Sub-exposure time resolution in wide-field time-correlated single photon counting (TCSPC) imaging

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Outline

- Background – fluorescence lifetime imaging (FLIM) microscopy
- Wide-field time-correlated single photon counting (TCSPC) with ultrafast cameras
- Photon arrival time from phosphor decay

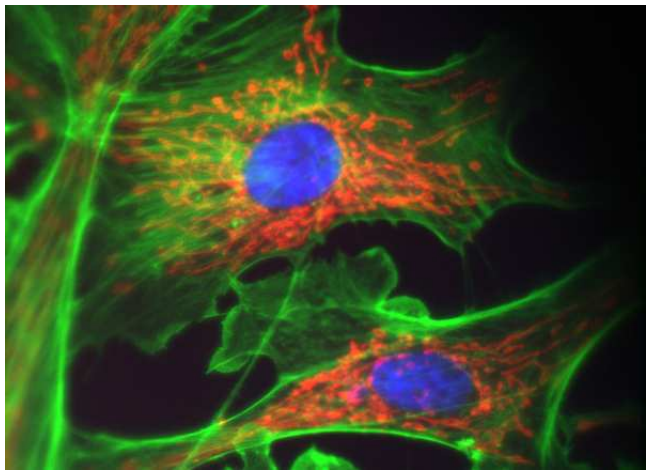
Poster 12: Electron-bombarded CCD (EBCCD) as a parallel-processing photoelectronic time-to-amplitude converter (TAC)

Fluorescence microscopy

- Rejection of reflected light $\rightarrow$ imaging inside cells and tissues
- Tagging of specific components of cell, sub-cellular resolution, single molecule sensitivity
- Visible wavelength $\rightarrow$ less damage, live cell dynamics & function

Fluorescence is multi-dimensional

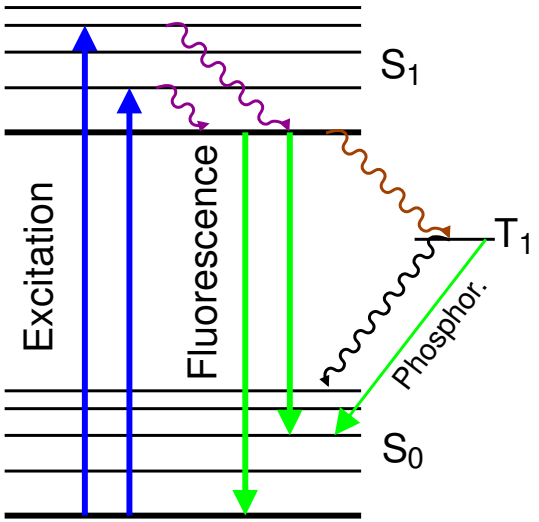
- Intensity
- Position
- Wavelength
- Polarisation
- Lifetime

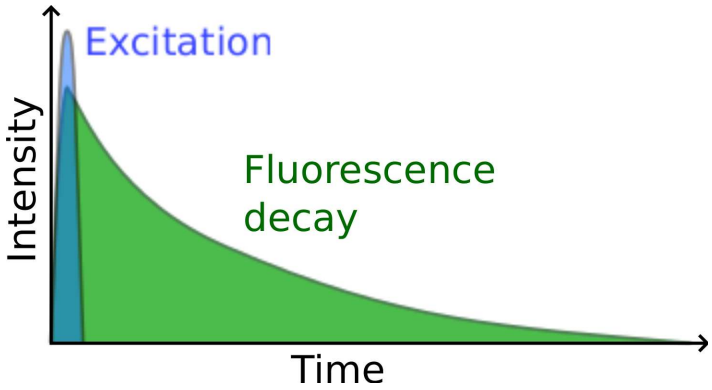


Nucleus – actin – mitochondria

Fluorescence Lifetime Imaging (FLIM)

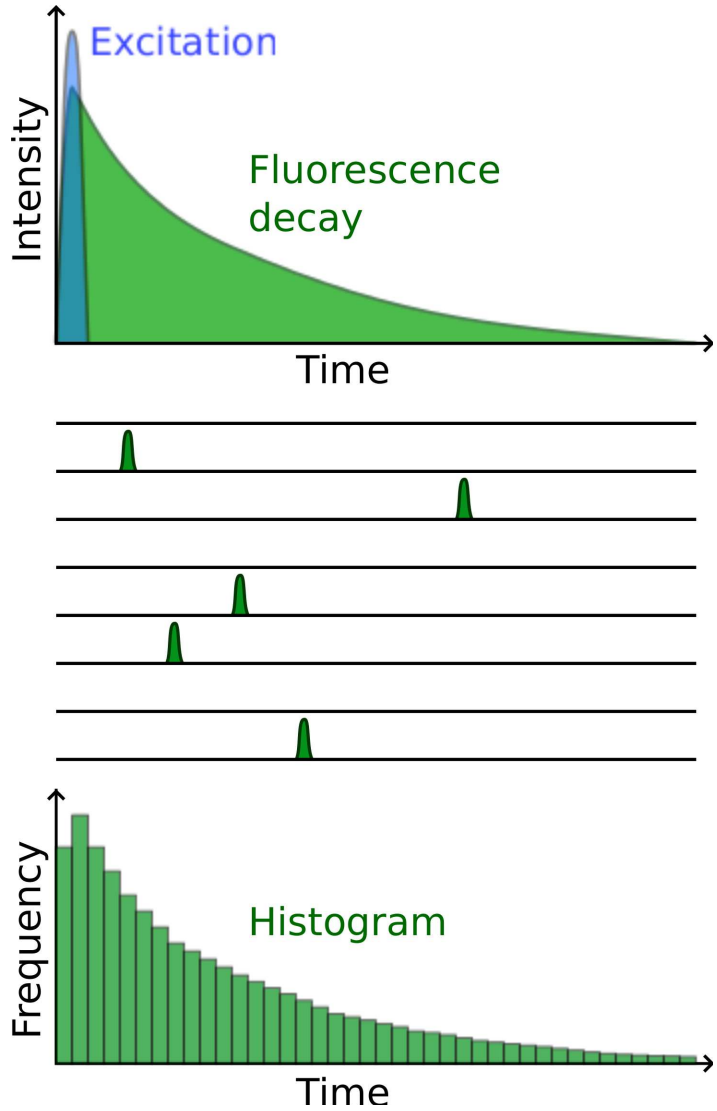
- Fluorescence lifetime τ
  = average time in excited state
- Independent of excitation intensity and fluorophore concentration
- Mapping of local environment:
  - Ion concentration (Na, Cl, Ca...)
  - Oxygen concentration
  - Acidity (pH)
  - Refractive index
  - Viscosity ...
- Applications: Cell biology, forensic science, art conservation, remote sensing, temperature sensing...





