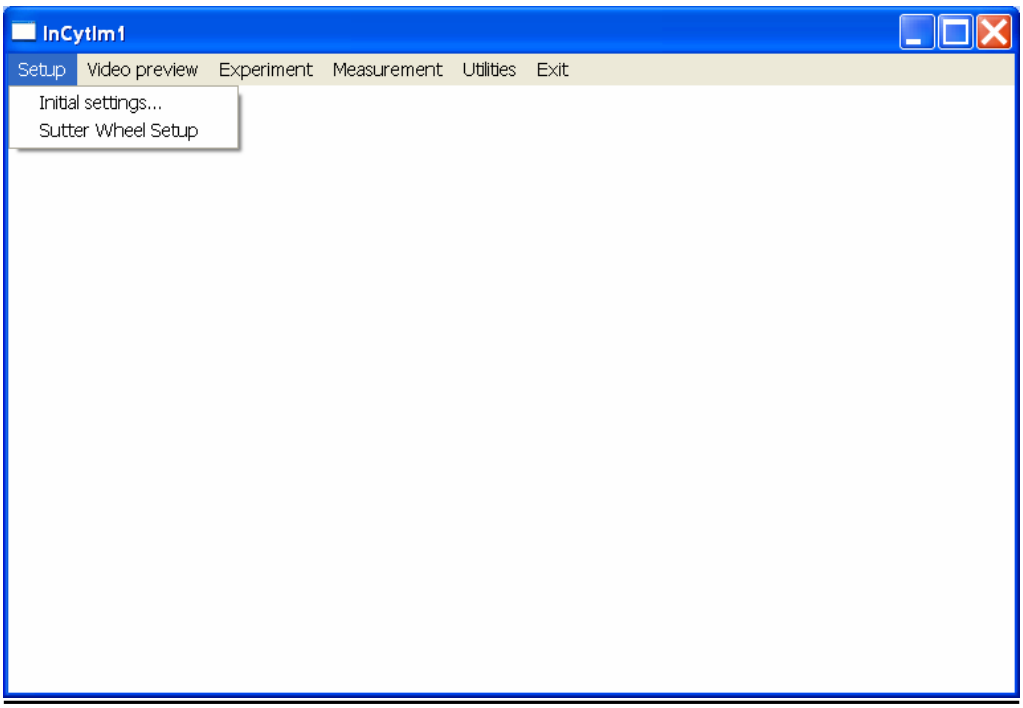
# InCyt Im1™ -- A Quick Overview of the Program

## Standard System with Sutter Wheel – Windows 10

The InCyt Im1™ program was designed as an easy-to-use tool for the research investigator to quickly analyze fluorescence changes in single-excitation probes & dyes during the course of an experiment. The program gives the user flexibility in determining how often measurements are taken and from where specifically in the sample measurements should be taken.

InCyt Im1™ is very similar to the InCyt Im2™ dual-wavelength (ratio) program, but even more simple to use. Obviously, there is only one wavelength to deal with, so there are no calibration, ratioing, or threshold settings. Below are screen shots from the Setup, Video Preview, Define Objects and Set Limits, Graph, and Animate sections of the program to give you a feel for the differences. Only options and selections that are different in corresponding InCyt Im2™ screen shots or dialog boxes have been covered. Program areas not covered here (Pseudocolor and Montage) are very similar to the corresponding areas in the InCyt Im2™ dual-wavelength program. Please note that there are no cell images presented in these screen shots. When the program is run the menu looks the same as InCyt Im2™:

