```
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//Revised 25 July 2018

//Revised 11 June 2021 (resolved issue with auto-generated scale data)

//Generates two borders, periosteal and endosteal, that can be used to calculate a cross-section's cortical area

macro "Cortical Area" {

//Generate Cortical Borders

endo = getBoolean ("Have you clipped the trabecular borders?");

if (endo == 1) {


image = getBoolean ("Is sample contrast strong?");

if (image == 1) {


//Generate Endosteal Border ROI for strong contrast image

name=getTitle;


//Duplicate original file to work off of

run("Duplicate...", "title=[]");

run("Set Scale...", "distance=0 known=0 unit=pixel");


//Adjust contrast

run("Brightness/Contrast...");

setMinAndMax(210,230);

run("Apply LUT");

run("Close");


//Remove noise

run("Remove Outliers...", "radius=15 threshold=50 which=Dark");
```

```
//Adjust contrast again
run("Enhance Contrast...", "saturated=20");
run("Brightness/Contrast...");
setMinAndMax(254,255);
run("Apply LUT");
run("Close");

//Convert to grayscale and threshold image
run("8-bit");
setAutoThreshold("Default");

//run("Threshold...");
setThreshold(0, 220);
setOption("BlackBackground", false);
run("Convert to Mask");

//Remove noise again
run("Remove Outliers...", "radius=10 threshold=50 which=Dark");

//FILL BACKGROUND
setForegroundColor(1, 1, 1);
floodFill(30, 3000);

//Create EA area for measurement
run("Invert");
run("Make Binary");

//Check if Invert held
makePoint(30, 3000);
```

```
run("Set Measurements...", "min redirect=None decimal=6");
run("Measure");
DOOM = getResult("Min");

//DOOM == 255 if the background remains black and must be inverted again
if (DOOM == 255){
	run("Invert");
	run("Options...", "iterations = 1 count =1 white");
	run("Close-");
	run("Fill Holes");

	//Create adjustable ROI
	run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
	roiManager("Show All with labels");
	roiManager("Show All");
	roiManager("Select", 0);
	run("Fit Spline");
	run("Interpolate", "interval=15");
	roiManager("Add");
	roiManager("Select", 0);
	roiManager("Delete");
	roiManager("Select", 0);

	//Close Results window
	if (isOpen("Results")) {
		selectWindow("Results");
		run("Close");
		}
```

```
        //Add ROI to original image
        close();
        roiManager("Show All");
}

else {
run("Options...", "iterations = 1 count =1 white");
run("Close-");
run("Fill Holes");

//Create adjustable ROI
run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
roiManager("Show All with labels");
roiManager("Show All");
roiManager("Select", 0);
run("Fit Spline");
run("Interpolate", "interval=15");
roiManager("Add");
roiManager("Select", 0);
roiManager("Delete");
roiManager("Select", 0);

//Close Results window
if (isOpen("Results")) {
        selectWindow("Results");
        run("Close");
        }
```

```
//Add ROI to original image
close();
roiManager("Show All");
}

//Generate Total Area ROI
name=getTitle;

//Duplicate original file to work off of
run("Duplicate...", "title=[]");
run("Set Scale...", "distance=0 known=0 unit=pixel");

//Adjust contrast
run("Brightness/Contrast...");
setMinAndMax(210,230);
run("Apply LUT");
run("Close");

//Remove noise
run("Remove Outliers...", "radius=15 threshold=50 which=Dark");

//Adjust contrast again
run("Enhance Contrast...", "saturated=20");
run("Brightness/Contrast...");
setMinAndMax(254,255);
run("Apply LUT");
run("Close");
```

```
//Convert to grayscale and threshold image
run("8-bit");
setAutoThreshold("Default");

//run("Threshold...");
setThreshold(0, 220);
setOption("BlackBackground", false);
run("Convert to Mask");

//Remove noise again
