Fish sorter instrument SOP

Last Updated:

Jan 20, 2026

Contents

Background

V2.0 of the fish sorter is a semi-automated zebrafish classification and selection instrument. It requires human labelled fish classifications and selection criteria. The fish sorter functions for both embryos and larvae. This operation is for use with the Fish Sorter instrument and using a napari interface for instrument control. This SOP is current for the python-fish-sorter software tagged v2.1.1.

Workflow

Load fish into an agarose array omnitray

See Casting agarose array v2 SOP

Loading a new pipette tip

Note that the tip can be reused many times

See Preparing pipette for Fish sorter v2 SOP

Setup the Fish Sorter Microscope

Setup microscope

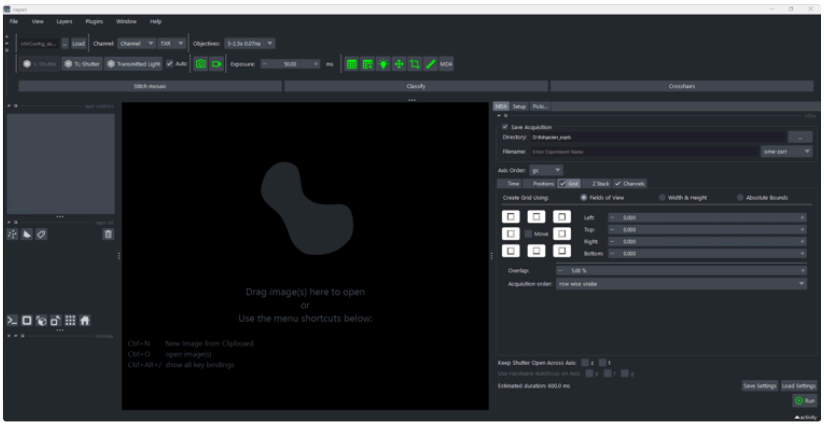
1. Turn on all hardware:
   a. light source
      i. open the shutter prior to operation

b. power strip that turns on
    i. camera
    ii. Leica controller
    iii. pressure box
        1. Set the vacuum to 25 inHg
        2. Set the pressure to 15 psi
2. Set up the destination plate holder
    a. Place the 6 well plate in the destination plate holder
    b. There is a set screw to hold the plate in place
        i. Use the allen key to loosen / tighten the screw against the plate
3. Place the omni tray containing the agarose array with fish into the plate holder on the stage

Start the Fish Sorter

Start software

1. Double click on the desktop shortcut `fish sorter`.
    a. This loads napari micromanager with the extended fish sorter hardware



Start up of the fish sorter software in napari micromanager

Set up Instrument to image fish

Pre-run setup

1. Set the desired file path and file name prefix
    a. Typical file format is:
        i. Folder name: `{fish age dpf or hpf}_{transgenic line}`
        ii. File name: `{fish age dpf or hpf}_{sort}{expt#}`
    b. Optional:
        i. Load previous settings then change the directory and file name
        ii. Unclick `include Z` and `Positions`

iii. Check the grid positions for the top left and bottom right
    1. Move to each position
    2. Recenter as needed
    3. Click the corner to update
iv. Double check the channel settings for exposure and channels

c. In some cases, it may be desirable to image with brightfield (for example, if the fluorescence is not bright enough to sufficiently detect fish)
    i. There are two methods to image in brightfield:
        1. With the brightfield condenser
            a. This method will not allow for full remote operation after imaging, because the pipette is not able to be positioned over the objective in this case
            b. Make sure the brightfield condenser arm is positioned over the objective
            c. Click the `Groups and Presets Table` button
            d. Adjust the `TL-Ap` (aperture) and `TL-Light` so that the array is detectable
            e. Note that when setting the MDA, the exposure and TL-Light values may need to be adjusted from the Preview values
            f. Once imaging is complete, rotate the condenser arm away from the objective, position the pipette over the objective and calibrate it
        2. Pseudobrightfield with the side LED
            a. Turn on the LED light
            b. Move the pipette over the objective and calibrate it for sorting
            c. Move the collection plate stage over the microscope for imaging
            d. Choose a suitable exposure time

d. In some cases, especially for embryos, it might be helpful to acquire images at higher magnification
    i. Change the objective with the Napari Micromanager `Objective` button
    ii. The rest of the setup is the same
    iii. It is important to do this before the Setup Picker tab
        1. If not, click `New Experiment` and repeat the setup

e. Note that we find it is faster to save the files locally to the computer and then transfer to the shared drive

