Introduction

SimplePCI **A**utomated **I**mage **C**apture-**S**tandard **D**evices (**AIC-SD**) includes all functionality of **AIC-CD** and increases flexibility of ***SimplePCI*** to control many devices while capturing images. Adding **AIC-SD** to ***SimplePCI*** provides all supported device drivers for automatic capture of color and monochrome images using motorized filter and shutter controls. A Scan Wizard simplifies collection of image sequences for XYZ or wavelength over time (time-lapse). Image sequences are easily viewed in a data file. **AIC-SD** supports data grouping for each pass of a data set.

Expand the functionality of ***SimplePCI*** and **AIC-SD** by adding the following optional modules:

- **AIC-AD/EP** for additional control of motorized microscopes
- **DIA**, dynamically measure intensity over time
- **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 **AIC-SD**. 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.

Example guides:

Add Camera to a New Profile

To accommodate multiple users on one system, it is possible to set up a profile for each user. Profile information is stored in the User Registry; therefore, the user must have Administrator or Standard User (Power user) access. Restricted user accounts will not allow storage of profile settings in the registry.

1. At File Menu, select **Manage Profiles** (**fig.1**)
2. Click Add (**fig.1.A**), enter a name for the new profile
3. Click **Properties** to add device and define file paths and settings (**fig.1.B**)
4. Assign Default File Paths by clicking on category (**fig.2.A**)
5. Browse to desired file path and make current (**fig.2.B**)
6. Click on **Device Control** to add cameras (**fig.2.C**)
7. Add a Camera by clicking on **Capture Devices** (**fig.3.A**)
8. **AIC-CD** supports cameras; other devices are not allowed and trying to add other devices will prompt the message (**fig.3.B**)
9. To add other devices, upgrade to **AIC-SD**, **AD** or **EP**
10. **AIC-CD** supports cameras from several manufacturers. Select a Camera by clicking on appropriate device (**fig.4**), which includes:
    - **D_CAM** supports Hamamatsu cameras
    - **PVCAM32** supports Photometric cameras
    - **SPOT32** supports Diagnostic Instruments cameras
    - **Q_CAM** supports QImaging Cameras
    - **DXM-1200** supports Nikon DXM 1200 camera
    - **Pixera** support for Pixera cameras
    - **TWAIN** generic driver for general use
11. Save Profiles by clicking on the **Save** icon (**fig.1.C**)

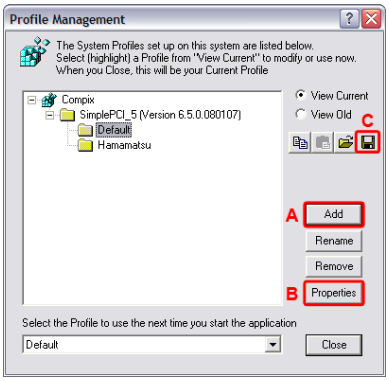

fig.1

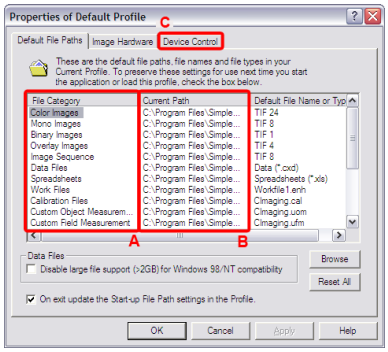

fig.2

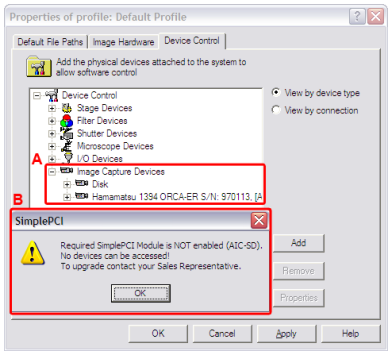

fig.3

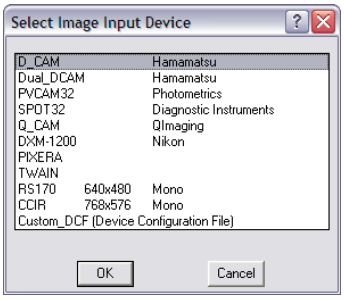

fig.4

Add Filter Wheels and Shutters

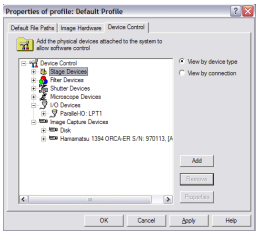

fig.1

1. Launch ***SimplePCI*** go to the File Menu > **Manage Profiles** > Select a Profile.
2. Click **Properties**. Click the **Device Control** Tab.
3. Highlight **Filter Devices** and Click **Add** (**fig.1**).
4. Select the type of filter being added and check the **Add Shutter** checkbox (**fig.2**).
5. Click the **Com-Port** tab and select the **COM-Port** where the filter wheel and shutter are connected.
6. Click the **Filter Setup** tab and click the **Apply** button.
7. Click the **Test** button to test communication and filter positions. You can step through each filter by selecting it. Click **OK**.

