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## DXR Raman Microscope Basic Overview



DXR User Training



# Introduction

## DXR Raman Microscope Basics

- Hardware
- Capabilities
- OMNIC
- “Go” Button
- Other presentations



# Overview of System Hardware

- DXR Raman Microscope



# Lasers, Filters, Gratings

- Lasers, filters, gratings



- iButton
  - Stores information about that particular component
  - Laser iButton has a battery to store lifetime information



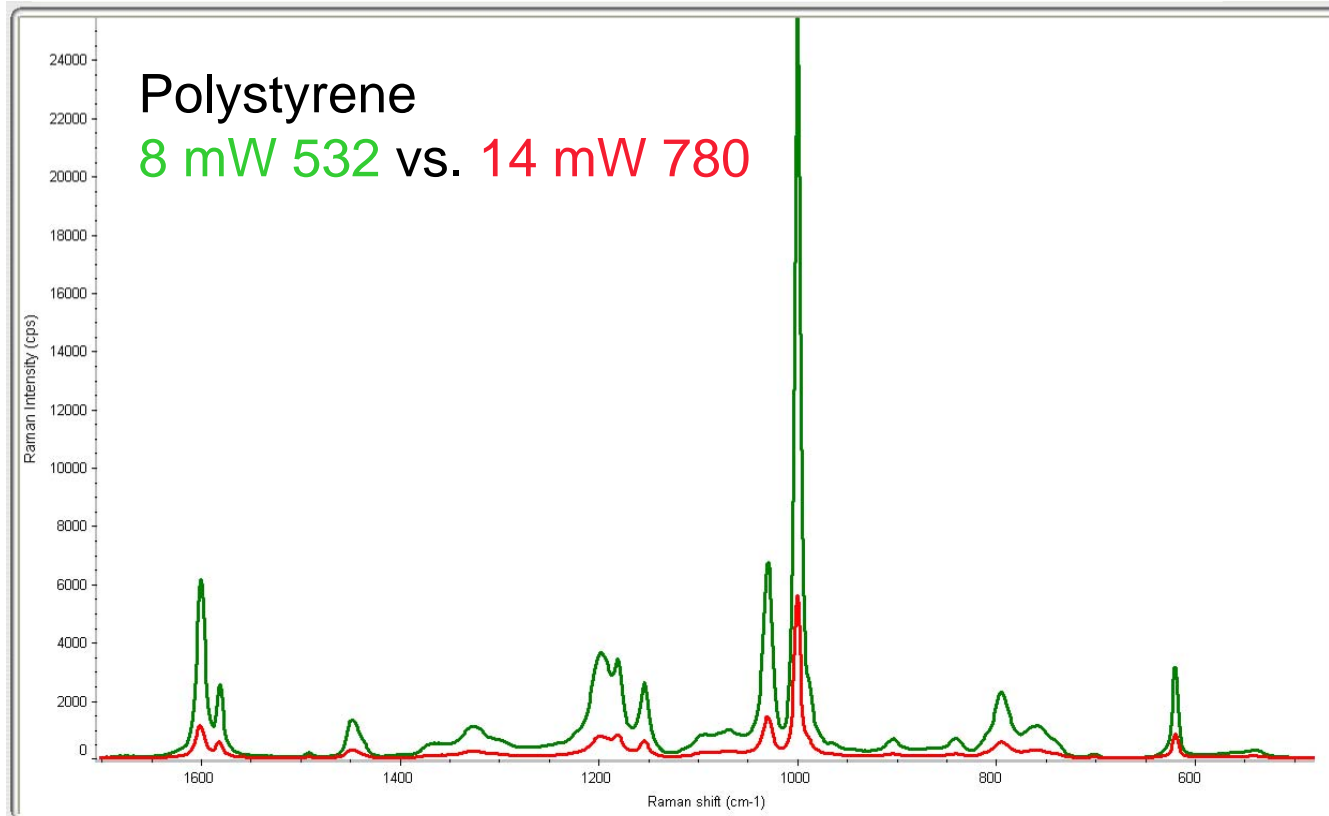
# Changing Excitation Lasers

- Why change the excitation laser wavelength?
  - Shorter wavelengths give a more intense signal
    - Raman emission is proportional to  $1/\lambda^4$
  - Less likely to see fluorescence interference at longer wavelengths
- Changing the laser wavelength means also changing the filter and the grating
  - All the DXR Raman Microscope lasers, gratings, and filters are user-exchangeable
  - Hot-swappable
  - SmartLock technology ensures reproducibility
  - Software recognizes the components
    - Alerts the user if component incompatibilities
    - Resets instrument and alignment to the settings last used with the component set



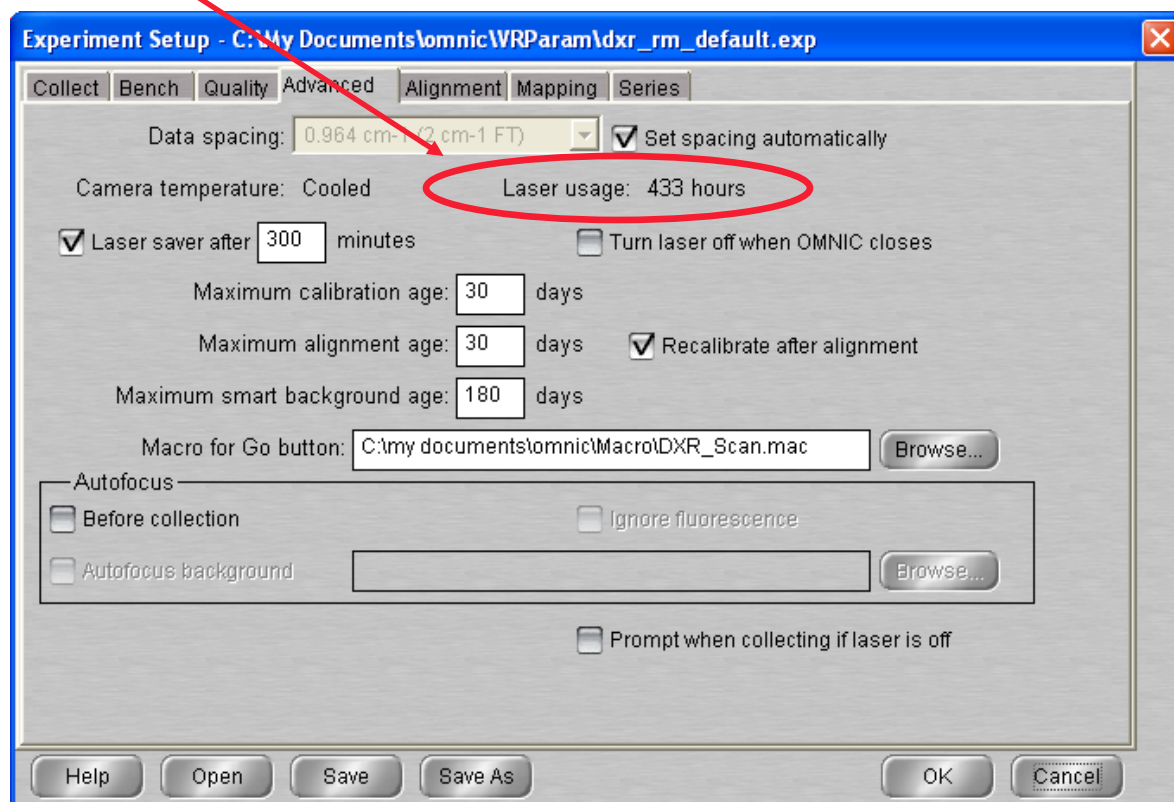
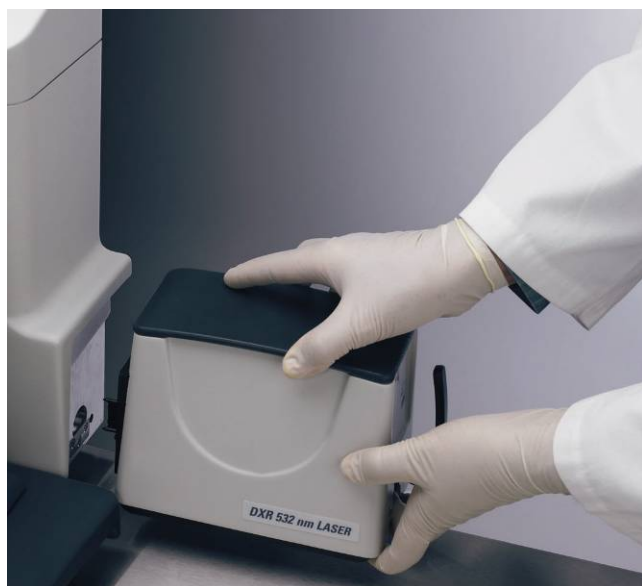
# Lasers

