UW Vision Research Core Facility Statement

The UW-Madison Vision Research Core has been funded by the National Eye Institute (NEI) since 2005. Core staff provide the expertise and laboratory space to provide these vision research services, enabling us to improve the efficiency and productivity in vision research and ultimately speeding the pace of progress in vision research. The Core also provides subsidies for service/equipment use for NEI R01 PIs and those who are submitting NEI R01 grants. Core instruments and services are located at the Medical Sciences Center, Clinical Sciences Center, and Wisconsin Institute for Medical Research. Subsidized services are located in various areas of the UW-Madison Campus.

Core 1 - Omics and Quantitative Molecular Biology
Multi-omic analysis is a powerful tool to identify pathways underlining disease-causing mechanisms and generating targets for new therapeutic approaches, and its use in basic and clinical science is growing. Researchers in the Department of Ophthalmology and Visual Sciences and other vision researchers on campus have increased their use of multi-omic approaches, and thus, there is a continuing need for support of such use. Once omics data is obtained, it is crucial to analyze it systematically to identify pathways and molecules to be used for understanding disease mechanisms and to generate novel therapeutic targets. To achieve this goal, all the processes from sample preparation, performing omic experiments, data analysis, and quantitative analysis to test the significance of results from omic analyses need to be supported. This core provides the necessary resources to support the flow of the entire process by combining our own quantitative core and facilitating the use of external resources such as Biotechnology Center Omics, Bioinformatics and Sequencing Services and UWCCC Flow Cytometry. The core is directed by Dr. Akihiro Ikeda and co-directed by Dr. Raunak Sinha.

Services

Primer Design

Investigators unfamiliar with PCR primer design can consult with the QMB CORE to obtain the primers best suited for PCR needs, including specialized primers for nested PCRs and primer design with restriction sites for sub-cloning. Primer design strategies to amplify homologous sequences from species where there may not be a published target sequence are utilized. Design criteria are now established to utilize MIQE guidelines.

Quantitative real time PCR (qPCR)

RT-qPCR is performed using the Quant Flex Studio 7 (QS7) RT PCR machines using Taqman SYBR Green I (as the primary dye) on 96-well standard, 96-well fast, 384-well standard, and 384 well microfluidic card platforms (microfluidic cards are designed by the user and custom synthesized by Applied Biosystems/ThermoFisher). Data generated from each run is exported into an Excel Spreadsheet

Highlighted Equipment

Zeiss PALM Microbeam Laser Capture Microdissection (LCM) system laser microdissection and analysis for DNA, RNA and protein isolation from live cells and slides. *This instrument* enables the precise isolation of cells for micro-genomic, -transcriptomic and -proteomic analyses.

Luminex MAGPIX multiplex analyzer performs up to 50 different tests for a single sample volume. Kits are available for immunology, immune response, metabolism/endocrinology, toxicity, for various species.

Agilent SeaHorse HS Mini Analyzer measures and reports energy metabolism and vital indicators of mitochondrial health, toxicity, and overall cellular function.

Subsidies for Service/Equipment Use for NEI R01 PIs and those who are submitting NEI R01 grants

Funding for fee based services or equipment on campus is available to NEI R01 investigators and PIs applying for NEI R01 grants. Funding for fee based services or equipment use will be determined each year and will be based on the number of qualified users and the level of use. Once the allocated amount is exceeded, the PI will have to provide their own funding. PIs requesting these funds should contact the Vision Core Director to determine the level of funding that may be available. Note, these subsidies are to be used for data needed for the PIs funded R01 project.

- Biotechnology Center Services – Mass Spectroscopy (Proteomics, Lipidomics, Metabolomics); Sequencing (Including RNAseq, NextGen Seq, and Bioinformatics)
- UWCCC Flow Cytometry (WIMR)
- Vision Core fee based instruments (MSC)
    - Zeiss laser capture microdissection scope – $30/hr
    - Luminex Magpix analyzer – $100/run
    - SeaHorse HS Mini Analyzer - $100/run

Core 2 - Pathology and Imaging

The Pathology and Imaging Core offers a number of services for investigators with funded NEI R01s and those planning on submitting an R01 to NEI. Among the services provided by the Pathology and Imaging Core are advanced histology

through the Translational Research Initiative in Pathology TRIP) lab. Assistance with light and electron microscopy, confocal microscopy, live cell imaging, image analysis, and quantitative morphometry of tissue sections and cultured cells is also available (see list of services below). The Core is directed by Dr. Donna Peters, and co-directed by Dr. Mrinalini Hoon.

Core Services

- Technical expertise and assistance with the processing of various eye tissues for immunocytochemical and histochemical analysis.

- Assists with the establishment of primary cultures and immortalized cell lines.

- Subsidizes user fees so Vision Core Researchers have access to State-of-the-Art imaging equipment available in campus facilities

- Assists with the acquisition, processing, and banking of ocular tissue for research.

TRIP LAB

The TRIP Lab provides routine histology, including paraffin and frozen processing, embedding, and sectioning. The histopathology services of the UW TRIP Lab are available at no charge to qualified NEI R01 investigators and investigators gathering preliminary data for their NEI R01 grant submissions.

Immunocytochemical and histochemical evaluations

Vision Core technical staff is also available to help embed samples in either paraffin, glycol methacrylate, Epon, OCT or gelatin. Our staff can help the investigator develop antigen retrieval procedures and post-fixation labeling studies. Expertise is available for a variety of labeling procedures, including live cell staining to visualize and quantify the viability of cells in cultured anterior segments.

Morphometry

The Pathology and Imaging Core can assist with qualitative and quantitative morphometry utilizing well established histologic procedures to measure ganglion cell loss, apoptosis, loss of cell contacts, changes in cell morphology or the organization of the cellular matrix. The core also has Zeiss Zen software for morphometry. This software allows the investigator to generate composite images of large objects, measure length, distance, area, circumference and angles and do spot counts either manually or automatically.

Tissue culture cell depository

In collaboration with the Lions Eye Bank and the UW Biobank, we have started a human eye tissue/cell depository. The goals of this service are to obtain human eye tissues and fluids (aqueous humor and vitreous) for PIs to use in their studies. We can train investigators to isolate human eye cells or to provide "hard to isolate" human eye cells.

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- Optical Imaging Core (WIMR)
- Electron Microscopy (WIMR)
- TRIP lab services involving IHC, Multispectral Imaging and Slide Scanning technology, and Cell line Authentication (STR) (WIMR)
- Tissue Availability from the Biocore Biobank (WIMR)
- Vision Research Core fee based instruments
    - X-Clarity tissue cleaning system (WIMR) – $100/run
    - Andor spinning disc confocal microscope (MSC) – $30/hr

Core 3 - Animal Models and Eye Organ Culture

The development of new therapies and improving our understanding of visual system function ultimately relies on transferring work done *in vitro* to animal models. The goal of the Animal Model and Eye Organ Culture Core is to provide users with assistance in using animal models. To facilitate the proposed research and to establish relevance to humans it is essential to have a resource that specializes in nonhuman primates. Preliminary to nonhuman primate studies, many similar research studies may be carried out in cats, rats or mice.

Within the Animal Model and Eye Organ Culture Core, expertise and services are provided to allow core users to assess the effects of agents, manipulations, and vectors/constructs on ocular function in rodents, cats and primates and on aqueous outflow in organ-cultured anterior segments. Models of experimental glaucoma, as well as inner and outer retinal lesions, can be produced. Central and retinal function as well as anterior segment physiology can be monitored. Photography and imaging of ocular structures are accomplished with scanning laser polarimetry, confocal scanning laser ophthalmoscopy, fundus photography, and angiography. Gene

delivery and detection of co-transduced reporter genes (GFP, RFP) is available. The Core is directed by Dr. Colleen McDowell and co-directed by Dr. Gillian McLellan.

Services

- Animal handling, anesthesia, procedure assistance and post-procedure monitoring
- Experimental glaucoma models and other ocular disease models
- Assess aqueous humor dynamics *in vivo*
- Assess retinal function using ERG and quantitative optomotor response testing (qOMR)
- IOP measurements, slit lamp and fundus photography
- Confocal scanning laser ophthalmoscopy with Spectralis HRA+ OCT
- Monitoring outflow facility non-invasively
- Tonometry to determine the effects of agents lowering intraocular pressure
- Delivery of therapeutics, stem cells, scaffolds, devices, and gene therapy constructs to the eye
- *Ex-vivo* eye organ culture