

# FlowCam Standard Operating Procedure

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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.fqbbmsn](https://dx.doi.org/10.17504/protocols.io.fqbbmsn)

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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

### Step 1.

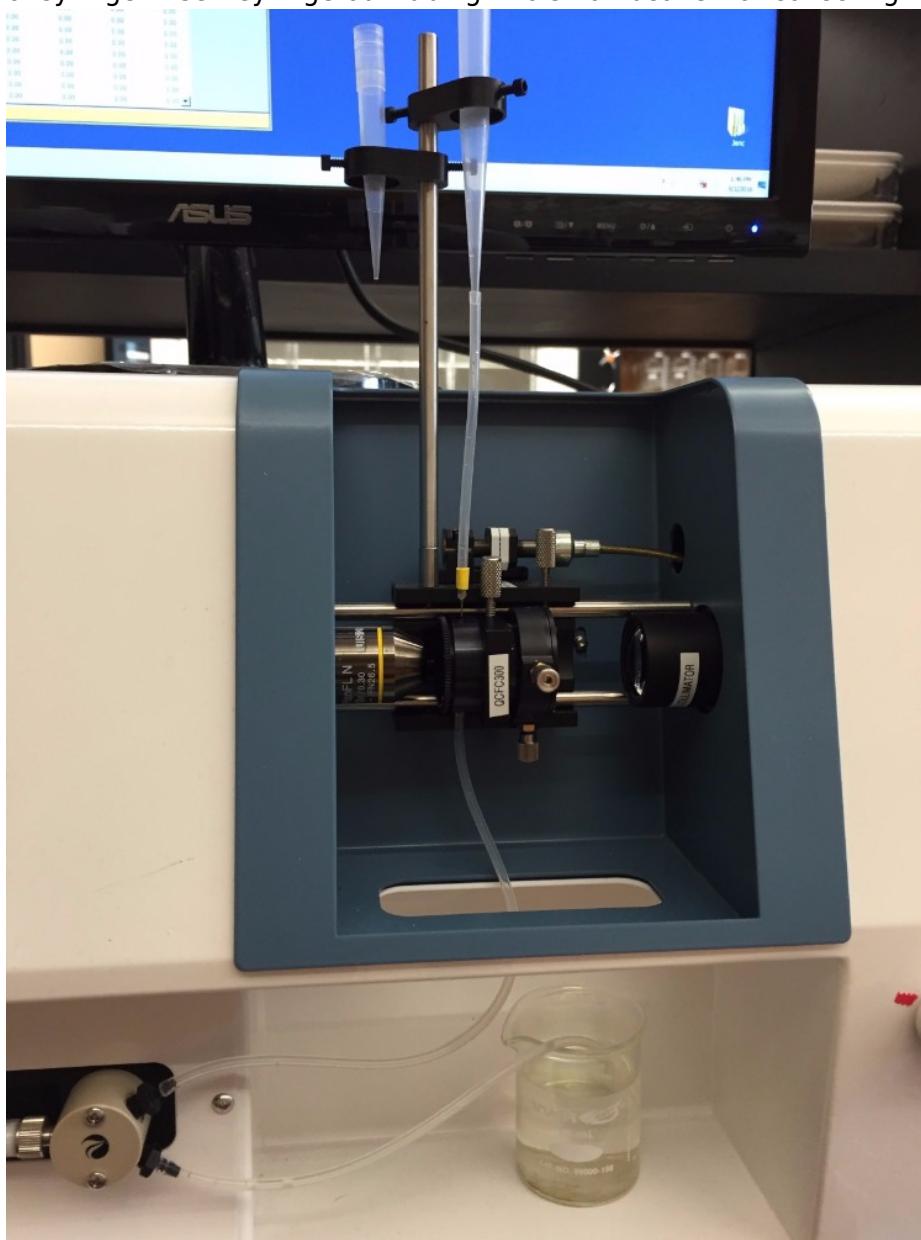
#### Start Up FlowCam Machine and Flow Cell Setup

