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Nanoplastics in zebra fish using Raman Imaging and Scanning Electron (RISE) microscopy.

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Background

- Plastic can degrade into particles between 5um and 0.1 um – similar size range to plankton.
- Main component of plastics are synthetic polymers.
- Possible identification methods:
 - Micro-Fourier transform Infra red spectroscopy
 - Spectrophotometry
 - Raman spectroscopy



Purpose of study

- Are nanoplastics absorbed by fish?
- Can nanoplastics be detected with Raman spectroscopy?

Methods

Zebra fish cultivated in 4 tanks

Polystyrene nanobeads introduced into 3 tanks:

30nm

50nm

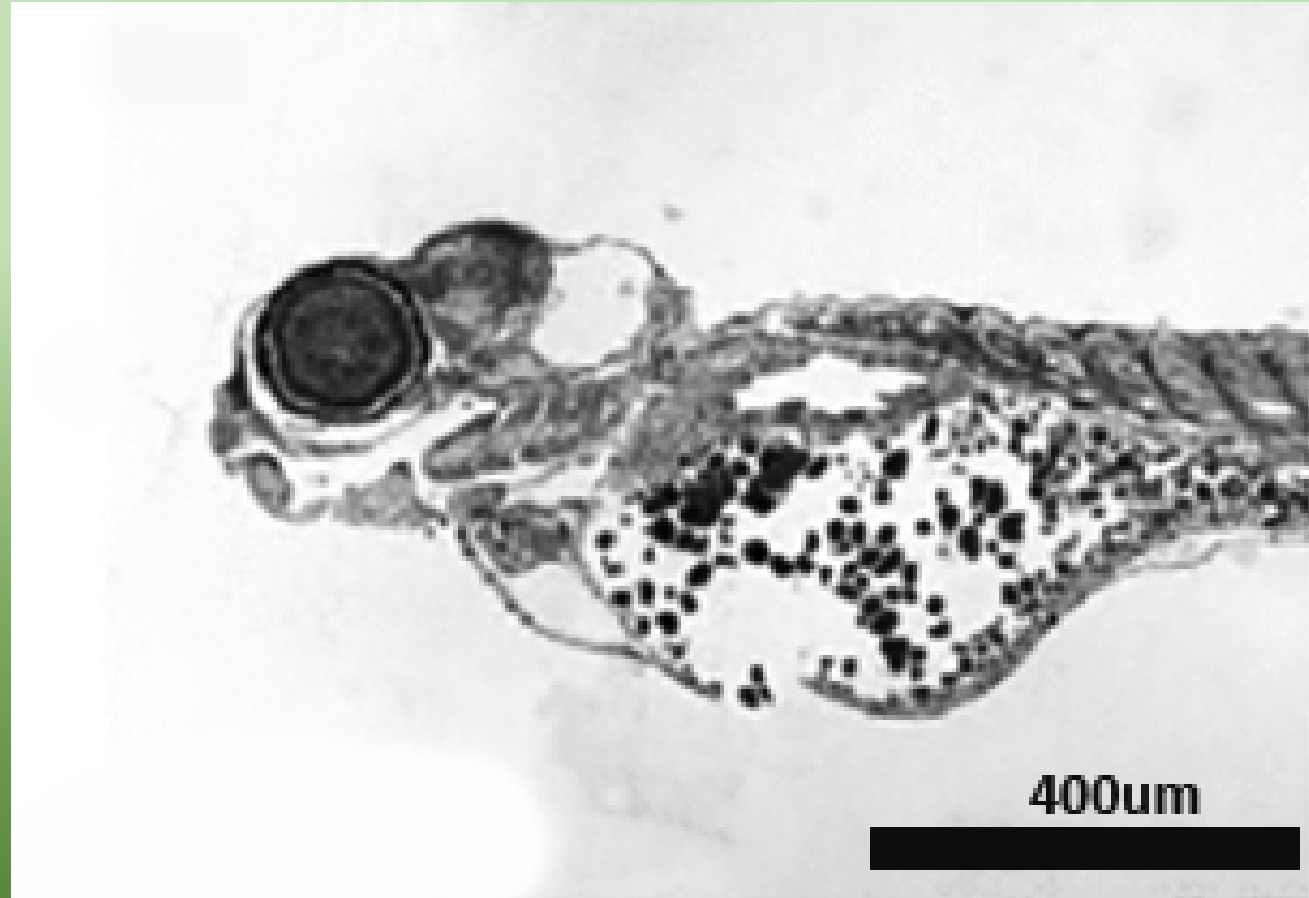
100nm

Two fish sacrificed each week for 6 weeks



Sample preparation

- Whole fish were frozen in OCT (Optimal Cutting Temperature compound)
- Sectioned – Frozen sections placed on glass slides
- OCT washed off with buffer
- Slides allowed to dry
- Viewed in the SEM under low vacuum conditions, using backscatter detector



