The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

310/1828 310/5

Workshop on Biomedical Applications of High Energy Ion Beams

Co-sponsored by: ICGEB and University of Surrey

12-16 February 2007

Venue:
Adriatico Guest House Giambiagi Lecture Hall
ICTP, Trieste, Italy

Cell And Tissue Imaging

Borivoj VOJNOVIC
Gray Cancer Institute, U.K.

Strada Costiera 11, 34014 Trieste, Italy - Tel. +39 040 2240 111; Fax +39 040 224 163 - sci_info@ictp.it, www.ictp.it

Workshop on Biomedical Applications of High Energy Ion Beams

Cell and Tissue Imaging

B. Vojnovic, University of Oxford, Gray Cancer Institute

vojnovic@gci.ac.uk

ICTP, Trieste 13th February 2007

Cell and Tissue Imaging

Presentation outline

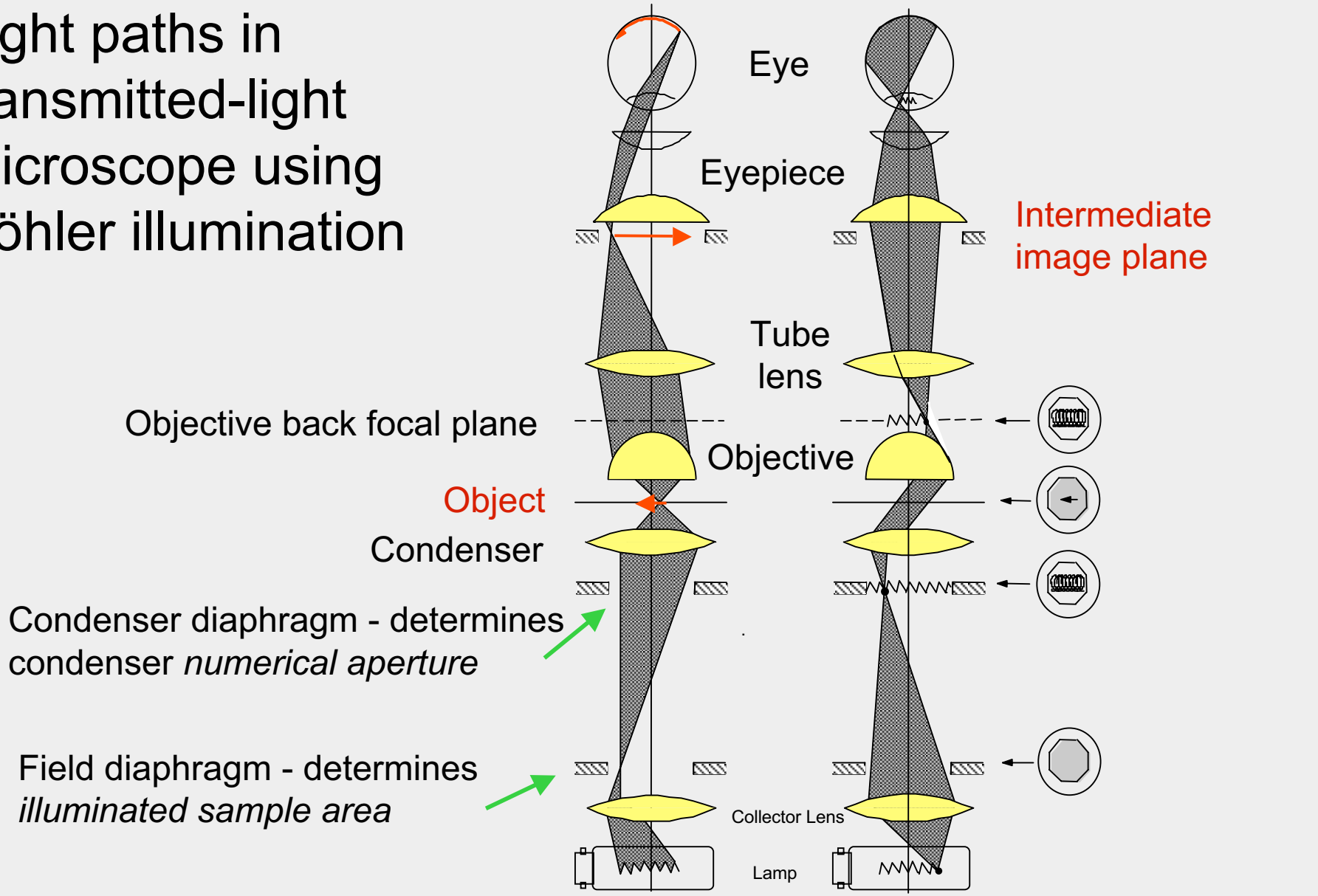
- The microscope and microscopy imaging methods
- Fluorescence microscopy and ‘deep’ imaging methods
- Widefield and beam-scanning methods
- Time-resolved and non-linear excitation methods
- Molecular imaging / protein interactions
- Image processing and segmentation
- Imaging methods applied to micro-irradiation

Light Microscopy basics….

We've probably all learnt about what a microscope does, but there is no harm in being reminded…

It does ***not*** 'make small objects larger', it shows us how small objects ***interact*** with light…

Light paths in transmitted-light microscope using Köhler illumination



Microscope resolution – Rayleigh criterion

Defined as distance between two adjacent points which are ‘just resolved’

When the objective numerical aperture matches that of the condenser, R is determined from the equation:

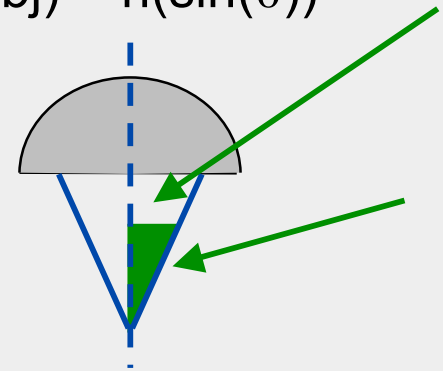
$R = 0.61 \lambda / NA(obj)$

λ: wavelength of light

NA(obj): numerical aperture of objective

Numerical aperture is determined by:

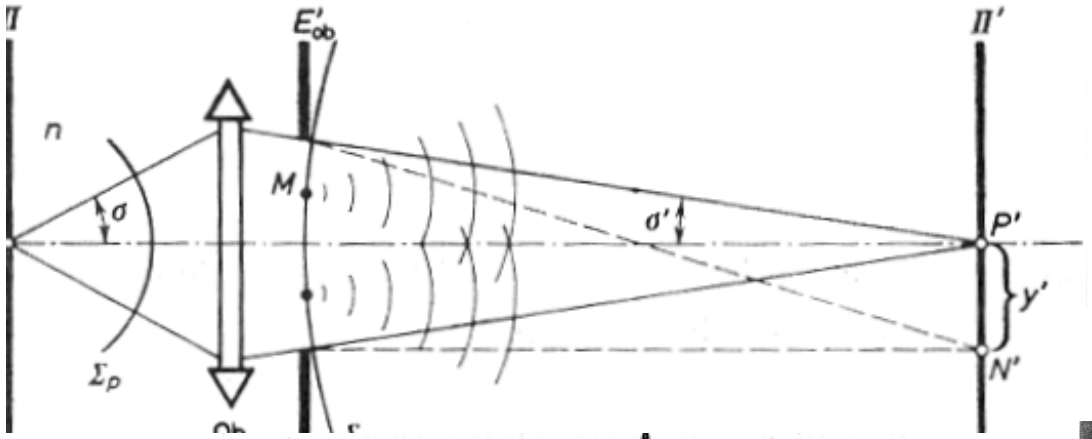
$NA(obj) = n(\sin(\theta))$

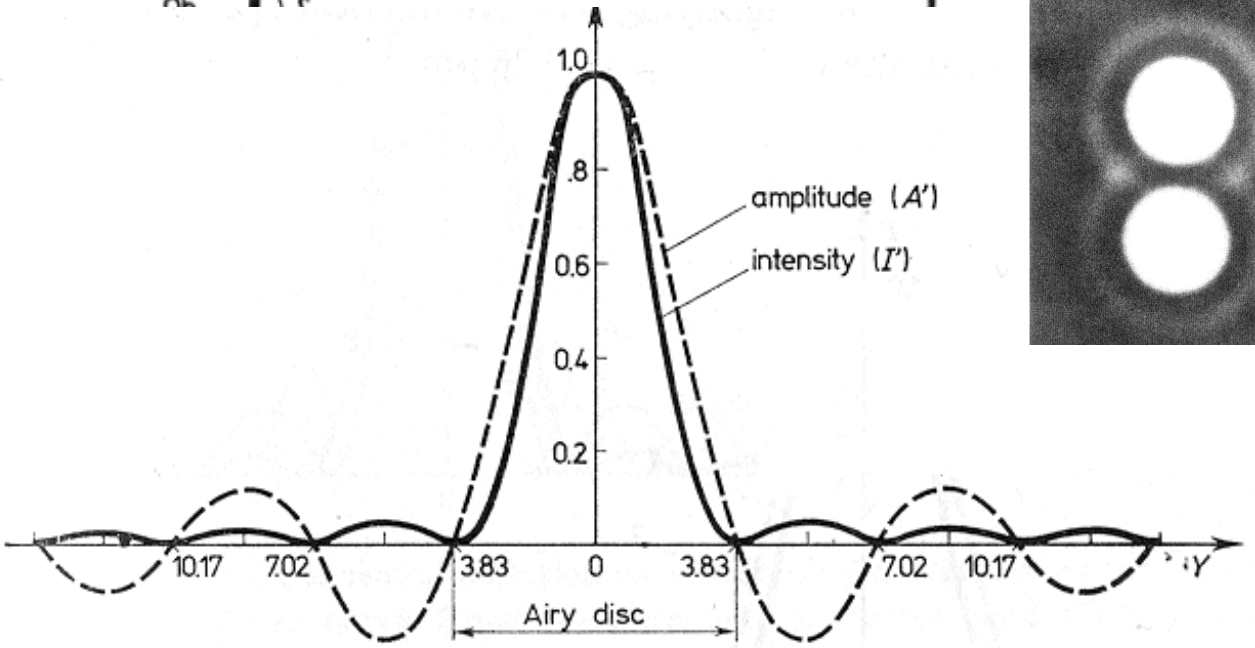


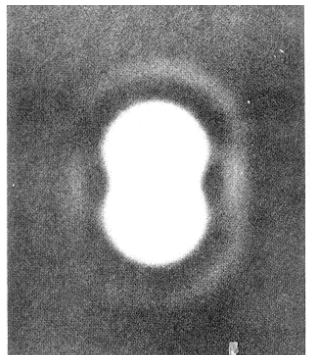
n = refractive index of the medium between the objective and the specimen (air, water, oil)

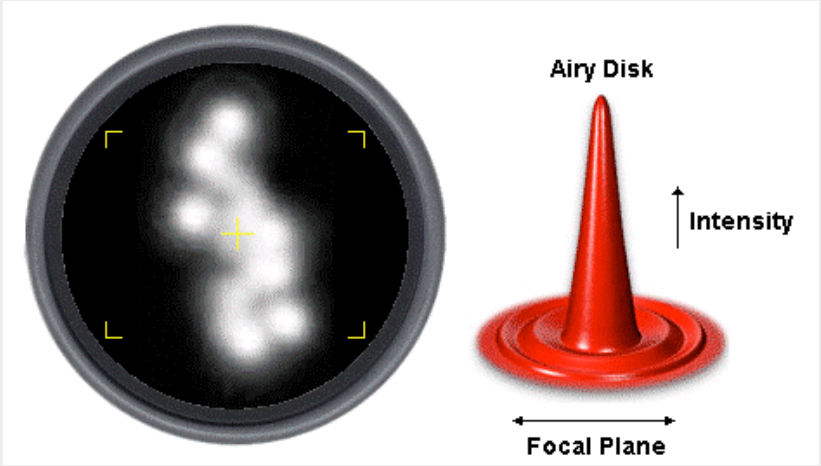
θ = the angle defining the half-cone of light captured

Microscope resolution

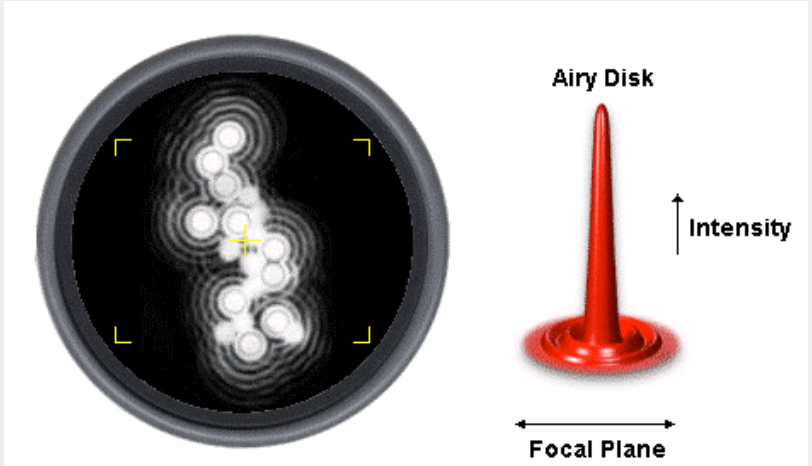




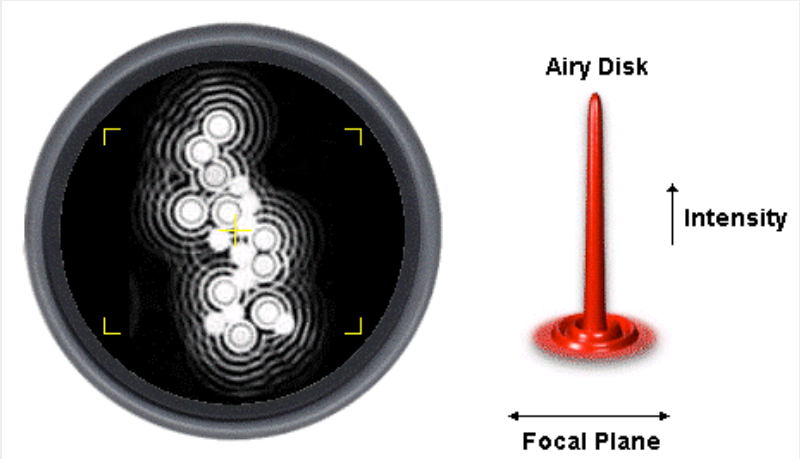




0.2 na

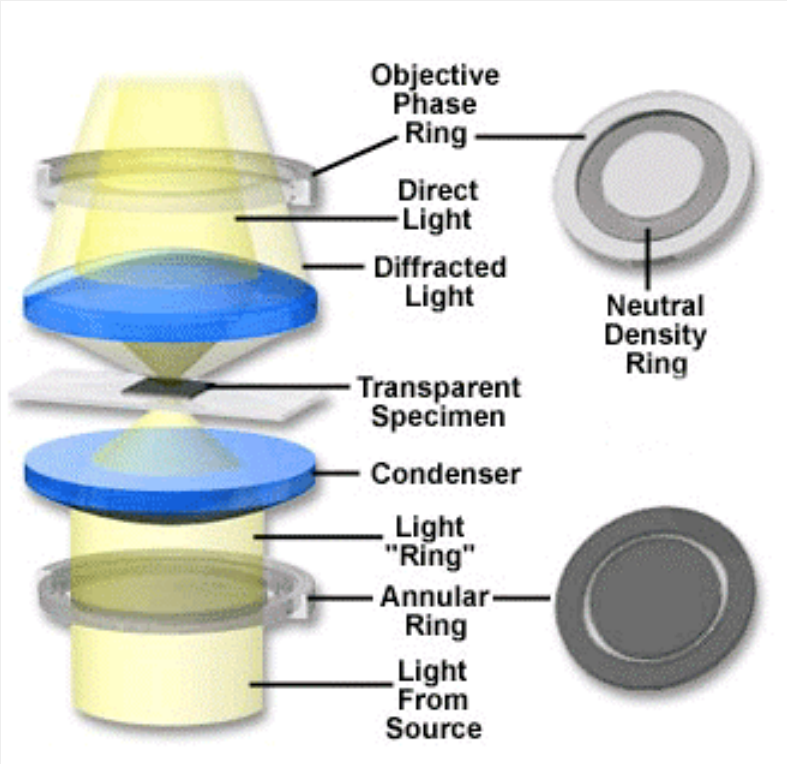


0.94 na



1.4 na

Imaging transparent objects

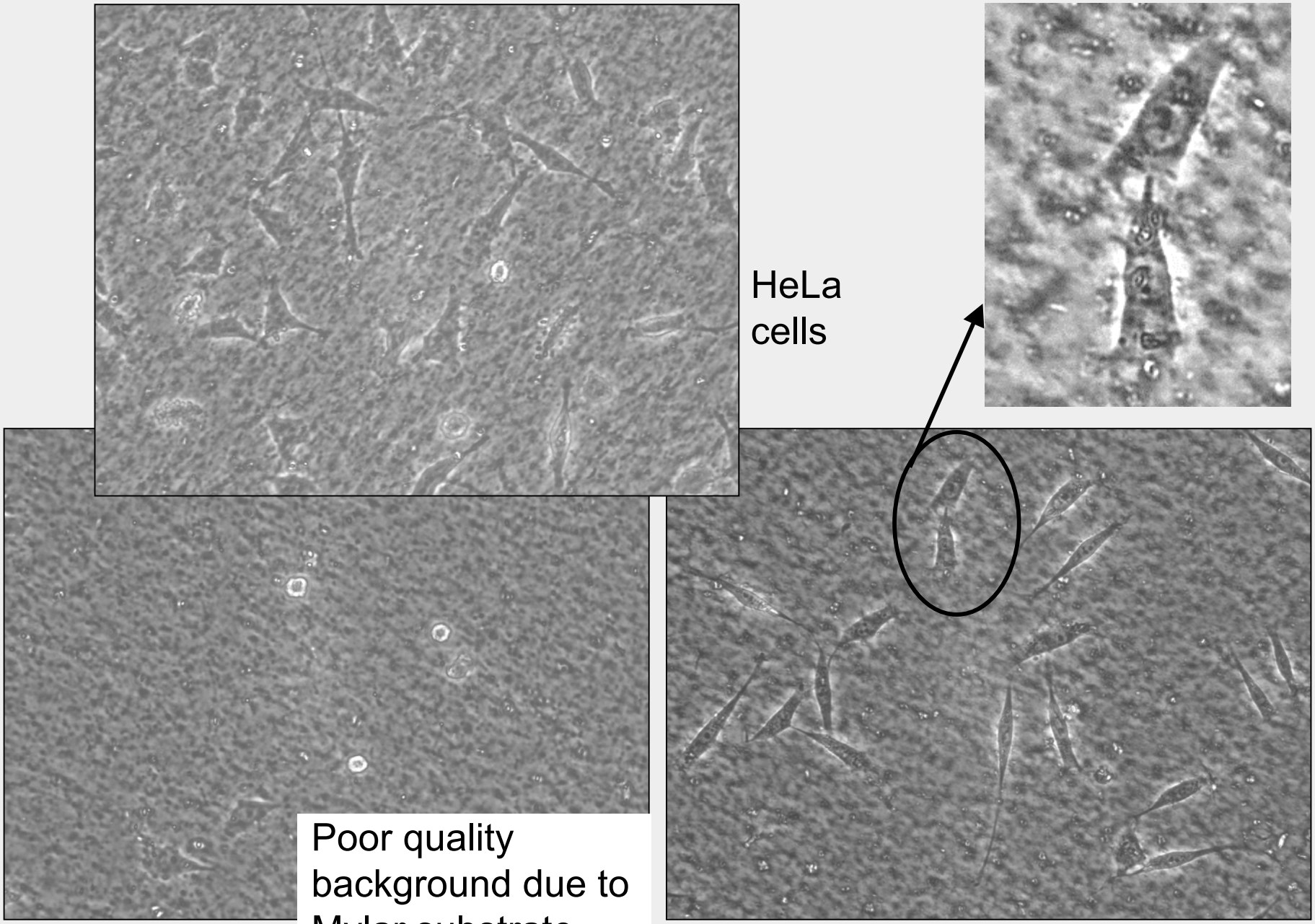


In **phase contrast microscopy**, a hollow cone of light emanating from the phase annulus (ring aperture) illuminates the specimen.

Small refractive index differences within cellular components generate image contrast.

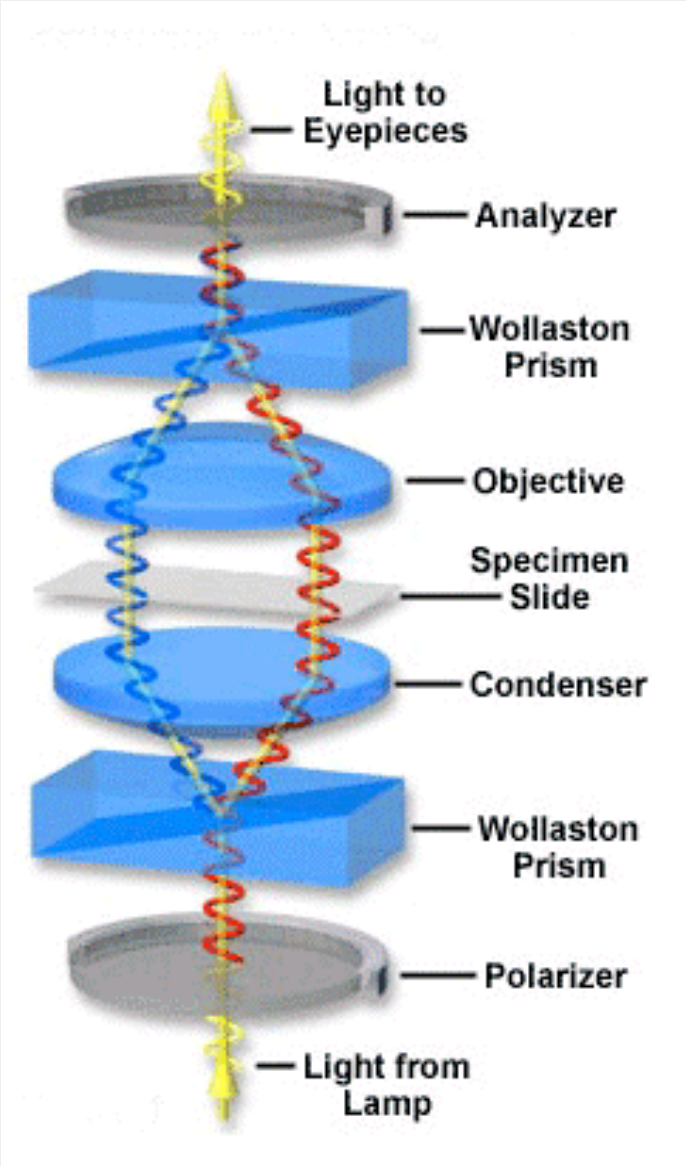
Light is either ***diffracted*** by cellular components, extending over all of objective rear focal plane or passes through ***undeviated*** through neutral density phase ring objective.

In the image plane, an interference pattern results, which produces **intensities proportional to the phase shift** induced by the specimen.



From G. Flaccavento

Differential Interference Contrast (DIC)



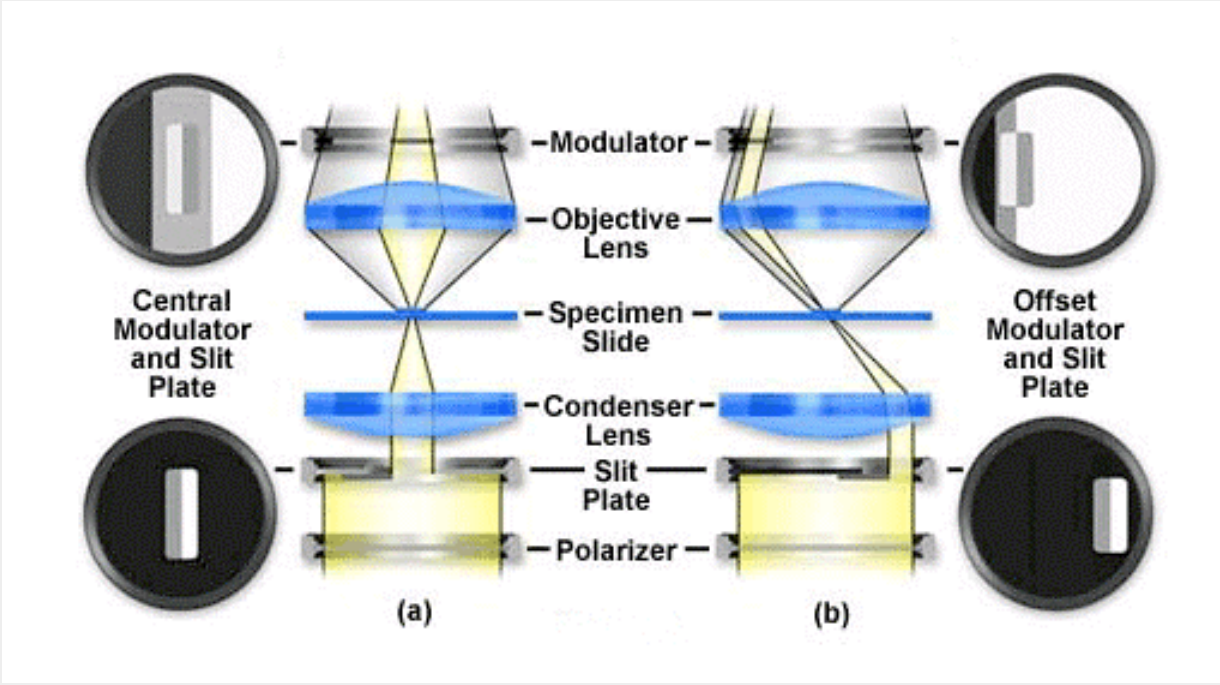
Illumination through a **polarizer** and a **Wollaston** prism that splits polarized light into two beams vibrating **perpendicular** to each other, travelling in slightly different directions. These are recombined in a second prism.

Colour and/or light intensity effects are related to **rate of change in refractive index**, **thickness**.

Orientation of specimen has pronounced effect on the relief-like appearance e.g. 180 degrees changes a hill into a valley or *vice versa*.

3-D appearance is **not a representation of the true geometric nature** of the specimen, but is an exaggeration based on optical thickness.

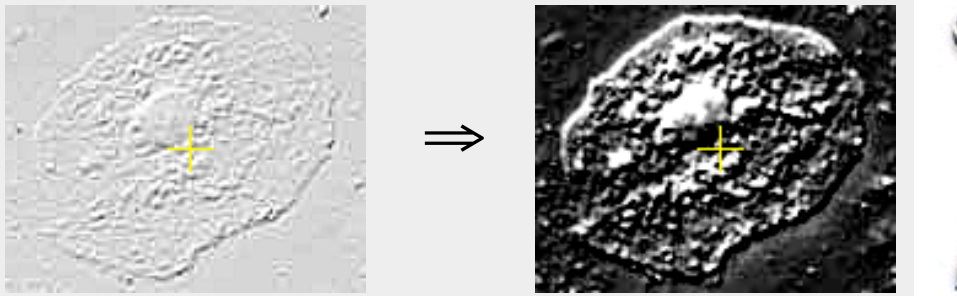
Hoffman modulation contrast

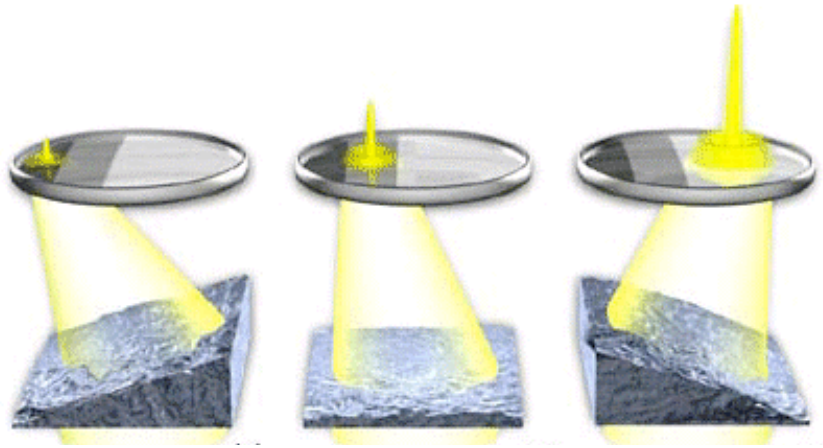


Simpler than DIC, relies on different optical thicknesses causing deviations of linearly polarised light through modulators with neutral density patterns.

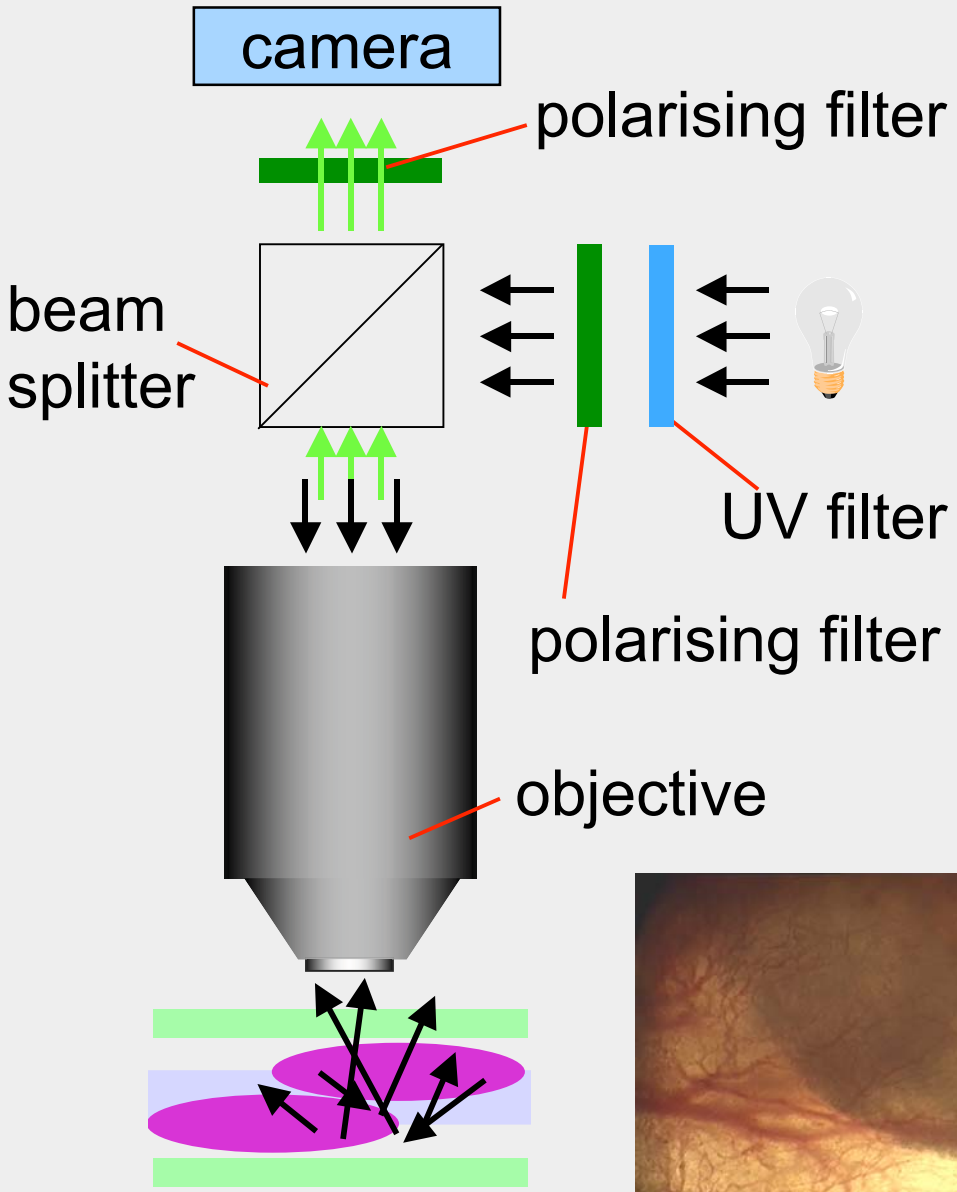
Contrast generated by phase gradients.

Δ phase ⇒ Δ amplitude



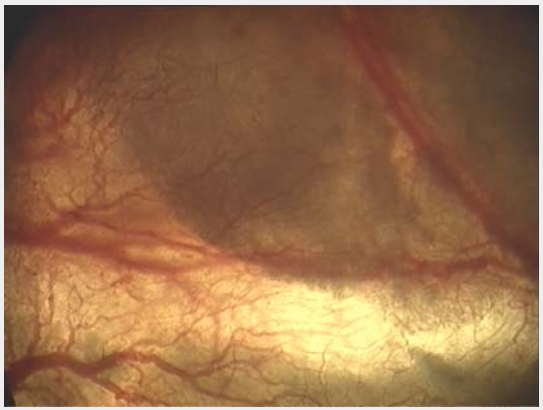


Polarised light sample epi-illumination



light is scattered in the sample and its plane of polarisation is altered

…so it effectively ‘trans-illuminates’ the preparation

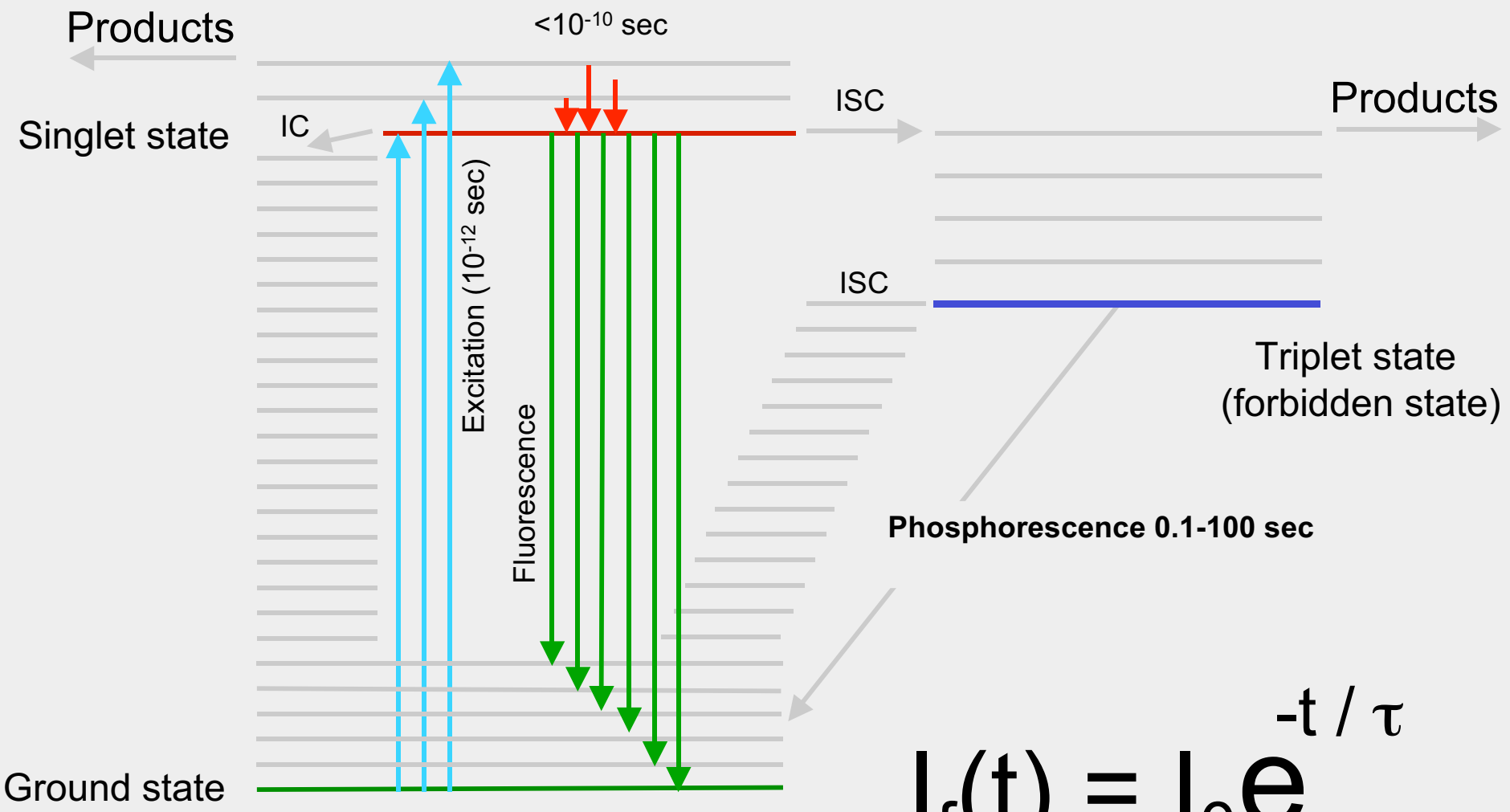


GRAY CANCER INSTITUTE

Fluorescence imaging basics….