1. Push silver button on the left of machine face to turn on computer and machine.
2. Select desired flow cell tubing based on flow cell size (for example, 100 um x 1 mm).
3. Insert glass flow cell into circular flow cell holder. Secure flow cell by gently screwing the holder until the flow cell is stationary. Be careful not to screw too tightly at the risk of breaking the

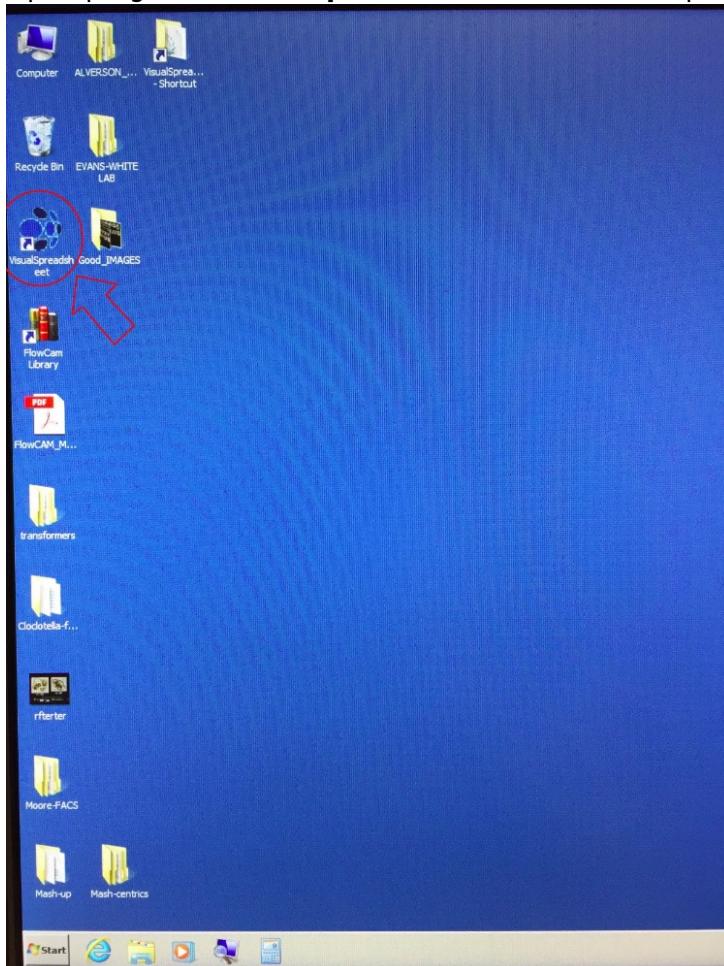


fragile flow cell.

4. 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.
5. 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.



6. 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.
7. Open program **Visual Spreadsheet** on the desktop.



8. Select the magnification based on which camera you are using, for example 10X if you are using the 10X camera lens.

## Step 2.

### 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<sub>2</sub>O. Depending on the culture, you may want to use media at a different salinity.
2. On the top menu bar, click **Setup > Pump > Flush**
3. Set to 4-5 cycles.
4. Click **Start**.

## Step 3.

### 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 appropriate (Be sure to note this in

- your files). Sieving is great for raw samples that might have detritus or zooplankton.
- 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.

## Step 4.

### Run Context

Click on **Setup > 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 **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.

