Emerging Clinical Applications of Fluorescence Using SPADs

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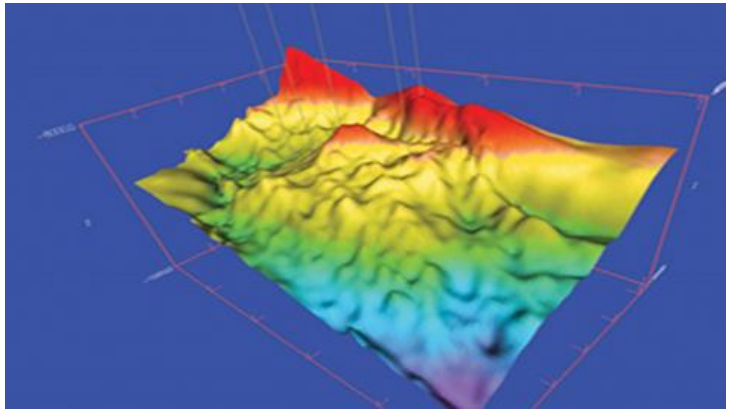
Visiting Professor of Applied Physics, ČVUT

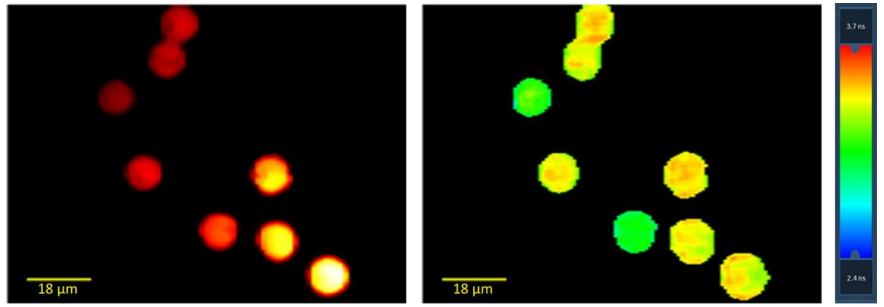
Summary of talk

1. **Introduction to fluorescence & its:**



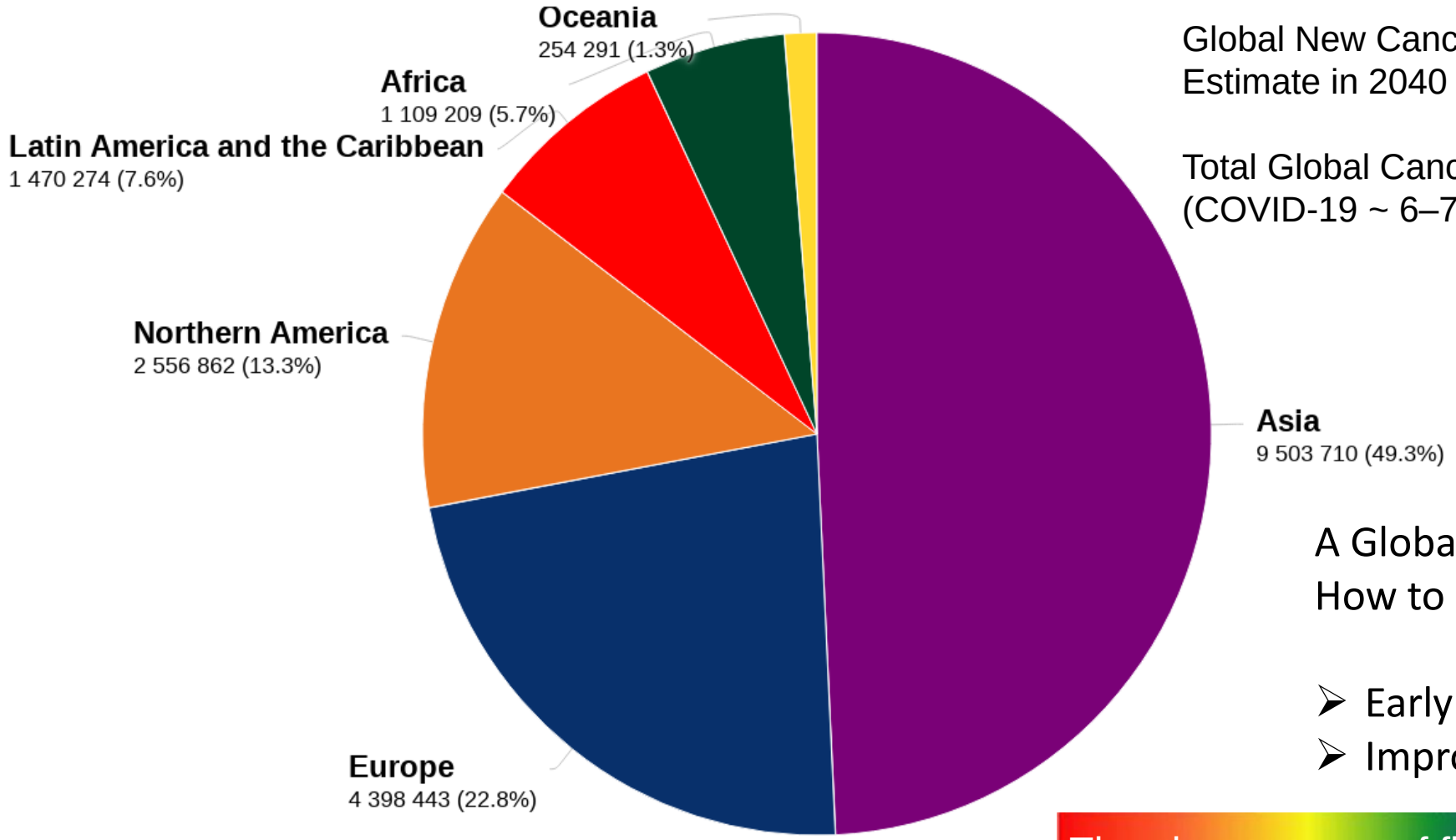
- Multidimensional fingerprint
- Lifetime
- Imaging using SPAD FLIM

2. **Emerging SPAD Applications to Cancer**



- Liquid biopsy screening for biomarkers
- Intraoperative guided surgery

Estimated number of new cases in 2020, all cancers, both sexes, all ages



Global New Cancer Cases in 2020 ~ 19m
Estimate in 2040 29m

Total Global Cancer deaths in 2020 ~ 10m
(COVID-19 ~ 6–7m to date)

A Global problem!
How to best manage?

- Early diagnosis
- Improved treatments

The phenomenon of fluorescence offers a wide spectrum of NEW opportunities

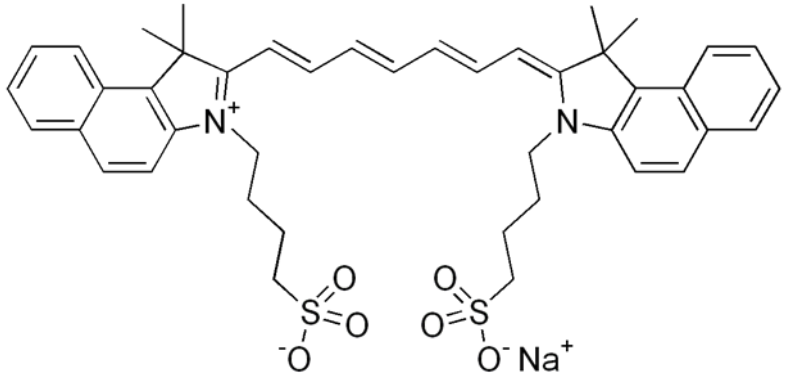
Data source: Globocan 2020
Graph production: Global Cancer Observatory (http://gco.iarc.fr)

International Agency for Research on Cancer
World Health Organization

Fluorescence

- The emission of light of one colour following absorption of light of a different colour

Dye fluorescence



Indocyanine green (ICG)

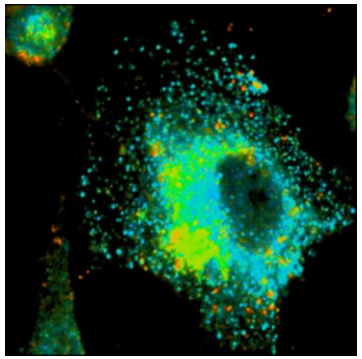
- <u>Not selective absorption and reflection</u> which gives rise to all the colours we usually see

Why is fluorescence so widely used across many molecular disciplines ?

