iCyte/iCys Revision 3.0 User Tutorial
Exercise 1

Exercise 1: One Primary Contour Scans

The purpose of this document is to provide the experienced iCyte user with an overview of the basic operation within iCyte software version 3.0

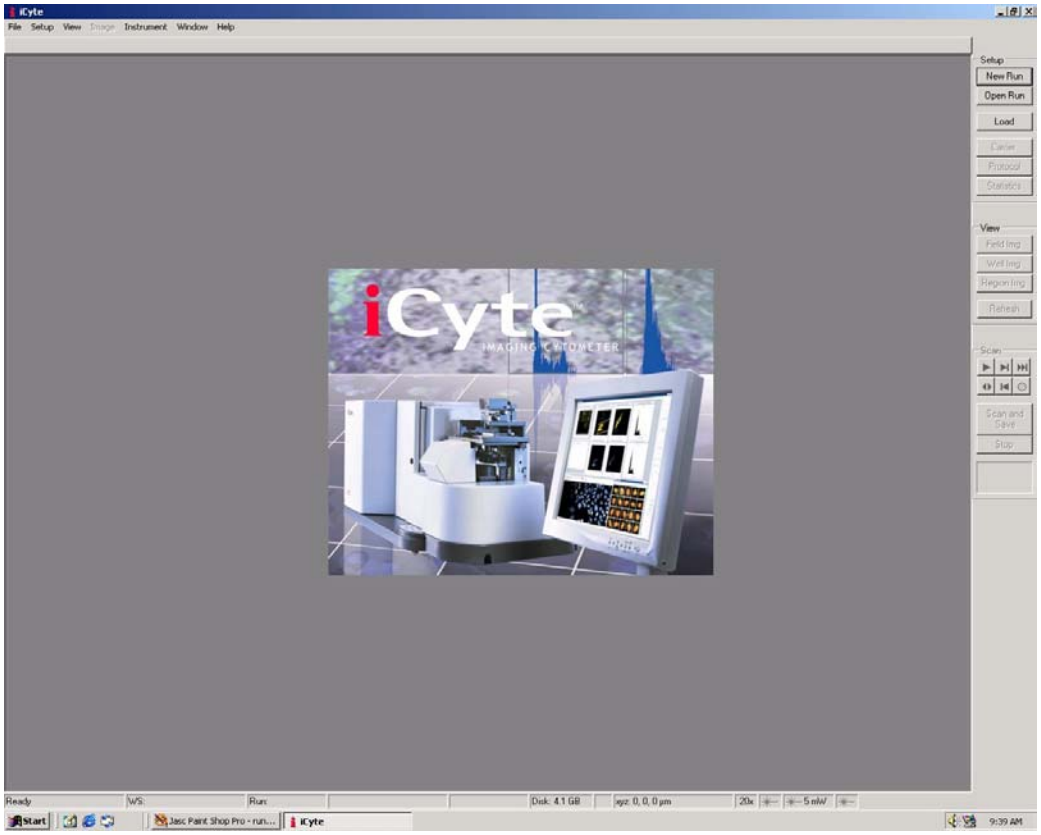
Before starting this tutorial ensure that iCyte revision 3.0 has been successfully installed.

Users should review the iCyte version 3.0 Release Notes before proceeding with this tutorial.

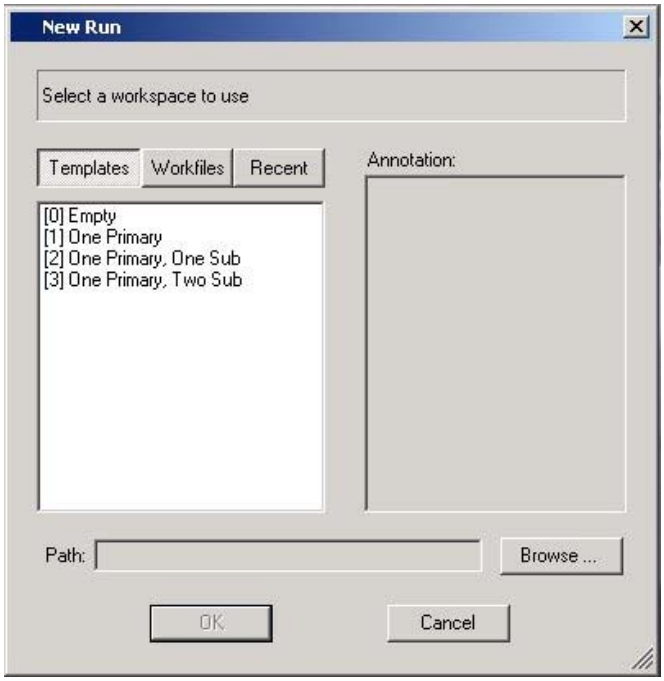
Start-up/Creating Workspaces

1. Turn on the iCyte main power and laser power.
2. Turn on the computer and all accessories.
3. Launch the iCyte application by double clicking on the iCyte desktop icon. The main iCyte screen will open.

