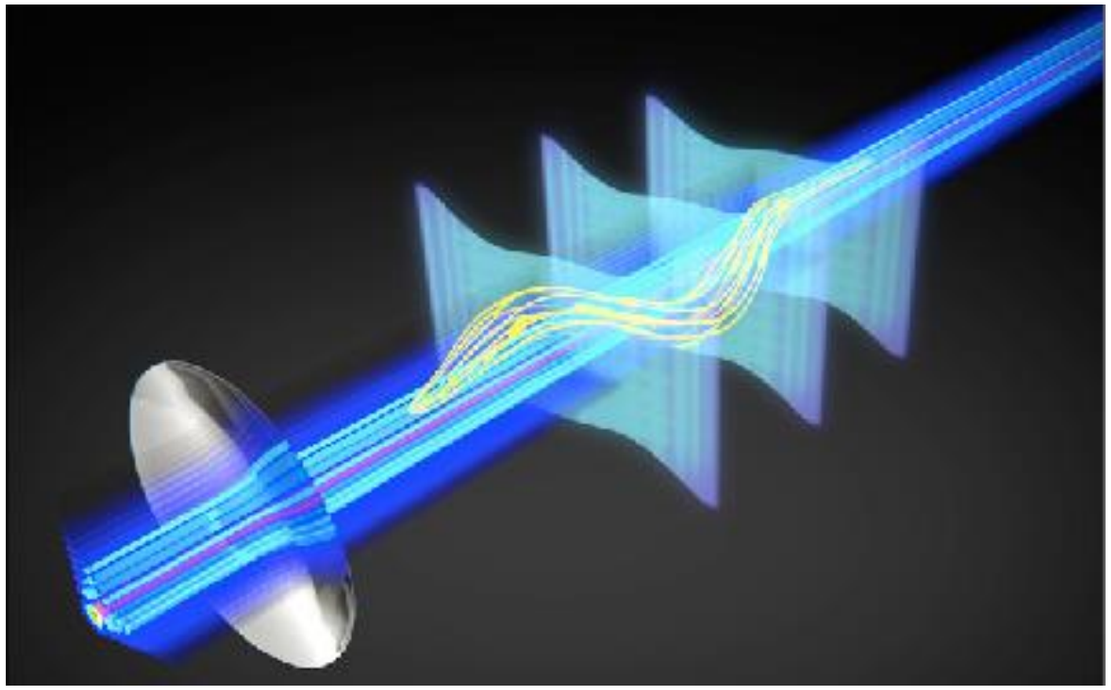
Challenges in Two-photon Excited Microscopy and in Light Sheet Microscopy



The Super resolution light microscopy
and nanoscopy lab (SLN)

Pablo Loza-Alvarez

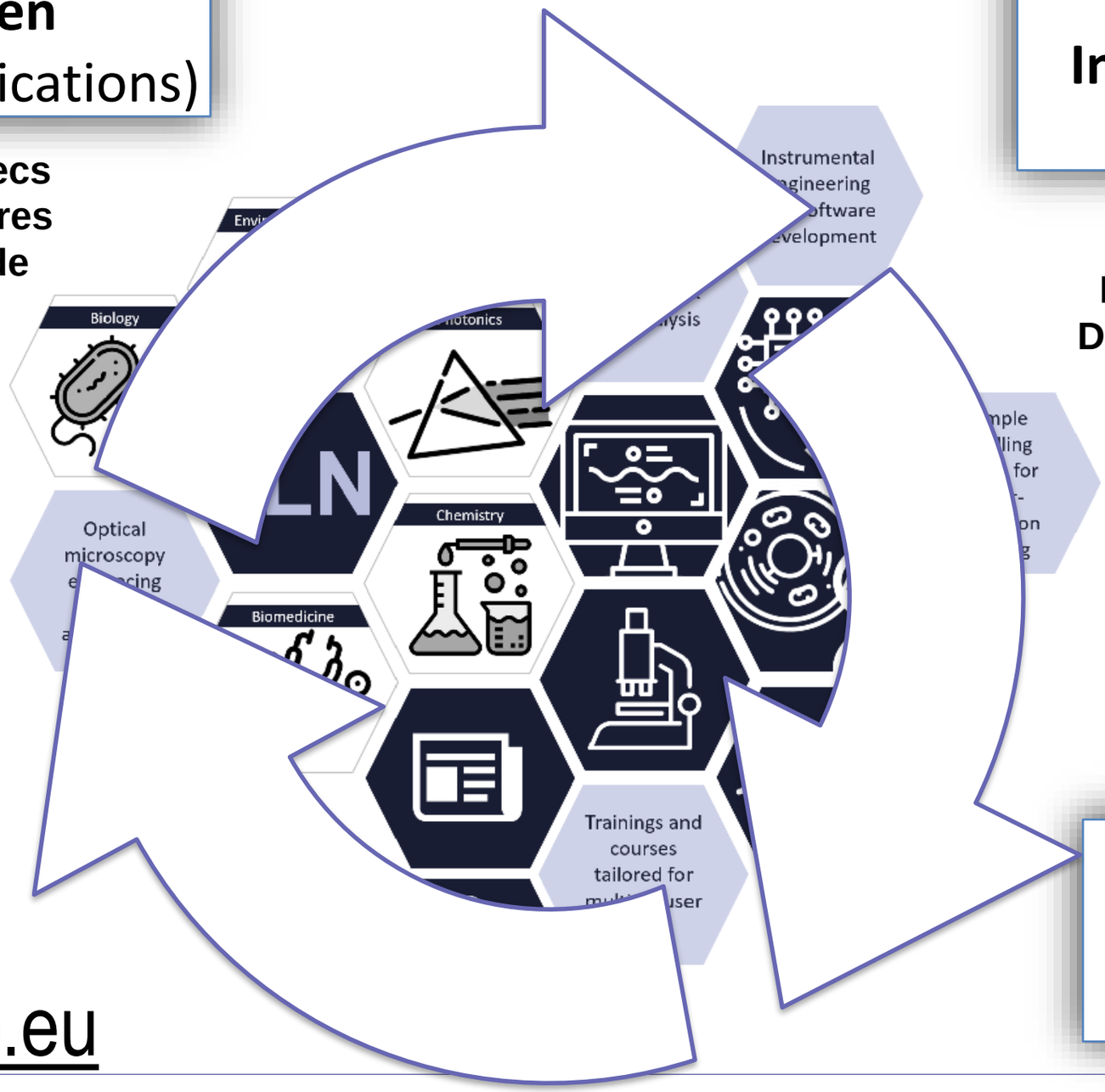
pablo.loza@icfo.eu

http://sln.icfo.eu

The Super resolution light microscopy and nanoscopy lab (SLN)

