

## Introduction

Organoids have emerged as powerful tools in preclinical research, and patient-derived organoids (PDOs) in particular are increasingly recognized as promising platforms for drug evaluation and personalized therapy development.

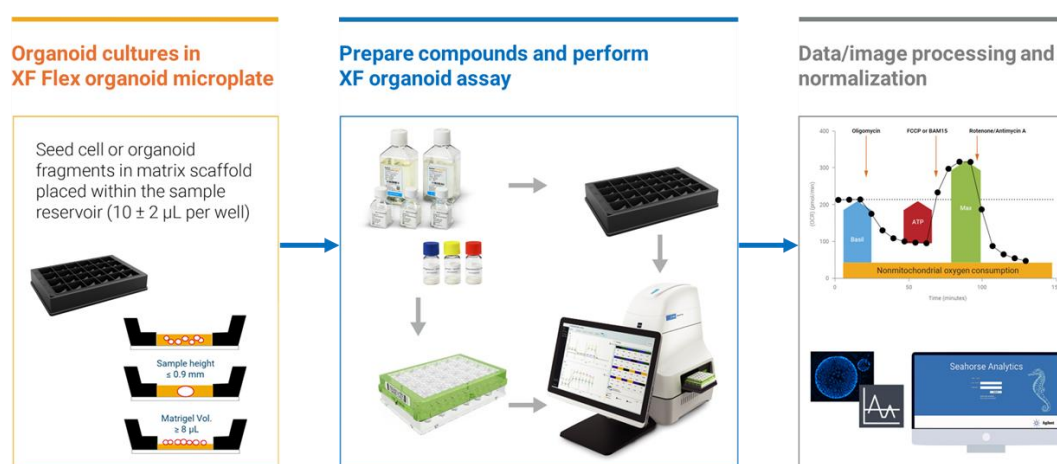
The U.S. Food and Drug Administration (FDA) supports the adoption of validated new approach methodologies (NAMs), including organoid-based systems to improve translational relevance in biomedical research.

In alignment with these FDA priorities, a novel assay workflow was developed using the **Agilent Seahorse XF Flex analyzer** in combination with the **Seahorse XF Flex organoid microplate**. This microplate enables long-term culture of organoids embedded in extracellular matrix (ECM) gels, such as Matrigel, for days prior to metabolic or imaging assays.

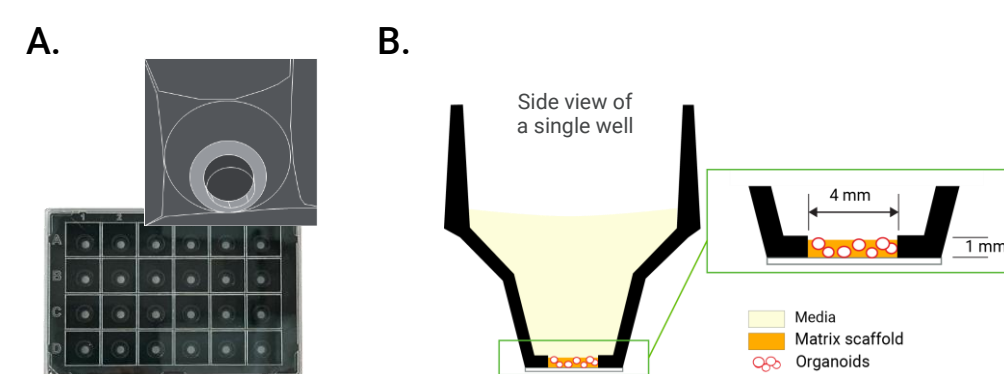
In this study, we introduce the **Seahorse XF Flex organoid assay** workflow to assess changes in mitochondrial function in colon cancer cell line-derived organoids in response to metformin treatment.

## Experimental

### Seahorse XF organoid assay<sup>1</sup>



**Figure 1.** Agilent Seahorse XF Flex organoid assay workflow using the Agilent Seahorse XF Flex organoid microplate. Cells or organoids embedded in Matrigel (10 µL per well) are cultured for days in an XF Flex organoid microplate to assess their metabolic profile using the Agilent Seahorse XF Flex analyzer. The intact organoids can subsequently be used for additional image analysis.

