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Photo: Laura Taverna

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ISTITUTO ITALIANO
DI TECNOLOGIA

Fluorescence Laser-Scanning Microscopy with SPAD Array Detector

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Molecular Microscopy and Spectroscopy (IIT),
and Genoa Instruments

EPIC
European Photonics Industry Consortium

Technology Meeting on Novel Photonic Solutions for Microscopy
28th June 2021, Online, 15.00 - 17.00 CEST

Who We Are?

Dr. S. Perego
Post Doc
Biophysicist

Dr. G. Tortarolo
Post Doc
Bioengineer

Dr. E. Slenders
Post Doc
Physicist

M. Scotto
Senior Technician
Physicist

A. Rossetta
PhD 3rd Year
Bioengineer
(with Nanoscopy)

A. Bucci
PhD 2nd Year
Physics-Engineer

Dr. M. Donato
Junior Technician
Physicist

F. Fersini
PhD 1st Year
Electronics-
Engineer

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DI TECNOLOGIA
MOLECULAR MICROSCOPY
AND SPECTROSCOPY

S. Zappone
Fellowship
Biotechnologist

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We are hiring
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Researcher

Marco Castello
Post Doc - CTO
Bioengineer

Simonluca Piazza
Post Doc - CEO
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erc

POLITECNICO MILANO

Prof. Alberto Tosi
Dr. Federica Villa
Dr. Mauro Buttafava

iit NANOSCOPY

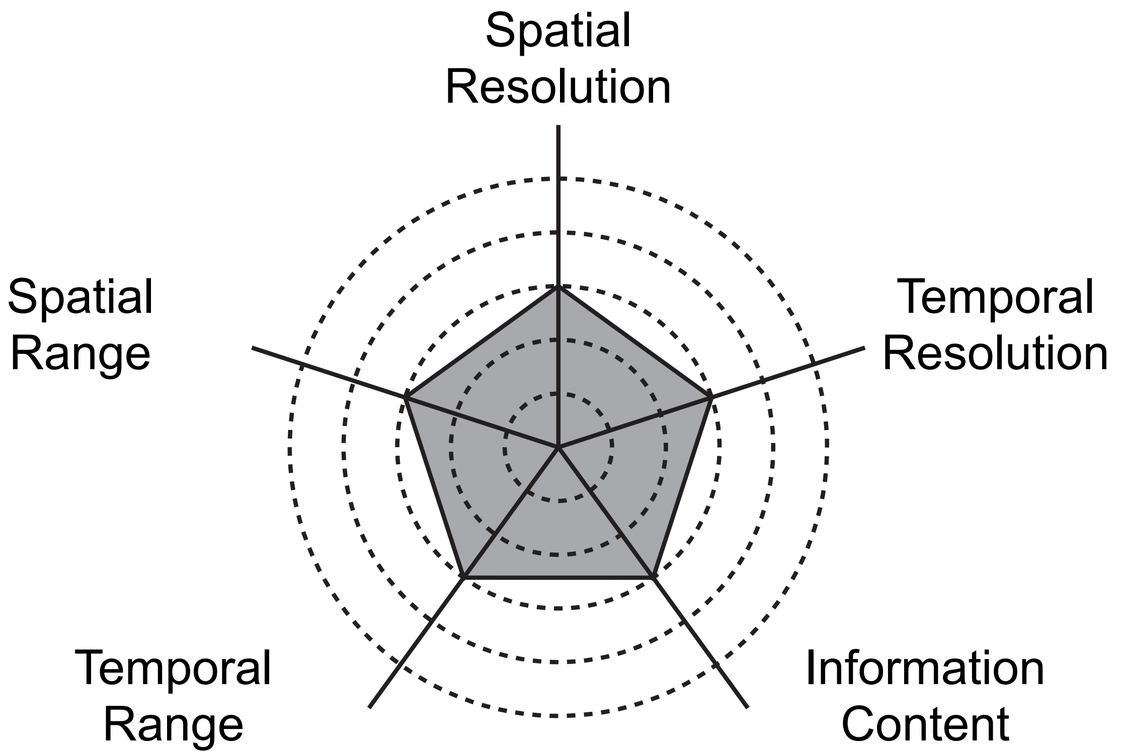
Prof. Alberto Diaspro
Prof. Colin Sheppard
Dr. Paolo Bianchini
Prof. Luca Lanzanò

