From 2D to 3D
組織與類器官
能量代謝分析

Jack Chou 周家豪
Product Manager
Level Biotechnology

Agilent
Trusted Answers

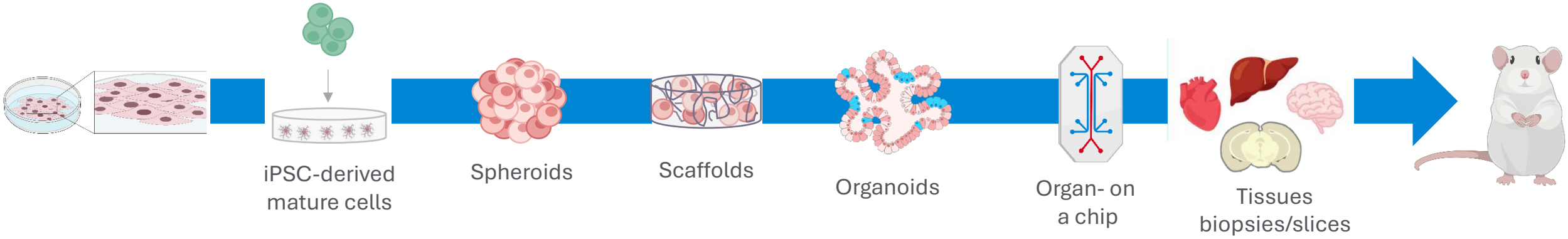
Biological Models Used in Biomedical Research

2D cell culture (monolayer)

3D cell culture

Ex vivo

In vivo



Complexity

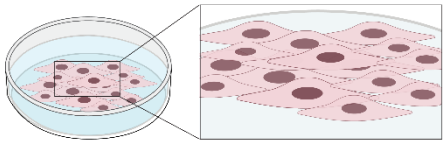
Biological Relevance

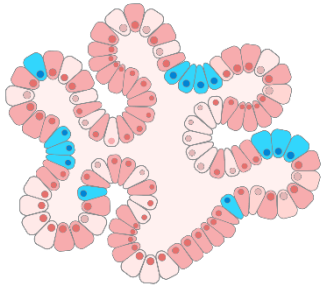
Low

High

[FDA News Release Promoting “Human-Based Lab Models”: FDA Announces Plan to Phase Out Animal Testing Requirement for Monoclonal Antibodies and Other Drugs]

2D vs 3D Cell Culture Comparison

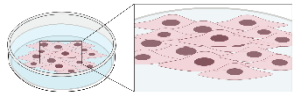
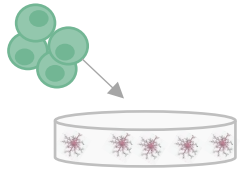
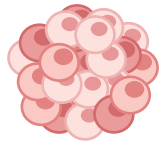
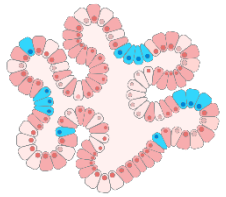
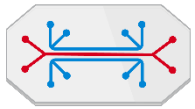




2D cell culture models	
PROS	CONS
• Simple to handle, low cost	• Limited correlation of results from 2D cultures to *in vivo*
• Easy to genetically manipulate to mimic disease models	• Limited cell-to cell or cell to ECM interactions
• Broad range of quantitative assays available	
• High reproducibility between experiments is achievable	

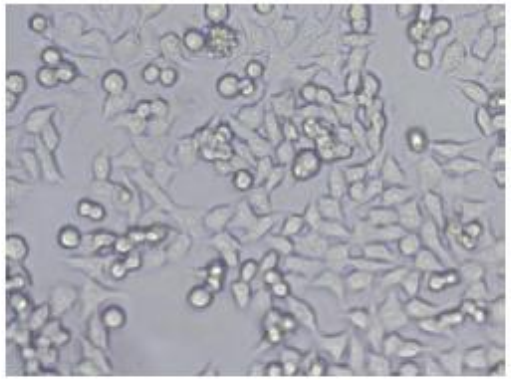
3D models	
PROS	CONS
• Recapitulate cell-cell and cell ECM interactions	• Higher cost and longer culture time
• Higher functionality and more accurate gene expression patterns	• Nutrients/drugs are harder to penetrate
• More accurate representations of gradients across structures	• Quantitative metrics harder to measure, lower reproducibility
• More reliable models for drug testing and toxicity studies	

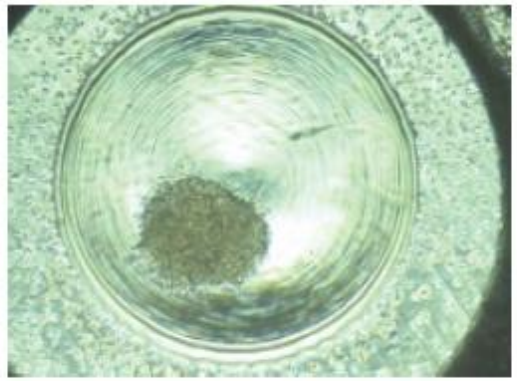
Comparison of Advanced Cell Culture Models

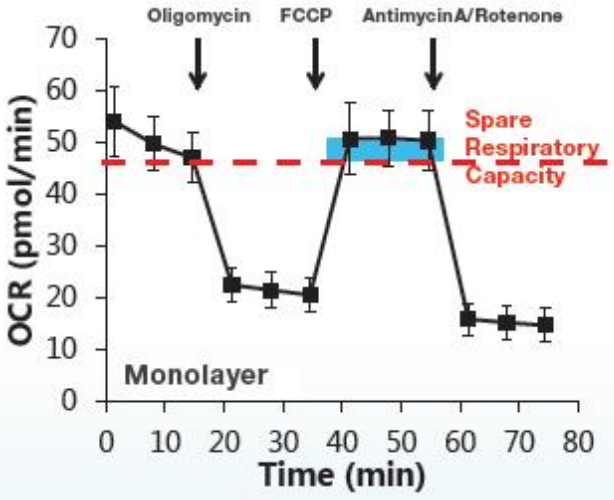
	2D standard cell culture	iPSC derived differentiated cells	Spheroids	Organoids	Organ-on-a-chip	Ex-vivo tissue
						
Culture handling	Very easy	• Days—week (cell type dependent) • Expensive culture medium/cofactors • Labor intensive	• Self-assemble into 3D structures • Relatively easy to generate and maintain	• Requires special media + scaffold • Technically challenge and time consuming	• Specialty microfluidic devices • Only some chips are designed for sample removal	• Requires access to live tissue • Limited viability ex vivo
Difficulty to genetically manipulate	Easy	Easy	Medium	Medium to difficult	Difficult	Difficult – require animal genetic manipulation
Quantitative assays available	High	High	Medium	Medium	Low	Low
Reproducibility between experiments	High	Medium	Medium	Medium (population heterogeneity)	Medium	Medium to Low
Recommended XF Plate	V7	V7 / V28	XF96 Spheroid plate	Organoid Plate	3D Capture plate	3D Capture plate

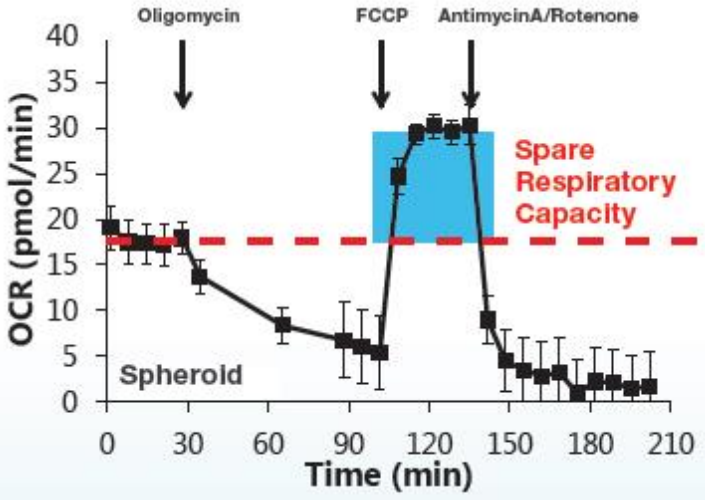
Spheroid Microplate for 3D analysis (XF Pro only)

HCT116 colon carcinoma cells









Protocol for Seahorse 3D Mito Stress assay in patient-derived pediatric brain tumor single neurospheres

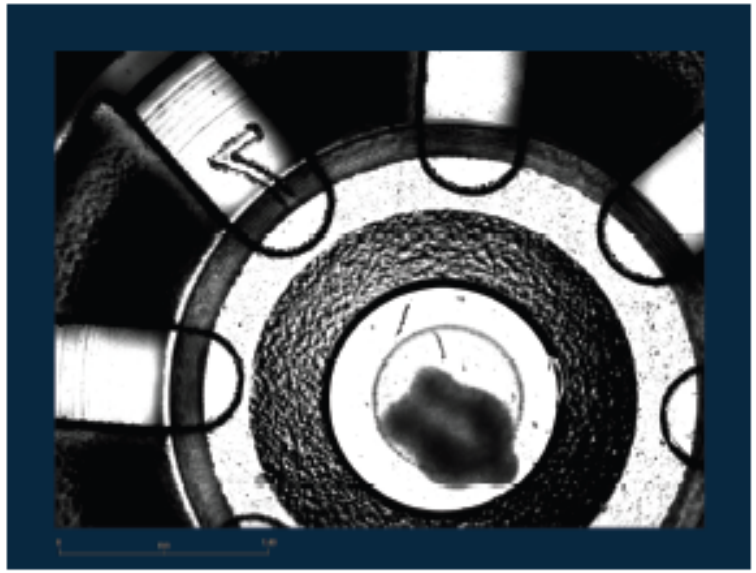
bioRxiv
THE PREPRINT SERVER FOR BIOLOGY

Stefania Tocci, Jessica W. Tsai

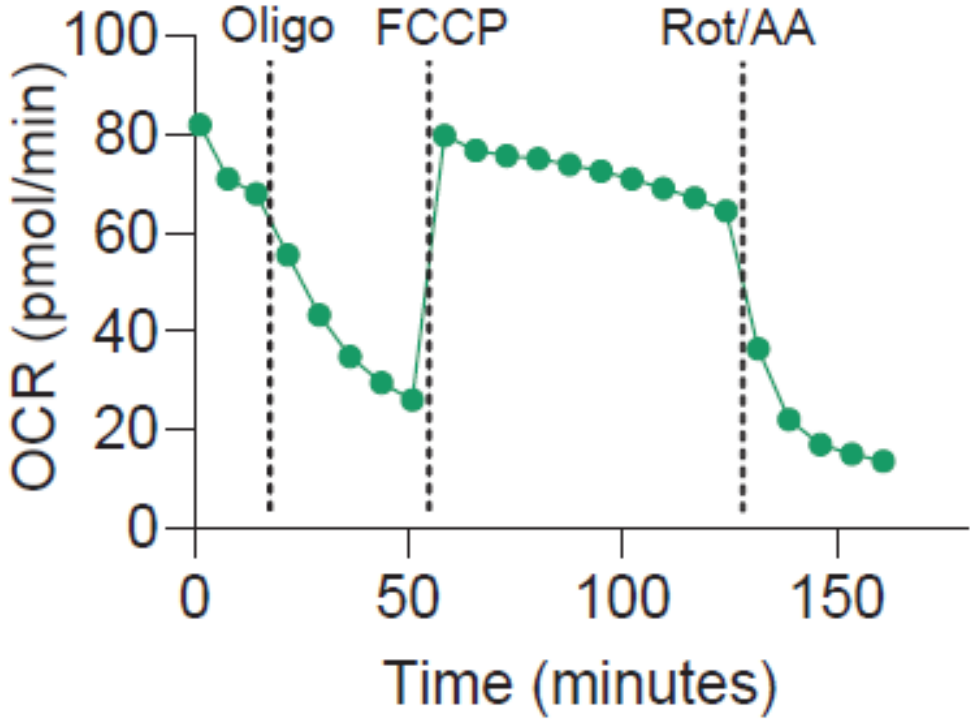
doi: https://doi.org/10.64898/2025.12.03.691986