- Raman emission – related to wavelength
  - Most efficient Raman emission
    - 532 is 4.59X stronger than 780, 1.99X vs. 633 (using the  $1/\lambda^4$  relationship)



# Changing Lasers

- Laser lifetime tracking



# Changing Filters

- Change when changing laser wavelength





# Changing Gratings

- Can change from standard resolution to high resolution
- Change when changing laser wavelength
- Grating door
  - Keep closed when not in use – keeps out dust and fingers



# Gratings

## ■ Standard Resolution

- 5  $\text{cm}^{-1}$  resolution
- Spectral range of 50 – 3500  $\text{cm}^{-1}$ 
  - 50 to 3300  $\text{cm}^{-1}$  for 780 nm
- Good for resolving peaks

## ■ High Resolution

- 3  $\text{cm}^{-1}$  resolution
- Spectral range of 50 – 1800  $\text{cm}^{-1}$
- Gains – Higher resolution
- Losses:
  - Spectral range is approximately half – “fingerprint region” emphasized
  - Signal to noise (S/N) is lower



# Alignment and Calibration

## ■ Alignment tool

- Plugged into left side of the microscope arm, by the nosepiece

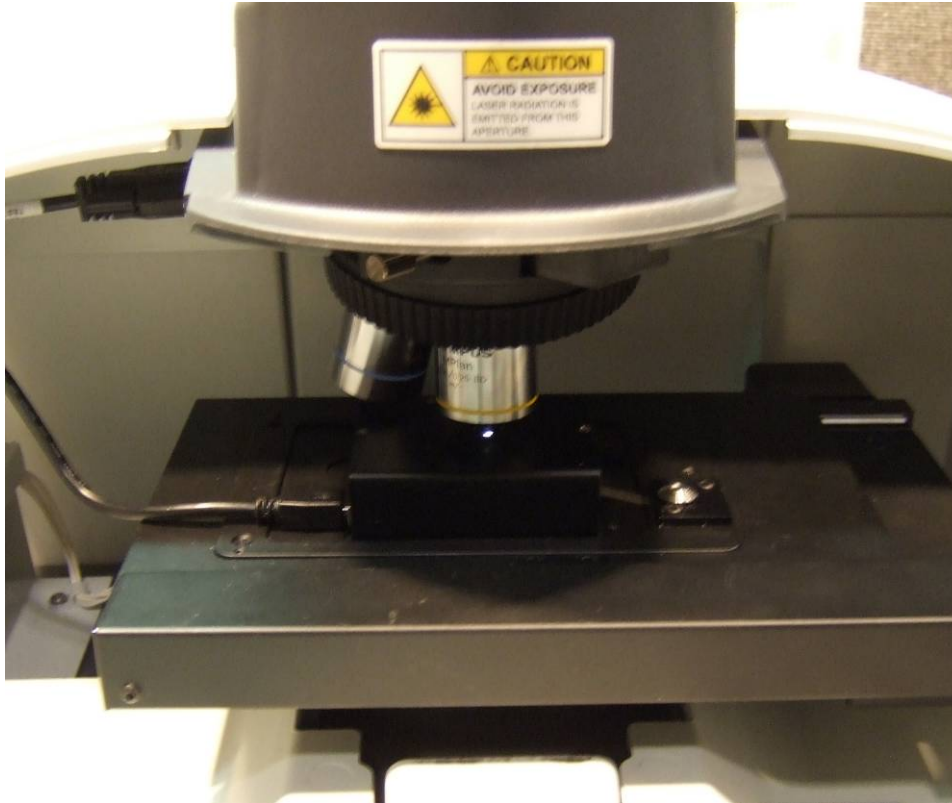
## ■ Process

- Place on microscope stage
- Focus on fiber with 10X Objective
- Run alignment – aligns 3 paths
  - Visual alignment – crosshairs in oculars
  - Aligns spectrograph
  - Aligns laser
- Run calibration
  - Calibrates: the detector, the laser, apertures, laser power, and automatic intensity correction