   You will note that there have been some changes made to the main screen particularly in the side Task Bar.



4. From the side Task Bar select **New Run**. The New Run window will open:

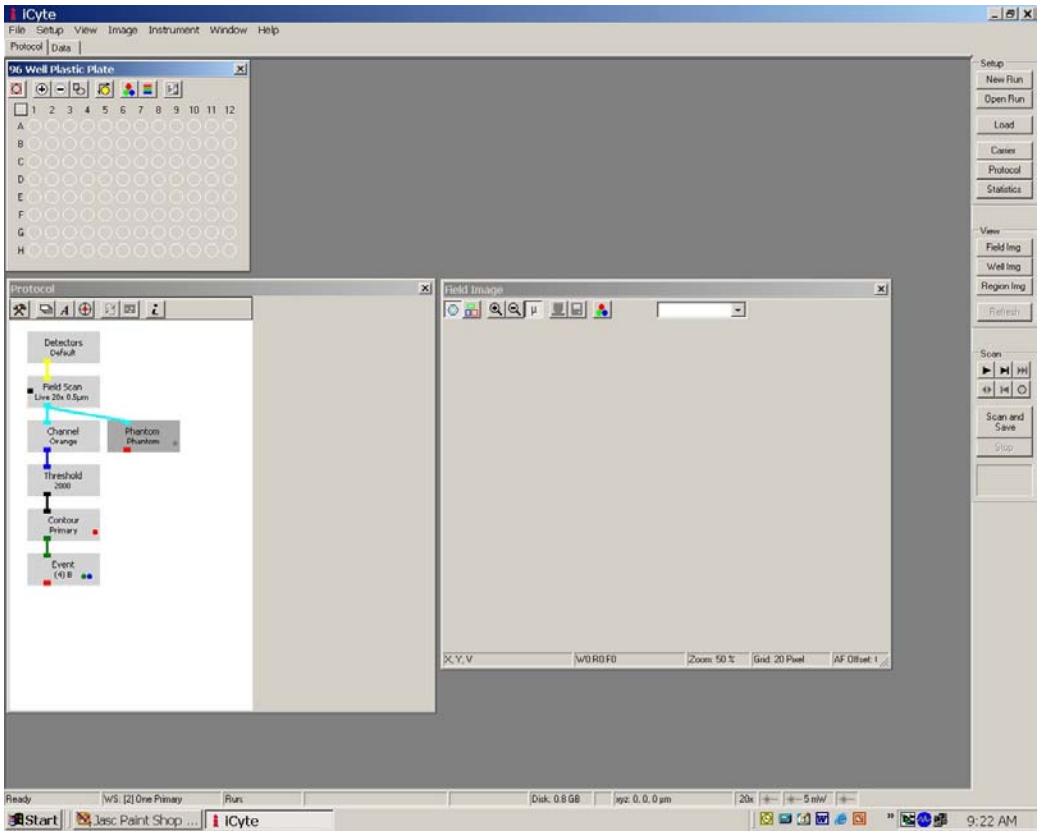


It is from this window that you will begin the process of setting up a workspace for scanning.

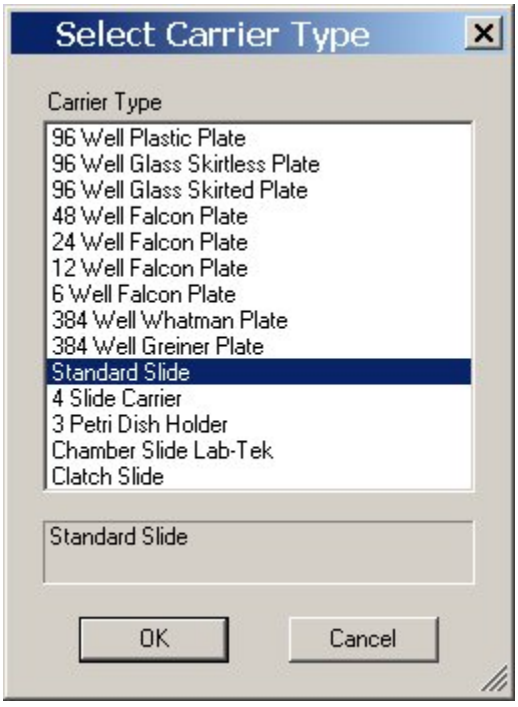
CompuCyte has provided a number of Templates that cover the most common scanning modes. These templates can be used as a starting point to build customized Workfiles.

Note: The above window is for iCyte/iCys instruments without the iNovator Application Development Tool Kit. Systems with iNovator will have additional templates.

