



Protocol September 2017

draft

Flashlapse Educational Package Required Materials:

- Flashlapse Units
- Flashlapse Software
- 90 mm Falcon Petri Dishes
- Filter paper
- Soaked Filter paper for Sowing
- Toothpick
- Micropore Tape
- Manual
- Packaging
- *A. Thaliana (Col-0)*

Optional:

- 70% Ethanol
- Parafilm
- Gloves

Biological Protocol for Gravitropic Assays/Preparation for Flashlapse

Pre-experiment sample preparation

1. Select *A. Thaliana* genotypes for a gravitropic assay.
2. Prepare media or filter paper, sow seeds.

Research Grade Space Media (Autoclave, fume hood, cold room)

Making Space Media

Materials needed:

- 3 g sucrose
 - 2.36 L&S Salts
 - 1 L deionized water
 - 10 g Phytogel
 - Large autoclave safe glassware
 - 90 mm circular Falcon petri dishes
- 1) Measure out 3g sucrose & 2.36g L&S salts into a container of 1L deionized water. Stir.
 - 2) Measure 10g of Phytogel, and put 5 g into 2 different containers. Pour the mixed solution into the bottles, 500 mL in each.
 - 3) Autoclave the bottles for 30 minutes. Let rest/cool for another 15 minutes.
 - 4) Pour out onto petri dishes in fume hood or sterile environment. Pour 35 mL in each dish. Take care to use heat resistant gloves as media is hot.
 - 5) Let the dishes set for 20 minutes.
 - 6) Tap side of dish to make sure it is set.
 - 7) Put lids back on plate and put in sterile bag, then put in cold room.

Media for Non-laboratory Environments (Classrooms, homes)

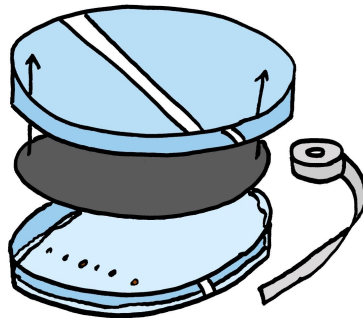
- Place prepared filter paper that came with Flashlapse into your Falcon dish.

Sowing Seeds

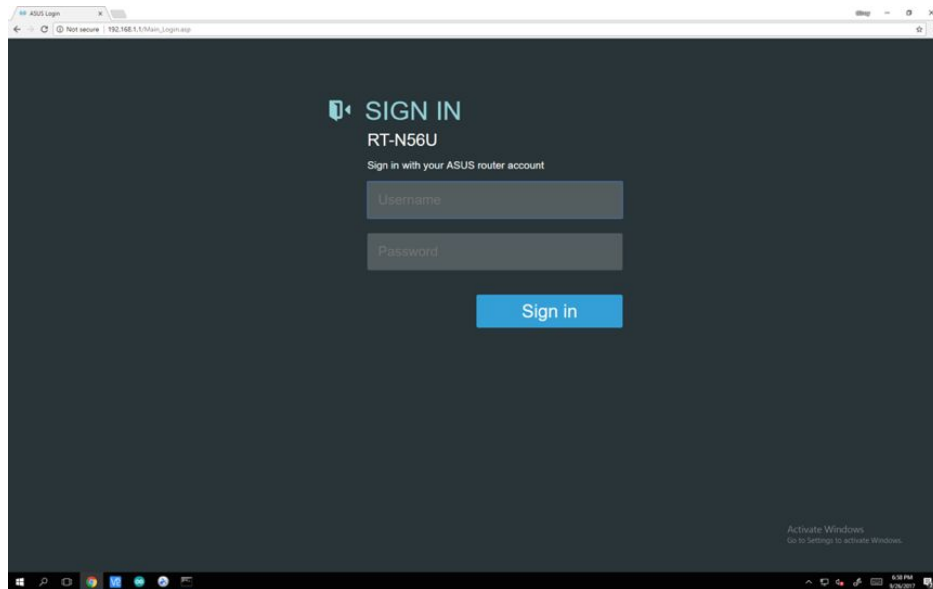
- Prepare a sterile environment to sow your seeds in. Ideally, this is a fume hood or other device designed to reduce contamination. A wipe down with 70% Ethanol is recommended. Wear gloves.
 - Put your *A. Thaliana* seeds in an Eppendorf tube with 70% Ethanol. If you have more than one genotype, separate them into different tubes.
 - Pour it out on filter paper, take care so no seeds get lost.
 - Soak a toothpick in 70% Ethanol and tap a seed to pick it up.
 - Evenly space your seeds on the filter paper/media
3. Sow *A. Thaliana* on space media/filter paper in selected petri dishes.
 4. Take care to keep the dishes upright for the remainder of the experiment.
 5. Use medical micropore tape to seal the dish for germination and 5 days growth under warm white LEDs.

