Non-invasive *in vivo* imaging for basic and translational research

From whole body to subcellular scale in health and disease

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Cancer Research UK, London Research Institute

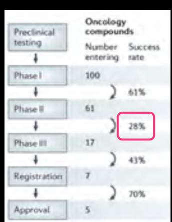
Research Associate
Centre for Advanced Biomedical Imaging (CABI), UCL

Animal models are needed
For basic and translational research

- ✓ Study development & homeostasis of normal tissues
- ✓ Physiopathology of human and animal diseases
- ✓ Validate new targets
- ✓ Test new therapeutic strategies
  - o PK
  - o PD
  - o Toxicity

… but predictive ones

Rates are for ten large pharmaceutical companies in the USA and Europe for the period 1991-2000

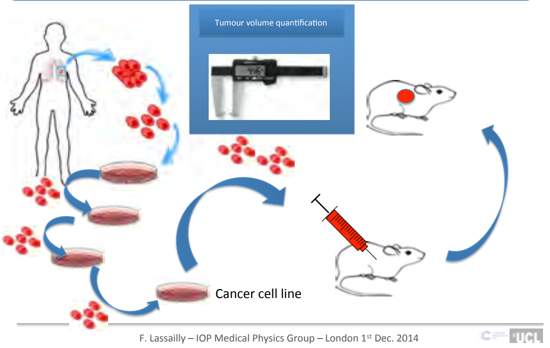


Efficacy !

95% attrition

Sharpless NE et al, Nat. Rev. Drug Discov 2006

Conventional preclinical model in oncology



The best model of a cat is a cat or, better , the cat itself
Norbert Weiner (1894-1964), Mathematician

Essentially, all models are wrong, but some are useful
George E. P. Box (1919- 2013), Statistician

What can we do?

1) Improve the (intrinsic) quality of animal models

2) Optimise the way we use animal models (extrinsic)

3) Develop best practice to maximise robustness and reproducibility

4) Report all studies appropriately

Select the best available model in oncology

Project dependent

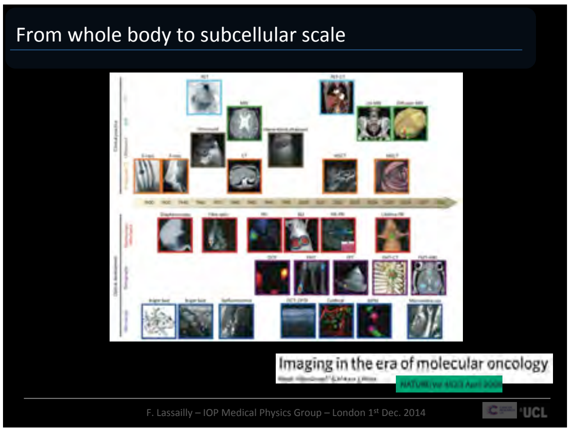
Mouse cancer cells	Human cancer cells
Syngeneic transplantation models Subcut, IP, IV, orthotopic	Xenograft of human cancer cell lines Subcut, IP, IV, orthotopic
Genetically engineered mouse models (GEMMs)	Patient derived xenografts Subcut, orthotopic

Orthotopic approaches should be preferred whenever possible

Imaging is required to follow orthotopic tumours

Hanahan D and Coussens LM, Cancer Cell 2012

In vivo imaging has long been indispensable in clinical practice


From whole body to subcellular scale
Imaging in the era of molecular oncology
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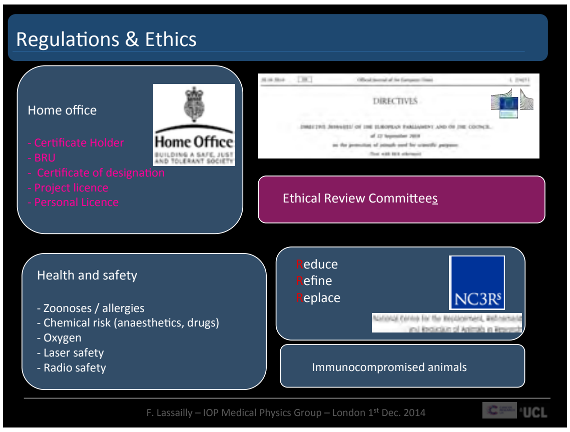
Regulations & Ethics
Home office
- Certificate Holder
- BRU
- Certificate of designation
- Project licence
- Personal Licence
Home Office
BUILDING A SAFE, JUST AND TOLERANT SOCIETY
DIRECTIVES
Ethical Review Committees
Health and safety
- Zoonoses / allergies
- Chemical risk (anaesthetics, drugs)
- Oxygen
- Laser safety
- Radio safety
Reduce
Refine
Replace
NC3Rs
Immunocompromised animals
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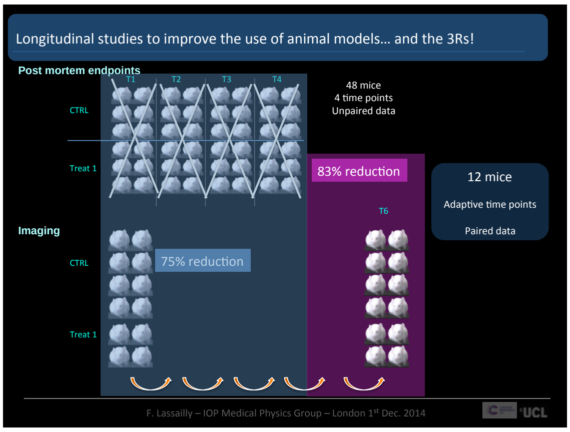
Longitudinal studies to improve the use of animal models… and the 3Rs!
Post mortem endpoints
T1
T2
T3
T4
CTRL
Treat 1
48 mice
4 time points
Unpaired data
83% reduction
12 mice
Adaptive time points
Paired data
T6
Imaging
CTRL
75% reduction
Treat 1
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Longitudinal studies to improve the use of animal models… and the 3Rs!

Minimise inter-individual variability
=> Improved sensitivity to detect small but biologically meaningful effects

“Staging” for subsequent randomisation before intervention

Enabling approach for exploring complex scenarios
- Resistance to treatment
- Residual disease
- Relapse
- Combination therapy

Disease progression correlates inversely with animal welfare

Imaging can be used to assess and REFINE experimental procedures

Dramatically reduce the need for survival studies

A strategic role for *in vivo* imaging facilities

Support:

- Study design
- Choice of animal models (including reporters etc)
- Choice of imaging technologies
  - Scanner
  - Probes
  - Contracting agents
  - Reporter systems
- Image acquisition
- Image analysis (segmentation/quantification)
- Data interpretation and preparation for publication
- Data storage and data sharing

- SOPs
- Blinded analysis
- Randomisation
- Reproducibility
- Relevance
- Best practice

CANCER RESEARCH UK | LONDON RESEARCH INSTITUTE

www.london-research-institute.org.uk

First Charity dedicated to cancer


42 Research groups
15 Core Technology platforms

In Vivo Imaging Facility @

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Some applications

- Example 1: Haematopoietic stem cells and acute myeloid leukaemia
  - Bioluminescence
  - Near Infrared fluorescence
  - Intravital microscopy

Example 2: Non Small Cell Lung Cancer (GEMM)
  - Micro-CT
  - Response to treatment
  - Relapse

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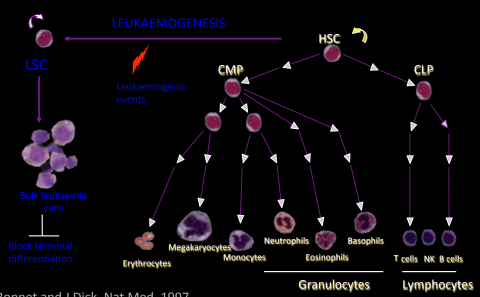
The haematopoietic hierarchy



