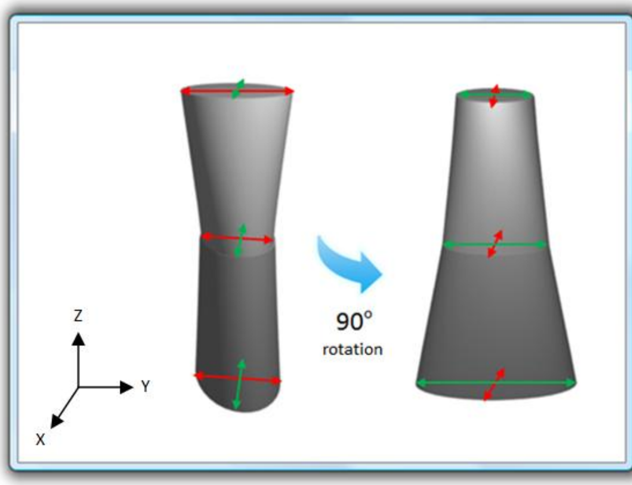
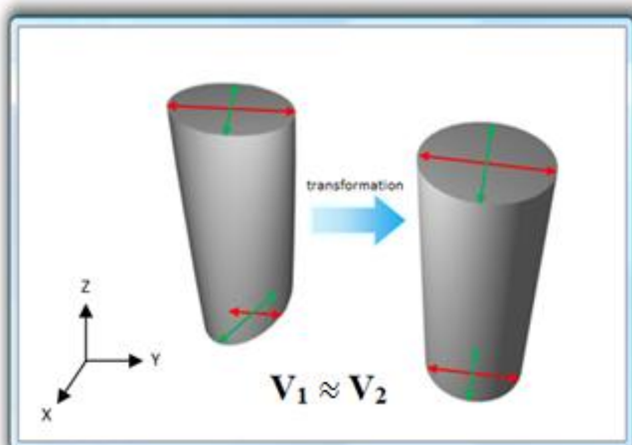
Supplement 1 – Segment Volume Calculations Methodology

What follows is our detailed methodology for performing the segment volume calculations, which are necessary for the neuron count calculations.

The bases of each segment can be approximated as ellipses with area $A = \pi(a/2)(b/2)$, defined by the transverse and sagittal diameters a and b. Note that the bases are not proportional to each other; for instance, the bottom of a segment can be longer or shorter in the transverse direction by a *different* factor than in the sagittal direction compared to the top of the segment; in other words, the dimensions of the top are not necessarily proportional to those of the bottom. Thus, even under this approximation, the segments are *not* simple truncated elliptic cones, but more complex solids. For example, consider the 3D rendering of two consecutive segments viewed from different perspectives in **Fig. 1a**.



a)



b)

Fig. 1. **a)** A geometrical approximation to spinal cord segments, based on *in vivo* measurements. Shown are two views of the same shape, rotated by 90° about the rostro-caudal *z*-axis. **b)** Transformation to regular truncated cones with circular bases of areas equivalent to the originals, to facilitate calculation.

The volumes of such geometrical shapes are difficult to calculate exactly (even with triple integration), so instead we use an isomorphic transformation to turn them into simpler solids whose volumes are much easier to determine. Consider a segment with diameters a_1, b_1 at the top and a_2, b_2 at the bottom. We can deform the shapes of the two elliptic bases however we wish; as long as we maintain the same areas, the volume will not change appreciably. Suppose we transform them both into circles, where R is the radius of the top circle and r is the radius of the bottom circle. Then the new, equivalent areas are $A_1 = \pi R^2$ and $A_2 = \pi r^2$, respectively. Therefore:

$$R = \sqrt{\left(\frac{a_1}{2}\right)\left(\frac{b_1}{2}\right)}, \qquad r = \sqrt{\left(\frac{a_2}{2}\right)\left(\frac{b_2}{2}\right)}.$$

The segment has now been transformed into a regular truncated cone; see **Fig. 1b**.

First, let us consider the scenario where $R > r$ **(Fig. 2a)**, meaning that the segment is larger at the top and smaller at the bottom. To find its volume, we need to know its length c and the distance d from the bottom of the cone to its imaginary apex if it was not truncated. Since the bases and the radii of a truncated cone are proportional, we establish the following proportions:

$$\frac{d}{r} = \frac{d + c}{R} \rightarrow d = \frac{c \cdot r}{R - r}.$$

The volume of the truncated cone is then the difference between the volume of the large cone (with height $c + d$) and the volume of the remaining cone (with height d):

$$V = \frac{\pi R^2(c + d)}{3} - \frac{\pi r^2 d}{3}.$$

Rewriting in terms of areas, we obtain:

$$V = \frac{1}{3}[A_1(c + d) - A_2 d] = \frac{1}{3}[A_1 c + (A_1 - A_2)d].$$

Plugging in for d and then for the radii, we have:

$$V = \frac{1}{3}\left[A_1 c + (A_1 - A_2)\left(\frac{c \cdot r}{R - r}\right)\right] = \frac{1}{3}\left\{A_1 c + (A_1 - A_2)\left[\frac{c\sqrt{\left(\frac{a_2}{2}\right)\left(\frac{b_2}{2}\right)}}{\sqrt{\left(\frac{a_1}{2}\right)\left(\frac{b_1}{2}\right)} - \sqrt{\left(\frac{a_2}{2}\right)\left(\frac{b_2}{2}\right)}}\right]\right\}.$$

