OSTEO

19.6.6

June Update

This documents contains Installation Instructions and Release Notes for the BIOQUANT OSTEO 19.6.6, 5/28/2019 update.

BIOQUANT®

JENOPTIK GRYPHAX® PROKYON CAMERA SUPPORT

BIOQUANT now supports the JENOPTIK GRYPHAX® PROKYON camera from Jenoptik.

NEW JENOPTIK CAMERA SUPPORT

JENOPTIK GRYPHAX® PROKYON

Jenoptik equips the JENOPTIK GRYPHAX® PROKYON microscope cameras with the latest CMOS technology. Short exposure times and high image refresh rates make the system time-saving and convenient to use. This means you can position and focus fast and easily.

BIOQUANT is currently offering a 20% discount on the JENOPTIK GRYPHAX® PROKYON camera for the trade in of your existing camera.

Features include:

- High image quality: 2.1, 9.2, or 20.7 megapixel image size. Pixel size 5.86 x 5.86 μm, 12 Bit, Dynamic range 73.3 dB, True color camera.

- Sensitivity: Exposure up to 60 seconds and Gain up to 30.

- Ease of use: Free JENOPTIK GRYPHAX® microscope camera software, large field of view 1/1.2", 60 fps @ 2.3 MPix and no external power supply needed.

- Stability: SONY Sensor – back illuminated, Made in Germany, 24 month warranty and software updates free of charge.

- Live integration with BIOQUANT. Live camera resoultion is1280 x 960 pixel (60 fps).

- In BIOQUANT, camera resolution in held mode is 1280 x 960 pixel, 2560x1920 pixel, or 3840 x 2880 pixel. All held image resolutions compatible with BIOQUANT live image calibration. Redraw tracings at any size. Zoom in to higher resolution at any point.

- In GRPHAX software, camera resolution in HELD mode 1920x1200 pixel, 3840 x 2400 pixel, and 5760 x 3600 pixel.

- USB 3.0 camera interface

THRESHOLD SLIDER BOX

The Threshold Sliders box allows the user to refine the threshold values for red, green, and blue, by moving slider bars in single digit increments from 255 to 0.

THRESHOLD SLIDERS BOX
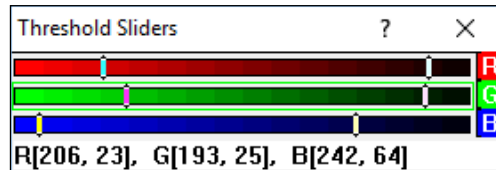
Move the high and low sliders to adjust the threshold range for that channel.
To turn channels on or off, click the boxes in the Threshold region.

The Threshold Sliders box is opened by clicking the Image menu and choosing "Open Threshold Sliders."

For detailed help, click the ? icon on the Threshold Sliders box.

MINOR FUNCTIONALITY CHANGES

The following minor change has been made.

1. Stage tracking and Redraw after opening an Image has been improved.

   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.

FEATURES IN OSTEO 19.2.6, 2-28-2019 RELEASE

All new features in the BIOQUANT OSTEO 2019, 19.2.6, 2-28-2019 Release are also included in the BIOQUANT OSTEO 2019, 19.6.6, 5-28-2019 Release.

OSTEO

19.2.6

Release Notes

BIOQUANT®

CONTENTS

BIOQUANT OSTEO: IMPROVEMENTS TO TUTORIALS

The OSTEO 2019 Tutorial Volume has new and improved Tutorial Sets. Each Tutorial Set has an associated guide and sample image(s). Follow the Guide steps to learn the protocol.

The Tutorials also often have procedures in the BIOQUANT OSTEO manual, which provide images or videos to help learn the protocol.

To see if the tutorial has an associated video, in the Measurement region, click the > button to open the OSTEO 2019 Video Handbook.

NEW TUTORIAL SETS

1. New Myofiber Tutorial: Myofiber BF H&E Regenerated

This new tutorial demonstrates myofiber analysis over multiple fields of view when central nuclei are plentiful and when myofibers are densely clustered. It is a good introduction to the editing tools introduced in this version.

2. New Measure Cells Tutorial: Measure Cells - 1 Cell Type

This new tutorial provides training in automatic cell counting using the Measure Cells measurement type. It introduces the basic topics of defining a boundary for cell counting and avoiding duplicate counts in overlapping fields of view.

3. New Measure Cells Tutorial: Measure Cells - Editing

This slightly more advanced version of the cell counting tutorial introduces some of the editing tools that help separate clusters of cells.

4. New Bone Tutorial: Bone Cortical Basic

A tutorial guide has been written for the analysis of cortical bone cross-sections. The template has always been available and the procedure was documented in the written manual, but there wasn't previously a guide available.

The guide describes the analysis of cortical bone shape and bone formation rate on both periosteal and endocortical surfaces.

TUTORIAL SET CHANGES

1. Tutorial Sets no longer save data when you exit the software.

To make the tutorials easily available to many people in the lab, BIOQUANT now cleans the data from the Tutorial Data Set between BIOQUANT sessions. This eliminates the need to delete all the data in the Tutorial Data Sets before training a new student.

2. All Tutorial Sets have been updated for new features.

Guides and Sample Images have also been updated.

3. The following tutorials have been renamed:

a. The Chondrocyte Proliferation tutorial has been renamed to Measure Cell - Proliferation.

b.   The Osseointegration tutorial has been renamed Bone Osseointegration.

c.   The microCT tutorial has been renamed Bone microCT.

d.   The No Guide - Cortical Basic tutorial has been renamed Bone Cortical Basic.

e.   The No Guide - Cortical Complete tutorial has been renamed Bone Cortical Complete.

BIOQUANT OSTEO: CHANGES TO TEMPLATES

The following templates have been added or modified.

NEW TEMPLATES

The following templates have been added to the OSTEO 2019 Template Volume.

1.   **Density - Avg Fluorescence - Immunofluorescent Staining**

This template collects stain intensity data for fluorescing samples. Data are collected for each of 3 color channels: red, green, and blue. Data units are "grayscale" and are reported on a scale from 0 to 255 where 0 is black and 255 is the maximum intensity for that color.

2.   **Density - Integrated Optical - Immunohistochemical Staining**

This data set is used in the quantitative analysis of immunohistochemistry data.

Integrated Optical Density is the sum of all the absorbance values for the pixels in an object. The absorbance data comes from Beer's Law: $A = -\log(I/I_o)$ where I is the intensity of the light coming through the sample and I_o is the intensity of the background light.

IOD data has also been used in the analysis of genome size by feulgen staining as IOD values correlate well with picograms of DNA in nuclei.

3.   **Measure Cells - 1 Cell Type**

This is a good template to start learning the Measure Cells Measurement Type. It uses the Measure Cells measurement type. Using this template, it's possible to (a) count cells only within a specified boundary and (b) to avoid double counting cells even when they reappear in adjacent and overlapping fields of view.

4.   **Measure Cells - 2 Cell Types**

This template is similar to the 1 cell type template, but is expanded to account for two classes of cells. It reports the total number of each class of cell, the numeric density of the cell populations, and proportion of each cell type to the total.

5.   **Density Wand Type - Cells**

This template is used with the Density Want measurement type. Using the density wand, circular or rectangular regions are marked on the image and the intensity of the stain is measured in those regions. Data are in units of "grayscale" and are reported on a scale of 0 to 255 where 0 is black and 255 is white. This is a handy way to analyze the stain intensity of cells when they are difficult to threshold individually.

6. **Density - Avg Brightfield**

    This template collects stain intensity data from brightfield stains. Data units are "grayscale" and are reported on a scale from 0 to 255 where 0 is black and 255 is white. This is most commonly used in conjunction with immunohistochemistry data so that both the area of the positively stained tissue and the intensity of the stain can be reported.

7. **Trace Type - Specimen Area**

    This specialized template is used for system which have either a motorized or encoded stage. It allows a very large area to be traced at high magnification when the whole area doesn't fit in one field of view. It is preferred that you scan your section over using the Trace Type. This template is provided for compatibility for older BIOQUANT users who have upgraded.

