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DXR Data Collection Introduction

DXR User Training

Scope

- Best practices
  - Laser power
  - Aperture
- Automatic operation
  - Auto focus
  - Auto exposure
- Optimizing performance
  - Exposure time vs. number of exposures
  - Sampling
    - Microscope objectives
    - Smart Raman accessories

Best Practices

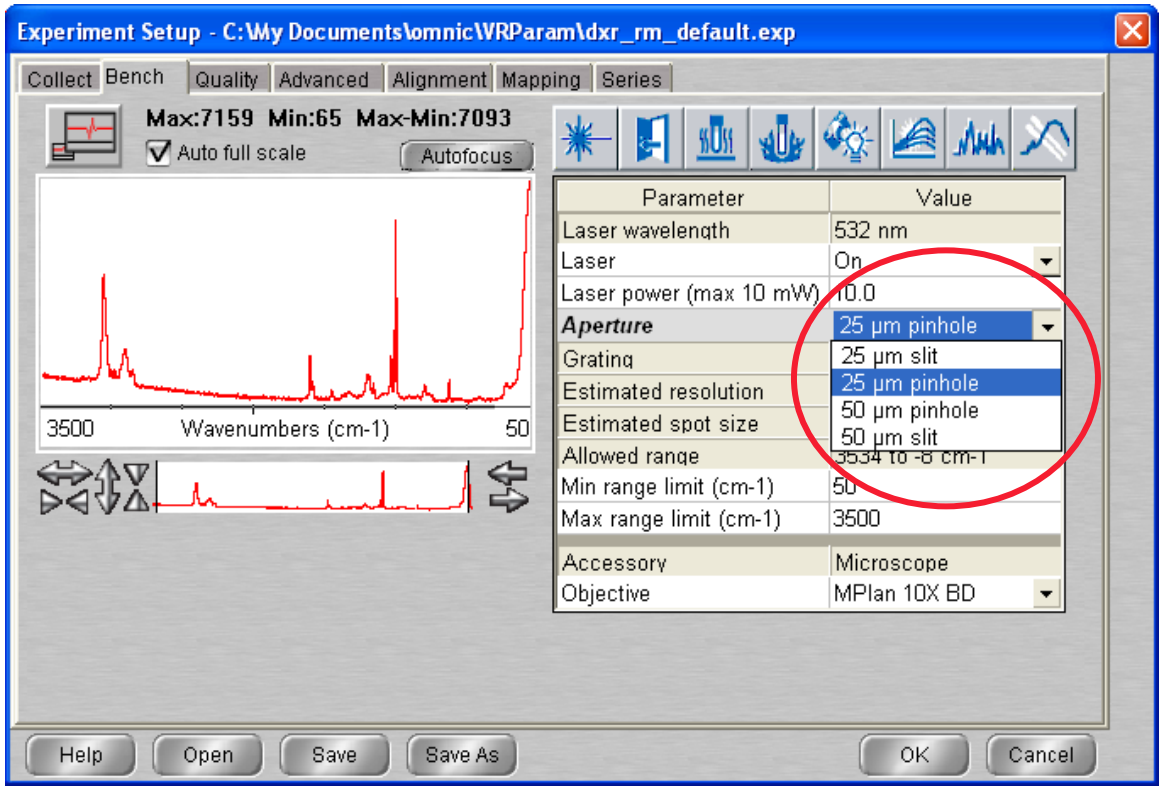
- Laser power
  - Use the maximum laser power whenever possible
  - Situations requiring less laser power
    - Sample changes
      - Burning – signal disappears
      - Heating – signal or spectrum changes
    - Fluorescence
- Aperture
  - Use a slit aperture whenever possible
    - Pinhole and slit yield the same spectral resolution
    - Pinhole is required only for confocal operation
  - Use a larger aperture if possible
    - Some loss of spectral resolution but it's not as bad as you may think
    - Try both aperture sizes and see if you loose any significant spectral resolution

Apertures

- Pinhole versus Slit
  - Definition – aperture: opening through which light will pass
    - Pinhole – small, sharp edged opening, good depth of field
    - Slit – rectangular opening, large length versus narrow width
  - Resolution – smaller opening, higher resolution, but loss of signal
  - Confocal – use a pinhole to reject light from regions that are not in focal plane
  - Applications
    - pinhole for microscopy work or small samples, for high resolution
    - slit for regular samples, bulk powders, liquids, for more signal

Apertures

- Apertures
  - 4 options
    - 2 pinholes and 2 slits
    - 25 and 50 micron
  - Software selected
  - Pinhole for confocal
  - Slit for bulk



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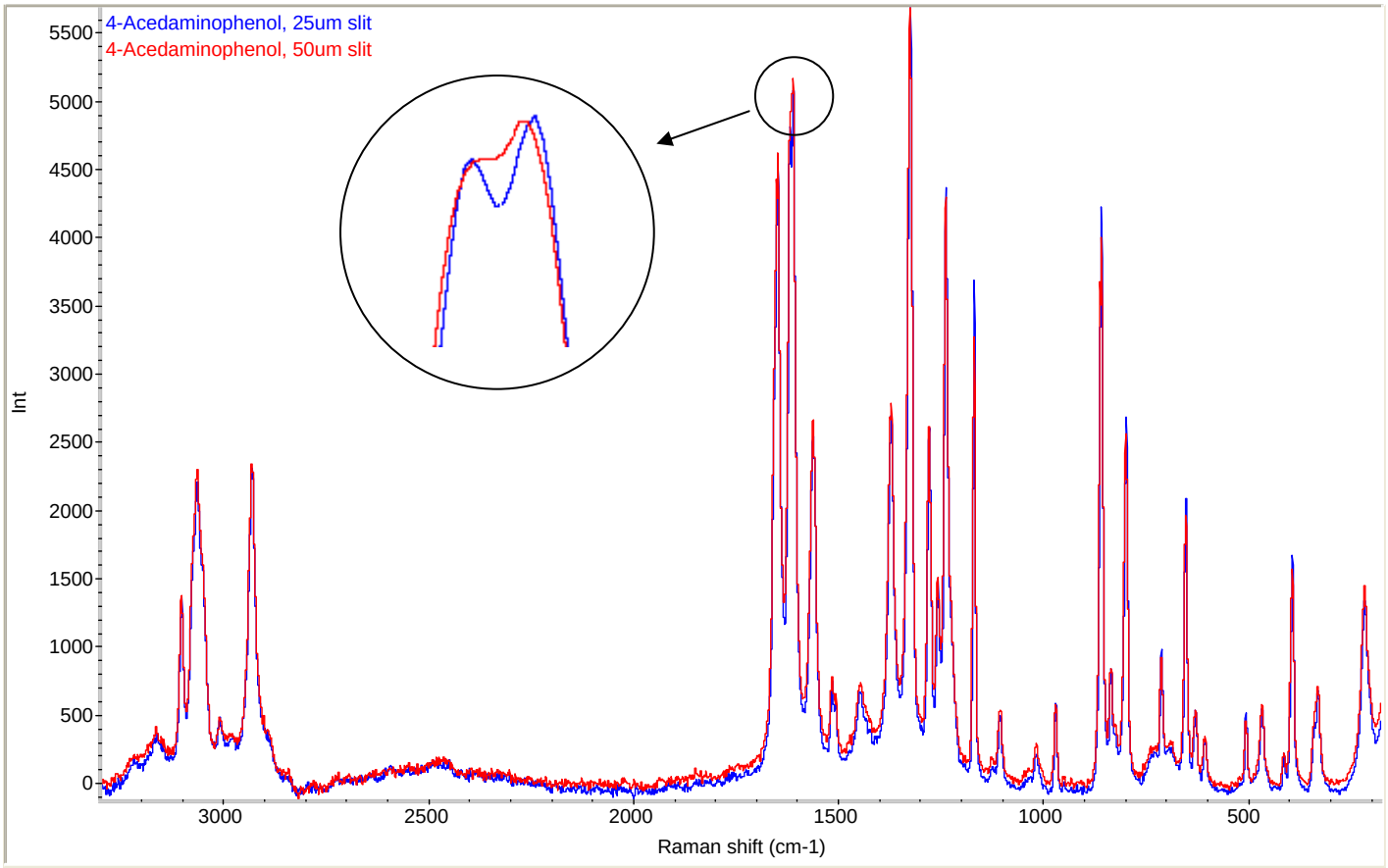
Effect of aperture size on spectral resolution