Figure 8

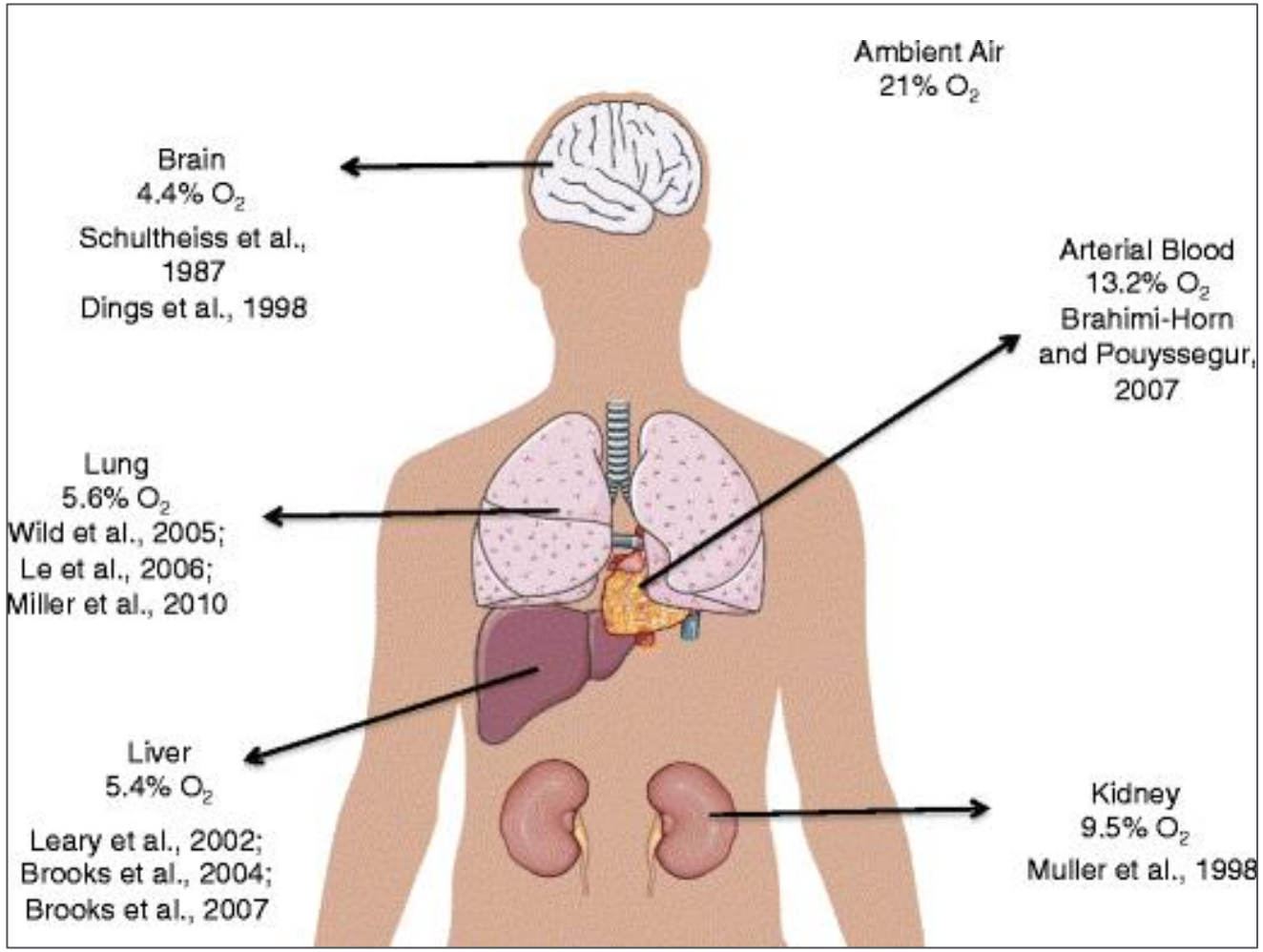
A

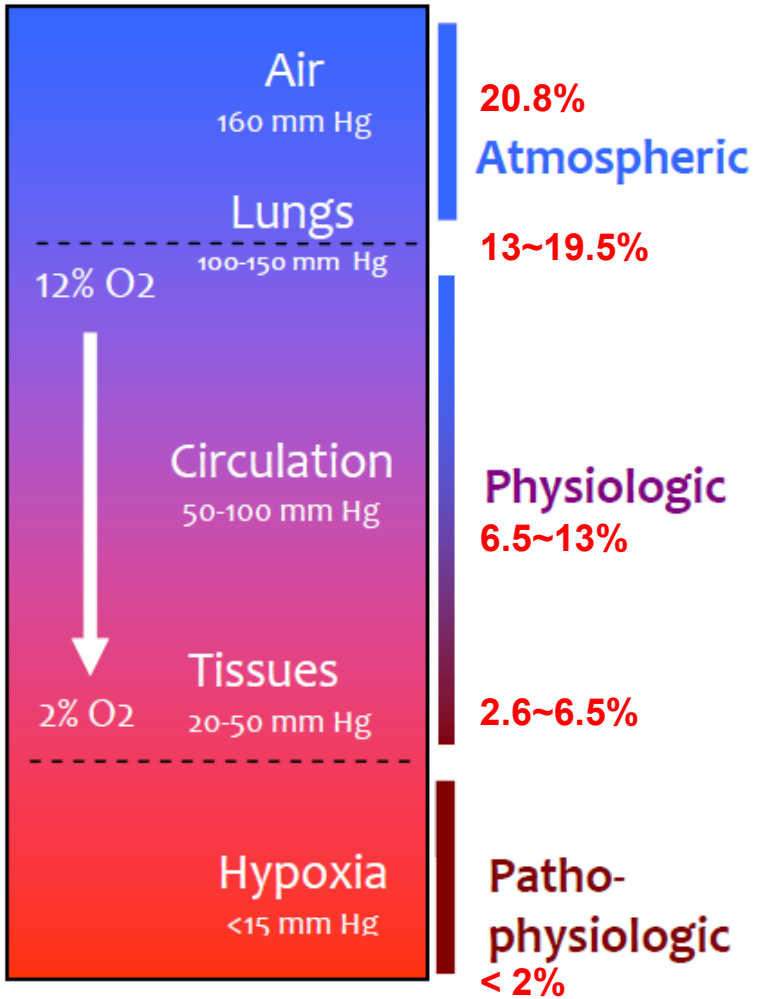


B



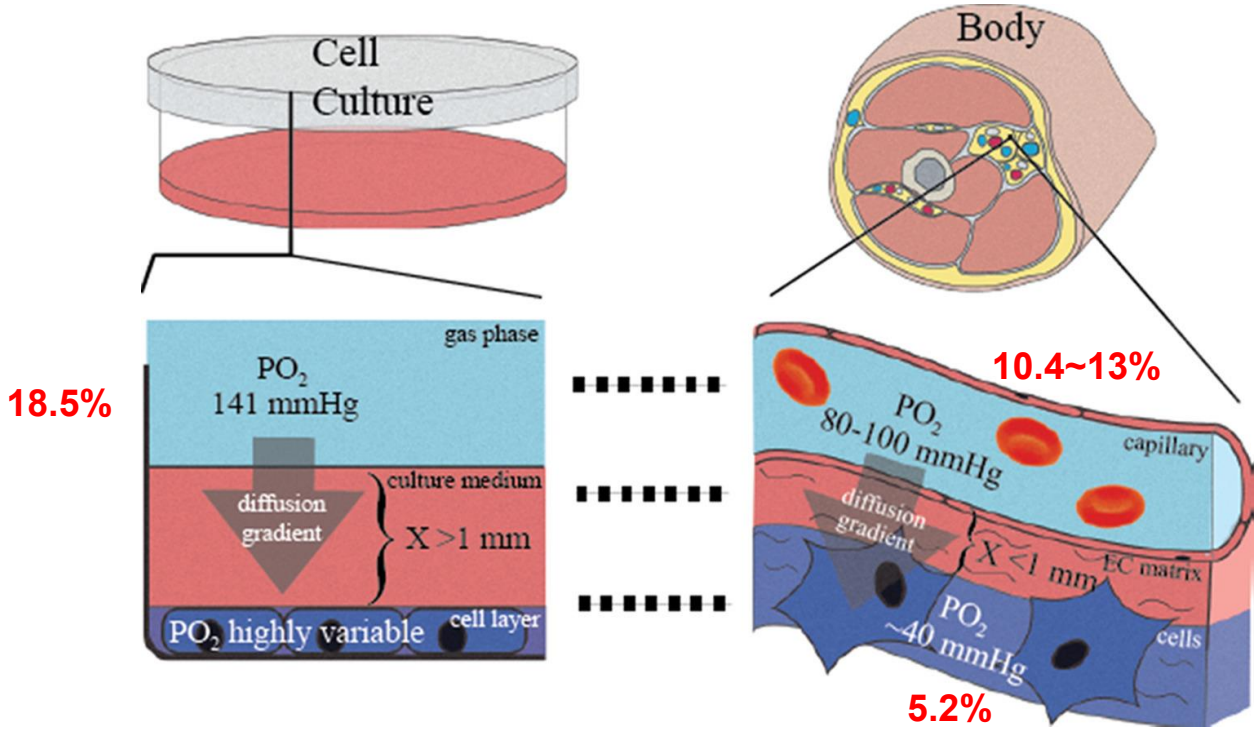
Oxygen Level of Physiology



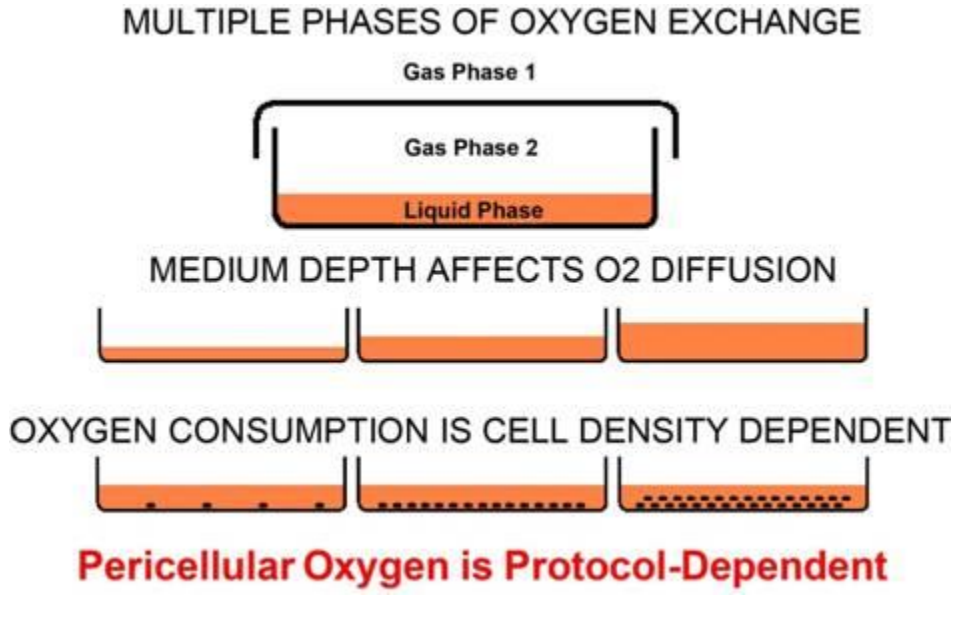


InVivo O_2 Levels.
From de Souza (2007)[8]

Comparison of Cell Culture and *in Vivo*



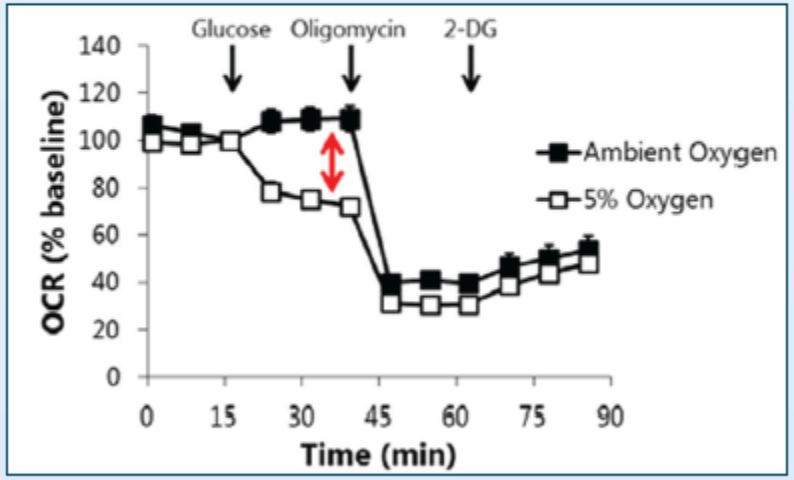
Free Radical Biology and Medicine 113 (2017) 311–322



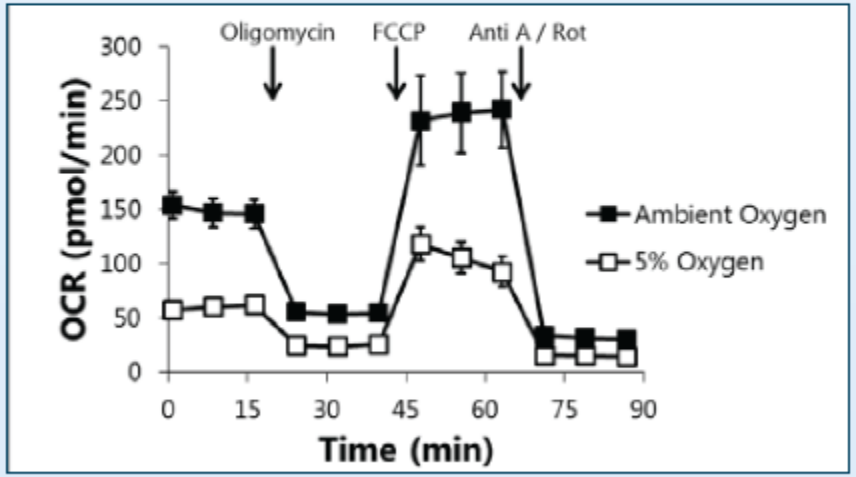
Metabolism Changed in hypoxia condition
(5% oxygen, the real *in vivo* condition)

Characterize metabolic phenotypes in hypoxia with XF Stress Tests

An XF assay conducted at 5% O_2 using the XF Glycolysis Stress Test reveals the Crabtree effect in tumor cell lines; whereas an XF assay conducted at 5% O_2 using the XF Cell Mito Stress Test reveals decreased mitochondrial function in tumor cell lines.

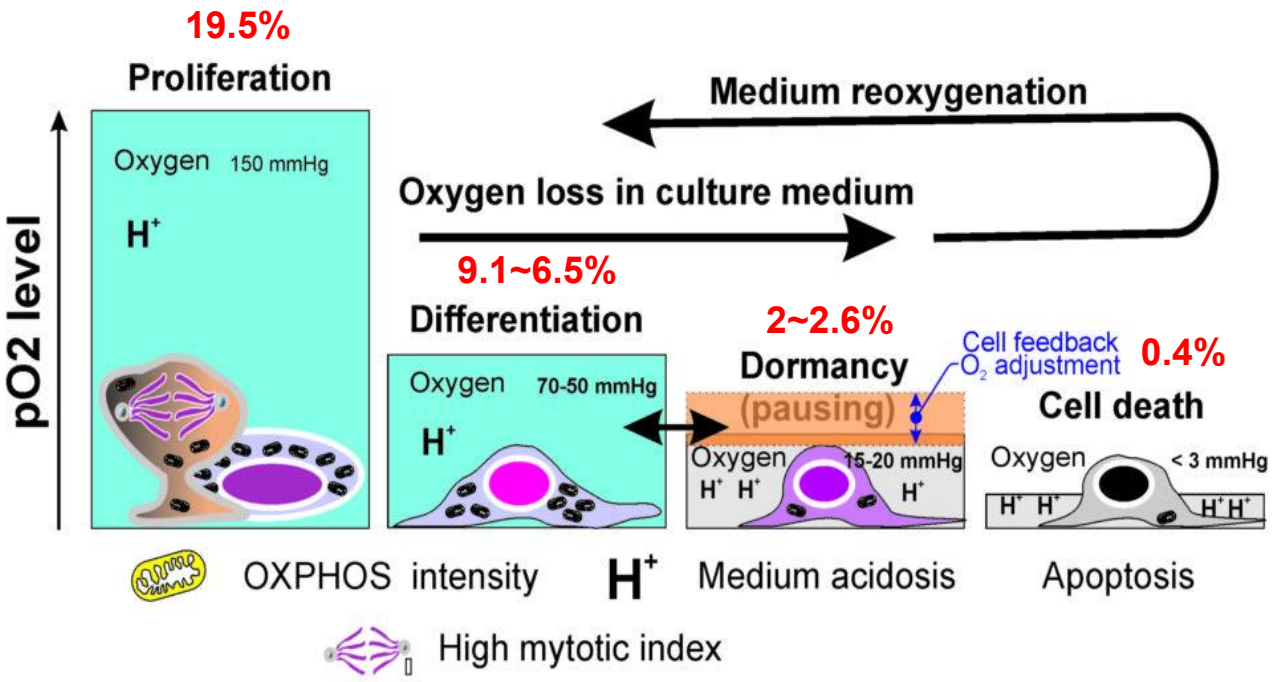


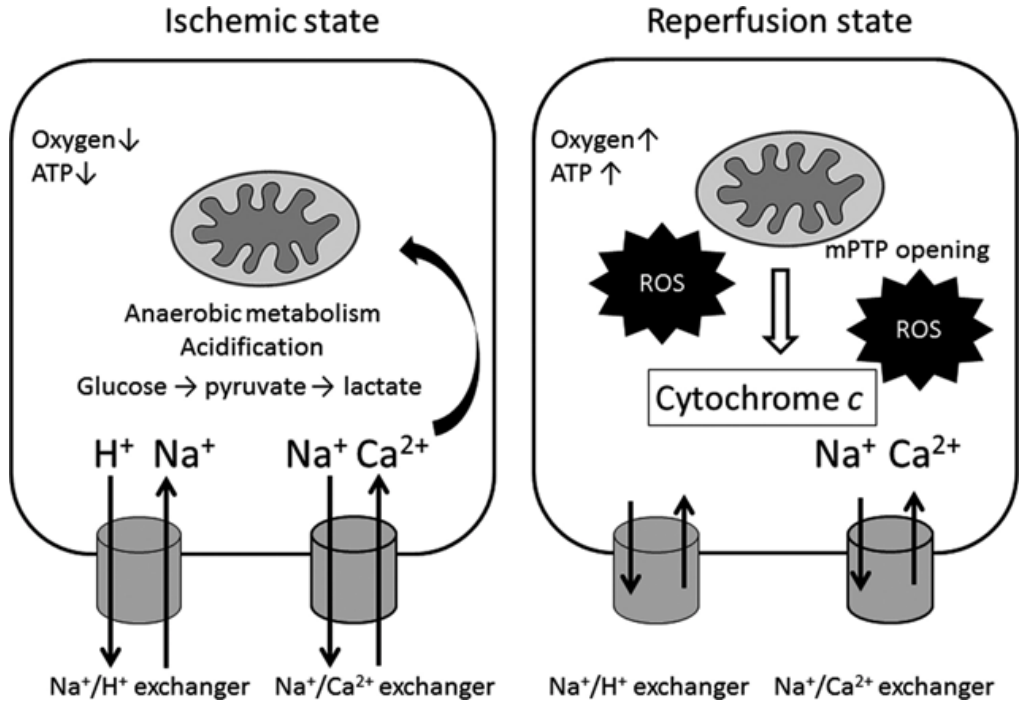
XF Glycolysis Stress Test (MCF-7 cells)



XF Cell Mito Stress Test (MCF-7 cells)

Hypoxia & Ischemia-Reperfusion



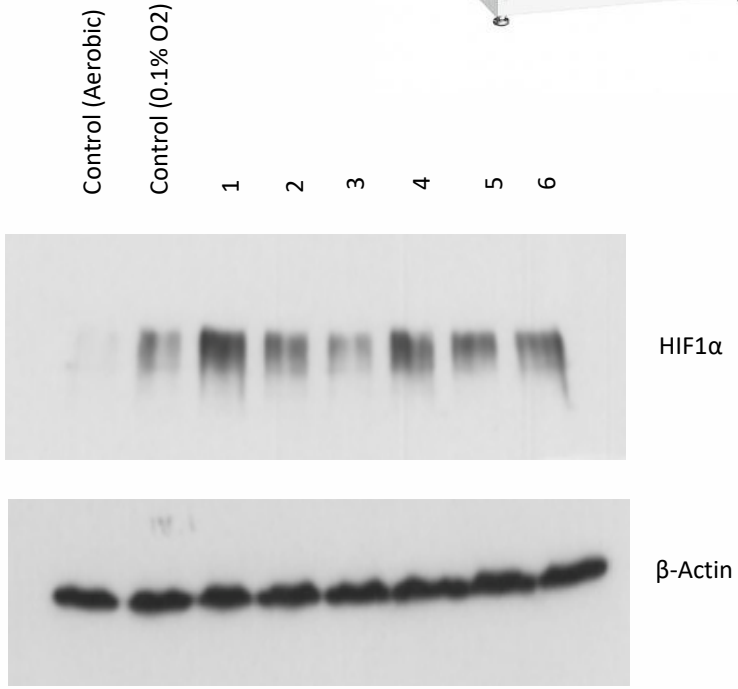


Analyze your cells in precisely controlled hypoxic conditions.

控制氧氣條件的重要性-3

那就重複實驗，
使用0.1% O_2的三氣培養箱 看HIF-1α 的表現

- HCT116 cell line 於0.1% O_2下，培養24hr後，移出培養箱，收cell lysate
- **做的更嚴謹，並且加快實驗動作**
- 透過Western blotting偵測HIF-1α



1. ***Control組的HIF-1α出現，***
2. ***其他實驗組也能觀察到HIF-1α表現***

但是....Bands 呈現smear...且強弱不一

不論動作多快，
系統中的任何氧氣都會導致 HIF-α 降解 !!!!

Data from Roger M Phillips
Professor of Cancer Pharmacology
University of Huddersfield

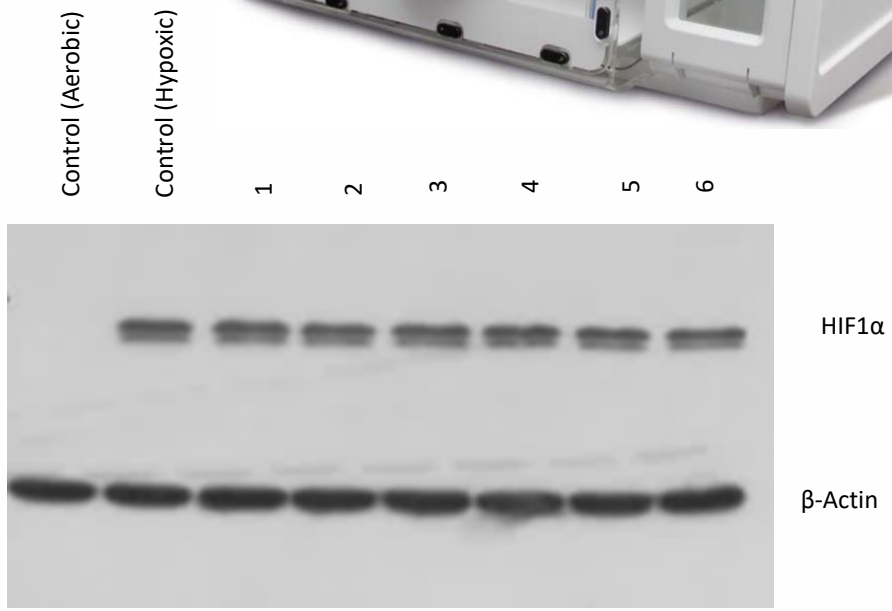
控制氧氣條件的重要性-4

實驗皆在H35 Hypoyxstation低氧工作站進行

HCT116 cell line 於0.1% O_2下，

培養24hr後在工作站內收cell lysate

- **過程皆無暴露在常氧環境中**



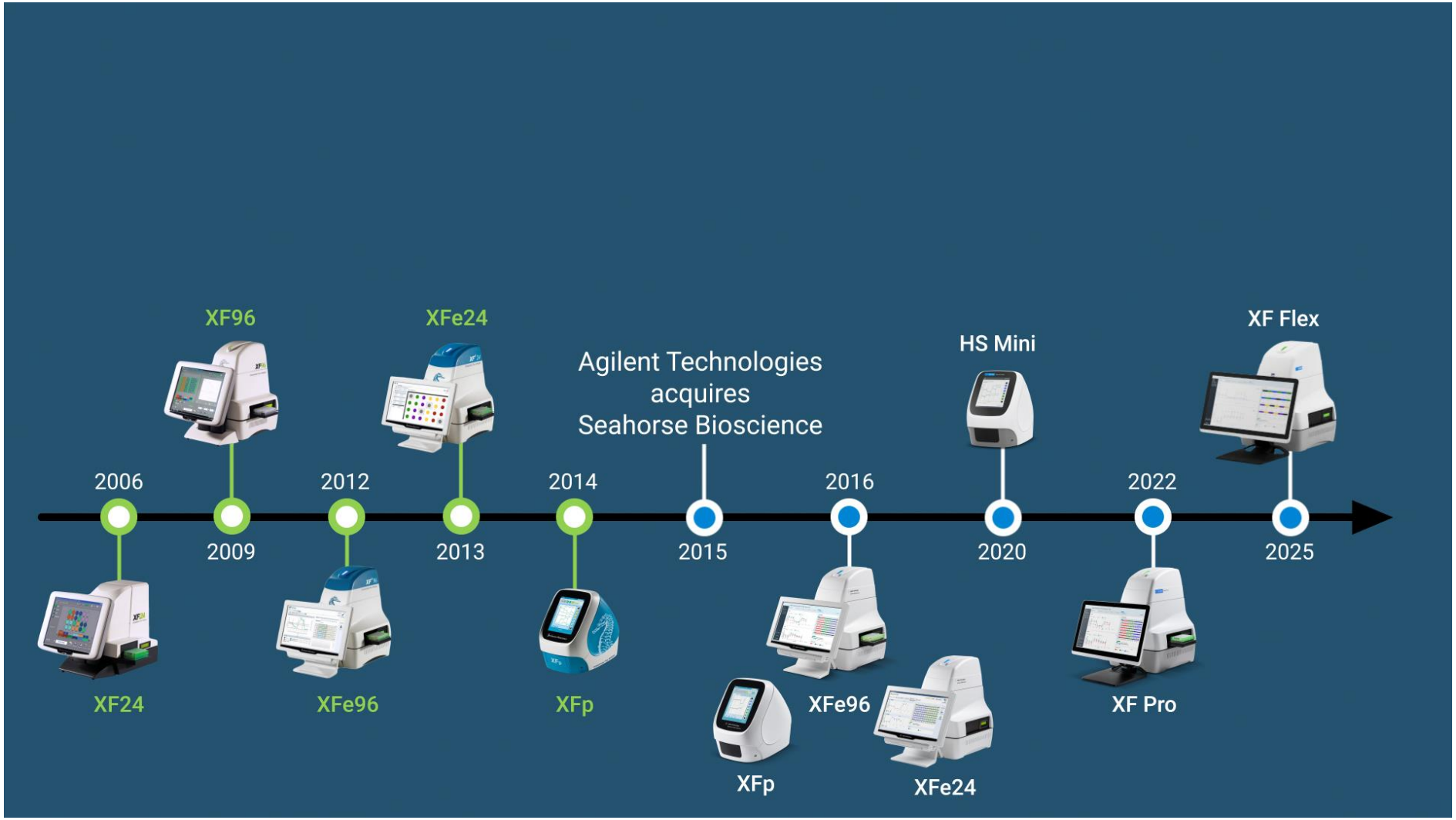
1. *Control組的HIF-1α 條帶清晰，*
2. *其他實驗組HIF-1α表現也穩定*

實驗中的任何氧氣都影響結果！！！！

甚至需要思考medium中的氧氣含量。

Data from Roger M Phillips
Professor of Cancer Pharmacology
University of Huddersfield

Seahorse XF Analyzers: 20 Years Enabling Metabolic Measurements

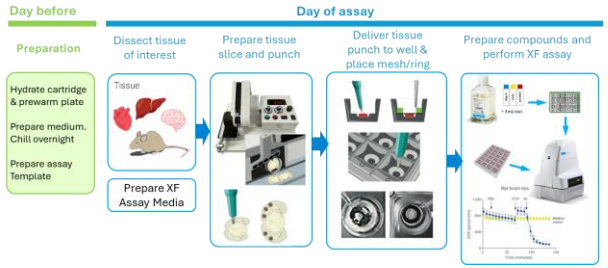


New Product Introduction

The Agilent Seahorse XF Flex analyzer, consumables and 3D workflow

New instrument/software

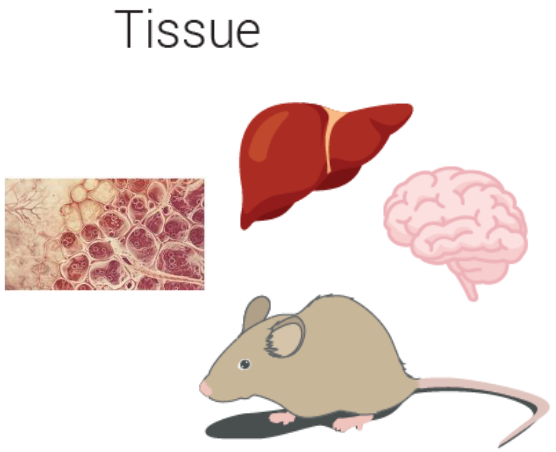
New 3D consumables



New 3D workflows

3D Models That Can Be Used With the 3D Capture Microplate-L

Tested Models



- Liver

Tech Overview: Improved Assay Workflow to Perform Metabolic Measurements with Live Tissue Samples

- Brain

App Note: A Superior System for Real-Time Metabolic Analysis with Brain Tissue and Other 3D Models

- Adipose

(beta tester)

- Tissue grown on a chip

(beta tester)

Other Potential Models

- C. Elegans
- Multiple spheroids
- Zebra fish embryos
- Organ-on-chip
- 3D printed tissue

* Be sure to consider sample chamber size when testing.