**Setup**



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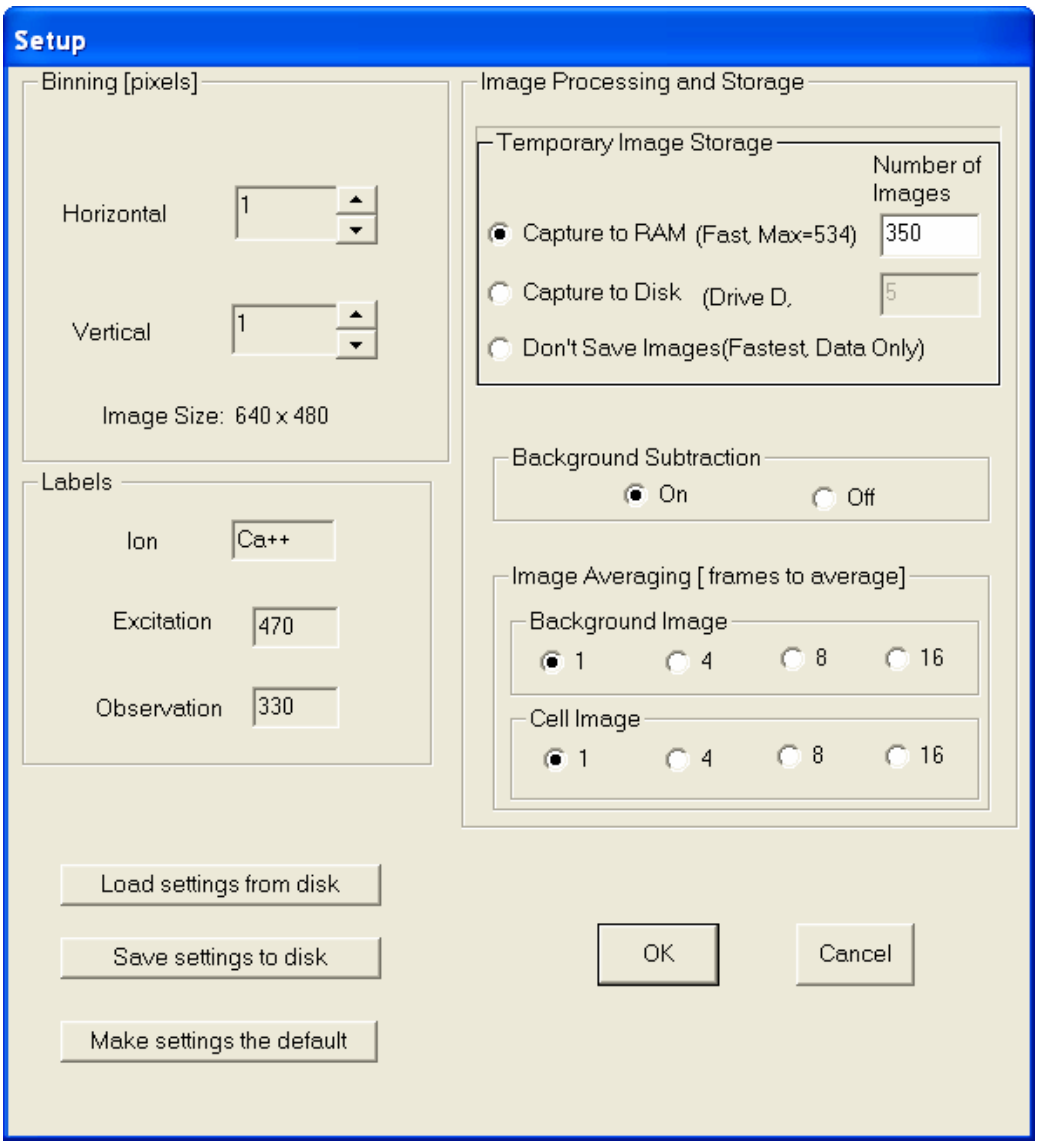
## Initial Settings

You start the experiment with this screen. Here you set image acquisition parameters (the size of the image, whether or not to subtract background light levels, and image averaging to reduce noise in the image), where you want to store images during the experiment (RAM or disk), and which filter you will use for excitation. Ion, Excitation (referred also as Measurement) and Observation (also an excitation) wavelengths are defined in the Sutter Wheel Setup. Emission wavelength is not mentioned in the program since it is installed in the microscope cube.



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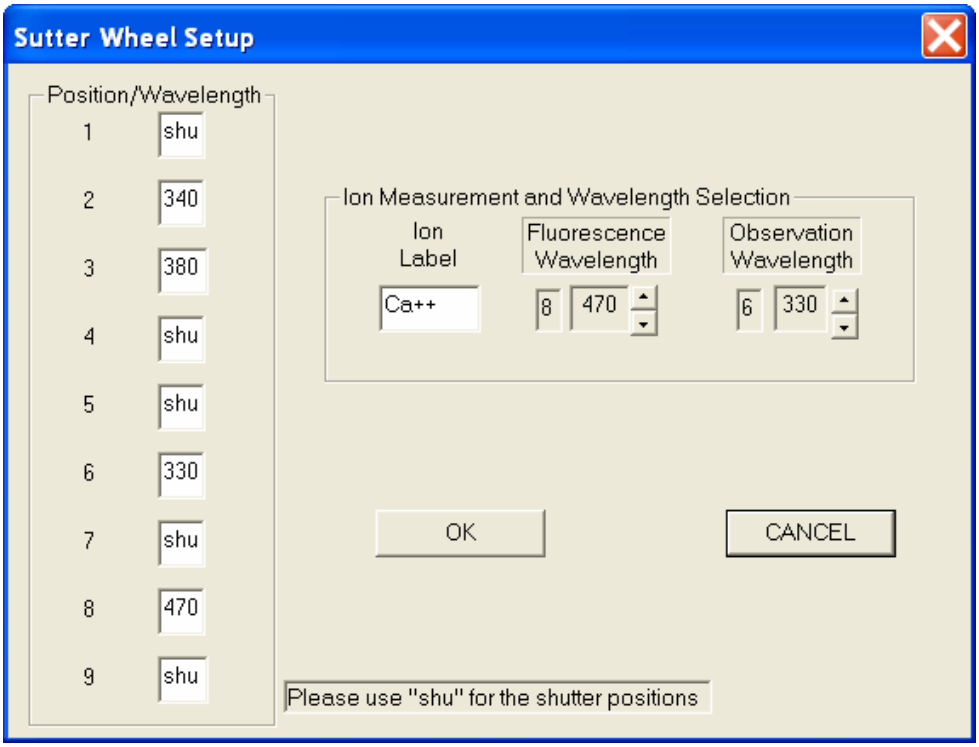
## Sutter Wheel Setup

