Automated **Analysis and Sorting** of 3D cell based Models

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Background & Aim

3D cell based models show promising potential to be next generation gold standard test systems for many fields of fundamental and applied life sciences. Despite the increasing interest, high scale production of suitable candidates for testing and further processing is limited by unstandardized protocols. Individual candidates may vary dramatically between different production batches and even within the same batch due to the inherited biological complexity of the model systems.

Therefore, efforts directed to 1) reduce batch heterogeneity by protocol improvements and 2) create automated methods to select suitable candidates are of major importance.

To overcome the technological gap in the production process, we developed low-cost classification and separation technologies for macroscopic spherical organisms (figure 1) with the following specifications:

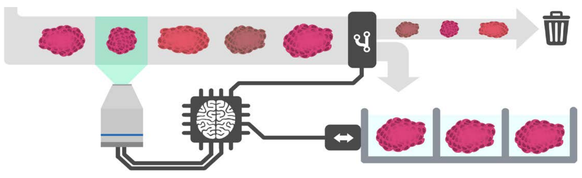

Figure 1: General process flow of image acquisition and sorting.

- Fluidic-based sphere trafficking
- Image acquisition via light microscopy
- AI-mediated candidate selection
- One candidate per second

The technologies are capable to isolate single entities from a pool and enable automated and un-biased transfer of candidates with desired morphology into collection tubes or MTPs of any format. The goal is to establish high-throughput analysis platforms that can be operated as benchtop systems or be incorporated into fully automated facilities to further increase processing rates.

Sorting of Spheroids and Organoids

3D cell aggregates strongly benefit from non-microfluidic sorting technologies to reduce shear forces that might interfere with the integrity or physiology. For that purpose, we developed the *ORGANICER*, a novel technology for **analysis of morphological properties and candidate selection** of spheroids and organoids (figure 2).

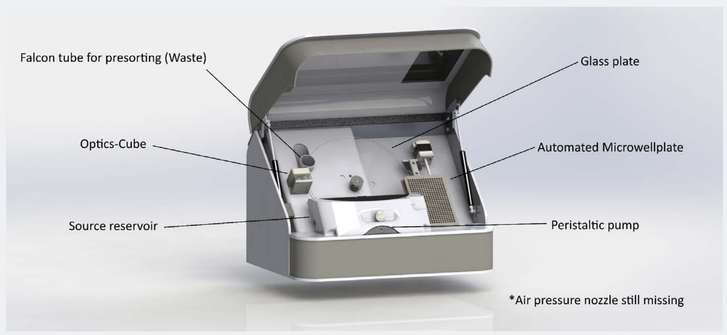

Figure 2: ORANICER demonstrator.

The spheres in suspension are introduced into a source reservoir and separated by a glass plate. **Each individual sphere is investigated** by light microscopy image acquisition in the optics-cube, followed by evaluation by the operator.

The spheres are ejected out of the system by an air-pressure pulse into target carriers, including flacon tubes as well as microwellplates of various formats (figure 3). In addition to sorting, the *ORGANICER* is capable to **isolate single spheres into each well of a microwellplate**, thus enables fast assay preparation.

Sorted spheres showed **no impact on integrity or physiology** compared to a control (figure 4). Initial shrinking was recovered after culturing the spheres for additional 24 hours after sorting and might be related to osmotic effects.

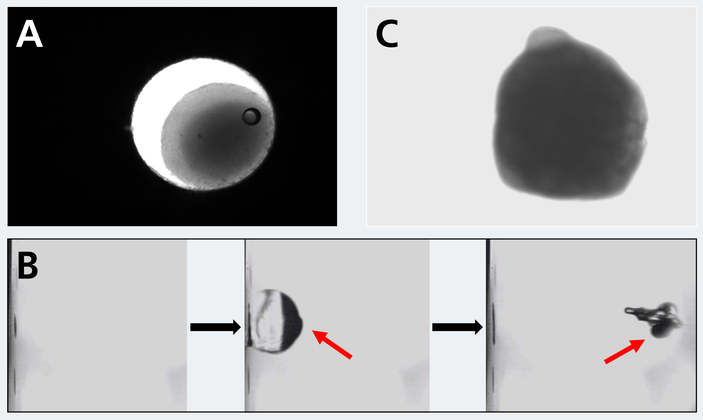

Figure 3: Sorting of organoids. (A) Organoid within the sorter. (B) Isolation process with organoid (red arrow) inside of a liquid droplet. (C) Integrity of isolated organoid.

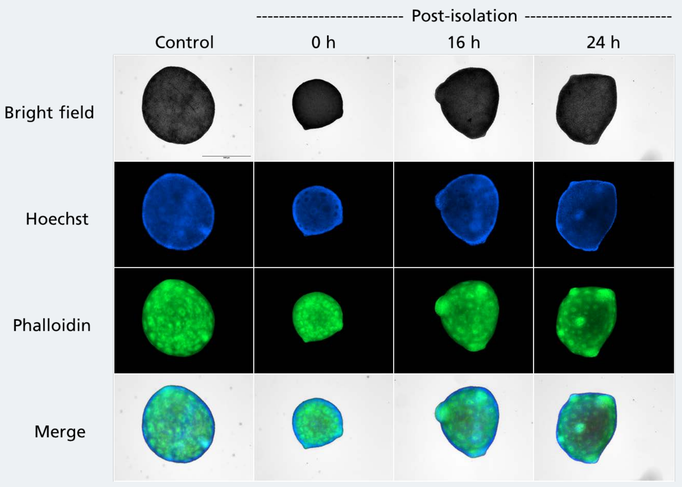

Figure 4: Integrity of isolated organoids compared to an unsorted control after fixation. Similar molecular patterns were observed in all samples (scale = 1.000 µm).