D. Bonnet and J Dick, Nat Med, 1997

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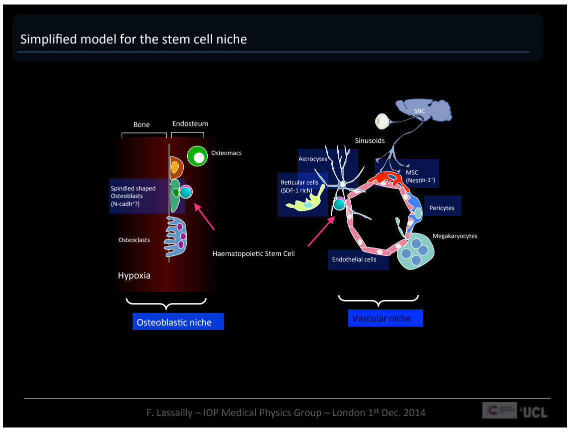
Simplified model for the stem cell niche
Bone
Endosteum
Osteomacs
Spindled shaped Osteoblasts (N-cadh+?)
Osteoclasts
Haematopoietic Stem Cell
Hypoxia
Osteoblastic niche
SNC
Sinusoids
Astrocytes
Reticular cells (SDF-1 rich)
MSC (Nestin-1+)
Pericytes
Megakaryocytes
Endothelial cells
Vascular niche
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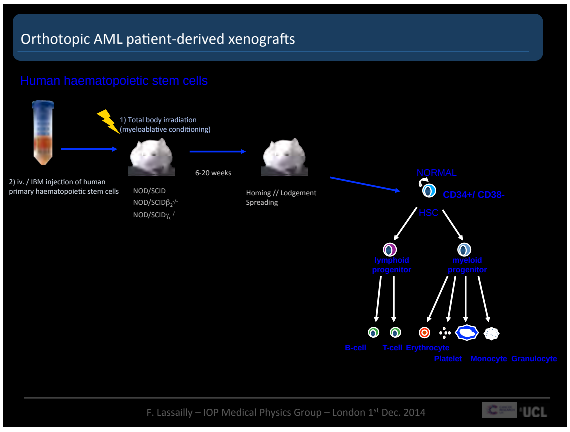
Orthotopic AML patient-derived xenografts
Human haematopoietic stem cells
1) Total body irradiation (myeloablative conditioning)
2) iv. / IBM injection of human primary haematopoietic stem cells
NOD/SCID
NOD/SCIDβ2-/-
NOD/SCIDγc-/-
6-20 weeks
Homing // Lodgement Spreading
NORMAL
CD34+/ CD38-
HSC
lymphoid progenitor
myeloid progenitor
B-cell
T-cell
Erythrocyte
Platelet
Monocyte
Granulocyte
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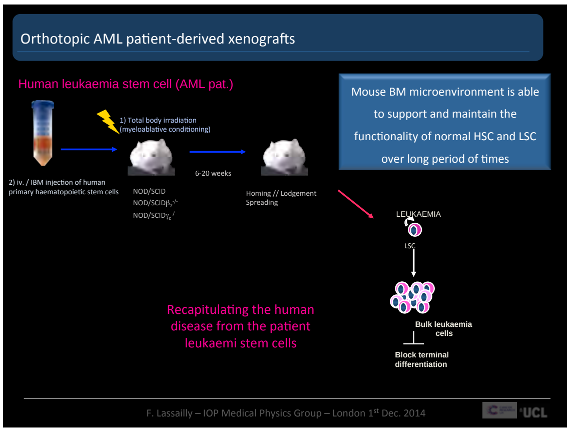
Orthotopic AML patient-derived xenografts
Human leukaemia stem cell (AML pat.)
Mouse BM microenvironment is able to support and maintain the functionality of normal HSC and LSC over long period of times
1) Total body irradiation (myeloablative conditioning)
2) iv. / IBM injection of human primary haematopoietic stem cells
NOD/SCID
NOD/SCIDβ2-/-
NOD/SCIDγc-/-
6-20 weeks
Homing // Lodgement Spreading
LEUKAEMIA
LSC
Bulk leukaemia cells
Block terminal differentiation
Recapitulating the human disease from the patient leukaemi stem cells
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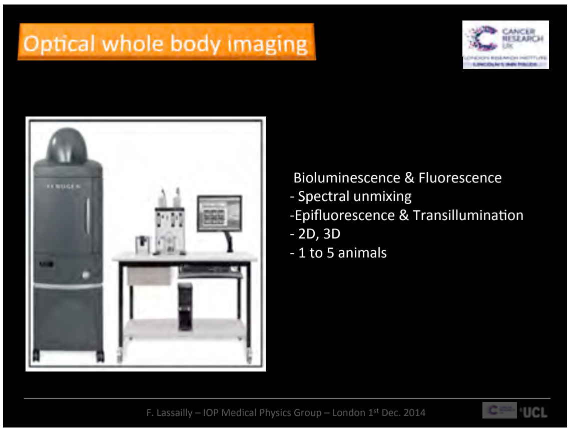
Optical whole body imaging
Bioluminescence & Fluorescence
- Spectral unmixing
-Epifluorescence & Transillumination
- 2D, 3D
- 1 to 5 animals
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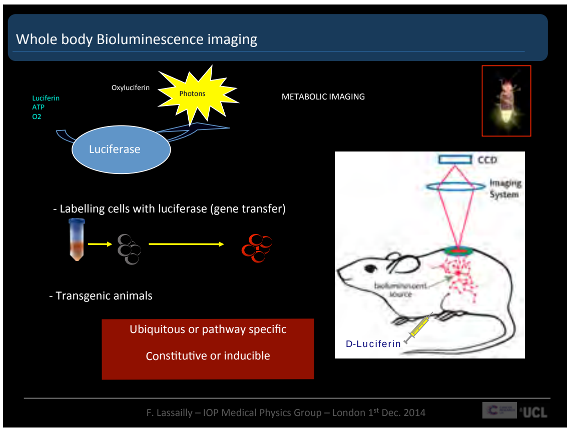
Whole body Bioluminescence imaging
Luciferin
ATP
O2
Oxyluciferin
Photons
Luciferase
METABOLIC IMAGING
CCD
Imaging System
D-Luciferin
- Labelling cells with luciferase (gene transfer)
- Transgenic animals
Ubiquitous or pathway specific
Constitutive or inducible
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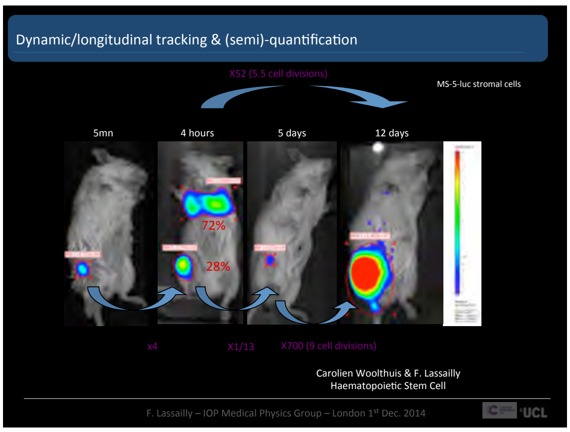
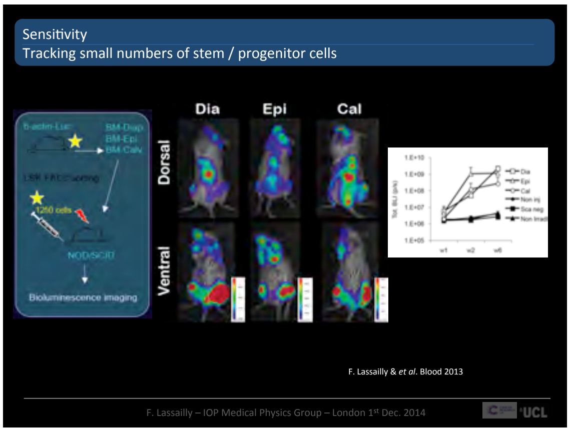
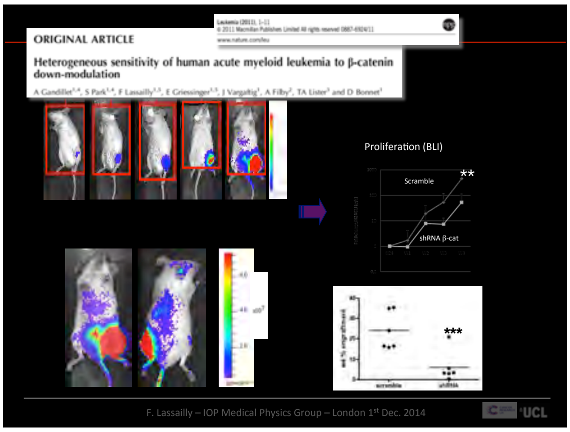

