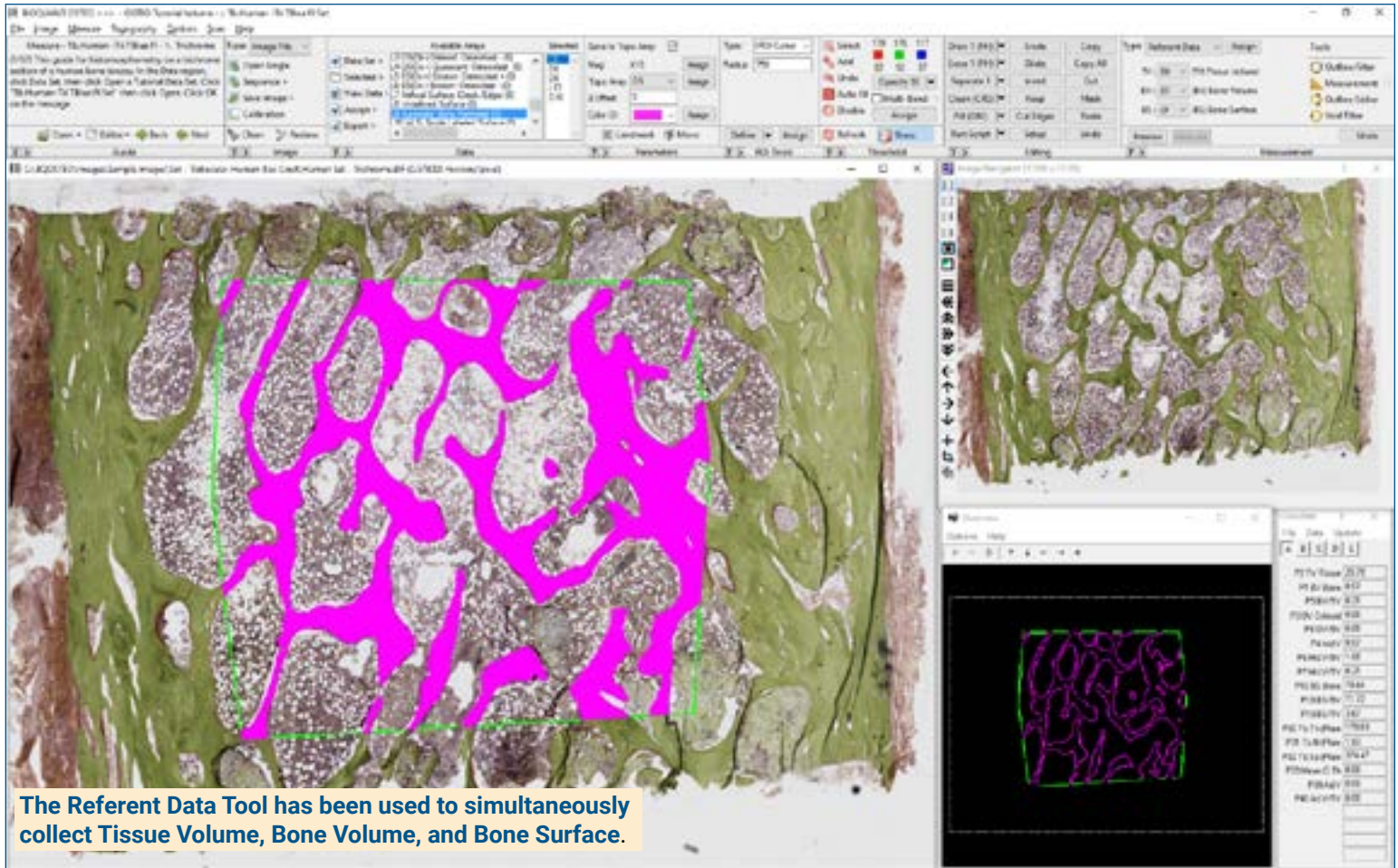


Human Bone Biopsy Protocol: Referent Data



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 cut edges.

Rectangle TV Tool

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

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.

