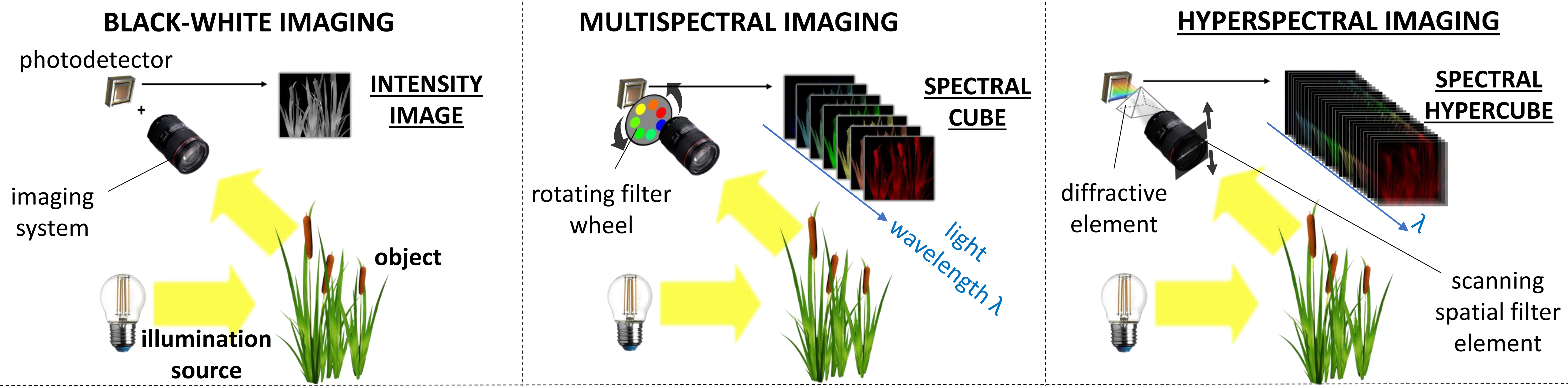
A multimodal widefield hyperspectral microscope

B. Ardini, G. Valentini, A. Bassi, A. Candeo, G. Cerullo, R. Vanna, C. Manzoni

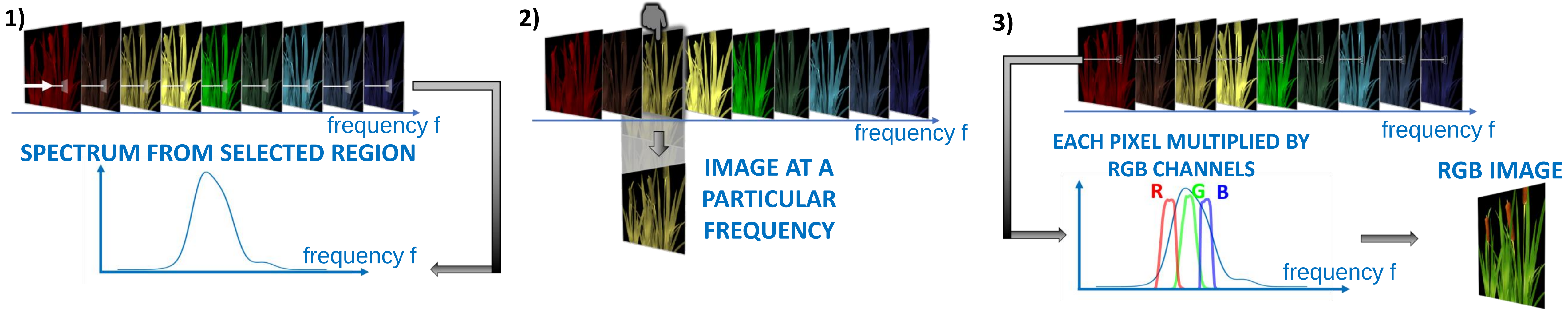
Outline

1) Hyperspectral imaging and microscopy
2) Fourier Transform (FT) spectroscopy
3) A novel ultrastable interferometer: TWINS
4) A Widefield FT hyperspectral microscope
5) Application and results

From Standard Imaging to Hyperspectral Imaging



SPECTRAL HYPERCUBE – INFORMATION AND ANALYSIS



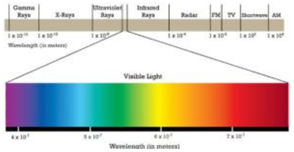
Hyperspectral microscopy

MICROSCOPE

- micrometric spatial resolution
- optimized light collection

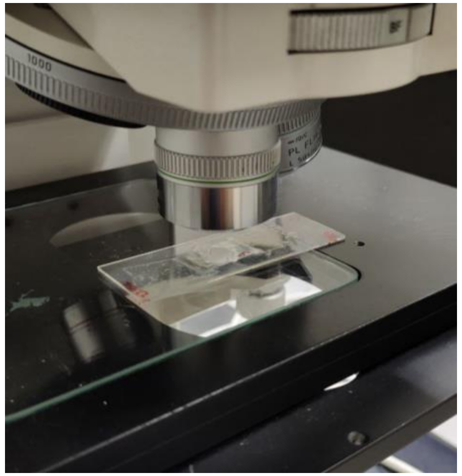
\+

SPECTROSCOPY

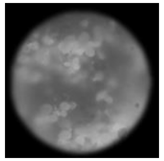


- Frequency dependent light-matter interaction

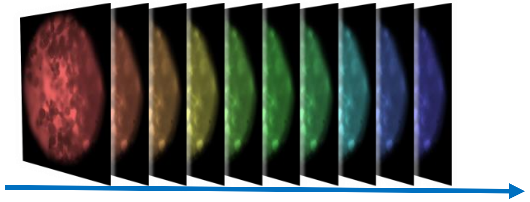
Very powerful toll for material science!



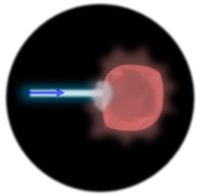
Field of View (FOV)



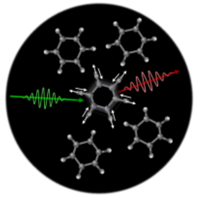
Spectral hypercube of the FOV



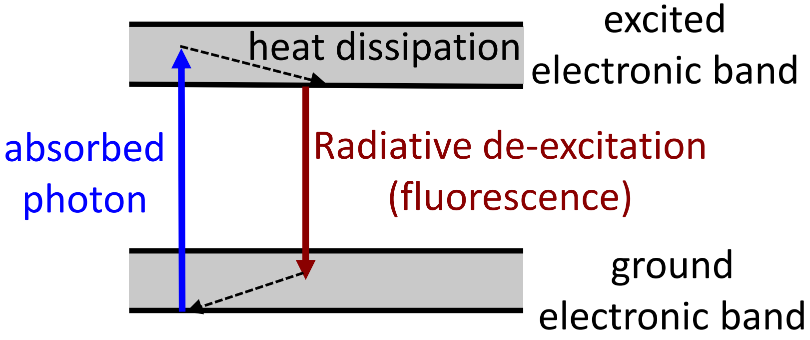
OPTICAL SIGNALS OF INTEREST FOR MATERIAL SCIENCE

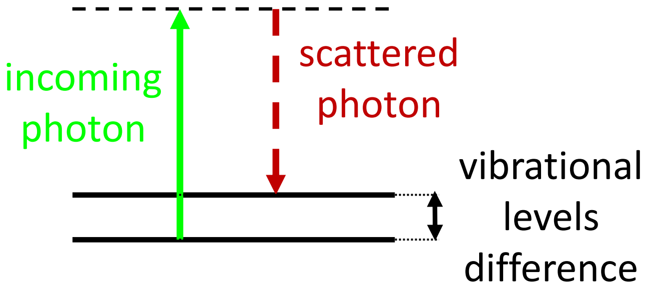


Fluorescence
↓
electronic transition

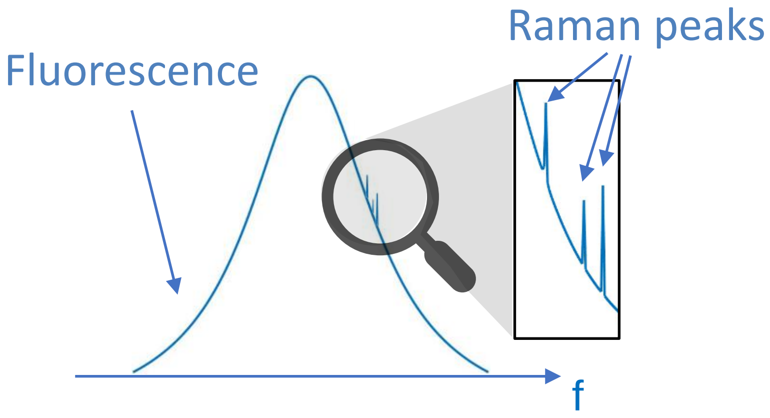


Raman
↓
molecule vibrations (chemical structure)





