HORIBA Scientific

Fluorescence

Graham Hungerford

Real time widefield TCSPC imaging of a model tumour system

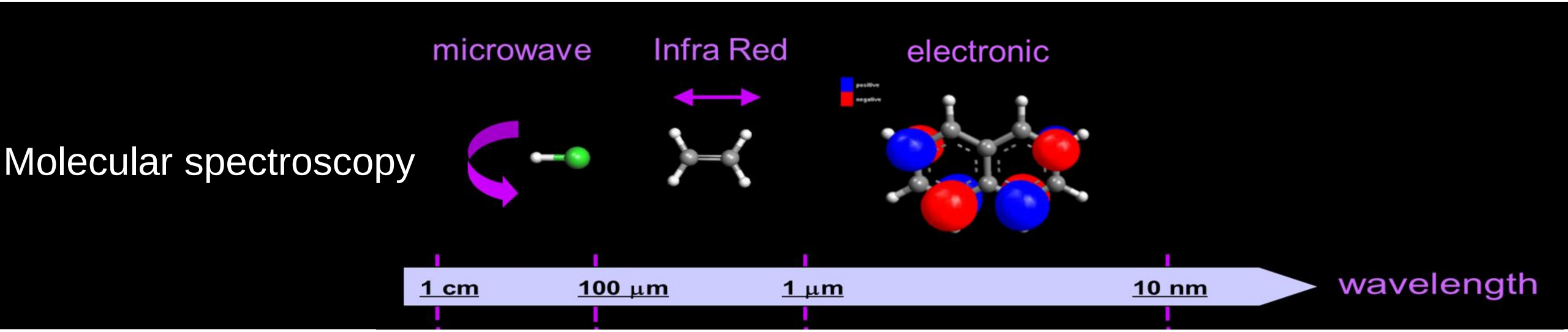
Lasers fighting Cancer
May 2021

Introduction

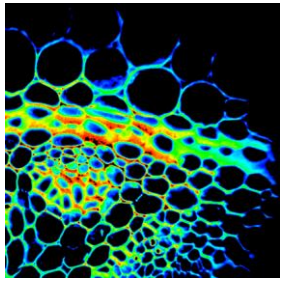
Fluorescence is the emission of light by a substance after previous absorption of light

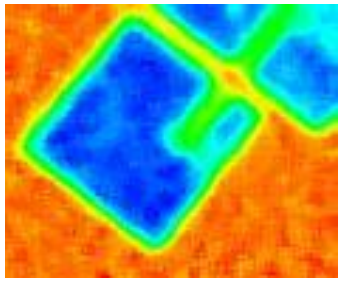
Usually at a longer wavelength (UV-NIR) and occurs on the ps to ns timescale

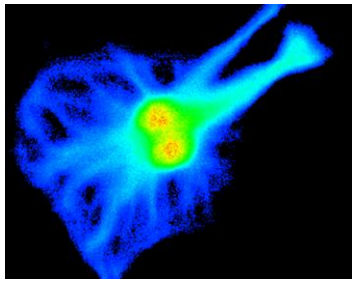
Molecular spectroscopy



What fluoresces?

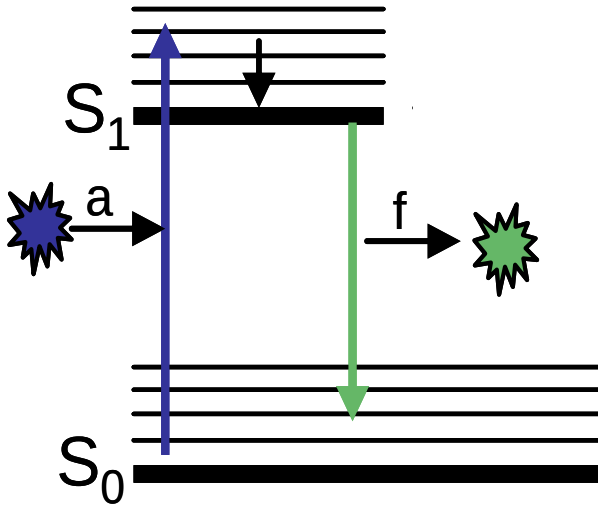




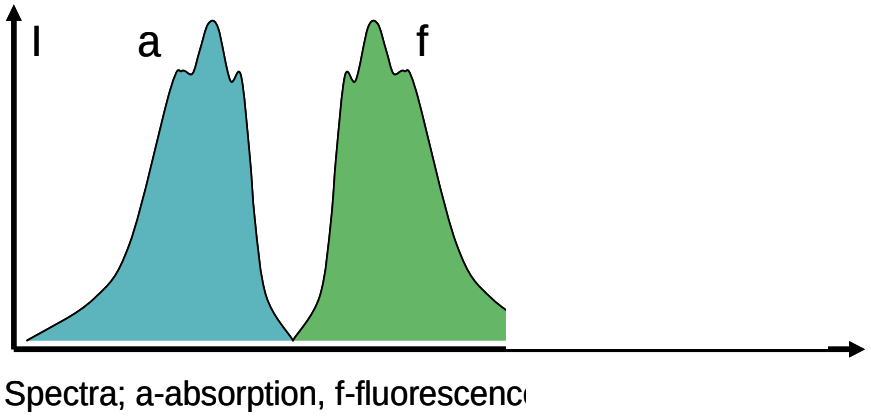


- Biological Sciences
  - proteins, membranes, photosynthesis, drugs, etc…
- Material Sciences
  - semiconductors, photovoltaics, lanthanides, OLEDs, diamonds, etc…
- Chemistry
  - dyes, caging groups, molecular electronics, security inks, etc…
- Food Industry
  - monitor presence of bioactive materials, toxins, microemulsions, etc…