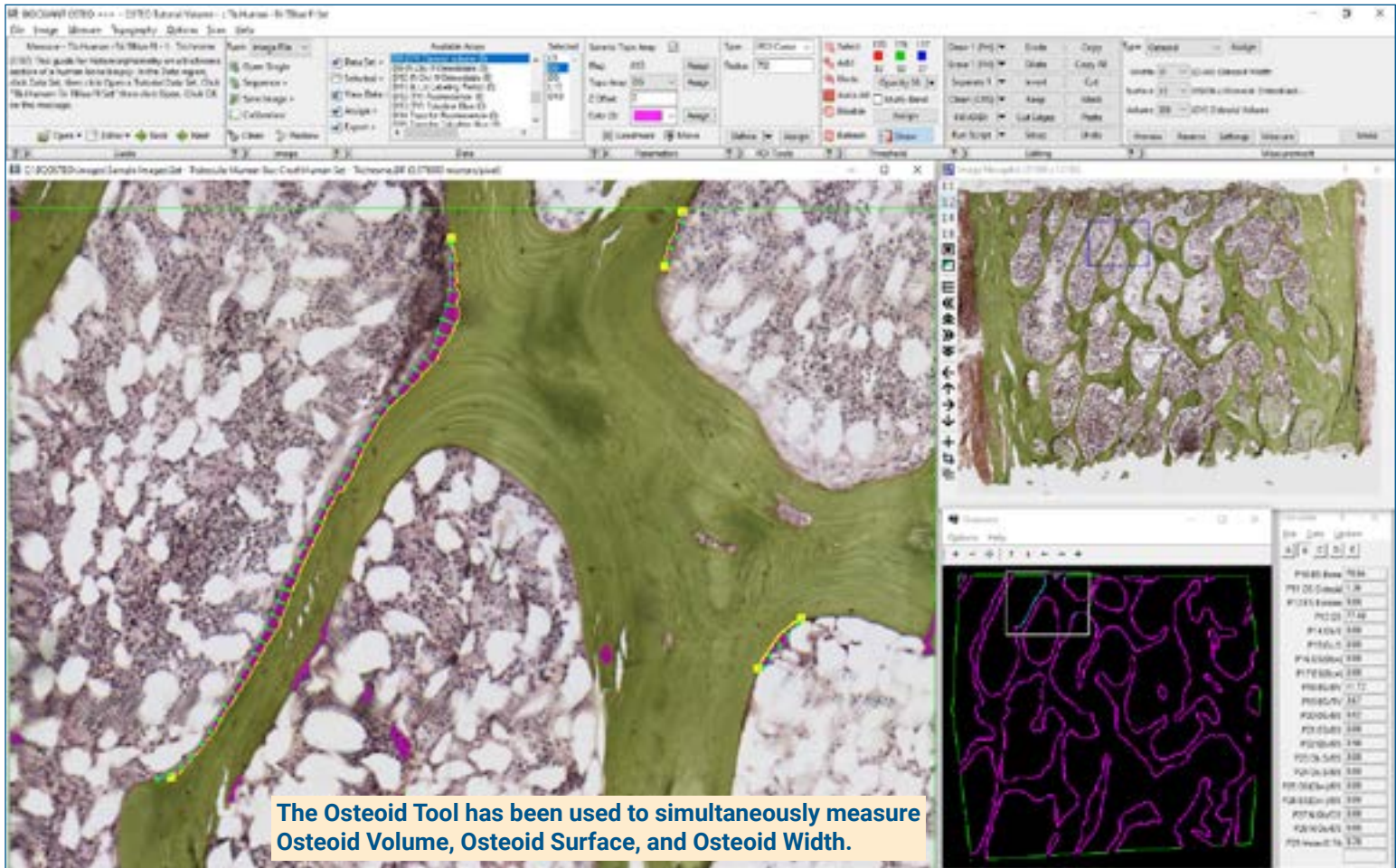
Use **Thresholding Editing** tools like Draw, Erase, Clean, Erode/Dilate, as necessary.

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.

Human Bone Biopsy Protocol: Osteoblast Data



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 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 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 seams, if necessary.

Click **Measure** to simultaneously record:

- OV, OS Ob+, OS Ob-, 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 osteoblasts.

