Dynamic super-resolution in optical microscopy

Webinar March 11th , 2020

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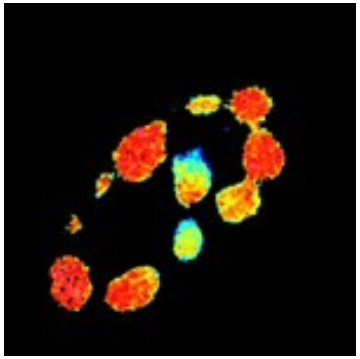
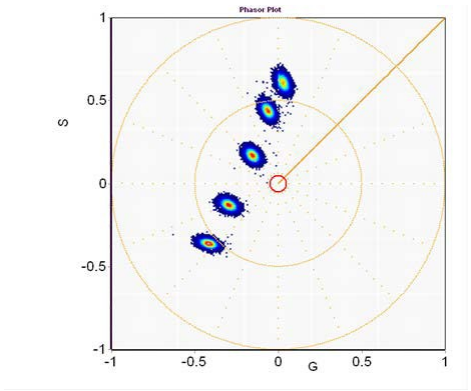


Polarization map of
Liquid-liquid phase separation
In the nucleus



Spectral phasor: Linear combination in the same pixel

Outline of the presentation

Optical super-resolution has been around for more than 10 years. Yet, superresolution is mainly applied to produce stunning images at the 10-20 nanometer scale of the interior of cell in tissues.

Super-resolution imaging has seen limited for applications to reveal the dynamics of molecular interactions at the nanoscale that is at the basis of life.

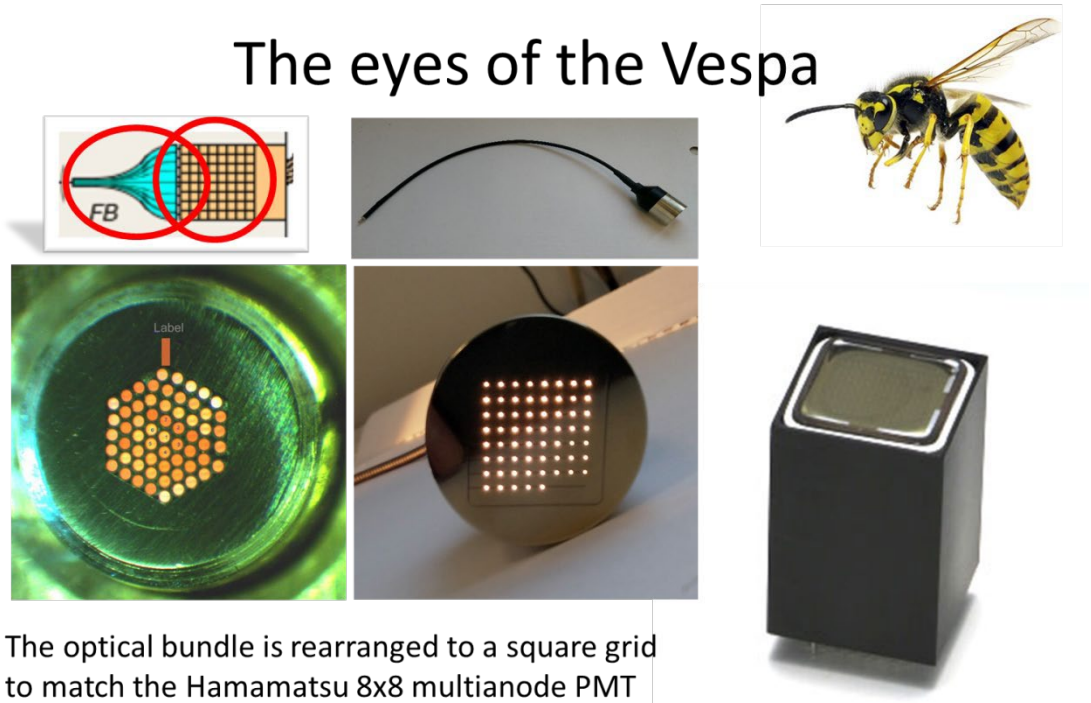
In our LFD P41 resource we have been working in filling this gap by developing enabling technologies that will open the potential of superresolution imaging to dynamic at the microsecond-millisecond-temporal scale.

In this **webinar** I will describe some of these enabling technologies and the discuss the impact the they could have in biology and in the clinical area.

