**Quantification of lysosome’s proximity to nucleus and periphery of the cell**

In order to quantify the locations of the lysosomes with respect to the nucleus border and the periphery of the cell, we introduce a single dimensionless measure as follows (**Figure S19**). Where cell’s periphery extends past the edge of the image, then edge of the image is viewed as the edge of the cell. First, periphery of the cell and the outer border of the nucleus are manually marked on the DIC image of the cell, as shown by orange and blue curves on the **Figure S19a**. Geometric center of mass of the nucleus is then found (yellow point on **Figure S19a**), taking uniform density within the nucleus border. To find perinuclearity measure for a given point , ray is cast from the nucleus center . We then find the point where ray intersects the nucleus border, as well as the point where ray intersects the cell’s periphery. If more than one intersection of with periphery is found, the intersection point that happens to be closer to the nucleus is used as point In practice, the nucleus border is always sufficiently convex and smooth to produce only one intersection of nucleus border with any possible ray . After the intersection points are found, point T itself may happen to be either on the interval OA or on the interval OB. Measure is defined such that interval OA is mapped linearly onto the interval of the values of , and interval AB is linearly mapped onto the interval :

if

if

This maps the entire area of the cell onto the range of possible values of : points outside the nucleus are mapped onto such that at the nucleus’s border and at the peripheral membrane; points inside the nucleus’s area are mapped onto the interval such that at the nucleus center.

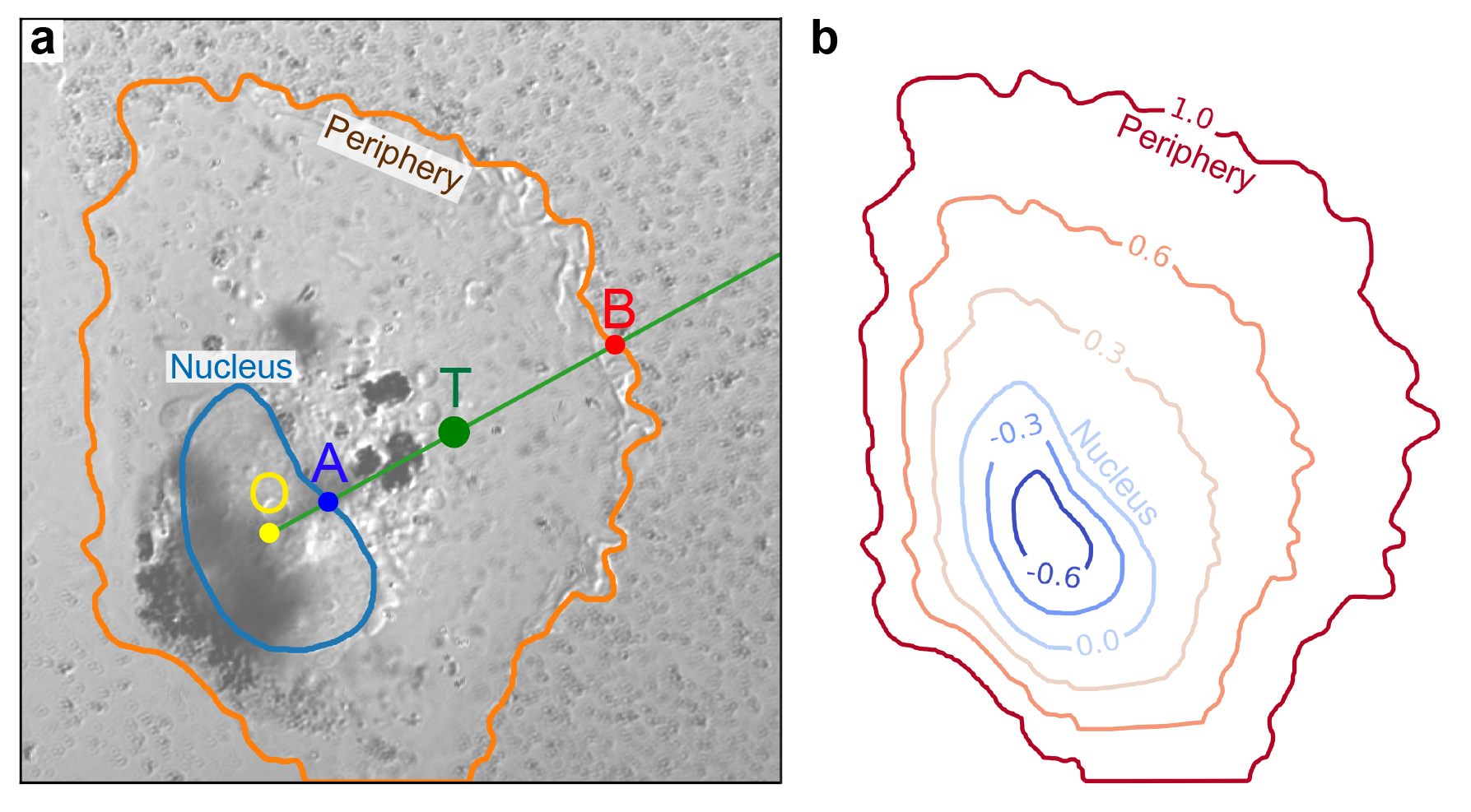
This mapping from the two-dimensional space of the image to the one-dimensional space of values is then applied to the fluorescence image of the labelled lysosomes as follows. First, the background level is subtracted from the fluorescence image such that signal is zero wherever no lysosomes are present. Then, for every pixel having non-zero fluorescent intensity at coordinates , measure was computed as described above. For a given single cell, this produced a distribution of over the values of . This resulting distribution was then normalized and plotted in the form of histograms or violin plots.

