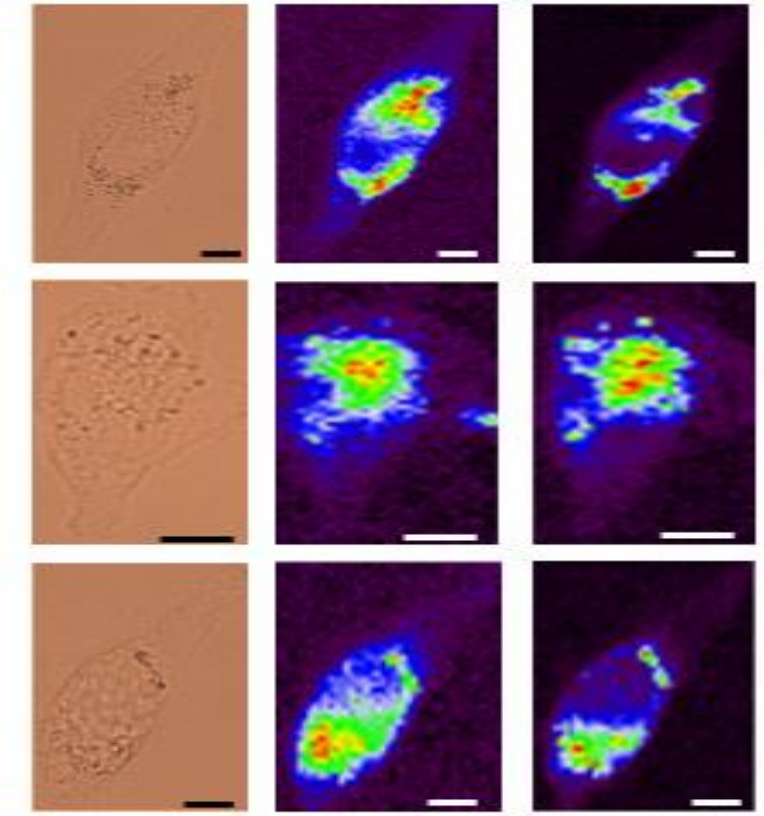
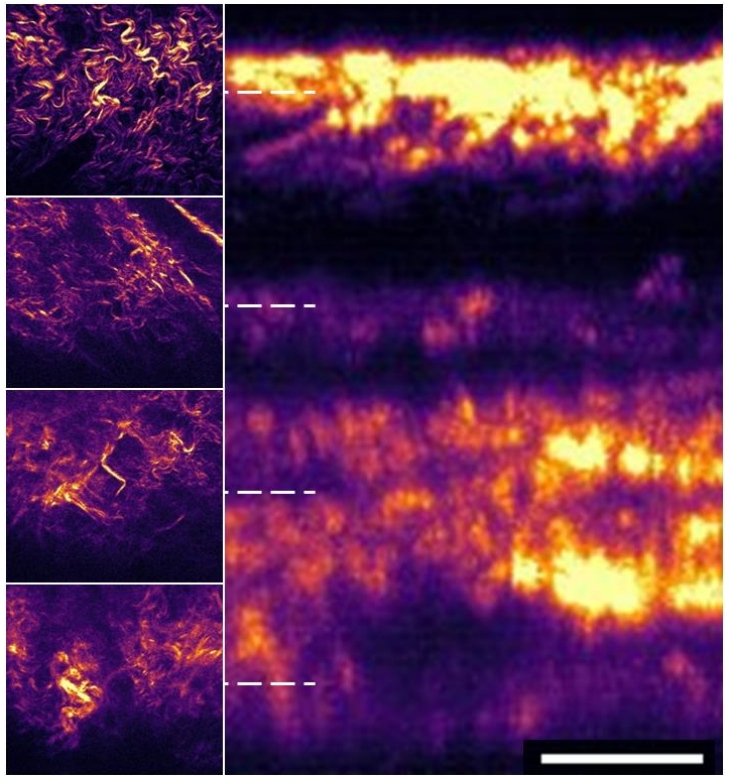
MICROSCOPY STRATEGIES FOR CANCER RESEARCH

The Super-resolution Light microscopy and Nanoscopy (SLN) lab

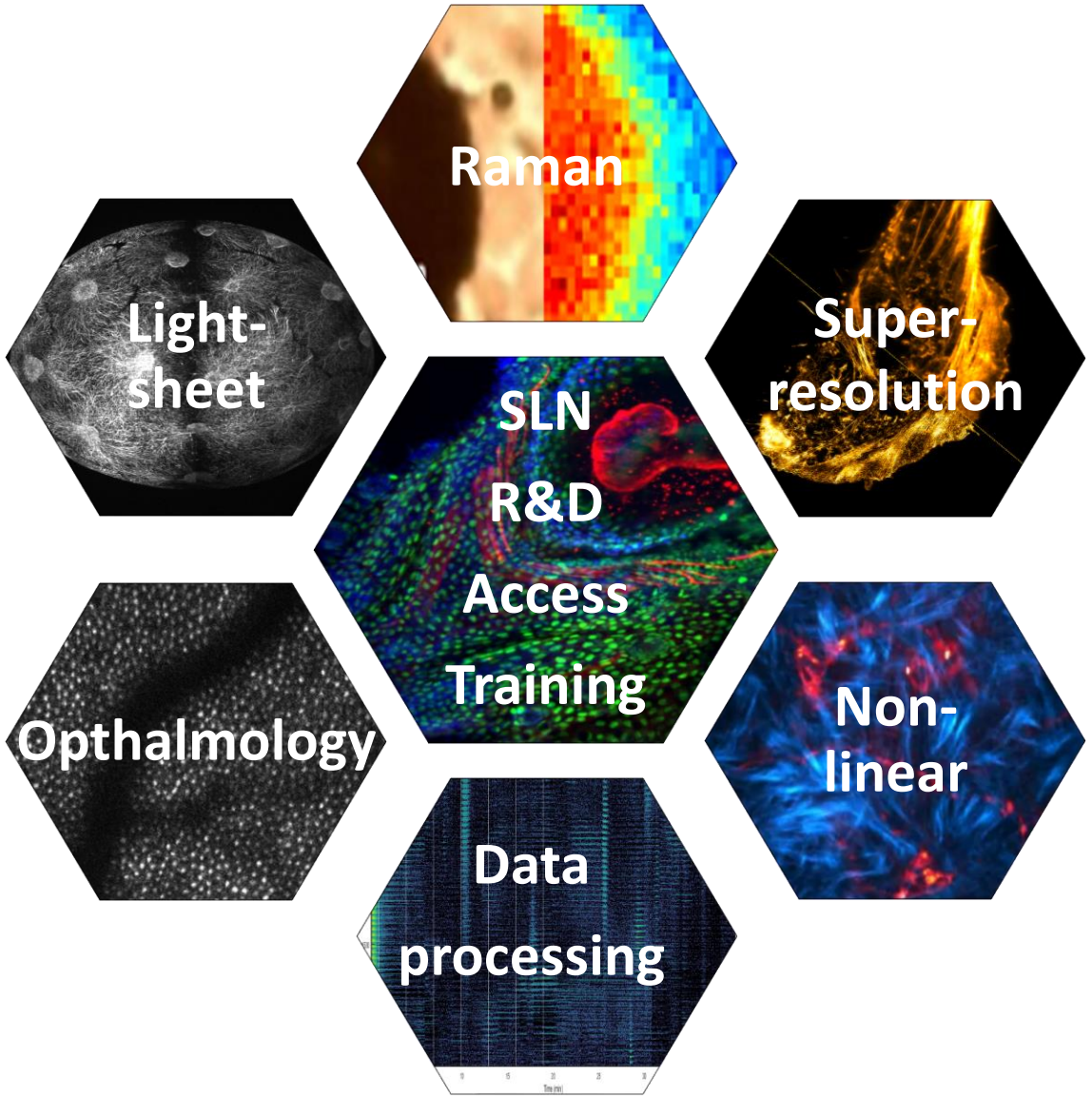
http://sln.icfo.eu





Pablo Loza-Alvarez
pablo.loza@icfo.eu

The Super-resolution Light microscopy and Nanoscopy (SLN) lab



We aim at providing **solutions** to the **unmet bio-medical needs** in microscopy

Our team
6 Staff members
4 Postdoctoral researchers
4 PhD students

Our statistics
11 imaging systems
80 users/year
+8000 hours/year

Collaborations with
Hospitals
Research centers
Industry
Universities

Raman spectroscopy in biomedicine

1) Raman spectroscopy can be implemented in vivo

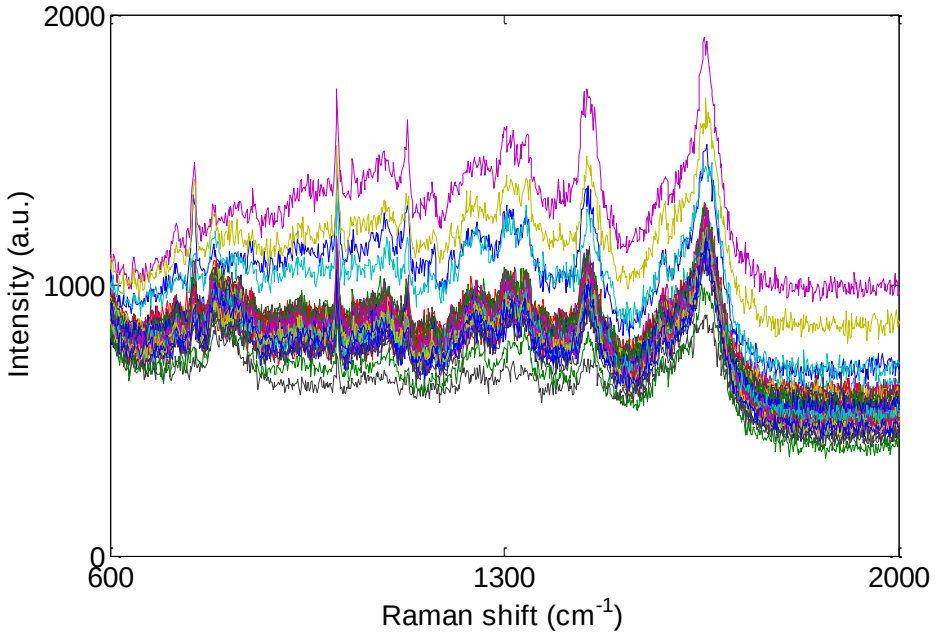
- Label-free
- In vivo applications

Microscopy + Raman spectroscopy

Lasers: 532nm, 785nm

2) Biochemical Raman spectra interpretation is challenging

- Difficult to extract all the chemical information



???

