

Bioquant 2019

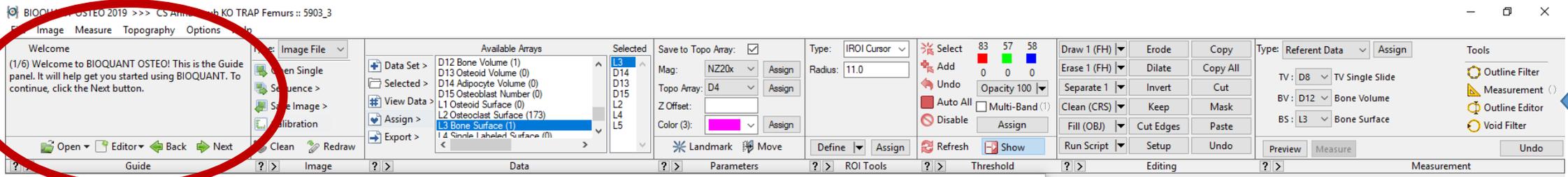
7/12/19

Outline

- Software overview
- 2019 updates to the software
- The use of calibration files
- Other hints for getting out quality data

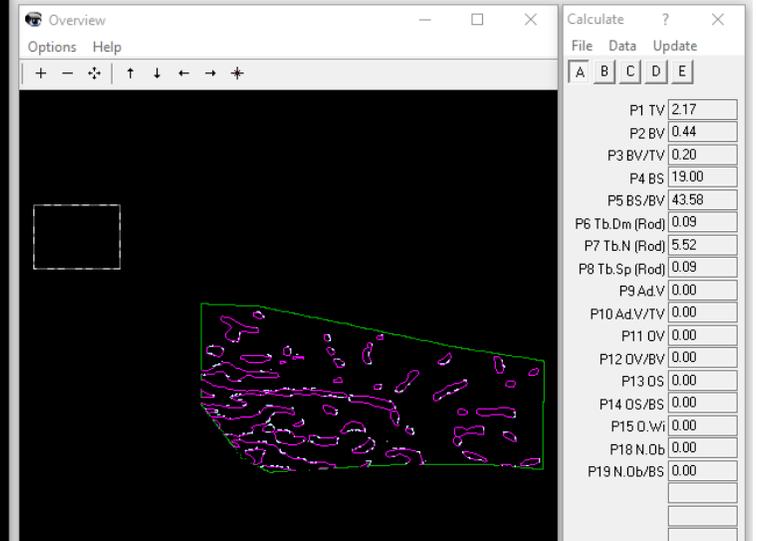
What does Bioquant measure?

- Anything on a 2D section
 - Dynamic histomorphometry (calcein/alizarin labels)
 - Counting cells
 - Muscle measurements
 - Area measurements (vessels, specific color of stains)
 - TRAP staining analysis



Tool ribbon

BQ 2019
-Tools have changed
-Help tutorials updated.



Tutorials are available – a quick way to get more information

Welcome

(1/6) Welcome to BIOQUANT OSTEO! This is the Guide panel. It will help get you started using BIOQUANT. To continue, click the Next button.

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  Guide

Welcome

(2/6) In addition to these guides, BIOQUANT has written and video documentation available in a PDF manual, the Help menu in BIOQUANT, and the help and video buttons in the different regions of this Tool Ribbon.

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Welcome

(3/6) The PDF Manuals are installed with your BIOQUANT software. You can find them in the \BQOSTEO2019\Manuals folder. They also have shortcuts on the desktop. The fastest way to open the OSTEO PDF Manual is to click on one of the Help icons on the ribbon.

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Welcome

(4/6) A list of videos associated with each region in the ribbon can be opened by click the Video ">" icon located in each region of the ribbon. The video procedures are also integrated into the PDF manuals and help.

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Welcome

(5/6) While you're learning BIOQUANT, if you have any questions, give us a call at 800-221-0549 or email support@bioquant.com to set up a Skype call.

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Welcome

(6/6) To continue with the BIOQUANT tutorials, click the Open button below and choose "Introduction to BIOQUANT OSTEO".

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  Guide

OSTEOSM

19.2.6



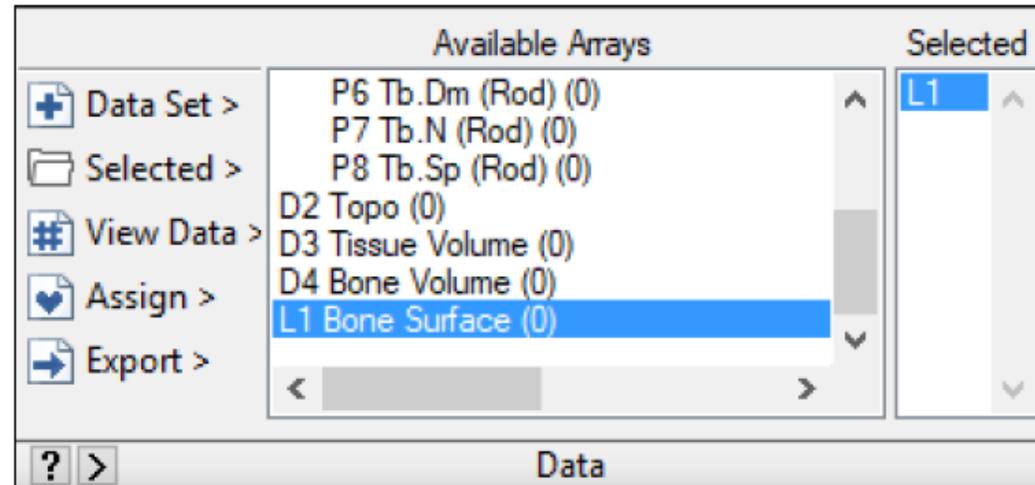
Release Notes

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

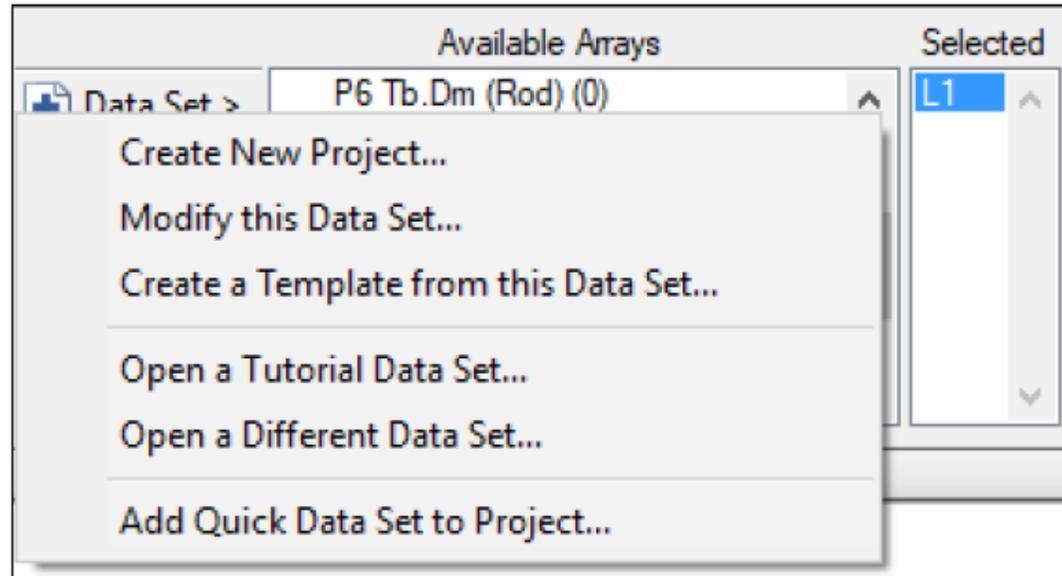


The Selected List region is now the Data region.

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.

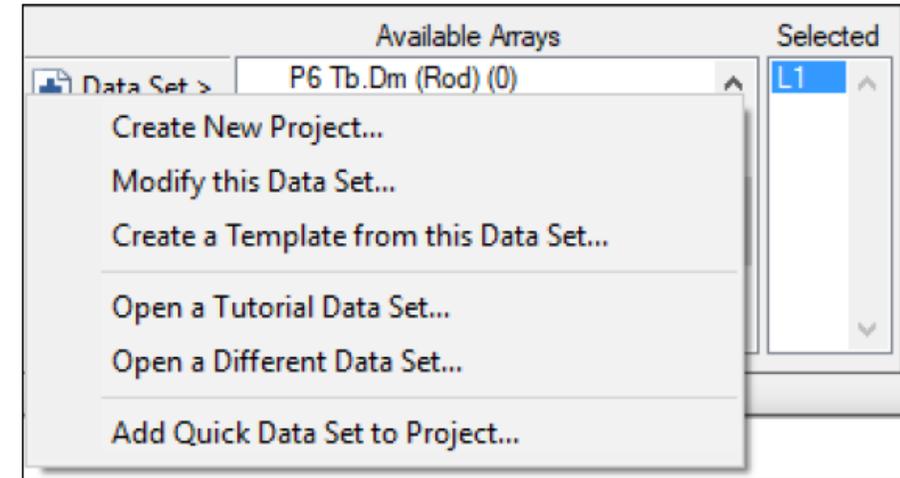
Previously, this box was opened by choosing “Open Data Set” from the File menu.

