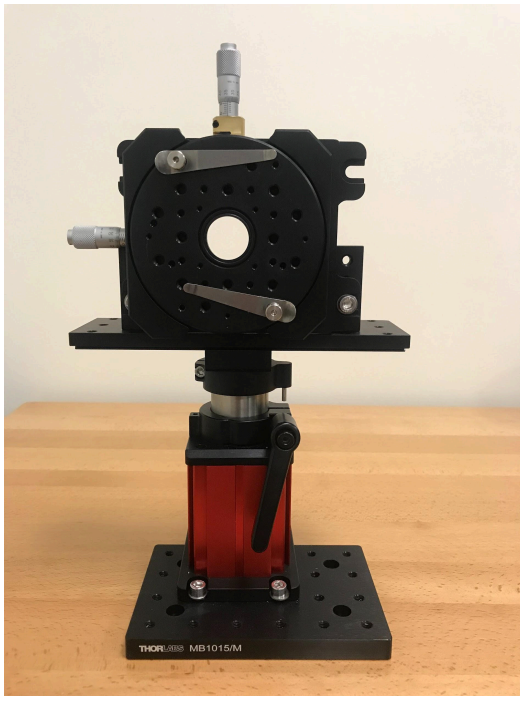
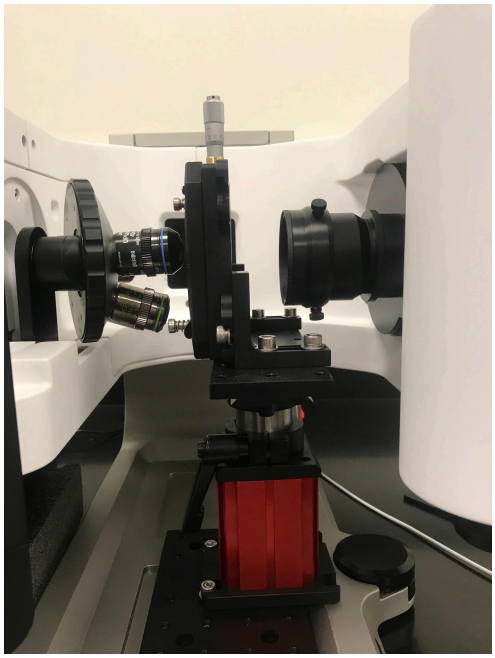
Microscopes are widely used in plant biology to study molecular processes. The problem with current microscopes is that you need to either ignore the effect of gravity or have a microscope placed on its side and dedicated to only gravitropic studies. In this study, we aimed to develop a method that would allow gravity studies on a microscope that was not dedicated for only that use.

We started with the Echo Revolve4 microscope as our base system due to its ability to rotate from inverted to upright on a solid base. The rotation allowed us to prop the microscope at a 180° angle and hold it in position with a 145 mm foam block. This position is checked every time using a bubble level to ensure a flat field of view.

The next obstacle we encountered is that a standard stage cannot be utilized when a microscope is positioned on its side. We, therefore, built a custom set up with standard Thor parts, that could be inserted when used for gravity studies. It was important for the stage to allow movement in the X-Y axis with coarse and fine adjustments as well as be able to rotate to allow for reorientation. We selected the XYR1/M Stage from Thorlabs with this in mind. It provides a 1” optical hole for transmitted light, 360° rotation, and thumbscrews for fine movements in the X-Y directions. On the Echo microscope, the objectives move to focus, so the fine and coarse focus knobs could still be utilized for focusing in the Z axis. The full list of parts and images for construction can be found below.

XYR1/M Stage with X/Y and rotational axis. 1” diameter optical hole	$627
MP-100 Adjustable stand with platform	$541
AB90C/M L-Brackets for mounting stage vertically	$52
MB1015/M 150mm width breadboard base	$42
SLH1/M Microscopy spring clips	$42
HW-KIT2/M M6 Screw kit for assembly	$112
Total	**$1416**