MINOR TEMPLATE CHANGES

The following reflects changes made to templates in the OSTEO 2019 Template Volume.

1. **Renamed Templates**

    These templates have been renamed to make them easier to find the in template list.

    a. Topo - For Export Topo Arrays (was Dataset of Topo Arrays)

    b. Bone Stereology Initial (was Bone Stereology Initial Templ)

    c. Bone Strut Analysis Template (was Strut Analysis Template)

    d. Bone Cortical Basic (was Bone Cortical Basic Template)

    e. Bone Cortical Complete (was Bone Cortical Complete Templ.)

    f. Bone microCT Template (was microCT Template)

    g. Bone Osseointegration (was Osseointegration Template)

    h. Measure Cells - Proliferation (was Chondrocyte Proliferation)

2. **Some Templates now have the threshold color assigned to arrays.**

    The threshold color can now be assigned to the current Selected array. This is useful when the default threshold color conflicts with the stain color.

3. **Deleted Templates**

    These templates have been removed from the template volume. They were not widely used. Key data computations from these have been incorporated in other templates. If you would like a copy for your own records, contact Technical Services at support@bioquant.com.

    a. Moments of Inertia Template

    b. Radius of Curvature Template

BIOQUANT OSTEO: IMAGING NEW FEATURES & ENHANCEMENTS

The Image Region and Image menu have the following new features and enhancements.

1. **Imaging Extensions are now part of BIOQUANT OSTEO**

   Tools which were previously part of the BIOQUANT Imaging Extensions add-on have been integrated into all versions of BIOQUANT.

   BIOQUANT SCAN remains a separate add-on. BIOQUANT SCAN is responsible for the automated control of motorized stages, the automatic focus correction of images, and the whole slide scanning of sections.

   ## FEATURES INCLUDE

   - Support for uncompressed TIFF and BMP images larger than 250MB.

   - The Imaging Toolkit

     ### THE IMAGING TOOLKIT CAN BE USED TO DO THE FOLLOWING

     - Capture sequences of live images
     - Generate a high resolution, calibrated scan of your specimen from a sequence of images captured in BIOQUANT (requires Microscope Scanning Upgrade Kit)
     - Open sequences of images captured with BIOQUANT or with third party source
     - Merge fluorescent or fluorescent and brightfield images
     - Capture time-lapse images

   - Dicom Imaging Support

     ### DICOM IMAGING SUPPORTS THE FOLLOWING

     - The DICOM Navigator, which can open and navigate through MicroCT image sequences.
     - 2D X-Ray Support.

   - Histograms

     A density histogram graphs the optical densities of picture elements (pixels) in an image. Histograms can be generated from the entire image, from special areas of interest, from thin lines, or from thick lines.

   - Pseudocolor

     Pseudocolor is artificial coloring that can be applied to dramatically illustrate density gradients within a specimen.

2. TWAIN Image Capture Support
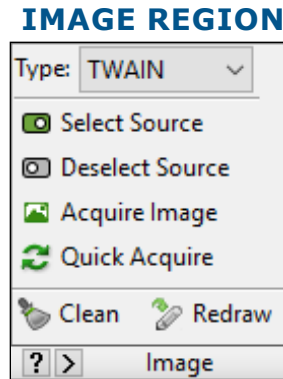
IMAGE REGION

Twain Type

In the Image region, a new Type has been added, TWAIN. TWAIN is a standards based way of transferring images between imaging devices and imaging software. If the camera or printer/scanner in the lab has a TWAIN interface, BIOQUANT can likely connect to it now.

SELECT SOURCE

The Select Source button opens the opens the Windows OS dialog box to choose your imaging device from a list of imaging devices installed on the computer.

DESELECT SOURCE

The Deselect Source button disconnects the BIOQUANT software from the imaging device when it's no longer needed.

ACQUIRE IMAGE

The Acquire Image button opens the control software for the imaging device. This software is provided by the device manufacturer, not BIOQUANT. Adjust the image using the software. When the image is correct, click a "Capture" button to transfer the image to BIOQUANT. The name of the "Capture" button varies from one manufacturer to another.

QUICK ACQUIRE

The Quick Acquire button is supported by some, but not all device manufacturers. Clicking this button should automatically transfer a new image to BIOQUANT using the same settings as the previous image. For some manufacturers this feature isn't implemented. In which case, use the Acquire Image button instead.

3. BIF Image Support: Display the Mag Factor

The magnification factor (the number of microns per pixel) in a BIF image is now displayed on the title bar of the Image window.

This makes it easy to check the numeric value of the calibration stored in BIF images.

Previously, it was only possible to see the text label associated with the mag value in the Parameters region.

BIOQUANT OSTEO: DATA REGION NEW FEATURES & ENHANCEMENTS

The Selected List region has been renamed to the Data region. The Arrays menu, the Edit menu, and parts of the File menu have been moved into the data region. Now, all the functions related to data are now found in the Data region.
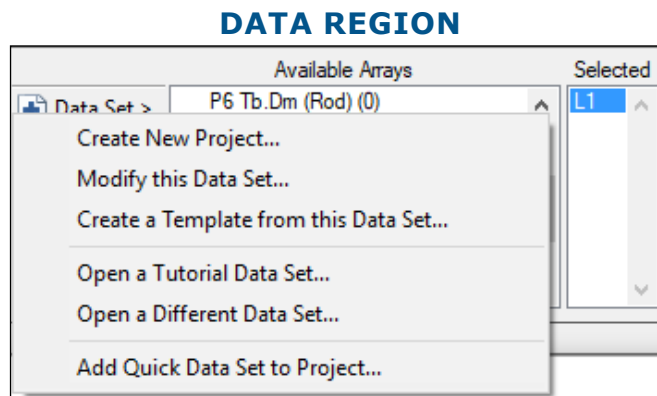
Notice the Export button. It is now possible to export data to the clipboard directly from BIOQUANT.
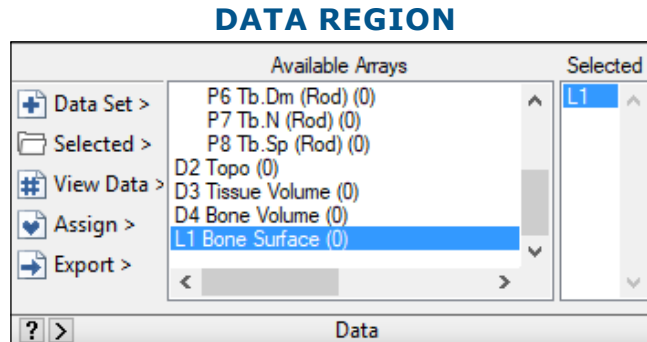
DATA REGION

The Selected List region is now the Data region.

INTERFACE CHANGES INCLUDE

1. **The Data Set Button Menu**

DATA REGION

The Data Set button menu.

CREATE NEW PROJECT

The "Create New Project" option opens the BIOQUANT New Project Wizard.

Previously, this was the BIOQUANT New Data Set Wizard and was opened by choosing "New Data Set" from the File menu.

MODIFY THIS DATA SET

The "Modify this Data Set" option opens the Modify Data Set box.

Previously, this box was opened by clicking the Modify button in the Selected List region or by choosing "Modify Data Set" from the Arrays menu.

CREATE A TEMPLATE FROM THIS DATA SET

The "Create a Template from this Data Set" option opens the Create Template box.

Previously, this box was opened by choosing "Create Data Set Template" from the File menu.

OPEN A TUTORIAL DATA SET

The "Open a Tutorial Data Set" option opens the Open a Tutorial Set box.

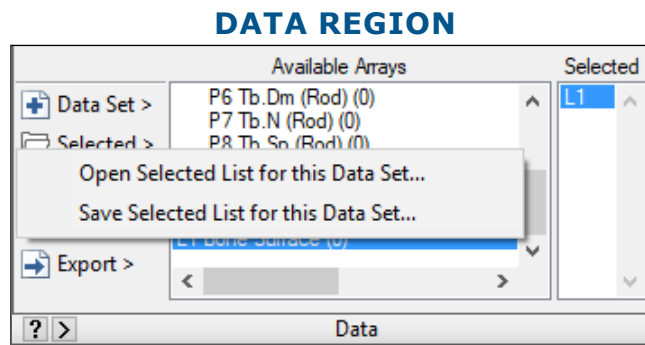Previously, this box was opened by choosing "Open Tutorial Set" from the File menu.