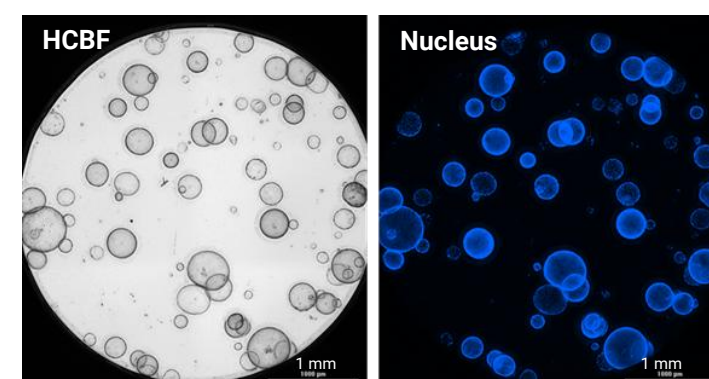


**Figure 2.** Illustration of the Agilent Seahorse XF organoid microplate. (A) The XF Flex organoid microplate is a 24-well microplate with a black side-wall and a clear/thin film bottom compatible with high-resolution imaging. (B) Organoids embedded in a matrix scaffold, such as Matrigel, are grown in the sample reservoir of 1 mm (height) x 4 mm (diameter) allowing for matrix volume of 10 ± 2 µL per well to be placed securely within the microchamber formed with the sensor probe.

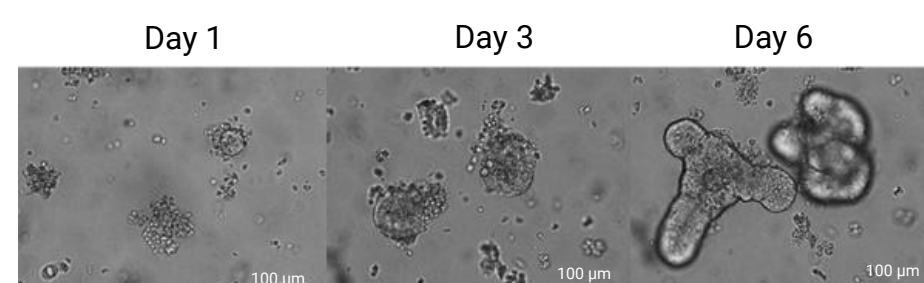
## Results and Discussion

### 3D organoid culture on Seahorse XF Flex organoid microplates

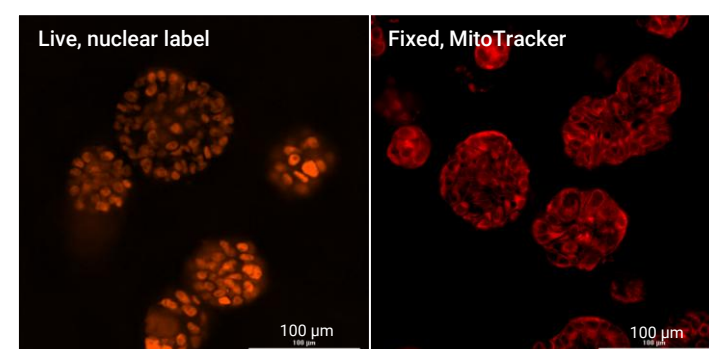
#### A. Mouse hepatic organoid (4x)



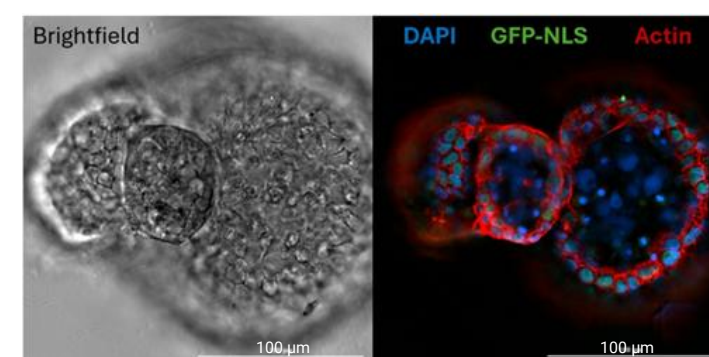
#### B. Mouse intestinal organoids (20x)