ACCESS
User driven
(biological applications)

Improved Specs
Unique Features
Custom made



R&D
Instrument development
(optics and photonics)

Adapt: **new technologies**
Merge: **imaging techniques**
Develop: **novel instrumentation**

New Generations
Different Backgrounds
According to Needs

TRAINING
Latest technologies
(all types of users)

http://sln.icfo.eu

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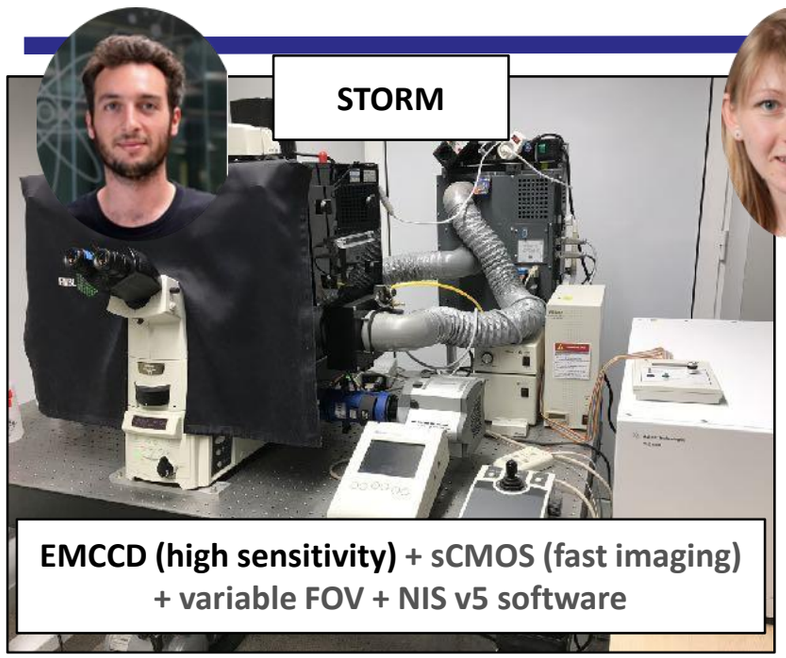
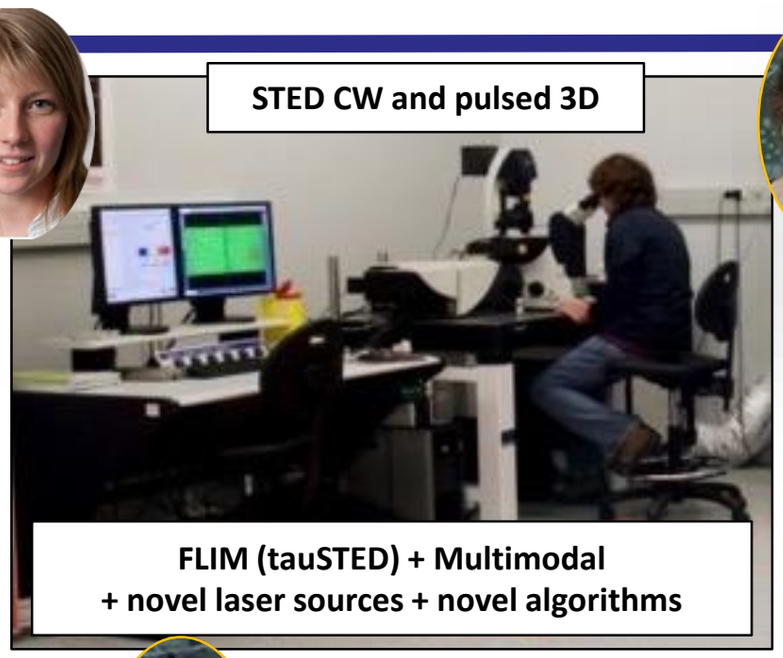
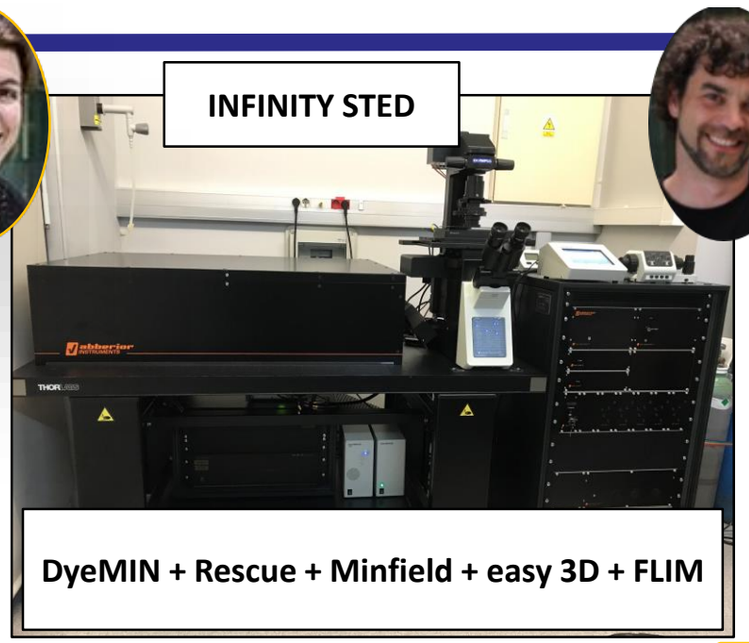
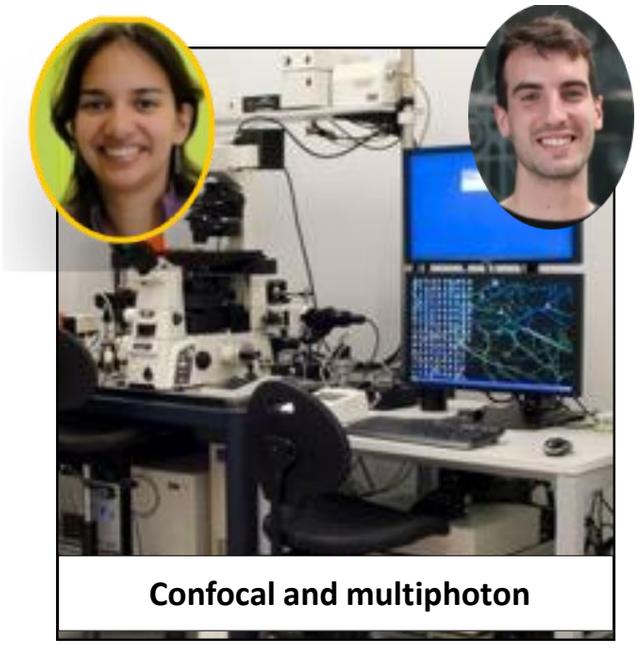
The SLN Team

