

CellXpress.ai Automated Cell Culture System: Automated workstation for reproducible organoid cultivation

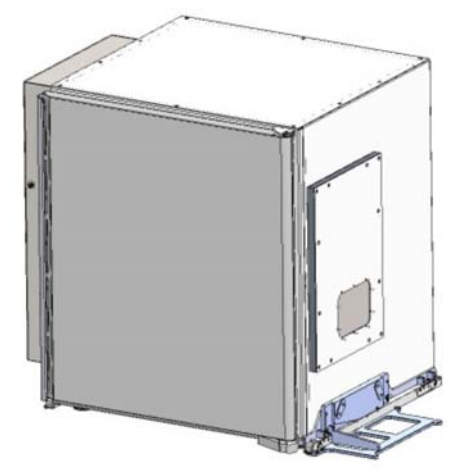
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Introduction

Within the past few years, many publications advocate the promise of organoids to improve clinical trial success and enable personalized medicine. However, organoid research is suffering from a lack of standardization and high organoid-to-organoid variability. These challenges, together with difficult cultivation techniques, are impeding the wider adoption of organoid technologies. To overcome these limitations, we have developed the CellXpress.ai™ Automated Cell Culture System. This highly integrated organoid generation and cultivation workstation integrates cutting-edge hardware and software technologies with advanced biological science to automate and standardize the 2D and 3D cell culture process. From maintenance, monitoring, and incubation, through imaging, analysis, and data processing, the CellXpress.ai cell culture system delivers consistent, unbiased, and biologically-relevant results at scale. To support scientists at every level of their organoid research, the system guides the user to set up and execute iPSC, tumoroid, and adult stem cell-derived organoid workflows. Key features of the CellXpress.ai cell culture system; (1) Automated cell monitoring using built-in incubation, liquid handling, media exchange, and imaging. (2) Machine learning-assisted automated decision-making to trigger events like feeding, passaging, or compound addition. (3) Automated quality control and sample tracking throughout the entire lifetime of an organoid or iPSC. (4) Minimized human error with assisted protocol creation, cell monitoring, and consumables handling. (5) Unified software package to control hardware, execute workflows, and acquire and analyze data. (6) Newly developed flexible scheduler to support multiple workflows in a multiuser environment and to track the cell journey, consumables usage, and media exchange.

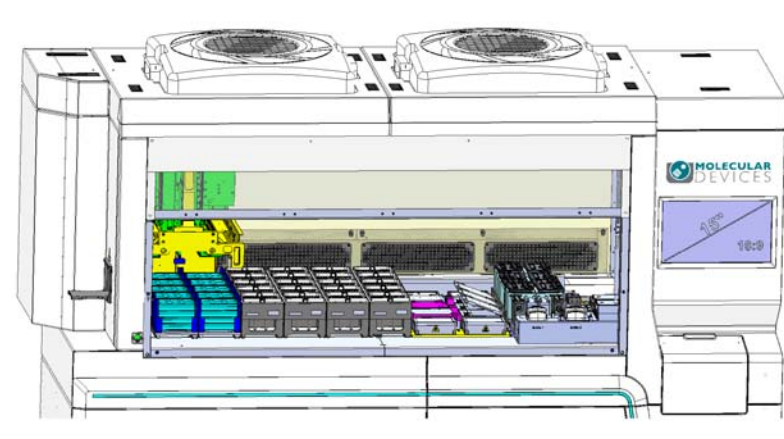
Hardware key features

Automated incubator



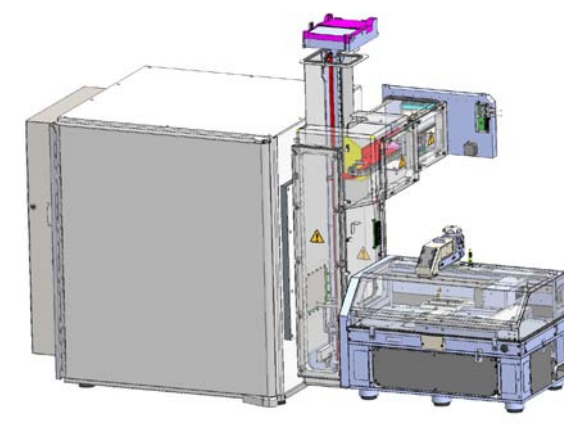
- Capacity between 44 or 154 plates
- Active humidity regulation
- Automatic decontamination system