5. Select **[1] One Primary** from the Template list and click on **OK**. The template will open and display 2 tabs (titled Protocol and Data). The Protocol tab will display 3 windows: Protocol, Field Image and 96 Well Plastic Plate (carrier).

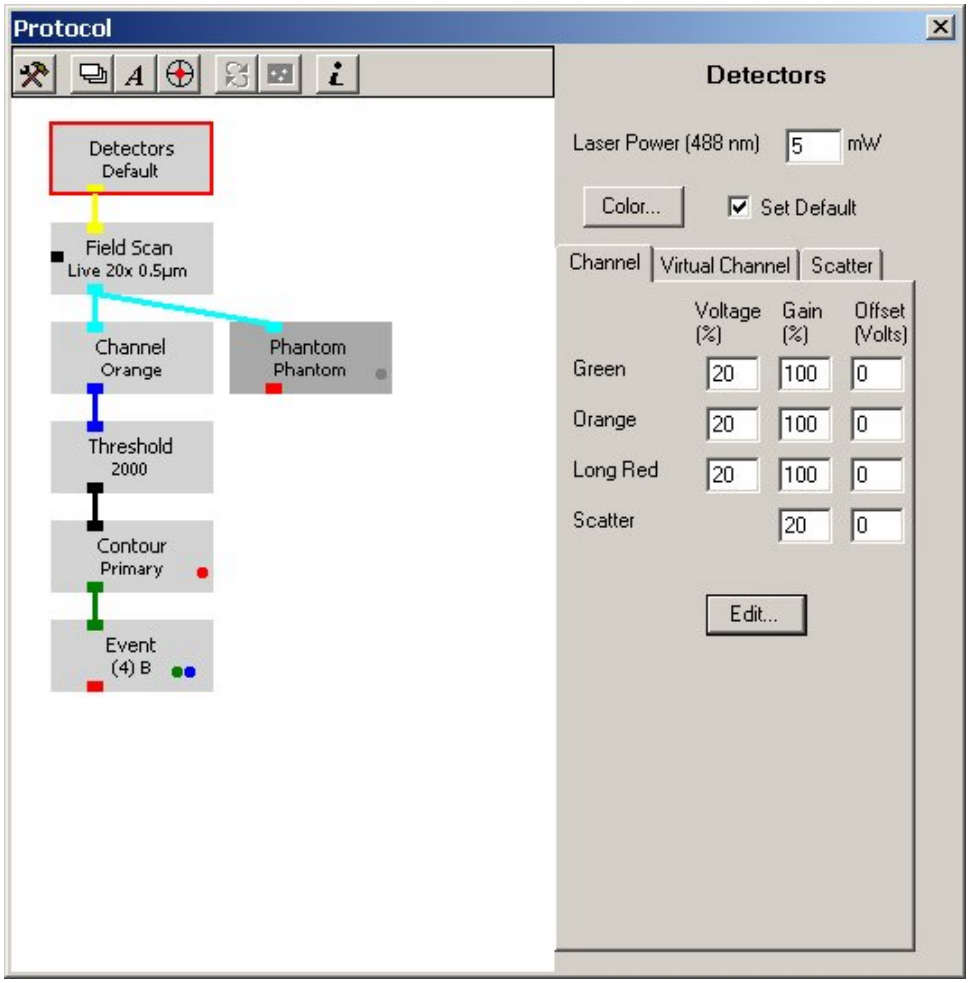


6. For this exercise the carrier type will need to be change to Standard Slide. To do this, click on the Select Carrier type icon  in the carrier window and select Standard Slide from the list.

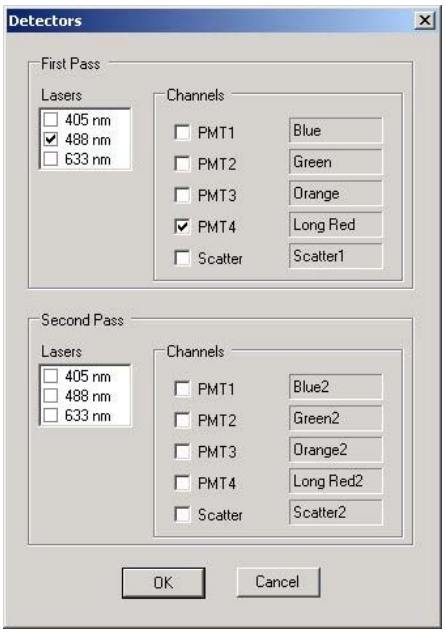


Detector Module

7. In the Protocol window, Click on the **Detectors** Module, the details of the Detector module will be displayed on the right portion of the window.

