



Edinburgh Cancer
Research UK Centre



CANCER
RESEARCH
UK



Edinburgh Cancer Discovery Unit (ECDU)

Dr Neil Carragher (n.carragher@ed.ac.uk)

Prof Margaret Frame

Dr Val Brunton

Edinburgh Cancer Research UK Centre

-Institute of Genetics & Molecular Medicine (IGMM)

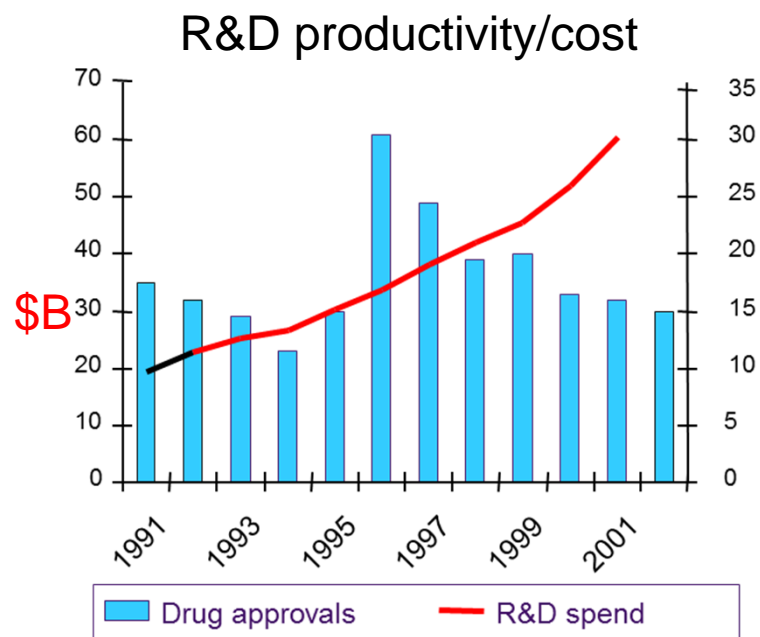
-College of Medicine & Veterinary Medicine

-University of Edinburgh



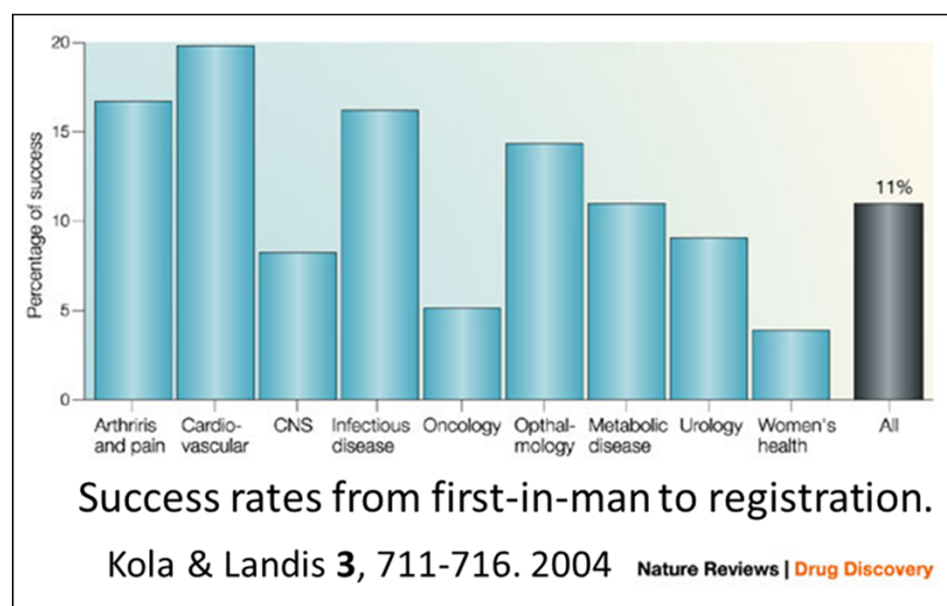
Drug Discovery Challenges:

High attrition; increasing R&D costs; patent expiration/competition



Source: Pharmaceutical Benchmarking Forum 1999-2003

All pharmaceutical companies face high attrition of compounds through discovery and development process



Edinburgh Cancer Discovery Unit Goals:

- Reduce attrition (enhance preclinical efficacy prediction/biomarkers)
- Reduce R&D costs (partnerships & agile chemistry strategies)
- Extend response duration (& patent life: franchise around combinations)



Alternative Drug Discovery Paradigms

Convention



Hit-to-lead

Selectivity
& potency



preclinical

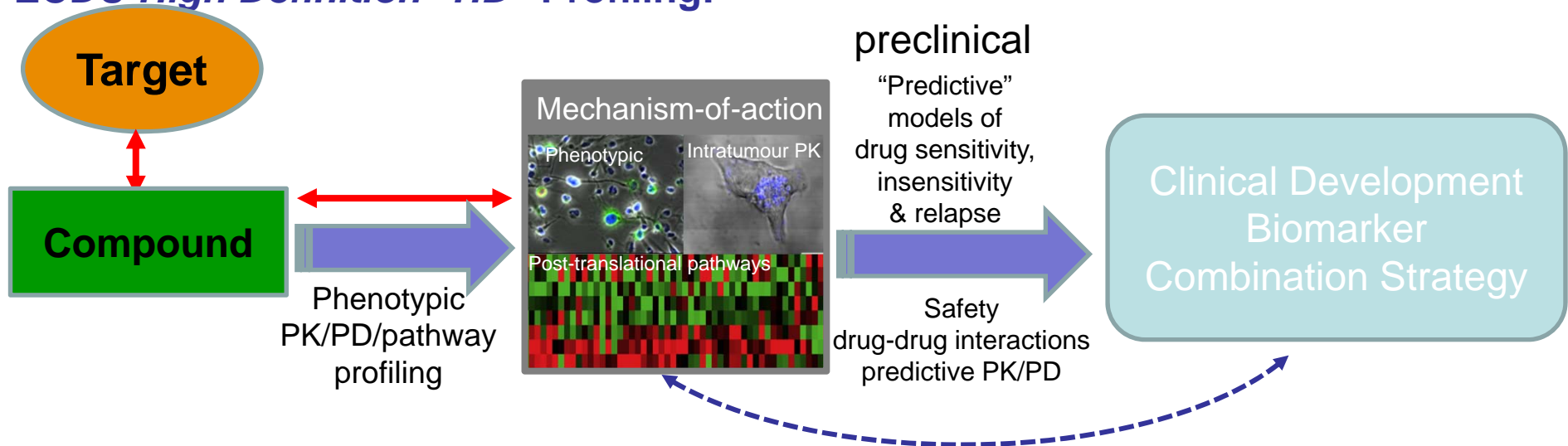
“efficacy”
(tum.vol-xenograft)
& safety



Knowledge Gap

Drug mechanism in complex biology/pathophysiology
No iterative learning

ECDU High Definition “HD” Profiling:





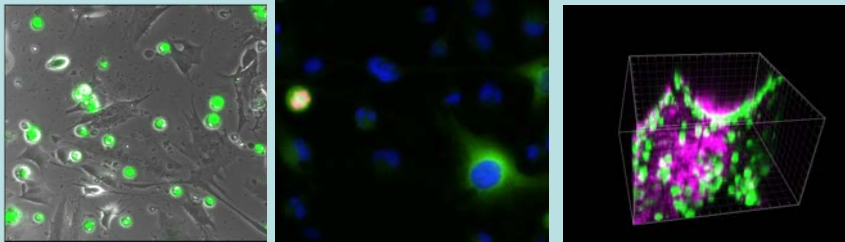
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Innovative “HD” Drug Discovery Capabilities:

Edinburgh Cancer Discovery Unit

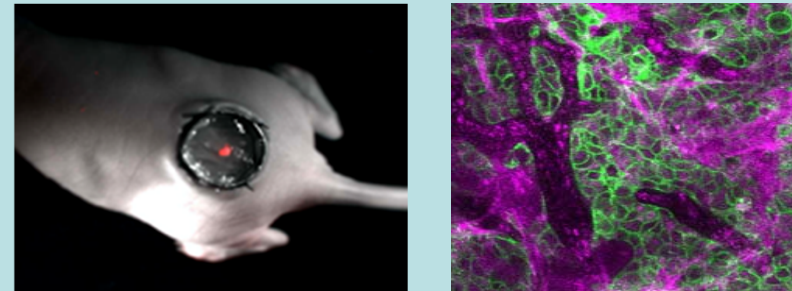
Phenotypic Screening Multiparametric Assays

High resolution phenotypic profiling of drug mechanism across suite of 2D and 3D models



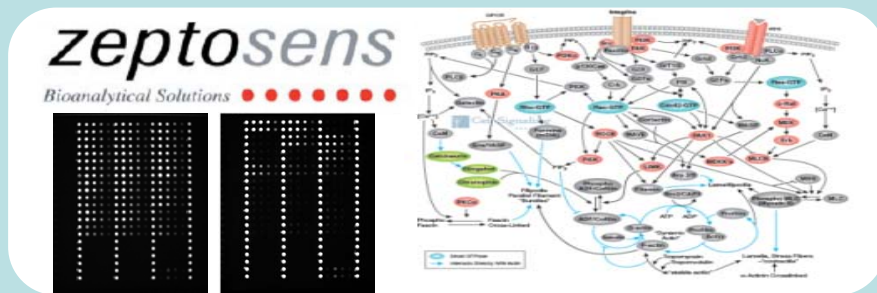
Quantitative intravital imaging

High resolution *in vivo* imaging of cancer biology, drug Pharmacology and Drug Phenotypes in live tumours



Zeptosens Reverse Protein Array Platform

Industry-leading Pharmacodynamic analysis of post-translational pathway biomarkers *in vitro* &/or *in vivo*



To complement: genomics/ mass-spec

Advanced preclinical models Access to clinical tissue



Target validation (new target classes)
Confirm: Drug/Target mechanism
Identify: Drug response biomarkers
Anticipate: Drug resistance mechanisms
Identify: Rationale Drug Combinations



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Edinburgh Cancer Discovery Unit



NHS Clinic



ImageXpress*



Wellcome Trust Clinical Research Facility

- Home
- Clinical Facilities
- Nursing Clinical
- Epidemiology & Statistics
- Education Programme
- Genetics
- Imaging
- Image Analysis
- IT
- Mass Spectrometry
- General Information
- Application Process
- Application Forms