ADD QUICK DATA SET TO PROJECT

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

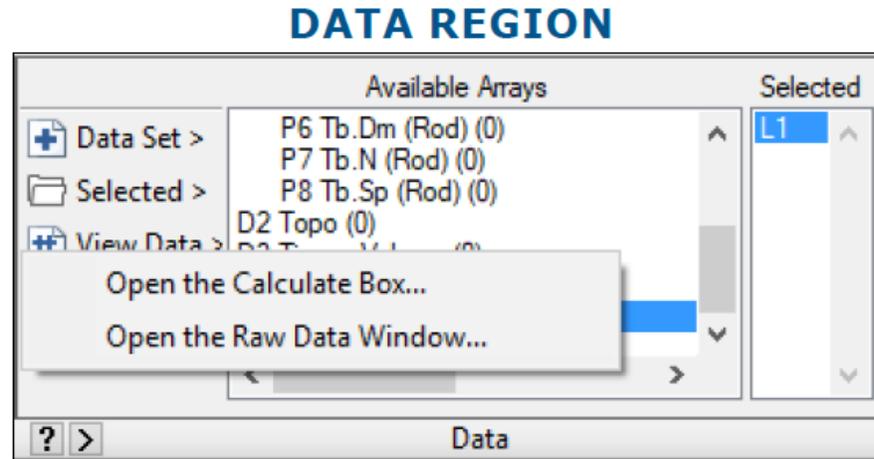
Previously, this box was named Quick Data Set and opened by choosing “Quick Data Set” from the File menu.

DATA REGION



The Data Set button menu.

3. View Data Menu



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.

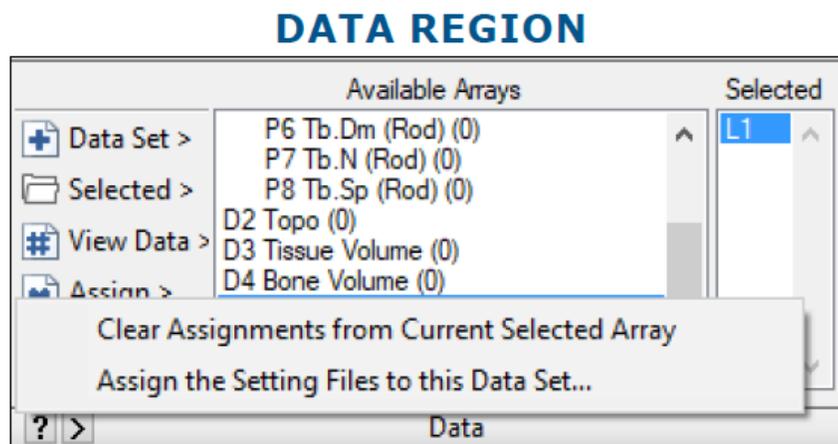
OPEN THE RAW DATA WINDOW

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



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.

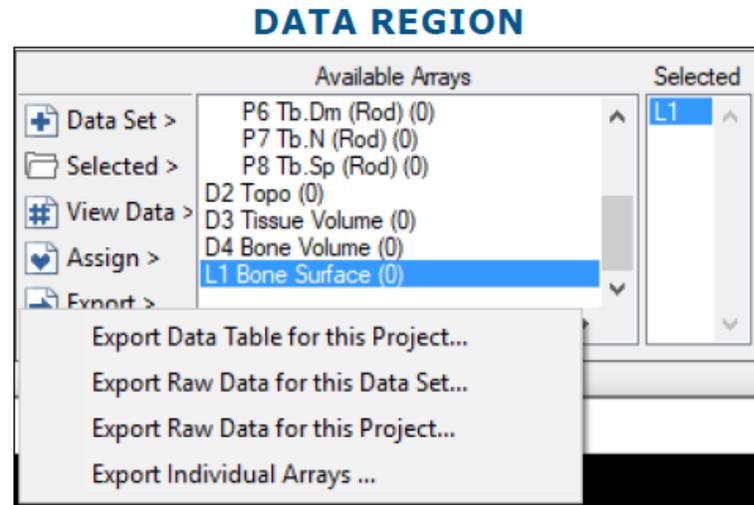
Previously, this feature was activated by choosing “Clear Assigned Comments” from the Arrays menu.

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



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.

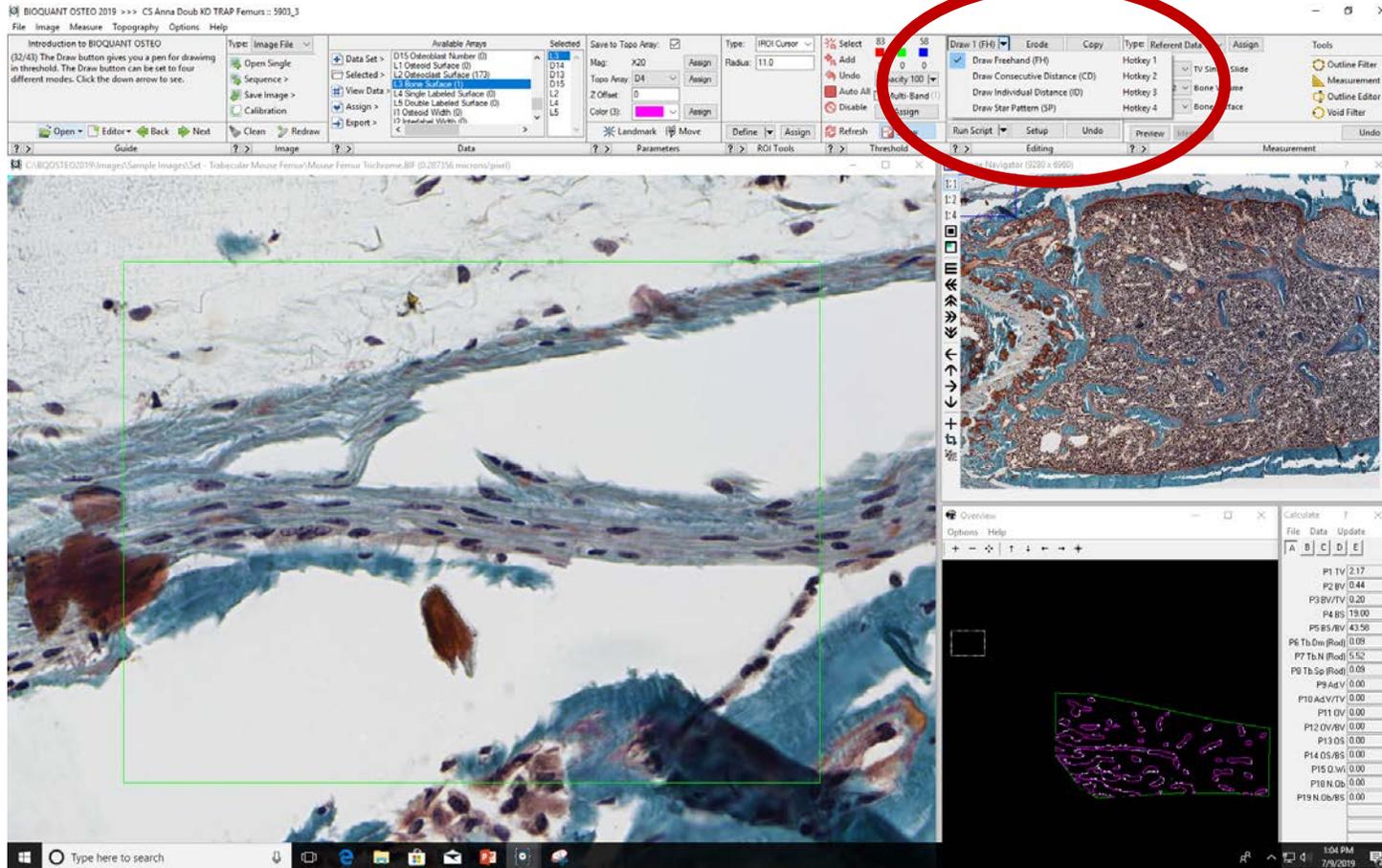
Export Data Table for this Project exports all the calculation arrays for the current data volume in a data table format to the clipboard. Then, you can click a cell in your spreadsheet program and paste.

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.

