Industrial-Scale Organoid Screening: Next Generation High Throughput Solution

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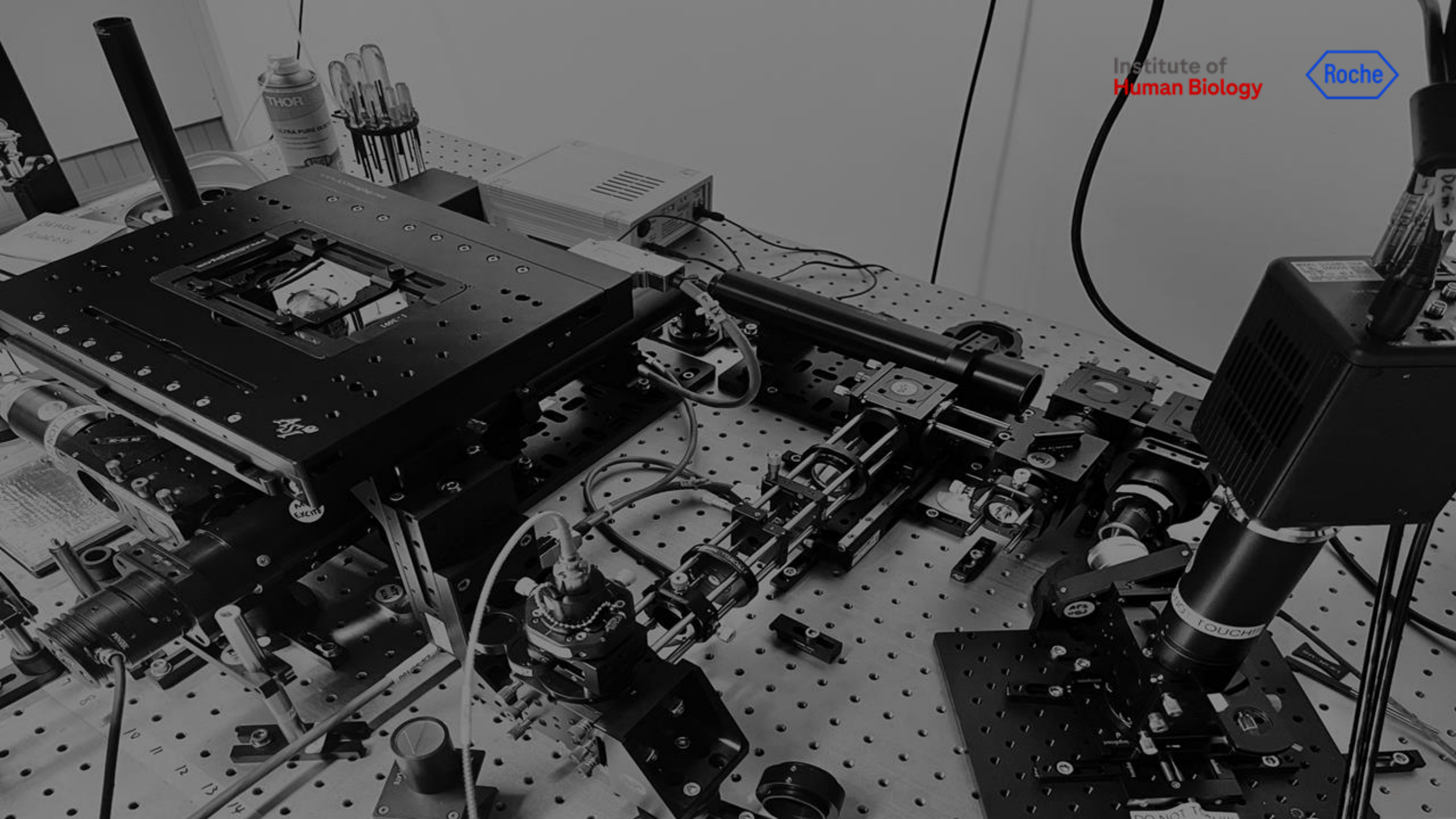
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Why organoids for screening (vs 2D/ animals)

Organoids bridge the gap between simple in vitro models and complex in vivo biology.

- 3D architecture, cell heterogeneity, tissue-like organization.
- Derived from primary tissue / iPSCs → patient-relevant.
- Capture structure + function (polarity, lumen, differentiation, etc.).
- Already used for: oncology drug response, toxicity, genetic disease modeling.