Welcome to WTCRF Edinburgh



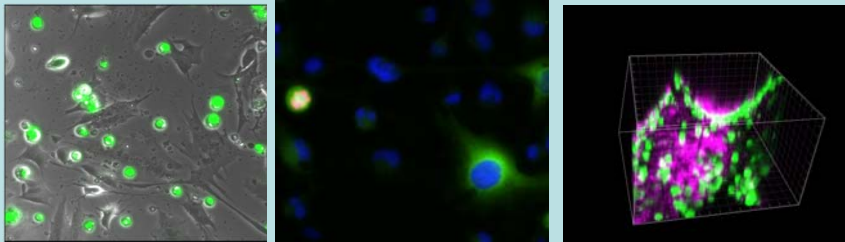
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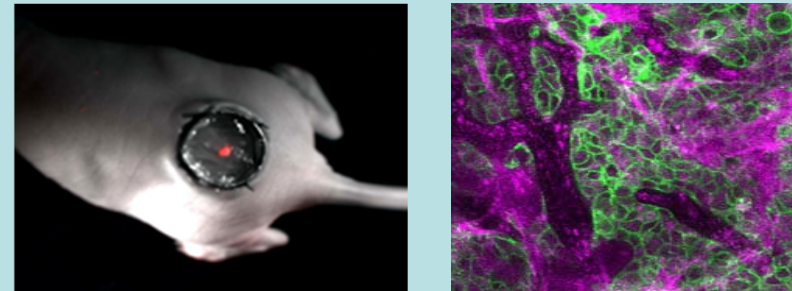
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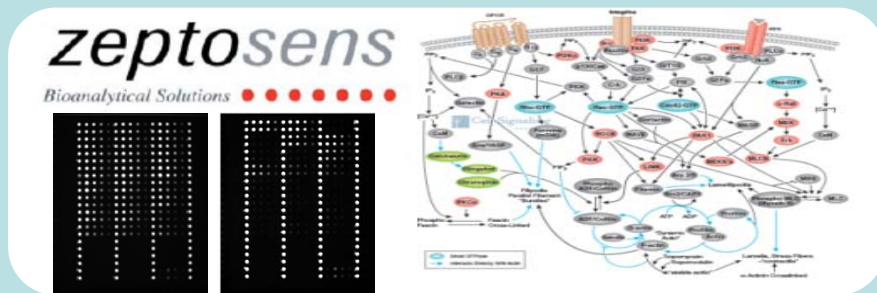
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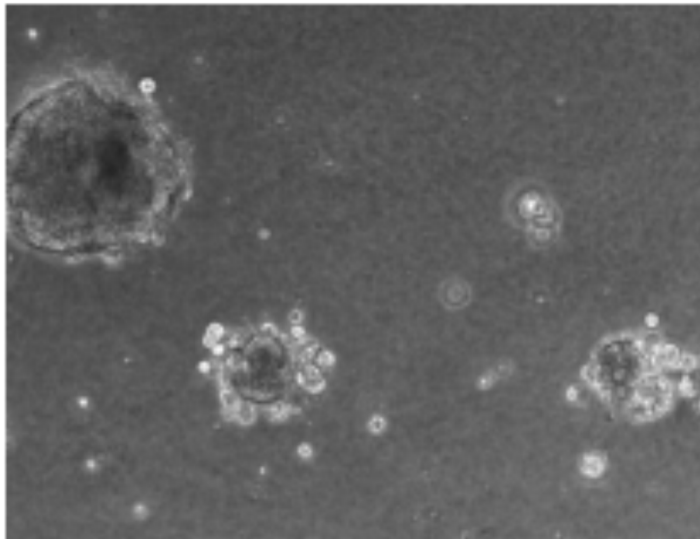
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Enhancing Predictivity:

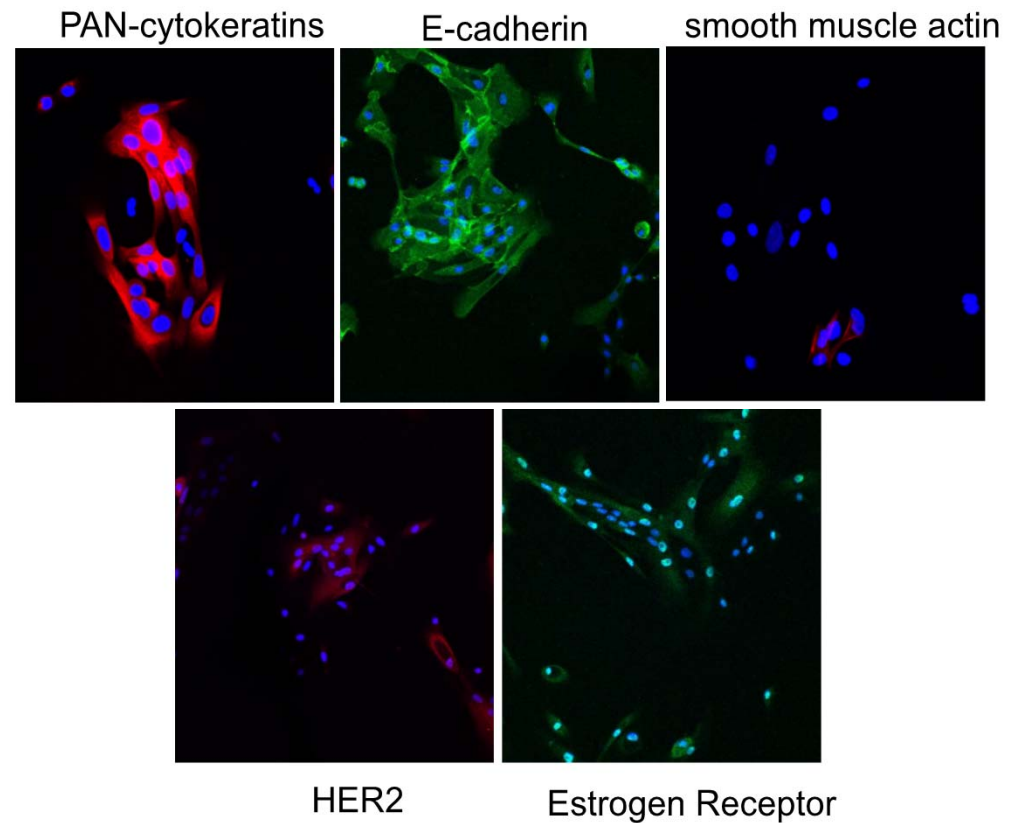
-Leveraging complex 3D, co-culture, primary ex-vivo tissue models

Generation & characterization of primary patient derived breast cancer cell lines. A. Images of fresh patient derived 3D breast cancer cell spheroids

A: 3D Spheroid culture



B: Image based phenotyping

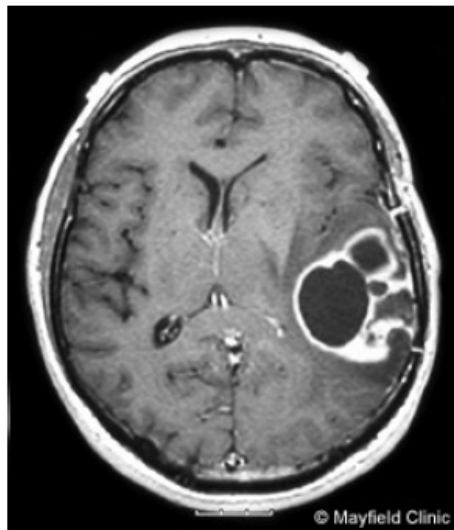




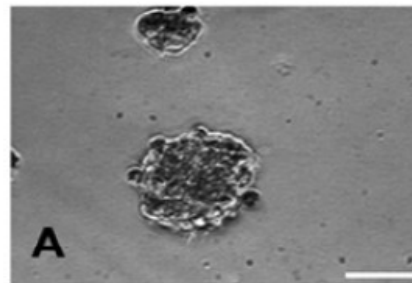
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Enhancing Predictivity Glioma Progenitor Like Cells (GPCs)

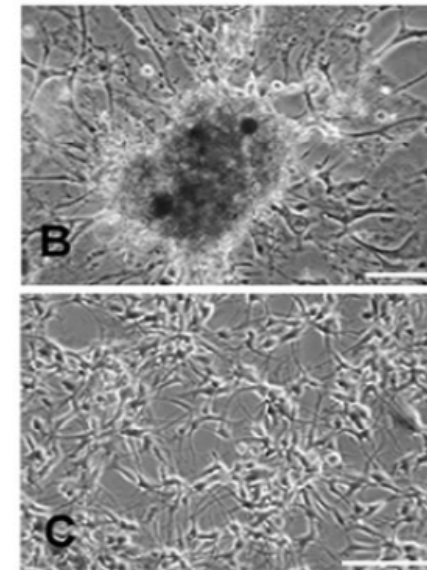
Tumour biopsy



Spheroid culture



Adherent culture



Serum free conditions,
hFGF, EGF

Matrigel coating
Serum free conditions
hFGF, EGF

P Brennan

(Talal M. Fael Al-Mayhani et al., 2008)



