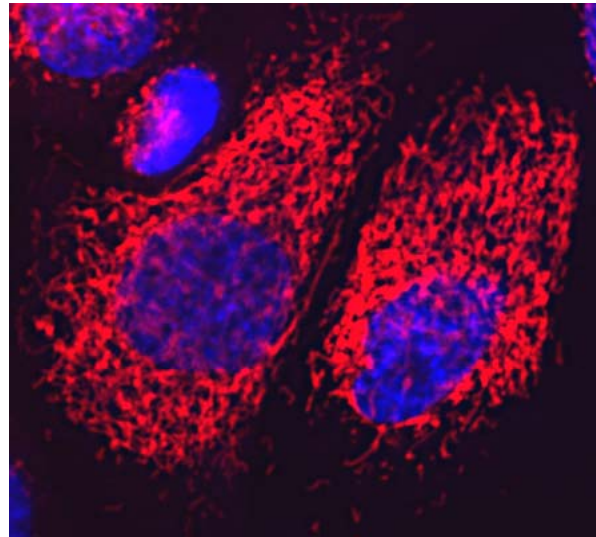
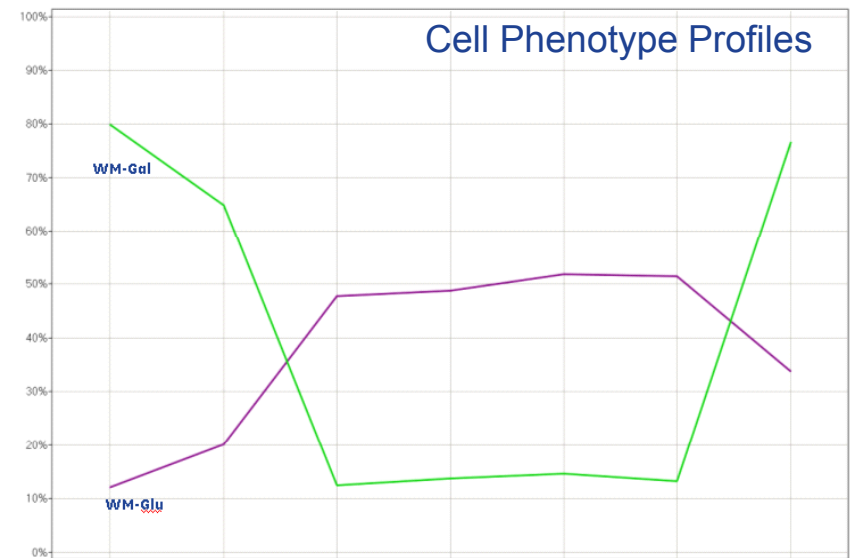


Models for Tumor Cell Drug Resistance

Monitoring Mitochondrial Status with HCA

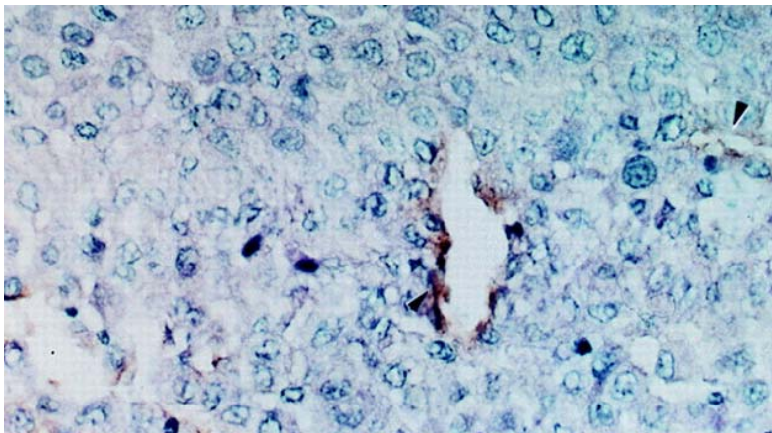
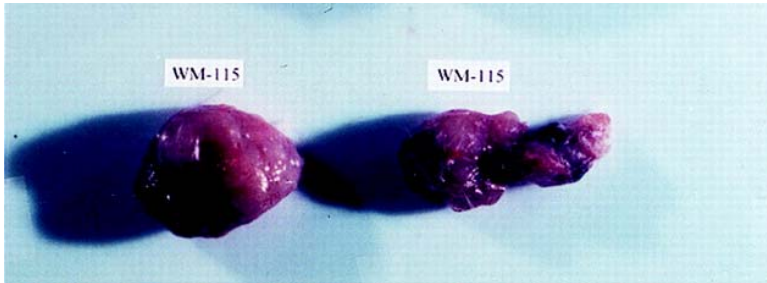


Robert Graves
Senior Applications Scientist
GE Healthcare
Piscataway, NJ, USA



Malignant melanoma models

Understanding mechanisms of drug resistance

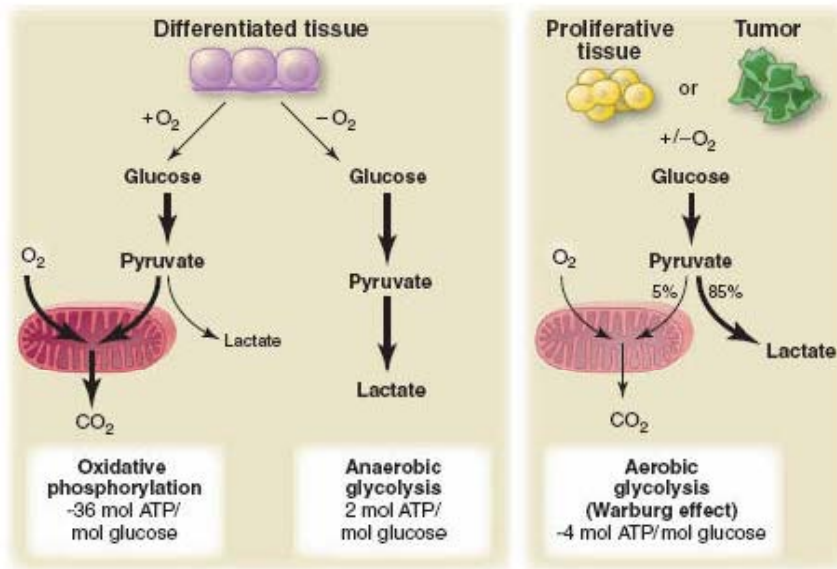


Images show human melanoma cell line WM115 grown as xenogeneic tumors in SCID mice. Taken from Keisuke Abe et al. Regulation of vascular endothelial growth factor production and angiogenesis by the cytoplasmic tail of tissue factor. *Proc. Natl. Acad. Sci USA* (1999) **96**:8663-8668

