

# FlowCam Standard Operating Procedure Version 3

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## Abstract

The FlowCam can be used to take quick cell density measurements of liquid samples.

**Citation:** Jacob Harris FlowCam Standard Operating Procedure. **protocols.io**

dx.doi.org/10.17504/protocols.io.hgjb3un

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## Before start

Before you begin, make sure that

1. the appropriate flow cell and syringe are mounted
2. appropriate objective and columnator, if needed, are in place

The above two usually default to the following configuration:

Flow Cell	100 um (fc100)
Syringe	0.5 mL
Objective	10x with columnator

## Protocol

### Turn on FlowCam

#### Step 1.

Push silver button on the left of machine to turn on the FlowCam and the computer.

### Install flow cell into flow cell holder

#### Step 2.

1. Select desired flow cell tubing based on application. We usually use the 100 um x 1 mm flow cells.
2. Use a kimwipe or sheet of lens paper to gently clean the glass portion of the flow cell.
3. Insert glass portion of the flow cell into the flow cell holder.
4. Secure flow cell by gently screwing the male threaded holder until the flow cell is stationary. Be careful not to screw too tightly or you risk breaking the fragile flow cell.



**Note:** There are usually multiple projects using the FlowCam, so be sure to select the flow cell labeled for each specific project.

#### ⊕ NOTES

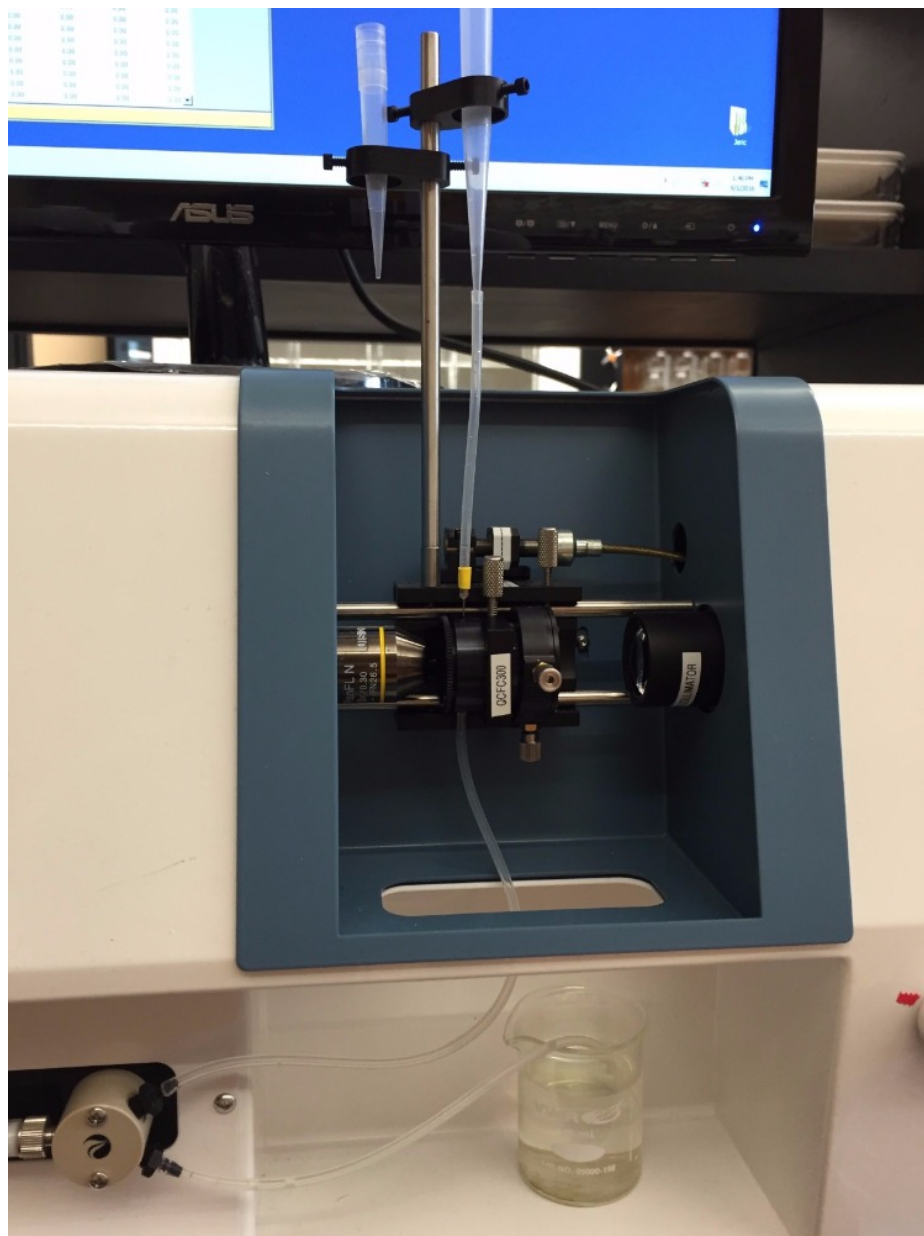
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- If this is the first time the flow cell is being used, cut the tubing above the flow cell to 10 cm and the tubing below to 20 cm.
- Measure from the tip of the flow cell that is inserted in the tubing.
- Be sure to record this information into the **Flow Cell** tab in the **Context** settings.