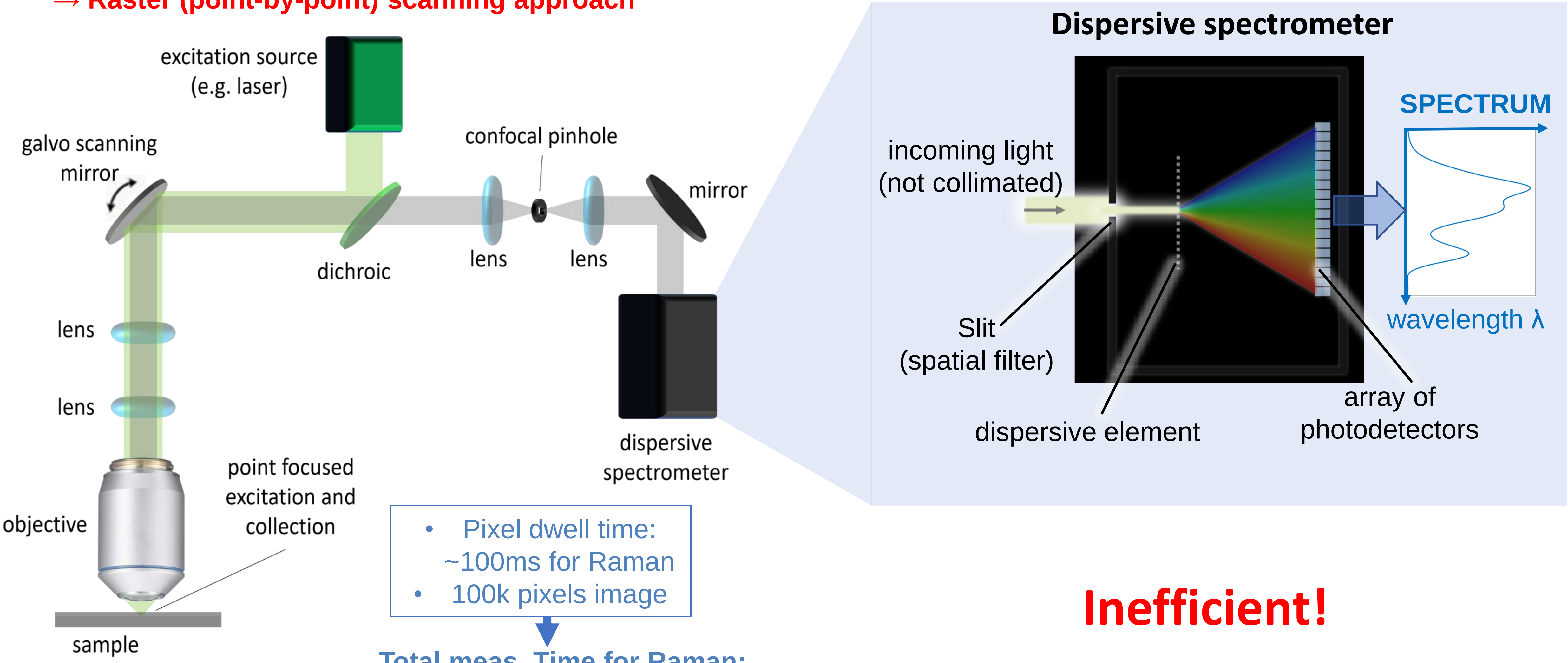
MEASURED SPECTRUM



Raman << Fluorescence ⟶ no multimodal systems

Typical hyperspectral microscopy scheme

→ **Raster (point-by-point) scanning approach**



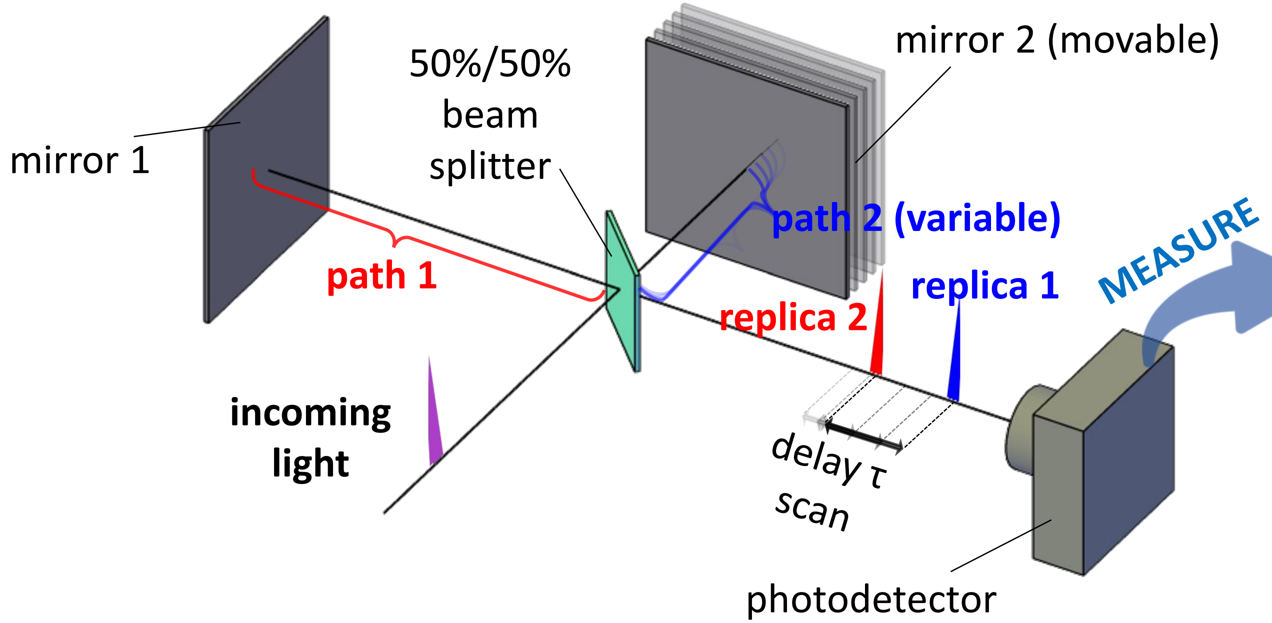
- Pixel dwell time: ~100ms for Raman
- 100k pixels image

Total meas. Time for Raman: ~3 hours!

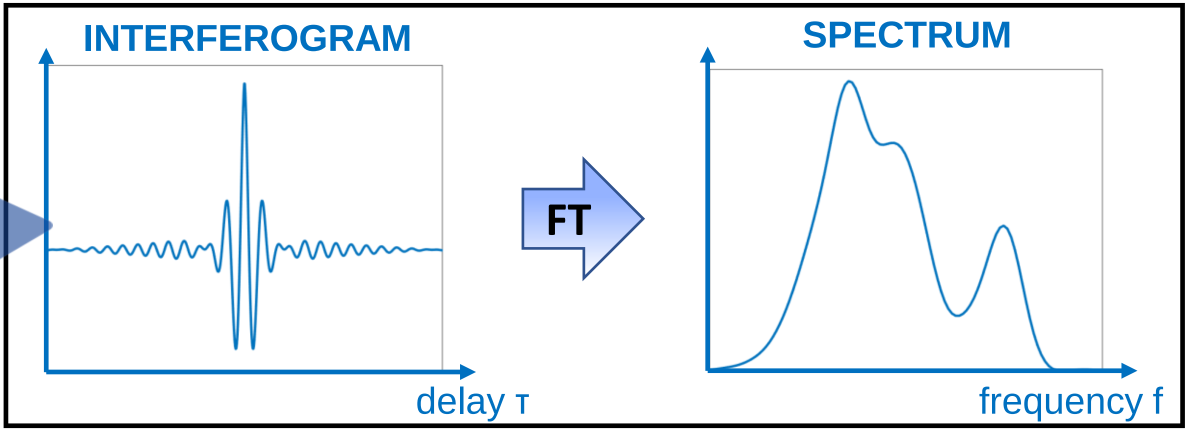
Inefficient!

Fourier Transform (FT) Spectroscopy

INTERFEROMETER (e.g. Michelson interferometer)



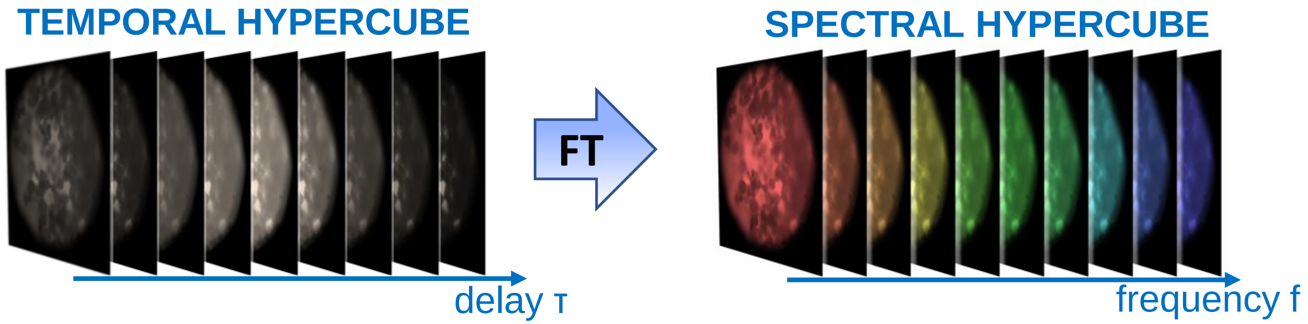
POST-PROCESSING OPERATION



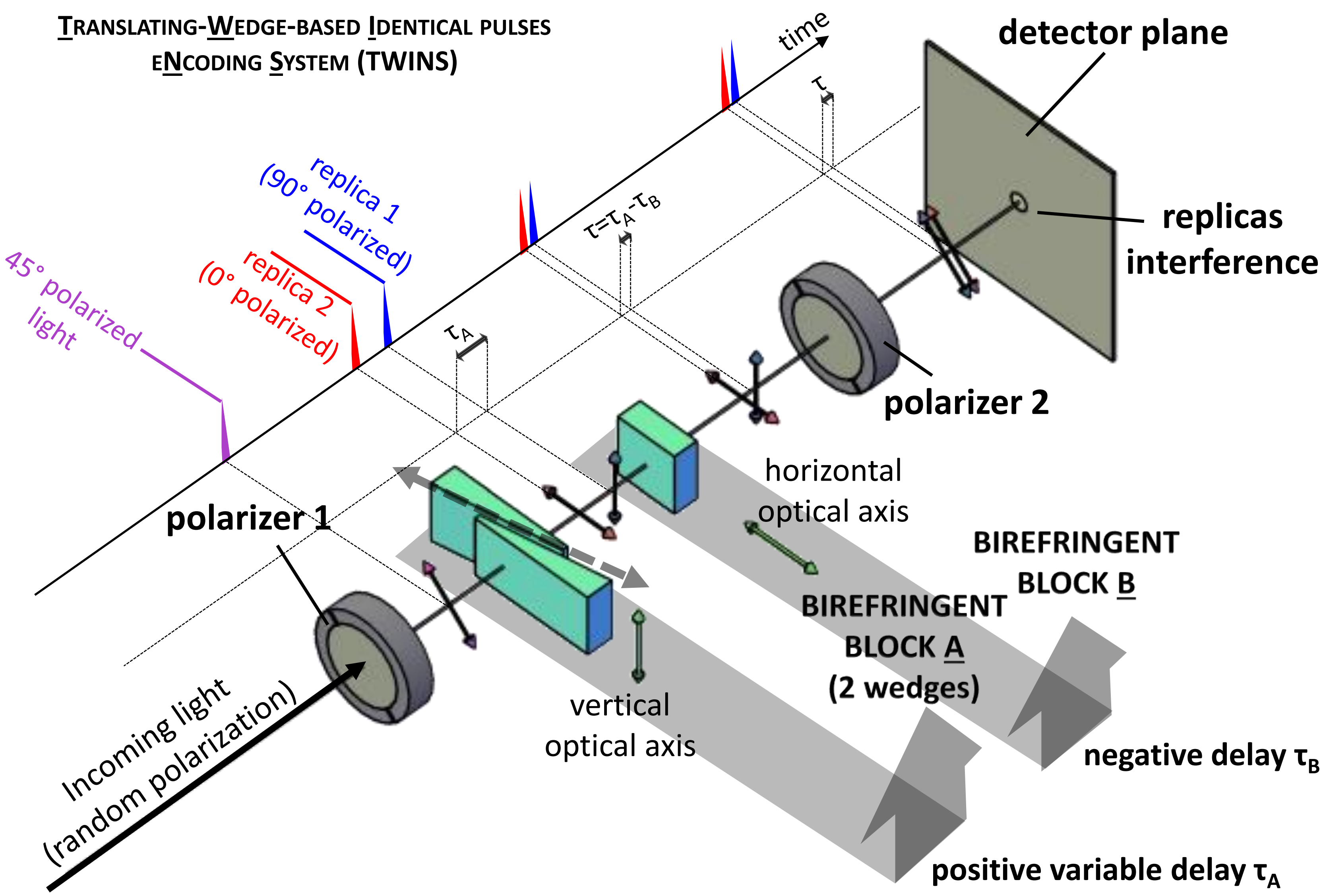
ADVANTAGES OF FT SPECTROSCOPY

- **high-throughput** (no slits needed)
- **possibility of parallel recording** (no light dispersion)

