RAMAN Spectroscopy of liver tissue samples

A pilot study

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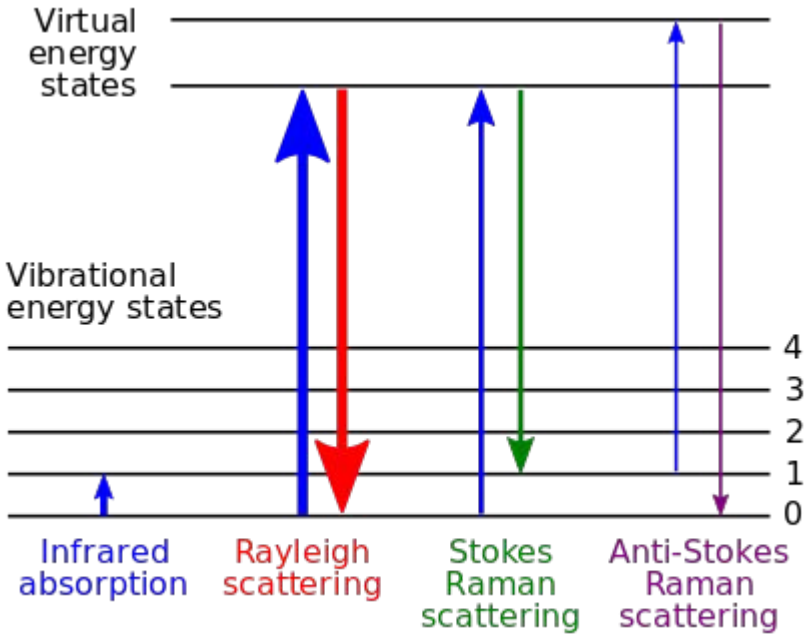
IMBB - FORTH

Raman principle



https://en.wikipedia.org/wiki/Raman_spectroscopy

Advantages

- Optical, non contact, non invasive, non destructive technique
- Vibrational spectroscopy technique specific to chemical bonds
- Provides a fingerprint to identify molecules (Label-free technique)
- Mapping possibilities through XYZ scanning
- Can provide reference spectra to CARS and SRS imaging for increased spatial resolution

Instrumentation

Horiba LabRAM HR

Located at IESL

Optimizations:

i) +785 nm laser source

ii) +9 high refl. mirrors

iii) obj lens 60X NA:1.2 W

iv) Trans illumination

v) temp stabilization

The study…

Samples:

- 1 normal liver slice (8um thickness on CaF2 substrate)
- 1 malignant liver slice (8um thickness on CaF2 substrate)

Questions:

- Can Raman spectroscopy differentiate them?
- What more can we learn from it?