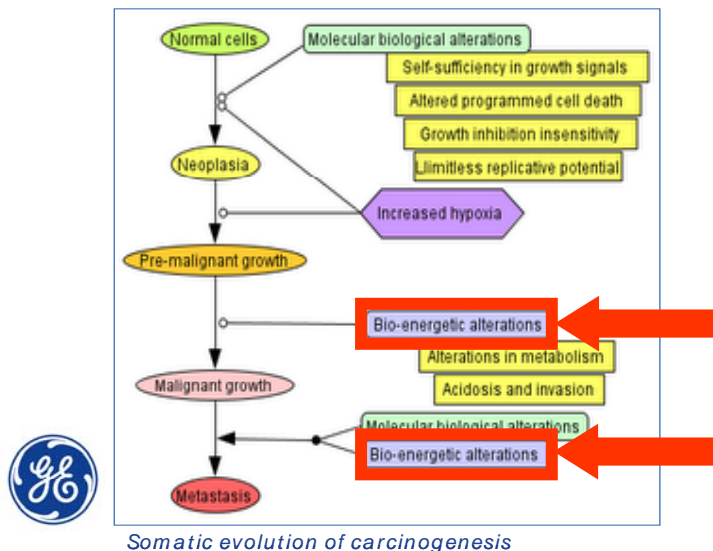
- Malignant melanoma - highly aggressive tumor that frequently resists chemotherapy.
- The search for better therapeutic agents is of great importance.
- Focus on strategies that exploit the unique properties of tumors or malignant cells. High glucose uptake utilized in PET scanning for melanoma
- Malignant melanomas (and many other tumor types) exhibit increased glycolysis (Warburg effect), which suggests a potential therapeutic window, but also makes cells resistant to mitochondrial toxicants.

Developing good model systems

Why the Warburg effect matters



- Warburg Effect: Glycolytic phenotype develops as part of the multi-step process of carcinogenesis
- Cells no longer depend on oxidative phosphorylation – more resistant to drugs that target mitochondria but also more vulnerable to drugs that exploit the glycolytic dependency
- Cultured cell models chronically exposed to high glucose maintain a similar preference for glycolysis
- Replacing glucose with galactose shunts cells toward Ox-phos, forcing cells to use their mitochondria

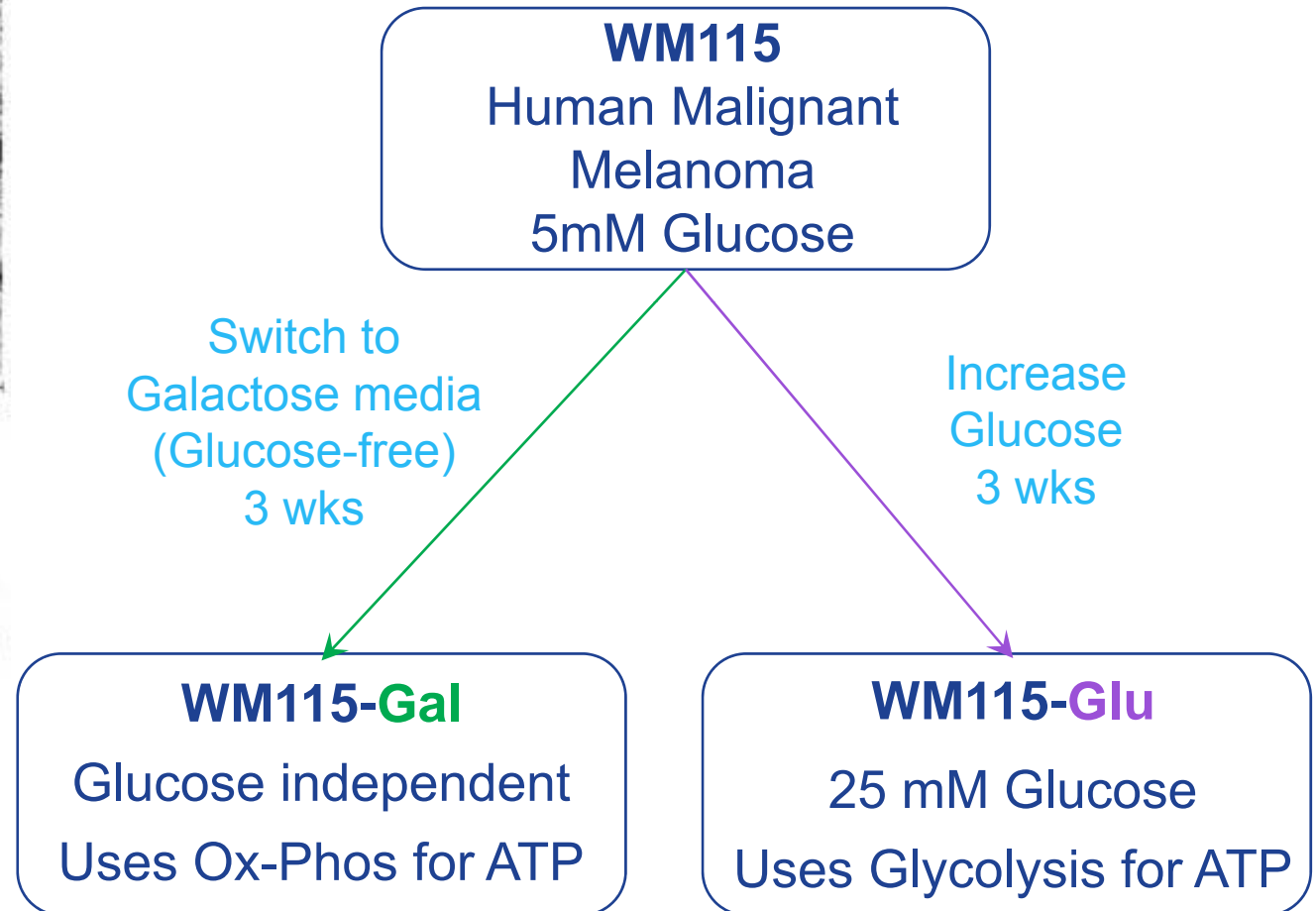
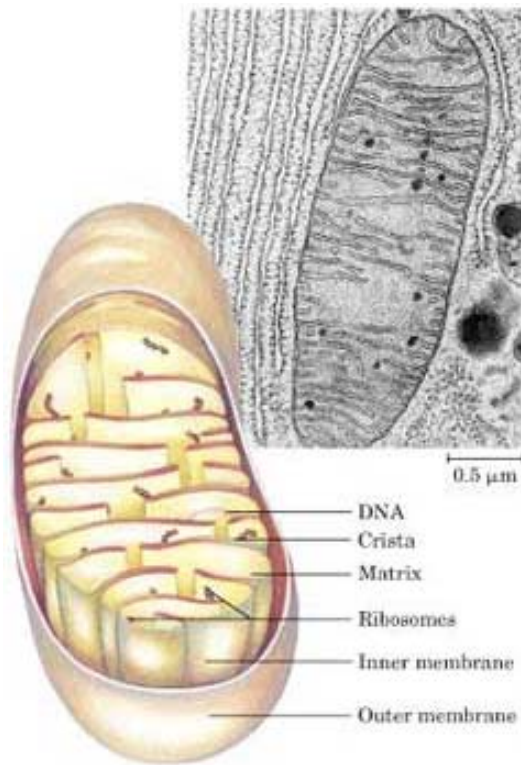