Optimized Seahorse XF Flex 3D Tissue Workflow

Day before

Preparation

Hydrate cartridge and prewarm plate

Prepare medium
Chill overnight

Prepare assay Template

Day of assay

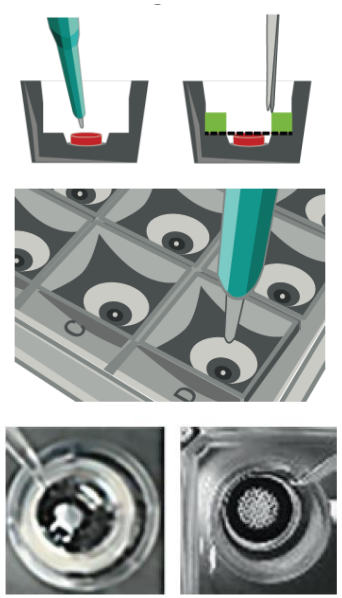
Dissect tissue of interest

Tissue

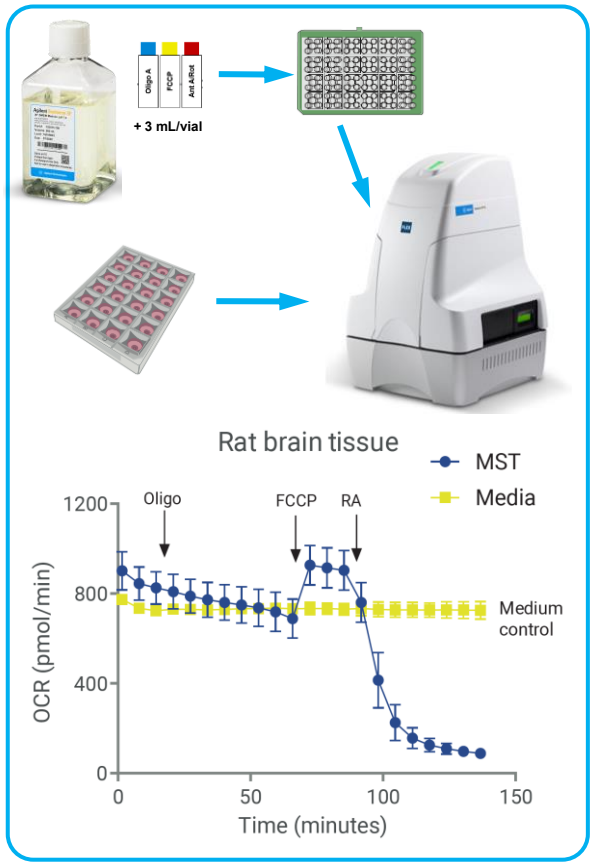
Prepare Seahorse XF assay media

Prepare tissue slice and punch

Deliver tissue punch to well and place mesh/ring



Prepare compounds and perform Seahorse XF assay

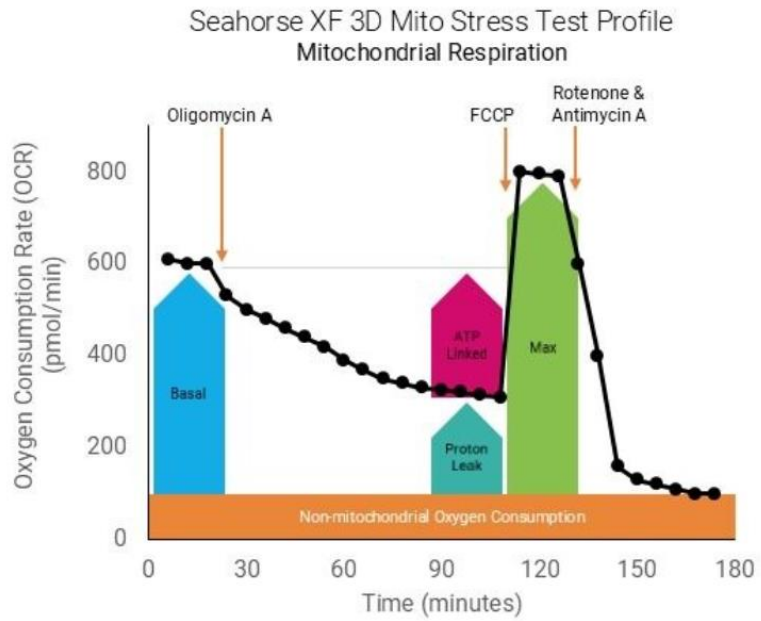


- ✓ Better sensitivity to ensure success
- ✓ Easy to get started for beginners
- ✓ Superior support from Agilent

Optimized 3D Tissue Workflow

Dedicated Consumables

XF 3D Mito Stress Test Kit



	XF Cell MST Kit	XF 3D MST Kit
	Low concentration	High concentration
Oligomycin	63 nmol	720 nmol
FCCP	72 nmol	540 nmol
Rotenone/Antimycin A	27 nmol	300 nmol
	Oligomycin mix	Oligomycin A only
	Needs further dilution (additional plastic tubes needed)	Ready to use
	Rubber top small vials	Screw cap 5 mL glass vials
	Tool needed to open vials	No tool needed
	Different resuspension volume	Same resuspension volume
	Different port loading volume	Same port loading volume

- ✓ Solutions tailored for 3D models
- ✓ Large vials and screw cap for simplified preparation steps
- ✓ Optimized protocol to minimize user errors

XF Flex 3D Capture Microplate-L vs XF24 Islet Capture Microplate

Parameter	Flex 3D Capture Microplate-L	XF24 Islet Capture Microplate
Ring	Black PET, easy to see and orient	Clear Polycarbonate
Mesh	285 µm PES	170 µm Nylon
Microchamber	8.5 µL	17 µL
Sample Chamber	250 µm × 3.15 mm	250 µm × 3.15 mm
Recommended Protocol	3 min mix-0 min wait-3 min measure Improved mixing and probe position parameters (automatically set by barcode)	3 min mix-2 min wait-3 min measure
Recommended Theoretical Rate Range	OCR 100-1500 pmol/min ECAR 15-267 mpH/min	None specified
Compatible Analyzer	XF Flex	XFe24

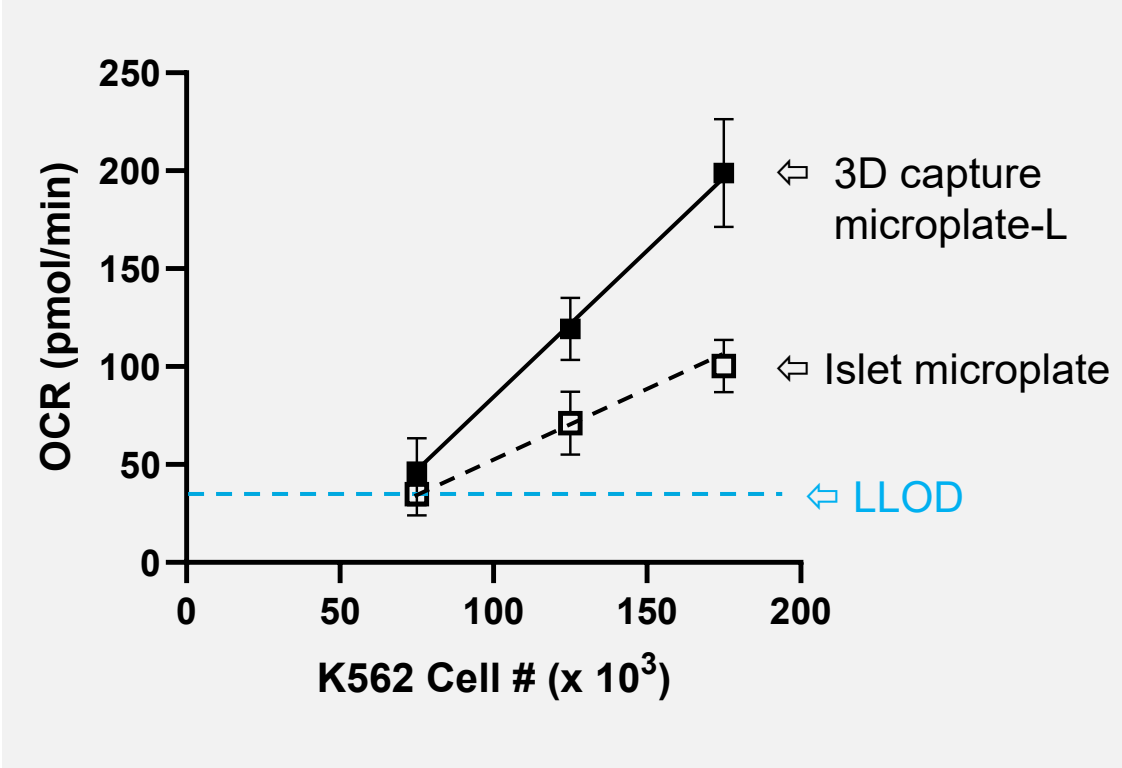
XF Flex 3D Capture-L

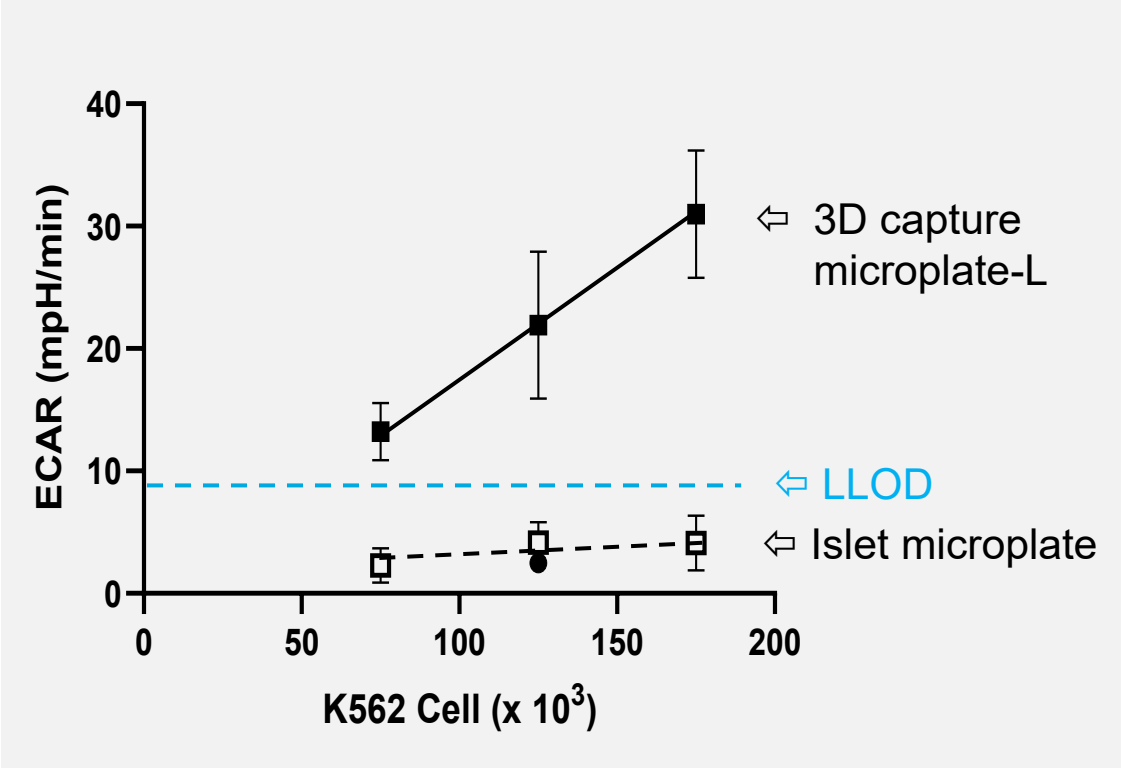
XF24 Islet Capture

Dedicated Consumables

Seahorse XF Flex 3D Capture Microplate-L

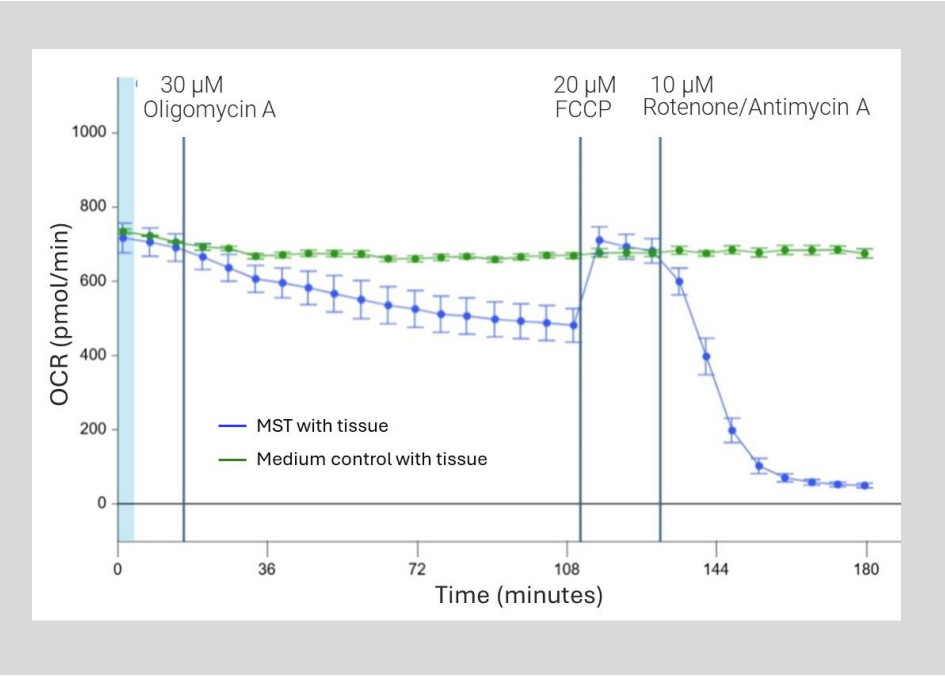
Demonstration of improved sensitivity



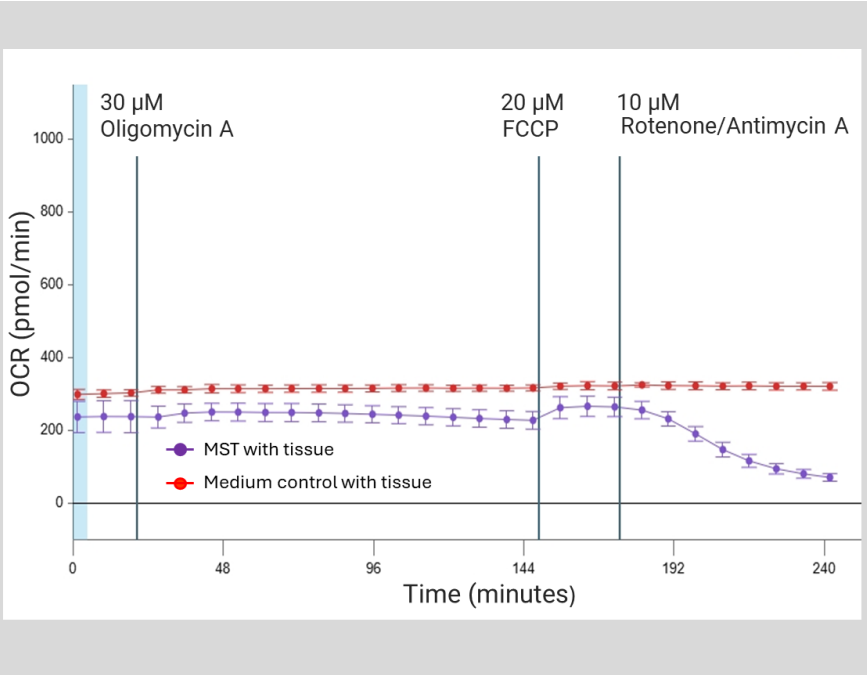


3D Mito Stress Test with the XF Flex 3D Tissue Workflow

XF Flex analyzer + Flex 3D capture microplate-L

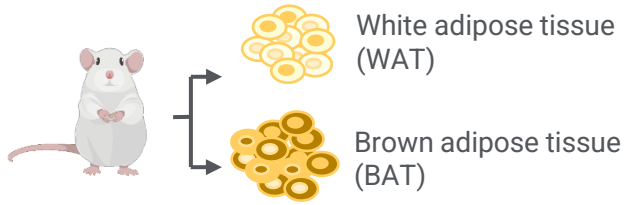


XFe24 analyzer + Islet microplate does not provide sufficient sensitivity.