Day Off Experiment Sample Preparation

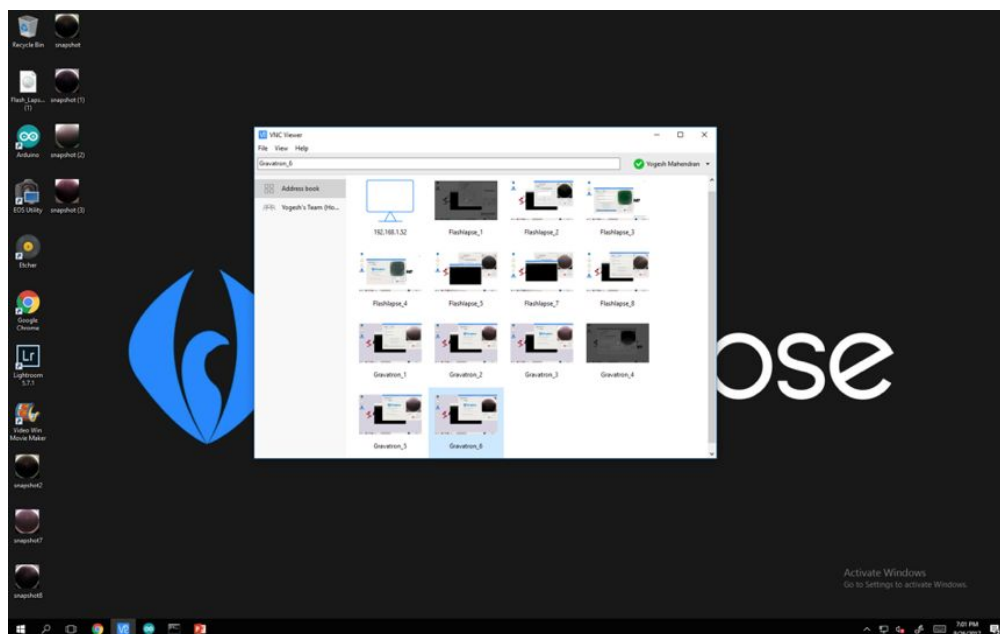
1. Open petri dish in side flow hood
2. Add black filter paper (THOMAS) (to prevent condensation from forming on the lid).
3. Reseal petri dish with micropore tape and then use parafilm for extra stability within the FlashLapse unit.
4. Place dishes in Flashlapse Units.
5. Activate software.



