

# BIOQUANT Life Science 2013, v.13.5.6

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The following release notes cover features in the release of BIOQUANT Life Science 2013 for Windows 7, v.13.5.60 software.

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## Combined Installation Program

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The BIOQUANT Life Science Installation program for the Windows 7 32-bit and Windows 7 64-bit versions have been combined. The installation program detects which Windows 7 version you have and installs the correct version of BIOQUANT Life Science.

Read the BIOQUANT Getting Started Handbook for directions on installing the BIOQUANT Life Science Program and the required BQ-V imaging board.

## BIOQUANT Plug-in Consolidation

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BIOQUANT Life Science's plug-ins have been consolidated and upgraded with more features.

### Topic Contents

- BIOQUANT Imaging Extensions add-on (page 3)
- BIOQUANT SCAN add-on (page 3)
- BIOQUANT Topograper add-on (page 4)
- BIOQUANT Life Science Core Software Change (page 4)

## BIOQUANT Imaging Extensions add-on

If you had the Imaging Toolkit plug-in, Densitometry Toolkit plug-in, and/or Stereology Toolkit plug-in, you have been upgraded to the BIOQUANT Imaging Extensions add-on. This add on contains:

- **Image Size Enhancements:** BIF and BMP images can be opened up to 4 GB in size

See “Image Size Enhancements” on page 28.

- **Imaging Toolkit box**

See “Imaging Toolkit Enhancements” on page 38.

- **MicroCT Navigator box**

See “Dicom Image Support / MicroCT Navigator” on page 29.

- **Densitometry Toolkit**

See “Densitometry Toolkit” on page 46.

- **Stereology Toolkit**

See “Stereology Toolkit” on page 39.

## BIOQUANT SCAN add-on

**Non-commercial License Users:** If you had the Automated Imaging Toolkit plug-in AND a motorized stage, you have been upgraded to BIOQUANT SCAN.

BIOQUANT SCAN performs automated imaging on fixed tissue sections on glass slides and fixed cells in well plates. It supports bright-field, wide-field fluorescence, darkfield, polarized, and phase contrast imaging. Scans are stored in the calibrated BIF image format for analysis and may be exported to the BMP format for publications.

BIOQUANT SCAN includes the BIOQUANT Imaging Extensions add-on.

BIOQUANT SCAN requires an ASI motorized stage. It can be used with a Ludl motorized stage, however the stitching quality will be degraded.

The Auto Sequential Imaging and Time Lapse Imaging boxes are no longer available. The functionality of Auto Sequential Imaging is found in BIOQUANT SCAN. Time Lapse Imaging can still be performed at one location using the Imaging Toolkit box.

## **BIOQUANT Topograper add-on**

The BIOQUANT Topograper add-on remains the same as in previous version. See “BIOQUANT Topograper Add-on Enhancement” on page 57.

## **BIOQUANT Life Science Core Software Change**

The BIOQUANT Topo Reader plug-in is no longer included with the BIOQUANT Life Science core software. The Topo Reader plug-in was used primarily to print and save just the 2D topography to a file. This can now be done in the Life Science core software by choosing Save Navigator Image plus Current Topo from the Save Single Image list in the Image Region.

## New Feature: Calculations Box

The Calculations box is used to dynamically display the results of Calculation arrays during data collection.

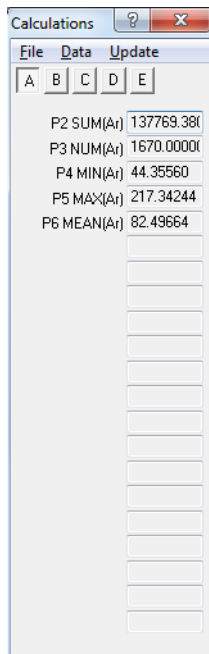
Although the Calculations dialog box will allow you to view the data of any array, it was designed for use with Parse My Comment arrays that are grouped to a Calculations (Object Count) array with AUTO; in its comment. Parse My Comment arrays grouped to the “Calculation” array in the BIOQUANT Life Science Templates are setup in this fashion.

Calculations can display up to 105 arrays (21 arrays on 5 pages).

All Life Science Template data sets have been updated to work with the Calculations box.

To see the Calculations box in action, you might walk through several of the tutorial guides in the Guides region. To see a list of tutorial guides, in the Guides region, click the Open button. A menu pops open that lists tutorial guides. Click on a guide to run it. Read the steps and follow the directions.

**Figure –Calculations**



## Raw Data Window Note

The Raw Data window no longer has an icon in the Selected List region. Raw Data can be opened in two ways:

- On the Calculations box, from the Data menu, choose Raw Data.
- On the main Life Science menu, from the Arrays menu, choose Raw Data.

## Life Science Template Changes

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The all Life Science Templates have been modified with calculation arrays compatible with the Calculations box. Each template has an corresponding Selected List and Calculations List. Many templates are also used in tutorial guides found in the Guides region.

Following is a list of the current Life Science Template data sets in the Life Science Template Volume. You may need to update your protocol if you are using one of these templates.

**Note:** The Manual Cell Count Template, Radius of Curvature Template, and Moments of Inertia Template have been removed from the Life Science Template Volume.

## Simple Templates for Initial Data Set Setup

- Calibration Template

Provides the arrays that are needed to test the accuracy of the current BIOQUANT calibration file.

Index	Name	Comment
D1	Topo	
I1	Distance Test	MEA6;ROI0;

- Initial Template

The Initial Template provides a single Area array, a Calculations array with the Total Structure Area grouped, and a Topo array.

D1	Topo	
D2	Calculations	AUTO;
P1	Tt.Ar (Total Structure Area)	SUM(A1)/1000000;mm2
A1	Structure Area	

- Basic Morphometry Template

The Basic Morphometry Template provides a Calculations array with Total Area, Total Labeled #, Labeled Density, Mean Labeled Size, Minimum Labeled Size, and Maximum Labeled Size grouped, a Structure Area array, a Labeled Area, and Topo array. It can be easily modified for many morphometry projects.

Index	Name	Comment
D1	Topo Structure	
D2	Topo Labeled Cells	
D3	Calculations	AUTO;
P1	Structure.Ar (Total Area)	SUM(A1)/1000000;mm2
P2	L.Number (Total Labeled #)	NUM(A2);#
P3	L.Density (Labeled Density)	P2/P1;#/mm2
P4	Mean L.Ar (Mean Labeled Size)	SUM(A2)/P2;micron2
P5	Min.L.Ar (Minimum LabeledSize)	MIN(A2);micron2
P6	Max.L.Ar (Maximum LabeledSize)	MAX(A2);micron2
A1	Structure Area	MEA0;COL11;TOPD1;
A2	Labeled Areas	MEA0;COL5;TOPD2;

- **Cell Counting Template**

The Cell Counting Template provides a Calculations array with Total Sampling Area, Total Cell #, Cellular Density, Mean Cell Size, Minimum Cell Size, and Maximum Cell Size grouped, a Sampling Area array, a Cell Area, and Topo array. It can be easily modified for many cell counting projects. Note: This template was previously called the Automatic Cell Count Template.

Index	Name	Comment
D1	Topo Structure	
D2	Topo Labeled Cells	
D3	Calculations	AUTO;
P1	Sampling.Ar (Sampling Area)	SUM(A1)/1000000;mm2
P2	Ce.Number (Total Cell Number)	NUM(A2);#
P3	Ce.Dn (Cellular Density)	P2/P1;#/mm2
P4	Mean Ce.Ar (Mean Cell Size)	SUM(A2)/P2;micron2
P5	Min.Ce.Ar (Minimum Cell Size)	MIN(A2);micron2
P6	Max.Ce.Ar (Maximum Cell Size)	MAX(A2);micron2
A1	Sampling Area	MEA0;COL11;TOPD1;
A2	Cell Area	MEA0;COL5;TOPD2;

- **Density, Avg Brightfield Template**

The Density, Avg Brightfield Template provides the arrays for initial data set setup for work with Average Density on bright field. Note: This template was previously called the Average Density Template.

Index	Name	Comment
D1	Topo	
D2	Calculations	AUTO;
P1	Mean OD	SUM(D3)/NUM(D3);grayscale
P2	Min OD	MIN(D3);grayscale
P3	Max OD	MAX(D3);grayscale
D3	Average Optical Density	DG;



- **Density, Avg Fluorescence Template**

The Density, Avg Fluorescence Template provides the arrays for initial data set setup for work with Average Density using fluorescence.

Index	Name	Comment
D1	Topo	
D2	Calculations	AUTO;
P1	Mean R Int.	SUM(D3)/NUM(D3);Grayscale
P2	Mean G Int.	SUM(D4)/NUM(D4);Grayscale
P3	Mean B Int.	SUM(D5)/NUM(D5);Grayscale
D3	Red Fluorescent Intensity	DR;
D4	Green Fluorescent Intensity	DG;
D5	Blue Fluorescent Intensity	DB;

- **Density, Integrated Optical Template**

The IO Density Template provides the arrays for initial data set setup for work with Integrated Optical Density. Note: This template was previously called the IO Density Template.

Index	Name	Comment
D1	Topo	
D2	Calculations	AUTO;
P1	Tt. IOD	SUM(D3);IOD
P2	IOD Count	NUM(D3);#
P3	Mean IOD	P1/P2;IOD
P4	Min.IOD	MIN(D3);IOD
P5	Max.IOD	MAX(D3);IOD
D3	Integrated Optical Density	IOD;DG;

- **Histogram Template (See “Histograms” on page 394.)**

The Histogram Template is designed to hold data when the Save Histogram Data feature is used on the Histogram Window. It provides three general purpose (Attribute) arrays that, in this case, are used to record the intensity value (density) of every pixel that contributes to a intensity histogram.

Index	Name
D1	Histo Data (Attribute array)
D2	Histo Data (Attribute array)
D3	Histo Data (Attribute array)

- **Auto Width Simple Template**

The Auto Width Simple Template provides the arrays for initial data set setup for work with the Auto Width Measurement Type.