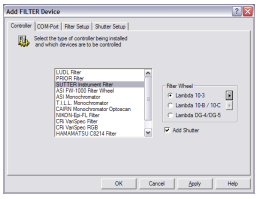

fig.2

8. Based on the filter position noticed on step 7, add or modify the name of the Filter list (**Fig.3**). To modify an item in the filter list, highlight it and change its name in the name field.

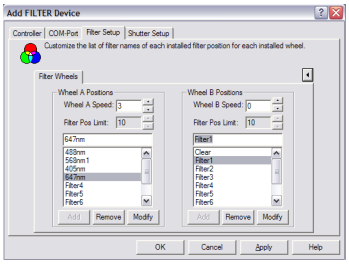

fig.3

9. Click on the **Shutter Setup** tab if a shutter is present. Click **Apply** > **Test** to test communication with the Shutter.
10. Click **OK** once all filter names have been added and devices are communicating properly. If a communication error message appears, ensure that the device is connected to the right **Com-Port**.
11. Highlight **Filter Devices** > click **Apply** > the **Automatic Filter and Shutter Control Settings** window comes up (**Fig.4**). Alternately you could click on **Filter Setup** in the Capture Menu.
12. Configure your shutter and filter settings. Click **Add** > Enter a filter name > **OK**
13. With the new name highlighted, select the filter wheel installed under the **Filter-Shutter** tab > double click on **Don't Care** or the name under **Position**. Select the filter position or wavelength from the pop-up window (**Fig.5**)
14. Select the Shutter Device installed and double click on **Don't Care** under Status to toggle between **Open/Close/Don't care**. Select **Open**. (**Fig.4**).

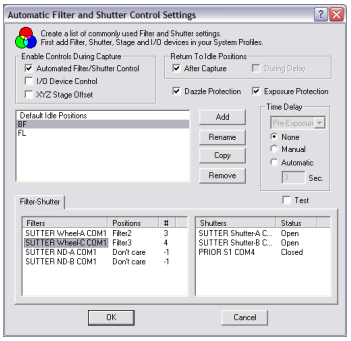

fig.4

15. Set the Default Idle Position for filter wheel to **Don't Care** and to **Close** for shutter.
    This is the position where the filter wheel and shutter will be after acquiring an image.

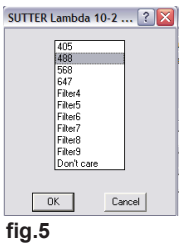

fig.5

16. Check the **Test** checkbox and select each of the filters in the name list to test their positions.
17. To minimize phototoxicity Check the **Exposure Protection** and **Dazzle Protection** checkbox (**Fig.4**). **Exposure Protection**: Shutter is closed after camera readout.
    Dazzle Protection: shutter close between filter position.
18. Click **OK** and **Close** to activate the filters and close the window.

Add and Calibrate an XYZ Stage

1. Launch ***SimplePCI*** and highlight your profile from the **File Menu** > **Manage Profiles** window.

2. Click **Properties** in the **Manage Profiles** window. Click the **Device Control** Tab.

3. Highlight **Stage Devices** and Click **Add** (**fig.1**).

4. Select the type of stage being installed (**fig.2**).

5. Click the **Com-Port** tab and select the **COM-Port** where the stage controller is connected.

6. Click the **Stage Setup** tab and click the **Apply** button to test communication between the stage and the computer (**fig.3**). If you get an error message make sure that you have selected the right **COM-Port**, and that you have connected everything properly. Click **OK**.

7. Calibrate* X,Y axis. Click **Step Size** (**fig.3**).
   ***Note**: before calibrating the stage, make sure you have loaded the correct calibration factor for the objective you are using and you have set the correct exposure for the image you will be using during calibration.
   See page 12 for calibrating the objective.

8. Center an object to the green crosshair box (**fig.4**) and click **OK**. The green crosshair will move to the bottom-right.

9. Center the same object to the middle of the bottom-right green crosshair (**fig.5**). Click **OK**.

10. If the object moved back to the middle of the top crosshair (as in step 8), the calibration for X, Y has been successful (**fig.6**), click **OK**.
    If it did not move to the same location, check the camera orientation to the microscope and repeat step 7-8.