run("Remove Outliers...", "radius=10 threshold=50 which=Dark");

//Fill in rib
run("Close-");
run("Fill Holes");

//Create adjustable ROI
run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
roiManager("Show All with labels");
roiManager("Show All");
roiManager("Select", 1);
run("Fit Spline");
run("Interpolate", "interval=15");
roiManager("Add");
roiManager("Select", 1);
roiManager("Delete");
roiManager("Select", 1);
```

```
//Add ROI to original image
close();
roiManager("Show All");
}

//If the image contrast is poor, the following version should be used
else if (image == 0) {

//Generate an endosteal area ROI
name=getTitle;

//Duplicate original file to work off of
run("Duplicate...", "title=[]");
run("Set Scale...", "distance=0 known=0 unit=pixel");

//Adjust contrast
run("Enhance Contrast...", "saturated=20");

//Adjust contrast
run("Brightness/Contrast...");
setMinAndMax(0,240);
run("Apply LUT");
run("Close");

//Remove noise
run("Remove Outliers...", "radius=10 threshold=25 which=Dark");

run("Brightness/Contrast...");
setMinAndMax(40,255);
```

```
run("Apply LUT");
run("Close");

run("Brightness/Contrast...");
setMinAndMax(20,200);
run("Apply LUT");
run("Close");

//Convert to grayscale and lighten background
run("8-bit");
run("Enhance Contrast...", "saturated=30");

run("Brightness/Contrast...");
setMinAndMax(0,254);
run("Apply LUT");
run("Close");

//run("Threshold...");
setAutoThreshold("Default");
setThreshold(0, 254);
setOption("BlackBackground", false);
run("Convert to Mask");

//Remove noise again
run("Remove Outliers...", "radius=8 threshold=50 which=Dark");

//FILL BACKGROUND
setForegroundColor(1, 1, 1);
floodFill(30, 3000);
```

```
//Create EA area for measurement
run("Invert");
run("Make Binary");

//Check if Invert held
makePoint(30, 3000);
run("Set Measurements...", "min redirect=None decimal=6");
run("Measure");
DOOM = getResult("Min");

//DOOM == 255 if the background remains black and must be inverted again
if (DOOM == 255){
	run("Invert");
	run("Options...", "iterations = 1 count =1 white");
	run("Close-");
	run("Fill Holes");

	//Create adjustable ROI
	run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
	roiManager("Show All with labels");
	roiManager("Show All");
	roiManager("Select", 0);
	run("Fit Spline");
	run("Interpolate", "interval=15");
	roiManager("Add");
	roiManager("Select", 0);
	roiManager("Delete");
```

```
        roiManager("Select", 0);

        //Close Results window
        if (isOpen("Results")) {
        selectWindow("Results");
        run("Close");
        }

        //Add ROI to original image
        close();
        roiManager("Show All");

}

else {
run("Options...", "iterations = 1 count =1 white");
run("Close-");
run("Fill Holes");

//Create adjustable ROI
run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
roiManager("Show All with labels");
roiManager("Show All");
roiManager("Select", 0);
run("Fit Spline");
run("Interpolate", "interval=15");
roiManager("Add");
roiManager("Select", 0);
```

```
roiManager("Delete");
roiManager("Select", 0);

//Close Results window
if (isOpen("Results")) {
	selectWindow("Results");
	run("Close");
	}

//Add ROI to original image
close();
roiManager("Show All");
}


//Generate a total area ROI
name=getTitle;

//Duplicate original file to work off of
run("Duplicate...", "title=[]");
run("Set Scale...", "distance=0 known=0 unit=pixel");

//Adjust contrast
run("Enhance Contrast...", "saturated=20");

//Adjust contrast
run("Brightness/Contrast...");
setMinAndMax(0,240);
run("Apply LUT");
```

```
run("Close");

//Remove noise
run("Remove Outliers...", "radius=10 threshold=25 which=Dark");

run("Brightness/Contrast...");
setMinAndMax(40,255);
