Retinal Fundus Flavoprotein Fluorescence in Age Related Macular Degeneration: New Insights from Multimodal Imaging

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Retinal Fundus Flavoprotein Fluorescence in Age Related Macular Degeneration: New Insights from Multimodal Imaging

Protocol Number: 2023-1715

Principal Investigator: Mihai Mititelu, MD, MPH

Date: November 6, 2024

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Protocol Version History

Protocol Version	Version Date	Description of Changes
1	03/06/2024	Initial version
2	11/06/2024	Updated to include sharing images and data with OcuSciences, Inc. for detailed image analysis

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1. Statement of Compliance

The signature below constitutes that the research will be conducted in accordance with the approved protocol, applicable regulations, laws and institutional policies.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitment.

PRINTED OR TYPED NAME	SIGNATURE	DATE
Principal Investigator(s)		

2. List of Abbreviations

AE	Adverse event
AMD	Age-related Macular Degeneration
ANOVA	Analysis of covariance
BCVA	Best Corrected Visual Acuity
CERU	Clinical Eye Research Unit
CFR	Code of Federal regulations
DOVS	Department of Ophthalmology and Visual Sciences
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	Fluorescein Angiography
FAF	Fundus Autofluorescence
FDA	Food and Drug Administration
FPF	Flavoprotein Fluorescence
GA	Geographic Atrophy
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Investigational Review Board
OCT	Optical Coherence Tomography
PI	Principal Investigator
SAE	Serious Adverse Event
UP	Unanticipated Problem

3. Study Summary

3.1 Synopsis

Full Title	Retinal Fundus Flavoprotein Fluorescence in Age Related Macular Degeneration: New Insights from Multimodal Imaging
Short Title	FPF in AMD
Protocol Number	2023-1715
Study Design	Single site pilot comparative imaging study
Objectives	The goal of this pilot project is to evaluate patterns of increased autofluorescence FPF in the setting of geographic atrophy, an advanced form of dry/non-exudative AMD in images taken with the OcuMet Beacon® compared to Heidelberg Spectralis autofluorescence images.
Main Inclusion Criteria	1. 50 years or older and may be either male or female and may be of any race. 2. Established diagnosis of GA due to AMD. 3. GA characteristics: GA area of between 1.25 mm² and 23 mm², with seventy percent of eyes having GA area ranging from 2.5 mm2 to 17.5 mm2. GA may be unifocal or multifocal. GA may be subfoveal or extrafoveal, with twenty-five percent of eyes having subfoveal GA. The presence of concurrent peripapillary atrophy will not exclude subjects from participation. 4. Willing to participate as evidenced by signing the written informed consent.
Main Exclusion Criteria	1. Unable to tolerate ophthalmic imaging. 2. Presence of neovascular AMD on OCT as confirmed by an ophthalmologist. 3. Presence of significant media opacity preventing adequate retinal imaging. 4. Presence of concurrent retinal disease which may confound assessment.
IND or IDE Number	The OcuMet Beacon® is a novel, non-invasive fundus camera with specialized filters that can detect, capture and assess flavoprotein fluorescence (FPF). The OcuMet Beacon's software displays a fundus image, the FPF image, a pupil image for alignment, and a quantitative measure of the patient's flavoprotein fluorescence measure. The Investigator feels that this device is nonsignificant-risk and is requesting an NSR determination from the UW HS-IRB.
Study Intervention	Patients will undergo FPF imaging using the OcuMet Beacon system.
Study Population	Patients 50 years of age or older with GA secondary to AMD who have been recruited for the COCO-GA study will be provided with the option to consent and enroll in the FPF in AMD Study.
Number of Subjects	We will take images of 60 eyes; we estimate that will be obtained from approximately 40 patients taking images of one or both eyes per patient.

4. Key Personnel

The following is a list of all key personnel and roles:

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5. Introduction

Age-related macular degeneration (AMD) is a disease that affects the central vision and is the leading cause of vision loss in older adults. There are two types of AMD, dry /non-exudative AMD, and wet AMD. Geographic atrophy (GA) occurs during the late stages of dry AMD, and there are limited treatments for the condition. The ability to detect AMD and specifically GA early is vital to preventing disease progression. The current standard imaging modality for this condition – well established as an end-point measurement in clinical trials – is fundus autofluorescence (FAF) [1], such as the one provided by Heidelberg Spectralis. As technology advances, new imaging modalities are being developed for this purpose.