Brief introduction to Raman spectroscopy

Sir C.V. Raman 1930 Nobel prize for physics

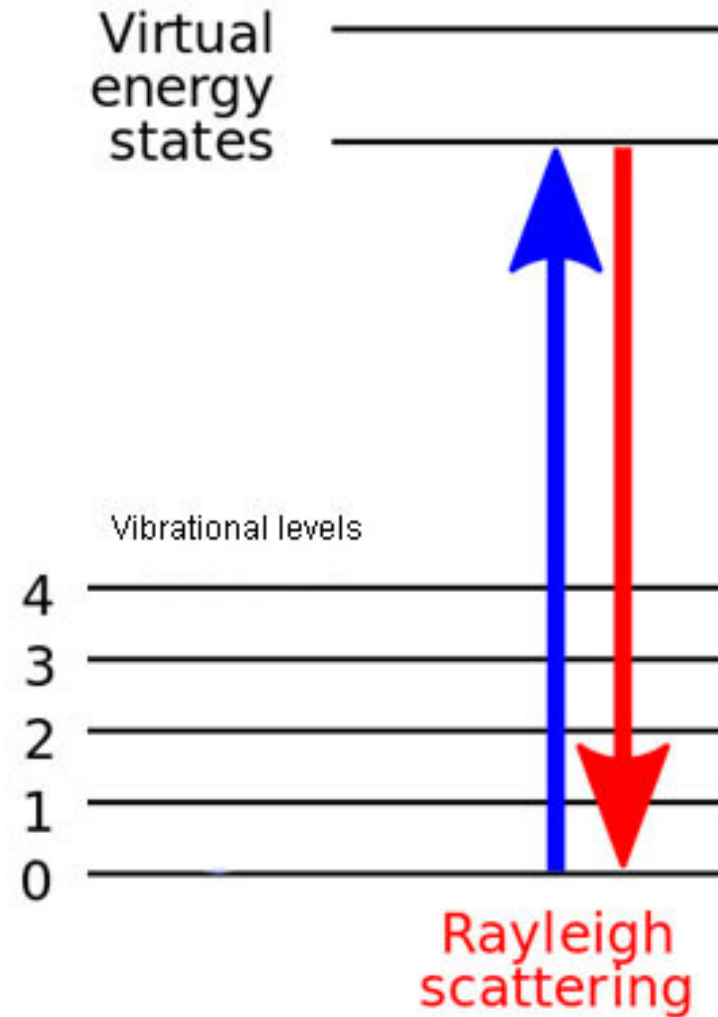
Raman Effect:

- **Inelastic scattering of monochromatic light, usually from a laser**
- **The laser light reacts with molecular vibrations, resulting in a change in the energy of the laser photons**
- **Can be used on liquids, gasses and solids**