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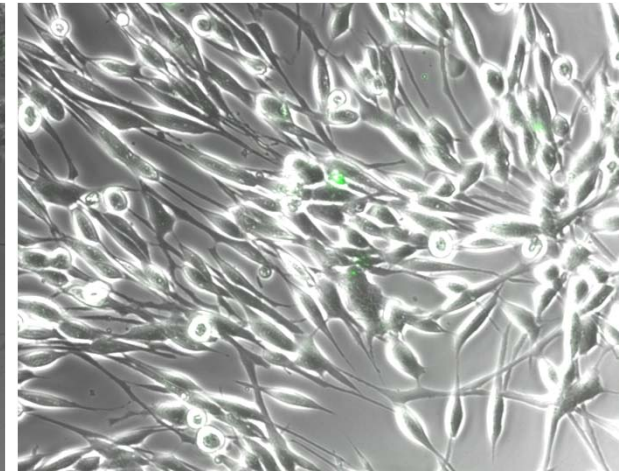
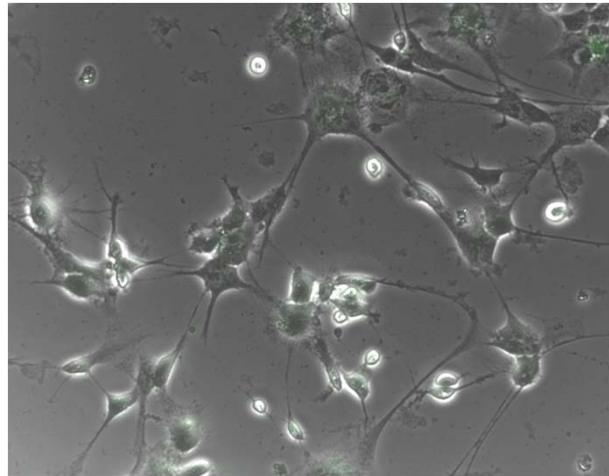
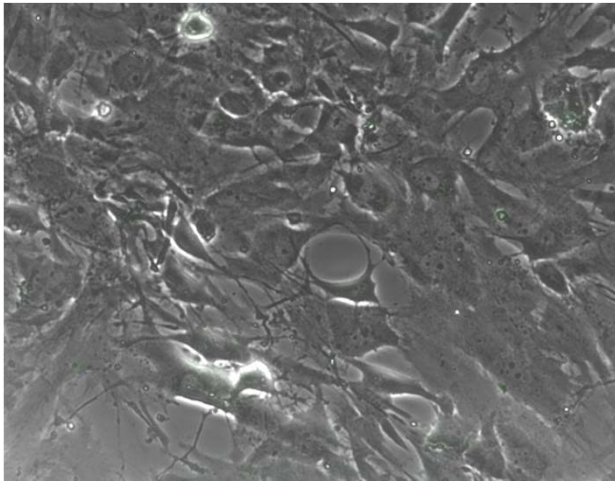
NucView Caspase biosensor

CC

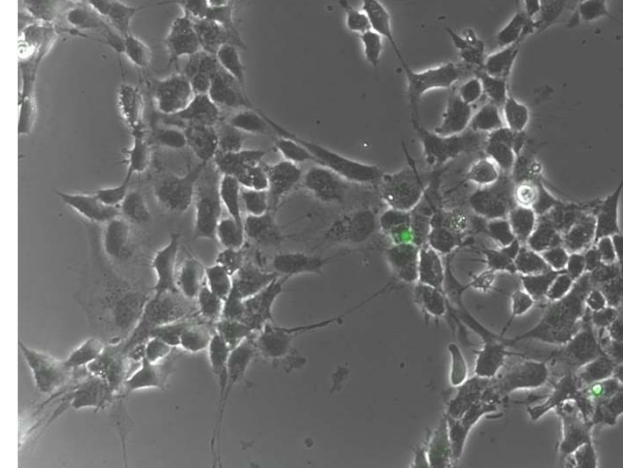
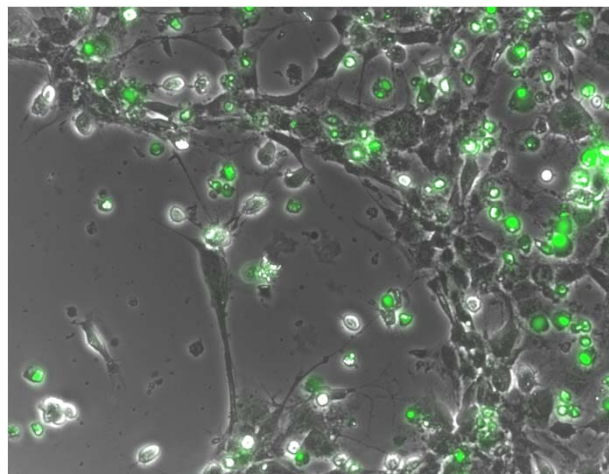
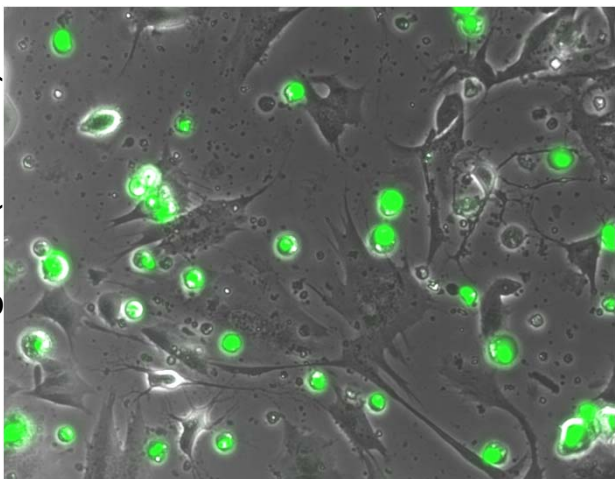
DD

X

control



Drug - X (50nM)

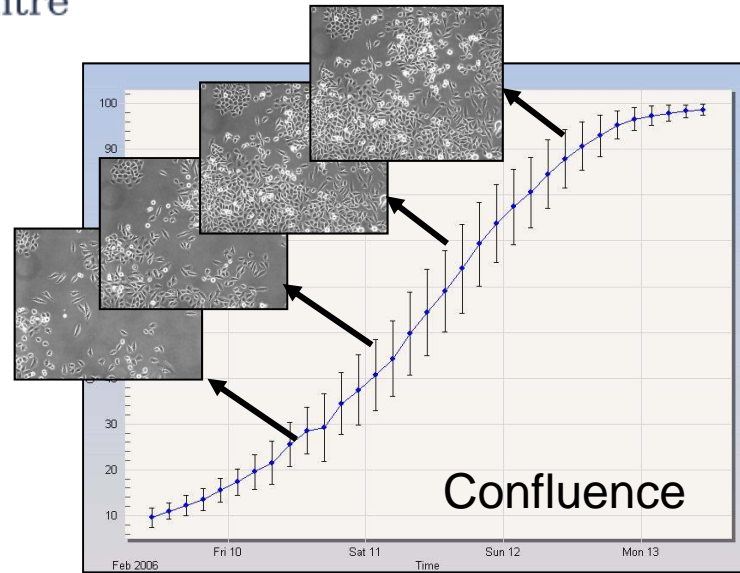




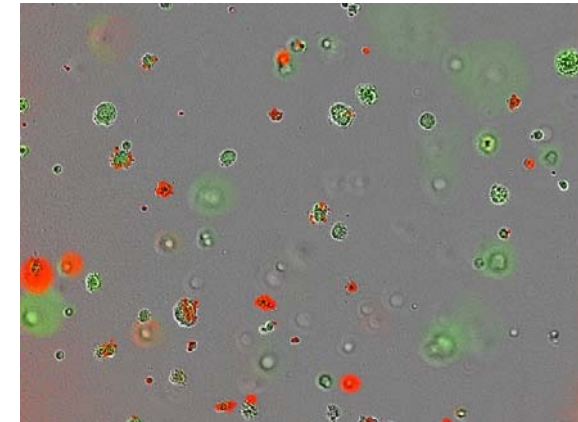
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Kinetic profiling phenotypic response 2D & 3D

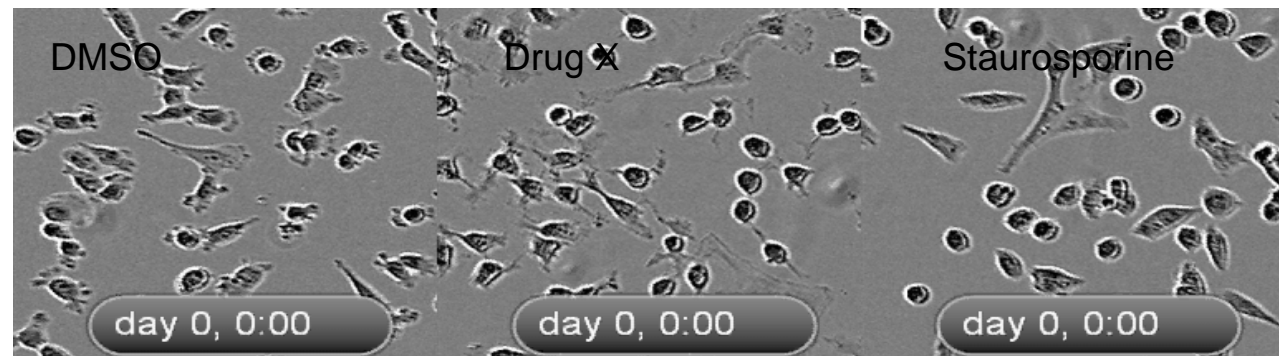
IncuCyte Zoom™



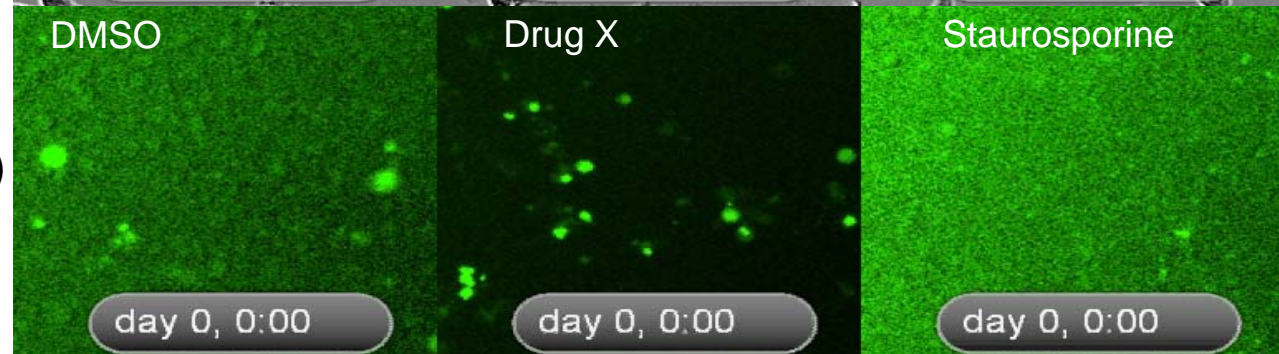
3D methycellulose colony
Live(green)/dead(red) assay