The Sutter Wheel Setup dialog box allows you to register the new filters that are installed in the filter wheel. Please read the Sutter Instruments Lamda 10-B, 10-C or 10-2 manual for instructions on how to install new filters.

1. Round aluminum blocking plates are installed in filter positions that should be blocked and are labeled “shu” that means shuttered position.
2. Wavelengths corresponding to filters that are installed are defined by the 3-digit wavelength number (e.g. 340, 380, 470, etc.).
3. Key in the appropriate wavelength or “shu” corresponding to the filter positions that need to be changed.
4. You may edit the Ion Label to a name that corresponds to the fluorophore used.
5. Select the “Fluorescence Wavelength” (called Excitation in Initial Settings and Measurement in Video Preview and experiment) and the “Observation Wavelength” by scrolling the up and down arrows beside the filter position and the wavelength number.
6. Clicking OK accepts and stores the new position assignments. Cancel retains old settings.



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**Video Preview**

Here you set the exposure time and lamp intensity for the experiment to achieve the desire image brightness. There are three main considerations in setting exposure time and lamp level:

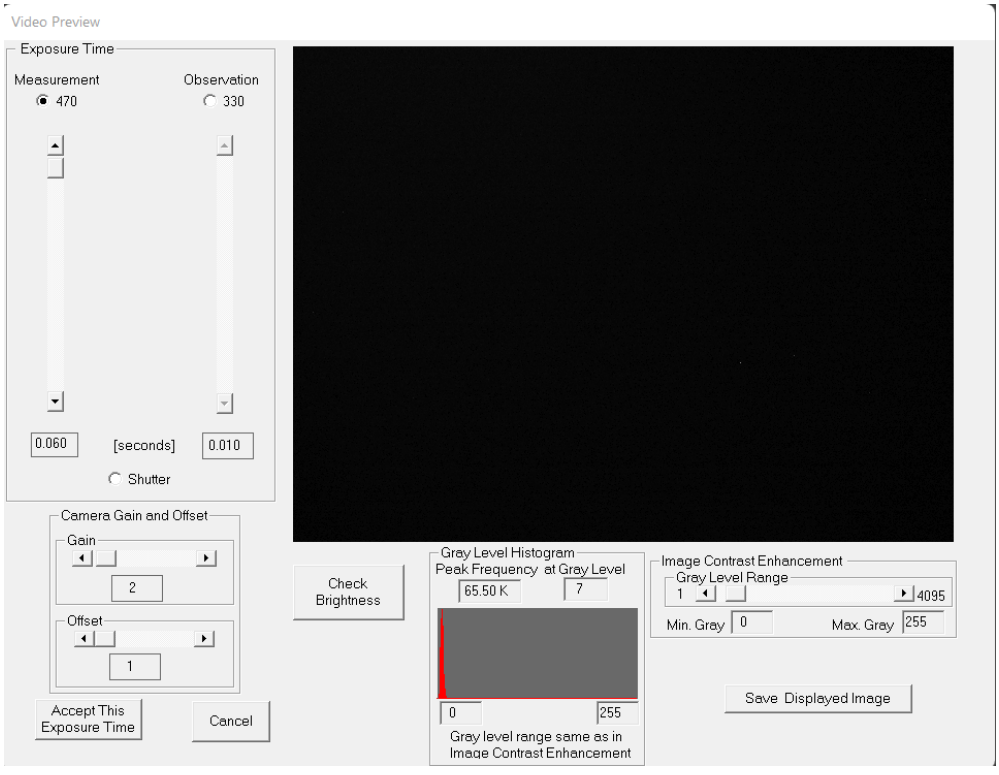
1. You want to set the exposure time at a level where you can easily see and focus the cells initially,