OPEN A DIFFERENT DATA SET

The "Open a Different Data Set" option opens the Open a Data Set box.

Previously, this box was opened by choosing "Open Data Set" from the File menu.

ADD QUICK DATA SET TO PROJECT

The "Add Quick Data Set to Project" option opens the Add Quick Data Set to Project box.

Previously, this box was named Quick Data Set and opened by choosing "Quick Data Set" from the File menu.

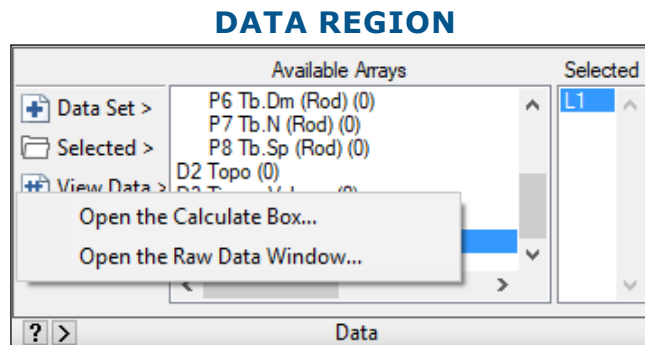2. **The Selected Button Menu**

The Selected Button Menu.

OPEN SELECTED LIST FOR THIS DATA SET

The "Open Selected List for this Data Set" option opens the BQ Open File box.

Previously, this box was opened by clicking the Open List button in the Selected List region.

SAVE SELECTED LIST FOR THIS DATA SET

The "Save Selected List for this Data Set" option opens the BQ Save File box.

Previously, this box was opened by clicking the Save List button in the Selected List region.

3.   View Data Menu

DATA REGION

The View Button Menu.

OPEN THE CALCULATE BOX

The "Open the Calculate Box" option opens the Calculate box.

Previously, this box was opened by clicking the Open List button in the Selected List region.

OPEN THE RAW DATA WINDOW

The "Open the Raw Data Window" option opens the Raw Data Window.

Previously, this window was opened by choosing Raw Data from the Array menu.

The Raw Data window can still be opened from the Calculate box by choosing "Open Raw Data" from the Data menu.

4.   Assign menu

DATA REGION
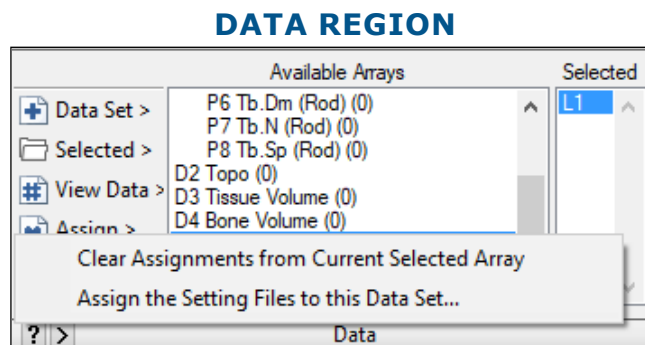
The Assign Button Menu.

CLEAR ASSIGNMENTS FROM CURRENT SELECTED ARRAY

The "Clear Assignments from Current Selected Array" option removes any parameters that have been assigned to the current Selected array, such as Mag, Color, ROI Type, Threshold, and Measurement Type. There is a warning message before the assignments are deleted. For a list of

exactly what is deleted, see the BIOQUANT OSTEO Manual.

Previously, this feature was activated by choosing "Clear Assigned Comments" from the Arrays menu.

ASSIGN THE SETTING FILES TO THIS DATA SET

The "Assign the Setting Files to this Data Set" option opens the Assign to Data Set box.

Previously, this box was opened by choosing "Assign to Data Set" from the File menu.

5.   **NEW: Export Menu**

DATA REGION
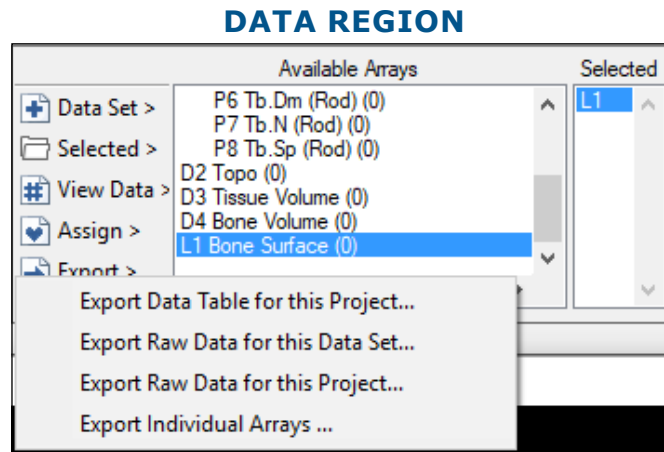
The Export Button Menu.

NEW: EXPORT DATA TABLE FOR THIS PROJECT

The "Export Data Table for this Project" option opens the Export Data Table for Volume box.

Export Data Table for this Project exports all the calculation arrays for the current data volume in a data table format to the clipboard. Then, you can click a cell in your spreadsheet program and paste.

This is ideal for projects that use the same data set template and apply it to many sections of tissue, usually using one data set per animal or patient. This is preferred because it allows you to easily audit your data and quickly track down problems.

Export Data Table for this Project gives you a simple way to create a data table. Each row contains the data for one animal and each column is a different index, either static or dynamic.

Exporting only the data in Calculation arrays, it creates a data table where each row is a data set and each column is one of the Calculation arrays.

NEW: EXPORT RAW DATA FOR THIS DATA SET

The "Export Raw Data for this Data Set" option opens the Export Data Set box.

Export Raw Data for this Data Set exports all the data in the current data set, except for Topo

arrays, to the clipboard. Then, you can click a cell in your spreadsheet program and paste.

If you need to export Topo arrays, use the Data Manager.

NEW: EXPORT RAW DATA FOR THIS PROJECT

The "Export Raw Data for this Project" option opens the Export Data Volume box.

Export Raw Data for this Project exports all the data in the current data volume, except for Topo arrays, to the clipboard. Then, you can click a cell in your spreadsheet program and paste.

If you need to export Topo arrays, use the Data Manager.

EXPORT INDIVIDUAL ARRAYS

The "Export Individual Arrays" option opens the Choose Arrays for Export box.

Previously, this was the "Available Arrays to Clipboard" box, which was opened by clicking the Edit menu and choosing Copy Data, then Choose Available Arrays.

BIOQUANT OSTEO: THRESHOLD ENHANCEMENTS

The threshold color can now be assigned the array. Then, whenever the array is selected in the Selected box on the Data region, the assigned threshold color is retrieved.

FEATURE

1. **The Threshold Color can now be assigned to the current Selected array.**

    Sometimes the threshold color and the stain color conflict and make it hard to see what is being thresholded.

    The threshold color can now be assigned to the current Selected array.

    #### PROCEDURE

    a. In the Data region, in the Selected box, click the array to which you would like to assign the new threshold color so that it is the current array.

    b. From the Options menu, choose Open Customizing.

    c. On the Customizing box, in the Color region, from the Threshold list, choose a new threshold color.

    d. Click "Yes" on the warning message.

    e. Close Customizing.

    f. In the Data region, in the Selected box, click the array index once to update BIOQUANT to match its assigned settings.

BIOQUANT OSTEO: EDITING REGION NEW FEATURES & ENHANCEMENTS

The Editing region has changed quite a bit in this version. The number of tools has increased substantially. Note in particular that there are now 4 kinds of Draw tools, 4 kinds of Erase tools, 2 new Separate tools, and 2 Clean tools. Many of these new tools help with separating touching cells and are useful with the Measure Cells and Myofibers Measurement Types.

