BIOQUANT® OSTEO

Skeletal Phenotying Protocol: Referent Data

The Referent Data Tool has been used to simultaneously collect Tissue Volume, Bone Volume, and Bone Surface.
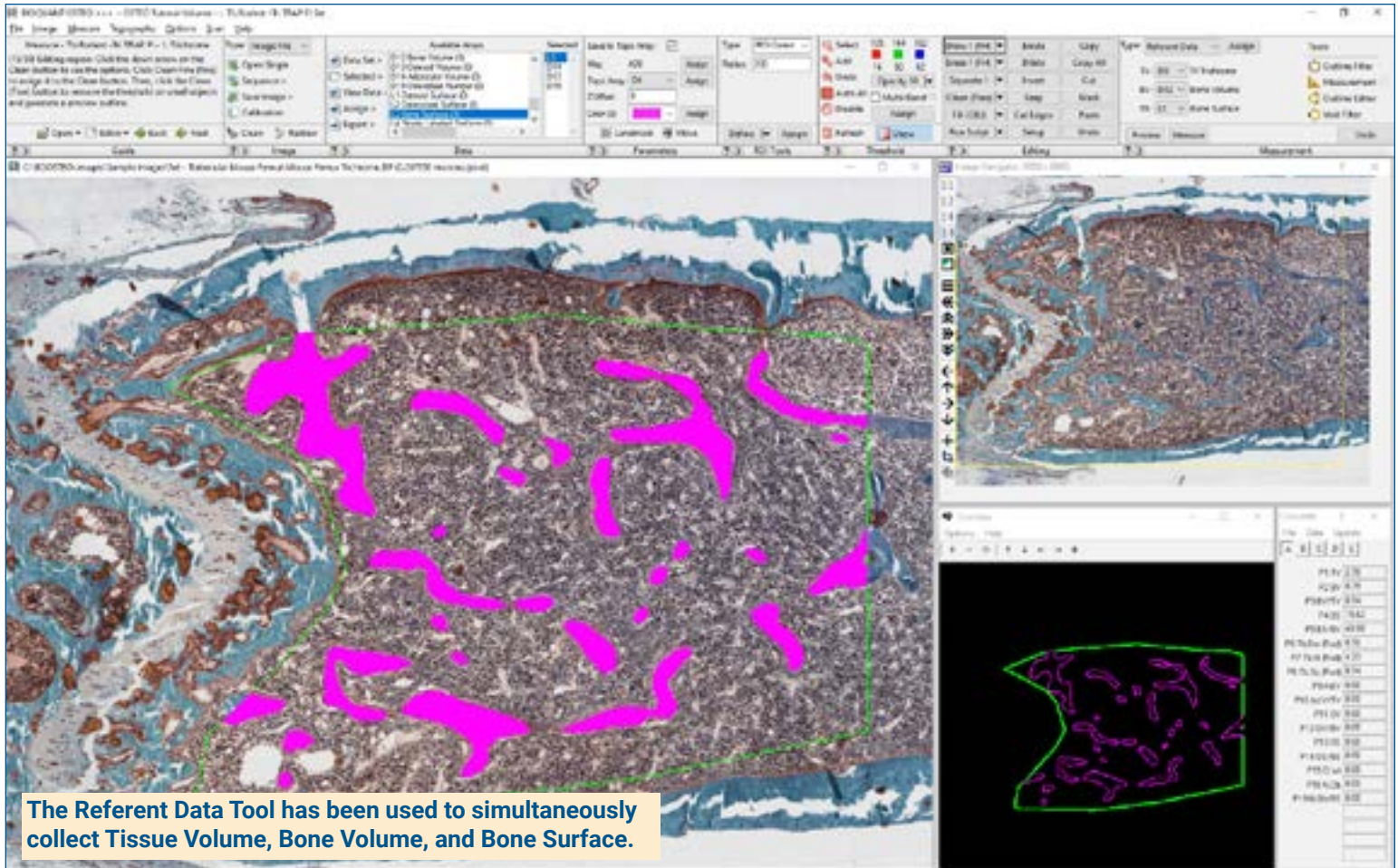
Get Image of TV

Up to **4 GB Scan** of Section:

- Scans can be generated manually by BIOQUANT OSTEO, automatically by BIOQUANT SCAN, or via a third party slide scanner.
- In BIOQUANT, zoom out so the TV fits in one field of view.