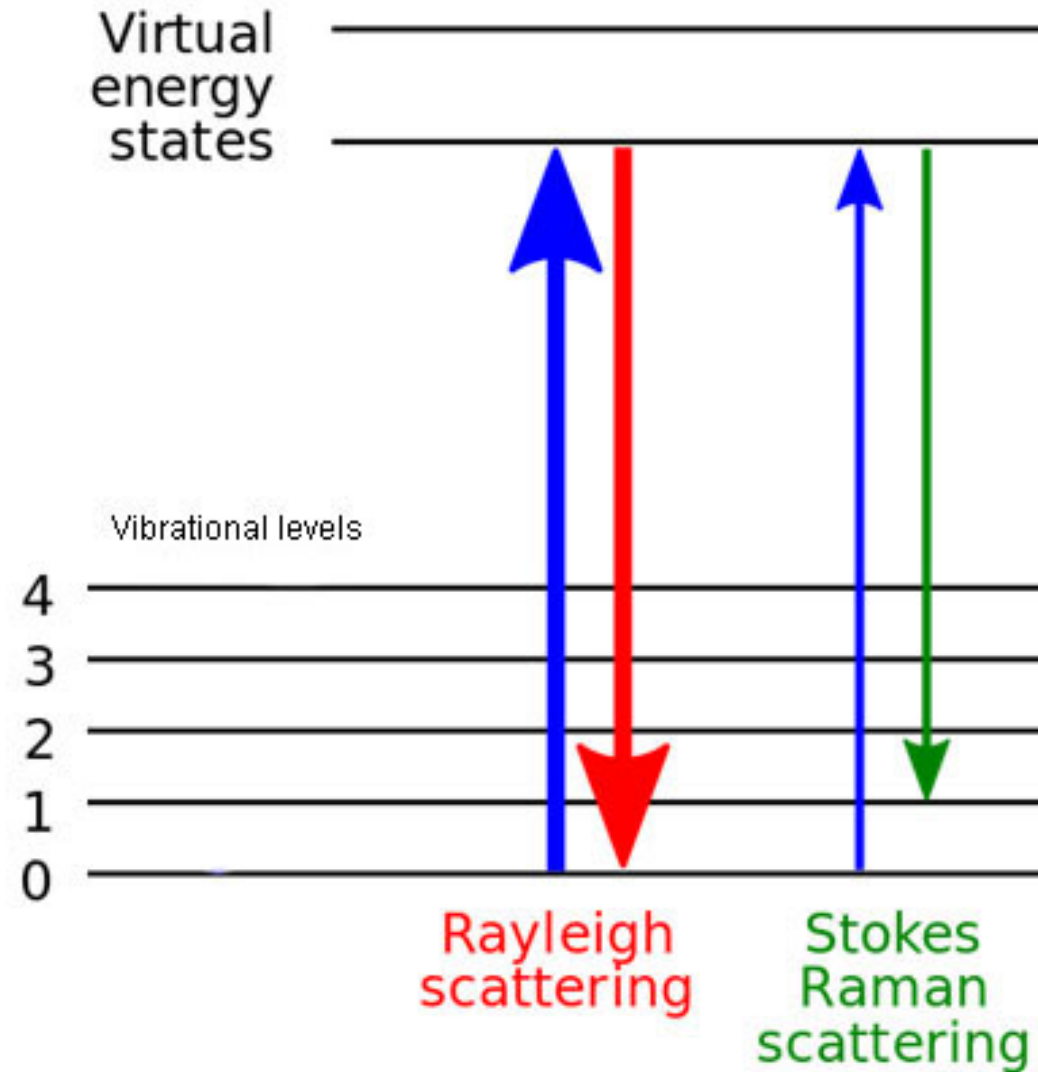
Basic principals of Raman spectroscopy

- Incident light interacts with photons in the sample
- Photons rise to a virtual energy state
- Photons fall back to the same level
- Rayleigh scattering



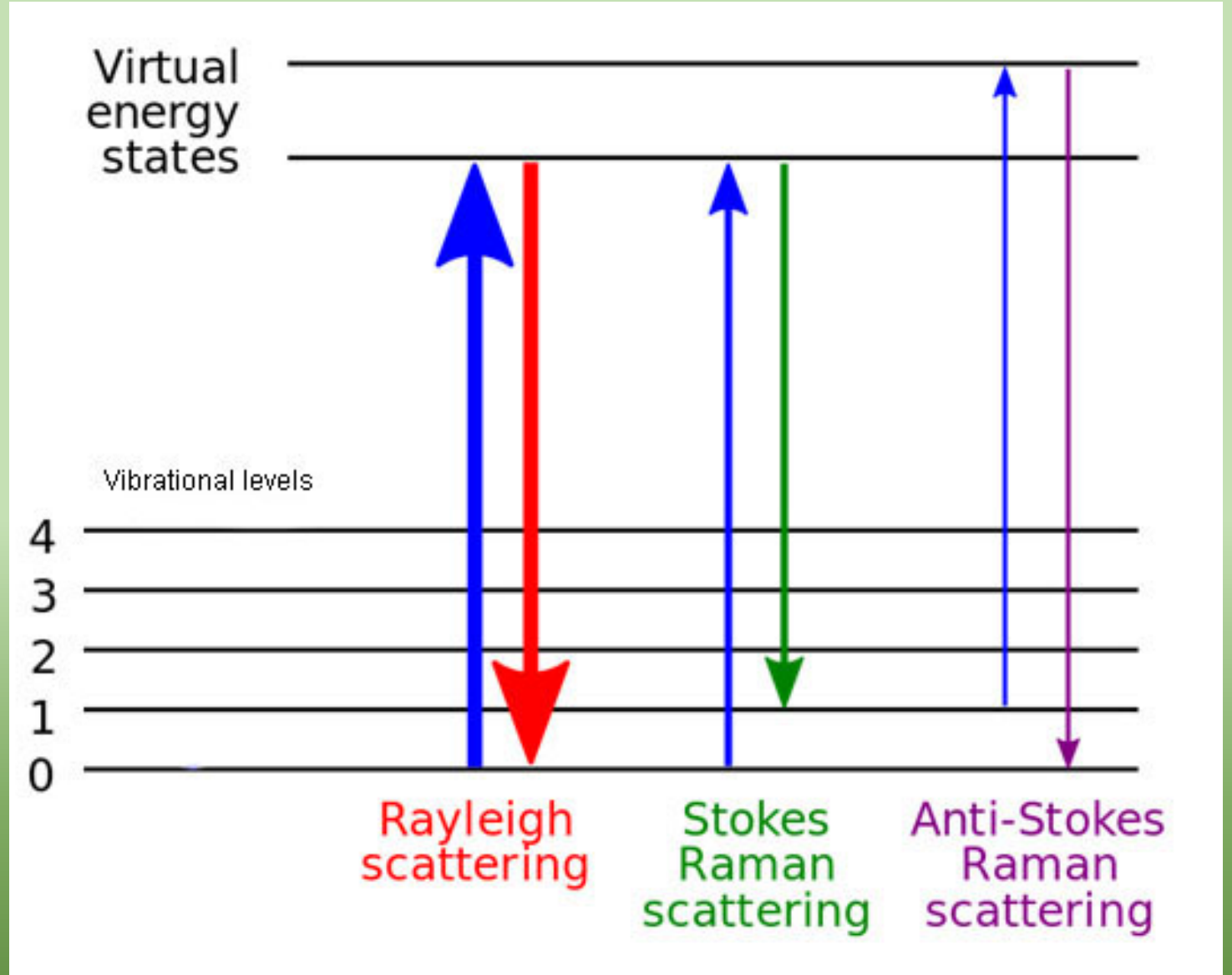
Basic principals of Raman spectroscopy

- Incident light interacts with photons in the sample
- Photons rise to a virtual energy state
- Photons fall back to a higher level
- Stokes Raman scattering

