Introduction

SimplePCI **D**ynamic **I**ntensity **A**nalysis (**DIA**) utilizes multi-threading for high speed processing and rapid measurement of cell intensity over time, while viewing images and data on a time graph. Measuring and plotting of data is available, on-line or off-line adding to the flexibility of this popular module. Event marking is possible during an experiment using interactive key presses or TTL signals for automatic tagging. **DIA** is ideally suited for live cell applications.

Expand the functionality of ***SimplePCI*** and **DIA** by adding the following optional modules:

- **AIC,** automated control and image acquisition
- **IPA**, develops icon-driven work files for automatic image analysis and processing
- **IPA-MTA**, track and analyze moving objects
- **QFA-FRET**, accurate FRET measurements and cross talk correction
- **VIS-MD**, provides rapid 3D visualization of multi-dimensional data sets
- **DNN**, Remove or Restore blur in images using fast algorithms
- **DNN-2D**, a Point Spread Function is derived and used in restoration

Getting Started

This **Quick Start Guide** contains examples of how to utilize **DIA**. For further assistance, refer to the online help, manual, or visit support at http://www.cimaging.net, for access to the latest **How to's** and frequently asked questions. Additional support is available at e-mail: support@cimaging.net, or Tel: 412-741-7920.

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Set up a Ratio Experiment

1. Click on **Camera** Icon to activate the Capture menu (**fig.1**).
2. Select your capture device from the top right drop-down menu. (**fig.1**) Select number of Channels for image capture, one channel for single wavelength and two for ratio(**fig.1**)
3. Set camera binning, bit depth in **Device Setup**.
4. Select Filter Wavelength from the Filter Setup drop down menu (**fig.1.**)
5. Define Regions for measurement, with ROI (**fig.1**) drawing tools and intensity threshold (**fig.2**).
   Set Exposure to 50% of min/max intensity to give best image. Hint; where possible, keep gain and exposure identical
6. Click on New Scan to Start **DIA** dialogue > choose Time Scan and Intensity Monitor > Save (**fig.3**)
7. Click **Display Setup** Tab to set up measurement parameters (**fig.4**)
8. Select intensity measurement by clicking on the **Measure** icon (**fig.4A**)
9. Choose one or two Intensity Graph Display (**fig.4B**), typically one display for single wavelength and two displays for ratio
10. Set X and Y axis properties for intensity plot display (**fig.4C**). Save display and resource parameters (**fig.4D**)
11. Click **Speed/Resource** Tab to set up time-lapse delay (**fig.5**)
12. **Acquisition Speed**: Enter user defined time intervals in Delay 1, 2 and 3, with options for Maximum speed (**fig.5A**)
13. User defined time intervals are available for dynamic selection during run (**fig.5B**).
14. **Image Display**: Select method of Image Display (**fig.5C**)
15. **Aquisition Control**: Define Time for total length of run (**fig.5D**)
16. Click **Save Scan** to save current settings (**fig.5E**)
17. Click **Intensity Plot** Tab to view graph (**fig.6**)
18. Click **Start** to begin image capture, measure and plot intensity over time (**fig.6**)
19. Click **Live Image** to pause sequence and adjust focus, or move ROIs
20. During run add event markers by clicking on the event button (**fig.6D**)