alumni

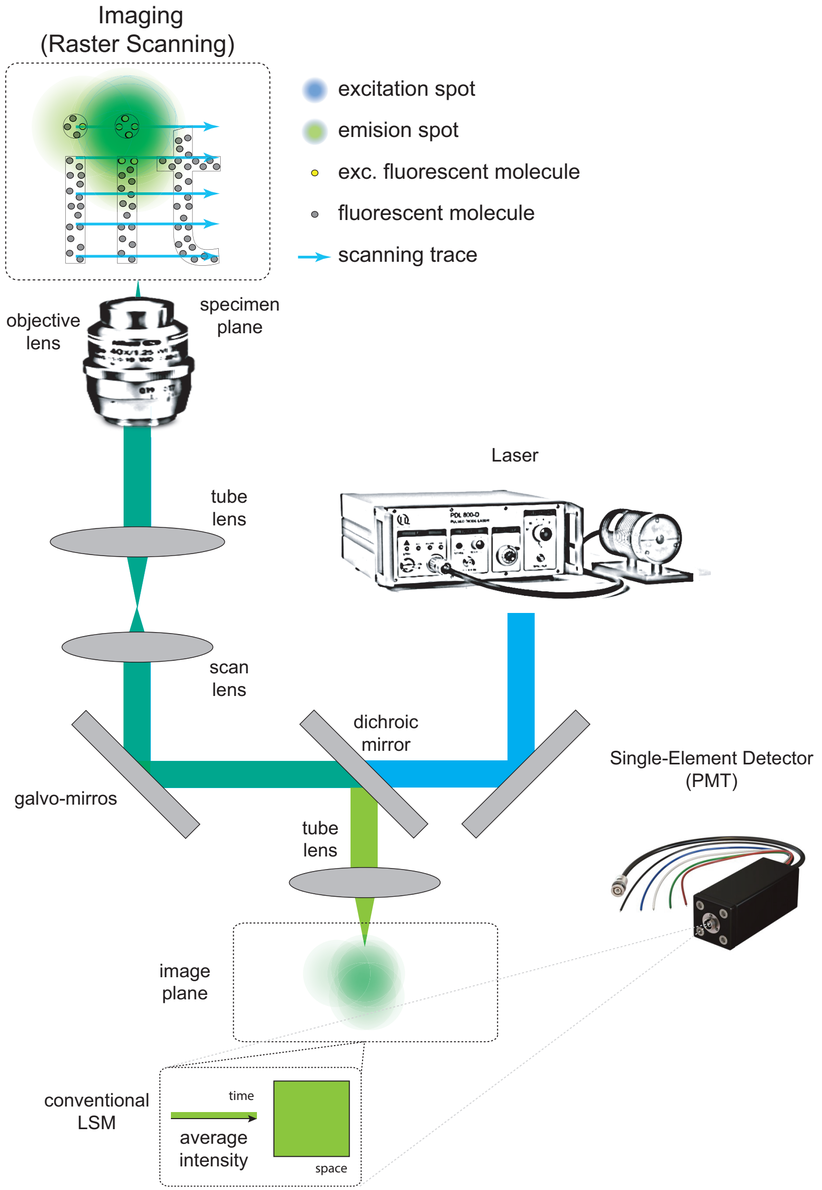
Dr. Sami V. Koho

What We Do?

The core research of the MMS lab is the design, development and validation of novel **optical and analytical tools** that allow the modern biologists **to observe biomolecule processes inside living biological systems with unprecedented temporal/spatial abilities and massive information content.**



Laser-Scanning Fluorescence Microscopy (LSFM)



- a beam is focused by the objective lens on a (usually diffraction-limited) region of the sample;
- the molecules inside this region emit fluorescence which is collected by the objective lens and imaged by the tube lens in the image plane;
- a single-element detector (usually a PMT) **integrates - across time and space - all the photons** in the image plane providing a single-intensity value;
- the region is scanned across the sample and all the intensity values registered during the different pixels dwell-time allow to build-up the image

by integrating photons (in time and space) a lot of useful information are lost;