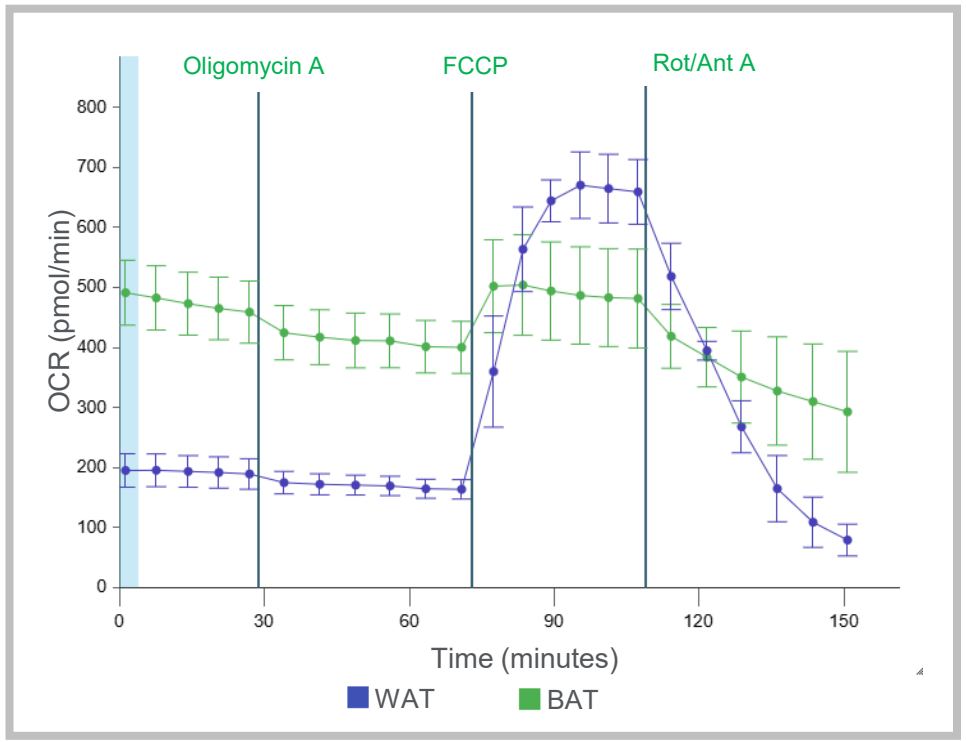


Example

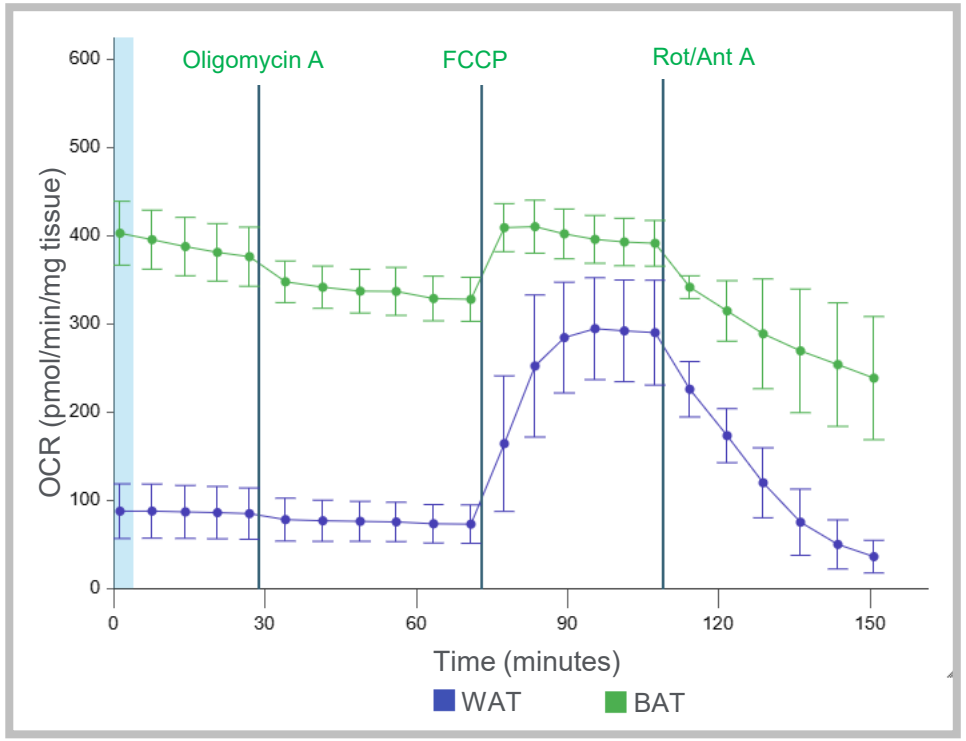
Mitochondrial function of white adipose and brown adipose tissue



Non-normalized



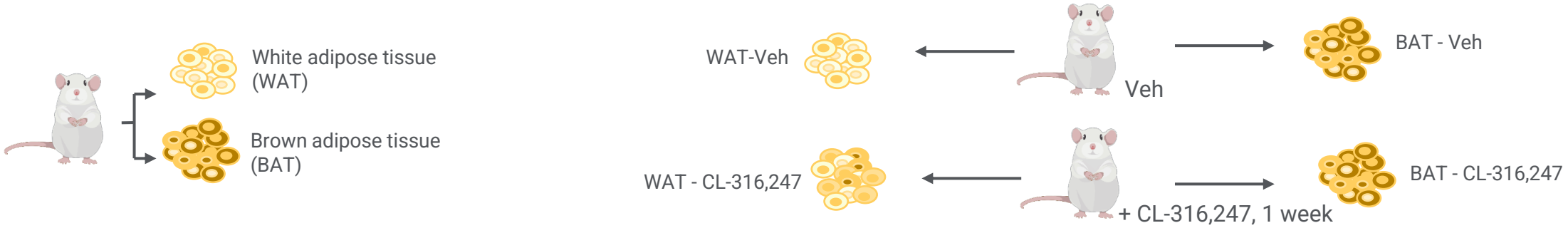
Normalized by weight

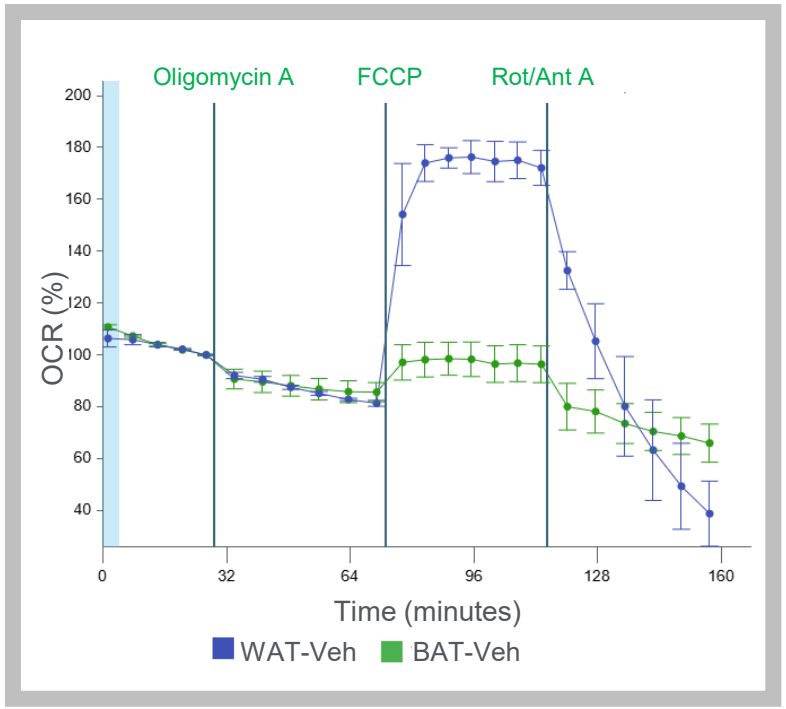


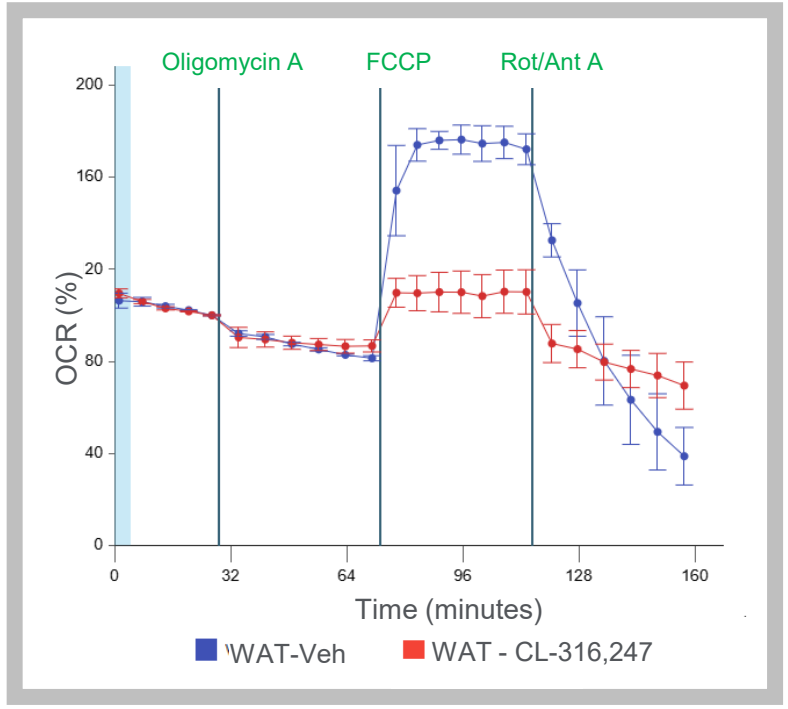
Dr. Yu (Aaron) An Lab – UT Health Science Center at Houston

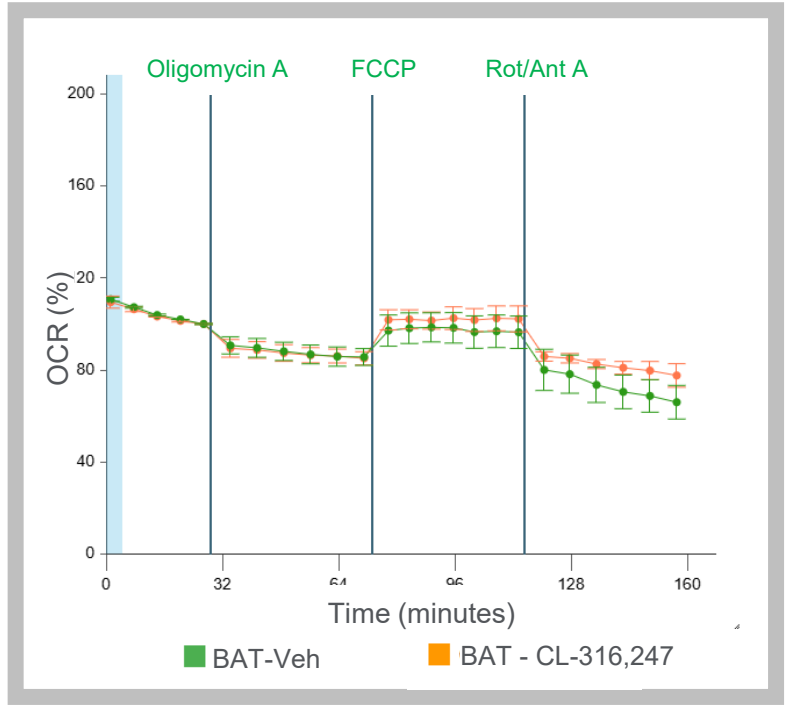
Example

CL-316,247 induces "brown-like" mitochondrial phenotype in WAT





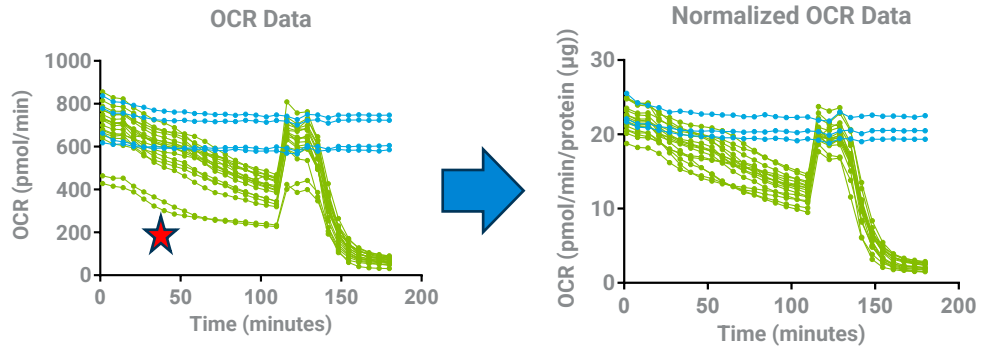




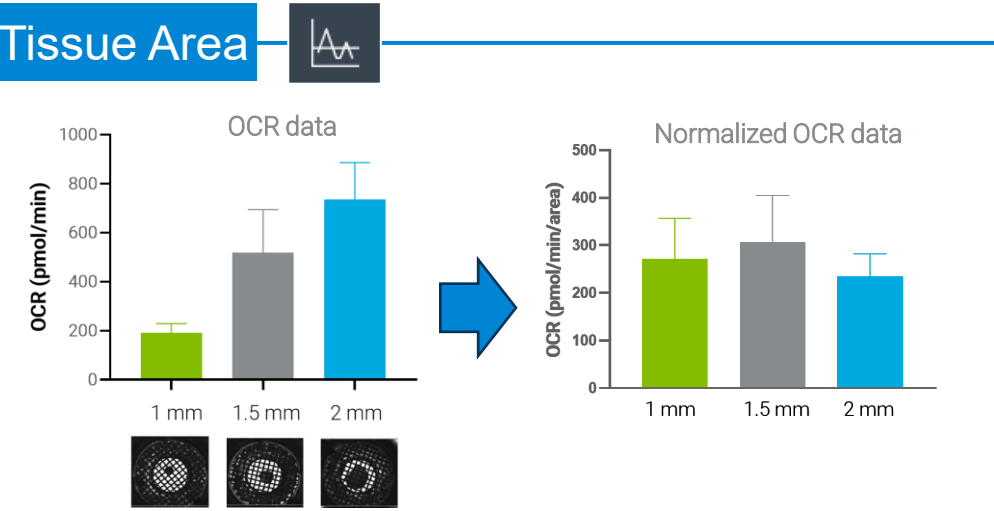
Dr. Yu (Aaron) An Lab – UT Health Science Center at Houston

Normalization Strategies for 3D Tissue Assays

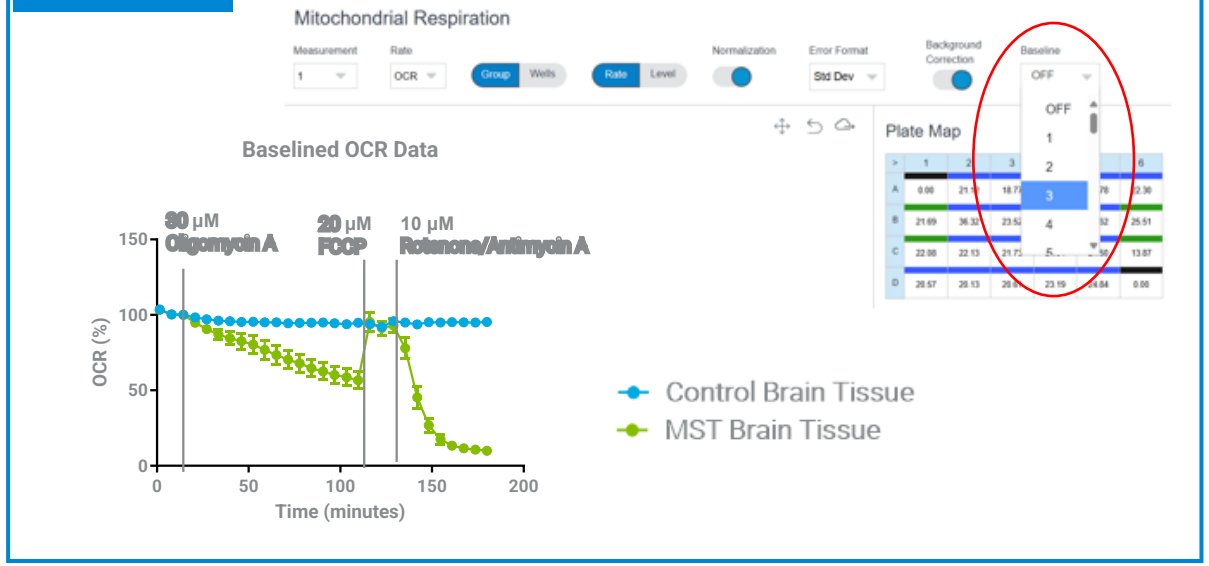
Protein Assay



Tissue Area



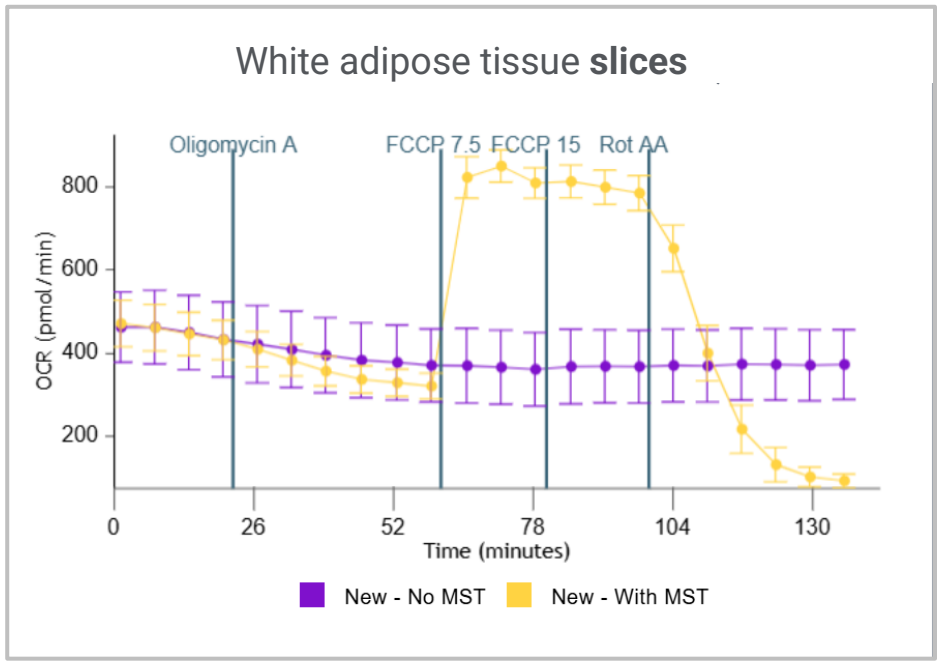
Baseline



- ✓ Several normalization options available depending on tissue type and study objective (protein, size, weight, baselining, DNA, etc.)

Customer Experience at Demos and Trainings

Adipose tissue slices (US institution)



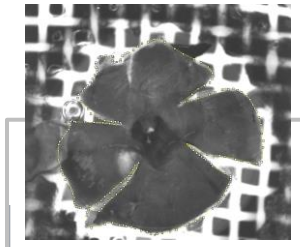
- ✓ Robust response to FCCP and Rot/AA
- ✓ Very high SRC
- ✓ A small but clear response to Oligomycin
- ✓ Variability is low – due to optimized tissue placement technique

Customer comments:

"Wow. The data is amazing. The error bars are smaller than my 2D culture assays."