Powerful chemometric techniques

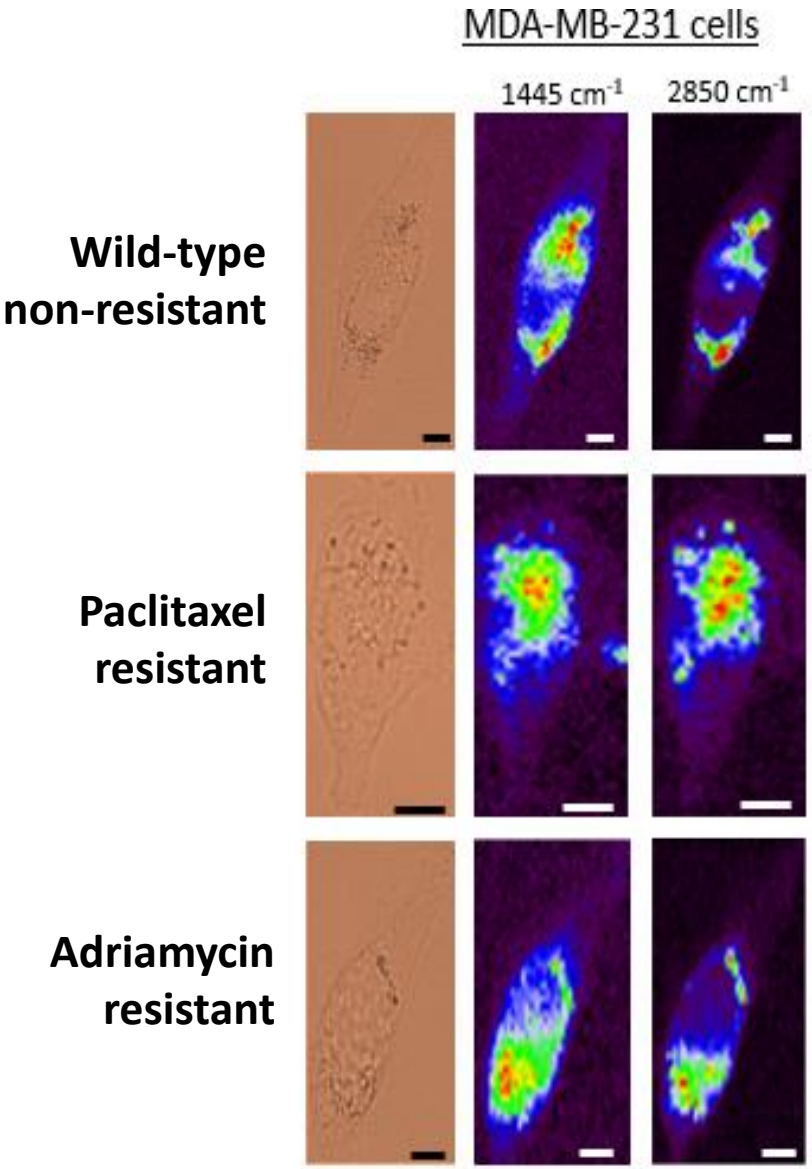
Multivariate Analysis + AI

BCC resistant to neoadjuvant treatments
- **Triple Negative**
- **HER2+**

Brest tumour localization in histology
- **Standard H&E staining vs RAMAN + AI**

Choroidal melanoma tissue

Comprehensive lipid droplet characterization of Triple Negative breast cancer cells resistant to neoadjuvant treatments



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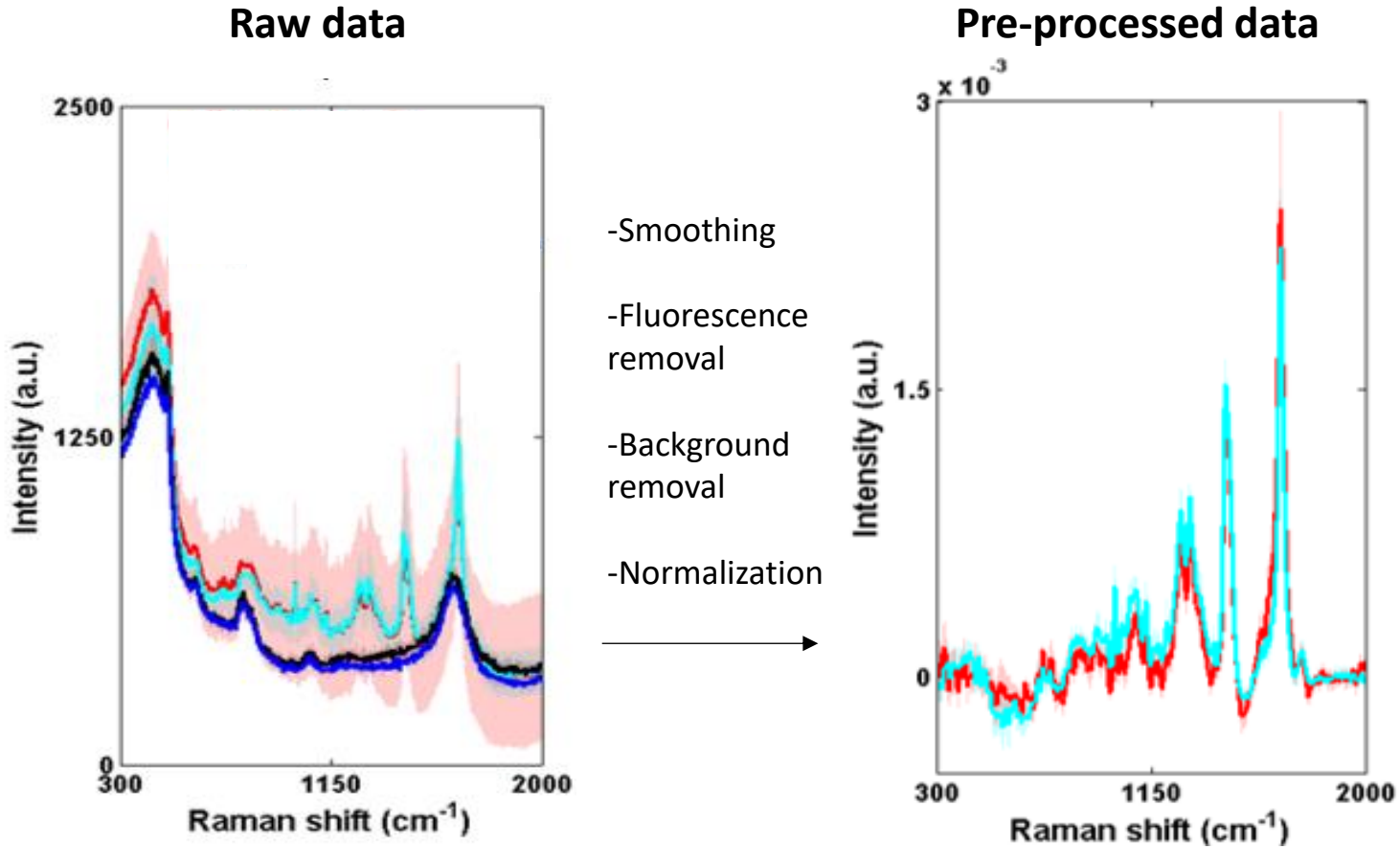
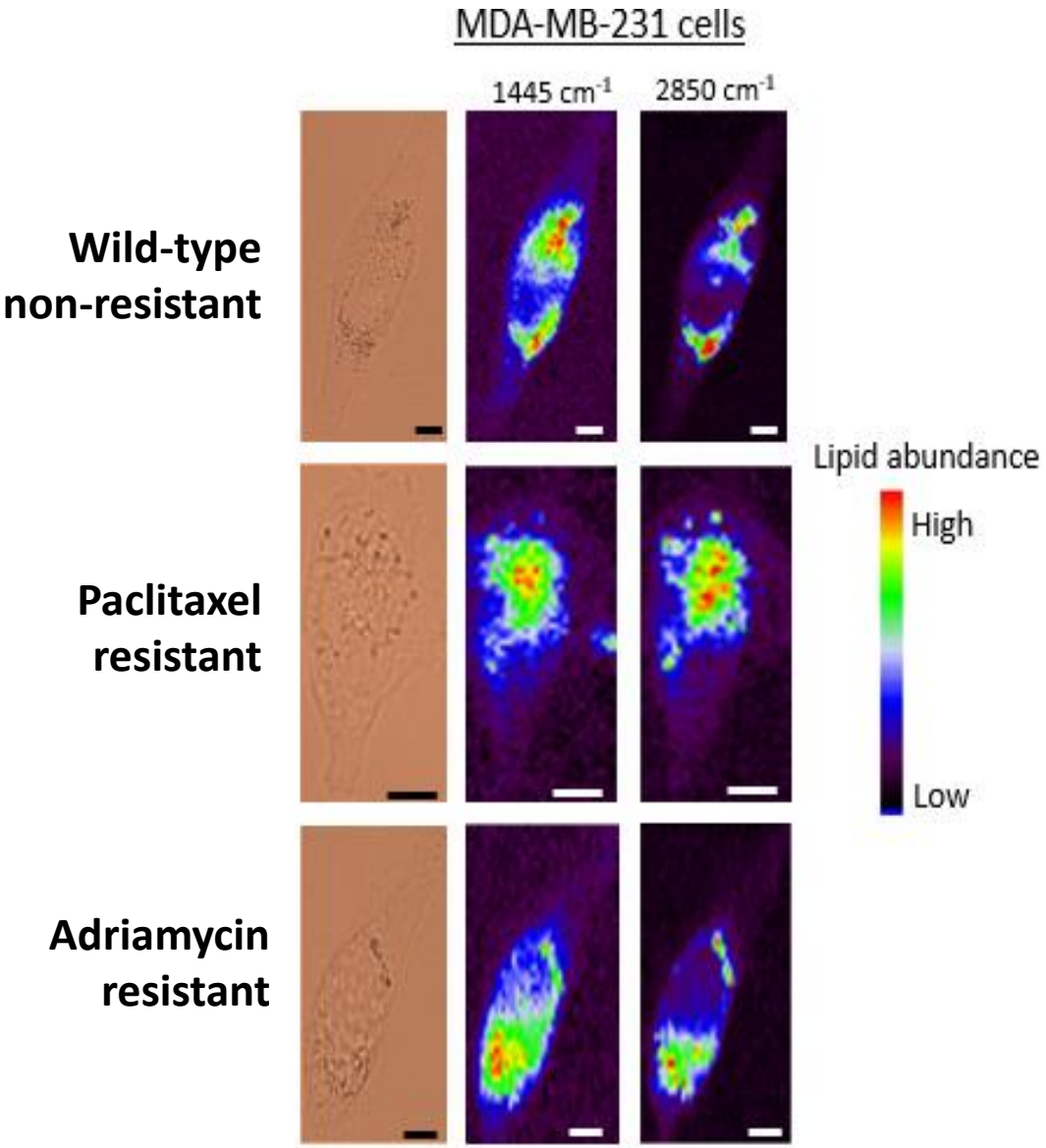


Comprehensive lipid droplet characterization of Triple Negative breast cancer cells resistant to neoadjuvant treatments



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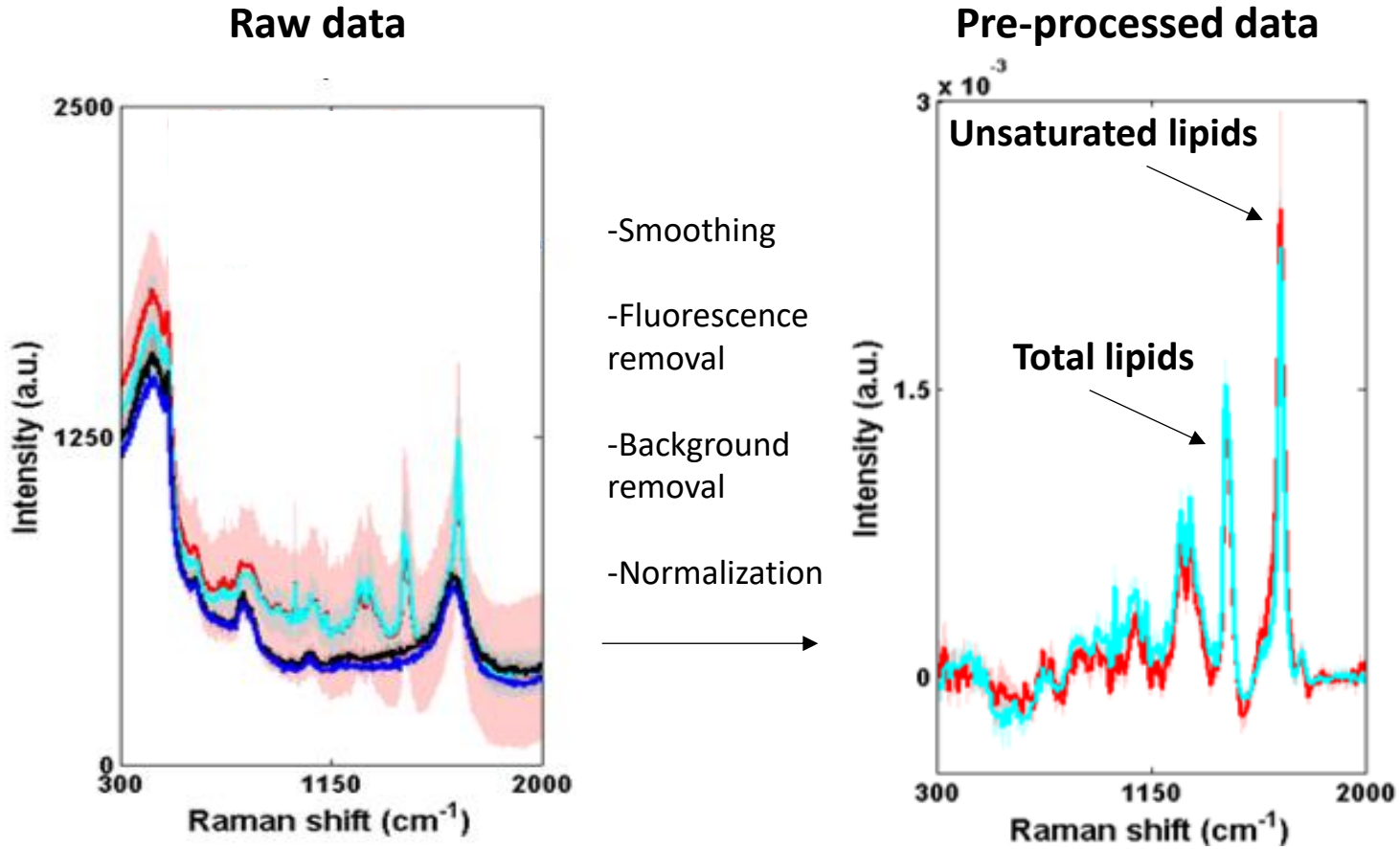

