

Incorporation of neighbourhood constraints to Fuzzy C-Means algorithm to improve the spectral histology of human tissue sections by Raman microimaging

Cyril GOBINET¹, Nathalie MAINRECK¹, Valérie UNTEREINER¹, Christophe EKLOUH-MOLINIER¹, Jayakrupakar NALLALA², Ganesh SOCKALINGUM¹, Olivier PIOT¹

¹Unité MEDyC, UMR CNRS/URCA 7369, UFR de Pharmacie, Université de Reims Champagne-Ardenne, 51 rue Cognacq-Jay, 51096 Reims cedex, France

²Biomedical Imaging and Biosensing, Biomedical Physics, School of Physics, University of Exeter, EX4 4QL, United Kingdom

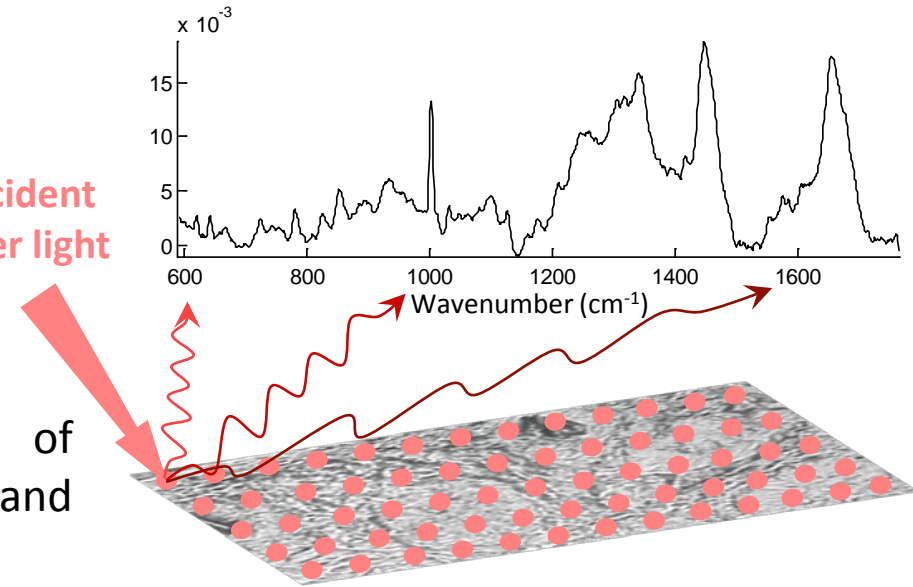


1) Raman micro-imaging

Raman spectroscopy

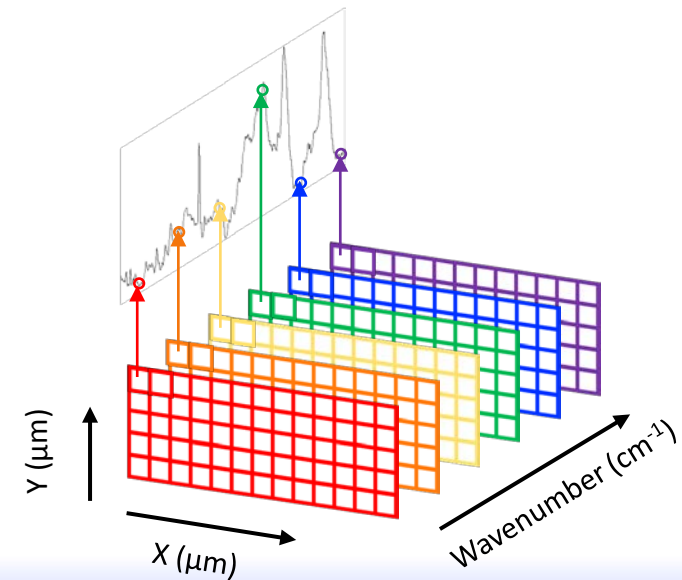
- Interaction between light and sample
- Vibrational information
- Molecular investigation
- Biological application: probe of biomolecular composition, structure and conformation of proteins, lipids
- No sample preparation, no staining, non invasive, non destructive

Incident laser light




Raman micro-imaging


- Coupling of microscopy, Raman spectroscopy and XY motorized stage
- Biomolecular investigation of complete samples
- Acquisition of hyperspectral images




2) Fuzzy C-Means (FCM)


 Aim: partition the N objects x_j , $1 \leq j \leq N$, into C clusters

 Each cluster is represented by its centroid v_i , $1 \leq i \leq C$

 Objective function: $J_m = \sum_{i=1}^C \sum_{j=1}^N u_{ij}^m \|x_j - v_i\|^2$

 u_{ij} is the membership value of the j^{th} object to the i^{th} cluster; $0 \leq u_{ij} \leq 1$; $\sum_{i=1}^C u_{ij} = 1$

 m is the fuzziness index

 Minimization of J_m according to u_{ij} and v_i


1) Fix C and m . Let $l = 0$ and initialize $U^{(l)} = [u_{ij}^{(l)}]$

2) Update $V^{(l)} = [v_i^{(l)}]$ using:
$$v_i^{(l)} = \frac{\sum_{j=1}^N (u_{ij}^{(l)})^m x_j}{\sum_{j=1}^N (u_{ij}^{(l)})^m}$$

3) Update $U^{(l+1)} = [u_{ij}^{(l+1)}]$ using:
$$u_{ij}^{(l+1)} = \left(\sum_{k=1}^C \left(\frac{\|x_j - v_i^{(l)}\|}{\|x_j - v_k^{(l)}\|} \right)^{2/(m-1)} \right)^{-1}$$

4) Let $l = l + 1$ and repeat steps 2 and 3 if $\|U^{(l)} - U^{(l-1)}\| \geq \varepsilon$. Otherwise, stop.