Enabling technology

Acquire more information about the number and location of molecular species in a small volume by exploiting fluorescence parameters such as multiple excitation, multiphoton imaging, fluorescence polarization, fluorescence lifetime decay, hyperspectral imaging and spatial correlation of fast fluctuations.

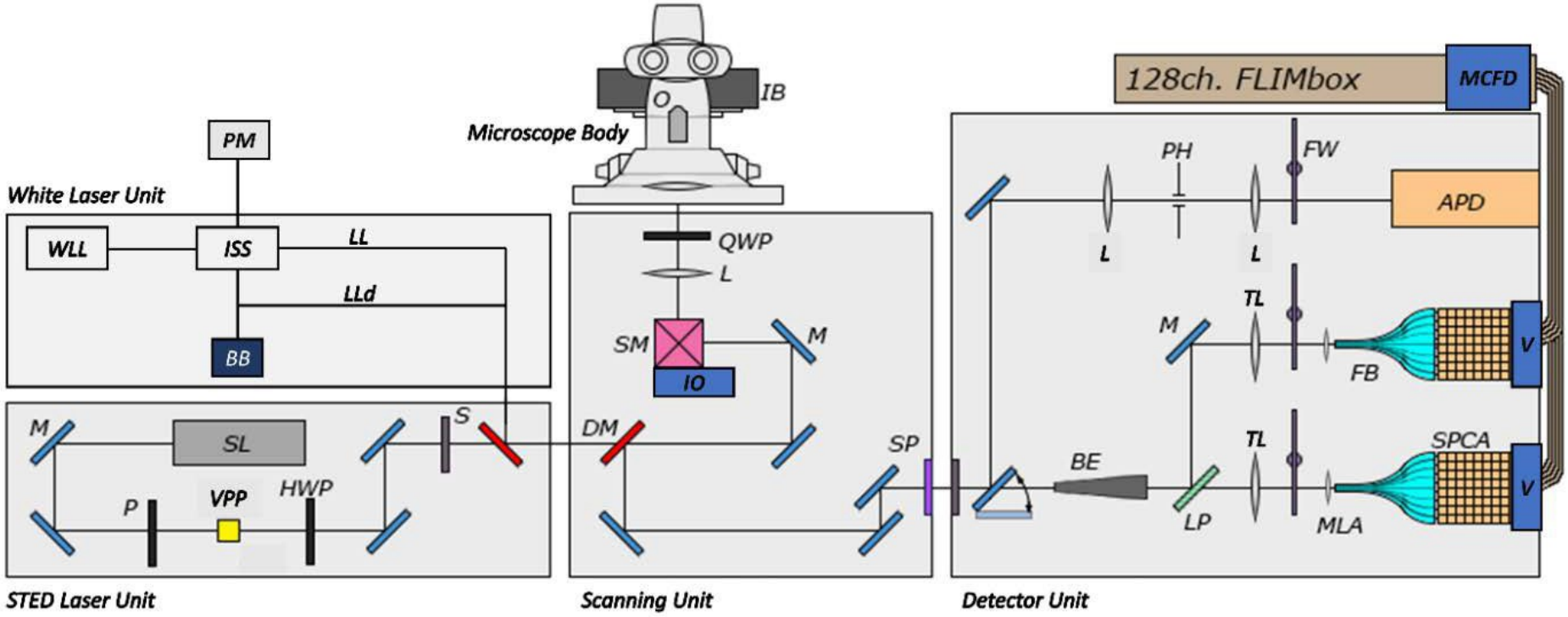
This technology is possible by the development of ultrafast cameras operating in the range of 1 million frames/sec, with hyperspectral and lifetime capabilities.



The optical bundle is rearranged to a square grid to match the Hamamatsu 8x8 multianode PMT
Fibers are bundled in a hexagonal grid (61 fibers)

Affordable technology gives fluorescence lifetime and fluorescence spectra at each of the 64 pixels of the multi-anode photomultiplier.

