Quantification of Tablets using Raman Imaging

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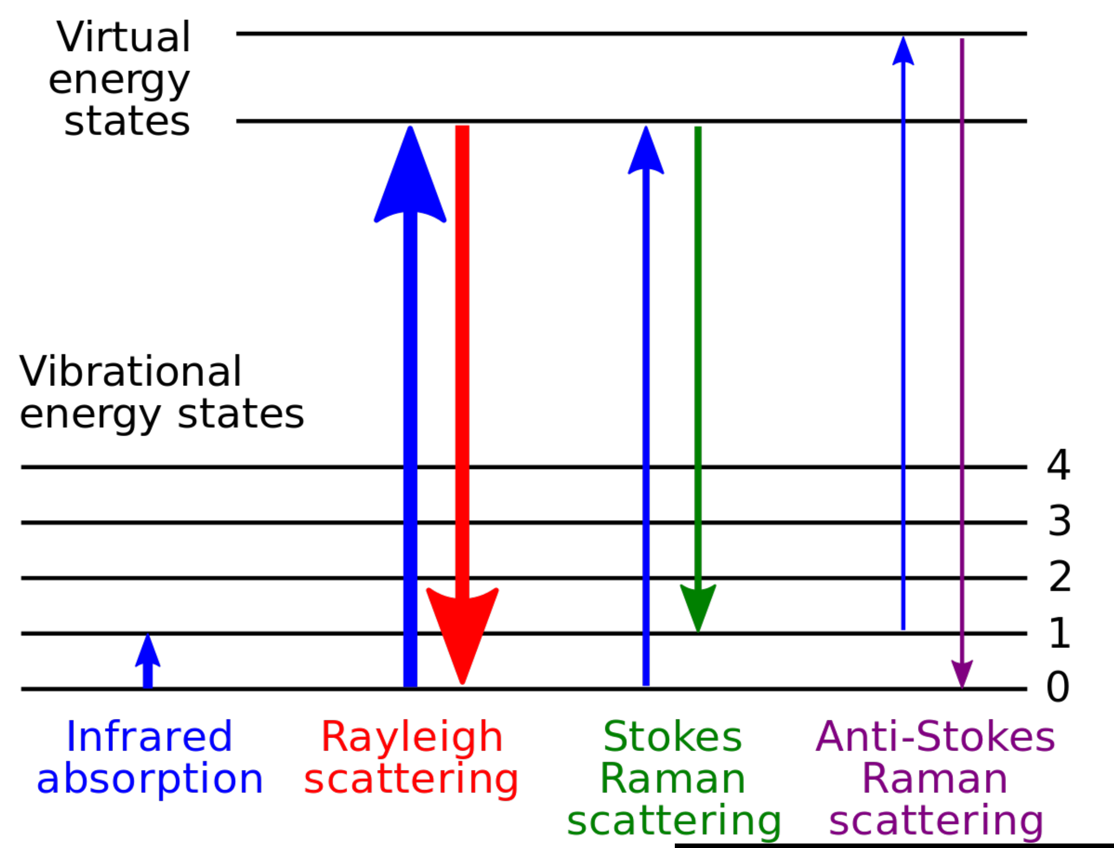
Background

What is Raman spectroscopy?

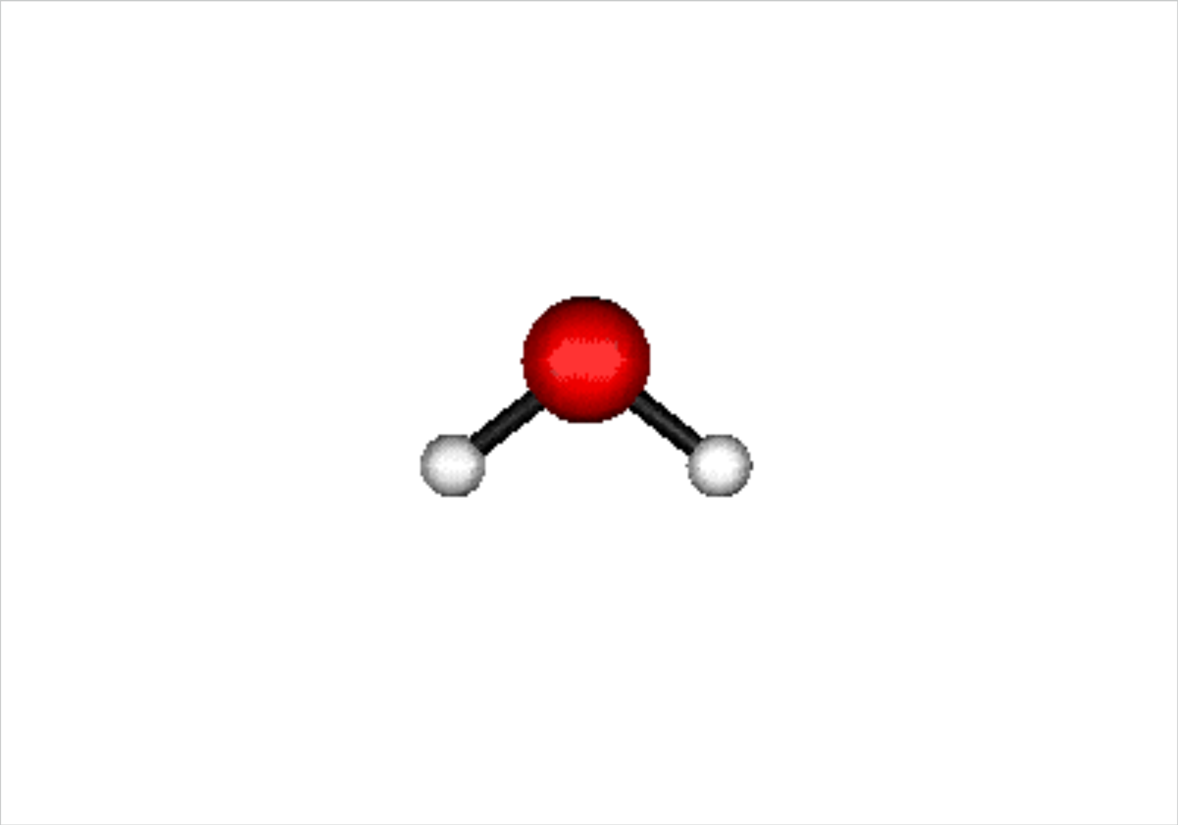
Raman Spectroscopy

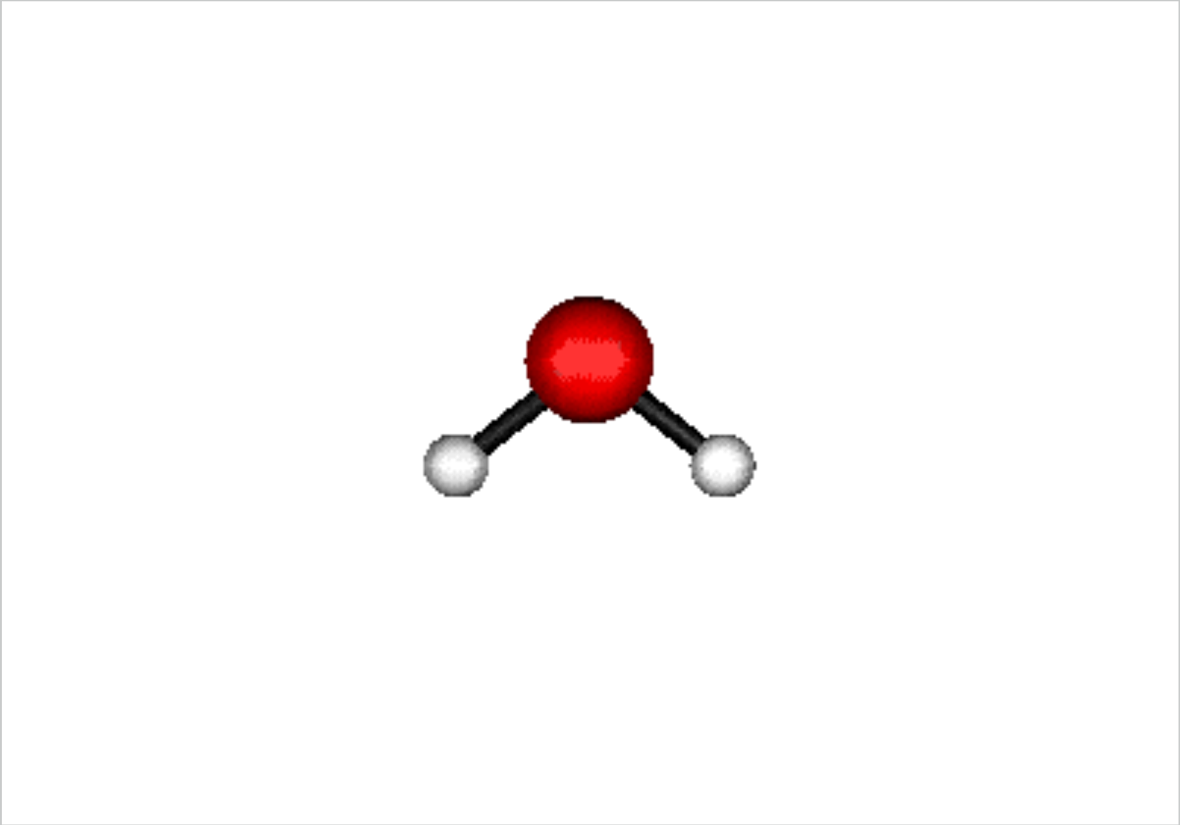
- A spectroscopic technique is used to observe vibrational, rotational and other low frequency modes in a system.
- Commonly used in chemistry to provide a **fingerprint** by which **molecules can be identified**.
- This latter portion (Stokes and anti-Stokes) is what we are interested in.



How A Molecule Reacts? (H_2O)

- Excited by the laser molecules reach a higher energy state.
- While returning to the stable (relaxed) state there is a release of energy indicative of the structure of the molecule.
- The difference in the wavelength is what Raman captures.



