## Measuring anuran snout-urostyle length (SUL) and digit length from digital images

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This method uses ImageJ<sup>™</sup> freeware available at <u>http://rsb.info.nih.gov/ij/</u>

Periodic measurement of snout-urostyle length (SUL) of frogs is necessary in captive populations to monitor growth and general health. Excessive handling can be stressful for frogs, and spread disease, and it is often difficult to obtain an accurate measure using callipers. It is possible to accurately measure frogs using digital images in conjunction with image analysis packages such as ImageJ<sup>TM</sup>, thus minimizing handling and stress to the individual. Note that the weight of the frog can be taken simultaneously by weighing the frog and the Petri dish together, and subtracting the weight of the Petri dish. (See <a href="http://zims.isis.org/aark/Research%20articles/Methods/Measuring%20caecilians.pdf">http://zims.isis.org/aark/Research%20articles/Methods/Measuring%20caecilians.pdf</a>).

Measuring SUL

 Mark a standard Petri dish with a bar of known length, eg. 40mm. This is used to scale the image in ImageJ<sup>™</sup>.





2. Place the frog in the Petri dish, replace the lid and hold against a white background. Take a digital image, ensuring the frog and the whole scale are visible. Make a note of the image number and which frog it correlates to (eg. tank number, individual number, code if tagged, etc.). NB. Often, dorsal patterning can be used to identify individuals as they grow.

- Once the image is downloaded to a computer, start up ImageJ<sup>™</sup> and open the image; File > Open
- 4. Select "straight line" from the icon bar. Draw a line along the scale bar. Choose Analyse > Set scale and enter the Known Distance (eg. 40) and the Unit of Length (eg. mm) and click "OK".





5. Again using the "straight line" tool, draw a line from the tip of the snout to the tip of the vent. Choose Analyse > Measure. The length of the line, and therefore the SUL, will appear in a "Results" window.

## Measuring digit length

This method can also be used to measure anura digit length, which can be used to investigate the effect of hormones on development through differences in length between males and females. By ensuring the feet are flat on the surface of the Petri dish, a digital image can be taken of the ventral side of the frog, making it possible to measure the length of the digits.

Digits are traditionally numbered with that nearest the body (the "thumb") as number 1, moving round to the outermost digit numbered 4 (forelimb) or 5 (hindlimb).





Again, the scale can be set using the same method as before (see above). The digits can then be measured by right clicking the "Straight line" tool in the tool bar and selecting "Segmented lines". This allows the straight line to bend round corners for more accurate measurement of digits. A line can then be drawn from the tip of the toe pad to the basal crease, and the length acquired by selecting Analyse > Measure.

The digits of larger frogs can be measured using the same method by holding the frog and pressing the foot flat against the Petri dish in line with the scale.