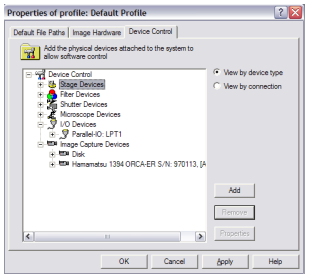

fig.1

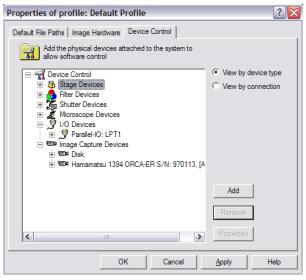

fig.2

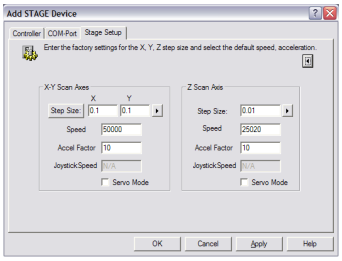

fig.3

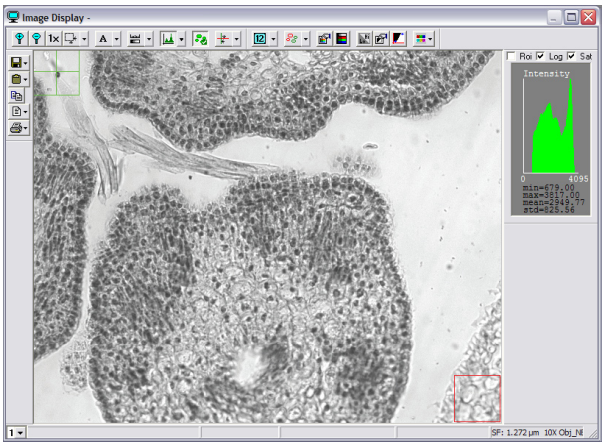

fig.4

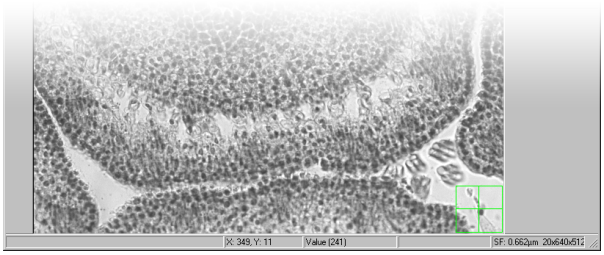

fig.5

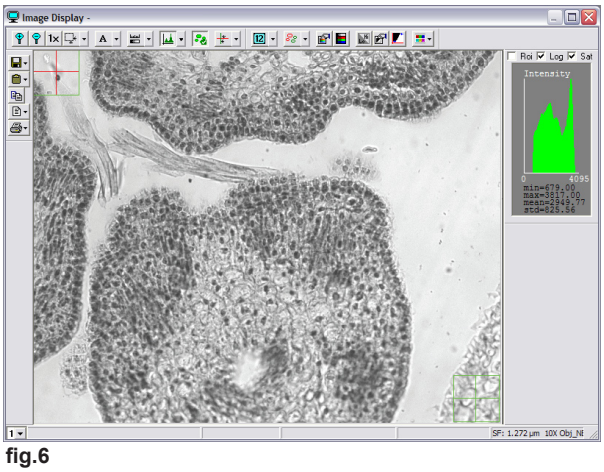

fig.6

Add Monochromator

1. Launch ***SimplePCI*** and highlight your profile from the **File Menu** > **Manage Profiles** window

2. Click **Properties** in the **Manage Profiles** window. Click the **Device Control** Tab.

3. Highlight **Filter Devices** and Click **Add** (**fig.1**).

4. Select the type of Monochromator being added (**ASI** or **T.I.L.L.**) (**fig.2**).

5. Click the **Com-Port** tab and select the **COM-Port** where the monochromator is connected.

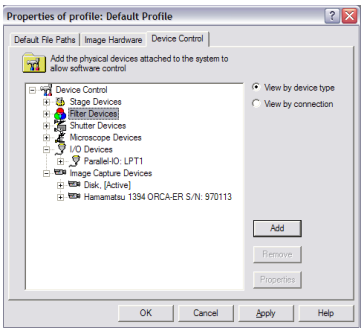

fig.1

6. Click the **Filter Setup** tab and click the **Apply** button.

7. Click the **Test** button to test communication between the computer and the monochromator. If you get an error message make sure you have selected the correct **COM-Port** and check all the connections. You can step through each filter by selecting it. Click **OK**.

8. Use the **Add**, **Remove**, and **Modify** buttons to create a list of wavelengths (**Fig.3**). You can also do this from the capture menu.