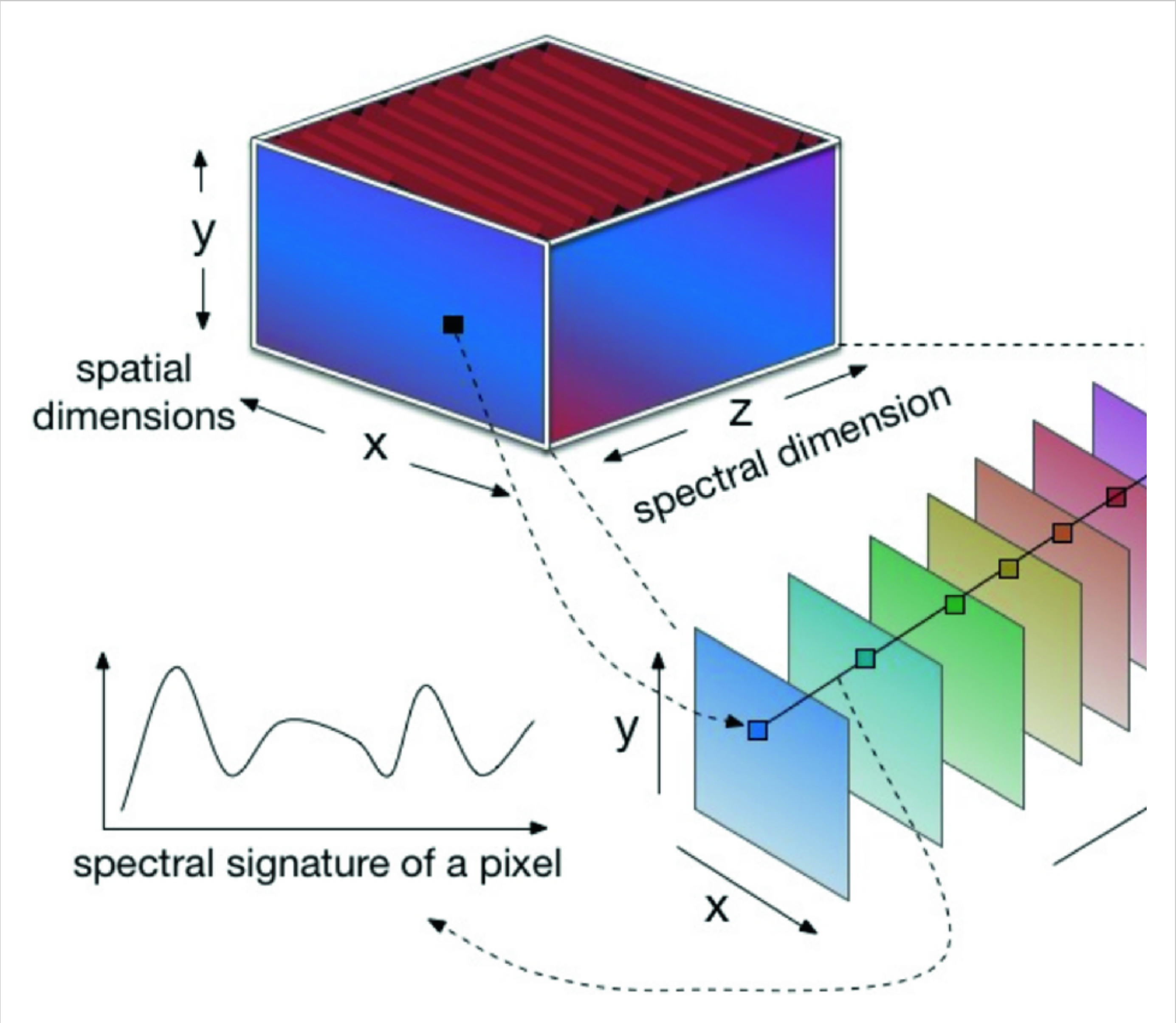


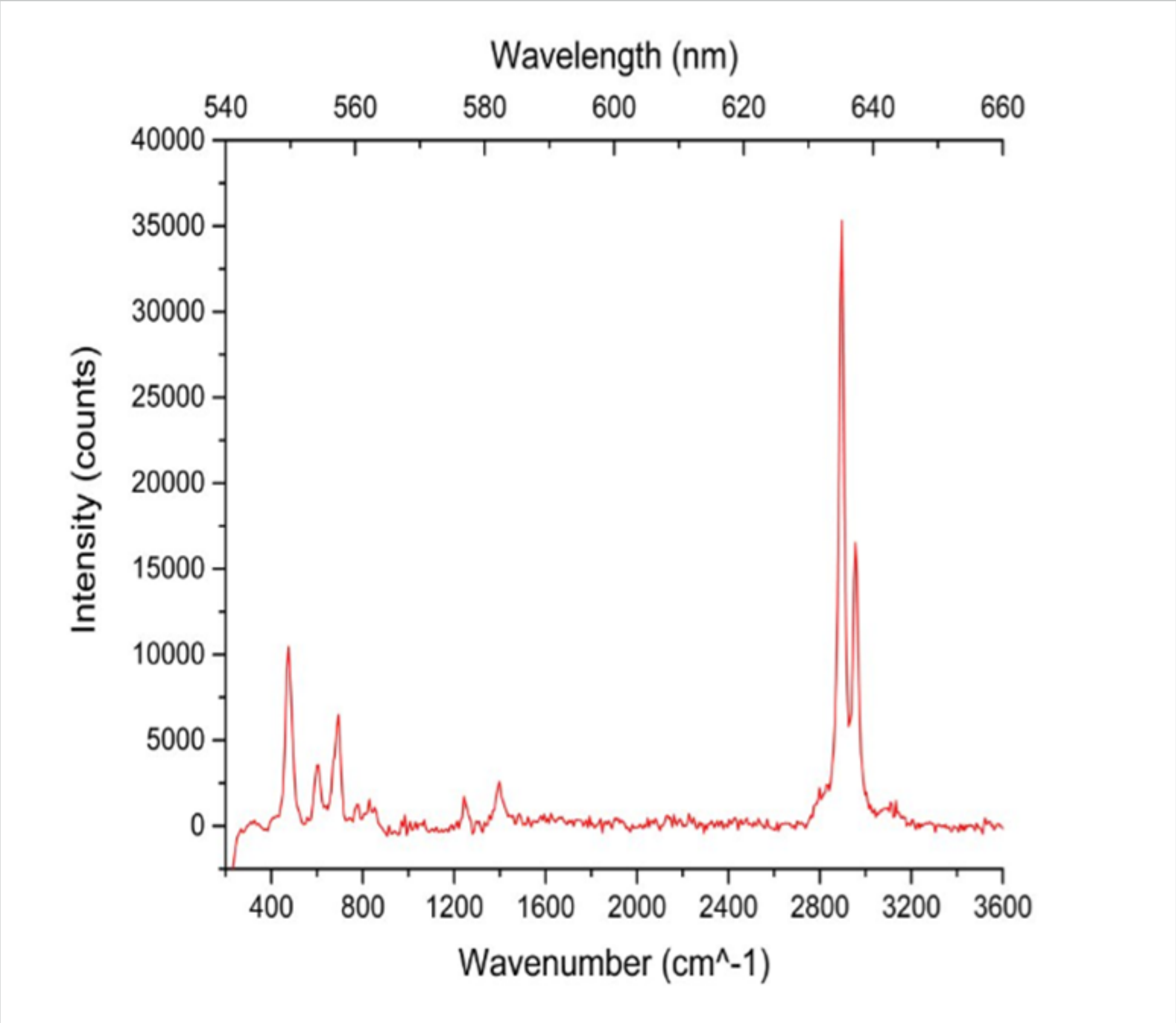
Raman Data

Handling Complex Hypercubes

Raman Data Hypercube

- The data acquired from Raman spectroscopy is a **3-dimensional hypercube**.
- The **spatial dimensions** (X, Y) indicate the location/co-ordinate in the sample.
- The **spectral dimension** (Z) contain the spectral signature i.e. the intensity measurements at varying incident wavelengths.





Sample Raman Spectrum

- X-axis: Wavenumber is the number of waves per unit distance.
- Y-axis: Intensity (counts) is the quantity of events the spectrometer detects for the particular Raman shift per second and is relative to the strength of light detected.

Pre-processing of Raman Spectra

Why?

Reduce the or eliminate irrelevant, random and systematic variations in the data.

Spike Removal

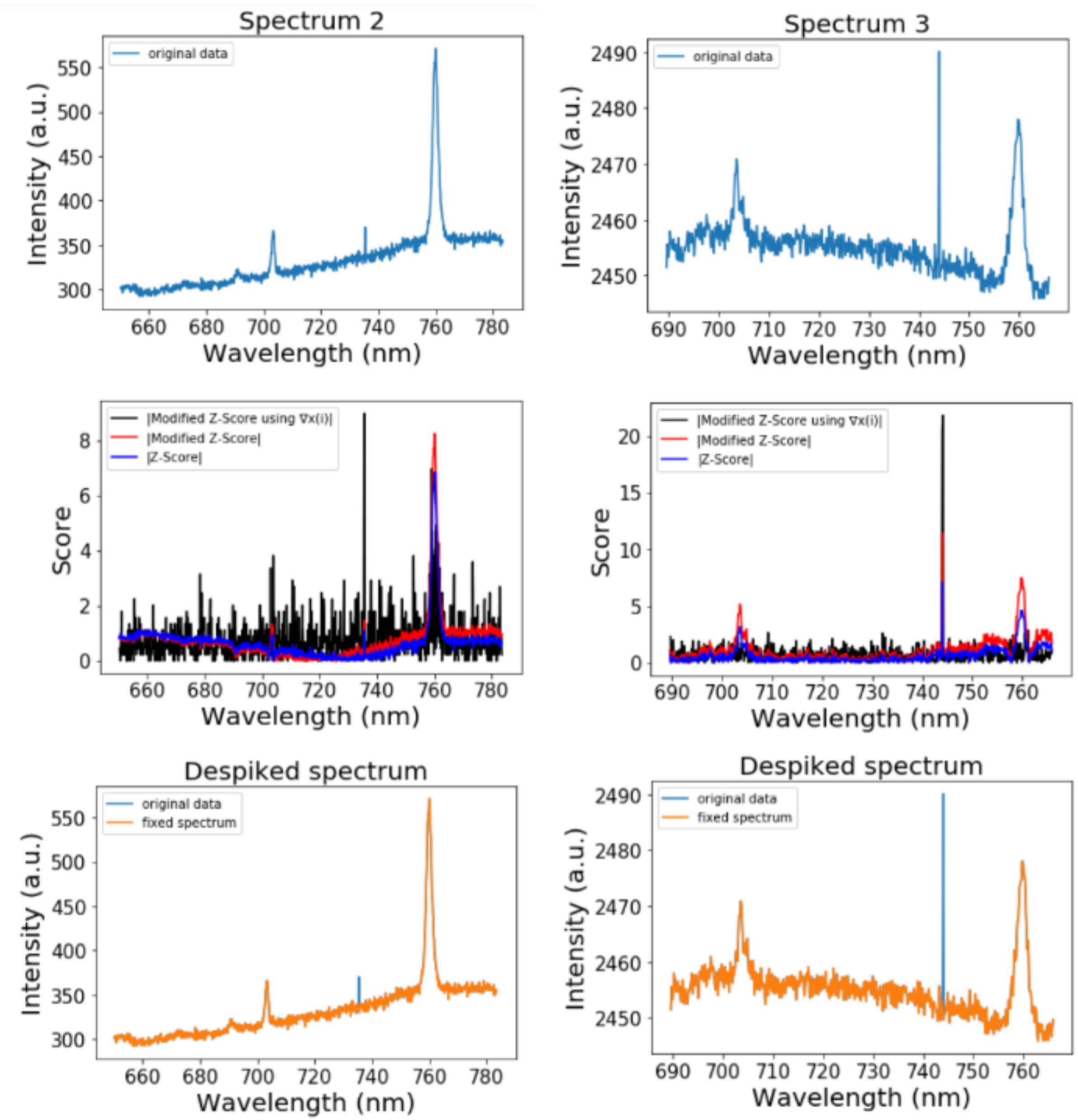
- Spikes:
  - Spurious nuisance events that typically appear at random positions and are present as positive, narrow bandwidth peaks.
  - Arise when the detector is truck by an errant high-energy particle.
- Despiked Spectra:
  - Interpolated values
  - $\bar{Y}_t = (\sum_{t-m}^{t+m} Y_t * I(\left|Z_t\right| < \tau))$/w ,

    where I is the indicator function

    and $w = \sum_{t-m}^{t+m} I(\left|Z_t\right| < \tau)$.

