BIOQUANT® *life science*

Chondrocyte Proliferation Protocol

The Irregular Tool has been used to define a Sampling Area.
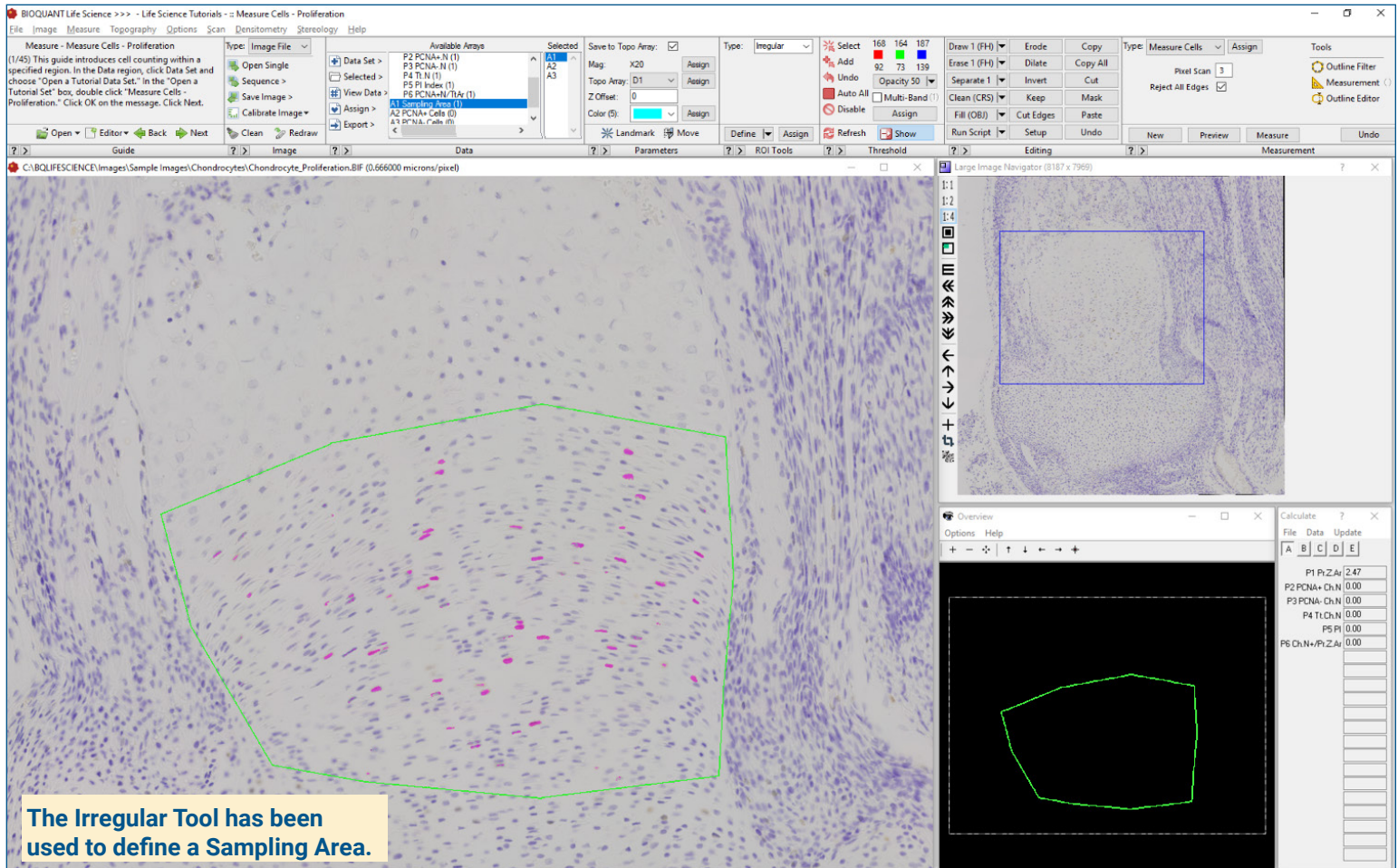
Get Image of the Section

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

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

Live Image from Microscope with Camera and tracking device:

- Use a low objective like 4X or 2X to display a live image of the Sampling Area to be counted in the BIOQUANT Image window.
- The Sampling Area does not need to fit within a single field of view.

Define Sampling Area

A Sampling area defines a region at low power on the section within which cells are measured at high power.

Irregular Tool

Create an irregular Sampling Areas that matches biological borders.

Rectangle Tool

Create a rectangular Sampling Area with known micron dimensions.

Measure the Sampling Area

Using the **Measure Object** tool, click the Measure button to automatically measure the area of the Sampling Area.

- Only **chondrocytes within the Sampling Area** will be measured.
- The Sampling Area data is used to **normalize** the number of proliferating cells by the size of the zone.
- At higher magnification, the **Sampling Area automatically redraws** as you pan and zoom the image at higher magnifications.
- Measurements are **bound by the Sampling Area** at all zoom levels.

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Measure Cells is used to measure PCNA+ and PCNA- cells within the Sampling Area.

Zoom In or Change Objective

Digital Scan in **Large Image Navigator**:

- Zoom in to higher magnification.
- Pan to the upper left corner of the Sampling Area. **Auto ROI** always keeps you within the Sampling Area.

Live Image with Microscope:

- Change to a higher objective
- Move to the upper left corner of the Sampling Area.
- The Sampling Area tracing redraws on the live image.

Use Measure Cells

Click **Preview** to automatically:

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

Click **Measure** to record PCNA+ data.

Repeat on this field for the PCNA- cells:

Repeat for all fields in the Sampling Area.

Proliferation Index and Other Data

As data are collected, BIOQUANT **automatically computes**:

- Pr.Zn.Ar (Proliferation Zone Area)
- PCNA+ Ch.N (+ Chondrocyte #)
- PCNA- Ch.N (- Chondrocyte #)
- Tt.Ch.N (Total Chondrocyte #)
- PI (Proliferation Index)
- PCNA+ Ch.N / Pr.Z.Ar

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/lifescience