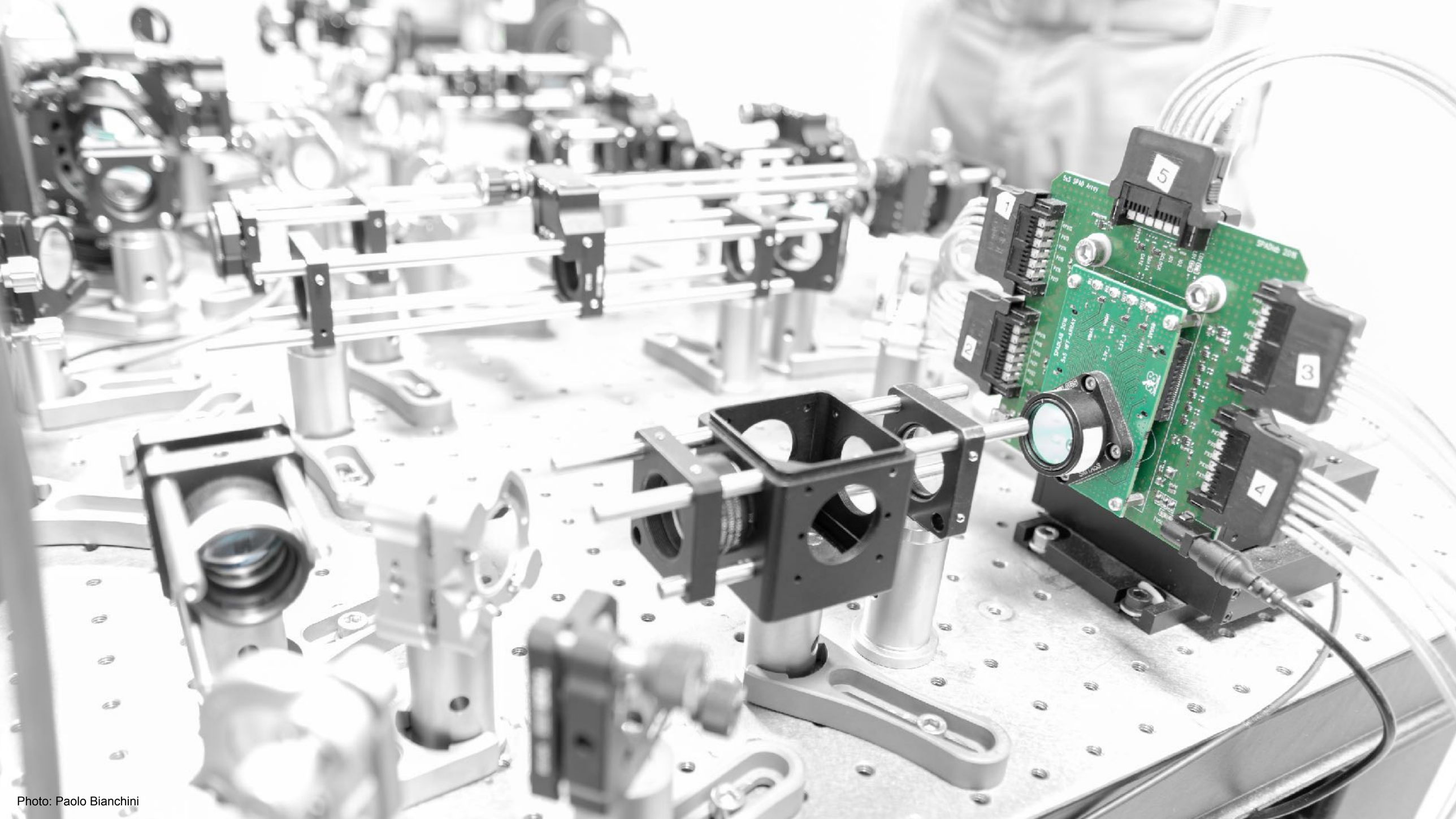
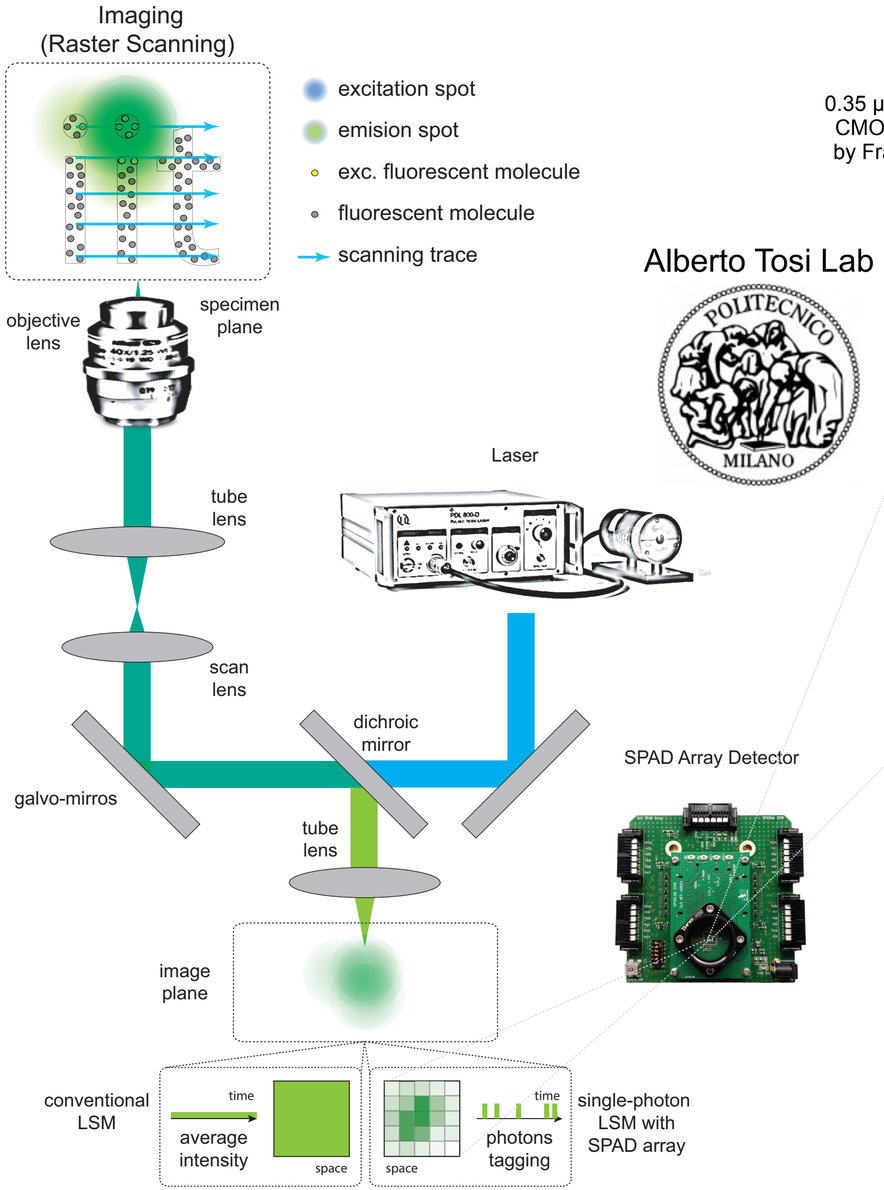
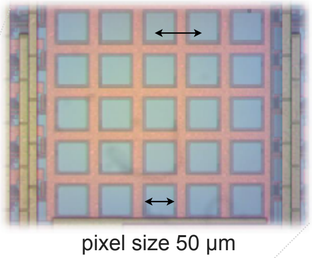


Photo: Paolo Bianchini

Single-Photon LSFM (SP-LSFM)





- 5×5 element fully parallel (asynchronous read-out).

The number of elements is high enough to guarantees Nyquist sampling of the focal region, and low enough to maintain realistic data transfer.

- 44% fill-factor.

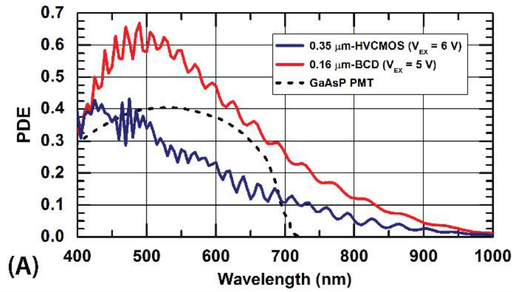
Fill factor enhanced with the BCD technology to 56%.