•Goal:

- Establish cell models representative of both high and low glucose conditions
- Use to compare metabolic status/mitochondrial function of the two phenotypes against the same genetic background
- Model for elucidating drug resistance and toxicity mechanisms

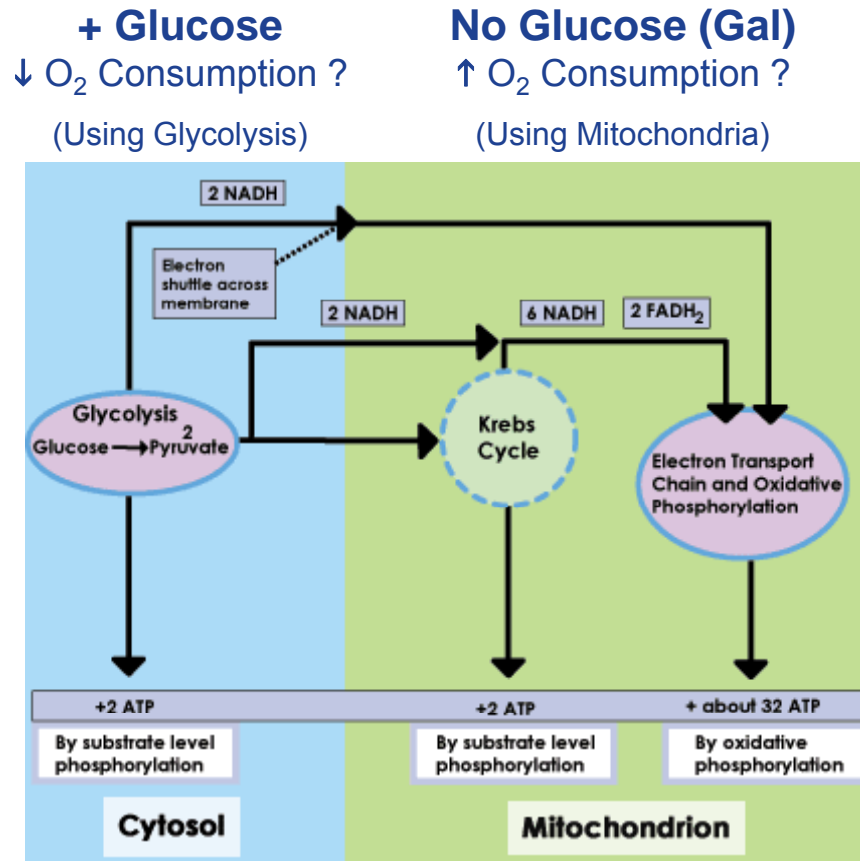
Development of model cell lines

Re-conditioning cells to use Ox-Phos or Glycolysis

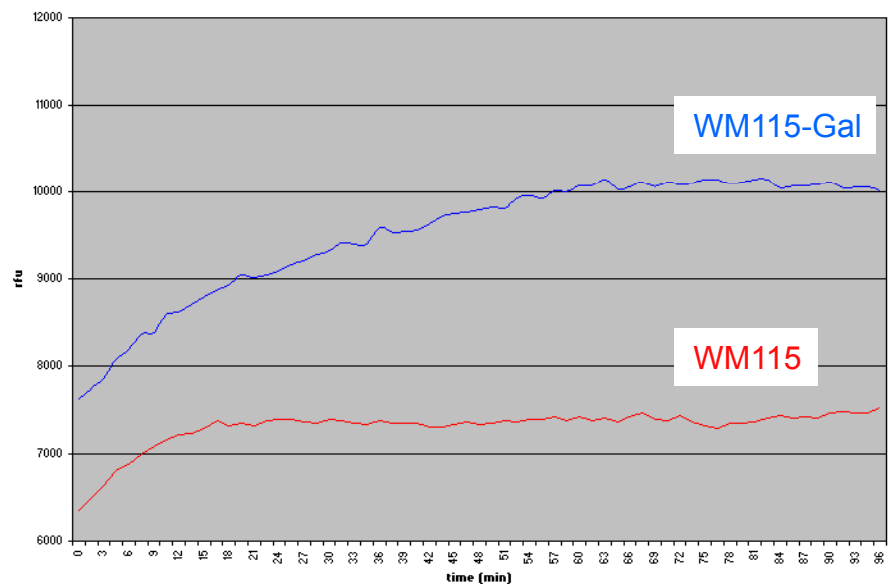


Cell Line Characterization

O₂ Consumption



**Glucose-Depleted Cells
Show Increased Respiration**
(Using Mitochondria rather than Glycolysis)



Oxygen sensitive probe (MitoXpress from Luxcel)

HCA Assay Workflow



Develop model cell lines:
Melanoma Cells (WM115)
conditioned into different media

Optimize imaging
conditions: HCA of
mitochondria

Characterize cell lines:
HCA and other approaches

Comparative study:
Challenge with drugs and
siRNAs (384-well study)

Analyze & Interpret:
Quantify differential
responses

Choice of objectives and
imaging modes

Multi-parametric profiles
of phenotype

Configuration for 384-wells,
automation, maximizing
speed, sufficient cell counts,
previewing results

IN Cell Investigator,
IN Cell Miner

High Content Analysis with IN Cell Analyzer 2000



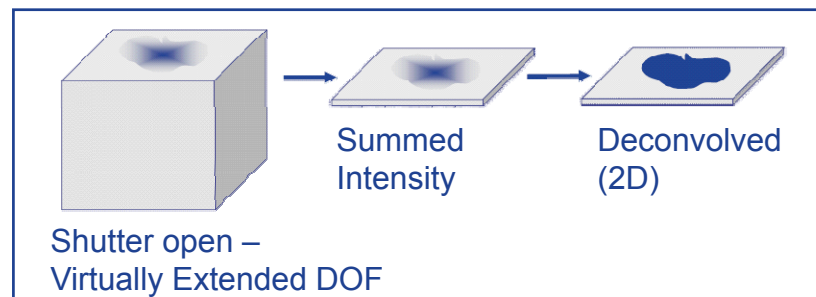
Feature	Details	Benefit
Illumination	Metal arc lamp, 200W	Long life (2000h min), good spectral distribution
Autofocus	HWAF (laser-based) and SWAF modes	Flexible automated focusing
Objectives	Wide range of choices from 2X – 100X	Automated turret
CCD Cameras	Standard and Large chip options	Large FOV with large chip
Transmitted Light	LED source; Bright-field, Phase & DIC	Live-cell studies, morphology
Slide Imaging	Capacity for 4 slides on the stage	Tissue samples
2D & 3D Imaging Modes	Image restoration, extended DOF	Enhance contrast and resolution
On-line Cell Counting	Variable fields, on-the-fly cell counting	Sample sufficient cell population with minimum number of fields
Preview Scanning	Rapid preview of any ROI	Quickly assess image data from any ROI – single well to whole plate
Manual Microscopy Mode	Manually pan, zoom, adjust settings	Optimize acquisition settings prior to initiating a run
Liquid Handling	On-board liquid addition	Run fast kinetic assays
Environmental Control	Variable temperature, humidified CO ₂	Live cell imaging over extended periods



