Honey and wine

2D correlation spectroscopy

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0 Outline

NUS National University of Singapore

1 Outline

1 Experiment

- FTIR spectrometer
- mid-IR range
- ATR accessory
- liquid samples

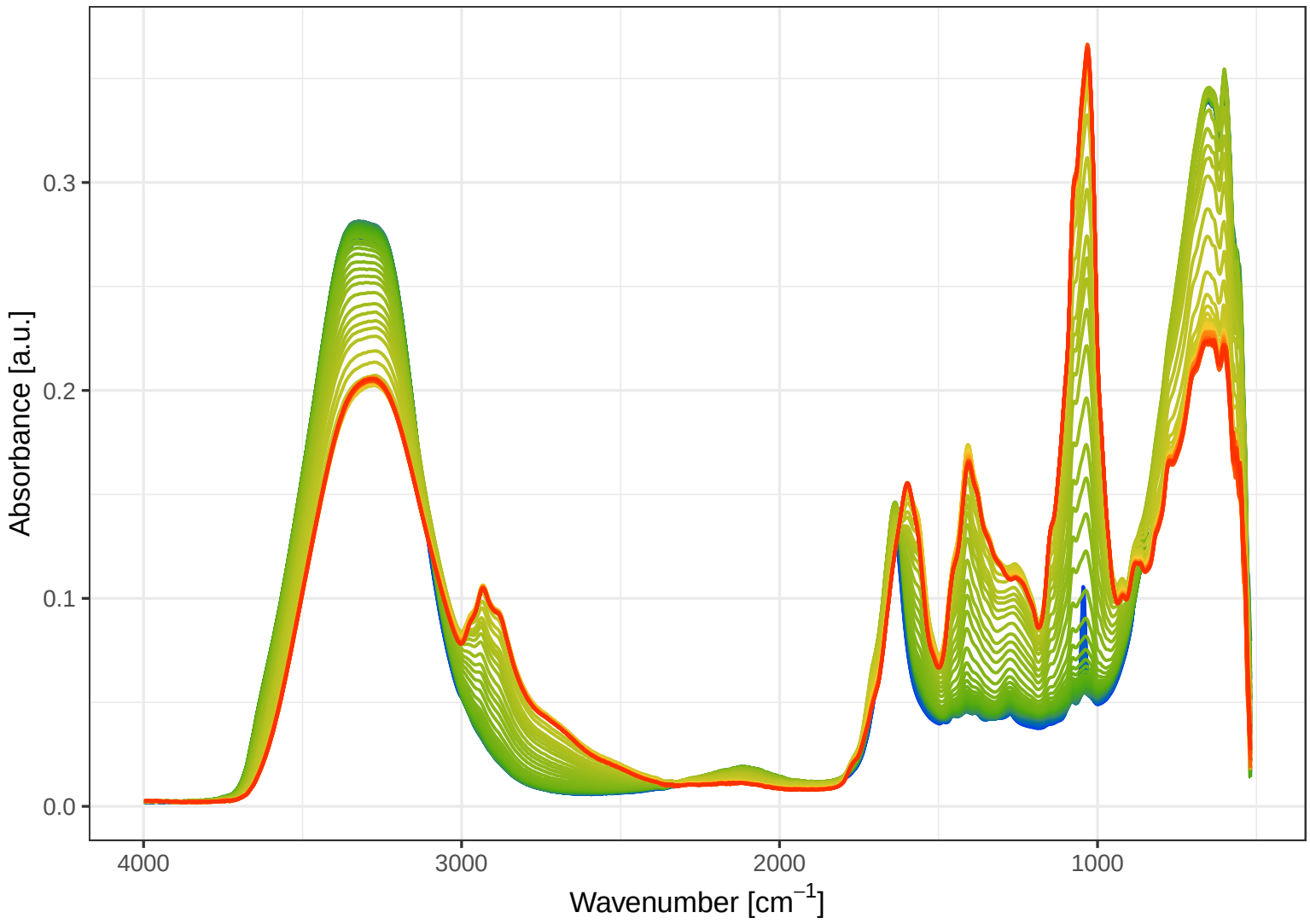
- macro-driven experiment
- sets of spectra with time-stamp in filename

1 Sample: Propolis

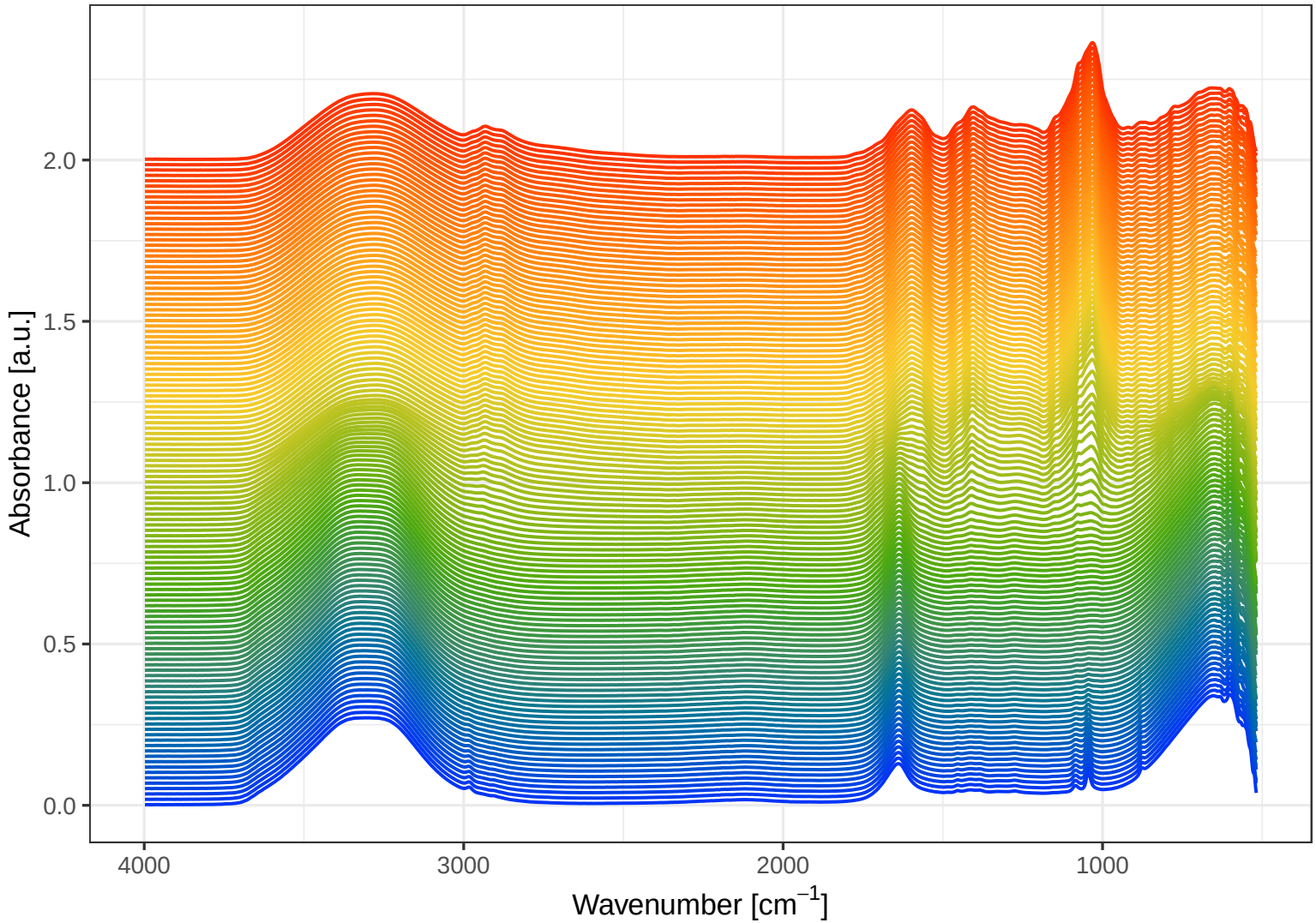
- derived from Greek to mean defense for *pro* and city or community for *polis*
- *bee glue* for sealing holes and cracks and for the reconstruction of the beehive and smoothing the inner surface of the beehive,preventing invasion by predators
- composed mainly of resin (50%), wax (30%), essential oils (10%), pollen (5%), and other organic compounds (5%) (phenolic compounds, esters, flavonoids, terpenes, beta-steroids, aromatic aldehydes, and alcohols)
- contains also important vitamins (B1, B2, B6, C and E) and useful minerals (Mg, Ca, K, Na, Cu, Zn, Mn and Fe)
- numerous applications in treating various diseases due to its antiseptic, anti-inflammatory, antioxidant, antibacterial, antimycotic, antifungal, antiulcer and anticancer