#### C. MCF10A (confocal, live, 40x)



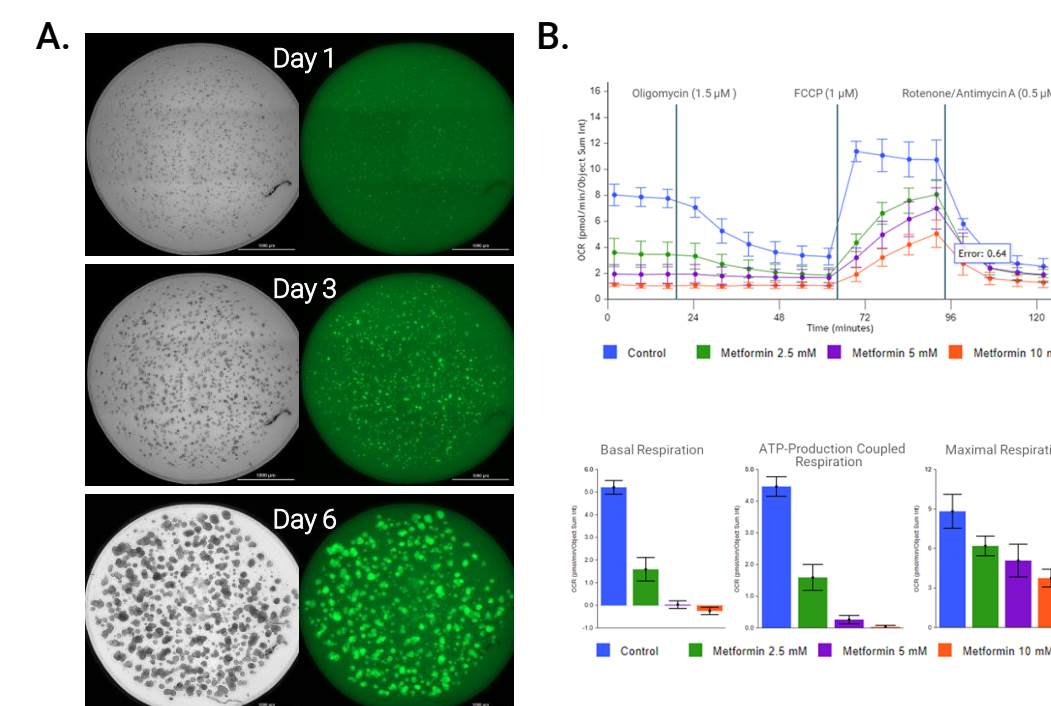
#### D. MCF10A (confocal, post-fixation, 60x)



**Figure 3.** Imaging of organoids cultivated in the Agilent Seahorse XF Flex organoid microplates. (A and B) Mouse hepatic organoids and intestinal organoids cultured from organoid fragments (STEMCELL Technologies) for four and six days respectively. The organoids were imaged by the Agilent BioTek Cytation 5. (C and D) MCF10A cell line-derived organoids imaged using the Agilent BioTek Cytation C10 confocal imaging reader. Organoids can be imaged live or after fixation.