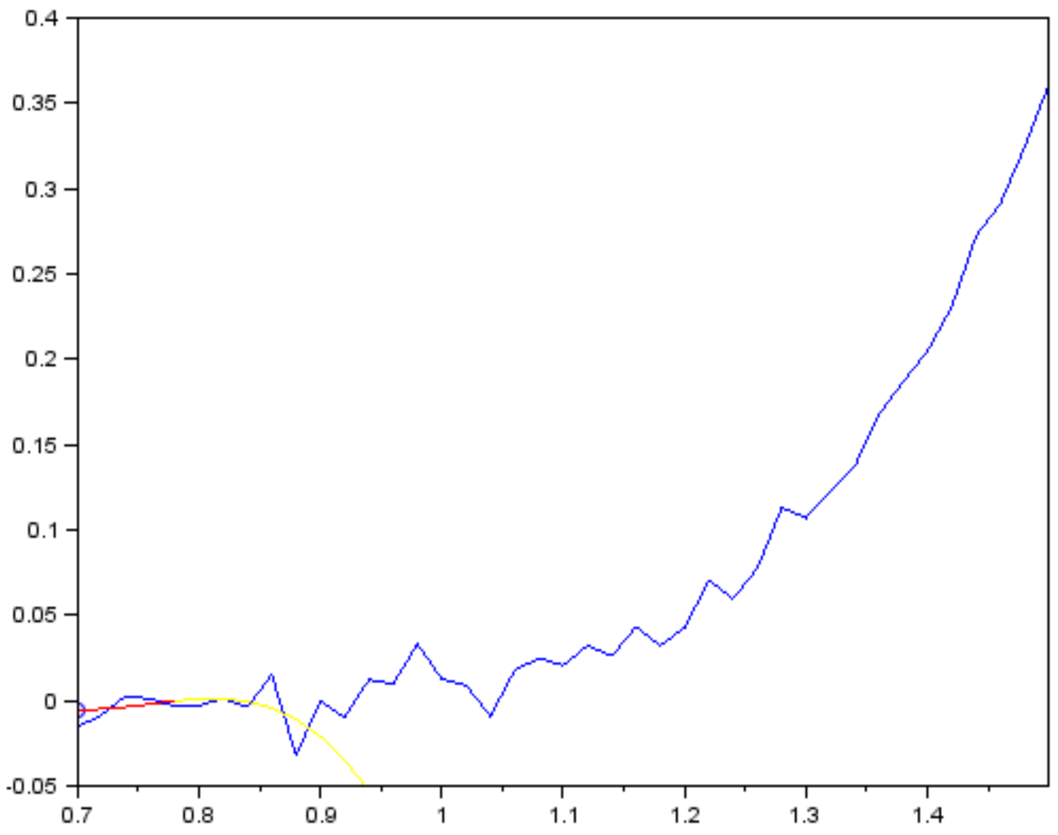
- Removal:
  - Let $Y_1, \ldots, Y_n$: values of Raman spectra equally spaces wavenumbers.
  - The detrended series:
  - $\nabla Y_t = Y_{\{t-1\}}$has the effect of annihilating linear and slow moving curve linear trends, however sharp peaks will be preserved.
  - Say M: median{∇Y_t} and MAD: median{$|\nabla Y_t - M|$}
  - Then the Z-scores can be defined as:
    - $Z_t = \left\{0.6745 * \left(\nabla Y_t - M\right)\right\}/MAD$
    - The criterion $\left|Z_t\right| > 3.5$ was proposed as guideline for outlier detection.

Spike Removal



Smoothing

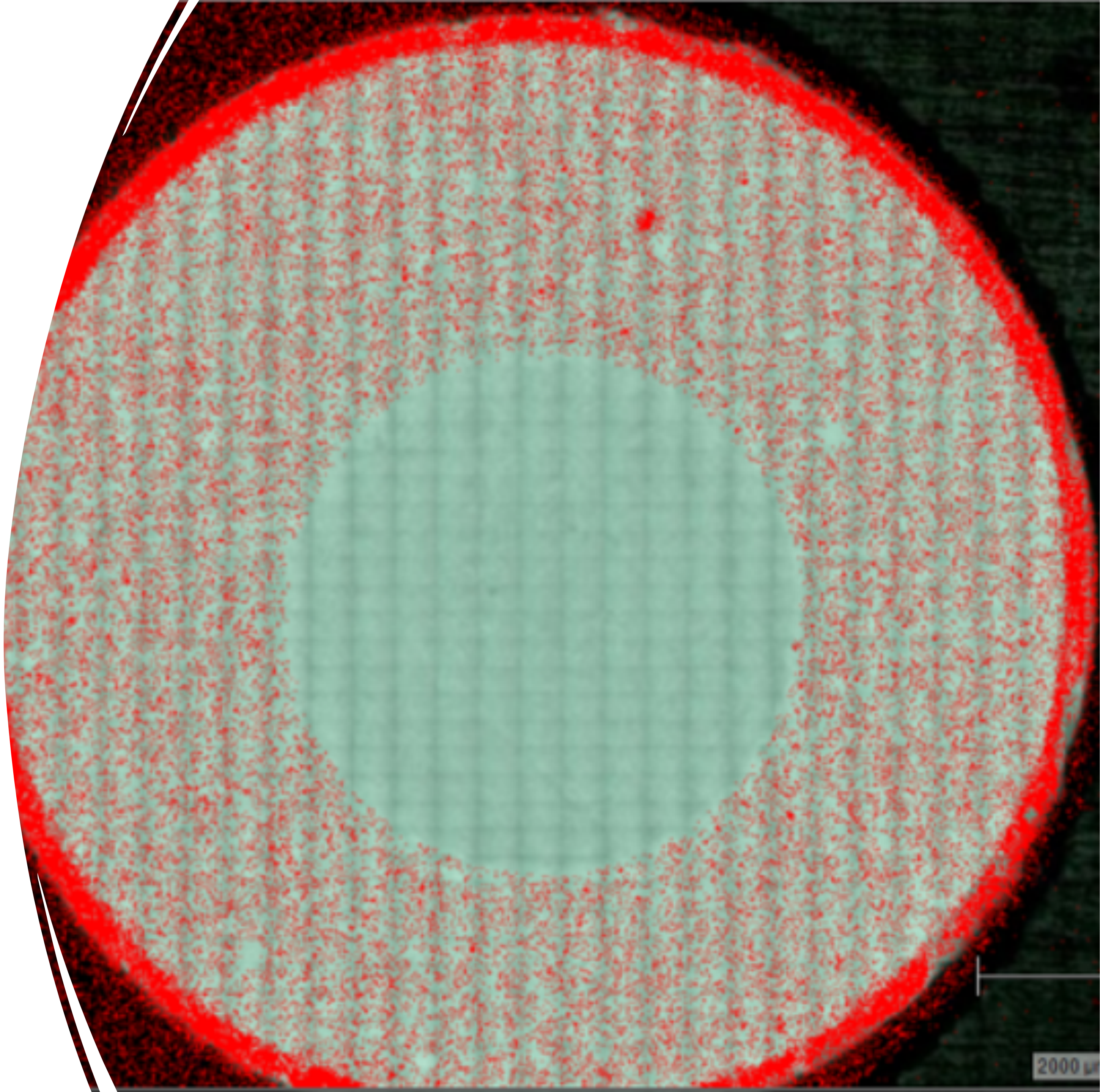
- Savitzky-Golay Filter:
  - Method of data smoothing based on **local least-squares polynomial approximation**.
  - Advantage: Reduces noise while ***maintaining the shape and height of waveform peaks***.
  - Say, $x[n]$ is the intensity at n^{th} wavenumber.
  - Considering a window of size 2M+1 centered at n=0, we obtain the coefficients of a polynomial –
    - $p(n) = \sum_{k=0}^{N} a_k n^k$



Quantification of Components from pre-mapped Raman Images

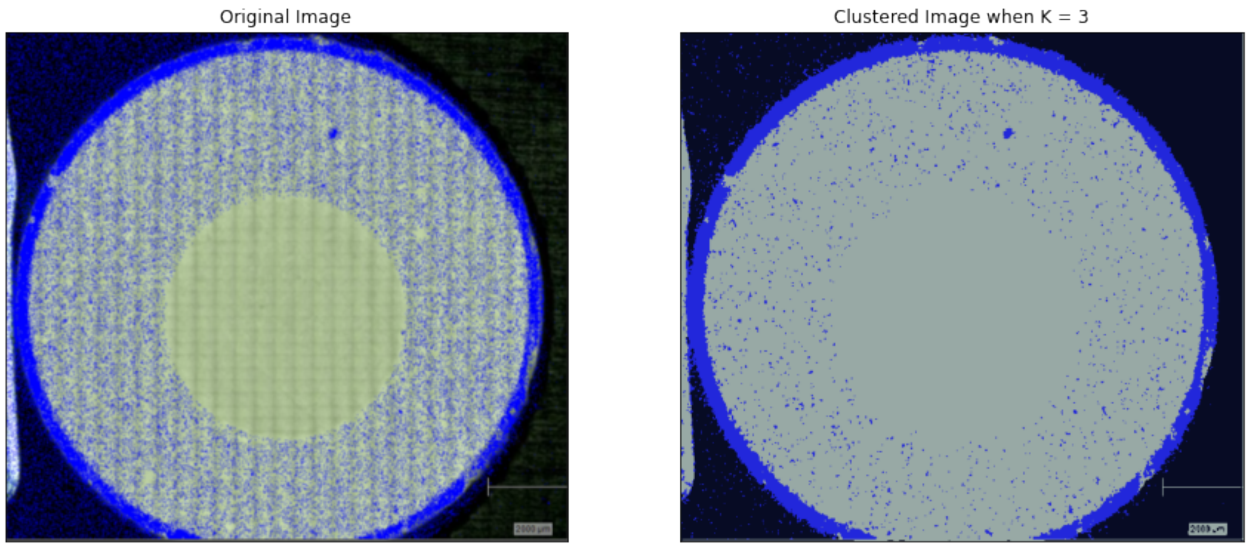
Estimation of the fractional quantity of Component-A in entire tablet

