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TM028 – Experimental process (mapping dedicated) WiRE™ 5

The inVia is great for high speed and high performance Raman mapping / imaging. Following the below suggested workflow will help optimise the speed, efficiency and information you can get from inVia.

Overview of the experimental process – specific for spatial analysis

Stage 1. Determining the suitable analysis option

- Determine the analysis option (see TM027)

Stage 2. Optimising the focal plane

Basic

- View the sample area of interest. Determine your map area.
  - A live video montage helps define a map area that is larger than the live video view.
- Move to different corners of the area to determine if the surface is flat, and level (double click on the white light montage to do this). If not, consider the Surface (TM007) or Focus Track (TM006) options, or level mechanically using an adjustable stage.
- Move to a location within the area you would like to analyse, and ensure the white light is in focus by looking for sharpness of the details in the live video view (or minimising the laser spot / line size).

Advanced

- Optimise the focal plane for Raman mapping by depth profiling at one location. This can then be used to set the Z value to be used for XY area mapping, or the Z range and step size for XYZ volume analysis:
  - ensure the geometry of the laser focus is the one you will use for mapping (spot focus for point mapping and StreamLineHR, line focus for StreamLine)
  - collect point spectra with the mapping objective—over the spectral range of interest—to determine the laser power the sample can tolerate without inducing photochemical or thermal damage.
  - set up a depth series measurement. For details, please read ‘Depth profiling’ (TM005):
    - specify the determined spectral range (which covers Raman bands of interest)
    - use the determined laser power
    - apply 1 s / spectrum exposure time (modify for stronger or weaker scatterers)
    - go to the *Depth series* tab, set-up the measurement so that it starts a few micrometres below the current z focus and finishes a few micrometers above the current z focus. For example, if the current z focus is at 0 µm, start at -3 µm, opt for 7 acquisitions at an increment of 1 µm, so that the measurement finishes at 3 µm. These values may vary depending on your sample.

  Review the spectra in the depth series to determine the optimal Z value by either:
  - cycling through the spectra while enabling the data label (right click on *Spectrum Viewer* window, scroll to *Labels*, select *Show dataset axis labels*). Determine the focal plane where the spectral quality is the highest (e.g. highest intensity at peaks of interest, lowest fluorescence baseline).

- or map review (e.g. intensity at point, signal-to-baseline, signal-to-axis) to generate profiles of peak intensities or integrated areas against z.

Stage 3. Optimising the exposure time and laser power

- Move to determined z plane, by using the track ball or typing the value in the z coordinate field of the xyz stage control.
- Collect point spectra using a laser power that does not damage the sample but is sufficiently high for exciting Raman scattering. Alter the exposure time until you find lowest exposure time that allows for the information you require to be gathered.
    - If any part of the sample is expendable, collect data at these regions first to determine the maximum power which can be used without sample damage
- Record the optimal laser power and exposure time (or minimum exposure time, for high speed imaging).

Stage 4. Define analysis region

- Enable live video viewing. Move the stage height back to the optimal white light focus plane if the view has become out of focus (blurred) using the track ball.
- For mapping experiments, determine the spatial area and step size for analysis.

Stage 5. Specify experimental parameters

- Specify the experimental parameters as determined in previous stages.

Optimising StreamLine imaging time

- If the sample has a damage threshold using a spot laser, the optimal exposure time may be estimated by the equation:

  $T = t*50/pb$

  T = exposure time$_{\text{StreamLne}}$

  t = exposure time$_{\text{spot focus or line focus single spectrum}}$

  p = Laser power increase factor

  b = y binning factor

  For example, if 1 s is optimal for a single spectrum acquired with the spot focus (for any given laser), but the laser power is limited to 10% before damage occurs, the exposure time for StreamLine imaging with binning factor of 1 is 1*50/100*1=0.5 s (typically the line focus power can be increased by at least a factor of 10 compared to the spot focus).

- If the sample does not have a damage threshold for the given spot laser, the optimal exposure time may be estimated by the equation:

  $T = t*50/b$

  For example, if 1 s is optimal for a single spectrum acquired with the line focus, the exposure time for StreamLine imaging with binning factor of 2 is 1*50/2=25 s.

Tips:

- match the step size in x to that in y if binning is applied to ensure the pixels are square
- apply *Slalom* if the information between the points is of interest.

The exposure time may be further altered. Once the mapping begins, the spectra will be updated on the screen as they are acquired. You can abort the experiment and alter the exposure time according to the spectral quality. (This is often the fastest and most practical approach)

Stage 6. Collect data

- Before the data collection begins, move the z plane to that determined in stage 3.
- Collect data.