Brightfield



NucView
(caspase biosensor)
Apoptosis



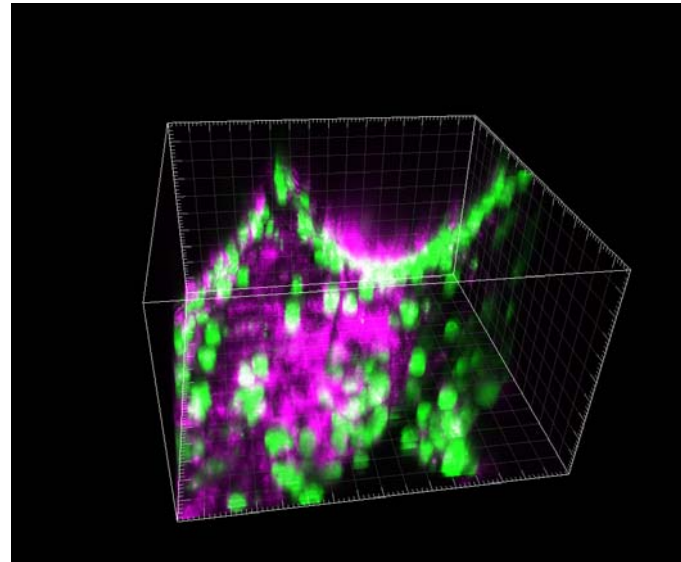
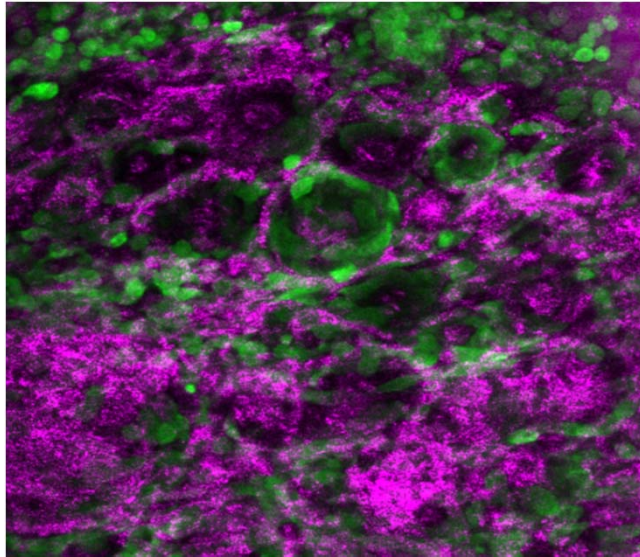


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Enhancing Predictivity:

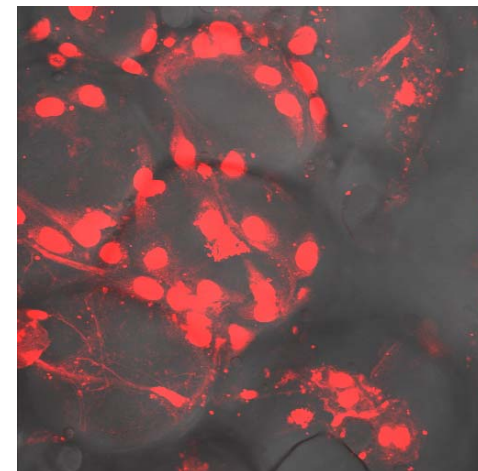
-Leveraging complex 3D, co-culture, primary ex-vivo tissue models

Pancreatic ex-vivo –Retroperitoneal model: 3D Panc1(GFP) (purple: connective tissue reflectance)



Ovarian ex-vivo model:

Confocal cross-section:
Red: Skov3 cells
seeded on omentum





Phenotypic Screening:

'Complex' but meaningful, predictive screen tests ...

1. cancer cell polarity
2. assembly of cancer integrin complexes
3. cadherin dynamic dysregulation
4. cancer cell proliferation
5. cytokinesis
6. pro-apoptosis
7. distinct modes of invasion
8. suppression of 'survival' autophagy
9. epigenetics (demethylation/heterochromatin)
10. dormancy
11. vascular modulation
12. interaction with 'host'



Drug candidate

targeting survival, invasion in metastatic niche and the
new Achilles heels !



proof-of-principle cancer modelling ...
quantitative dynamic imaging in vivo ...



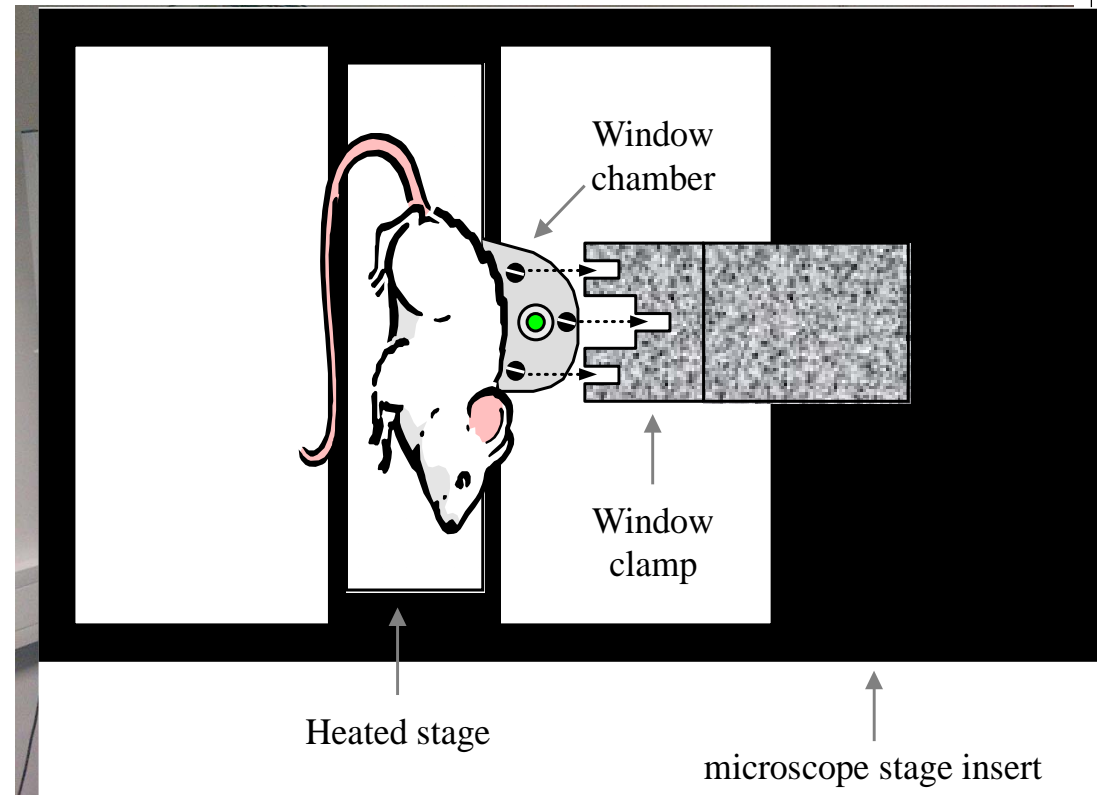
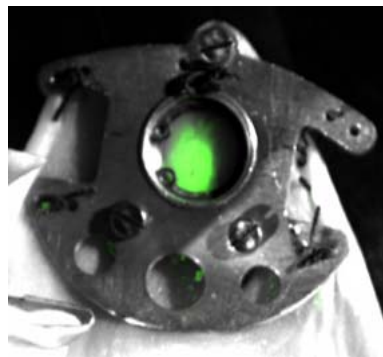
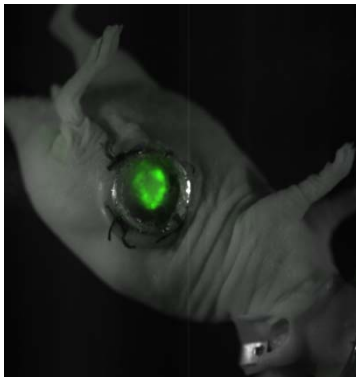
... **multi-parametric** activity readouts
.....understand **combinations**
.....**matrix** testing - genetic ?
..... **chemical biology**
... frontload **Predictive Biology**



Quantitative pre-clinical cancer imaging

intravital imaging : optical windows

- optical window reduces problem of light scattering
- removal of skin increases resolution



Alan Serrels, M Canel, V Brunton, M Frame

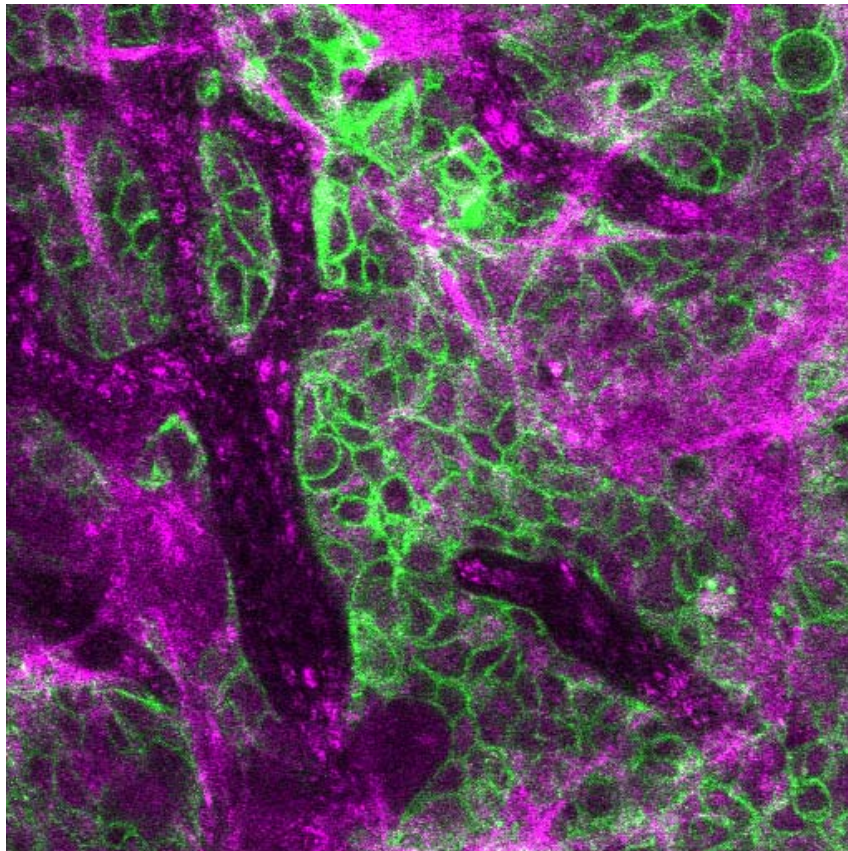
quantitative endpoints are vital



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Quantitative pre-clinical cancer imaging

