Single Photon Microscopy

Only including section generated by Avery Books
This was made for EE218A @ UC Berkeley
with Prof. Kante
Speaker Notes: Notion

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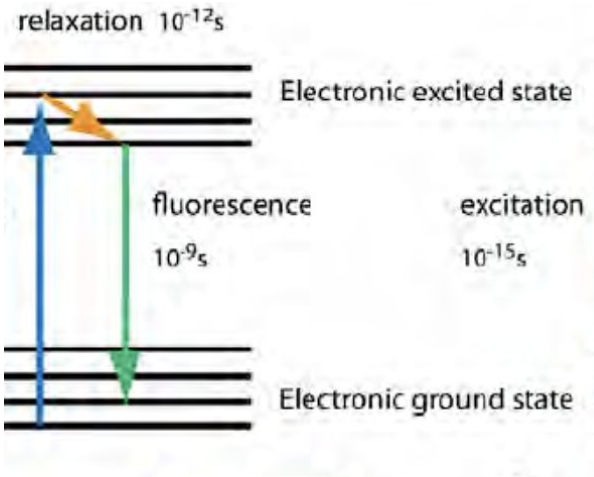
How Single Photon Microscopy Works

What is Single Photon Microscopy?

Uses an external light source to **pump** specimen that is embedded with **single-photon emitters** that absorb a photon from the pump, excite and de-excite it's electron, and emit single photons which are detected by the microscope array.

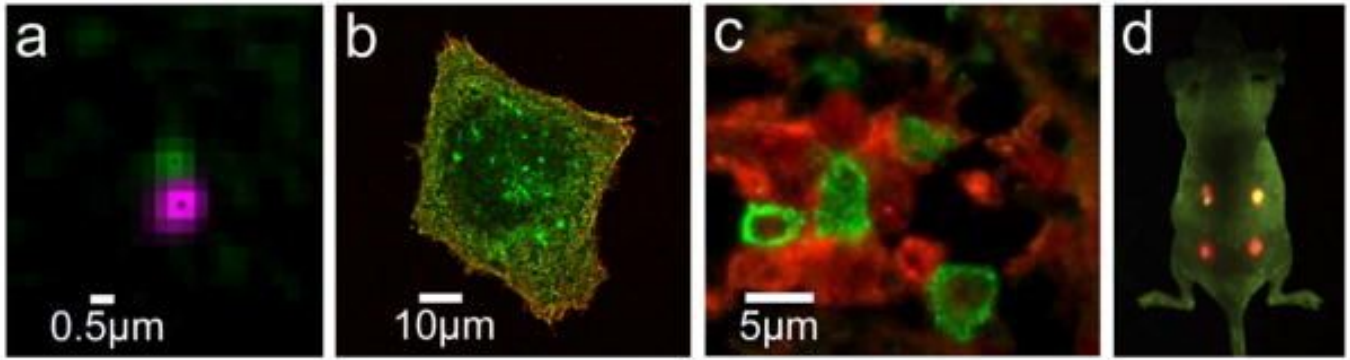
- Single Photon Sources - Quantum Dots, Organic Dyes, Fluorescent Proteins
- Engineering of single photon sources
- Applications of single photon
- Brief comparison to another method



Source: [14]

Single Photon Sources: Why Important?

