The proportionator: a new sampling tool in stereology

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The viewing, sampling and estimation process of cells and organelles can be improved by introducing automatic and computerized image analysis into existing uniform and non-uniform stereology procedures. In most commonly available computer aided stereology sampling systems, the computer performs the splitting of the images into uniformly sampled units (fields of view), displays the sampled fields of view on the screen, and saves the counts.

Here we present powerful and unbiased methods for uniform and non-uniform sampling of cells and organelles. These methods are strictly unbiased, improve precision, reduce workload and utilize the computer image analysis and computational capabilities. The benefit is not only visible in sparse or non-homogeneous distributions, but also in homogenous distributions. The Horvitz-Thompson formula for providing an unbiased number estimate requires known sampling probability and an unbiased count in each and every sampled unit. The estimate of each unit is the unbiased count divided by the sampling probability of that unit. The common systematic uniform random sampling (SURS) method, which has been commonly used in the past years, is based on uniform probability. The fields of view have a uniform probability to be chosen, which is inversely proportional to the sampling period. The precision of the described method can be improved by integrating the smooth protocol. The smoothing protocol is based on reordering the fields of view according to an arbitrary associated variable before SURS. The associated variable should have a potential positive correlation between its value in each field of view and the count in the unit. The computer, in low magnification, acquires the value of the associated variable automatically. When looking for blue stained cells, the associate variable for a field of view may be the number of blue pixels in that field of view. Applying the smooth protocol before doing SURS increase precision by a factor of 2 to 3 (evaluated by computer simulation).

The proportionator [1, 2], which is a combination of the associate variable and probability proportional to size sampling, is even more beneficial. Accumulating the values of the associate variables over the whole population of fields of view followed by SURS on the accumulated values gives a benefit of approximately 12 times compared to the commonly used SURS (evaluated by computer simulation). The proportionator gives a (known) non-uniform sampling probability to every field of view (SURS period divided by the associate variable value). Using Horvitz-Thompson the estimate is strictly unbiased, regardless of the associate variable value and the biased image analysis. An additional benefit of the proportionator is the ability to provide a direct and unbiased estimate of the coefficient of error of the estimate, without any extra user workload.

The proportionator was tested on three biological examples, estimating total number of granule cells in rat cerebellum, estimating total number of green fluorescence protein orexin neurons in transgenic mice brain, and estimating area fraction of insulin β islet cells in

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dog pancreas. The combined efficiency of time and precision has been increased 8 to 27 times with the proportionator when compared to the traditional SURS.

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