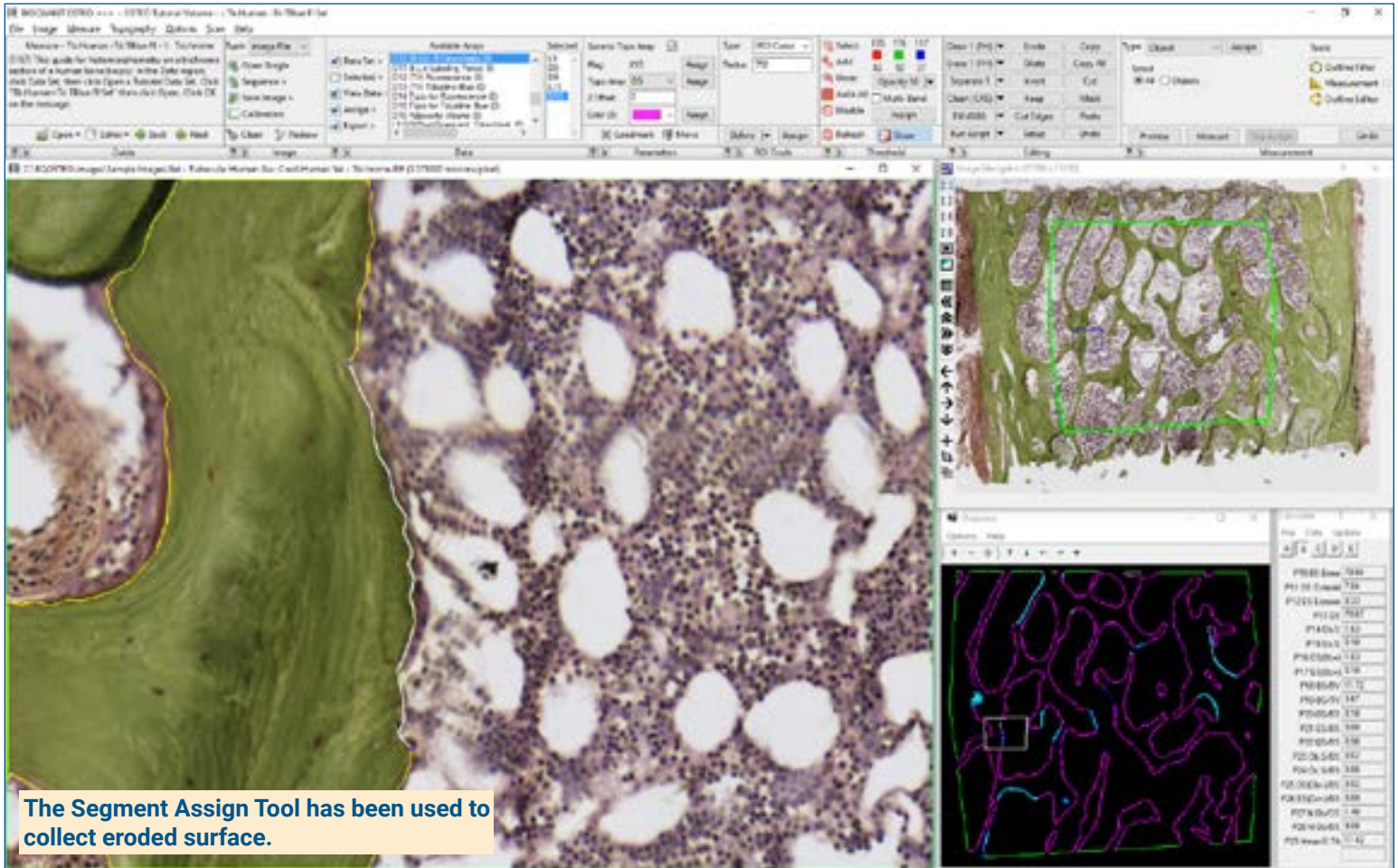
Click **Measure** to record:

- 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.

Human Bone Biopsy Protocol: Osteoclast Data



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 eroded surface or 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 eroded surface or osteoclasts within the Tissue Volume.

Use the Segment Assign Tool

Click **Preview**, BIOQUANT automatically:

- Thresholds the bone surface.
- Traces the bone surface

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

Click the beginning and end of a segment of eroded surfaces to measure:

- ES Oc+, ES Oc-
- ES Oc+/BS, ES Oc-/BS, ES/BS

Pan through the Tissue Volume and measure other eroded surfaces. Redraw of measurement tracings **prevents duplicate measurement**.

