OSTEO

22.5.6

Release Notes

BIOQUANT®

CONTENTS

RELEASE NOTES OVERVIEW

DEVELOPMENT THEMES

A major focus of the work in 2021/2022 has been on continued improvement of BIOQUANT by using more of the remarkable features in the Jenoptik digital cameras. That work supports other improvements that shorten measurement procedures and speed up BIOQUANT SCAN.

JENOPTIK CAMERA INNOVATION CONTINUES

The speed and computational power in the Jenoptik cameras make some really great features possible.

Most exciting is Live Correction. The live image of the camera is continuously corrected for uneven lighting and other imperfections of the microscope. It's like looking at your samples with a brand new microscope.

Configure your Jenoptik camera for brightfield imaging with one click. A new automatic brightfield setup algorithm makes it wonderfully simple to properly adjust your camera for accurate imaging.

Background adjustment for fluorescence imaging has arrived too. The fluorescence black balance parameter suppresses autofluorescence to make the background of fluorescent images pure black.

LEGACY QIMAGING CAMERA WORK

We are pleased to continue legacy QImaging camera support in this version. At the same time, many labs are upgrading to Jenoptik cameras and substantial trade-in discounts are available.

Some of the work we did with Jenoptik cameras to simplify background correction was applicable to QImaging cameras. Check the detailed notes below for the simplified background correction user interface.

EXPANDING BIOQUANT SCAN

The introduction of Scan by Topo Array in 2021 was a huge success. Nearly every lab switched their protocol to adopt the new methodology. Some labs are even experimenting with using the Tissue Volume tracing as the Scan area tracing for added efficiency. We're continuing to work on new ways to bring BIOQUANT SCAN to more people.

In 2022, we've addressed one of the biggest bits of feedback: completely customizable labels for each scan area when using Scan by Topo. Previously, files were named using a slide id and sequential numbers.

TRANSITION TO WINDOWS 10 / NEW JENOPTIK CAMERAS

Microsoft discontinued support of Windows 7 on January 14, 2020. As of 2020, BIOQUANT requires Windows 10 64-bit.

AS OF 2020: BIOQUANT OSTEO NO LONGER SUPPORTS WINDOWS 7

As of 2020, BIOQUANT OSTEO no longer supports Windows 7. Microsoft discontinued support of Windows 7 on January 14, 2020. BIOQUANT OSTEO 2021 requires Windows 10 64-bit.

UPDATED COMPUTER REQUIREMENTS

When upgrading to BIOQUANT OSTEO 2022, consider whether your computer should be updated. Although older computers might run BIOQUANT OSTEO 2022, those that don't meet current specifications will be slower, especially when considering core clock speed.

Here are the current computer requirements and recommendations:

OPERATING SYSTEM REQUIREMENTS

- Windows 10 64bit Pro, Education, or Enterprise. (Windows 8, Windows 7, Windows XP, Mac OS not supported.)

MINIMUM COMPUTER SPECIFICATIONS

- Dell Precision Series Mini-Tower Chassis
- Multi-core Intel Processor: I7 processor or better
- 3.5 GHz core clock speed
- NVIDIA Quadro K620 GPU or better
- 16 GB RAM
- Chassis with room for 1/2 height & 1/2 length PCIe 1X board
- Monitor that supports a 1920x1200 desktop resolution

RECOMMENDED COMPUTER SPECIFICATIONS

- Dell Precision 5000 Series Mini-tower Chassis
- Multi-core Intel Processor: Xeon processor
- 4.0 GHz or higher core clock speed
- NVIDIA Quadro P1000 GPU or better
- 32 GB RAM or more
- Chassis with room for 1/2 height & 1/2 length PCIe 1X board
- Monitor that supports a 1920x1200 desktop resolution

LEGACY QIMAGING CAMERA SUPPORT

QImaging does not support their FireWire cameras under Windows 10.

BIOQUANT will make every effort to support legacy QIMAGING cameras with the current BIOQUANT OSTEO 2022 release. However, please be aware that our capacity to help is limited due to those outside constraints. In addition, new imaging features, such as Live Correction, are only being added for the Jenoptik cameras.

For the long-term stability of your system, we encourage you to consider upgrading to one of the recommended Jenoptik Gryphax cameras listed below.

JENOPTIK NEXT-GENERATION SCMOS CAMERA SUPPORT

NEW JENOPTIK CAMERA SUPPORT

JENOPTIK GRYPHAX® CAMERAS

We are excited to announce support for the **biggest camera technology upgrade** since the introduction of the first high resolution digital cameras.

BIOQUANT now supports the PROKYON, KAPELLA, and SUBRA cameras from Jenoptik.

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

These cameras can deliver a full 1920x1200 resolution image at between 30 and 60 frames per second. This is **2X to 4X faster** than current cameras at **4X the resolution**.

In BIOQUANT, supported Jenoptik cameras provide a live image at **1280x960** to the Image Window with optional **1920x1200** image capture. The PROKYON camera model adds additional capture sizes of **3840 x 2400** and **5760 x 3600**.

THREE AVAILABLE MODELS TO FIT ANY BUDGET

The flagship **Prokyon** model offers a special 20 megapixel mode through pixel-shifting in addition to the native live image at 1920x1200 60 fps with fan-less cooling. The **Kapella** camera keeps the fan-less cooling but saves money by removing the 20 megapixel imaging mode. The **Subra** model is even more economical than the Kapella by removing the fan-less cooling.

	JENOPTIK PROKYON	JENOPTIK KAPELLA	JENOPTIK SUBRA
Model Number	KIT1-PRO	KIT1-KAP	KIT1-SUB
Sensor Specifications			
Sensor Model	Sony IMX174	Sony IMX174	Sony IMX174
Sensor Design	Scientific CMOS	Scientific CMOS	Scientific CMOS
Sensor Size	1920x1200	1920x1200	1920x1200
Frame Rate	60fps	60fps	30fps
Pixel Size	5.8 microns	5.8 microns	5.8 microns
Image Specifications			
Capture Resolutions	5760x3600 3840x2400 1920x1200	1920x1200	1920x1200
Exposure Control	26 us to 60 s	26 us to 60 s	26 us - 30 s
Cooling	Fanless	Fanless	N/A
Gain Control	1X - 30X	1X - 30X	1X - 10X
Exposure Control	Auto Exposure	Auto Exposure	Auto Exposure
Color Correction	Auto White Balance	Auto White Balance	Auto White Balance
Data and Power Interface Specifications			
Bit Depth	8bits / channel + HDR	8bits / channel + HDR	8bits / channel + HDR
Bus Type & Power	USB3.0 + 4.5W	USB3.0 + 4.5W	USB3.0 + 4.5W
Physical Specifications			
Mount Type	C-Mount	C-Mount	C-Mount
Dimensions	85mm x 75mm x 50mm	85mm x 75mm x 50mm	85mm x 75mm x 50mm
Warranty Information			
Warranty Term	24 months	24 months	24 months

Contact us for Pricing and Upgrade Discounts.