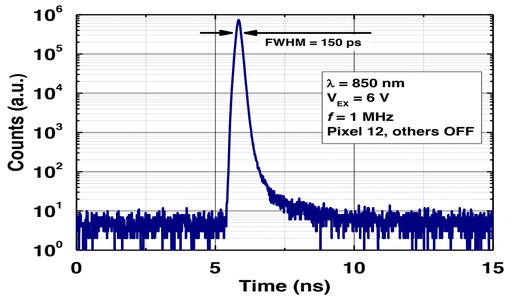
- < 1.5% cross-talk.

- PDE comparable to the typical GaAsP-PMT.

PDE doubled with the BCD technology (blue curve).

- 100 cps dark count @25°.





- 200 ps photon-timing jitter (FWHM).

Photon-timing jitter reduced to 100 ps with the BCD technology.

- tuneable hold-off time down to 20 ns.

50 MHz, < 10% after-pulsing.

Buttafava, M.,…, Tosi, A., Optica, 7(7):775-765 (2020)

SP-LSFM - A New Paradigma

From other groups:

[1] Tenne, R.,…, Oron D., Nat. Photonics 13:116–122 (2019)
[2] Sroda A.,…, Lapkiewicz R., Optica 7:1308-1316 (2020)
[3] Scippioni L.,…, Gratton E., Nat. Methods 18(5):542-550 (2021)

From other groups:

[4] Castello M.,…, Vicidomini G., Nat. Methods 16, 175–178 (2019)
[5] Tortarolo, G.,…, Vicidomini , G., bioRxiv 741389; doi:10.1101/741389 (2020)
[6] Koho, S. V.,…, Vicidomini G., Biomed. Opt. Express,11(6): 2905-2924 (2020)
[7] Slenders, E.,…, Vicidomini, G., Light Sci Appl., 10: 31 (2021)
[8] Rossetta, A.,…, Vicidomini, G., *Submitted* (2021)

- via image scanning microscopy (ISM)[4];
- via stimulated-emission-depletion ISM (STED-ISM)[5];
- via quantum microscopy (Q-ISM)[1];
- via super-resolution optical fluctuation imaging (SOFISM)[2];
- via image deconvolution[6];
- via single-molecule imaging.

Conventional LSM

Single-Photon LSM with SPAD array

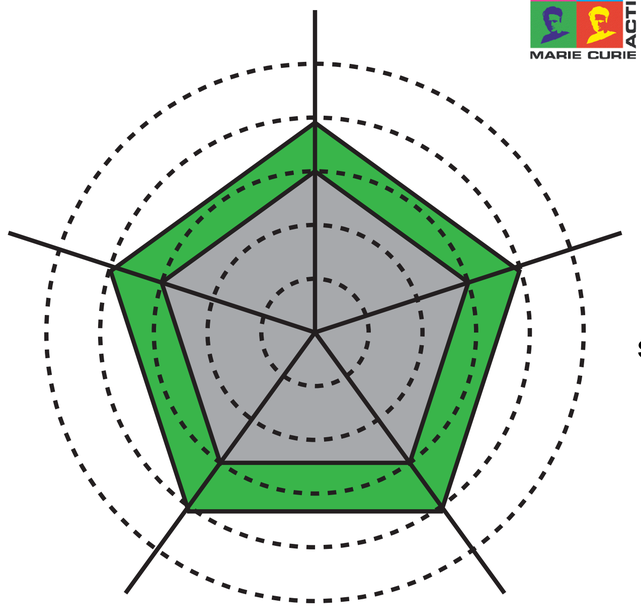
Spatial Resolution

Spatial Range

- via adaptive-optics;
- via two-photon-excitation ISM (TPE-ISM)[6];
- via out-of-focus rejection.

Temporal Resolution

- via ISM with resonant-mirror;
- via fluorescence fluctuation spectroscopy (e.g., FCS, RICS)[7,8];
- via real-time single molecule tracking.



Temporal Range

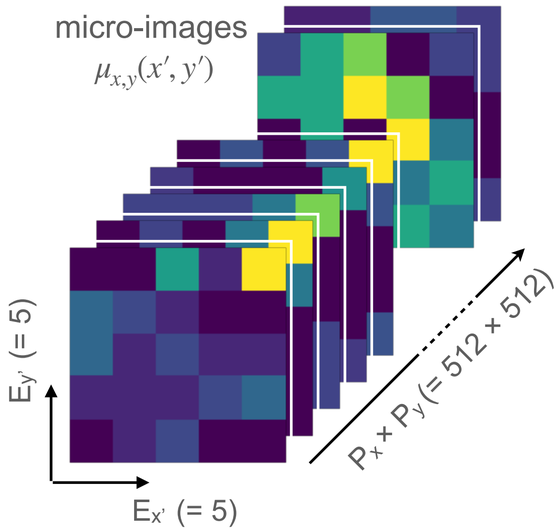
- via image scanning microscopy (ISM)[4];
- via time-resolved STED-ISM;
- via image deconvolution;
- via deep-learning.