Note that the normalized distribution of over dimensionless measure is invariant with respect to any affine transformations of the cell (including translation, rotation, scale and shear), is not affected by the absolute magnitude of the fluorescence intensity , and does not rely on localization of individual lysosomes. For the latter reason, the measure does not suffer from any artefacts of localization algorithms, since no such algorithms are employed.

For the purposes of this research project, only the values were considered in subsequent analysis. Values were ignored, as these corresponded to lysosomes located above or below the nucleus in the direction normal to the sample plane, and including these lysosomes into the analysis would have been ambiguous.

Distributions obtained for individual cells were combined into a single distribution for the entire cell line in question, and the resulting violin plots are shown in Figure SXX [THE FIGURE YOU PUT THE VIOLIN PLOTS INTO].

Our Python code for computing the perinuclearity measure as defined above and producing plots in Figure SXX [THE FIGURE YOU PUT THE VIOLIN PLOTS INTO along with the input raw data can be downloaded from <https://github.com/yaroslavsobolev/perinuclearity>

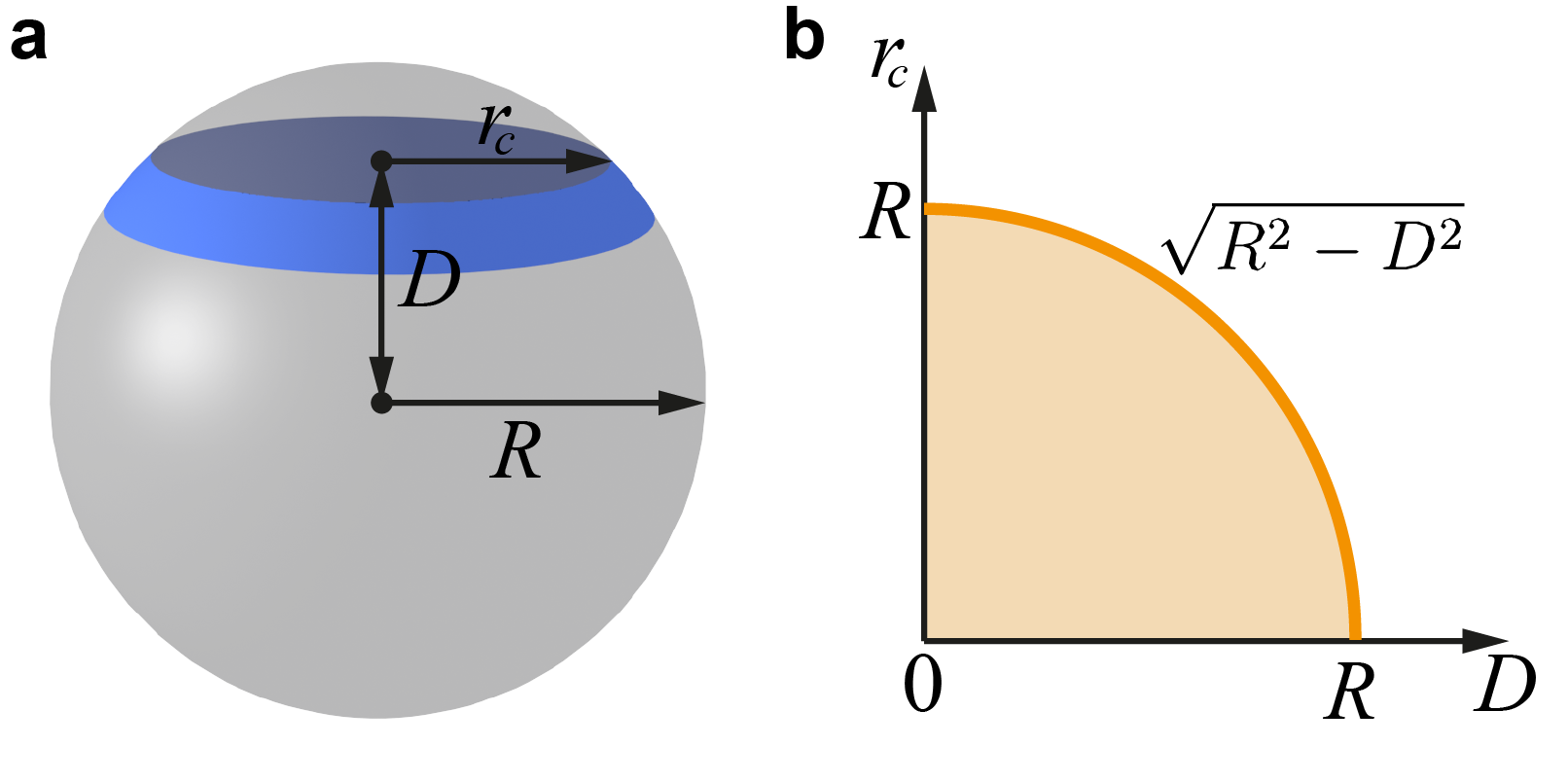


**Figure S19**. **Illustration to the definition of dimensionless measure of lysosome locations with respect to the nucleus and the periphery of the cell**. **a**, DIC image of the cell with marked nucleus border (blue) and cell periphery (orange). For a given point , ray (green) is cast from the nucleus center of mass (yellow) and intersects nucleus border at point and cell periphery – at point . Real-valued perinuclearity measure for point is then defined such that the interval maps onto range [-1,0] of , and interval maps onto interval [0, 1] of . **b**, Isolines of different levels of (indicated at contours) are computed for the cell image from **a**. at the cell periphery, at the nucleus border, and at the nucleus center.

**Estimation of the number of nanoparticles per lysosome from TEM images**

To estimate the number of nanoparticles per single lysosome, we used TEM images of 80-nm-thick microtome slices. First, the volume fraction of NPs inside a lysosome within a microtome slice were estimated using a procedure described in Note 2 and **Figure S14**.