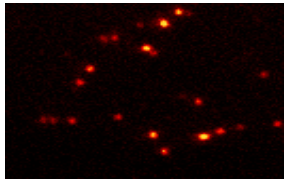
- Its colour & intensity are influenced by changes in molecular interactions
- It is a spy on a secret molecular world that we cannot see

– including the biological world within ourselves

- just like a spy we can control it to go where we want & do what we want



- & what's more - Everything fluoresces under the right conditions!
- Even single molecule sensitivity -

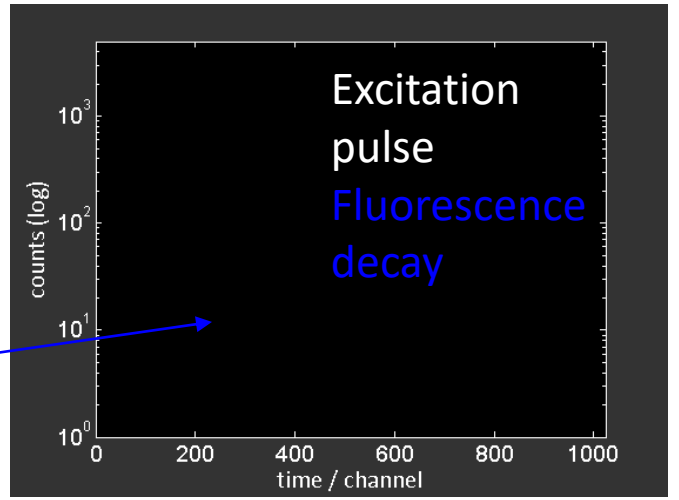


- Fluorescence lifetime (~ ns) provides complementary contrast information to intensity –
  - important at the higher data rates SPADs offer microscopy & imaging

Fluorescence lifetime τ_f

$$i(t) = i(0)exp-(t/\tau_f)$$

$$Log_e\,[i(t)/i(0)] = -t/\tau_f$$



Time Correlated Single Photon Counting

- **Comparison with fluorescence intensity:**
- Fluorescence intensity is a difficult and inaccurate measurement
  - depends on constant excitation intensity & dye concentration

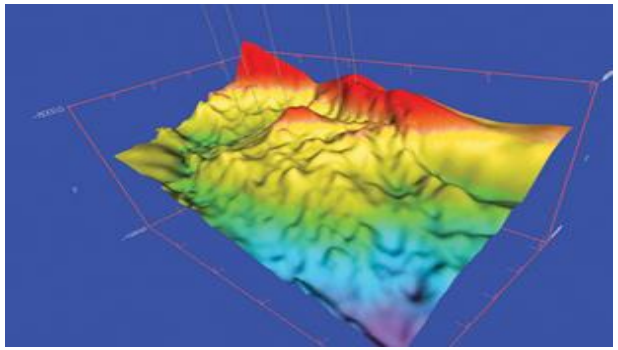
  whereas τ_f is:
- Independent of intensity & dye concentration – overcomes dye bleaching
- Easier measurement to calibrate (ns), accurate & absolute
- Can discriminate against background fluorescence, scattered excitation etc
- Unique to a fluorescent molecule <u>& its environment</u>

Fluorescence is a multidimensional fingerprint/contour – BUT at present we only access small slices

SPADs offer the best opportunity to SIMULTANEOUSLY access more of the contour

& parameters can be combined e.g. FLIM

Fluorescence Lifetime Imaging Microscopy



$$\text{Fluorescence} = f(I, \lambda_{exc}, \lambda_{em}, \bar{p}, \bar{r}, t)$$

Measurands

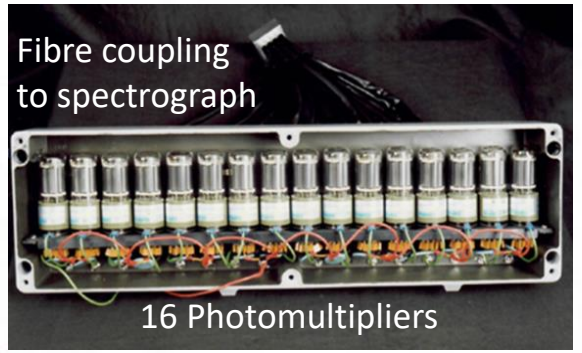
I = intensity	$\Rightarrow$ Quantum yield
λ_{exc} = excitation wavelength	$\Rightarrow$ Absorption spectrum
λ_{em} = emission wavelength	$\Rightarrow$ Fluorescence spectrum
$\bar{p}$ = polarisation	$\Rightarrow$ Fluorescence anisotropy
$\bar{r}$ = position	$\Rightarrow$ Fluorescence microscopy
t = time	$\Rightarrow$ Fluorescence decay lifetime

16 Channel TCSPC Multiplexing

Multiplexed single-photon counting 1:
A time-correlated fluorescence lifetime camera.
D McLoskey, D J S Birch, A Sanderson, K Suhling, E Welch & P J Hicks,
Rev. Sci. Instrum. 67, 2228-37, 1996

History of IBH products in
Fluorescence in Industry,
Springer Series on Fluorescence,
Vol 18, Ch. 3, 103, 2019 (Ed. B Pedras)

Limit to number of detectors

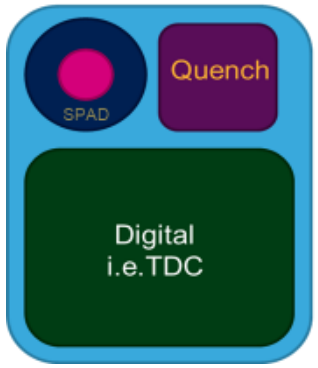


Model 5000W SAFE - Simultaneous polarization measurements

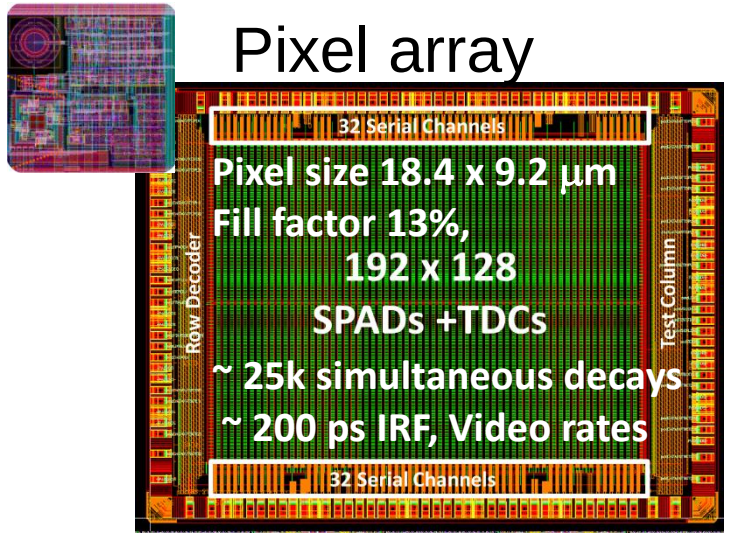
Multiplexed Wavelength Array J.Phys. E. 1988, Chem. Phys. Letts. 1988

The way forward!! The *SPEED* of TCSPC SPAD arrays

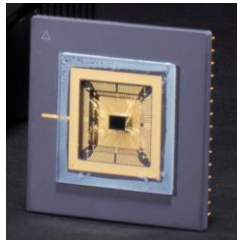
Pixel



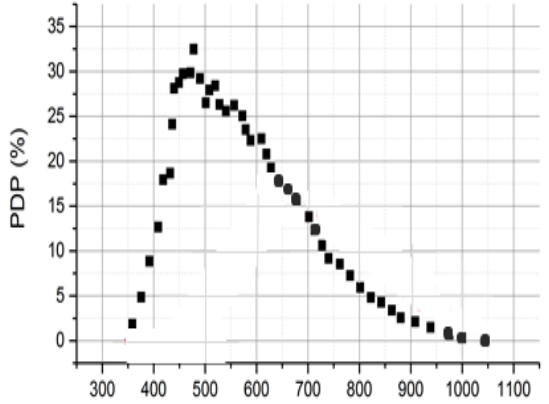
40 nm CMOS Pixel array



Quanticam Chip ⇨ FLIMera™ TCSPC camera



λ response



A 192×128 Time Correlated SPAD Image Sensor in 40-nm CMOS Technology.