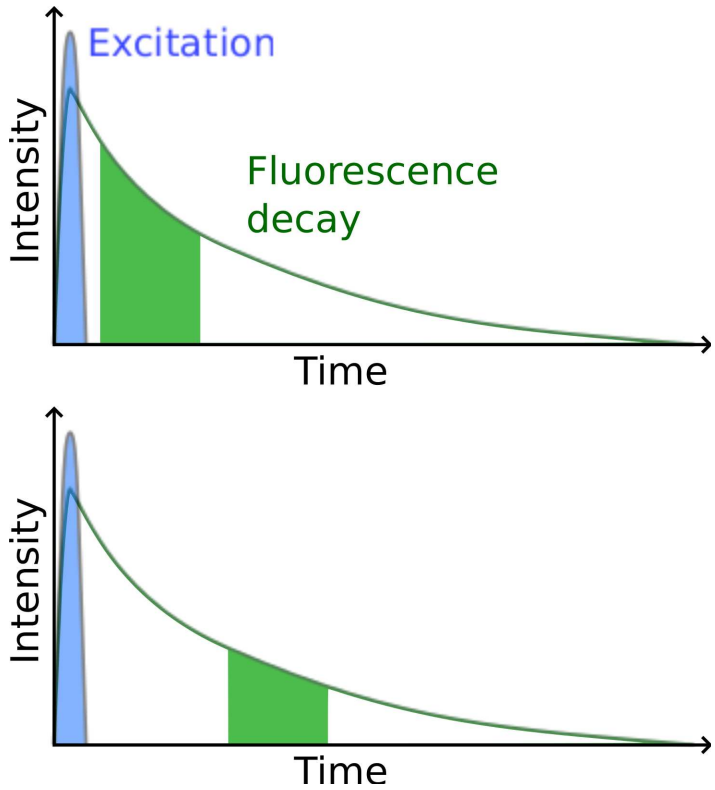
FLIM implementations (time-domain)

- Scanning TCSPC
  - Image build one pixel at a time
  - Max one photon per pulse
  - High rep-rate lasers
  - Nano/picosecond time resolution
- Wide-field gated detection
- Wide-field TCSPC with ultrafast camera



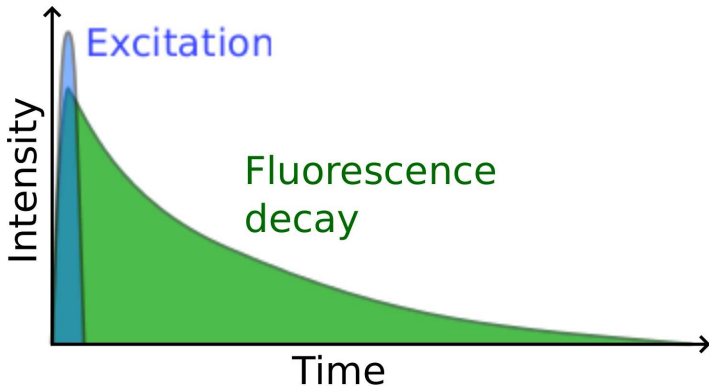
FLIM implementations (time-domain)

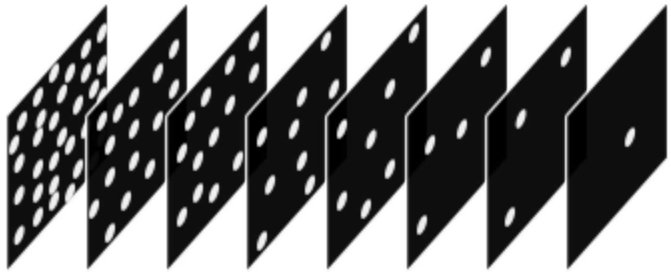
- Scanning TCSPC
- Wide-field gated detection
  - Fast (all pixels parallel)
  - Loss of photons
  - No single photon sensitivity
  - Photobleaching and intensity fluctuations affect measurement
- Wide-field TCSPC with ultrafast camera



FLIM implementations (time-domain)

- Scanning TCSPC
- Wide-field gated detection
- Wide-field TCSPC with ultrafast camera
  - All pixels parallel
  - No loss of photons
  - Unlimited dynamic range
  - Insensitive to photobleaching and intensity fluctuations
  - Time resolution determined by frame rate (microseconds)