- 25um aperture spectrum superimposed on a 50um aperture spectrum (acetaminophen tablet)



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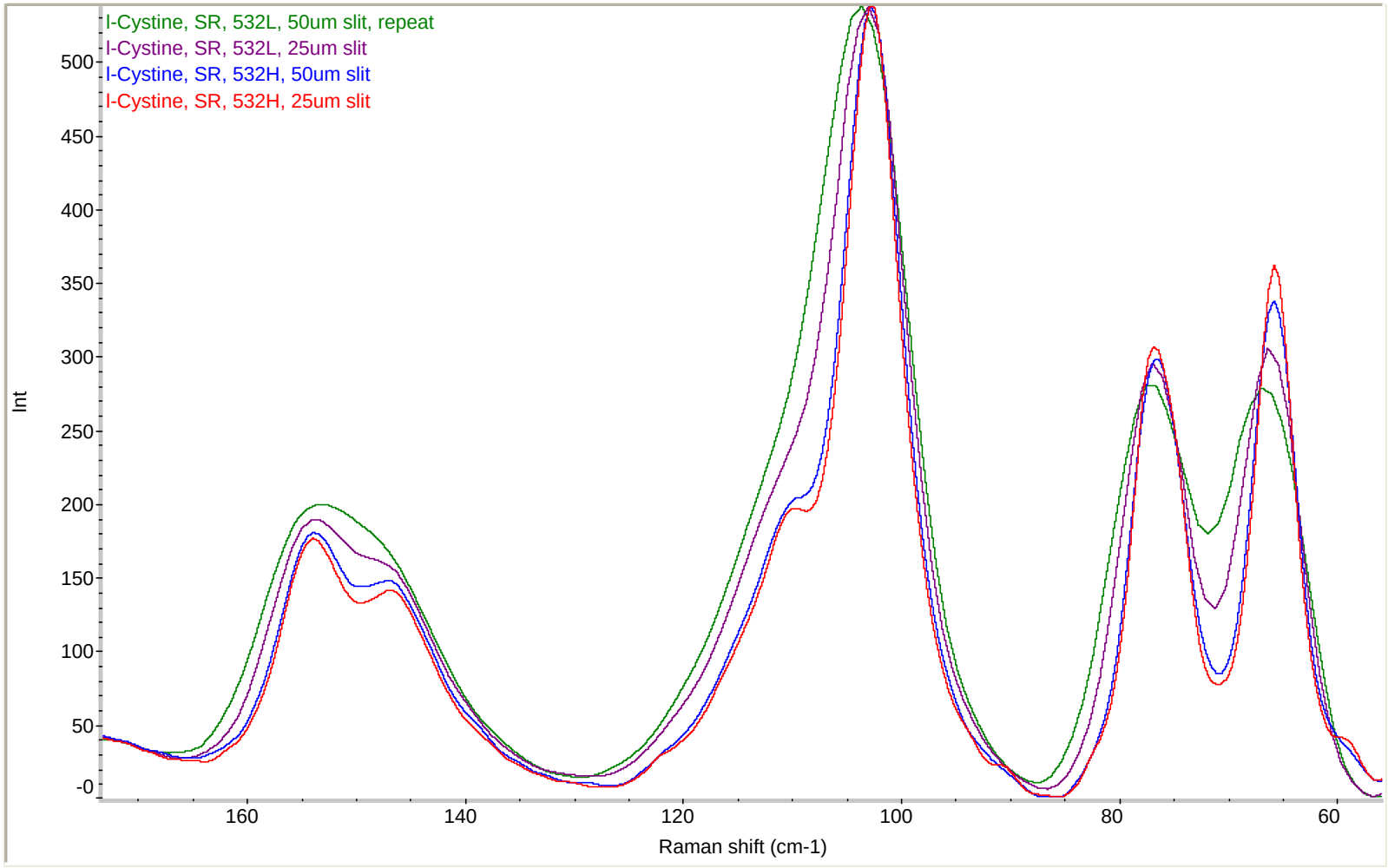
DXR Spectral resolution comparison

- L-Cystine, 532 nm laser excitation



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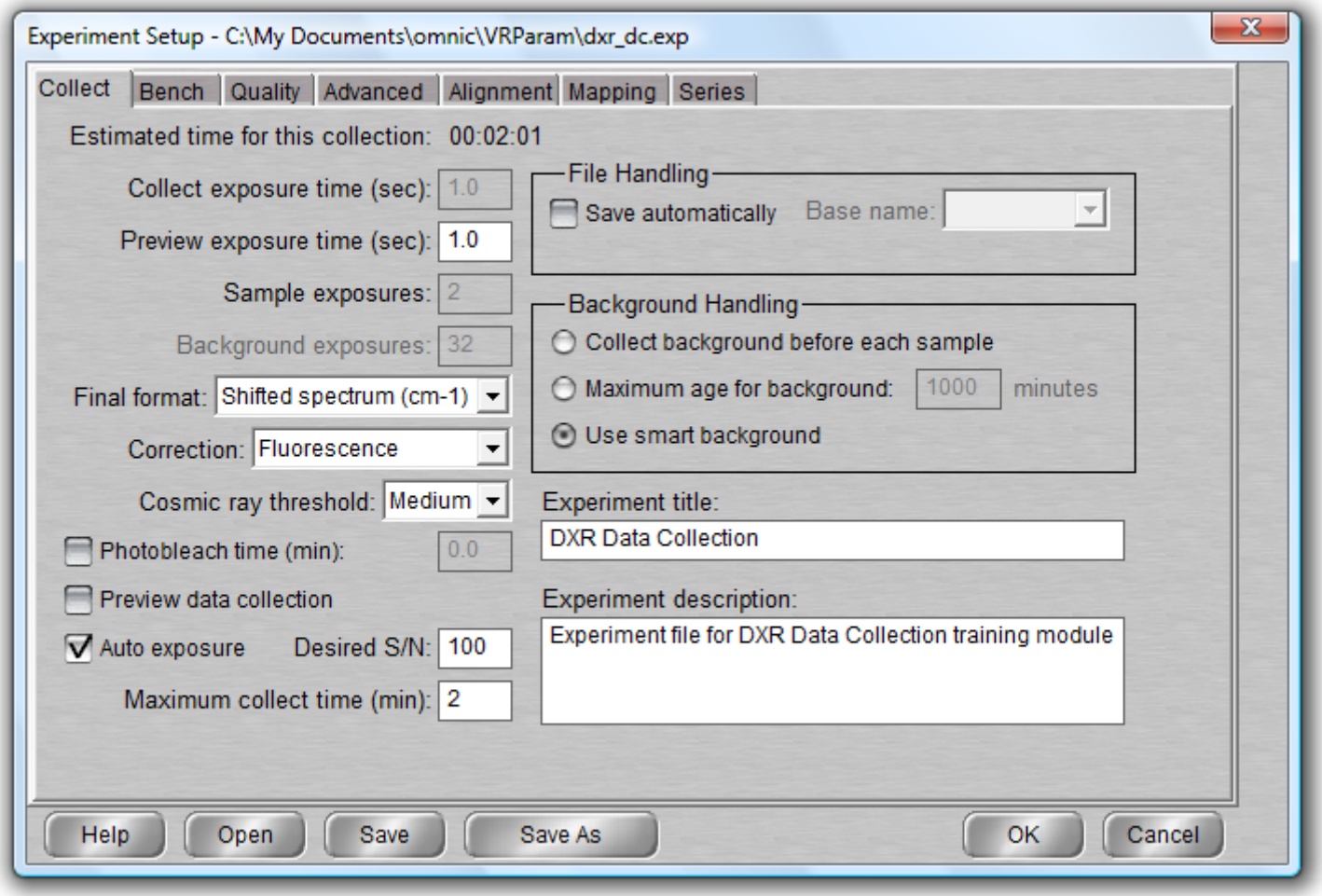
Auto exposure



