A highly automated microscopy analytics pipeline for high-throughput organ-on-chip platforms

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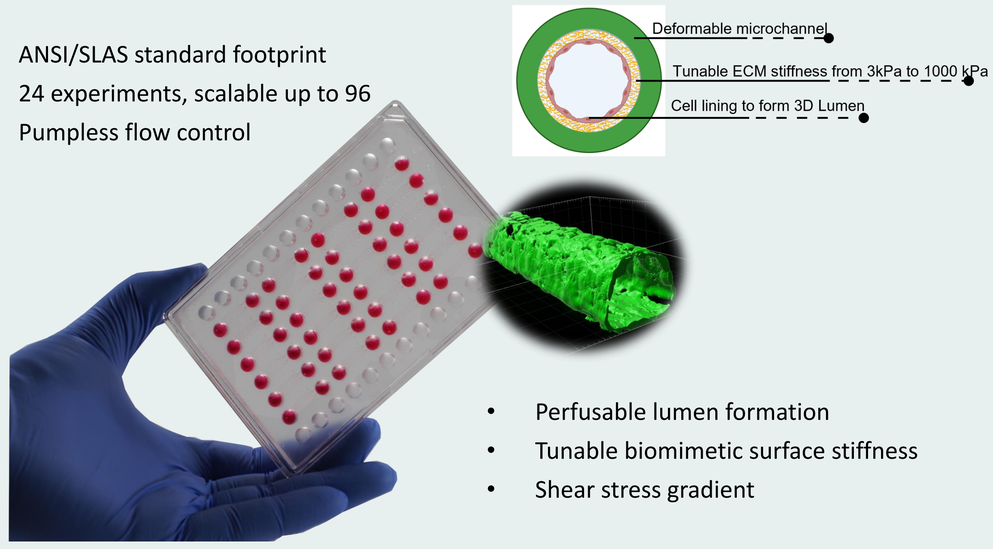
INTRODUCTION

Organ-on-Chip (OoC) technologies have been becoming a much-needed alternative for 2D cell cultures and even animal studies. These OoC systems are used to establish increasingly complex 3D tissue models. Often it is of interest to gather visual data from these models using microscopy coupled with appropriate fluorescent markers. However, many state-of-the-art OoC systems are not compatible with high-throughput microscopy. In continuation, there would be a need to process the generated data, but there is no such option readily available for research use. To address these difficulties, this research aims to establish a proof-of-concept highly automated image analytics pipeline using available tools. This is coupled with a high-throughput vessel-on-chip platform, termed AKITA plate, to establish an in vitro disease model.

- ANSI/SLAS standard footprint
- 24 experiments, scalable up to 96
- Pumpless flow control

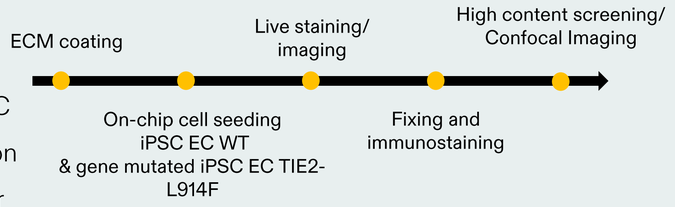


- Perfusable lumen formation
- Tunable biomimetic surface stiffness
- Shear stress gradient

METHODS

Generation of on-chip in vitro vascular model

Assessing the morphological differences of WT iPSC-derived endothelial cells (iPSC ECs) and iPSC ECs expressing vascular malformation causative TIE2 mutation L914F under the effects of flow