Fluorescence – molecular process

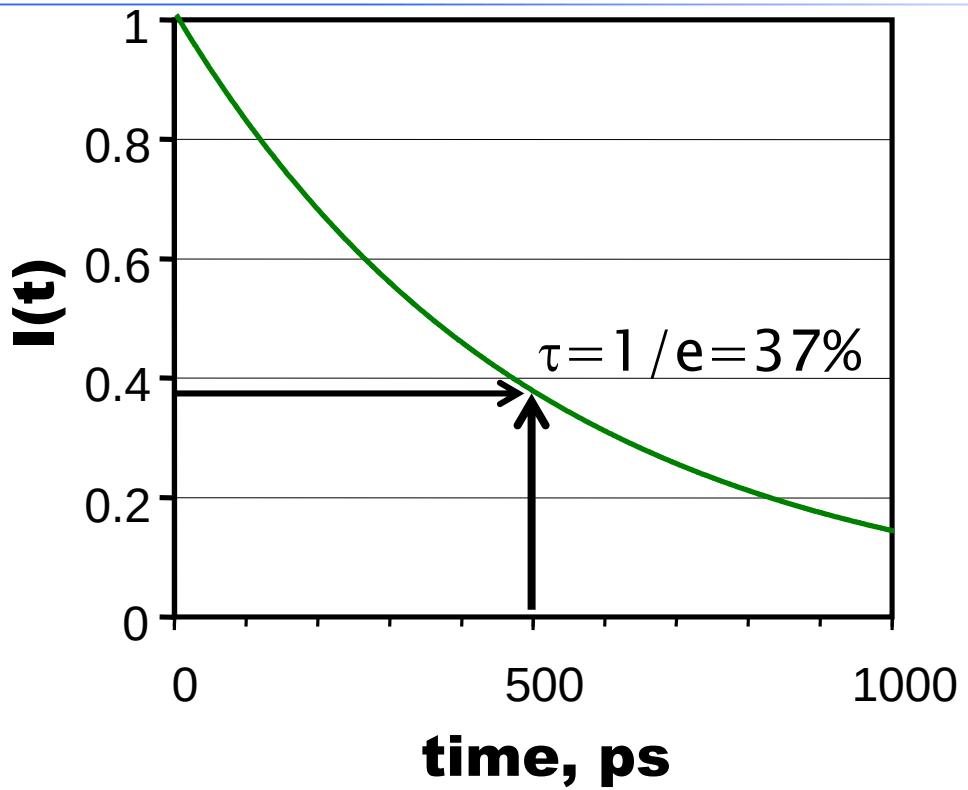


Steady state



Spectra; a-absorption, f-fluorescenc

Lifetime



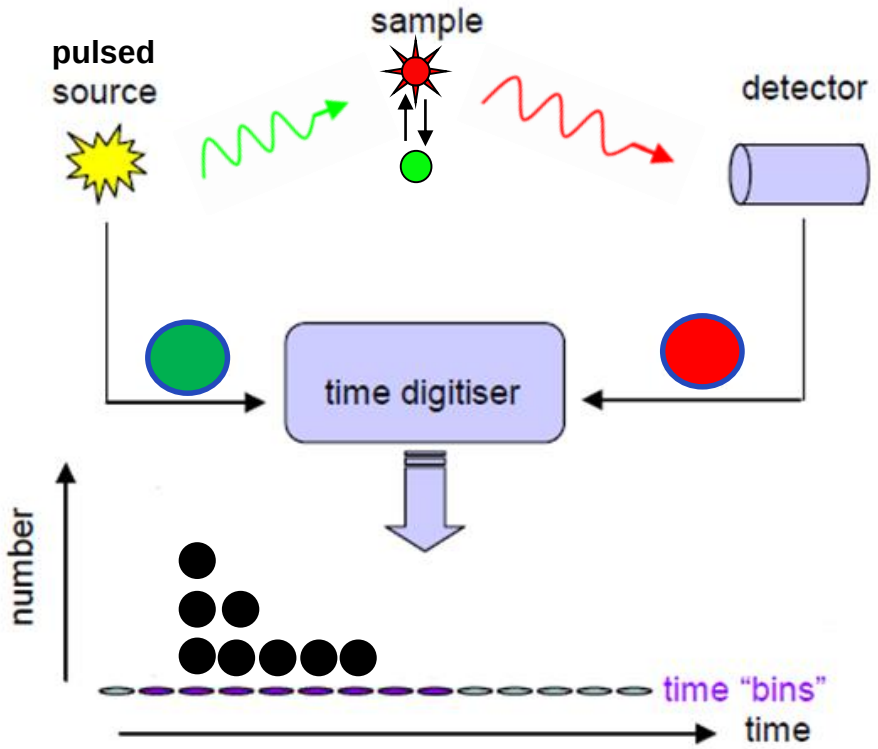
$$I(t) = I_0 \exp(-t/\tau)$$

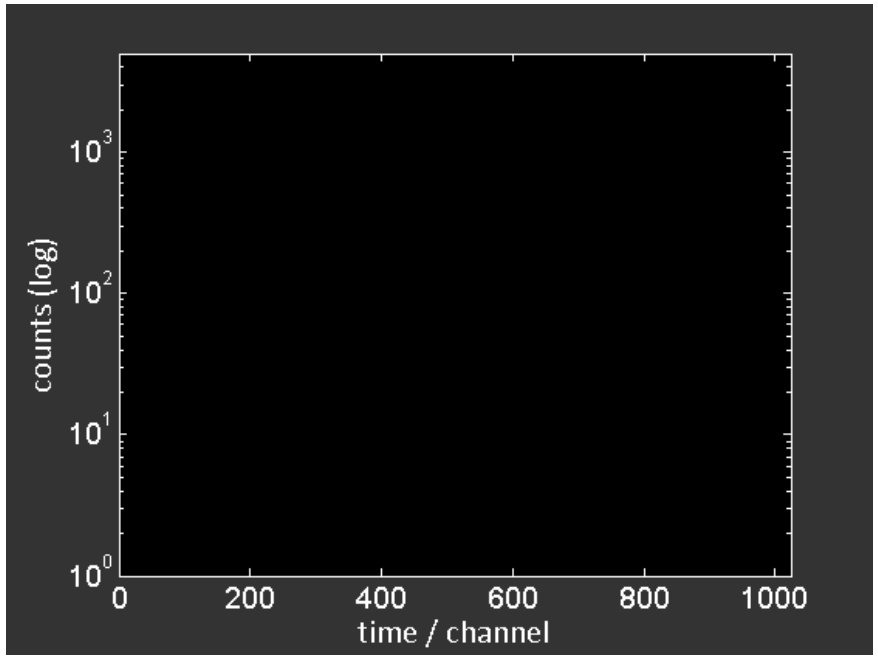
$$\tau = \frac{1}{k_r + k_{nr}}$$

Fluorescence = *f* (*I*, λ_{ex}, λ_{em}, *p*, *x*, *t*)