Dynamic/longitudinal tracking & (semi)-quantification
X52 (5.5 cell divisions)
MS-5-luc stromal cells
5mn
4 hours
5 days
12 days
72%
28%
x4
X1/13
X700 (9 cell divisions)
Carolien Woolthuis & F. Lassailly
Haematopoietic Stem Cell
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014


Sensitivity
Tracking small numbers of stem / progenitor cells
Dia
Epi
Cal
Dorsal
Ventral
Bioluminescence imaging
F. Lassailly & et al. Blood 2013
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
UCL


ORIGINAL ARTICLE
Heterogeneous sensitivity of human acute myeloid leukemia to β-catenin down-modulation
Proliferation (BLI)
Scramble
shRNA β-cat
**
***
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
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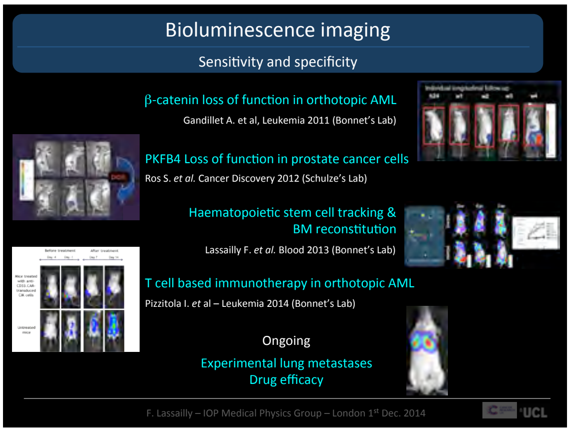
Bioluminescence imaging
Sensitivity and specificity
β-catenin loss of function in orthotopic AML
Gandillet A. et al, Leukemia 2011 (Bonnet's Lab)
PKFB4 Loss of function in prostate cancer cells
Ros S. et al. Cancer Discovery 2012 (Schulze's Lab)
Haematopoietic stem cell tracking & BM reconstitution
Lassailly F. et al. Blood 2013 (Bonnet's Lab)
T cell based immunotherapy in orthotopic AML
Pizzitola I. et al – Leukemia 2014 (Bonnet's Lab)
Ongoing
Experimental lung metastases
Drug efficacy
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Bioluminescence imaging

Sensitivity and specificity

Low resolution

Quantification affected by depth of the signal

Need for gene transfer

⇒ Not suitable for routine tracking of primary AML or solid tumours from human origin (PDX)

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Optical whole body imaging

Bioluminescence & Fluorescence
- Spectral unmixing
- Epifluorescence & Transillumination
- 2D, 3D
- 1 to 5 animals

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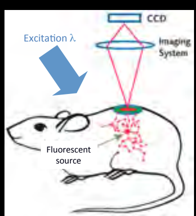
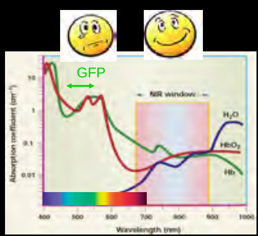
Whole body Fluorescence Imaging (VIS / NIR)





Adapted from Weissleder R, Nat. Biotech 2001

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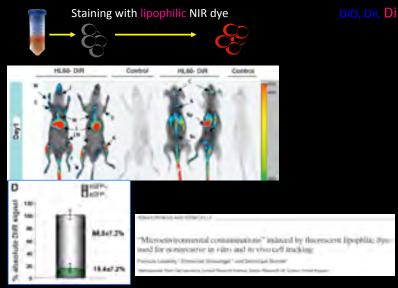
Applications: tracking fluorescently labelled leukemic cells

Staining with lipophilic NIR dye

DiO, DiI, DiD, DiR, PKH26 etc…



Lassailly et al, Blood 2010

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Fluorescent / fluorigenic probes (compatible microscopy)

Target	Application	Type
Blood pool agents	Angiogenesis / BVD / leakiness	Non specific
Perfusion	Hypoxia / metabolism	Non Specific
Integrins (avb3)	Neoangiogenesis, tumours	Targetted
CAix		
VEGF-R		
Glucose Transp		
Annexin V		
Caspases		
MMP-2, -3, -9, -		
Cathepsin B, L, S		
Cathepsin K	Osteoclasts	Activatable
Hydroxy Apatite	Bone remodelling	Targetted

How about your own probe(s)?

Antibodies

Peptides

Drugs

Lassailly F. *et al.* Blood 2013

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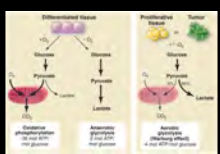
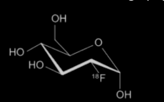
Imaging glucose metabolism



Positron Emission Tomography (PET)



[18F]fluoro-2-deoxy-D-glucose (FDG)

Could NIR-2-Deoxy-glucose (2DG) be used to monitor human leukaemia development non-invasively?

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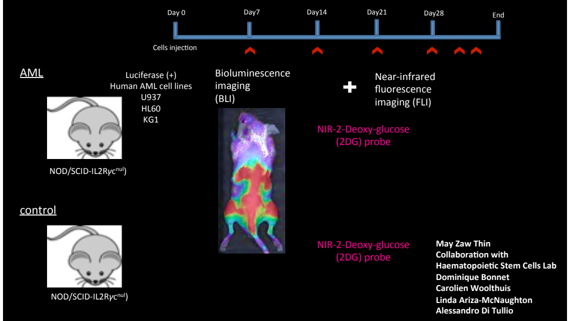
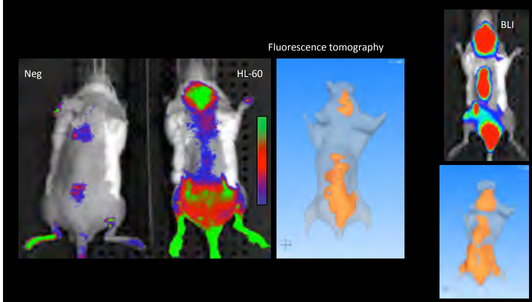
Study design



Near-infrared (non-radioactive) 2-Deoxy-glucose (2DG) imaging



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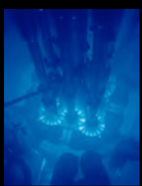
New developments: Cerenkov Luminescence Imaging



Cerenkov light:

Electromagnetic radiation emitted when a charged particle (such as an electron) passes through a dielectric medium at a speed greater than the phase velocity of light in that medium.

