

CALCULATING IRREGULAR LEAF AREA FOR GAS EXCHANGE WITH THE LICOR6800:

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Background: In some instances you will want to measure some species that have leaves that don't completely fill the leaf chamber for the LICOR6800. The inability to fill the leaf chamber can lead to biases/errors in estimates of gas exchange. Although the area can be estimated under the "Constants" tab, it is still a good idea to measure the leaf area after measurements have been taken to ensure the most accurate data possible. For non-destructive measurements where leaves are to be kept on the plant after gas exchange measurement, leaf area can be calculated with the following procedure:

- 1) While a leaf is inside the LICOR6800, mark 4 points around the perimeter of the leaf chamber.
- 2) Remove the leaf from the chamber, then take a picture of the leaf on a contrasting background making sure to include some sort of scale.
 - a. Try to avoid shadows of leaf onto the background in the picture as this can lead to potential trouble when thresholding the leaf later in ImageJ
- 3) Import an image into ImageJ, but before doing so, make sure the "Concentric Circles" plugin is downloaded and installed.
- 4) Set the scale in your image.
- 5) After setting the scale in your image, calculate the number in of pixels equivalent to the area of the circle created by the perimeter of the outer gasket of the leaf chamber, and the inner edge of the leaf chamber gasket (i.e. a donut shape).
- 6) Open the select and open the Concentric Circles plugin. Enter "2" for the number of concentric circles you'd like to make representing the outer and inner edges of the leaf chamber gaskets, then enter the area for the inner and outer areas represented by the gasket.
 - a. If you may be able to determine the center of the circle created by the gasket, but I didn't bother learning how to do this
- 7) Select the circle tool and make a circle that perfectly overlaps with the circle representing the outer edge of the leaf chamber gasket. Perfect circles can be made by holding down the "shift" key while making the circle with the mouse/trackpad.
- 8) Move the circle that was just made using the circle tool to the region of the leaf that you marked with 4 points and align the perimeter of your circle to these points
- 9) Once the 4 marked points and the circle are aligned, go to Edit>Clear outside to remove the image outside of the circle.
- 10) Once clear, make a circle that perfectly overlaps the circle represented by the inner edge of the leaf chamber gasket, and move this circle so that it sits exactly within the current circular image (this may have to be "eye-balled").
- 11) Once the circle representing the inner edge is appropriately placed, clear the surrounding image by selecting Edit>Clear Outside.
- 12) With this remaining image, threshold the image and calculate leaf area following standard procedures.