

Introduction

Automated Image Capture-Experiment Planner (AIC-EP) module provides you the ability and flexibility to design multi-dimension experiments without having to write complex macros or journals. Design your experiment by simply dragging and organizing your device resources into a document. Automatically capture multi-color images using several motorized devices. Design complex multi-dimension image sequences with several wavelength positions over time (time-lapse). The data is then stored and organized in a data document which contains a Tree View detailing the contents of the file.

Expand the functionality of **AIC-EP** by adding the following optional modules:

- **DIA**, dynamically measures intensity changes on- or off-line
- **IPA**, develops icon-driven work files for automatic image analysis and processing
- **IPA-MTA**, track and analyze moving objects
- **VIS-MD**, provides rapid 3D visualizations of multi-dimensional data sets
- **QFA-FRET**, accurate FRET measurements and cross talk correction
- **DNN**, removes out-of-focus haze from optical images using fast algorithms

Getting Started

This **Quick Start Guide** contains examples of how to utilize **AIC-EP**. 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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Design a Nine Color Time-Lapse Acquisition

1. Click the **New** icon or press **Ctrl+N** /Select **Log Document** from the **New** window Click **OK (fig.1)**. The **Log Document** appears (**fig.2**).
2. Add the acquisition events (**fig.2**). Rename the **New Experiment** event folder to **Nine Color**. Right-click on the **Nine Color** event folder and select **Add Sub-event** from the drop-down menu. Click the **+** icon to expand the event folder. A sub-event folder named **Event_1** appears, rename this event folder **Loops**. Right-click on the **Loops** event folder and select **Add sub-events...** from the drop down menu. Enter the number of events to be added. For this example we need to add ten event folders, nine folders for each fluorochrome, and one for time delay. Enter 10 in the pop-up window and click **OK**. Note that you can add or delete additional folders. Click the **+** sign next to the **Loops** event folder to expand the folder and reveal the additional ten event folders. Rename the folders as shown in figure 3.

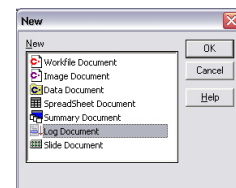


fig.1

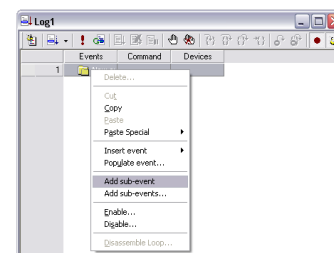


fig.2

3. Add how many times you want to loop through the events (**fig.3**). In the **Command** column, double click on the cell across the **Loops** event folder and enter the number of loops; for example 50. This is how many times the acquisition will be repeated.
4. Drag required device resources to the **Log Document (fig. 4 & 5)**. Go to the **View** menu and select **Device resources...** Drag **Time** from the Device resources window onto the **Device** column in the **Log Document**. When you see a **+**, release to add the device resource. Drag the **Acquisition** and **Storage** folders in the same manner.

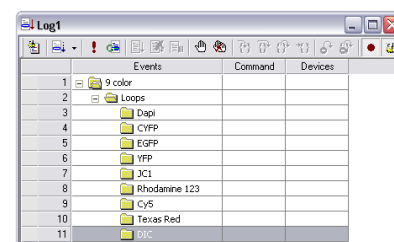


fig.3

It's important to organize the resources in the order they are going to be executed. In this example, we have a delay, then acquisition, and finally storage of the data.

5. Setup acquisition (**fig.6 & 7**). Under the **Setup** column, double click on the cell across each fluorochrome, a setup capture icon appears in the cells. Click the icon with the three dots (...), see figure 6. The capture window appears. Set the capture window (**fig.7**) to monochrome, and under filter setup select the filters for your fluorescence image. For example, for GFP select the GFP filter from the **Filter Setup** drop-down list. Set the correct exposure by clicking **Auto Exposure**. Click **OK**. Repeat the same step for the additional fluorochromes. Under the **Capture** column, double click on the cells across the camera setup icon, a camera icon appears (**fig.7**). This icon activates the camera.