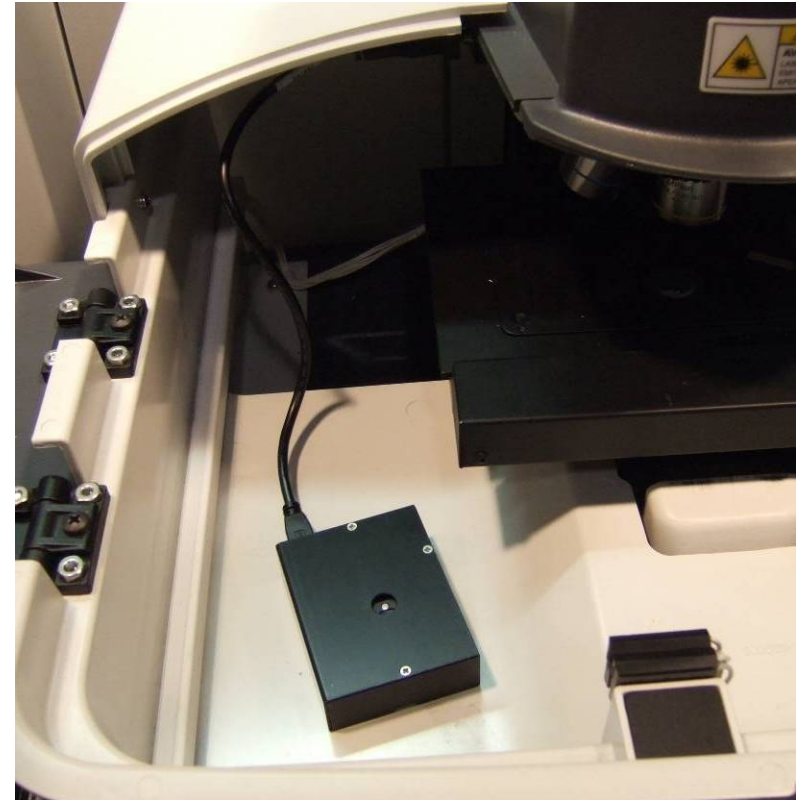


# Alignment and Calibration

- Alignment tool placement



Align tool on stage



Storing align tool



# Illuminators

- Microscope illuminator – two types



## Brightfield (BF)

Uses long life LED

Power control knob on top of illuminator



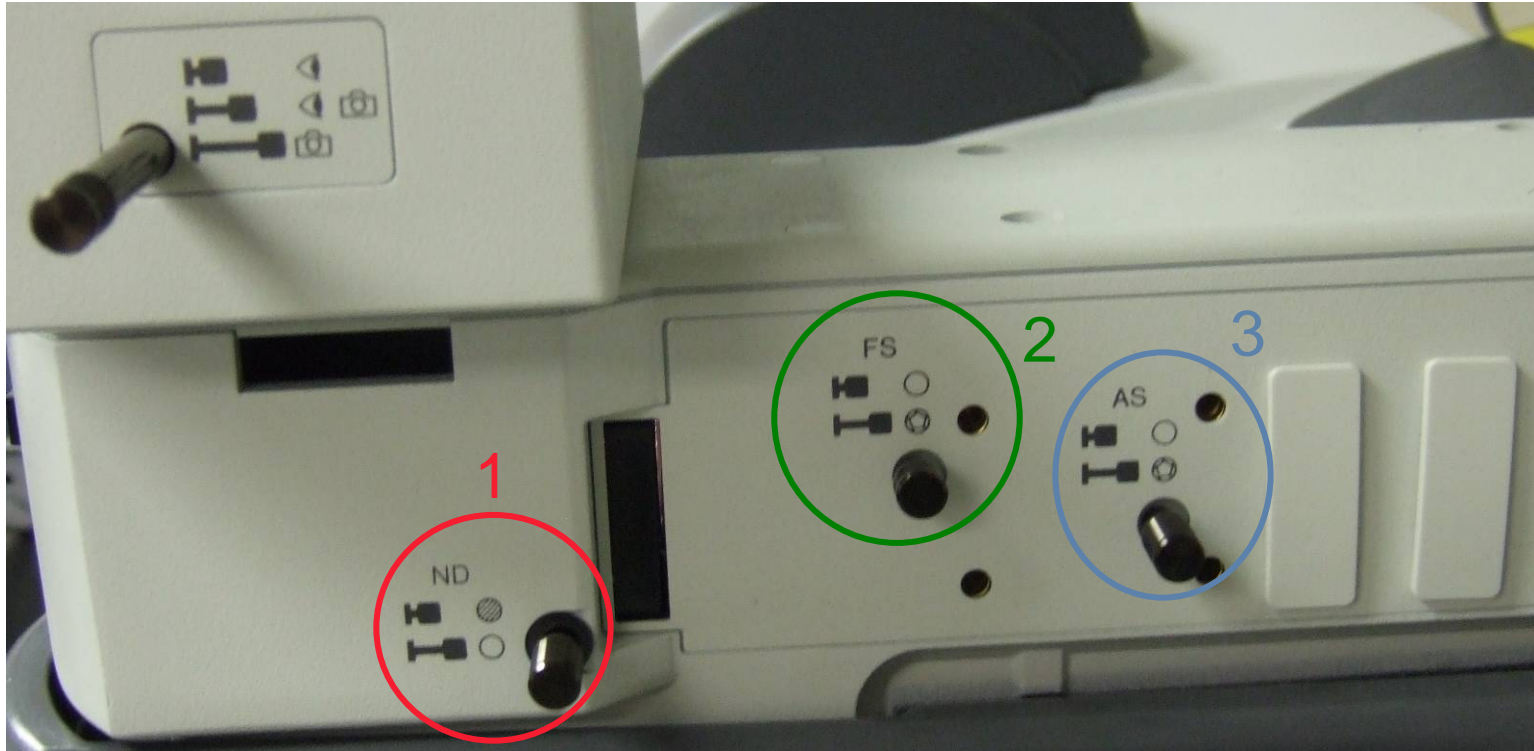
## Brightfield/Darkfield (BF/BD)

Uses powerful tungsten/halogen

Separate controller and power supply

# Brightfield/darkfield Illuminator

- Some options on the brightfield/darkfield illuminator

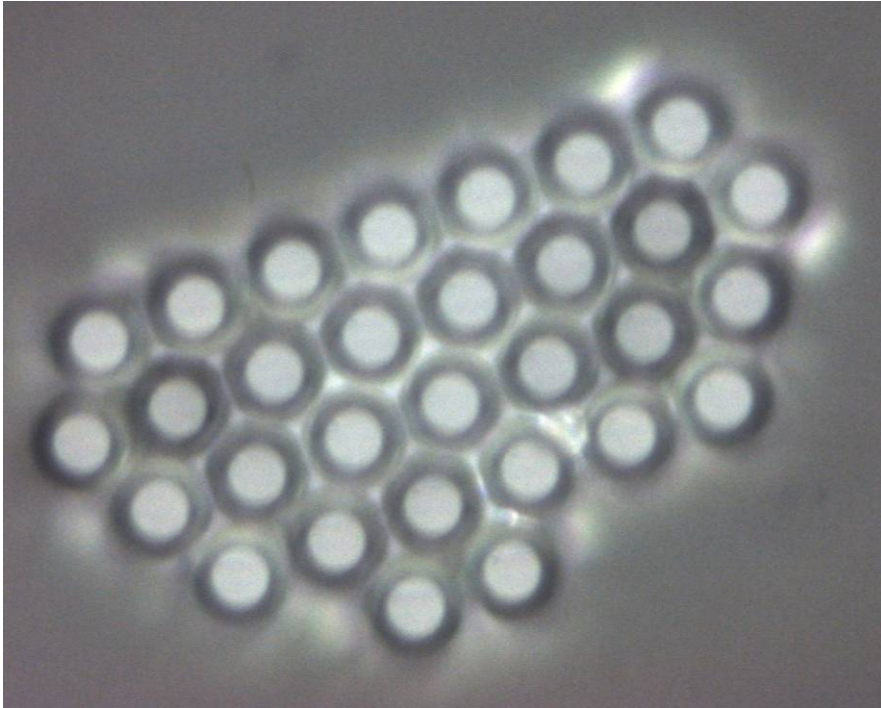


- 1 Darkfield illumination – for indirect sample illumination
- 2 Field stop – for when careful focusing on a sample is wanted
- 3 Aperture stop – for when contrast adjustment is needed

