Background: Animals must be able to navigate their environment in order to survive and thrive. Across the animal kingdom, vision is a guiding sense for many organisms, and requires precise processing of light from the natural world. Light itself is an electromagnetic wave. Humans detect its wavelength as color and its amplitude as luminance (brightness). Light waves also have another feature, the angle of the waves relative to the direction of propagation, known as polarization (Fig. 1), which humans cannot perceive. However, polarization vision is found throughout the animal kingdom, particularly in invertebrates. How the brain processes polarized light has been studied using a variety of different organisms, including drosophila [1], as well as aquatic species such as the fiddler crab and the crayfish. In the fiddler crab, polarization processing occurs separately but in parallel to luminance, and is suggested to provide a wider range of contrast in their environment. [2] On the other hand, in the crayfish polarization information is processed using the same channel as luminance, and aids in their vision of motion. [3]

Fig 1. Light naturally comes in at all angles of incidence. The directionality of the light can be filtered to only allow certain orientations through.

Cephalopods have tremendous visual capabilities, such as high visual acuity and the ability to identify shapes based solely on polarization.[4] Previous studies have recorded the electrophysiological response properties of their photoreceptors to polarized light [5] and others have demonstrated behaviorally that they can distinguish between different polarizations of light.[6] *However, it remains unknown how their visual system processes this fundamental information about their environment*. To determine how *Octopus bimaculoides* encode polarization information, I will measure neural activity in the optic lobe, the central brain structure where visual processing takes place in the octopus, in response to visual stimuli that vary in first luminance and then polarization angle. I will then determine how this information travels to different downstream brain regions. Using standard visual stimuli, I have successfully executed the experiments needed to measure neural responses in the octopus optic lobe. I propose to use these skills to determine how this critical but unexplored light feature, polarization, is processed in this convergently evolved visual system.

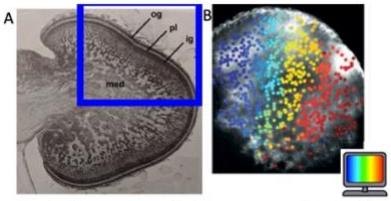

Fig 2. **A**) Anatomy of the octopus optic lobe. [8] **B**) Retinotopic organization of visual responses in the optic lobe, in a region corresponding to blue outline in A. *Preliminary data.*

Aim 1 *Determine how neurons in the optic lobe encode polarization information using 2-photon calcium imaging of neural activity in response to visual stimuli that vary in either luminance or polarization angle.* This aim will be the first to measure the visual encoding of polarization in the central visual system of a cephalopod. Investigating the divergence or convergence of visual system function and organization between cephalopods and other species with polarization vision will highlight essential features of the evolution of this visual capability.

Aim 2 *Identify the neural pathways that process polarized light, by using retrograde tracing together with the imaging of neural responses.* This aim will be the first to identify the pathways that carry polarization information in the octopus to downstream brain areas associated with specific functions. This will demonstrate how polarization information is used for visual behavior.

Approach: To record neural activity in response to visual stimuli, I will record from the optic lobe using calcium imaging. Our method was originally established in the zebrafish optic tectum by my advisor, Dr. Cristopher Niell.[7] In brief, I dissect the octopus to access the optic lobe and inject a fluorescent calcium indicator that increases in fluorescence when neurons are active. The preparation is then placed in a chamber that allows the presentation of visual stimulus on one side, under a 2-photon microscope. The visual stimulus consists of white or black circles presented at different locations on the screen, to allow me to determine retinotopy of responses to luminance variances. Figure 2 demonstrates the measurement of the retinotopic map in the optic lobe, where the response within

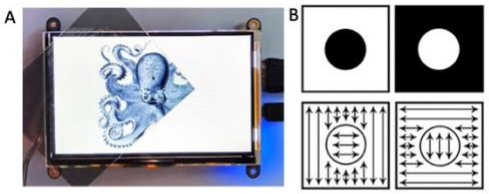

Fig 3. **A**) LCD screen with polarizing filter on the left side, showing an image of an octopus, and no filter on the right, where the screen appears uniformly white due to our inability to detection polarization. **B**) Luminance stimuli (top) vs polarization stimuli (bottom).

the lobe correlates to the location of the luminance stimulus shown on the screen. To present a stimulus that can vary in either polarization or luminance, I modified an LCD screen to allow me to switch between these two modes by inserting or removing a polarizing filter (Fig. 3).