The ultrafast camera microscope



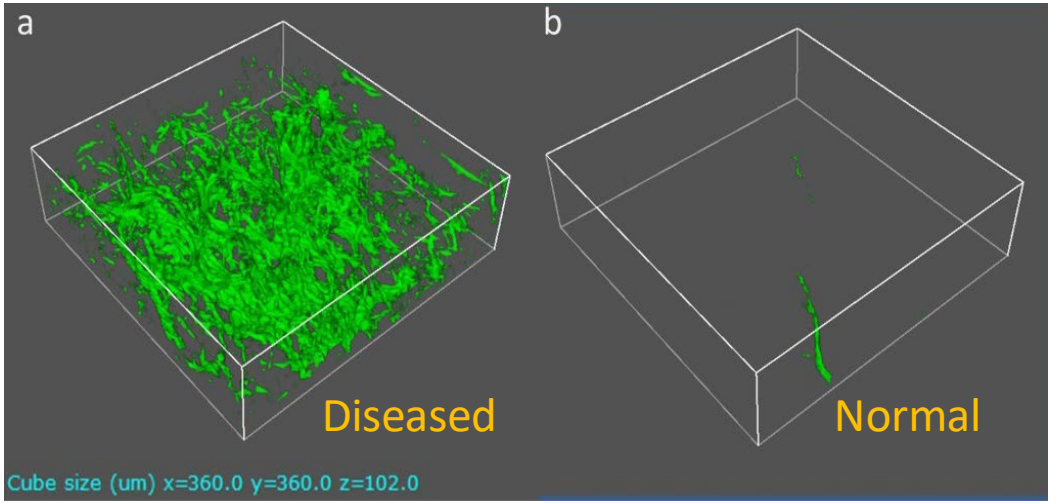
Benefits of the dual channel ultrafast camera (10^6 frame/s):

1. Superresolution using structural illumination
2. Fluorescence decay at each pixel
3. Fluorescence spectrum at each pixel
4. Image fluctuations among all pixel and cross correlations between channels
5. All commercial parts and software available at our web site

Biological/clinical examples: Content superresolution based on spectral and lifetime data (about 10-20 molecular species per pixel). Important for potential clinical applications . (Collaboration with Dr. Moshe Levi and Dr. Suman Ranjit, Georgetown University)

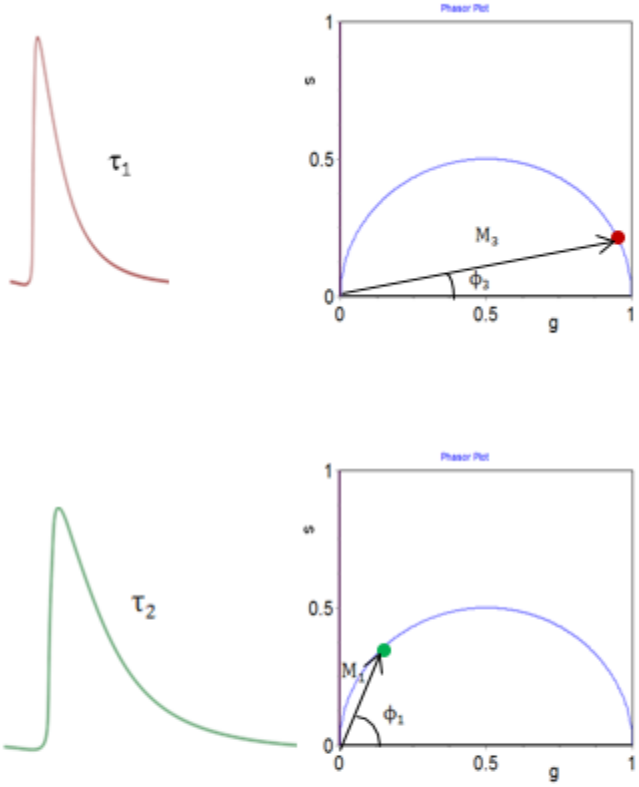
Collagens have a combination of SHG and fluorescence. By judicious combination of emission filters we can separate different types of collagens

