Introduction

SimplePCI **I**mage **P**rocessing and **A**nalysis (**IPA**) broadens ***SimplePCI,*** to customize icon driven work-files (macros) for multiple applications. Innovative interactions between image objects, graphs, and tables provide instant user feedback. The user can select and customize over 150 measurements to quantify count, size, shape, position, intensity and color of objects in an image.

Directly load images into ***SimplePCI IPA*** and select the functions to solve the application. The procedure is methodical, where the operator selects each option interactively. Save or view the steps in a work-file (macro), and load the work-file for automated data collection with immediate statistical analysis.

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

- **AIC**, automated control and image acquisition
- **DIA**, dynamically measure intensity over time
- **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 **IPA**. 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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Create a Workfile

A workfile is the basic mechanism for automating image acquisition, processing and measurement operations. The workfile contents can be considered as a macro language, without the user having to deal with complicated textual interface. The workfile is created and modified by the user selecting the imaging icons from the Task Toolbar (**fig.1**). Each icon in the Task Toolbar will open a dialog for the manipulation of the current image on display using a variety of image capture, processing and measurement tools. The options selected are saved as a Workfile.

Task Toolbar/use these icons to create a Workfile

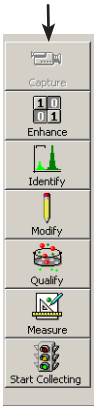


fig.1

1. Click on the New icon  to create a new **Workfile Document**. Select **Workfile Document** and click **OK**.

2. Click the **Capture** icon  to activate the **Capture** window (**fig.2**) and the **Image Display** (**fig.3**).

3. In the **Capture** window, select **Disk** from the top-right dropdown menu and set the number of color to Auto Depth.

Open image

4. Click on the **Open** icon (**fig.2**) in the **Capture** window, locate and select the image, click **Open**. The image will appear in the Image Display (**fig.3**).
If you would like to automate an image capturing event, select a camera device from the drop-down list, top-right. Make all selections and procedures as you normally do for image acquisition. And follow the steps described below.

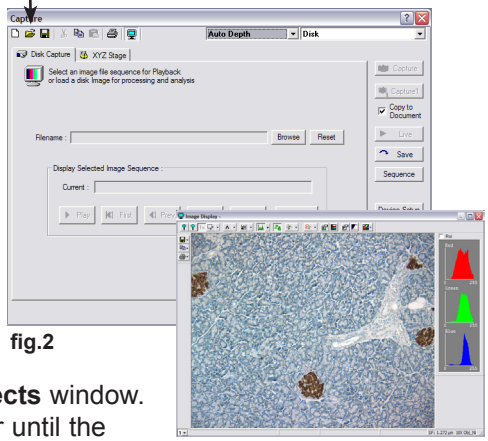


fig.2

fig.3

5. Click the **Enhance** icon in the **Task** toolbar if the image needs any type of image processing for easy identification. If the image does not need any enhancement, go to the next step.

6. Click the **Identify** icon in the **Task** toolbar to open the **Indentify Objects** window. Set the threshold of your image by adjusting the **Min.** and **Max.** slider until the objects of interest are covered by a green binary overlay (threshold). Click **OK**.

7. Click the **Modify** icon in the **Task** toolbar if you need to modify your binary image with a binary filter, such as **Erode**, **Dilate**, **Close**, **Open**, etc.

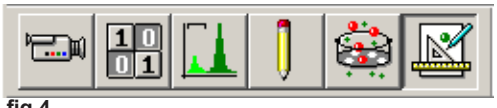


fig.4

Workfile

8. Click the **Qualify** icon in the **Task** toolbar to reject unwanted objects from your binary overlay.

9. Click the **Measure** icon in the **Task** toolbar to open the **Select Measurement** window. Check or customize the desired measurements. Click **OK**.

10. Save the **Workfile** (**fig.4**). **File menu > Save As >** assign a file name **> Save** (**fig.5**).

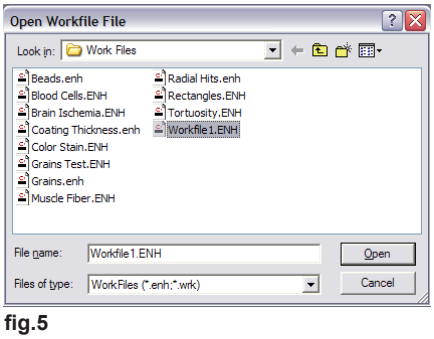


fig.5

Run a Workfile for Image Analysis on Existing Images

1. Open the **Workfile** if it is not open. **File > Open > Workfile Document >OK > Select file > Open** (**fig.6**).

2. Open the ***cxd** file to be analyzed. **File > Open > Data Document > OK > Select file > Open.** Note: To run the workfile on non-cxd image files, go to step 4.

3. Click the **Start Collecting** icon (**fig.7**) in the **Data Document** toolbar **>** assign a file name **> Save >** Check all the data components (**fig.8**) to be included in the measurements **> OK >** Click **Start** in the **Task** toolbar.

4. If you have non cxd-files, follow the following steps. *Note that if you have a sequence of images, you can import them as a cxd file allowing to run the workfile as described above.

5. Click on the **Start Collecting** icon (traffic light) (**fig.9**) in the **Task Toolbar >** Assign file name **> Save > Scan >** select **Time Scan** and **Continous > Finish > Record**, check all data components **> Start**.

6. Open image file. Click the **Open** icon in the **Capture** window (**fig.10**) and open an image file. **Open > select image > Open**, the image will appear in the **Image Display**. Make sure to have **Runtime Menu** checked in the **Capture** window (**fig.10**). Runtime menu pauses the workfile to open an image or change settings **>Click OK.**

7. After the workfile has finished running, the **Capture** window will appear again allowing you to open the next image.

8. Click **Stop Collect** when finished (**fig.10**). A new data file ***cxd** will have a data tree with several folders containing all the measurements and image details (**fig.11**).