Editing tools



Draw 1 (FH) ▼	Erode	Copy
Erase 1 (FH) ▼	Dilate	Copy All
Separate 1 ▼	Invert	Cut
Clean (CRS) ▼	Keep	Mask
Fill (OBJ) ▼	Cut Edges	Paste
Run Script ▼	Setup	Undo
? > Editing		

Draw 1 (FH) ▼	Erode	Copy	Type: Refer
✓ Draw Freehand (FH)			Hotkey 1
Draw Consecutive Distance (CD)			Hotkey 2
Draw Individual Distance (ID)			Hotkey 3
Draw Star Pattern (SP)			Hotkey 4

Pictures are gone,
words and dropdowns
are new

Check through the 19.2.6 release notes for more changes

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.

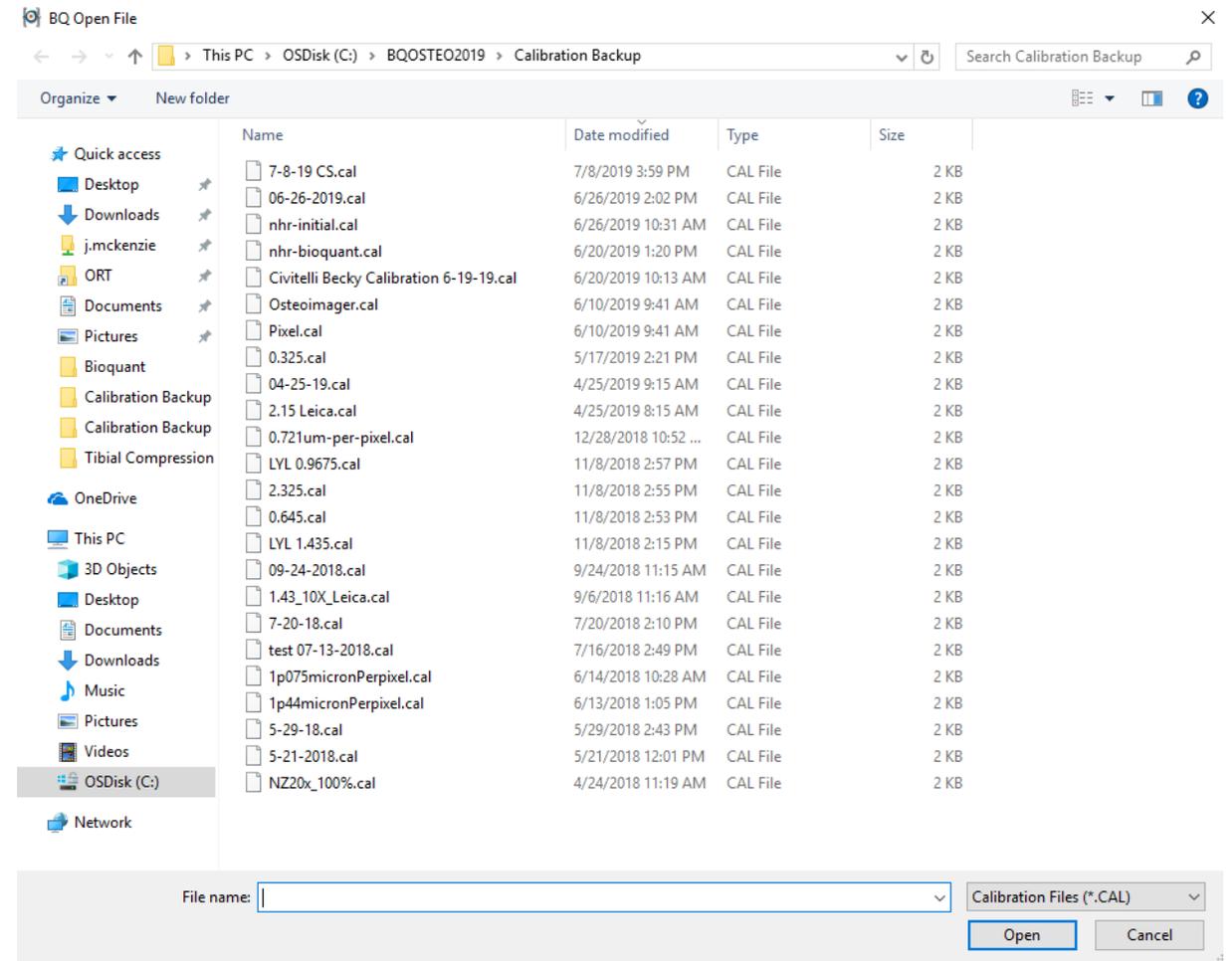
Calibration

Why?

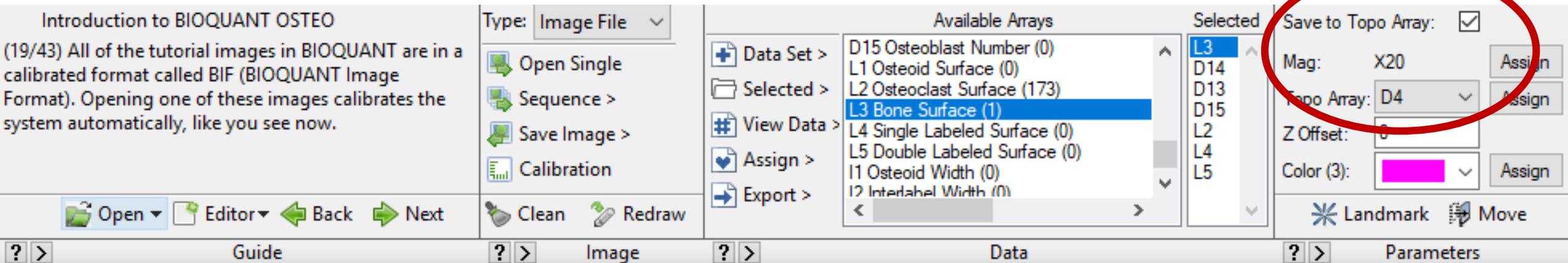
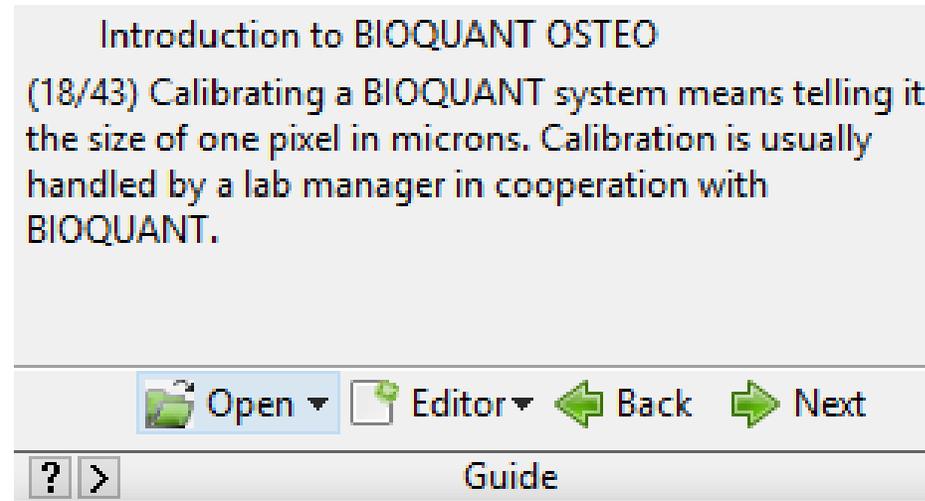
How?

Current calibration file problems

- We have a lot of calibration files
- Nomenclature varies
- Each .cal file contains MULTIPLE calibration options
- Many of these files are identical
- You are REQUIRED to save .cal file anytime a change is made to calibration (this is why we have so many)



Images accepted: .bmp, .tif, .jpg, .bif



Problems: Magnification shown does not give you full info. Could be X20 for a different scope. It is possible for someone to accidentally change the value of your pixel magnification.

