Demonstrating the effects of area normalization, using a Raman hyperspectral image of wood fibers in the cross-section of a hybrid aspen stem as an example

The panels show representative steps of the workflow, using non-normalized (left column) and area normalized (right column) data. Other pre-processing steps were applied uniformly (AsLS baseline correction, lambda = 30,000; p=0.001; Savitzky-Golay smoothing, order = 1, frame = 3)

a and b) Pure component (C1-C5) distribution maps (concentration profiles), following MCR-ALS (5 components, iteration limit: 50, convergence limit: 0.1). The white diamonds mark the location of the purest pixel for each component. These locations do not change, but the distribution maps are markedly different in intensity. The non-normalized maps (left) contain larger intensity fluctuations due to fluorescence (especially noticeable for C3 and C4)

c and d) The corresponding pure spectral profiles (C1-5). Notice that the spectral profiles are identical, i.e. the normalization had no effects on the spectral profiles (no distortions).

e and f) Segmentation maps, following k-means clustering with 4 clusters, based on the concentration profiles in a and b, respectively. Please note that k-means clustering arbitrarily assigns the cluster numbers, thus they do not match in e and f. This results in a coloring difference between the two plots. Nevertheless, the different zones are almost identical, except for a few borderline pixels (pixels 1, 12, 17 and 21).

g and h) The corresponding centroid profiles, showing the contribution of different components to each cluster. The effect of normalization is very obvious, with more absolute amounts in the non-normalized centroid plots, and more relative (proportional) amounts in the normalized centroid plots. When signal intensity is comparable (cluster 2 for the non-normalized data and cluster 4 for the normalized data), the component profiles are very similar. In other cases, the normalization "stretches" the contributions and discrepancies occur (c.f. cluster 1 in both cases).