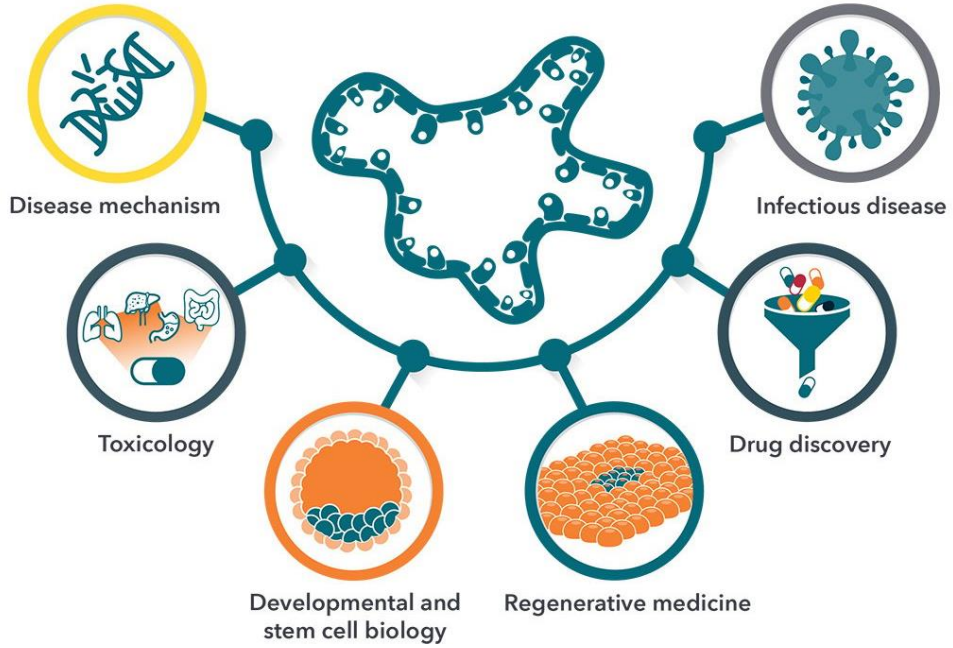
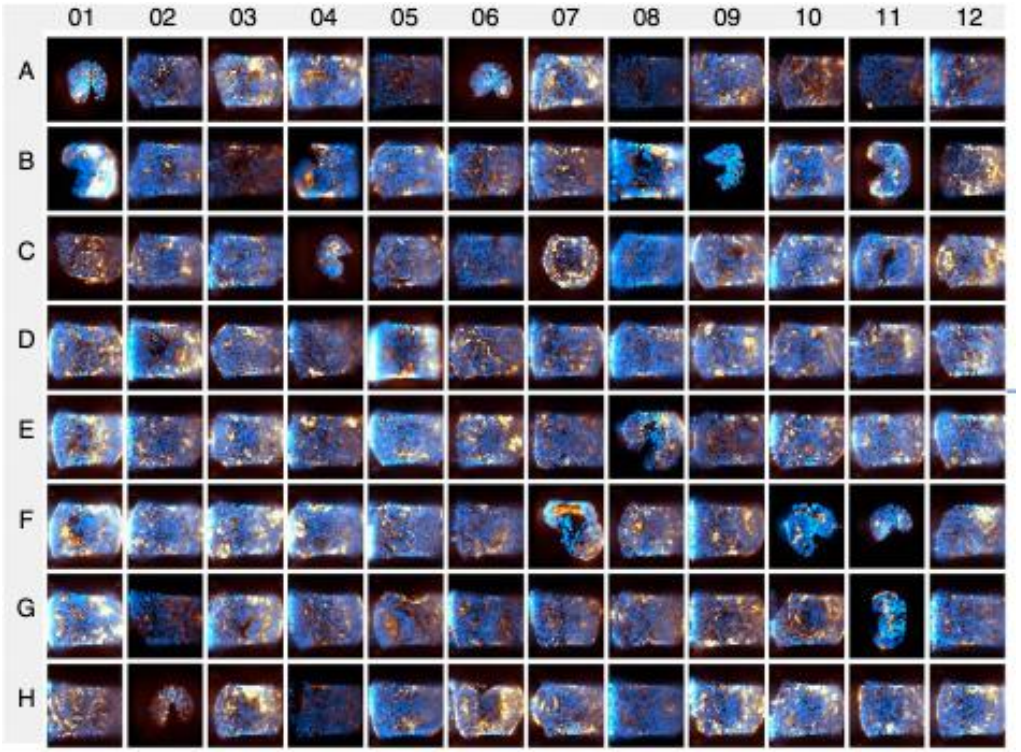


Image from https://blog.crownbio.com/key-applications-of-organoids

Organoid screening as a “factory” pipeline

Organoid screening in pharma is an industrial pipeline with multiple bottlenecks.

Biopsy / iPSC	Biopsy or iPSC derivation
Expansion & banking	Organoid expansion & banking
Plate setup (96/384 → 1536)	Assay miniaturization in multiwell plates / microfluidics
Drug library (robotics + liquid handler)	Compound library addition (primary screen + follow-up)
Readouts (FIS / ACC / HCI / LSFM)	Imaging / readout
Data analysis	Data integration (omics, clinical, etc.)

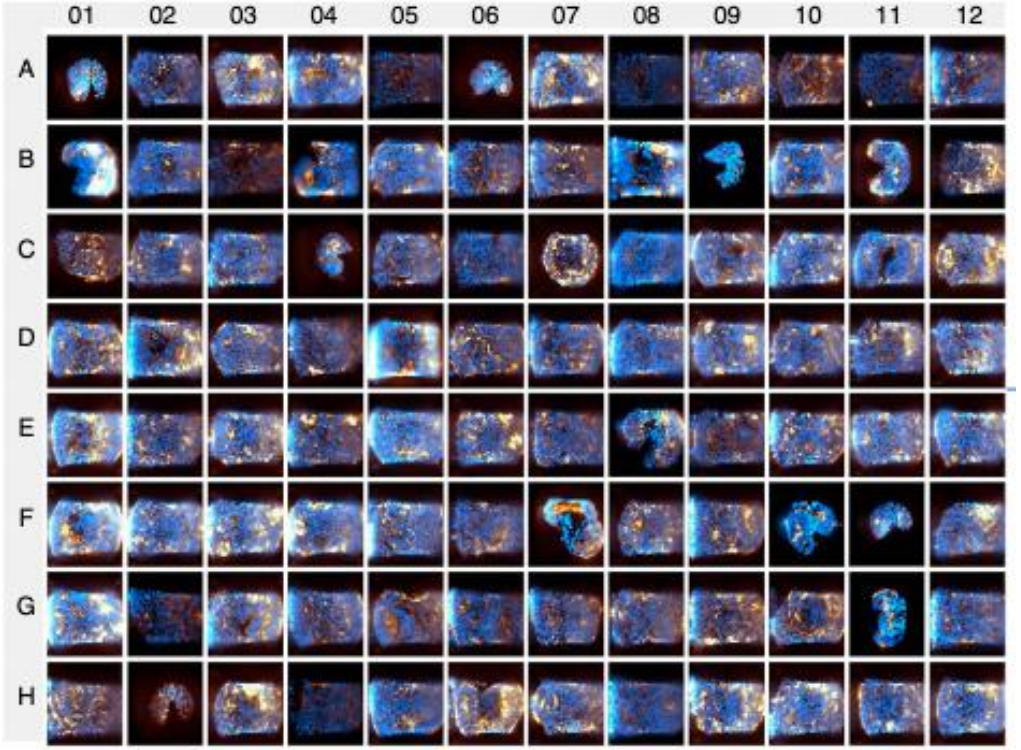


Beghin, A. *et al. Nat Methods* 1–12 (2022)

Roche aims to use organoids to enhance the drug discovery pipeline

But organoids screening impose technical challenges in data acquisition, data analysis and data interpretation.

