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# Release Notes

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**BIOQUANT**<sup>®</sup>



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# OS & Camera Support

As of BIOQUANT Life Science 2023, Windows 11 Pro 64-bit is supported in addition to continued support for Windows 10 Pro 64 bit. Windows 7 is not supported by BIOQUANT Life Science 2023.

## BIOQUANT LIFE SCIENCE 2023 SUPPORTS WINDOWS 11 & WINDOWS 10

BIOQUANT Life Science 2023 now supports Windows 11 64-bit. Windows 10 64-bit is also supported

As of 2020, BIOQUANT Life Science no longer supports Windows 7. Microsoft discontinued support of Windows 7 on January 14, 2020. BIOQUANT Life Science 2021 and later require Windows 10 64-bit.

## UPDATED COMPUTER REQUIREMENTS

When upgrading to BIOQUANT Life Science 2023, consider whether your computer should be updated. Although older computers might run BIOQUANT Life Science 2023, those that don't meet current specifications will be slower, especially when considering core clock speed.

### OPERATING SYSTEM REQUIREMENTS

- Windows 11/10 64bit Pro, Education, or Enterprise. (Windows 8, Windows 7, Windows XP, Mac OS not supported.)

### MINIMUM COMPUTER SPECIFICATIONS

- Dell Precision Series Mini-Tower Chassis
- Multi-core Intel Processor: I7 processor
- 3.5 GHz core clock speed
- NVIDIA Quadro K620 GPU
- 16 GB RAM
- 1 TB HDD (Solid State Drive is better, but not required)
- Chassis with room for 1/2 height & 1/2 length PCIe 1X board
- Monitor that supports a 1920x1200 desktop resolution

### RECOMMENDED COMPUTER SPECIFICATIONS

- Dell Precision 5000 Series Mini-tower Chassis
- Multi-core Intel Processor: Xeon processor
- 4.0 GHz or higher core clock speed
- NVIDIA Quadro T1000 GPU or better
- 32 GB RAM or more
- 2 TB Solid State Drive
- Chassis with room for 1/2 height & 1/2 length PCIe 1X board
- Monitor that supports a 1920x1200 desktop resolution

## LEGACY QIMAGING CAMERA SUPPORT

QImaging does not support their FireWire cameras under Windows 10.

BIOQUANT will make every effort to support legacy QIMAGING cameras with the current BIOQUANT Life Science 2023 release. However, please be aware that our capacity to help is limited due to those outside constraints. In addition, new imaging features, such as Live Correction and the Virtual Stage Controller, are only being added for the Jenoptik cameras.

For the long-term stability of your system, we encourage you to consider upgrading to one of the recommended Jenoptik Gryphax cameras listed below.

## JENOPTIK NEXT-GENERATION SCMOS CAMERA SUPPORT

### NEW JENOPTIK CAMERA SUPPORT



The graphic features the Jenoptik logo at the top center. Below it, the text 'PROGRES GRYPHAX® PROKYON' is displayed. To the right, three camera models are shown: a large blue camera with a lens, a smaller blue camera, and a small blue cube-shaped camera. Below the cameras, the text 'Technical data' is followed by a list of 'KEY FACTS':

- True color microscope camera 20.7 | 19.2 | 2.3 MPix
- 60 fps at 2.3 MPix
- Pixel size 5.86 x 5.86 µm
- Dynamic range 73.3 dB
- 1/1.2" SONY sensor (back-illuminated)
- Global shutter
- Software included

At the bottom left of the graphic, it says 'Explore the micro universe with the flagship.'

### JENOPTIK GRYPHAX® CAMERAS

We are excited to announce support for the **biggest camera technology upgrade** since the introduction of the first high resolution digital cameras.

BIOQUANT now supports the PROKYON, KAPELLA, and SUBRA cameras from Jenoptik.

These Jenoptik cameras all use a next generation **scientific CMOS** sensor that eliminate the compromises inherent in all the prior CCD sensor cameras.

These cameras can deliver a full 1920x1200 resolution image at between 30 and 60 frames per second. This is **2X to 4X faster** than current cameras at **4X the resolution**.

In BIOQUANT, supported Jenoptik cameras provide a live image at **1280x960** to the Image Window with optional **1920x1200** image capture. The PROKYON camera model adds additional capture sizes of **3840 x 2400** and **5760 x 3600**.

## THREE AVAILABLE MODELS TO FIT ANY BUDGET

The flagship **Prokyon** model offers a special 20 megapixel mode through pixel-shifting in addition to the native live image at 1920x1200 60 fps with fan-less cooling. The **Kapella** camera keeps the fan-less cooling but saves money by removing the 20 megapixel imaging mode. The **Subra** model is even more economical than the Kapella by removing the fan-less cooling.

	JENOPTIK PROKYON	JENOPTIK KAPELLA	JENOPTIK SUBRA
<b>Model Number</b>	KIT1-PRO	KIT1-KAP	KIT1-SUB
<b>Sensor Specifications</b>			
<b>Sensor Model</b>	Sony IMX174	Sony IMX174	Sony IMX174
<b>Sensor Design</b>	Scientific CMOS	Scientific CMOS	Scientific CMOS
<b>Sensor Size</b>	1920x1200	1920x1200	1920x1200
<b>Frame Rate</b>	60fps	60fps	30fps
<b>Pixel Size</b>	5.8 microns	5.8 microns	5.8 microns
<b>Image Specifications</b>			
<b>Capture Resolutions</b>	5760x3600 3840x2400 1920x1200	1920x1200	1920x1200
<b>Exposure Control</b>	26 us to 60 s	26 us to 60 s	26 us - 30 s
<b>Cooling</b>	Fanless	Fanless	N/A
<b>Gain Control</b>	1X - 30X	1X - 30X	1X - 10X
<b>Exposure Control</b>	Auto Exposure	Auto Exposure	Auto Exposure
<b>Color Correction</b>	Auto White Balance	Auto White Balance	Auto White Balance
<b>Data and Power Interface Specifications</b>			
<b>Bit Depth</b>	8bits / channel + HDR	8bits / channel + HDR	8bits / channel + HDR
<b>Bus Type &amp; Power</b>	USB3.0 + 4.5W	USB3.0 + 4.5W	USB3.0 + 4.5W
<b>Physical Specifications</b>			
<b>Mount Type</b>	C-Mount	C-Mount	C-Mount
<b>Dimensions</b>	85mm x 75mm x 50mm	85mm x 75mm x 50mm	85mm x 75mm x 50mm
<b>Warranty Information</b>			
<b>Warranty Term</b>	24 months	24 months	24 months

Contact us for Pricing and Upgrade Discounts.

## LIMITED TIME CAMERA TRADE-IN DISCOUNT PROMOTION

BIOQUANT and Jenoptik offer a trade-in discount for QIMAGING cameras toward the purchase of a new Jenoptik camera. Every QIMAGING camera sold with a BIOQUANT system, and many others, qualifies for this trade-in discount promotion. Discount amounts vary depending on the Jenoptik camera model purchased. Please act quickly, this is a limited time promotion.

# BIOQUANT Life Science New Features

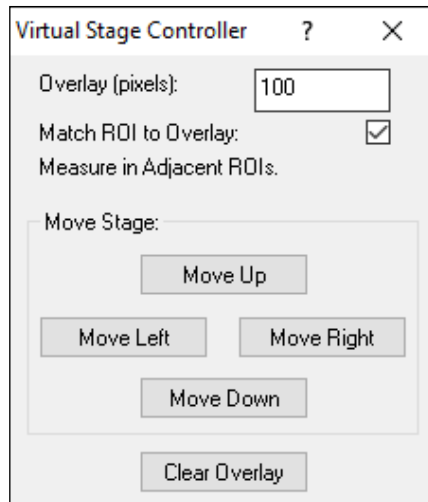
## Lab Without Motorized/Encoded Stage? New Virtual Stage Controller .....

With the Virtual Stage Controller, BIOQUANT uses software features to simulate the movement of a motorized stage when measuring at a single magnification field to field in a raster pattern. This makes it easier to measure field to field at 10x or 20x on a live image without a tracking device, like a motorized stage or stage encoders.

The Virtual Stage Controller can also be used to collect images from the microscope field to field in a raster pattern that can then be montaged into a high resolution, calibrated BIF image.

The Virtual Stage Controller requires a Jenoptik camera.

### THE VIRTUAL STAGE CONTROLLER BOX



The Virtual Stage Controller can be opened from the Topography menu.

### FEATURES

- **A simple control pad is used to guide the user in moving the stage in regular steps around the section.**
- **A semi-transparent overlay of the previous field of view is used to align the image to the current field of view.**
- **As an added confirmation, the measurement tracings made on previous fields of view reappear in the overlapping areas between fields of view.**
- **The Overview “You Are Here” box updates on a map of your section to show your current position relative to the entire section.**

## VIDEO TUTORIALS

For in-depth video tutorials on the Virtual Stage Controller, in the Parameters region, click the “>” Open Video Tutorials button.

Videos include:

- [Using the Virtual Stage Controller with Field Type at High Mag](#)
- [Using the Virtual Stage Controller with Measure Cells at High Mag](#)
- [Using the Virtual Stage Controller with Imaging Toolkit to Manually Capture a Scan](#)

## PROCEDURE

When using the Virtual Stage Controller, it is best to have it open BEFORE you start collecting data on the first field in the series.

**1. On the microscope go to the objective for collecting field to field data in a raster pattern.**

**2. In the Parameter region, choose the matching Mag.**

The system must be properly calibrated for the stage to track topography correctly.

**3. Setup the lighting for this objective.**

For brightfield, follow the “Jenoptik Camera: Quick Brightfield Setup Procedure.”

For fluorescence, follow the “Jenoptik Camera: Quick Fluorescence Setup Procedure.”

**4. Position the stage so that the first field of view to be measured is in the Image window. Then, go to held image.**

This is often the field that represents the upper right of the Sampling Area.

DO NOT physically move the stage after this step.

**5. In the Parameters region, before you begin your measurement protocol, do the following:**

- Check Save to Topo array and designate a Topo Array in the list.

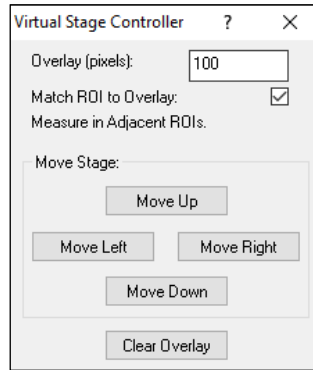
Most bone templates already have the Topo array designated.

- If this is a new section, set a Landmark.

Once the landmark has been set, you can not move the stage without using the Virtual Stage Controller procedure.

**6. From the Topography menu, choose Open Virtual Stage Controller to open the Virtual Stage Controller box.**

## THE VIRTUAL STAGE CONTROLLER BOX



The Virtual Stage Controller can be opened from the Topography menu.

### 7. **Decide whether to check Match ROI to Overlay.**

#### **CHECKED**

When checked, the Overlay outline is used as the rectangle ROI on each field of view.

This is the preferred choice if the tissue to be measured does not overlap, field to field. An example is measuring % area of positive immunohistochemical staining at higher magnification, such as 10x or 20x, field to field.

Typical Measurement Type Used: Field Type or any Measurement Type besides Measure Cells or Myofibers.

#### **UNCHECKED**

When unchecked, the Overlay outline is not used as the rectangle ROI on each field of view.

This is the preferred choice if the tissue to be measured does need to overlap, field to field. An example is measuring cells or muscle at higher magnification field to field. Since previously measured cells are excluded from measurement, this allows all cells to be measured in the sampling area without getting cut in half by edge fields.

Typical Measurement Type Used: Measure Cells or Myofibers

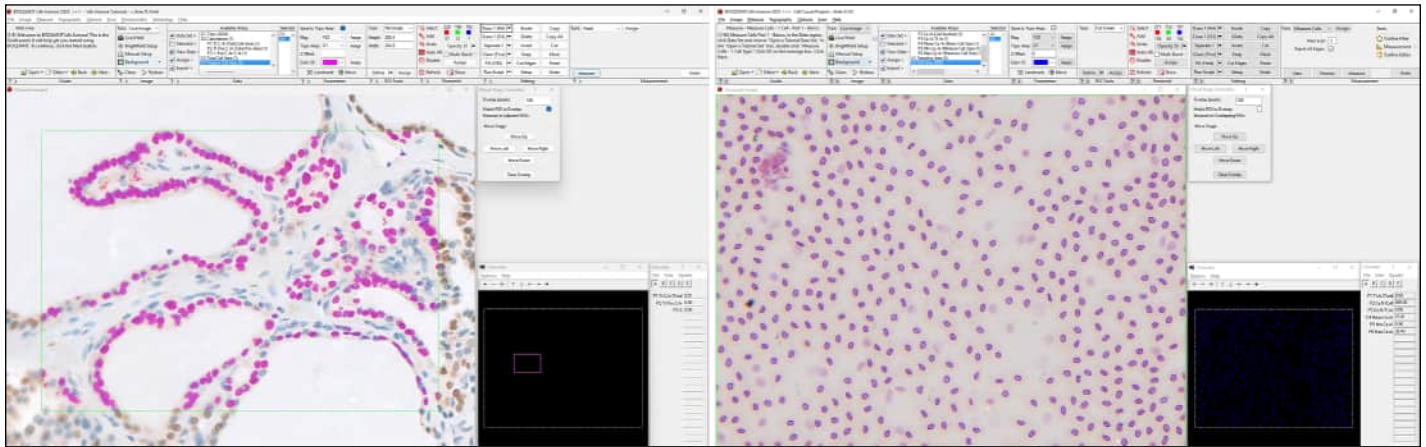
### 8. **Preview, edit threshold, and measure the current field of view.**

Use the measurement protocol of your choice.

Do NOT physically move the stage during this step.



## THE VIRTUAL STAGE CONTROLLER BOX



If "Match ROI to Overlay" is checked, measure within the rectangle ROI. (left)  
If "Match ROI to Overlay" is not checked, measure within the full screen ROI. (right)

### 9. When you are ready to move to the next field, do the following::

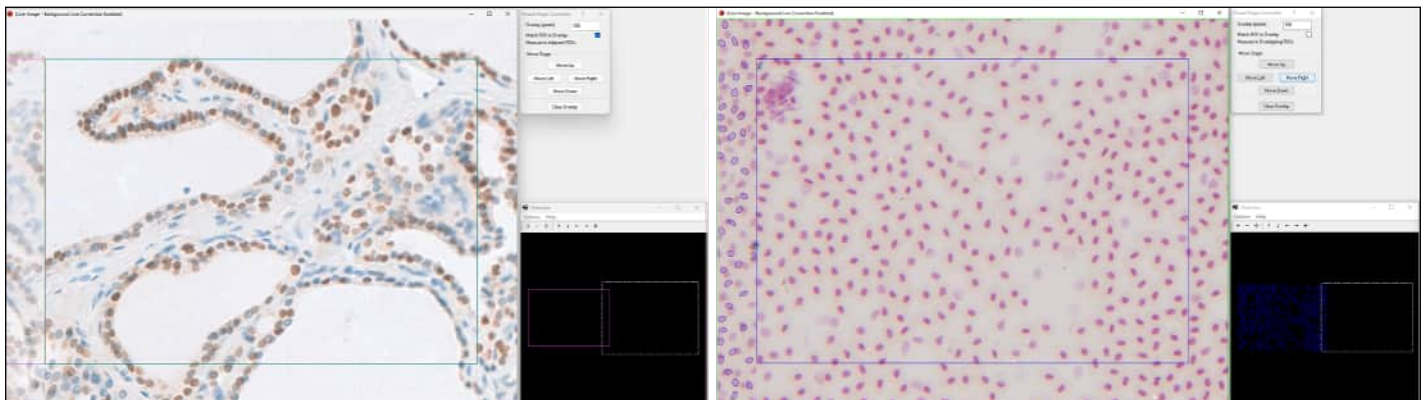
- a. To move in a raster pattern, on the Virtual Stage Controller, click the Move Right button.

The image goes live and the semi-transparent overlay region shows up on the left part of the Image window.

For Measure Cells or Myofibers Measurement Types, if any objects were measured in this overlay region, their redraw tracings also show up.

Additionally, the Overview preview moves the "You are Here" box to the next field of view.

## THE VIRTUAL STAGE CONTROLLER BOX



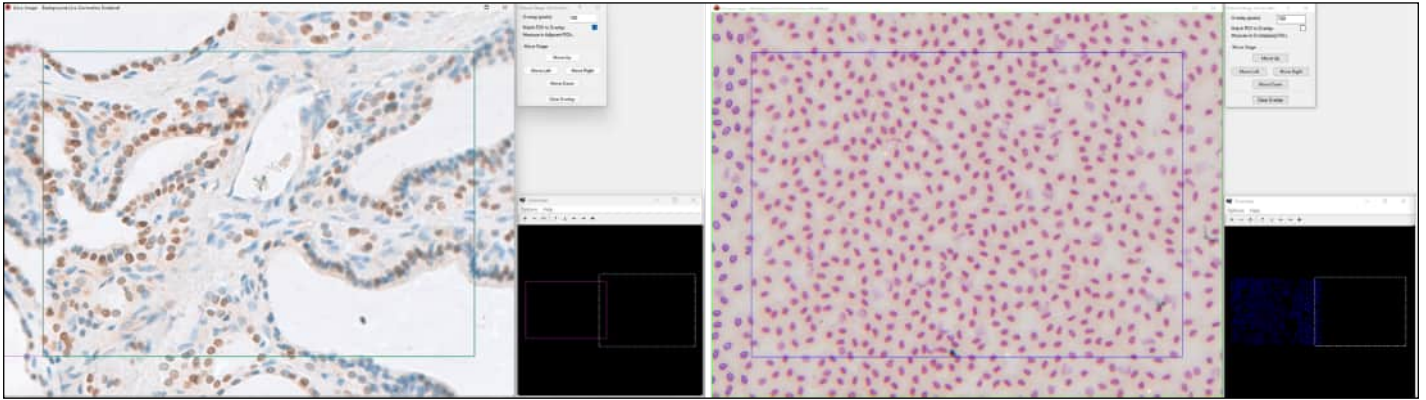
The semi-transparent overlay shows up on the left part of the Image window and the Overview "You are Here" box moves to the next field of view.

- b. Move the microscope stage so that the tissue previously at the right portion of the Image window aligns with the overlap on the left edge.

Before you move, it is useful if you study the tissue/cells between the right overlay edge and the right edge of the Image window. This is the portion of the image that you will align with the overlay at the left edge of the Image window.

If using Measure Cells or Myofibers Types, you can also use the Redraw tracings to help you align.

## THE VIRTUAL STAGE CONTROLLER BOX



Move the stage to the right so that the live image lines up with the image overlay and tracings.

- c. Click the Clear Overlay button to make the overlay go away.

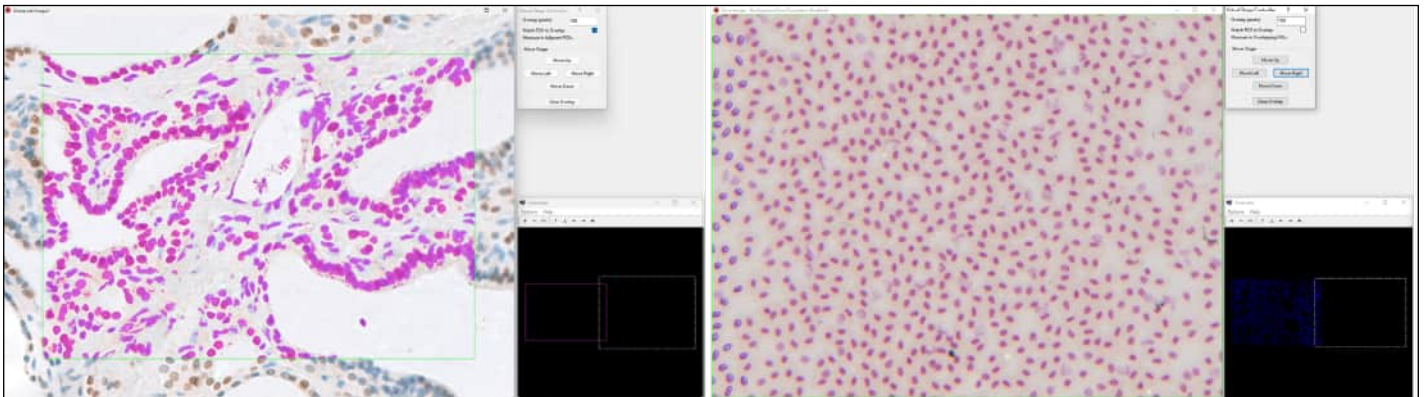
Clear Overlay removes the image overlay at the left edge of the screen.

Alternatively, you can use other actions to clear the overlay, like Refresh on the Threshold region or Preview in the Measure region.

At this point, if “Match ROI to Overlay” is checked, the Overlay region becomes a rectangle ROI.

If “Match ROI to Overlay” is unchecked, the ROI is the Full Screen.

## THE VIRTUAL STAGE CONTROLLER BOX



After Clear Overlay:

If “Match ROI to Overlay” is checked, the Overlay box becomes the Rectangle ROI. (left)

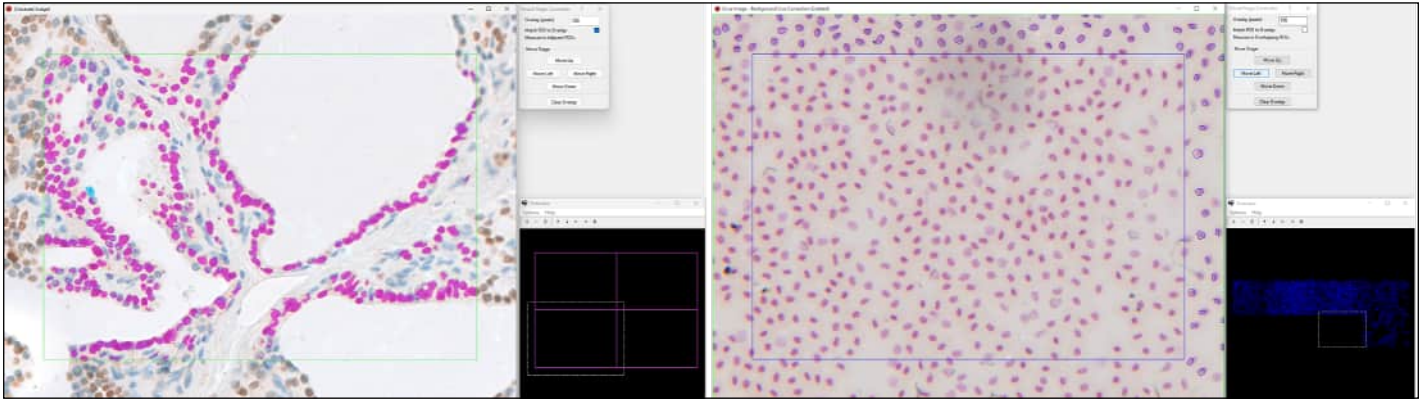
If “Match ROI to Overlay” is not checked, the full screen ROI becomes the Region of Interest. (right)

10. Repeat measurements on this field of view.

11. Repeat using the Virtual Stage Controller to move to the next field.

Use Move Right until you reach the desired number of fields. Then, use Move Down to move the stage down a field. Then, use Move Left to move the stage to the left. Repeat in a raster pattern until the entire sampling area has been measured. Track your measurement progress in the Overview window.

## THE VIRTUAL STAGE CONTROLLER BOX



Left: After moving right one field, down one field, and to the left one field.  
Right: After moving right five fields, down one field, and to the left one field.

12. **When the entire sampling area has been measured, you are finished. At this point, close the Virtual Stage Controller to stop tracking stage movement.**

## Want a Scale Bar on the Scan? New Save Navigator BIF Image Plus Stamp

Previously, calibration scale bars could be saved only on images from the Image Window. This was done through Save Image > Save Image Window plus Graphics.

Now, calibration scale bars can be saved on the entire BIF scan, regardless of size. This makes it possible to annotate whole slide scans so the micron per pixel calibration factor is easy to determine when exporting scans to TIF, an uncalibrated format.

To save the stamp on the BIF image in the Image Navigator, a new Save Image is available, “Save Image Navigator BIF plus Stamp.”

### FEATURE PARAMETERS

- The image currently open in the Image Navigator must be a BIF image.
- The stamp should be placed on the image in the Image window at 1:1 ratio. This means that the maximum width of the scale bar is the maximum width of the Image window at 1:1.
- If using a very high resolution image, consider increasing the Scale Bar Height on the Stamp so it is easier to see.
- The stamp uses the Parameters region Color drop list to set the color.
- The “Save Navigator Image Plus Stamp” option can be used to save a BIF as a BIF, BMP, or TIF. The image saved is the entire high resolution image in the Image Navigator with the scale bar embedded.
- After saving with the “Save Navigator Image Plus Stamp” option, the saved image is opened in the Image window. If you saved to TIF or BMP, before measuring this image, make sure the system is calibrated.

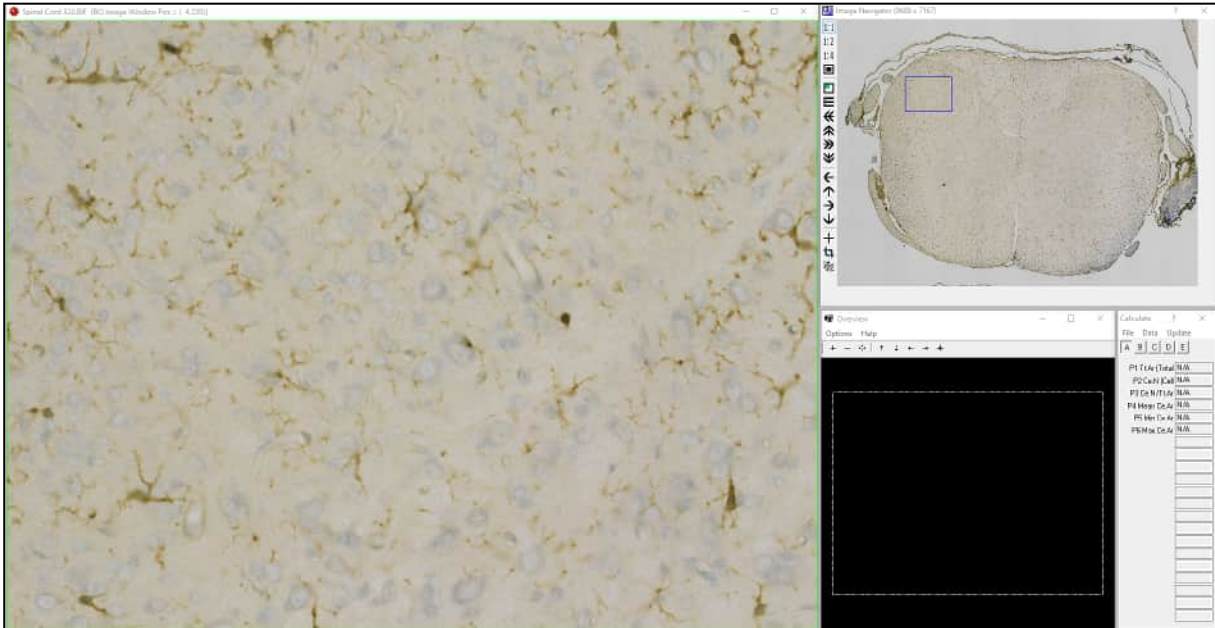


## PROCEDURE

1. In the Image region, use **Open Single Image** to open the **BIF** image that you would like to save with an embedded scale bar, or other stamp, into the **Image Navigator**.

Any BIF image that is larger than 1280 x 960 pixels automatically opens into the Image Navigator. To save an image with a stamp that is less than 1280 x 960 pixels, use **Save > Save Image Window with Graphics** instead.

### BIF IMAGE IN IMAGE NAVIGATOR

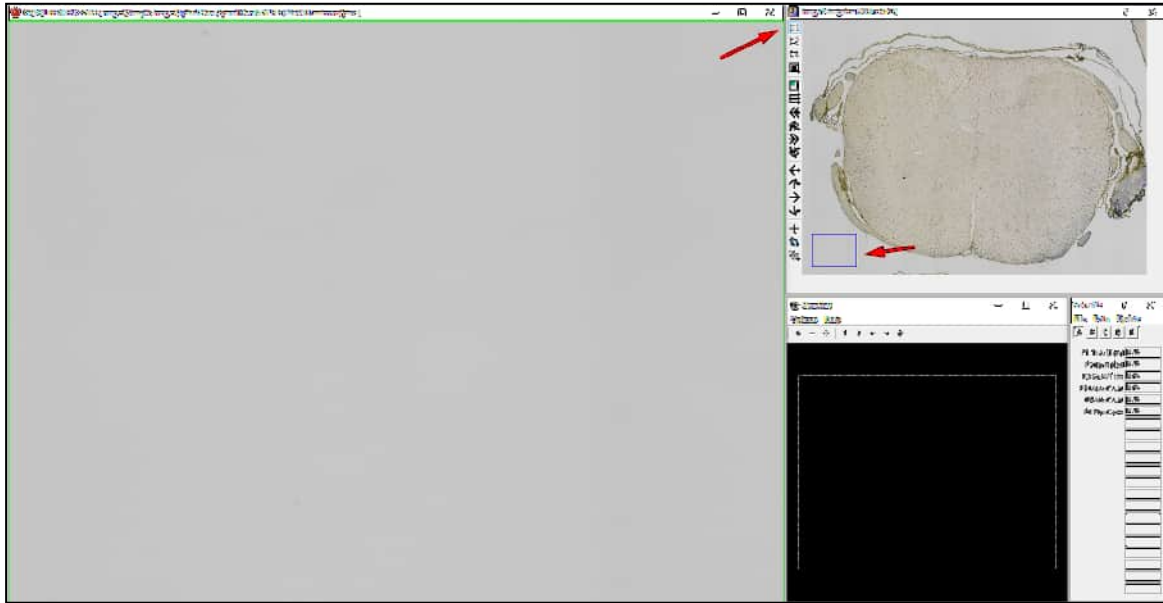


A BIF image has been opened into the Image Navigator.

2. In the **Image Navigator**, choose the **1:1** button and move the **Pan** box to the desired location for the stamp so that the portion of the image is in the **Image** window.

The stamp should be placed on the image in the Image window at 1:1 ratio. This means that the maximum width of the scale bar is the maximum width of the Image window at 1:1. If placing a scale bar, consider increasing the Scale Bar Height on the Stamp so that it is easier to see at other ratios.

## BIF IMAGE IN IMAGE NAVIGATOR



Make sure you are using 1:1 and move the Pan box to the field that will hold the stamp.

3. From the Image Menu, choose “Open Stamp Tools...” to open the Stamp box.
4. On the Stamp box, do the following:

### STAMP BOX

Stamp ? X

Type: Scale Bar v

Length (Microns):  
500

Text:  
500 Microns

Scale Bar Height (Pixels):  
20

Stamp Erase All

For this example, we will use the Scale Bar stamp.

- a. From the Type list, choose the stamp type. In this example, we will choose Scale Bar.
- b. In the Scale Bar type, in the Length (Microns) box, type the length of the stamp.

Since the stamp should be placed on the image in the Image window at 1:1 ratio, the maximum width of the scale bar is the maximum width of the Image window at 1:1.

You might need to experiment. After you place the stamp, if it is too short or long, click the Erase All button to erase it, then adjust the Length and place the stamp again.

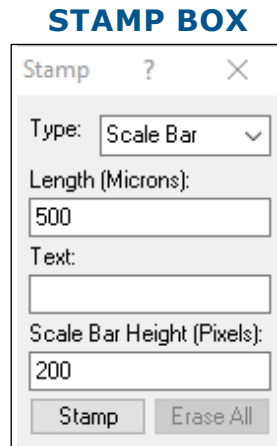
- c. In the Text box, type the Text that will appear under the stamp.

Note: If your image has very high resolution (for example if the 1:4 or 1:8 pan boxes are offered), the

text may be too small to read at resolutions other than 1:1. In this case, consider removing the text and making a policy of always using a set scale bar length for your image series so that you know what it is when you distribute images.

- d. In the Scale Bar Height (Pixels) box, type the height in pixels of the stamp.

If the image is very high resolution, consider increasing the height to a large number, so the scale bar shows up at different pan box sizes and/or on the shrunk image.



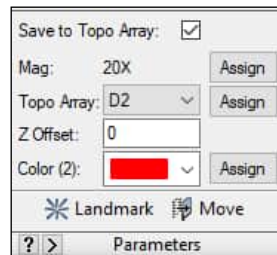
For this example, we are using a length of 500 microns.

The Text label is empty as the image is high resolution so we won't be able to see it well zoomed out.

The Scale Bar Height is 200 microns so we will be able to see it zoomed out as well.

- e. In the Parameters region of the ribbon, in the Color box, choose a color for the stamp.

### PARAMETERS REGION



For this example, we will use Red for the stamp.

- f. Click the Stamp button.

The cursor enters the Image window.

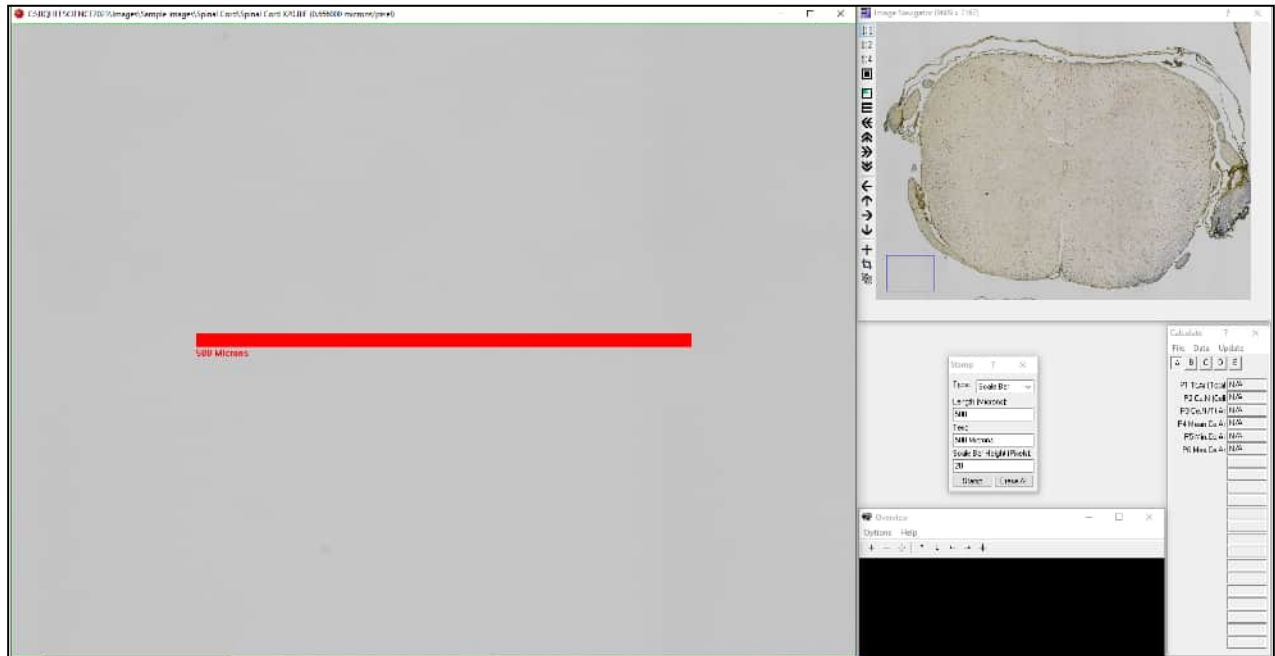
The cursor is a straight line, the length of the stamp.

If you increased the height of the stamp, the cursor represents the bottom of the stamp.

If the length entered does not fit within the Image window, you will get an error message. Adjust the number in the Length box and try again.

- g. Click in the Image window to place the stamp.

## BIF IMAGE IN IMAGE NAVIGATOR

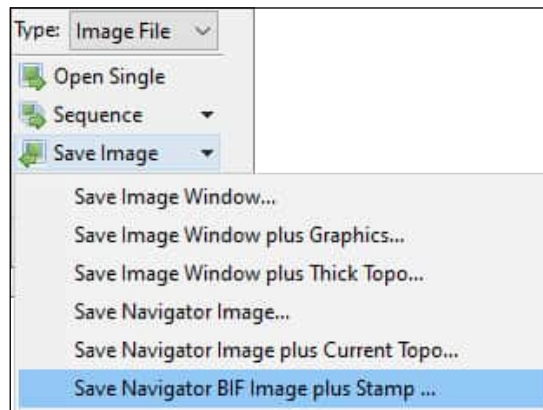


The stamp has been placed. In this example a stamp of 500 microns was used with a height of 200 pixels. Since the image is very high resolution, a text label was not used.

- h. If the stamp is too big, too small, needs its height adjusted, or needs to be replaced, on the Stamp box, click the Erase All button and repeat until you are happy with the stamp.

### 5. In the Image region (Image File Type), from the Save Image drop list, choose “Save Navigator BIF Image plus Stamp”

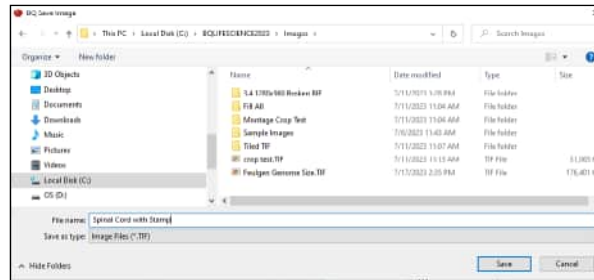
#### IMAGE REGION: SAVE IMAGE OPTIONS



Choose Save Navigator BIF Image plus Stamp.

- a. In the BQ Save Image box, in the File name edit box, enter a name for the image.
- b. In the Save as Type box, choose .BIF, .BMP, or .TIF.

## BQ SAVE IMAGE



In this example, the image is saved as a TIF image.

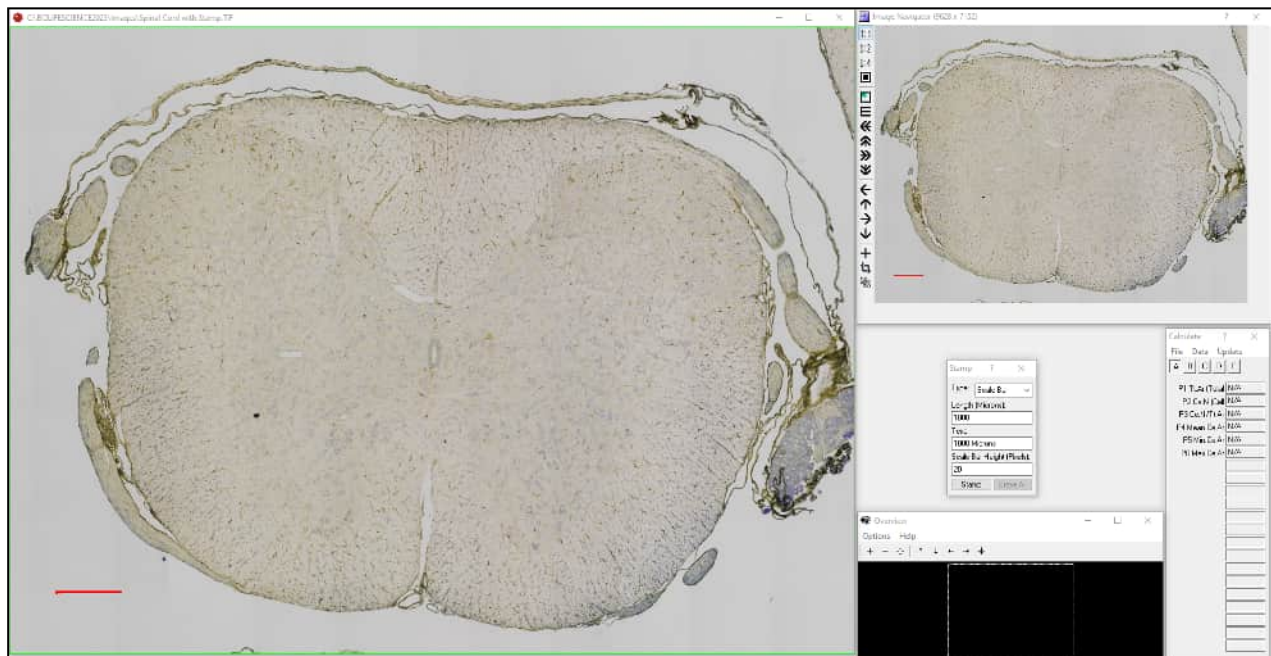
- c. Click the Save button.

The image is saved with the embedded stamp. It immediately reopens into the Image Navigator.

At 1:1, move the Pan box to the scale bar field to see it. If your scale bar height was tall enough, it will also be visible at other Zoom levels with Shrink to Fit.

If you saved to TIF or BMP, before measuring this image, make sure the system is calibrated

## BIF IMAGE IN IMAGE NAVIGATOR



The image with embedded scale bar has opened into the Image Navigator.

Move the Pan box to see the scale bar location, or click Shrink to Fit to see the entire image.

If the scale bar is only visible at 1:1, consider increasing your Scale Bar Height on the Stamp box.



## Want to Fill All Holes in the Threshold? New Editing Tool: Fill (ALL)

Previously, there were two kinds of fill: fill one hole and fill all the holes in a single object. In some contexts, this was inefficient.

A new fill tool has been added: Fill All Holes. This tool can quickly fill small holes in bone when measuring with the Auto Surface Measurement Type. Additionally, this tool is helpful when measuring with pixel based arrays, such as VC Area, Density, and Pixel Cont, and using unstained or partially stained tissue where the surfaces of interest must be drawn by hand.

The Fill (All) tool is used to fill all holes in the thresholding that are smaller than the Fill All Operator minimum size specified on the Editing Setup box.

### WHAT IS HAPPENING

Fill (All) does the following 1) Inverts the Threshold 2) Cleans threshold smaller than the Fill All Operator on Editing Setup 3) Inverts the Threshold.

With the default Fill All Operator setting of 50000 pixels, this in effect, fills all small to medium holes in the threshold.

### USING FILL (ALL) TO FILL SMALL TO MEDIUM HOLES IN THRESHOLD

With default settings, Fill (All) fills all small to medium holes in the threshold.

This is ideal for filling holes threshold when using stains like TRAP and measuring Osteoclasts with the Auto Surface Type.

### USING FILL (ALL) TO FILL LARGE HOLES CREATED BY HAND DRAWN OUTLINES.

If you have unstained sections, sometimes you are unable to threshold structures of interest.

In this case, you can use the Editing Draw tool to trace the outlines of the unstained structures. If you are using geometric based arrays, such as Area or Length, you are done, as the preview outlines are used to compute the data.

However, if you are using pixel based arrays, such as VC Area, Pixel Count, or Density, only the thresholded pixels within the preview tracings are measured. In this case, the Editing Fill (All) tool can be used to fill the very large holes created by these outlines with threshold. Then, if any internal structures, such as valid void space are thresholded, they can be erased.

### NOT NECESSARY FOR AREA ARRAY MEASUREMENTS

If you use the Area array, Fill (All) is not necessary as all pixels within the preview outline created from the hand drawn structure are measured.

## GENERAL PROCEDURE: USING FILL (ALL) TO FILL SMALL TO MEDIUM HOLES

When you notice that the threshold in the Image window has many small or medium sized holes you would like to fill at once, do the following:

1. **In the Editing region, click the down arrow on the Fill button and choose “Fill (All)” to assign Fill (All) to the button.**

**2. Click the Fill (All) button.**

The system does the following: 1) Inverts the Threshold 2) Cleans threshold smaller than the Fill All Operator on Editing Setup 3) Inverts the Threshold.

**3. Examine the image and consider the following pointers.**

**POINTERS**

- **HIDE THE THRESHOLD:** In the threshold region, click the Show button so that it is up to hide the threshold. This allows you to see the tissue underneath. Do the preview outlines line up? If not, at this point use the Editing tools to adjust the threshold.
- **CHECK FOR VOID SPACE:** Did Fill (All) fill valid void space? Use the Erase 1 (FH) tool in Editing to erase the valid void space.
- **SOME THRESHOLDED OBJECTS DID NOT FILL:** If some thresholded objects did not fill: 1) first click Undo 3 times in the Editing region to get back to before Fill (All). 2) Then, click Setup in the Editing region and increase the Fill All Operator Min Size. Click OK to close Editing Setup. 3) Then, click Fill (All) again.
- **AUTO PREVIEW:** If the system does not automatically generate preview outlines after clicking Fill (All), check the “Preview after Editing” box on the Editing Setup box.
- **FIELD TYPE - NO PREVIEW OUTLINE:** Remember that Field Type does not generate preview outlines. In this case, all threshold within the region of interest will be measured.

**SPECIALIZED PROCEDURE: USING FILL (ALL) TO FILL LARGE HOLES CREATED BY HAND DRAWN OUTLINES**

Pixel based arrays, such as VC Area, Pixel Count, and Density, measure only thresholded pixels within the preview outline. In this case, a quick way to fill all holes in the threshold may be useful.

An example of when VC Area is used is when measuring the area of trabecular bone. In this case, the valid void space should be excluded from the area measurement, so the VC Area array is used. With VC Area, as with all pixel based arrays, only thresholded pixels within the preview outline are measured. When the tissue can be thresholded, then the void spaces would automatically be excluded, as they would not be thresholded. However, with unstained bone, the user often needs to hand trace the bone as it can not be thresholded. In this case, a fast way to fill hand drawn structures is convenient. Then the few valid void space threshold can be erased and will not be included in the total area.

**AREA ARRAY - FILL (ALL) NOT NECESSARY**

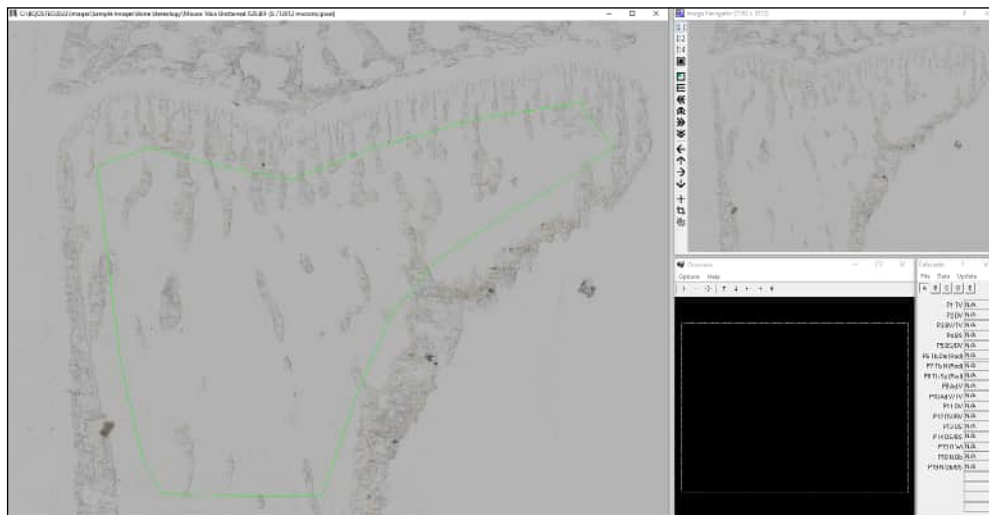
If you use the Area array, Fill (All) is not necessary as all pixels within the preview outline created from the hand drawn structure are measured.

**1. Follow your normal data collection protocol until you are ready to hand draw the outlines of the bone.**

At this point the Image window should contain the image of the bone that you need to hand trace.

If you are measuring within a Sampling region, the Sampling region of interest has also been defined.

## UNSTAINED IMAGE



In this example, the iROI Cursor has been used to create a Sampling Area region of interest..

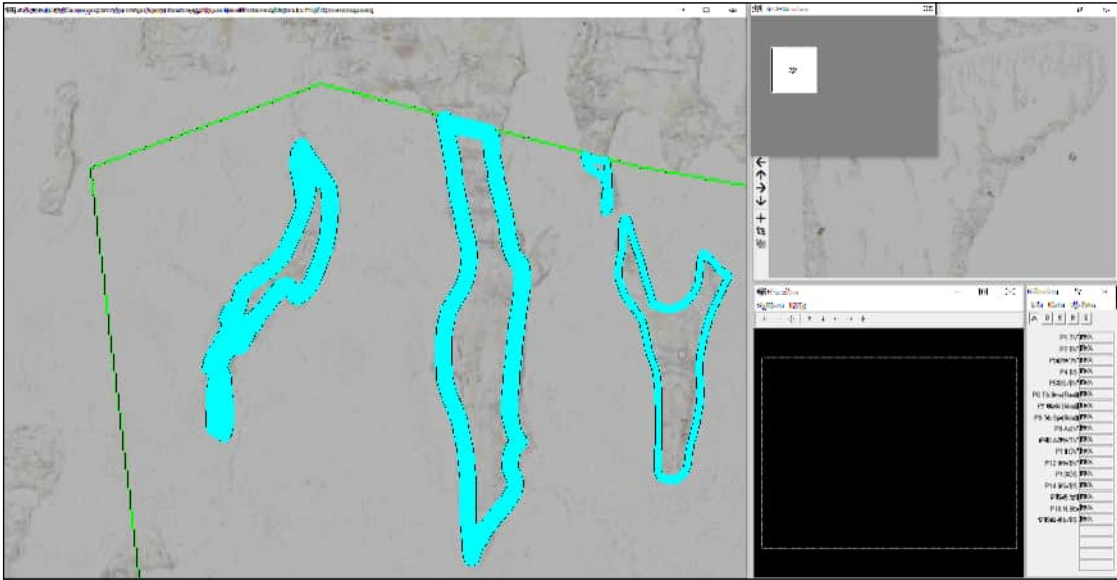
### 2. In the Editing region, click the Draw 1 (FH) button.

The pointer changes to the Draw 1 cursor and enters the Image Window.

### 3. Use these pointers to hand trace the bone:

- Use the mouse scroll wheel to increase or decrease the cursor size. The cursor size can be increased and decreased to best fit the bone you are tracing.
- Using the left mouse button, click and drag to outline the bone.
- Make sure that all outlines are closed polygons. (Fill All only fills holes).
- For bone that crosses the Sampling Area region of interest boundary, make sure to trace along the region of interest to create a closed polygon.
- You can zoom and draw. To zoom, center the cursor on where you want to zoom in. Then, on the keyboard, tap the Z key to zoom. Tap again for 2x. Tap a third time to return to original zoom level. You can draw on all zoom levels, however, you should not tap Z while tracing.

## UNSTAINED IMAGE



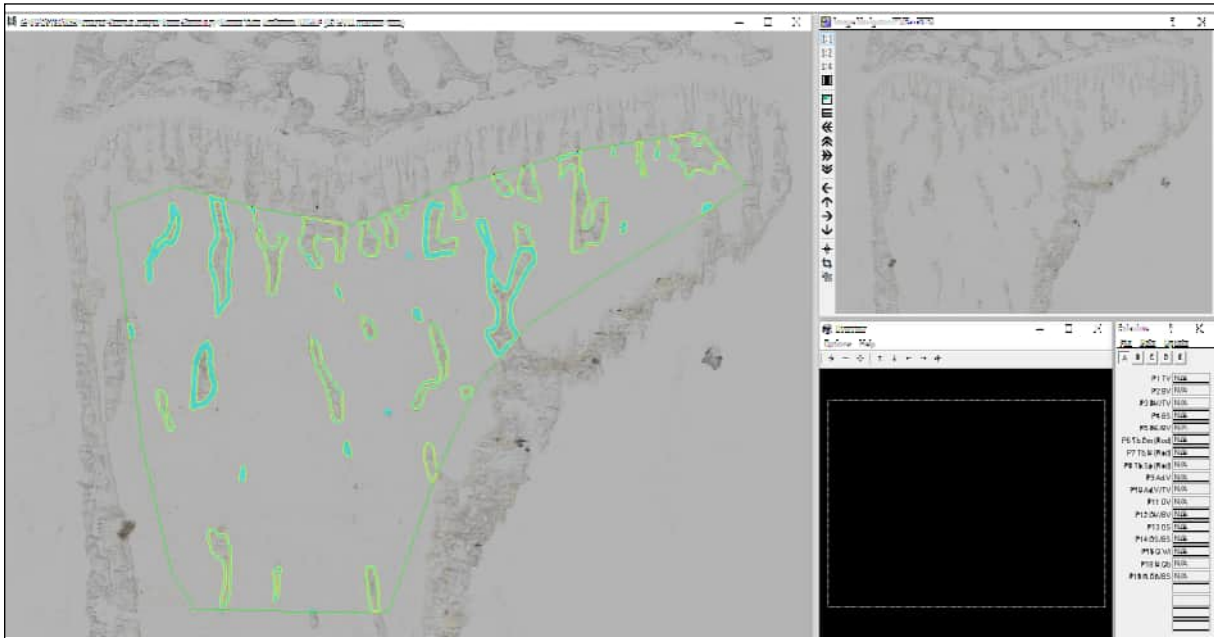
In this example, we are drawing at 2x zoom. We have adjusted the cursor size to best fit different shapes. We have made sure to close polygons, including along the ROI boundary.

- If you make a mistake, do not worry. You can use the Erase tool late to adjust boundary tracings.

#### 4. When finished, right click to exit the Image window.

At this point the exteriors of the threshold tracings are outlined and interior void space is also outlined.

## UNSTAINED IMAGE



In this example, all bone has been traced. The preview tracings are outlining all interior holes as void space at this point.

5. **Click the Fill (All) button.**

Fill (All) does the following 1) Inverts the Threshold 2) Cleans threshold smaller than the Fill All Operator on Editing Setup 3) Inverts the Threshold.

**UNSTAINED IMAGE**



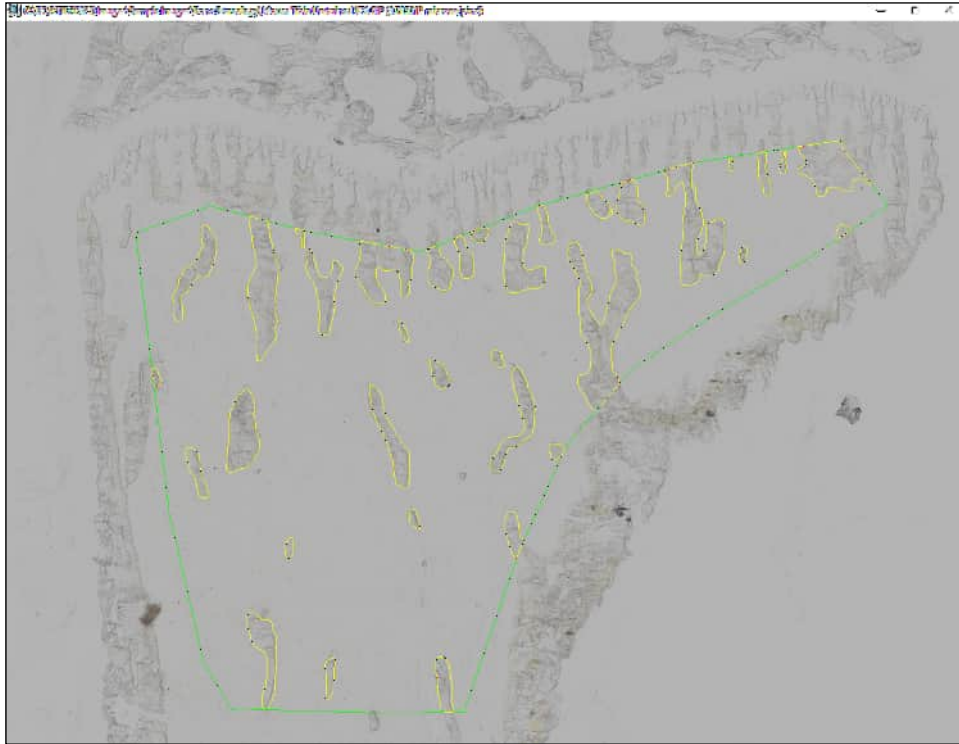
Now all the outlines have been filled.

6. **Examine the image and continue editing with the following pointers in mind:**

**POINTERS**

- **HIDE THE THRESHOLD:** In the threshold region, click the Show button so that it is up to hide the threshold. This allows you to see the bone underneath. Do the preview outlines line up? If not, at this point use the Editing tools to adjust the threshold.

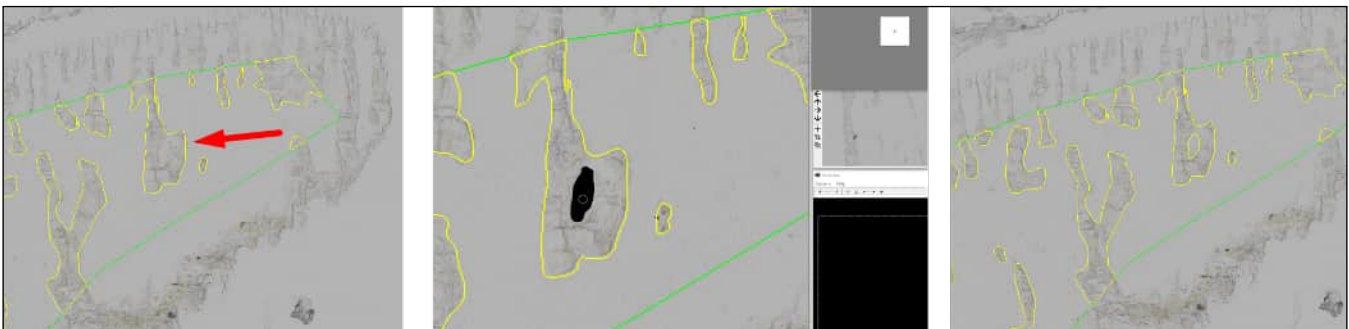
## UNSTAINED IMAGE



Toggling Show off shows the bone under the threshold.

- **CHECK FOR VOID SPACE:** Did Fill (All) fill valid void space? Use the Erase 1 (FH) tool in Editing to erase the valid void space.

## UNSTAINED IMAGE



Left: Void space is not outlined.

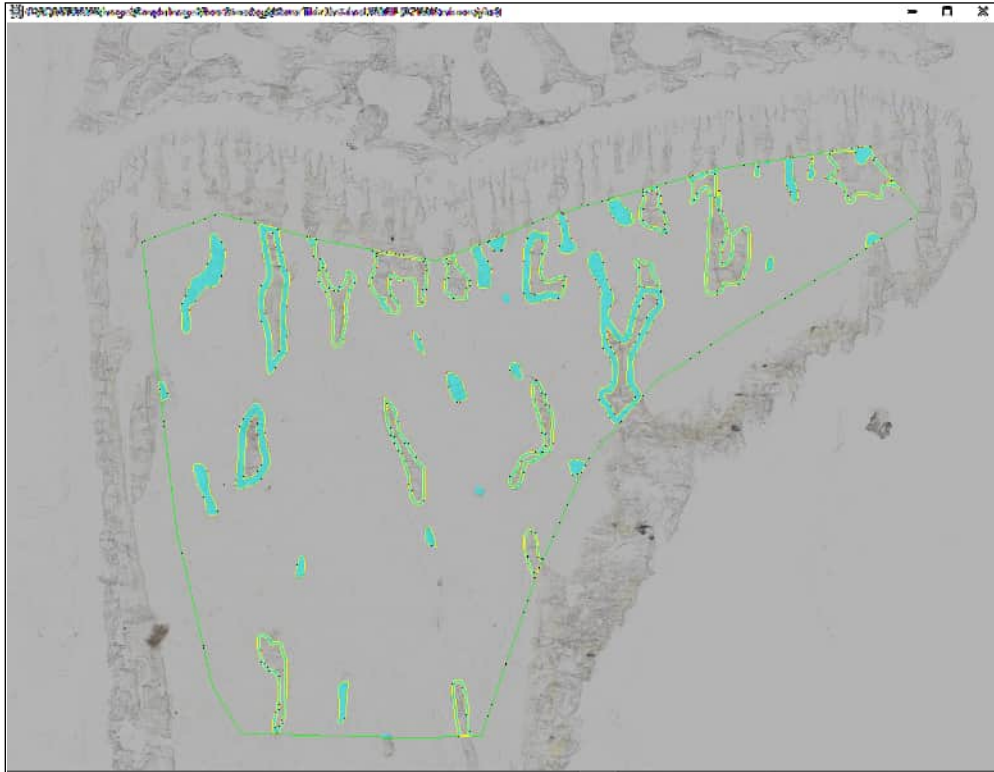
Middle: Erase tool is used to erase void space.

Right: Void space is now outlined.

- **SOME THRESHOLDED OBJECTS DID NOT FILL:** If some thresholded objects did not fill: 1) first click Undo 3 times in the Editing region to get back to before Fill (All). 2) Then, click Setup in the Editing region and increase the Fill All Operator Min Size. Click OK to close Editing Setup. 3) Then, click Fill (All) again.

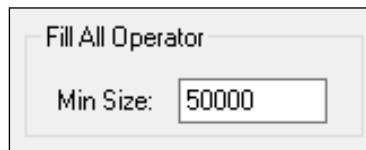


## UNSTAINED IMAGE



In this case, the Fill All Operator Min value had been set to 500. Smaller outlines filled, but large outlines did not fill. It needs to be increased to fill all the outlines.

### EDITING SETUP: FILL ALL OPERATOR REGION



The Fill All Operator Min Size has been set to 50000.

#### 7. Continue on with your measurement protocol.

## Want to Create a Precise Sampling Area Shape? Use New Stamp Protocol

Previously, there were two methods to standardize the shape of the sampling area region of interest: (1) Create a rectangle of known height and width that is a known distance from a point on the tissue section (2) Create a polygon shape that is a known distance from surfaces in the tissue section. These two tools are not always flexible enough.

BIOQUANT now includes a comprehensive set of annotation tools that can be used to create Sampling Area shapes in any necessary shape, when used with the iROI Cursor Type.

The Stamp tools are unique because they can be set to a precise micron length. An Arrow line, Diagonal line, and Scale Bar are available.

These lines and arrows of known micron length can be used to annotate the image. The user places them along structures of interest where the Sampling area should be a set distance from the surface. Multiple set distances of

different micron lengths can be defined.

Then, the iROI Cursor type can be used to draw the Sampling area using the annotations as a guide.

### VIDEO AVAILABLE

.....

This procedure has an associated tutorial video that visually walks the user through the steps. On the ROI Tools section of the ribbon, click the > button to open the Video Tutorials. Then click “iROI Cursor: Using Stamp Tools to Create Precise Sampling Area Shapes.”

## QUICK PROCEDURE

This procedure can be easily adapted to create any precise Sampling Area shapes that can be standardized across a project.

- 1. In the Image window, get an image of the region where you would like create a Sampling Area region of interest.**
- 2. From the Image menu, choose Open Stamp Tools to open the Stamp box.**
- 3. On the Stamp box, use the Arrow line, Diagonal line, and/or Scale Bar to annotate the Image.**

### POINTERS

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- The precise micron distance (Diagonal line, Arrow line) and micron length (Scale Bar) is set on the Stamp box. This value can be changed between placing annotations, making it useful to mark varying micron distances from different surface structures.
  - To change the color of the Stamp annotations, use the Color drop list in the Parameters region.
  - Multiple stamp types can be placed on the image.
  - To start over, on the Stamp box click Erase All.
  - In the Image region, if you accidentally Clean the stamps, click Redraw to bring them back.
  - Stamps remain on the image as long as the Stamp box is open. They are cleared when the Stamp box is closed.
- 4. Keep the Stamp box open. In the ROI Tools region, choose the iROI Cursor from the Type list.**

The annotations temporarily clear from the Image. They will return when the Define button is clicked. The annotations only return when defining an irregular region of interest with the iROI Cursor Type.
  - 5. In the ROI Tools region, the radius of the iROI Cursor can also be set to a set micron distance. If you would also like to use a known radius, enter the micron length in the Radius field now.**
  - 6. In the ROI Tools region, click the Define button. The cursor enters the Image window.**
  - 7. In the Image window, the stamp annotations are now visible. Click along the annotations to define the irregular region of interest.**

### POINTER

.....

- The size of the iROI Cursor radius can be adjusted dynamically by scrolling the mouse wheel



up or down.

**8. Once finished, right click to exit the Image window.**

The region of interest has been defined.

At this point, if you want a visual record of the markup and the ROI, in the Image region, click the Redraw button to redisplay the arrows. Then, in the Image region (Type: Image File), from the Save Image drop list, choose Save Image Window + Graphics.

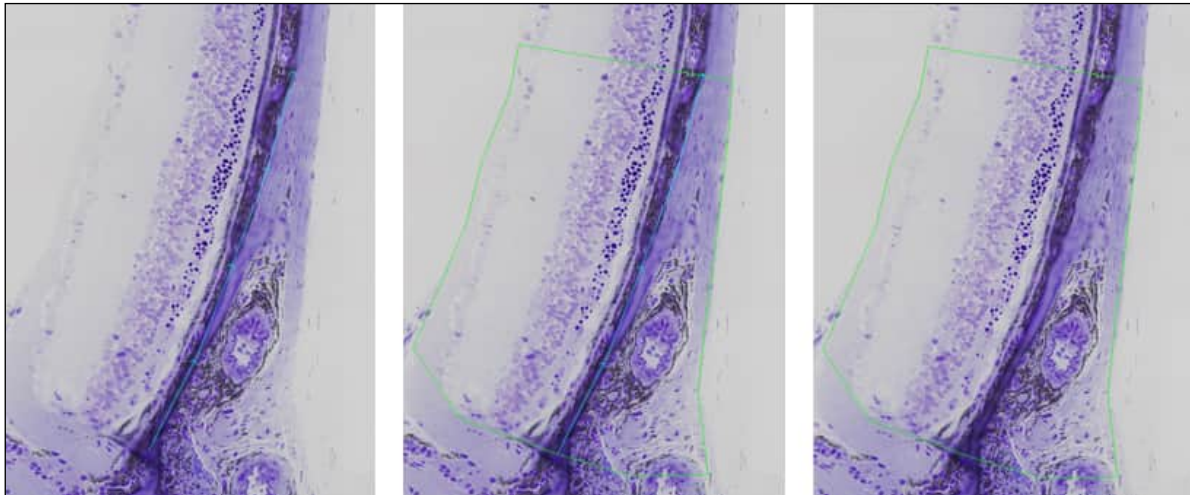
You're ready to use the irregular region of interest to collect your data!

### **EXAMPLE: CURVED SAMPLING AREA**

In this example, the end user wanted an irregular region of interest that extended 500 microns along the retina, upwards from the optic nerve.

**1. First the Arrow line Stamp tool was used to place annotations on the image in the Image window.**

#### **STAMP TOOLS ANNOTATIONS WITH IROI TV EXAMPLE**



Left: The Stamp Tools were used to draw 5 consecutive arrows of 100 microns each to create a curved line 500 microns long.

Middle: The iROI cursor was used to draw an irregular ROI along the anatomical borders, to a distance of 500 microns from the Optic Nerve.

Right: The resulting ROI as used as a Sampling Area for measurement.

**2. Next, while keeping the Stamp box open, the iROI Cursor Type in the ROI Tools region was used to draw an irregular ROI, using the annotation to determine the distance from the optic nerve.**

**3. When finished, the resulting region of interest was used to limit data collection to within its boundaries.**

### **EXAMPLE: SAMPLING AREA REQUIRING MULTIPLE MICRONS DEPTHS FROM DIFFERENT SURFACES**

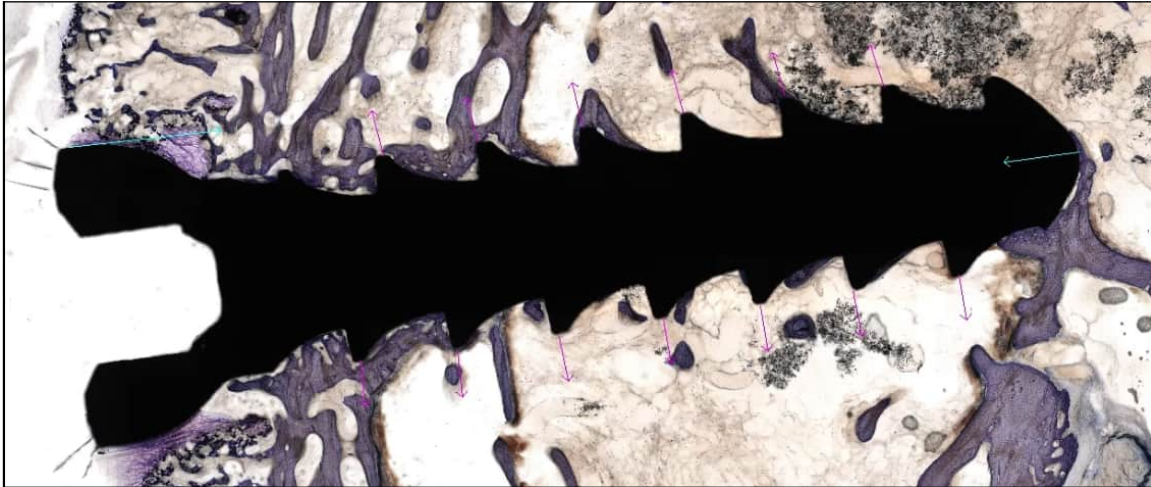
In this example, the user wanted a Sampling Area to measure bone to implant contact surface that is within 150

microns of the screw shaft, starting 1000 microns below the periosteal surface, and ending 150 microns from the screw tip.

**1. First the Arrow line Stamp tool was used to place annotations on the image in the Image window.**

The Annotations were color coded to indicate their micron length and purpose. (To change the color of the Stamp annotations, use the Color drop list in the Parameters region.)

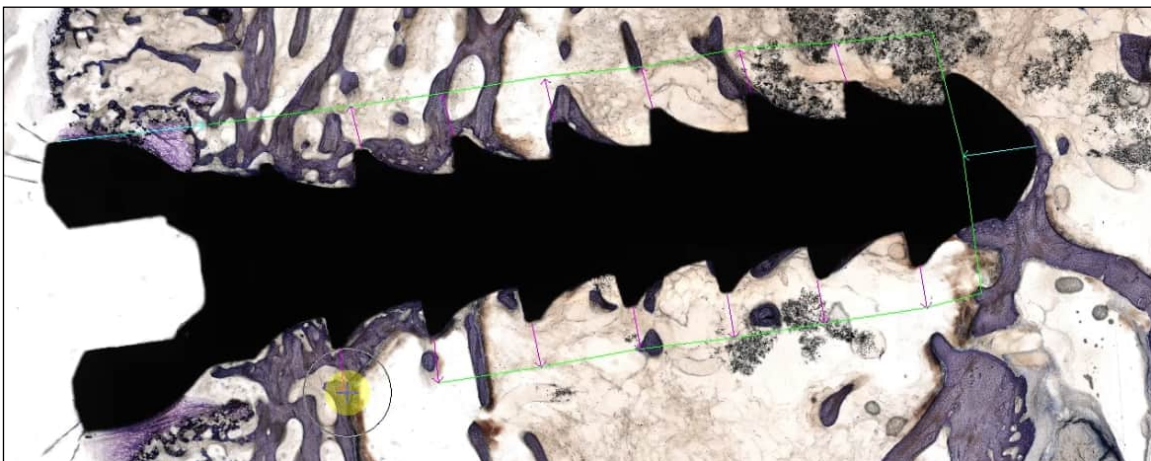
**STAMP TOOLS ANNOTATIONS WITH IROI TV EXAMPLE**



The user placed a 1000 micron blue arrow from the periosteal surface, pointing in the direction of the screen. They placed a 500 light blue arrow at the tip of the screw, pointing in the direction of the screw. Finally, they placed 500 micron pink arrows from the set of the tips of the screw threads, pointing away from the screws.

**2. Next, while keeping the Stamp box open, the iROI Cursor Type in the ROI Tools region was used to draw an irregular ROI, clicking from annotation to annotation.**

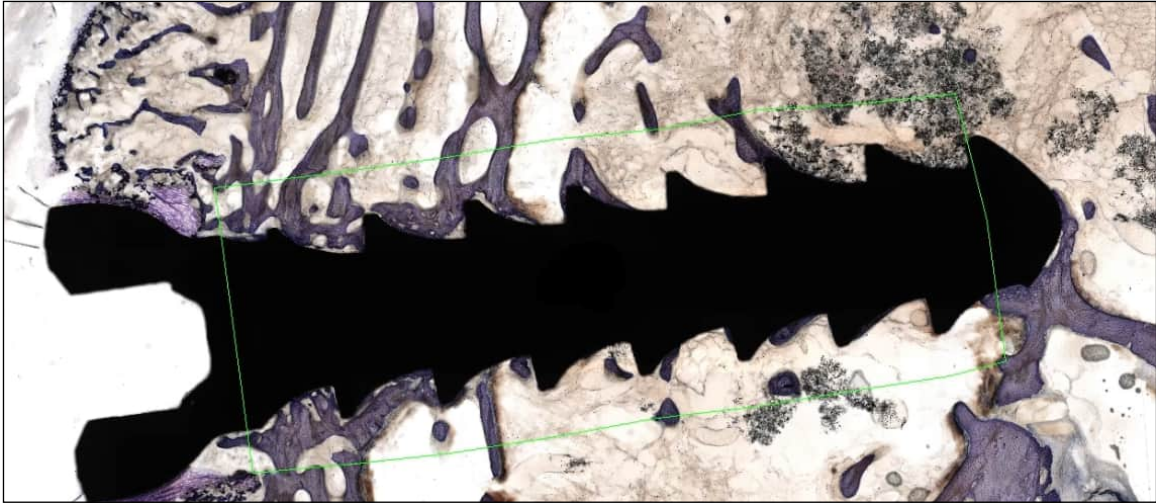
**STAMP TOOLS ANNOTATIONS WITH IROI TV EXAMPLE**



The iROI cursor was used with the stamp annotations to draw an irregular region of interest a precise micron distances from key anatomical structures.

**3. When finished, the resulting region of interest was used to limit data collection to within its boundaries.**

## STAMP TOOLS ANNOTATIONS WITH IROI TV EXAMPLE



# BIOQUANT Life Science & Data Manager Enhancements

## New Project Wizard: Data Volume Name Now Matches Explorer Folder .....

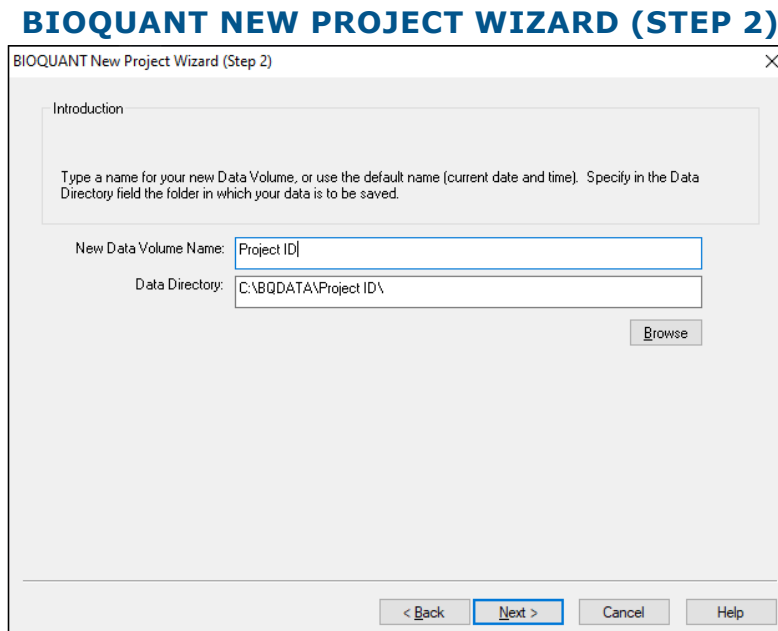
Previously, projects were placed in automatically numbered folders. For example C:/BQDATA/DATA57/. This makes it hard to find your project later.

Now, projects are stored in folders with the same name as the project name. For example: C:/BQDATA/My Project/. This makes it easier to find projects for data export and in the Data Manager.”

In practice, on Step 2 of the New Project Wizard, the Data Volume Name now automatically appends to the Data Directory path.

### PROCEDURE EXCERPT

1. In the Data region, click “Create New Project” to open the BIOQUANT New Project Wizard (Step 1).
2. On the BIOQUANT New Project Wizard (Step 1), choose the “Create New” button and click Next. Create New will create a new data volume, or project, instead of using the current one.
3. On the BIOQUANT New Project Wizard (Step 2), do the following:



Type a name for the new Data Volume.

- a. In the New Data Volume Name field, type a name for the new volume. (The default name is the current date and time.)

Note that the New Data Volume Name is automatically appended to the Data Directory path. This makes it easy to find your Data Volume on the hard drive.

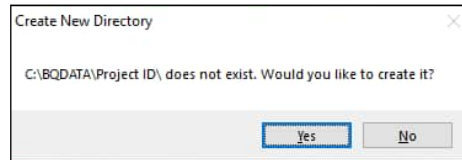
If you do not want to store the Data Volume in C:\BQLifeScience2023, then use the Browse button to



navigate to a different path. Note that the New Data Volume Name is automatically appended to the new Data Directory path.

- b. Click the Next button. On the Create New Directory box, click Yes.

## BIOQUANT NEW PROJECT WIZARD (STEP 2)



Click Yes to create the new directory.

4. On the BIOQUANT New Project Wizard (Step 3), in the list, click to choose the template for your Data Set and click Next.
5. On the BIOQUANT New Project Wizard (Step 4), in the Data Set Name box, type a name for your data set and click Finish.

## Export Data Table: Now Exports Formulas for Computed Values

Previously, in the Export Data Table box exported the data from the Calculate box, the Array names, and the Data Set names. It did not include the formulas used in the calculation of the data.

Now, underneath the Array name row, is a new row which contains the formulas used to compute the data in that column. These formulas are found in the Comment field of each array. This helps improve the audit trail for data collected with BIOQUANT since the formulas used to compute data are now part of the data table.

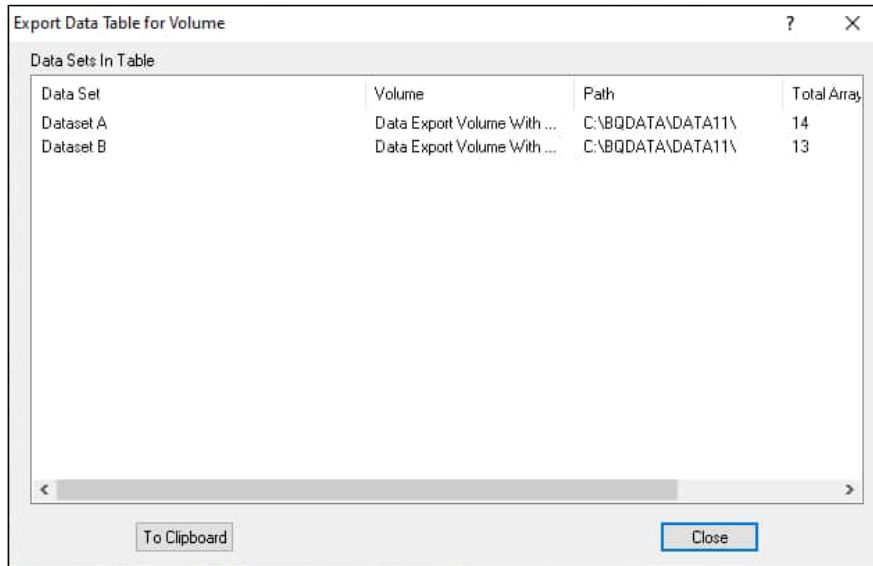
This feature is supported both in “Export Data Table for This Dataset” in the Data Region of BIOQUANT Life Science and in “Export Data Table” in the Data Manager.

### PROCEDURE

1. On the BIOQUANT Life Science title bar, note the current data volume and data set. The data volume is the project that will be exported.  
All the calculations in all the data sets in this data volume will be used to create a data table.  
If this is not the correct data volume, open a data set that is in the correct data volume.
2. In the Data region, click Export and choose “Export Data Table for this Project” to open the Export Data Table for Volume box.
3. In the Data Sets in Table list, confirm that the correct data sets and data volume are listed. If they are not, close the box and then open the correct data set.

If this is not the correct data volume, open a data set that is in the correct data volume.

## EXPORT DATA TABLE FOR VOLUME



Confirm you are in the correct data volume.

4. Click the “To Clipboard” button.
5. Open the spreadsheet program into which you would like to paste.
6. Click the top left cell in the spreadsheet.
7. Click Edit and choose Paste.

The data volume calculation data is pasted in data table format.

Row 1 contains the Array Index.

Row 2 contains the Array Name.

Row 3 contains the Array Comment.

Row 4 and down contain the data values.

## SPREADSHEET

	A	B	C	D	E	F	G	H	I
1		P1	P2	P3	P4	P5	P6	P7	P8
2		TV Tissue Volume	BV Bone Volume	BV/TV	BS Bone Surface	BS/BV	Tb.Dm (Rod)	Tb.N (Rod)	Tb.Sp (Rod)
3		SUM(D3)/1000000; mm2	SUM(D4)/1000000; mm2	P2/P1; %	SUM(L1)/1000; mm	P4/P2; 1/mm	4*(P2/P4); mm	SQRT(4/3.141*P2/P1)*(1/P6); 1/mm	(SQRT(3.141/4*P1/P2)-1)*P6; mm
4	Dataset A	2.111736	0.26809	0.126953	12.982863	48.427206	0.082598	4.867951	0.122827
5	Dataset B	2.111736	0.329618	0.156089	13.195366	40.032291	0.099919	4.462027	0.124194
6									

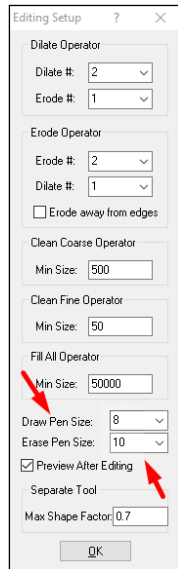
Paste the data into your spreadsheet.

## Editing Region: Draw & Erase Faster with Larger Brush Sizes

Previously, the maximum radius of the Draw and Erase brushes was 30 pixels. This was a bit small for editing high magnification images of bone.

Now, the maximum radius of the Draw and Erase brushes is 60 pixels. That allows for brushes that are up to 4 times larger than before. This should make editing the threshold to identify bone at high magnification faster.

## EDITING SETUP



Draw and Erase Pen Size can now be set up to 60 pixels.

### TO CHANGE THE DEFAULT PEN SIZE FOR THE DRAW CURSORS:

- In the Editing region, click the Setup button to open Editing setup. On Editing Setup, from the Draw Pen Size drop list, choose the desired pen size. Then click the OK button to close Editing Setup.

### TO CHANGE THE DEFAULT ERASE PEN SIZE FOR THE ERASE CURSORS:

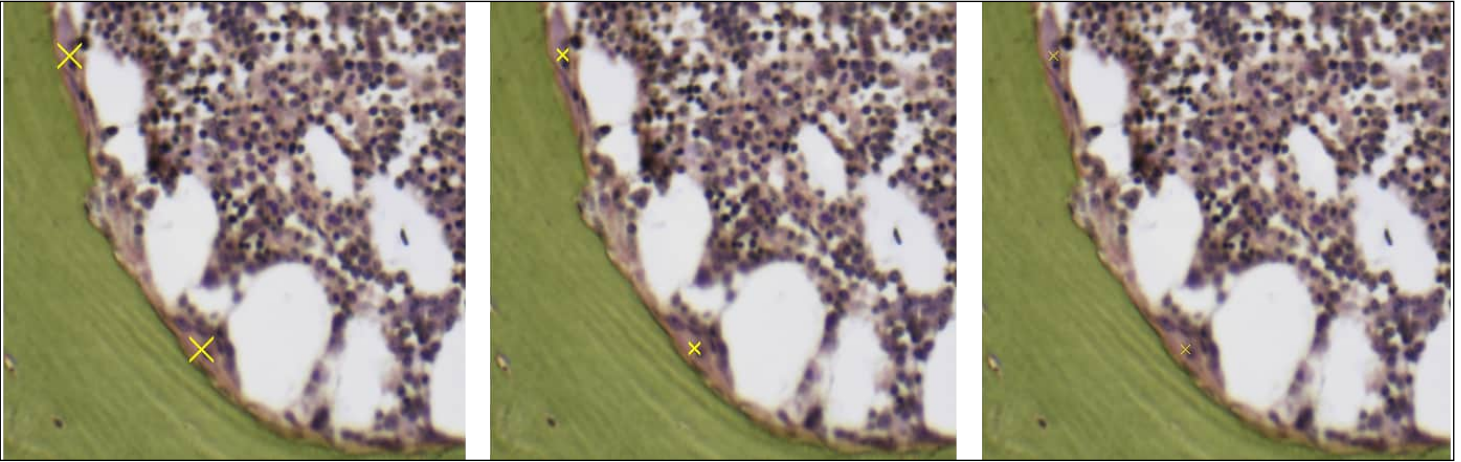
- In the Editing region, click the Setup button to open Editing setup. On Editing Setup, from the Erase Pen Size drop list, choose the desired pen size. Then click the OK button to close Editing Setup.

## The “x” Marker Length & Thickness Options Have Increased.....

Previously, when counting objects using Object Count arrays, the software marked to location of each counted object with an X that was between 1 and 9 pixels in size. These marks were sometimes hard to see.

Now, the X markers have been made more visible. X markers can now be as large as 23 pixels and for markers 11 pixels and larger, the thickness of the arms of the X are thicker. The default marker size has been changed to 11 which will make it easier to see the marks while counting.

## OBJECT COUNT MEASUREMENT



Left: Point Marker Size 23. Middle: Point Marker Size 11, Right: Point Marker Size: 9

The size of the Object Count or other point markers is set on the Customizing box. In addition to Object Count arrays, the Landmark, and Stereology grid marks also use Point Marker Size.

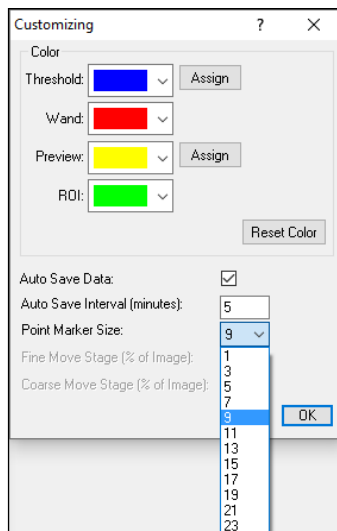
If the Point Marker Size is 9 or less, the marker is drawn as an “x” single pixel thick. If the Point Marker Size is 11 or more, the maker is drawn as an “x” with arms 3 pixels thick.

The Point Marker size is saved when you exit and return to BIOQUANT.

## PROCEDURE

1. From the Options menu, choose “Open Customizing” to open the Customizing box.
2. On the Customizing box, from the Point Marker Size list, choose the desired size.

### CUSTOMIZING BOX



Choose a new Point Marker Size from the list.

3. Click the OK button to close.



## Batch Scripts: Now Supports Sequencing Through 5D Navigator Planes .....

Confocal and lightsheet data are becoming increasingly important for bone and muscle research. BIOQUANT is continuing to expand its support for the automated analysis of this data.

For 5D image sequences that can use the same region of interest, are very easy to threshold without editing, and automate nicely with Preview/Measure, Next Image, Repeat, the 5D Navigator now supports batch measurement using the Batch Script Editor.

Note that Batch Scripts can also be used interactively to walk the user through a sequence of steps, by adding Pause messages to give the user time to do editing, or by adding threshold editing commands themselves. In this case, the Batch Script does the routine steps and pauses to check in with the user to see if threshold editing is needed before measuring.

New Batch Script command that loads the next 5D Image Z Plane:

<b>COMMAND NAME</b>	<b>COMMENT</b>
Load Next 5D Z Plane	Z Plane Right Arrow Button 5D Image Navigator

### FOR MORE INFORMATION

- In the Measurement region, click on ? “Open PDF Manual” button to open the BIOQUANT Life Science Manual. Then, in the Chapter Contents, choose “The Measure Menu: Batch Script Editor.”

## Threshold Select/Add: New “Ctrl” + Scroll For Faster Thresholding .....

To speed the process of thresholding using Select and Add, a keyboard modifier has been added to the scroll up/down process. When you scroll the mouse button to add or remove threshold, the system increases or decreases the threshold range by 2. Now, if you hold down the “Ctrl” key while scrolling, the system will increase or decrease the threshold range by 20.

### QUICK PROCEDURE

1. In the Threshold region of the ribbon, click Select.

The cursor enters the Image window.

2. Choose from the following:

#### CHOOSE THE INITIAL COLOR

- With the left mouse button, click an initial color on one of the objects of interest.

The pixel clicked and any other pixels in the Image Window that match the selected pixel’s red, green, and blue intensity values are highlighted.

#### AUTO GENERATE INITIAL COLOR

- Immediately scroll up and the software will pick the initial color for you based on the colors in the image.

Auto Generate works best on single stained slides, like TRAP.

**3. Scroll the mouse wheel up to add threshold automatically.**

If you add too much, scroll the mouse wheel down to remove threshold automatically.

**MOUSE BUTTON SCROLL NOTE**  
.....

When you scroll the mouse button to add or remove threshold, the system increases or decreases the threshold range by 2.

If you hold down the “Ctrl” key while scrolling, the system will increase or decrease the threshold range by 20.

**4. Now, click a second color. The threshold will expand to include second color. Now scrolling the mouse wheel will add colors similar to the expanded color range.**

If you add too much, scroll the mouse wheel down to remove threshold automatically. Remember, you can hold down the Ctrl key to expand or contract the threshold range in larger steps.

**5. When you are happy with the threshold, right click to exit the Image window.**

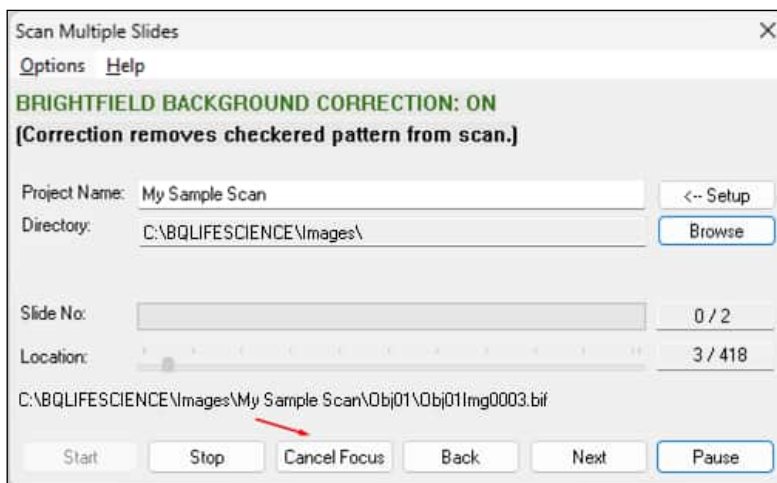
# BIOQUANT SCAN Enhancements

## Scan: New “Cancel Focus” Button Lets You Override Auto Focus

Currently, automatic focus correction is either enabled or disabled in the beginning setup for a series of scans. Normally, it is on. However, it's not always easy to predict if a tissue section is going to be compatible with the automatic focus correction. Some very wavy sections or some very thick sections don't work well with autofocus.

To help address this situation, a “Cancel Focus” button has been added that is available while the scan is running. Now, if the auto focus controls are not working well with a scan, you can click the “Cancel Focus” button to revert the scan to Manual Focus Correction without interrupting the scan. The scan will continue as configured, now pausing periodically for manual focus correction.

### SCAN MULTIPLE SLIDES BOX



New Cancel Focus button.

## PROCEDURE EXCERPT

1. Click the Start button to start the scan.
2. If you notice a field of view out of focus, click the Pause button.  
This stops the stage.
3. After the stage stops, click the Back button until you reach the out-of-focus field.
4. Refocus the image.

## WHEN TO USE CANCEL FOCUS

The Cancel Focus button is active once the project is started.

Automatic focus is either enabled or disabled in the beginning setup for a series of scans. It is defaulted to ON. However, it's not always easy to predict if a tissue section is going to be compatible with the automatic focus correction. Some very wavy sections or some very thick sections don't work well with autofocus.

During the scan, if the user notices the auto focus correction is not working well, the user can click Pause to

pause the scan, then click the Cancel Focus button to turn off auto focus and revert to manual focus. From that point on, the scan will continue as configured, now pausing periodically for manual focus correction, using the Manual Focus Method above.

Since Cancel Focus does mean that the user would need to manually focus for the remainder of the slide, only use it if the auto focus stops working. If you can manually adjust the focus on one blurry field and then auto focus can find the focal depth again, it is faster to keep it on.

**5. Click Continue to start scanning again from the current location.**

# BIOQUANT Life Science & Data Manager Issue Resolutions

The following minor enhancements have been made to the BIOQUANT Life Science and Data Manager.

## Exporting Data Issue Resolutions

A number of issues related to exporting data have been addressed. They include: defaulting to 6 decimal places for data export in all contexts, adding and removing multiple array simultaneously from the export individual arrays box, allowing the export of more than 21 arrays using the export individual arrays box. This should make exporting data simpler and more predictable.

### EXPORT DATA: IDENTICAL NAME ISSUE

In both BIOQUANT Life Science and Data Manager, if the Data Set Names in the Export Raw Data box were identical, the second Data Set name would not export

Additionally, in Data Manager, if the Data Volume names in the Export Raw Data box or Export Topo Arrays box were identical, the second Data Volume name would not export.

These issues have been resolved in both BIOQUANT Life Science Data Region (Export>Export Raw Data for This Data Set) and BIOQUANT Data Manager (Export Raw Data and Export Topo Arrays).

### EXPORT RAW DATA: CRASH ISSUE

Export Raw Data crashed with certain data sets. The algorithm has been improved.

This issue has been resolved in both BIOQUANT Life Science Data Region (Export>Export Raw Data for This Data Set) and BIOQUANT Data Manager (Export Raw Data).

### EXPORT INDIVIDUAL ARRAYS: PRECISION ISSUE

In BIOQUANT Life Science, in the Data Region, Export>Export Individual Arrays used the precision set on the Calculate box when exporting data. However, this precision defaults to 2 and users want to export more decimal places. All the other Export features exported with 6 decimal places.

Export Individual Arrays has been changed to export with 6 decimal places.

### EXPORT INDIVIDUAL ARRAYS: BACK BUTTON ISSUE

On the Choose Arrays for Export box, the Back <<< button would only move back 1 array at a time, even if multiple are selected. This issue has been resolved.

To open the Choose Arrays for Export box, in BIOQUANT Life Science's Data region, choose Export, then Export Individual Arrays.

## **EXPORT INDIVIDUAL ARRAYS: 21 ARRAYS LIMITATION ISSUE**

On the Choose Arrays for Export box, you could not add more than 21 arrays to the Destination clipboard box if you have ever removed an array from the Destination clipboard box previously. This issue has been resolved.

## **Large Image Navigator Issue Resolutions**

The Image Navigator has been enhanced to streamline functionality with Crop Image.

### **LARGE IMAGE NAVIGATOR: CROP IMAGE: TRACINGS DON'T ALIGN ISSUE**

A rare issue related to the update of the calibration data after cropping images at certain zoom levels has been addressed. Previously, if the cropped version of an image was automatically reloaded after cropping the original, it was necessary to change the zoom on the Image Navigator to update the calibration data. This has been resolved.

#### **PROCEDURE THAT CAUSED ISSUE:**

- a. Crop from a zoom level 1:1, 1:2, 1:4, 1:8, 1:16. Do not use shrink to fit.
- b. Then on the cropped image, measure data at 1:1.

After changing the zoom level to 1:2, 1:4, 1:8, or 1:16, the data values were too big and tracings made at 1:1 were not the right size.

The problem was not visible if you stayed on 1:1 and toggled Shrink to Fit off and on.

This issue has been resolved.

### **LARGE IMAGE NAVIGATOR: CROP IMAGE: CLEAR THE CROP BOX ISSUE**

The Crop box is now cleared from the Image Window after the cropping if “Reload After Save” is Off.”

## **Image Region Issue Resolutions**

The Image Region has been improved with several issue resolutions.

### **CONVERT IMAGE SEQUENCE TO BIF: SOME CONVERSIONS BROKE ISSUE**

An issue with reviewing images created by the TIF to BIF batch conversion has been resolved. Sometimes, after converting a set of TIF images to BIF images, it was necessary to restart BIOQUANT before the new images could be opened. This is no longer necessary.

### **OPEN TILED TIF IMAGE: INCORRECT RGB CHANNEL ORDER ISSUE**

BIOQUANT has expanded its support for TIF images created by the export functions of some digital pathology systems. Specifically, some digital pathology systems export TIFs with the data structured in tiles rather than strips. Previously, when tiled TIF images were open, the R and B channels were exchanged. These tiled TIF images are now supported in BIOQUANT.