FlowCam Standard Operating Procedure Version 2

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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**
https://www.protocols.io/view/flowcam-standard-operating-procedure-f2tbqen
Published: 18 Oct 2016

Before start

Before you begin, make sure that

1. the appropiate flow cell and syringe are mounted
2. appropiate 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

Step 1.

Turn on FlowCam

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

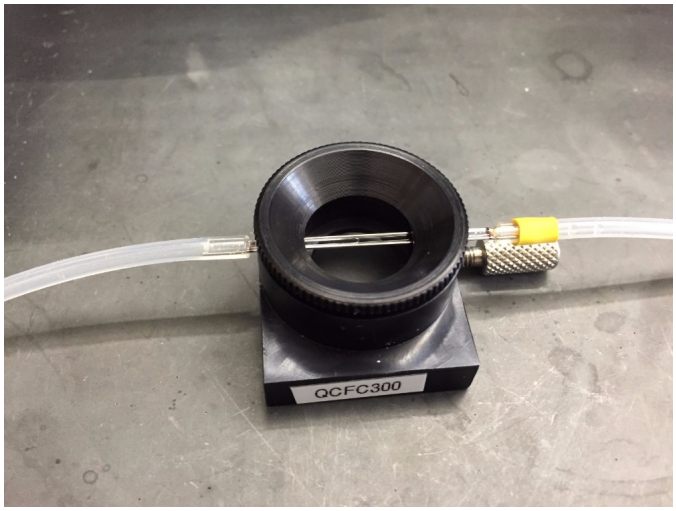
Install Flow cell

Step 2.

Instal flow cell into the flow cell holder

1. Select desired flow cell tubing based on application. (In general we use the 100 um x 1 mm flow cell).
2. Use a kimwipe or 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 at the risk of breaking the fragile flow cell.



Note: Since there are multiple projects using the FlowCam be sure to select the flow cell labeled for each specific project.

NOTES
Jeric Harper 06 Oct 2016

- If this is the first time the flow cell is being used cut the tubing above the flow cell to 10cm and the tubing below to 20cm.

- Measure from the tip of the flow cell that is inserted in the tubing.