9. Click **OK** once all filter names have been added.

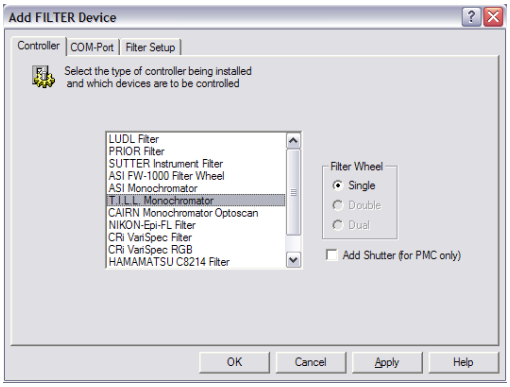

fig.2

Using Monochromator

1. Select **Monochromator** tab from Capture dialogue.

2. Switch between saved wavelengths (**fig.3**).

3. To switch to a specific wavelength, type wavelength into the **Current** field.

4. To set scan limits, type the **Top**/**Bottom** limit and check the appropriate box (**fig.4**).

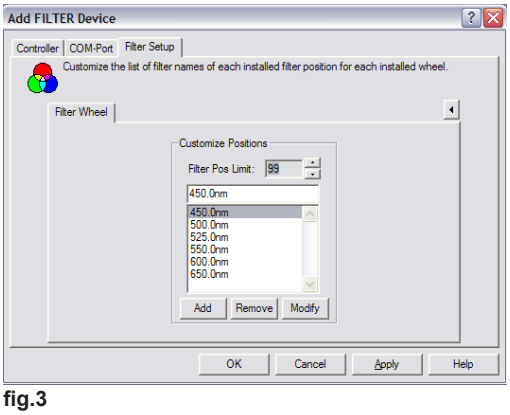

fig.3

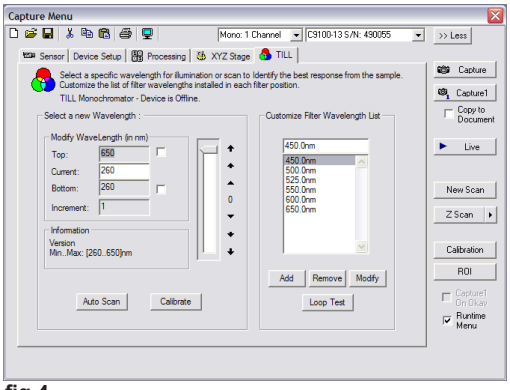

fig.4

Scan a Z-series

1. Click the **Camera** icon (**fig.1**), the capture menu appears (**fig.2**).

fig.1

2. Select the camera and the number of colors to be captured from the top-right drop-down list (**fig.2**).

3. Click **Device Setup** to configure the capture device to the appropriate image depth, resolution and binning mode.

4. Select the filter(s) from the filter drop-down list (**fig.2**).

5. Click **Autoexposure** to let the software determine the best exposure.
Note: If you can't see an image, make sure that the light path to the camera sideport is open and the correct filter cubes are in place.

6. Click **Live** and move the microscope stage to a field of interest.

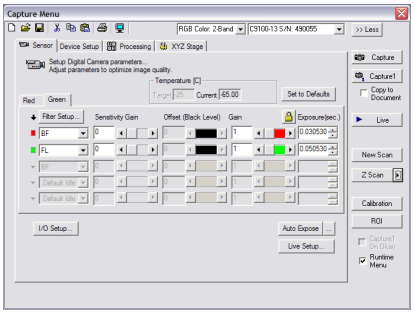

fig.2

7. Set your Z scan range. Click on the **X,Y,Z Stage** Tab. Click **Live**.
Open the **Z Focus** dialog by clicking on the arrow to the right of the Z button(**Fig.3**).
Set the current position to zero by clicking on the **0** button (**fig.4**) and clicking on **Set to 0.** Focus to the bottom of the sample and check the Bottom check box. Focus to the top and check the Top check box.
Click **OK** to close the Z focus window. Stop capturing by clicking on the **Stop** or Abort button in the Capture Menu.

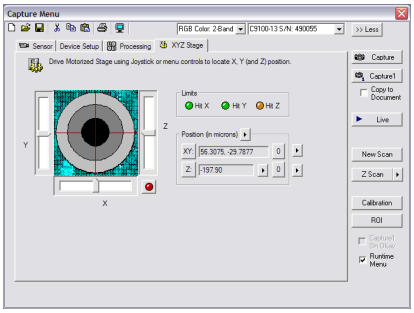

fig.3

8. Select **Z Scan** from the **Time Scan** pull down menu in the **Capture Menu**. Click **Z Scan**.

9. Retrieve the Z top and bottom position (**fig.5**). Click on the Top->Bottom button or on the Bottom -> Top button depending on which direction you want the Z-scan to start from. Enter the increment between slices in the **Increment** field name. Click **Next**, the Scan List window appears.

10. Click **Finish**. Specify file name and path and click **Save**.

11. Set field delay and number of passes and Click **Start** (**fig.6**). ***SimplePCI*** will start scanning the Z-series.

12. View saved Data Set (**fig.7**). Export as an AVI. File Menu > Export Image Sequence > Display Image > select AVI for file type.