Imaging Considerations

Maximizing Information Content

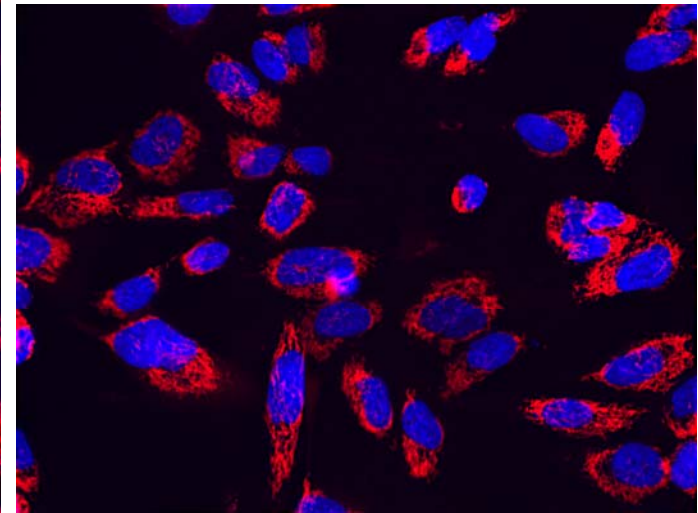
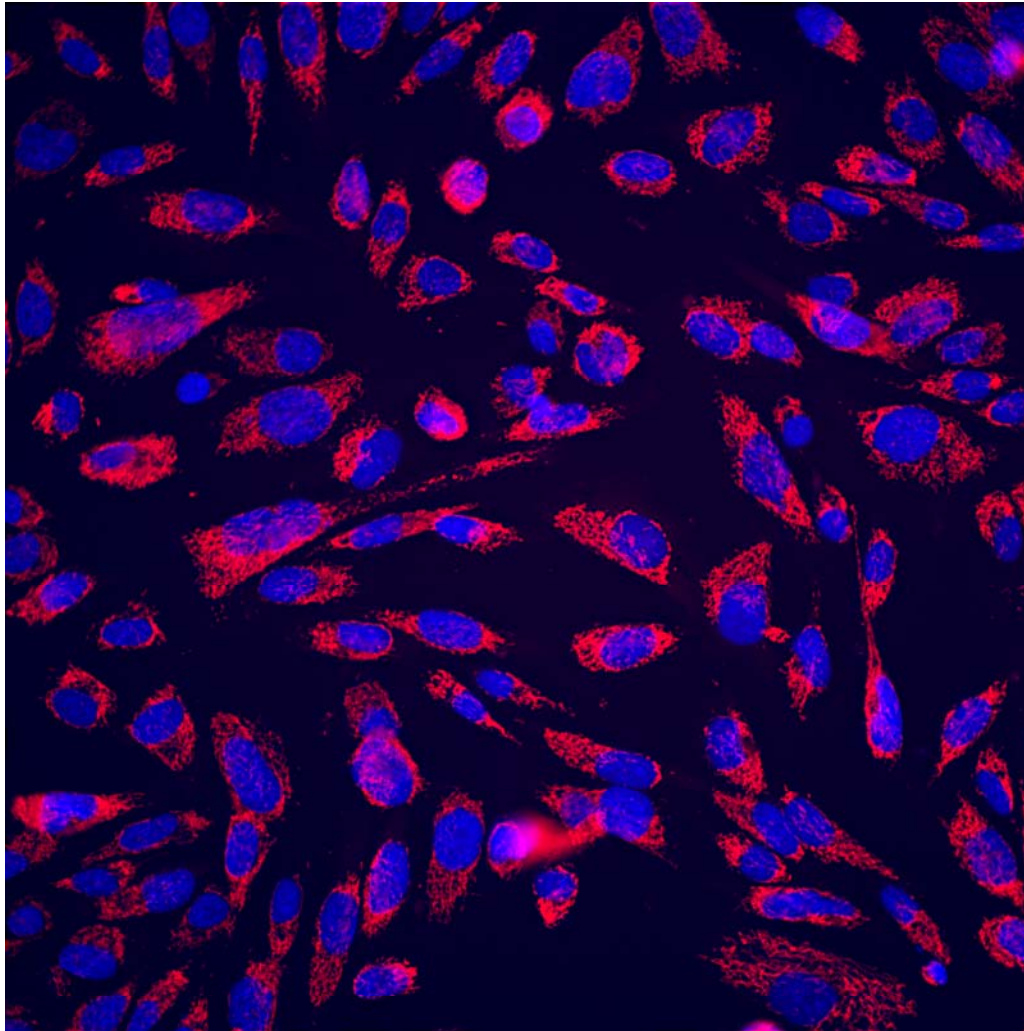
<u>Factor</u>	<u>Choice</u>	<u>Reason</u>
Live vs. Fixed	Live	Preserve mitochondrial morphology Avoid any other fixation artifacts Enable live-cell stains (e.g. Calcein)
Probes/Sensors	CMXRos Calcein AM Hoechst	Mitochondrial membrane potential, fixable Cell viability, segmentation, normalization Cell count, nuclear morphology
Magnification	40X/0.6NA Objective	Excellent resolution of mitochondrial morphology
Imaging Modes	2D Deconvolution + Virtually extended DOF ("2.5D Decon")	Maximize x-y resolution, no time penalty Quantify mitochondrial mass



