

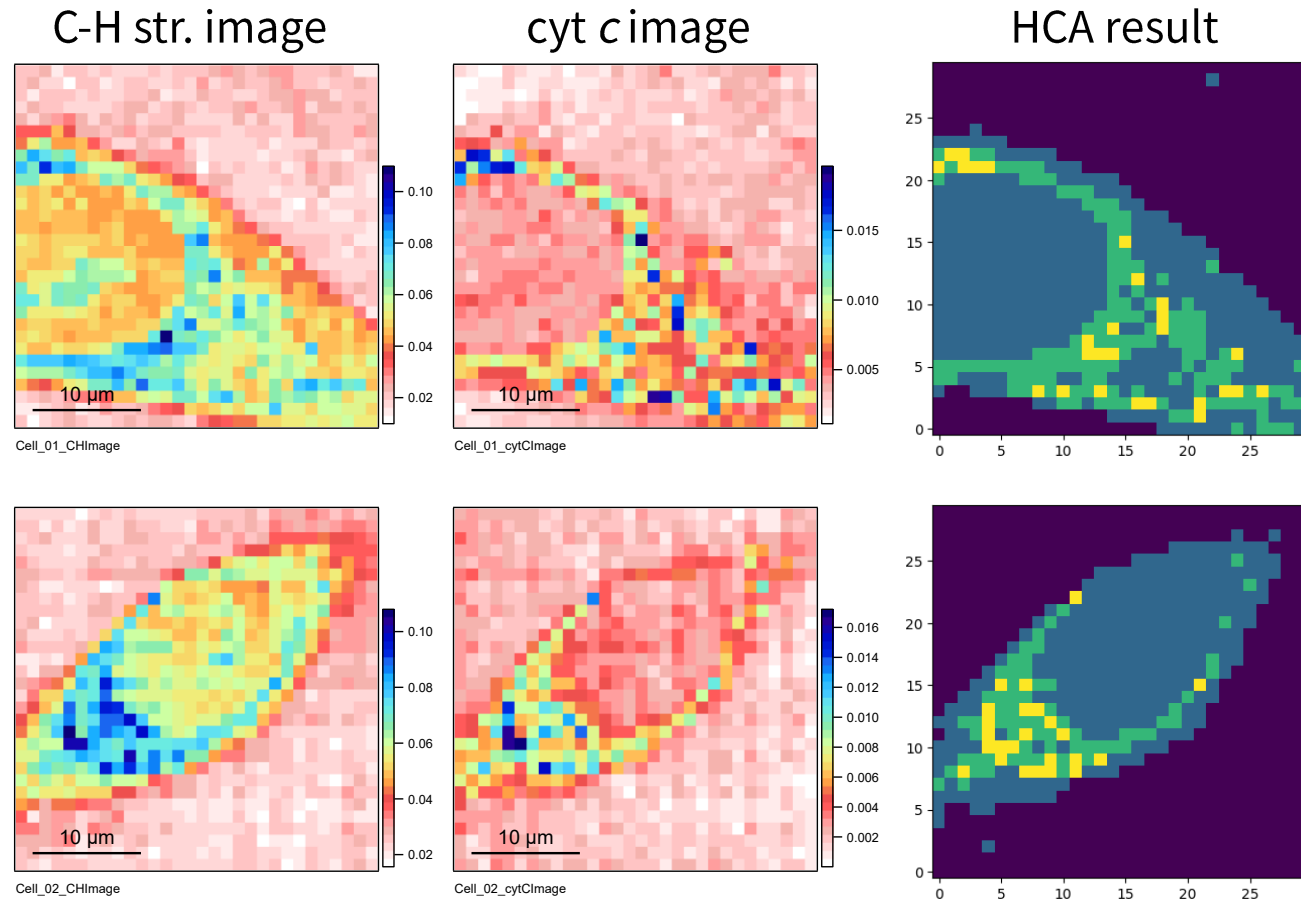
Theory of Hyper Spectral HCA (HSHCA)

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The Challenges of HCA in Raman Imaging Analysis of Cells

Hierarchical Clustering Analysis (HCA) enables automatic classification of intracellular regions (cytoplasm, nucleus, etc.) in Raman imaging of cells



Utilized algorithms:

- Ward's method
- Euclidean distance

- There are instances where the periphery of the cytoplasm is misclassified as the nucleus, leading to incorrect classification

Improvements to the Hierarchical Clustering Analysis Algorithm

In Raman imaging, adjacent points are likely to belong to the same intracellular region

→ Introducing the concept of **real-space distance** into distance definition in HCA

$$d(a, b) = d_{\text{spectral}}(a, b) + \lambda \cdot d_{\text{spatial}}(a, b)$$

Distance Between Spectra

(e.g., sum of squared differences in spectra)

Real-Space Distance (μm)

$$\sqrt{(x_a - x_b)^2 + (y_a - y_b)^2}$$

Note: The two distances are in different dimensions → Introduce a coefficient (λ) to scale them

