Pore Extractor 2D v. 2.0: New Utilities for Mapping Collagen Fiber Orientation and Cortical Pore Distribution

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Introduction

- Quantifying porosity in bone tissue can inform forensic interpretations of skeletal trauma, age-at-death, and bone tissue quality and fragility[1,2].
- We previously developed a custom ImageJ macro toolkit, Pore Extractor 2D[3], to expedite the identification and automate the morphometric analysis of cortical pores on histological images.
- This new release provides tools to quantify the effect of cortical porosity on cross-sectional:
  1. Collagen fiber orientation (**CFOTool**)
  2. Whole-bone strength calculated relative to the centroid and bending planes (**CPSTool**)

Pore Extractor 2D Materials & Methods

- Pore Extractor 2D provides computer-assisted image contrast and noise removal (**Fig. 1A**), cortical border identification (**Fig. 1B**), and isolation of probable pore spaces (**Fig. 1C, 1D**), followed by automated morphometric analyses by pore type and region.
- Freehand manual correction of pore borders can split single pixels (**Fig 2**), which is corrected by the morphometric analysis tool. This sub-pixel correction is also extended to manually modified pore sets uploaded directly to the new CFOTool or CPSTool.

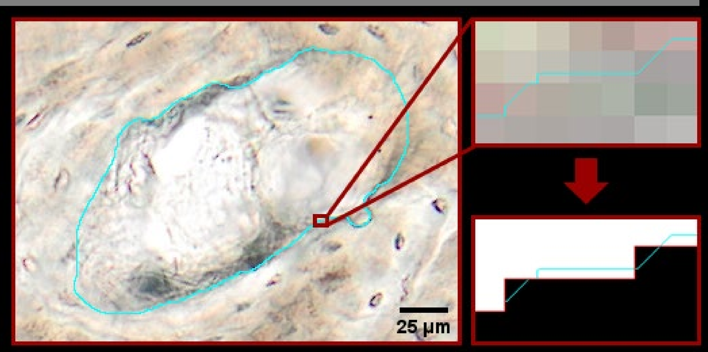
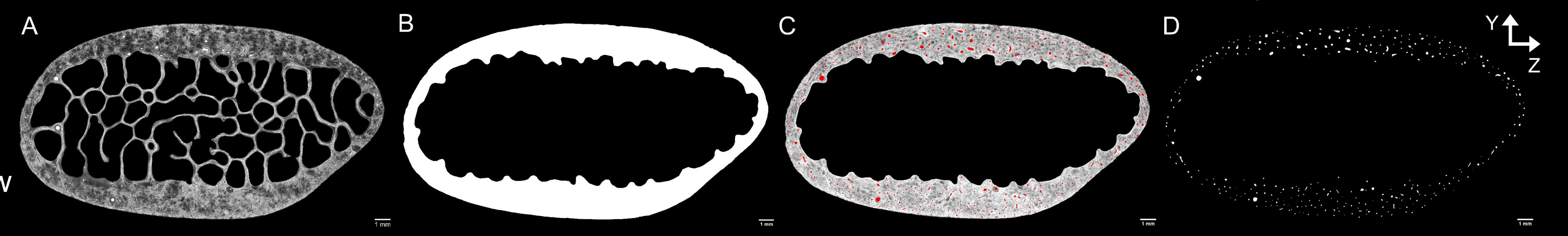


Fig. 2: Sub-pixel splitting by a manually modified pore border

Fig. 1: Pore Isolation Workflow



Collagen Fiber Orientation Tool (CFOTool)

- Collagen fibers are highly birefringent, refracting light that can pass through circular polarizing filters[4] (**Fig 3**).
- Collagen fiber orientation (CFO) is thought to reflect the section's localized mechanical loading history (**Fig 4**)[5,6].

  Dark → Fibers Longitudinal to Section → Resist Tension

  Bright → Fibers Transverse to Section → Resist Compression
- CFO has forensic potential to help explain fracture locations by identifying tissue regions poorly optimized to resist different strain modes and loading directions[7].
- The predominant CFO of a tissue cross-section or region can be quantified from the Weighted Mean Gray Level (WMGL)[8] of an 8-bit (0 – 255) grayscale image:

$$\mathrm{WMGL} = \sum_{i=\mathrm{GL}_{min}}^{255} \frac{A_i \mathrm{GL}_i}{A_t}$$

GL_{min} = minimum graylevel

A_i = area of *i*th graylevel

GL_i = *i*th graylevel

A_t = total area imaged

- Current methods[4–8] account for porosity by setting a minimum graylevel to exclude dark pixels. However, this also removes dark tissue regions, over-estimating WMGL compared to excluding discrete pore spaces (**Fig 5**).
- CFOTool can automatically calculate WMGL with:
  (1) No pore removal
  (2) Excluding a dark pixel range (default: 0—28)
  (3) Removing pore spaces identified by Pore Extractor 2D
- CFOTool also provides optional regional calculation of WMGL for long bone quadrants, rib cutaneous/pleural halves, or user-created regions (**Fig 6**).
- CFOTool exports a default CFO-specific colormap[7] but can also use ImageJ lookup tables or any custom subdivision created by the user with the ImageJ Lookup Table Editor (**Fig 7**).

