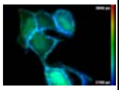
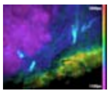
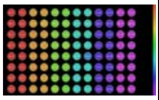
Imperial College London

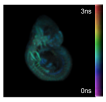
Multidimensional fluorescence imaging & metrology

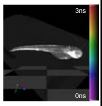
- for cell biology, drug discovery and clinical diagnosis











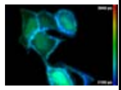
Paul French

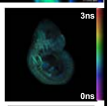
Photonics Group,
Physics Department
Imperial College London

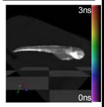
MDFI across the scales

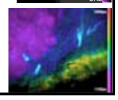
Imperial College London

- **Fluorescence lifetime imaging and metrology**
- **→ higher spatial resolution**
  - *STED (3-D & FLIM)*
- **→ higher speed/throughput FLIM & FRET**
  - *high-speed & automated Nipkow FLIM for HCA*
  - *implementation on IN Cell 1000 multiwell plate reader*
- **→ higher physiological relevance (⇒ *in vivo*)**
  - *microconfocal TCSPC FLIM endoscope*
  - *tomoFLIM (FLIM-OPT) of mouse, zebrafish*
  - *tomoFLIM (FLIM-DFT) of mouse*
  - *label-free FLIM of tissue autofluorescence*
    - *ex vivo* FLIM of cancer, osteoarthritis, atherosclerosis
    - *in vivo* AFL measurements of cancer, heart disease
    - *in vivo* clinical multiphoton multispectral FLIM



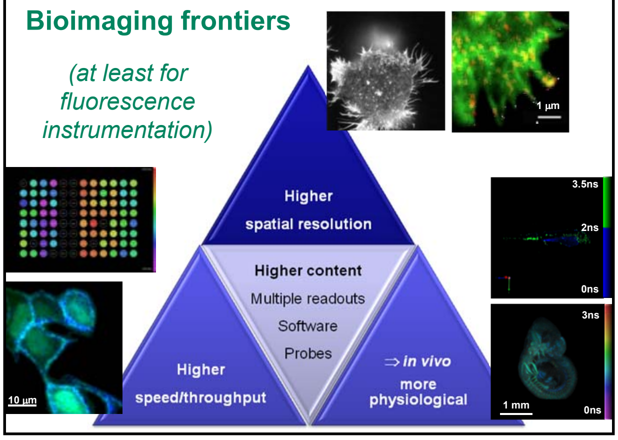






Bioimaging frontiers

(at least for fluorescence instrumentation)



Multidimensional fluorescence imaging

Imperial College London

Dominic Alibhai, Natalie Andrews, Lingling Chen, Sergio Coda, *Pieter de Beule*, *David Grant*, Douglas Kelly, Romain Laine, *Hugh Manning*, *Dylan Owen*, *Stephane Oddos*, Rakesh Patalay, *Tom Robinson*, Hugo Sinclair, Hugh Sparks, Sean Warren,, Laurence Bugeon, *Neil Galletly*, Yuriy Alexandrov, *Egidijus Auksorius*, *Alice Brown*, Sunil Kumar, *Peter Lanigan*, *Martin Lenz*, Anca Margineanu, *Ewan McGhee*, Ian Munro, *Jose Requejo-Isidro*, Gordon Kennedy, *Daniel Stuckey*, *Paul Tadrous*, *Harriet Taylor*, *Khadija Tahir*, Clifford Talbot, **James McGinty, Chris Dunsby, Mark Neil, Paul French**

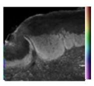
Imperial College London
Biology, Chemistry (ICB), Medicine, Physics

Praveen & Uma Anand
Geoff Baldwin
David Carling
Anthony Chu
Margaret Dallman
Dan Davis
Andrew deMello
Dan Elson
Mike Ferenczi
Jo Hajnal
Yoshifumi Itoh
Eric Lam
Alexander Lyon
Jonathon Lamb
Tony Magee
Ken MacLeod
Nicholas Peters
David Phillips
Guy Rutter
Ann Sandison
Alex Sardini
Gordon Stamp
Ed Tate
Andrew Thillainayagam..

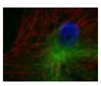
Support from:
BBSRC, BHF, TSB, EPSRC, EU, MRC, NIHR, Royal Society, Wellcome Trust…AstraZeneca, GE Healthcare, GSK, JenLab Kentech Inst., Leica, Mauna Kea Tech., Perkin Elmer, Pfizer …

Fluorescent opportunities

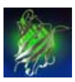
- **Endogenous fluorophores (*autofluorescence*)**
  - *label-free molecular contrast*

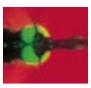


- **Extrinsic fluorophores (fluorescent *labels*)**
  - *"conventional staining", convenient but toxic labels*

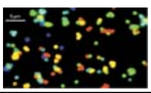


- **Genetically engineered fluorophores**
  - *"intrinsic" GFP labels for in vivo imaging*





- **Nano-engineered fluorophores**
  - *e.g CdSe/ZnS quantum dots*



λ_{exc} = 360 nm:

Multidimensional fluorescence imaging

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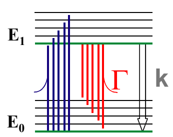
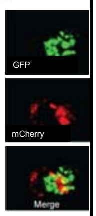
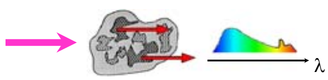


Intensity ~ f{η}, $\eta = \Gamma/(\Gamma + k)$

Wavelength, $\lambda \sim hc/(E_1 - E_0)$



Problems: *[fluorophore], heterogeneity, scattering and background fluorescence*



Difficult to make absolute intensity measurements

Solution*: *ratiometric measurements

Multidimensional fluorescence imaging

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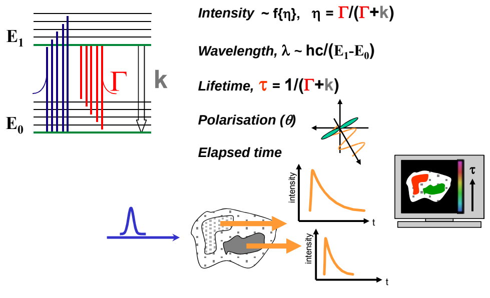

***Solution**: ratiometric measurements*

Multidimensional fluorescence imaging

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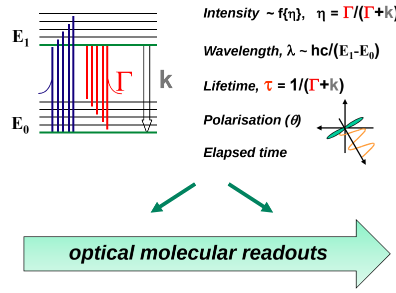

Molecular Biology **Drug Discovery** **Clinical imaging**

Multidimensional fluorescence imaging

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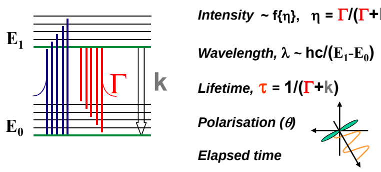
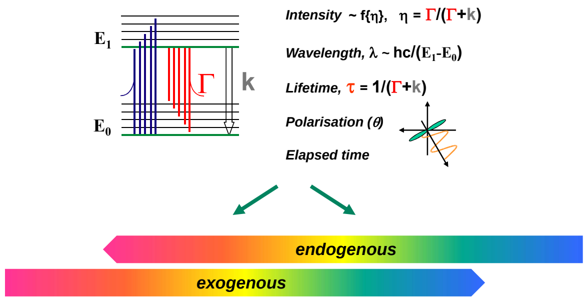


Application of ultrafast laser and photonics technology to biomedical imaging

*- maximise **information** from fluorescence signal*

Multidimensional fluorescence imaging

Imperial College London



Molecular Biology **Drug Discovery** **Clinical imaging**

Multidimensional fluorescence imaging

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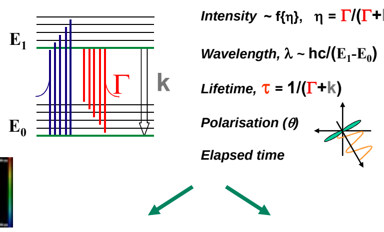
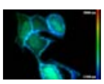
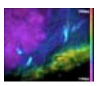