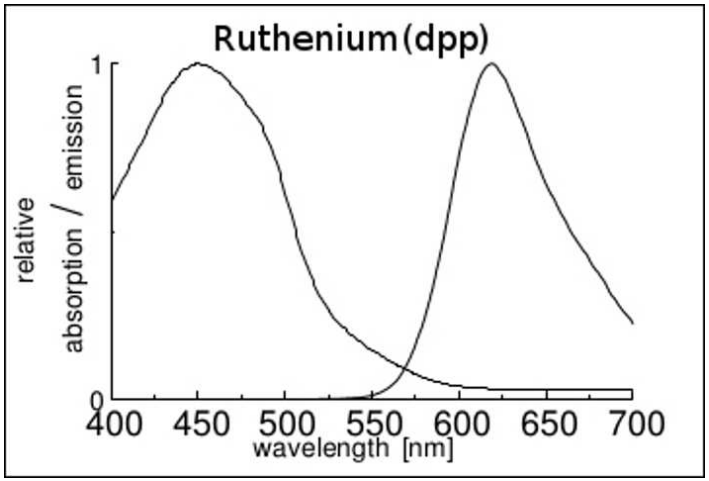




Long lifetime probes

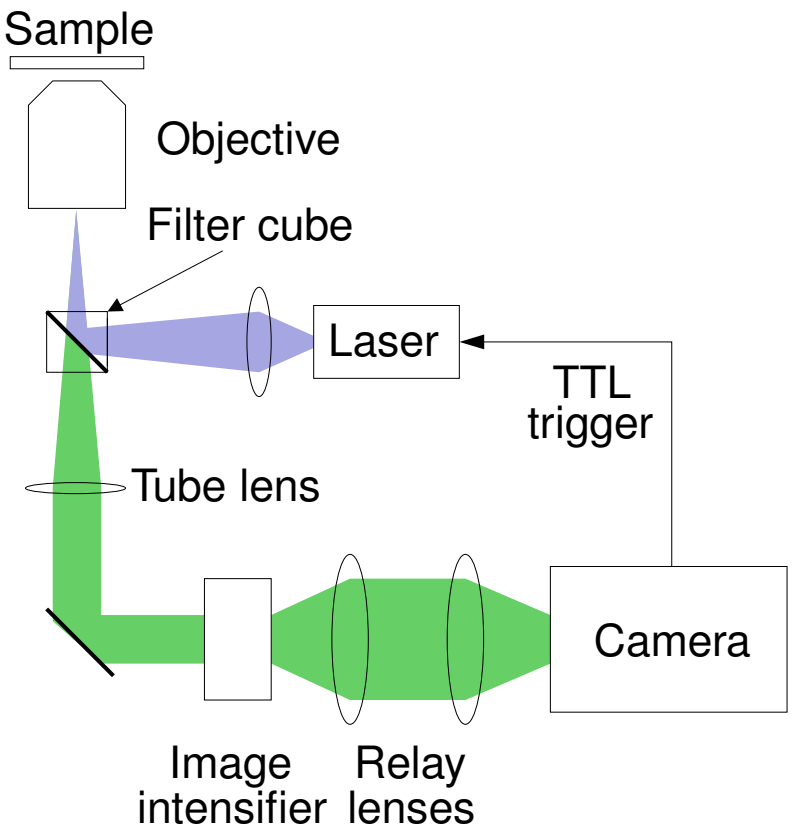
Transition metals

- Ru, Ir, Pt, Pd, Rh, Re, ...
- μs lifetimes
- Long Stokes shift, bright
- Chemically stable, water soluble
- Visible excitation wavelength



- Lanthanide compounds (Eu, Tb, ...): ms lifetimes
- Nanodiamonds, Quantum dots, ...