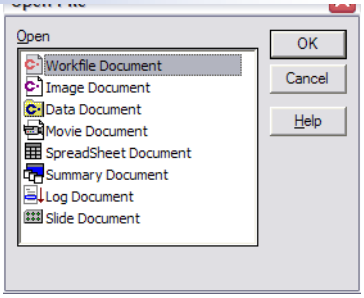


fig.6

fig.7

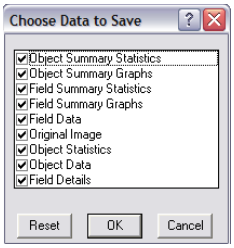


fig.8

fig.9

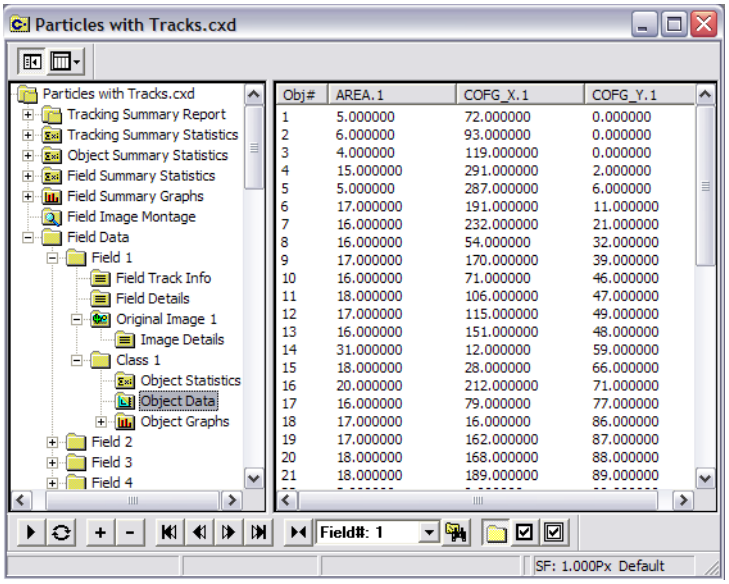


fig.11

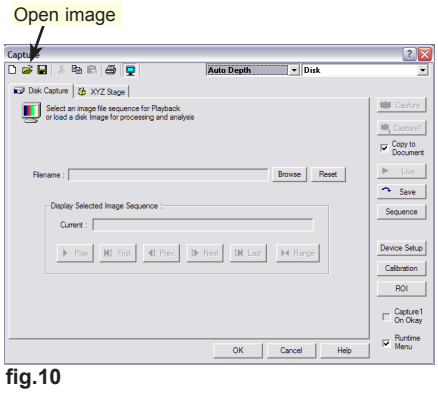


fig.10

Create Custom Measurements

1. Click the **Measure** icon in the **Task Toolbar** (**fig.12**).
2. Click **Define** in the **Select Measurements** window (**fig.13**).
3. Click **Add** in the **Custom Object Measurement Menu** and assign a name to your custom measurement. E.g. Ratio (**fig.14**)
4. Select the desired Object Measurements or Field Measurements, and Operators. E.g. **M8:Mean_ Red/M14: Mean_Green**.
5. Save the custom measurement. Click on the **Save** icon at the bottom-left of the **Custom Object Measurement Menu**, and click **OK**.
6. Select the custom measurement from the **Select Measurement** window.
7. To re-open the custom measurement, click **Measure > Define >** click the folder icon at the bottom-left of the **Custom Object Measurement Menu > select file > Open**.

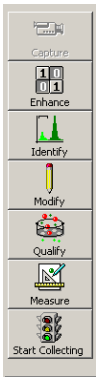


fig.12

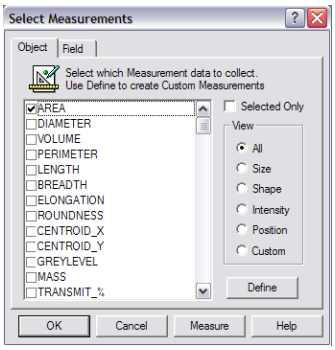


fig.13

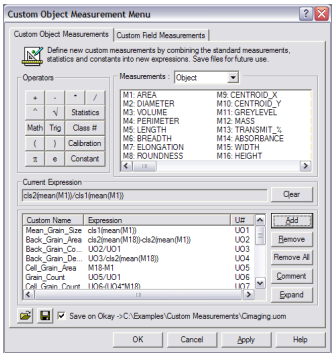


fig.14

Analyze Objects Inside a ROI

1. Click the **Camera** icon.
2. Click the **Open** icon in the **Capture** window and open an image file. **Open > select image > Open**, the image will appear in the **Image Display**.
3. Click the **ROI** button in the **Capture** window.
4. Draw **ROIs > OK >**Close the **Capture** window.
5. Click the **Identify** icon and set the threshold of your image (**fig.15**). A green overlay will appear in the area outside and inside the **ROIs** (**fig.16**).
6. Click the **Qualify** icon in the **Task** toolbar
7. Click the **ROI Edge Object** icon (**fig.17**)
8. Select **Cut off objects outside the edge** (**fig.18**).

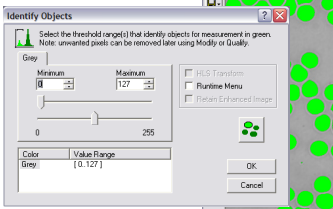


fig.15

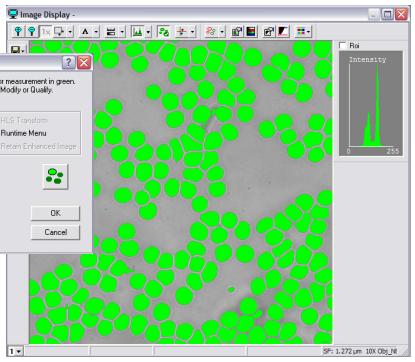


fig.16

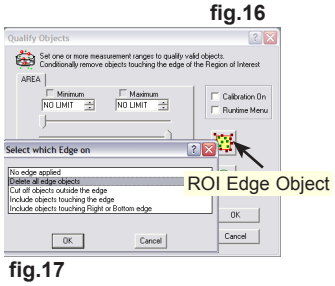


fig.17

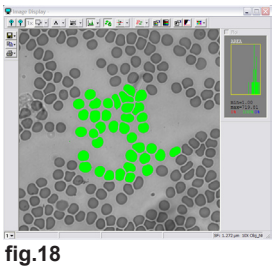


fig.18

Blood Cell Example

1. Create a new **Workfile**. **File > New > Workfile Document > OK**

2. Click the camera icon to activate the **Capture** window and the **Image Display** (**fig.19**)

3. Select **Disk** from the top-right drop-down menu. Click the **Open** icon in the **Capture** window. Locate **Blood Cells.tif** in the example CD. **Examples/Images/ Blood Cells.tif > Open**. The image will appear in the **Image Display** (**fig.19**).