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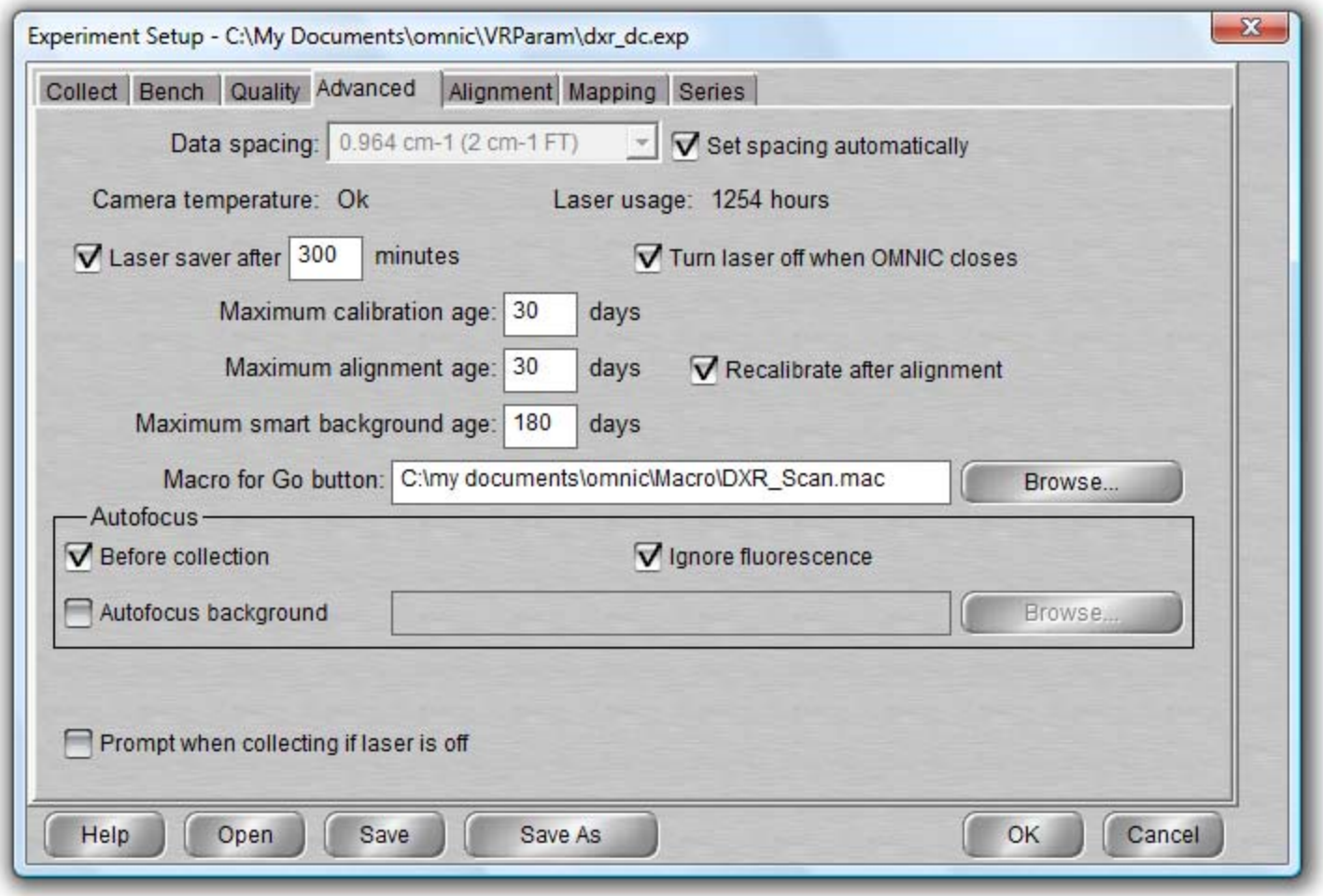
Autofocus



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Optimizing performance

- Exposure time vs. number of exposures
  - Increasing the exposure time reduces the noise level
    - The signal level stays constant (counts per second)
  - Increasing the number of exposures reduces noise level
    - Random noise is reduced by signal averaging
- Both reduce noise; which is best?

Optimizing performance

- Exposure time vs. number of exposures
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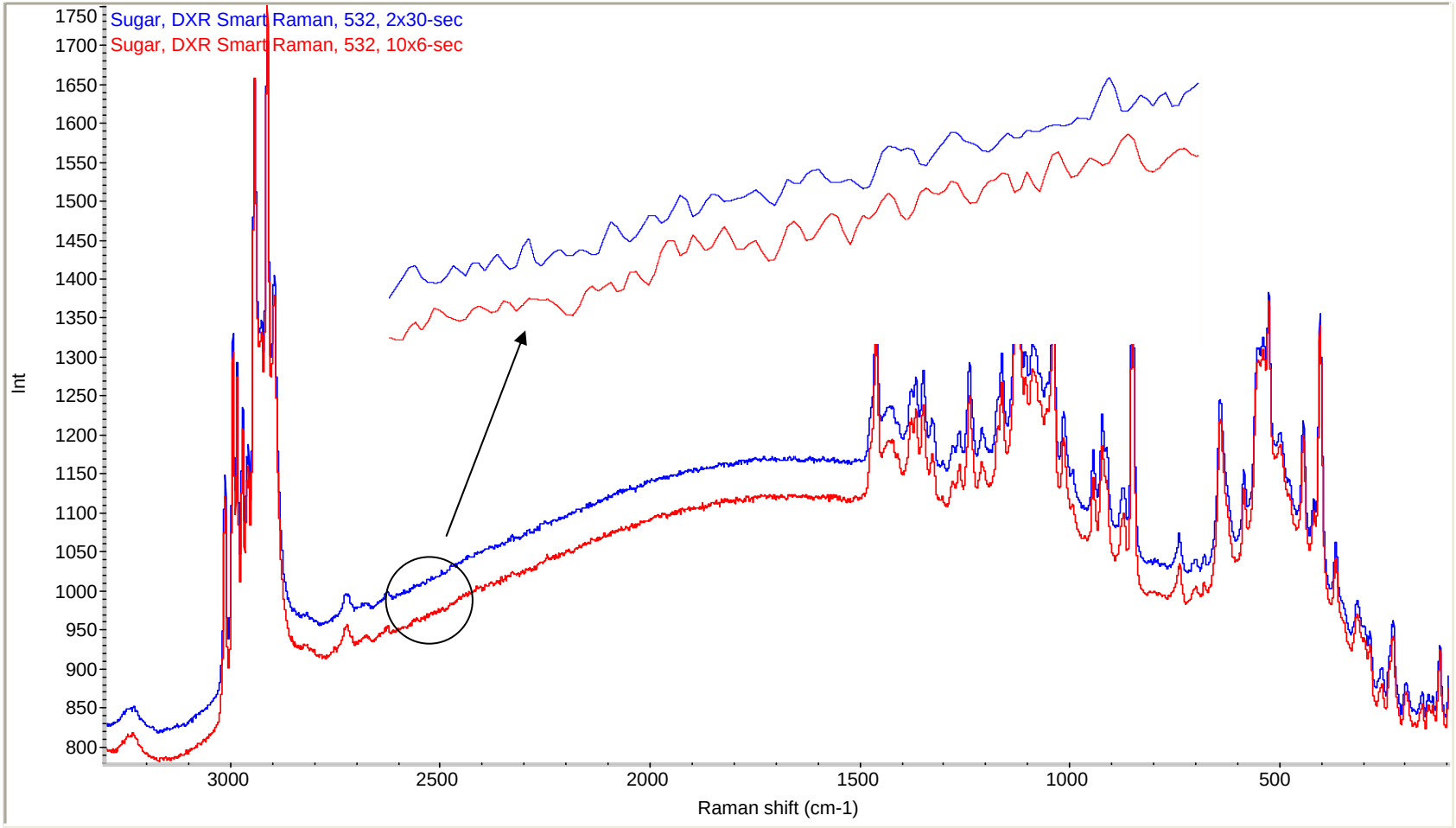
    - The signal level stays constant (counts per second)
  - Increasing the number of exposures reduces noise level
    - Random noise is reduced by signal averaging
- Both reduce noise; which is best?