Live Image from Microscope with Camera:

- In BIOQUANT, see a live image of the tissue directly in the Image Window at a low objective like 4X or 2X.
- The TV does not have to fit in one field of view.

Define Tissue Volume

Draw the boundary of the TV in the Image window:

iROI Cursor Tool

Makes it easy to draw the irregular Tissue Volume boundary a fixed distance from the cortical shell and growth plate.

Rectangle TV Tool

Defines a rectangular Tissue Volume with known micron dimensions a consistent distance from the cortical shell and growth plate.

Use the Referent Data Tool

Click **Preview** to automatically:

- Threshold the bone by color.
- Fill in small holes and cracks.
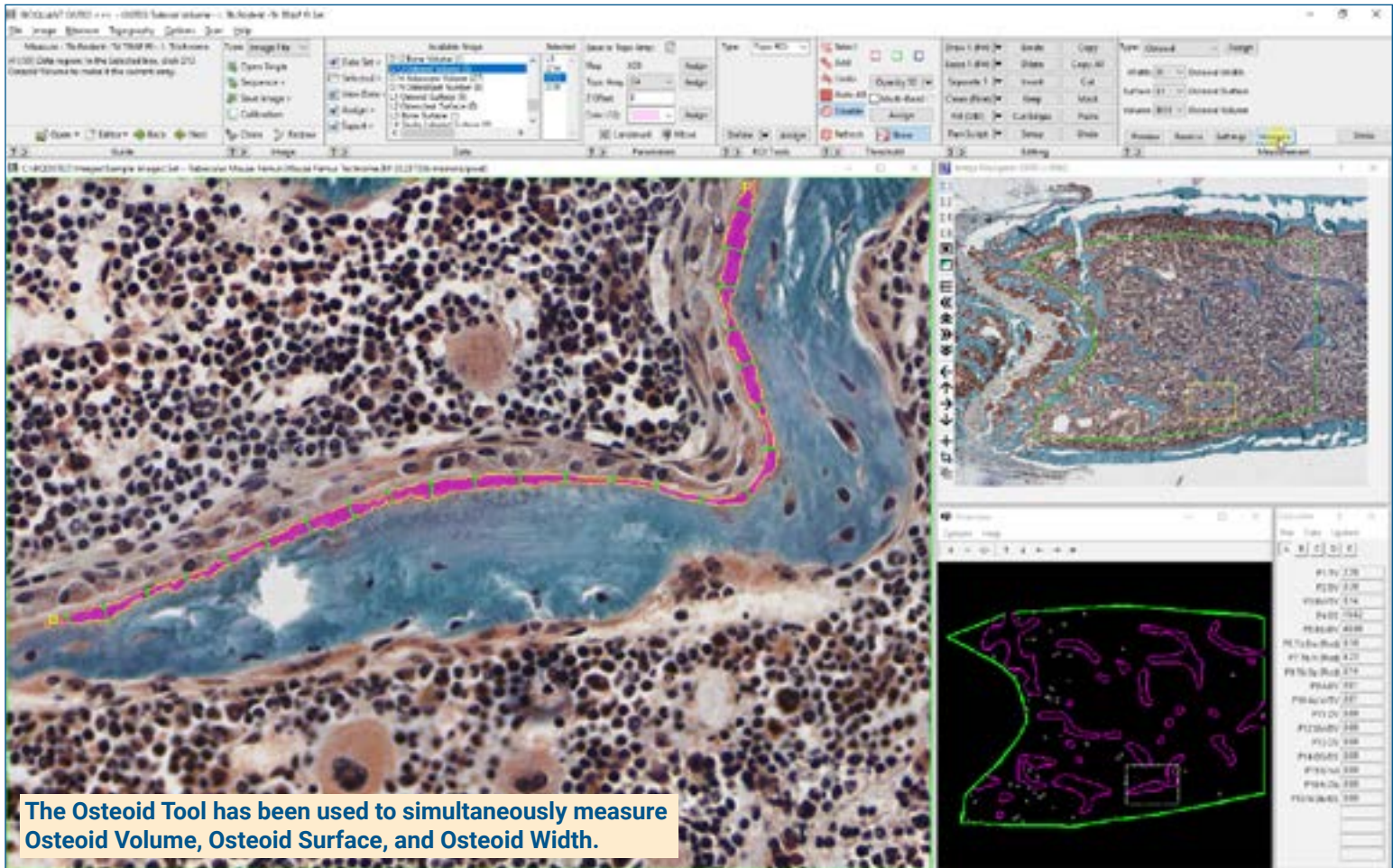- Preserve void spaces.
- Trace the bone.