Information Content

- via fluorescence lifetime imaging[4,8];
- via photon coincidence correlation;
- via fluorescence lifetime imaging and spectral imaging[3];

Super-Resolution SP-LSFM (via Image Scanning Microscopy)

raw dataset (micro-images or scanned-image)



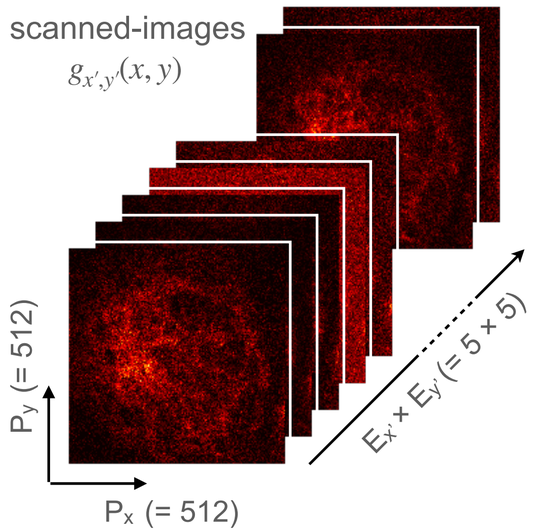
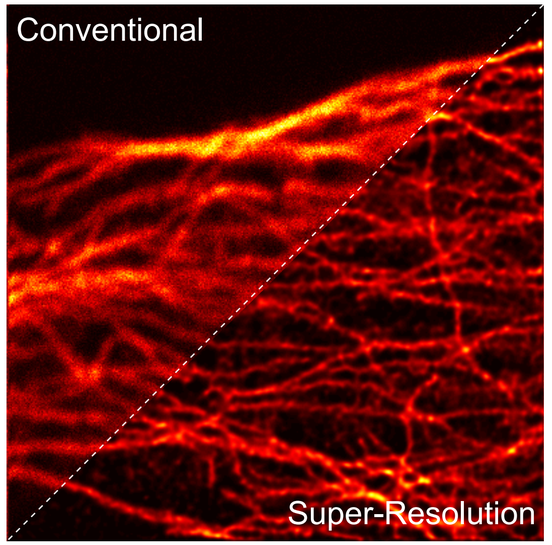


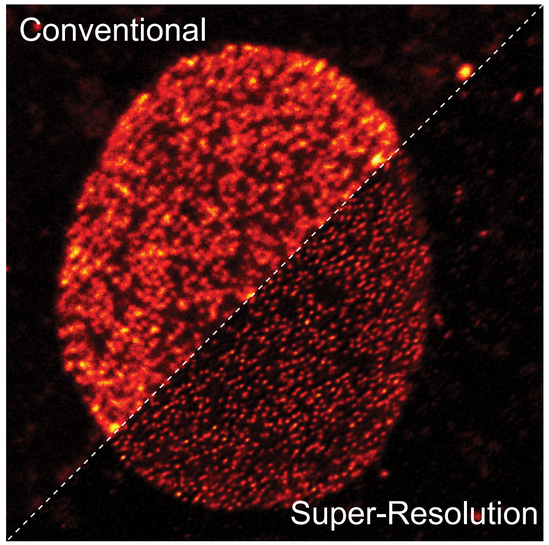
image reconstruction
(pixel-reassignment, deconvolution)

image scanning microscopy (ISM)