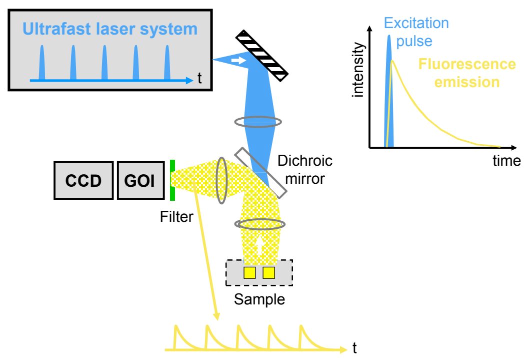


Sensing: imaging molecular (probe) ***environment***

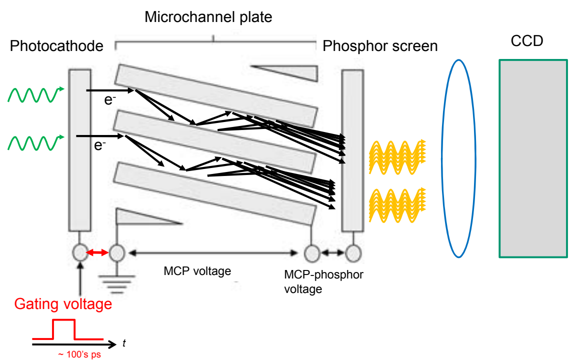
Label-free molecular contrast

Molecular Biology **3D, *in vivo* models** **Clinical imaging**

Fluorescence lifetime imaging (FLIM)



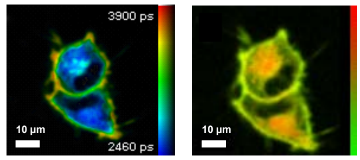
Gated optical image intensifier (GOI)



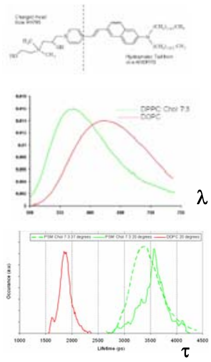
TCSPC FLIM sensing fluorophore environment

FLIM reporting lipid "rafts"
(membrane lipid microdomains)

Di-4-ANEPPDHQ membrane staining dye to probe lipid order
(Obaid et al., J Neurosci Meth 2004)

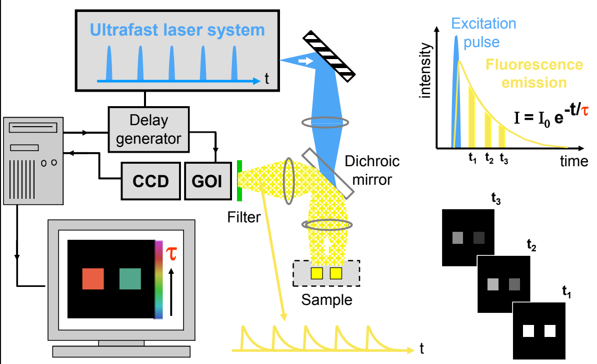


FLIM image   Spectral image



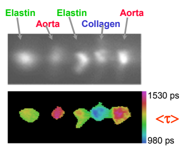
Owen et al., Biophys. J. (2006)

Fluorescence lifetime imaging (FLIM)



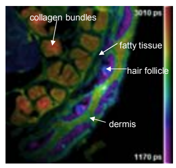
Label-free FLIM of tissue autofluorescence

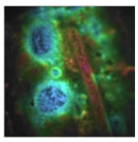
415 nm excitation



Dowling et al, Opt Lett . (1998)

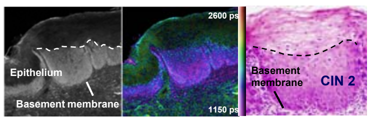
355 nm excitation





Lee et al, Biophys. J. (2001)

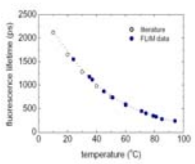
740 nm multiphoton excitation

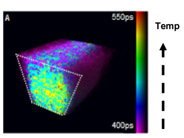


Elson et al, Reviews in Fluorescence 2006

FLIM of molecular environment in microfluidics

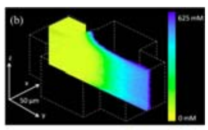
Rhodamine B reports temperature in microfluidic devices for PCR



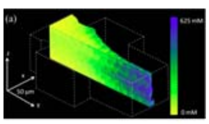


Benninger et al., Anal. Chem. (2006)

3-D FLIM of fluorescein quenching by NaI in continuous flow mixer



CFD simulation of [NaI]

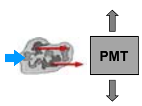


Experimental [NaI] from FLIM data

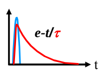
Robinson et al., Opt. Lett. (2008)

How to FLIM?

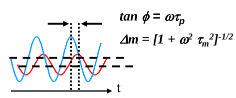
Single channel measurement (scanning FLIM)



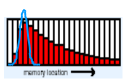
Time-domain direct measurement
e.g. streak camera



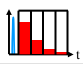
Frequency-domain (heterodyne detection)



Time-correlated single photon counting (TCSPC)

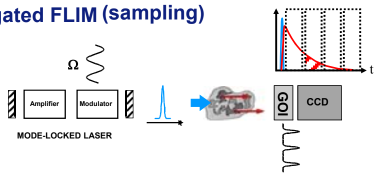


Time-binning (LIMO)

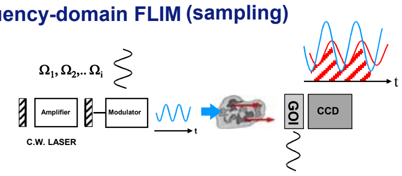


How to FLIM?

Wide-field time-gated FLIM (sampling)

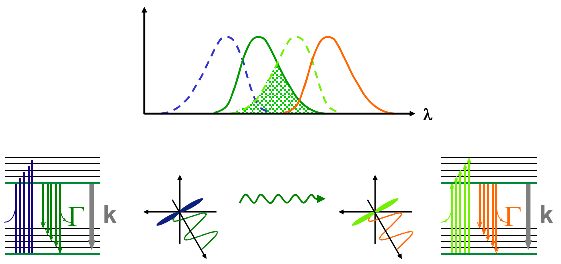


Wide-field frequency-domain FLIM (sampling)

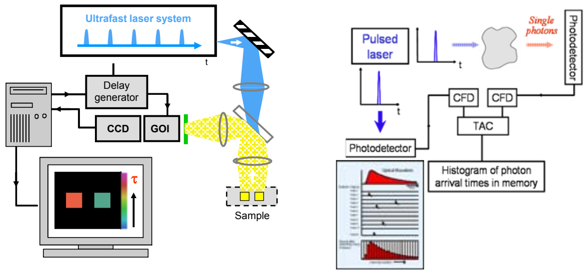


FLIM with exogenous fluorophores

FRET: *Colocalisation and the spectroscopic ruler*



(Time domain) FLIM technology



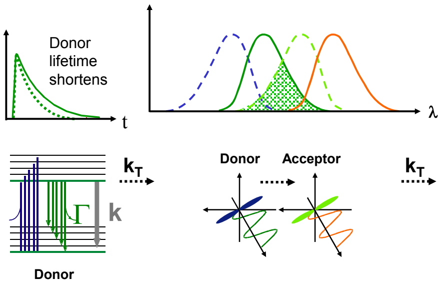
Wide-field time-gated imaging | **Confocal/MP TCSPC**

Highest accuracy/second | ***Highest accuracy/photon***

FLIM with exogenous fluorophores

FRET: *Colocalisation and the spectroscopic ruler*

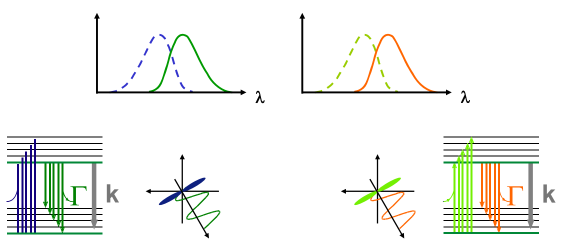
Förster Resonant Energy Transfer between fluorescent molecules over short (< 20 nm) distances : *dipole-dipole interaction (NO PHOTONS)*



$$k_T = \frac{1}{\tau_{0D}}\left(\frac{R_0}{R}\right)^6$$

FLIM with exogenous fluorophores

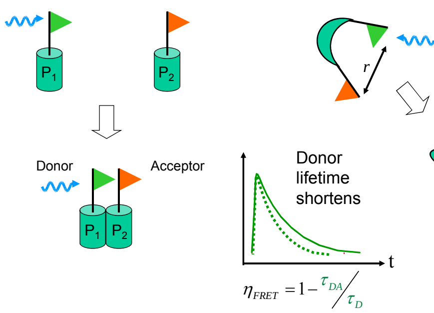
FRET: *Colocalisation and the spectroscopic ruler*