Index	Name	Comment
D1	Topo	
D2	Calculations	AUTO;
P2	Avg Width	SUM(I1)/NUM(I1);
I1	Widths	MEA4;

- **Area % Wand Simple Template**

The Area % Wand Simple Template provides the arrays for initial data set setup for work with the Area % Wand Measurement Type.

Index	Name	Comment
D1	Topo	
D2	Calculations	AUTO;
P2	Tt.Ar	SUM(D3);microns2
P3	Tt.Th.Ar	SUM(D4);microns2
P4	Tt.%Th	P3/P2;%
D2	Wand Area	COL5;MEA3;
D3	Thresholded Area	
P1	% Thresholded	D4/D3;%

- **SegAssign Simple Template**

The Segment Assign Simple Template provides the arrays for initial data set setup for work with the Segment Assign Measurement Type.

See Guide: Measure Segment Assign - Cerebellum

Index	Name	Comment
D1	Topo	
D2	Calculations	AUTO;
P1	Tt.Ln.1	SUM(L1);microns
P2	Tt.Ln.2	SUM(L2);microns
P3	Tt.Ln.3	SUM(L3);microns
P4	Tt.Ln.4	SUM(L4);microns
P5	Tt.Ln	P1+P2+P3+P4;microns
A1	Contour Area	SEG=L1,L2,L3,L4;COL1;MEA7;
L1	Segment Type 1	COL3;
L2	Segment Type 2	COL4;
L3	Segment Type 3	COL5;
L4	Segment Type 4	COL8;

- Dataset of Topo Arrays

The Dataset of Topo Arrays Template is used by the Data Manager's Create Dataset of Topo Arrays feature to create a data set with only Topo arrays.

Index	Name
D1	Topo

## Procedural Data Set Templates

Many of these data set templates have Guides that walk you through a protocol. To see a list of guides, in the Guides region, click the Open button and choose a guide from the list. The guides will walk you through opening a Sample Dataset, loading a Sample Image, and through a Sample Protocol for the procedure.

- Auto Width Cortex Layers Template

The Auto Width Cortex Layers Template is used to generate width measurements between layers of the cortex.

Guide: Measure Auto Width - Cortical Layers

Protocol in Life Science Manual: Auto Width between Consecutive Layers

Index	Name	
D1	Topo	
D2	Calculations	AUTO;
P1	Lay 1 Avg.Wi	SUM(I1)/NUM(I1);microns
P2	Lay 2 Avg.Wi	SUM(I2)/NUM(I2);microns
P3	Lay 3 Avg.Wi	SUM(I3)/NUM(I3);microns
P4	Lay 4 Avg.Wi	SUM(I4)/NUM(I4);microns
P5	Lay 5 Avg.Wi	SUM(I5)/NUM(I5);microns
P6	Lay 6a Avg.Wi	SUM(I6)/NUM(I6);microns
P7	Lay 6b Avg.Wi	SUM(I7)/NUM(I7);microns
I1	Cortex Layer 1 Width	COL10;
I2	Cortex Layer 2 Width	COL9;
I3	Cortex Layer 3 Width	COL8;
I4	Cortex Layer 4 Width	COL5;
I5	Cortex Layer 5 Width	COL4;
I6	Cortex Layer 6a Width	COL3;
I7	Cortex Layer 6b Width	COL1;MEA4;

- mRNA Grain Analysis Template

The mRNA Grain Analysis Template is designed to work with the mRNA Grain Analysis procedure, using the Area % Wand Measurement Type.

Guide: Measure Area % Wand -Grain Analysis

### Protocol in Life Science Manual: mRNA Grain Analysis Specific Procedure

Index	Name	Comment
D1	Topo	
D2	Topo	
D3	Topo	
D4	Calculations	AUTO;
P1	Est. Gr.Ar	SUM(D6)/NUM(D6);microns2
P2	Est. Bg.Gr.#	SUM(D8)/FIRST(P1);#
P3	Bg.Sa.Ar	SUM(D7);microns2
P4	Est. Bg.Gr.Dn	P2/P3;#/microns2
P5	Min.Ce.L	MIN(P10);%
P6	Max.Ce.L	MAX(P10);%
P7	Mean Ce.L	SUM(P10)/NUM(P10);%
D5	Sample Single Grain Area	TOPD1;COL3;MEA3;
D6	Single Grain Area	
D7	Background Grain Sample Area	TOPD2;COL12;MEA3;
D8	Background Grain Area	
D9	Cell Area	TOPD3;COL5;MEA3;
D10	Cellular Grain Area	
P8	Est. Cellular Grain Number	D10/FIRST(P1);#
P9	Est. Cellular Grain Density	P8/D9;#/microns2
P10	Est. Cellular Grain Labeling	P9/FIRST(P4);%

- **Muscle MyHC Isoform Analysis**

The Muscle MyHC Isoform Analysis Template reflects current protocols.

**Guide: Application - Muscle Isoform Analysis**

Index	Name	Comment
D1	Topo	
D3	Calculations	AUTO;
P8	Mean Myocyte Area	SUM(A2)/NUM(A2);
P9	Mean Myocyte Diameter	SUM(P1)/NUM(P1);
P10	Satellite Cell Count	NUM(A3);
P11	Neuromuscular Junction Count	NUM(A4);
A1	Whole Muscle Area	COL1;CHA7;MEA2;
A2	Myocyte Area	COL3;CHA7;MEA0;
P1	Myocyte Diameter	2*SQRT(A2/3.141);
P2	Myocyte X-Center	
P3	Myocyte Y-Center	
D2	Isoform Mean Optic Density	DG(255,0);
A3	Satellite Cell Area	COL4;CHA0;MEA0;
P4	Satellite Cell X-Center	
P5	Satellite Cell Y-Center	
A4	Neuromuscular Junction Area	COL5;CHA0;MEA0;
P6	Neuromuscular J X-Center	
P7	Neuromuscular J Y-Center	

- **Stereology Template**

This template is used only with systems that have the BIOQUANT Imaging Extensions add-on.

Note: Stereology Optical Fractionator Project users do not use Create Date Set to create Data Volumes and Data Sets. Instead they use the Optical Fractionator Project Manager.

Protocol in Life Science Manual: Imaging Extensions: Stereology Toolkit

Index	Name	Comment
D1	Sampling Area Topo	DFH9999;DFL5;SMO2;
D2	Fractionator Grid Topo	
D3	Director Location Topo	
D4	Count Per Disector	
D5	Estimated Cell Volume	
I1	Tissue Thickness	
A1	Sampling Area	

- **Lung Stereology Template**

This template is used only with systems that have the BIOQUANT Imaging Extensions add-on.

This template uses the Sine-weighted Lines Probe to simultaneously estimate volume fractions of air space and tissue as well as alveolar surface in lung tissue.

Protocol in Life Science Manual: Sine-weighted Lines Probe

Index	Name	Comment
D1	Sampling Area Map	
D2	Grid Map	
D3	Disector Location Map	
D4	Number of Points in Frame	
D5	Tissue Hits	COL8;
D6	Air Space Hits	COL9;
D7	Septum / Duct Intercepts	COL10;
D8	End Point Count	COL11;
D9	Triple Point Count	COL12;
D10	Calculations	AUTO;
P1	Tt.Points	SUM(D4);#
P2	Tt.Tissue Hits	SUM(D5);#
P3	Tt. Air Hits	SUM(D6);#
P4	% Tissue	P2/P1;%
P5	% Air Space	P3/P1;
A1	Sampling Area	COL1;

- **Liver Fibrosis Template**

This template is used to generate % Fibrosis Area.

Index	Name	Comment
D1	Topo	
D2	Calculations	AUTO;
P2	Tt T.Ar	SUM(D3);microns2
P3	Tt.Fb.Ar	SUM(D4);microns2
P4	Tt.Fb.%	P3/P2;%
D3	Tissue Area	COL12;MEA1;
D4	Fibrosis Area	
P1	% Fibrosis	D4/D3;%

- **Stent Analysis Template**

This template provides arrays and array comments to measure stented blood vessels.

#### Guide: Application - Blood Vessel Analysis

Index	Name	Comment
D1	Vessel Map	
D2	Calculations	AUTO;
P1	St.Lm.Di	SUM(I1)/NUM(I1);microns
P2	EEL.Ar	SUM(A1)/1000000;mm2
P3	IEL.Ar	SUM(A2)/1000000;mm2
P4	Lm.Ar Lumen Area	SUM(A3)/1000000;mm2
P5	Md.Ar Medial Area	P2-P3;mm2
P6	It.Ar Intimal Area	P3-P4;mm2
P7	% Stenosis	P6/P3;%
P8	Tt.St.Ar Total Stent Area	SUM(A4)/1000000;mm2
P9	Tt.Sc.Ar Total Scar Area	SUM(A5)/1000000;mm2
A1	External Elastic Lamina (EEL)	COL1;MEA0;CHA0;
A2	Internal Elastic Lamina (IEL)	COL3;
A3	Lumen Area (Lm.Ar)	COL4;CHA7;
A4	Stent Area (St.Ar)	COL5;SMO2;CHA7;
A5	Scar Area (Sc.Ar)	COL9;CHA7;
I1	Strut-Lumen Distance St.Lm.Di	COL6;MEA6;

- **MRI Brain Template**

This template is used only with systems that have the BIOQUANT Imaging Extensions add-on.

This template provides a starting point for data analysis of MRIs of the brain. For this template, you would use Modify Data Set to enter the step size into the comment of P1 Step Size.