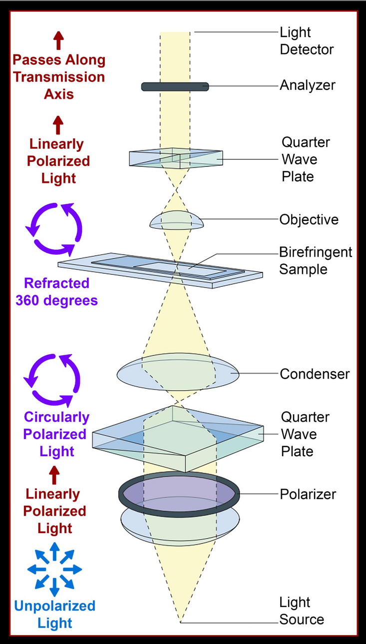


Fig. 3: Circular Polarization

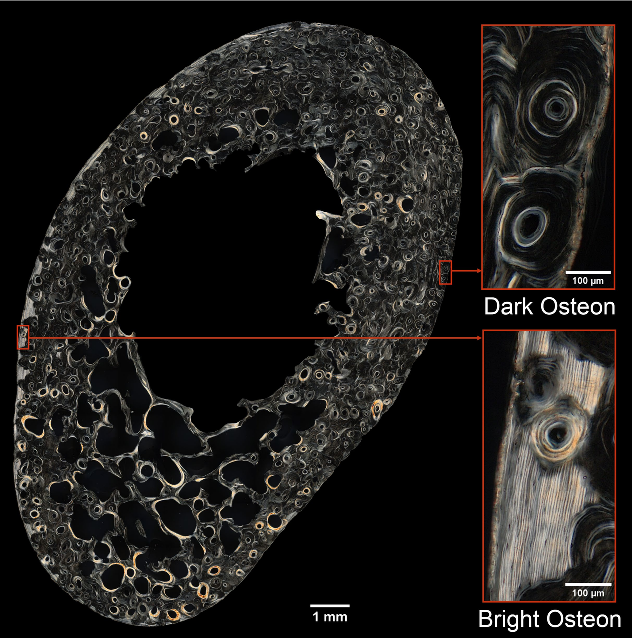


Fig. 4: CFO in a Human Clavicle Cross-Section

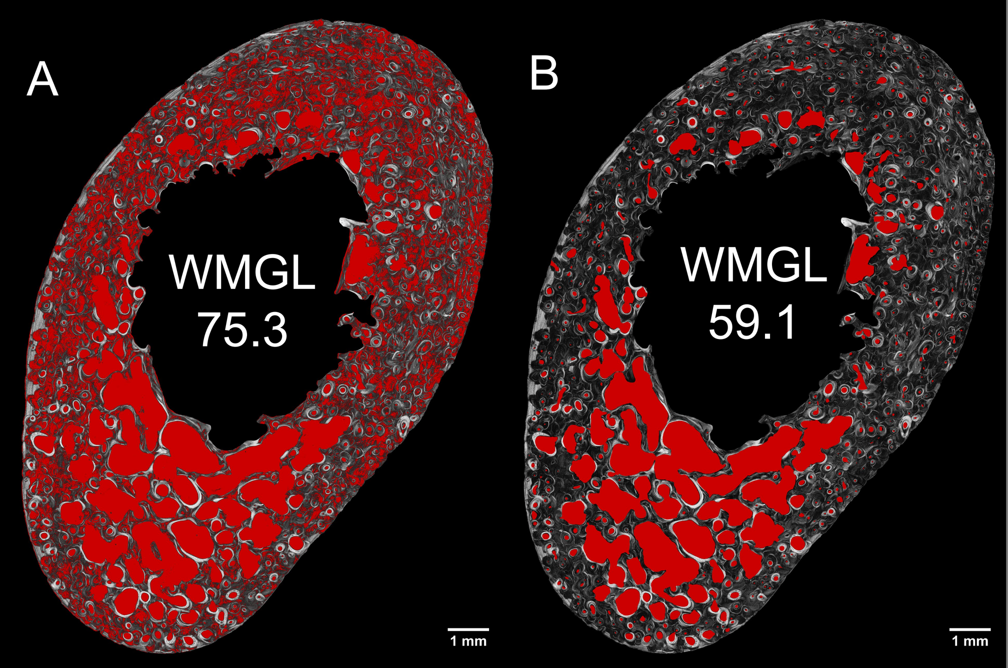


Fig. 5: Pixels excluded (red) by (A) minimum