Install the flow cell holder into the FlowCam

#### Step 3.

1. Make sure the objective lens and collimator are compatible with each other. For example, when using the 10X lens, use the corresponding 10X collimator.
2. Use a sheet of lens paper to gently clean the objective lens and collimator.
3. Install flow cell holder (screw pointing up) into the flow cell holder mount located in the middle of the camera apparatus. Tighten screw on top of holder to secure it to the mount.
4. Insert tubing on top of flow cell into the bottom of sample funnel. Insert bottom tubing into tip of syringe. Insert syringe-out tubing into small beaker for waste collection.

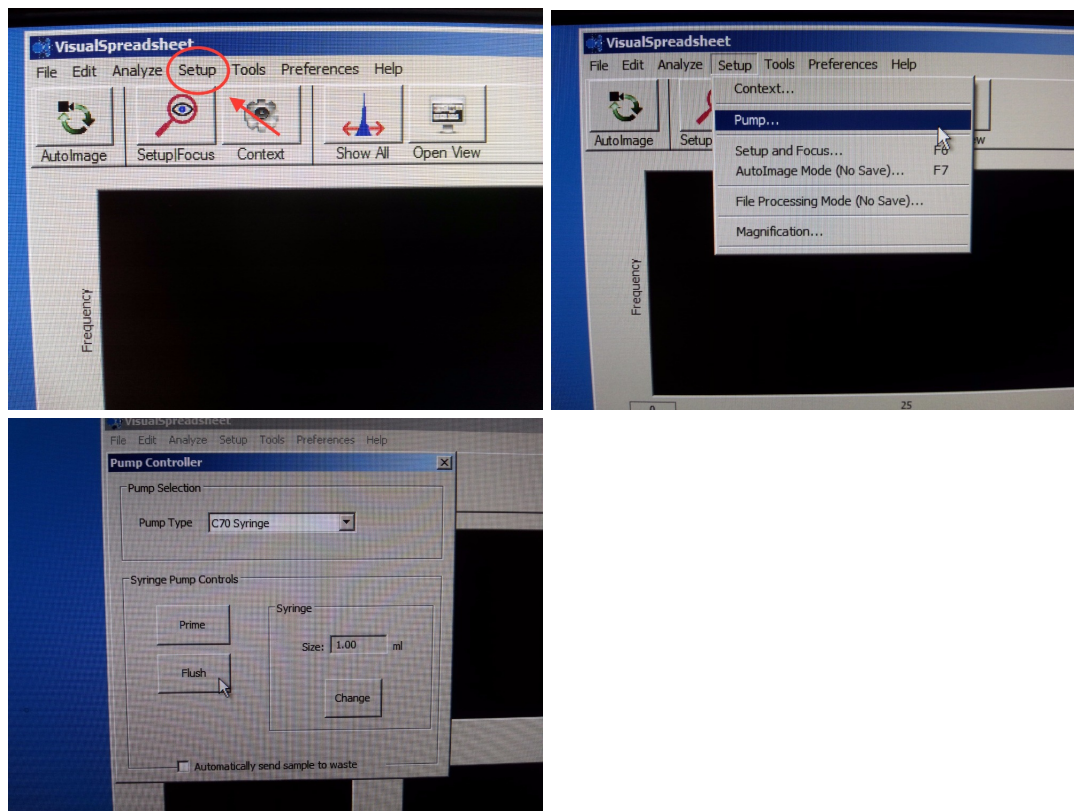