#### Guide: Measure DICOM - Brain MRI

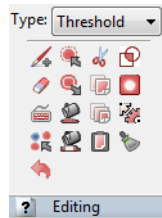
Index	Name	Comment
D1	Vessel Map	
D2	Calculations	AUTO;
P1	Step Size	Replace_Text_With_Step_Size;um
P2	FL.V Frontal Volume	SUM(A1)*P1/1000000000;mm3
P3	PL.V Parietal Volume	SUM(A2)*P1/1000000000;mm3
P4	OL.V Occipital Volume	SUM(A3)*P1/1000000000;mm3
P5	TL.V Temporal Volume	SUM(A4)*P1/1000000000;mm3
P6	CrblV Cerebellum Volume	SUM(A5)*P1/1000000000;mm3
A1	Frontal Lobe Area	COL1;SMO10;MEA0;
A2	Parietal Lobe Area	COL2;SMO10;
A3	Occipital Lobe Area	COL3;SMO10;
A4	Temporal Lobe Area	COL4;SMO10;
A5	Cerebellum Area	COL5;SMO10;

## New Feature: Threshold Editing: Clean

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There is a new Threshold Editing tool named  Clean.

**Figure –Editing Region: Threshold Type**



The Clean button is the bottom button in the 4th column.


Clean deletes all thresholded objects less than a user-specified minimum size. This makes it easier to remove non-specific thresholding.


## Topic Contents

- Quick Procedure (page 17)
- Adjusting the Clean Tool (page 17)



## Quick Procedure

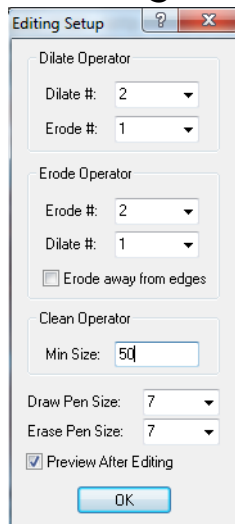
1. Threshold the image.
2. Click the  Clean button.

When the user clicks the  Clean button, the system deletes small thresholded objects.

## Adjusting the Clean Tool

To adjust the size of the thresholded objects that are cleaned:


**Figure –Editing Setup box**




Adjust the Clean Operator Min Size.

1. In the Editing region (Threshold Type), click the Threshold Edit Setup button.
2. On the Editing Setup box, increase or decrease the Clean Operator Min Size.

This value is in pixels and is remembered across BIOQUANT sessions.

3. Click OK to close the Editing Setup box.
4. In the Threshold region, click Refresh to restore the original threshold.
5. In the Editing region (Threshold Type), click the  Clean button again to test the new settings.

**Note:** Once the Clean Operator has been adjusted for your sample, you can assign  Clean to a Quick Batch Script, along with other threshold editing features. The Quick Batch Script can be run before clicking Preview in the Measurement region (General Type). See “Batch Measurement Enhancements” on page 18.

## Batch Measurement Enhancements

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Batch Measurement has several enhancements in this version.

### Topic Contents

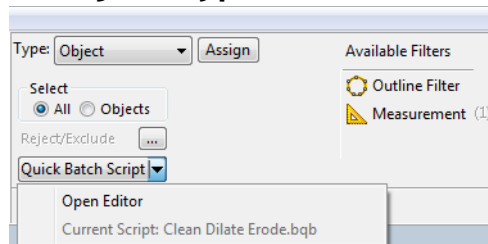
- Measurement Region: Batch Script Enhancements (page 19)
- Batch Measurement Editor Enhancements (page 20)

## Measurement Region: Batch Script Enhancements

The Measurement region, Object Type, has the following Batch Measurement enhancements.

- A new Quick Batch Script button/arrow combo has been added to the Object Type.

**Figure –Object Type: Quick Batch Script**



Click the arrow to open a list.

Click the button to run the Quick Batch Script.

- If you click the Quick Batch Script button directly, BIOQUANT immediately runs the batch script assigned as the Quick Batch Script.
- If you click the Quick Batch Script arrow, a drop list opens with the “Open Editor” option. If you choose the Open Editor open, the Batch Measurement Editor box opens.
- The current batch script assigned as the Quick Batch Script is listed next to “Current Batch Script” on the drop list.

To see this menu item, click the Quick Batch Script arrow to open a drop list.

## Batch Measurement Editor Enhancements

The Batch Measurement Editor box has the following enhancements.

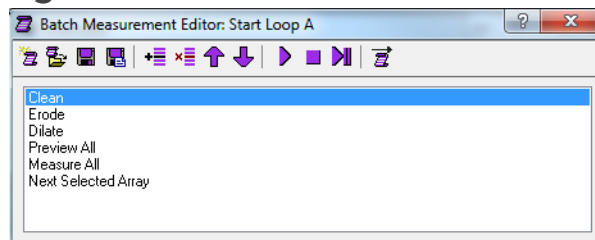
### Topic Contents

- New Batch Script Steps (page 20)
- Assign to Quick Batch Script (page 21)

### New Batch Script Steps

The following steps can now be added to a Batch Script:

**Figure –Batch Measurement Editor**



- Editing: Threshold: Clean
- Editing: Threshold: Keep Objects
- Editing: Threshold: Draw Threshold
- Editing: Threshold: Erase Threshold
- Navigation: Load Next Dicom Image

Available with the BIOQUANT Imaging Extensions add-on.

## Assign to Quick Batch Script

The current Batch Script can be assigned as the Quick Batch Script that is associated with the Quick Batch Script button in the Measurement region (General Type).

### Procedure Snippet

- a. On the Batch Measurement Editor box, open the Batch Script that is to be assigned to the Quick Batch Script button.

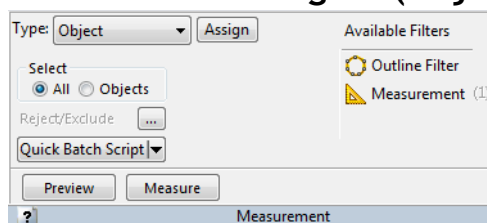
NOTE: If you have just created a new batch script, save it to a Batch Script file before assigning it.

- b. On the Batch Measurement Editor, click the  Assign to Quick Batch Script button.

The Batch Measurement Editor box title bar now has the Batch Script name with “>>> Quick Batch” appended.

- c. Now in the Measurement region (Object Type), click the Quick Batch Script button to run the batch script.

### Figure –Measurement region (Object Type)



Click the Quick Batch Script button to run the script.

## QImaging Enhancements

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The QImaging Camera has several enhancements.

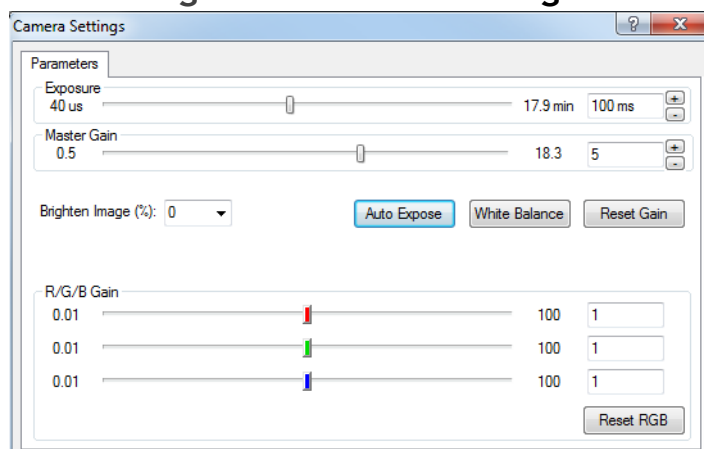
### Topic Contents

- Camera Settings: R/G/B Gain (page 22)
- QImaging EXi Aqua Camera Support (page 23)

### Camera Settings: R/G/B Gain

Using the new R/G/B Gain controls, the brightness of different fluorescent labels can be adjusted in the image. This makes measuring interlabel width easier with multiple fluorochromes like calcein and alizarin red labeling since red labels often appear dimmer than green labels.

**Figure –Camera Settings**



**To optimize an image of multi-channel fluorescence, we suggest:**

1. Turn the output of your excitation source to maximum.

2. Set the exposure time of the camera to 100ms (milliseconds).
3. Set the master gain to 5.
4. Set the individual color gains, red, green, and blue, to 1. Generally speaking labels which emit green light are very bright and it's not necessary to raise the green gain above 1.
5. If the green labels are too strong, turn down the strength of the excitation source. Many fluorescent lamps have a dial to attenuate the strength of the light and help preserve the sample.
6. Raise the gain levels for red and blue to balance those labels with the green labels.
7. Close the Camera Settings box and proceed as before.

## QImaging EXi Aqua Camera Support

BIOQUANT now has QImaging EXi Aqua camera support. The EXi Aqua provide regulated cooling to 0 degrees Celsius and a 14-bit dynamic range.

## Image Region / Image Menu Enhancements

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The following enhancements have been made to items on the Image region and Image menu.

### Topic Contents

- Image region (Image File Type): Open Single / Open Sequence Changes (page 24)
- Image region (Image File Type): Save Image Changes (page 25)
- Image region (Live Image Type): Background Enhancements (page 25)
- Image menu: Background Box Enhancements (page 26)

### Image region (Image File Type): Open Single / Open Sequence Changes

- PNG files are now supported and have been added to the File Type list.
- Imaging Extensions add-on: The DCM format is now in the File Type list.

DCM is the Dicom image format.

To load a single DCM image, use Open Single.

To load a series of DCM images, use Open Sequence.

If you are loaded a series of DCM images, the image sizes must be 1280x960 or smaller to load into the MicroCT Navigator.

Otherwise, the first image in the series will load into the Large Image Navigator.



- The TGA image format is no longer supported and has been removed from the File Type list.

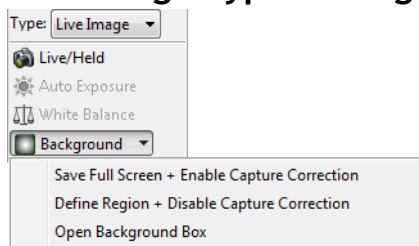
## Image region (Image File Type): Save Image Changes

- The TGA image format is no longer supported and has been removed from the File Type list.