LIMITED TIME CAMERA TRADE-IN DISCOUNT PROMOTION

BIOQUANT and Jenoptik offer a trade-in discount for QIMAGING cameras toward the purchase of a new Jenoptik camera. Every QIMAGING camera sold with a BIOQUANT system, and many others, qualifies for this trade-in discount promotion. Discount amounts vary depending on the Jenoptik camera model purchased. Please act quickly, this is a limited time promotion.

NEW FEATURES: LIVE IMAGE TYPE FOR JENOPTIK CAMERA

REDESIGNED: IMAGE REGION (TYPE: LIVE IMAGE) FOR JENOPTIK CAMERA

The Image Region (Type: Live Image) has been redesigned for Jenoptik Camera users.

THE IMAGE REGION (TYPE: LIVE IMAGE)
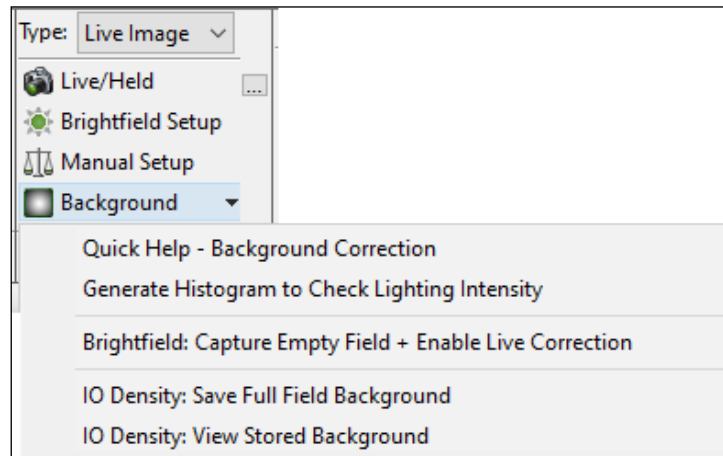
The buttons and menus on the Image region have changed.

LIVE/HELD BUTTON

The Live/Held button toggles from a live image (down) to a held image (button up).

LOAD PRESET BUTTON

The [image] Load Preset button opens the BQ Open File box so that camera presets can be opened.

You can open default camera presets for brightfield or fluorescence, which is useful if your camera is acting strangely.

You can also save your own camera preset files using the Manual Camera Setup box.

NEW: BRIGHTFIELD SETUP BUTTON

The Brightfield Setup button uses a new automatic setup routine for brightfield imaging unique to the Jenoptik camera.

Review the "New: Brightfield Setup & Live Correction Protocol (Jenoptik)" below for a complete protocol on setting up for Brightfield imaging with the new Live Correction feature.

NEW: MANUAL SETUP BUTTON

The Manual Setup button opens the Manual Camera Setup box for the Jenoptik camera. Review the "New: Manual Camera Setup Features for Jenoptik Camera" section below to learn about new features on the Manual Camera Setup box.

NEW: BACKGROUND BUTTON MENU

The Background button opens a menu, which now has new menu items.

On this menu, you can get help on background correction, generate a histogram to check lighting intensity, enable or disable Live Correction, and save or view IO Density background reference images.

Review the "New: Brightfield Setup & Live Correction Protocol (Jenoptik)" below for a complete protocol on setting up for Brightfield imaging with the new Live Correction feature.

The Background button menu includes:

QUICK HELP - BACKGROUND CORRECTION

This menu item opens the PDF Manual help for Background Correction. Read this help to learn about how to correctly prepare your camera for brightfield setup, how to enable/disable Live Correction, and how to prepare the system for IO Density data collection.

GENERATE HISTOGRAM TO CHECK LIGHTING INTENSITY

This opens a Full Field Histogram window with a histogram of the Image window. If the histogram does not generate, on the Full Field histogram window, from New Histo, choose Full Field.

Before clicking this menu you should have already done the Brightfield setup procedure. You should be on a held image of a blank, empty portion of the slide.

The histogram lines should be between 192 and 250. If they are not, read through the Quick Help mentioned above for troubleshooting advice.

BRIGHTFIELD: CAPTURE EMPTY FIELD + ENABLE LIVE CORRECTION

This menu is used on a live image to capture an empty field of the slide, then turn on the "Live Correction" feature.

Before clicking this menu you should have already done the Brightfield setup procedure and checked a histogram for lighting intensity. You should be on a live image of a blank, empty portion of the slide.

DISABLE LIVE CORRECTION

Once the "Brightfield: Capture Empty Field + Enable Live Correction" menu item has been clicked, the menu item changes to "Disable Live Correction."

Clicking "Disable Live Correction" turns off the "Live Correction" feature.

THE IMAGE REGION (TYPE: LIVE IMAGE)
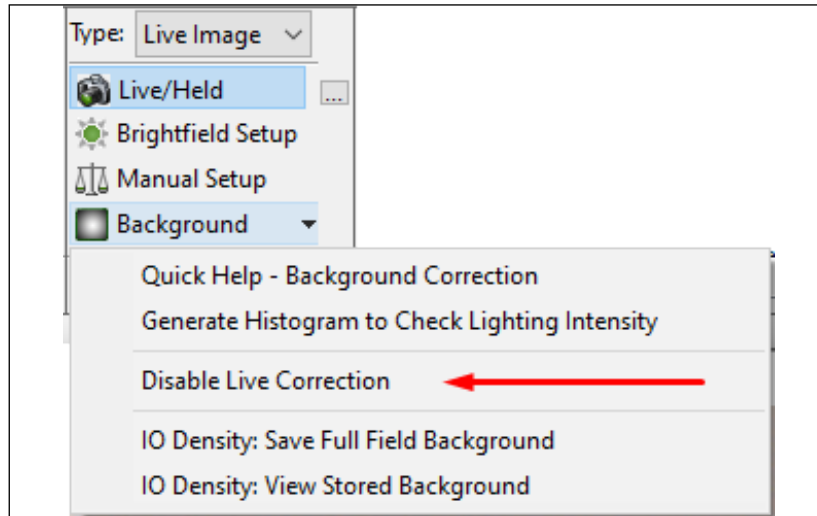
Disable Live Correction menu item.

IO DENSITY: SAVE FULL FIELD BACKGROUND

This menu item is used to save the full field in the Image window as the IO Density background reference image.

Unlike in previous versions, this image can be corrected using Live Correction, or the original, uncorrected image. If the corrected image is saved, then Live Correction should be on when capturing fields for IO Density measurement. If the original, uncorrected image is saved, then Live Correction should be off when capturing fields for IO Density measurement.