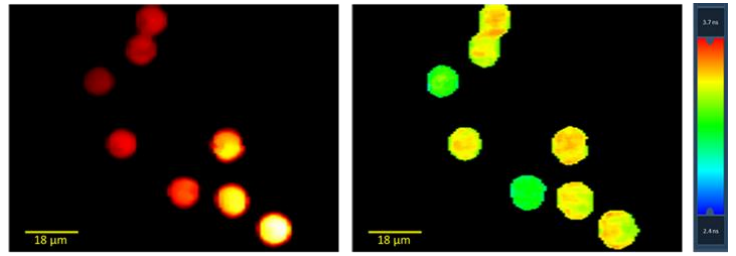
R K Henderson et. al. IEEE J Solid-State Circuits. 54, 1907-1916, 2019.

But SPADs are not without their limitations for fluorescence:

Limitations less important for microscopy & imaging

- Poor UV response
- Wavelength dependent temporal response limits fl. lifetime resolution
- Small area (~ 100 µm^2) – lower sensitivity than photomultipliers

SPAD FLIM Cancer Applications
- still in preclinical development



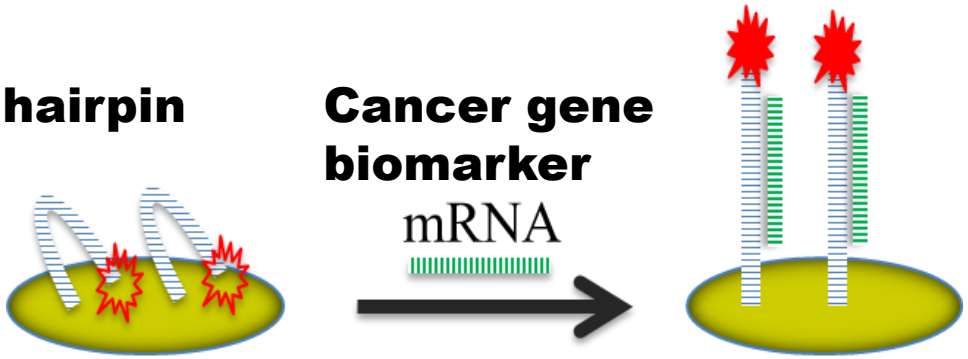
1. Liquid biopsy screening – to replace tissue biopsy
   early detection increases survival rates
   - need to detect down to 1 circulating tumour cell/ml
   & 1000s cells/sec

2. Intraoperative guided surgery –
   to improve tumour margin estimation
   - need 1 mm precision

1. Liquid biopsy screening:
Cancer-specific Intracellular Gold Nanoprobe

Cancer-specific DNA hairpin with dye attached

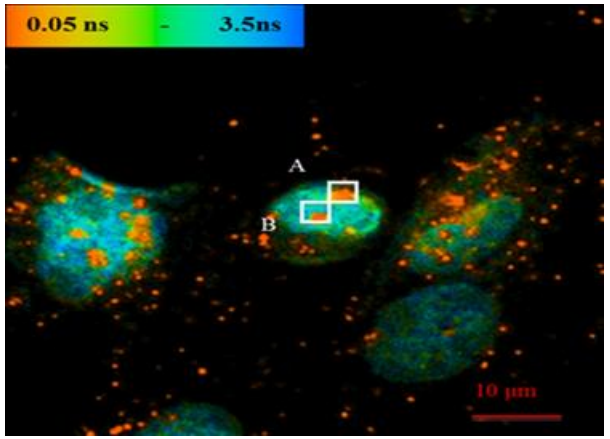
Cancer gene biomarker



DNA-mRNA binding releases hairpin & removes quenching

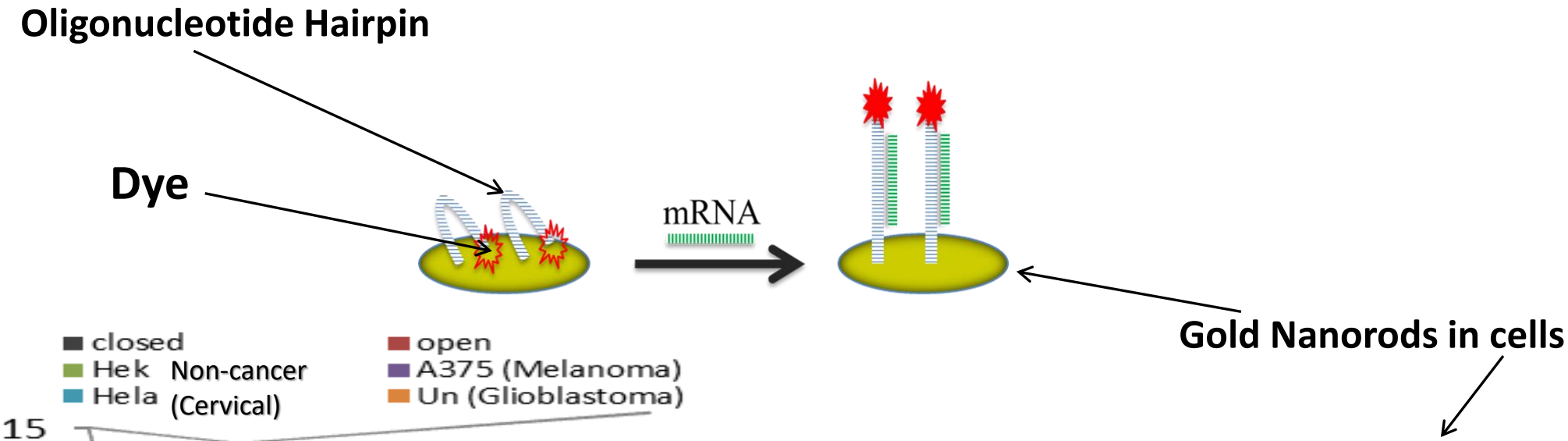
Fluorescence intensity & lifetime increase

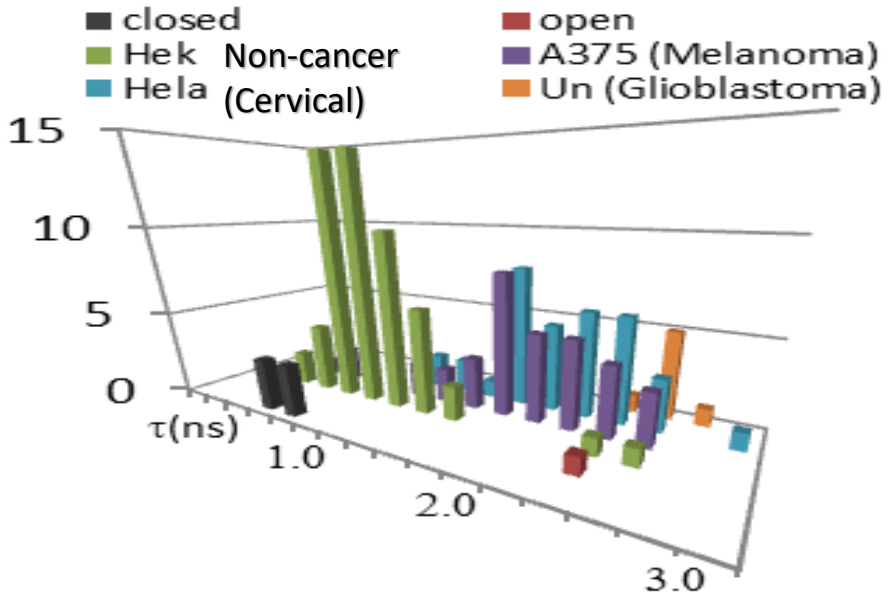
Gold nanorod (~ 10x50 nm)
Plasmonic quenching of fluorescence

