

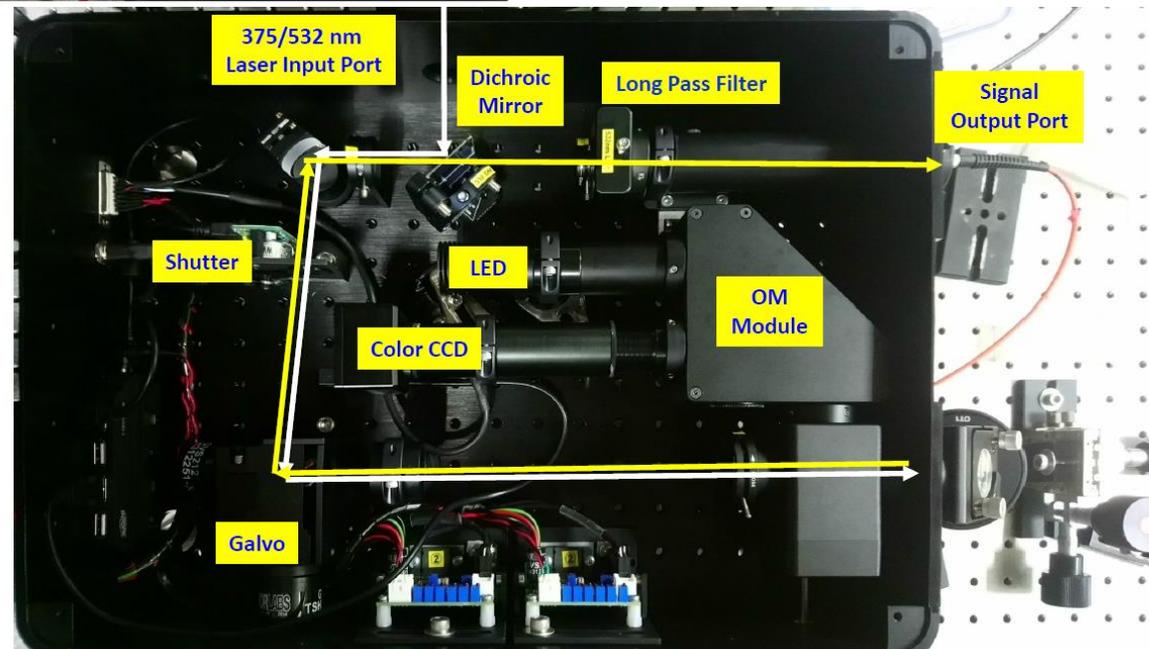
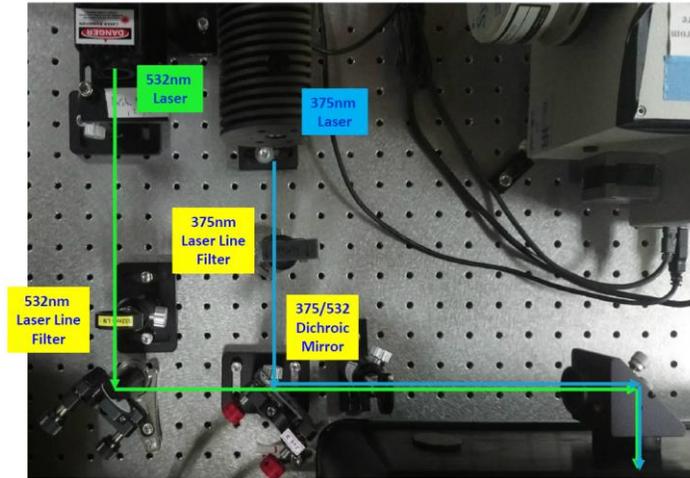
2023光電工坊教育訓練課程@NTOU

雷射掃描共軛焦光譜顯微鏡

2023/11/28

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Optical Layout of LSCM 4.0



開機流程

1. 開啟各項設備電源

- 檢查各儀器間的連線狀態

2. 開啟軟體

- 執行Scanner4.3軟體
- 檢查參數檔
- 檢查DAQ和Shutter連接狀態
- 確認PMT訊號源設定
- 檢查Galvo是否正常工作，置中Galvo
- 檢查COM和CCD連接狀態
- CCD降溫
- 設定與微調光譜儀