Liquid handling deck



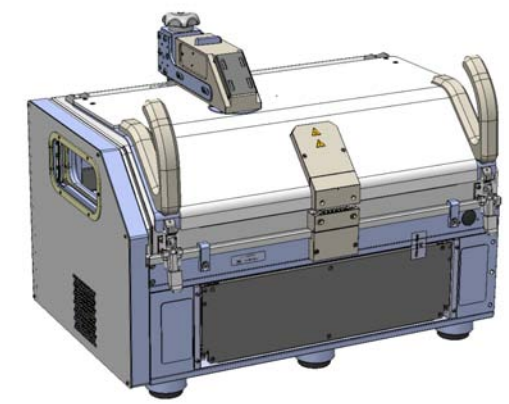
- 8 independent channels
- On-deck storage: 20 plates and 20 tip boxes
- 4x titling, 2x heating-cooling, 2 heating positions
- Hepa filtered incoming air
- UV-decontamination system

Internal robotics



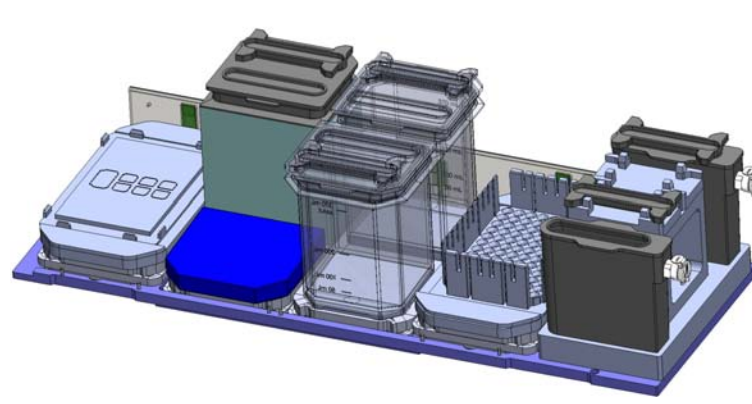
- Robust internal robotics
- Design for reliability
- 2 ports for external integration

Automated microscope



- TL, FL widefield automated imaging system
- 6-channel FL illumination
- 2x–40x objectives
- CO₂ and temperature regulation

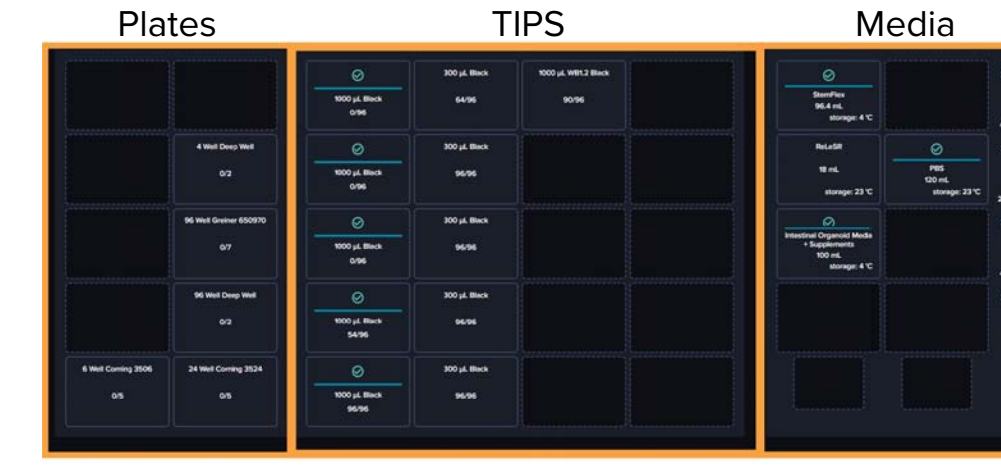
Smart media module



- 8 on-deck-vessel media positions
- 2x2L bulk media storage
- Automatic heating, cooling and stirring
- Adapter for reservoir plates
- Robot-friendly media vessels