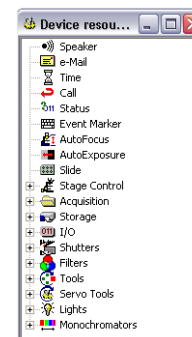


fig.4

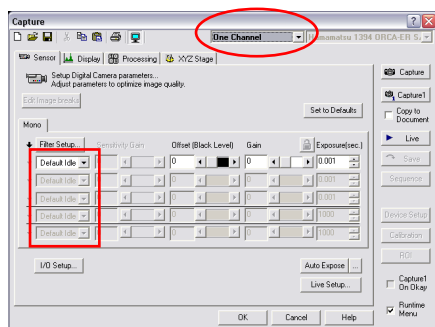


fig.7

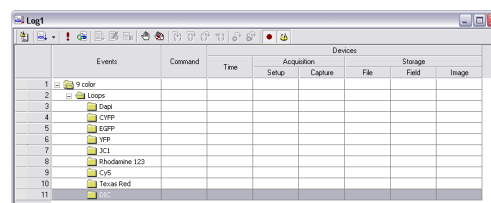


fig.5

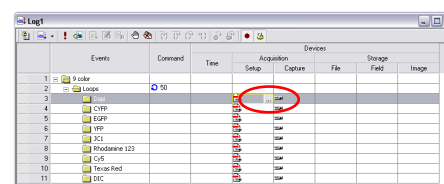


fig.6

6. Add time delay (**fig.8**). Under the **Time** column, double click on the cell across the delay folder and enter the time delay, for example **5m** (5 minutes). Note: Time starts counting down from the beginning of the first image. To start counting down from the last image, **Reset** the time then add a time delay. Right click on the time delay and select **Reset** from the drop-down menu. Then add a second Time column from the device resources window and set the time interval as we did before to 5m as shown in figure 9.

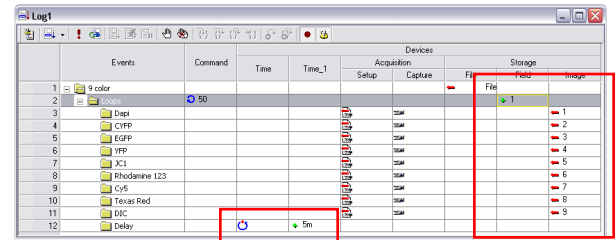


fig.8

7. Name data file (**fig.8**). Under the **File** column, double click on the cell across the first folder, **Nine Color**. Click on the three dots icon (...) and name the cxd file. Under the **Field** column double click on the first cell, a **+1** icon appears. Click on the **+1** and select **Set** and enter **0** (**fig.10**). In the **Field** column (**fig.9**) double click on the cell across the Fluorescence and DIC folder, a **+1** appears which indicates the field will be incremented by one frame. Right click on the **Collection** and **Image** column and select **Delete** since we are not using them in this example.

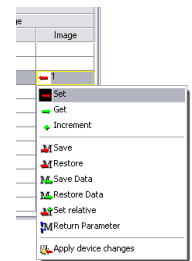


fig.9

8. Add Event icons to each event (**fig.11**). Press the action tag icon and click on the column to the left of each event as shown in figure 11 to mark the action.

Note: without the action tag icon the events are not executed. Also, It's a good idea to debug the acquisition before running it to make sure everything is working properly. Click on the first column to the left of the first folder (**Nine Color**), the breakpoint icon appears (brown circle) (**fig.11**). Press the **Running line** icon and press the **Run** icon. The program will be run in the debugging mode. Click on the **Step in** icon to test each of the events. When finished debugging, click the **Abort** icon and remove all the breakpoints by clicking the **Remove all breakpoints** icon.