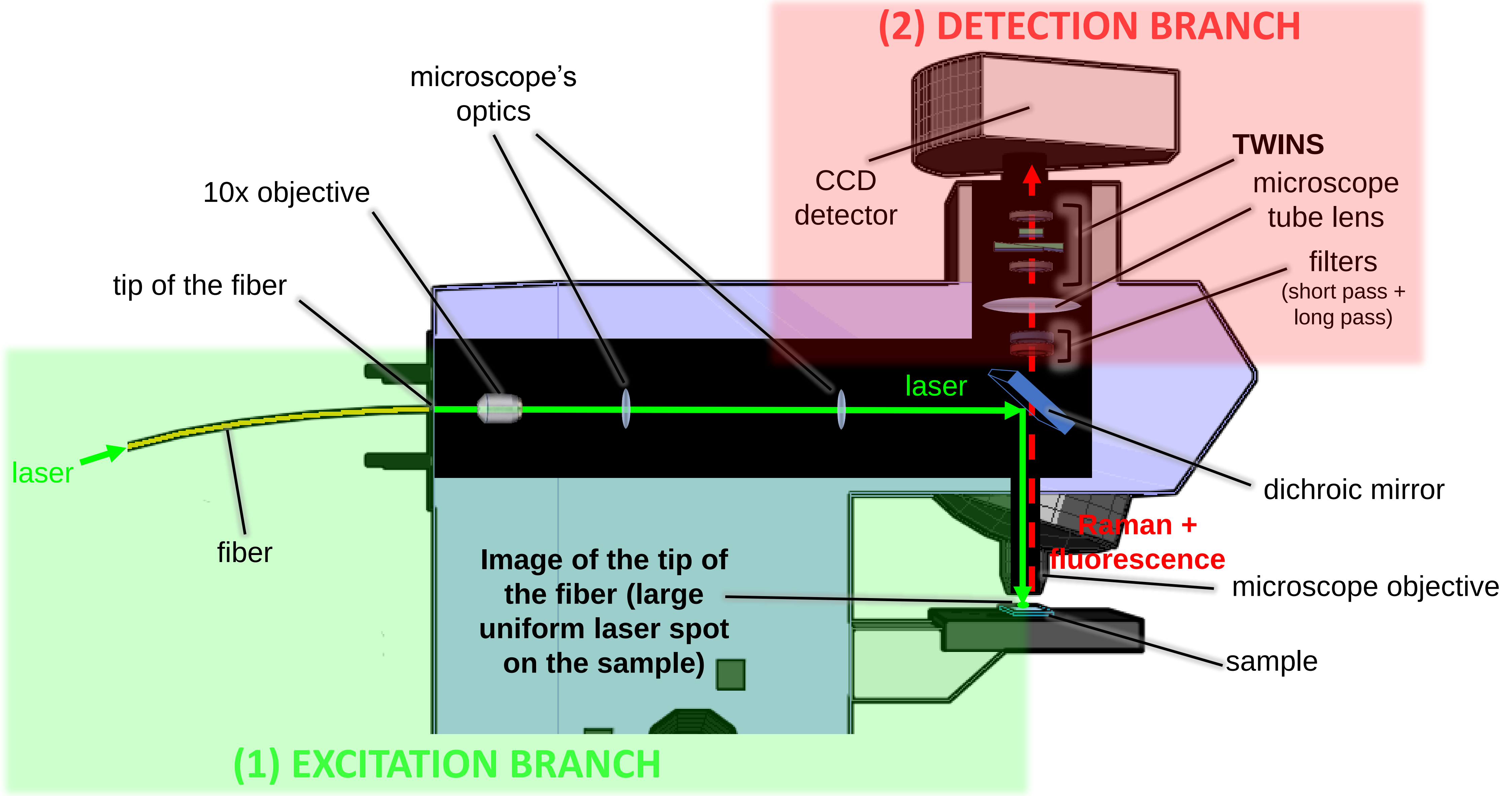
PARALLEL ACQUISITION (WIDEFIELD APPROACH)



An ultrastable common-path interferometer: TWINS

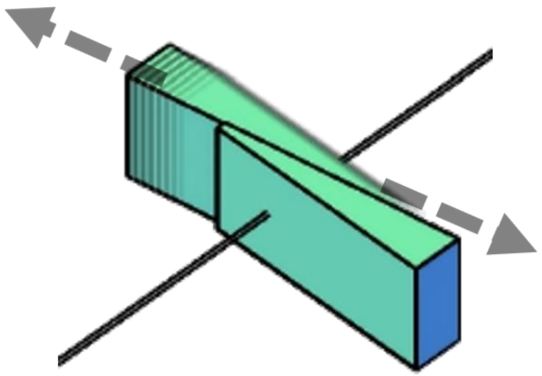


Widefield hyperspectral microscope for Raman and Fluorescence



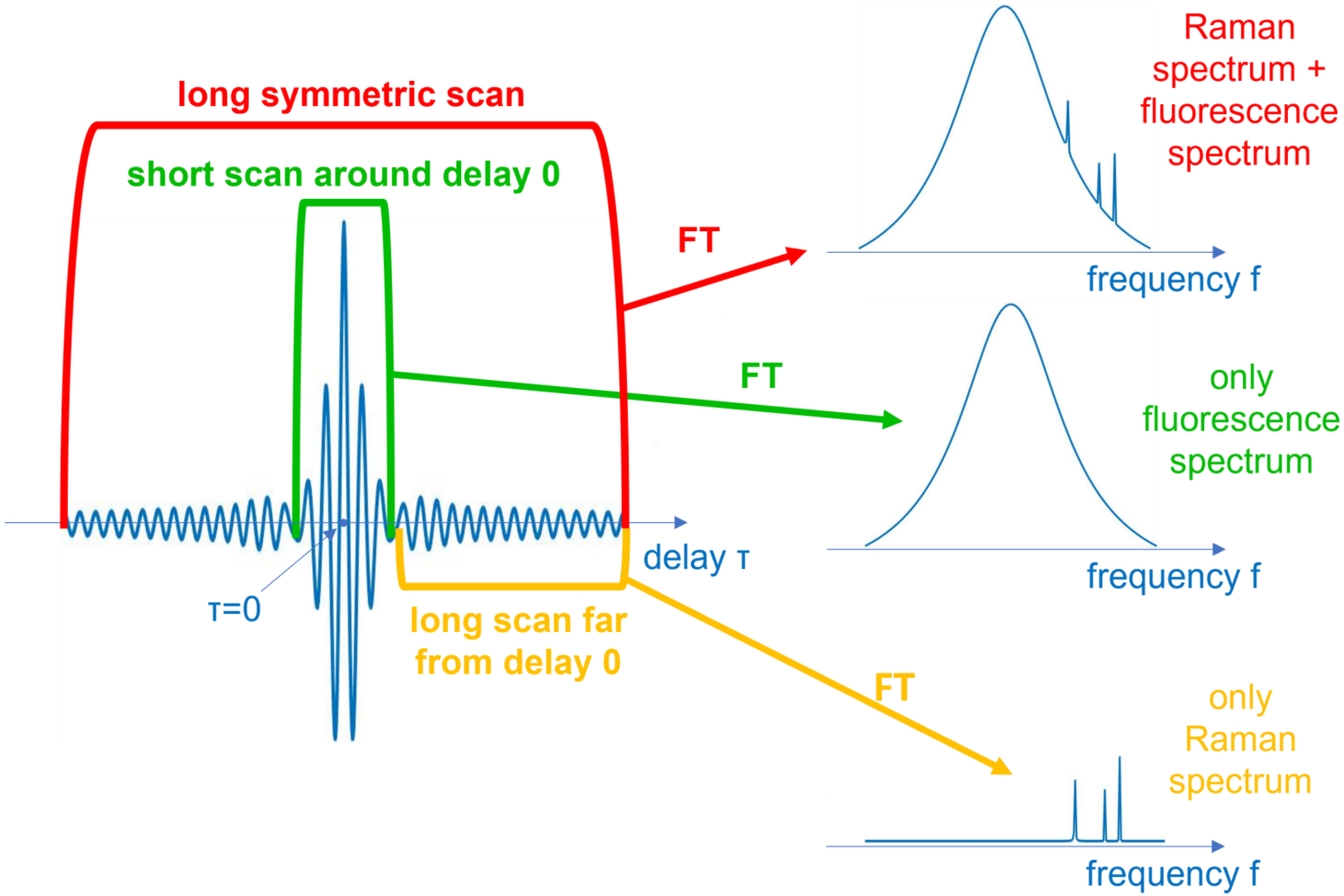
Fluorescence-Raman uncoupling

WEDGE SCAN



ACQUIRED INTERFEROGRAM

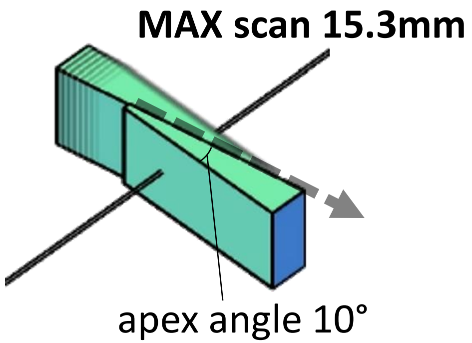
SPECTRUM

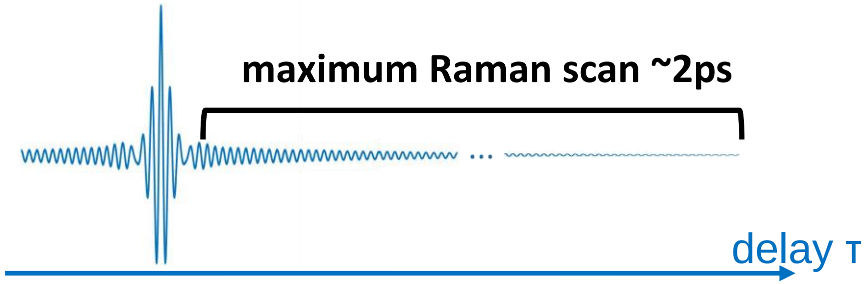


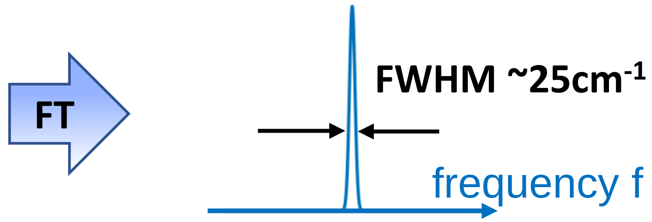
Increase the spectral resolution

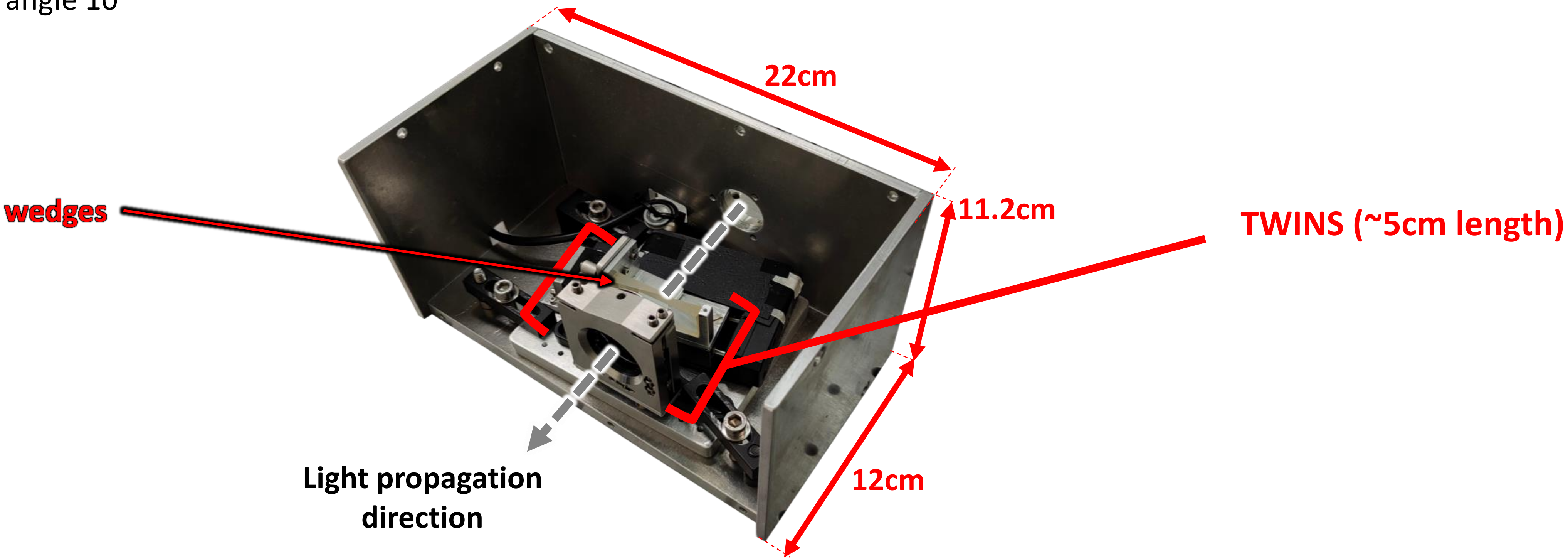
YVO_4 WEDGES

Birefringence ($\|n_o-n_e\|$)	Scan length	Maximum delay scan	Spectral resolution
0.2253	15.3 mm	2 ps	~25 cm^{-1}



