

# OSTEO<sup>SM</sup>

# 20.8.6



## August Update

This documents contains Installation Instructions and Release Notes for the BIOQUANT OSTEO 20.8.6, 8/31/2020 update.

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BIOQUANT Image Analysis Corporation  
Version 20.8.6, 8-31-2020

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## MINOR ISSUE RESOLUTIONS

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The following minor changes have been made.

- Issue Resolved: BIOQUANT default Path Gets Broken if Image is Opened or you go Live/Held**  
The default path information for the Selected Lists and Tutorial Volumes was getting broken when certain imaging actions were taken, for example, going Live/Held or opening an image. The largest effect of this issue is that the path to the Selected List memorized to the data set was forgotten, so the user had to open the Selected List on their own. This issue has been resolved.
- Issue Resolved: “Save to Topo” unchecks if you go to Modify Dataset without saving data first**  
In previous versions, if you had measured data, and then went to Modify Dataset without saving the data first, after you clicked Apply to leave Modify Dataset, “Save to Topo” in the Parameters regions would uncheck. This issue has been resolved.
- Issue Resolved: Background Correction: Histogram Issue**  
If you’re on a live image, when you click the Full Field Histogram button on Background box, the image goes held after the histogram is generated so the histogram is actually based on the prior held image, not the held image currently in the Image window. This could cause issues with determining the Correction Level. This issue has been resolved.
- Issue Resolved: 5D Navigator Calibration Issue**  
The 5D Navigator had a calibration issue that caused the Measurement Types to always record 0, and the Redraw, ROI, and Auto ROI to perform poorly. The Y aspect ratio was broken. This issue has been resolved.
- Issue Resolved: 5D Navigator Setup box: Coarse Move Step (Cells/Fibers) used Navigator Setup Value**  
The Coarse Move Step (Cells/Fibers) value on 5D Navigator Setup was ignored. Instead the Coarse Move Step (Cells/Fibers) value on the Navigator Setup box was used. This issue has been resolved.

## FEATURES IN OSTEO 2020 20.5.6, 5-31-2020 RELEASE

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All new features in the BIOQUANT OSTEO 2020 20.5.6, 5-31-2020 Release are also included in the BIOQUANT OSTEO 2020, 20.8.6, 8-31-2020 Release.

OSTEO<sup>SM</sup>

20.5.6



# Release Notes

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# WINDOWS 10 REQUIRED. WINDOWS 7 NOT SUPPORTED.

Microsoft discontinued support of Windows 7 on January 14, 2020. BIOQUANT OSTEO 2020 no longer supports Windows 7.

## MAJOR NEW FEATURES

### JENOPTIK NEXT-GENERATION SCMOS CAMERA SUPPORT

#### NEW JENOPTIK CAMERA SUPPORT



The advertisement 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 square-shaped camera, a smaller cube-shaped camera, and a larger cube-shaped camera. The text 'Technical data' is on the left, and 'KEY FACTS' is on the right, listing technical specifications.

**JENOPTIK**

PROGRES GRYPHAX® PROKYON

Technical data

Explore the micro universe with the flagship.

KEY FACTS

- True color microscope camera 70 71 9 21 2 3 MPix
- 60 fps at 7 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

#### JENOPTIK GRYPHAX® CAMERAS

BIOQUANT is 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, SUBRA cameras from Jenoptik.

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

Preliminary support for the Jenoptik Prokyon camera was first included in the BIOQUANT OSTEO 2019 June Update. Prokyon capture sizes of 3840 x 2400 and 5760 x 3600 are new to BIOQUANT OSTEO 2020.

## 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 fanless cooling. The **Kapella** camera keeps the fanless cooling but saves money by removing the 20 megapixel imaging mode. The **Subra** model is even more economical than the Kapella by removing the fanless 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 range from **\$690 to \$1990** depending on the Jenoptik camera model purchased. Please act quickly, this is a limited time promotion.