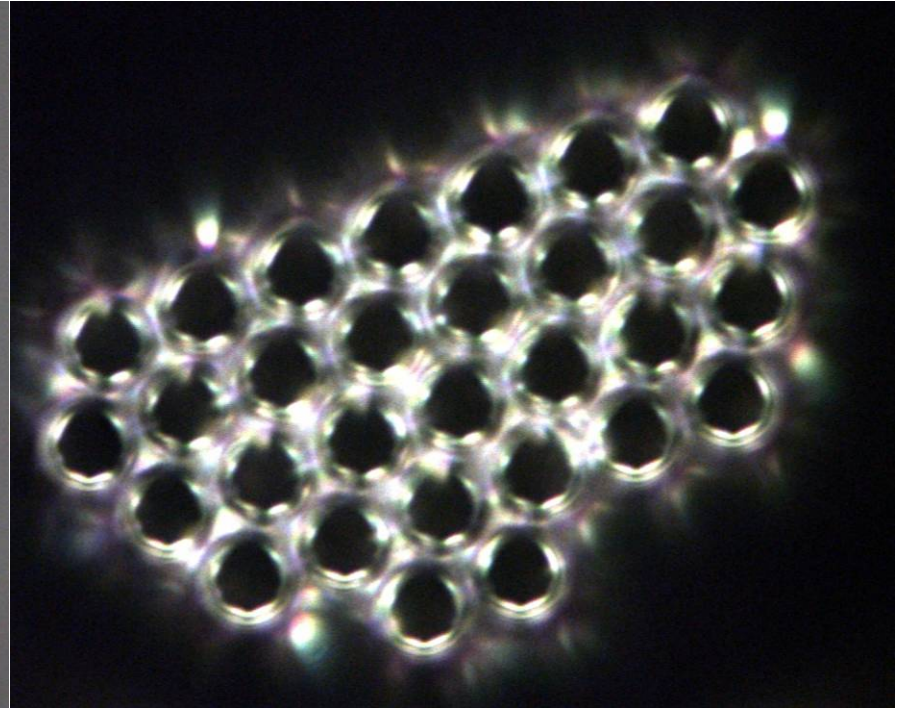


# Illuminators

- Brightfield versus Darkfield Images



Brightfield



Darkfield

10 micron polystyrene beads, 100X magnification

# Nosepieces and Objectives

- Nosepieces

- Brightfield (BF)
- Brightfield/darkfield (BF/BD)



- Objectives

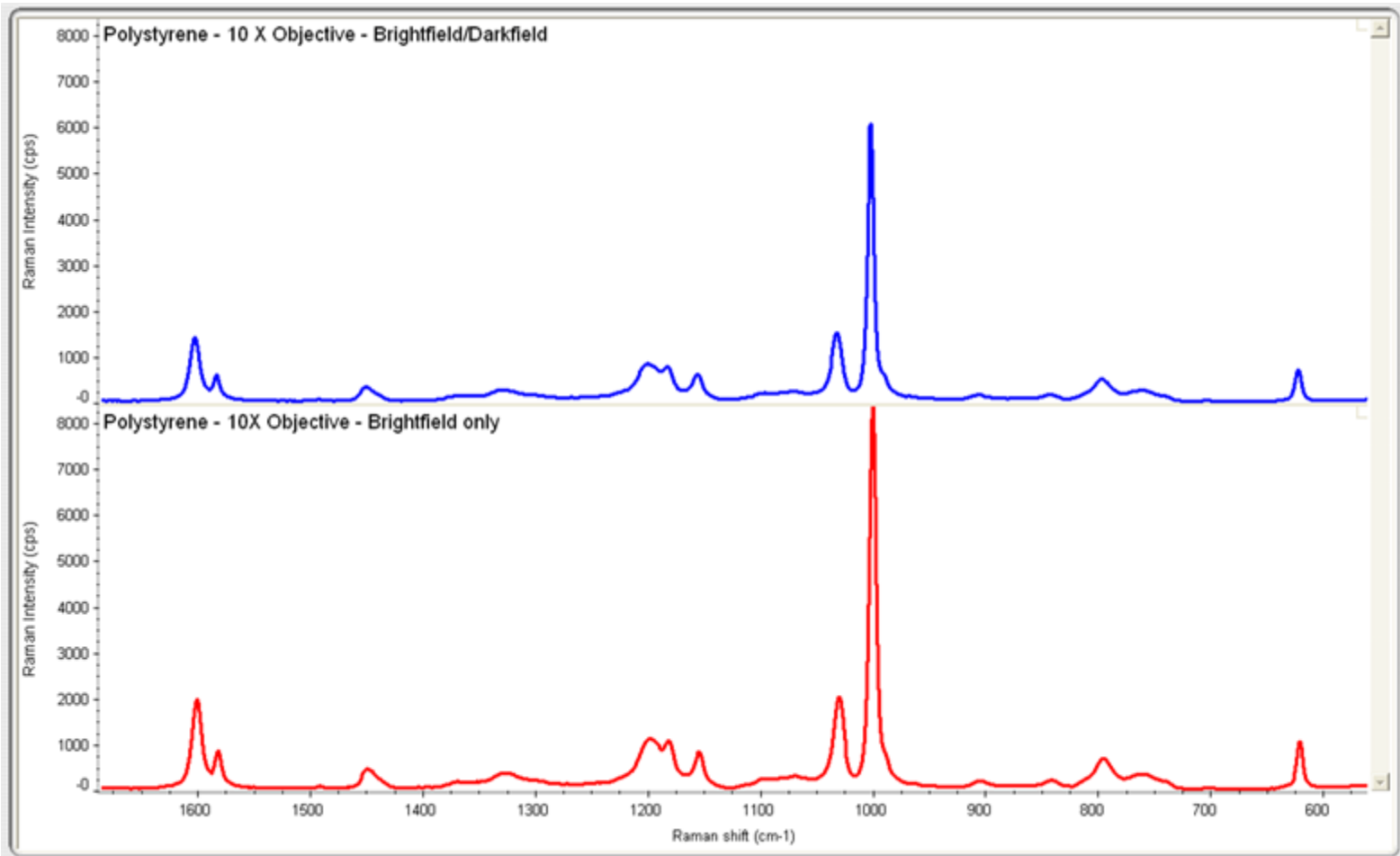
- Brightfield and brightfield/darkfield
- Adapter – to use BF objectives on BF/BD nosepiece





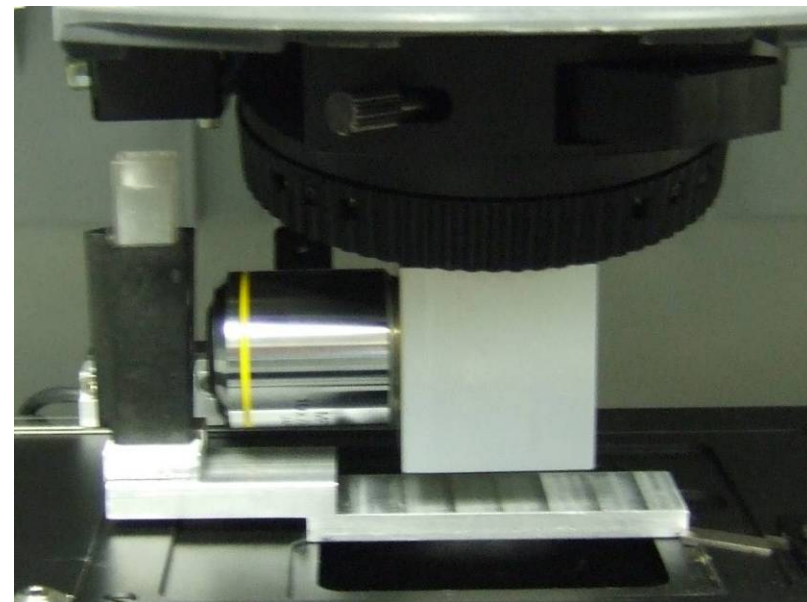
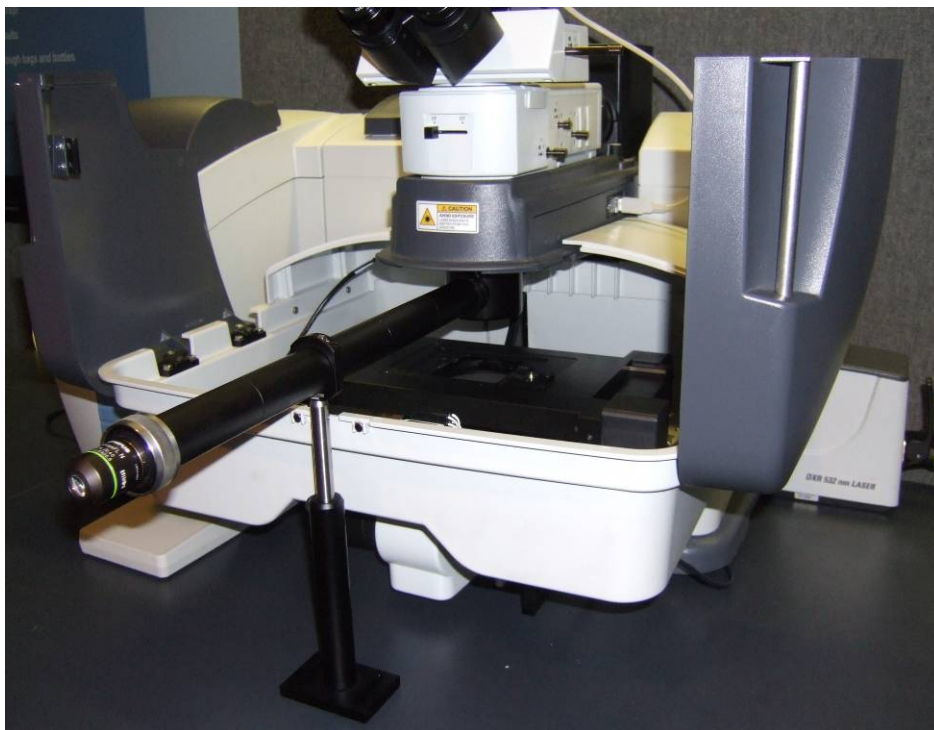
# Microscope Objectives

