Supplemental Material for Venkatareddy et al, JASN 2013. Estimating podocyte number and density using a single histologic section.

Derivation of a quadratic equation relating apparent mean caliper diameter d, true mean caliper diameter D, section thickness T and nuclear shape k that can be adapted for spreadsheet calculations.

It is well known that the average number of nuclei in a finite thickness section is given by the expression

$$N = nA(T + D),$$

for a solid of arbitrary geometry (e.g. Hilliard, 1967) (36). To derive this expression, consider an arbitrary shaped object whose orientation is described by Euler angles (α, β, γ) in a box cross sectional area A and length L, aligned with the z-axis. (To remove box-edge effects we can formally identify the edges of the box to form a 3-torus.) Let the caliper diameter in the z direction be $D(\alpha, \beta, \gamma)$, which is a function of the object orientation. The probability that the object overlaps a rectangular section of the box thickness T and area A lying normal to the axis of the box is

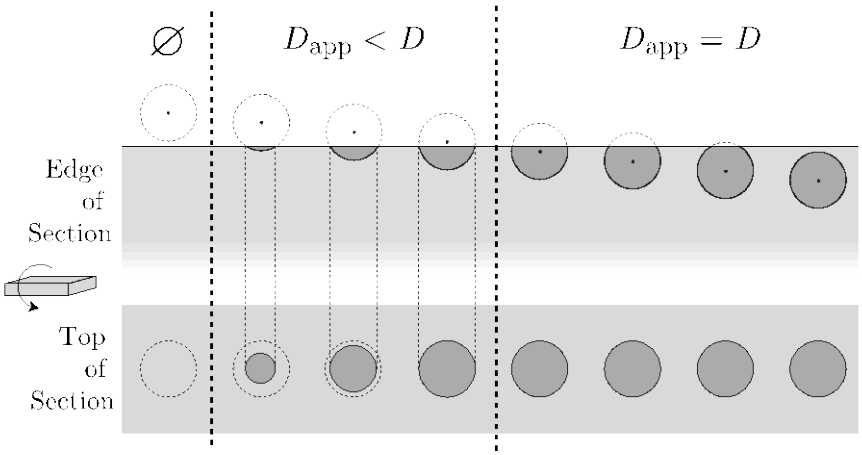
$$N = nA\big(T + D(\alpha, \beta, \gamma)\big).$$

If the object is randomly oriented, we must average over all orientations and we recover the equation written above.

Spherical nuclei. Let N_0 be the total number of podocyte nuclei whose geometrical centers lie within a section area A and thickness T and volume $V = A\,T$. The nuclei are assumed to be randomly distributed within the section and have number density

$$n = \frac{N}{V}.$$

Due to the finite thickness of the nucleus, some nuclei are themselves sectioned in the cutting process. First, we will assume that the nuclei are spherical with identical volumes. Nuclei whose geometrical centers lie within the section will have the true mean caliper diameter (D) whereas nuclei whose centers lie within a nuclei radius above or below the section boundaries are still observed, but are observed to have sections of smaller diameter than the true mean caliper diameter. (See Supplemental Figure 1).



Supplemental Figure 1: *Diagrammatic illustration for how spherical nuclei at the edge of the section whose centers lie above the section boundary will appear to have a smaller diameter than the true mean caliper diameter (D), while those whose centers lie within the tissue section will have true mean caliper diameters (D).*

It is straightforward to determine the mean observed diameter for this sub-population that are in this boundary region:

$$D_b \equiv \frac{2}{R}\int_0^R dz\,\sqrt{R^2 - z^2},$$

where R is the radius of the spherical nuclei ($2R = D$). Integration gives the expression:

$$D_b = \frac{\pi}{4}D = k\,D,$$

where we have introduced the geometrical parameter $k = \pi/4$. The average summed caliper diameter for all nuclei observed in a section includes both nuclei with centers lying within the volume of the section (numbering $N_0 = n\,A\,T$) and nuclei in the boundary regions (numbering $N_b = n\,A\,D$):

$$\langle \sum_i D_i \rangle = N_b D_b + N_0 D = nA(kD^2 + TD).$$

If we divide this sum by the number of observed nuclei, we compute the apparent mean caliper diameter (d), and

$$d = \frac{DN_0 + D_b N_b}{N_0 + N_b}.$$

Eliminating the Ns in terms of the number density and dividing out common factors of number density and area can simplify this expression to:

$$d = \frac{DT + kD^2}{T + D},$$

which is independent of the area and number density. This equation can be rearranged to give a quadratic equation D, the true mean caliper diameter:

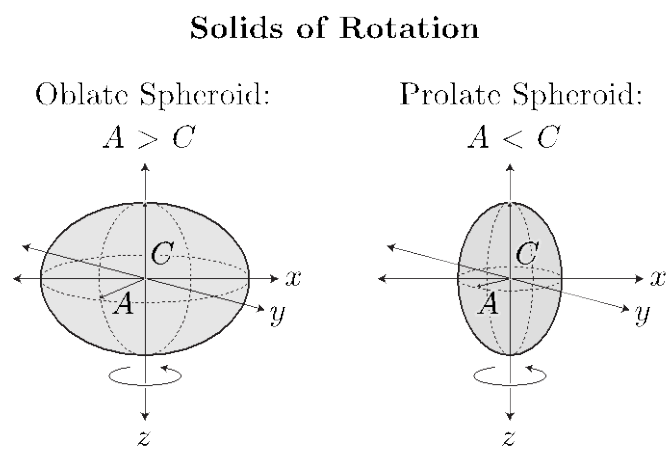
$$0 = kD^2 + (T - d)D - dT.$$

The observed mean caliper diameter (d) is smaller than the true mean caliper diameter (D) due to the presence of the nuclei lying in the boundary region. This equation can be solved for the true diameter D:

$$D = \frac{d - T + \sqrt{(d-T)^2 + 4kdT}}{2k},$$

using the quadratic formula, keeping only the positive root.

Ellipsoidal Nuclei. Next, we generalize the derivation to a more general case of ellipsoidal nuclei. We consider the quite realistic geometries of oblate and prolate spheroids: ellipsoids with equatorial lengths (A, B, C) where $A = B$. For a prolate spheroid, $C > A$ whereas an oblate spheroid has $C < A$. An equivalent sphere is defined as a sphere with a volume equal to that of the ellipsoid.



Supplemental Figure 2: *Oblate and prolate spheroids are defined as solids of rotation generated from ellipses. For a prolate spheroid A<C whereas an oblate spheroid has A>C.*

Ellipsoids have volume:

$$V = \frac{4\pi}{3} ABC.$$

The radius of an equivalent sphere is therefore:

$$A_{sphere} = \sqrt[3]{ABC}.$$

For sections thicker than maximum diameter of the nuclei, the average sum of the caliper diameters of all observed nuclei can still be written

$$\langle \sum_i D_i \rangle = nA(kD^2 + TD),$$

where geometrical factor k is a dimensionless constant that depends on the shape of the solid, but not its physical size. This form is simply the result of dimensional analysis and the observation that this sum has both linear and constant terms in T, the thickness of the section. (Note that the form is more complicated for T smaller than the largest caliper diameter of the nuclei.) For an oblate or prolate spheroid, k depends on the ratio A/C or equivalently A/A_{sphere}.

To compute k explicitly, we carried out a simulation to compute the average sum of the caliper diameter. In short: We generated a series of ellipsoids with $A = B$, $ABC = 1$, varying A, centered at the origin. We approximated the boundary of the ellipse with roughly 1000 points. To average over orientations, we performed a Monte Carlo integration with $N = 10{,}000$ samples, rotating the ellipse to random orientations. We then scanned a segment-boundary plane along the z-axis and measured the sum of the caliper diameters of the resulting segmented ellipses. Using the equation quoted above, we varied the thickness of the section (T) to determine D, and then computed the constant offset (with respect to T) to determine k. The computed values of k are shown in Supplemental Figure 3, Panel A and Supplemental Table 1. As a check, we both (i) recover the known value of k for the sphere and (ii) the known values of the true mean caliper diameter D for which there is an analytic expression (Supplemental Figure 3 Panel B) (36):

