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Raman imaging of plant metabolite crystals

Our understanding of plant and crop biochemical mechanisms is fundamental to our ability to maintain ecosystems and provide sustainable nutrition. Metabolite production is a key marker to correlate to environmental, nutritional, and mechanical stresses which allow the plant health and development to be effectively studied[1].

Raman imaging is an ideal technique for studying plant tissue in a non-destructive and non-invasive way. Raman images spatially resolve chemical and structural information, and can be acquired in the presence of water and in vivo. Here the spatial distribution of plant metabolites and tissue structure are revealed using the Renishaw inVia™ Qontor® confocal Raman microscope.

Fast imaging of plant sections

Raman spectral data of English oak leaf and wood sections were acquired using a Renishaw inVia Qontor Raman microscope, equipped with 785 nm laser excitation. Renishaw's StreamHR™ fast mapping was used to measure both sections at high spatial resolution. Raman images of each section were generated using analysis based on the comparison of the chemical components with reference spectra, and the Raman band intensity.

Calcium oxalate hydrates (whewellite and weddellite) are observed in most plant tissues and organs, including leaves, stems, roots, seeds and flora. The crystals are formed in calcium oxalate crystal idioblasts. The amount of calcium oxalate crystals, and their shape, size and function are defined by a combination of genetic and environmental factors[2,3]. These crystals perform various functions, including regulation of calcium levels in plant tissues and organs; protection against herbivory; detoxification of heavy metals; strengthening of the tissues; and the gathering and reflecting of light for photosynthesis[3].

Raman images enable us to observe the spatial distribution of the calcium oxalate crystals, and their hydration state, in the leaf section of English oak (Figure 1). The Raman image shows the variation in the Raman band intensity corresponding to the lignin band at 1602 cm^{-1} (orange). It highlights the compound middle lamella (CML) and cell corners (CC), where the concentration of lignin is higher. Large calcium oxalate metabolite crystals are present in the corners of the leaf cells.

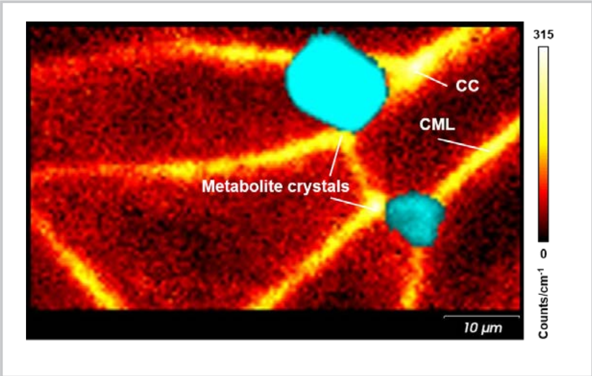

Figure 1. Raman image of a cross section of a leaf showing the intensity changes of the lignin Raman band at 1602 cm^{-1} (orange), and the presence of calcium oxalate (cyan).

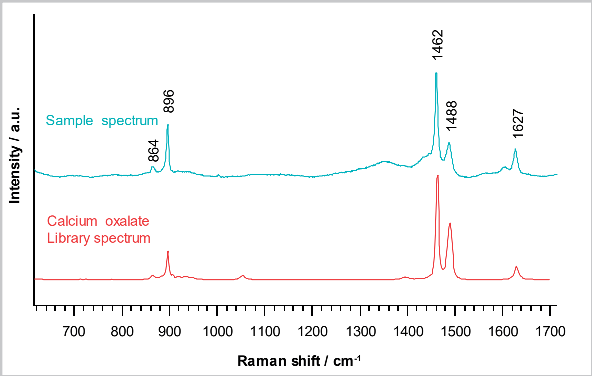

Figure 2. Raman spectrum of calcium oxalate metabolite from Raman image vs library spectrum.