Mitochondrial dysfunction in the setting of oxidative stress results from the accumulation of various reactive oxygen species that cause cellular damage. The spectral properties of flavoproteins have previously been demonstrated to be different from those of other retinal fluorophores, such as lipofuscin. In diseased states, flavoproteins linked to mitochondrial enzymes become oxidized and emit a green autofluorescence when stimulated by blue light.[2]

This represents an important step in the pathogenesis of various posterior pole conditions such as AMD, diabetic retinopathy (DR), retinal vein occlusion (RVO) or central serous retinopathy (CSR). [3] Thus, mitochondrial flavoprotein fluorescence (FPF) can serve as a marker of mitochondrial oxidative stress potentially serve as a non-invasive measure of metabolic dysfunction present within the human retina and the optic nerve. [4,5,6]

The OcuMet Beacon® is a novel fundus camera that can detect, capture, and assess FPF. [7] This imaging modality may prove to be an easier and faster way to evaluate macular health in GA compared to Heidelberg Spectralis, which requires separate, formal measurements of the areas of macular disease, whereas the results of the FPF machine are calculated automatically and provided on the machine itself at the end of the imaging session. If the results of this study suggest FPF OcuMet Beacon produces sufficient agreement with FAF, FPF may streamline the diagnostic process, increase efficiency, and lower the burden for patients, while offering clinicians a rapid, reliable method to assess the progression of geographic atrophy.

Protocol 2023-0958, COmparison of Clarus and Optos Ultrawide Field Imaging Systems for Geographic Atrophy (COCO-GA), is a prospective, comparative study of GA area measurements in FAF images taken using the Heidelberg Spectralis, and Zeiss Clarus and Optos ultrawide field imaging systems being conducted by Wisconsin Reading Center investigators. [8] Participants in 2023-0958 are recruited from the clinical practices of the UW Health University Station Clinic and will also be invited to participate in this study. The Spectralis images taken for protocol the COCO-GA Study will be compared to the FPF images taken for this study.

6. Study Objectives

6.1 Purpose

To evaluate patterns of increased FPF in the setting of geographic atrophy, an advanced form of dry/non-exudative AMD.

6.2 Objectives

The objectives for the study are:

- Primary Objective: To estimate the agreement between the average areas of hypofluorescent GA area on images obtained by the Heidelberg Spectralis autofluorescence to those of mitochondrial flavoproteins on the OcuMet Beacon machine in the setting of GA.
- Secondary Objective: To compare the cross-sectional associations between FPF intensity and BCVA to FAF area at the time of measurement.

6.3 Endpoints

The endpoints for the study are:

- Primary Endpoint: The limits of agreement between the FAF area of GA on images taken with the Heidelberg Spectralis to the FPF area of GA with images taken with the OcuMet Beacon.
- Secondary Endpoint: The correlation of the area of abnormal FPF intensity on Beacon images and BCVA scores to FAF area.

7. Study Design

7.1 General Design

This is a single site, pilot study in which subjects participating in protocol 2023-0958 will have additional images taken with the OcuMet Beacon. The imaging will occur either immediately after a 2023-0958 study visit or participants may return at another time after the 2023-0958 study visit.

7.2 Rationale for Intervention

Although historically retinal damage has been evaluated by structural changes, as shown by changes in autofluorescence in images taken with the Heidelberg Spectralis camera, this approach shows the end-result of metabolic disturbances but does not capture real-time fluctuations in oxidative stress. The OcuMet Beacon is a relatively novel type of camera capturing functional imaging of mitochondria which provides in vivo quantitative information of FPF.

7.3 Study Risk/Benefit Assessment

This section describes the overall study risk/benefit assessment.

7.3.1 Known Potential Risks

Participation in this study poses minimal to no risk to subjects.

Imaging Device: The OcuMet Beacon fundus camera is a standard imaging device previously described in the literature and used in IRB approved clinical trials and poses no risk to the subject. Sensitive participants may experience some discomfort or light sensitivity due to imaging. This should be mild and brief.

Pupillary Dilation: It is expected that most participants will enroll and complete the study on the same day as their clinical appointment. Pupillary dilation is a standard procedure of a clinical appointment and is necessary to obtain quality imaging of the back of the eye. The FDA-approved and standard of care drugs used for dilation are phenylephrine hydrochloride (2.5%) and tropicamide (1%). If the participant chooses to complete the study on the same day, dilation will not need to be repeated. However, if a participant chooses to complete the study on a different day, phenylephrine hydrochloride (2.5%) and tropicamide (1%) will be used to dilate the pupil of the participant's eye.

A concern about the use of these drugs is that they can precipitate acute angle closure in a very small percentage of patients (~1:20,000). This would result in a painful eye, blurry vision, and possibly nausea. In addition, a small proportion of the population can develop conjunctivitis in response to topically applied eye drops. This is a benign condition that is self-limited upon discontinuing the drops and will either spontaneously resolve in one to three days without treatment or can be treated with mild anti-inflammatory medications if needed.

Confidentiality Breach: There is a risk that patient information could be unintentionally shared with others (e.g. an unlawful data breach). To minimize this risk strict adherence to the IRB protocol will be maintained.

7.3.2 Potential Benefits to the Subjects

There is no known potential benefits to subjects.

7.3.3 Potential Benefits to Society

If this study is able to show that FPF imaging is an easier and faster way to evaluate macular health society and the community at large would benefit.

7.3.4 Risk Minimization

To minimize the risk of an acute angle closure event, we will ascertain whether a subject has previously had their eyes dilated or not. Successful previous dilation eliminates the prospective risk of an acute angle closure event. Fortunately, subjects who are susceptible to this problem can be identified by an ophthalmic exam prior to administering the drug. Before beginning this study, every subject will be examined to ensure they are not susceptible to such an acute angle closure event. The exam will be administered under the supervision of an ophthalmologist at the UW Health Eye Clinics. We may use a slit lamp or a pen light test to check for the presence of an open angle. In addition, we will carefully instruct the subject that if they experience any of the symptoms (painful eye, blurry vision, and possibly nausea) to let us know right away.

To minimize the risk of conjunctivitis associated with pupillary dilation, personnel administering drops will wear gloves prior to handling and administering the eye drops.

To minimize the risk of a breach of confidentiality, research procedures will be conducted in a private room. Subject interactions will be held to the UWHC clinical privacy standards. In addition, sensitive information will be limited to the amount necessary to achieve the aims of the research. The imaging procedures performed in this study do not include any facial features and these images cannot be used to identify a subject. Additional information regarding data management and confidentiality are described in **Section 12 Data Management and Recordkeeping**.

To minimize the risk of a breach of confidentiality due to sharing images and data, only coded images and data will be shared. The images and data will be transferred to OcuSciences using one of the UW-Madison Office of Compliance Approved Tools for Exchanging and Storing PHI (e.g., Globus). Images and data will be shared, used, and maintained in compliance with the approved Data Transfer and Use Agreement (DTUA) between the UW-Madison study team and OcuSciences

8. Subject Selection

Patients 50 years of age or older with GA secondary to AMD who have been enrolled in protocol 2023-0958 will be provided with the option to consent and enroll in this study.

8.1 Inclusion Criteria

To be eligible to participate in this study, an individual must meet all of the following criteria:

1. 50 years or older and may be either male or female and may be of any race.
2. Established diagnosis of GA due to AMD.
3. GA characteristics: GA area of between 1.25 mm^2 and 23 mm^2, with seventy percent of eyes having GA area ranging from 2.5 mm^2 to 17.5 mm2. GA may be unifocal or multifocal. GA may be subfoveal or extrafoveal, with twenty-five percent of eyes having subfoveal GA. The presence of concurrent peripapillary atrophy will not exclude subjects from participation.
4. Enrolled in protocol 2023-0958 and willing to participate as evidenced by signing the written informed consent.

8.2 Exclusion Criteria

An individual who meets any of the following criteria is not eligible to participate in this study and is excluded:

1. Unable to tolerate ophthalmic imaging.
2. Presence of neovascular AMD on OCT as confirmed by an ophthalmologist.
3. Presence of significant media opacity preventing adequate retinal imaging.
4. Presence of concurrent retinal disease which may confound assessment.

8.3 Recruitment

Participants will be recruited at a regular UW Health - Eye clinic visit by the clinician responsible for their care. Participants will be recruited based on information contained in their private/protected records (e.g., medical records – a diagnosis of GA due to AMD with certain characteristics) and their enrollment in protocol 2023-0958. During the consent discussion and in the consent form for protocol 2023-0958, participants will be provided with information on the optional study activities of being contacted for future studies and using data and images for future studies. If participants are interested in these optional activities, the study team member obtaining consent will provide participants with information about the FPF Study. Consent for the FPF study will be obtained by staff who are study team members on both protocol 2023-0958 and the FPF Study.

8.4 Retention Strategies

Subjects will receive $20 compensation for participating in the study/completing the FPF images.

8.5 Early Termination and Withdrawal

Subjects are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a subject from the study for the following reasons:

- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject
- Disease progression which requires discontinuation of the study intervention

- If the subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

Subjects who sign the informed consent form and are randomized but do not complete the study imaging may be replaced.

9. Study Intervention

9.1 Study Intervention Description

9.1.1 Imaging Devices

Images from two systems will be utilized for this study, the Heidelberg Spectralis and the OcuMet Beacon.

The current gold standard in FAF is the FDA-approved Spectralis FAF imaging from Heidelberg Engineering. Spectralis is a scanning laser ophthalmoscope which uses a blue light excitation wavelength of 488 nm and a 500 nm barrier filter to produce FAF images by evaluating abnormal fluorophore signal. The Spectralis camera images 20-55 degrees of the retina, and in this study a 30-degree imaging field will be used. One single 30-degree FAF image will be obtained from each eye with GA in the COCO-GA Study (Protocol 2023-0958). Those images will be used in this study.

The OcuMet Beacon imaging system is an investigational device where the macula is illuminated using a narrow bandwidth blue light (455 – 470 nm) and fluorescence is recorded using a narrow notch filter to match the peak emission of flavoproteins from 520 to 540 nm. The device captures infrared images of 60° x 21.5° and an FPF image of 17° x 21.5° field of view. It uses a 1-ms flash of blue light (455 – 470 nm) to capture fluorescence from 520 - 540 nm. FPF is primarily expressed as two parameters known as FPF intensity and FPF heterogeneity. The former reflects global signal strength by measuring average pixel intensity over a 5.5 mm-diameter region centered at the macula.

A total of four, non-overlapping fundus areas will be imaged (using both FPF and Near Infrared Reflectance modalities) in each eye: Nasal, Central Nasal, Central and Temporal. (Figure 1). This will ensure that we capture the largest extent of GA lesions. Images will be saved in a de-identified fashion on the machine using consecutive numbers that identify each participant (FPF #1, FPF #2, etc.)

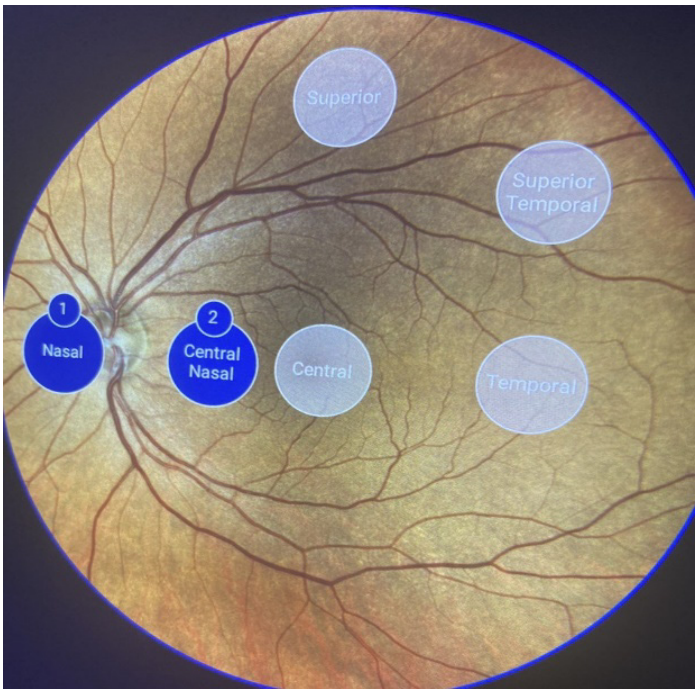


Figure 1. Four FPF fields (Nasal - blue, Central Nasal - blue, Central – gray, and Temporal - gray) will be imaged on the OcuMet Beacon® fundus camera. A wider field of view using NIR will also be obtained on the same fundus camera for each eye

Nonsignificant Risk Designation

For this study, a nonsignificant risk designation is requested from the University of Wisconsin-Madison Health Sciences IRB. The OcuMet Beacon system does not present a potential for serious risk to the health, safety, or welfare of subjects, is not an implant, and is not used in supporting or sustaining human life. The Beacon is a diagnostic device but is not being used for a substantial importance in diagnosing, curing, mitigating or treating disease or otherwise preventing impairment of human health. The focus of this study is to test the nonsignificant risk device for feasibility compared to an established diagnostic tool (Heidelberg Spectralis).

9.1.2 Pupil Dilation

Phenylephrine hydrochloride (2.5%) and tropicamide (1%) are FDA-approved and standard of care drugs used for pupil dilation. Pupillary dilation is necessary to obtain quality imaging of the back of the eye. If a participant completes the study on the same day of a clinical visit and COCO-GA Study (Protocol 2023-0958) procedures, dilation will not need to be repeated. However, if a participant chooses to complete the imaging for this study on a different day, phenylephrine hydrochloride (2.5%) and tropicamide (1%) will be used to dilate the pupil of the participant's eye.

In a very small percentage of patients (~1:20,000) these medications can precipitate acute angle closure. If this occurs, patients may experience a painful eye, blurry vision, and possibly nausea. In addition, a small proportion of the population can develop conjunctivitis in response to topically applied eye drops. This is a benign condition that is self-limited upon discontinuing the drops and will either spontaneously resolve in one to three days without treatment or can be treated with mild anti-inflammatory medications if needed.

Phenylephrine hydrochloride (2.5%) and tropicamide (1%) will be used according to their intended, FDA approved use. This study is not intended to test these medications and they are not being evaluated for investigational use. Data regarding the use of the dilation medication in this study will not be collected and thus will not be reported to the FDA, submitted to support a significant change in the advertising of

the product. The route and dosage are administered according to their approved use and therefore do not increase any risks, and will be used in compliance with current marketing limitations.

9.2 Source

The OcuMet Beacon was provided to the CERU by an industry sponsor for use in another clinical trial (2022-1044). Dr. Mititelu received approval from the sponsor to use the Beacon in this study.

9.3 Packaging and Labeling

The OcuMet Beacon is labeled with the following information: the name and place of business of the manufacturer, the name of the device and the following statement: "CAUTION - Investigational device. Limited by Federal (or United States) law to investigational use."

10. Study Visits and Procedures

10.1 Screening and Enrollment

The Screening and Enrollment visits and procedures are described in detail below.

10.1.1 Informed Consent

Potential patients for protocol 2023-0958 will be introduced to the study by their clinician. Interested patients will be referred by the clinician to a study team member for a consent discussion. During the informed consent process for protocol 2023-0958, patients will be provided with a brief overview of this FPF study and asked if they are interested in learning more about the FPF study.

Consent Process
Informed consent will be obtained for this study. A member of the study team will obtain informed consent in a private room within the Clinical Eye Research Unit (CERU) office suite in the UW Health – University Station Eye Clinic. The FPF study consent process is in addition to the 2023-0958 consent process and will be conducted face to face and provided to participants in person. If participants need additional time to consider the study, they may return for a separate research visit. The study will be discussed at an appropriate language level, all questions will be answered, and the study team member will ensure that the potential participant understands the information. Participants will be encouraged to ask any questions throughout the consent process and study participation. The study team will follow HRP-090 - SOP - Informed Consent Process for Research.

Waiver of Signed Consent
We are requesting a waiver of signed consent to obtain consent to share coded images and related-data with the investigational camera manufacturer, OcuSciences, Inc.(see section 12.5 Data Sharing). We will obtain consent verbally via telephone call using an IRB-approved telephone script. Verbally obtained informed consent will be documented on the telephone script and in the electronic medical record. If participants provide verbal consent, we will mail each participant an IRB-approved information letter that contains the description of the image and data sharing information. We feel the waiver of signed consent is appropriate for this instance because the study is non-significant risk and the details of the change are easily conveyed via telephone conversation. Additionally, there is only one clinic visit for this study. Due to participants' age (50 or older) and possible vision impairment due to AMD/GA, it is impractical to ask participants to return to clinic to complete written consent.

For participants who are visually impaired, the study team will ensure that an impartial witness is present during the consent discussion to fulfill the requirements described in section 5.1.4 of HRP-090.

Process to Document Consent in Writing
The study team will be following HRP-091 - SOP - Written Documentation of Consent.

10.1.2 Screen Failure and Re-enrollment

Individuals who do not meet the criteria for participation in this trial (screen failure) may be rescreened. Rescreened subjects should be assigned a new subject identification number when they are re-screened.

10.2 Study Visit

There is one visit for this study. FPF images will be taken. After informed consent has been obtained, the estimated length of the study visit is one hour.

11. Data Collection

The following data will be collected and obtained for protocol 2023-0958 and this study. The data will be collected once and shared with both study teams.

- Informed Consent: A member of the study team obtains documented consent from each potential subject prior to participating in the study, and before any study procedures are performed. Consent is documented on an IRB approved consent form and is signed and dated by the potential subject and the person conducting the consent discussion. The subject will be informed of any new information relevant to the subject's willingness to participate in a timely manner. The new information will be provided to the subject in a new consent form or addendum to the original consent. The informed consent process will adhere to all applicable IRB, institutional, state, and federal requirements, guidance, laws, and regulations.
- Demographics: Demographic information will be collected from the subject by a member of the study team.
- Medical History: The subject's medical history will be obtained by a member of the study team. Medical history will include all active conditions and any past conditions that are considered to be clinically significant by the Investigator.
- Visual Acuity: The subject's most recent visual acuity will be obtained by a member of the study team from the subject's medical record.
- Lens Status: The subject's most recent lens assessment will be obtained by a member of the study team from the subject's medical record.

12. Data Management and Recordkeeping

12.1 Data Confidentiality

Researchers will take the following steps to secure the data (e.g., training, authorization of access, password protection, encryption, physical controls, certificates of confidentiality, and separation of identifiers and data) during storage, use, and transmission.

- Data will be coded and the "key" linking identities to codes will be kept separate from the data.
- Only CERU study team members have access to the "key."

12.2 Data Storage

Data will be stored and maintained in compliance with the UW-Madison Policy UW-4032 Data Stewardship, Access, and Retention. Data will be stored and maintained on:

- Paper documents will be kept in a locked cabinet within the Clinical Eye Research Unit at 2880 University Avenue, Madison, WI 53705.
- Electronic image files will be stored in a HIPAA-compliant secure platform (e.g., SecureBox) and will only be accessed by the study team.

12.3 Management of Identifiers

When subjects are enrolled, they will be assigned a study identification number. A “key” linking identities to codes, will be stored on paper, and secured in a locked filing cabinet or drawer inside a locked office in the CERU office suite. Identifiers will be destroyed at study closure or at the time of publication.

12.4 Provisions to Protect the Privacy Interests of Participants

The following steps will be taken to protect participants’ privacy interests.

- Subject identifying information and subject ID will be maintained by CERU staff and only shared with DOVS staff.
- Procedures will be performed in a private area where others cannot see the procedures being performed or overhear the conversation between subjects and researchers.
- All members of the study team are up to date on their institutional HIPAA training.
- The study is not collecting information that could pose legal or reputational risks to participants.

12.5 Data Sharing

Data will be shared with the DOVS staff conducting protocol 2023-0958. Data will be stored and maintained on a secure, department server.

Images and data will also be shared with the device manufacturer, OcuSciences, Inc., for the purpose of detailed image analysis. The images and data provided to OcuSciences will be coded. The images and data will be transferred to OcuSciences using one of the UW-Madison Office of Compliance Approved Tools for Exchanging and Storing PHI (e.g., Globus). Images and data will be shared, used, and maintained in compliance with the approved Data Transfer and Use Agreement (DTUA) between the UW-Madison study team and OcuSciences.

Individual results will not be reported in participants’ health records.

Study results may be submitted for peer-reviewed publication in a scientific journal. Patient identifiers will be removed from the manuscript.

12.6 Records Retention

Records will be retained according to UW-Madison Policy UW-4032 Data Stewardship, Access, and Retention.

13. Data and Safety Monitoring

13.1 Incidental Findings

During informed consent, subjects will be informed that the FPF images are considered experimental, are only being done for research purposes and have no clear meaning for healthcare. Therefore, subjects will not be informed of any results or unexpected findings.

13.2 Adverse Event (AE) Definition

Adverse event (AE) means any untoward or unfavorable medical occurrence in a human subject or others that happens during or after participation in a research study.

13.3 Serious Adverse Events (SAEs)

An adverse event is considered "serious" if, in the view of either the investigator or sponsor, meets any of the following criteria:

- Results in death
- Is life-threatening
- Requires an inpatient hospitalization or prolongation of an existing hospitalization
- Results in persistent or significant disability or incapacity
- Results in a congenital anomaly/birth defect
- A medical event, based on appropriate medical judgment, that is believed to jeopardize the subject and/or requires medical or surgical intervention to prevent one of the outcomes defining a SAE. For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home, convulsions that may not result in hospitalization

13.4 Classification of an Adverse Event

13.4.1 Severity of Event

All AEs will be assessed by the clinician using the following guidelines to describe severity.

Mild (Grade 1)	Events require minimal or no treatment and do not interfere with the subject's daily activities.
Moderate (Grade 2)	Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
Severe (Grade 3)	Events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.
Life Threatening (Grade 4)	The subject was at risk of death at the time of the event.
Fatal (Grade 5)	The event caused death.

13.4.2 Relationship to Study Intervention(s)

For all collected AEs, the clinician who examines and evaluates the subject will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

Definitely Related	Clearly related to the study procedures/intervention and other possible contributing factors can be ruled out.
Probably Related	Likely related to the study procedures/intervention and the influence of other factors is unlikely.
Possibly Related	Possibly related to the study procedures/intervention and there are other factors that could be equally likely.
Unlikely to be related	Doubtfully related to the study procedures/intervention and there is another likely cause.
Unrelated	Clearly not related to the study procedures/intervention and/or evidence exists that the event is definitely related to another cause.

13.4.3 Expectedness

The PI will be responsible for determining whether an AE is expected or unexpected in relation to the study procedures and intervention(s) (as applicable).
An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described in the clinical protocol, device manual, investigator's brochure, the package insert(s), the IRB application, or the informed consent document. Expectedness is recorded for both study procedures and interventions.

13.5 Reporting AEs and SAEs

All AEs and SAEs will be reported in accordance with applicable IRB, institutional, state and federal guidance, regulations, and laws.

13.6 Unanticipated Problem

An unanticipated problem (UP), as defined by the DHHS Office for Human Research Protection (OHRP), is any incident, experience, or outcome that meets all of the following criteria:

- The incidence, experience, or outcome is unexpected given the research procedures described in protocol-related documents (e.g., the study protocol, the informed consent documents, the Investigator's Drug Brochure) and the characteristics of the subject population being studied. An event may be considered unexpected if it exceeds the nature, severity, or frequency described in the study-related documents, Investigator's Drug Brochure, product labeling, or package insert.
- The incidence, experience, or outcome is related or probably related to participation in the research study. "Probably related" means the incidence, experience, or outcome is more likely than not to be caused by the research study procedures.

The occurrence of the incidence, experience, or outcome suggests that the research places subjects or others at a greater risk of harm (physical, psychological, economic, or social) than was previously known or recognized.

13.7 Unanticipated Adverse Device Effect

An unanticipated adverse device effect (UADE) means any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

An investigator shall submit to the sponsor and to the reviewing Institutional Review Board (IRB) a report of any UADE occurring during an investigation as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect (21 CFR 812.150(a)(1)).

A sponsor who conducts an evaluation of an UADE shall report the results of such evaluation to the FDA and to all reviewing IRBs and participating investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter the sponsor shall submit such additional reports concerning the effect as FDA requests (21 CFR 812.150(b)(1).

13.8 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. Noncompliance will be reported to the IRB as applicable and within the required time frame(s).

13.9 Study Monitoring

Sponsors/sponsor-investigators are responsible for ensuring that independent study monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that reported study data are accurate and credible, and that the study is conducted in compliance with the protocol and applicable regulatory requirements, guidelines, laws and institutional policies.

For this study, monitoring will be conducted by independent compliance/regulatory staff within the Department of Ophthalmology and Visual Sciences (DOVS). DOVS has several qualified staff members within CERU and the Wisconsin Reading Center (WRC) that will be performing monitoring based on availability and workloads. The monitoring staff have an average of 15 years of regulatory, FDA-regulated research compliance, auditing and monitoring experience.

On-site, centralized monitoring will be conducted throughout the study to ensure the safety of research participants and data integrity by confirming that study is implemented in a manner consistent with the IRB approved protocol and in compliance with applicable regulations and institutional policies. A comprehensive (100%) review of consents and eligibility determinations will be performed. A comprehensive (100%) review of adverse events, safety and any UADEs will also be conducted to ensure proper documentation and reporting as necessary.

14. Study Analysis

14.1 Sample Size Justification

Using an equivalence test for a clustered trial comparing two continuous variables, a sample size of 26 participants - assuming two eyes per participant - would yield 81% power to detect equivalence between two means. We assume the interval of equivalence is between -2.5% and 2.5 with a mean bias between devices

of 0.5% and a between-participant standard deviation of 3%. To account for the within-participant (between-eye) correlation we assume a moderate-to-high intraclass correlation of 0.7. The statistical test is based on a one-sided two-sample t-test with alpha set to 0.05 and the degrees of freedom based on the number of participants. Power calculations were performed using nQuery version 9.3.1.

14.2 Statistical Methods

The primary objective of our statistical analysis is to compare two continuous variables measured by different devices in a clustered setting (assuming two eyes from each participant). We aim to establish equivalence between these measurements, accounting for within-participant correlation due to data being collected from both eyes of each participant. To account for the clustered nature of our data, we will fit a mixed-effects linear model. This model will include a random intercept for each participant to handle the intra-individual correlation between measurements from both eyes. A post-hoc equivalence test will be conducted using the Two One-Sided Tests (TOST) procedure. This approach will test if the difference in measurements between the two devices falls within a pre-specified equivalence range, denoted as (-2.5%, 2.5%). The equivalence test will be performed using the estimated fixed effect coefficient and standard errors derived from the mixed-effects model, where the null hypothesis is that the difference in measurements between the two devices is outside the equivalence bounds of -2.5% to 2.5%. The alternative hypothesis is that the difference lies within these bounds, indicating equivalence.

The secondary objective is to estimate the correlation between three continuous variables, accounting for within-person correlations due to data being collected from both eyes of each participant. Specifically, we seek to explore whether FPF intensity or BCVA is more correlated with AMD disease severity as measured on Spectralis FAF. BCVA will be converted to logMAR for a continuous variable. We will employ mixed-effects linear modeling to appropriately handle the non-independence of observations within the same individual with random intercepts, as each participant provides data for both eyes. Two separate models will be performed, one where the independent variable is FPF intensity and the other where the independent variable is BCVA. We will extract the fixed effect coefficients from each model to provide an estimate of the relationship between AMD severity. Since each model will have the same degrees of freedom, we will test the difference between the two fixed effects coefficients to estimate the strength of association, where the null hypothesis is that there is no significant difference in the strength of association between FPF intensity and BCVA with AMD disease severity.

15. References

1. Massamba N, Sellam A, Butel N, Skondra D, Caillaux V, Bodaghi B. Use of Fundus Autofluorescence Combined with Optical Coherence Tomography for Diagnose of Geographic Atrophy in Age-Related Macular Degeneration. Med Hypothesis Discov Innov Ophthalmol. 2019 Winter;8(4):298-305. Epub 2019 Oct 1. PMID: 31788492; PMCID: PMC6778681.
2. Ahsanuddin S, Rios Hernan A., Otero-Marquez O, Macanian J, Zhou D, Rich C, Rosen RB. Flavoprotein fluorescence elevation is a marker of mitochondrial oxidative stress in patients with retinal disease. Frontiers in Ophthalmology, Volume 3 (2023). Doi: 10.3389/fopht.2023.1110501. ISSN=2674-0826
3. Feher J, Kovacs I, Artico M, Cavallotti C, Papale A, Balacco Gabrieli C. Mitochondrial alterations of retinal pigment epithelium in age-related macular degeneration. Neurobiol Aging. 2006 Jul;27(7):983-93. doi: 10.1016/j.neurobiolaging. 2005.05.012. Epub 2005 Jun 23. PMID: 15979212.
4. Masuda T, Shimazawa M, Hara H. Retinal Diseases Associated with Oxidative Stress and the Effects of a Free Radical Scavenger (Edaravone). Oxid Med Cell Longev. 2017;2017:9208489. doi: 10.1155/2017/9208489. Epub 2017 Jan 18. PMID: 28194256; PMCID: PMC5286467.
5. Chen AX, Conti TF, Hom GL, Greenlee TE, Raimondi R, Briskin IN, Rich CA, Kampani R, Engel R, Sharma S, Talcott KE, Singh RP. Functional imaging of mitochondria in retinal diseases using flavoprotein fluorescence. Eye (Lond). 2021 Jan;35(1):74-92. doi: 10.1038/s41433-020-1110-y. Epub 2020 Jul 24. PMID: 32709959; PMCID: PMC7852520.
6. Zhou DB, Castanos MV, Geyman L, Rich CA, Tantraworasin A, Ritch R, Rosen RB. Mitochondrial Dysfunction in Primary Open-Angle Glaucoma Characterized by Flavoprotein Fluorescence at the Optic Nerve Head. Ophthalmol Glaucoma. 2022 Jul-Aug;5(4):413-420. doi: 10.1016/j.ogla.2021.12.006. Epub 2021 Dec 28. PMID: 34968754.
7. Muste JC, Russell MW, Chen AX, Seth K, Iyer AI, Valentim CCS, Wu AK, Kuo BL, Kalur A, Sastry R, Hom GL, Conti TF, Rich CA, Talcott KE, Sharma S, Singh RP. Functional Imaging of Mitochondria in Age-Related Macular Degeneration Using Flavoprotein Fluorescence. Ophthalmic Surg Lasers Imaging Retina. 2023 Jan;54(1):24-31. doi: 10.3928/23258160-20221214-03. Epub 2023 Jan 1. Erratum in: Ophthalmic Surg Lasers Imaging Retina. 2023 Dec;54(12):732. PMID: 36626211.
8. Domalpally, A. ***CO***mparison of ***C***larus and ***O***ptos Ultrawide Field Imaging Systems for Geographic Atrophy (COCO-GA). Wisconsin Reading Center Imaging protocol. Internal DOVS document. October 2023.