For more details see the "New: IO Density with Live Correction (Jenoptik)"below.

IO DENSITY: VIEW STORED BACKGROUND

This menu item is used to view the current background reference image that has been saved for IO Density.

NEW: MANUAL CAMERA SETUP FEATURES FOR JENOPTIK CAMERA

The Manual Camera Setup box is now opened by clicking the Manual Camera Setup button in the Image region (Type: Live Image). There are several new features for the Jenoptik camera: Color Skew and Fluorescent Black Background.

MANUAL CAMERA SETUP BOX FOR JENOPTIK CAMERA
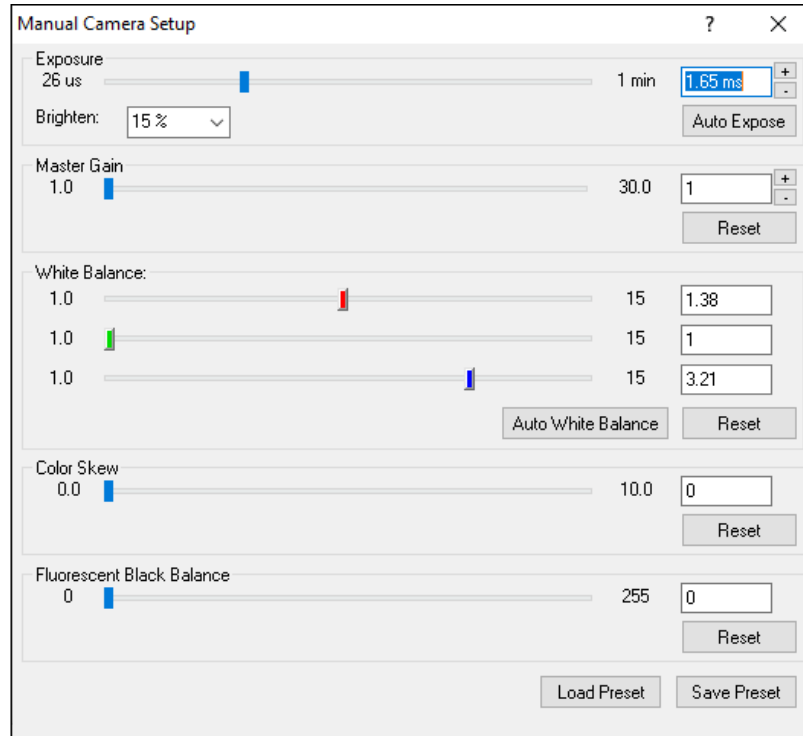
Color Skew and Fluorescent Black Background are new features.

COLOR SKEW

Color Skew tints the live image so that it more orange, which better matches eye piece view of the halogen light from the microscope. It is kind of the opposite of White Balance, which corrects the "yellow" cast of the halogen light to white. This optional feature might be preferred when capturing images for publication.

If Color Skew is adjusted for use with measurement, it should be set at the beginning of a study and kept at the same level for the entire study, as it affects thresholding and intensity (density) values.

Color Skew changes can be saved to a Preset file and reopened in a new BIOQUANT session.

To use, slowly increase the color skew value by moving the slider to the right until the live image looks more like the color you see in the microscope. To reset the value back to 0, click the Reset button.

The Brightfield Setup button will revert Color Skew back to 0. Use Auto Expose and Auto White Balance on the Manual Camera Setup box instead of Brightfield Setup if you want to retain a custom color skew value.

FLUORESCENT BLACK BALANCE

Fluorescent Black Balance suppresses autofluorescence in the background of slides. It applies to both the live and held images. Its effect is to make the background of fluorescent images blacker.

If Fluorescent Black Balance is used with optical density measurements, it should be set at the beginning of a study and kept at the same level for the entire study, as it affects thresholding and intensity (density) values.

The Fluorescent Black Balance value can be saved to a Preset file and reopened in a new BIOQUANT session.

To use this feature, on a live image, slowly increase the fluorescent black balance value by moving the slider to the right until the background is the desired black level. To reset the value back to 0, click the Reset button.

NEW: BRIGHTFIELD SETUP & LIVE CORRECTION PROTOCOL (JENOPTIK)

BIOQUANT has implemented a quick Brightfield Setup procedure and added Live Correction for the Jenoptik Camera.

Live Correction automatically corrects the **live** image for the uneven lighting.

Additionally, the Live Correction can be used for Integrated Optical Density, unlike Capture Correction with QImaging camera..

This protocol walks you through Brightfield Setup and Live Correction for the Jenoptik camera connected to your microscope.

PROTOCOL

1. **On the Microscope put a slide on the stage and focus the sample.**

2. **Change objectives until you're at the magnification you want to use.**

3. **Check that the microscope condenser is in Kohler Illumination.**

    There's a tutorial video for that or check with your lab manager.

4. **Adjust the brightness of the microscope to about 80% of the maximum.**

    Some microscopes have an imaging button on the base that sets the brightness to a predetermined level for imaging.

    Cameras need more light than human eyes, so it's normal if the eyepieces seem a little too bright.

5. **In the Image region, from the Type drop list, choose Live Image.**

    This connects to the camera and shows a live image in the Image window.

6. **Move your stage to a blank part of the slide.**

7. **Now, in the Image Region (Type: Live Image), click the "Brightfield Setup" button.**

    This sets up the exposure time and color correction.

8. **Next we will enable Live Correction.**

    Every microscope is a little brighter at the center than it is at the edges. Live Correction uses a reference

"background" image of the blank part of the slide to even out lighting for pictures of the sample.

With Jenoptik cameras,this correction is dynamically applied to the live image. We call this Live Correction.

If you have upgraded from a QImaging camera, Live Correction is different from the traditional background correction that you used, which did not apply the correction until the camera went held.