- Increasing exposure time reduces the noise level quicker than increasing the number of exposures

- For best results:
  - Leave # exposures = 2 (for cosmic ray rejection)
  - Increase the exposure time until CCD overflow

Comparison of exposure time and number of exposures

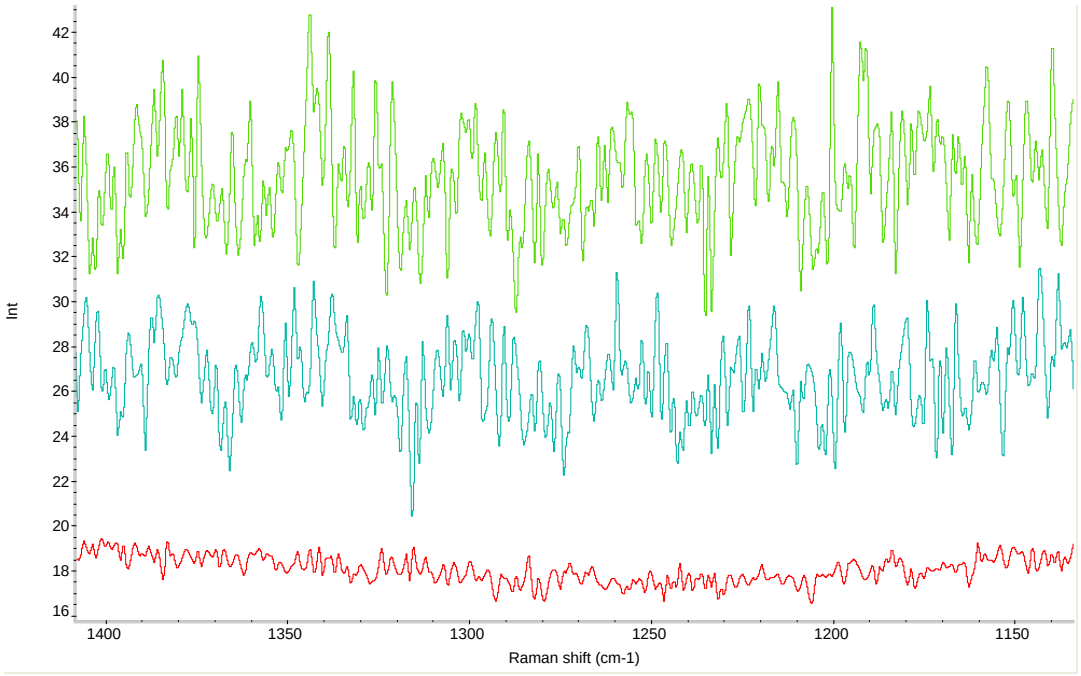
- Sugar, 532, 10x6-sec (red) vs. 2x30-sec exposure (blue)



Noise reduction without fluorescence

- Silicon, DXR Smart Raman, 532 nm laser excitation

- 60x1-sec (top, green)
- 30x2-sec (middle, blue)
- 2x30-sec (bottom, red)



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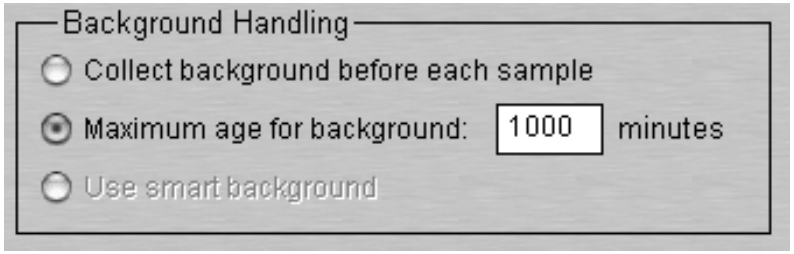
Backgrounds

- Raman is an emission experiment so the concept of a background is much different than FT-IR which is (usually) an absorbance experiment
- A Raman background is a measure of the detector dark signal
  - Dark signal is the product of CCD temperature and exposure time
  - For the DXR, the detector temperature is constant
  - Measured with no light entering spectrograph (dark)
- Raman backgrounds are saved and reused until they expire
  - Background Handling control is on Collect tab of Experiment Setup