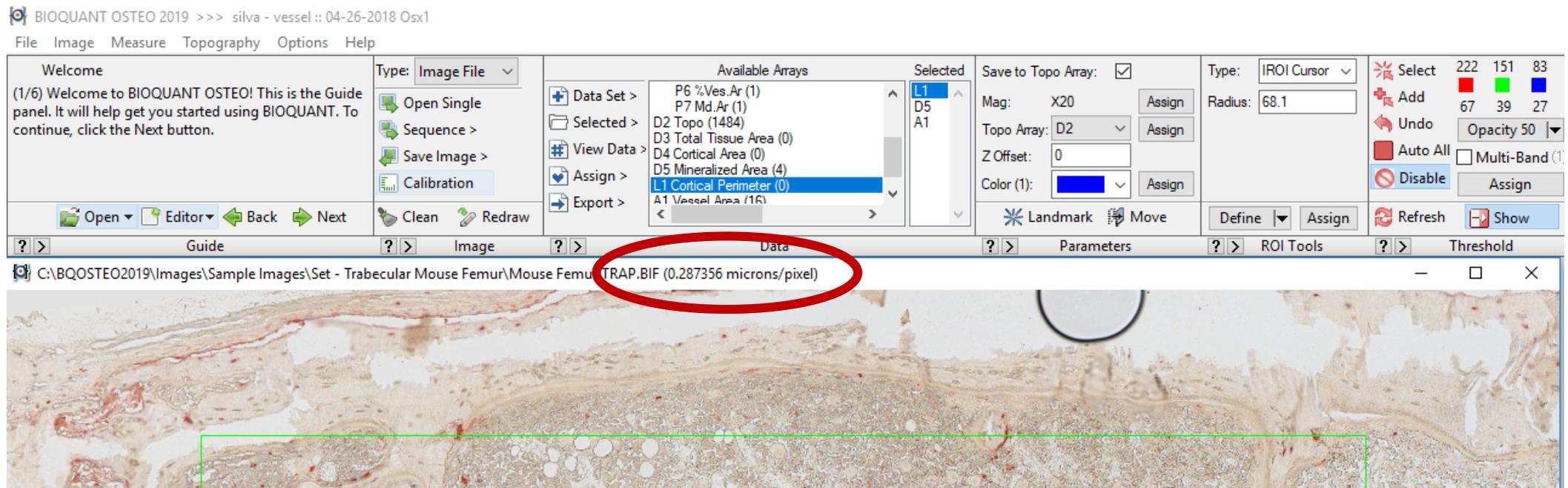
3. BIF Image Support: Display the Mag Factor

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

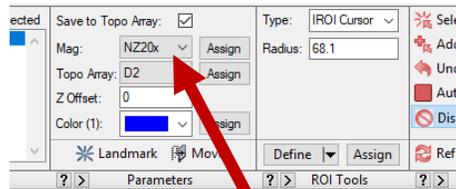
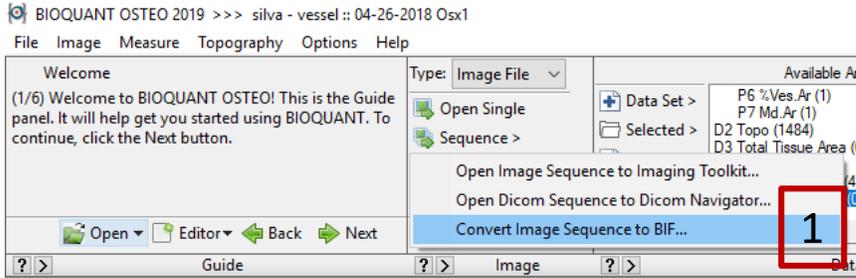
This makes it easy to check the numeric value of the calibration stored in BIF images.

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

- Save .bif files to folder on desktop (delete folder when finished with analysis)
- Magnification noted in image file heading for .bif files. This is the most reliable way to analyze your images in bioquant



Converting images to .bif files



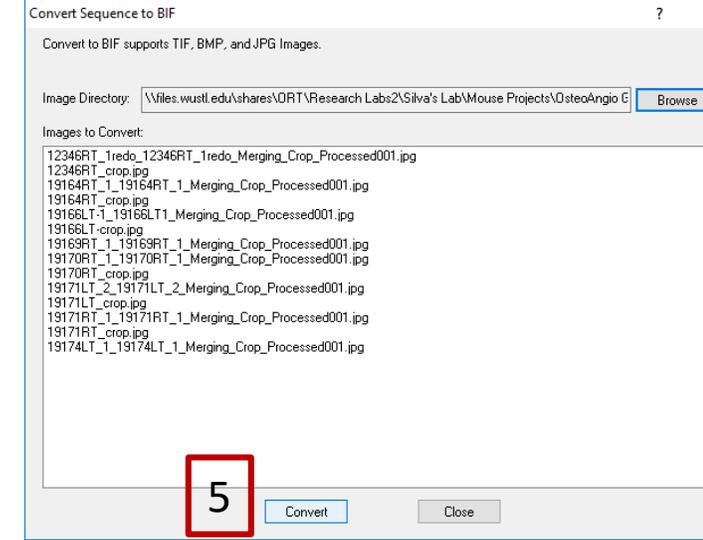
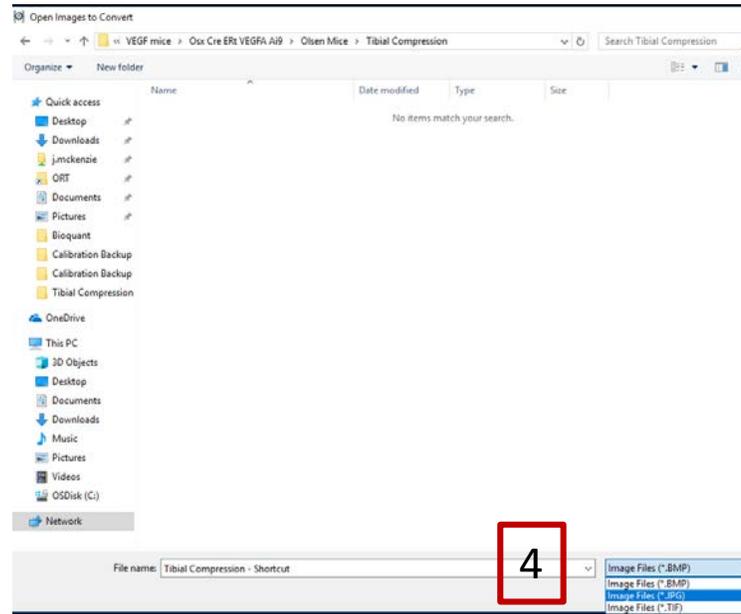
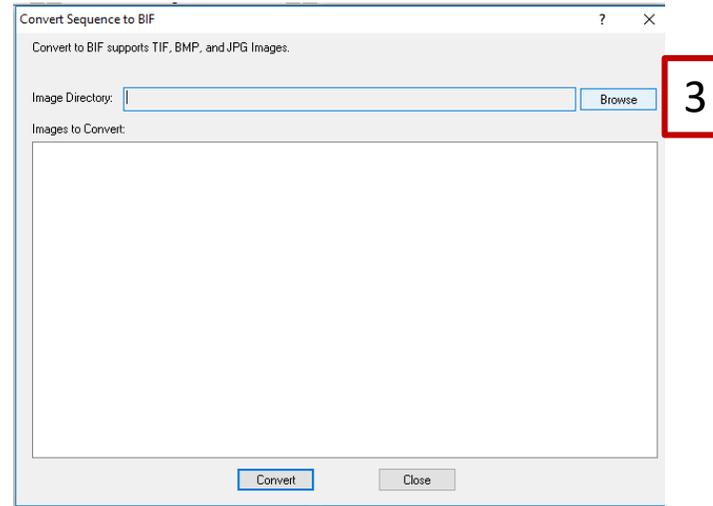
Verify this is correct



! Convert Sequence to BIF requires BIOQUANT to be accurately calibrated. Make sure that the system is calibrated for the TIF, JPG, or BMP images you are converting BEFORE using this feature. This feature will use the current selection in the Mag drop list in the Parameters region as the calibration for your BIF images. Do you want to continue?

2

Yes No



1. Open Sequence>Convert...
2. VERIFY magnification (and pixel value)
3. Browse for your images
4. Change file type if needed
5. Convert images

- Microscope calibration slide available (ask Michael Brodt)
- Zeiss, Leica scopes may output pixel factor (no calibration slide needed)
- Images of 20x nanozoomer already on bioquant computer
- **ALWAYS** DOUBLE CHECK before you start analysis.