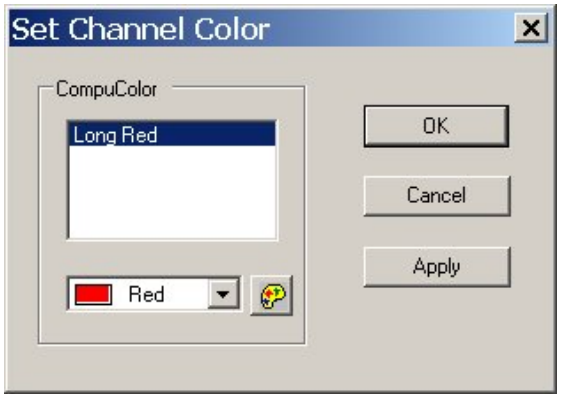


8. Select **Edit**, the Detector selection window will open, here is where you select the laser(s) and detectors for the scan. In the One Primary template, the 488 laser and Green, Orange, Long Red and Scatter channels are selected. For this exercise, deselect the Green, Orange and Scatter channels.



Note: Any combination of lasers can be selected for either pass, for example, the 633nm laser can be used in the first pass and the 405 nm or 488 nm laser used in the second pass.

9. Select **OK** to close the detector selection window.
10. Click on the **Color** box in the Detector window to view the CompuColor selection for the Long Red Channel.

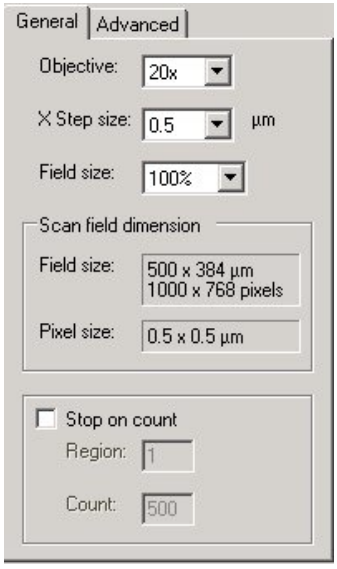


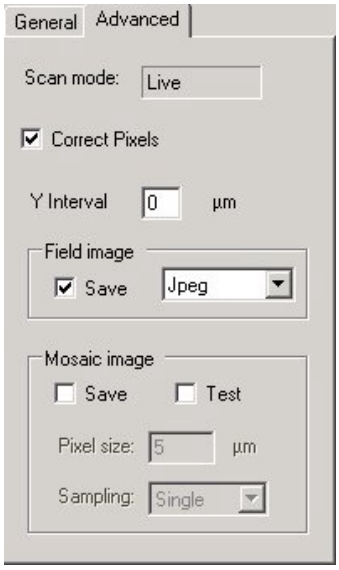
The iCyte application has the following CompuColor defaults set.

Channel	Default CompuColor
Green	Green
Long Red	Red
Scatter	Gray

Field Scan Module

11. Click on the **Field Scan** Module to display the scan parameter.
12. Select the **General Tab** and select the 20x objective, and X step size of 0.5 µ, and a Field Size of 100%.
13. Select the **Advanced Tab** and ensure Correct Pixel and Save Field Image boxes are selected.





Channel Module

14. Click on the **Channel** Module to select the contouring channel.
15. Click on the Channel drop down menu to select Long Red as the contouring channel.

Phantom Module

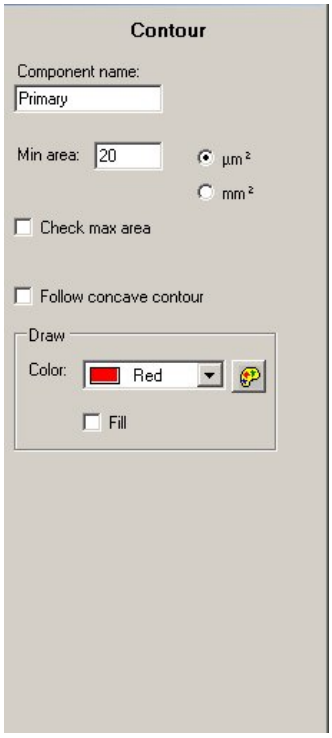
16. The **Phantom** Module is deselected (darker gray) and not active in this template.

Threshold Module

17. Click on the **Threshold** Module, select manual and enter a value of 1000. Note that the value in the Threshold Module box changes to indicate the threshold value.

Contour Module

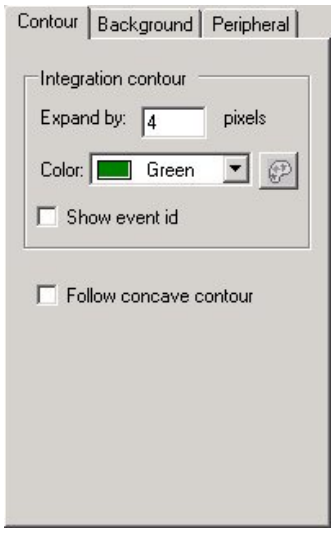
18. Click on the **Contour** Module, Enter Primary as the Component Name, a minimum area of 20 μ^2 In the Draw box the color of the Primary contour is set to red (iCyte default).