Software key features

Simple deck configuration



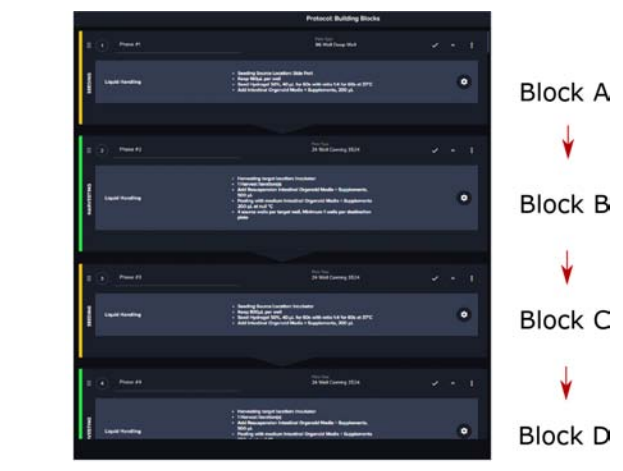
- Simple deck configuration
- Missing consumables are visualized
- Media area overview

Graphical scheduler



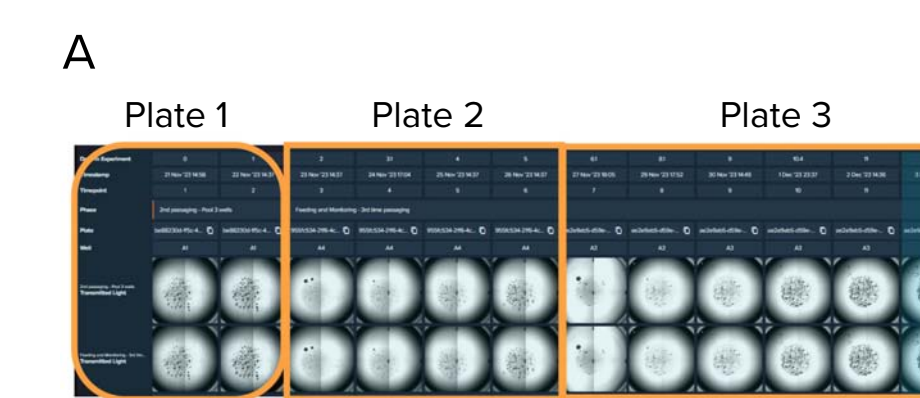
- Graphical display of experiments
- Different subsystems displayed
- Conflict visualization
- Experiment comparison

Building blocks



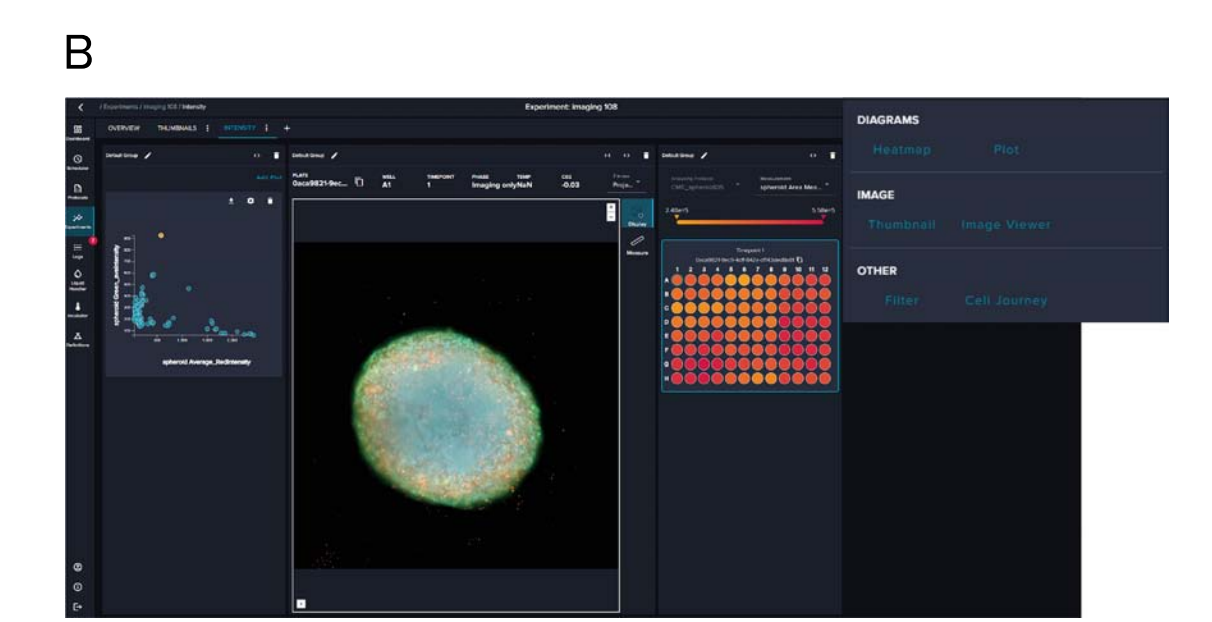
- Experiment building blocks
- Blocks can be chained
- Decision making for "phase-transition"