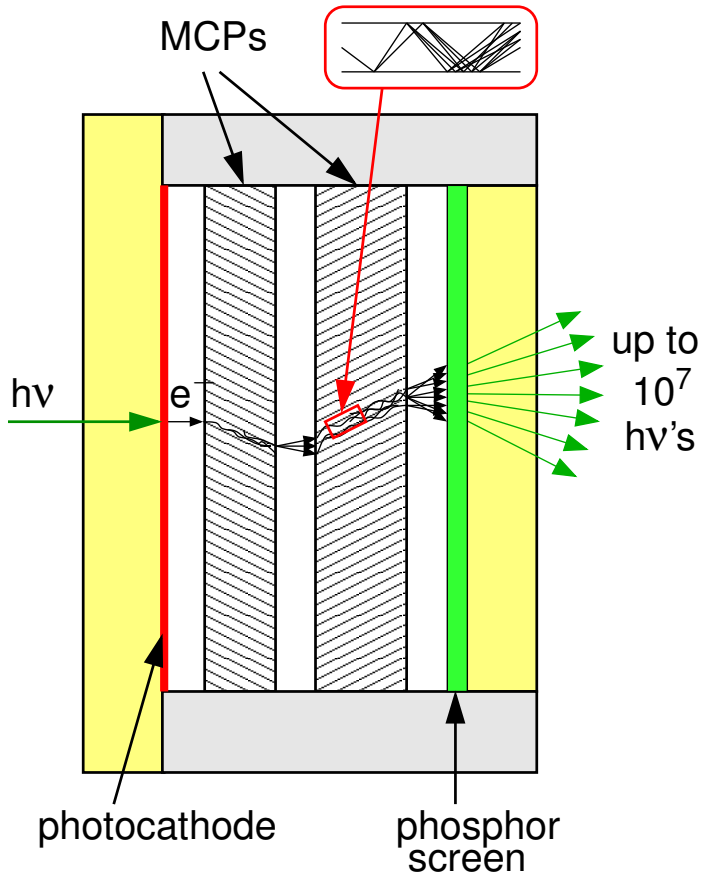
Microscope setup



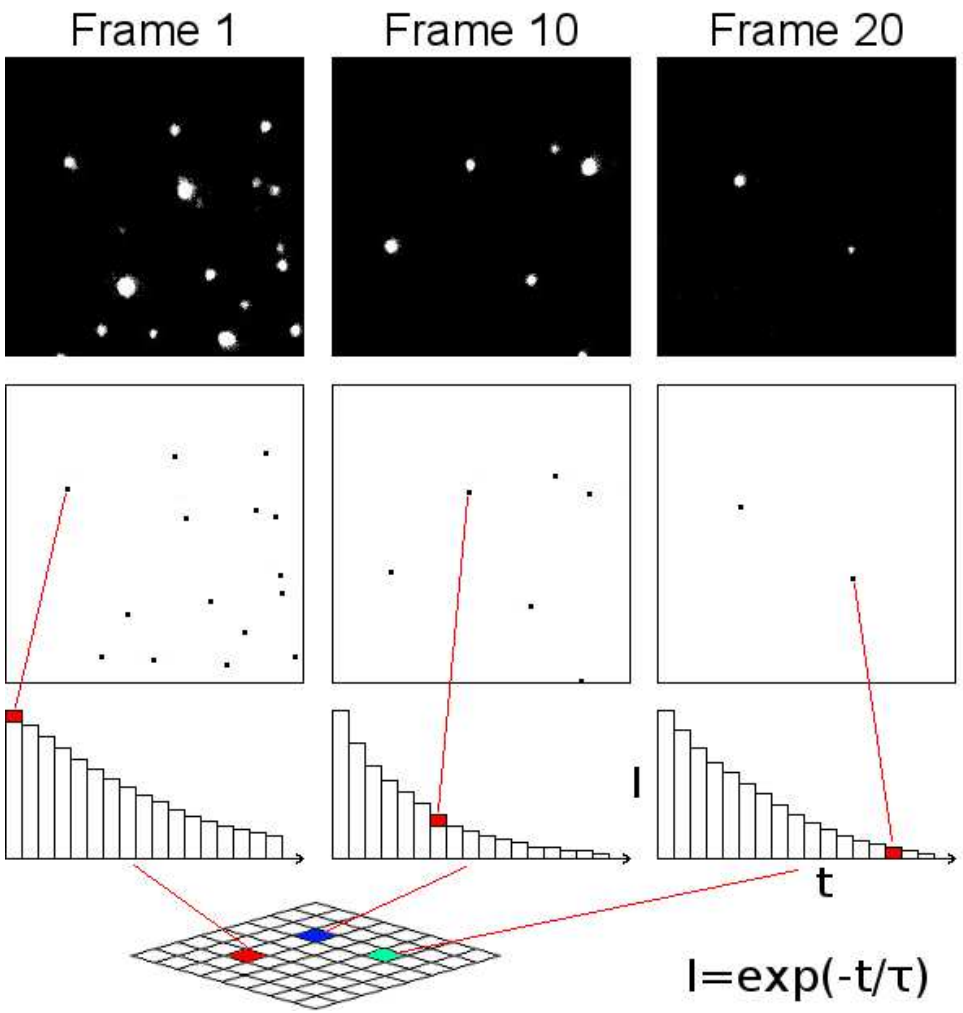
- Nikon TE inverted microscope
- Light sources:
  - Picosecond pulsed diode laser
  - Pulsed LED
  - Mercury lamp with chopper
- Camera: Photron SA5 / SA1.1
  - 1 MHz frame rate: 16x64 pixels
  - 54 kHz frame rate: 320x264 pixels
- Camera and light source synchronised

Image intensifier

- Single photon sensitivity
- Photon creates a photoelectron, multiplication of electrons, conversion back to photons
- Our intensifier: 3 MCPs, P20 phosphor: long decay (μs), green



Data processing



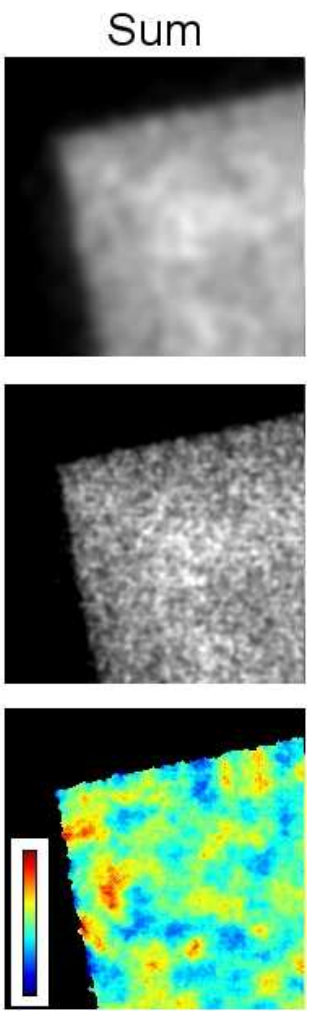
Raw data

↓

Threshold & centroiding

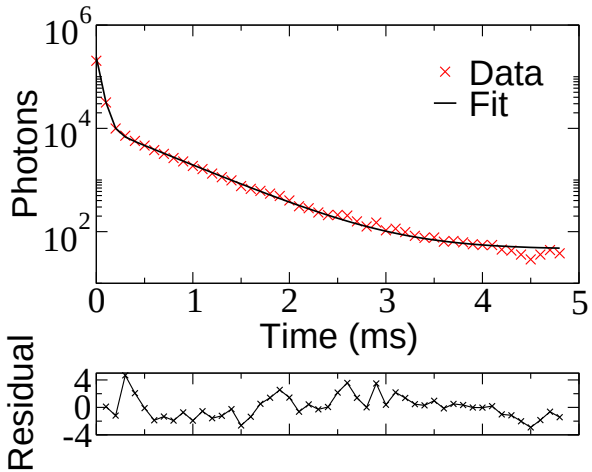
↓

Histogram & analysis

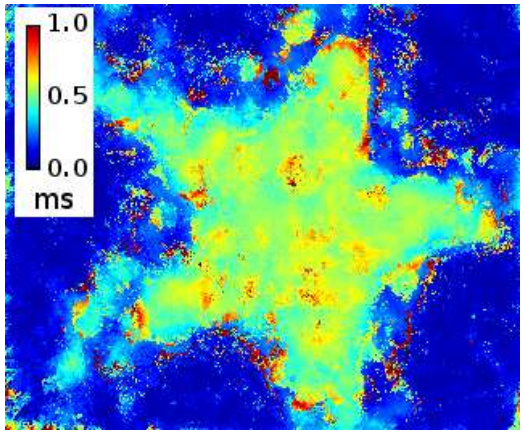


Euro note

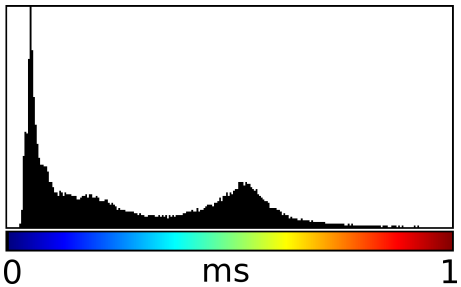
- Star in €20 note
- Lifetimes (biexponential):
  - 568±4 μs
  - 43±1 μs