We've probably all learnt it but how far can we take it?

Jablonski diagram of fluorescence processes



Emitted light always of longer wavelengths than the excitation light (**Stokes' Law**).

$$I_f(t) = I_0 e^{-t/\tau}$$

Photobleaching significantly reduces or stops fluorescence altogether.

Reduction of emission intensity is also called **fading**.

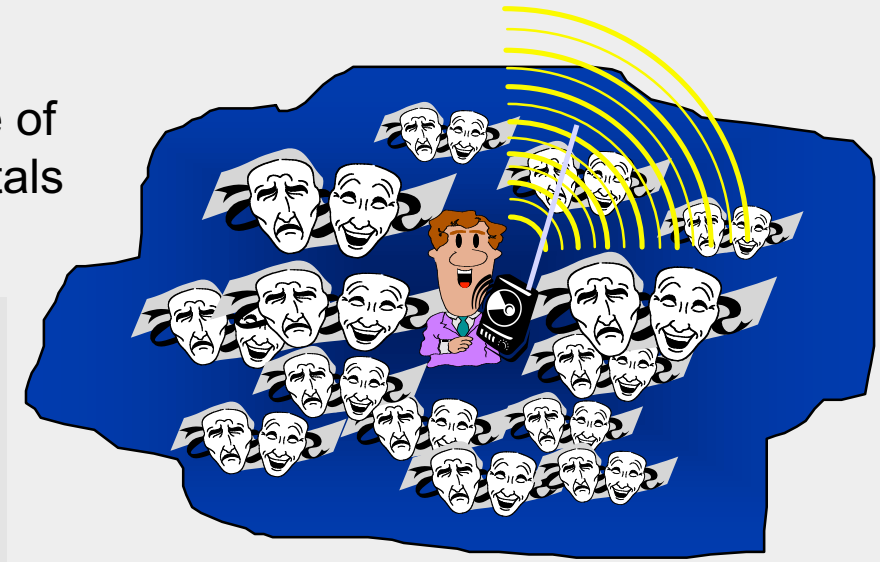
This is subdivided into **quenching** and **bleaching**.

Bleaching is the irreversible decomposition of the fluorescent molecules in the presence of molecular oxygen.

Quenching also results in reduced fluorescence intensity and frequently comes about as a result of the presence of oxidizing agents or of salts of heavy metals or halogen compounds.

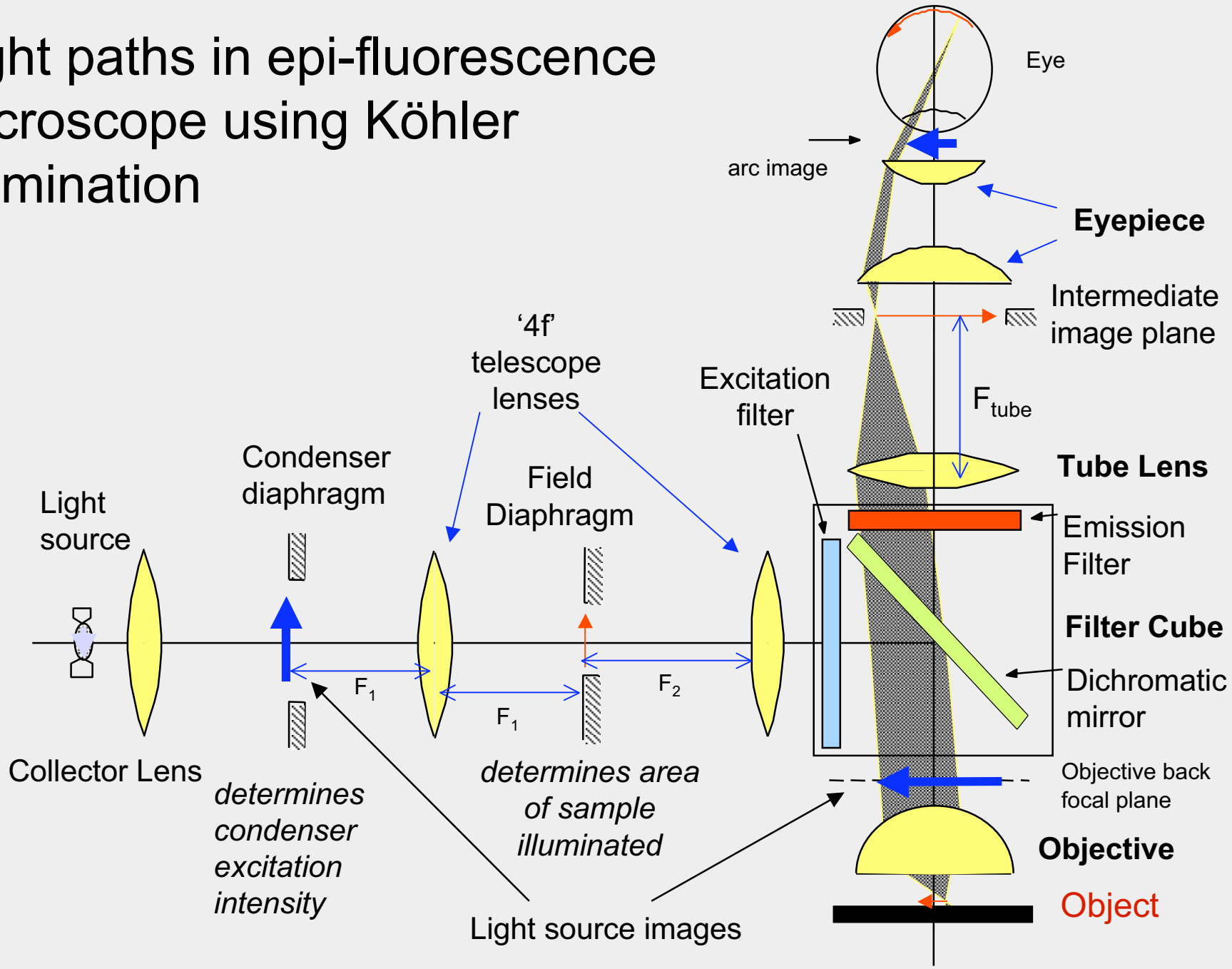
Quenching can also result from the *transfer of energy* to other so-called acceptor molecules, physically close to the excited fluorophores:

Resonance Energy Transfer. This is the basis for a new techniques of measuring distances far below the lateral resolution of the light microscope.

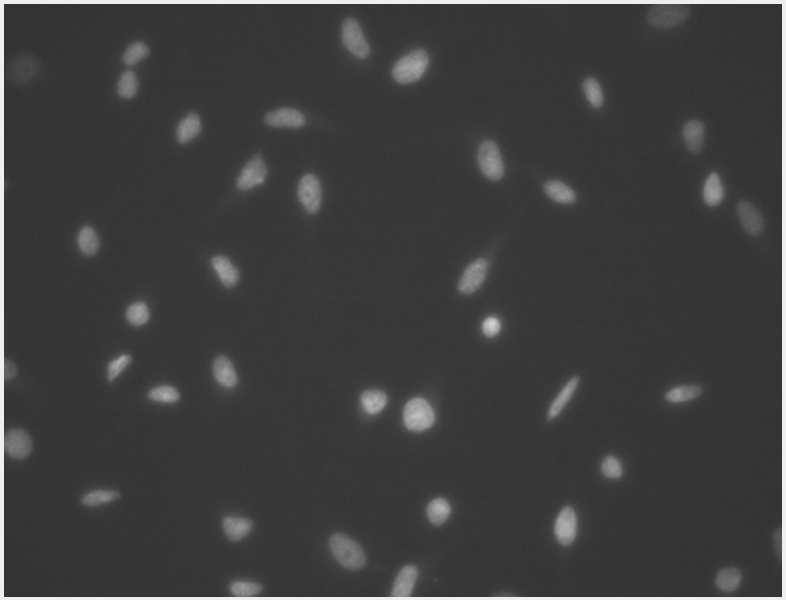


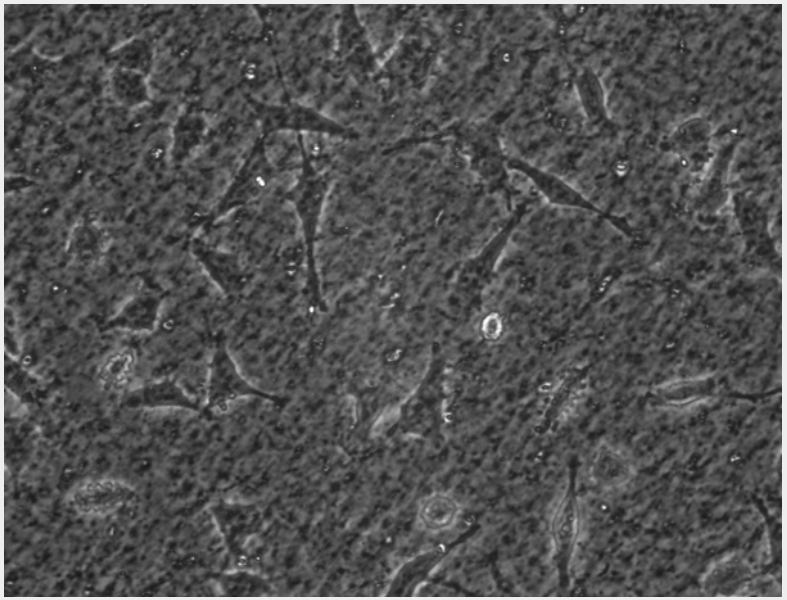
Fluorescent probe molecule reports 'classified' information on behaviour of surrounding molecules

Light paths in epi-fluorescence microscope using Köhler illumination



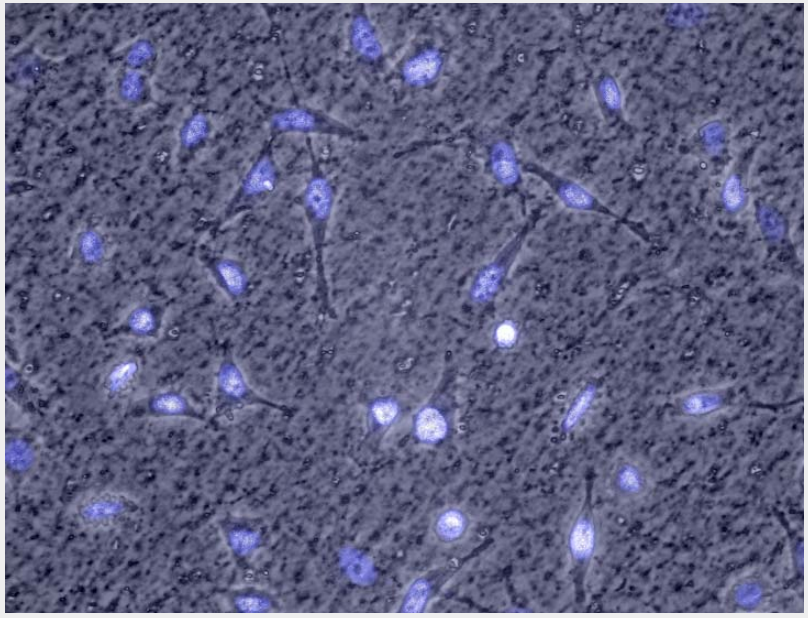
HeLa cells





Fluorescence

Intercalating DNA stain



phase contrast

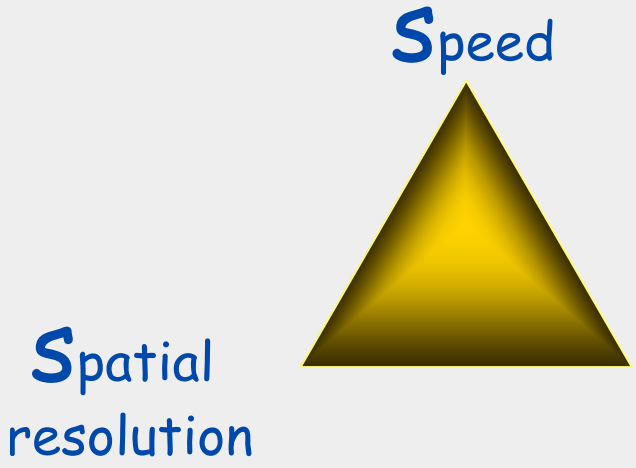
Poor quality background due to Mylar substrate

From G. Flaccavento

GRAY CANCER INSTITUTE

Fluorescence photon budgets

- Fluorophore molecule can emit typ. **10^3 - 10^4 quanta**



The three Ss or the 'eternal fluorescence triangle'

- Any of the three can be enhanced at the expense of the other two
- Available quanta can be resolved in time or in wavelength, but only up to max. available

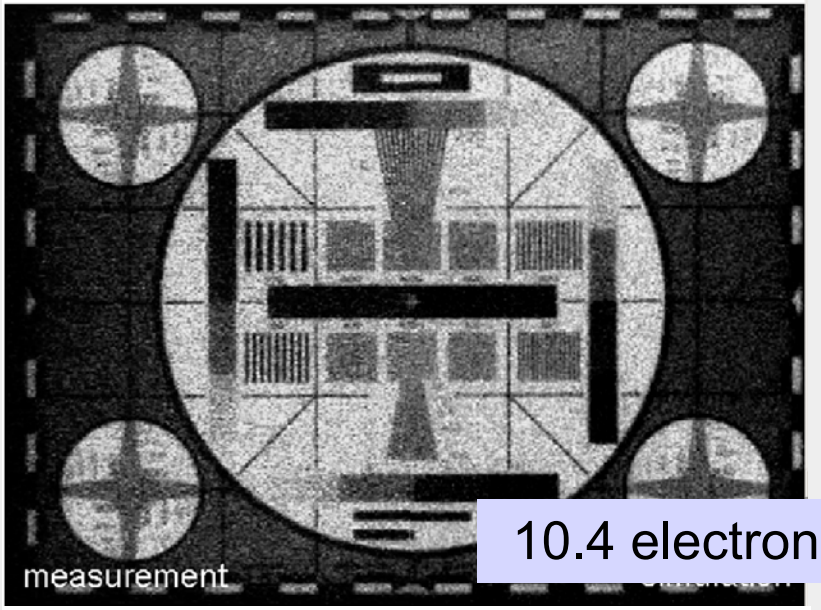
Fluorescence photon budgets

- 10^6 molecules @ 1 μM cell ≈ 1 pl
- 10^{10} photons **per cell** maximum
- At max. excitation ≈ 10^{13} photons per sec
  - Fluorescence lifetimes ≈ 10^{-9} - 10^{-8} sec typical
- Collection, QE limitations ≈ 1-10% photons 'used'
- 10^8 -10^9 detected quanta **per cell** practical
- At max. excitation ≈ 10^{11} -10^{12} photons per sec
  - s/n ratio ≈ 10^4 -10^5 for steady-state signal

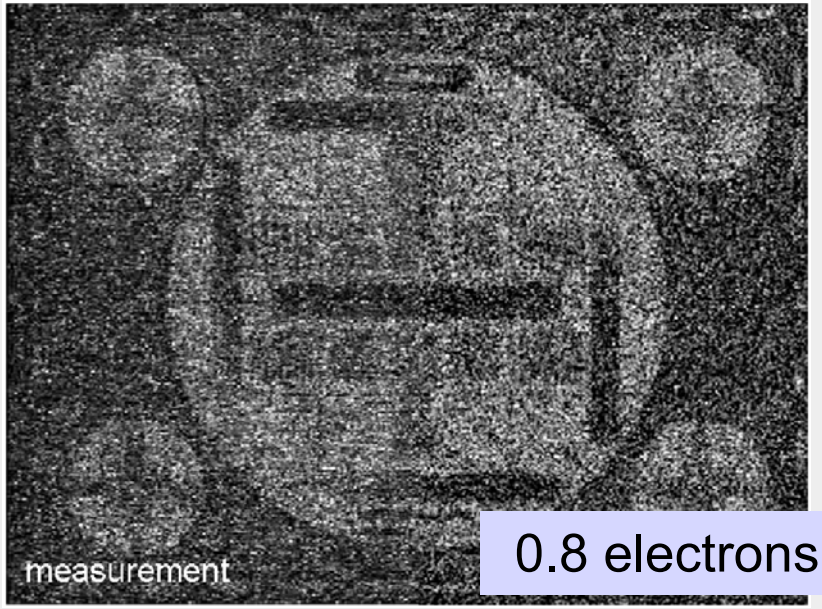
Distributed over time, space

GRAY CANCER INSTITUTE

The ‘ultimate’ camera? EMCCD

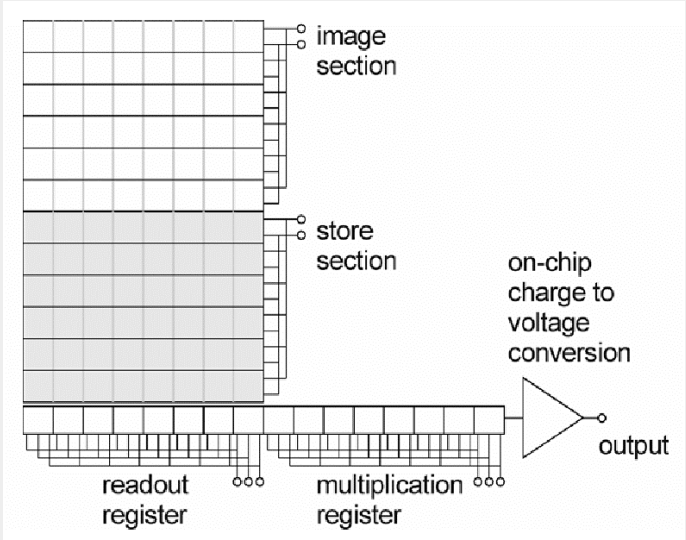


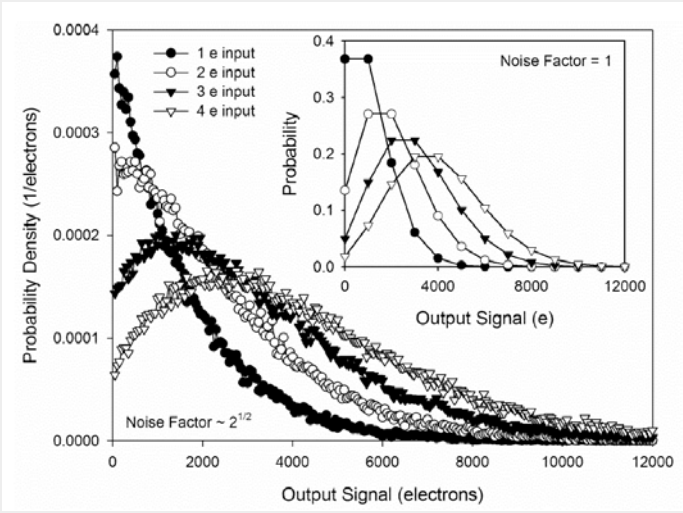
10.4 electrons pixel^{-1}



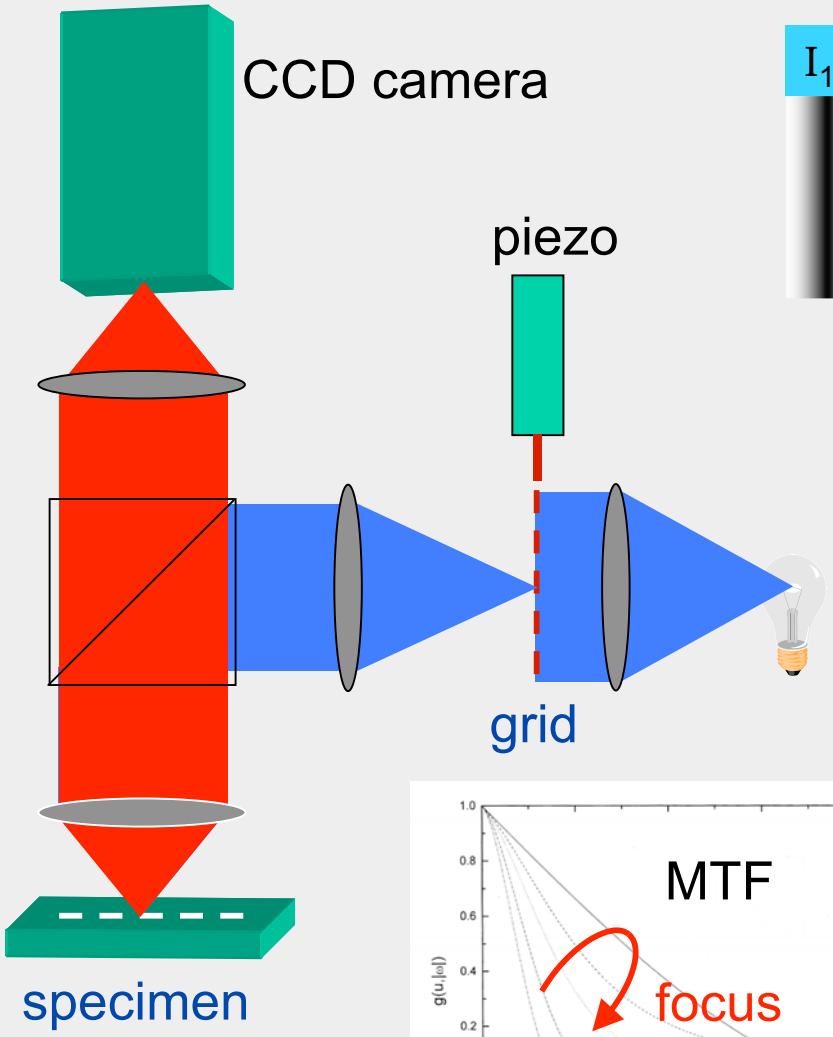
0.8 electrons pixel^{-1}

Electron multiplication – typ. x1000

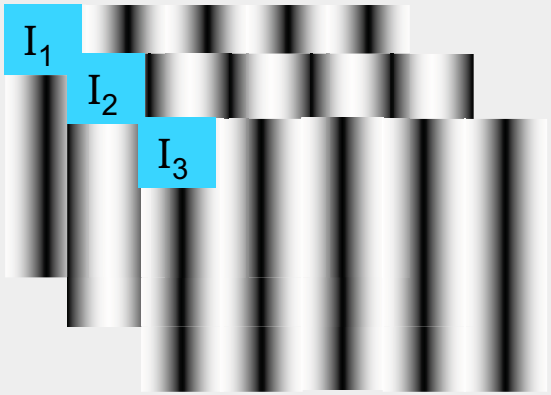




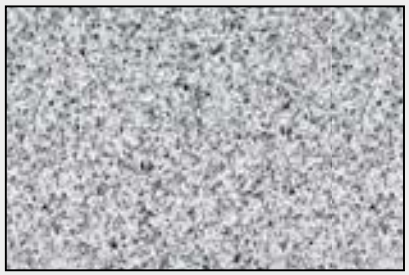
Fast optical sectioning



⇨ Capture images at 3 grid phases



$$I_p = \left[(I_1 - I_2)^2 + (I_1 - I_3)^2 + (I_2 - I_3)^2 \right]^{1/2}$$



Optically sectioned image

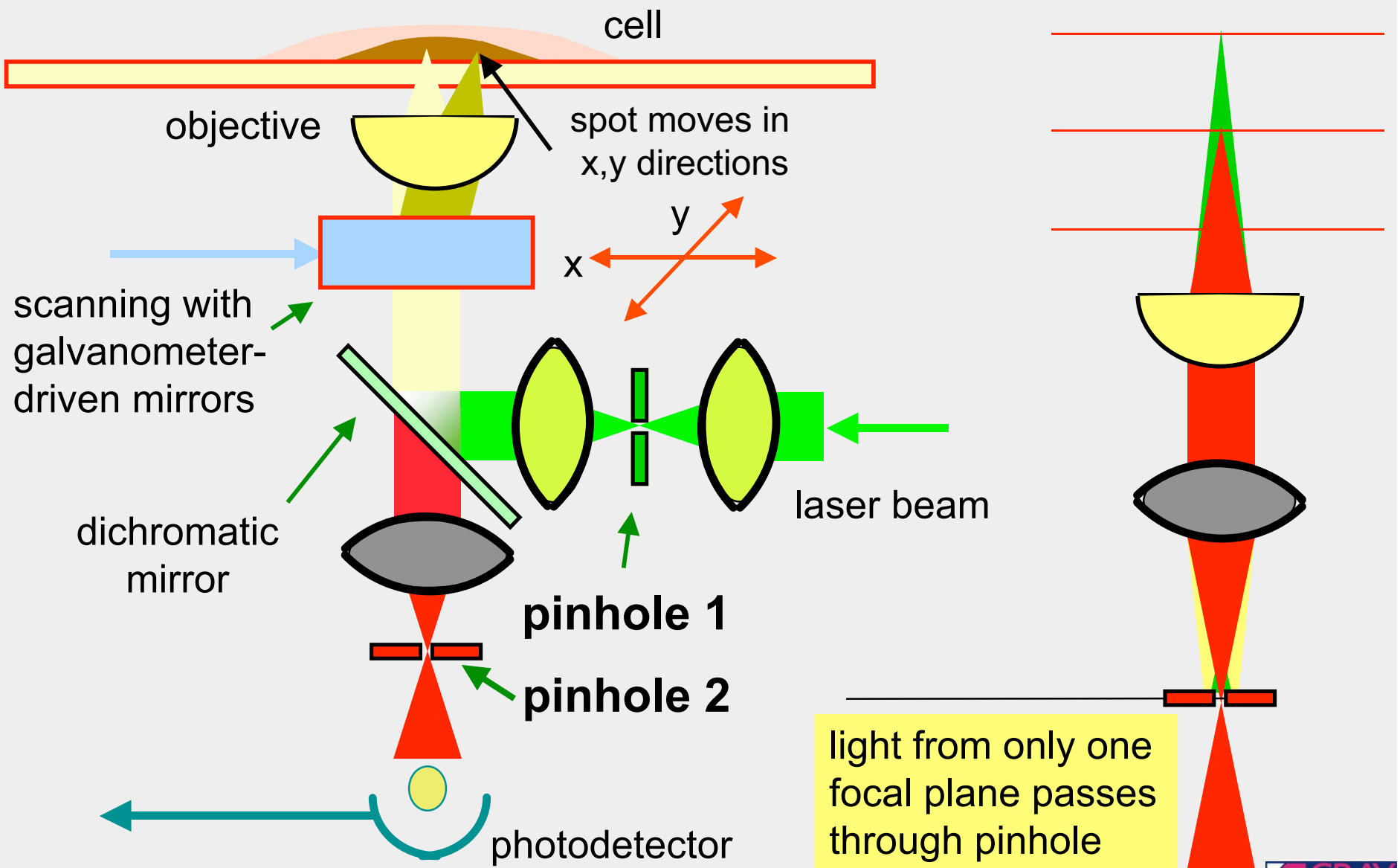
- Conventional image also available

T. Wilson Group, Oxford

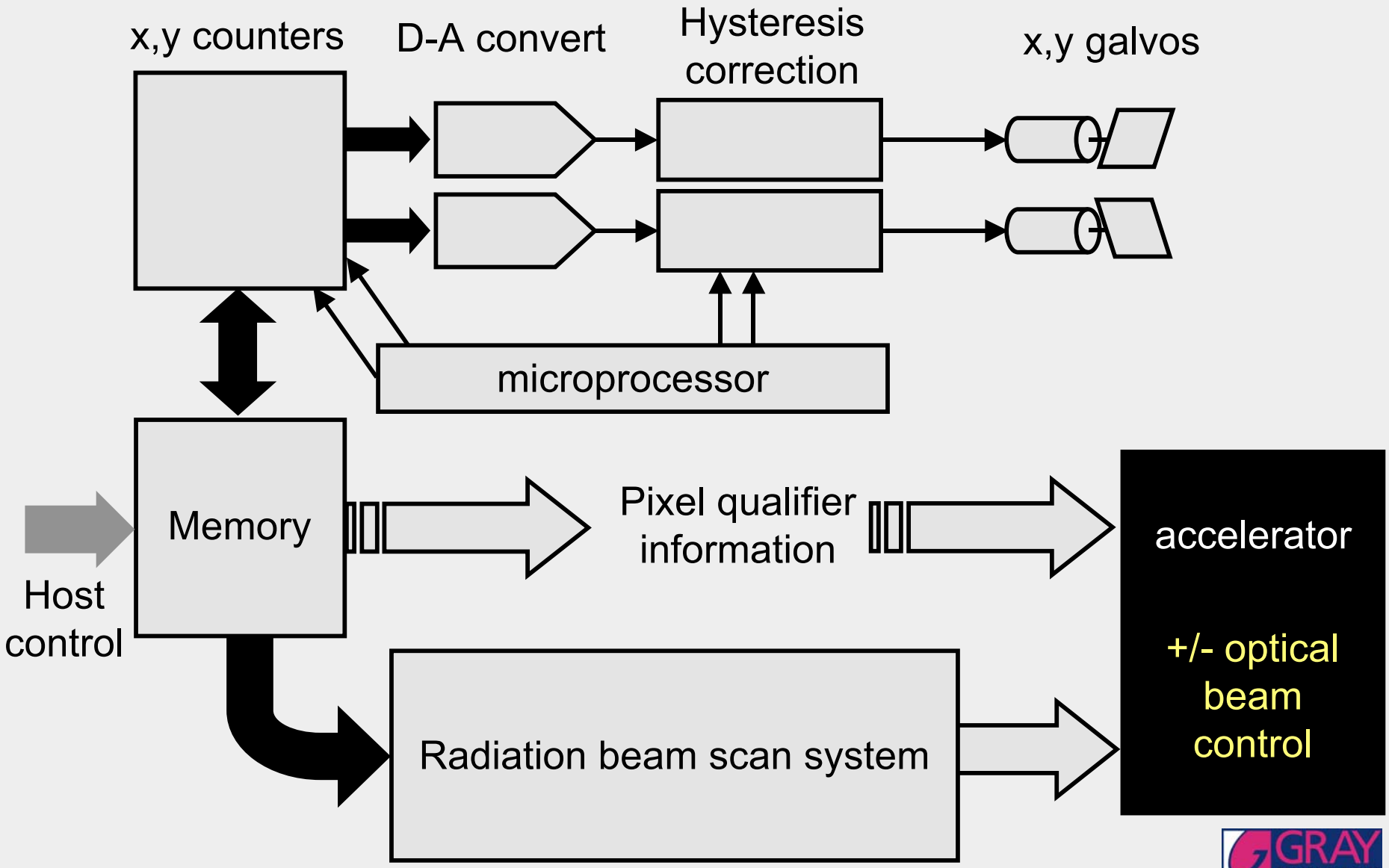
Scanning and Confocal Microscopy

…..able to provide optical sectioning

The laser-scanning confocal microscope



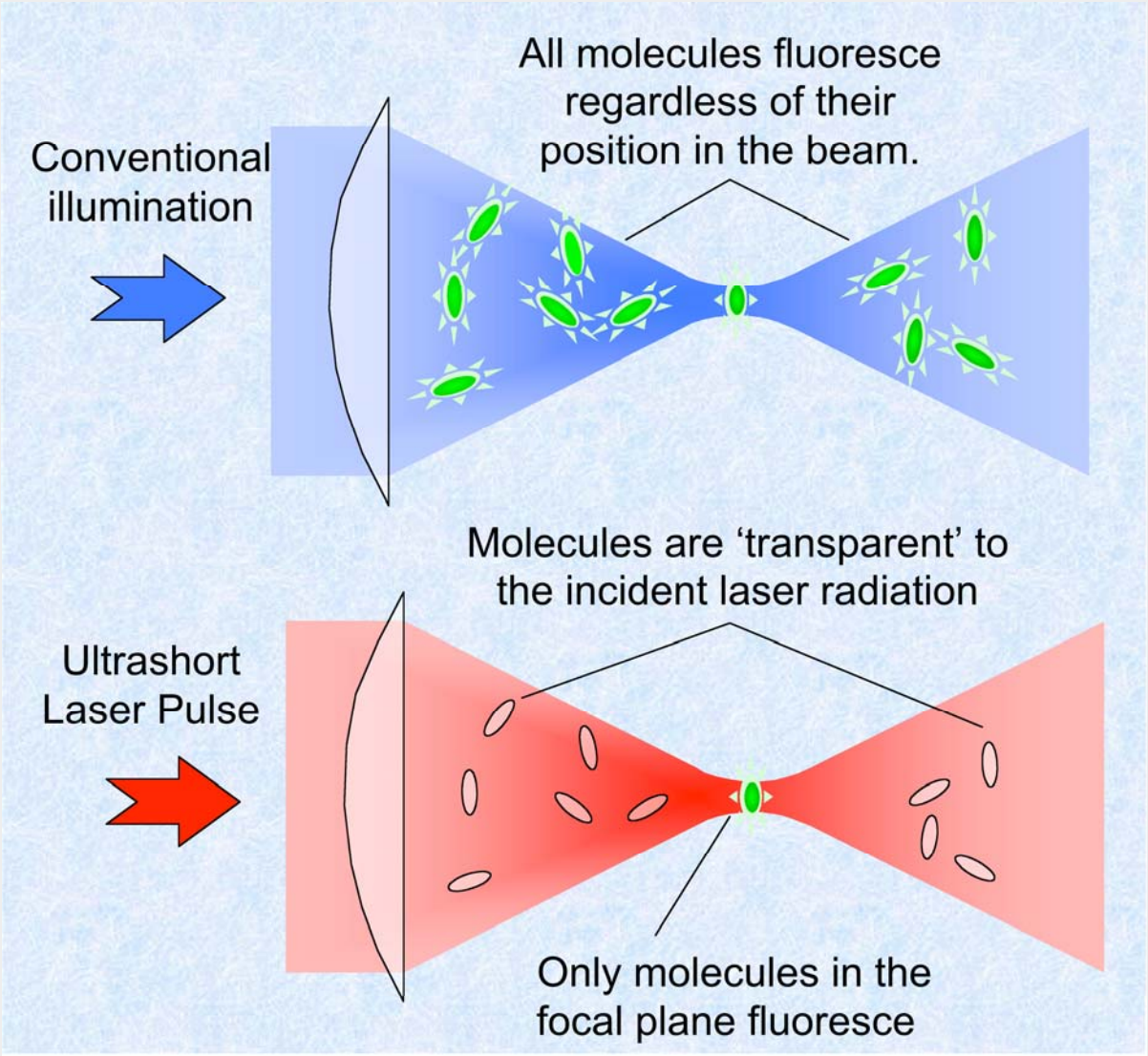
Maintaining optical scan registration



Scanning microscopy exploiting absorption of two or more photons

…..a better way to provide optical sectioning

Multi-photon excitation – principle



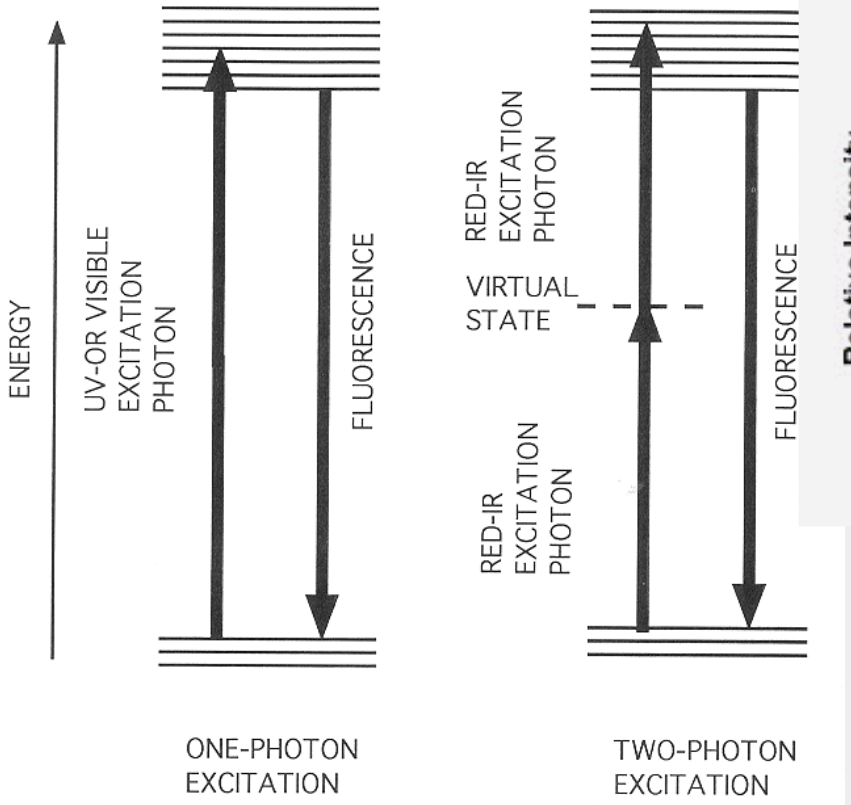
An *ultrashort* laser pulse is used for excitation – very high peak power, very low average power, …100 fs in 10 ns…