Sorting of Zebrafish Eggs

Fertilized eggs from Zebrafish *D. rerio* are used in fundamental research to study developmental biology in vertebrates. However, **only a fraction of total eggs are successfully fertilized** during insemination and isolation of fertilized candidates from unfertilized or damaged eggs is an essential but time consuming process, which has to be performed at the very early stage during embryonic development. By light microscopy, each individual egg has to be separated and investigated manually for characteristic morphology and transferred into an assay plate for further processing (figure 5).

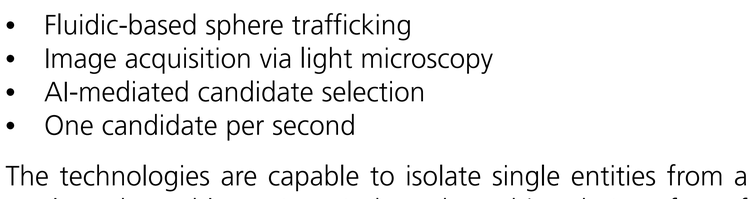

Figure 5: Different morphologies of Zebrafish eggs after insemination. (A) Damaged egg with disrupted yolk. (B) Unfertilized egg with intact yolk (orange circle). (C) Fertilized egg with two cells (red circle). (D) Fertilized egg with four cells.

We developed a new technology based on microfluidics, light microscopy and an AI machine learning algorithm to **analyse and sort Zebrafish eggs according to their fertilization state** in a fully automated and high throughput process (figure 6).

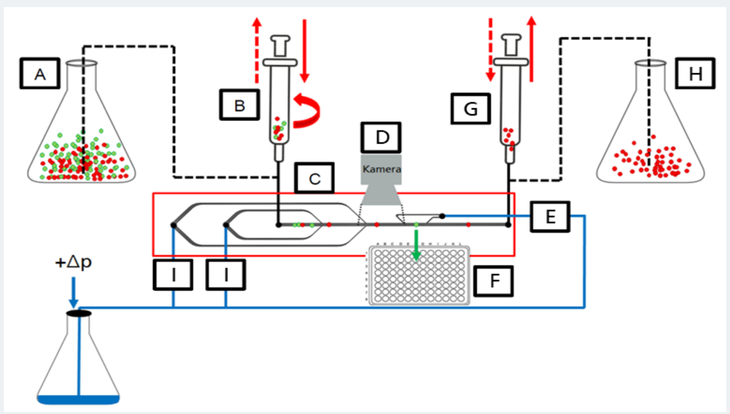

Figure 6: Principle of the Zebrafish egg sorter. (A) Heterogeneous pool of fertilized (green) and unsuitable (red) eggs. (B) Pumping syringe for dispersion of the pool. (C) Microfluidics. (D) Camera unit for light microscopy image acquisition. (E) Ejection port for the target carrier. (F) Target carrier. (G) Pumping syringe for dispersion of the waste. (H) Waste container. (I) Pressure valves.

Once the algorithm is trained for morphologies of interest, heterogeneous pools of Zebrafish eggs coming from insemination are introduced into the setup, followed by **image acquisition and AI-mediated classification**. Suitable candidates are sorted out of the pool and may also be individually isolated into a target microwellplate for automated assay preparation.

We integrated the technology into a benchtop demonstrator and developed a user-friendly software for experiment setup (figure 7).

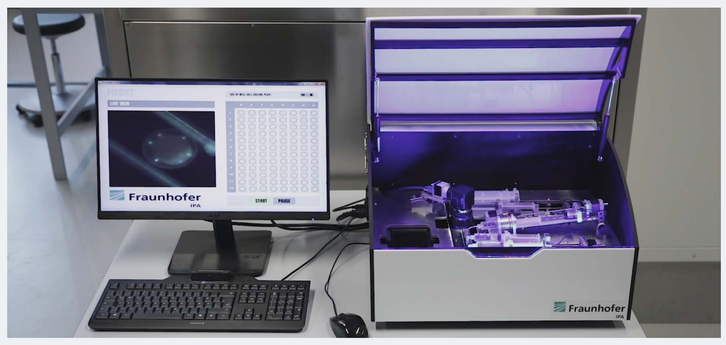

Figure 7: Benchtop demonstrator of the Zebrafish egg sorter.

Conclusion & Next Steps

By early exclusion of abnormal samples based on parameters such as size, shape and texture, we obtain homogeneous cultures to reach comparable read-outs in later applications and reduce material costs as well as labour time.

In this poster, we present novel technologies for the automated and gentle isolation of 3D macroscopic spheres from suspension based on light microscopy image acquisition in a high throughput pace. By using these technologies, we are able to isolate fertilized candidates from heterogeneous batches of Zebrafish eggs or analyse and sort multicellular spheroids and even organoids according to the morphological profile.

By addressing the overall integrity and physiology after isolation, we demonstrate suitable workflows to identify and isolate candidates of various different biological model systems for further processing, including but not limited to the model systems presented.

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