9. **First, click the "Background" button to open a menu. Click the first menu item "Quick Help: Background Correction."**

THE IMAGE REGION (TYPE: LIVE IMAGE)
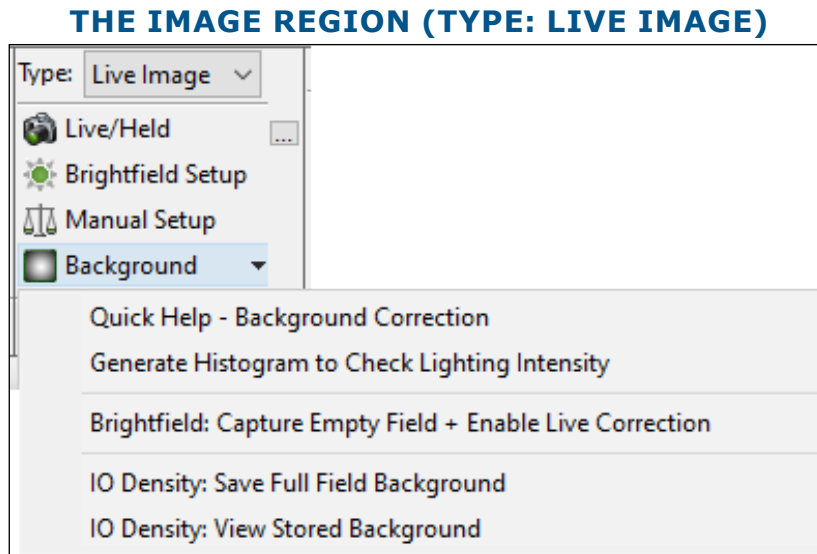
The Background button opens a menu. Choose "Quick Help - Background Correction."

This opens help for Background Correction. Figuring out how to get the best image for your camera and microscope can make a huge difference with thresholding and scans. It is worth a read-through.

10. **Now let's check the lighting intensity of the microscope. Make sure you are still on a blank part of the slide and click the "Live/Held" button to go to a held image.**

11. **Then click the "Background" button and click "Generate Histogram to Check Lighting Intensity."**

This opens a Full Field Histogram window with a histogram of the Image window.

If the histogram does not generate, on the Full Field histogram window, from New Histo, choose Full Field.

The histogram lines should be between 192 and 250. If they are not, read through the Quick Help: Background Correction mentioned above for pointers.

12. **Now let's activate Live Correction. Click the "Live/Held" button to return to a live image.**

13. **Click the "Background" button and choose "Brightfield: Capture Empty Field + Enable Live Correction."**

This evens the illumination in the image, improving the quality of scans and densitometry data.

14. **To see how Live Correction works over the tissue, move the stage back to the tissue. Notice how even the**

illumination is on the live image.

The Brightfield Setup and Live Correction settings remain accurate as long as you stay on this objective and don't adjust the lighting.

If you do change objectives or adjust the lighting, you will need to repeat this procedure: 1) move to a blank field 2) click "Brightfield Setup" 3) click the "Background" button, then click "Brightfield: Capture Empty Field + Enable Live Correction."

NEW: IO DENSITY WITH LIVE CORRECTION (JENOPTIK)

The Integrated Optical (IO) Density protocol with Jenoptik Camera has improved.

Previously, the IO Density array could not be used with Background Correction ON. That is no longer the case for Jenoptik camera users with Live Correction.

With Live Correction enabled, a background image can now be stored with Live Correction ON. Then, the corrected image of the tissue can be used to collect IO Density data.

PROTOCOL

This protocol saves the current, empty full field in the Image window as the IO Density background reference image.

It is designed to use with live imaging using the Jenoptik camera.

1. **Make sure you understand what IO Density is and how to use it.**

   In the Image region (Type: Live Image), click the Background button to open a menu and then click "Quick Help - Background Correction" to open the PDF help files. Read the section on IO Density with a Jenoptik Camera.

2. **Follow the "New: Brightfield Setup & Live Correction Protocol (Jenoptik)" above to setup for brightfield measurement, check your lighting intensity, and enable Live Correction.**

   If you are measuring IO Density across slides and across BIOQUANT sessions, it is important that the lighting is consistent from section to section, slide to slide, and day to day. Use the same presets and check your intensity histogram to ensure that the reference background (empty field) is consistent. Read the PDF help for guidance, or give us a call.

3. **In the Image region (Type: Live Image), click the Background button, then click "IO Density: Save Full Field Background."**

THE IMAGE REGION (TYPE: LIVE IMAGE)
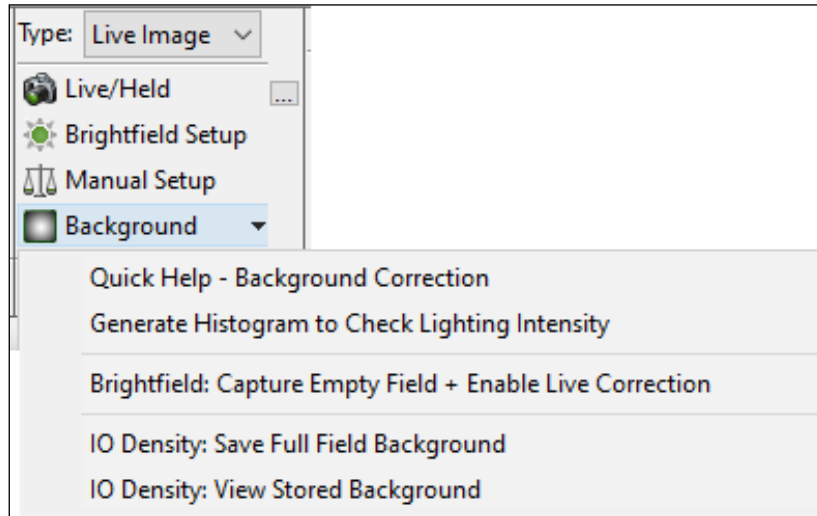
The Background button opens a menu. Choose "IO Density: Save Full Field Background."

TO VIEW REFERENCE BACKGROUND

* To view your reference background, click the Background button, then click "IO Density: View Stored Background.

4. **You are now ready to take IO Density measurements.**

Since you captured your IO Density background reference image with Live Correction ON, make sure Live Correction is ON when you make measurements.

Make sure you have used a template with an IO Density array, or have read the help to properly created your own IO Density array.

Also, remember that IO Density considers all pixels within the region of interest (Field Measurement Type), preview outline (Object Measurement Type), manual tracing (Manual Measurement Type), or wand shape (Density Wand Type).

The Brightfield Setup, Live Correction, and IO Density Reference Background settings remain accurate as long as you stay on this objective and don't adjust the lighting.

If you do change objectives or adjust the lighting, you will need to repeat this procedure.

For more information, in the Image Region (Type: Live Image), click the Background button and then click "Quick Help - Background Correction."

NEW: FLUORESCENT IMAGE SETUP FOR JENOPTIK CAMERA

This guide walks you through getting a fluorescent image from the camera connected to your microscope.

PROTOCOL

1. **Using a low power objective, such as 4x, set up the microscope for brightfield viewing.**

2. **Once the specimen is in focus, turn off the brightfield light and turn on the fluorescent light.**

3. **Switch to the objective that will be used for fluorescent imaging.**

4. **In the Image region, change the Type to Live Image and get a live image.**

    It will probably be very dark.

5. **Image Region (Live Image Type):**

    a.   Click the [...] button next to the Live/Held button to open the BQ Open File window.

    a.   In the BQ Open File window, click the Fluorescence.cps preset file.

    a.   Click the Open button.