Measurement settings

Laser source: 532nm, 100mW

Objective lens: x50, NA 0.50, air

Spot size: 1.3um spatial, 1.6um axial

Energy on the sample: 13mW

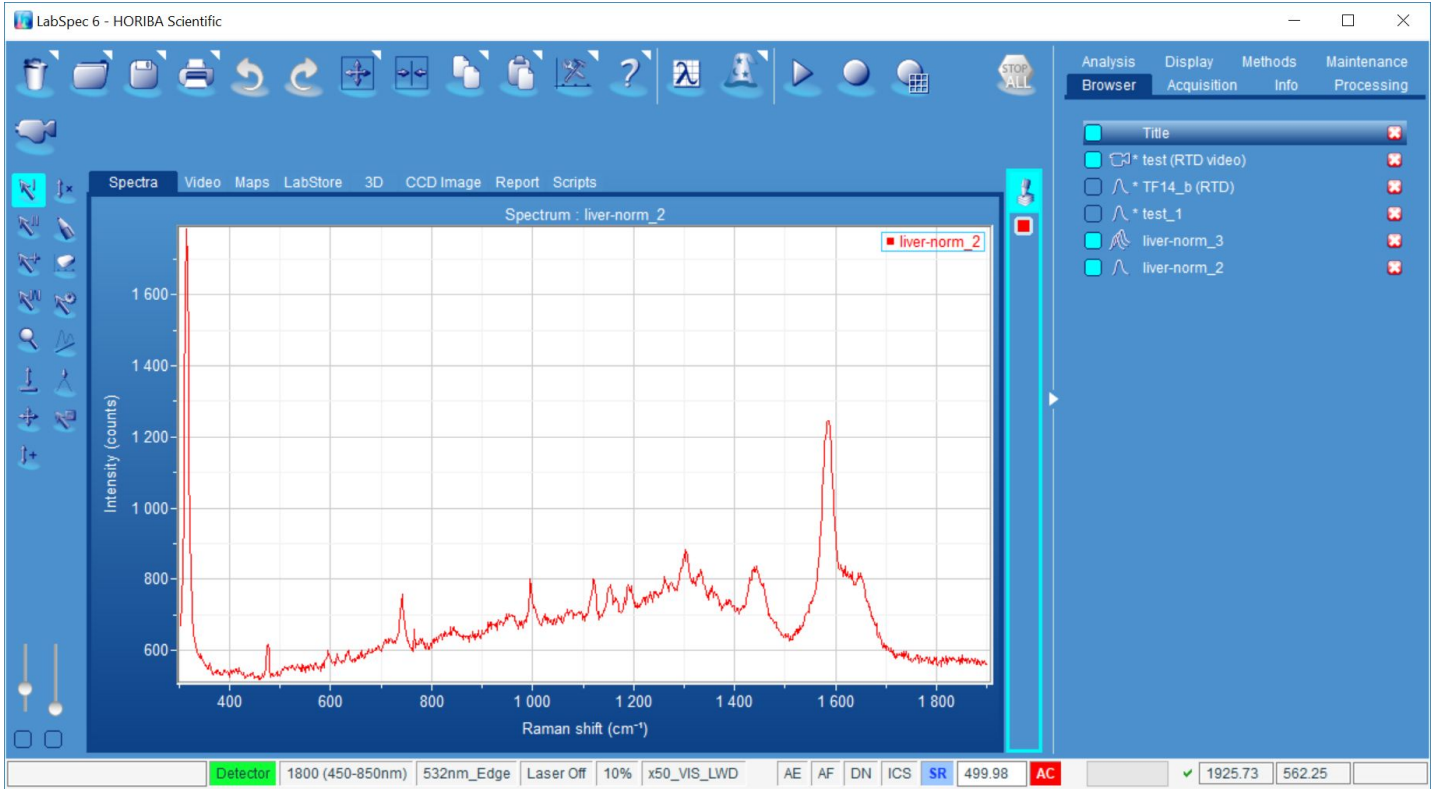
Exposure time: 30 sec/point, 9 accumulations

