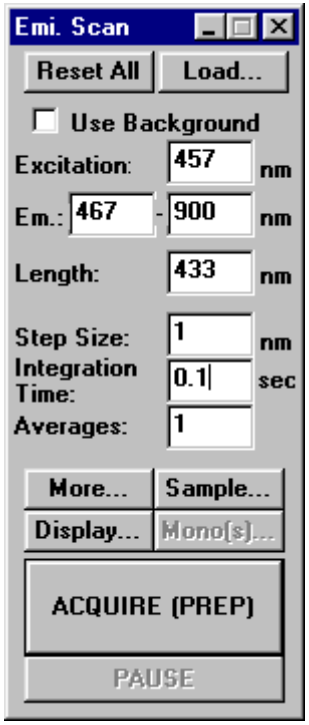
Setting Up Excitation Mono II

1. Open reference source gain window
2. Set excitation wavelength around 450 nm
3. Set the slits to 1 mm (2 full turns)
4. Adjust the gain slider so that the reference signal is 1V
5. Enable source reference
6. **<u>Do not change reference gain setting afterwards</u>**



Setting Scan Parameters

- Setting the scan parameters:
  - Set an appropriate excitation wavelength (your sample must absorb light to produce PL, maximum of the absorption band is preferred)
  - Set the emission scan range starting at least 10 nm away from the excitation wavelength
  - **Never allow the emission mono to scan across the excitation wavelength. This can kill the detector!!!**
  - Integration time affects signal/noise ratio, but not the signal magnitude, since the photon count rate is computed rather than the number of detected photons. For evaluation purposes and bright samples 0.1 seconds is sufficient.

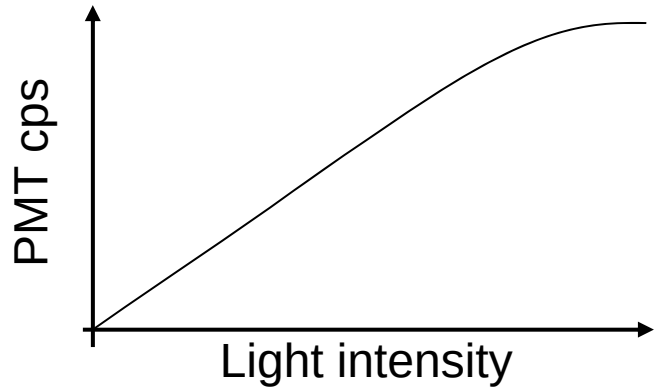


Emission Mono Setup

- The signal should be measurable with a good signal/noise ratio
- PMT detector should not be saturated

- Set the excitation mono and scan parameters.
- Set emission mono slits to 1 nm resolution
- Use the brightest sample for the slits tuning.
- Disable source reference (or use raw PMT signal)
- Scan the PL spectrum
- The reasonable S/N ratio is achieved for signals with count rate greater than 10,000 counts/s, for count rates greater than 2×10^6 cps saturation or even damage to the detector is possible.

