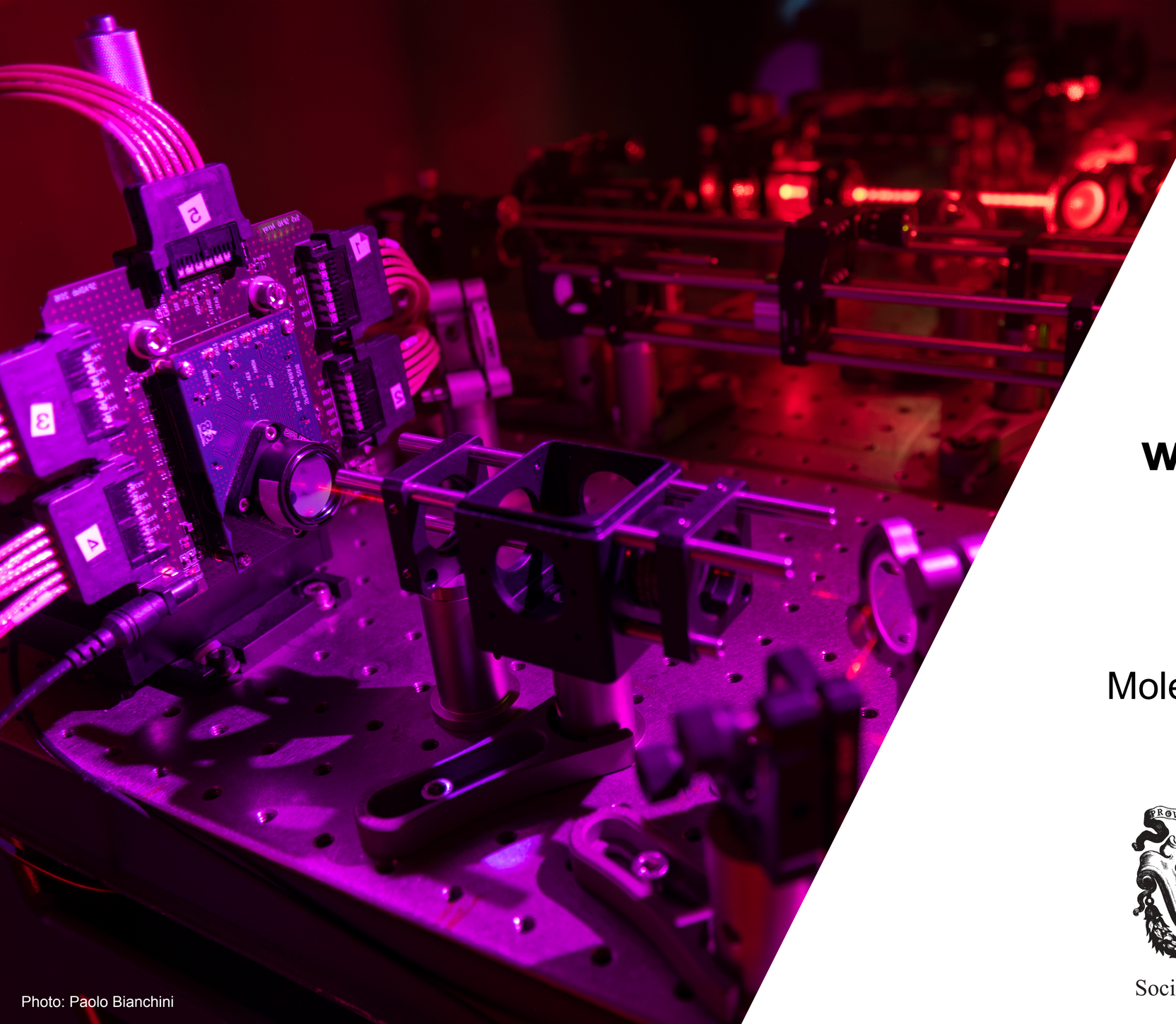

Photo: Paolo Bianchini

ISTITUTO ITALIANO
DI TECNOLOGIA

Laser-Scanning Microscopy with SPAD Array Detector

Giuseppe Vicidomini
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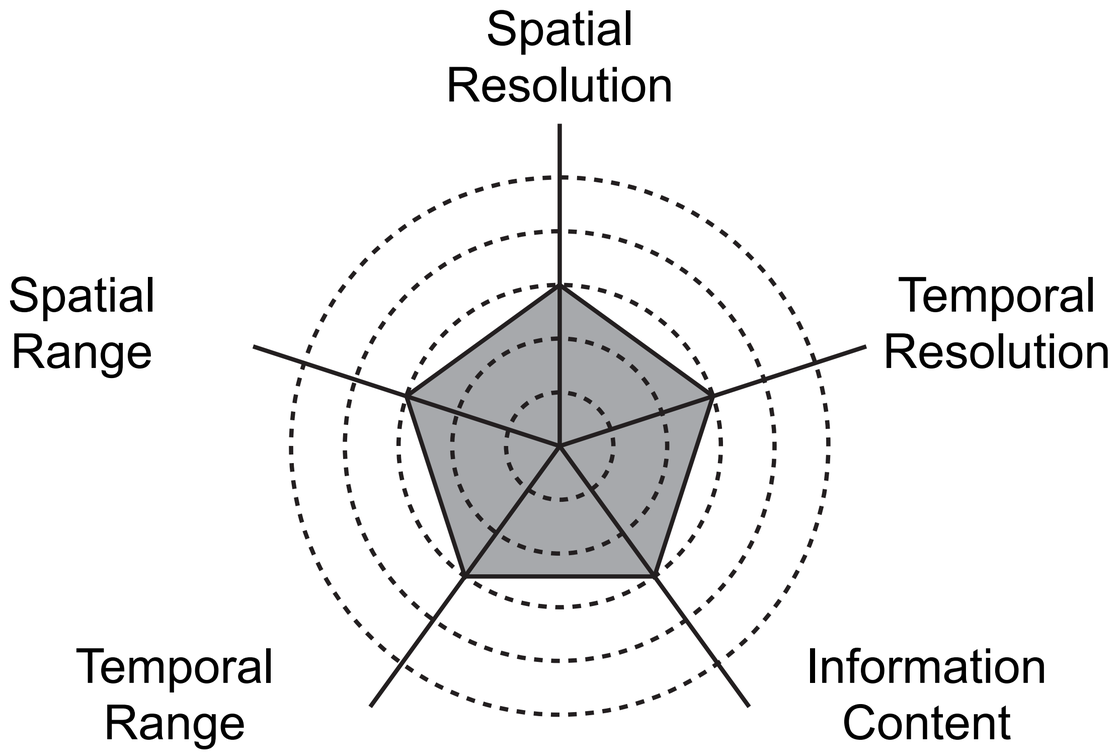
Società Italiana di Fisica

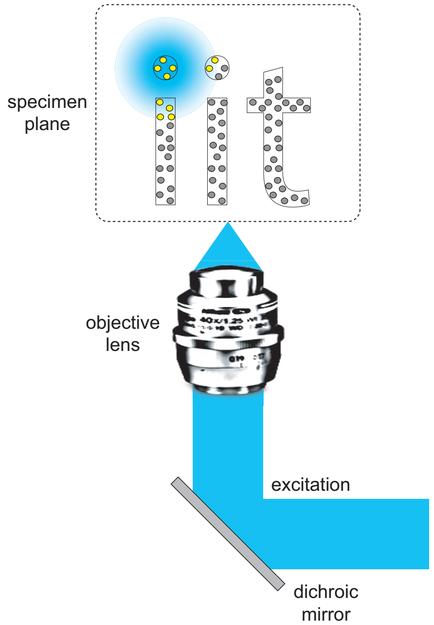
Società Italiana di Fisica
106° Congresso Nazionale
14-18 Ottobre 2020

Laser-Scanning Microscopy

laser-scanning microscopy is one of the most popular optical microscopy architecture in Life-Sciences and Material-Science

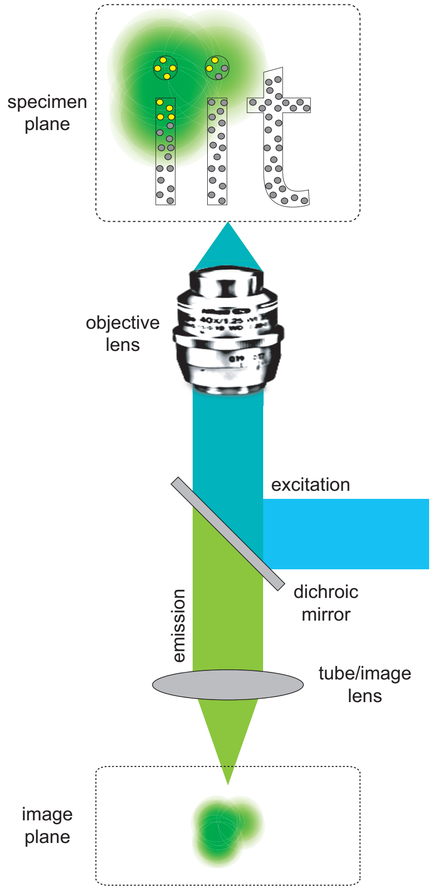


Laser-Scanning Fluorescence Microscopy



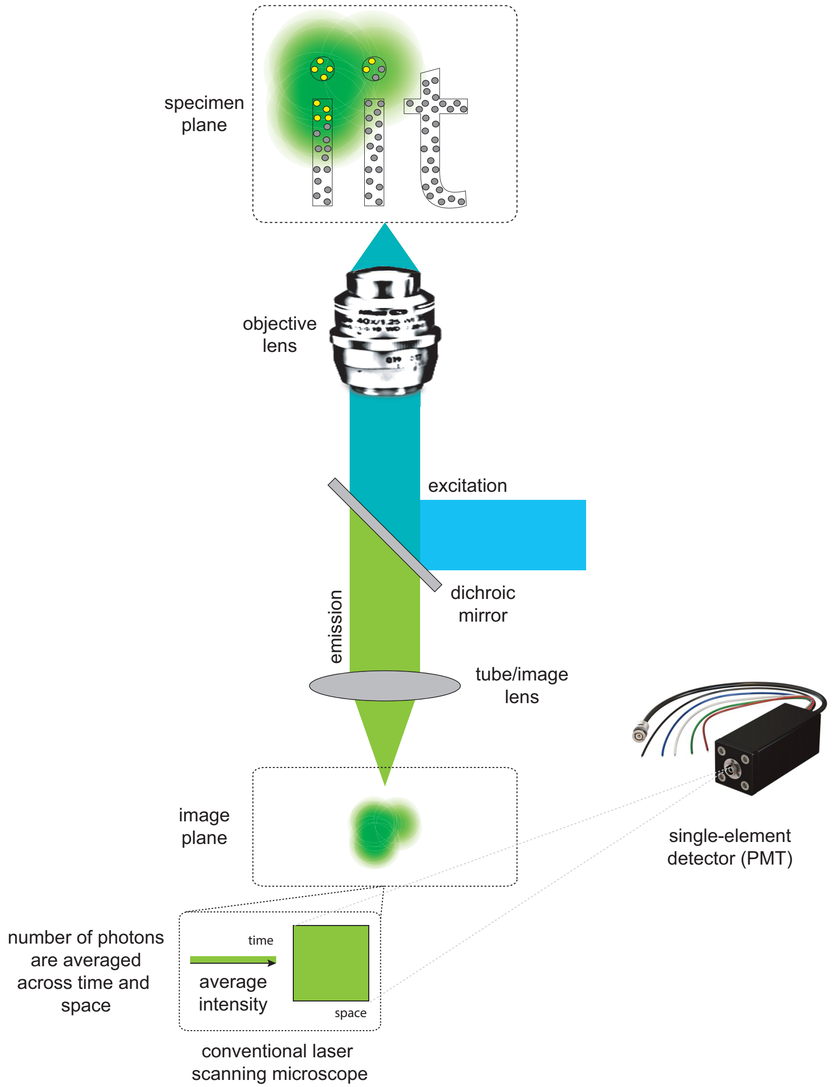
- a beam is focused by the objective lens on a (usually diffraction-limited) region of the sample;

Laser-Scanning Fluorescence Microscopy



- 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;