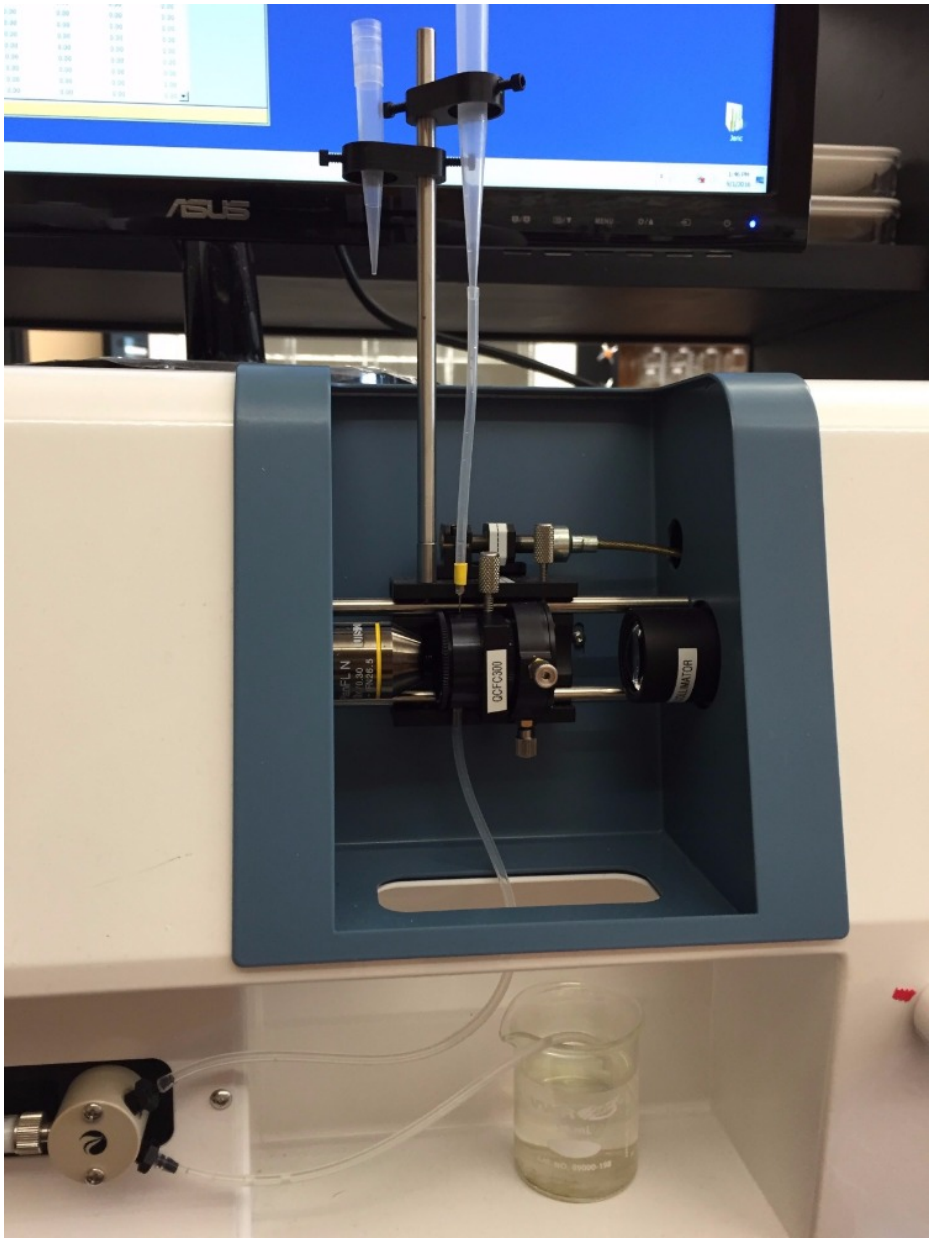
- Be sure to record this information into the **Flow Cell** tab in the **Context** setings.

Install Flow cell

Step 3.

Instal the flow cell holder into the FLowCam

1. Make sure that 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 kimwipe or lens paper to gently clean the objective lens and collimnator.
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 collecting waste.



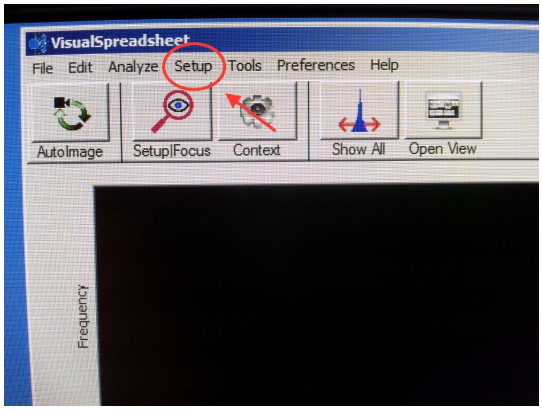
Pre-run Setup

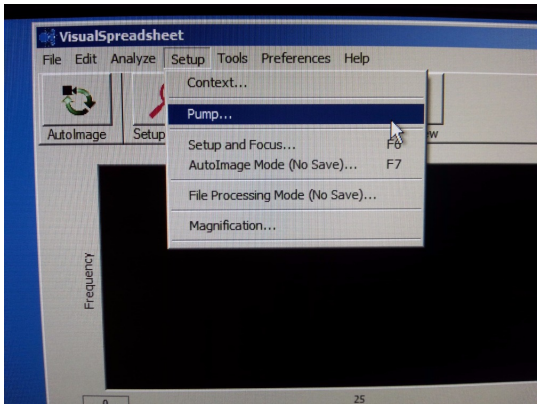
Step 4.