2. If starting a new calibration Turn on `Preview` and `Crosshairs`

    a. Calibrate the Top left and Bottom Right corners
        i. Move to each position and click the specific corner when calibrated
    b. Make sure the pipette is over the objective if using fluorescent channels for locating calibration points
3. Click the `Setup` tab
    a. Select the imaging and dispense plate arrays and fish type
    b. Make sure the objective is correct prior to clicking `Setup Picker`
    c. Click `Setup Picker`
    d. Note that this will save the MDA acquisition settings automatically here
        i. It is important that the magnification not be changed after this point
            1. If it needs to be changed, click the `New Experiment` button and adjust the setup parameters
4. On the `Picking` tab
    a. Select `Move to Swing`
    b. Move the pipette over
    c. Tighten the swing arm to lock the pipette in place
    d. Confirm the pipette stage arm is ready for software control:
        i. Check that the LED light at the top of the stage is blue, and not blinking red
            - If is blinking red, gently rotate the knob to move the stage until you feel it click into a set position
5. Calibrate the pipette
    a. Adjust the pipette up or down using the move pipette button and the input distance
    b. Ensure the pipette is centered
    c. For the the pick position, navigate to an area between wells in the agarose array
    d. Move the tip to just touch the top of the agarose array
    e. Move up 50 um, move to a well, move back down 50 um
    f. Move down 500 um and set the tip position
    g. Click the `calibrate pick position`
6. Calibrate the dispense position
    a. Move the dispense plate ensure the tip height for dispense makes sense
7. Bring to the center of the plate and focus on a fish
8. Move the destination plate between the microscope and the pipette tip

a. This prevents reflection of the excitation off of the pipette tip and holder
b. Click under the `Picking` tab `Move stages to image`

9. Start imaging
    a. Turn off the preview by clicking the `Preview` button
    b. Close the curtain and attach the automation in progress sign up
    c. Click the `MDA` tab
    d. Select `Run`

At this point, the system is walk-away and the remainder of the workflow can be completed using remote computer control.

Stitch Mosaic and Classify Fish

Classification

1. Mosaic stitching and loading the classification GUI will automatically run after the imaging finishes. If you need manual controls, follow the steps below:
    a. Click `Stitch Mosaic`
        i. It takes a little bit, so wait or check the command line for status
    b. Click `Classify`
        i. At any point it is possible reset the view to the full mosaic by clicking the `home button` to center everything
2. After the classification GUI has loaded, there should be only layers for the mosaics of the imaged channels and Well Locations layer
3. Click on the layers to change the contrast fluorescence intensity
    a. Right click the contrast bar to make a big slider bar
4. Find Fish
    a. Choose either a specific channel, or the sum of the intensity
    b. Set the Sigma to ~1 and click `Find Fish`
        i. Check whether it found the fish well enough or not
        ii. Repeat if needed if not all of the fish are selected or there are too many wells selected by adjusting the Sigma, typically reducing the value
5. Start to classify fish
    a. Make sure you select the `Well Locations` layer
    b. Click in the Classification feature widget
    c. Start to classify based on the hot keys
    d. Notes:
        i. Double check the head orientation for larvae
        ii. You can select a well point and it will load that well crop image

iii. If you hit empty and multiple it will deselect the well, e.g. if you navigate backward it will not cycle through that position under the found fish toggle condition

iv. Classify whether the slot is empty, has multiple fish, or only one fish (singlet)

1. Hotkeys: `e`, `m`, `s`

v. If the fish has fluorescence features, label it appropriately

1. Hotkeys: Numeric (`1`, `2`, ...)

vi. Navigate through the wells

1. It is not required to use the Find Fish algorithm to classify fish. With the navigation mode set to all wells you can navigate through all wells and classify.
2. Hotkeys: Right/left arrow
3. Press `T` to switch between navigating through all of the fish or the subset of fish found by the fish finding algorithm
4. Under all well navigation mode, the right/left arrow keys move to the next/previous well
    a. If a feature classification is selected for a well, the well will be automatically classified as a singlet
5. It is possible to lasso several wells at once and bulk classify
6. Under singlet navigation mode, the right/left arrow keys move to the next/previous fish marked as a singled
    a. Note: If a fish or well is reclassified as anything that is not a singlet (empty, multiple, etc) it will be removed from the singlet navigation group. The next well with a singlet fish will be immediately displayed

vii. Press the `Reset` button to remove all found fish and classifications

6. Hit `Save Classification` when done
    a. This is at the bottom left in the same tab group as `Finding Nemo`
    b. This saves an auto-generated classification for each well (`{file prefix}_classifications.csv`) in the data saving folder