- Challenges:
  - Drug libraries can contain tens of thousands compounds, which in turns means screening over hundred of thousand organoids.
  - Different to adherent cells (long-standing preferred model for drug screening), organoids are three dimensional in nature.
  - Given the complexity of organoids (i.e. different cell types), multichannel acquisition is necessary.



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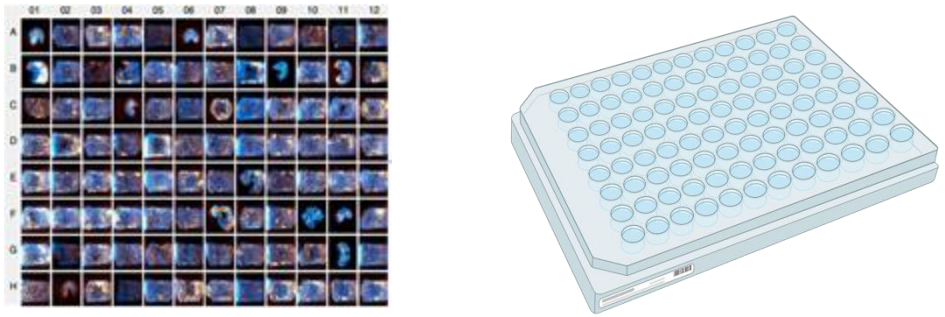
We need better technologies to increase the organoid phenotyping and screening throughout

Until now scientist have relied largely on confocal microscopy to perform screening using imaging methods, but it is not realistic for an organoid-based screening.



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If we consider an easy experiment:

- 1 organoid per well
- 4 channels
- 500 x 500 x 500 um
- 100 plates = 9600 volumes
- 5 um Z step size
- 3,840,000 single images

Solution:

- Yokogawa CV8000
- Several weeks of experiment
- 800,000 Dollars

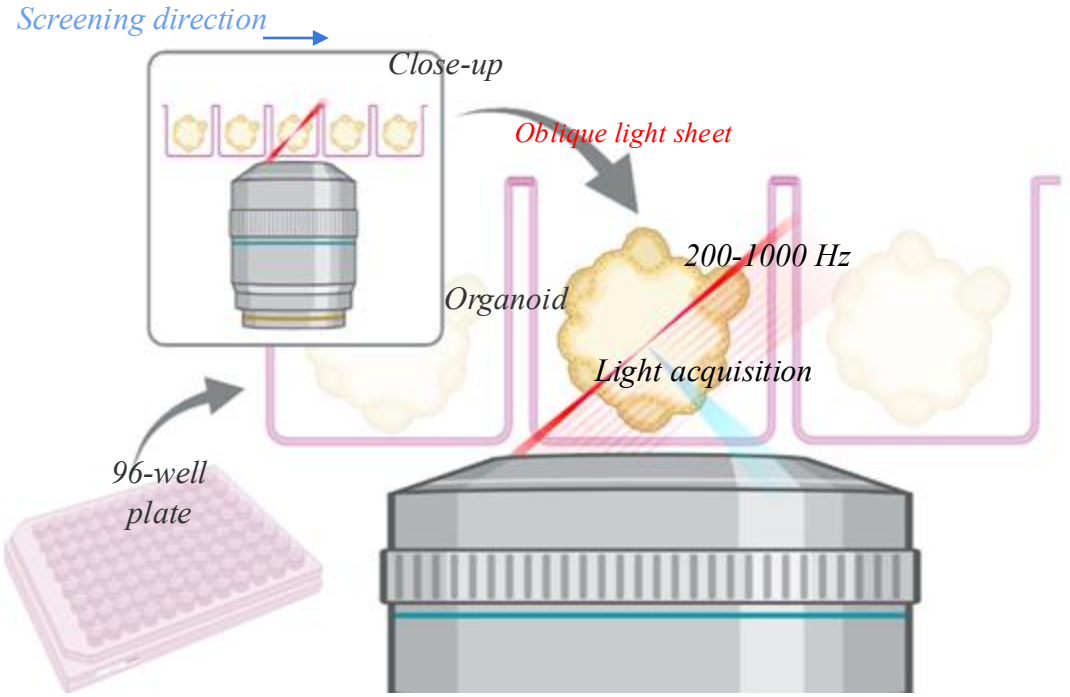
OPM is a promising technology that aims to increase significantly the throughput of volumetric imaging.

Oblique Plane Microscopy (OPM) is a light sheet fluorescent microscopy technique that uses a single high numerical aperture microscope objective to both illuminate a tilted plane within the specimen and to collect fluorescence from the tilted illuminated plane.