Spatial resolution: 3X9, 5um step

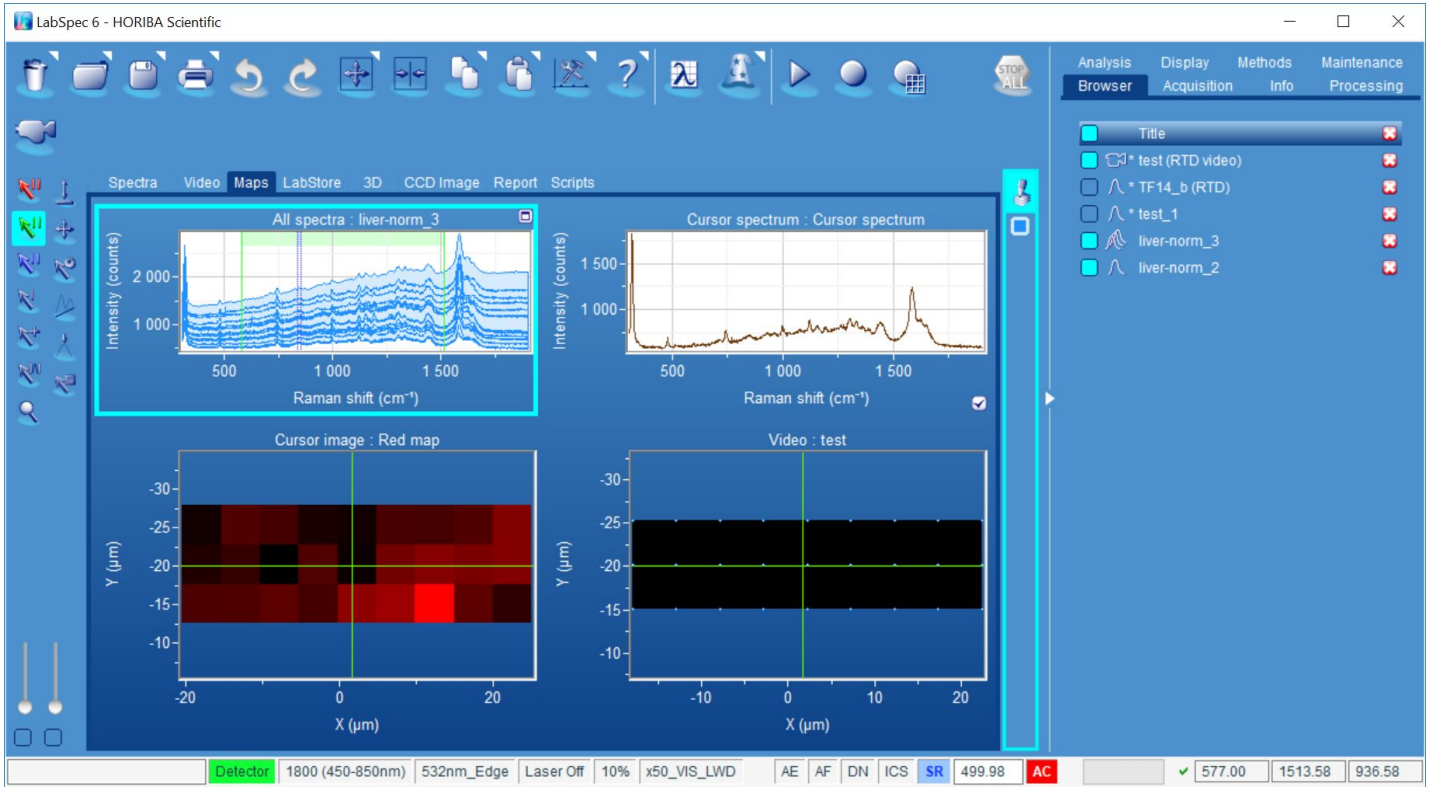
Measurement time: 2 hours

Measurement

Raman spectrum from normal liver tissue

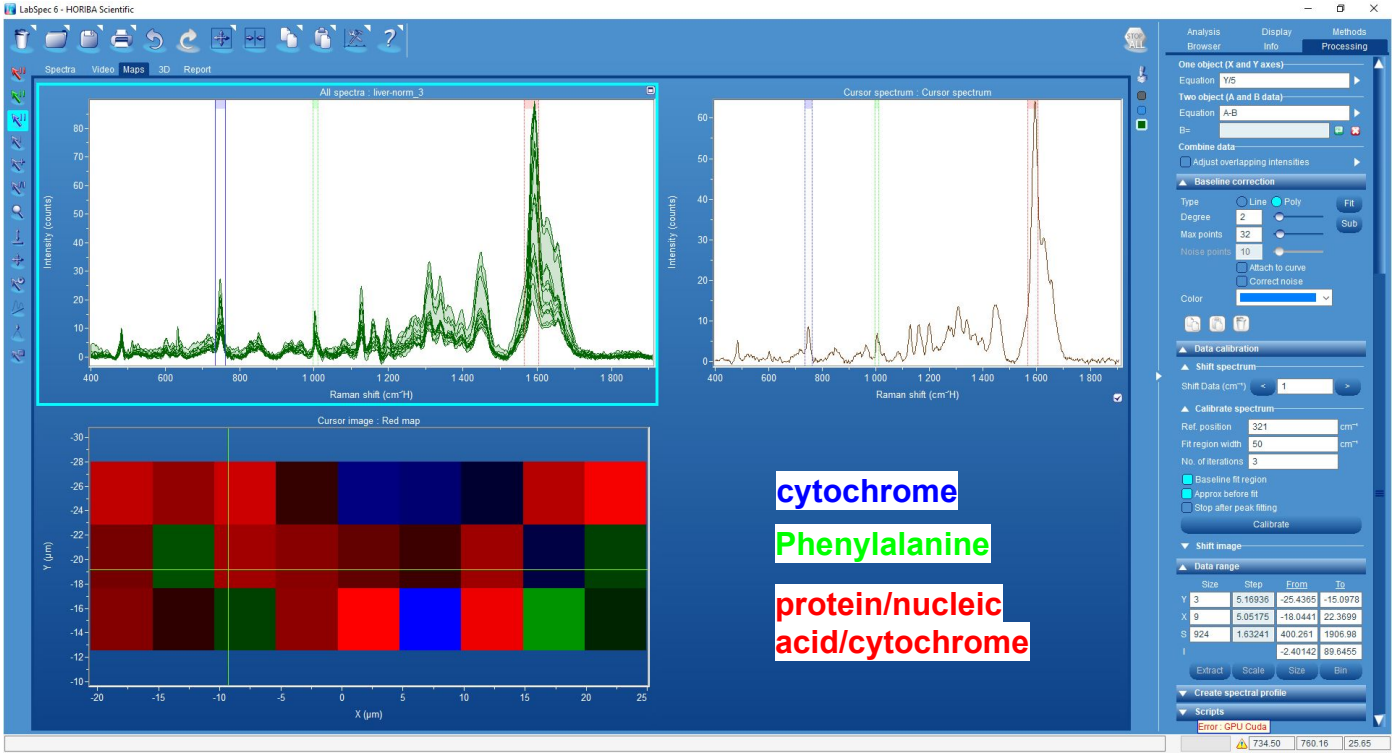


Mapping


LabSpec 6 - HORIBA Scientific
Analysis
Display
Methods