FLIM FRET to read out molecular dynamics

Förster Resonant Energy Transfer between fluorescent molecules over short (< 20 nm) distances : *dipole-dipole interaction (NO PHOTONS)*

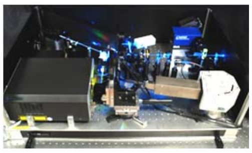
e.g. protein binding | e.g. change in conformation

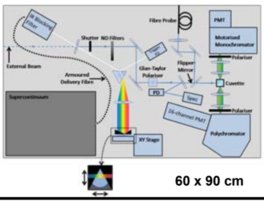


$$r = R_0\left(\frac{1}{\eta_{FRET}} - 1\right)^{1/6}$$

$$\eta_{FRET} = 1 - \frac{\tau_{DA}}{\tau_D}$$

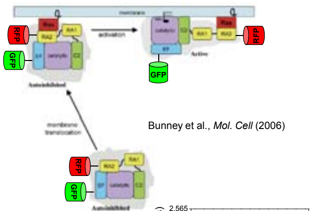
Multidimensional fluorometer with supercontinuum - for cuvette & fibre-optic probe measurements





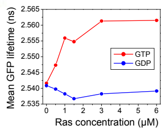
60 x 90 cm

Conformational change of PLCε upon h-Ras binding



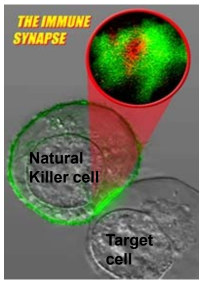
Bunney et al., *Mol. Cell* (2006)

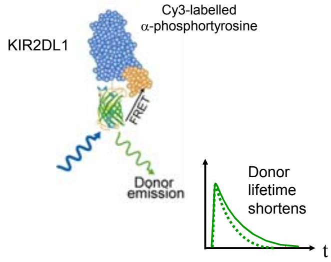
Now multiwell plate reader



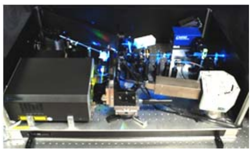
Imaging KIR2DL1 phosphorylation by FRET

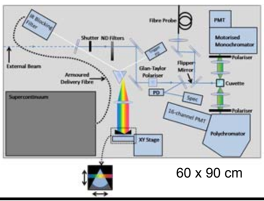
(studying immune system)





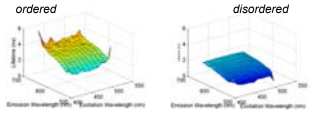
Multidimensional fluorometer with supercontinuum - for cuvette & fibre-optic probe measurements





60 x 90 cm

Membrane probe (di-4-ANEPPDHQ) of lipid order

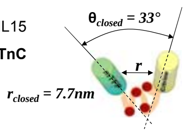


Excitation, emission, lifetime in unilamellar vesicles in the ordered (DPPC7:Chol3) and disordered phase (DOPC).

Improved [Ca²⁺] FRET probe for FLIM readout

Troponin TNL15

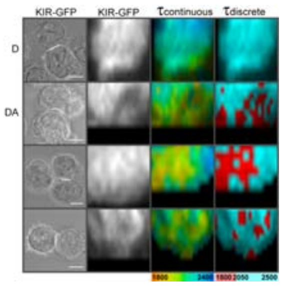
→ **TFP-TnC**



Imaging KIR2DL1 phosphorylation by FRET

(studying immune system)

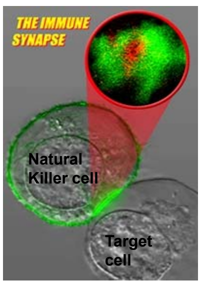
Treanor et al, J. Cell Biol., 2006

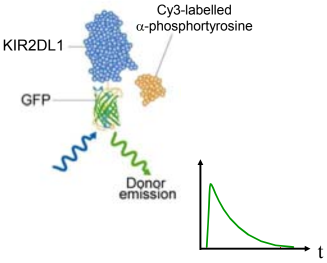


- FLIM/FRET can image phosphorylation of receptors, including KIR at the IS
- KIR phosphorylation:
  - *requires a Src family kinase,*
  - *is spatially restricted to the NK cell IS*
  - *lasts several minutes*
  - *can occur at multiple synapses.*
- KIR phosphorylation is not uniformly distributed at the synapse but occurs in nanoclusters.

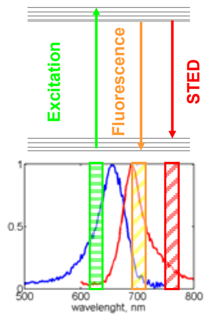
Imaging KIR2DL1 phosphorylation by FRET

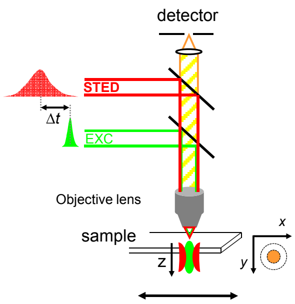
(studying immune system)



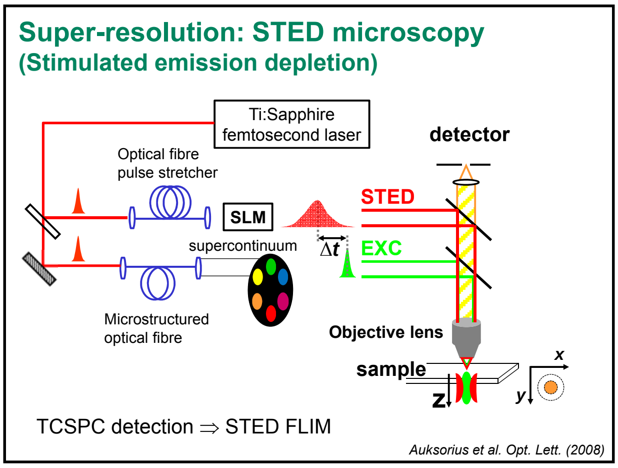


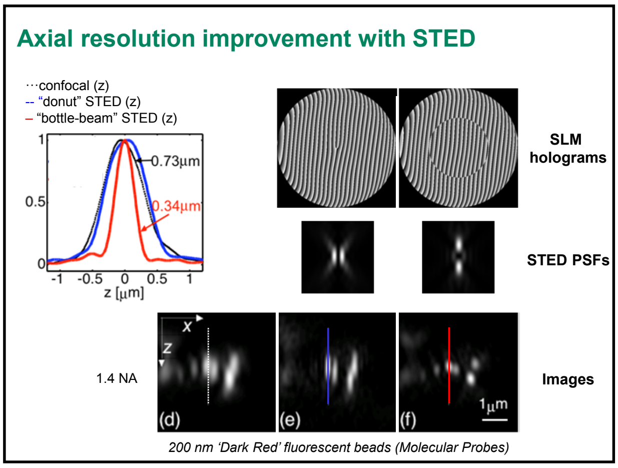
Super-resolution: STED (Stimulated emission depletion)

