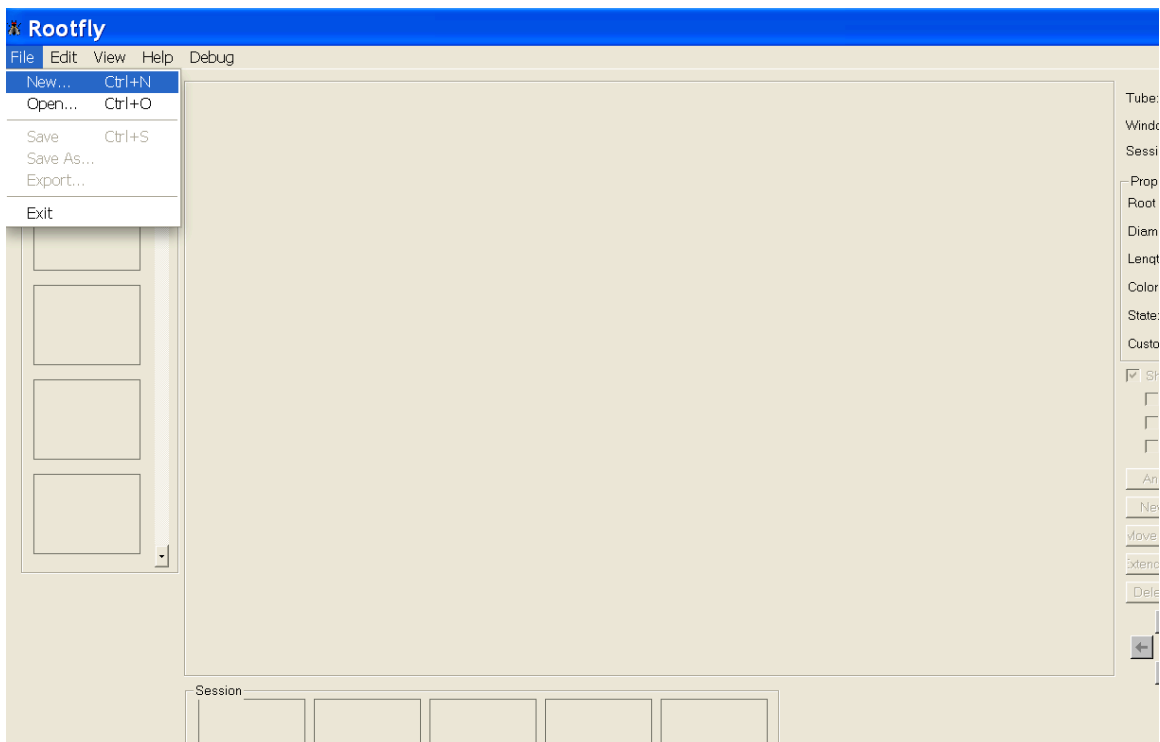


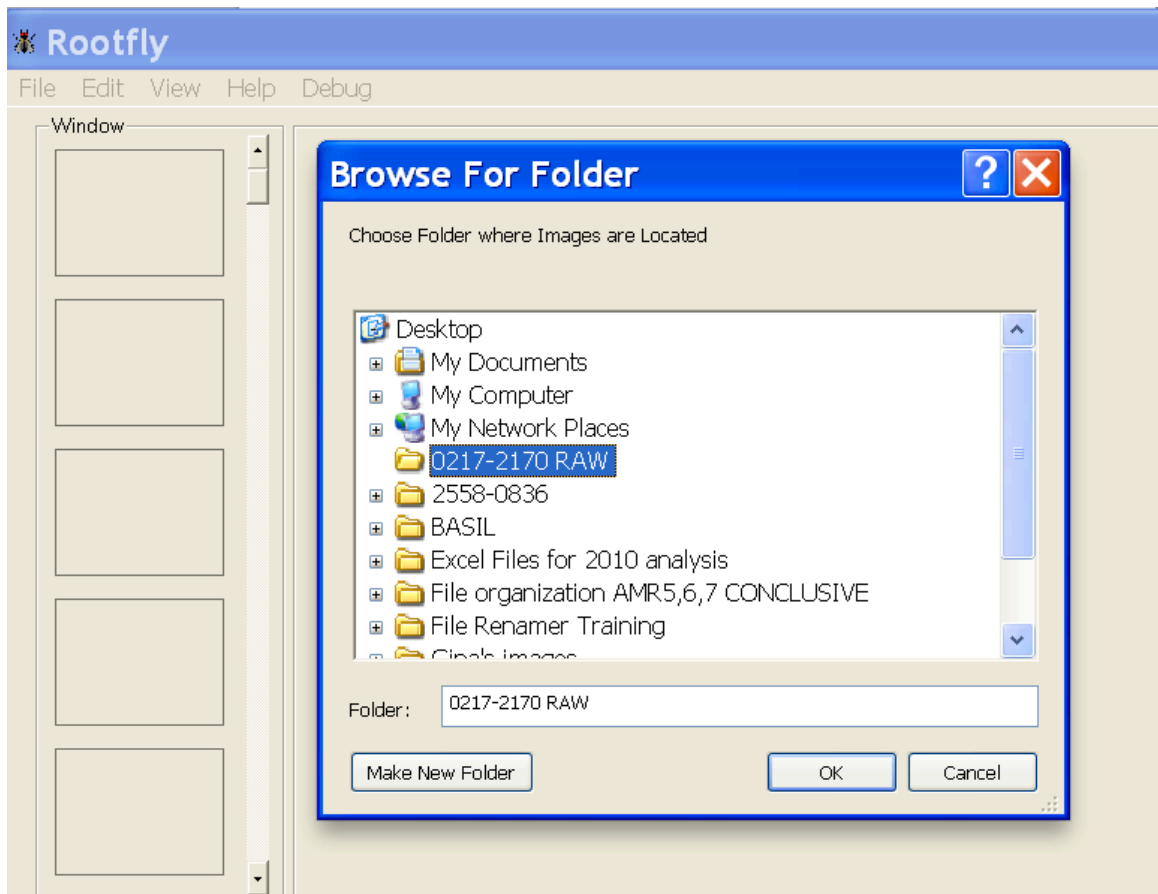
How to Set Up Images with Rootfly

