**Thermotaxis Arena Worm Tracking System Setup Checklist**

Modified: 08/3/23

Startup Sequence

*If using heating elements (e.g. thermotaxis assays or thermotaxis + odor assays):*

1. Turn on circulating water bath under the counter. Make sure water bath is set to the correct temperature, which will vary depending on the specific thermal gradient you want. Before moving on to the next step, allow the water bath to reach its new temperature (at least 30 minutes). The water bath can be turned on hours before starting the next step. If raising the water bath temperatures to 30C and above, try to turn it on at least an hour before step 2, so that it has time to heat the metal plate.

2. [No more than 1 hour before you want to start your assays] Turn on surge protectors to the right and left of the shed. This will turn on the ATEC302/FTC100D controllers as well as the LED array.

*If NOT using heating elements (e.g. odor alone assays or salt gradient assays):*

Unplug the ATEC302/FTC100D power cables from the surge protectors. Turn on the surge protector, making sure power is going to the LED array and the camera(s).

To set the thermal gradient

1. On the computer, open the ATEC302/FTC100D Software. Select the desired Com (Communication) port. The baud rate is always 9600. Hit “Confirm”. The correct com port numbers are:   
   Com Port 4 = “Hot side” on left  
   Com Port 7 = “Cold side” on right  
   *\*\*as of 8/3/23, note that these may change if the USB ports connecting the computer to the Accuthermo deviced are changed).*

Example gradient settings (as of 8/3/23 on the Bryant lab thermotaxis arena):

|  |  |  |  |
| --- | --- | --- | --- |
| Gradient | Cold Side SV | Hot Side SV | Water Bath Temp |
| 16-20 | 12.5 | 20.5 | 10 |
|  |  |  |  |
|  |  |  |  |

Example gradient settings (as of 11/3/22 on the Hallem Lab thermotaxis arena):

|  |  |  |  |
| --- | --- | --- | --- |
| Gradient | Cold Side SV | Hot Side SV | Water Bath Temp |
| 20.6-24.6 | 18.5 | 27.5 | 15 |
| 21-34 | 16.5 | 46.5 | 25 |
| 16-20 | 12.5 | 22.0 | 10 |
| 12-22 | 5 | 26 | 5 |

*\*Note: these parameters are subject to change as the ability of the 4 TECs to maintain temperature changes over time/use. If you haven’t run assays in a while, check with recent users to see if there are new parameters. In addition, track your own parameters in your notes.*

To change the temperature parameters, hit the Parameters button. Type in a new desired temperature in the SV window (top left). If the bottom right wheel is not set to “On”, set it to “On”.

1. Repeat for second temperature. Wait ~30 minutes for the system to reach equilibrium; this time is approximate, please check by eye for your specific gradient. Longer for hotter gradients (esp. the gradients that involve temperatures near core body temperature).
2. Place a small amount of 70-100% glycerol on the aluminum surface of the thermotaxis setup and then set down a thermotaxis dish. The dish should “lock” into place, seated firmly on the inner moat edges. Try and avoid air bubbles. Wait ~20 minutes with the lid off. You can do this as soon as you set up the temperature gradient if you want.
3. Use the laser thermometer to confirm the desired temperatures have been achieved at the “hot” and “cold” ends of the thermotaxis agar plate.

To image worm migration using the camera

*To take images of the thermotaxis system requires 2 components: The camera (takes the photos at pre-determined intervals) and pylonViewer software (triggers image acquisition at desired intervals, controls camera shutter settings, receives images from camera and saves them).*

1. Open pylon Viewer software
   1. In the Devices panel, select the appropriate camera listed under USB connections. With the device selected, use the toggle button to Open Device (toggle will go from red to green).
   2. Use the Recording Settings panel to set up a “Sequence of still images” acquisition.
      1. Output format = Tiff
      2. Recording buffer size = 100 frame(s)
      3. Record a frame every = <your desired frame rate>
         1. For tracking adult worms, record a frame every 5 seconds
         2. For tracking iL3s, record a frame every 2 seconds
         3. For quantifying general worm preferences without detailed tracking, record a frame every 1 minute.
      4. Stop recording after = <your assay duration>
      5. Scroll down the panel to navigate the output folder. Redirect folder to documents >[your name]>New folder>Rename UID folder for experiment.
   3. Use the Features – Basic panel to set the exposure time. For the thermotaxis arena, <71500 us has been good.
   4. To view a live image of the plate, At the top of the window click the blue video camera icon.

*At this point, the camera is set up and ready to acquire images. When I place the worms on the assay, I like to have the camera in live mode, but not saving images. That way I can confirm that the worms are showing up in the images, before starting the image acquisition.*

1. If assays include odors, place odors at desired locations.
2. *If doing a population assay:* place 3-5 uL of worms at the desired temperature (measured using the laser thermometer). Spread the droplet using a glass worm spreader.

– OR –

*If doing a single worm/small population assay*: place worms in <3 uL at the desired temperature (measure using the laser thermometer. Using the camera’s live imaging mode, watch until you see worms emerging from the drying water droplet (can take ~5 minutes for a 3-5 uL drop to dry, depending on the starting temperature).

1. When ready to begin acquiring images, with the shed door closed, hit the red record button.
2. Set a timer for the duration of your experiment then go do something else.
3. After the experiment has finished, remove the agar-filled plate from the shed, adding iodine to kill parasitic worms, then scraping the agar from the dish into biohazard trash. Rinse the iodine/glycerol from the dish in the sink; set aside for additional washing in a dishwasher followed by cleaning with 90% ethanol.

*Note that the Basler cameras tend to overheat, so at the end of the experiment the pylon Viewer software may close automatically. Data will be saved; you can open the camera again when necessary to take more images. To prevent overheating, stop live imaging (and/or Close Device) whenever possible.*

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