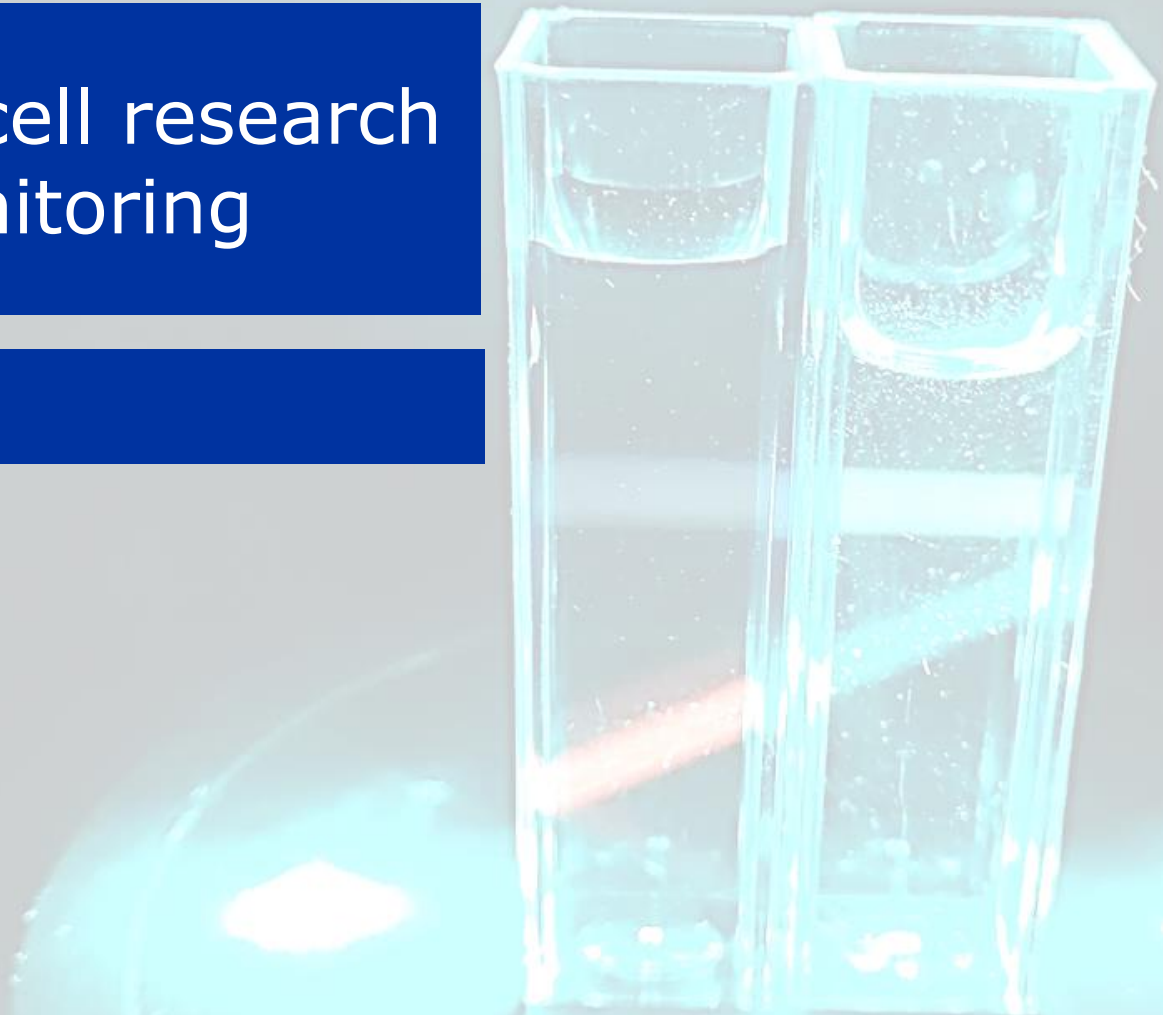
Lab-on-a-chip: from cell research to environmental monitoring

Tatevik Chalyan



VUB B-PHOT BRUSSELS PHOTONICS

Trieste, Italy 10 - 21 February 2014

The Abdus Salam
International Centre
for Theoretical Physics

UNIVERSITY
OF TRENTO - Italy

Doctoral dissertation
in fulfillment of the requirements for the degree of
Doctor of Philosophy in the subject of Physics

OPTICAL BIOSENSORS FOR MYCOTOXIN DETECTION IN MILK

Supervisor:
Prof. Lorenzo Pavesi

Ph. D. candidate:
Tatevik Chalyan

NanoScience Laboratory
Department of Physics

XXX PhD cycle in Physics
July, 2018

VRIJE
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BRUSSEL

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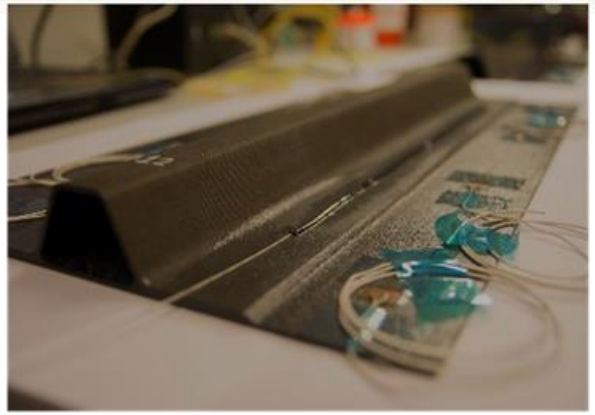
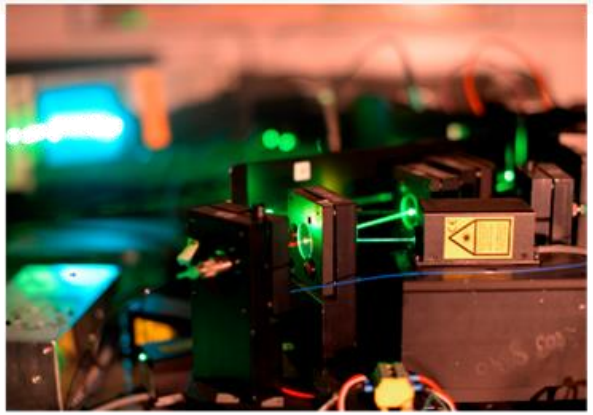
BRUSSELS
PHOTONICS

AMBASSADORS

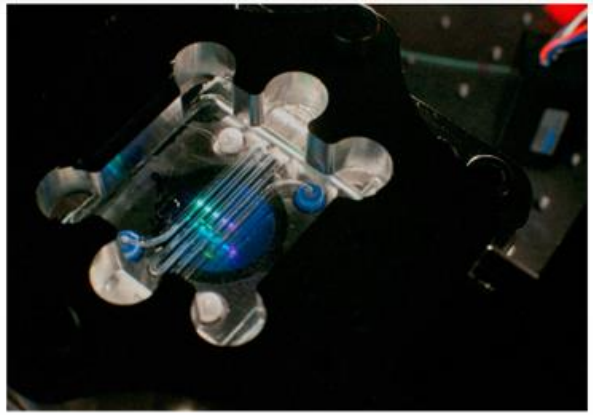
B-PHOT's Research Tracks



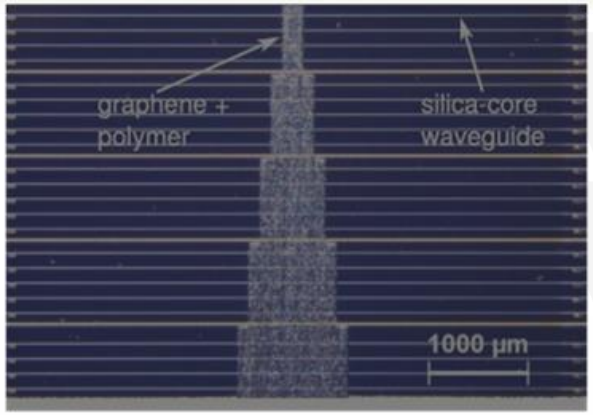
Optical Fiber Sensors



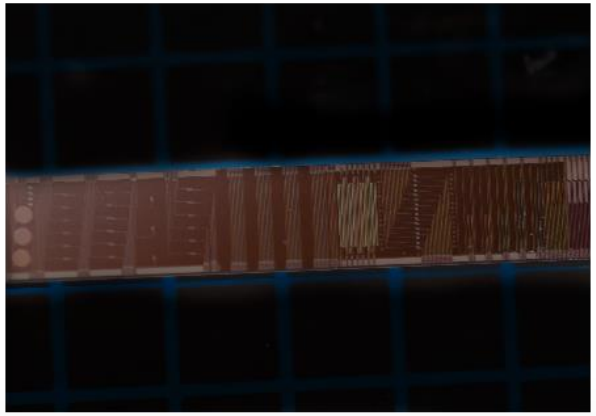
Optical Spectroscopy



Photonics lab-on-chip

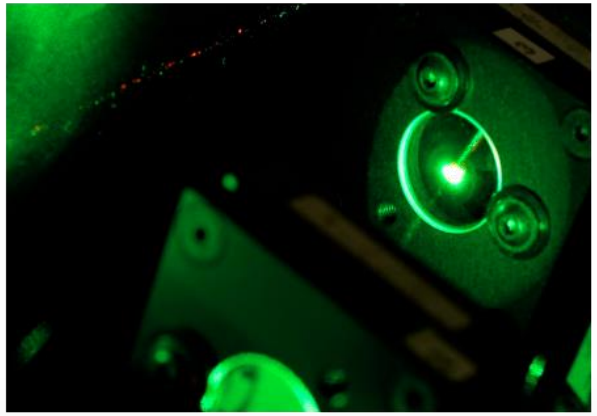


Nonlinear Photonics



Photonic Integrated Circuits

Freeform Optics



Laser Dynamics

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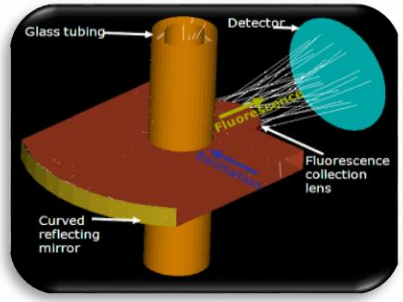
B-PHOT's technology supply chain for disruptive photonics innovation

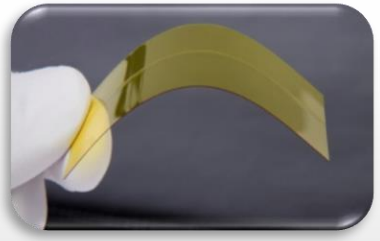
Mastering and Prototyping Technologies

Optical Measurement and Characterization

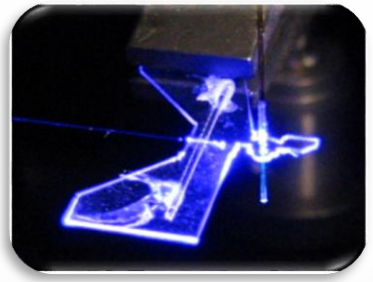
Low-Cost Low-Volume Replication



Optical Modelling

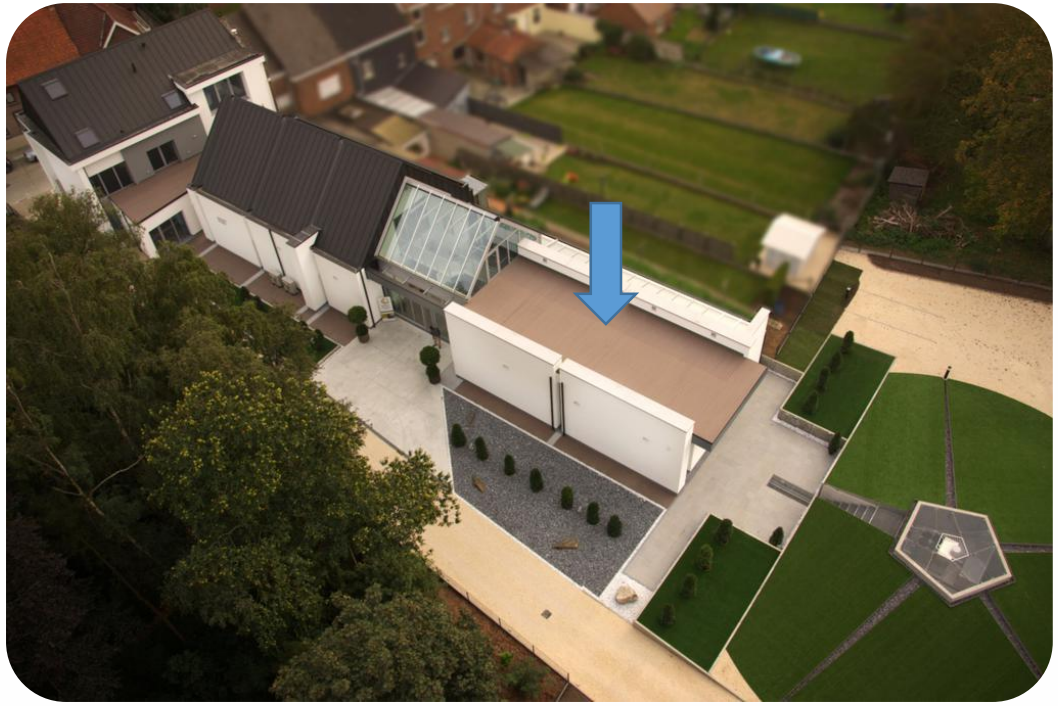


Advanced Materials

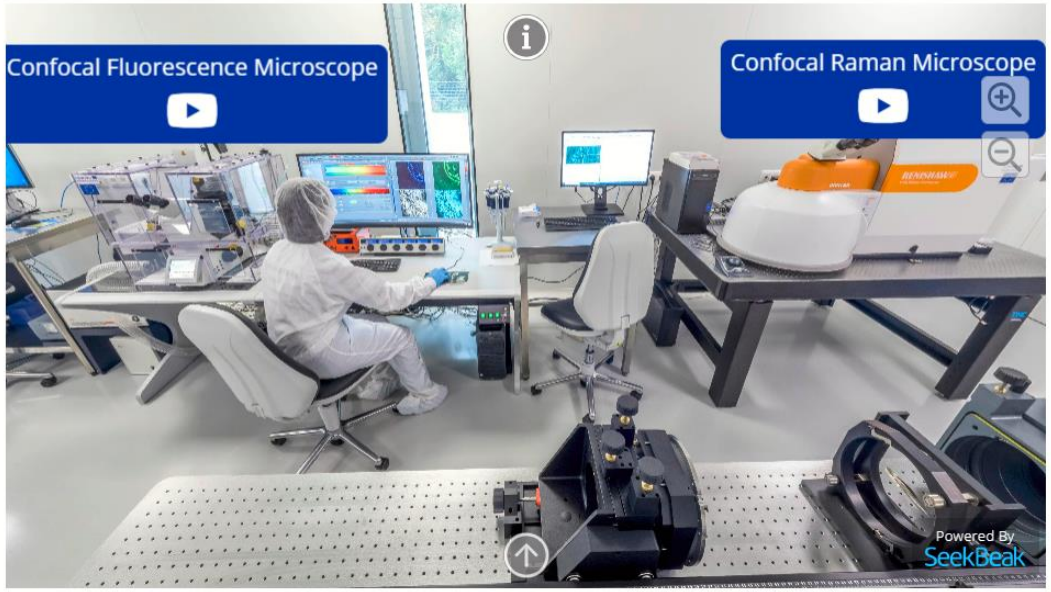


Demonstrators and Prototypes

B-PHOT's Photonics Innovation Center opened on 4 October 2013

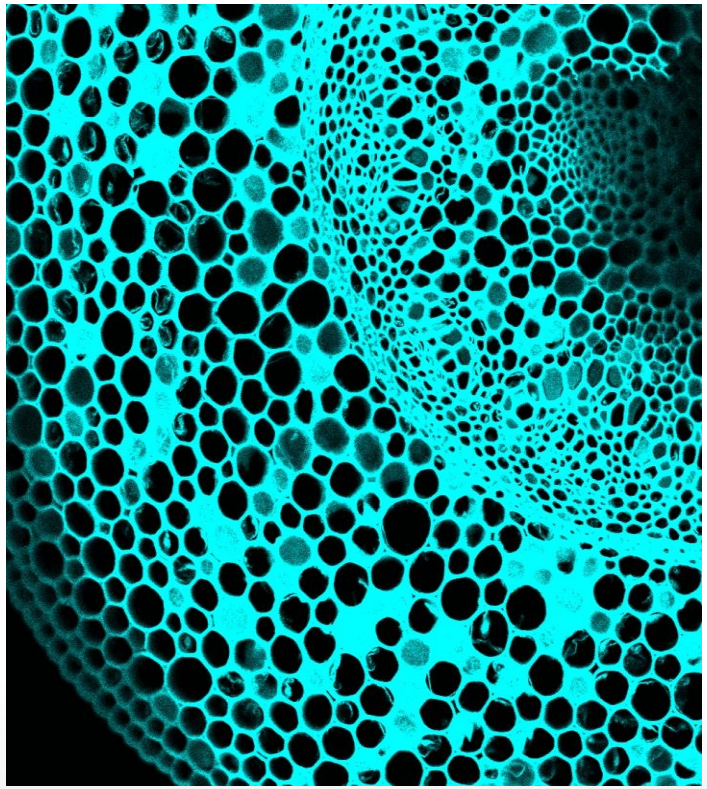


Cleanroom

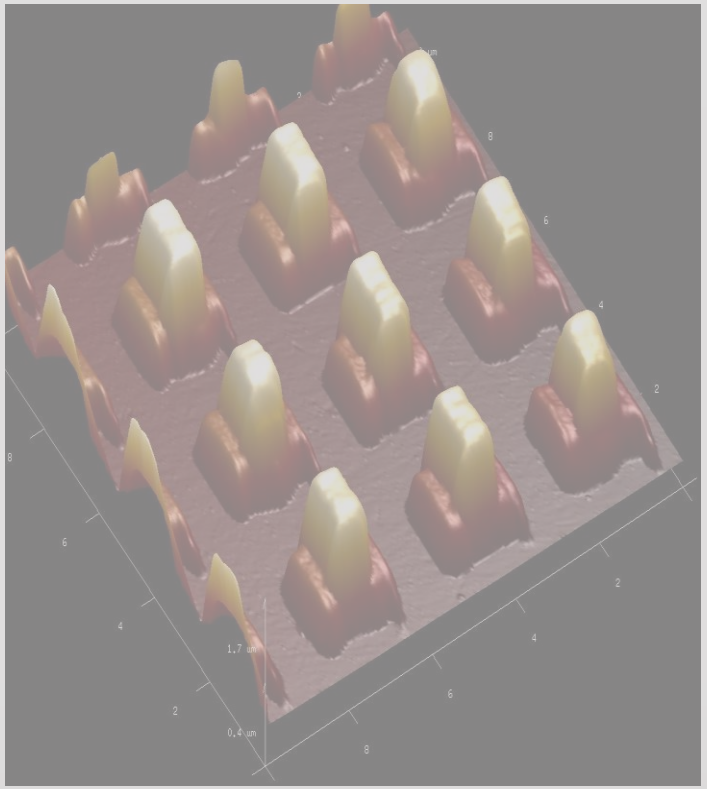


Outline

Towards Compact, Multimodal Spectroscopic Devices For The Read-out Of Microfluidic Organs-on-chip