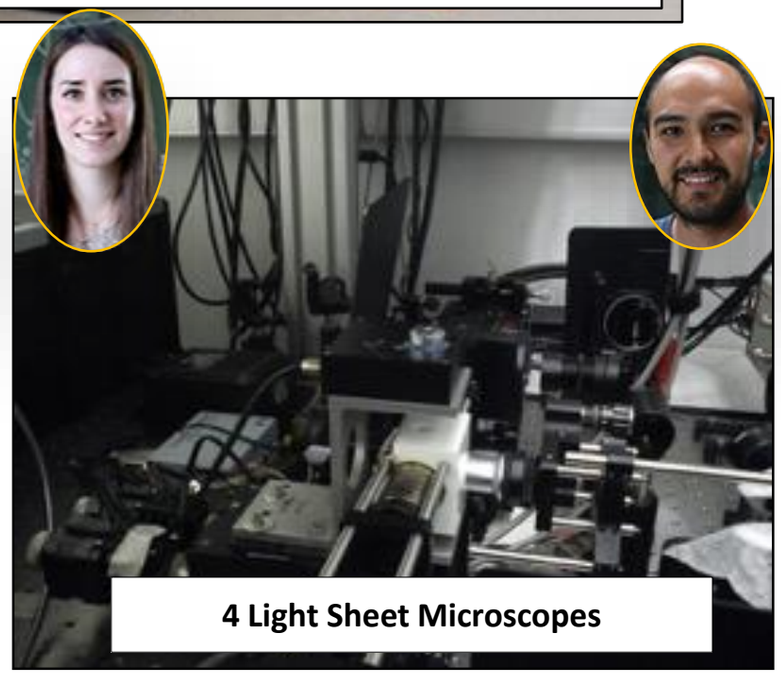




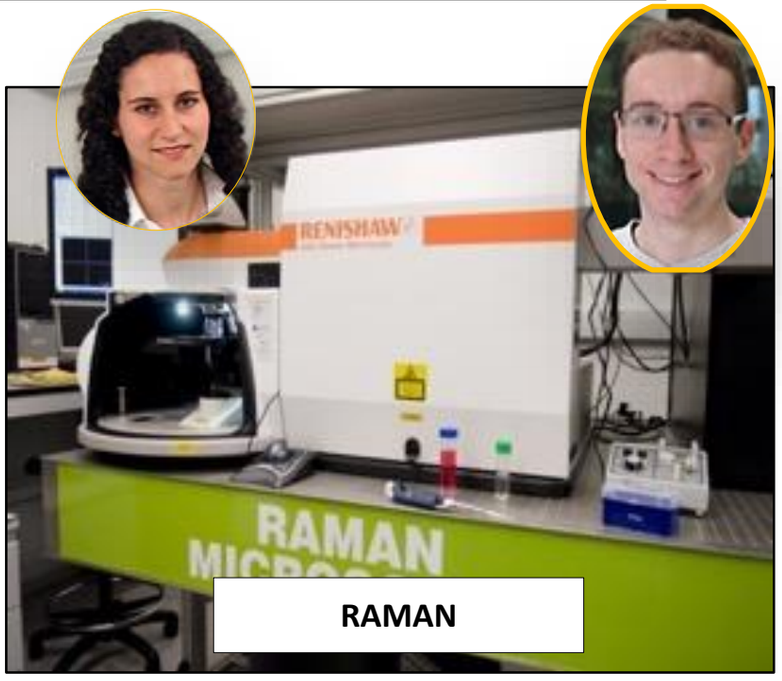




Spectral Confocal+
TPEF +SHG+THG (nonlinear)
+AOSLO+ Photomanipulation



Linear, nonlinear, RAMAN
High throughput, Fast 3D imaging



Advanced Mathematical and
Statistical analysis

- 10 researchers:
- 3 Permanent Staff
- 4 Postdocs
- 3 PhD students

- EU, National and Regional projects
- Private and public funded
- ~10+ Q1 pub/year

- 11 imaging systems
- <80 Internal and External users/year
- Usage/year: 8K-9K hrs

