FDG uptake in a heterogeneous microenvironment: A single-cell study

JOINT AAPM-WMIS SYMPOSIUM:
METABOLIC IMAGING OF CANCER

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Radiation Oncology & Medical Physics
August 4th 2016

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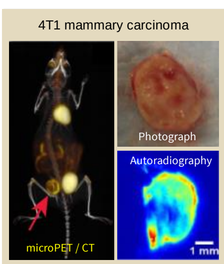
Heterogeneity of FDG uptake in tumors grafts



- **FDG uptake factors:**
  - Tissue perfusion
  - Viable cancer cell density
  - Immune cell infiltration
  - Stromal cells
  - Cancer cell metabolism
- **Two distinct microenvironments: Core and periphery**
  - Impacts metabolism
  - Oxygen, nutrients, pH, etc.

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Goals of the study

QUESTION 1: IS THE METABOLISM OF CANCER CELLS IN THE CORE OF THE TUMOR DIFFERENT THAN IN THE PERIPHERY?
- … or is the difference in FDG uptake simply due to viable cell density and tissue perfusion?

QUESTION 2: IF SO, WHAT CAUSES THIS DIFFERENCE?
- What specific microenvironmental factors can alter tumor cell metabolism?

QUESTION 3: WHAT IS THE MALIGNANT POTENTIAL OF CELLS IN THE CORE?
- Should we worry about these cells?

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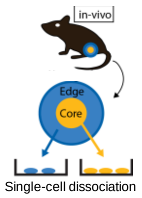
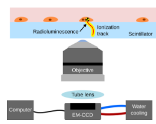
Experimental design

Tumor graft



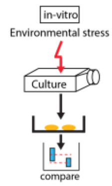
Single-cell imaging



Radioluminescence:
- Single-cell FDG uptake

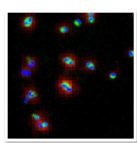
Bioluminescence:
- Viability / cancer

Cell culture



Radioluminescence microscopy

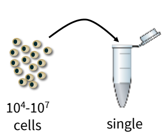
MULTIMODAL TOOL FOR SINGLE CELL ANALYSES

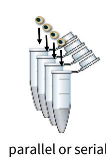


Biological assays

Conventional analysis
- Pooled samples
- low cost, high sensitivity

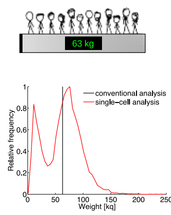
Single-cell analysis
- Differences between single cells



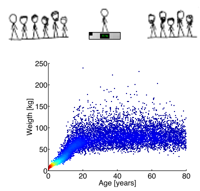


Why differences matter?

Conventional analysis

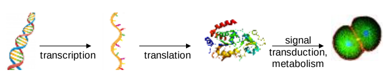


Single-cell analysis



data from CDC

The Central Dogma of biology

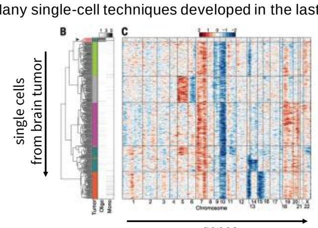


DNA	**RNA**	**Protein**	**Phenotype**
Gene sequencing In-situ hybridization	RNA-seq RT-PCR Microarrays	Western blot Mass spec Immunostaining	Functional assays

- New assays to measure the genome, transcriptome, proteome, and phenotype of **single cells**!

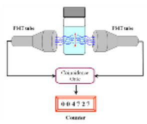
Single-cell transcriptomics

Many single-cell techniques developed in the last 5 years

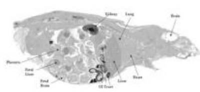


Science **344**, 1396-1401 (2015)

Radionuclides and single cells?



Liquid scintillation counting



Autoradiography

MicroPET

http://departments.agri.huji.ac.il
http://imaging.bme.ucdavis.edu/
http://www.qpsinfo.com

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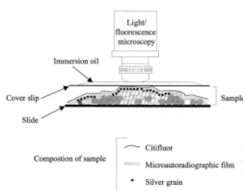
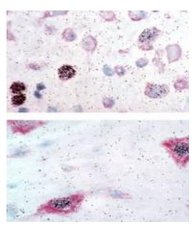
Microautoradiography (film)



- Fixed tissue
- Technically challenging
- Low energy (^{3}H = 19 keV e$^-$)
- Non-linear



^{3}H-labeled vitamin D

J Pharmacol Toxicol Methods **51**, 25-40 (2005)
Appl. Environ. Microbiol. **65**, 1289-1297 (1999)

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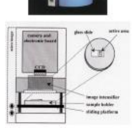
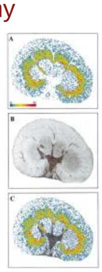
Digital autoradiography