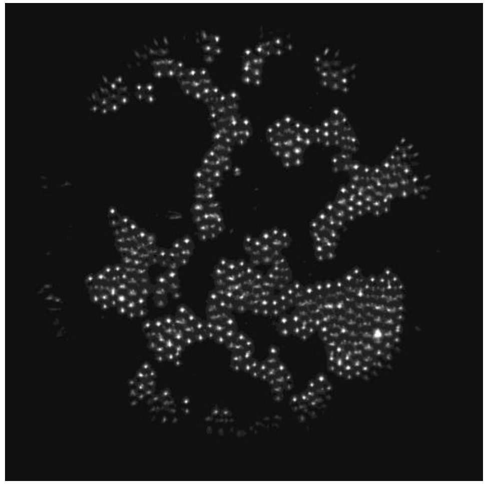




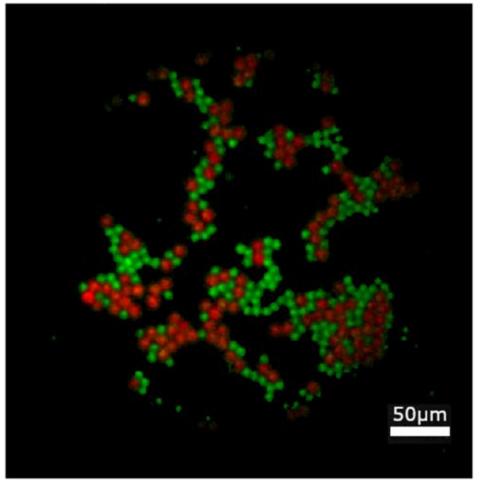


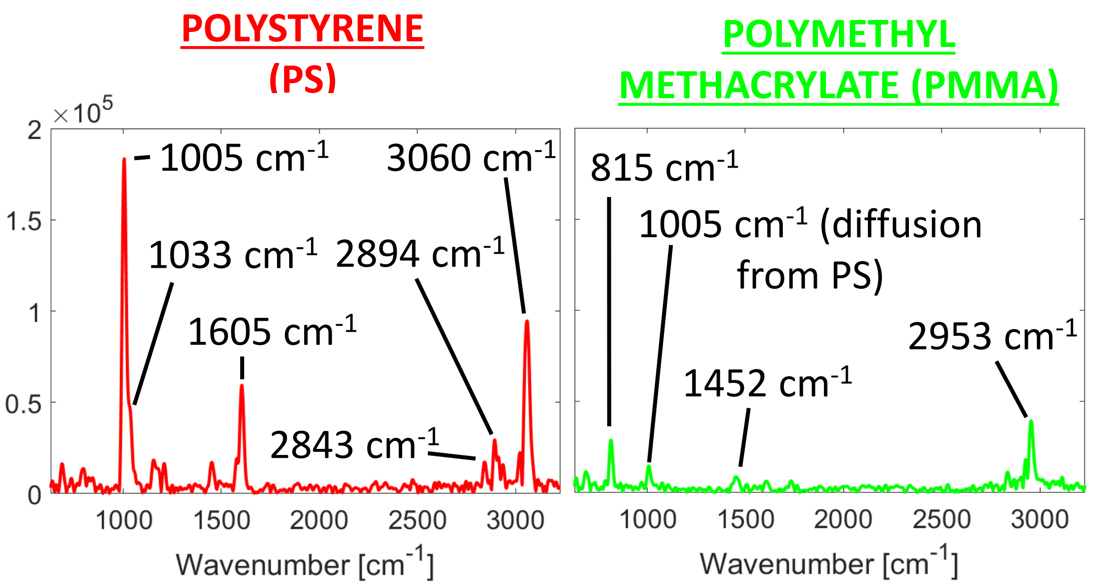
Raman measurement on PMMA & PS beads

REFLECTIVITY IMAGE

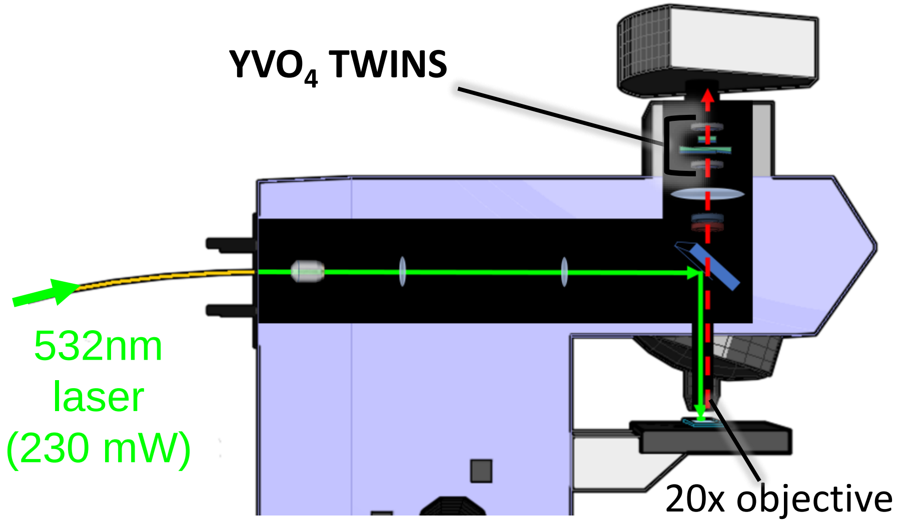


RAMAN MEASUREMENT RESULT





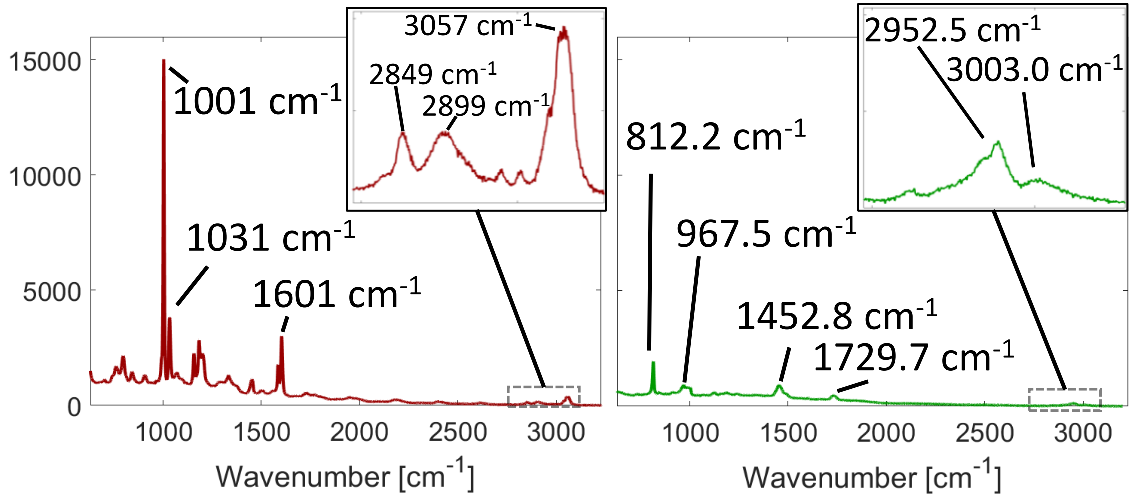
MEASUREMENT SETUP



PARAMETERS

CCD integration per frame	N° of acquired frames
2.5 s	806
Total measurement time	**N° of pixels in the image**
38 minutes	250 kP

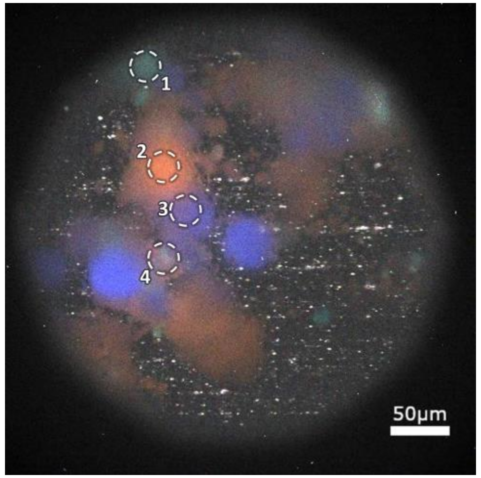
SPECTRA WITH STANDARD RAMAN SPECTROMETER
(785nm EXCITATION)

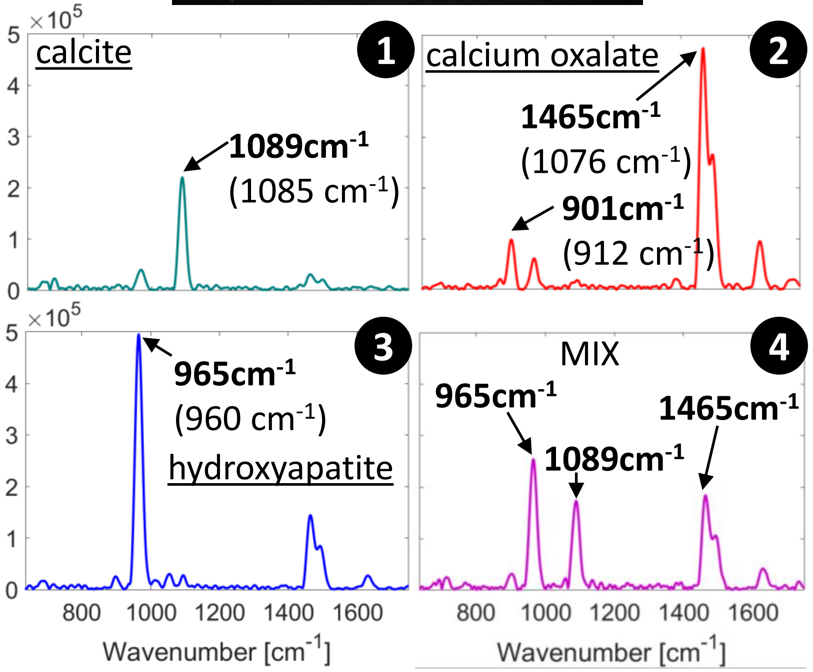


Example of multimodal application on minerals' grains

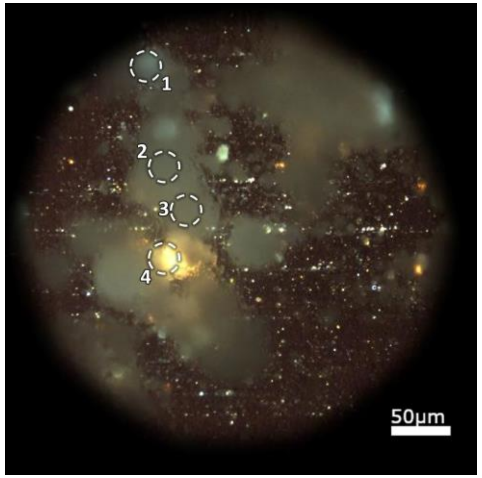
REFLECTIVITY IMAGE

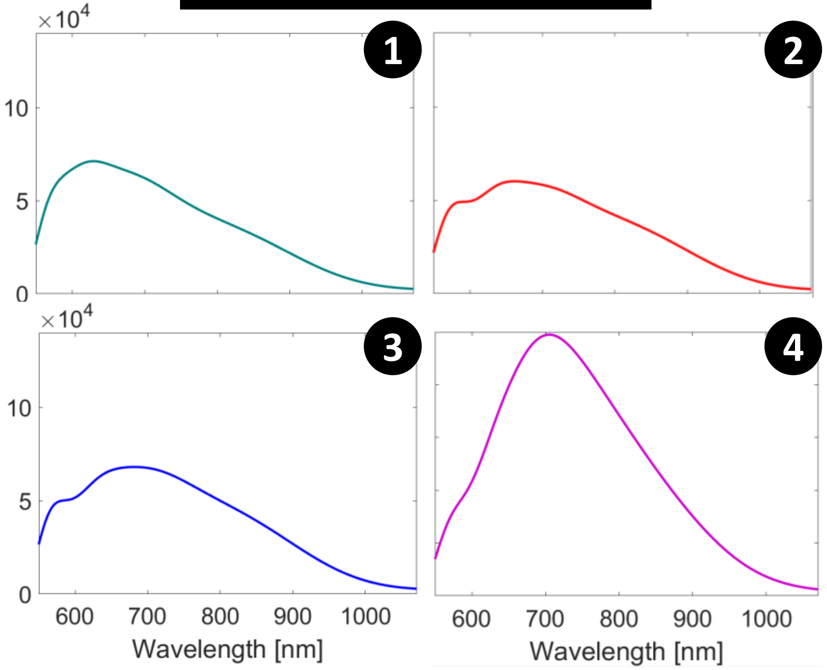
RAMAN (FALSE COLOUR RGB)