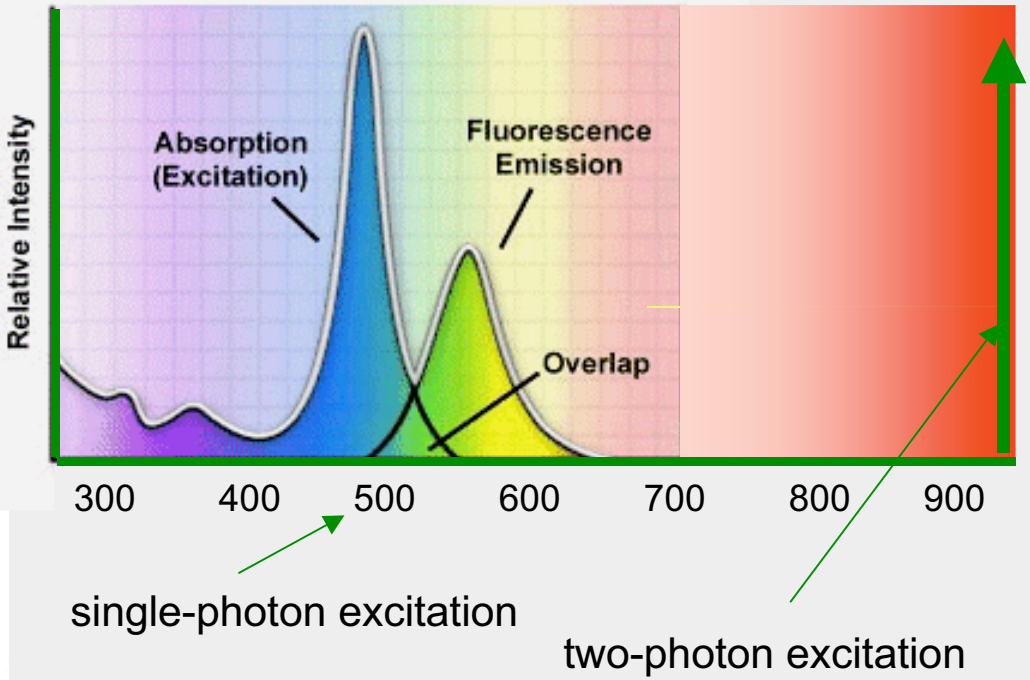
Only a single voxel is excited at any one time – photon density appropriate only at focus

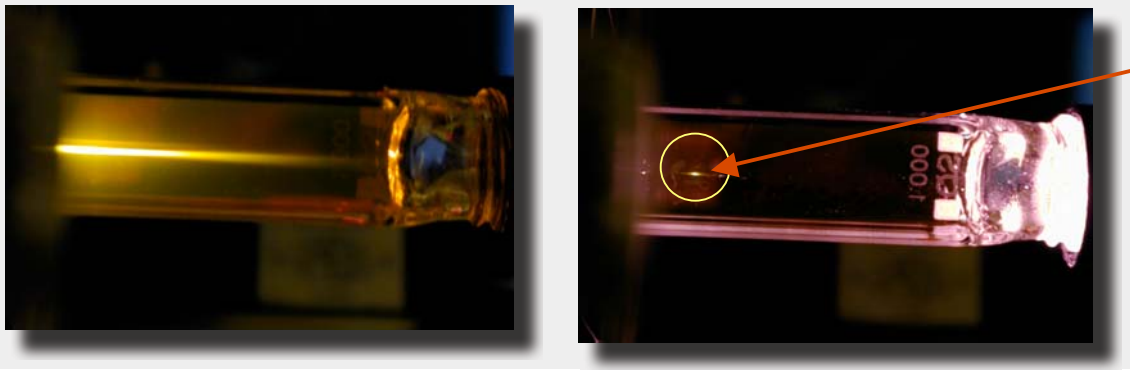
This excitation point is scanned in x,y and z to build up 3-D image

GRAY CANCER INSTITUTE

Multi-photon excitation - principle







Two-photon fluorescence excitation

Number of photons $\mathbf{n_a}$ absorbed per **laser pulse** through two-photon excitation

$$n_a \sim \frac{(p_0^2 \delta)}{(\tau_p f_p^2)} \quad [A^2/2\hbar c\lambda]^2$$

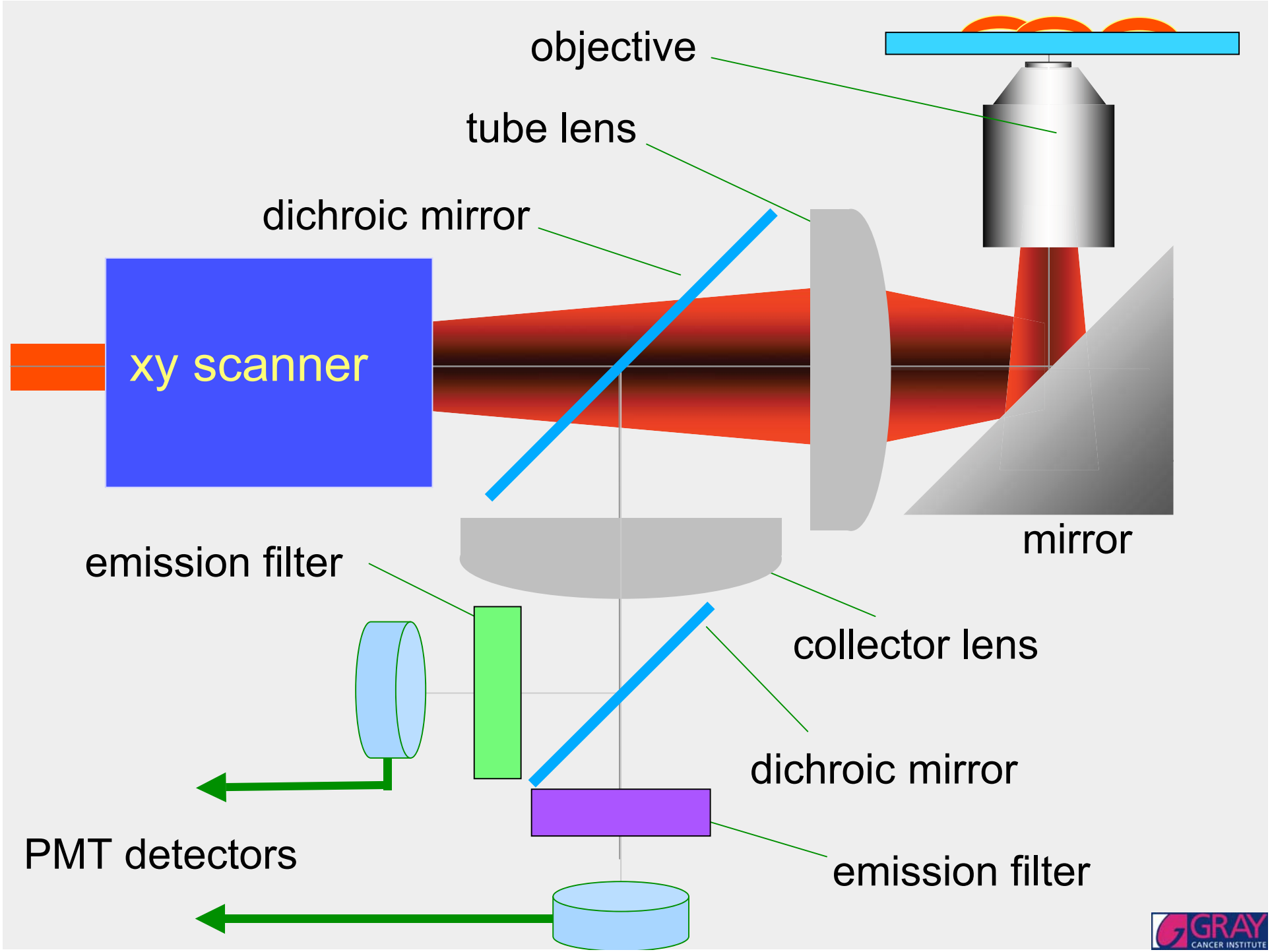
p_0 average laser power
δ 2-photon absorption x-section
τ_p laser pulse duration
f_p laser repetition rate
A objective numerical aperture
$\hbar$ Planck's constant
λ wavelength

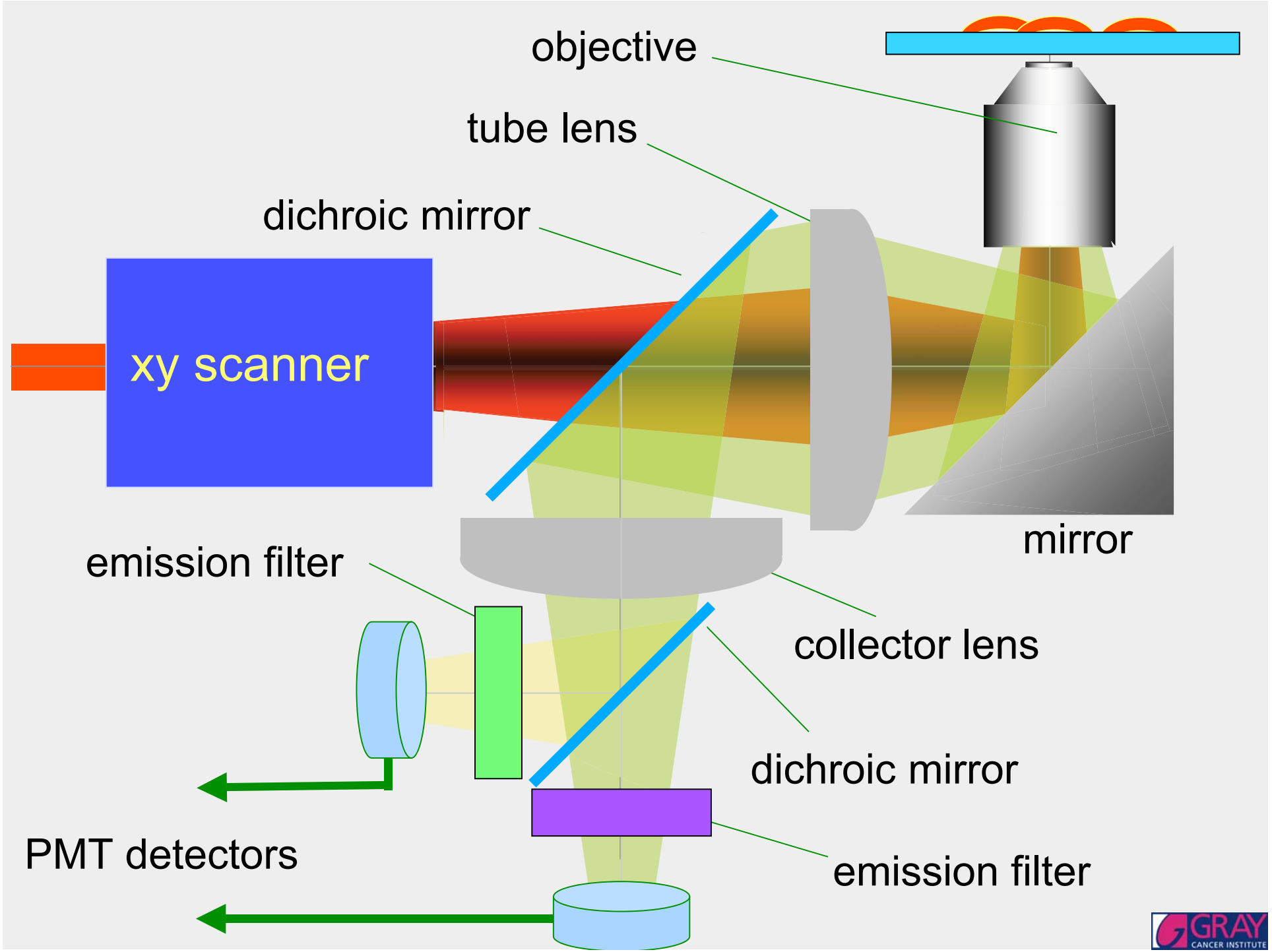
Peak 10 kW+ per pulse
10^{10} photons per 100 fs pulse
mW average power

Typ. repetition rates 80 MHz
Typ. pixel dwell time 10 μs

Only a few photons per **laser pulse** are generated

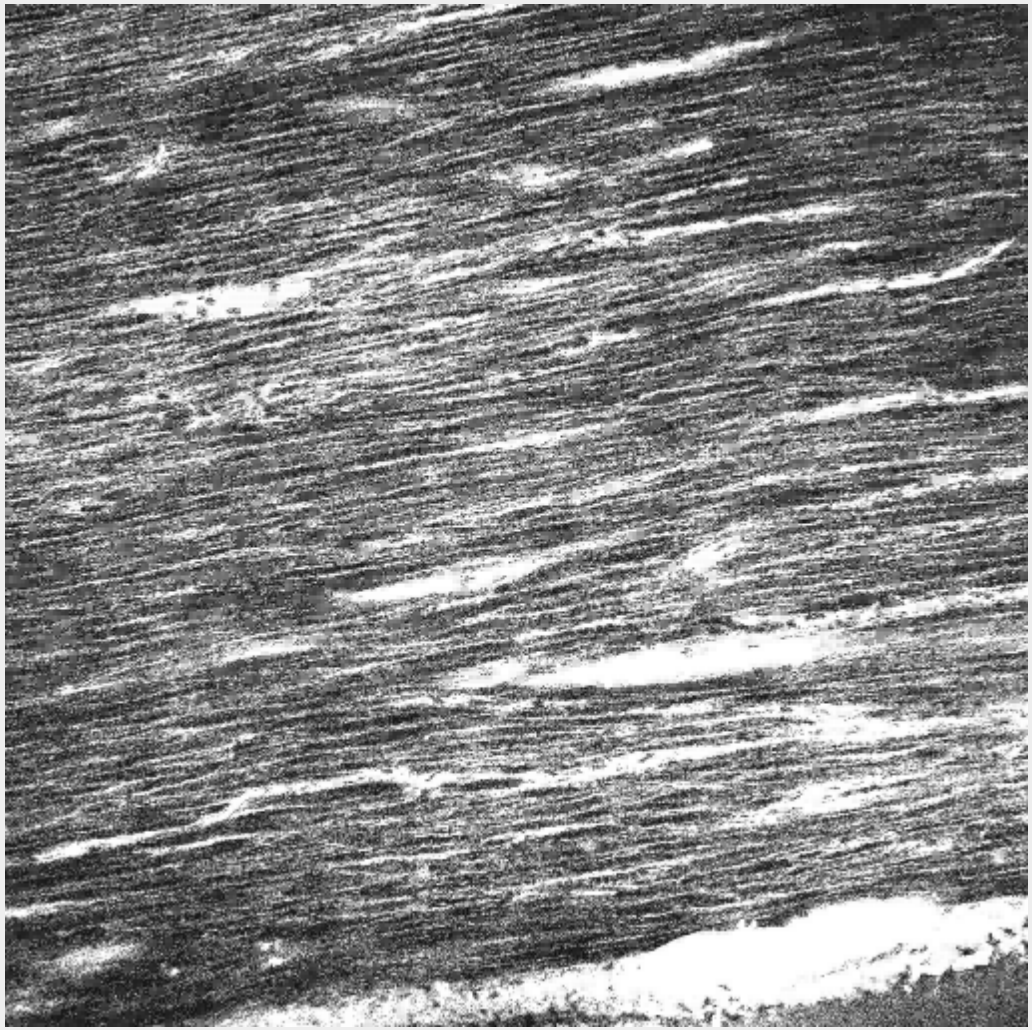
GRAY CANCER INSTITUTE


objective
tube lens
dichroic mirror
xy scanner
mirror
emission filter
collector lens
dichroic mirror
PMT detectors
emission filter
GRAY
CANCER INSTITUTE

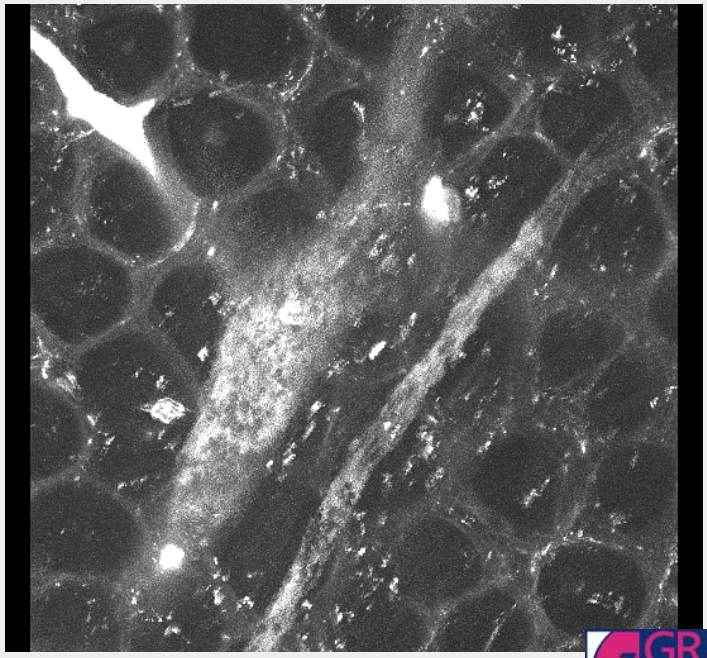

objective
tube lens
dichroic mirror
xy scanner
mirror
emission filter
collector lens
dichroic mirror
PMT detectors
emission filter
GRAY
CANCER INSTITUTE

Ex vivo imaging - rat gut

Image sequence of successive optically-sectioned layers

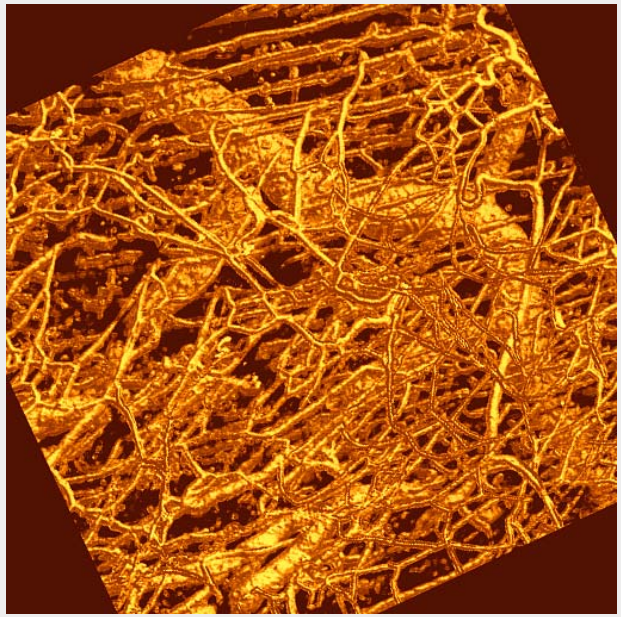


With MP excitation, it is possible to image deeply into specimens, (>>100 microns)

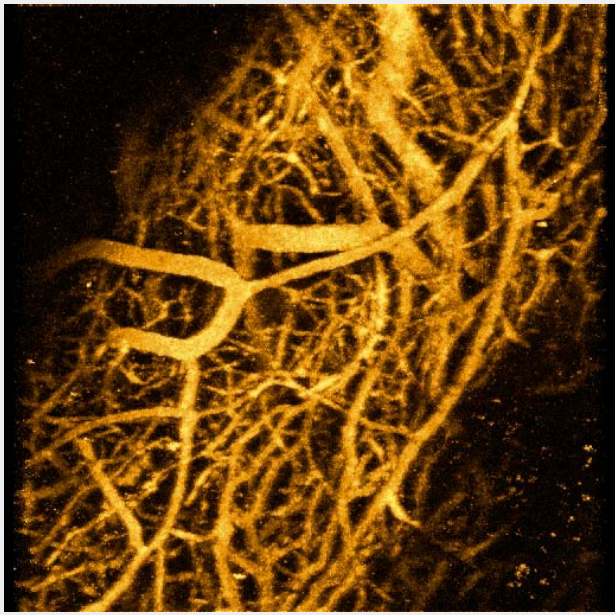


GRAY CANCER INSTITUTE

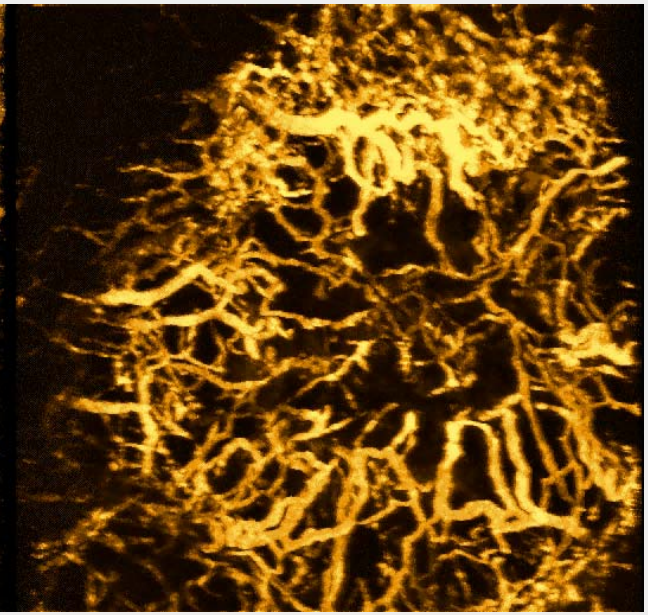
Two-photon fluorescence imaging - *in vivo*



muscle vasculature
CBA mouse



P22 fibrosarcoma
BD9 rat

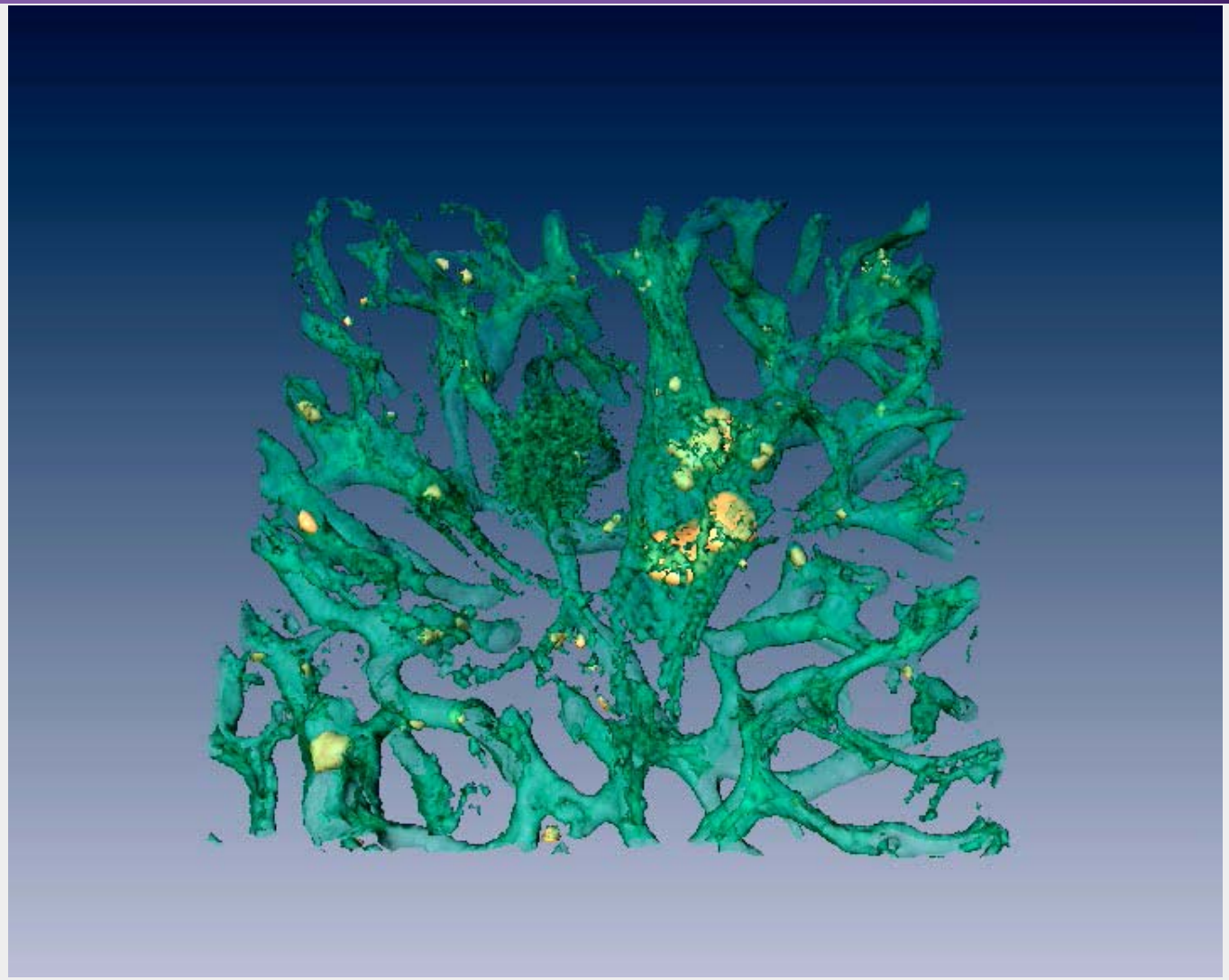


HT29 human colon carcinoma
SCID mouse

GRAY CANCER INSTITUTE

With G. Tozer, V. Prise

High resolution, microvessel imaging



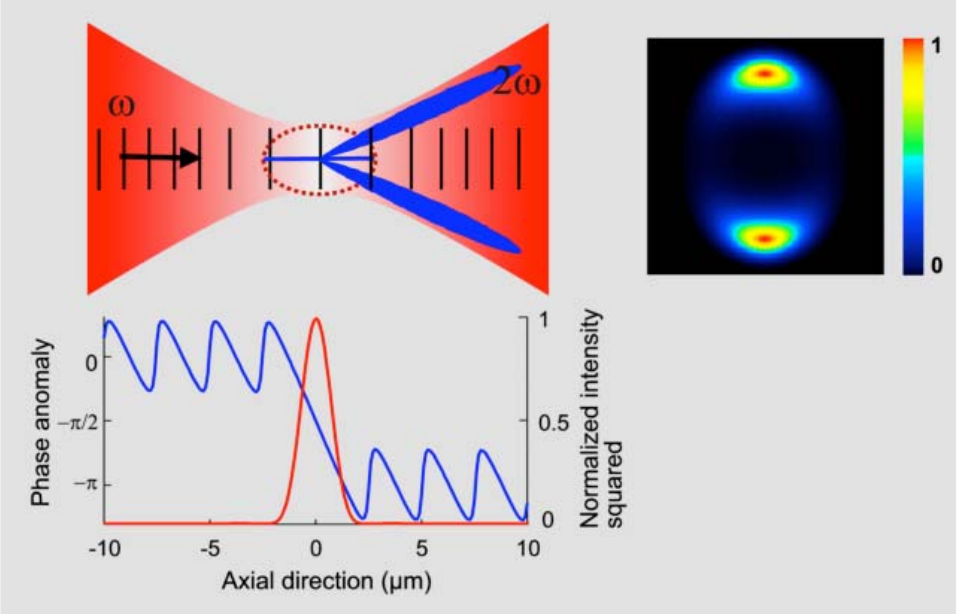
Liver metastasis formation

GFP tumour cells

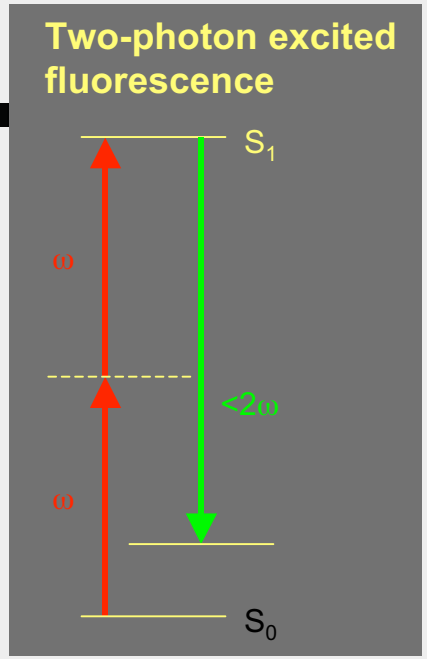
Collaborations with Jaehong Im, Ruth Muschel, ROBU, Oxford

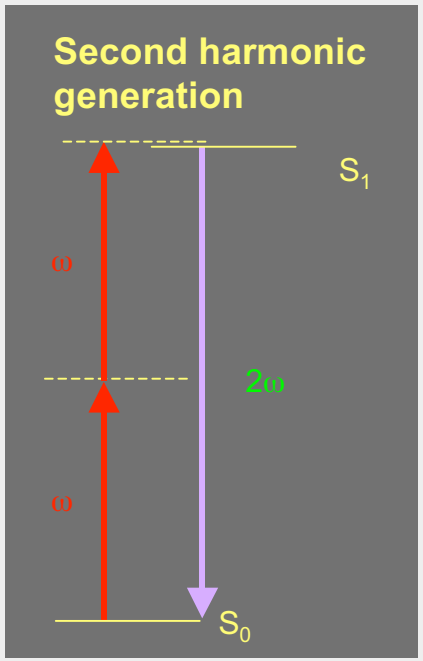
Second harmonic generation

Laser field induces a non-linear, second order, polarization in the assembly of chromophores, producing a coherent wave at exactly twice the incident frequency (or half the wavelength)

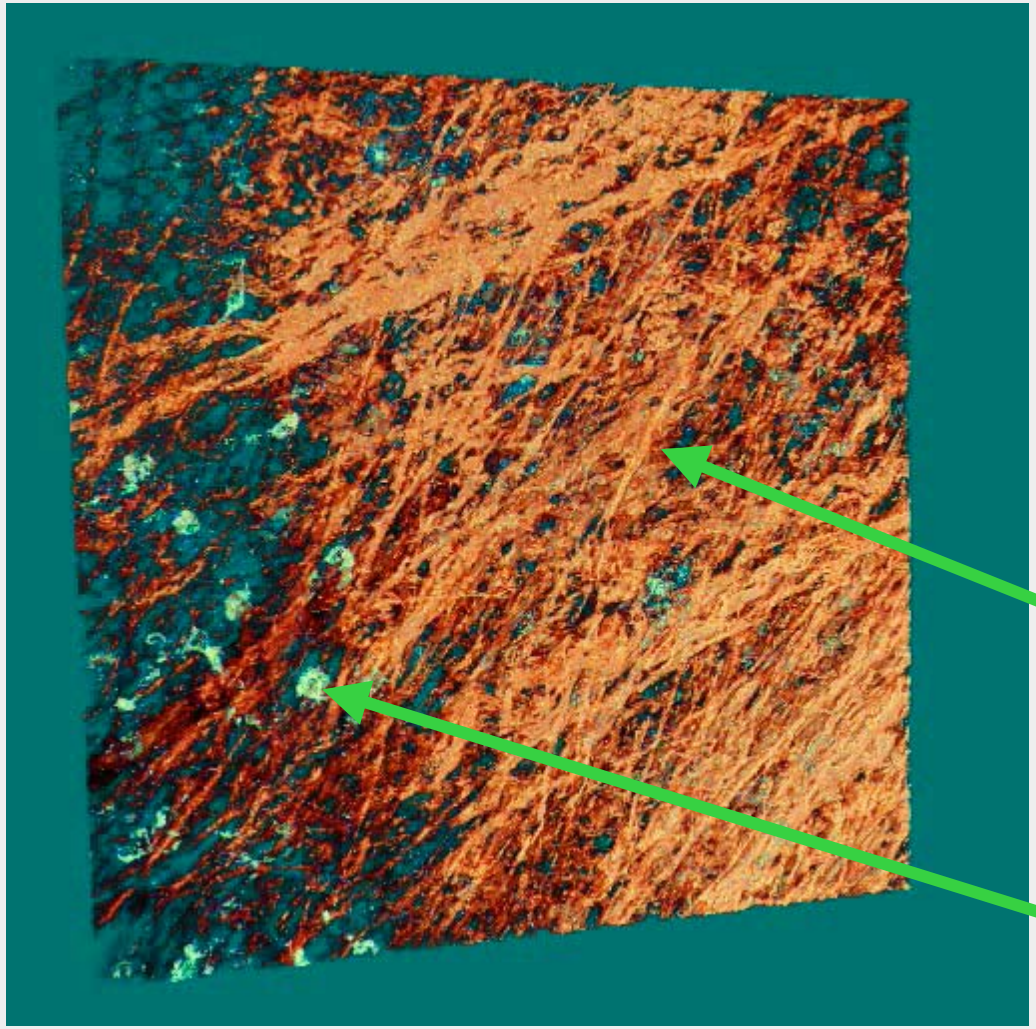


SHG can only be produced from regions lacking inversion symmetry, since in centro-symmetric environments the polarization sums to zero.