3) Incorporation of spatial constraint to Fuzzy C-Means:

 Aim: to take into account the neighbourhood in order to compensate the sensitivity of Fuzzy C-Means to noise

 Proposed solution:



Analysis of the update rule of u_{ij} :

$$u_{ij}^{(l+1)} = \frac{1}{\left(\underbrace{\|x_j - v_i^{(l)}\|^{2/(m-1)}}_{\text{Influential term}} \sum_{k=1}^C \underbrace{\left(\frac{1}{\|x_j - v_k^{(l)}\|} \right)^{2/(m-1)}}_{\text{Normalizing term}} \right)}$$

$$u_{ij}^{(l+1)} = \frac{1}{(I_t \times N_t)} \text{ with } I_t = \|x_j - v_i^{(l)}\|^{2/(m-1)} \text{ and } N_t = \sum_{k=1}^C \left(\frac{1}{\|x_j - v_k^{(l)}\|} \right)^{2/(m-1)}$$

3) Incorporation of spatial constraint to Fuzzy C-Means



Analysis of the **influential term** of u_{ij} :

$$x_j \text{ is close to } v_i^{(l)} \Rightarrow \left\| x_j - v_i^{(l)} \right\|^{2/(m-1)} \text{ small} \Rightarrow u_{ij}^{(l+1)} \cong 1$$

$$x_j \text{ is far from } v_i^{(l)} \Rightarrow \left\| x_j - v_i^{(l)} \right\|^{2/(m-1)} \text{ high} \Rightarrow u_{ij}^{(l+1)} \cong 0$$



Spatial constraint added to the **influential term** of the update rule of u_{ij}

$$I_t = \left(\left\| x_j - v_i^{(l)} \right\|^2 + \alpha \frac{1}{\sum_{m=1}^{N_j} u_{im}^{(l)}} \right)^{1/(m-1)}$$

with: N_j the number of neighbours of object x_j

$\sum_{m=1}^{N_j} u_{im}^{(l)}$ the sum of the membership values of the neighbours of object x_j

α the spatial constraint weight



Analysis of the **spatial constraint**:

$$\text{the } x_m \text{ are close to } v_i^{(l)} \Rightarrow \sum_{m=1}^{N_j} u_{im}^{(l)} \text{ is high} \Rightarrow \frac{1}{\sum_{m=1}^{N_j} u_{im}^{(l)}} \text{ is small}$$

$$\text{the } x_m \text{ are far from } v_i^{(l)} \Rightarrow \sum_{m=1}^{N_j} u_{im}^{(l)} \text{ is small} \Rightarrow \frac{1}{\sum_{m=1}^{N_j} u_{im}^{(l)}} \text{ is high}$$

Similar behaviours



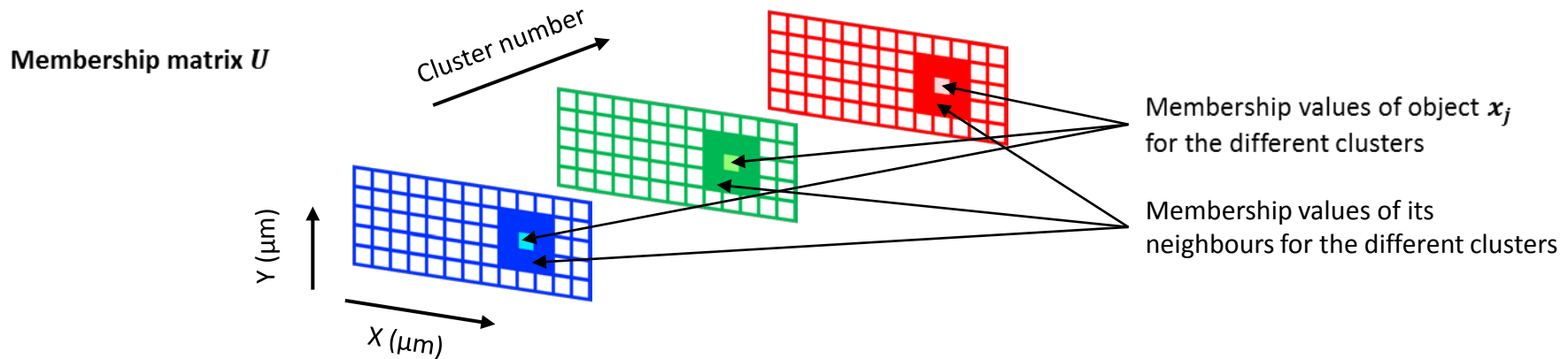
3) Incorporation of spatial constraint to Fuzzy C-Means

$$u_{ij}^{(l+1)} = \frac{\left(\|x_j - v_i^{(l)}\|^2 + \alpha \frac{1}{\sum_{m=1}^{N_j} u_{im}^{(l)}} \right)^{-1/(m-1)}}{\sum_{k=1}^C \left(\|x_j - v_k^{(l)}\|^2 + \alpha \frac{1}{\sum_{m=1}^{N_j} u_{km}^{(l)}} \right)^{-1/(m-1)}}$$

New update rule for u_{ij} :

New parameters to manage:

N_j the number of neighbours $\Rightarrow N_j = 8$ in the following (a 3×3 window centered on object x_j)



α the spatial constraint weight to be chosen in function of the signal-to-noise ratio of the processed image

The remaining of the algorithm is identical to classic FCM algorithm

New algorithm = Fuzzy C-Means Extended with Spatial Constraint (FCM-ESC)