FLUORESCENCE (FALSE COLOUR RGB)



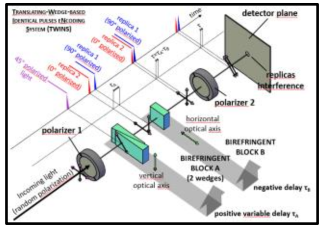


MEASUREMENTS PARAMETERS

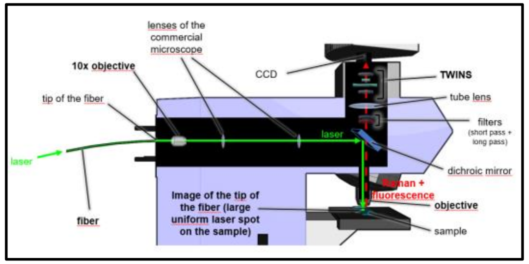
MEAS.	CCD int. time per frame	N° of acquired frames	Total meas. time	N° of pixels in the image
Fluo.	2 s	200	**7 minutes**	250 kP
Raman	8 s	806	**1h 51 minutes**	250 kP

Conclusion

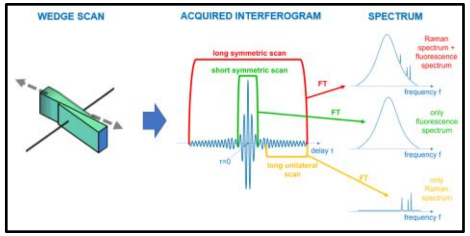
- An ultrastable common-path interferometer: TWINS



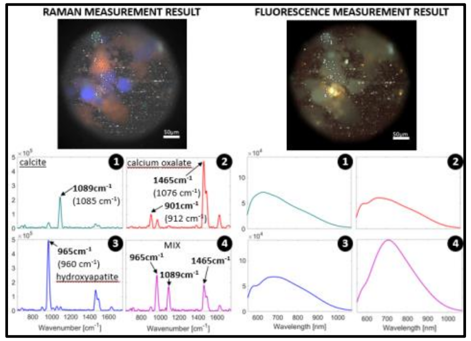
- Scheme of a widefield multimodal hyperspectral microscope



- Uncoupling of Raman and Fluorescence



- Example of multimodal (Fluorescence & Raman) microscopy
  - ➢Optimal spatial resolution: ~1µm
  - ➢Good spectral resolution: ~25cm^{-1}
  - ➢Fast measurements : ~40 min VS. ~ 3 h (raster scanning) for ~ 100k pixels

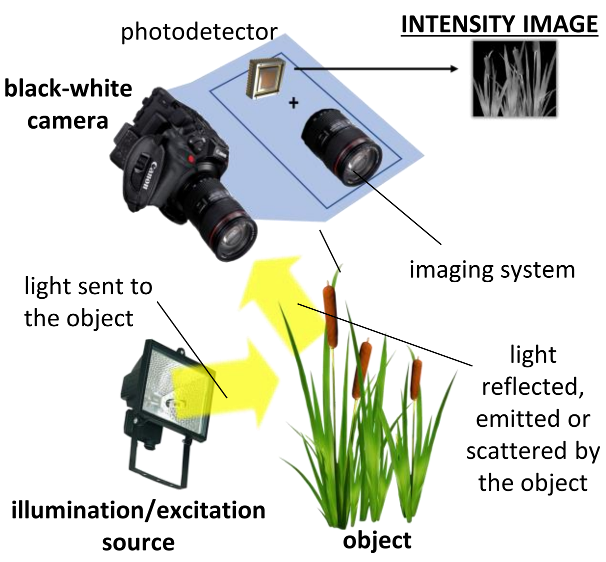


Thanks for your attention!

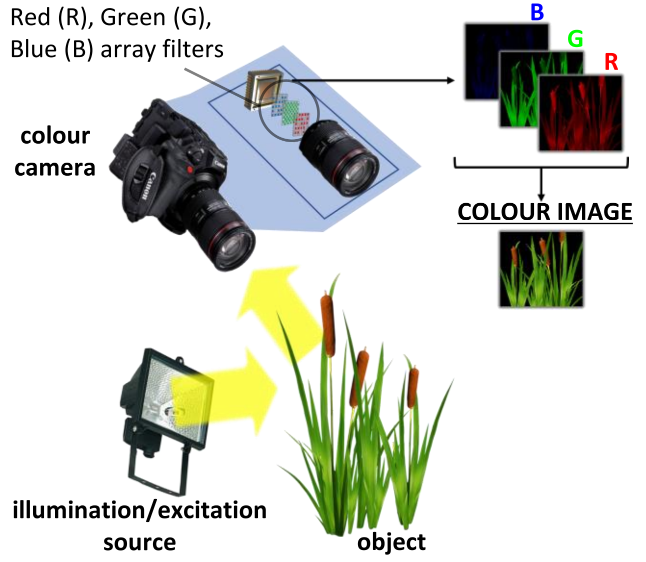
Back-up slides

From Standard Imaging to Hyperspectral Imaging

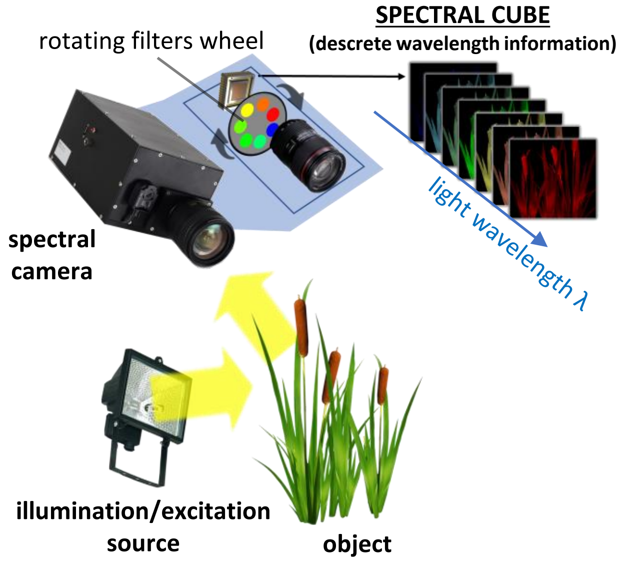
BLACK-WHITE IMAGING



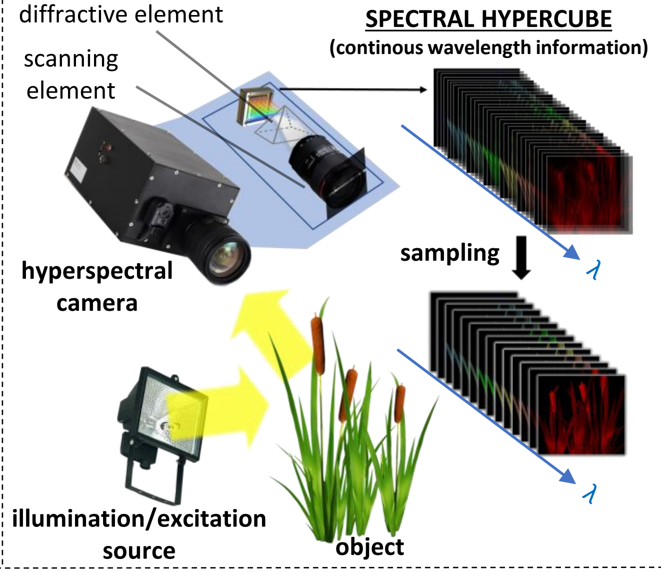
RGB COLOUR IMAGING



MULTISPECTRAL IMAGING

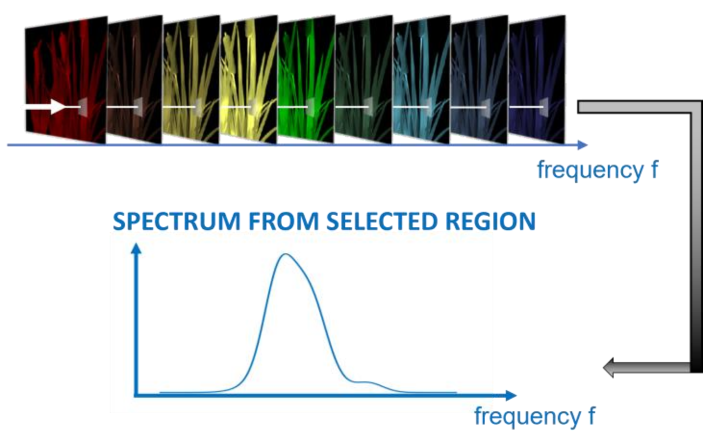


HYPERSPECTRAL IMAGING

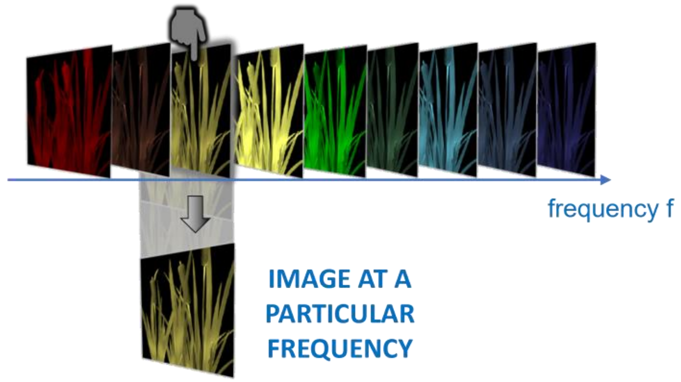


SPECTRAL HYPERCUBE – INFORMATION AND ANALYSIS

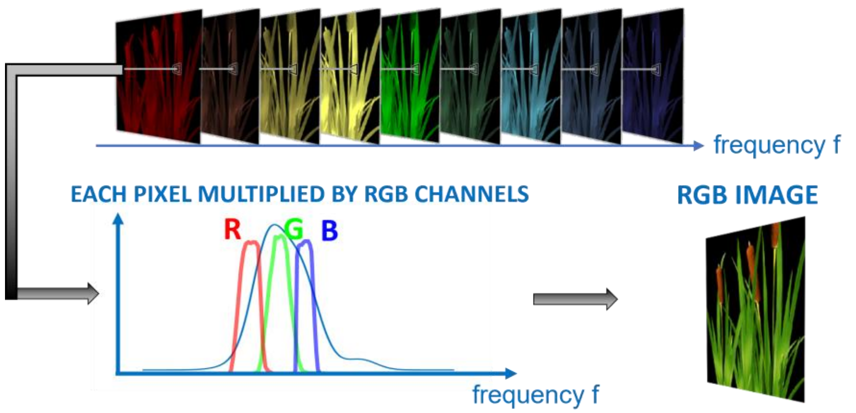
1)