Count Osteoclasts

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

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

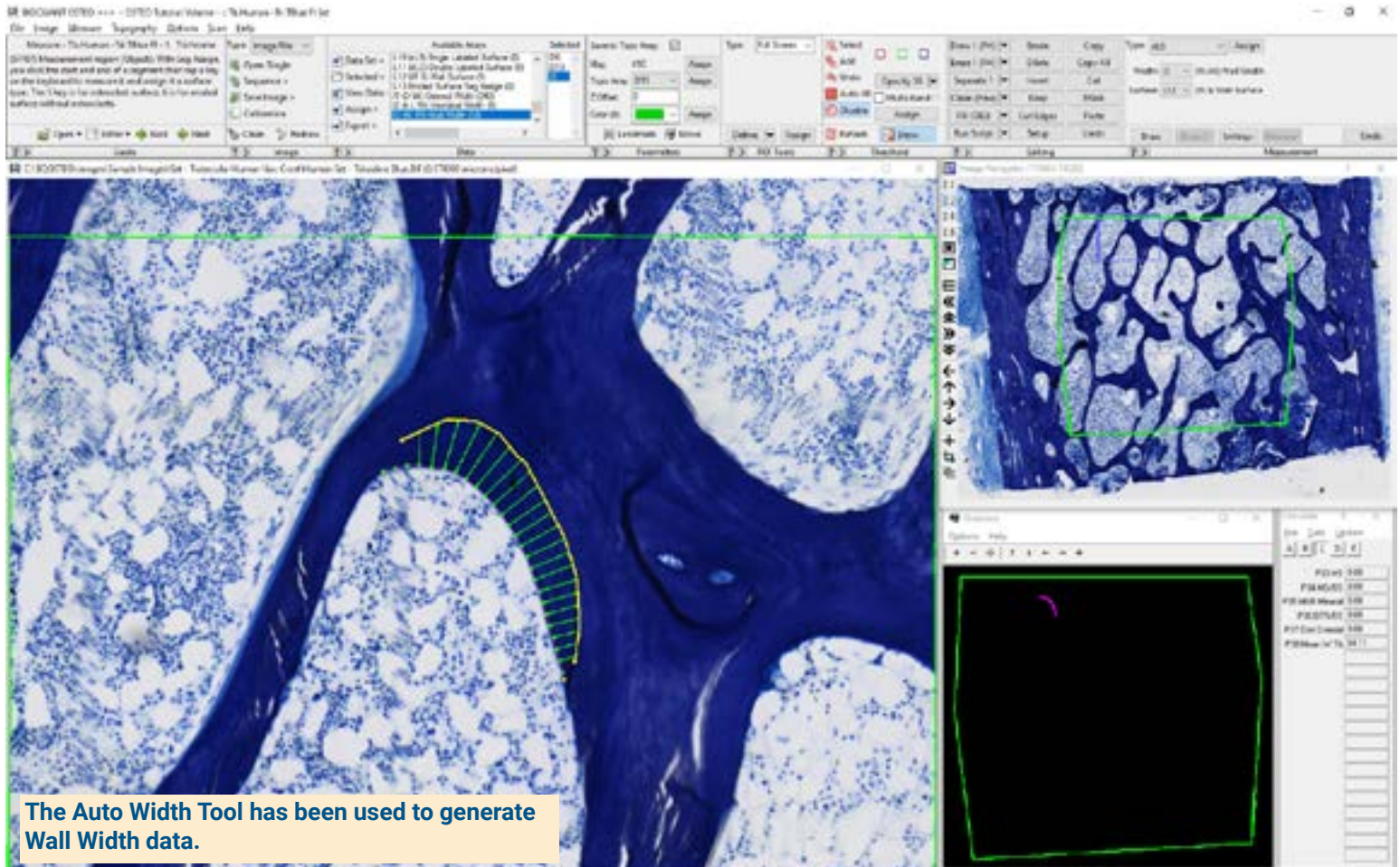
Click **Measure** to record:

- N.Oc, N.Oc/BS

Continue to pan through the Tissue Volume looking for more osteoclasts.

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

Human Bone Biopsy Protocol: Wall Width Data



Get Image of Wall Width Section

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.

Standardize the TV and Zoom In

At low power, standardize the TV:

- Recall the TV from the Trichrome Section.
- TV boundaries can be saved and loaded back.
- Alternatively, use iROI Cursor Tool to quickly draw the TV boundary.

Then, zoom in or change objective:

- Zoom in on a digital scan.
- Switch objectives with a live image from the microscope.
- Move to a field with remodeling surface.

Use the Auto Width Tool

Using Auto Width, click **Draw**, then:

- Click along the bone surface. Right click.
- Click along the cement line. Right click.