2. You need to set the exposure time so that you can capture data quickly enough to measure the anticipated response, and

3. You need to set the lamp intensity low enough so you do not photobleach the dye nor photodamage your cells.

Increasing the Gain on the camera will increase the sensitivity of the camera. This may allow you to decrease the exposure time or lower the lamp intensity to get the same image brightness. However, you the image may be noisier/grainier with the higher Gain.

Reducing the Grey Level Range will increase the brightness of the image without changing the dynamic range of the camera. However, if you have to reduce the Gray Level Range too much to observe your sample, small, inconsequential changes in fluorescence may cause a lot of noise in the measurements.



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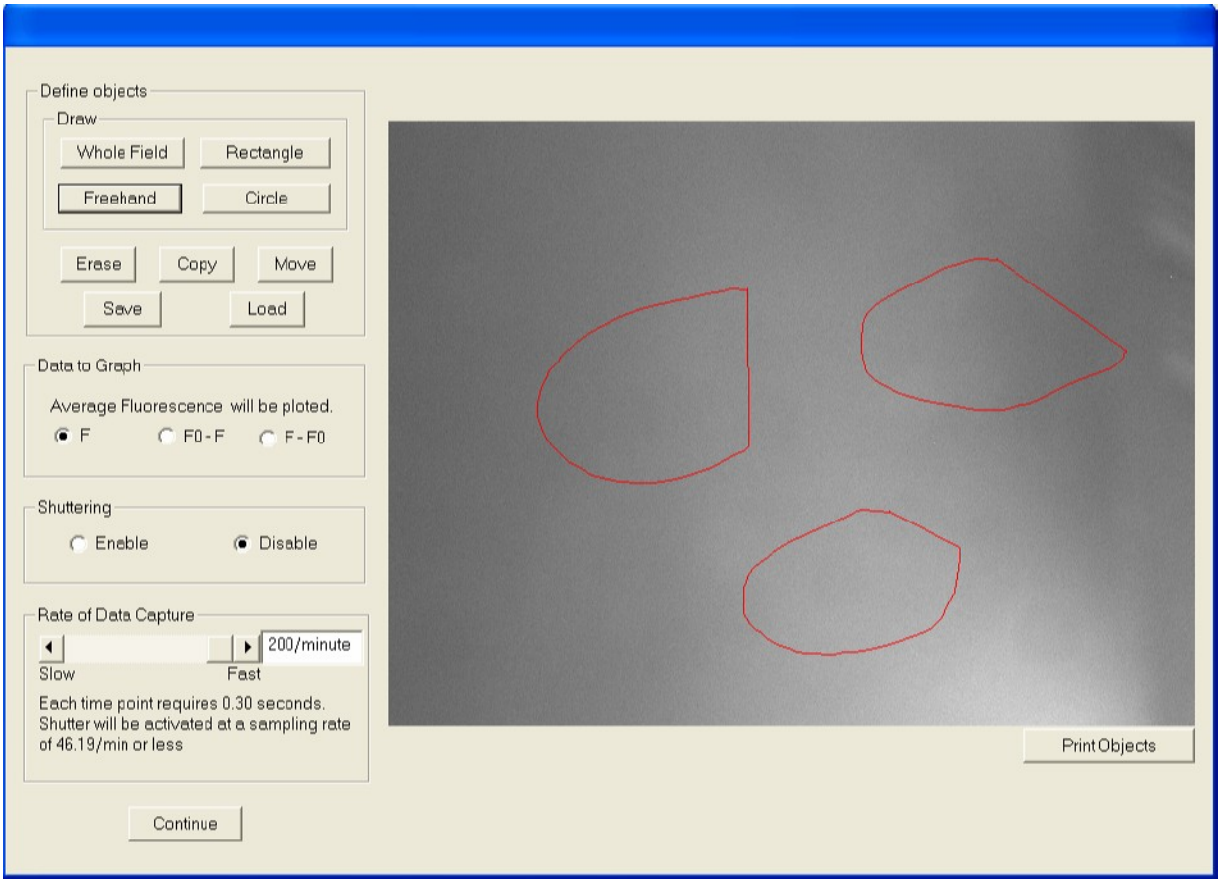
**New Experiment/Define Objects**

In the Define Objects dialog box in the InCyt Im1™ program, we have the selection of what parameter to graph. F is the average fluorescence at any time point, F0 is the baseline or the average fluorescence at time zero, and F-F0 and F0-F are fluorescence differences relative to F0.