Direct detection of fibrosis in the kidney

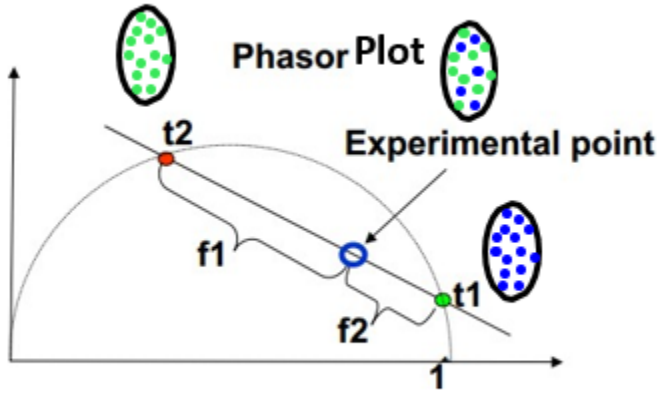


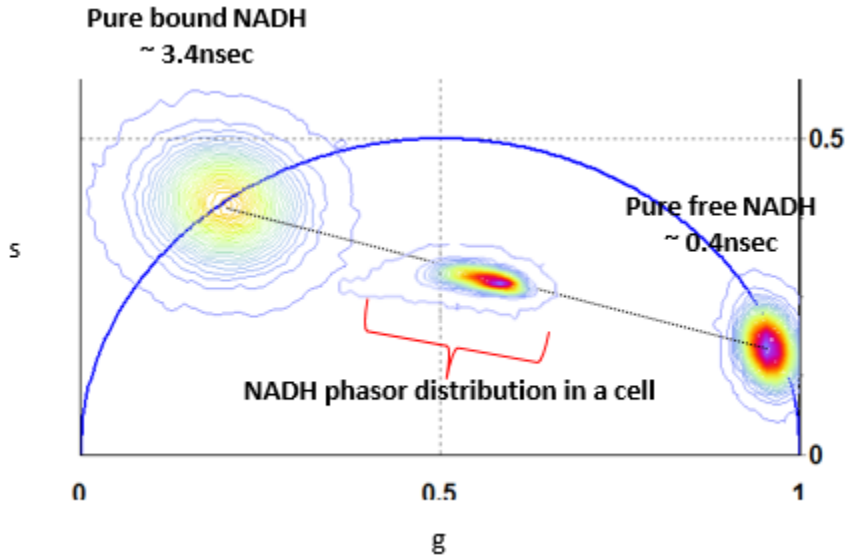
Introduction to phasors

Phasor plots



Law of linear addition of phasors:

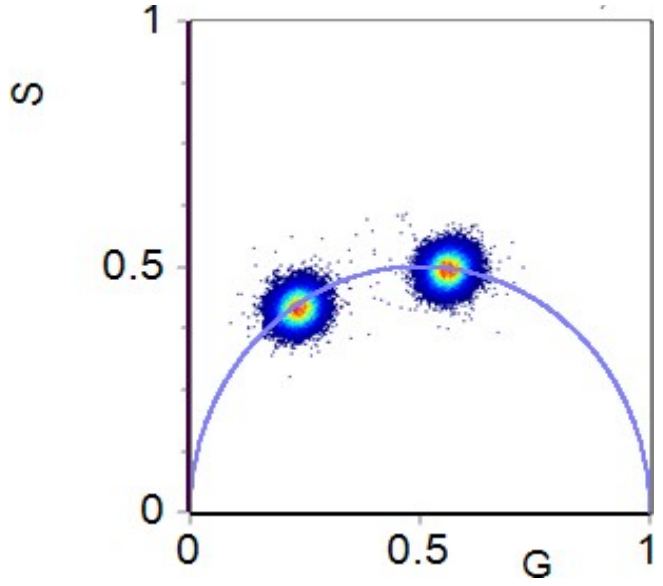




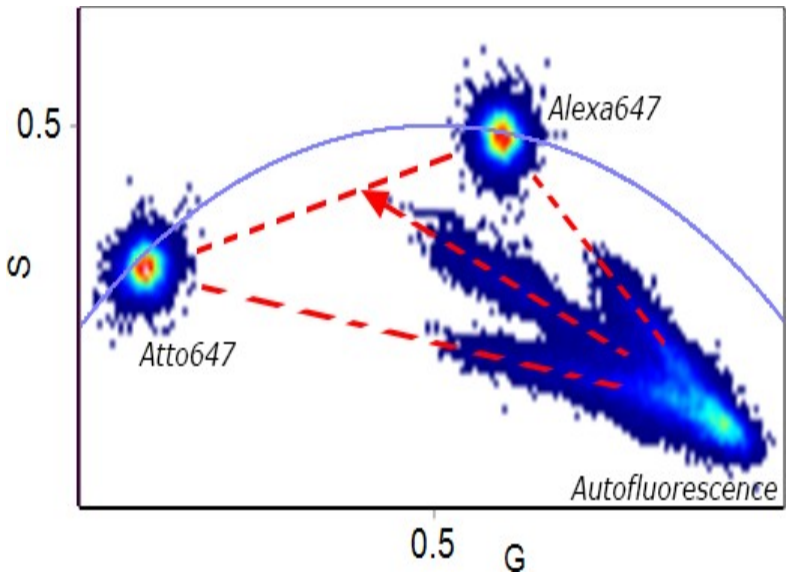
At every pixel of an image we measure the decay and then the decay is transformed to the phasor representation

Multiplexing several species (3 or more) in every pixel of an image and in 3D is within our reach. For tissue analysis (multiple cells, cancer, embryos and other applications) we have a number of species that can be recognized by fluorescence spectra and lifetime in the same pixel (About 20 with current technologies, 5 spectral and 4 lifetimes) and dynamically.