- Collection efficiency



# Adapters

- Macro adapter
  - Good for cuvettes
- Extended reach macro adapter



For larger samples that won't fit on the stage or in the enclosure

# Microscope Stages

- Manual
- Motorized
  - Optiscan – 1 micron steps
  - ProScan – 0.1 micron steps
  - Z-focus
    - Joystick



Manual



OptiScan

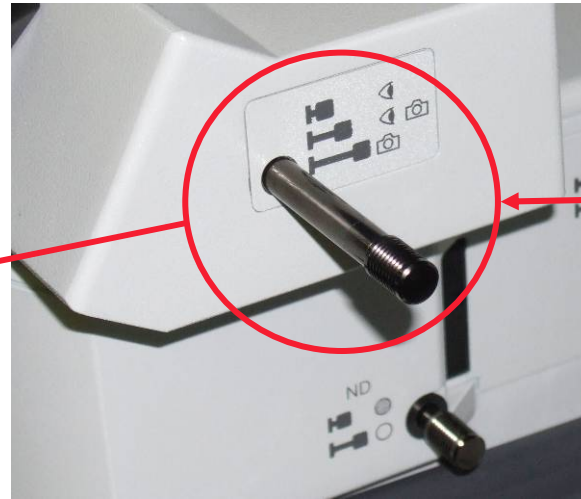


ProScan



# Video Camera

- Video camera
  - Video microscopy features image capture for documentation and reports, and allows specimen viewing at high resolution on the computer monitor.

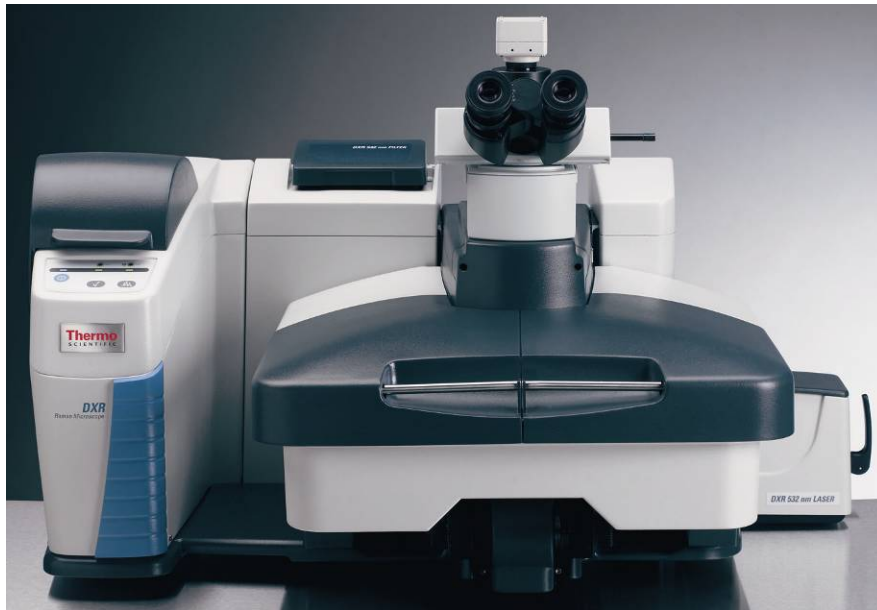


Slider set to middle position for video and ocular viewing

- Can view your sample using  $\mu$ View or Atl $\mu$ s software
  - Capture images for presentations or reports