$$\frac{\Delta N}{\langle N \rangle} = \frac{1}{\sqrt{\langle N \rangle}}$$



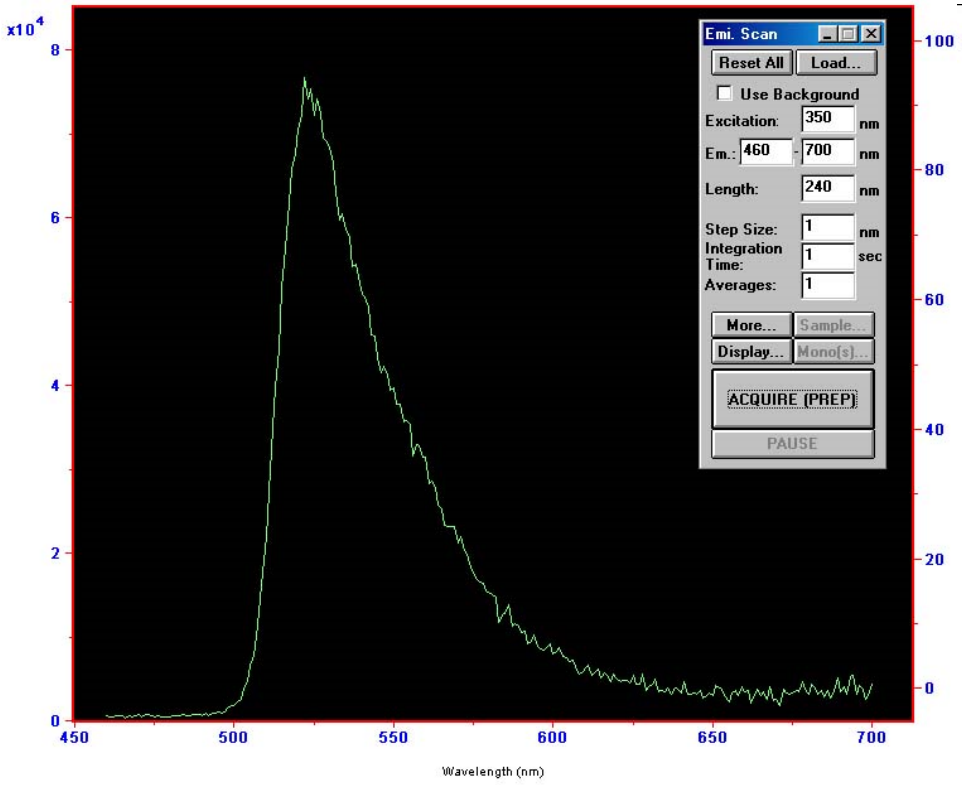
Emission Mono Setup II

- Signal too strong (CPS > 2.0×10^6)
  - Try closing emission monochromator slits
  - Insert neutral density filters into excitation or emission channels
  - Tune excitation mono to a wavelength corresponding to less absorption of the sample.

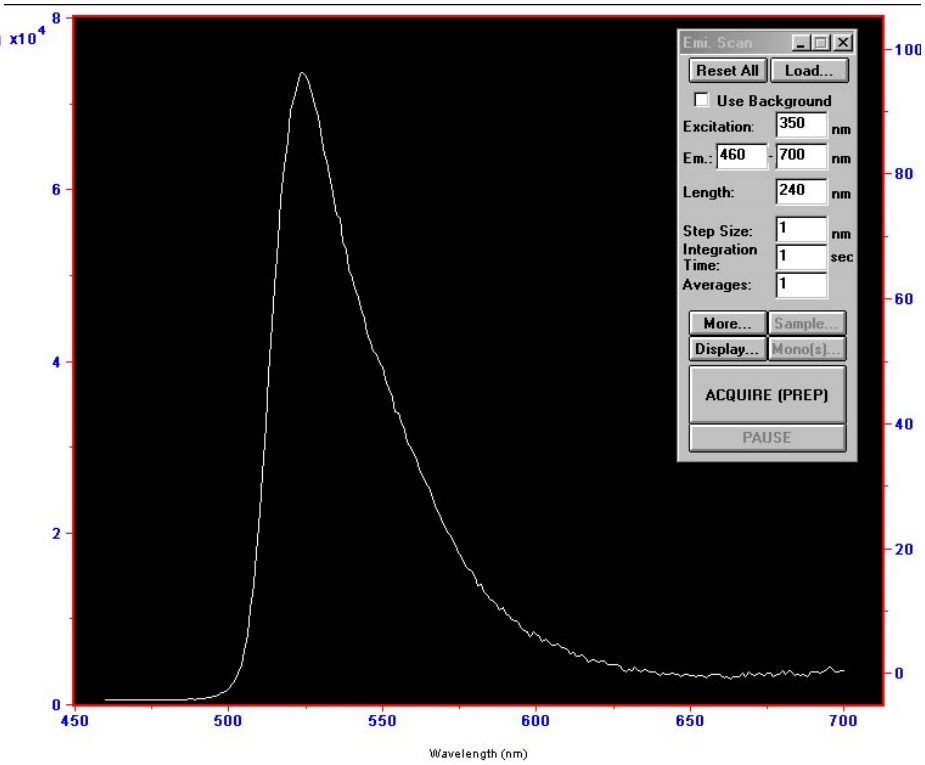
- Signal too weak (CPS < 1×10^4)
  - The most correct remedy is to improve S/N ratio by increasing the integration time or by averaging several spectra
  - You may trade off spectral resolution for signal by opening emission mono slits. Look for stray light and excitation light leakage.
  - Opening of the excitation mono slits is not recommended if quantitative measurements are required. Look for stray light and excitation light leakage.

