Instruction manual for phenotyping leaf disc using Zeiss AxioZoom microscope (easy version) and ZenBlue 3.0

Basic microscope setup

- Turn on the computer
- Turn on the microscope – EMS 3
- Turn on the Gooseneck lights; position them in a 45° angle to the table
- Set 0.5 magnification lens
- Attach the LED ring light to the magnifying lens and turn on
- Start Zen 3.0 Blue edition
- Perform stage calibration (calibration of the table; mandatory)
- Follow on screen instructions for focal calibration of the microscope (mandatory)

Leaf disc imaging

1. Switch from “locate” tab to acquisition tab
2. Select the pre-imported available template as default – leaf disc plate
3. Click on “Live”
4. Position the phenotyping plate on the table and align the A1 tile to the tile on microscope screen(A1)
5. Once overlapping
   → Double click on A12 to ensure linear travel over the centres of samples
6. Repeat the previous step for H12 and H1.
7. Once the microscope is moving to the centre of the four tiles your done
   → Double click on A1 (list of 96 individual leaf disc; Table should move to position)
8. Click verify tile region
   - → click on run “autofocus and set Z”
   - → right click on “A1” and click “set current Z for all point”
   - → and then click on “set all points as verified” (we assume that the agar is even)
   - → close
   - Go to “Channels set” tab
     → Set exposure time manually, by clicking measure (Note: At this step Gooseneck light, LED ring light and back light need to be on! Make sure that the estimated exposure time is always roughly the same (+/- 2 ms) for all experiments; important for the CNN; with the mentioned light setup 19 – 21 ms are the usal)
9. Start experiment
10. Once image capturing is done click on processing
    → Click on method
    → click on image export
    → set file type to JPEG
    → set export folder
    → set prefix (optional*) ex: default date, experiment number etc
    → choose input and click “Apply”.