Laser-Scanning Fluorescence Microscopy



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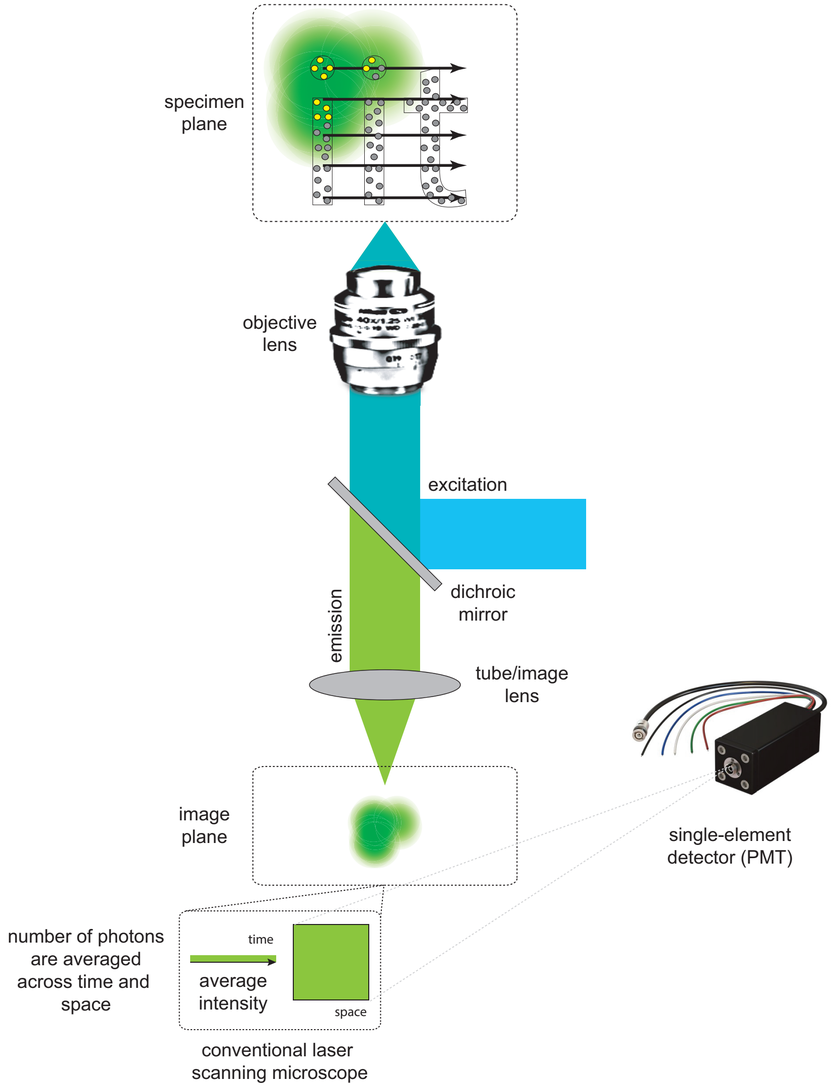
Laser-Scanning Fluorescence Microscopy



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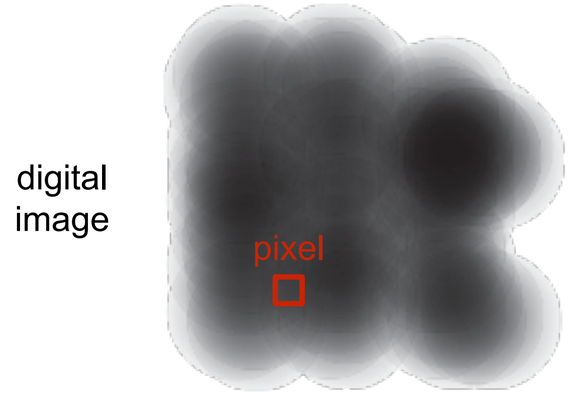
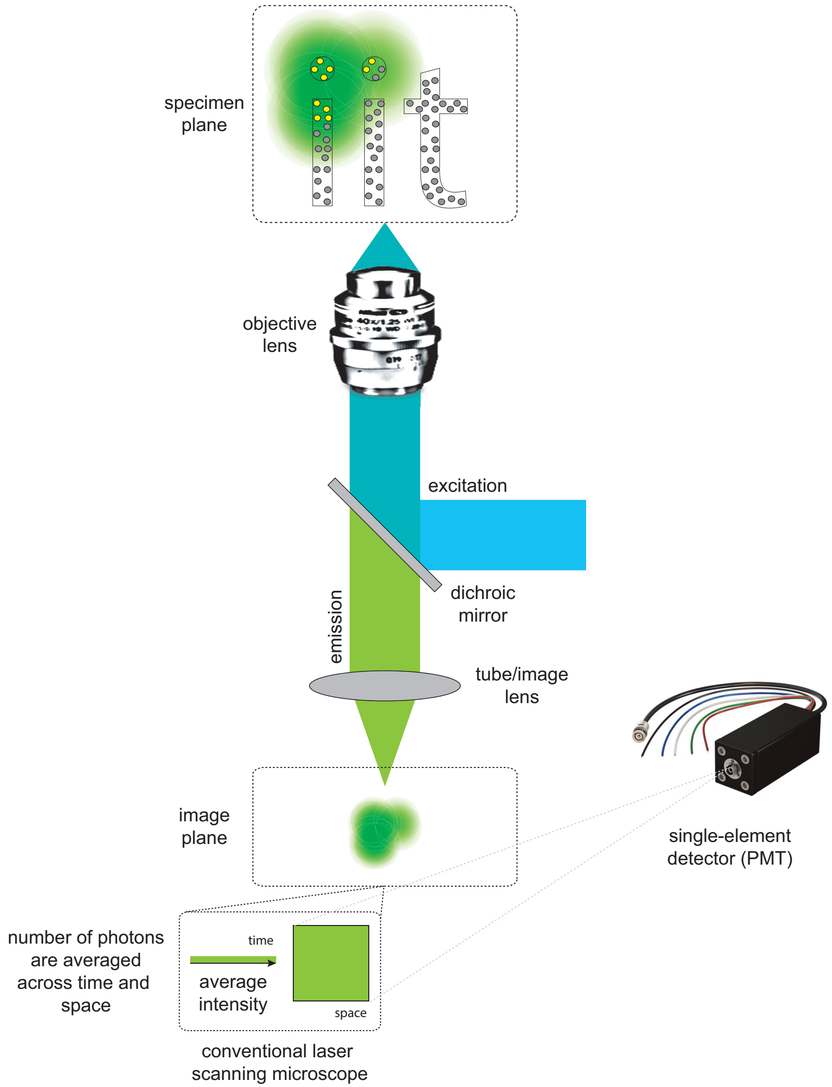


Laser-Scanning Fluorescence Microscopy



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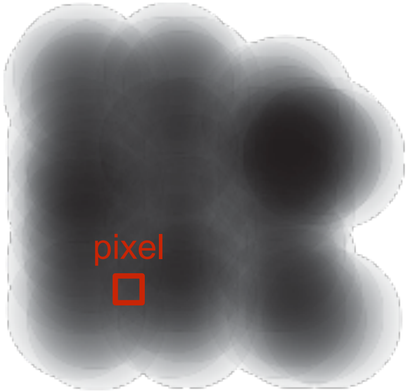
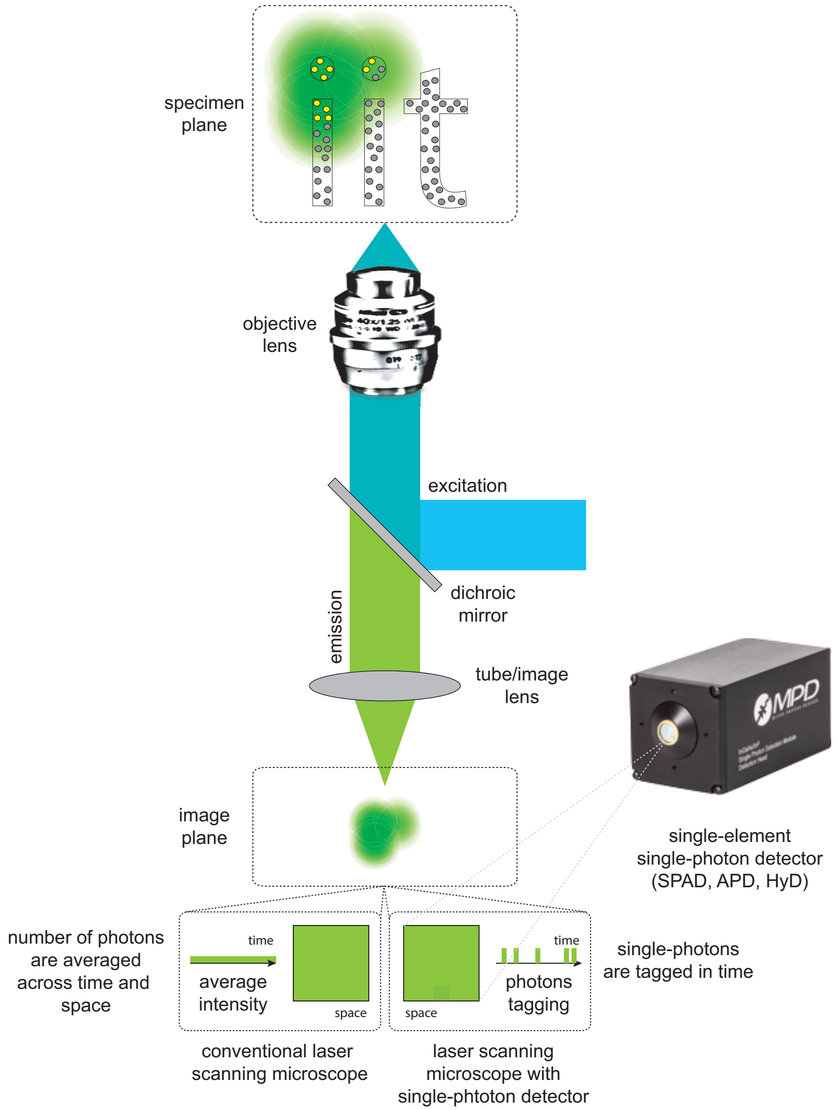
By integrating photons (in time and space) a lot of useful information are lost;

digital image



Laser-Scanning Fluorescence Microscopy



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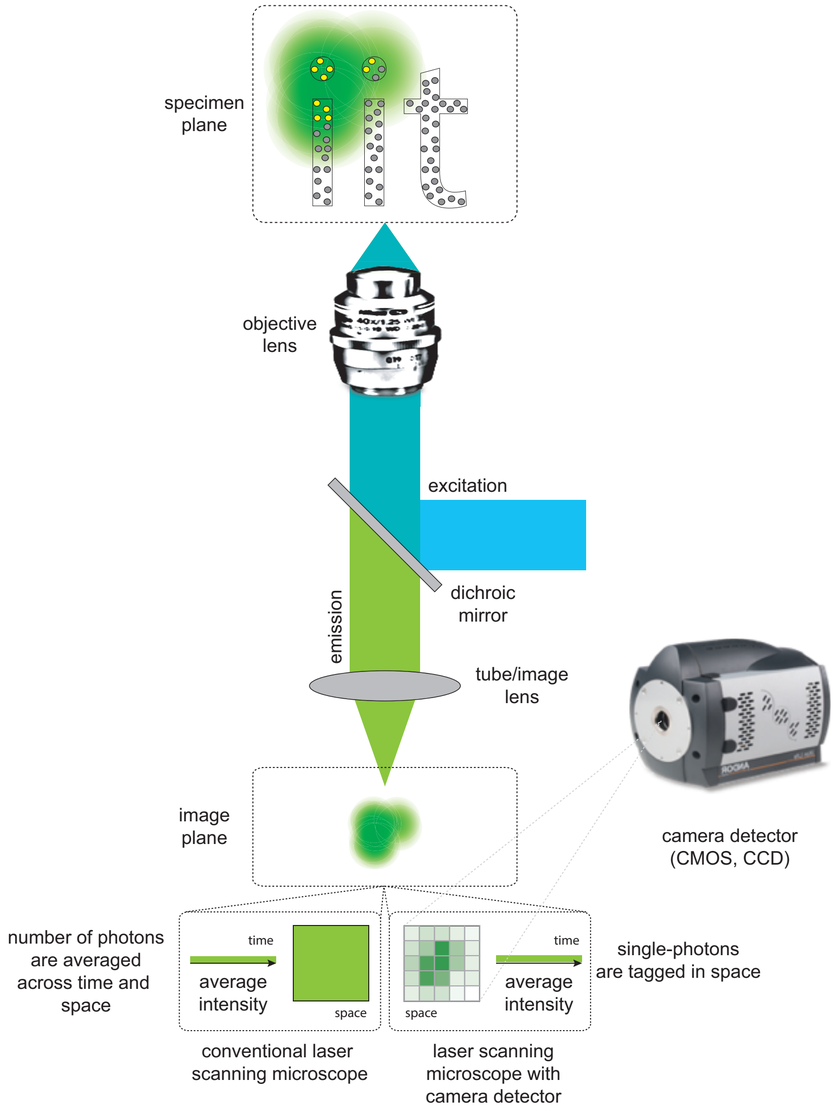
Laser-Scanning Fluorescence Microscopy



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

- single-photon detectors (such as APD, SPAD, HyD) stream the data in photons but lose spatial information as well;
- pixelated detector (such as CCD, EM-CCD, CMOS) register the photon's position but integrate over the time.