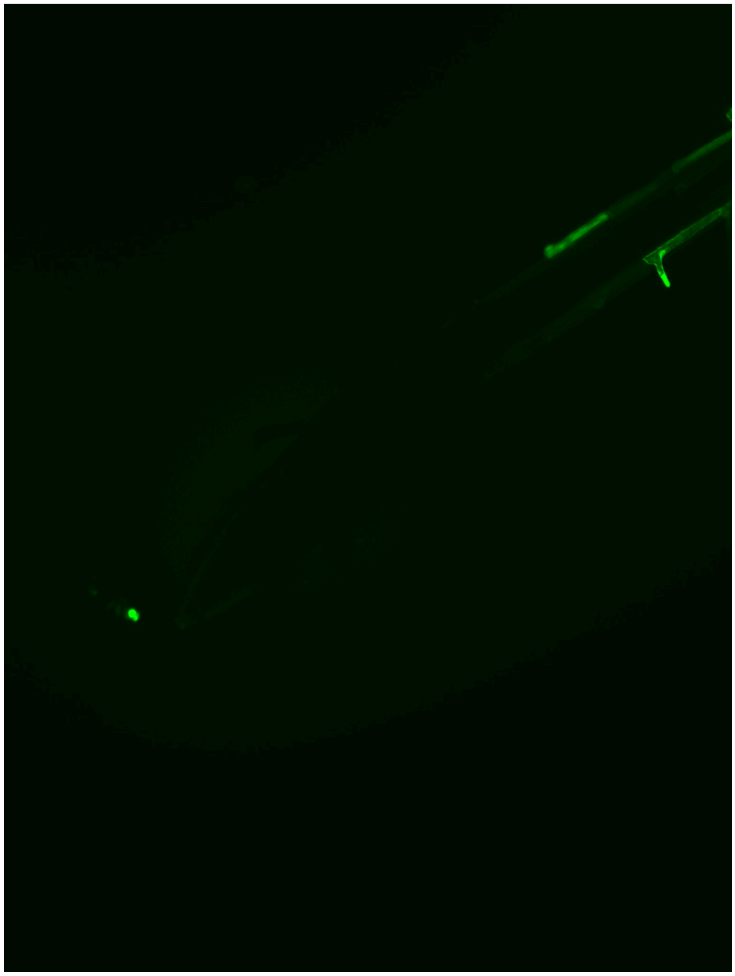




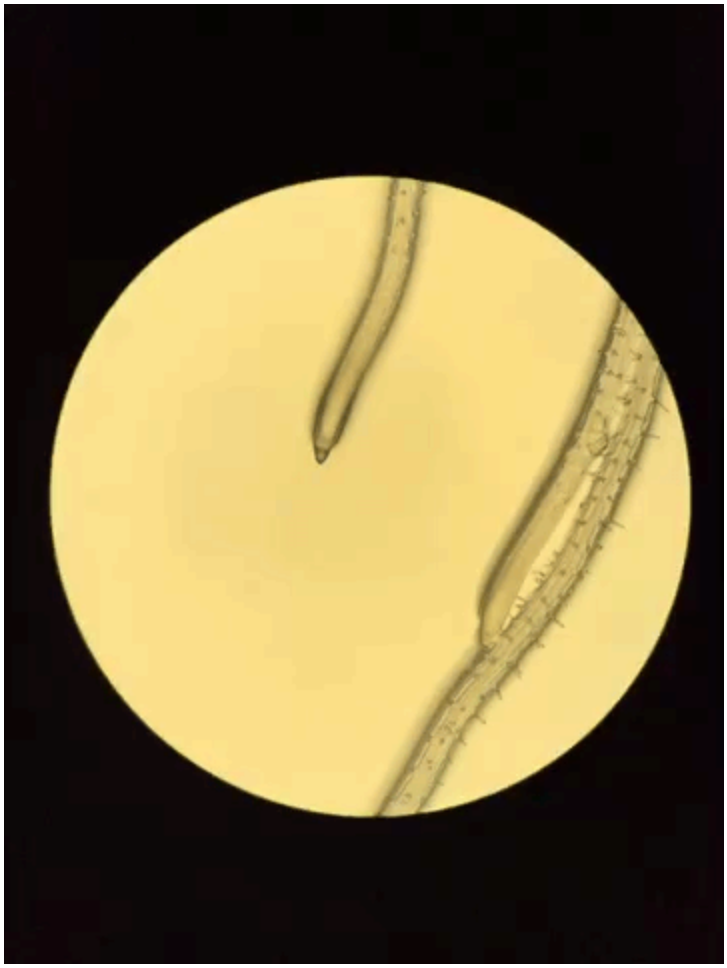
Results

We performed two different experimental setups utilizing this vertical stage setup: fluorescent manual timelapse and a brightfield automated timelapse. There is currently no way to control the fluorescent LEDs and monochrome camera without using the Revolve software. Therefore, manual timelapse is the only option if your experiment requires fluorescence. We used Arabidopsis seedlings that were stained with DCF and mounted on slides to visualize changes in ROS during root re-orientation. Images were captured every 3 minutes for 30 minutes and then made into a timelapse with photoshop. Using this method we were able to observe asymmetrical patterns of ROS expression (Figure xx).

Next, we performed automated timelapse utilizing the iPad app "LapseIt." The seedlings remained on their growth plates and were mounted to the custom stage. The transmitted light can be turned on manually at the base of the Revolve4 microscope and remained on for the duration of the experiment. Images were captured every 10 minutes overnight and results can be seen in Figure xx.



(Manual Time Lapse of ROS in re-oriented root with 4x objective)



(Time lapse utilizing LapseIt App, 1.25x objective with brightfield)