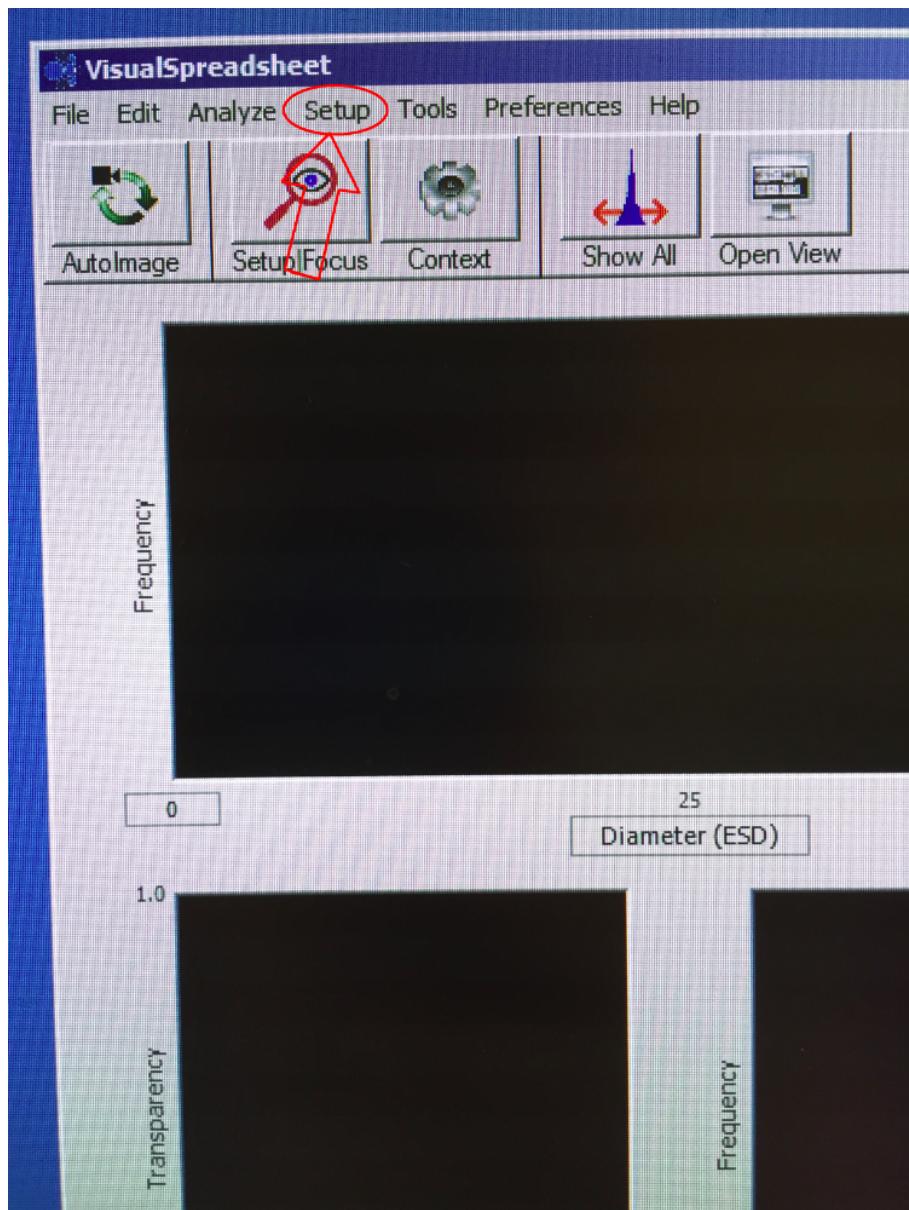
You can save these settings and load them as needed.