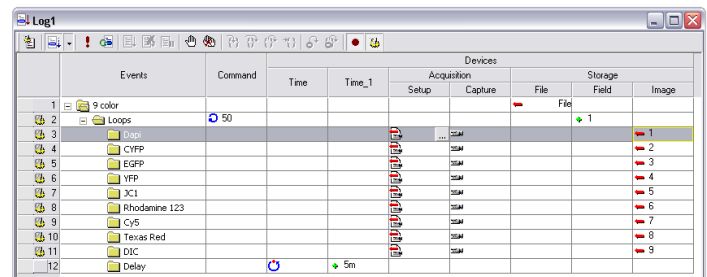


fig.10

9. Save the **Log Document** for future experiments. Go to the **File** menu and click **Save**, assign a name and click **Save**. Your final **Log Document** should look like the one in figure 12.

10. Run the acquisition. Press **Running line** icon to see the program executing the events. Press the **Run** icon to run the program.

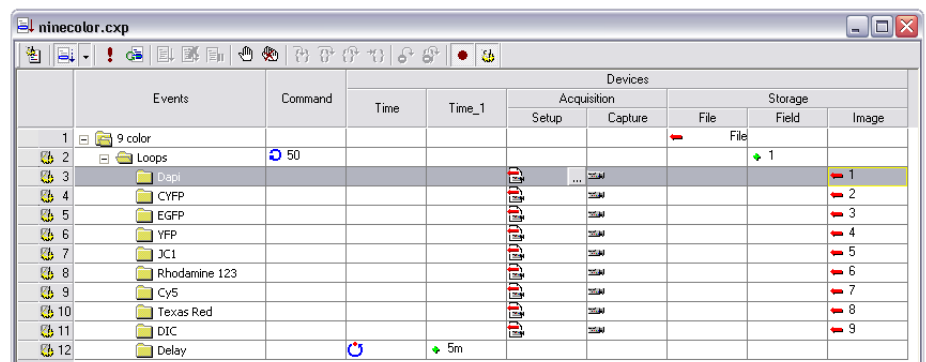


fig.11

Multi-Site Z scan Template Slide Example

1. Set up the Camera and automated devices for the specimen



fig.12

Use the **Capture** icon (**fig.12**) to select the number of channels for the image to be stored if using a single monochrome or multi-color image at each location. For additional images at each location, see the section at the end of the document.

Ensure all automated devices are operating normally. Select the matching calibration for the current objective in the **Calibration Dialog**. Adjust the Exposure/Gain and Filter/Shutter combinations for each image channel. If using Auto Exposure for fluorescence imaging, select the **Dark field** mode to reduce the number of exposures and avoid saturating small bright objects. Note for color sequential images the camera settings for the currently selected Tab are used in EP Feedback mode and Auto focus.

2. Open the Slide document

Use **File|Open** to select Slide documents and open the file “Multi-Site Z CS Template.sld”.

This Slide document contains a circular cover slip, 25mm in diameter with a 2mm border. The Slide image can be created or replaced using the **Slide|Create Slide** menu option.

The Slide document display shows a two-dimensional view of the slide on the microscope stage, a reticule of the microscope coordinates, and the locations of View Fields to be located when the slide is run (**fig.13**).

The Slide document contains additional log documents used for this application, specifically the **Internal Startup Event Log** and the **Default View Field Event Log**.

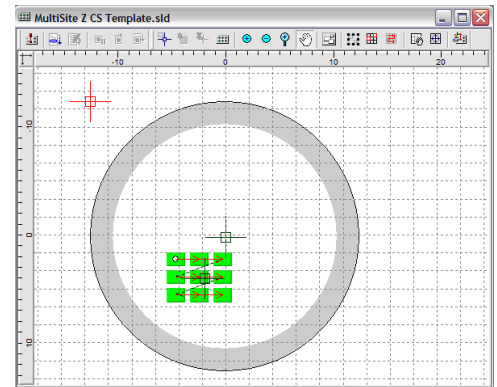


fig.13

The Startup Log contains the commands for timing and loop control, file creation and naming. It also contains the name of the slide to be processed.

The Default Event log is used to control the actions at each View Field location visited when running the slide.