2 Outline

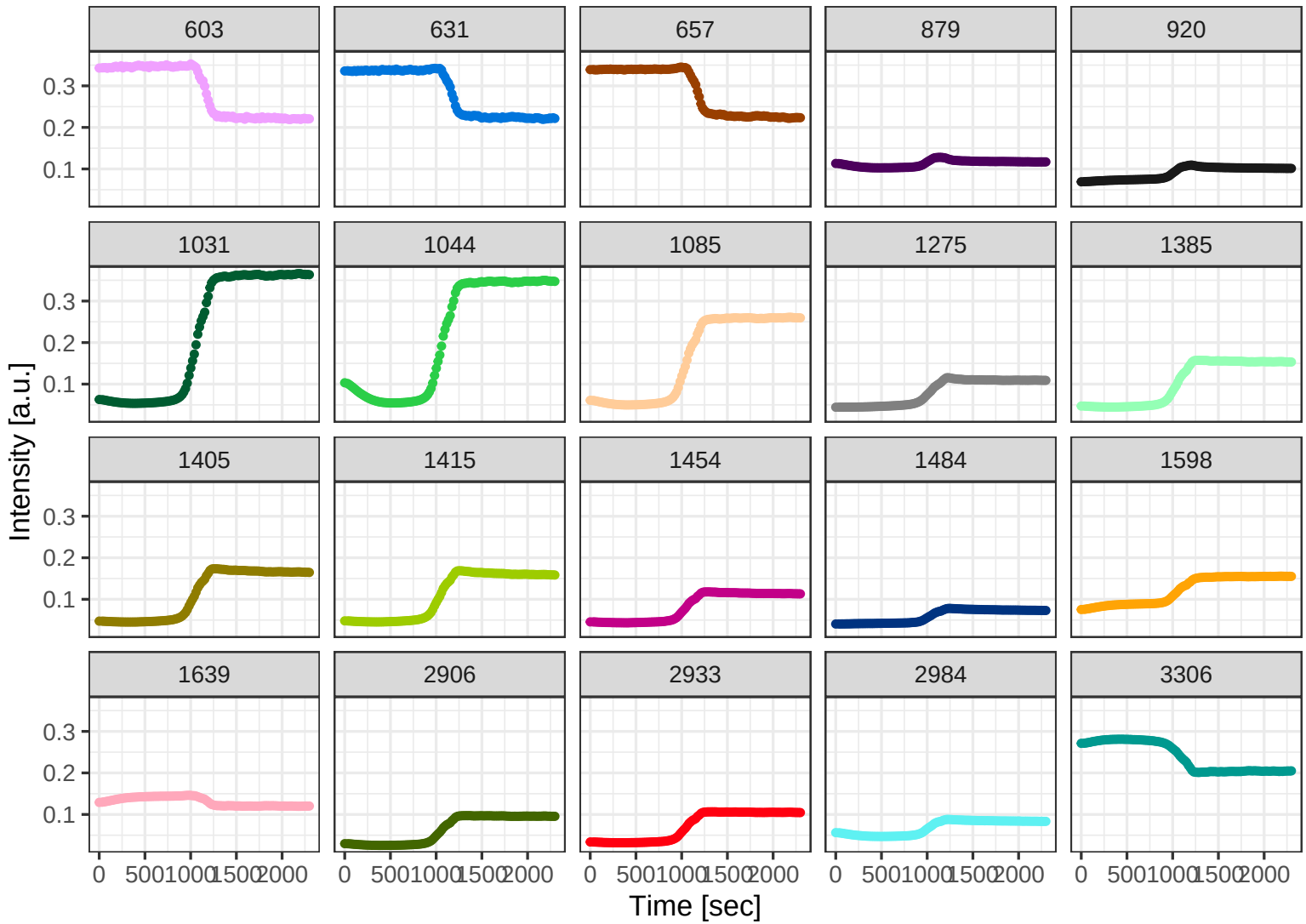
2 All spectra: from blue to red



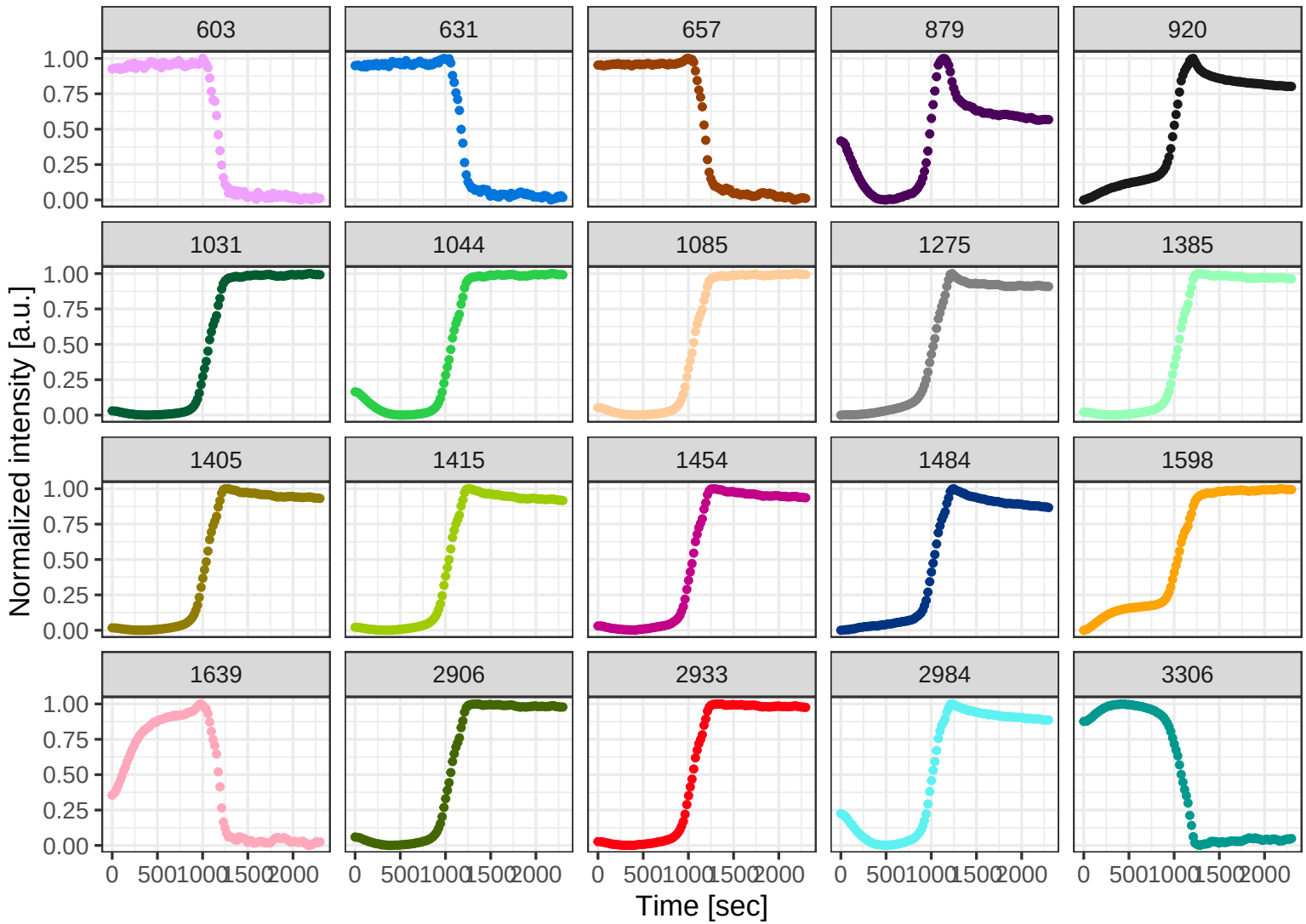
2 All spectra with shift



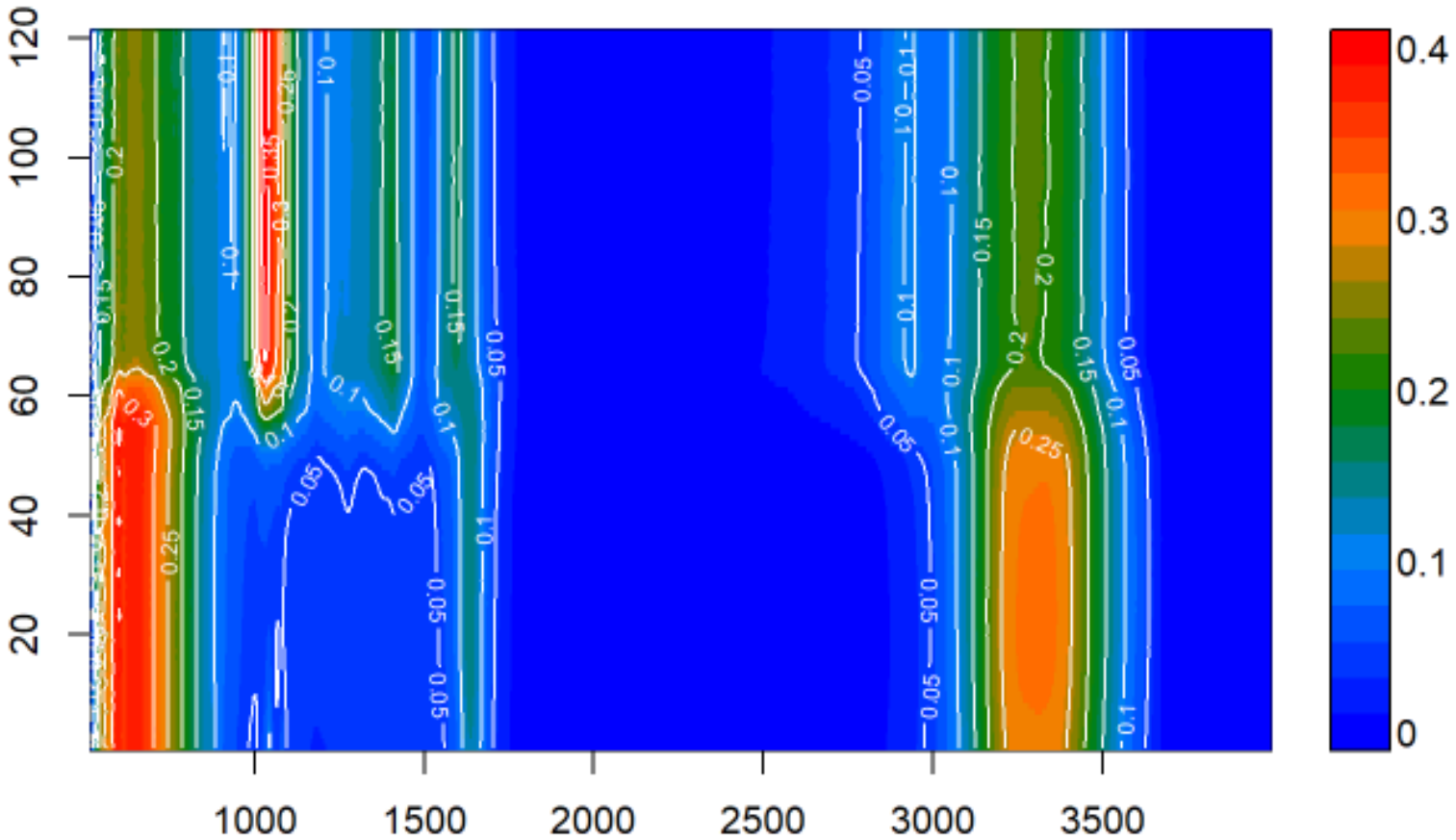
2 Raw Spectra



2 Normalized Spectra

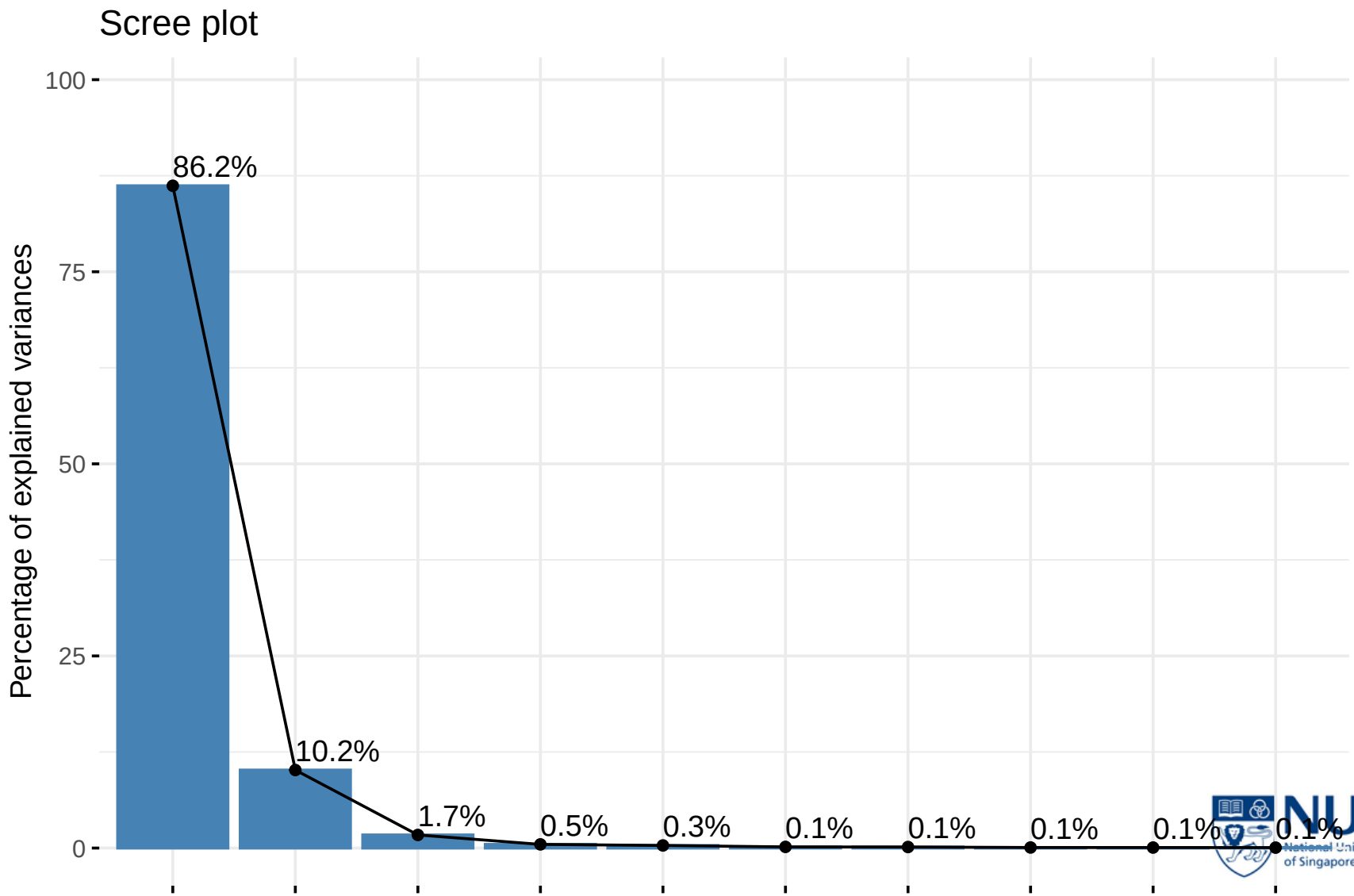