Flashlapse VNC and GUI Protocol for Gravitropic Assays



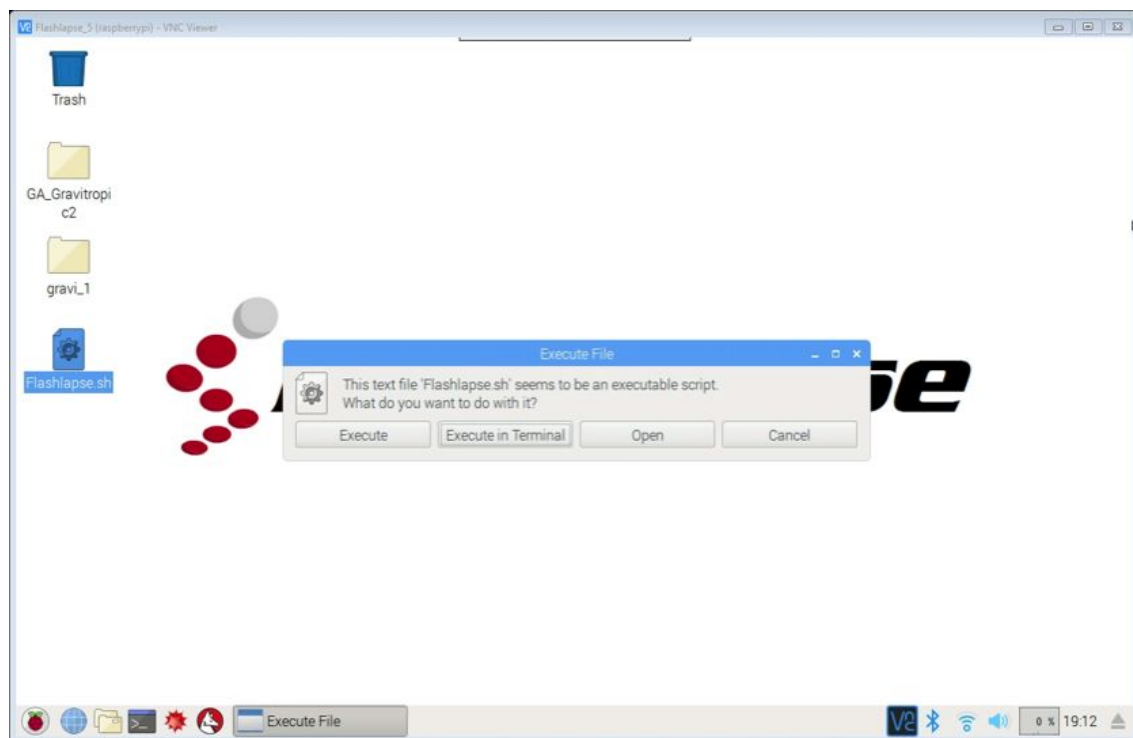
Sign in to your router provider to view which Flashlapse devices are connected.



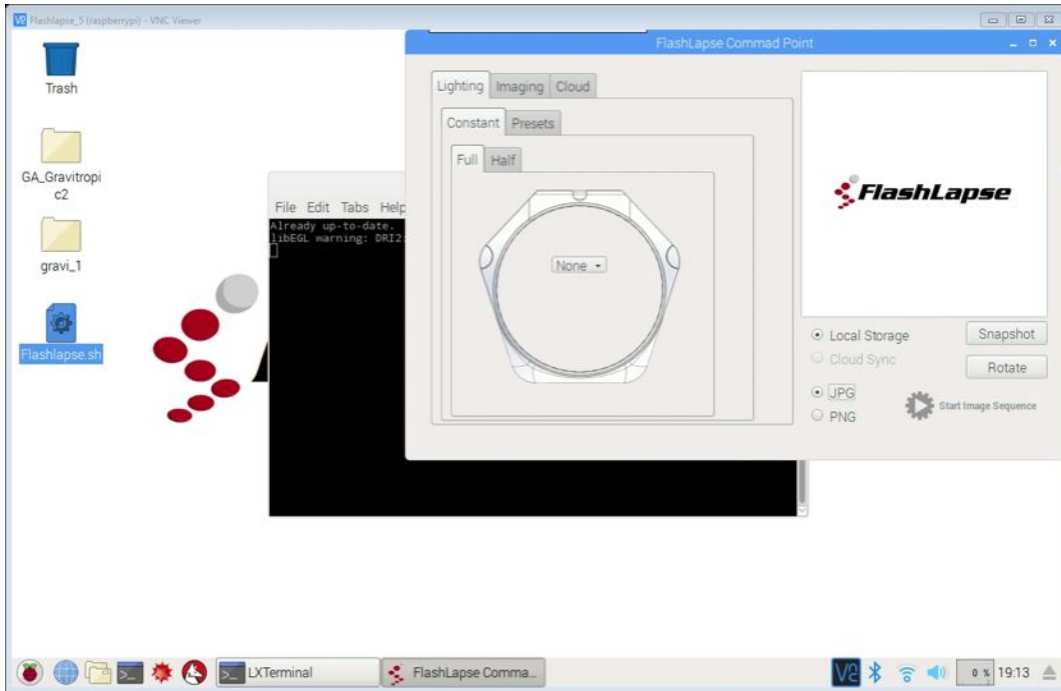
Open the Flashlapse VNC Viewer and click on one of your connected Flashlapse devices.