S/N Ratio Improvement

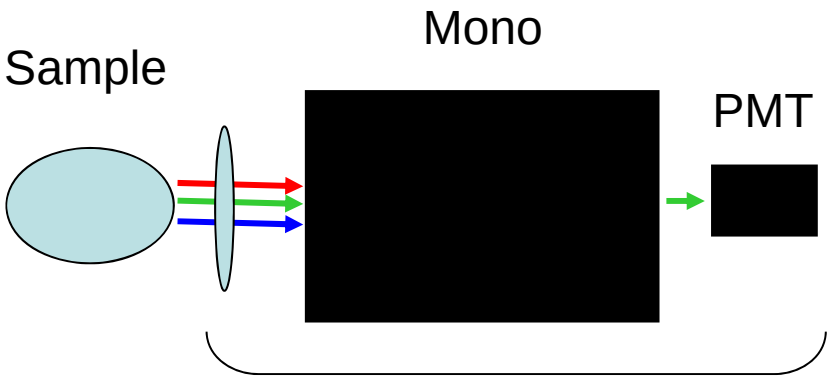
0.1 s



1 s



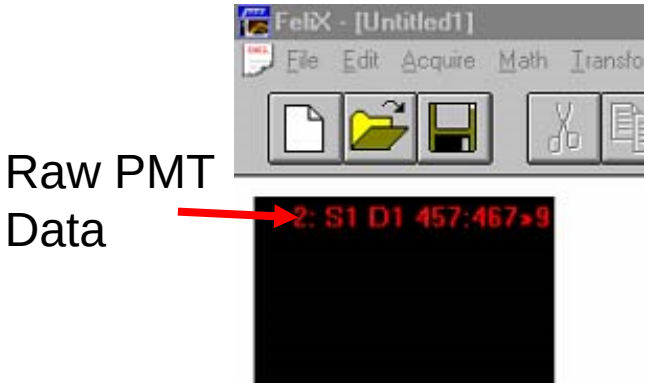
Emission Spectra Correction

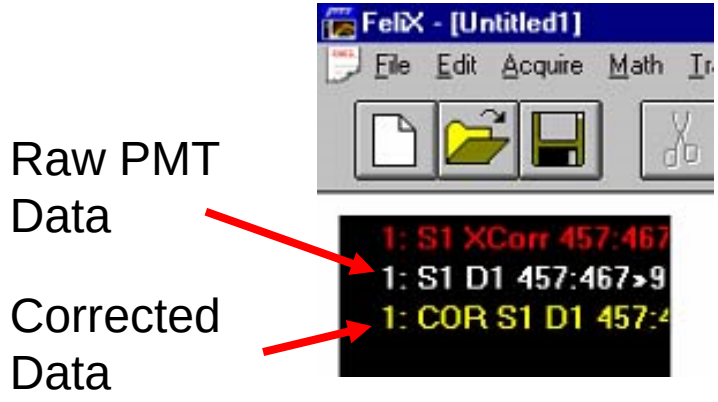


$$I_{det}(\lambda) = T(\lambda)F(\lambda)I(\lambda)$$

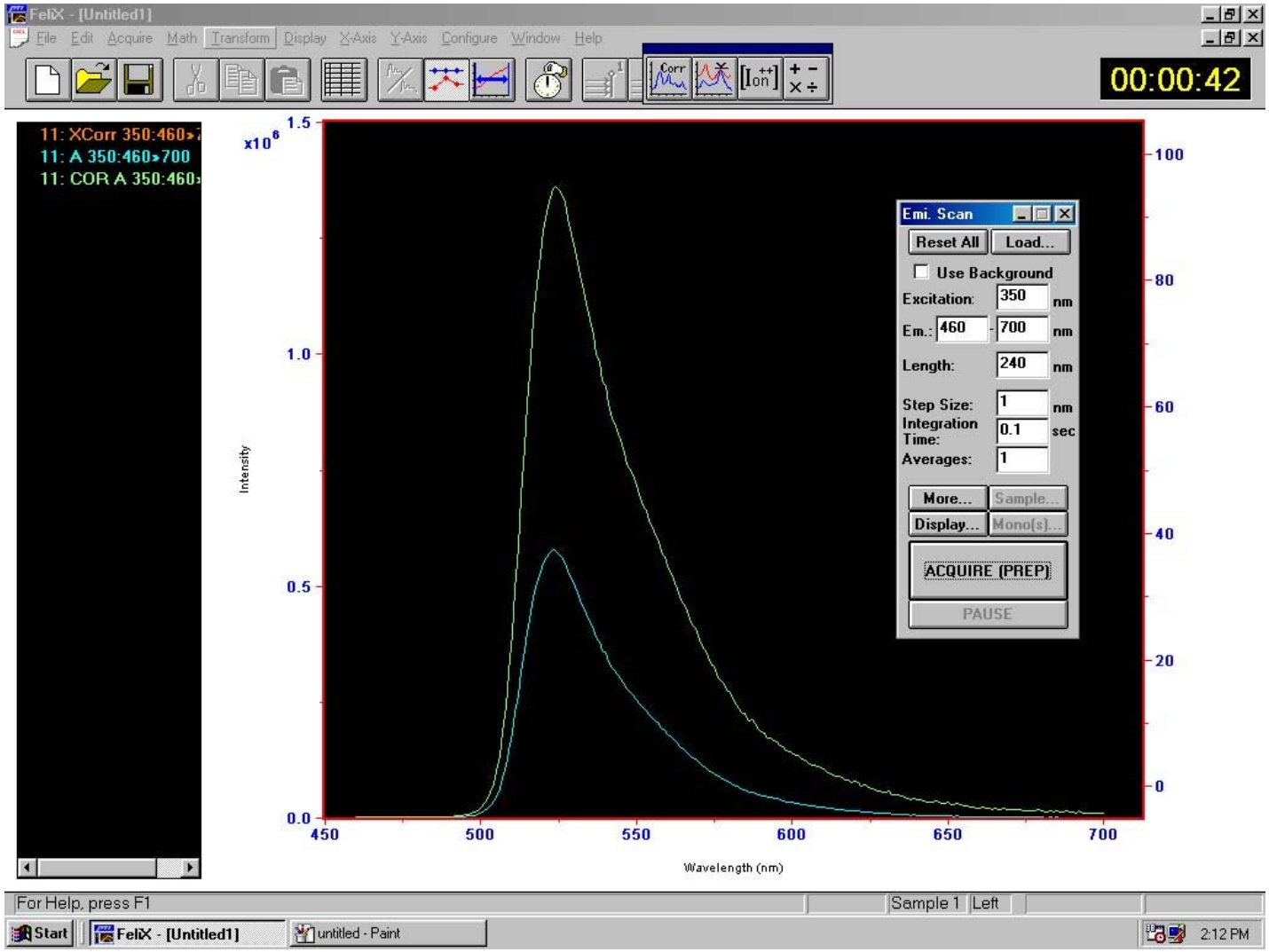
$$I(\lambda) = I_{det}(\lambda)/C(\lambda) = I_{det}(\lambda)/T(\lambda)F(\lambda)$$

- To eliminate the instrument-related artifacts and obtain true fluorescence spectra the acquired data have to be corrected
- **<u>This correction is enabled when the source reference gain option is active</u>**





