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.
Tutorials are available – a quick way to get more information
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.

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.
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.
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

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

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

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
How to change calibration

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 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

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.
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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Magnification</th>
<th>Pixel Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZ20</td>
<td>10x at 1.0 zoom, 1024x1024</td>
<td>1.075 Leica confocal - 10x digital zoom 1.0 resolution 1024x1024</td>
</tr>
<tr>
<td>Nanozoomer</td>
<td>10x at 1.5 zoom, 2048x2048</td>
<td>0.718 Leica confocal - 10x digital zoom 1.5 resolution 2048x2048</td>
</tr>
<tr>
<td>Zeiss slide scanner - 20x</td>
<td>10x at 1.0 zoom, 512 x 512</td>
<td>1.43 Leica confocal - 10x digital zoom 3.0, 512x512 (need to verify)</td>
</tr>
</tbody>
</table>

What other scopes/magnifications do we need?
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

1. Open the software and navigate to the data set you wish to assign.
2. Click on "Assign to Data Set" and select the desired lists:
   - **Selected List**: Silva - Vessel + Wo Count.bqa
   - **Calculation List**: Silva - Vessel + Wo Count.bqa

3. Click "OK" to apply the assignments.
Verify as you go: look at the raw data file
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.