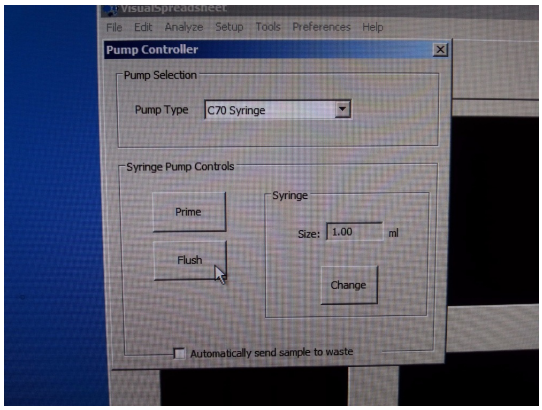
Flush Flow Cell

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

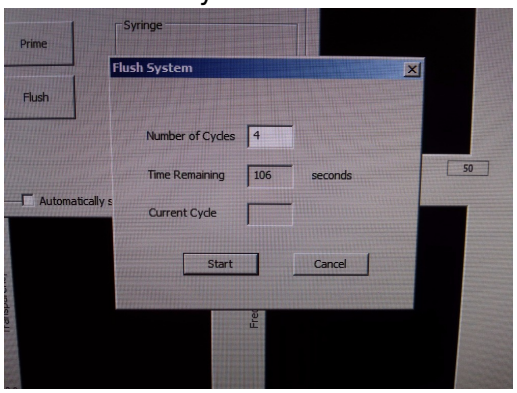
1. Load the funnel with ddH_2O.
2. On the top menu bar, click **Setup** -> **Pump** -> **Flush**







3. Set to 4-5 cycles.



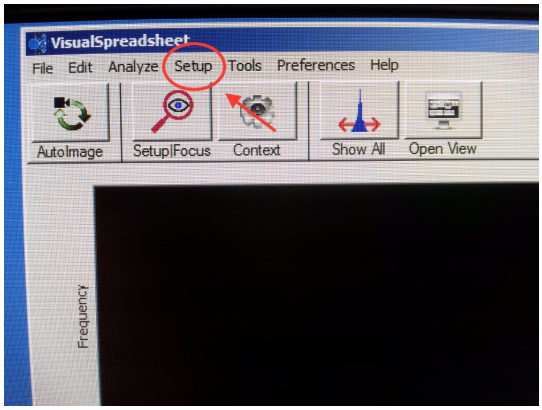
4. Click **Start**

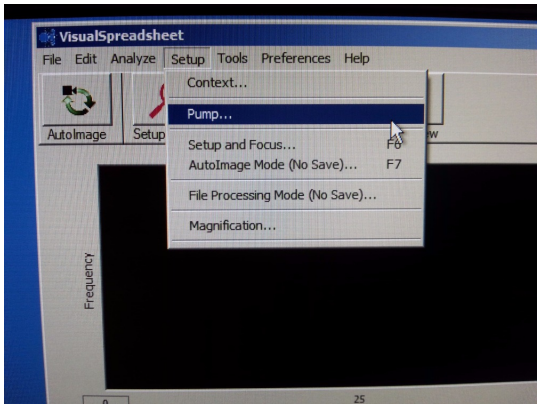
Pre-run Setup

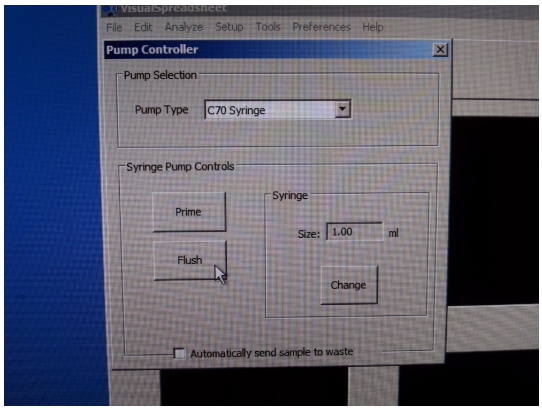
Step 5.

