Morphometric imaging of organoids for toxicology and assessment of therapeutic efficacy.

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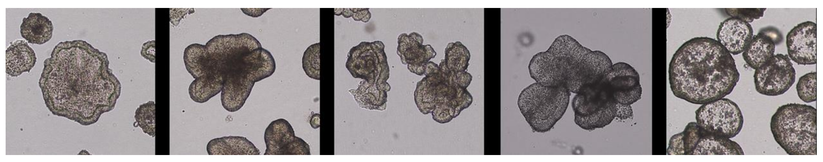
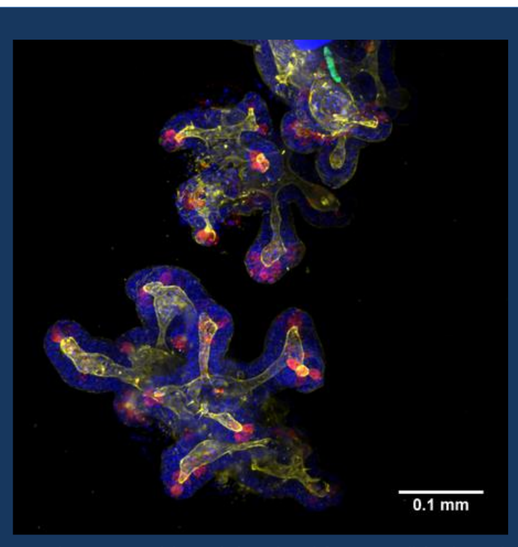
ORGANOIDS

Research with patient-derived colorectal tumour organoids shows that the therapeutic outcomes of the patients mirrored the effects on their corresponding organoids [1]. Organoids, organ on a chip and other multicellular models are also being assessed for a range of different toxicology and drug development applications.

Can quantitative structural imaging of organoids be used to measure therapeutic efficacy?

*[1] Vlacchogiannis G., et al., Science, **359**, 920-926 (2018)*





Intestine organoid model stained for DNA (blue), actin (yellow), Paneth cells (red), sample prepared by Dr Sylvie Le Guyader from the Karolinska Institutet.

APPLICATIONS

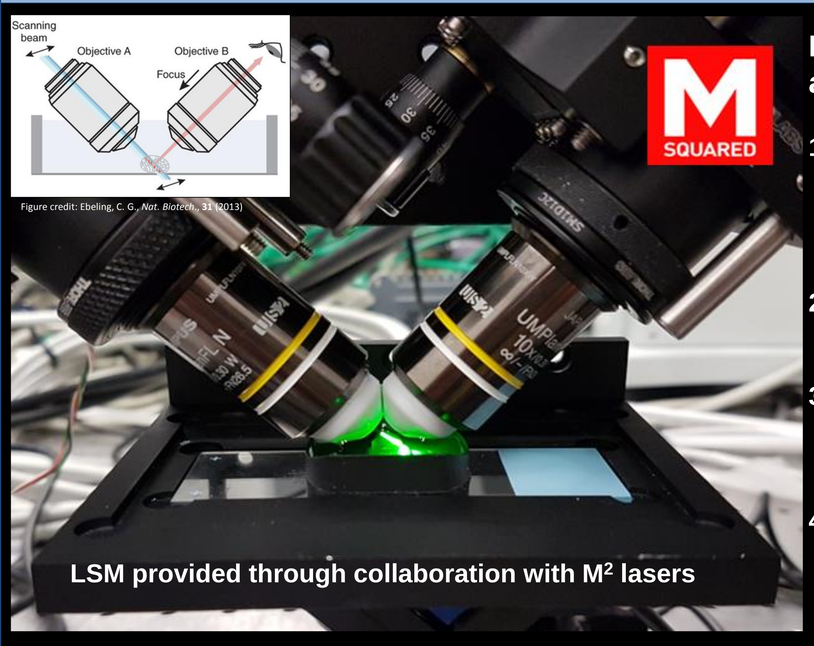
- Examine organ development and tissue morphogenesis
- Model diseases
- **Test drug sensitivity and toxicity**
- Potentially form complex tissues for transplantation

CHALLENGES

- Traditional in vitro cellular end-point, or biochemical assays may fail to capture subtle phenotypic responses.
- To acquire, analyse and compare data-rich images.