2 Level plot

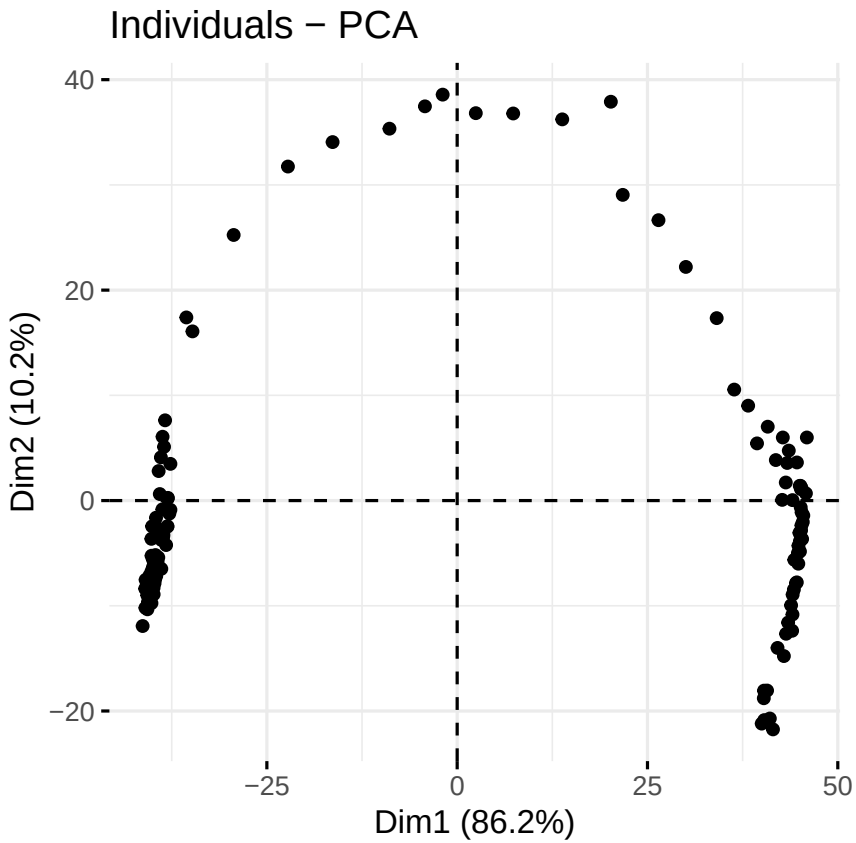


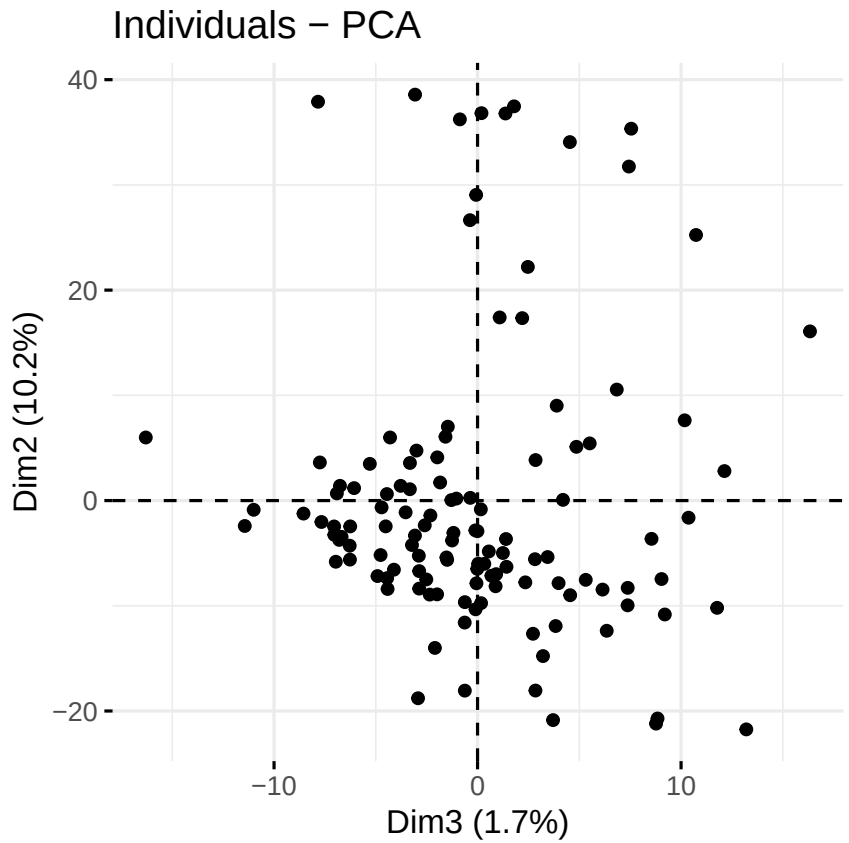
3 Outline

3 PCA Screeplot

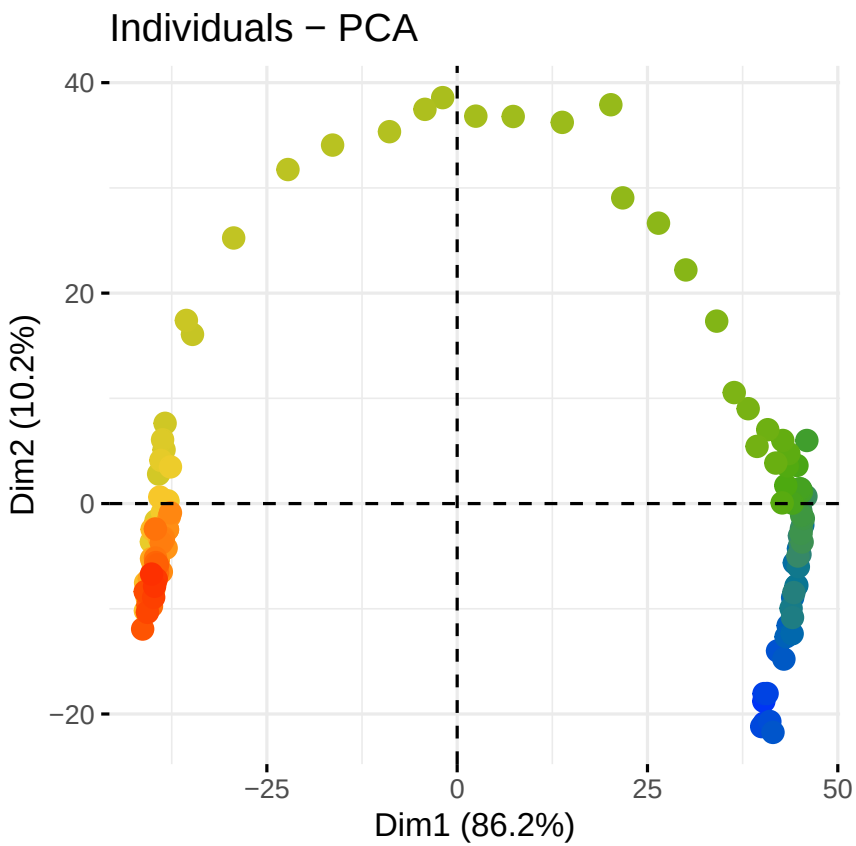


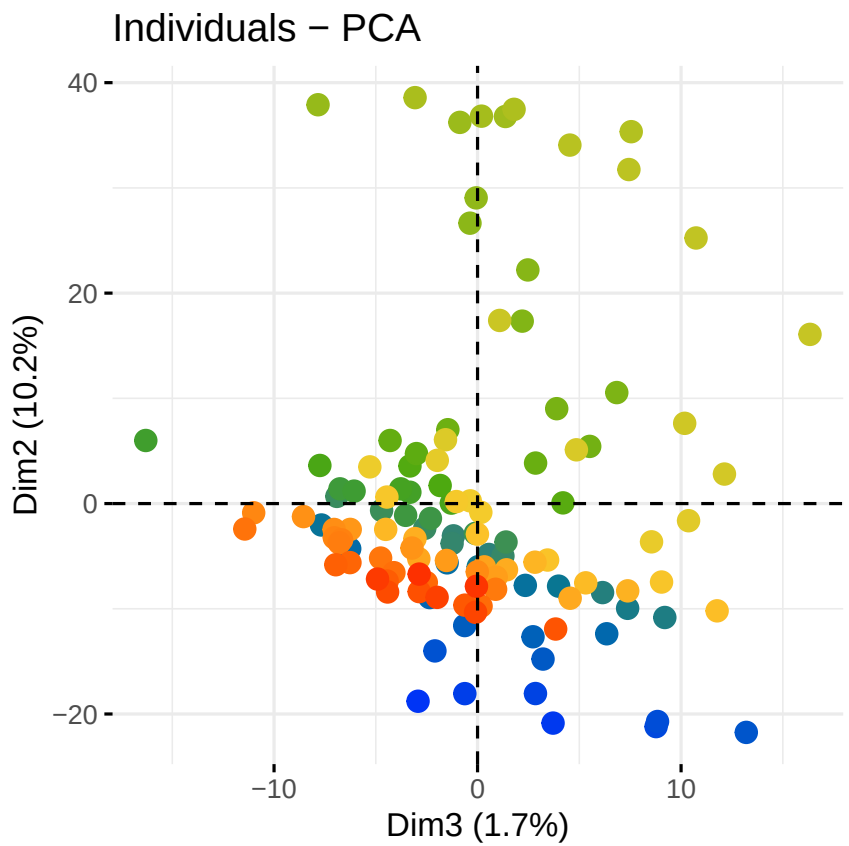
3 PCA Scoreplots



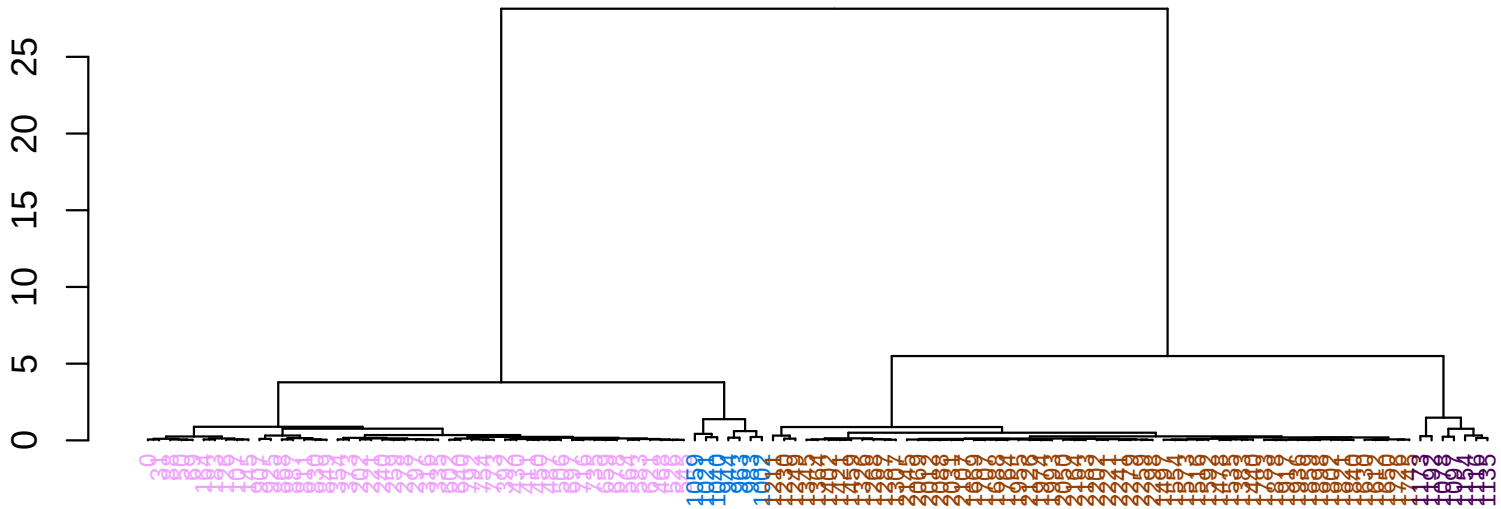