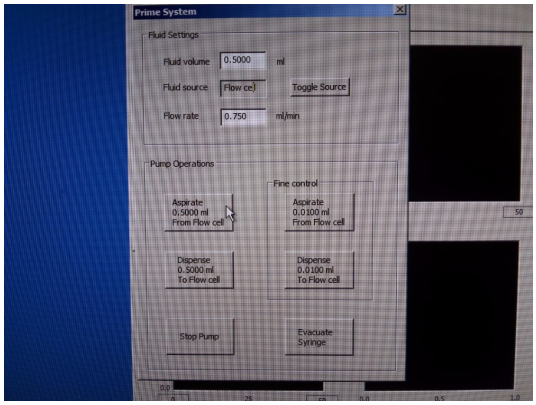
Load Sample

1. Use pipet to draw sample from your container. Usually about **0.2-0.5 mL** of homogenized sample is sufficient. For dense cultures, a dilution may be appropiate (Be sure to note this in your files). Sieving is great for raw samples that might have 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 0.500mL from Flow Cell**









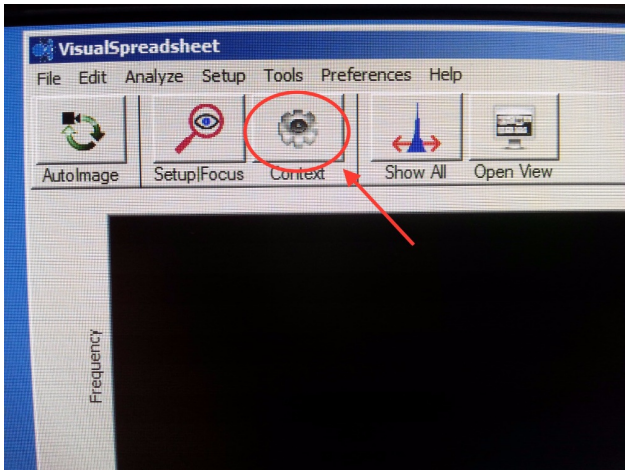
4. Click **Stop Pump** when the sample volume has reached the flow cell

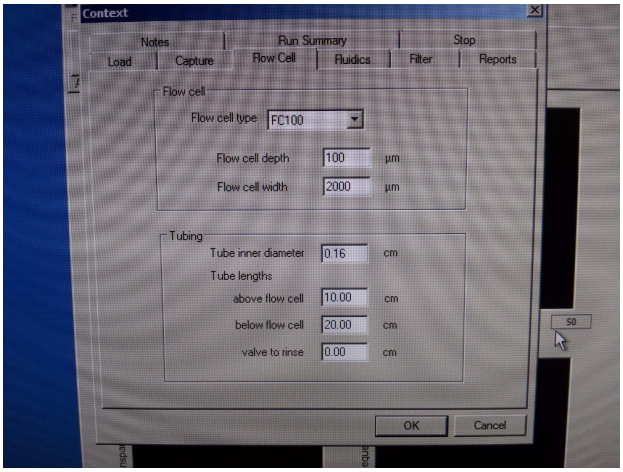
Pre-run Setup

Step 6.

Run Context

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





- **Notes Tab** - any relevant metadata. There is a file on the **Desktop** that can be pasted and modified as needed. This file includes lines for date, experiment, strain, species, magnification, flow cell, syringe, dilution, etc.
- **Fluidics tab** - Adjust here 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. By default it is at **0 cm**. 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.

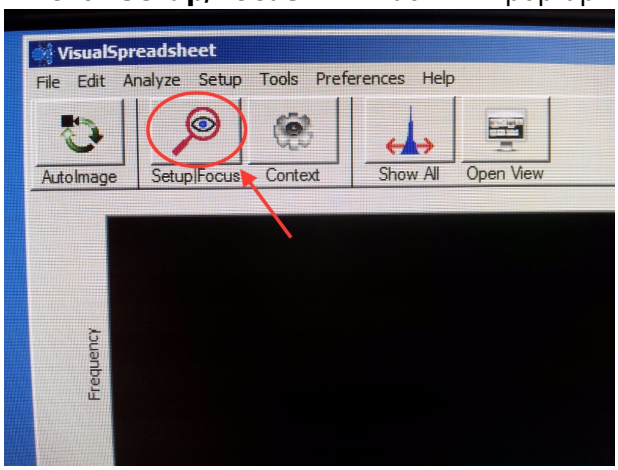
You can save these settings and load them as needed.