2)

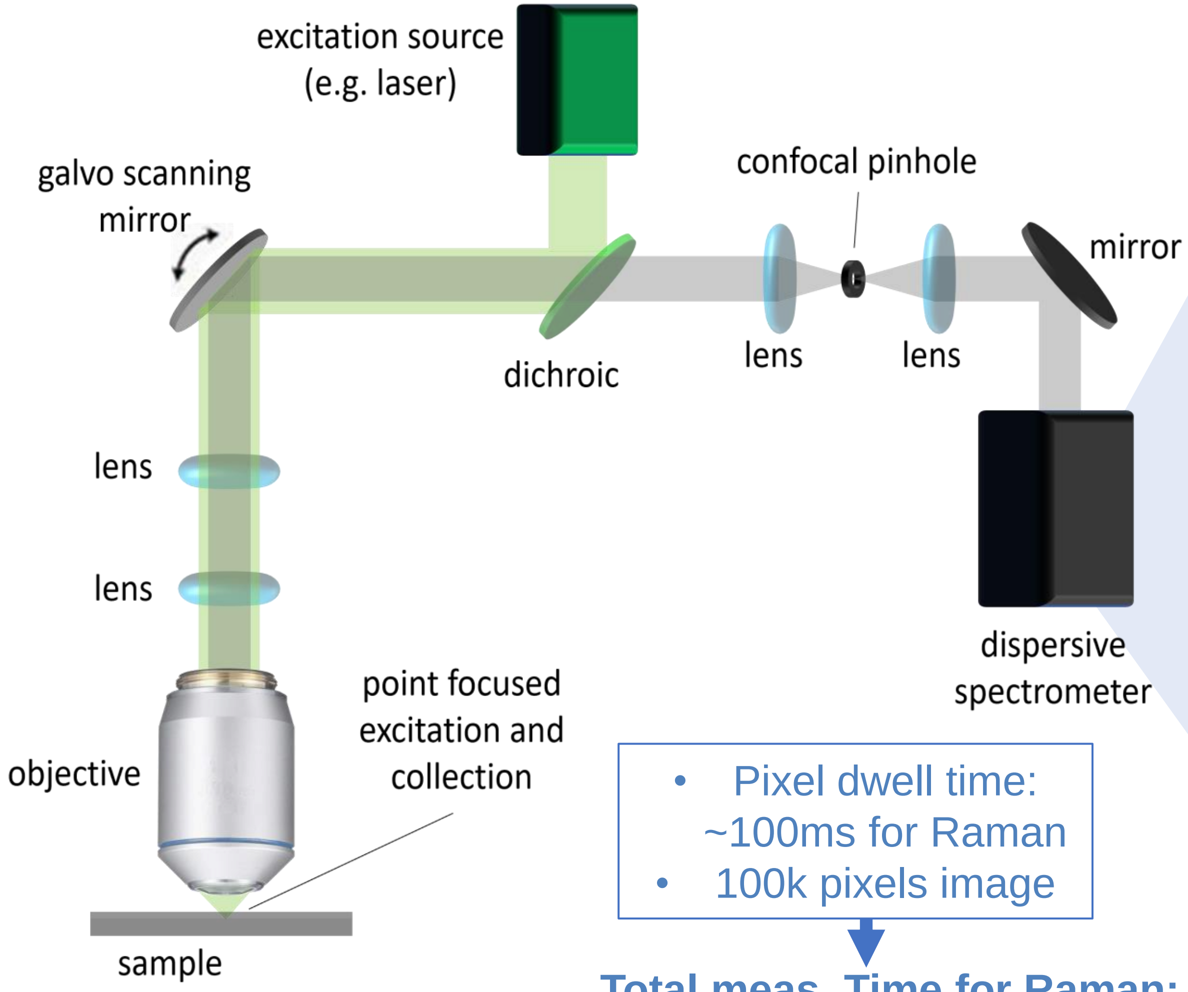


3)



Typical hyperspectral microscopy scheme

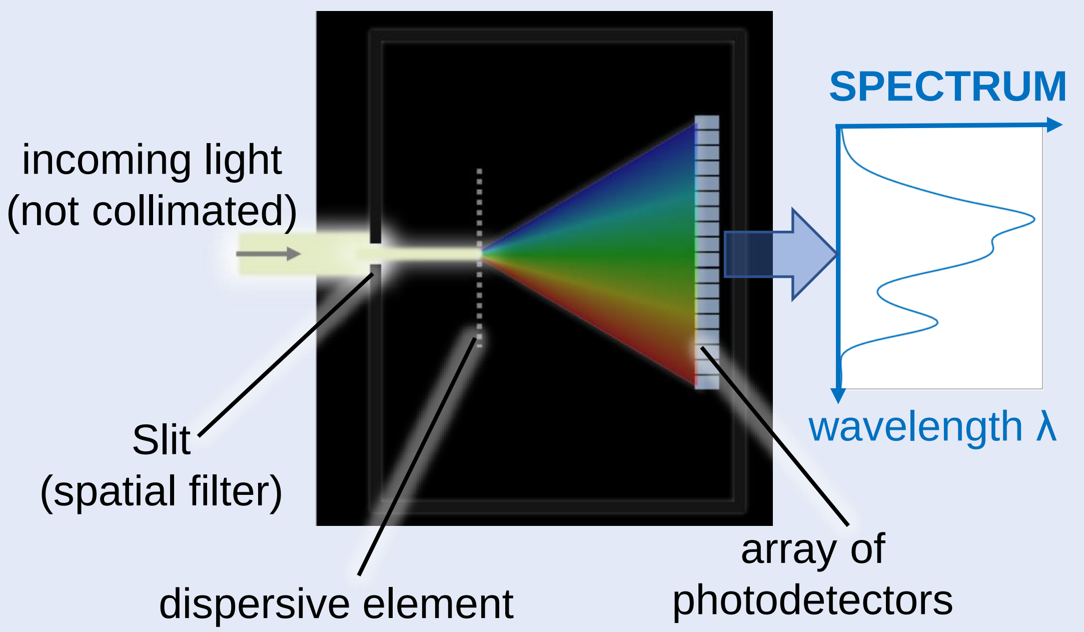
→ Raster (point-by-point) scanning approach



- Pixel dwell time: ~100ms for Raman
- 100k pixels image

Total meas. Time for Raman: ~3 hours!

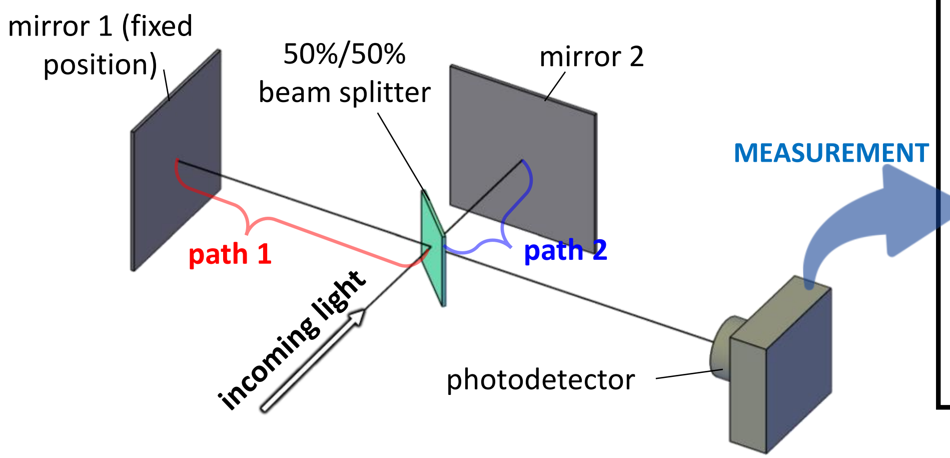
Dispersive spectrometer



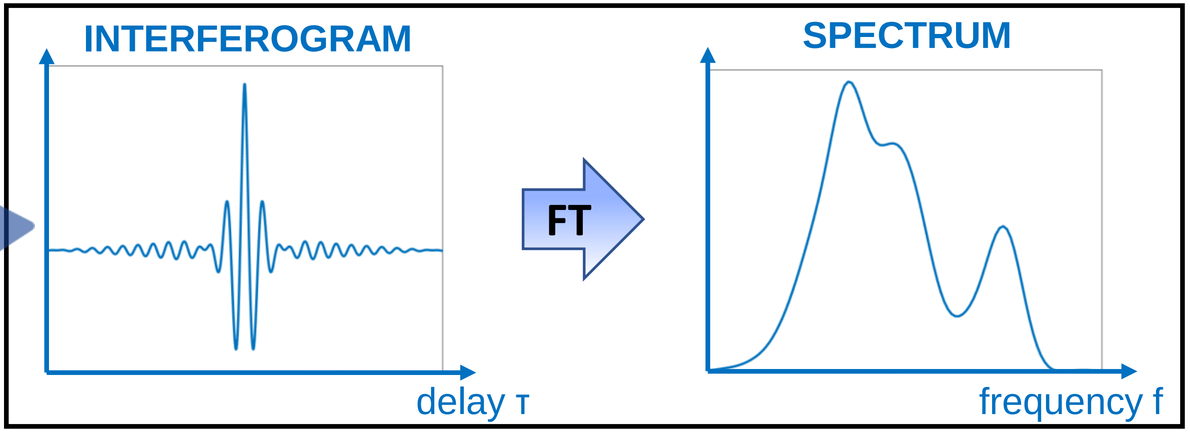
- Spatial filtering (slit) → **low light-throughput**
- Light dispersion on photodetector array → **No possible parallel acquisition** (only one spectrum at a time)

Fourier Transform (FT) Spectroscopy

INTERFEROMETER (e.g. Michelson interferometer)



POST-PROCESSING OPERATION

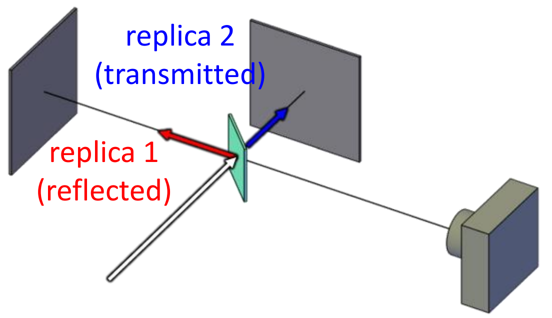


ADVANTAGES OF FT SPECTROSCOPY

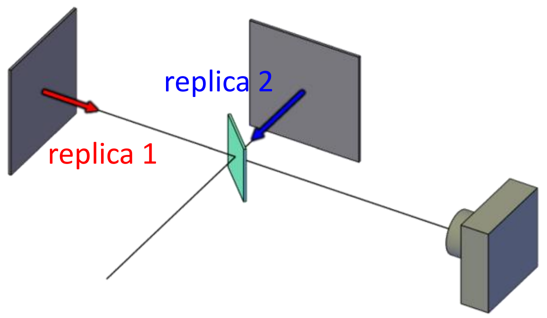
- **high-throughput** (no slits needed)
- **possibility of parallel recording** (no light dispersion)

