



## INTRODUCTION

# There is no rationale to still rely on outdated, biased tools for quantitative morphology in pulmonary research

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**P**ulmonary research was once the scientific area that most significantly contributed to conceptual and methodological progress in the development of quantitative morphology. The pioneering work of WEIBEL and co-workers [1–5] provided the basis for what is now called design-based stereology. The basic methods of lung fixation, of sampling strategies ensuring that specimens, sections and microscopic fields of view representative of the whole organ (*i.e.* systematic uniform random samples) are collected, and of designing appropriate test systems for the analysis of volume, surface area and length of a structure of interest, were established during those early days. These tools have successfully been used in pulmonary research to gain insights into important structure–function relationships, aspects of lung development, pulmonary function and pathogenetic mechanisms in lung diseases (see [5–8] for references to original work).

The next breakthrough in quantitative morphology came in 1984 when a new stereological tool, the disector (“di-sector” because it consists of two sections a known distance apart), was introduced, which for the first time allowed particle (cell) number and size to be counted without the need for model assumptions about, for example, shape or orientation [9]. Further new developments included methods designed to obtain unbiased quantitative data taking into account the anisotropy of structures (vertical sections, orientator, isector), such as the bronchial tree, and volume changes due to tissue shrinkage (fractionator, smooth fractionator), and to provide unbiased estimates of mean particle size (rotator, nucleator, point-sampled intercepts) [10–12]. Notably, these developments were no longer achieved in the area of pulmonary research but in neurosciences and nephrology, which may in part explain why these tools are still used by only a minority of lung scientists.

Design-based stereological methods have been discussed in the area of neuroscience, for

example, in *Trends in Neurosciences*, one of the top neuroscience journals [13, 14]. Awareness of the importance of design-based stereology prompted the editors of the *Journal of Comparative Neurology* to be the first to establish a policy that “stereologically-based unbiased estimates are always preferable for establishing absolute counts or densities of structures in tissue sections,” and further that they “expect that any papers that use simple profile counts, or assumption-based correction factors, will provide adequate justification for these methods, which will stand up to critical review” [15, 16]. The *Journal of the American Society of Nephrologists*, the number one journal in urology and nephrology, went one step further and requested from their authors “that appropriate stereologic methods be used to quantify structures in tissue sections in all manuscripts submitted to the Journal” [17]. Although no similar policies have been established by leading respiratory journals, the increasing number of related debates highlight the need for the respiratory community to update the methodological armamentarium used in pulmonary research [18–20]. Furthermore, awareness of the benefit of design-based stereology for quantitative morphological studies is increasing within the respiratory science community [5, 21, 22].

Consequently, the European Respiratory Society supported two stereology courses dedicated to “Quantitative Morphology in Pulmonary Research”, which were held in 2004 and 2006 at a beautiful venue, the Castle of Rauischholzhausen near Marburg, Germany. The courses were considered to be an introduction to the theoretical principles of and practical solutions offered by design-based stereology and were aimed at graduate students and scientists who wished to implement design-based stereological tools into their own research projects. We, Hans Joergen G. Gundersen, Dallas M. Hyde, Jens R. Nyengaard, Matthias Ochs and Heinz Fehrenbach, the lecturers involved in these courses, particularly appreciated the invitation to contribute to part I of this issue

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of the *European Respiratory Review (ERR)* dedicated to “Quantitative morphology in pulmonary research”, and it is a personal pleasure for me to serve as Guest Editor. Recognising that most of the scientists in pulmonary research (including ourselves) are thinking in terms of disease-related studies, both in clinical studies and in studies of animal models, the aim of the present issue of the *ERR* is to first give a general introduction into design-based stereology [12] and then to demonstrate how these tools have already contributed or could potentially contribute to the understanding of a specific disease, *i.e.* acute lung injury [23], allergic bronchial asthma [24] and pulmonary emphysema [25], by studying appropriate animal models. Realising that we could not adequately cover some areas that significantly rely on quantitative morphology, we were happy that Connie C.W. Hsia, Prescott G. Woodruff, and Anh L. Innes agreed to contribute with their expertise in compensatory lung growth [26] and in the study of bronchial biopsies [27], an approach that directly relates stereology to the study of human disease. Although some of the major lung diseases, *e.g.* pulmonary fibrosis and lung cancer, are not specifically addressed in this issue, we believe that the collection of papers presented here will provide a sound basis for the implementation of stereological tools in studies aiming to quantify morphological parameters in pulmonary research in general. To facilitate the implementation of design-based stereology in future projects, each paper highlights some of the most important and useful parameters for the assessment of the characteristics of the specific disease (model).

Progress in science depends both on innovative concepts of thinking and on the development of pioneering methodological tools. It is our strong belief that continuing to use a method only because it has already been used for decades is one of the weakest arguments in science. The choice of a specific method should be guided by aspects of appropriateness, lack of bias, efficiency and reproducibility, which we believe are best met in the area of quantitative morphology in pulmonary research by tools of design-based stereology. There is no rationale to further rely on outdated, biased tools for quantitative morphology in pulmonary research.

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