Pick fish

Pick

1. Click the `Pick Selection` tab
    a. Populate the rows according to the fish feature(s) you'd like sorted in each `dispenseWell`
    b. Add rows for each additional picking condition

c. Click `Save` to save the pick selections
    i. This saves an auto-generated picking file (`{file prefix}_pickable.csv`) in the data saving folder
2. (If not done already, or needed) adjust the pick and dispense heights and click their corresponding buttons to update calibrate picking and dispense position in `Pick` tab
    a. If necessary, recalibrate the picking and dispense position by adjusting the pick and dispense heights and clicking their corresponding buttons to update
3. If you want to watch live
    a. Move dispense stages to home
    b. Click the `preview` button and hide the other layers
        i. Make sure to turn on one of the fluorescence channels for pseudo brightfield
    c. Click the `crosshairs` and zoom to the desired size
4. Click `Full Pick!`
5. If you need to pause picking, click the `Pause Picking` button, and then `Resume` when desired
    a. This will restart from the current location in the pick list
6. If you need to stop picking, click the `Stop Picking` button
    a. If you click `Full Pick!` again, it will start from the beginning of the pick list

Collect Fish and Shut down

Shutdown

1. Move the pipette to the Swing position
2. Exit the program
3. Shut off the lamp
4. Toggle the power switch button
5. Remove the plates of picked fish and agarose mold
6. Rotate the pipette arm away from the objective
7. To keep dust off the objective, place a blank plate in the plate holder
8. Return the agarose mold to the refrigerator after removing any remaining fish

Troubleshooting / Reset

From almost every step in the process it is possible to recover from any issue that arises. In most cases this does not require reloading the fish sorter napari window.

Hardware:

Hardware issues

- If the dispense stages fail to move properly, or are not responding, press the `Move Dispense Stages to Home` and then `Move Dispense Stages to Image`.
  - If they still do not move, click `Reset Hardware` and see if the stages move then
  - Check that the LED lights at the back of the stage are lit
- If the pipette fails to move properly, is only moving in one direction, or fails to respond
  - Check that the LED light at the top of the stage is blue, and not blinking red
    - If is blinking red, gently rotate the knob to move the stage until you feel it click into a set position
    - Check if it now will move with the software
  - If it will not move down but will move up
    - Turn off the live preview, if it is on
    - Click the `Pipette Down` button
    - Toggle to the terminal window and read the current position setting
      - If it is at 75 mm, then the pipette is at the travel limit
      - Contact BioE for support
  - For all else, click the `Reset Hardware` button and see if the pipette moves.
- If the pneumatics are not operating properly
  - If the fish are not being picked properly, check the pick height position
    - Make sure that the pipette will slightly drag on the top surface of the agarose. There should be some lag in the movement of the pipette as the stage moves
    - Check the pick height is 500 um below the height of the agarose
      - If you moved the pipette up 50 um to move into the well, be sure to move the pipette 550 um down from that point
  - Use the `Single Pick` option to check if a fish can be picked
    - Navigate to a fish
    - Click Move to Pick Position
    - Click Single Pick
    - The fish should be aspirated
    - At this point, either
      - dispense the fish into the destination plate

        - Click the `Move to Dispense Position`
        - Click `Expel` twice
      - move the stage to the far edge of the plate, outside of the well array
        - Click `Expel` twice
    - If the fish are still not picked properly, rotate the pipette away from the objective
      - Click the `Toggle Pressure Valve`
      - Ensure that there is a steady air stream coming from the tip
      - Click `Toggle Pressure Valve` again to stop the flow
      - If there was a steady stream of air, check picking a single fish again
    - Check the Draw / Expel by moving to the outside of the well array
      - Move the pipette to the liquid surface
      - Click `Draw`
      - Check that liquid was aspirated
      - Move the pipette up
      - Click `Expel`
      - Check that the liquid was expelled
    - If it is still not possible to aspirate / dispense fish, replace the tip
  - Note that `Reset Hardware` maintains all experiment parameters, and does not require `New Experiment` setup