When the Shutter Enable radio button is selected the program switches to shutter after every time point. When it is in Disable, the program will not go to shutter unless the rate of data capture is reduced (using the Rate of Capture slider) so that the time between data points is greater than or equal to 1 second. Commonly this program has been used with the setting kept at Disable and shuttering is left to depend on the rate of data capture.



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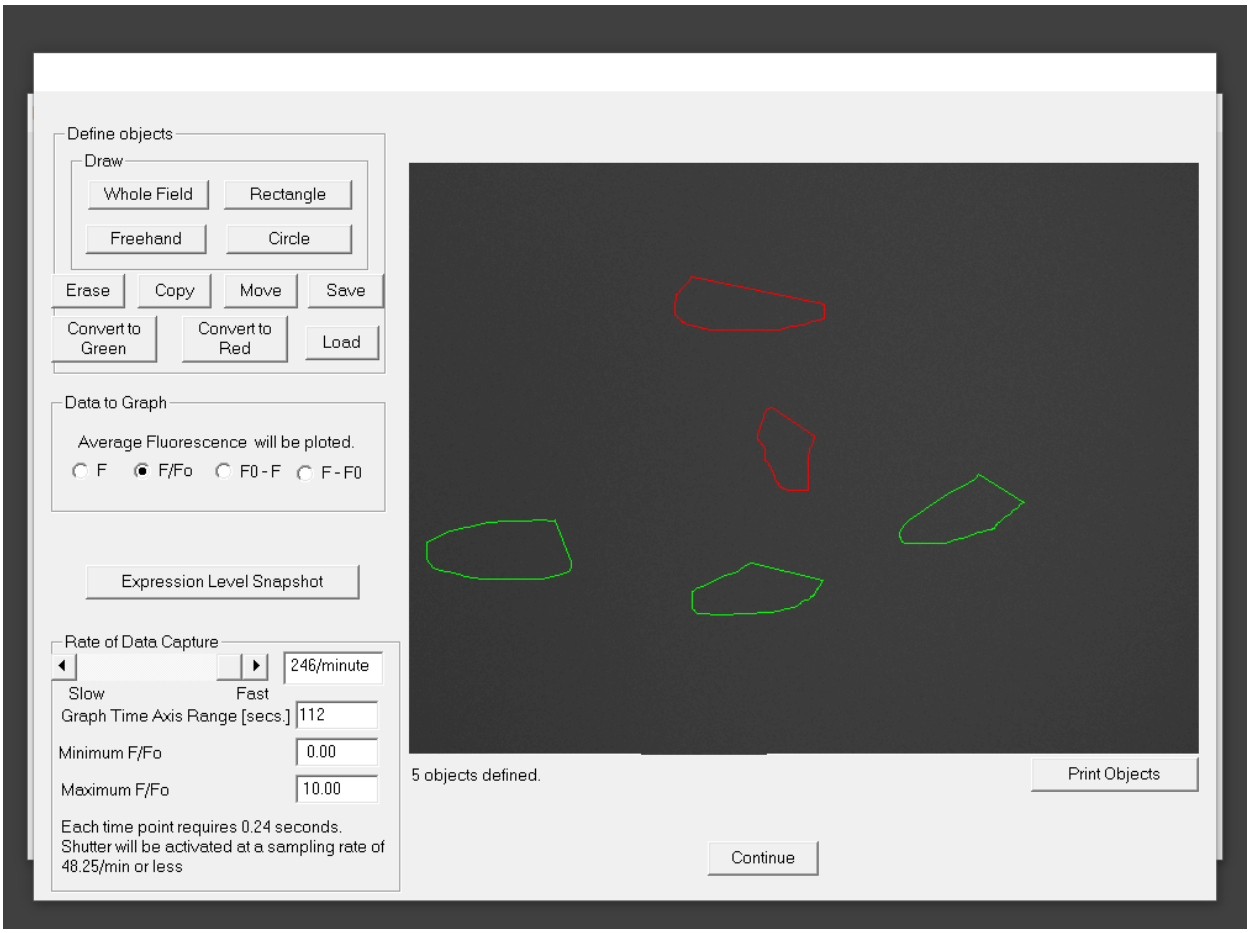
## New Experiment/Define Objects

In some versions of the program, you have two additional capabilities:

1. The measurement F/F0 is also available. This puts all objects on the same baseline, no matter how bright they are at the beginning. For the graph to be meaningful during the experiment, be sure to set your Maximum F/F0 to a level that is likely to reveal the changes you anticipate during the experiment.