Flush the flow cell

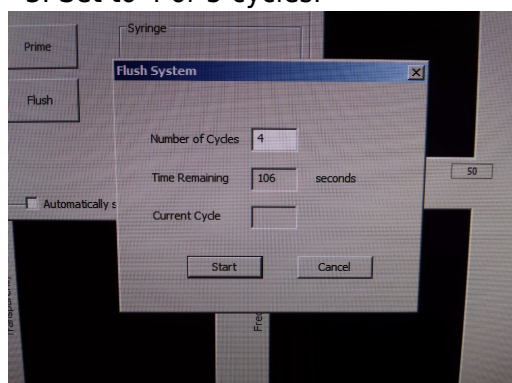
#### Step 4.

Make sure to flush the flow cell before beginning to prevent any previous cellular material or debris from showing up in your sample.

1. Load the funnel with ddH<sub>2</sub>O.
2. On the top menu bar, click **Setup > Pump > Flush**.



3. Set to 4 or 5 cycles.



4. Click **Start**.

## Load sample

### Step 5.

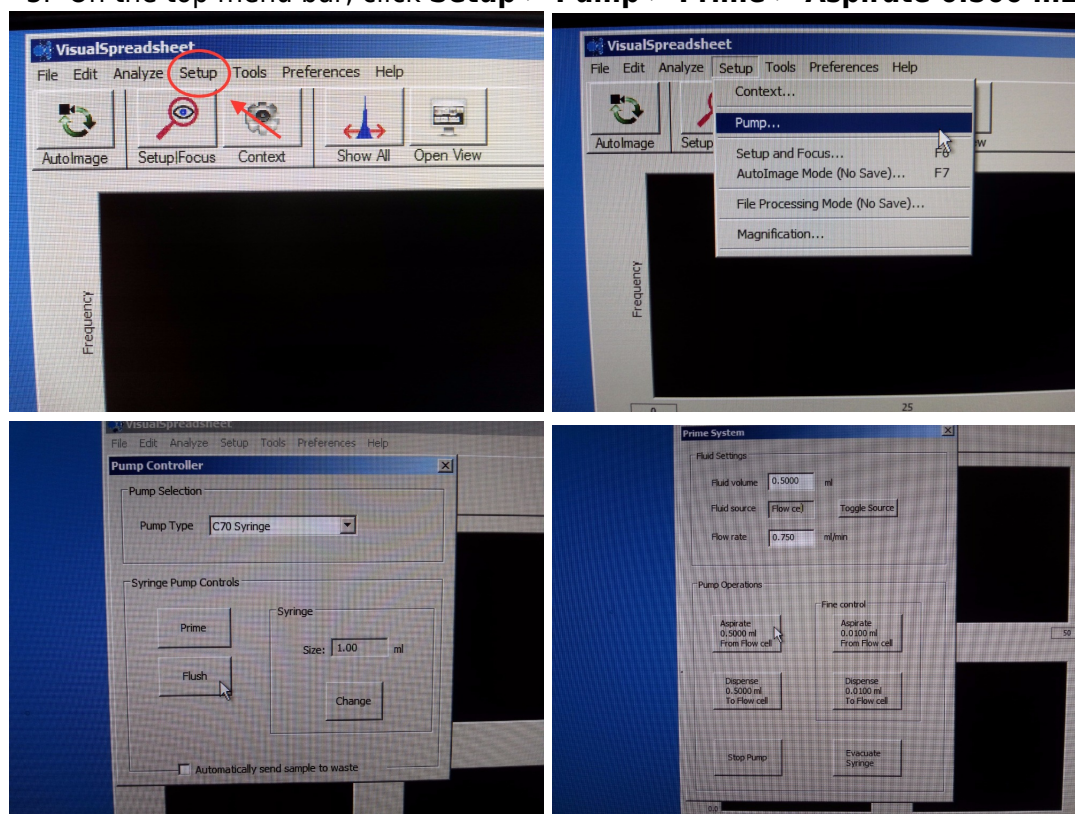
1. Use a pipet to draw sample from your container. Usually about **200-500 uL** of homogenized sample is sufficient. For dense cultures, a dilution may be appropriate (be sure to record this in your notes). You can sieve raw samples that have high levels of detritus or zooplankton.