Example of the Spectra

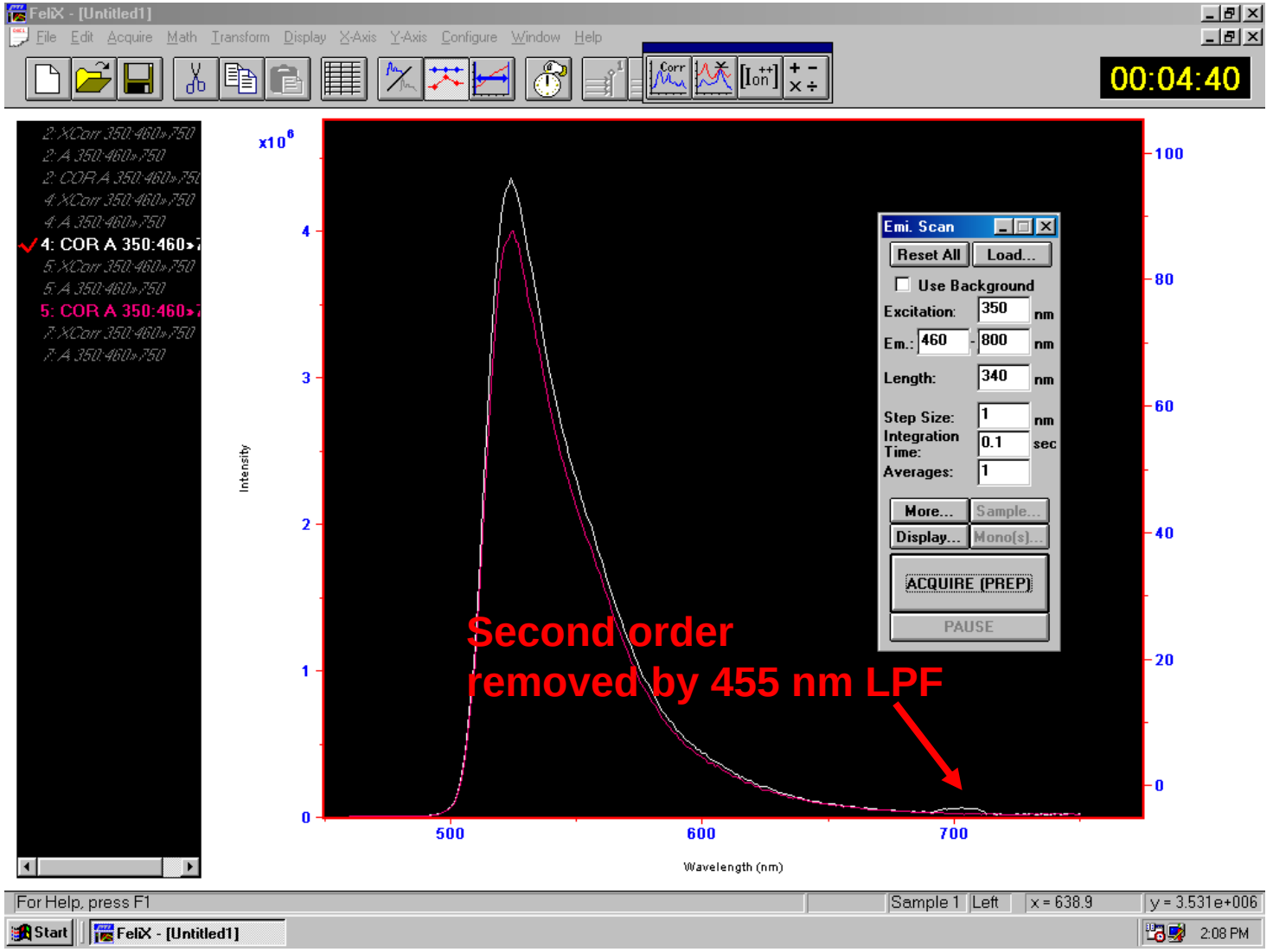


PL Artifacts I

$$d(\sin\theta_m(\lambda) - \sin\theta_i) = m\lambda$$

- Usually the first (*m=1*) order of diffraction is used
- However, the diffraction angle Θ_m will be the same for *m=1* and wavelength λ, and *m=2* and wavelength $\lambda/2$
- **Simultaneous detection of two different wavelength is possible!**