Maintenance
Browser
Acquisition
Info
Processing
Title
* test (RTD video)
* TF14_b (RTD)
* test_1
liver-norm_3
liver-norm_2
Spectra
Video
Maps
LabStore
3D
CCD Image
Report
Scripts
All spectra : liver-norm_3
Intensity (counts)
Raman shift (cm⁻¹)
Cursor spectrum : Cursor spectrum
Cursor image : Red map
Video : test
Y (µm)
X (µm)
Detector
1800 (450-850nm)
532nm_Edge
Laser Off
10%
x50_VIS_LWD
AE
AF
DN
ICS
SR
499.98
AC
577.00
1513.58
936.58

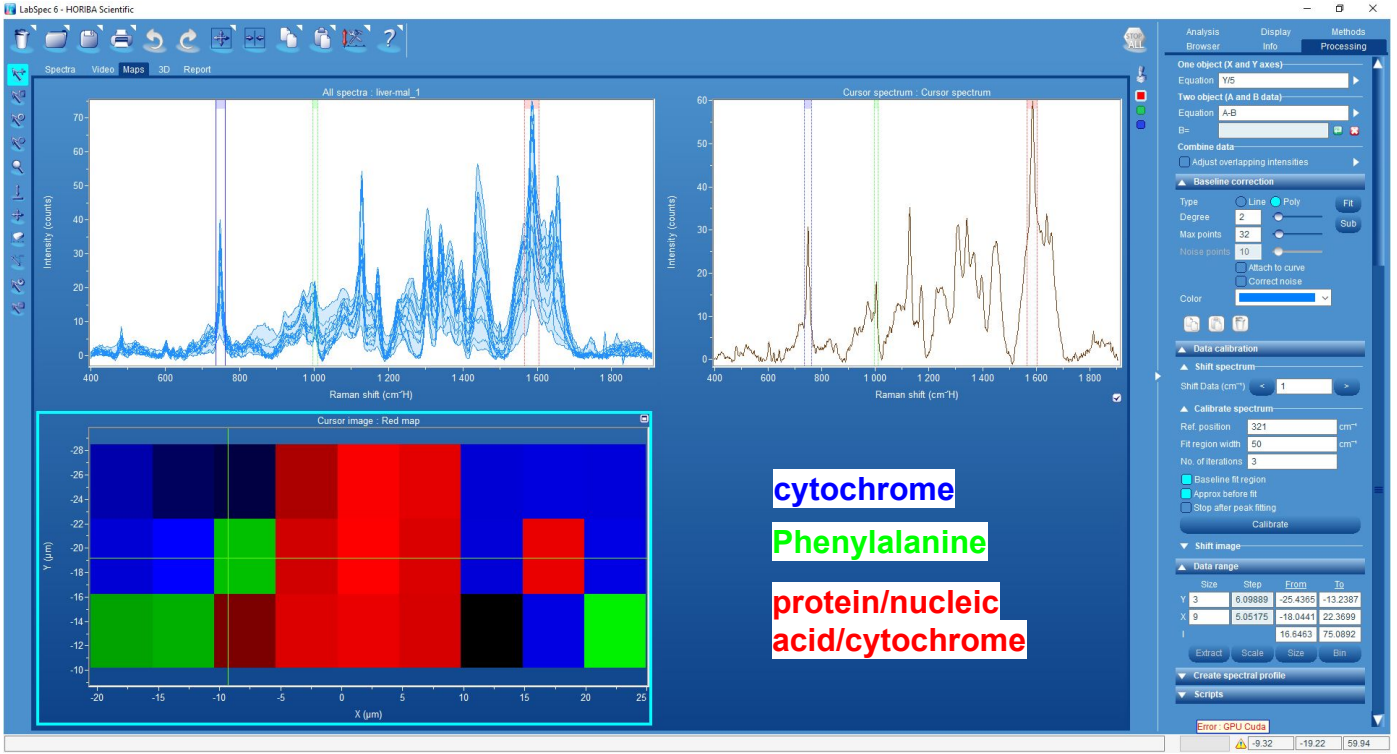
Pseudocolor imaging on individual tissue samples

