In-vivo spektroskopisches Imaging von Pflanzeninhaltsstoffen

Zedler[1], N. Vogler[1,2], A. Walter[1], I. Weißflog[1,2], U. Münchberg[1], K. Grosser[3], L. Wagner[4], P. Rösch[1], **M. Schmitt[1]**, B. Dietzek[1,2],
K. Voigt[4], G. Pohnert[3], E. Kothe[4], W. Boland[5], **J. Popp[1,2]**

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[3]IAAC, Bioorganic Analytics, Friedrich-Schiller-Universität Jena, Lessingstr. 8, 07743 Jena

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Overview

- Motivation - molecular imaging
- Raman spectroscopic characterization of the spatial distribution of secondary algal metabolites
- Visualization of mitochondria activity via cytochrome localization in hyphal tip cells by means of resonance Raman and CARS microspectroscopy
- Raman spectroscopic characterization of the oil composition in single intact hyphae
- CARS microscopy for the characterization of leaf components

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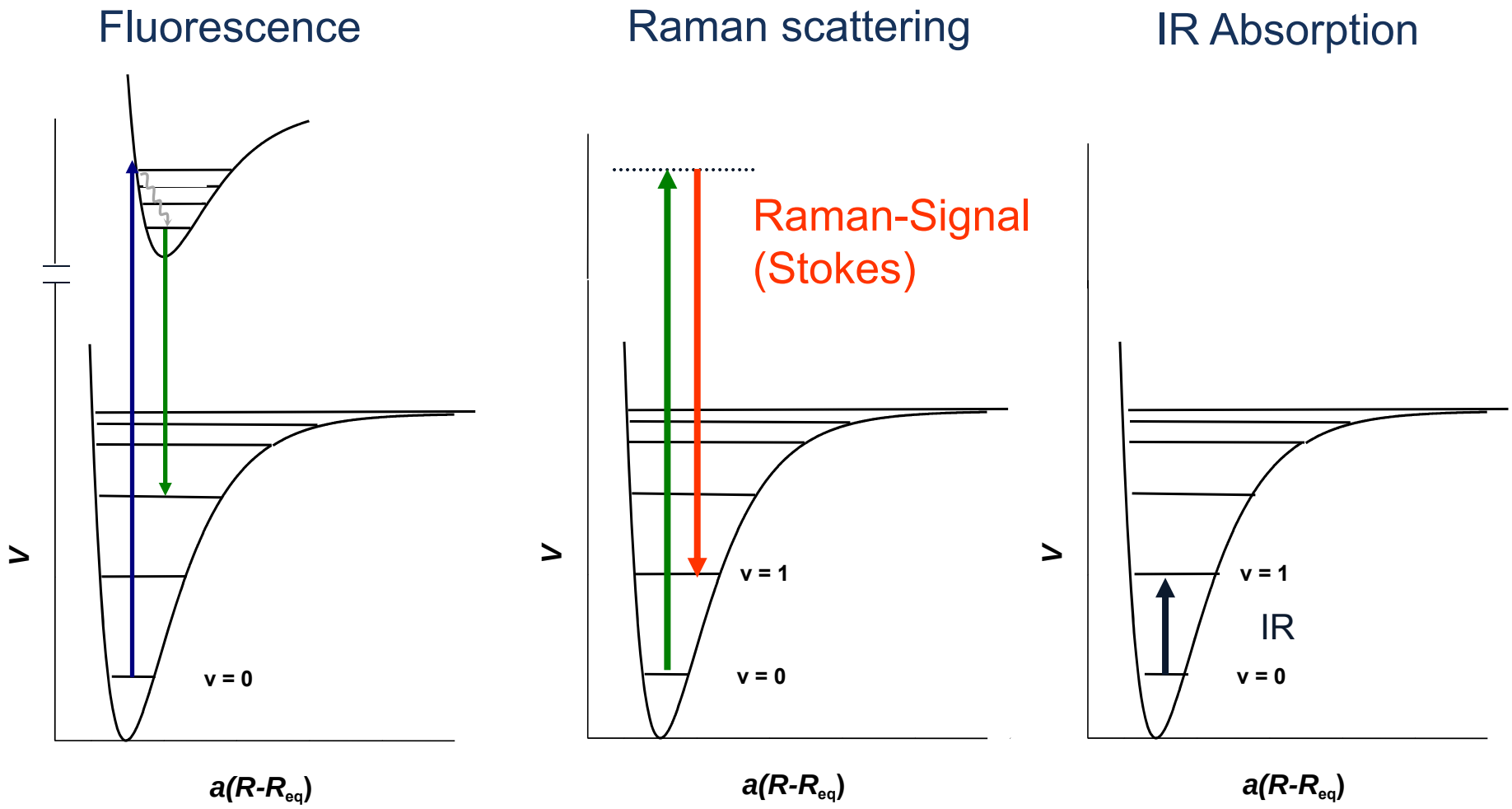
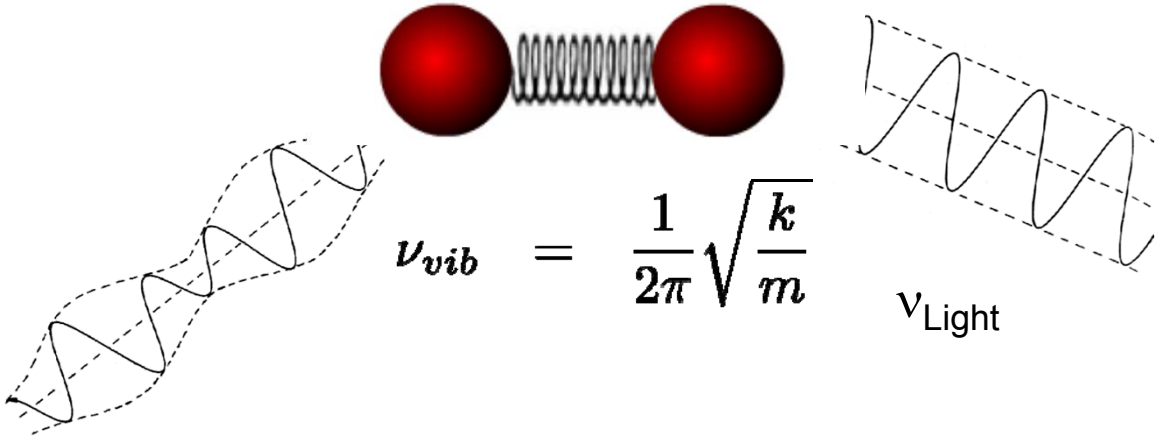
Molecular microspectroscopy for biomedical imaging: molecular / chemical contrast



Raman – a brief introduction

The vibrational Raman effect – classical description



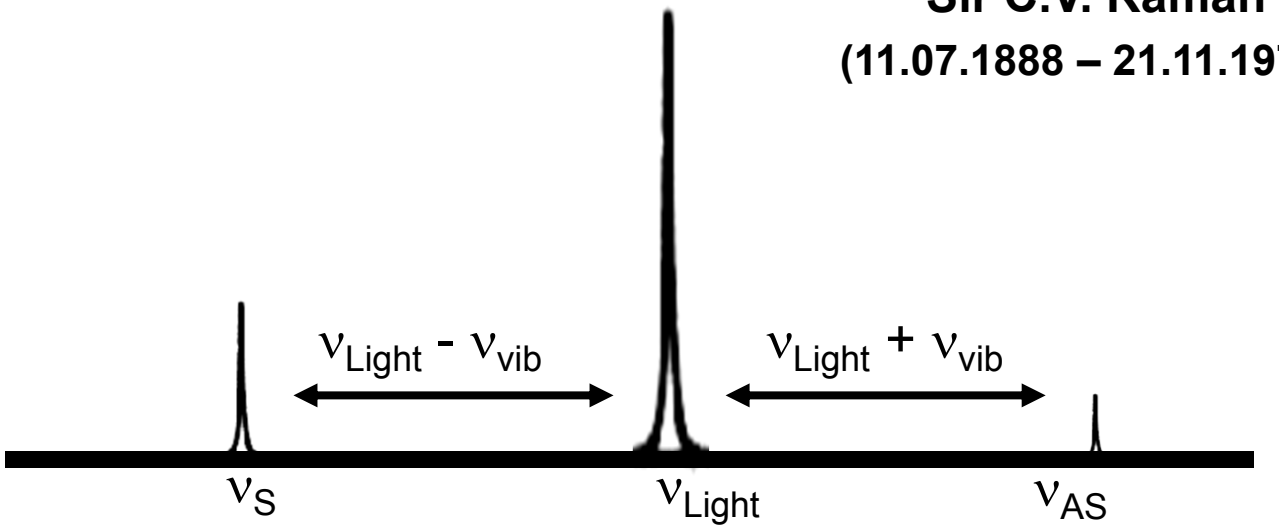
$$\nu_{vib} = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$$

$\nu_{Light} \pm \nu_{vib}$

ν_{Light}

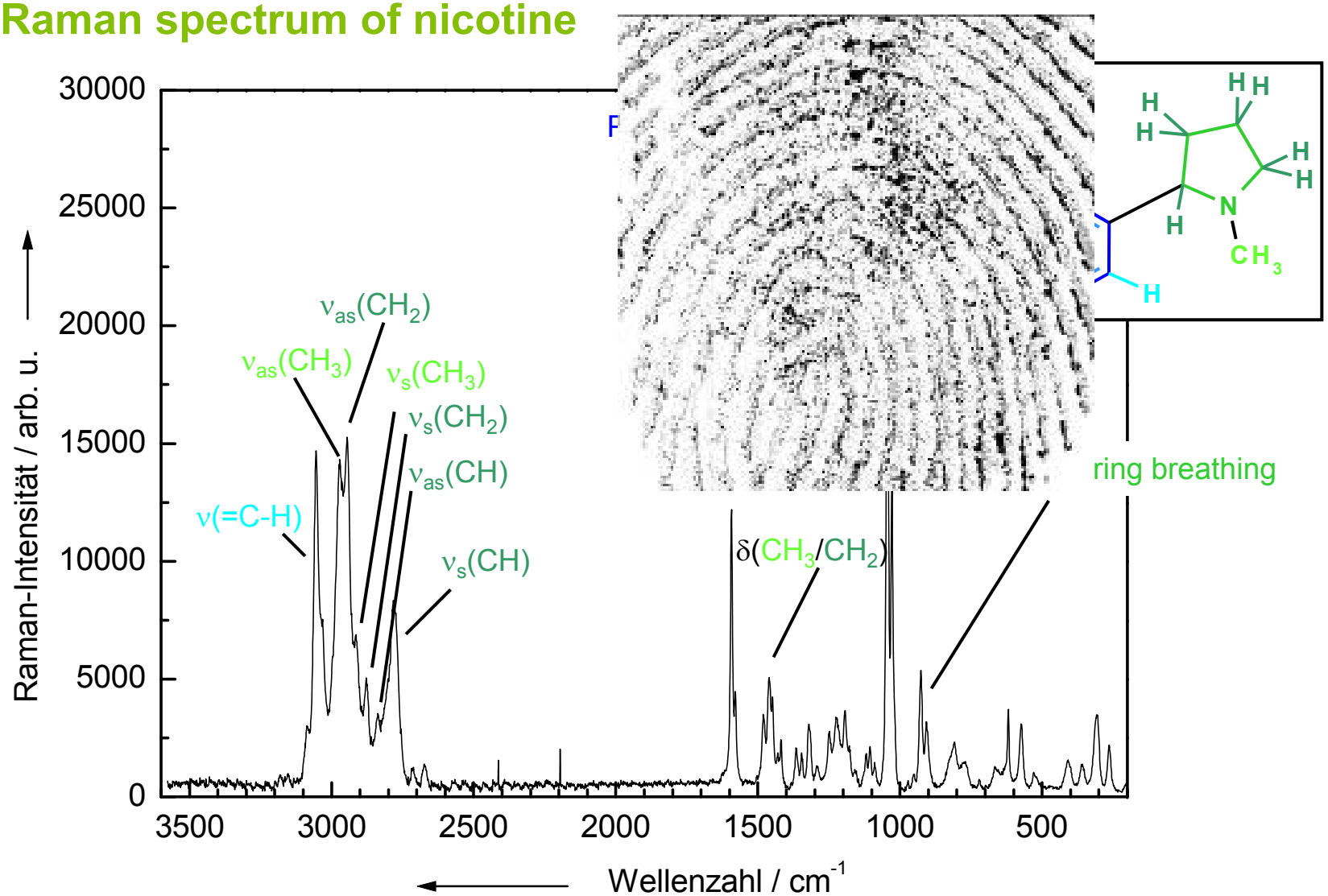
Sir C.V. Raman

(11.07.1888 – 21.11.1970)