4) Results on real Raman micro-images

a) Ulcerated nodular basal cell carcinoma

Image of the unstained slice used for Raman acquisition

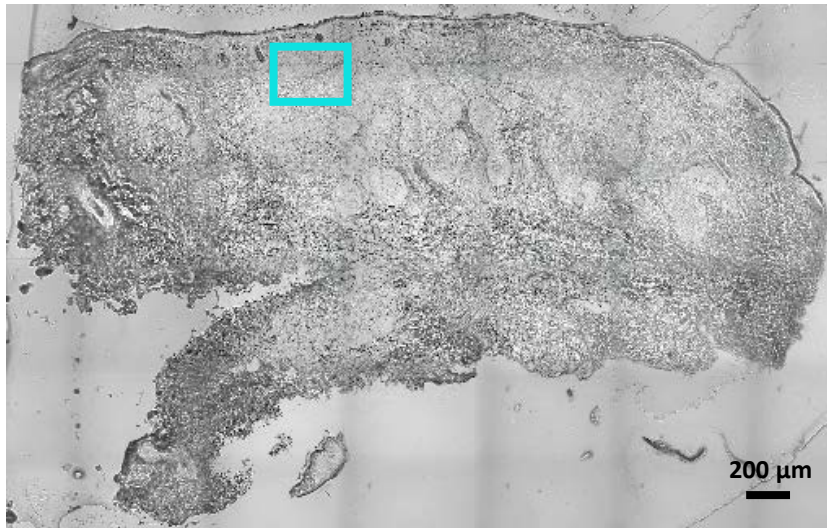
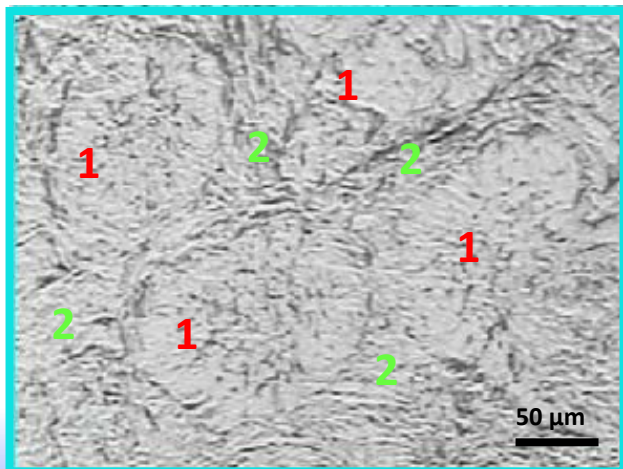
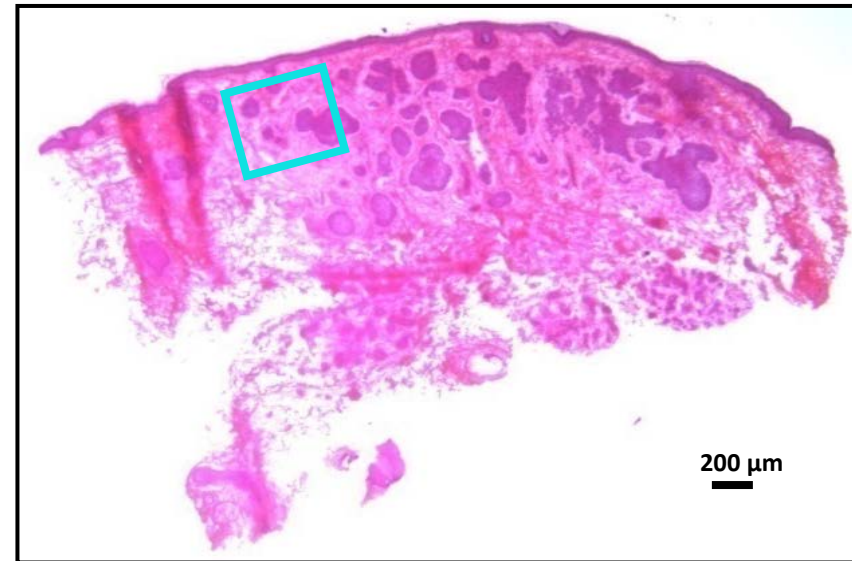
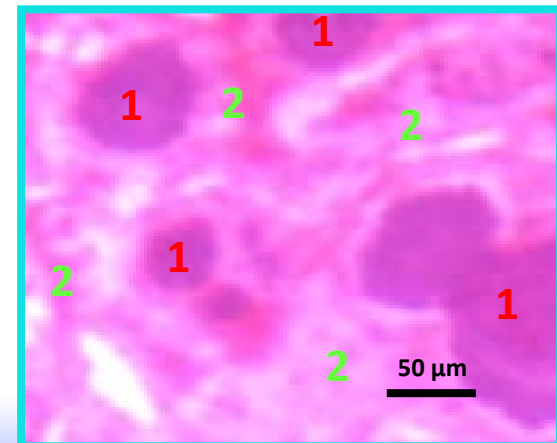


Image of the H&E stained adjacent slice for histopathological analysis



1 = Tumor
2 = Dermis

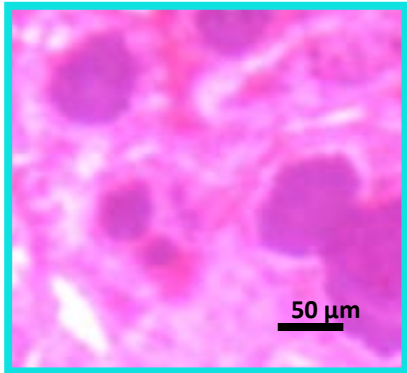


4) Results on real Raman micro-images

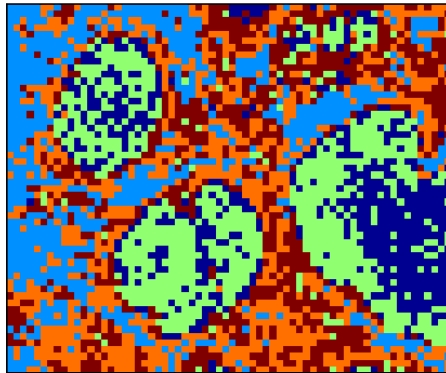
a) Ulcerated nodular basal cell carcinoma