graylevel (0—28)[7] versus (B) removing discrete pore spaces identified by Pore Extractor 2D

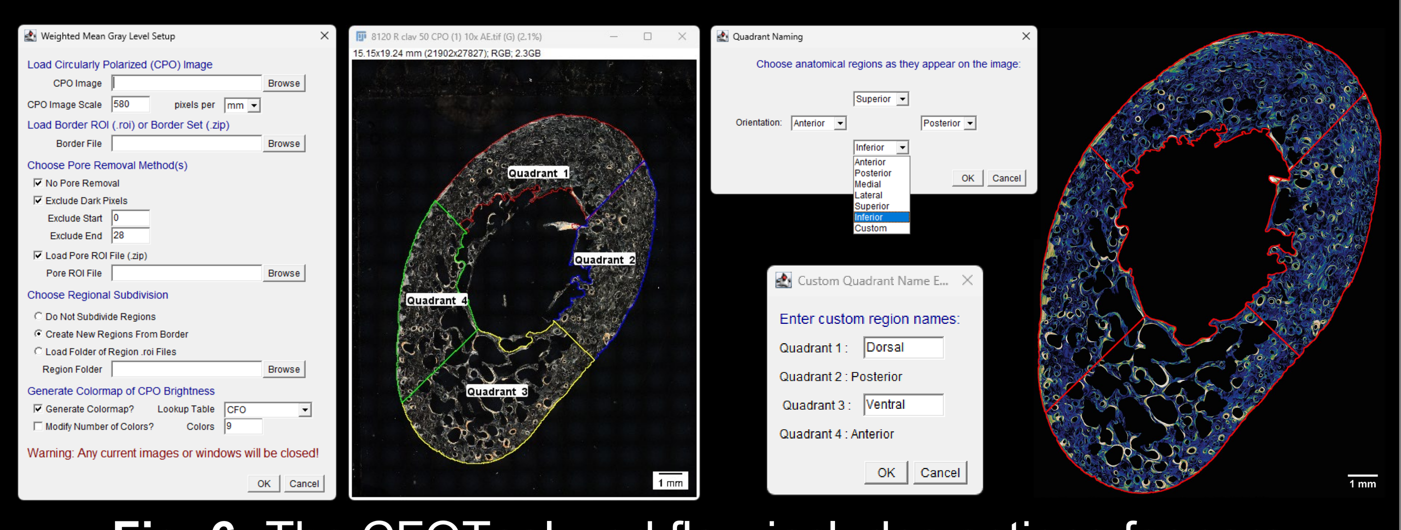


Fig. 6: The CFOTool workflow includes options for pore removal, regional subdivision, and custom region names