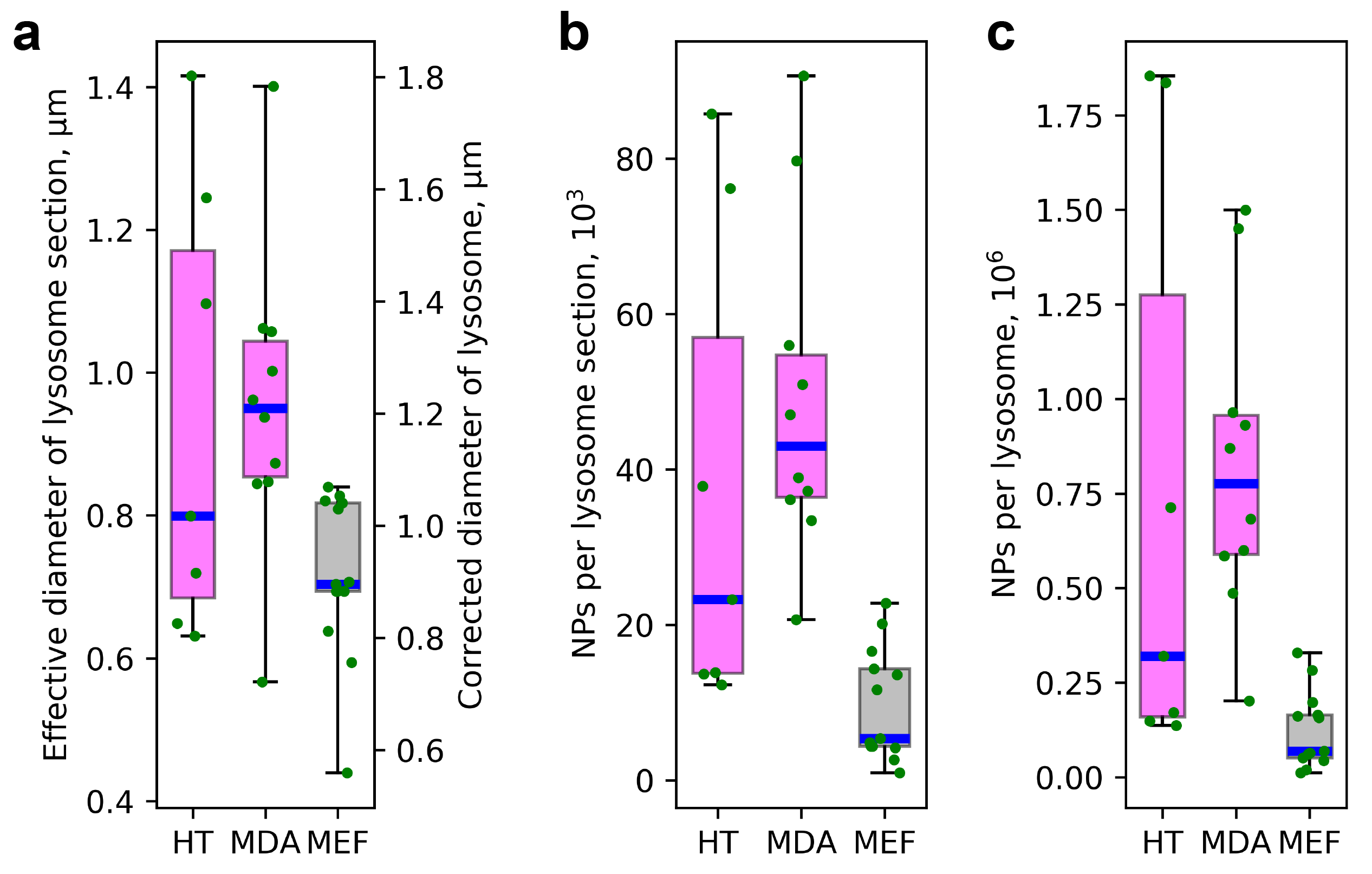
Next, the volume of the lysosome in question must be estimated. This is done by assuming the lysosome to be spherical, and taking into account the fact that the radius of random cross-section of a sphere (in our case – the microtome slice) is always smaller than the radius of that sphere (**Figure S20a**). More precisely, the average value is equal to . This can be derived by observing that the distance from random cross-section plane to the sphere center is uniformly distributed between and , while at a given is . Thus, the average , since the integral is equal to the area of one fourth of a circle, the area under the graph in **Figure S20b**.



**Figure S20. Derivation of the geometric correction factor for the effective radius of lysosome**.

Presence of this geometric correction also means that the effective apparent radius of lysosome’s microtome section, as computed from lysosome’s apparent area in TEM image, will differ by a factor from the effective radius computed using confocal fluorescence microscopy, because axial size of confocal collection volume is similar to typical lysosome diameters (around , **Figure S21a**), while microtome slice (80 nm) is significantly thinner than that.

Thus, if lysosome’s apparent radius from TEM image is , then unbiased estimate of this lysosome’s radius is , and its volume is . Volume fraction of NPs calculated within the imaged microtome slice (**Figure S14**) is assumed to be representative of the