



# Protocol for Tree Ring Marking via ImageJ

## 1. Drag the Stem Image into ImageJ

## 2. Set Measurements:

- Analyze / Set Measurements
- Select: Area, Perimeter

## 3. Use Drawing Tools

- Oval Selection 
- Draw an Oval (or circle) that is bigger than the stem disc.
- Right Click the “Oval Selection” icon, choose “Selectin Brush Tool” 
- Use the brush to shape the circle to Tree Ring Structure.

## 4. Load ROI Manager

- Navigate to: **Analyze / Tools / ROI Manager**
- Once the Area is selected, click “**Add**” or type “t”
- Rename the Object name by “**Rename...**”  
Rename them as Ring- “RING NUMBER” (From Bark to pith),  
For example, the 1<sup>st</sup> Tree ring as Ring-01; Bark (if marked) as Bark.
- In ROI Manager:  
“Show All” allows you to see all the regions we have created.  
“Labels” allows us to see the object ID.  
“Measure” generates the results (which we have set before).

## 5. For Outer Tree Ring Marking (Saved as TIF Image):

- Follow Section 3 & 4.1-4.3 to mark the outer-most tree ring (not the bark) and add it in ROI Manager (**ROI Manager / Add**).
- **Select the ROI Object** (outer-most tree ring) in the ROI Manager
- Navigate to: **Edit / Selection / Create Mask**
- Save the pop-up image (Black as Background/ White as Foreground) as a tiff object: **File / Save as / Tiff...**
- Name the file as “**04\_OuterRing\_Mask.tif**”
- Please ensure that the Mask image file is saved in the same folder as the targeted image file.

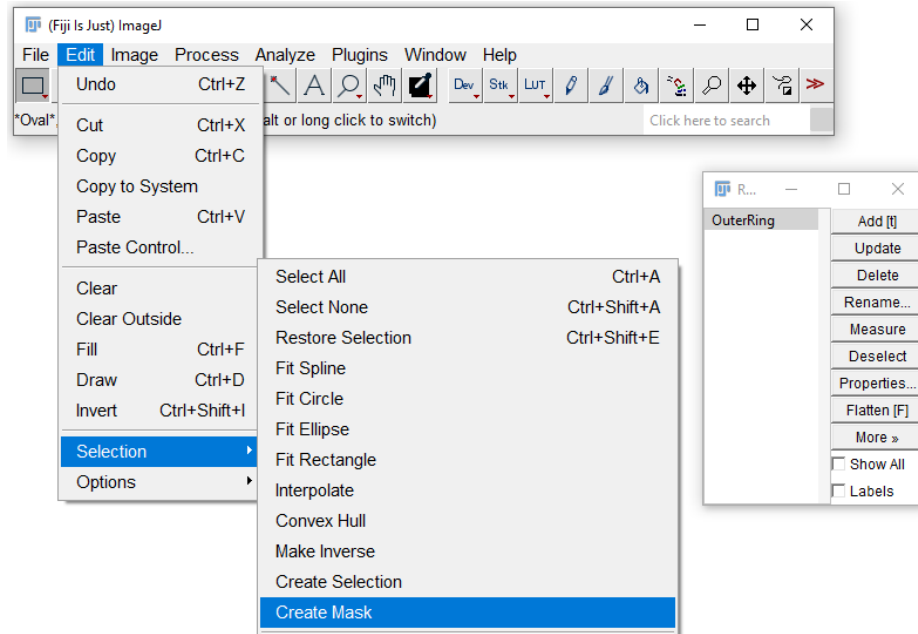


Fig.01 How to create an OuterRing Mask in ImageJ.

## 6. For Manual Tree Ring Marking (Saved as ROI Objects):

- Repeat Section 3 & 4.1-4.3 until all tree-rings are marked and added in ROI Manager.
- Once the 1<sup>st</sup> Tree ring is drawn and added into ROI Manager, you can always use this created ring as the start reference and use “Selectin Brush Tool” to modify this ring to the neighboring ones. Please note that, once the new tree ring is done, always click “Add” to add the new tree ring into ROI Manager. If the “Update” is not clicked, the one used for the reference start will not be influenced.
- You can always tick “Show all” to ensure the previous works are still there in the ROI Manager.
- If bark is marked for personal purposes, please name it as “Bark” to allow R scripts recognize it.
- **ROI Manager / More >> / Save**
- Name as **ROI-IMAGE NAME**, for example, ROI-La-01. The saved file will be a “.zip” file with all ROI objects that specifies each marked tree ring positions.