Imaging molecular structure of plant cells by Confocal Raman microscopy

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Abstract

During the last years Confocal Raman microscopy evolved as a powerful method to get insights into chemistry and structure of plant cells and cell walls with a spatial resolution of around 300 nm. Two-dimensional spectral maps can be acquired of selected areas and Raman images calculated by integrating the intensity of characteristic spectral bands or by using multivariate data analysis methods. This enables direct visualization of changes in the molecular structure and analyzing the spectra laying behind the chemical images reveals detailed insights into cell wall chemistry and structure [1-4].
Insights have been gained into the design of plant cell walls to achieve movement in wooden parts of trees or in roots by means of gelatinous fibers. Plant cell walls are based on cellulose microfibrils embedded in a matrix of hemicelluloses and lignin. The orientation of the cellulose microfibrils (alignment with respect to the fiber axis) on the nanolevel, the arrangement of different layers on the microlevel, as well as the amount of lignin determine mainly properties and functionalities. These parameters are elucidated in-situ in context with the microstructure and reveal thus the design of e.g. so called gelatinous fibers. Almost pure cellulose has been identified as the main swelling core of this fibers, functionalized by a small outer lignified layer with high microfibril angle [4-7].
Recently the potential of method has also been shown on the algal model system *Micrasterias denticulata.* The changes in the molecular structure within the different cell organelles and structures can be followed as well as the changes in the outer cell wall during growth (Figure1).

References

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Figure1: Raman spectroscopic image of *Micrasterias denticulata* (160 x 160 µm, 0.5 µm step size,532 nm WITec300RA). Based on the 102 400 Raman spectra images were calculated with the help of non-negative matrix factorization (NMF), a method to evaluate distribution maps of different components and demixed basis spectra. The different colours represent the different basis spectra (components). The blue colour represents the outer cellulosic cell wall, which is more highlighted in the old half of the cell (lower part of the image) due to higher cellulose amount and crystallinity than in the newly formed young part (upper smaller side). In the inner part the red colour corresponds to starch and highlights the small round pyrenoids in the older cell half, which are embedded in the chloroplast. Proteins, pectins and fats are coloured in green and yellow.