P. Laniece et al. *J Neurosc Meth* (1998)
http://www.biospacelab.com

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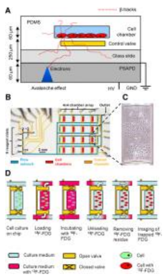
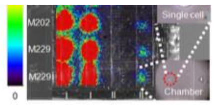
Microfluidics beta camera





□M202 ■M229

Single cell / chamber possible
16 chambers

NT Vu *et al. J Nucl Med* (2011)

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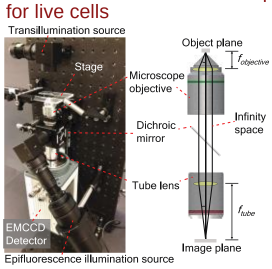
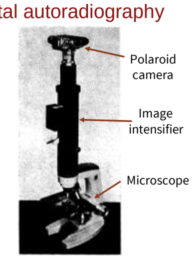
Radioluminescence microscopy: digital autoradiography for live cells



Radioluminescence microscope
PLOS One 7, *10* (2012)



Scintillomicroscope
Rev. Sci. Instr. *39, 298* (1968)

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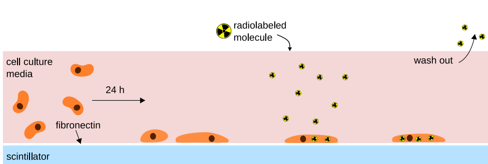
Preparing cells for imaging



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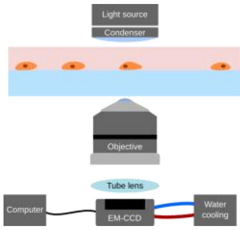
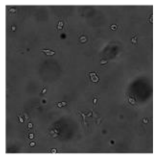
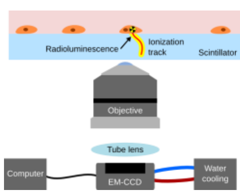
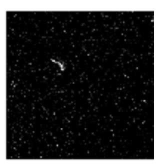
Brightfield imaging



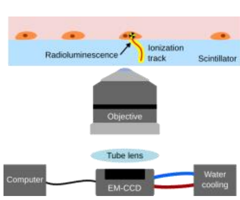


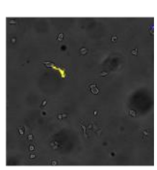
Radioluminescence imaging



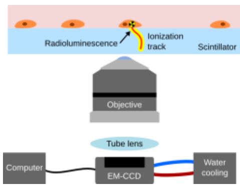


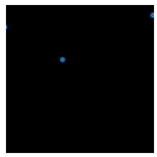
Combined brightfield / radioluminescence





Reconstruction





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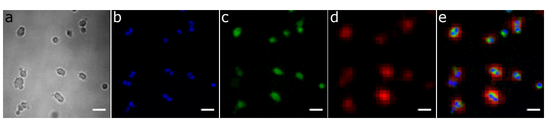
Four modalities in one instrument



Brightfield Fluorescence Bioluminescence Radioluminescence Overlay

System performance

- Spatial resolution:
  - 30 μm [$CdWO_4$]
  - 20 μm [Lu_2O_3 thin film]
- Sensitivity:
  - 21% [$CdWO_4$]
  - 40% [Lu_2O_3 thin film]

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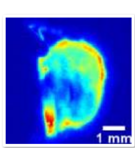
FDG uptake in a heterogeneous microenvironment

A SINGLE CELL STUDY



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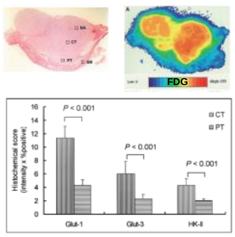
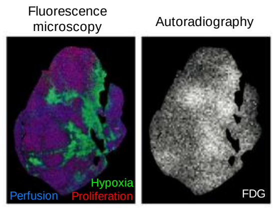
Impact of the microenvironment on FDG uptake

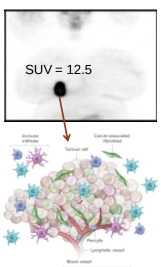


S. Zhao *et al.* J Nucl Med *46 (4)* 2005



A. Pugachev *et al.* IJROBP *62(2)* 2009

Limitations of measuring FDG uptake with PET

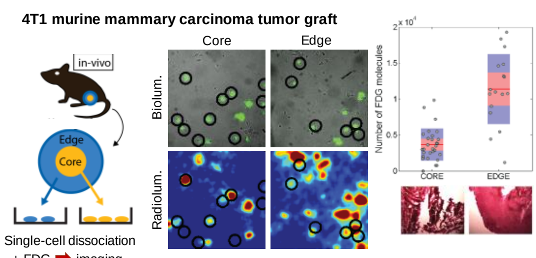


- Many different cell types:
  - Immune cells (FDG uptake in macrophages)
  - Stromal cells (reverse Warburg effect)
  - Cancer cells (Warburg effect)
- Density of viable cells
- FDG perfusion (tumor vasculature)

How to measure effect of microenvironment on cancer cell metabolism, independantly of the other factors?

