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DXR Data Collection Introduction







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





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



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

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

Collect Bench Quality Advanced Alignment Mapping Series Max:7159 Min:65 Max-Min:7093 Image: Collect Bench Image: Colle	Experiment Setup - C:\My Documents\omnic\VRPar	am\dxr_rm_default.exp	Þ
Max:7159 Min:65 Max-Min:7093 Auto full scale Autofocus Autofocus Autofocus Autofocus Autofocus Autofocus Autofocus Parameter Value Laser wavelength Laser ower (max 10 mW) 0.0 Aperture Statinated resolution Estimated resolution Estimated spot size Allowed range Min range limit (cm-1) Max range limit (cm-1) Stoto Accessory Objective Melan 10X BD	Collect Bench Quality Advanced Alignment Mapp	ing Series	
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Accessory Microscope Objective MPIan 10X BD -		Max range limit (cm-1) 3500	
Objective MPIan 10X BD		Accessory Microscope	
		Objective MPIan 10X BD 🚽	



Effect of aperture size on spectral resolution

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





DXR Spectral resolution comparison

L-Cystine, 532 nm laser excitation





Auto exposure

Experiment Setup - C:\My Documents\omnic\VRParam\dxr_dc.exp	x
Collect Bench Quality Advanced Alignment Mapping Series	
Estimated time for this collection: 00:02:01	
Collect exposure time (sec): 1.0 File Handling	
Preview exposure time (sec): 1.0	
Sample exposures: 2 Background Handling	
Background exposures: 32 O Collect background before each sample	
Final format: Shifted spectrum (cm-1) - O Maximum age for background: 1000 minutes	
Correction: Fluorescence	
Cosmic ray threshold: Medium 💌 Experiment title:	
Photobleach time (min): 0.0 DXR Data Collection	
Preview data collection Experiment description:	
Auto exposure Desired S/N: 100 Experiment file for DXR Data Collection training module	
Maximum collect time (min): 2	
Help Open Save Save As OK Can	cel





ollect Bench Quality Advance	ced Alignment Ma	ping Series
Data spacing: 0.96	4 cm-1 (2 cm-1 FT)	Set spacing automatically
Camera temperature: Ok	Lase	usage: 1254 hours
✓ Laser saver after 300 r	ninutes	✓ Turn laser off when OMNIC closes
Maximum calib	ration age: 30 c	ys
Maximum align	nment age: 30 c	ys 🗹 Recalibrate after alignment
Maximum smart backg	round age: 180 c	ys
Macro for Go button:	C:\my documents\o	inic\Macro\DXR_Scan.mac Browse
Before collection		☑ Ignore fluorescence
Autofocus background		Browse
Prompt when collecting if la	ser is off	OK Cancel



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
 - 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?
- 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)





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

Background Handling	
Collect background before each sample	
Maximum age for background: 1000 minutes	
O Use smart background	



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

Experiment Setup - C:\My Documents\omnic\VRP	aram\dxr_dc.exp	x
Collect Bench Quality Advanced Alignme	ent Mapping Series	
Estimated time for this collection: 00:02:0	01	
Collect exposure time (sec): 1.0	File Handling	
Preview exposure time (sec): 1.0		
Sample exposures: 2	Background Handling	
Background exposures: 32	Collect background before each sample	
Final format: Shifted spectrum (cm-1) 💌	O Maximum age for background: 1000 minutes	
Correction: Fluorescence	O Use smart background	
Cosmic ray threshold: Medium 💌	Experiment title:	
Photobleach time (min): 0.0	DXR Data Collection	
Preview data collection	Experiment description:	
Auto exposure Desired S/N: 100	Experiment file for DXR Data Collection training module	
Maximum collect time (min): 2		
Help Open Save S	Save As OK Cancel	D



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

• Corrections

- Cosmic ray rejection
- Preview collect

Photobleaching



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

Experiment Setup - C:\My Documents\omnic\VRPa
Collect Bench Quality Advanced Alignme
Estimated time for this collection: 00:02:0
Collect exposure time (sec): 1.0
Preview exposure time (sec): 1.0
Sample exposures: 2
Background exposures: 32
Final format: Shifted spectrum (cm-1) 💌
Correcti Raman spectrum (nm)
Cosmic Shifted spectrum (cm-1)
Photobleach time (min): 0.0
Preview data collection
Auto exposure Desired S/N: 100
Maximum collect time (min): 2
Help Open Save S





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

None

- Raman efficiency
 - Generally not needed advanced technique
 - Applies v^4 factor to Y-axis intensity
- Fluorescence
 - Removes fluorescence background using a polynomial fit or a reference spectrum
 - Recommended setting (order=5)

Experiment Setup - C:\My Documents\omnic\\	/Rł
Collect Bench Quality Advanced Alig	nm
Estimated time for this collection: 00:0)2:)
Collect exposure time (sec): 1.0	
Preview exposure time (sec): 1.0	٦
Sample exposures: 2	
Background exposures: 32	
Final format: Photoluminescence (nm	•
Correction: Fluorescence	•
Cosmic ra Raman efficiency	
Photobleach tin Fluorescence	
Preview data collection	
Auto exposure Desired S/N: 100	
Maximum collect time (min): 2	٦
Help Open Save	



Fluorescence Correction Example





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





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





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

Experiment Setup - C:\My Documents\omnic\VRP
Collect Bench Quality Advanced Alignme
Estimated time for this collection: 00:00:0
Collect exposure time (sec): 1.0
Preview exposure time (sec): 1.0
Sample exposures: 2
Background exposures: 32
Final format: Shifted spectrum (cm-1) 💌
Correction: Fluorescence
Cosmic ray threshold: Medium 💌
Photobleach time (min):
Preview data collection
Auto exposure Desired S/N: 100
Maximum collect time (min): 2
Help Open Save



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





- 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





Choose Collect > Collect Sample from the OMNIC menu







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



