## Statement of research interests and career goals

Multiphoton imaging of retinal structure and function in hypoxia

My career goal is to continuously gain scientific and clinical skills to improve and restore human vision. I believe non-invasive functional imaging and characterization of tissue function will constitute a major paradigm shift in eye care. My medical and engineering training uniquely positions me to apply novel biophotonic methods to investigate tissue function within the context of disease.

I am a board certified ophthalmologist and practicing vitreoretinal surgeon. Patients in my clinic present with varying retinal diseases for which we are dependent upon imaging tools that, while enormously valuable, are limited to providing mostly structural information. Dual wavelength two-photon excited fluorescence (dwTPEF)[(1)], fluorescence lifetime imaging microscopy (FLIM), and hyperspectral imaging microscopy (HSpec)[(2)] are non-invasive, live imaging tools for characterizing cellular structure, metabolism and molecular distributions.

This proposal is to develop my foundation in biophotonics and its application to the visual system. I concluded my graduate studies 8 years ago. Despite leaving protected research training to be a full time clinical trainee from 2009 to 2017, I have persevered to grow as a basic scientist. In 2017 I published the only research on the use of multiphoton lifetime imaging to describe metabolic changes in developing retinal organoids.[(2)] Multiphoton FLIM of retina has never been demonstrated *in vivo* [(3)] and I intend to achieve this landmark in zebrafish and ultimately, humans.

During the training period, the majority focus of my time will be toward research. My faculty teaching responsibilities are tied into my clinical and surgical practice. Therefore, clinical and educational components are combined into a single element freeing my time for research.

My mentors comprise a multidisciplinary team. Bruce Tromberg PhD provides the backbone in biophotonic training and his talents make UC Irvine one of the only places where training of this nature is possible. Magdalene Seiler is a stem cell biologist studying retinal organoid implantation in animal models and will provide laboratory access and organoid populations. Thomas Schilling is the chair of Developmental and Cellular Biology whose expertise in zebra fish biology will guide my *in vivo* microscopy training.

**Research Plan**

I propose to develop advanced biophotonics principles in the study of **oxygen dependent metabolism in retinal tissues**. Oxygenation is important to both the developing and the mature retina. Pediatric retinopathy of prematurity has taught us that oxygen concentration is critical to retinal development.[(4)] The majority of adult retinal pathology is comprised of retinal vascular diseases including diabetic retinopathy, retinal vein occlusion, arterial occlusion and hypertension.

Organoids and fish respectively provide avascular and vascular models for studying retinal oxygenation. Retinal organoids are generated from human stem cells and self-assemble into layered retinal architecture. Organoid oxygenation is entirely dependent upon oxygen concentration of the tissue culture media. Developing zebrafish tissues are dependent on diffusion mediated oxygenation from the environment.[(5)] In adult fish, blood oxygenation determines retinal tissue oxygenation. These *in vitro* and *in vivo* systems allow control of oxygenation to the retinal tissues. Multiphoton microscopy to characterize intrinsic fluorophores and metabolism[(2)] will be optimized to study these model systems in the context of normoxia and hypoxia.

***Aim 1: To determine the effect of oxygen concentration on developing retina structure and metabolism.***

*Hypothesis 1a: Differential oxygen control during early retinal organoid development will affect inner layer metabolism and survival.*

*Hypothesis 1b: Normal and hypoxic conditions will affect zebrafish retinal development and this will be observable by changes in the metabolic signature of developing retinal layers.*

***Aim 2: To determine the effect of oxygen concentration on mature retina metabolism.***

*Hypothesis 2a: Hypoxic tissue culture of mature retinal organoids will results in: (i) a detectable change in structure and metabolic signature of the retinal organoid, (ii) cell death will begin in the inner layers of the retinal organoid.*

*Hypothesis 2b: Ischemia of adult zebrafish will result in inner retinal cell death characterized by an observable switch from oxidative phosphorylation to glycolytic metabolism.*

**Materials and methods:**

Retinal organoids will be initiated using established protocols.[2; 6] Immature organoids and mature organoids will be maintained at 5% and 20% incubator oxygen concentrations *ceteris paribus*. Non-pigmented Crystal strain[7] zebrafish will be stressed with hypoxia during development and in adulthood using a hypoxic water[8] and cobalt chloride.[9] The metabolic signature of the developing and adult retinal tissues under varied oxygenation will be followed using dwTPEF, FLIM and HSpec to characterize metabolism and molecular distributions. Anatomical correlation via H&E and immunofluorescence microscopy will be obtained at different time points.

**Translation**:

The results from this work will confer new skills, reveal tissue function in models for common blinding conditions, serve as a spring board to K08/K23/R01 applications and present first step toward a clinical device for metabolic imaging.

**References**

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