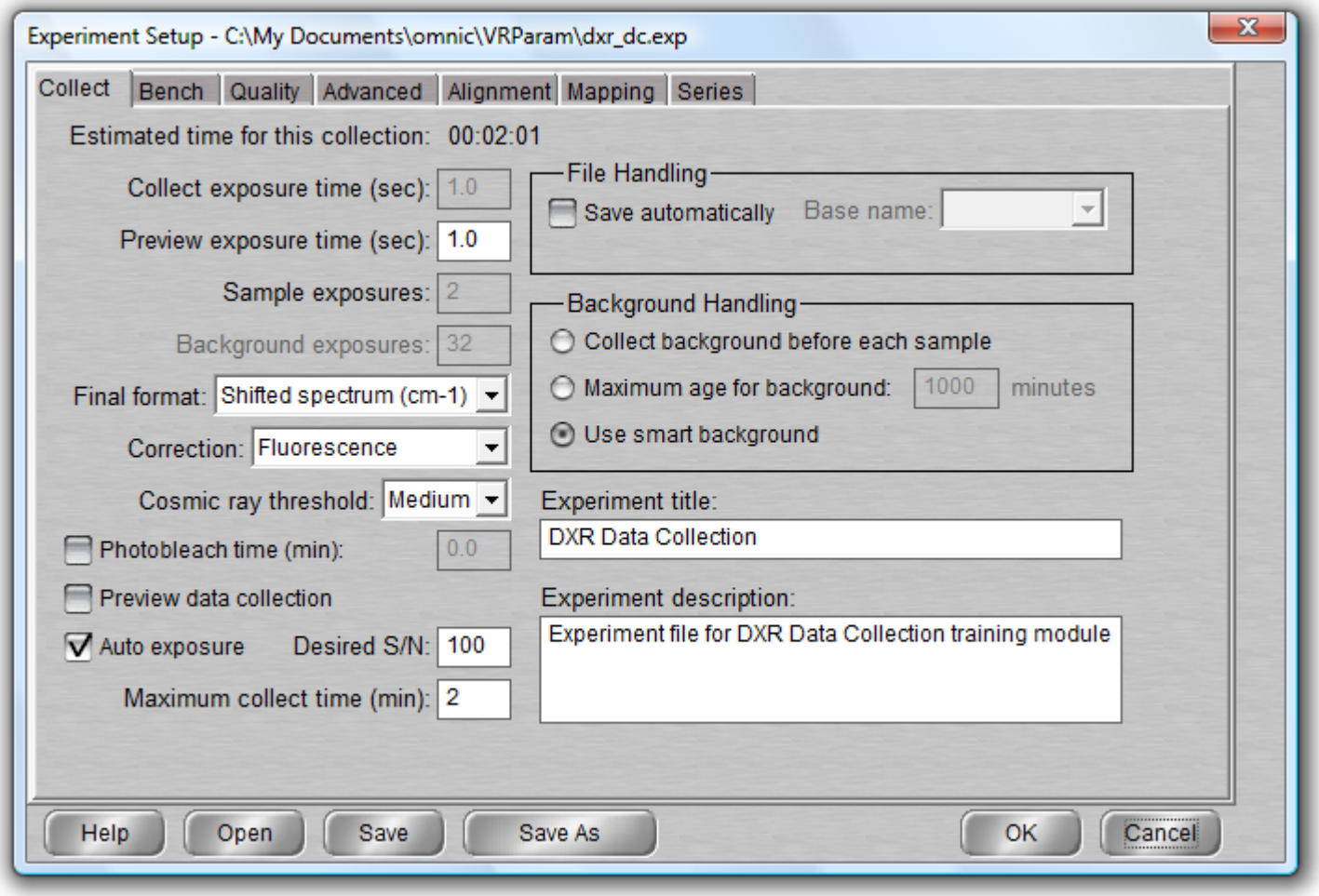


Smart Backgrounds

- Patented technology that measures backgrounds at various exposure times from 1 to 60 seconds. A mathematical model is constructed which allows us to predict the background response for any exposure time

- Smart backgrounds
  - Eliminates need to collect backgrounds each time we change exposure time
  - Allows Auto Exposure capability based on *any* exposure time
  - Takes approximately 13 hours (overnight) to collect data for the model

Background handling controls



Other topics

- We have covered the primary features contributing to data collection quality
  - Exposure time
  - Number of exposures
  - Aperture
  - Laser power
  - Autofocus

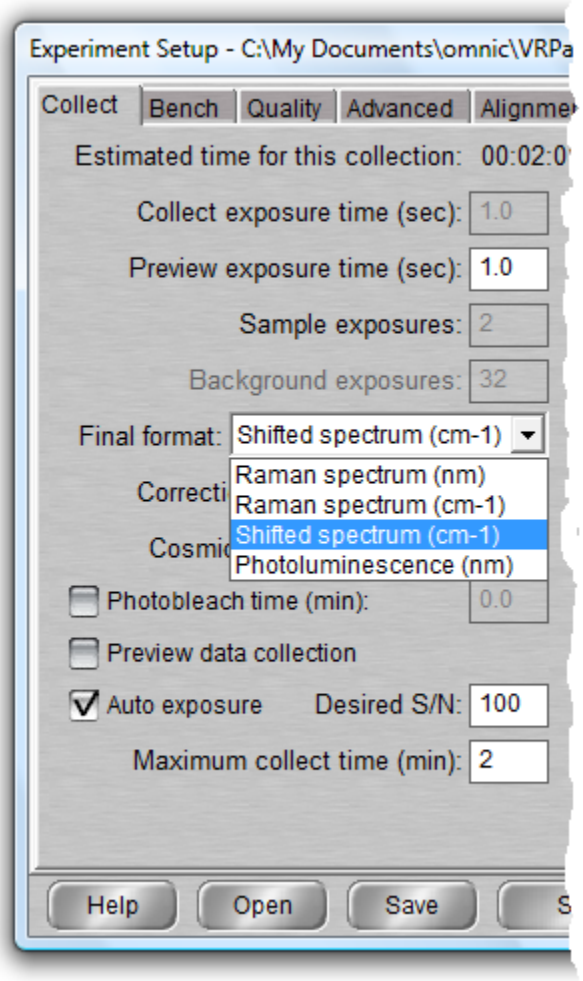
- The rest of this module will explore a variety of topics related more to operation and convenience
  - Final format
  - Cosmic ray rejection
  - Photobleaching
  - Corrections
  - Preview collect

Final format

- Selects the format for the collected spectrum

- Shifted spectrum (cm^{-1})
  - Raman shift
  - Typical format for Raman spectra
  - *Use this setting*

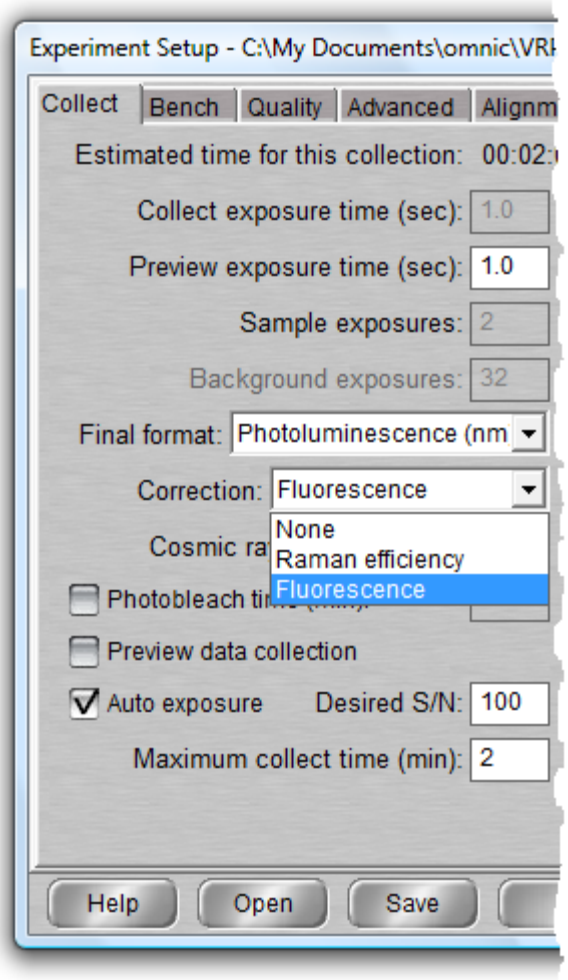
- Raman spectrum
  - Unshifted X-axis in either nm or cm^{-1}
  - Raman spectra collected with different lasers will not overlap
- Photoluminescence (nm)
  - X-axis in nanometers, Y-axis in emission units



Corrections

- Selects a spectral operation to apply automatically after collect and before displaying result

- None
- Raman efficiency
  - Generally not needed – advanced technique
  - Applies ν^4 factor to Y-axis intensity
- Fluorescence
  - Removes fluorescence background using a polynomial fit or a reference spectrum
  - *Recommended setting (order=5)*