## Image region (Live Image Type): Background Enhancements

The Background feature on the Image region (Live Image Type) has been enhanced. The Background Correct button has been renamed Background and is now a drop list that contains the following:

**Figure –Live Image Type: Background list**



Click Background to open a list.

- **Save Full Field + Enable Capture Correction**

Saves the current Image window as a background reference image. Also enables capture correction. Afterward, whenever Live/Held is clicked, the image will be corrected using the Background reference image before it is placed in the Image window.

This feature is used to correct the captured image for uneven lighting. It is also required for accurate Average Density measurements.

- **Define Region + Disable Capture Correction**

Puts the cursor into the Image window so that the user can define a background sample region. Also disables capture correction.

The feature is used to save a background reference image when working with densitometry on images from another source.

This feature is required for Integrated Optical Density.

- **Background Box**

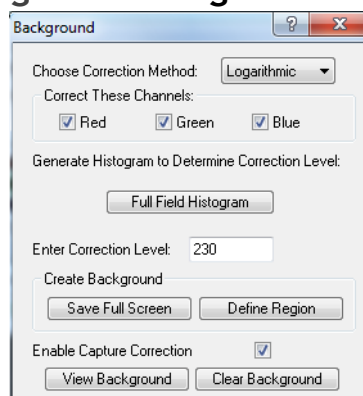
Opens the Background box. The Background box contains the settings for the Save Full Field and Define Region Background features.

## Image menu: Background Box Enhancements

The Background box can be opened by choosing Background from the Image menu. Alternatively, it can be opened by going to the Image region (Live Image Type) and choosing Open Background Box from the Background list.

The Background box has the following enhancements.

**Figure –Background box**



- Several items have been renamed.
  - “Choose Correction Source” has been renamed “Create Background.”
  - “Save Full Field” has been renamed “Save Full Screen.”
  - “Build Partial Field” has been renamed “Define Region.”
  - “Background Correct Image?” has been renamed “Enable Capture Correction.”
- The Correction Level has been defaulted to 230 and is now remembered across BIOQUANT sessions if changed.

## General Enhancements and Issue Resolutions

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The following general enhancements and issue resolutions to the BIOQUANT Life Science core software have been made.

- Number of Data Sets Limit: An error message has been added if the user tries to add more than 1000 data sets to a data volume.
- General interface improvements have been made, specifically dialog box sizes have been standardized.
- The Overview window now updates dynamically when the Segment Assignment feature is used.

## BIOQUANT Imaging Extensions Add-on Enhancements

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The BIOQUANT Imaging Extension add-on contains image size enhancements, the Imaging Toolkit, MicroCT Navigator, Densitometry Toolkit, and Stereology Tools.

### Topic Contents

- Image Size Enhancements (page 28)
- Dicom Image Support / MicroCT Navigator (page 29)
- Imaging Toolkit Enhancements (page 38)
- Stereology Toolkit (page 39)
- Densitometry Toolkit (page 46)

### Image Size Enhancements

The BIOQUANT Imaging Extensions add-on has special algorithms that allow the user to open BIF or BMP images up to 4 GB in size.

In BIOQUANT Life Science, BMP, TIF, JPG, and PNG images can be opened up to around 1 GB, depending on system resources. BIF images up to 4 GB can be opened.

## Dicom Image Support / MicroCT Navigator

The BIOQUANT Imaging Extensions add-on now supports the dicom image format. Single images, such as X-rays, can be opened into the Large Image Navigator. Series of images, such as a MicroCT image set, can be loaded into the MicroCT Navigator.

- New MicroCT support files:
  - A new MicroCT Template has been added to the Life Science Template Volume.
  - Sample Images can be found in Sample Images\DICOM 2-D X-ray and in Sample Images\DICOM microCT.
  - A guide that walks you through a simple MicroCT protocol can be found in the Guides region by clicking the Open button and choosing Measure - MicroCT.
- Dicom single images, such as X-rays, are opened using Open Single in the Image region (Image File Type).

NOTE: If the Dicom image is larger than 1280x960, it will open in the Large image Navigator.

- Dicom series of images, such as an image set from a MicroCT, are opened using Open Sequence in the Image region (Image File Type).

To open the Dicom image set, in Open Image Sequence, click the first file name in the set, then click Open.

The Dicom series of images is loaded into the MicroCT Navigator.

NOTE: The MicroCT Navigator can current load series of images with up to 1280x960 resolution. If you try to load an image series with resolution larger than 1280x960, the first image will open in the Large Image Navigator.

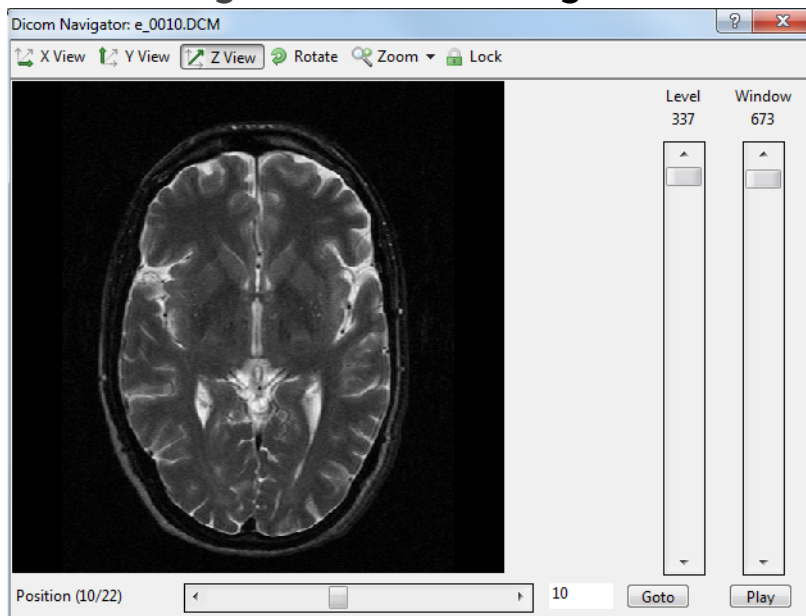
- The MicroCT Navigator has the following features:

The MicroCT Navigator allows the BIOQUANT system to view and analyze trabecular and cortical structure from MicroCT data exported as serial 2D Dicom files. Dicom is a cross-vendor format used by many microCT manufacturers for compatibility with other software systems. Calibration data, when available, will be read from the DICOM file and the Parameters region will display "Dicom" for the magnification. Also, the Z position of tracings will be stored in the measurement tracing topography.

The MicroCT Navigator is used to sequence through a Dicom series of images generated by a MicroCT. The X view, Y view, and Z view of the image stack can be rotated. Level and Window adjustments can be made at any view.

When the user has positioned the image stack at the desired location, the settings can be locked and the image moved to the Image window for measurement.

**Figure –MicroCT Navigator**

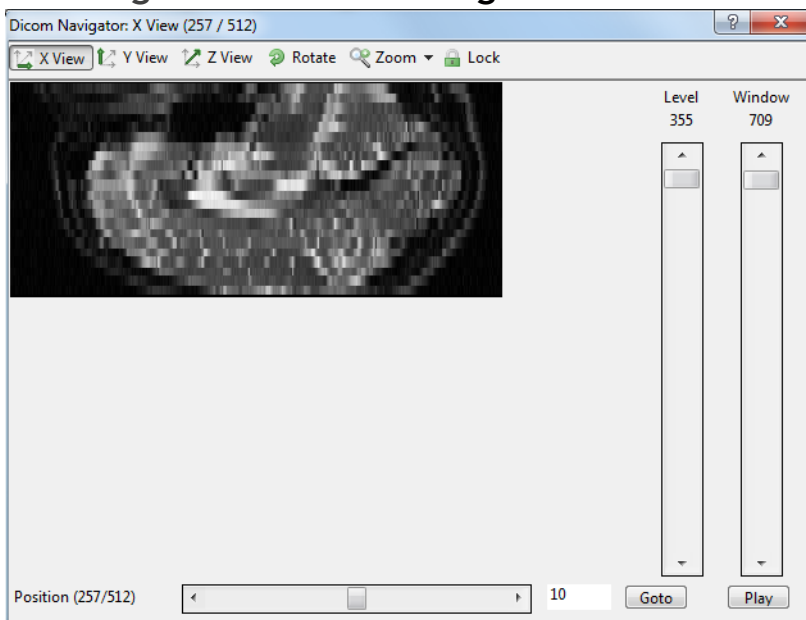


- X View

View the microCT volume from along the X axis, perpendicular to the Y and Z view. The anatomical orientation of the sample in X View will depend on the orientation of the sample when it was scanned.

This view requires BIOQUANT to recalculate the image planes so performance may be slower.

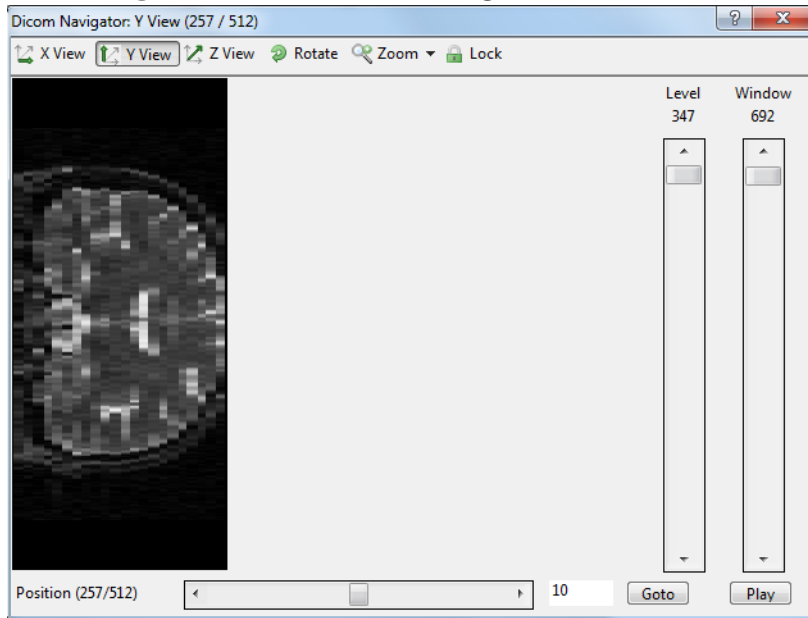
**Figure –MicroCT Navigator - X View**



- Y View

View the microCT volume along the Y axis, perpendicular to the X and Z view. This view requires BIOQUANT to recalculate the image planes so performance may be slower.