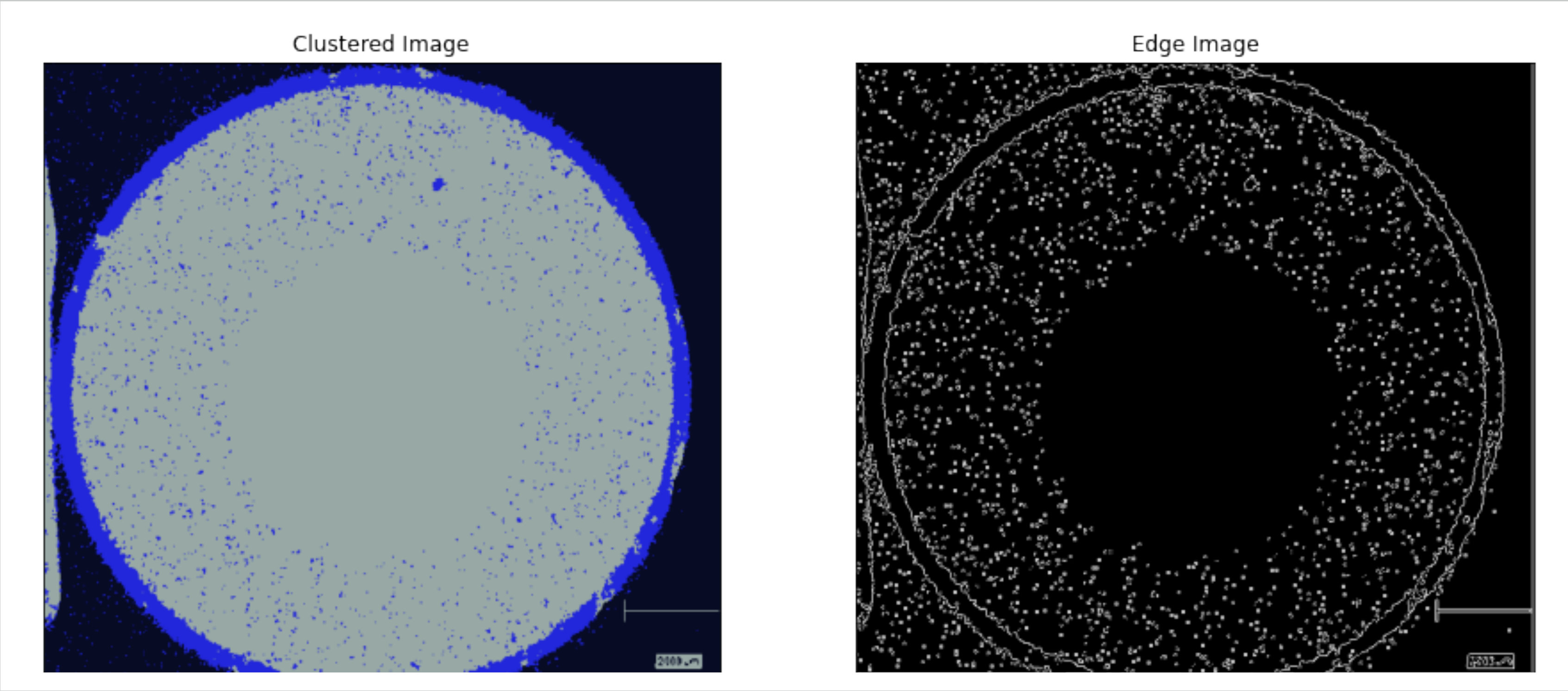
- Input Image: An overlay of Component-A (color mapped as Red) on the white light image of the entire tablet.
- Methodology used:
  - ROI selection and cropping
  - K-means clustering
  - Edge Detection
  - Morphological Transformation
  - Contour Detection
  - Contour Area Computation



K-means Clustering

- Choice of K = 3 since:
    - 1 – Background outside the tablet
    - 2 – Background on the tablet
    - 3 – Component A
- The clustering algorithm can identify the small regions within the circular area. However due to the close gradient of the background (2) some of the points seem to be missed out.



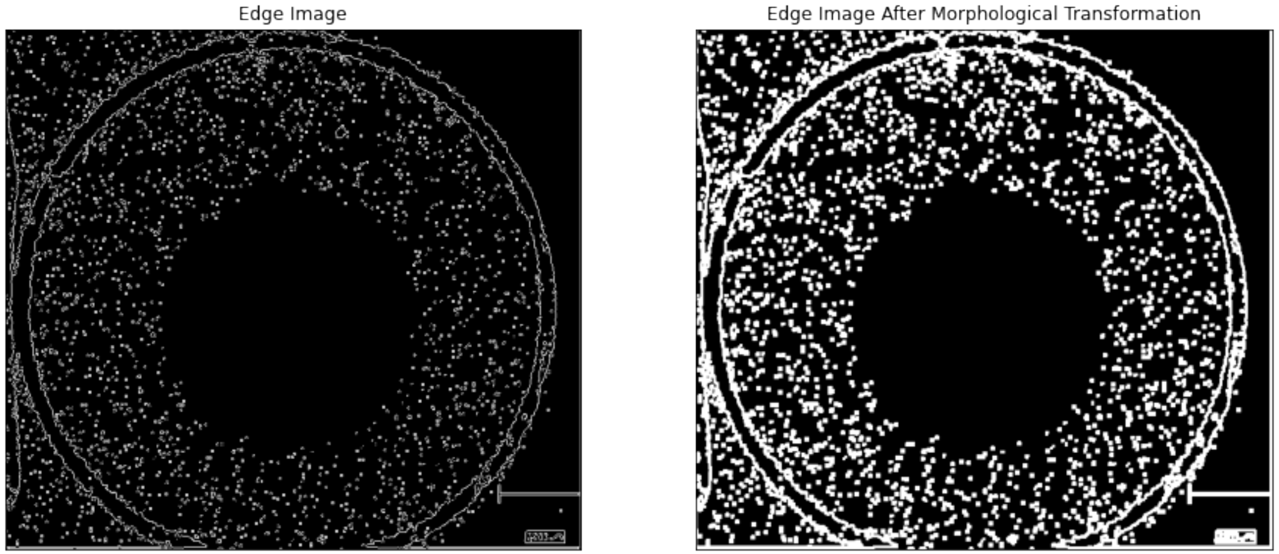


Edge Detection

- In order to get an idea of the area covered by the component we begin by finding the edges over the clustered image.

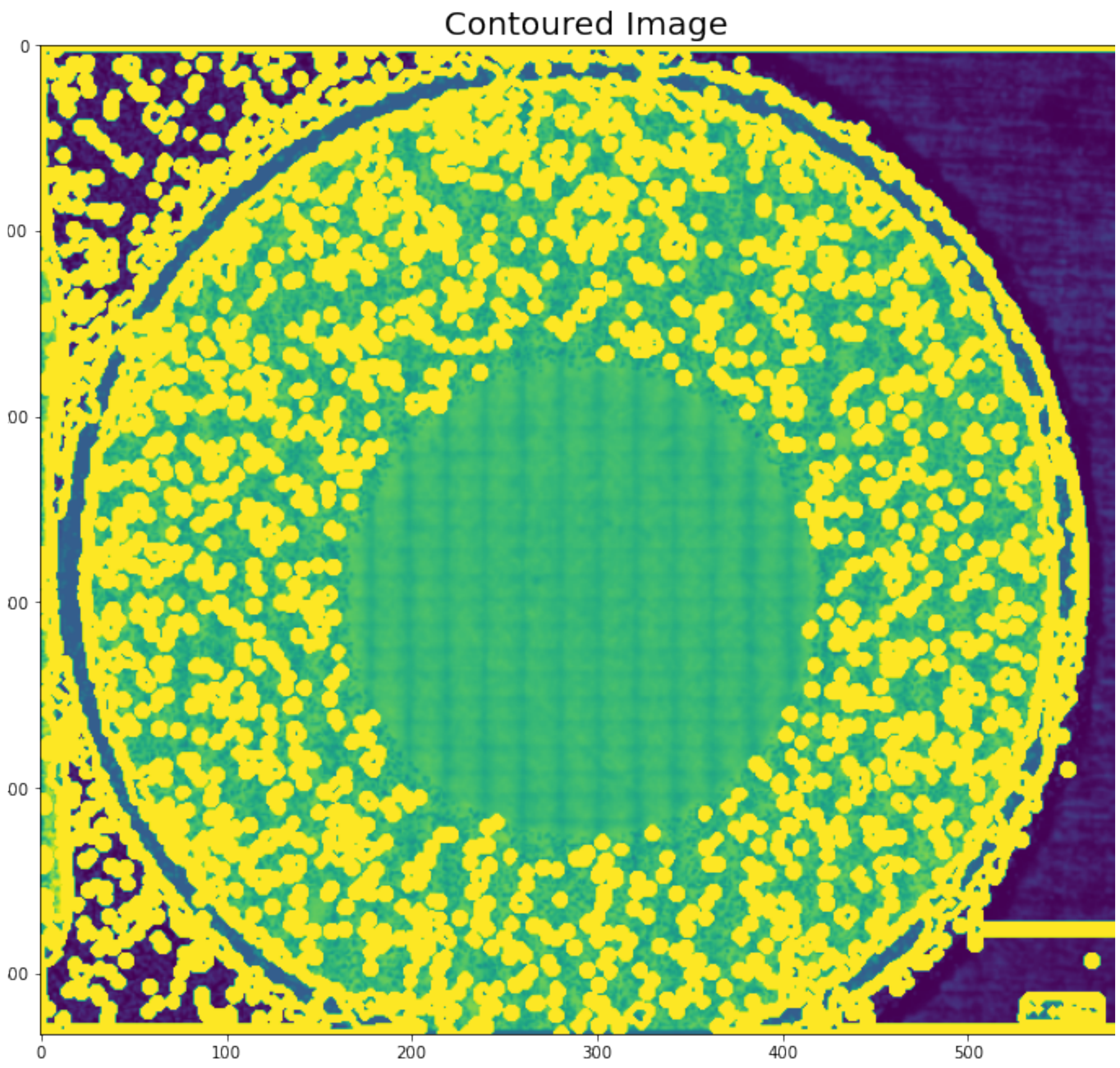
Morphological Transformation

- Since the edges detected are very fine some of the regions that are supposed to be together are broken.
- In order to overcome this, we perform a morphological operation: **Dilation**.
- Dilation **turns a pixel value of the original edged image to 1 if at least one of the pixels under the kernel is 1**.
- Thus, results in joining of many broken regions which will consequently contribute to the area covered by a component.



Contour Detection & Area Computation

- The contours detected show some alarms:
    - There are a few contours outside the ROI that require to be removed.
    - Since the image currently used is a screenshot the entire image box is taken as a contour that requires to be addressed.
- Once the contours are filtered, we compute the contour area .



