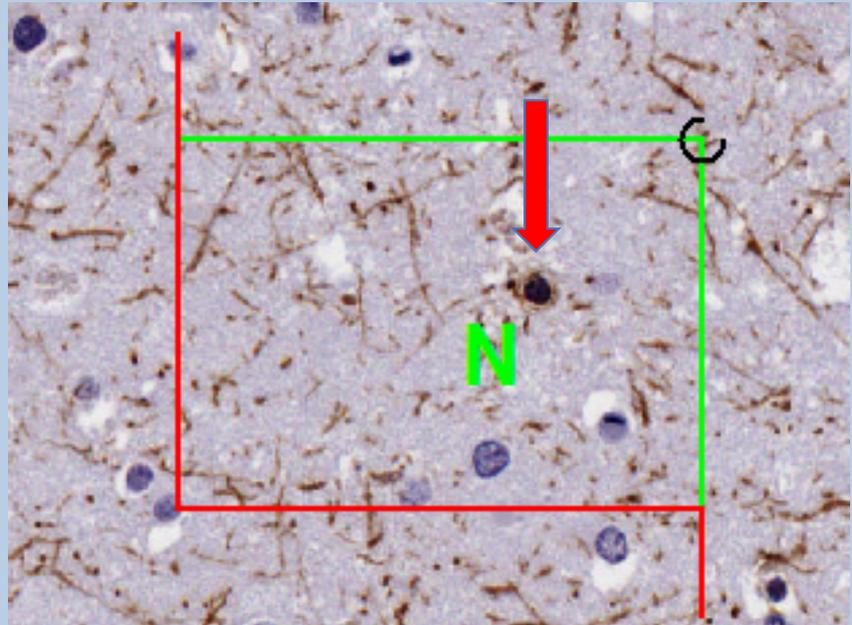


- A stereology toolbox for High Through-put analysis

Stereology software developed in close collaboration with inventors of modern design-based stereology, Prof. Hans Jørgen G. Gundersen and Prof. Jens R. Nyengaard from the Stereology Research Laboratory, University of Aarhus.



Whole Slide Stereology combines the benefits of whole slide imaging (digital slide systems) and stereology.

By using Visiopharm's High Through-put (HT) Whole Slide Stereology software, stereological analysis significantly faster and more cost-effective than microscope-based systems.

Image acquisition and stereological analysis are separated into two independent processes, which allows you to do the analysis on your laptop at any time and location.

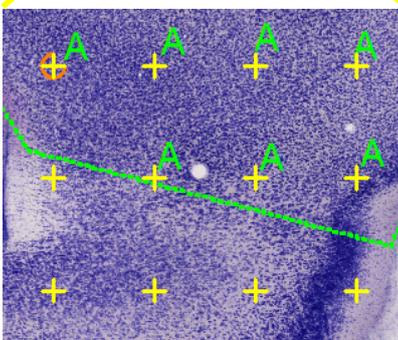
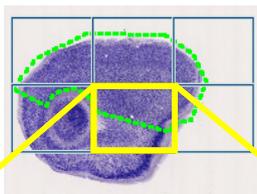
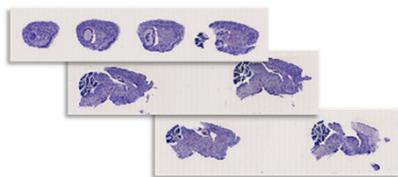
Images are archived in a database and accessible for sharing. Furthermore, you avoid laborious challenges with the microscope setup, e.g. camera, motorized stage and calibration issues.

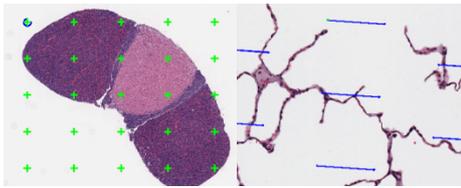
In Whole Slide Stereology, the entire workflow is more efficient, overview images are generated instantly, regions-of-interest can be outlined automatically, images are calibrated and in focus, which all result in a significant reduction in work hours. This enables laboratories with a need for high through-put analysis to apply stereological techniques.