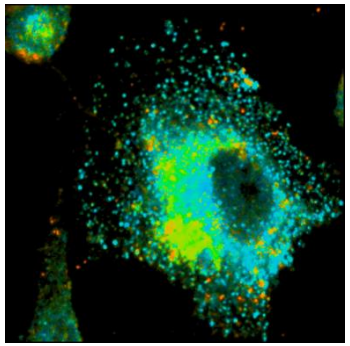


FLIM image of 2-photon excited gold nanorods in cells

Hairpin-dye gold plasmonic quenching removed by hybridisation with cancer gene C-Myc mRNA target





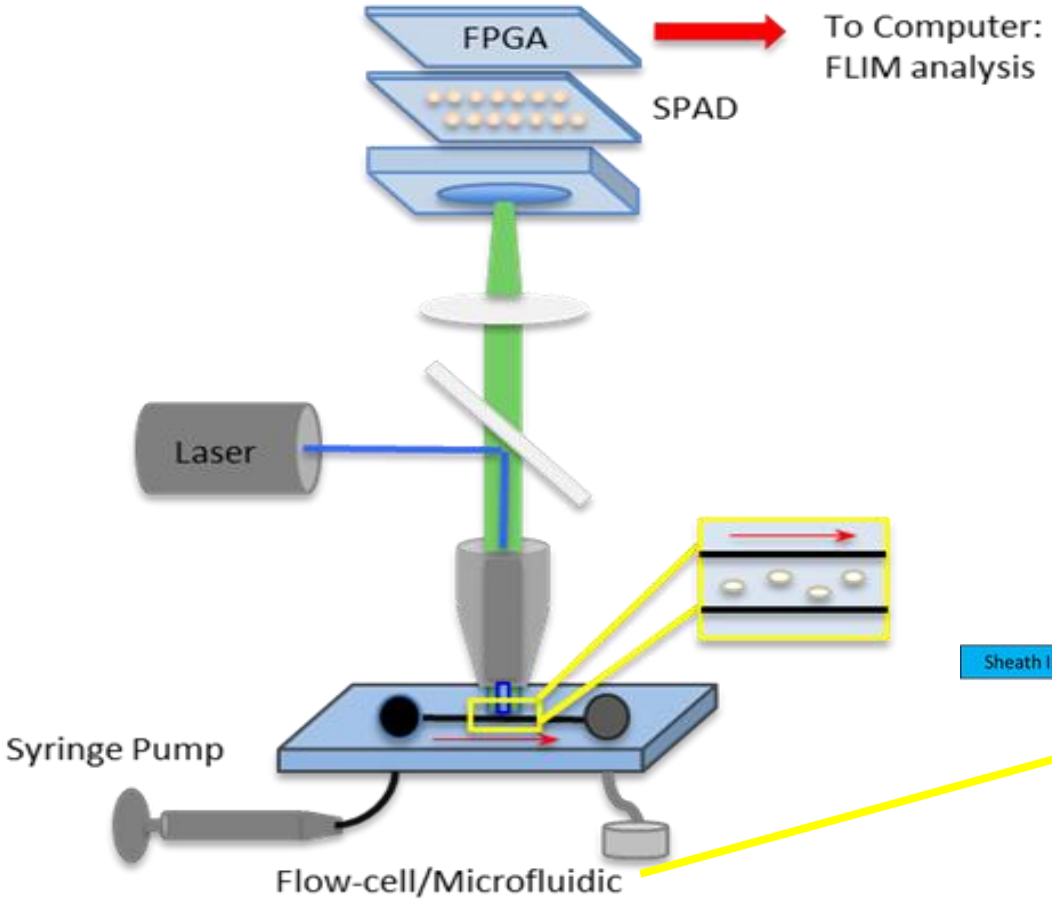


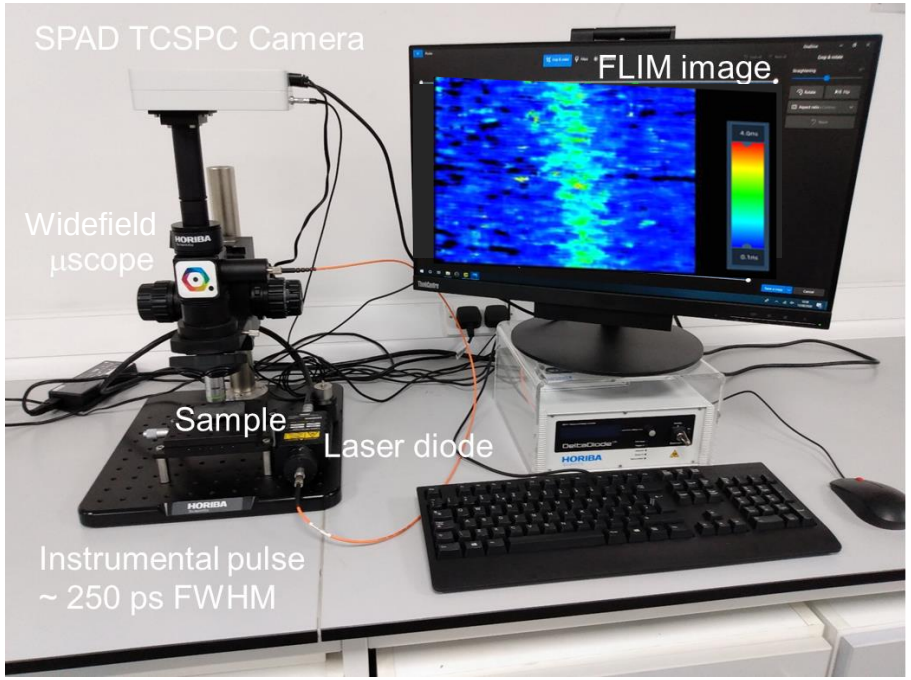
2-photon excited dye stained HeLa cells, incubated with AuNRs for 60mins.

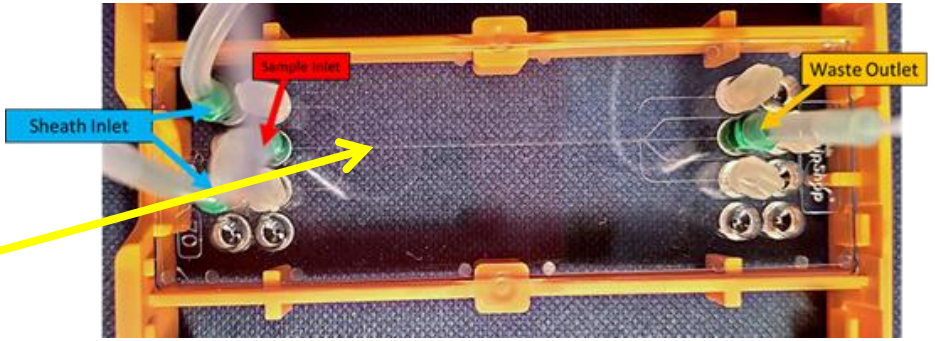
Y. Zhang, G. Wei, J. Yu, D. J. S. Birch and Y. Chen, *Faraday Discussion* 178, 383 (2015)

G. Wei, D. Simionesie, J. Sefcik, J. U. Sutter, Q. Xue, J. Yu, Y. Wang, D. J. S. Birch and Y. Chen, *Opt. Lett.* 40, 5738 (2015)