Surface Enhanced Raman Spectroscopy for Biosensing



Microplastic Detection In Water: MONPLAS

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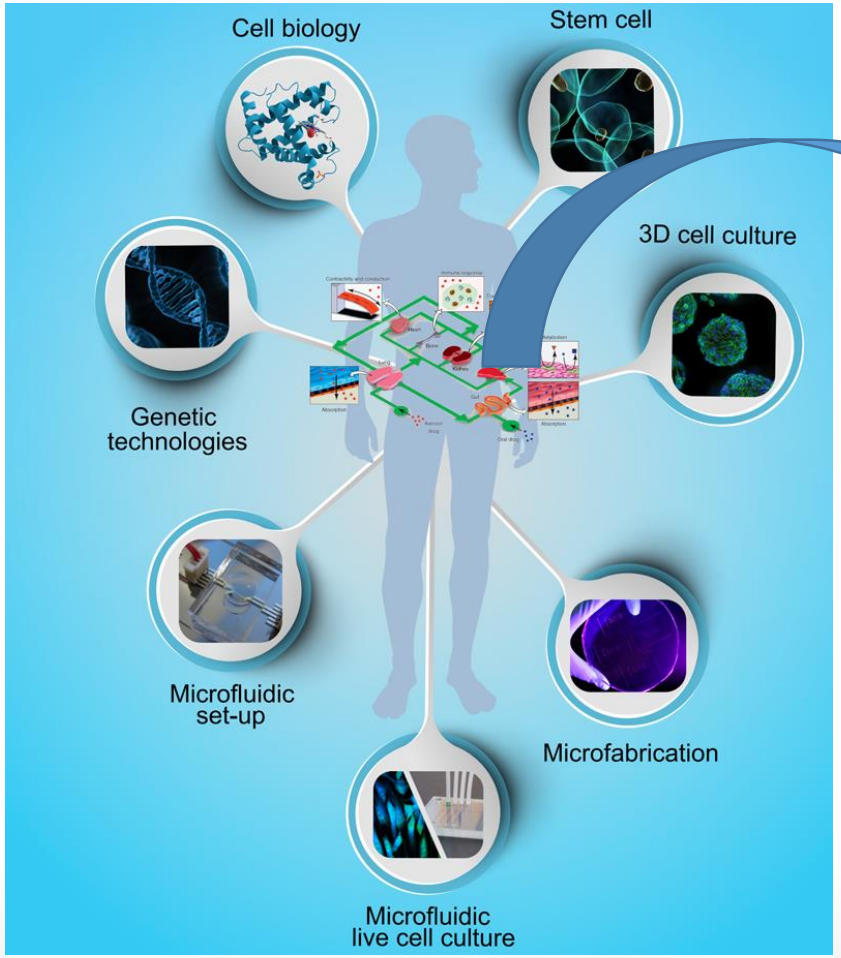
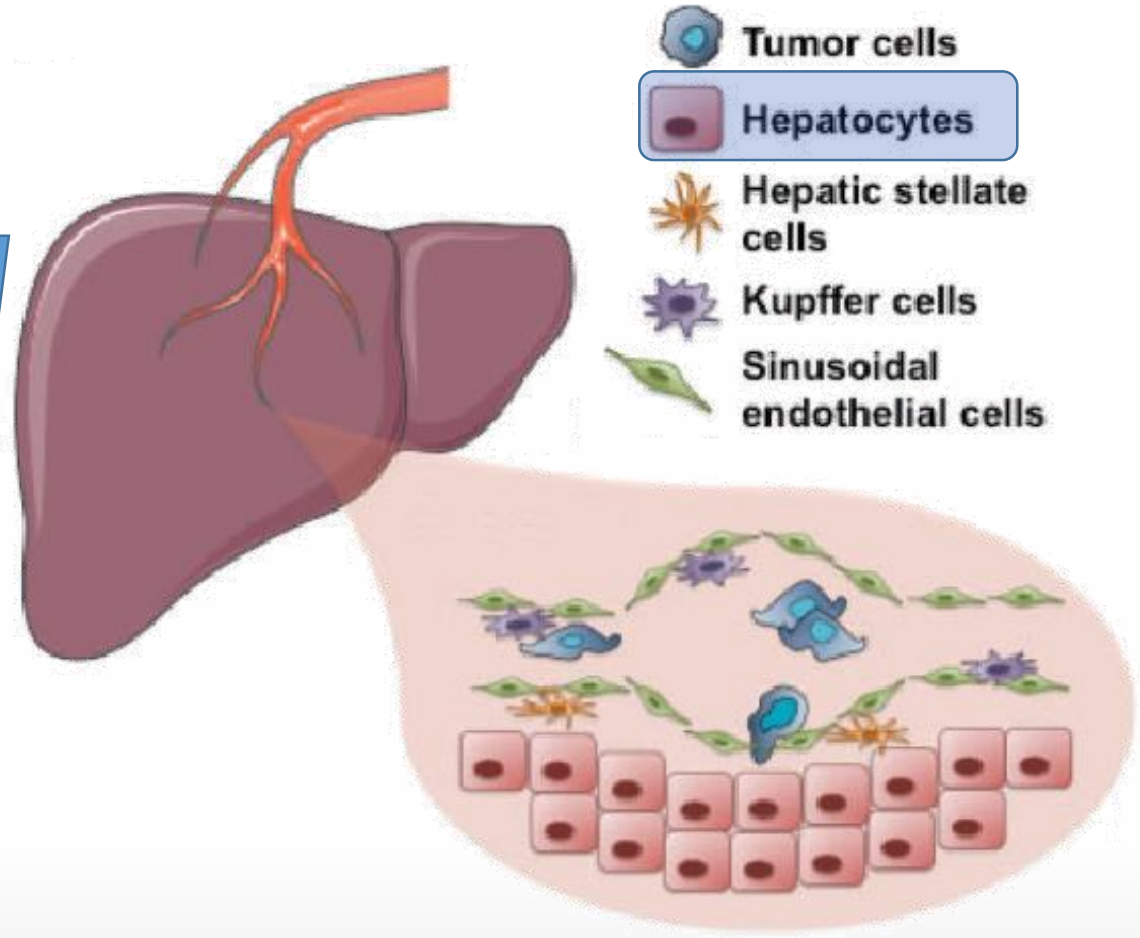
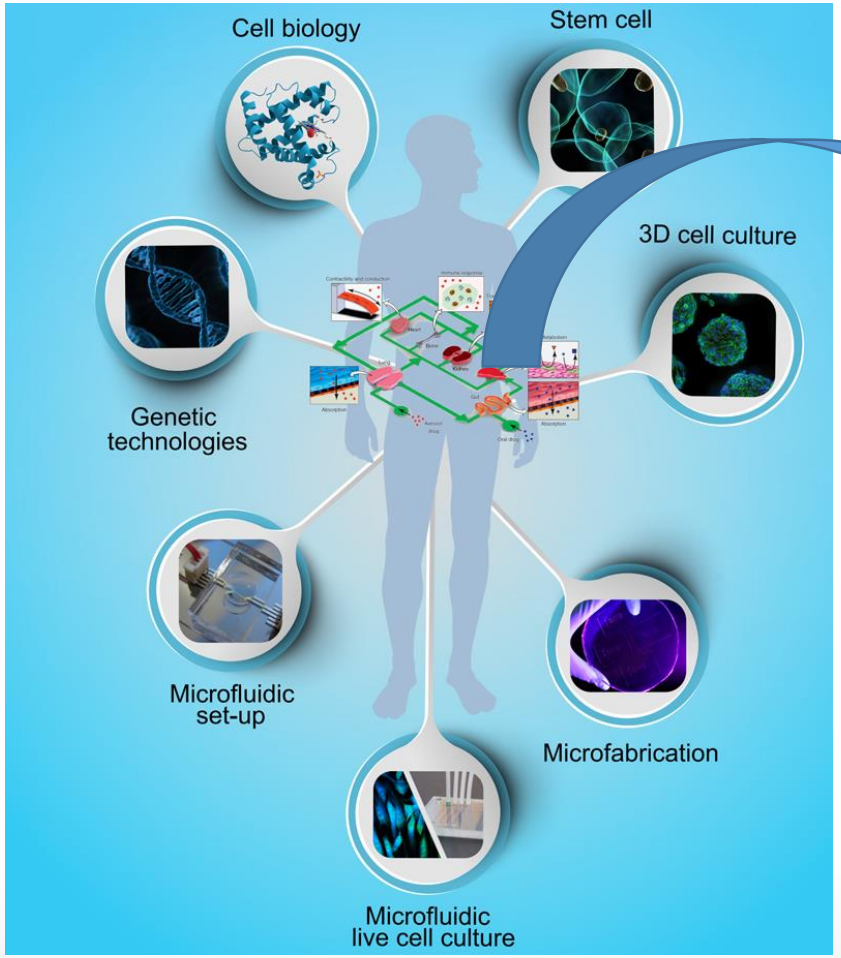
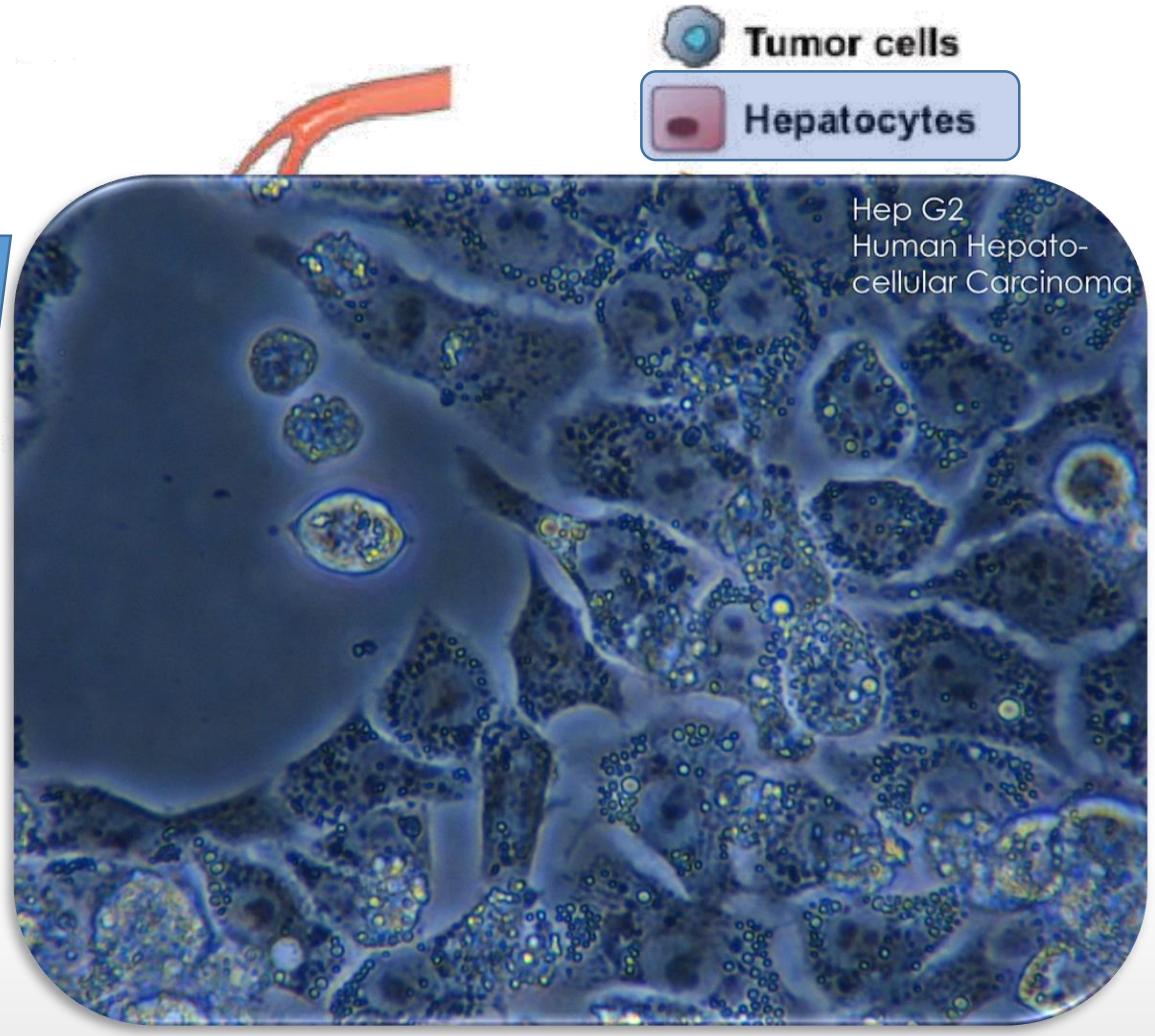
Organs on chip





Organs on chip





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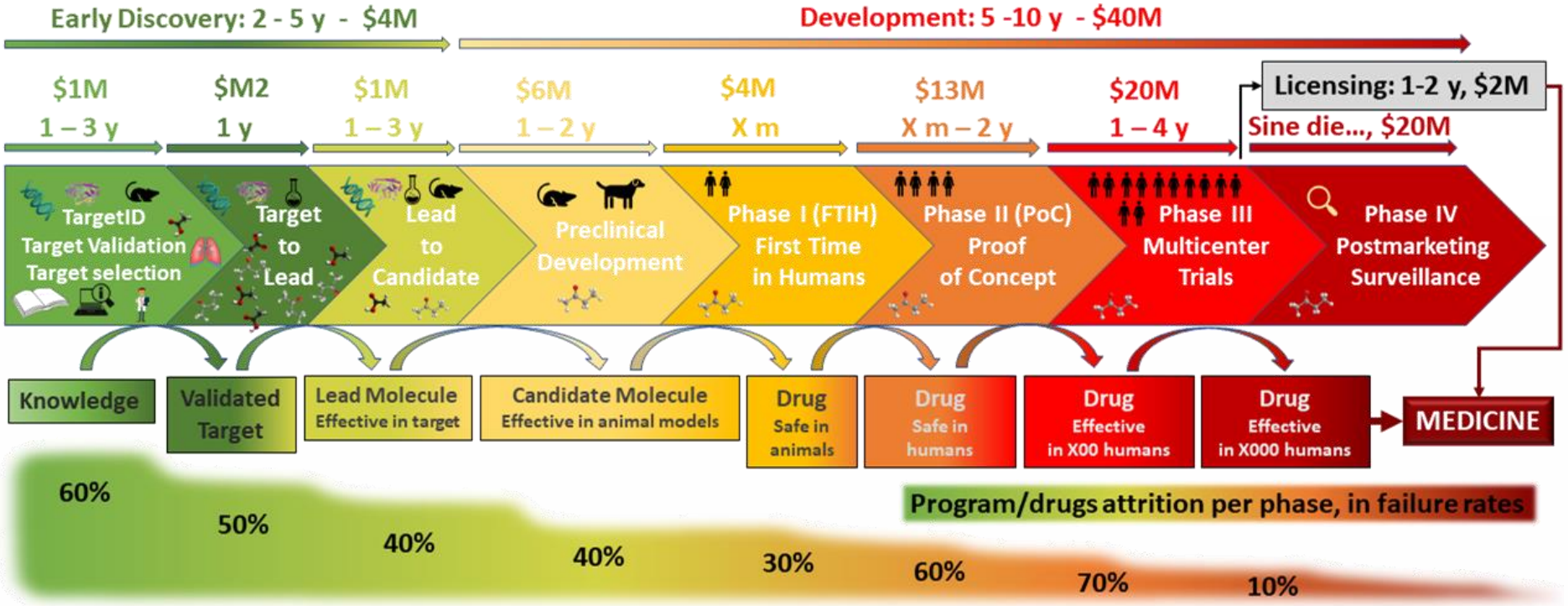
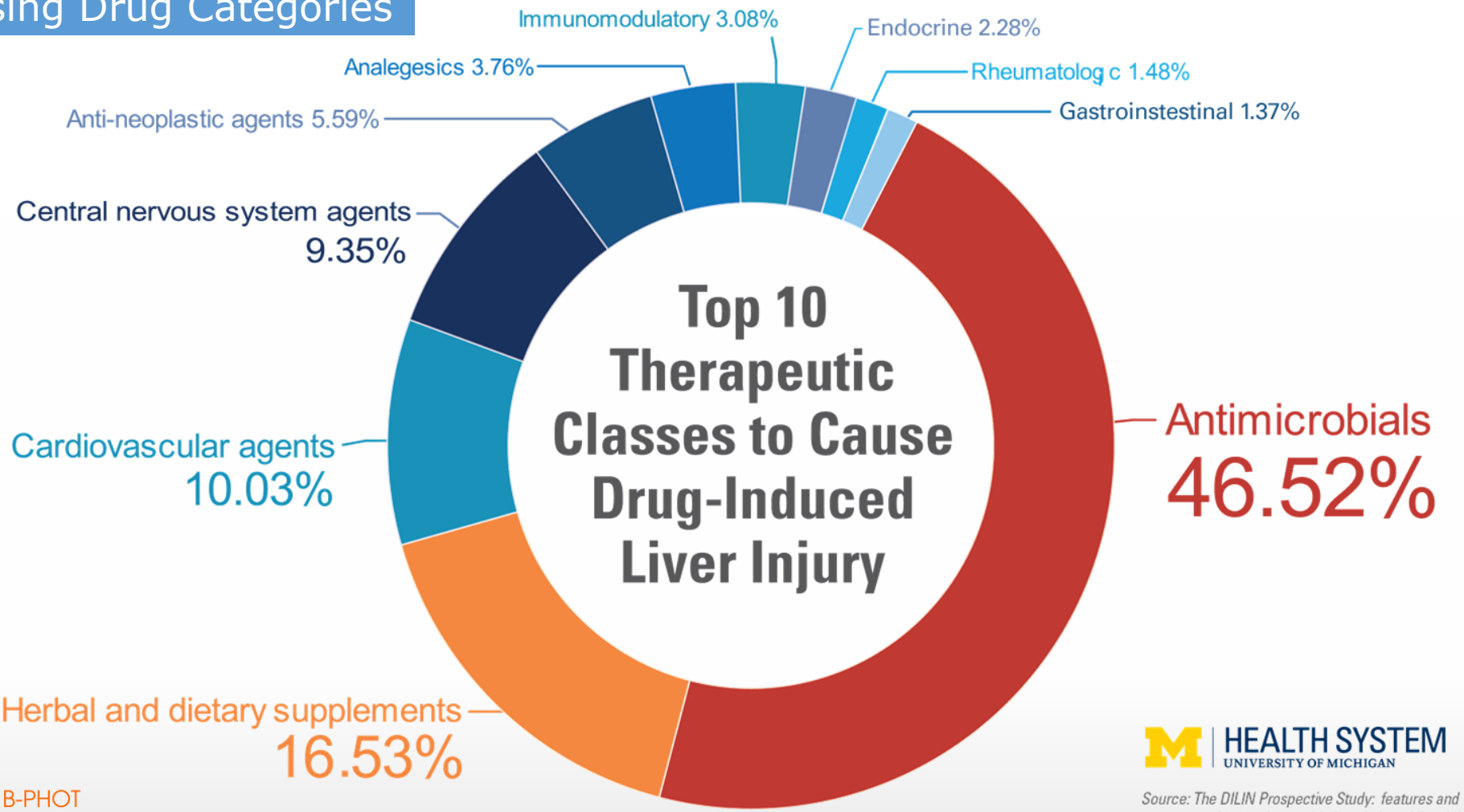
Drug development process



Drug-induced liver injury

DILI Causing Drug Categories



HEALTH SYSTEM
UNIVERSITY OF MICHIGAN

Source: The DILIN Prospective Study: features and outcomes of 899 patients with drug-induced liver injury

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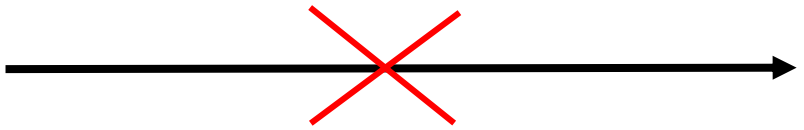
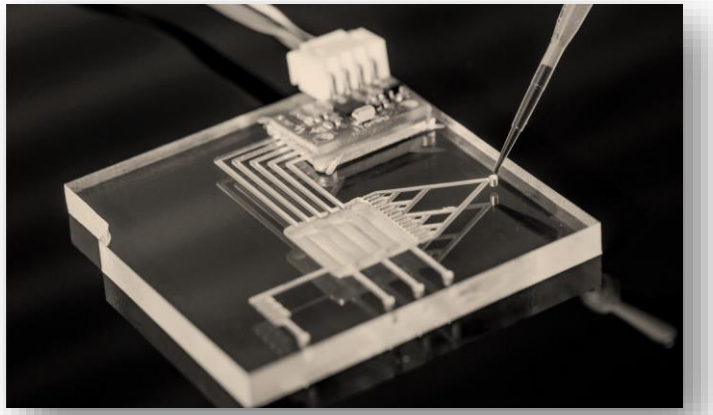
Research objectives