Pre-run Setup

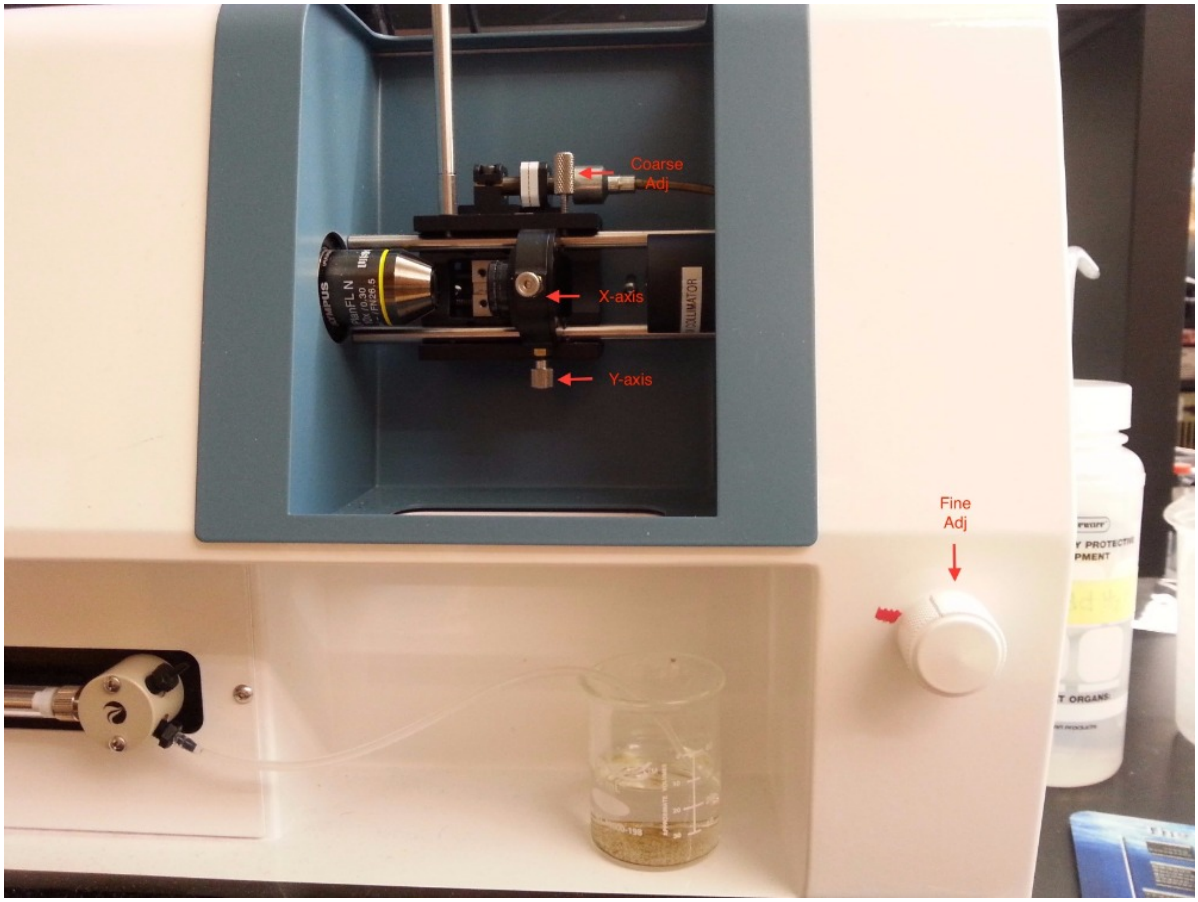
Step 7.

Setup and Focus

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.