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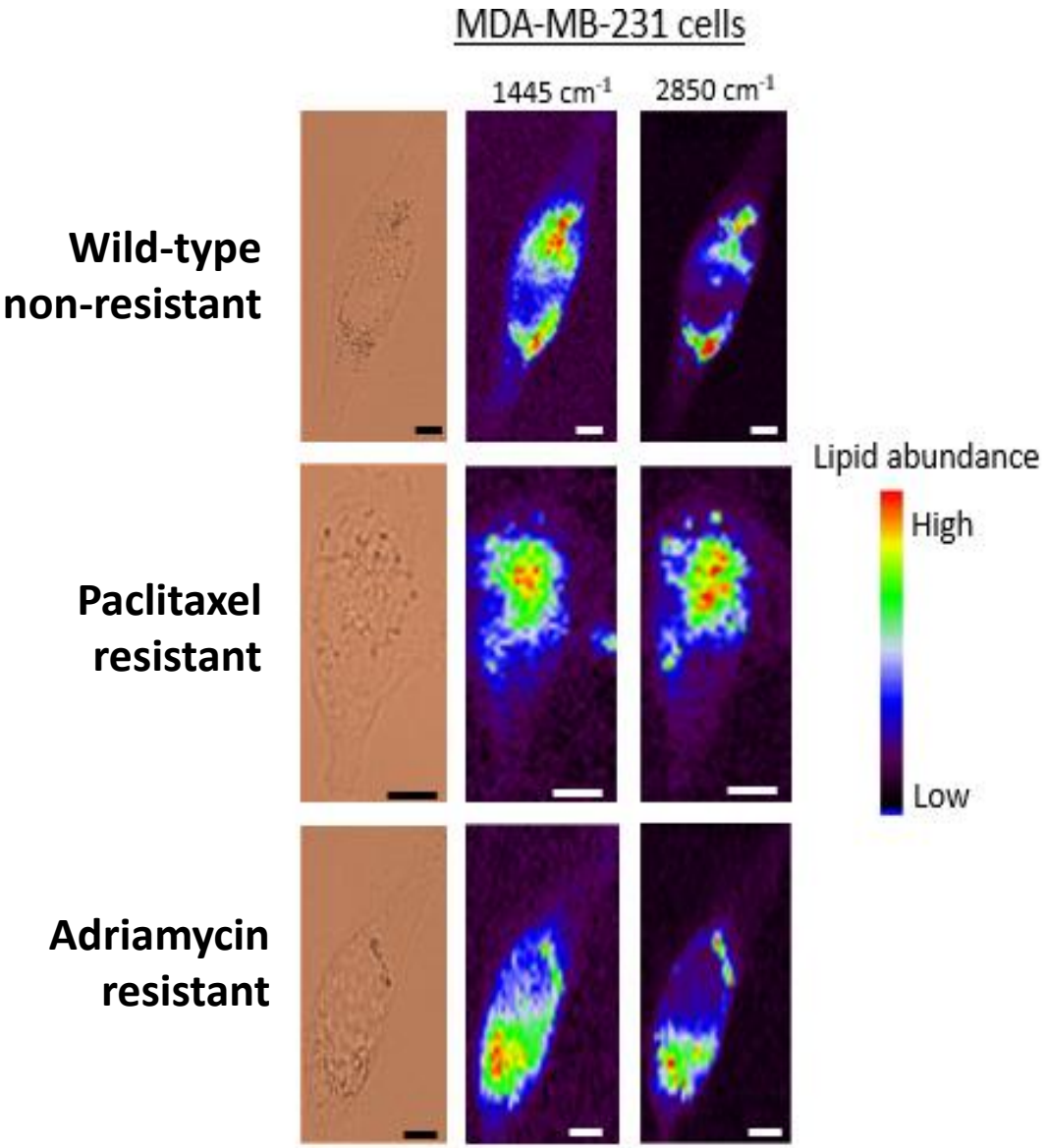

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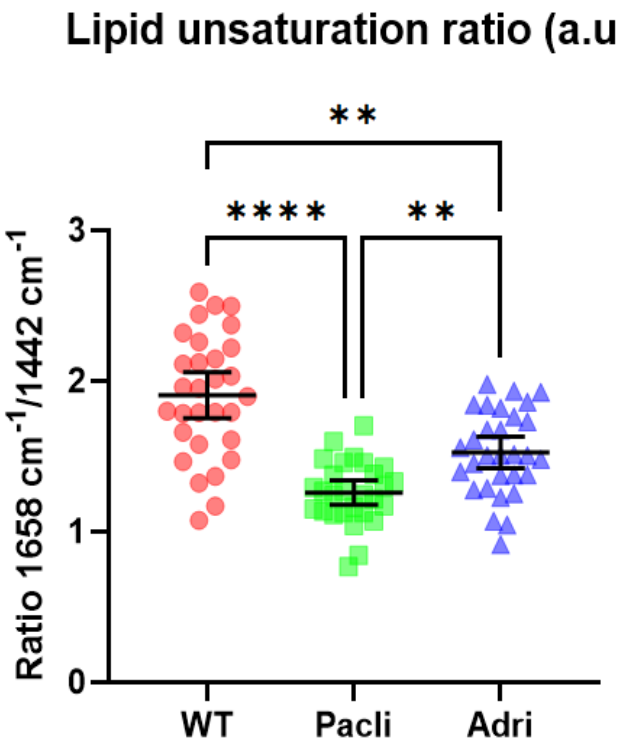
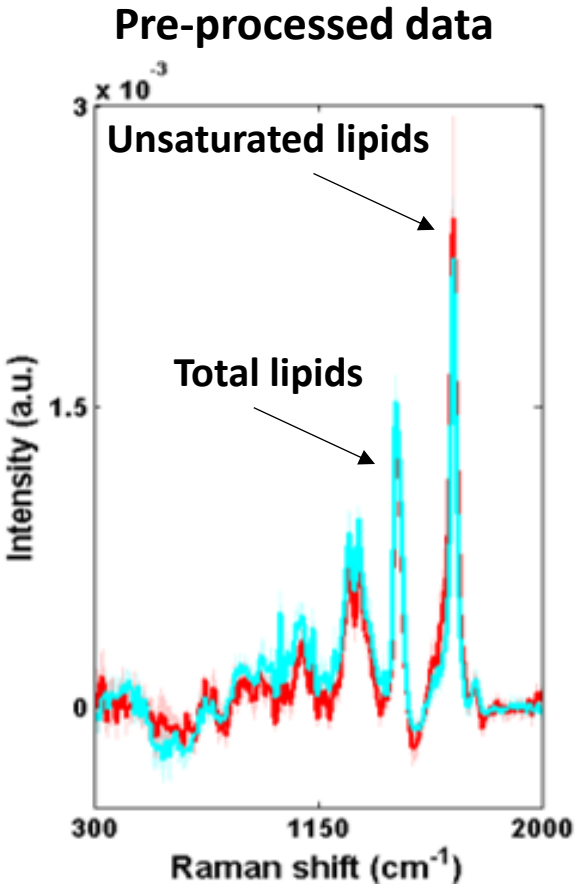
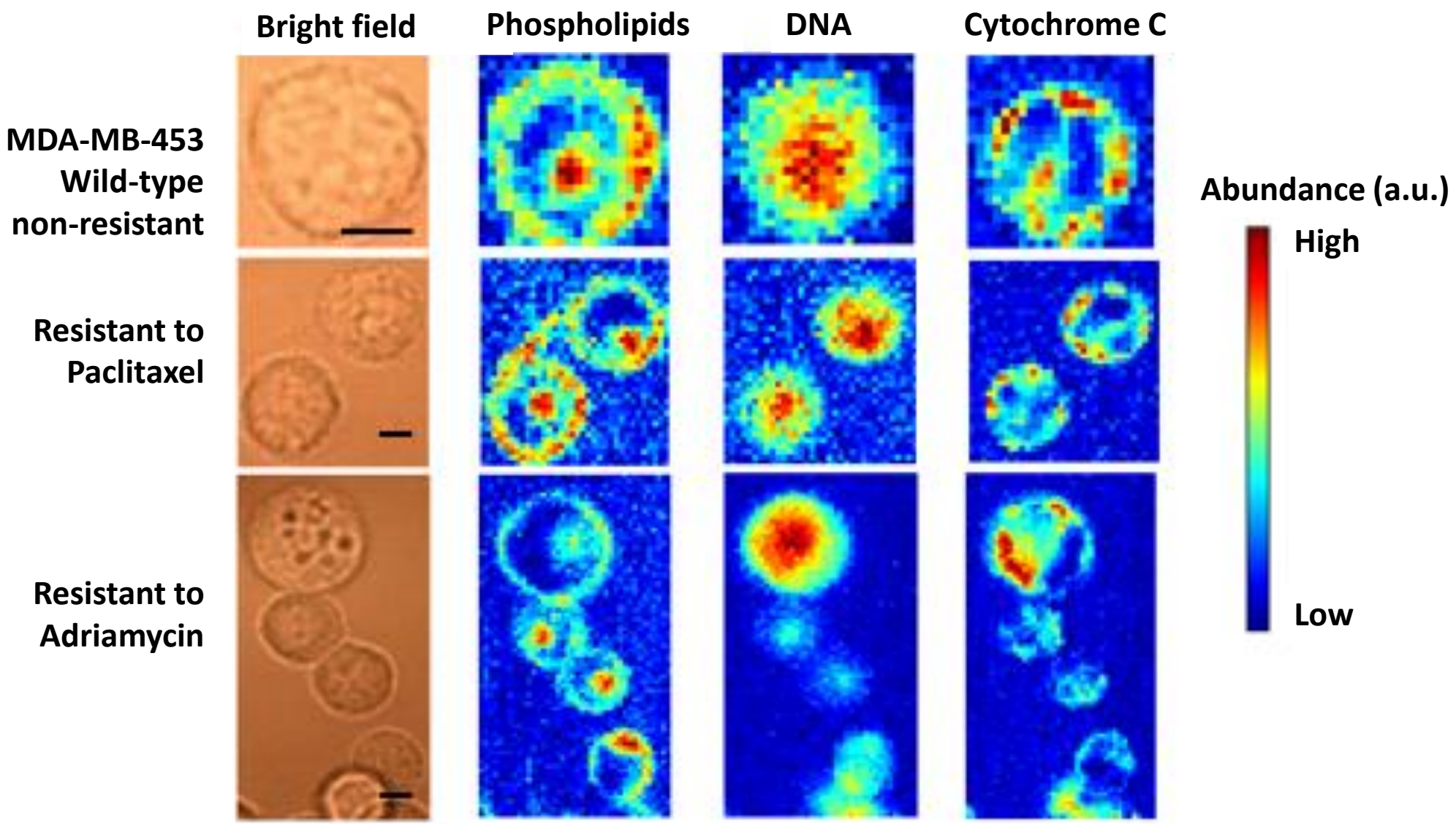