By Renee Wang

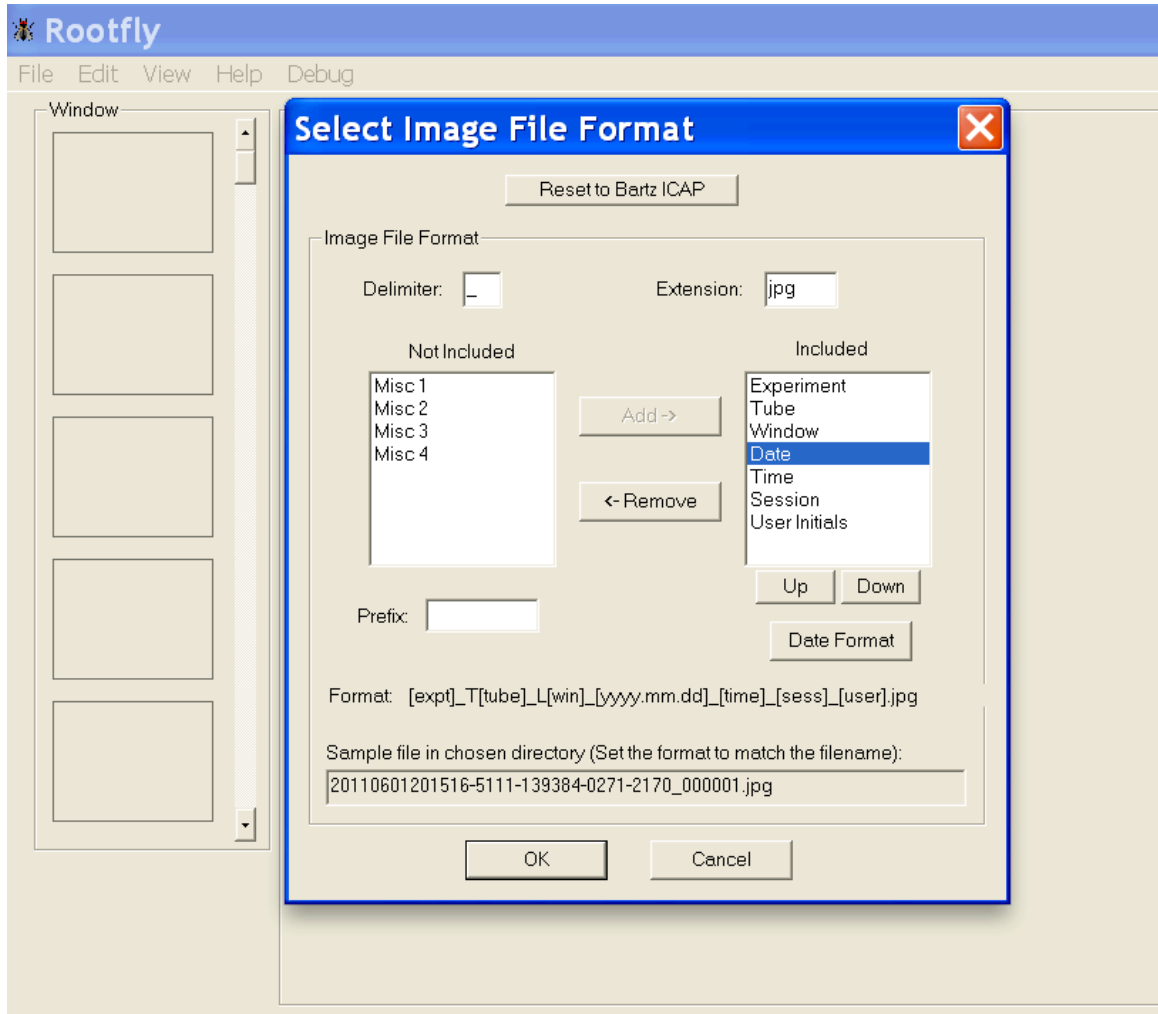
1. Open Rootfly on Windows XP
2. Click **File** then select **New**