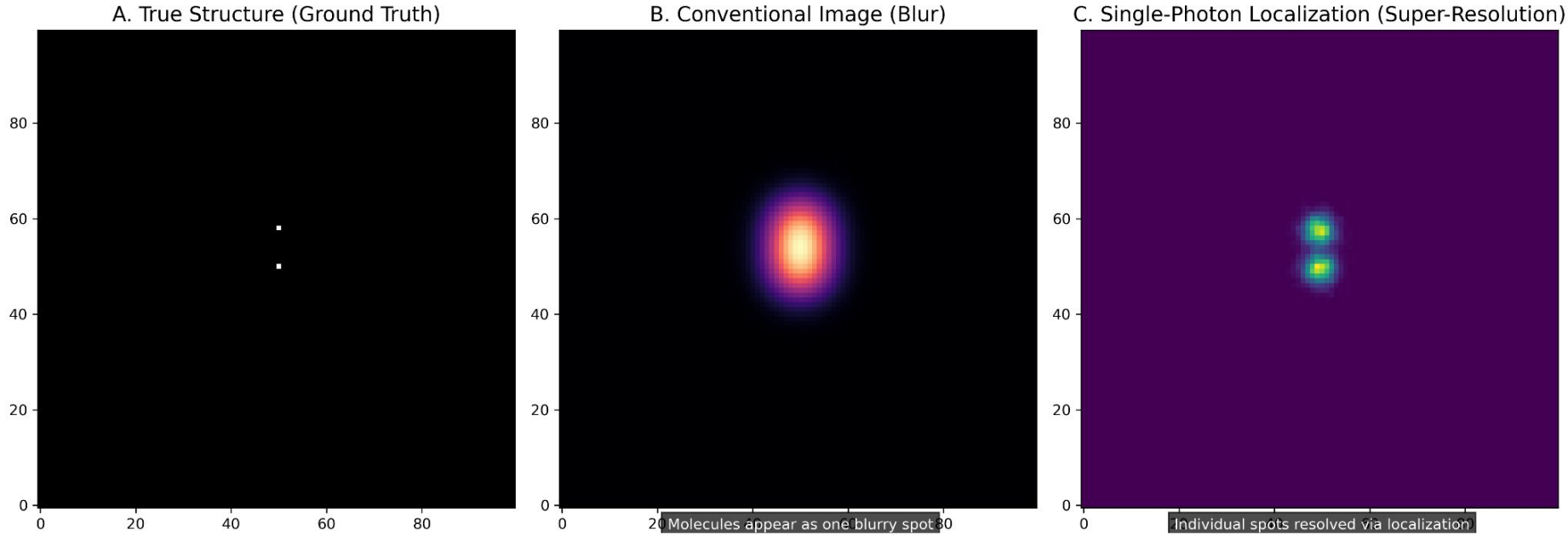
1. Break resolution limit by isolation of PSFs
2. Sources integrated into object



	Single Molecule	Single Cell	Tissue	In vivo
Modality	widefield confocal TIRF	widefield confocal TIRF	widefield confocal spectral imaging	whole animal intravital two-photon
Spatial resolution	nm	μm	μm	μm-mm
Temporal resolution	ms	ms-min	N/A	s-hr

[2] Vu, T. Q., et al. (2015). Quantum dots for quantitative imaging: from single molecules to tissue.

Python Simulation - Comparison with Std. Microscopy



Single Photon Sources: Types

Quantum Dots (QDs)

0-dimensional nanoscale semiconductor crystal that “blinks” and behaves as an artificial atom

Made via “wet chemistry”

0-dimensional

- Electrons spatially constricted s.t. 0 d.o.f.
- Forces electron to discrete energy levels

“Blinking”

- Inherent stochastic binary states of fluorescence
- Sparse random subset of probes in the dot are “on” at any given point and are required to locally isolate

Single Photon Sources: Types

Organic Dyes

Small Molecule dyes that are conjugated to antibodies or other agents that blink due to chemical buffers

Chemical Buffers → Requires optimized and cytotoxic imaging buffers to induce blinking

Optical properties → Less photo stable and less total photons than QDs before photobleaching

Optical properties → Small size minimizes linkage errors -> better map of location

Single Photon Sources: Types

Fluorescent Proteins

Genetically encoded labels that are produced by cell

- Photoactivatable → Exist in “off” state and turn “on” when excited (irreversible)
- Photoconvertible → Change emission color after activation by particular wavelength
- Properties
  - Endogenous and biocompatible
  - Low photon count and photostability

Fig from:

Interventions for preventing or controlling health care–associated infection among health care workers or patients within primary care facilities: A scoping review

Lucyna Gozdzielewska, PhD, et. al.

	QDs	Organic Dyes	Fluorescent Proteins
Size	1		
Photostability		2	2
Broad excitation	3		
Multiplex imaging	4		
Brightness	5		6
Flexible conjugation	7		
Genetically expressible	8	8	
Electron dense		9	9
Sample fixation requirements	10		
Continuous emission	11		
Accessible time/length scales	12	13	13
In vivo imaging	14		15

Spontaneous Emission

Quantum Mechanics & Electrodynamics govern the emission parameters:

Dirac Theory of Radiation → Transition of states is viewed as interaction with vacuum fluctuations of QEM field (random phase)

Fluorescence Parameters →

- Molar Extinction Coefficient (probability to absorb)
- Fluorescence Quantum Yield (brightness)
- Fluorescence Lifetime (avg. time in excited state)

Comparison to Differential Interference Contrast (DIC) Microscopy

Single Photon:

1. Incoherent Fluorescence
2. Chemical Map: shows precise location of specific labeled molecules
3. Intensity
4. Limited by Diffraction (not as much)

Differential Interference Contrast

1. Phase shifts cause by sample's refractive index
2. Shows surface contours and optical density
3. Phase converted to pseudo - 3D shadow cast images
4. Limited by diffraction

Sources

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