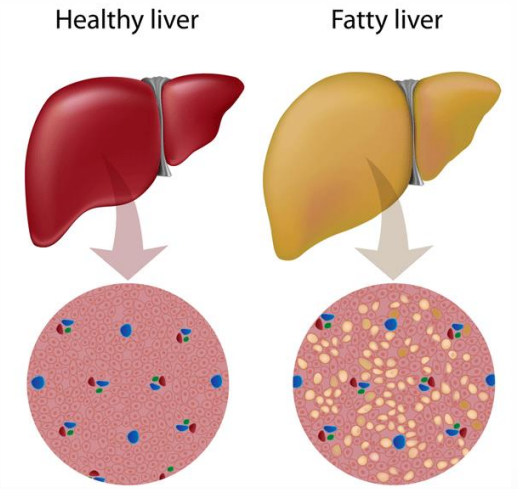


Drug – induced liver Injury

(= Hepatoxicity)



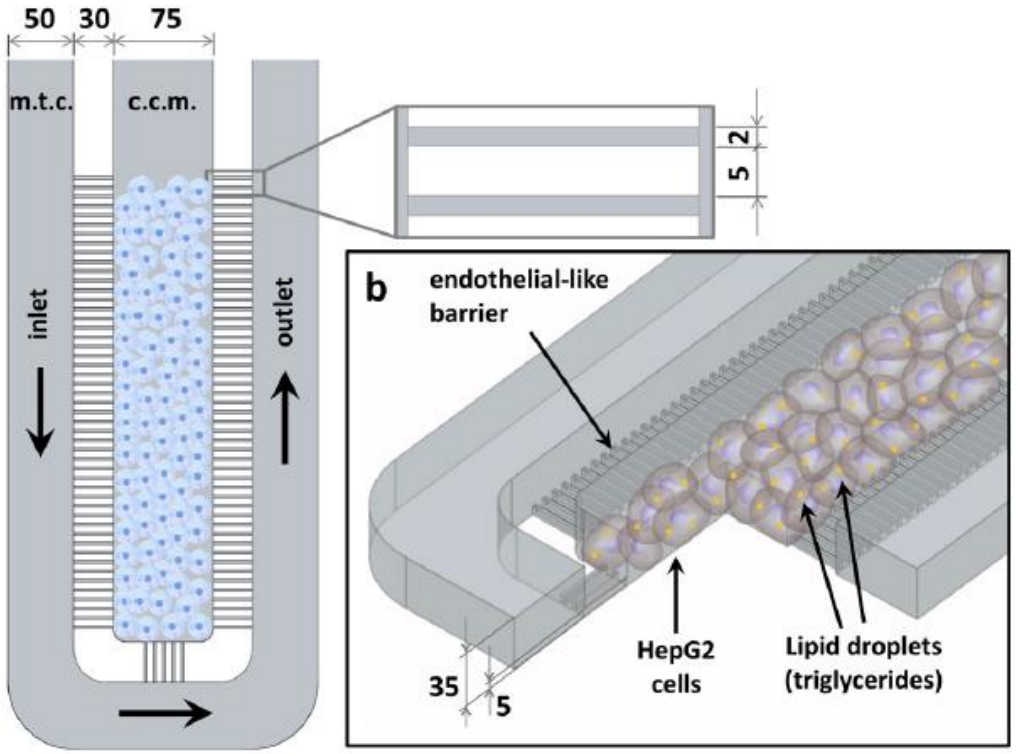
- High-content screening
- Minimize animal testing



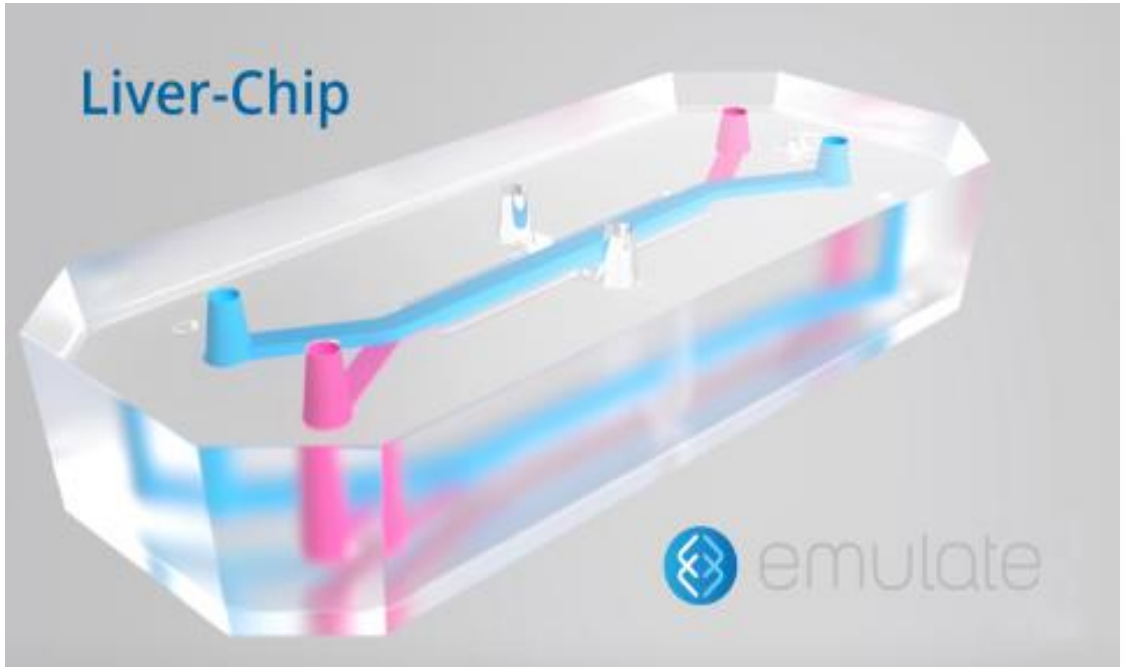
Eg Steatosis

Sources:
1) https://stock.adobe.com/ee/search?k=headache+cartoon [online]
2) https://www.shutterstock.com/nl/search/thermometer+cartoon [online]
3) https://www.freeimages.com/nl/premium/yellow-orange-capsule-978384 [online]
4) **https://murphyshockeylaw.net/uncategorized/1682/global-lab-on-a-chip-market-2020-top-industry-players-as-becton-dickinson-and-company-agilent-technologies-perkinelmer/** [online]
5) https://www.healthline.com/nutrition/fatty-liver [online]

State of the art: Liver on chip

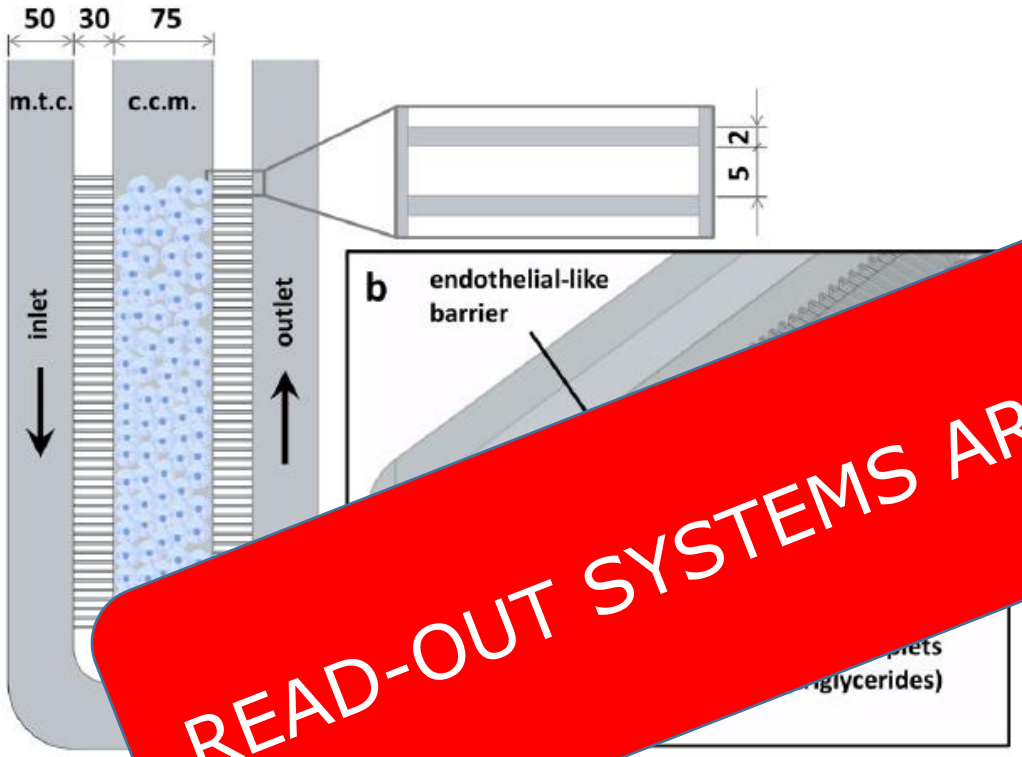


July 20, 2016
https://doi.org/10.1371/journal.pone.0159729



November 06, 2019
https://stm.sciencemag.org/content/11/517/eaax5516

State of the art: Liver on chip



READ-OUT SYSTEMS ARE BULKY AND EXPENSIVE

http…1371/journal.pone.0159729

November 06, 2019
https://stm.sciencemag.org/content/11/517/eaax5516

Towards Compact, Multimodal Spectroscopic Devices For The Read-out Of Microfluidic Organs-on-chip







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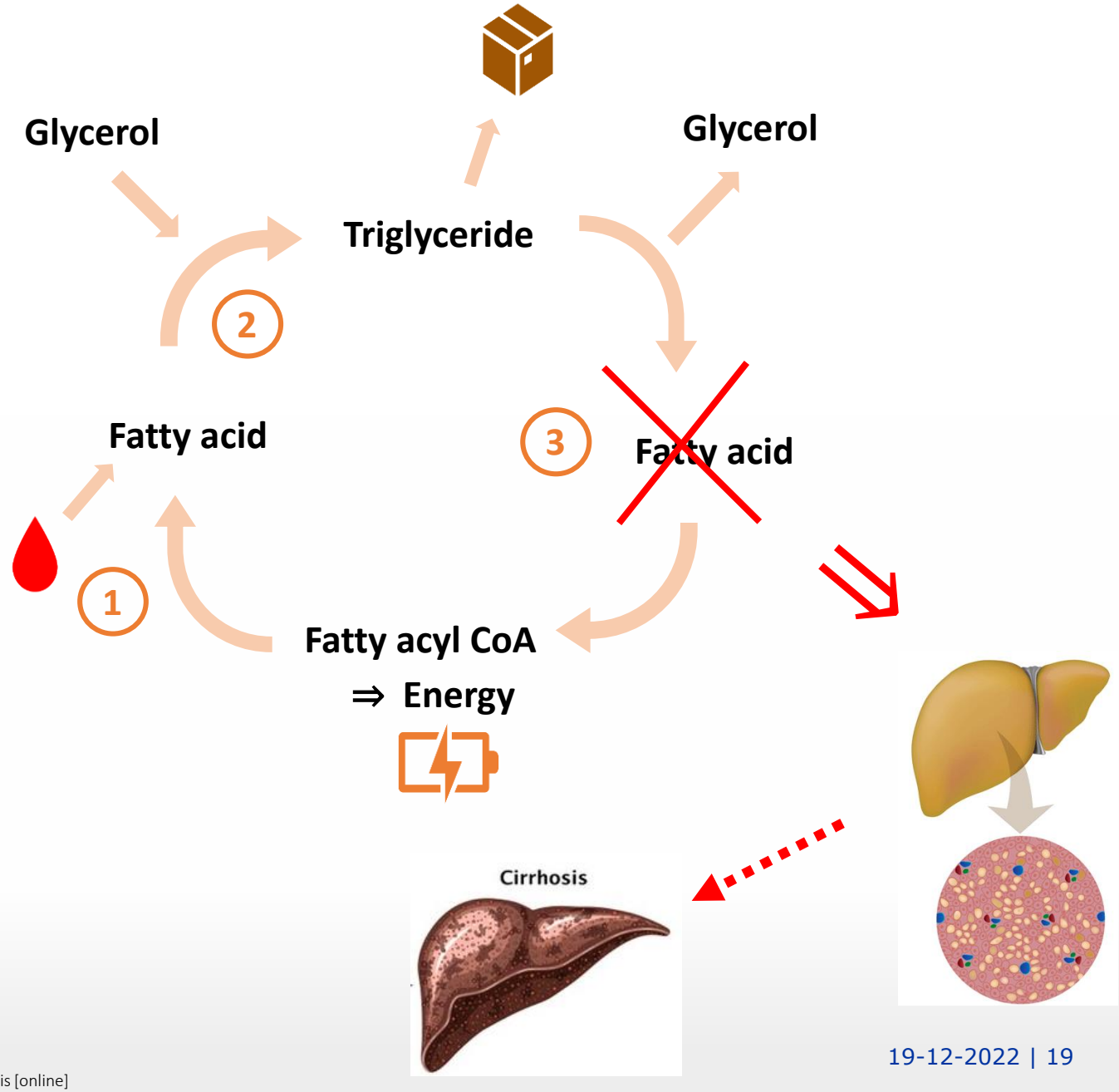
Background on topic

1 Steatosis

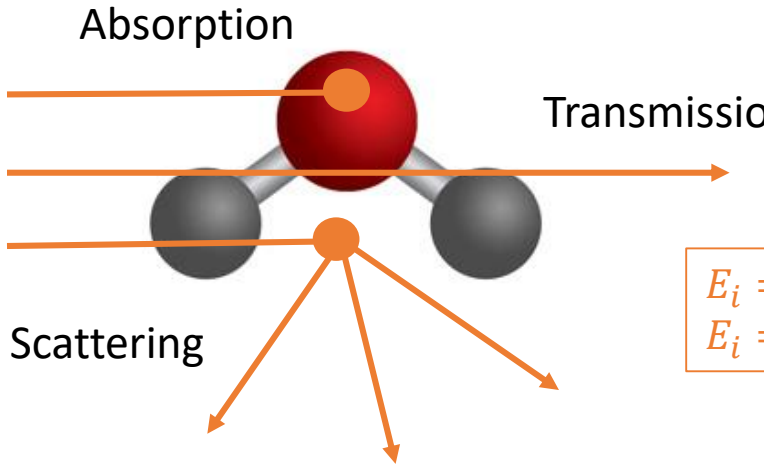


Sources:
1) https://www.shutterstock.com/nl/search/cirrhosis [online]

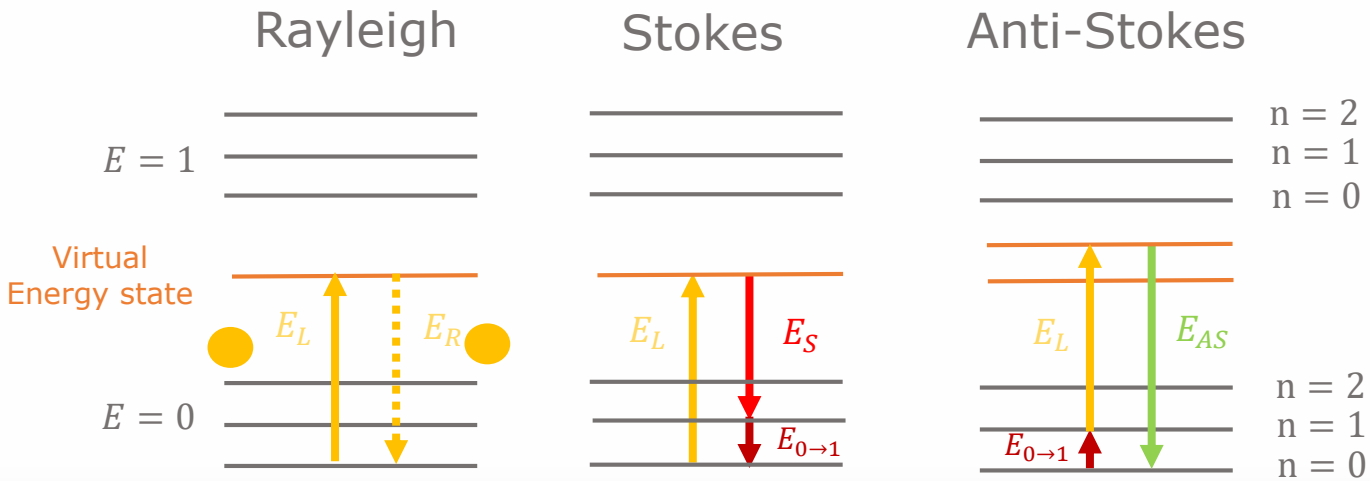
Background on topic

1 Steatosis

2 Raman spectroscopy



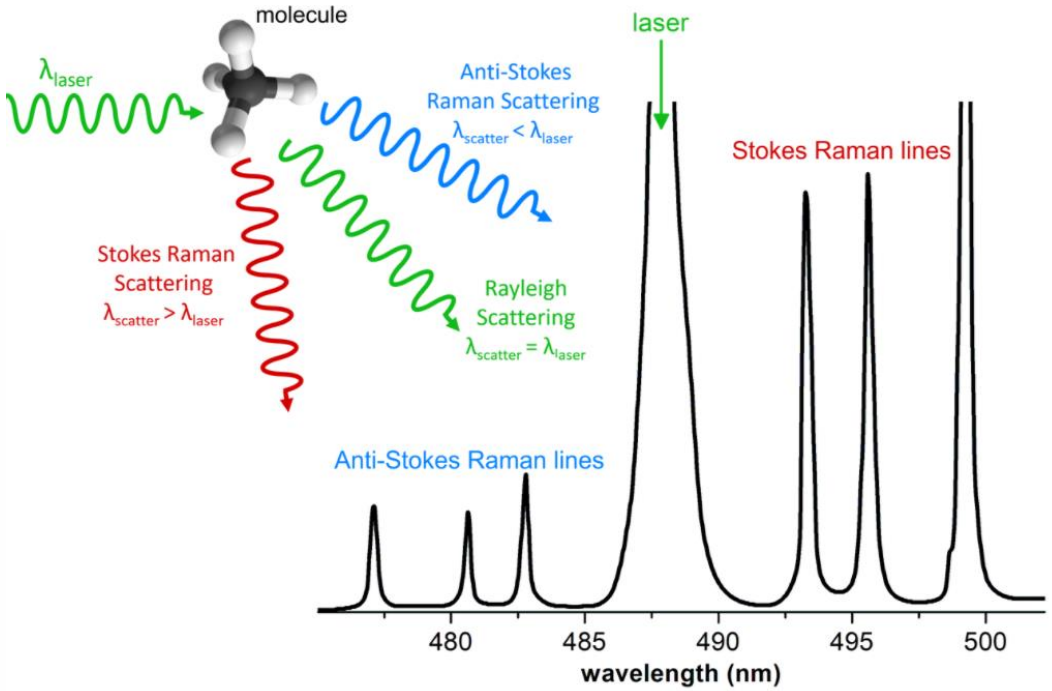
$E_i = E_s$ Rayleigh scattering
$E_i \neq E_s$ Raman scattering



$E_L = E_R$ $E_S = E_L - E_{0\to1}$ $E_{AS} = E_L + E_{0\to1}$

Sources:
1) https://www.middleschoolchemistry.com/lessonplans/chapter2/lesson2 [online]

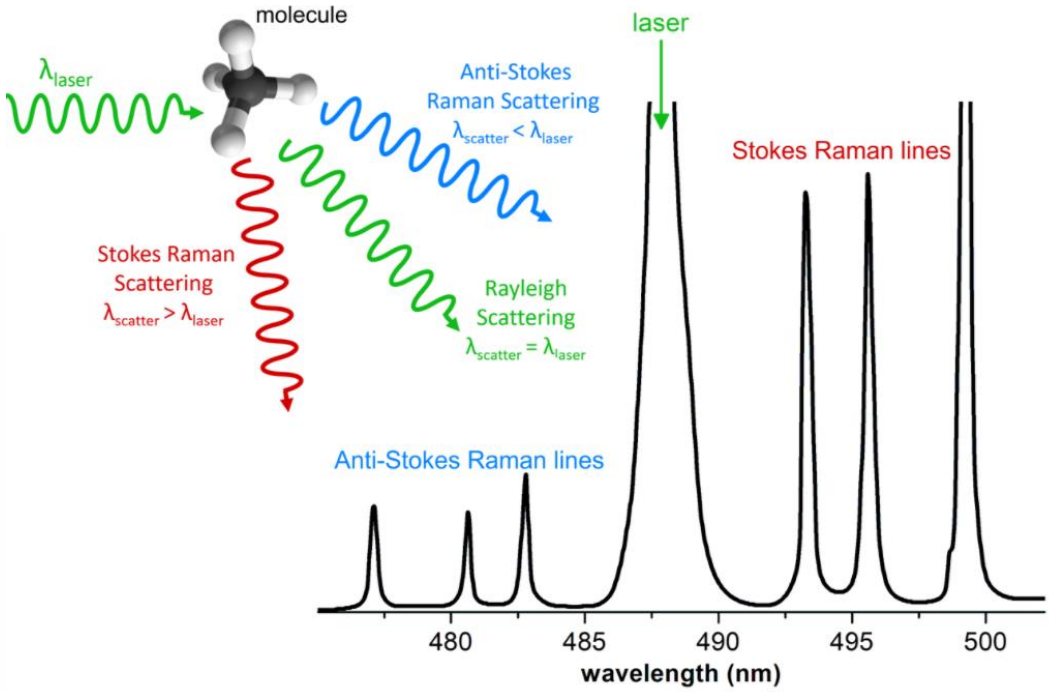
Raman Spectroscopy



$$I = Kl\alpha^2\omega^4$$

This is a very weak phenomenon such that only one in every 10^6 – 10^8 photons may experience it in a short time on the order of a femtosecond.