Cell journey images



Cell journey: Software tools to follow organoids over time and over multiple plates. (A) Visualization of intestinal organoids during a multi-week, multi-plate experiment including passaging. Selected wells are displayed during the entire duration of the experiment. (B) Widgets for image visualization, different visualizations and graphs can be added.

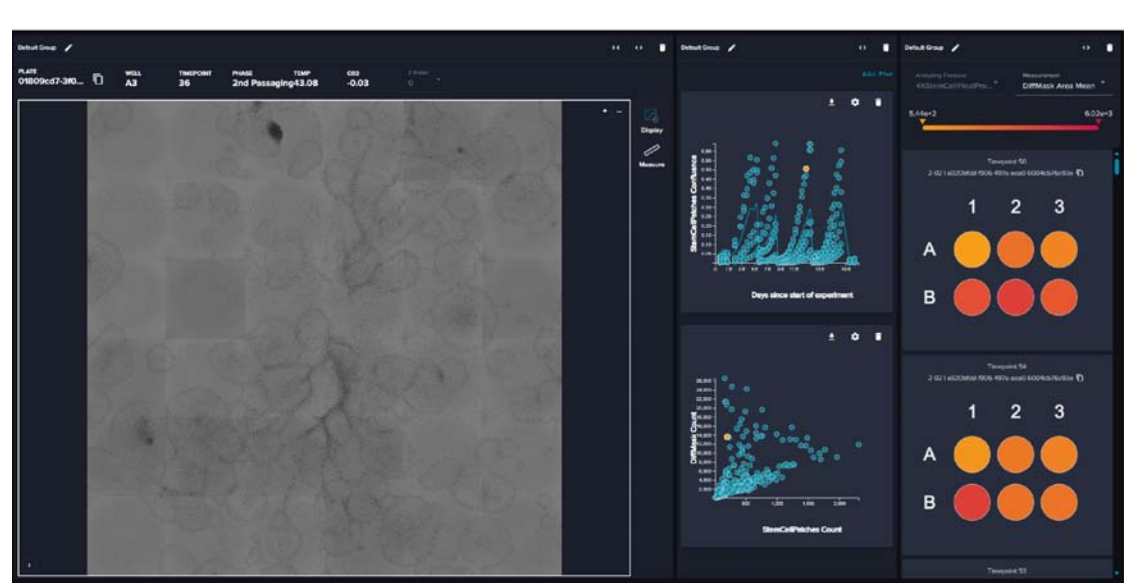
Widgets for data visualization



Workflow overview

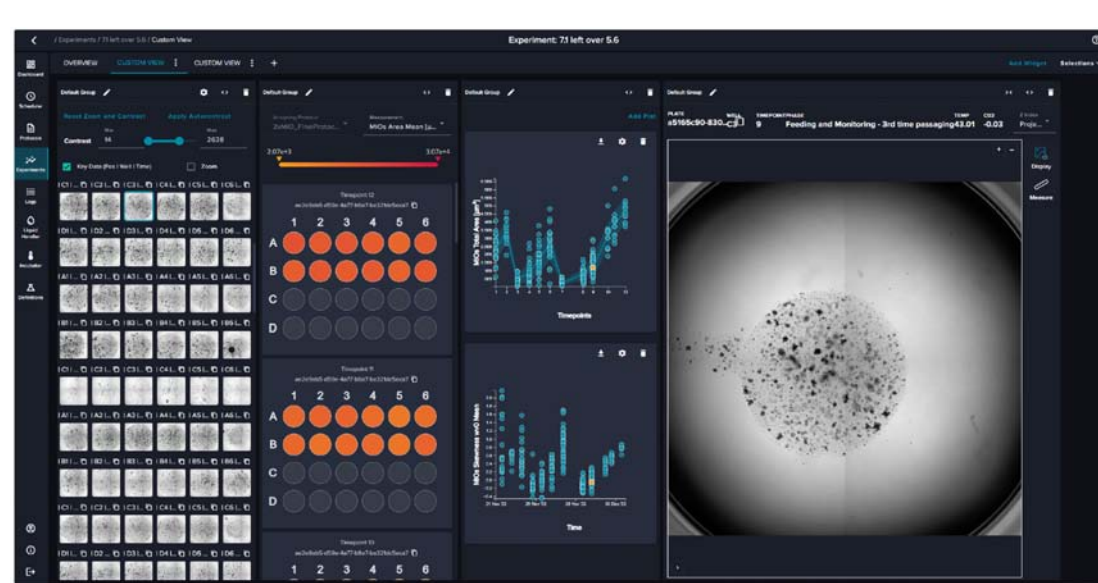
