Developing and Validating OrganoSeg2 for Improved Organoid Analysis

Addressing Patient Concerns for Informed Consent in Organoid Research

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Introduction

My research will focus on how the scientific community can better prepare to use organoids as a research tool. Organoids are three-dimensional *in vitro* (outside a living organism) models derived from stem cells or tissue samples which grow to mimic organs or other biological tissues. In research settings, organoids often exist at the scale of tens-hundreds of microns in diameter (Yao et al., 2020), but can grow up to millimeters in the case of brain organoids (Hofer & Lutolf, 2021) or transplantation models (Watanabe et al., 2022). Organoids recapitulate *in vivo* (inside a living organism) behavior better than other *in vitro* models, given their ability to form complex, self-organizing structures, and exhibit diverse cell types and interactions (Tang et al., 2022). Additionally, organoids can capture inter-patient heterogeneities that are critical to providing appropriate care or understanding variability in biological states. Patient derived organoids have been shown to mimic *in vivo* development (Fujii et al., 2016) and be positive predictors of treatment response (Yao et al., 2020) in cancer, helping to personalize medicine and tackle patient heterogeneities. Organoids have also been shown to model disease states to allow for research on potential perturbations (Schwank et al., 2013), or screen for drugs against infections (Zhou et al., 2017). Organoids have been established as a promising direction for biomedical research, but the standards for organoid use are still developing, from the social protocol surrounding the generation of organoids from donors, to the image analysis methods for collecting data that allows scientists to extract meaning from organoids. Failing to solidify these methods could lead to distrust among patients and a loss of information gained from organoids. I aim to address this problem by evaluating the concerns of patients and professionals in regards to informed consent for organoid donations, and by developing and testing the generalizability of an accessible platform for organoid segmentation.

Technical Research

I am developing OrganoSeg2, an updated graphical user interface (GUI) for organoid segmentation. OrganoSeg uses conventional techniques to segment brightfield images of organoids, showing anywhere from a single organoid to several hundred, and reports data on organoid size and shape. Published in 2018 by Borten et al., OrganoSeg was reported to be more accurate for organoid segmentation than alternatives ImageJ, MorphoLibJ, and CellProfiler. Those platforms are popular for cell segmentation, but OrganoSeg's edge in organoid segmentation accuracy demonstrated that the three-dimensional setting in which organoids grow benefits from an organoid specific segmentation platform (Borten et al., 2018).

Organoid specific alternatives have since developed, with a focus on machine learning (Keshara et al., 2022). Schröter et al. (Schröter et al., 2024) compared OrganoSeg with three platforms, one using conventional methods and two using machine learning, in segmenting brain organoids across 30 days and two labs. SegFormer, a deep learning model, scored the highest, but "require[s] programming and deep-learning experience" (2024, p. 3). Additionally, 80% of the 1,400 images were used for training in each split. While machine learning platforms have some advantages in accuracy, they are less accessible to people without related experience, and often intimidating/inefficient due to the training data required. OrganoSeg still showed accurate recording of organoid growth for the majority of the experiment without training data, and its GUI makes it accessible to a wide audience.

Efficient segmentation and collection of data assesses organoid response to therapeutics so that it can be applied to research and treatment planning (Morelli et al., 2022; Yao et al., 2020).

OrganoSeg remains a popular tool, having been cited over 100 times in the past 5 years, indicating the importance of continued development to improve performance and match user needs. We rebuilt the GUI to make OrganoSeg2, addressing limitations in efficiency, accuracy, and functionality by seeking feedback on the original application from users within our lab. My research problem is that this platform has not proven to be generalizable across different research settings, which could limit the ability of researchers in heterogeneous settings to collect meaningful data. Schröter et al. (2024), show OrganoSeg's limitations with boundary recognition in one organoid environment, and other limitations may appear elsewhere. I will conduct this research by obtaining images of other organoid types or environments and evaluating OrganoSeg2's performance, recording defects by my own observations or the accounts of users. Evidence will involve statistically comparing the segmentation performance of the new platform to the original, or to other existing platforms. Additionally, new functionality of the app will be tested in a proof of concept. We incorporated fluorescence analysis and individual organoid tracking in OrganoSeg2. This will be applied to track the death response (marked fluorescently) of breast cancer organoids exposed to irradiation, where individual tracking gives more power to recognize trends than population level data alone. The overall goal of this research is to generalize OrganoSeg2 and show its enhanced ability for organoid analysis so that it may become publicly available, providing an accessible and accurate means for assessing organoid growth.