2. As in the similar version of InCyt Im2™, you have the ability to distinguish different groups of objects by mask color. When you use the Observation Wavelength to draw object masks, the default color for the mask will be green. This is often used to identify cells transfected with a fluorophore that is observable with the Observation Wavelength. The default mask color when using the Measurement Wavelength is red. One can switch the color of individual masks if a mistake was made.




When drawing objects using the Observation Wavelength, one can take an <Expression Level Snapshot>, which records in a text file (extension .ESS) the fluorescence of red and green object under the excitation of the Observation Wavelength (see example file next page).

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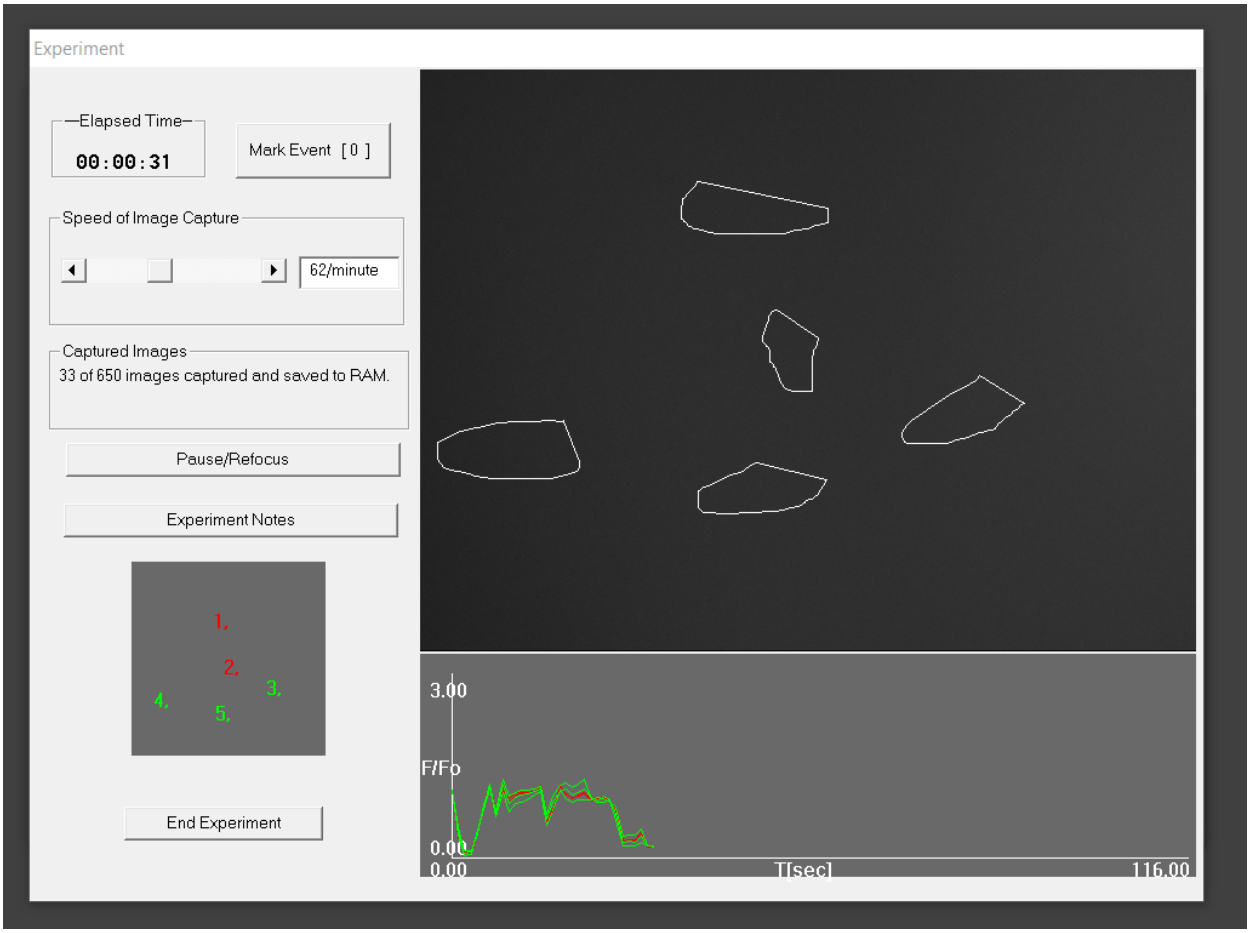
incytim1 Demo ESS.ess

```
D:\InCytIm1\incytim1 Demo ESS.ess
Red_Objects=     2       Green_Objects=   3
Ion:                              Ca++
Measurement_Wavelength:                   340
Observation_Wavelength:                   485
Measurement_Exposure_Time:                50
Observation_Exposure_Time:                10
DATA_AFTER_THIS_LINE
Object Number     Area     Total Fluorescence        Average Fluorescence
Red Objects
    1            2521          907560                        360
    2            3246         1032228                        318
Green Objects
    3            2584          767448                        297
    4            5552         1893232                        341
    5            2734          822934                        301
```