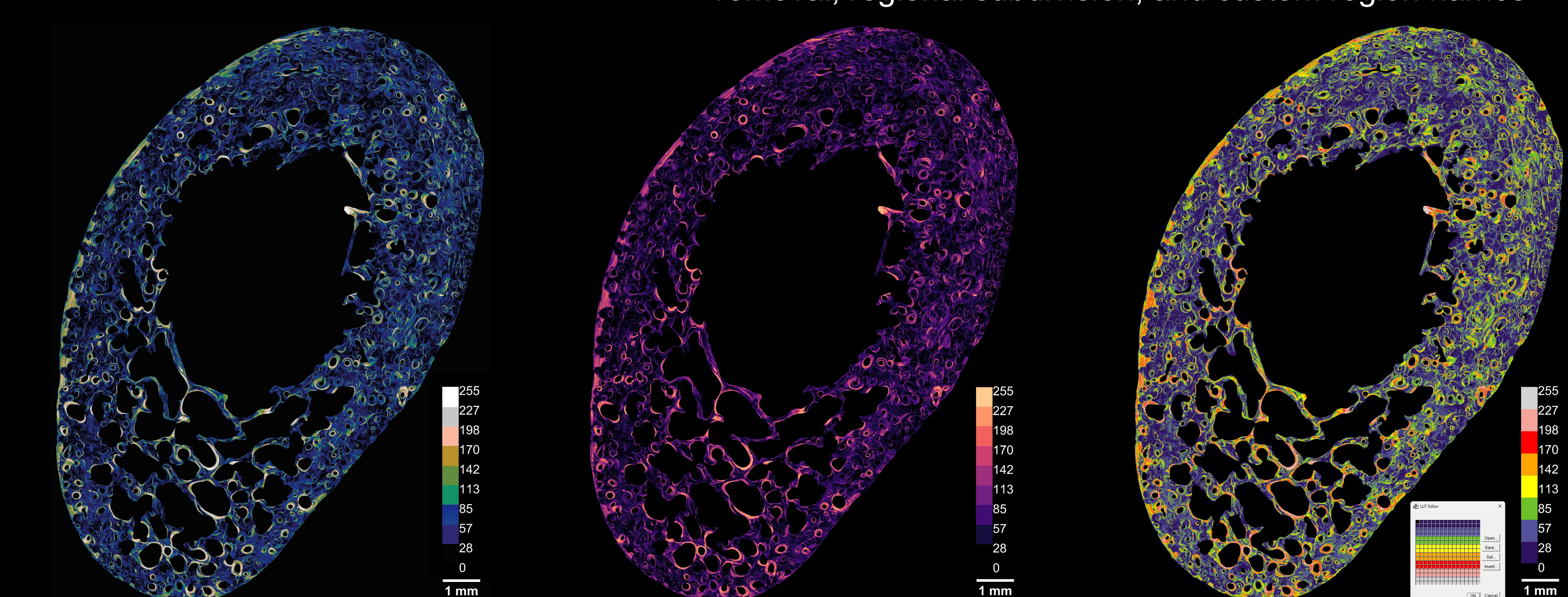


Fig. 7: Default CFO Lookup Table[7] ImageJ Lookup Table 'Magma' User-Created Lookup Table[4,5]

Cortical Pore Score (CPSTool)

- A cross-section's centroid shifts and its major and minor axes rotate when pore spaces are represented as voids (**Fig. 8**).

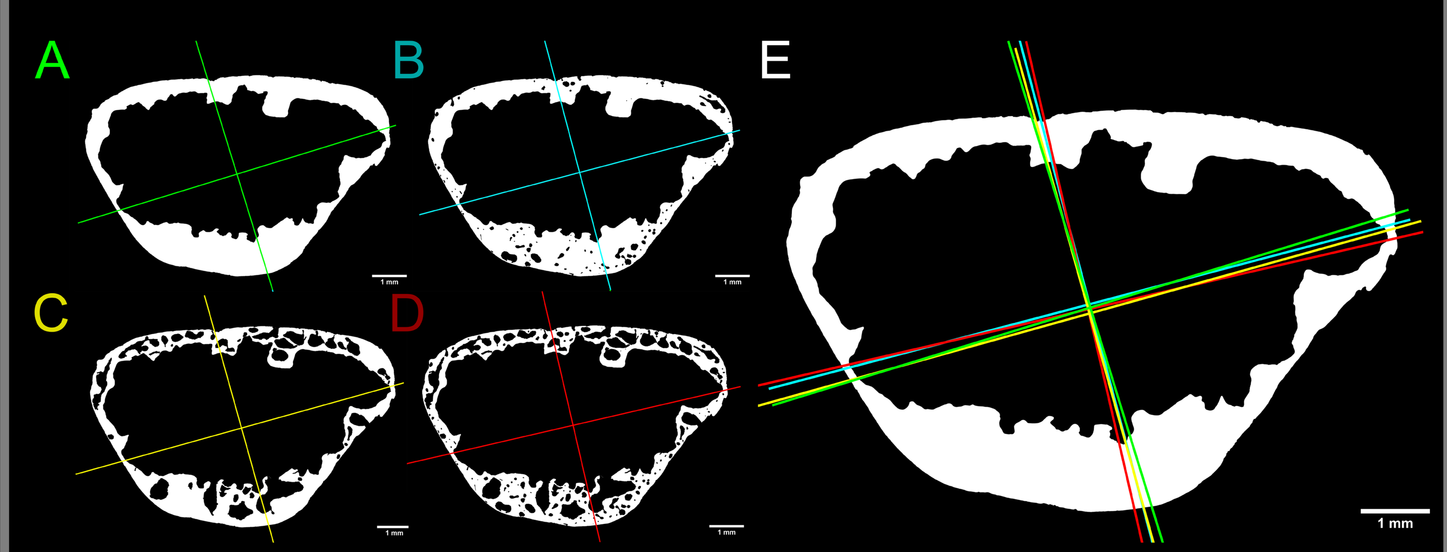


Fig. 8: Major and minor axes of the solid cortex (A) shift when representing (B) cortical, (C) trabecularized, or (D) all pores