- **Model system: Colorectal tumour organoids (Cellesce)**
- **Treatment at 2 days: Dabrafenib, Vemurafenib, 5-FU, G007-LK**
- **Organoids fixed and stained at 7 days with DAPI and F-actin**

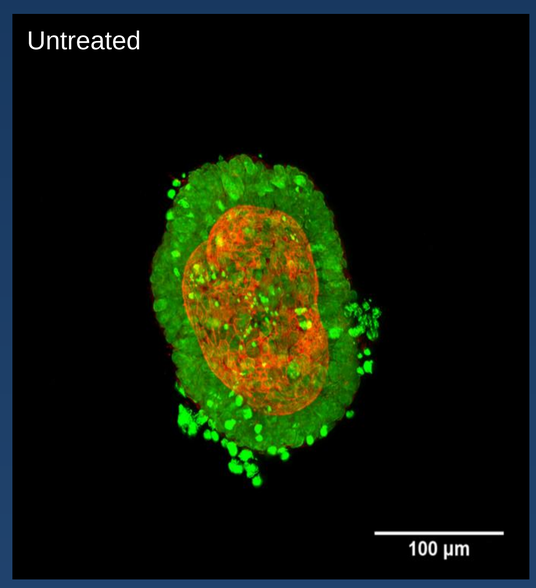
LIGHT SHEET MICROSCOPY (LSM)

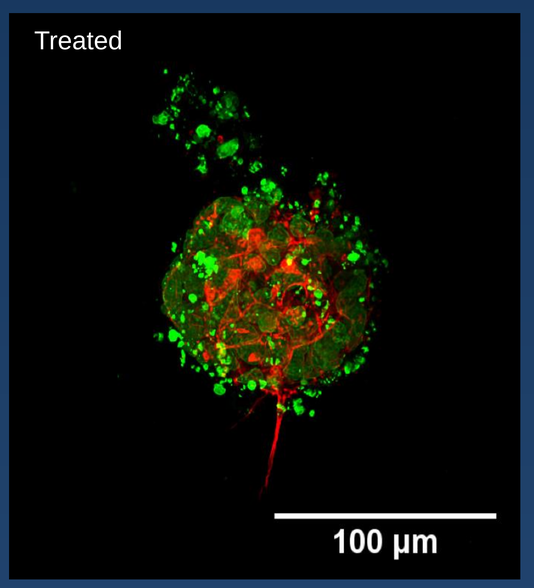


LSM imaging advantages:

1. **Only illuminates in-focus plane - ↓ phototoxicity & photobleaching.**
2. **Fast volumetric imaging.**
3. **Beam shaping can increase field of view.**
4. **Mm³ sized working volume with sub µm spatial resolution**

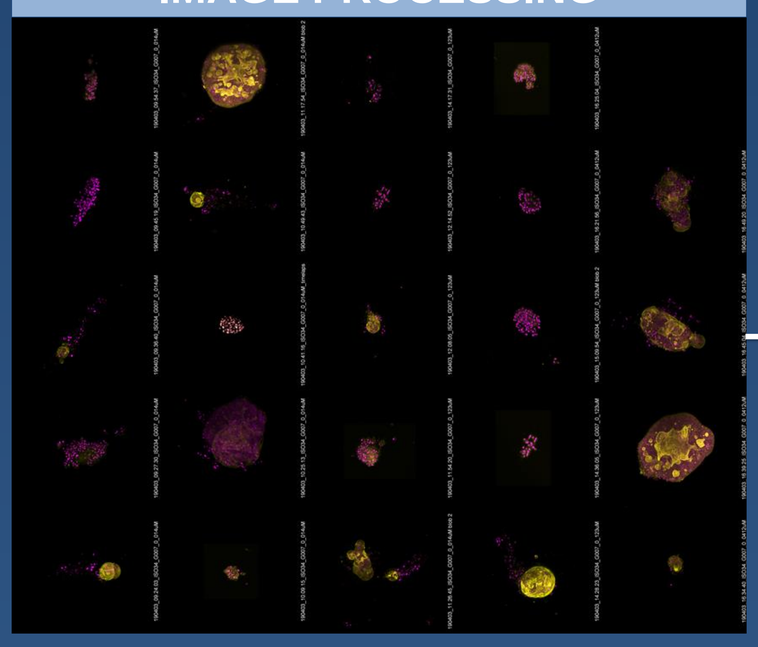
Rapid imaging of organoids without photodegradation





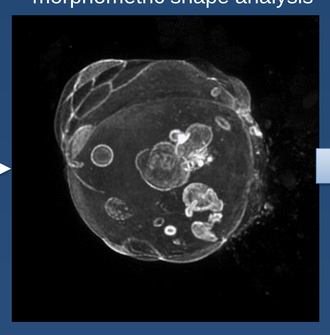
- **Software developed to:**
  - **Segment organoids and nuclei**
  - **Measure morphometric parameters**
  - **Detect and quantify effects of therapeutic treatment**

IMAGE PROCESSING

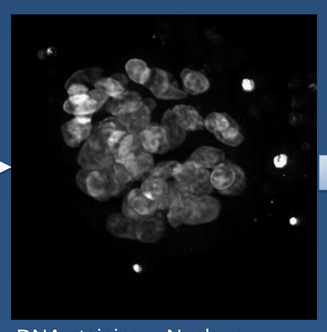


Examples morphologies of drug treated organoids.