6. **Examine the image in the Image window. If necessary, adjust the excitation source.**

    If the image is too dim or too bright, it's best to adjust the excitation source.

    Some excitation sources have a global brightness adjustment, some have brightness adjustments for individual channels. The scope may also have neutral density filters to dim the excitation source.

    Some excitation sources aren't adjustable. Check with your lab manager or call BIOQUANT if you have questions.

7. **In the Image region (Live Image Type), click the Manual Setup button to open the Manual Camera Setup box.**

8. **If the output of the excitation source is not adjustable or it is at maximum, increase or decrease the Master Gain parameter.**

    This will brighten or dim the image.

    If the Master Gain value must go above 10 to get sufficient brightness, there may be some other problem with the fluorescence excitation source and it may be best to consult with your microscope support.

    If your sample has multiple labels, and you r excitation source had adjustable channel brightness, use that to equalize the brightness of the labels.

    Alternatively, try increasing the Red or Blue gain values to brighten the corresponding label. Green labels are usually the brightest so there's no need to adjust the green gain.

    If the excitation source is adjustable, change its settings before adjusting the setting on Manual Setup.

9. **The last adjustment to make is the Fluorescent Black Balance. The default value is 25. Adjust this value to remove the autofluorescence background from the live image, making it easier to see the sample.**

10. **To save these new camera settings as a preset for future use, click the Save Preset button and save a file in the Camera Presets folder.**

NEW FEATURES: LIVE IMAGE TYPE FOR QIMAGING CAMERA

The QImaging Camera has new protocols for Brightfield Setup and Background Correction. IO Density users also have an updated protocol when used with live imaging and the QImaging camera.

REDESIGNED: BACKGROUND BUTTON MENU FOR QIMAGING CAMERA

The Background button menu for the QImaging camera has been enhanced.

THE IMAGE REGION (TYPE: LIVE IMAGE) FOR QIMAGING CAMERA
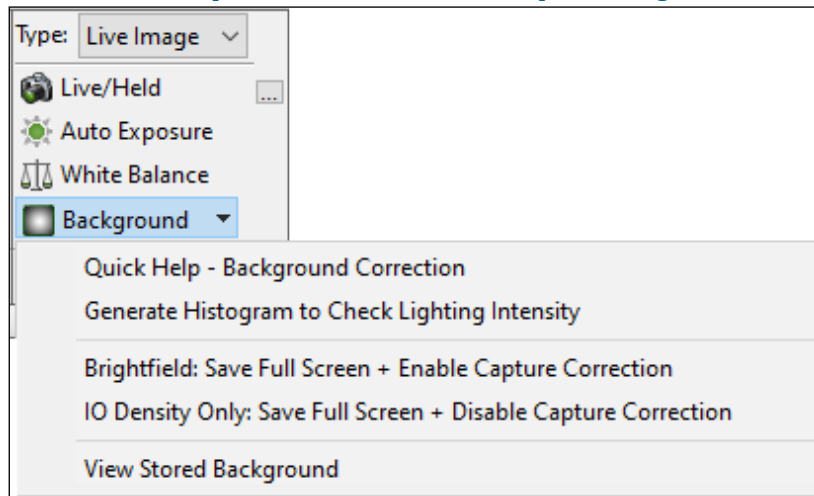
Clicking the Background button opens a menu.

On this menu, you can get help on background correction, generate a histogram to check lighting intensity, capture and enable capture correction for a brightfield image, save a reference background for IO Density, and view the current background image.

Review the "Redesigned: Image Region (Type: Live Image) for Jenoptik Camera" procedure below for a complete protocol on setting up for Brightfield imaging with the Background Correction. Alternatively, if you are measuring Integrated Optical Density, review the "New: IO Density Background Image Protocol (QImaging Cameras)" procedure below.

The Background button menu includes:

QUICK HELP - BACKGROUND CORRECTION

This menu item opens the PDF Manual help for Background Correction. Read this help to learn about how to correctly prepare your camera for brightfield setup, how to enable/disable Capture Correction, and how to prepare the system for IO Density data collection.

GENERATE HISTOGRAM TO CHECK LIGHTING INTENSITY

This opens a Full Field Histogram window with a histogram of the Image window. If the histogram does not generate, on the Full Field histogram window, from New Histo, choose Full Field.

Before clicking this menu you should have already done the Brightfield setup procedure for your camera. You

should be on a held image of a blank, empty portion of the slide.

The histogram lines should be between 192 and 250. If they are not, read through the Quick Help mentioned above troubleshooting advice.

BRIGHTFIELD: SAVE FULL SCREEN + ENABLE CAPTURE CORRECTION

This menu is used on a live image to capture an empty field on the slide, then turn on the "Background Correction" feature.

Before clicking this menu you should have already done the Brightfield setup procedure for your camera and checked a histogram for lighting intensity. You should be on a live image of a blank, empty portion of the slide.

IO DENSITY ONLY: SAVE FULL SCREEN + DISABLE CAPTURE CORRECTION

For QImaging cameras, measuring Integrated Optical (IO) Density requires a background reference image that has not been corrected for the background. Before clicking this menu you should have already used Auto Exposure and White Balance, plus checked the histogram for consistent lighting intensity across sessions.

This menu captures the empty full screen image and saves it as the background reference e image. It also disables Background Capture Correction.

VIEW STORED BACKGROUND

This menu is used to view the current reference background image.

NEW: BRIGHTFIELD SETUP AND CAPTURE CORRECTION (QIMAGING)

To get the best quality image, it is recommended that you follow the Brightfield Setup Protocol and activate Capture Correction.

This protocol walks you through getting a brightfield image from the QImaging camera connected to your microscope and enable Capture Correction.

If have a QImaging camera and are collecting Integrated Optical Density, then do not use this procedure. Inside, see "New: IO Density Background Image Protocol (QImaging Cameras) on page 20."

PROTOCOL

1. **On the Microscope, put a slide on the stage and focus the sample.**

2. **Change objectives until you're at the magnification you want to use.**

3. **Check that the microscope condenser is in Kohler Illumination.**

   There's a tutorial video for that or check with your lab manager.

4. **Adjust the brightness of the microscope to about 80% of the maximum.**

   Some microscopes have an imaging button on the base that sets the brightness to a pre-determined level for

imaging.

Cameras need more light than human eyes, so it's normal if the eyepieces seem a little too bright.

5. **In the Image region, from the Type drop list, choose Live Image.**

    This connects to the camera and shows a live image in the image window.