EGFP *in vivo*; *ex vivo* imaging



890 nm excitation

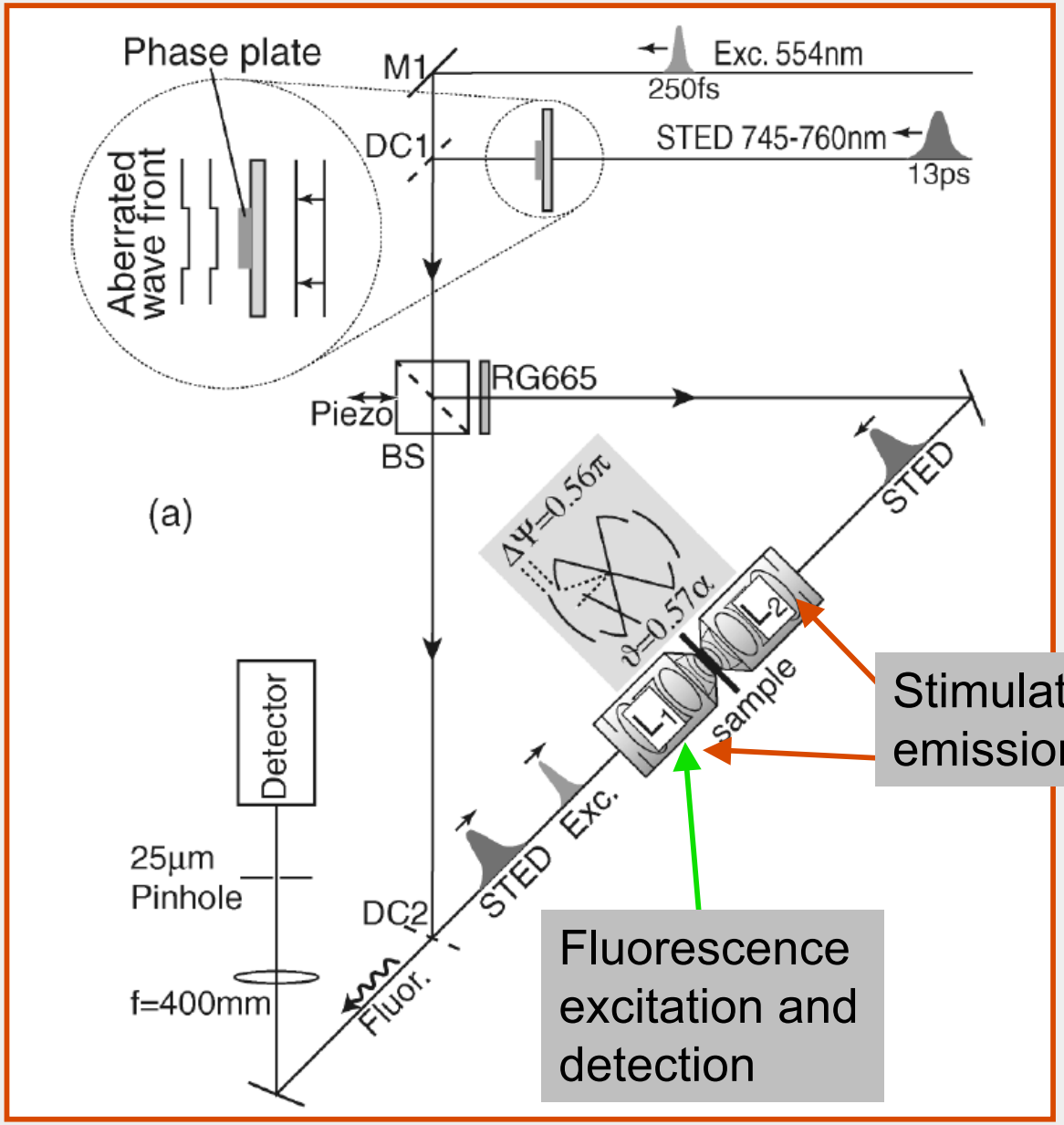
Collagen fibres
SHG signal @445 nm

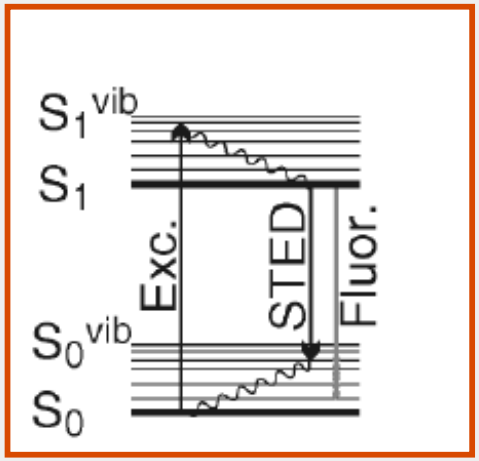
EGFP-expressing areas
Fluorescence signal @500 nm

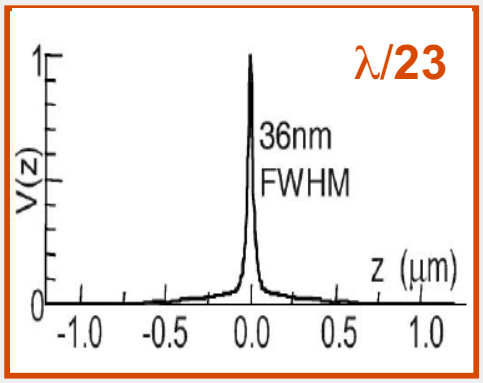
With B. Pedley, J Dearling, UCL

GRAY CANCER INSTITUTE

STED – 4π microscopy







Stimulated emission depletion microscopy

S. Hell Group

Image generation

…..can we improve on classical methods

Imaging challenges

- ***Fast*** methods of imaging/scanning ***large*** areas
- ***Fast*** target identification methods
- ***Automated*** imaging + irradiation methods
- ***Cell signaling*** visualisation methods
- ***Deep / 3D*** imaging methods

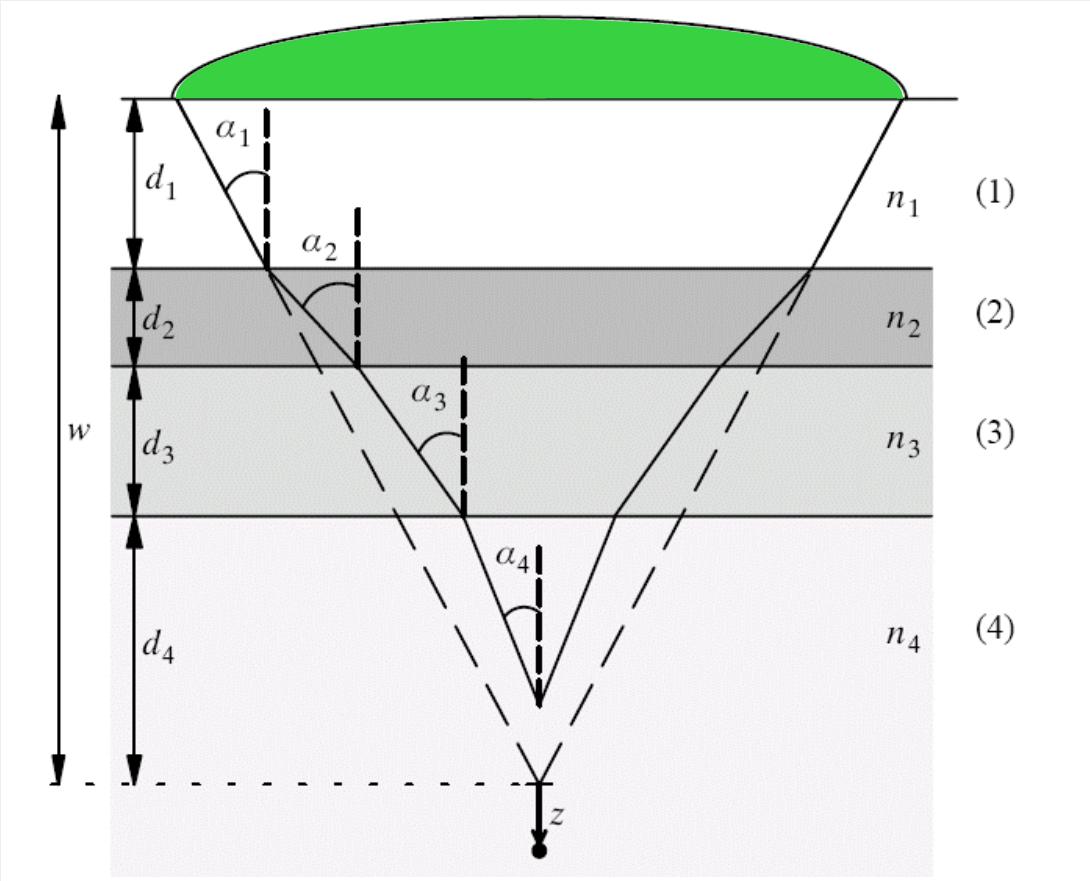
GRAY CANCER INSTITUTE

Imaging at depth

- Good image = good point spread function
- Good point spread function = control of optical wavefront
- 2P/MP methods → maintenance of excitation pulse width
- Samples *always* modify the wavefront
- Objectives are designed to ‘work’ at a given depth

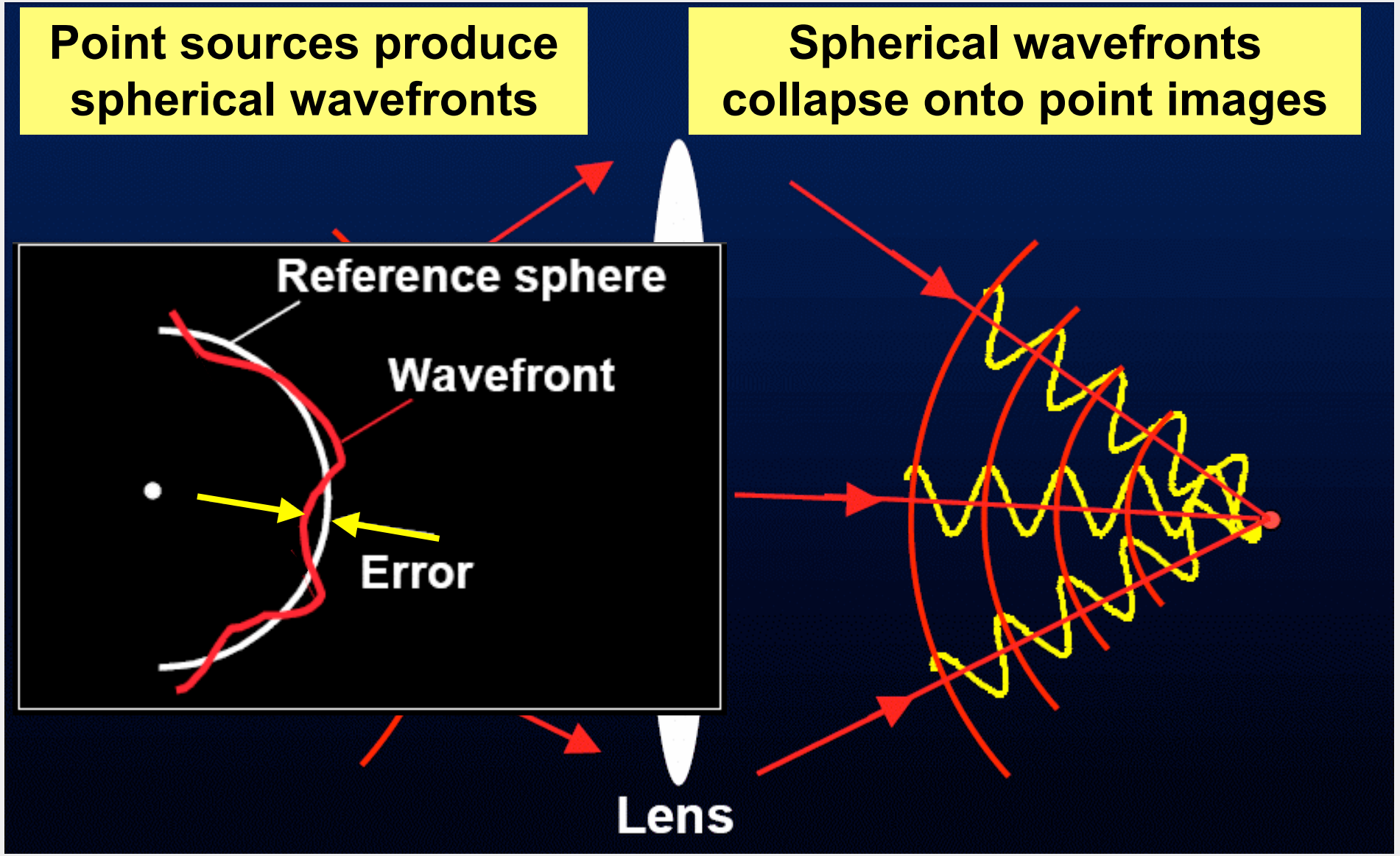
Aberrations in multi-layer media

- Aberrations introduced when focussing through layers of different refractive indeces
- Aberration depends on focus depth
- Variable, automated correction required
- Use of adaptive optics

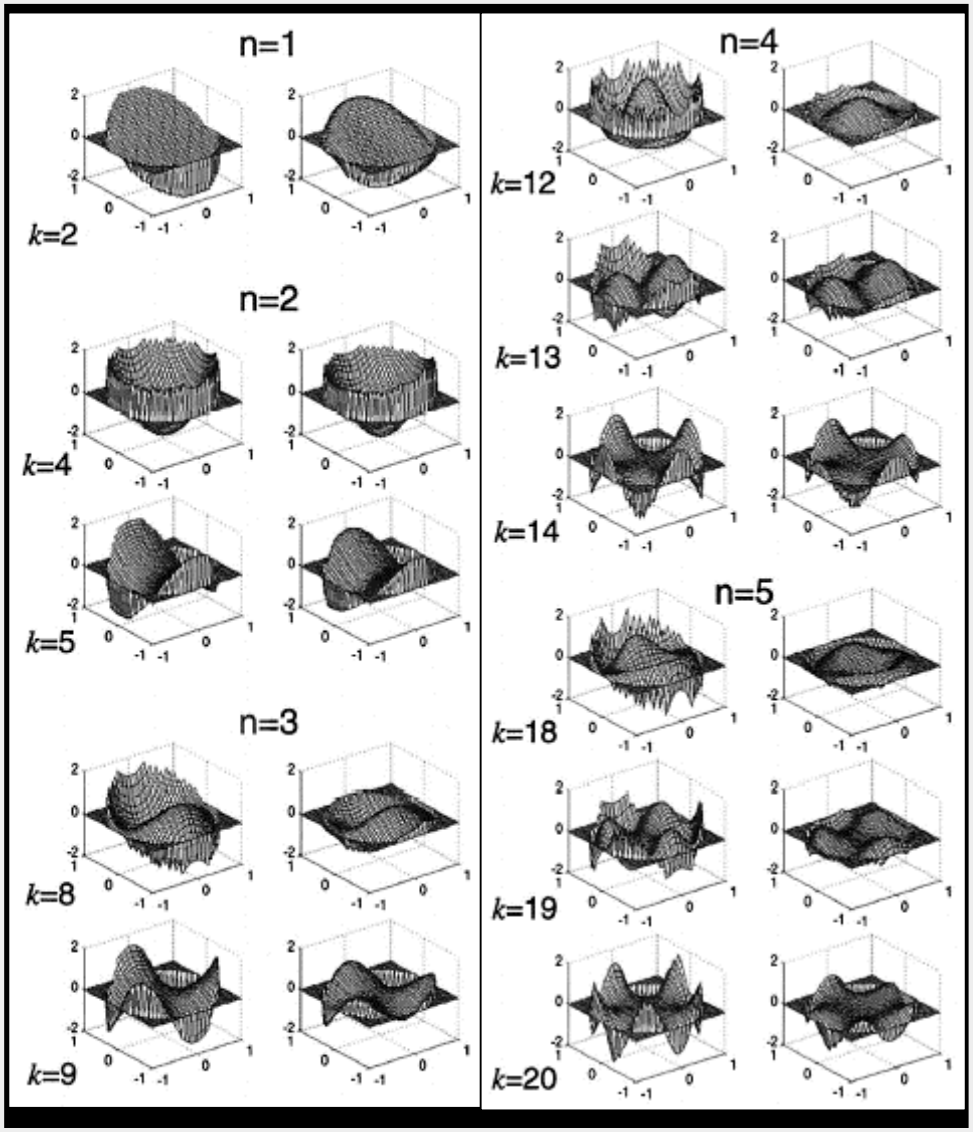


$$\Psi(r) = \frac{2\pi}{\lambda} n_1 \sin\alpha_1 \sum_{p=1}^{P} d_p \left[\sqrt{\csc^2\alpha_p - r^2} - \sqrt{\csc^2\alpha_1 - r^2} \right]$$

The perfect wavefront



Zernike modes



Zernike mode description is mathematically convenient, where the wavefront is described in terms of normalised polynomials of different order, frequency, harmonics

Example of polynomials describing two common aberrations

order frequency

Astigmatism: $Z_2^{+2} = \sqrt{6}\, r^2 \cdot \cos(2\theta)$

normalization constant polynomial harmonic

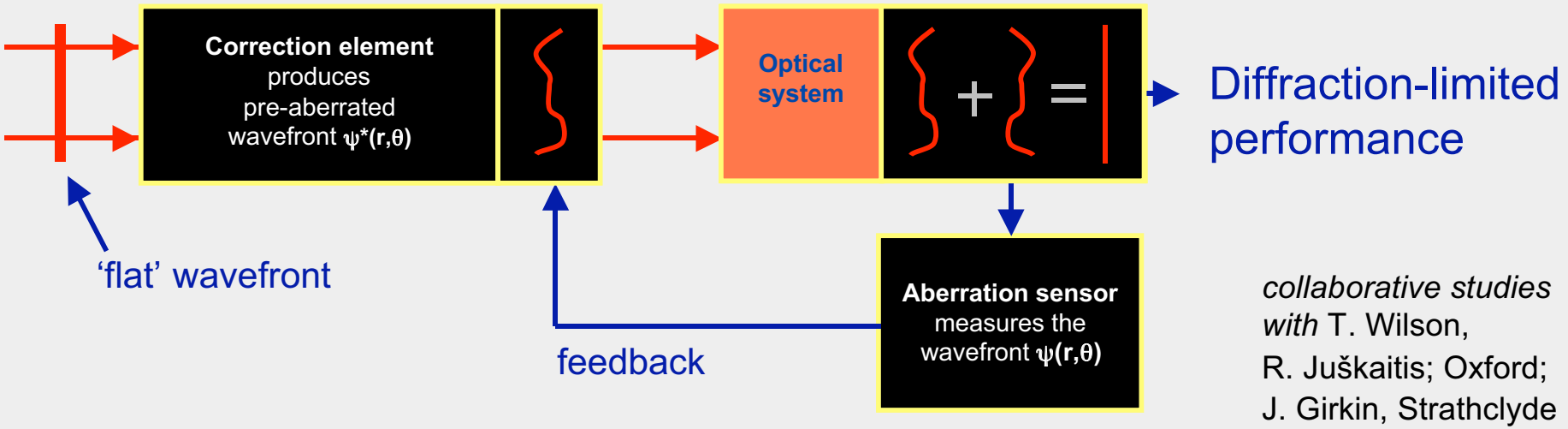
Coma: $Z_3^{-1} = \sqrt{8}\,(3r^3 - 2r) \cdot \sin(\theta)$

GRAY CANCER INSTITUTE

Correcting aberrations: Imaging Adaptive control

- What do we require from an Adaptive Optical system?
  - Ability to shape the wavefront of an incoming laser beam
  - Ability to control and optimise the wavefront
  - Ability to perform corrections dynamically

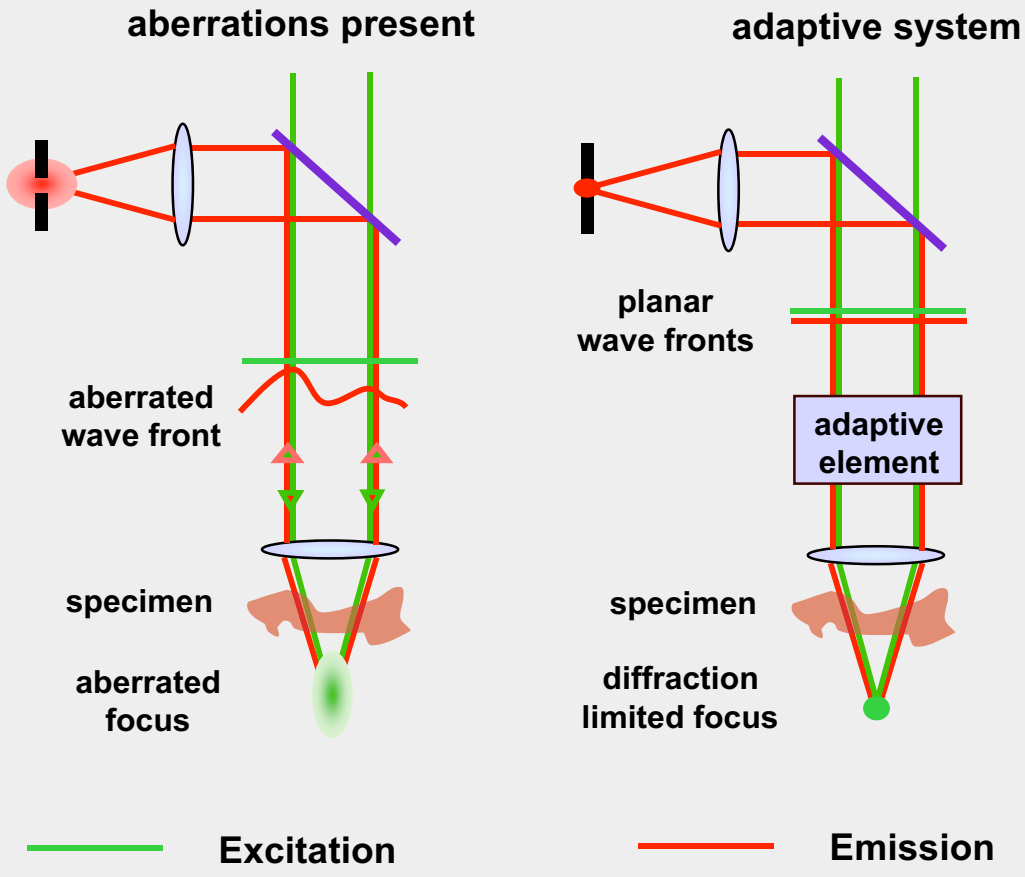
Adaptive optics system: wavefront sensor
aberration correction element
feedback loop



collaborative studies with T. Wilson, R. Juškaitis; Oxford; J. Girkin, Strathclyde

Scanning microscope AO

Configuration of ‘adaptive confocal microscope’



- **Aberration correction required in both paths for confocal**
- **Axial selectivity needed for wave front sensing – use confocal signal**
- **Sequential adaptation of adaptive element**
- **Separate wave front sensor not needed**

T. Wilson and M. Booth; Oxford

Wavefront control

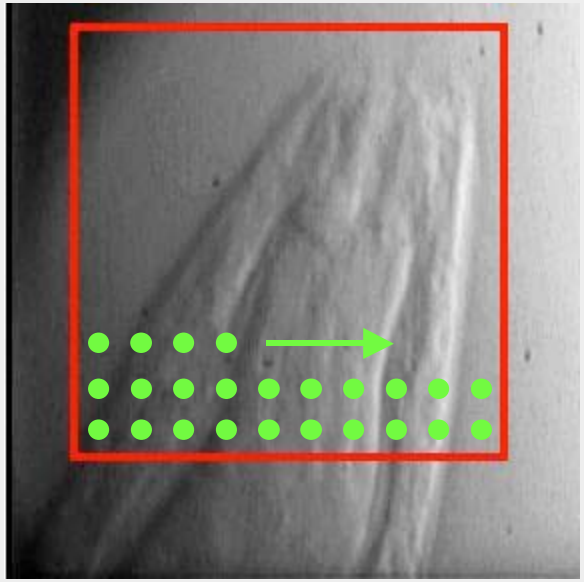
How?

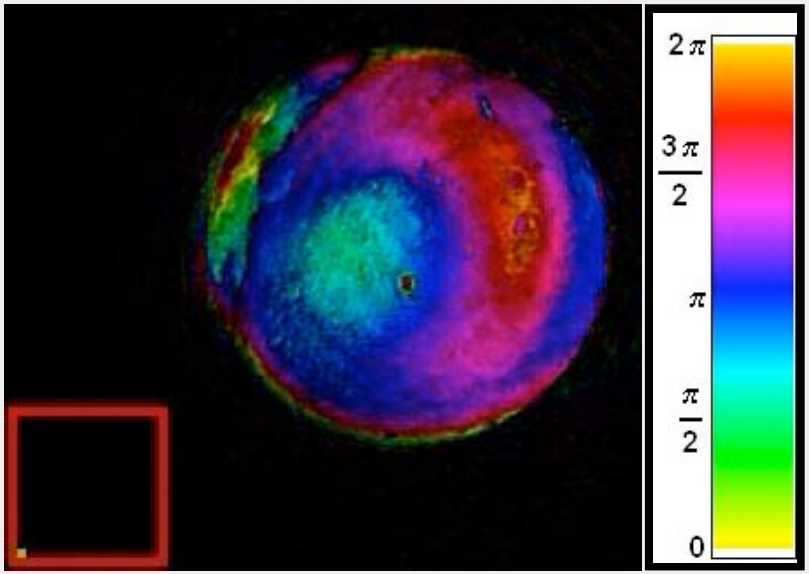
Deformable mirror technologies in imaging paths

Exploiting non-linear effects in 2P and controlling the propagation of ultrafast excitation pulse

Understanding how the sample affects the propagation of the excitation (and emission) wavefronts – determination of phase delays

Example of measured wavefront data

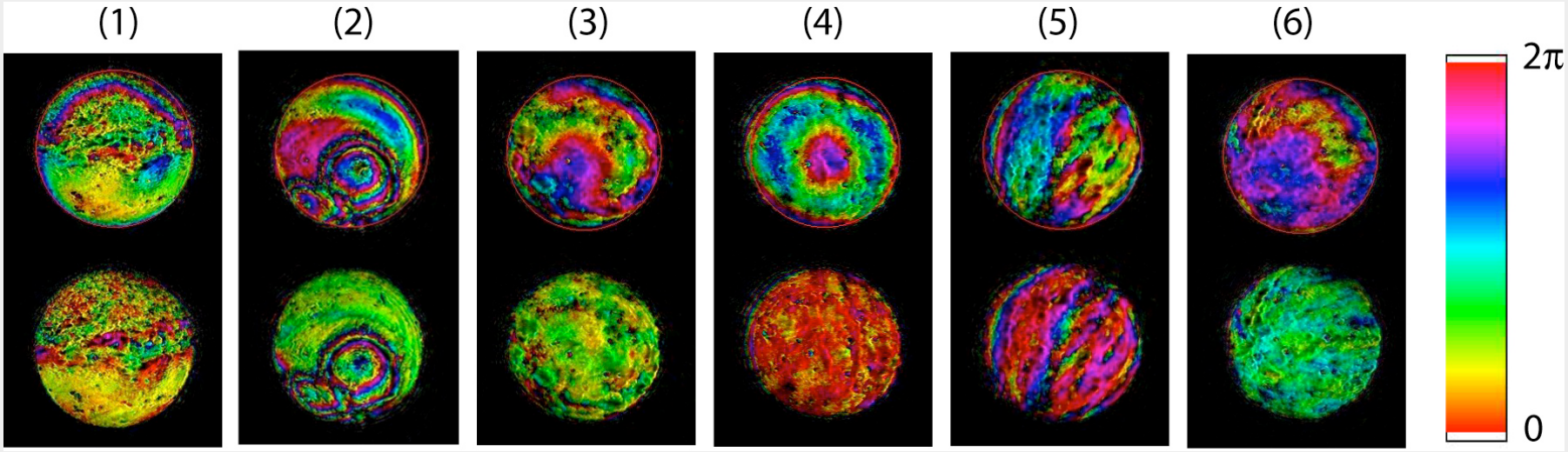




Specimen: c. elegans
Scanned region: 50 x 50 microns
wavefronts measured on 16 x 16 grid of spots
Objective NA = 1.2

Schwertner *et al.*, OPTICS EXPRESS 6548 Vol. 12, No. 26, 27 December 2004

Simulation of Zernike-based modal correction

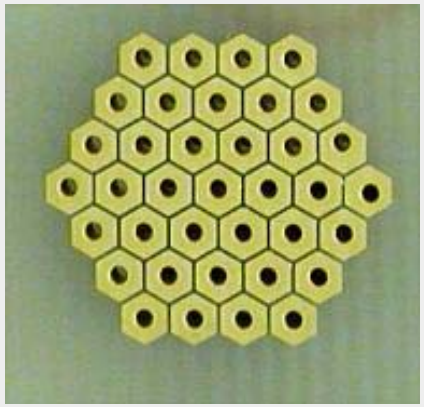


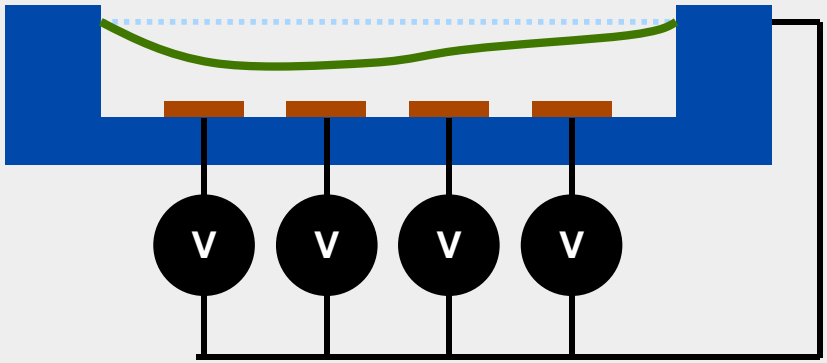
Top: original individual wavefront, bottom: correction up to Zernike mode 22

Specimen #	1	2	3	4	5	6
Description	Brain	Oocyte	Liver tiss.	Muscle (str.)	C. Elegans	VAS
Thickness (microns)	30	80	20	40	40	30
Initial mean Strehl	0.40	0.47	0.32	0.48	0.46	0.47
Corrected mean Strehl	0.65	0.62	0.65	0.81	0.71	0.72
Mean Signal improvement	**757 %**	**627 %**	**784 %**	**335 %**	**965 %**	**280 %**

Deformable membrane mirror

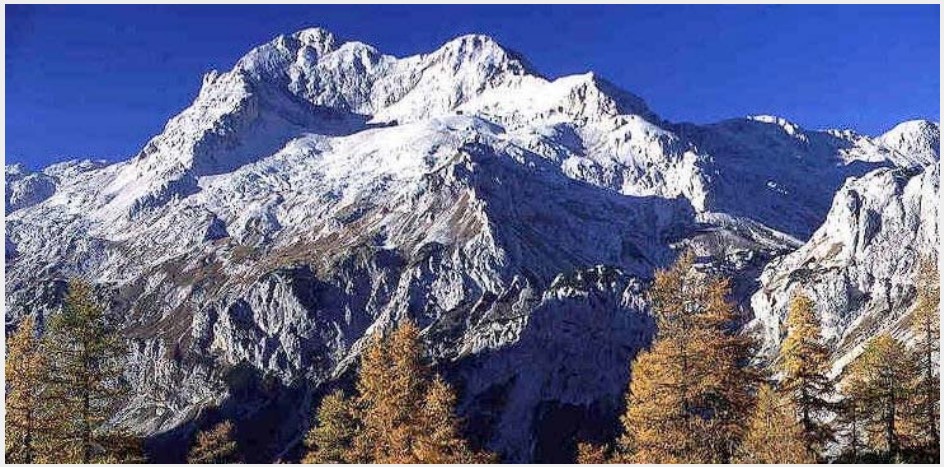
- Al or Au coated SiN membrane above electrode structure
- Applied voltages cause membrane to deform by electrostatic forces





- 37 control electrodes, 15 mm diameter, bound edge
- High reflectivity, continuous phase function, bandwidth ~1 kHz
- Correction range limited to a few wavelengths