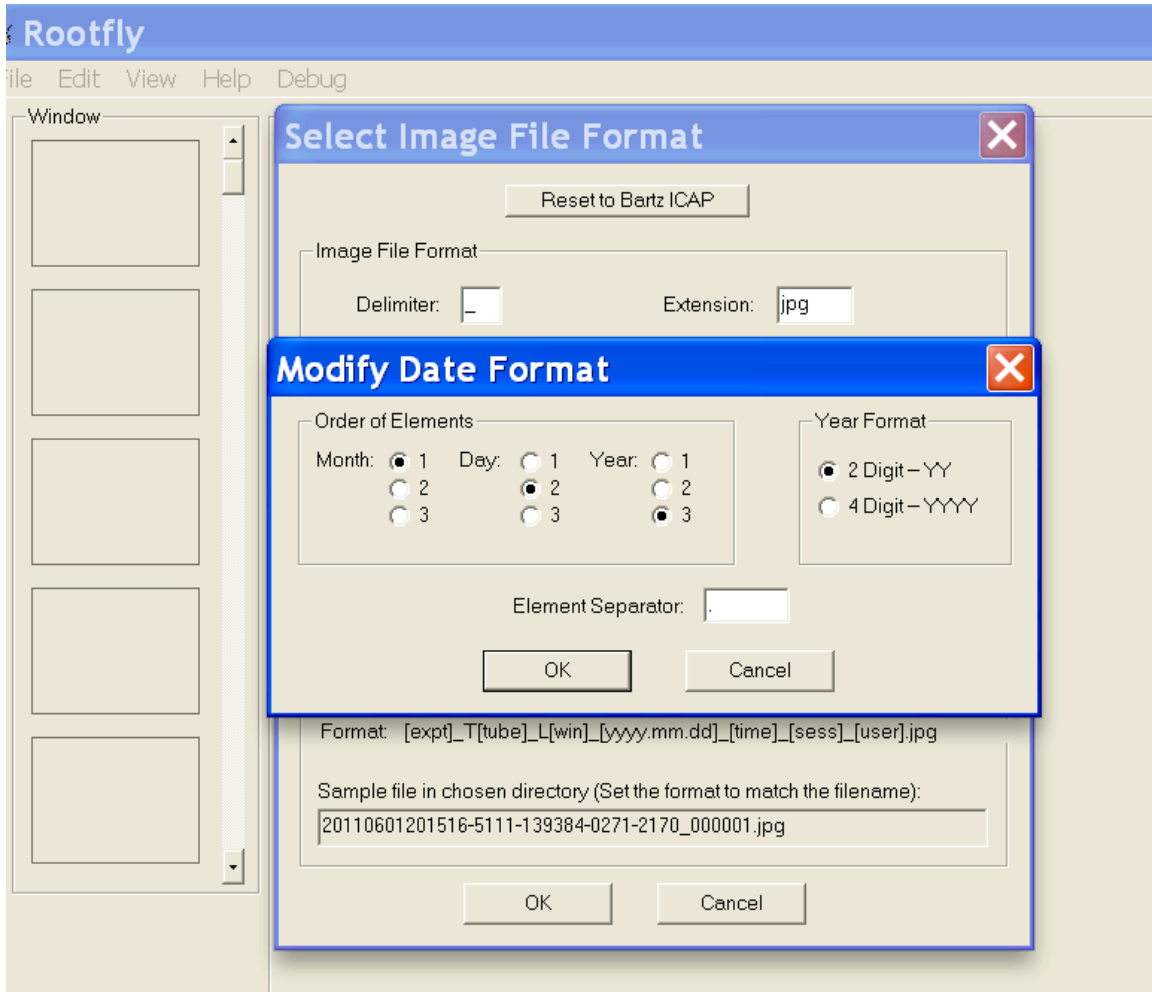
3. Select folder, a pop up window “**Select Image File Format**” will show up