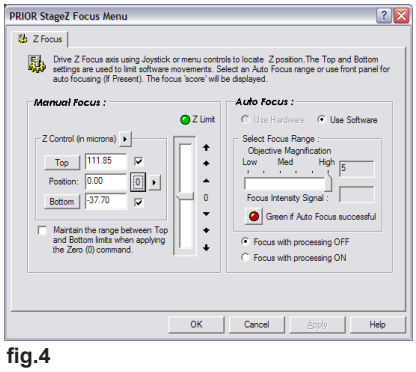

fig.4

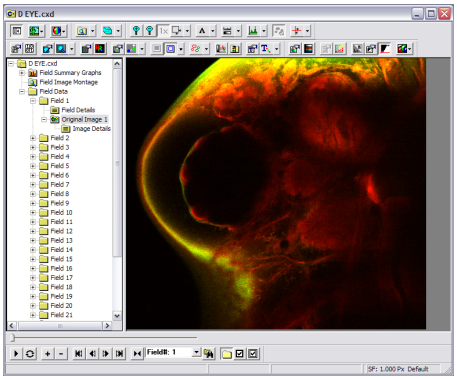

fig.7

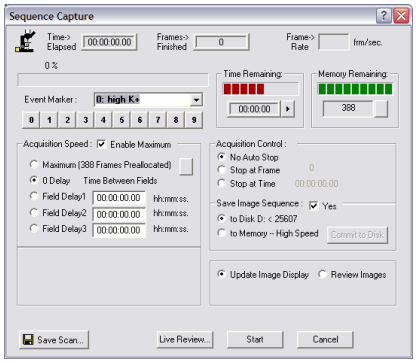

fig.6

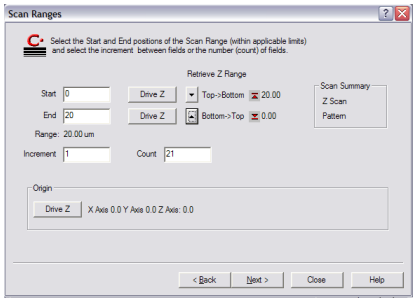

fig.5

Create Multiple XY Position, Z-scan with Time-Lapse

1. Click on **Camera** Icon (**fig.1**) and enter Capture Menu window (**fig.2**).
2. Select Camera and choose number of Channels for image capture, one channel for single wavelength and two, three, four or five for multi-color images (**fig.2**).
3. Set camera binning, bit depth in Device Setup (**fig.2**).
4. Select filter(s) from filter drop-down list (**fig.2**).
5. Adjust Exposure manually or automatically (**fig.2**) for best Image Display; view intensity distribution in histogram.
6. Click on New Scan (**fig.2**) > choose **XY(Z) Scan** and **List Entry** (**fig.3**) > **Next**.
7. Click **Add**, (**fig.4**) to access the **Stage Control** menu. A live image appears in the Image Display. Select positions of interest and click **Apply Add** > **OK**; repeat for all positions to add to the list.
8. Set a range for **Z-scan** to be applied at every XY coordinate (**fig.5**) Check **Add sub-Z Range** > Select current Z position to be Middle, Top or Bottom> Enter Z increment and the desire number of Z slices.
9. Enter number of passes or check for a **Continuous** scan.
10. Select **One file per Position** to create a **cxs** which contains a cxd per location. (**fig.8**) Select **To a Single File** to create a cxd for all the locations (**fig.7**). Click **Finish >** Assign file name and file path **> Save > Sequence Capture** window appears (**fig.6**).
11. Set time interval between passes. Click on **Save Scan** to save scan settings for future use. Click **Start** (**fig.6**).
12. A data document is created with data grouping per XY position (**fig.7,8**).