Univariate Methodologies

Intensity based Color Map from Raman spectral data

Intensity - Concentration Relation

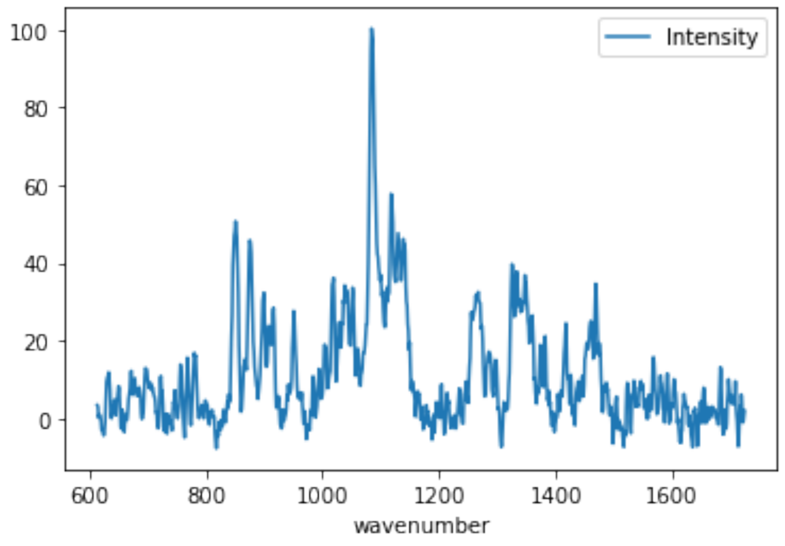
- Quantitative Raman measurements utilize the following relationship between **signal**, $\boldsymbol{S\gamma}$, at a given wavenumber, γ, and the **concentration of the sample**, $\boldsymbol{C}$ –

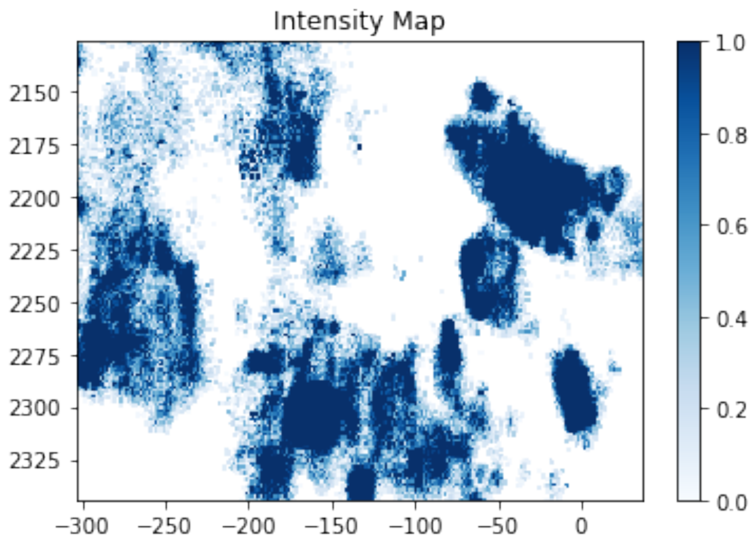
  - $\boldsymbol{S_\gamma = K\sigma_\gamma\gamma_L\left(\gamma_L - \gamma_\beta\right)^3 P_0 C}$
    - K: Constant that depends on laser beam diameter, collection optics, sample volume and temperature.
    - σ_γ: Raman cross-section of the particular vibrational mode.
    - γ_L: Laser wavenumber
    - γ_β: Wavenumber of the vibrational mode.
    - P_0: Laser power.

- From the above equations, it is apparent that peak signal is directly proportional to concentration.
- On the basis of this relationship we are trying to quantify the components.

Intensity At A Point

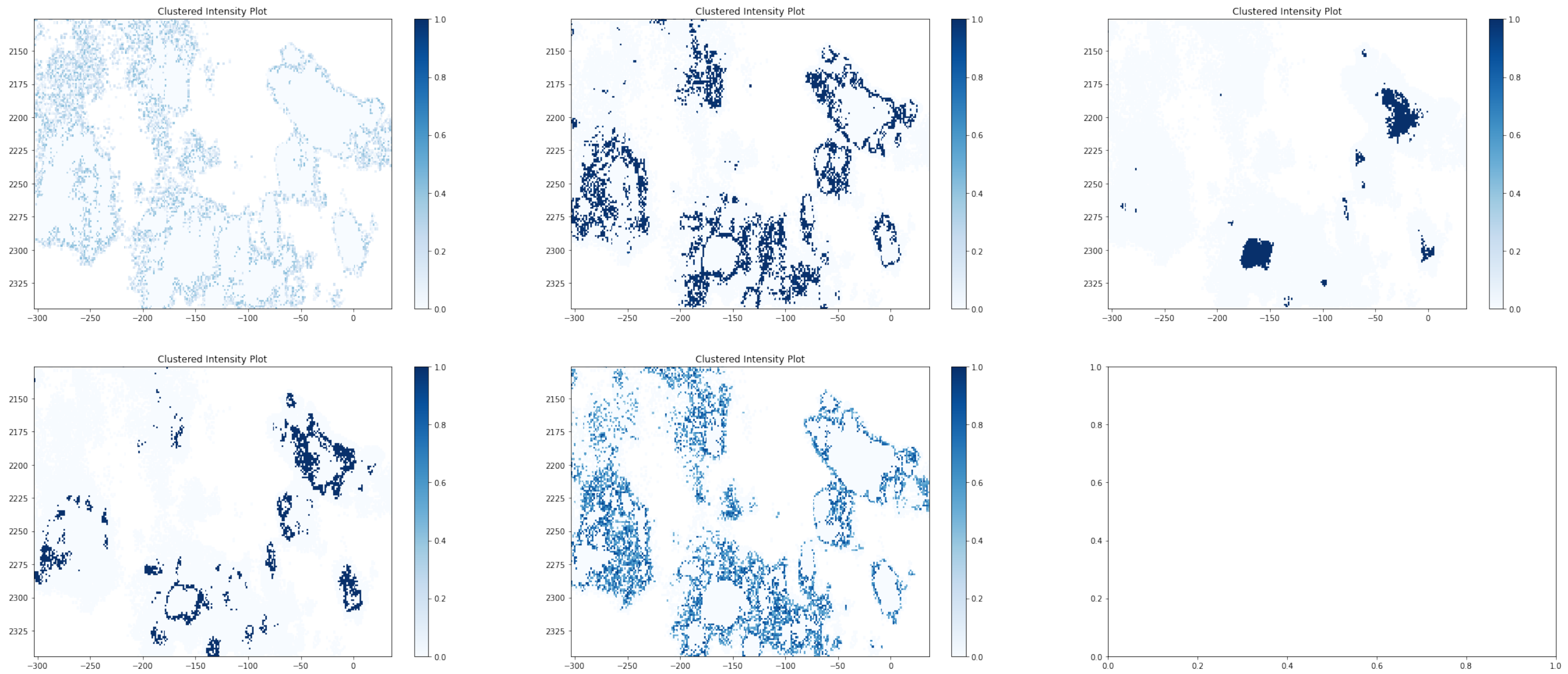
- Data: Acquired Raman spectra at every co-ordinate.
- Corresponding to each component the **unique peak intensity** is extracted within the interval provided by the domain expert.
- Burn outs and background is filtered out by thresholding over a certain value of intensity. [here it was taken to be 500 arbitrary units.]
- Pre-processing: The unique peak intensities at every co-ordinate is normalized.
- Color mapping is based on these normalized intensities.





Clustered Intensity Maps

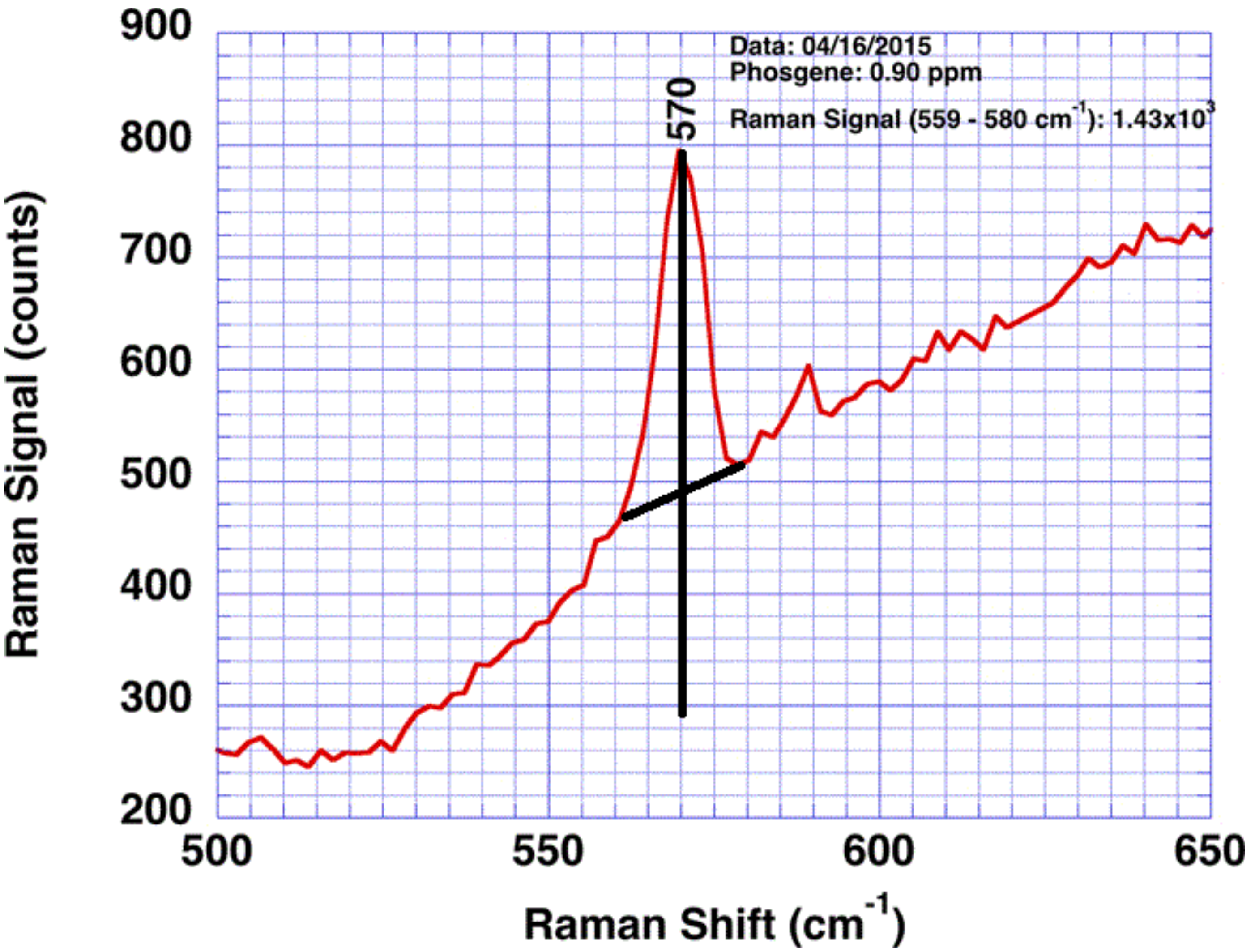
Deeper the color implies higher the intensity, thereby indicating higher concentrations.