2. Lower tip of pipet into bottom of P1000 sample funnel. Forcibly squeeze pipet to ensure that



sample ends up as one liquid mass and doesn't stick to the walls of the funnel.

3. On the top menu bar, click **Setup > Pump > Prime > Aspirate 0.500 mL from Flow Cell**.

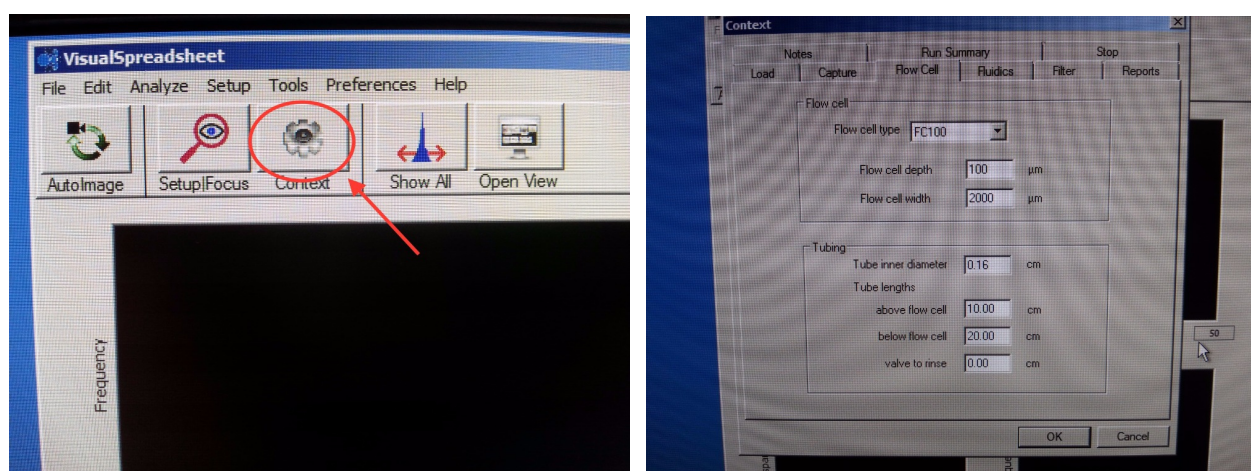


4. Click **Stop Pump** when the sample volume has reached the flow cell.

Run context

**Step 6.**

Click on **Context**. A window with several tabs will pop up.



- **Notes tab:** Relevant metadata. There is a file on the **Desktop** that can be modified as needed.

This file includes lines for date, experiment, strain, species, magnification, flow cell, syringe, and dilution factor.

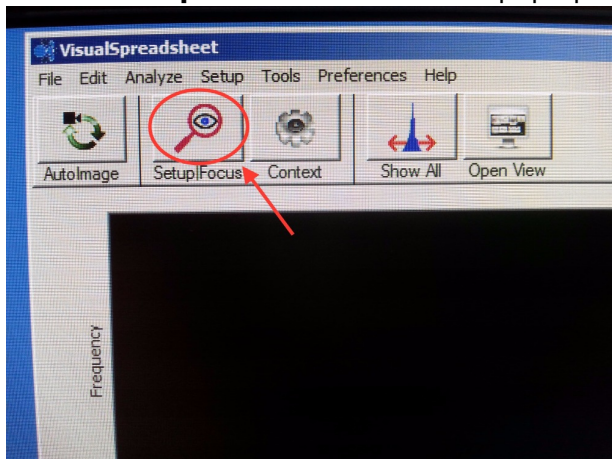
- **Fluidics tab:** Adjust the **sample volume** (usually 1 mL), relative to the **stop rule** (see below). Also, make sure the **efficiency** is near 25%.
- **Flow cell tab:** Adjust the tube length below the flow cell. This is set to **0 cm** by default. Our flow cells are cut to have **10 cm** tubing above and **20 cm** tubing below.
- **Stop tab:** Stop rule for terminating the run. Usually after **100 uL (0.1 mL)** of sample has been imaged. This works well with 1 mL of sample and a short priming step (see Setup and Focus below).
- **Reports tab:** Check the **export data** and **export summary data** checkboxes. This saves the measurements of all captured particles. The **list** file with collages of images are saved by default upon starting a run.

