# NSA SERVICES: IMAGE ANALYSIS

VOLUMETRIC CALCULATIONS

NSA offers multiple methods for calculating volumes depending on the researcher's budget and data endpoint needs. These techniques include the ellipsoid method and Simpson's Rule method.

The ellipsoid method can be implemented if the area of interest (AOI/ROI) is roughly ellipsoidal in shape. By taking a handful of measurements, a volume estimate can be calculated with ease.
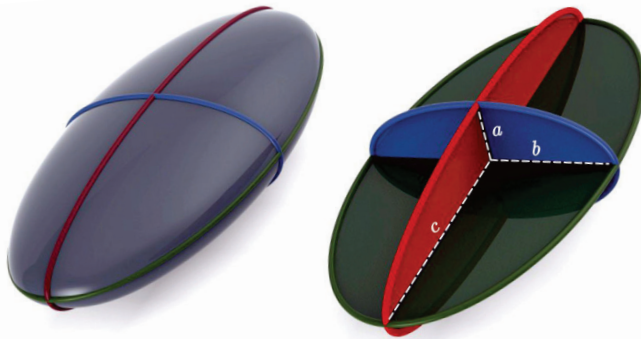
The following formula is used to calculate the volume of ellipsoid-shaped regions of interest:

$$V = {}^4\!/3\ \pi \ x \ {}^1\!/2(a + b + c)$$

Where

V = Volume
a = Height of AOI/ROI
b = Width of AOI/ROI
c = Length of AOI/ROI

The more exact measure of volume calls for using an application such as Image J/FIJI to measure the areas of interest. Eleven (or more but an odd number of) uniformly spaced measures must be made to comply with the application of Simpson's rule. More levels yields greater accuracy. Simpson's rule is a power series used to approximate definite integrals as well as areas under curves.
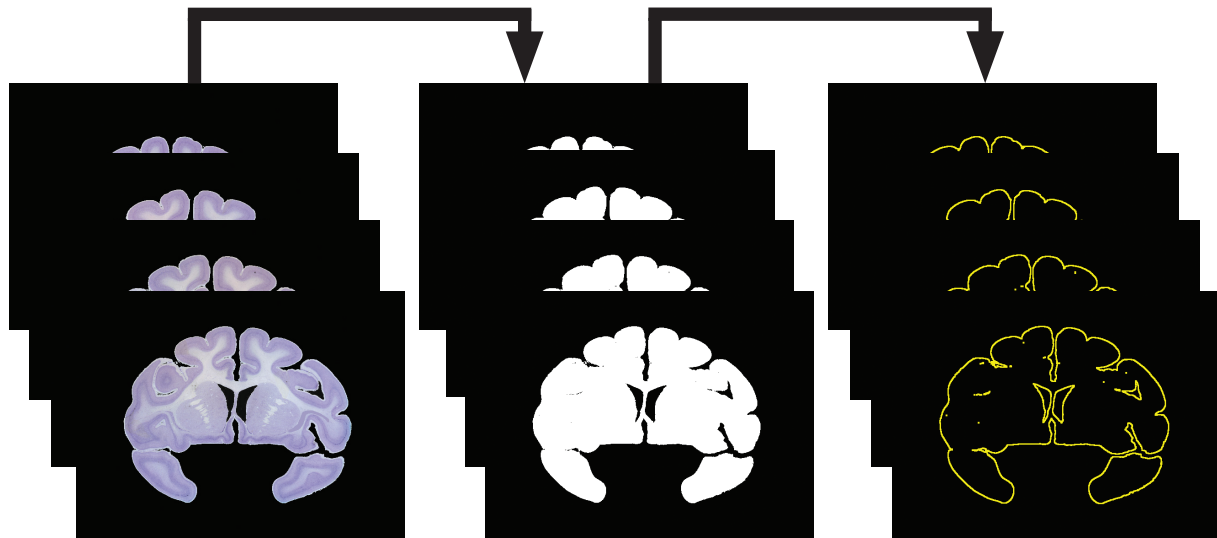
Original images are taken through a series of processing steps (abbreviated above) to allow for thresholding and area of interest (AOI/ROI) selection. Stained images are converted to binary (black and white) by applying an appropriate thresholding filter. A selection is made from these binary images (shown by the yellow outline in the third column of images). It should be noted that the AOI/ROI selection method is dependent upon the AOI/ROI and the staining of tissue.   Each analysis case presents unique challenges regarding tissue and staining, therefore different methods are implemented based on these factors. After AOI/ROI are defined, they are saved and measured (area measurements shown in the bottom right images).   These measurements are then processed using Simpson's rule for approximating definite integrals (formula shown below).

	Label	Area
1	Stack0001.tif:Level 1	715.36
2	Stack0001.tif:Level 2	820.34
3	Stack0001.tif:Level 3	885.92
4	Stack0003.tif:Level 4	880.59
5	Stack0004.tif:Level 5	922.19
6	Stack0001.tif:Level 6	958.18
7	Stack0006.tif:Level 7	1030.32
8	Stack0007.tif:Level 8	1074.40
9	Stack0008.tif:Level 9	1076.94
10	Stack0009.tif:Level 10	1143.04
11	Stack0010.tif:Level 11	1103.69
12	Stack0011.tif:Level 12	1157.26
13	Stack0012.tif:Level 13	1171.04
14	Stack0013.tif:Level 14	1192.71
15	Stack0014.tif:Level 15	1194.92
16	Stack0015.tif:Level 16	1165.95
17	Stack0016.tif:Level 17	1187.08
18	Stack0017.tif:Level 18	1212.10
19	Stack0018.tif:Level 19	1158.52

$$\int_a^b f(x)dx \approx (f(x_0) + 4f(x_1) + 2f(x_2) + 4f(x_3) + 2f(x_4) + \cdots$$

$$+2f(x_{n-2}) + 4f(x_{n-1}) + f(x_n))\frac{\Delta x}{3}$$