Clustering method: Gaussian Mixture Model

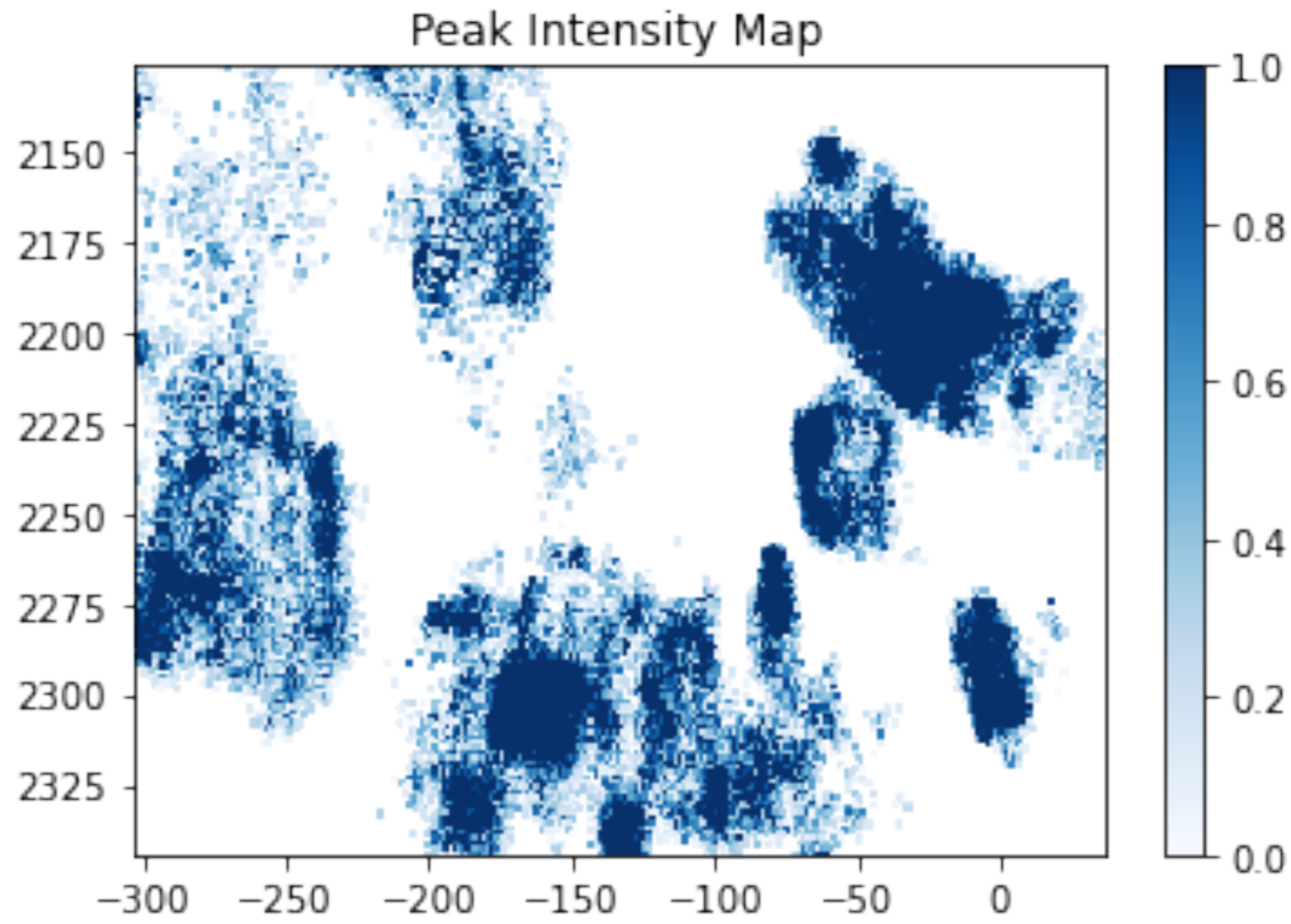
Peak Intensity

- At the given interval [a, b] we obtain a straight line.
- The maximum intensities -
- [w, i] extracted from the Raman spectral data correspond to another straight line.
- Intersection of these two straight lines – max intensity = Peak Intensity.



Source: https://aip.scitation.org/doi/full/10.1063/1.4942109

Peak Intensity Based Color Map



Quantification Results

Weighted Proportion

Weights correspond to the cluster means obtained by clustering over the normalized intensities.

Pixels with similar intensity-based color maps are clubbed.

Proportion: 45 - 46.5 %

Pixel Count Proportion

The count of the number of pixels with normalized intensity values > 0 are considered as the presence of a component.

Count of pixels with values < 0 are that of non-components.

Proportion: 42 - 43 %

Multivariate Approaches

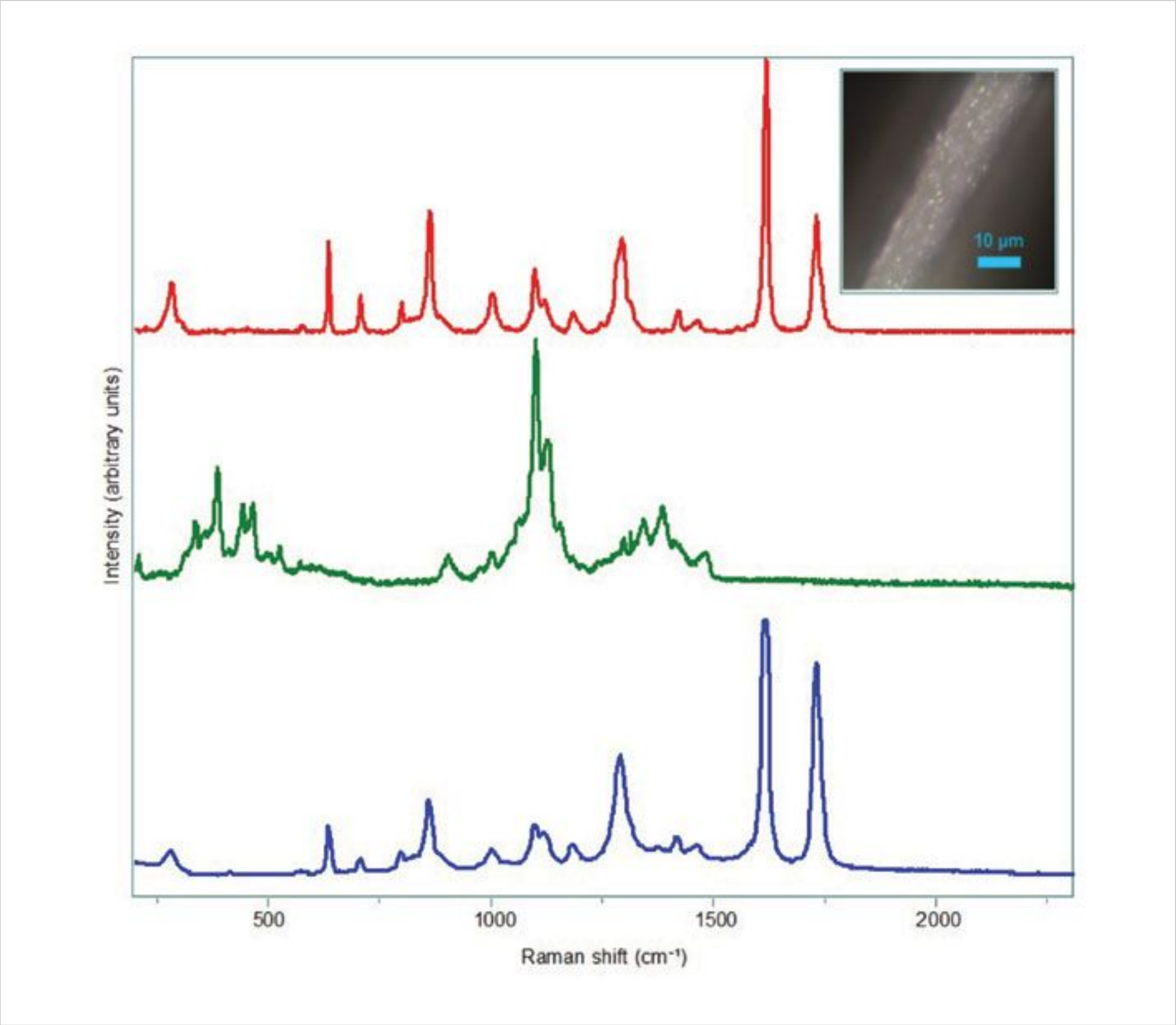
Full spectra Intensity based Color Map from Raman spectral data

Ordinary Least Squares

Regression based Color Map from Raman spectral data

Classical Least Squares

- The method of linear regression using ordinary least squares finds the **linear combination of spectra** from the **pure components** making up the sample **that most closely matches the acquired Raman spectrum** of the sample.
- <u>**The regression coefficients are proportional to the concentration of the respective components**</u>.
- Assumption: The pure spectrum of a component in a sample spectrum remains unchanged when mixed with pure spectrum of another component.