Automated culturing and passaging of iPSC lines and adult stem cell-derived organoids, as well as seeding and culturing tumoroids. The system automatically performs feeding and image acquisition. Passaging is triggered by "rules" like confluency, number of differentiated cells, or phenotypic classification. Media is automatically heated prior to usage and cooled afterwards.

iPSC workflow



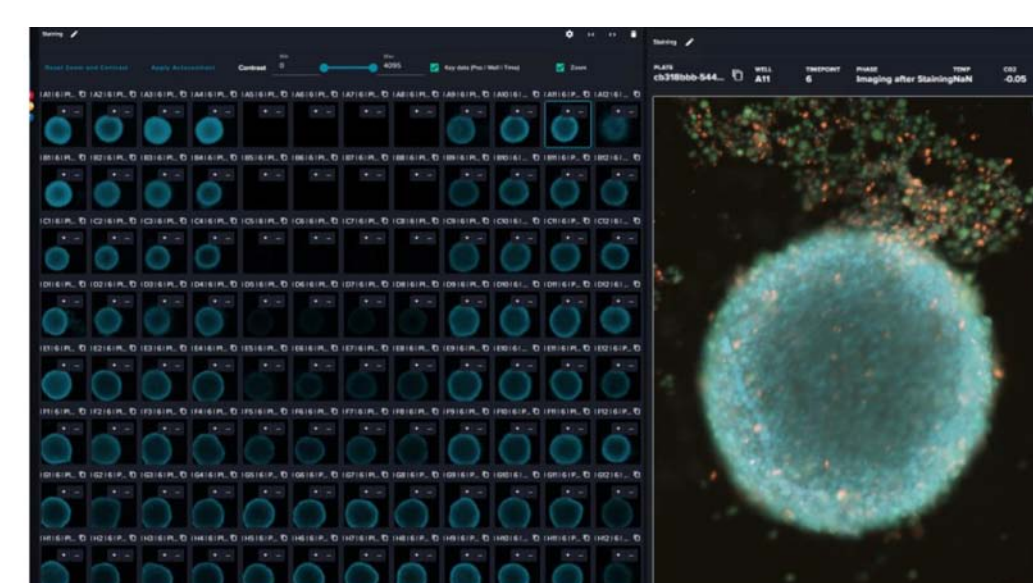
iPSC-derived stem cells were cultured for 30 days on the CellXpress.ai system. Cells were monitored daily and passaging was triggered by confluency measurement.