HT Whole Slide Stereology is compatible with most whole slide image file formats on the market — and works with both brightfield and fluorescence.

The use of Visiopharm stereology software is widely cited in the scientific literature.

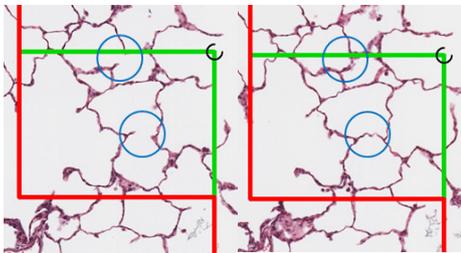
Visiopharm products are not for clinical diagnostic use in the U.S.



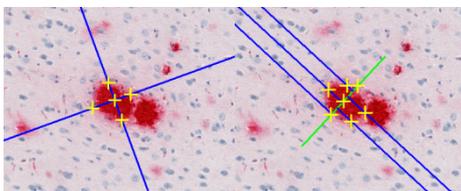


Point probe for volume estimation

Line probe for surface estimation



Counting frame for number and length estimation



Nucleator for local vol /area estimation

Rotator probe for local vol estimation

Life Science Stereology

provides efficient practical techniques for obtaining 3D quantities from 2D sections – such as cell number and volume. The methods are statistically proven and the study is consequently unbiased by design - i.e. there are no assumptions, models or correction factors involved. The Visiopharm newCAST Whole Slide Stereology software includes methods for estimating: volume, surface area, length, and number of objects.

About High Through-put Whole Slide Stereology™

HT Whole Slide Stereology software provides a complete set of well-documented stereology tools allowing the researcher to accurately estimate relevant end-points through the use of:

- Points, lines, counting frames
- Physical disector and fractionator
- Local estimators – nucleator and rotator
- Unique, unbiased method for handling artificial edges

All probes are scientifically well-documented and have been rigorously tested by Prof. Hans Jørgen G. Gundersen and Prof. Jens R. Nyengaard from the University of Aarhus, Denmark.

Automated Physical Disector

HT Whole Slide Stereology software makes it possible to work fast and efficiently with the physical fractionator principle by automating the most time-consuming aspects of the physical disector.

The patented Autodisector module in the HT Whole Slide Stereology software allows for automated alignment of paired tissue sections and unattended sampling in the matched disector pairs. The sampled disector pairs are stored in the system database as corresponding digital images and the whole sampling can be accessed from remote workstations. This makes it possible for a single imaging station to serve several researchers who can download their studies to remote analysis stations.

Proportionator - efficient sampling

The proportionator is the most efficient unbiased stereological sampling method. The proportionator samples with Probability Proportional to Size (PPS), which means that fields of view are not sampled with the same probability. Rather, fields of view with high content of the feature in question, has a higher (known) probability of being selected. The principle improves efficiency of the estimation process up to 25 times as empty fields of view become rare events.

The Proportionator™ sampling method can be combined with all available probes in the HT Whole Slide Stereology software.

With the Visiopharm HT Whole Slide Stereology software, it is possible to design the optimal setup for research projects, combining well-documented sampling strategies and stereological probes with an increased flexibility.

All collected data (sampling parameters, probe data, and analysis results) are seamlessly recorded for calculation of the final results, and are immediately available for export to MS Excel.

