Fourier-Transform Infrared (FTIR) Microscopic Focal Plane Array (FPA) Imaging of Nematode Worms

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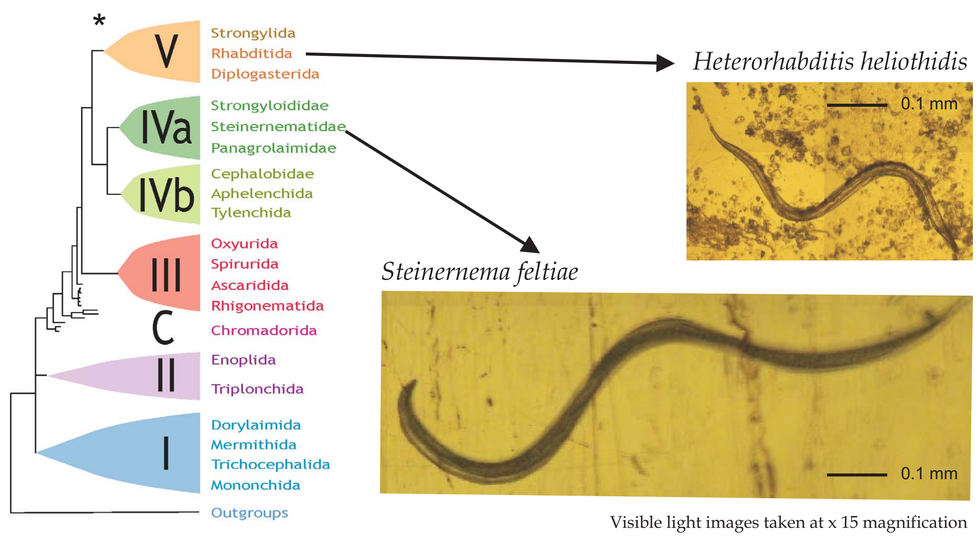
Nematode Worms

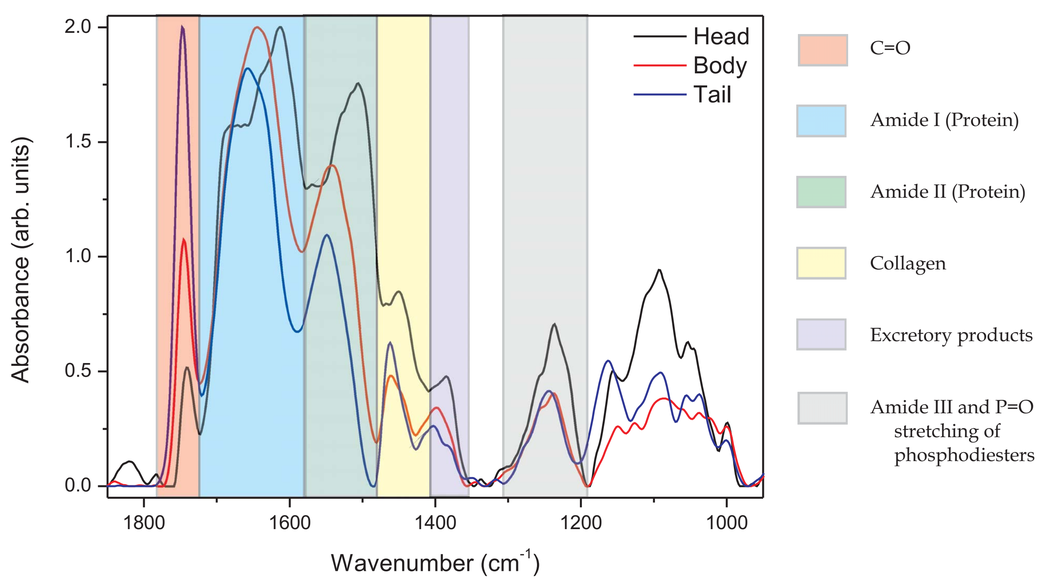
Nematode worms or 'roundworms' are found in all biotic environments on earth. More than 80,000 species have been identified with approximately 15,000 of these noted to be parasitic. Plant parasitic nematodes can be responsible for crop failures while animal parasitic nematodes can infect cattle. Human parasitic nematodes are responsible for many clinical conditions, such as *Brugia malayi* which causes elephantiasis. Other nematodes can be beneficial; predetory nematodes kill garden pests and can be used as a form of organic pest control. One nematode, *Caenorhabditis elegans*, is used extensively in molecular biology as a 'model organism' providing insight into a wide range of biological phenomena ranging from cell differentiation to nicotine dependence.