Intestinal organoids

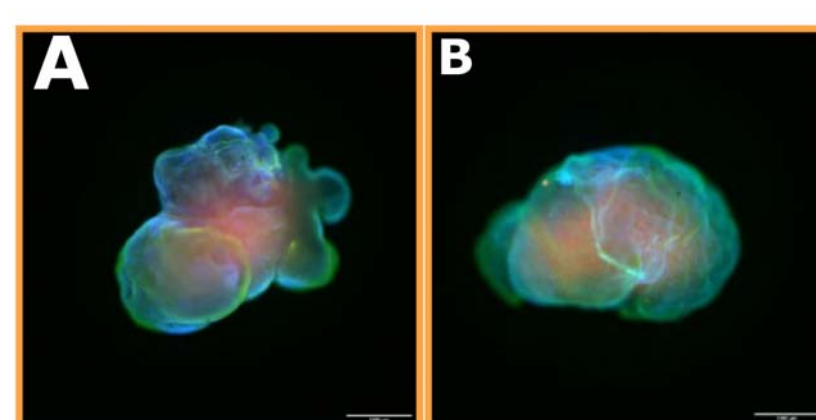


Representative picture of an intestinal organoid dome. Mouse intestinal organoids were successfully cultured for 4 weeks on the CellXpress.ai system.

Tumoroids



10K cells were seeded into 384w u-bottom plates using the CellXpress.ai system. Images were acquired every 24h using a 4x objective. Drug addition, staining, image acquisition, and analysis was conducted on the CellXpress.ai system.

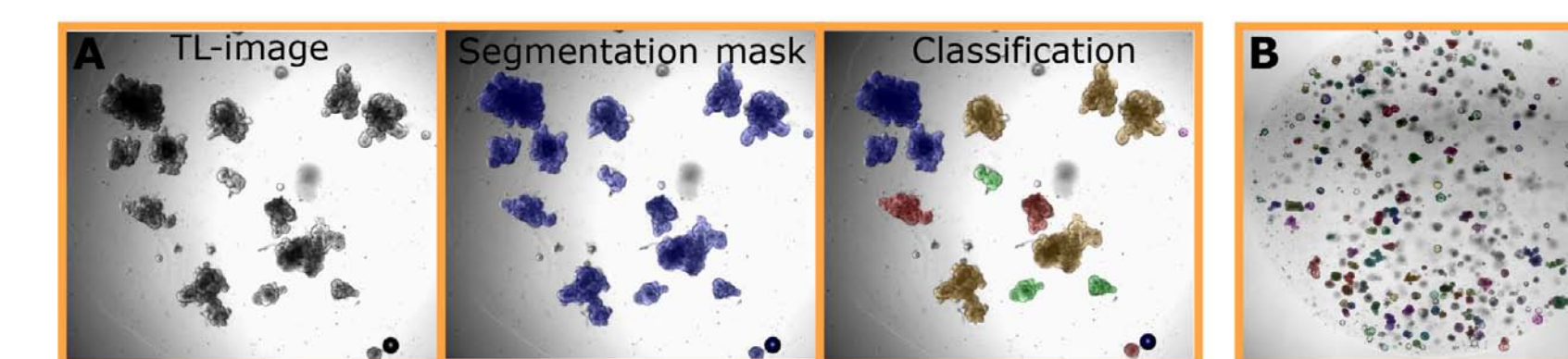


(A, B) Whole mount stain of cerebral organoids, images acquired using 2x objective.

Built in image analysis

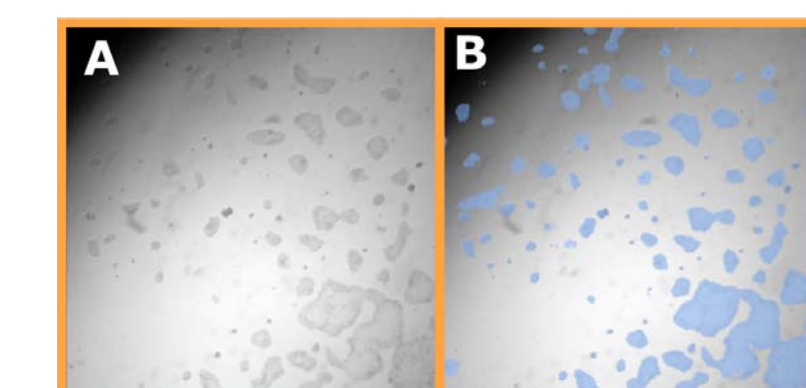
Advanced image analysis: Automatic image analysis using the CellXpress.ai automated cell culture system software package. Neuronal networks that were trained offline can be imported into the CellXpress.ai system software. Phenotypic classification can be used to build "rules" to trigger "phase-transition".