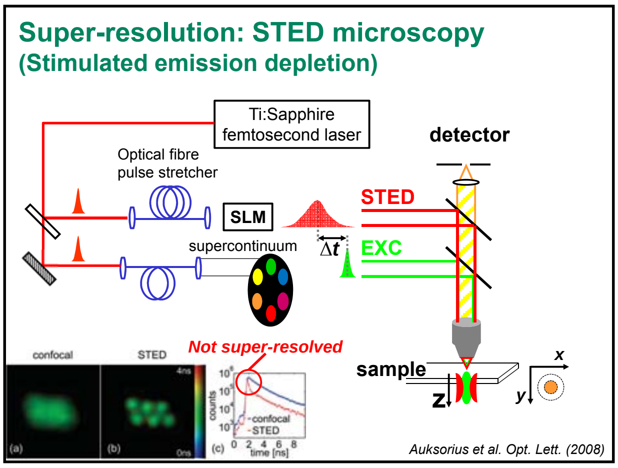


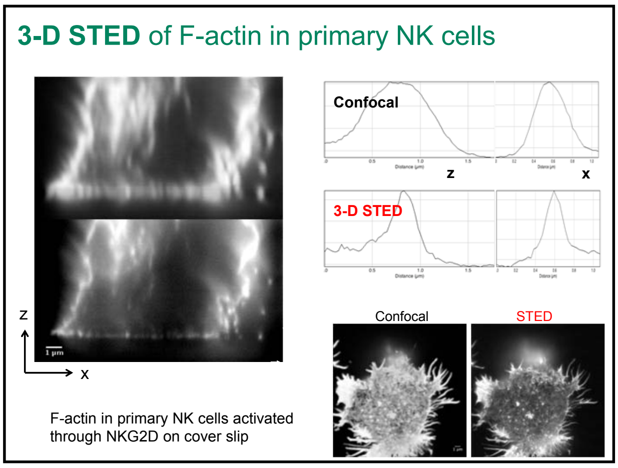


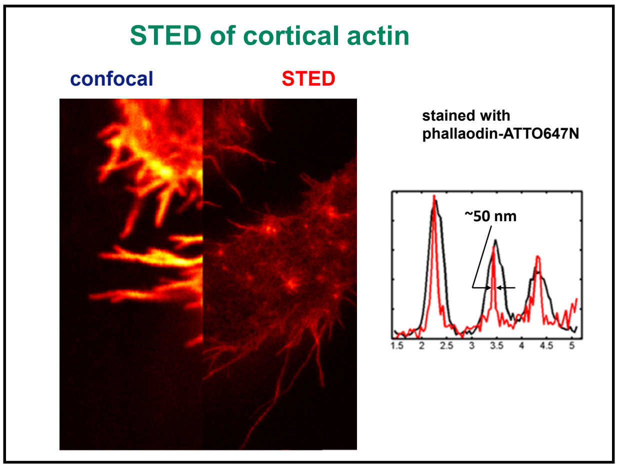
scan to acquire super-resolved image

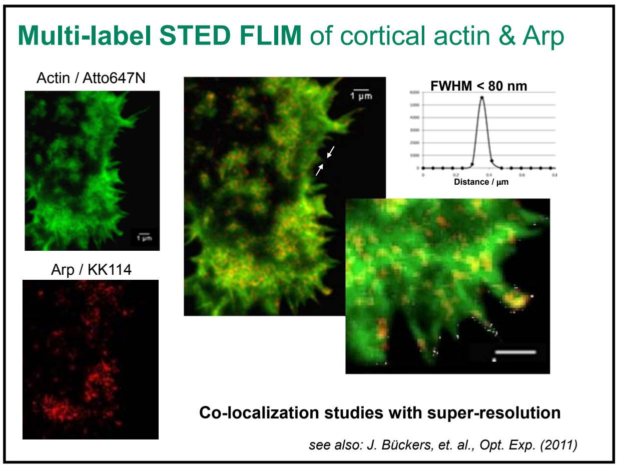

Super-resolution: STED microscopy
(Stimulated emission depletion)
Ti:Sapphire femtosecond laser
detector
Optical fibre pulse stretcher
SLM
STED
supercontinuum
Δt
EXC
Microstructured optical fibre
Objective lens
sample
x
y
z
TCSPC detection ⇒ STED FLIM
Auksorius et al. Opt. Lett. (2008)


Axial resolution improvement with STED
confocal (z)
"donut" STED (z)
"bottle-beam" STED (z)
0.73µm
0.34µm
z [µm]
SLM holograms
STED PSFs
1.4 NA
(d)
(e)
(f)
1µm
Images
200 nm 'Dark Red' fluorescent beads (Molecular Probes)


Super-resolution: STED microscopy
(Stimulated emission depletion)
Ti:Sapphire femtosecond laser
detector
Optical fibre pulse stretcher
SLM
STED
supercontinuum
Δt
EXC
confocal
STED
Not super-resolved
confocal
STED
time [ns]
sample
x
y
z
Auksorius et al. Opt. Lett. (2008)

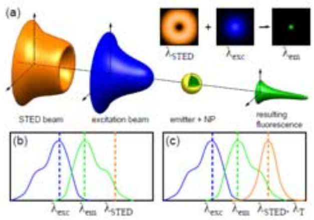

3-D STED of F-actin in primary NK cells
Confocal
z
x
3-D STED
Confocal
STED
z
x
F-actin in primary NK cells activated through NKG2D on cover slip


STED of cortical actin
confocal
STED
stained with phallaodin-ATTO647N
~50 nm


Multi-label STED FLIM of cortical actin & Arp
Actin / Atto647N
Arp / KK114
FWHM < 80 nm
Distance / µm
Co-localization studies with super-resolution
see also: J. Bückers, et. al., Opt. Exp. (2011)

NP-STED?


ACS NANO
Article
Subscriber access provided by Imperial College London | Library
Nanoparticle-Assisted Stimulated-Emission-Depletion Nanoscopy
Yonatan Sivan, Yannick Sonnefraud, Stephane Kena-Cohen, John B. Pendry, and Stefan A. Maier
ACS Nano, Just Accepted Manuscript • DOI: 10.1021/nn301082g • Publication Date (Web): 24 Apr 2012



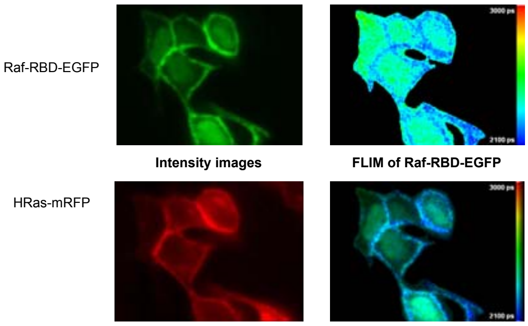
Near field plasmonic resonance enhancement of stimulated emission *and* triplet decay rate

⇒ reduced power in STED beam

⇒ reduced photobleaching

Nipkow FLIM-FRET of Raf RBD/Ras-mRFP

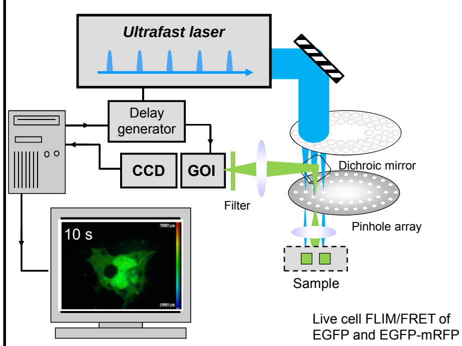
Imaged after 10 minutes stimulation with EGF (5 s FLIM acquisition)



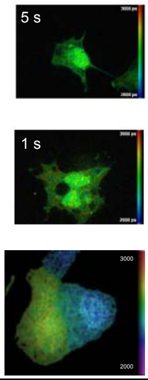
Grant et al. Opt. Exp. (2007)

(RBD = Ras Binding Domain)

Wide-field optically-sectioned FLIM



FLIM of live COS cells with EGFP

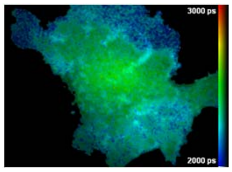


Live cell FLIM/FRET of EGFP and EGFP-mRFP imaged at 5 frames/s:

Nipkow FLIM-FRET of Raf RBD/Ras FRET

High-speed *⇒ 3-D imaging and/or time lapse imaging*

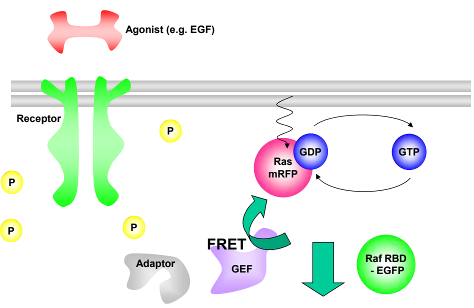
COS 7 cell expressing H-Ras-mRFP and Raf-RBD-EGFP imaged in 100 s after EGF stimulation