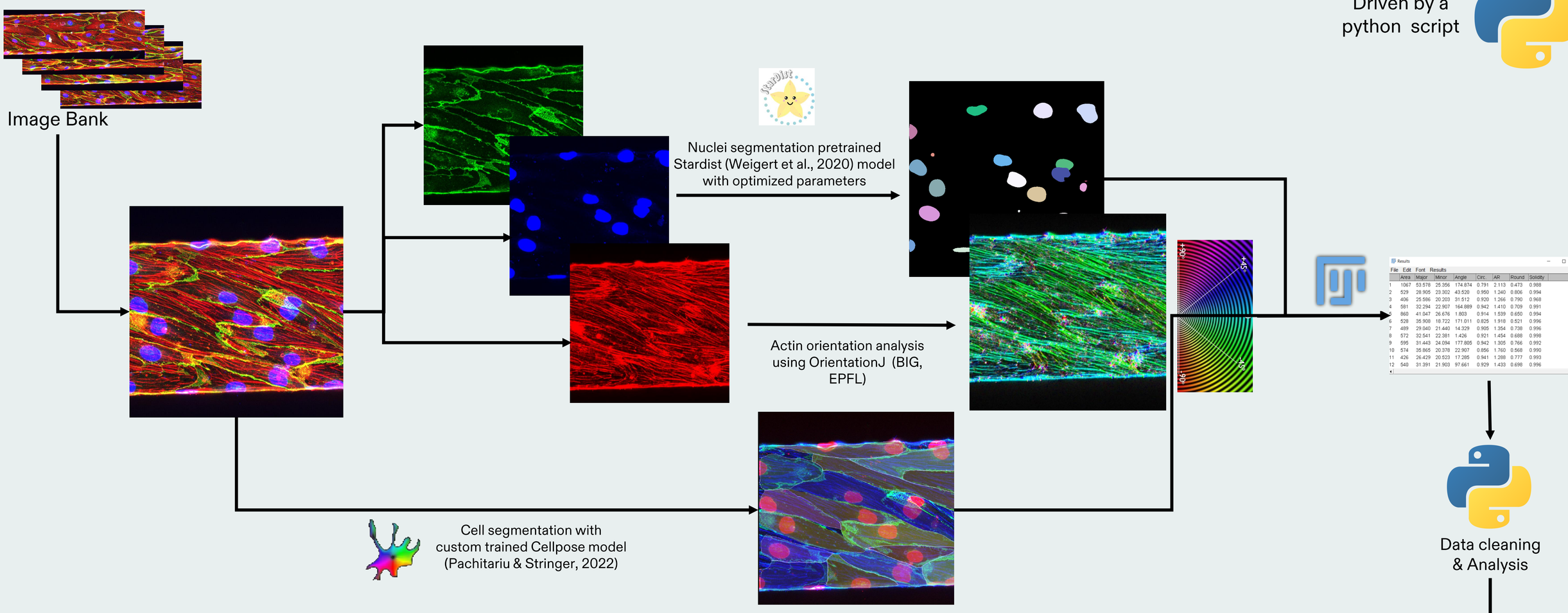


Goals of established pipeline

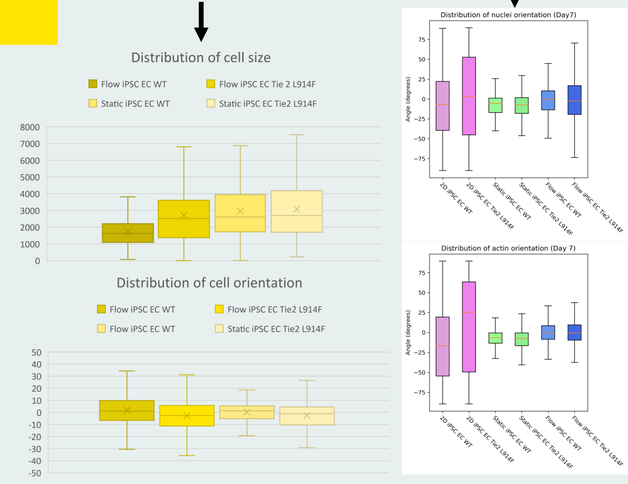
- Automated image acquisition (24 culture units, 3 zones of interest each)
- Established processing and analytics predominantly with open-source tools
- Minimize human intervention beyond set-up
- Acquire results on cell shape descriptors, nuclei descriptors, and actin orientation through the pipeline to identify differences in established models
- Identify possible drawbacks of the method

Simplified visual workflow of the automated image analytics pipeline

Driven by a python script



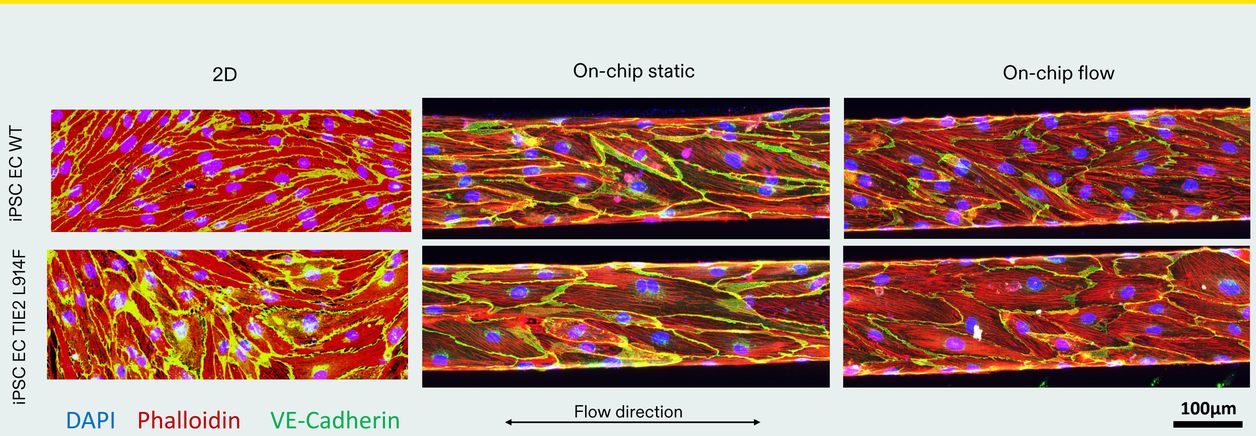
Export for custom analysis



Graphs were generated from inputted images after user-defined parameters were set. Custom analysis was possible from exported results

RESULTS

The on-chip cell cultures differ from 2D cultures in terms of morphology and cell alignment. Confined microfluidic channels help align the cells along the flow direction and the cells in 2D are largely arranged randomly. The TIE2 mutation shows more elongated and dispersed cells and less intact cell-cell junctions. In the absence of flow on the static chips, wider actin and nuclei orientation distribution and nuclei elongation.



CONCLUSIONS

We were able to establish a relevant high-throughput in vitro model. On this model we were able to showcase the ability to acquire automated images with minimal human intervention from different zones up to 24 replicates at a time. Additionally, we showcased a proof-of-concept pipeline for analyzing acquired images that was able to show differences between established conditions. This was done omitting analysis in 3D space that was seen as a challenge. Decrease in accuracy was observed due to overlapping ROIs and semi-circular channel of the model causing overlapping of ROIs.

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