To see three documents at once, do the following:

- 1) Select the Slide document and use the menu command **Slide|Default View Field Event Log|Open**.
- 2) Select the Slide document and use the menu command **Slide|Internal Startup Event Log|Open**.
- 3) Select the menu command **Window** and choose **Tile**. Adjust the column widths by dragging the right column border of any cell in the Log documents.
- 4) Use the **Show All** button (**fig.14**) in the Slide toolbar to fit the slide and all View Fields in the visible frame.



fig.14

3. Align the Slide view to the microscope stage

The virtual slide view on the screen can be synchronized with the stage positions so that the View Fields can be placed accurately.

To make sure the correct calibration information is used with the Slide:

1) Use the **Slide|View Field Properties** Menu item to view the current calibration.

2) Check the **Use Calibration Info** box to use the calibration info from the Calibration Dialog.

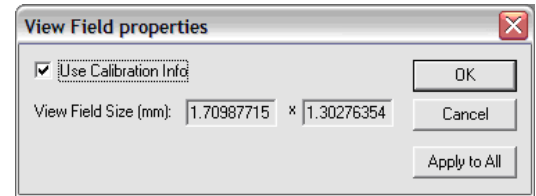


fig.15

Select the **Stage Marker** button (fig.16) in the Slide Toolbar. This activates the **Stage Marker** Feedback mode using the current Capture settings to provide a live image from the microscope. It also shows the current position of the stage displayed on the Slide (fig.17):



fig.16

*While in this mode new markers can be placed on the slide at the current stage position by pressing the **Create Marker** button (fig.18). When not in **Stage Marker** Feedback mode, new Markers can be created at any position in the slide by using the Right Click menu item **Create Marker**.

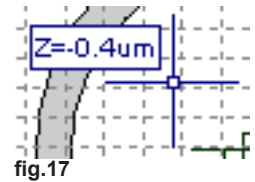


fig.17

In this Slide Document a dark green Marker has been created in the center of the cover slip (fig.19).

To align the stage to this marker, drive the stage to the center of the cover slip *while looking at the microscope*.



fig.18

When the stage is centered in the cover slip, press the **Adjust Slide** button (fig.20) on the Slide toolbar. Right-Click on the Marker corresponding to the current stage position, and select **Snap XY to Stage** and... **Set All Z**. This will synchronize the stage position with the Slide position, so that all movements of the stage are aligned with the Slide (fig.21).

Unpress the Adjust Slide button when done.

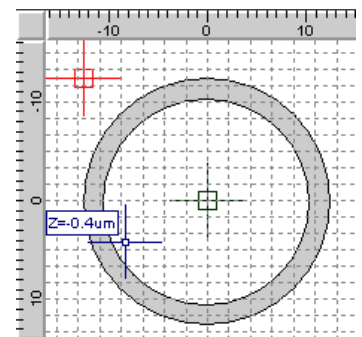


fig.19



fig.20

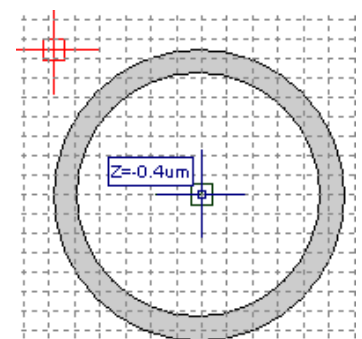


fig.21

4. *Create randomly placed View Field locations for the XY scan*

View Fields can be created anywhere in the slide at any time by using the Right Click menu item **Create View Field**.

To locate View Fields by scanning the slide for interesting specimens, activate the **Stage Marker** Feedback mode and drive the stage looking in the Image Display for areas of interest (**fig.22**).

To add a View Field simply click the **Create View Field XYZ** button (**fig.23**) in the Slide toolbar. This stores the XY location to be revisited later when the slide is run. The Z location is also stored, so take care to focus at the required position on each field.

View Fields can be removed by Right-Clicking on the View Field and using the menu item **Remove** (**fig.24**).

View Fields can be modified when the Edit View Fields button (**fig.25**) in the Slide toolbar is pressed. Left click to select a View Field. The Right-Click menu contains several options. In addition, the selected View Field can be dragged to a new position on the slide.