Peak finding

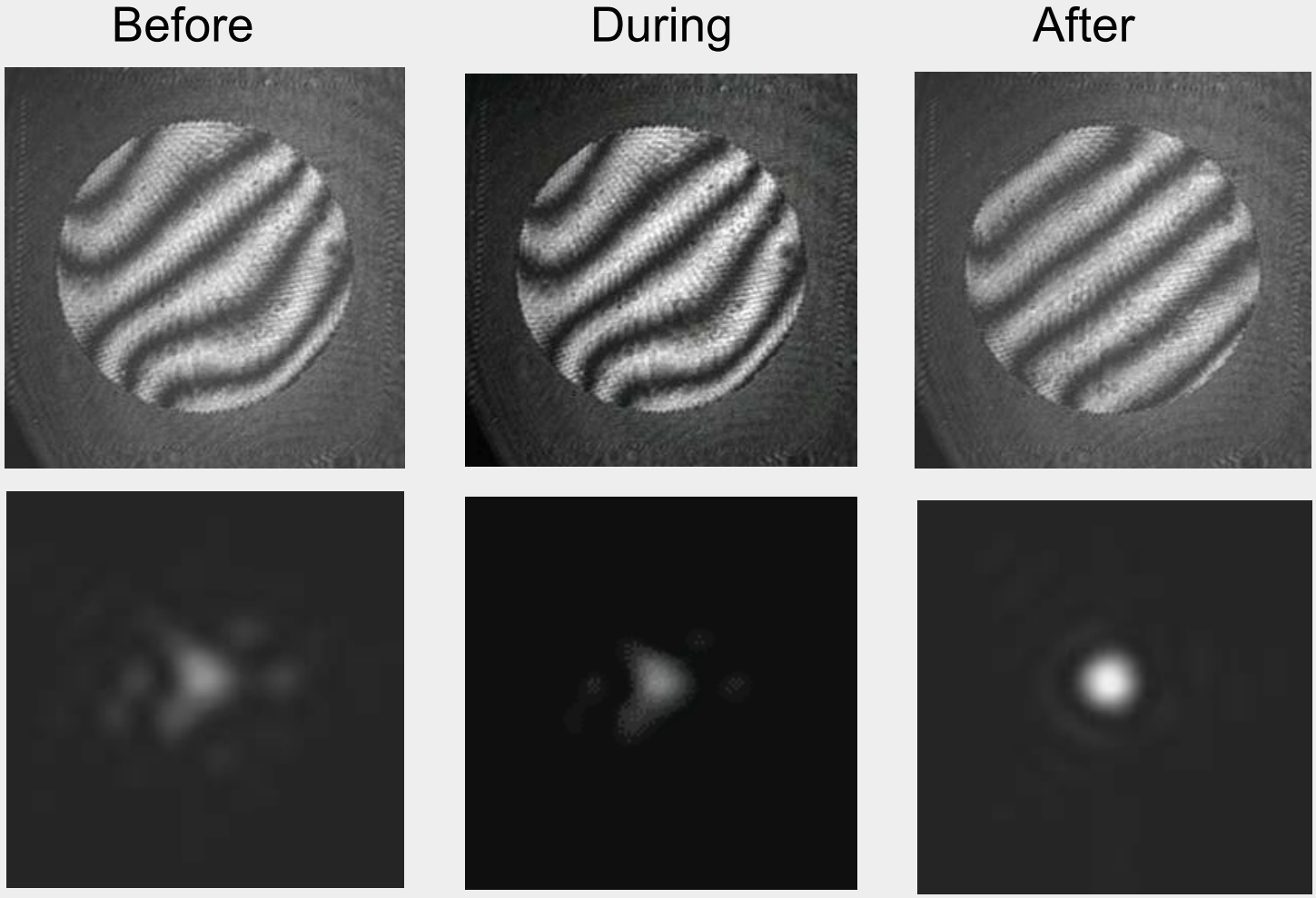


Several optimisation algorithms could be used

- Modified Hill Climbing
- Genetic Algorithm
- Random Search
- Adapted random search
- Simulated annealing
- …….
- Model-based searches

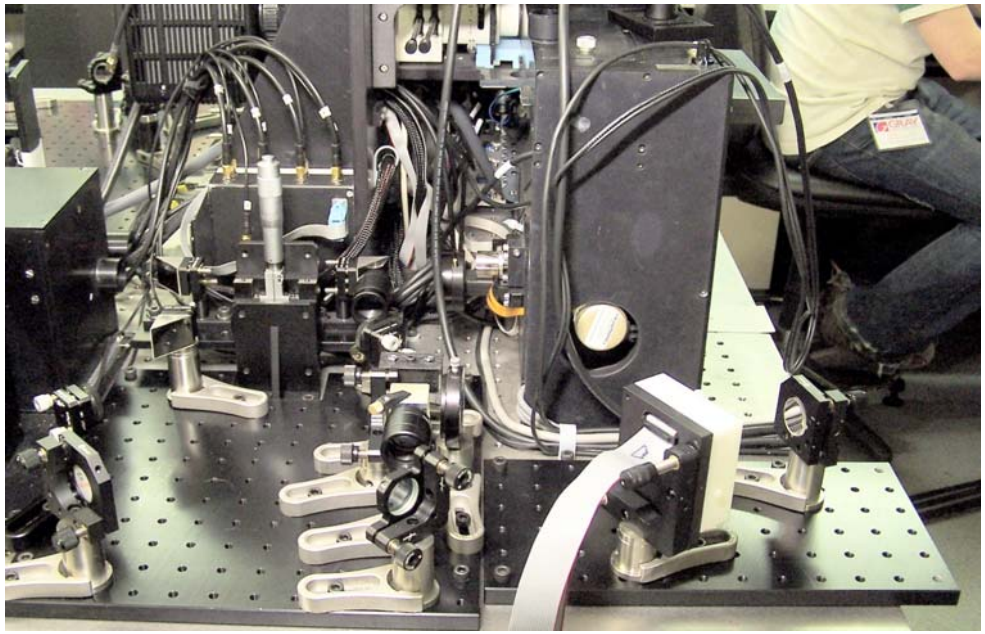
Sequential correction using deformable mirror

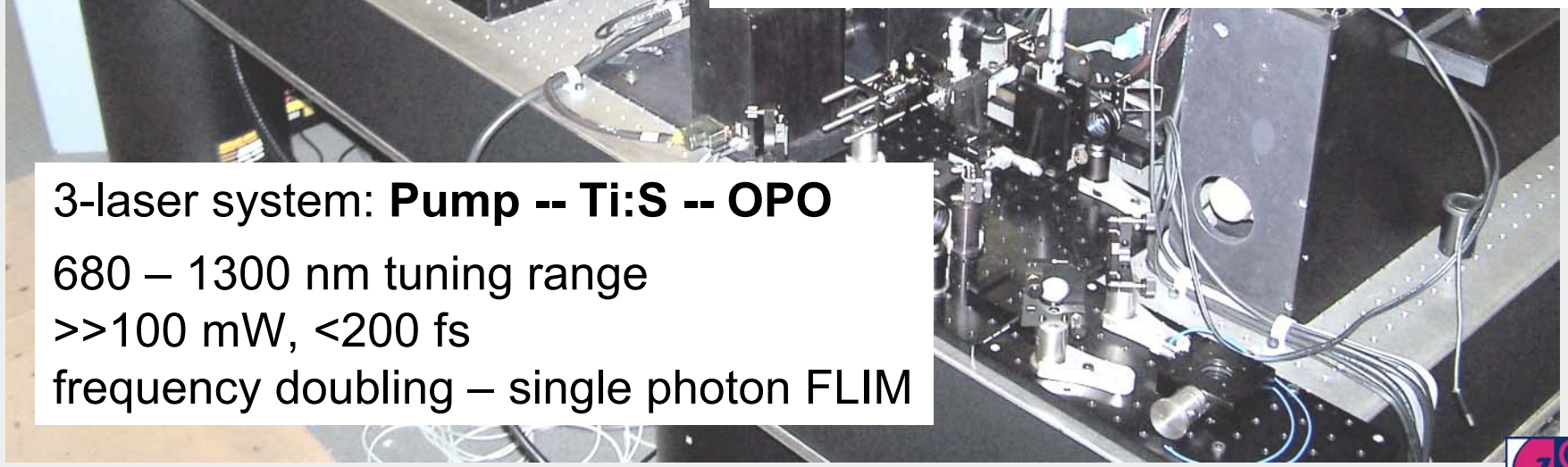
OKO membrane mirror, pupil plane interferograms and focal plane images



Booth *et al*., Wilson Group, Oxford

‘High resolution’ imaging at depth



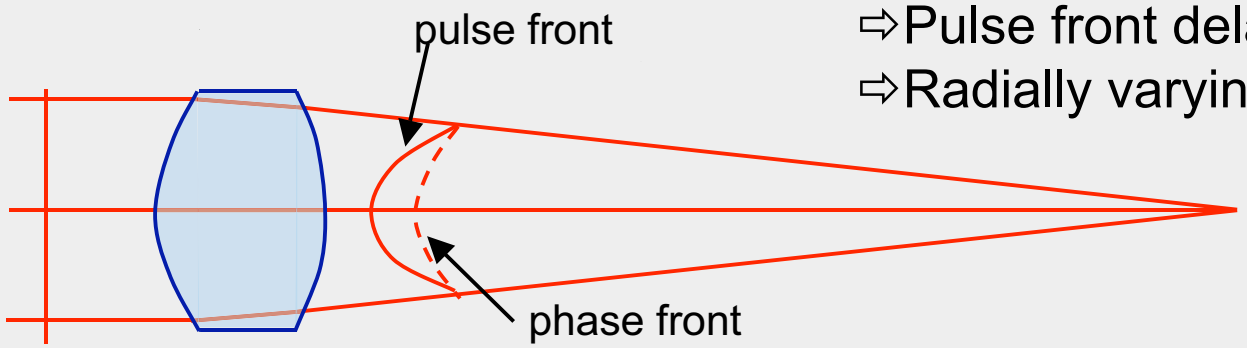


3-laser system: **Pump -- Ti:S -- OPO**

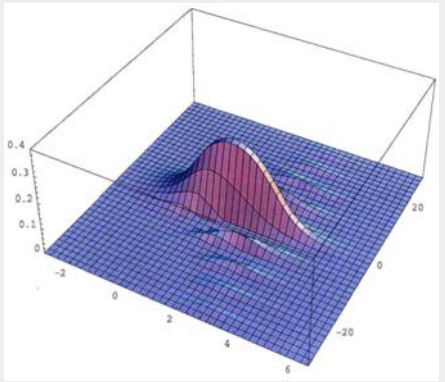
680 – 1300 nm tuning range
>>100 mW, <200 fs
frequency doubling – single photon FLIM

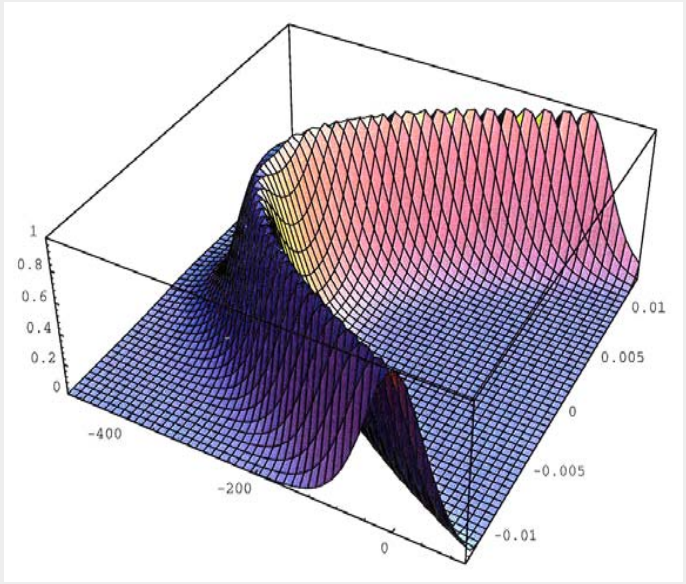
GRAY CANCER INSTITUTE

Pulse broadening in lenses



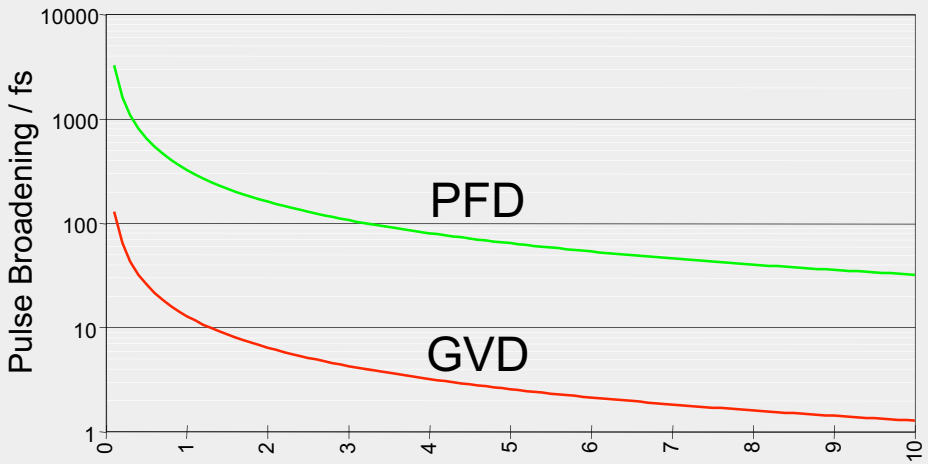
Lens introduces
⇨Pulse front delay (PFD)
⇨Radially varying pulse stretch (GVD)





NO radial dependence to GVD in achromatic optical system

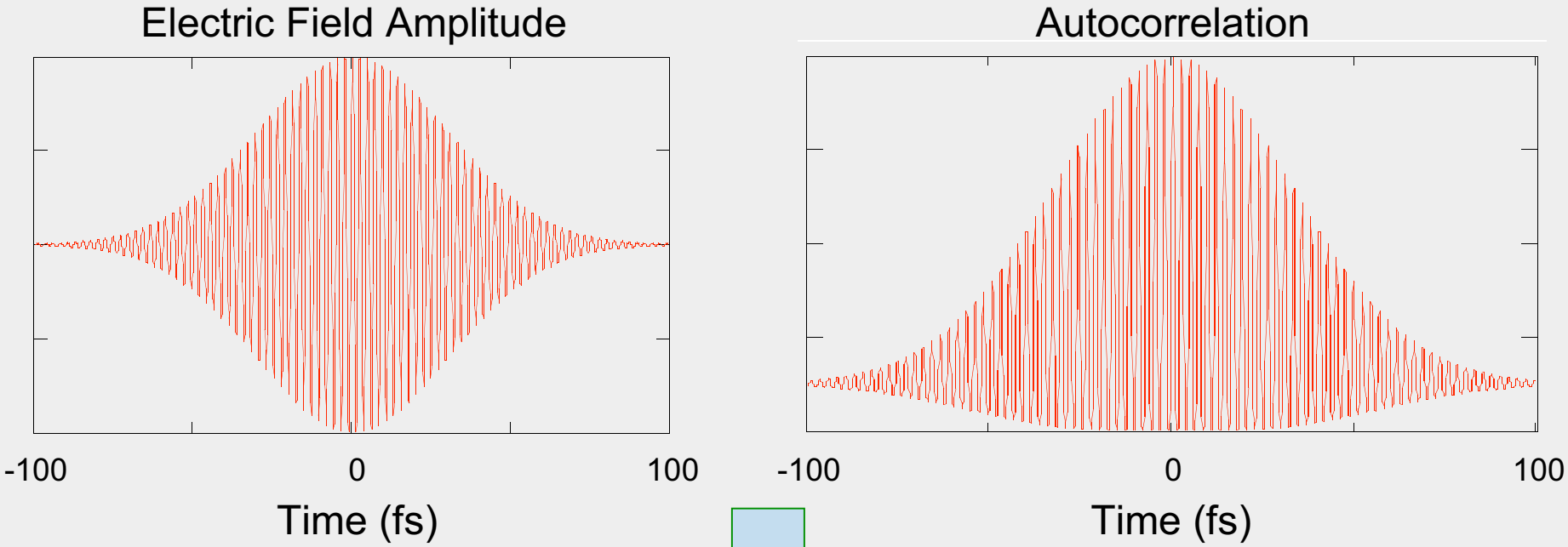
PFD >> GVD

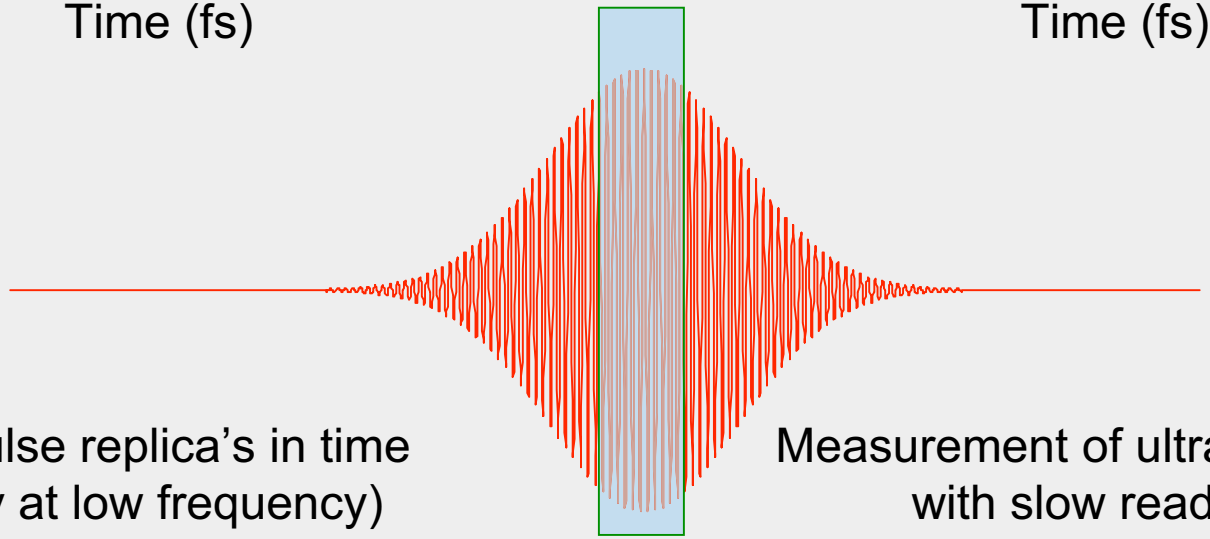


Increasing NA

Ultrashort pulse measurement

50 fs pulse at 800 nm





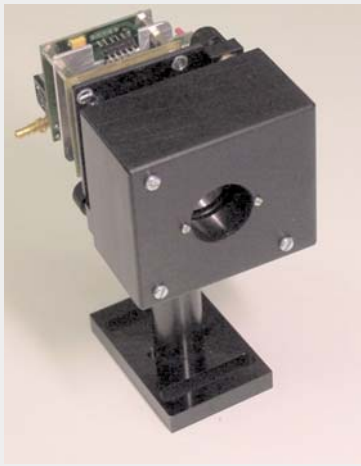
Sweep pulse replica's in time
(typically at low frequency)

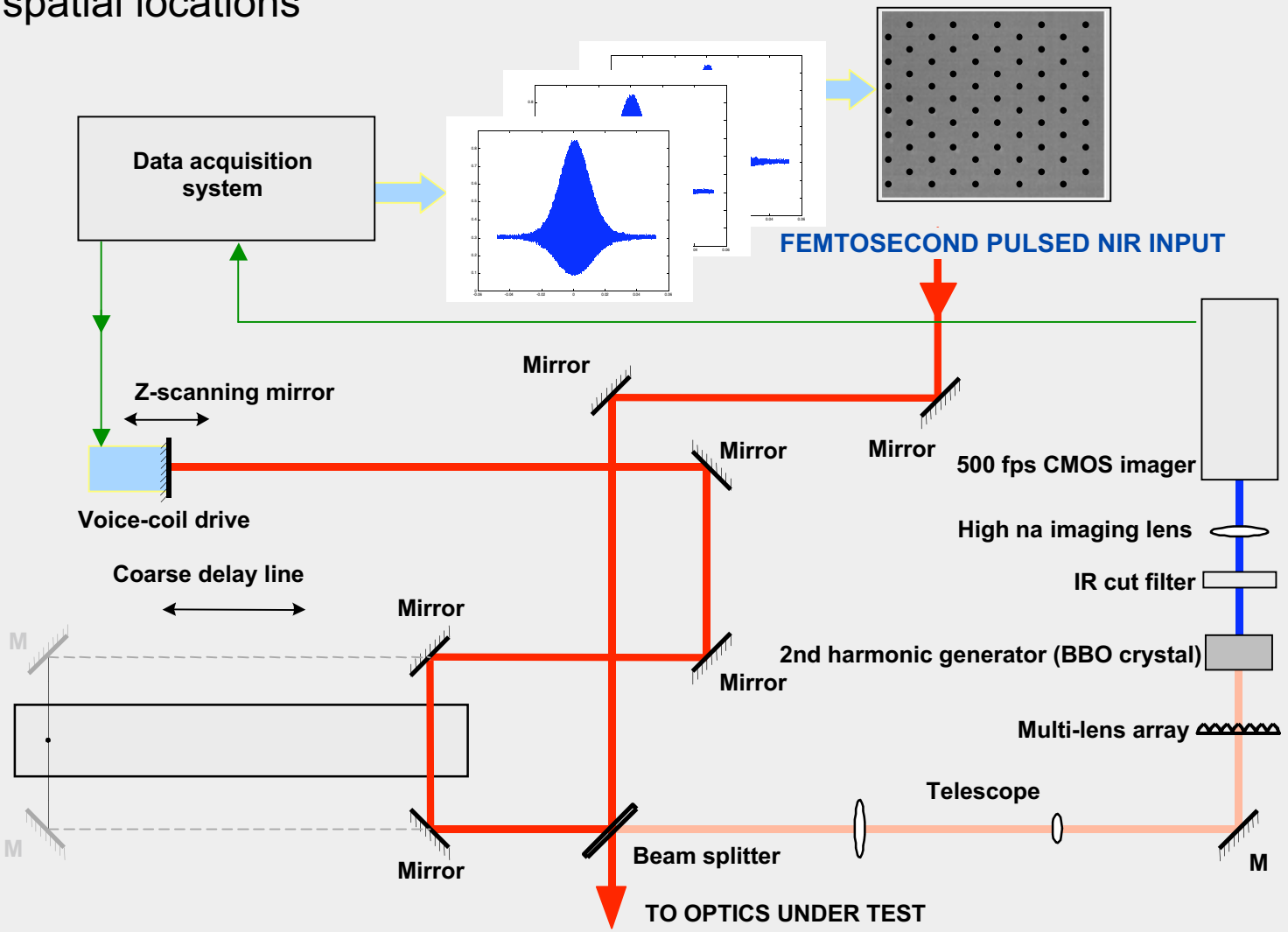
Measurement of ultrafast pulse
with slow read-out

GRAY CANCER INSTITUTE

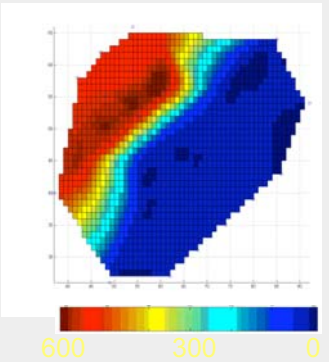
Pulse front determination

Determination of *amplitude* and *phase* of electric field of the pulse at a large number of spatial locations





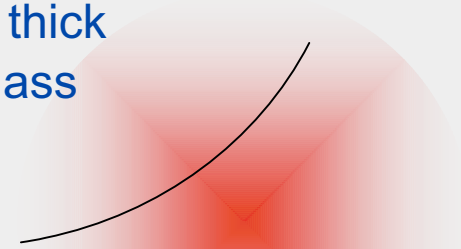
Pulse front delay (fs)

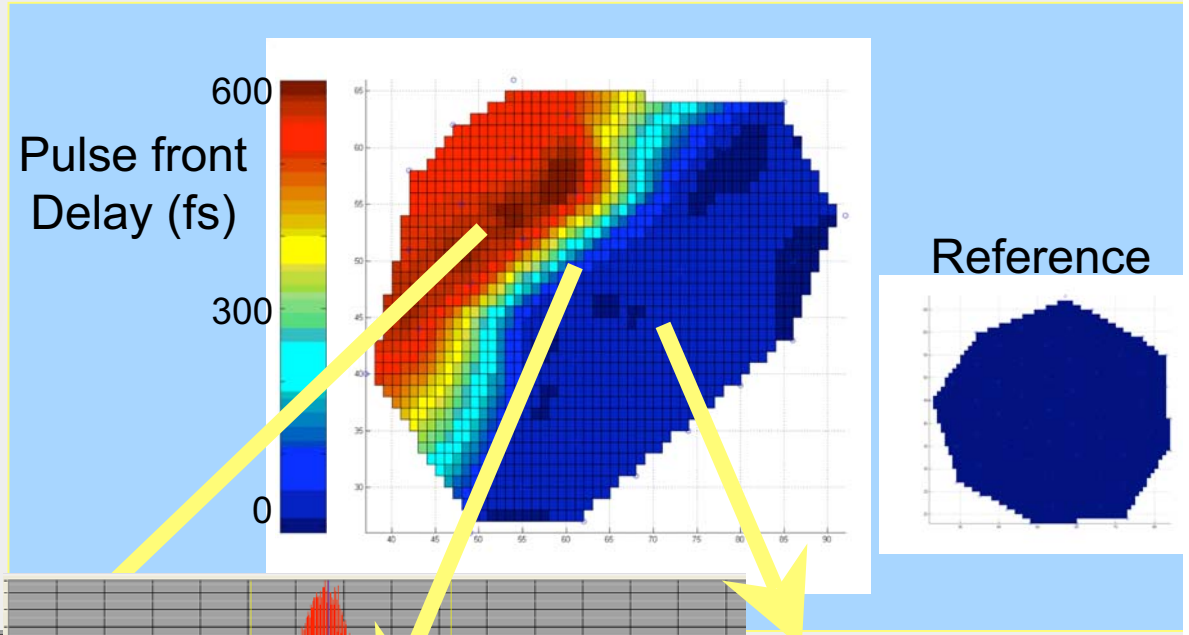


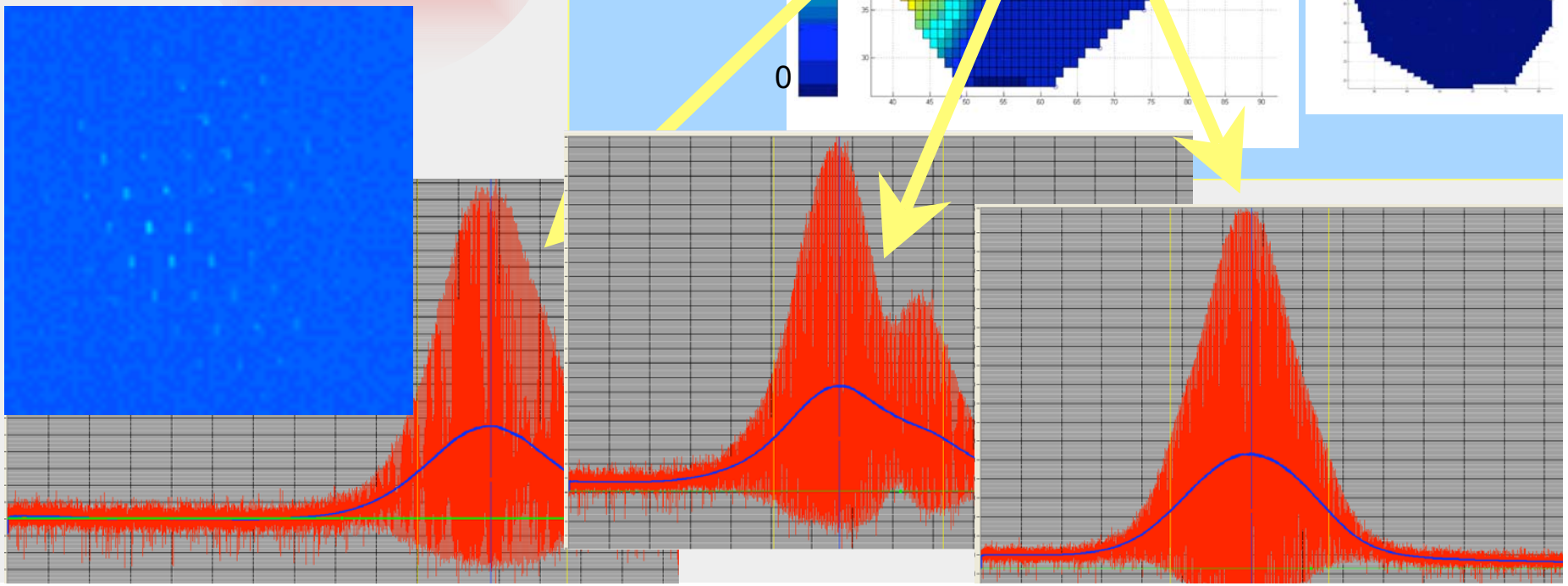
Characterisation of ICC

Effect of inserting dislocation

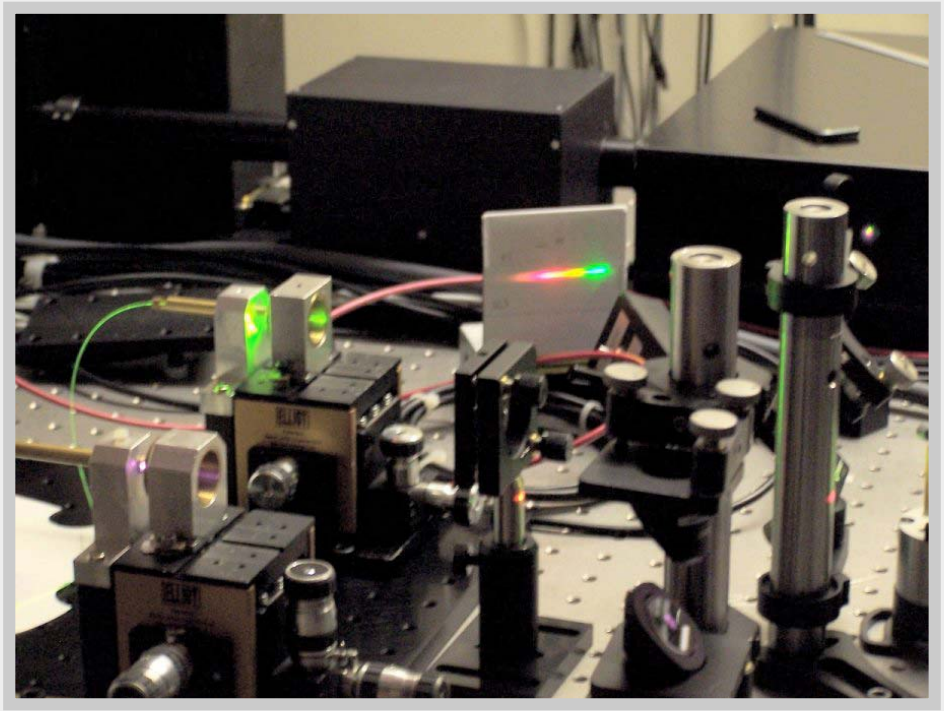
170 μm thick coverglass

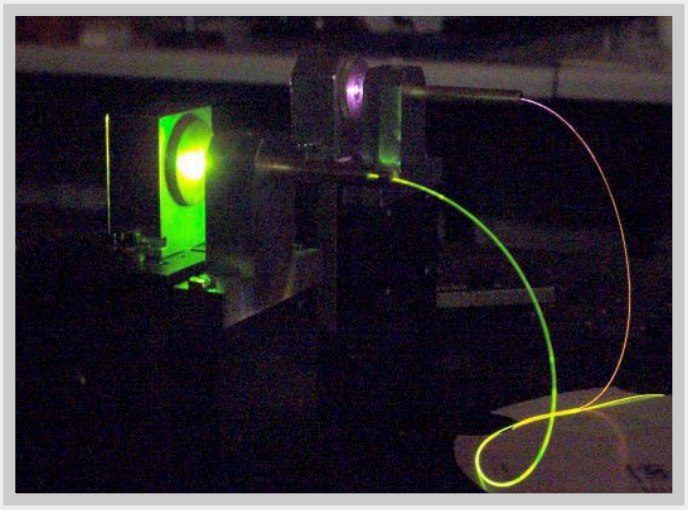






‘Continuum’ generation





Excitation wavelength selection through continuum generation

Use of photonic fibres

NIR pulse in…..
……‘white light’ pulse out
(>>mW average, ps pulses)

Spectroscopy
Wavelength agility
Single-photon time-resolved

collaborative studies with
J. Girkin, G. McConnell

INSTITUTE OF PHOTONICS

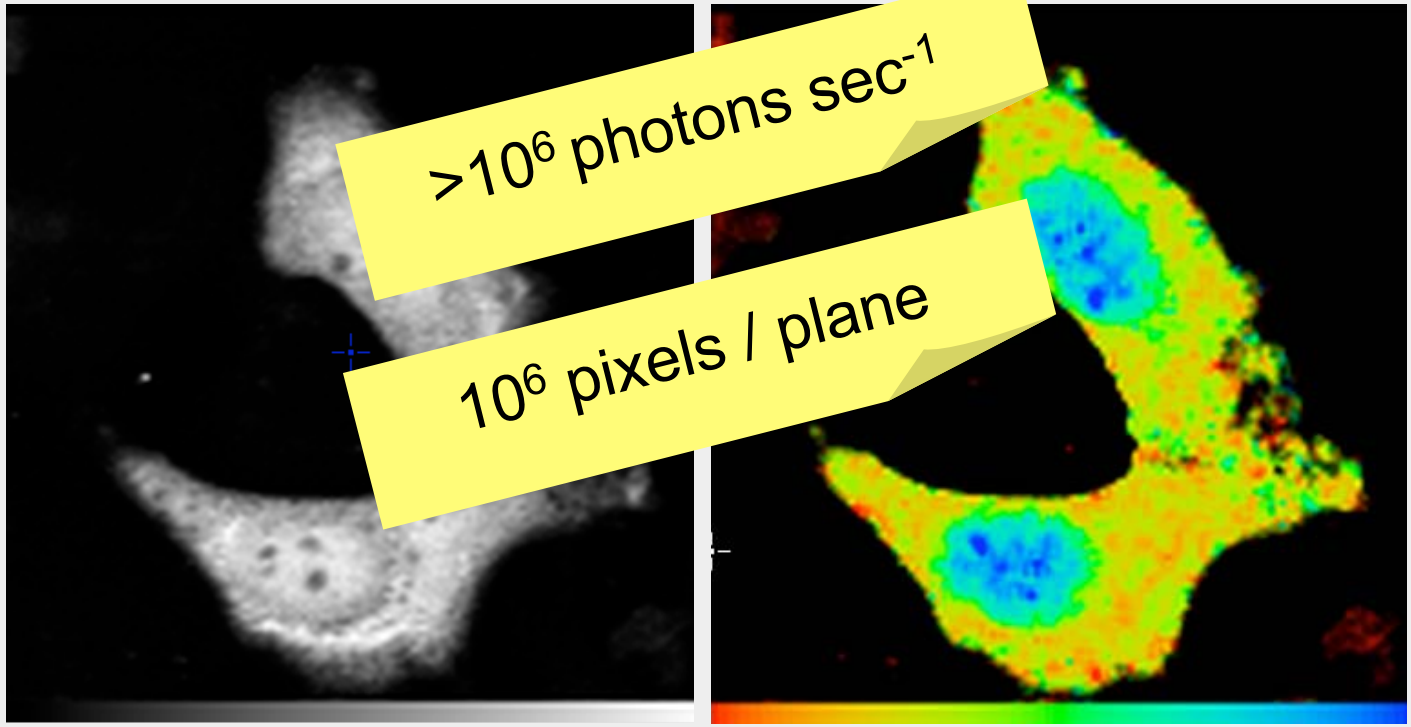
Fluorescence lifetime imaging

…..able to image molecular interactions – and applied to far-field methods with near-field performance