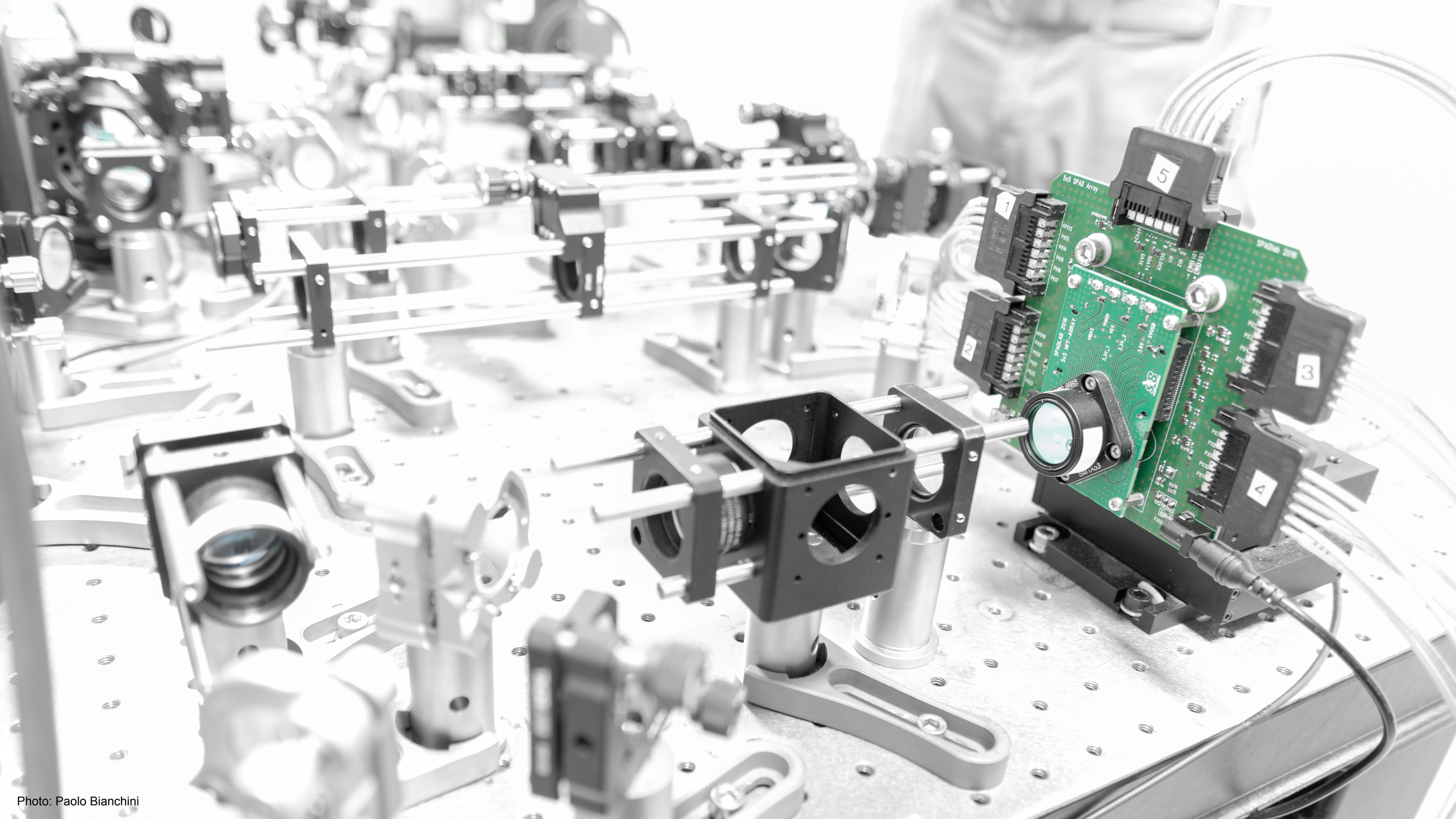
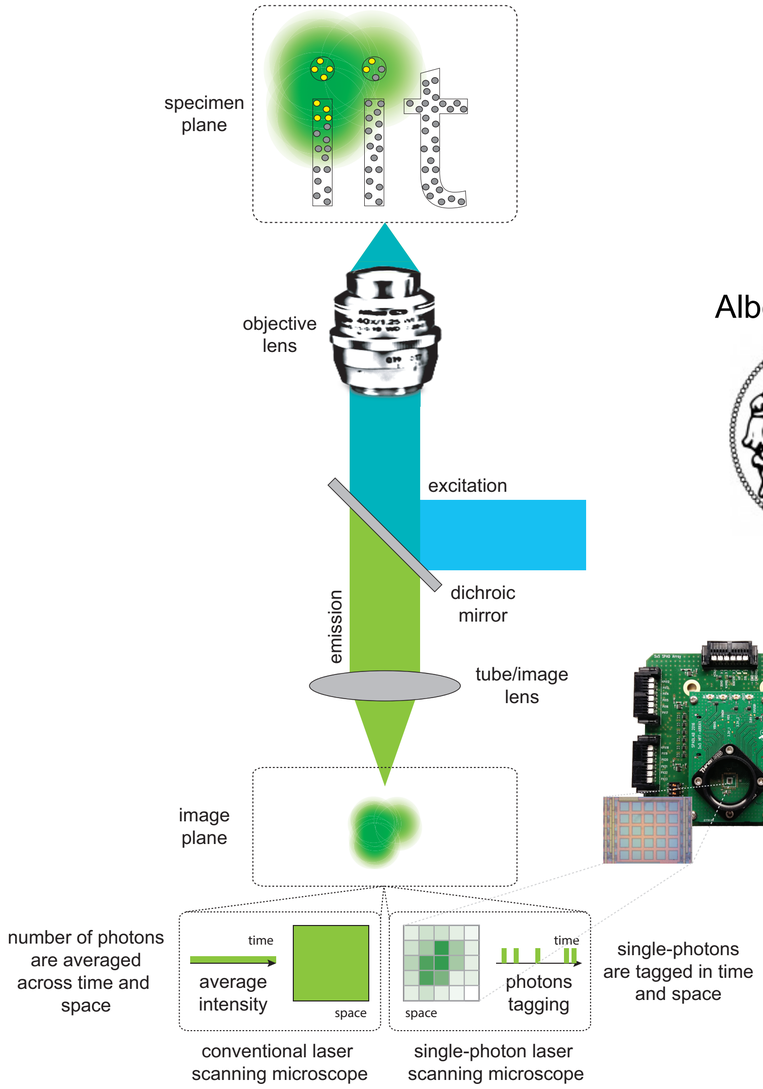


Photo: Paolo Bianchini

Laser-Scanning Fluorescence Microscopy

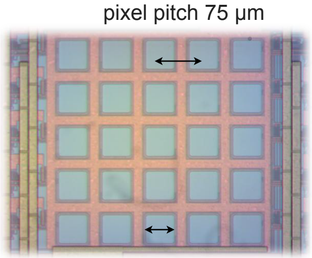


Alberto Tosi Lab

single-photon-avalanche-diode (SPAD) array detector

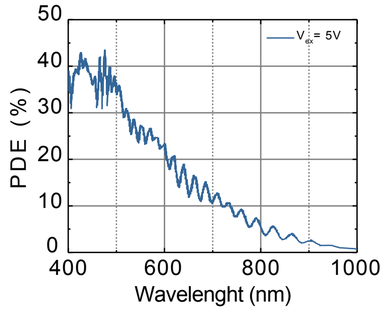
Spatial Characteristics

0.35 µm high-voltage CMOS technology by Fraunhofer IMS



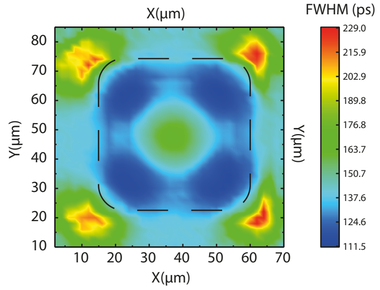
- 5×5 pixels;
- ~ 50% fill-factor (pixel pitch 75 µm and pixel size 50 µm);
- <1% cross talk probability (first neighbours orthogonal);

Spectral and Noise Characteristics



- 45% @450 nm, 15% @650 nm, 5% @850 nm PDE;
- < 100 cps dark count @25° (with 5% hot pixels) ;
- < 2% after pulsing @100 ns hold-off time.

Temporal Characteristics



- asynchronous-readout (5×5 pixels fully parallel);
- < 200 ps photon-timing jitter (FWHM);
- tuneable hold-off time down to 25 ns (40 MHz, < 10% after-pulsing);

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

LSFM with SPAD Array - A New Paradigma

Conventional laser-scanning microscope

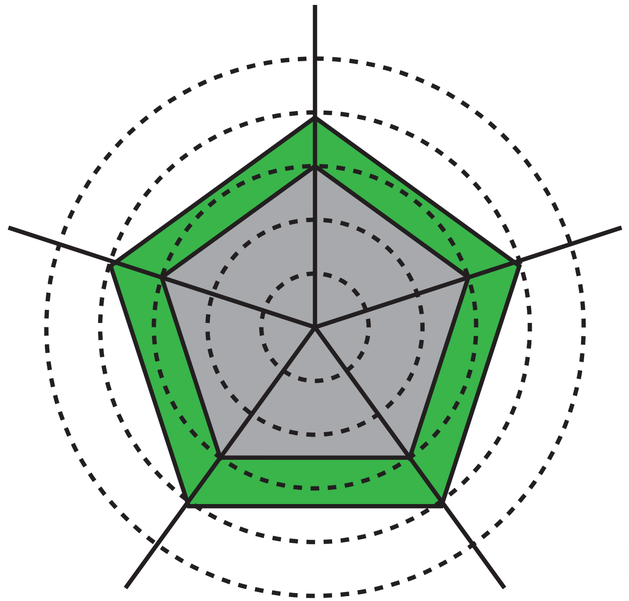
Laser-scanning microscope with SPAD array

- via image scanning microscopy (ISM);
- via STED-ISM;
- via quantum microscopy;
- via image deconvolution;

Spatial Resolution

Spatial Range

- via adaptive-optics;
- via two-photon-excitation (TPE) microscopy;



Temporal Resolution

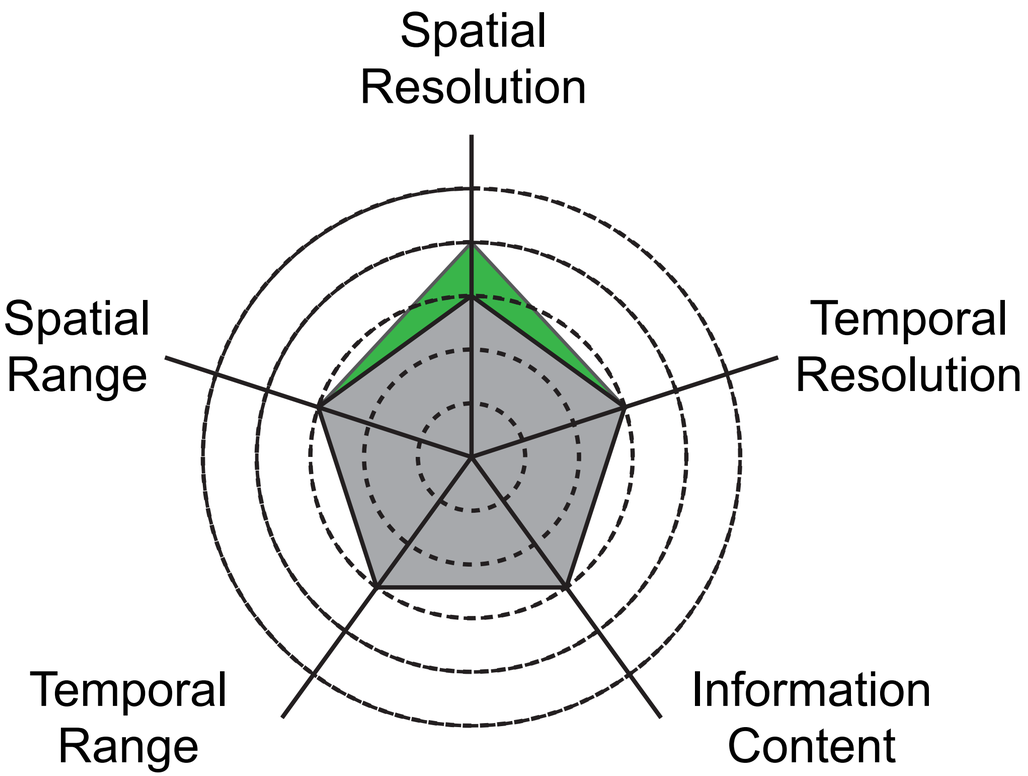
- via resonant-mirror scanning;
- via fluorescence fluctuation spectroscopy (e.g., FCS, RICS);
- via real-time single molecule tracking.