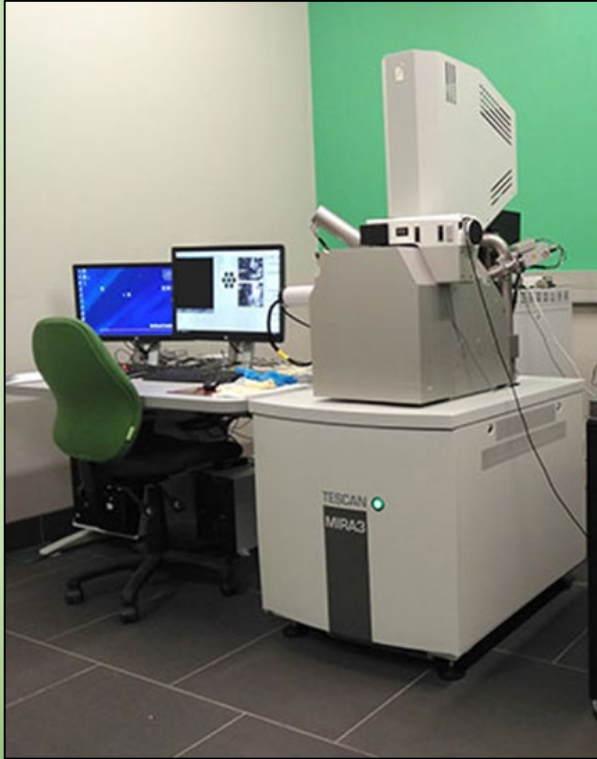


Basic principals of Raman spectroscopy

- Incident light interacts with photons in the sample
- Photons rise to a virtual energy state
- Photons fall back to a lower level
- Anti-Stokes Raman scattering

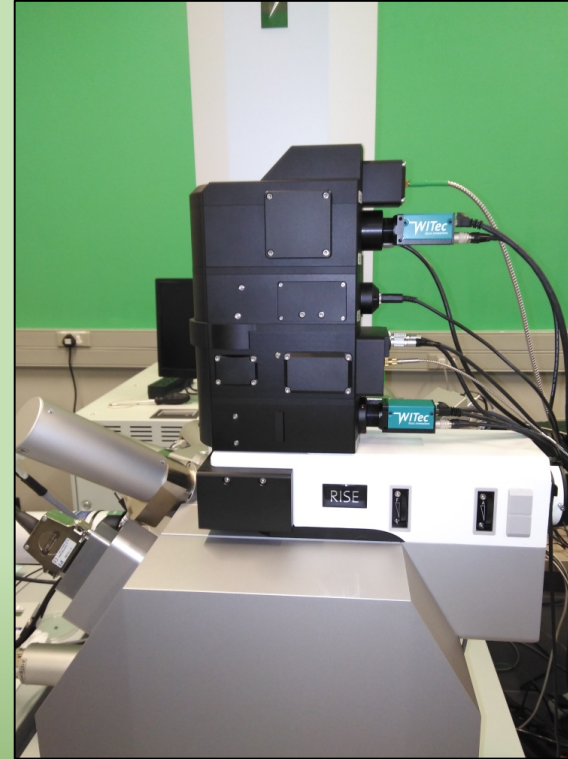
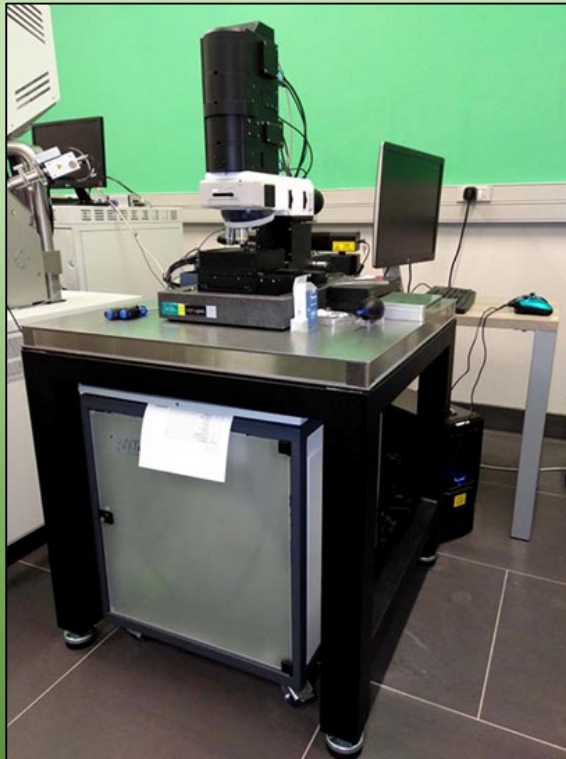