4. Close the **Capture** window. Click **Identify** in the **Task** toolbar. Adjust the minimum and maximum sliders until the objects of interest on the image are covered with green overlay (**fig.20**).

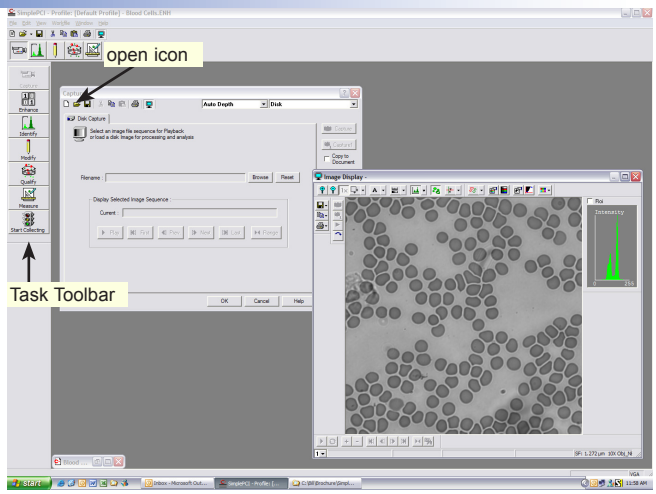


fig.19

5. Click on the **Qualify** icon in the **Task** toolbar. Reject unwanted objects by moving the top or bottom slider until unwanted objects appear red (**fig.21**)

6. Click **Measure** in the **Task** toolbar. Check **Area** and **Diameter** (**fig.22**). Click **OK**.

7. Close the **Image Display** and save the **Workfile**. **File > Save As > assign a file name > Save**

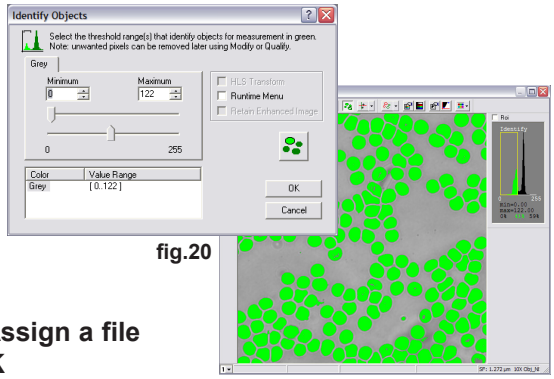


fig.20

8. Run the **Workfile**. Click **Start Collecting** in the **Task** toolbar **> Assign a file name > Save > Check Data Components to be recorded > OK**

9. Click **Start**. The **Capture** window and the **Image Display** appear. Open the **Blood Cells.tif image** if it's not open. Click **OK** on the **Capture** window. The **Workfile** will complete one cycle of the process and return to the Capture window. Other images may be open for multiple field analysis. In this example, there is only one image; therefore, click on the **Stop Collect** button to close the capture window and exit the operation loop. Close the **Image Display**.

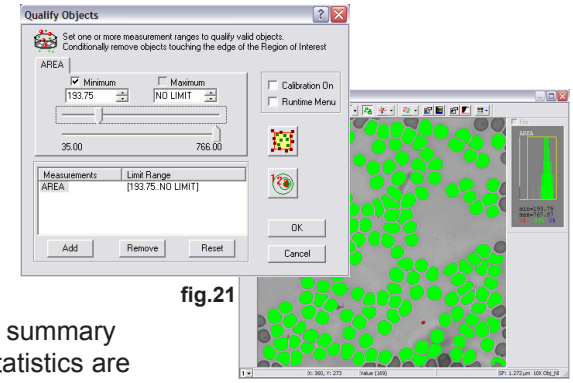


fig.21

10. Review the ***cxd** file. The ***cxd** file will display object summary statistics, object summary graphs, field summary statistics, field summary graphs, field image montage, and field data. In this case, the statistics are reported for **Area** and **Diameter** of the blood cells.

11. The data can be easily displayed in spreadsheet form. Figure 23 shows a Spreadsheet, which indicates the **Area** and **Diameter** for all of the black dots.

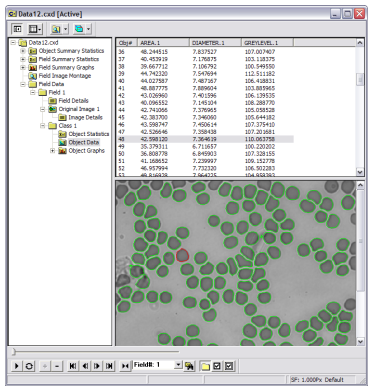


fig.23

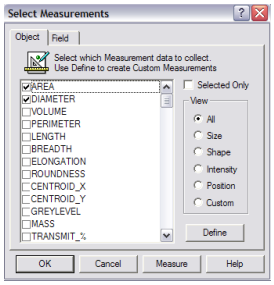


fig.22

Coating Example

1. Create a new Workfile. **File > New > Workfile Document > OK**

2. Click the camera icon to activate the **Capture** window and the **Image Display** (**fig.24**)

3. In the Capture window select **Disk** from the top-right dropdown menu (**fig.24**) Click the **Open** icon. Locate **Coating Thickness.tif** in the example CD. **Examples/ Examples/Images/Coating Thickness.tif > Open**. The image will appear in the image display.