STS Research

My STS research addresses informed consent in organoid research. Given organoids' relative newness, there is less certainty about what donors agree to when their biospecimens are used.

Donors may also have closer ties to organoids than other biospecimens due to their complex structure (de Jongh et al., 2022). Finally, biobanks store organoids and associated patient information for long-term clinical or research applications, but present complications around privacy, ownership, and commercialization (Boers et al., 2016). Using organoids for research without representing the values of donors will lead to distrust of their use, reducing sources and support for organoid research. I would like to understand the complications that organoids present for patients and researchers, and how to address these concerns when establishing donor consent.

Biospecimens have already seen much debate, most noticeably with the case of Henrietta Lacks, whose cells were unknowingly distributed without informing or compensating Lacks's family (Beskow, 2016). Beskow (2016) discusses the current state of handling biospecimen donations, governed by the Common Rule, as well as the lessons to be learned from the story of Henrietta Lacks and from public opinion regarding informed consent. Considering the context of informed consent regulation as well as relevant (bio)ethical frameworks such as reciprocity and solidarity (Beskow, 2016) is important to developing recognizing patient rights while using organoids.

A systematic review by de Jongh et al., (2022) of organoid ethics presents informed consent as a topical issue. This source highlights the most common areas of concern, e.g., respecting privacy without losing biological information, but also those which are inadequately emphasized, e.g., organoid transplantation. While not clinically applied yet, transplantation exemplifies the uncertain possibilities to consider in a consent model (de Jongh et al., 2022). One proposed model is consent for governance, guided by a set of conditions that will be followed with patient

donations. Boers & Bredenoord (2018) emphasize the presentation of the context of the research, in regards to organoid management, privacy, benefits, ongoing participation, and ethical considerations. Lewis & Holm (2021) stress the need for autonomy, allowing donors to make an authentic decision based on their own morals, without coercion. While models such as governance aim to inform the patient, Lewis & Holm suggest this emphasizes values inauthentic to the donor, and the donor instead should receive only "value-sensitive information" (2021, p. 751), based on their own values. Despite invoking possible additional barriers, both agree that properly recognizing patients' rights will enable, not hinder, organoid research by gaining trust among donors (Boers & Bredenoord, 2018; Lewis & Holm, 2022)

The theoretical framework I will use is the Collingridge Dilemma, or the "social control of technology" (Collingridge, 1980). Early on with a technology, there is less known about what should be controlled, but later, the practices surrounding it are ingrained and difficult to change (Genus & Stirling, 2018). Kudina & Verbeek (2019) discuss strategies to address this dilemma, balancing anticipation and concurrent regulation. They propose "technological mediation," examining interactions between humans, technology, and the environment, and how related values influence one another at the early stage of a technology. This mediation could be applied to organoids, with some applications theorized but not yet fully integrated at a clinical level, and generally a consent model that is still in discussion (de Jongh et al. (2022). Genus & Stirling (2018) discuss how this dilemma addresses holes in responsible research and innovation (RRI). They suggest that RRI often settles on one trajectory for a technology, which ultimately limits responsiveness. Focusing on Collingridge's ideas, such as openness to a variety of ideas, taking incremental steps, and emphasizing criticism on ideas that have become "entrenched", may result

in more successful control of technology, they suggest. For organoids, there are discussions on what consent models are appropriate, but no changes to the Common Rule in over half a decade (Protections (OHRP), 2017). Heeding these ideas could assist getting regulation around organoid consent implemented.

Insights from both Bollinger et al.'s account of patients (2021) and Lensink et al.'s of professionals (2021) will be primary evidence for understanding balancing concerns and shaping an appropriate consent model. These will be compared to elements of current regulation (Common Rule), as well as proposed models (Boers & Bredenoord, 2018; Lewis & Holm, 2022) in order to see which concerns are fulfilled or not. Similarly, I plan to examine consent documents in settings such as university research or commercial biobanks to see how the informed consent agreements may influence users' values, reducing autonomy. If possible, I hope to find evidence of rates of donor consent, which when examined alongside the language and conditions of the consent, might identify key values to the donor.