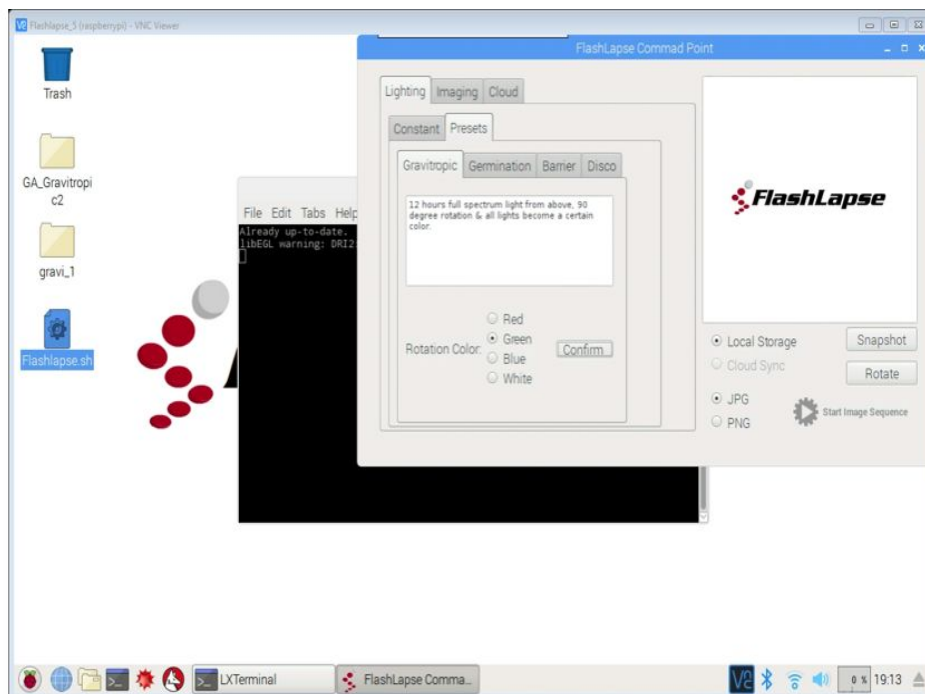
Click the Flashlapse.sh file



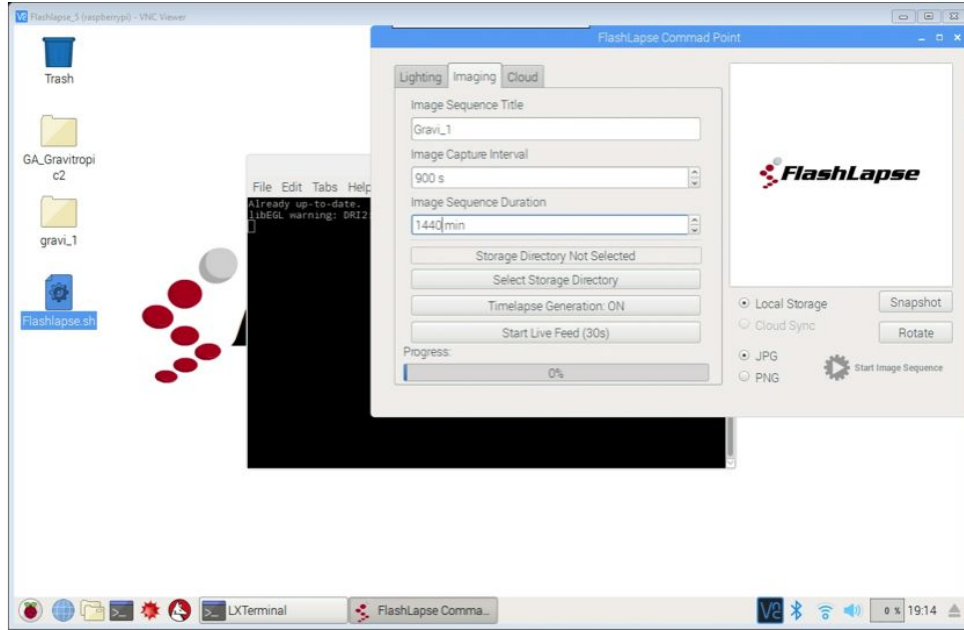