Raman – a brief introduction

Raman spectrum of nicotine

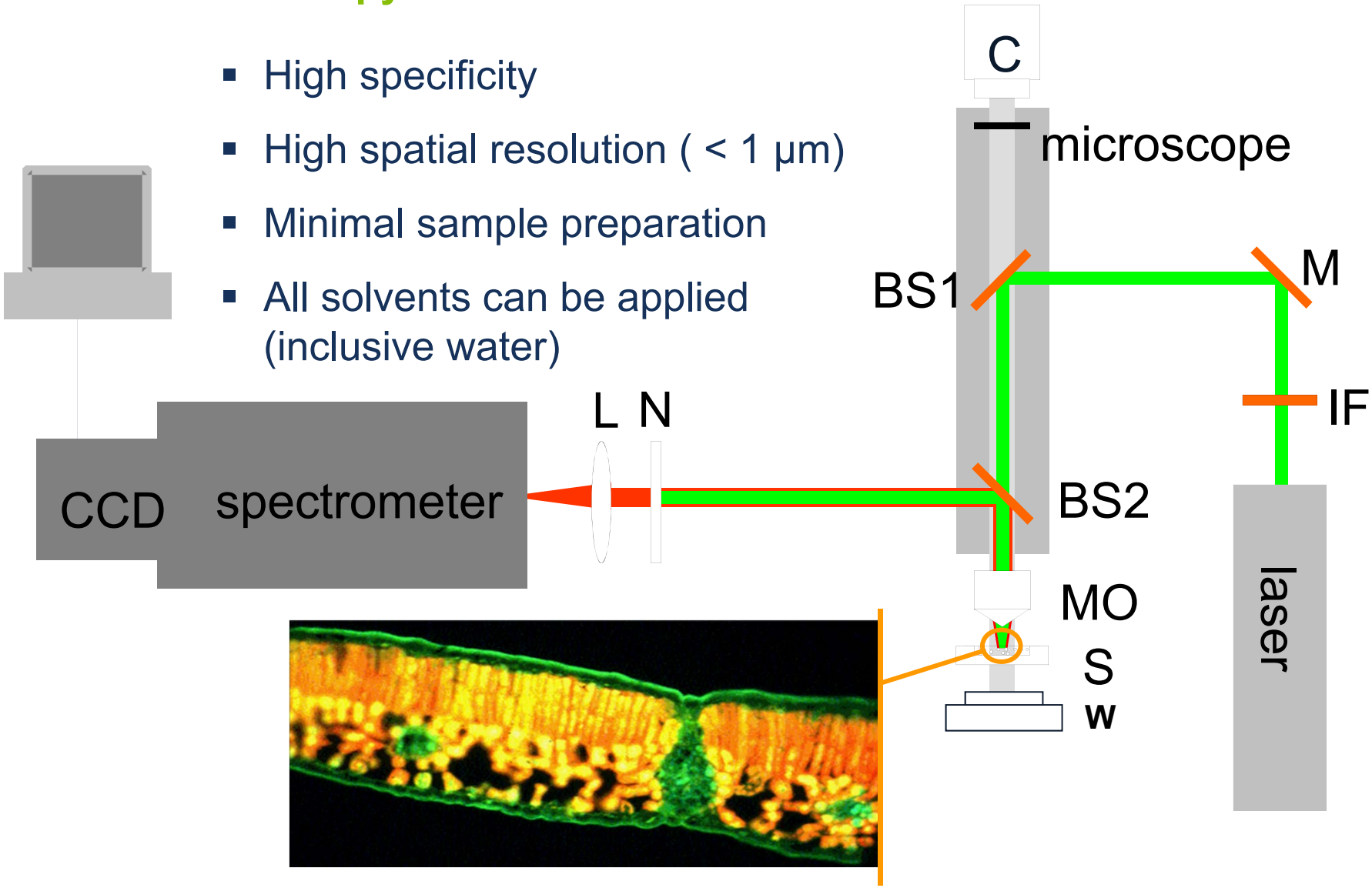


➡ Raman yields molecular fingerprint information

Raman – a brief introduction

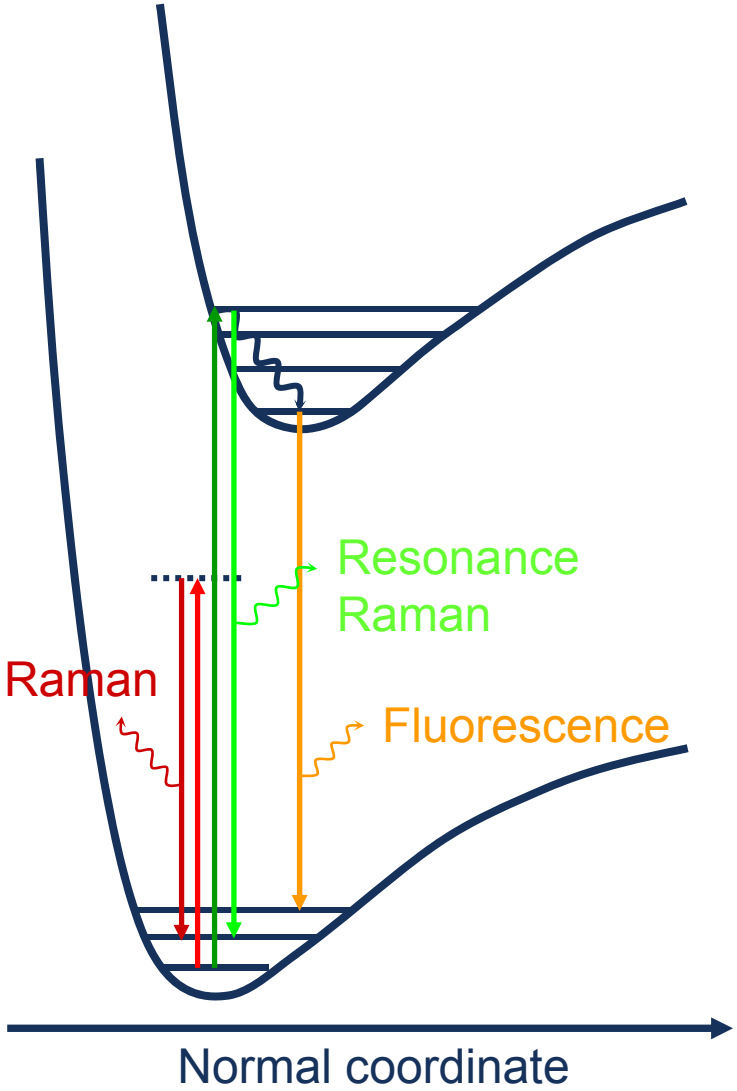
Raman microscopy

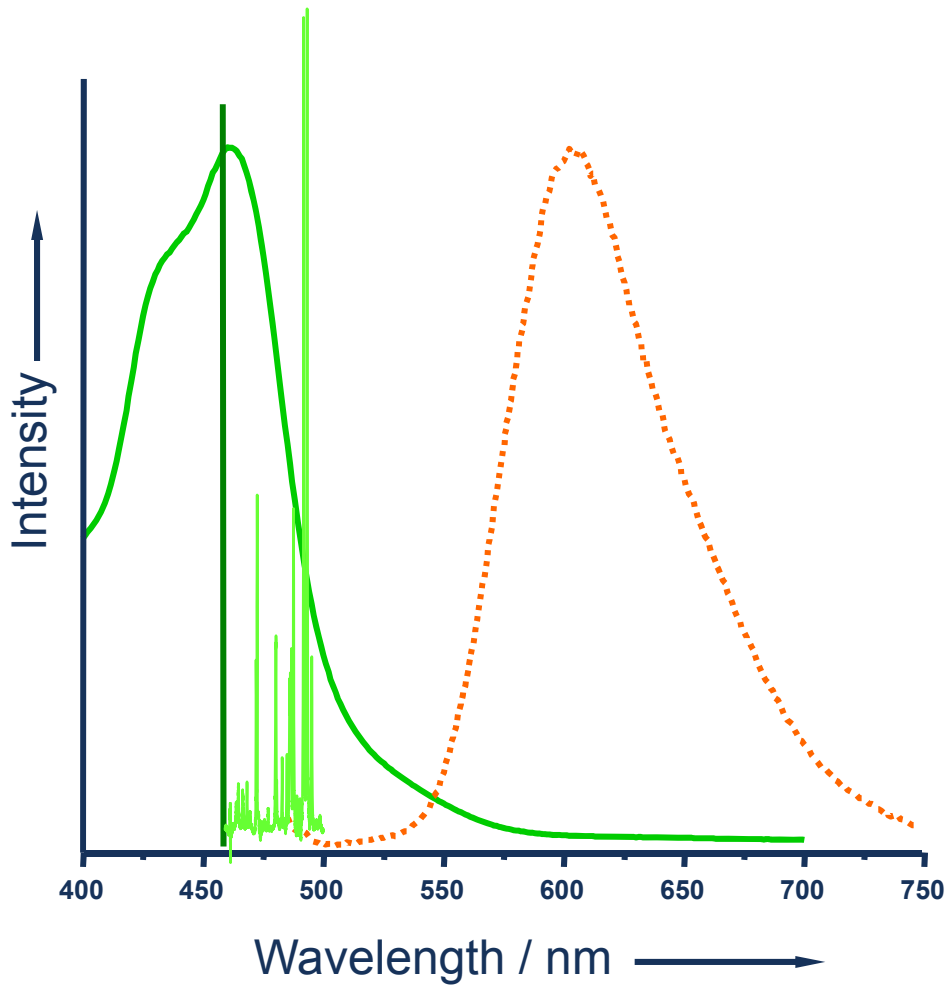
- High specificity
- High spatial resolution (< 1 µm)
- Minimal sample preparation
- All solvents can be applied (inclusive water)



Raman – a brief introduction

Resonance Raman spectroscopy





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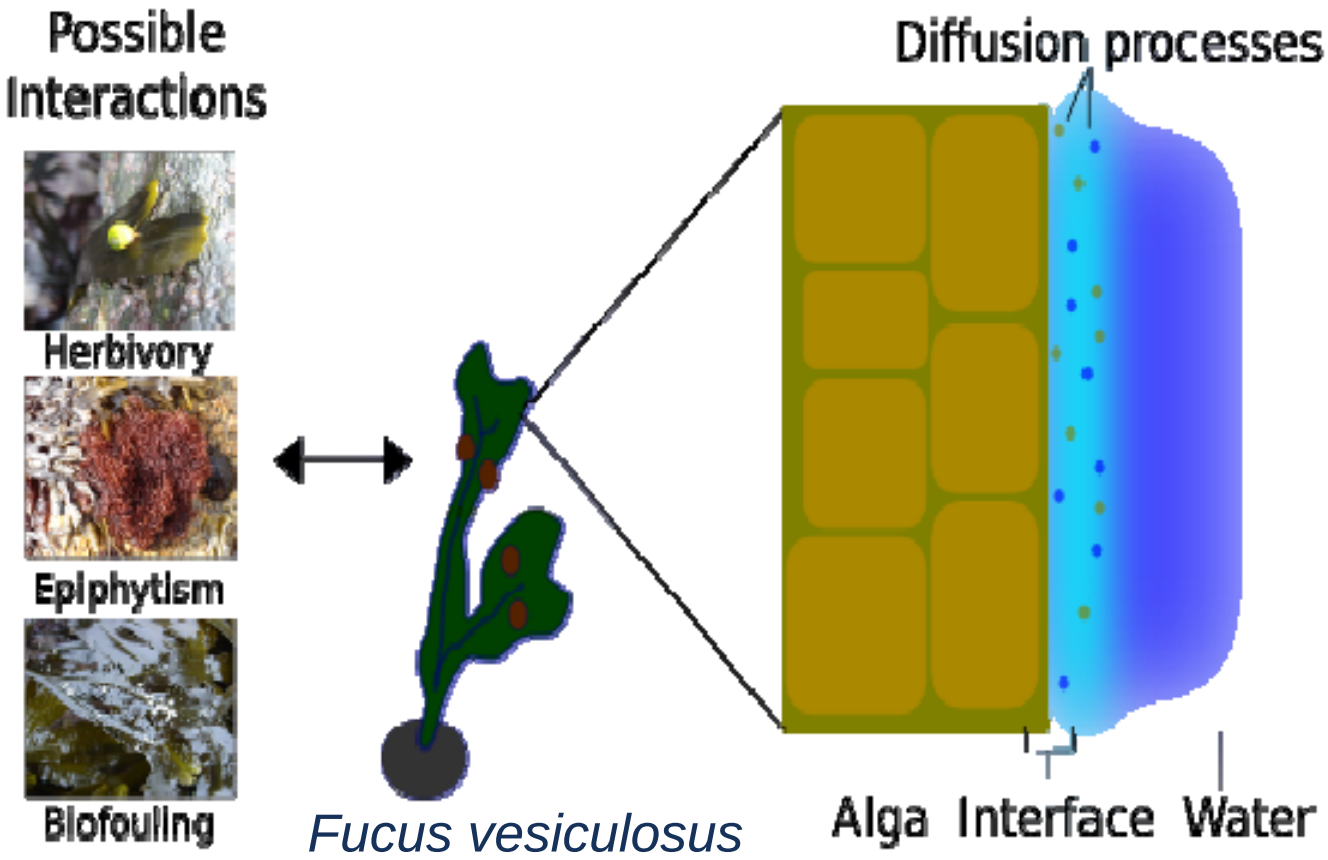
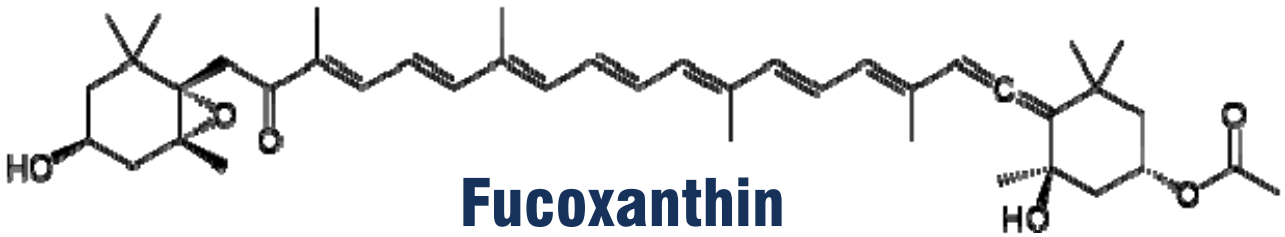
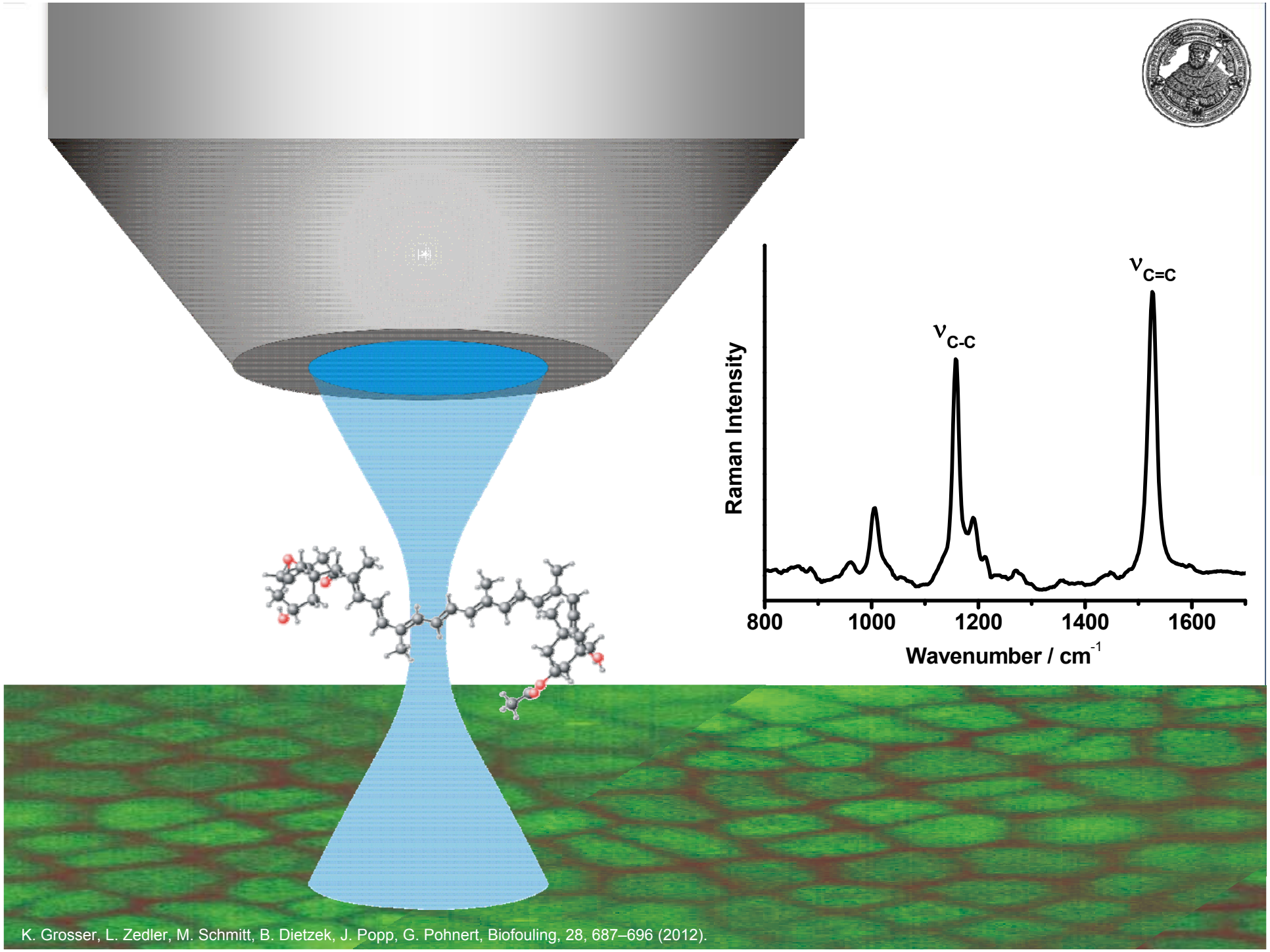
Resonance Raman microscopy: determination of metabolites around algal surfaces