MICHELSON INTERFEROMETER WORKING PRINCIPLE

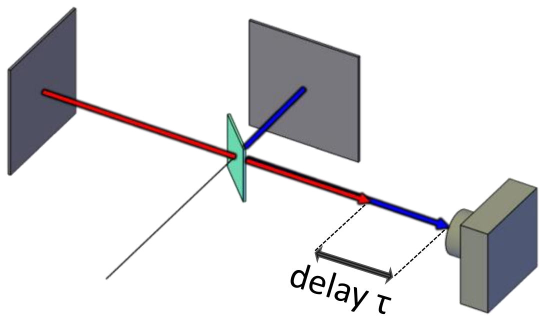
(1) Incoming light splitted into 2 replicas in 2 paths



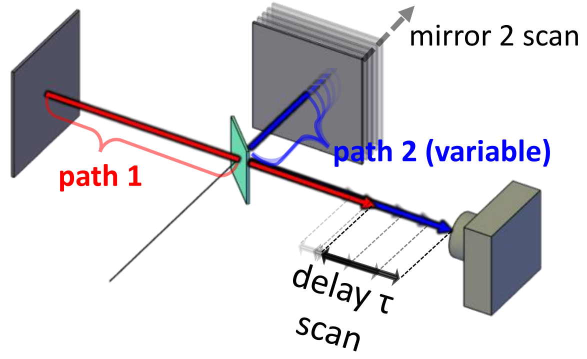
(2) The 2 replicas reflected by the mirrors



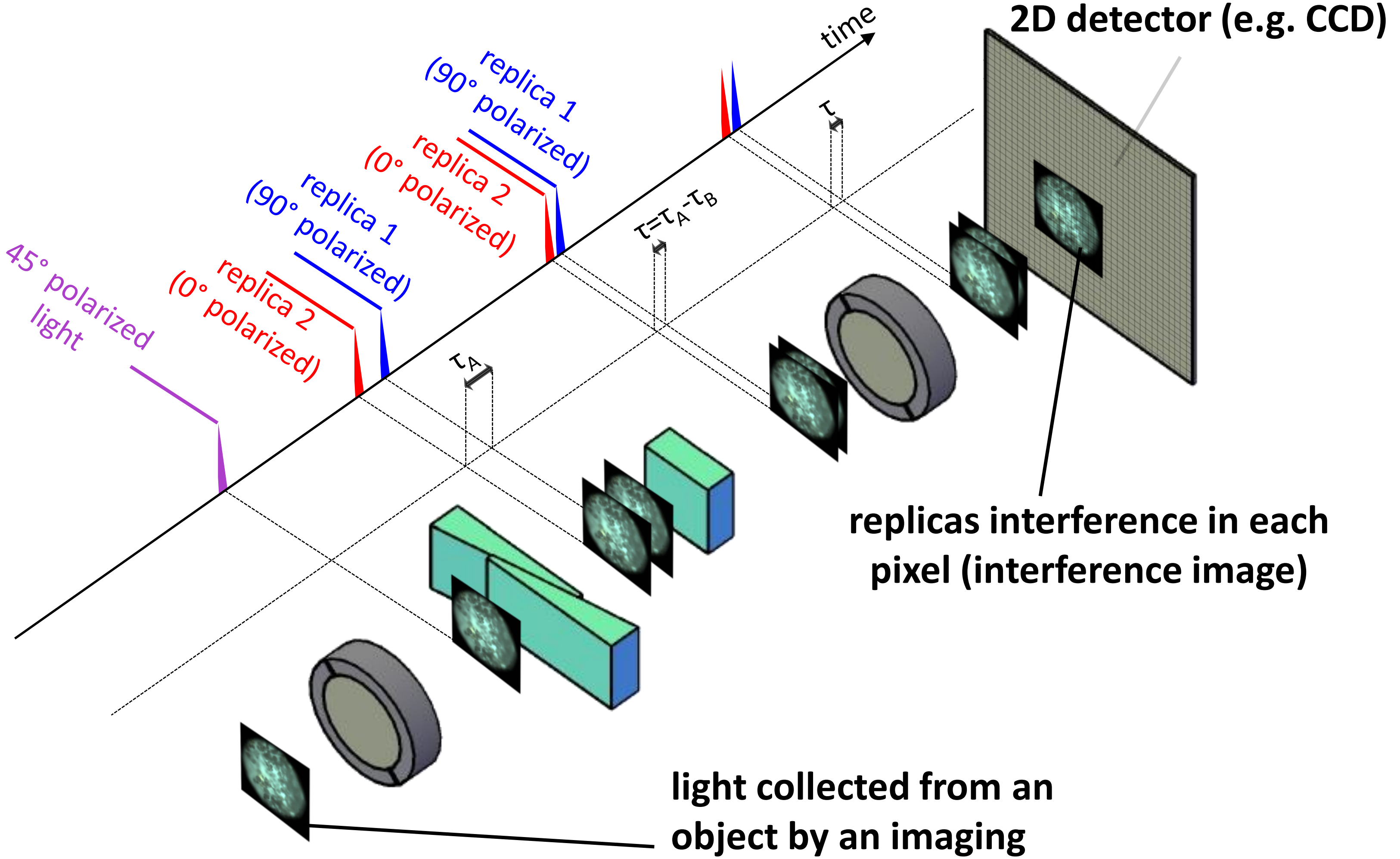
(3) The 2 replicas recombine and interfere on the detector



MEASUREMENT: move mirror 2 and acquire light for each position scan

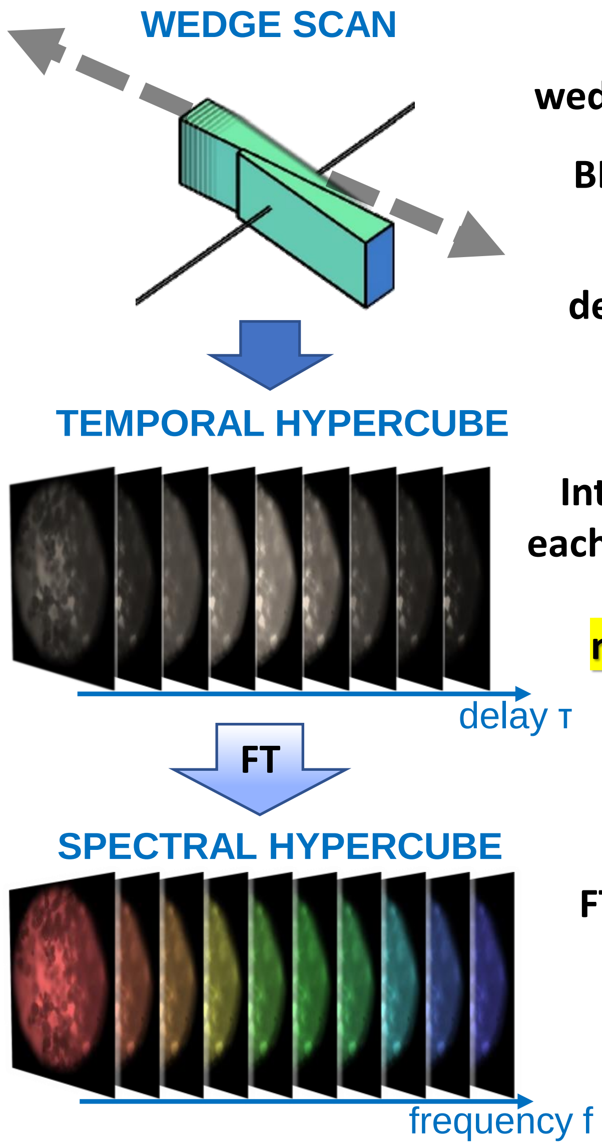


TWINS for Widefield hyperspectral imaging



replicas interference in each pixel (interference image)

light collected from an object by an imaging system (e.g. microscope)



wedge translation
↓
BLOCK A width change
↓
delay τ change

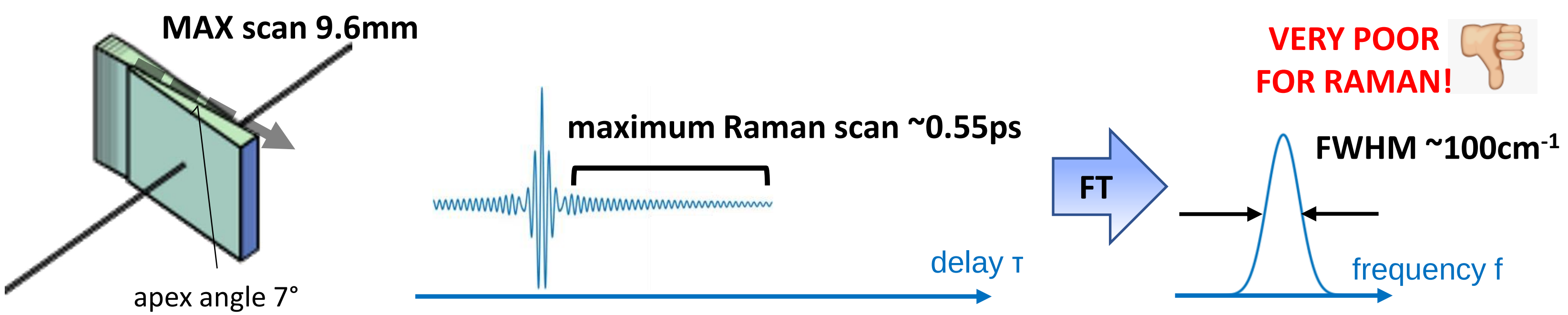
Interferogram for each pixel in parallel: **WIDEFIELD measurement**

FT of temporal hypercube

Increase the spectral resolution

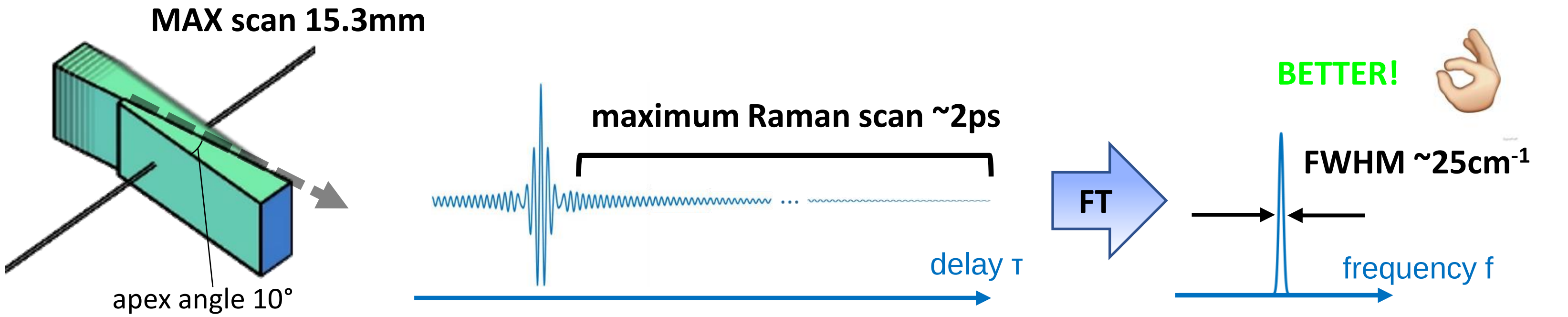
α-BBO WEDGES

Birefringence (|n_o-n_e|)	Scan length	Maximum delay scan	Spectral resolution
0.1403	9.6 mm	0.55 ps	~100 cm^{-1}

