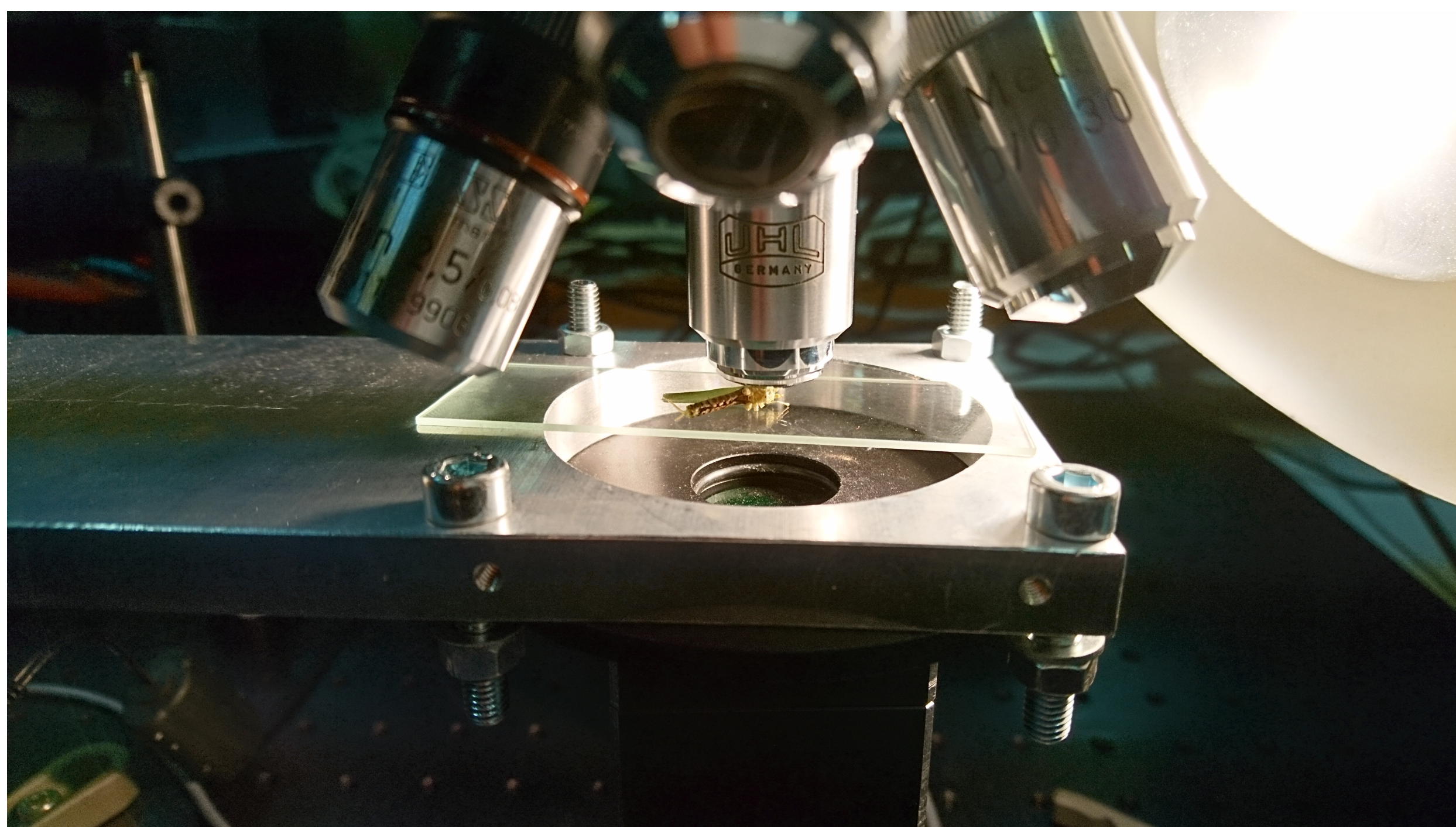


Motivation & Research Goals

Aim of the project: Get 3D info from focused/defocused microscopic images. The task was to capture microscope images using a camera while the object is moved through a plane of focus using a motorized Zaber stage. The images contain various focal planes, which can be later merged into a fully focused image through post-processing using algorithms. To achieve automated image capture, I wrote a Python code that controls both the motor and the camera. This code allows the camera to take pictures automatically as the object stage follows a predefined path.