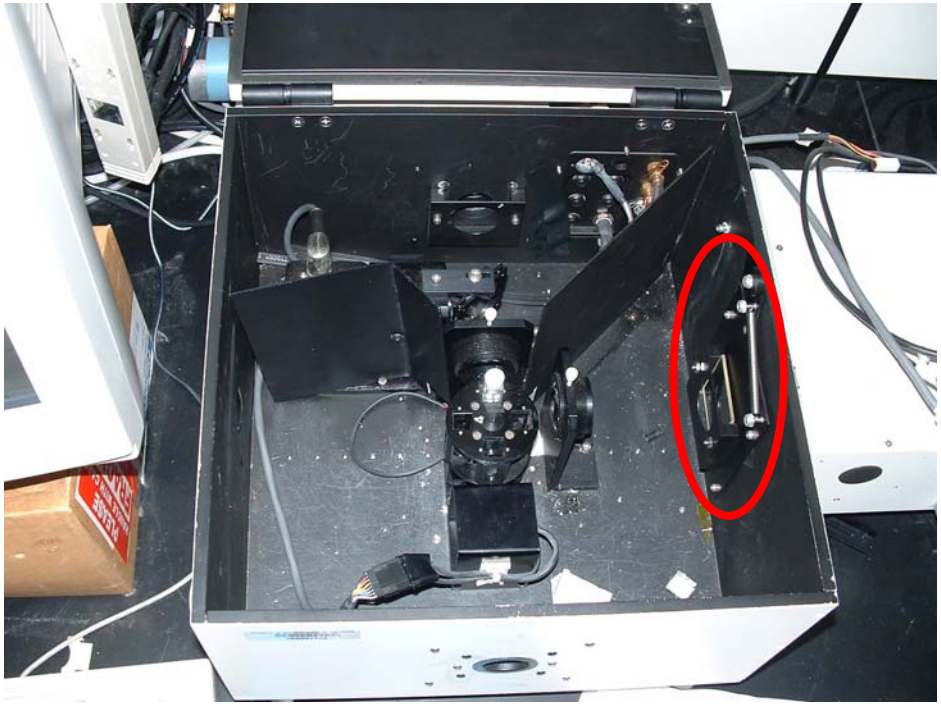
Second Order Detection



PL Artifacts II

How to avoid diffraction orders mixing?

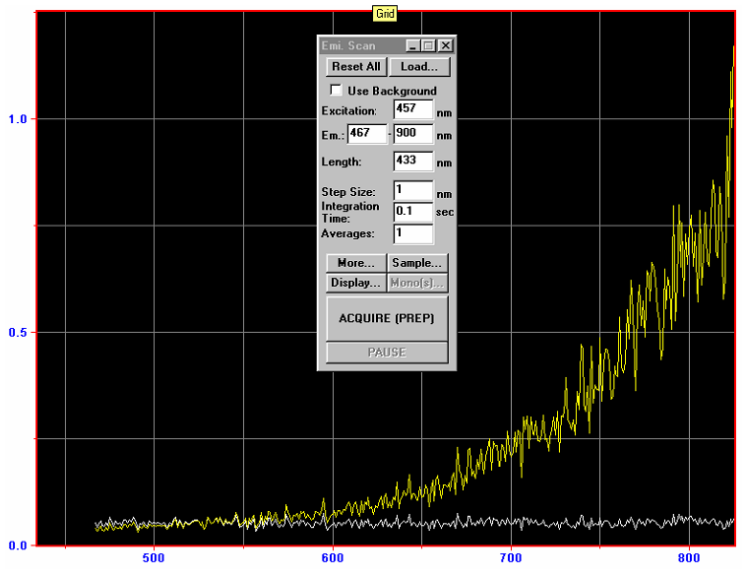
- Use $\lambda_{max} < 2\lambda_{min}$, if possible.
- Use order sorting filters (usually for suppression of excitation light)

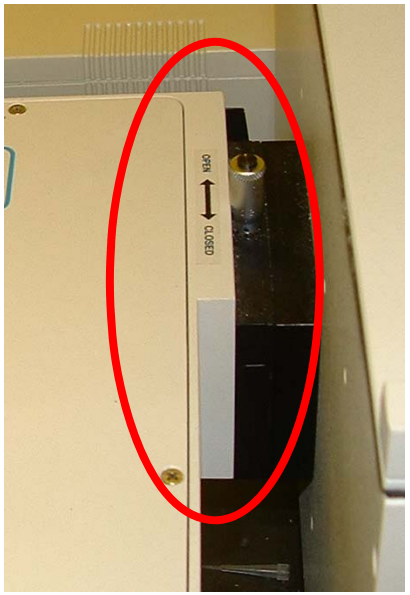


- Use long-wavelength-pass filters ($T=0$, if $\lambda<\lambda_0$)
- $\lambda_{exc} < \lambda_0 < \lambda_{min}$
- If high accuracy is required, measure the filter transmission spectrum T(l) on UV-VIS and correct the PL spectrum: $I_{corr}(\lambda) = I(\lambda)/T(\lambda)$