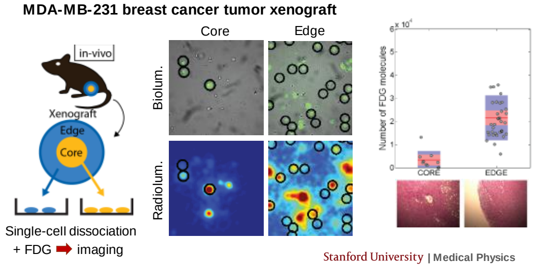
FDG uptake of single dissociated cells

4T1 murine mammary carcinoma tumor graft

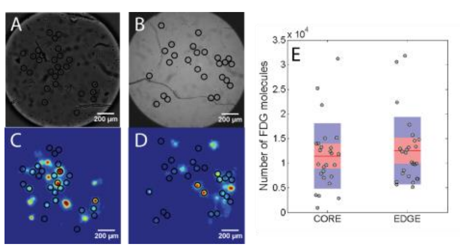


FDG uptake of single dissociated cells

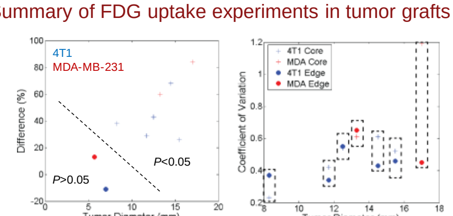
MDA-MB-231 breast cancer tumor xenograft



Cells revert to their original phenotype after 3 weeks



Summary of FDG uptake experiments in tumor grafts

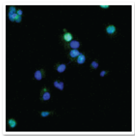


- FDG uptake higher in core of large tumors
- FDG uptake more heterogeneous in core
- Observed in both tumor models

$$CV = \frac{\text{standard deviation}}{\text{mean}}$$

Microenvironmental determinants of cancer cell metabolism

HYPOXIA, LACTATE, OR PROLIFERATION?



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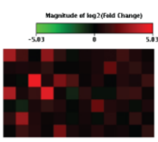
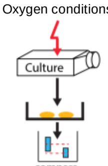
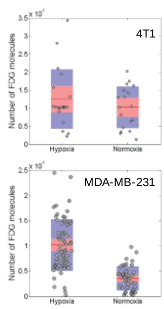
Condition tested: Hypoxia



Hypoxia gene expression (core vs. periphery)

Oxygen conditions





- Core more hypoxic
- Hypoxia promotes FDG uptake
- **Hypoxia not driving factor**

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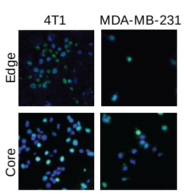
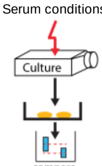
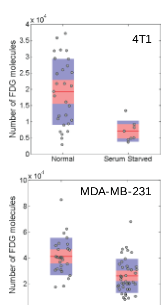
Condition tested: Cell proliferation



EdU staining

Serum conditions





- Proliferation promotes FDG uptake
- **No difference in proliferation between core and periphery**

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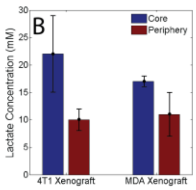
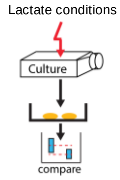
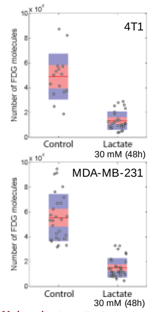
Condition tested: Lactate

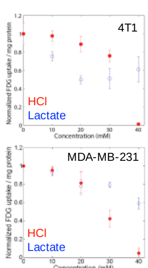


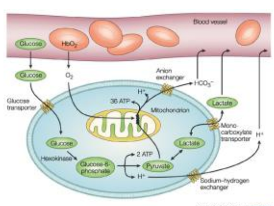




- Lactate builds up in the tumor core
- Lactate inhibits FDG uptake
- **Best candidate**

Inhibition of FDG uptake by lactate





Goals of the study

QUESTION 1: IS THE METABOLISM OF CANCER CELLS IN THE CORE OF THE TUMOR DIFFERENT THAN IN THE PERIPHERY?

- Metabolism is decreased by 40 ±30% in the core, compared to the periphery.

QUESTION 2: IF SO, WHAT CAUSES THIS DIFFERENCE?

- Lactate buildup in the core is the most likely driving factor.

QUESTION 3: WHAT IS THE MALIGNANT POTENTIAL OF CELLS IN THE CORE?

- Cells can revert to their original phenotype within 3 weeks
- No significant difference in proliferation
- Lactate promotes cancer invasion

Conclusions

New methodology for testing effect of microenvironment on cancer cells

- Single-cell analysis
- Independent of other confounding factors

FDG uptake is sensitive to lactate accumulation

- Clinical relevance
- Radiation dose painting
- Tumor response monitoring

Other microenvironmental factors modulate FDG uptake

- Proliferation, hypoxia

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Acknowledgments

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