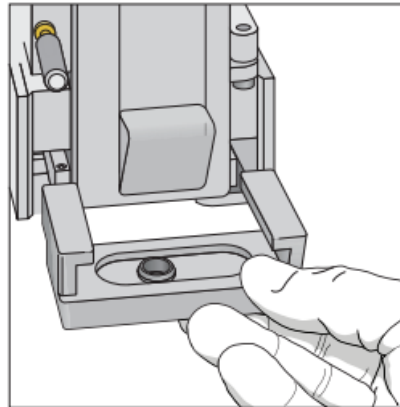


Fusion Jon – Setting Up for Plate Sorting

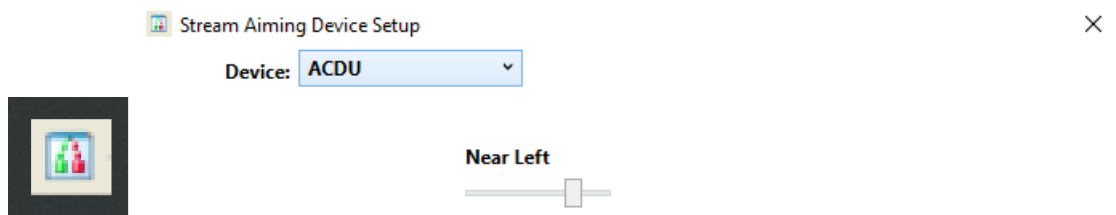
To calibrate the plate stage for plate sorting, we recommend using the Accudrop Drop Delay Experiment Template and Accudrop Beads.

When using Fusion Jon to sort in plates, please calibrate the plate stage using the standard 384 and 96 well Falcon plate format. The following document guides you step-by-step on how to calibrate the plate stage using the home device function.

1. Install the Splash Shield by sliding into the fitting beneath the sort aspirator drawer.



2. In the computer taskbar, launch the **SortDeviceControl** app and change the guide to **ACDU**. Please pay attention: the Stream Aiming Device Setup tells you to use the near-left voltage slider to sort in plates, but that is an error, and one must always use the far-left voltage slider!

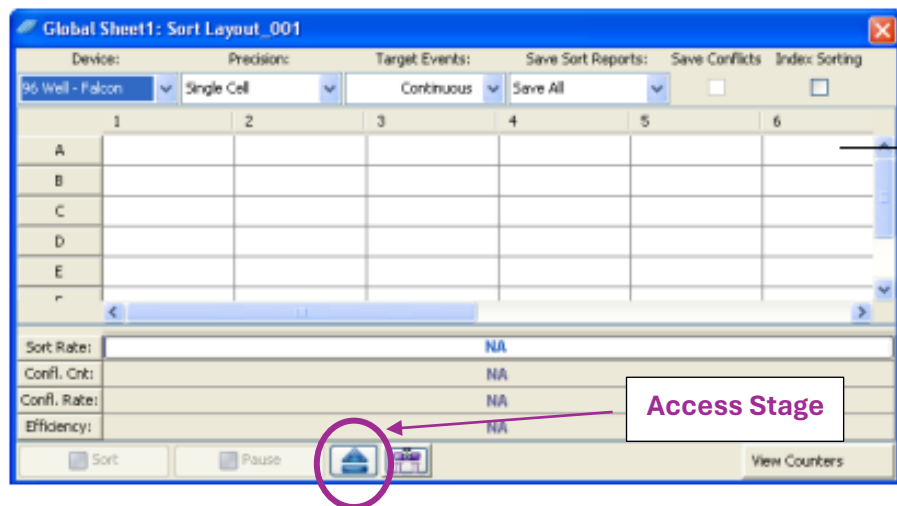


SortDeviceControl App Icon and Stream Aiming Device Setup window

If sorting with the 100 μ m nozzle, set the **far-left voltage slider** to a value of **18^a**, all the remaining 3 voltage sliders should be kept at 0.

3. Open the Accudrop Drop Delay Experiment.
 4. Create a new Sort Layout under the Accudrop Drop Delay Experiment Tube:
 - a. If sorting in a 384 well plate, in the Device drop-down menu select **384 well – Falcon**.
 - b. If sorting in a 96 well plate, in the Device drop-down menu select **96 well – Falcon**.
- a) The value of 18 is optimised only for Fusion Jon with the 100 μ m configuration. Other configurations/sorters will have different optimized values for the % charge applied to the side stream.