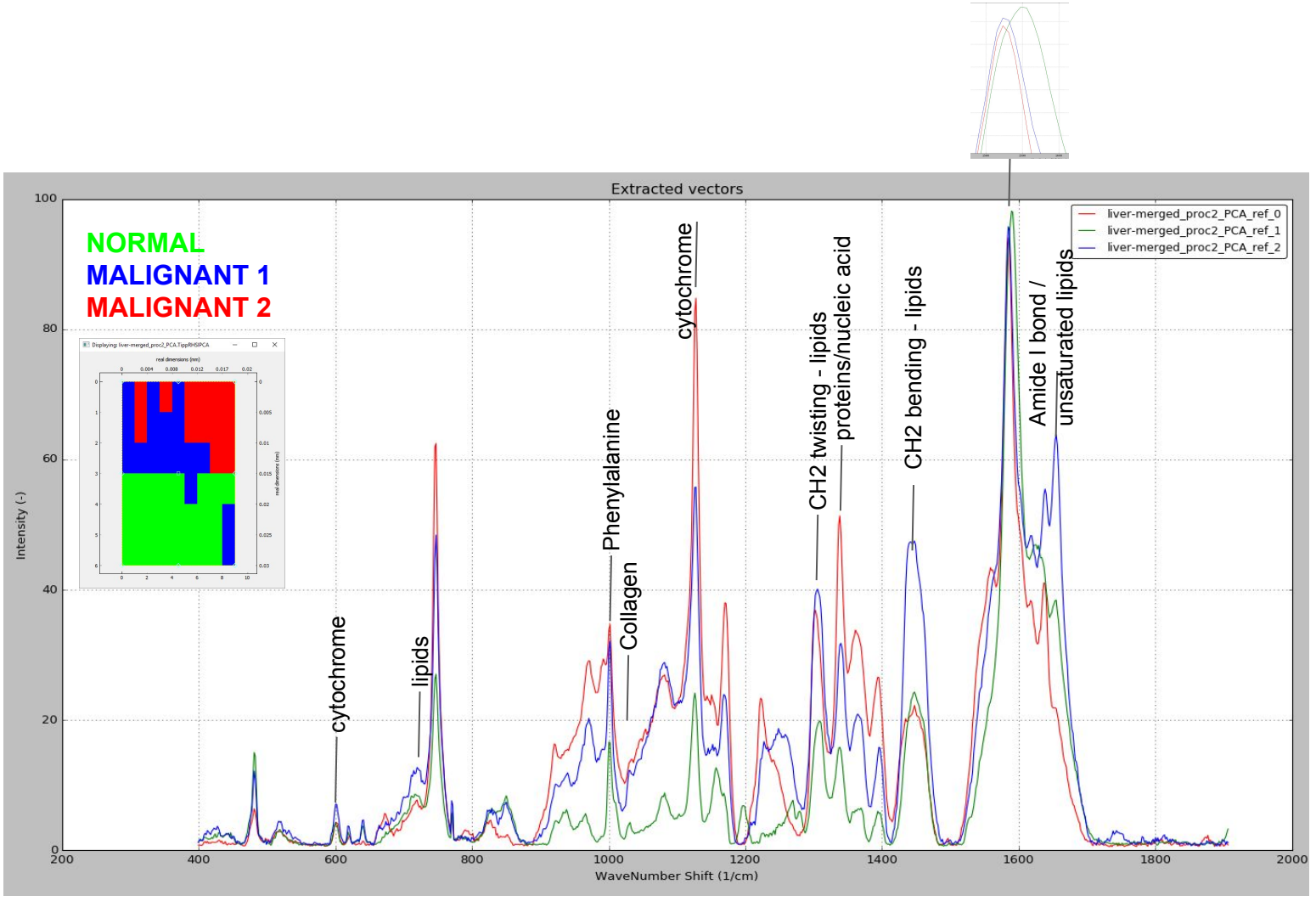
Normal



Pseudocolor imaging on individual tissue samples

Malignant




Extracted vectors
NORMAL
MALIGNANT 1
MALIGNANT 2
liver-merged_proc2_PCA_ref_0
liver-merged_proc2_PCA_ref_1
liver-merged_proc2_PCA_ref_2
cytochrome
lipids
Phenylalanine
Collagen
cytochrome
CH2 twisting - lipids
proteins/nucleic acid
CH2 bending - lipids
Amide I bond /
unsaturated lipids
Intensity (-)
WaveNumber Shift (1/cm)

Solutions

- Laser line at 785 nm (minimize autofluorescence <70%) - to be tested soon
- Water immersion objective lens (increase signal by 3.5 times) - under testing
- Surface tracing algorithms - in progress
- Develop advanced machine learning algorithms - under testing
- Develop the calibration methodology - in progress
- Develop the reference spectra database - in progress

Problems, problems, problems...

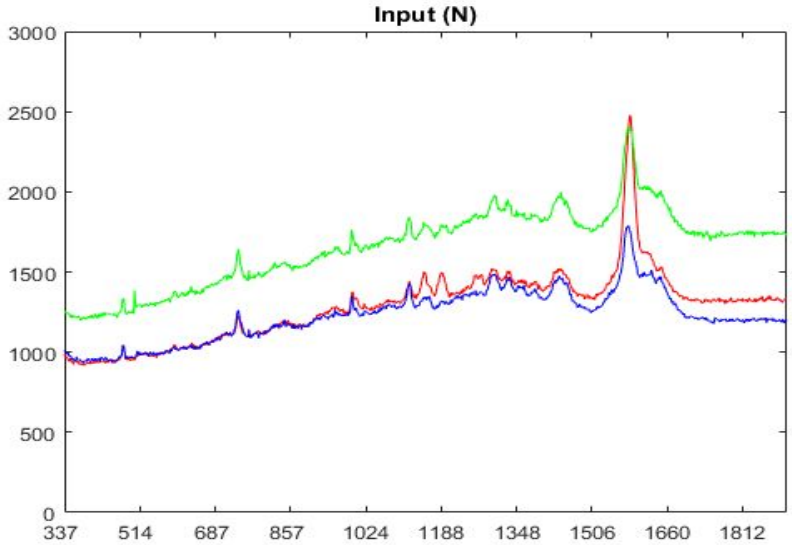
1. Raman is 10E-6 of the emitted signal so everything matters
2. Focal distance affects signal intensity
3. Background is different on every point due to AF
4. There is no calibration process to secure quantitative measurements
5. There is no specific reference database
6. Classification is currently performed on peaks comparison, manually