Maximizing the number of cells acquired

Large Camera

WM115-Glu Cells Imaged Live
40x/0.6NA Objective



Standard Camera

220 mm x 170 mm FOV

Cell count: 40

Hoechst

CMXRos

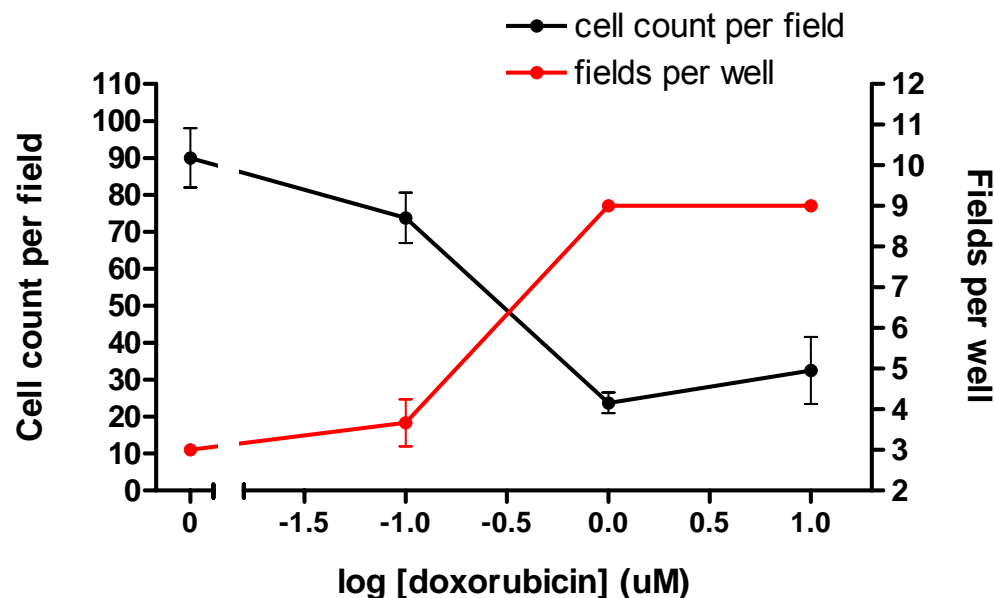
Large Camera

380 mm x 380 mm FOV

Cell count: 116

Ensuring sufficient cell count with minimum fields

On-line cell counting

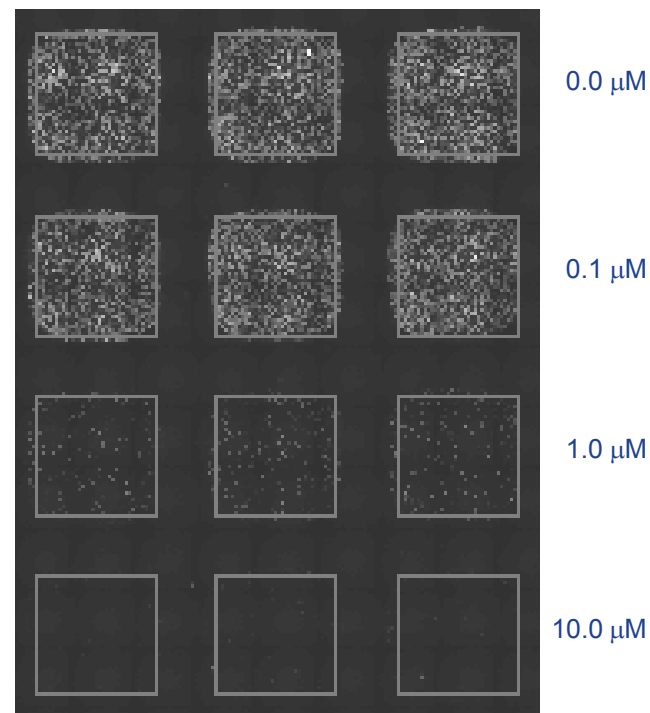


User-set threshold: 200 Cells, or 9 Fields Max, per well

Plate reads as much as 3-4 times faster with OLCC

- Saves time – minimal number of images
- Ensures sufficient cell count for every treatment
- Minimizes storage requirements – no extra images

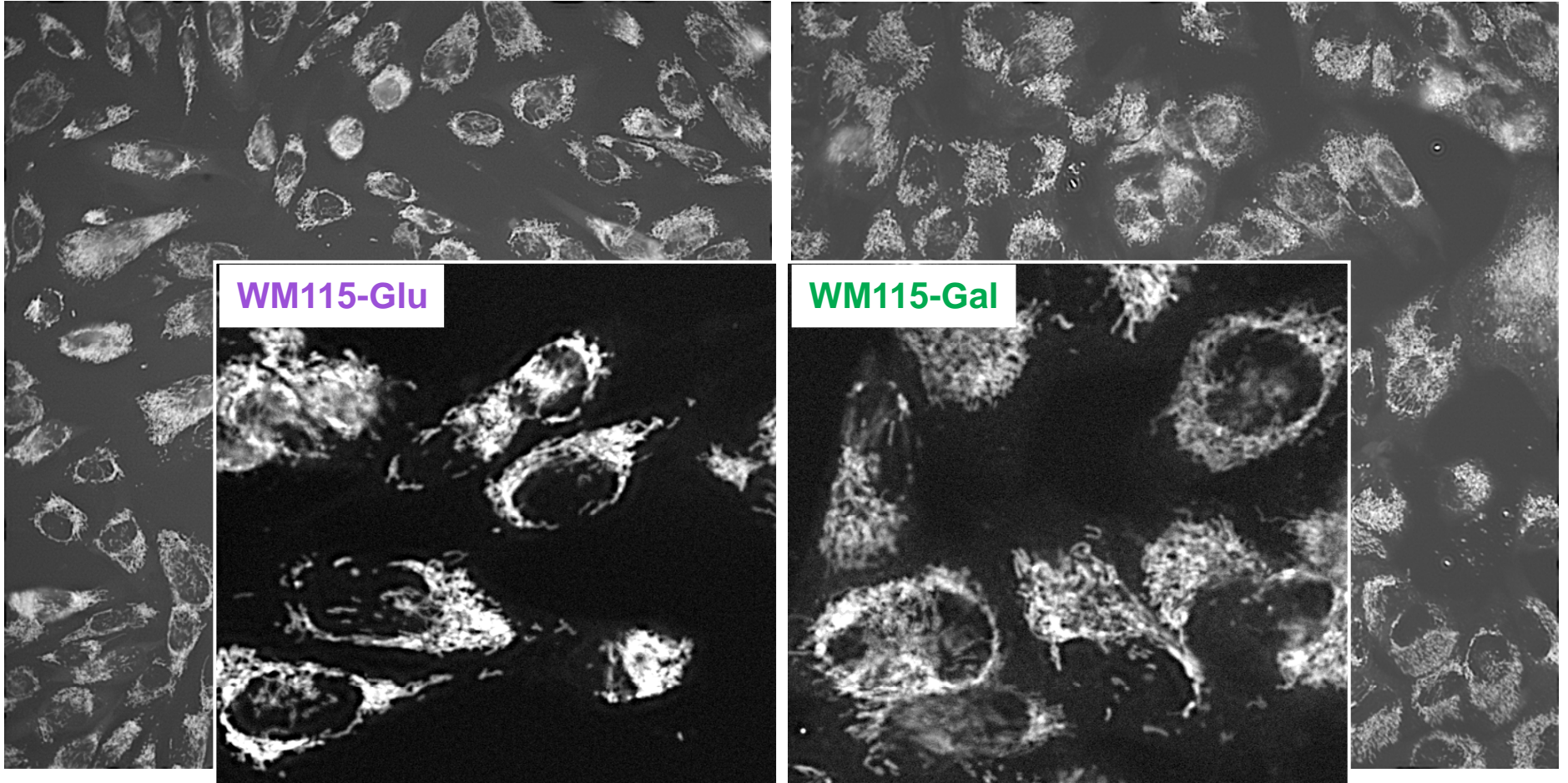
Doxorubicin Dose-Response (WM115-Glu Cells)



Preview Scan, nuclear channel

Oxidative Phosphorylation vs. Glycolysis

Distinct Multi-Parameter Phenotypes



WM115-Glu
(Glycolysis)