Cancer screening with liquid biopsy

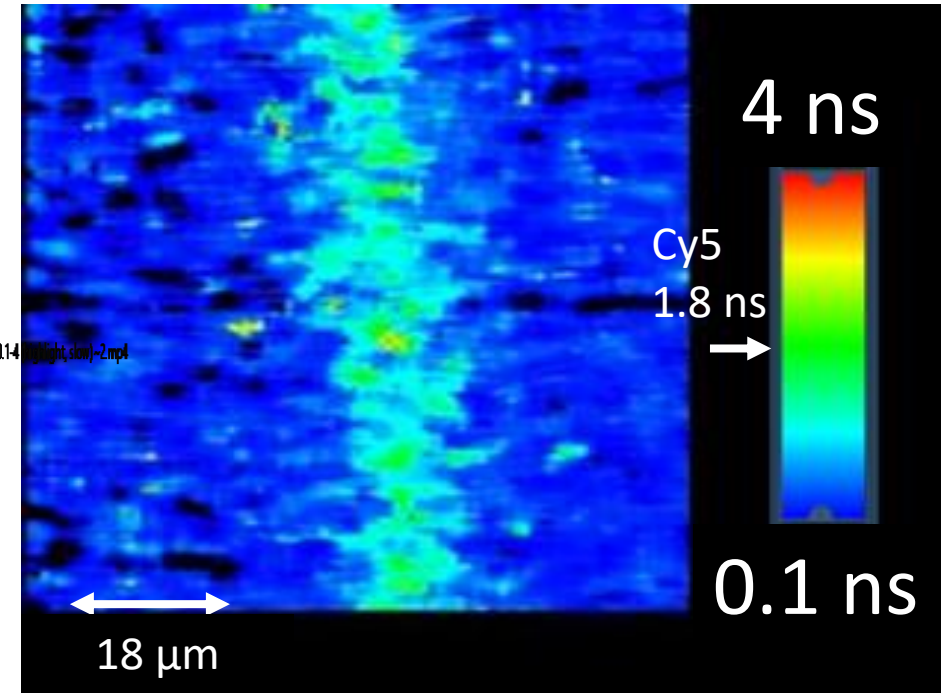


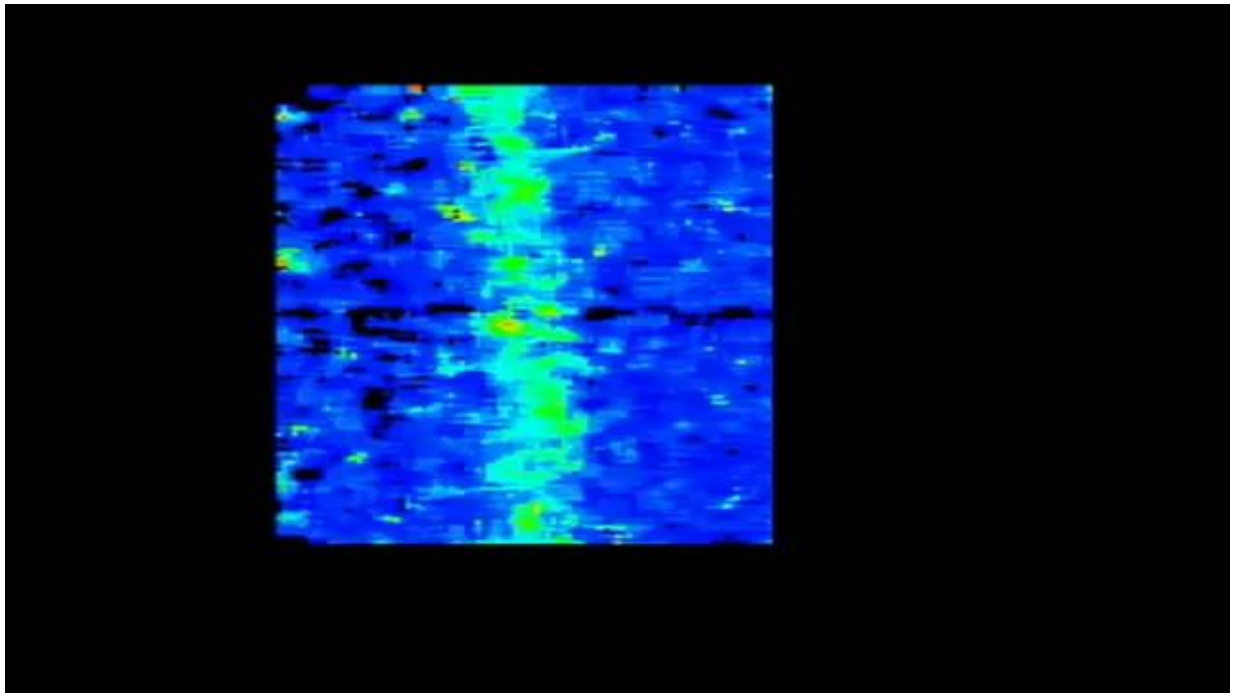




Prototype flow cell

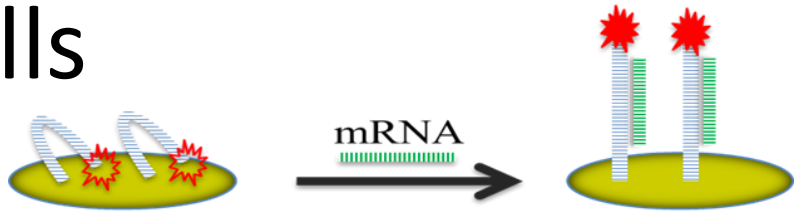
SPAD FLIM Detection of Flowing PC3 Prostate Cancer Cells labelled with C-MYC CY5 Nanoprobe





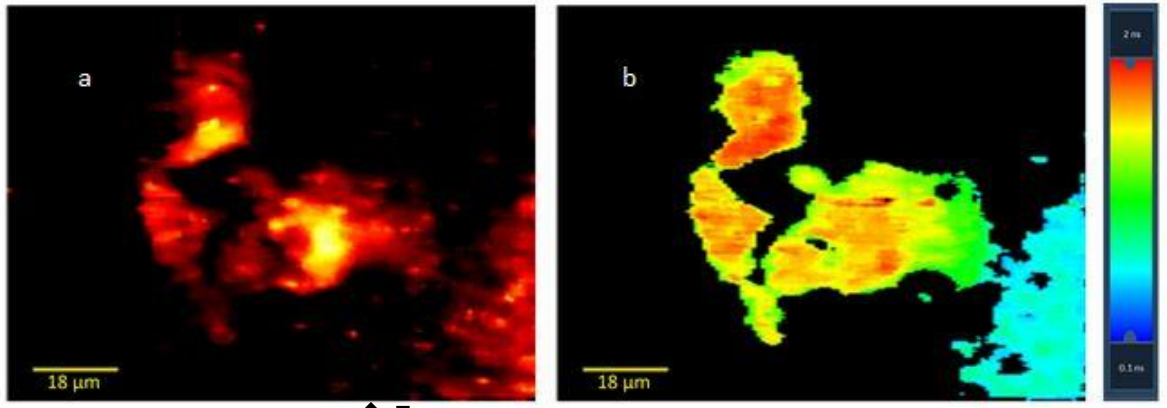
Video rate SPAD array FLIM at 30 frames/sec –
~ 60x faster than conventional single point translational scanning FLIM