The advantages are:

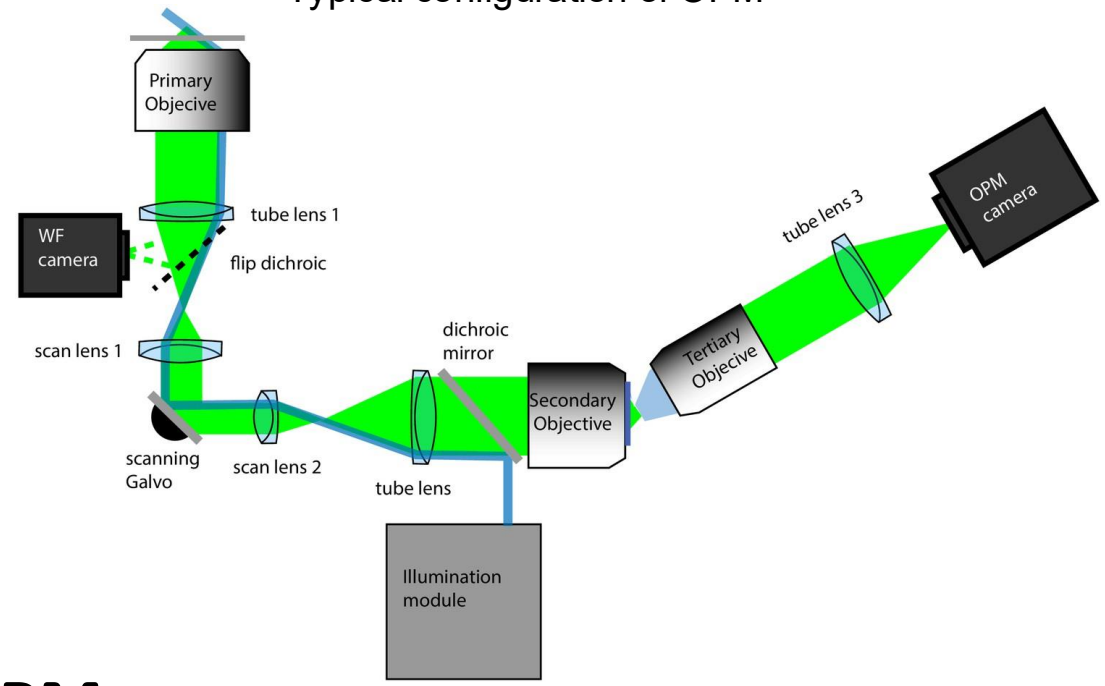
1. High throughput
2. Tailored for standard labware (eg. 384 well-plate)
3. Low phototoxicity
4. Low photobleaching
5. High signal to noise ratio
6. High NA objective of O1 is possible
7. In some cases quasi-isotropic resolution

Single objective light sheet microscope concept for high-throughput screening

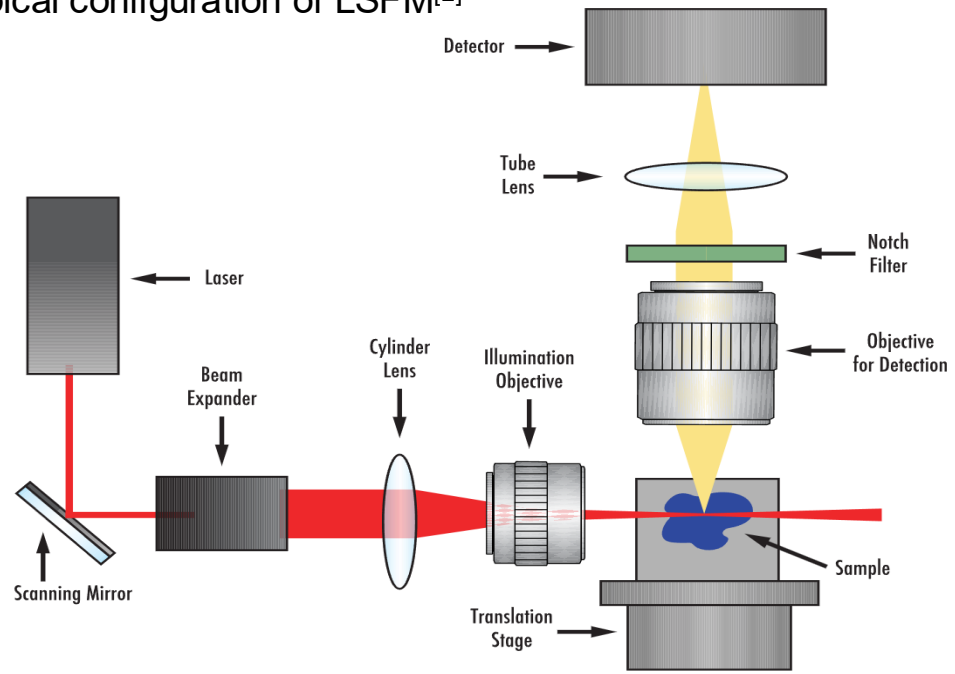


Comparison Between LSFM(Light sheet fluorescence microscopy) and OPM

Typical configuration of OPM[1]



Typical configuration of LSFM[2]



OPM

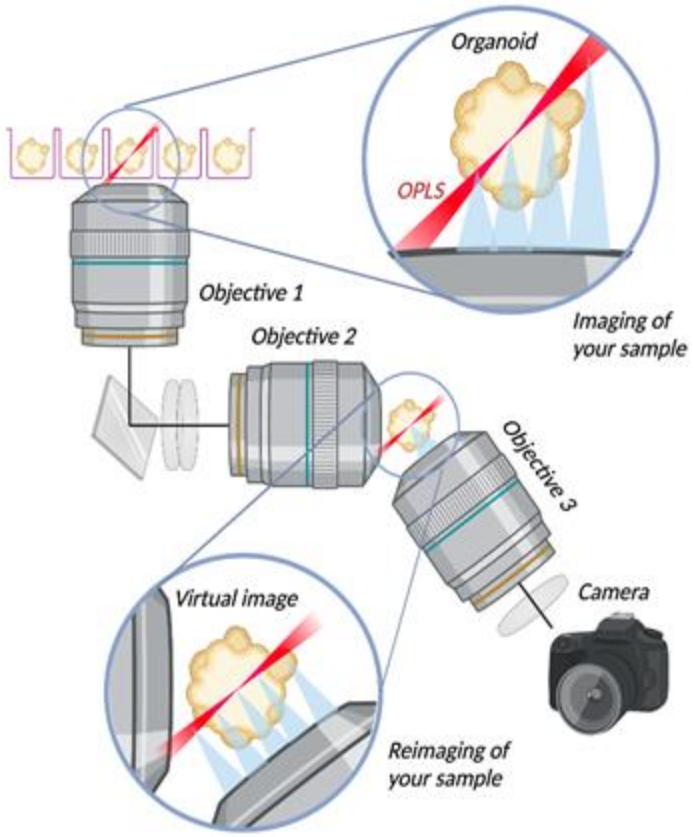
- Able to use **standard labware**, long scanning is possible
- Single objective for both illumination and detection
- Complex system for alignment
- Deskew is needed to reconstruct the original 3D structure