Studying drug treatment resistance on HER2+ breast cancer cells



Scalebar: 10 µm

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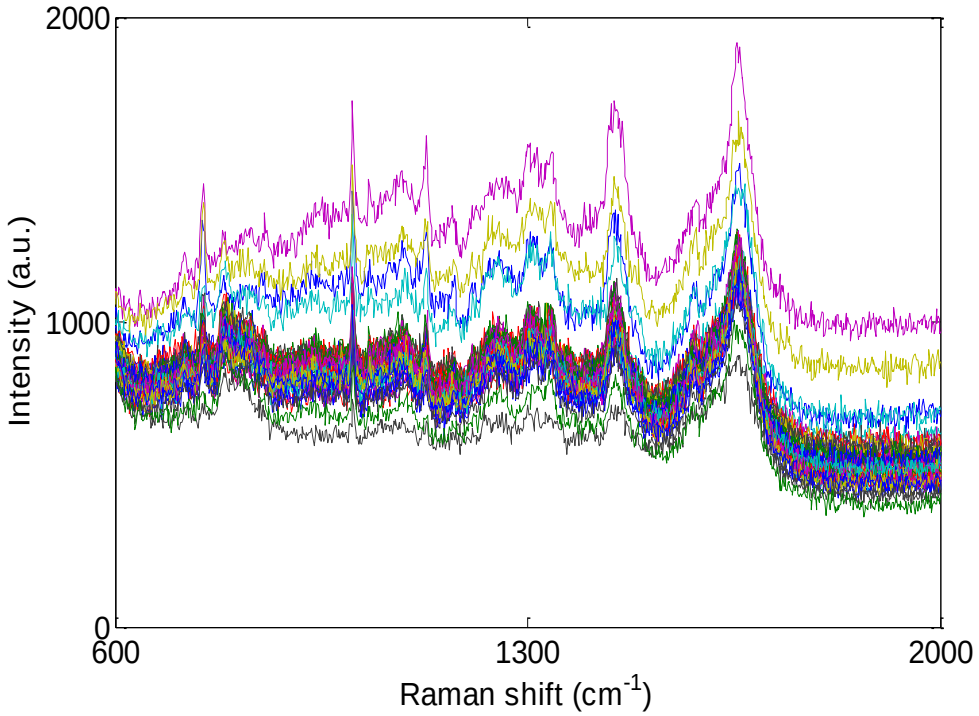
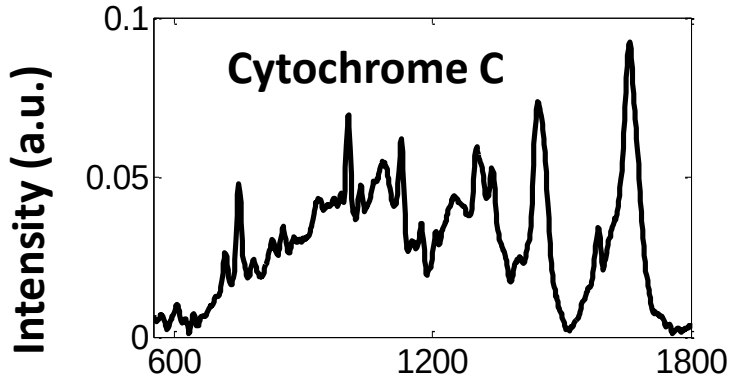
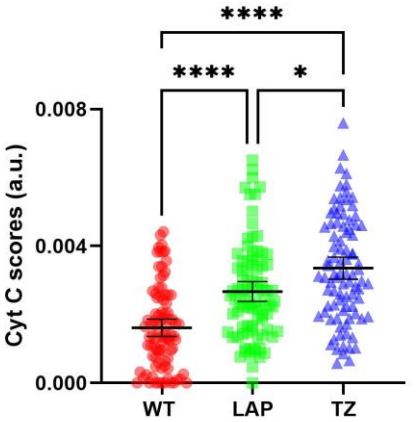
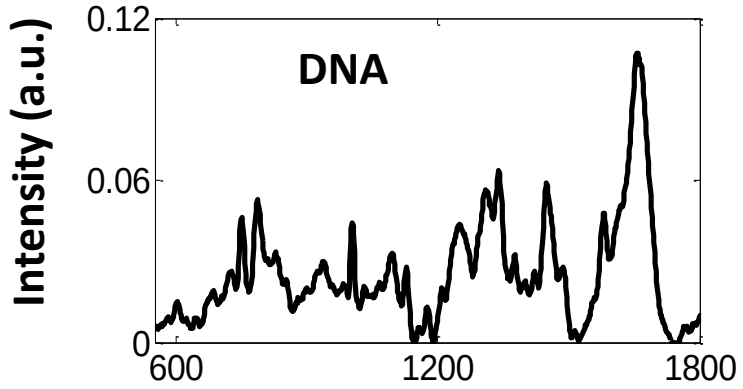


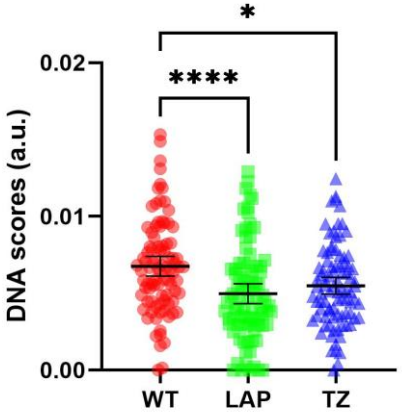
Difficult to interpret by naked eye:

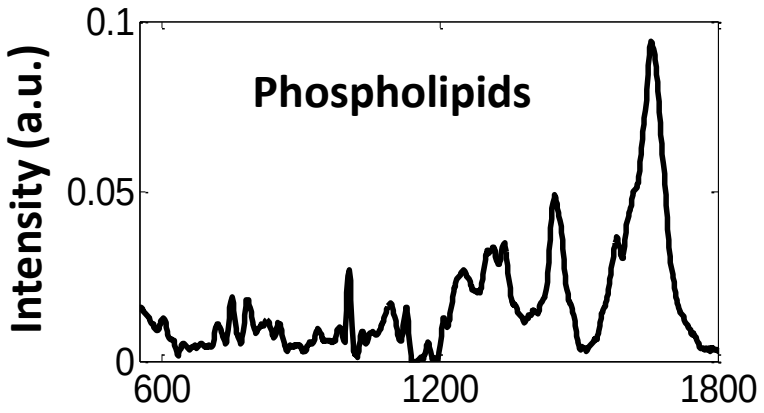
- Fluorescence
- Weak Raman signal
- **Mixture of different molecules**

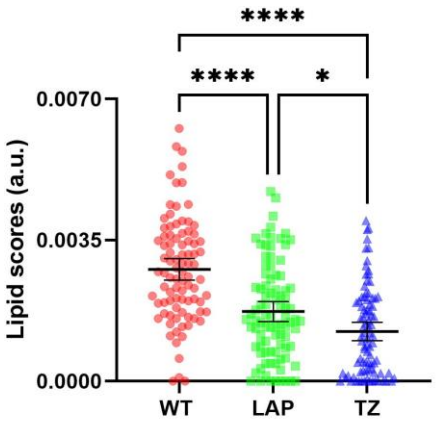






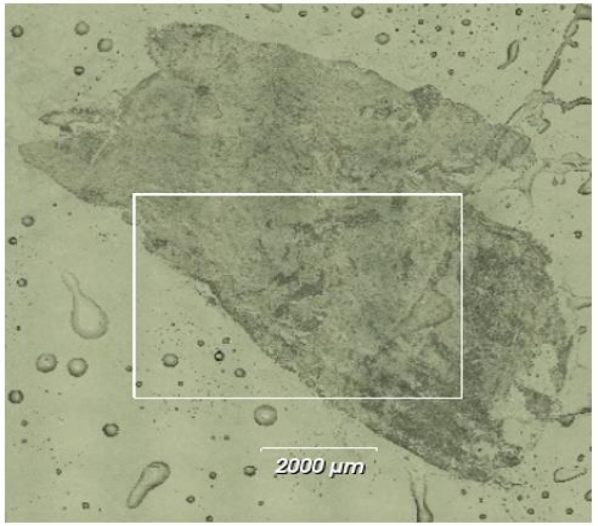




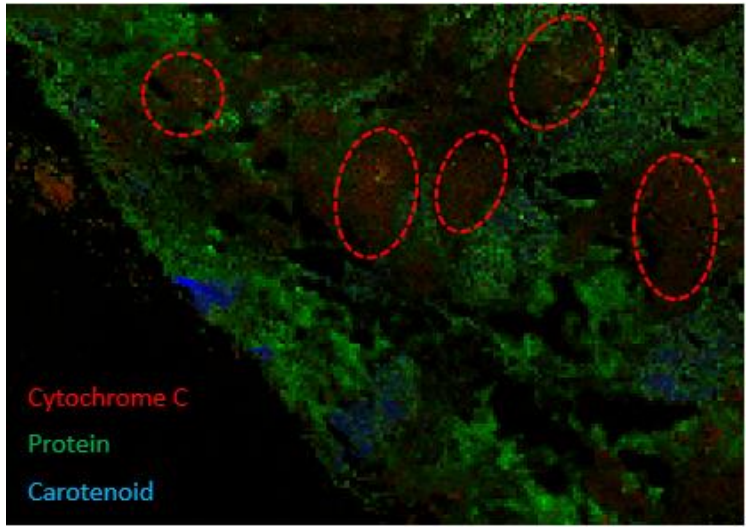


Breast tumour localization by Raman spectroscopy: validation with standard H&E staining