Visible light images taken at x 15 magnification

IR Spectroscopy of Micro-organisms

Infrared spectroscopy can provide insight into the biology of living cells and organisms. Such information can be useful both for the identification of species and for the monitoring of biological processes. The figure below shows three IR spectra obtained from different regions of an individual *Steinernema feltiae* nematode, showing the potential differences in spectral profile within an individual, as well as highlighting the main regions of biological interest.



Sample Preparation

Nematodes were purchased dried from biohelp GmbH (Austria). A small scoop of dried nematodes were rehydrated in distilled water and filtered using a NanoSep filtration device (Pall Corporation, USA) to remove any contamination from the packaging process. The nematodes were then resuspended in distilled water, spotted onto ZnSe plates and allowed to air dry. IR measurements were taken immediately after drying.

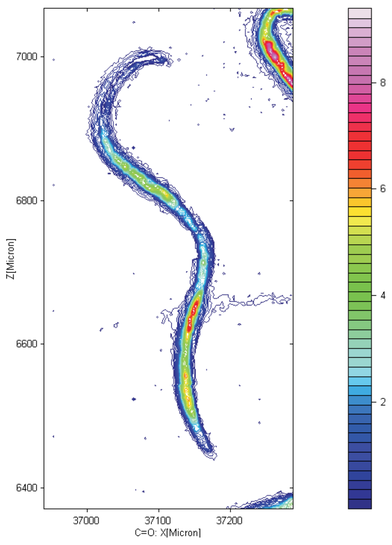
All IR measurements were taken using a Bruker Hyperion 3000 imaging microscope operating in transmission mode with a resolution of 4 cm^{-1}. Single point spectra were collected using 32 scans while the FPA measurements were collected for 256 scans.

Steinernema feltiae

Visible Light Image

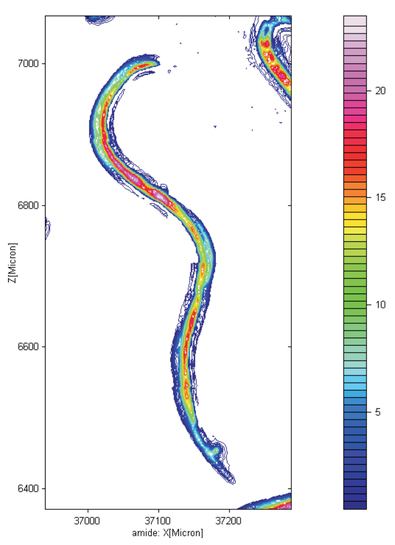
Visible light image taken at x 15 magnification.

Lipids C=O



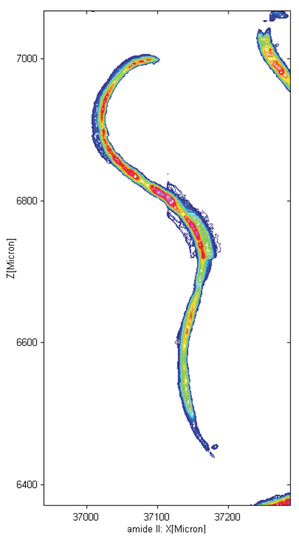
The lipid content of the nematode is concentrated along the centre of the organism, especially towards the tail.

Amide I



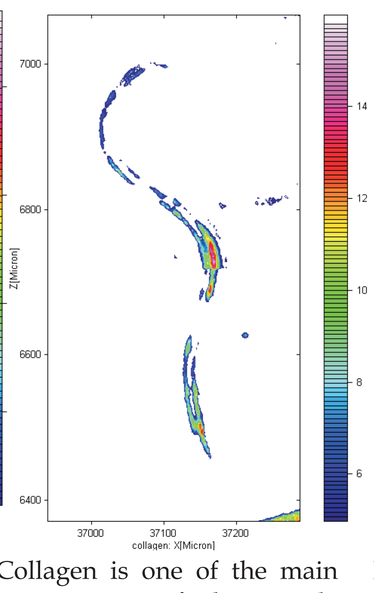
The proteins identified by the Amide I IR band are found at the head, along the centre of the body below the pharynx and along the intestine.