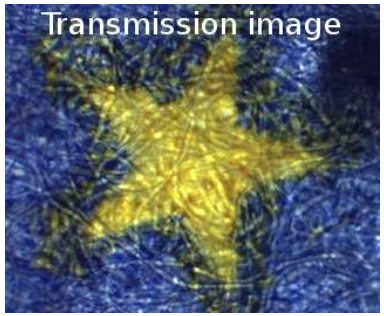


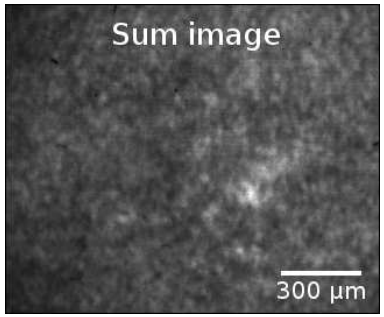
Lifetime image

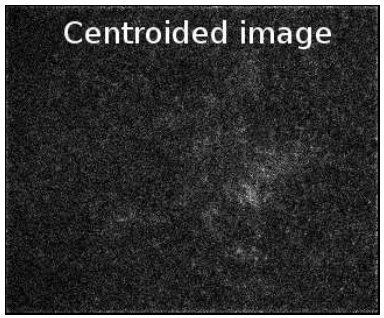


Lifetime histogram







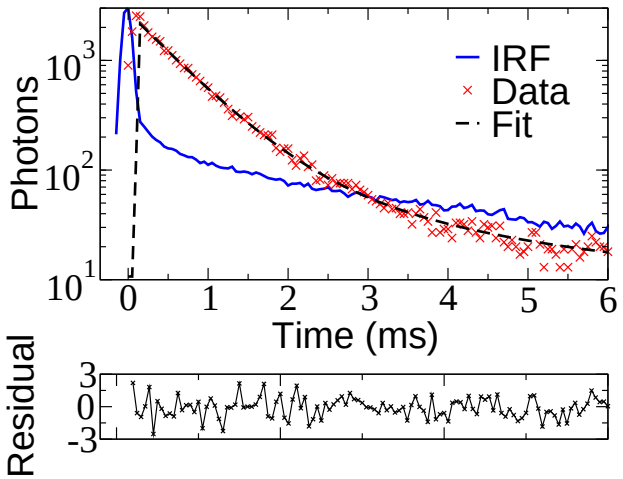


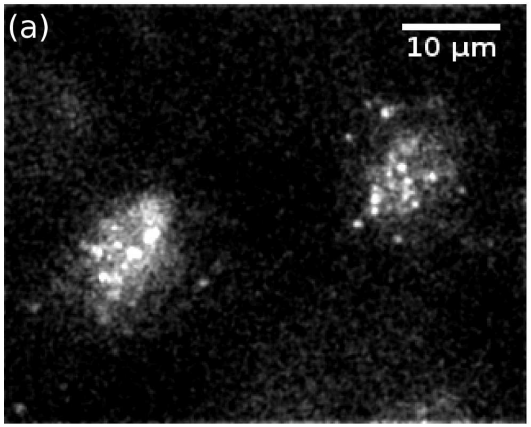
Acquisition: 10000 frames/s, 50 frames/pulse, ∼135000 frames, ∼60 sec;

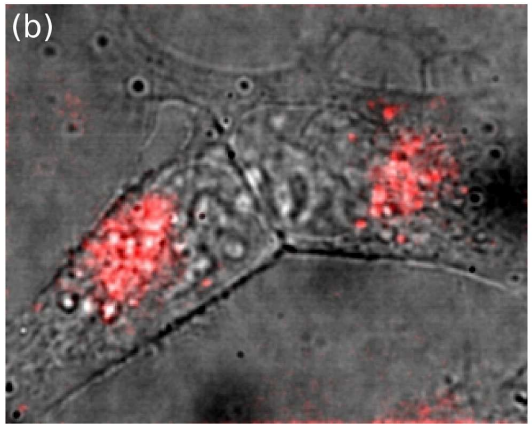
Exc: UV LED (365 nm) @ 50 Hz; Em: 515nm LP filter; 4× air objective

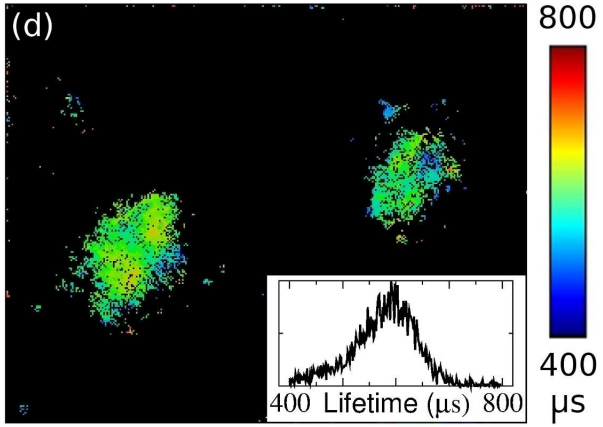
Europium in living HeLa cells

- 40 nm Europium beads in living HeLa cells.
- 320x256 pixels, 20 kHz frame rate, 92.3 photons/pulse.
- Data collection time: 3.5 seconds.
- Lifetime $\sim$ 570 μs.