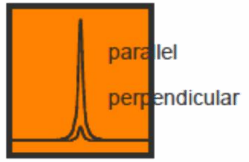
Raman Spectroscopy



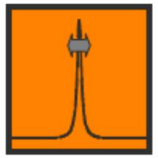
Information from Raman Spectroscopy

characteristic Raman frequencies → composition of material

changes in frequency of Raman peak → **Stress/strain state**



polarisation of Raman peak → crystal symmetry and orientation



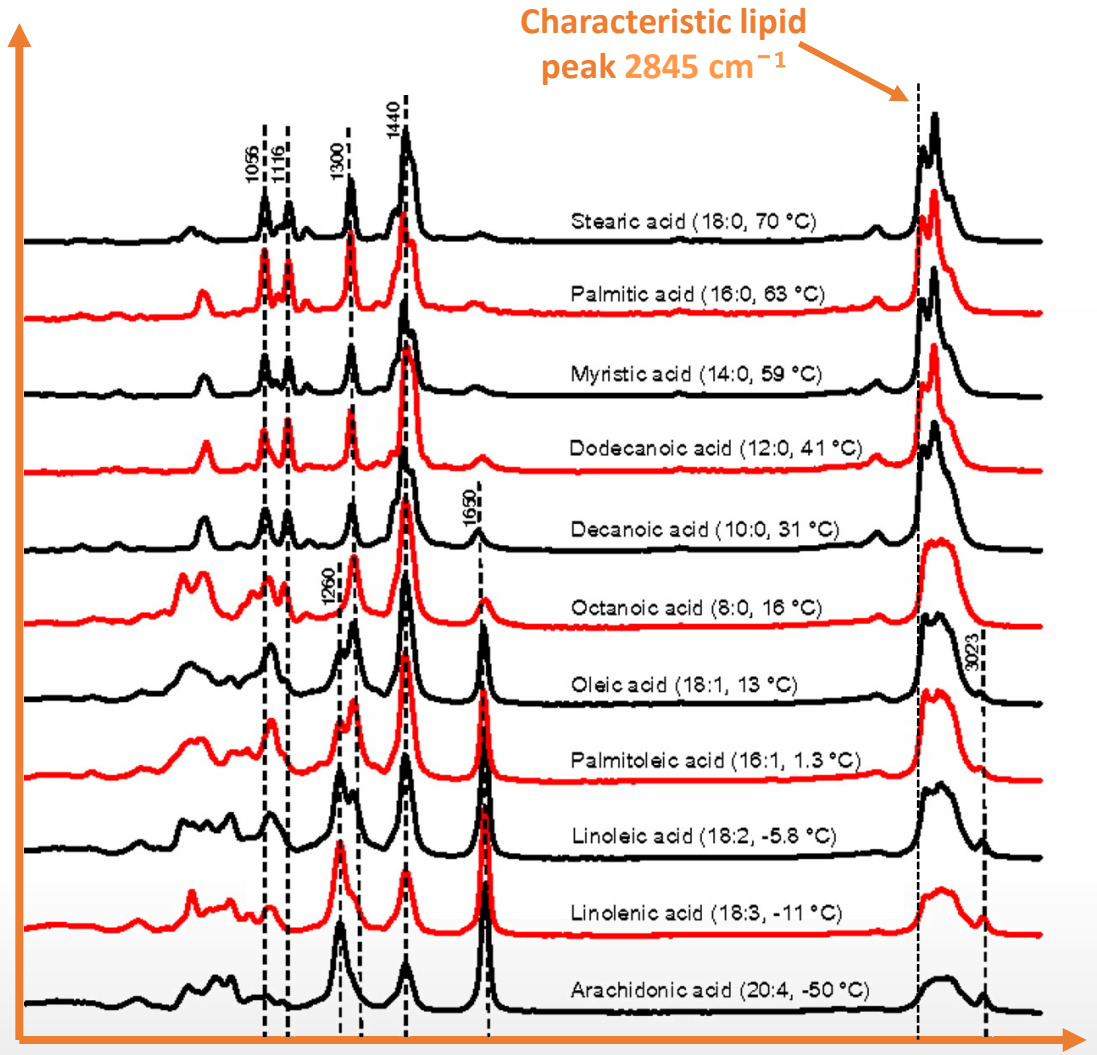
width of Raman peak → quality of crystal

intensity of Raman peak → amount of material

https://sisu.ut.ee/heritage-analysis/book/32-raman-spectroscopy

Background on topic

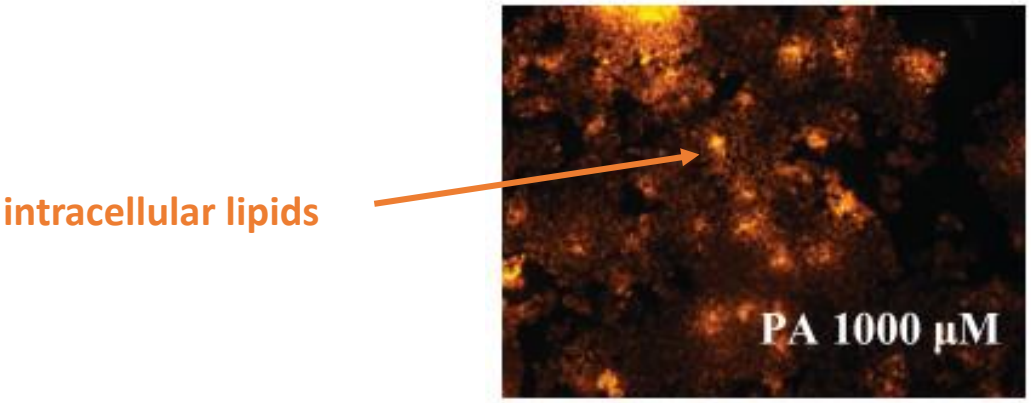
1	Steatosis
2	Raman spectroscopy

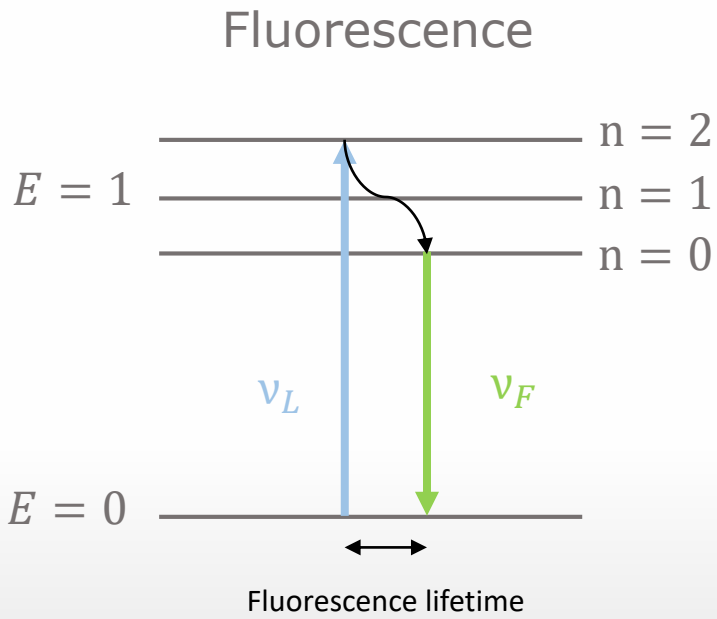


Sources:
1) NAJBJERG, Heidi, et al. Monitoring cellular responses upon fatty acid exposure by Fourier transform infrared spectroscopy and Raman spectroscopy. *Analyst*, 2011, 136.8: 1649-1658.

Background on topic

1	Steatosis
2	Raman spectroscopy
3	Fluorescence microscopy





Sources:
1) IZDEBSKA, Magdalena, et al. The beneficial effects of resveratrol on steatosis and mitochondrial oxidative stress in HepG2 cells. *Canadian journal of physiology and pharmacology*, 2017, 95.12: 1442-1453.