LSFM

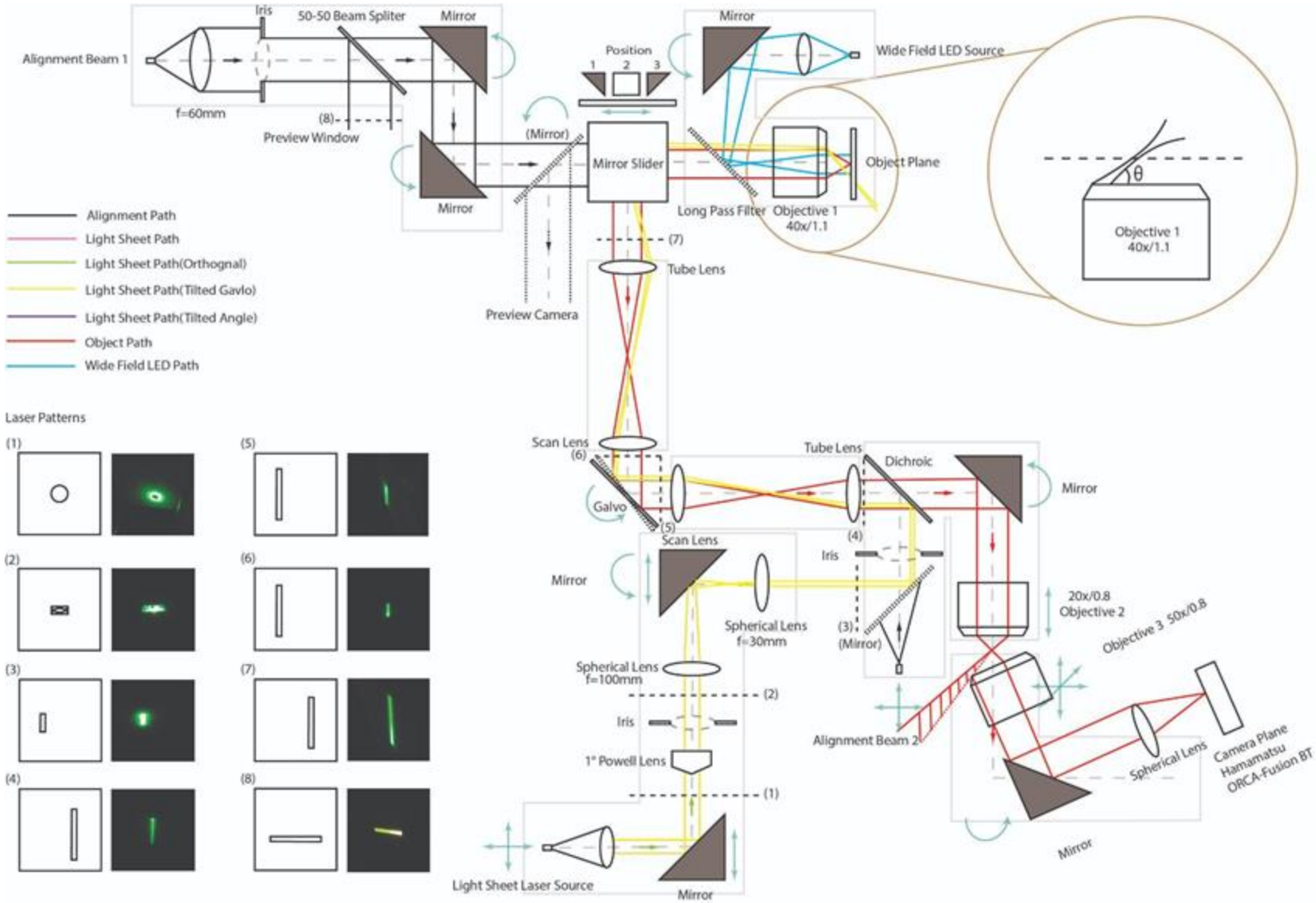
- Two objectives are needed
- Multiple commercial system is available on market
- Intuitive FoV for 3D reconstruction
- Very time consuming for sample mounting

[1]OPM figure from A versatile oblique plane microscope for large-scale and high-resolution imaging of subcellular dynamics. eLife, 9:e57681, nov 2020.
[2]LSFM figure from :Light-sheet microscopy: a tutorial. Adv. Opt. Photon., 10(1):111–179, Mar 2018.