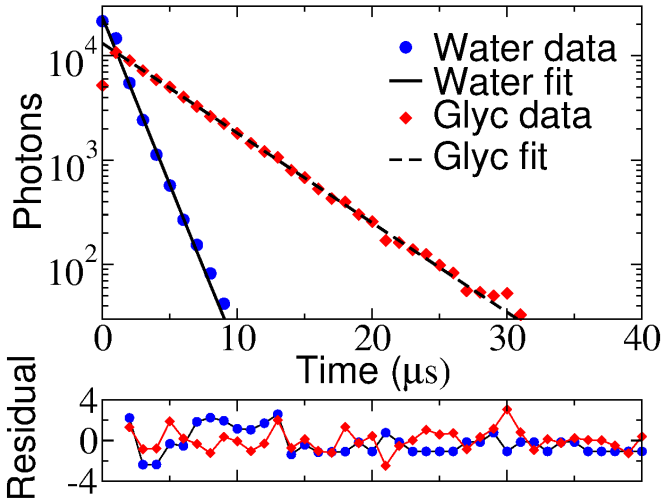


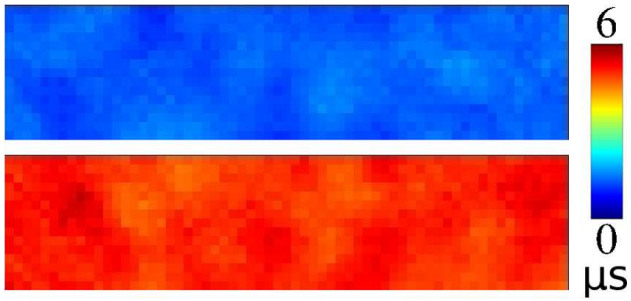


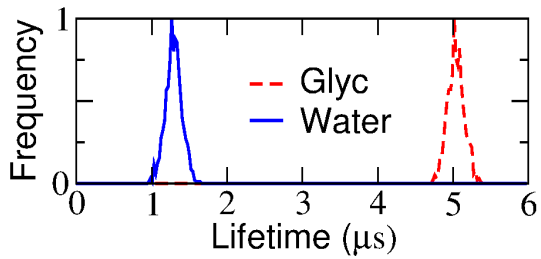
Eu intensity Eu (red) + transmission (grey) Lifetime image

Ruthenium in solution

- Ru(dpp) in water and glycerol
- Lifetimes (monoexponential):
  - Water: 1.35 μs
  - Glycerol: 5.05 μs
- Different lifetimes due to different oxygen diffusion rates







Acquisition: 1,000,000 frames/s, 50 frames/pulse, ∼800,000 frames, <1 s;

Exc: Diode laser (467 nm) @ 100 kHz; Em: 515nm LP filter; 10× air objective

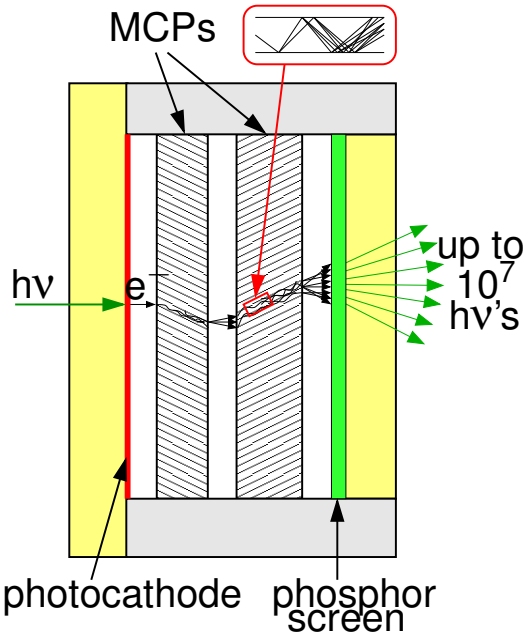
Summary (1st part)

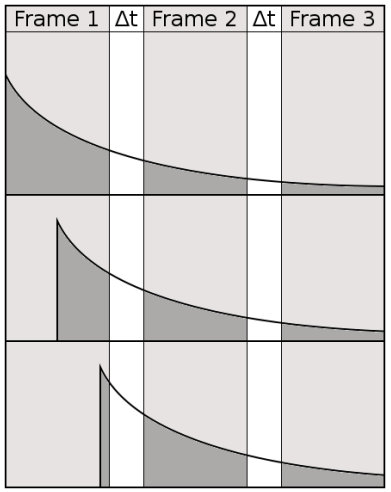
- Image intensifier + ultrafast camera allows wide-field TCSPC imaging.
- Collection of up to 100's of photons / pulse.
- Time-resolution limited by camera frame rate to $\sim \mu$s.

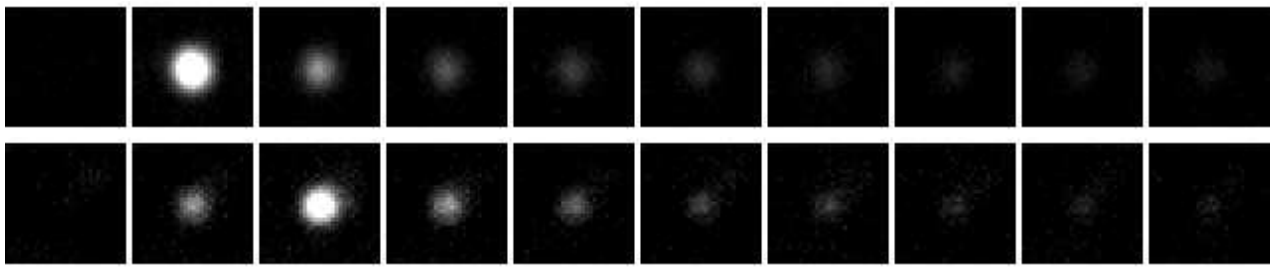
- How to improve time resolution ?

Sub-exposure time resolution from phosphor decay

- Image intensifier phosphor screen has an afterglow.
- Decay time depends on the type of phosphor (ns to ms).
- P20: multi-exp, $\sim 250\mu s$
- Intensity ratio of first and second frame yields photon arrival time.