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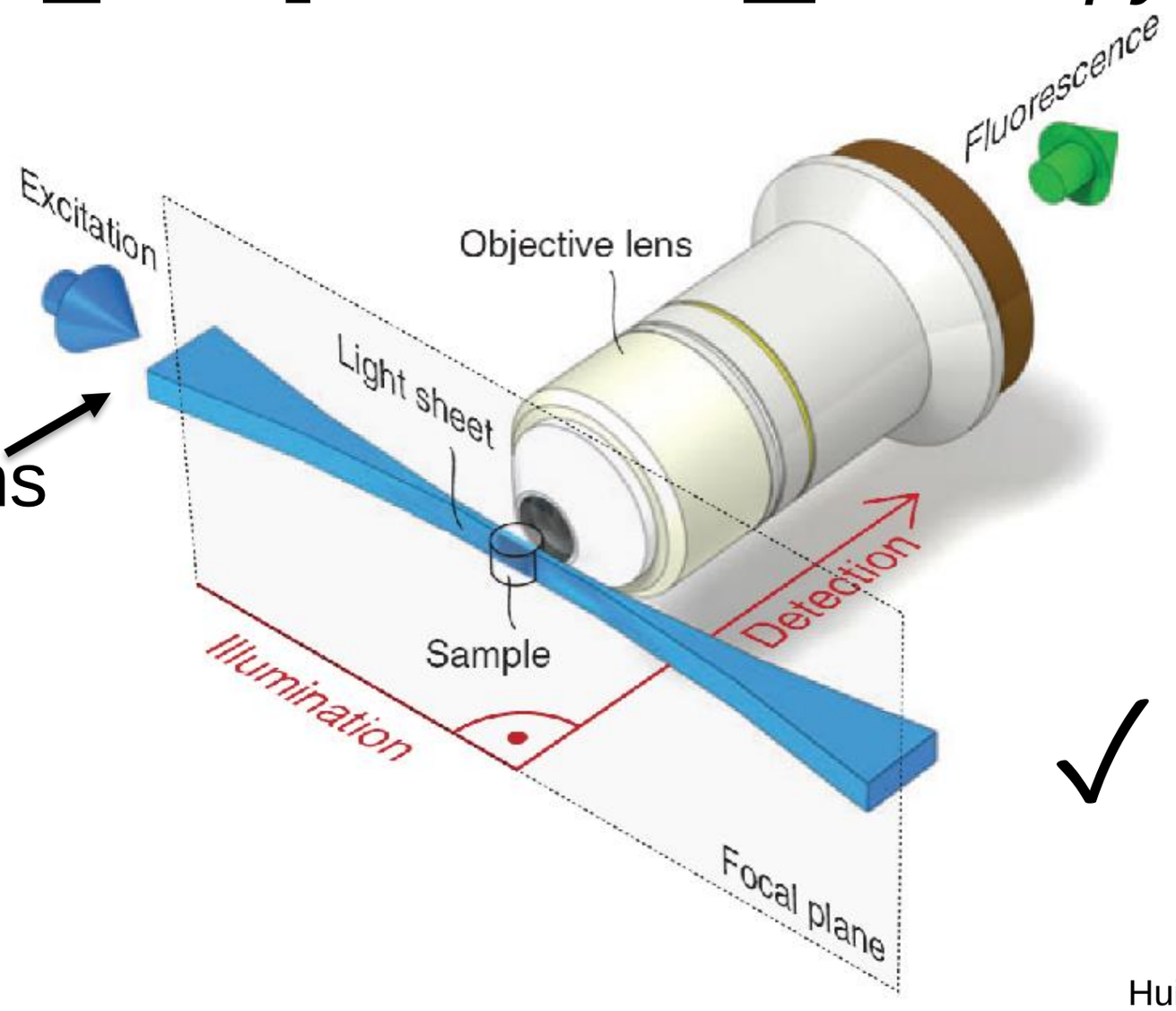
Light-Sheet fluorescence Microscopy

Selective Plane Illumination Microscopy (SPIM)

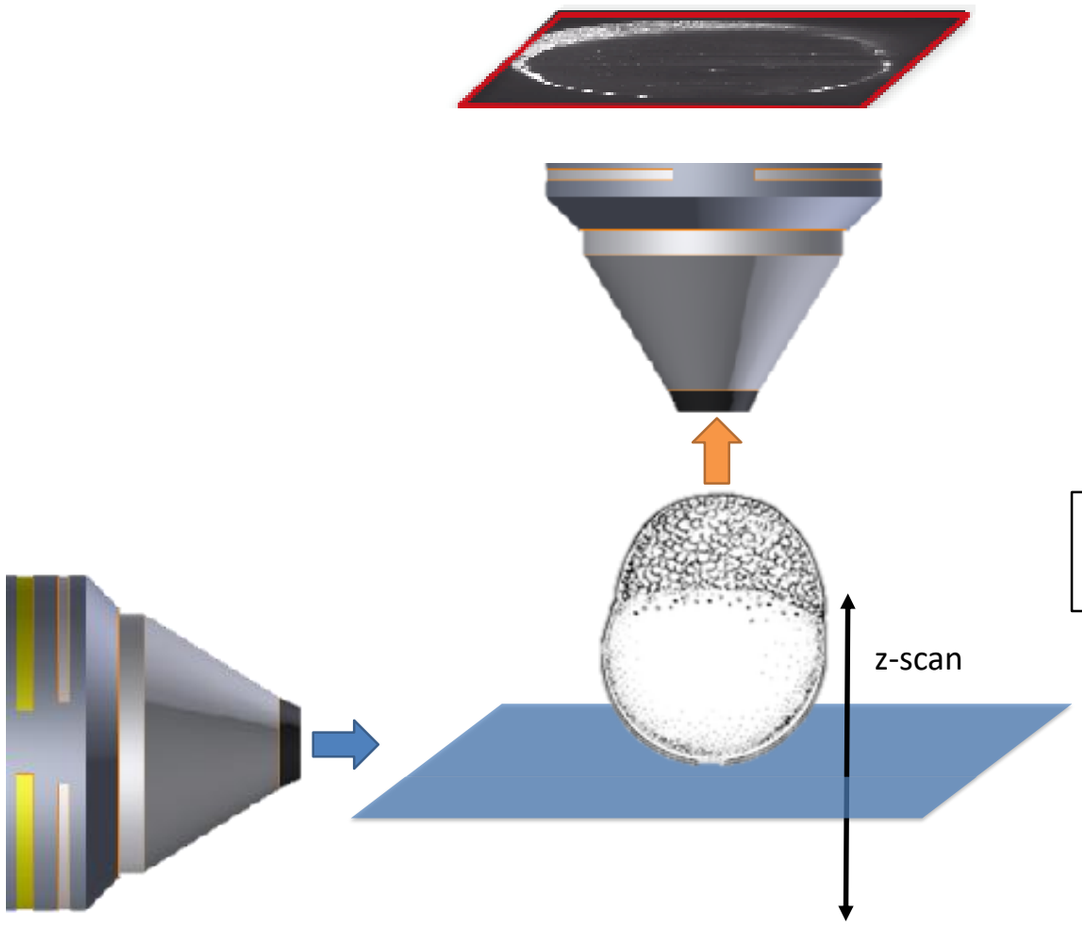


Cylindrical Lens

✓ FAST imaging

Adapted from
Huisken, J. et al., Development (2009)