Technical Characteristics of OPM — Optical Path



Simplified optical path for illustration



Optical path of OPM

A Video to show How a customized microscope is being built

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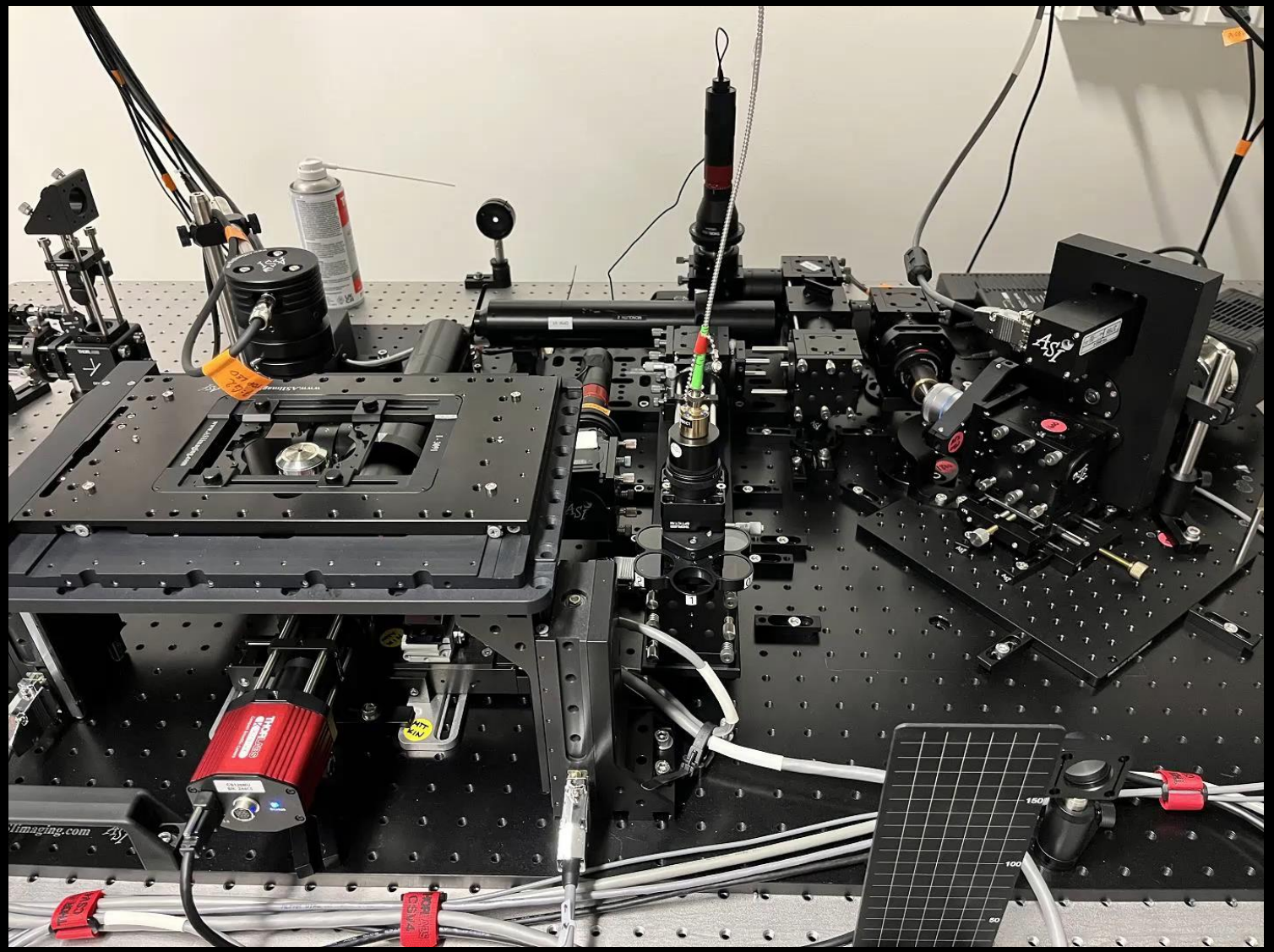


…2-3 months of work in 45 seconds…

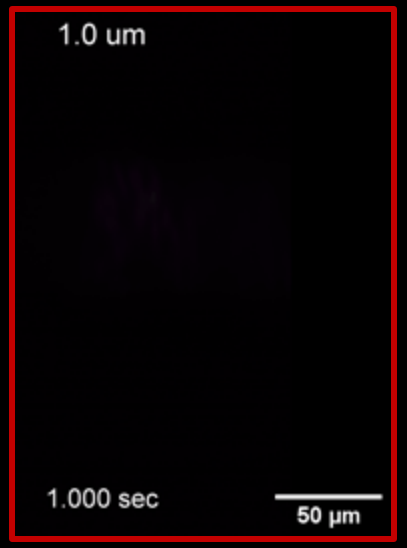
Sample data – fix cells(we can also imaging live cells)

- A few experiment were performed on multiple different organoids: colon organoids, lung organoids and retinal organoids