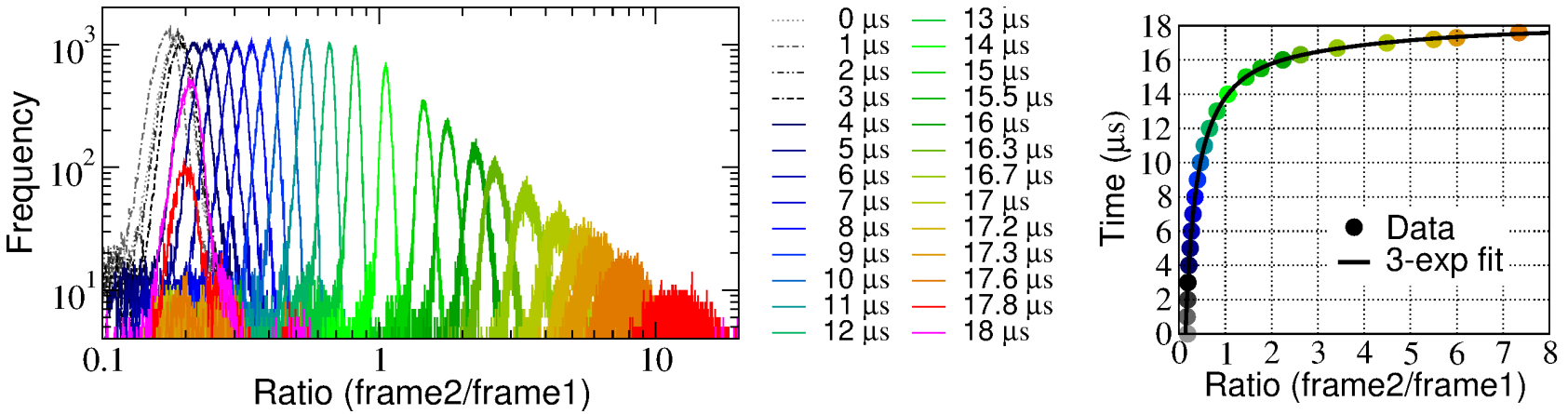






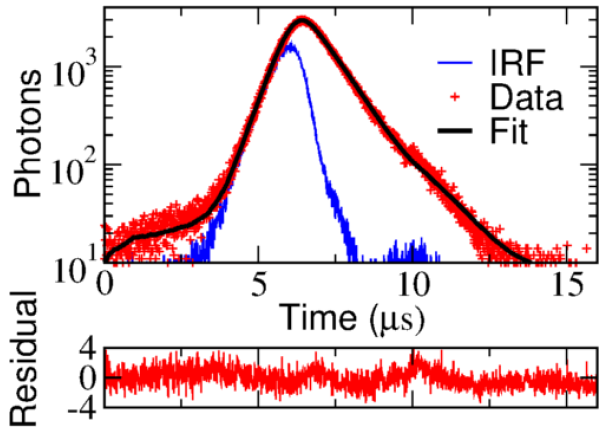
Intensity ratio to time conversion

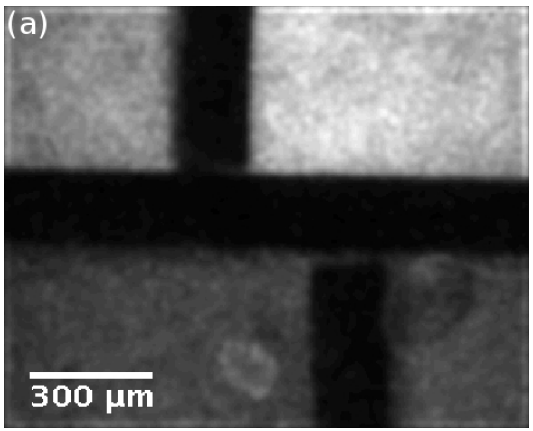
- Convert intensity ratio frame2/frame1 to photon arrival time.
- Measured by reflection and varying the time delay between the frame start time and the laser trigger pulse.
- 54 kHz frame rate (18.5 μs frame exposure time).



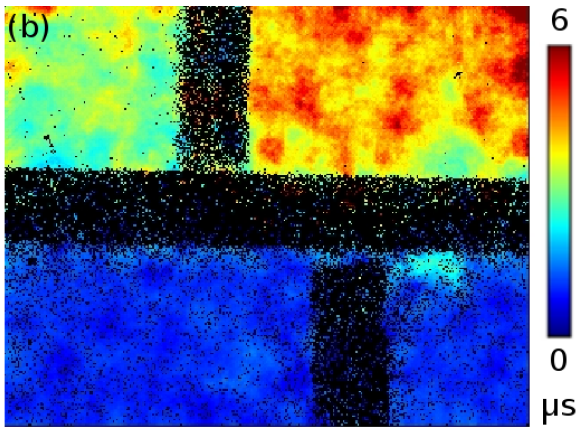
Ruthenium in water and glycerol mixtures

- 4 solutions: 100%, 50%, 20% and 0% glycerol mixed with water
- 54 kHz frame rate, 2.6 s
- Decay for each pixel of image
- Ru(dpp) lifetime contrast due to oxygen diffusion