Conclusion

I aim to contribute to the scientific community's potential for the use of organoids in medicine. I hope to further develop an application that can effectively collect data on organoids, while keeping in mind what users of different backgrounds want. Validation aims to show the potential to use this technology in a variety of contexts and to uncover previously difficult to identify trends in patient derived organoid behavior so that it may be incorporated into patient-oriented pipelines. I also hope to better understand the reservations that patients have in regards to organoid use, and how past examples of informed consent should be used to guide organoid

regulation. Ideally this will respect the values of non-scientists so that they are excited and willing to donate biospecimens for organoid research, without introducing significant hurdles to the research process. These goals work towards making the collection of information from organoids easier and more responsible, improving our ability to understand human disease and discover favorable treatments.

References

Beskow, L. M. (2016). Lessons from HeLa Cells: The Ethics and Policy of Biospecimens. *Annual Review of Genomics and Human Genetics*, *17*(Volume 17, 2016), 395–417. https://doi.org/10.1146/annurev-genom-083115-022536

Boers, S. N., & Bredenoord, A. L. (2018). Consent for governance in the ethical use of organoids. *Nature Cell Biology*, *20*(6), 642–645. https://doi.org/10.1038/s41556-018-0112-5

Boers, S. N., van Delden, J. J., Clevers, H., & Bredenoord, A. L. (2016). Organoid biobanking: Identifying the ethics. *EMBO Reports*, *17*(7), 938–941. https://doi.org/10.15252/embr.201642613

Bollinger, J., May, E., Mathews, D., Donowitz, M., & Sugarman, J. (2021). Patients' perspectives on the derivation and use of organoids. *Stem Cell Reports*, *16*(8), 1874–1883. https://doi.org/10.1016/j.stemcr.2021.07.004

Borten, M. A., Bajikar, S. S., Sasaki, N., Clevers, H., & Janes, K. A. (2018). Automated brightfield morphometry of 3D organoid populations by OrganoSeg. *Scientific Reports*, *8*(1), Article 1. https://doi.org/10.1038/s41598-017-18815-8

Collingridge, D. (1980). *The Social Control of Technology*. St. Martin's Press.

de Jongh, D., Massey, E. K., Berishvili, E., Fonseca, L. M., Lebreton, F., Bellofatto, K., Bignard, J., Seissler, J., Buerck, L. W., Honarpisheh, M., Zhang, Y., Lei, Y., Pehl, M., Follenzi, A., Olgasi, C., Cucci, A., Borsotti, C., Assanelli, S., Piemonti, L., … the VANGUARD consortium. (2022). Organoids: A systematic review of ethical issues. *Stem Cell Research & Therapy*, *13*(1), 337. https://doi.org/10.1186/s13287-022-02950-9

Fujii, M., Shimokawa, M., Date, S., Takano, A., Matano, M., Nanki, K., Ohta, Y., Toshimitsu, K., Nakazato, Y., Kawasaki, K., Uraoka, T., Watanabe, T., Kanai, T., & Sato, T. (2016). A Colorectal Tumor Organoid Library Demonstrates Progressive Loss of Niche Factor Requirements during Tumorigenesis. *Cell Stem Cell*, *18*(6), 827–838. https://doi.org/10.1016/j.stem.2016.04.003

Genus, A., & Stirling, A. (2018). Collingridge and the dilemma of control: Towards responsible and accountable innovation. *Research Policy*, *47*(1), 61–69. https://doi.org/10.1016/j.respol.2017.09.012

Hofer, M., & Lutolf, M. P. (2021). Engineering organoids. *Nature Reviews Materials*, *6*(5), 402–420. https://doi.org/10.1038/s41578-021-00279-y

Keshara, R., Kim, Y. H., & Grapin-Botton, A. (2022). Organoid Imaging: Seeing Development and Function. *Annual Review of Cell and Developmental Biology*, *38*(1), 447–466. https://doi.org/10.1146/annurev-cellbio-120320-035146

Kudina, O., & Verbeek, P.-P. (2019). Ethics from Within: Google Glass, the Collingridge Dilemma, and the Mediated Value of Privacy. *Science, Technology, & Human Values*, *44*(2), 291–314.