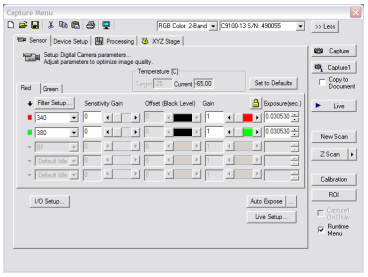

fig.1

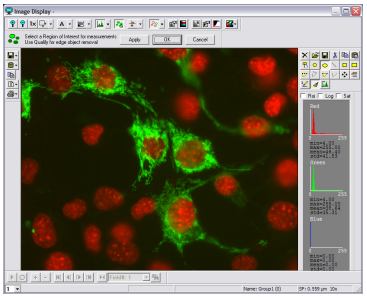

fig.2

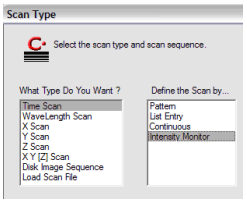

fig.3

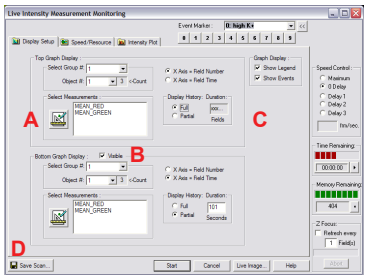

fig.4

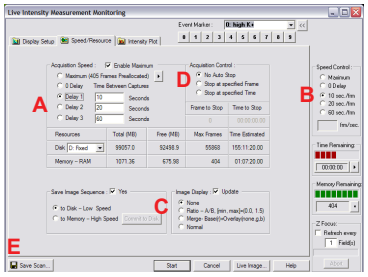

fig.5

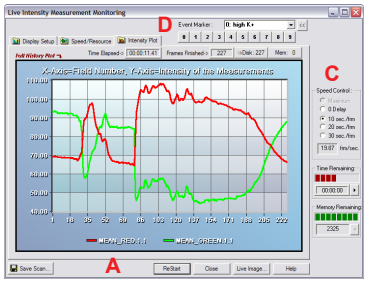

fig.6

Analyze Single Channel Data Off-line

1. Open Data File.
2. Expand Field Data and select **Original Image** (**fig.1.A**)
3. Click the ROI icon  to activate the drawing tools. Select a drawing tool (**fig.1.C**) and draw regions. Or select the Intensity threshold (**fig.1.D**) icon to threshold regions of interest.
4. Click **Intensity Measurement** icon  (**fig.1.E**) and select intensity measurements, for example **MEAN_GREY**. Click **Measure** to analyze ROI's through out the image sequence.
5. Select **MEAN_GREY** under **Group 1** in **Object Summary Graphs** (**fig.2**)
6. Choose **All** or **Average** ROI's from **Obj** drop-down menu (**fig.2**)
7. Zoom in/out of graph by clicking + dragging a selected area. Pan graph by dragging over a zoomed area. Click on the graph to return to normal view.
8. View Data in a form by selecting **Spreadsheet View** from the **Graph View**  drop down menu

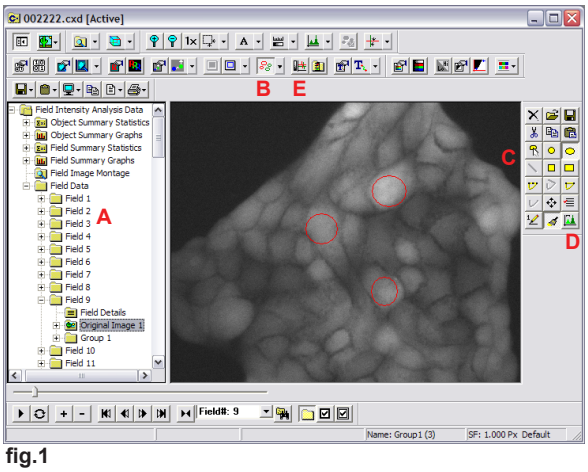

fig.1

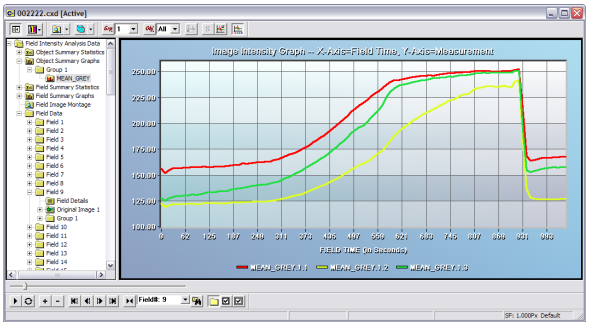

fig.2

Export Data into an Excel Format

1. Open Data File
2. Expand and highlight folder Object Summary Graph, to view **MEAN_GREY** (**fig.1**)
3. Go to the **Edit** menu and select **Copy to Excel. Excel** will launch and a copy to the spreadsheet will appear.

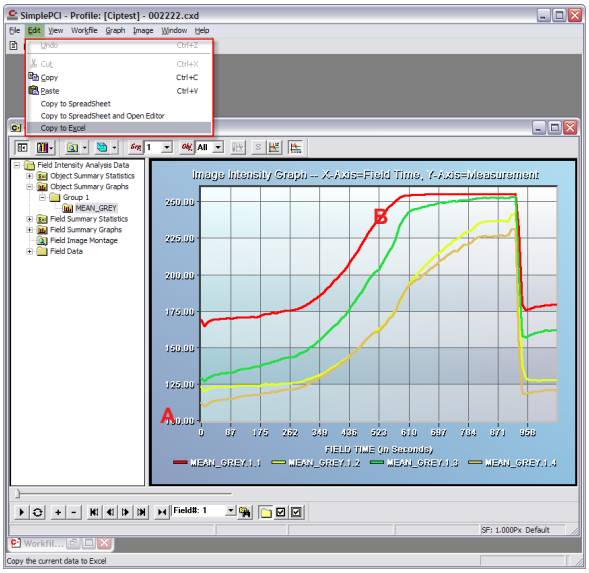

fig.1

Analyze Ratio Data Off-line

1. Open Data File.

2. Expand Field Data and select **Original Image** (**fig.1.A**)

3. Click the ROI icon  (**fig.1.B**) to activate the drawing tools. Select a drawing tool (**fig.1.C**) and draw regions. Or select the Intensity threshold (**fig.1.D**) icon to automatically threshold regions of interest.