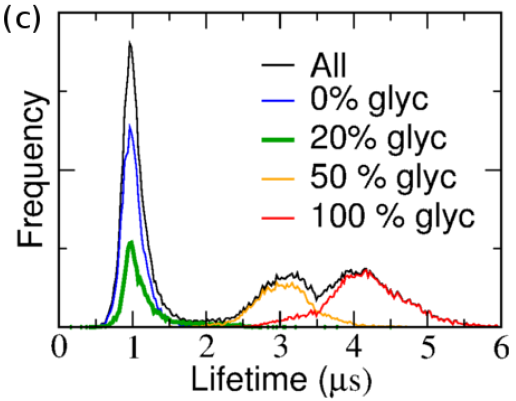




Intensity image



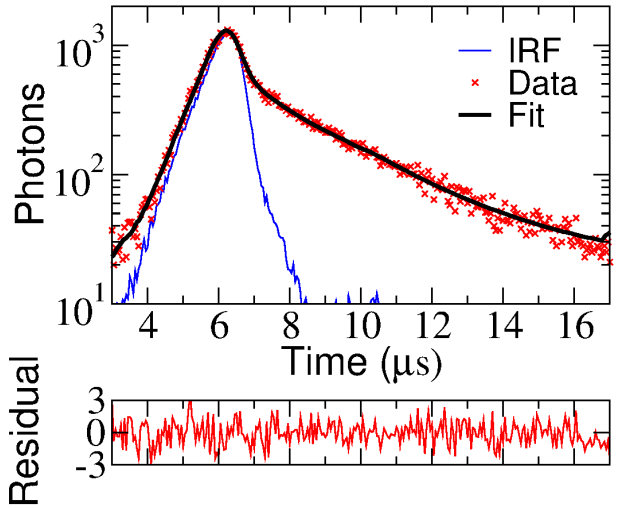
Lifetime image

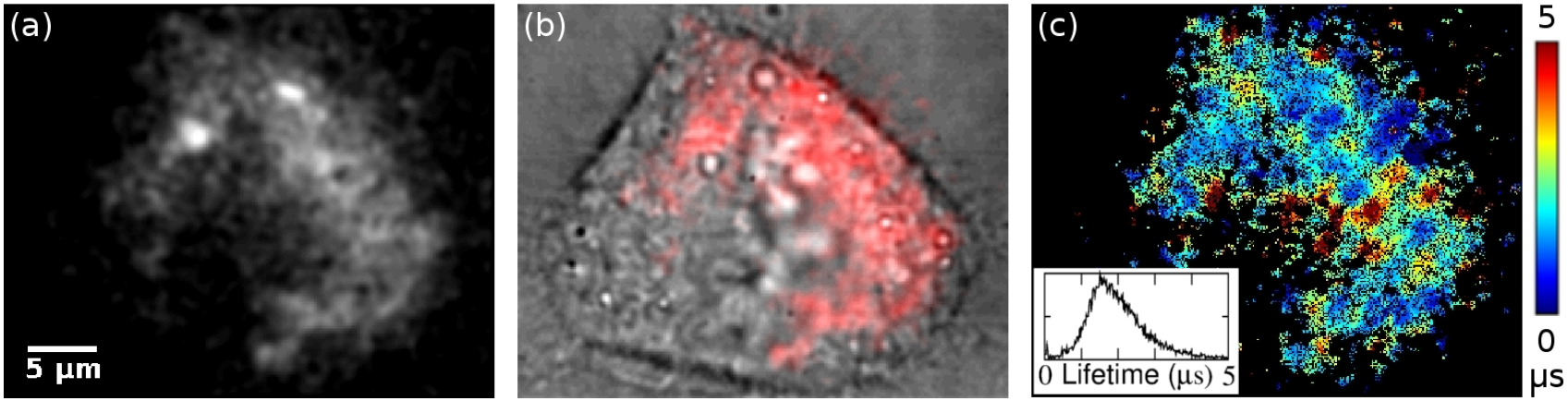


Lifetime histogram

Ruthenium in living HeLa cells

- Ru(dpp) in living HeLa cells
- 54 kHz frame rate, 1.3 s
- Average lifetime from 2-exp fit: 2.7 μs (+ fast component 0.1 μs)
  $\rightarrow$ Partial protection from quenching by molecular oxygen

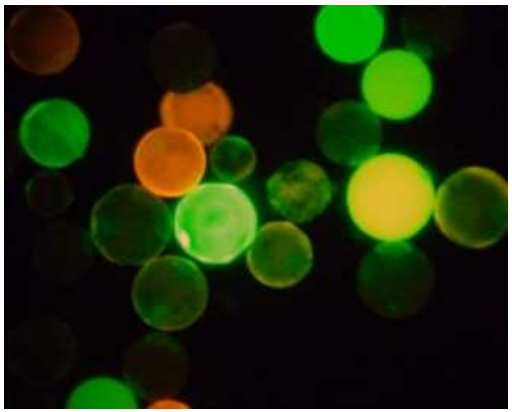




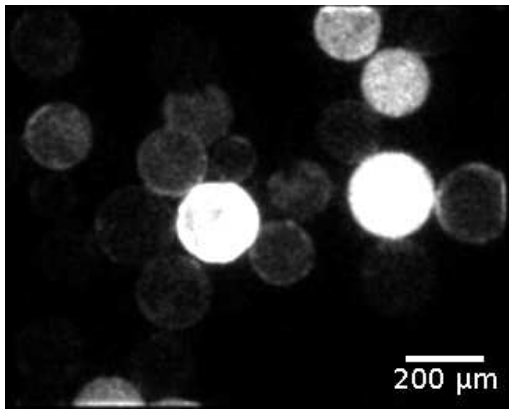
Ru intensity Ru (red) + transmission (grey) Lifetime image

Iridium beads

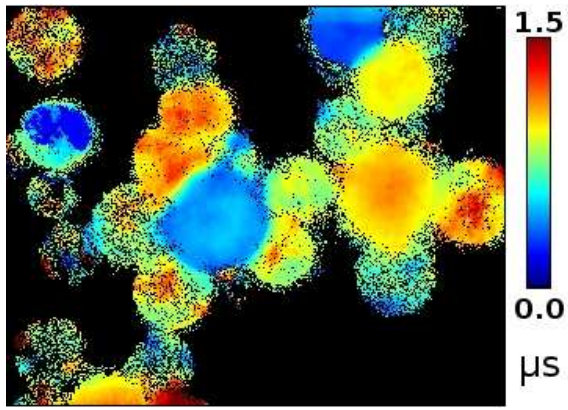
- Beads mix: $Ir(ppy)_3$, Ir(BMes) phos, $Ir(fppy)_3$ phos, Pd(OEP), green fluorescence
- 54 kHz frame rate (18.5 μs frame exposure time)



Colour photo



Intensity image

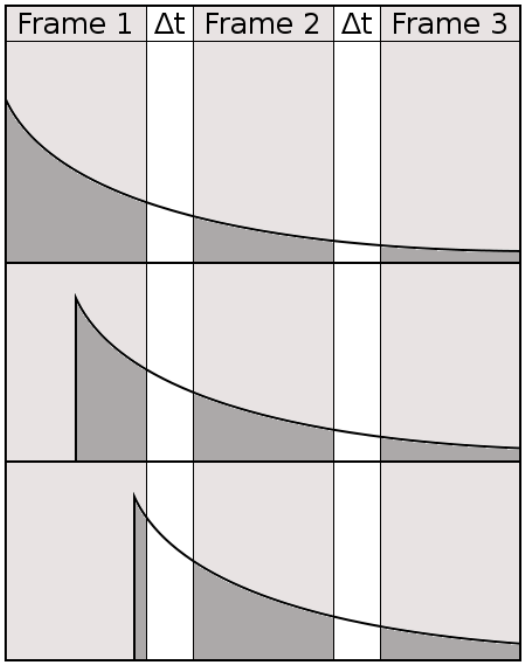


Lifetime image

Summary

- Image intensifier + ultrafast camera allows wide-field photon counting imaging.
- Time-resolution limited by camera frame rate to microseconds.
- Sub-exposure time resolution from image intensifier phosphor decay.
- Demonstrated with 18.5 μs exp time and sub-μs lifetimes (P20 phosphor).

- *Faster phosphor and frame rate for nanosecond time resolution?*



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