Actin staining – Organoid morphometric shape analysis

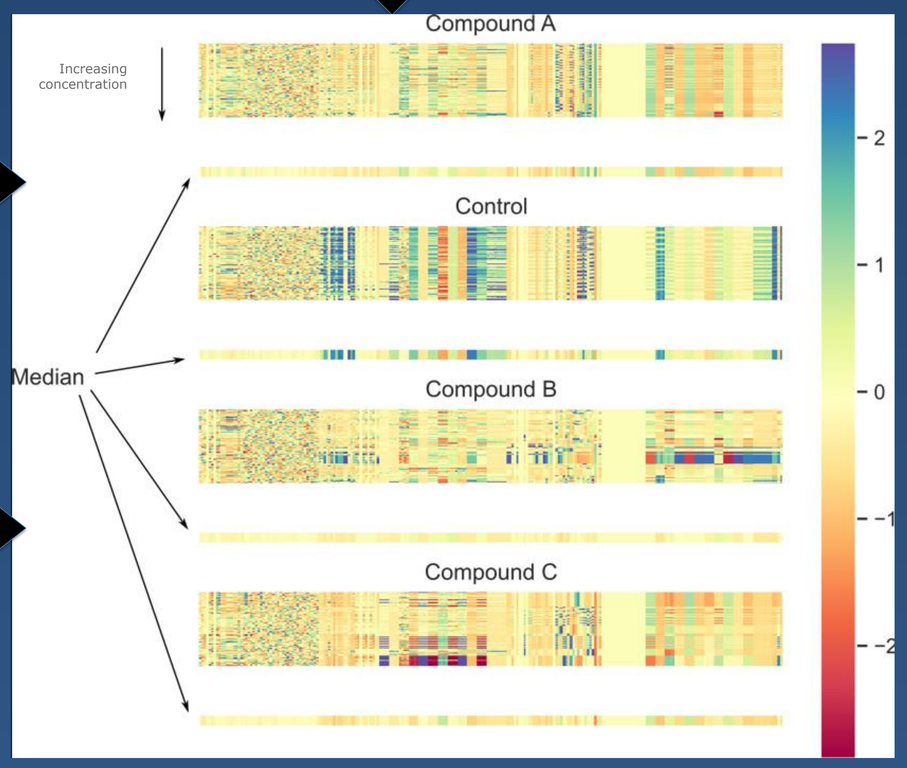


1. Measure variation in shape parameters with therapeutic dose



DNA staining – Nucleus morphometric shape and texture descriptors

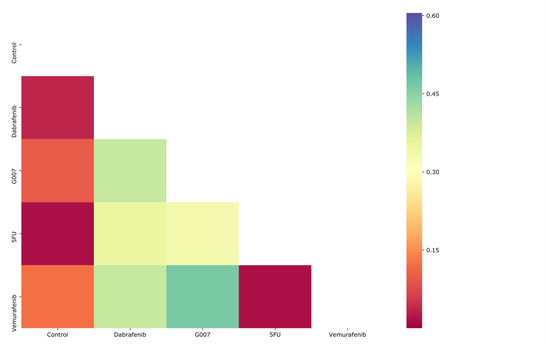
1. Compute shape and texture parameters
2. Classify and search for patterns to characterise the response



Compound dose response data

DRUG SIMILARITY

- For each drug we trained a NN to classify response for remaining 3 compounds.
- Over 95% accuracy in predicting which drug the organoid has been exposed to.
- Similarity = fraction of cells which are identified as having been treated with a given drug.



CONCLUSIONS

1. Light sheet techniques are particularly well suited to fast volumetric imaging of 3D cell cultures, spheroids, organoids and model organisms.
2. Morphological image analysis offers a way to quantify therapeutic effects in organoids.

FURTHER APPLICATIONS

- Morphometric imaging is being applied to study respiratory toxicity of aerosols under the EMPIR AeroTox project (http://empir.npl.co.uk/aerotox/) using various lung models including: air-liquid interface; repopulated lung scaffolds and lung organoids.
- Tissue imaging (including biodistribution of therapeutics).
- Organ on a chip.
- Collaborations, proof of concept studies sought for the application of morphometric imaging.

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