Fluorescence lifetime measurement

Generic scheme for fluorescence lifetime determination
using Time-Correlated Single-Photon Counting





TCSPC is most sensitive method to obtain fluorescence lifetimes

Typical TCSPC components (HORIBA)

Pulsed (ps) semiconductor and fibre amplified lasers (up to 100MHz, λ ~355nm to 1310nm) – “**DeltaDiode**”

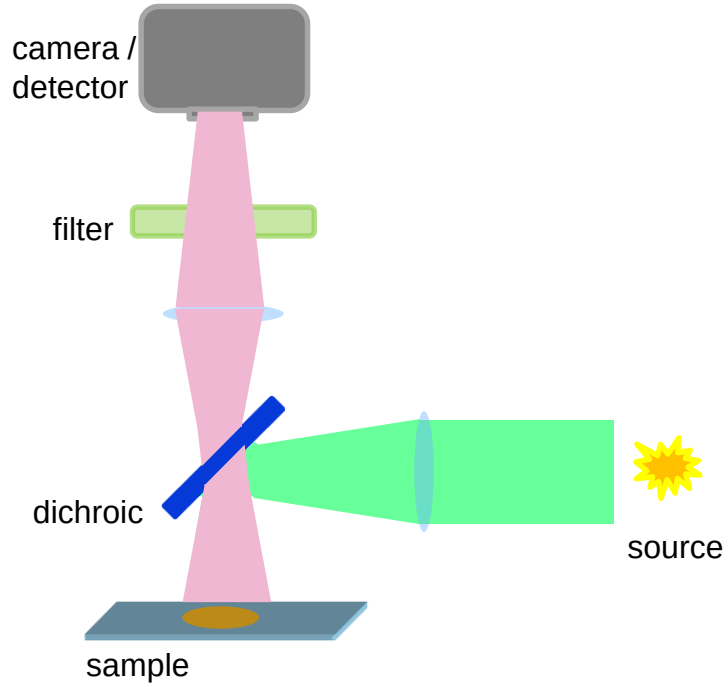
Hybrid detectors and Picosecond detection modules (λ ~230nm to 920nm) – “**HPPD**” & “**PPD**”

Timing electronics (Histograms, Photon streaming -*Time tagging*-) lifetimes from ps to seconds – “**FiPho**”

Fluorescence imaging

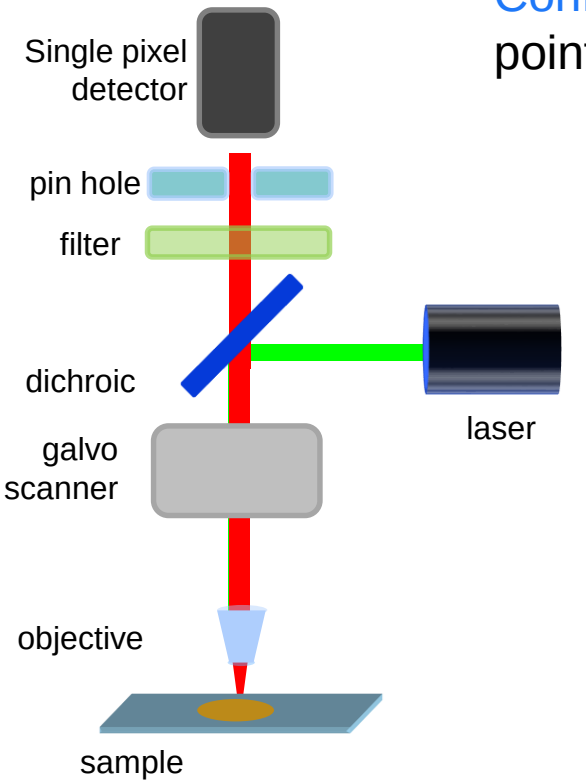
Fluorescence = f (I, λ_{ex}, λ_{em}, p, x, t)

Wide field



+ fast
- out of focus fluorescence

Confocal



+ optical sectioning
- slow

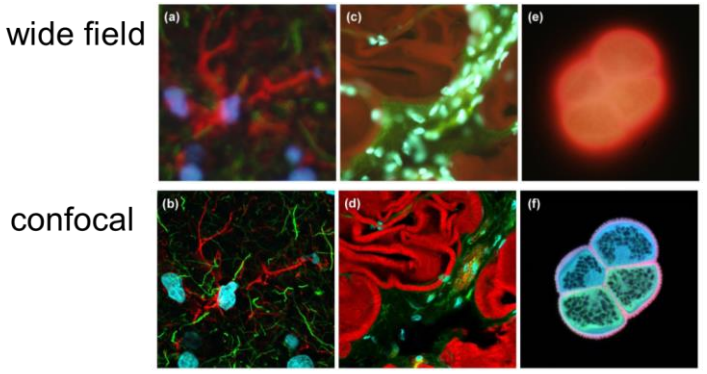
Wide field – whole sample illumination and image collection (camera)
Confocal – laser scanned over sample, point by point data collection (detector)

Resolution (point spread function)
confocal psf = wide field psf^2

$$\Delta r = 0.44\lambda / NA$$

$$\Delta r = 0.61\lambda / NA$$

Radial resolution



from N. Claxton et al confocal microscopy

Fluorescence lifetime imaging (FLIM)