3. 檢查/優化系統光路

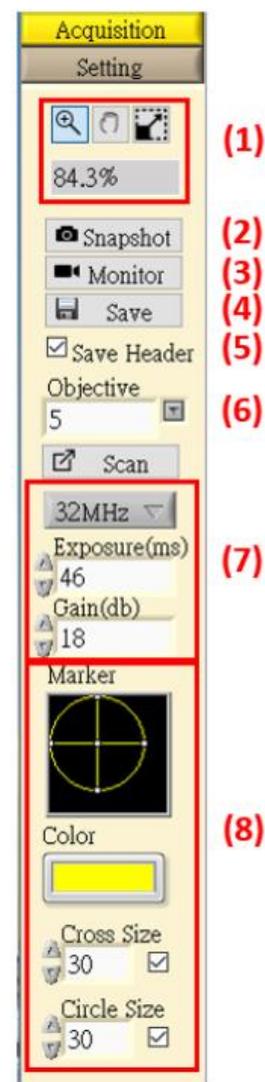
- 切換雷射光路
- 使用pinhole檢查光路，必要時微調外部鏡子
- 使用標準樣品測試系統效率
- 優化共軛焦點（樣品和收光端）

量測: 1) OM影像

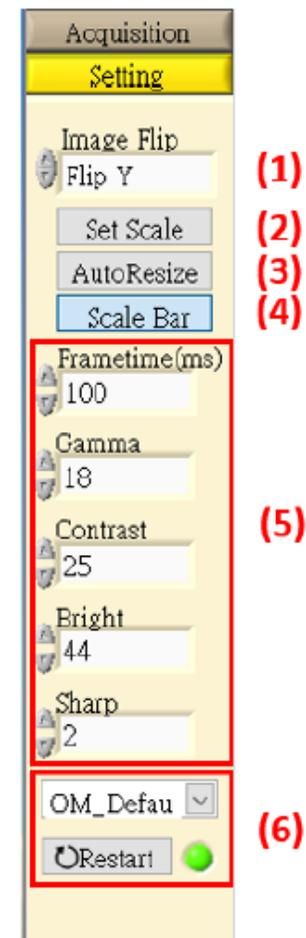
- 開LED燈
- 切換雷射/OM光路
- 調整樣品聚焦和移動視野
- 設定各參數
- 存取