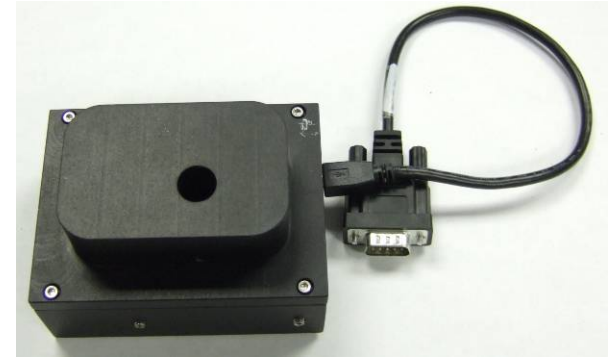
# Safety Features

- Class 1 enclosure
  - Also reduces stray light
- Beam blockers



# Fiber probes

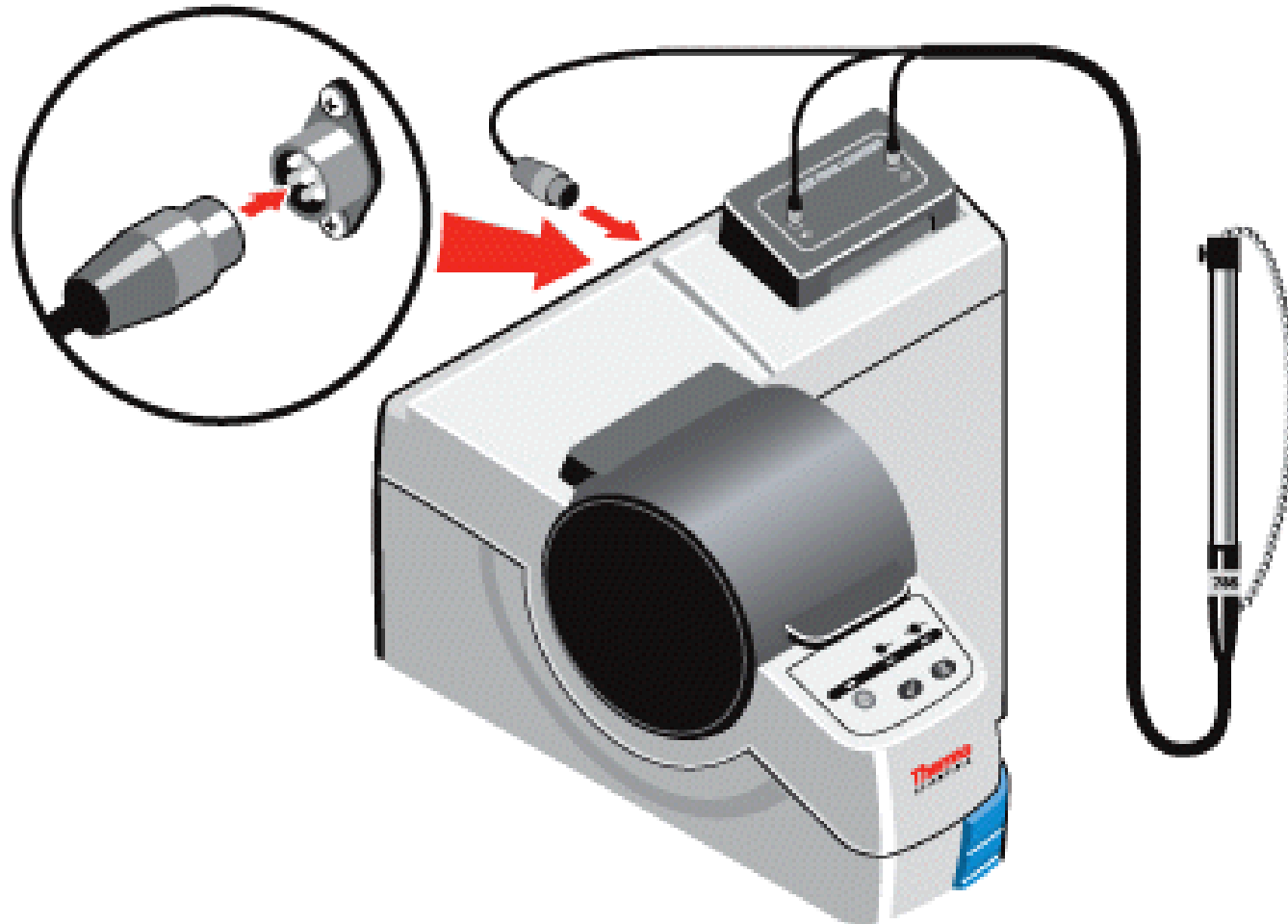
- Fiber launcher
- Cables
- Alignment and Calibration
- Class 3B operation – safety eyewear





# Fiber probe

- Installation of the fiber launcher and probe





# Fiber probe

- Using the alignment and calibration tool for the fiber probe.







# System Maintenance

- Alignment
- Calibration
- Checking magnets
- Cleaning optics
- Longer, separate presentation



# Overview of System Capabilities

- Single Point Sampling
- Mapping
- Confocal Capabilities
- Depth Profiling
- Array Automation



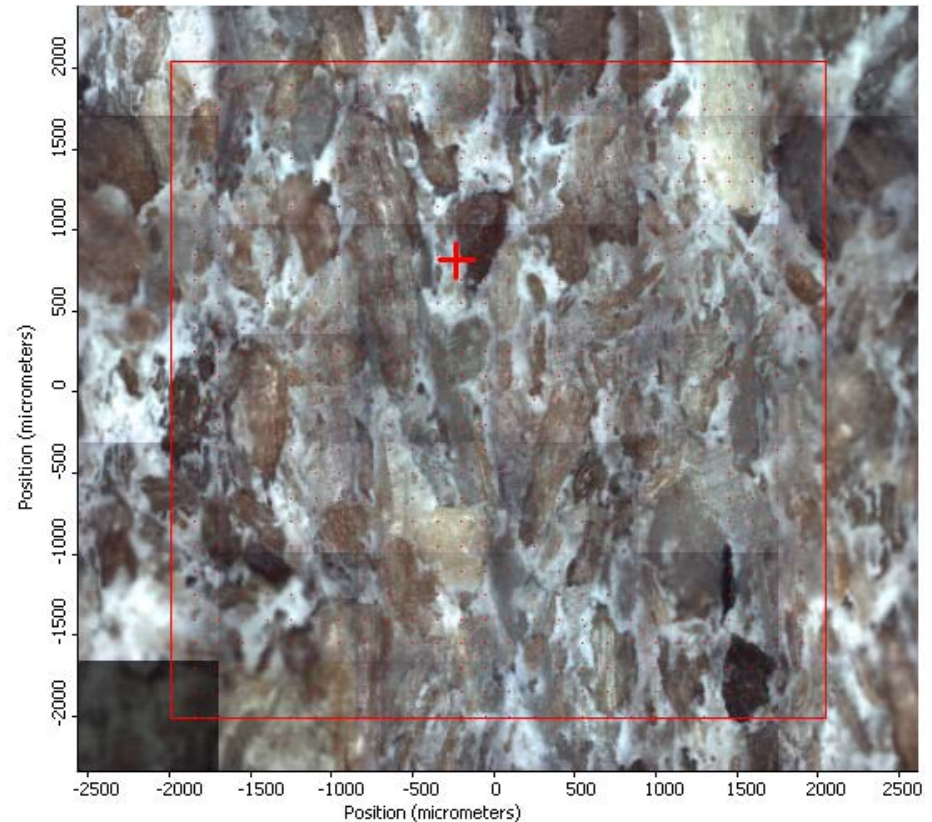
# Apertures

- 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

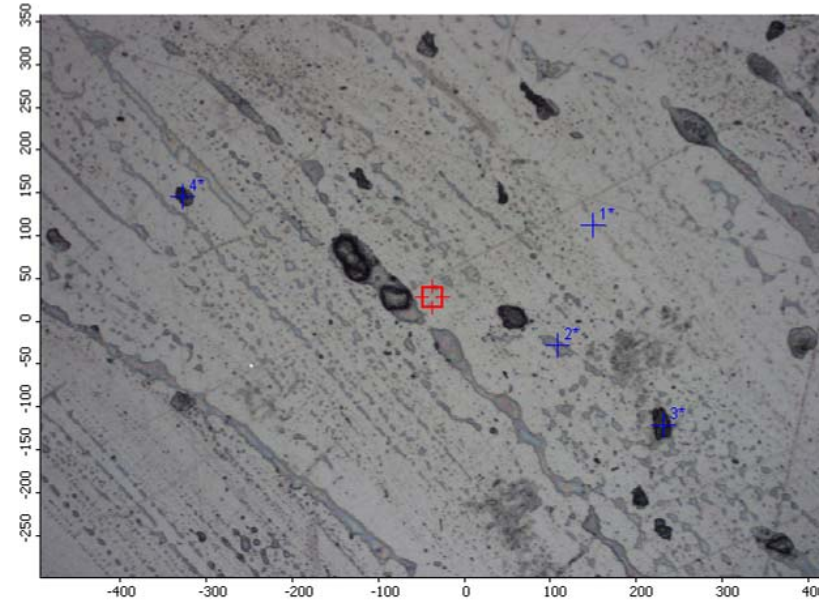
# Single Point Mapping

- Select a point or points to analyze
- Set collection parameters
- Collect
- Analyze