K. Grosser, L. Zedler, M. Schmitt, B. Dietzek, J. Popp, G. Pohnert, Biofouling, 28, 687–696 (2012).



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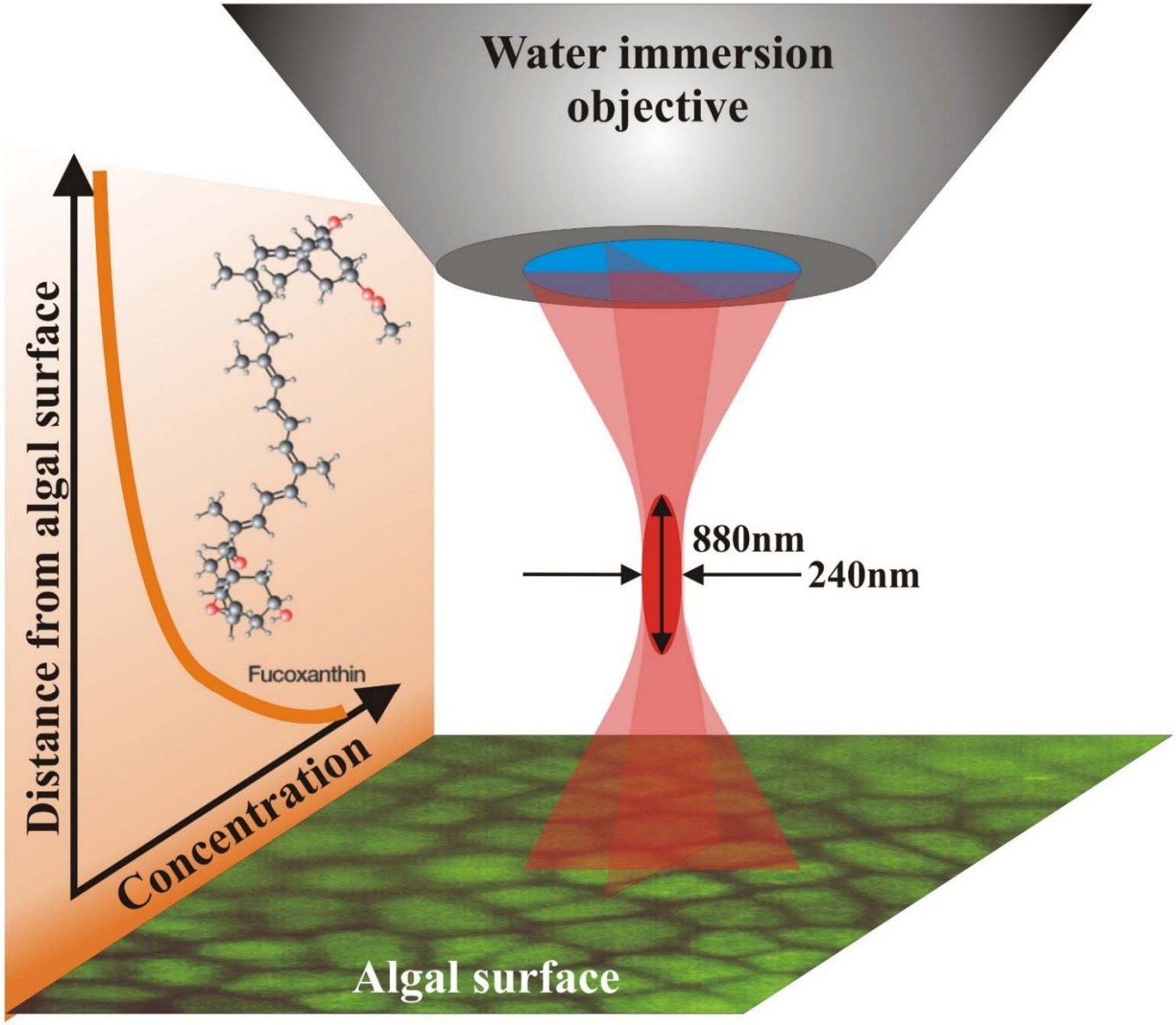
Resonance Raman microscopy: determination of metabolit gradients around algal surfaces



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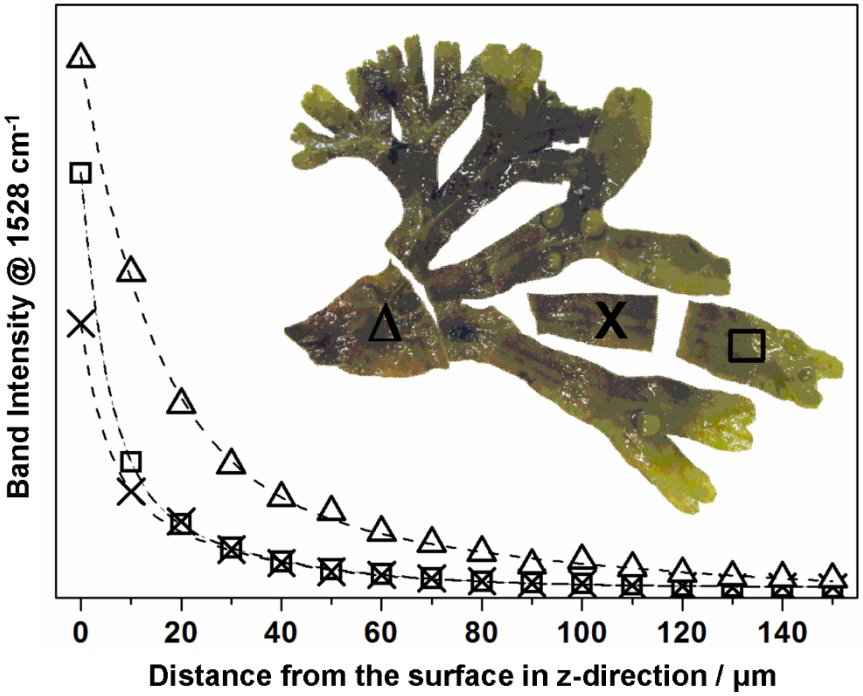
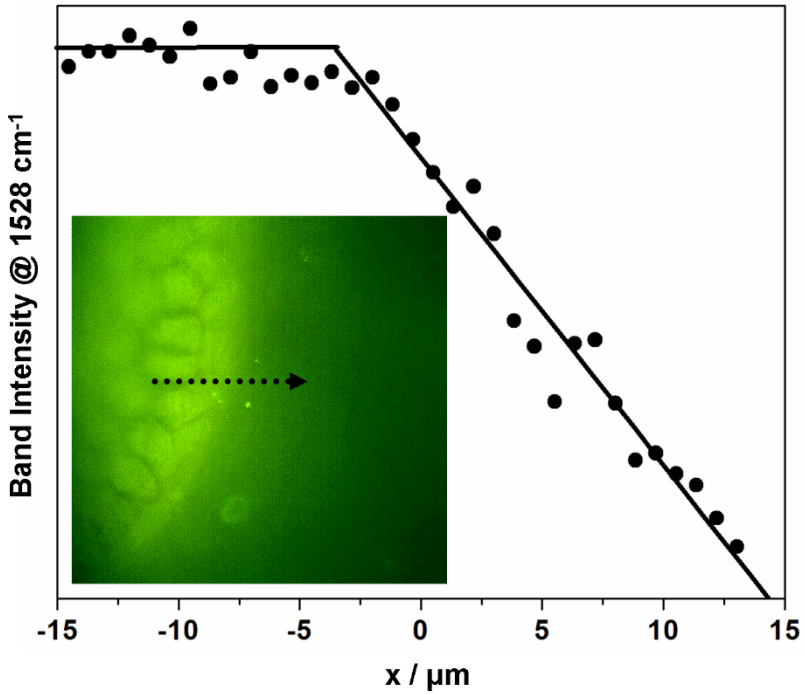


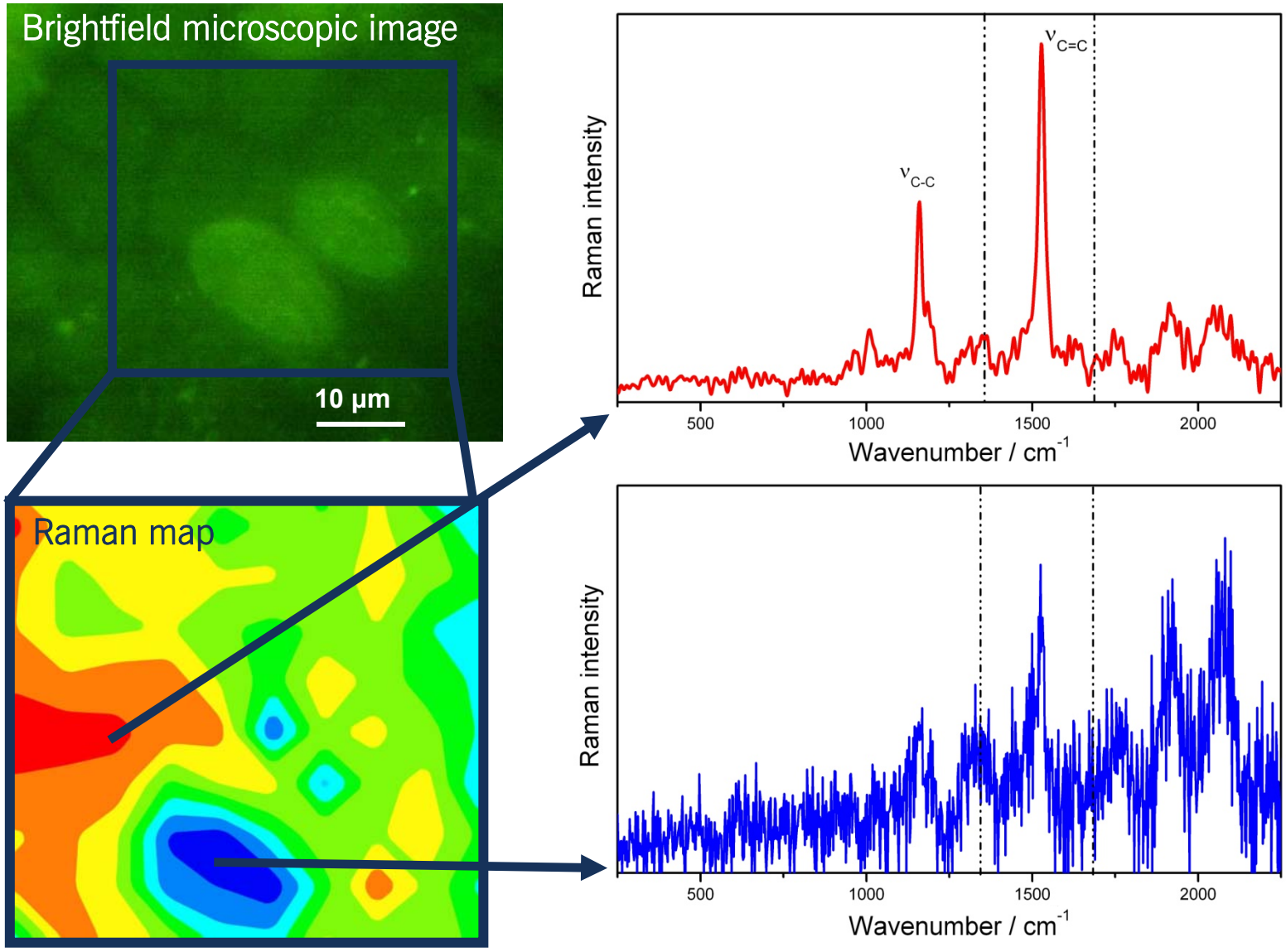


- Decay of the signal intensities for different parts of the algae
  - Gradient of metabolite fucoxanthin
- Results prove the active release of the unpolar metabolite fucoxanthin

- Lateral fucoxanthin distribution
- constant band intensity across the algal surface
- Sudden decay of the band intensity when the algal border was reached