SPAD FLIM identifying cancer cells



Intensity image Lifetime Image

PC3 Cells –
Prostate cancer
+ gold nanoprobe



2 ns

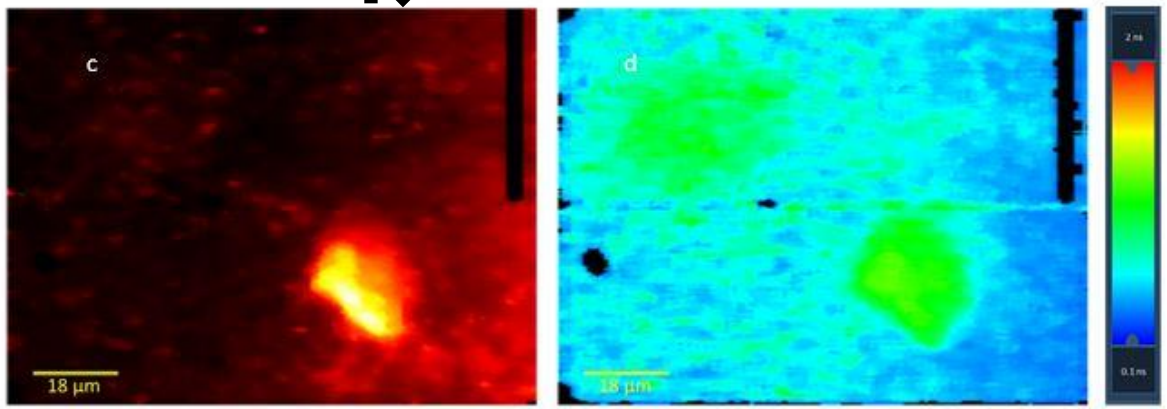
Cy5 dye

τ_f ~ 1.8 ns

0.1 ns

No difference

HeK Cells –
not cancer
+ gold nanoprobe



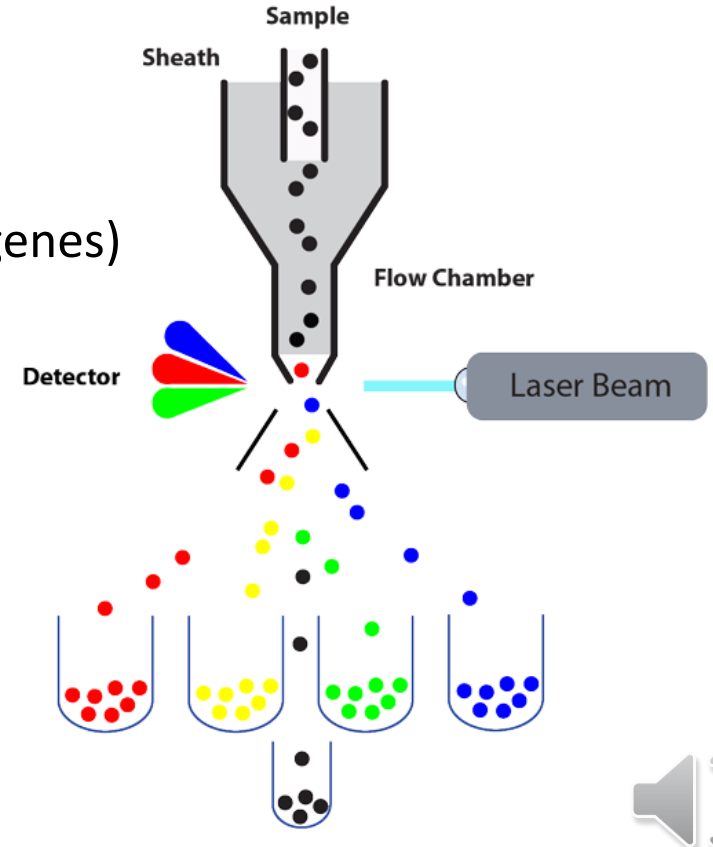
2 ns

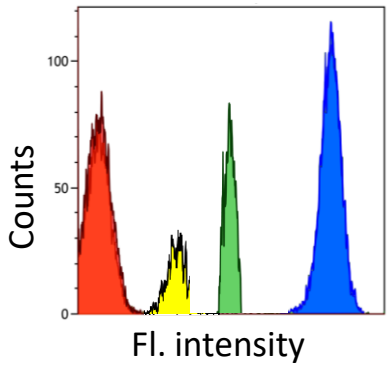
0.1 ns

Liquid Biopsy: Further Opportunities

- Help determine tumour origin, progression & response to chemotherapy
- Be applicable to specific diagnosis of other diseases – e.g. infections, genetic disorders
- Open up further applications in life science research,

  e.g. transcriptomics in drug discovery (analysis of actively expressed genes)
- Combine with fluorescence lifetime activated cell sorting in flow cytometry*

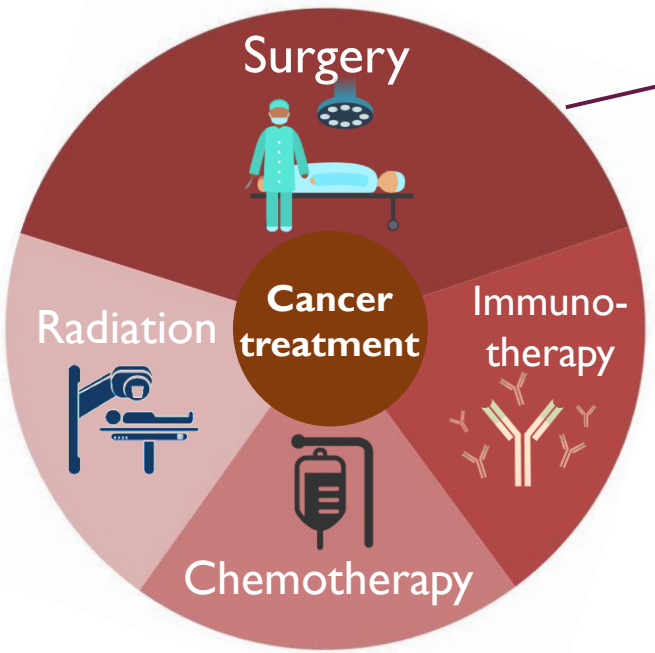




*Houston J.P. et al Cytom. A. **77A**, 861,2010

Nedbal J. et al Cytom. A. **87A**, 104, 2015

2. Intraoperative cancer surgery



Surgery is one of the **primary** treatment options for tumour removal

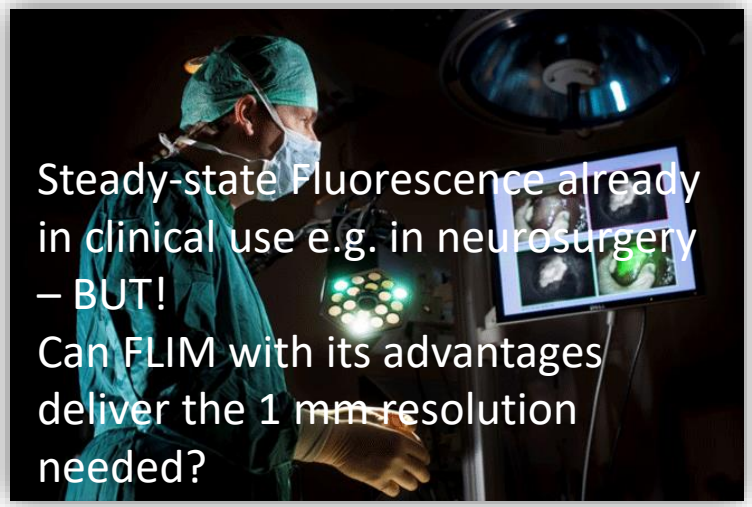
Almost **half** of UK patients will undergo surgery for treatment[1]

Many require repeat surgery – need for improved precision

Standards:	Drawbacks:
✓ Eye	✗ Bulk tumour only
✓ Touch	

Fluorescence guided surgery[2,3]:

- ✓ High contrast and sensitivity
- ✓ Can reveal hidden structures
- ✓ High specificity
- ✗ Limited FDA approved dyes
- ✗ In pre-clinical stages for margin estimation



