# Manual for generating a leaf disc phenotyping template using a Zeiss AxioZoom microscope with motorized table

## 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)

## 1. Basic Tile setup

- Switch from “locate” tab to acquisition tab
- Set RGB imaging as default
- Click “Live”?
- Switch on the “Tiles” option ☑
- Go to “Tiles” tab
    - switch on the sample carrier
      → import “leaf disc plate” as template
      → select the “leaf disc plate”
      → and finally switch on the carrier well container
    - Click on advanced setup
        - click on tab carrier
          → select all the position (96 tiles)
          → go to create/remove tile regions for the selected carrier container
          → finally click on create
        - Fill factor setup
          → fill factor 65%

## 2. Microscope manual setting – SYCO3 joystick screen

- Click on microscope
  → set magnification to 10x
- Click on aperture
  → set it to 37% (smallest possible; highest focal depth)

## 3. Software autofocus

- Go to “Software autofocus” tab
    - Mode: Contrast
    - Quality: Default
    - Search: Smart
    - Sampling: Default

## 4. Focus strategy setup

- Go to “ Focus strategy” tab
    - Select “Software autofocus” in dropdown menu
    - Checkmark ☑

→ Reference channel and offset to bright and zero
- Checkmark ☑
  → stabilization event repetitions and frequency
  → set it to expert
- Select tile and repeat to each 1 of the tiles

## 5. Channels setup

- Go to "Channels set" tab
  - Checkmark ☑
    → bright
  - Uncheck auto exposure
  - 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 usual)

## 6. Calibrating the sample plate and positioning

- Go to "Tiles" tab
  - click on tile sample carrier
    → click on calibrate
    - By using "SYCO3 joystick" , manually move the "moving table" to extreme top left corner (move maximum distance to left and move maximum distance to top)
    - Place sample plate on with template and leaf discs on ta
    - Align ⊕ (x, y) axis symbol present on the microscope screen to the symbol present on the left top corner of phenotyping plate.
    - By using "SYCO3 joystick" check if the travel is linear along the X-axis and Y-axis respectively. (Takes a few tries)
    - Click next once it's aligned
      → Move table again to extreme top left corner
      → set the axis to zero (0, 0)
    - Click next
      → Click on "set current x, y"
    - Click finish
  - Go to tile region
    → double click on A1 (list of 96 individual leaf disc; Table should move to position)
    → Move between A1 and A12 to ensure linear travel over the centres of samples
      - 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"
        → close.
- Start experiment

## 7. Image file export

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”.