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Design-based stereology: Planning, volumetry and sampling are crucial steps for a successful study

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SUMMARY

Quantitative data obtained by means of design-based stereology can add valuable information to studies performed on a diversity of organs, in particular when correlated to functional/physiological and biochemical data. Design-based stereology is based on a sound statistical background and can be used to generate accurate data which are in line with principles of good laboratory practice. In addition, by adjusting the study design an appropriate precision can be achieved to find relevant differences between groups. For the success of the stereological assessment detailed planning is necessary. In this review we focus on common pitfalls encountered during stereological assessment. An exemplary workflow is included, and based on authentic examples, we illustrate a number of sampling principles which can be implemented to obtain properly sampled tissue blocks for various purposes.

1. Introduction

Design-based stereology represents a set of tools which can be employed to quantify three-dimensional structures using two-dimensional (e.g. microscopic) sections, based on a sound statistical and stochastic background. It has been recommended as the method of choice for quantification of structure in kidney (Madsen, 1999), lung (Hsia et al., 2010) and brain research (Saper, 1996). However, performing a sophisticated design-based stereological assessment is demanding and prone to numerous pitfalls which have to be identified and avoided well in advance, ideally before starting to harvest the organs. This review will focus on the most common pitfalls an investigator might encounter. Some of these pitfalls will be analyzed in detail and appropriate action will be presented. Various ways of performing volumetry and sampling of tissue for multiple purposes (e.g. light microscopy, electron microscopy and immunohistochemistry) will be presented.

1.1. Organizational environment

Experience shows that conceptual planning is often underestimated in microscopic quantification. Quantitative results obtained by an intuitive approach can be biased, may be misinterpreted, or are simply wrong (Mendis-Handagama and Ewing, 1990). Careful preliminary planning of the entire stereologic workflow is the most important prerequisite to obtaining unbiased quantitative data from microscopic images. Hence, the key to a successful stereological study is the deliberation made during the planning phase, before even touching any tissue or material. A best-case scenario would be a core unit within a scientific organization that can provide stereological know-how and support in step with actual practice.

As an example, our units do not offer a full stereology service, whereby scientists would supply animals and receive final numeric results. We expect interested scientists to be closely involved in the entire process of sampling and probing, since their specific knowledge of the biological problem being investigated is fundamental. Ideally a scientist intending to conduct a quantitative evaluation would contact a stereologist during the initial planning stage of the experiment. At this early time point the stereological study can be optimally designed (see details in Section 1.2 and Fig. 1). The stereologist would then accompany the investigator through all the steps of the experiment.

1.2. Study design

In stereology, all steps of the quantification workflow are interdependent as each step builds on the previous one. The

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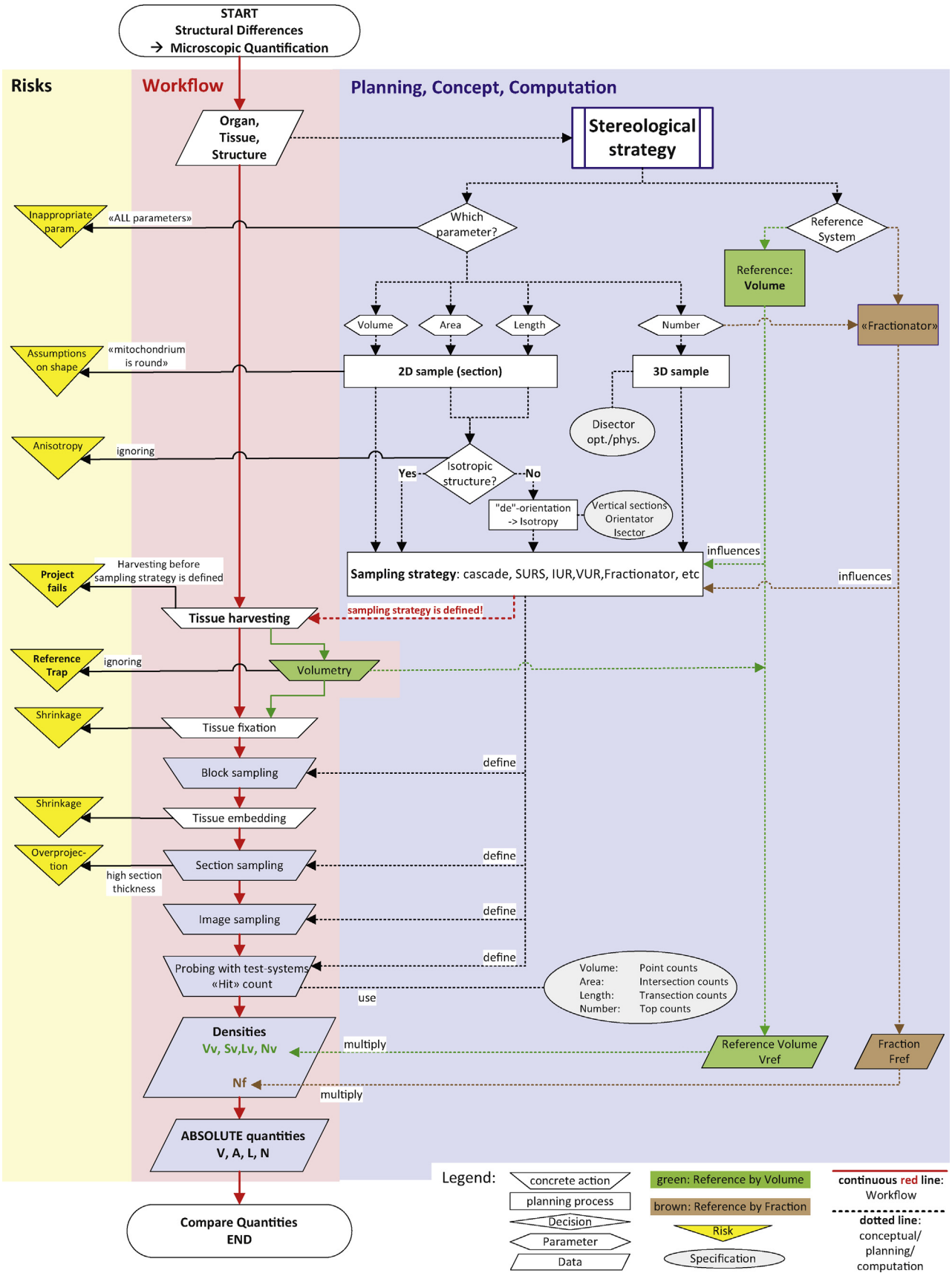

Fig. 1. Workflow of a stereological assessment including the planning phase, organ harvest, volumetry, tissue sampling and processing. Typical pitfalls are indicated.

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