Superresolution obtained with structured illumination (about 120 nm) and with STED (about 15 nm).



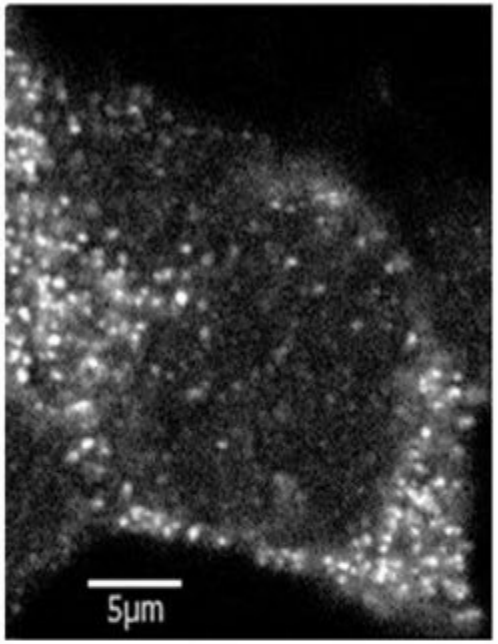
Measured phasor positions/lifetime of free dye in solutions

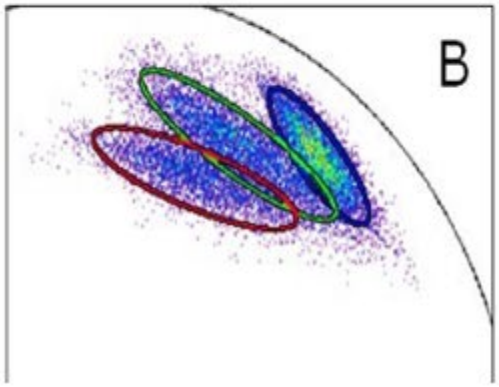


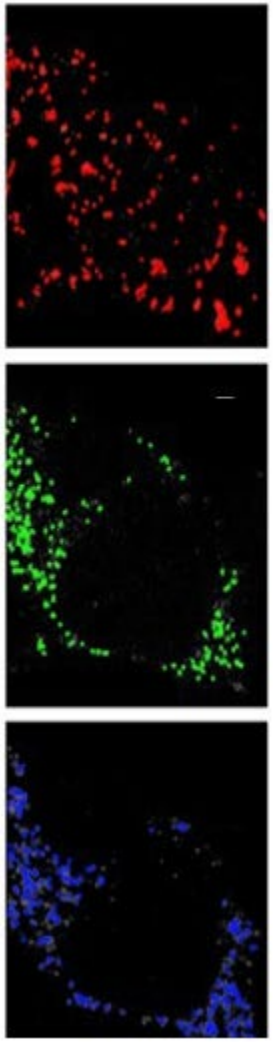
Measured phasor positions/lifetime of free dye in solutions
Change → mix them together in the phasor plot and show where expected lifetime is (linear combination)

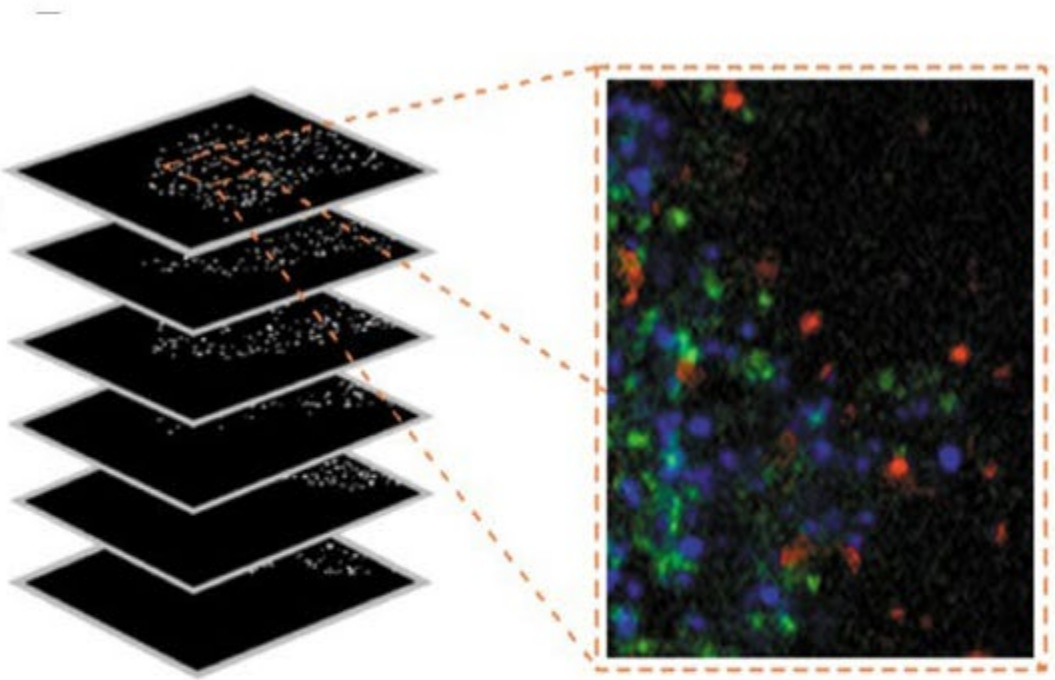
Biological example: observing gene expression in single cells at superresolution in one washing step: exploiting multiplexing