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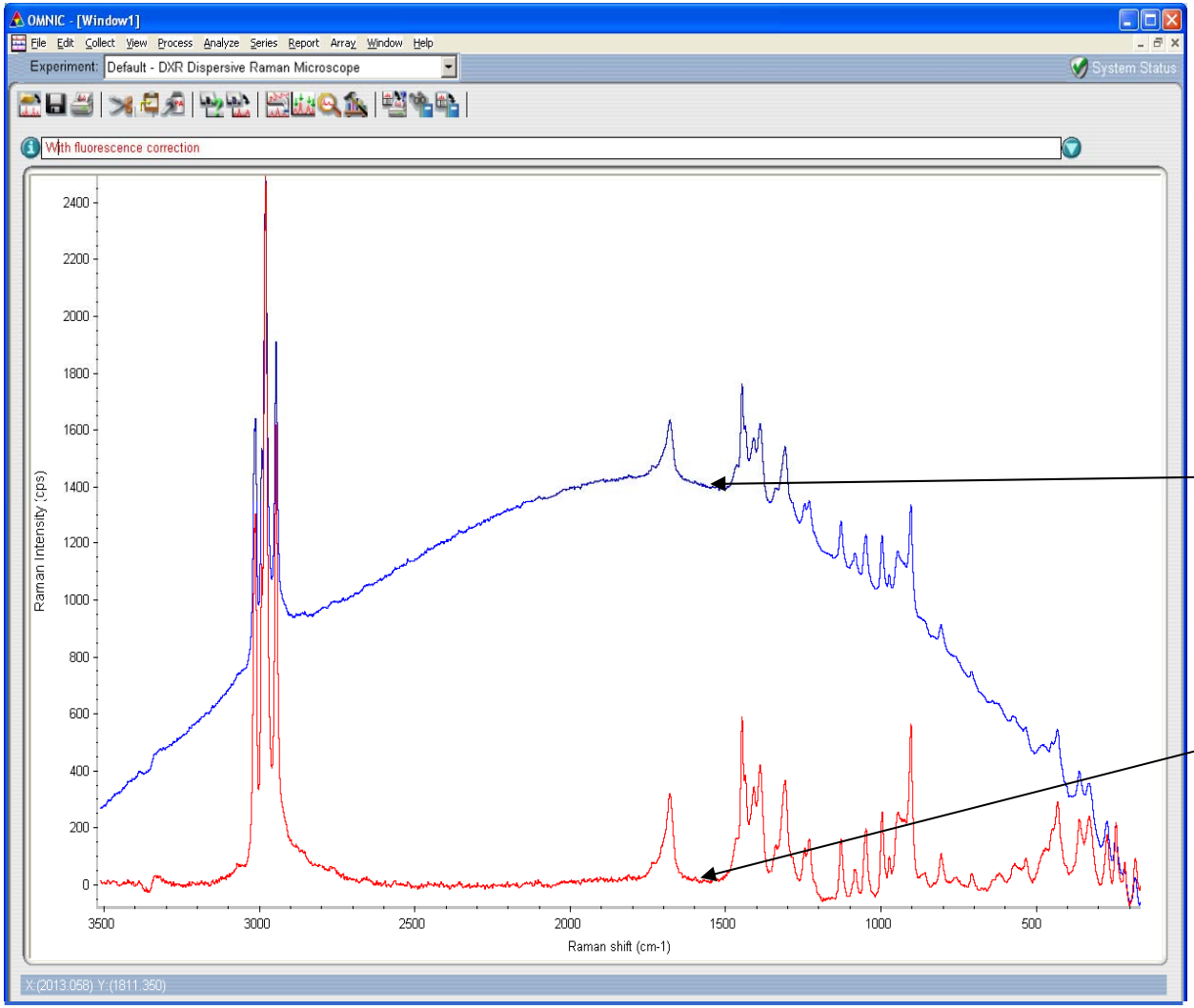
Fluorescence Correction Example



Uncorrected spectrum

Spectrum after automated fluorescence correction applied

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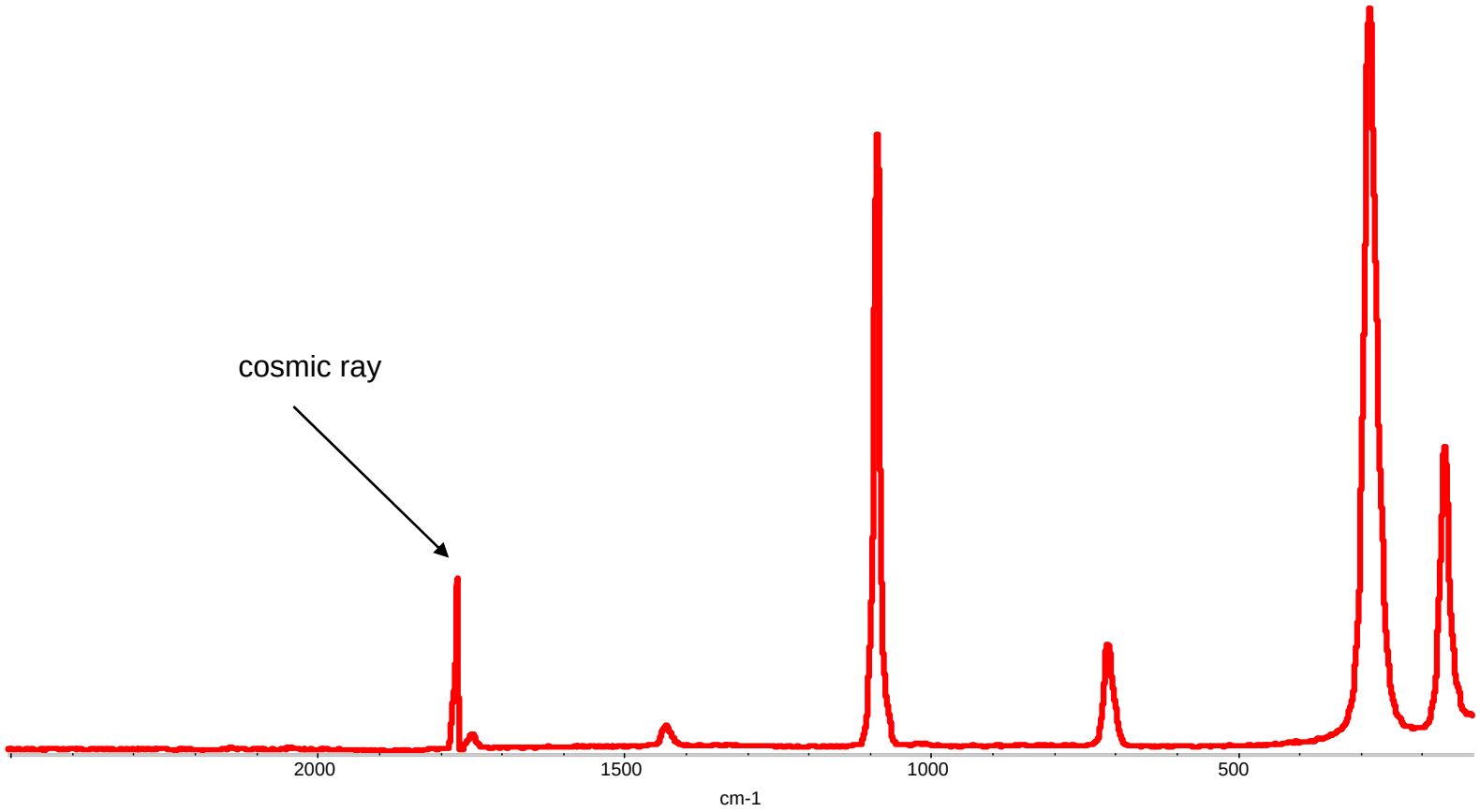
Cosmic ray threshold

- Cosmic rays are natural events detected by sensitive integrating detectors such as a CCD.
  - Random, unpredictable events – not a specific wavelength
  - Typically show up as spikes in Raman spectra or backgrounds
- Rejection
  - Requires two exposures
  - Exposures are compared to detect and repair events
  - Turn-off (Threshold = None) to collect just one exposure
- Sensitivity threshold
  - Low is most sensitive (detects low energy events)
  - *Medium is recommended setting*