K. Grosser, L. Zedler, M. Schmitt, B. Dietzek, J. Popp, G. Pohnert, Biofouling, **28**, 687-696 (2012)

Resonance Raman microscopy: determination of metabolites on algal surfaces

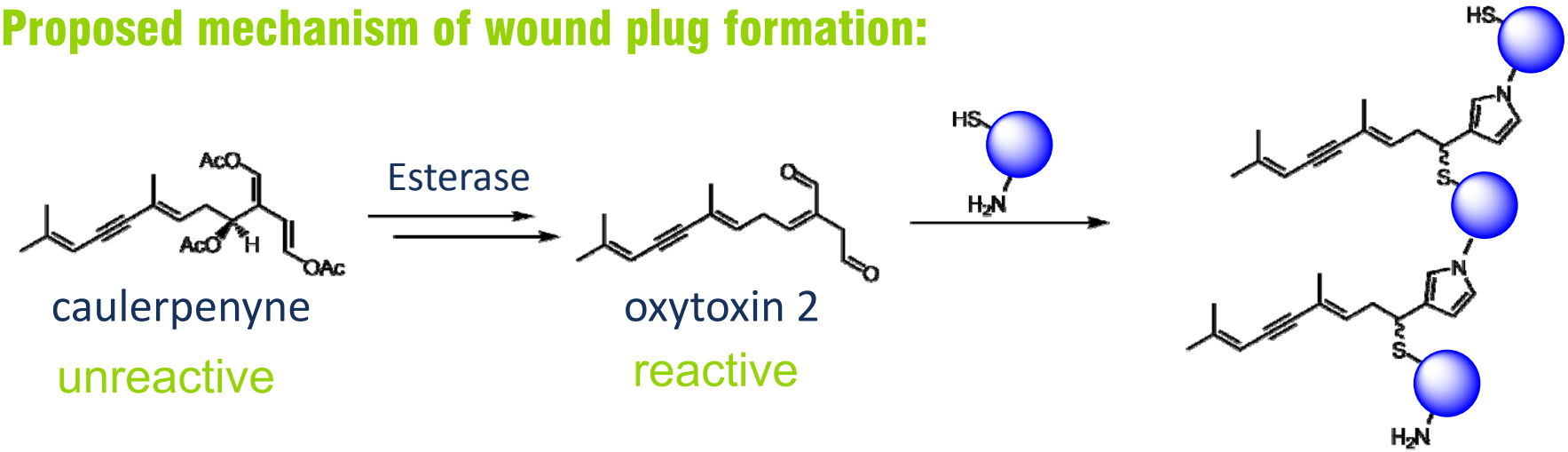


- Carotenoid content on the surface higher than on the associated diatoms

K. Grosser, L. Zedler, M. Schmitt, B. Dietzek, J. Popp, G. Pohnert, Biofouling, **28**, 687-696 (2012)

Raman microscopy: determination of chemical gradients within the wound plug of Caulerpa taxifolia

Proposed mechanism of wound plug formation:



caulerpenyne

unreactive

oxytoxin 2

reactive

- Tissue disruption → Transformation of caulerpenyne to the 1,4-dialdehyde Oxytoxin
- Oxytoxin acts as an efficient protein cross linker →fast closure of the wound
- Investigation of the caulerpenyne distribution within the algal cell by means of FT-Raman spectroscopy

S. Adolph, V. Jung, J. Rattke, G. Pohnert, *Angewandte Chemie-International Edition* **2005**, *44*, 2806.
Schröder H,C. et al., Environ Toxicol Phar, 5: 119-126 (1998)

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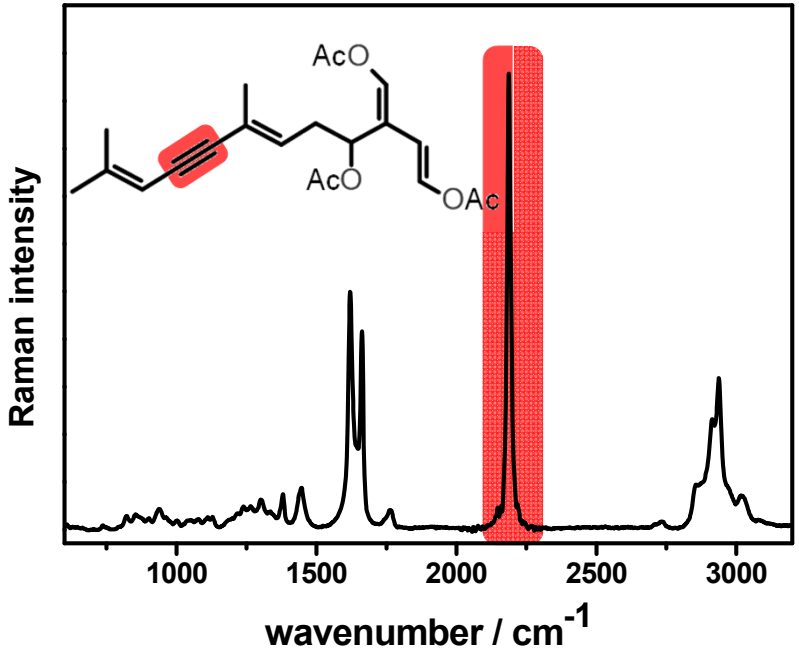
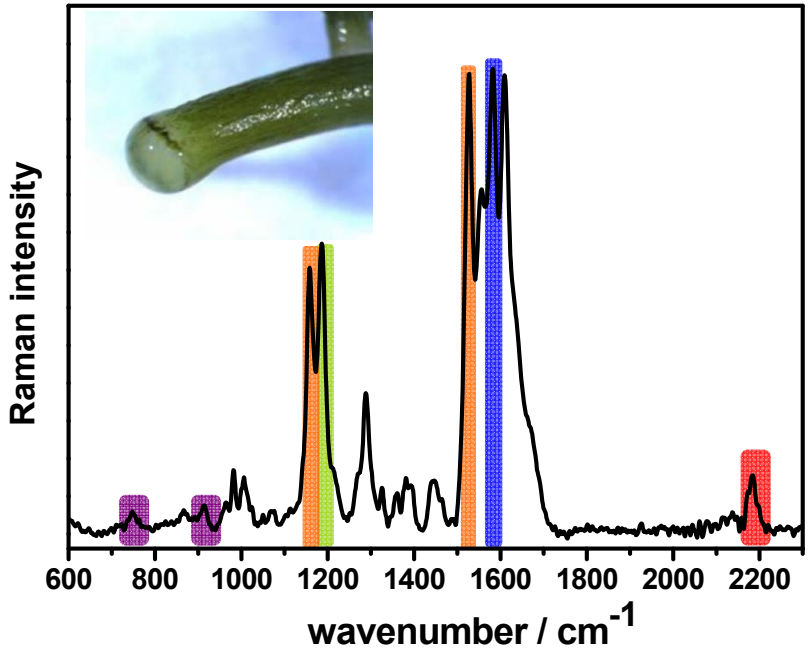
FT-Raman spectrum of Caulerpenyne



- Main secondary metabolite
- Triple bond
  - → rare in biology
  - → strong Raman activity

FT-Raman spectrum of wound plug



Caulerpenyne: $\nu_{C\equiv C}$ (~2185 cm^{-1})

Wound plug: ~1582 cm^{-1}

Sulfated polysacch: $\nu(SO_2)$ (~1188 cm^{-1})

Carbohydrates: 747 cm^{-1} + 916 cm^{-1}

β-Carotene: 1158 cm^{-1} + 1528 cm^{-1}

I. Weissflog, K. Grosser, M. Bräutigam, B. Dietzek, G. Pohnert, J. Popp, ChemBioChem, 14, 727 – 732 (2013).

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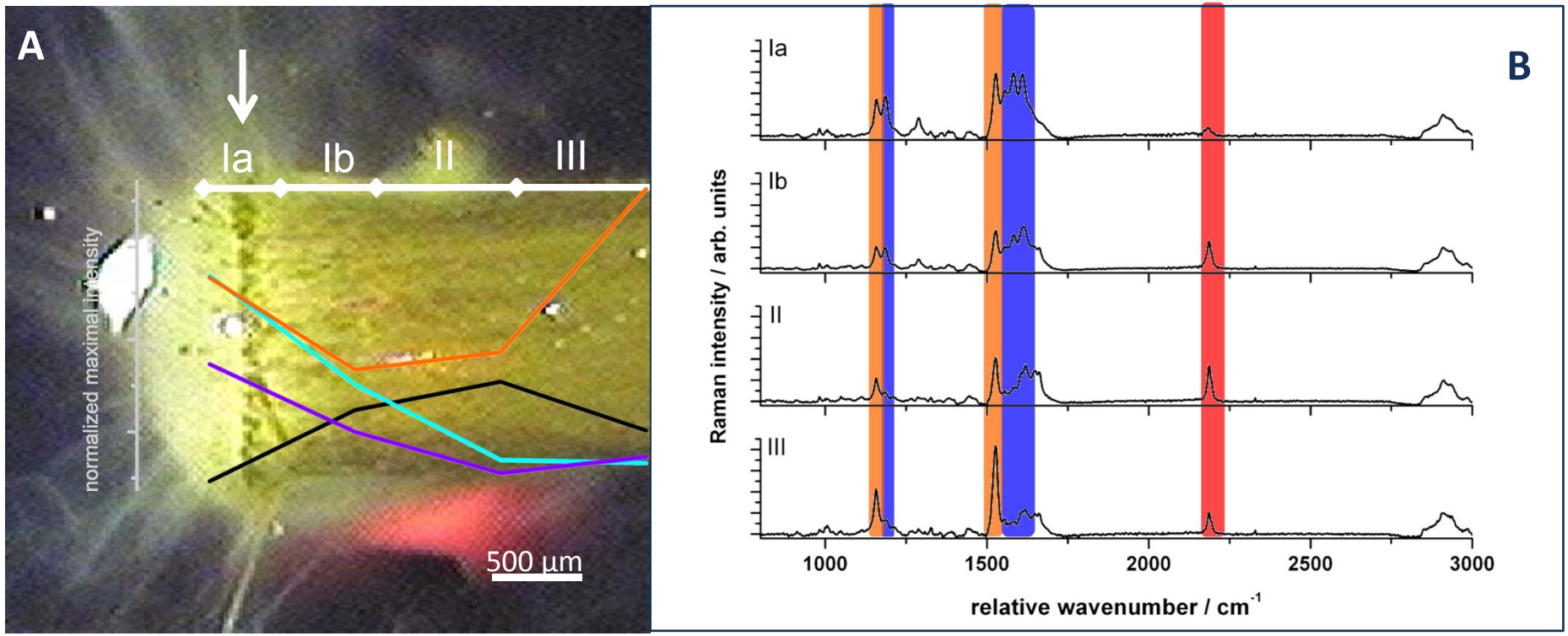

Zone Ia, Ib (wound plug):	spectra are dominated by amide bands and bands of the cross linked protein (metabolic product of caulerpenyne)
Zone II (area of retreat):	most intensive signals of caulerpenyne and β-carotene, less intense Raman signals of the wound plug components
Zone III (intact tissue):	strong contributions of β-carotene, decreased signal intensity of caulerpenyne

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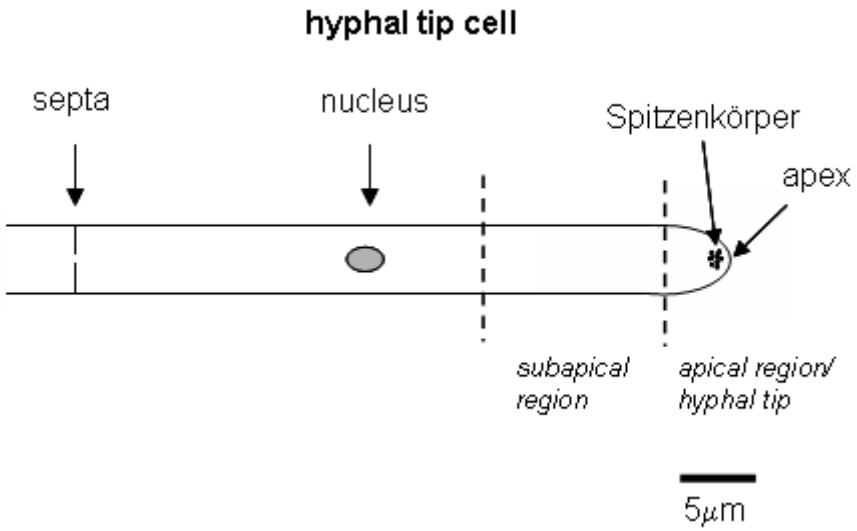
Raman microscopy: cytochrome localization in hyphal tip cells

Scheme of fungal hypha tip cell

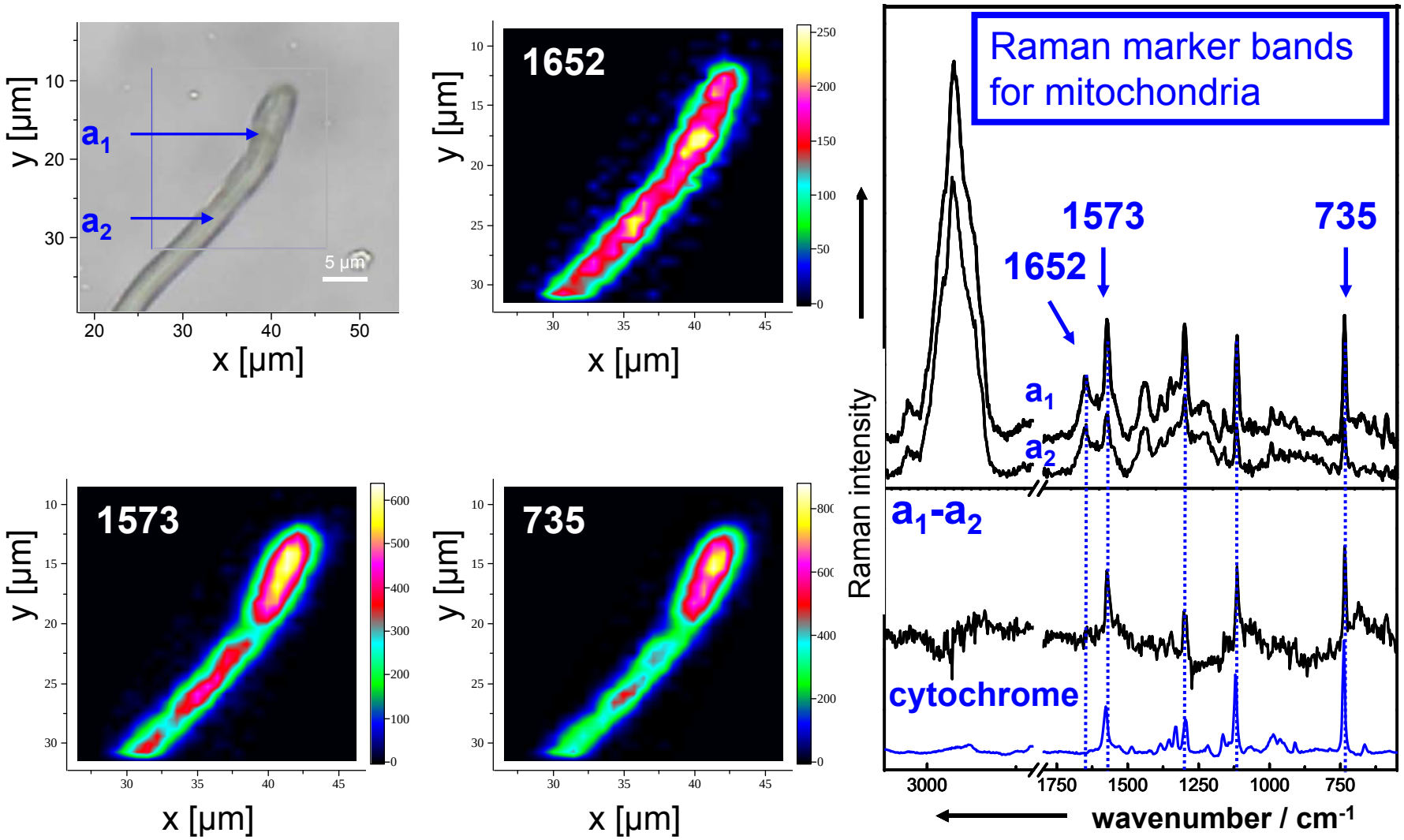


Fruit bodies of *Schizophyllum commune* on wood.