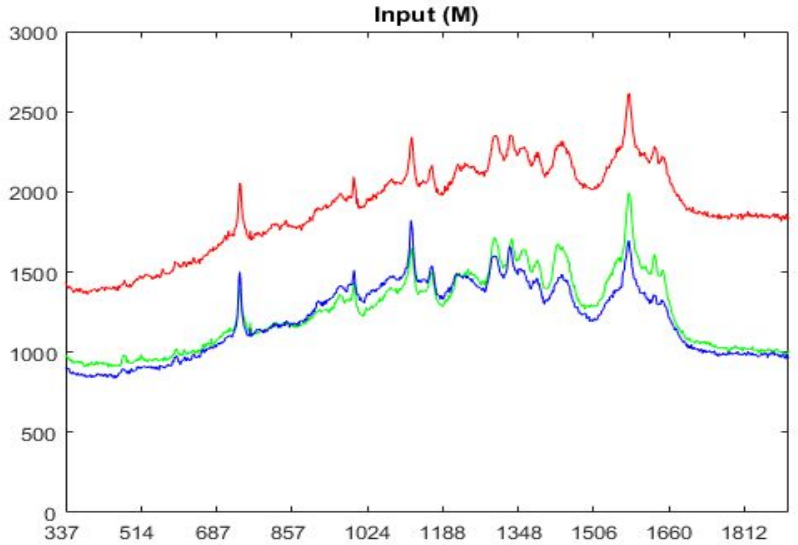
Preliminary results from machine learning algorithms

Work on progress...

Robust PCA (advanced signal processing)

Machine learning algorithms to extract repetitive information from large datasets.

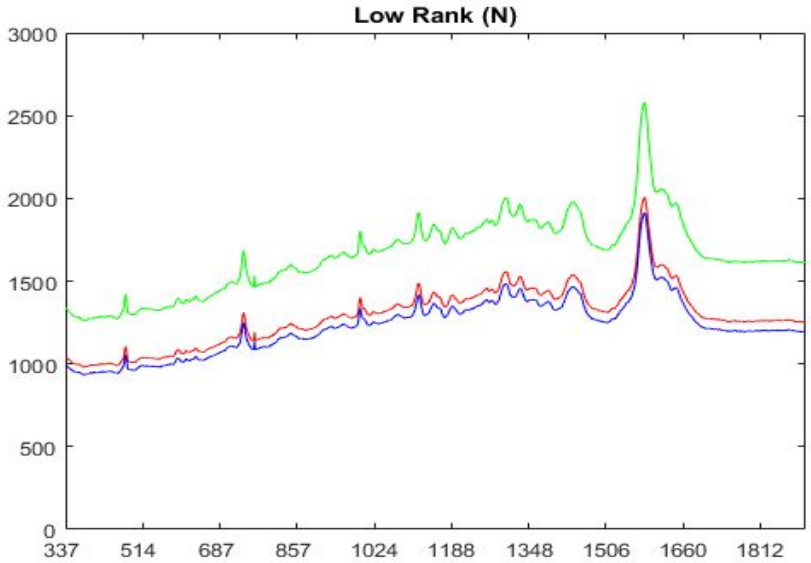


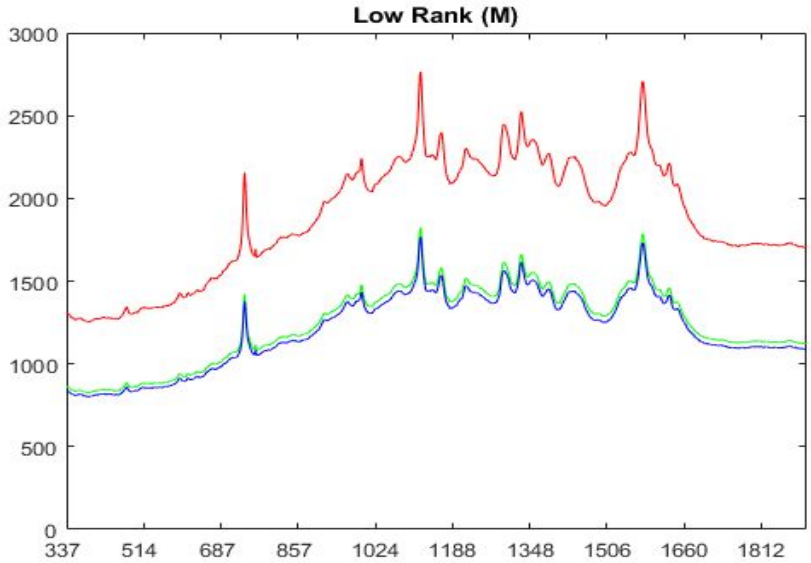


Each color line represents a random point on the image map

Robust PCA (advanced signal processing)

