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The Art of Counting

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Fifteen years have passed since the publication of an unusual article that was to change the science and methods of stereology forever. The article was titled, "The Unbiased Estimation of Number and Size of Arbitrary Particles Using the Disector." It was published in the *Journal of Microscopy*, one of the leading journals in the field of biologic application of stereology (1). It was written by a certain D. C. Sterio, a name that later turned out to be a nom de plume for a now well known stereologist. The article described a new stereologic method or counting tool called the "disector" that could be used to obtain unbiased estimates of the number of objects in a tissue. Since this seminal publication by Sterio in 1984, a relatively small group of investigators has developed and described a large number of new stereologic tools that have revolutionized the world of stereology. These "new" stereologic methods are the focus of the review article by Jens Nyengaard in this issue of the *Journal of the American Society of Nephrology* (2).

A considerable number of manuscripts submitted to the Journal include quantitative morphologic data based on counts and measurements of profiles observed in tissue sections or projected images. Quite often these so-called morphometric analyses are based on assumptions and approximations that cannot be verified and therefore may be incorrect. Moreover, many manuscripts have insufficient descriptions of the sampling procedures and statistical analyses in the Methods section, or it is apparent that inappropriate (biased) sampling techniques were used. Because of the availability today of many new and some old stereologic methods and tools that are not based on undeterminable assumptions about size, shape, or orientation of structures, the Editors of the Journal believe that it is time to dispense with the old, often biased, model-based stereology and change the way we count and measure.

The article by Jens Nyengaard in this issue of the Journal describes the application of stereologic methods in the biologic sciences with particular emphasis on kidney research. The review focuses on the newer stereologic methods that are free of assumptions regarding size and shape, but also includes a discussion of important older design-based methods.

Stereologic methods are used to obtain unbiased quantitative estimates of structures from profiles in tissue sections or projections of sections. Because these methods are based on distinct mathematical and statistical principles, careful experimental design and the use of unbiased sampling procedures are essential. Therefore, one section of the article is devoted to the discussion of proper sampling techniques. Another section discusses the new unbiased counting methods and describes the use of the disector to obtain unbiased estimates of numbers of structures from real or optical sections. Because of the importance of obtaining total numbers, volumes, or surface areas as opposed to densities of structures, a special section is devoted to the description of the Cavalieri principle and its use for the estimation of total volume, which can subsequently be used to calculate absolute values from estimated densities. Finally, a special section is devoted to the estimation of error variance with the purpose of identifying the sampling step with the greatest variance. Analysis of error variances should enable the investigator to optimize sampling efficiency and thus, "do more less well," which would ultimately also result in greater cost effectiveness.

With the review article by Jens Nyengaard in this issue, the Editors of the *Journal of the American Society of Nephrology* introduce a new policy that will potentially affect all **quantitative** morphologic studies submitted to the Journal. Beginning with this issue, we request that appropriate stereologic methods be used to quantify structures on tissue sections in all manuscripts submitted to the Journal. In particular, precise descriptions of sampling procedures and statistical analyses will be required. Authors will be expected to be familiar with the new stereologic tools, and unbiased counting rules should be used whenever possible. An example of the use of appropriate stereologic methods, including some of the newer tools such as the disector, can be found in the article by Cobo *et al.* published in this issue of the Journal (3).

To establish a uniform standard in the Journal, we will attempt to have all papers containing quantitative morphology reviewed by a referee with expertise in stereology. In situations in which it may be difficult or impossible to use unbiased sampling and counting methods, the authors will be requested to provide adequate justification and demonstrate that they are aware of the potential problems and limitations associated with the use of biased sampling and counting procedures.

We would like to point out that other journals, particularly in the field of neuroscience, have established similar policies that emphasize the importance of using unbiased counting methods. It is time to do the same in nephrology.

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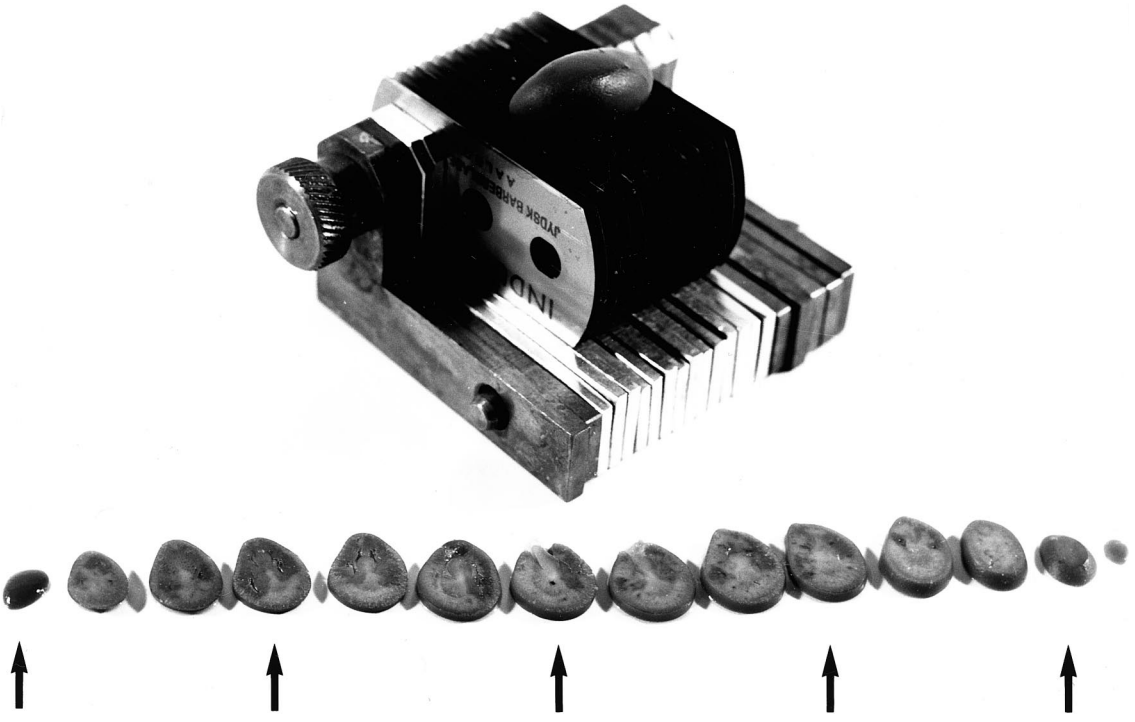

Figure 1. Illustration of systematic, uniformly random sampling of a rat kidney using a slicing device with parallel and equidistantly spaced razor blades. The systematic component is the decision to choose a sampling periodicity of 3. The random sampling component is to look up a random number between 1 and 3; in this case #1. The arrows indicate the slices (1, 4, 7, 10, and 13) that were sampled using this procedure. (Courtesy of Thomas F. Bendtsen and Jens R. Nyengaard).