Temporal Range

- via ISM;
- via time-resolved STED-ISM;
- via image deconvolution;
- via deep-learning;

Information Content

- via fluorescence lifetime imaging;
- via **photon** coincidence correlation;

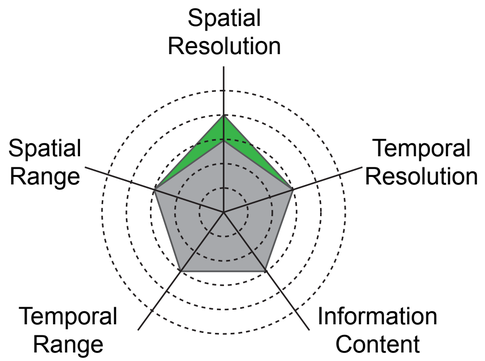


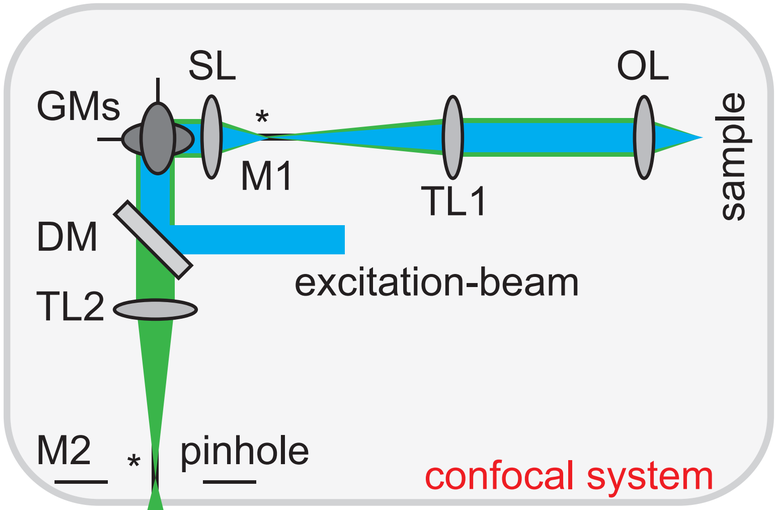
Improving Spatial Resolution *via* Image Scanning Microscopy

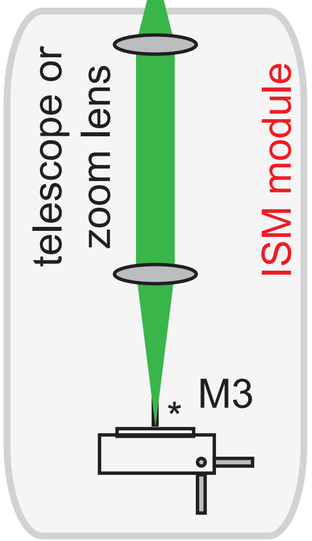
(and Extra-Spatial Information)

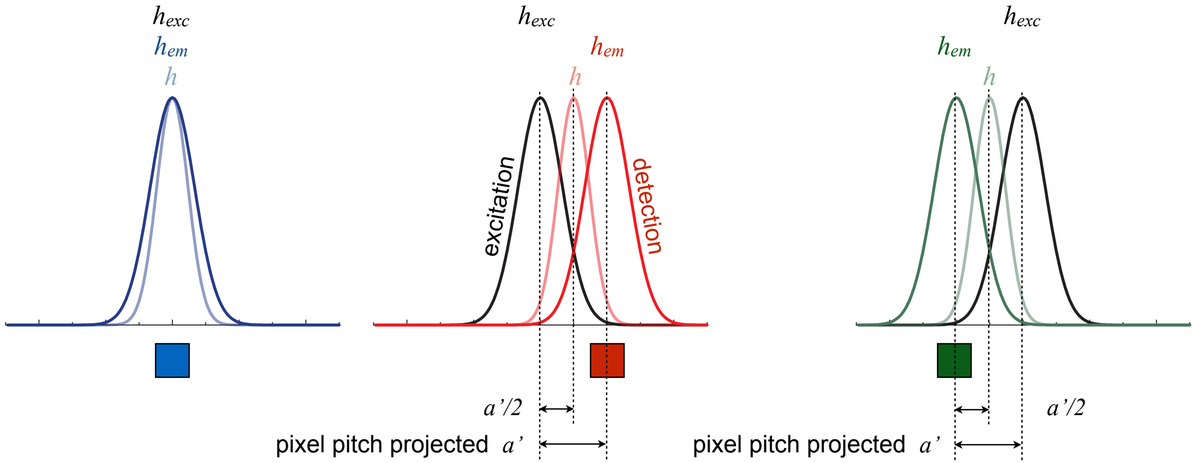
Image Scanning Microscopy (ISM)

$$h(i) = h_{exc} \times h_{em}(i)$$

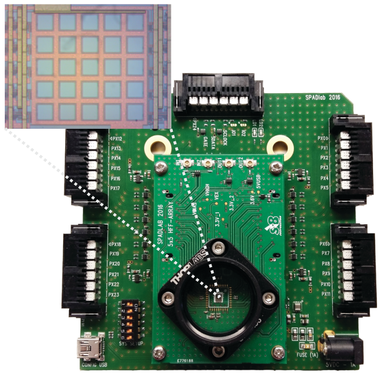


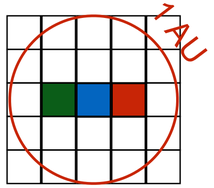




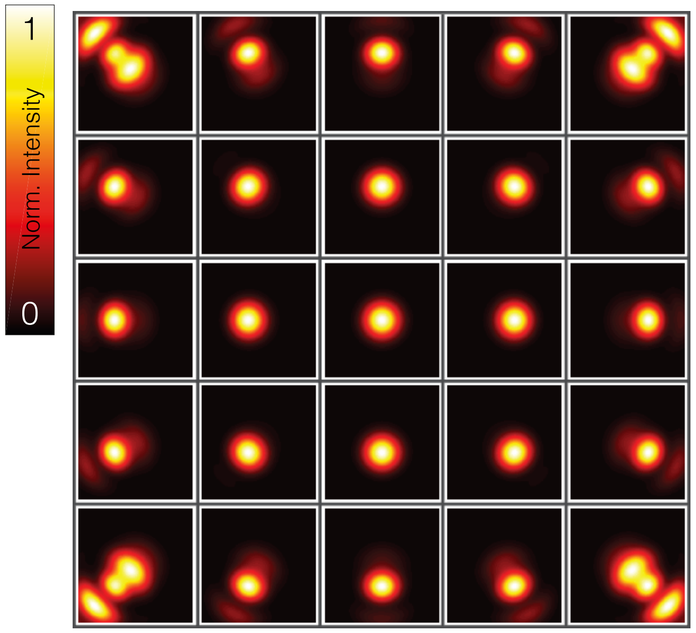


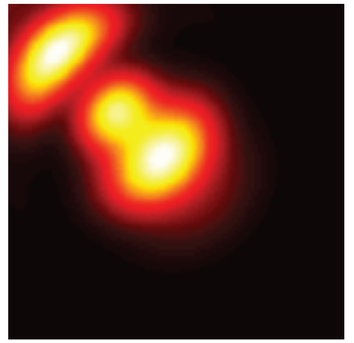
single-photon-avalanche-diode (SPAD) array detector





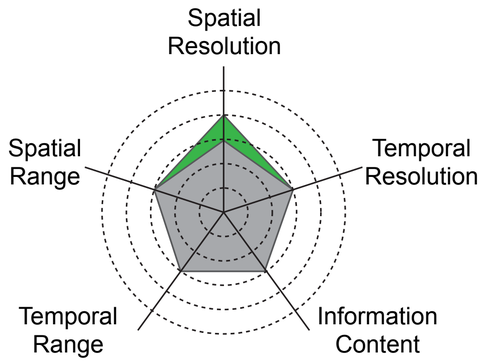
Detector Array

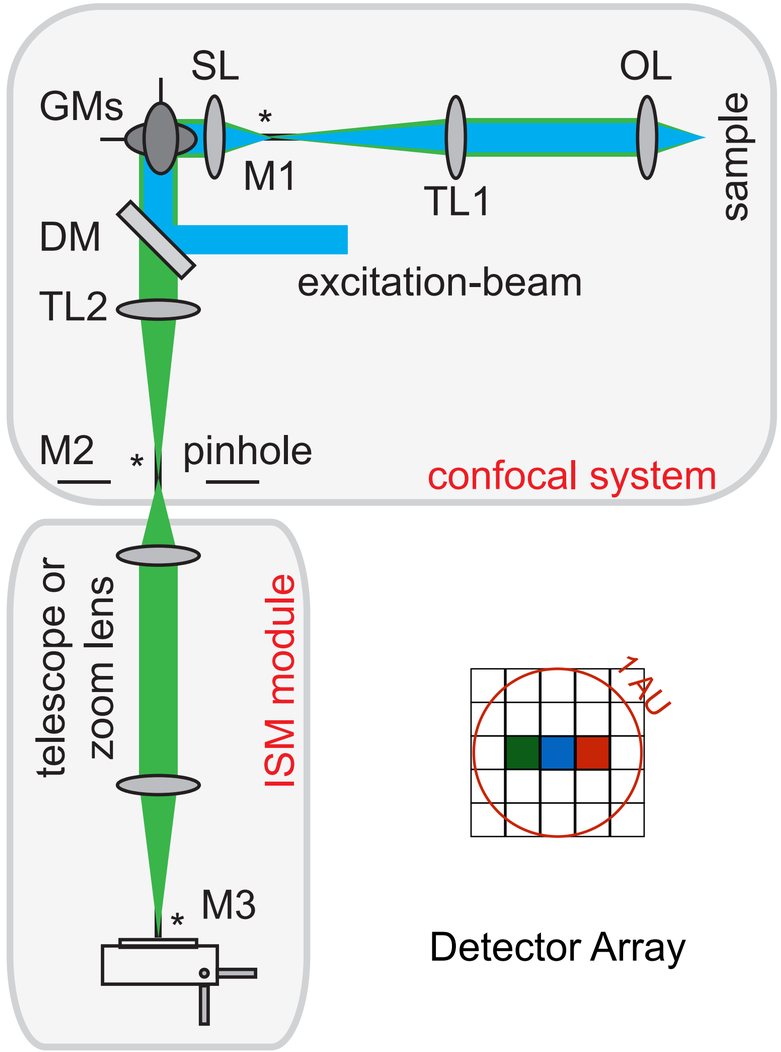


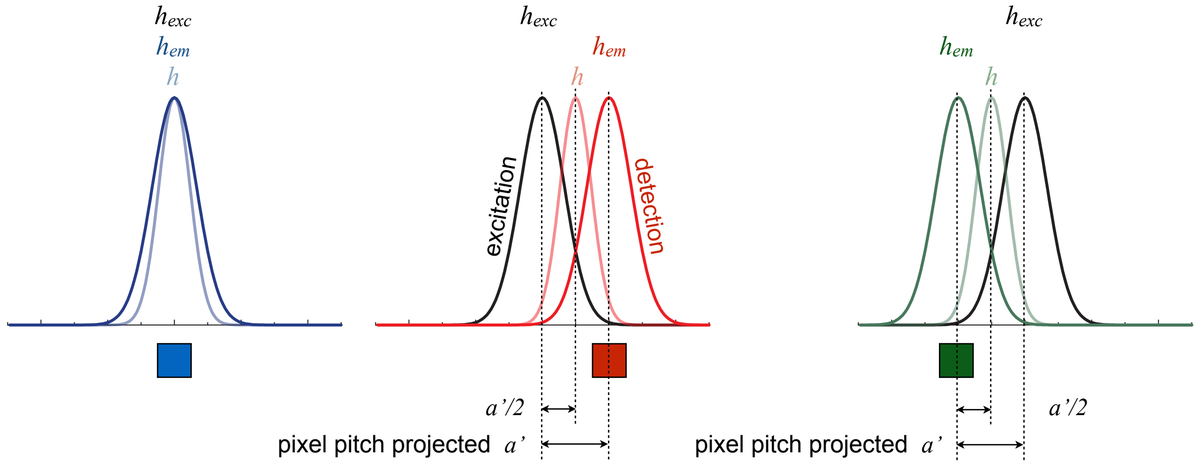


ISM via Pixel-Reassignment (PR-ISM)

$$h(i) = h_{exc} \times h_{em}(i)$$







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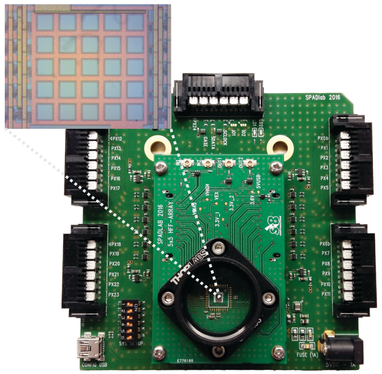