Plugging in for A_1 and A_2, we can now write the volume in terms of only the original known dimensions of the segment, a_1, b_1, a_2, b_2, and c:

$$V = \frac{1}{3}\left\{\left[\pi\left(\frac{a_1}{2}\right)\left(\frac{b_1}{2}\right)\right]c + \left\{\left[\pi\left(\frac{a_1}{2}\right)\left(\frac{b_1}{2}\right)\right] - \left[\pi\left(\frac{a_2}{2}\right)\left(\frac{b_2}{2}\right)\right]\right\}\left[\frac{c\sqrt{\left(\frac{a_2}{2}\right)\left(\frac{b_2}{2}\right)}}{\sqrt{\left(\frac{a_1}{2}\right)\left(\frac{b_1}{2}\right)} - \sqrt{\left(\frac{a_2}{2}\right)\left(\frac{b_2}{2}\right)}}\right]\right\}.$$

For the other scenario, where $R < r$ **(Fig. 2b)**, we proceed in a similar fashion.

Based on the proportions, we find the formula for d:

$$\frac{d}{R} = \frac{d + c}{r} \rightarrow d = \frac{c \cdot R}{r - R}.$$

The volume is thus:

$$V = \frac{\pi}{3}[r^2(c + d) - R^2 d] = \frac{1}{3}[A_2 c + (A_2 - A_1)d].$$

Plugging in for d, then for r and R:

$$V = \frac{1}{3}\left[A_2 c + (A_2 - A_1)\left(\frac{c \cdot R}{r - R}\right)\right] = \frac{1}{3}\left\{A_2 c + (A_2 - A_1)\left[\frac{c\sqrt{\left(\frac{a_1}{2}\right)\left(\frac{b_1}{2}\right)}}{\sqrt{\left(\frac{a_2}{2}\right)\left(\frac{b_2}{2}\right)} - \sqrt{\left(\frac{a_1}{2}\right)\left(\frac{b_1}{2}\right)}}\right]\right\}$$

Plugging in for A_1 and A_2, the volume can then be written as:

$$V = \frac{1}{3}\left\{\left[\pi\left(\frac{a_2}{2}\right)\left(\frac{b_2}{2}\right)\right]c + \left\{\left[\pi\left(\frac{a_2}{2}\right)\left(\frac{b_2}{2}\right)\right] - \left[\pi\left(\frac{a_1}{2}\right)\left(\frac{b_1}{2}\right)\right]\right\}\left[\frac{c\sqrt{\left(\frac{a_1}{2}\right)\left(\frac{b_1}{2}\right)}}{\sqrt{\left(\frac{a_2}{2}\right)\left(\frac{b_2}{2}\right)} - \sqrt{\left(\frac{a_1}{2}\right)\left(\frac{b_1}{2}\right)}}\right]\right\}$$

Simplifying these algebraic expressions, we can rewrite them together as the following piecewise function:

$$V(a_1, b_1, a_2, b_2, c) = \begin{cases} \frac{\pi c}{12}\frac{\left[(a_1 b_1)^{3/2} - (a_2 b_2)^{3/2}\right]}{\left(\sqrt{a_1 b_1} - \sqrt{a_2 b_2}\right)}, & \text{if } R > r. \\ \frac{\pi c}{12}\frac{\left[(a_2 b_2)^{3/2} - (a_1 b_1)^{3/2}\right]}{\left(\sqrt{a_2 b_2} - \sqrt{a_1 b_1}\right)}, & \text{if } R < r. \end{cases}$$

Alternatively, we can write them as a single function:

$$V(a_i, b_i, a_j, b_j, c) = \frac{\pi c}{12} \frac{\left[(a_i b_i)^{3/2} - \ a_j b_j)^{3/2}\right]}{\sqrt{a_i b_i} - \sqrt{a_j b_j})},$$

where *i*,*j* = 1,2 if *R* > *r*, and 2,1 if *R* < *r*.

We disregard the theoretical case *R* = *r*, as the biological reality is that none of the segments can be equated to perfect cylinders.

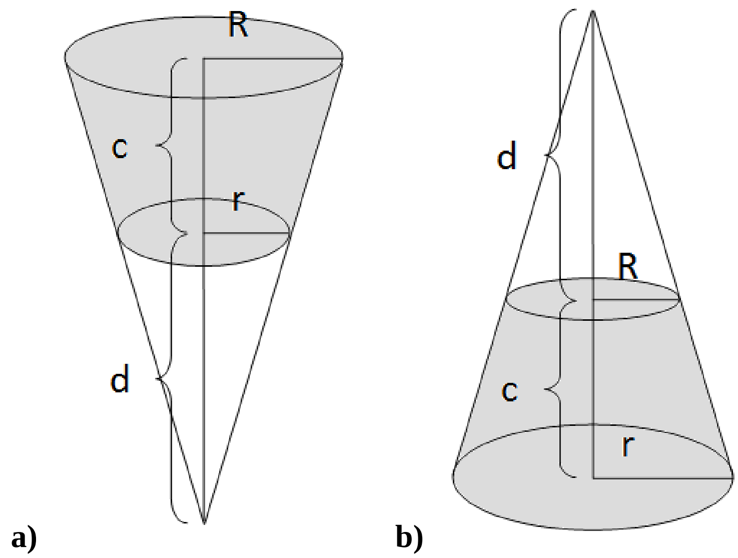


Fig. 2. a) The *R* > *r* case. **b)** The *R* < *r* case.

A less rigorous method would be to approximate each segment as an elliptical cylinder, with both bases defined by the proximal diameters. The actual difference between these two approaches is relatively small, but treating the segments as truncated cones ensures the most accurate volume estimates that are currently possible.