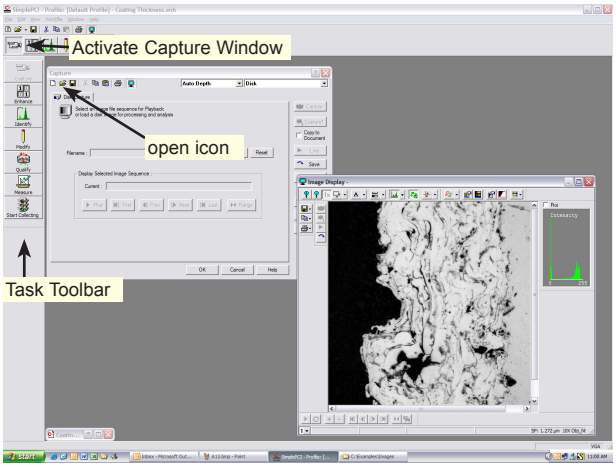


fig.24

4. Close the **Capture** window. Click the **Enhance** icon in the **Task** toolbar (**fig.24**). Apply a **Kirsh** and a **Smooth** filter to the image. The **Kirsh** filter uses a grey slider intensity gradient of a 3x3-pixel neighborhood in any direction. It enhances the current image to highlight object boundaries (**fig.25**).

5. Click the **Identify** icon in the **Task** toolbar (**fig.24**). Adjust the minimum to 27 and the maximum to 255 or move the sliders until the enhanced image of the coating is covered with a green overlay (**fig.26**).

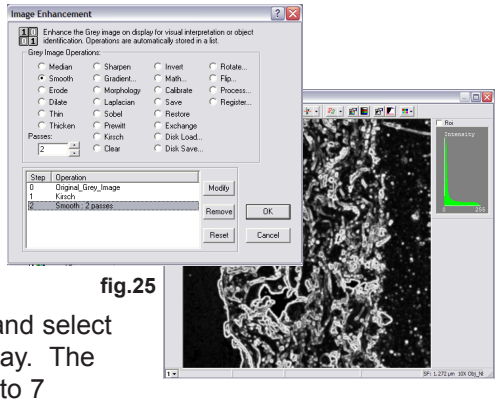


fig.25

6. Click the **Modify** icon in the **Task** toolbar. The **Modify** window appears (**fig.27**). Click **Draw Grid >** Select **Horizontal Grid** and enter 255 for the Y spacing to create two horizontal lines **> Fill Holes** to join the green overlay **> Open** to remove unwanted artifacts **>** Click **Save** to save the current overlay in memory for use later **>** Click **Clear** to remove the current overlay **> Draw Grid > Select Horizontal Grid** and enter 3 for the Y spacing **>** click **Math** and select **AND** to accept the lines where they match the previously saved overlay. The complete process may be observed by clicking through the Steps # 0 to 7 (**fig.27**).

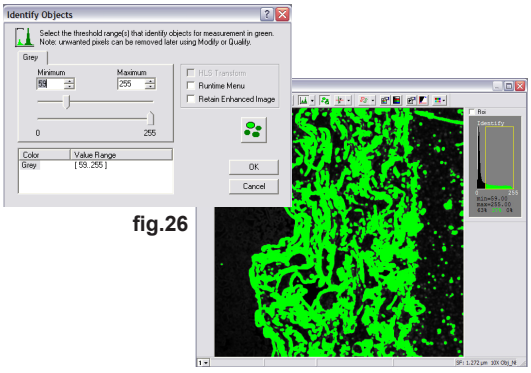


fig.26

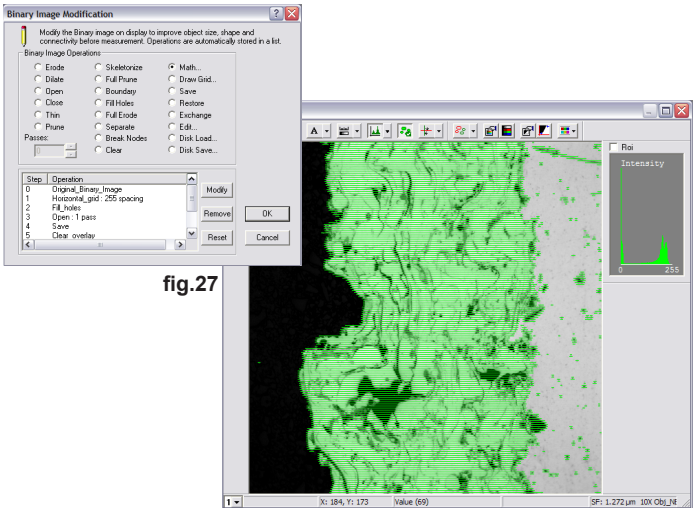


fig.27

7. Click on the **Qualify** icon in the **Task** toolbar. Reject unwanted objects by moving the top slider to 150, depending on the calibration factor loaded, or until unwanted objects appear red (**fig.28**).

8. Click the **Measure** icon in the **Task** toolbar (**fig.29**). Check **Width** to measure the width of the metal coating.

9. Close the **Image** display and save the **Workfile**. **File > Save As > assign a file name > Save**

10. Run the workfile. Click **Start Collecting** in the **Task** toolbar **> Assign a file name > Save >** Check **Data Components** to be recorded **> OK**

11. Click the **Start** icon (**fig.30**) in the **Task** toolbar. The **Capture** window and the **Image Display** appear. Open the **Coating Thickness.tiff** image if it's not open. Click **OK** on the **Capture** window. The **Workfile** will complete one cycle of the process and return to the **Capture** window. Other images may be open for multiple field analysis. In this example, there is only one image; therefore, click on the **Stop Collect** button to close the **Capture** window and exit the operation loop. Close the **Image Display**.