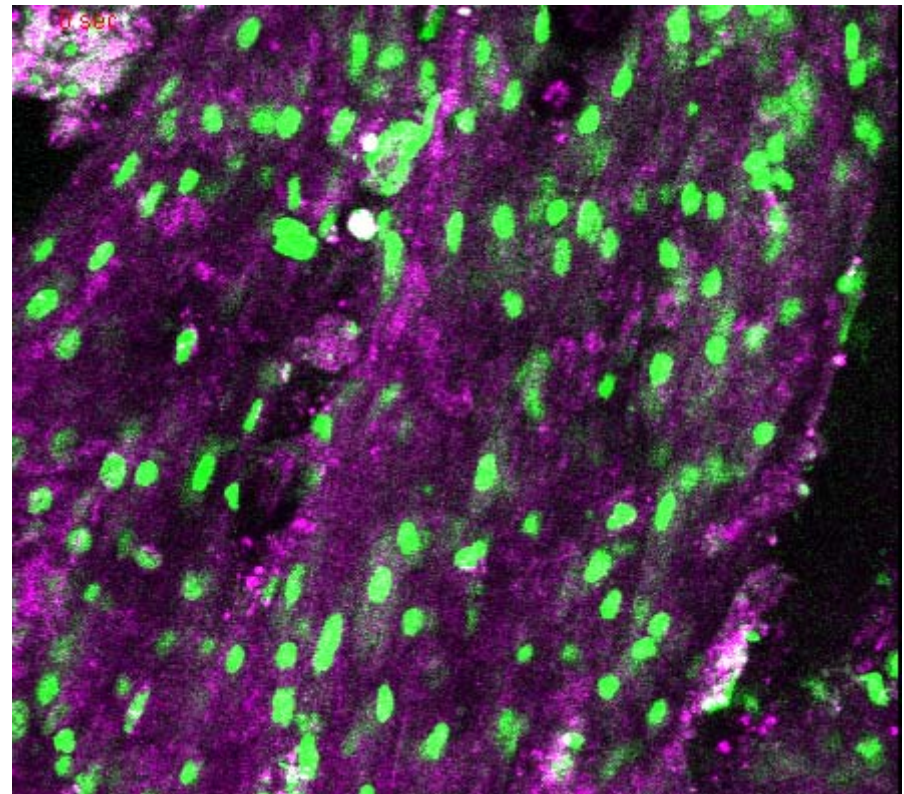
- inside the 'mouse' -
complex in vivo environment



Alan Serrels

Problems ::

- cell migration is relatively rare *in vivo*
- difficult to quantify accurately

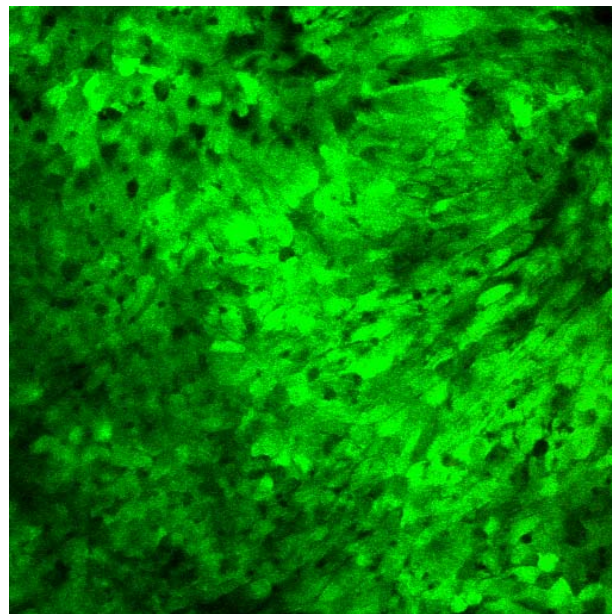




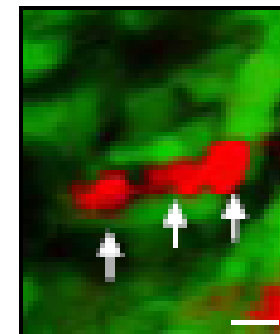
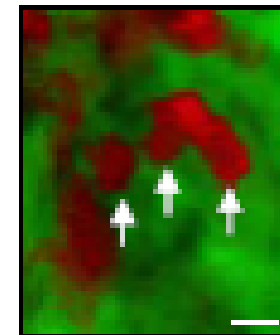
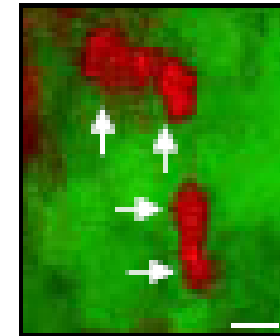
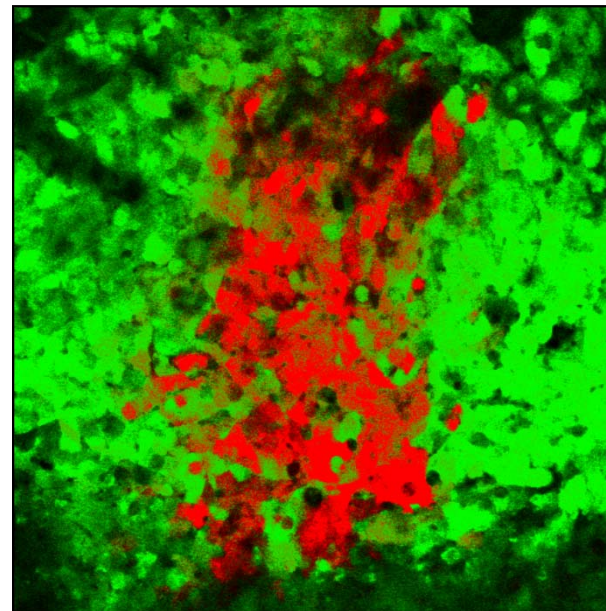
Quantifying invasion *in vivo*: *photo-convertible proteins*



In vivo $T = 0$



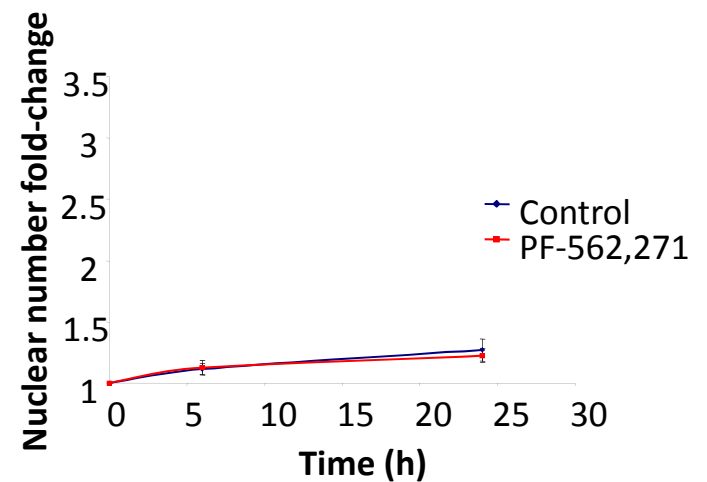
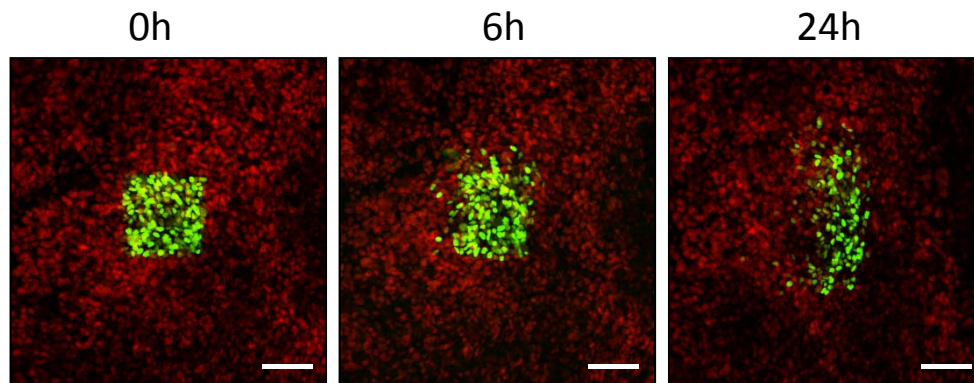
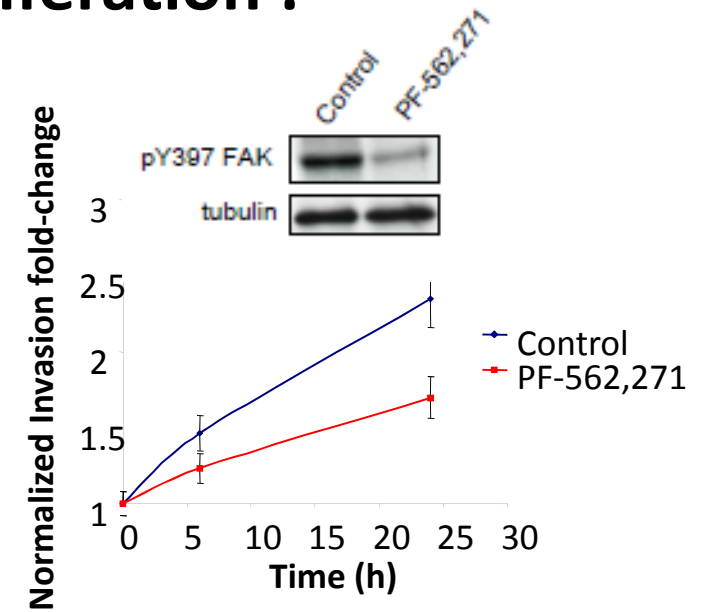
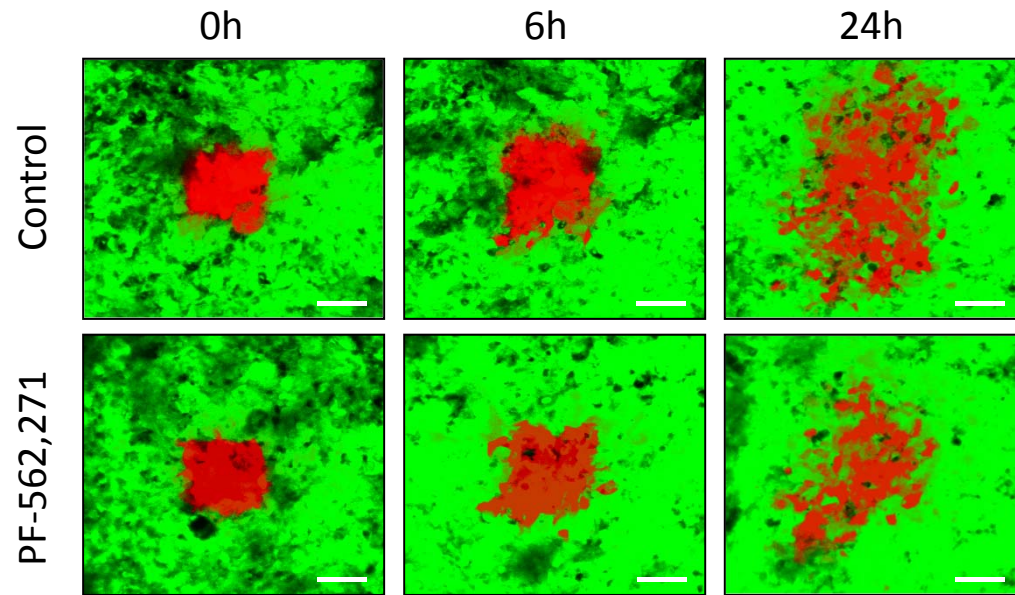
24 hours