fig.1

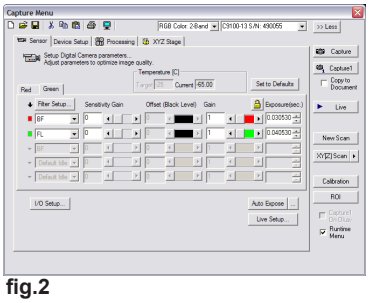

fig.2

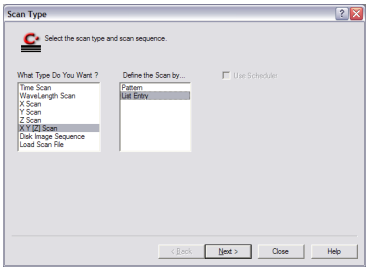

fig.3

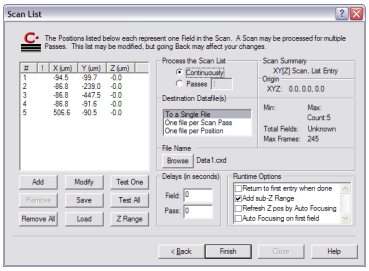

fig.4

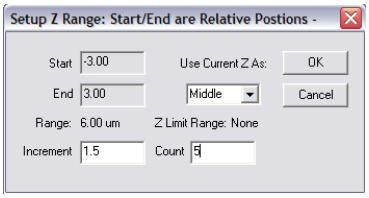

fig.5

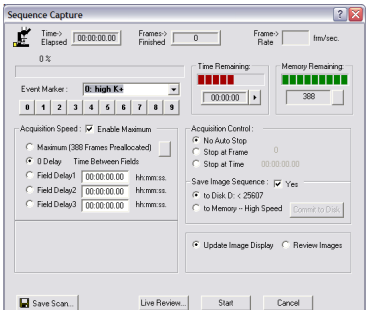

fig.6

To a Single File

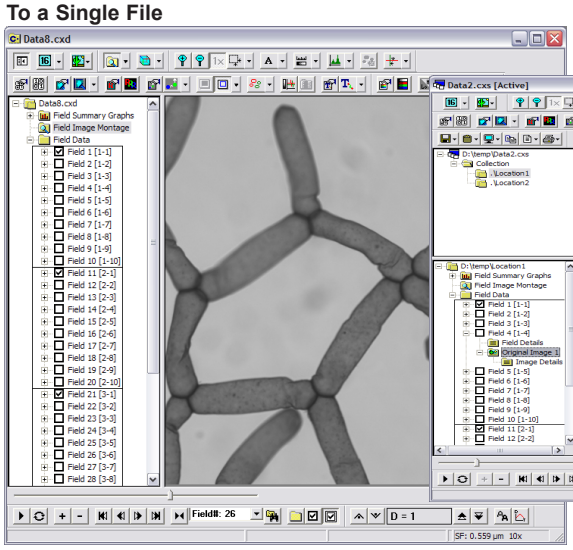

fig.7

One File per Position

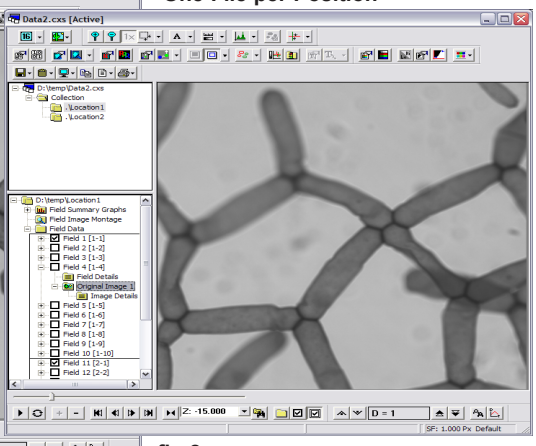

fig.8

Create Mosaic Scan

1. Make sure your stage is calibrated. Refer to page 4 of this guide. Click the **Camera** icon (**fig.1**), the Capture Menu appears (**fig.2**).

2. Select the camera and the number of colors to be captured from the top-right drop-down list (**fig.2**).

3. Click **Device Setup** (**fig.2**) to configure the capture device to the appropriate image depth, resolution and binning mode.

4. Select the filter(s) from the filter drop-down list (**fig.2**).

5. Click **Autoexposure** to let the software determine the best exposure(**fig.2**). **Note:** If you can't see an image make sure that the light path to the camera port is open, and the correct filter cubes are in place.

6. Load the correct calibration file. Click **Calibration** (**fig.2**). Open the correct calibration file for the objective and resolution you are using. Highlight the correct calibration and click **OK**.

7. Click on **New Scan** (**fig.2**).

8. Select **XY[Z] Scan** and **Pattern**. Click **Next** (**fig.3**).

9. Click **Drive Pattern** (**fig.4**), the **XYZ Stage Control Menu** appears (**fig.5**), and a live image appears in the image Display.

10. Drive the stage to the top-left corner of the image and click **Apply Add**. Now drive the stage to the bottom-right corner of the sample and click **Apply Add**. The software calculates all the steps between the two corners. Click **OK** (**fig.5**).

11. Select type of focus correction. Select **Surface Approximation** (**fig.4**). Check **Use Auto Focus** or **Focus Manually** and enter 2 x 2 for pattern size; you can enter larger pattern size for more accurate focus. Click **Focus/Start/OK**.

12. Assign a name and a file path to your new Data File. Click **Save**.

13. Click **Next /Finish/Start** (**fig.5**)

14. Create a montage from the sequence. Please refer to page 6 in the ***SimplePCI Quick Start Guide*** for more details on how to create a montage.