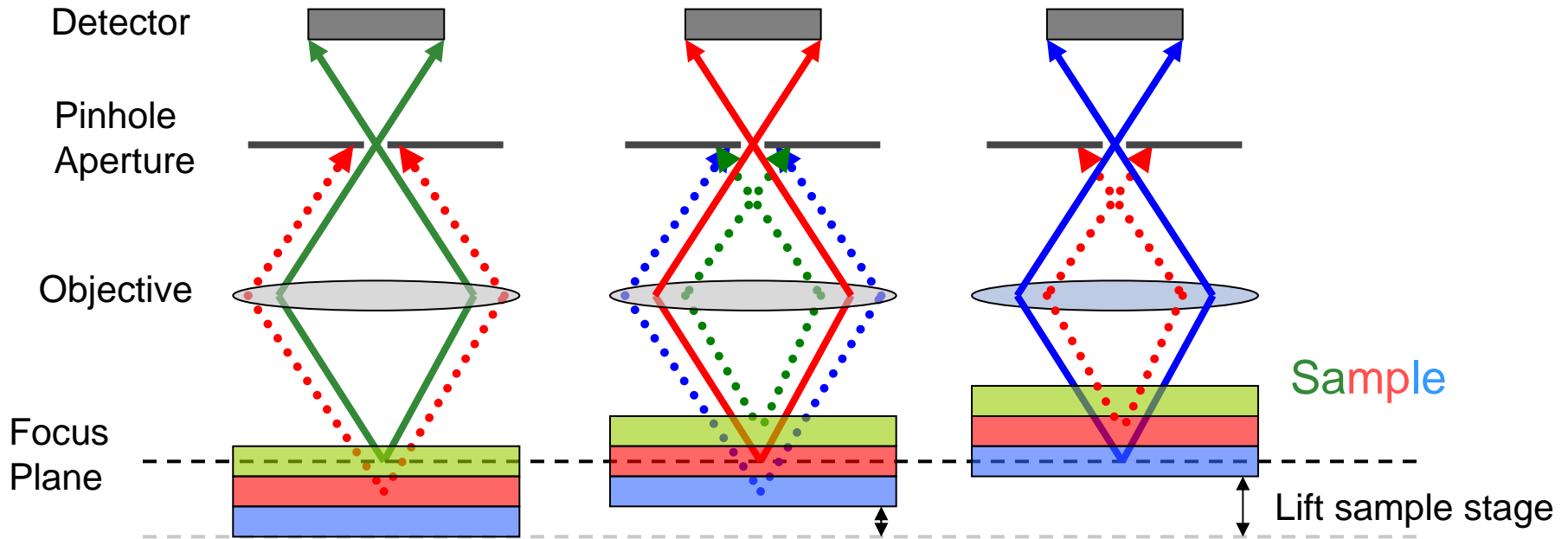
# Mapping

- Multi-point mapping
- Line mapping
- Two dimensional mapping
  - X-Y mapping
  - Cross sectional mapping – down into the sample, Z-axis
- Separate Atl $\mu$ s presentation that covers mapping and software in depth



# Confocal Capabilities

- Raman signal from focused laser point
- Non-destructive depth analysis
  - Depth selected by focusing Z-axis of microscope stage
- Depth resolution better than 2 microns achievable





# Depth Profile

- Select point for the Depth Profile
- Check Collection parameters
- Check Mapping parameters
- Collect Map

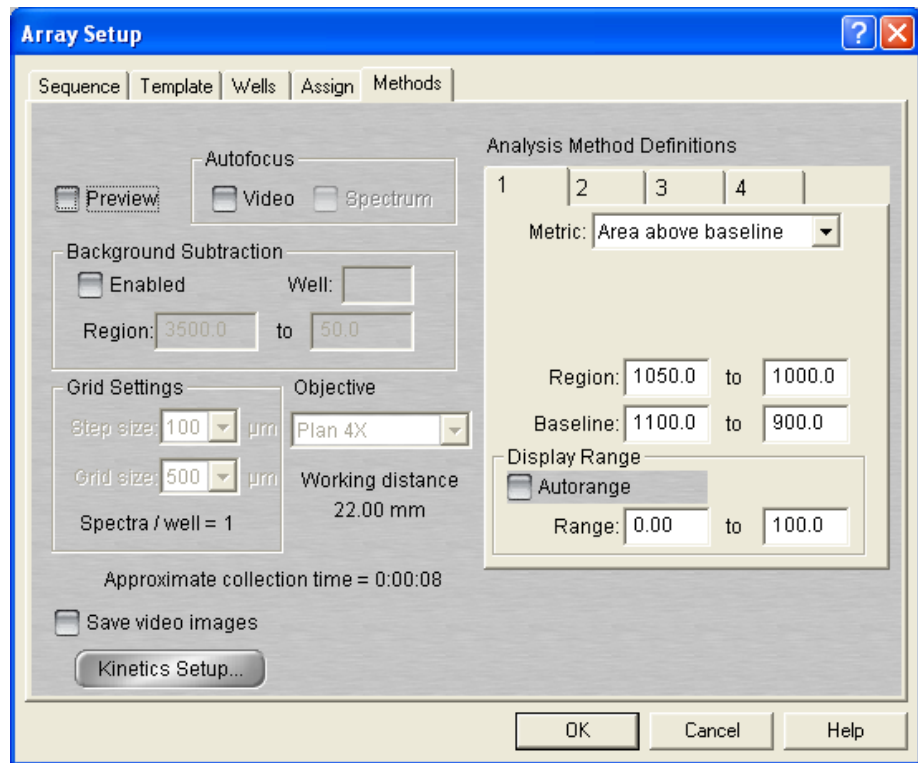
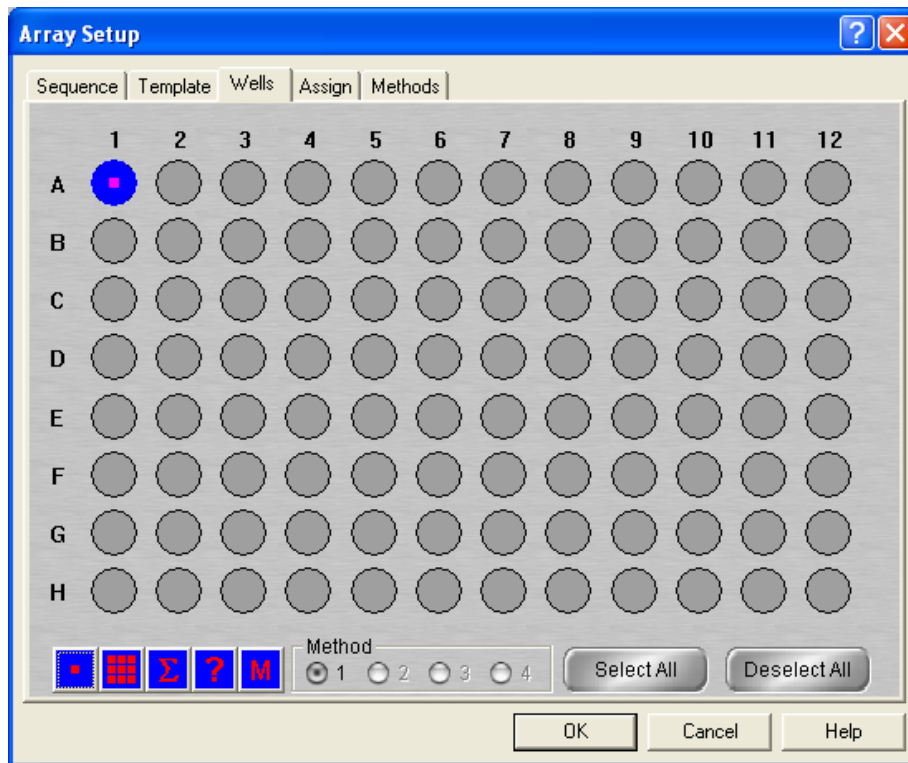
3 micron inclusions