You can save these settings and load them for future runs as needed.

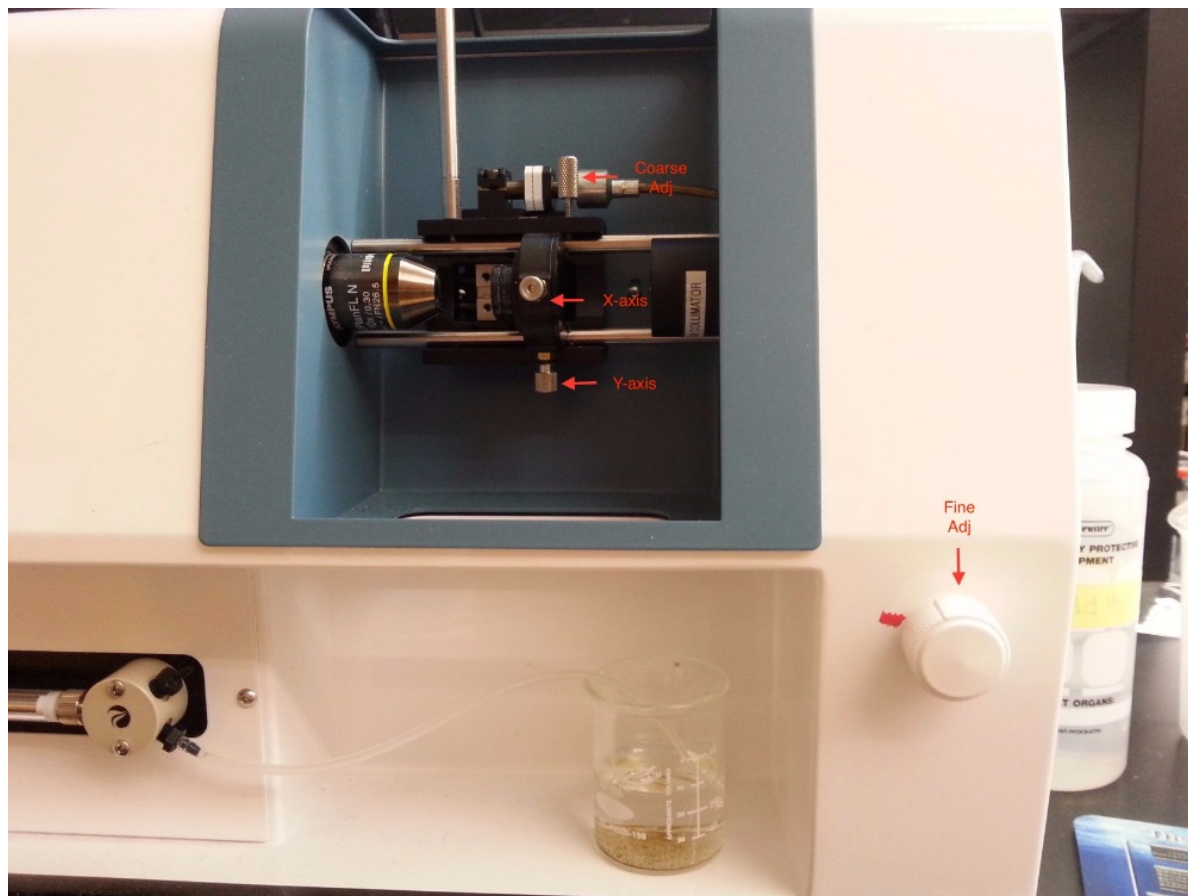
## Setup and focus

### Step 7.

1. Click **Setup/Focus**. A window will pop up with a live camera view of the flow cell.



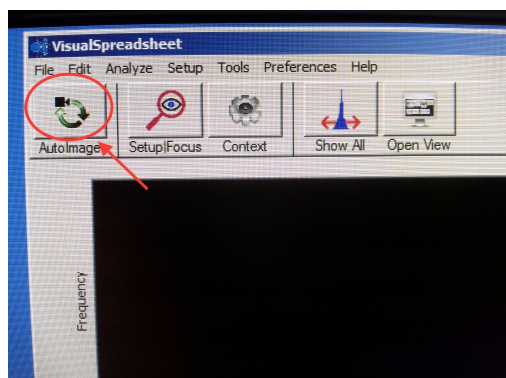
2. Use the X-axis and Y-axis adjustment knobs to center the flow cell.
3. Use the Coarse and Fine adjustment knobs to bring the sample into focus.