Customer Experience at Demos and Trainings

Retinal tissue (US institution)

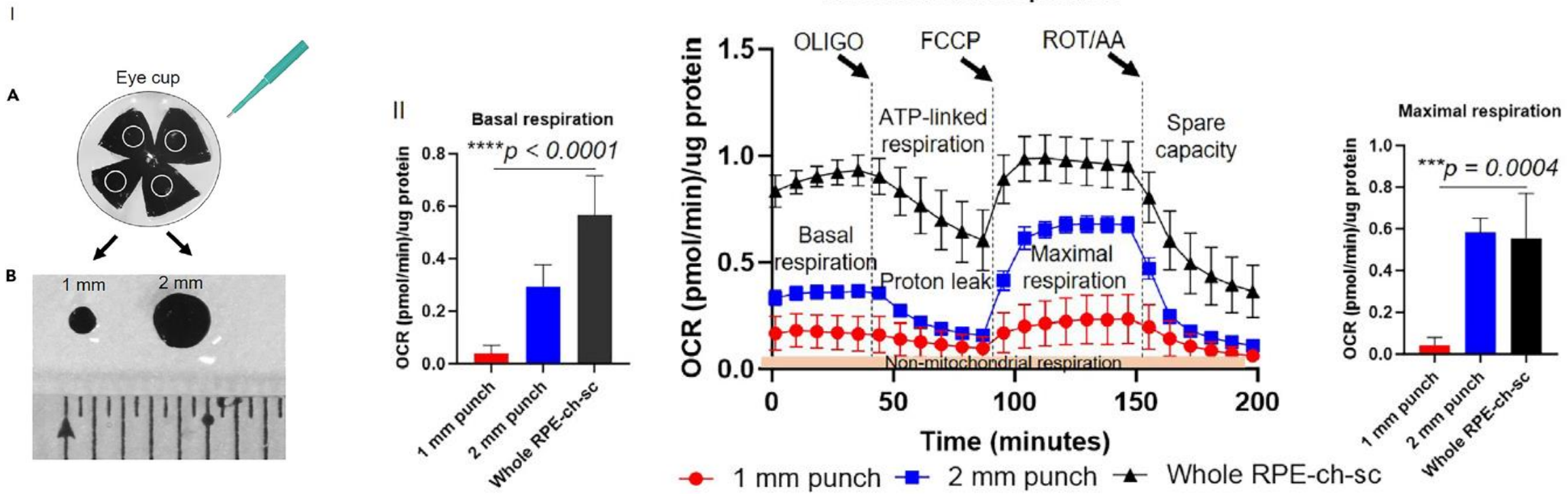


- ✓ Robust response to all modulators
- ✓ Decent SRC
- ✓ Variability is low – due to optimized tissue placement technique

STAR Protocols

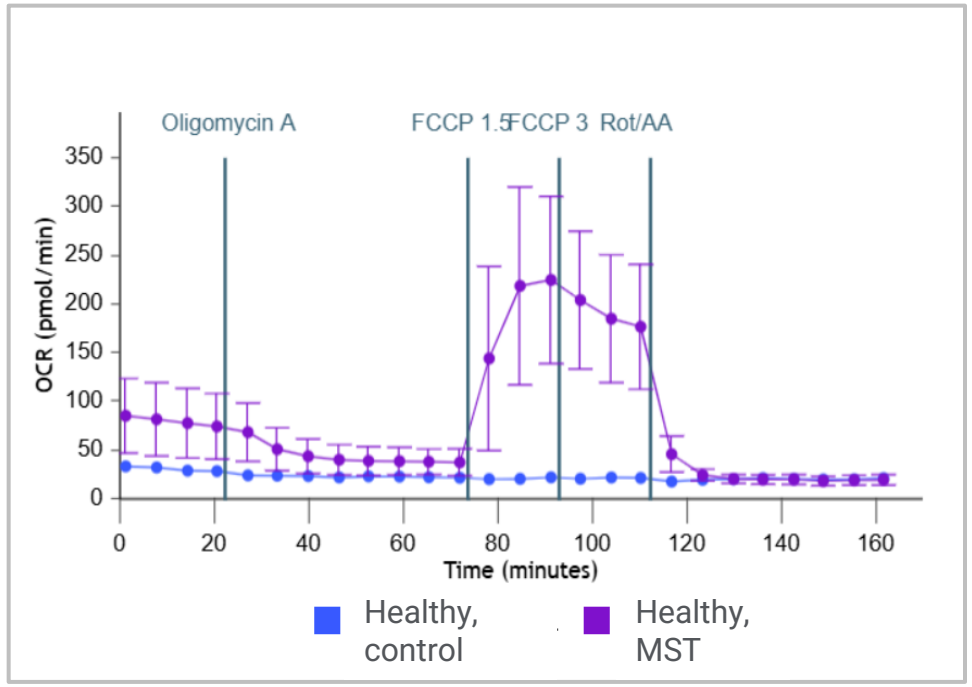
CellPress
OPEN ACCESS

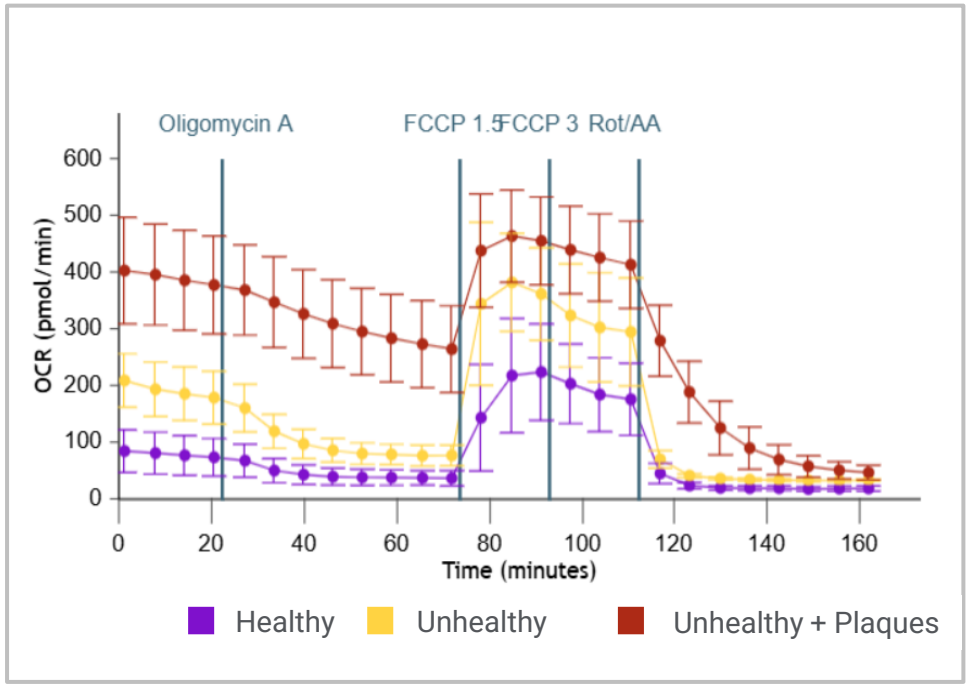
Protocol for real-time measurement of mitochondrial respiration in the mouse ocular posterior pole using a Seahorse XFe24 analyzer



Customer Experience at Demos and Trainings

Aorta tissue punches (US institution)

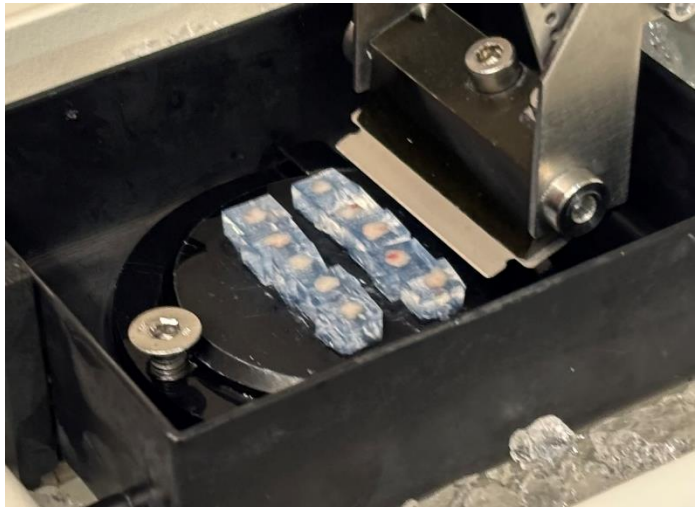


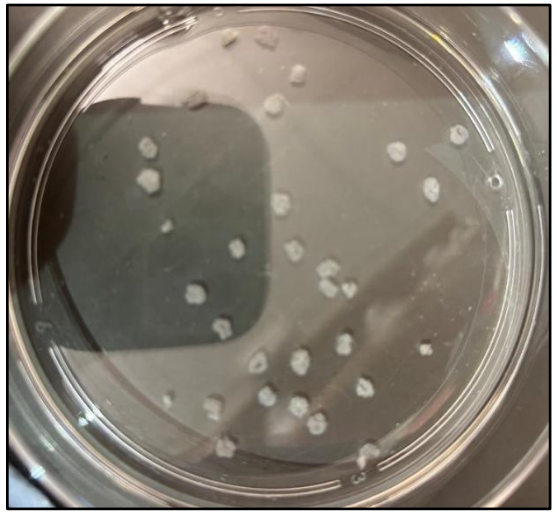


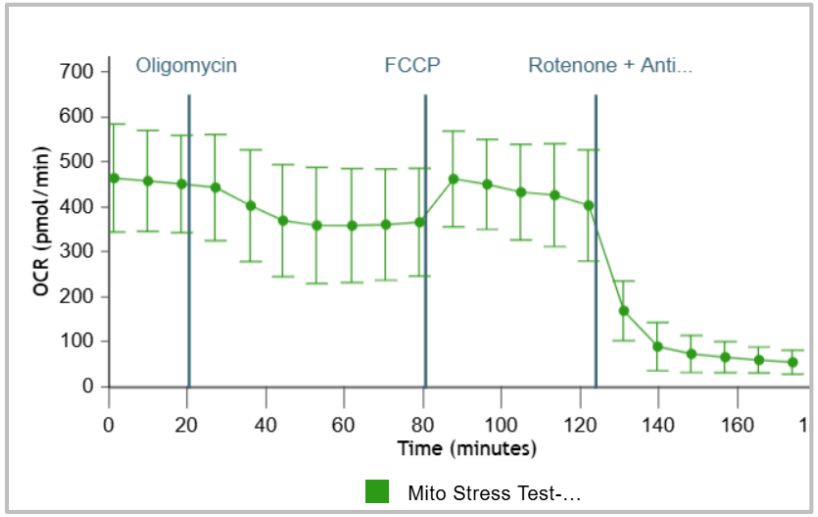
- ✓ Robust response to all modulators
- ✓ Very high SRC
- ✓ Variability is on the high end

Customer Experience at Demos and Trainings

Pancreatic tissue slice punches (US institution)





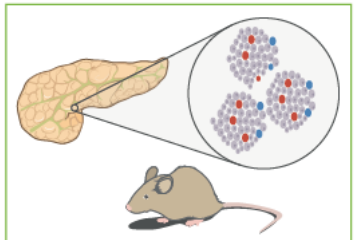


- ✓ Robust response to all modulators
- ✓ Very little SRC
- ✓ Variability is high – futher optimization is needed

Seahorse XF Flex Pancreatic Islet Workflow

Day before assay

Isolate islets and prepare assay



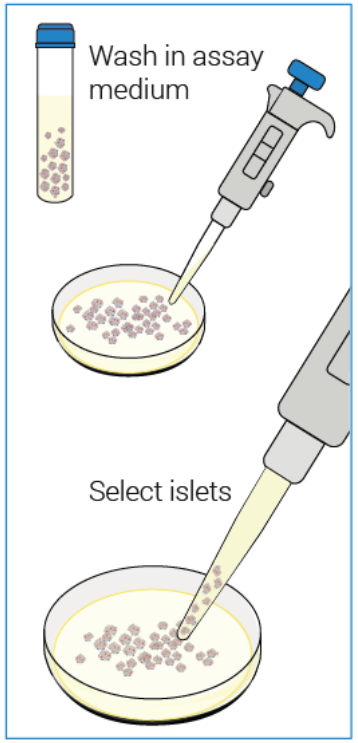
Recover 37 °C,
5% CO_2 overnight

Hydrate cartridge and prewarm plate

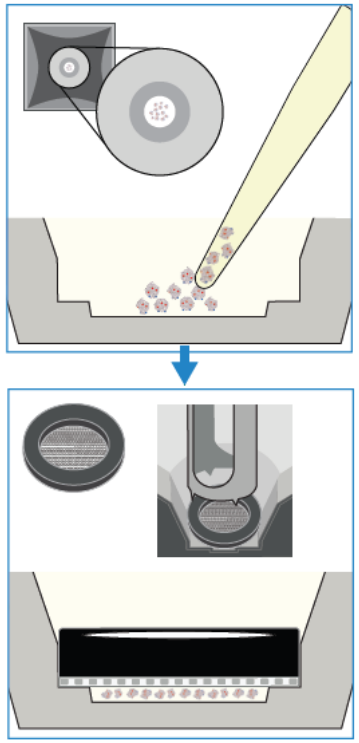
Prepare assay template

Day of assay

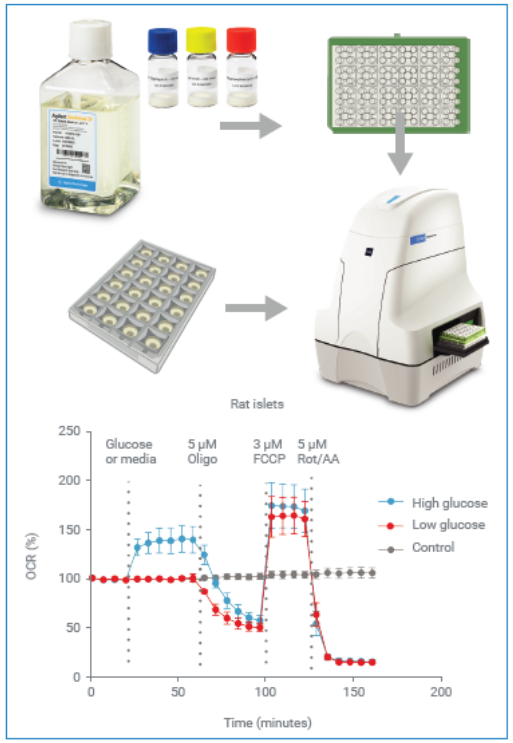
Prepare media and collect islets



Deliver islets to well and install capture screen



Prepare compounds and perform XF assay



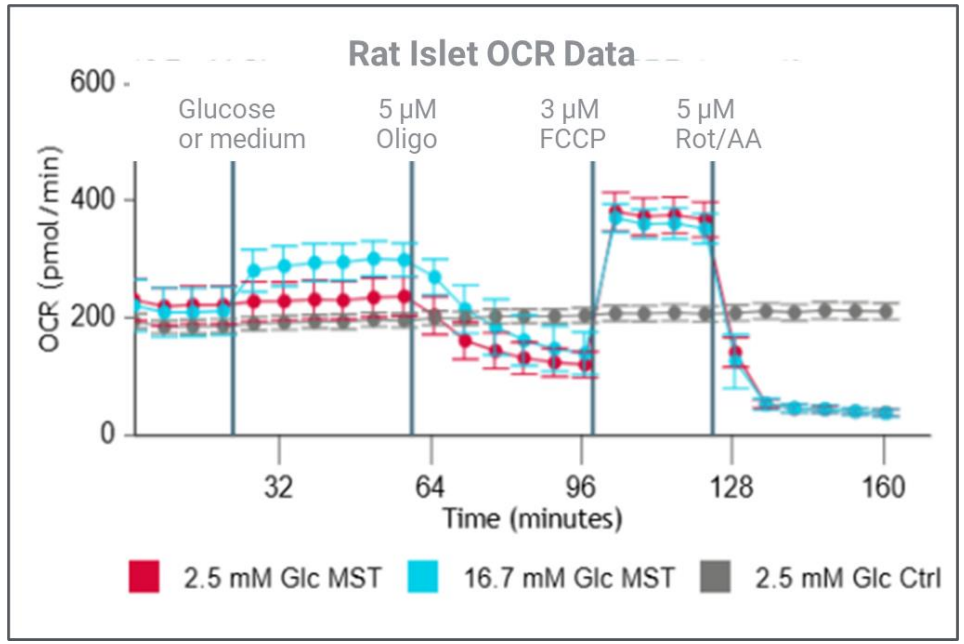
XF Flex 3D Capture Microplate-S/L vs XF24 Islet Capture Microplate

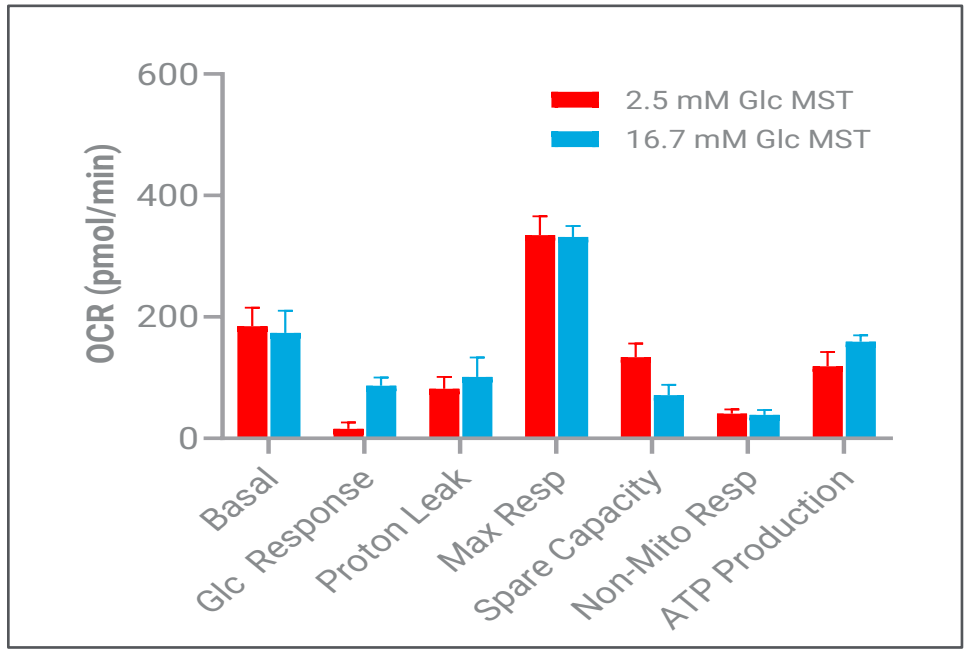



	XF Flex 3D capture microplate-S	XF Flex 3D capture microplate-L	XF24 islet capture microplate
Ring	**Black** PET, easy to see and orient		Clear polycarbonate
Mesh	**170 µm PES**	**285 µm PES**	170 µm nylon
Imaging with mesh	**Compatible with Hoechst staining post assay**	Auto fluorescent, not compatible with Hoechst staining post assay	
Microchamber	**8.5 µL**		17 µL
Sample chamber	250 µm × 3.15 mm		250 µm × 3.15 mm
Recommended protocol	3 min mix - 0 min wait - 3 min measure Improved mixing and probe position parameters (automatically set by barcode)		3 min mix - 2 min wait - 3 min measure
Recommended theoretical rate range	OCR 100-1500 pmol/min ECAR 15-267 mpH/min		None specified
Compatible analyzer	XF Flex		XFe24

Example Data

Rat pancreatic islets with high and low glucose

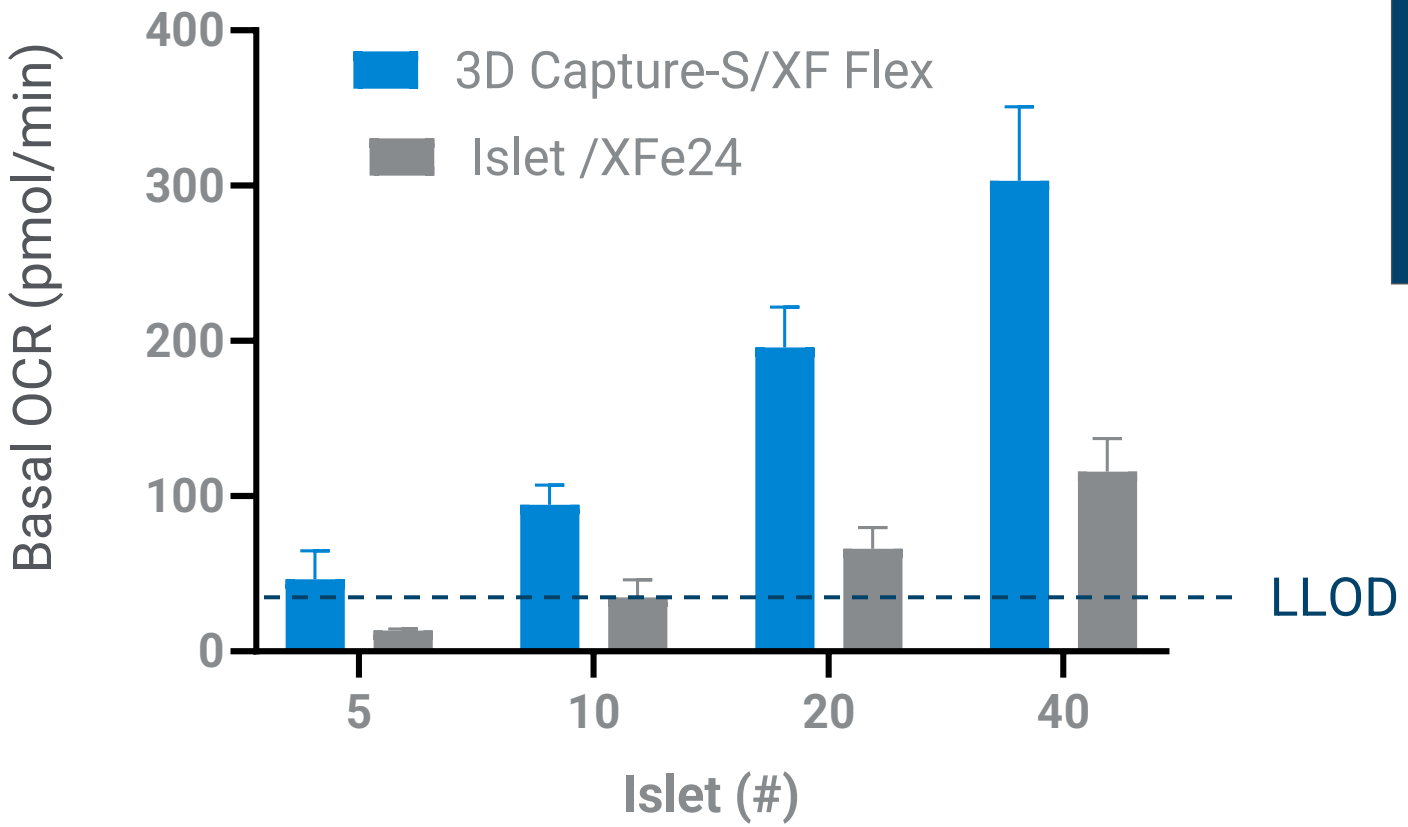




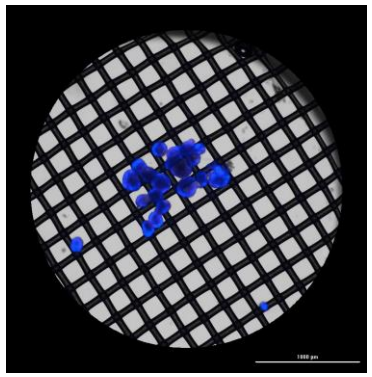
- ✓ Typical Islet assay evaluating high and low glucose followed by Mito Stress Test assay
- ✓ Stable OCR with islet media control demonstrates health of islets in course of assay
- ✓ SHA provides output of multiple mitochondrial parameters that are indicative of mitochondrial function