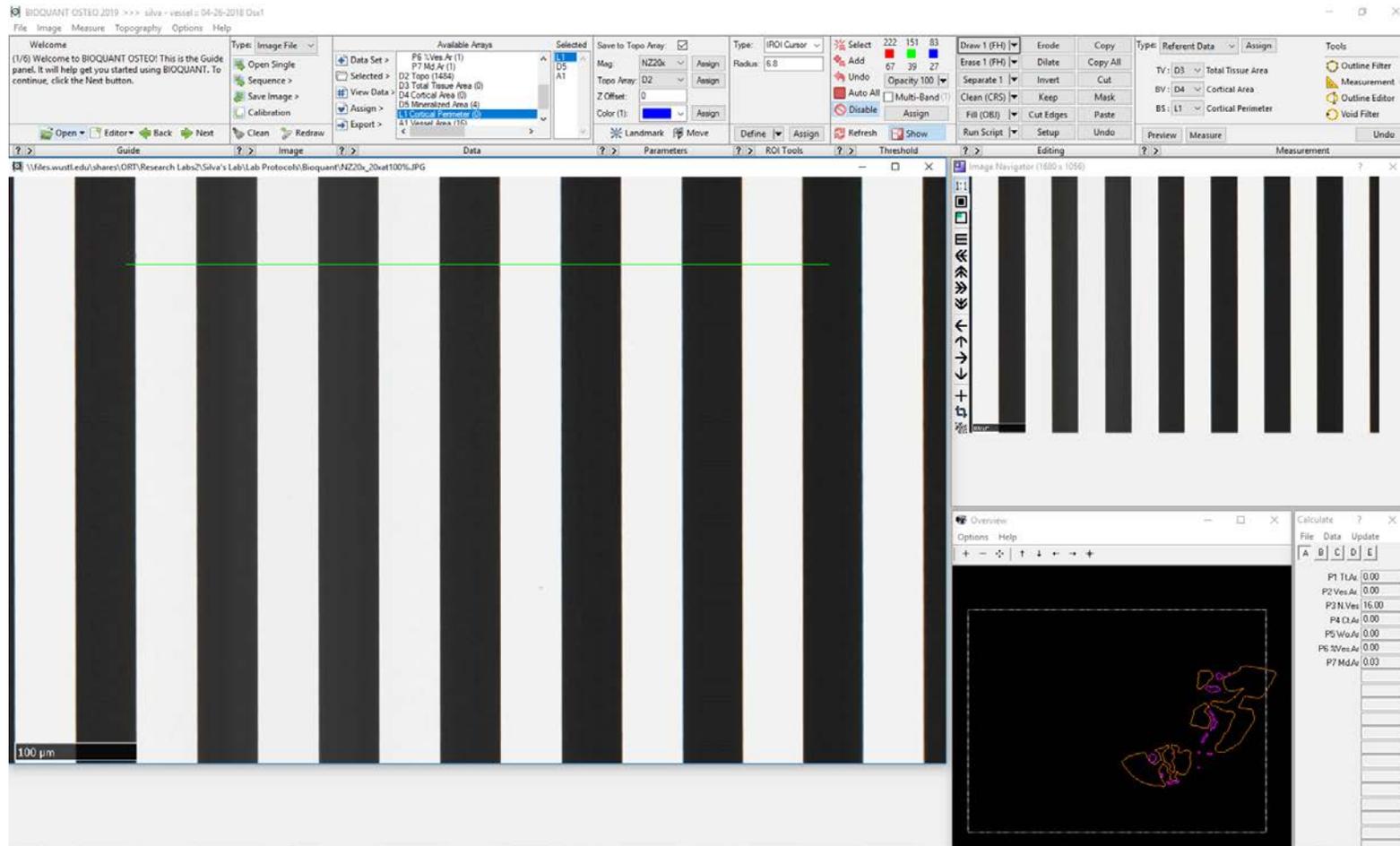
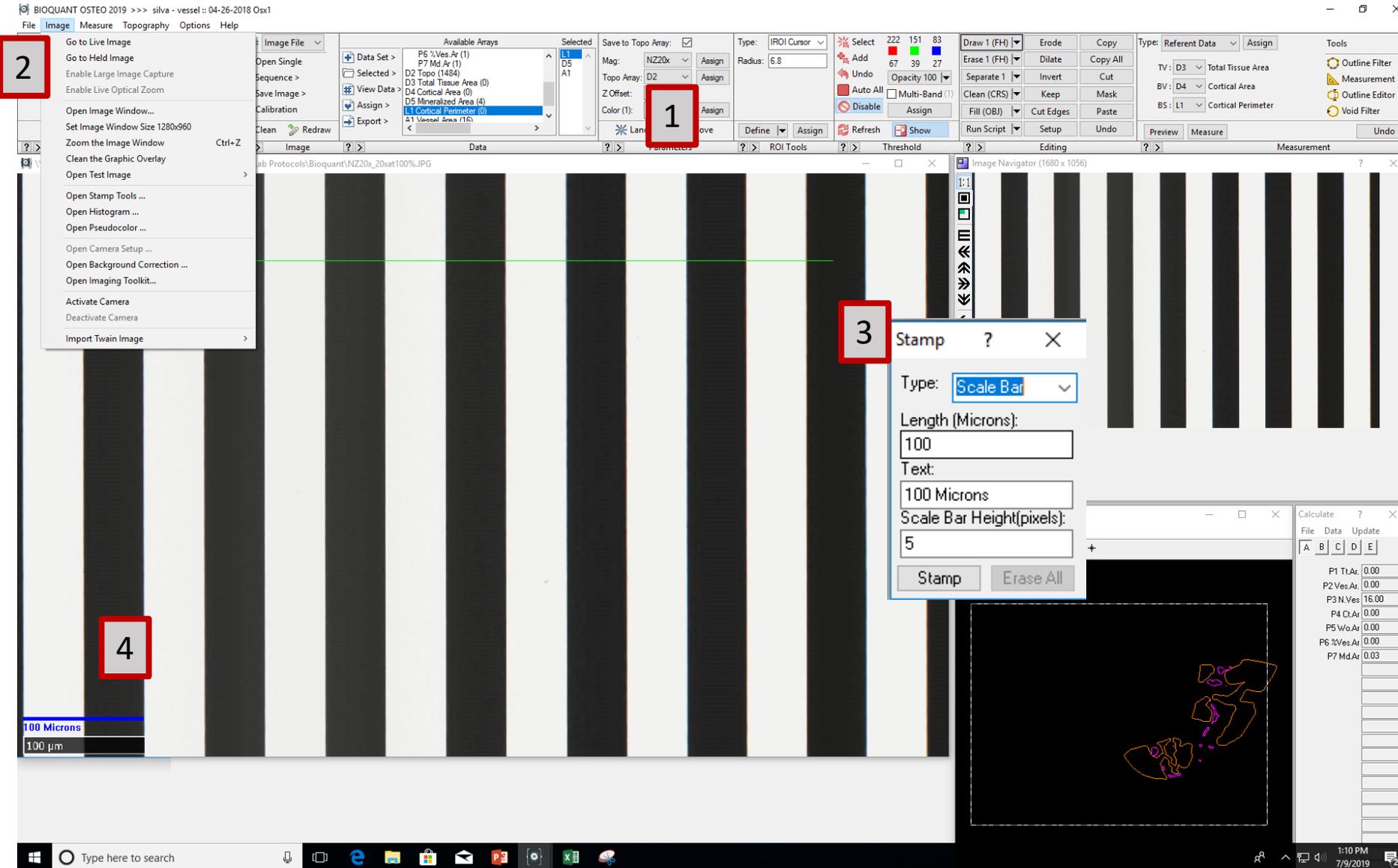


Image of calibration slide – each 2 bar set is 100 microns

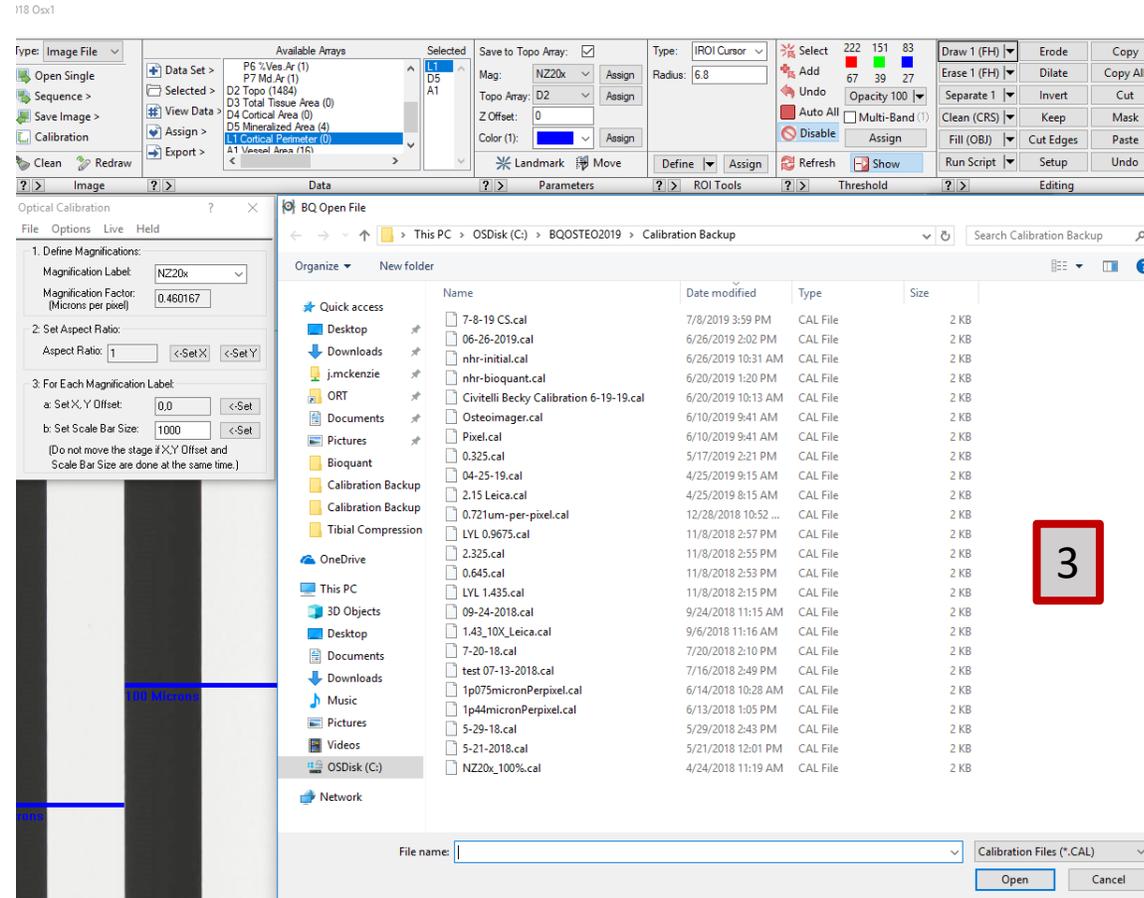
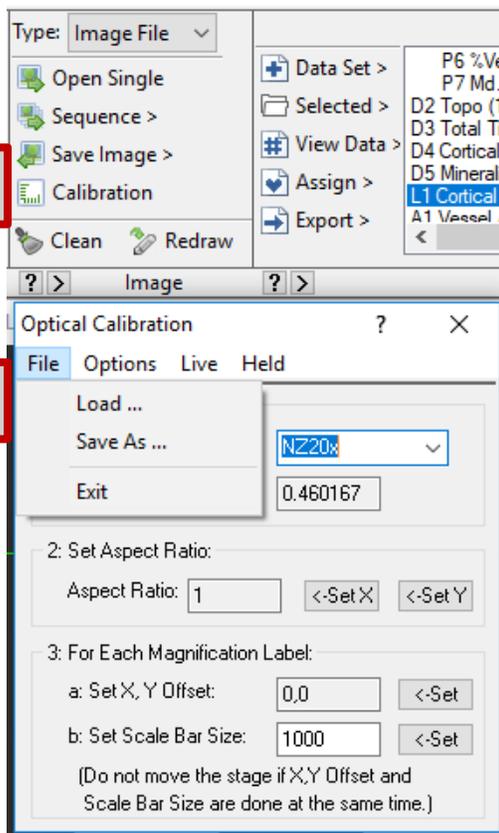
File	Data	Update
P1 Tt.Ar	0.00	
P2 Vv.Ar	0.00	
P3 N.Vv	16.00	
P4 Ct.Ar	0.00	
P5 Wv.Ar	0.00	
P6 Sv.Vv.Ar	0.00	
P7 Md.Ar	0.00	