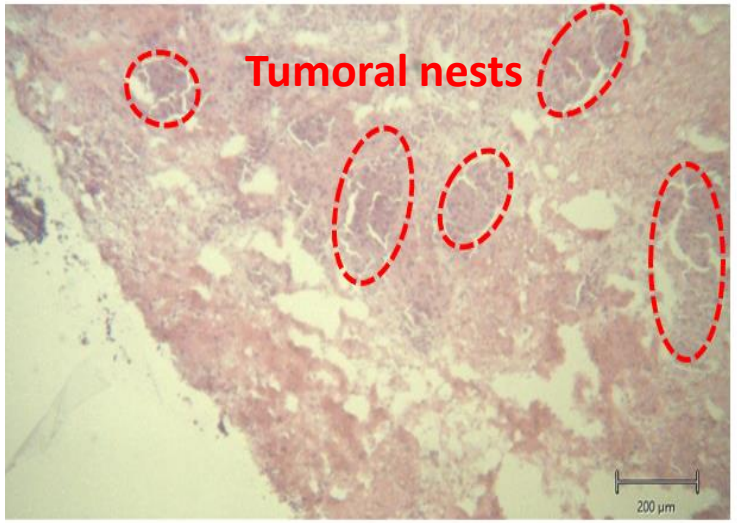
Unstained histological cut



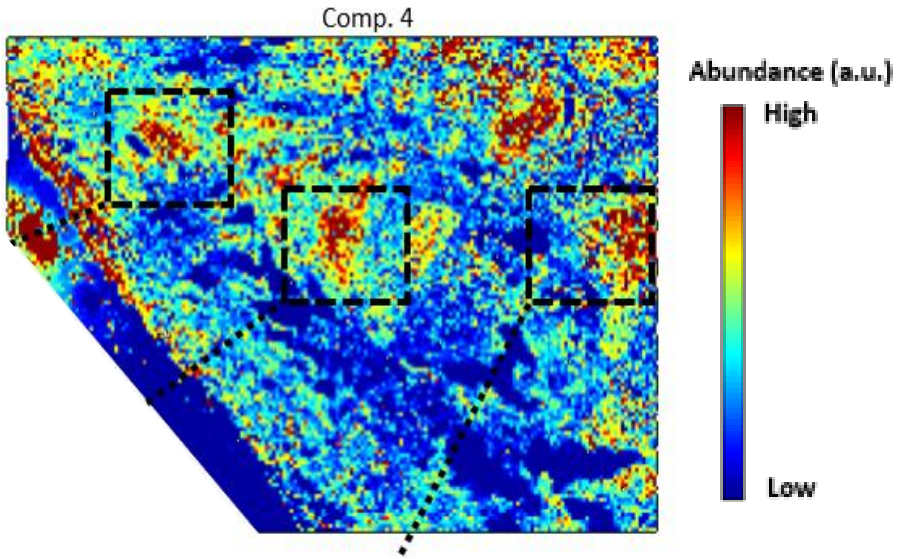
Raman image



H&E staining



Cytochrome C is in higher abundance in tumoral nests



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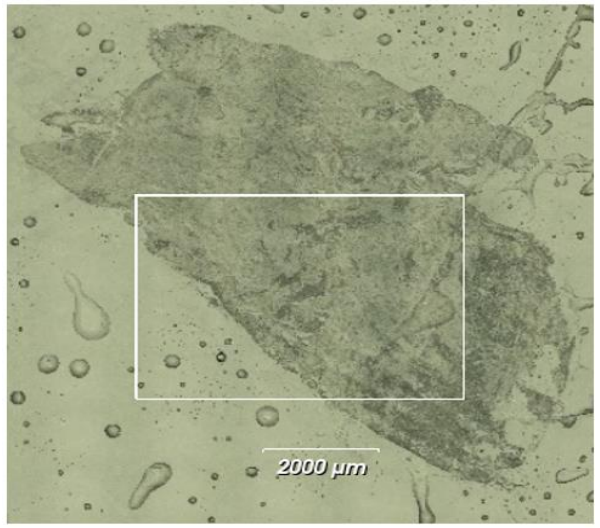
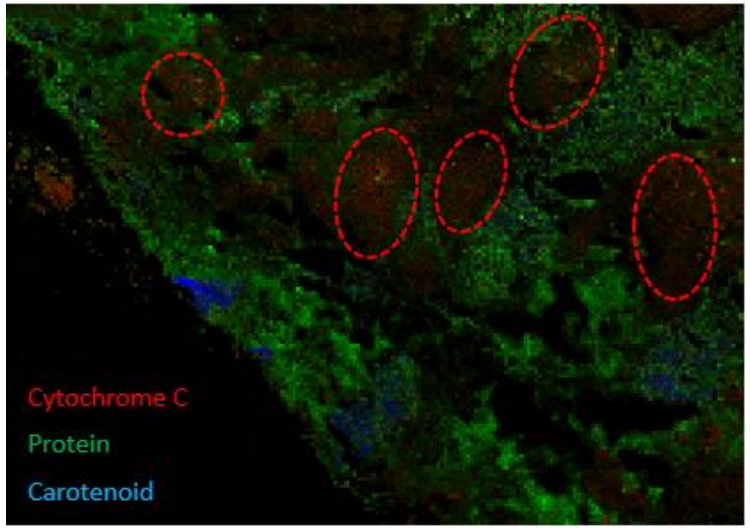
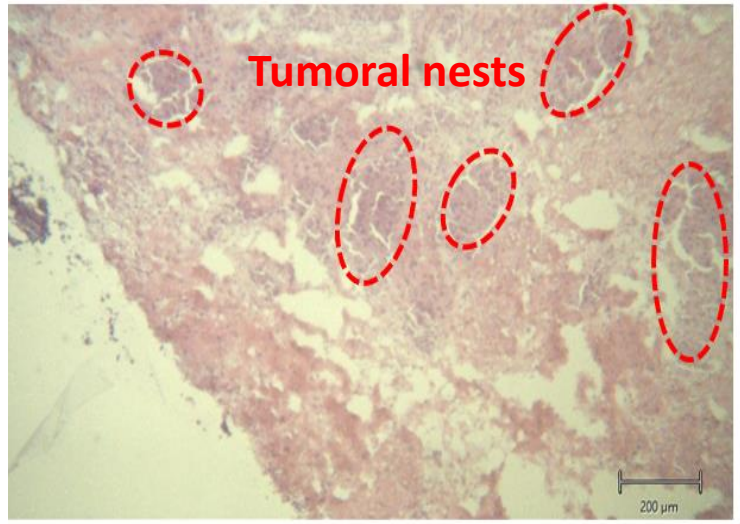


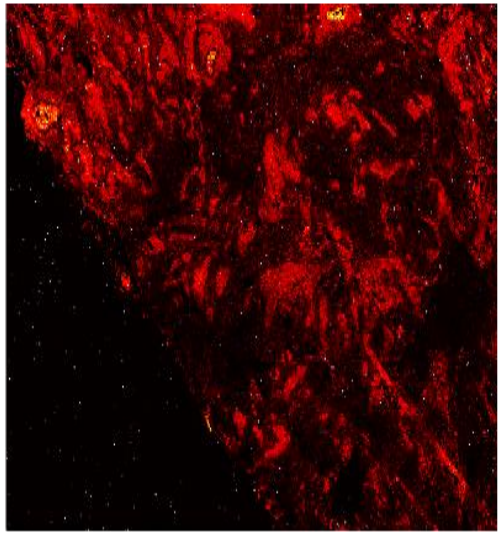
Raman image



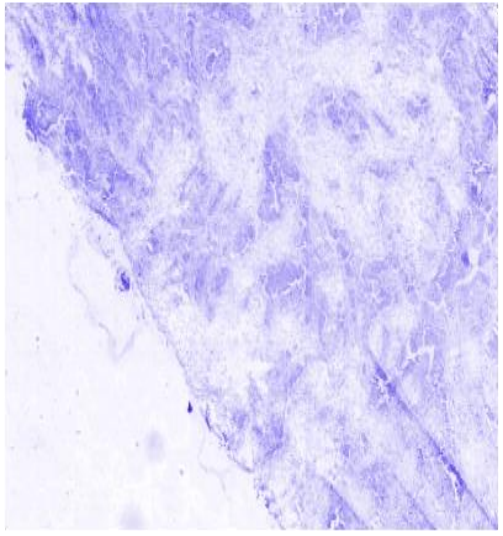
H&E staining



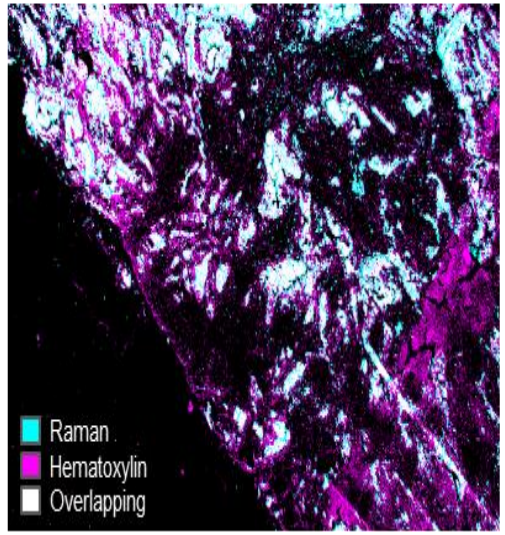
A) Raman map



B) Hematoxylin staining



C) Overlapping

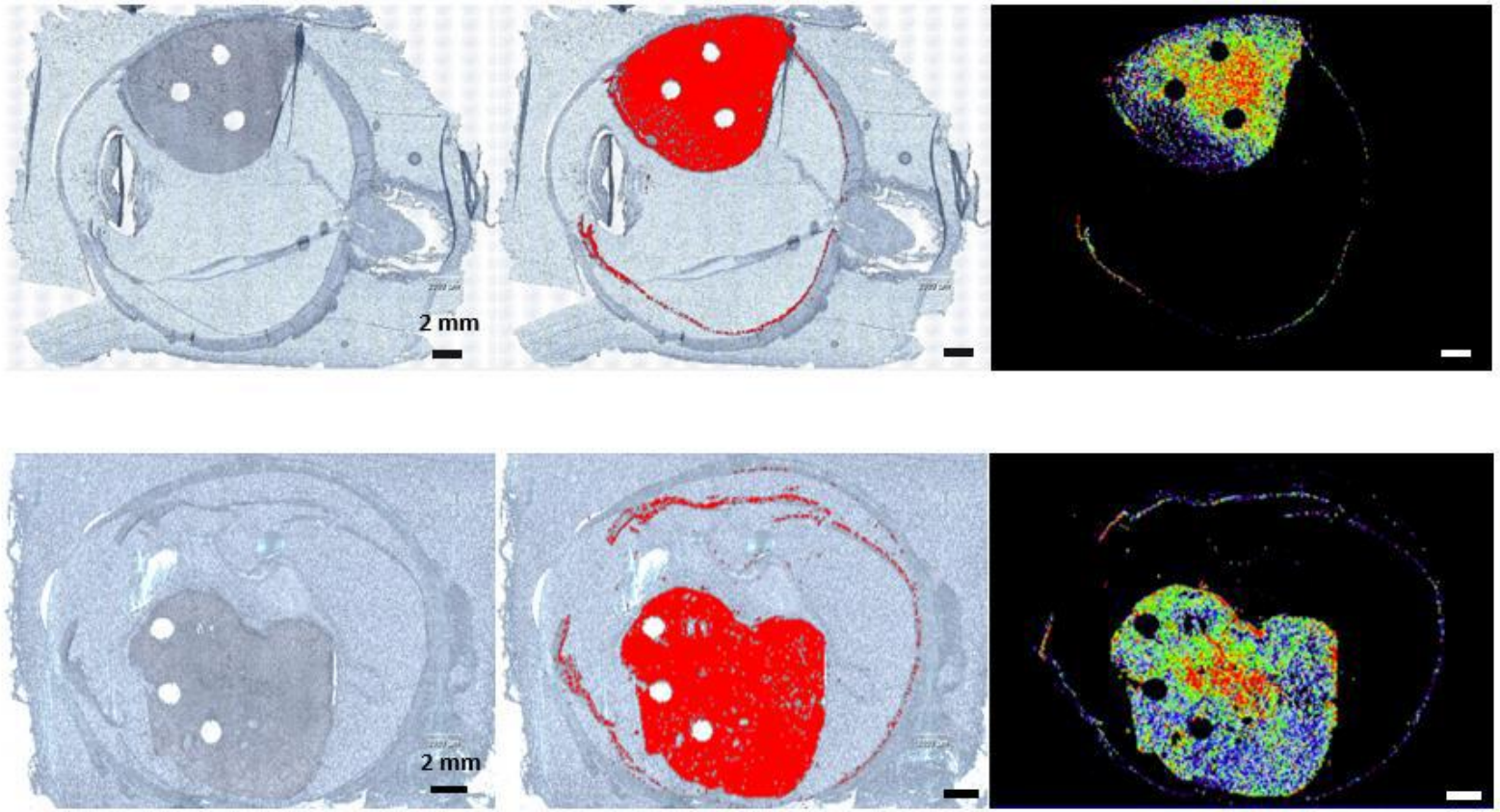


Identification and characterization of choroidal melanoma tissue (IR laser 785nm)