In addition, there are new hotkeys to swap between Editing buttons while you are editing in the Image window. Even better, the "U" key will undo the last threshold edit made while the cursor is still editing in the Image window.
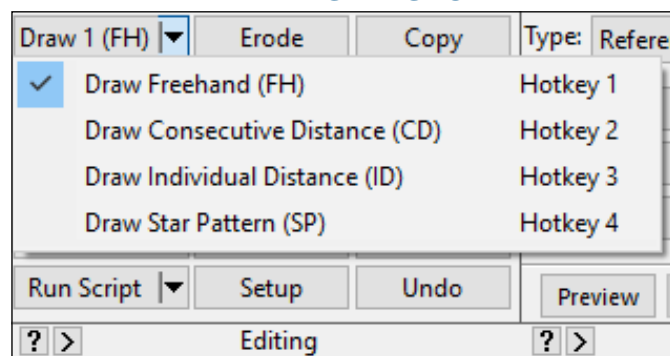
EDITING REGION
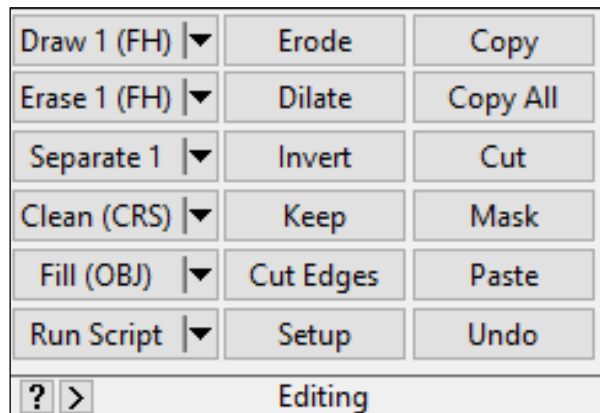
The Editing region has new buttons.

1. **The Draw button now has four modes.**
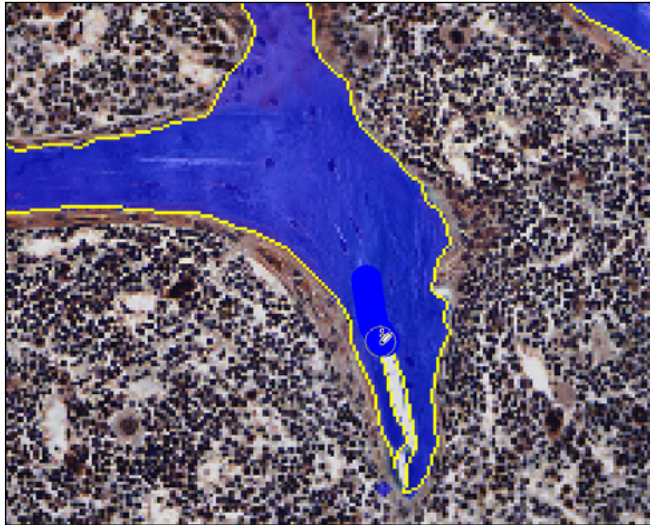
EDITING REGION

The Draw Button Menu.

DRAW FREEHAND (FH)

DRAW 1 (FH)

Click and drag to draw threshold.

Draw Free Hand (FH) enables the user to draw threshold free hand in the Image window. Click and hold the mouse button down while drawing.

To switch the Draw button to Draw (FH), click the down arrow on the Draw button and choose "Draw Free Hand (FH) Hotkey 1."

If you click the Draw button in any other Draw mode and you want to switch to the Draw (FH) mode while in the Image window, tap the 1 key.

DRAW CONSECUTIVE DISTANCE (CD)

DRAW 2 (CD)
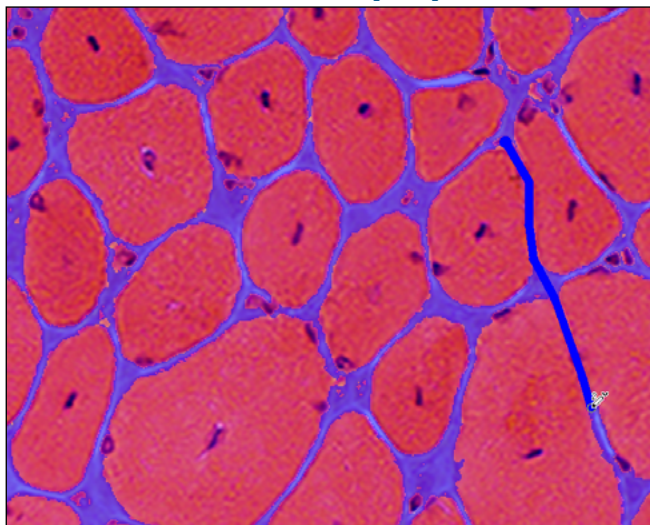
Click consecutive points to connect with threshold.
Tap the space bar to complete the series.

Draw Consecutive Distance (CD) enables the user to click consecutive points in the Image window to draw threshold. When finished with a consecutive distance series, tap the space bar to close it. Then, you can continue to click to define a new series.

To switch the Draw button to Draw (CD), click the down arrow on the Draw button and choose "Draw Consecutive Distance (CD) Hotkey 2."

If you click the Draw button in any other Draw mode and you want to switch to the Draw (CD) mode while in the Image window, tap the 2 key.

DRAW INDIVIDUAL DISTANCE (ID)

DRAW 3 (ID)
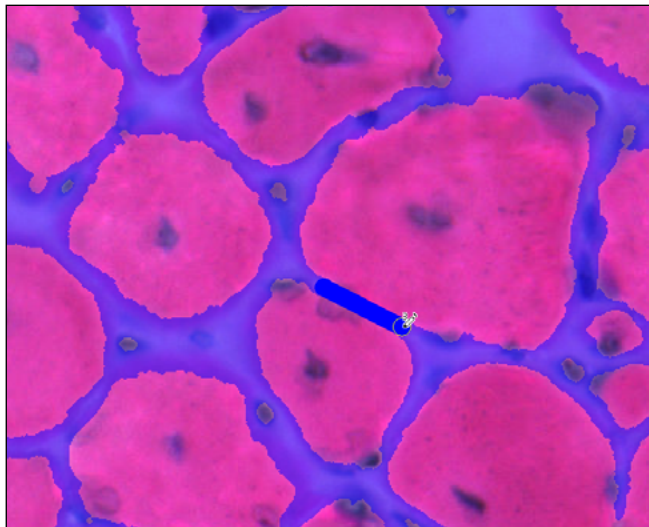
Click two points to connect with threshold.

Draw Individual Distance (ID) enables the user to click two points in the Image window to draw threshold between them.

To switch the Draw button to Draw (ID), click the down arrow on the Draw button and choose "Draw Individual Distance (ID) Hotkey 3."

If you click the Draw button in any other Draw mode and you want to switch to the Draw (ID) mode while in the Image window, tap the 3 key.
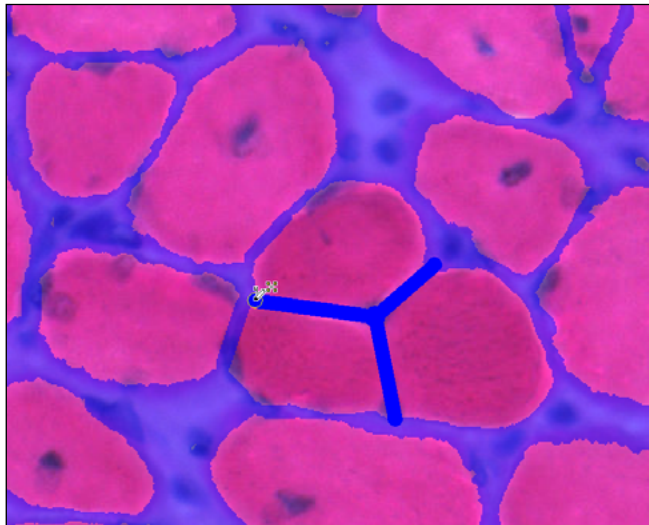
DRAW STAR PATTERN (SP)

DRAW 4 (SP)
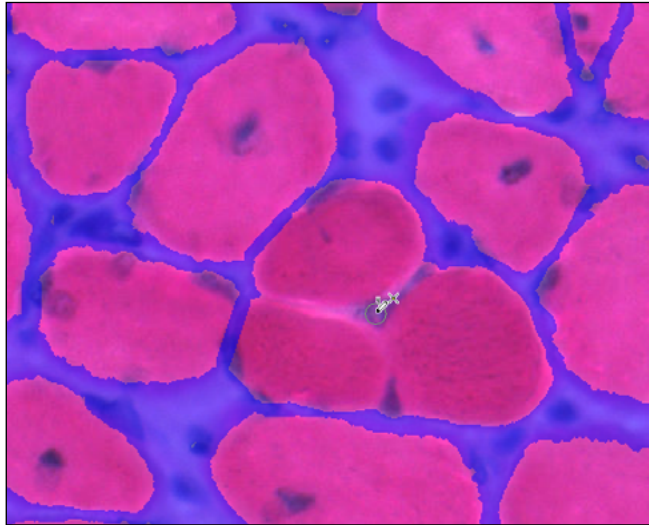
First, click a central point.