OM分頁

Acquisition tab

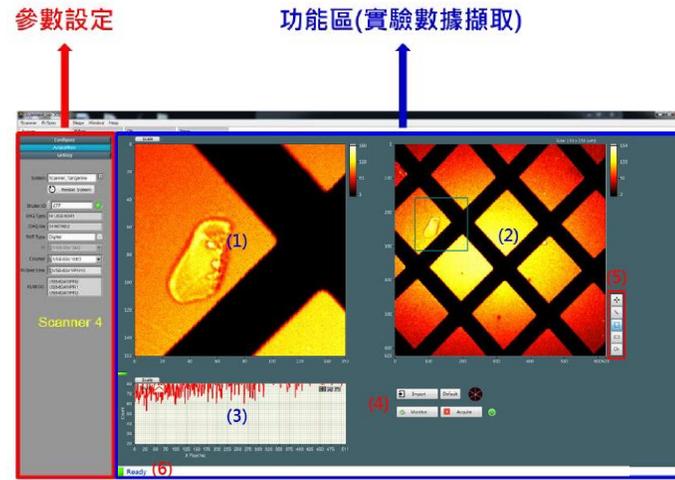


Setting tab

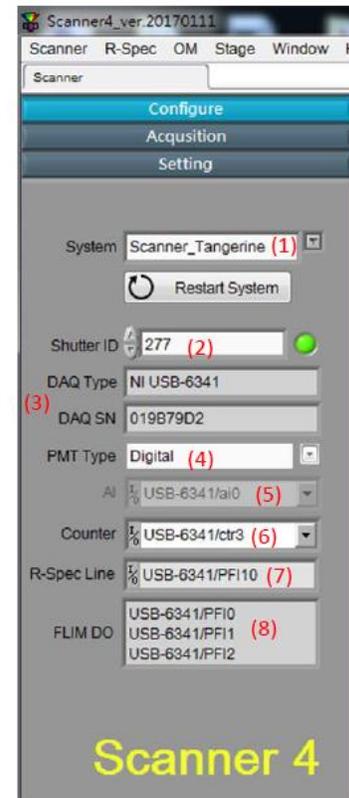


量測: 2) PMT掃描

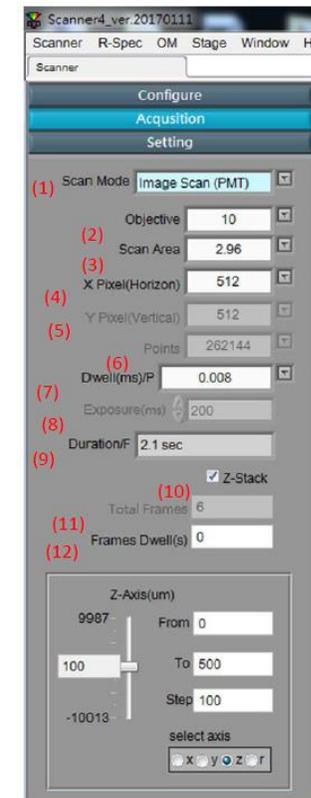
- 切換雷射/OM光路
- 連接光纖到PMT
- 開啟PMT
- 設定各參數
- 開始掃描 (Monitor) ! 注意: 避免PMT過曝
- 調整樣品聚焦
- 根據掃描影像品質進一步調整各參數
- 各種掃描模式
- Import reference 影像



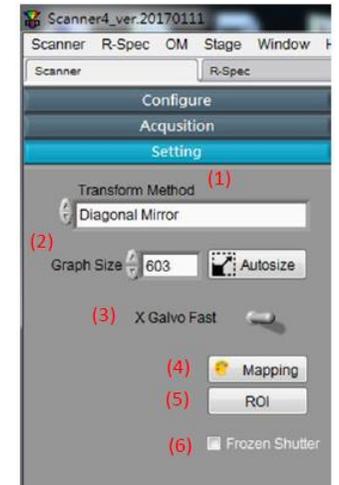
Configure tab



Acquisition tab

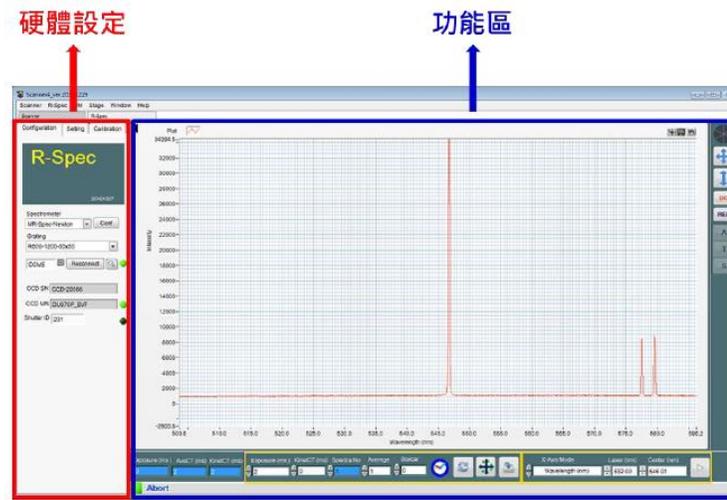


Setting tab



量測: 3) 單點光譜

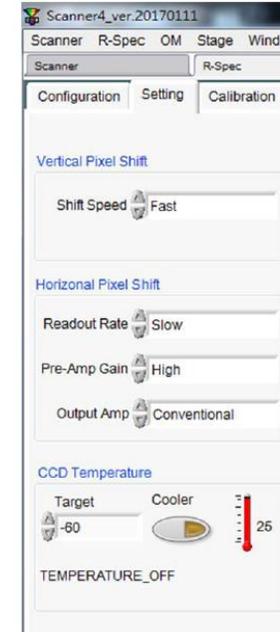
- 連接光纖到光譜儀
- 設定量測中心波長 (Center)
- 設定積分時間 (Exposure)
- 連續單點光譜量測 (Monitor) ! 注意: 避免CCD過曝
- 調整樣品聚焦
- 根據光譜品質進一步調整各參數
- 去除背景光譜
- 各種光譜擷取模式