3 PCA Scoreplots with Colours

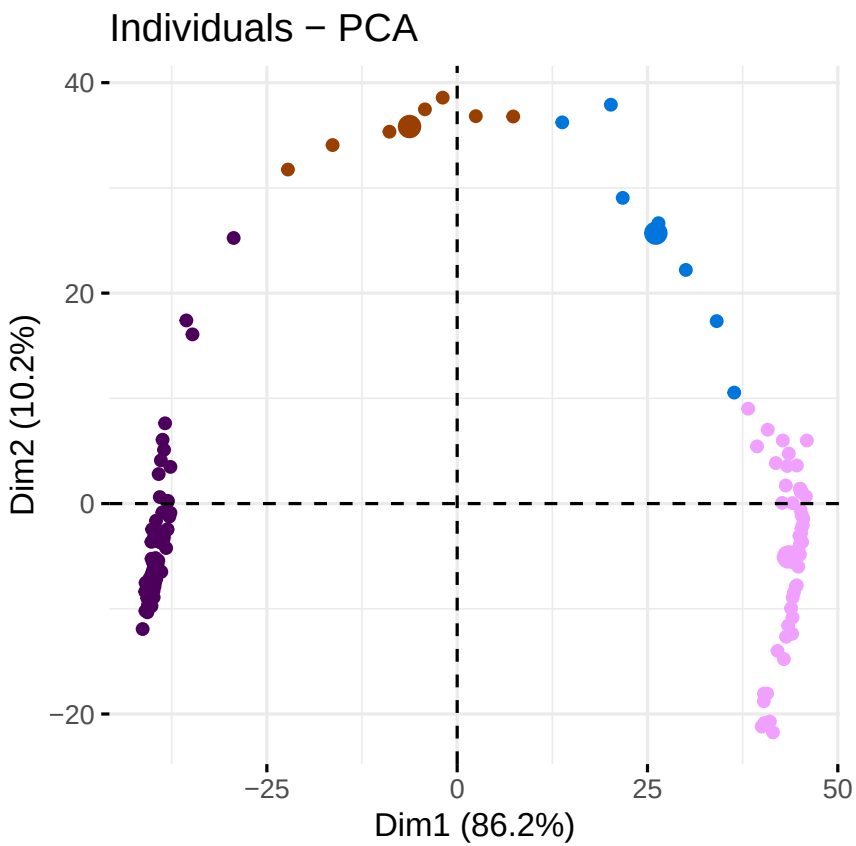


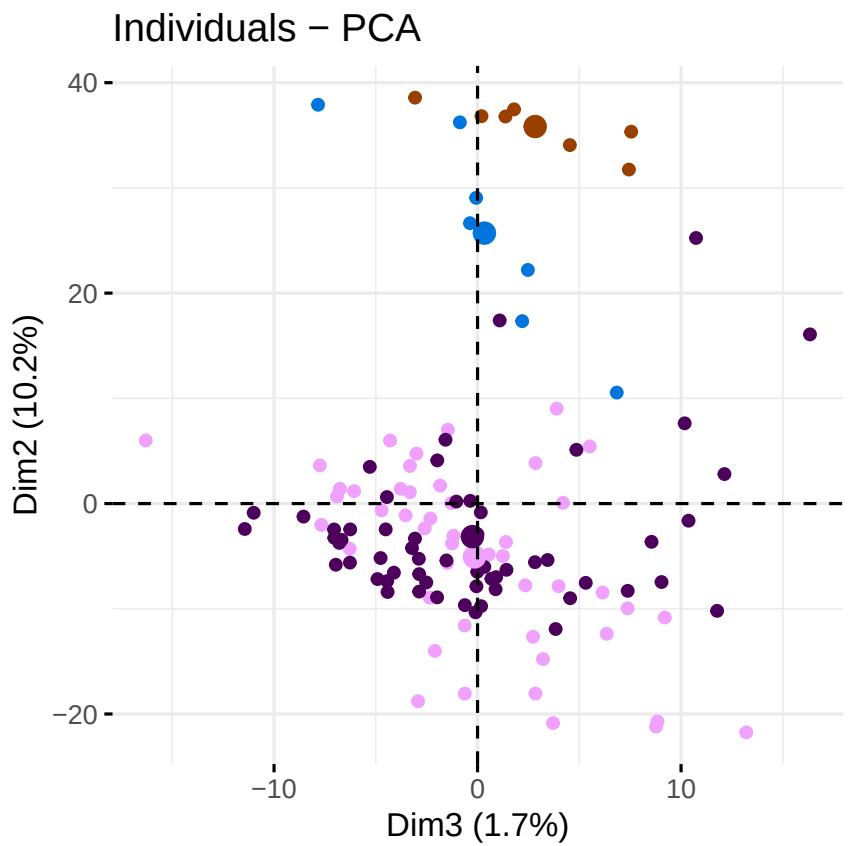


3 HCA Dendrogram 4 Clusters

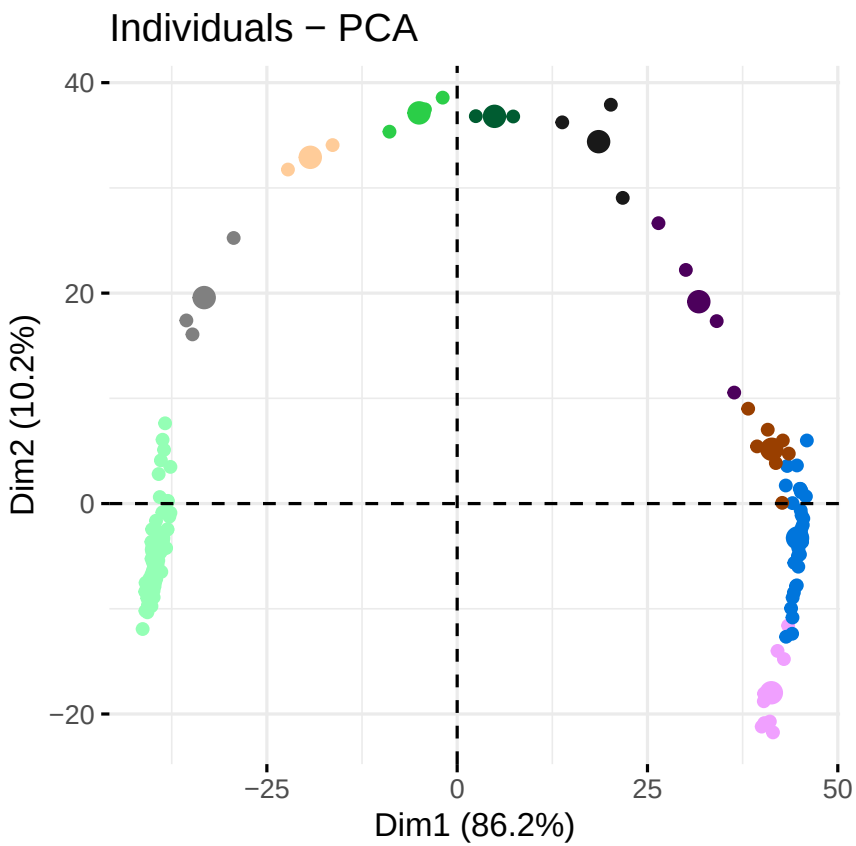


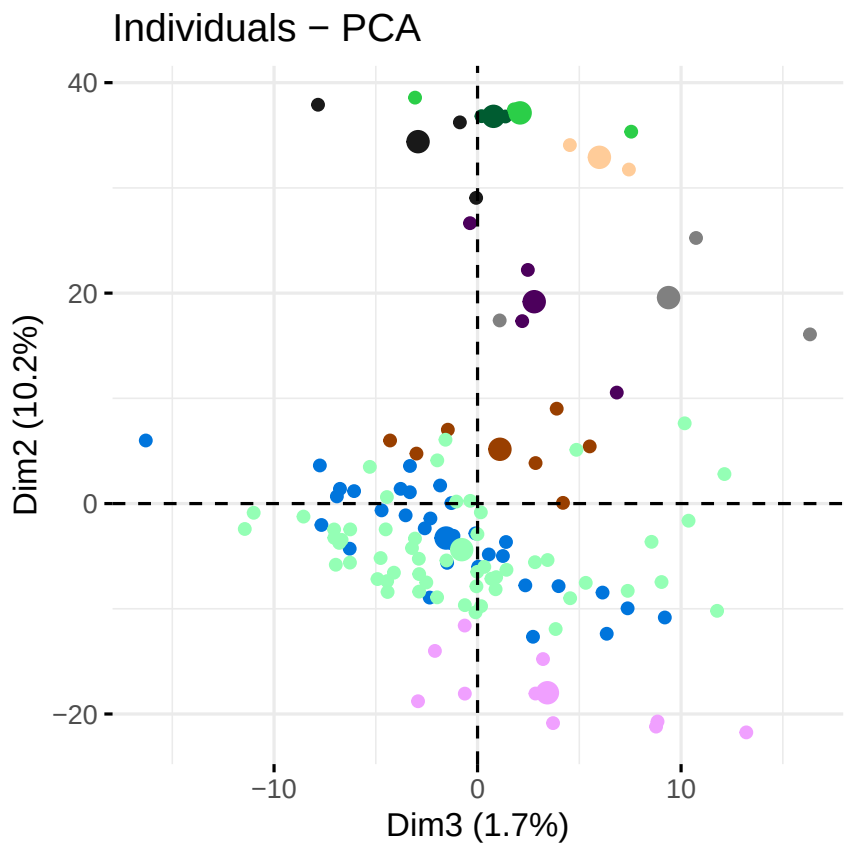
3 PCA Scoreplots with 4 Clusters Membership