Classic FCM results obtained on the Raman image: C = 5; m = 1.7

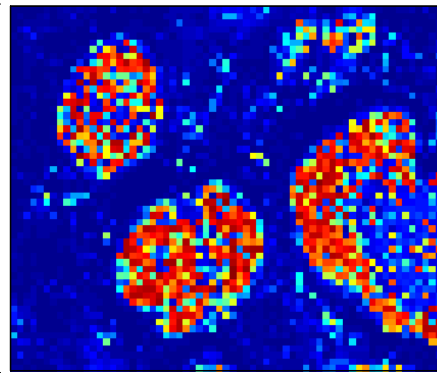
H&E stained image



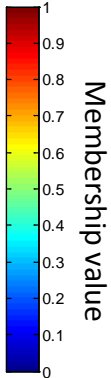
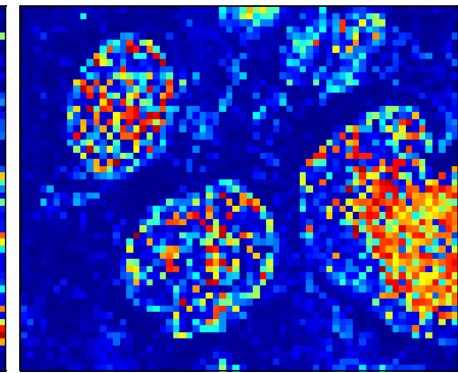
Defuzzificated image



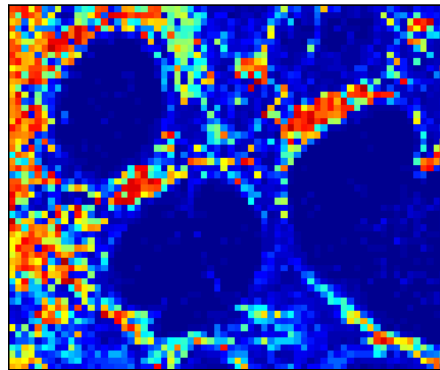
Cluster 1: tumor



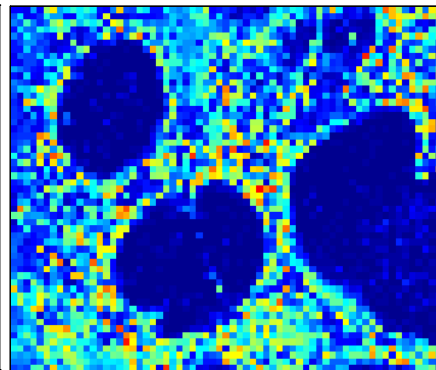
Cluster 2: tumor



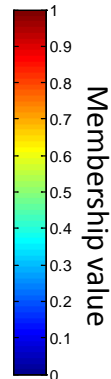
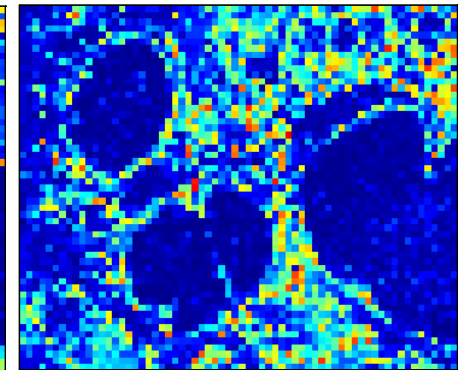
Cluster 3: dermis



Cluster 4: dermis



Cluster 5: dermis

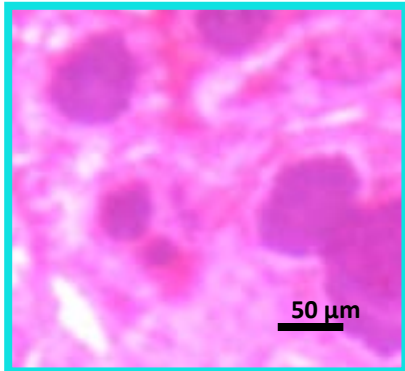


4) Results on real Raman micro-images

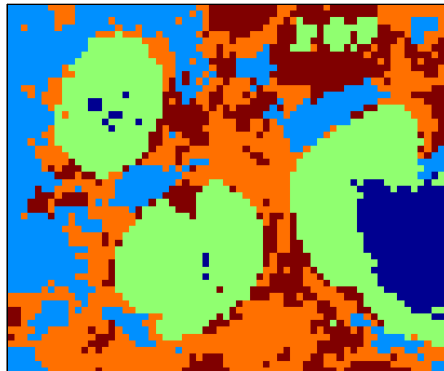
a) Ulcerated nodular basal cell carcinoma

FCM-ESC results obtained on the Raman image: $C = 5$; $m = 1.7$; $\alpha = 10$

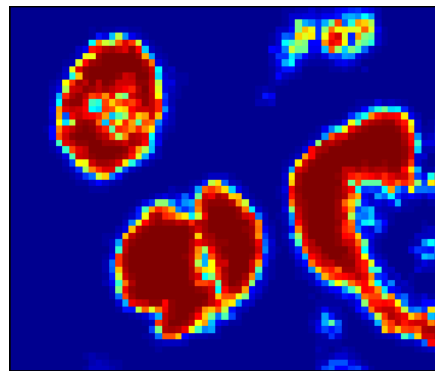
H&E stained image



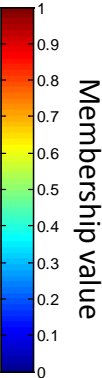
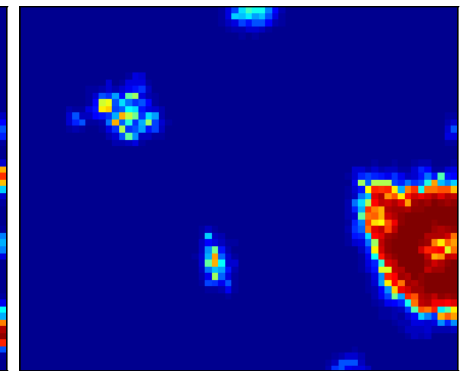
Defuzzificated image