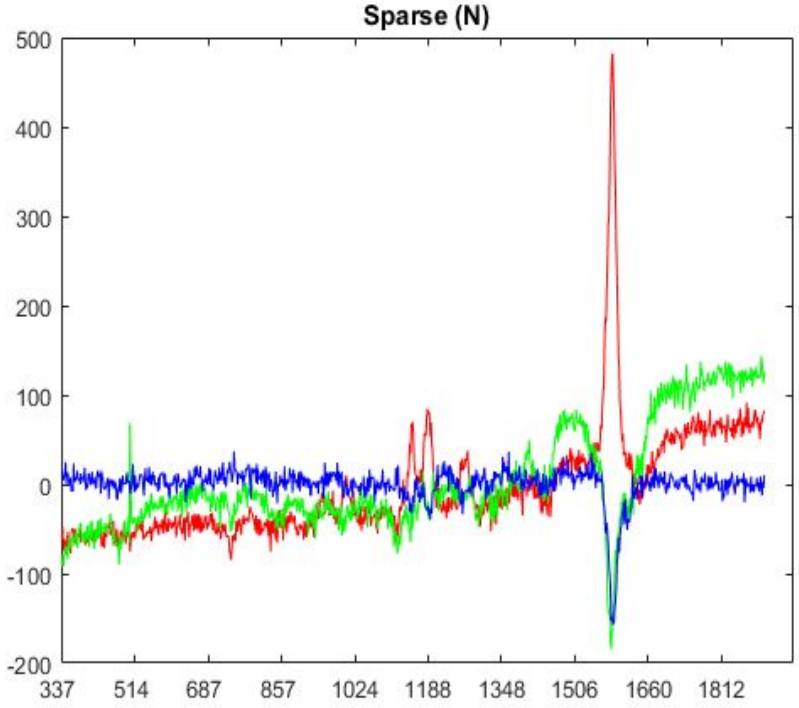
Identification of common Raman signal within the 27 spatial points of the map

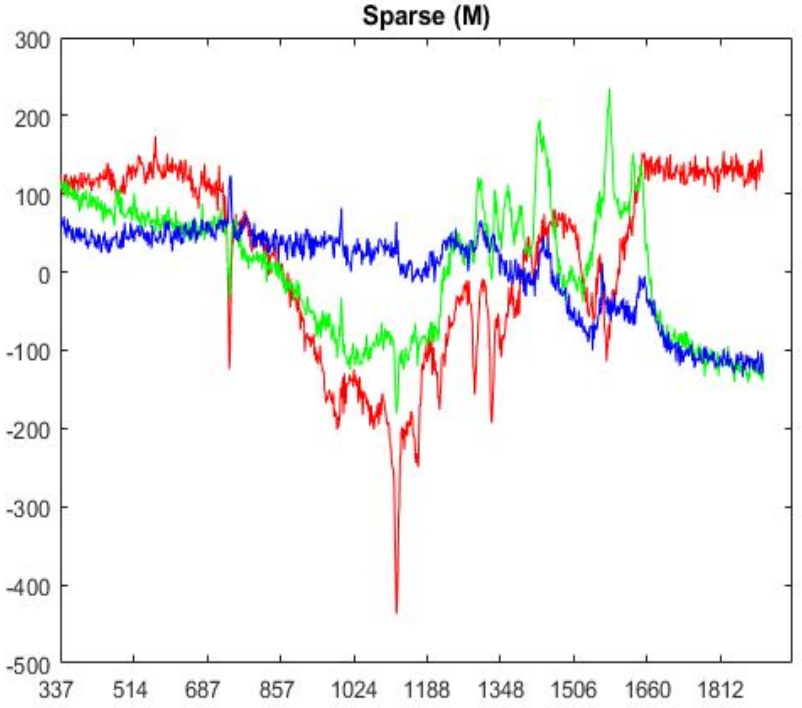




Robust PCA (advanced signal processing)

Raman signal differences between the 27 spatial points of the map





Thank you for your attention!