4. Click **Intensity Measurement** icon  (**fig.1.E**) and select intensity measurements, for example **MEAN_ Red**, and **MEAN_Green**. Click **Define** to activate the **Custom Intensity Measurement Menu**, and customize measurements. If you don't need to create custom measurements go to step 6.

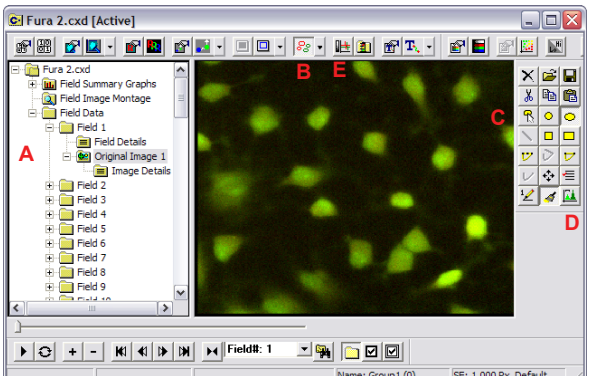

fig.1

5. **Custom Intensity Measurements Menu** (**fig.2**) allows you to customize **DIA** measurements to suit your application. For example, for a fura experiment, **Mean_Red** can be called **340nm**. Click **Add** > type a name, 340nm, click on the expression **M9:Mean_Red**. **Mean_Green** can be called **380nm**. Click **Add** > type 380nm, click on the expression **M14:Mean_Green** (**fig.2**).
Custom Measurement can be saved by clicking on the **Save** icon at the bottom left. Close the **Custom Intensity Measurement Menu**.

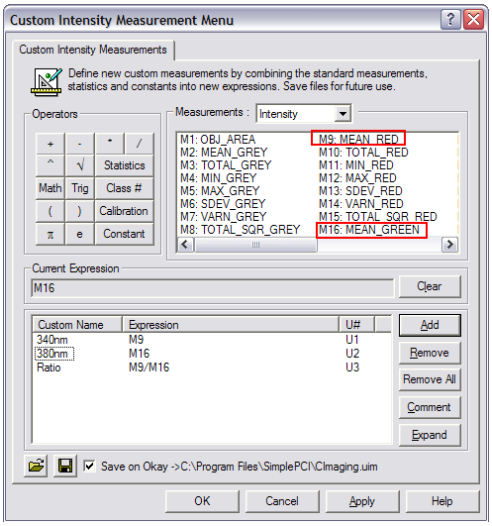

fig.2

6 Select the created custom Measurements. Check **Custom** > check the custom measurements (**fig.3**)

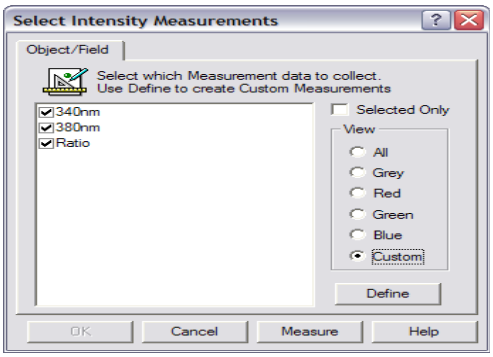

fig.3

7. Click **Measure** to analyze ROI's throughout the image sequence

8. Select **340nm**, **380nm** or **Ratio** under Group 1 in Object Summary Graphs (**fig.4**)

9. Choose **All** or **Average** ROI's from **Obj** drop-down menu. Click on any of the graph to split the data in dual view (**fig.4**). You can also click + drag on any part of the graph to zoom in.