## Step 5.

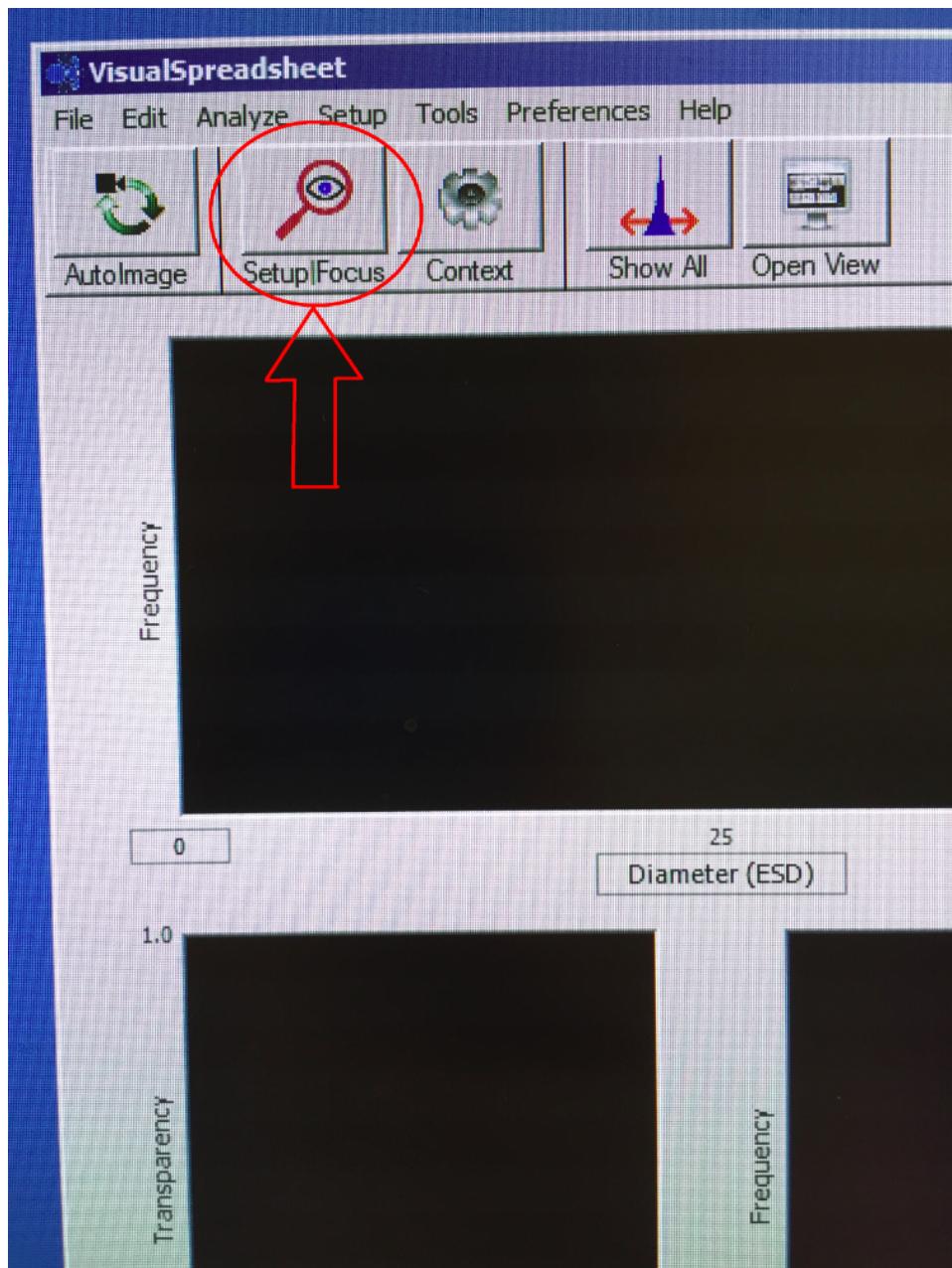
### Setup and Focus

This makes sure that the sample reaches the flow cell before a run begins. You can also adjust the focus of the camera.