12. The data document (**fig.31**) will be displayed, giving access to object summary statistics, object summary graphs, field summary statistics, field summary graphs, field image montage and field data. In this case, the data are reported for the **Width** of the coating.

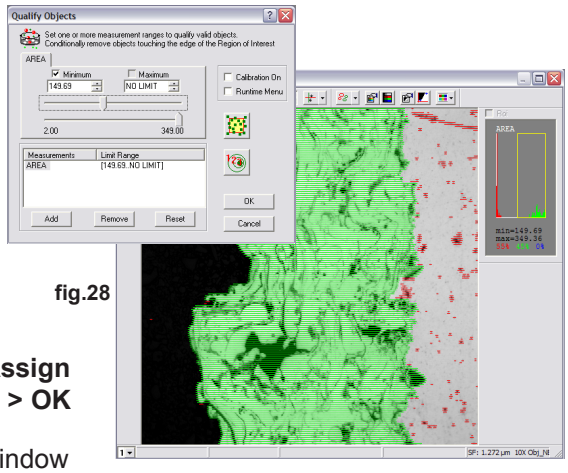


fig.28

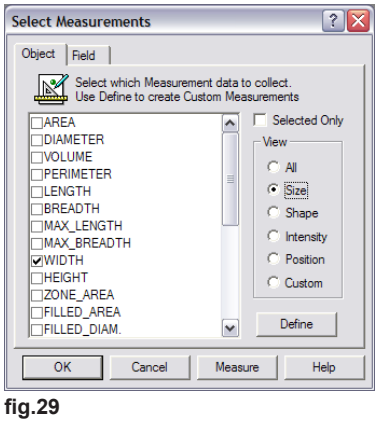


fig.29

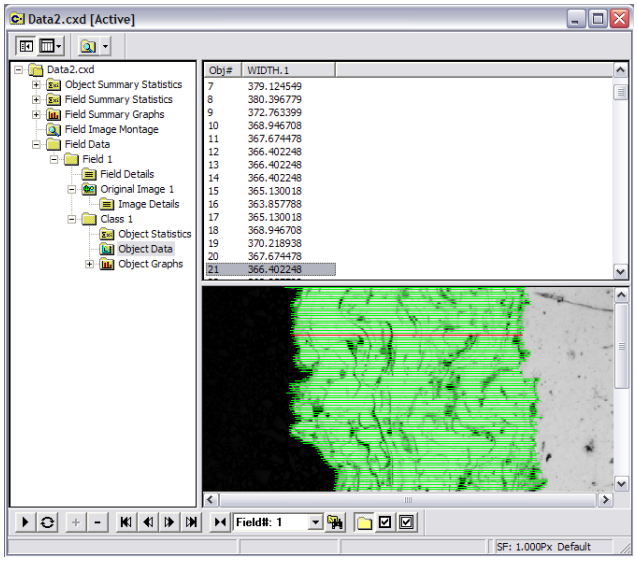


fig.31

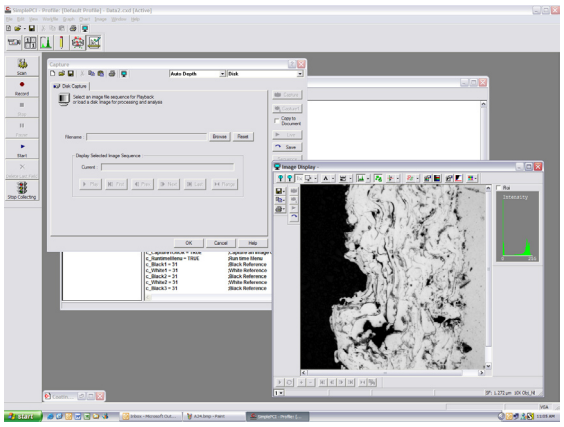


fig.30

Muscle Fiber Example

1. Create a new **Workfile**. **File > New > Workfile Document > OK.**

2. Click the camera icon to activate the **Capture** window and the **Image Display** (**fig.32**).

3. Select **Disk** from the top-right dropdown menu (**fig.32**) Click the **Open** icon in the **Capture** window. Locate **muscle.tiff** in the example CD. **Examples/Examples/ Images/muscle.tiff > Open**. The image will appear in the **Image Display**.

4. Close the **Capture** window. Click the **Enhance** icon in the **Task** toolbar (**fig.32**). Apply a **Kirsh** and two passes of the **Smooth** filter to the image (**fig.33**). The **Kirsh** filter will hightlight fiber boundaries by using a gray slider intensity gradient within a 3x3-pixel neighborhood of any direction.

5. Click the **Identify** icon in the **Task** toolbar (**fig.32**). Adjust the minimum to 0 and the maximum to 68 or move the sliders until the muscle fiber is covered with a green binary overlay (**fig.34**).

6. Click the **Qualify** icon in the **Task** toolbar. Reject unwanted objects by moving the top slider to 1545 or until unwanted objects appear red (**fig.35**).

7. Click the **Modify** icon in the **Task** toolbar. The **Modify** window appears (**fig.36**). Click **Edit >** press on the **Open polygon shape** and the **Erase tool >** Erase any connection between the fibers by drawing a line across the connection **>** Click **Apply** to preview and **OK** when finish. In the **Binary Modification** window, click **Open** to remove small links between the fibers **> Fill Holes** to ensure each fiber is solid **> Open**, apply two passes.