RISE system



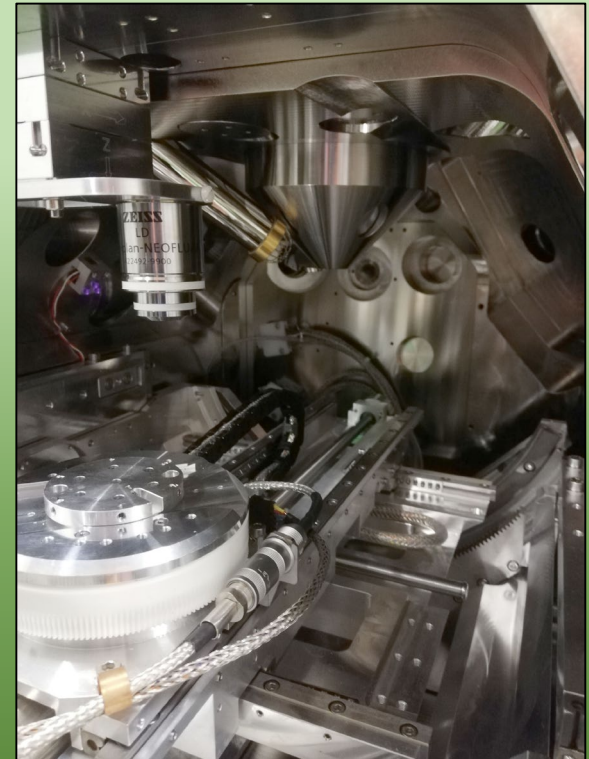
SEM
Tescan MIRA3 GMU

Raman
WiTec Alpha 300

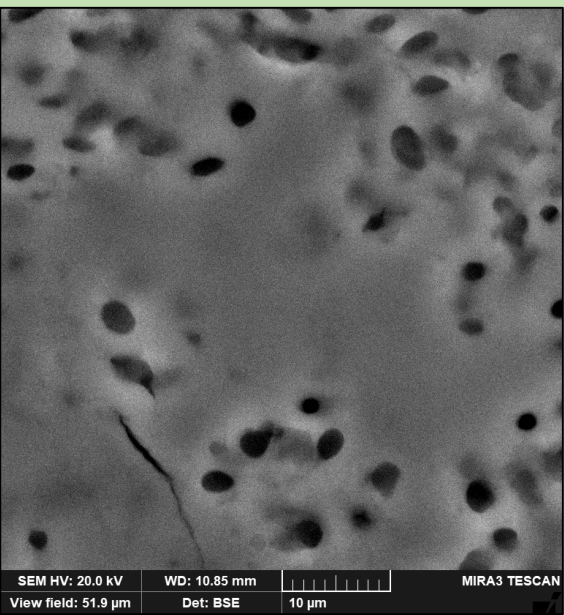


Raman tower
connected to SEM

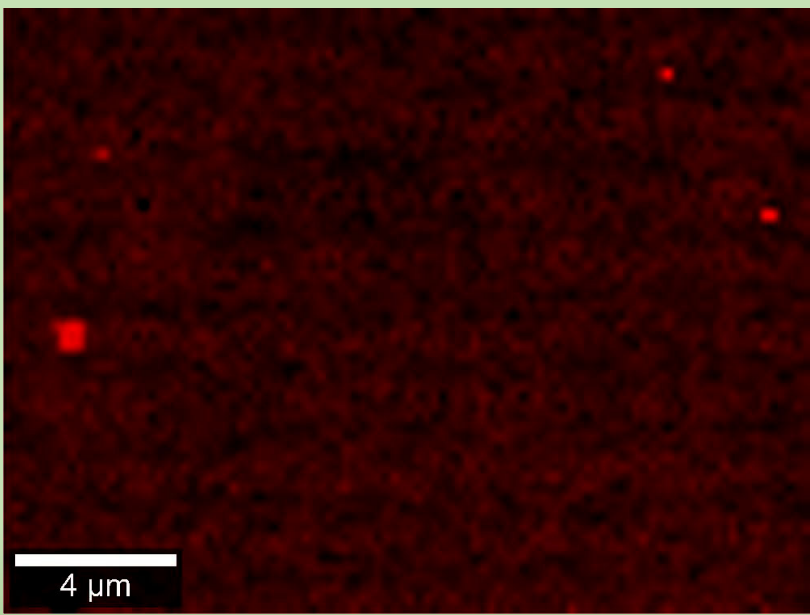
MIRA chamber



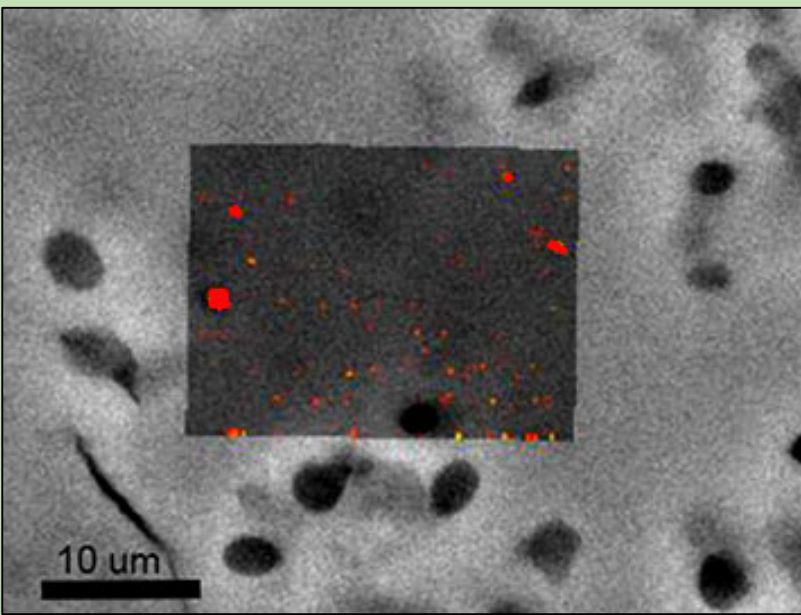
Results: Control sample: Brain, 6 weeks



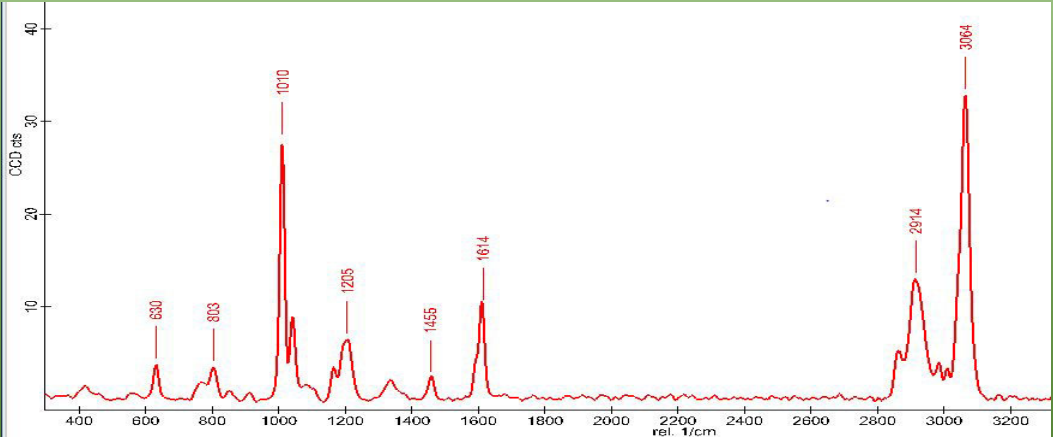
SEM image



Raman map of polystyrene