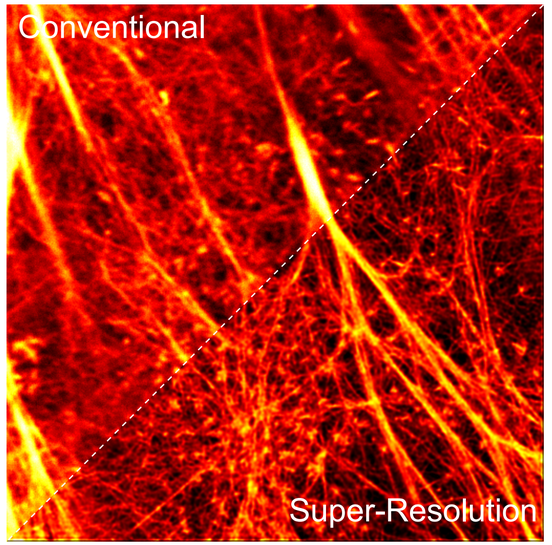
Tubulin



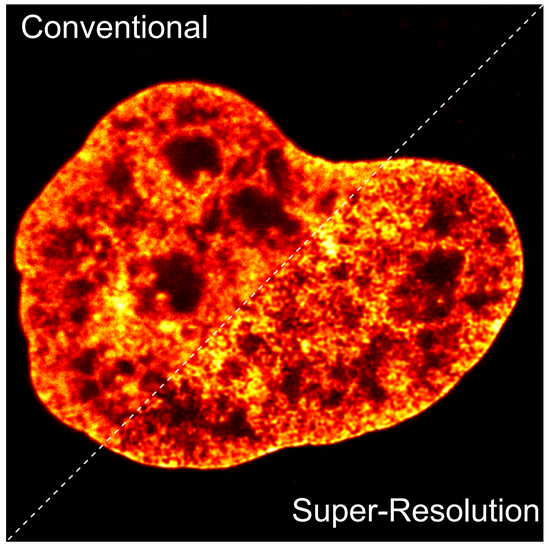
Nuclear Pore Complex



Actin

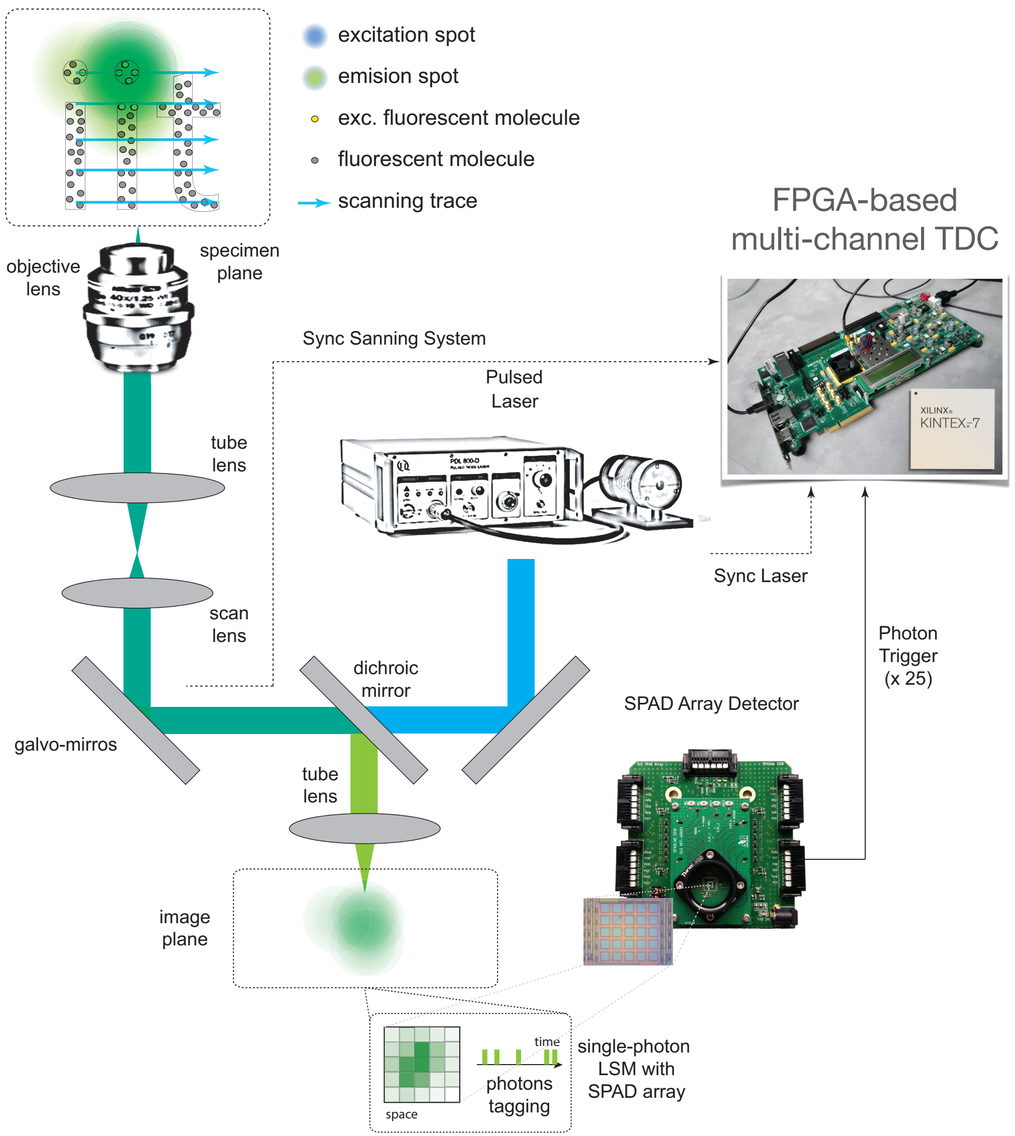


Chromatine



Castello M.,…, Vicidomini G., Nat. Methods 16, 175–178 (2019)

BrightEyes-TTM: Open Source Time-Tagging Module



Open-Source BrightEyes-TTM Project

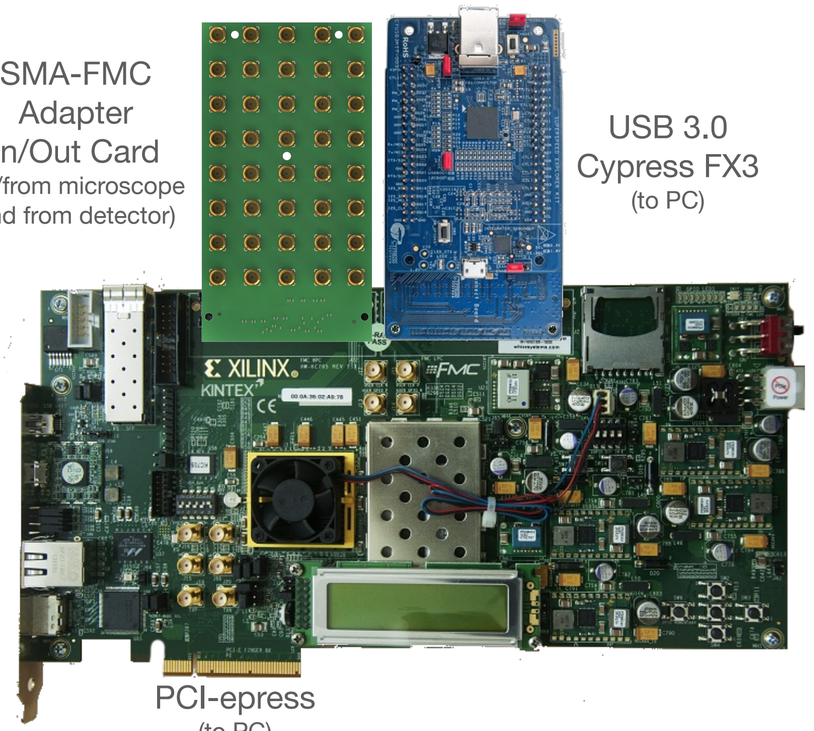
BrighEyes-TTM is based on cheap FPAGA evaluation board and electronics components available to buy all over the world.