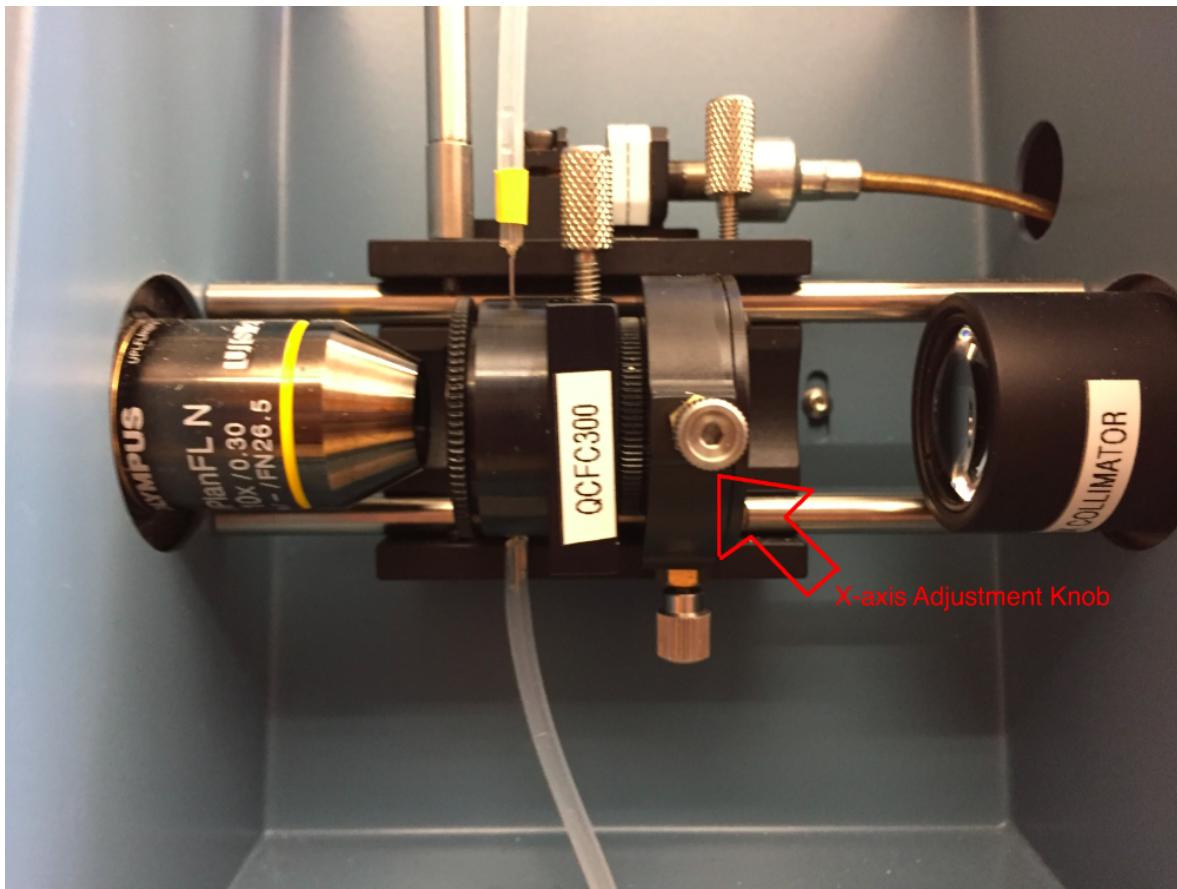
- Click **Menu>Setup>Pump>Prime**.



2. Click **Aspirate 0.500 mL from container** to start the pump. Wait until the sample reaches the flow cell. Click **Stop pump** once sample has just reached flow cell. Close pump control window.
3. Click **Setup/Focus**. A window will pop up with a live camera view of the flow cell.



4. Use the X-axis adjustment knob (small knob to the right of flow cell holder) to center the view of flow cell if necessary.

