Organoid live imaging analysis with AI

Presentation of the research team

Lab: Vision Institute

Team: Live imaging in patients and cells

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Presentation of the research project

Inherited retinal dystrophies (IRD) that cause definitive loss of photoreceptors typically result in permanent visual impairment. Retinitis Pigmentosa (RP) is the most common form of IRD, with a prevalence of 1 out of 3500–5000, for more than 1.5 million cases worldwide. Mutations in Rhodopsin (RHO) lead to the death of rod photoreceptors, followed by a secondary death of cone photoreceptors. In France, the P347L (Class I mutation) appears to be the most prevalent[1] while the P23H mutation (Class II) is the most common mutation in the USA[2] . Class I RHO mutants fold normally in cells but affect post Golgi trafficking and outer segment targeting contributing to photoreceptor cell death[3]. In contrast, Class II mutants cause RHO misfolding resulting in retention in the endoplasmic reticulum (ER) causing an ER stress leading to cell death[4]. Although the mechanisms of photoreceptor death carrying a mutation in RHO are not yet fully defined, preventing and rescuing the retina is a major challenge for which pharmacotherapy can be an answer[5,6]. In fact, neuroprotection has emerged as a strategy for delaying photoreceptor death and preserving vision[7]. One advantage of the neuroprotection strategy is that it can have the capacity to slow photoreceptor degeneration regardless of the underlying causative pathway and may even be generalizable to other retinal neurodegenerative diseases[7]. Currently, there are no approved treatments for IRD. Despite the blossoming of recent progress in gene and cell therapies, the use of neuroprotective agents remains a front-line approach for a spectrum of neurodegenerative diseases, including IRD disorders caused by photoreceptor death[8]. The approved neuroprotectants have documented pharmacokinetics, bioactivities and demonstrate minimal adverse effects, facilitating long-term administration and compliance that are normally required for the treatment of chronic neurological diseases[9].

Today, the possibility to make a “Disease-in-a-Dish” with patient-based cell models - using induced pluripotent stem cell (hiPSC)-derived retinal cells - represents a chance for drug discovery. These highly relevant cellular models offer a unique opportunity for studying the effects of specific gene defects in the human context to better understand the disease and

find anti-degenerative treatment[5,10]. Sacha Reichman's team at the Vision Institute is working on identifying neuroprotective compounds in hiPSC-derived retinal organoid disease models[11]. To image these organoids live, Kate Grieve and Olivier Thouvenin's research groups have pioneered a novel, label-free imaging technique called Dynamic Full-Field Optical Coherence Tomography (DFFOCT)[12-15]. This method detects all living cells within complex samples and measures their local activity, offering valuable insights into cell metabolism[12,13], stress[15], mitosis[13], and apoptosis[12]. DFFOCT has already demonstrated its utility in long-term imaging of retinal organoids over several weeks, without any sign of phototoxic effects[13]. However, while DFFOCT contrast relies on the intrinsic optical and biophysical properties of tissues, its specificity remains limited and interpretation can be challenging. We hypothesize that biological specificity can be enhanced through a multi-scale analysis, combining information on cell morphology, activity, metabolism, and scattering properties. By incorporating machine learning and AI, we aim to achieve virtual staining of samples [16,17], offering contrast similar to fluorescence imaging without the need for labelling. Our team has previously published on AI analysis of DFFOCT data in the context of cancer biopsies, and would now like to translate this to retinal organoid data [17]. The primary scientific objective of the PhD project is to validate DFFOCT as a versatile and cost-effective method for label-free, longitudinal imaging of patient- derived organoid models. Our goal is to demonstrate that DFFOCT, combined with AI-driven analysis, can create relevant numerical twins of organoids to predict which drugs will be most effective and least toxic for individual patients.

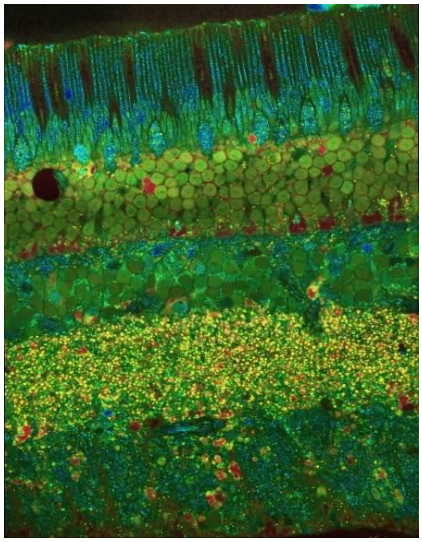

Fig. 1. High resolution dynamic FF-OCT imaging of a retinal explant showing all retinal cells. The color codes for metabolic-related information

To achieve this, the ORGAI project will take the following steps:

- Patient-based models of organoids will be developed by Sacha Reichman's team at the Vision Institute. This step develops retinal organoids from patients with inherited retinal dystrophies and tests neuroprotective molecules identified by the Vision Institute to assess structural and functional restoration.

- High throughput label-free microscopes developed by Kate Grieve's team at the Vision Institute will be used to image the organoids. DFFOCT has proven useful to follow cell viability and cell stress in retinal cell organoids over several weeks. The retinal organoid models of RP undergoing degeneration and with the drug screenings for neuroprotection will be followed with DFFOCT, forming an image database.

- Data Analysis and algorithm development. With DFFOCT, we can quantify the morphology and viability of all cells in the organoids. But AI and automatic data analysis are required to transfer such data into interpretable metrics and to perform multiscale analysis. Steps will involve segmentation and analysis of 3D spatial interactions to quantify organoid health at different stages; time prediction to forecast the outcome of long-lasting toxicity and efficacy

drug testing and compressed sensing to improve DFFOCT speed and reduce data volume; and finally aggregation of data from multiple organoids under different conditions to build several models.

We anticipate that digital tools developed in the ORGAI project in the specific context of identifying neuroprotective compounds in hiPSC-derived retinal organoid disease models may be generalizable to other samples imaged with DFFOCT label free live microscopy and could therefore beyond this project be applied to imaging with other groups involved in the DIM C-BRAINS network.

Main missions of the PhD student:

The student will learn to use the DFFOCT microscope to image retinal organoids and build the image database for analysis. In parallel, the student will be responsible for the development of the AI and machine learning tools for analysis of the data. The student will interact with the multidisciplinary project team to feedback on results and interpretation. We would like to recruit a student with a primary experience in computer science, programming, and image processing. Secondary experience and/or interest in optics, microscopy and biology would be appreciated.

The PhD project will include the following steps, with some being in parallel:
1. Use of the DFFOCT microscope (M1-M12)
2. Handling retinal organoids and obtaining good images of them (M1-M12)
3. Managing image database (M1-M30)
4. (Main focus): development of AI and machine learning tools (M6-M24)
5. Data analysis using developed tools and feedback to the project team (M12-M30)
6. Dissemination of results (M25-M36)

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