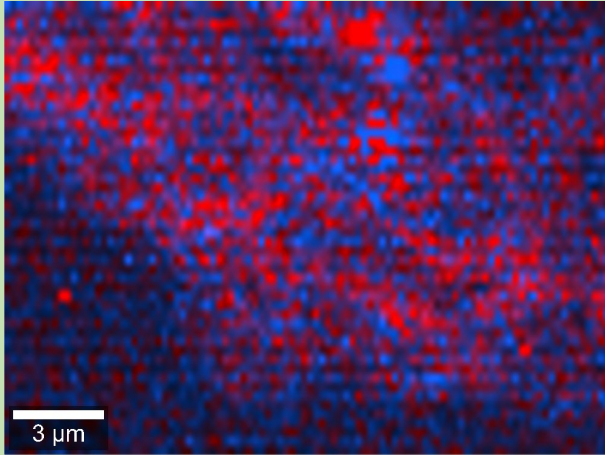


SEM and map combined

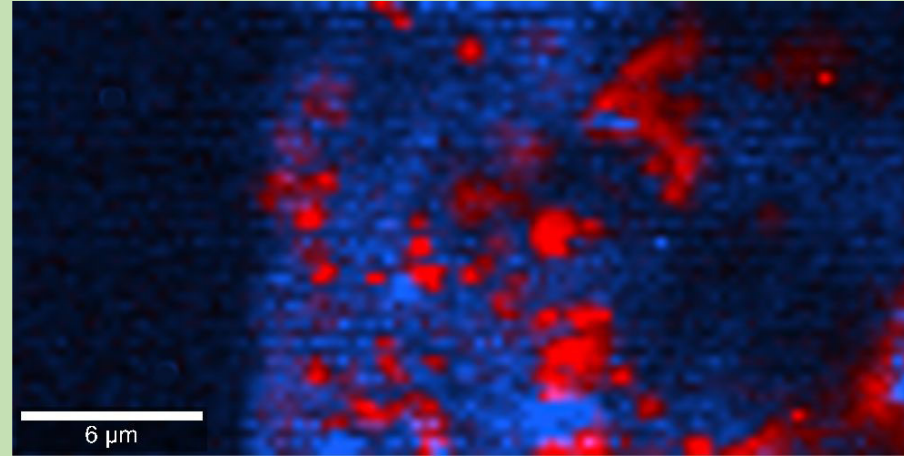


Raman spectrum for polystyrene

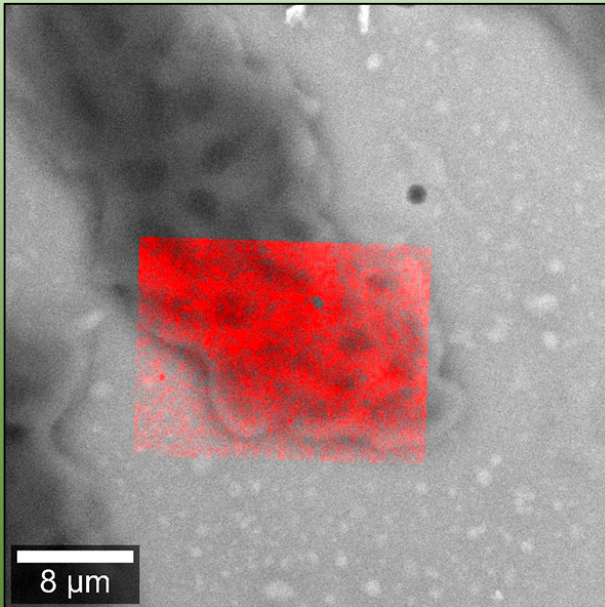
Results: 1 week 30nm beads



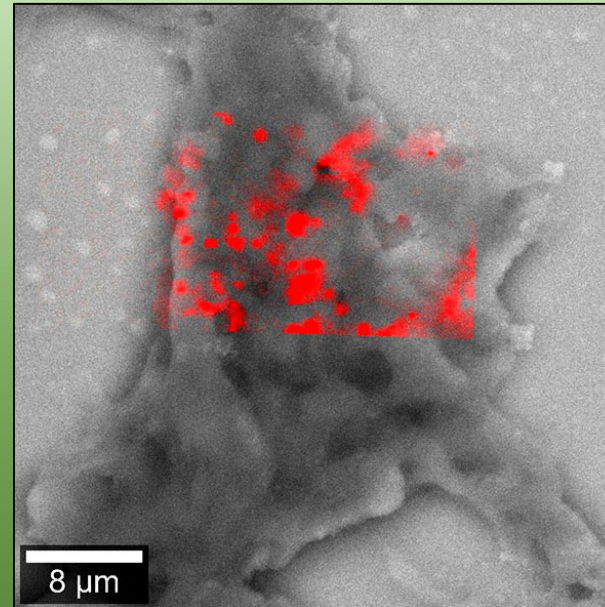
Gut Raman map



Brain Raman map

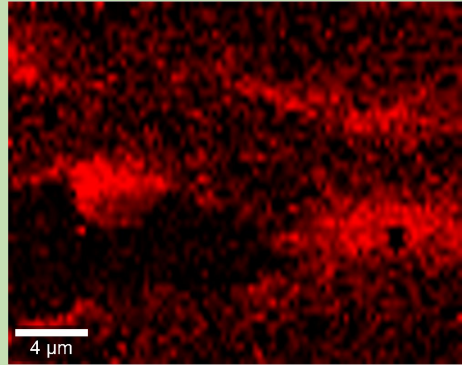


Polystyrene map overlaid
on
SEM image

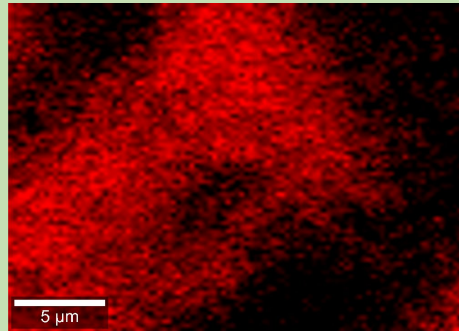


Results: 2-6 weeks brain 30nm beads

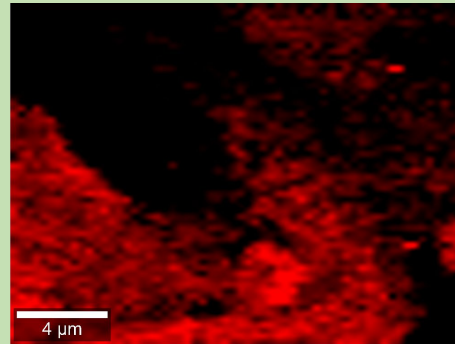
Raman maps for polystyrene



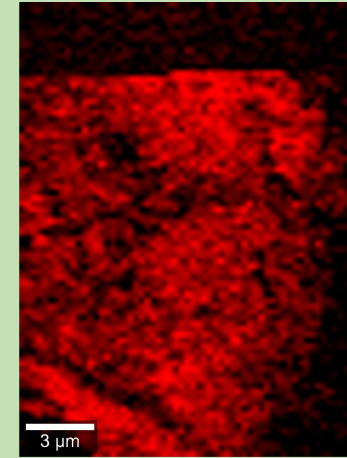
2 weeks



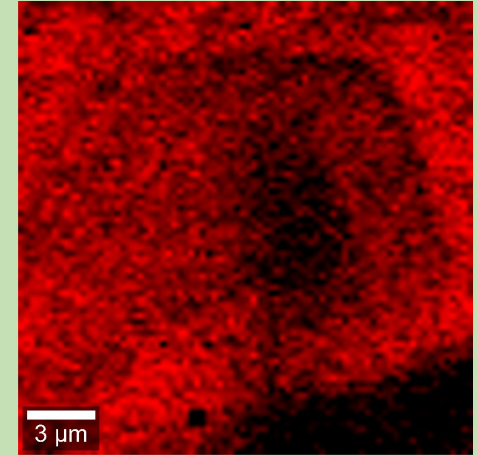
3 weeks



4 weeks

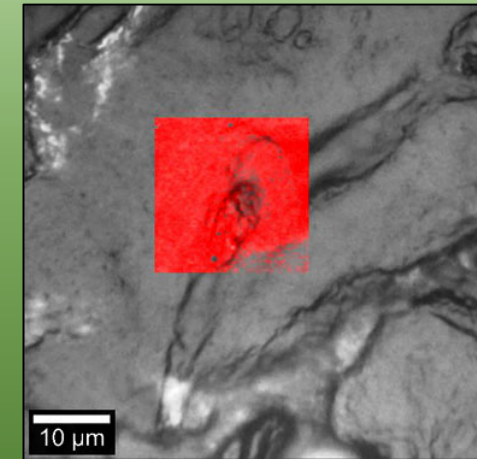
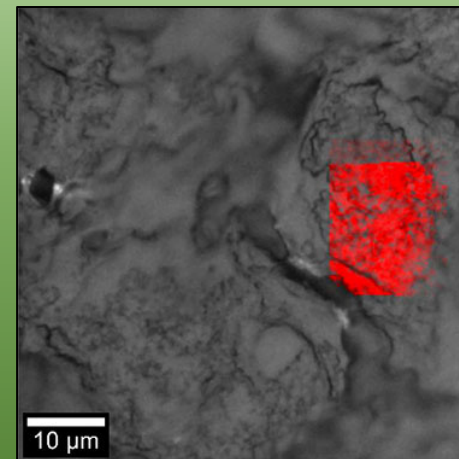
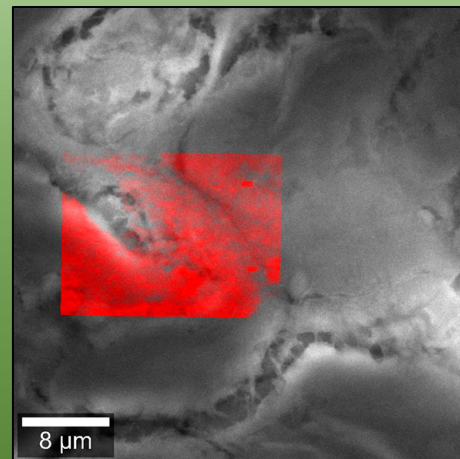
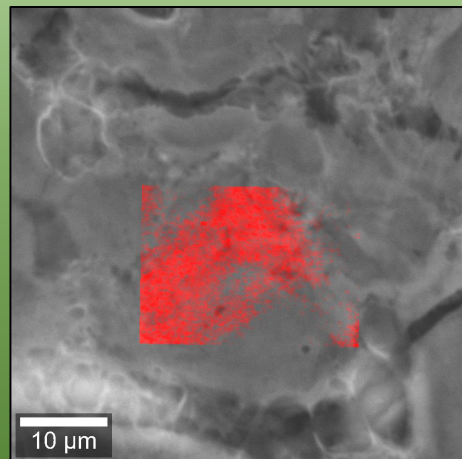
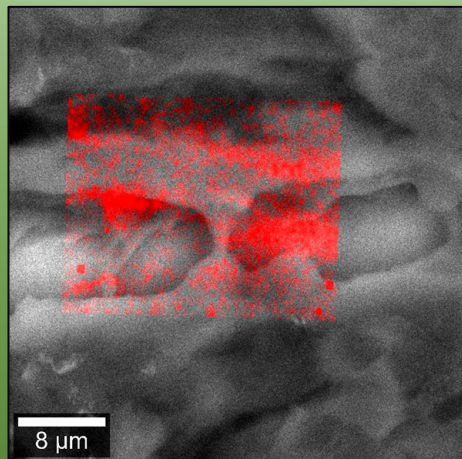


5 weeks



6 weeks

Polystyrene maps superimposed on SEM images



Conclusions

- Polystyrene beads are incorporated into tissue in 1 week
- RISE can be used to give information about nanoplastics in fish tissue

Further studies:

- Technique is being applied to wild caught shark brains



Acknowledgements:

- NRF
- University of Cape Town
- Wirsam Scientific