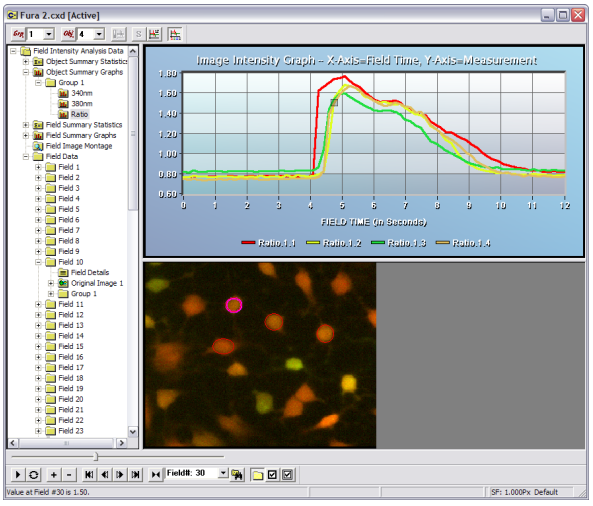

fig.4

Display Crossover of 340nm and 380nm in a Fura-2 Experiment

1. Open Data File
2. Select **Group 1** in **Object Summary Graphs** (**fig.1.A**)
3. Choose **Average** ROI's from **Obj** drop-down menu (**fig.1B**)
4. Click on **Select Measurement** Icon (**fig.1.C**) and check 340nm and 380nm (**fig.2**) to view 340nm and 380nm average profiles.

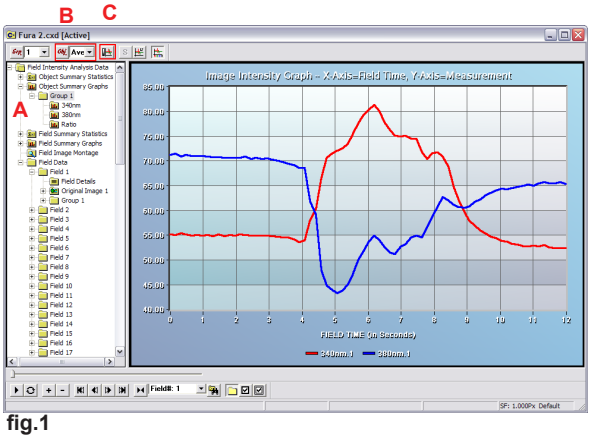

fig.1

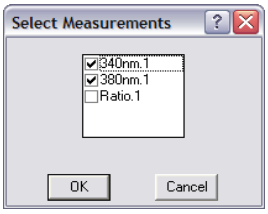

fig.2

Create Ratio ImageFura-2 Experiment

1. Open Data File
2. Expand Field Data and select **Original Image** (**fig.1**)
3. Click **Merged Display Properties** icon select **A/B Ratio** from the **Merge Display** pull-down. Enter Min Max value for best ratio display
4. Click **Contrast Display Properties** Select **Pseudocolor Spectrum** (**fig.2**) from the drop-down list.
5. Adjust min/max contrast levels for best image display (**fig.3**)

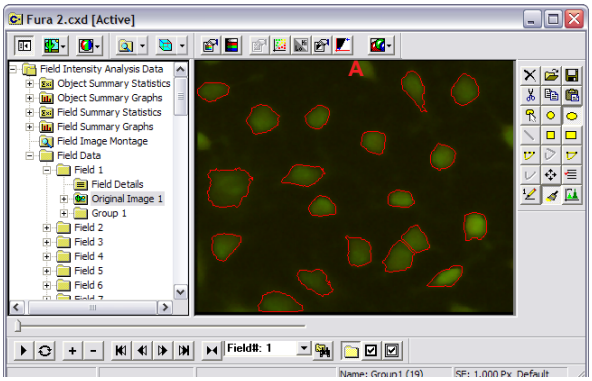

fig.1

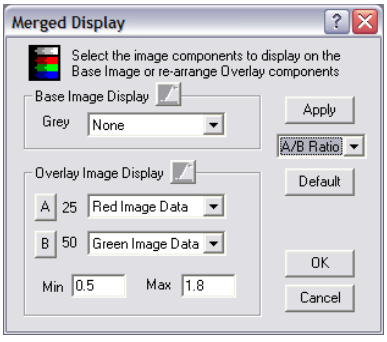

fig.2

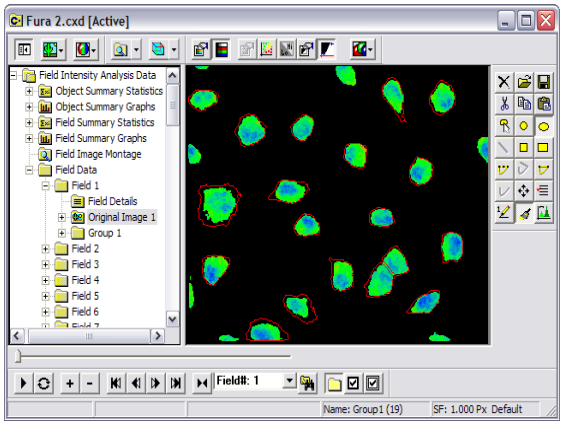

fig.3

Correct for Image Background in a Ratio Experiment

1. Open Data file

2. Expand Field Data and select **Original Image** (**fig.1.A**)

3. Click the ROI icon  (**fig.1.B**) to activate the drawing tools. Select a drawing tool (**fig.1.C**) and draw regions. Or select the Intensity threshold (**fig.1.D**) icon to automatically threshold regions of interest. The first regions are automatically classified as Group 1.