Fluorescence lifetime imaging - FLIM

Kinetic trace at every pixel acquired (time resolution 130 ps)
Kinetics analysed at every pixel to derive lifetime (τ)
Lifetime mapped in (false colour) x (intensity)

Analysis of the excited state lifetime of a population of fluorescent probe molecules + Spatially resolved acquisition of data

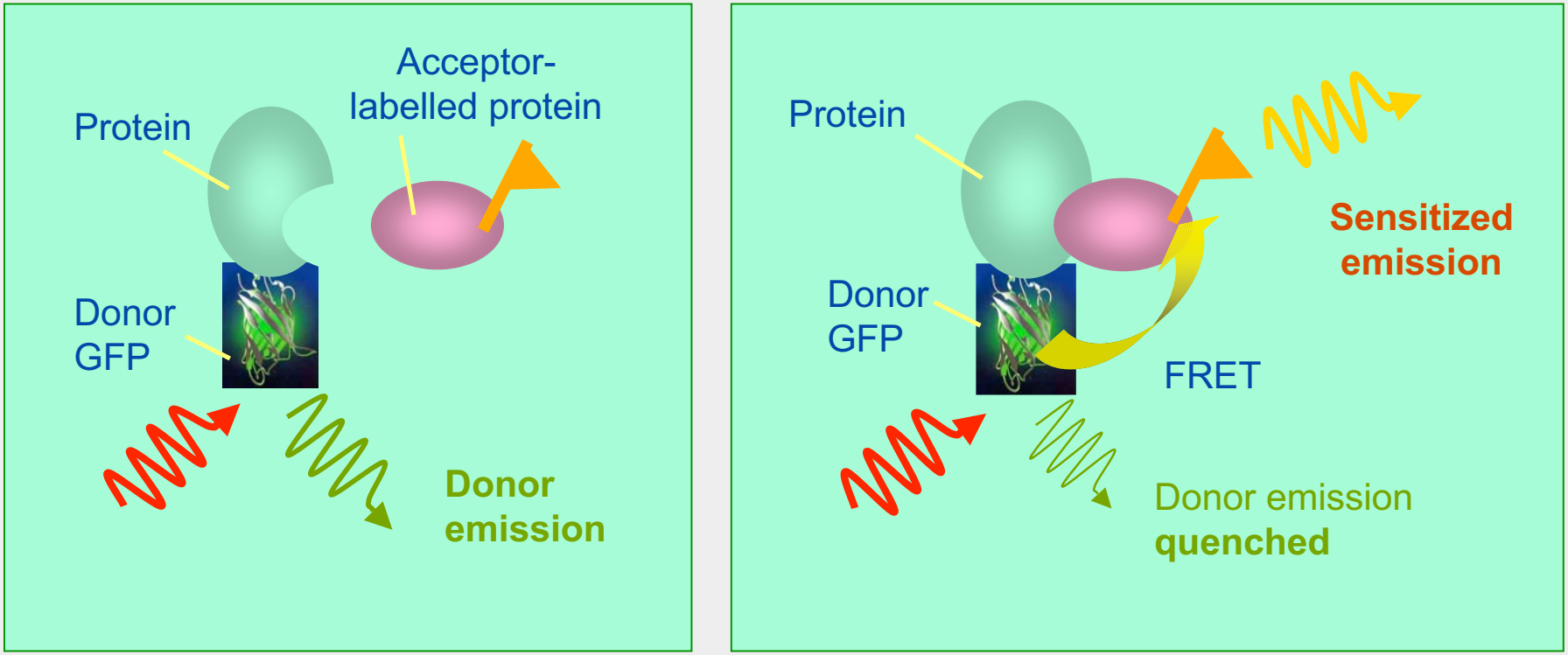


Intensity image **Lifetime image**

Informs on molecular environment

GRAY CANCER INSTITUTE

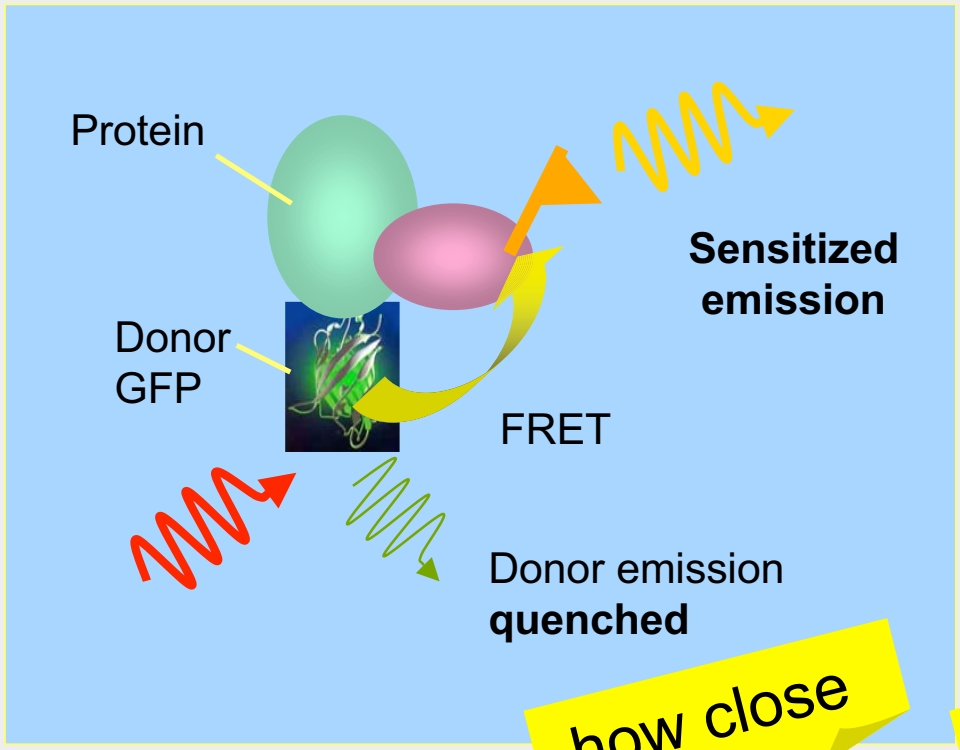
Förster Resonance Energy Transfer (FRET)

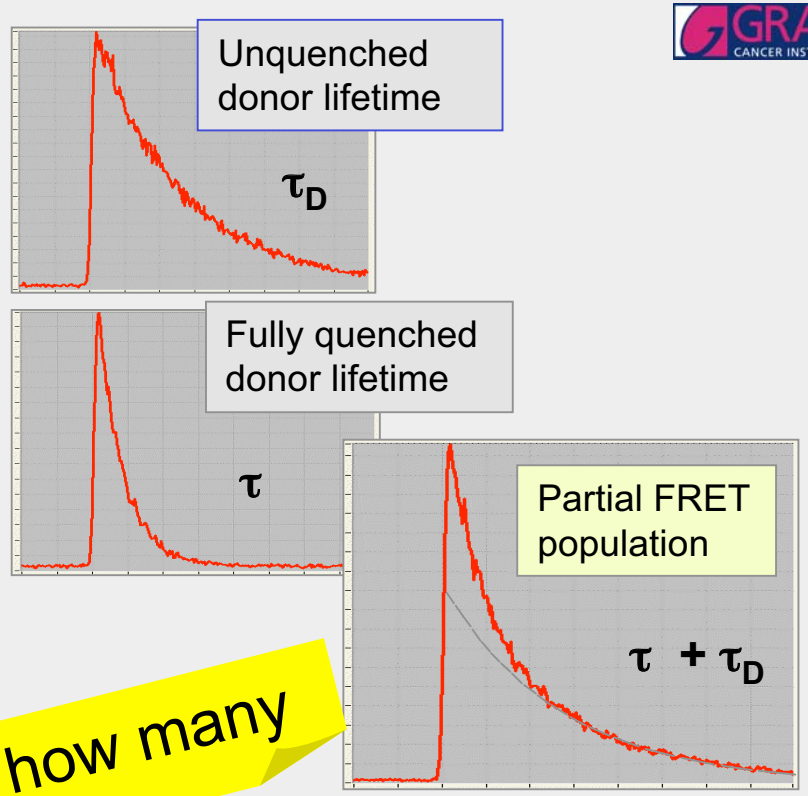


- When donor emission spectrum **overlaps** with acceptor absorption spectrum
- When dipole donor and acceptor moments are **aligned**
- Probability of FRET∞ [separation]$^{-6}$ (0.1-10 nm range)

GRAY CANCER INSTITUTE

Förster Resonance Energy Transfer (FRET)





how close

how many

FRET efficiency
$= (\tau_D - \tau) / \tau_D$

Population of FRET species
$= A1 / (A2+A1)$

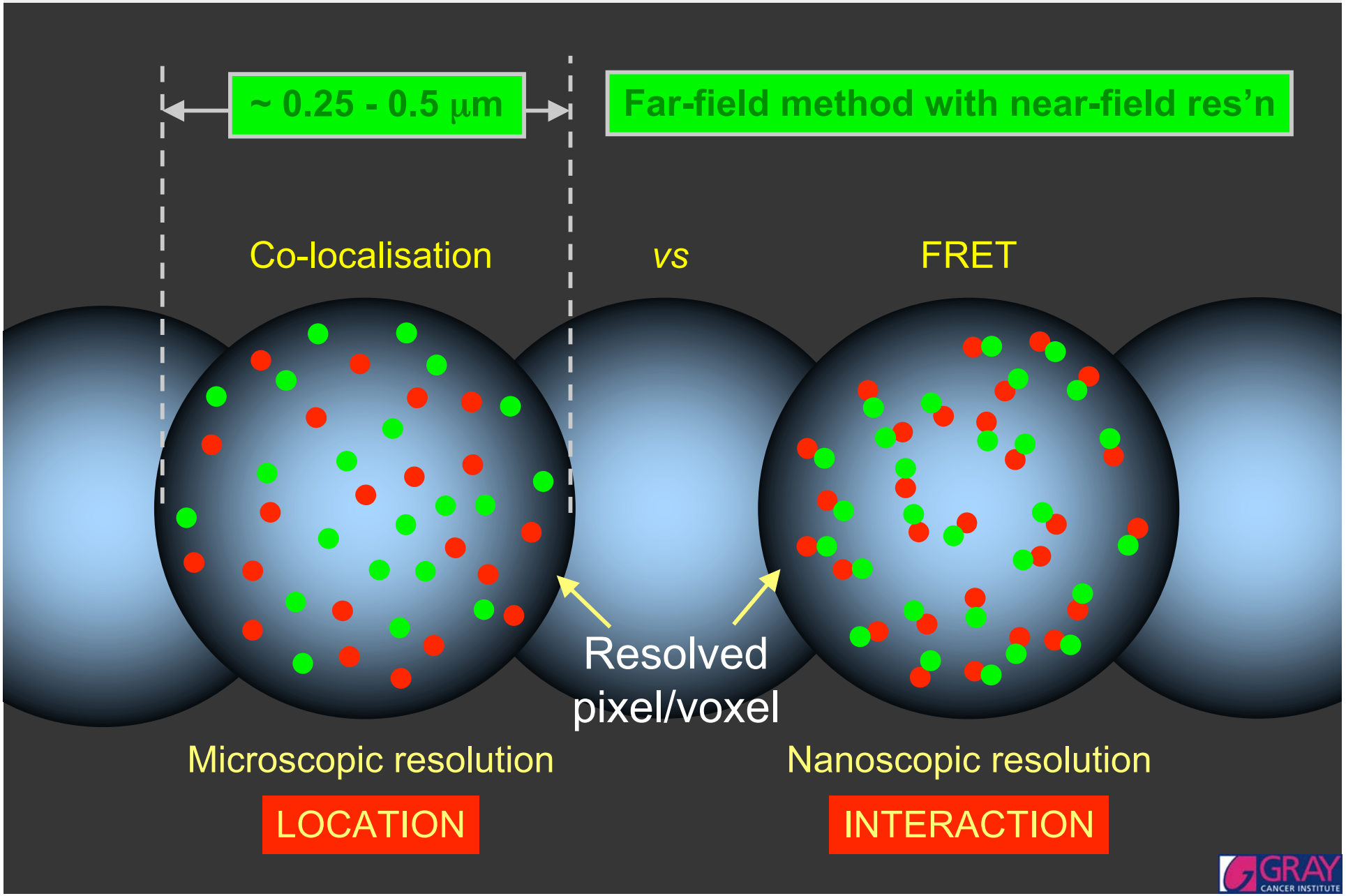
τ_D = control donor lifetime

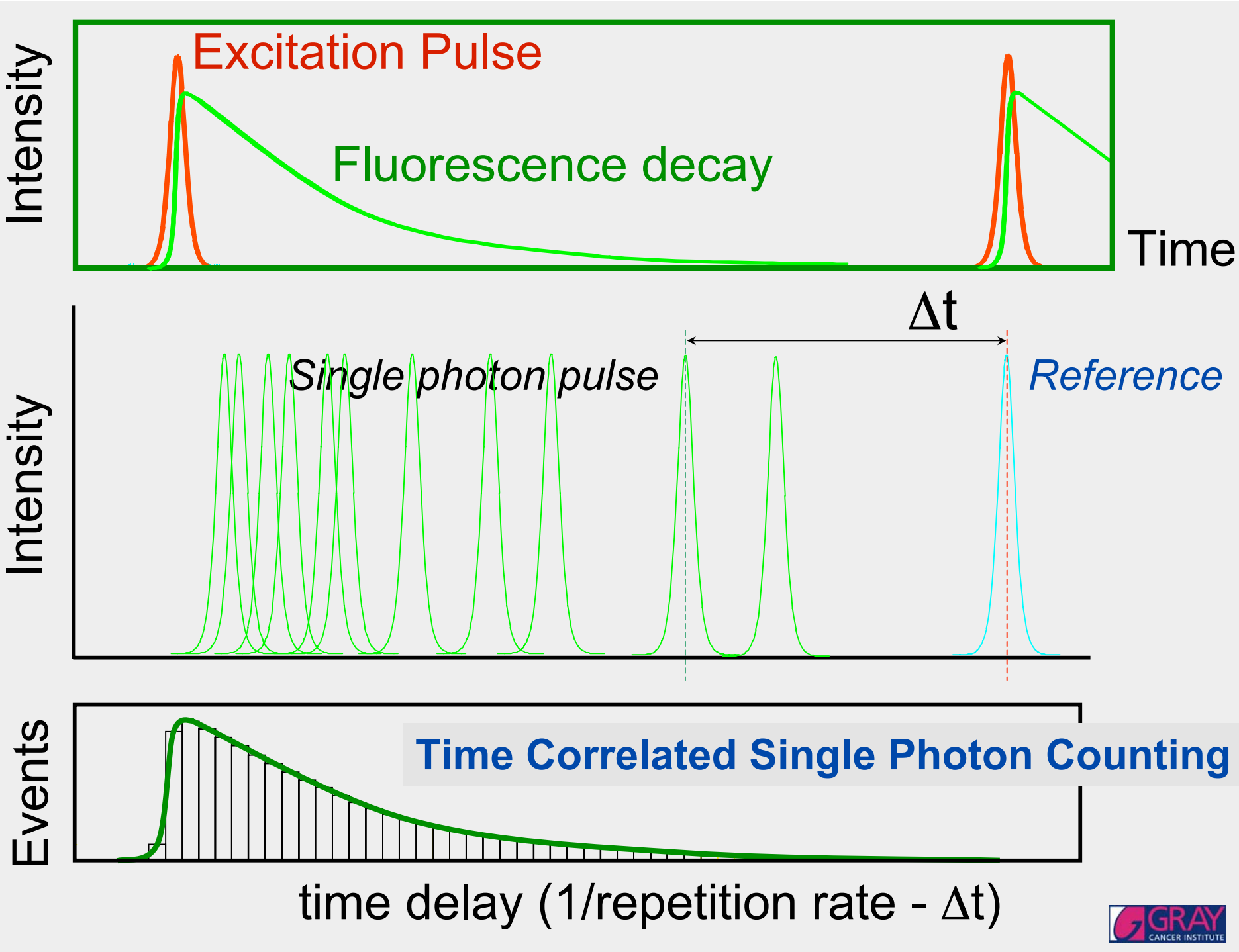
τ = quenched donor lifetime

A1 = amplitude of quenched donor lifetime

A2 = amplitude of control donor lifetime

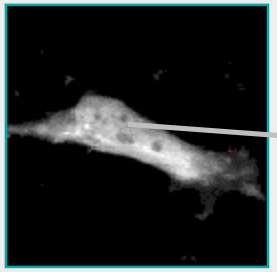
FRET *vs.* Co-localisation

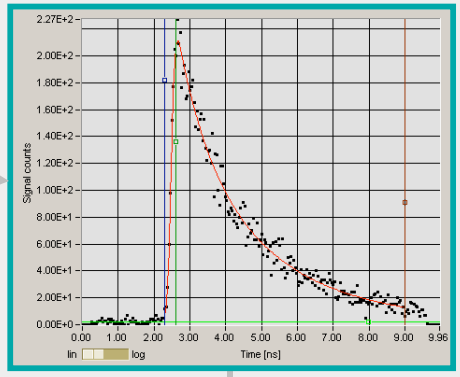



Intensity
Excitation Pulse
Fluorescence decay
Time
Intensity
Δt
Single photon pulse
Reference
Events
Time Correlated Single Photon Counting
time delay (1/repetition rate - Δt)
GRAY
CANCER INSTITUTE

Pixel-by-pixel analysis

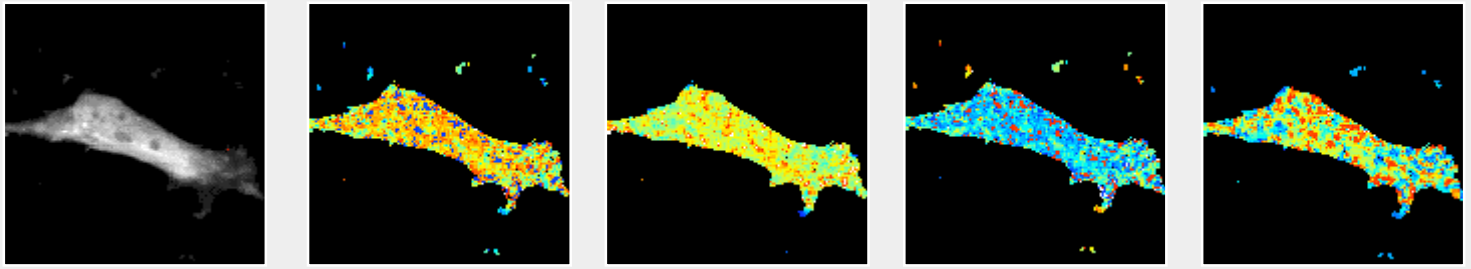
Time-resolved image





$$I(t) = Z + I_0\left(\alpha_1 \exp(-t/\tau_1) + \alpha_2 \exp(-t/\tau_2)\right)$$

I_0 α_1 τ_1 α_2 τ_2



GRAY CANCER INSTITUTE

Global analysis

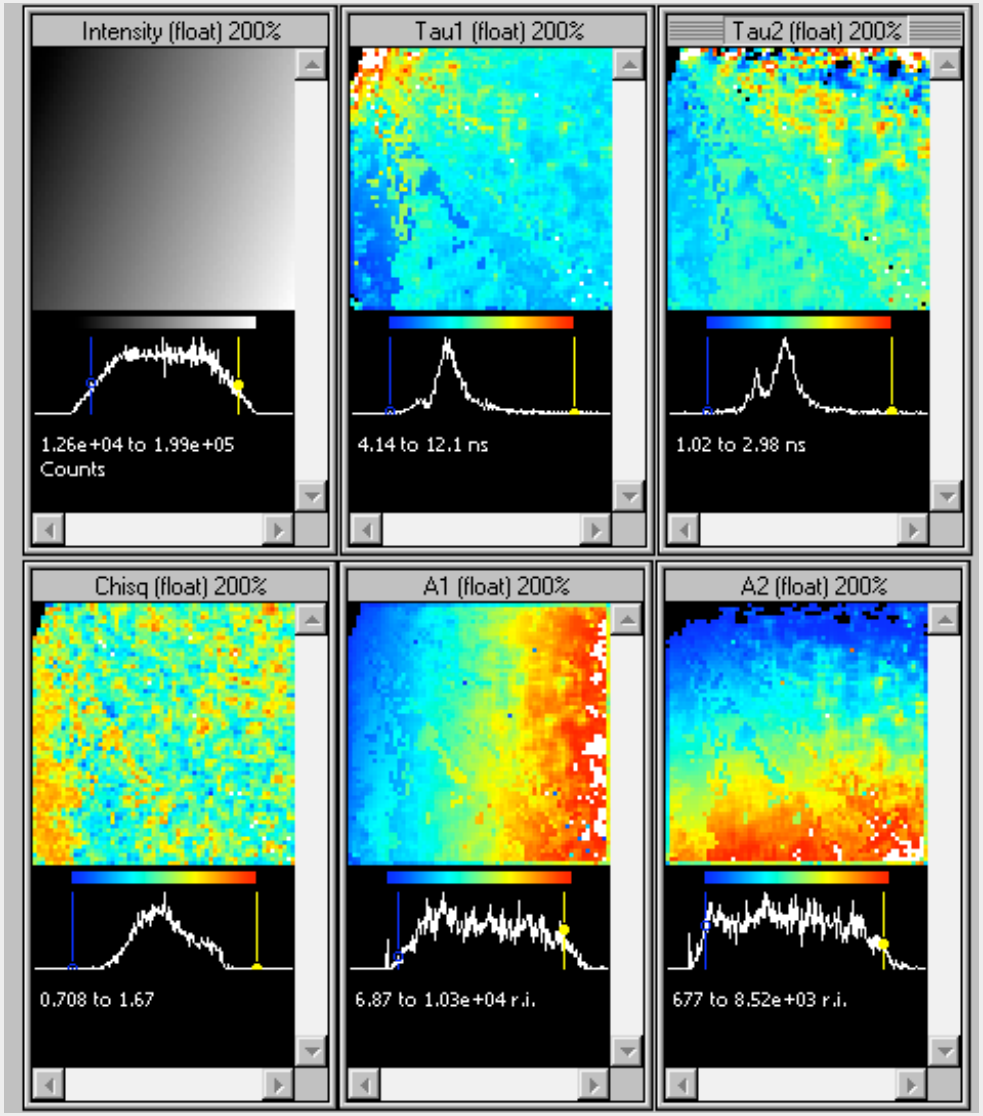
- Sum transients over all pixels and determine component lifetimes
- Make **relative amplitudes** free parameters for each pixel
- Fit across all pixels with same τ_1 and τ_2
- Determine overall χ^2 (error map)
- Vary τ_1 and τ_2 (same at all pixels) and relative amplitudes until the overall χ^2 is minimised

- Faster computation (fewer free parameters) than free pixel fitting
- More accurate fits with poor signal-to-noise ratios
- Reduced loss of spatial resolution

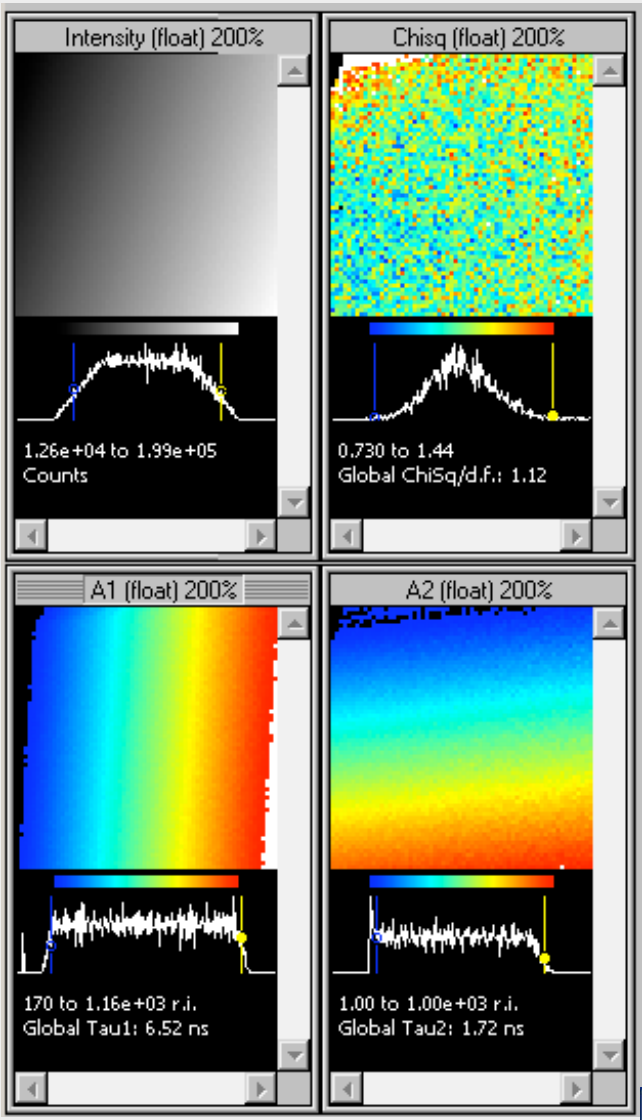
GRAY CANCER INSTITUTE

Analysis of simulated image – pixel and global

Marquardt individual pixel fitting

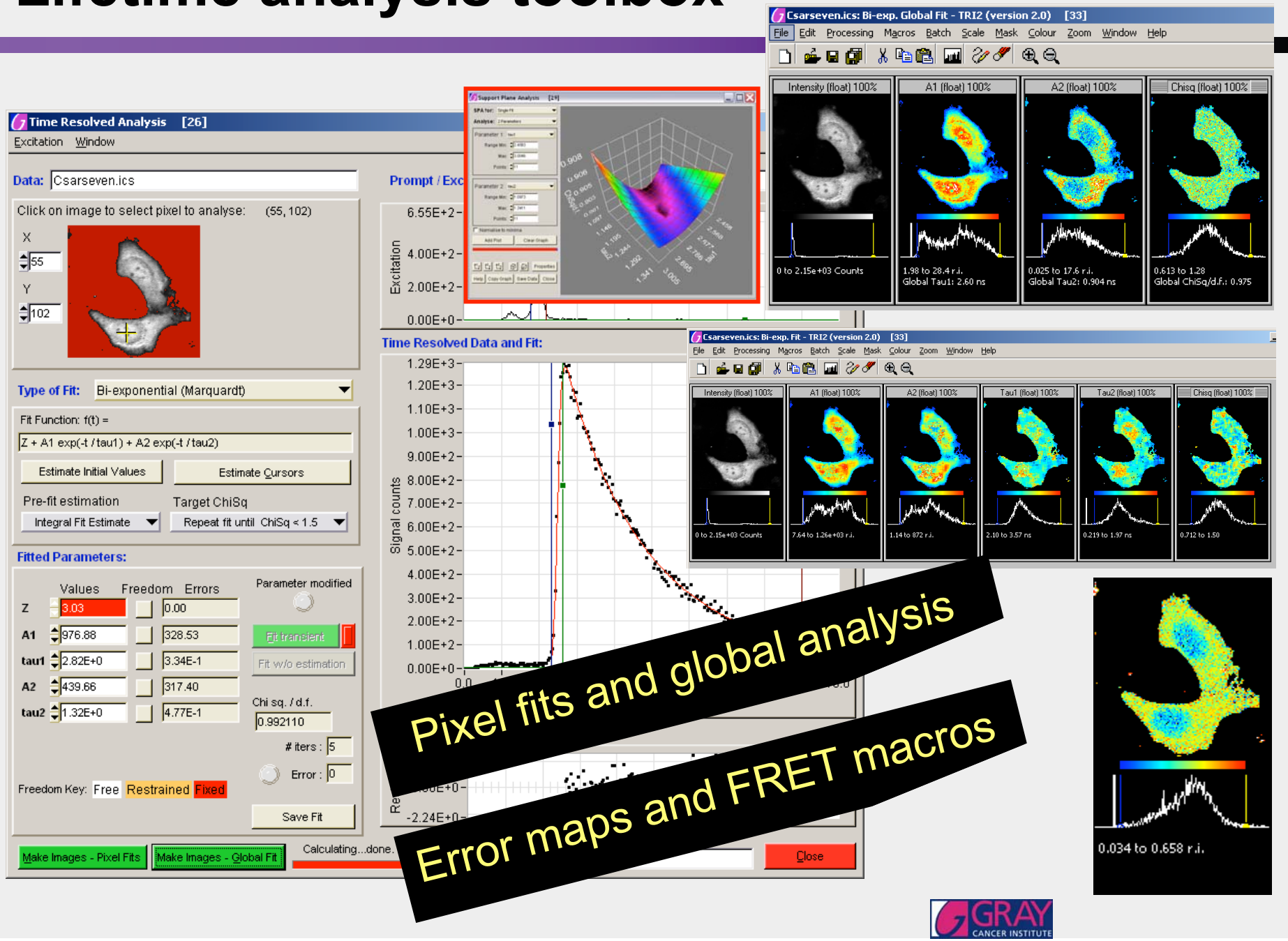


Global analysis

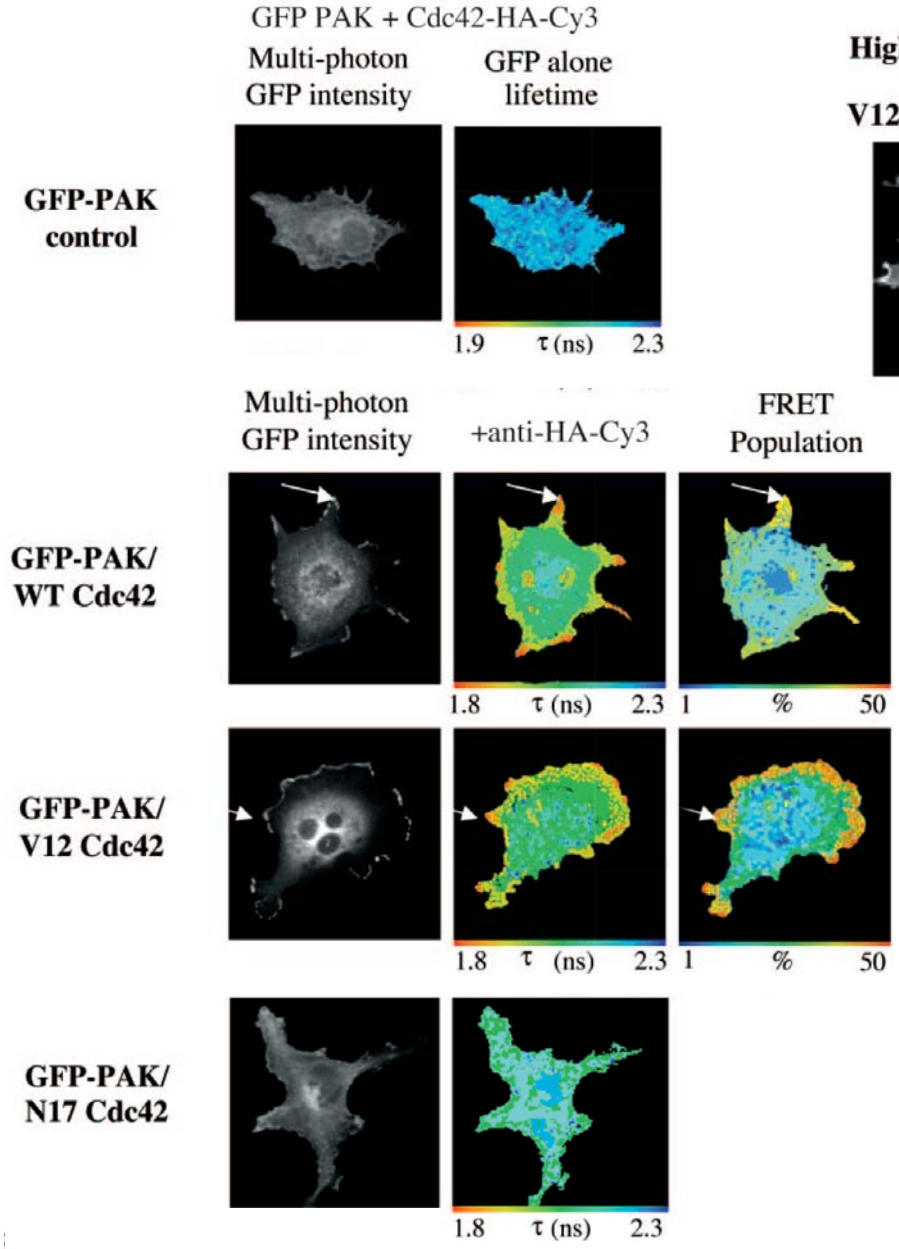


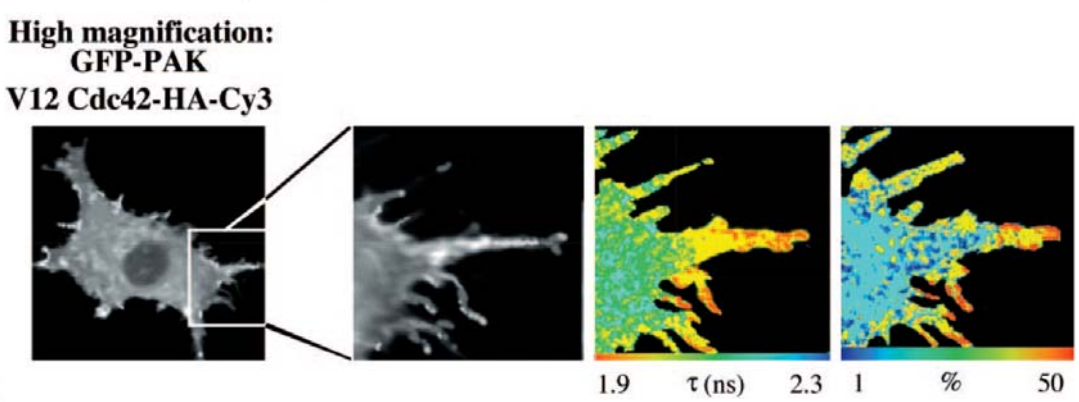
GRAY CANCER INSTITUTE

Lifetime analysis toolbox



FLIM/FRET example: GFP donor, Cy3 acceptor





Location of site of PAK1-Cdc42 interaction.

MP FLIM used to determine the extent of FRET between the GFP-PAK1 donor and anti-HA-Cy3 acceptor in cells co-expressing GFP-PAK1 and HA-tagged Cdc42 (WT and N17 variants).