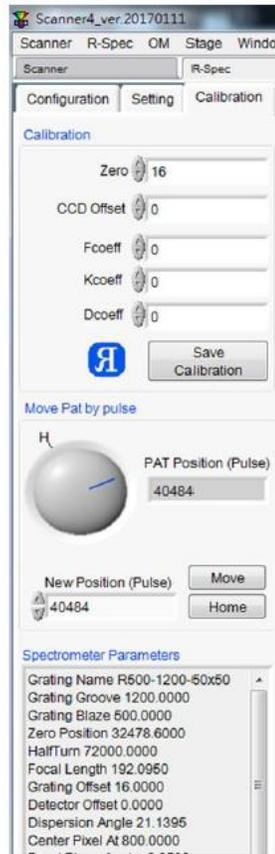
Configuration



Setting



Calibration



量測: 4) 光譜掃描

- 根據單點光譜量測確定量測參數後，切換到Scanner主頁
- Scan Mode切換到Spectral Scan
- 設定各量測參數
- 開始光譜掃描 (Acquire)
- 各種掃描模式

Acquisition tab

The screenshot displays the 'Acquisition tab' of a software interface. At the top, there are tabs for 'Scanner', 'R-Spec', 'OM', 'Stage', and 'C'. Below these, there are sub-tabs for 'Scanner' and 'R-Spec'. The main content area is divided into sections: 'Configure', 'Acquisition' (highlighted in blue), and 'Setting'. Under the 'Acquisition' section, the following parameters are visible:

- Scan Mode: Spectral Scan (dropdown menu)
- Objective: 5 (dropdown menu)
- Scan Area: 1.00 (dropdown menu)
- X Pixel(Horizon): 32 (dropdown menu)
- Y Pixel(Vertical): 32 (dropdown menu)
- Points: 1024 (dropdown menu)
- Dwell(ms): 0.008 (dropdown menu)
- Exposure(ms): 200 (input field)
- Duration/F: 17 min 44.8 sec (input field)
- Special Mode: None (dropdown menu)
- Merge Time-Lapsed Spectral Mapping
- Total Frames: 1 (input field)
- Frames Dwell(s): 0 (input field)

At the bottom, there is a section for 'X-Axis()' with a vertical slider and input fields for 'From 0', 'To 100', and 'Step 20'. Below this, there is a 'select axis' dropdown menu with options 'x', 'y', 'z', and 'r'.