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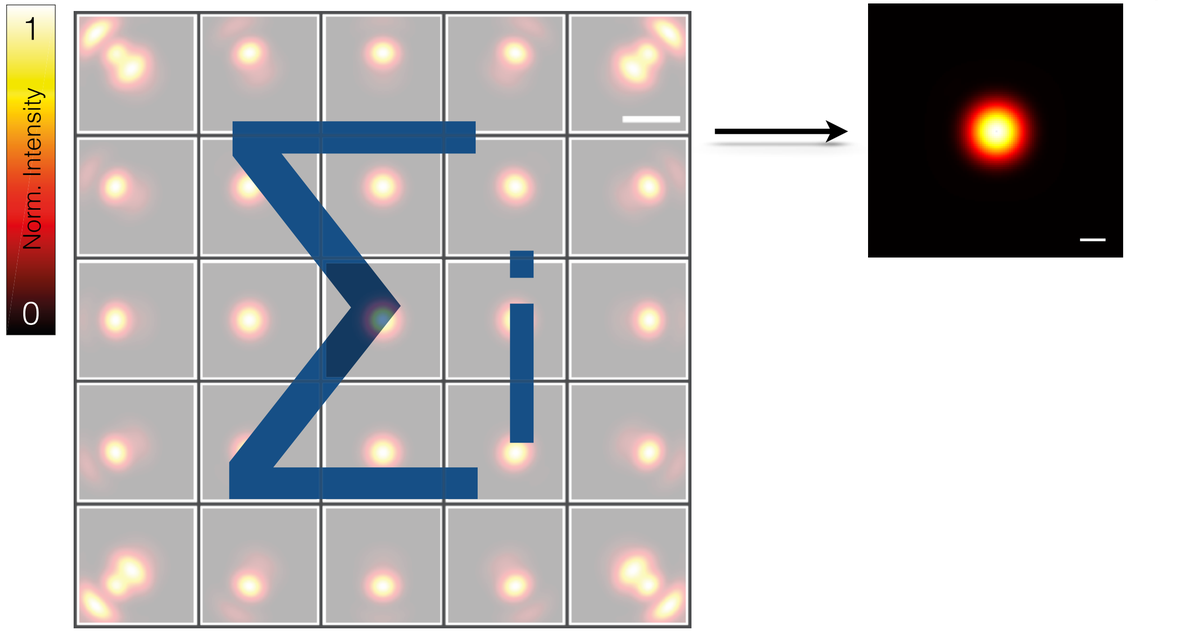

Castello M.,…, Vicidomini G., Nat. Methods 16, 175–178 (2019)

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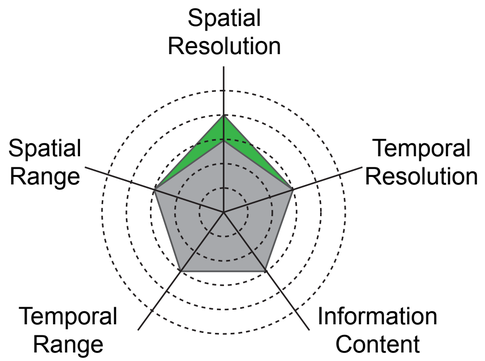

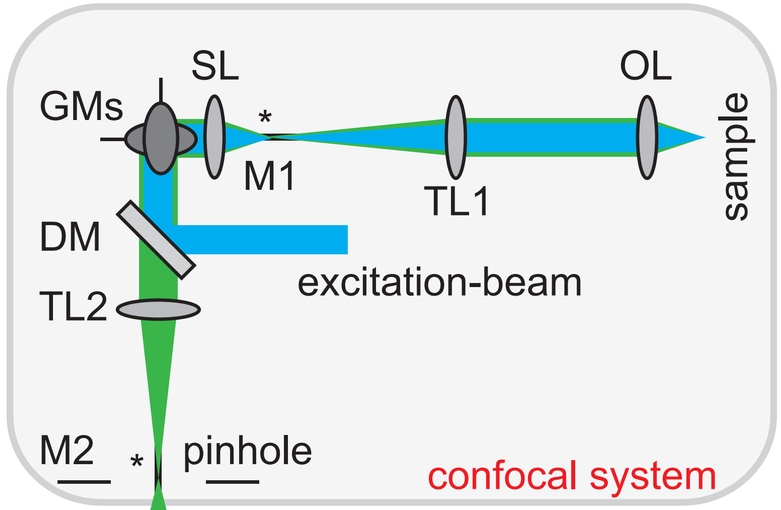

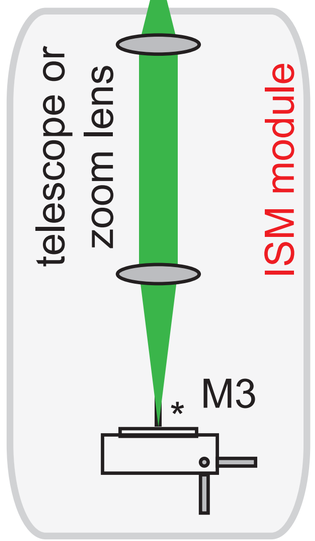

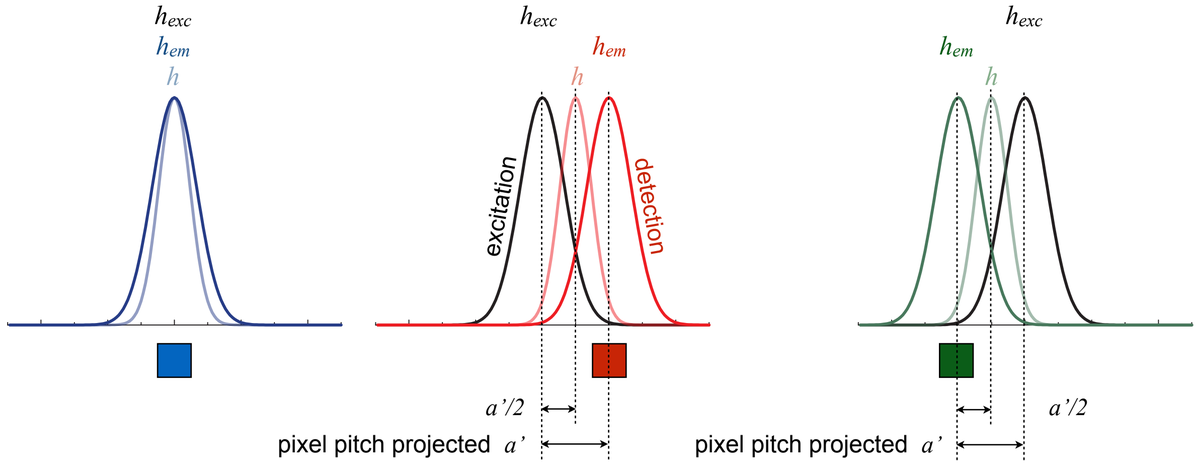

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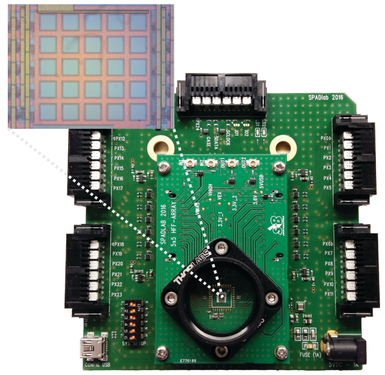

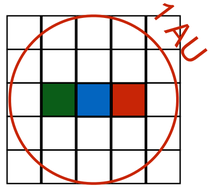

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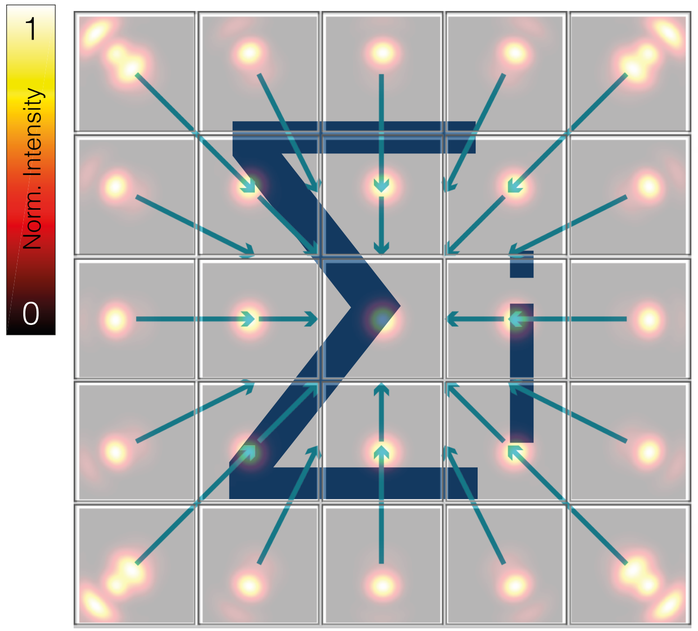
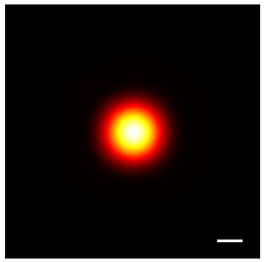
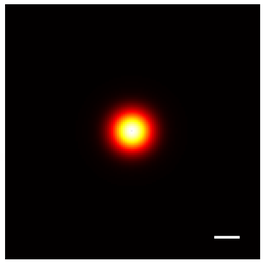


sum (conventional)



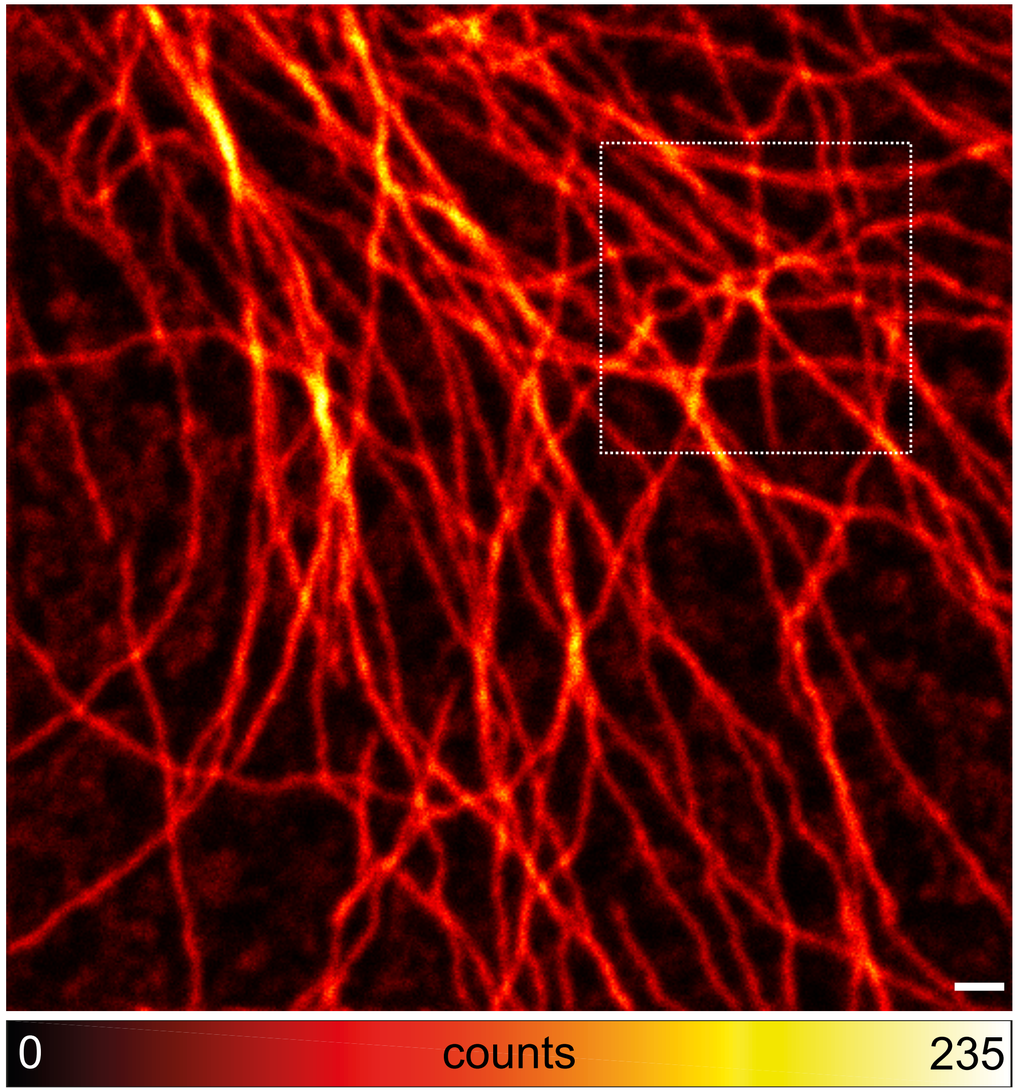
pixel reassignment (PR)
shift-back and sum