4. Define ROI for the background in Group 2. Click on the **Select Group** icon  in the ROIs toolbox. Select **Add New Group** > OK > Pick a color for the new group > OK

5. Draw a ROI on the background. Note: Increasing the image contrast will help define a good background region.

6. Click **Intensity Measurement** icon  Click **Define** to activate the **Custom Intensity Measurement Menu**, and customize measurements.

7. Click **Add** in the **Custom Object Measurement Menu** and assign a name to your custom measurement. E.g. **Corrected_Ratio** (**fig.2**) .

8. Create the following formula: **(M9-cl2(mean(M9))** for the corrected 340nm or (Mean Red);and create the following formula **(M16-cl2(mean(M16))** for the corrected 380 by clicking on the appropriate object measurements and operators (fig.2).

9. **M8** is the Mean intensity in the Red channel, subtracted by **-cls2(mean(M9)),** the Mean intensity in the RED channel of class 2 or second group (**fig.2**)

10. Click the **Save** icon to save the custom measurement and click **OK**.

11. Select the created custom Measurements. Check **Custom** > check the custom measurements (**fig.3**)

12. Click **Measure** to analyze ROI's throughout the image sequence (**fig. 3**)

13. Select **Corr_340nm**, **Corr_380nm** or **Corr_Ratio** under Group 1 in **Object Summary Graphs** (**fig.4**)

14. Choose **All** or **Average** ROI's from **Obj** drop-down menu (**fig.4**)

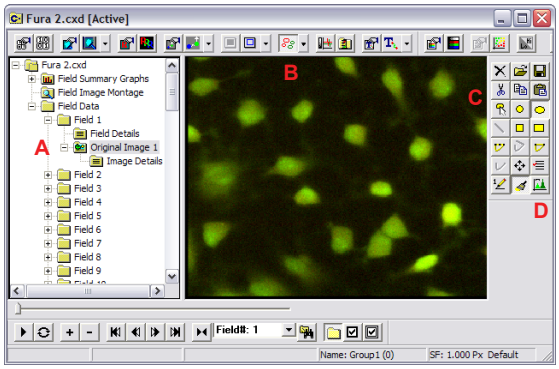

fig.1

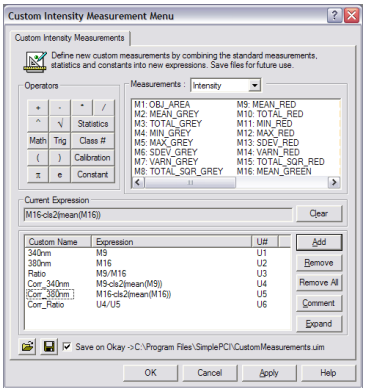

fig.2

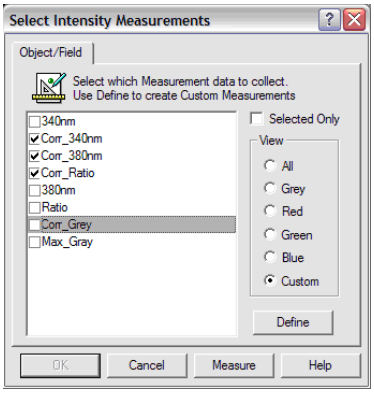

fig.3

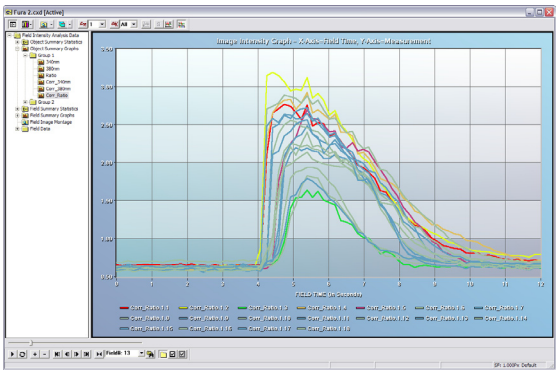
fig.4