Z stack

Grant et al. Opt. Exp. (2007)

Raf RBD-EGFP with Ras-mRFP FRET



(RBD = Ras Binding Domain)

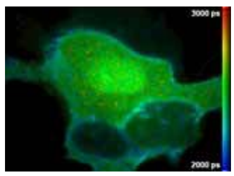
Imperial College London

The Institute of Cancer Research

Nipkow FLIM-FRET of Raf RBD/Ras FRET

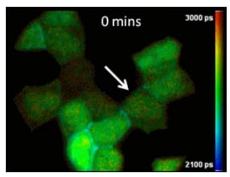
High-speed *⇒ 3-D imaging and/or time lapse imaging*

COS 7 cell expressing H-Ras-mRFP and Raf-RBD-EGFP imaged in 100 s after EGF stimulation



Z stack

HRas-mRFP with Raf-RBD-EGFP in MDCK cells following EGF stimulation



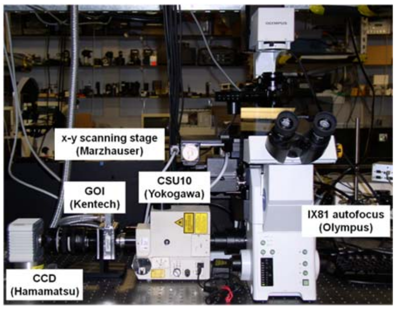
Time lapse

6 s per FLIM image

Grant et al. Opt. Exp. (2007)

Automated Nipkow FLIM plate reader

(1st prototype)



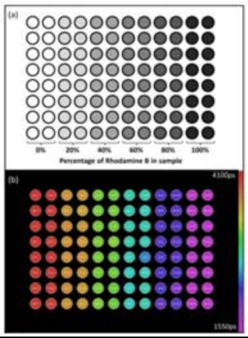
Tunable continuum source (Fianium)

dti AstraZeneca PerkinElmer gsk GlaxoSmithKline Imperial College London Kentech Instruments Ltd

Automated FLIM multiwell plate reader

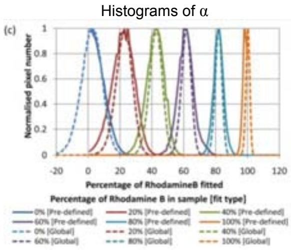
(based on GE Healthcare IN Cell 1000)

Mixtures of RhB/Rh6G



$$I = I_0\left(\beta e^{-t/\tau_B} + (1-\beta)e^{-t/\tau_{6G}}\right)$$

Histograms of α



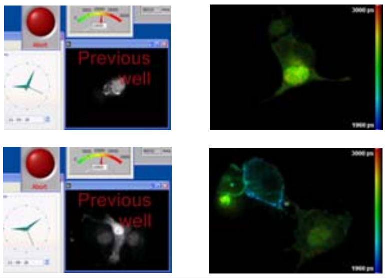
Kumar et al. ChemPhysChem (2011)

Automated Nipkow FLIM multiwell plate reader

- Implemented on Olympus IX81 with autofocus and motorised stage
- Auto-find and auto-exposure algorithms
- Tested on live cells cotransfected with EGFP-Raf RBD and K-Ras mRFP
- Stimulation with EGF induces Raf binding to K-Ras

Control

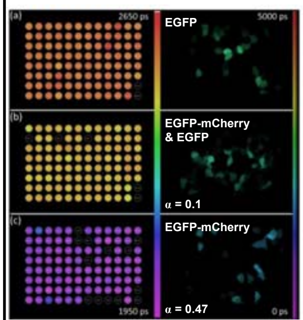
FRET



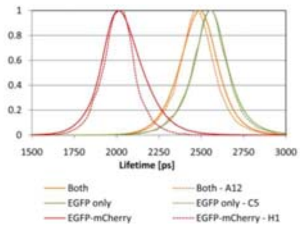
Talbot et al., J. Biophotonics (2008)

Automated FLIM multiwell plate reader

(based on GE Healthcare IN Cell 1000)



Lifetime histograms



$$I_{GFP} = I_0\left(\beta e^{-t/\tau_{FRET}} + (1-\beta)e^{-t/\tau_{free}}\right)$$

τ_{free} = 2516 ps; τ_{FRET} = 1068 ps

Kumar et al. ChemPhysChem (2011)

Technology Strategy Board
Driving Innovation

Ultrafast photonics for fluorescence imaging and time-resolved assays – TP:16401 – Proj:100297

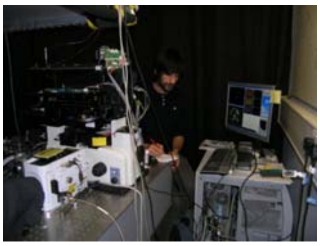
TSB project: prototype FLIM multiwell plate reader

(based on GE Healthcare IN Cell 1000)

Established wide-field multiwell plate reader

- + *Yokogawa CSU-X (more efficient)*
- + *wide-field time-gating*
- + *supercontinuum excitation source*
- + *FLIM/segmentation analysis*
- + ***prescan mode***

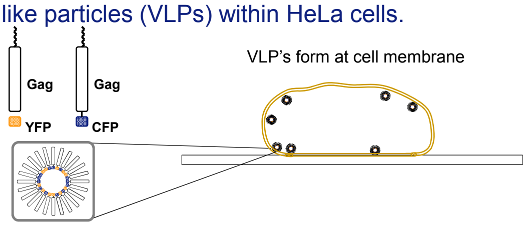
⇒ <~15 min/96 well plate for FLIM of FP-labelled live cells



AstraZeneca GE Healthcare gsk GlaxoSmithKline Imperial College London Kentech Instruments Ltd

FLIM-FRET of HIV-1 Gag Aggregation

- HIV-1 Gag protein oligomerisation in HIV-1 virus-like particles (VLPs) within HeLa cells.

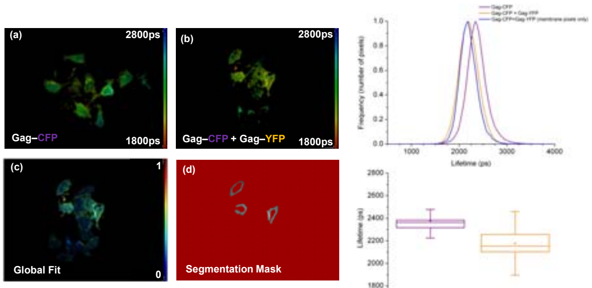


Stochastic labelling of Gag with both CFP and YFP should produce FRET

⇒ readout of HIV-1 formation

Automated FLIM-FRET of protein oligomerisation

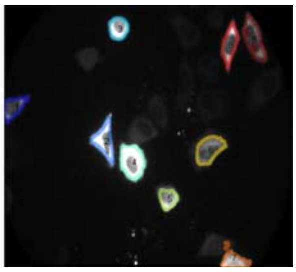
HIV-1 Gag-CFP and Gag-YFP in VLPs in fixed HeLa cells (using modified Nipkow FLIM IN Cell 1000)



τ_{free} = 2625 ps
τ_{FRET} = 1113 ps

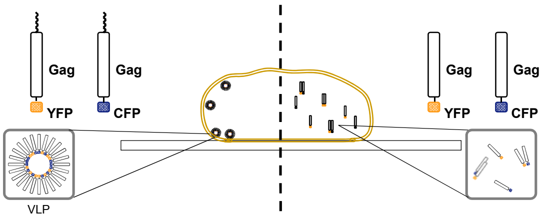
HIV-1 Gag Assay: Automatic image segmentation for improved contrast

- VLP formation occurs at the cell plasma membrane.
- Use automatic segmentation routines to create membrane regions of interest (ROIs).
- Each ROI is binned to a single decay.
- Increases S/N.
- Results in single (average) lifetime value per cell membrane.



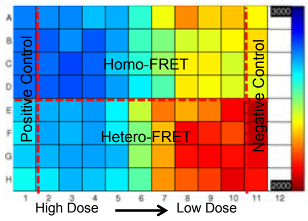
HIV-1 Gag Assay: Disruption of NMT enzymes

- HIV-1 Gag membrane binding requires endogenous NMT enzymes to add myristic acid moiety to N-terminus.
- Through use of an inhibitor of the NMT enzymes we disrupted HIV-1 Gag protein's ability to form VLPs.