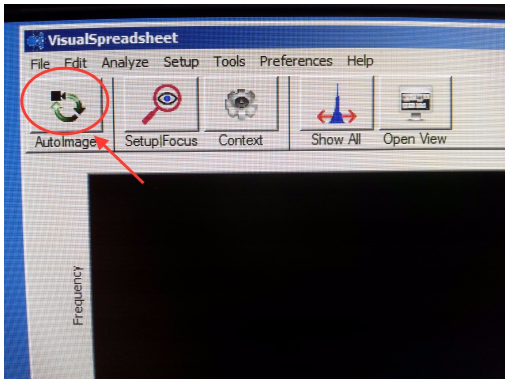


Running a Sample

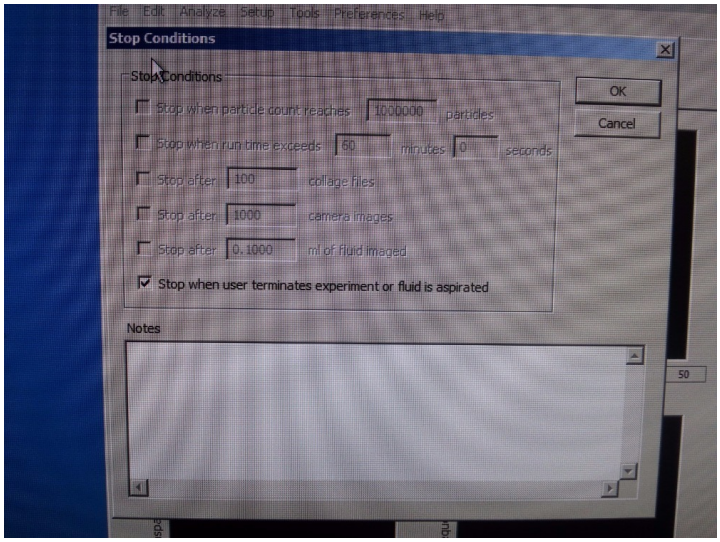
Step 8.

Run Sample

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. # of events, volume or time) or leave the 'Stop when user terminates...' button checked and manually stop.



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** are reached or the user terminates the run by pressing the **Autoimage** button which will now display a red stop symbol.

NOTES

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

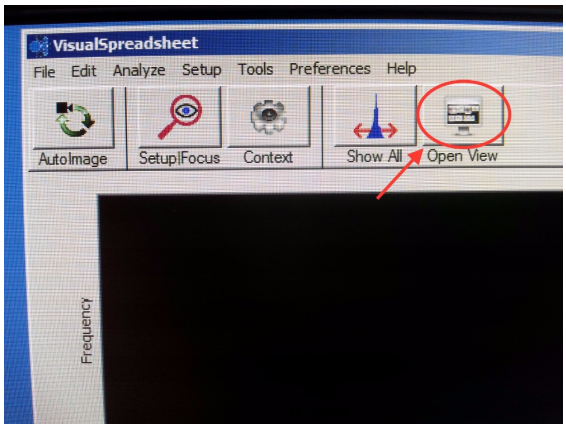
- Ensure that the efficiency meets the criteria for the flow cell being used (for example, the efficiency for the 100 um x 1 mm flow cell is between 20-25%).

Pre-run Setup

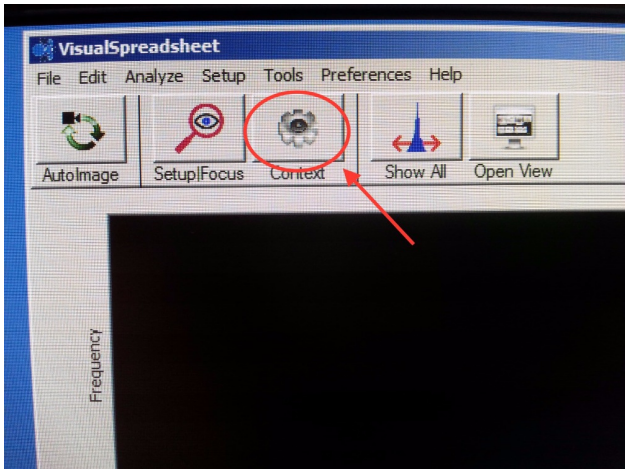
Step 9.