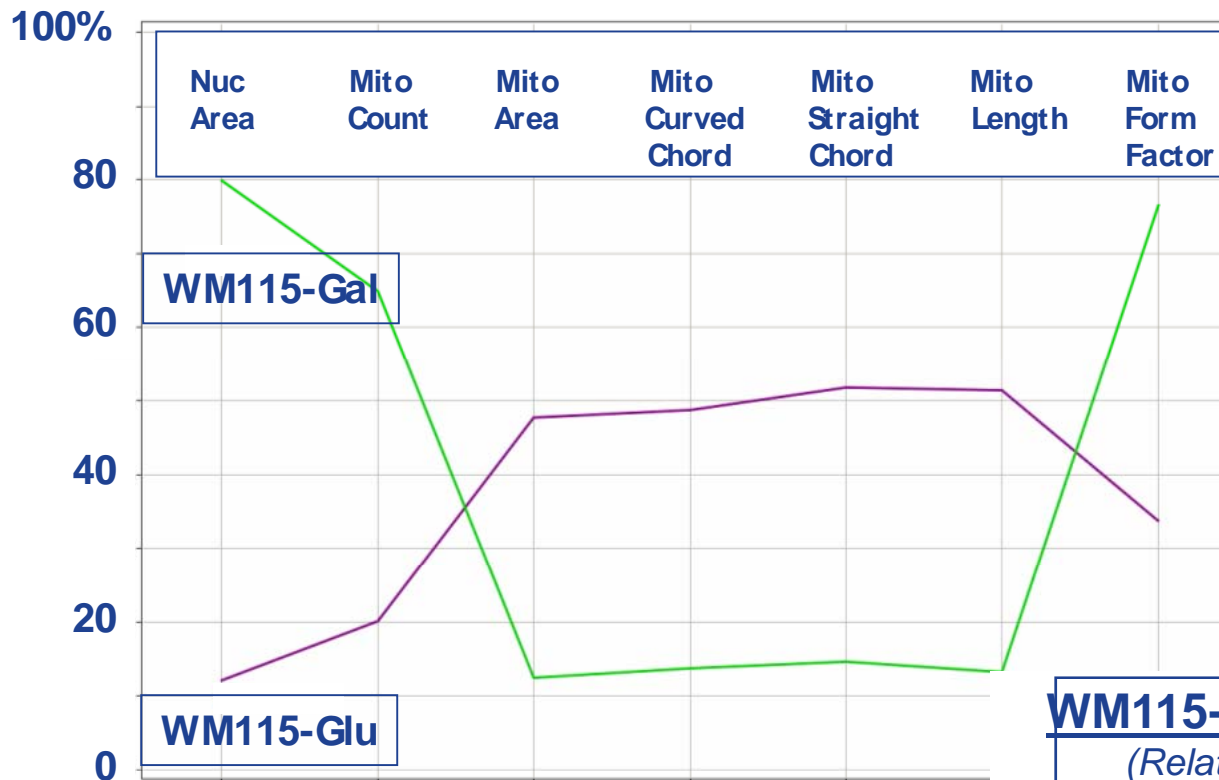
WM115-Gal
(Ox-phos)



imagination at work

Oxidative Phosphorylation vs. Glycolysis

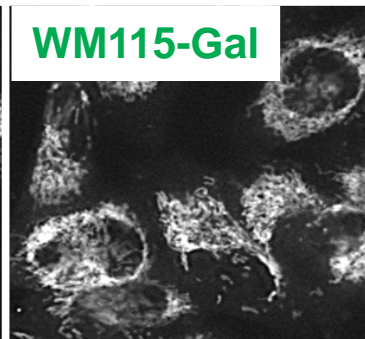
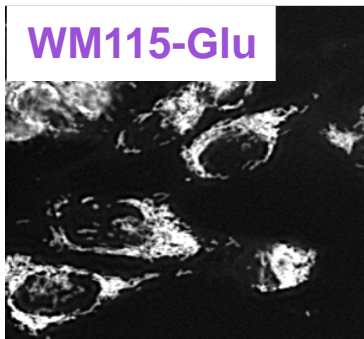
Distinct Multi-Parameter Phenotypes



WM115-Gal Phenotype – Ox-Phos

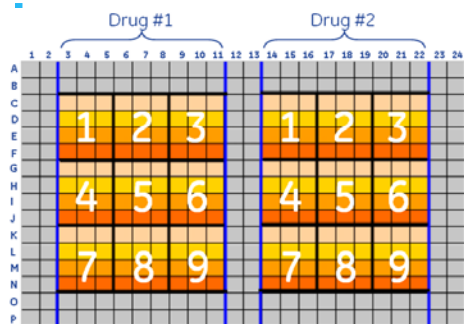
(Relative to WM115-Glu - Glycolysis)

- ↑ Nuclear Area
- ↑ Number of Mitochondria
- ↑ Mitochondrial Form Factor
- ↓ Mean mitochondrial Area
- ↓ Mean mitochondrial Length
- ↓ Chord Features



Preliminary Validation - Comparative Study

Differential Responses to Challenge with Drugs, siRNAs?