To see a live image at the same time in order to better align the View Field with the specimen, or to adjust the focus position, select the View Field and use the Right-Click menu option **Feedback**. This will initiate Feedback mode and drive the stage to the selected View Field position, displaying the live image in the Image Display. While in this mode the stage movements will move the View Field position and show these changes on the Slide.

Click off the **Edit View Fields** button when done.

Click off the **Stage Marker** Feedback button when done.

5. *Create a Path of travel between View Fields*

In order to visit the View Fields they must be connected with a Path. View Fields that do not have a path are shown in light green, connected View Fields are shown in bright green.

To create the desired path of travel between View Fields, press the **Edit Path** button (**fig.26**) in the Slide toolbar. Then left click on each View Field in turn to link the View Fields together. Click away from a View Field when finished. The path is shown as red connecting arrows (**fig.27**).

Click off the **Edit Path** button when done.

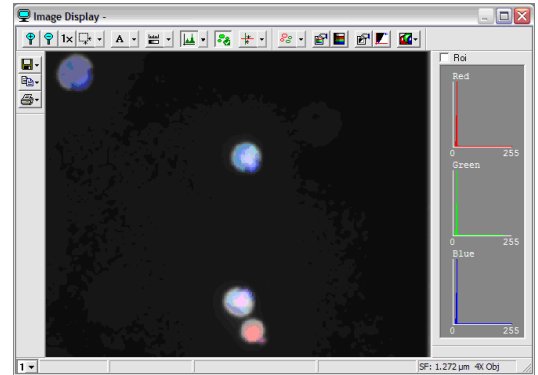


fig.22



fig.23

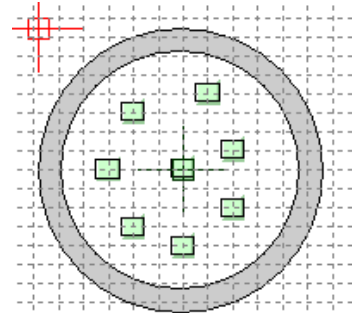


fig.24



fig.25



fig.26

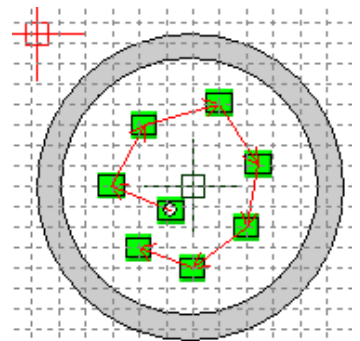


fig.27

6. Create a pattern of View Field locations for the XY scan

View Fields can be created in a rectangular pattern. Spacing of the View Fields is determined by the Grid displayed on the Slide.

The Grid location can be controlled when the **Move Grid** button (fig.28) is pressed in the Slide Toolbar. Locate the cursor near a grid intersection, click and drag the grid to the required position. Click off the **Move Grid** button when done.



fig.28

The Grid spacing can be controlled when the **Grid Spacing** button (fig.29) is pressed in the Slide Toolbar. Locate the cursor near a grid intersection and click and drag the grid to the required spacing. Click off the **Grid Spacing** button when done.



fig.29

For locating adjacent View Fields at a spacing equal to the size of the image, use the **Grid|Reset to View Field Size** Menu item. This will match the Grid spacing to the current View Field size using the currently selected calibration and the pixel size of the image. *Note: for high magnifications the grid lines can be very closely spaced. Control the view of the Slide display using the **Control** buttons (fig.30) in the Slide Toolbar (**Zoom In, Zoom Out, Zoom Tool, Pan** and **Show All**).



fig.30

Once the grid is sized and located, view fields can be created as a group using the **Group Selection** tool (fig.31) in the Slide Toolbar. In this mode, click and drag to create a rectangle enclosing the grid intersections for the View Fields required, this creates a Group Box (fig.32). Then right click in the Group Box and select **View Fields|Create** from the right click menu. This will create a View Field at each intersection (fig.33). To create a Path between the View Fields, right click and select the **Path|Create** menu item. This will use the **Auto Trace Properties** to determine the route of the path and display it as a series of red arrows connecting the View Fields (fig.34). Use the right click menu item **Path|AutoTrace** Properties dialog to change the route selection (fig.35).