Seahorse XF Flex 3D Capture Microplate-S

Demonstration of improved sensitivity using islets

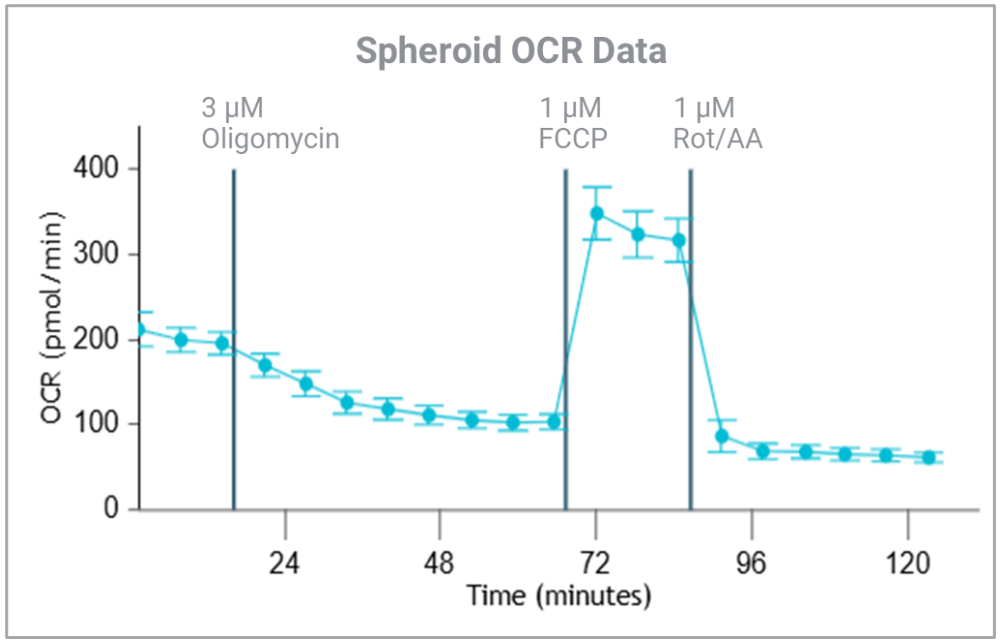


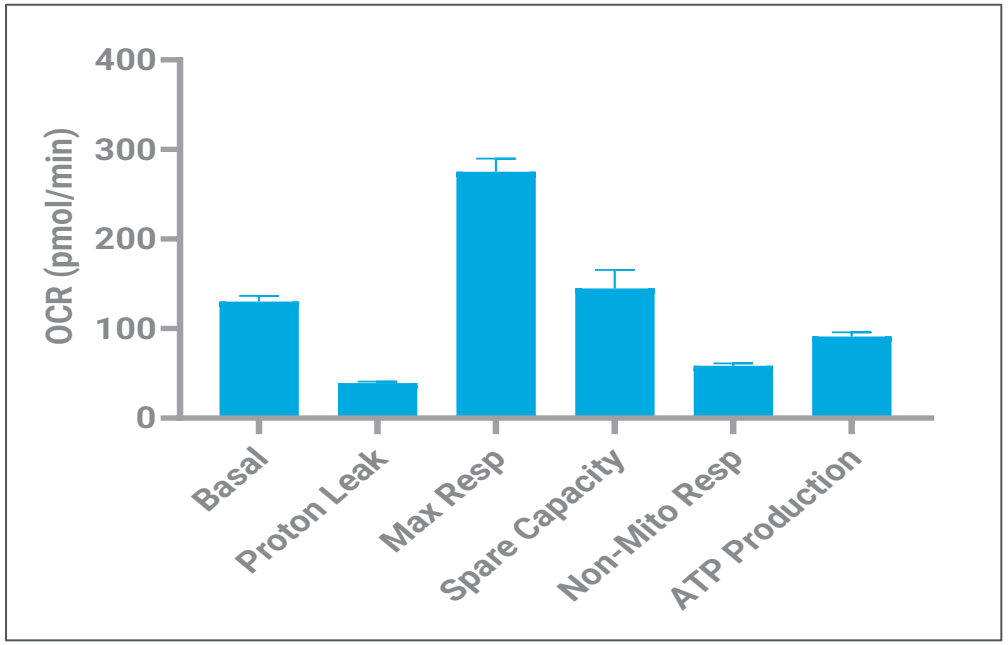
- ✓ Marked sensitivity improvement enables researchers to work with ~25% the number of islets previously needed
- ✓ Researchers harvest limited samples



20 islets/well

Example Data: HCT116 Cancer Spheroids

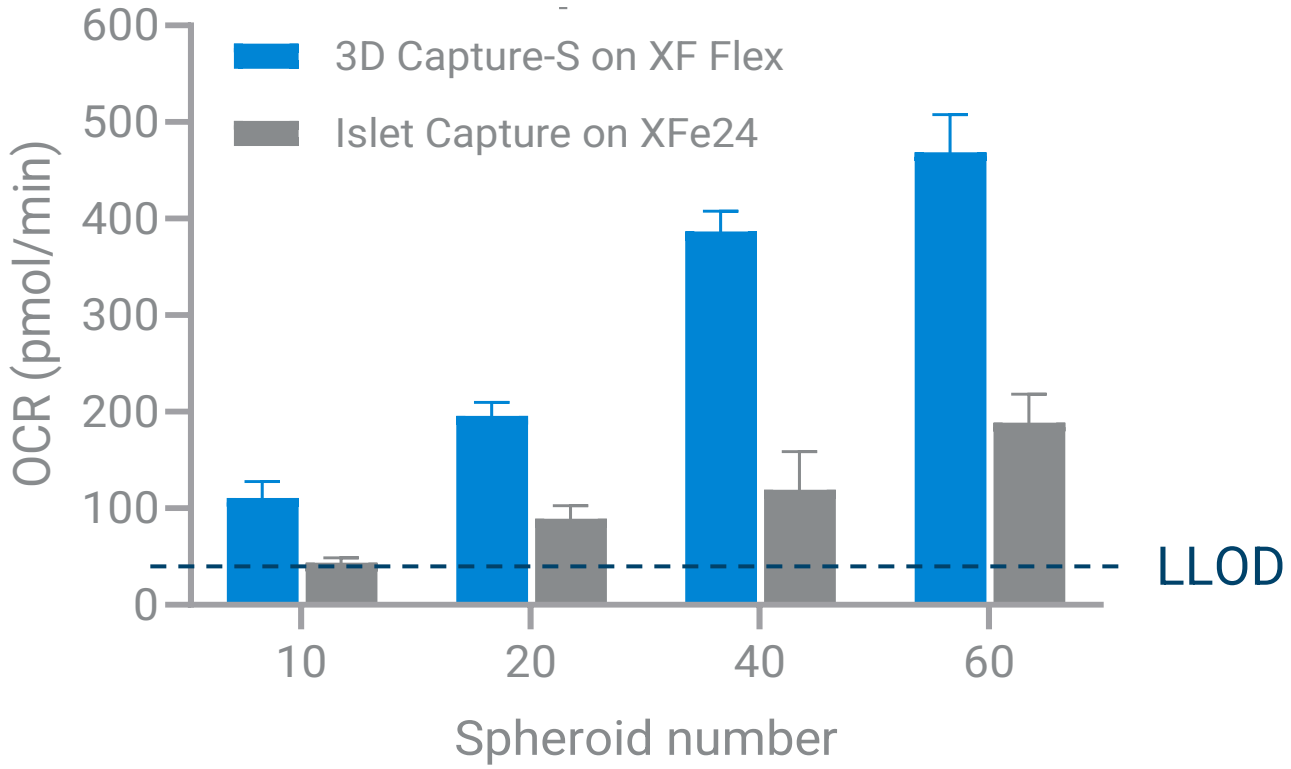




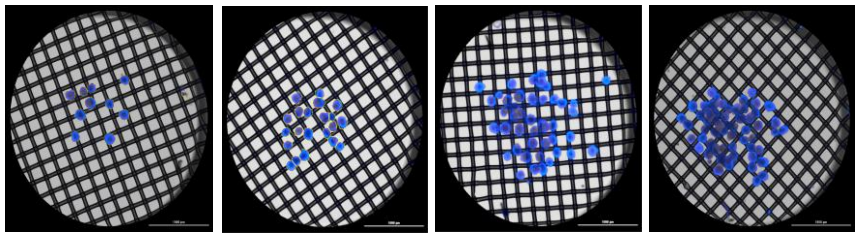
- ✓ MST assay with 20 HCT116 spheroids per well (Day 7, about 150 µm diameter).
- ✓ Robust basal OCR and clear responses to all 3 modulators
- ✓ SHA 3D Mito Stress Test companion view provides output of multiple mitochondrial parameters

Seahorse XF Flex 3D Capture Microplate-S

Demonstration of improved sensitivity using spheroids



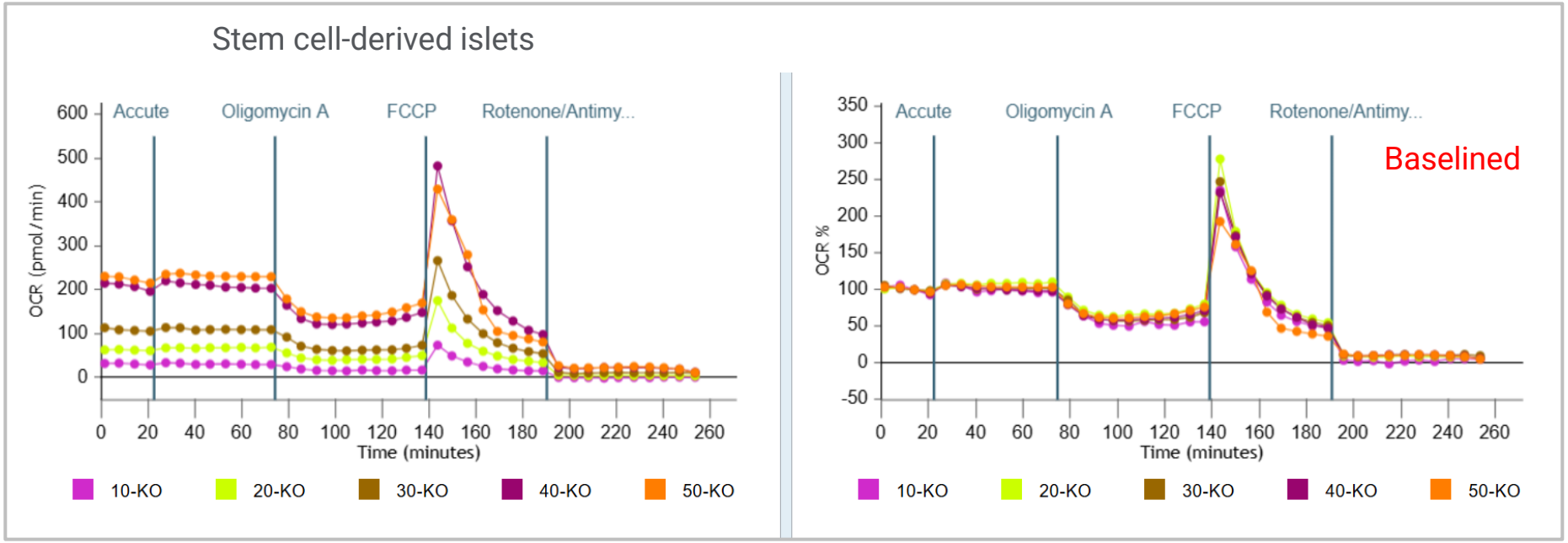
- ✓ 3-4x more sensitive
- ✓ Marked improvement in data quality (smaller error bars relative to signal)



10/well 20/well 40/well 60/well

Customer Experience at Demos and Trainings

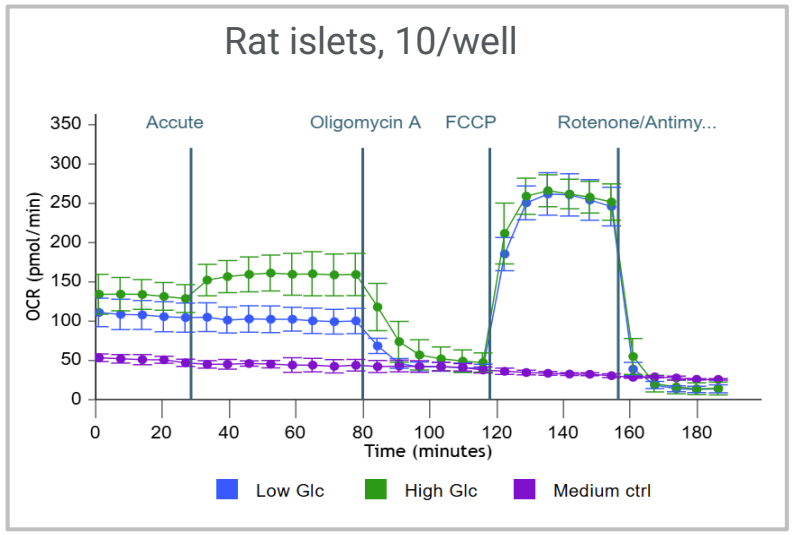
Stem cell-derived islets (European institution)



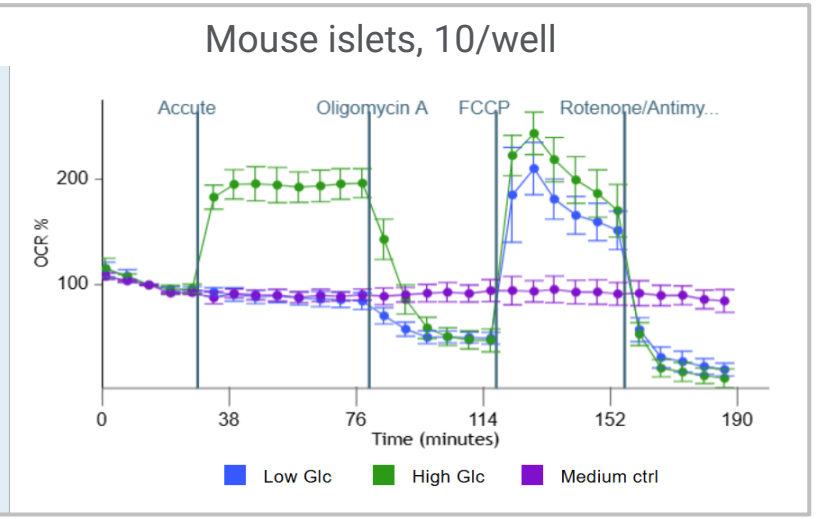
- ✓ Robust response to all modulators
- ✓ Clear seeding density-dependency

Customer Experience at Demos and Trainings

Rat and Mouse Islets (US Institution)

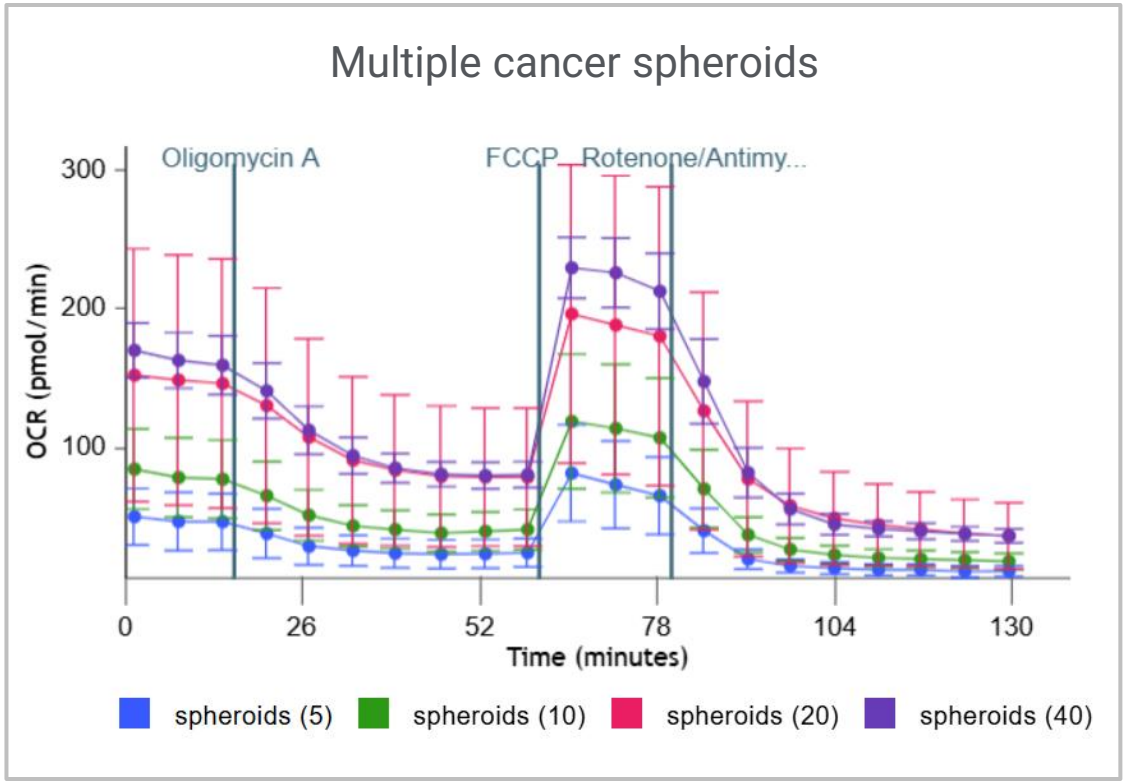


- ✓ Robust response to all modulators
- ✓ Variation for mouse islet is high – more optimization on islet placement technique is needed



Customer Experience at Demos and Trainings

Multiple cancer spheroids (US institution)



- ✓ Robust response to all modulators
- ✓ Clear seeding density-dependency
- ✓ Variation is high – more optimization on spheroid placement technique is needed

Customer comments:

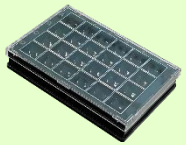
"We have tried with another system before but were not able to see any responses."