How to verify your pixel size



1. Check current magnification (change if necessary)
2. Go to Image>Open Stamp tools
3. Edit length (if needed), click 'Stamp'
4. Verify scale bar

How to load a different magnification file



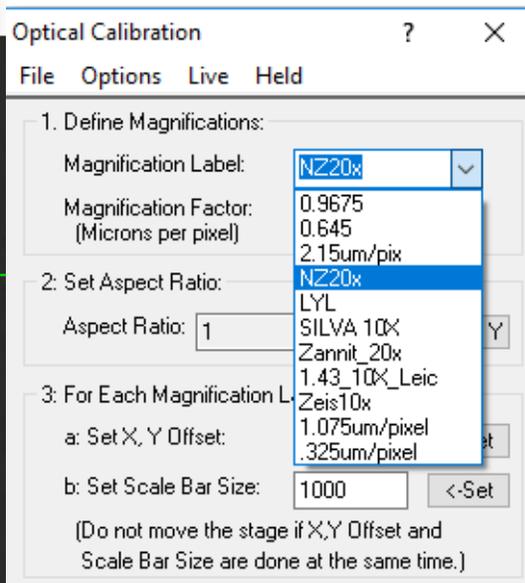
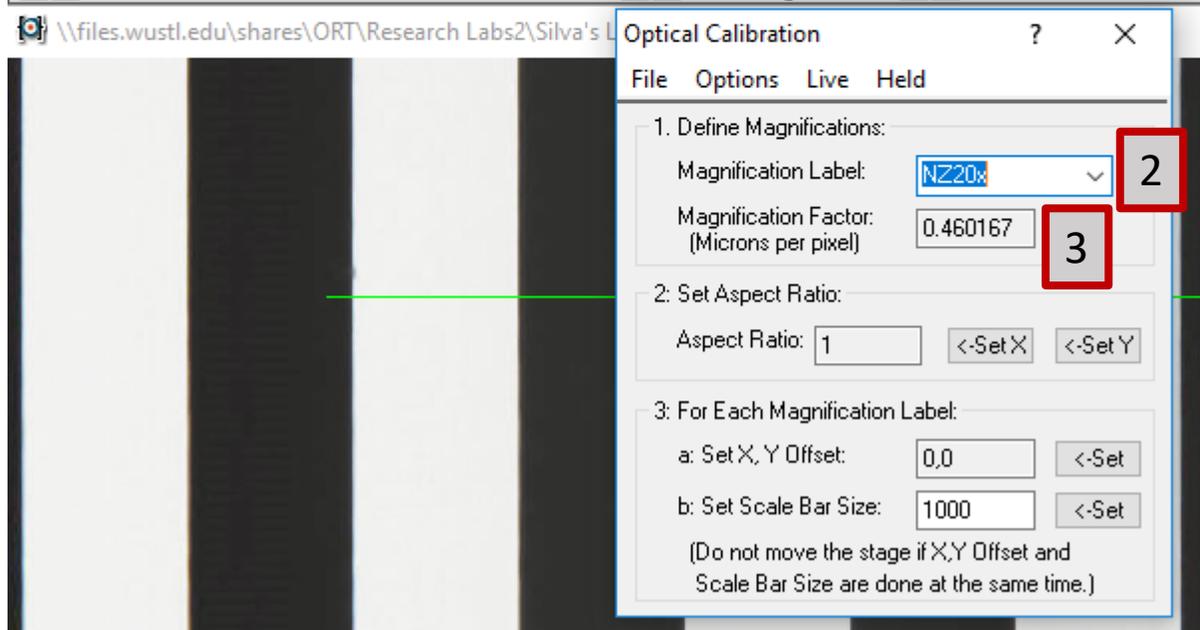
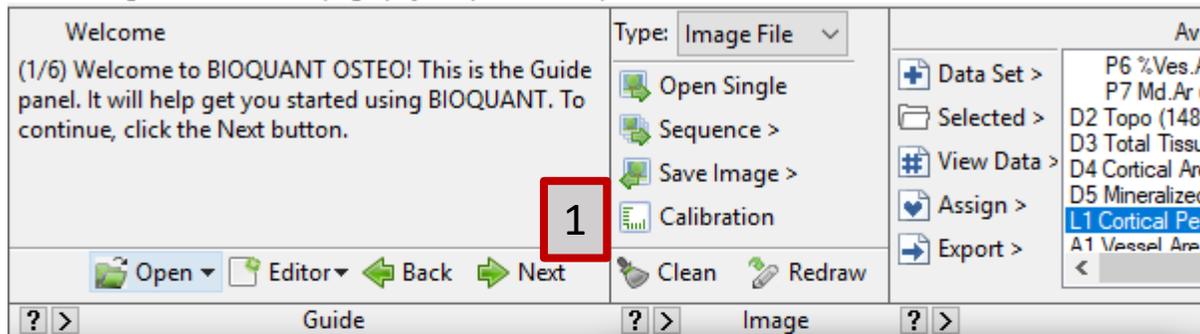
1. Open Calibration
2. File>Load
3. Select .cal file with newest date

Calibration Files: C:\BQOsteo2019\Calibration Backup

How to change calibration

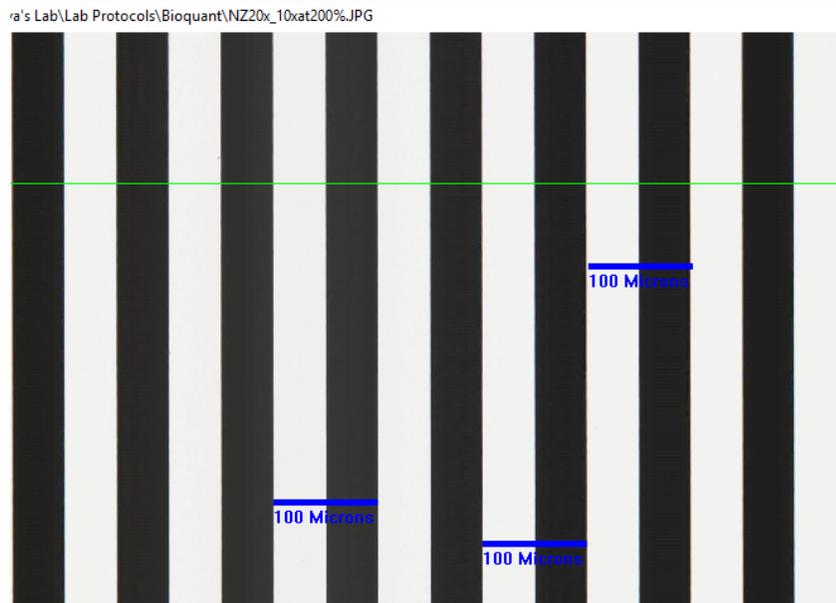
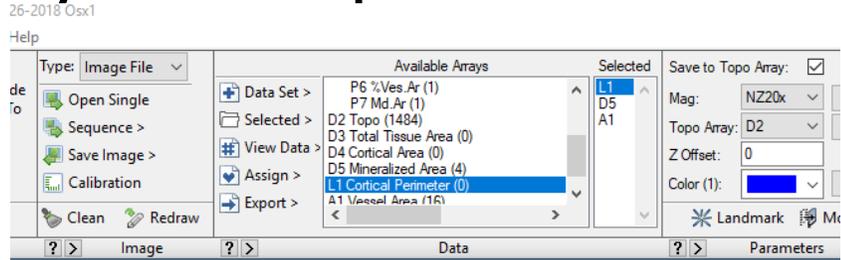
BIOQUANT OSTEO 2019 >>> silva - vessel :: 04-26-2018 Osx1