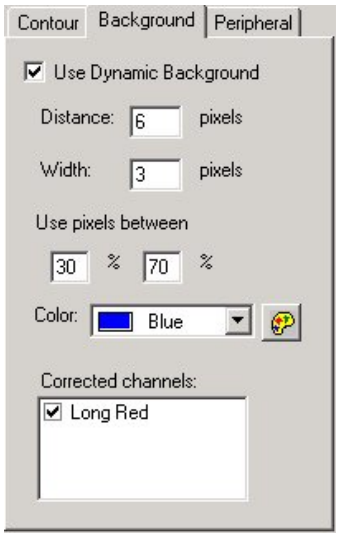


Note that in the lower right portion of the Contour Module a small red circle appears indicating the color of the event contour.

Event Module

19. Click on the **Event** Module, and then click on the **Contour** Tab. To set the Integration contour, enter a value in the expand by field, the iCyte default value is 4. The color of the integral contour is set to green (iCyte default).
20. Select the **Background** Tab, check Use Dynamic Background and enter a distance value of 6 and a width value of 3 in the appropriate fields. The color of the background contour is blue (iCyte default).
21. Select the Long Red channel in the Corrected channels box.



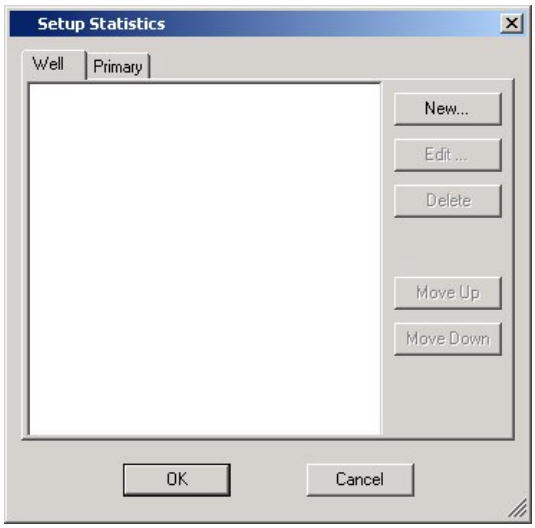


Notice that small green and blue circles appear an the lower right portion of the Event Module indicating the colors of this contours.

22. Initialize the protocol by clicking on the  in the Protocol Window icon bar.

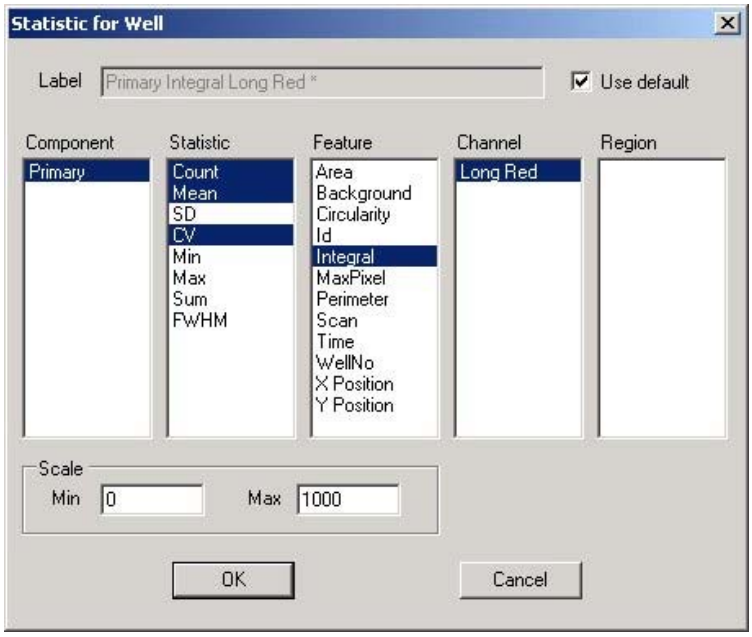
Run Statistics

23. Click on the Statistics box in the side Task Bar to set-up the Run Statistics (formerly Well Features).
24. Select the Well Tab and New to create Run Statistics.