Methods



Algorithmically achievable aspects are:

1. Controlling the object stage using motorized control.
2. Capturing images using the camera.

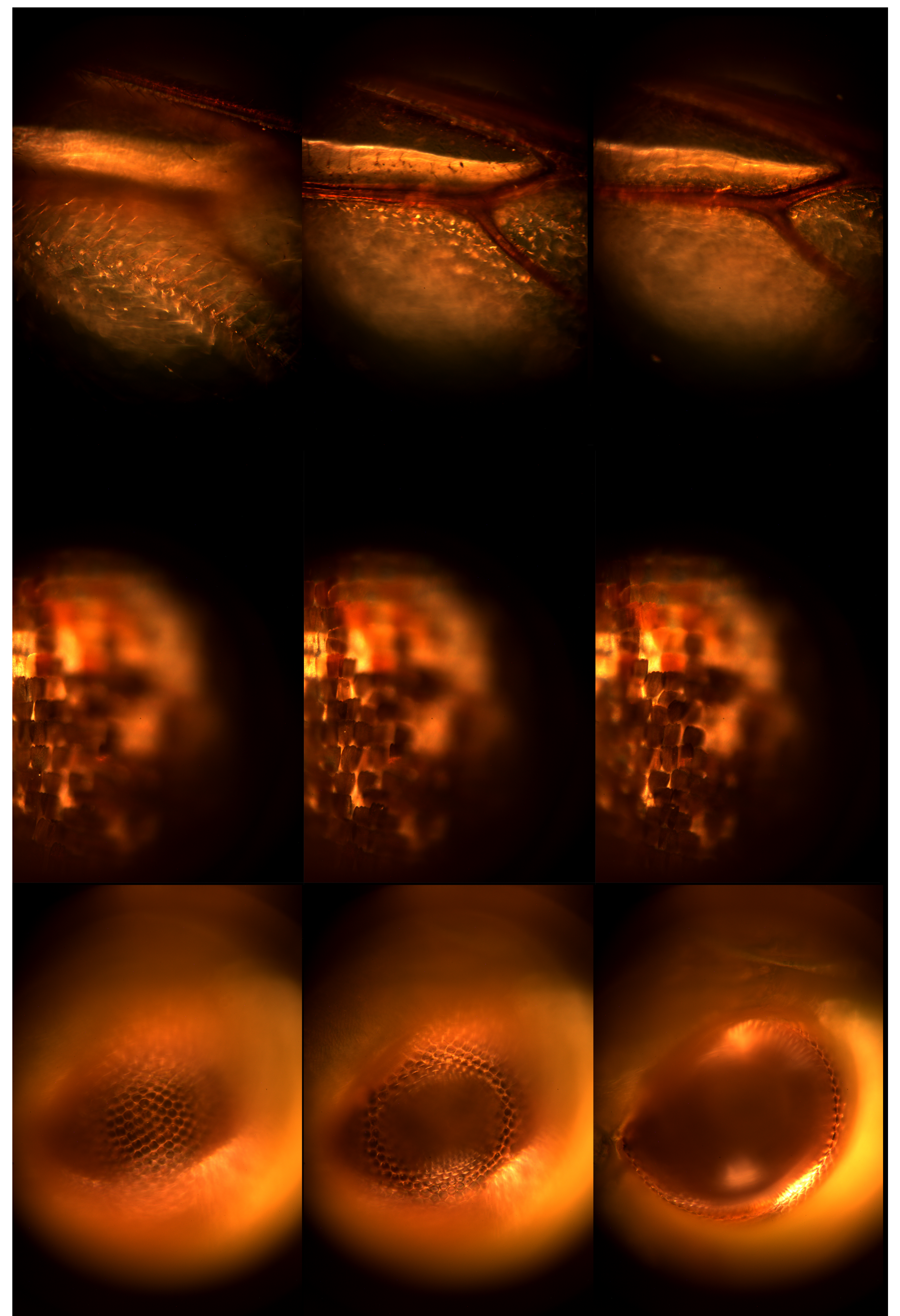
Algorithmically, the task is to combine both aspects:

1. Move the object stage to a reference position. Incrementally move the stage towards the lens, capturing an image at each step. Return the stage to the reference position and move it a certain distance away from the lens.

