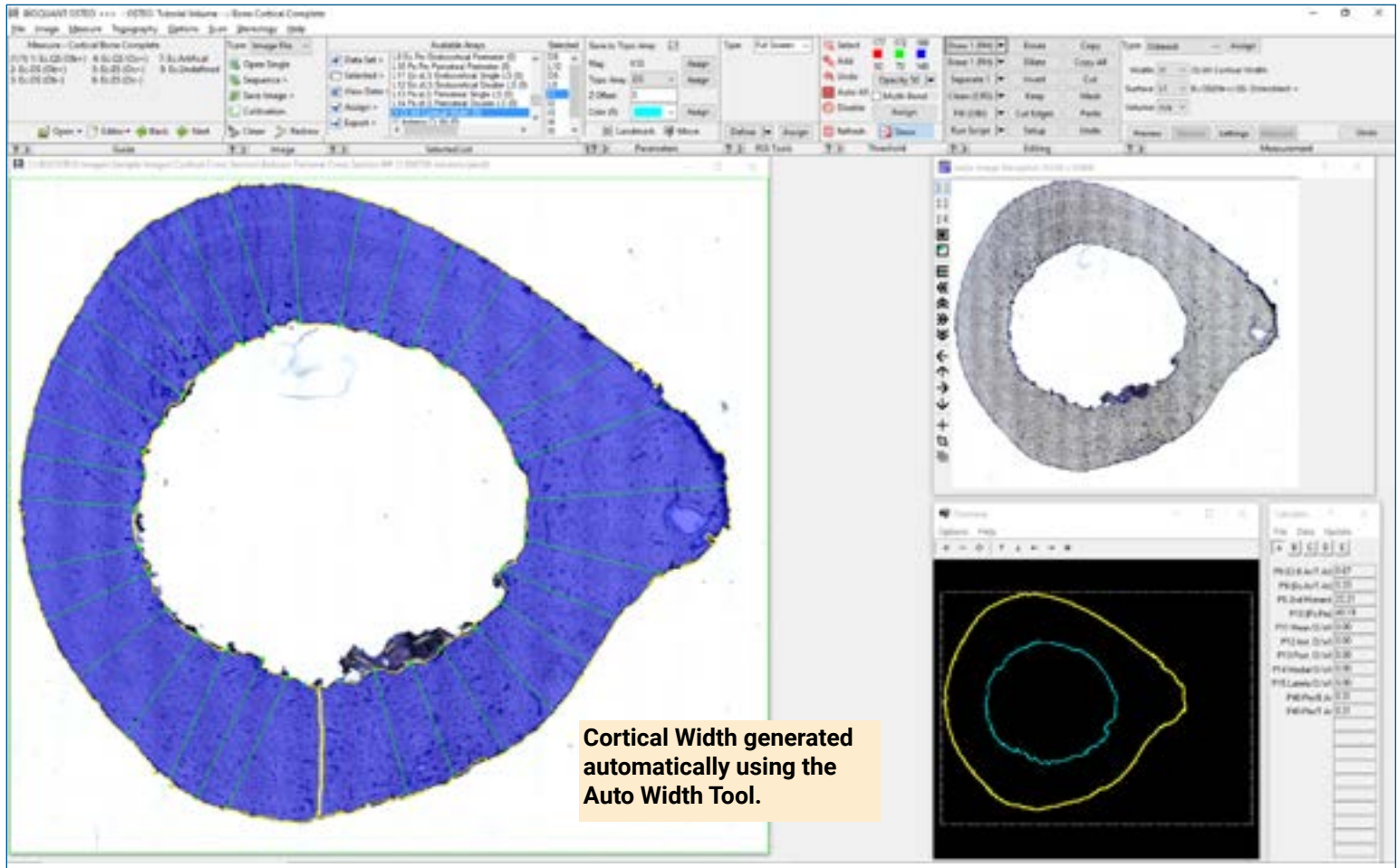


Cortical Bone Protocol: Low Power Indices



Get Image of Cortical Bone

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 entire Cortical Bone fits in one field of view.

Live Image from Microscope with Camera and tracking device:

- 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.

Collect Low Magnification Data

Based on a threshold of the cortical bone, BIOQUANT uses the **Object Measurement Tool** to automatically collect:

- Ct.Ar Cortical Bone Area
- Ps.Pm Periosteal Perimeter
- Ec.Ar Endocortical Area
- Ec.Pm Endocortical Perimeter

The **Auto Width Tool** is used to auto generate Ct.Wi Cortical Width.

Individual Distance is used to collect Anterior Ct.Wi, Posterior Ct.Wi, Medial Ct.Wi, and Lateral Ct.Wi.