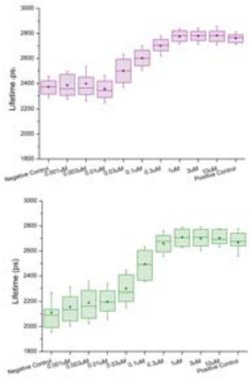


LOW DOSE → HIGH FRET | HIGH DOSE → LOW FRET

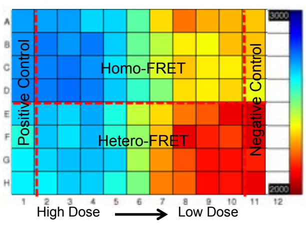
HIV-1 Gag Assay: Using high speed FLIM for HCA



- Image segmentation leads to improvement in assay performance:
  - Z'$_{Homo\text{-}FRET}$ = 0.75
  - Z'$_{Hetero\text{-}FRET}$ = 0.67



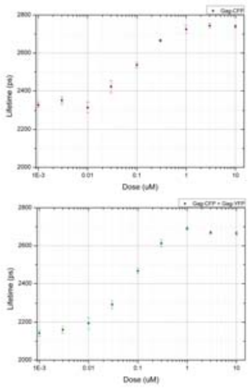
HIV-1 Gag Assay: Using high speed FLIM for HCA



- Assay performance measured by Z' parameter:

$$Z' = 1 - \left(\frac{3 * [\sigma_{pos} + \sigma_{neg}]}{\mu_{pos} - \mu_{neg}}\right)$$

Z'$_{Homo\text{-}FRET}$ = 0.63
Z'$_{Hetero\text{-}FRET}$ = 0.62



Label-free FLIM of metabolites in live cells

***Autofluorescence from* NAD(P)H** (excited in u.v.) and **flavoproteins**, e.g. FAD (excited in blue)

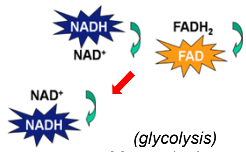
Complex decay profiles → approximate to double exponential

Can read out changes in metabolic pathways
e.g. aerobic → anaerobic respiration (e.g. hypoxia, cancer)

NADH **intensity** increases
NADH τ_1 ~ constant
NADH τ_2 ~ decreases
NADH τ_{mean} ~ decreases

FAD **intensity** decreases
FAD τ_{mean} ~ decreases?

***aerobic** respiration (oxidative phosphorylation)*



(glycolysis) ***anaerobic** respiration*

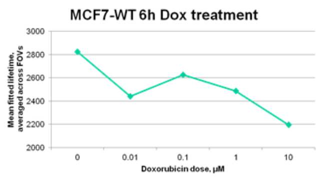
Label-free toxicology: Multiwell plate FLIM of NADH

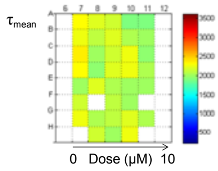
Doxorubicin is cardiotoxic

- linked with decline in oxidative phosphorylation

FLIM of live MCF7 cells treated with Doxorubicin (360 nm excitation)

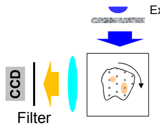
	7	8	9	10	11
A	0	0.01	0.1	1	10
B	0	0.01	0.1	1	10
C	0	0.01	0.1	1	10
D	0	0.01	0.1	1	10
E	0	0.01	0.1	1	10
F	0	0.01	0.1	1	10
G	0	0.01	0.1	1	10
H	0	0.01	0.1	1	10

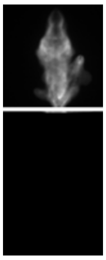




Optical Projection Tomography

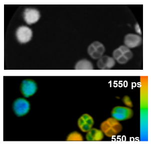
Sharpe et al, Science (2002)
Optical analogue of X-ray CT
- transparent samples (chemically cleared)
- size <1cm diameter



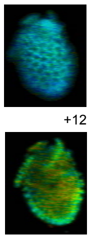


Mouse embryo neurofilament labelled with Alexa-488 conjugated antibody

TCSPC FLIM with a confocal endomicroscope

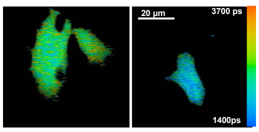


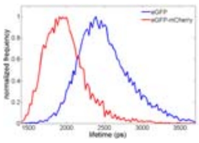
FLIM of pollen grains



Kennedy et al., J Biophotonics (2009)

FLIM FRET in live Cos cells with eGFP and eGFP-mCherry





1 s FLIM acquisitions

Kumar et al. ChemPhysChem (2011)

Optical Projection Tomography

Sharpe et al, Science (2002)
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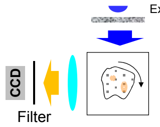

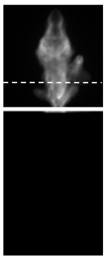

Mouse embryo neurofilament labelled with Alexa-488 conjugated antibody

Optical Projection Tomography

Sharpe et al, Science (2002)
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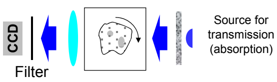
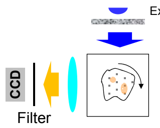
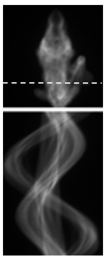
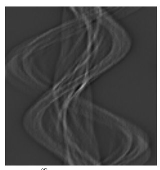


Optical Projection Tomography

Mouse embryo neurofilament labelled with Alexa-488 conjugated antibody



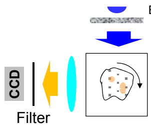


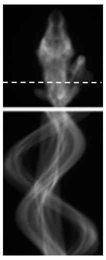


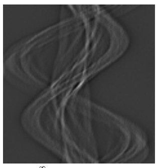
$$\int_{-\infty}^{\infty} S_\theta(w)|w|dw$$

Optical Projection Tomography

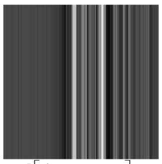
Mouse embryo
neurofilament labelled
with Alexa-488
conjugated antibody







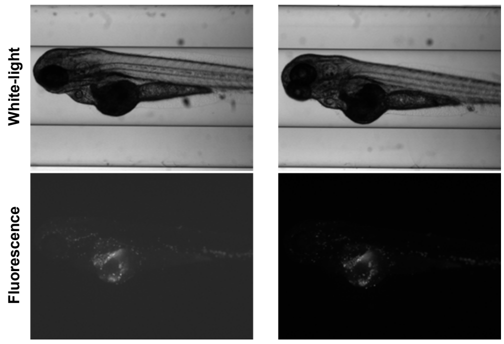
$\int_{-\infty}^{\infty} S_\theta(w)|w|dw$



$\int_0^\pi \left[\int_{-\infty}^{\infty} S_\theta(w)|w|dw \right] d\theta$

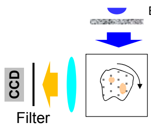
Video & OPT of live (lysC:GFP) zebrafish

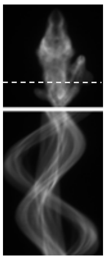
Video acquired at 20fps **OPT acquisition**

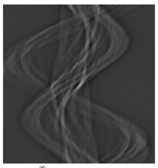


Optical Projection Tomography

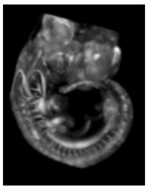
Mouse embryo
neurofilament labelled
with Alexa-488
conjugated antibody





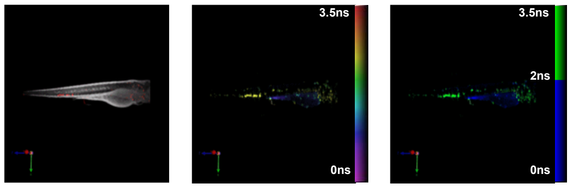


$\int_{-\infty}^{\infty} S_\theta(w)|w|dw$



FLIM OPT of live (lysC:GFP) zebrafish

Intensity (absorption: white, fluorescence: red) | **FLIM** (continuous scale) | **FLIM** (discrete scale)

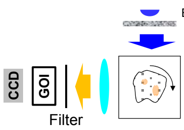


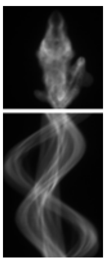
The FLIM data acquired with 6 time-gates every 4 degrees with an integration time of 2s per image (total acquisition time ~20 minutes).