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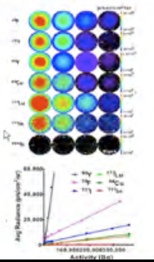
New developments: Cerenkov Luminescence Imaging

Common radionuclides for molecular imaging

	Half-life	β+	β-	Gamma rays
18-F	109.80 min	x		
13-N	9.97 min	x		
64-Cu	12.70 hrs	x	x	
32-P	14.26 days		x	
90-Y	2.67 days		x	
177-Lu	6.65 days		x	
131-I	8.03 days		x	x
99m-Tc	6.01 hrs			x



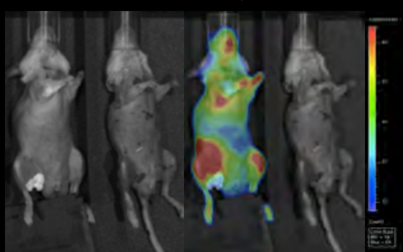
Hongguang Liu *et al.* PlosOne 2010

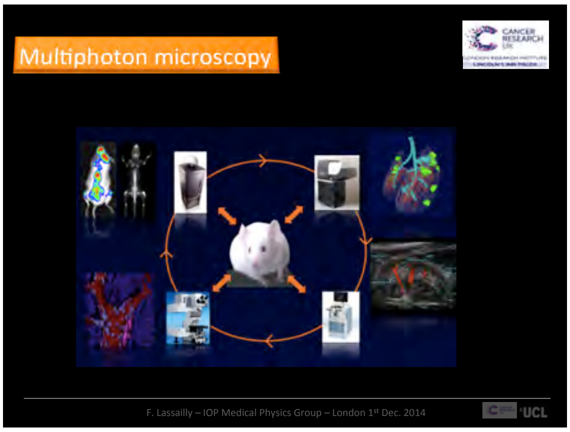
Preliminary test with (18)F-Fluoride (bone)

Tony Gee

Preliminary test with (18)F-Fluoride (bone)

30MBq Ctrl




Multiphoton microscopy
CANCER RESEARCH UK
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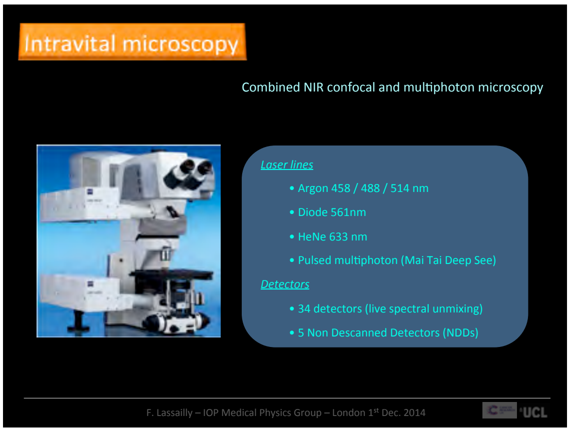
Intravital microscopy
Combined NIR confocal and multiphoton microscopy
Laser lines
• Argon 458 / 488 / 514 nm
• Diode 561nm
• HeNe 633 nm
• Pulsed multiphoton (Mai Tai Deep See)
Detectors
• 34 detectors (live spectral unmixing)
• 5 Non Descanned Detectors (NDDs)
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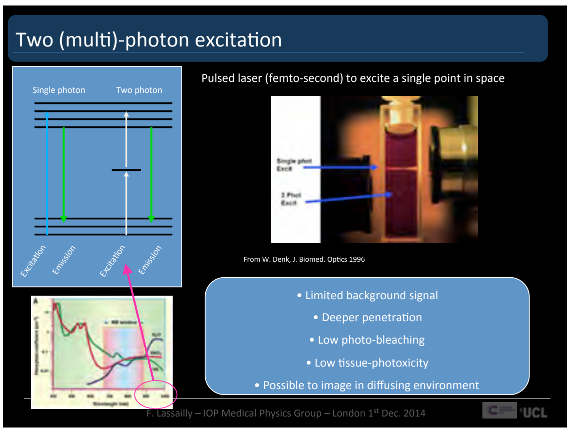
Two (multi)-photon excitation
Single photon
Two photon
Excitation
Emission
Excitation
Emission
Pulsed laser (femto-second) to excite a single point in space
From W. Denk, J. Biomed. Optics 1996
• Limited background signal
• Deeper penetration
• Low photo-bleaching
• Low tissue-photoxicity
• Possible to image in diffusing environment
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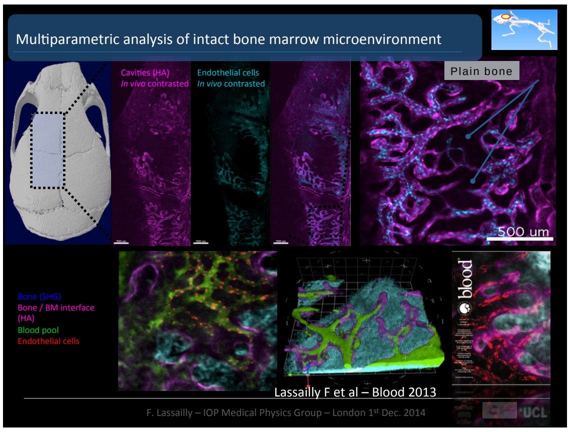
Multiparametric analysis of intact bone marrow microenvironment
Cavities (HA)
In vivo contrasted
Endothelial cells
In vivo contrasted
Plain bone
500 um
Bone (SHG)
Bone / BM interface (HA)
Blood pool
Endothelial cells
blood
Lassailly F et al – Blood 2013
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
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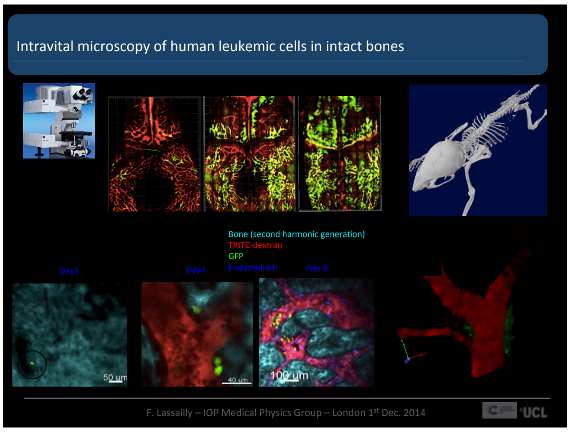
Intravital microscopy of human leukemic cells in intact bones
Bone (second harmonic generation)
TRITC-dextran
GFP
Endothelium
Day1
Day4
Day 8
50 um
40 um
100 um
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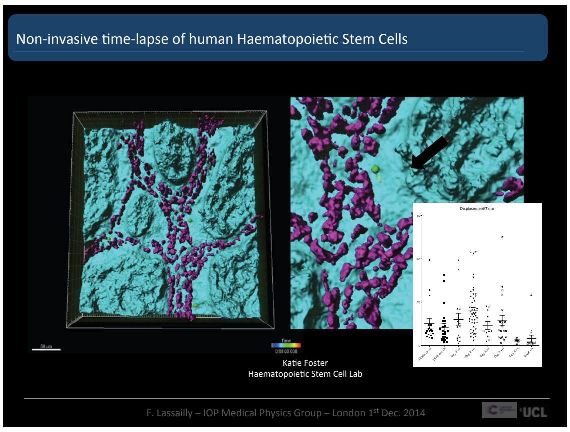
Non-invasive time-lapse of human Haematopoietic Stem Cells
Katie Foster
Haematopoietic Stem Cell Lab
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
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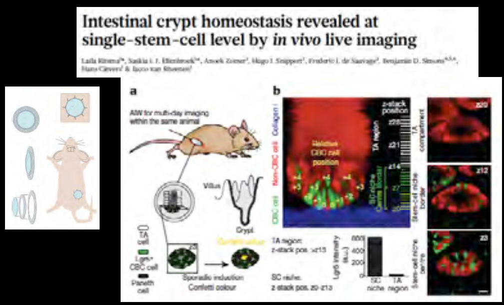
Microscopic imaging of deep tissues