Renishaw plc
Spectroscopy Products Division
New Mills, Wotton-under-Edge,
Gloucestershire GL12 8JR
United Kingdom

T +44 (0) 1453 524524
F +44 (0) 1453 524901
E raman@renishaw.com

www.renishaw.com/raman

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Figure 2 shows the Raman spectrum from a cyan area of the image. The Raman bands located at 896 cm^{-1}, 1462 cm^{-1} and 1488 cm^{-1} correspond to the ν(C-C) stretching mode and to two C-O symmetric stretching vibrations, respectively. These bands are characteristic of calcium oxalate monohydrate; the stable hydration state of calcium oxalate[4].

The Raman data (Figure 3) also revealed ellagic acid. This acid belongs to a large group of polyphenolic compounds named tannins, considered secondary metabolites[5], used to protect against attack from diverse microorganisms, such as bacteria and fungi.

Figure 3 demonstrates the distribution of ellagic acid crystals within the English oak wood cells. The image shows the changes in the Raman band intensity corresponding to lignin at 1596 cm^{-1}. This highlights the shape of the wood cells, consisting of compound middle lamella (CML) and corners (CC). Different size crystals of ellagic acid are observed inside the wood cells. The size and shape of the crystals can be potentially correlated to environmental changes.

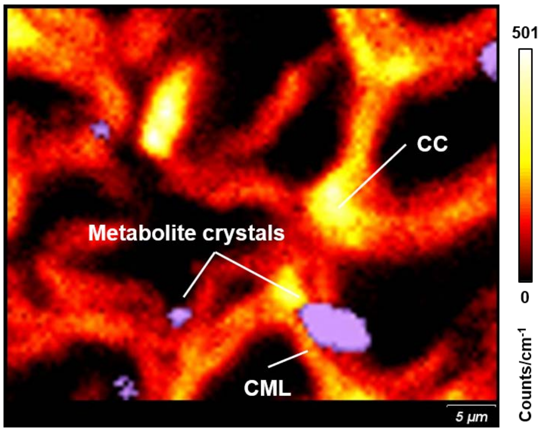

Figure 3. Raman image of cross section of wood showing intensity changes of the lignin Raman band at 1596 cm^{-1} (red/orange) and ellagic acid (violet).

Conclusion

High spatial resolution Raman images, of various parts of English oak tissues, enable details within the cell structure, and the presence of metabolites and their hydration state to be determined. The high specificity of Raman spectroscopy confirmed the presence of two different metabolites within the oak, calcium oxalate monohydrate in leaves and ellagic acid in wood. A combination of chemical and structural information, with data on metabolite distribution, can support the understanding of the impact of biotic and abiotic factors on the development of plants.

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References

1. H.J. Butler, M.R. McAinsh, S. Adams, F.L. Martin, *Application of vibrational spectroscopy techniques to non-destructively monitor plant health and development*, **Anal. Methods**, 7 (2015) 4059-4070 (https://doi.org/10.1039/C5AY00377F)
2. V.R. Franceschi, P.A. Nakata, *Calcium Oxalate in Plants: Formation and Function*, **Annu. Rev. Plant Biol.** 56 (2005) 41-71 (https://doi.org/10.1146/annurev.arplant.56.032604.144106)
3. M.N. Islam, M. Kawasaki, *Morphological Changes and Function of Calcium Oxalate Crystals in Eddo Roots in Hydroponic Solution Containing Calcium at Various Concentrations*, **Plant Prod. Sci.** 17 (2014) 13-19 (https://doi.org/10.1626/pps.17.13)
4. C. Frausto-Reyes, S. Loza-Cornejo, T. Terrazas, M. de la Luz Miranda-Beltrán, X. Aparicio-Fernández, B.M. López-Macías, S.E. Morales-Martínez, M. Ortiz-Morales, *Raman Spectroscopy Study of Calcium Oxalate Extracted from Cacti Stems*, **Appl. Spectrosc.** 68 (2014) 1260-1265 (https://doi.org/10.1366/14-07485)
5. L. Sepúlveda, A. Ascacio, R. Rodríguez-Herrera, A. Aguilera-Carbó, C.N. Aguilar, *Ellagic acid: Biological properties and biotechnological development for production processes*, **Afr. J. Biotechnol.** 10 (2011) 4518-4523

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