Run the sample

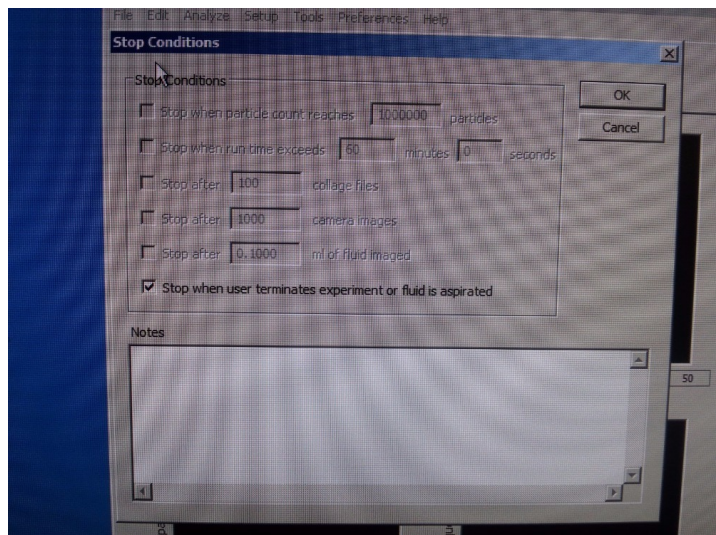
## Step 8.

1. Click **Autoimage**.



2. A pop-up with your **Notes** and **Stop rule** (Context) will show up. You can either enter a value to automatically stop the run (e.g., number of events, volume, or time) or leave the 'Stop when user terminates' button checked if you prefer to manually stop the run.





3. A window to create a folder for the run will pop up next. Make an informative folder name with sample info in the title (date, strain info, magnification, flow cell size, dilution, etc.) and click **Save**. Once folder has been named, the sample run will begin.
4. The sample will run until the criteria of the set **Stop Conditions** have been met or the user terminates the run by pressing the **Autoimage** button, which will display a red stop symbol.

#### ⊕ NOTES

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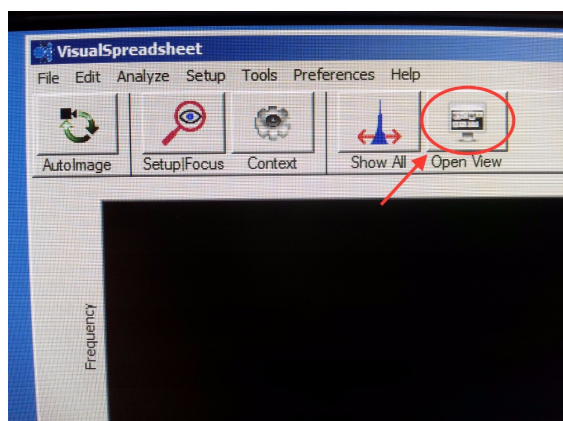
Note: If using manual termination allow the sample to run for at least 10,000 events.

Note: Ensure that the efficiency meets the criteria for the flow cell being used (for example, the target efficiency for the 100 um x 1 mm flow cell is between 20-25%).