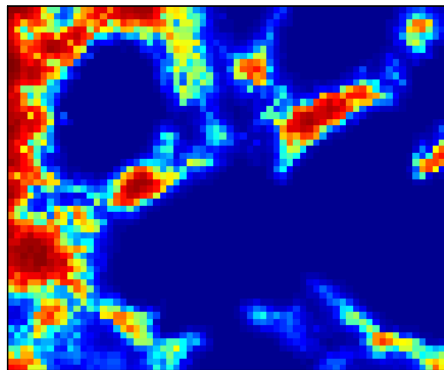
Cluster 1: tumor



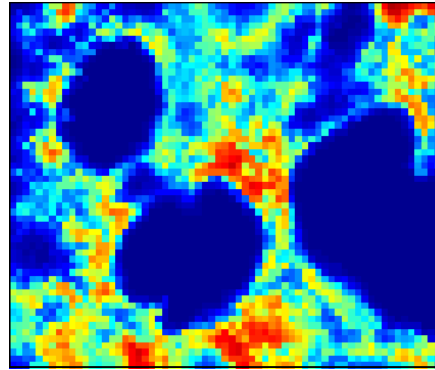
Cluster 2: tumor



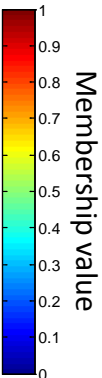
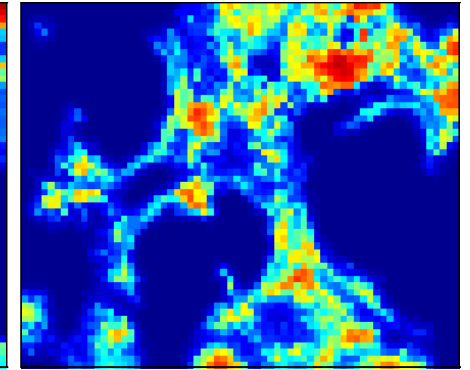
Cluster 3: dermis



