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Deciphering biological processes by multiscale fluorescence microscopy and nanoscopy @CISUP

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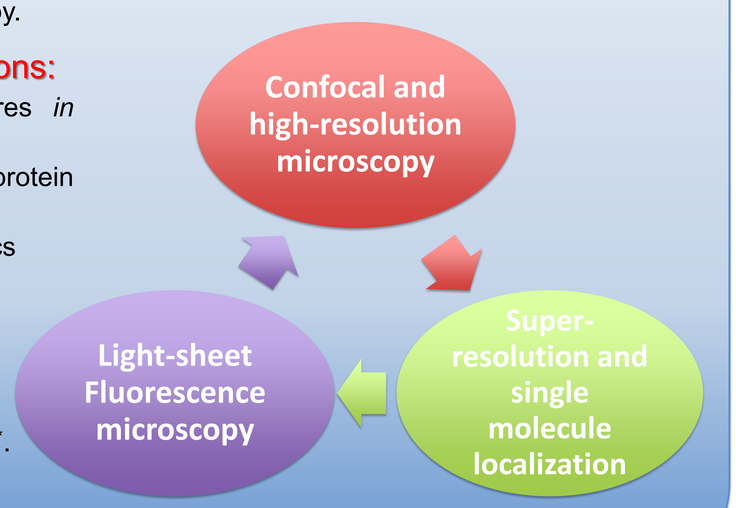
ABSTRACT

Fluorescence Microscopy and Nanoscopy systems are available at the Flagship Imaging Facility @CISUP. Different imaging techniques with complementary characteristics are integrated within the CISUP environment, spanning from super-resolution, single-molecule localization microscopy, AiryScan confocal microscopy and light-sheet illumination microscopy.

Requirement and applications:

- ✓ Observe subcellular structures *in vitro* and *in vivo*
- ✓ Characterize nanoscale protein distribution
- ✓ Study interaction and dynamics

The fluorescent imaging techniques available exhibits complementary features to high content screening techniques @CISUP (Operetta)*.



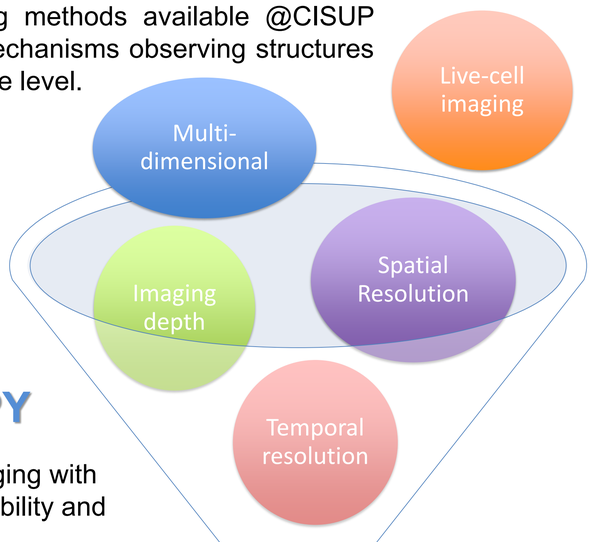
*see poster "Unlocking the Full Potential of High-Content Screening with Operetta CLS High-Content Analysis System"

KEY POINTS AND MAIN CHARACTERISTICS

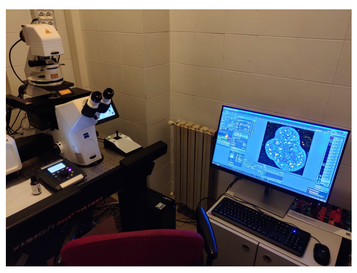
The different characeristics of the imaging methods available @CISUP provide a toolbox to unveil the biological mechanisms observing structures ranging from the molecular scale to the tissue level.