關機

- CCD回溫
- 關閉所有軟體
- 關閉所有儀器電源（CCD溫度回到室溫後）

雷射

波長影響：

- 螢光效應
- 激發效率
- 共振效應
- 損壞樣品

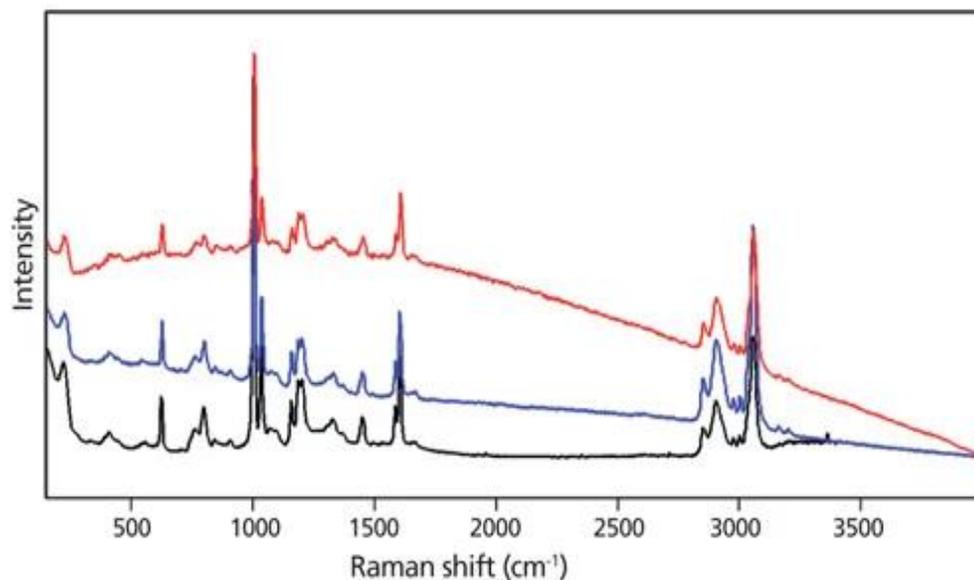
頻寬影響：

- 光譜解析度
- 低頻拉曼光譜

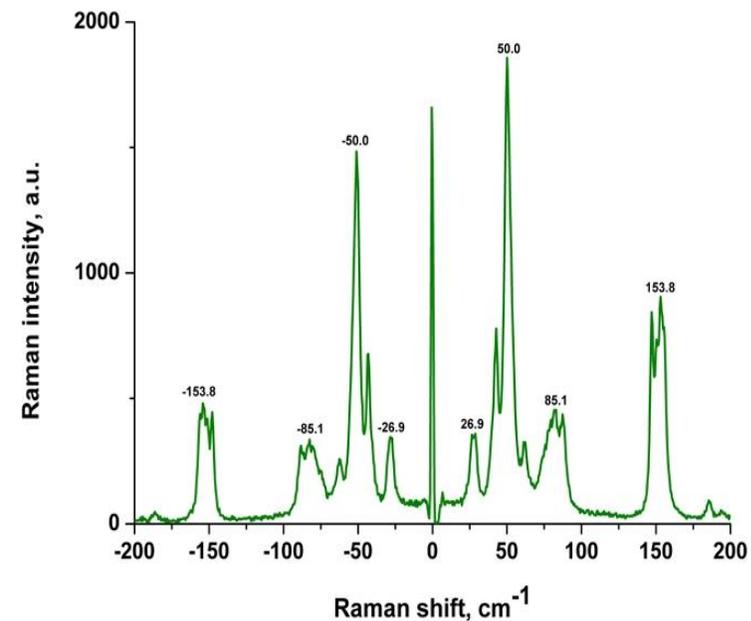
功率影響：

- 損壞樣品

Raman spectrum of polystyrene under different excitation wavelength



Raman spectrum of sulfur

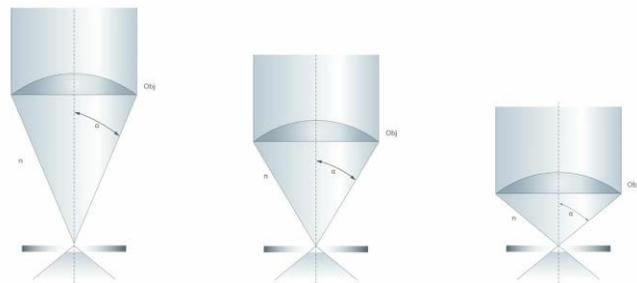


光學濾片種類

- **Laser line filter**
- **Dichroic mirror/Beamsplitter**
- **Long-pass filter**
- **Notch filter**
- **ND filter**

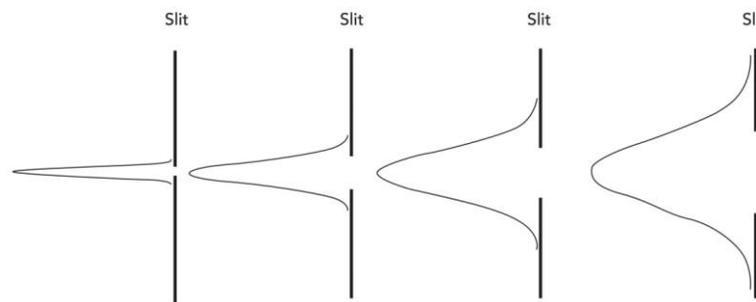
物鏡影響

- 激發強度
- 掃描解析度



光譜儀影響

- 狹縫寬度：光譜解析度
- 光柵：光譜解析度, 光譜頻寬, 分光效率

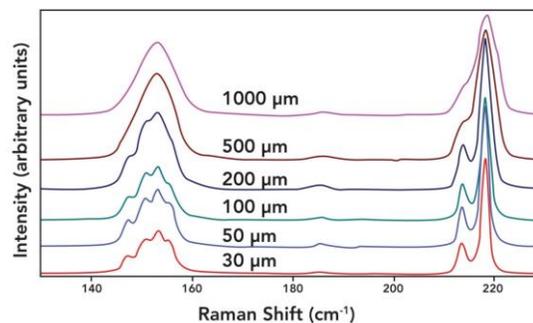


光偵測器 (PMT/CCD) 影響

- 感光效率
- 光譜解析度 (CCD)

光纖孔徑大小影響

- 訊號強度
- 光譜與掃描解析度



Reader2

- 讀取Scanner4.3存取的影像和光譜
- 把檔案轉存成ASCII檔

1. File -> Load
2. Reference影像
3. 光譜或訊號強度
4. 檔案資料
5. Scale
6. 切換ROI
7. 改變影像顏色