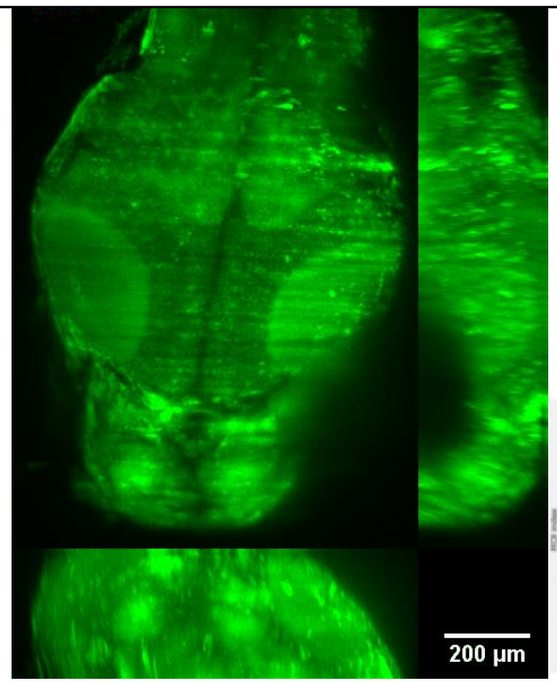
Light-Sheet fluorescence Microscopy



A 3D image is formed by:

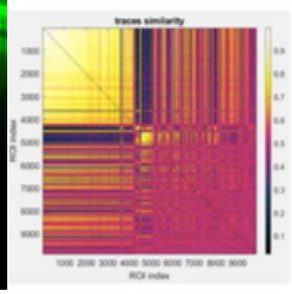
MOVING the SAMPLE
through
the light sheet

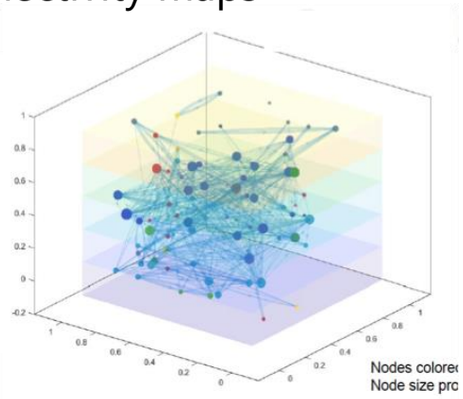
Image speed ~ 1 hertz



Zebra fish brain activity
Top, lateral and frontal projections

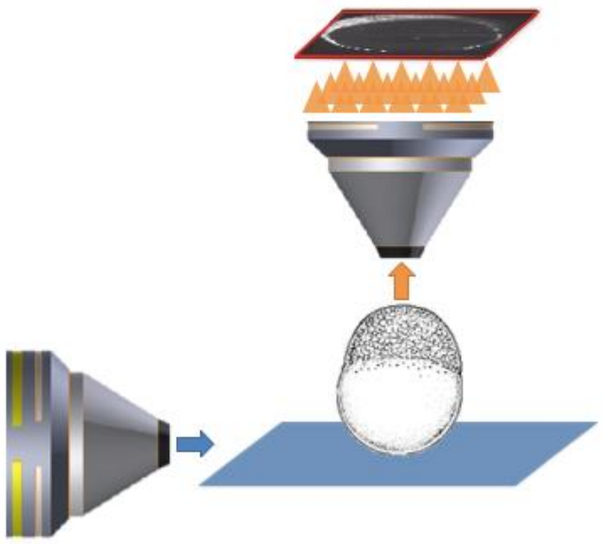
3D Connectivity maps





Important dynamics my be lost!

Fast volumetric imaging in LSFM



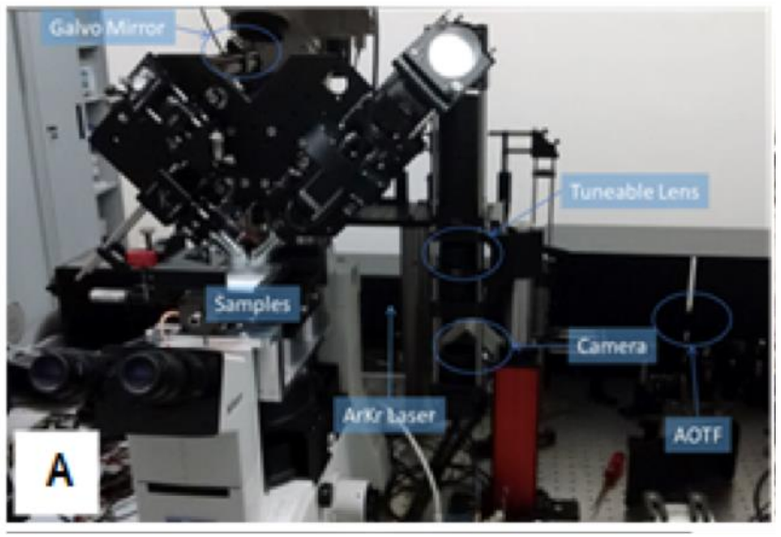
A 3D image is formed by:

MOVING the SAMPLE
<u>through</u>
the light sheet

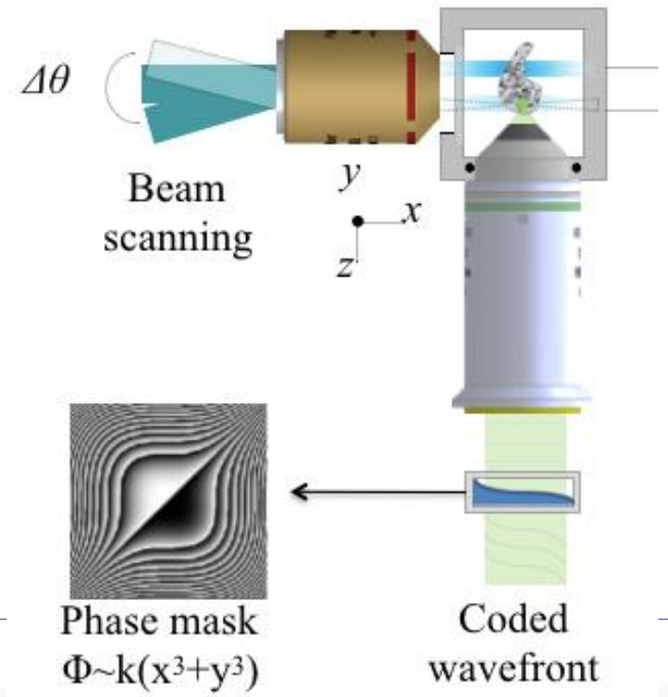
SOLUTION:

ONLY Move the light sheet

I: Use Electrically tunable lenses

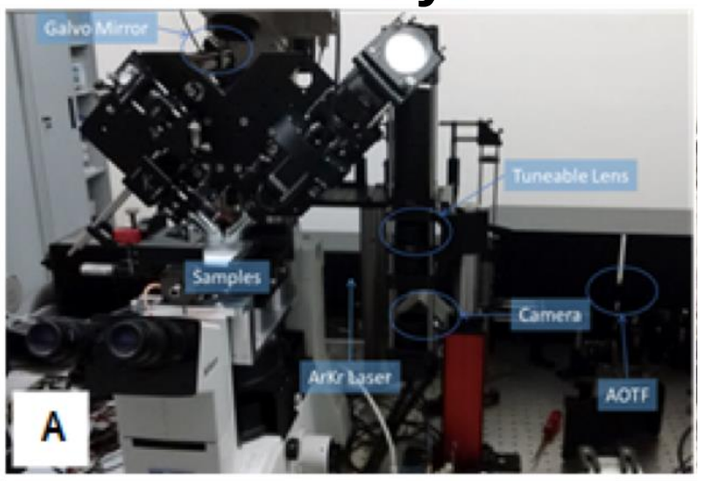


II: Extending Depth of field (wave front coding)



Fast volumetric imaging in LSFM

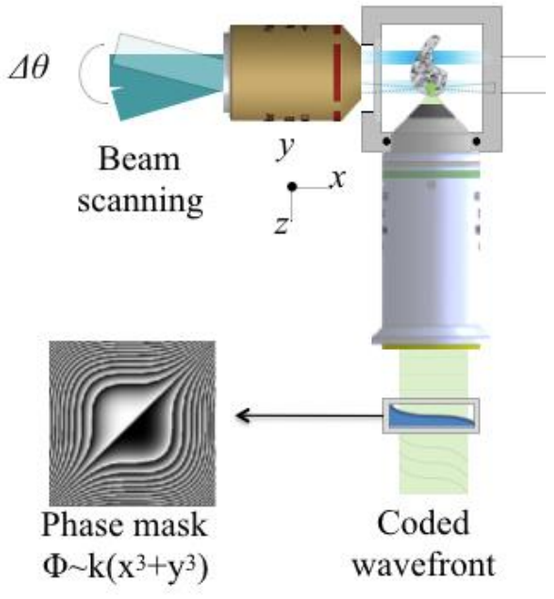
I: Use Electrically tunable lenses



25 vols/sec
Small volumes
1Vol < 10 planes

<u>At fast speeds</u>
Loss of linearity
Loss of amplitude

II: Extending Depth of field (wave front coding)



Potential to reach 100 V/s

Needs calibration (PSF)
Requires deconvolution (high SNR)

<u>CHALLENGES</u>

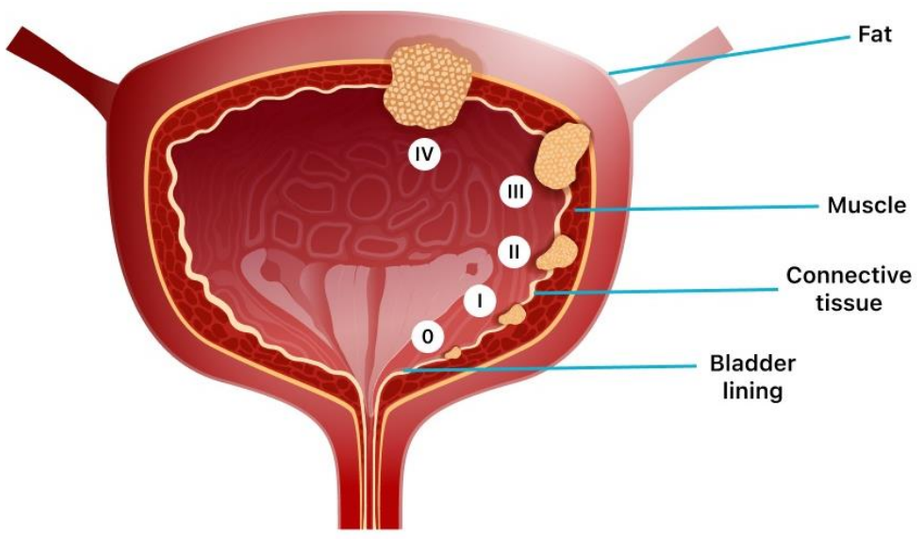
- Brighter Fluorescent markers
- Faster 2D array detectors
  High resolution (small pixels)
  High sensitivity
- Large amount of data (~0.5 TB/hr)
  Storage
  Processing
  Accessibility

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

www.amplitude-imaging.com

Bladder Cancer

To identify the first stages (0, I) of bladder cancer



5-year survival rate decreases with increasing stage:

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

<u>Bladder thickness ~ 3mm,</u>

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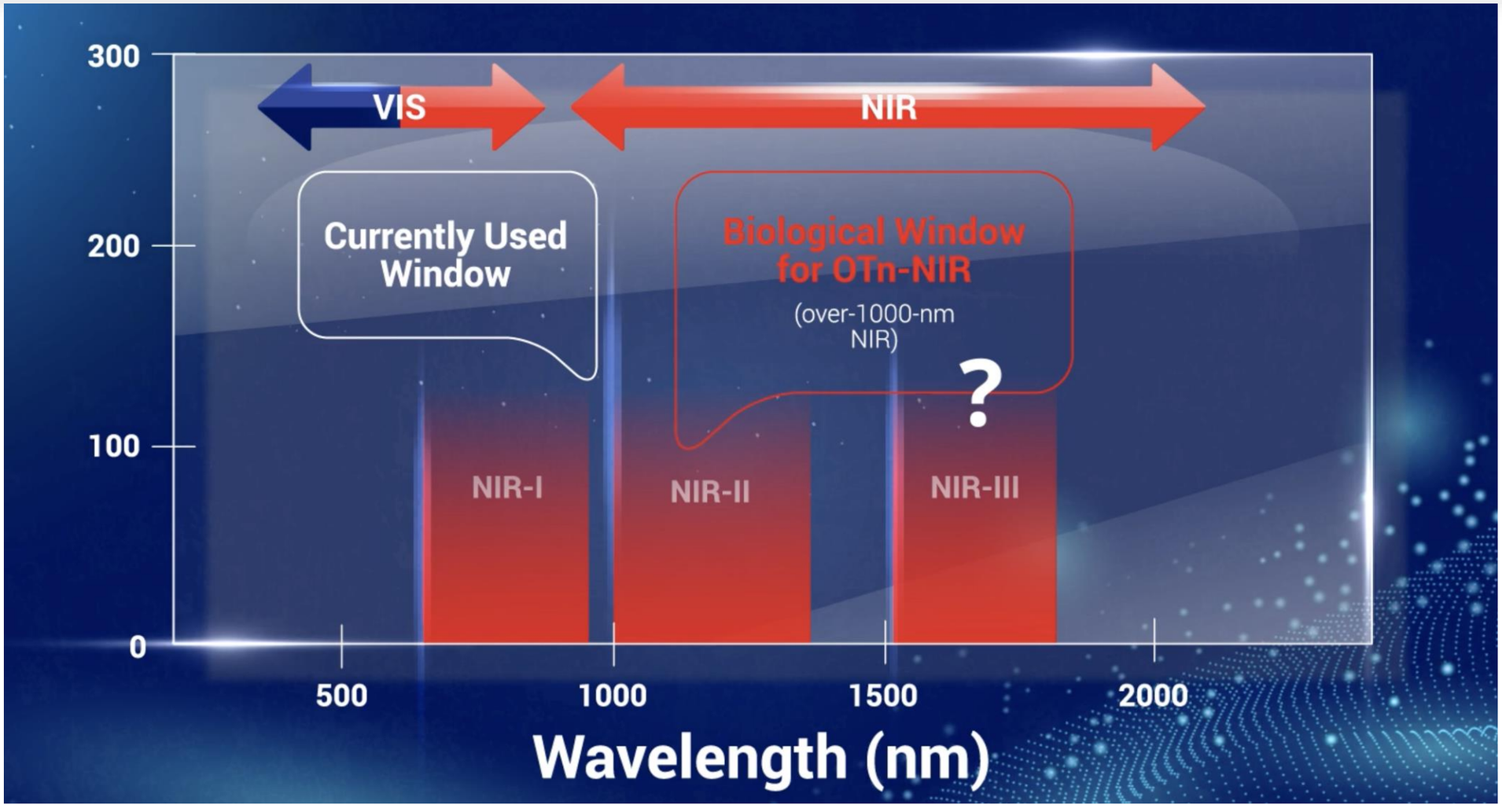
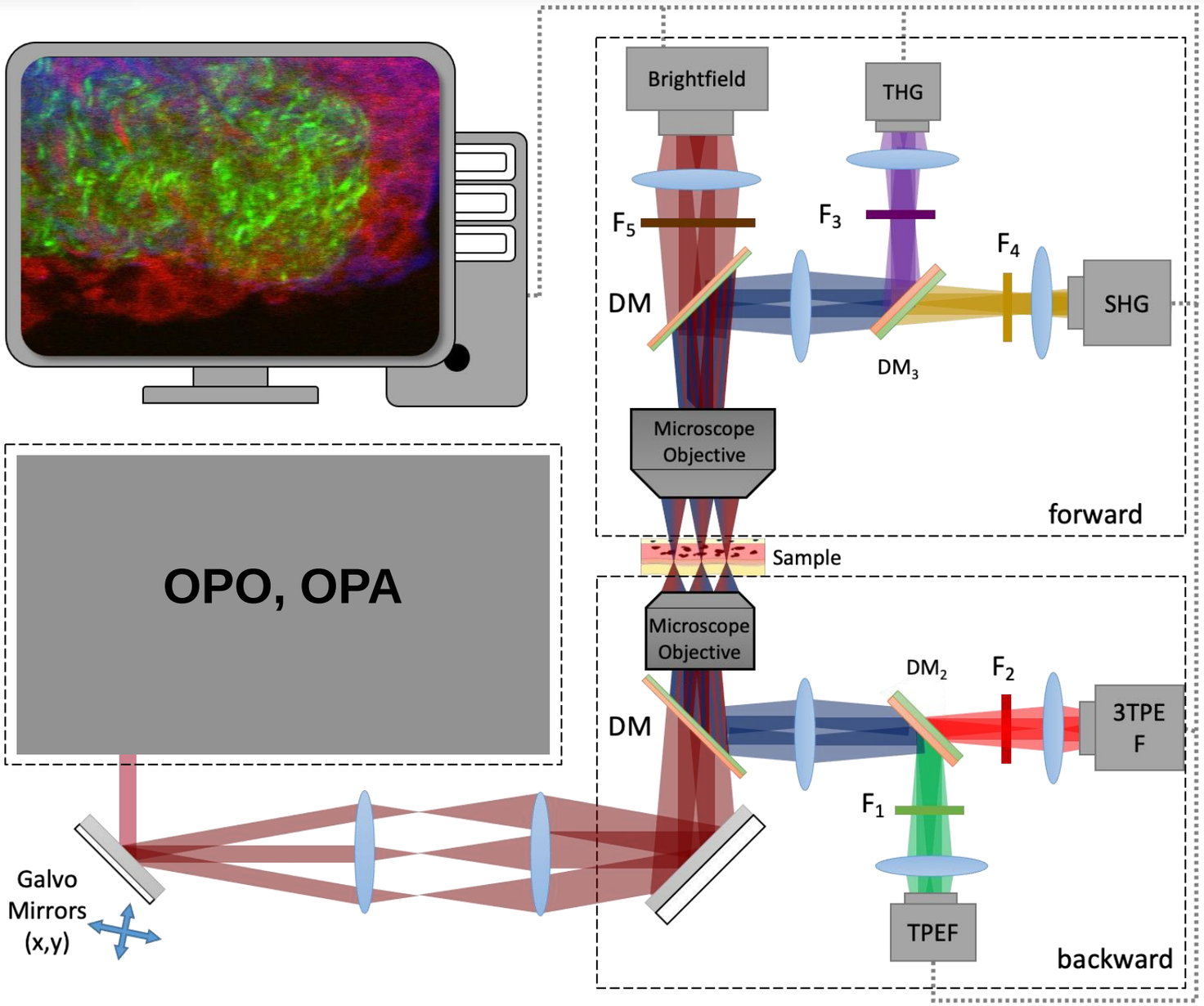
Explore the third optical window for imaging at large penetration depths