Seahorse XF Flex Organoid Workflow

Days before

Prepare organoid cultures in XF organoid plate

1. Seed cells or organoid fragments in matrix scaffold in sample reservoir (10 µL/well)
2. Grow organoids (up to 10 days)
3. Refresh medium as needed



1 Day before

Preparation cartridge

1. Hydrate sensor cartridge (1 mL/well)
2. Incubate at 37°C, non-CO_2

Day of assay

Wash organoid plate, prepare compounds, and perform XF assay

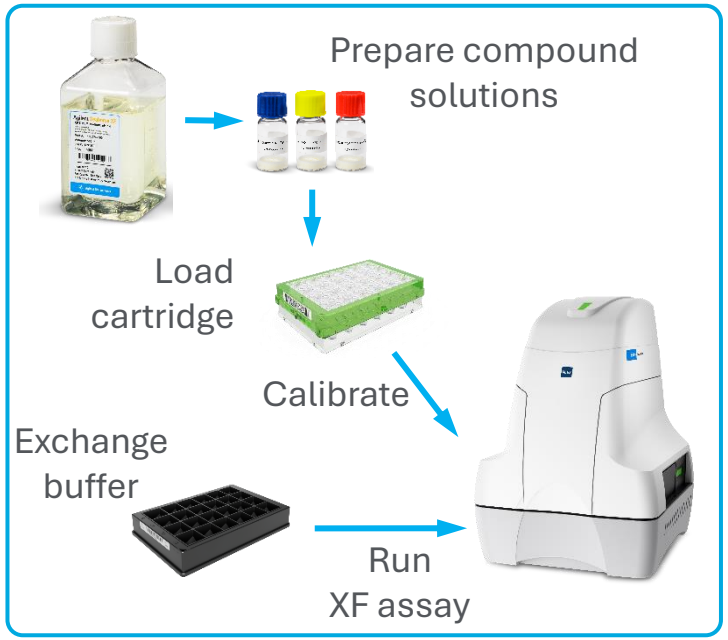
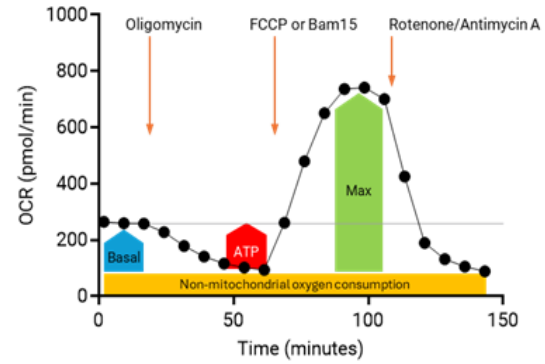


Image organoid culture and analyze XF assay result



XF Seahorse Analytics

- ✓ Work with 2 plating strategies
- ✓ Support several organoid structures
- ✓ Provide serval normalization options and dedicated data analytics

Seahorse XF Flex Organoid Microplate Product Overview

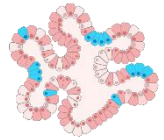
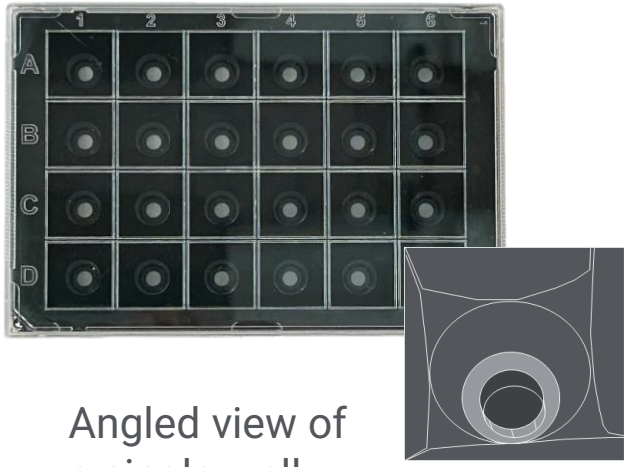
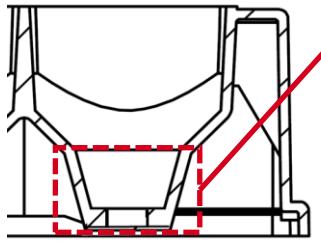


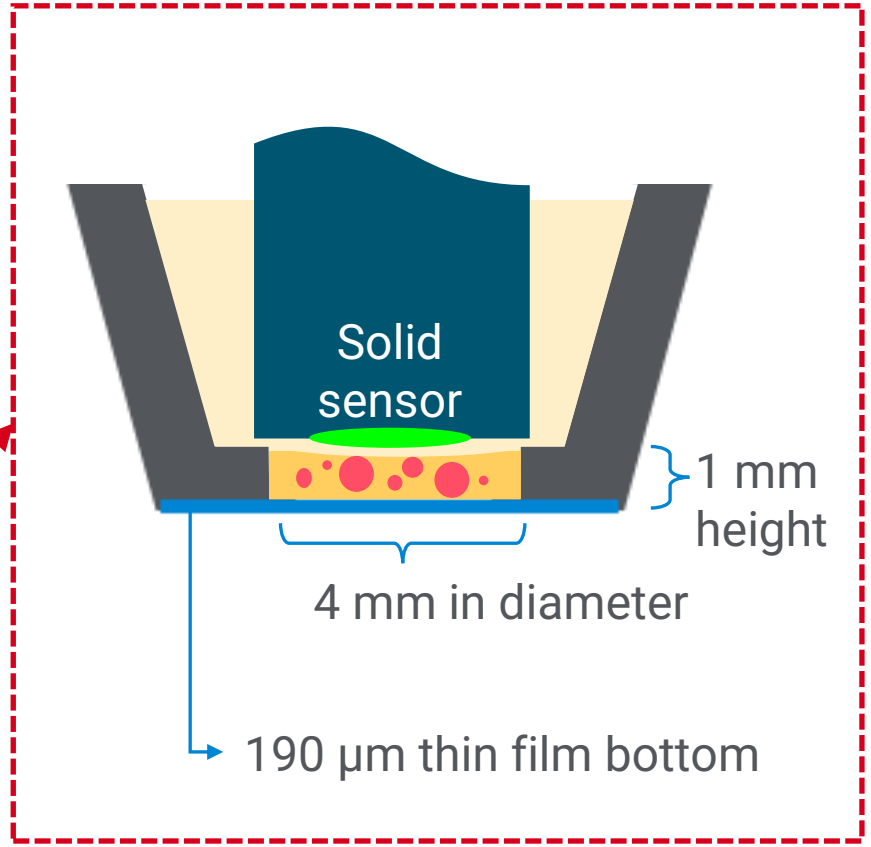
Plate top view



Angled view of a single well from top

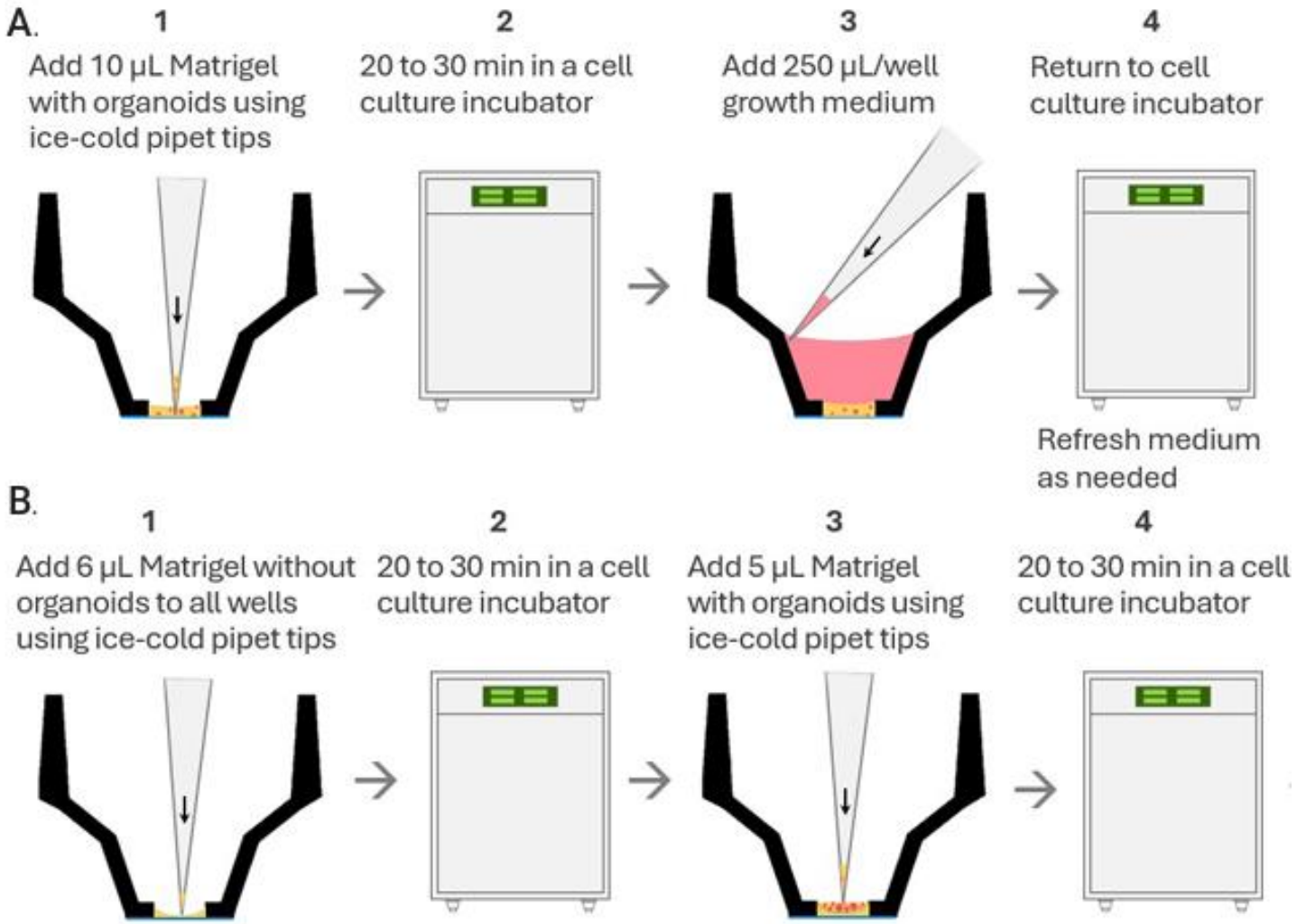
Side view of a single well





- ✓ Sample reservoir size: 1 mm (H) x 4 mm (Dia.), suitable for plating with 10 µL sample in matrix (min= 8 µL; max =12 µL)
- ✓ Thin film bottom enabling high-resolution imaging
- ✓ Flexible – supports several types of organoid cultures
- ✓ Robust workflow
- ✓ Only compatible with XF Flex (not with XFe24)

Work with 2 plating strategies



Single Step

- ✓Easy to handle
- ✓Small and many organoid

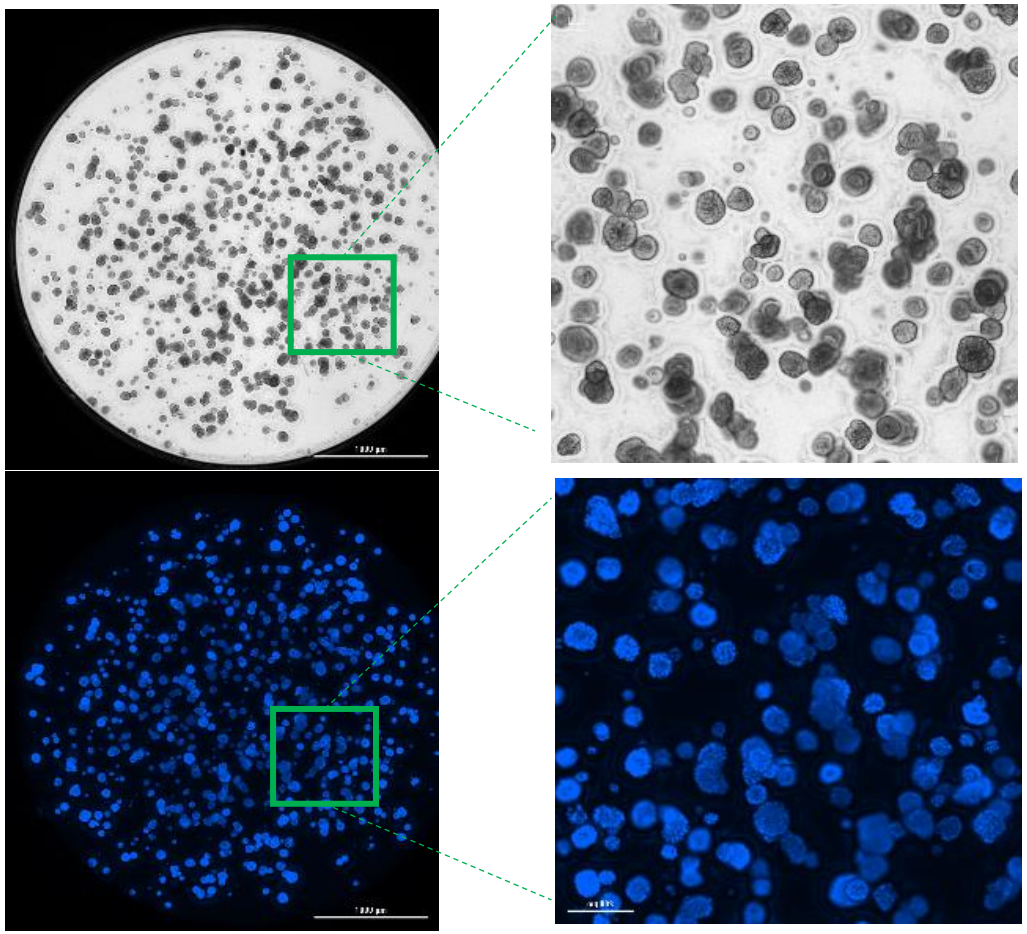
Two Step

- ✓Advanced handle
- ✓Large and less organoid

Example Data – MCF10A Organoid Culture (8-day culture)

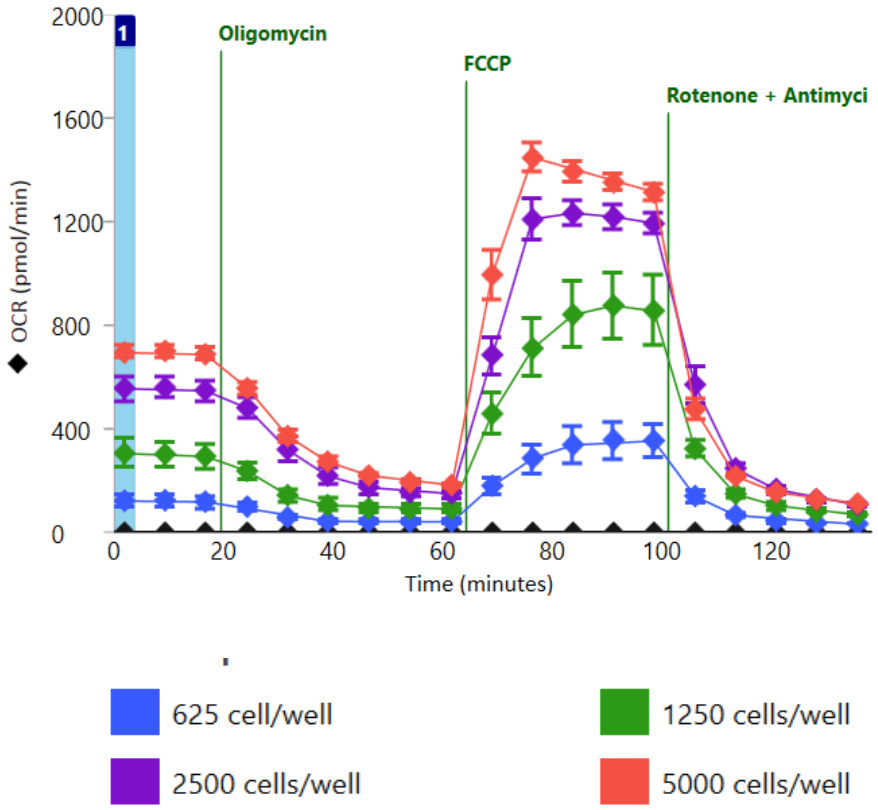
Cytation 5

4x, Brightfield



4x, Fluorescence, Hoechst

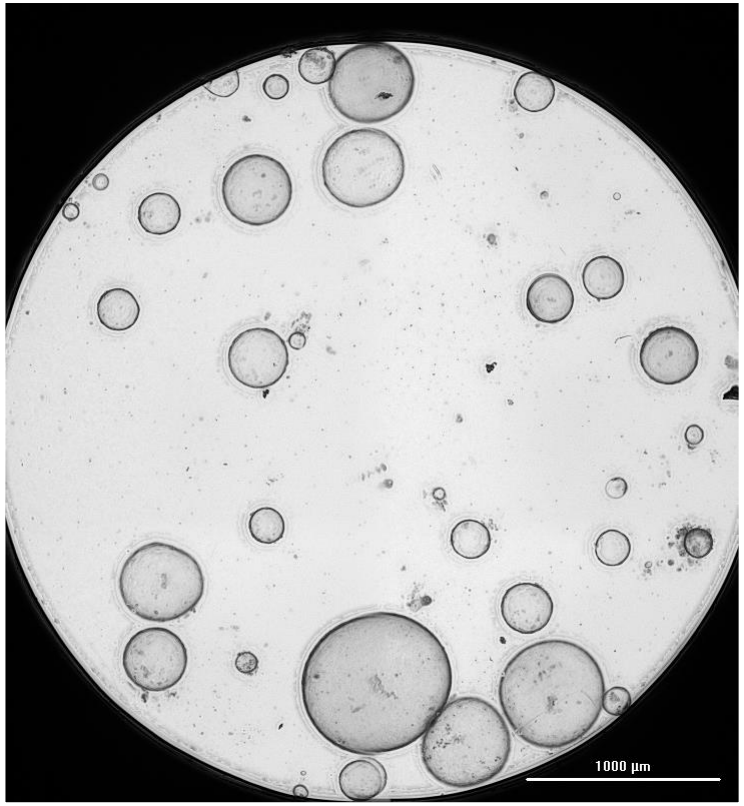
Typical XF Mito Stress Test Profile



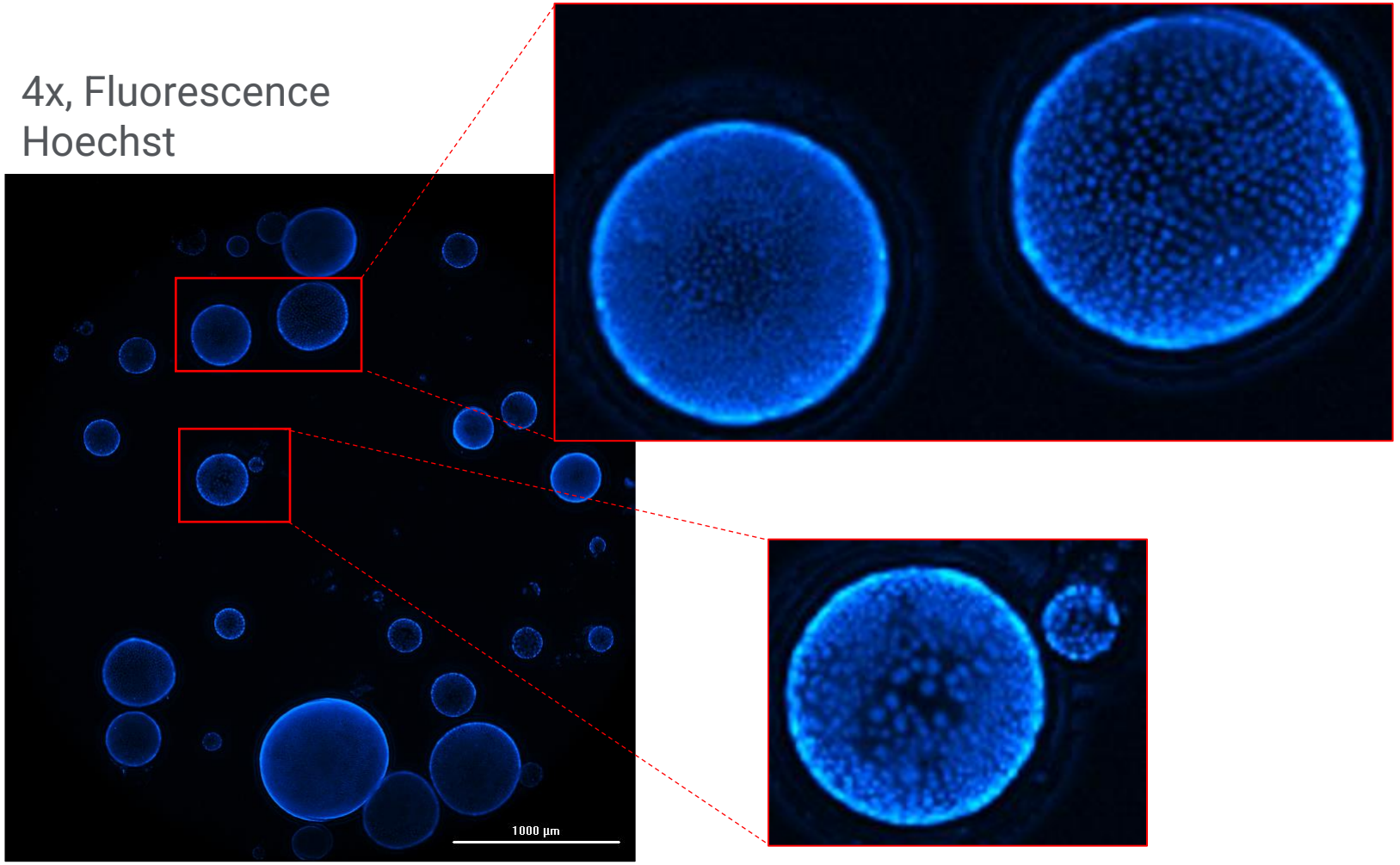
Example Data: Stem Cell-Derived Mouse Liver Organoids (3-day culture)

