# Using the Attune cytometer

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**If you are the first user of the day (the Attune is off):**

1. Turn on the cytometer by pressing the power button. Some startup noises are normal.
2. Give the machine about 5 min to go through it's system check.
3. The computer screen probably says "shutdown complete". Hit OK and Logout.
4. Then quit the Attune software and log out the last user from windows completely. (The shutdown was run under the last person's login).
5. Log yourself in.
6. Double click on the Attune software icon on the desktop.
7. Click the button on the upper right hand that says **"startup"**. Give the machine about 10-15 min. to warm up the lasers and go through it's startup.
8. Login with your username and password.
9. Click "**performance test**".
10. Take a FACS tube (5mL small "snap-cap round-bottom falcon" tube) and add **1mL of 1X PBS**. Take beads from refrigerator and shake tube well. Add one drop to the 1mL PBS. (If you accidentally add two drops, that is fine too, but try not to do this routinely as the beads are expensive).
11. Load the beads.
12. Click **"run performance test"**. Give the machine about 20 minutes to complete the test. (****currently all of our beads have the same lot # so there is no need to *ever* change the lot #****)

**If you are *not* the first user of the day (ie. the Attune is already on):**

1. Check the date of the last performance test. This will be showing with a green checkmark below the login box. If it was today, you may login and **proceed**. If not, **go back to step 8 in the above section**.

2. If the system has passed the test, please login, this will bring you to the main menu.
3. Your templates should appear in the menu at the bottom half of the screen.
4. Double click on your template and enter your # of samples. (I usually only have one "specimen" (this indicates the *type* of run ie. KC cells or live wing cells) but several "samples" (ie. genotypes plus controls) I usually allow the date to remain the filename and keep a table in my notebook of what I ran on the Attune with each date, so that I can easily locate my desired data files by the date.
5. Check the paramters for the acqusition on the left. You should usually be running at "standard sensitivity"; collecting at least 10,000 in R1 (for KC cells) or 10,000 GFP+ (R1,R2,R4) for live dissected wing or eye analysis; and

running 500uL when you have 1mL of solution in your Facs tube or 300uL when you have 500uL (which is the normal situation for dissected tissues).

6. Double click on your first sample for the day (be sure a little arrow is to the left of your 1st sample name in the window in the right.) Right click to rename your sample (ie. en-gal4 ctrl, en-gal4 E2F etc.)
7. Load your sample. The green run arrow on the right should light up. Click run. Your cells should fall close if not already within the pre-drawn gates in your template. In general, you should not need to adjust voltages with our pre-designed templates.
8. You may adjust gates slightly if needed. **Click record**.
9. Once the sample has finished running if you pressed record the data will be saved. If you forgot to press record, the machine will ask if you want to save the data. If you do, press yes.
10. Double click on your next sample, making sure the little arrow points to the correct next sample. If you adjusted any parameters, the machine will say something like "your worskpace has changed do you want to save". The answer is yes. Rename the next sample if needed.
11. Run & record.

**Shutdown of the Attune:**

1. Once you have run and recorded your last your last sample, check to see if anyone else needs the machine that day. If not, proceed with shutdown.
2. Go to main Menu (listed after clicking the circle with AB at the top left)
3. Logout.
4. Click shutdown
5. Prepare a FACS tube with 3mL of 10% bleach. This is ready-made in a ble-capped Falcon tube and/or a small bottle labeled bleach next to the Attune. I find it is easiest to pour from the small bottle into the FACS tube until about 80% full. You can re-fill the small bottle from the blue-capped conical tune labeled "bleach".
6. Follow instructions on screen. We use standard wash (10 cycles).
7. Once you have started the shutdown operation, walk away, the full shutdown + wash takes about 30 min. and shuts off the lasers. The Attune cannot be re-started for at least 1 hour after a shutdown cycle, (which is why we always check if someone else will use it that day). The next person can log you out, or you can come back and log yourself out about 30 min. later.

**Notes on using the Attune:**

You may need to refill solution bottles. The machine will tell you when this is needed.

All of the needed solutions are next to the Attune. To add solution, press on the textured portion of the reagent access door (below the power button) to open the door. Pull out the bottle slightly without disconnecting it. Unscrew the white cap and refill the bottle until about ½ full. <u>MAKE SURE YOU ADD THE CORRECT SOLUTION TO THE CORRECT BOTTLE!</u>

**Note that the SHUTDOWN SOLUTION is 10X, and therefore must be diluted to 1X with autoclaved dH2O before pouring into shutdown bottle. There is a conical 50mL tube for this dilution (pour in 5mL of 10X, add dH2O to the 50mL mark – mix and pour into bottle)**

The waste bottle is the ONLNY bottle you should EVER disconnect. To empty waste, press down on black tube to release, pull out second connector. Carry to sink, open white cap and empty into sink. Re-close. To reconnect, press down on acceptor to firmly and snugly reconnect black tube. Re-attach second connector.