fig.31

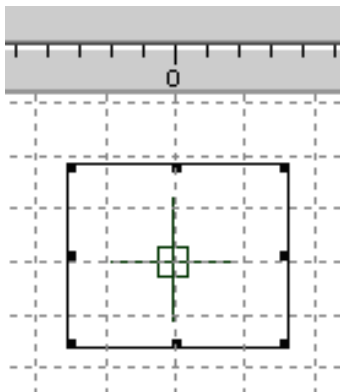


fig.32

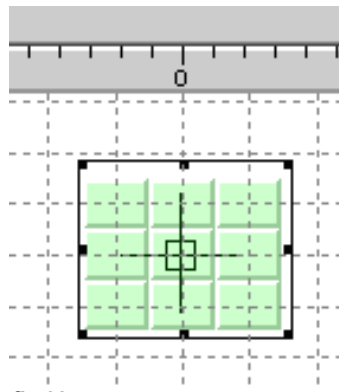


fig.33

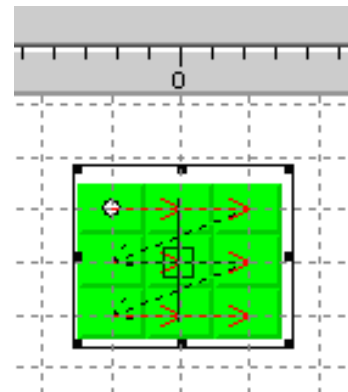


fig.34

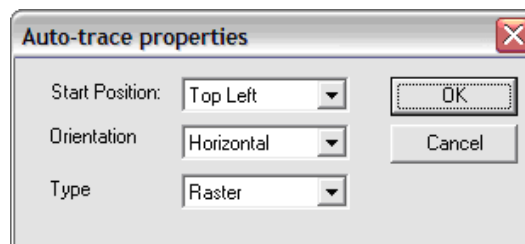


fig.35

To discard the current Group Box and draw a new one, first click outside the Group Box and then click and drag the new Group Box.

7. *Moving single View Field locations on the Slide*



fig.36

Individual View Fields can be moved when the **Edit View Fields** button (**fig.36**) in the Slide toolbar is pressed. Left click to select a View Field and drag it to the new position. The Right-Click menu contains several options. In addition, the selected View Field can be dragged to a new position on the slide.



fig.37

8. *Moving multiple View Field locations on the Slide*

View Fields can be selected as a group using the **Group Selection** tool (**fig.37**) in the Slide Toolbar to create a **Group Box**.

All View Fields within the Group Box can be moved retaining their relative positions by pressing the Ctrl key and dragging the Group Box to a the new position. The View Fields are moved when the mouse click is released. A Group of View Fields within a Group Box can be aligned with the grid intersections using the right click menu item **View Fields|Snap to Grid**. Click off the **Group Selection** button when done.

9. *Saving changes to the Slide Document*

After making changes to the Slide document you may want to save the new Slide document for re-use. To save the changes to the same file, use the **File|Save** menu item.

Select the Slide Document and use the **File|Save** menu item to update the Slide document on disk. You will be asked to save any changes to the Startup Log and the Default Event Log.

If you save to a different name, you must update the reference to the Slide document in the Internal Startup Event Log (see step 10, line 4, described below).