Example of application of automatic recognition of spectral and lifetime simultaneous operation using AI clustering methods. Three different lifetime barcoding methods are used to spatially profile over 140 transcript using a 3 barcoding scheme composed of multiplexing combinations of 16 fluorophores





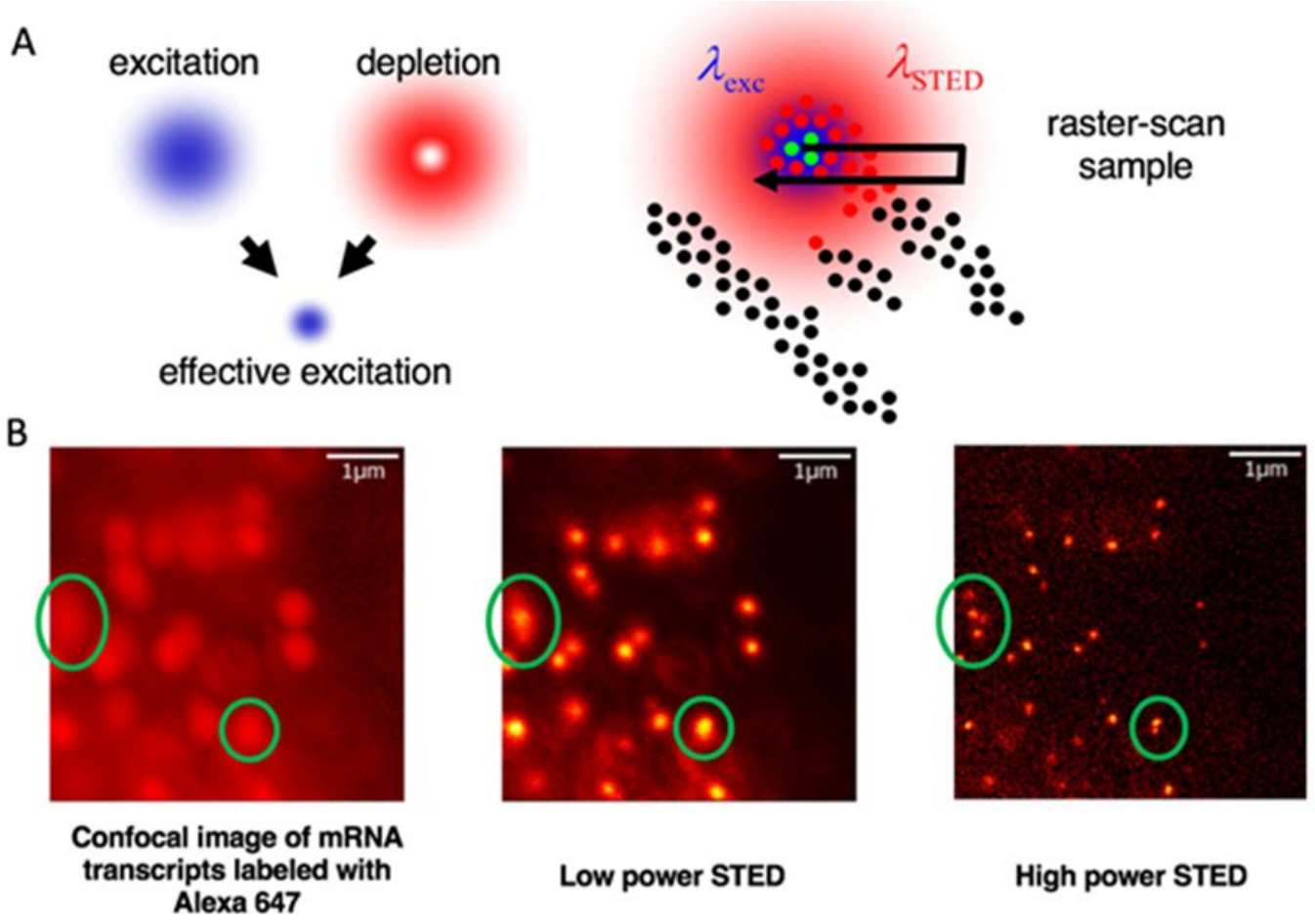




CELL ID	CH1 #	CH2 #	CH3 #
1	104	155	47
2	108	121	98
3	131	117	91
4	96	146	85
5	94	120	44
6	122	139	50

Cell ID	Puncta ID	Puncta Intensity (RFU)	Punctal Spectra (nm)	Puncta Lifetime (ns)	X, Y, Z Coordinates (um)	Identified Gene	Encoded Label
1	1	1500	647	1.7	15, 19, 20	UBC	Alexa 647
1	2	1651	647	3.6	16, 19, 20	POLR2A	Atto 647