DRAW 4 (SP)

Then, click "arms." Threshold connects the arms to the central point.

Draw Star Pattern (SP) enables the user to click a central point in the Image window, then click points around the central point to add "arms." Threshold is added between all the "arms" and the central point.

To switch the Draw button to Draw (SP), click the down arrow on the Draw button and choose "Draw Star Pattern (SP) Hotkey 4."

If you click the Draw button in any other Draw mode and you want to switch to the Draw (SP) mode while in the Image window, tap the 4 key.
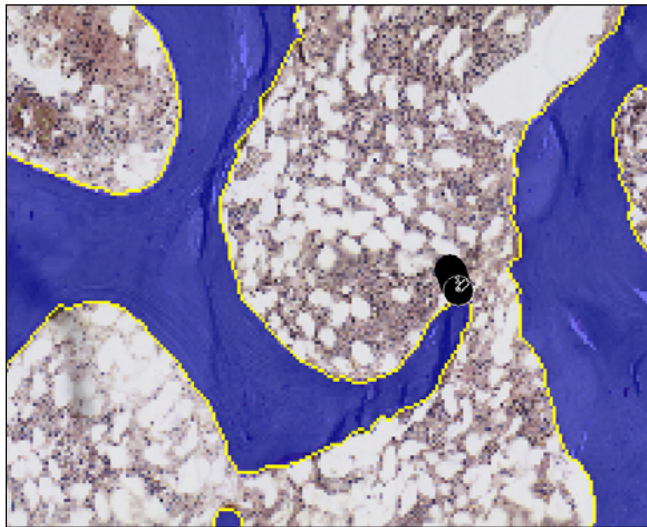
2. **The Erase button now has four modes.**

EDITING REGION
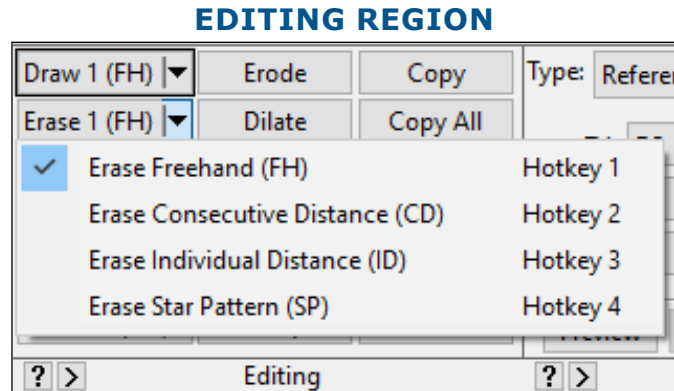
The Erase Button Menu.

ERASE FREEHAND (FH)

ERASE 1 (FH)

Click and drag to erase threshold.

Erase Free Hand (FH) enables the user to erase threshold free hand in the Image window. Click and hold the mouse button down while erasing.
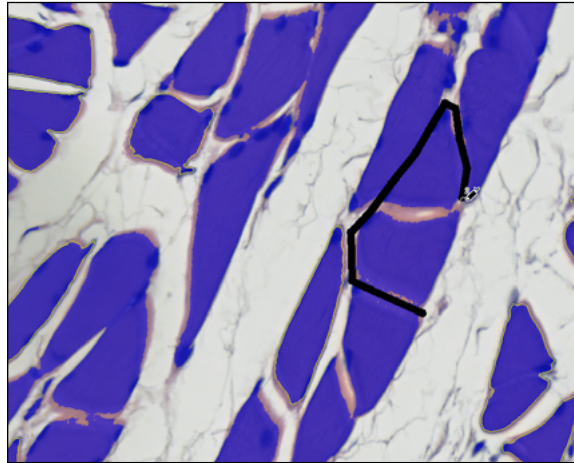
To switch the Erase button to Erase (FH), click the down arrow on the Draw button and choose "Erase Free Hand (FH) Hotkey 1."

If you click the Erase button in any other Erase mode and you want to switch to the Erase(FH) mode while in the Image window, tap the 1 key.

ERASE CONSECUTIVE DISTANCE (CD)

ERASE 2 (CD)

Click consecutive points to erase threshold between them.
Tap the space bar to complete the series.

Erase Consecutive Distance (CD) enables the user to click consecutive points in the Image window to erase threshold. When finished with a consecutive distance series, tap the space bar to close it. Then, you can continue to click to define a new series.

To switch the Erase button to Erase (CD), click the down arrow on the Erase button and choose "Erase Consecutive Distance (CD) Hotkey 2."

If you click the Erase button in any other Erase mode and you want to switch to the Erase (CD) mode while in the Image window, tap the 2 key.

ERASE INDIVIDUAL DISTANCE (ID)

ERASE 3 (ID)
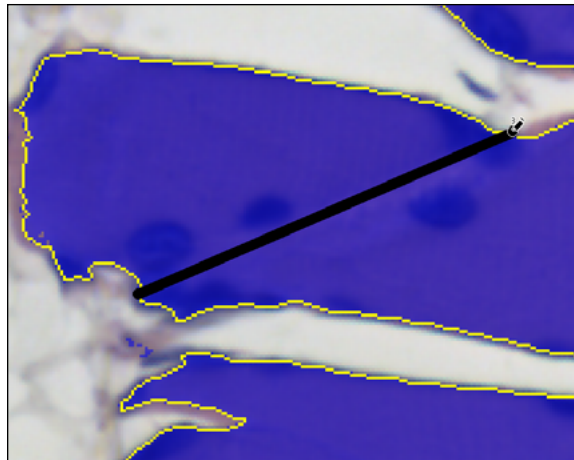
Click two points to delete threshold between them.

Erase Individual Distance (ID) enables the user to click two points in the Image window to erase threshold between them.
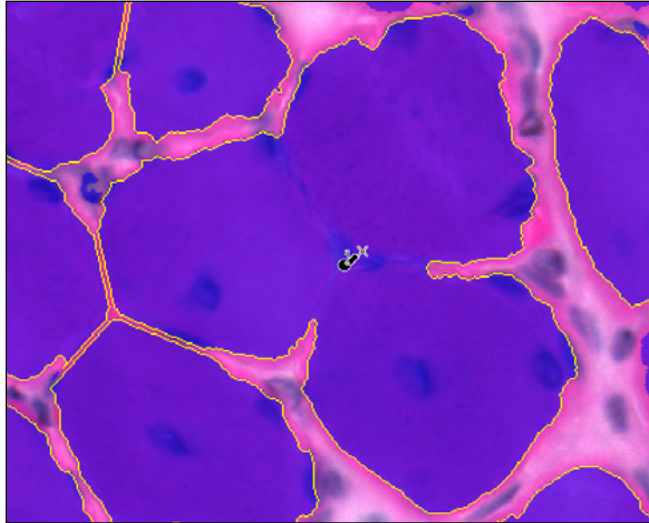
To switch the Erase button to Erase (ID), click the down arrow on the Erase button and

choose "Erase Individual Distance (ID) Hotkey 3."