File Image Measure Topography Options Help

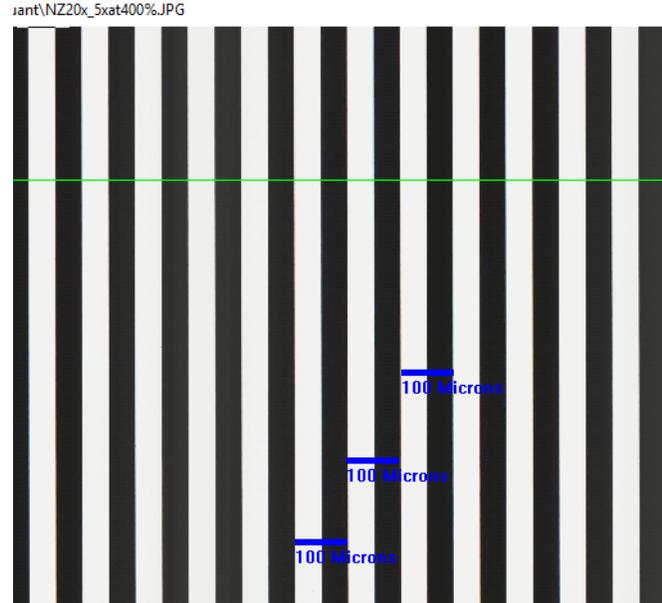
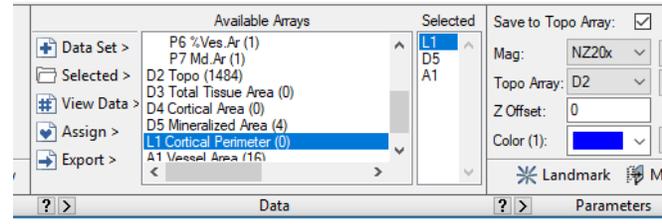


1. Open Calibration
2. Change to a different label
3. Verify Magnification factor (microns/pixel)
 - ~1 for 10x image
 - ~0.5 for 20x image
 - ~0.25 for 40x imageIf you don't know – use PIXEL (=1). With some work you can edit your results in excel later

Nanozoomer: magnification depends on how you export the image



Zoom to 10x, exported at 200%



Zoom to 5x, exported at 400%

- Do the same thing for all images in a study.
- Make sure you know how images were exported.
- Put scale bars on images to help verify

Zoom to 20x, exported at 100%

All of these have the same calibration pixel value. If you do a different zoom/export combination your pixel value changes.

All of these calibration scale bar images are available on the bioquant computer.

The screenshot shows a Windows File Explorer window titled "Calibration Images". The address bar indicates the path: "This PC > OSDisk (C:) > BQOSTEO2019 > Calibration Backup > Calibration Images". The left sidebar shows the "Quick access" pane with "This PC" selected. The main pane displays a list of 15 items, all of which are JPG files. The list is organized into columns for Name, Date modified, Type, and Size.

Name	Date modified	Type	Size
NZ20x_2p5xat100%	7/10/2015 7:52 AM	JPG File	309 KB
NZ20x_2p5xat200%	7/10/2015 7:52 AM	JPG File	818 KB
NZ20x_2p5xat400%	7/10/2015 7:52 AM	JPG File	2,204 KB
NZ20x_2p5xat800%	7/10/2015 7:53 AM	JPG File	6,753 KB
NZ20x_2p5xat1600%	7/10/2015 7:56 AM	JPG File	21,814 KB
NZ20x_5xat100%	7/10/2015 7:48 AM	JPG File	234 KB
NZ20x_5xat200%	7/10/2015 7:49 AM	JPG File	626 KB
NZ20x_5xat400%	7/10/2015 7:49 AM	JPG File	1,909 KB
NZ20x_5xat800%	7/10/2015 7:50 AM	JPG File	5,829 KB
NZ20x_10xat100%	7/10/2015 7:50 AM	JPG File	160 KB
NZ20x_10xat200%	7/10/2015 7:50 AM	JPG File	482 KB
NZ20x_10xat400%	7/10/2015 7:51 AM	JPG File	1,474 KB
NZ20x_20xat100%	7/10/2015 7:51 AM	JPG File	121 KB
NZ20x_20xat200%	7/10/2015 7:51 AM	JPG File	369 KB
NZ20x_40xat100%	7/10/2015 7:51 AM	JPG File	92 KB

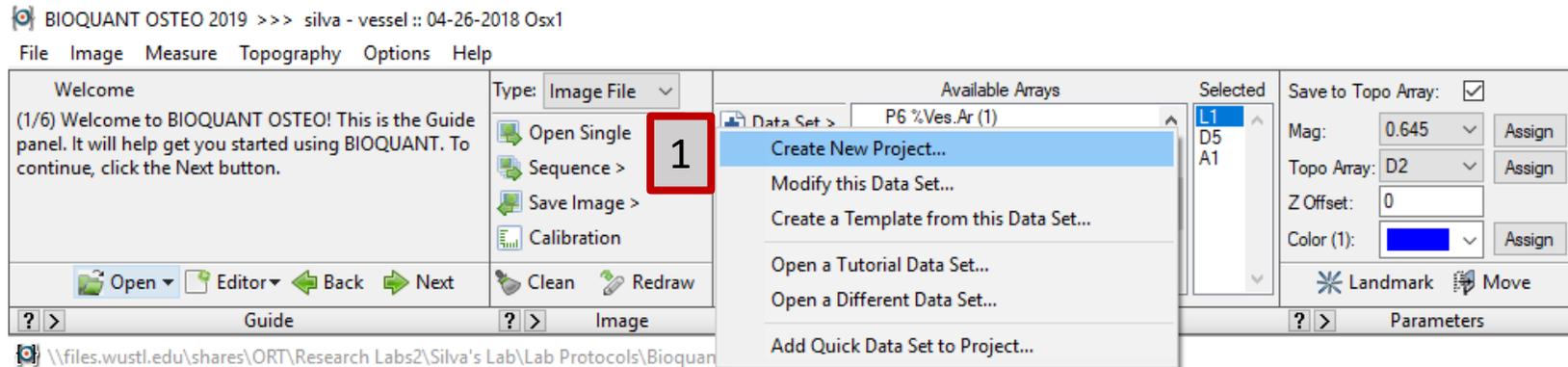
What to do about calibration files going forward?

- Put all current .cal files into an 'archive' folder
- Make a calibration file for each microscope
 - Leica confocal
 - Zeiss slidescanner at WUCCI
 - Nanozoomer
 - Others?
- Label each .cal file with microscope name and date
- If new magnification added (with help of Bioquant support staff), move previous .cal version into archive folder