S. Erdmann, 2008-Feb

Raman microscopy: cytochrome localization in hyphal tip cells

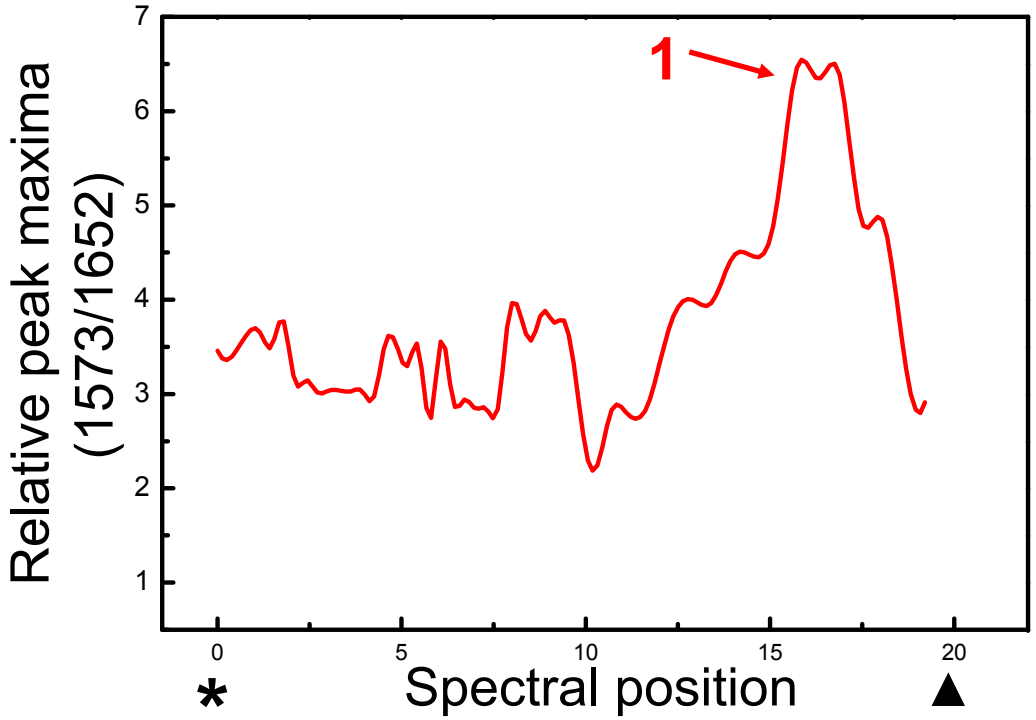
Schizophyllum commune



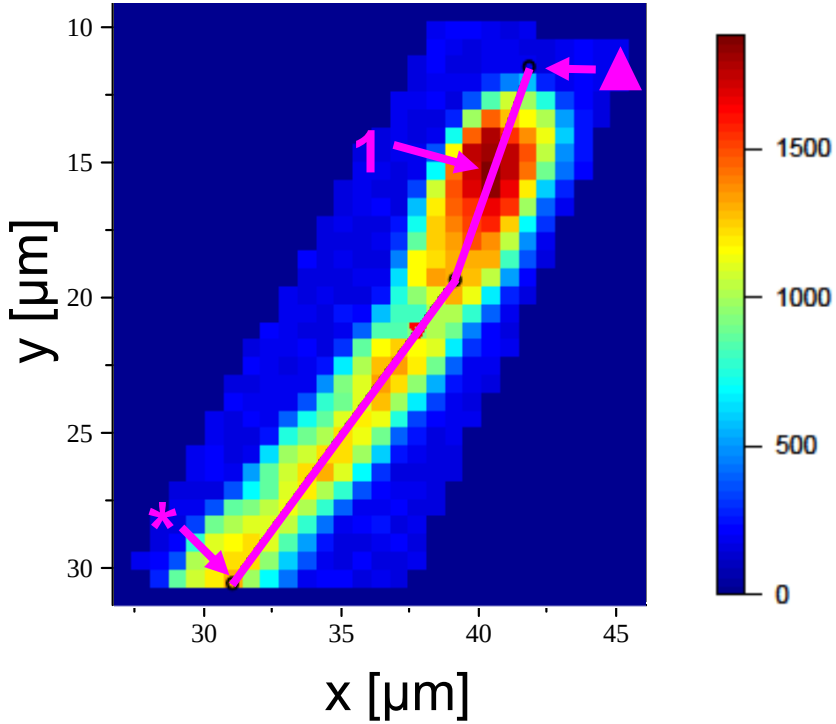
A. Walter, S. Erdmann, T. Bocklitz, E.-M. Jung, N. Vogler, D. Akimov, B. Dietzek, P. Rösch, E. Kothe, J. Popp, Analyst, 135, 908–917 (2010).

Raman microscopy: cytochrome localization in hyphal tip cells

Schizophyllum commune: cytochrome content



Hyphae tip



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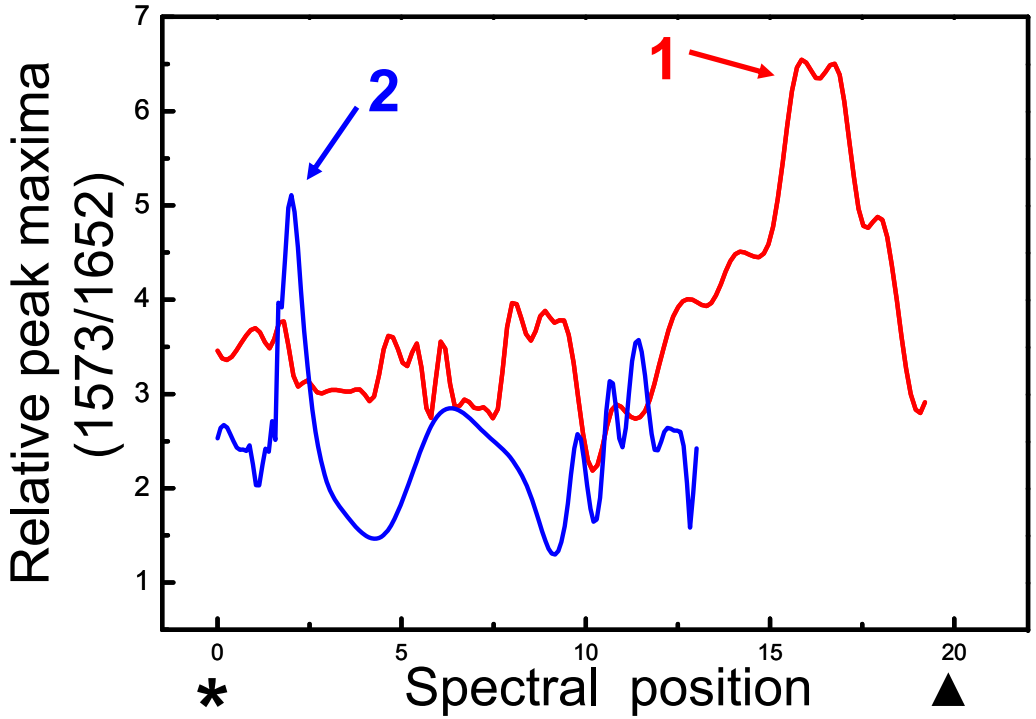

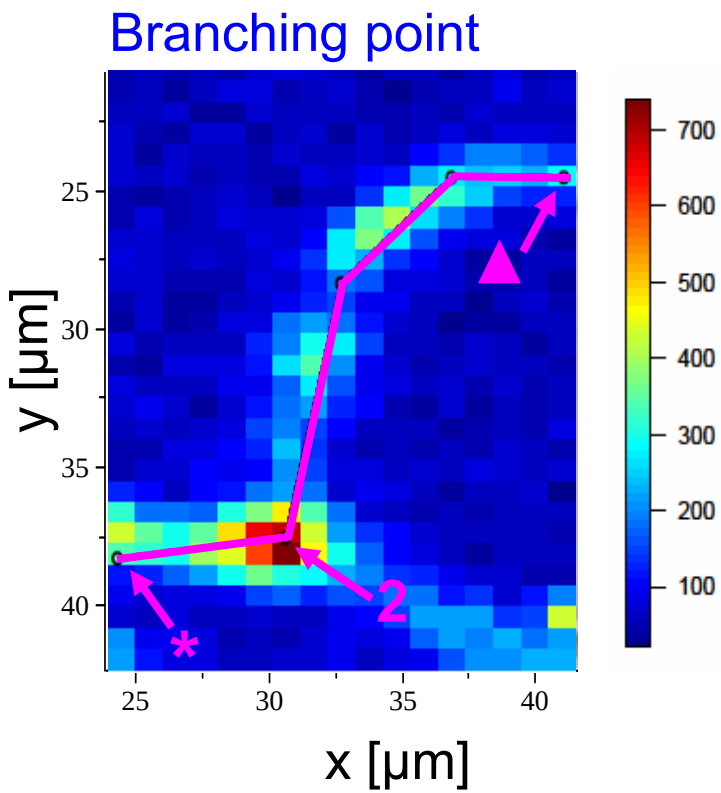

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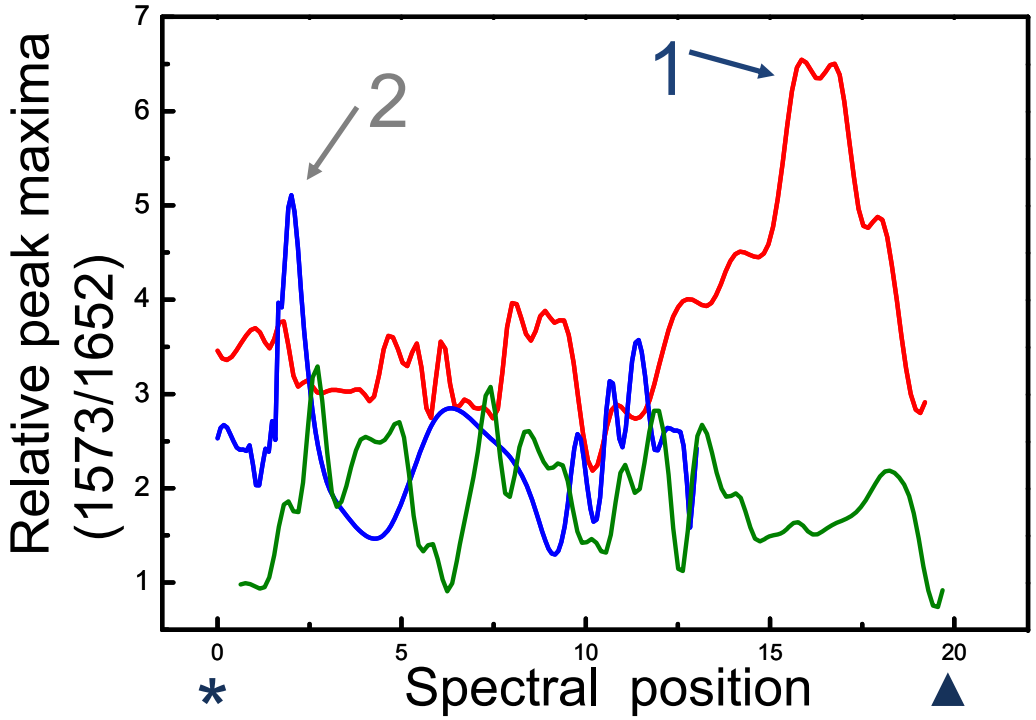
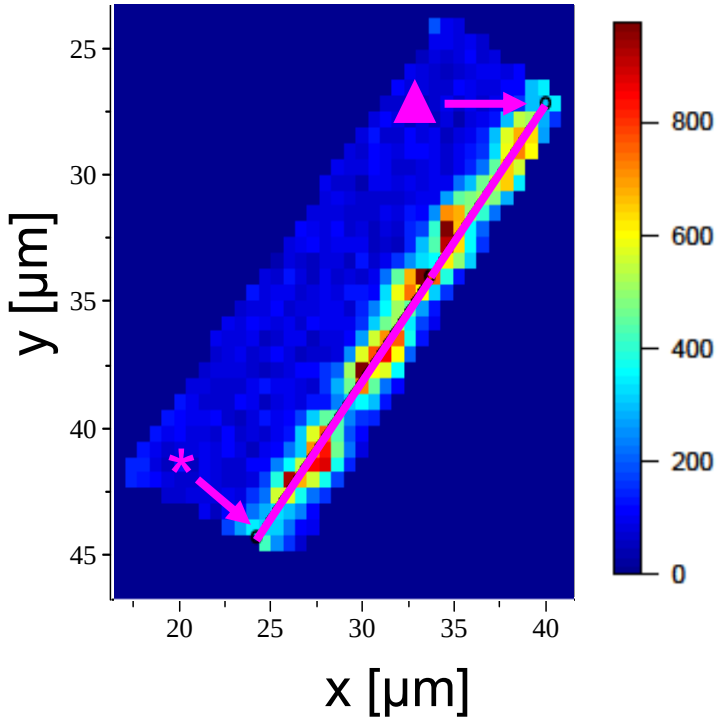