Execute Flashlapse.sh in Terminal or simply click “execute.”



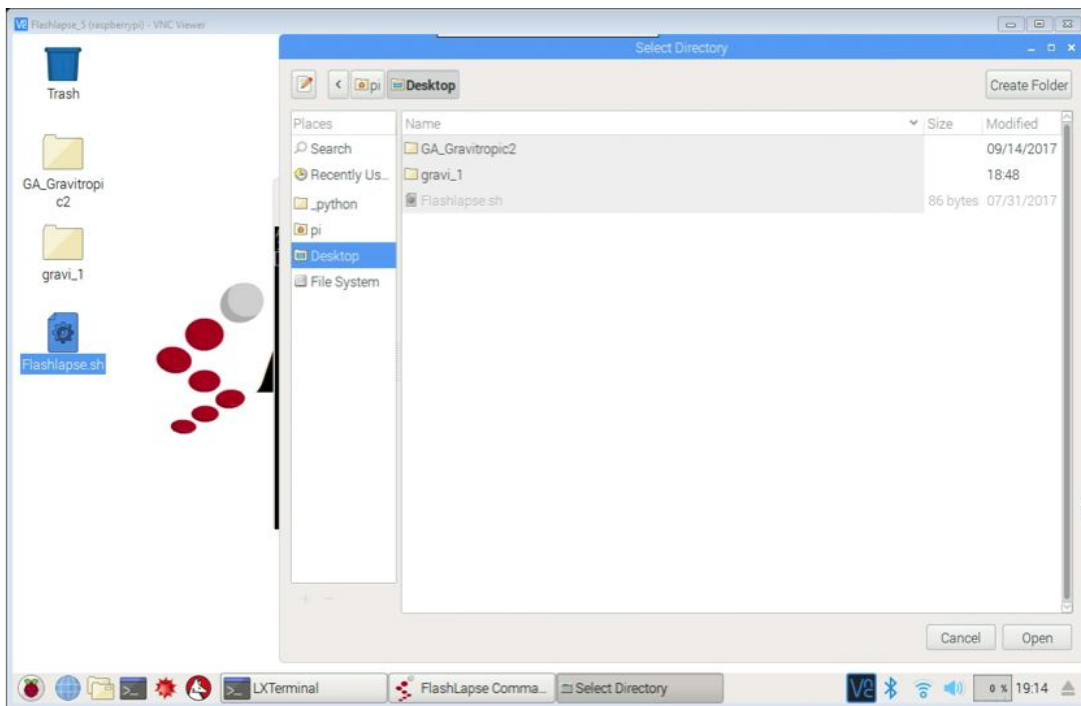
This GUI should pop up.