General Restart / Next Experiment:

General

- Click the `New Experiment` button
  - This will clear all layers, close any fish sorter tabs, clear the pipette calibration positions, and clear all fish sorter setup parameters
- If you have not collected images yet, proceed as in a new experiment above Fish sorter v2 SOP | Set up Instrument to image fish
  - If the MDA settings were correct reload from the yaml file
    - Directory: `{experiment data folder}`
    - Filename: `{file prefix}_settings.yaml`
    - Unclick the `Positions` button in the MDA
- If the mosaics are correct, follow Fish sorter v2 SOP | Restart from Classification:
- If you have collected images, but something was wrong

- Delete the previous `ome.zarr` folder and mosaics (anything marked `.tif`)
- If the MDA values are desired,
  - Reload the MDA settings from the yaml file
    - Directory: `{experiment data folder}`
    - Filename: `{file prefix}_settings.yaml`
  - Make adjustments, as needed, to the MDA settings
  - Unclick the `Positions` button in the MDA
  - Delete the `.ome.zarr` from the filename
    - It will reappear, but if it is not deleted it can get appended a second time
  - Input the setup sorter parameters and click `Setup Picker`
  - If you had previously calibrated the pipette, you can recalibrate by pressing the `Move to Pick Position` / `Move to Dispense Position` and once the pipette is done moving press the `Calibrate Pick Postion` / `Calibrate Dispense Position` button respectively
  - Click `Run` on the MDA
- Otherwise, setup the MDA values as above Fish sorter v2 SOP | Set up Instrument to image fish
  - If for the new experiment the directory and filenames stay the same as before, delete the `.ome.zarr` from the filename
    - It will reappear, but if it is not deleted it can get appended a second time

Restart from Classification:

Classification restart

1. Click the `New Experiment` button or restart the program
2. Reload the MDA settings from the saved settings in the `{experiment data folder}`
   a. `{file prefix}_settings.yaml`
3. Input the setup sorter parameters and click `Setup Picker`
4. Drag in the mosaics saved as `{imaging channel}.tif` in the `{experiment data folder}`
   a. Change the `Blending` to `additive`
   b. Adjust the `Contrast Limits` as need
      i. If you need higher resolution on the Contrast bar, right click on the bar

5. Now click the `Classify` button and wait for it to load
6. Proceed through classification as above
7. Note that if you had previously calibrated the pipette, you can recalibrate by pressing the `Move to Pick Position` / `Move to Dispense Position` and once the pipette is done moving press the `Calibrate Pick Postion` / `Calibrate Dispense Position` button respectively

Restart for Picking:

Picking restart

1. Navigate to the `{experiment data folder}`
   a. Confirm that the classification and pick selection files exist and contain accurate information
      i. If so, continue on
      ii. If not, follow the steps for Fish sorter v2 SOP | Restart from Classification:
2. Click the `New Experiment` button
3. Reload the MDA settings from the saved settings in the `{experiment data folder}`
   a. `{file prefix}_settings.yaml`
4. Input the setup sorter parameters and click `Setup Picker`
5. Click `Classify`
   a. Note that it is not required to load the mosaics, if the classification and pick selection parameters (csv files) already exist
   b. This loads the array plate location settings and is required for picking
6. Calibrate the Pipette
   a. If you had previously calibrated the pipette, you can recalibrate by pressing the `Move to Pick Position` / `Move to Dispense Position` and once the pipette is done moving press the `Calibrate Pick Postion` / `Calibrate Dispense Position` button respectively
7. Click `Full Pick`

Exported/saved data

Data

Images: `{data folder}`

- All images are saved to the data folder defined in the MDA tab
- Mosaics are saved as `{imaging channel}.tif`

- Data is saved locally and then can be moved to BRUNO, which should be `/hpc/instruments/bioe.fishsorter`

Classification data: `{data folder}/{date_time}_{file prefix}_classifications.csv`

- CSV with classification based on user selection
- Headers/feature names are generated based on `features` defined in `pick_type_config.json`

Picking parameters `{data folder}/{date_time}_{file prefix}_pickable.csv`:

- CSV specifying dispense well for each set of fish features based on GUI inputs
- Headers/feature names are generated based on `features` defined in `pick_type_config.json`

Picking results: `{data folder}/{date_time}_{file prefix}_picked.csv`

- CSV with classification based on user classification and picking parameters
- Each time `Full Pick!` is run a new file will be generated