Sample preparation

Healthy HepG2 cells

Fatty HepG2 cells

BSA

PA/EtOH

BSA

PA/EtOH

Control Sample

BSA

EtOH

BSA

EtOH

Lipidox Green

Hoechst 33342

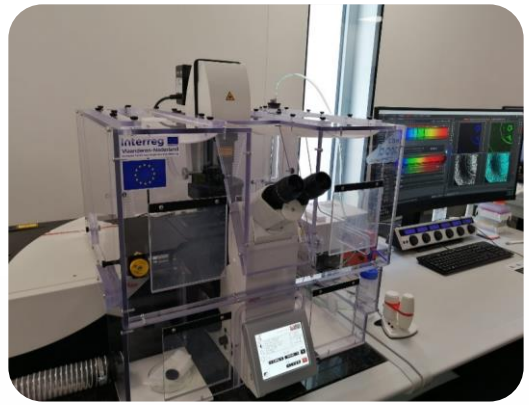
Texas red Phalloidin

2x PBS

25°C

~45 min

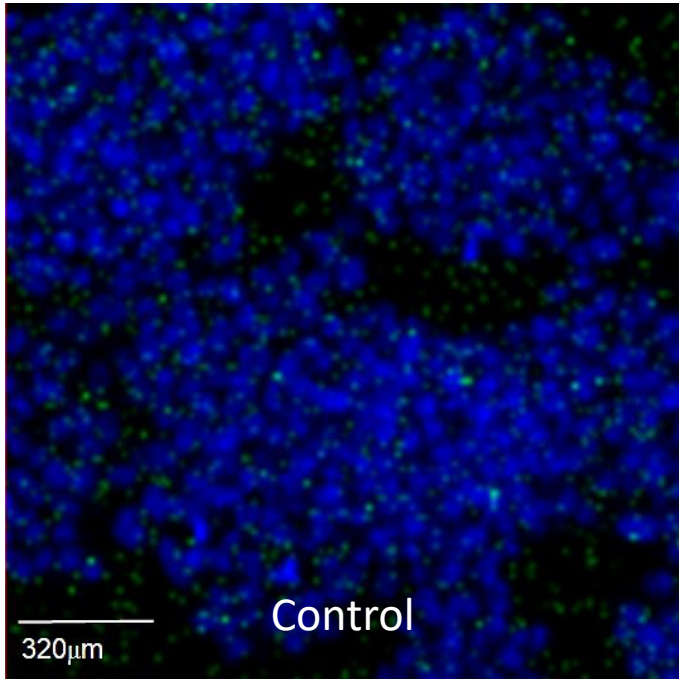
Confocal Fluorescence microscopy

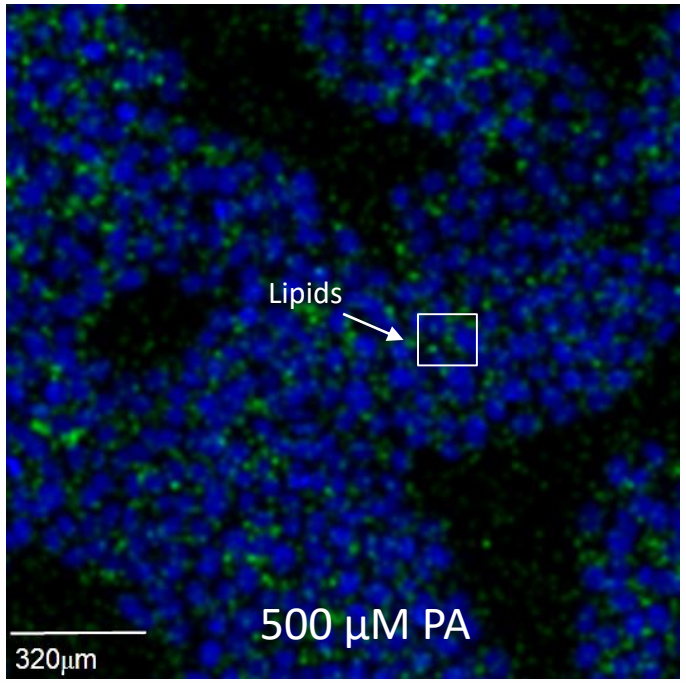


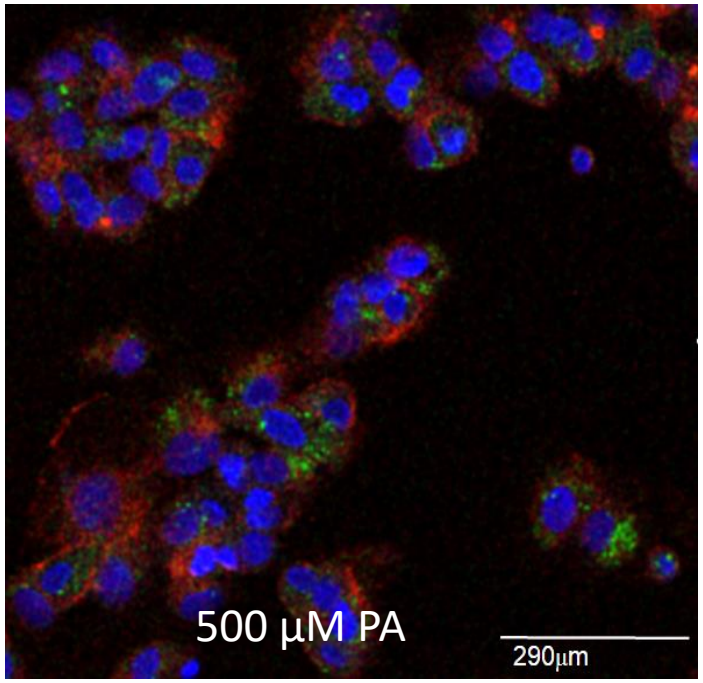
Confocal Raman spectroscopy

- 532 nm
- 785 nm

Intracellular lipid accumulation in control and PA-induced cells





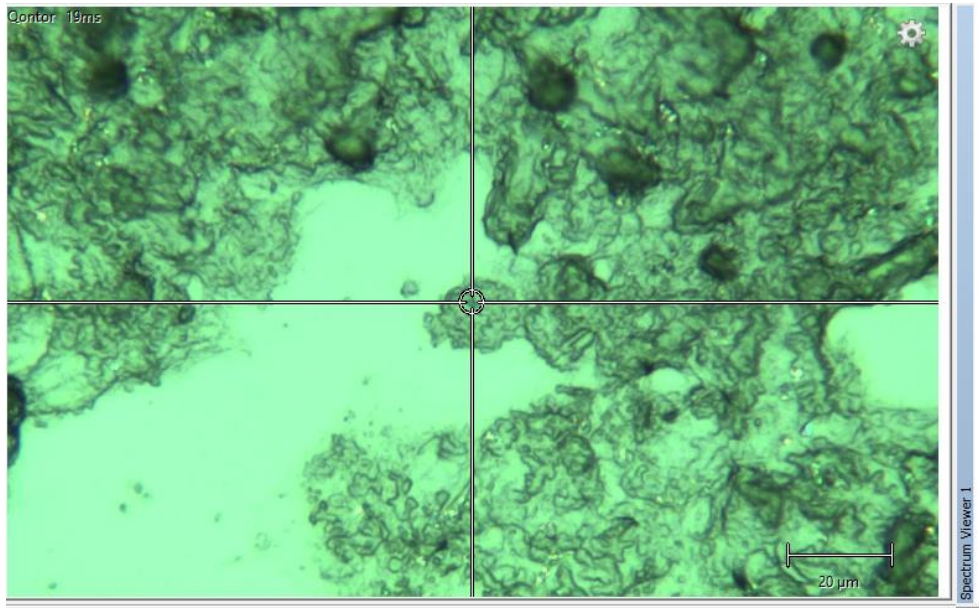


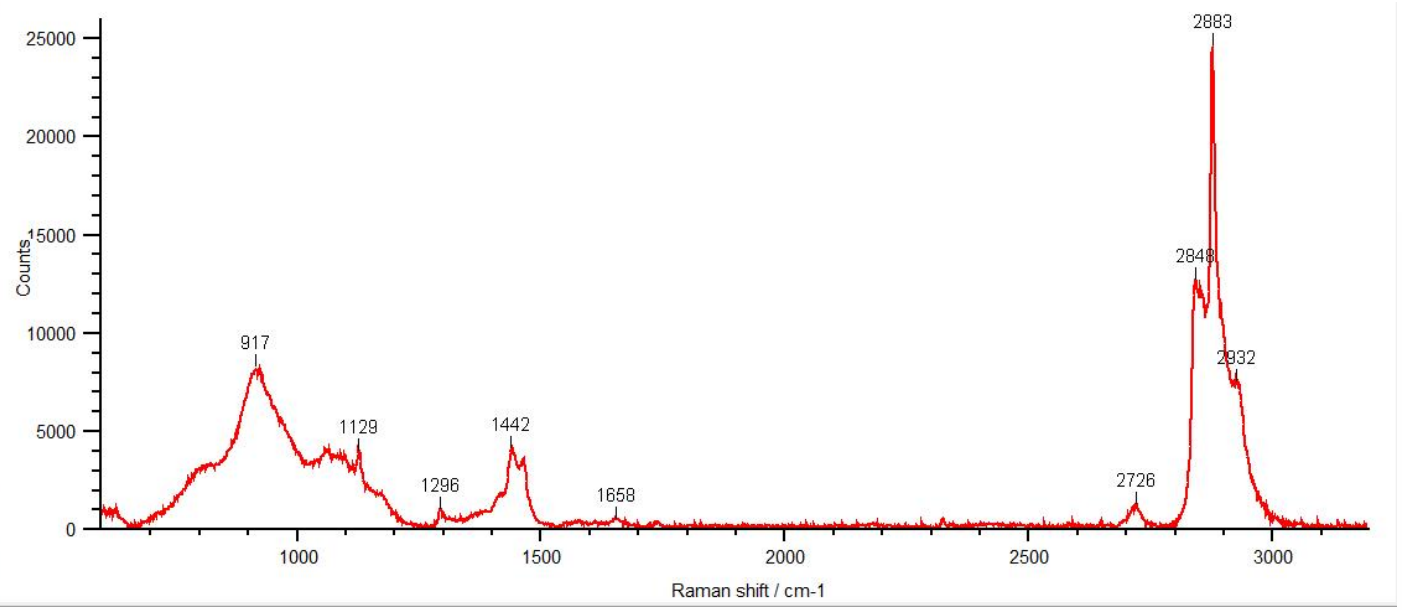
Lipid droplets

Cell nucleus

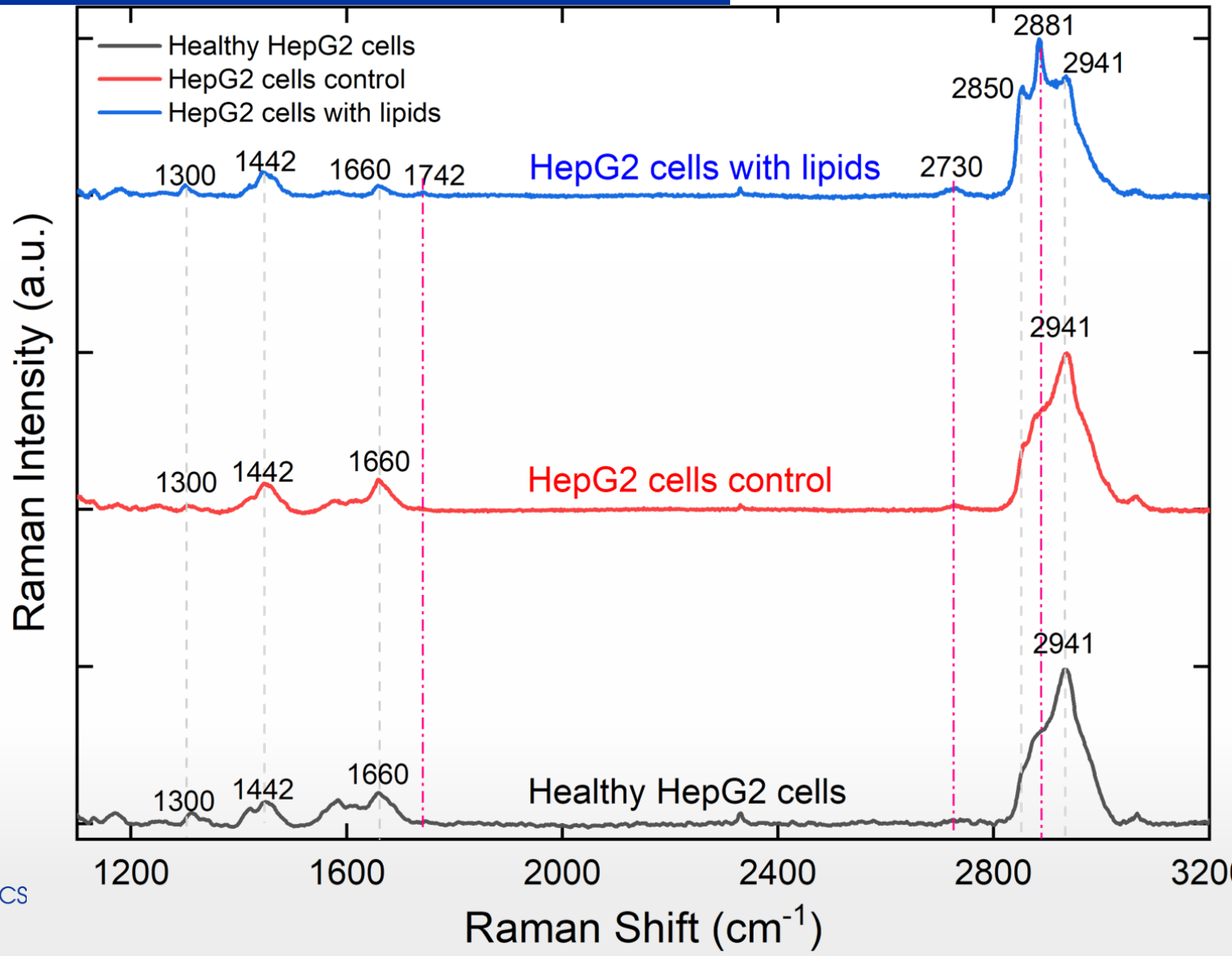
Cell volume

Raman spectroscopy of living cells

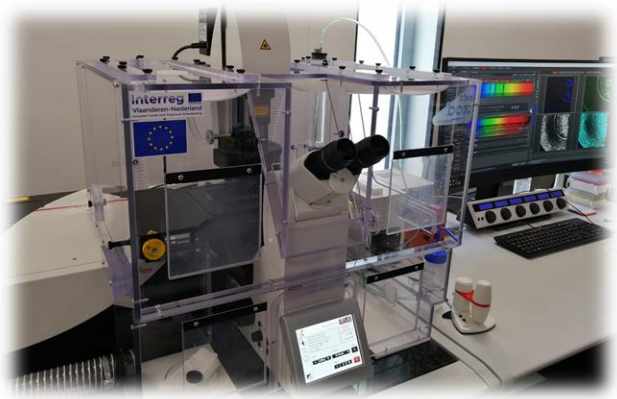




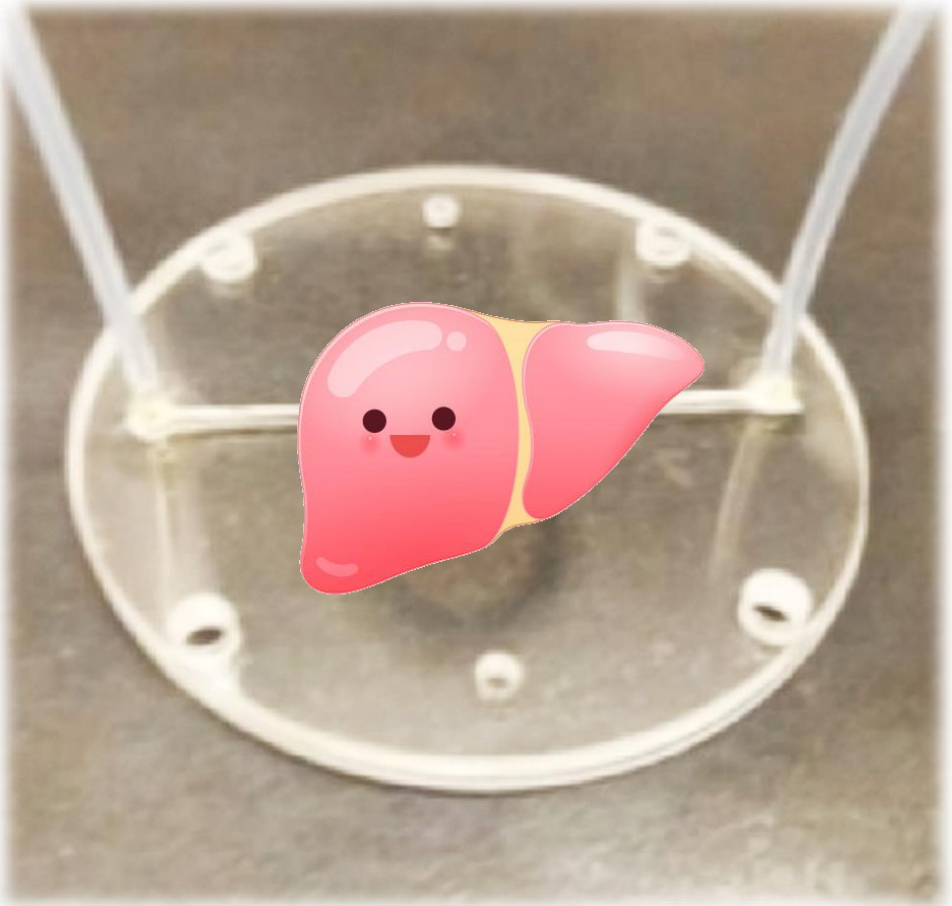
Raman spectroscopy of living cells

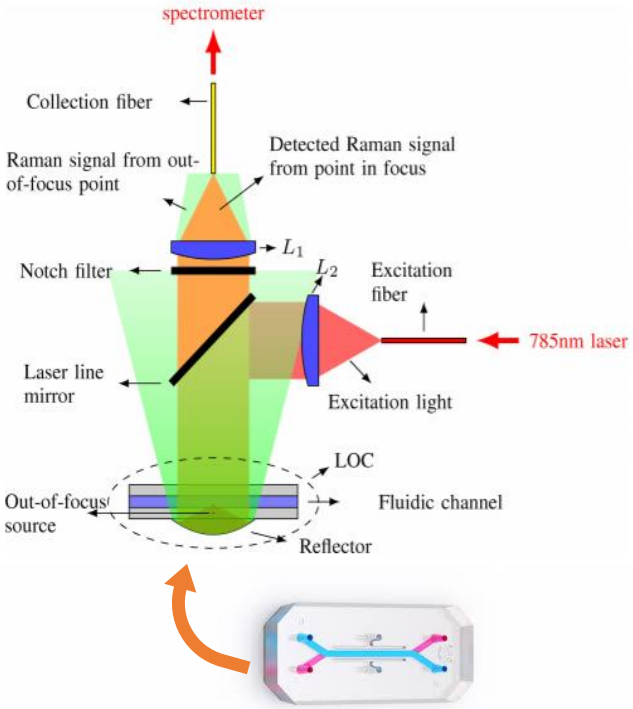


What next?




RENISHAW




spectrometer
Collection fiber
Raman signal from out-of-focus point
Detected Raman signal from point in focus
L_1
L_2
Notch filter
Excitation fiber
785nm laser
Laser line mirror
Excitation light
LOC
Out-of-focus source
Fluidic channel
Reflector

Outline

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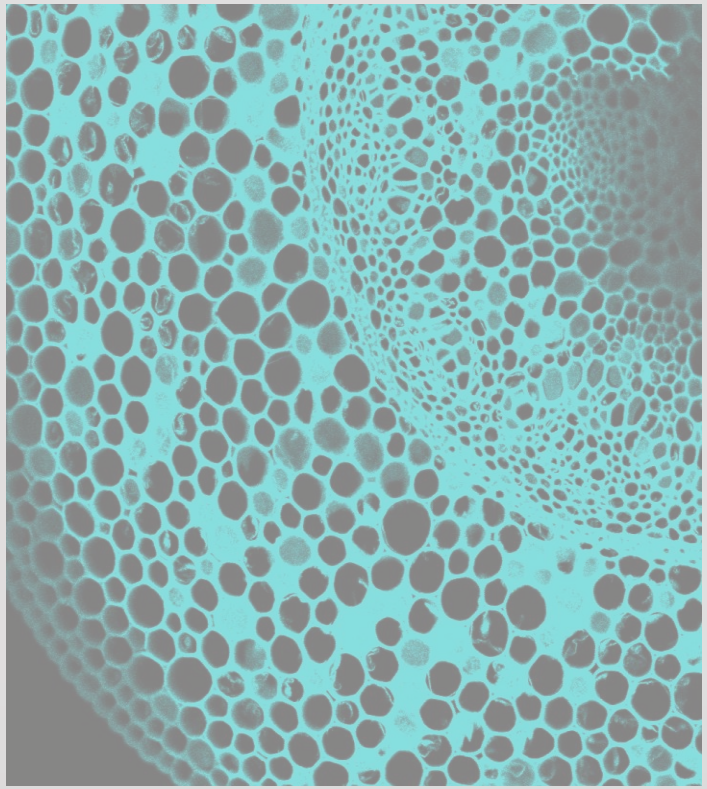
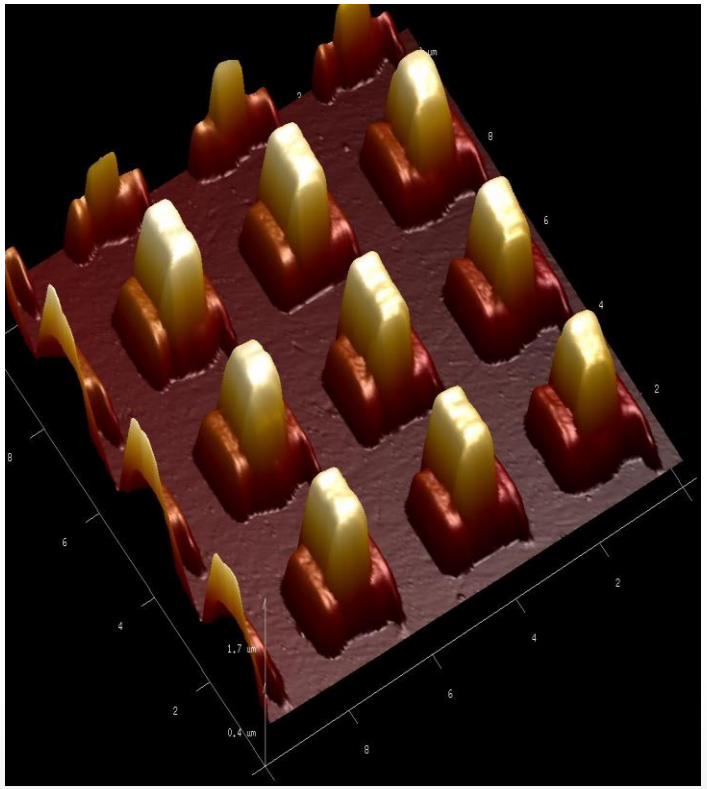
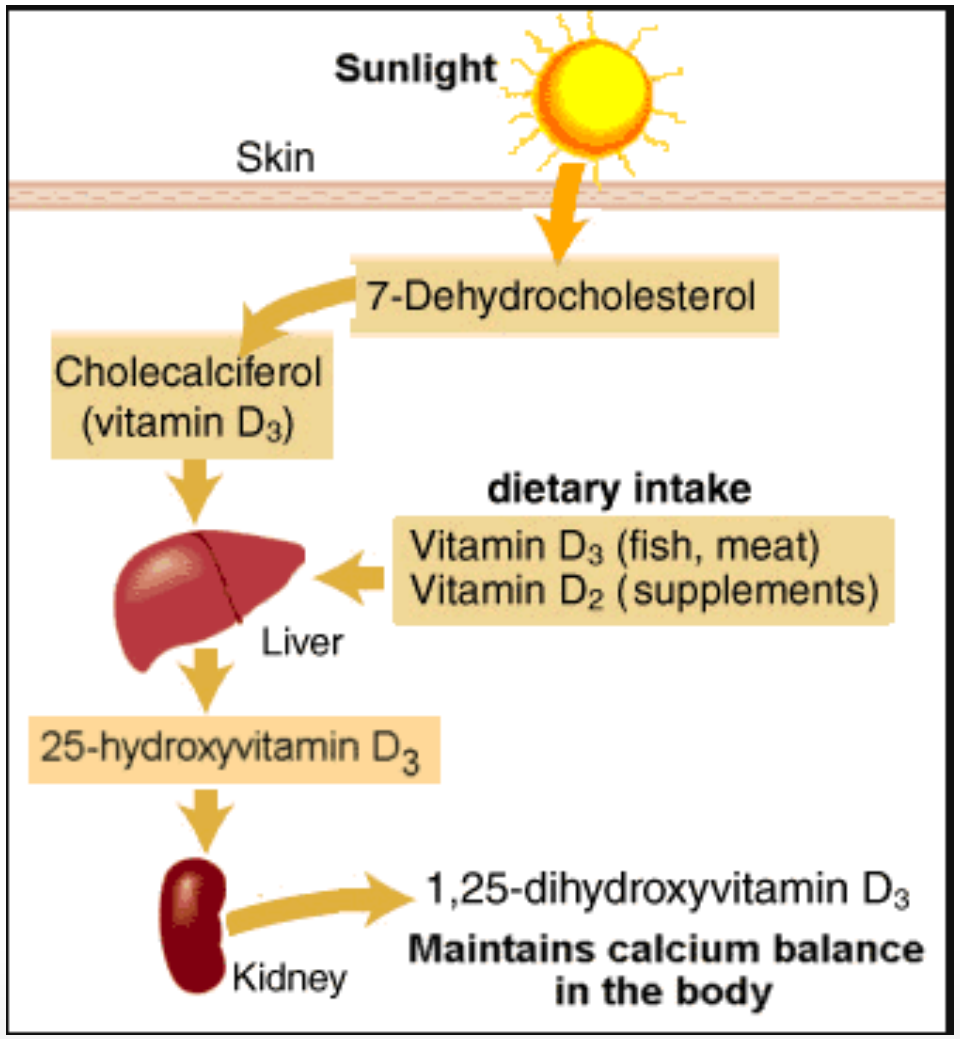


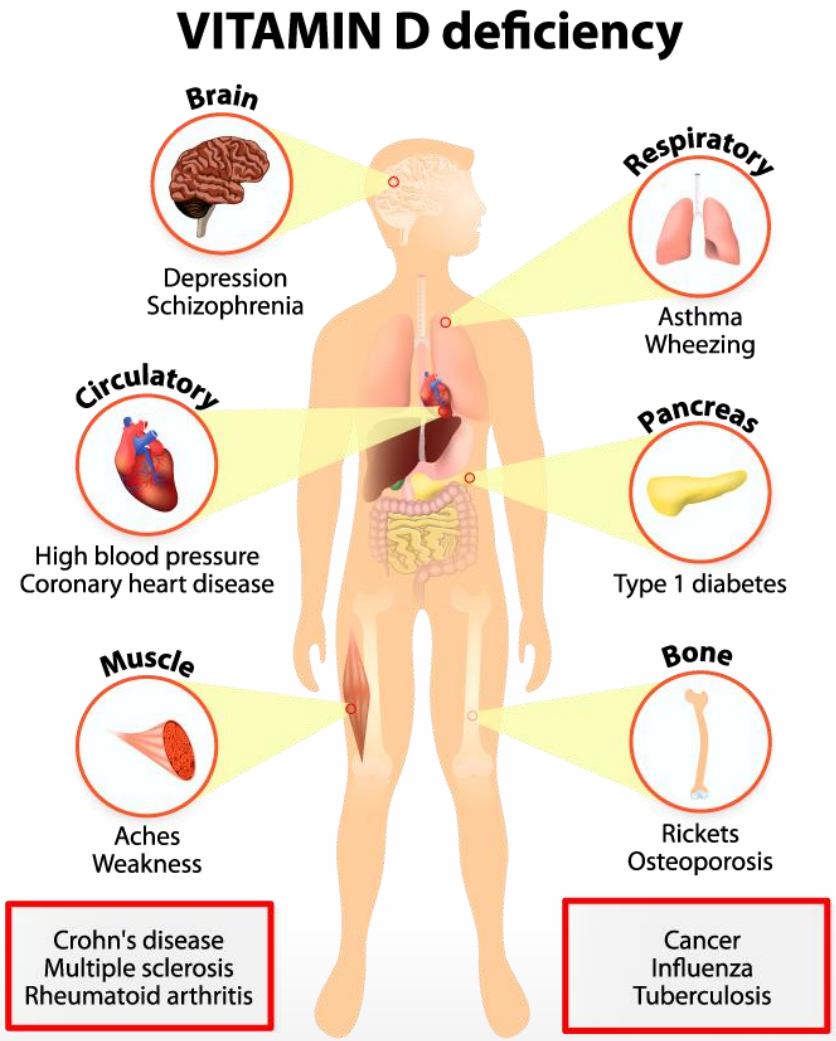
Surface Enhanced Raman Spectroscopy for Biosensing



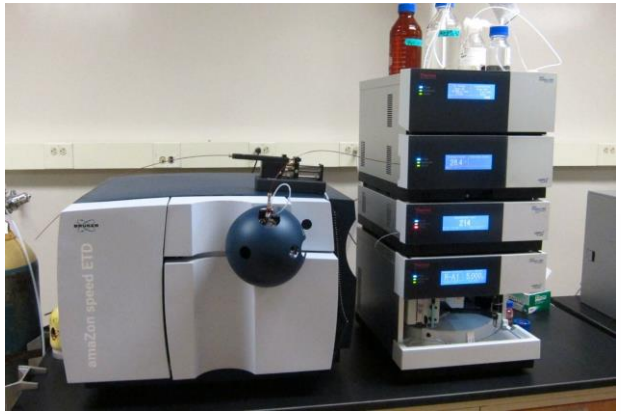
Microplastic Detection In Water: MONPLAS