10. *Running the Slide Document*

To run the Slide document, it is suggested to do the following steps:

- 1) Align the Slide to the Stage.
- 2) Position the View Fields to be visited in XY and Z.
- 3) Open Internal Startup Event Log and Default View Field Event Log, and select **Window|Tile** to see all three documents together.
- 4) In the Startup Event Log, Customize the destination Summary Document and Data Document paths and filenames for the current specimen, and the number of Loop passes and Loop timing.
- 5) In the Default View Field Event Log, Customize the Z scan range as required (number of Z steps, step size, offset)
- 6) Use **File|Save** to Save changes to the Slide Document (**fig.38**).
- 7) Select **Running Line** in the Slide Document (**fig.39**) to trace the execution through the log documents by highlighting the active line.
- 8) Select **Run Slide** from Slide Document (**fig.40**).
- 9) Open Summary Document and review the Data Documents.



fig.38



fig.39



fig.40

11. Multi-Site Z scan Template Slide document - Internal Startup Event Log

The **Internal Startup Event Log** is the first Event Log that is called when the **Run Slide** is selected in the Slide Toolbar . The Startup Event Log typically contains settings to name the destination Summary Data Document files and control the timing and number of passes to process the View Fields contained in Slide Document. To view the Startup Event Log select the Slide document and use the menu command **Slide|Internal Startup Event Log|Open**. The Startup Event Log is stored internally to the Slide document for convenience.



fig.41

The **Internal Startup Event Log** contained in the example Multi-Site Z scan Template Slide Document

	Events	Command	Time	Slide	Devices			
					Collection	Storage		Image
						File	Field	
1	New experiment - Init Field parameter buffer						0	
2	Name files, Init Field number				C:\Pro...\MultiSite_ZD.cxs	\TargetSite_ZD.cxd	0	
3	Loop count and time Control, reset File name	3	20s			0	20	
4	Run slide (refer to parent slide filename)			template.sld				
5	Save last Field to parameter buffer							

fig.42

The columns represent the devices in use and the rows contain actions for each device.

Here is a brief description of each step:

- 1) In step 1, we initialize the value of the Field Parameter Buffer; also, used in line 5. This functions like the memory on a simple calculator and provides a way to recall a value for later use.

See below in the Default View Field Event Log for more details.

- 2) Initialize names for the Summary Document (.cxs) which contains a list of the Data Document (.cxd) files in this experiment for easy retrieval.

Before this log is run for a new specimen these names and file paths should be changed to suit.

Overwrite protection is active when an Event Logs is run and confirmation is requested before any existing files are replaced.

When each pass of the Slide is run the Field number is incremented at each location. On this line we also initialize the Field number to 0.

- 3) This line uses the **Loop Command** to repeat the Slide 3 times. The number of passes can be changed by selecting and editing the value.

A **Time Delay** is specified between each Loop. The Time delay is set as seconds and can also be changed. Valid values are formatted like this: 1h 20m 30s.

The **File Storage** number is initialized to 0. To do this, click in the Storage File cell and select the **Set** operation. The value will be changed to "File" to change this to a numerical value, right click and select **Type|Number** the value will become "1". Select the cell to edit and change the value, in this case to "0".

The Field Storage has a **Get** operation, which will update the Field number as the Slide is run to view the progress.

11. *Multi-Site Z scan Template Slide document - Internal Startup Event Log (continued)*

- 4) This line includes a **Process Slide** command, which references the Slide to be run each time the **Loop Command** is repeated.

This must refer to the correct Slide Document. The Startup Log file can refer to several Slide Documents, and so this reference must be explicit.

Note that if changes are made to a Slide Document and saved to a new file name, the reference must be updated in the Startup Event Log to refer to the new Slide Document. Click on the [...] button to change the name.

Before this Slide Document is run, check that the Slide Document name is correct.

- 5) Field Storage action - **Save** Field number to Parameter Buffer.

When the Slide document is run, each View Field is located on the microscope and a View Field Event Log is run (see below for details). The View Field Event log controls the actions at this location, and in our case includes operations to do a scan of the Z axis collecting and saving images at each Z location. These images are stored in the Data Document sequentially with automatically incrementing Field numbers. This requires that each time we move to the next View Field location in the Slide and prepare to save images to the file for that location, the Field number must be reset to the correct starting number.

This line includes just one action, where the last Field number used at the last XY location is used to generate the starting number for the next Pass of the Slide.

This operation is critical to maintain correct sequential numbering within each View Field. It also allows us to run the Slide for any number of Loop passes.