6. **On the microscope, move your stage to a blank part of the slide.**

7. **In the Image region (Live Image Type), click the Auto Expose button and then the White Balance button.**

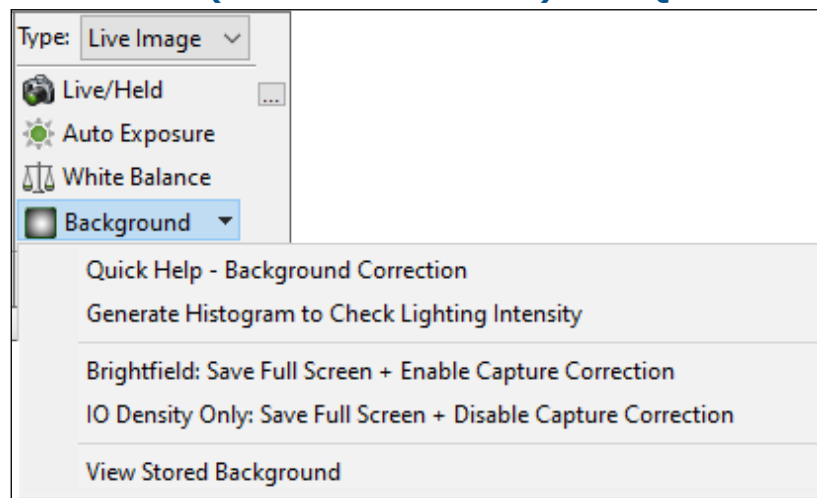   This sets up the exposure time and color correction.

8. **Now we will enable Background Correction.**

    Every microscope is a little brighter at the center than it is at the edges. Background Correction uses a reference image of the blank part of the slide to even out lighting for pictures of the sample.

    Only the captured image is corrected. The live image remains uncorrected.

9. **First, click the Background button to open a menu. Click the first menu item "Quick Help: Background Correction."**

THE IMAGE REGION (TYPE: LIVE IMAGE) FOR QIMAGING CAMERA

The Background button opens a menu. Choose "Quick Help - Background Correction."

This opens help for Background Correction. Figuring out how to get the best image for your camera and microscope can make a huge difference with thresholding and scans. It is worth a read-through.

10. **Now let's check the lighting intensity of microscope. Make sure you are still on a blank part of the slide and click the Live/Held button to go to a held image.**

11. **Then click the Background button and click "Generate Histogram to Check Lighting Intensity."**

    This opens a Full Field Histogram of the Image window. If the histogram does not generate, from NewHisto, choose Full Field.

    The histogram lines should be between 192 and 250. If they are not, read through the help document we

opened for troubleshooting advice.

12. **Now let's enable Capture Correction. Click the Live/Held button to return to a live image.**

13. **Click the Background button and choose "Brightfield: Save Full Screen + Enable Capture Correction."**

    This evens the illumination in the image, improving the quality of scans and densitometry data.

14. **To see how Background Correction works over the tissue, move the stage back to the tissue. Then, toggle from a live image to held image. Notice how even the illumination is on the held image.**

    The Auto Exposure, White Balance, and Capture Correction remain accurate as long as you are on this objective and don't adjust the lighting.

    If you do change objectives or adjust lighting, repeat this procedure: 1) move to a blank field 2) click "Auto Expose" and "White Balance" 3) then click Background and "Brightfield: Save Full Screen + Enable Capture Correction."

NEW: IO DENSITY BACKGROUND IMAGE PROTOCOL (QIMAGING CAMERAS)

This protocol saves the current, empty full field in the Image window as the IO Density background reference image.

It is designed to use with live imaging using the QImaging camera.

PROTOCOL

1. **Make sure you understand what IO Density is and how to use it.**

   In the Image region (Type: Live Image), click the Background button to open a menu and then click "Quick Help - Background Correction" to open the PDF help files. Read the section on IO Density with a QImaging Camera.

2. **On the Microscope, put a slide on the stage and focus the sample.**

3. **Change objectives until you're at the magnification you want to use.**

4. **Check that the microscope condenser is in Kohler Illumination.**

   There's a tutorial video for that or check with your lab manager.

5. **Adjust the brightness of the microscope to about 80% of the maximum.**

   Some microscopes have an imaging button on the base that sets the brightness to a pre-determined level for imaging.

   Cameras need more light than human eyes, so it's normal if the eyepieces seem a little too bright.

6. **In the Image region, from the Type drop list, choose Live Image.**

   This connects to the camera and shows a live image in the image window.

7. **On the microscope, move your stage to a blank part of the slide.**

8. **In the Image region (Live Image Type), click the Auto Expose button and then the White Balance button.**

This sets up the exposure time and color correction.

9. **Now let's check the lighting intensity of microscope. Make sure you are still on a blank part of the slide and click the Live/Held button to go to a held image.**

10. **Then click the Background button and click "Generate Histogram to Check Lighting Intensity."**

This opens a Full Field Histogram of the Image window. If the histogram does not generate, from NewHisto, choose Full Field.

The histogram lines should be between 192 and 250, and a consistent number across BIOQUANT sessions. If they are not, read through the help document we opened for troubleshooting advice.

If you are measuring IO Density across slides and across BIOQUANT sessions, it is important that the lighting is consistent from section to section, slide to slide, and day to day. Use the same presets and check your intensity histogram to ensure that the reference background (empty field) is consistent. Read the PDF help for guidance, or give us a call.

11. **In the Image region (Type: Live Image), click the Background button, then click "IO Density: Save Full Screen + Disable Capture Correction"**

This saves the empty field as your background reference image for IO Density. It also disables Capture Correction. For QImaging cameras, data is collected on uncorrected images, as the background reference image is uncorrected.

THE IMAGE REGION (TYPE: LIVE IMAGE) FOR QIMAGING CAMERA
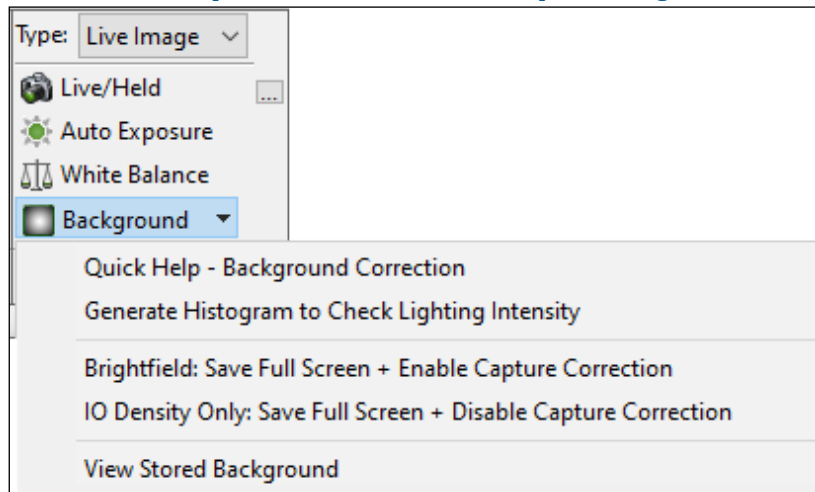
The Background button opens a menu. Choose "IO Density Only: Save Full Screen + Disable Capture Correction"

TO VIEW REFERENCE BACKGROUND

- To view your reference background, click the Background button, then click "View Stored Background.