Autodisector –automation of the physical disector

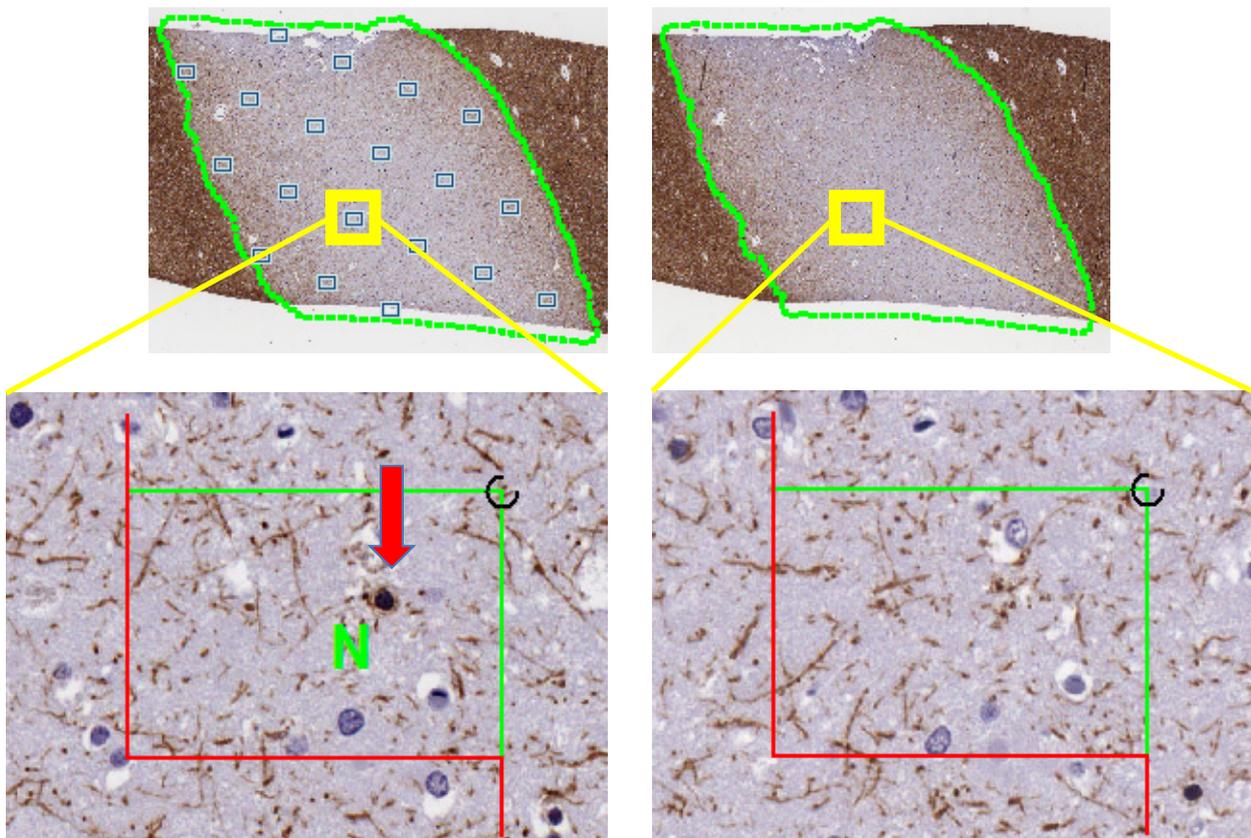
The Autodisector module in the HT Whole Slide Stereology software makes it possible to work fast and efficiently with the Physical Fractionator Principle by automating the most time-consuming aspects of the physical disector.

How does the Autodisector module work?

1. Performs an automated detection of all tissue sections on all slides
2. Allows the operator to "pair" identified tissue sections whether on the same or on different slides
3. Performs initial match of tissue sections (translation, rotation, and local deformation)
4. Allows the user to define the regions for sampling (defined on the reference sections)
5. Performs a fully unattended sampling and storage of perfectly matched disector pairs at any working magnification (tissue independent)
6. Allows for off-line analysis at remote analysis stations.
7. Enables history of countings for personnel training and regulatory documentation.