To achieve Aim 1, I will apply the same 2-photon calcium imaging approach used to show the organization of response to luminance contrast stimuli with my modified LCD apparatus to vary polarization. I hypothesize that polarization information is processed independently of luminance information during the initial stages of visual processing, and then is integrated to supplement contrast information in later stages of the visual processing system. To address Aim 2, I will use retrograde tracers to label cells in specific octopus brain regions, such as the vertical lobe that facilitates learning and the middle suboesophageal mass that controls a subset of chromatophores[8], to establish the neuronal cell types that project from the optic lobe to these brain areas. I will then use imaging, as described for Aim 1, to determine which of these projection neuron types actually convey polarization information.

These aims are a novel extension of ongoing research on the octopus visual system in the Niell lab. As part of the team, I have designed this original research project that contributes to the overall project by examining the neural coding of polarized light. The Niell lab has successfully completed the required protocols and has a steady supply of octopuses that we receive from the Marine Biological Laboratory. Furthermore, the methods to measure visual responses are well-established in the lab, and I have consistently performed this full recording procedure, from imaging to analysis. I can now perform these experiments independently, so I do not anticipate any significant technical obstacles.

Intellectual Merit: *This project will be the first to characterize the functional organization and cellular organization that mediates polarization vision in cephalopods*. I hypothesize that the octopus processes polarized information separately but in parallel to luminance information, to add contrast to their perception of their environment. My preliminary data on the large-scale organization of neural activity in the optic lobe provide a basis for the proposed experiments to quantify neural coding of polarization at the level of individual neurons. They suggest that responses to polarization stimuli will be retinotopically organized as luminance information is, and that there will be distinct, though adjacent, spatial activation patterns to luminance and polarization stimuli.

The data I have already collected to measure neural responses to luminance will be included in "Visual Response Properties and Functional Organization of the Octopus Optic Lobe", a paper in preparation from our lab for which I will be a co-author. I also plan to present my preliminary results on the visual response of polarization light at the Society of Integrative and Comparative Biology conference in 2023.

Broader Impacts: Cephalopods evolved a highly capable visual system that contains eyes that are like those of vertebrates, with a complex brain similar to that of invertebrates. Their system evolved independently from all other vision-reliant species, yet their visual system is not well studied. By studying their evolutionarily distinct neural circuitry, I am actively expanding the field of vision science. This work will add context to the fundamental question of how neural circuits evolve, assemble, and function.

Outside of lab work, I use the charismatic allure of octopuses to engage with the community through octopus demonstrations. By sharing how I got into research, and the novel research I am currently doing, I hope to encourage young students to be curious and creative when confronted with scientific questions.

I will continue to do octopus demonstrations throughout my graduate career, with an emphasis on serving underrepresented communities in STEM. I currently have multiple future demonstrations planned, including an open house for SPICE, a program that serves female middle schoolers in STEM, and a presentation for the Women in STEM seminar series at UO. My future goal for the octopus demonstrations is to build a program through the Institute of Neuroscience DEI initiative that serves racially underrepresented students monthly, so I can make a larger and more lasting impact on them, and so that there is an established program for other students to continue such demonstrations after I graduate.

[1] Hardcastle, B. J., et al. (2021). ELife. [2] Smithers, S. P., et al. (2019). Science Advances. [3] Glantz, R. M., & Schroeter, J. P. (2006). J Comp Phys A. [4] Pignatelli, V., et al. (2011). Phil Trans of Royal Soc B: Bio Sci. [5] Saidel, W. M., et al. (1983). Nature. [6] Rowell, C. H. F. & Wells, M. J. J. exp. Biol (1961). [7] Niell, C. M., & Smith, S. J. (2005). Neuron.[8] Young, J. Z. (1971). Clarendon Press.