Quantitative pre-clinical cancer imaging

Invasion versus proliferation :

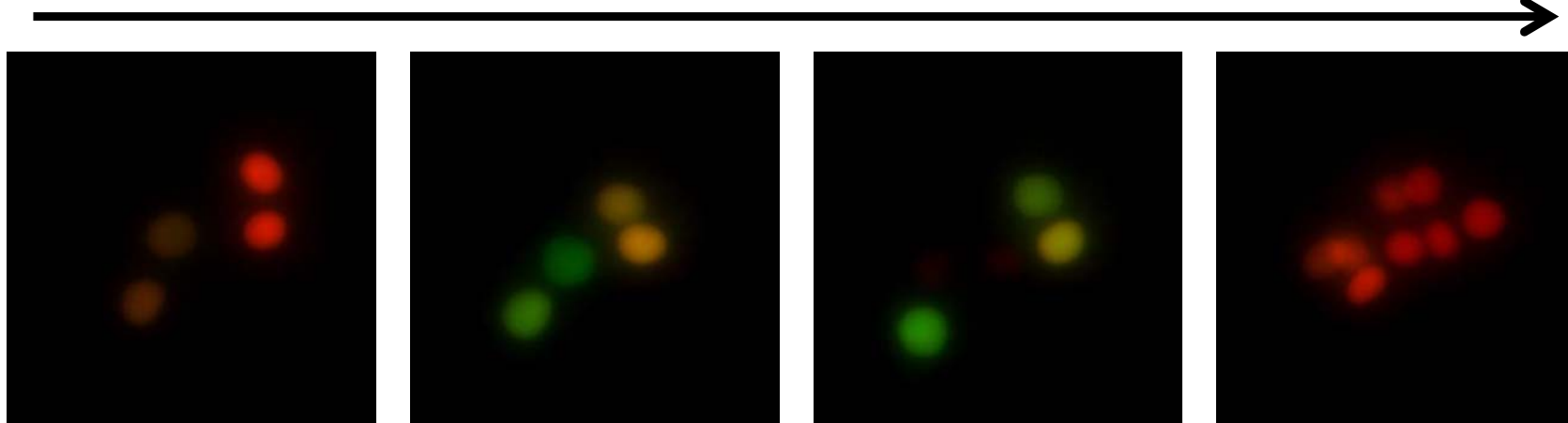




Imaging cell cycle distribution *in vitro* & *In vivo*

Fucci Probes: *In vitro*

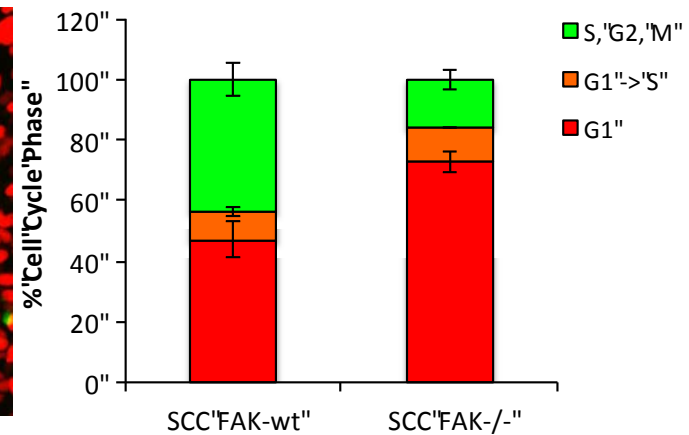
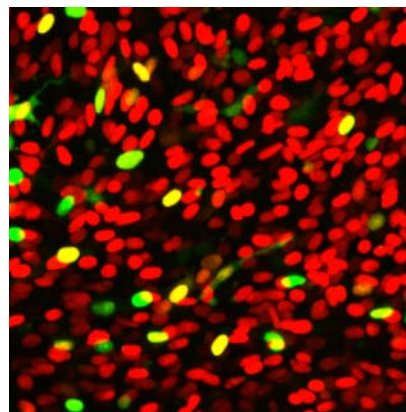
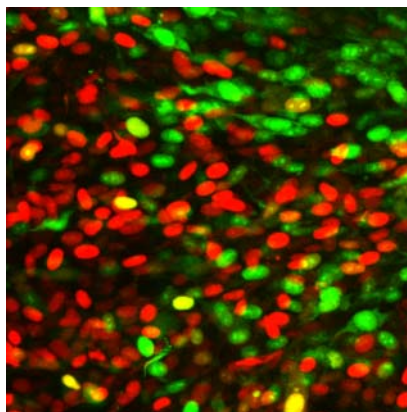
Time



Fucci Probes: *In vivo*

SCC FAK-wt

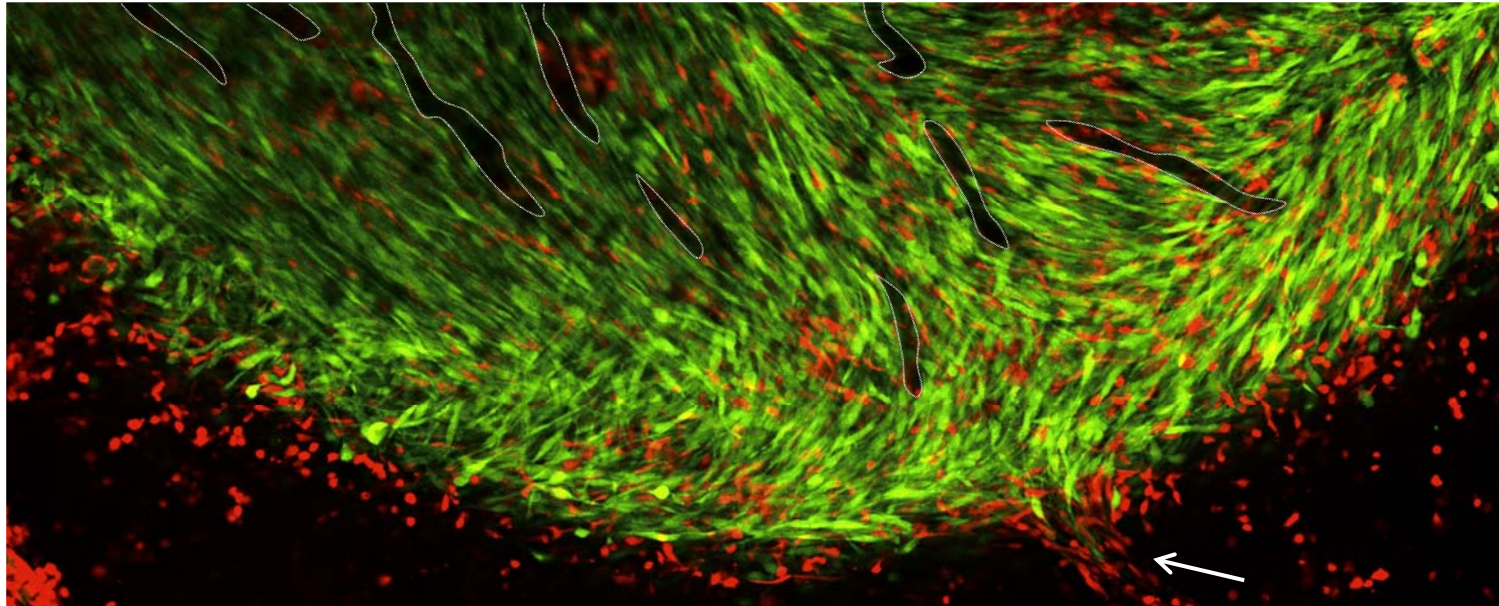
SCC FAK-/-



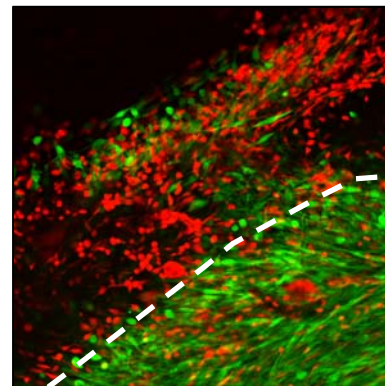
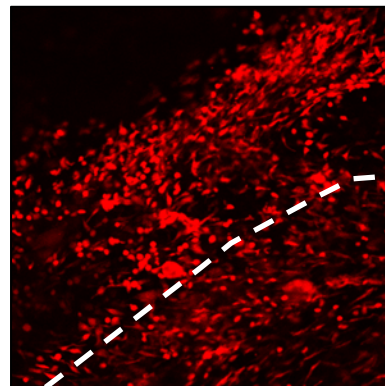
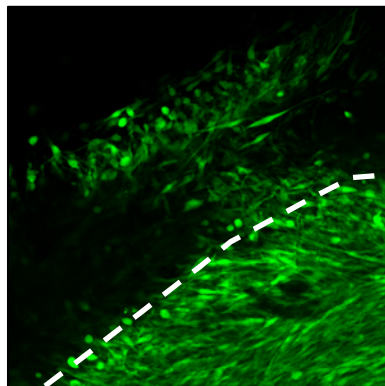