Cluster 4: dermis



Cluster 5: dermis

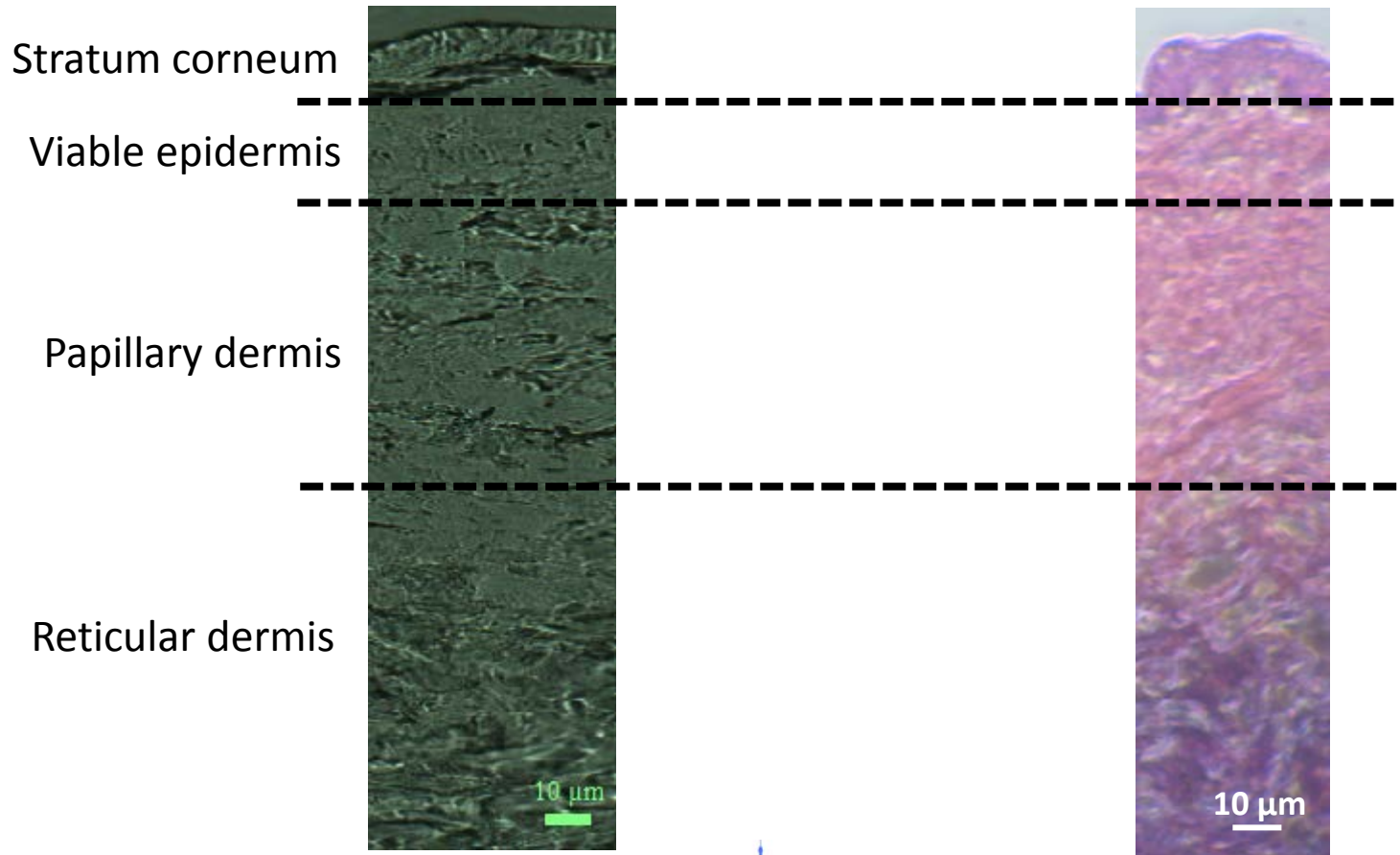


4) Results on real Raman micro-images

b) Normal human skin

Image of the unstained slice
used for Raman acquisition

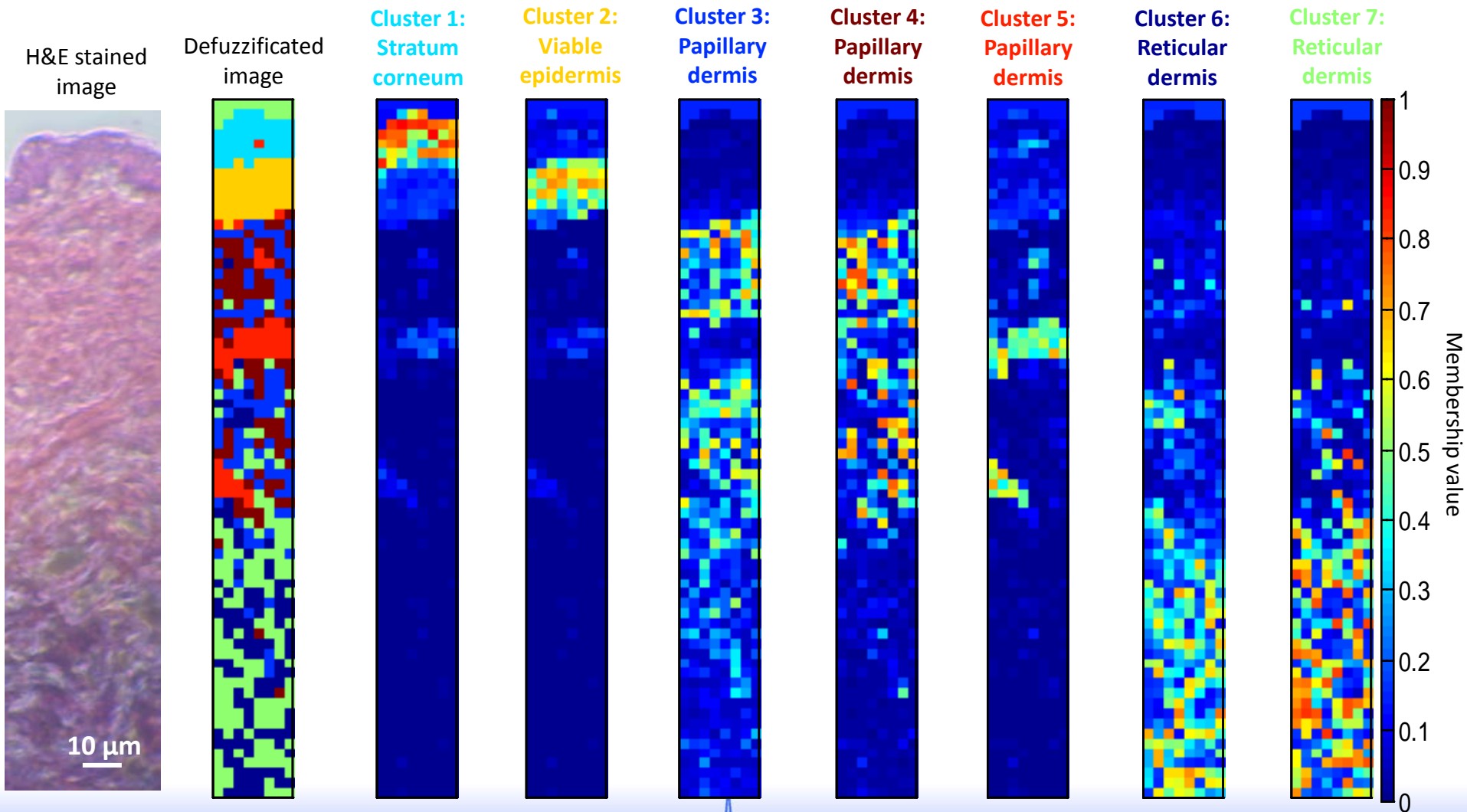
Image of the H&E stained adjacent
slice for histological analysis