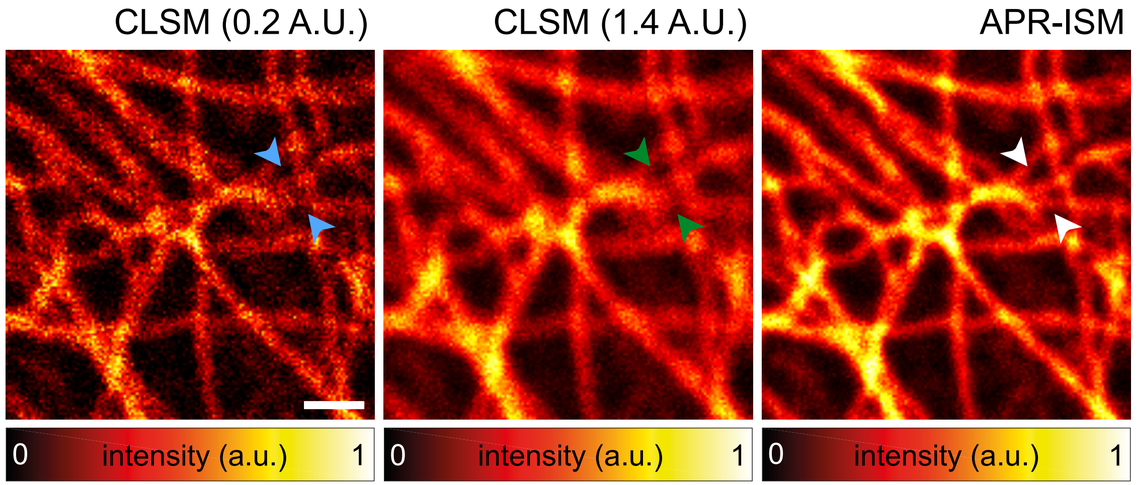


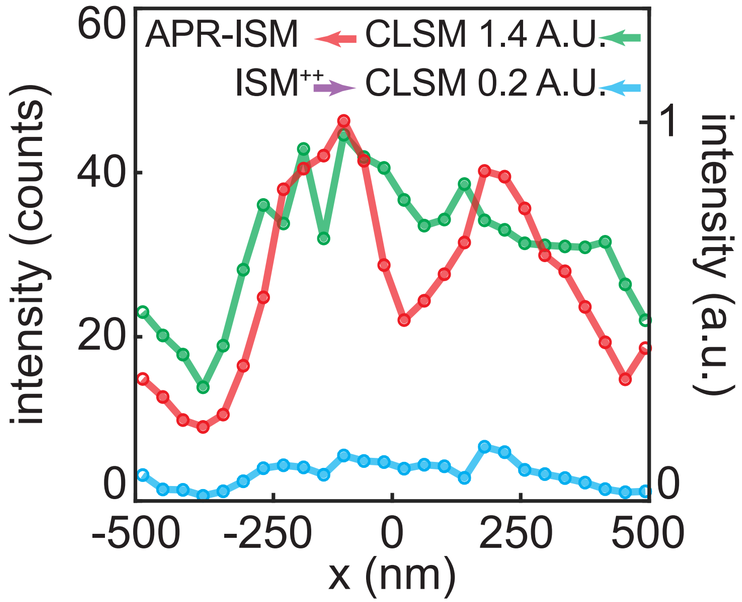
Castello M.,…, Vicidomini G., Nat. Methods 16, 175–178 (2019)

Tubuline Network Imaging

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



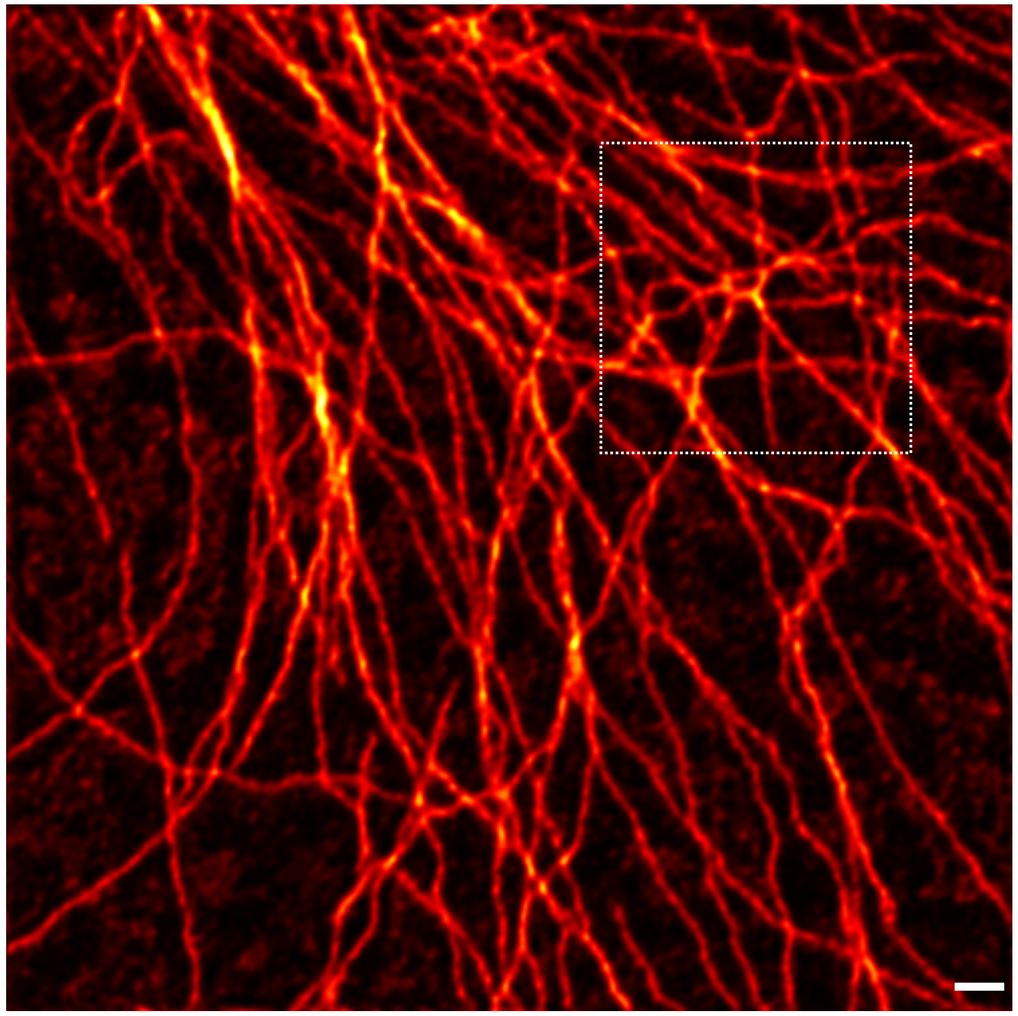




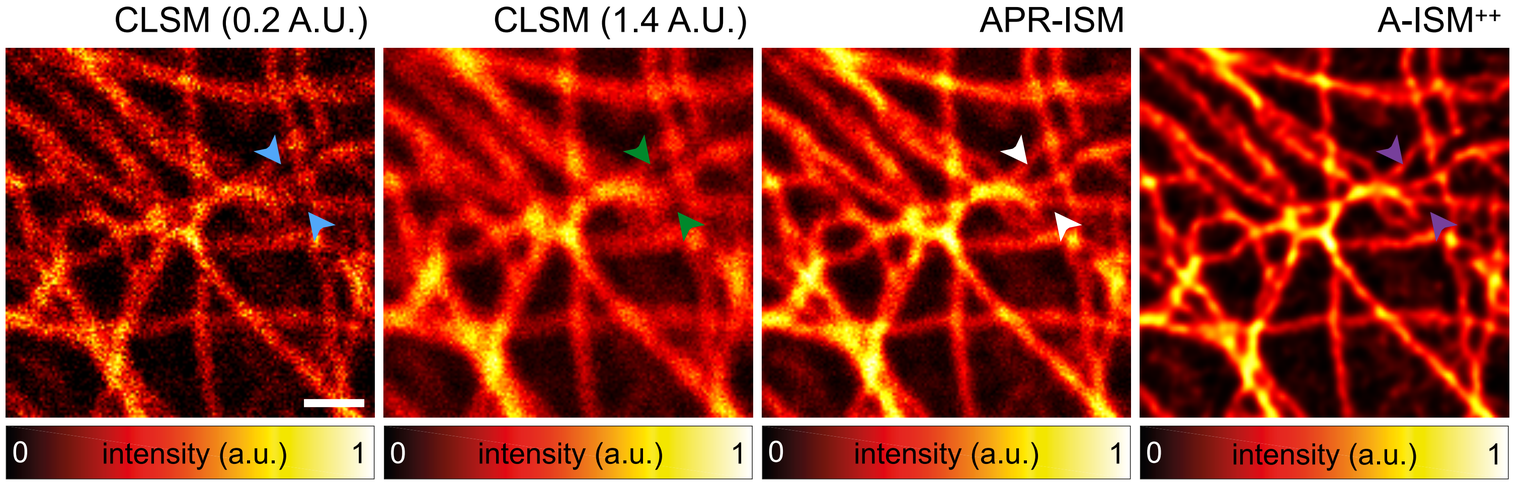
Imaging of ATTO647N immunolabelled tubuline network, 60x 1.4 Nikon oil objective lens, scaler bars 1 µm

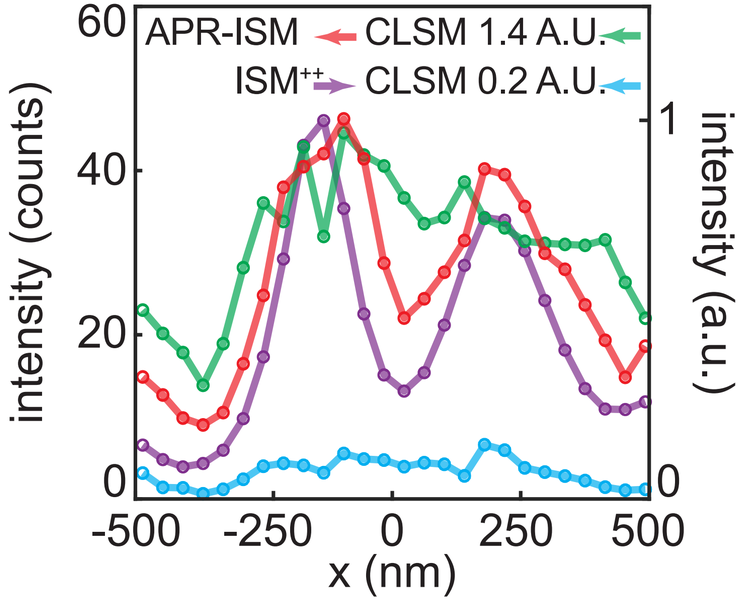
Improving Spatial Resolution *via* Adaptive Deconvolution

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



Imaging of ATTO647N immunolabelled tubuline network, 60x 1.4 Nikon oil objective lens, scaler bars 1 µm



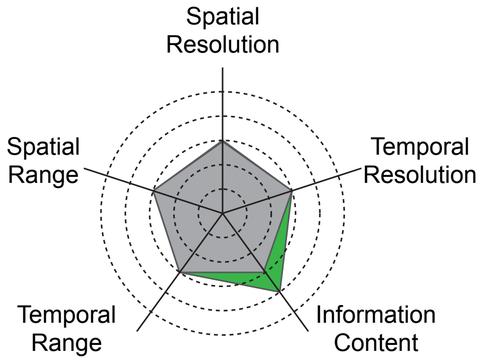


Richardson-Lucy-based multi image deconvolution algorithm with adaptive PSFs (A-ISM++)