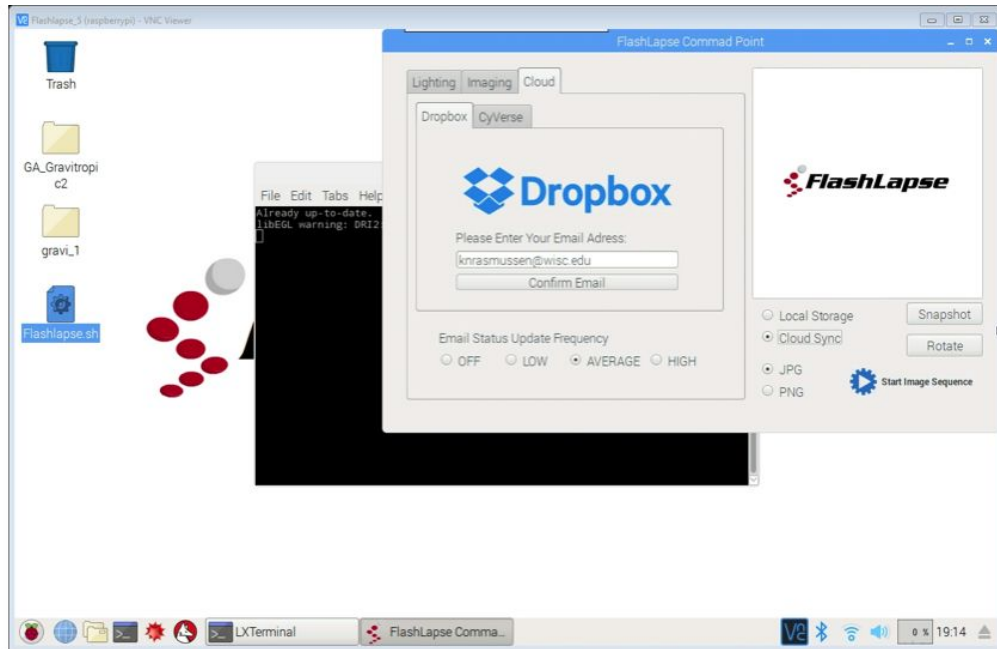
Under “Lighting” go to the presets for Gravitropic and select what color you want to run your experiment under.



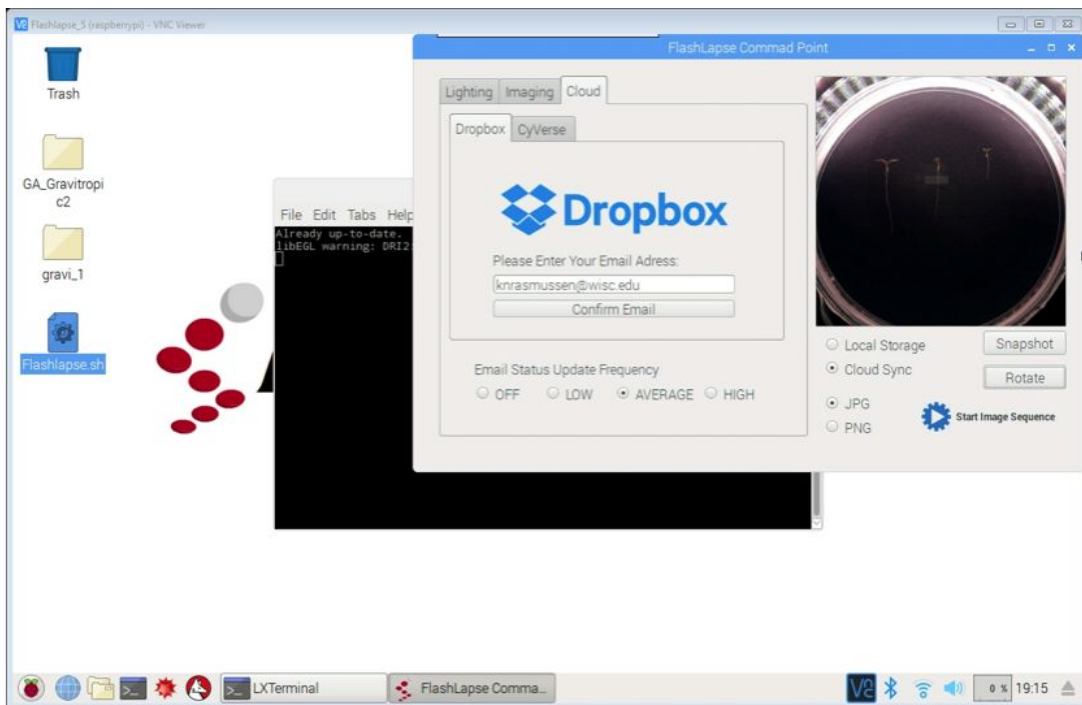
Under “Imaging”, specify your file name and make sure your file names are consistent with other Flashlapse devices. **They must be unique and it is highly recommended that your file name includes your genotype/ecotype/treatment.** Specify duration between taking pictures and also total experiment duration.