McGinty et al., Biomed Opt Express (2011)

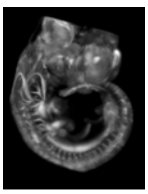
Fluorescence Lifetime OPT

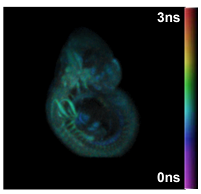
Mouse embryo
neurofilament labelled
with Alexa-488
conjugated antibody





fixed and cleared in BABB



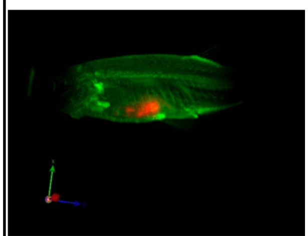


McGinty et al., J. Biophotonics (2008)

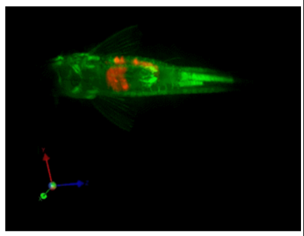
OPT of live 30 dpf zebrafish

Caspar (FLi:GFP) with mCherry tumour

Fish 1

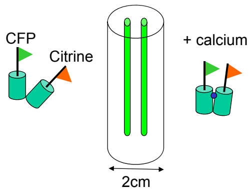


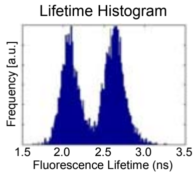
Fish 2

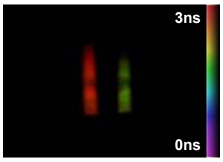


tomoFRET in transparent phantoms
- wide-field time-gated OPT

Silicone phantom containing two wells with cytosolic preparation of HEK293 cells expressing Troponin-C (TN-L15)







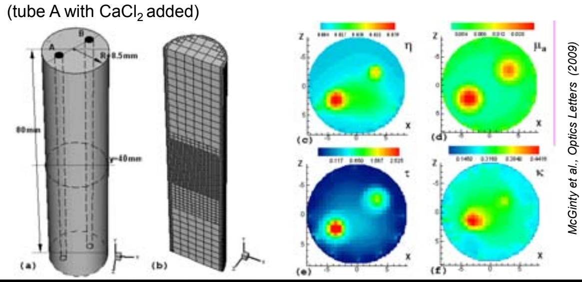
tomoFRET in scattering phantoms
- wide-field time-gated DFT

Wide-field time-gated imaging with supercontinuum excitation

Frequency domain reconstruction using Telegraph equation

Cells labelled with troponin-based [Ca^{2+}] CFP/YFP FRET sensor

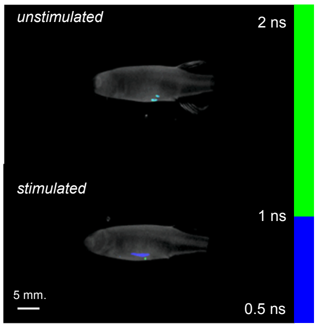
(tube A with $CaCl_2$ added)



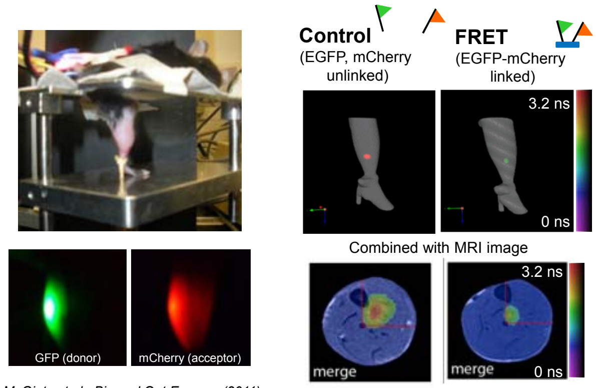
McGinty et al., Optics Letters (2009)

FLIM FRET in live 100 dpf zebrafish

Live 100 dpf Caspar Fli:GFP zebrafish injected with HEK293 cells expressing FLAIR FRET biosensor for Rac



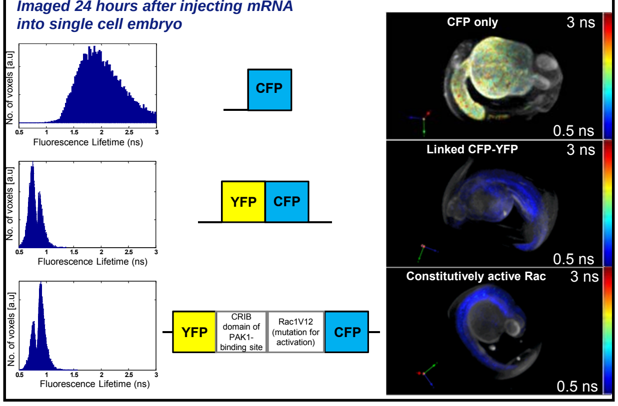
In vivo FLIM FRET tomography



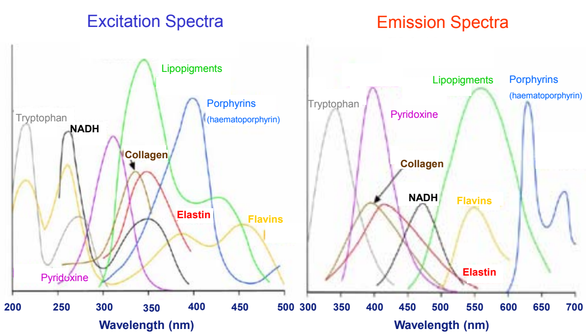
McGinty et al., Biomed Opt Express (2011)

In vivo FLIM-OPT of Rac FRET biosensor

Imaged 24 hours after injecting mRNA into single cell embryo



Endogenous Tissue Fluorophores

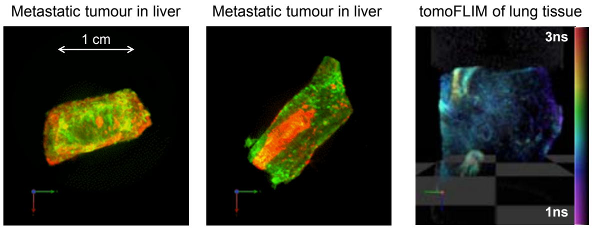


adapted from Wagnieres et al, Photochem. Photobiol. ***68*** *(1998) 605*

OPT of unstained clinical resections

Application to rapid clinical histopathology – label-free?

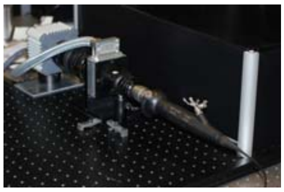
Fixed in formalin, dehydrated and cleared in BABB

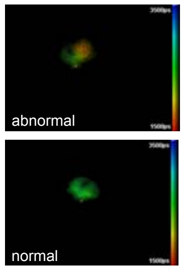


Green – fluorescence λ > 500 nm, excited at 460 nm
Red – white light absorption

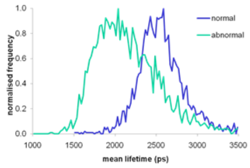
Wide-field FLIM endoscope

Karl Storz Flex X ureteroscope



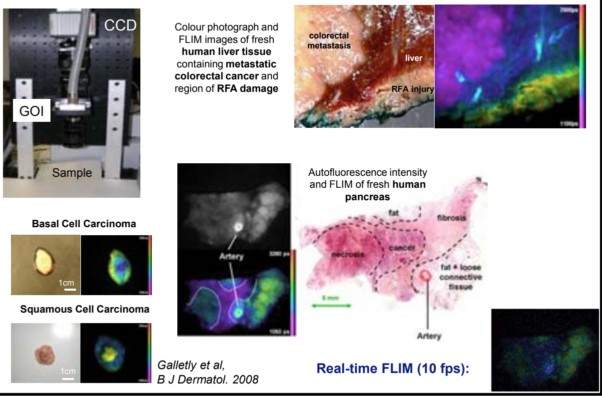


Ex vivo FLIM of normal & cancerous biopsies



405nm excitation, 430nm long pass filter

Clinical wide-field FLIM of fresh human tissue

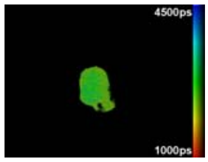


Galletly et al, B J Dermatol. 2008

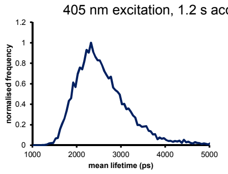
Real-time FLIM (10 fps):

