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Spontaneous Raman and Stimulated Raman spectroscopy for the analysis of adipocytes

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CONTEXT

In-situ investigation cell cultures



Current methods

- Mass Spectroscopy
- Fluorescent microscopy
- Chromatography etc...

Current methods

Destructive
Label needed

Raman
Non Destructive
Label free,
Molecular level

Aim of the study:

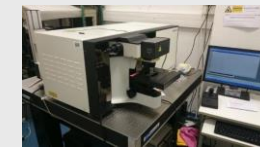
Pros and cons of Standard and Stimulated Raman spectroscopy on adipocyte in-vitro model

SAMPLES & METHODS

Cell culture

Murine OP9 stromal cells differentiated 17 days on CaF2 substrates, 2 conditions:

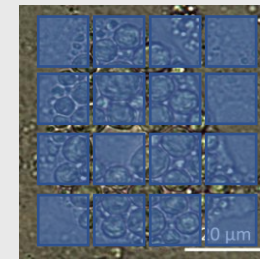
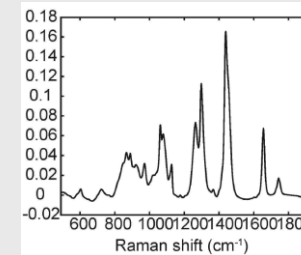
- Spontaneous (Spont.): spontaneous adipogenesis, 10% FBS (non-supplemented medium)
- Induced (Ind.): adipogenesis induced with complete alpha-MEM, dexamethasone , IBMX (3-isobutyl-1-methylxantine), 10% FBS and insulin



Spontaneous Raman

Microspectrometer confocal
LabRAM HR800 (HORIBA) $\lambda=785$ nm

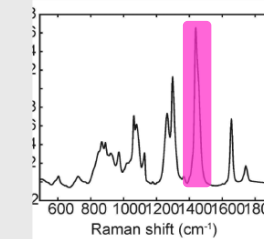
Raman imaging 2D



Stimulated Raman

LEICA-STELLARIS 8: Microscope confocal
Volume analyzed 100×100×40 μ m

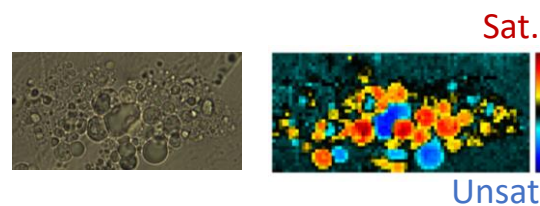
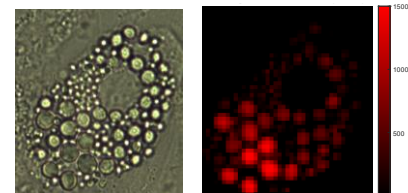
Raman imaging 3D



RESULTS & DISCUSSION

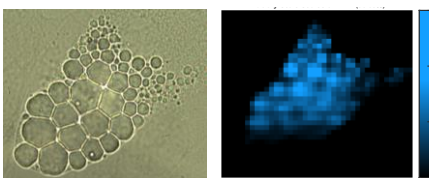
Spontaneous Raman spectroscopy

Spont. BMAds



Spont. BMAds

Ind. BMAds

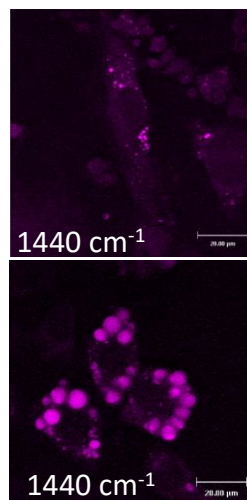


- Raman imaging 2D
- 100×100 μ m
- Fingerprint spectral region
- Long acquisition \sim 4h

Ind. BMAds

Stimulated Raman spectroscopy

Images 2D



Images 3D

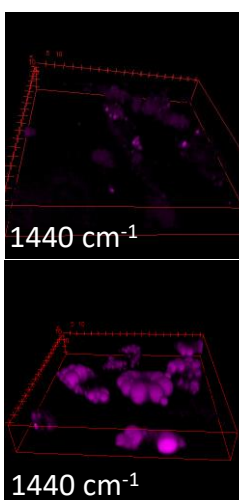
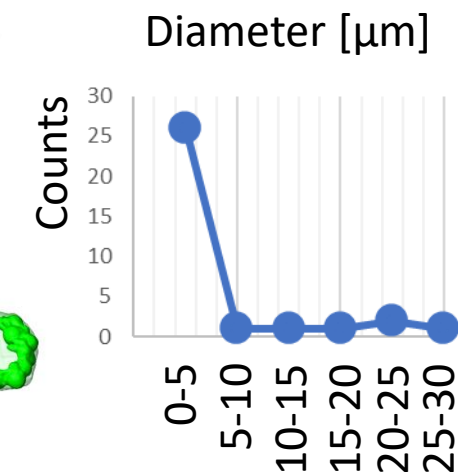
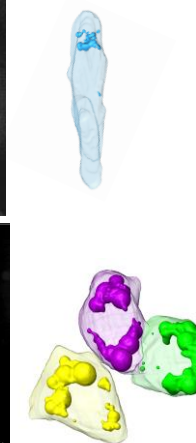
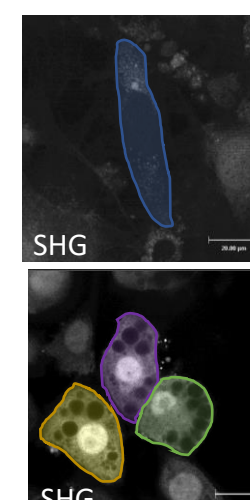


Image Analysis 3D volumes



TAKE HOME MESSAGE

A Raman technique as function of yours needs...

- Spontaneous Raman
- Fingerprint regions
 - Spectral detailed analysis
 - Long acquisition times
- Stimulated Raman
- Fast analysis
 - Molecular images 3D
 - 3D parameters (Diameter, area and volume)