Set Up Imaging
Conditions

Image Acquisition

Data Analysis

384-Well Plates

Automated dispensing of cells, media & reagents

6 Test Compounds (2 per plate)

4 Concentrations per compound: 0, low, med, high

3 Replicates per Concentration

6 Strategically targeted siRNAs, plus scrambled
& non-transfected controls

Same regime for both WM115-Glu and WM115-Gal
lines

Manual Microscopy Mode

Preview Scan

2.5 D Imaging Mode (Extended DOF, Deconvolution)

On-Line Cell Counting

Large Camera

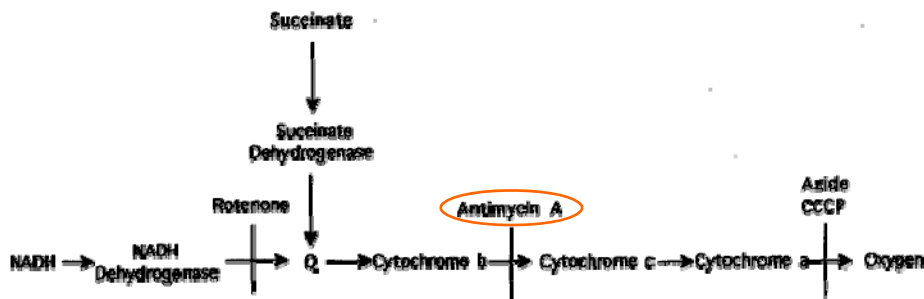
In Progress

Respiring cells are susceptible to Antimycin A

Using Preview Scan to assess samples before the run

Test Compound: Antimycin A

Acts at Respiration Complex II of the Mitochondrial Electron Transport Chain

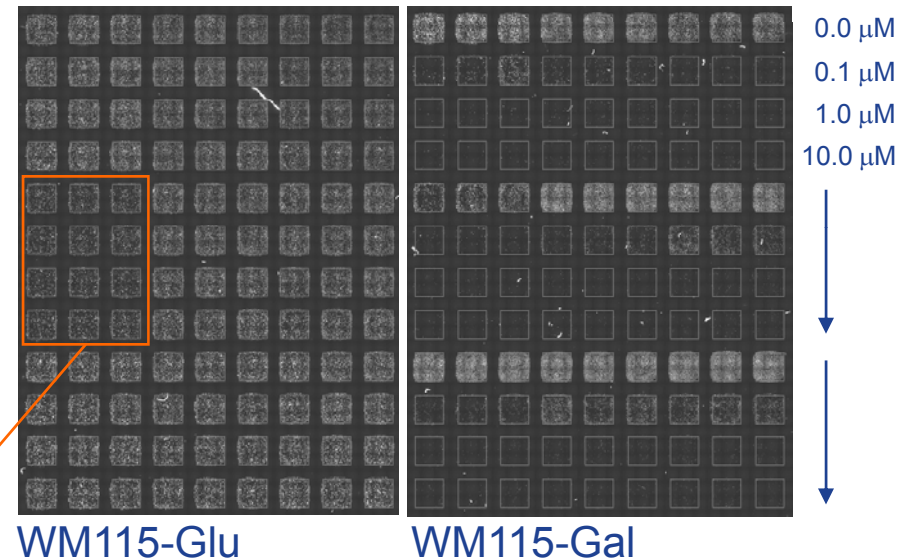


Will either cell line be susceptible?

Example knockdown effect - siABCB8

Decreased cell count observed following knockdown of mitochondrial ATP-Binding Cassette (ABC) transporter protein ABCB8 – to be investigated further

Antimycin A Treatment (Nuclear Channel)



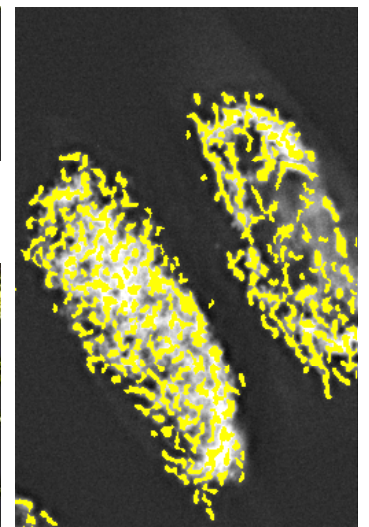
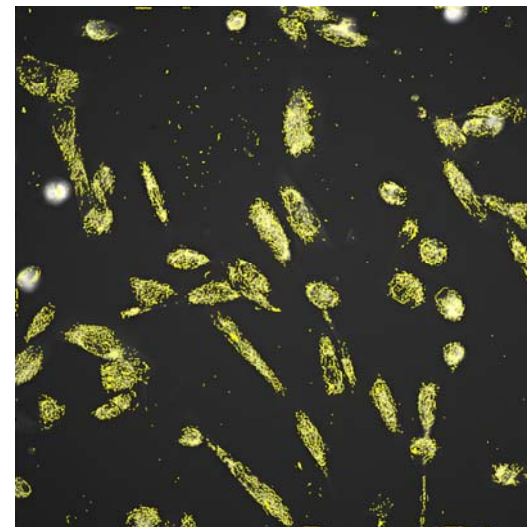
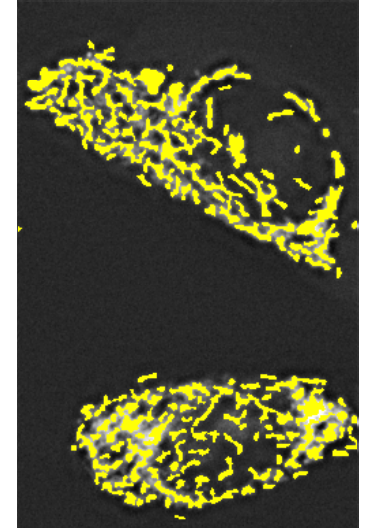
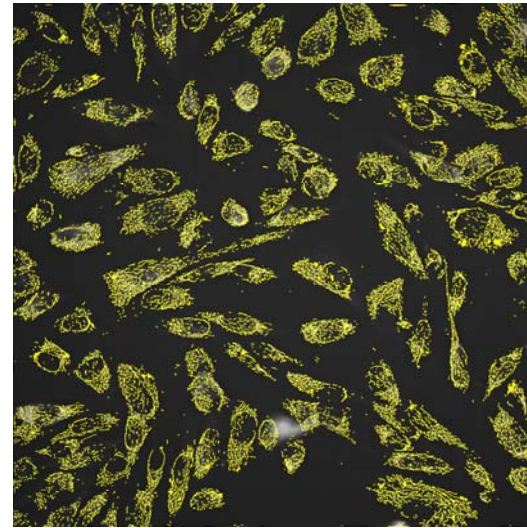
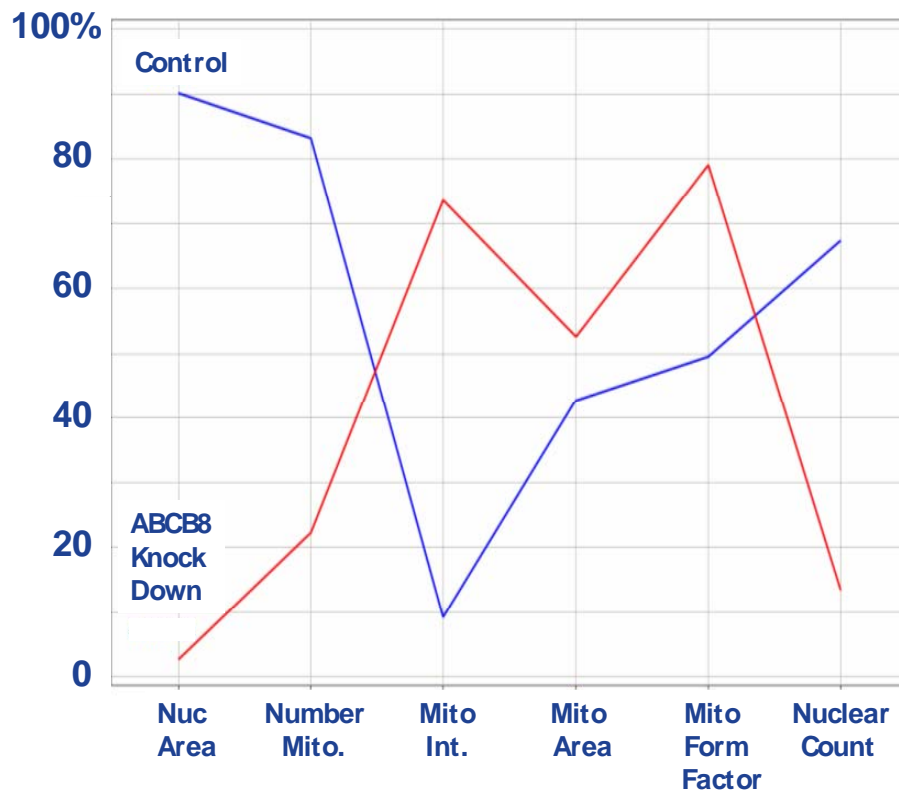
Glycolytic cells (WM115-Glu) are resistant, but Respiring cells (WM115-Gal) are **susceptible** to Antimycin A