If you click the Erase button in any other Erase mode and you want to switch to the Erase (ID) mode while in the Image window, tap the 3 key.

ERASE STAR PATTERN (SP)

ERASE 4 (SP)

First, click a central point.

ERASE 4 (SP)

Then, click "arms." Threshold erases between the arms and the central point.

Erase Star Pattern (SP) enables the user to click a central point in the Image window, then click points around the central point to add "arms." Threshold is erased between all the "arms" and the central point.

To switch the Erase button to Erase (SP), click the down arrow on the Erase button and choose "Erase Star Pattern (SP) Hotkey 4." If you click the Erase button in any other Erase mode and you want to switch to the Erase (SP) while in the Image window, tap the 4 key.
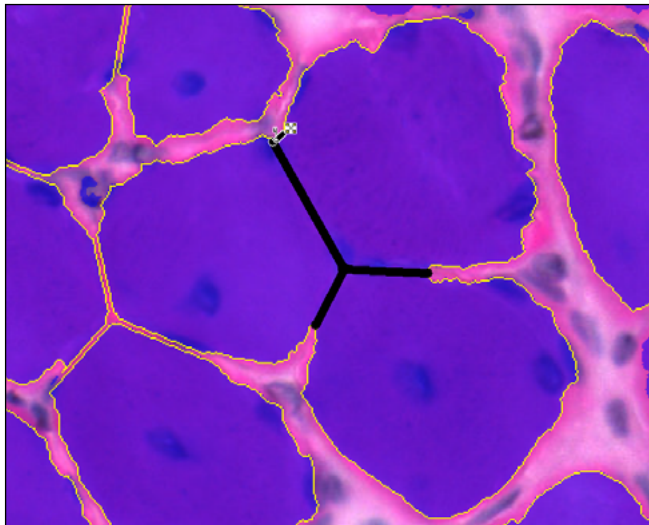
3.   NEW: The Separate button has two modes
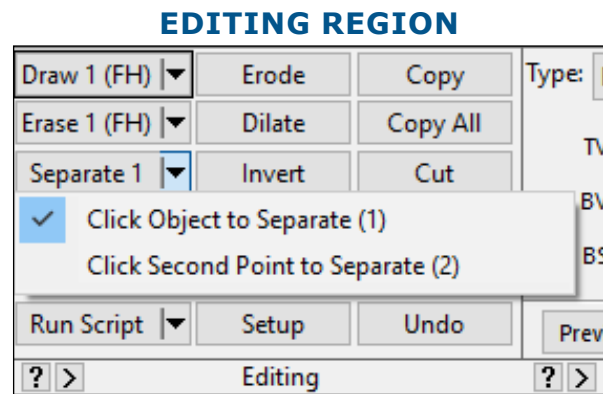
The Separate Button Menu.

SEPARATE 1: CLICK OBJECT TO SEPARATE

SEPARATE 1
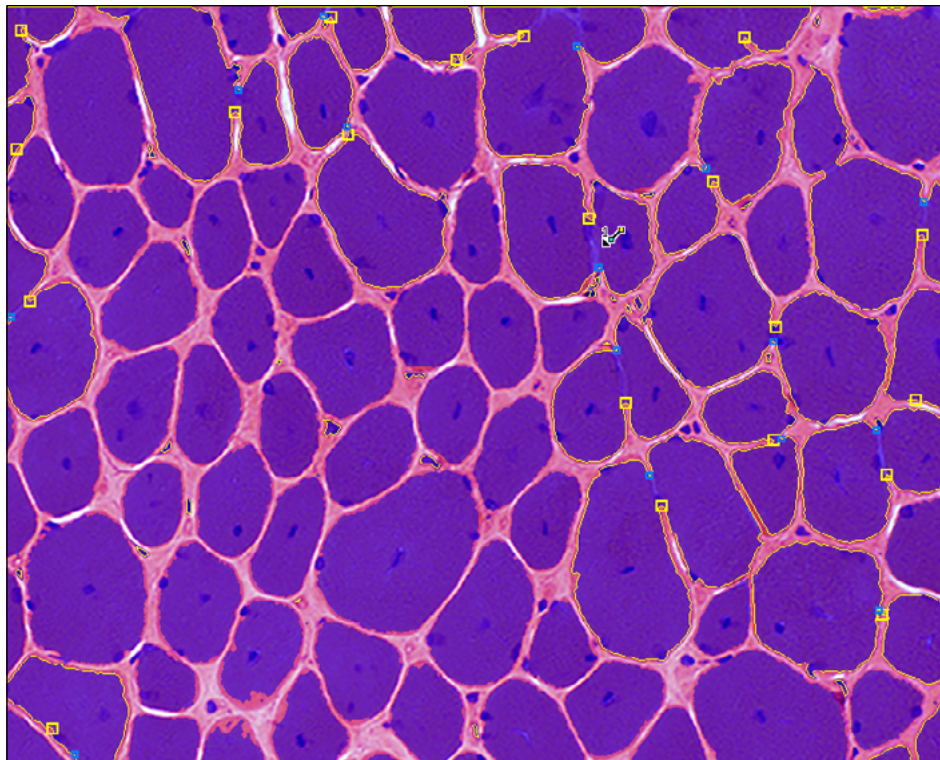
For objects correctly detected, click them to separate.

Click Object to Separate 1 is used to separate touching thresholded objects when the system can detect both separation points. When the user clicks the Separate 1 button, the system detects touching cells. It puts a yellow box and blue box at either end of separation locations. When the user clicks on accurately detected cells, they separate.

- The Separate tools work best at higher magnification.

- The Separate tools work best with a Smoothing Limit of 0 or 1.

- To speed things up, the filters and edge rejection do not work when the cursor is in Separate mode. However, as soon as you right click to exit, they are activated again. Don't spend time separating cells that will be filtered.

SEPARATE 1

Use Separate 1 to sequence through a series of touching cells.

Separate 1 can be used to separate a cluster of touching cells. As you click on cells, they automatically separate and new cells to separate are detected. This allows you to separate cells that are touching other cells in a sequence. The system detects the first separate location, you click to separate, the preview outlines update and the system detects the next separate location. You repeat the sequence until all cells are separated.

To switch the Separate button to Separate 1, click the down arrow on the Separate button and choose "Click Object to Separate 1."