Cosmic Ray

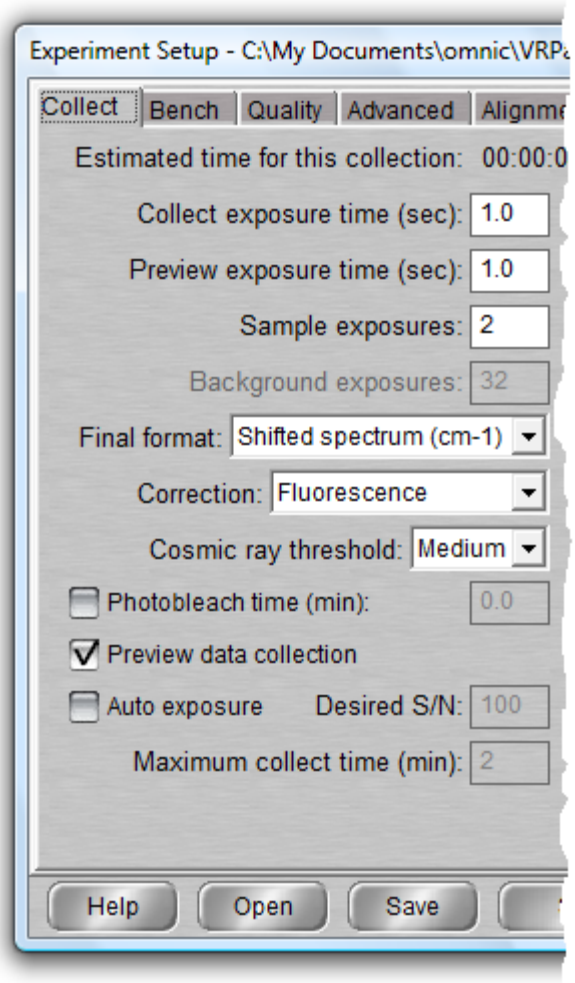
- Here is an example of a spectrum that has a cosmic ray



Calcite spectrum

Preview collect

- Preview is a live display that displays a Raman spectrum so you can adjust sample focus or placement
  - Bench tab
  - Preview collect window before Collect
- Preview exposure time (sec):
  - Applies only to preview
  - 0.1 – 10 sec
- Preview data collection
  - Select (check) this option to automatically display a Preview Collect window before every data collection



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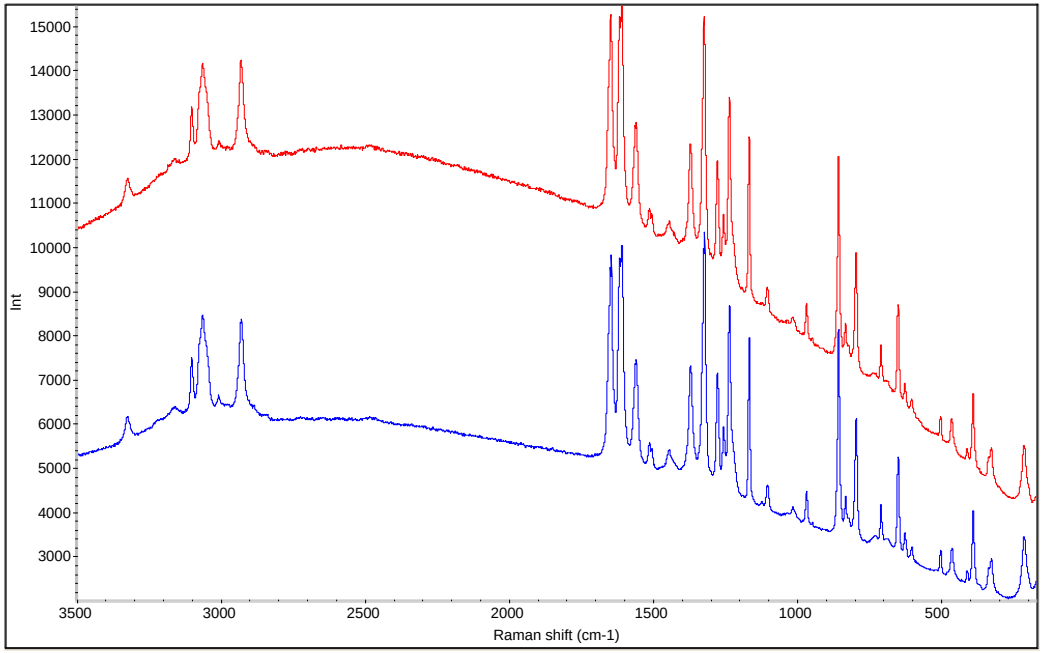
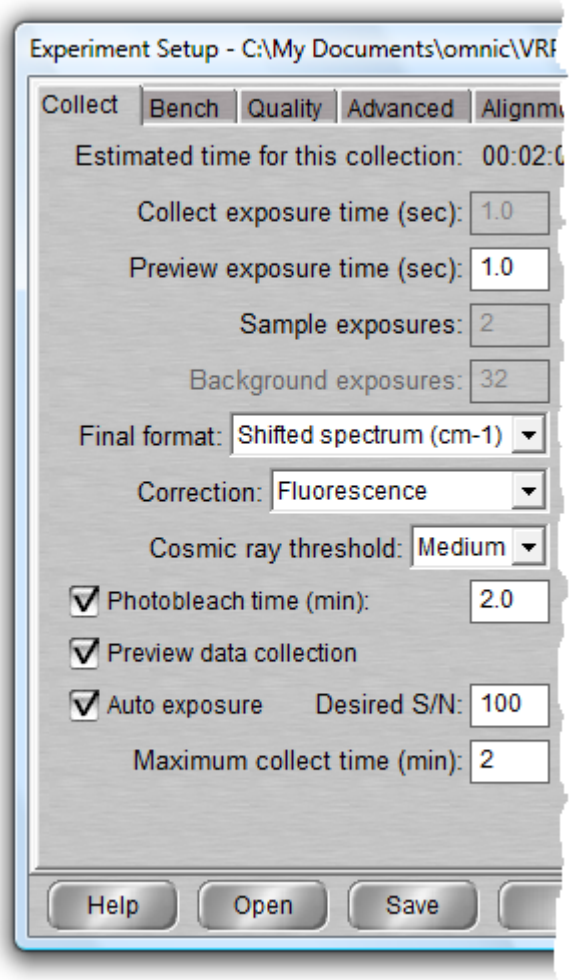
Photobleaching

- Photobleaching exposes the sample to laser for a specified amount of time before starting data collection
- Reduces fluorescence by “bleaching” impurities causing the fluorescence





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Alignment and calibration

- Two important but different processes for optimal performance

- Alignment is the process of bringing the laser beam and spectrograph sampling point into agreement
  - For the DXR Raman microscope, a third constraint is the visual crosshair which designates the sampling point
  - Alignment needed for best spatial resolution of microscope

- Calibration assures the accuracy of the spectrum wavelength (x-) axis and intensity (y-) axis
  - Software operations using known reference materials
  - Neon and polystyrene for the x-axis
  - White light for the y-axis

Calibrate Instrument

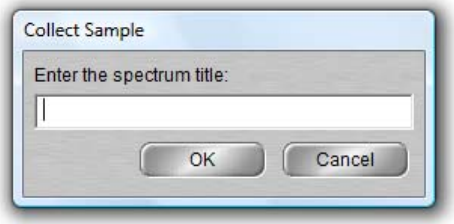
- The system is aligned. Next step: Calibration