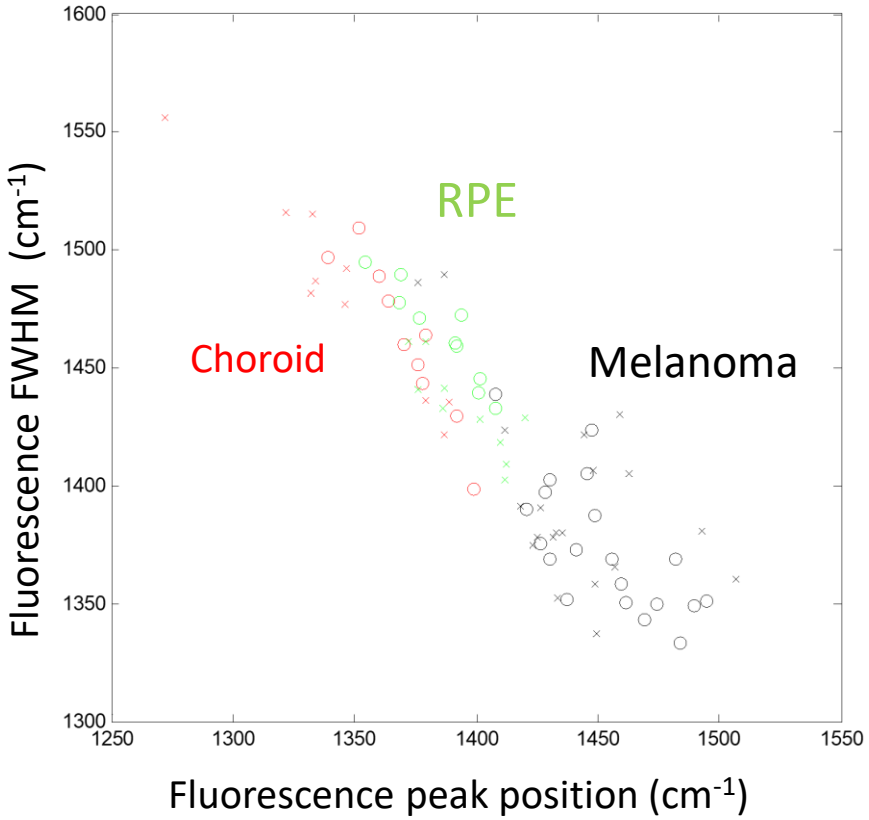
Bright field biopsy image

Melanin localization (K-means clustering)

Melanin abundance (Multivariate curve resolution)



Distinguishing melanin pigmented regions



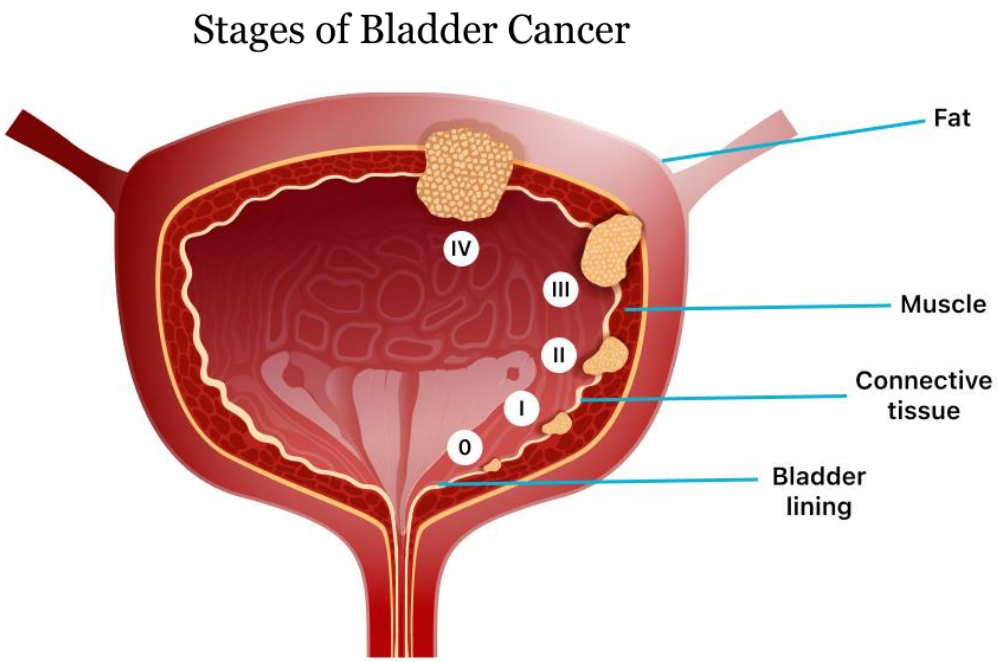
Scalebar: 2 mm

ADVANCED MULTIMODAL PHOTONICS LASER IMAGING TOOL FOR UROTHELIAL DIAGNOSIS AND ENDOSCOPY (AMPLITUDE)

www.amplitude-imaging.com

Bladder Cancer

To identify the first stages of bladder cancer: 0, I



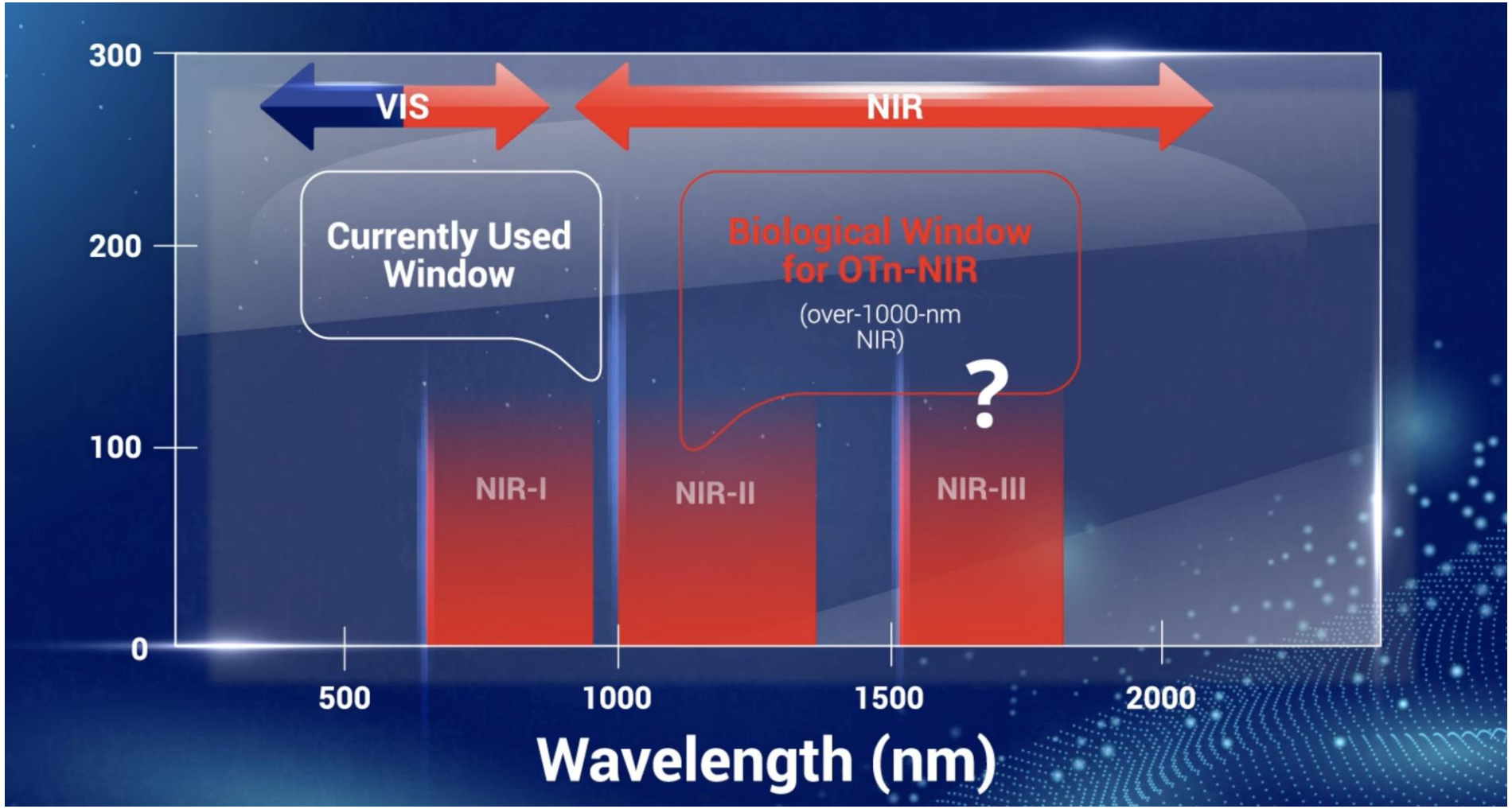
5-year survival rate decreases with increasing stage:

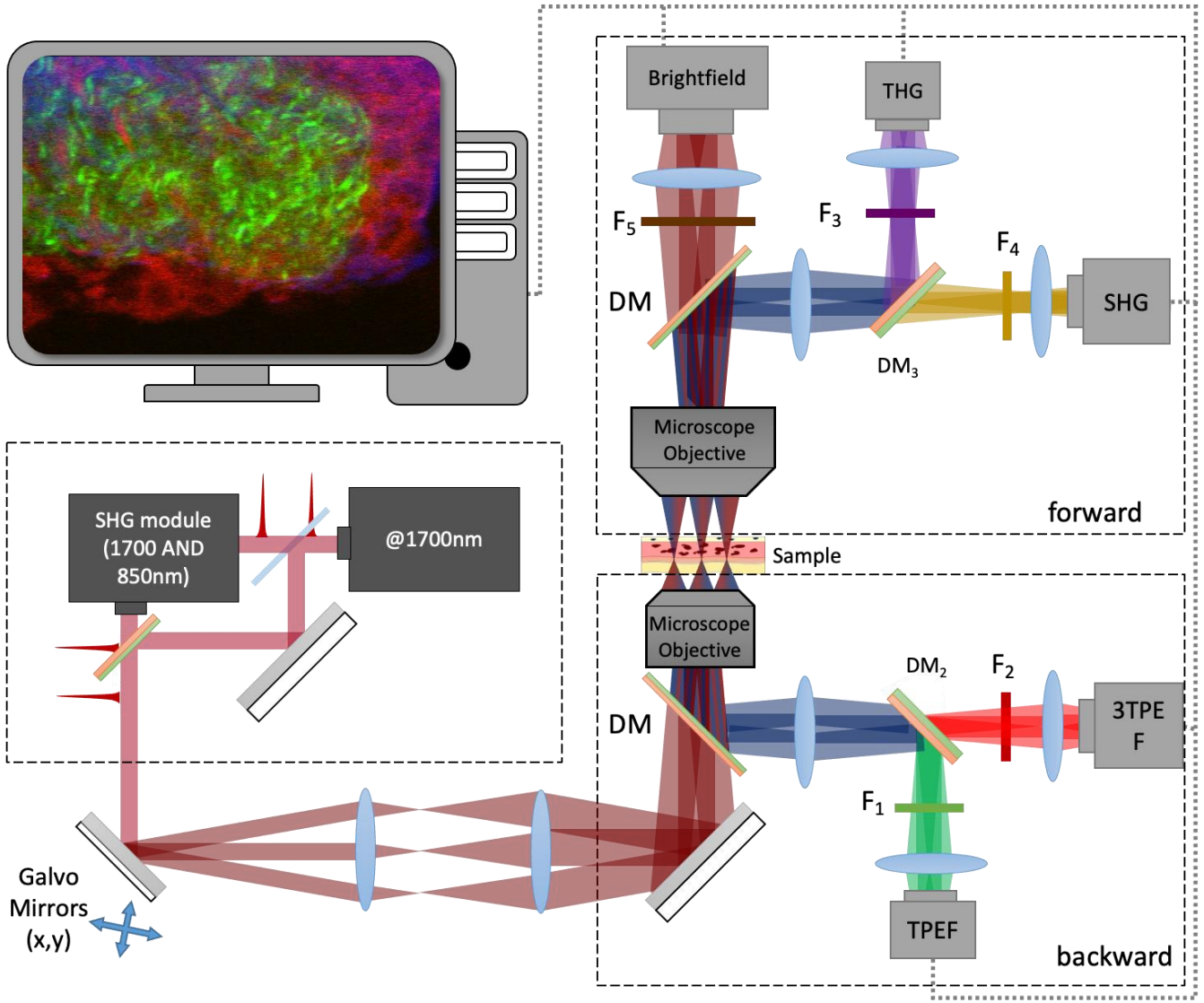
0, I –> 82-100%;
II –> 63-83%;
III –> 17 71;
IV –> 0-22% *(despite active or radical treatment)*