# 5D IMAGE NAVIGATOR FOR CONFOCAL AND LIGHTSHEET IMAGE SUPPORT

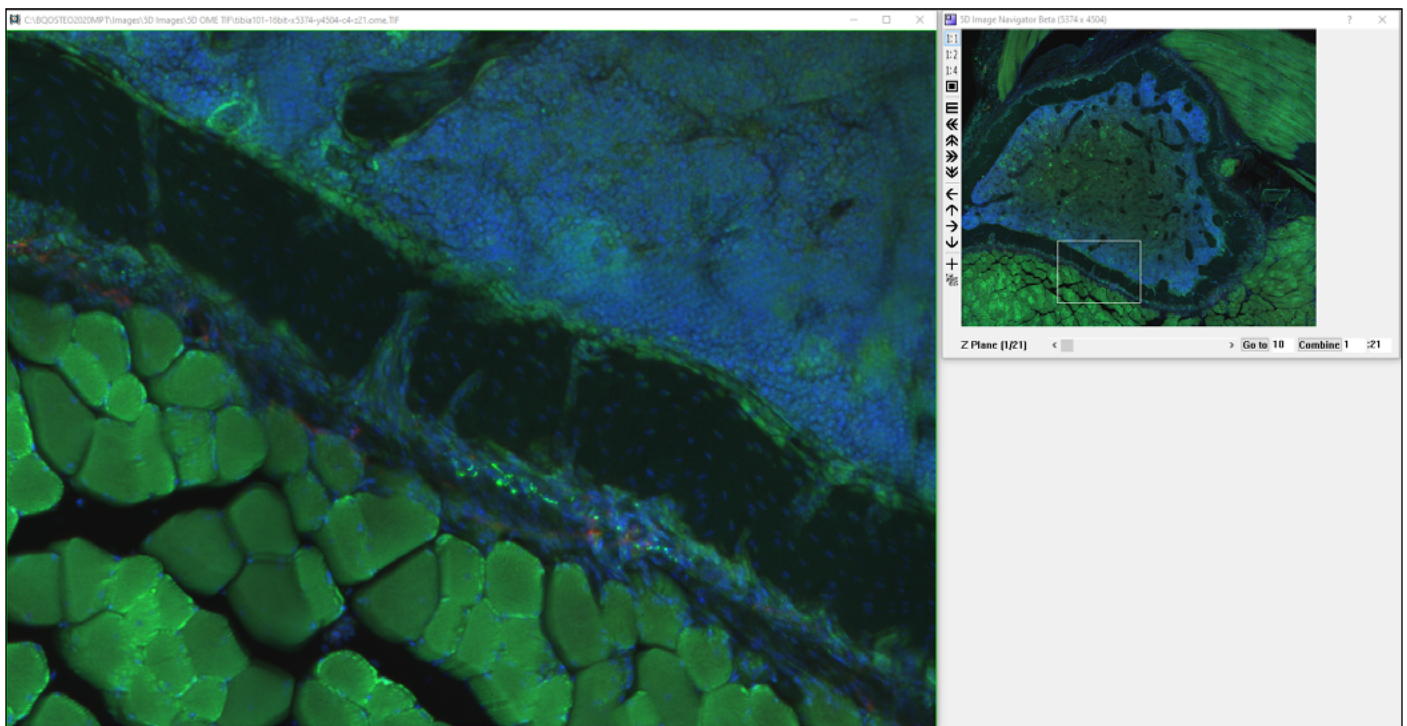
## CONFOCAL AND LIGHTSHEET DATA FOR MUSCULOSKELETAL RESEARCH

BIOQUANT is pleased to announce support for a new dimension in musculoskeletal research. BIOQUANT OSTEO now supports multi-dimensional image data from confocal and lightsheet microscopes.

New optical clearing techniques for in-tact bone have made the use of confocal microscopy possible in previously inaccessible bone samples. Labs are now leveraging confocal microscopy in bone to explore solutions to previously intractable questions.

Simultaneously, a new generation of microscope systems called Lightsheet microscopes are starting to collect enormous image data sets of entire bones with multiple fluorescent labels and multiple Z depths at sub-micron resolutions.

### 5D IMAGE NAVIGATOR - CORTICAL BONE INNERVATION.OME.TIF\*



A high magnification view of a cortical bone cross-section showing nerve distribution along the periosteum and across the cortical bone. Composite image of 5 Z planes and 3 different fluorescent labels.

#### \* CORTICAL BONE INNERVATION.OME.TIF

Multi-channel Confocal Stack of Cortical Bone Innervation by Erica Scheller and Washington University in St. Louis (<https://sites.wustl.edu/schellerlab/>) is licensed under CC BY 4.0. To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0>.



## THE DETAILS OF 5D IMAGING

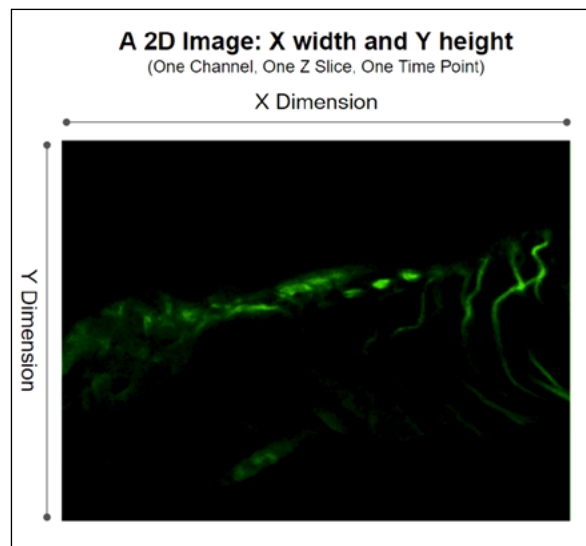
### NEED AN INTRODUCTION TO 5D IMAGING?

Watch the “Introduction to 5D Imaging Video.” In the Image region, click the > button to open the Tutorial Videos.

5D imaging is a strategy for organizing images of fluorescent samples. The 5 dimensions we’re talking about are x width, y height, c fluorescent channel, z focal plane, and t time point.

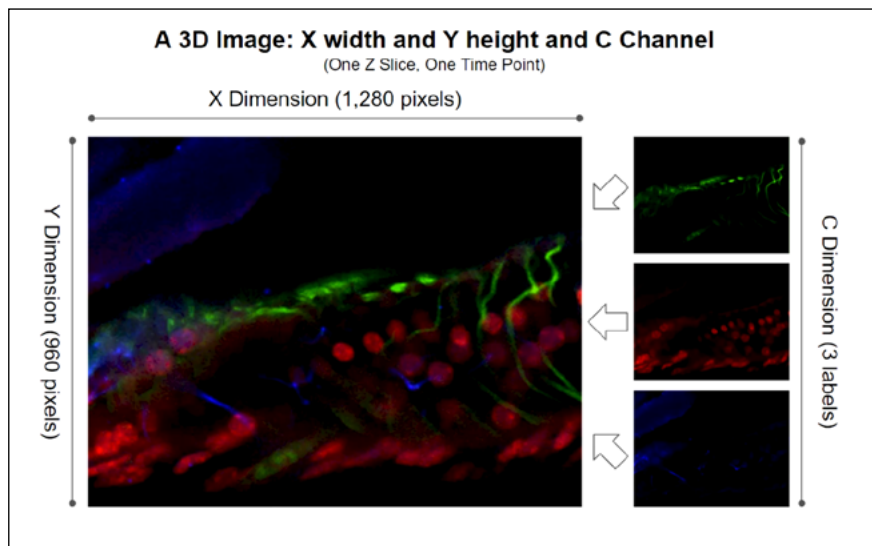
The simplest images is an area of a sample with a single fluorescent label at a single focal plane at a particular time. As you add more fluorescent channels, add more Z planes, and add more time points, the number of dimensions expands from 2 to 5. The following series of diagrams helps to explain.

### DETAILS OF 5D IMAGING: A 2D IMAGE



A single fluorescent label at a single focal point at a particular time creates a 2D image.

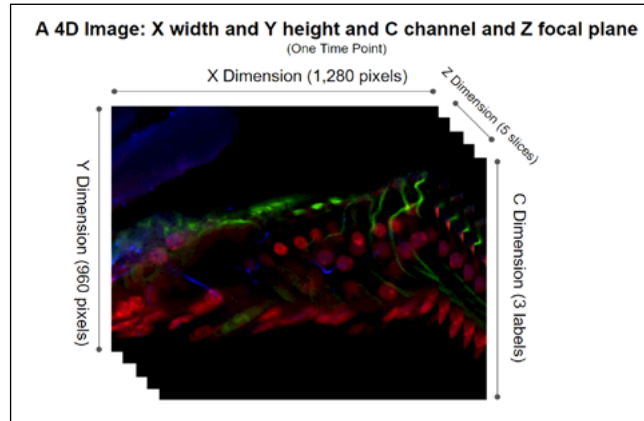
### DETAILS OF 5D IMAGING: A 3D IMAGE



Three fluorescent labels at a single focal point at a particular time create a 3D image.

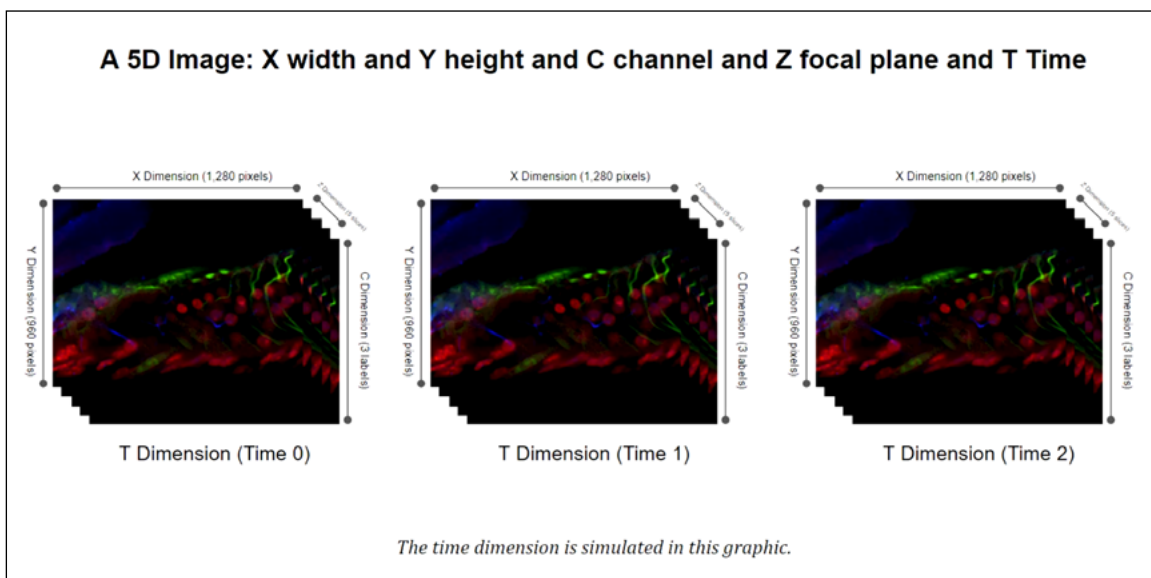


## DETAILS OF 5D IMAGING: A 4D IMAGE



Three fluorescent label at a five focal points at a particular time creates a 4D image.

## DETAILS OF 5D IMAGING: A 5D IMAGE



Three fluorescent label at a five focal points at 3 distinct time points creates a 5D image.

Confocal and lightsheet microscopes can collect many images of a sample each representing different combinations of XYZ, and T. These images can easily reach multiple gigabytes in size.

To simplify moving all that data around, a few different solutions have emerged.

- **First, confocal and lightsheet companies made their own proprietary file formats.**
- **Then, ImageJ created Hyperstacks using the TIF format. Hyperstacks are a way to store 5D data in TIF files. TIF was an easy choice because the TIF file standard actually supports storing many different images in a single file.**

What was missing from ImageJ's approach was a more complete way to store the necessary information describing all the various images in the TIF file. So the folks at the Open Microscopy Environment project created OME-TIFF.

- **OME-TIFF uses the basic idea of storing multiple images in a single TIF file, but adds a formal framework for storing all the necessary information about those images in the**

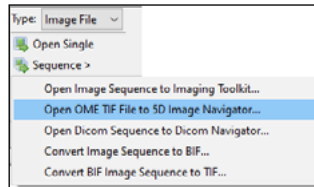
**same TIF file. This information is called metadata.**

The BIOQUANT 5D Image Navigator supports the OME-TIFF format.

We chose this format because the Open Microscopy Environment project also made a free ImageJ plugin called BIO-FORMATS which can convert just about every other 5D format into OME-TIFF. That allows BIOQUANT to support the broadest array of 5D data.

## BIOQUANT SUPPORTS THE OME-TIFF FORMAT

### IMAGE REGION: SEQUENCE BUTTON



New “Open OME TIF File to 5D Navigator” option.

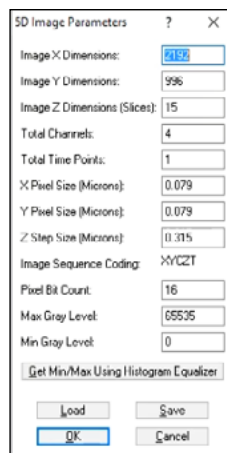
Multi-dimensional image data in the OME-TIFF format is loaded into the 5D Navigator using with the new “Open OME TIF File to 5D Image Navigator” menu item on the Sequence button in the Image region

If your confocal or lightsheet software cannot save to OME-TIFF, the BIO-FORMATS plug-in to ImageJ can be used to convert it to the OME-TIFF format. We have a procedure that walk you through using ImageJ to covert to OME-TIFF, contact us!

Currently BIOQUANT opens single OME-TIFF files up to 4 GB. If future versions, BIOQUANT will support sequences of multiple OME-TIFF files which will allow the maximum image data size to expand beyond 4GB. If you are interested in beta testing this feature, contact BIOQUANT Support.

When loading 5D data into BIOQUANT, you’ll see the OME-TIFF metadata displayed on a configuration box. This gives you chance to double check that the data are accurate and add any missing data.

### 5D IMAGE PARAMETERS



Displays OME-TIF metadata.

# FLUORESCENCE CHANNEL MIXING AND DEPTH OF FIELD ADJUSTMENT

## 5D IMAGE SETUP



Use this box to change the 5D Image Navigator settings.

There are 2 major controls in this box:

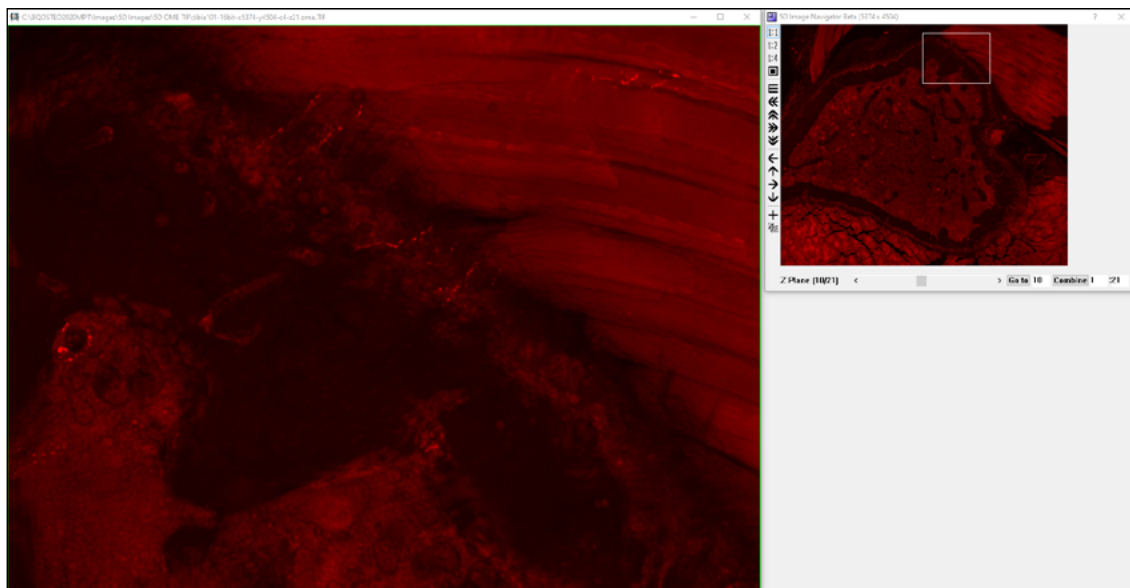
- **Assignments for different fluorescent labels (channels) to different colors.**

By default, the first channel is set to red, the second to green, and the third to blue. Channels can be assigned to the preferred color. If there are more than 3 channels in the image data, only three can be visualized at one time.

- **Adjustment of the depth of field in the Image Window.**

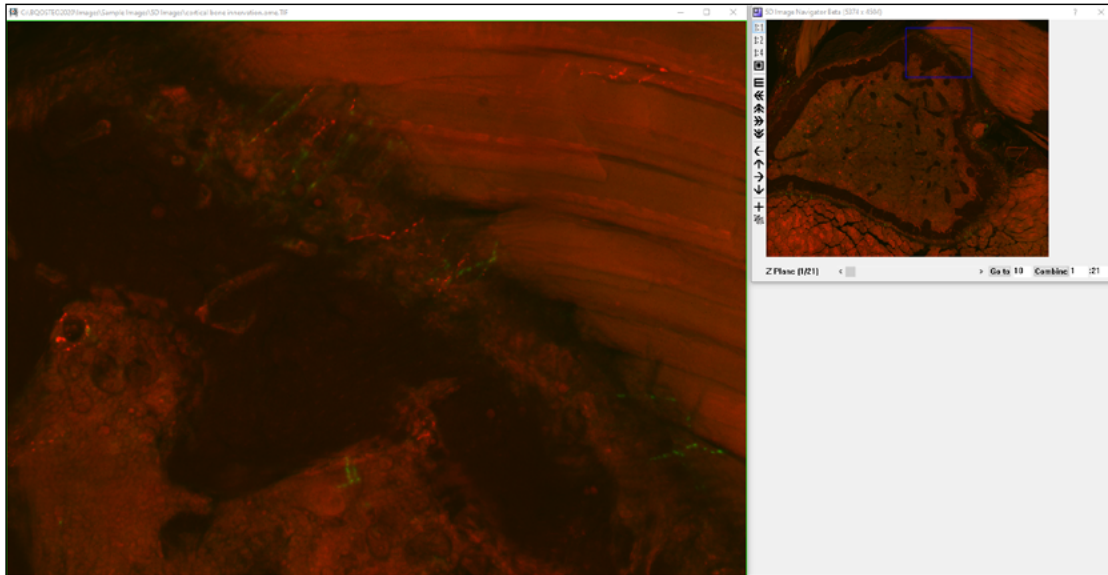
By default, the Image Window displays only the current Z position on the 5D Image Navigator Z position slider. But the depth of field can be expanded using a maximum intensity algorithm. Increase the Expand Depth of Field value to indicate how many additional Z planes above and below the current position should be merged into the image.

## 5D IMAGE NAVIGATOR - CORTICAL BONE INNERVATION.OME.TIF\*



In this example, channel 2, which labels one kind of nerve, and is mapped to Red.

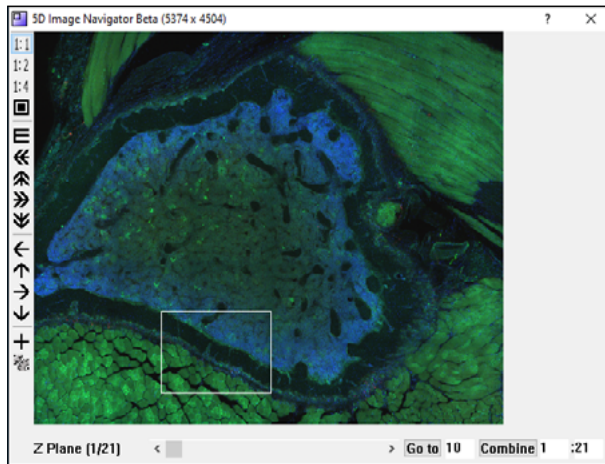
## 5D IMAGE NAVIGATOR - CORTICAL BONE INNERVATION.OME.TIF\*



In this example, channel 2 is mapped to red and channel 4 is mapped to green. These channels label two different types of nerve.

## Z PLANE NAVIGATION

### 5D IMAGE NAVIGATOR - CORTICAL BONE INNERVATION.OME.TIF\*



In this example, there are 121 Z Planes.

The OME-TIFF images often contain multiple Z Planes. The Z Plane slider on the 5D Image Navigator is used to sequence through the Z planes. The Go To box is used to go to a specific Z plane.

The Combine button is used to combine all Z planes or a subset of Z planes into a single image using a maximum intensity projection algorithm.

Once the final image is in the achieved, you can measure data as usual in BIOQUANT. Or you can save the image to share with others or measure on a BIOQUANT Satellite System.

## FOR HELP USING THE 5D IMAGE NAVIGATOR

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- a. In the Image region, click the ? Help button to open the Image Region help.
- b. Then, under Contents, click “Image Region: Image File Type” to open the Image File Type help.
- c. Then, under Contents, click “Image Region: Sequence Button Options.”
- d. Finally, click the link for “Open OME TIF File to 5D Image Navigator.”
- e. Once you have read about how to open your OME-TIF file into the 5D Navigator, read the chapter called “5D Image Navigator.”

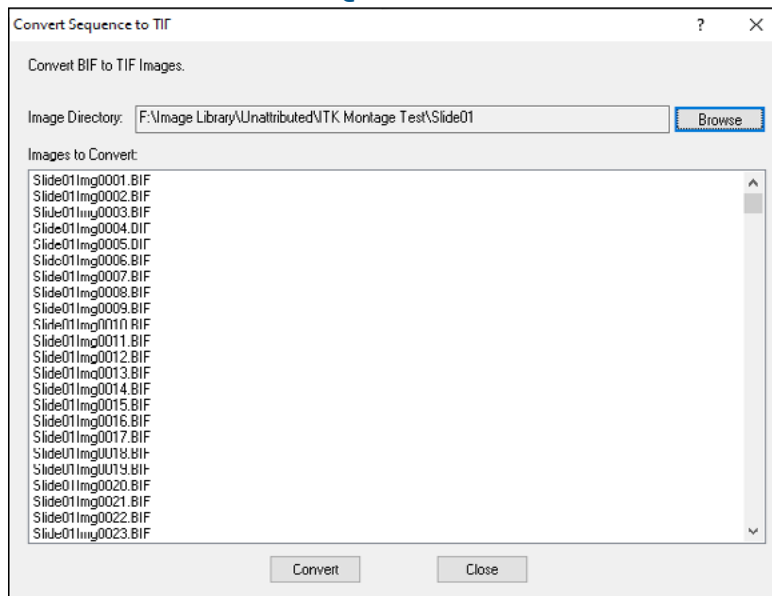
## USER REQUESTED ENHANCEMENTS

### CONVERT BIF SEQUENCE TO TIF

---

The Convert BIF Sequence to TIF feature is used to convert a series of BIF images to the TIF format.

#### CONVERT SEQUENCE TO TIF BOX



Click Convert.

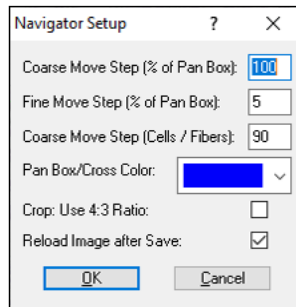
This is useful if BIOQUANT SCAN or the Imaging Toolkit has been used to save a series of calibrated BIF images that the end user would like to also have in a format that other programs can use.

To find this feature, in the Image region, click the Sequence button and choose “Convert BIF Sequence to TIF” from the pop-up menu to open the “Convert Sequence to TIF” box.

For detailed help, click the ? button on the Covert Sequence to TIF box.


## CROP TOOL CAN NOW QUICKLY SAVE MULTIPLE REGIONS FROM A SCAN .....

### NAVIGATOR SETUP



Navigator Setup has new options for Crop.

The crop tool can now save part of a scan without reloading the saved image.

A “Reload Image after Save” check box has been added to the Navigator Setup box. To open the Navigator Setup box, on the Image Navigator, click the  Navigator Setup button.

If this box is not checked, the cropped image is saved to the hard drive and the original image remains in the Image Navigator. This makes it easier to save multiple regions out of a large scan.

If this box is checked, the cropped image is opened into the Image Navigator after it is saved. The original image is still on the had drive, but is no longer open.

Note: This feature is not available with the BIOQUANT OSTEO Satellite System. In this case, the cropped image is saved to the hard drive and the original image is left open in the Image Navigator. Use Open Single Image if you would like to see the cropped image.

For procedures on how to crop an image, click the ? button on the Image Navigator box. Then, click “Crop Image.”

## CROP TOOL CAN CROP AUTOMATICALLY TO 4:3 RECTANGLE .....

A new “Crop 4:3 ratio” check box has been added to the Navigator Setup box.

If this box is checked, then when you draw the crop box, it will be forced to a 4:3 ratio. This is the same ratio as the Image window and the Image Navigator Pan box. This makes it easy to use the Coarse Move Pan Box tool to navigate in a comb pattern through the Image Navigator at high zoom level.

To open the Navigator Setup box, on the Image Navigator, click the  Navigator Setup button.

If this box is not checked, then when you draw the crop box, you can draw free hand.

## **SUPPORT FOR LZW COMPRESSED TIF IMAGES**

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Open Single and the Open Image Sequence now automatically uncompress and open LZW compressed TIFs.

When the system detects and LZW compressed TIF, it automatically creates an uncompressed version and opens it in BIOQUANT. Consequently, it will bit longer than opening an uncompressed TIF.

The uncompressed version of the TIF is saved in the same directory with the same name as the compressed TIF, with “uncompressed” appended.

In prior versions of BIOQUANT, LZW compressed TIF images could not be opened.

## **OPEN DATA SET DEFAULTS TO CURRENT VOLUME AND DATA SET**

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Data Region: Data Set> Open a Different Data Set

The Open a Different Data Set dialog box now opens with the current Data Volume and current Data Set already selected. This makes it easier to open other data sets in the same volume.

If a Tutorial Set is open, then use the Data Set>Open a Tutorial Data Set menu instead of Open a Different Data set to open another Tutorial Set.

## **BIOQUANT SCAN NOW DELETES SOURCE FILES AFTER A SCAN IS MADE**

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A “Delete Source Files after Montage” option has been added to Scan Multiple Slide Setup.

On the Scan Multiple Slides box, on the Options menu, there is a new menu item, “Delete Source Files after Montage.”

If it is checked, the individual field of view source files used to create the montage will be deleted after the montage has been generated. Generally this option will stay checked on.

Delete Source Files after Montage is default to ON when BIOQUANT is installed. If you uncheck it, it will remain unchecked across BIOQUANT sessions.



# MINOR ENHANCEMENTS / ISSUE RESOLUTIONS

## IMAGE REGION ENHANCEMENTS

---

1. **Open Single and the Open Image Sequence can now open Jpeg images with either the “.jpeg” or “.jpg” extension.**

Previously the extension had to be “.jpg.”

2. **Open Image Sequence now supports BMP and TIF images up to 4GB.**

Previously they were limited to 250MB each.

3. **Open Single and the Open Image Sequence no longer support PNG images.**

Previously, PNG support in BIOQUANT was limited to less than 100MB. Given modern image size requirements and the wide support for TIF, PNG support has been removed.

4. **Open Image Sequence and Open Single Image now show only supported Image types.**

The File Type “All Files” has been removed.