FPGA firmware and Python software for data analysis are open-source

European Research Council
Established by the European Commission

BrightEyes

XILINX **Kintex-7 FPGA KC705 Evaluation Kit**



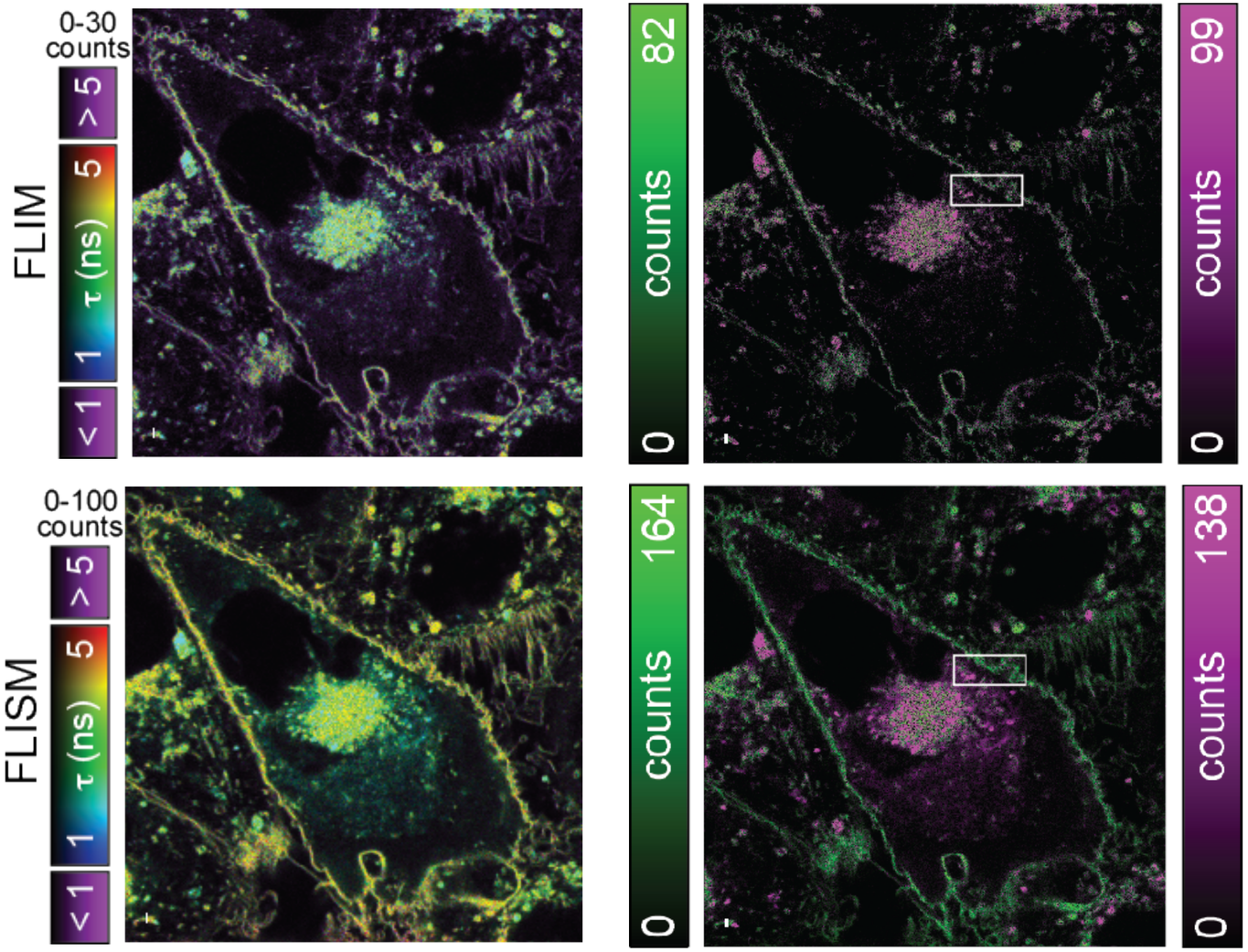
The FPGA allows for:
- 21 TDCs with 30 ps precision;
- reprogrammability and scalability;
- fast prototyping;
- low cost.

The evaluation board provides:
- already implemented fast serial connectors;
- FMC connectors for integrating any asynchronous read-out array detectors;
- a series of memory.

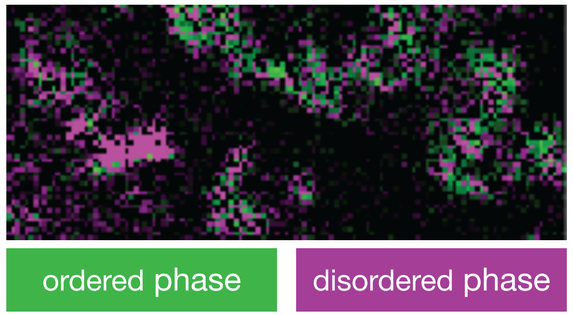
[8] **Rossetta, A.,…, Vicidomini, G., *Submitted* (2021)**

Super-Resolution Fluorescence Lifetime Imaging

Live-Cell Imaging (nanoWatt illumination power)



Castello M.,…, Vicidomini G., Nat. Methods 16, 175–178 (2019)



ordered phase | disordered phase

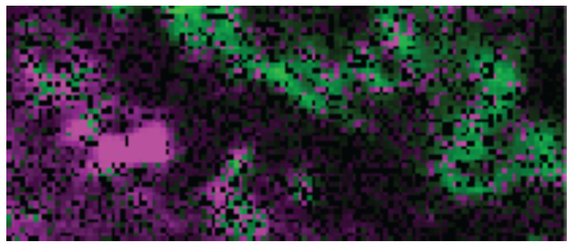
Conventional FLIM
Low-Resolution and Low-Precision

The most gentle and fast super-resolved fluorescence lifetime imaging technique.
Fully combative with any previous fluorescence lifetime analytical tool and sample protocols.