Lung Organoid
2 channels @ 60 Hz

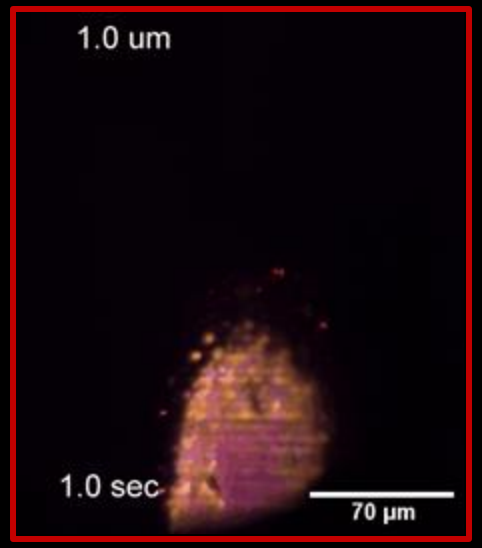
448 slices / volume

Gut Organoid
3 channels @ 90 Hz



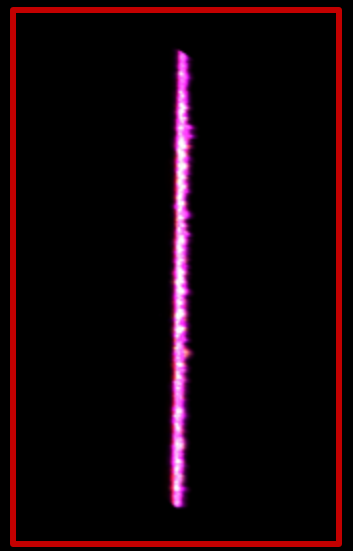
1644 slices / volume

Retinal Organoid
4 channels @ 120 Hz



2800 slices / volume

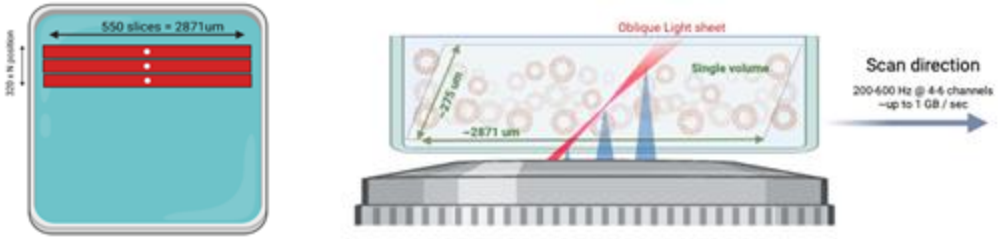
Assembloid CRC
4 channels @ 120 Hz



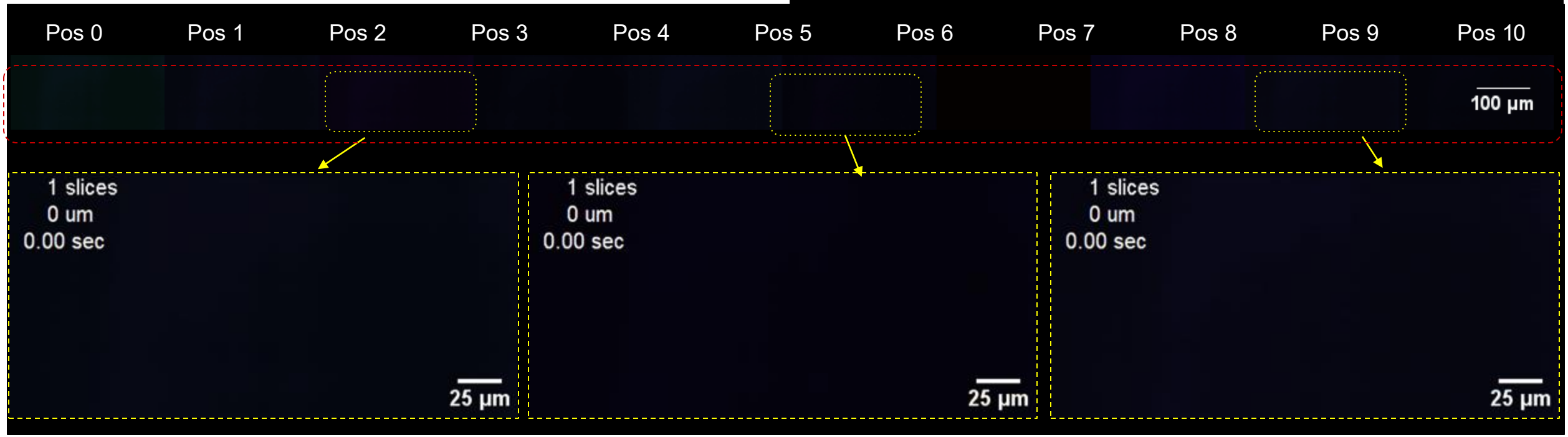
4000 slices / volume

How does an OPM scan a well plate

Results: We are able to screen organoid screening units (wells) using the OPM with a significant increase of speed when compared to the state of the art screening microscopes.

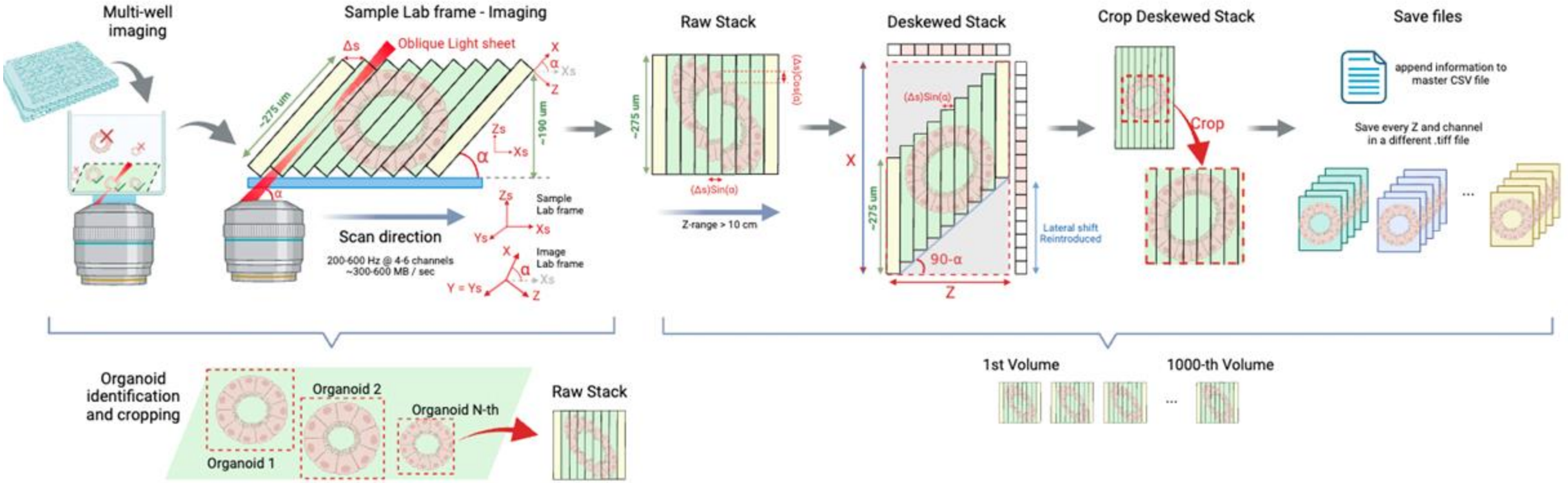


14850 slices : 260 x 2300 um x 2245 um
12.5 ms/slice/channel : 80Hz : 160 seconds/well
3 channels on a single chip
1x1 binning : 16 bit : 3.3 MB / image : 4.3 GB OPM line scan : 47.3 GB per well: 18.2 TB per late (E)
110 seconds/well ~ 17 hours per plate (E)