run("Apply LUT");
run("Close");

run("Brightness/Contrast...");
setMinAndMax(20,200);
run("Apply LUT");
run("Close");

//Convert to grayscale and lighten background
run("8-bit");
run("Enhance Contrast...", "saturated=30");

run("Brightness/Contrast...");
setMinAndMax(0,254);
run("Apply LUT");
run("Close");

//run("Threshold...");
setAutoThreshold("Default");
setThreshold(0, 254);
setOption("BlackBackground", false);
run("Convert to Mask");
```

```
//Remove noise again
run("Remove Outliers...", "radius=8 threshold=50 which=Dark");

//Fill in rib
run("Close-");
run("Fill Holes");

//Create adjustable ROI
run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
roiManager("Show All with labels");
roiManager("Show All");
roiManager("Select", 1);
run("Fit Spline");
run("Interpolate", "interval=15");
roiManager("Add");
roiManager("Select", 1);
roiManager("Delete");
roiManager("Select", 1);

//Add ROI to original image
close();
roiManager("Show All");
}}

else if (endo == 0) {}
}
```

```
//Generates an endosteal border, which measures the medullary area
macro "Endosteal Area" {
//Generate Endosteal Border
endo = getBoolean ("Have you clipped the trabecular borders?");
if (endo == 1) {

image = getBoolean ("Is sample contrast strong?");
if (image == 1) {

name=getTitle;

//Duplicate original file to work off of
run("Duplicate...", "title=[]");
run("Set Scale...", "distance=0 known=0 unit=pixel");

//Adjust contrast
run("Brightness/Contrast...");
setMinAndMax(210,230);
run("Apply LUT");
run("Close");

//Remove noise
run("Remove Outliers...", "radius=15 threshold=50 which=Dark");

//Adjust contrast again
```

```
run("Enhance Contrast...", "saturated=20");
run("Brightness/Contrast...");
setMinAndMax(254,255);
run("Apply LUT");
run("Close");

//Convert to grayscale and threshold image
run("8-bit");
setAutoThreshold("Default");

//run("Threshold...");
setThreshold(0, 220);
setOption("BlackBackground", false);
run("Convert to Mask");

//Remove noise again
run("Remove Outliers...", "radius=10 threshold=50 which=Dark");

//FILL BACKGROUND
setForegroundColor(1, 1, 1);
floodFill(30, 3000);

//Create EA area for measurement
run("Invert");
run("Make Binary");

//Check if Invert held
makePoint(30, 3000);
run("Set Measurements...", "min redirect=None decimal=6");
```

```
run("Measure");
DOOM = getResult("Min");

//DOOM == 255 if the background remains black and must be inverted again
if (DOOM == 255){
	run("Invert");
	run("Options...", "iterations = 1 count =1 white");
	run("Close-");
	run("Fill Holes");

	//Create adjustable ROI
	run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
	roiManager("Show All with labels");
	roiManager("Show All");
	roiManager("Select", 0);
	run("Fit Spline");
	run("Interpolate", "interval=15");
	roiManager("Add");
	roiManager("Select", 0);
	roiManager("Delete");
	roiManager("Select", 0);

	//Close Results window
	if (isOpen("Results")) {
	selectWindow("Results");
	run("Close");
	}
```

```
        //Add ROI to original image
        close();
        roiManager("Show All");

} else {
run("Options...", "iterations = 1 count =1 white");
run("Close-");
run("Fill Holes");

//Create adjustable ROI
run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
roiManager("Show All with labels");
roiManager("Show All");
roiManager("Select", 0);
run("Fit Spline");
run("Interpolate", "interval=15");
roiManager("Add");
roiManager("Select", 0);
roiManager("Delete");
roiManager("Select", 0);

//Close Results window
if (isOpen("Results")) {
selectWindow("Results");
run("Close");
}

//Add ROI to original image
```

```
close();
roiManager("Show All");
}}

//For poor contrast images, the following version should be used