12. Multi-Site Z scan Template Slide document - Default View Field Event Log

The **Default View Field Event Log** is an Event Log that is called when each View Field is activated while processing the Slide. This log typically contains actions related to capturing and storing images, and in this case also includes actions to perform a Z scan. To view the Default Event Log, select the Slide document and use the menu command **Slide|Default View Field Event Log|Open**.

The **Default View Field Event Log** and other View Field Event Logs are stored internally to the Slide document for convenience.



fig.44

The **Default View Field Event Log** contained in the example MultiSite Z scan Template Slide Document looks like this:

	Events	Command	Stage Control	Devices						
				Z	Setup	Capture	Collection	File	Field	Image
1	Move stage half scan size from start pos		↕ -20um							
2	Get Z, Increment File for new ViewFi		↗ -60.35um					↕ 1	↕ 1	
3	Z Loop control	↻ 10								
4	Apply Brightfield Settings and		↗ -24.35um						↕ 1	↕ 1
5	Fluorescence, (Double click o		↔ 24um							↔ 2
6	Increment Z and apply physici		↕ 4um						↗ 740	
7	Show ending field/file							↗ 9	↗ 740	

fig.43

The columns represent the devices in use and the rows contain actions for each device.

Here is a brief description of each step through **fig.43**:

Line 1) **Increment Z Stage** device +20 um. This value is half of the total travel for the Z scan and allows the user to position XYZ of the View Field in the center of the object to be scanned.

*Note: this line has the Physical Action Tag (**fig.44**) on the left side. This causes the new settings to be applied to the hardware directly.

Line 2) The **Get** command for the Z device will retrieve the current Z position, and in this view displays the position after moving +20 microns from the XY(Z) View Field location. It represents the starting position for the Z scan, including an offset for half of the Z scan. The **Increment File +1** command increments the File number for each View Field.

Remember in step 3 of the Startup Event Log the File number was reset to 0 on each pass of the Slide? This increment starts the first View Field with the File number 1 for each pass. The File Storage device uses the root file name specified in the Startup Log and appends the File number as /path/rootname_#.cxd when data is written to the file. The Field Storage action Restore will read in the Field Device Parameter Buffer at the beginning of this log so that each file starts at the same number. This Parameter Buffer is updated after the Slide is completed in line 5 of the Startup Log so that the next pass of the Slide starts at the correct Field number.

*Note: this line does NOT have a Physical Action Tag, and so no data is written to the file at this time.

12. Multi-Site Z scan Template Slide document - Default View Field Event Log (continued)

Line 3) The **Loop** command controls the number of Z steps for the Z scan.

Line 4) The **Get** command for the Z device will retrieve the current Z position, and in this view displays the position of each Z step as the Z scan takes place.

The Acquisition Setup device would use settings saved in this cell, but this device is disabled. This causes the Capture Settings from the Capture Dialog to be used by the Acquisition Capture device for the image Capture. The Capture command activates the image acquisition and includes any camera related settings, Filter/Shutter selection and will cycle through the images as specified in the Capture Dialog. The Field Storage **Increment** command selects the next Field number and the Image Storage **Set** command forces the image to be stored in Image 1 in the Data Document.

This line includes the Physical Action Tag to acquire the image and write a Field and image details to the Data Document.

This line includes additional Acquisition Device operations to load Capture Settings, Capture an image and save Image 2 to the Data Document in the same Field. The Physical Action Tag (**fig.44**) causes the new settings to be applied to the hardware directly.

Line 5) This line has been disabled. To enable or disable an entire line, right click on the Event title and select Enable/Disable from the menu.

Line 6) The Z Device **Increment** command moves the stage a fixed distance to the next Z position in the Z scan.

The Field Storage **Get** command will display the current Field number as the Loop is run.

Line 7) The File Storage **Get** command (set to type Number) will display the File number, and the Field Storage Get command will display the Field number after the Loop command has completed 10 passes.

In this case, 740 Fields have been collected as the Slide has been repeatedly processed. The File number gets up to 9, as there are 9 XY locations.