PCT/IB2019/050595(EP,CN,USA,JP)

PATENT PENDING

ordered phase | disordered phase



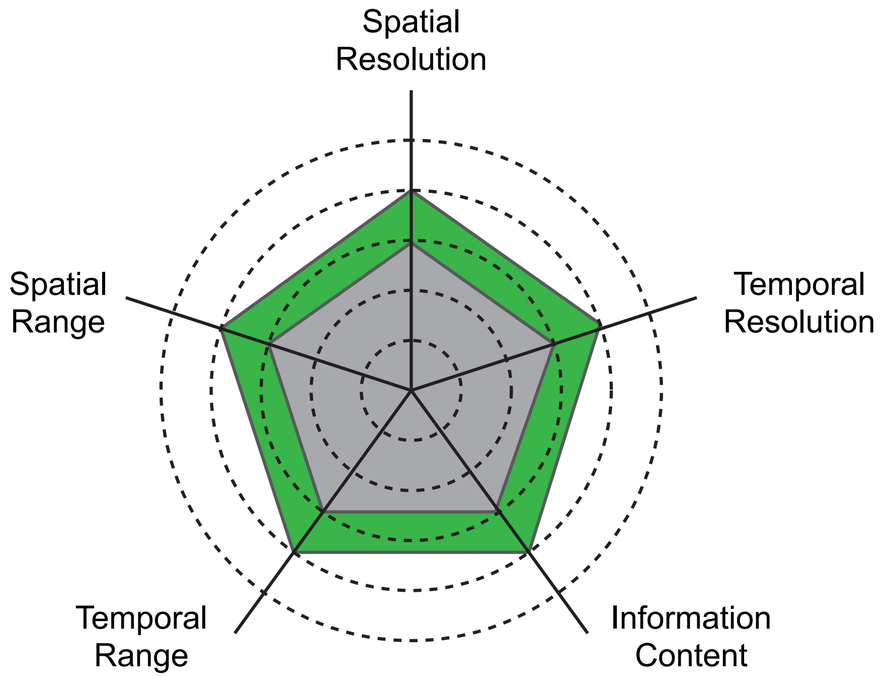
Super-Resolved FLISM
Low-Resolution and Low-Precision

Polarity sensitive membrane probe ANEP in Prostate Carcinoma (PC-3) live-cells. Pixel-size: 62.5 nm. Image format: 800 × 800 pixels. Excitation power Pexc = 3 µW. Scale bars: 1 µm.

Conclusions

Conventional Laser-Scanning Microscopy

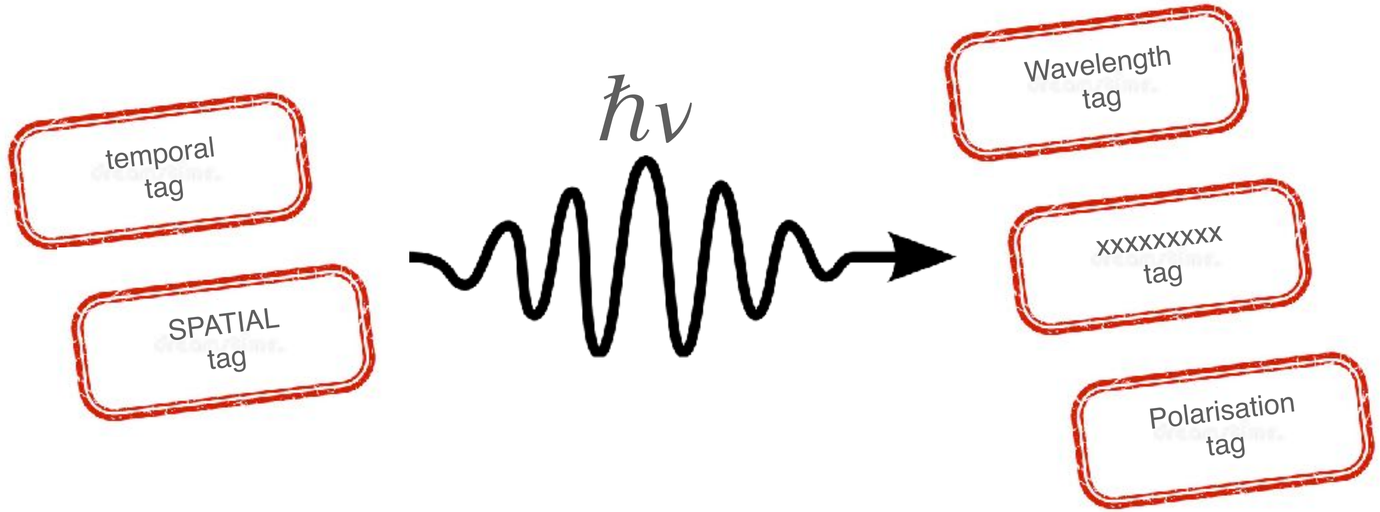
Laser-Scanning Microscopy with SPAD array



Further info at:
https://vicidominilab.github.io
https://www.genoainstruments.com

The single-photon microscopy revolution

Asynchronous readout SPAD array detector allows leveraging the spatiotemporal information carry out by single-photons to implement a new set LSM techniques



How to support the revolution

improved SPAD array detector
improved DAQ system
cheap super-continuum pulsed laser and AO system
new data analysis and storage tools