12. You are now ready to take IO Density measurements.

Make sure you have used a template with an IO Density array, or have read the help to properly created your own IO Density array.

Also, remember that IO Density considers all pixels within the region of interest (Field Measurement Type), preview outline (Object Measurement Type), manual tracing (Manual Measurement Type), or wand shape (Density Wand Type).

The Auto Exposure, White Balance, and IO Density Reference Background settings remain accurate as long as you stay on this objective and don't adjust the lighting.

If you do change objectives or adjust the lighting, you will need to repeat this procedure.

For more information, in the Image Region (Type: Live Image), click the Background button and then click "Quick Help - Background Correction."

NEW: IMAGE FILE TYPE: CALIBRATE IMAGE BUTTON MENU

There is a new Calibrated Image button on the Image Region (Type: Image File).

The new Calibrate Image button opens a menu that is used to access the Calibration box, for optical calibration, as well as the Integrated Optical (IO) Density Background Calibration menus.

IMAGE REGION (IMAGE FILE TYPE)
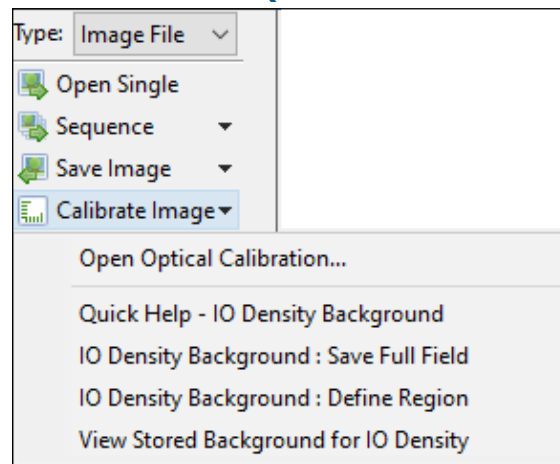
The new Calibrate Image menu.

NEW: OPEN OPTICAL CALIBRATION MENU ITEM

The "Open Optical Calibration..." menu item opens the Optical Calibration box so that you can calibrate BIOQUANT for images opened from your hard drive. The Optical Calibration procedure has not changed.

NEW: IO DENSITY MENU ITEMS FOR IMAGE FILES

The menu items under the horizontal line are related to calibrating the image file for Integrated Optical Density measurements.

IO Density integrates the size of the object (total number of significant pixels) and the intensity of pixels in the object. The intensity of each pixel in the object is brightness-corrected based on the intensity of a corresponding pixel in a stored background image.

QUICK HELP - IO DENSITY BACKGROUND

This menu item opens the PDF Manual help for collecting IO Density on an image file. Read this help file so that you understand what IO Density is and how it is collected on image files in BIOQUANT.

IO DENSITY BACKGROUND: SAVE FULL FIELD

This menu item saves the current, empty full field in the Image window as the IO Density background reference image.

It is designed to use with large scans that have opened in the Image Navigator.

To use:

1. **Open the scan image file on which you would like to measure IO Density.**

    The scan opens into the Image Navigator.

2. **On the Image Navigator, click the Pan box size that matches the size to be used for measurement.**

3. **Then, move the Pan box so that an empty, blank field is in the Image window.**

4. **In the Image region (Type: Image File), click the Calibrate Image button, then click "IO Density Background: Save Full Field."**

    The empty, blank field is saved as the IO Density background reference image.

5. **You are now ready to take IO Density measurements.**

    Make sure you have used a template with an IO Density array, or have read the help to properly created your own IO Density array.

    Also, remember that IO Density considers all pixels within the region of interest (Field Measurement Type), preview outline (Object Measurement Type), manual tracing (Manual Measurement Type), or wand shape (Density Wand Type).

    For more information, in the Image Region (Type: Image File), click the Calibrate button and then click "Quick Help - IO Density Background."

IO DENSITY BACKGROUND: DEFINE REGION

This menu item creates a background reference image based on a user defined region n the Image window and saves it as the IO Density background reference image.

It is designed to use with image files that open in the Image window, but are not large enough for the Large Image Navigator, or when the Pan box can not be moved to an empty, blank field in the Large Image Navigator.

To use:

1. **In the Image region (Type: Image File), click the Calibrate Image button, then click "IO Density Background: Define Region."**

   The cursor moves to the Image window.

2. **In the Image Window, trace a representative region of empty background space.**

   The cursor exits the Image window.

   A background reference image has been created for IO Density based on the traced region.

3. **You are now ready to take IO Density measurements.**

   Make sure you have used a template with an IO Density array, or have read the help to properly created your own IO Density array.

   Also, remember that IO Density considers all pixels within the region of interest (Field Measurement Type), preview outline (Object Measurement Type), manual tracing (Manual Measurement Type), or wand shape (Density Wand Type).

   For more information, in the Image Region (Type: Image File), click the Calibrate button and then click "Quick Help - IO Density Background."

VIEW SAVED BACKGROUND FOR IO DENSITY

This menu puts the background reference image that is currently being used for IO Density measurements into the Image window. It is used to validate that the background reference image is correct.

ENHANCED OSSEOINTEGRATION PROTOCOL

The Osseointegration Tutorial Set, guide, and protocol have been improved.