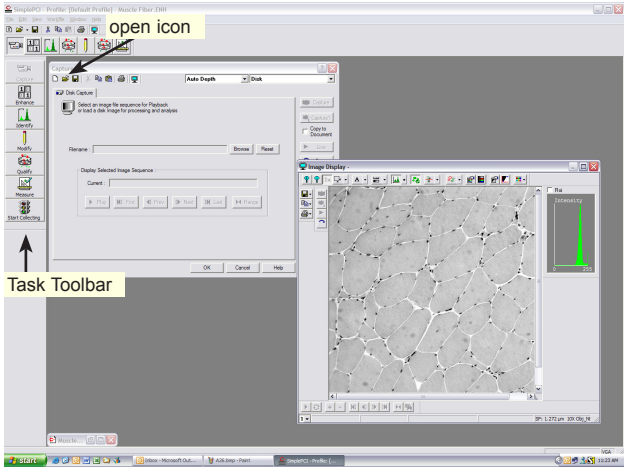


fig.32

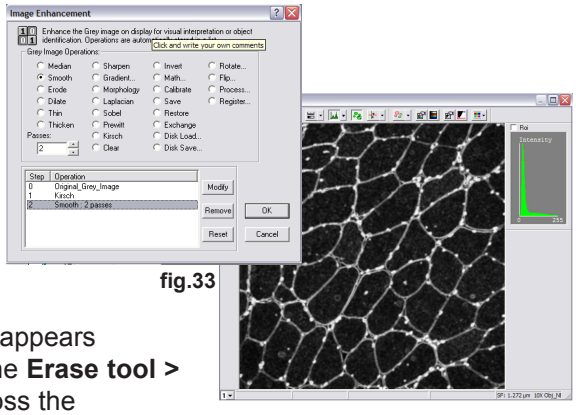


fig.33

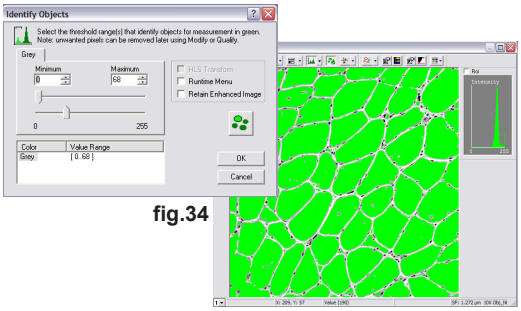


fig.34

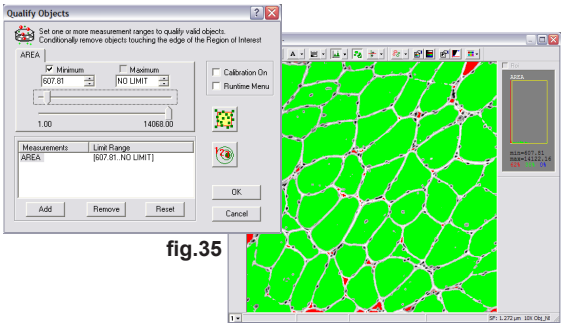


fig.35

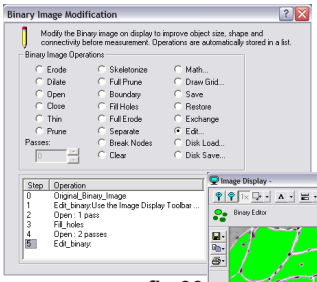
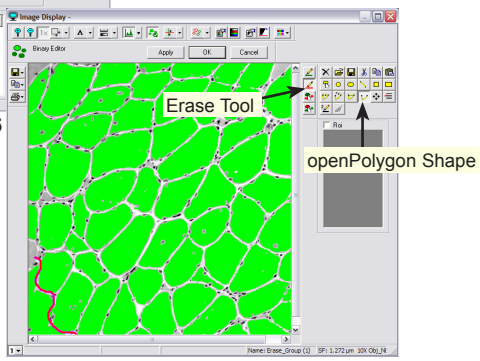


fig.36



8. Click on the **Qualify** icon. Reject unwanted objects by moving the top slider to 896 or until unwanted objects appear red (**fig.37**). Remove objects touching the edges. Click on the **ROI Edge Object** icon and select **Delete All Edge Objects** otherwise select **No Edge Applied**.

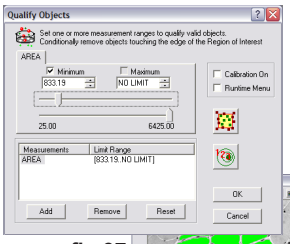
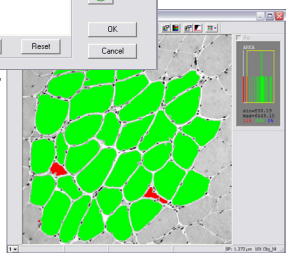


fig.37



9. Click the **Measure** icon in the **Task** toolbar (**fig.38**). Check **Width** to measure the width of the metal coating.

10. Close the Image Display and save the **Workfile**. **File > Save As > assign a file name > Save**

11. Run the **Workfile**. Click **Start Collecting** in the **Task** toolbar **> Assign a file name > Save >** Check **Data Components** to be recorded **> OK**

12. Click the **Start** icon (**fig.39**) in the **Task** toolbar. The **Capture** window and the **Image Display** appear. Open the **muscle.tif** image if it's not open. Click **OK** on the **Capture** window. The **Workfile** will complete one cycle of the process and return to the **Capture** window. Other images may be open for multiple field analysis. In this example, there is only one image; therefore, click on the **Stop Collect** button to close the **Capture** window and exit the operation loop. Close the **Image Display**.

13. The data document (**fig.40**) will be displayed, giving access to object summary statistics, object summary graphs, field summary statistics, field summary graphs, field image montage and field data. In this case, the data are reported for the **Area, Max Length, and Max Breadth** of the muscle fibers.