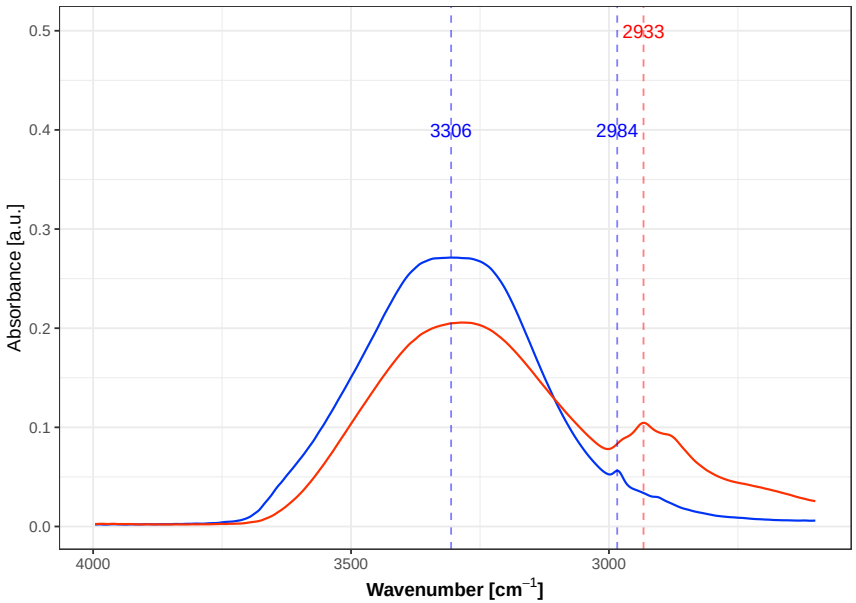
3 PCA Scoreplots with 10 Clusters Membership