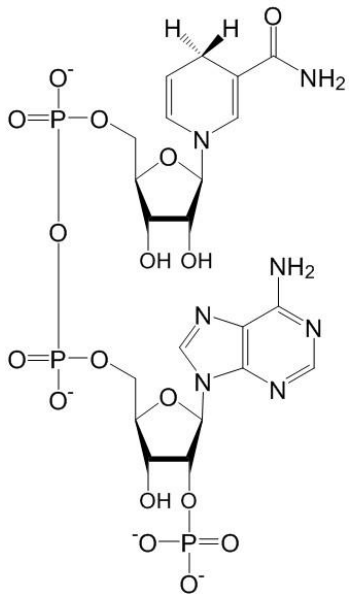
[1]Cancer Research UK, *Cancer statistics for the UK*: http://www.cancerresearchuk.org/health-professional/cancer-statistics

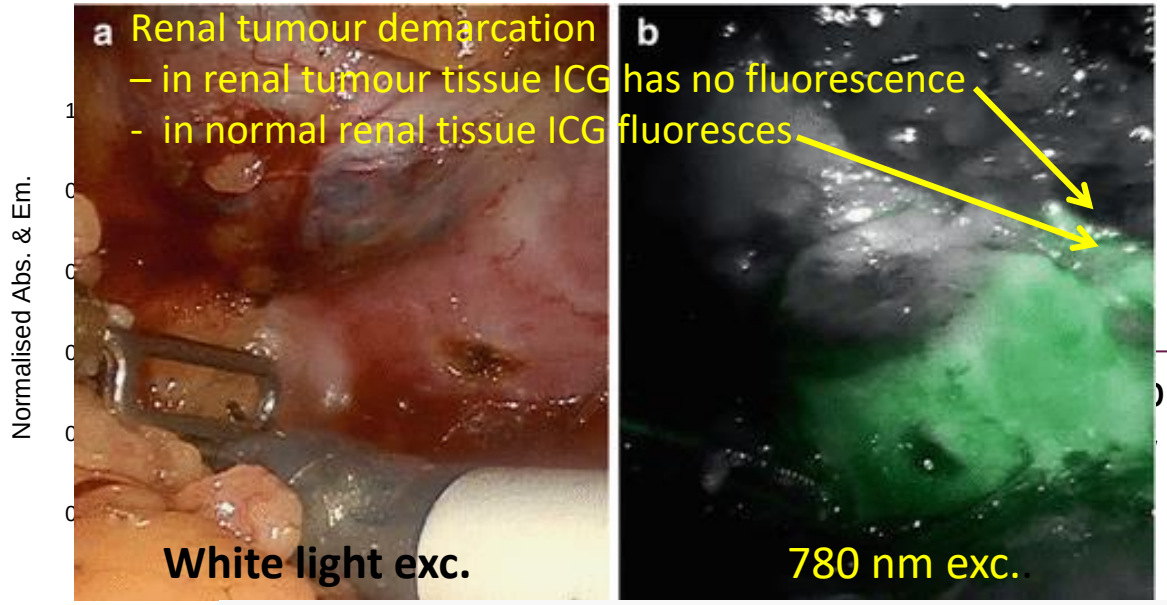
[2]A. L. Vahrmeijer et. al., *Nat. Rev. Clin. Oncol.,* 2013, **10**, 507-518.

[3]H. L. Stewart & D. J. S. Birch, Methods Appl. Fluoresc., 2021, **9**, 042002.

Fluorescent Probes for Tumour Margin Estimation

Intrinsic: NAD(P)H





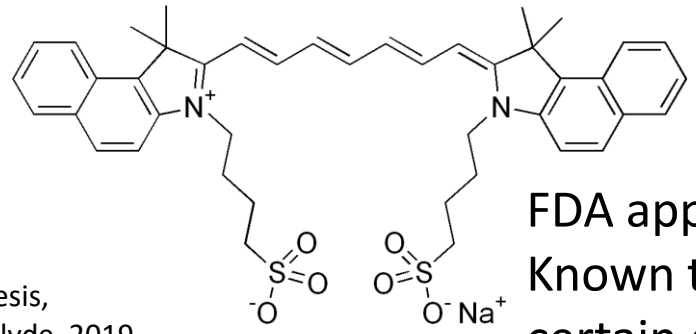
- NADPH production **increased** in cancer ce

ble with

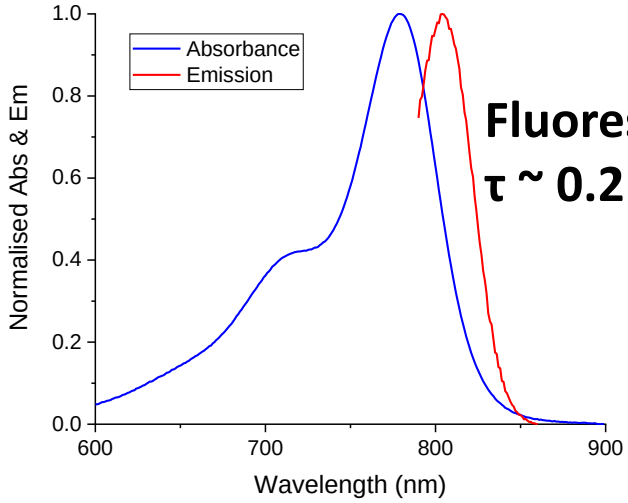
h

Abdominal Key, *Fluorescence Image-Guided Robotic Surgery* (2016): https://abdominalkey.com/fluorescence-image-guided-robotic-surgery/

Extrinsic: Indocyanine Green (ICG)



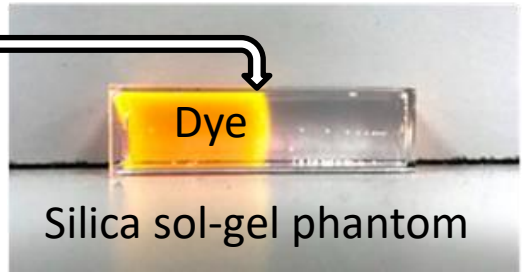
FDA approved **fluorescent dye**
Known to accumulate in certain cancerous tissues