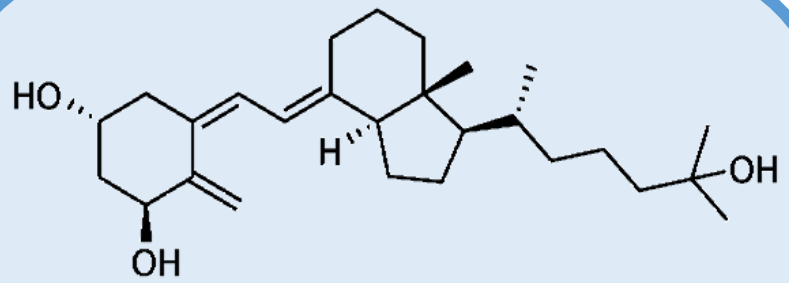
Vitamin D importance in our life





Vitamin D importance in our life





$75 - 125\ nmol/L$

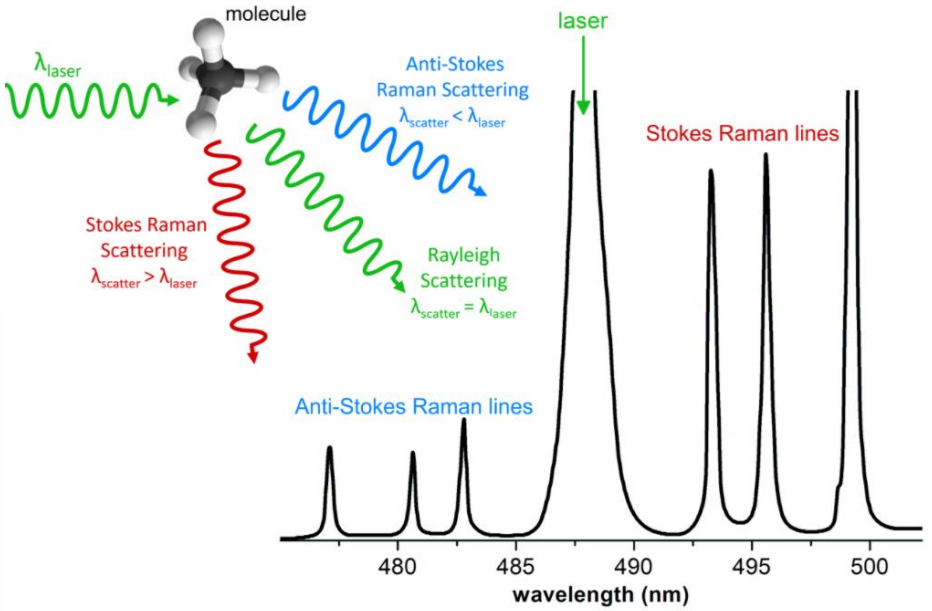
Standard measuring techniques (LC, MS)

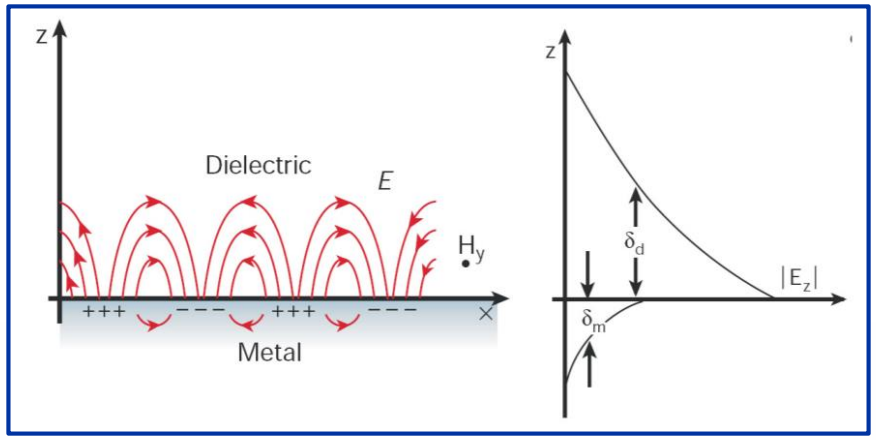
Heavy, expensive and non-user friendly

Detection with **SERS**

Smaller, cheaper and faster

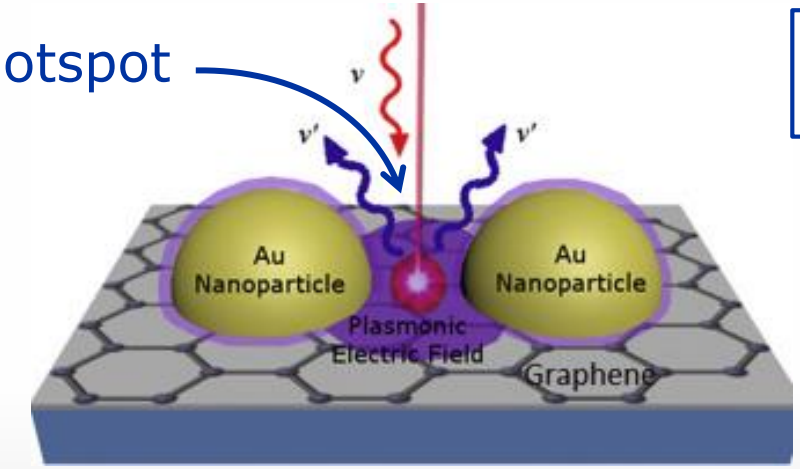
Surface enhancement Raman spectroscopy





PROBLEM → Weak signal
(1 of ~10^6 photons)

Hotspot



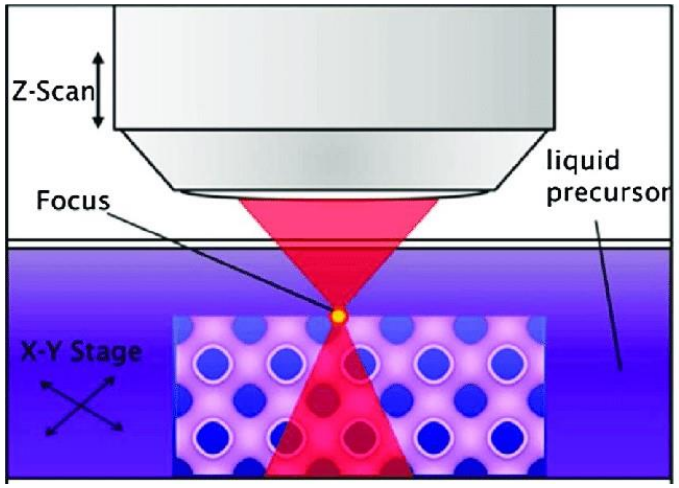
$$EF \cong \frac{|E_{loc}(\omega_i)|^4}{|E_o(\omega_i)|^4}$$

SERS Substrates

SERS substrate	EF	Synthesis	Dimensions
Roughened electrodes	$10 - 10^6$	Electrochemical ORC Chemical etching	$25 - 500nm$ (surface protrusion)
Colloidal nanoparticles, nanocore-shells	$10^4 - 10^9$	Chemosynthesis	$10 - 300nm$ (diameter)
Metal island films	$10^2 - 10^{12}$	Thermal evaporation Sputtering Electrochemical deposition	$5 - 200nm$ (thickness)
Periodic nanostructures	$10^4 - 10^9$	Lithography 2PP Chemical deposition Chemical etching Etc.	$10 - 500nm$ (diameter, pitch)

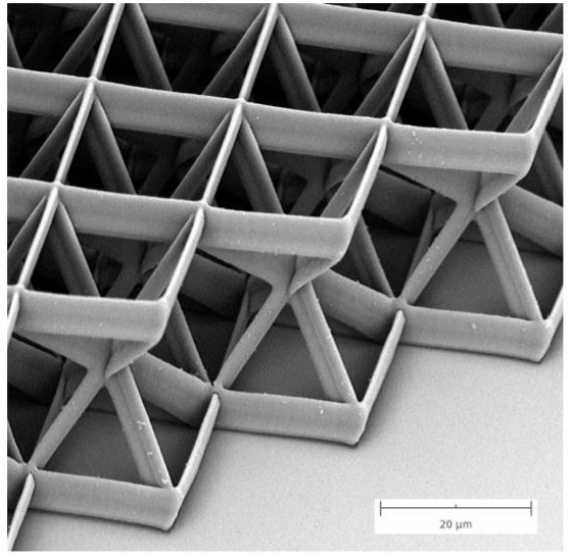
Freeform-based optofluidic devices towards surface-enhanced Raman spectrosocopy (SERS), Qing Liu, April 2020.

Two photon polymerization



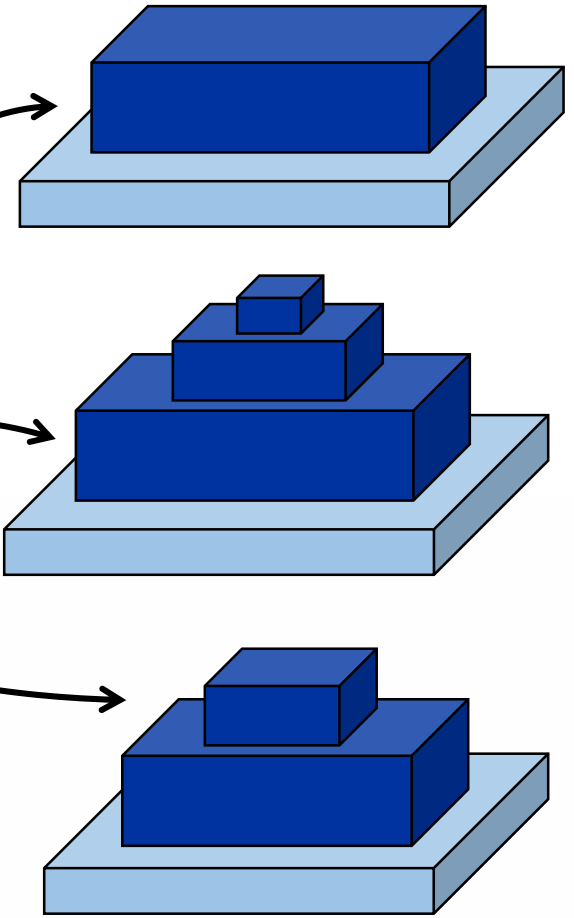
Result

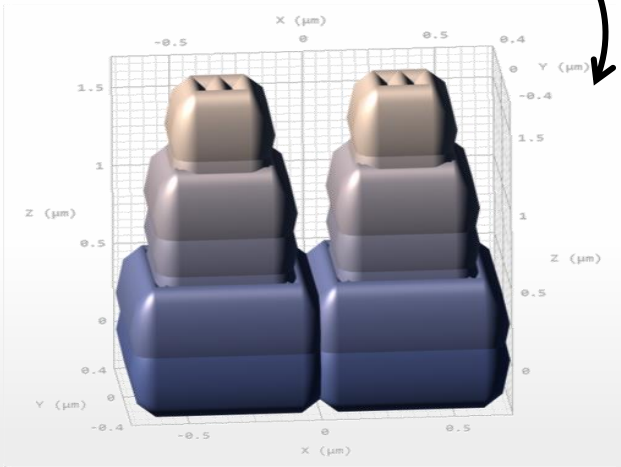
Two photon polymerization



Two photon polymerization fabrication

Structure geometry	Name	Lateral length L	Height h	Pitch p	Fabrication
Rectangular nanopillar	Rectangle 1	867nm	300nm	38nm	Normal
Rectangular nanopillar	Rectangle 2	867nm	300nm	160nm	Normal
Three stair pyramid	Three stairs 1	600nm	1400nm	86nm	Normal
Three stair pyramid	Three stairs 2	600nm	1400nm	140nm	Normal
Three stair pyramid	Three stairs 3	600nm	1400nm	180nm	Normal
Two stair pyramid	Two stairs 1	867nm	600nm	38nm	Normal
Two stair pyramid	Two stairs 2	867nm	600nm	100nm	Normal
Two stair pyramid	Two stairs 3	867nm	600nm	160nm	Normal
Two stair pyramid	Contour	867nm	600nm	38nm	With contour