Ritsma L et al. Nature 2014

Some applications

- Example 1: Haematopoietic stem cells and acute myeloid leukaemia
  - Bioluminescence
  - Near Infrared fluorescence
  - Intravital microscopy

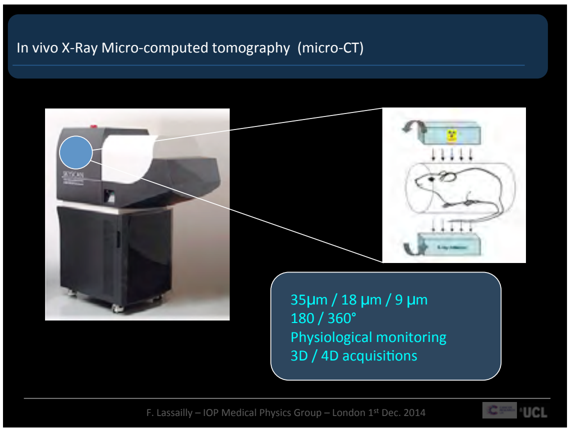
Example 2: Non Small Cell Lung Cancer (GEMM)
- Micro-CT
- Response to treatment
- Relapse

X-ray micro-CT

SkyScan 1176 (SkyScan/Bruker microCT)

micro-CT (3D X-ray)

Lung tumors & metastasis
Bone biology
Vascular biology


In vivo X-Ray Micro-computed tomography (micro-CT)
35µm / 18 µm / 9 µm
180 / 360°
Physiological monitoring
3D / 4D acquisitions
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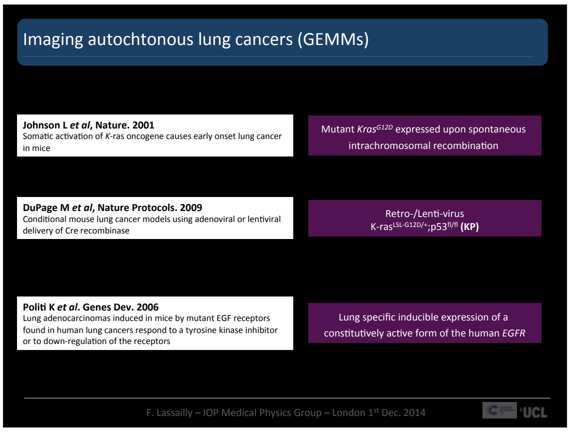
Imaging autochtonous lung cancers (GEMMs)
Johnson L et al, Nature. 2001
Somatic activation of K-ras oncogene causes early onset lung cancer in mice
Mutant Kras G12D expressed upon spontaneous intrachromosomal recombination
DuPage M et al, Nature Protocols. 2009
Conditional mouse lung cancer models using adenoviral or lentiviral delivery of Cre recombinase
Retro-/Lenti-virus
K-ras LSL-G12D/+;p53 fl/fl (KP)
Politi K et al. Genes Dev. 2006
Lung adenocarcinomas induced in mice by mutant EGF receptors found in human lung cancers respond to a tyrosine kinase inhibitor or to down-regulation of the receptors
Lung specific inducible expression of a constitutively active form of the human EGFR
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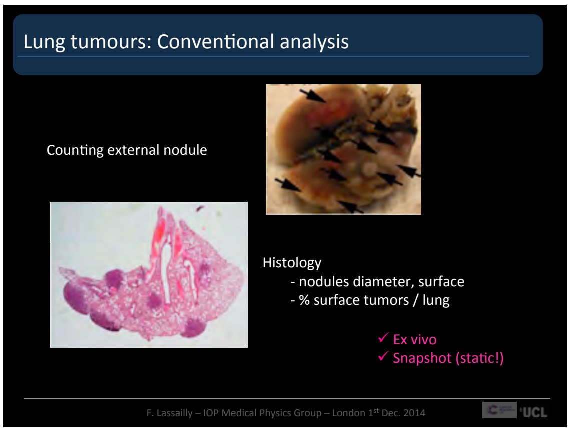
Lung tumours: Conventional analysis
Counting external nodule
Histology
- nodules diameter, surface
- % surface tumors / lung
✓ Ex vivo
✓ Snapshot (static!)
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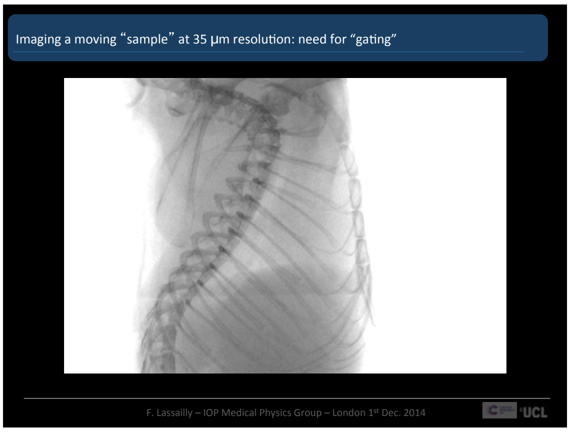
Imaging a moving "sample" at 35 µm resolution: need for "gating"
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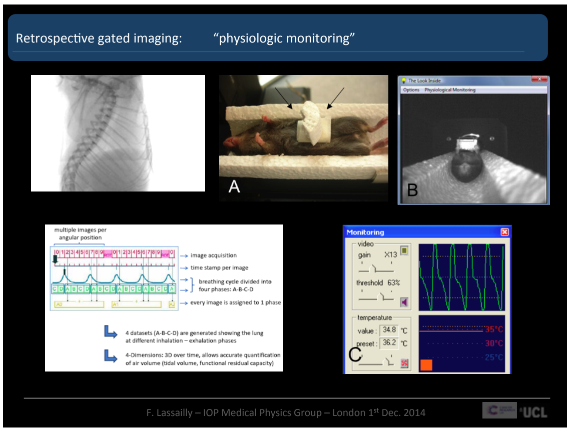
Retrospective gated imaging: "physiologic monitoring"
A
B
multiple images per angular position
image acquisition
time stamp per image
breathing cycle divided into four phases: A-B-C-D
every image is assigned to 1 phase
4 datasets (A-B-C-D) are generated showing the lung at different inhalation – exhalation phases
4-Dimensions: 3D over time, allows accurate quantification of air volume (tidal volume, functional residual capacity)
Monitoring
video
gain X13
threshold 63%
temperature
value : 34.8 °C
preset : 36.2 °C
35°C
30°C
25°C
C
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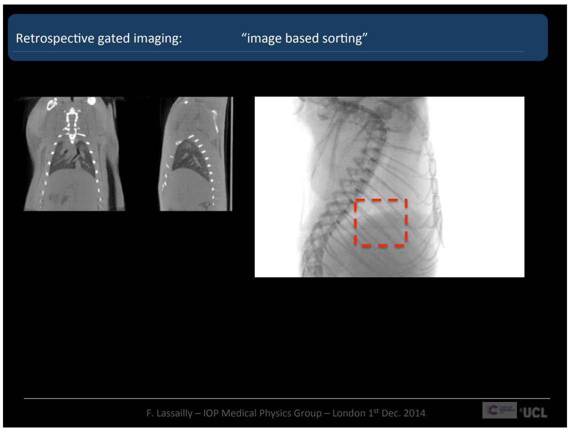
Retrospective gated imaging: "image based sorting"
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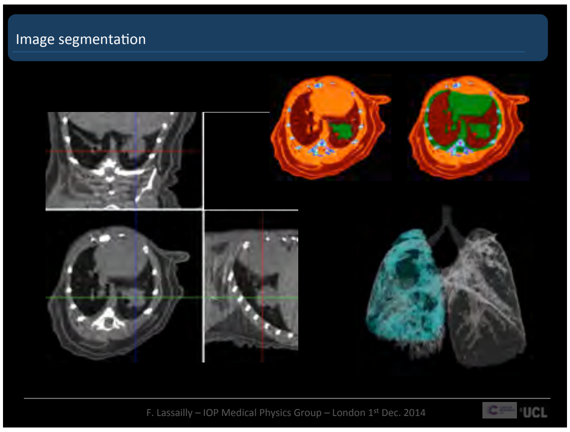
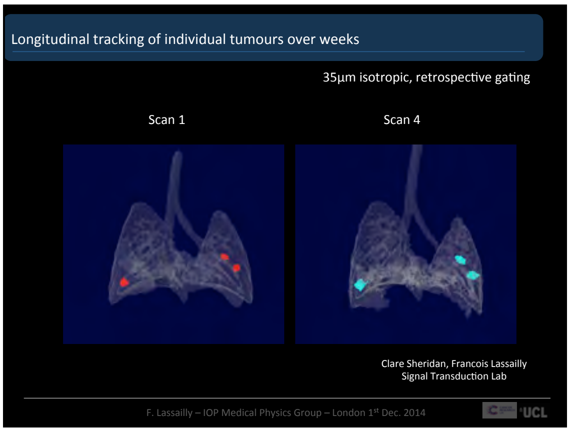
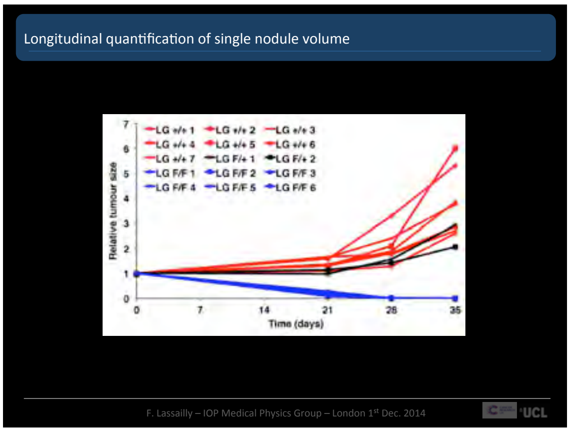