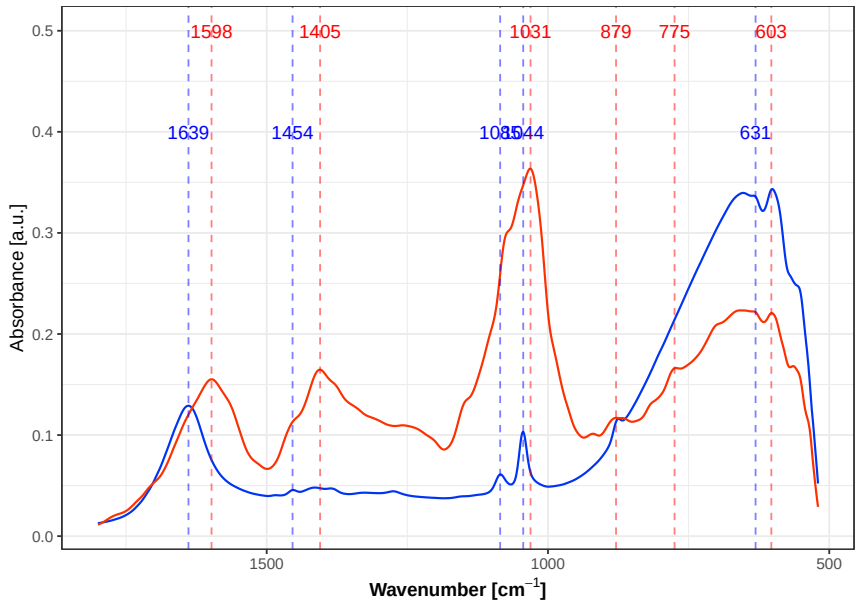




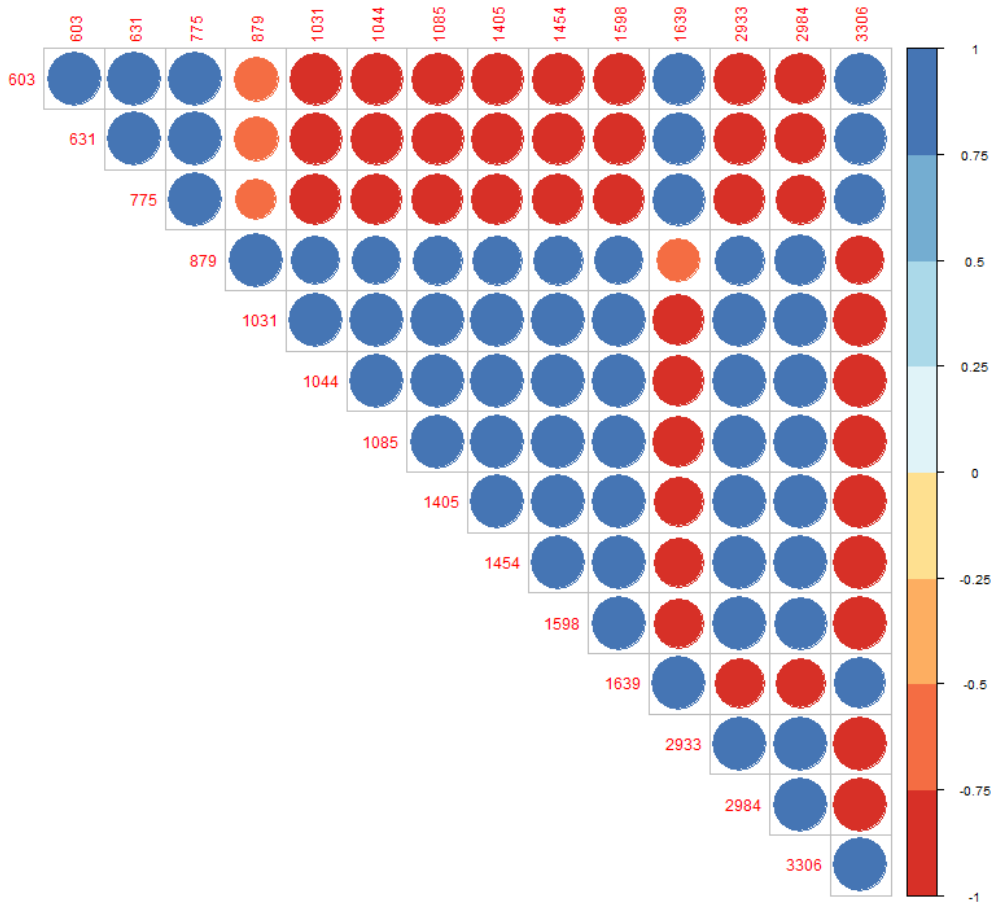
4 Outline

4 Spectra Inspection

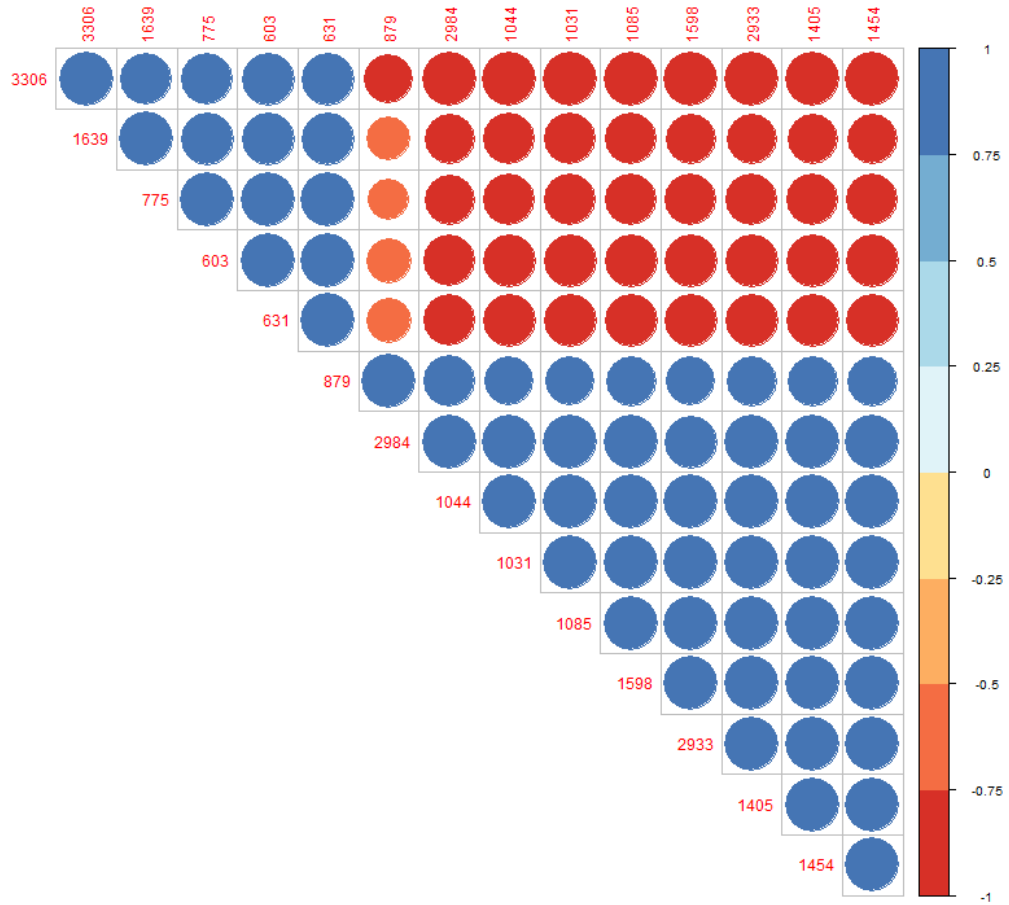




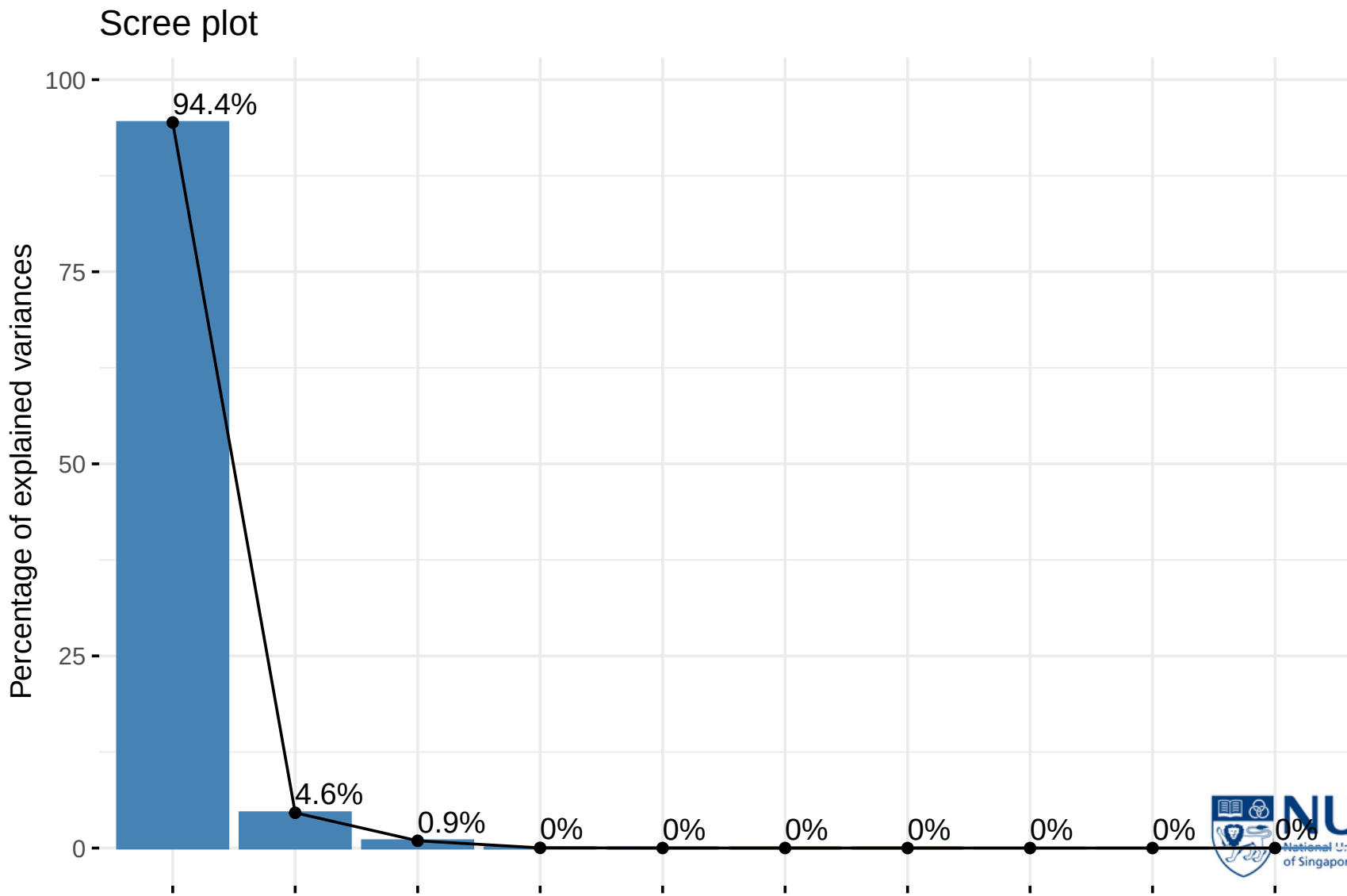
4 Correlation Matrix - Original Order



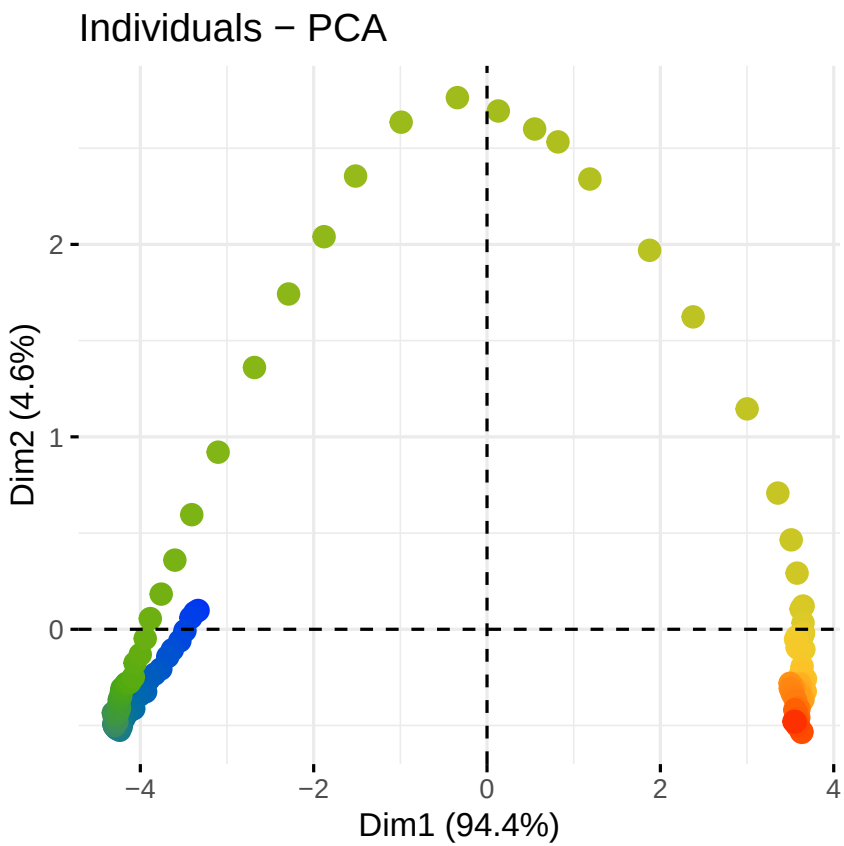
4 Correlation Matrix - Clustering Order

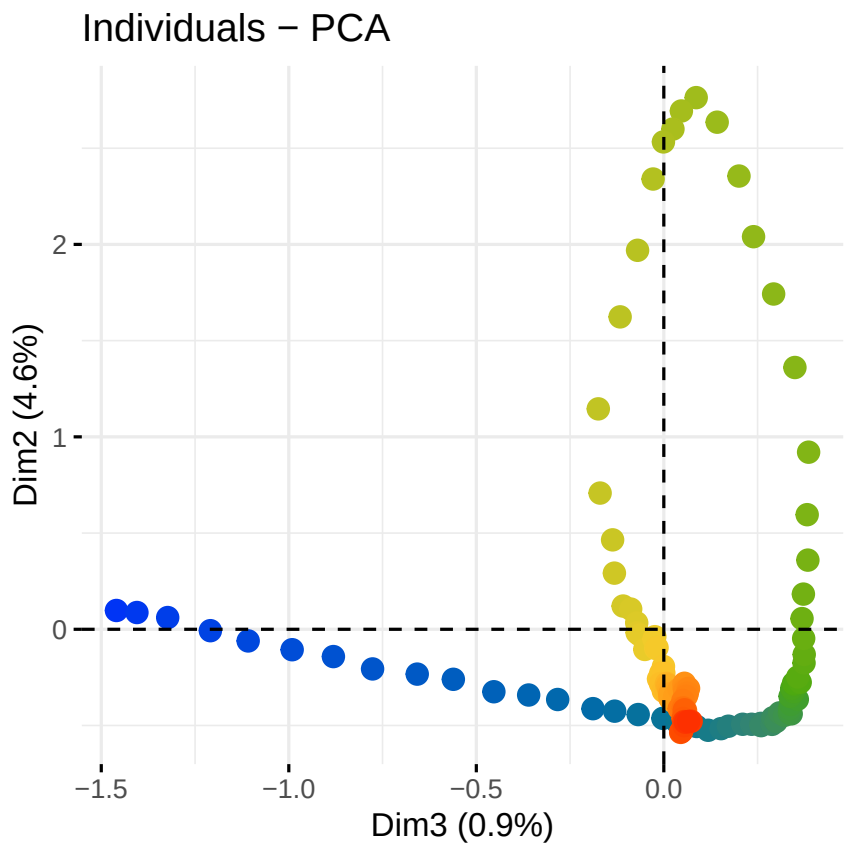


4 PCA on selected wavenumbers

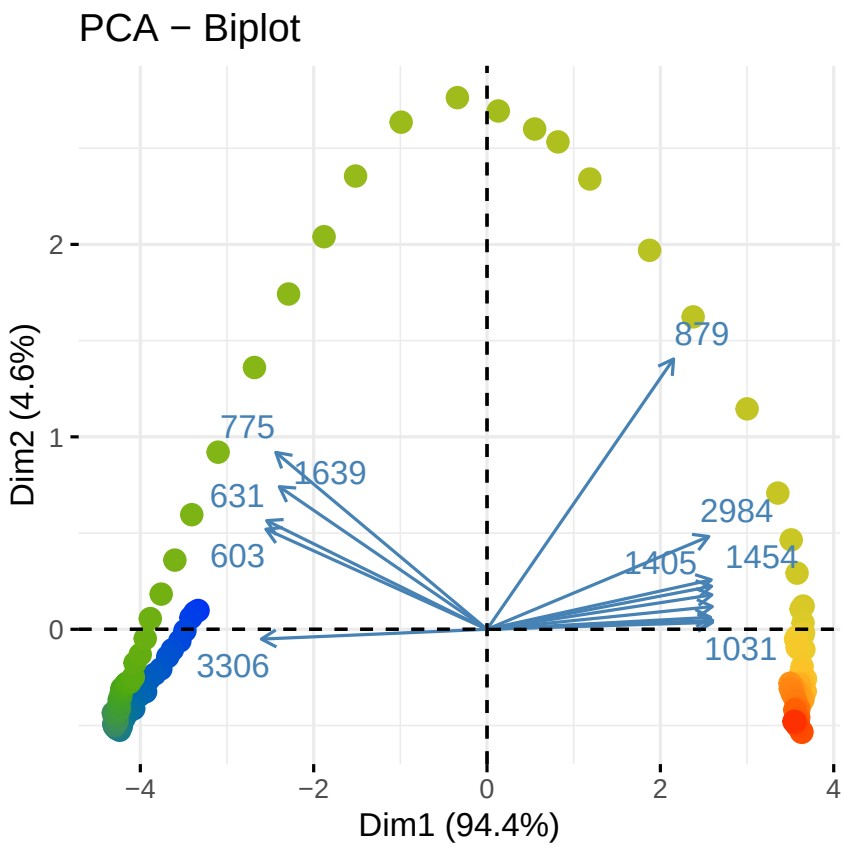


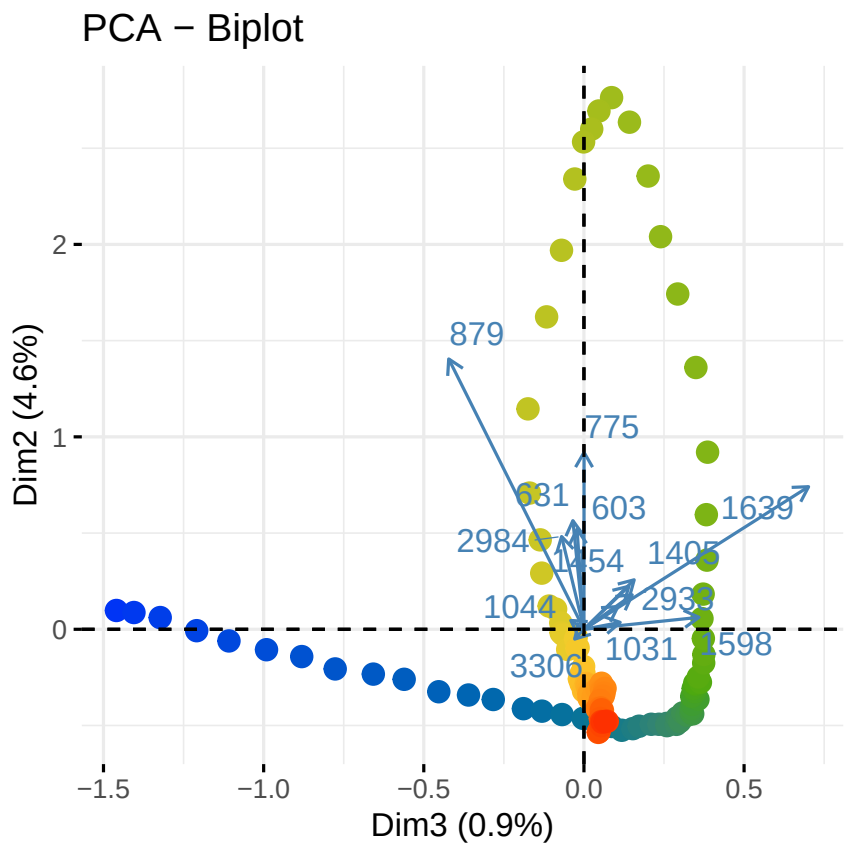
4 PCA Scoreplots with Colours





4 PCA Biplots

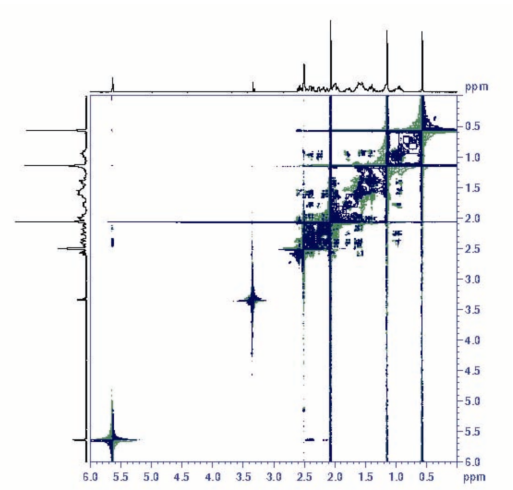




5 Outline

5 Some history

- 1971 Jean Jeener AMPERE Summer School Basko Polje (Yugoslavia)
- 1976 Richard Ernst first experimental demonstration of the technique
- 1980 Isao Noda sinusoidal perturbations based 2D spectroscopy
- 1993 generalized 2D correlation analysis based on Fourier transformation
- 2D correlation analysis is used for the interpretation of many types of spectroscopic data (including XRF, UV/VIS spectroscopy, fluorescence, infrared, and Raman spectra)



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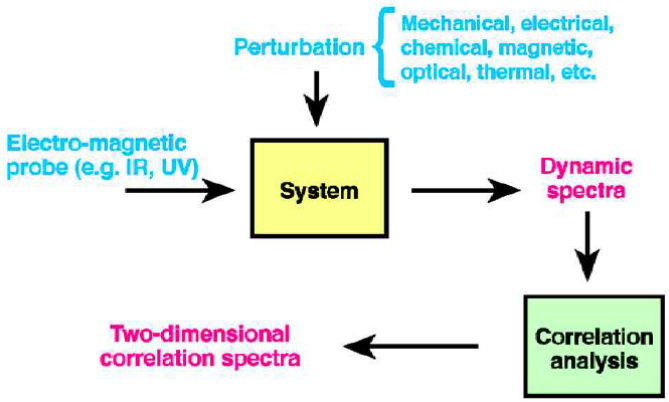
5 Idea

- mathematical technique used to study changes in measured signals
- Spectra measured with perturbation
  - time
  - temperature
  - pressure
  - incidence angle
  - magnetic field
  - position in space
- measured spectra show systematic variations that are processed with 2D correlation analysis for interpretation
- synchronous and asynchronous matrix



5 Applications

- determine the events that are occurring at the same time (in phase) and those events that are occurring at different times (out of phase)
- determine the sequence of spectral changes
- identify various inter- and intra-molecular interactions
- band assignments of reacting groups