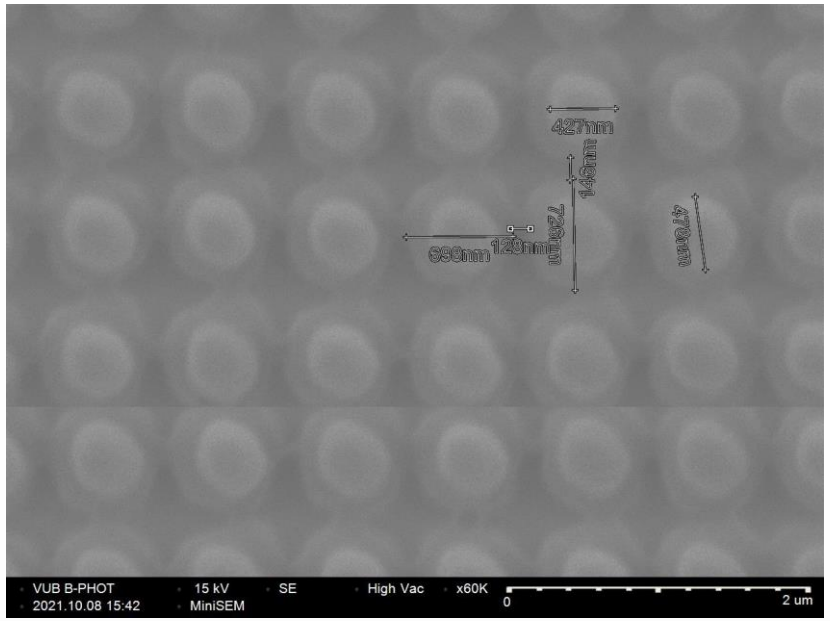




SERS measurements

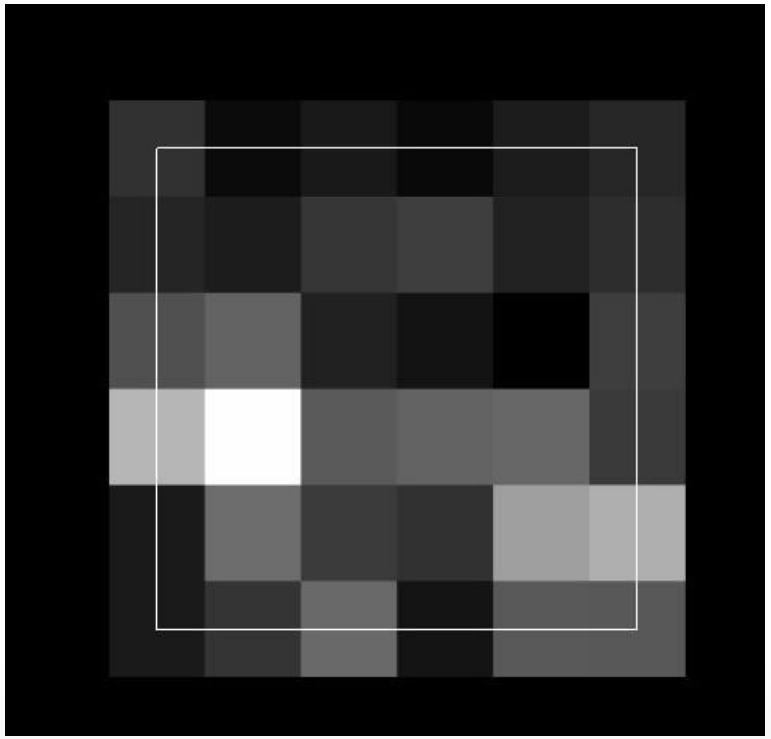
SERS substrate

$(50 \times 50 \mu m^2)$

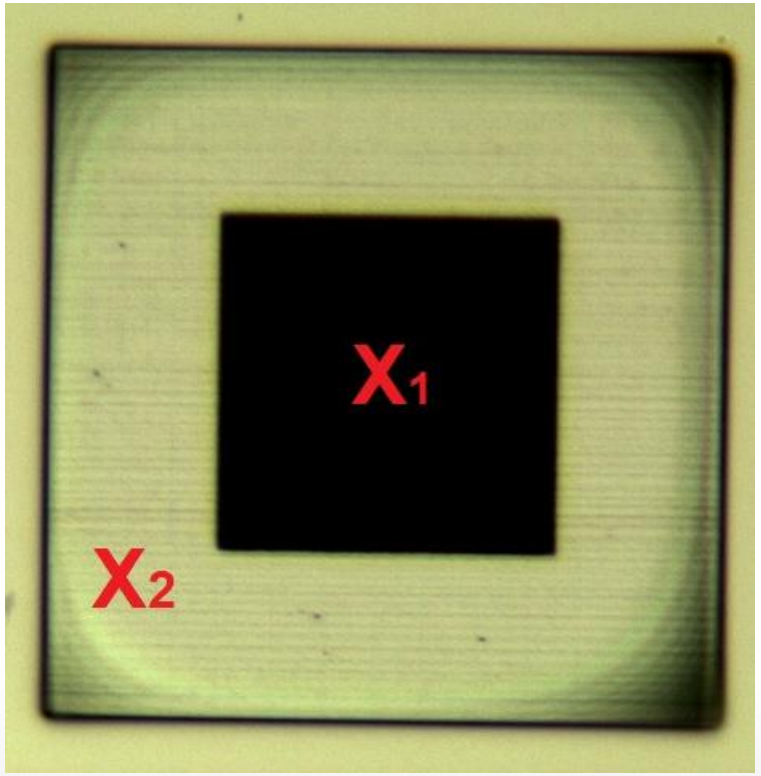


Map measurements

$(5 \times 5 \mu m^2)$

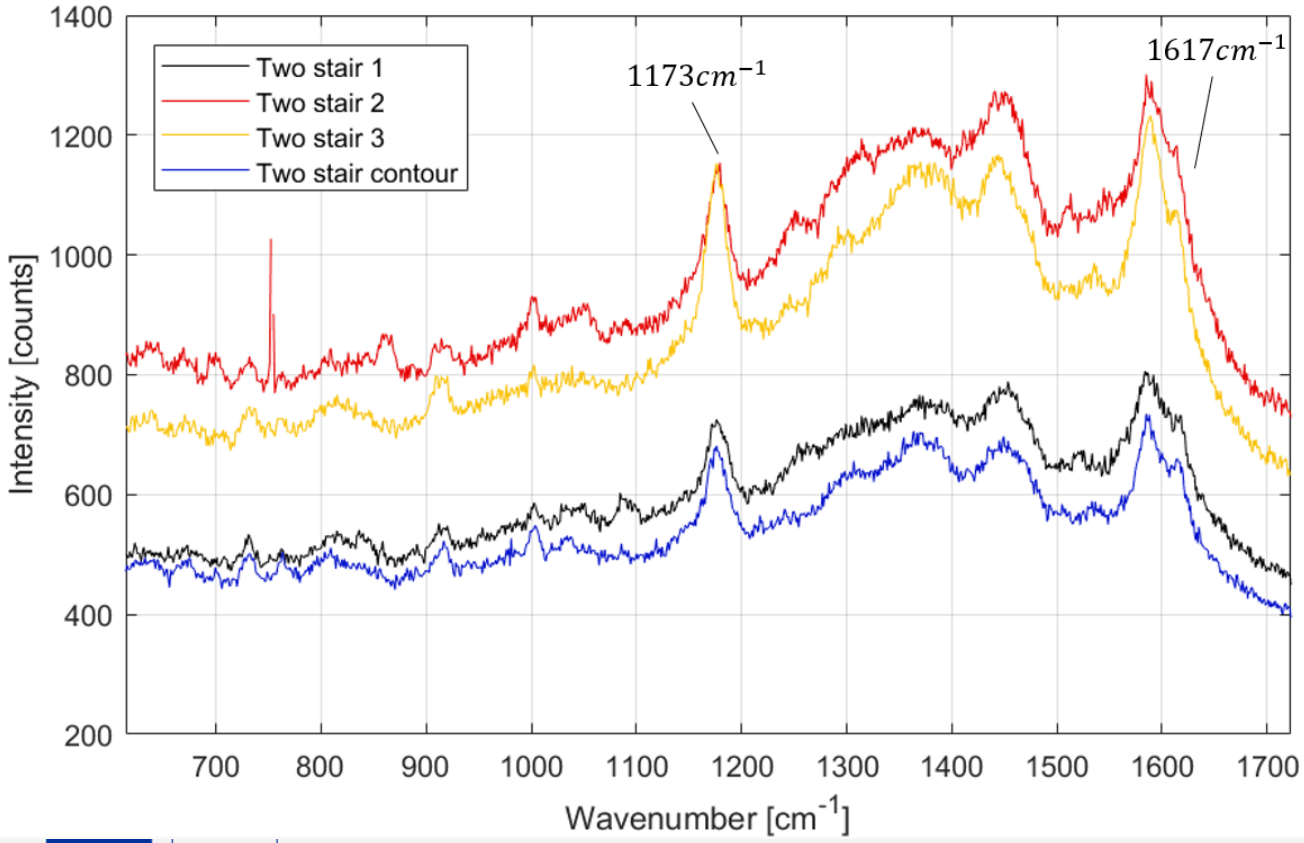


Single point measurements



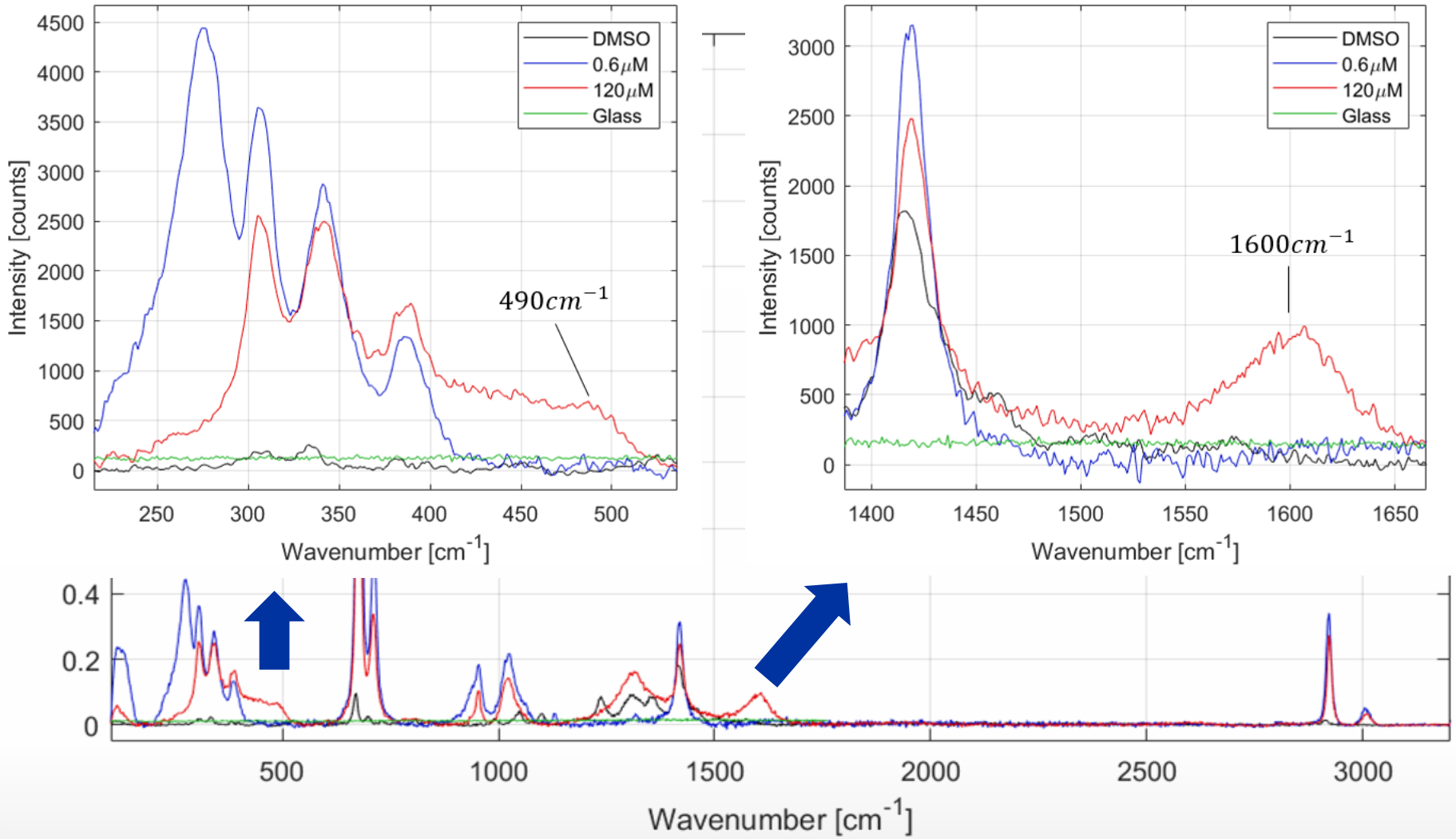
Crystal violet measurements

Two stair pyramid



- Rectangular nanopillar: no peak
- Three stair pyramid: sucessful detection
- Two stair pyramid: sucessful detection

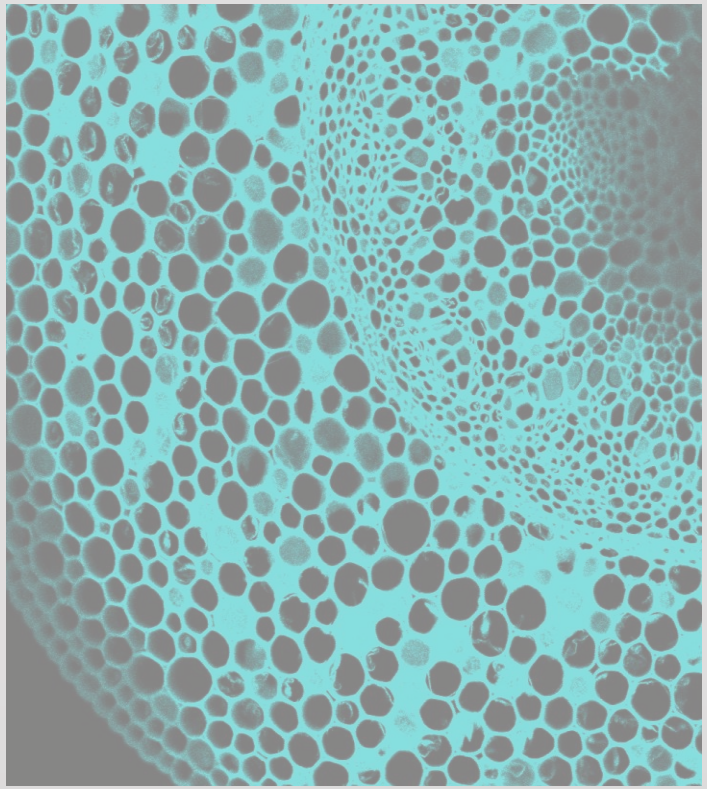
Vitamin D_3 measurements



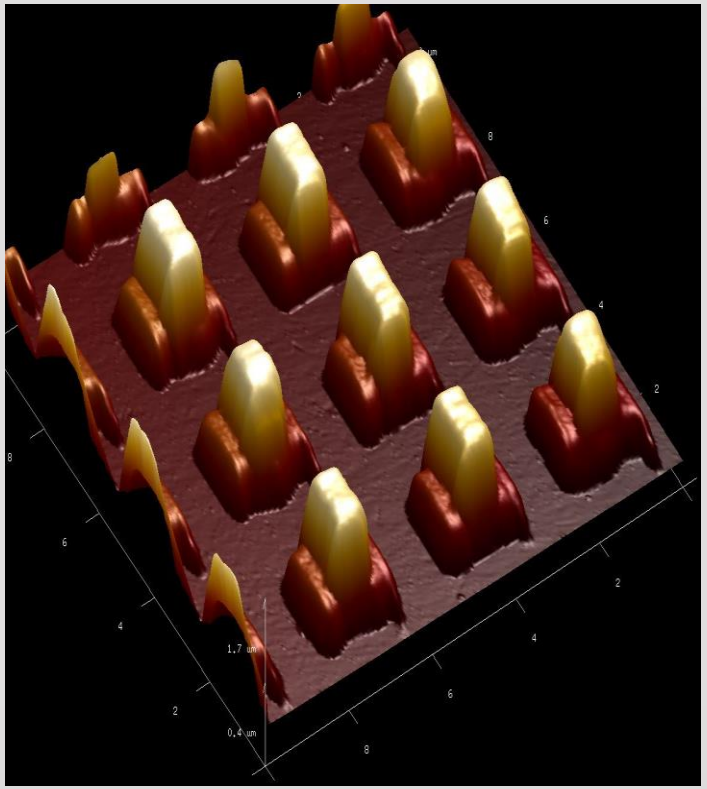
Optimisation is in progress…

Outline

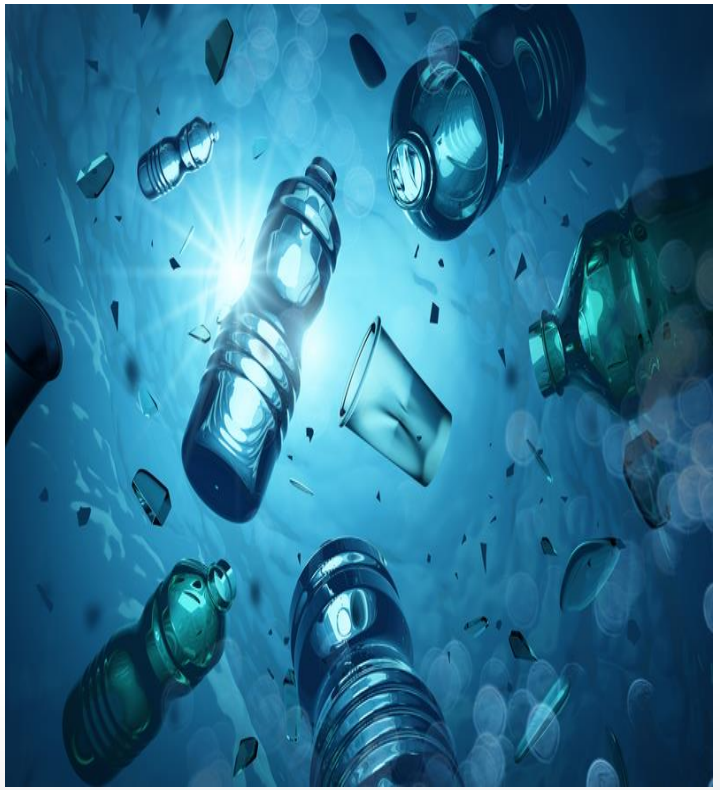
Towards Compact, Multimodal Spectroscopic Devices For The Read-out Of Microfluidic Organs-on-chip



Surface Enhanced Raman Spectroscopy for Biosensing



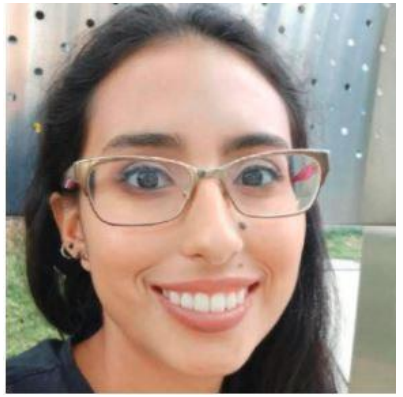
Microplastic Detection In Water: MONPLAS



Microplastic Detection In Water: MONPLAS







Microplastics

Terminology	Size range
i. Macroplastics	>2.5 cm
ii. Mesoplastics	0.5 – ≤ 2.5 cm
iii. Large microplastics	1 – ≤5 mm
iv. Small microplastics	1 μm – ≤ 1000 μm
v. Nano plastics	1nm – ≤ 1 μm

Polystyrene (PS)

Polypropylene (PP)

Polyethylene (PE)

Polyethylene terephthalate (PET)

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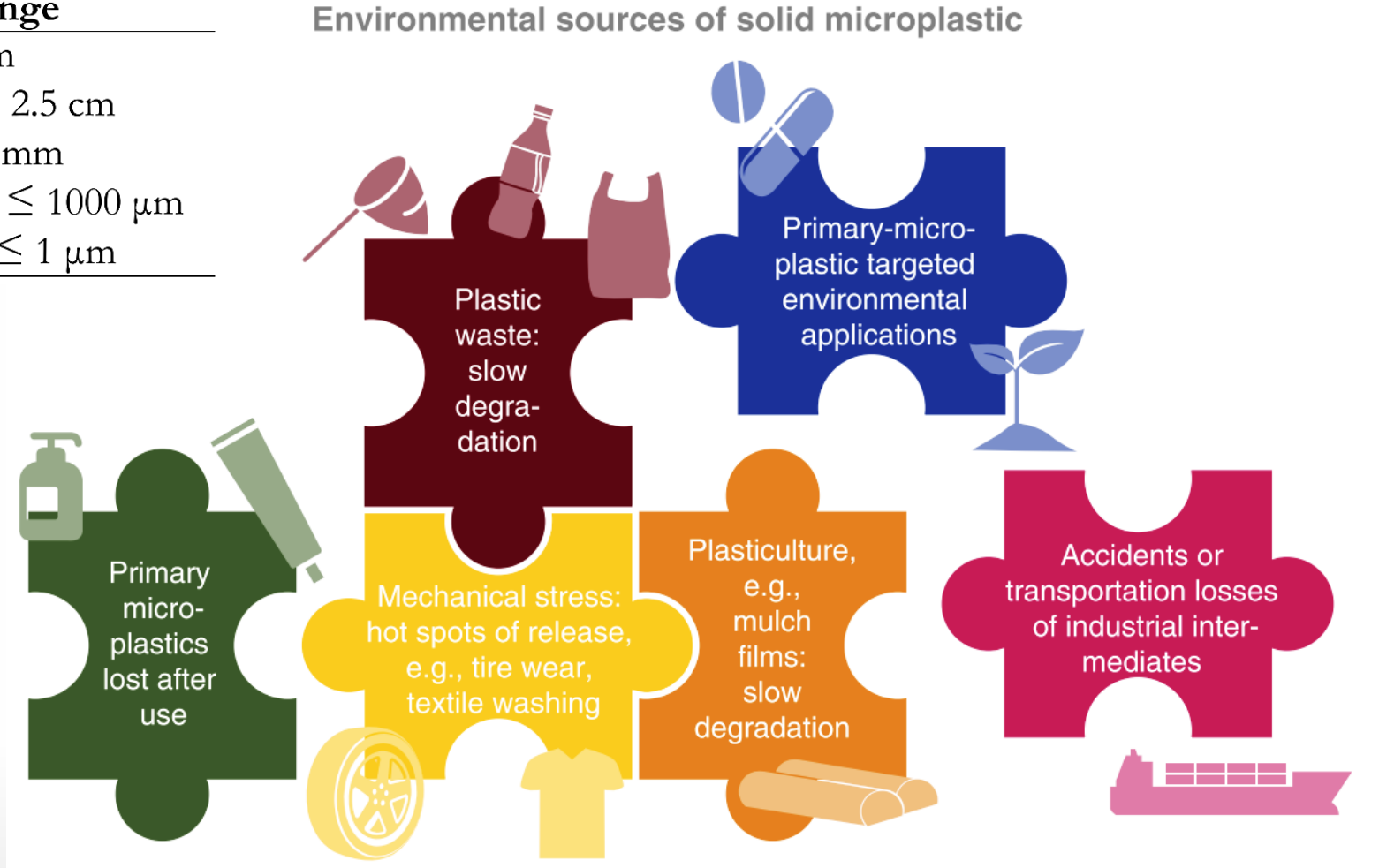


Gago, J. et al. 2019; Denise M. et al. 2020

Microplastics are ubiquitous in the environment. They have been found in **marine water, wastewater, fresh water, food, air and drinking-water, both bottled water and tap water**. (WHO, 2019)

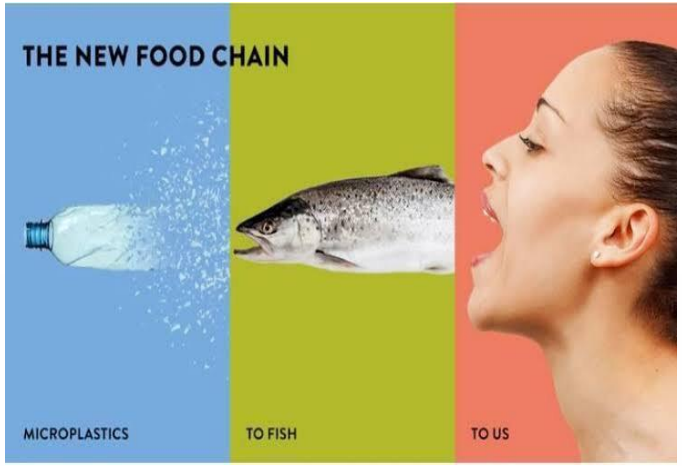
Microplastics revealed in the placentas of unborn babies

Health impact is unknown but scientists say particles may cause long-term damage to foetuses

Bottle-fed babies swallow millions of microplastics a day, study finds

Exposure is far higher than previously thought and also affects plastic food containers

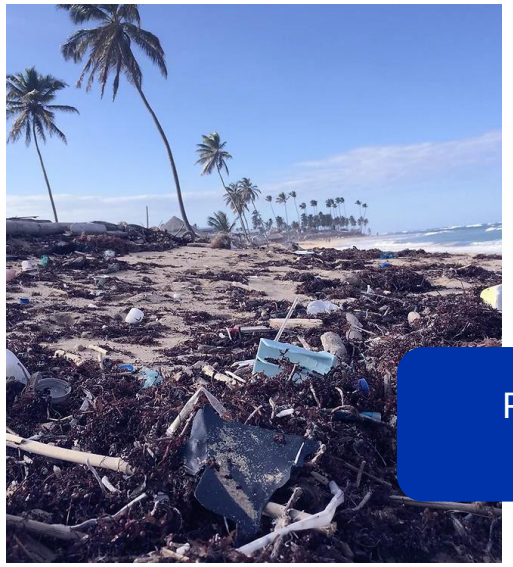
Microplastics are ubiquitous in the environment. They have been found in **marine water, wastewater, fresh water, food, air and drinking-water, both bottled water and tap water**. (WHO, 2019)



"Without any action, there will be <u>12 billion tonnes</u> of plastic in landfills and the environment by 2050."