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

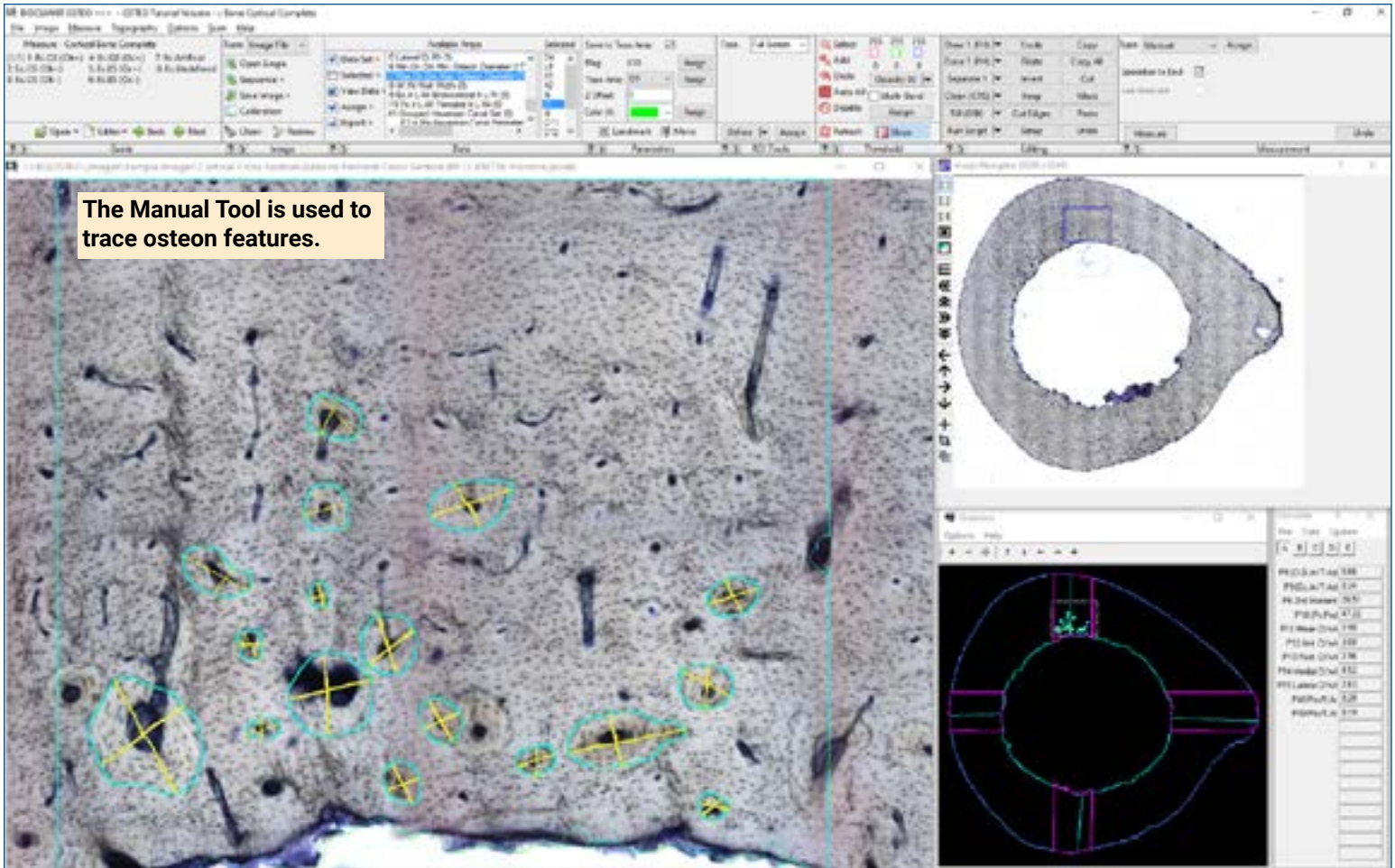
Define Sampling Areas

Region of Interest tools help define standardized Sampling Areas at low magnification of:

- anterior
- posterior
- medial
- lateral

We will measure within these Sampling Areas at high magnification.

Cortical Bone Protocol: High Power Indices



Zoom In or Change Objective

Digital Scan in **Large Image Navigator**:

- Zoom in to higher magnification.
- Pan to a field inside one of the Sampling Areas.

Live Image with Microscope:

- Change to a higher objective.
- Move to a field inside one of the Sampling Areas.

Use the Manual Tool

Find and Measure Osteons

- On.Ar Osteonal Area
- On.Pm Osteonal Perimeter
- On.N Osteon Number
- OPD Osteon Population Density
- Ha.Ar Haversian Canal Area
- Ha.Pm Haversian Perimeter
- On.Fg.Ar Osteon Fragment Area
- On.Fg.N Osteon Fragment Number
- On.Ar/Sa.Ar, Ha.Ar/Sa.Ar, On.Fg.Ar/Sa.Ar
- Po.Ar Pore Area and Porosity

Measure field to field, using **Redraw** to prevent duplicate measurement.