Specific features:

- ✓ Spatial resolution (2D and 3D)
- ✓ Temporal resolution
- ✓ 3D volumetric imaging
- ✓ Lifetime and functional measurements



CONFOCAL AND HIGH-RESOLUTION MICROSCOPY



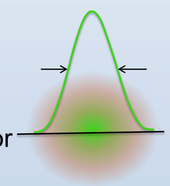
Confocal 4D imaging with high spectral flexibility and super-resolution (Zeiss LSM 900 Airyscan 2).

Unveil molecular processes in live cells

NANOSCOPY AND SUPER-RESOLUTION

Single molecule localization microscopy

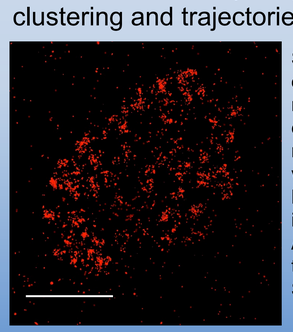
The super-resolution system Nikon STORM 5.0 provides a reliable microscopy method to achieve super-resolution images (lateral resolution ~20nm and axial resolution ~50nm)[1].



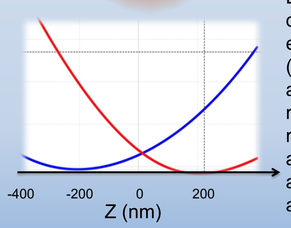
$$\sigma_{x,y}^2 = \frac{s^2 + \frac{a^2}{12}}{N} + \frac{8\pi s^4 b^2}{a^2 N^2}$$

Features:

- ✓ Widefield, HILO, TIRF illumination with CMOS detector
- ✓ Software for single-molecule localization, image reconstruction and post-processing (rendering, clustering and trajectories analysis)



Super-resolution images of DNA damage and repair proteins after conventional radiotherapy exposure. γH2AX proteins were labeled with indirect immunostaining with Alexa647, Exposure time 30ms, 2000 frames. Scale bar 2μm.



Localization precision[2] dependence from the experimental parameters (PSF, N Photons, pixel size and background). 3D super-resolution imaging is reached performing astigmatic PSF engineering and calibration (axial accuracy ~50nm).

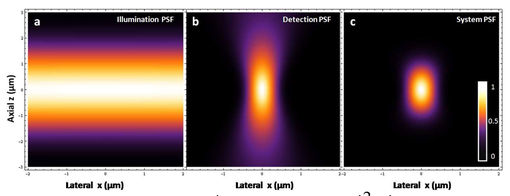
Main Applications:

- ➢ Molecular nanoscale organization studies and clustering
- ➢ Stoichiometry and supra-molecular organization

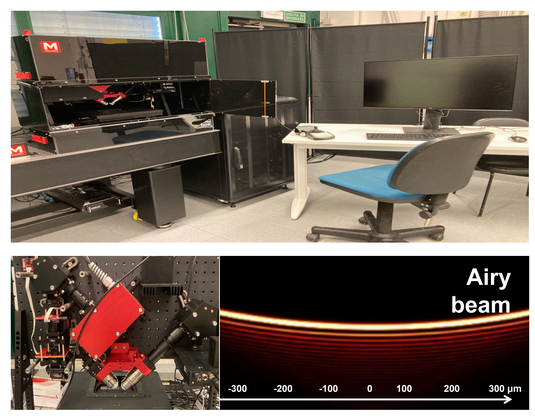
LIGHT-SHEET ILLUMINATION MICROSCOPY

The Light-sheet illumination concept:

Orthogonal illumination and detection paths with a planar illumination volume improve the imaging depth and reduce light-sample interaction[3].



$$PSF(x,y,z)_{SPIM} = \left|h_{ill}(-z,y,x)\right|^2 \cdot \left|h_{\det}(x,y,z)\right|^2$$



Features:

- ✓ Inverted configuration
- ✓ Fast 3D imaging with optical sectioning (100 frames/sec),
- ✓ 10mm working distance