Central hyphae



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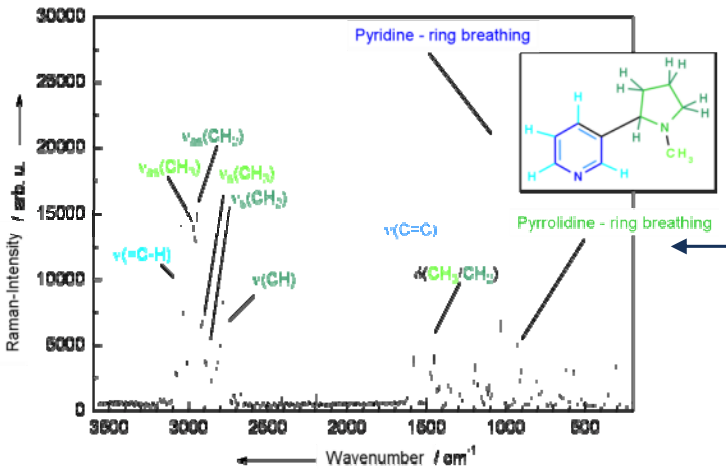
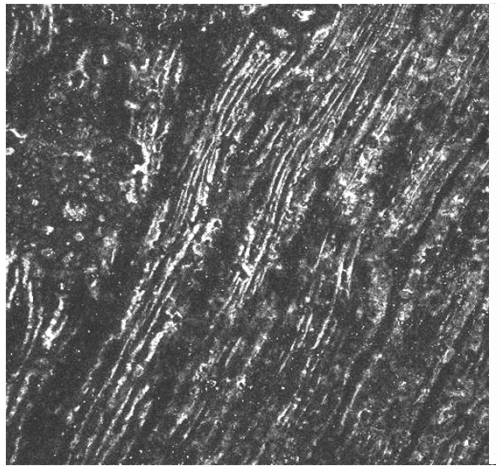
Raman – a brief introduction

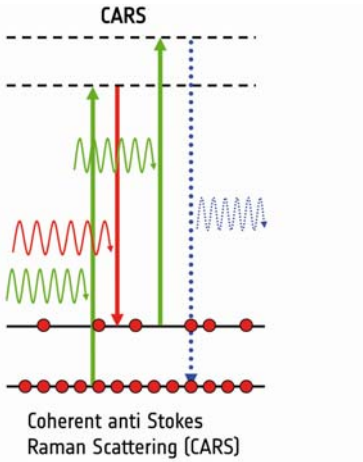
Label free chemical imaging – CARS

Raman spectroscopy



CARS microscopy





Complete spectral fingerprint of sample

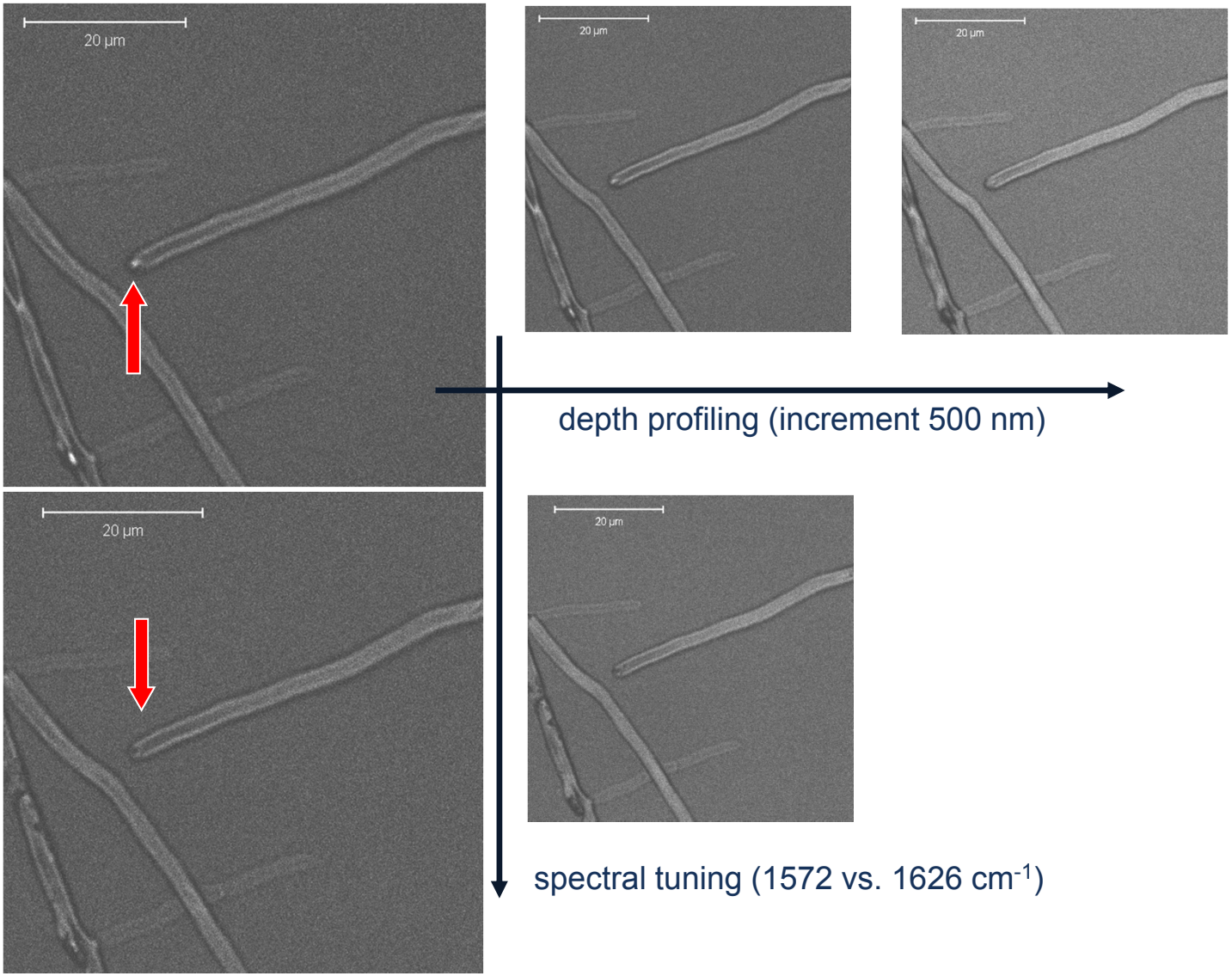
Fast imaging of large areas using preselcted spectral marker bands

CARS-microscopy allows to record chemical images online with method-intrinsic confocality

- ⇨ *single-band*-CARS images represent univariant results
- ⇨ CARS images represent part of the entire microspectrocopic information

Raman microscopy: cytochrome localization in hyphal tip cells

CARS microscopy: towards online localization of



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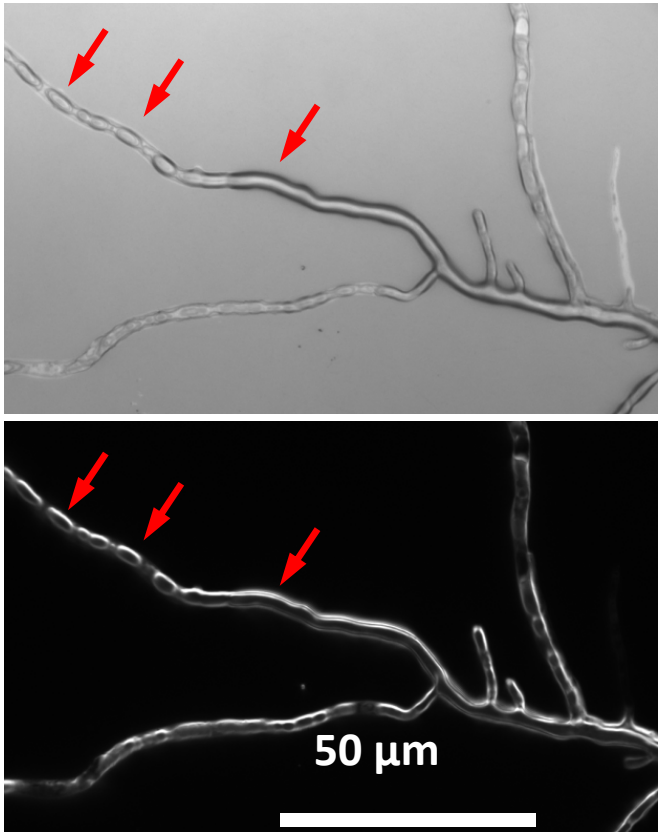
Raman microscopy: characterization of the oil composition in single intact hyphae

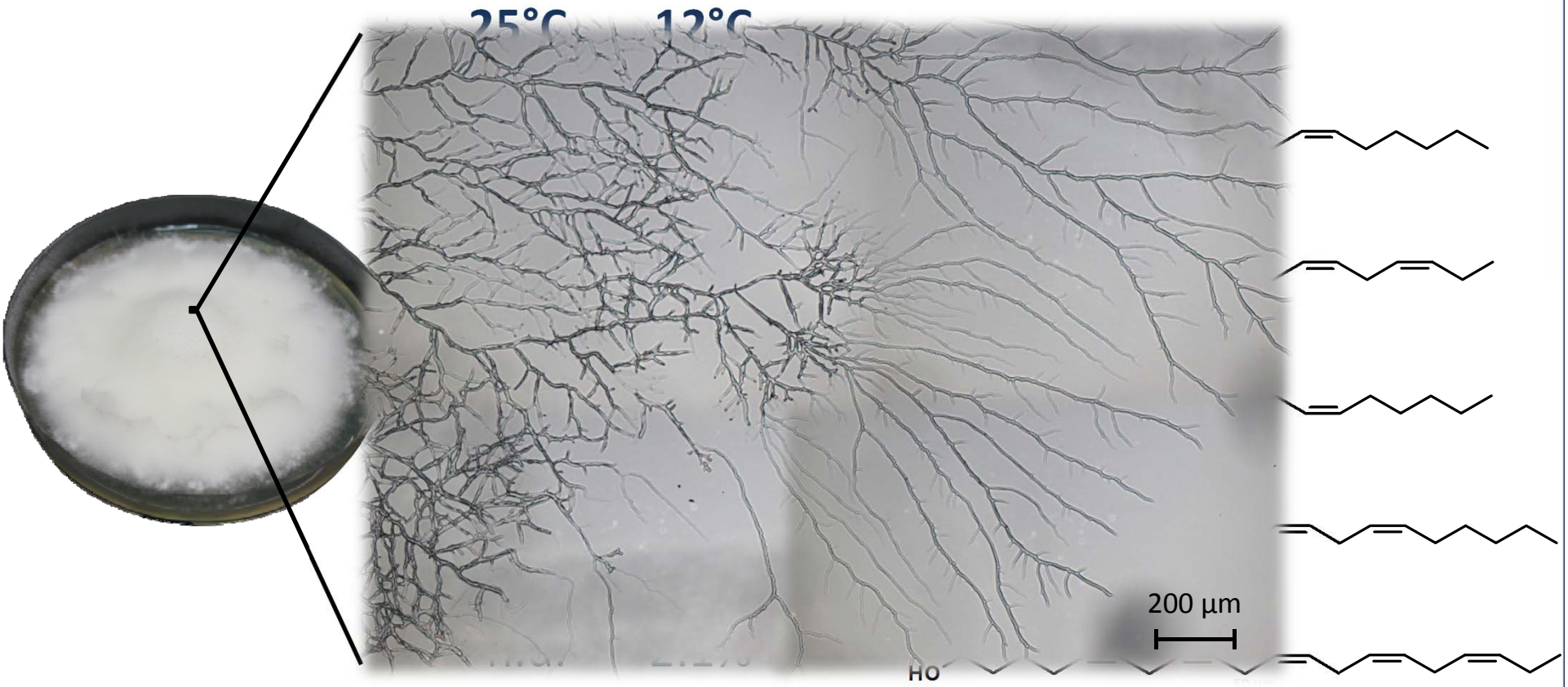
The oleaginous fungi *Mortierella*



U. Münchberg, L. Wagner, E. T. Spielberg, K. Voigt, P. Rösch, J. Popp, Biochimica et Biophysica Acta 1831 341–349 (2013).