Parsons, M., Monypenny, J., Ameer-Beg, S.M., Millard, T.H., Machesky, L.M., Peter, M., Keppler, M.D., Schiavo, G., Watson, R., Chernoff, J., Zicha, D., Vojnovic, B. and Ng, T. (2005) 'Spatially Distinct Binding of Cdc42 to PAK1 and N-WASP in Breast Carcinoma Cells', *Mol. Cell. Biol.* 25, 1680-1695.

GRAY CANCER INSTITUTE

The next generation of instruments….

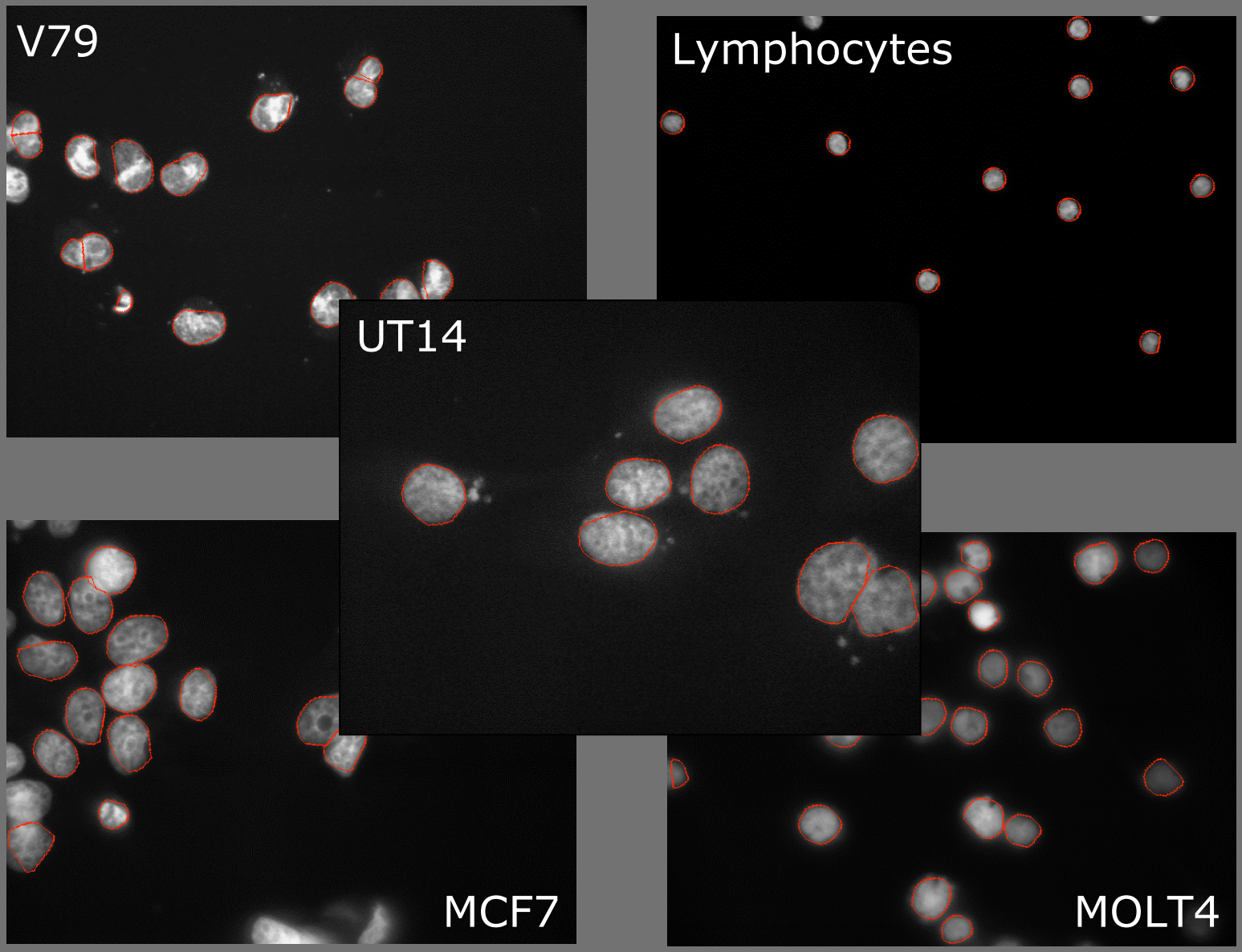
- Disparate but complementary technologies required
- ‘Individual’ techniques – e.g. micro-irradiation or FLIM/FRET proven and available
- **Convergence of high throughput, high content imaging and high resolution targeting methods**
- High degree of automation and control
- High speed FLIM will be essential + TCSPC ‘gold standard’
- Data processing, analysis and appropriate biology experiment design likely to be challenges

GRAY CANCER INSTITUTE

Image processing

…..a subject in its own right and with numerous applications in microscopy

Finding cell nuclei



GRAY CANCER INSTITUTE

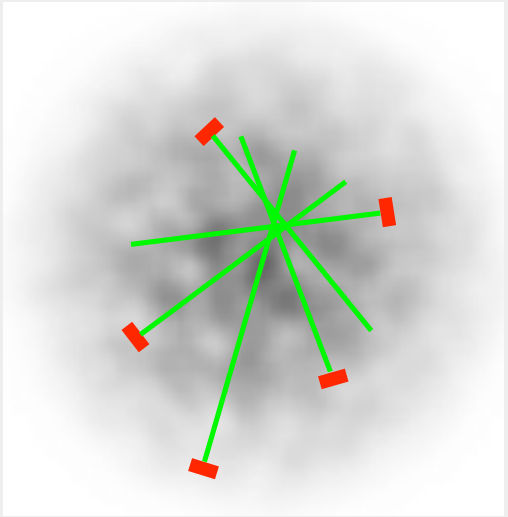
with Kai Rothkamm, GCI

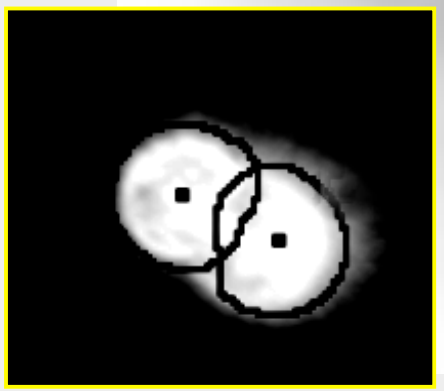
Image processing –finding cell nuclei

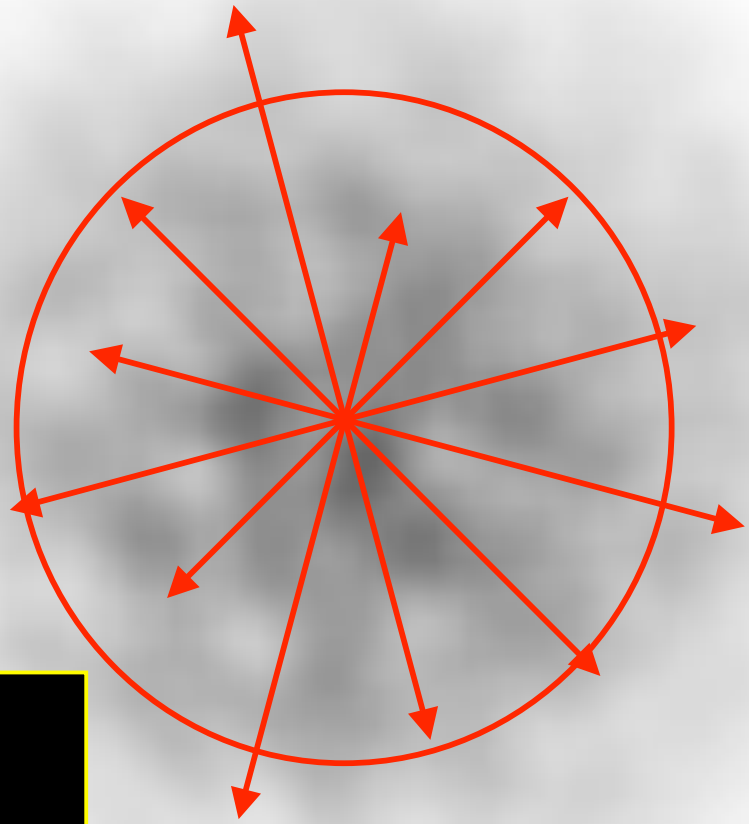
Compact Hough Transform

Search outwards
Local area mapping

- Independent of intensity
- Not too sensitive to shape
- Overlapping targets identified





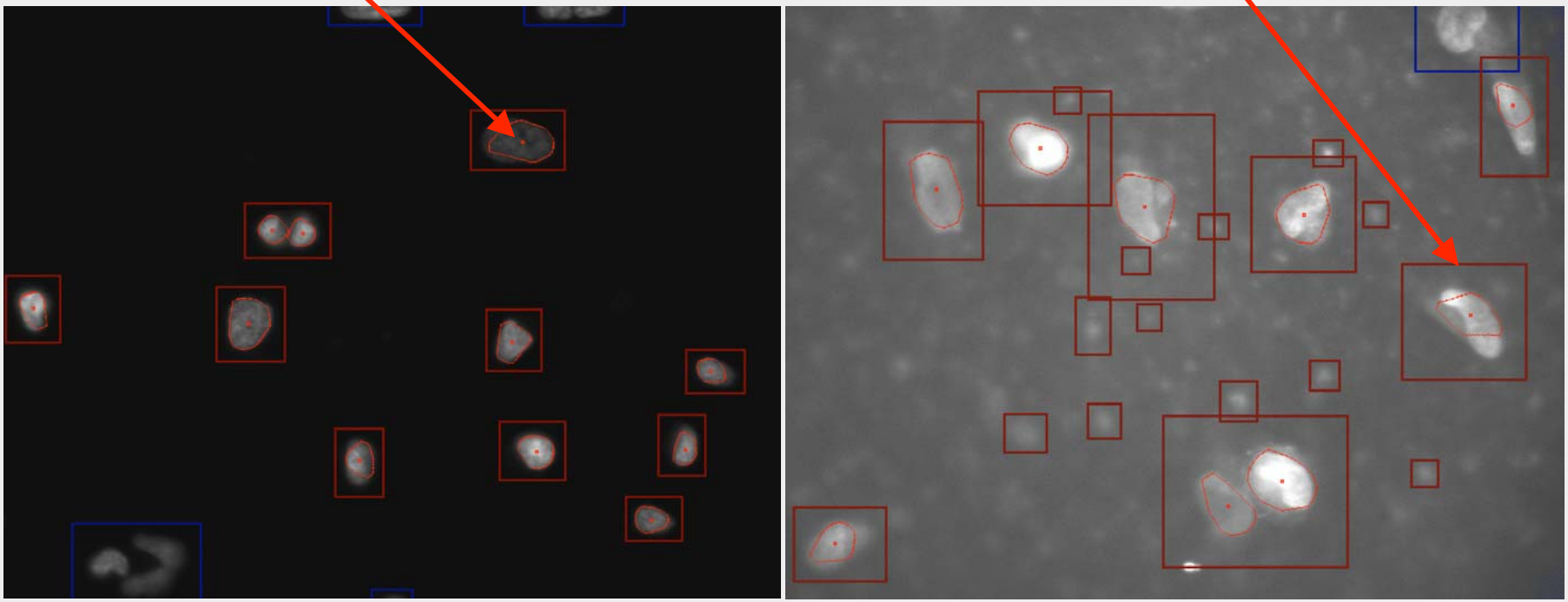


Compact **H**ough transform **A**nd **R**adial **M**apping

Target identification – CHARM algorithm

Target centre identified

Potential target
- pre-processing



high-contrast - HeLa nuclei

poor-contrast, noisy - T98G nuclei

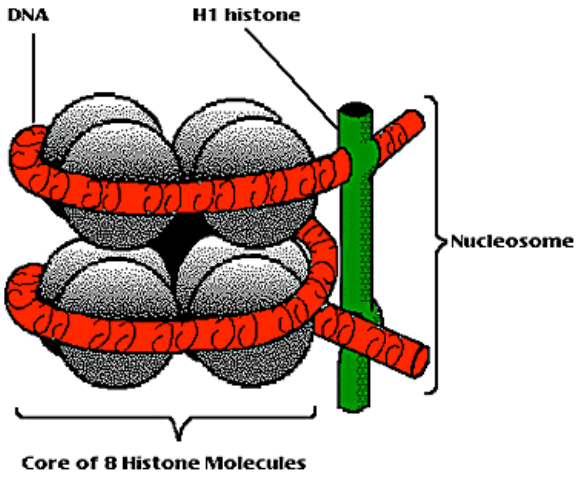
Hoechst 33342 intercalating DNA stain

Barber, P.R., Vojnovic, B., Kelly, J., Mayes, C.R., Boulton, P., Woodcock, M. and Joiner, M.C. (2001) Automated Counting of Mammalian Cell Colonies. *Physics in Medicine and Biology* 46, 63-76.

GRAY CANCER INSTITUTE

Measurement of γH2AX foci

- H2AX represents 2 – 10% of the **H2A subfamily** of histone proteins in chromatin
- **Phosphorylated** rapidly in response to DSB at serine 139
- ~1% of the H2AX phosphorylated per Gy and number of **γH2AX foci** ≈ number of DSB
- Acts as major **recruiter** of repair enzymes
- Kinases involved in the phosphorylation step are **ATM/ATR**
- Cellular response to **dsb intermediates** in V(D)J recombination, meiotic recombination

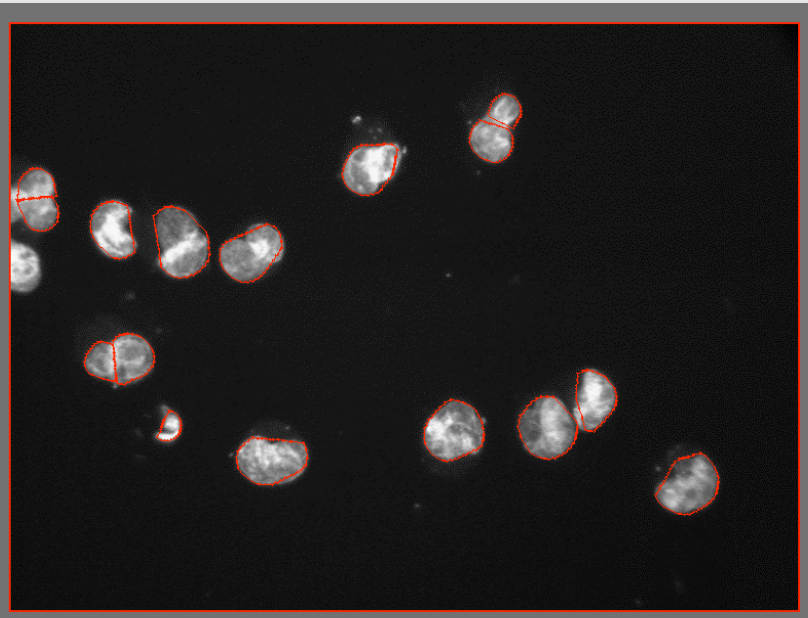


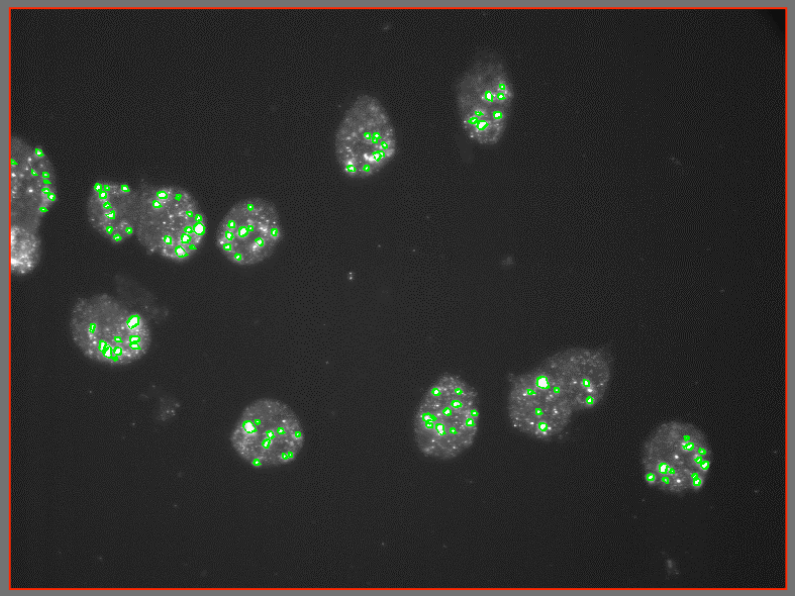
Nucleosome

2 x histone 2A
2 x histone 2B
2 x histone 3
2 x histone 4

γH2AX foci – CHARM algorithm

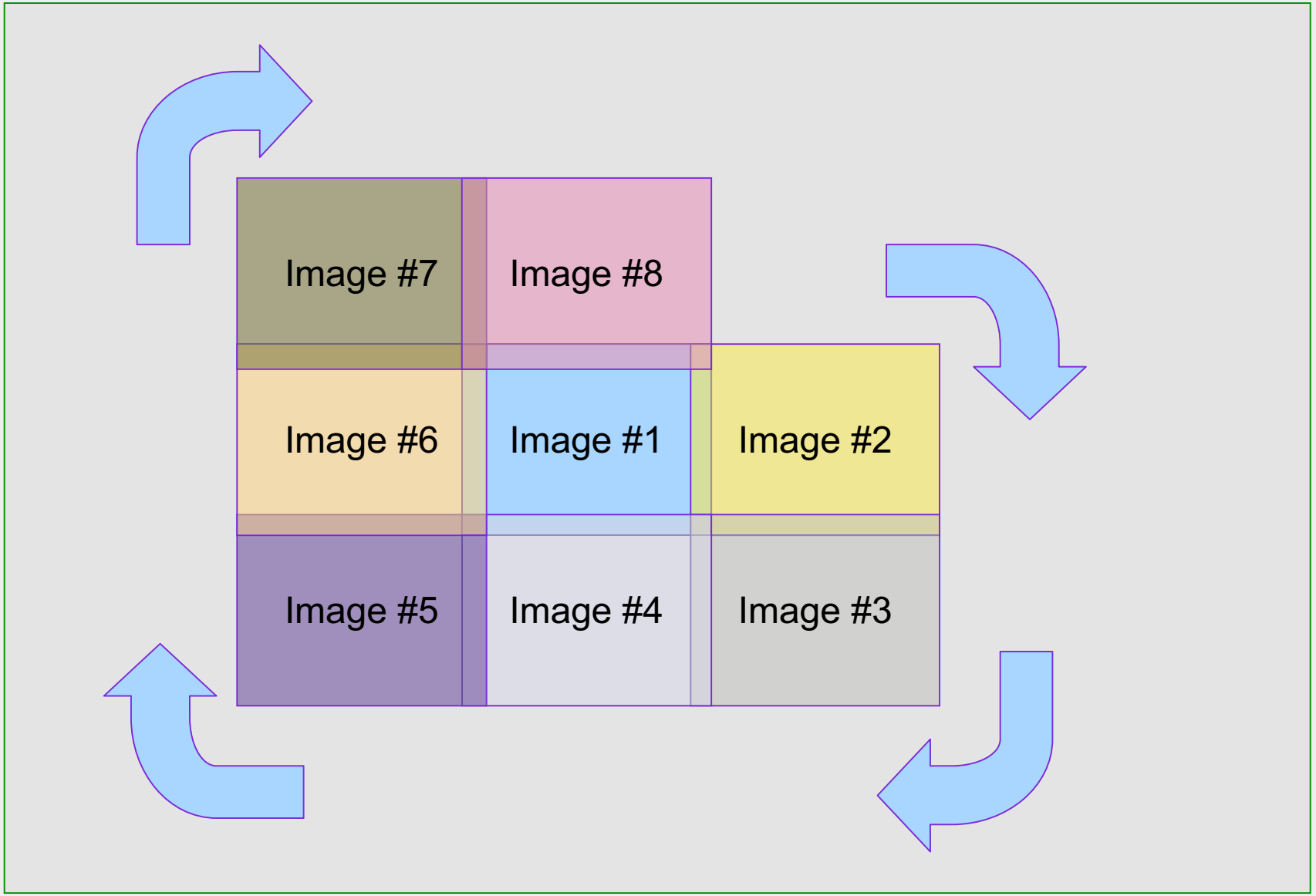
Apply same algorithm in two-step process:
find nuclei, find foci within nuclei – different size criteria

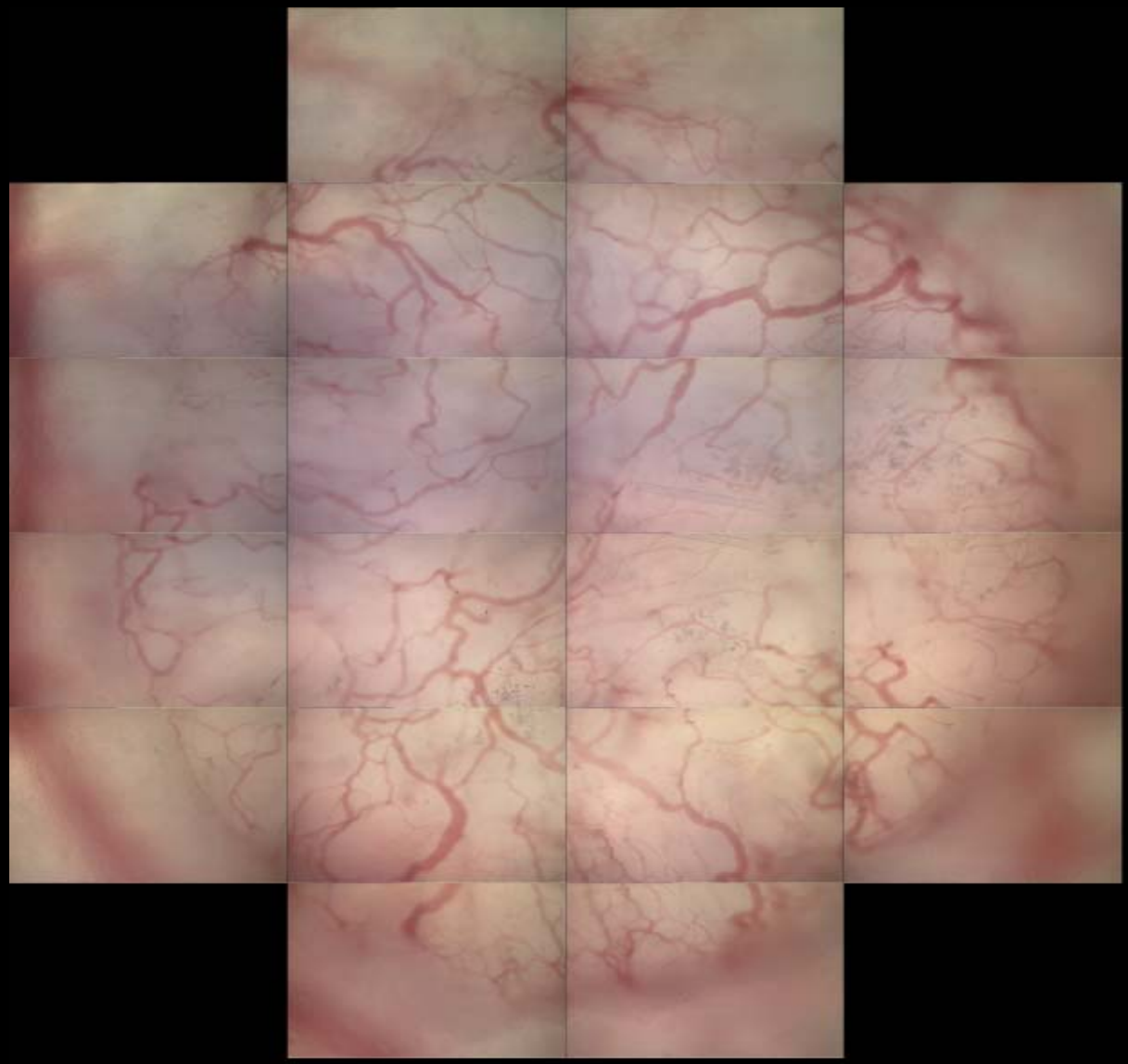




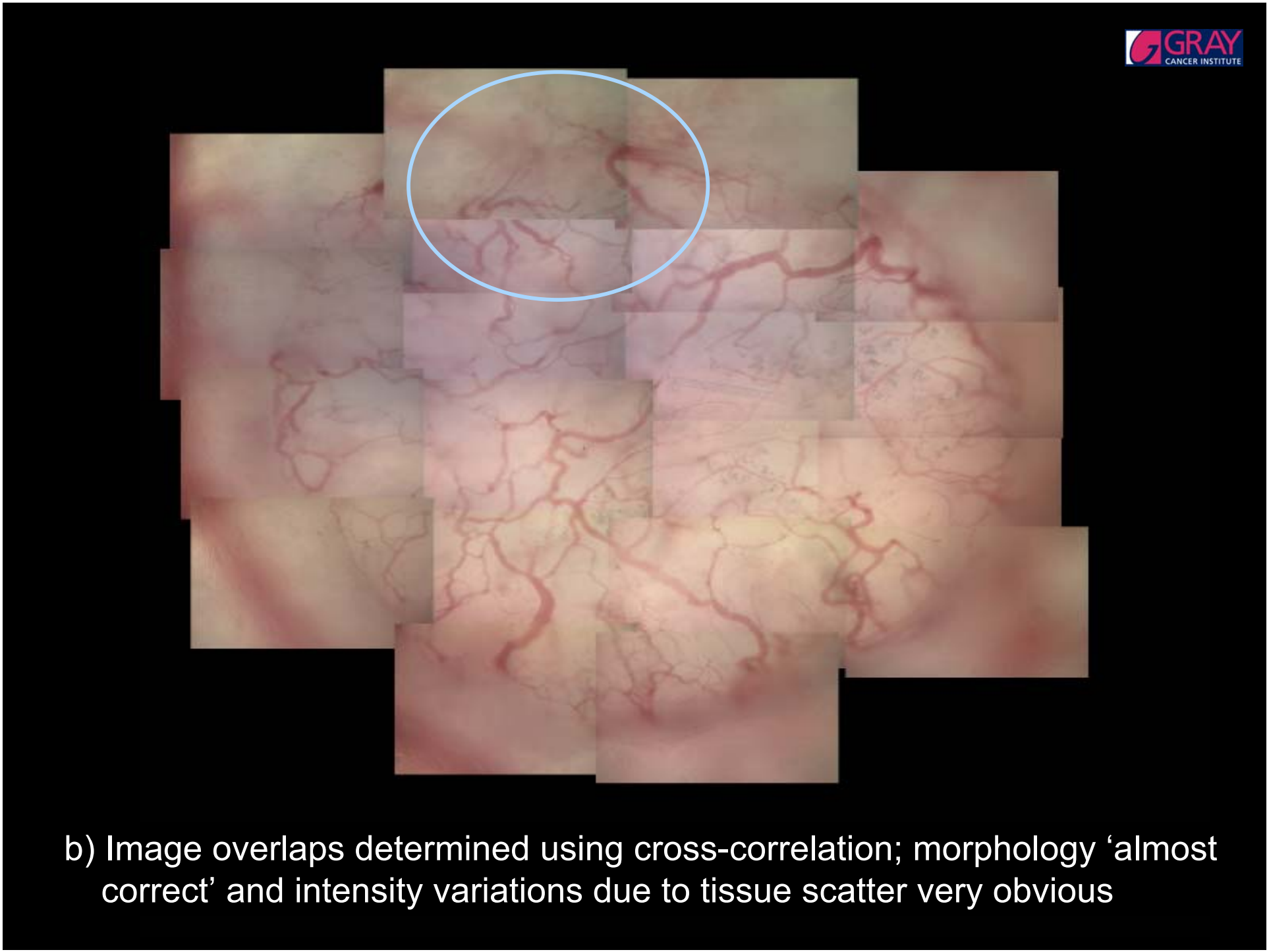
With Kai Rothkamm, GCI

Image processing large areas

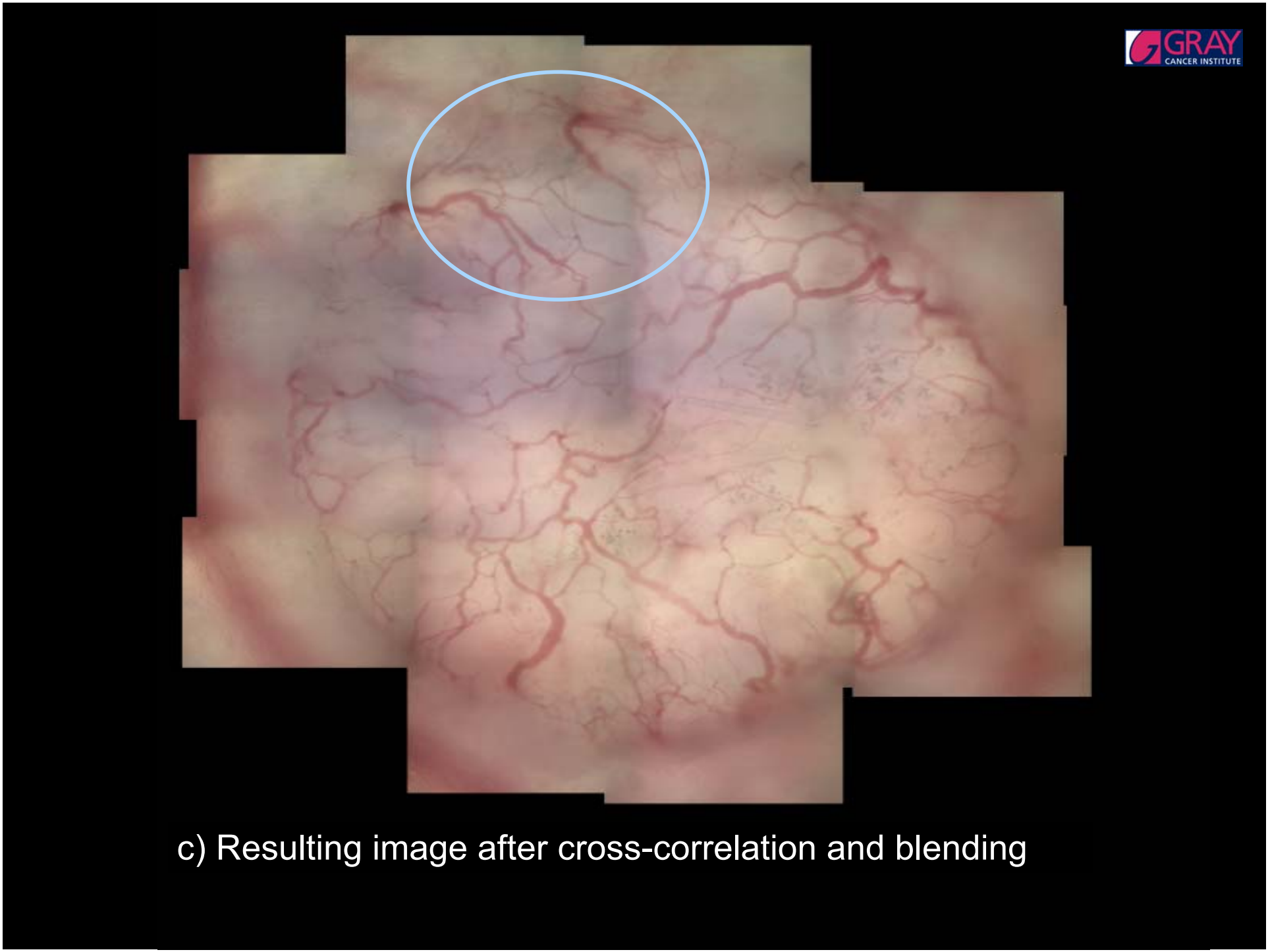




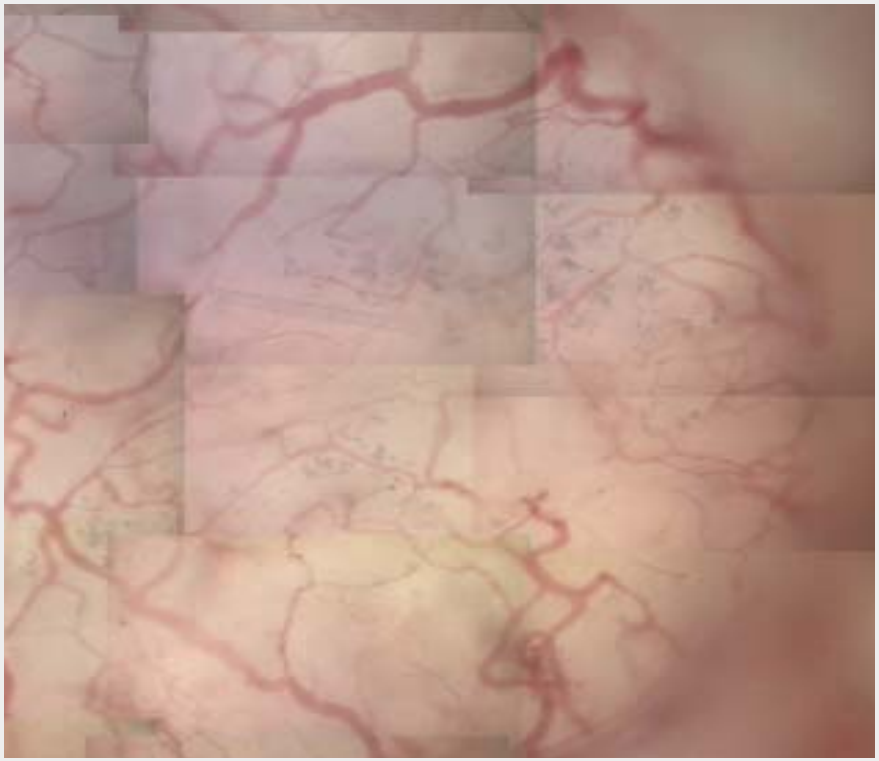
a) Unprocessed composite image, images in ‘correct’ sequence but image overlaps are incorrect