During the experiment, the red and green objects will be graphed in different colors.



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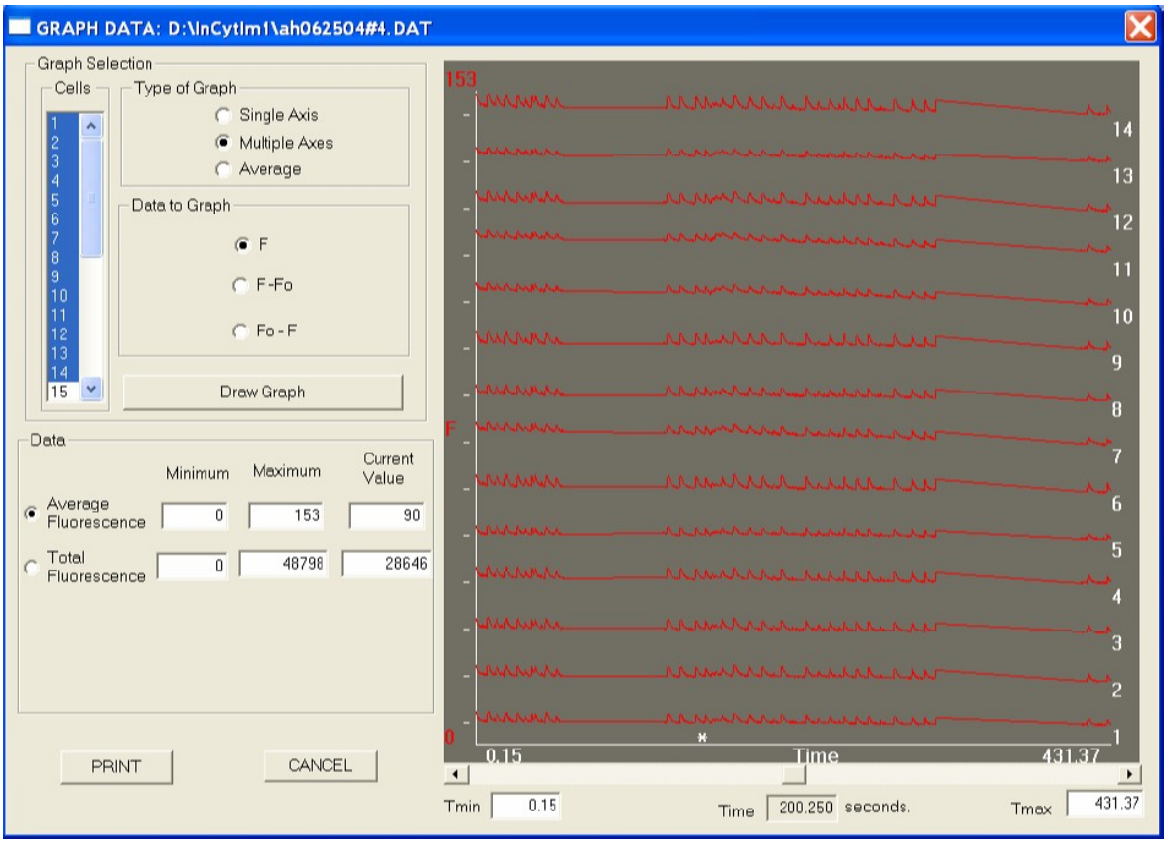
## Graph Data

The user can graph fluorescence data either as fluorescence/area or total fluorescence. The "fluorescence/area" is the average fluorescence level per pixel in the object of interest, expressed in terms of gray value (0-1023 for an 10-bit camera; 0-4095 for a 12-bit camera). Total fluorescence is the total of all the gray values in the object.

The user has the choice of graphing fluorescence in three ways: straight fluorescence (F), subtracting the base fluorescence level from subsequent images ($F-F_0$), or subtracting the fluorescence level from the base image ($F_0-F$).