Bladder thickness ~ 3mm,

Stretched ~1mm → optically accessible at 1700nm

Explore the third optical window





Explore the use of the 2 lasers

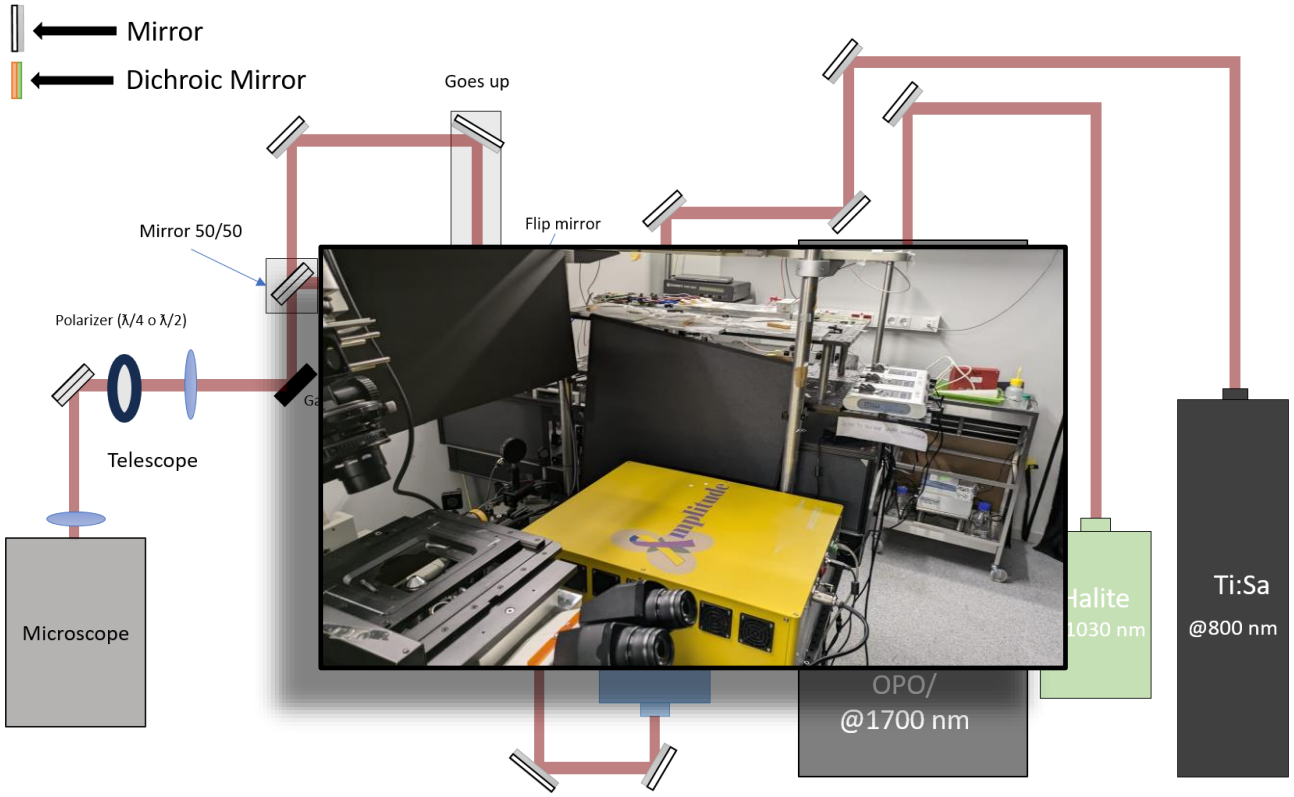
Laser source at 1700nm:

- THG (566nm),
- 3PEF (autofluorescence),
- TPEF (autofluorescence),
- SHG (850nm),
- Elastic scattering

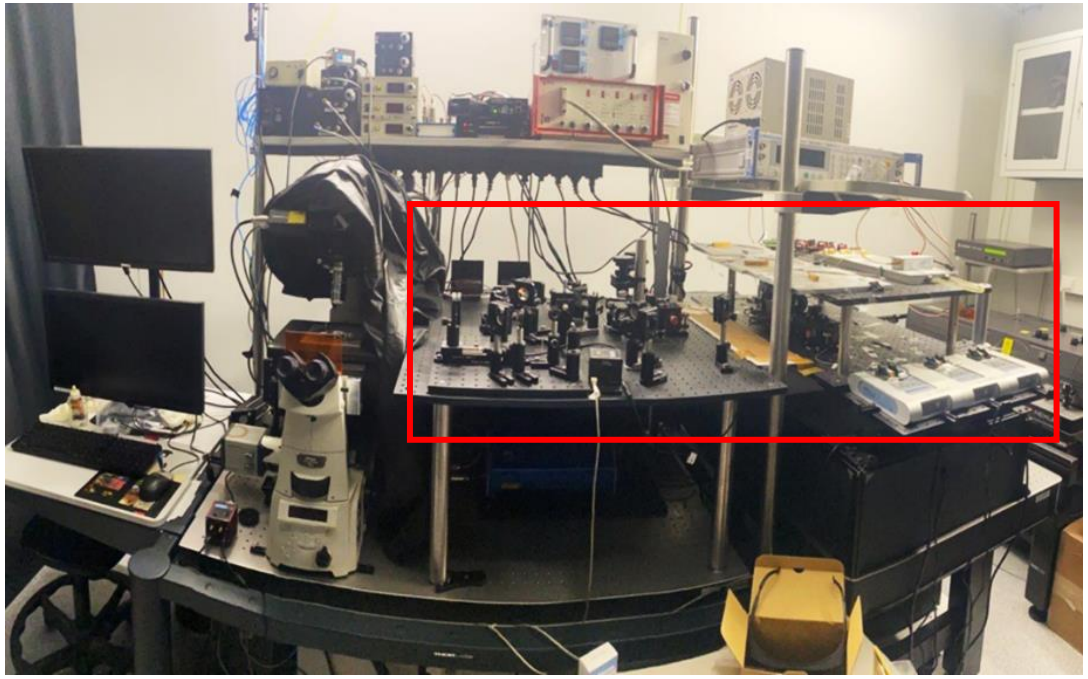
Frequency doubled laser source at 850 nm

- TPEF (autofluorescence),
- SHG (425nm),
- elastic scattering

Coupling the **laser** to multimodal microscope



Multimodal microscope platform prepared in a plug and play fashion to allow for testing of different laser excitation sources

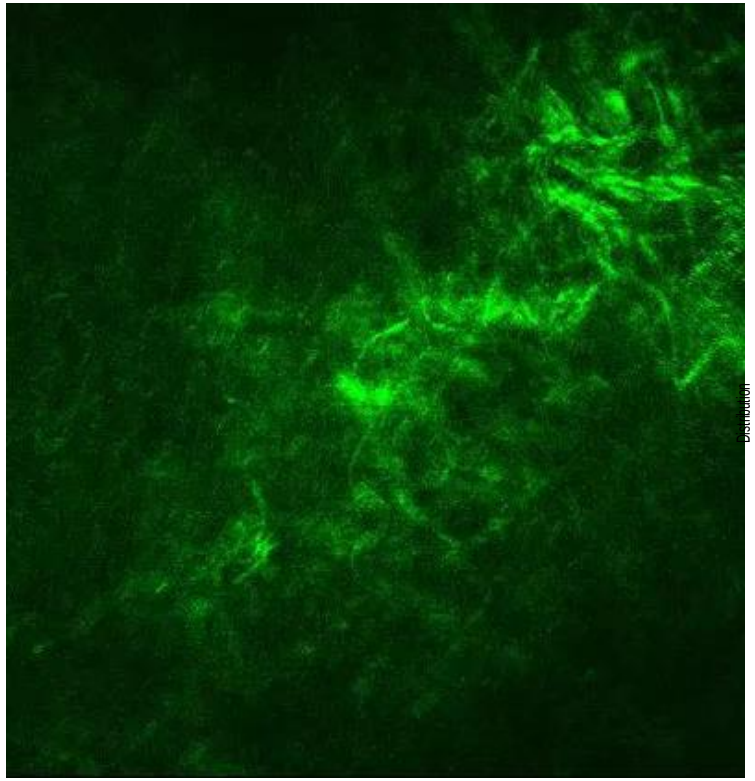


Coupled of the **laser** to the multimodal microscope at ICFO

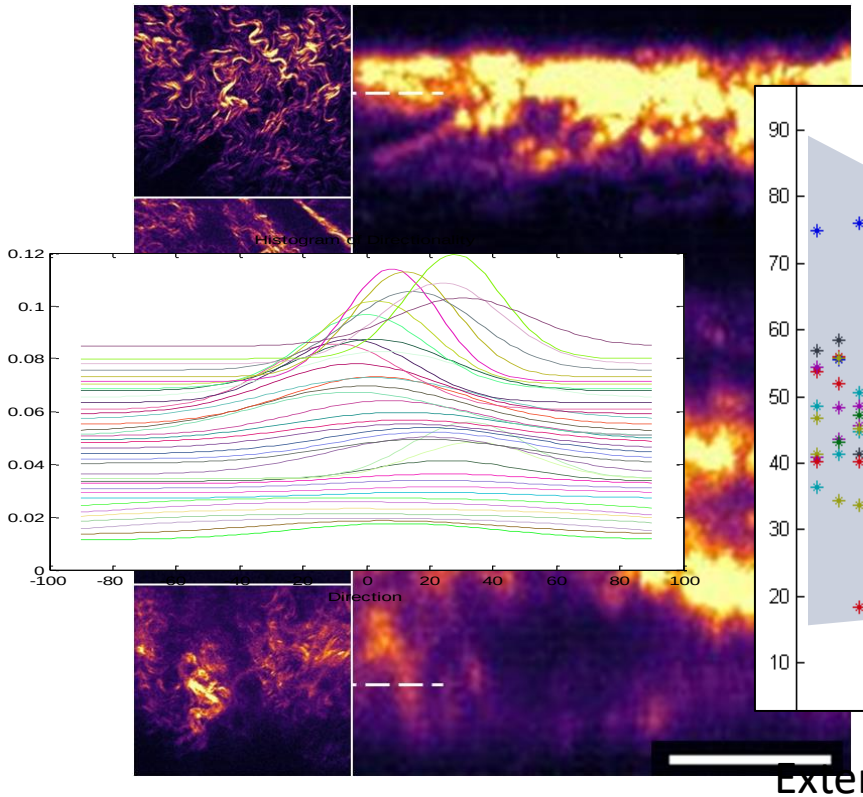
Bladder characterization through SHG imaging