fig.1

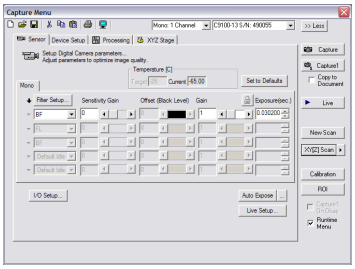

fig.2

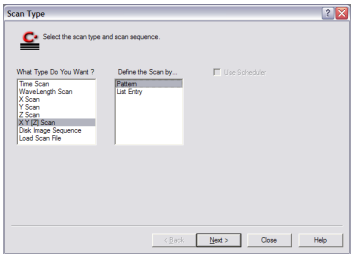

fig.3

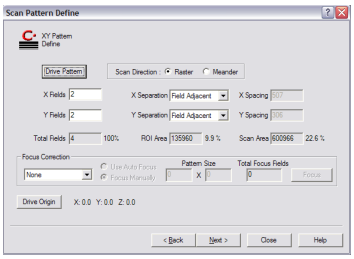

fig.4

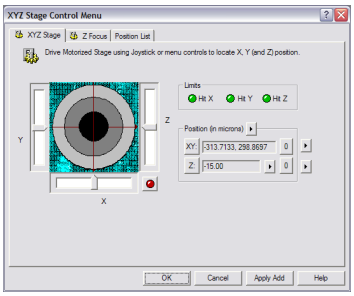

fig.5

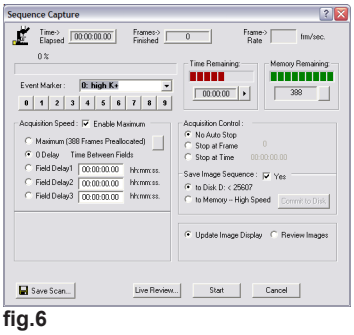

fig.6

Calibrate an Image from Pixels to Microns

1. Capture an image of a micrometer or reticle of a known distance.
2. Click **Calibration** in the Capture Menu, the Calibration window appears (**fig.1**).
3. Enter a Title for the calibration file, e.g., 10X. Select the Units and symbol from the Pull-down menu, and customize if required (**fig.1**).
4. Click **Calibrate** in the Calibration window (**fig.1**). Move the Calibration window to the side so you can see the image. Move the cursor to the start of a known distance; click and drag a line to span the distance you wish to measure, as shown in (**fig.2)**
5. Enter the known distance of the line you have drawn and click **OK** (**fig.3**). The Calibration Factor in the Spatial Calibration Menu will be updated.
6. Save the Calibration file. Click **Save** in the Calibration window and click **OK**.

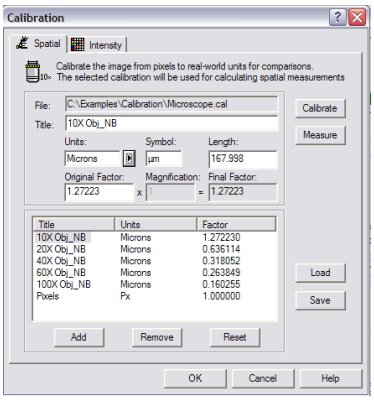

fig.1

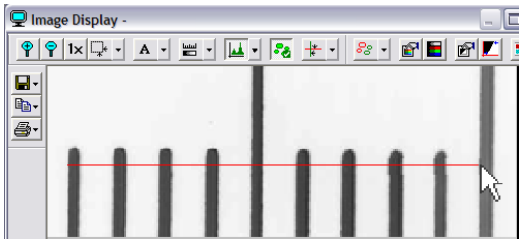

fig.2

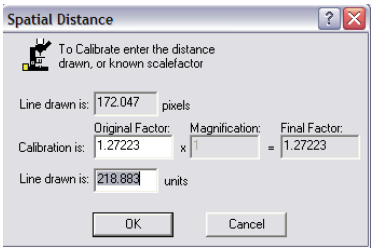

fig.3

Align the Optical Insights MulitSpec Micro-Image

1. Click the **Camera** icon, the capture menu appears (**fig.1**).
2. Select the camera and set it to monochrome from the top-right drop-down list (**fig.2**)
3. Set the **Micro-Imager** to **Split View**. Image a slide with a grid to ensure proper alignment between the camera and the microscope. Click **Autoexposure** to determine exposure automatically or enter the correct exposure manually. Click **Focus** and rotate the camera or the **Micro-Imager** until the camera is parallel to the microscope. Click **Stop** upon finishing.
4. Align the split image. Activate the **Processing** Tab and check **Split Subtraction/Addition** under Split Image Registration (**fig.2**).
5. Register the split image. Click **Live** to get a live image. Use the levels on the **Micro-Imager** to register the split image until it is correctly registered. Click **Stop** upon finishing.
6. Turn Processing Off. Check Off from the Processing Tab (**fig.1**).
7. Click **Sequence/Save data file/Time Scan/Continuous/Finish/Start**