Panoramic view: Imaging tumour heterogeneity

macrophage clustering at the invasive edges of SCC syngenic tumours



Green = GFP labeled SCC tumour cells; Red = c-fms ECFP



← = tumour / stromal margin



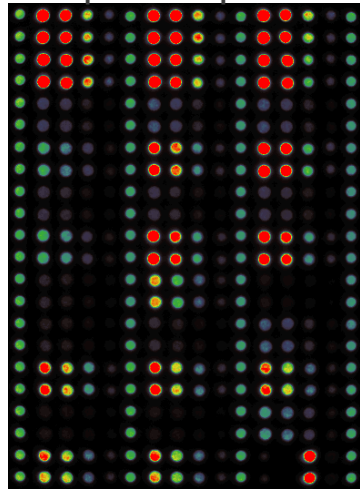
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zeptosens
Bioanalytical Solutions

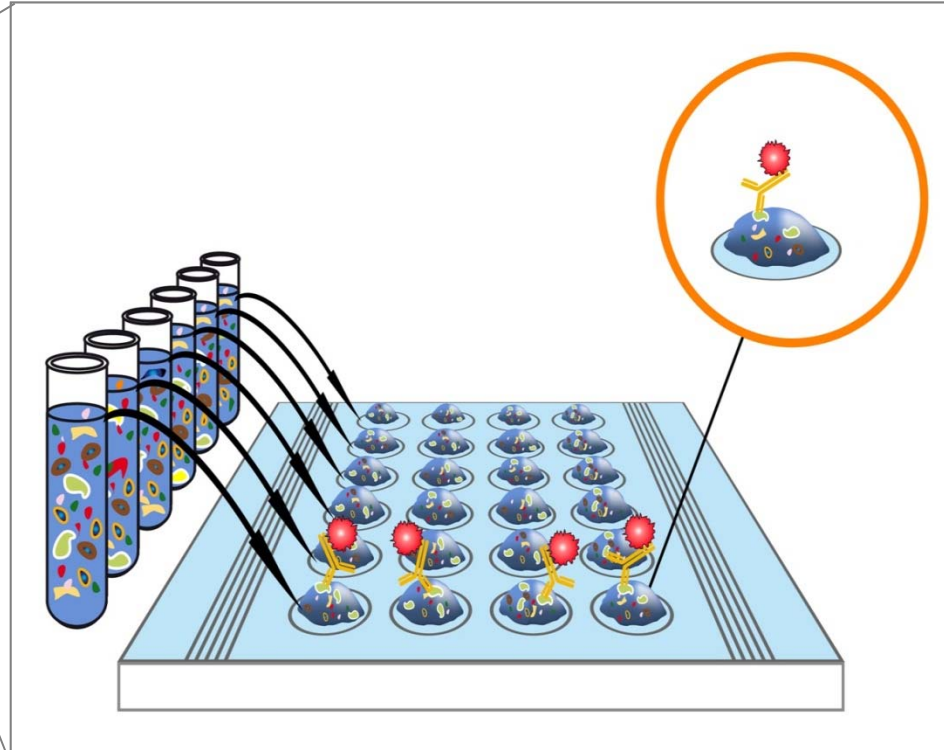
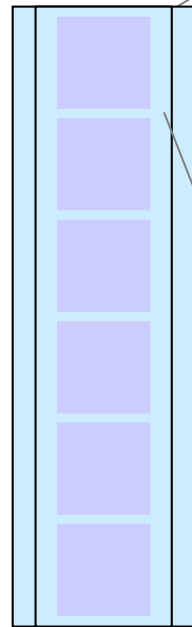
**High Throughput Pathway Profiling
Protein biomarkers**

Reverse Phase Protein MicroArray (RPPMA) Platform:

1536 sample/chip
400pL sample vol



6 Arrays/Chip



- No antibody cross-reactivity: unlimited multiplexing
- > 300 protein expression level and post translational markers validated:
(125 phospho-prot, 30 histone modifications (Met,Ac))
- Small sample / antibody volumes required – Cost effective & ultra-sensitive

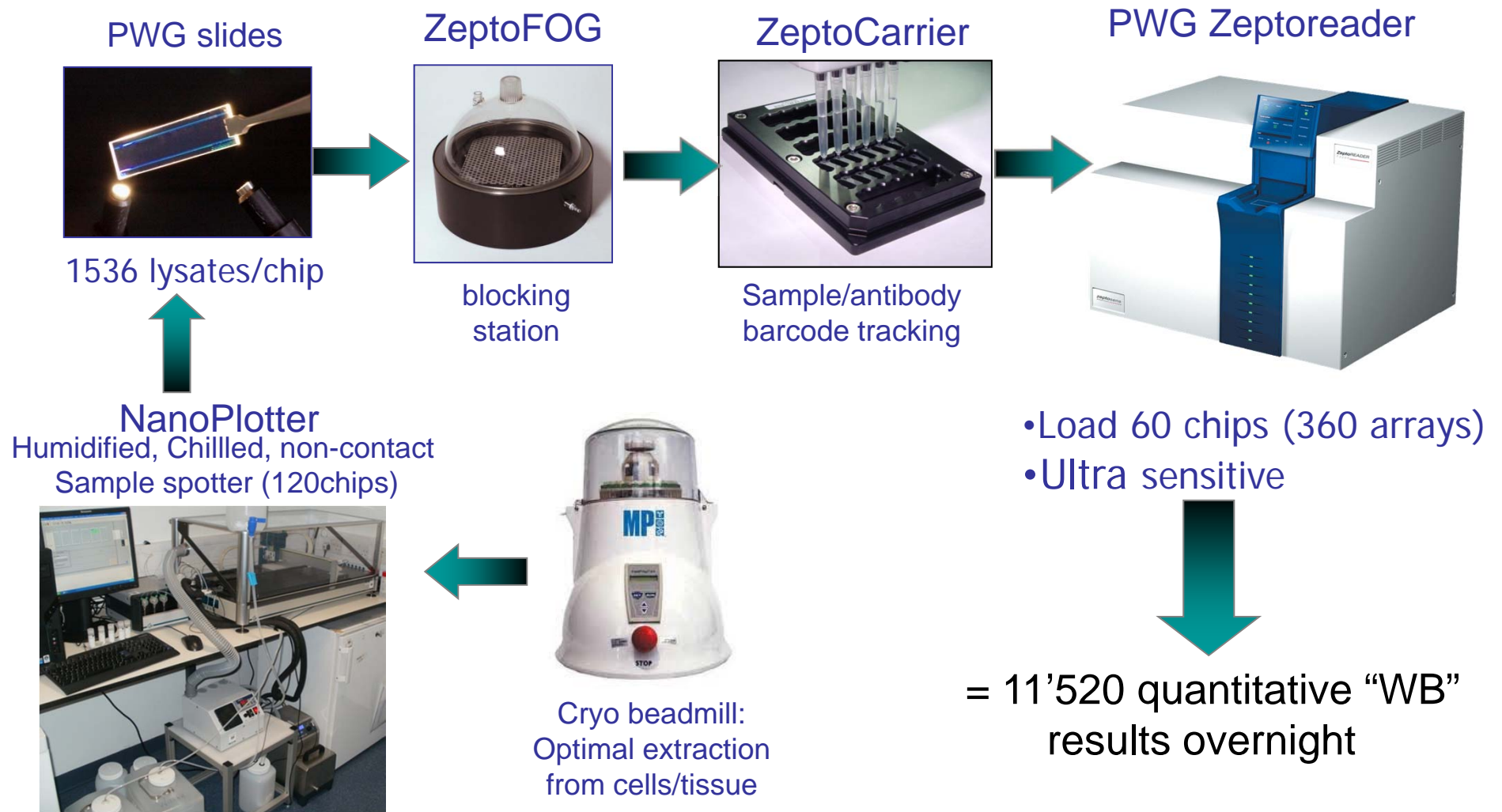


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Bioanalytical Solutions

*High Throughput Pathway Profiling
Protein biomarkers*

Fully dedicated/integrated Reverse Protein MicroArray Platform in U.K:





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Bioanalytical Solutions

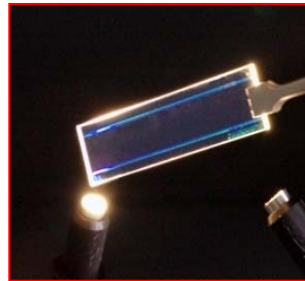
**High Throughput Pathway Profiling
Protein biomarkers**

Planar Waveguide (PWG) technology -industry-leading sensitivity:

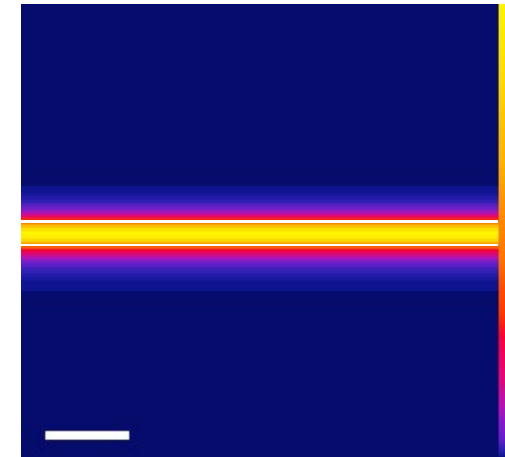
PWG Zeptoreader



PWG slides



“Planar Waveguide Technology”



~ no background
Evanescent excitation
Maximizes emission detection

Small samples:

1. Cell culture: 30,000 cells (96well plates)
2. Tissue: 1mg, FNA, LCM
3. Body fluids (serum, plasma, CSF) -20 μ l

Zeptomol Sensitivity:
(600 proteins) per spot

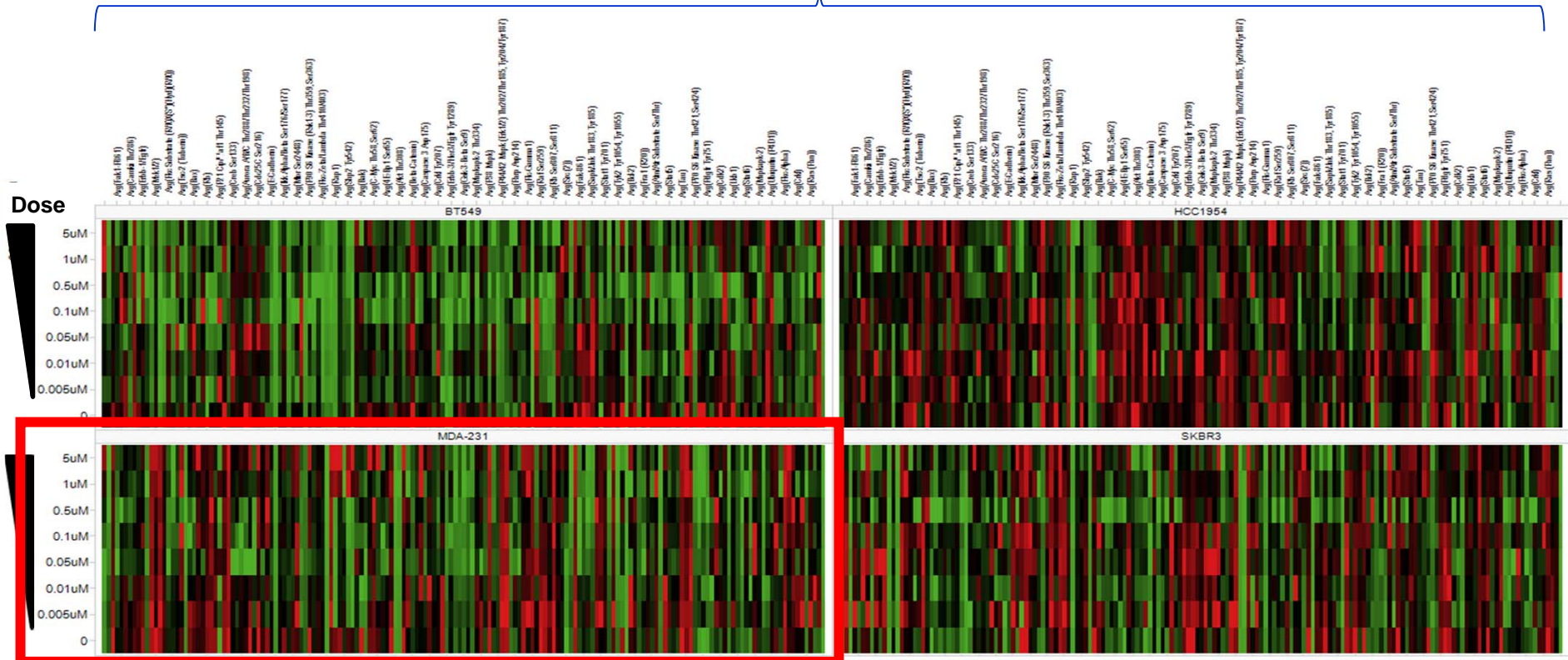
... and measure hundreds of proteins

Proteomics: RPA = Optimal throughput, sensitivity and cost for routine application



Compound Profiling: Temporal- and Dose-Response Pathway Profiling Across Breast Cancer Cell panel

Pathway Markers



Filter Settings
- time: (3Hrs)

Max
Average
Min

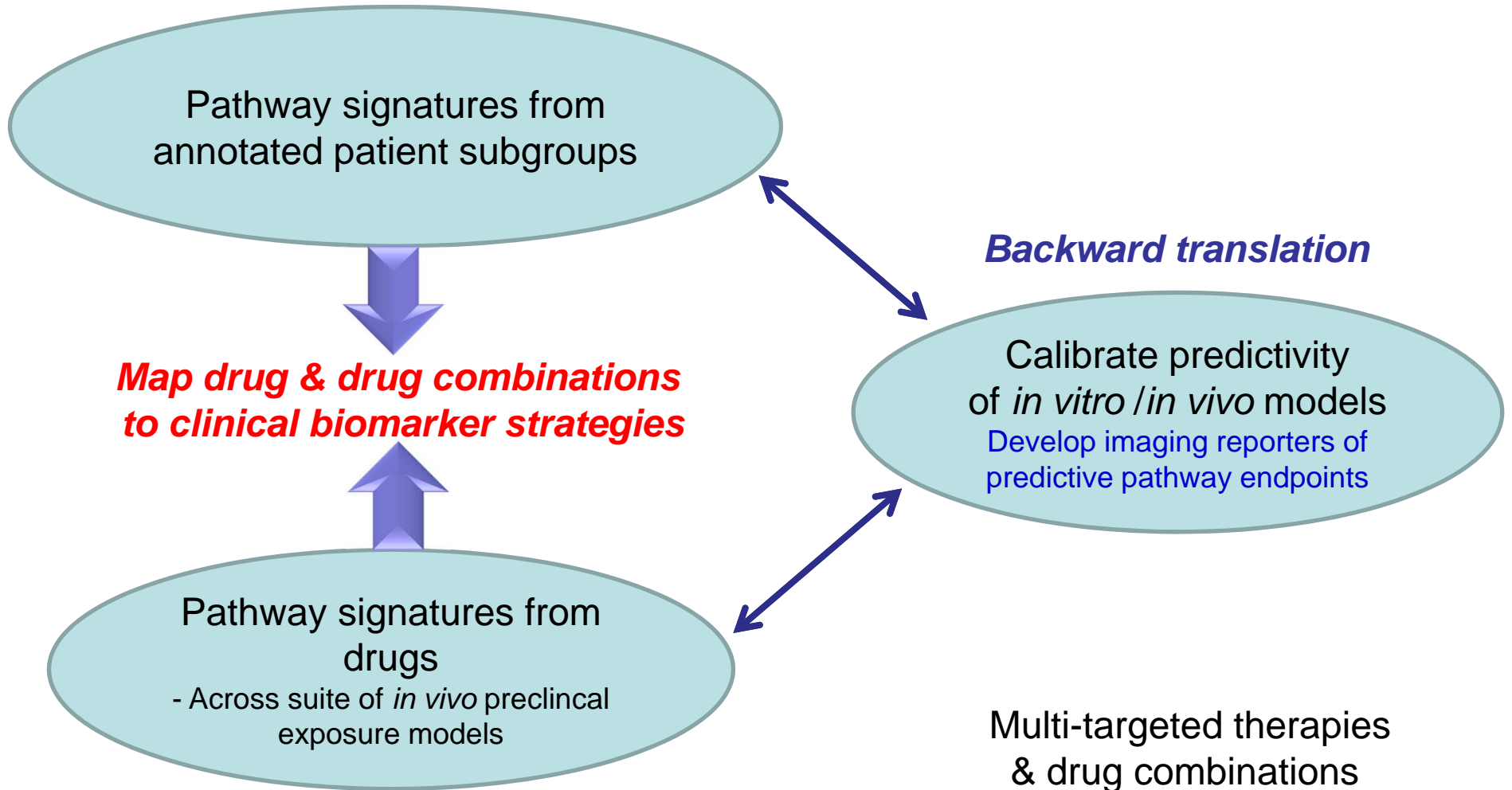


* *

* Map compensatory or resistance pathways to drug/target database = rational combination hypothesis



Reducing the gap between disease mechanism and drug mechanism





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Reverse Phase Protein Microarray: High-throughput profiling at post-translational level



INSTITUTE OF GENETICS
& MOLECULAR MEDICINE



2nd REVERSE PHASE PROTEIN ARRAY GLOBAL WORKSHOP

Edinburgh, Scotland, UK,
12th -13th November, 2012

For more information please visit: www.ecrc.ed.ac.uk
Venue: John McIntyre Conference Centre, Edinburgh

"Bringing together Industry and Academia to
discuss the needs, opportunities and challenges of
RPPA"



Sponsors:











Organising committee:
Lance Liotta: George Mason University, Washington DC, USA
Emanuel Petricoin: George Mason University, Washington DC, USA
Gordon Mills: MD Anderson, Houston, USA
Ulrike Korf: DKFZ Heidelberg, Germany
Michael Pawlak: NMI at the University of Tubingen, Germany
Leanne De Koning: Institut Curie, Paris, France
Bryan Serrels: University of Edinburgh, UK

RPPA Advances:

- High-throughput printing
- Enhanced sensitivity
- Antibody validation
- Clinical application

Outcomes:

- Global RPPA society
- White paper
- 2013 Mtg in Japan





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- **Future work**

- ECDU expansion: Edinburgh College of Medicine **“Translational Pharmacology Unit”**: incorporate Medicinal Chemistry & ADMET profiling and **Extend remit across disease area**

- **Education**

- Institute of Genetics & Molecular Medicine (IGMM) Graduate School training
 - Several joint PhD studentships
 - Teaching BSc Honours Cancer Biology Course
 - MRC funded clinical training (ECAT) program

- **Collaboration**

- University of Warwick; University of Strathclyde; University of St Andrews; University of Glasgow; Beatson Institute for Cancer Research; Patterson Institute for Cancer Research; University of Ulm (Germany); Umeå University (Sweden); Garvin Institute (Australia) and the Friedrich Miescher Institute for Biomedical Research (Switzerland).

- **Commercial activities (i.e. spinout companies, services offered etc)**





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Acknowledgements:

Margaret Frame
Val Brunton
Alan Serrels
Bryan Serrels

John Dawson
Kenny Macleod
Dahlia Doughty-Shenton
Emily Russell

Chemistry:

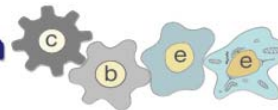
Asier Unciti Broceta, Craig Fraser, Jason Weiss (Chemistry)
Scott Webster (Chemistry and IP development Lab)

Clinical:

Paul Brennan (Honorary Specialist Registrar Neurosurgery)
Mark Duxbury (Honorary Consultant Surgeon; Hepatic-Pancreatico-Biliary Surgical Services)
Charlie Gourley (Honorary Consultant in Medical Oncology: "Platinum Resistant Ovarian cancer")
Mike Dixon, David Cameron (Breast Cancer Program: pre- and on-treatment profiling)



Centre for Biomedical Engineering at Edinburgh



Alastair Elfick; Andy Downes