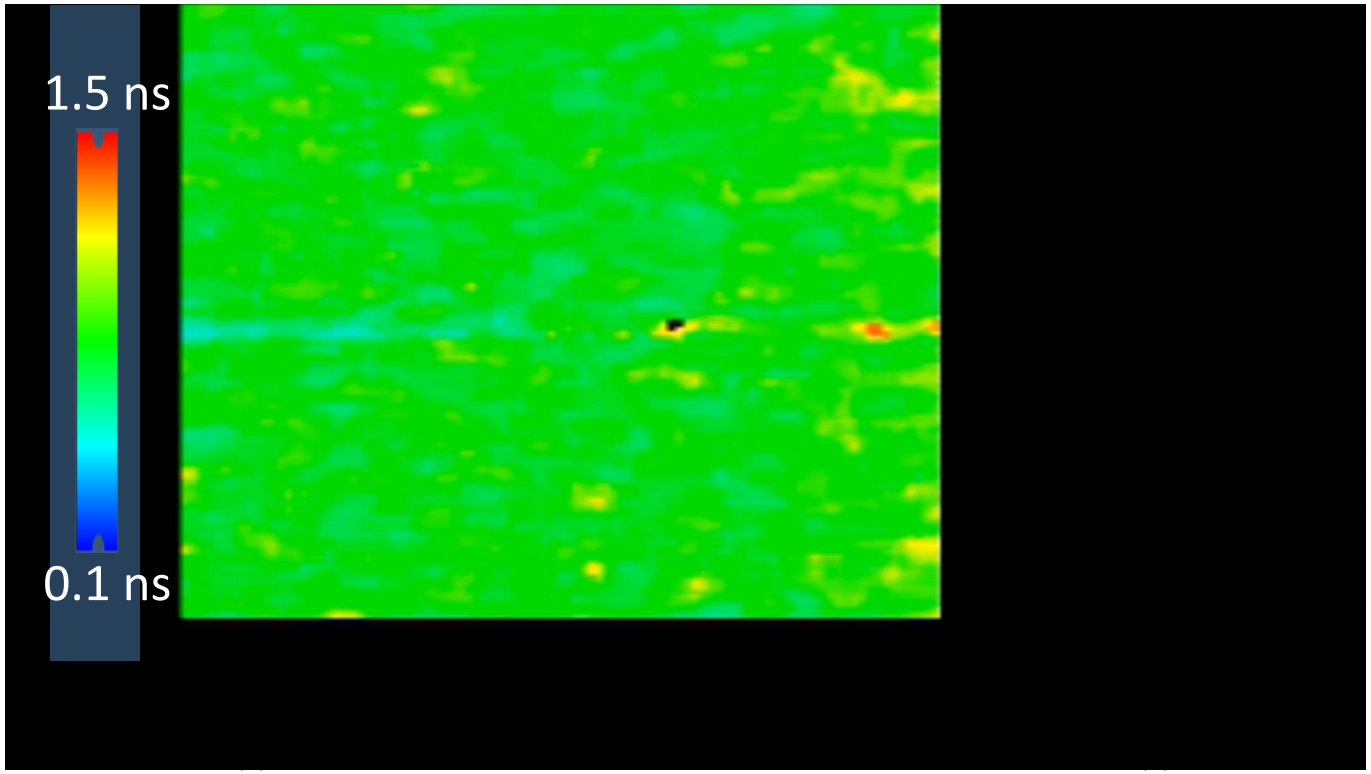
Fluorescence lifetime
τ ~ 0.2 ns

H L Stewart, PhD Thesis, University of Strathclyde, 2019

SPAD FLIM Camera Margin Microscope Estimation with ICG*



*H L Stewart, G Hungerford and D J S Birch. Meas. Sci. Technol. 31, 125701, 2020



Attractions of SPADs in fluorescence guided surgery:

Real time imaging
Operate in dimmed ambient light
Excellent near IR response e.g. for ICG
Cellular resolution when combined with microscopy

ICG FLIM video.mp4

Commercial steady-state near IR fluorescence guided surgical systems compatible with ICG

FLIM advantages yet to be widely implemented in FGS….but likely will!

Novadaq
SPY Elite

Hamamatsu
PDE

Novadaq
SPY PHI

Fluoptics
fluobeam

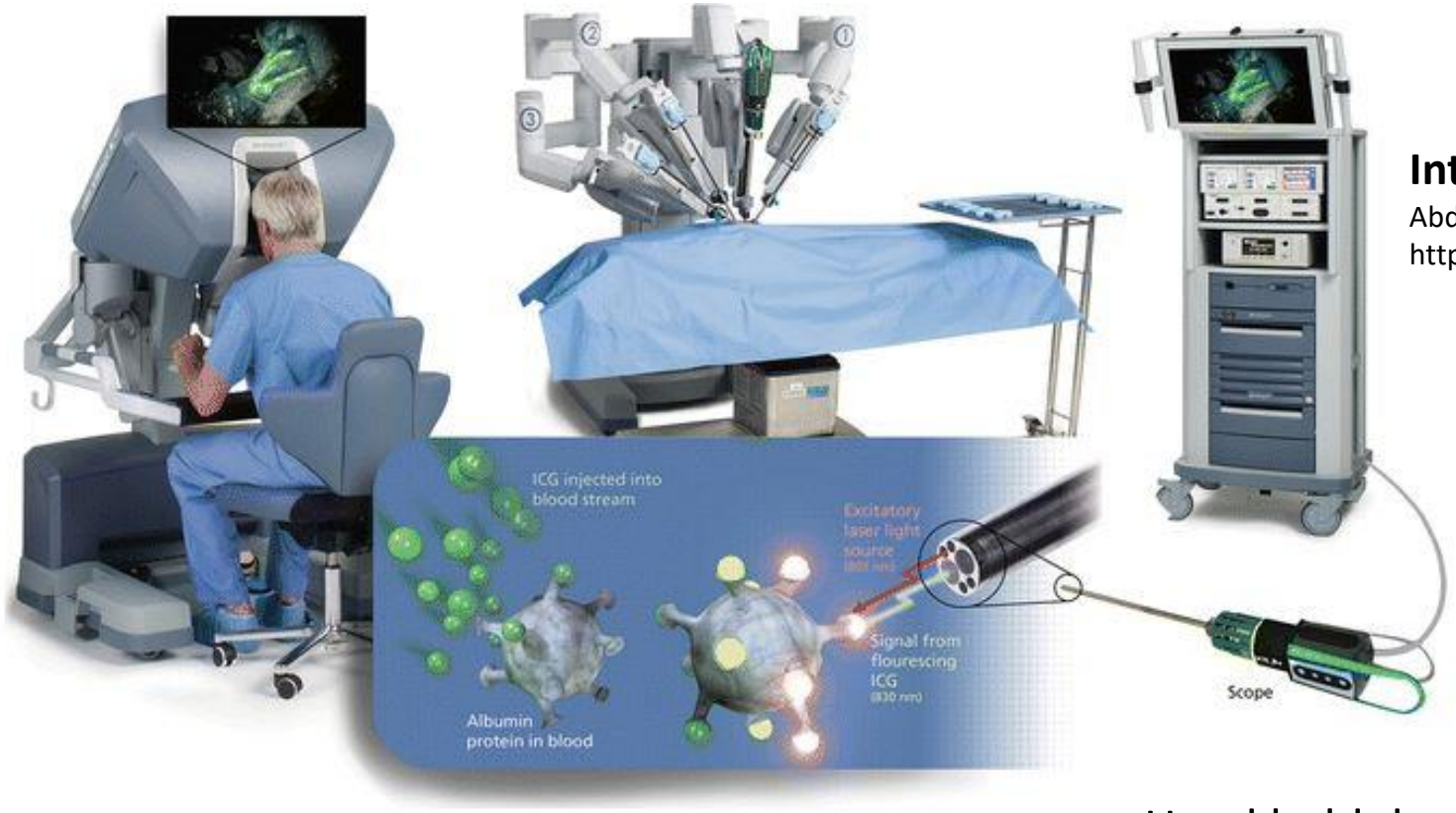
Quest medical
spectrum

Iridium
vision sense

Karl storz
vitom ICG

Intraoperative biophotonic imaging systems for image-guided interventions
S Sajedi, H Sabet, H S Choi, Nanophotonics, 8, 99-116, 2018.

The future looks bright ! Robotic surgery…

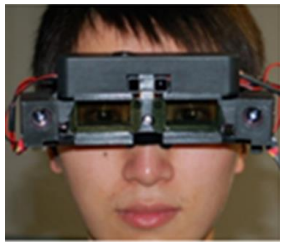


Intuitive da Vinci Platform

Abdominal Key, *Fluorescence Image-Guided Robotic Surgery* (2016): https://abdominalkey.com/fluorescence-image-guided-robotic-surgery/

Stryker SPY-PHI

Hand held dual white light/fluorescence cameras & augmented reality goggles



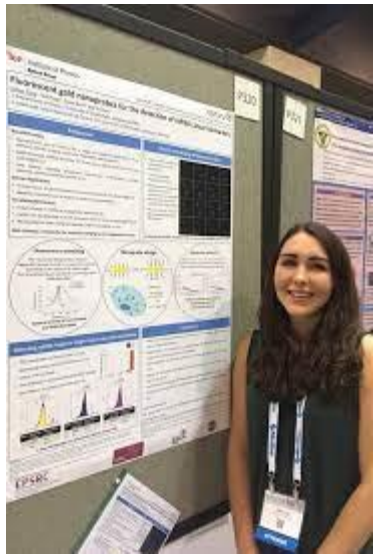
Zhu, N. *et al. J. Biomed. Opt.* **20**, 096010 (2015).

Acknowledgements

Applications:

Yu Chen
Gold nanoprobe

Gillian Craig
Liquid biopsy



Natakorn Sapermsap
Liquid biopsy

Hazel Stewart
Tumour margin

SPADs

Robert Henderson

Graham Hungerford

David McLoskey

Sponsors: