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TITLE: Adaptive Optics Retinal Imaging in Multiple Sclerosis

PRINCIPAL INVESTIGATOR: Daniel M. Harrison

CONTRACTING ORGANIZATION: University of Maryland, Baltimore, MD

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14. ABSTRACT Attached is the first technical report for this study, which was funded by the MSRP to perform an evaluation of Adaptive Optics Retinal Imaging in Multiple Sclerosis. In this report you will find an update on technical progress as to our development and finalization of an imaging protocol and study mechanism. You will find an update as to our progress in recruitment of study participants (13 thus far). You will also find samples of images that have been acquired and proposed methods for analysis. You will also find our plans for the coming year.					
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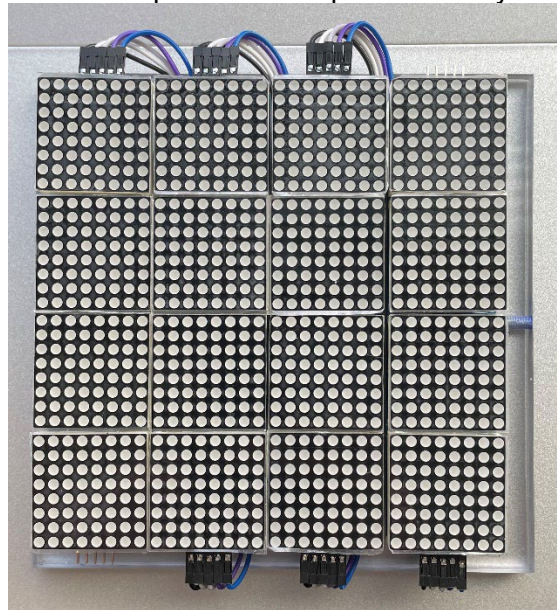
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1. **INTRODUCTION:** In this study, we are utilizing adaptive optics OCT (AO-OCT) to provide cellular level resolution of the retina in multiple sclerosis (MS). We expect to visualize MS-related changes in three specific retinal cell types/structures that are known to be affected by MS: RGCs, axons in the RNFL, and retinal macrophages. Our specific aims were: Specific Aim 1: To demonstrate that AO imaging provides in vivo quantification of neuronal loss in patients with MS. Specific Aim 2: To demonstrate that AO imaging provides in vivo quantification of axonal loss in patients with MS. Specific Aim 3: To use AO imaging to evaluate the link between innate immunity and neuronal and axonal loss in patients with MS. To achieve these aims, 30 MS patients with a history of optic neuritis (ON), 30 MS patients without ON, and 20 healthy controls will undergo AO retinal imaging, in addition to ophthalmologic and neurologic data collection. Aim 1 will be achieved by quantifying and comparing RGC soma density and diameter in each study group. Aim 2 will be achieved by quantifying and comparing RNFL axonal bundle density and volume in each study group. Aim 3 will be achieved by quantifying and comparing ILM macrophage density and motility measurements in each study group, and by relating these values to those found in Aims 1 and 2.
2. **KEYWORDS:** Multiple Sclerosis, Optical Coherence Tomography, Adaptive Optics, Neurodegeneration, Optic Neuritis
3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.*
 - **What were the major goals of the project?**
 - Aims 1 – 3, Major Task 1: To perform the study activities involving subjects.
 - Aim 1, Major Task 1: Analyze data obtained in the study to meet the goals of Aim 1.
 - Aim 2, Major Task 1: Analyze data obtained in the study to meet the goals of Aim 2.
 - Aim 2, Major Task 1: Analyze data obtained in the study to meet the goals of Aim 3.
 - **What was accomplished under these goals?**
 - Aims 1 – 3, Major Task 1: To perform the study activities involving subjects.
 - *Major Activities:* After receipt of funding, we proceeded to final testing of the FDML Adaptive Optics machinery and the associated imaging protocol being used in this study. This testing revealed the need to develop a novel fixation target for the optic nerve head, which required engineering work to be performed to develop a series of manually controllable LED lights in locations that would allow visualization of the optic nerve head. The FDA AO-FDML system was designed with an internal fixation target (FT) and stimulus port to achieve a range of 30° across the retina, centered on the fovea. Practically, this range is limited to ~13-14° on either side of the fovea. With this range, it is not possible to image and fully map the optic nerve head (ONH) region, which for most subjects, starts 12-15° nasally from the fovea and extends to 20-25°. Therefore, we constructed a light emitting diode (LED)-based display board to be used for fellow-eye fixation, to image the region around the ONH. The spacing between individual LEDs was measured so that the distance from the FT to the eye is such that a 3x3 array of locations with 4.5° field-of-view (and ~25% overlap) would fully image a ~10°x10° range with the ONH in the center. These scans are designed to collect a map of microglia density around the ONH. The temporary LED board has to be moved on the optical table when we are imaging the left or right eyes of our volunteers.

We have proceeded to recruit subjects for the study and perform study activities using the temporary ONH fixation target described above. These activities

include the ophthalmologic and neurologic examinations and the AO-OCT scanning procedures. The first subject in the study underwent AO-OCT scanning on 4/5/2023. To date, 13 subjects have completed their study visits. Four more are scheduled in the coming weeks. Thus far, we have had no screen failures. These activities were described in the original SOW to be ongoing and continue throughout the course of the study. We believe we are on track to meet the goals as planned.

- *Specific Objectives:* The specific objectives of this major goal are to recruit subjects, perform study activities, and document data from study activities in the database.
- *Significant Results:* As stated above, we thus far have scanned 13 subjects. This includes 1 healthy volunteer and 12 MS subjects. All data has been entered into the database, as planned.
- *Other Achievements:* In addition to the manually controlled fixation target, we have also begun work on a more automated and finely controlled fixation target. For a more permanent solution, we designed and constructed a new FT using an Arduino microprocessor control board and 32x32 LED array printed circuit boards mounted to a plexiglass plate. Two boards were constructed, which will be mounted above the AO system (at a distance from the eye to achieve the same angular range as the temporary FT) and activated and controlled from the computer for more seamless operation of volunteer fixation control. The new FTs are fully assembled and will be fully integrated into the FDA AO-FDML system this Fall. A picture of the planned array is below.



- Aim 1, Major Task 1: Analyze data obtained in the study to meet the goals of Aim 1.
 - *Major Activities:* As scans were being obtained, we worked to formalize and finalize custom Matlab and R code processing scripts for proper processing of raw image data into analyzable formats. After working through some software glitches/bugs, we have accomplished this goal and now have a processing script that converts all images into 3D volume stacked tiff files, which can be utilized in image review software. We also have tested utilization of these images in the process of automated RGC segmentation and analysis, with manual review. After successful completion of this testing, we have now recently begun to submit processed images to this analysis software for quantification. We also have

developed analysis pipelines for evaluation of Spectralis OCT scans obtained during the ophthalmologic visit. This includes scripting to extract layer segmentation values from the Heidelberg Eye Explorer software package and convert them into analyzable values in a spreadsheet. After each study visit is completed, this script is run to extract values for ganglion cell layer thickness, among others.

- **Specific Objectives:** Specific objectives for this goal included AO image processing, RGC cell counting, clinical OCT analysis, statistical analysis, and manuscript preparation. Only the first 3 were expected to begin during this grant period and will be ongoing, with the last 2 occurring later.
- **Significant Results:** Below are screenshots of AO-OCT images obtained in this study, which demonstrate the ability to visualize RGCs, as planned, along with examples of RGC analysis. More detailed results will only be available after a larger number of subjects are recruited.

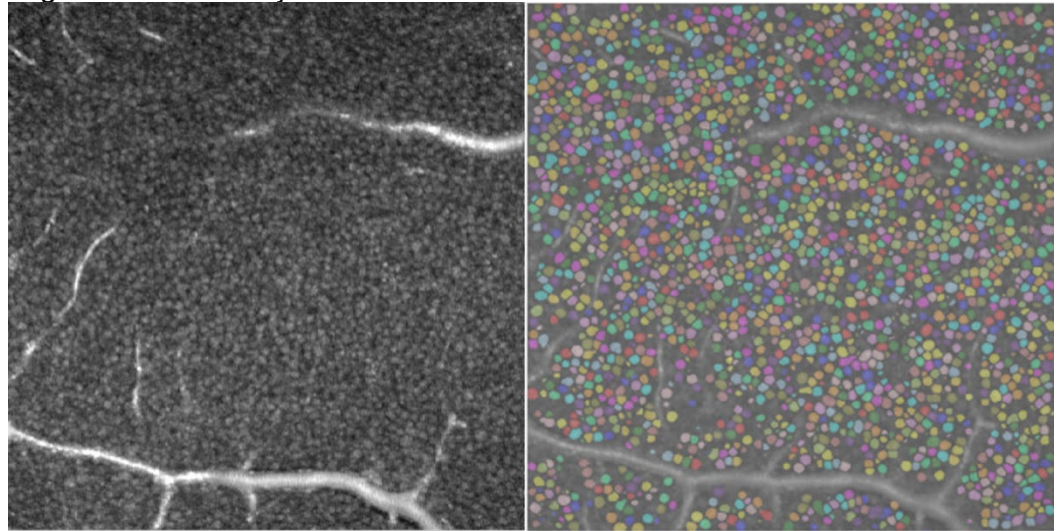


Figure 1: Retinal Ganglion Cells (RGC): RGCs imaged on an MS patient in this study. Left shows raw image showing individual RGCs and associated vasculature. Right shows segmentation of individual RGCs via automated software.

- **Other Achievements:** N/A. We believe overall we are on track to meet the goals of this Aim.
- Aim 2, Major Task 1: Analyze data obtained in the study to meet the goals of Aim 2.
 - **Major Activities:** Most of the major activities described for Aim 1 overlap with the activities of this Aim. See above. In addition to extraction of ganglion cell layer thickness, the processing script for extraction of data from the Heidelberg Spectralis scans also extracts both optic nerve head and macular nerve fiber layer thickness. We also have initiated work to investigate if a more automated means by which to analyze nerve fiber bundles can be accomplished. In the original grant, we proposed manual fiber counting and volume derived from inpainting of nerve fiber bundles using semi-automated region filling tools in MIPAV. We are also now currently exploring development of more automated segmentation methodologies using custom scripting.
 - **Specific objectives:** The specific objectives for this Aim included AO image processing, nerve fiber bundle quantification, clinical OCT analysis, statistical analysis, and manuscript preparation. Only the first 3 were expected to begin during this grant period and will be ongoing, with the last 2 occurring later.

- **Significant Results:** Below are screenshots of AO-OCT images obtained in this study, which demonstrate the ability to visualize nerve fiber bundles, as planned. More detailed results will only be available after a larger number of subjects are recruited.

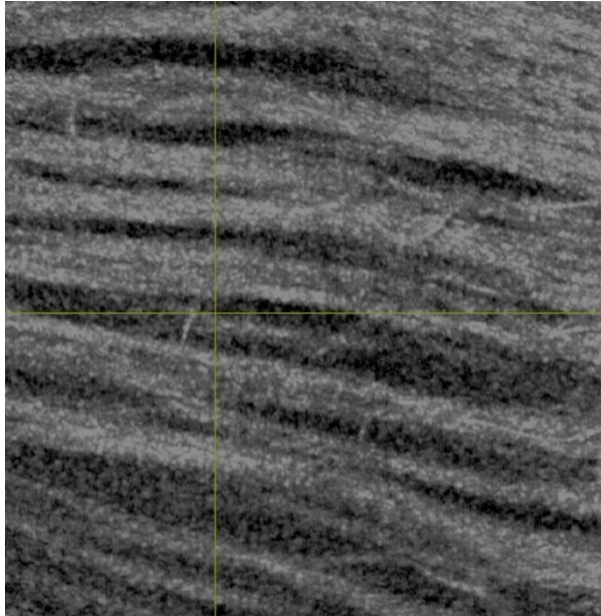


Figure 2: Nerve Fiber Layer (NFL) Axonal Bundles: Shown are images from AO-OCT images from an MS patient in the study, with individual NFL axonal bundles shown.

- **Other Achievements:** N/A. We believe overall we are on track to meet the goals of this Aim.
- Aim 3, Major Task 1: Analyze data obtained in the study to meet the goals of Aim 3.
 - **Major Activities:** Most of the major activities described for Aim 1 overlap with the activities of this Aim. See above.
 - **Specific objectives:** The specific objectives for this Aim included AO image processing, macrophage quantification, statistical analysis, and manuscript preparation. Only the first 2 were expected to begin during this grant period and will be ongoing, with the last 2 occurring later.
 - **Significant Results:** Below are screenshots of AO-OCT images obtained in this study, which demonstrate the ability to visualize macrophages, as planned. More

detailed results will only be available after a larger number of subjects are recruited.

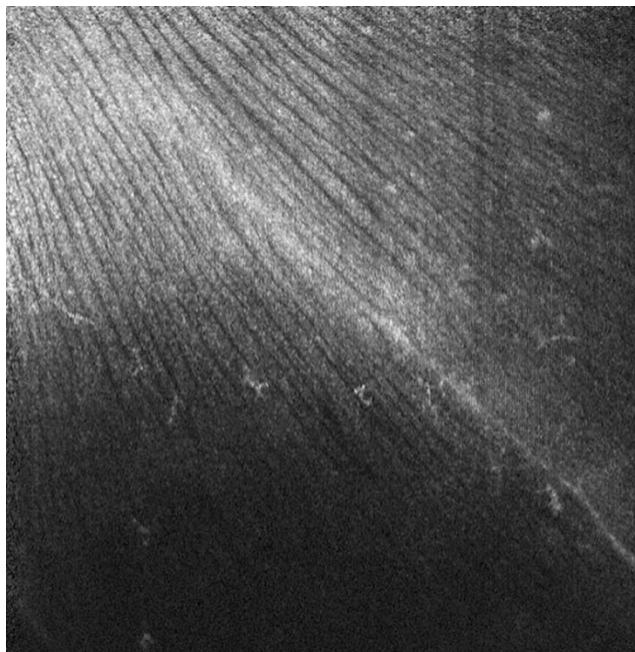


Figure 3: Inner Limiting Membrane (ILM) Macrophage-like Cells: Shown is an image from AO-OCT on one subject in the MS study at the level of the ILM. Scattered macrophage-like cells are seen and available for counting/motility analysis.

- *Other Achievements:* N/A. We believe overall we are on track to meet the goals of this Aim.
- **What opportunities for training and professional development has the project provided?**
 - Nothing to report
- **How were the results disseminated to communities of interest?**
 - Nothing to report
- **What do you plan to do during the next reporting period to accomplish the goals?**
 - During the next reporting period we will continue to recruit subjects, at an accelerated pace. We will work to recruit the majority of the MS subjects during this period. Towards the end of the period, we will begin recruiting healthy volunteers that approximately age/sex match the MS subjects recruited to date at that point. As scans are completed, we will continue to process all of the AO-OCT and Spectralis OCT images. We will work iteratively as each scan is completed to perform RGC counting and analysis, nerve fiber bundle quantification, and macrophage counting and motility analysis. All of the data derived from each of these tasks will be integrated with the clinical and demographic data collected on the day of the study visit to build the study database to the point where statistical analyses can begin, which will occur in earnest in the following reporting period. We will continue to work on the controlled fixation target and continue to explore automated methods for retinal nerve fiber bundle analysis.

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**

- Nothing to report
- **What was the impact on other disciplines?**
 - Nothing to report
- **What was the impact on technology transfer?**
 - Nothing to report
- **What was the impact on society beyond science and technology?**
 - Nothing to report

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
 - Nothing to report
- **Actual or anticipated problems or delays and actions or plans to resolve them**
 - Development of a fixation target for optic nerve head was an unanticipated problem. This caused a minor delay in launching the study. This has already been resolved.
- **Changes that had a significant impact on expenditures**
 - Nothing to report
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
 - Nothing to report
- **Significant changes in use or care of human subjects**
 - Nothing to report
- **Significant changes in use or care of vertebrate animals.**
 - Nothing to report
- **Significant changes in use of biohazards and/or select agents**
 - Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
Report only the major publication(s) resulting from the work under this award.
 - **Journal publications.** *Nothing to report*
 - **Books or other non-periodical, one-time publications.** *Nothing to report*
 - **Other publications, conference papers, and presentations.** *Nothing to report*
- **Website(s) or other Internet site(s)**
Nothing to report
- **Technologies or techniques**
Nothing to report
- **Inventions, patent applications, and/or licenses**
Nothing to report

- **Other Products**
Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Daniel Harrison
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0001-8707-2004
Nearest person month worked:	1.8
Contribution to Project:	<i>Dr. Harrison has overseen the project, performed study visit activities, and participated in image analysis.</i>
Funding Support:	<i>This award</i>

Name:	Daniel Hammer
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	0000-0003-0532-1914
Nearest person month worked:	1.2
Contribution to Project:	<i>Dr. Hammer has overseen the AO-OCT components of this project, performed AO-OCT scans, and participated in image analysis.</i>
Funding Support:	FDA internal funds

Name:	Zhuolin Liu
Project Role:	Co-I

Researcher Identifier (e.g. ORCID ID):	000-0001-8019-2054
Nearest person month worked:	1.4
Contribution to Project:	<i>Dr. Liu has overseen the AO-OCT components of this project, performed AO-OCT scans, developed analysis software, implemented software components of the study, and participated in image analysis.</i>
Funding Support:	FDA internal funds

Name:	Udayakumar Karuppanan
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	0000-0002-2174-838X
Nearest person month worked:	8
Contribution to Project:	<i>Dr. Karuppanan has been the main staff member working on the AO-OCT part of the project and has been involved in all aspects.</i>
Funding Support:	This grant

Name:	Yuxin Zeng
Project Role:	Research Analyst
Researcher Identifier (e.g. ORCID ID):	None
Nearest person month worked:	4
Contribution to Project:	<i>Data management and organization and processing of Spectralis OCT scans.</i>

Funding Support:	This grant
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Name:	Kerry Naunton
Project Role:	Research Nurse
Researcher Identifier (e.g. ORCID ID):	none
Nearest person month worked:	3.6
Contribution to Project:	<i>Ms Naunton has organized all study visits, performed study visits, assisted with recruitment, and has managed the study database.</i>
Funding Support:	This grant

Name:	Osamah Saeedi
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	0000-0002-3082-4604
Nearest person month worked:	0.6
Contribution to Project:	<i>Dr. Saeedi has performed the ophthalmologic exams and advised on analysis.</i>
Funding Support:	This grant

Name:	Grace Forbes
Project Role:	Research Coordinator

Researcher Identifier (e.g. ORCID ID):	0000-0002-5807-0939
Nearest person month worked:	0.45
Contribution to Project:	<i>Grace has performed study visit activities at the ophthalmologic exam visit and assisted with extraction of eye exam data for entry into the database.</i>
Funding Support:	This grant

Name:	Shaiza Mansoor
Project Role:	Research Coordinator
Researcher Identifier (e.g. ORCID ID):	None
Nearest person month worked:	0.15
Contribution to Project:	<i>Shaiza has performed study visit activities at the ophthalmologic exam visit and assisted with extraction of eye exam data for entry into the database.</i>
Funding Support:	This grant

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
 - Nothing to report
- **What other organizations were involved as partners?**
 - **Organization Name:** Food and Drug Administration
 - **Location of Organization:** Silver Spring, Maryland, USA
 - **Partner's contribution to the project** (*identify one or more*)
 - **In-kind support:** Drs. Hammer and Liu provide analysis time, advisory time
 - **Facilities:** The AO-OCT device is located at the FDA campus. The use of the device and associated computing and computer network.

- **Collaboration:** Drs. Hammer and Liu and members of their lab collaborate on this project.
- **Personnel exchanges:** Udayakumar Karuppanan works both at the laboratory of Dr. Harrison at UMB and has a volunteer appointment at the FDA campus.

8. SPECIAL REPORTING REQUIREMENTS

- N/A

9. APPENDICES: *None*