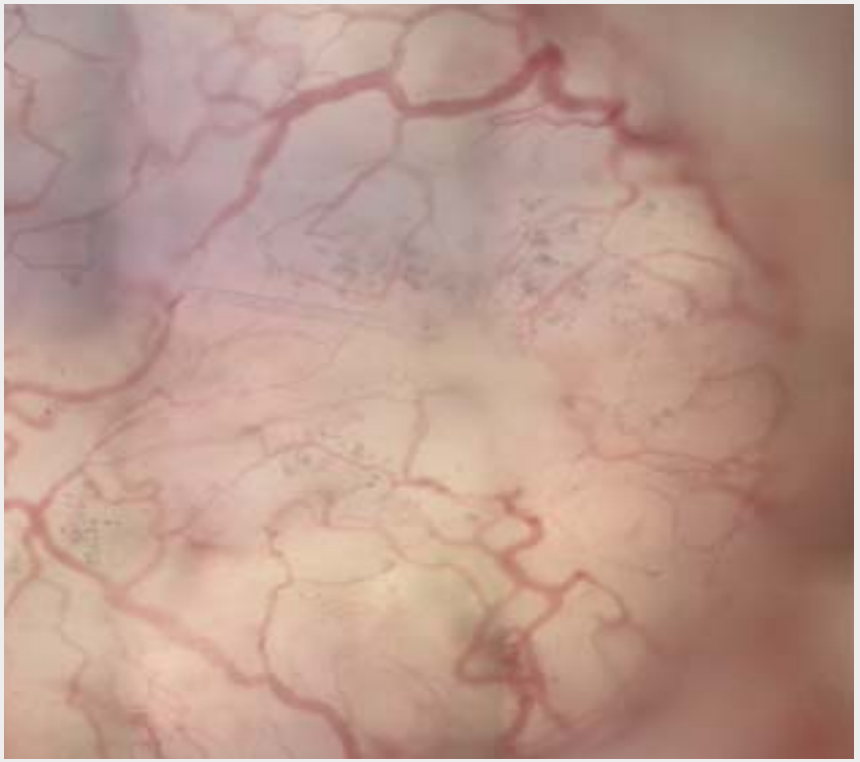


b) Image overlaps determined using cross-correlation; morphology ‘almost correct’ and intensity variations due to tissue scatter very obvious



c) Resulting image after cross-correlation and blending

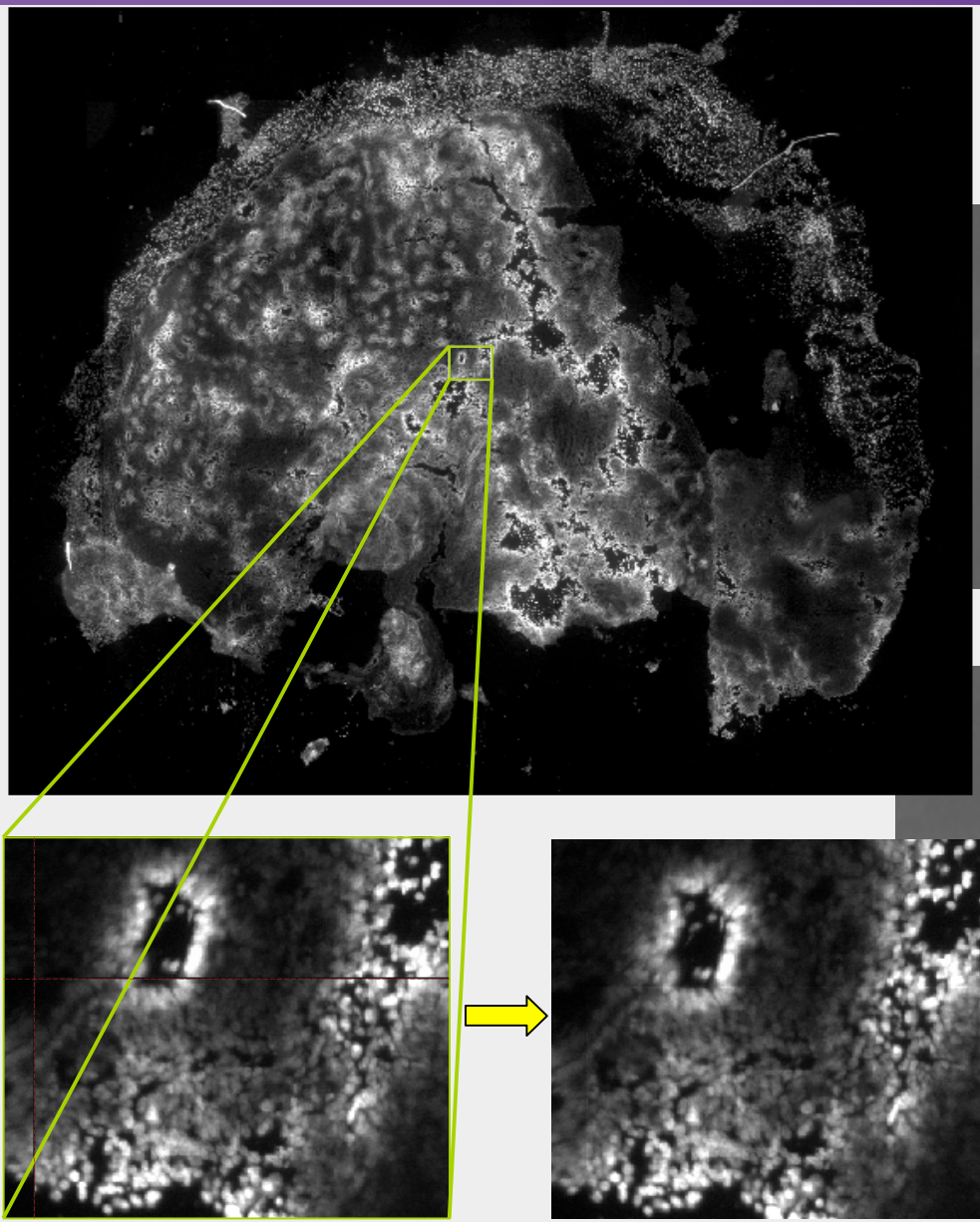




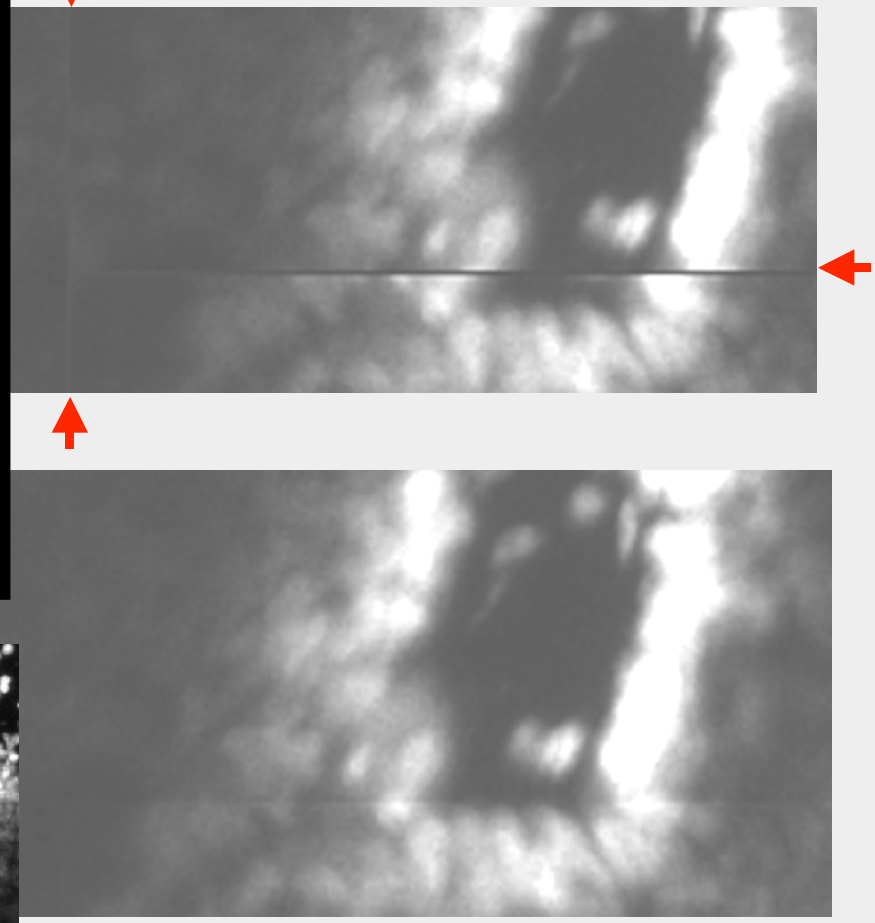
Before and after blending process

“Rubbish in – rubbish out” applied to computers not always true!...

Large area example - fluorescence

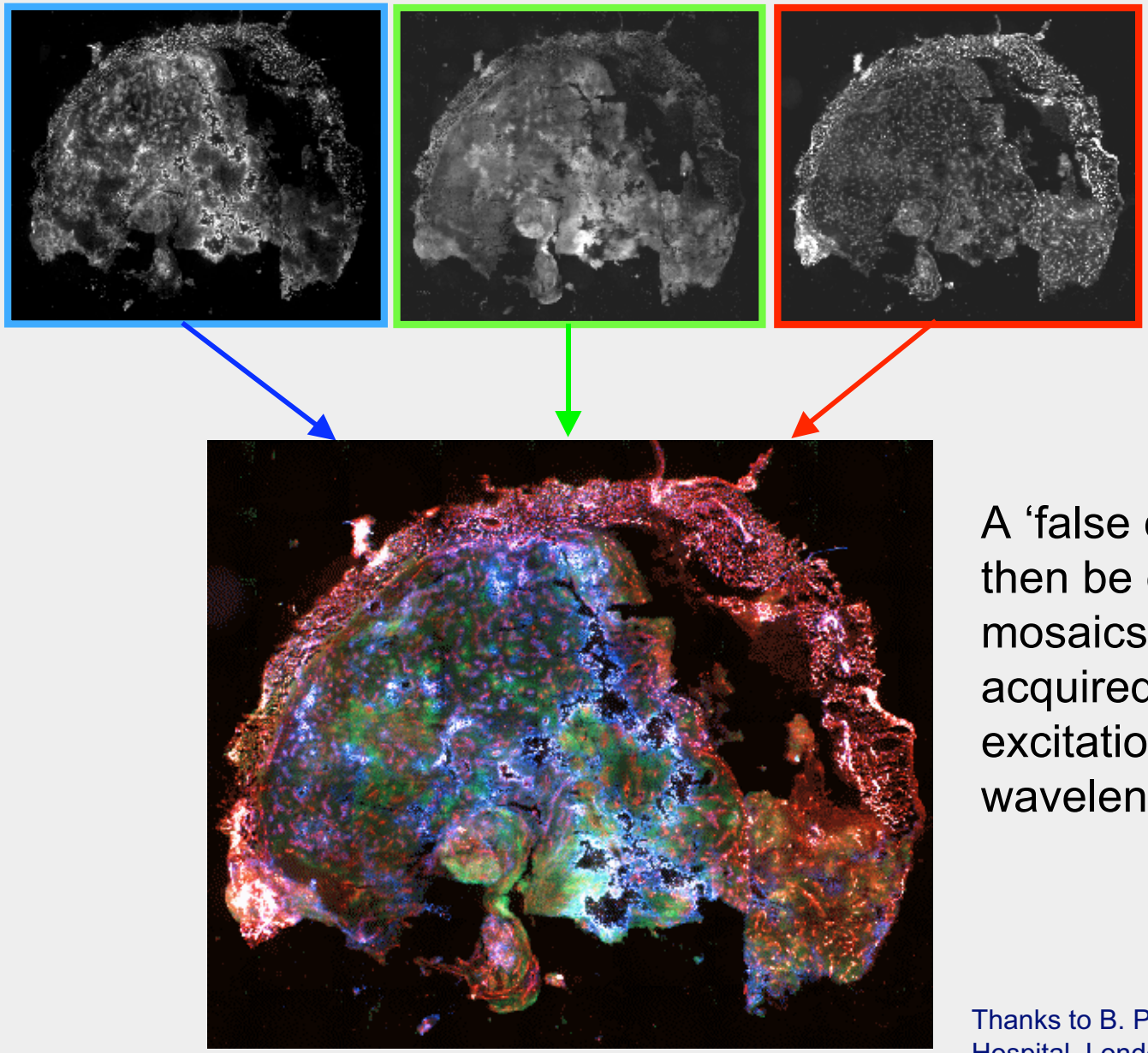


Widefield fluorescence excitation



GRAY CANCER INSTITUTE

Large area example - fluorescence



A ‘false colour’ image can then be composed from mosaics, individually acquired at different excitation and emission wavelengths

Thanks to B. Pedley (Royal Free Hospital, London) for samples

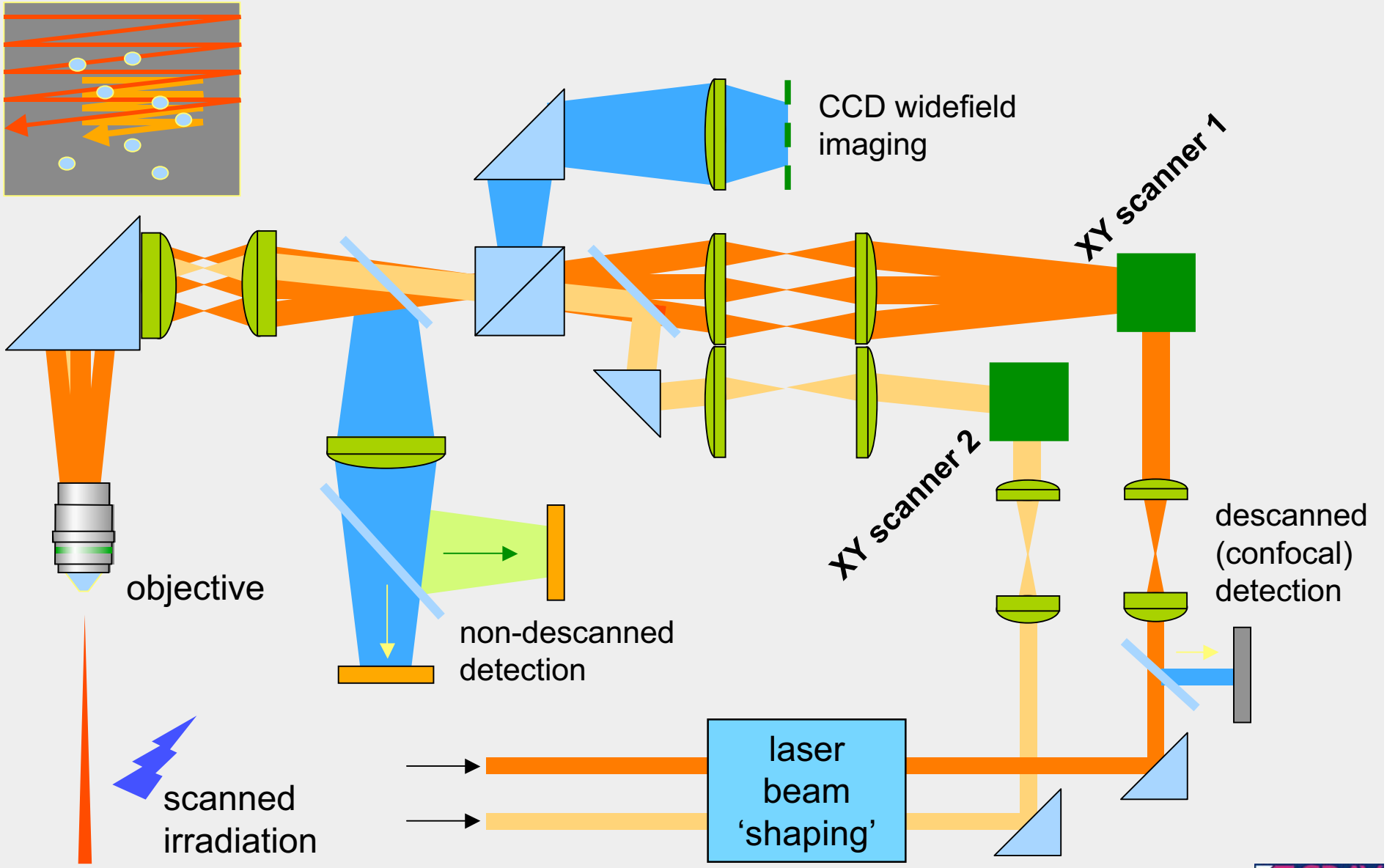
Image processing ….has a long way to go

I cdnuolt blveiee taht I cluod aulaclty uesdnatnrd waht I was rdenieg. The phaonemneal pweor of the hmuan mnid Aodccrnig to a rscheearch at Cmabrigde Vinervtisy, it dnsoe't mttaer in waht oredr the ltteers in a wrod are, the olny iprmoatnt tihng is taht the frist and lsat ltteer be in the rghit pclae. The rset can be a taotl mses and you can sitll raed it wouthit a porbelm. Tihs is bcuseae the hmuan mnid deos not raed ervey lteter byi stlef, but the wrod as a wlohe. Azmanig huh? yaeh and I awlyas tghuoht slpeling was ipmorantt!

Applications to micro-irradiation

…..what do we need to have in an ideal system?

Imaging – irradiation workstation



Scanning focussed vertical ion nanobeam **UK facility for cell irradiation and analysis**

Multipurpose imaging system

- Widefield transillumination modes
- Widefield fluorescence – imaging down to ‘single’ photons
- Scanned beam fluorescence imaging, 1PE and 2PE
- Scanned beam time-resolved fluorescence imaging, 1PE and 2PE
- Ion beam particle counting
- Imaging of samples *in vacuo* and samples held on substrates
- Deep tissue imaging modes
- Long-term / time lapse imaging modes

GRAY CANCER INSTITUTE

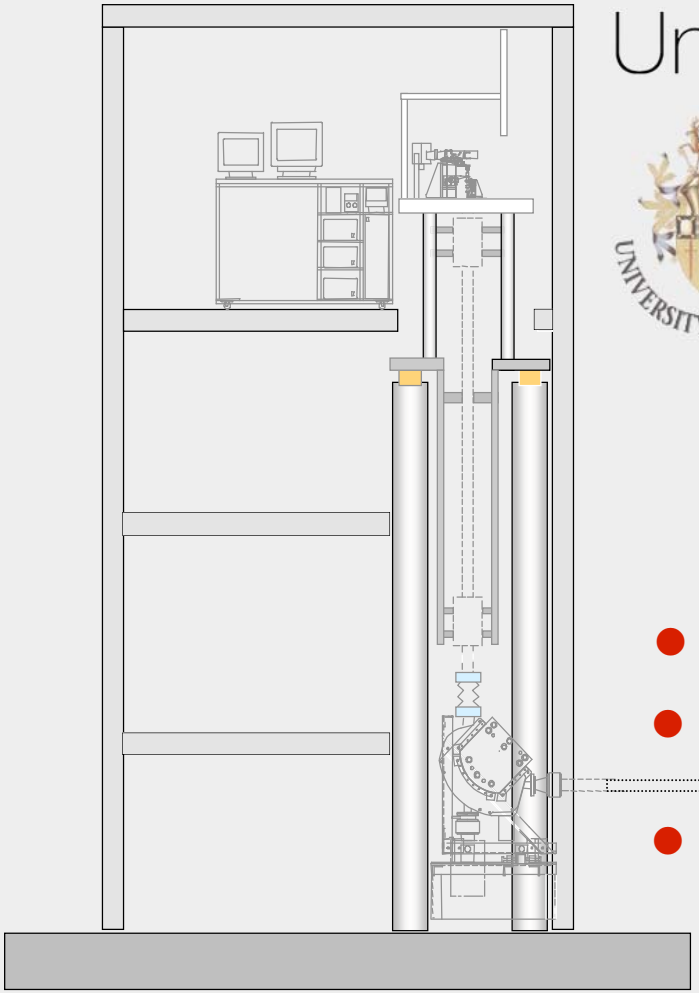
Scanning focussed vertical ion nanobeam **UK facility for cell irradiation and analysis**

Why imaging flexibility….complexity?

- Some experiments require irradiation of large numbers of targets
- 10^5 targets per hour aimed for: competing requirements in apportioning time between imaging/finding and irradiation…and sample movement (5-10 cm^2)….≈ 35 ms
- Some experiments require irradiation of few targets (1?) and following of effects on neighbouring biology
- Some experiments require irradiation of few (10's-100's) targets and following of signalling processes within target and/or neighbours

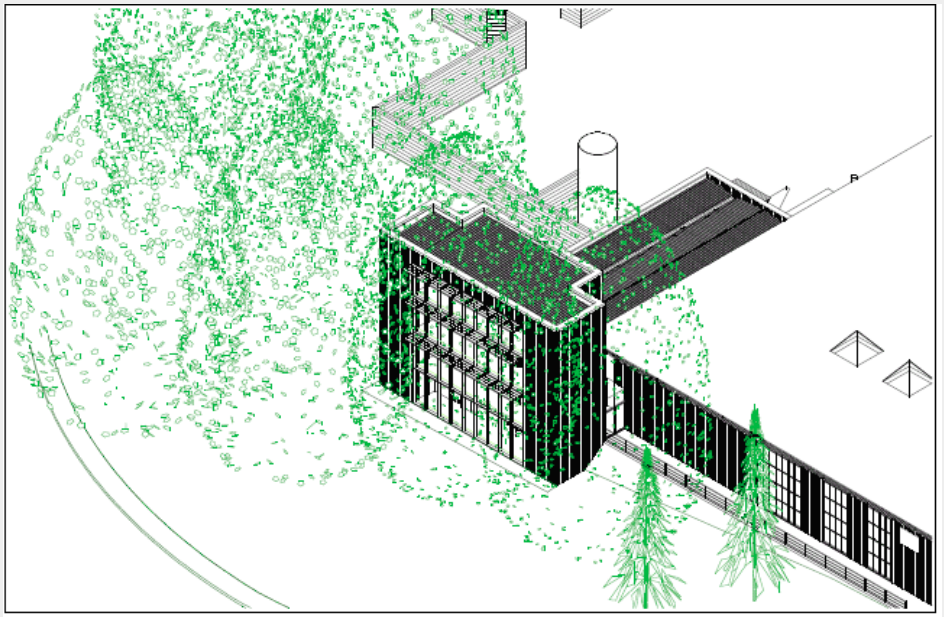
GRAY CANCER INSTITUTE

Scanning focussed vertical ion nanobeam
UK facility for cell irradiation and analysis



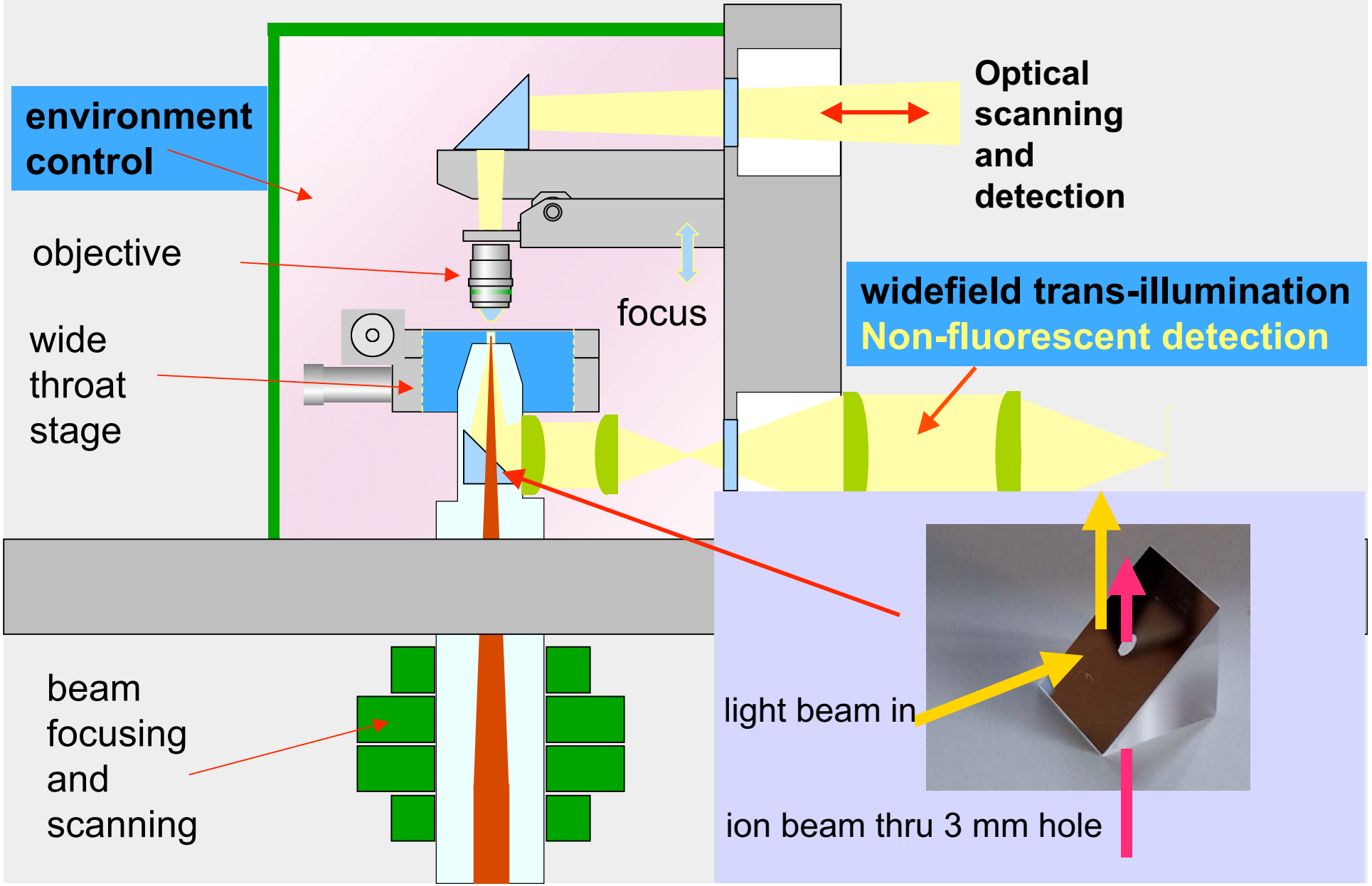
UniS

UNIVERSITY OF SURREY

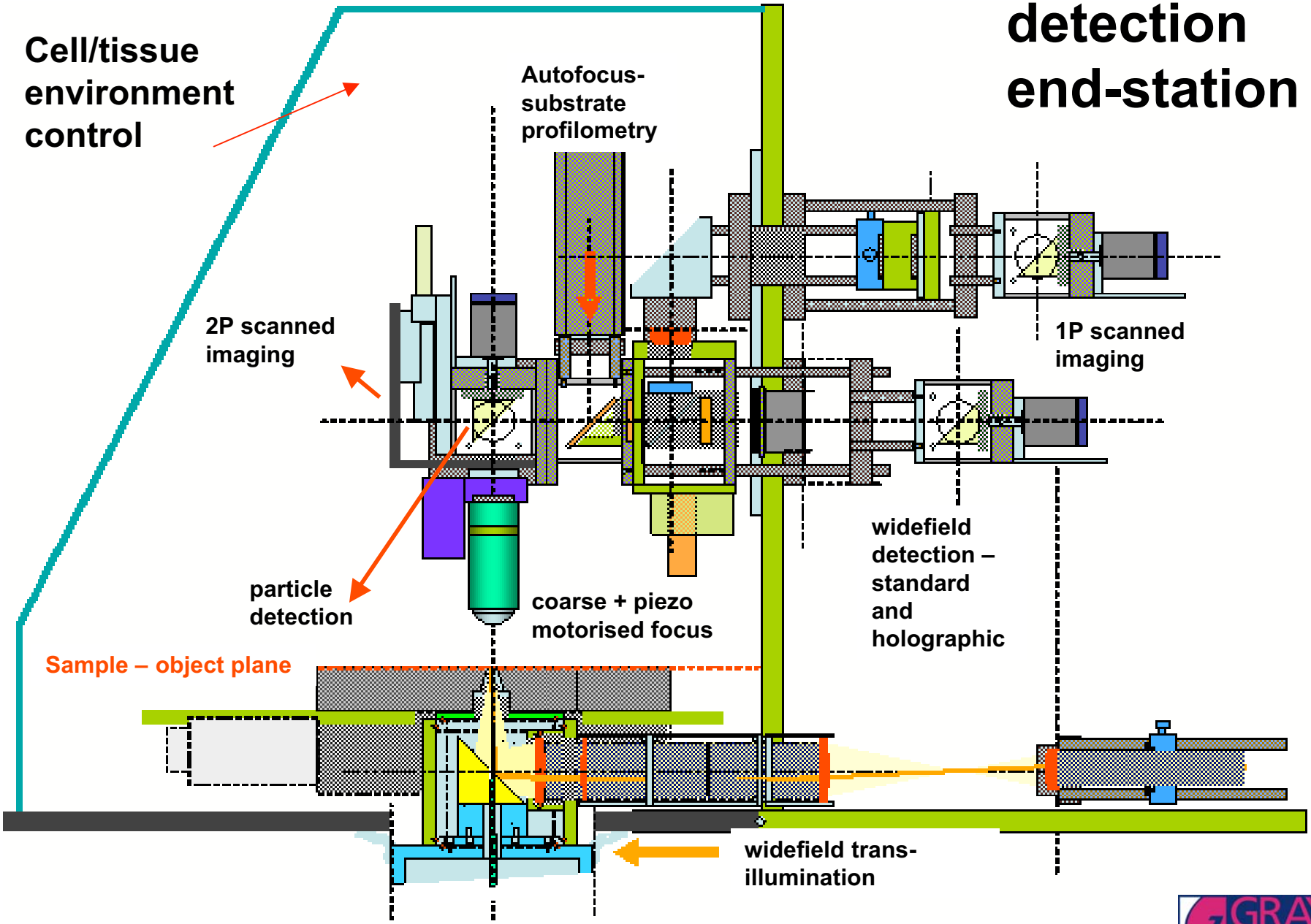


- **Based on horizontal 2 MV tandetron beam line(s)**
- **Vertical beam line extension in new building**
- **Biological sample nano- and micro-irradiation, elemental analysis, focussed ion beams, wide range of ions**

Optical detection end-station

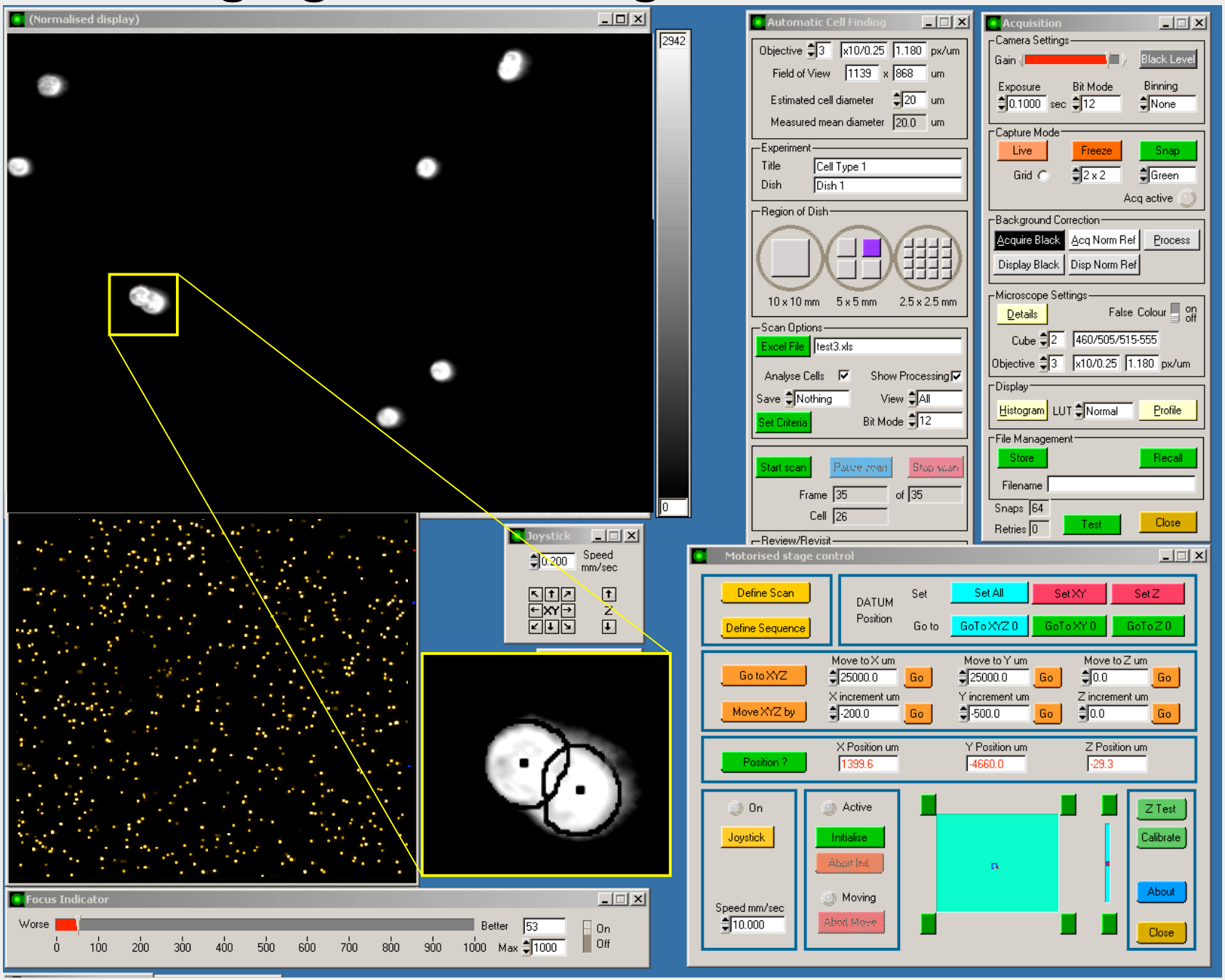


Optical detection end-station



GRAY CANCER INSTITUTE

Cell imaging / cell finding



Summary

- Numerous cell and tissue imaging methods are available and new ones are evolving
- To understand biological processes associated with low dose exposures microbeam approaches can be combined with imaging methods
- Imaging techniques based on 2PE, 1PE FLIM can be used to obtain 3-D, 2-D information on molecular/protein interactions and signalling events, will be key future technologies
- Imaging without integrated image processing and image analysis is not an option!
- Automation and high throughputs are essential for future developments
- High cost and development cost of customised solutions dictates flexibility of new designs, combining several imaging modalities

GRAY CANCER INSTITUTE

Acknowledgements

Imaging

S Ameer Beg
PR Barber
R Edens
J Gilbey
J Girkin
RJ Hodgkiss
R Juškaitis
RJ Locke
G McConnell
RG Newman
V Prise
S Roberts
G Tozer
T Wilson

Microbeams

PR Barber
M Folkard
G Flaccavento
S Gilchrist
G Grime
K Kirkby
RJ Locke
RG Newman
BD Michael
KM Prise
P Schettino
R Sunderland
L Tartier

FLIM/FRET

S Ameer Beg
PR Barber
F Festy
RJ Locke
RG Newman
T Ng
M Parsons
N Edme
M Keppler
M Peter

Collaborators…..

M Booth, University of Oxford
R Juškaitis, University of Oxford
J Girkin, Strathclyde University
G Grime, University of Surrey
K Kirkby, University of Surrey
B Larijani, LRI, London
T Ng, King's College, London
P Parker , King's College, London
M Parsons, King's College, London
K. Rothkamm, University of Oxford
M Keppler, King's College, London
T Wilson, University of Oxford