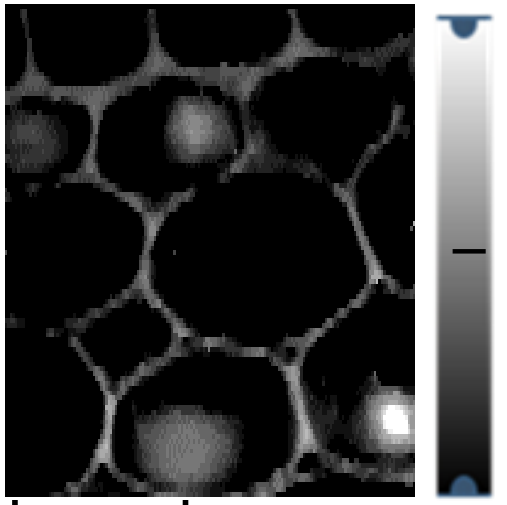
Multiparameter signal

Fluorescence = $f(I, \lambda_{ex}, \lambda_{em}, p, x, t)$

The use of the fluorescence lifetime advantageous;

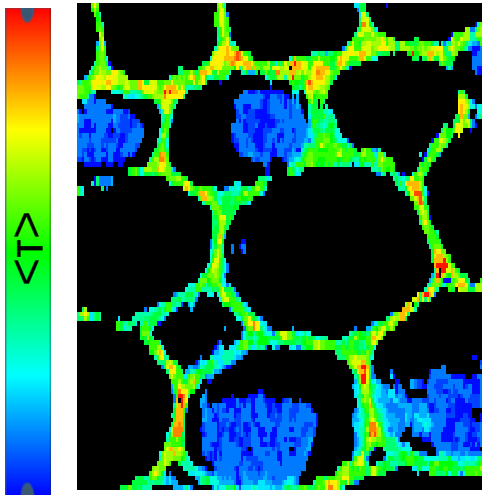
- Absolute measure
  (concentration independent)
- Intensity is a relative measure
  (affected by photobleaching)
- Lifetimes measured using TCSPC
  (most sensitive method for lifetime determination)

Means to provide extra image contrast and elucidate interactions



intensity

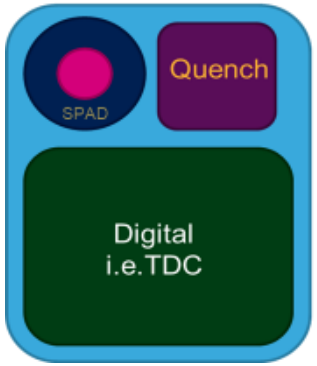
lifetime



Flimera TCSPC camera (detection & timing)

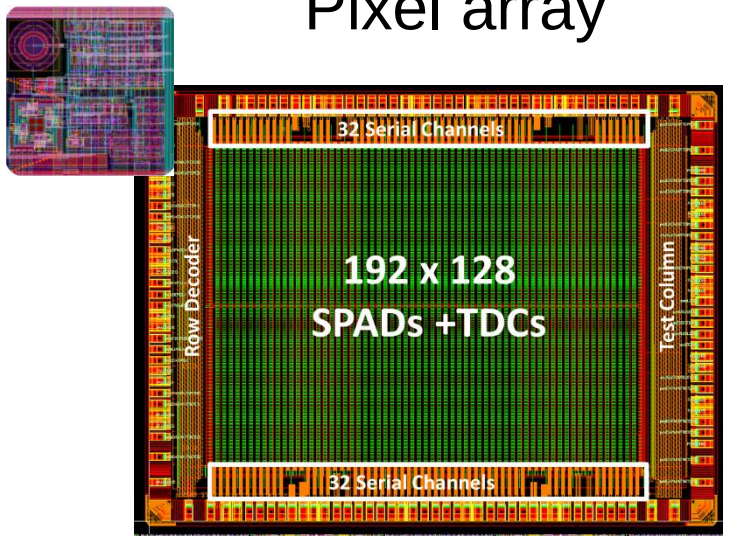
Business Innovation Award 2019
IOP Institute of Physics

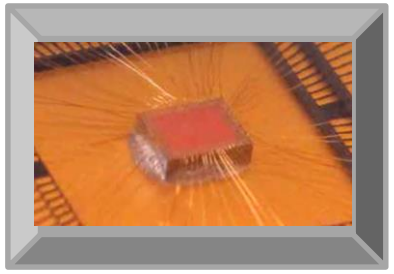
Pixel

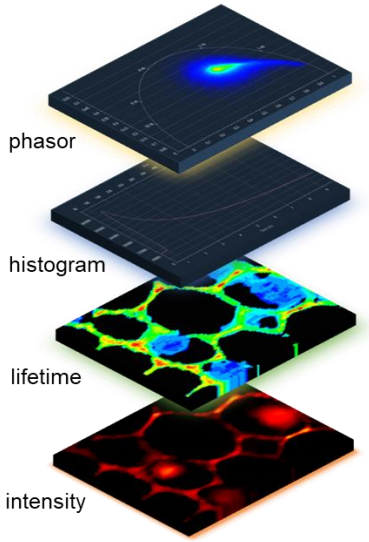


Pixel ~ 18µm x 9µm

Pixel array







In-pixel photon detection and timing!

Each of the 24,576 pixels has a detector and timing
→ parallel TCSPC acquisition (widefield FLIM)