If you click the Separate button in Separate 2 mode and you want to switch to Separate 1 mode while in the Image window, tap the 1 key.
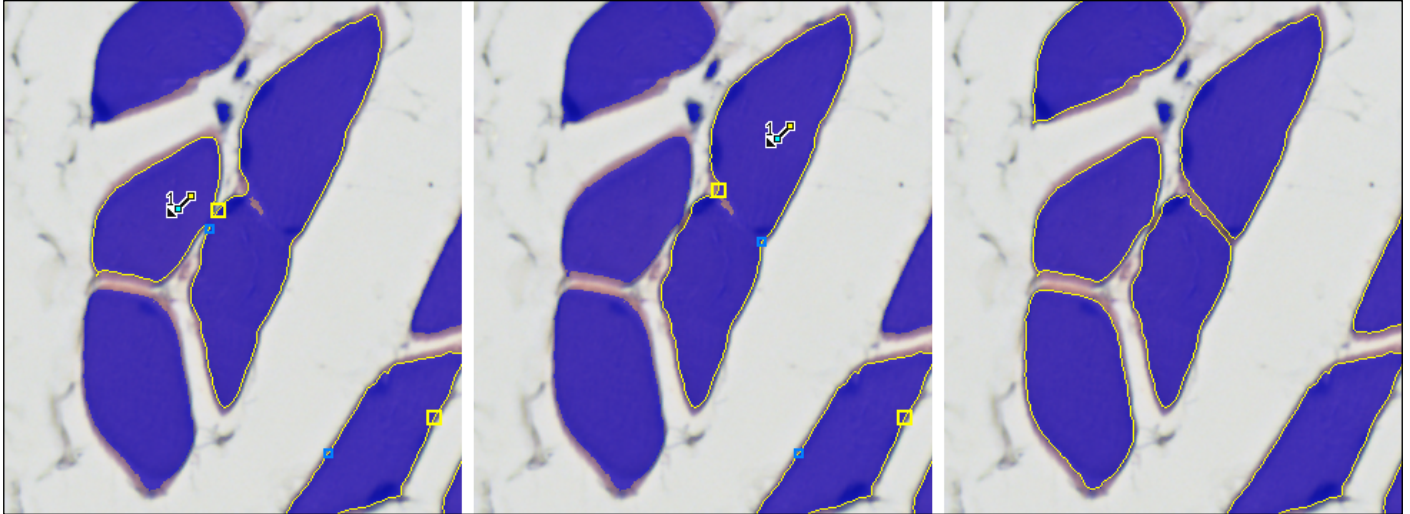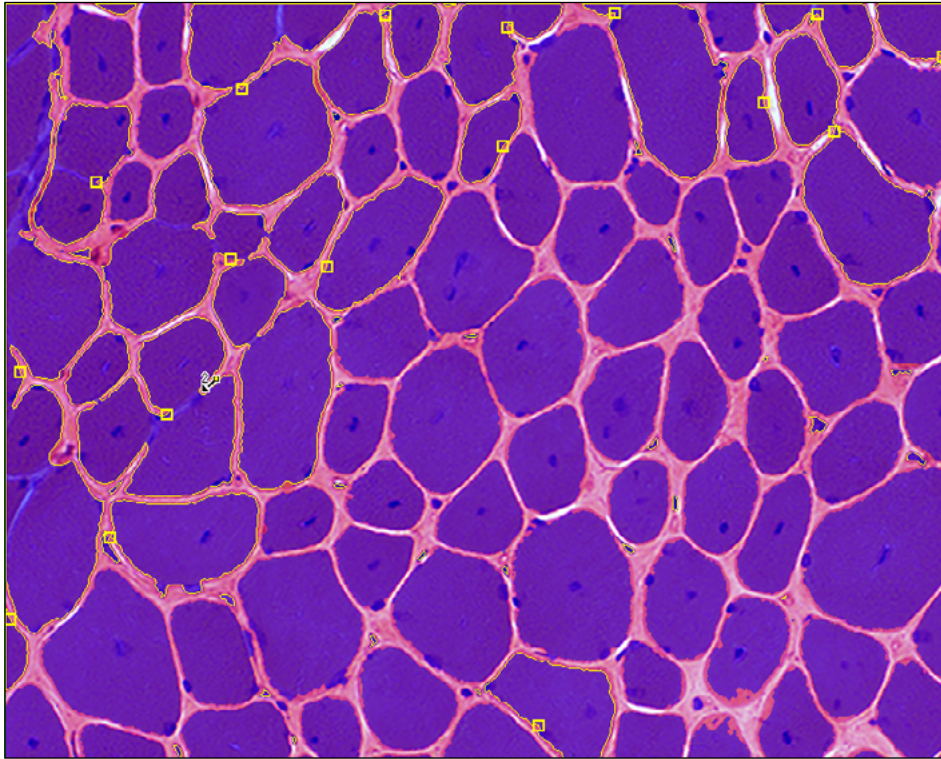
SEPARATE 2

The system detects the first separation point, you indicate the second point.

Click Second Point to Separate 2 is used to separate touching thresholded objects when the system is unable to detect both separation points. When the user clicks the Separate 2 button, the system detects touching cells. It puts a yellow box on one end of the separation location. When the user clicks on second point of separation, the cell separates.

POINTERS
. . . .

- The Separate tools work best at higher magnification.

- The Separate tools work best with a Smoothing Limit of 0 or 1.

- To speed things up, the filters and edge rejection do not work when the cursor is in Separate mode. However, as soon as you right click to exit, they are activated again. Don't spend time separating cells that will be filtered.

To switch the Separate button to Separate 2, click the down arrow on the Separate button and choose "Click Second Point to Separate 2."

If you click the Separate button in Separate 1 mode and you want to switch to Separate 2 mode while in the Image window, tap the 2 key.

SEPARATE 2
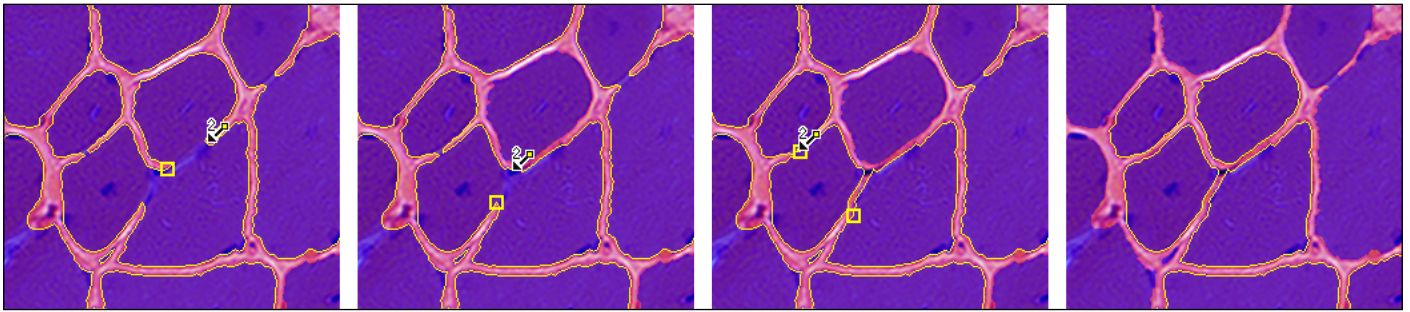
Use Separate 2 multiple times to separate clusters of cells.

As you click on cells, they automatically separate and new cells to separate are detected. This allows you to separate cells that are touching other cells in a sequence. The system detects the first point of the first separate location, you click the second point, and the preview outlines update. Then, the system detects the next first point of the next separate location. You repeat the sequence until all cells are separated.

As you separate large clusters of cells, it will make it easier for Separate 1 to automatically detect the cells. To switch to Separate 1 mode while in Separate 2 mode, tap the 1 key. Separate 1 detects both separation points.

4.   **The Clean button has two modes.**

The Clean tool removes small pieces of threshold. This makes it easier to improve the threshold with Dilate and Erode because the "noise" has been removed from the threshold.

The Clean tool has been split into Clean Fine and Clean Coarse, each with their own minimum size setting on the Editing Setup box.

Both Clean Fine and Clean Coarse can be used in Batch Scripts.

EDITING REGION
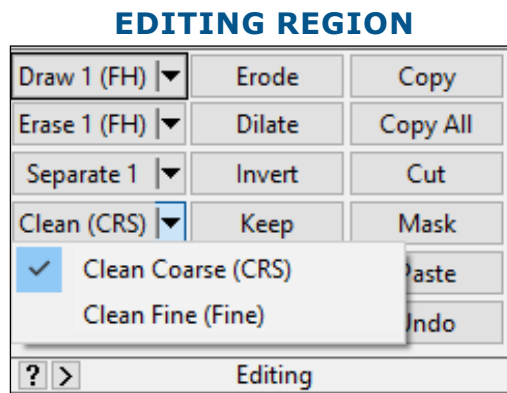
The Clean Button Menu.

CLEAN COARSE (CRS)

Clean Coarse (CRS) deletes all thresholded objects less than a user-specified minimum Clean Coarse size. The default minimum size is 500 pixels. The minimum size can be changed on the Editing Setup box.

CLEAN FINE (FINE)

Clean Coarse (Fine) deletes all thresholded objects less than a user-specified minimum Clean Fine size. The default minimum size is 50 pixels. The minimum size can be changed on the Editing Setup box.