Image segmentation
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
UCL


Longitudinal tracking of individual tumours over weeks
35µm isotropic, retrospective gating
Scan 1
Scan 4
Clare Sheridan, Francois Lassailly
Signal Transduction Lab
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
UCL


Longitudinal quantification of single nodule volume
LG +/+ 1
LG +/+ 2
LG +/+ 3
LG +/+ 4
LG +/+ 5
LG +/+ 6
LG +/+ 7
LG F/+ 1
LG F/+ 2
LG F/F 1
LG F/F 2
LG F/F 3
LG F/F 4
LG F/F 5
LG F/F 6
Relative tumour size
0
1
2
3
4
5
6
7
0
7
14
21
28
35
Time (days)
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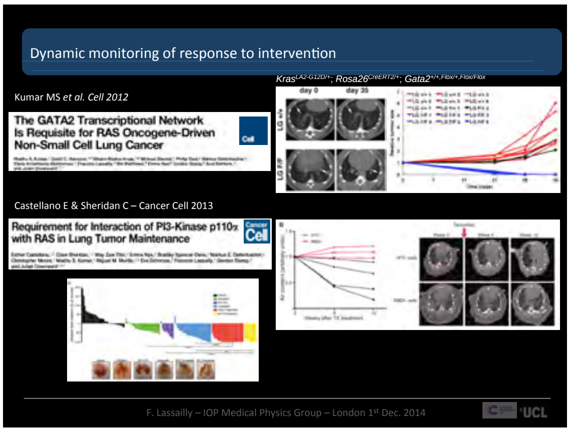
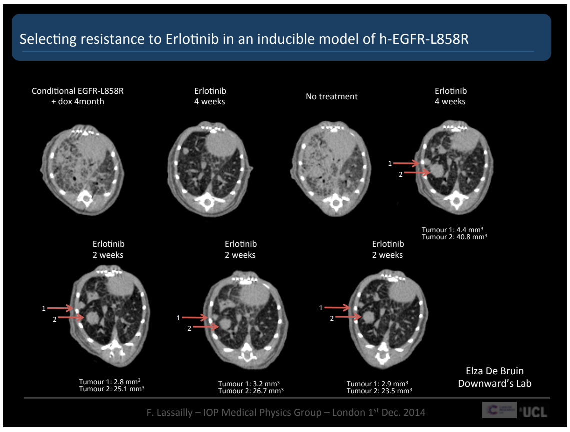


Dynamic monitoring of response to intervention
Kumar MS et al. Cell 2012
The GATA2 Transcriptional Network Is Requisite for RAS Oncogene-Driven Non-Small Cell Lung Cancer
Castellano E & Sheridan C – Cancer Cell 2013
Requirement for Interaction of PI3-Kinase p110α with RAS in Lung Tumor Maintenance
Cancer Cell
day 0
day 35
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014