NP volume fraction in the entire lysosome’s volume. Therefore, total number of NPs in a given lysosome can be estimated as , where is the volume of a single NP calculated from NP radius . **Figure 21c** shows the numbers of NPs calculated this way for the HT, MDA, and MEF cell lines conditioned for 24 hours with 80:20 mix-charge NPs [PLEASE WRITE IT IN SOME MORE BIO-COMPATIBLE WAY THAN I DID] (these are the same boxplots as in Figure XXX [WHEREVER MAGDA HAVE PUT THESE BOXPLOTS]) in comparison to numbers of NPs within the microtome section only (**Figure 21b**).



**Figure S21. Estimation of the number of nanoparticles inside a single lysosome**. In all panels, marks on the horizontal axis correspond to cell lines conditioned for 24 hours with 8020 NPs[PLEASE WRITE IT IN SOME MORE BIO-COMPATIBLE WAY THAN I DID]. Boxplot elements are: dataset minima and maxima (whiskers), 25% and 75% percentiles (box edges), and medians (blue lines). Green dots are overlaid data points. **a**, Lysosome’s effective diameter (i.e. diameter of a circle that has the same area as the given lysosome) within its microtome cross-section imaged by TEM (left axis). Right axis differs from the left one by the factor (see text) and corresponds to estimated true diameter of lysosome. **b**, Number of nanoparticles within the lysosome’s microtome section (cf. **Figure S14**). **c**, Estimated number of nanoparticles inside the whole lysosome (same data was used in Figure XXX [WHEREVER MAGDA HAVE PUT THESE BOXPLOTS])