- to detect correlations between spectra of different techniques, for example infrared spectroscopy and Raman spectroscopy

$$C_{\text{auto}}(\tau) = \int_{-\infty}^{\infty} f^*(u) \cdot f(u+\tau) du \tag{1}$$

$$C_{\text{cross}}(\tau) = \int_{-\infty}^{\infty} f^*(u) \cdot g(u+\tau) du \tag{2}$$

$$\widetilde{y}(\nu,t) = \begin{cases} y(\nu,t) - \overline{y}(\nu) & \text{for } T_{\min} \leq t \leq T_{\max} \\ 0 & \text{otherwise} \end{cases} \tag{3}$$

$$\overline{y}(\nu) = \frac{1}{T_{\max} - T_{\min}} \int_{T_{\min}}^{T_{\max}} y(\nu,t)dt \tag{4}$$

$$\widetilde{Y}(\nu,\omega) = \mathcal{F}(\widetilde{y}(\nu,t)) = \int_{-\infty}^{\infty} \widetilde{y}(\nu,t)\cdot e^{-i\omega t}dt \tag{5}$$

$$\Phi(\nu_1,\nu_2) + i\Psi(\nu_1,\nu_2) = \frac{1}{2\pi(T_{\max}-T_{\min})}\int_{-\infty}^{\infty} \widetilde{Y}(\nu_1,\omega)\cdot\widetilde{Y}^{*}(\nu_2,\omega)d\omega \tag{6}$$

$$\Phi(\nu_1, \nu_2) = \frac{1}{m-1} \sum_{j=1}^{m} \widetilde{y}(\nu_1, t_j) \cdot \widetilde{y}(\nu_2, t_j) \tag{7}$$

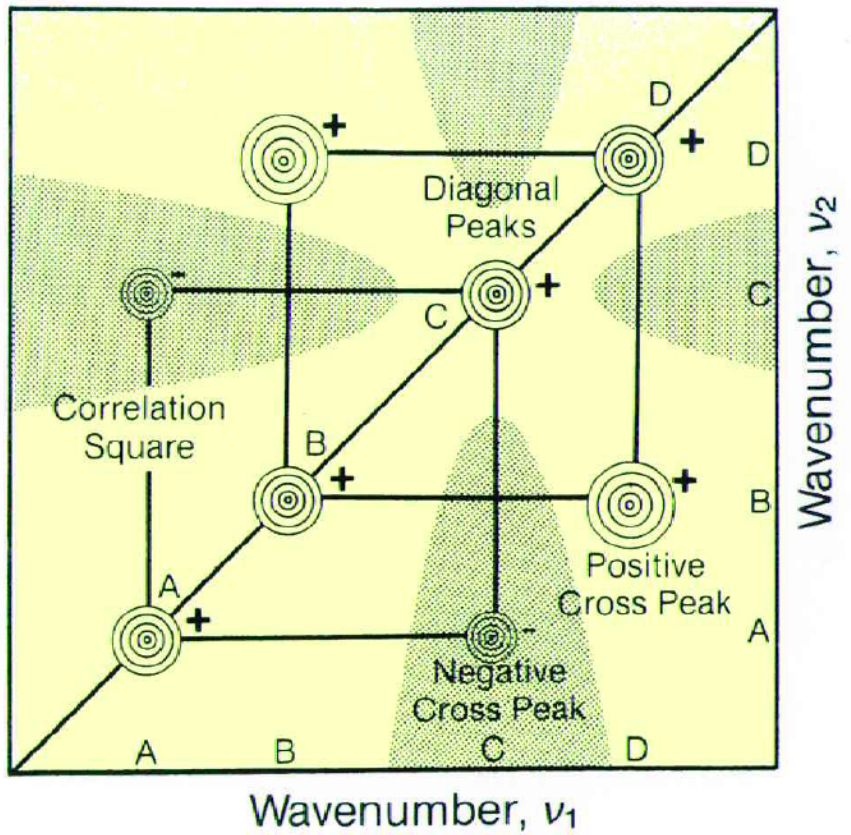
$$\widetilde{z}(\nu_2, t_j) = \sum_{k=1}^{m} N_{jk} \cdot \widetilde{y}(\nu_2, t_k) \tag{8}$$

$$N_{jk} = \begin{cases} 0 & \text{if } j = k \\ \frac{1}{\pi(k-j)} & \text{otherwise} \end{cases} \tag{9}$$

$$\Psi(\nu_1, \nu_2) = \frac{1}{m-1} \sum_{j=1}^{m} \widetilde{y}(\nu_1, t_j) \cdot \widetilde{z}(\nu_2, t_j) \tag{10}$$

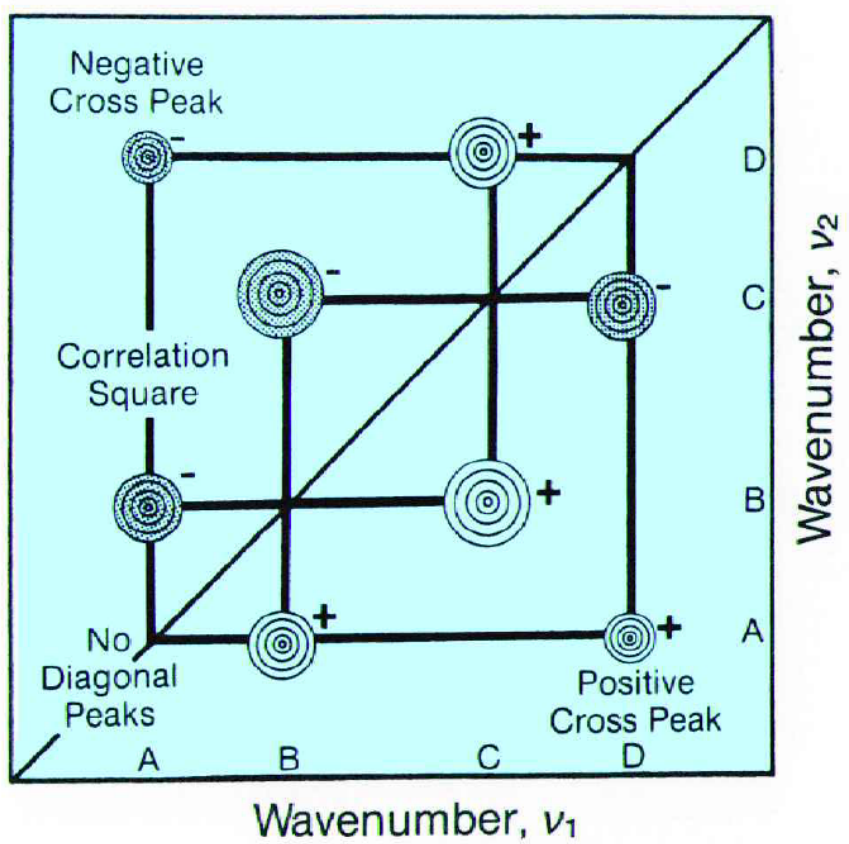
5 Synchronous Spectrum

- peaks at diagonal - auto-correlation function
- magnitude of autopeaks (>0) - the overall intensity variation
- off-diagonal cross peaks - simultaneous changes of spectral intensities for two spectral variables
- the sign of synchronous cross peaks is positive if the intensities at two different spectral variables are either increasing or decreasing together