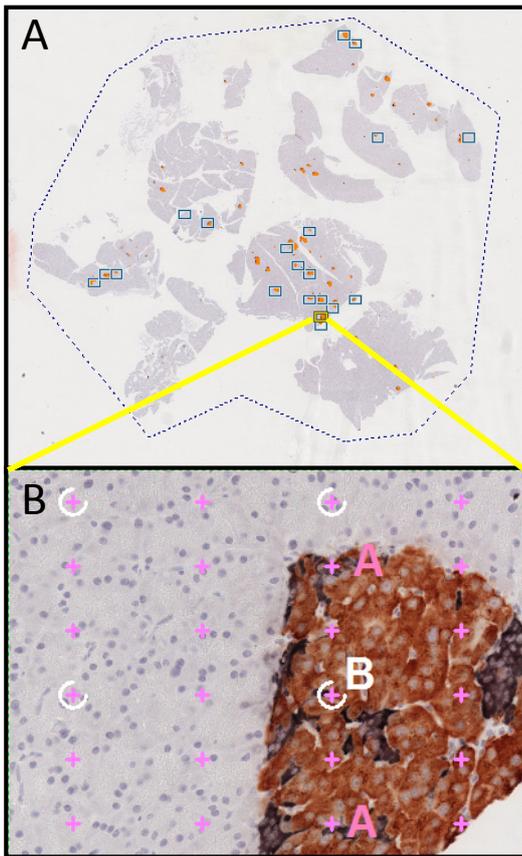
Count of oligodendrocytes using the automated physical disector

The number of oligodendrocytes in post mortem brain is estimated using the automated physical disector on pairs of serial sections. The Autodisector module automatically aligns the tissue sections at low magnification *and* aligns the fields of view at high magnification. Below, an aligned tissue pair (upper images). Systematic uniform random sampling is performed (upper left) and the sampled fields of view are aligned and counted (lower images).



*Left section with example field of view.
Oligodendrocyte count marked with red
arrow and green N.*

*Right section with corresponding field of view
aligned to field of view from left section.*



Proportionator - efficient sampling

The proportionator is the most efficient unbiased stereological sampling method.

To the left, beta cell volume (mass) in the pancreas has been estimated.

Figure A:

- Region of interest is delineated at 1x
- Beta islets are detected by image analysis (orange) for Proportionator™ sampling
- Proportionator™ sampling is performed within region of interest resulting in only 20 fields of view showing mostly areas with islet content, and very few empty areas

Figure B

- Field of view at 20x magnification
- Beta cell volume is estimated by point probes
- After counting, the unbiased estimate can be calculated using weight-corrected counting results
- CE of the estimate can be directly calculated from the sampling data

Technical features:

General

- Separate image acquisition and stereological analysis process allows analysis on laptop at any location
- Software contains database for images and sampling parameters, probe data and analysis results
- Possibility for image sharing with other labs
- Support of whole slide image formats: Olympus, Hamamatsu, Aperio, Leica, 3-D Histech/Zeiss, and standard images formats as tiff and jpeg
- All data can be exported to MS Excel
- Detailed user manual implemented in the software

Sampling

- Methods: Systematic uniform random sampling (SURS) based on either fractional area, X,Y - stepper or number of sample
- Sampling can be performed on the entire whole slide image or on user defined region-of-interests
- Overview image displays region-of-interest (ROI) and sampling progress within ROI during the sampling process
- Stereological designs: Cavalieri, Isotropic uniform random (IUR) sampling, Vertical sections (VUR), Physical Disector/Fractionator

Geometrical probes and estimators:

- Point and line probes
- 2-D and 3-D counting frames

- Rotator, 2-D and 3-D Nucleator
- Point sampled intercepts

Physical disector and fractionator:

- Visual or automated alignment of section pairs
- Live or unattended sampling of paired fields of view
- Customized extended field of view in look up section
- Conn Euler analysis
- Handling of artificial edges

Proportionator:

- Non-uniform random sampling of fields based on the content of selected feature(s)
- Can be combined with all available probes
- Automated calculation of the weight-corrected counting result

Non-stereological probes:

- Mean cord length
- Mean linear intercept
- Perimeter
- 2-D distance measurement

Counting:

- Define up to 30 different counting marks
- Count different objects simultaneously
- Automatic logging of count and sampling data during the counting process