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High resolution confocal Raman imaging of a *Steinernema kraussei* nematode

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Introduction

- Nematodes are key model organisms for understanding the functions of genes and proteins, and human diseases, such as HIV and Alzheimer's Disease
- Genotype-phenotype investigation in genetically modified nematodes has provided important information on gene and protein functions
- Current biological assays mostly rely on using stains, dyes or labels, and/or invasive techniques
- For the first time, Raman spectroscopic imaging has been explored as a tool for analysing a nematode, non-invasively and without labelling
- inVia's StreamLine imaging reveals chemico-structural information of the imaged area at high spatial resolution
- The resultant images provide a powerful and visual method for assessing phenotypic changes in nematodes at the sub-micrometre scale

Experimental

S kraussei nematodes were washed, deposited on a quartz slide, and air-dried. Raman measurements were carried out using an inVia Raman microscope (Renishaw plc, UK) coupled to a 532 nm laser excitation source, at high resolution (diffraction limit). The sample was immersed in 0.9% NaCl solution, and a 50× (NA 0.75) water immersion microscope objective (Nikon, Japan) lowered into the liquid surface.

Complete nematode
- StreamLine Plus imaging
- Step size 1.3 µm
- ~ 84,800 spectra
- Collection time ~ 1 hr

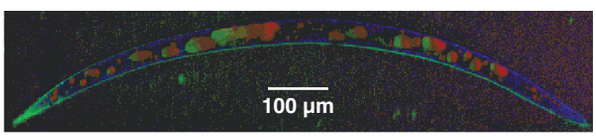
Partial worm sections
- StreamLineHR imaging
- Step size 0.5 µm
- Middle section ~ 41,500 spectra
- Collection time < 6 hrs

Data analysis

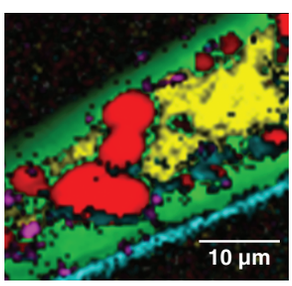
Principal component analysis (PCA) was applied to the data sets using WiRE™ 3 software (Renishaw, UK) and Matlab® (MathWorks, MA, USA). PCs were assigned to physiological domains based on the PC loadings. Raman images are presented based on Raman spectral assignment.

Results

Complete nematode



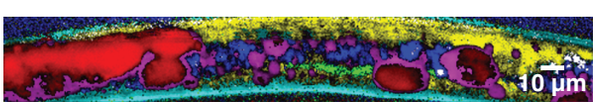
The three major components of a whole nematode; lipid rich domains (red), collagen and carotenoid rich domains (green), and protein rich domains (blue).



Tail

Pure lipid domains (red), muscular domains (yellow), outer cuticle (cyan), globins (purple), main protein and lipid worm body (green).

Middle section



PC	Assignment	Colour
1	Background	Black
2	Lipid rich (weak protein presence)	Magenta
3	Pure lipid	Red
4	Outer cuticle	Cyan
5	Unassigned	Blue
6	Muscular	Yellow
7	Collagen and carotenoids	White
8	Lysed cells, origin unassigned	Lime

Conclusions

This work presents the first example of resolving an unprecedented level of chemico-structural detail within a nematode, in a label-free and non-invasive manner.

The richness of information present in the Raman data enables a wide range of biochemical molecules to be investigated in a single sample. StreamLine Raman imaging has the potential to be a major analytical tool for assessing phenotypic changes in model organisms, and could play a key role in the search for cures for human diseases.