OSSEOINTEGRATION PROTOCOL VIDEO
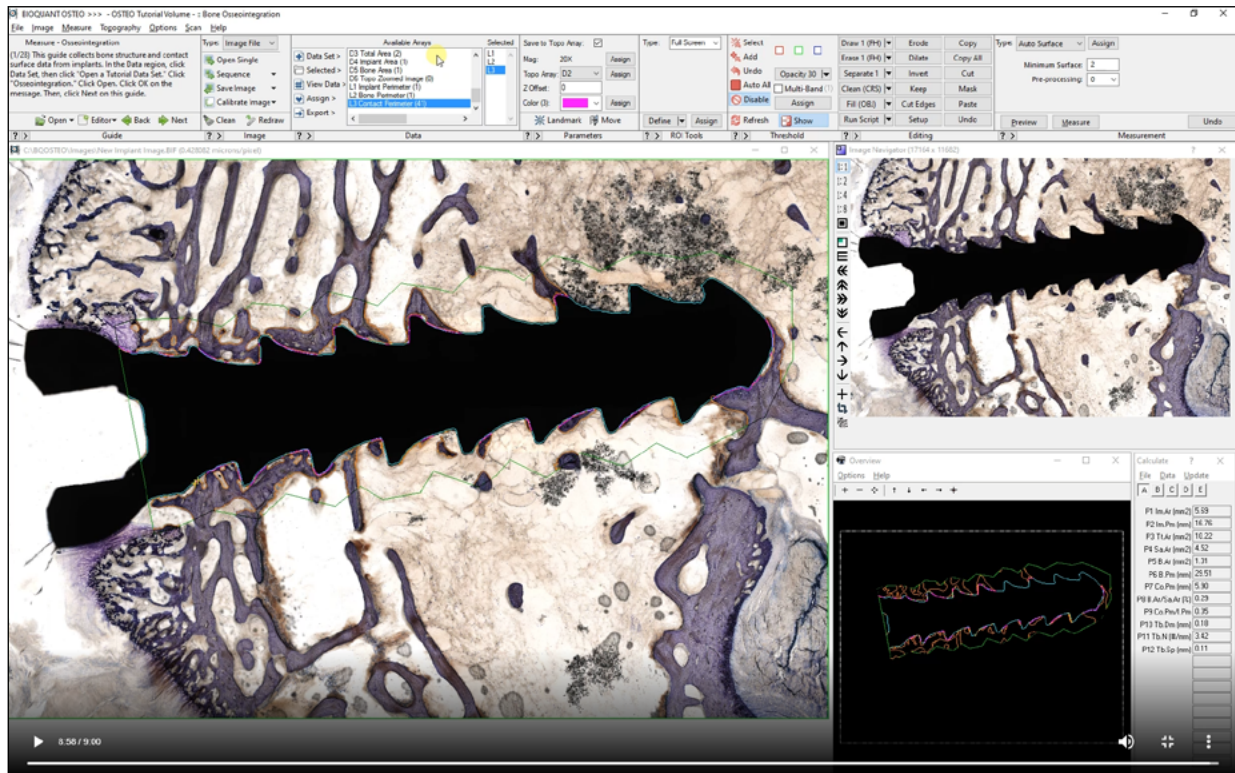
The new Calibrate Image menu.

TO LEARN THIS PROTOCOL

- **Video Tutorial**

  To review the updated Osseointegration Protocol as a video, click the > button in the Measurement region to open the BIOQUANT OSTEO Video Tutorials for the Measurement region. Then, under Bone Complete Protocol Videos, click Osseointegration Protocol.

- **PDF Help Procedure**

  To view the updated Osseointegration Protocol in the PDF manual, click the ? button in the Measurement region to open the Osseointegration Protocol in the BIOQUANT OSTEO Manual.

- **Guide Region and Tutorial Data Set**

  To follow a guide in BIOQUANT, in the Guides region, click Open, then click Measure - Osseointegration. This guide uses the Bone Osseointegration Tutorial Set and the dental implant.BIF sample image.

MINOR ENHANCEMENTS / ISSUE RESOLUTIONS

The following minor enhancements and issue resolutions have been made to the software.

BIOQUANT OSTEO: MINOR ENHANCEMENTS

The following minor enhancements have been made to the BIOQUANT OSTEO.

JENOPTIK CAMERA: THE OVERVIEW WINDOW IS MORE RESPONSIVE.

The Overview window is more responsive and now updates dynamically when the Jenoptik Camera image is live.

JENOPTIK AND QIMAGING CAMERAS: LIVE/HELD TIMING ISSUES RESOLVED.

In previous versions, sometime when on a live image, and the user clicks a button that requires a held image, there would be a timing issue and the system would preform the buttons' function of the previous held image before the new held image could be captured. This would result in confusing previous outlines, threshold mismatches, and other distortions. The user would then have to go live, then held, and then click the button to proceed correctly.

This timing issue has been resolved.

THE BACKGROUND BOX HAS BEEN REMOVED.

The Background box has been removed. It is replaced by the new Background button menu items in the Image region (Type: Live Image), and by the new Calibrate Image button menu items in the Image region (Type: Image File).

BIOQUANT SCAN: MINOR ENHANCEMENTS

The following minor enhancements have been made to the BIOQUANT Scan Add-on to BIOQUANT OSTEO.

ENHANCEMENT: SCAN BOX: BACKGROUND NOTIFICATION IMPROVED

The Background Correction notification has improved based on the new Background Correction algorithm. It no longer mentions IO Density, as Integrated Optical Density could be taken on the background corrected scan using the new IO Density options. See "New: IO Density Menu Items for Image Files on page 22."

Background Correction (either Live Correction with the Jenoptik camera or Capture Correction with the QImaging camera) is recommended when capturing images with BIOQUANT SCAN as it removes the "checker" pattern caused by uneven microscope lighting.

ENHANCEMENT: SCAN MULTIPLE SLIDES: SET BY: DEFAULT PARAMETERS

Now, when the user switches between Set By types, default parameters for the chosen type are loaded instead of keeping the prior type's parameters. This reduces confusion on the starting point for these parameters.

ENHANCEMENT: SCAN MULTIPLE SLIDES: SCAN BY TOPO: CUSTOMIZE LABELS

On the Scan Multiple Slides box, when using the Scan by Topo Type, up to 8 labels names can now be customized.

ENHANCEMENT: SCAN MULTIPLE SLIDES: SCAN BY TOPO: AUTO FOCUS

Focus is now checked at more evenly distributed locations across the scan area.

The Focus Pattern options (Once / Two Fields and Once / Three Fields) now count correctly based on fields of view within the scan area. Previously, the count could get off because it was based on fields of view in the rectangle that contained the scan area.

ENHANCEMENT: SCAN MULTIPLE SLIDES: SCAN BY TOPO: CHANGING SECTIONS

The auto focus is better able to find the correct starting focus when changing scan areas.

Auto focus now checks a large Z range at the first scan position. Previously, it used the center of the scan area, which for irregularly shaped scan areas, is not necessarily over the tissue.

ENHANCEMENT: SCAN SETUP: UPDATED INTERFACE FOR SHUTTER CONTROL

The "Photofluor" region is now called "Light Control" with Automatic On/Off check boxes, as this region now supports both the Photofluor and CoolLed excitation sources.

ENHANCEMENT: THE OPEN CALIBRATE FOCUS FEATURE HAS BEEN MOVED.

Under the Scan menu, you can now click the "Open Calibrate Auto Focus … " menu item to open the Calibrate Focus box.

Calibrate Focus is used when the motorized stage has the Auto Focus option. It is a one time calibration. It made more sense to move it to its own menu item rather than keep it on the Scan by Setup boxes that are used frequently.

The Calibrate Focus buttons have been removed from the Scan By Setup boxes.