### Analyzing the run data

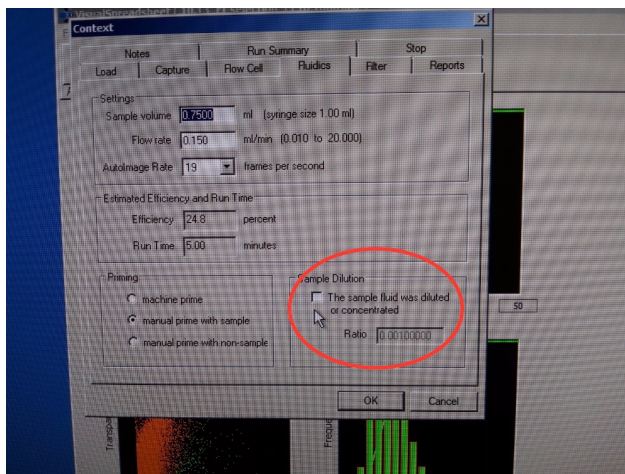
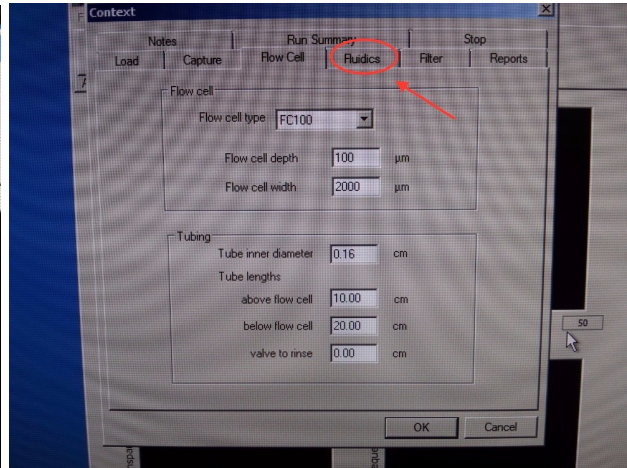
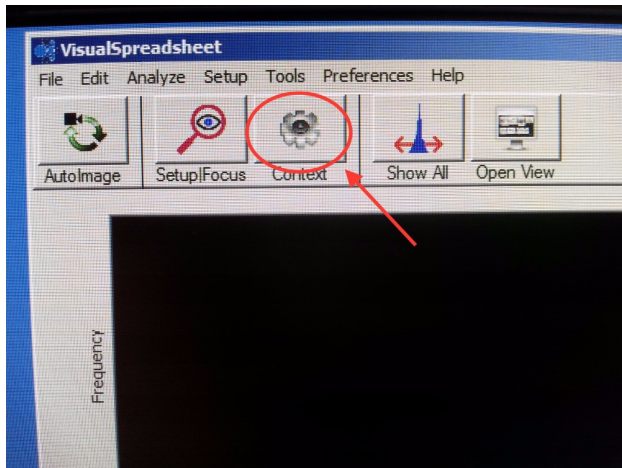
#### Step 9.

- Data will be displayed for each filter on the Visual Spreadsheet page.
- Select your data, either by clicking on the desired filter or highlighting portions of the graphs and selecting **Open View** to evaluate the images.

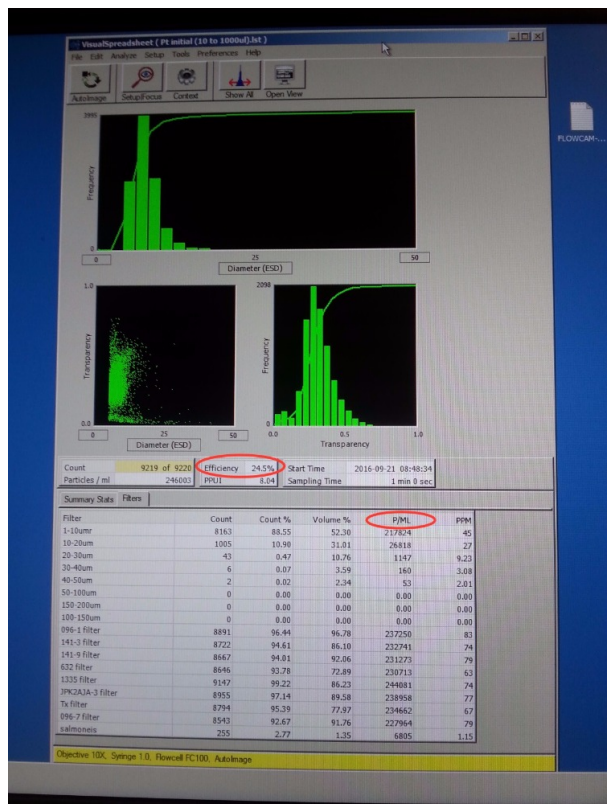




- Adjust for any dilution used in **Context > Fluidics** and checking the **Sample Dilution** box. Enter in the ratio of the dilution used (volume of sample / total volume)



- Particles / mL is the cell count
- Make sure the efficiency is within the target range for the flow cell that was used.



IMPORTANT: Be sure to flush the flow cell between samples.