Inclusion



# Array Automation

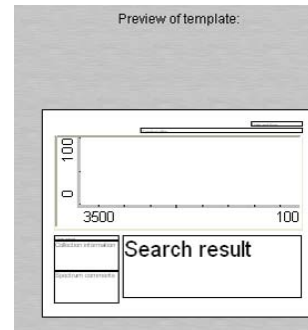
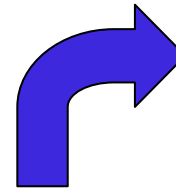
- Array automation software package add-on
  - Automated analysis of well-plates, capillary tubes, or tablets (using custom holder)



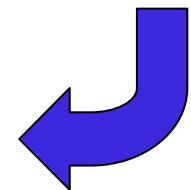
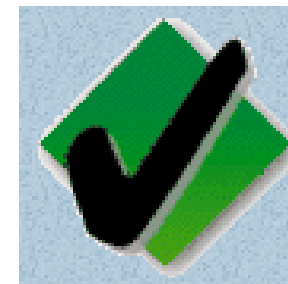
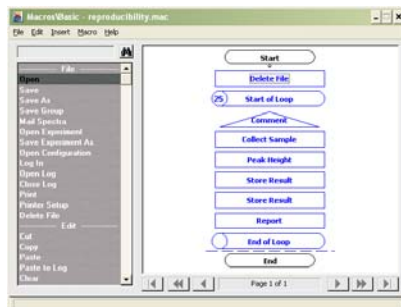
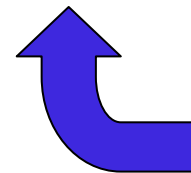
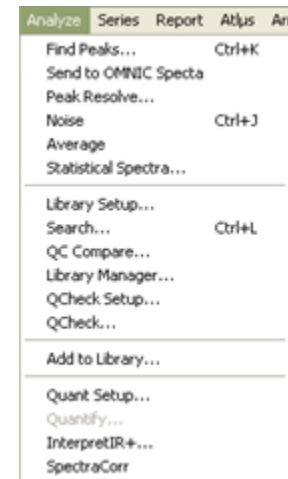
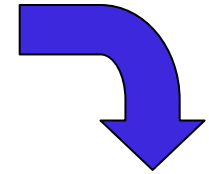


# OMNIC 8 Software for Dispersive Raman

- Fully featured software
  - Collection
    - Complete data collection
  - Processing
    - Corrections, smoothing, math
  - Analysis
    - Library searching, peaks, quant.
  - Reporting
    - Customized reporting features
  - Macros can do all the above

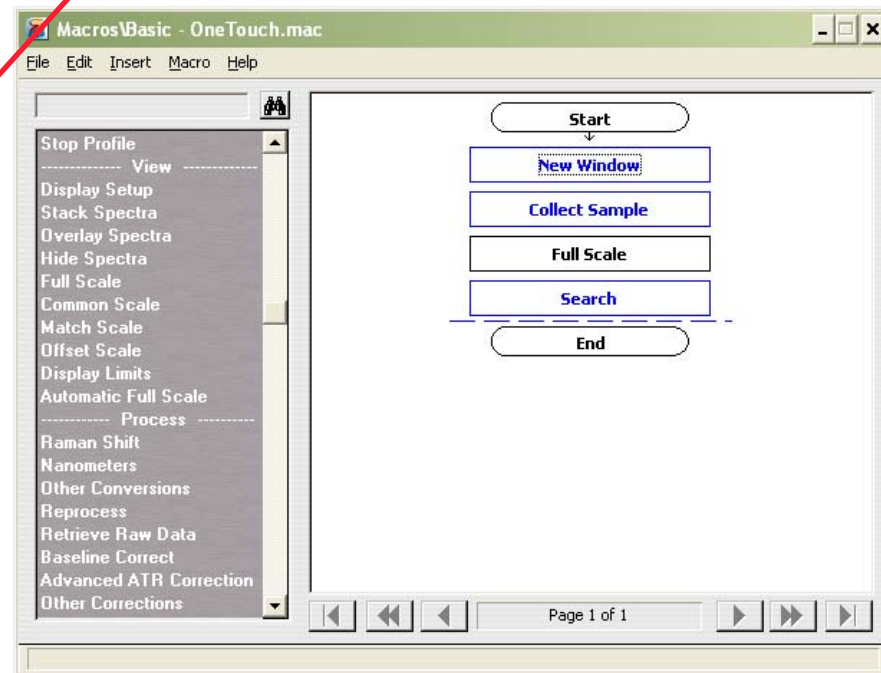
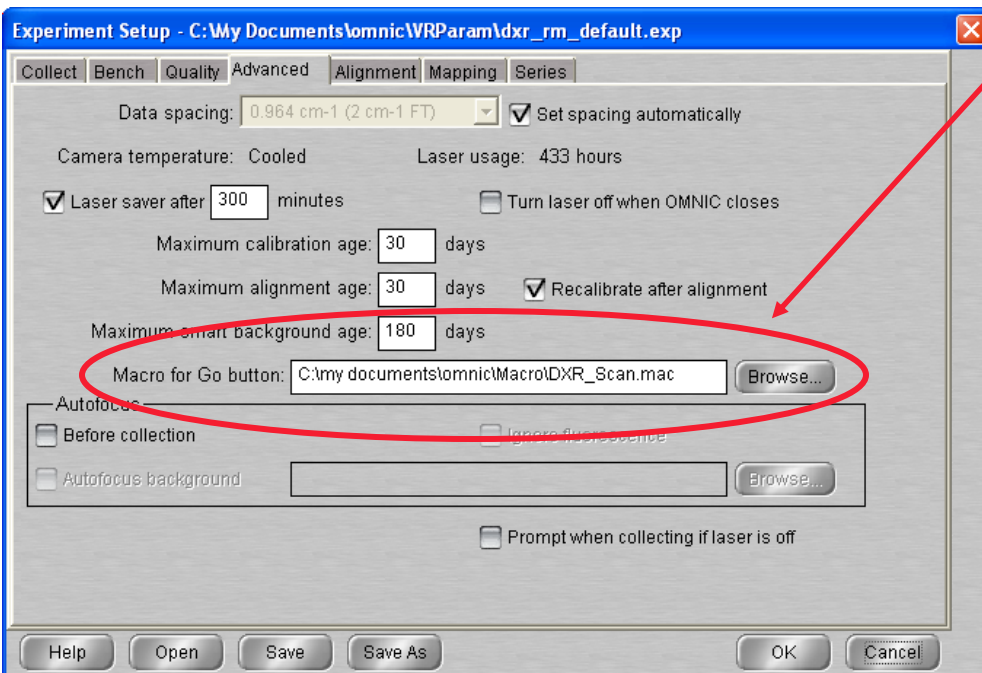


OMNIC



# “Go” Button

- Where to set it up
- How to set it up
- Macros Basic
  - Separate presentation





# Follow-up Presentations

- Data collection
- Atl $\mu$ s
- Setting up and using Macros
- Instrument Maintenance