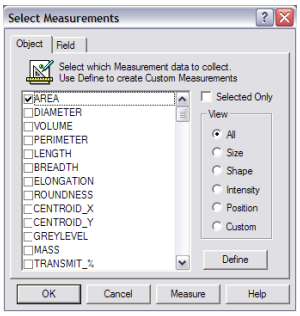


fig.38

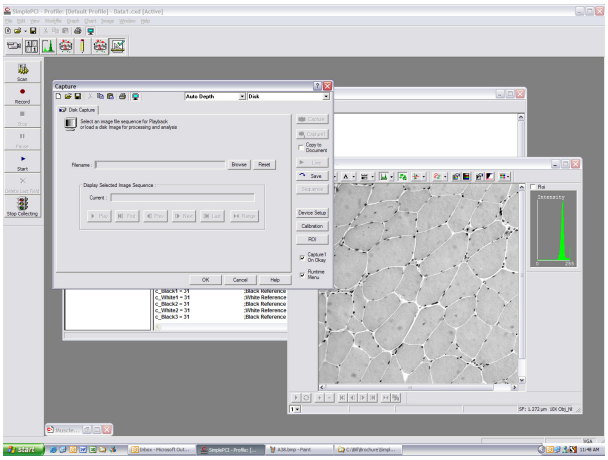


fig.39

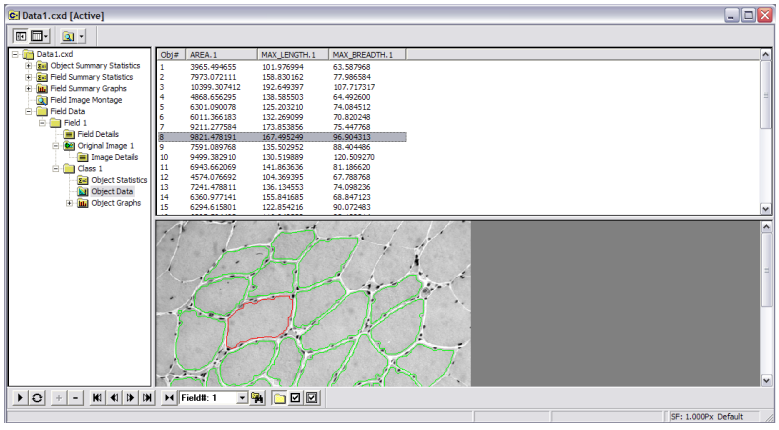


fig.40

Brain Ischemia Example

Objective:

To determine the amount of infarct area of the rat's brain.

1. Create a new Workfile. **File > New > Workfile Document > OK.**

2. Click the camera icon to activate the **Capture** window and the **Image Display** (**fig.41**).

3. Select **Disk** from the top-right drop-down menu (**fig.41**). Click the **Open** icon in the Capture window. Locate **Brain Ischemia.tif** in the example CD. **Examples/Examples/Images/ Brain Ischemia.tif > Open**. The image will appear in the image display.

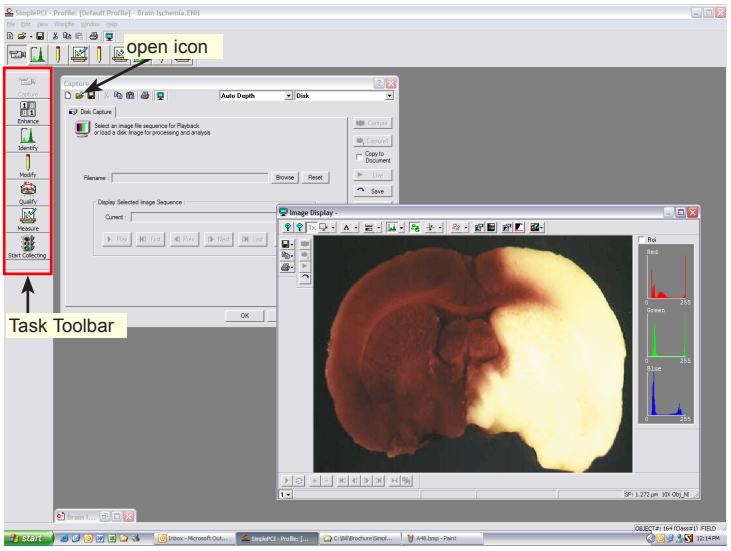


fig.41

4. Close the **Capture** window. Click **Identify** in the **Task** toolbar and set the threshold to identify the full brain. Set the minimum slider for Red to 62, Green to 27 and Blue to 16, and set the maximum slider for the Red, Green and Blue channel to 255, or adjust the sliders until the full brain section is covered with a green overlay as shown in **figure 42**.

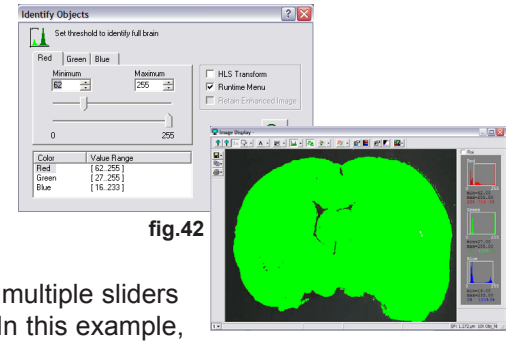


fig.42

5. Click on the **Modify** icon. Apply Fill Holes to fill any hole in the green overlay. Apply Open to remove any small artifacts. **> OK** (**fig.43**).

6. Click **Measure > Define** (**fig.44**), the Custom Object Measurement Menu appears (**fig.45**). Click on the Custom Field Measurement Tab **> Add > Type Full_Brain_Area > OK > Class #**, enter 1. When multiple sliders of measurements are made, they are referenced as Class numbers. In this example, the first measured Area is Class 1. **>** Press **F2: OBJ_Area** in the measurements field. The custom measurement should look like **figure 45**. **>** Press **OK.**

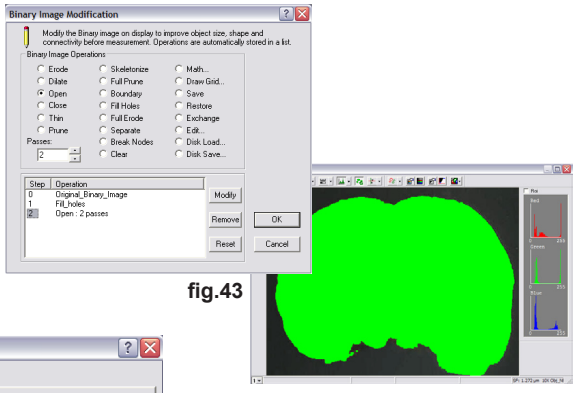


fig.43

7. Check **Area** in the **Object** tab and the custom measurement, **Full_Brain_Area** in the **Field** tab of the **Select Measurement** window and press **OK** (**fig.44**).