**Figure –MicroCT Navigator - Y View**

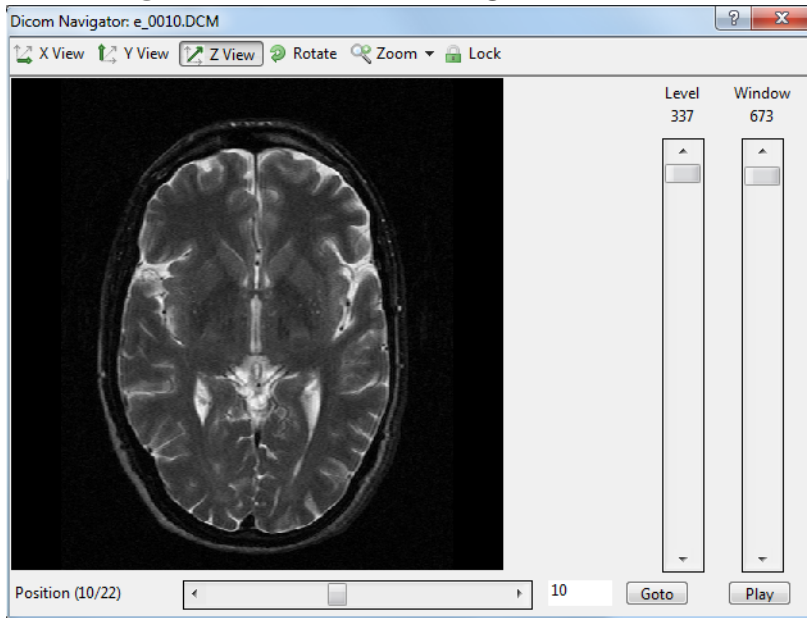




- Z View

View the microCT volume along the Z axis. This is the native plane of section of the images and offers the fastest performance.

**Figure –MicroCT Navigator - Z View**



- Rotate

Turn the image clockwise in 90 degree increments.

If in Z View, the image will remain rotated as you sequence through image positions in the series.

In X View or Y View, the image will revert to the original rotation when you sequence to a different image position.

**Figure –MicroCT Navigator - Rotate**



- Zoom

Increase the size of the image sent to the Image window by 200%, 300%, or 400%. The maximum size of the image in the

Image window is 1280x960. For zoom levels larger than 1280x960, the image is cropped equally from all edges.

**Figure –MicroCT Navigator - Zoom**

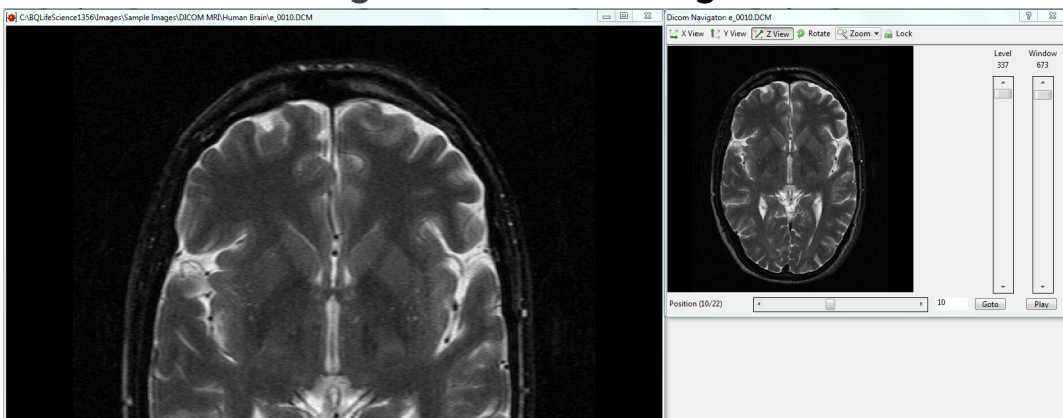


- Lock

Send the current image in the MicroCT Navigator to the Image window.

If the Dicom file header contained calibration information, in the Parameters region, the Mag will say “Dicom” after you lock the image on the MicroCT Navigator.

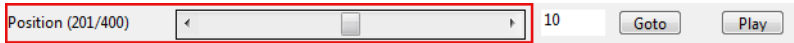
**Figure –MicroCT Navigator - Zoom**



- **Position Slider**

Quickly move through the volume. Click on either side of the moving box to jump 10 images forward or backward. Click the left or right arrow buttons to move one image at a time. Click and drag the slider to move to a specific position.

**Figure –MicroCT Navigator - Position Slider**



- **Goto**

Type in a specific image number then click the Goto button to jump to a specific image.

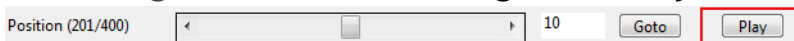
**Figure –MicroCT Navigator - Goto**



- **Play/Pause**

Click Play to move sequentially through the sample along the selected axis. Click Pause to stop. When the end of the sample is reached, play is continued in the reverse order.

**Figure –MicroCT Navigator - Play**

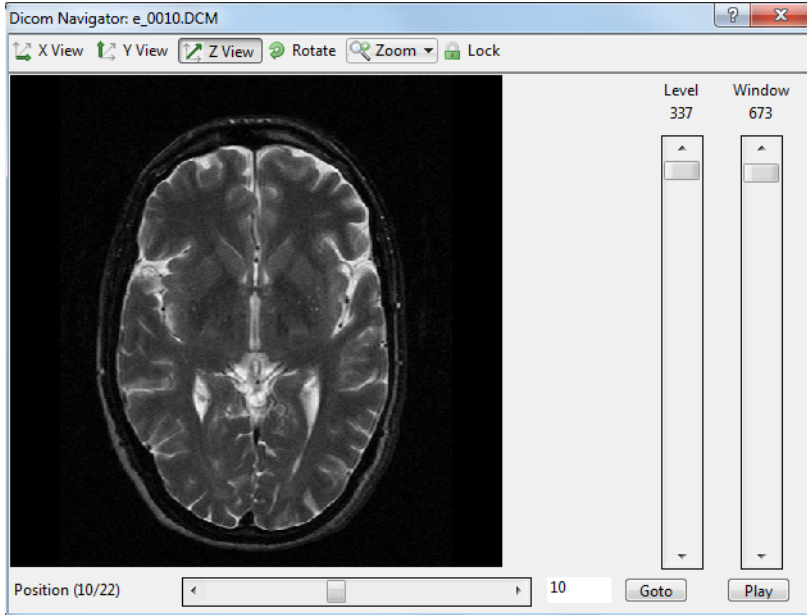


- **Level**

The level slider temporarily adjusts the brightness of an image in the microCT volume. The optimal level value is read from the

Dicom header or calculated if the header does not contain a level value. Moving to the next image will reset the level value.

**Figure –MicroCT Navigator - Level**

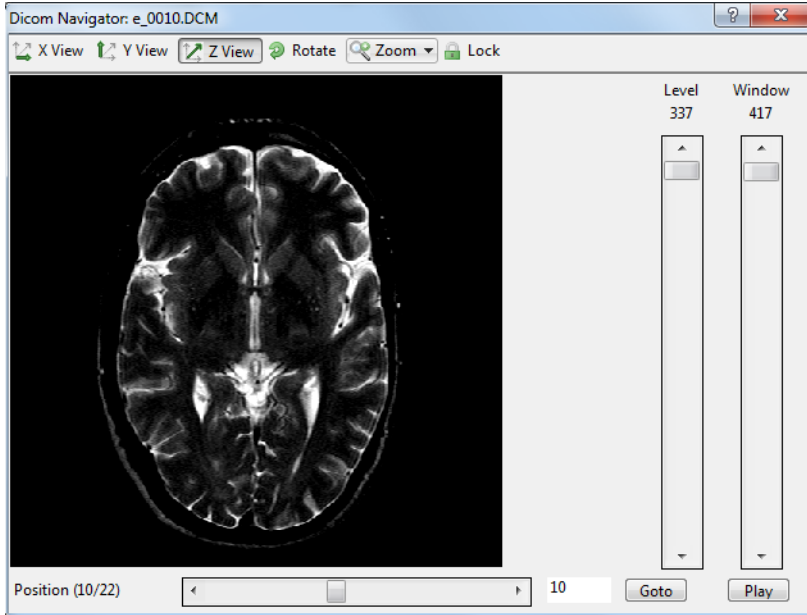


- Window

The window slider temporarily adjusts the contrast of an image in the microCT volume. The optimal window value is read from the Dicom header or calculated if the header does

not contain a window value. Moving to the next image will reset the window value.

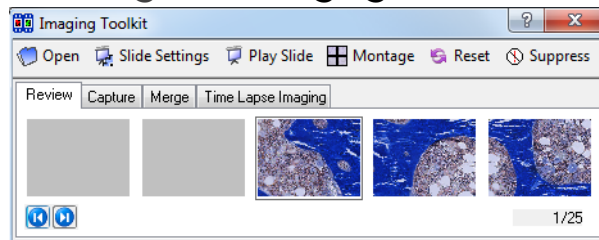
**Figure –MicroCT Navigator - Window**



## Imaging Toolkit Enhancements

The Imaging Toolkit has been added to the Imaging Extensions add-on. The Imaging Toolkit box has the following enhancements:

**Figure –Imaging Toolkit**



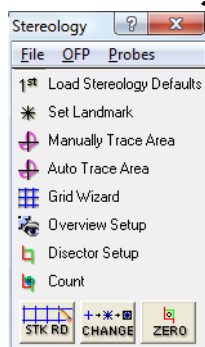
- The interface has been improved. Buttons have been given text labels and the number of thumbnails visible has increased from three to five.