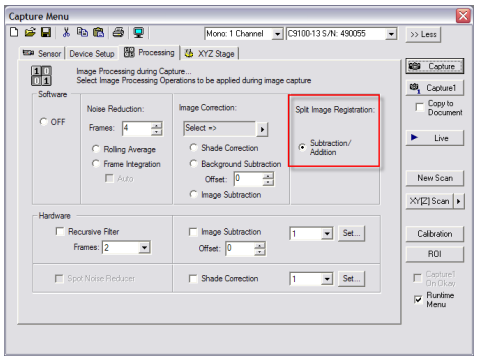

fig.1

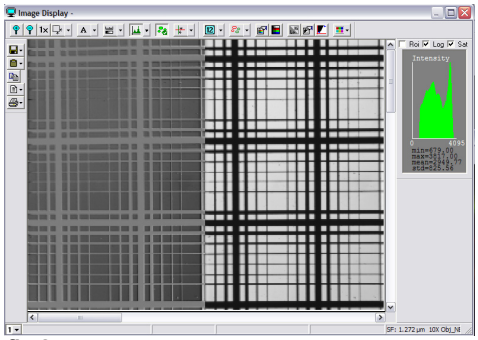

fig.2

Merging Dual View Images

1. Open Data File if it's not open.

2. Expand the data tree files and select **Original Image**. Right click on the image and select **Original Image** from the **Export Image Sequence** sub-menu (**fig.1**).

3. Select a file type from the drop-down list. Click **Browse**, assign a file name and click **Save**. Check **Convert 1-plane to 2 Color Planes** and **Horz/Vert** if image is split vertically (right-left), uncheck **Horz/Vert** if image is split horizontally (top-bottom). Click **OK** (**fig.2**).

4. A merged CXD file is created (**fig.3**).

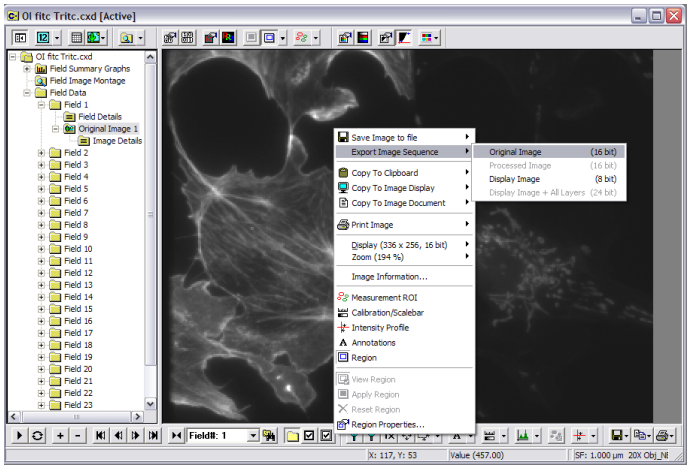

fig.1

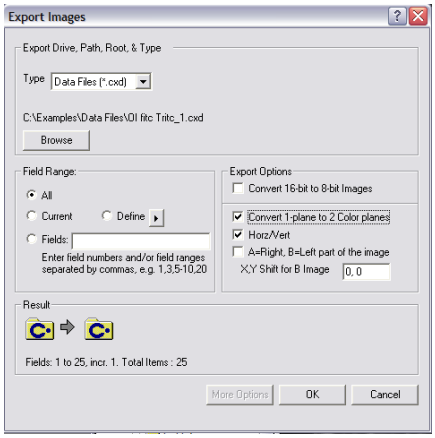

fig.2

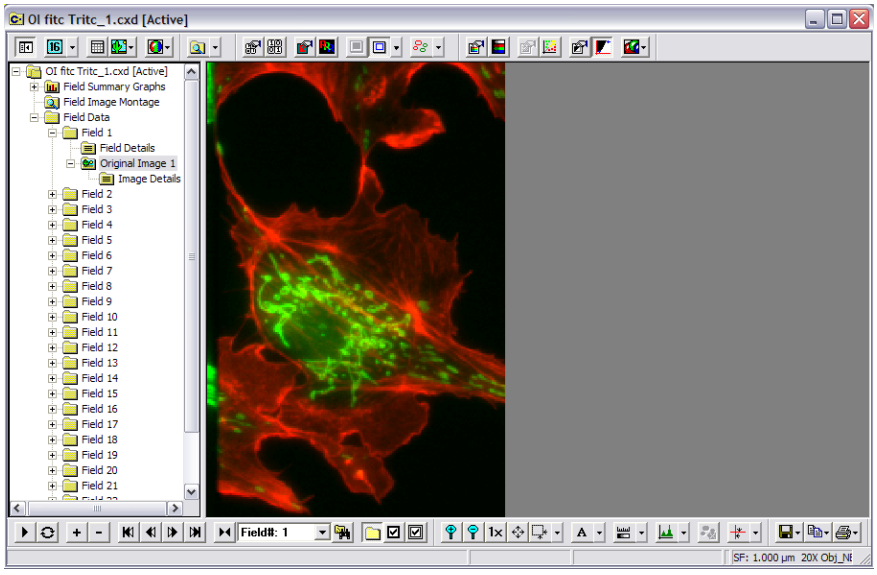

fig.3