4. Click on **“Date”** under **“Included”** box, then select **“Date Format”**

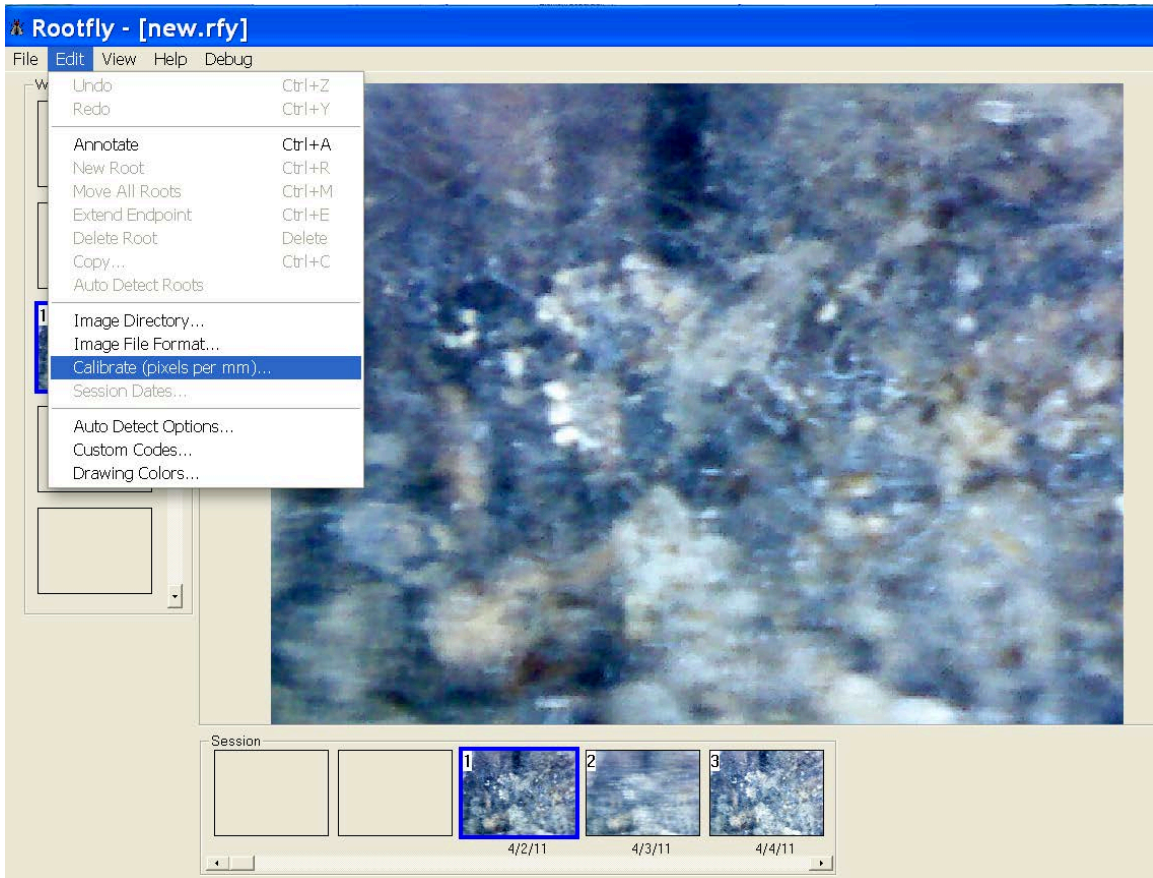


5. “Order of Elements” will be mm/dd/yy.
With “Year Format” in 2 digits. Then select “ok”
twice.