- The thumbnails generated from large unsaved montages are no longer scrambled.

## Stereology Toolkit

The Stereology Toolkit has been added to the BIOQUANT Imaging Extensions add-on. For information on how to use the Stereology Toolkit, see the BIOQUANT Life Science Manual.

**Figure –Stereology box**



## Topic Contents

- Optical Fractionator Project Protocol (page 39)
- Cavalieri Stereology (page 44)
- Lung Stereology (page 45)

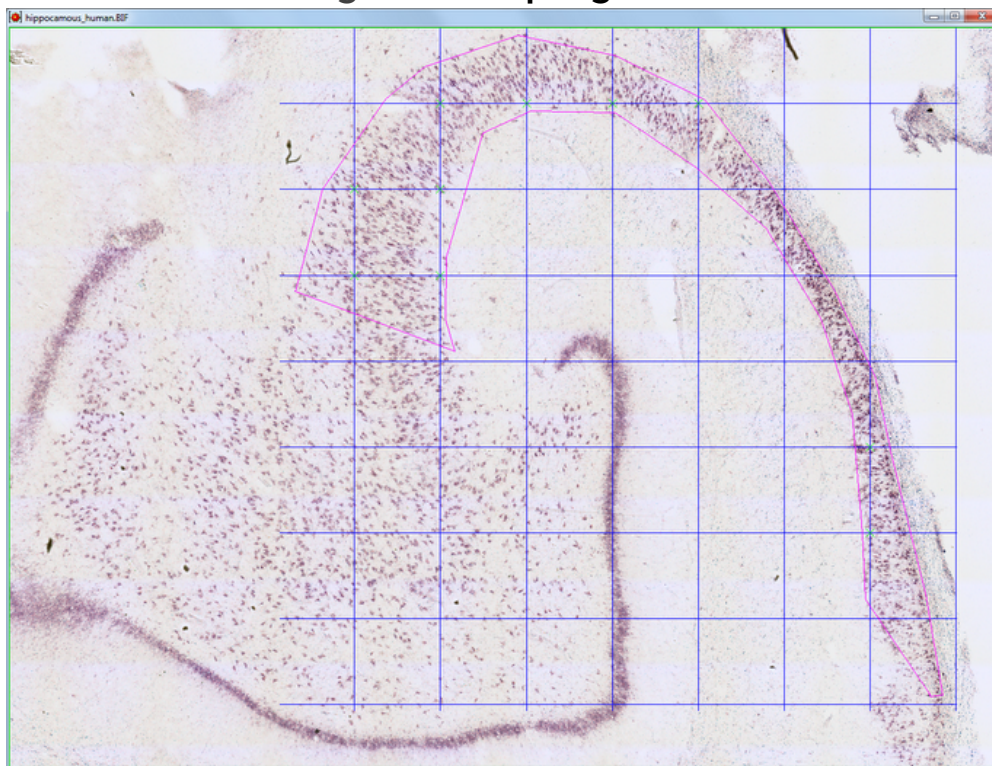
## Optical Fractionator Project Protocol

The BIOQUANT Stereology Toolkit plug-in for BIOQUANT Life Science provides an reliable set of tools for the stereologic estimation of number, length, surface area, and volume.



## Define Sampling Area

Figure –Sampling Area

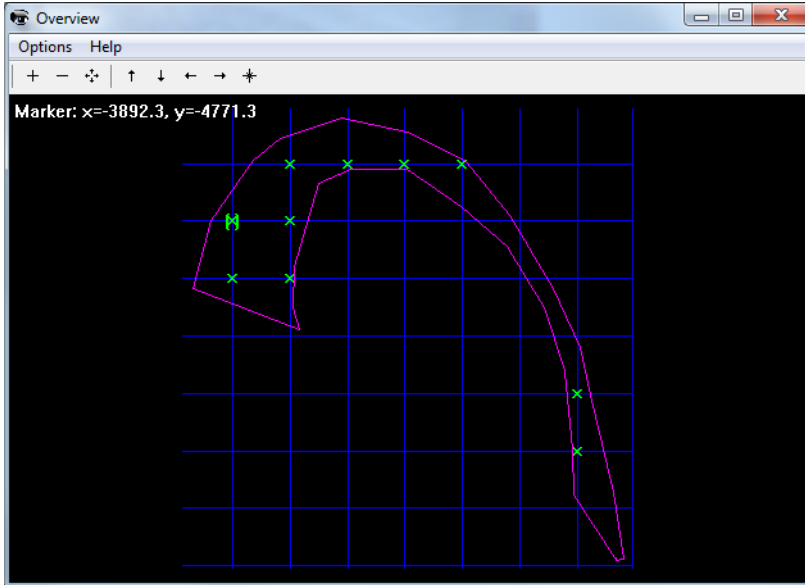


Either on a montage or at low magnification, the sampling area is traced. The grid is automatically bound to the sampling area and sampling locations within the sampling area are automatically marked.



## Track Position with Overview window

Figure –Overview window

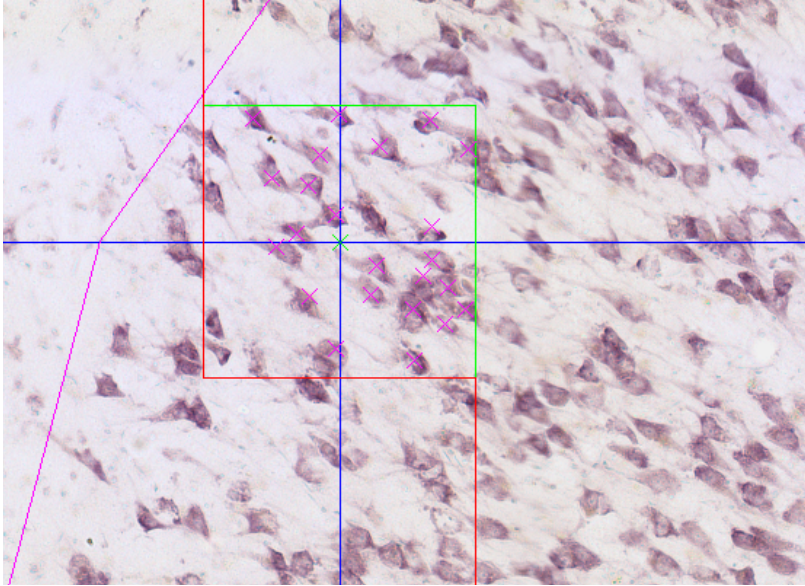


The Overview window shows progress through the section. Clicking a sampling point on the map either drives the motorized stage to that location or displays coordinates for manual measuring stages.

This method is used to navigate to sampling areas within the grid at high magnification. Within the specimen sampling area, measured grid intersections are marked with an X and unmeasured intersections are marked with an O.

## Define Counting Frame

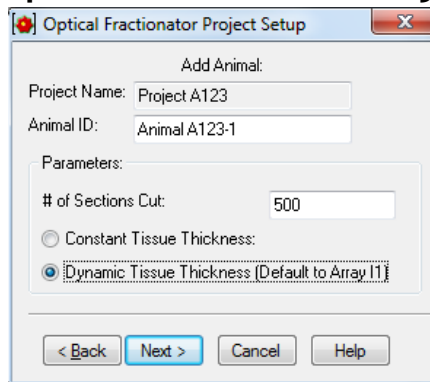
Figure –Counting Frame



At high magnification the counting frame is drawn on the live image making it easy to count neurons within the counting space. The rotator or nucleator probe can also be used at this point to estimate the mean cell size and population of cells.

## Easy Wizard to Add Animals and Sections

Figure –Optical Fractionator Project Setup

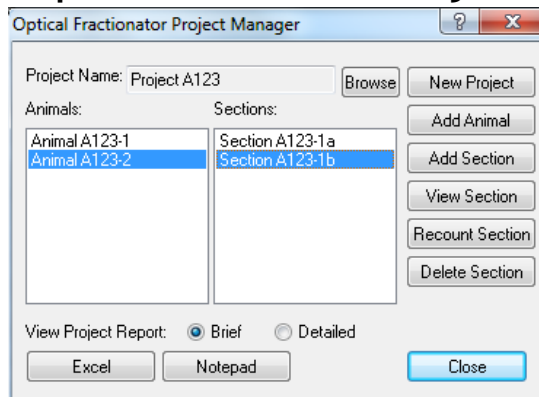


The Optical Fractionator Project Wizard walks the user through setting up the project. Quick Add Animal and Quick Add Section

options are also available. The software gives the user the choice between using a constant tissue thickness or having the software calculate the dynamic tissue thickness at each disector location.