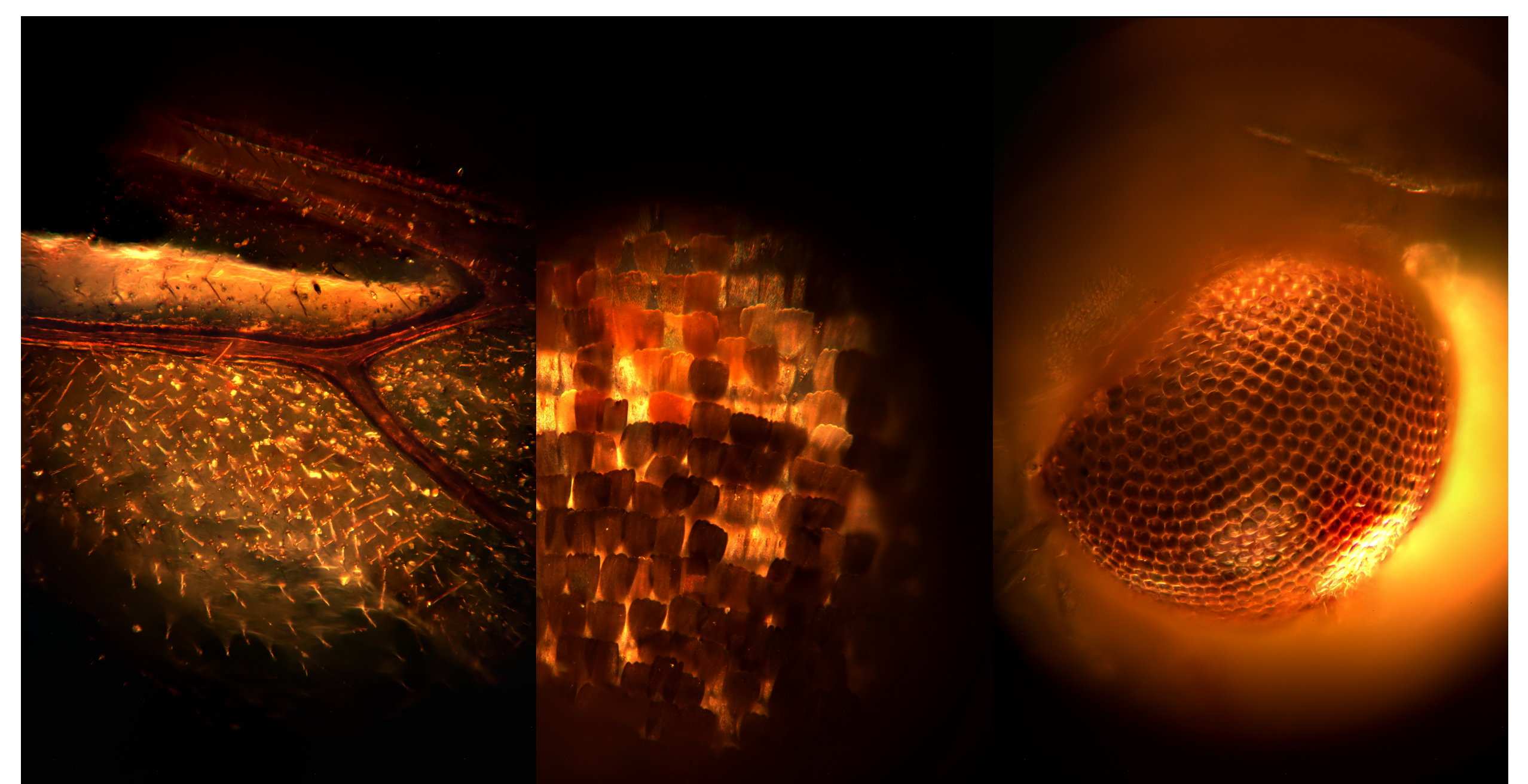
The algorithm follows these steps:

1. Set values in the main program for parameters to be sent to the stage, such as reference position, maximal, and minimal positions.
2. Define parameters for stack size and discretization, including step count, step size (e.g., 1 or 5 micrometers), and speed.
3. In the image capture function, input camera-specific values like exposure time and ISO. Initiate Micromanager for camera communication.
4. Start the main program: Step 1: Move the stage to the home position at a predefined speed. Step 2: Move to the reference position and wait until reached. Step 3: Create timestamped folders for image storage, including subfolders for specific data. Step 4: Capture the first image at the reference position with a stack counter. Step 5: Take images while incrementally moving towards the lens. Repeat until desired steps are covered. Return the stage to the reference position at a different speed. Step 6: Capture images while moving away from the lens in the opposite direction. Once images are captured, return the stage to the home position.

Selected Results




I've used this approach to capture stacked images of a bumblebee wing (top), butterfly wing (middle) and grasshopper eye (bottom) using the highest magnification lens. The final fully focused images were created using Photoshop.




A video showcasing the project can be viewed at the following link:



References

[1]  Zhang, Biomed. Opt. Express 12, 5544-5558 (2021)

[2]  Guo, Opt. Lett. 45, 260-263 (2020)