A Critical Comment on a Recent Publication Using Parenchymal Airspace Profiling

To the Editor:

With interest, we have read the recent contribution by Xiao and colleagues in this *Journal* entitled "Parenchymal Airspace Profiling: Sensitive Quantification and Characterization of Lung Structure Evaluating Parenchymal Destruction" (1). We appreciate the general goal of the authors to "overcome shortcomings" of "traditional lung morphometry," which according to the authors' perception suffers from simplistic data interpretation, subjective data acquisition, and bias due to tissue shrinkage, just to name a few "shortcomings."

However, many of the "shortcomings" that the authors introduce as a motivation for their study have comprehensively been addressed in the American Thoracic Society/European Respiratory Society (ATS/ERS) statement on quantitative assessment of lung structure, and clear recommendations have been made (2). The overall problem we see with the study by Xiao and colleagues is the fact that these recommendations by the ATS/ERS were not taken into consideration, so the described method is far from overcoming such "shortcomings" as mentioned above.

As an example, the study claims to overcome a bias due to "scaling, differences in degree of inflation, and tissue shrinkage during tissue collection and processing." However, if formalin fixation and paraffin embedding are used, as in the presented study, unpredictable tissue deformation and, above all, shrinkage will occur and introduce a bias for every kind of image analysis (3).

Also, based on size analyses of distal airspaces on 2D sections, parenchymal airspace profiling was used to differentiate alveoli from alveolar ducts, and the data were further processed to determine, e.g., the "alveolar count" or "alveolar size." However, this approach does not take into account the complexity of alveolar architecture in 3D and the fact that alveoli have entrances that connect them with alveolar ducts. When one takes a closer look at the images provided in the paper, it becomes obvious that structures that can clearly be identified as alveolar airspaces have been labeled as ductal airspaces by the parenchymal airspace profiling. Hence, simple determination of the 2D area of a distal airspace is not appropriate to separate alveoli and ducts. Moreover, for simple reasons based on stochastic geometry, it is impossible to estimate the number of objects in a 3D space (such as the number of alveoli) without bias by analyzing only single 2D thin sections. This problem can only be solved by 3D methods, such as disector (4). These considerations challenge the meaning of the data provided in the article, and it is obvious that the described methodology does not measure what it is supposed to measure.

In summary, the described method is prone to generate biased instead of accurate data, in particular because the ATS/ERS recommendations from 2010 are not taken into consideration. Unfortunately, these limitations of the study are not discussed in the paper. It is somewhat disappointing to see a paper that ignores even the most basic principles of the ATS/ERS recommendations published in this *Journal*, even more so because they were introduced to its readers by an editorial in 2010 (5). Quantitative assessment of lung structure is demanding and requires careful planning of experiments, regardless of whether stereology or automated image analyses are performed. The ATS/ERS guidelines from 2010 represent an extremely valuable basis for accurate assessment of lung structure, which is essential for good laboratory practice. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Reply: Parenchymal Airspace Profiling Has Its Merits and Is a Valuable Addition to Existing Methods for Lung Morphometry

From the Authors:

We thank Drs. Knudsen and Ochs for their interest and insightful comments. The American Thoracic Society/European Respiratory Society statement on quantitative assessment of lung structure was taken into consideration before the conception of the parenchymal airspace profiling (PAP) method and was cited in our article (1). We used formalin fixation, paraffin embedding, and errors in pixel scales to test the robustness of the PAP method, and are not in any way encouraging researchers to deviate from the guidelines set forth by the American Thoracic Society and European Respiratory Society.

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Our motivation for initially undertaking these studies was to develop a more feasible approach to lung morphometry for smaller laboratories. Although they are precise and all-inclusive, the guidelines are impractical for investigators who do not have the manpower to perform the extensive sectioning and processing required. We intended to develop a method that could overcome certain biases from different protocols while allowing rapid, large-scale analyses of the lung.

The authors are correct that unpredictable tissue deformation and shrinkage will introduce bias in any image analysis. Although the overall shrinkage as a result of histologic processes can be somewhat unpredictable, ranging from 24% to 36% for different species (2), the linear correction factor repeatedly discussed in the article also suggests that these changes are nearly linear or proportional to the original unprocessed tissue. As demonstrated in our article, PAP-derived parameters, such as the ductal/destructive fraction, stayed unbiased with proportional changes by $\pm$ 20% of the original size, whereas the mean linear intercept did not. Nevertheless, the development of a robust method to withstand some of these biases would be beneficial and a great addition to previously utilized methods.

Another criticism made by the authors is regarding our definition of terms, such as the "count." We fully recognize the complexity of lung structure, and defined parameters in the manuscript pertaining to our methodology, such as the count per unit area (#/2D). The counts in the article are indeed 2D section based and clearly labeled with the unit of $1/mm^2$. We have no intention to suggest that the count ($1/mm^2$) is an unbiased representation of objects in a 3D space. As for the argument regarding the difference between "alveolar airspaces" and "ductal airspaces," the "ductal/destructive airspaces" defined by the PAP method will always refer to those airspaces that are centered by the second Gaussian distribution based on the area-weighted size distribution. This will facilitate future advancements in this area of research, as it will allow researchers to exactly reproduce the results, carefully examine them, and make corrections/improvements as needed.

The criticism that Drs. Knudsen and Ochs made regarding certain aspects of the PAP method is duly noted; however, the 3D method "disector" is less than ideal, as this method is not widely used. As a matter of fact, since the introduction of the disector method in 1984 by Sterio (3), there have been very limited follow-up publications, as revealed by a recent PubMed search. While looking forward to high-resolution 3D scans in the future, we should still pay attention to the most widely used technology that is currently available. The fact that 3D structures can be reconstructed from numerous 2D sections suggests that 2D-based methodologies are the foundation of future 3D technologies. We will further dedicate time and effort to develop a 3D-based method that we hope will resolve some of the limitations of the 2D method and become more widely utilized by researchers. ■

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