EZ TIME Image FLIM software

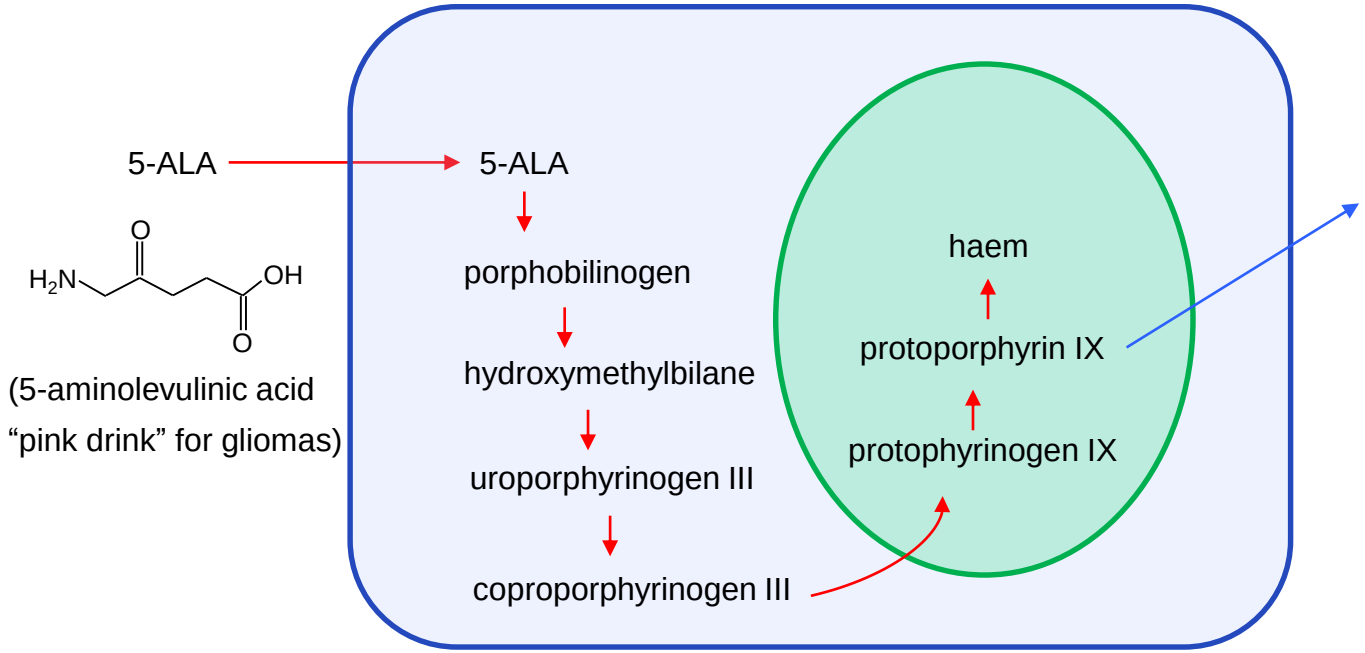
"Fluorescence Lifetime Acquisition by Simultaneous Histogramming"
(FLASH – FLIM)

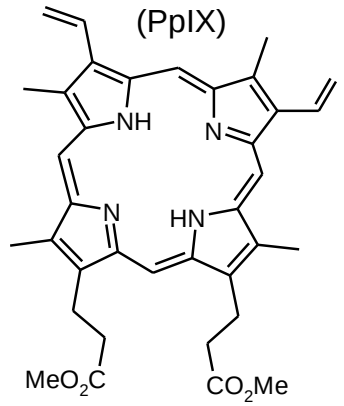
R.K. Henderson, N. Johnston, H. Chen, D.D.-U. Li, G. Hungerford, R. Hirsch, D. McLoskey, P. Yip and D.J.S. Birch. IEEE J. Solid-State Circuits. **2019**, 54, 1907.

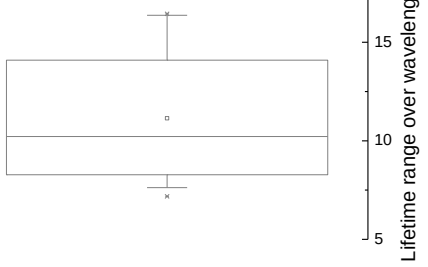
Can fluorescence lifetimes improve tumour margin demarcation?

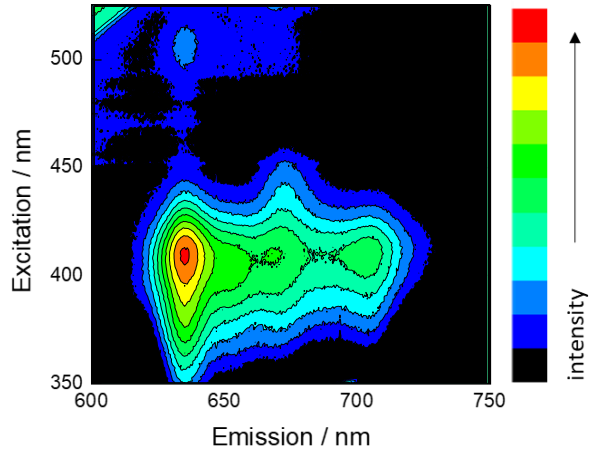
Model system based on Protoporphyrin IX (PpIX)

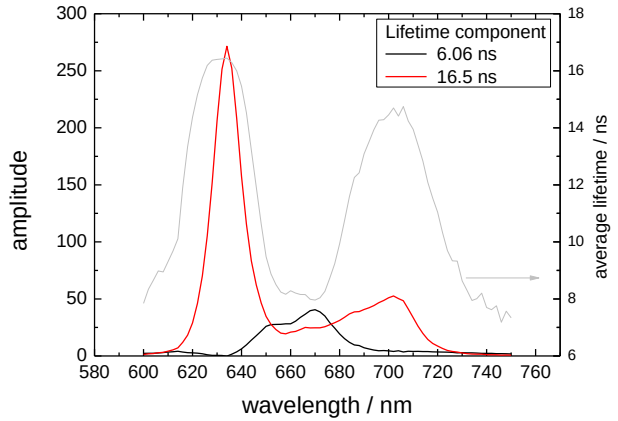
- PpIX is a naturally occurring (haem pathway) fluorophore facilitated by administration of 5-ALA,
  - located in cells & can be used in fluorescence guided surgery & PDT (singlet oxygen)
- Gellan gum (polysaccharide) host
- Intralipid (fat emulsion; droplet size 25-625 nm), optical scatter