4) Results on real Raman micro-images

b) Normal human skin

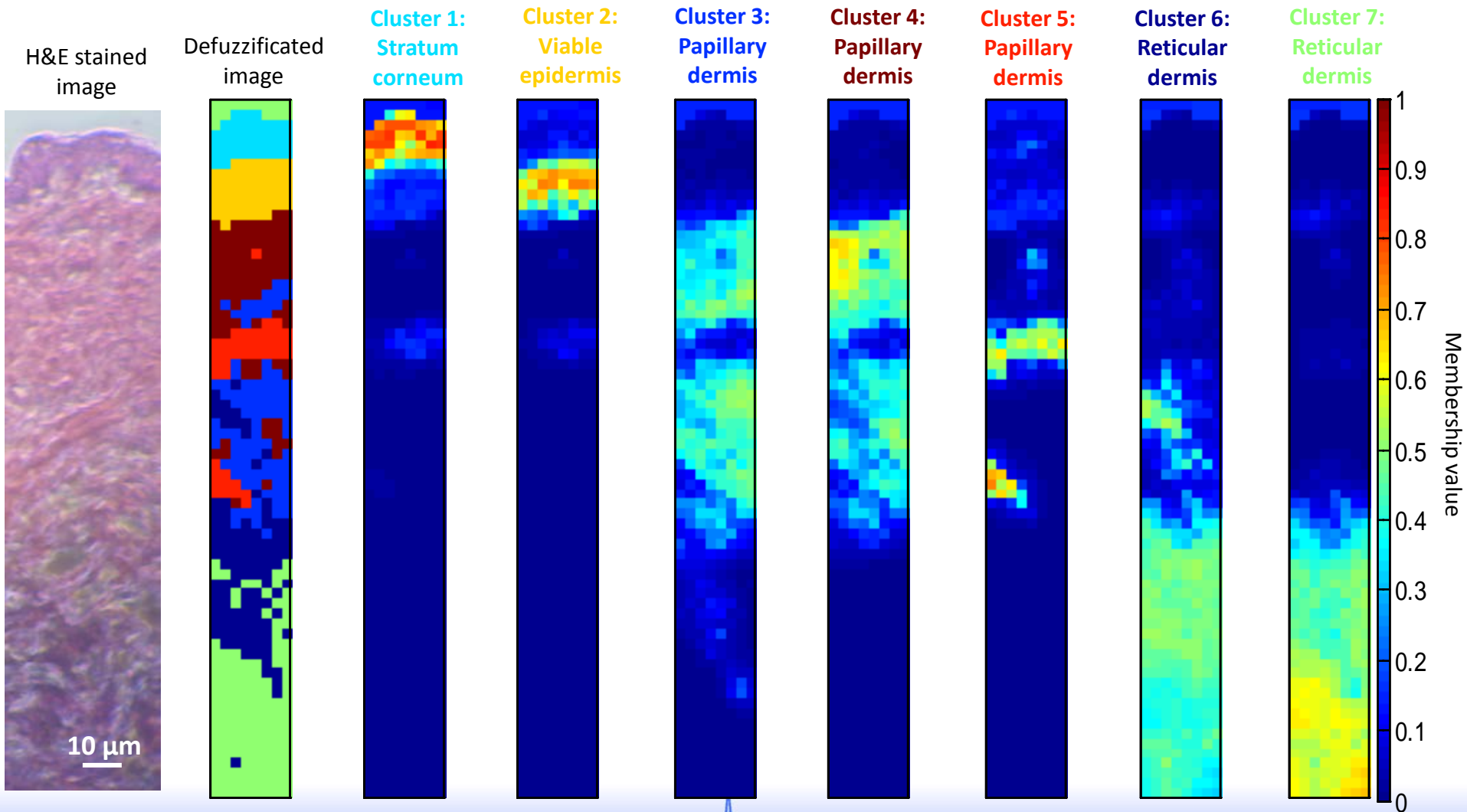
Classic FCM results obtained on the Raman image: $C = 7$; $m = 1.8$



4) Results on real Raman micro-images

b) Normal human skin

FCM-ESC results obtained on the Raman image: $C = 7$; $m = 1.8$; $\alpha = 10$



4) Results on real Raman micro-images

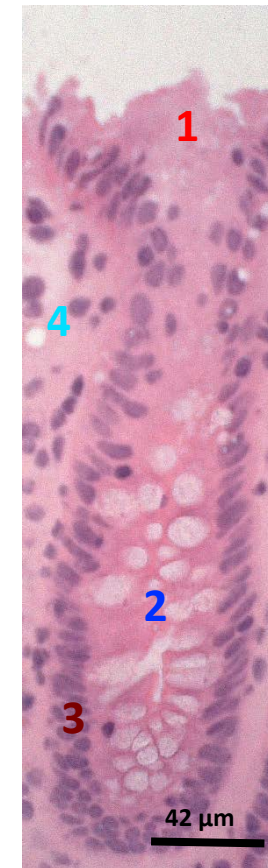
c) Normal human colon tissue

Image of the unstained slice
used for Raman acquisition



- 1 = secreted mucus
- 2 = mucinous inner region of the crypt
- 3 = outer region of the crypt (epithelial cells)
- 4 = connective tissue

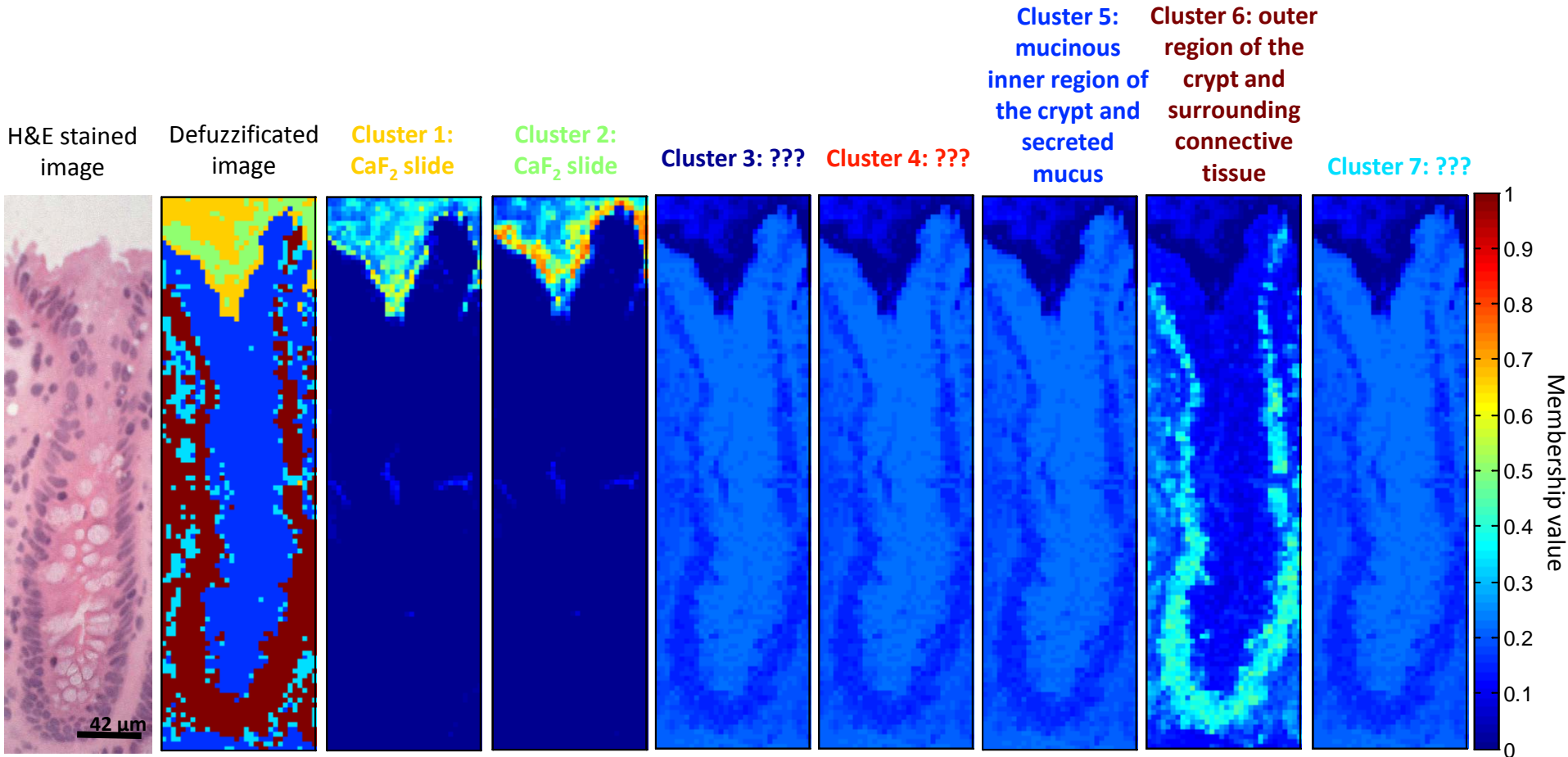
Image of the H&E stained adjacent
slice for histological analysis