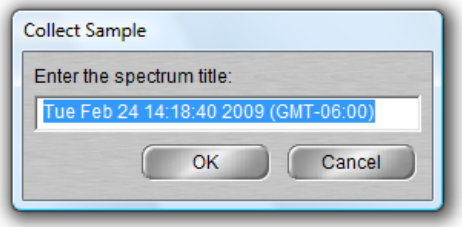
- Calibration assures the accuracy of the spectrum wavelength (x-) axis and intensity (y-) axis
  - Software operations using known reference materials
  - Neon and polystyrene for the x-axis
  - White light for the y-axis

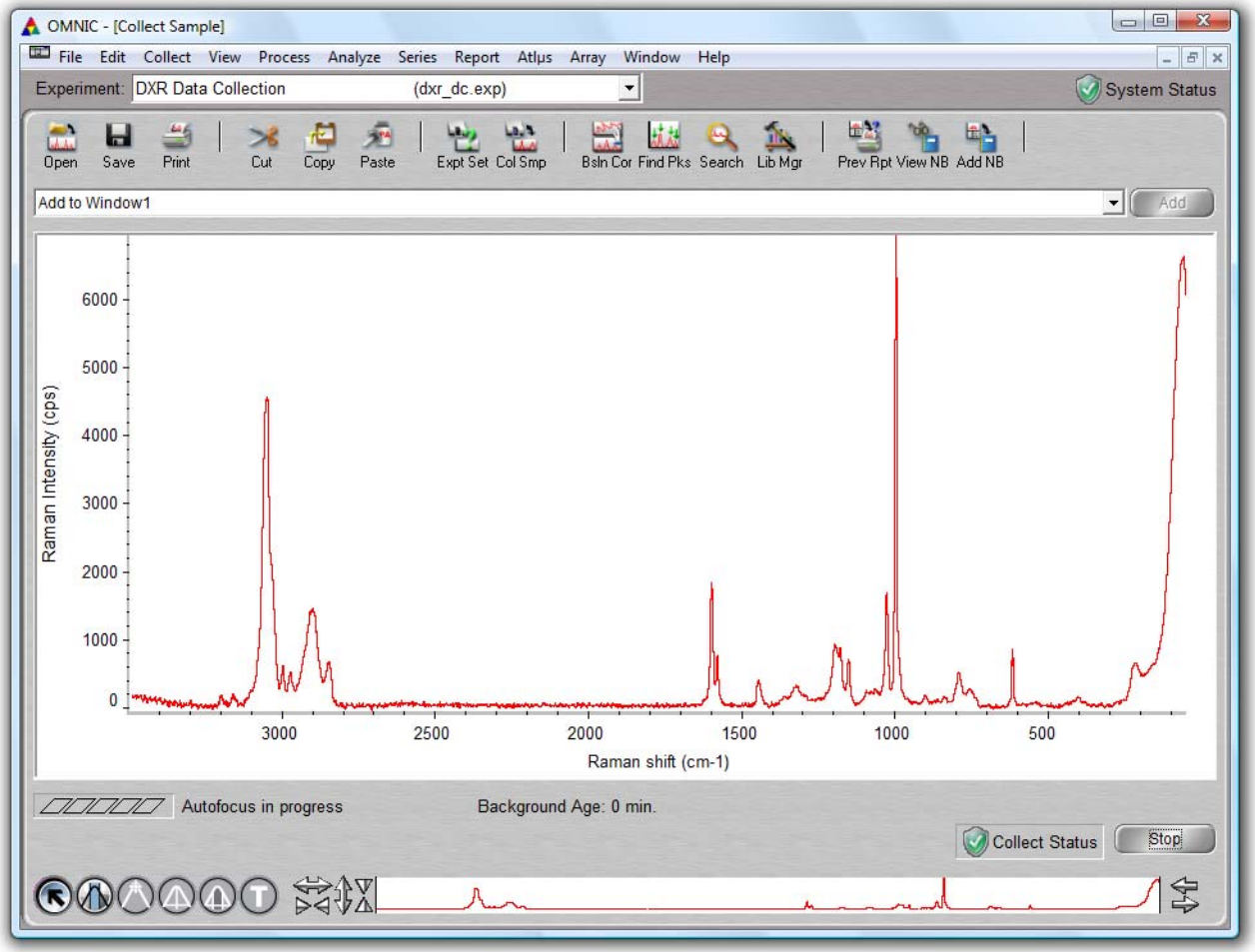
- Choose Collect > Calibrate Instrument from the OMNIC menu
- Place the alignment tool on the microscope stage and focus on the pinhole -or-
- Place the calibration accessory into the Smart Raman

Collect Sample

- Choose Collect > Collect Sample from the OMNIC menu







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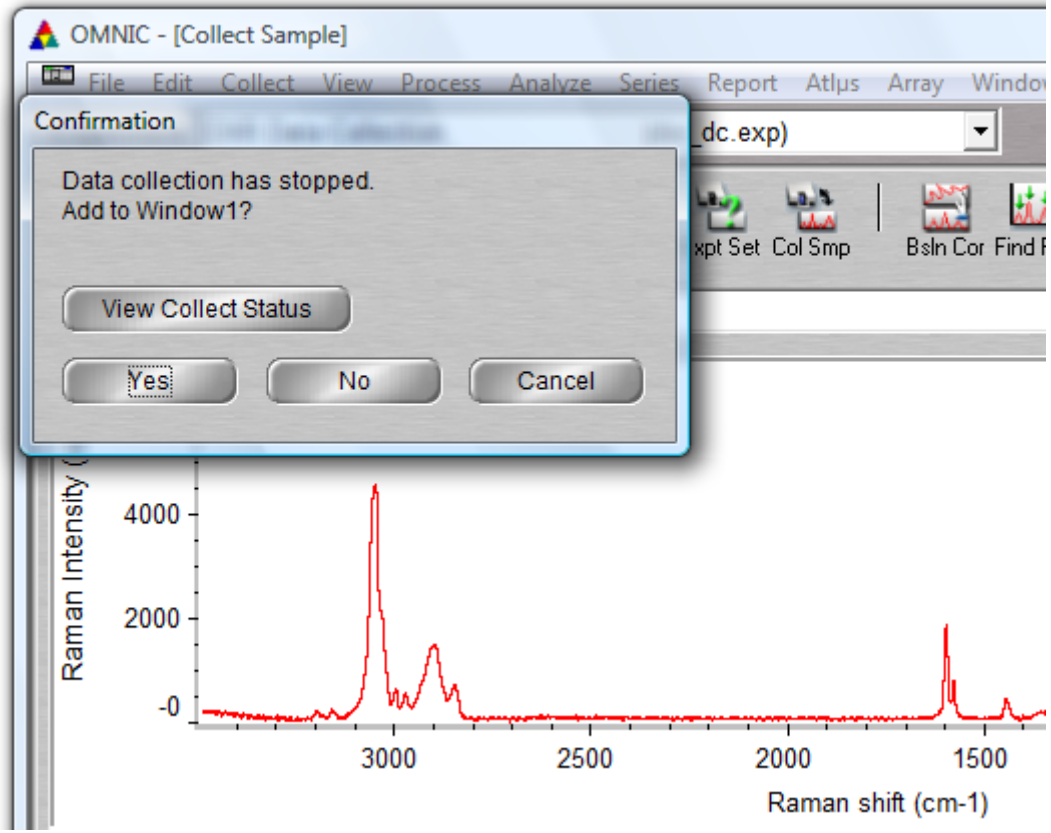
Add collect result to window

- The Confirmation dialog is displayed when the collect is finished
- The spectrum is still in the [Collect Sample] window
- If the result looks OK, press the Enter key or click the Yes button
- The spectrum is added to the active spectrum window



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