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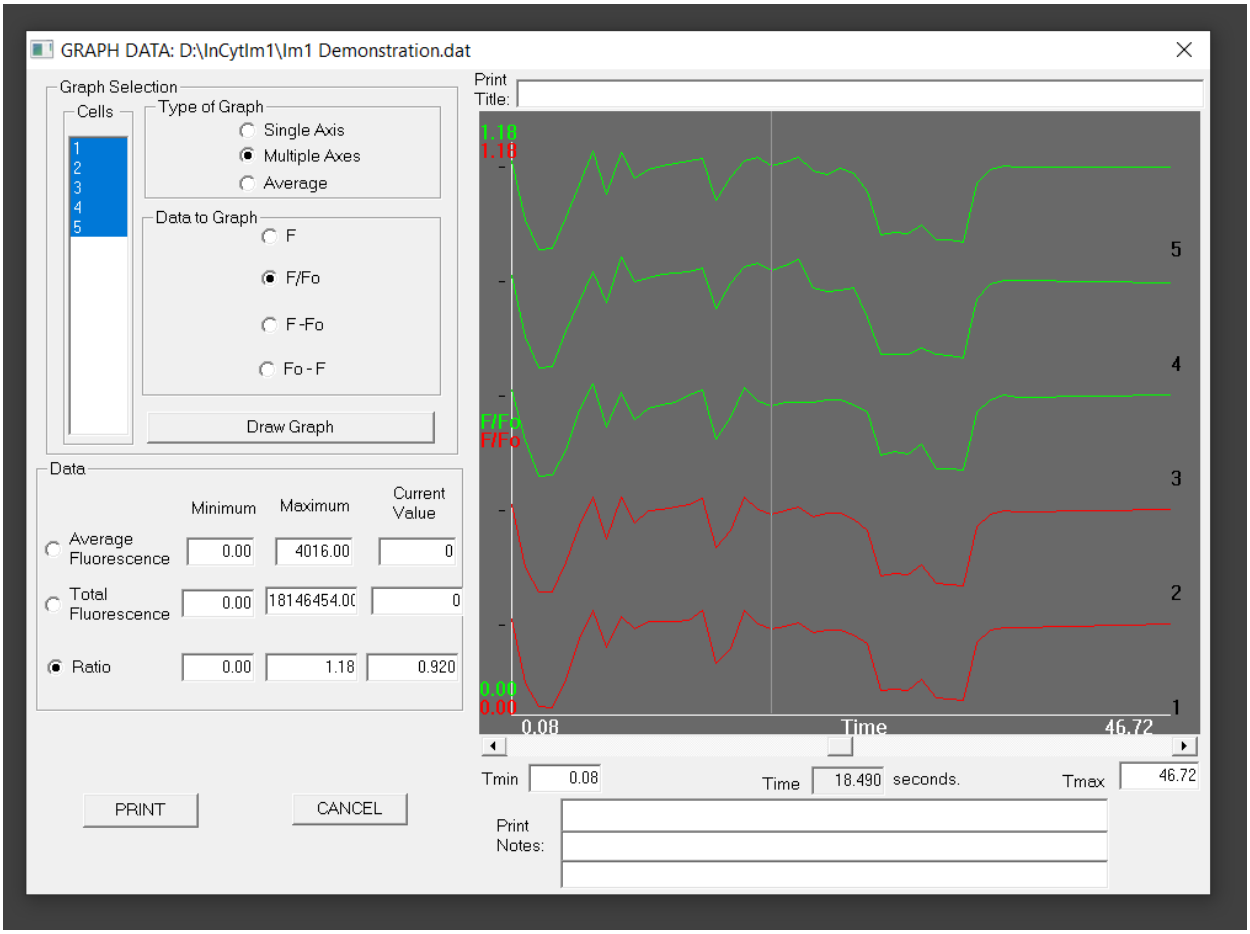
## Graph Data

In the programs versions where red and green object mapping is allowed, they will be graphed separately in the <Graph Data> screen, as shown below. In this version of the program, graphing $F/F_0$ is also available.



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**Animate**

In this section, you can play back the time-series of fluorescence images collected during the experiment. As with graphing, you have three options in displaying the images (F, F-$F_0$, $F_0$-F). All images are monochrome in their original form, but you can apply pseudocolor to these images (assigning different colors to different gray values), and display them here.

You can save and print individual images from this screen. You can also save the entire sequence that can be played back as a movie.

F/F0 measurement is not available in the <Animate>, <Pseudocolor> or <Montage> functions of the program.