Raman microscopy: characterization of the oil composition in single intact hyphae

Poly unsaturated fatty acids in *Mortierella* lipids



data for *M. alpina*, from Jang, **2005**, *Biores. Techn.*, pp. 1633

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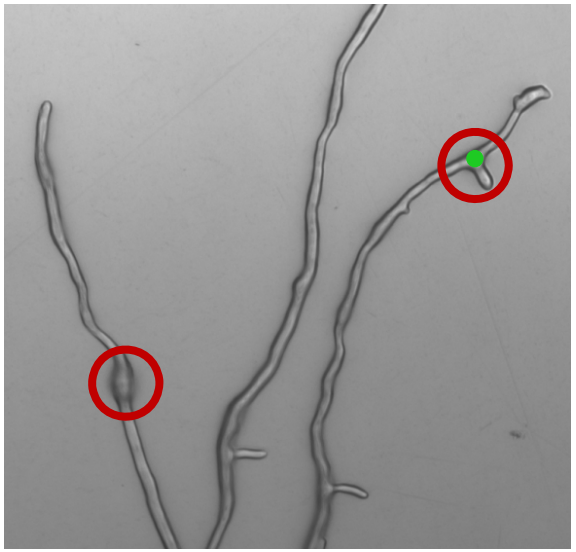

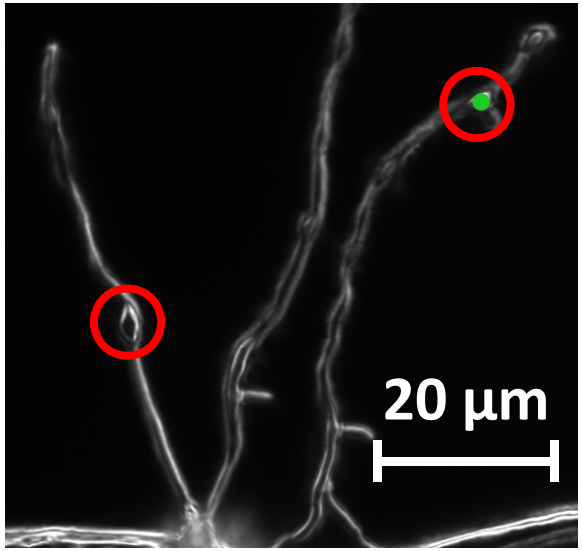

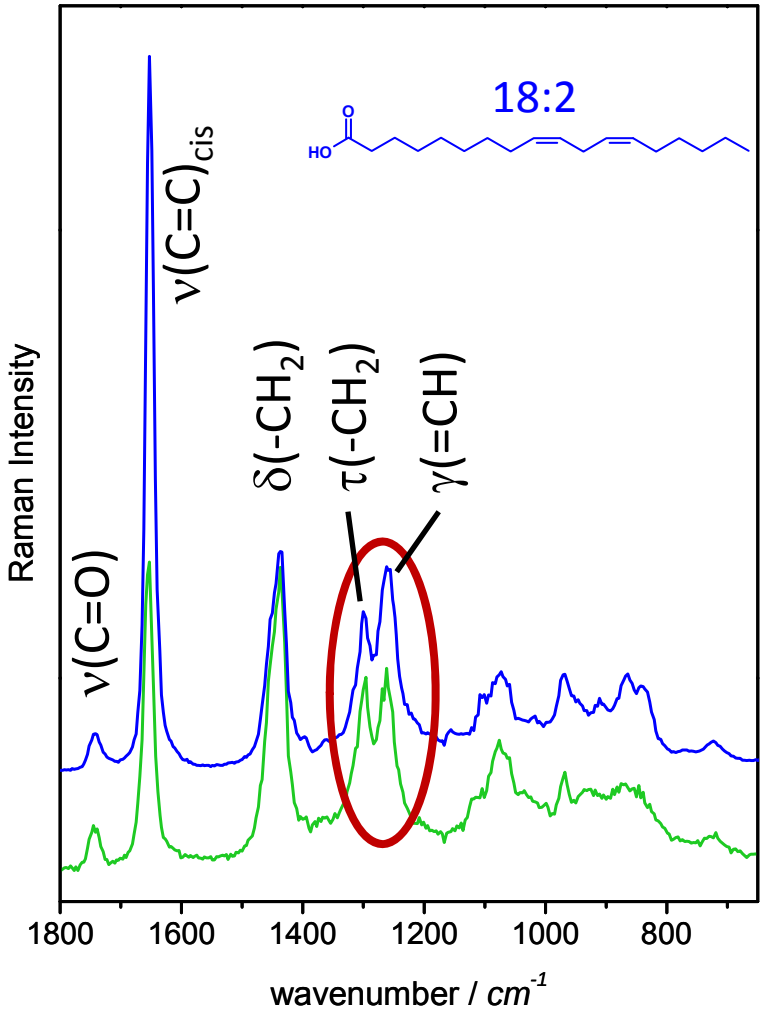

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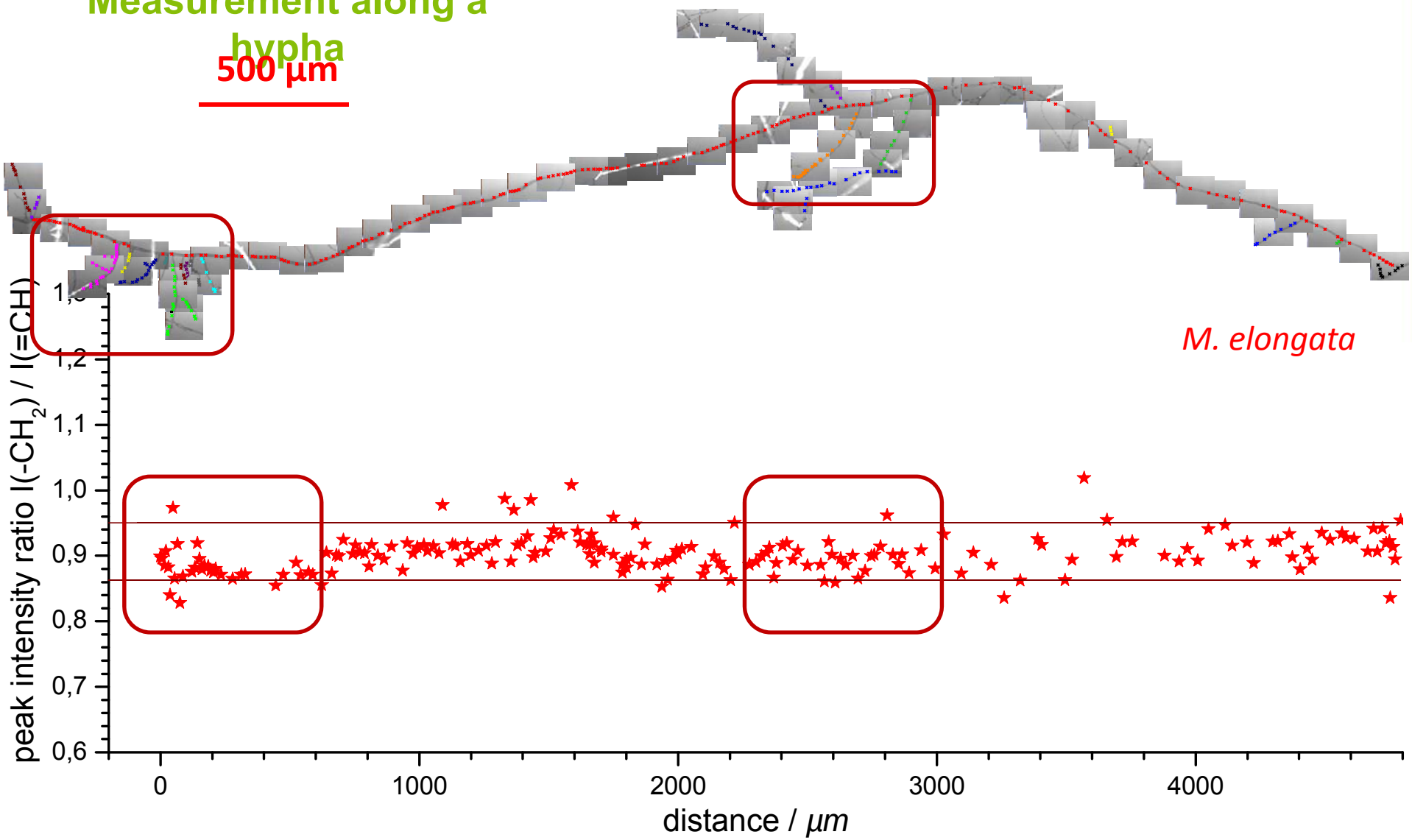

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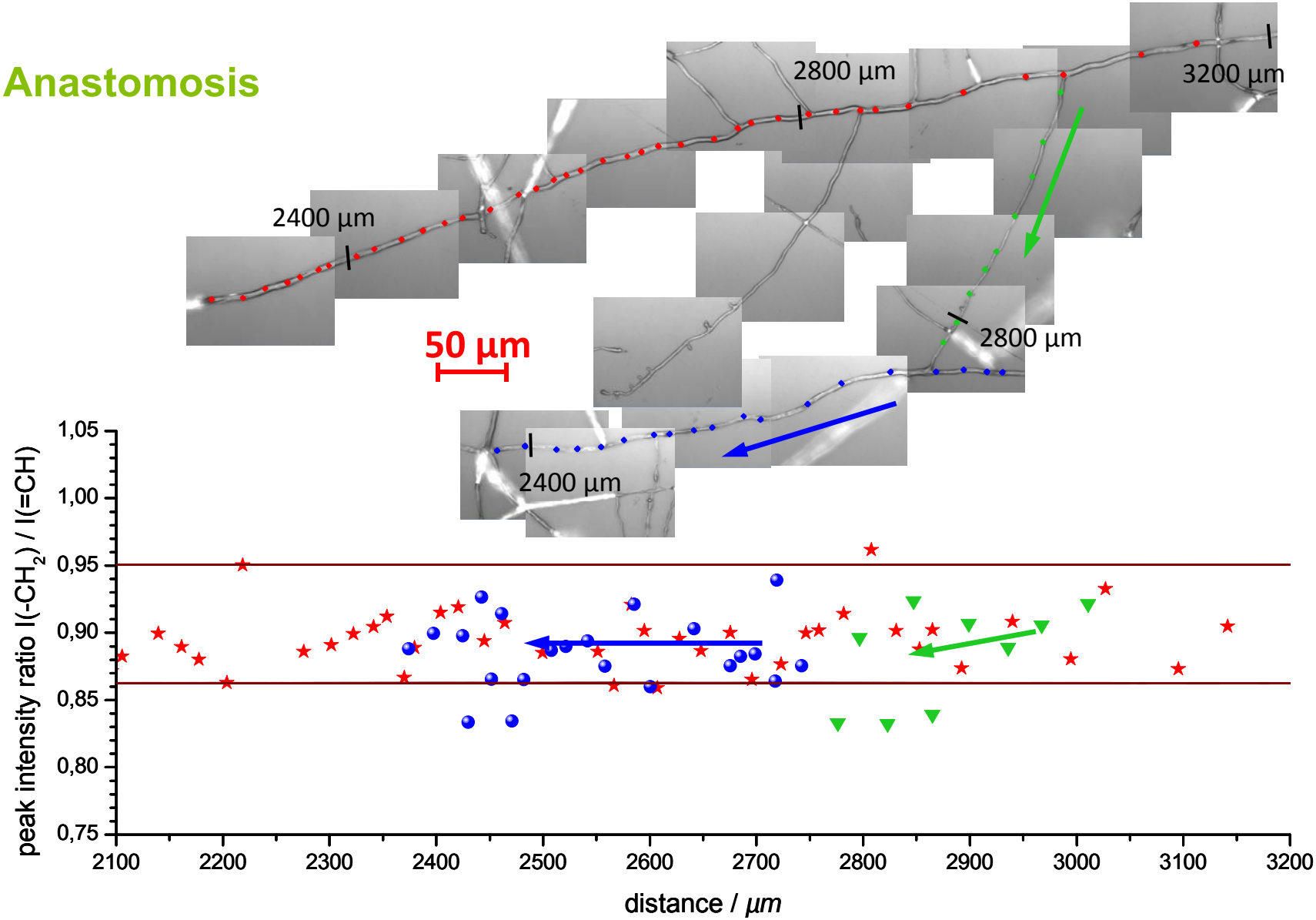
Anastomosis



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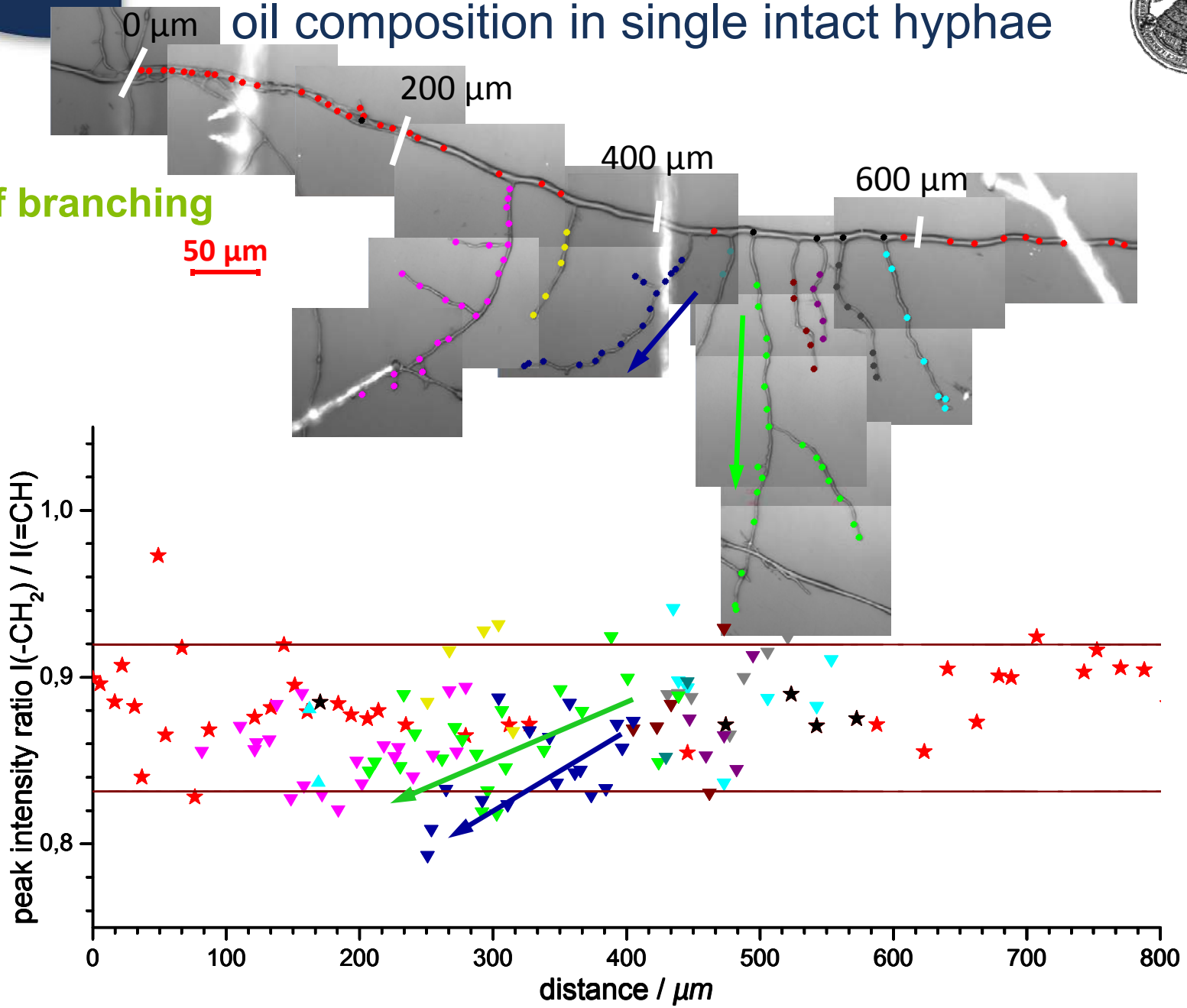
Raman microscopy: characterization of the oil composition in single intact hyphae