## NOTES

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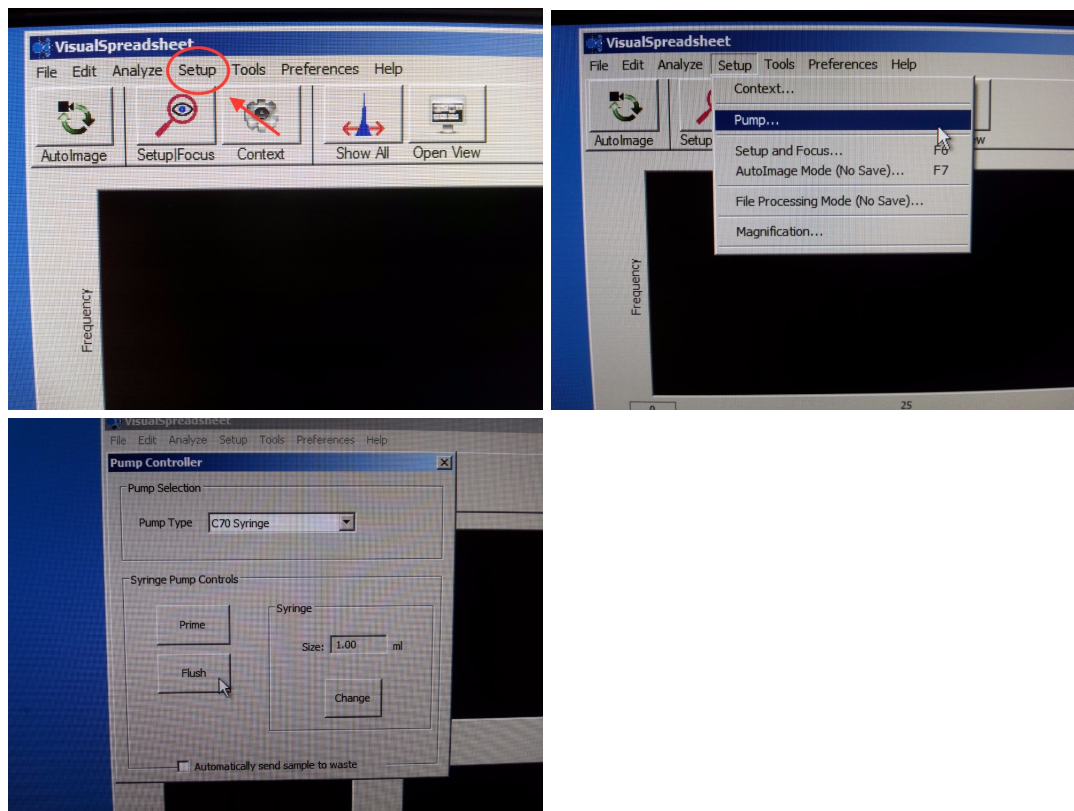
Note: The data you get out is only as good as the time you spend building a good filter.

## Flush the flow cell

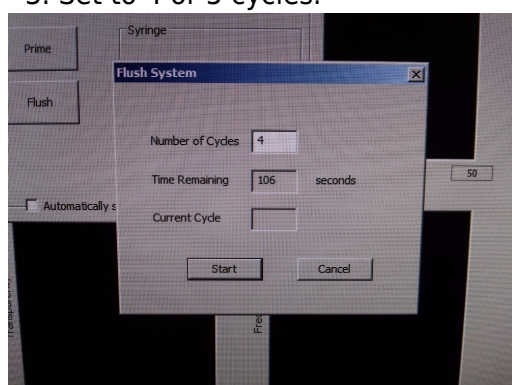
### Step 10.

Make sure to flush the flow cell between samples to prevent any previous cellular material or debris from showing up in your sample.

1. Load the funnel with ddH<sub>2</sub>O.
2. On the top menu bar, click **Setup > Pump > Flush**.



3. Set to 4 or 5 cycles.



4. Click **Start**

Continue running or shut down the instrument

### Step 11.

- To continue running samples repeat steps 5-10 (you can skip steps 6 & 7 since the settings are already saved).
- Continue to step 12 to shut down the instrument.

Disassemble and shut down

### Step 12.

1. Remove tubing from the syringe tip and sample funnel.
2. Loosen the flow cell holder screw and remove the flow cell holder and flow cell.
3. Remove the flow cell from the flow cell holder.

4. Put the flow cell back in its protective tube.
5. Close the program and shut down computer.

#### NOTES

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**Note:** Since there are multiple projects using the FlowCam be sure to label the flow cell being used for each specific project.