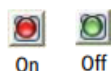
5. In the **Target Event** field box, set the number of events to be sorted to 50.
Add Population P1 to the 4 wells at each corner of the plate and to 4 wells in the center of the plate.
6. Click on the **Access Stage** button on the Sort Layout to bring the plate stage to the front of the Collection Chamber.
Connect the plate stage to the refrigerated circuit if you wish to keep your plate and collected samples at 4°C.



7. Insert the plate (with lid or film on) on the plate stage. A1 well should be placed in the front left position.
8. Select **Sort > Home Device**
9. **First Check: Side Stream is passing the Splash Shield.**

In the side stream window, turn the **Voltage** button ON, and OPEN the **Waste Drawer**.

Voltage button

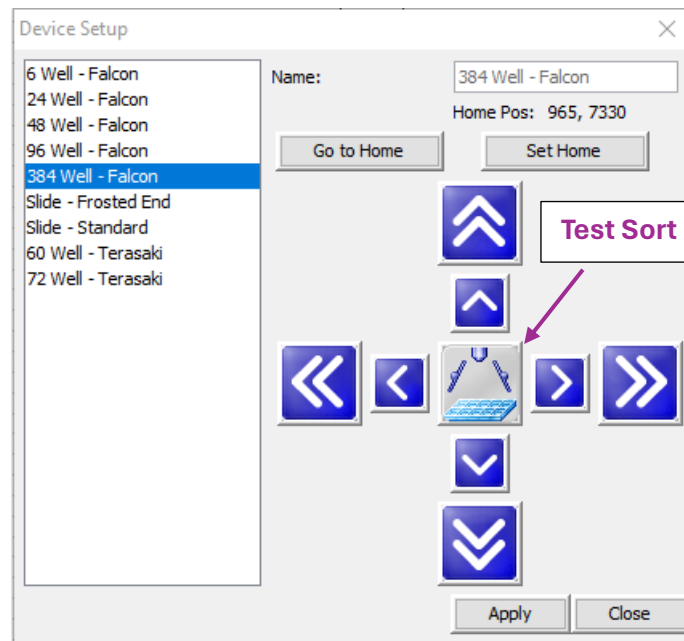


Waste Drawer button



In the Device Setup Window, select the custom device you are using (*384 well - Falcon* or *96 well - Falcon*) and press the **Test Sort** button.

Inspect visually the plate and the side stream window to check that the side stream is passing the Splash Shield. If it so, stop the Test Sort, wipe the plate and move to Step 10. If you don't see any liquid passing the Splash Shield, or if you see a "splash" line in the side stream window, stop the Test Sort, correct the voltage slider and repeat Step 9. Remember that you want the voltage slider value to be as low as possible with the stream passing through the splash shield.



10. **Second Check: Set the Home Position.**

In the Device Setup Window, click on Go To Home button to send the plate stage to the current Home Position (Well A1) coordinates.

In the side stream window, turn the **Voltage** button ON, and OPEN the **Waste Drawer**.

In the Device Setup Window, press the **Test Sort** button ON & OFF quickly to deposit a drop in the Home Position.

Inspect visually the plate to check if the Home Position corresponds to the center of A1 well.

- a. If so, move to Step 11.
- b. If the Home Position is not centered on well A1, adjust its coordinates by pressing the appropriate arrow button (the arrows indicate the direction the plate stage will move; large arrow = 5 steps, small arrow = 1 step) to move the plate stage. After any change, click on **Set Home** and **Apply** to save the new coordinates. Repeat Step 10 until calibration is correct.

11. **Last Check: Confirm plate stage calibration sorting Accudrop Beads.**

In the Device Setup Window, click on **Go to Home** button.

Load a tube with Accudrop beads and select the appropriate sort layout (see Step 4).

Start the sort.

When the sort is completed, inspect visually the plate.

- a. If the plate stage alignment is correct, you can proceed with sorting your sample.
- b. If there is any misalignment, go back to the Device Setup Window, click on **Go to Home** and then make further adjustments in the coordinates for the Home Position as described in Step 10. Repeat Step 11 until calibration is correct.

12. Create an experiment for your sort and prepare to sort your sample.

Please remember before starting a plate sort (& even between plates) to always click on **Go to Home on the Device Setup Window, otherwise the plate stage will lose its alignment.**