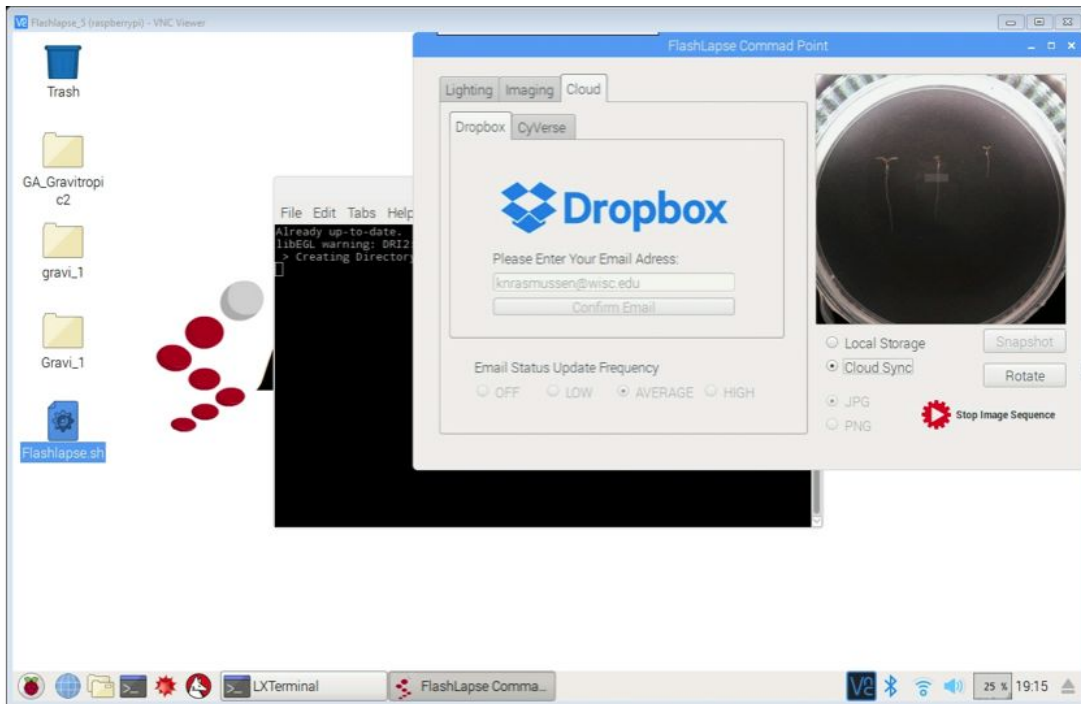
Click “Select Directory” and choose where you want to save your Flashlapse files.



Enter your email address to receive snapshots to the cloud.
Make sure you select CLOUD SYNC.



Finally click SNAPSHOT to make sure lighting on the Flashlapse is on.
Click START IMAGE SEQUENCE to begin.



Flashlapse is now running.

Exit out of the larger window, DO NOT EXIT OUT OF THE FLASHLAPSE COMMAND POINT. THIS WILL END THE EXPERIMENT.

When experiment is complete download the images.
Split the image series into 2 sections, straight growth and reorientation.
Then save them in CyVerse for analysis.