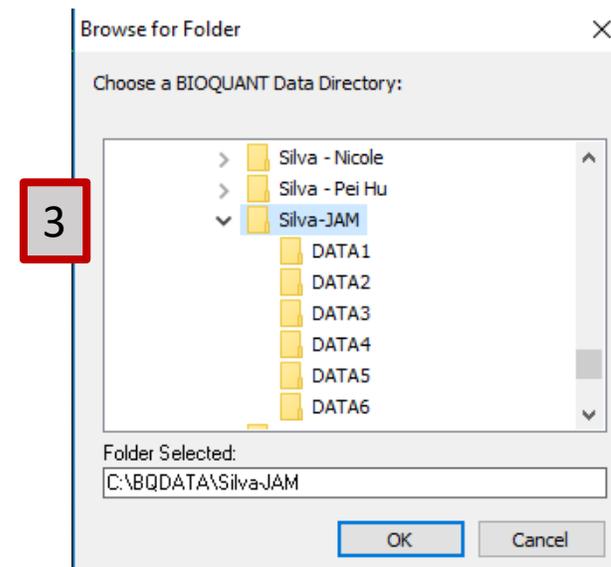
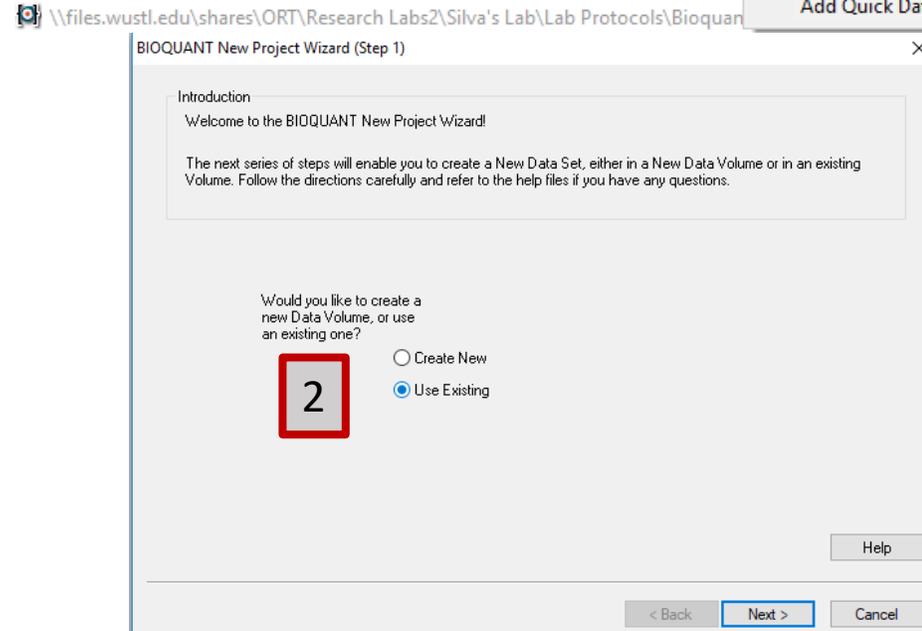
Other Hints

- 'Ctrl+F10' to clear if stuck in analysis window
- If program seems frozen check to see if second window open in background (new data set box, etc)
- 'Undo' will take you one step back in measurements.
- 'R' when inside image window will redraw
- 'Ctrl + R' will redraw contours when outside of imaging box
- Save sporadically during long samples (autosave set at 5min)
- If you are someone different than folder 'dyn histo' was selected from – open file, verify info then start your own data volume (new set instead of quick set)
 - Once you are in your own
 - folder you can do quicksets

You want to do the same data analysis as your lab mate, but save in a different location



1. Open Data Set>Create New Project
2. Select 'Use Existing'
3. Navigate to new folder (start folder with your PI name)



How to check/assign selected and calculation lists

The screenshot displays the BIOQUANT OSTEO 2019 software interface. The main window shows a grayscale image of a vessel with a green horizontal line. A red box labeled '1' highlights the 'Assign the Setting Files to this Data Set...' option in the 'Data Set' menu. An 'Assign to Data Set' dialog box is open in the foreground, with a red box labeled '2' highlighting the 'Selected List' dropdown menu. The dialog box contains the following fields:

- Current Data Set: silva - vessel :: 07-09-2019 test1
- Current Data Set Assignments
 - Guide: None
 - Selected List: Silva - Vessel + Wo Count.bqa
 - Calculation List: Silva - Vessel Wo Count.bqc
 - Measurement Filter: None
 - Multi-Band Threshold: None
- Remove Current Data Set Assignments

Buttons for 'OK' and 'Cancel' are visible at the bottom of the dialog box. The Windows taskbar at the bottom shows the system clock as 1:20 PM on 7/9/2019.

Verify as you go: look at the raw data file

The screenshot displays the BIOQUANT OSTEO 2019 software interface. The main window shows a histology image with a green horizontal line indicating a region of interest. A 'Raw Data: Current Array' window is open, showing a list of data points with the number '2' highlighted in a red box. A 'Calculate' window is also open, showing a list of parameters and their values, with the 'Open Raw Data ...' button highlighted in a red box and labeled '1'. The software interface includes a menu bar (File, Image, Measure, Topography, Options, Help), a toolbar, and various panels for data management and analysis.

Available Arrays:

- P6 %Ves.Ar (1)
- P7 Md.Ar (1)
- D2 Topo (1404)
- D3 Total Tissue Area (0)
- D4 Cortical Area (0)
- D5 Mineralized Area (4)
- L1 Cortical Perimeter (0)
- A1 Mineral Area (16)

Selected: L1, D5, A1

Save to Topo Array:

Type: IROI Cursor

Mag: 0.645

Radius: 68.1

Topo Array: D2

Z Offset: 0

Color (1): [Blue]

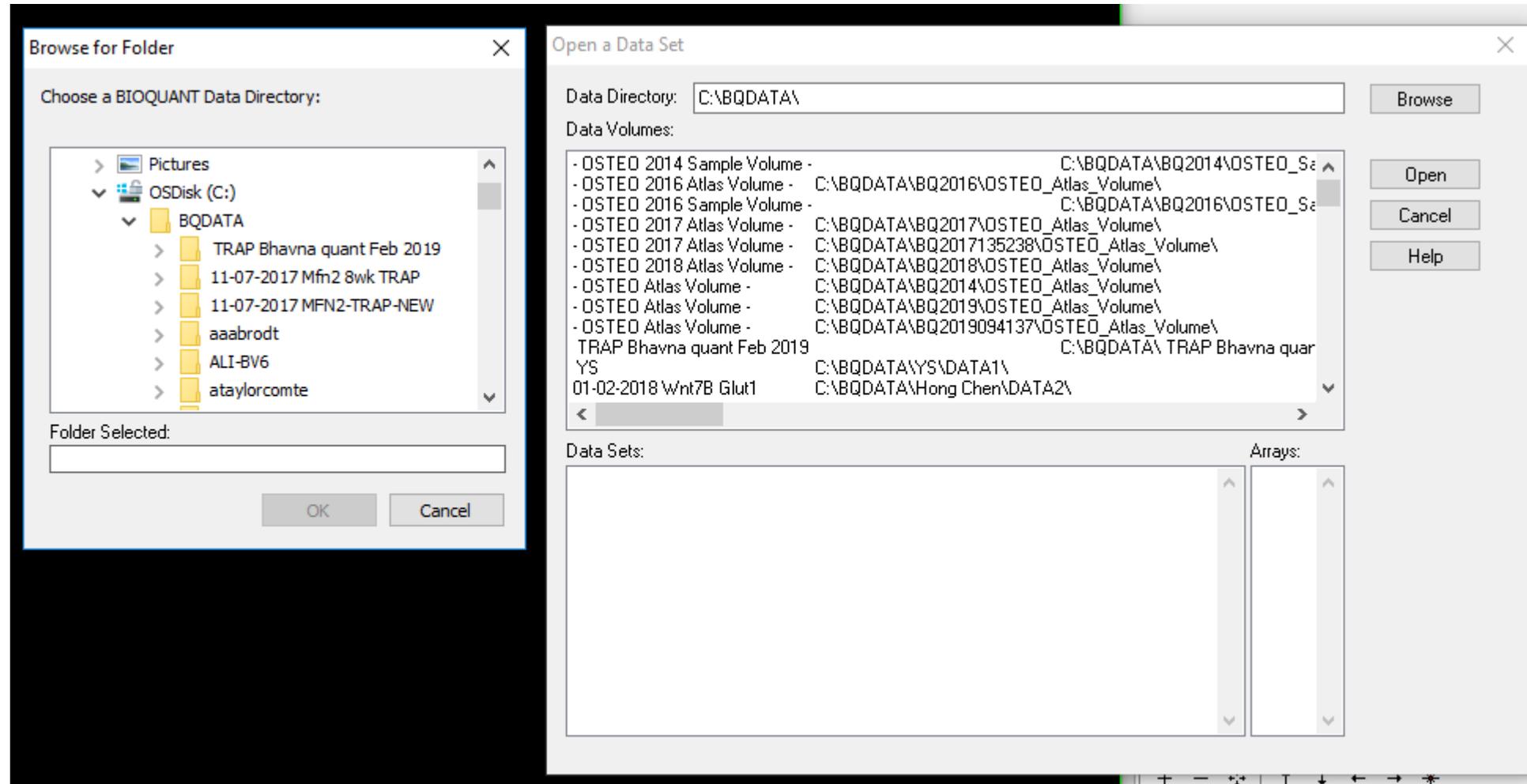
Tools:

- Outline Filter
- Measurement
- Outline Editor
- Void Filter

Calculate:

- P2 Ves.Ar: 0.00
- P3 N.Ves: 16.00
- P4 Ct.Ar: 0.00
- P5 Wc.Ar: 0.00
- P6 %Ves.Ar: 0.00
- P7 Md.Ar: 0.03

All files located in C:\BQDATA\... You can make an individual lab folder (recommended to start with your PI name)



We all have access to everyone's files – don't open things that aren't yours.

Make a screenshot tutorial guide for your analysis

- You WILL forget how you did something.
- Take 10 min and do this for each project.

Questions?

- Contact Bioquant Support

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