Effect of branching



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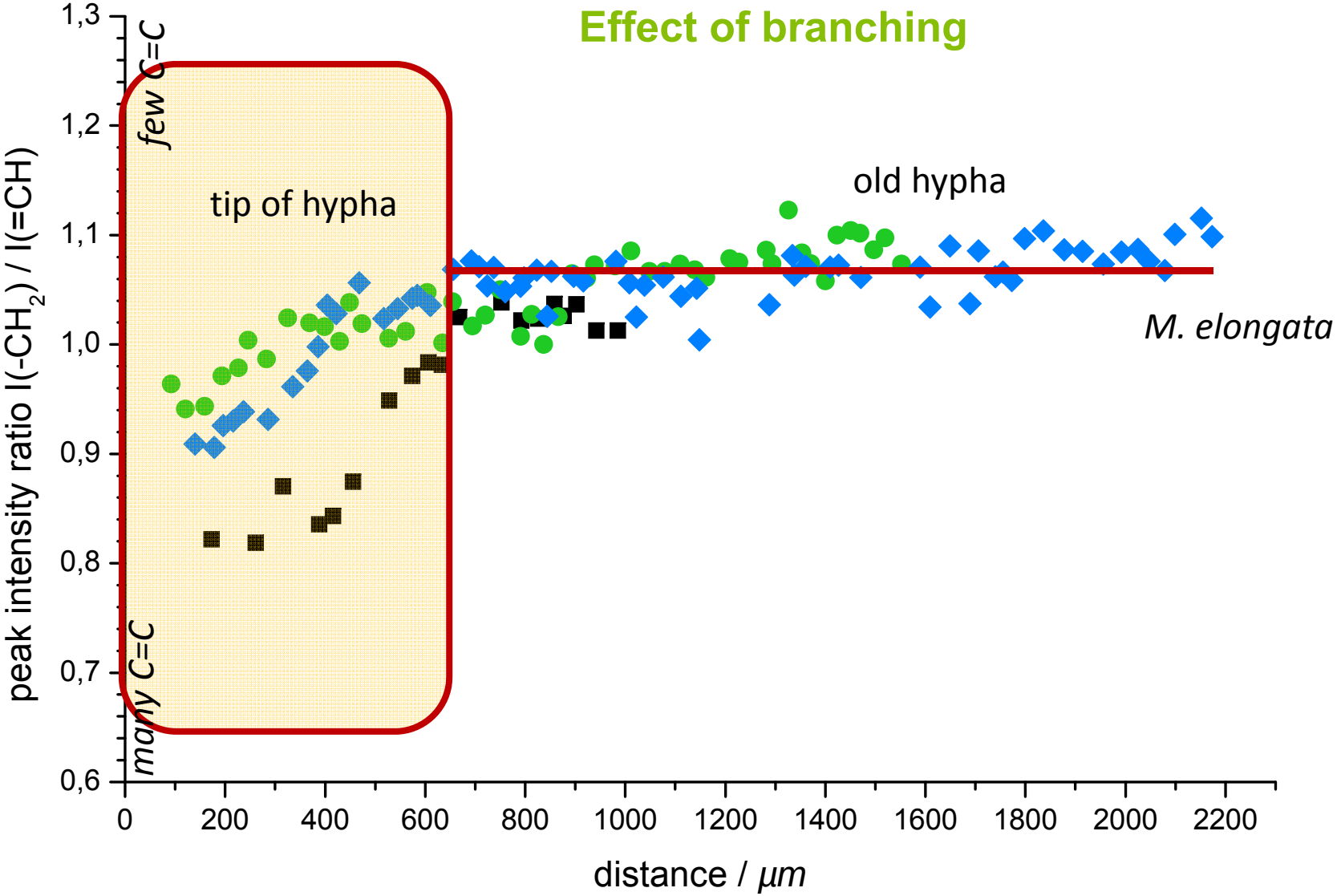

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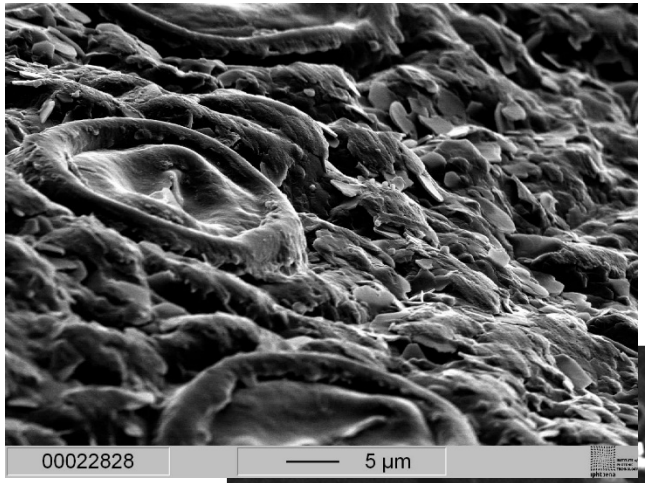
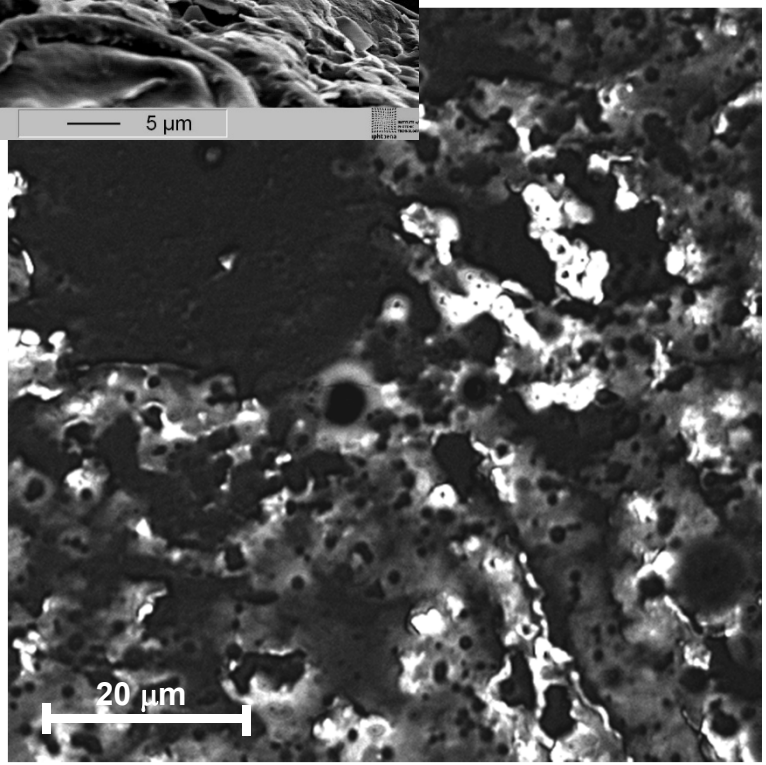
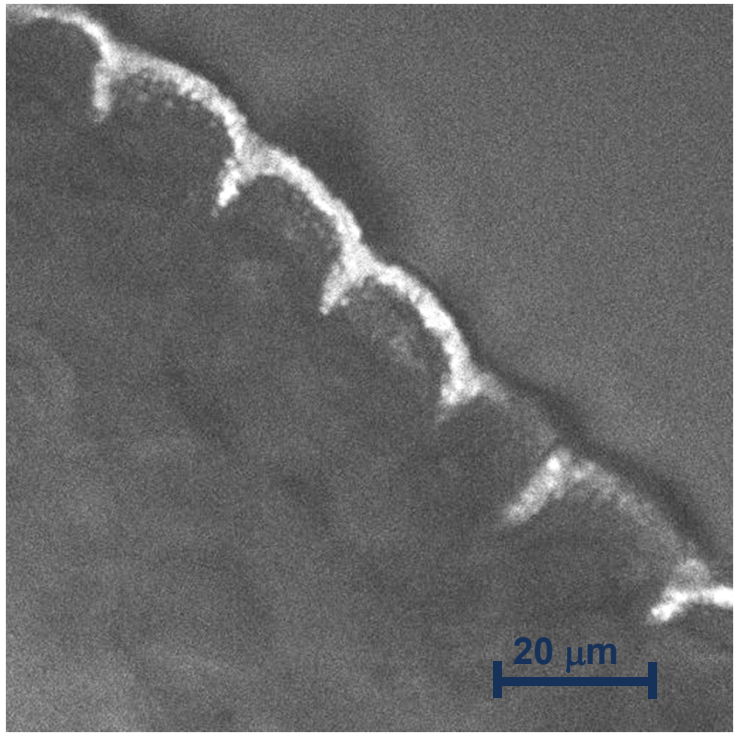
CARS microscopy: spatial distribution of epicuticular waxes

Cuticular waxes - Prunus Laurocerasus



SEM upper leaf surface



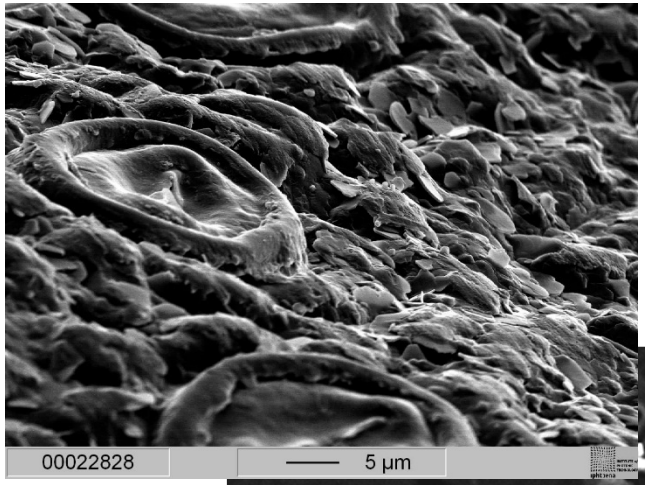


Lower side of a *Prunus laurocerasus Leaf*. Image recorded at 2846 cm^{-1}.

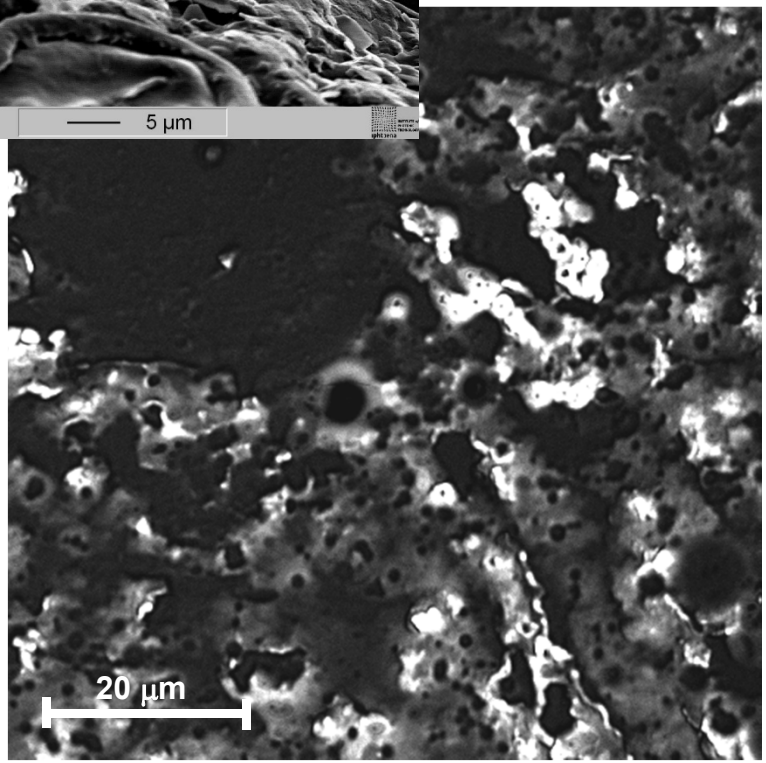
I. Weissflog, N. Vogler, D. Akimov, A. Dellith, D. Schachtschabel, A. Svatos, W. Boland, B. Dietzek, J. Popp, Plant Physiology, 154, 604–610 (2010).

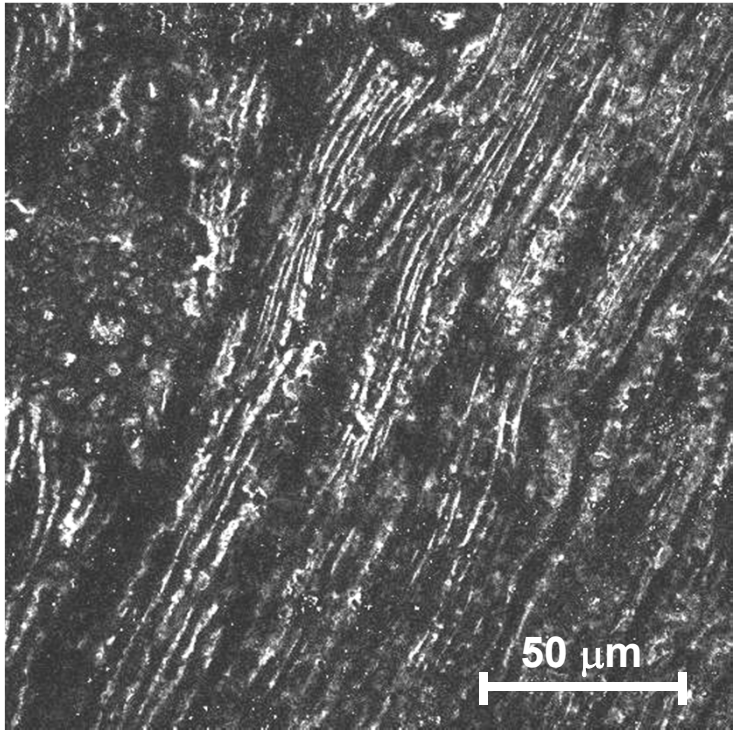
CARS microscopy: spatial distribution of epicuticular waxes

Cuticular waxes - Prunus Laurocerasus



SEM upper leaf surface





main leaf vein imaged at 2862 cm^{-1}

I. Weissflog, N. Vogler, D. Akimov, A. Dellith, D. Schachtschabel, A. Svatos, W. Boland, B. Dietzek, J. Popp, Plant Physiology, 154, 604–610 (2010).

Conclusion

- Linear and non-linear Raman imaging approaches are powerful bioanalytical approaches:
  - non-invasive surface and tissue studies
  - high spatial resolution
  - direct visualization of metabolite distribution at concentrations in the µM range
  - Employing the joint strengths of Raman (multivariate results) and CARS (univariate results, fast, confocal) enables insight not only into tissue composition but also into the dynamics of processes.

Thanks!

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