25. In the Run Features for Well window that opens, select Primary as the Component.

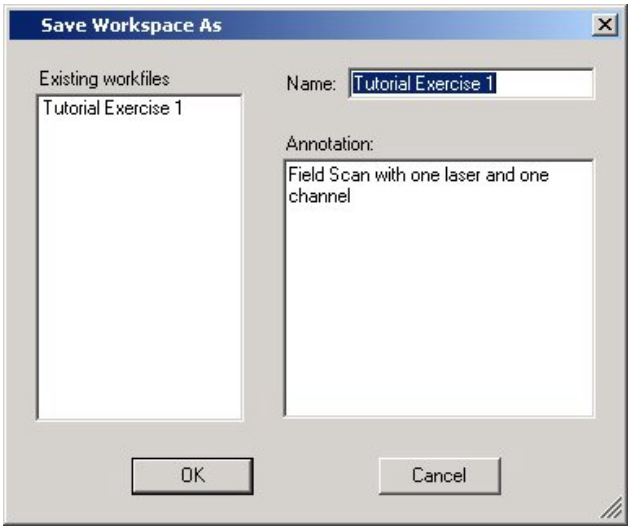
26. In the statistics column select Count, Mean and CV. Multiple statistics can be selected at the same time by pressing the Ctrl key and highlighting the desired statistics.
27. In the Features column, select Integral.
28. In the Channel column select Long Red.



Data Display

29. Select the Data tab at the top of the window; this Template does not have any analysis widows created so the tab will be blank.
30. Select **View** from the top menu bar then New Scattergram.
31. Double click in the field of the scattergram to open its properties.
32. Configure the scattergram to show the Long Red Max Pixel on the X-axis and the Area on the Y-area.
33. Create 2 rectangular regions (R1 and R2) in the scattergram just created.
34. Create a second scattergram showing the Long Red Integral on the X-axis and the Area on the Y-axis.
35. Create a Histogram for the Long Red Integral.
36. Create a region (R3) in the Long Red Integral histogram.
37. Create a new Tab by selecting **Setup** then **New Tab** from the top menu bar.
38. Enter Galleries as the new tabs name and click on OK
39. Highlight the Galleries Tab and Select View then **New Gallery** from the top menu bar. Create a Gallery for Regions 1 and 2.

40. Select File and Save Workspace as: The save window will open providing you with an opportunity to name this workspace and notations about the workspace.

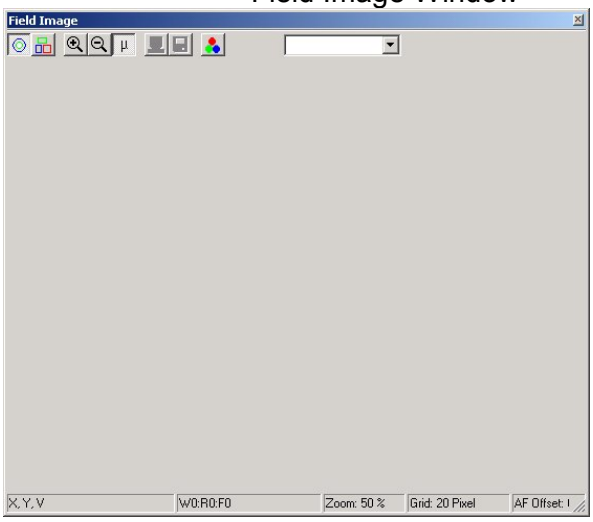


Test Scanning

Note: Although this tutorial uses a raw data file, steps 1 – 4 describe how the PMT were set.

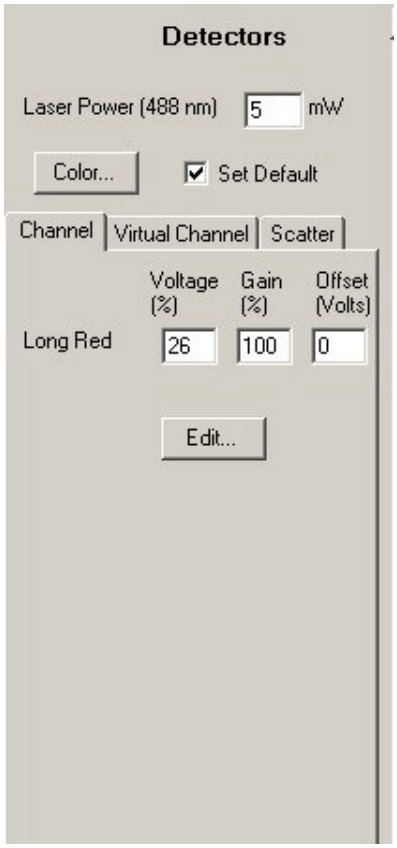
1. Select Field Image from the side Task Bar, this will open the Field Image window This window is essentially the same as the Scan View window in software version 2.6, with one notable exception; the scan control keys have been removed from the Field Scan window and placed in the side Task Bar.

Field Image Window

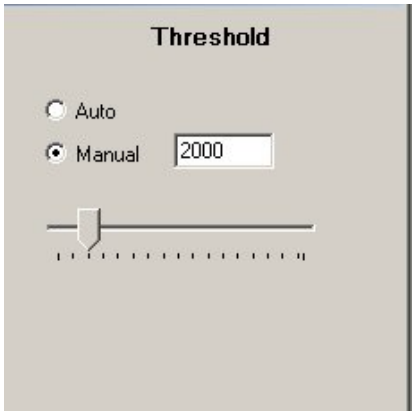


Scan Controls

2. The process of setting the PMT's to achieve optimal fluorescence intensity in the Field Scan window remains the same. A test scan is initiated using the Test Scan or Single Scan control in the Scan side Task bar.
3. The Detector module in the Protocol window is opened and adjustments are made to the PMT Voltage (%) and Offset (volts).