Use **Thresholding Editing** tools like Draw, Erase, Clean, Erode/Dilate, as necessary.

Click **Measure** to simultaneously record:

- TV, BV, BS
- BV/TV, BS/BV
- Tb.N, Tb.Dm, Tb.Sp

If the TV didn't fit in a single field of view, repeat for remaining fields of view.

The Osteoid Tool has been used to simultaneously measure Osteoid Volume, Osteoid Surface, and Osteoid Width.

Zoom In or Change Objective

Digital Scan in **Image Navigator**:

- Zoom in to higher magnification.
- During measurement, you're free to zoom in or zoom out as needed.
- Pan to a field with osteoid or osteoblasts.
- Auto ROI always keeps you within the Tissue Volume.

Live Image with Microscope:

- Change to a higher objective.
- During measurement, you're free to change objectives as needed.
- Move to a field with osteoid or osteoblasts within the Tissue Volume.

Use the Osteoid Tool

Using the Osteoid Measurement tool, click **Preview**, then click on each osteoid.

BIOQUANT automatically:

- Thresholds the osteoid seams based on the color of their stain.
- Traces the osteoid seams.

Use **Thresholding Editing** tools like Draw, and Erase to clean steams, if necessary.

Click **Measure** to simultaneously record:

- OV, OS, O.Wi
- OV/BV, OS/BS

Osteoid Width is measured directly, without estimation.

Draw and Count Osteoblasts

Use **Draw Threshold** to mark each osteoblast. You are welcome to zoom in.

Click **Preview**. BIOQUANT automatically traces the osteoblasts.

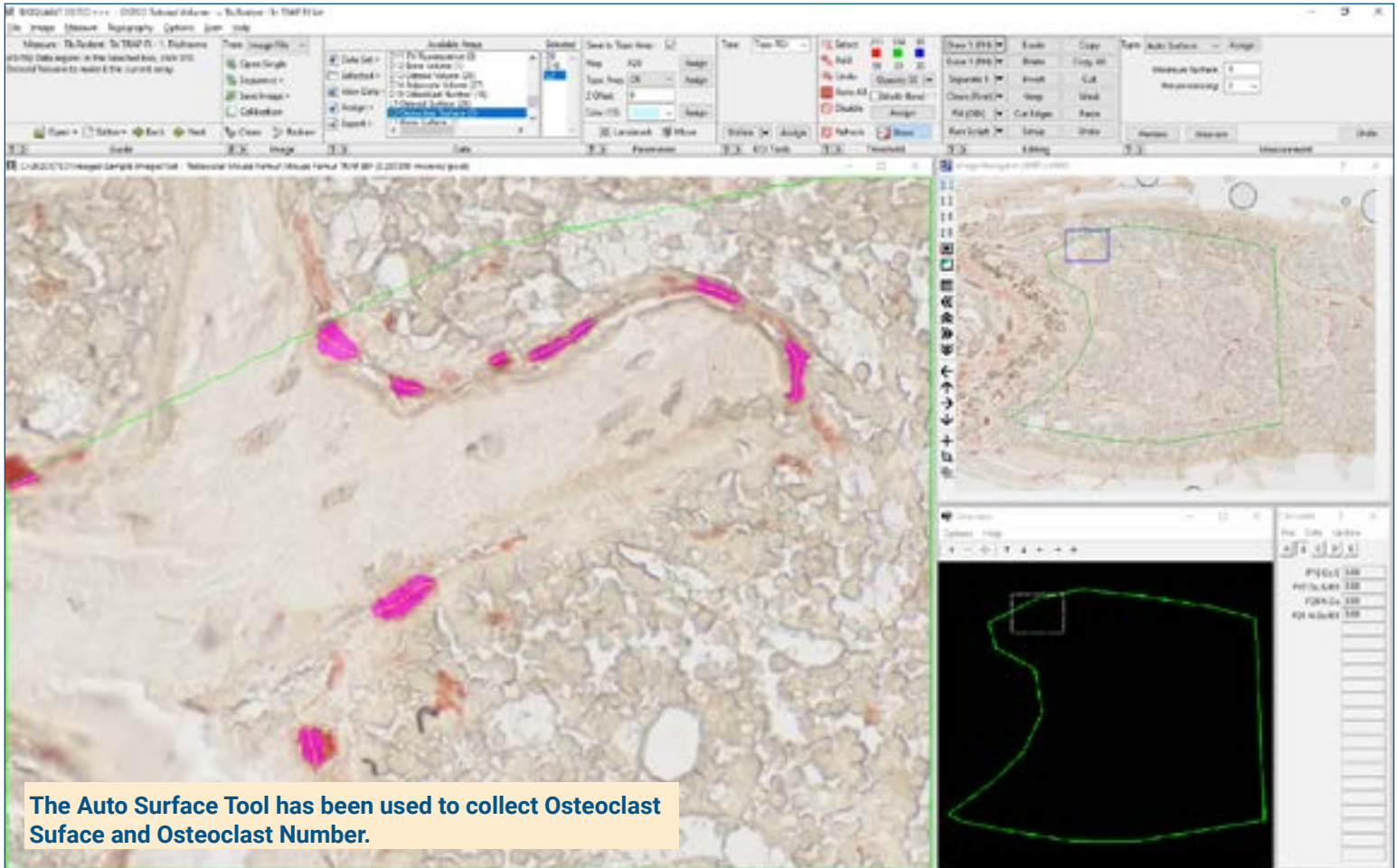Use **Thresholding Editing** tools like Draw and Erase to add or remove threshold.