else if (image == 0) {

//Generate an endosteal area ROI
name=getTitle;

//Duplicate original file to work off of
run("Duplicate...", "title=[]");
run("Set Scale...", "distance=0 known=0 unit=pixel");

//Adjust contrast
run("Enhance Contrast...", "saturated=20");

//Adjust contrast
run("Brightness/Contrast...");
setMinAndMax(0,240);
run("Apply LUT");
run("Close");

//Remove noise
run("Remove Outliers...", "radius=10 threshold=25 which=Dark");

run("Brightness/Contrast...");
setMinAndMax(40,255);
run("Apply LUT");
```

```
run("Close");

run("Brightness/Contrast...");
setMinAndMax(20,200);
run("Apply LUT");
run("Close");

//Convert to grayscale and lighten background
run("8-bit");
run("Enhance Contrast...", "saturated=30");

run("Brightness/Contrast...");
setMinAndMax(0,254);
run("Apply LUT");
run("Close");

//run("Threshold...");
setAutoThreshold("Default");
setThreshold(0, 254);
setOption("BlackBackground", false);
run("Convert to Mask");

//Remove noise again
run("Remove Outliers...", "radius=8 threshold=50 which=Dark");

//FILL BACKGROUND
setForegroundColor(1, 1, 1);
floodFill(30, 3000);
```

```
//Create EA area for measurement
run("Invert");
run("Make Binary");

//Check if Invert held
makePoint(30, 3000);
run("Set Measurements...", "min redirect=None decimal=6");
run("Measure");
DOOM = getResult("Min");

//DOOM == 255 if the background remains black and must be inverted again
if (DOOM == 255){
	run("Invert");
	run("Options...", "iterations = 1 count =1 white");
	run("Close-");
	run("Fill Holes");

	//Create adjustable ROI
	run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
	roiManager("Show All with labels");
	roiManager("Show All");
	roiManager("Select", 0);
	run("Fit Spline");
	run("Interpolate", "interval=15");
	roiManager("Add");
	roiManager("Select", 0);
	roiManager("Delete");
	roiManager("Select", 0);
```

```
        //Close Results window
        if (isOpen("Results")) {
        selectWindow("Results");
        run("Close");
        }

        //Add ROI to original image
        close();
        roiManager("Show All");

}

else {
run("Options...", "iterations = 1 count =1 white");
run("Close-");
run("Fill Holes");

//Create adjustable ROI
run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude
add");
roiManager("Show All with labels");
roiManager("Show All");
roiManager("Select", 0);
run("Fit Spline");
run("Interpolate", "interval=15");
roiManager("Add");
roiManager("Select", 0);
roiManager("Delete");
```

```
roiManager("Select", 0);

//Close Results window
if (isOpen("Results")) {
	selectWindow("Results");
	run("Close");
	}

//Add ROI to original image
close();
roiManager("Show All");
}}

else if (endo == 0) {}
}}




//Generates a periosteal border, used to measure the total area
macro "Perioseal Area"{

//Generate Periosteal Border
image = getBoolean ("Is image contrast strong?");
if (image == 1) {
name=getTitle;

//Duplicate original file to work off of
```

```
run("Duplicate...", "title=[]");
run("Set Scale...", "distance=0 known=0 unit=pixel");

//Adjust contrast
run("Brightness/Contrast...");
setMinAndMax(210,230);
run("Apply LUT");
run("Close");

//Remove noise
run("Remove Outliers...", "radius=15 threshold=50 which=Dark");

//Adjust contrast again
run("Enhance Contrast...", "saturated=20");
run("Brightness/Contrast...");
setMinAndMax(254,255);
run("Apply LUT");
run("Close");

//Convert to grayscale and threshold image
run("8-bit");
setAutoThreshold("Default");

//run("Threshold...");
setThreshold(0, 220);
setOption("BlackBackground", false);
run("Convert to Mask");

//Remove noise again
```

```
run("Remove Outliers...", "radius=10 threshold=50 which=Dark");

//Fill in rib