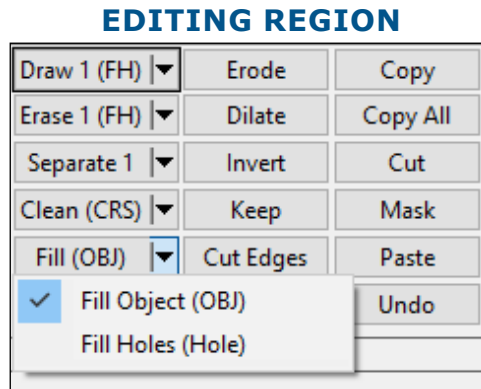**5. The Fill button has two modes.**

The Fill Object and Fill Holes buttons have been combined into a single Fill button. Use the drop down menu to switch between them. The functionality of the tools has not changed.

EDITING REGION

Draw 1 (FH) ▼	Erode	Copy
Erase 1 (FH) ▼	Dilate	Copy All
Separate 1 ▼	Invert	Cut
Clean (CRS) ▼	Keep	Mask
Fill (OBJ) ▼	Cut Edges	Paste
✓ Fill Object (OBJ)		Undo
Fill Holes (Hole)		

The Fill Button Menu.

FILL OBJECT (OBJ)

Fill Object (OBJ) fills all holes in the designated thresholded object. Click on the thresholded object to fill.

FILL HOLES (HOLE)

Fill Holes (Hole) fills specific holes in the thresholded object. Click inside the holes to fill.

6. The Run Script button has been moved from the Measurement region to the Editing region.

The Run Script button has been moved from the Measurement region to the Editing region. This better reflects the use of batch scripts in BIOQUANT, since most of the commands supported by batch scripts are from the Editing region.
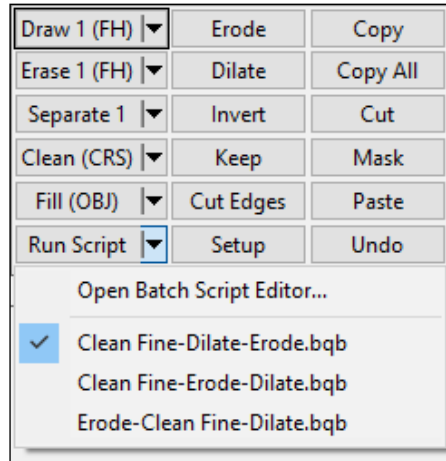
The Run Script Menu.

A batch script can be assigned to the Run Script button by default. This makes it possible to simply click the button and run the default script. If, for example, the lab has found a particular series of editing commands improve the threshold of trabecular bone for the measurement of referent data, these commands can be stored in a batch script and attached to the Run Script button.

The Run Script button also has a drop down menu that shows the 5 most recently used batch scripts. This makes it easy to switch the behavior of the button among several helpful scripts. Three are pre-assigned at installation.

7.   **The Editing Setup box has new features.**

CLEAN OPERATORS

The Clean Operators are used to adjust the size of the thresholded objects that are removed when using the Clean Coarse and Clean Fine tools.

The Clean Coarse Operator Min Size designates the minimum size of a thresholded object in pixels that is allowable. All thresholded objects with fewer than this number of pixels will be removed when Clean (CRS) is clicked.

The Clean Fine Operator Min Size designates the minimum size of a thresholded object in pixels that is allowable. All thresholded objects with fewer than this number of pixels will be removed when Clean (Fine) is clicked

SEPARATE TOOL SHAPE FACTOR

When you click Separate 1 or Separate 2, the system preview outlines cells that it thinks are touching. Part of the algorithm that is uses is a Shape Factor value. This value can be adjusted in the Separate Tools region of the Editing Setup box.

The shape factor of an object is the ratio of the object's area to the area of a circle with identical perimeter. Effectively this is a measurement of an object's roundness on a scale from 0 (straight line) to 1 (perfect circle).

The Max Shape Factor value indicates the maximum "roundness" that is allowed for previewing cells when Separate 1 or Separate 2 are clicked. The default value is 0.7.

8.   **The Editing region now supports keyboard hotkeys.**

EDITING REGION HOTKEYS: V B N M

KEYBOARD

The V B N and M keys.

Editing tool hot keys have been expanded. These keys, accessible in a single row with the left hand, switch among the Separate (V), Draw (B), Erase (N), and Fill (M) buttons. These keys were chosen because of their convenient location, not necessarily because of they matched the command names.

DRAW AND ERASE MODE HOTKEYS: 1 2 3 4

KEYBOARD

The 1 2 3 4 keys.

These keys, accessible in a single row with the left hand, switch among the different modes of the of the Draw and Erase tools. Freehand (1), Consecutive Distance (2), Individual Distance (3), Star Pattern (4).

These keys, accessible in a single row with the left hand, switch between the two modes of the Separate tool. Click Object to Separate (1) and Click Second Point to Separate (2).

While using the editing tools in the Image window, it's now possible to tap the U key to undo the most recent change to the threshold.

Previously, it was necessary to exit the Image window and click the Undo button, which would reset the threshold to its appearance when the cursor last entered the Image window.

BIOQUANT OSTEO: MINOR CHANGES

The following minor changes have been made.

MINOR FUNCTIONALITY CHANGES

1. **Improvements to Myofibers Type**

   The algorithms used in the Myofibers Measurement Type have been improved. The algorithm is better able to detect myofiber cells.

2. **Invert has been improved to handle edge pixels with certain ROIs.**

   For some ROI shapes, the pixels along the edge of the ROI were not inverted while the rest of the pixels in the threshold were. Previously, this caused confusing behavior with the Measure Cells and Myofiber Measurement Types. Cells which appeared to touch the edge of the ROI were, in fact, one pixel away from the ROI.

3. **Calculate Box: Select Calculation Arrays box has improved.**

   The Select Calculation Arrays box has been redesigned to make it easier to organize long lists of data. This box is opened by choosing "Select New Arrays for Calculate" or "Modify Calculate List" on the Calculate box File menu.

4. **The Batch Script Editor has been improved.**

   - The Step by Step button now consistently moves through steps in the a guide. Previously, the Step by Step would get confused if the length of the guide changed or if the guide was interrupted while in Step by Step mode.

   - Clean Fine is now a Batch Script Command.

   - Clean Coarse is now a Batch Script Command.

   - Backward Compatibility: If you have an existing Batch Script with the "Clean" command, the system will do the "Clean Fine" command. In the future use Clean Fine in batch scripts.

MINOR INTERFACE CHANGES

1. **The Large Image Navigator has been renamed to Image Navigator.**

2. **The General Measurement Type has been renamed to the Object Measurement Type.**

3. **The Spacebar to End button has been removed from the Editing region.**

    This functionality has been replaced with the Draw /Erase Consecutive Distance button options.

4. **These menus have been changed:**

    a. Menu items that have been moved to the ribbon are no longer on the menus.

    b. Menu items names have been renamed to better match what they do. For example, the Image>Live menu is now Image>Go to Live Image.

    c. The File > Print and File > Print Setup menu items have been removed. This functionality is no longer supported.

        Other File menu items have been moved to the Data region buttons.

    d. The Edit main menu no longer exists and these Edit menu items have been discontinued:

        •   Edit>Cut

        •   Edit>Copy>Current Selected Array

        •   Edit>Copy>All Selected Arrays

        •   Edit>Copy>Choose Available Arrays

            This functionality has been moved to the Data Region. Click the Export button and choose "Export Individual Arrays."

        •   Edit>Paste

        •   Edit>Delete All Data

            We strongly recommend that you DO NOT delete all the data in the data set. If this is part of your protocol, call us so that we can help you adapt it. Now, to delete data in the data set, use the Raw Data window.

    e. The Arrays main menu no longer exists. Its menu items have moved to the Data region buttons.

    f. The Stereology Tools menu item has moved to the Measure main menu.

    g. The Stereology main menu no longer exists.

    h. The Topo main menu has been renamed to the Topography main menu.

    i. The Stage menu item has been renamed "Activate Stage" and is now the first item on the

Topography menu.

j.   The Motorized Stage Controls menu item has been renamed "Open Motorized Stage Toolbar" and is now the second item on the Topography menu.

k.   The Topographer main menu item has been moved to the Topography menu and renamed "Open Topographer add-on."

The BIOQUANT Topographer is an add-on to BIOQUANT OSTEO. You might not have this menu item.

l.   The Topographer main menu no longer exists.