Use Measure Cells

Click **Preview** to automatically:

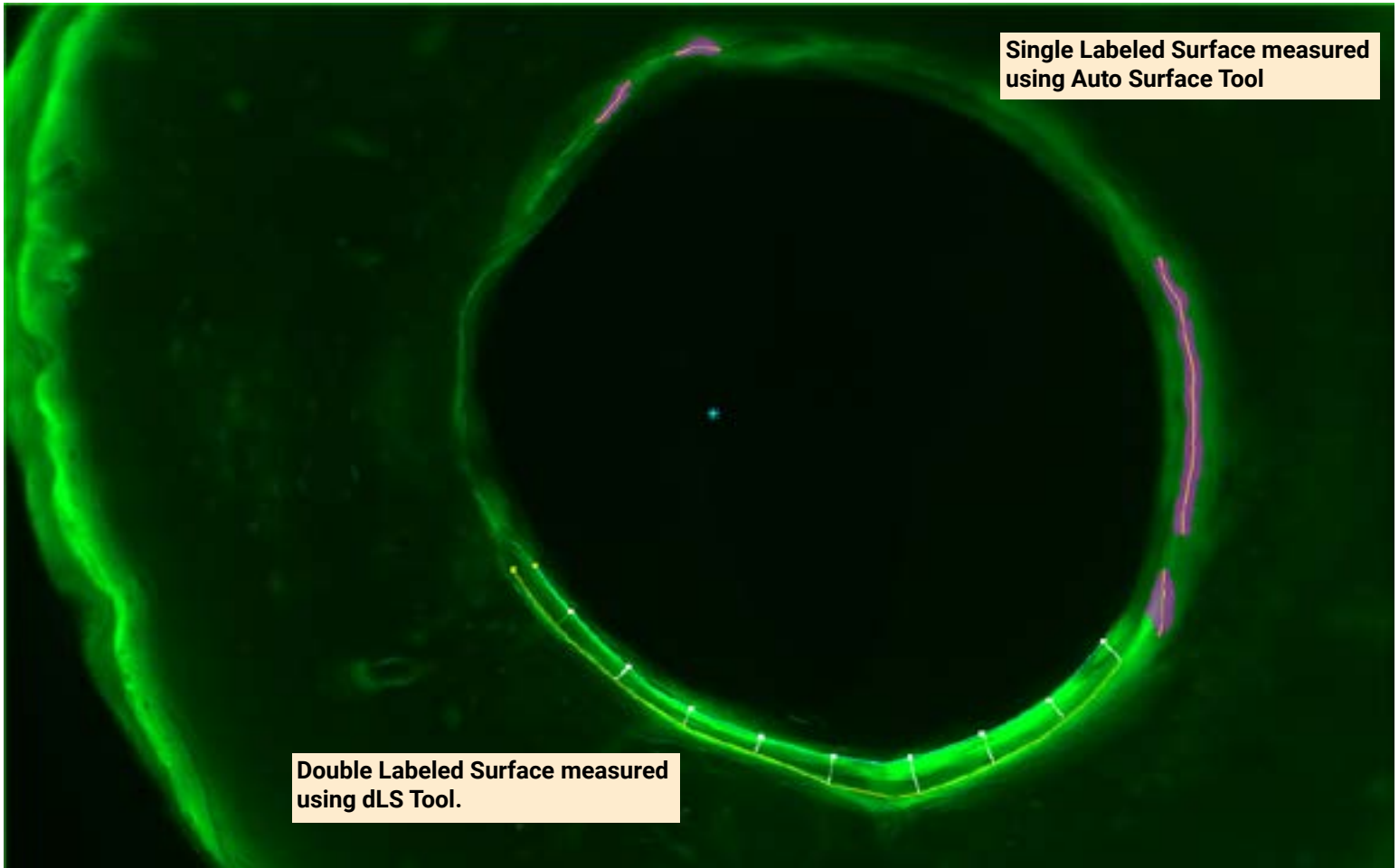
- Threshold the osteocytes.
- Preview outline the osteocytes.
- Osteocytes already measured on previous, overlapping fields of view are **not** preview outlined.
- Osteocytes cut by the region of interest are **not** preview outlined.

Click **Measure** to simultaneously record:

- (Oc.N) Osteocyte Number
- (Oc.Dn) Osteocyte Density
- (Oc.Ar) Osteocyte Area

Continue to scan measure field to field within the Sampling Area.

Cortical Bone Protocol: Dynamic Indices



Get Image of Calcein Section

Open up to [4 GB Scan](#) of Section.

Or get [Live Image](#) from Microscope with Camera.

[Zoom in or Change Objectives](#) and move to a field with Endocortical Single Labeled Surface.

Use Auto Surface Tool

Using the Auto Surface tool, click [Preview](#), BIOQUANT automatically:

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

Use Thresholding Editing tools like Keep and Erase to isolate the Ec.sLS.

Click Measure to record Ec.sLS Endocortical Single LS.

Repeat for Ps.sLS Periosteal Single LS.

Use dLS Tool

Using the dLS tool, click Draw, then:

- Click along surface one and two.

The double labeled surface and Interlabel Widths preview outline.

Click Measure to simultaneously record:

- Ec.Ir.L.Wi Endocortical Ir.L.W
- Ec.dLS Endocortical Double LS
- Ec.MS, Ec.MAR
- Ec.BFR/BS, Ec.BFR/BV, Ec.BFR/TV

Repeat for the Periosteal 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