Selecting resistance to Erlotinib in an inducible model of h-EGFR-L858R
Conditional EGFR-L858R
+ dox 4month
Erlotinib
4 weeks
No treatment
Erlotinib
4 weeks
Tumour 1: 4.4 mm³
Tumour 2: 40.8 mm³
Erlotinib
2 weeks
Erlotinib
2 weeks
Erlotinib
2 weeks
Tumour 1: 2.8 mm³
Tumour 2: 25.1 mm³
Tumour 1: 3.2 mm³
Tumour 2: 26.7 mm³
Tumour 1: 2.9 mm³
Tumour 2: 23.5 mm³
Elza De Bruin
Downward's Lab
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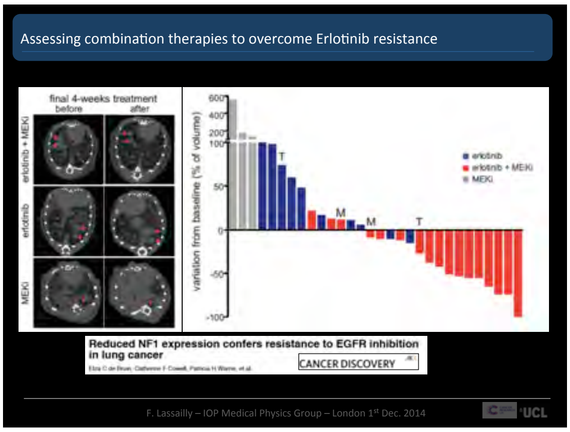
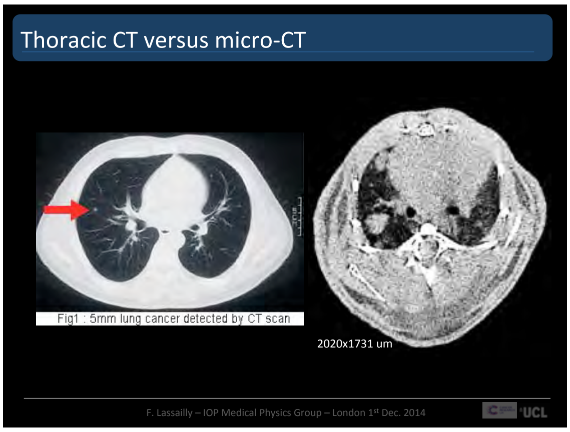
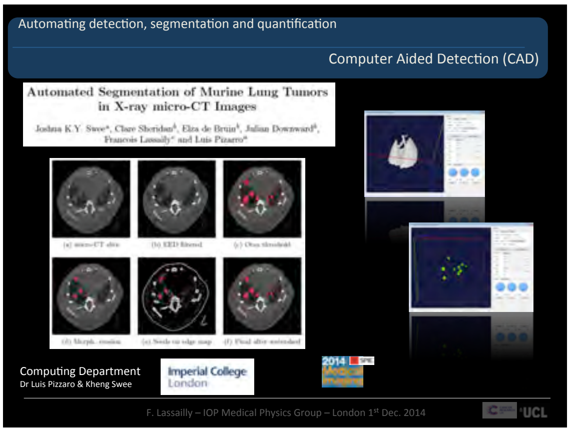
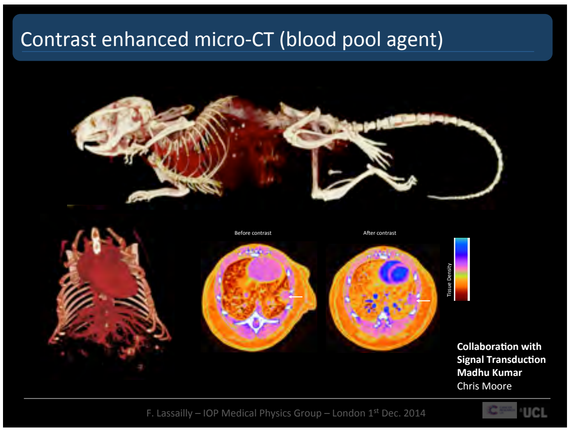
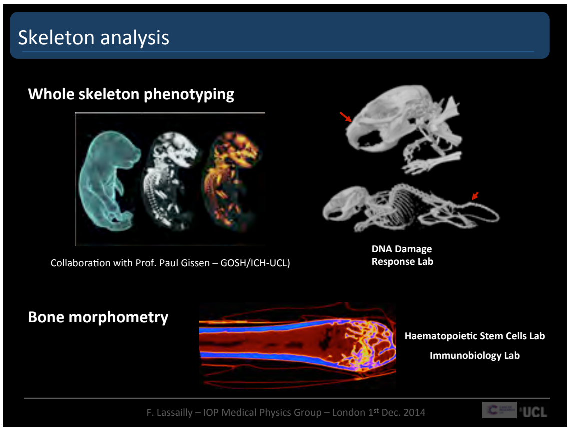


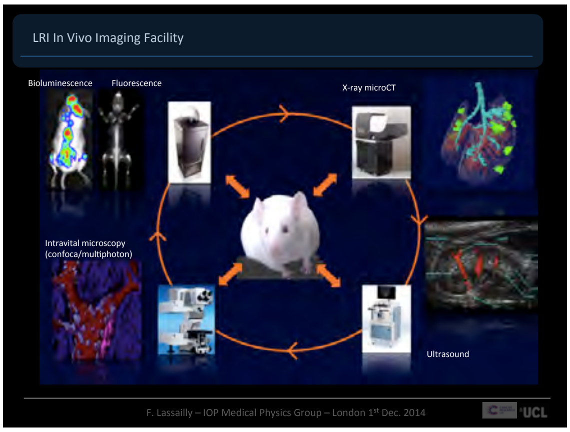
Assessing combination therapies to overcome Erlotinib resistance
final 4-weeks treatment
before
after
erlotinib + MEKi
erlotinib
MEKi
variation from baseline (% of volume)
erlotinib
erlotinib + MEKi
MEKi
Reduced NF1 expression confers resistance to EGFR inhibition in lung cancer
CANCER DISCOVERY
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014


Thoracic CT versus micro-CT
Fig1 : 5mm lung cancer detected by CT scan
2020x1731 um
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
UCL


Automating detection, segmentation and quantification
Computer Aided Detection (CAD)
Automated Segmentation of Murine Lung Tumors in X-ray micro-CT Images
Computing Department
Dr Luis Pizzaro & Kheng Swee
Imperial College London
2014
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
UCL


Contrast enhanced micro-CT (blood pool agent)
Collaboration with
Signal Transduction
Madhu Kumar
Chris Moore
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
UCL


Skeleton analysis
Whole skeleton phenotyping
Collaboration with Prof. Paul Gissen – GOSH/ICH-UCL)
DNA Damage
Response Lab
Bone morphometry
Haematopoietic Stem Cells Lab
Immunobiology Lab
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
UCL


LRI In Vivo Imaging Facility
Bioluminescence
Fluorescence
X-ray microCT
Intravital microscopy
(confoca/multiphoton)
Ultrasound
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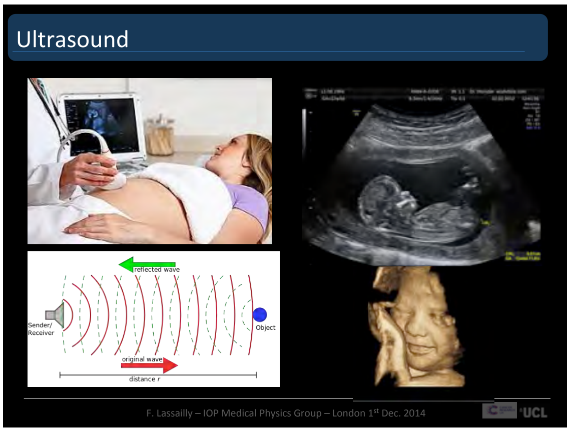
Ultrasound
reflected wave
Sender/
Receiver
Object
original wave
distance r
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Ultrasound (US)
Vevo 2100 (Visual sonic)
Motor for 3D
Oncology
- tumor/mets volume measurement
- vasculature
Abdominal anatomy
Embryology
Cardiovascular
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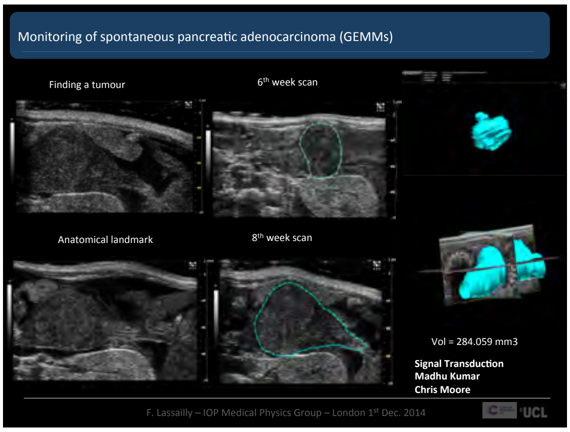
Monitoring of spontaneous pancreatic adenocarcinoma (GEMMs)
Finding a tumour
6th week scan
Anatomical landmark
8th week scan
Vol = 284.059 mm3
Signal Transduction
Madhu Kumar
Chris Moore
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014


Director: Sir Paul Nurse: 2001 Nobel Prize in Physiology or Medicine
www.crick.ac.uk
Imperial College London
wellcome trust