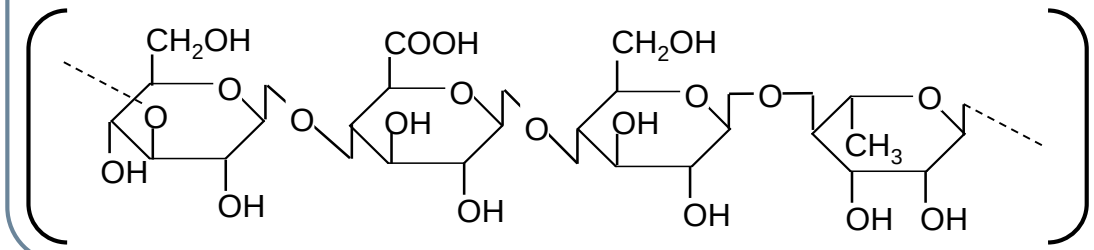


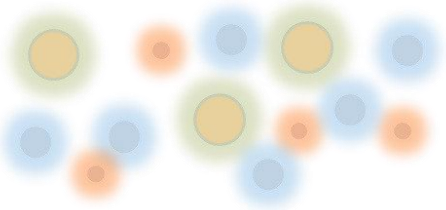


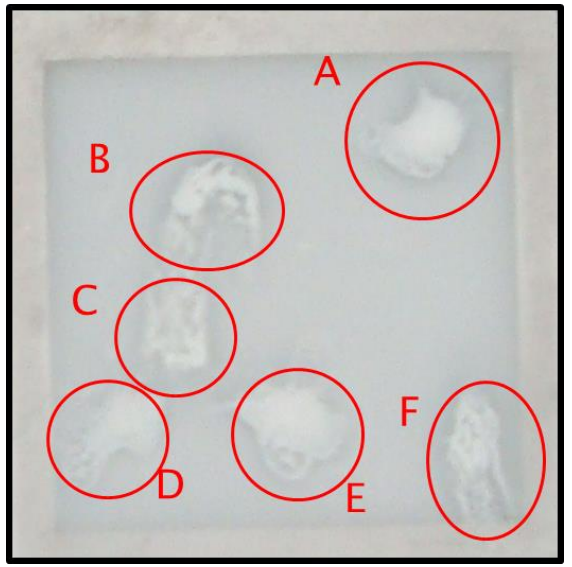
Can fluorescence lifetimes improve tumour margin demarcation?

Model system based on Protoporphyrin IX (PpIX)

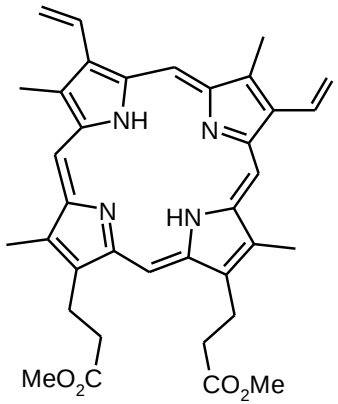
Construct matrix – Gellan gum + intralipid

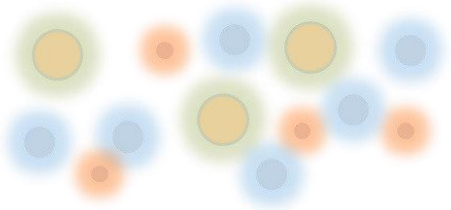






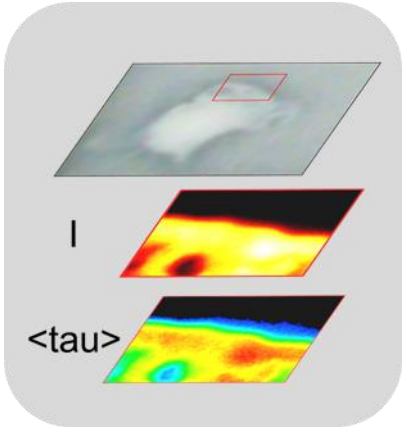
“tumour” mimic
–PpIX+ intralipid

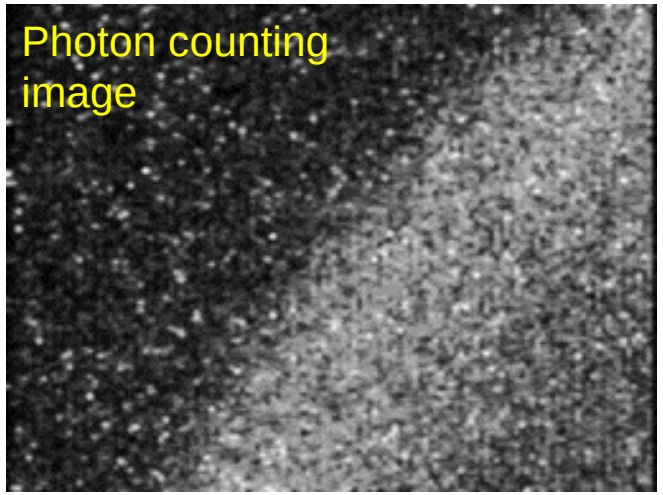


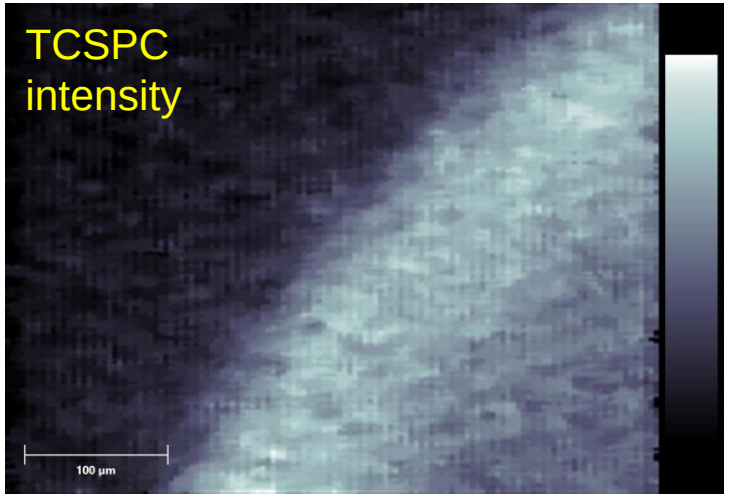


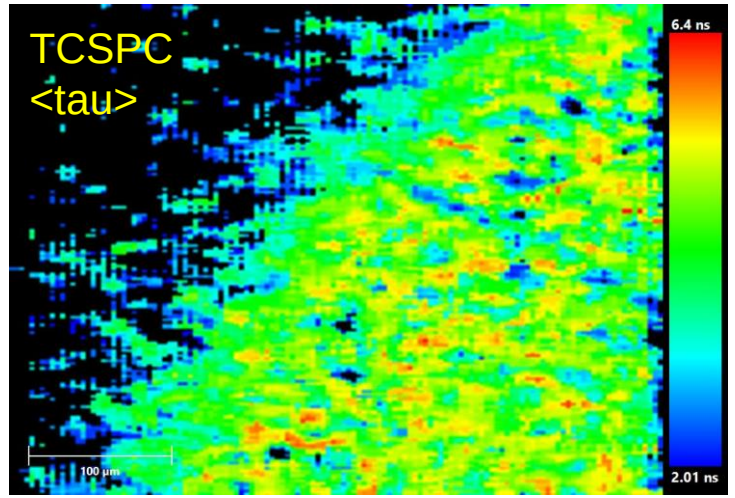
Can fluorescence lifetimes improve tumour margin demarcation?