Main Applications

- ➢ Live 3D volumetric imaging
- ➢ Tissue and whole organisms imaging
- ➢ Observing human brain organoids, neuronal populations in murine and human tissues, cellular arrangement in human-relevant models of liver and barriers

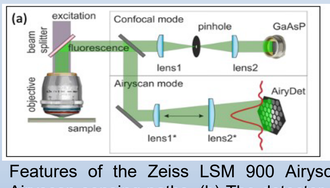
Airy beam illumination[4] improves the field of view (FOV) and innately yields up to a ten-fold larger FOV:

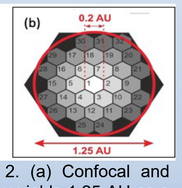
$$FOV_{Gaussian} \approx 4\frac{\lambda n}{NA^2} \qquad FOV_{Airy} \approx 6\frac{\alpha\lambda / n}{1-\sqrt{1-\left(NA/n\right)^2}}$$

APPLICATIONS: SUPER RESOLUTION ON LIVING SAMPLES @CISUP

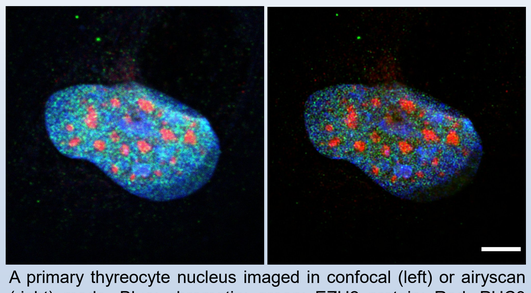
Confocal microscopy and the ISM concept

Image Scanning Microscopy (ISM) overcomes the resolution/SNR compromise of typical confocal systems by replacing the single point detector of a confocal microscope with a detector array[4].





Features of the Zeiss LSM 900 Airyscan 2. (a) Confocal and Airyscan sensing paths. (b) The detector array yields 1.25 AU as a whole, while each element behaves as a 0.2 AU pinhole. Credit: ref. 5 and 6



A primary thyreocyte nucleus imaged in confocal (left) or airyscan (right) mode. Blue: chromatin; green: EZH2 protein; Red: PHC3 protein. No deconvolution was applied to the airyscan image, and the resolution enhancement was exactly for all three channels. The ISM approach has been recently applied to investigate functional polycomb protein colocalization in adenocarcinoma lung cells[7]. Scale bar: 5μm

Features:

- ✓ resolution 120-140 nm
- ✓ higher SNR than confocal
- ✓ compatible with standard fluorophores/labelling and multicolor imaging *of living cells*.

Main Applications

- ➢ Live cell super resolution, including diffusion maps
- ➢ Correlative imaging with electron microscopy

HOW TO JOIN AND ACCESS:

Policies, access and booking procedures and are available at https://cisup.unipi.it
Prices* for instruments use:

Microscope	Internal users (UNIPI)	External users (Public entities)	External users (Private entities)
Nikon STORM 5.0 (single molecule)**	40€/h	90€/h	160€
Light-sheet Aurora **	40€/h	90€/h	160€
Zeiss LSM 900 Airyscan 2 (Confocal/ISM)	10€/h	30€/h	40€/h

**Prices include training and support. For autonomous users a 50% reduction will be applied
*Prices VAT excluded

REFERENCES AND LITERATURE:

[1]Scalisi, S., et al. *Microscopy Research and Technique*, **1**– 11 (2023).
[2]Deschout, H., Zanacchi, F., *et al. Nat Methods* **11**, 253–266 (2014)
[3]Stelzer E.H.K.*et al. Nat Rev Methods Primers* **1**, 73 (2021).
[4]Castello, M. *et al. Nat Methods* **16**, 175–178 (2019)
[5]Huff, J. *Nat Methods* **12** (2015)
[6] Korobchevskaya et al. *Photonics* **4** (2017)
[7]Nepita I. et al. *Appl. Sci.* **13**(3), 1556 (2023)

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