## Manage with Optical Fractionator Project Manager

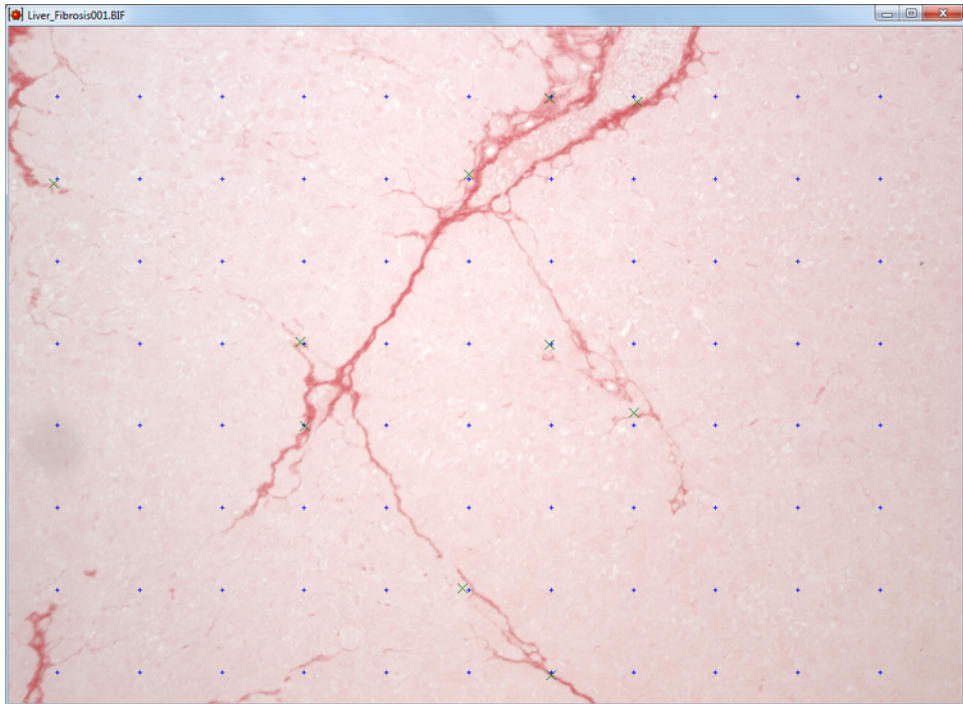
**Figure —Optical Fractionator Project Manager**



The Optical Fractionator Project allows the user the add animals, add sections, view sections, recount sections, or delete sections. The Project Report dynamically updates stereology calculations for the animal and exports directly to Excel.

## Cavalieri Stereology

Figure –Cavalieri Grid



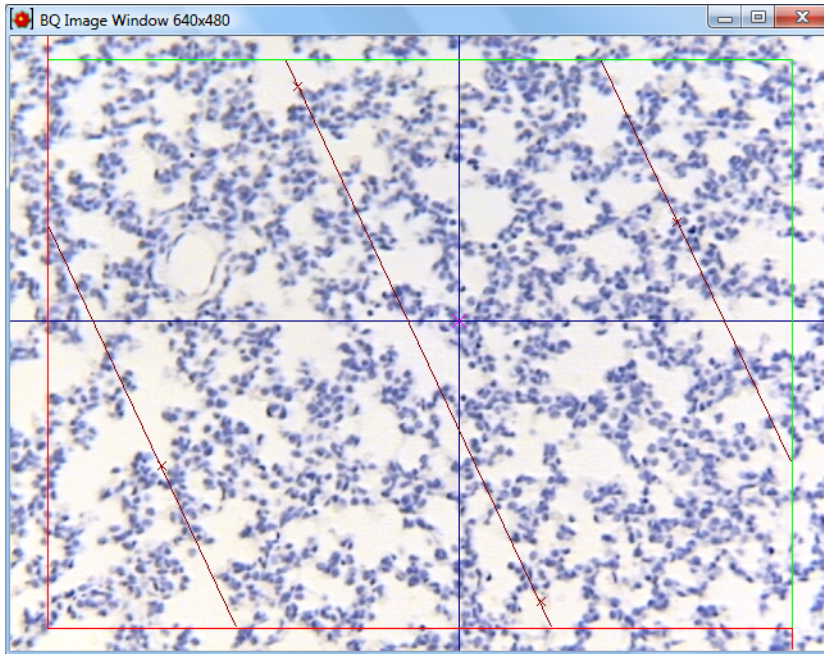
The Cavalieri estimator generates unbiased estimates of cross sectional area and volume ratios.

### Sample Protocol

- Place Grid on Tissue
- On High Magnification Touch Count Tissue Area of interest.
- Export Data to BIOQUANT Excel template to compute the tissue volume estimate and CE estimate.

## Lung Stereology

Figure –Disector with Probes



### Lung Stereology Protocols

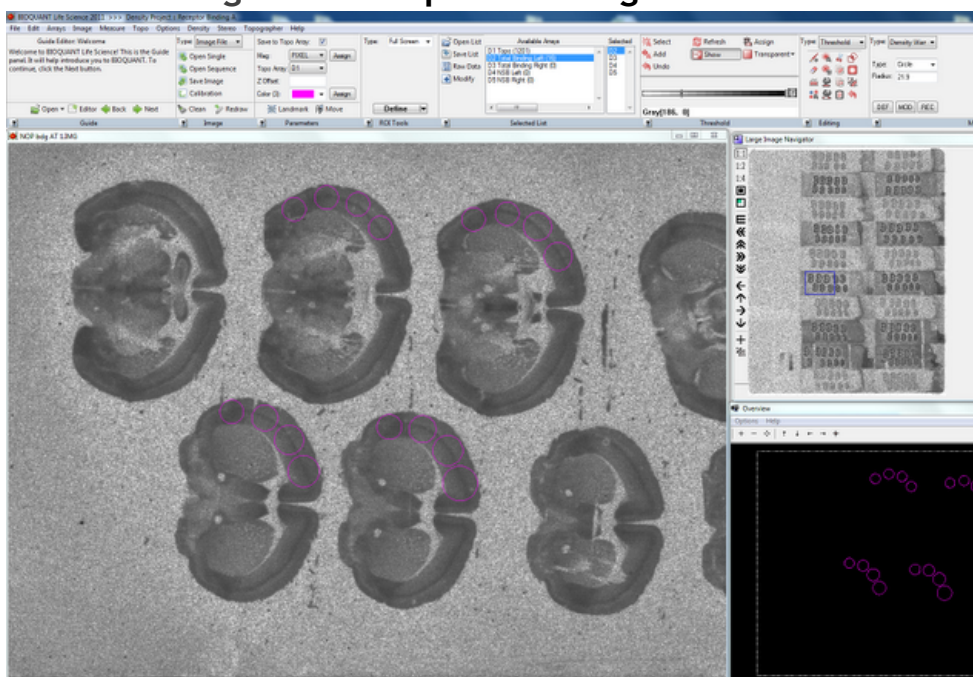
- Trace Tissue (manual or automatic)
- Place Grid on Tissue
- On High Magnification Add Sine Weighted Probes
- On High Magnification Touch Count Air Space and Tissue
- Touch Count Septum / Duct Intercepts along the lines, End Points, and Triple Points
- Export Data to compute the alveolar surface per unit volume and the mean interalveolar septal wall distance as well as other morphometric parameters.



## Densitometry Toolkit

The Densitometry Toolkit has been added to the BIOQUANT Imaging Extensions add-on. For information on how to use the Densitometry Toolkit, see the BIOQUANT Life Science Manual.

**Figure –Receptor Binding Protocol**



### Selected Software Features

- Curve Fitting for Density Calibrations using Seven Types of Curves including Exponential, Linear, Logarithmic, Power, and N-degree polynomial
- Calibration Curve Shows R2 for the Curve and Relative Error for each Standard Value
- Data, Histograms, and Pseudocolor Palettes Reported in Calibrated Units
- Pseudocolor Palettes Automatically Generated from Standards

- Easily Save Pseudocolor Images with Pseudocolor Calibration Bars
- Place, Move, & Erase Sampling Areas in Preview Mode Before Saving Data
- Automatic Mode Records Data for Objects that Meet Threshold and Geometric Filter Criteria

## Representative Applications

- Receptor Binding Assays
- Quantitative Autoradiography
- In-situ Hybridization Histochemistry
- Whole-body Autoradiography
- Genome Size Quantification by Feulgen Staining

## Main Supported Hardware

- Use Fuji BAS 5000 Phosphorimager to generate IMG + INF files that can be opened in BIOQUANT.
- Capture direct, live monochrome images using the QImaging RETIGA EXI Monochrome Camera.
- Digitize film with high-resolution, 4+ OD scanner to generate a 16-bit high resolution TIFF files that can be opened in BIOQUANT.

## BIOQUANT SCAN Add-on Enhancements

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BIOQUANT SCAN includes support for multi-slide high resolution image scanning. It supports either the user's microscope with Scanning Upgrades or the BIOIMAGER scanning microscope.

### Topic Contents

- SCAN Enhancements (page 48)
- BIOIMAGER Enhancements (page 57)

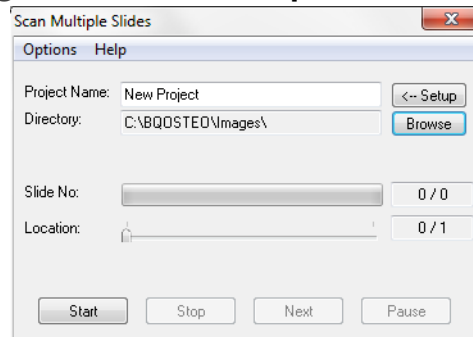
### SCAN Enhancements

The BIOQUANT SCAN program has been enhanced in many ways. For an updated Scan Multiple Slides or Scan Well Plate protocol, see the BIOQUANT Life Science Manual.

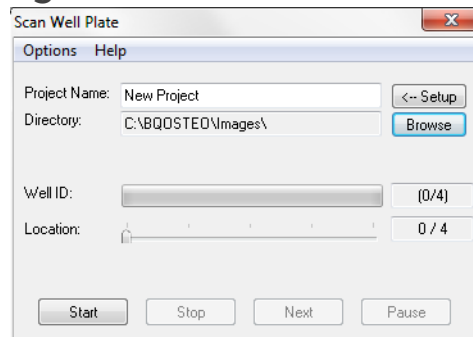


## Scan Multiple Slides / Scan Well Plate Box

**Figure –Scan Multiple Slides Box**



**Figure –Scan Well Plate Box**



- The Create Montage After Image Capture checkbox has been removed. This feature is now on the new Options menu. From Options choose Montage After Capture. This feature is checked by default.

Uncheck this option only if you are making very large montages and you want to eliminate the time it takes to generate the large montage before capturing the next montage.