Amide II



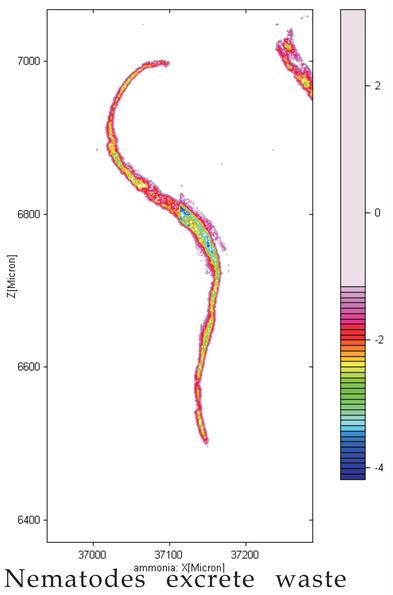
The protien content identifies by the Amide II band is most prominent in the head and upper body regions, with less intensity observed along the intestine than for the Amide I region.

Collagen



Collagen is one of the main contsituents of the cuticle proteins on the outer surface of the nematode. As such, the collagen is mainly distributed around the 'edges' of the nematode.

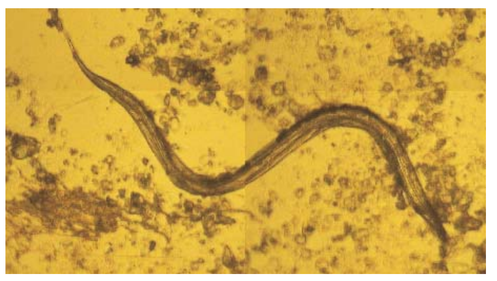
Nitrogen based excretion products



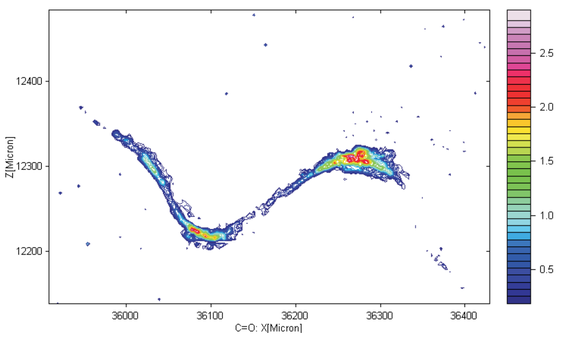
Nematodes excrete waste products through the cuticle. Therefore, the most intense areas for these excretion products are along the cuticle of the nematode.

Heterorhabditis heliothidis

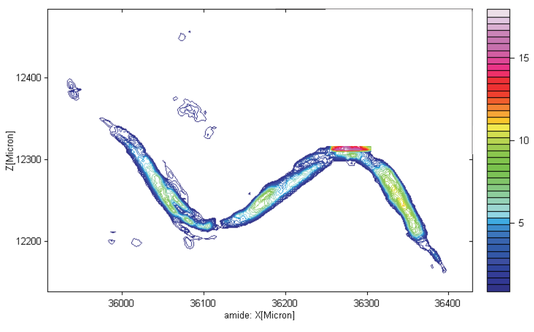
Visible Light Image



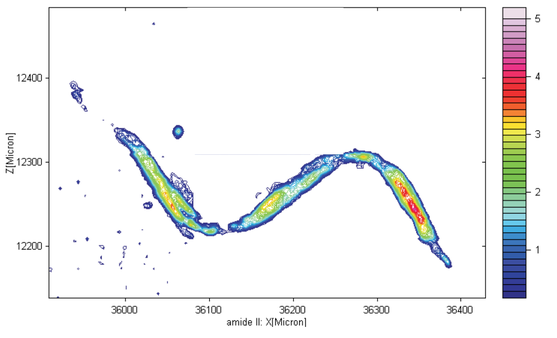
Lipids C=O



Amide I



Amide II



This individual *Heterorhabditis heliothidis* nematode did not give rise to intense bands arising from collagen or from nitrogen based excretors products, suggesting a significant difference between this and *Steinermema feltiae*.

The lipid content for this nematode is most concentrated in the upper part of the body. The Amide I and Amide II bands suggest the concentration of proteins is highest in the upper part of the nematode body with slightly reduced levels in the head and intestine areas compared to *Steinermema feltiae*.

Conclusion

The results presented here highlight the effectiveness of IR imaging spectroscopy for the identification of the distribution of biological molecules within different nematode species, illustrating the use of this technique for the study of multicellular organisms.

* Diagram courtesy of Mark Blaxter