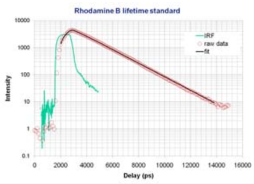
Handheld FLIM arthroscope

Tru-cut bladder biopsy (~3 mm)



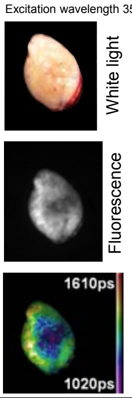
405 nm excitation, 1.2 s acquisition

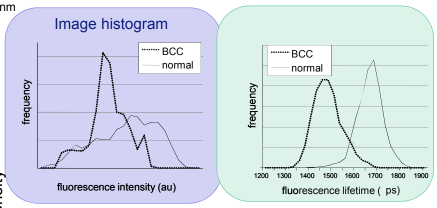




Wide-field FLIM of freshly resected human skin

Excitation wavelength 355nm



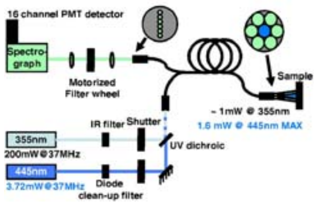


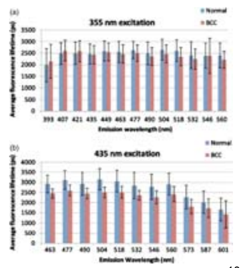
(n=25)	Mean fluorescence intensity	Mean fluorescence lifetime
BCC	104 ± 23 au	1400 ± 130 ps
difference (normal - BCC)	10 ± 23 au	150 ± 70 ps***

Galletly et al., B J Dermatol. 2008

Clinical hyperspectral fluorescence lifetime fibre-optic probe

Preliminary study of 23 *ex vivo* human skin samples (NOT fixed)



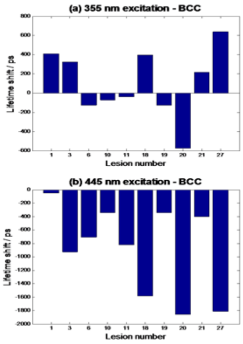


De Beule et al, Rev. Sci Instr. (2007)

n=10

Clinical hyperspectral fluorescence lifetime probe

***In vivo* measurements**
(27 clinically diagnosed skin lesions at Lund University hospital)



Now in clinical trial for GI cancer at Charing Cross Hospital

Thompson et al, J Biophotonics (2012)

4 Channels – *ex vivo* images

Dysplastic naevus imaged with the multichannel FLIM detector unit

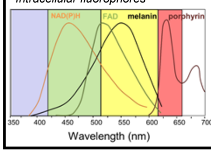
<415nm(ch3)
collagen, **SHG**

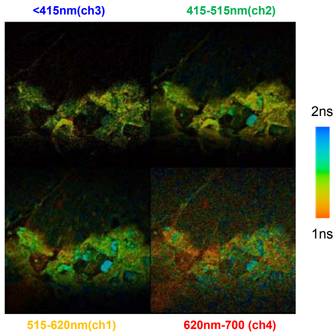
415-515nm(ch2)
collagen, elastin, FAD, keratin, melanin **NAD(P)H**,

515-620nm(ch1)
elastin FAD, keratin, **melanin**

620nm-700 (ch4)
melanin, porphyrins

Intracellular fluorophores





Clinical FLIM of human tissue in vivo

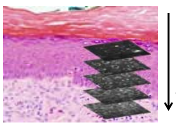
DermaInspect clinical multiphoton microscope

Imperial College London
Multispectral FLIM

http://www.jenlab.de/DermaInspect-R.29.0.html

SKINSPECTION
Multimodal Skin Inspection with Hybrid Acoustic & Optical Spectroscopic Imaging

JenLab - Kibero – IBMT - Imperial - Modena - tp21

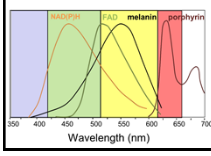


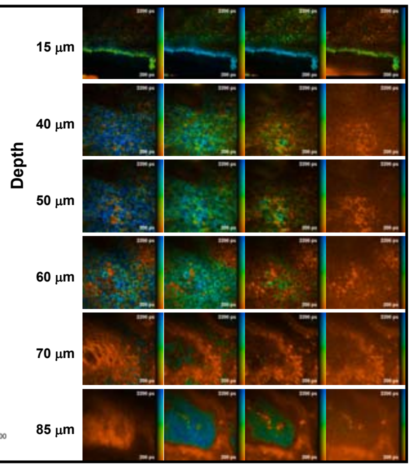
50 µm in vivo
100 µm in vivo

4 channel FLIM – *in vivo* imaging

Normal skin - medial forearm

Acquisition Time 25.5s/depth
Excitation @ 760nm
Images 177µm x 177µm

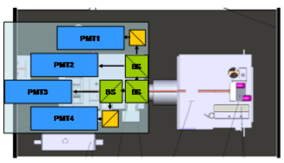


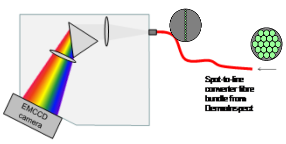


Clinical FLIM of human tissue in vivo

DermaInspect clinical multiphoton microscope

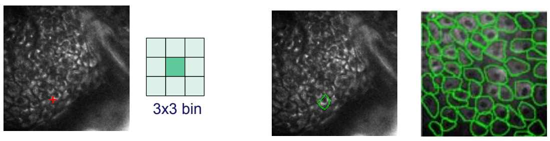
Imperial College London
Multispectral FLIM

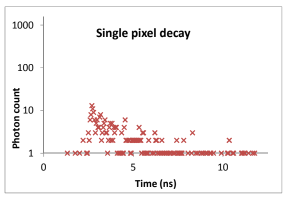


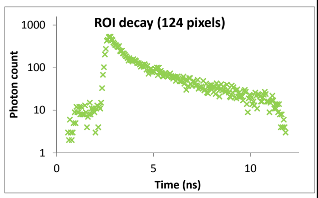


IRF measured using gold nanorods: broadband impulse *(Talbot et al, Opt Exp 2011)*

Segmentation ⇒ cellular autofluorescence lifetime







Clinical skin FLIM

Discrimination analysis

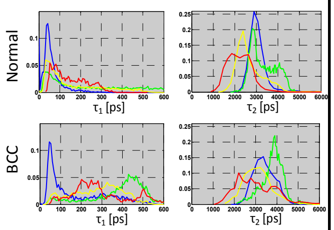
Per cell analysis, **BCC v. Normal**
Top 5 spectral/lifetime/morphological parameters ranked by discrimination

Diagnosis	Patients	Cells	%
BCC	10	2377	27.1
Melanoma	6	809	9.2
Naevus	15	2905	33.1
Normal (in vivo)	14	2696	30.6
Total	45 patients	8787	100.0

Channel/Lifetime component

Red/Short	AUC = 0.82
Blue/Short	AUC = 0.80
Yellow/Short	AUC = 0.77
Red/Long	AUC = 0.74
Green/Long	AUC = 0.73

Cellular morphology: AUC ≤ 0.66
Spectral components: AUC ≤ 0.63



Multidimensional fluorescence imaging

Imperial College London

"Thank you"

The Institute of Cancer Research

AstraZeneca · GE Healthcare · gsk GlaxoSmithKline · PerkinElmer · Pfizer · JenLab · STORZ · Mauna Kea Technologies · Kentech Instruments · THE ROYAL SOCIETY CELEBRATING 350 YEARS · BBSRC bioscience for the future · EPSRC Engineering and Physical Sciences Research Council · MRC · wellcome trust · Technology Strategy Board Driving Innovation · dti · NHS National Institute for Health Research