If this option is unchecked, use the Imaging Toolkit in the core BIOQUANT Life Science system or the BIOQUANT Life Science Satellite system to load the image series and then create the montage.

- Buttons have been renamed:

Start Grab is now Start.

Stop Grab is now Stop.

Grab Next is now Next.

- The File menu and Tools menu have been removed.

The File menu had one menu item, Exit. Use the close button in the upper right corner of the box to close the box.

The Tools menu had one menu item, Create Montage. Use the Imaging Toolkit to create the montage if Montage After Capture is not on.

- The Scan Multiple Slides box and Scan Welled Plate Box will be closed if a BIF image is loaded or if the Topo>Stage option is turned off.

## Scan Multiple Slides / Scan Well Plate Setup Box

Figure –Scan Multiple Slides Setup Box

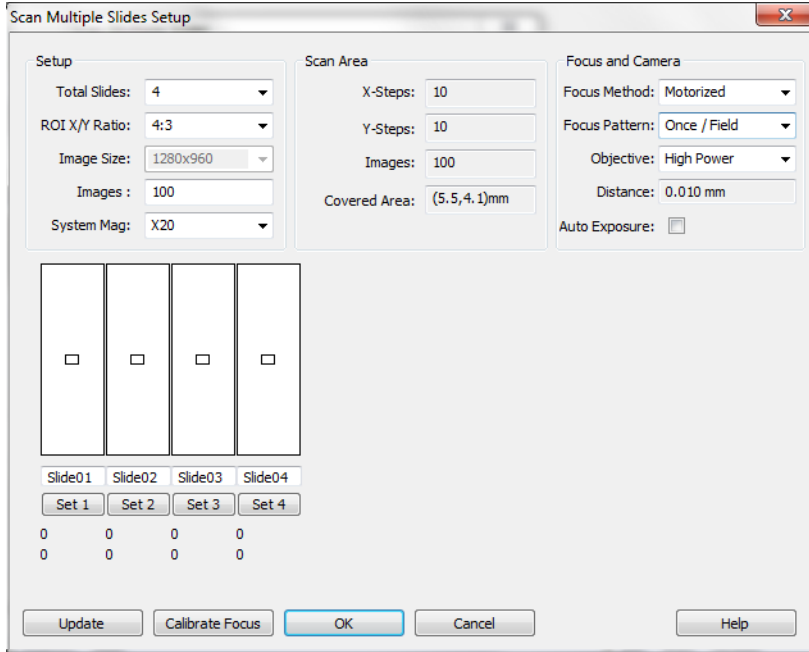
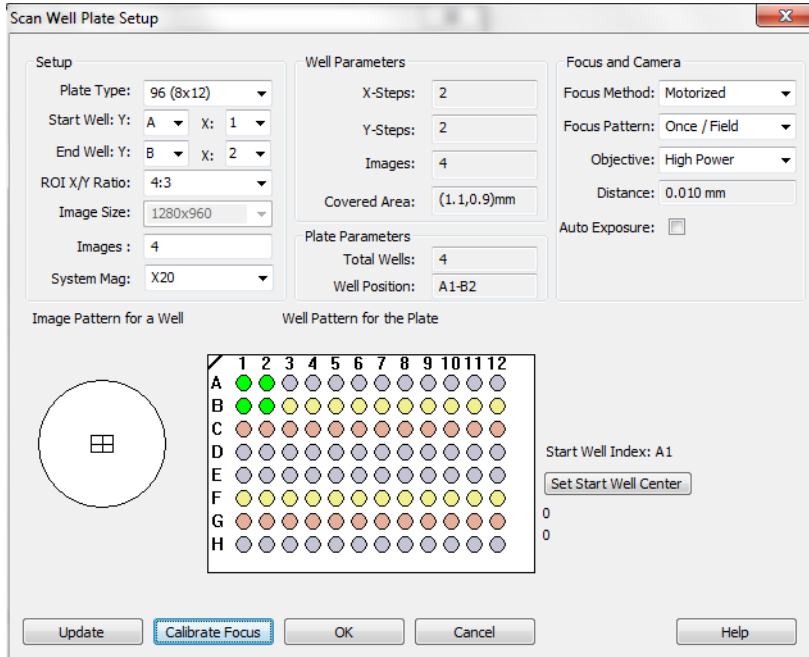


Figure –Scan Well Plate Setup Box



- **New Focus and Camera region:**

There is a new Focus and Camera region on both the Scan Multiple Slides Setup and Scan Well Plate Setup boxes. This region offers enhanced options for choosing auto focus patterns and allowing the user to manually focus on field based on a focus pattern. It also activates the Auto Exposure box.

This region replaces the Auto Focus and Pre Focus check boxes from the previous version.

## **Focus Method**

Choose from the following:

- **Motorized**

Use the motorized auto focus attached to the microscope or Life ScienceIMAGER scanning microscope. The Auto Focus hardware module is required for this option.

- **Manual**

The user will manually focus the microscope whenever it stops. Stop locations are based on the Focus Pattern (see below). This is a good choice if you have an XY motorized stage without the Auto Focus module.

- **None**

No focus method will be used. This is a good choice if your specimen is flat and really doesn't need focus. This option makes image capture faster than choosing Motorized or Manual.

## Focus Pattern

Choose from the following:

- **Once/Field**

Motorized: Perform auto focus on every field captured.

Manual: Stop once per field and wait for the user to focus. When the user clicks the Next button, continue with capture.

None: Focus Pattern is grayed out.

- **Once / 2 Fields**

Motorized: Perform auto focus every other field captured.

Manual: Stop on every other field and wait for the user to focus. When the user clicks the Next button, continue with capture.

None: Focus Pattern is grayed out.

- **Once / 3 Fields:**

Motorized: Perform auto focus every third field captured.

Manual: Stop on every third field and wait for the user to focus. When the user clicks the Next button, continue with capture.

None: Focus Pattern is grayed out.

- **Once / Slide or Once / Well**

Motorized: Perform auto focus once per slide/well.

Manual: Stop once per slide/well and wait for the user to focus. When the user clicks the Next button, continue with capturing the entire slide/well.

None: Focus Pattern is grayed out.

## Objective

Objective is used to set the search range for auto focus. On a high power lens, the auto focus uses a faster, shorter z axis search range because the depth of field is short. On a low power lens, the auto focus uses a longer, slower z axis search range because the depth of field is deeper.

This option is ignored if the Focus Method is Manual or None.

Choose from the following:

- High Power

Choose this option if you are capturing images using a high powered objective with N.A. of 0.2 or higher. Often this means the condenser cap is in.

- Low Power

Choose this option if you are capturing images using a low powered objective with N.A. of 0.1 or lower. Often this means the condenser cap is out.

## Distance

Distance displays the size of the search range for the auto focus. This value changes based on the Objective designated above.

## Auto Exposure

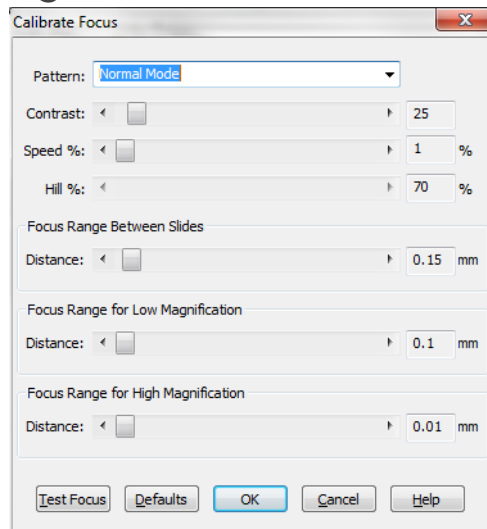
- To perform an Auto Exposure every time an image is captured, check the Auto Exposure box.

This option defaults to OFF. Auto Exposure will change the lighting across the montage. If it is OFF, the lighting will be more consistent in the montage.

This option might be turned ON if there is a large hole in the middle of the section would be overexposed without Auto Exposure.

- The Auto Focus Setup button has been renamed Calibrate Focus.
- The Calibrate Focus box has been enhanced:

**Figure –Calibrate Focus Box**



## Pattern

**Normal Mode:** Use for everything except deep, thick cell cultures.

**Hill Detect:** Use for deep, thick cell cultures. It will focus on the top layer of cells.

## Contrast

This value controls how sensitive the auto focus is to changes in the focus of the sample. The lower the value, the more sensitive the auto focus is and the higher the chance of a false positive due to dust or other debris. The higher the value, the more tolerant the auto focus is of minor changes in the image focus and the more likely the system is to leave the Z position unchanged. The default value is 25. Useful ranges are between 10 and 200.

## Speed %

This value controls the speed of the Z axis while searching for optimal focus. The default is 1%, which is the slowest, most precise setting.

## Hill

This value controls the sensitivity of the hill detect mode. Lower values make the algorithm more sensitive to individual cells on the surface of the culture. Higher values allow the algorithm to ignore individual cells and find the larger culture surface. The default is 70%.

## Focus Range

Between Slides Distance: This value controls how far the auto focus will search when changing slides in order to find the next slide. If the system can not find the next slide when switching slides increase this value. The useful range for this value is between 100 and 500 microns. The default value is 150 microns.

For Low Magnification Distance: This value controls how far the auto focus will search when moving between adjacent



fields on the same section if Objective is set to Low Power on the Setup dialog box. The default value is 100 microns.

For High Magnification Distance: This value controls how far the auto focus will search when moving between adjacent fields on the same section if Objective is set to High Power on the Setup dialog box. The default value is 10 microns.

## BIOIMAGER Enhancements

- Software control over the bright field light source has been added. From the Scan menu, choose either:
  - Turn Light On
  - Turn Light Off
- Software control over the objective lens selection has been added. From the Scan menu, choose either:
  - Move to Next Objective
  - Move to Previous Objective

## BIOQUANT Topographer Add-on Enhancement

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- When you go to Topographer with Save Topo Array checked, when you return it is still checked.