4. Contours are set through adjusting the Threshold value in the Threshold module in the Protocol window.

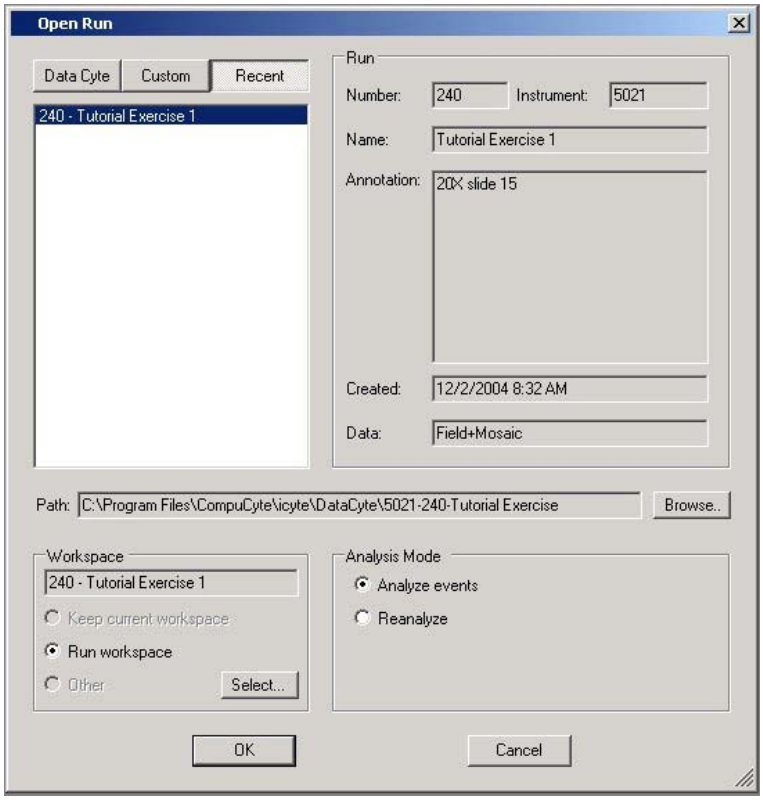


5. The test scan can be stopped by clicking on the stop icon in the Scan area of the side Task Menu bar.

Opening Runs

For this part of the tutorial, insert the iCyte/iCys Revision 3.0 Tutorial data disk into the CD-ROM drive of the computer.

1. Select **Open Run** from the side Task Bar, the Open Run window will be displayed

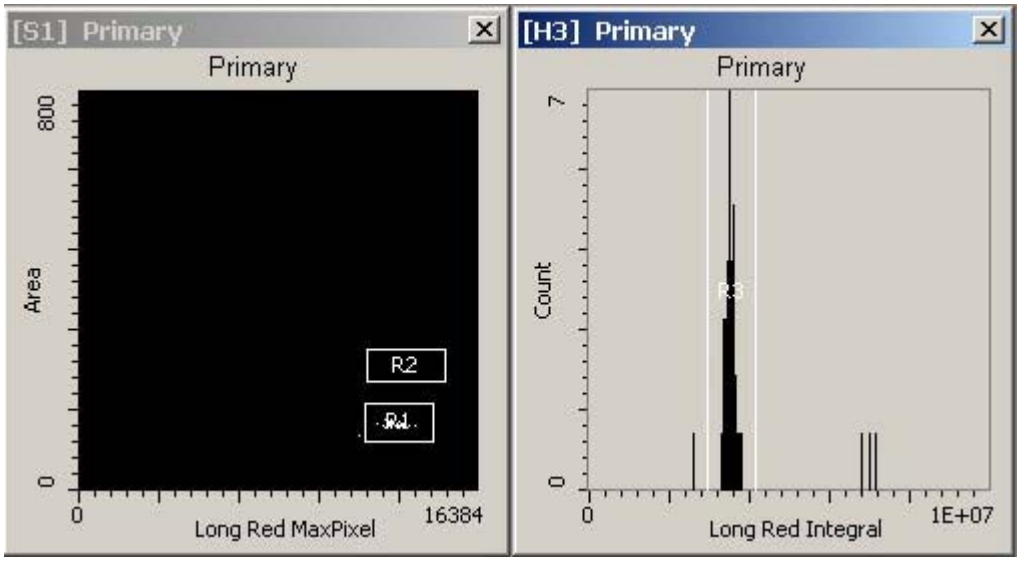


The Open Run window makes it easy to import a Run.xml file from a number of a different locations. Data files located in the DataCyte folder as well as a customized location can be displayed by clicking on the appropriate box.

