## Setting the scale for scanner or camera-based imaging platforms

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By default, RhizoVision Explorer will report lengths, areas, and volumes in pixel units. To return physical units, the user must provide information about the physical size of a pixel in their image. In the case of flatbed scanning, this is equivalent to the DPI (dots per inch) the user selects to scan their sample. The DPI can be used directly in RhizoVision Explorer, which will internally convert and report units in millimeters. However, the pixel to millimeter conversion when using a camera depends on the sensor size, the lens, the focus, and the distance of the camera to the sample (the working distance).

Therefore, we recommend taking an image of an object of known size before an imaging session. Circular objects may be used, or if rectangular, the object should be 'straight' across rather than diagonal to get a good measurement. If using a backlit system like RhizoVision Crown, the silhouette can be used, or a clear ruler with markers can be used. We do not currently recommend keeping an object for scale in every photo, but rather to keep track of the metadata for an imaging session. If the scale object is desired, position it such that the sample can be cropped out using the same Region of Interest (ROI) during analysis. Since we recommend using the same pixel to millimeter conversion factor for all images, this means zoom and working distance should remain constant with use of a tripod or other physical setup (such as in RhizoVision Crown).

To determine the 'pixels to mm ' conversion, take a look at the screenshot below:


An ROI was drawn from 400 to 200 mm (red numbers at bottom). Use of ROI is described in the PDF manual included with the software download. To the right, in the ROI Manager, you can see the width of this box is 1568 pixels, which would be in pixels since no physical conversion was set. So now, we know 1568 pixels over 200 mm , thus pixels per mm would be 1568/200= 7.84 pixels per mm, which is what should be set here:

4 Image pre-processing
Analysis mode Broken roots
$\sqrt{V}$ Convert pixels to physical units
7.84 Pixels per mm

Image thresholding $\frac{\text { DPI }}{\text { Pixels per mm }}$