Rapid Preview Scan – 4X binning, 10x objective
~5X Faster than full acquisition time

Characterizing the ABCB8 Knockdown Phenotype

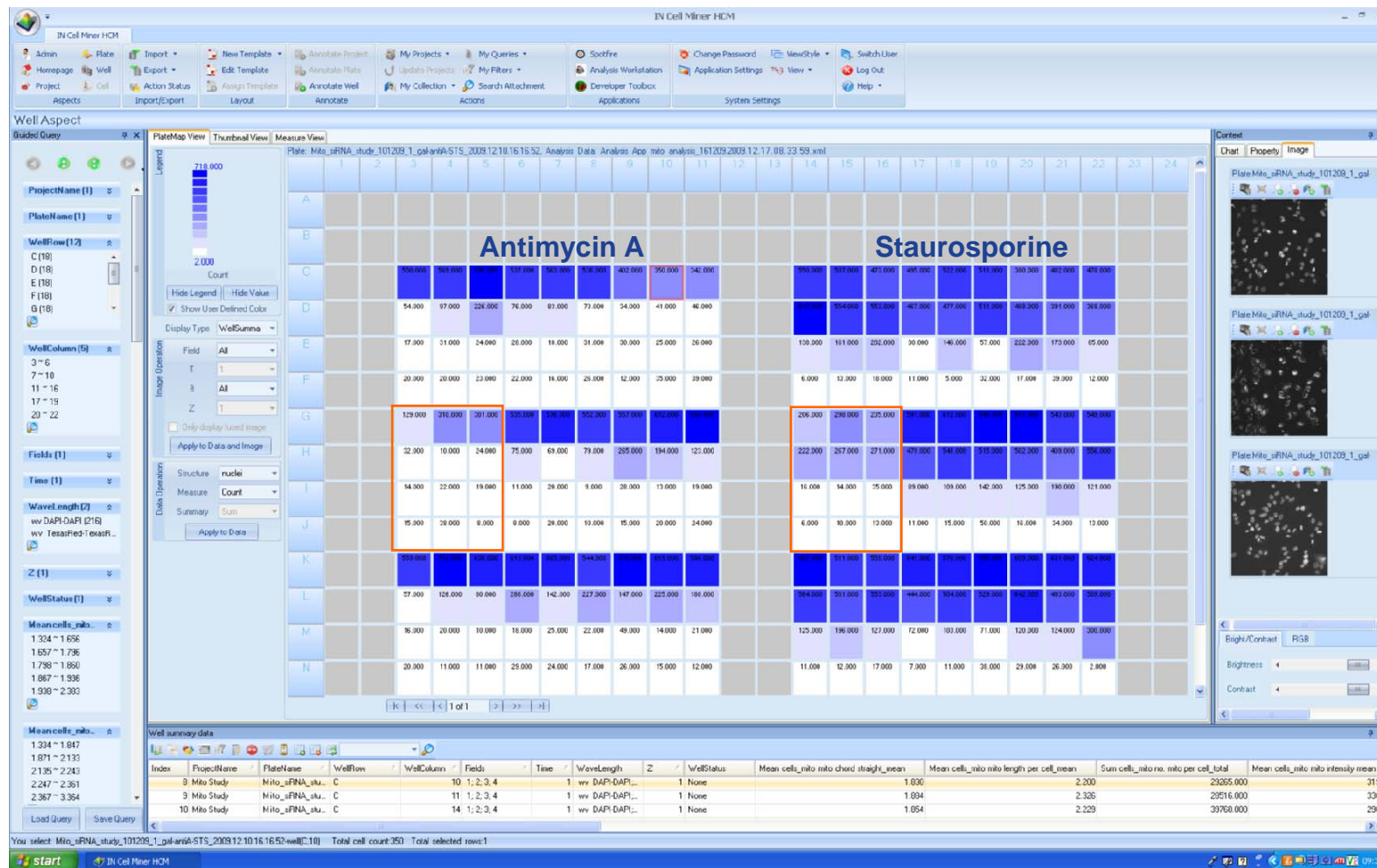
Multi-parameter profile plots reveal phenotype changes

WM115-Glu - control



Mining image and analysis results

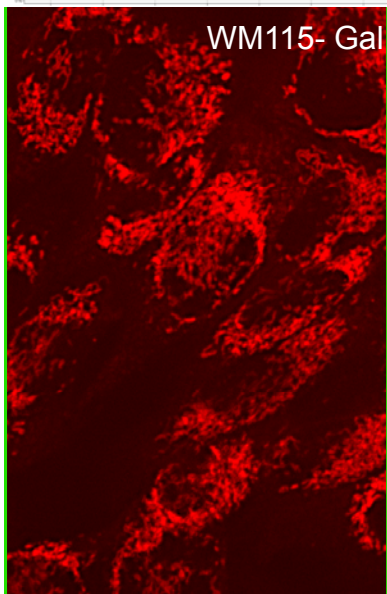
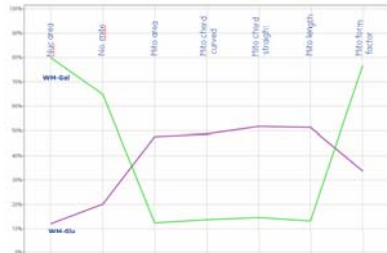
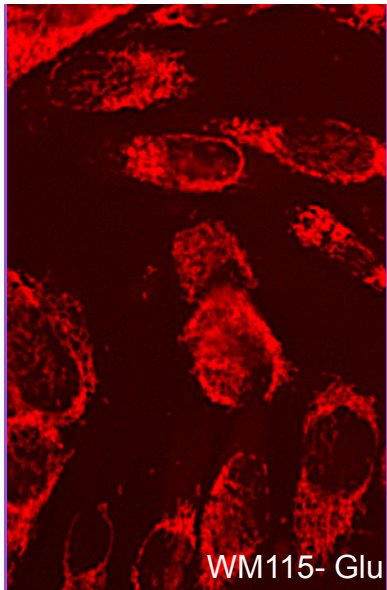
IN Cell Miner



Nuclear count heatmap for WM115-Gal cells

Highlighted area is ABCB8 knockdown

Summary



- Differentially conditioned melanoma cell lines have been developed as models for elucidating mechanisms of drug resistance
- The cell lines differ in respiration rates and phenotypic HCA profiles
- WM115-Gal cells are sensitized to the mitochondrial poison Antimycin A, consistent with their dependence on oxidative phosphorylation
- Preview scanning aided rapid confirmation of control treatment effects, and quickly flagged up potential toxicity of various test compounds and siRNAs
- Detailed multi-parametric HCA of the entire data set is underway – many intriguing observations to investigate further
- *IN Cell Analyzer 2000* ensured generation of robust, high-quality data and improved the overall workflow for the HCA study



Acknowledgements

Cath Hather

Albie Santos
Liz Richardson

Thank You

Nick Thomas

Bethan Lloyd-Lewis

Val Millar



imagination at work

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