Click **Measure** to record:

- N.Ob, N.Ob/BS

Continue to pan through the Tissue Volume looking for more osteoid seams and osteoblasts.

Measure at any zoom level, previous tracings redraw to prevent duplicate measurement.

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Skeletal Phenotying Protocol: Osteoclast Data

The Auto Surface Tool has been used to collect Osteoclast Suface and Osteoclast Number.

Get Image of Trap Section

Open up to **4 GB Scan** of Section:

- In BIOQUANT, zoom out so the TV fits in one field of view.

Get **Live Image** from Microscope with Camera:

- In BIOQUANT, see a live image of the tissue directly in the Image Window at a low objective like 4X or 2X.
- The TV does not have to fit in one field of view.

If your sections align, you can use the same Tissue Volume as the region of interest for the TRAP section.

Zoom In or Change Objective

Digital Scan in **Large Image Navigator**:

- Zoom in to higher magnification
- During measurement, you're free to zoom in or zoom out as needed
- Pan to a field with osteoclasts.
- Auto ROI always keeps you within the Tissue Volume.

Live Image with Microscope:

- Change to a higher objective
- During measurement, you're free to change objectives as needed.
- Move to a field with osteoclasts within the Tissue Volume.

Use the Auto Surface Tool

Using the Auto Surface tool, click **Preview**, BIOQUANT automatically:

- Thresholds the osteoclasts.
- Uses size filters to remove small TRAP positive monocytes.
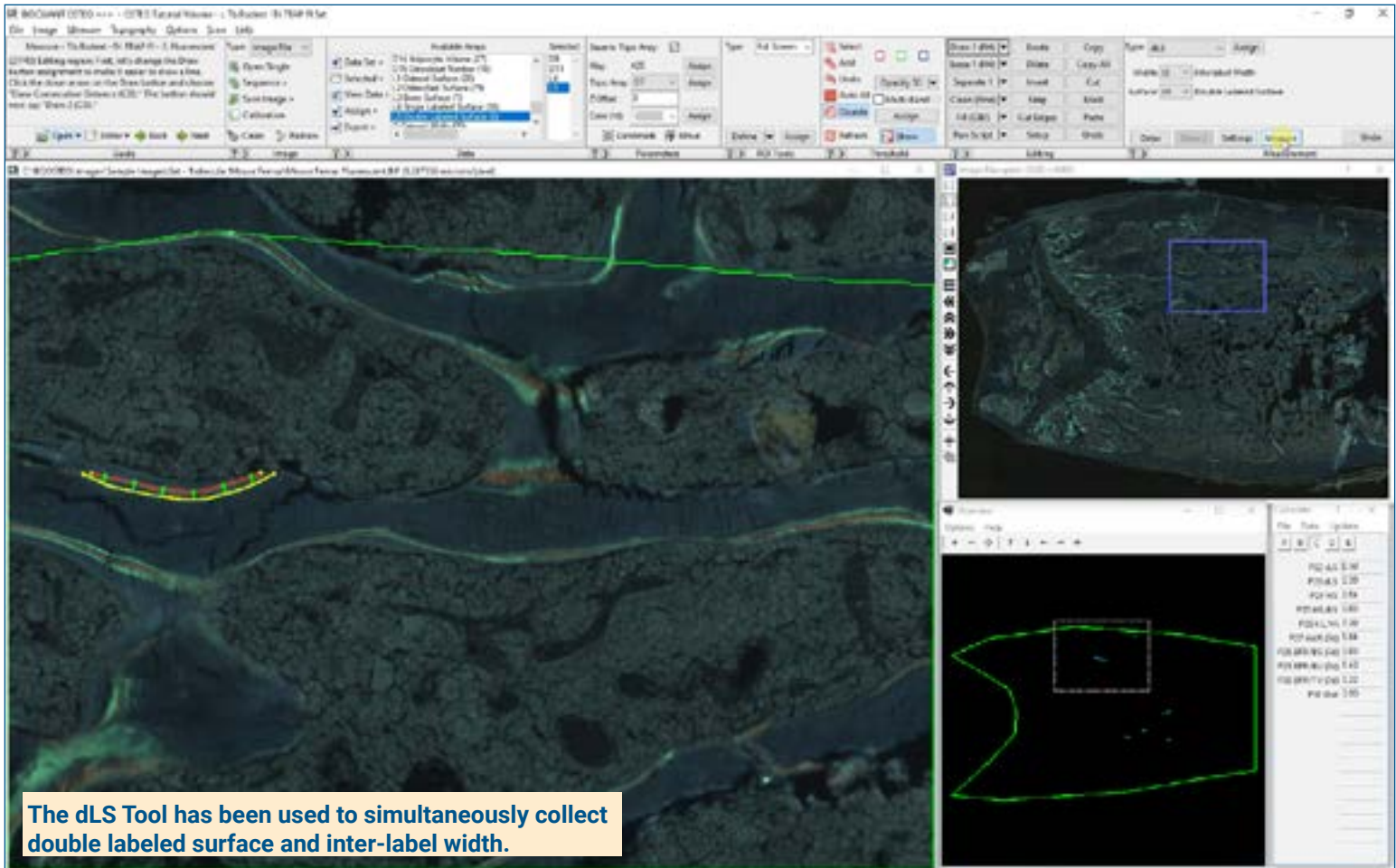- Traces the osteoclasts.

Use **Thresholding Editing** tools like Draw, and Erase to edit threshold if necessary.

Click **Measure** to simultaneously record:

- Oc.S, Oc.N.
- Oc.S/BS , N.Oc/BS

Pan through the Tissue Volume and measure more osteoclasts. Redraw **prevents duplicate measurement**.

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Skeletal Phenotying Protocol: BFR Data

The dLS Tool has been used to simultaneously collect double labeled surface and inter-label width.

Get Image of Calcein Section

Open up to **4 GB Scan** of Section.

Or get **Live Image** from Microscope with Camera:

If your sections align, you can **use the same Tissue Volume** as the region of interest for the calcein section.

Zoom in or Change Objectives and move to a field with single or double labeled Surface.

Auto ROI always keeps you within the Tissue Volume.

Use the Auto Surface Tool

Using the Auto Surface tool, click **Preview**, BIOQUANT automatically:

- Thresholds the labels.
- Uses size filters to remove small calcein labels.
- Traces the labels.

Use **Thresholding Editing** tools like Keep and Erase to isolate single labeled surface.

Click **Measure** to simultaneously record:

- sLS

Use the dLS Tool

Using the dLS tool, click **Draw**, then:

- Click along surface one. Right click.
- Click along surface two. Right click.

The double labeled surface and Interlabel Widths preview outline.

Click **Measure** to simultaneously record:

- dLS, Ir.L.Wi, MS, MS/BS, MAR (7d), Omt, BFR/BS, BFR/BV, BFR/TV (7d)

Move to the field in the TV and repeat for sLS and dLS.

This is a sample protocol. It is easily modified for different animals, tissues, embedding methods, and stains.

All parameters can be renamed. New calculations can be added. Both live imaging and scans are supported.

Measure at the magnification that is best for your histology. Find out more: www.bioquant.com/osteo