6. Go to the first image by click on it at the
bottom of the page.

7. Click on “**Edit**”, and select “**Calibrate image**” from the drop down menu.



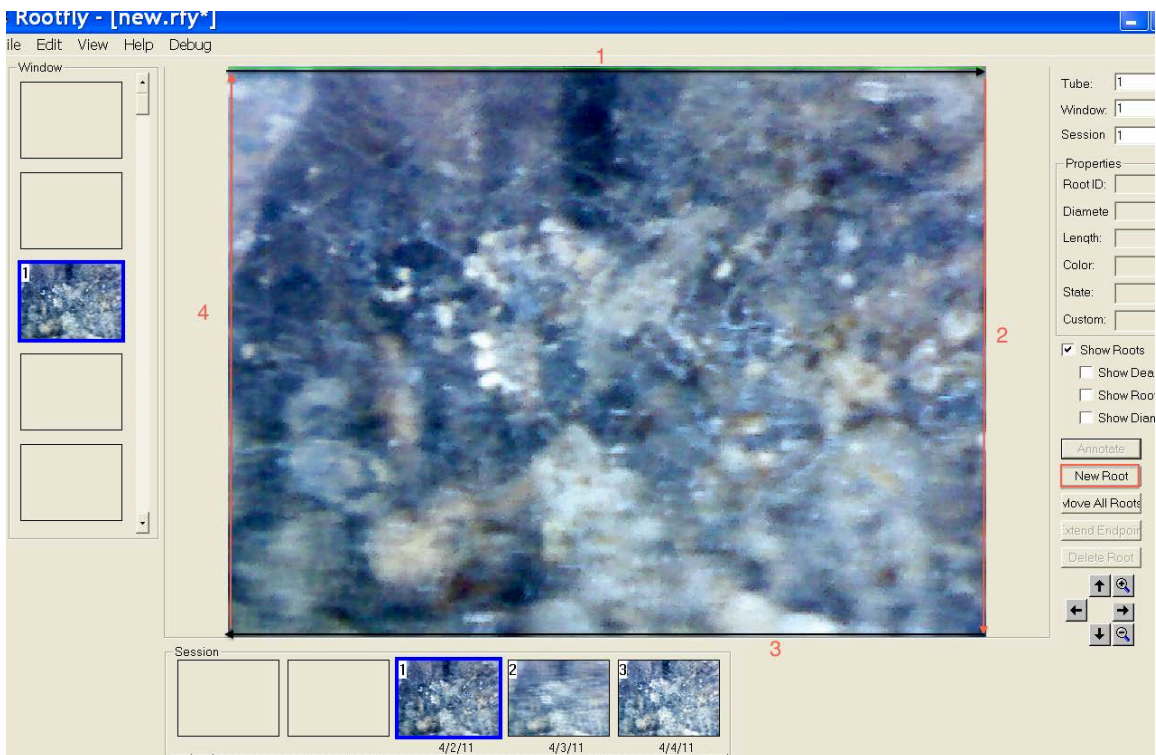
8. You folder will pop up and you can click open any image in that folder for calibration. Remember, you only need to calibrate the images **ONCE** at the beginning of the analysis!

9. Once you open an image for calibration, use the green cursor box and draw a horizontal line across the image. Make sure there is no breaks in the green line and it show be aligned with the borders on the right and left. Then, change the pixel number at the bottom to **30.1mm!!!!!!!!!! (VERY IMPORTANT)**. Once done calibrating, click “OK”.



10. Click on “**Annotate**” on the right, and begin digitizing. You need to annotate each image, as it will copy all the hyphae you traced from the previous image to the next.

11. To begin tracing, we need to click on “**New Root**” and we trace around the borders of the image to create a “Field-of-view”. We ONLY trace hyphae and roots activities inside the field-of-view. Make sure to click on “**New Root**” for EACH border. Also, lines should be drawn in order (see image below).



12. Now that you have the field-of-view, you make start tracing. Record the length and diameter on your sheet. Make sure your first root is #5 because root #1-4 are borders for field-of-view.



13. **DO NOT FORGET TO SAVE YOUR WORK!!** (Format for saved document should be File name_mm.dd.yy_initial)

01-25-2012