FLASH – FLIM





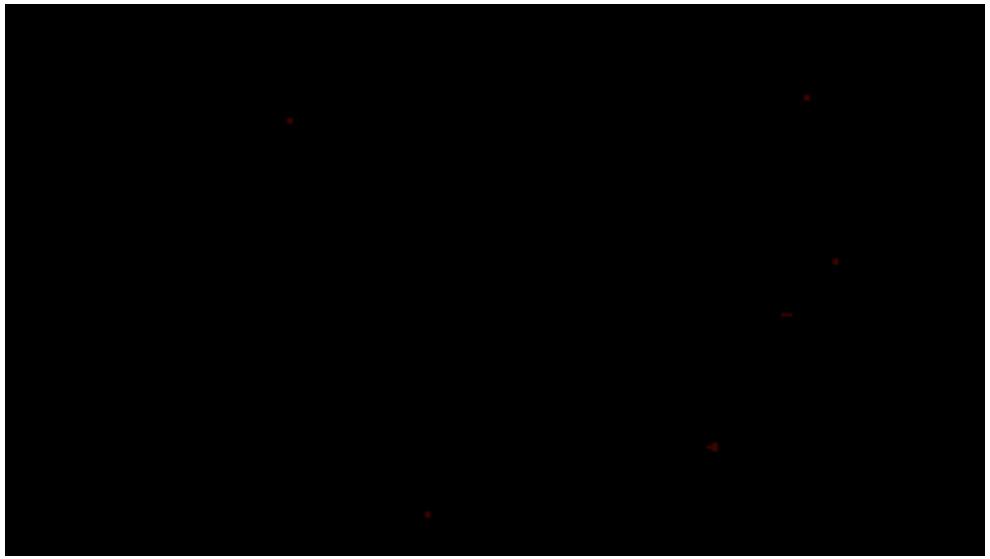


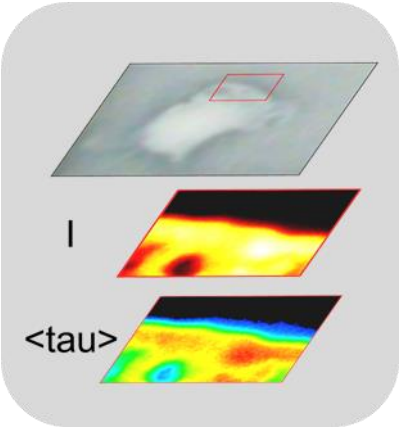


Acquisition time 1 second

Can fluorescence lifetimes improve tumour margin demarcation?

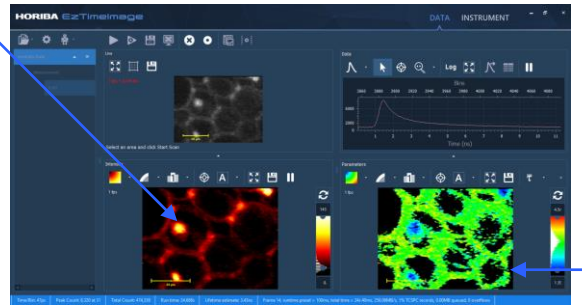
FLASH – FLIM 6fps traversing PpIX inclusion





