# Bioquant 2019 <sub>7/12/19</sub>

# 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



## BQ 2019 -Tools have changed -Help tutorials updated.

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© Overview		
Options       Help       File       Data       Update         + -        ↑ ↓ + + → +       A       B       C       D       E         P1 TV       217       P2 BV       0.44       P3 BV/TV       0.20       P4 BS       19.00         P5 BS/RV       4358       P6 Tb.Dm (Rod)       0.03       P7 Tb.N (Rod)       0.03       P7 Tb.N (Rod)       0.03         P7 Mol V/TV       0.00       P10 AdV/TV       0.00       P10 AdV/TV       0.00       P11 OV (0.00)         P12 0V/RV       0.00       P13 NO 0.00       P13 NO 0.00       P19 NO 0.00       P10	🐨 Overview — 🗆 🔿	× Calculate ? ×
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Tool

ribbon

## 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.	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.	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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? > Guide	? > Guide	? > Guide

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

	Available Arrays		Selected					
Data Set >	P6 Tb.Dm (Rod) (0)	~	L1 🔥					
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Modify th	is Data Set	- 1						
Create a T	Create a Template from this Data Set							
Open a Tu	Open a Tutorial Data Set							
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Add Quic								

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**

	Available Arrays									
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Modify th	is Data Set									
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Open a Tu	utorial Data Set									
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Add Quic	k Data Set to Project									

The Data Set button menu.

#### 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.

# Editing tools



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Draw Star Pattern (SP)

Pictures are gone, words and dropdowns are new

Hotkey 4

## 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)

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Calibration Files (\*.CAL)

Cancel

File name:

# 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. <u>This is the most reliable</u> way to analyze your images in bioquant



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

# Converting images to .bif files



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- 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

## How to verify your pixel size



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

## How to load a different magnification file

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

Calibration Files: C:\BQOsteo2019\Calibration Backup

)18 Osx1

## How to change calibration

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

File Image Measure Topography Options Help



1. Open Calibration

3.

2. Change to a different label

Verify Magnification factor (microns/pixel) ~1 for 10x image ~0.5 for 20x image ~0.25 for 40x image If 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



- 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 10x, exported at 200%

Zoom to 5x, exported at 400%

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.

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# 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

# List of calibration names, pixel values to be posted next to computer.

-													 		
4	NZ20		0.460167	Nanozoom	er										
5	1.075um/pix	10x at 1.0 zoom, 1024x1024	1.075	Leica confo	cal - 10x o	digital zoor	n 1.0 resol	ution 1024	x1024						
6	0.718um/pix	10x at 1.5 zoom, 1024x1024	0.718	Leica confo	cal - 10x (	digital zoor	n 1.5 resol	ution 1024	x1024						
7	0.537um/pix	10x at 1.0 zoom, 2048x2048	0.537	Leica confo	cal - 10x o	digital zoor	n 1.0 resol	ution 2048	x2048						
8	0.359um/pix	10x at 1.5 zoom, 2048x2048	0.359	Leica confo	cal - 10x o	digital zoor	n 1.5 resol	ution 2048	x2048						
9	Wucci Zeiss slide scanner - 20x	Wucci Sl	0.325	Zeiss slides	canner at	t WUCCI- H	eather, Tay	ylor							
10	1.43	10x at 1.0 zoom, 512 x 512	1.43	Leica confo	cal - 10x (	digital zoor	n 1.0, 512x	512 (need	to verify)						
11	Nanozoomer	2.5x at 100%	3.681336	(images \\	files.wus	tl.edu\sha	res\ORT\R	esearch La	bs2\Silva'	s Lab\Lab P	rotocols\B	oquant)			
12	Nanozoomer	2.5x at 200%	1.840668												
13	Nanozoomer	2.5x at 400%	0.920334												
14	Nanozoomer	2.5x at 1600%	0.2300835												
15	Nanozoomer	5x at 100%	1.840668												
16	Nanozoomer	5x at 200%	0.920334												
17	Nanozoomer	5x at 400%	0.460167												
18	Nanozoomer	5x at 800%	0.2300835												
19	Nanozoomer	10x at 100%	0.920334												
20	Nanozoomer	10x at 200%	0.460167				What	other	scon	es/ma	agnific	ations			
21	Nanozoomer	10x at 400%	0.2300835				······	other			9				
22	Nanozoomer	20x at 100%	0.460167				do we	e need	35						
23	Nanozoomer	20x at 200%	0.2300835												
24	Nanozoomer	40x at 100%	0.2300835												
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## 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'
- Navigate to new folder (start folder with your PI name)

# How to check/assign selected and calculation lists

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panel. It will help get you started using BIOQUANT. To continue, click the Next button.	P / Md.Ar(0)     D5     Selected > D2 Topo (0)     A1     Topo Array: D2 ✓ Assign	→ Undo Opacity 100 ▼ Se	arate 1 V Invert Cut	D3 V Total Tissue Area Measurement ()	
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## Verify as you go: look at the raw data file

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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

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.