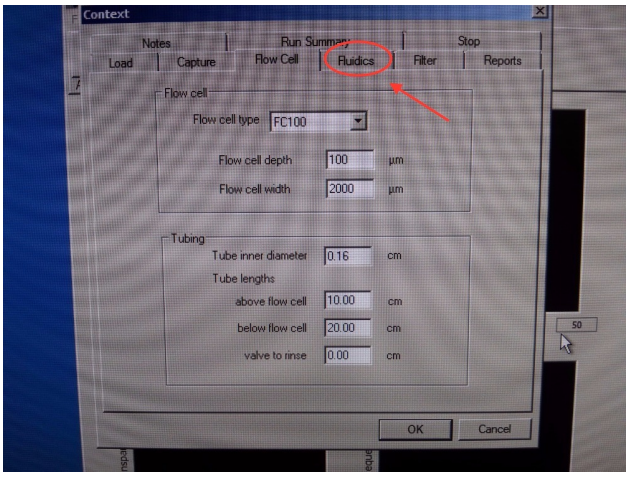
Data

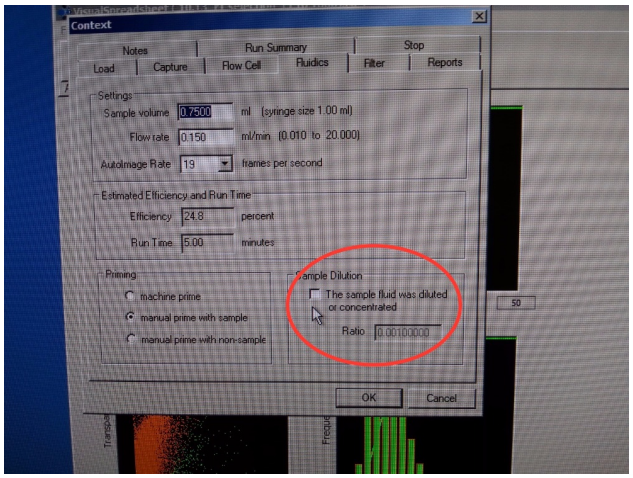
- The data will be displayed for each filter on the Visual Spreadsheet page.
- Select your data (either by clicking on the desired filter or highlighting portions of the graphs and selecting **Open View** to evaluate the images.



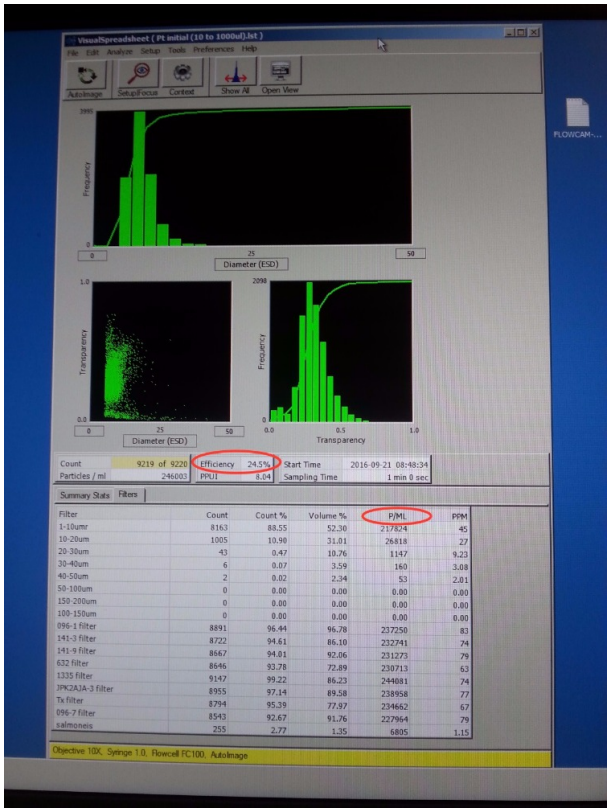
- Adjust for any dilution used in **Context -> Fluidics** and checking the **Sample Dilution** box. Enter in the ratio of the dilution used (volume of sample / total volume)







- Particles / mL is the cell count
- Make sure the Efficiency is within the range of the flow cell being used.