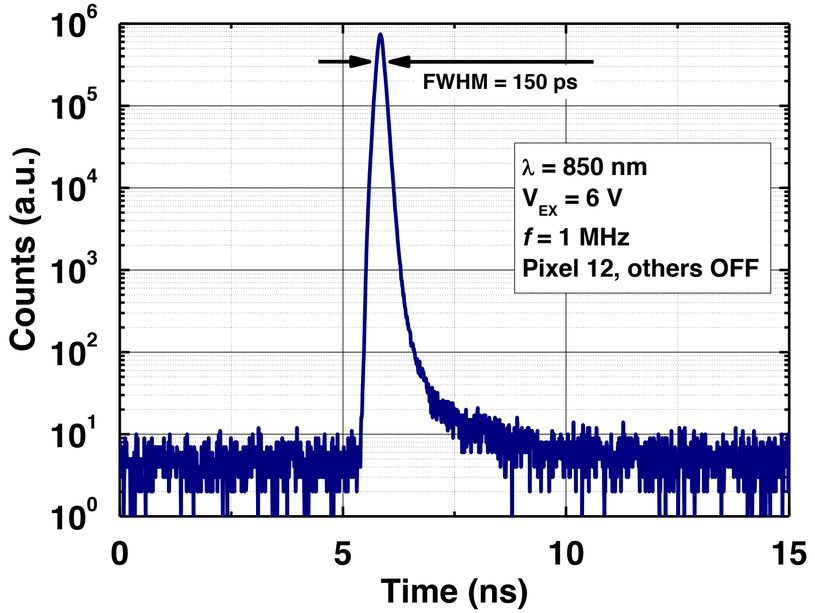
$$f^{k+1} = f^{k} \sum_{(i,j)} \left(w_{i,j}^{-1} h_{i,j} \star \frac{g_{i,j}}{h_{i,j} * f^{k}} \right)$$

Castello M.,…, Vicidomini G., Appl. Phys. Lett., 105:(23):234106 (2014)

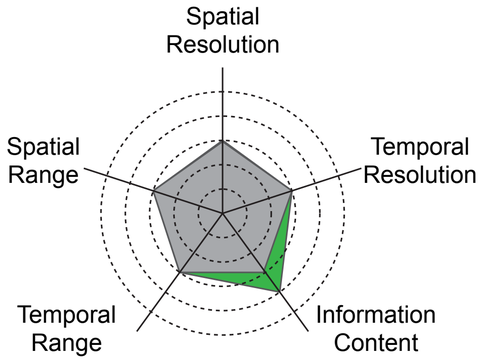
Single-Photon Timing Ability



impulse response function (IRF)



Fluorescence Lifetime ISM (FLISM) *via* SPAD Array



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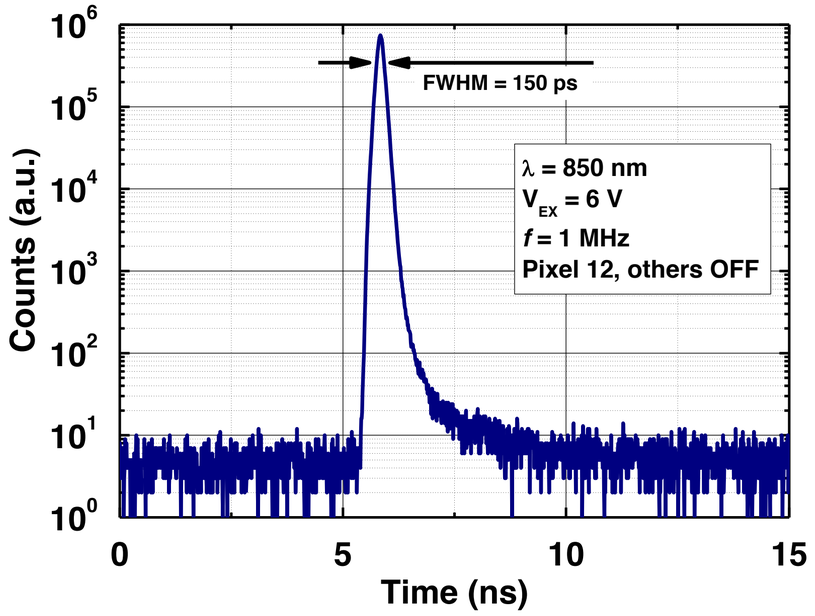
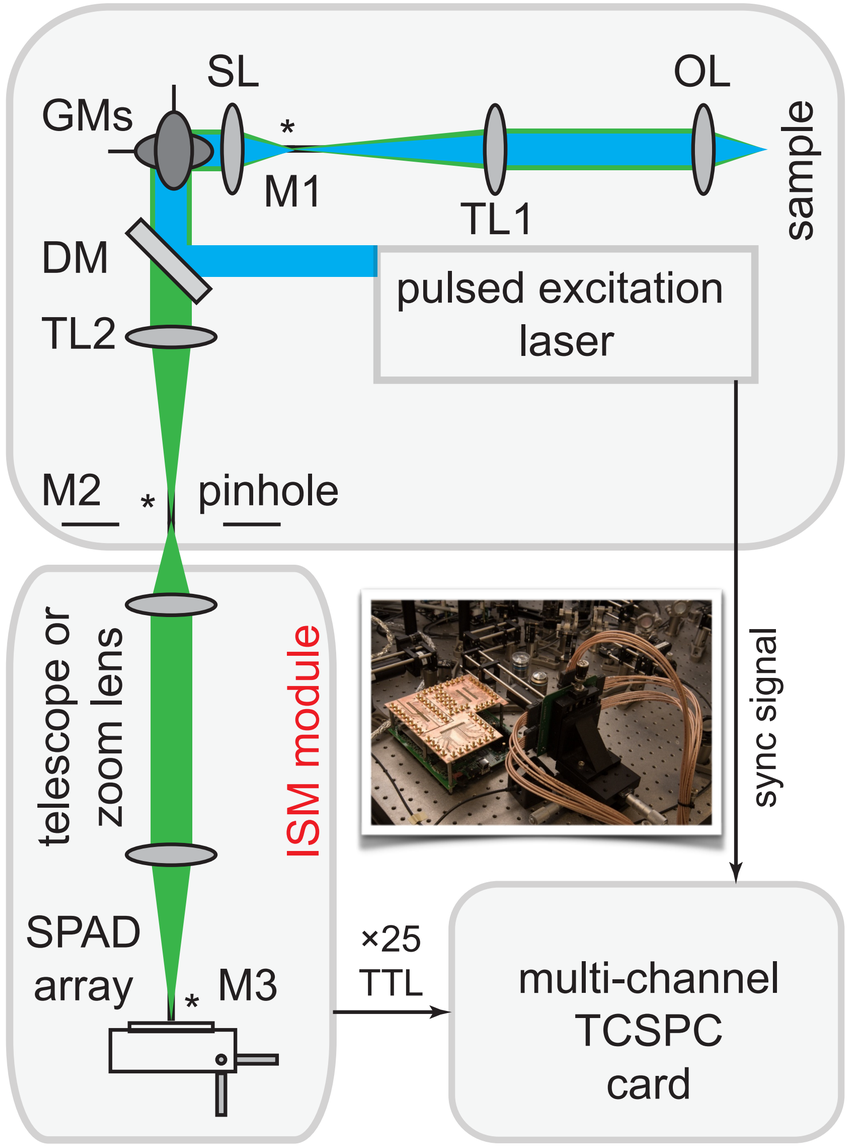
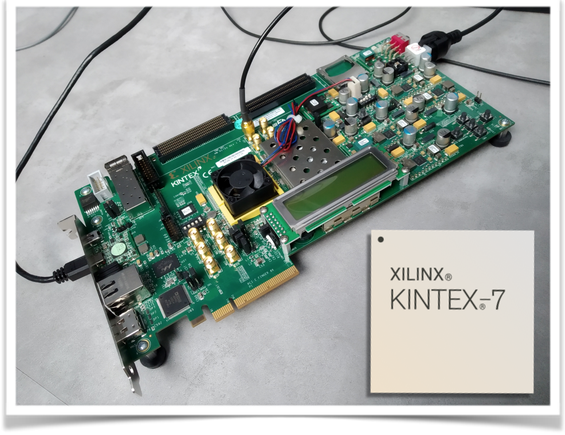


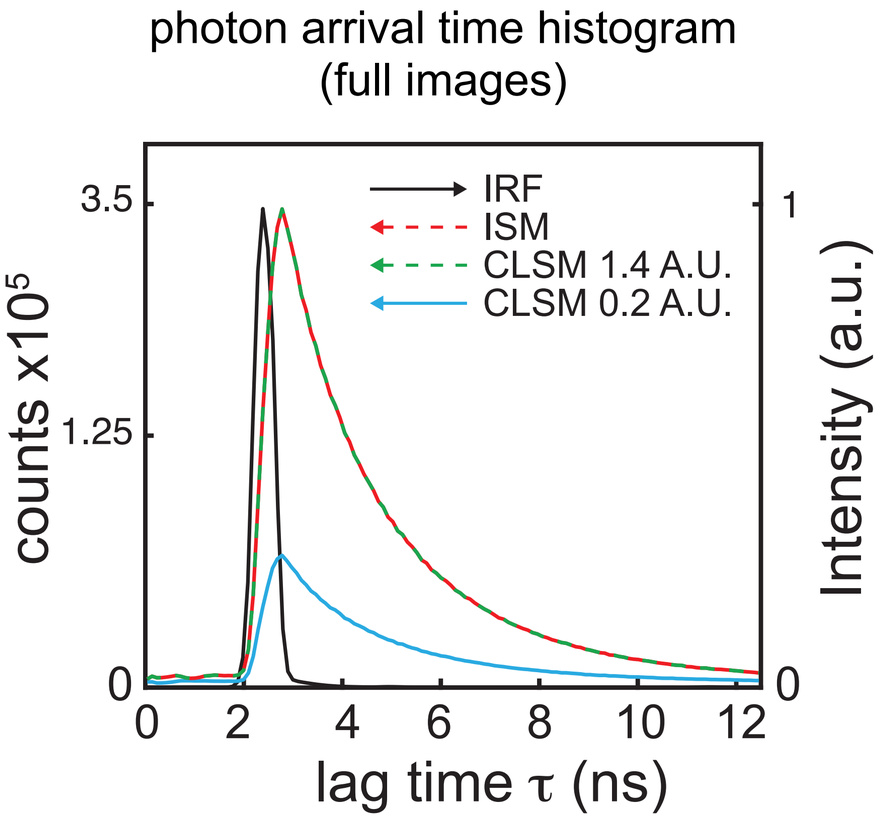


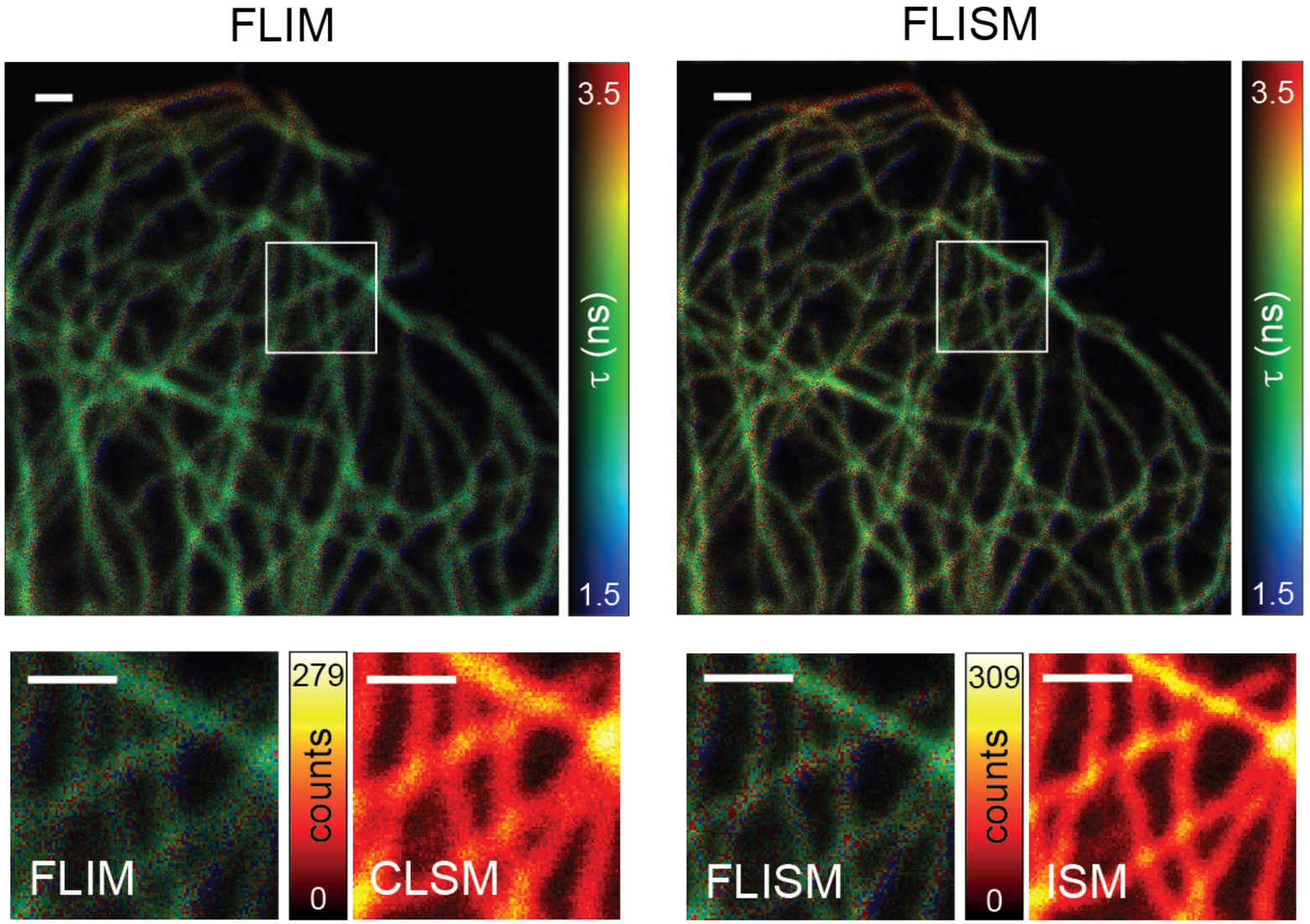
Picosecond Laser Diode
80 MHz repetition rate
80 ps pulse-width