MONPLAS: **MON**itor concentrations of micro and nano**PLAS**tics in water for their presence, uptake and threat to animal and human life

Background



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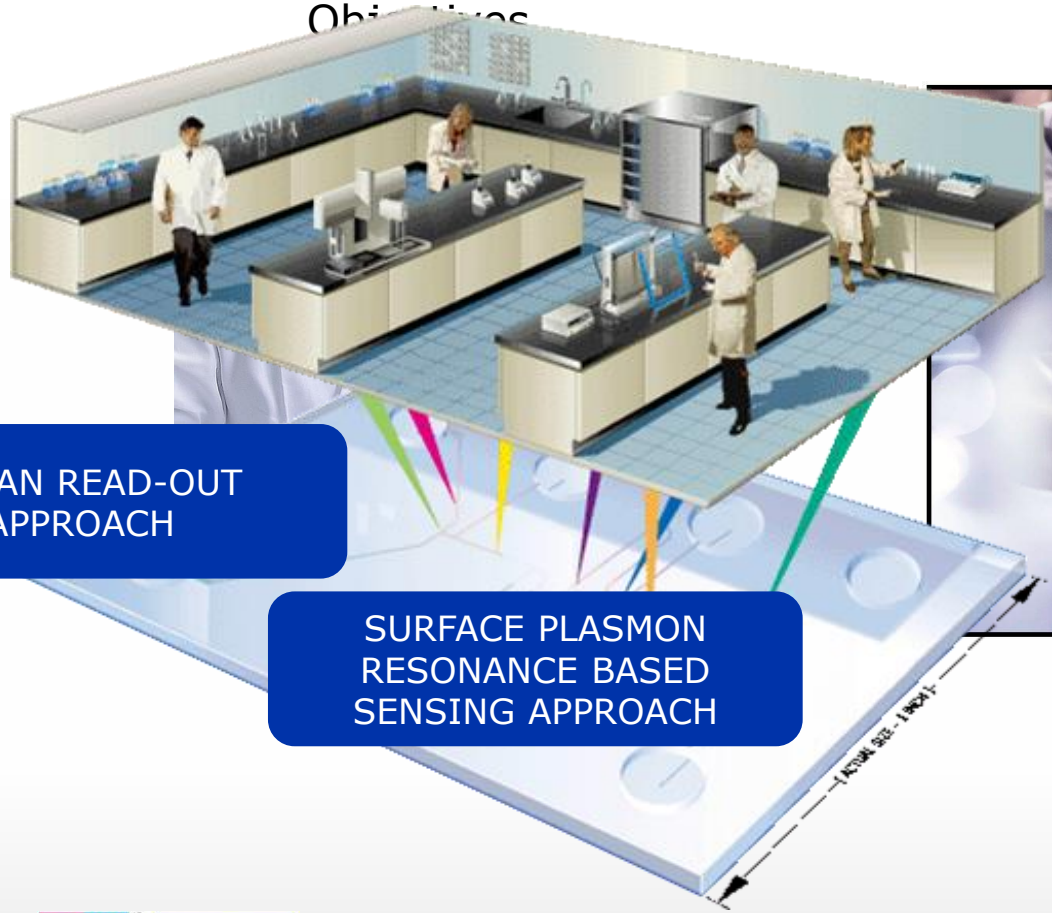


RAMAN READ-OUT APPROACH

SURFACE PLASMON RESONANCE BASED SENSING APPROACH

Innovation

Microplastic detection methods

Visual (microscopic) inspection

~500 µm

Py-GC-MS

~50/100 µm

- ✓ **Shape**
- ✓ **Size**
- ✓ **Number**
- ✓ **Mass**
- ✓ **Composition**

µ-FTIR

~10 µm

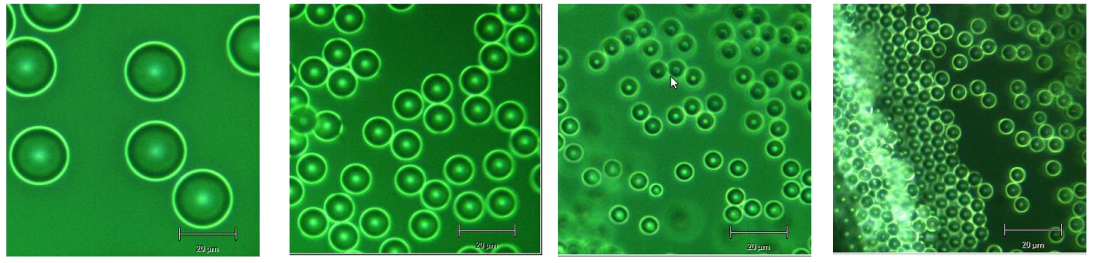
µ-Raman spectroscopy

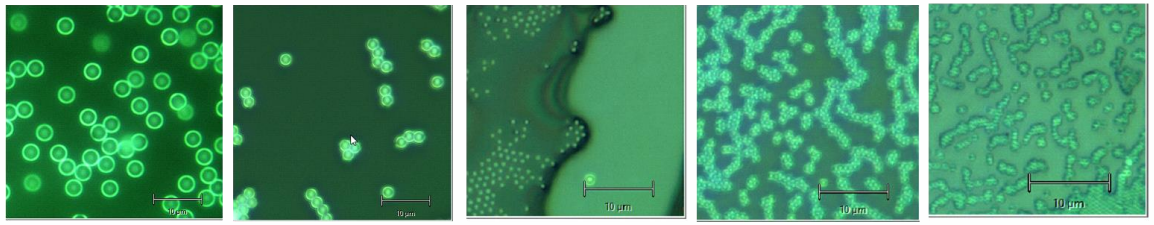
~1 µm

B-PHOT BRUSSELS PHOTONICS

L. Lv. et al. 2019; Claudia et al. 2020; Elkhatib et al. 2020.

WHAT NEXT?





Polystyrene (PS)

Polypropylene (PP)

Polyethylene terephthalate (PET)

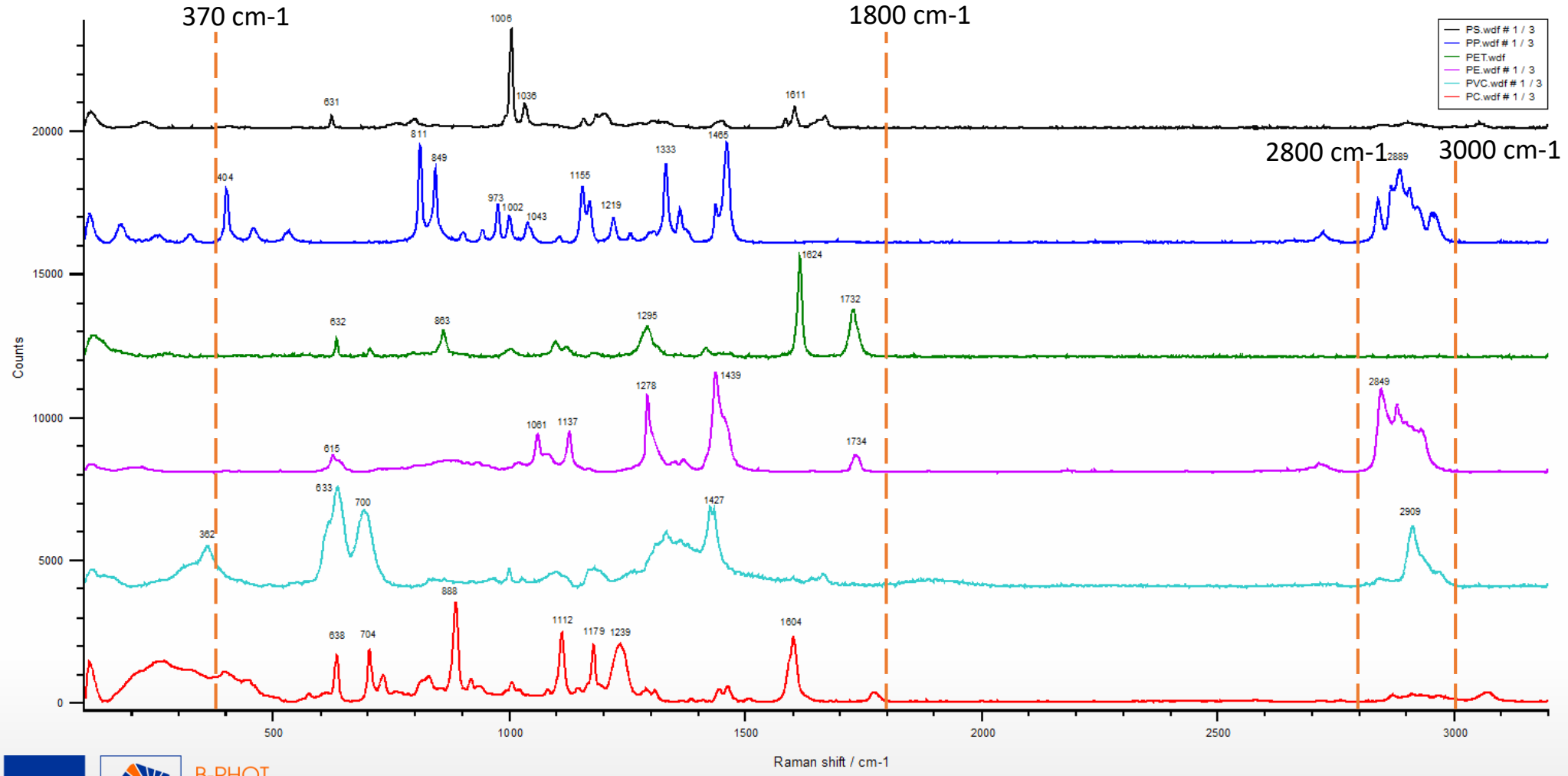
Polyvinyl chloride (PVC)

White colored Polycarbonate (PC)

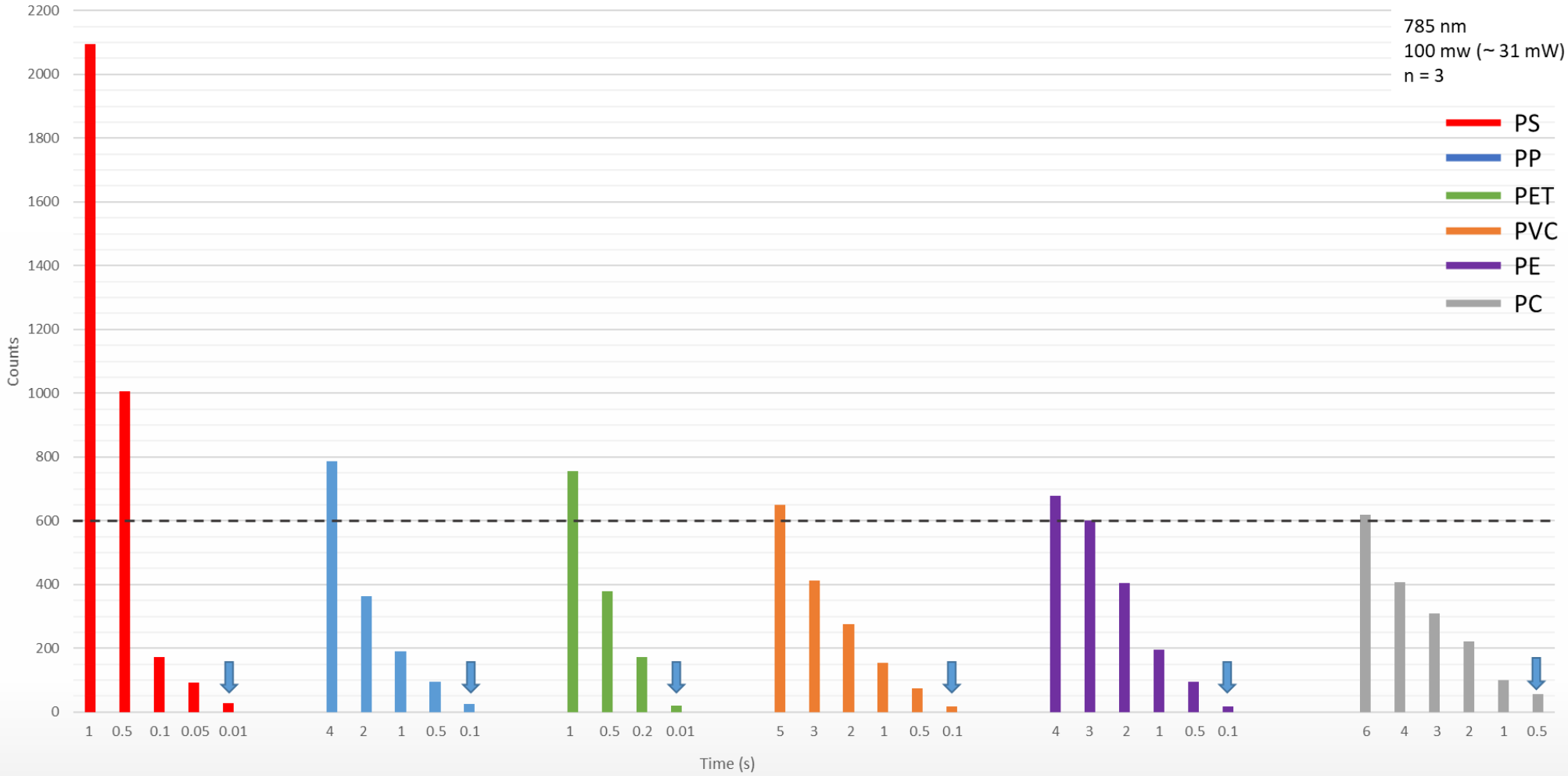
Polyethylene (PE)

VUB B-PHOT BRUSSELS PHOTONICS

Region of interest



Optimal parameters for various plastics



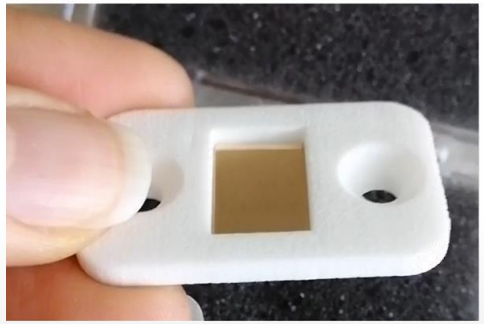
Our solutions to the problem

Raman spectroscopy:

Identification of microplastics

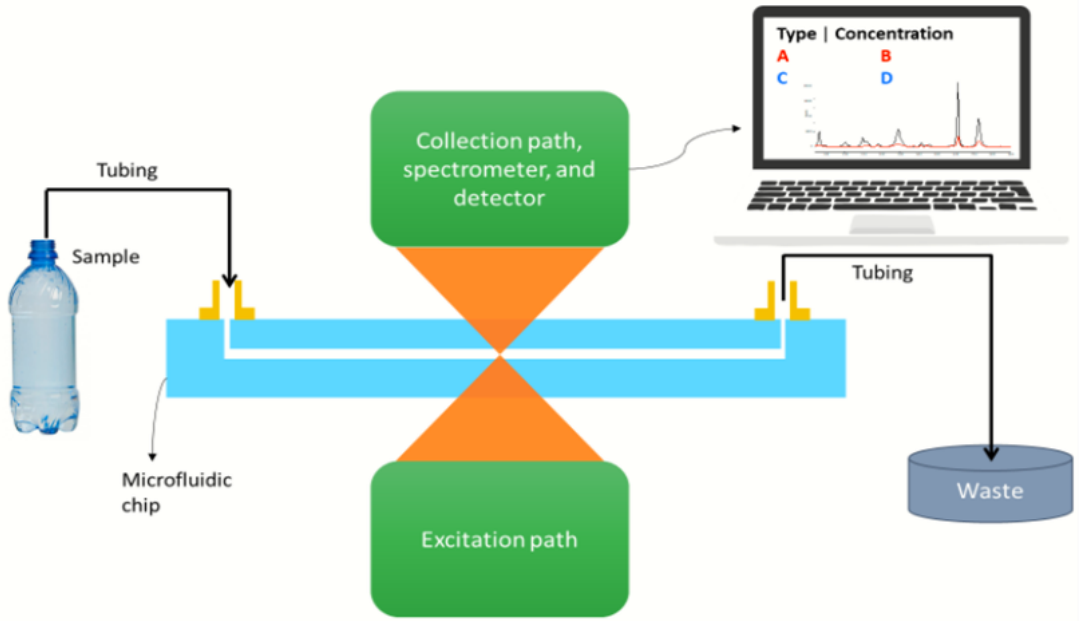
Scatterometry:

Size and concentration measurement



Surface Plasmon Resonance based sensor:

Type and concentration measurement



Conclusions

1. Raman Spectroscopy and fluorescence microscopy are able to detect lipid presence in fatty liver cells

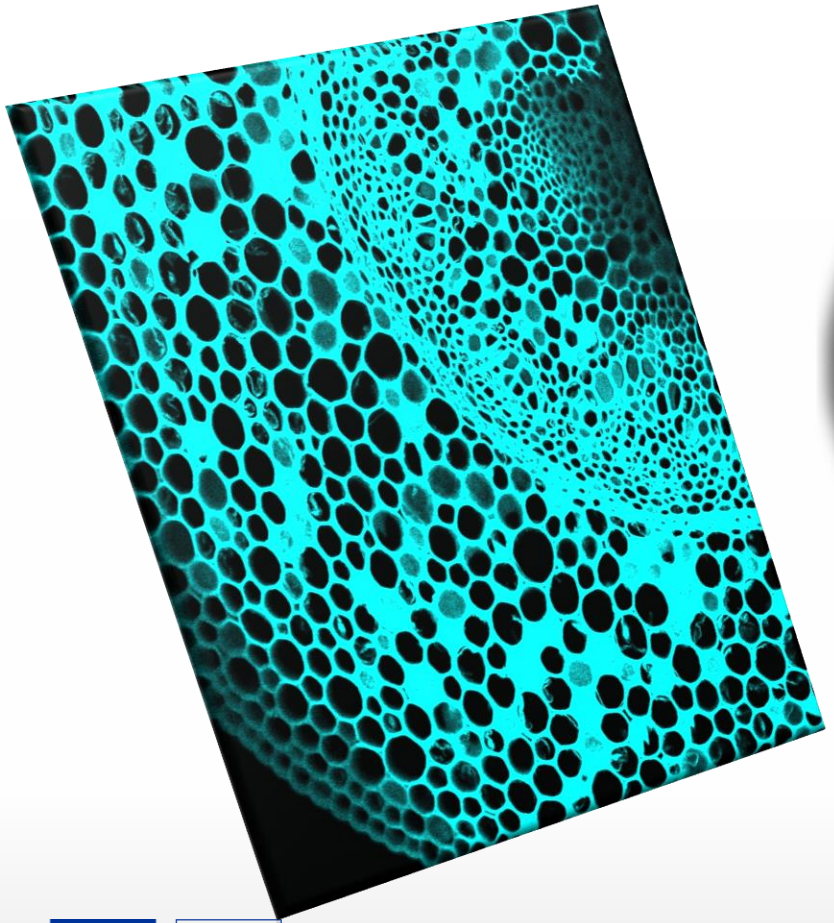
2. A clear difference in Raman peaks can be observed between healthy HepG2 cells and fatty HepG2 cells
   - HepG2 cell peak: 2930 cm^{-1}
   - Lipid peak: 2854 cm^{-1}, 2887 cm^{-1}

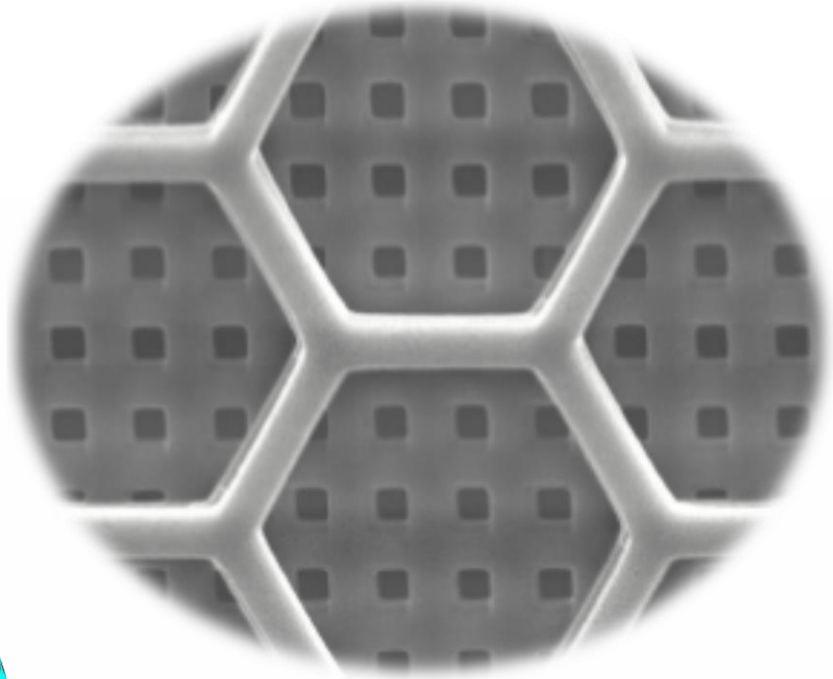
3. Nanostructures fabricated by 2PP can serve as SERS substrates for biosensing application

4. The regions of interest for the analysis of MPs are 370 – 1800 cm^{-1} and 2800 – 3000 cm^{-1}.

5. To avoid saturation and loss of signal in the extended mode, the power of excitation should not go above 10 mW and below 1 mW, respectively. Moreover, based on our setup and samples, 0.5 s is the shortest exposure time that can be used to analyse MPs with 785nm laser.

THANK YOU





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