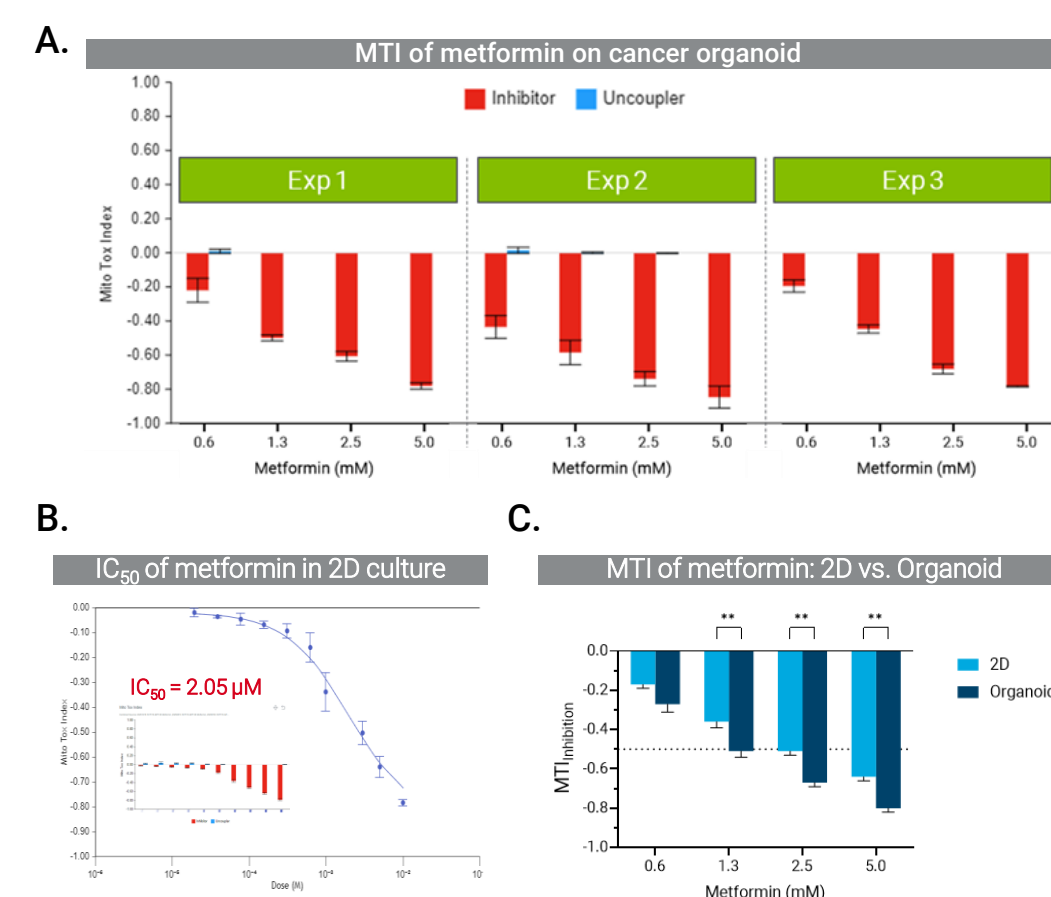
## Results and Discussion

### The assessment of mitochondrial function in cancer organoids<sup>3</sup>



**Figure 4.** Cancer organoid culture on Agilent Seahorse XF Flex organoid microplates and assessment of the effect of metformin on their mitochondrial function. (A) HCT116-H2B-GFP cancer organoid formation embedded in Matrigel within the Seahorse XF Flex organoid microplate. Brightfield (left) and GFP fluorescence images (right) were captured by an Agilent BioTek Cytation 5 cell imaging multimode reader using a Gen5 protocol. (Scale bar = 1,000 µm). (B) Changes in OCR kinetic data and key bioenergetic parameters in response to the inhibitory effect of metformin after overnight treatment.

### Mito Tox Index (MTI) measurement of cancer organoids<sup>2,3</sup>



**Figure 5.** Inhibitory effect of metformin on cancer cells in 2D and 3D organoid culture conditions. (A) HCT116 organoids were exposed to metformin overnight at four different doses and the MTI was assessed by three independent XF Mito Tox assays. Data is presented by means ± S.D. (B) XF Mito Tox dose-response assay data using 2D culture condition was combined to assess the IC<sub>50</sub>. (C) The inhibitory MTIs of metformin obtained in 2D, and 3D organoid culture conditions were compared. The combined data in (B) and (C) are presented by means ± S.E.M. of three independent assays.

## Conclusions

This study demonstrates the use of a robust workflow for metabolic profiling of cancer organoids and the assessment of drug impact on mitochondrial function, using the **Agilent Seahorse XF Flex analyzer** and **Agilent Seahorse XF Flex organoid microplate**.

The Seahorse XF Flex organoid microplate supports long-term culture of a wide range of matrix-embedded organoids while securely retaining 3D samples during real-time metabolic analysis and high-resolution imaging.

Cancer cell derived organoids could be reliably generated within the plate format, enabling direct real time metabolic analysis of matrix embedded cancer organoids, which may include PDO models.

In this proof-of-concept study, the **Mito Tox Index (MTI)**, measured using the **Seahorse XF Mito Tox Assay<sup>2</sup>**, is demonstrated as a quantitative metric for evaluating anti-cancer drug-induced mitochondrial dysfunction in a organoid model.

MTI-based toxicity quantification revealed enhanced sensitivity of organoids to metformin, providing a more direct and sensitive readout of mitochondrial dysfunction across different culture systems, including comparisons between 2D cultured cells and 3D organoids.<sup>3</sup>

Overall, the **Seahorse XF Organoid assay** workflow is readily adaptable to PDO-based studies, supporting its potential application in translational and precision medicine research.

## References

<sup>1</sup>Measuring Mitochondrial Function of Matrix-Embedded Organoids using the Agilent Seahorse XF Flex Analyzer. *Agilent Technologies technical overview*, 5994-8742EN, 2025.

<sup>2</sup>Kam, Y.; Rogers, G. W.; Winer, L.; Schwalfenberg, M.; Romero, N. A Customized XF Workflow for Detection and Characterization of Mitochondrial Toxicity. *Agilent Technologies application note*, 5994-4778EN, 2022.

<sup>3</sup>Kam, Y.; Winer, L.; Romero, N. Metabolic Profiling Cancer Organoids Cultured in the Agilent Seahorse XF Flex Organoid Microplate. *Agilent Technologies application note*, 5994-8888EN, 2026.