1	3	1890	647	2.7	16, 28, 20	MNEONG	Alexa 647 + Atto 647
1	4	1570	647	1.7	19, 45, 20	UBC	Alexa 647
1	5	1479	647	3.6	27, 33, 20	POLR2A	Atto 647

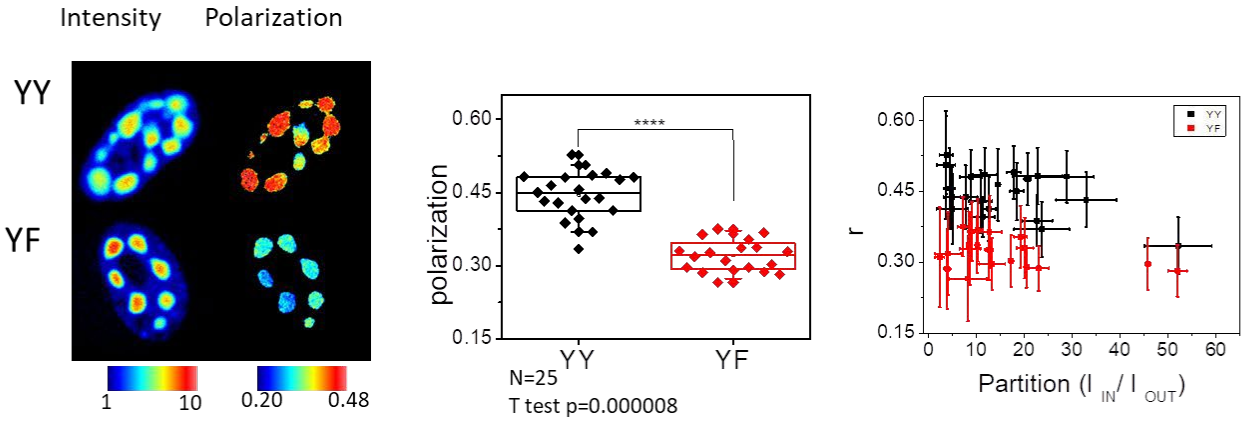
Tracking biological dynamical processes at superresolution.
Other examples not shown in this webinar: Gene expression at the polymerase junction, traffic through the nuclear pore complex. Also important for clinical application.

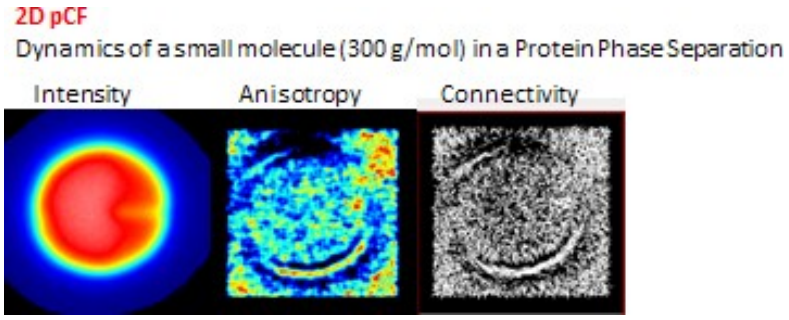


Principle of STED and application to FISH. (A) STED mechanism. **(B)** Confocal image of mRNA labeled with Atto647 (left). Corresponding STED images at low (middle) and high (right) depletion beam powers. The lateral spatial resolution of the STED images is 15nm.