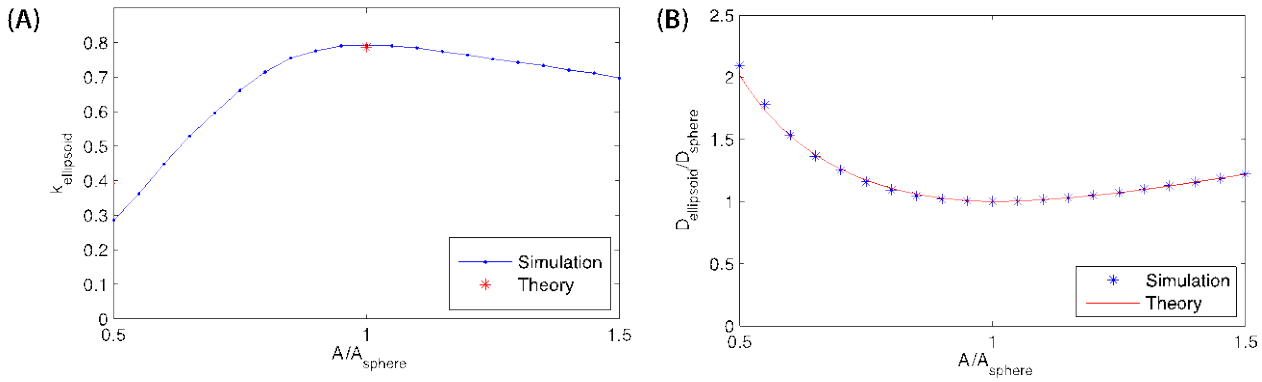
$$\frac{D_{ellipsoid}}{D_{sphere}} = \begin{cases} \alpha^{-2} + \frac{\alpha}{\varepsilon}\sin^{-1}\varepsilon, & \alpha > 1\ (oblate) \\ 1, & \alpha = 1\ (spherical) \\ \alpha + \frac{1}{\alpha^2\varepsilon}\sinh^{-1}\varepsilon, & \alpha < 1\ (prolate) \end{cases}$$

where we have used eccentricity of the ellipse (ε) for which the spheroid is surface of revolution:

$$\varepsilon \equiv \begin{cases} \sqrt{1-\alpha^{-6}}, & \alpha > 1\ (oblate) \\ 1, & \alpha = 1\ (spherical) \\ \sqrt{1-\alpha^{6}}, & \alpha < 1\ (prolate) \end{cases}$$

(note that we have corrected a sign error in this definition from Hilliard, 1967) and defined α as the ratio of the spheroid axis A to the radius of the equivalent sphere A_{sphere}:

$$\alpha \equiv \frac{A}{A_{sphere}}.$$



Supplemental Figure 3: Panel A: *Geometical factor k as a function of ellipsoid shape. The ratio of the equitorial axis A to the radius of the equivalent sphere for an ellipse with axes (A, A, C). Ellipsoids with $A < A_{sphere}$, are prolate spheroids whereas ellipsoids with $A > A_{sphere}$ are oblate spheroids. Geometrical factor k is maximized for the sphere, where $k = \frac{\pi}{4}$ (red star). The dependence of k on geometry is not particularly strong meaning that small changes in the shape of the nuclei will not have a strong effect on the estimate of the true mean caliper diameter D.* ***Panel B:*** *Mean caliper diameter in simulations compared with analytic results. There is an excellent match between the simulated mean caliper diameter (blue) and the theoretical expression (red).*

Supplemental Table 1: Computed values of geometrical factor k for various spheroid geometries.

Spheroid Geometry Parameter $\alpha \equiv \frac{A}{A_{sphere}}$	**Geometrical factor** k
0.50	0.28
0.55	0.36
0.60	0.45
0.65	0.53
0.70	0.60
0.75	0.66
0.80	0.72
0.85	0.75
0.90	0.78
0.95	0.79
1.00	0.79
1.05	0.79
1.10	0.78
1.15	0.77
1.20	0.76
1.25	0.75
1.30	0.74
1.35	0.73
1.40	0.72
1.45	0.71
1.50	0.70