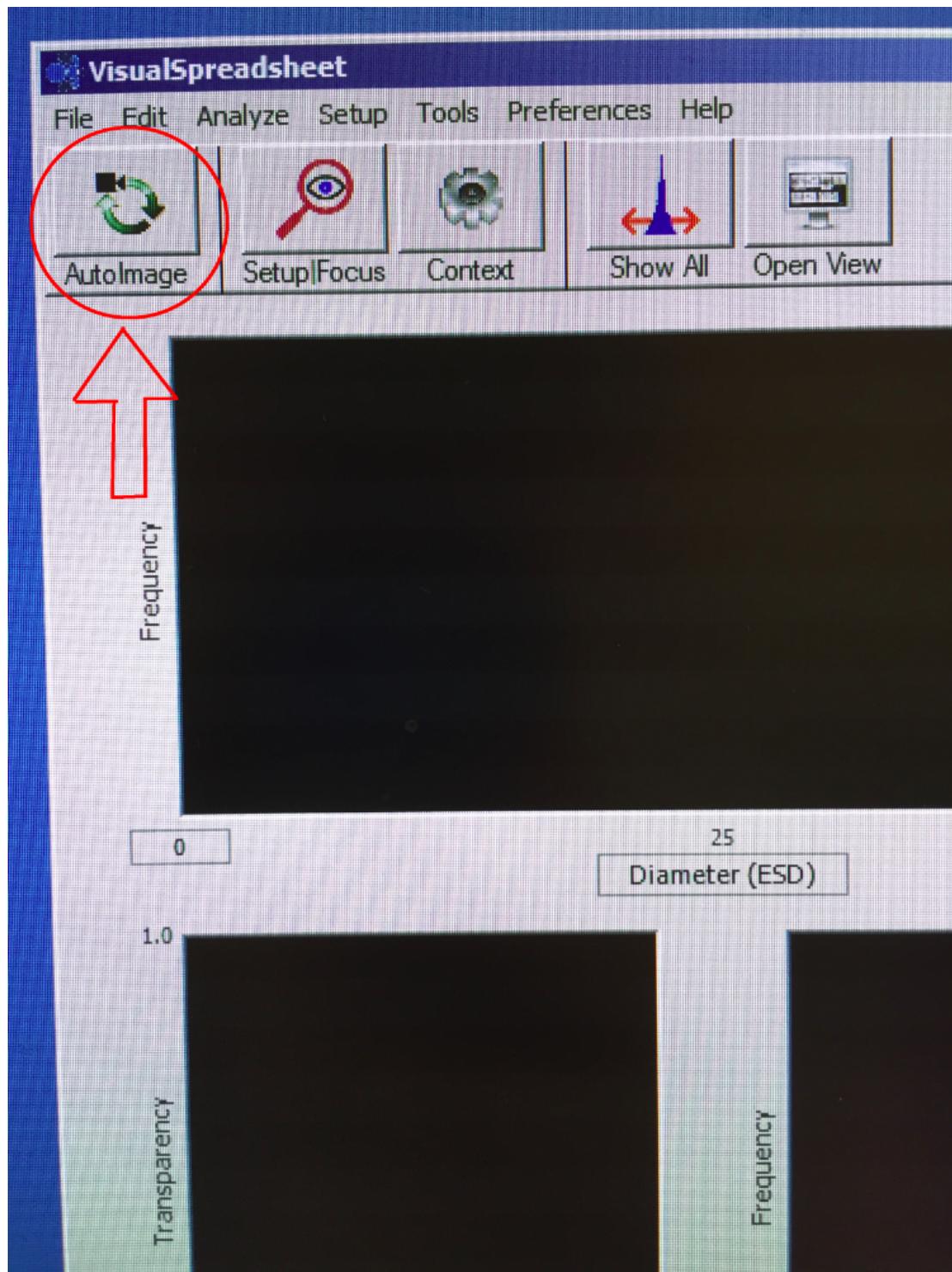


5. Use white knob on the right side of machine face to focus image of samples on screen.
6. When images are centered and focused, close live camera window.

## Step 6.

### Run Sample

1. Click **Autoimage**.



2. A pop-up with your **Notes** and **Stop rule** (Context) will show up. Click through.
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 (strain info, magnification, flow cell size, dilution, etc.) and click through.
4. Once folder has been named, the sample run will begin.
5. Sample will run until the protocols of the set Stop rule, usually either when sample has been run through or when user hits stop button.
6. Allow sample to run for at least 1 minute. Check that efficiency is between 20-25%.
7. When finished with data collection, click on **Autoimage** button which will display a red stop symbol.

Images are now saved in set folder as a collage that can be sorted through and sorted based on

numerous characteristics.

## Step 7.

### Finishing Up

When finished running samples, make sure to properly clean up and shut down FlowCam.

1. Ensure that all files are safely saved in desired folders.
2. Flush flow cell using the same procedure as noted in **Step 2**.
3. Remove tips of tubing from the sample funnel and bottom syringe. Unscrew flow cell holder screw to unmount flow cell.
4. Gently unscrew flow cell holder to release flow cell. Store flow cell away in plastic tube to protect the glass cell from scratches.
5. Close VisualSpreadsheet program. Turn off computer by pushing silver button on the left side of machine face.
6. Place plastic bag on top of sample funnel to protect machine apparatus from dust.