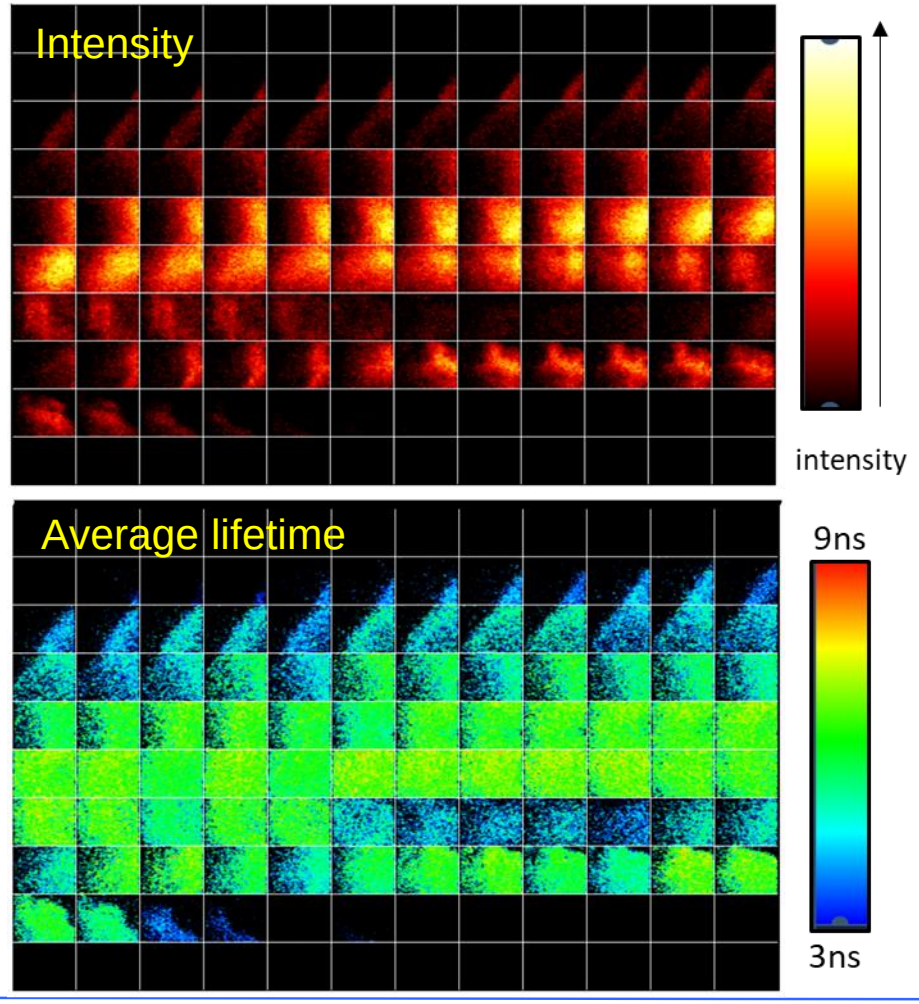
intensity

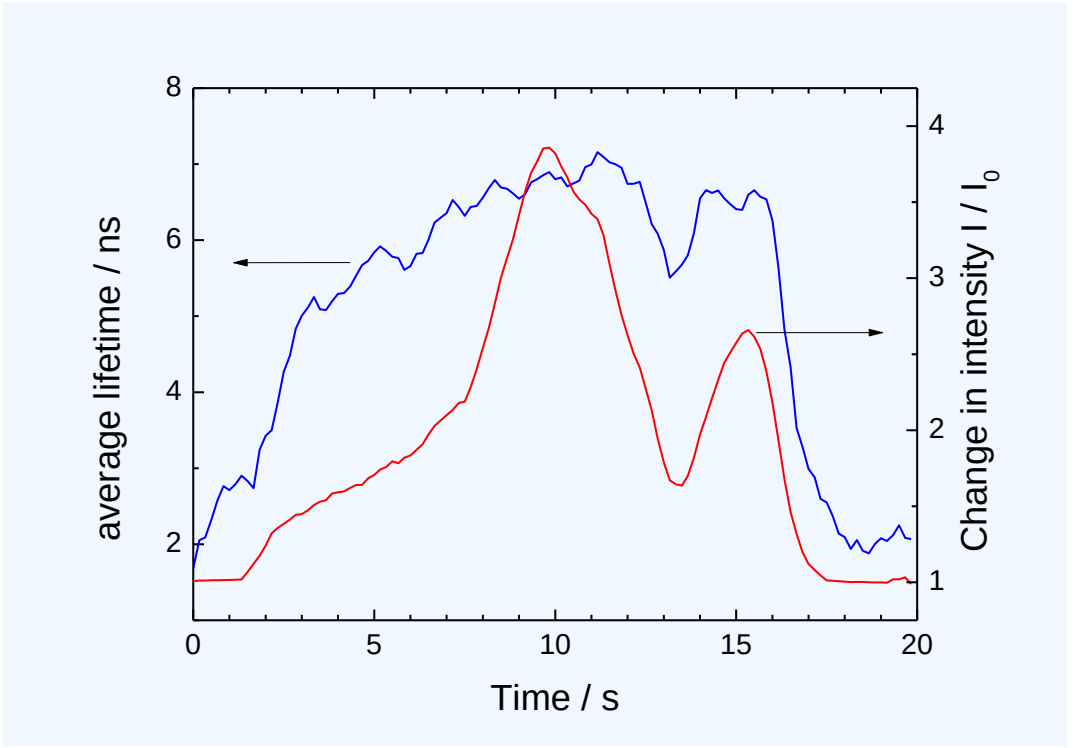
lifetime



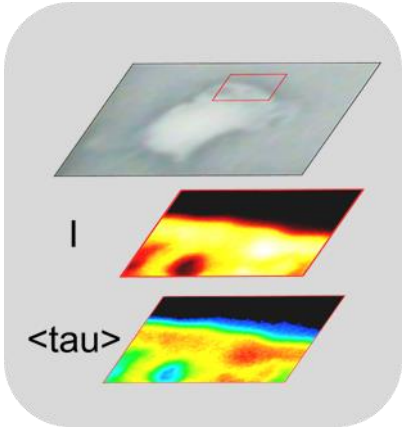
Can fluorescence lifetimes improve tumour margin demarcation?

FLASH – FLIM 6fps





Change in TCSPC parameters



K. Sagoo, N. Cumberbatch, A. Holland and G. Hungerford, **2021**. Methods Appl. Fluoresc. 9, 015002.

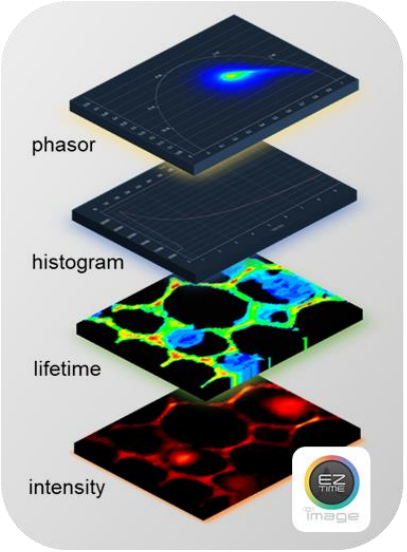
Summary

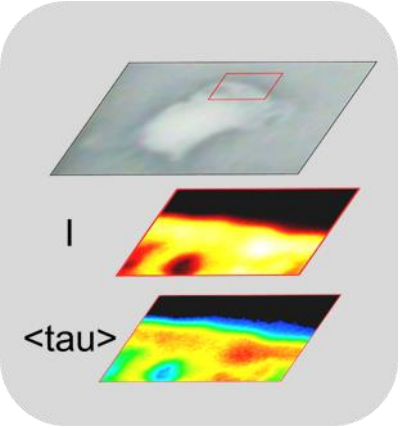
Fast (~video rate) FLIM achievable with a CMOS based SPAD array in real time

Uses the sensitivity of TCSPC

Complementary to confocal techniques

Use of fluorescence lifetime shows good potential for tumour demarcation





Thank you

감사합니다

Cảm ơn

ありがとうございました

Dziękuję

धन्यवाद

Grazie

Merci

谢谢

நன்ற

ขอบคุณครับ

Obrigado

Σας ευχαριστούμε

شُكْرا

Tack ska ni ha

Большое спасибо

Danke

Gracias