run("Close-");
run("Fill Holes");

//Create adjustable ROI
run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
roiManager("Show All with labels");
roiManager("Show All");
roiManager("Select", 0);
run("Fit Spline");
run("Interpolate", "interval=15");
roiManager("Add");
roiManager("Select", 0);
roiManager("Delete");
roiManager("Select", 0);

//Add ROI to original image
close();
roiManager("Show All");
}

//For poor contrast images, the following version should be used
else if (image == 0) {
name=getTitle;

//Duplicate original file to work off of
```

```
run("Duplicate...", "title=[]");
run("Set Scale...", "distance=0 known=0 unit=pixel");

//Adjust contrast
run("Enhance Contrast...", "saturated=20");

//Adjust contrast
run("Brightness/Contrast...");
setMinAndMax(0,240);
run("Apply LUT");
run("Close");

//Remove noise
run("Remove Outliers...", "radius=10 threshold=25 which=Dark");

run("Brightness/Contrast...");
setMinAndMax(40,255);
run("Apply LUT");
run("Close");

run("Brightness/Contrast...");
setMinAndMax(20,200);
run("Apply LUT");
run("Close");

//Convert to grayscale and lighten background
run("8-bit");
run("Enhance Contrast...", "saturated=30");
```

```
run("Brightness/Contrast...");
setMinAndMax(0,254);
run("Apply LUT");
run("Close");

//run("Threshold...");
setAutoThreshold("Default");
setThreshold(0, 254);
setOption("BlackBackground", false);
run("Convert to Mask");

//Remove noise again
run("Remove Outliers...", "radius=8 threshold=50 which=Dark");

//Fill in rib
run("Close-");
run("Fill Holes");

//Create adjustable ROI
run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
roiManager("Show All with labels");
roiManager("Show All");
roiManager("Select", 1);
run("Fit Spline");
run("Interpolate", "interval=15");
roiManager("Add");
roiManager("Select", 1);
roiManager("Delete");
```

```
roiManager("Select", 1);


//Add ROI to original image
close();
roiManager("Show All");
}}




//Generates adjustable nodes on a polygon selection
//When the adjustable ROI portion of an area macro fails, select the ROI in the ROI Manager and run the "Nodes" macro
//When the code finishes, delete the originally selected ROI, leaving only the new adjustable ROI
macro "Nodes" {
	run("Set Scale...", "distance=0 known=0 unit=pixel");
	run("Fit Spline");
	run("Interpolate", "interval=20");
	roiManager("Add");
	}




//Creates adjustable nodes after a selection has been edited with the selection brush, which renders it a nonpolygon
//User must indicate the number of ROIs currently in the ROI Manager (1 or 2) to ensure proper execution
macro "Adjustable ROI" {
```

```
	input_num = getNumber("How many borders, 1 or 2?", 1);
	if (input_num == 1) {

	run("Set Scale...", "distance=0 known=0 unit=pixel");
	roiManager("Select", 0);
	run("Create Mask");
	run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
	roiManager("Select", 1);
	run("Fit Spline");
	run("Interpolate", "interval=20");
	roiManager("Update");
	close();

	roiManager("Select", 0);
	roiManager("Delete");
}

else {run("Set Scale...", "distance=0 known=0 unit=pixel");
	roiManager("Select", 0);
	run("Create Mask");
	run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
	roiManager("Select", 2);
	run("Fit Spline");
	run("Interpolate", "interval=20");
	roiManager("Update");
	close();
```

```
	roiManager("Select", 1);
	run("Create Mask");
	run("Analyze Particles...", "size=500000-Infinity circularity=0.00-1.00 show=[Overlay Outlines] exclude add");
	roiManager("Select", 3);
	run("Fit Spline");
	run("Interpolate", "interval=20");
	roiManager("Update");
	close();

	roiManager("Select", 0);
	roiManager("Delete");

	roiManager("Select", 0);
	roiManager("Delete");
}
}
```