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

NOTES

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

- See Jeric if you need to build a filter, library or classification.

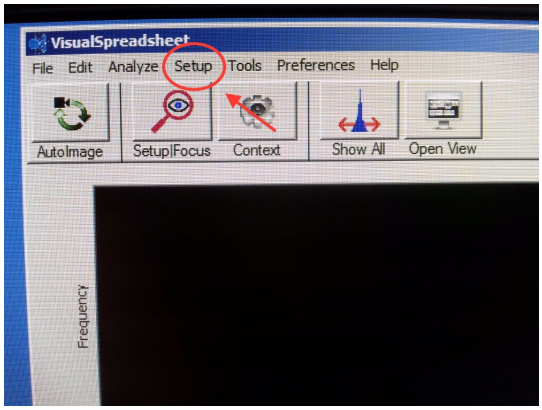
Running a Sample

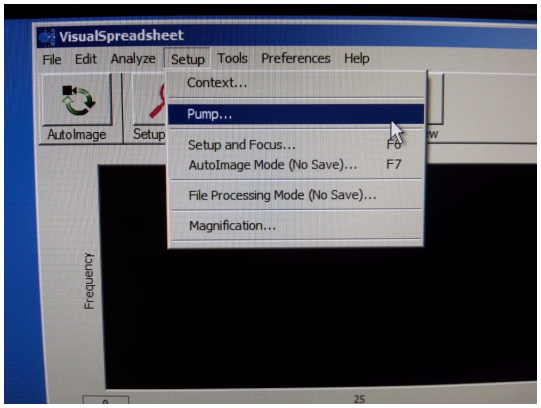
Step 10.

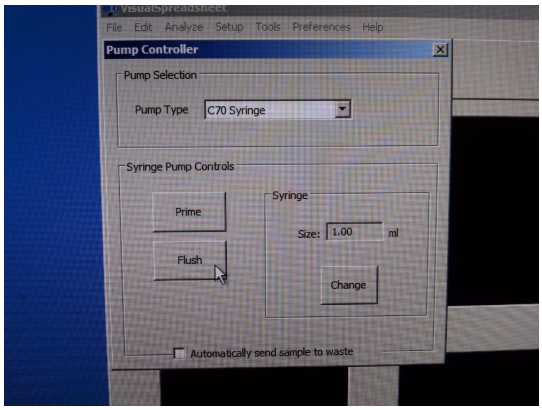
Flush Flow Cell

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

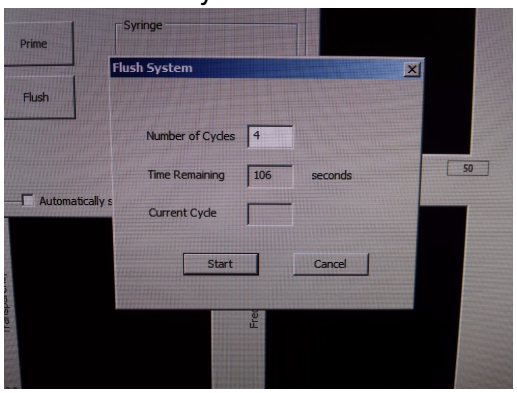
1. Load the funnel with ddH_2O.
2. On the top menu bar, click **Setup** -> **Pump** -> **Flush**







3. Set to 4-5 cycles.



4. Click **Start**

Step 11.

Continue running or Shut down

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

Shut down

Step 12.

Dissasembly

1. Remove the tubing from the syringe tip and the 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

Jeric Harper 11 Oct 2016

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