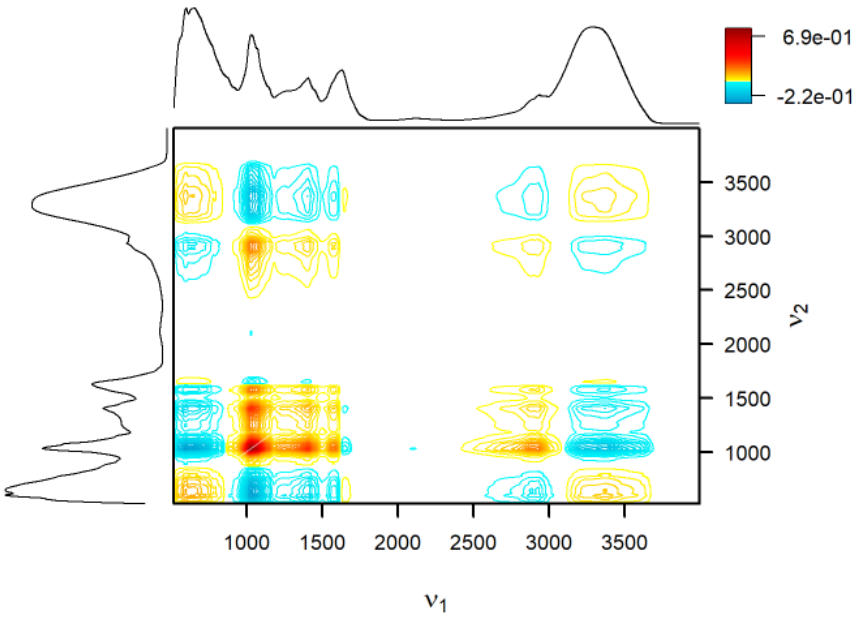
5 Asynchronous Spectrum

- antisymmetric with respect to the diagonal line
- asynchronous peak develops only if the intensities of two spectral features change out of phase (delayed or accelerated) with each other
- the sign of an asynchronous peak becomes positive if the intensity change at ν_1 occurs predominately before ν_2 in the sequential order of t

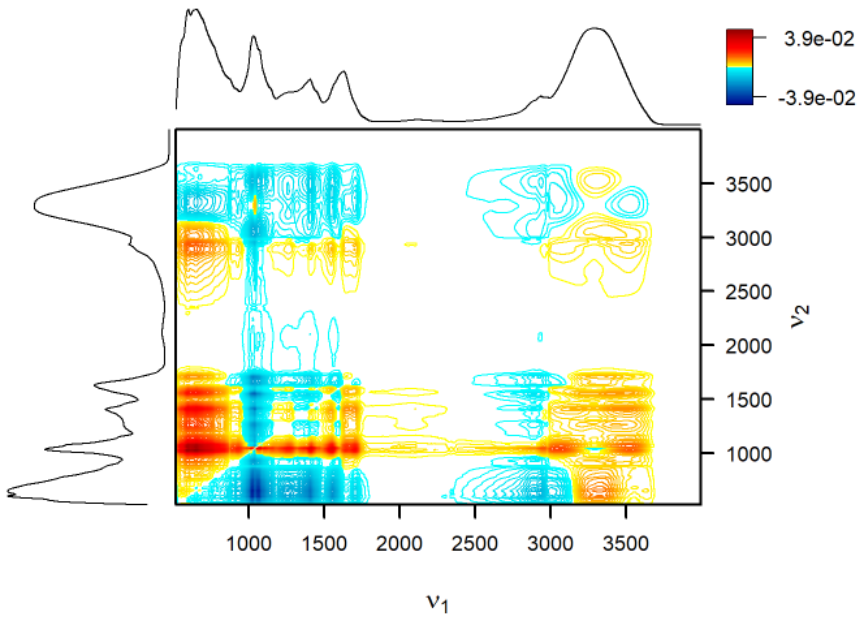


5 Synchronous and asynchronous matrix for propolis

Synchronous

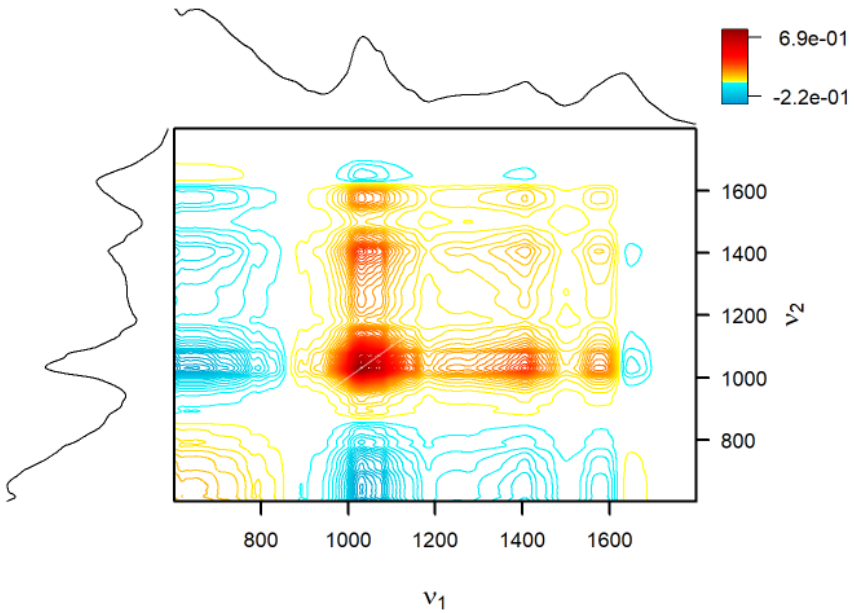


Asynchronous

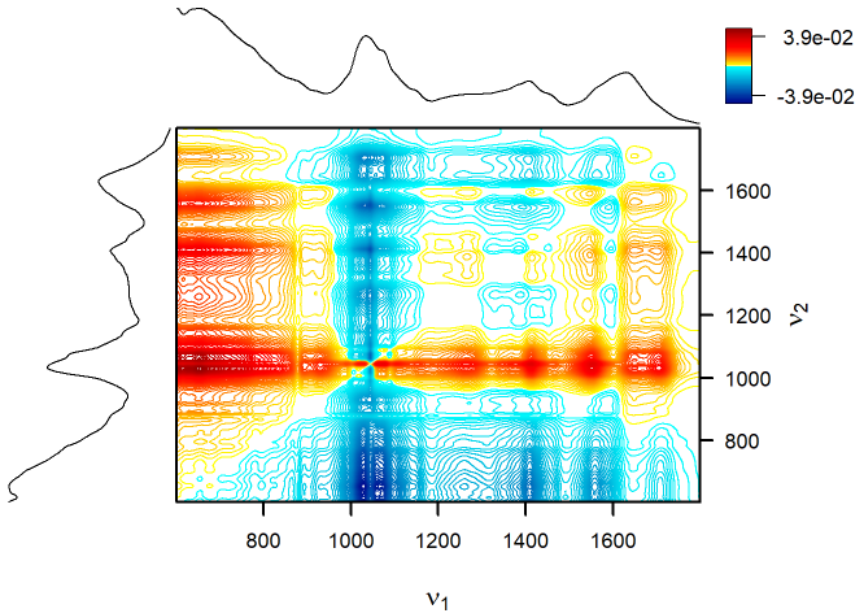


5 Close-up

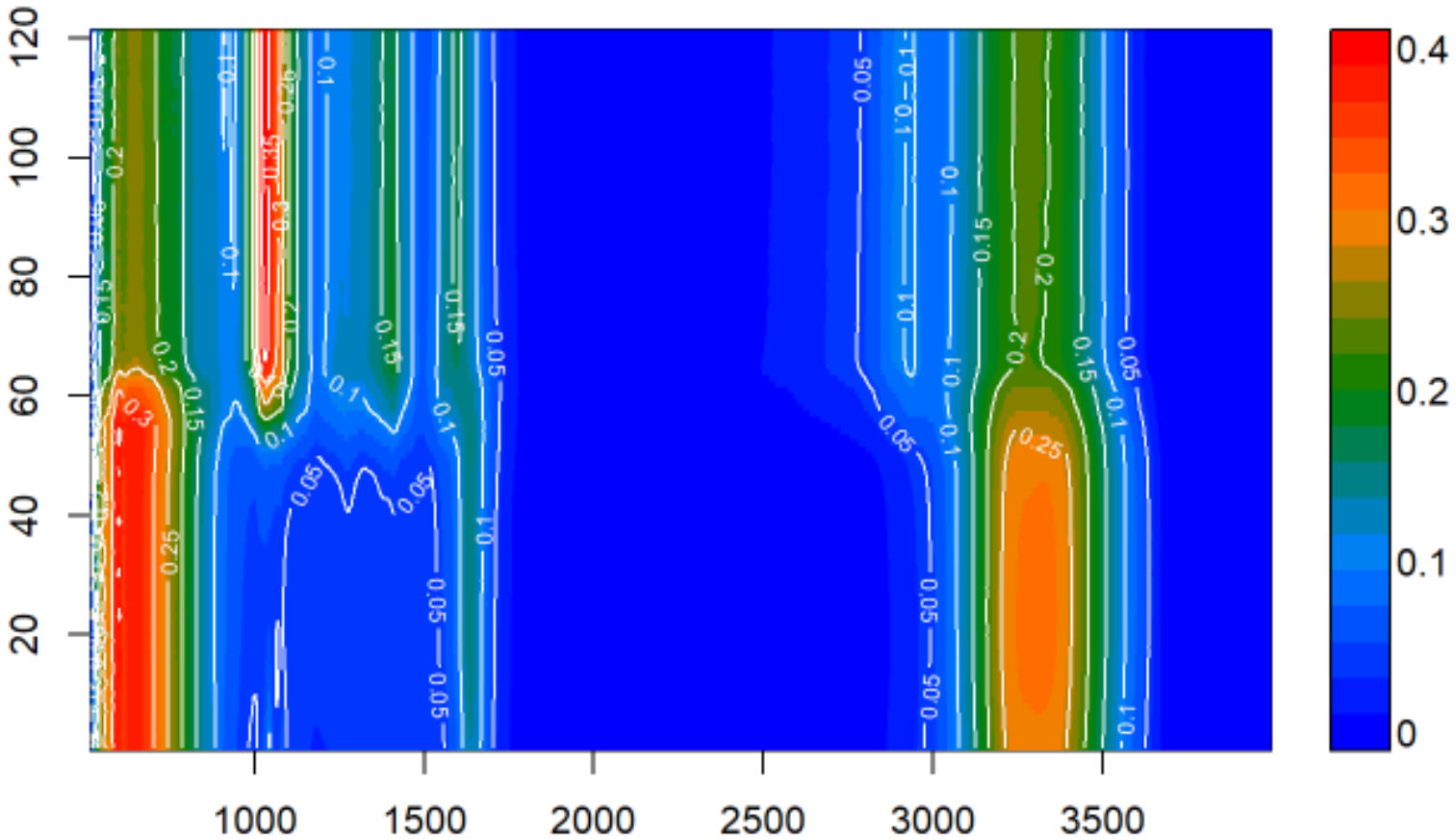
Synchronous



Asynchronous



5 Level plot



6 Outline

6 Summary

- for the spectral datasets measured with external perturbation (time, temperature, pressure, pH, concentration, position in space) we can look for correlations
- dimension reduction is helpful in finding the important wavenumbers (energies, frequencies)
- 2D correlation analysis and investigation of synchronous and asynchronous matrices may help in finding relationships between spectral bands and clearly visualise the changes under the perturbation


Thank you!

$$\Phi(\nu_1,\nu_2) = \frac{1}{T_{\max} - T_{\min}} \int_{T_{\min}}^{T_{\max}} \widetilde{y}(\nu_1,t) \cdot \widetilde{y}(\nu_2,t) dt \tag{11}$$

$$\widetilde{z}(\nu_2,t) = \mathcal{H}(\widetilde{y}(\nu_2,t)) = \frac{1}{\pi} \quad PV \int_{-\infty}^{\infty} \frac{\widetilde{y}(\nu_2,t')}{t'-t} dt \tag{12}$$

$$\Psi(\nu_1,\nu_2) = \frac{1}{T_{\max} - T_{\min}} \int_{T_{\min}}^{T_{\max}} \widetilde{y}(\nu_1,t) \cdot \widetilde{z}(\nu_2,t) dt \tag{13}$$