Stretched ~1mm **Optically accessible at 1700nm**

Label free imaging: NON LINEAR



Explore the use of the OPO at 1700nm for

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

Frequency doubled OPO source (at 850 nm)

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

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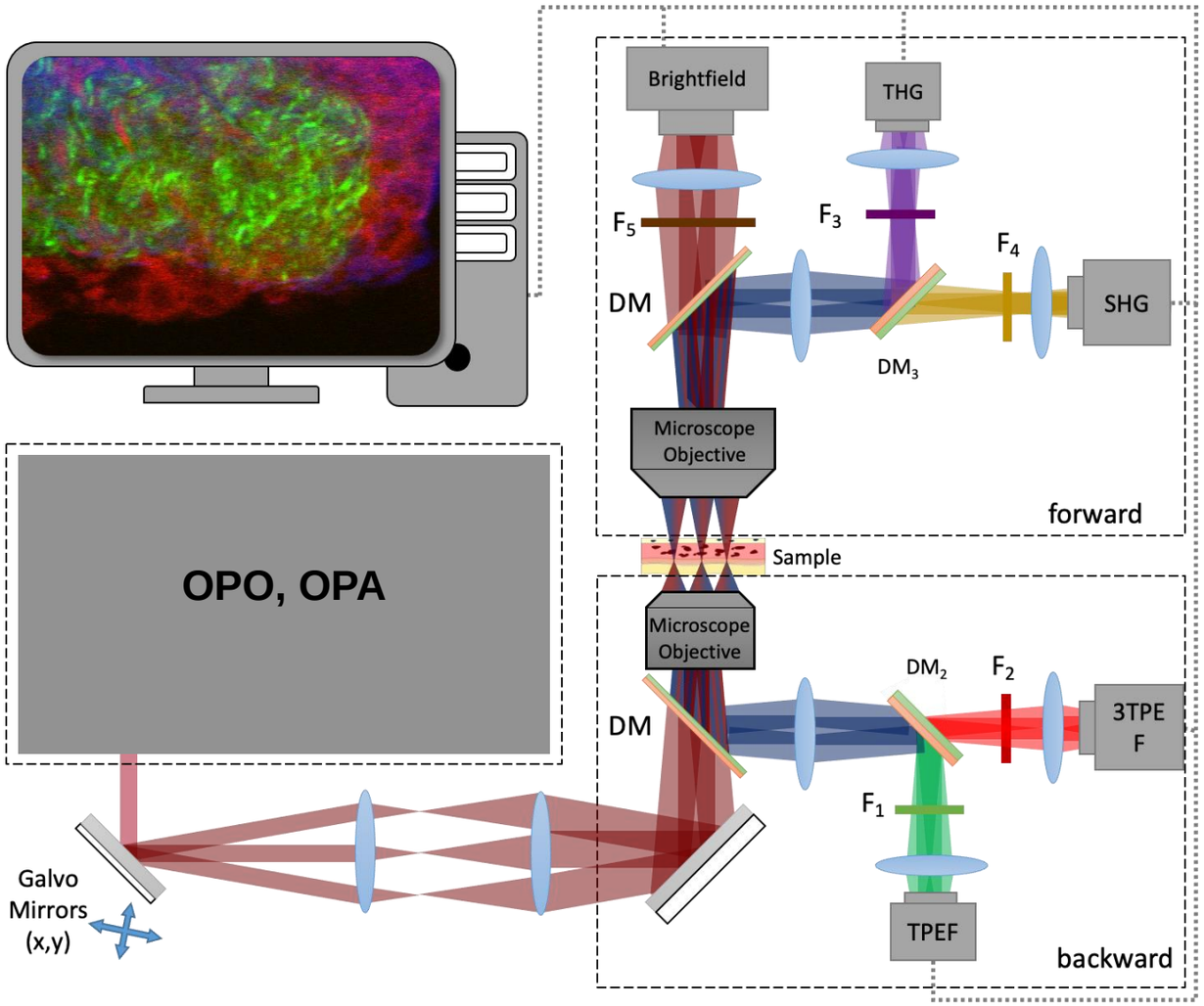
Challenges and opportunities

CHALLENGES



OPPORTUNITIES

Thank you!


GOBIERNO
DE ESPAÑA
MINISTERIO
DE CIENCIA
E INNOVACIÓN


LaserlabEurope


PREMIUM


MesoBrain
FET 2020


MERLIN


"la Caixa"


Fundació
La Marató de TV3


Fundació
CELLEX
Barcelona


ACT
PHAST


MARIE CURIE ACTIONS
HORIZON 2020


BIST
THEIA


mplitude


ImageInLife


CORBEL


EURO-BIOIMAGING


MARIE CURIE ACTIONS
HORIZON 2020