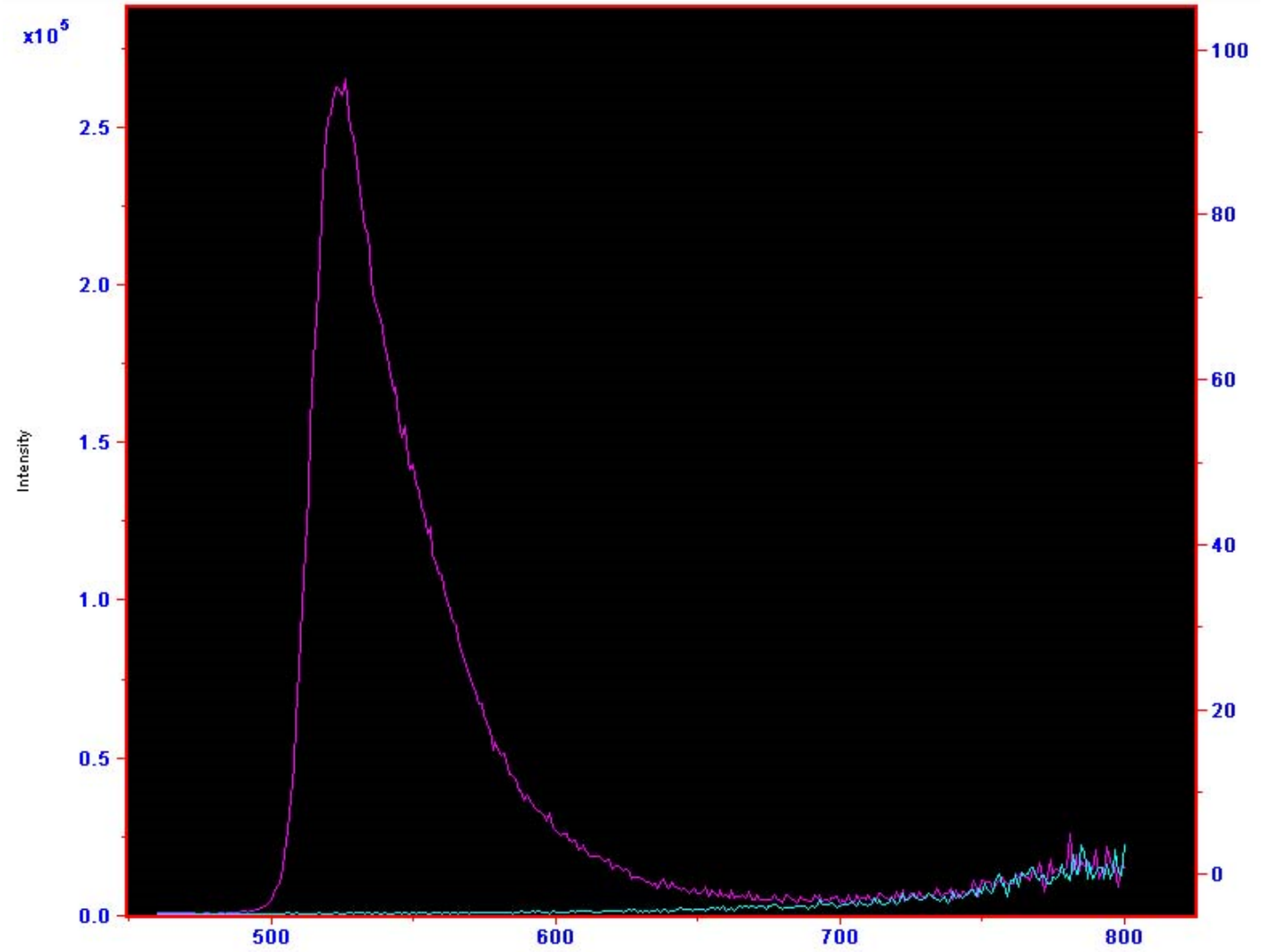
PL Artifacts III

- Baseline artifacts are seen only if the emission correction is enabled
- They manifest themselves as a false increase of the intensity at the red end of the spectrum
- They appear because the software compensates for the detector sensitivity drop and artificially increases the dark counts level.
- Dark counts – signal coming from PMT when it is not illuminated
- **Remedy: block emission PMT or use a non-fluorescent sample and record dark count spectrum with enabled spectral correction. Subtract the corrected dark count spectrum from PL.**





PL Artifacts IV



Few Remarks

Solid films measurements:

- When measuring do not mount them at 45 degree with respect to emission and excitation monochrometers. This increases the amount of stray excitation light getting into the detection optics
- Measurements in solid films provide only qualitative information, since the emission pattern can change from sample to sample

Know your sample absorption spectra:

- Measure absorption spectrum on UV-VIS before measuring PL.
- Avoid using concentrated sample. High optical density does not allow to excite PL evenly in the sample. Internal filter effects (reabsorption of PL) are possible.
- OD ~ 0.1 is recommended
- If measurements in in concentrated solution are required, use microvolume cell
- Use quartz cells.