Highlighting the data file will display the information about the data file in the Run field.

Users can also select a Workspace other than the run files Workspace to view the data with by selecting the desired Workspace in the Workspace field.

2. For this exercise click on the **Browse** button and navigate to the computers CD-ROM drive, select Tutorial Exercise 1 and **Open**.
3. Select Run.xml and **Open**.
4. Select **Reanalyze** in the Analysis Mode box. This is analogous to selecting the Active Data – Raw data in software version 2.6. Selecting Analyze Events is similar to setting the active data to Event data in version 2.6.
5. Select **OK**, the Workspace from the run will open with the run information.
6. Select File > Open Workspace from the top menu bar. Select No to save protocol question.
7. Highlight the workfile that was created and **OK**.
8. Select **Single Field** from the side Task bar to start the reanalysis of the data file, the Field Image window will display the first scan field.
9. Display the event contours and check that all events in the scan field are contoured, if not, click on the Threshold Module and adjust so that all beads in the scan field are contoured.
10. Click on the **Data** tab and reposition R1 on the single bead population and R2 on the double bead population in the Long Red Max Pixel vs. Area scattergram.
11. Reposition R3 in the Long Red Integral histogram on the single bead peak.

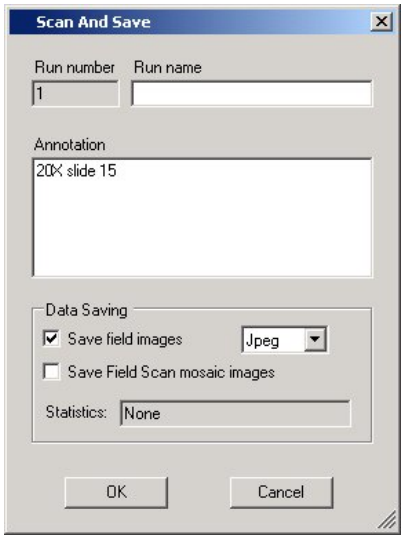


12. Stop the Test scan.

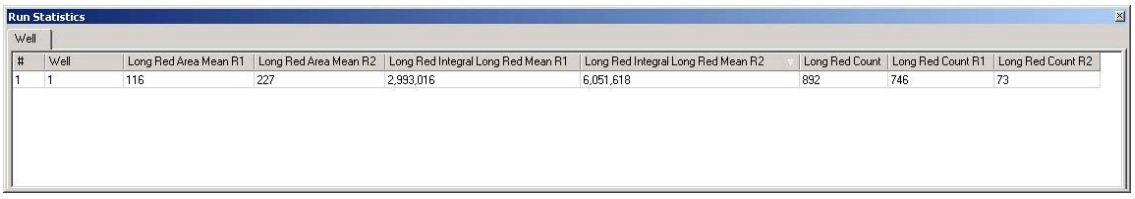
iCyte/iCys Revision 3.0 User Tutorial
Exercise 1

Scan and Save

1. To start a scan Select **Scan and Save** from the side Task Bar. The Scan and Save window will open.



2. Enter a Run name in the Run name field.
3. Enter any notations about the specimen or scan in the Annotation field.
4. Select how you want the raw data to be saved, either Jpeg or Flat, these remain the same as in revision 2.6 software.
5. Select **Save Field Scan Mosaic Image** if you would like to save a mosaic image of all scan fields together (Well Image)
6. Click OK to initiate the scan and save.
7. When the scan is completed the Scan Complete window will appear. Click on OK to clear the window.
8. Select View – Run Statistics to display the well statistics specified earlier in the run set-up.



9. Select View – Region Statistics will display the statistics for each region as in software version 2.6.

Region Statistics

Region	Label	Param	Mean	CV %	STD	FWHM %
S1 (945:99%)		Long Red Max	13233.47	6.82	903.04	3.17
		Area	152.82	40.17	61.39	5.34
R1 (794:83		Long Red Max	13217.84	3.19	421.98	2.97
		Area	133.42	4.19	5.59	2.92
R2 (84:8%)		Long Red Max	13604.42	2.62	356.06	2.26
		Area	255.52	5.02	12.83	3.17
S2 (918:97%)		Long Red Inte	3882927.02	30.75	1193852.65	4.08
		Y Position	13578.77	9.75	1323.42	13.27
H3 (918:97%)		Long Red Inte	3882927.02	30.75	1193852.65	4.08
R3 (791:83		Long Red Inte	3520919.76	3.07	108247.66	2.96

10. Adjustments can be made to regions and the data in both the Run Statistics and Region Statistics windows will be updated.
11. Click on the Galleries tab to view the galleries from Region 1 and Region 2. Note that both the Long Red detector and CompuColor images are saved.