Lensink, M. A., Boers, S. N., Jongsma, K. R., Carter, S. E., van der Ent, C. K., & Bredenoord, A. L. (2021). Organoids for personalized treatment of Cystic Fibrosis: Professional perspectives on the ethics and governance of organoid biobanking. *Journal of Cystic Fibrosis*, *20*(3), 443–451. https://doi.org/10.1016/j.jcf.2020.11.015

Lewis, J., & Holm, S. (2022). Organoid biobanking, autonomy and the limits of consent. *Bioethics*, *36*(7), 742–756. https://doi.org/10.1111/bioe.13047

Morelli, M., Lessi, F., Barachini, S., Liotti, R., Montemurro, N., Perrini, P., Santonocito, O. S.,

Gambacciani, C., Snuderl, M., Pieri, F., Aquila, F., Farnesi, A., Naccarato, A. G., Viacava, P., Cardarelli, F., Ferri, G., Mulholland, P., Ottaviani, D., Paiar, F., … Mazzanti, C. M. (2022). Metabolic-imaging of human glioblastoma live tumors: A new precision-medicine approach to predict tumor treatment response early. *Frontiers in Oncology*, *12*. https://www.frontiersin.org/articles/10.3389/fonc.2022.969812

Protections (OHRP), O. for H. R. (2017, January 17). *Revised Common Rule* [Page]. https://www.hhs.gov/ohrp/regulations-and-policy/regulations/finalized-revisions-common-rule/index.html

Schröter, J., Deininger, L., Lupse, B., Richter, P., Syrbe, S., Mikut, R., & Jung-Klawitter, S. (2024). A large and diverse brain organoid dataset of 1,400 cross-laboratory images of 64 trackable brain organoids. *Scientific Data*, *11*(1), 514. https://doi.org/10.1038/s41597-024-03330-z

Schwank, G., Koo, B.-K., Sasselli, V., Dekkers, J. F., Heo, I., Demircan, T., Sasaki, N., Boymans, S., Cuppen, E., van der Ent, C. K., Nieuwenhuis, E. E. S., Beekman, J. M., & Clevers, H. (2013). Functional Repair of CFTR by CRISPR/Cas9 in Intestinal Stem Cell Organoids of Cystic Fibrosis Patients. *Cell Stem Cell*, *13*(6), 653–658. https://doi.org/10.1016/j.stem.2013.11.002

Tang, X.-Y., Wu, S., Wang, D., Chu, C., Hong, Y., Tao, M., Hu, H., Xu, M., Guo, X., & Liu, Y. (2022). Human organoids in basic research and clinical applications. *Signal Transduction and Targeted Therapy*, *7*(1), 1–17. https://doi.org/10.1038/s41392-022-01024-9

Watanabe, S., Kobayashi, S., Ogasawara, N., Okamoto, R., Nakamura, T., Watanabe, M., Jensen, K. B., & Yui, S. (2022). Transplantation of intestinal organoids into a mouse model of colitis. *Nature Protocols*, *17*(3), 649–671. https://doi.org/10.1038/s41596-021-00658-3

Yao, Y., Xu, X., Yang, L., Zhu, J., Wan, J., Shen, L., Xia, F., Fu, G., Deng, Y., Pan, M., Guo, Q., Gao, X., Li, Y., Rao, X., Zhou, Y., Liang, L., Wang, Y., Zhang, J., Zhang, H., … Hua, G. (2020). Patient-Derived Organoids Predict Chemoradiation Responses of Locally Advanced Rectal Cancer. *Cell Stem Cell*, *26*(1), 17-26.e6. https://doi.org/10.1016/j.stem.2019.10.010

Zhou, T., Tan, L., Cederquist, G. Y., Fan, Y., Hartley, B. J., Mukherjee, S., Tomishima, M., Brennand, K. J., Zhang, Q., Schwartz, R. E., Evans, T., Studer, L., & Chen, S. (2017). High-Content Screening in hPSC-Neural Progenitors Identifies Drug Candidates that Inhibit Zika Virus Infection in Fetal-like Organoids and Adult Brain. *Cell Stem Cell*, *21*(2), 274-283.e5. https://doi.org/10.1016/j.stem.2017.06.017