Further developments for the Francis Crick Institute
CABI
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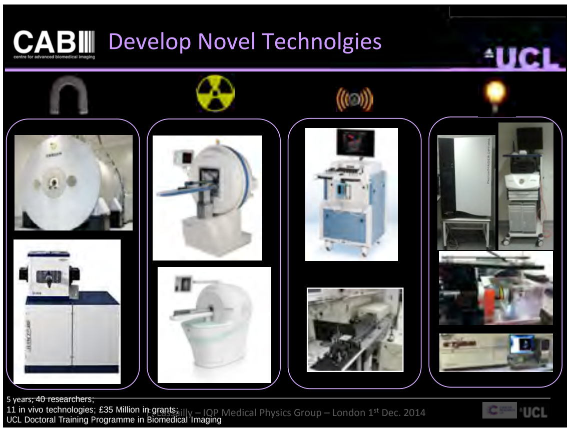
Centre for Advanced Biomedical Imaging
Imaging Techniques
MRI
Photoacoustics
SPECT/PET
In Vivo Microscopy
Ultrasound
Confocal_Endoscopy
Bioluminescence
In Vivo Irradiation
OPT
Research Areas
Neuroscience
Cardiovascular MRI
Phenotyping
Stem Cell Tracking
Cancer Imaging
Professor Mark Lythgoe
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
UCL


CABI
Develop Novel Technolgies
UCL
5 years; 40 researchers;
11 in vivo technologies; £35 Million in grants;
UCL Doctoral Training Programme in Biomedical Imaging
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
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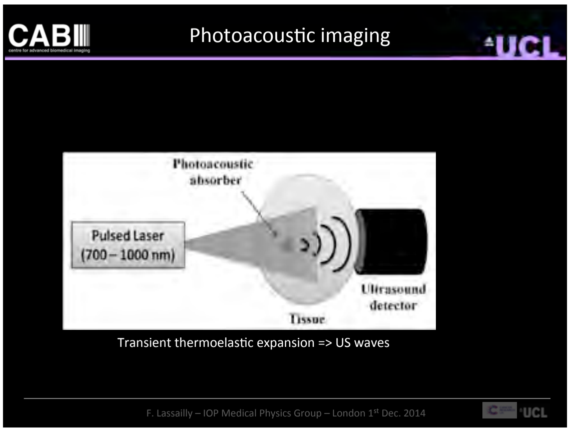
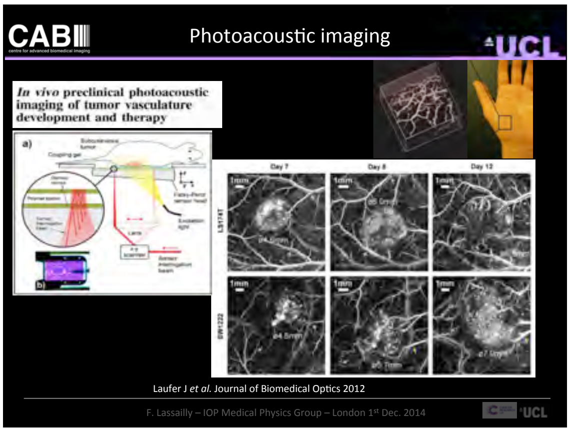
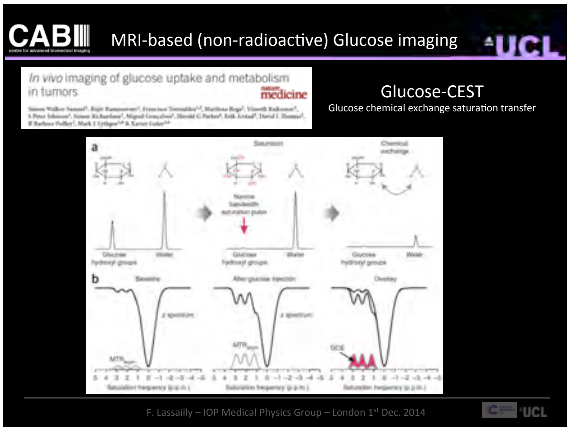
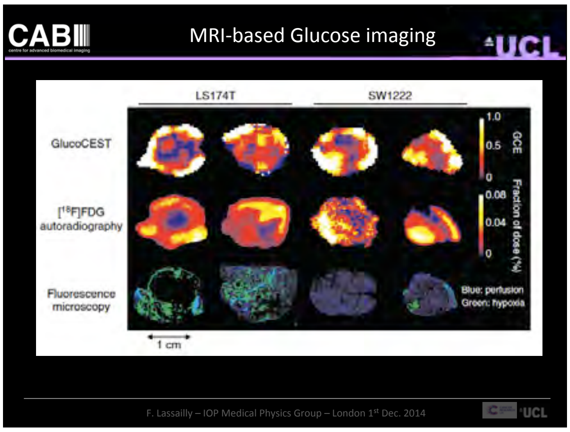
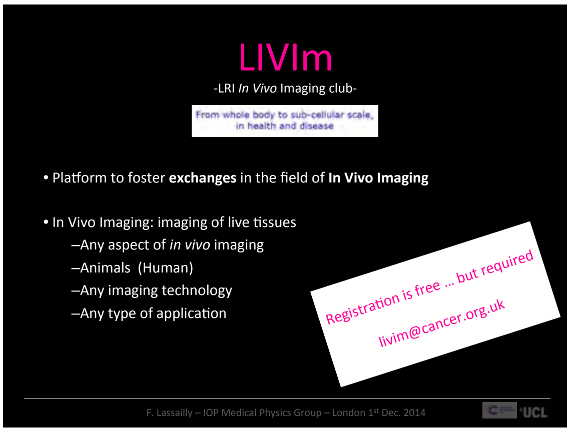

CABI
Photoacoustic imaging
UCL
Photoacoustic absorber
Pulsed Laser (700 – 1000 nm)
Ultrasound detector
Tissue
Transient thermoelastic expansion => US waves
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
UCL


CABI
Photoacoustic imaging
UCL
In vivo preclinical photoacoustic imaging of tumor vasculature development and therapy
Laufer J et al. Journal of Biomedical Optics 2012
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
UCL


CABI
MRI-based (non-radioactive) Glucose imaging
UCL
In vivo imaging of glucose uptake and metabolism in tumors
nature medicine
Glucose-CEST
Glucose chemical exchange saturation transfer
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
UCL


CABI
MRI-based Glucose imaging
UCL
LS174T
SW1222
GlucoCEST
[18F]FDG autoradiography
Fluorescence microscopy
GCE
Fraction of dose (%)
Blue: perfusion
Green: hypoxia
1 cm
F. Lassailly – IOP Medical Physics Group – London 1st Dec. 2014
UCL


LIVIm
-LRI In Vivo Imaging club-
From whole body to sub-cellular scale, in health and disease
• Platform to foster exchanges in the field of In Vivo Imaging
• In Vivo Imaging: imaging of live tissues
–Any aspect of in vivo imaging
–Animals (Human)
–Any imaging technology
–Any type of application
Registration is free … but required
livim@cancer.org.uk
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MANY THANKS !
Together we will beat cancer
May Zaw Thin In Vivo Imaging Facility
Julian Downward Signal Transduction Lab
Dominique Bonnet Haematopoietic Stem Cell Lab
Ilaria Malanchi Tumour Host Interaction
Erik Sahai Tumour Cell Biology
Simon Boulton DNA Damage Lab
Caetano Reis e Sousa Immunobiology Lab
Axel Behrens Mammalian genetics Lab
Charles Swanton Translational Cancer Therapeutics Lab
NC3Rs
THE FRANCIS CRICK INSTITUTE
Lymphocyte Interaction
Andreas Bruckbauer
Facundo Batista
Computing Department
Luis Pizzaro
Kheng Swee
Imperial College London
Crick – BRF work package group
Kathleen Mathers
Gary Childs
Biological Resource Unit
Experimental Histopathology
Flow cytometry
Division Imaging Sciences
Tony Gee
Centre for Advanced Biomedical Imaging
Mark Lythgoe
Bernard Siow
Adam Badar
Tammy Kalber
UCL
Tumour Modelling Core
Paul Mackin
LI-COR
Caliper