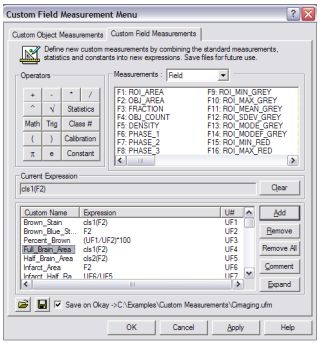


fig.45

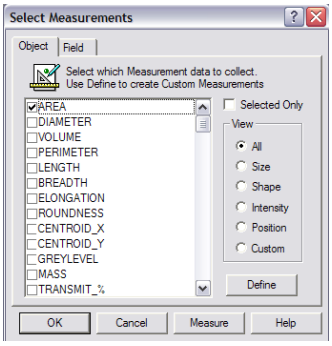


fig.44

8. Click **Modify** in the **Task** toolbar. Apply **Boundary** to outline the infarct area (**fig.46**) **> Edit**, draw a midline, press the **Open Polygon Shape** and **Draw** icon. Click and drag to draw a mid-line. **> Click Apply >** Press the **Erase** icon and the **Open Polygon Shape** to erase the top and bottom where the midline intercepts as shown in **figure 47 >** Click **Apply** and ensure that the line is separated from the other half **> OK >** Apply **Fill Holes > Open** 2 passes (**fig.46**).

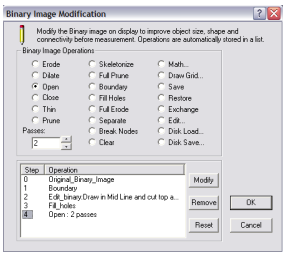


fig.46

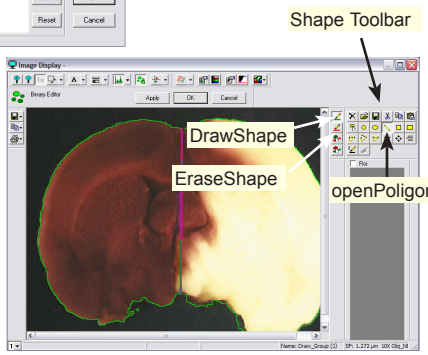


fig.47

9. Click the **Measure** icon in the **Task** toolbar. Click **Define** (**fig.48**) and create the following custom measurement as shown in figure 9: **Half_ Brain_Area: CI2(F2)**. Click **OK.** See step 7 of this example or page 3 of the SimplePCI Quick Guide on how to add custom measurement. Check the custom measurement in the **Select Measurement** window > Click **OK** (**fig.48**).

10. Click **Identify**. Set the Minimum slider for Red to 216, Green to 122 and blue to 85 and leave the maximum slider to 255, or adjust the slider until the infarct section of the brain is covered by a green binary overlay (**fig.50**).

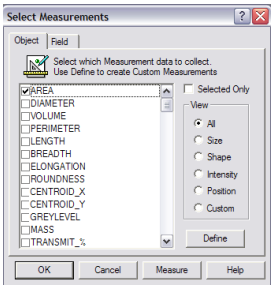


fig.48

11. Click the **Measure** icon in the **Task** toolbar. Click **Define** (**fig.51**) and create the following custom measurements as shown in **figure 49**: **Infarct_Area: F2 and Infarct_ Half_Ratio: UF3/UF2 (UF3= Infarct Area, UF2= Half_Brian Area)**. Click **OK.** Check the custom measurement in the Select Measurement window **>** Click **OK** (**fig.52**).

12. Close the Image Display and save the Workfile. **File > Save As > assign a file name > Save.**

13. Run the **Workfile**. Click **Start Collecting** in the **Task** toolbar **> Assign a file name > Save > Check Data Components** to be recorded **> OK.**

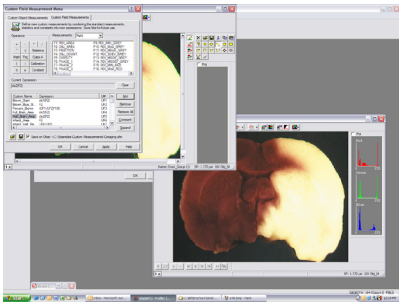


fig.49

14. Click the **Start** icon in the **Task** toolbar. The **Capture** window and the **Image Display** appear. Open the **Coating Thickness.tif** image if it's not open. Click **OK** on the **Capture** window. The **Workfile** will complete one cycle of the process and return to the **Capture** window. Other images may be open for multiple field analysis. In this example, there is only one image; therefore, click on the **Stop Collect** button to close the **Capture** window and exit the operation loop. Close the **Image Display**.

15. The data document will be displayed, giving access to object summary statistics, object summary graphs, field summary statistics, field summary graphs, field image montage and field data. In this case the data are reported for the **Width** of the coating.

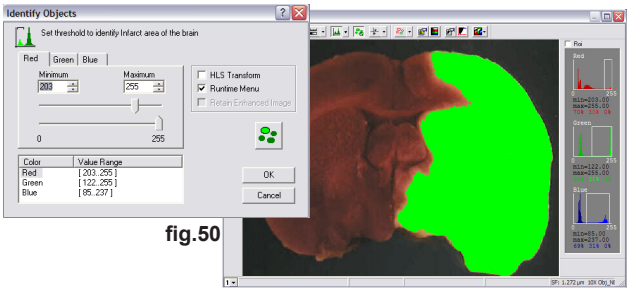


fig.50

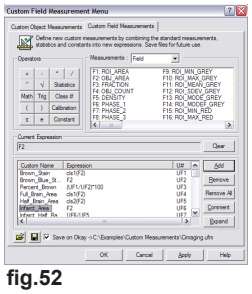


fig.52

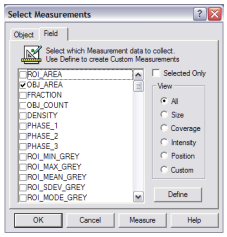


fig.51