4) Results on real Raman micro-images

c) Normal human colon tissue

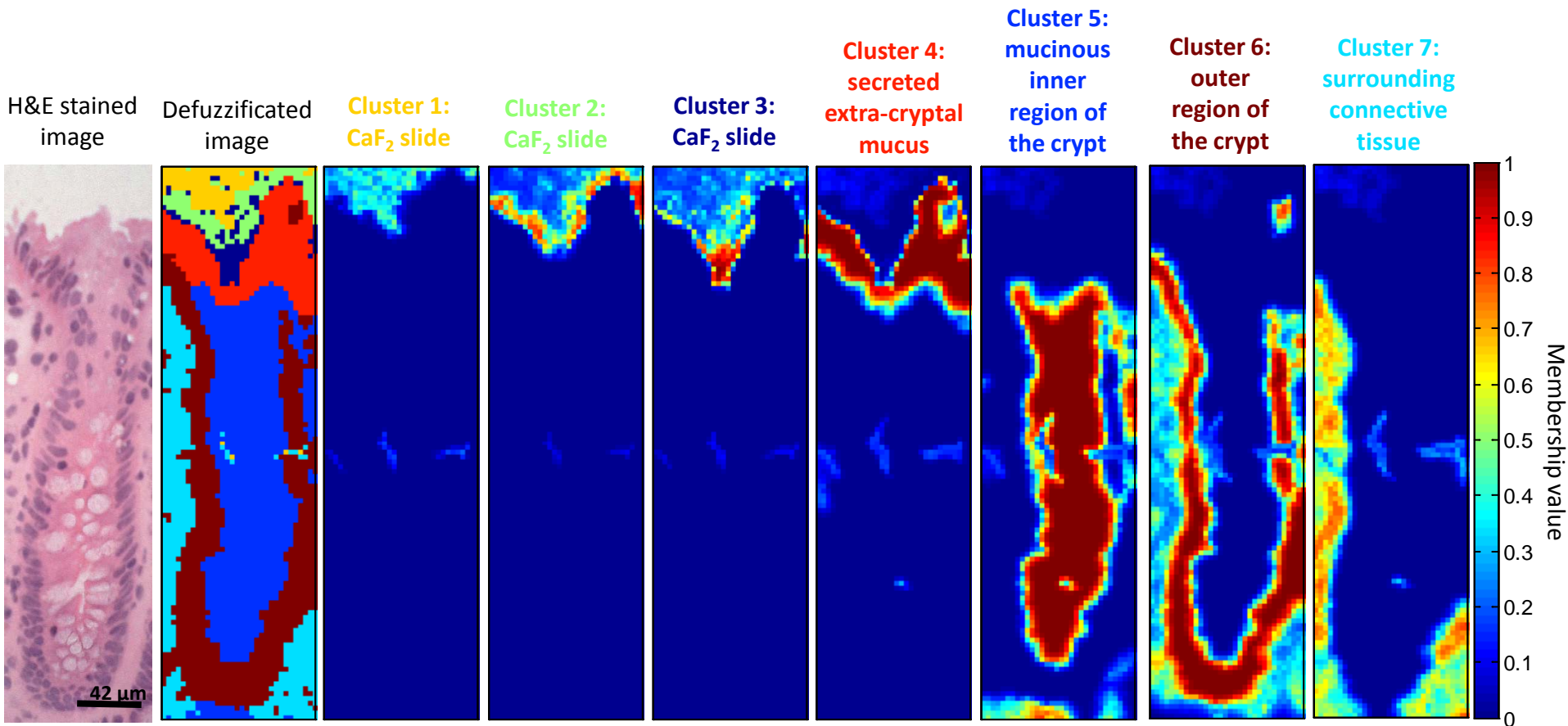
Classic FCM results obtained on the Raman image: C = 7; m = 1.5



4) Results on real Raman micro-images




c) Normal human colon tissue

FCM-ESC results obtained on the Raman image: $C = 7$; $m = 1.5$; $\alpha = 20$







6) Conclusions and perspectives

Conclusions:

-  Sensitivity of classic Fuzzy C-Means to noise \Rightarrow difficulty to interpret Raman images
-  New Fuzzy C-Means algorithm incorporating spatial constraint
-  Efficiency of this new algorithm on noisy Raman images

Perspectives:

-  Empirical algorithm \Rightarrow need of mathematical proof
-  Preliminary results \Rightarrow confirm the results on more Raman images
-  Choice of α by try-and-error procedure \Rightarrow toward an automatic estimation of α
-  Comparison of our algorithm to those previously developed in literature

