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Autofluorescence variability in maize stems by multispectral image analysis of series of large images at the macroscopic scale

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The composition, structure and relative proportions of plant tissues are under biological control and determine the nutritional quality and use of the plant. In case of lignocellulosic biomass like maize stems, histological studies are of major importance for understanding the recalcitrance of plant material to enzymatic degradation¹. In such plants, tissues can be observed without any labelling thanks to the autofluorescence of their cell wall components, i. e. lignin and hydroxycinnamic acids. Recent imaging equipments allows multispectral fluorescence imaging at the macroscopic scale with large fields of view (> 1 cm²) and a resolution below 3 µm per pixel. Such devices enable the study of large samples and take into account the statistical variability by analysing together sets of multispectral mosaic images.

In the present work, the variability of maize stem sections was investigated. Large images containing more than 4000 x 4000 pixels x 12 multispectral channels were acquired and usual chemometric approaches had to be adapted to account for the huge volume of data. Principal Component Analysis was implemented to assess common loadings from series of large images. An unsupervised multispectral image segmentation was retained to reveal fluorescence variability without any *a priori*. A multi-scale representation of images using image pyramids was combined with k-means clustering methods in order to take into account the whole volume of data².

We present here the analysis of a set of six cross sections of maize stem images taken from two stems showing different lignin fluorescences. The four first principal components revealed respectively fluorescent tissues, lignin and hydroxycinnamic acid fluorescences, the two phenotypes of lignin fluorescence, two regions of parenchyma that were related to different lignification. The multiscale k-means clustering model made it possible to segment the main tissues of the stem: sclerenchyma sheaths in vascular bundles in the pith and in the rind, epidermis, fibers of xylem, lignified and non-lignified parenchyma (Figure 1). A large variability in fluorescence properties was observed within and between stem sections.

The study demonstrate the potential of macrofluorescence imaging combined with appropriate chemometric methods to analyse together series of large samples. The method open the way to modelling fluorescence variability at the scale of the organ.

References

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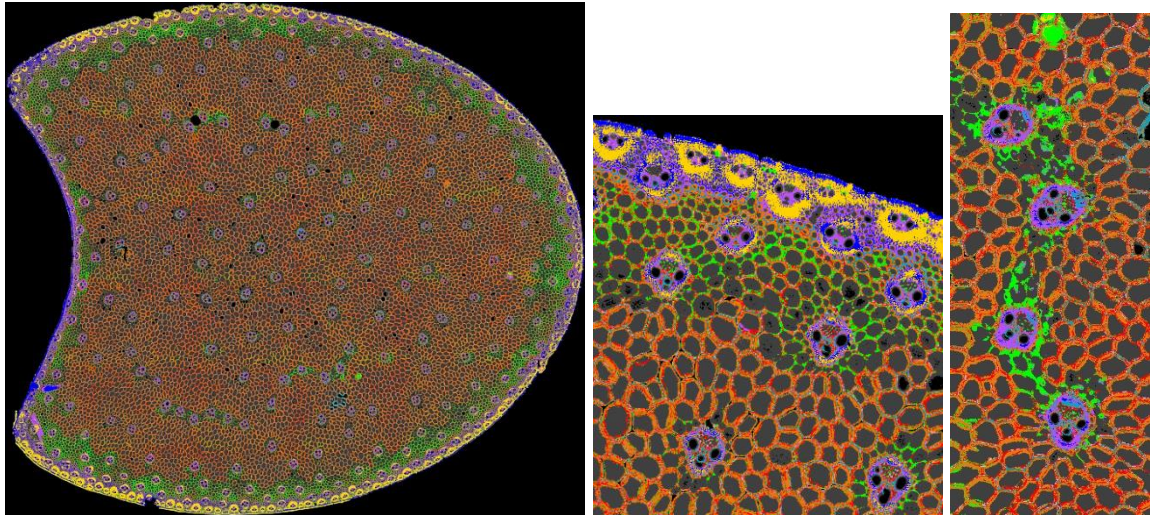


Figure 1. Example of multispectral fluorescence image segmentation. Left whole image (initial size 6397 x 5387 pixels $\approx 17 \times 14 \text{ mm}^2$. Middle and right: selected regions: rind and centre of the stem. (700 x 800 pixels $\approx 1.9 \times 2.1 \text{ mm}^2$ and 1000 x 400 pixels $\approx 2.7 \times 1.1 \text{ mm}^2$).