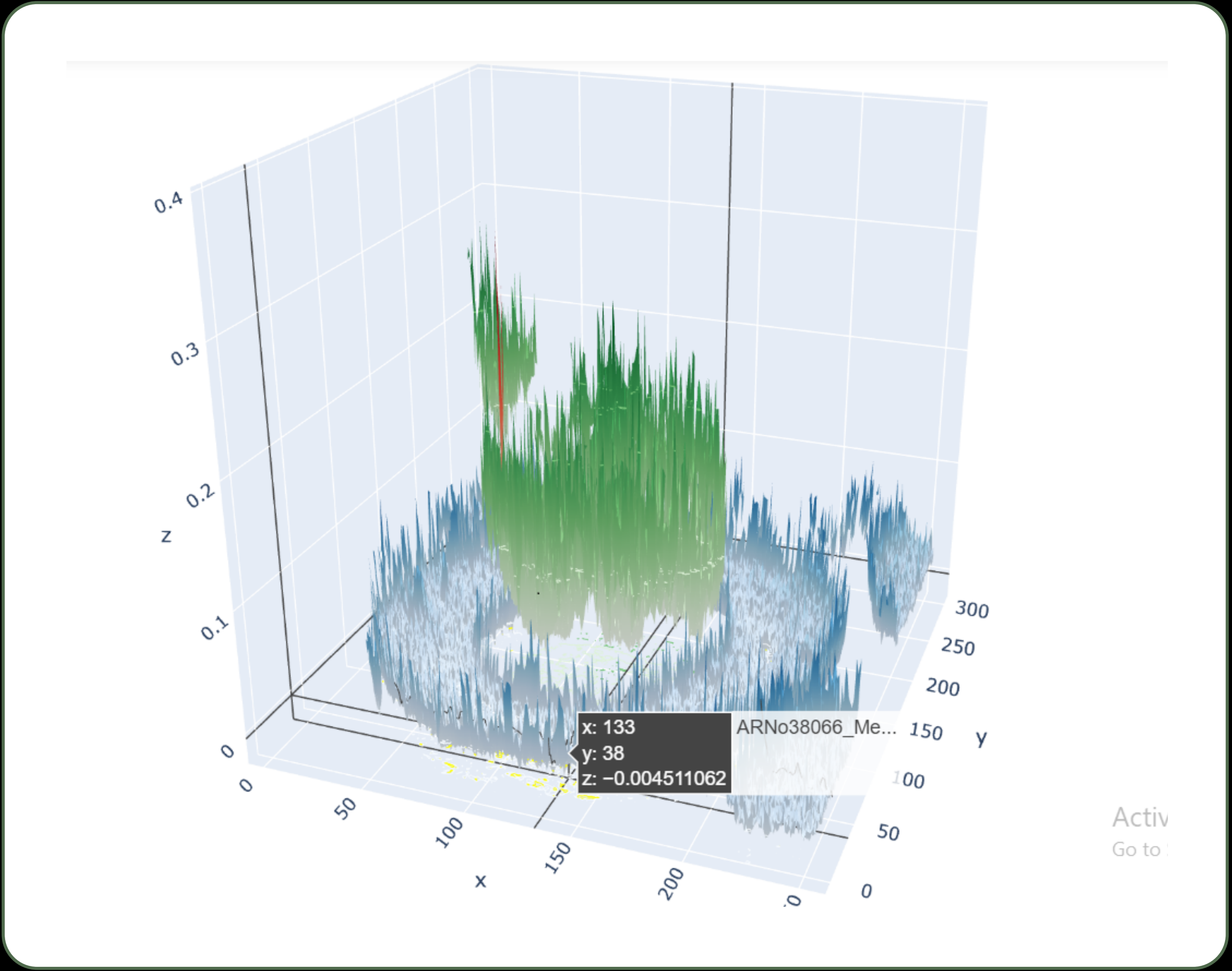


Single Component Linear Regression

- At every pixel location the algorithm performs a linear regression with each component spectral signature as regressed variable (separately).
- Following this we incorporate two filters:
- 1. F-test:
  - H0: Regression is significant
  - Use the p-value of the F-statistic based on the sum of squared errors.
- 2. R^2:
  - All the regression pixel locations having an adjusted R^2 value lesser than specified are discarded.

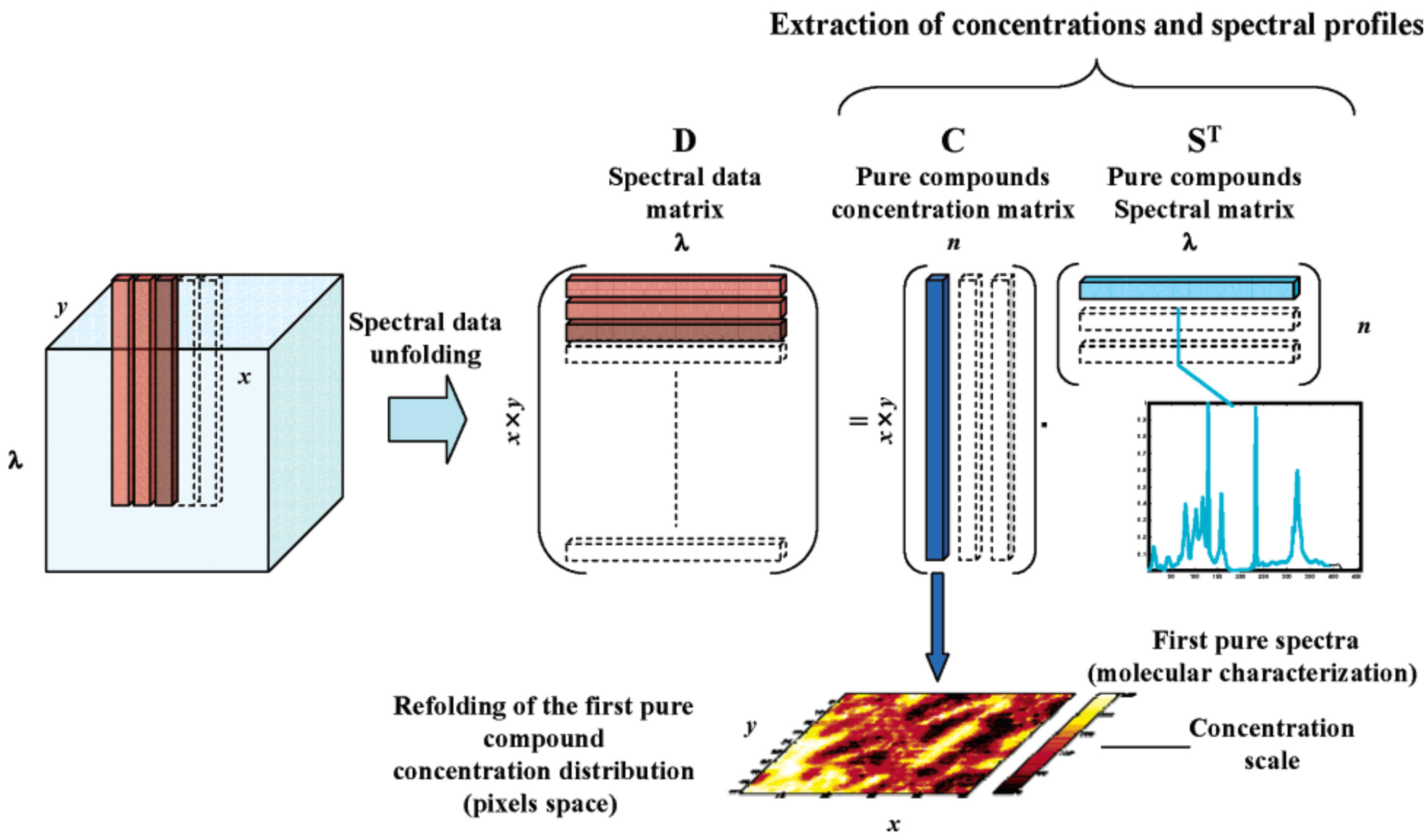
Problem: Negative Regression Coefficient

- The regression model used is of the form:
  - $y = \beta_0 + \beta_1 x + \epsilon$
- Since we are estimating the quantity of the component **x** using the regression coefficient β_1, a **negative value is of no physical significance**.
- In order to address this problem two approaches have been utilized:
  - **Savitzky-Golay** filter on the spectrum
  - Dropping the constant term and modelling as:
  - $y = \beta_1 x + \epsilon$



Multivariate Curve Resolution

Supervised Abundance Maps – Matrix Factorization



Multivariate Curve Resolution

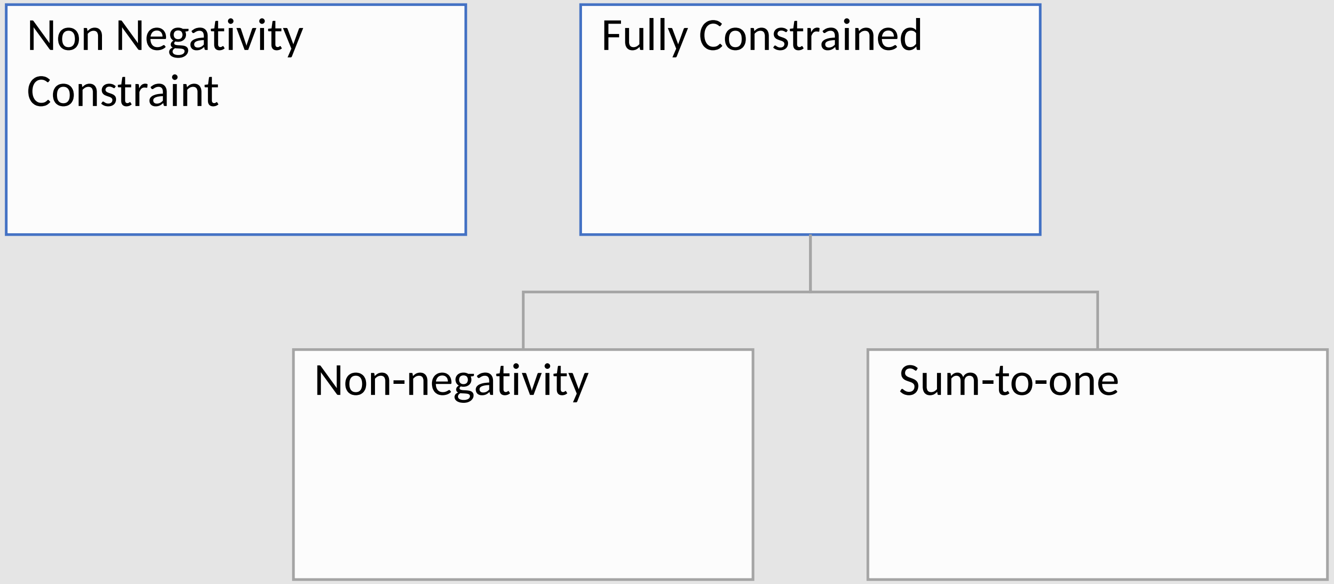
- The spectral data matrix (D) can be **factorized into two separate matrices C (concentration) and S (spectra)** with the inclusion of a new parameter n, which is the rank of factorization.
- Here n is the number of components.
- Thereby for each pixel -

$D_{ij} = u_i * p_j$, intuitively assuming each pixel is composed of all the n components.