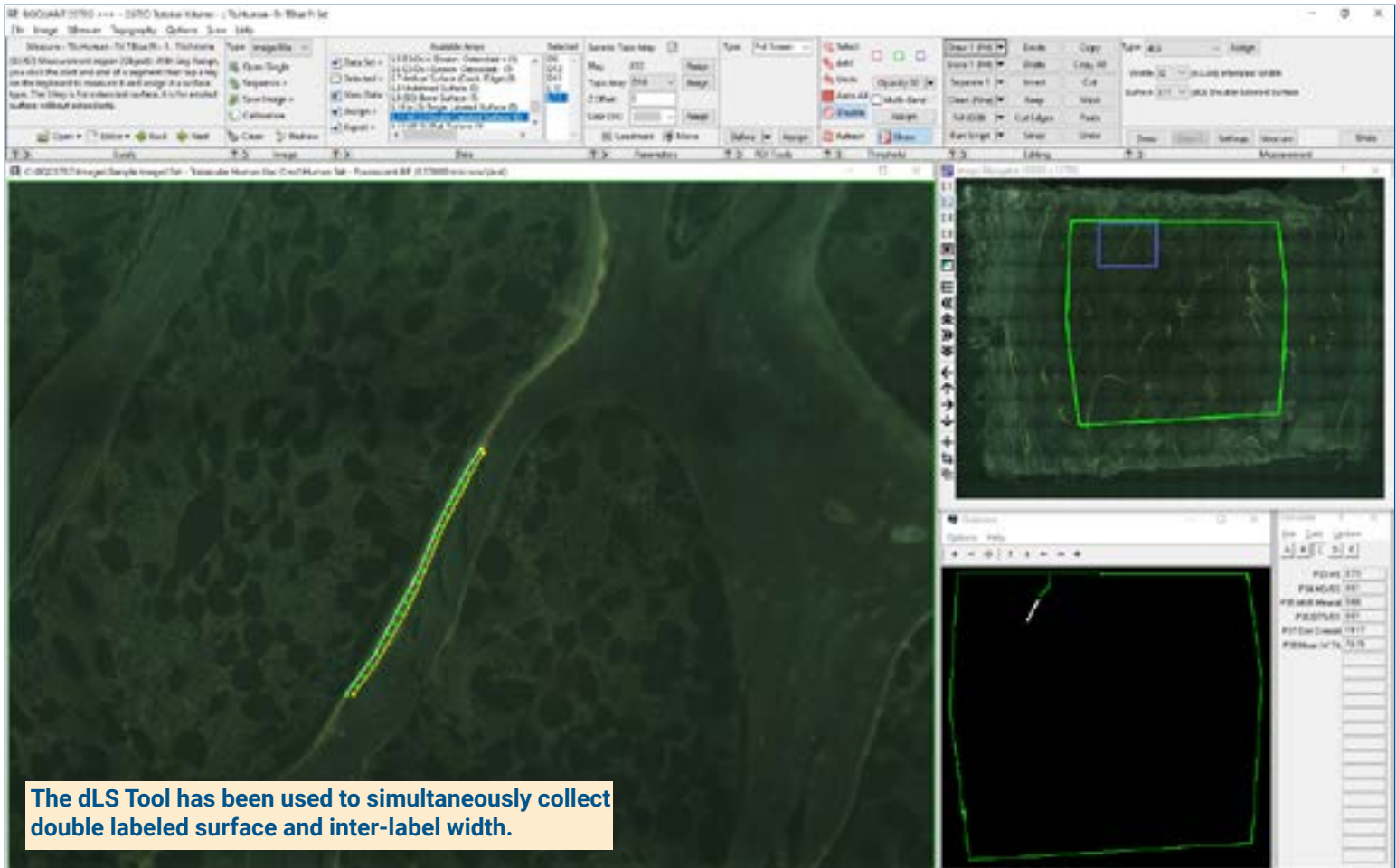
The Wall Width previews are generated.

Click **Measure** to simultaneously record:

- W.Wi: The width of the remodeling site at regular intervals along the surface.
- The length of the surface of the remodeling unit.

Repeat for other remodeling surfaces on this field, and then at other locations within the Tissue Volume.

Human Bone Biopsy Protocol: BFR Data



Define Calcein TV

Get an Image of the Calcein Section

- Open up to 4 GB Scan of Section.
- Or get Live Image from Microscope with Camera:

Standardize the TV

- Recall the TV from the Trichrome Section.
- TV boundaries can be saved and loaded back.
- Alternatively, use iROI Cursor Tool to quickly draw the TV boundary.

Use the Auto Surface Tool

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

- Thresholds the labeled surface.
- Traces the labeled surface.

Use **Thresholding Editing** tools like Keep and Erase so only single labeled surface is identified.

Click **Measure** to 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)

Pan the TV and repeat for sLS and dLS, using Redraw to prevent duplicate measurement.

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