Open Source Microscopy and Automation for Scalable Tissue Culture

Ryan Crane, Mechanical Engineering
Mentor: Dr. Benjamin Bartelle, Assistant Professor
Ira A. Fulton Schools of Engineering

Introduction

Human stem cell derived tissue and organoid models have been generated for every vital organ and are increasingly used in biomedical research **[1]**. For all their potential, generating organoids remains laborious, taking months of continuous attention by a highly skilled human operator. This project aims to develop an epi-fluorescence microscope to monitor cells within a 96-well plate with automated image acquisition and processing for tissue culture health and morphological upkeep using the UC2 open-source architecture **[2]** and Opentrons OT2 pipetting robot. This method overcomes the challenges of manual cell culture by improving reliability, scalability, and parallelization. This work will accelerate research breakthroughs in disease modeling, developmental biology, regenerative medicine, and other tissue culture applications afforded by organoid culture, through lab automation.

***Figure 1**: Opentrons OT2*

Organoid Culture Methods

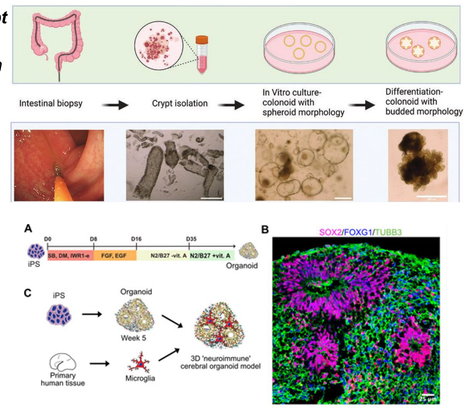


Figure 2 (top right): Intestinal Crypt Organoid. *Primary cells are harvested from mice or biopsied from human samples. Stem cells are isolated and cultured in vitro over 2 weeks for spontaneous formation of organoids with high yield.*

***Figure 3 (bottom right): Human Cortical Organoid and Forebrain Assembloid. A**) Embryonic or induced pluripotent cells are differentiated for 5 weeks into an organoid. **B**) Early cortical developmental markers indicate neural stem cells and glia. **C**) Assembly with microglia for immune competence and continued culture.*

Optics Design

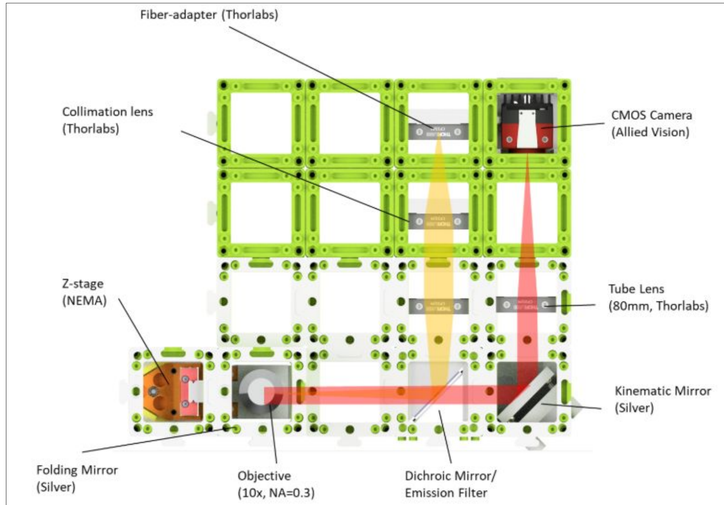


***Figure 4: Schematic of microscope light path**. The UC2 microscope platform has a cube architecture, with each functional element set for fluorescence microscopy or DIC imaging. 3 channel laser light is projected through a dichroic mirror, to a 10X objective and to a CMOS camera for data acquisition using the ImJoy imaging pipeline.*

Robotics Layout

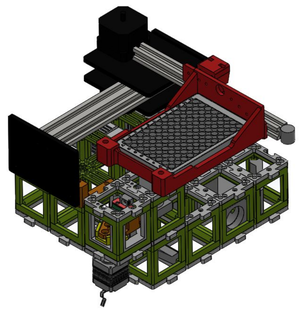
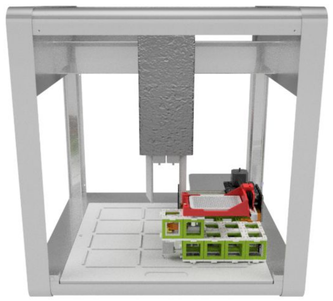


***Figure 5 (above):** Robotics layout with XY stage for 96 well and optics layout below with a Z stage for the objective*

***Figure 6 (below)**: Robotics layout within the Opentrons OT2*



Software Design

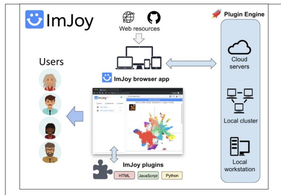
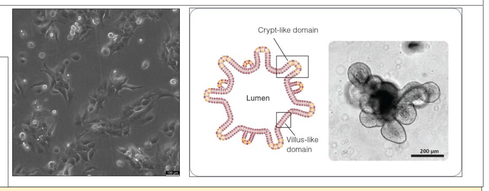


***Figure 7 (top left): ImJoy Imaging Infrastructure.** Data collection from UC2 platform is uploaded to a local or cloud server. A machine vision based AI completes real time image analysis for well by well decisions or information is databased for longitudinal analysis of organoids at high throughput.*

***Figure 8 (bottom right): Machine Vision.** UC2 data consists of cellular imaging, or morphometry based object recognition, such as formation of villi in a crypt organoid vs cell death.*



Future Work

Develop and test autonomous protocols for organoid culture:

1. **Media Change and Assay:** (UC2) Acquire brightfield well image, (ImJoy) Locate organoid and calculate well position, (OT2) Move pipette to a safe position, exchange media and retain media for metabolic analysis
2. **Tissue Culture Passage:** (UC2) Acquire fluorescence well image, (ImJoy) Quantify fluorescence and calculate the dilution factor for passage, (OT2) Aspirate old media, dissociate, and passage into fresh media.
3. **Proliferation/Viability Assay:** (UC2) Acquire brightfield well image, (ImJoy) Count number of organoids, categorize organoid health based on shape, and mark dead wells, (OT2) Exchange media in healthy wells only.

Acknowledgements

I would like to thank Dr. Benjamin Bartelle for giving me the opportunity to pursue this project within his lab, and Dr. Douglas Shepherd and Steven Sheppard for their invaluable microscopy advice.

References

[1] Kim, Jihoon, et al. “Human Organoids: Model Systems for Human Biology and Medicine.” *Nature News*, Nature Publishing Group, 7 July 2020
[2] W. Ouyang, R. W. Bowman, H. Wang, K. E. Bumke, J. T. Collins, O. Spjuth, J. Carreras-Puigvert, B. Diederich, An Open-Source Modular Framework for Automated Pipetting and Imaging Applications. *Adv. Biology* 2022, 6, 2101063. https://doi.org/10.1002/adbi.202101063

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Ira A. Fulton Schools of Engineering
Arizona State University