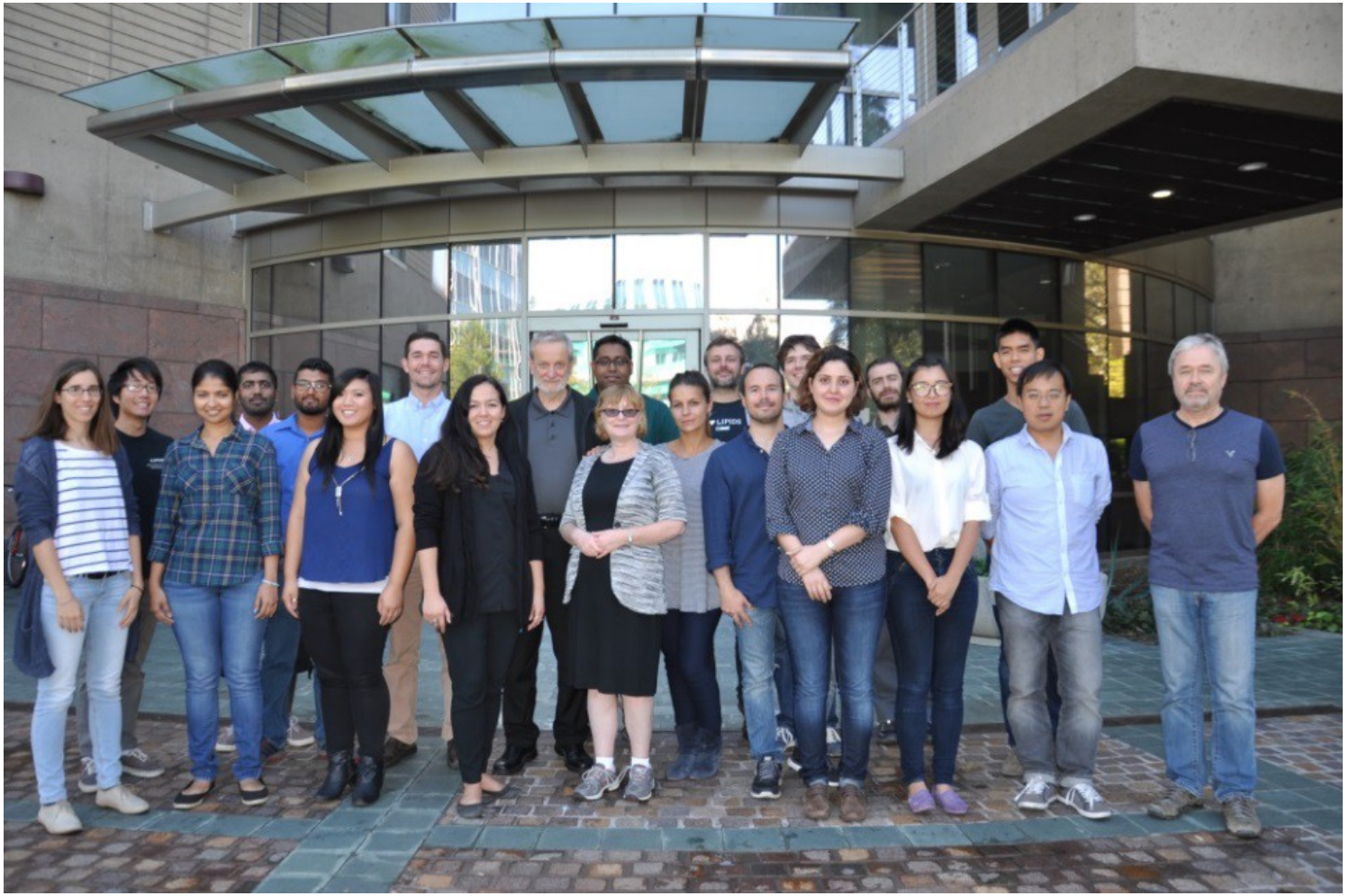
Biological example: observing and tracking liquid phase separation in the nucleus
Collaboration with Jiang, Hao, University of Virgina

Polarization allowed us to observed different protein aggregation for YY and YF on the phase separated spots





If we could have at the same time 3D tracking of some features in a cell, we could have superresolution and dynamic information of the structure we track.



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