Cytation 5

4x, Brightfield



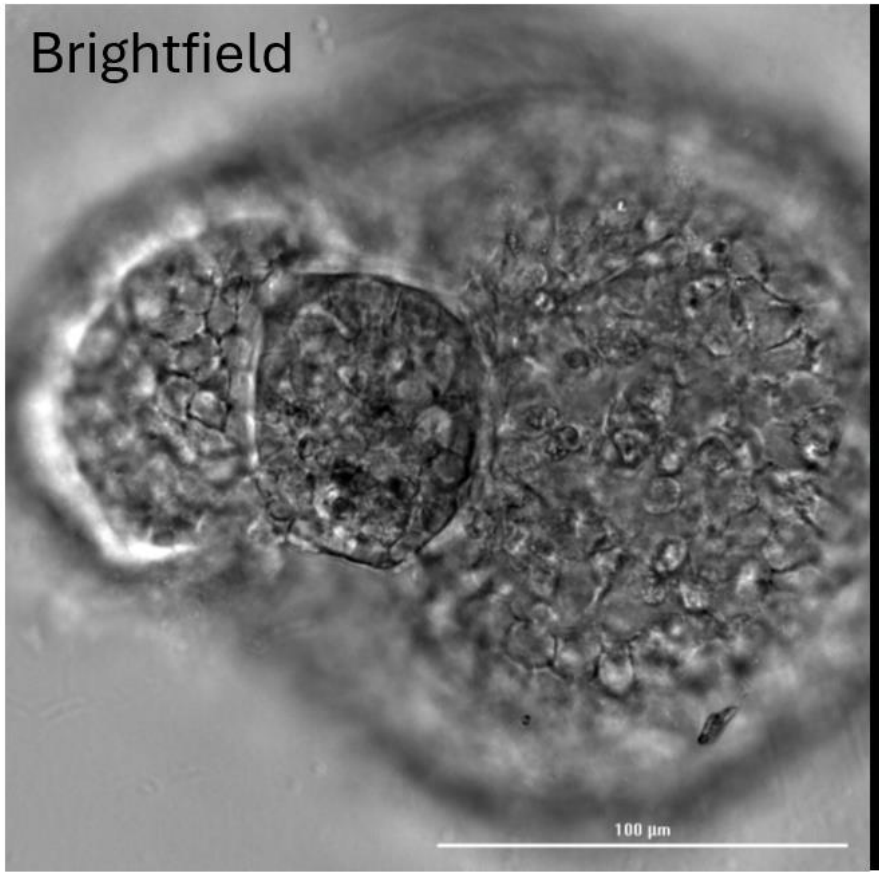
4x, Fluorescence
Hoechst



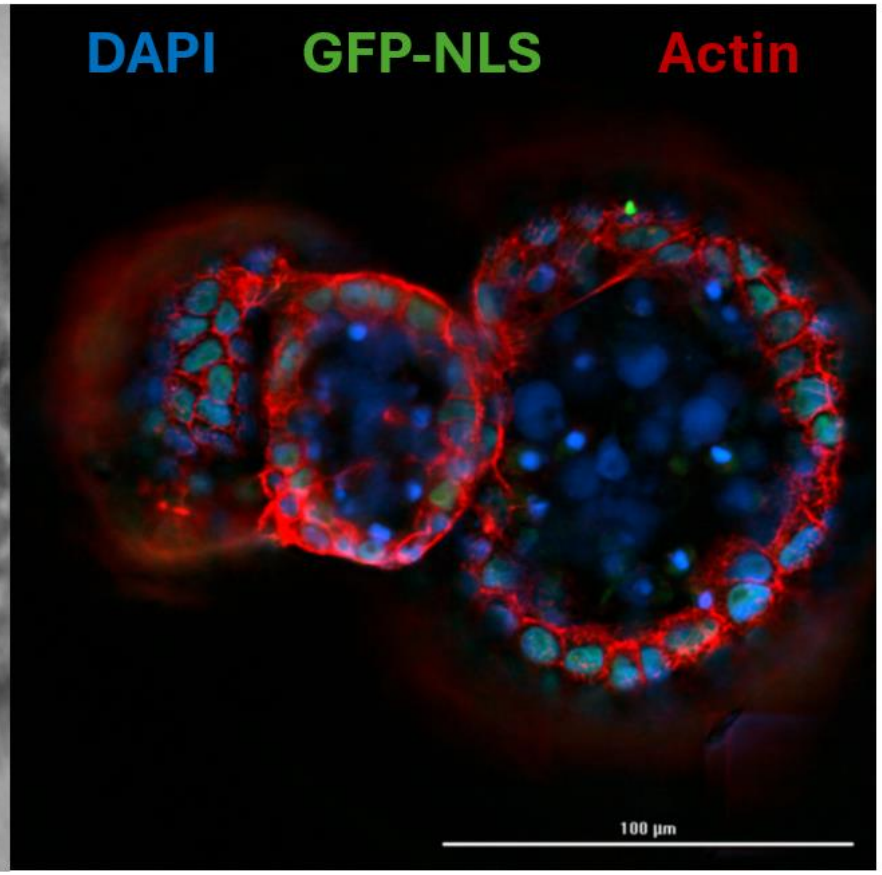
Example Data: MCF10A Breast Organoid Culture (6-day culture)

Cytation C10

60x, Brightfield

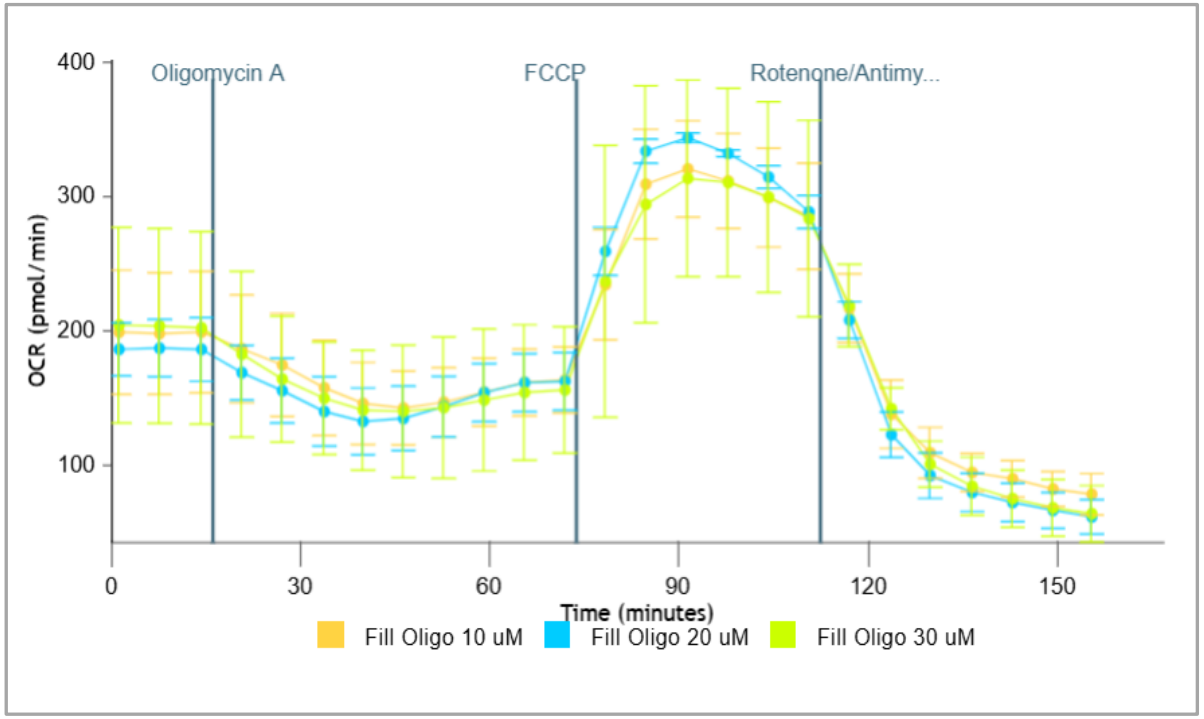


60x, Fluorescence, fixed sample



Customer Experience – Beta Testing

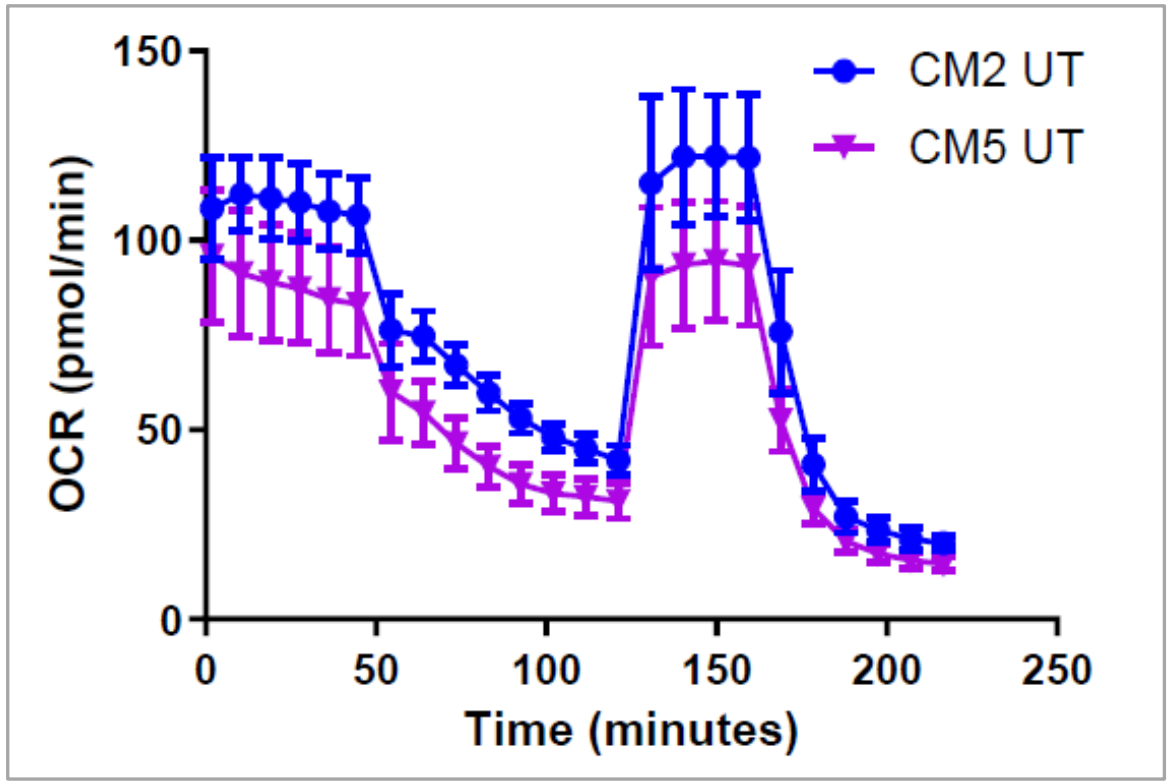
Intestinal Organoids (European Institution)



- ✓ Workflow easier and more consistent than previous methods (droplet/dome of embedded organoid material)
- ✓ Our normalization methods that do not require pooled samples from each experimental group are great. (Normalization options are being trialed at this site.)

Customer Experience at Demos and Trainings

Brain Organoids (US Institution)

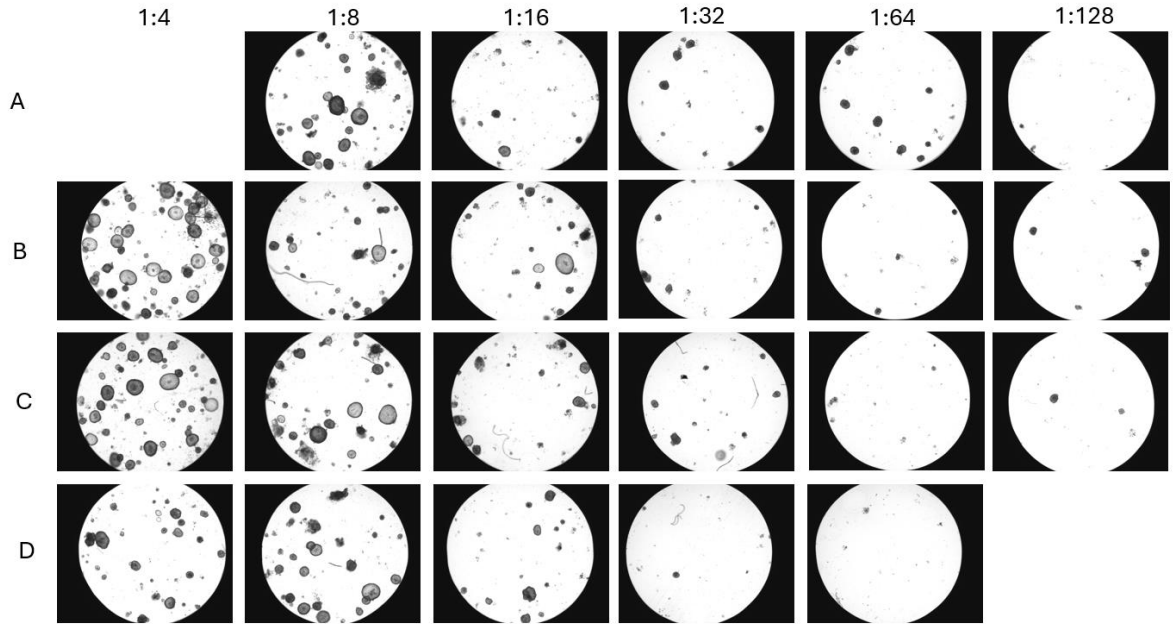


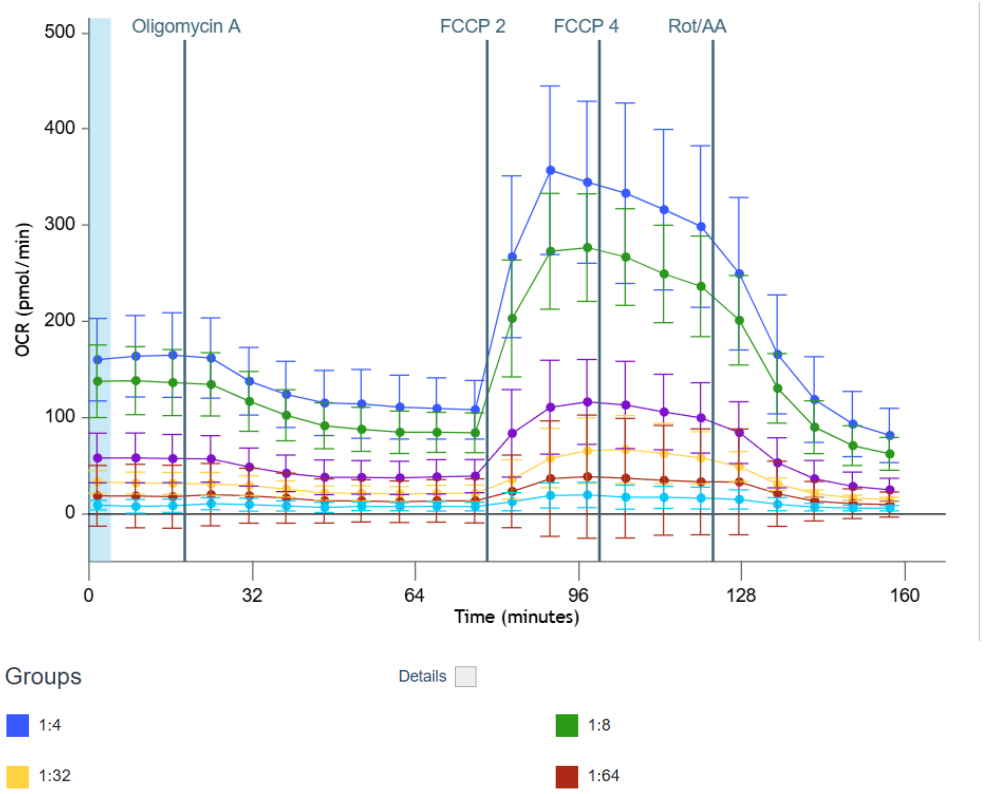
- ✓ Robust response to all modulators
- ✓ Very little SRC
- ✓ Variability is relatively high – optimization on organoid placement technique is needed

Note: Brain organoids were grown in another device and transferred to XF Flex Organoid Plate

Customer Experience at Demos and Trainings

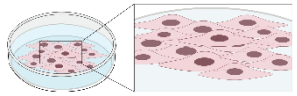
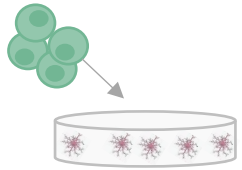
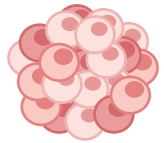
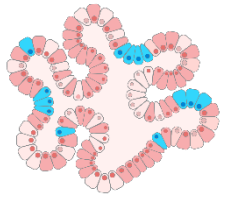
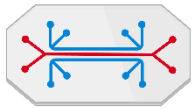
Intestinal Organoid (US Institution)





- ✓ Successful growth and imaging of organoids
- ✓ Robust response to all modulators
- ✓ High SRC

Comparison of Advanced Cell Culture Models

	2D standard cell culture	iPSC derived differentiated cells	Spheroids	Organoids	Organ-on-a-chip	Ex-vivo tissue
						
Culture handling	Very easy	• Days—week (cell type dependent) • Expensive culture medium/cofactors • Labor intensive	• Self-assemble into 3D structures • Relatively easy to generate and maintain	• Requires special media + scaffold • Technically challenge and time consuming	• Specialty microfluidic devices • Only some chips are designed for sample removal	• Requires access to live tissue • Limited viability ex vivo
Difficulty to genetically manipulate	Easy	Easy	Medium	Medium to difficult	Difficult	Difficult – require animal genetic manipulation
Quantitative assays available	High	High	Medium	Medium	Low	Low
Reproducibility between experiments	High	Medium	Medium	Medium (population heterogeneity)	Medium	Medium to Low
Recommended XF Plate	V7	V7 / V28	XF96 Spheroid plate	Organoid Plate	3D Capture plate	3D Capture plate

Build Your Cell Analysis Lab with Agilent

Investigate dynamic cancer cell strategies with innovative cell analysis technologies

BioTek Liquid Handling

BioTek Automated Imaging Systems

Seahorse XF Real-Time Metabolic Cell Analysis

BioTek Multimode Microplate Reading

NovoCyte Flow Cytometry

xCELLigence Real-Time Cell Analysis