- The impact of individual pores on whole bone strength depends on pore area and pore distance to the bending plane, or to the section centroid if a loading direction is not assumed (**Fig. 9**)[9].
- Cortical pore score[9] quantifies porosity's cumulative effect as:

$$\mathrm{CPS} = \sum_i A_i d_i^2$$

A_i = area of *i*th pore

d_i = distance of *i*th pore centroid to section plane or centroid

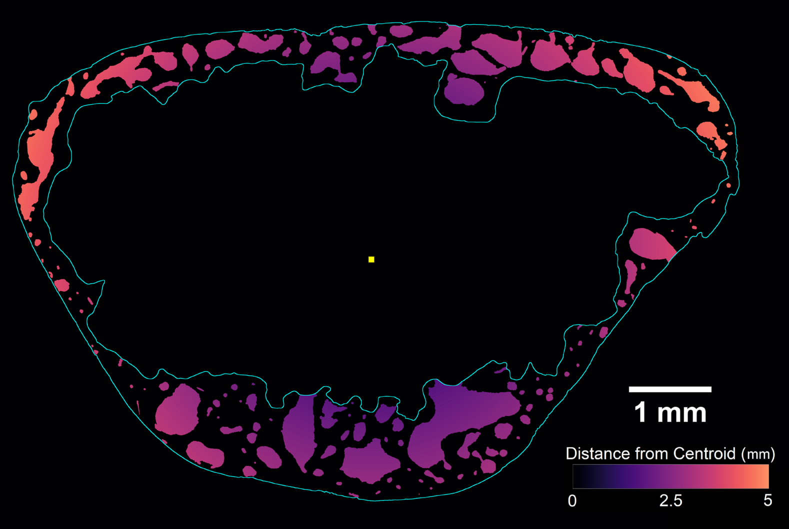


Fig. 9: Pore distances to the centroid

- CPSTool calculates CPS relative to the section centroid, major and minor axes, and bending axes (**Fig. 10**).
- CPSTool also calculates the percentage change in area moments of inertia (I_{max}, I_{min}) when pores are removed from the solid cortex.

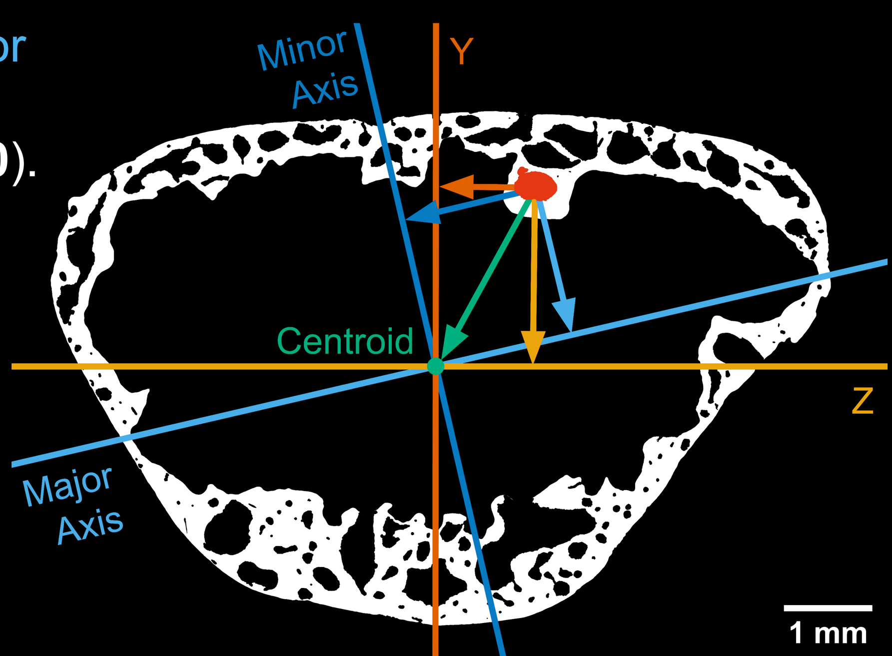


Fig. 10: CPSTool Measurement Options

Discussion & Conclusions

- Pore Extractor 2D can now account for porosity when using collagen fiber orientation or cross-sectional geometry to predict bone tissue's mechanical response.
- Future tools will incorporate machine learning for improved identification of probable pore spaces.

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