λ_{ex}= 1030nm

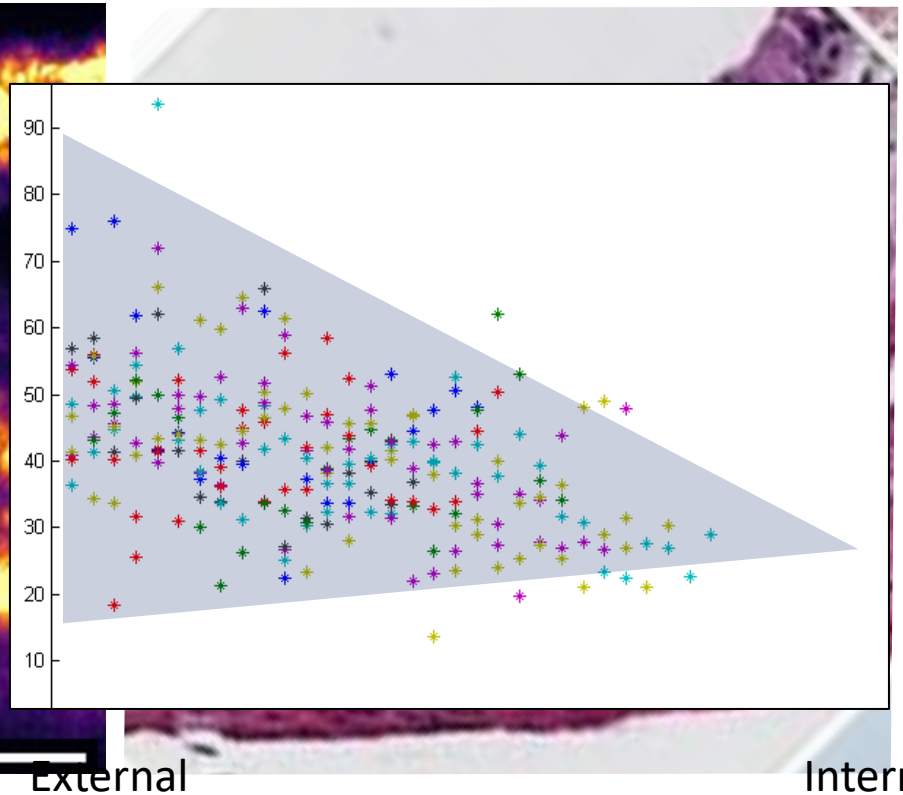
- **Tumours leverage extracellular matrix remodelling to create a microenvironment that promotes tumourigenesis and metastasis***



Rat bladder collagen Z-stack



Lateral projection

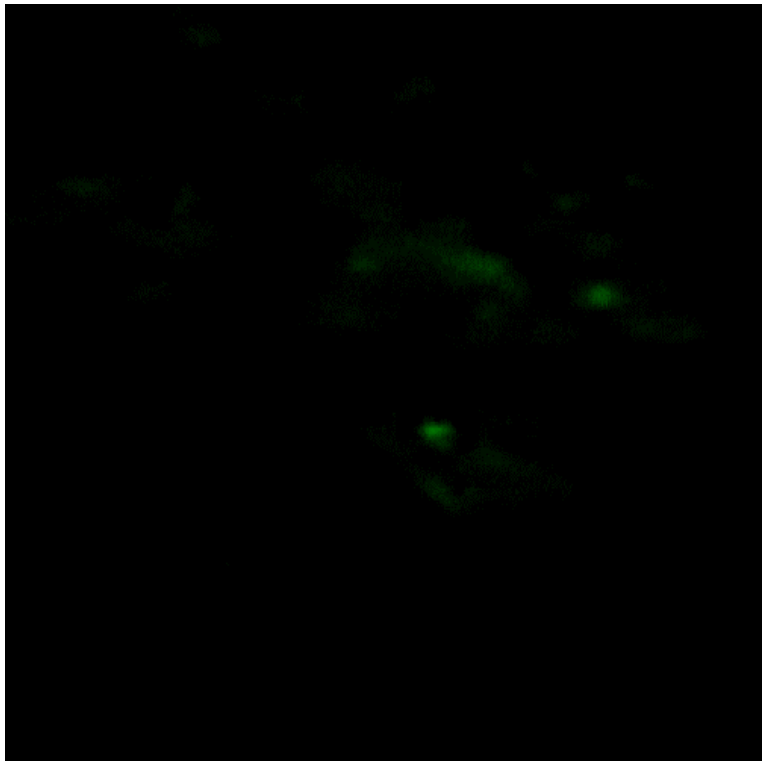


Histological section

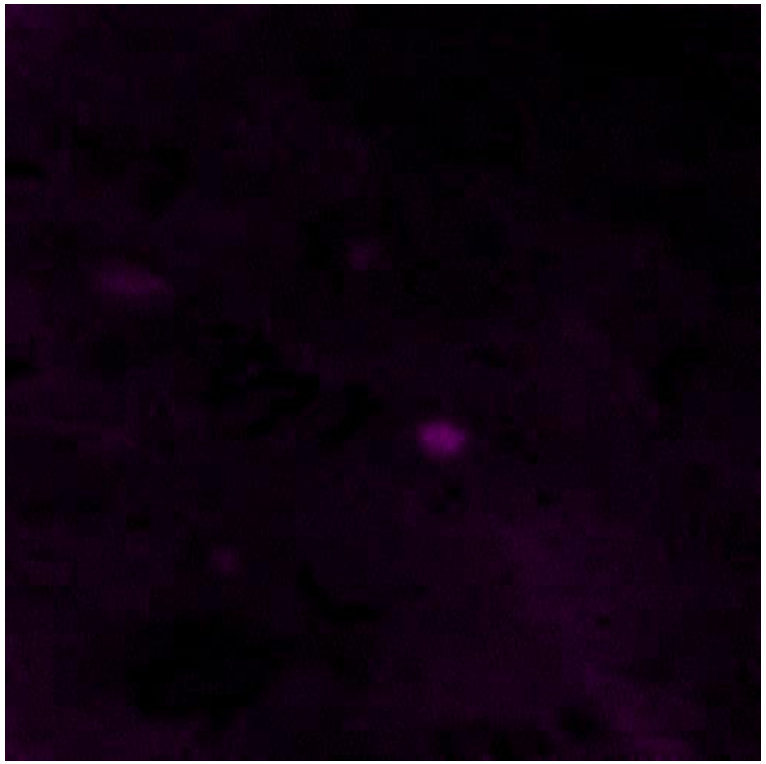
*Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nat Commun* (2020).

Penetration depth in Muscles
Preliminary results at 1675

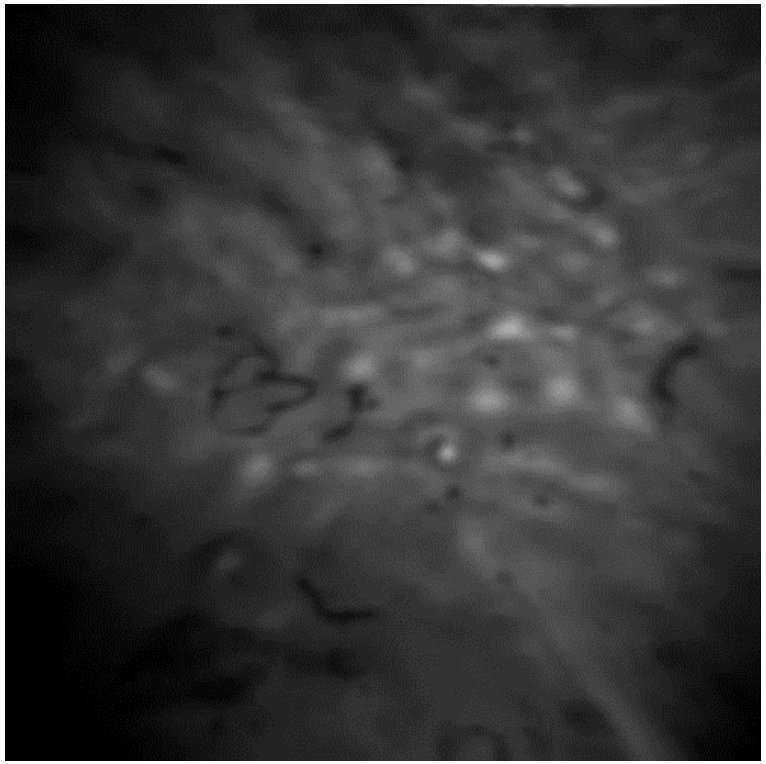
λ_{ex}= 1675nm



SHG



THG

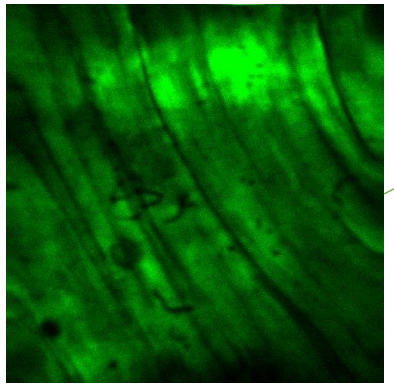


Transmission

FoV = 400 um
Objective= 20x NA=0.75

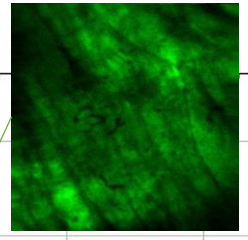
Preliminary results

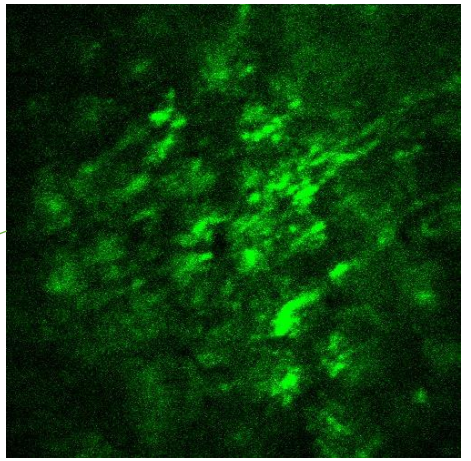
λ_{ex}= 1650nm



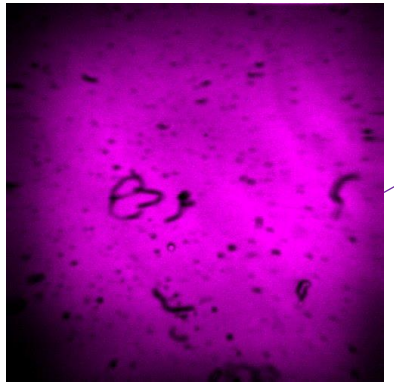
muscle

SHG intensity profile



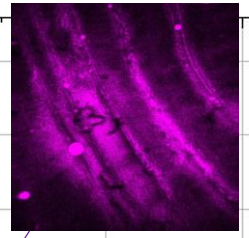


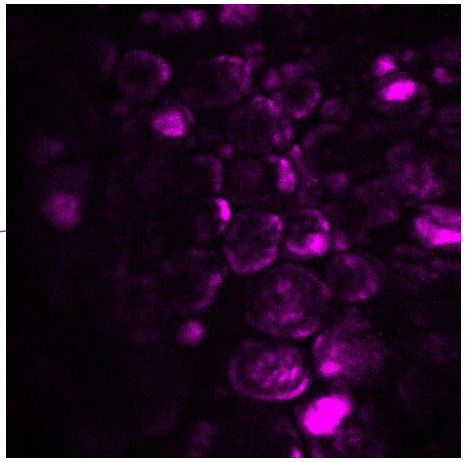
Extra cellular matrix



glass

THG intensity profile





Adipose tissue

Conclusion

- Non-invasive techniques like Raman, TPEF, and SHG can extract important data from tissue samples.
- Multiphoton images offer better tissue penetration, resolution, and reduced photodamage.
- Combining multiple optical techniques can overcome individual limitations, improving cancer diagnosis.
- Photonics-based methods could enhance diagnostic accuracy.

Acknowledgements

The SLN Group

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MERLIN
"la Caixa"
ImageInLife
Fundació CELLEX Barcelona
Fundació La Marató de TV3
MARIE CURIE ACTIONS
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Amplitude
EURO-BIOIMAGING
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