Intestinal organoid segmentation



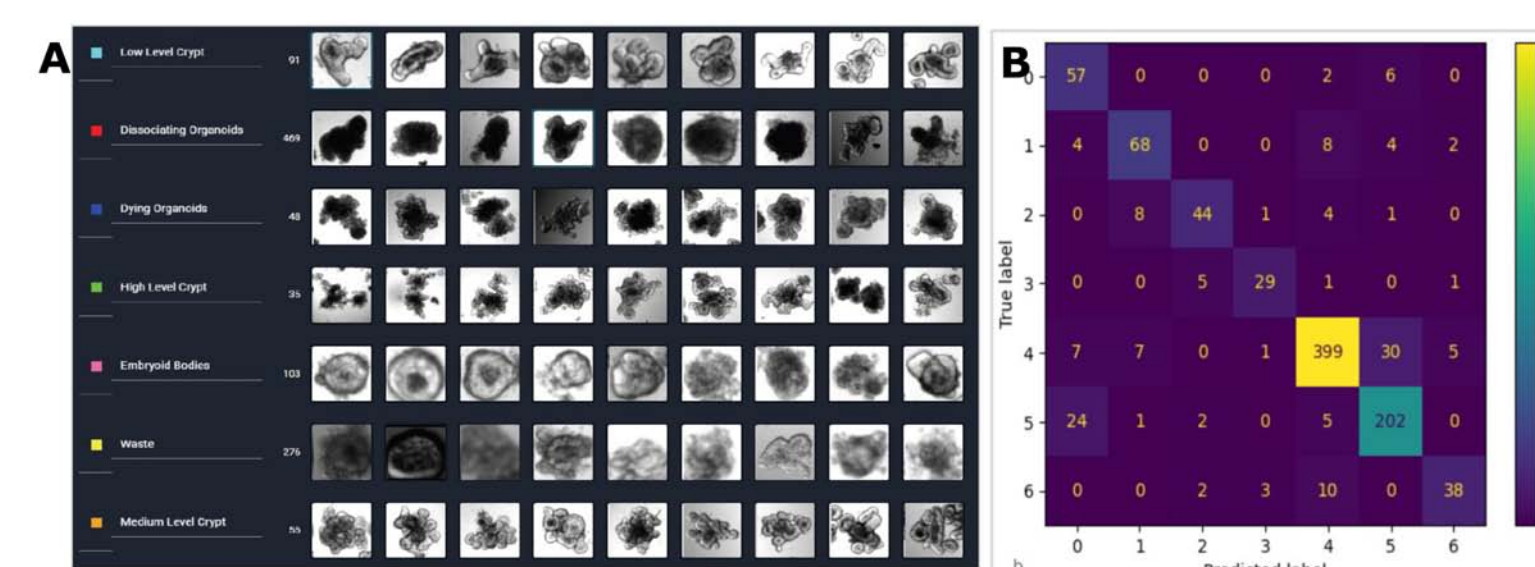
Label free segmentation and classification of organoids. (A) TL-Image of intestinal organoid, segmented and classified. (B) Representative image of a large intestinal organoid dome.

Stem cell segmentation



Label-free segmentation of stem cells. (A) Image of iPSC colonies, dark field visible. (B) Segmentation of colonies, despite the dark-field, caused by the meniscus of the media.

Label-free organoid classification



A) Intestinal organoids were acquired and segmented using the CellXpress.ai automated cell culture system. Objects were classified based on label-free features using Phenoglyps. **(B) Manually annotated dataset was compared with an annotation calculated by phenoglyps.** Diagonal values indicate good classification results. 4x objective, TL-illumination, 40 µl matrigel domes, 150 organoids/dome.