Optimization process – Alternating Least Squares

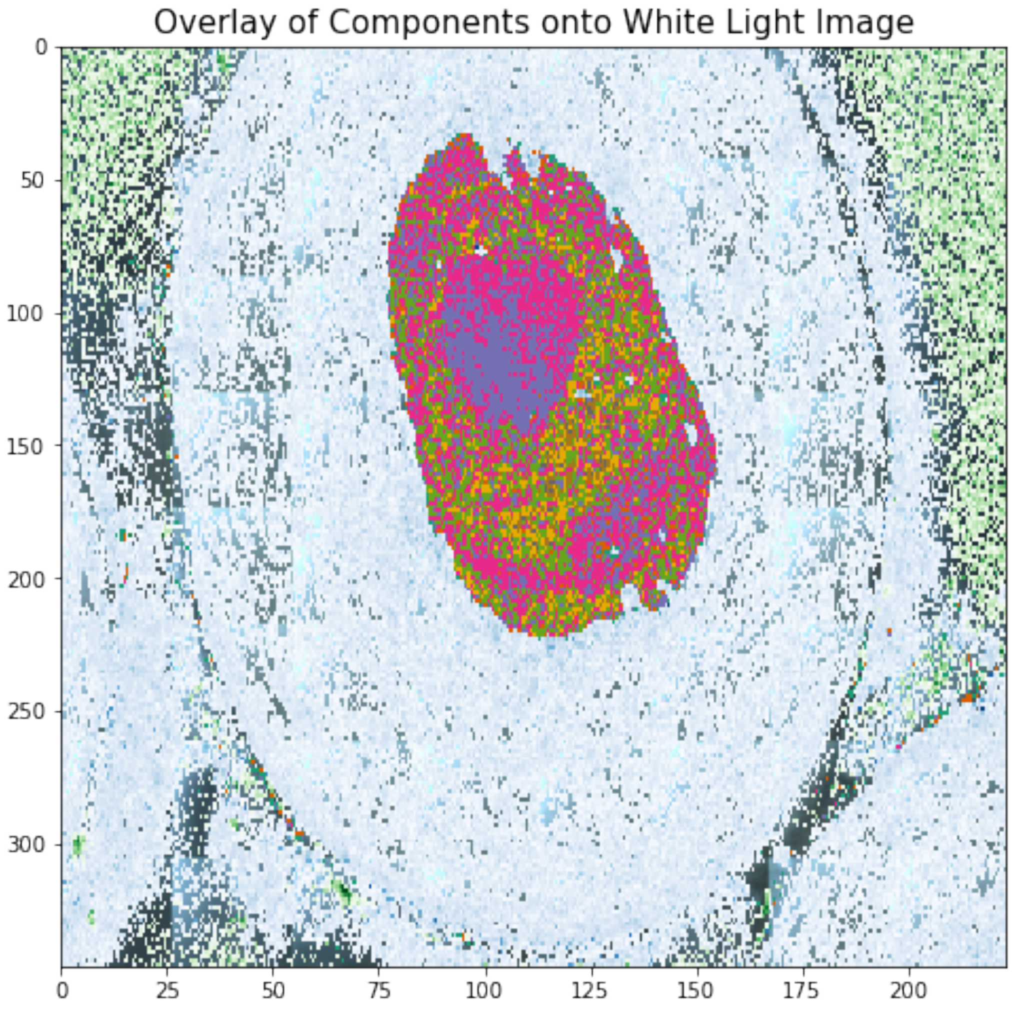
- The method aims to approximate the data matrix with the two matrices C and S by minimizing the loss function.
    - $J = \left\| D - C * S^T \right\|_2 + \lambda(\left\| C \right\|_2 + \left\| S \right\|_2)$
- The loss function appears to be learning two types of variables – C and S. Thus ignoring the regularization term at the moment it is clear that the loss –
    - $\left\| D - C * S^T \right\|_2 = \sum_{ij} \left(D_{ij} - C_i * S_j \right)$ is **not convex**.
- We fix any one of them C or S, it is a simple means squared error loss which is convex with an exact analytical solution. Moreover this guarantees a minimal MSE.
- Thus an iterative two-step process can be employed (fixing one at a time) until convergence.

Constrained Least Squares



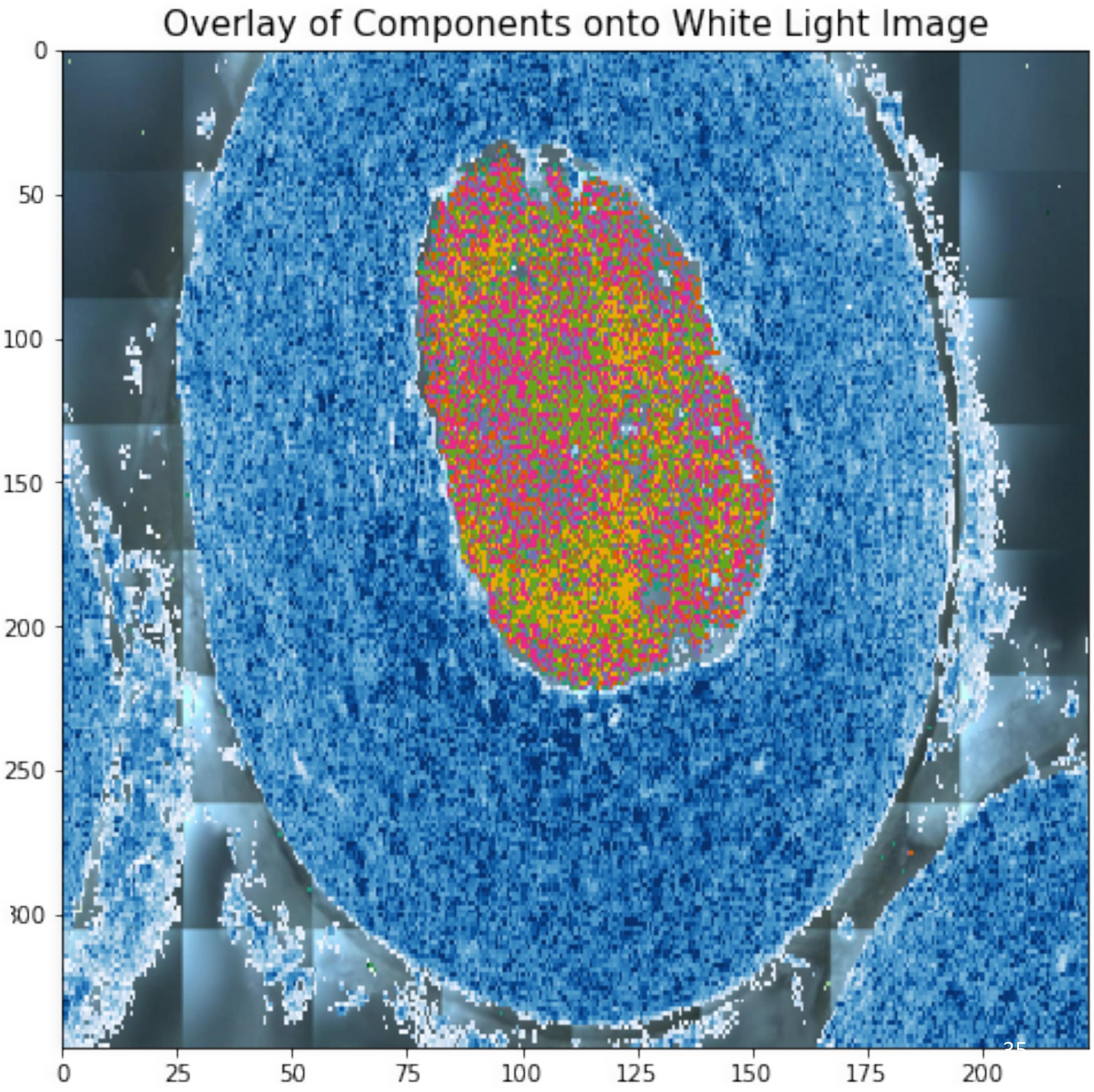
Non Negative Least Squares

- A type of constrained least squares problem where the search space of the coefficients is confined to the non-negative region.
- Given a design matrix $\boldsymbol{A}$ and a vector of response variables $\boldsymbol{y}$, the goal is to find
  - $\operatorname*{argmin}_{x} \| Ax - y \|_2 \; subject\ to\ x \geq 0$
- The above problem can be written as a quadratic programming problem:
  - $\arg\min_{\{x \geq 0\}} \frac{1}{2} x^T Q\, x + c^T x$
- where $Q = A^T A$ and $c = -A^T y$.



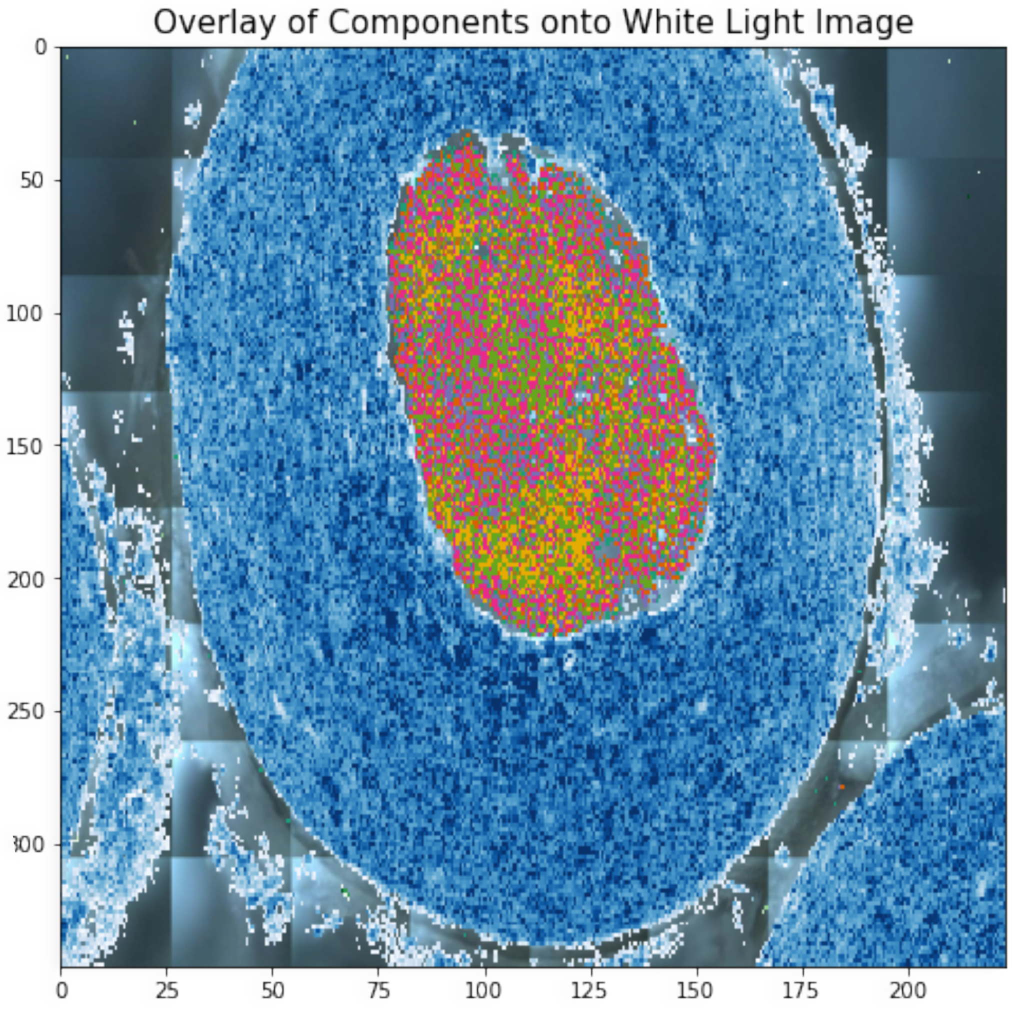
Fully Constrained Least Squares

- The search space of the regression coefficients is further constrained with:
  - $\boldsymbol{x} \geq \boldsymbol{0}$
  - $\sum \boldsymbol{x} = \boldsymbol{1}$
- Thus the problem of minimizing the least squares boils down to –
- $\min_{x} \frac{1}{2} \left\| Ax - y \right\|_2^2$
- $subject\ to\ \boldsymbol{x} \geq \boldsymbol{0}\ and\ \sum \boldsymbol{x} = 1$



Problems: Exterior of a Tablet

- A quick glance - reveals abundance map of the blue component (Mesalamine) **outside the circular boundary** of the tablet.
- In terms of abundance mapping on the white light image of the entire ROI the exterior maps are relevant and important to be taken into consideration.
- However, while quantifying the amount of Mesalamine in the tablet, this **<u>exterior maps can cause significant changes in the estimate of the amount</u>**.
- Also, an advantage of masking the exterior can be to get **rid of the background noise**.



Thank You