Open Single Image: Supported file types: BIF, BMP, JPG/JPEG, TIF/TIFF, DCM

Open Image Sequence: Supported file types: BIF, BMP, JPG/JPEG, TIF/TIFF

Use “Open Dicom Sequence to Dicom Navigator” to open Dicom Sequence files (DCM).

Use “Open OME TIF File to 5D Image Navigator” to open multi-page OME TIF. See documentation for the exact format supported.

5. **For the QIMAGING PV1 camera, the Master Gain “None” has been removed.**

Previously, some QImaging PV1 cameras showed the Master Gain “None” option caused the Live image to distort. The right half of the image would be brighter than the left half of the image. The “None” option has been removed by default. If your camera did not experience this issue and you would like the “None” option active, contact BIOQUANT Support.

This change was first introduced in the BIOQUANT OSTEO 2019 June update.

6. **Camera Setup Brighten Image Value Saved for Jenoptik and QImaging FireWire Cameras**

The Brighten Image parameter on Camera Setup is now remembered across BIOQUANT sessions. The QImaging PV1 camera does not have the Brighten Image parameter.

Previously, it reset to 0 each time BIOQUANT started.

7. **Save Navigator Image plus Current Topo: Updated Color Assignments for SVG**

When exporting to SVG, the SVG color assignments now match the BIOQUANT color palette.

## TOPO COLOR INDEX VALUE = TEXT COLOR NAME FOR SVG FILE

---

- 1 = blue (no change)
- 2 = red (no change)
- 3 = magenta (no change)
- 4 = lime (no change)
- 5 = aqua (no change)
- 6 = yellow (no change)
- 7 = dodgerblue(was gray)
- 8 = darkorange (was navy)
- 9 = darkviolet (was maroon)
- 10 = darkturquoise (was purple}
- 11 = green (no change)
- 12 = lightpink (was teal)
- 13 = lightcyan (was olive)
- 14 = lightgray (no change)
- 15 = white (no change)

### 8. **Image Region: Save Image Window options no longer save to the JPG format.**

The Save Image Window options can save to BIF, BMP, and TIF.

## DATA REGION ENHANCEMENTS

---

### 1. **Assign to Data Set Stability Enhanced**

The Selected List and Calculations List data set assignments are now more stable after the data sets has been modified.

Previously, going to Modify Data Set would sometimes “break” the data set assignments.

### 2. **Export> Export Individual Arrays Issue Resolution**

The Choose Arrays for Export box no longer limits you to 21 arrays at a time.

### 3. **AUTO; Comments Issue Resolution**

If the array has the AUTO; comment, it will no longer store additional comments.

This ensures that the Calculations box will run smoothly.

The AUTO; comment is used when grouping calculation (parse my comment) arrays to an array. It triggers the Calculations box to update as data are collected.

## MEASUREMENT REGION ENHANCEMENT

- **Measurement Filter Issue Resolution**

Measurement Filters 2 through 9 now work when Measurement Filter 1 is off.

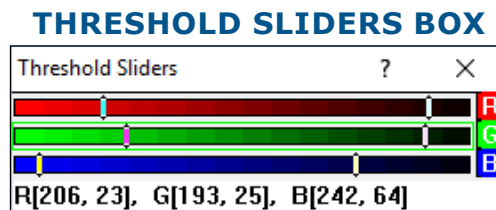
Previously, if Measurement Filter 1 was off, the other measurement filters would not work, even if they were on.

## THRESHOLD REGION ENHANCEMENT

- **Manual Control over Threshold Color Ranges**

From the Image menu, choose Open Threshold Sliders to access the new Threshold Sliders box. For detailed help, click the ? icon on the Threshold Sliders box.

This box allows the user to refine the threshold color ranges for red, green, and blue, by moving slider bars in single digit increments from 255 to 0.



Move the high and low sliders to adjust the threshold range for that channel.

To turn channels on or off, click the boxes in the Threshold region.

This feature was introduced in the BIOQUANT OSTEO 2019 June update.

## STAGE TRACKING ENHANCEMENT

- **Stage Tracking and Redraw after opening an Image Improvement**

Returning to live imaging with stage tracking after opening an image from the hard drive has improved. Previously you would have to reset the landmark, re-select the Mag label, and turn the stage back on.

Now, after opening a BIF image you only need to turn the stage back on after going live.

After opening a non-BIF image, you don't need to do anything as the stage remains on.

Note: This improvement was also in the BIOQUANT OSTEO 2019 June and September Updates.

## OSTEOIMAGER ENHANCEMENT

- **OSTEOIMAGER users with an 89 North Photofluor Illuminator**

There is a new stage statement for the OSTEOIMAGER users who have the original ASI controller and 89 North Photofluor. It will be included with your installation set.