

Proportionator™ fact sheet

Proportionator™ - a new sampling principle in stereology

The Proportionator™ sampling principle is a new general sampling principle in stereology that uses image analysis to perform a non-uniform sampling. Sampling of fields is done based on their content of a selected feature – in principle a biased method – but the sampling probability of each field is known, allowing for calculation of unbiased estimates.

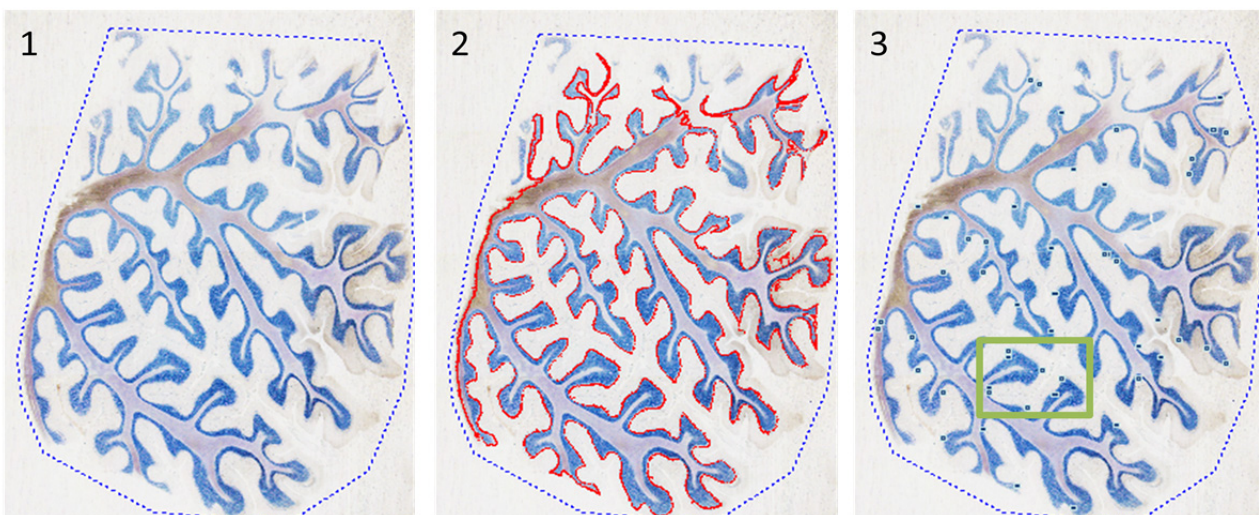
The Proportionator™ has similarities to the smooth fractionator sampling principle in that it greatly increases sampling efficiency and estimate precision, but is different in giving that the possible sampling fields holds different sampling probabilities range from very low to very high.

References

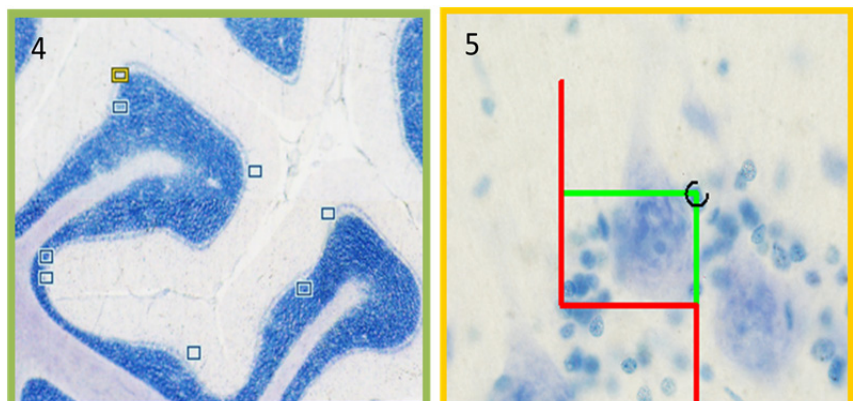
Gardi, J.E, Nyengaard, J.R. and Gundersen, H.J.G. (2008). The proportionator: Unbiased stereological estimation using biased automatic image analysis and non-uniform probability proportional to size sampling. *Computers in Biology and Medicine* 38: 313 – 328

Gardi, J.E, Nyengaard, J.R. and Gundersen, H.J.G. (2008). Automatic sampling for unbiased and efficient stereological estimation using the proportionator in biological studies. *J. Microsc.* 222: 242 – 250.

Proportionator 1 – 2 – 3: Estimation of the total number of Purkinje Neurons in the cerebellum



- 1) A Navigator image of the slide is created, and the region of interest is delineated.
- 2) An image analysis protocol identifies light blue stained areas close to a border of a more densely stained region (marked in red). Inside the region the relative amount of blue is measured for use in the Proportionator™ sampling.
- 3) The Proportionator™ sampling of fields of view is performed, giving a higher probability of sampling for fields with a high relative amount of blue. Purkinje neurons have a pale blue staining, and there is an increased probability of counting Purkinje neurons in the pale blue areas.
- 4) The sampling pattern is clearly different from systematic uniform random sampling
- 5) Counting of Purkinje neurons in optical disectors.



How does it work?

Proportionator™ for online sampling in newCAST™

A Proportionator™ sampling in newCAST™ always starts by acquisition of a low magnification Superimage in the Superlens Navigator.

This generates an overview of the slide contents, which can be used for application of the image analysis protocol the forms the basis for the Proportionator™ sampling.

Several Superimages representing several slides may be created (for slide loaders).

When defining the regions of interest with the mask tool, several regions of interest or tissue sections may be defined on each slide.

Prior to starting the sampling, the magnification and probes for the stereological analysis is selected.

The Proportionator™ sampling set up is opened. This allows for selection of a Visiomorph™ image analysis protocol that has been created for the application.

The feature for the sampling is detected, and the relative amount of feature, termed “weight”, is calculated. The sampling period is defined in a “weight”-number. For pilot studies, the number of sampling positions may be selected.

Then the computer performs a lot of calculations: It divides the region of interest into a 100% sampling of small fields of view, sorts the fields of view based on their weight value, and performs the sampling from the sorted fields. This gives fields with a high “weight” value a higher probability of being sampled.

For each sampled field the computer automatically logs the weight value and the sampling probability.

For the sampling the total weight of the region of interest is logged together with the sampling weight period.

All the user experiences when starting the sampling is that the computer performs some calculations and then shows the selected sampling positions in the Superimage in the Superlens Navigator.

Upon starting the sampling, the stage is automatically moved to the first sampling position in the first of the regions of interest selected for the sampling.

Now the stereological analysis is conducted as usually. The Proportionator™ sampling method is a generally applicable sampling method and can be combined with all probes available in newCAST™.

Data are logged for each sampling position (field of view) together with the sampling probability for the field. This results in an automated calculation of the weight-corrected counting result inside the newCAST™ data sheet.

After finalizing the analysis/counting procedure, the unbiased estimate can be calculated using the weight-corrected counting results. The CE of the estimate can be directly calculated from the sampling data.

Proportionator™ for sampling of image series automated imaging modules

The Proportionator™ sampling method may be applied when performing sampling of series of images for later stereological analysis.

Perform sampling of series of images from individual tissue sections using the Microimager™, paired tissue sections using the Autodisector™, or from several matching sections using the Section assembler™.

The Proportionator™ sampling method is added to the list of sampling methods in the autosampling module.

The Proportionator™ may be used for sampling from whole slide images (virtual slides) using WSManger™.

Create, store and load image analysis protocols for identification of the feature used to perform the sampling.