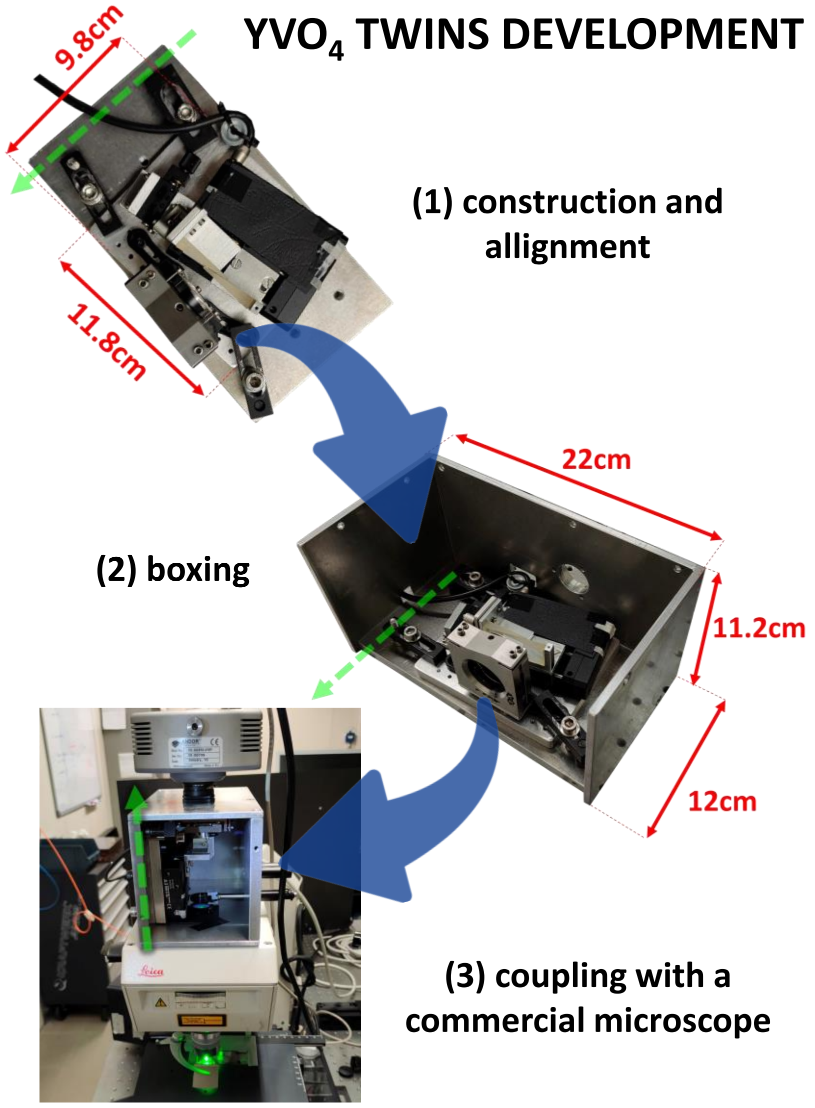


YVO_4 WEDGES

Birefringence (|n_o-n_e|)	Scan length	Maximum delay scan	Spectral resolution
0.2253	15.3 mm	2 ps	~25 cm^{-1}

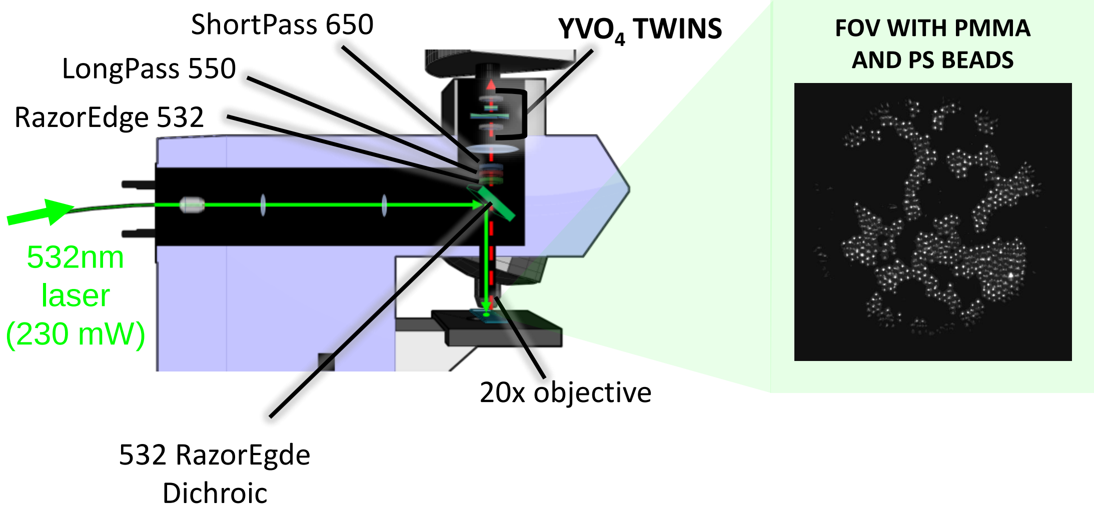


YVO_4 TWINS DEVELOPMENT



Raman measurement on PMMA & PS beads

RAMAN & FLUORESCENCE SETUP

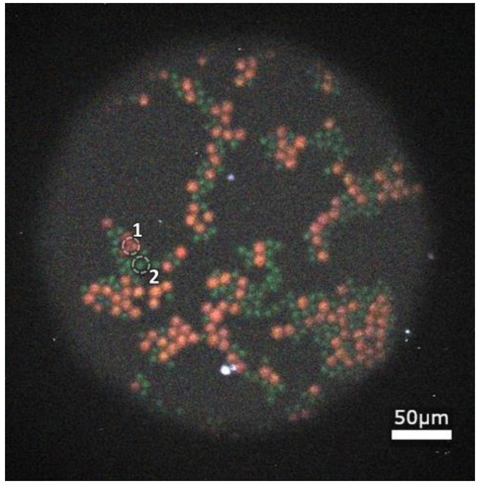


MEASUREMENT PARAMETERS

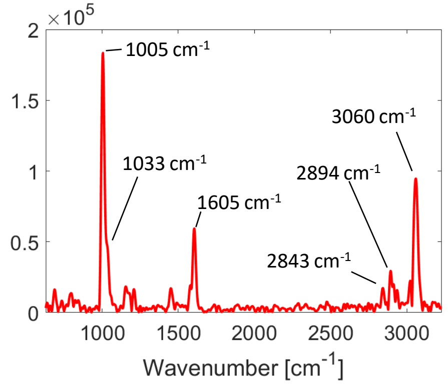
CCD integration per frame	N° of acquired frames	Total measurement time	N° of pixels in the image
2.5 s	806	38 minutes	250 kP

RESULTS

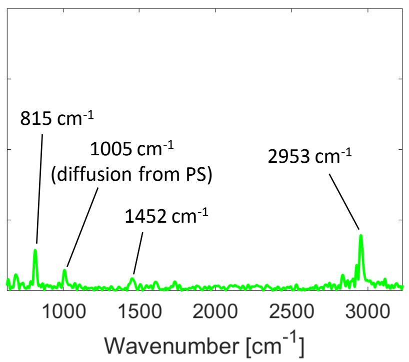
FALSE COLOUR RGB 2797cm^{-1}-3172cm^{-1}



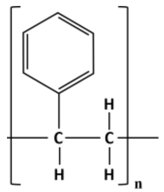
1 POLYSTYRENE (PS)



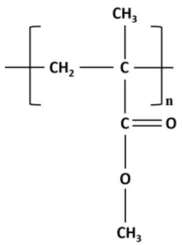
2 POLYMETHYL METHACRYLATE (PMMA)



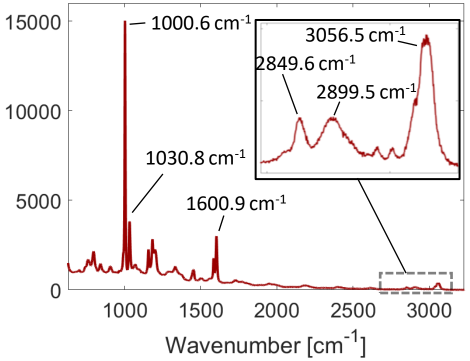
POLYSTYRENE (PS)

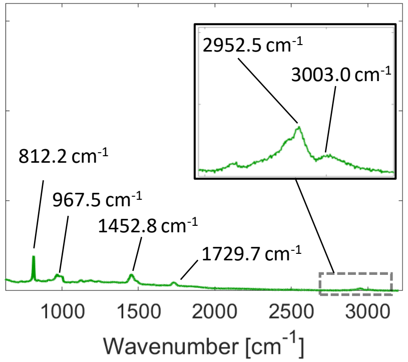


POLYMETHYL METHACRYLATE (PMMA)



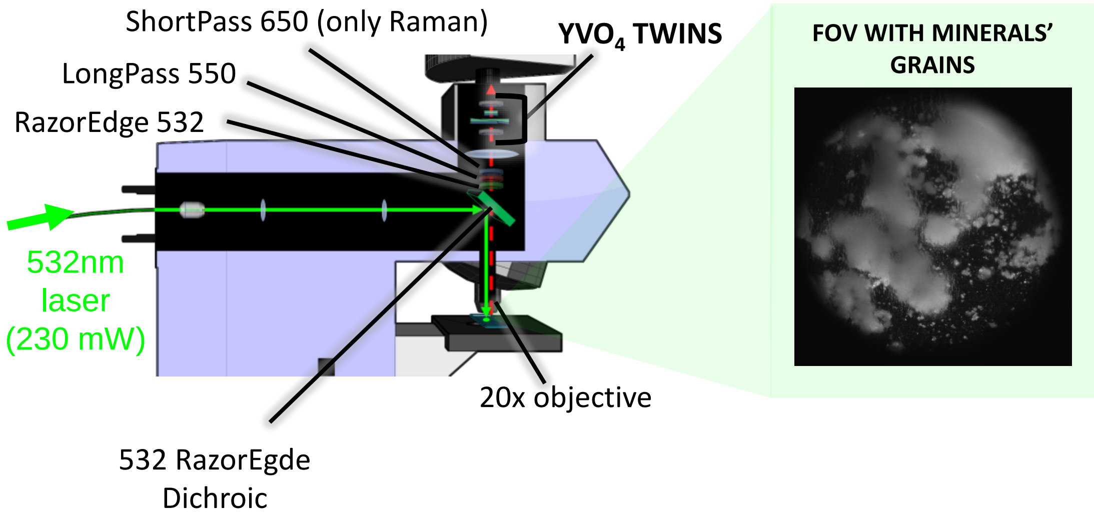
SPECTRA WITH STANDARD RAMAN SPECTROMETER AND 785nm EXCITATION LASER





Example of multimodal application

RAMAN & FLUORESCENCE SETUP



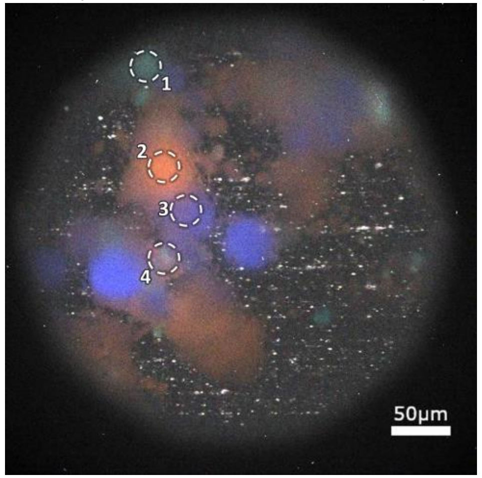
MEASUREMENT PARAMETERS

MEASUREMENT	CCD integration per frame	N° of acquired frames	Total measurement time	N° of pixels in the image
Fluorescence	2 s	200	**7 minutes**	250 kP
Raman	8 s	806	**1h 51 minutes**	250 kP

RESULTS

RAMAN

(FALSE COLOUR RGB 615cm^{-1}-1847cm^{-1})



FLUORESCENCE

(FALSE COLOUR RGB 550nm-750nm)