FPGA-based
multi-channel TDC
40 ps timing precision



Fluorescence Lifetime Imaging *via* SPAD Array

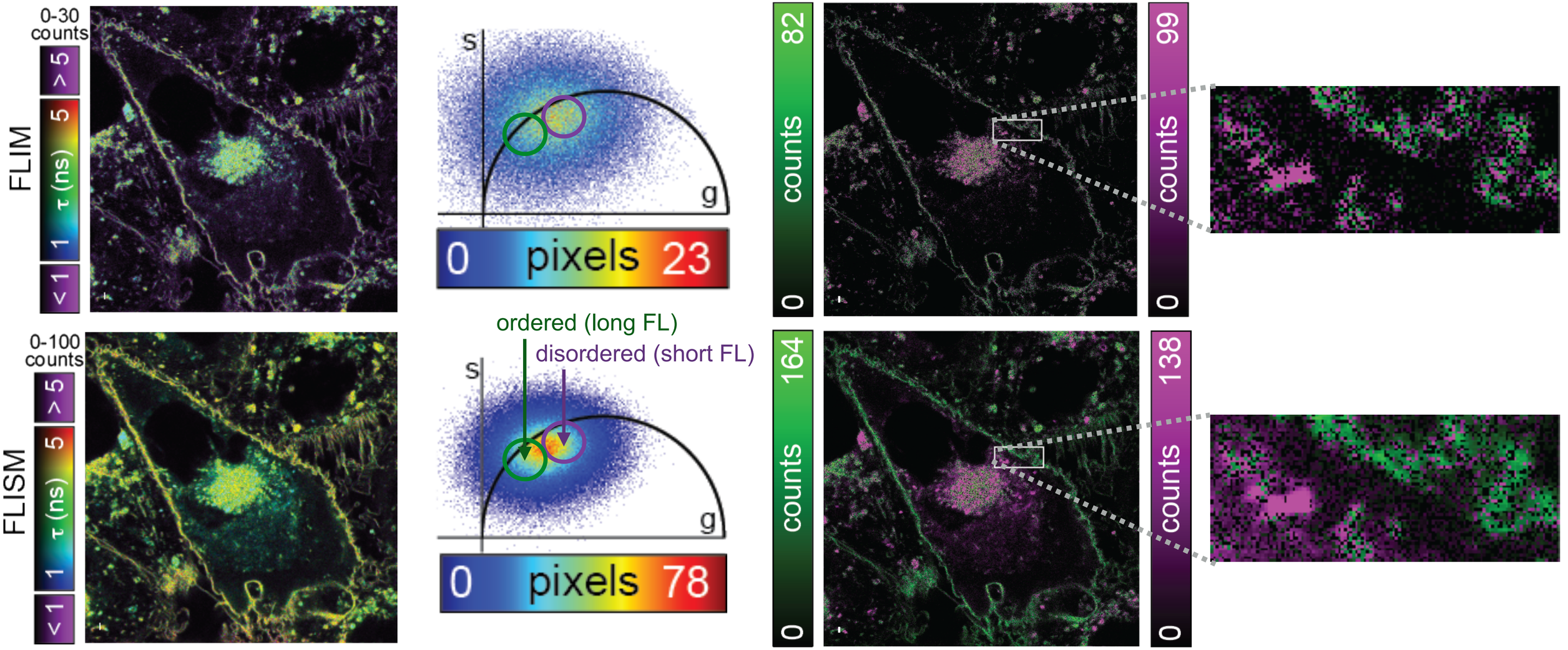




Castello M.,…, Vicidomini G., Nat. Methods 16, 175–178 (2019)

Fluorescence Lifetime Imaging *via* SPAD Array

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



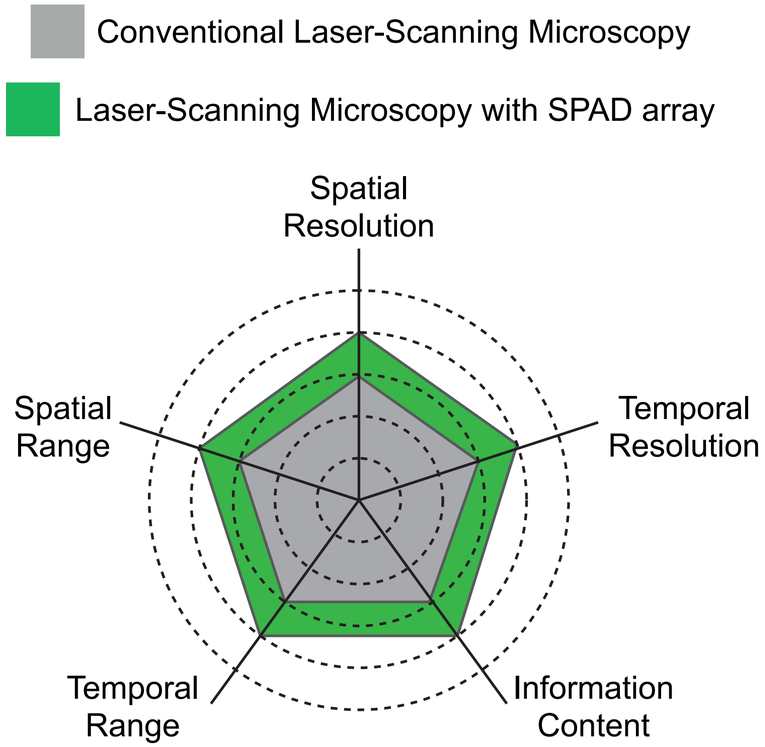
Polarity sensitive membrane probe ANEP in Prostate Carcinoma (PC-3) live-cells. Pixel-size: 62.5 nm. Image format: 800 × 800 pixels. Excitation power Pexc = 3 µW. Scale bars: 1 µm.

Conclusions

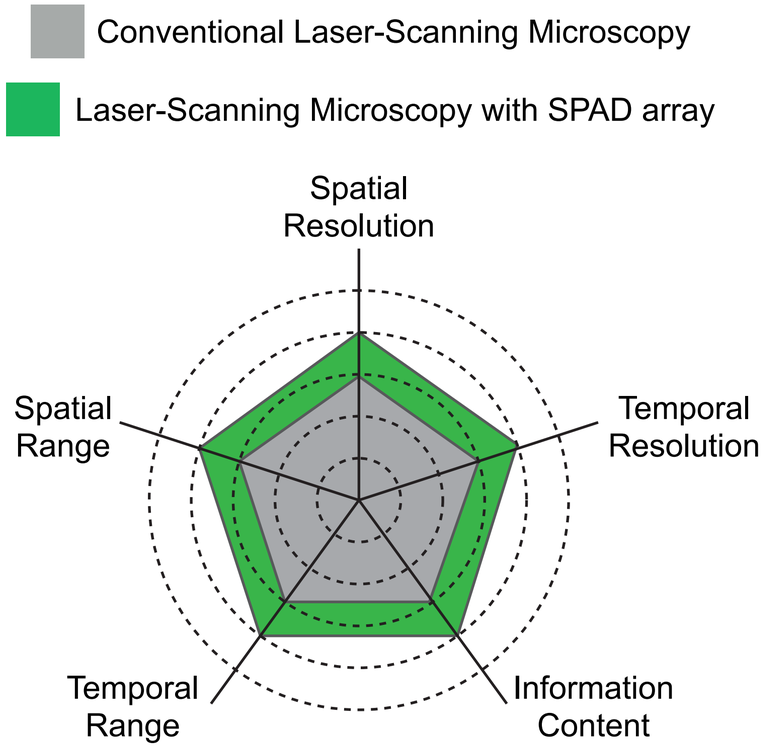
By **simply replacing** the typical single-element detector of a laser-scanning fluorescence microscope with an **“event-driven” SPAD array detector** it is possible to improve:

- Spatial Resolution (via ISM);

- Information Content (via FLIM)



Conclusions



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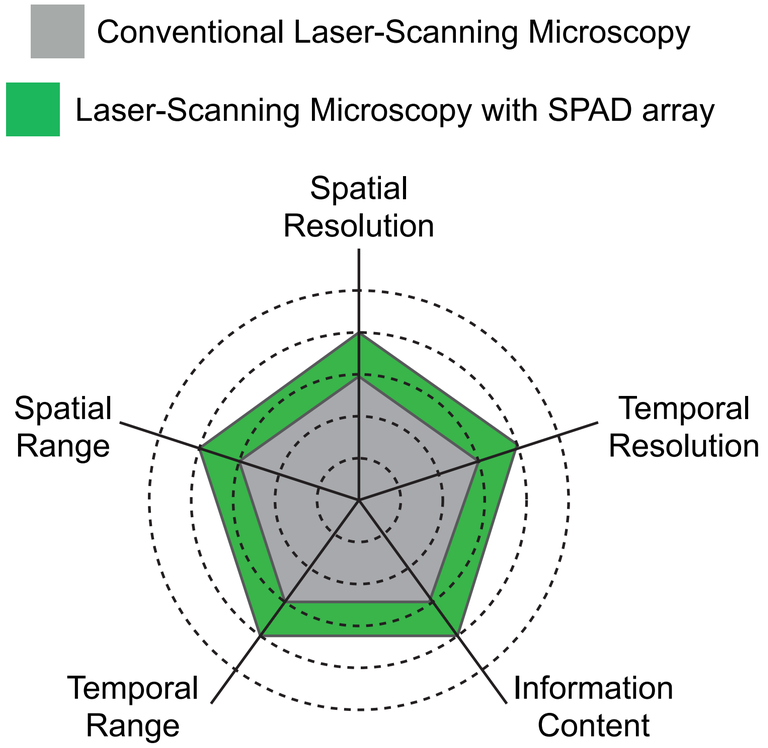
and more
(in combination with STED,
FCS, TPE, SMT, resonant-scanner,……)

Castello M.,…, Vicidomini G., Nat. Methods 16, 175–178 (2019)
Buttafava, M.,…, Tosi, A., Optica, 7(7):775-765 (2020)
Koho, S. V.,…, Vicidomini G., Biomed. Opt. Express,11(6): 2905-2924 (2020)
Tortarolo, G.,…, Vicidomini , G., bioRxiv 741389; doi:10.1101/741389 (2020)

Conclusions



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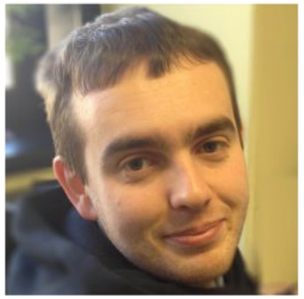
This LSM implementation can explore all the spatial ad temporal information carry out by any single photon, which is the minimum granularity of light

Towards Single-Photon Microscopy

Molecular Microscopy and Spectroscopy

Giorgio Tortarolo
PhD Student



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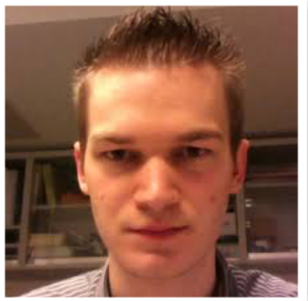

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We are hiring Fellowships, Ph.D. Students, PostDocs, and Researchers