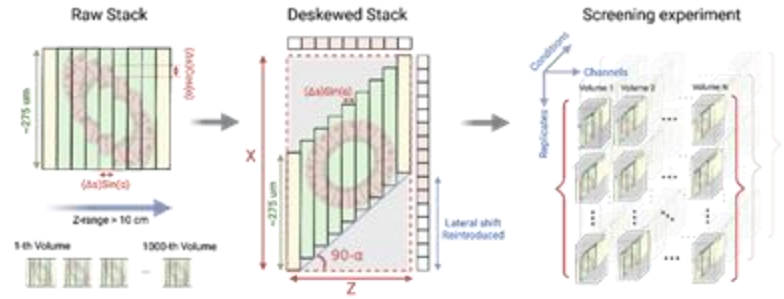


Data Pre-processing

Results: To make the data compatible to the screening pipeline of the Organoid Phenotyping imaging pipeline every organoid needs to be identified, extracted, deskewed, rotated, and mapped it into the Imaging pipeline format.



EPFL


Raw Stack
Deskewed Stack
Screening experiment
~275 um
(Δx)Sin(α)
Z-range > 10 cm
1 th Volume
1000-th Volume
X
Z
90-α
Lateral shift
Reintroduced
Channels
Replicates

The next step – Machine Learning Segmentation/Classification

Multi-level segmentation & tracking

- Organoid + lumen segmentation
- Nuclei / cells segmented by DL (OrganoID-like)
- Track organoids and/or cells over time → trajectories & lineage trees

Feature extraction (per organoid & per condition)

- **Structure:** volume, surface, sphericity, lumen volume, number of buds
- **Dynamics:** growth rate, lumen opening/closing, budding dynamics
- **Cell fate:** division rate, apoptosis onset, spatial pattern of dying cells
- **Heterogeneity:** variance between organoids in the same condition

Drug response modelling

- Compress features into **phenotypic scores** for each drug/dose

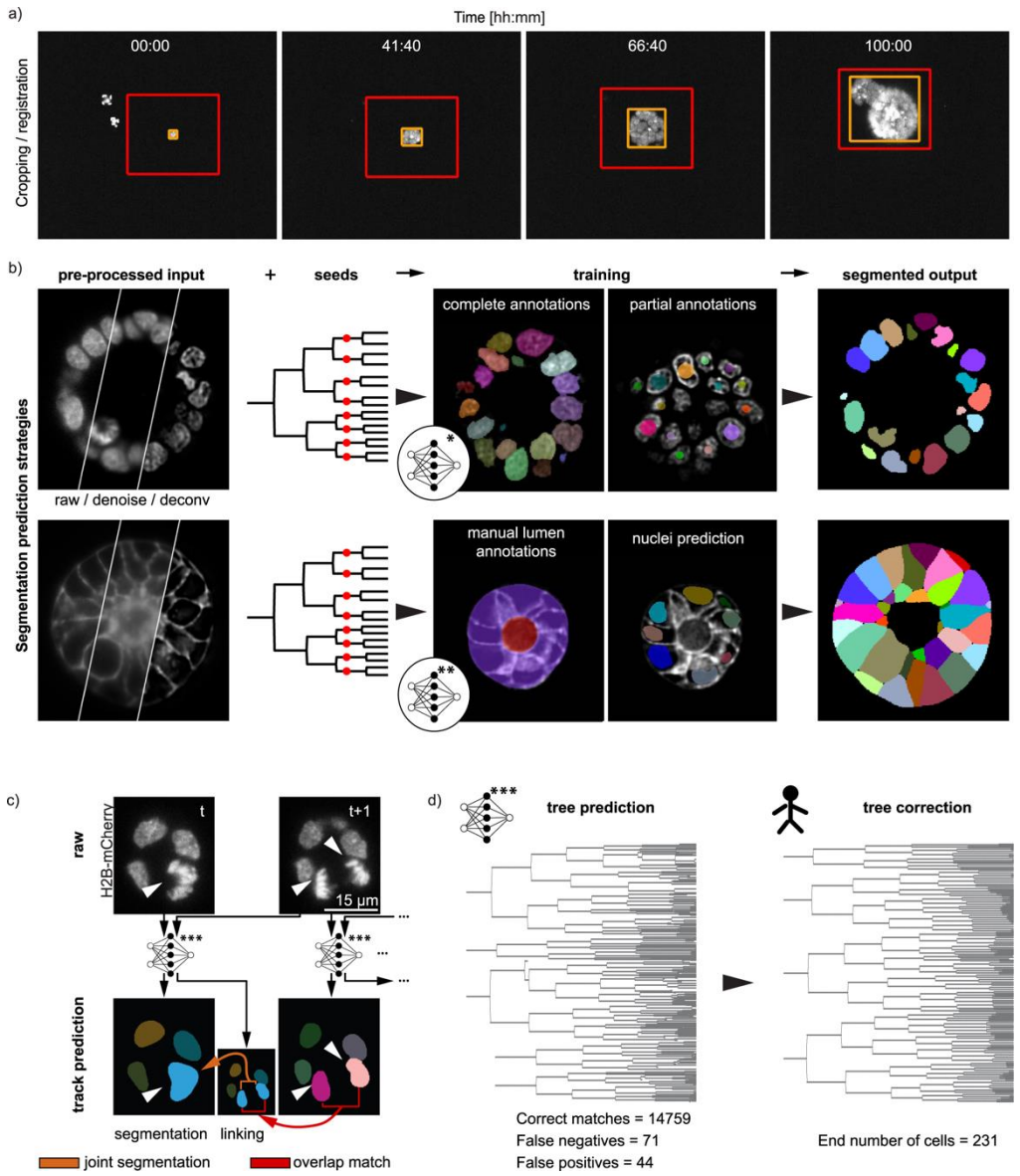


Image from: https://www.nature.com/articles/s41467-022-32465-z

Thanks for listening

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