

The new Computer Assisted Stereology Toolbox

– *newCAST™ by Visiopharm*



Unbiased quantification of tissue changes

Quantitative microscopy is a powerful tool in research and clinical settings, when a detailed analysis of tissue microstructure is essential to understand pathological or natural changes.

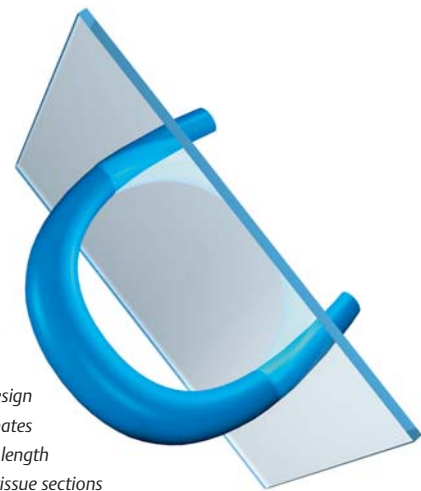
Today, stereology is the method of choice for quantification of anatomical changes of physiological importance, and can be used for answering research questions such as:

- Has the number or size of neurons changed in a specific brain region?
- Has the alveolar surface area been reduced in a model of lung disease?
- Have the total bone volume and trabeculation of bone changed?
- Has there been a change in the number, size or length of nerve fibers?

In a growing number of scientific communities, stereology is recognized as the only scientifically valid method to perform this type of quantitative analysis.



Quantification of individual 2-D profiles is a widely used method for obtaining information about tissue structure and composition. For biological structures of varying size, shape, and orientation this often provides severely biased results. Design based stereology ensures unbiased estimates of structural information (volume, area, length and number) in 3-D obtained from 2-D tissue sections



Complete, well-documented, and innovative

newCAST™ 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 and optical disectors and fractionators
- Virtual planes
- Local estimators – nucleator and rotator
- Unique, unbiased method for handling artificial edges

Implemented in close collaboration with inventors and innovators of modern design-based stereology, Prof. Hans

Jørgen G. Gundersen and Prof. Jens R. Nyengaard from the University of Aarhus, all probes supported by the software are scientifically well-documented and have been rigorously tested. The probes may be combined, creating a versatile tool for unbiased, stereological analyses in virtually all fields of biomedical research.

newCAST™ can be further extended with new, innovative tools and methods that increase efficiency and reduce the amount of manual labor, such as the Autodisector™ and Proportionator™.

Sample - Count - Document - Calculate - Publish

Sample: newCAST™ can control most state-of-the-art microscopes, cameras, stages, encoders, and is compatible with most whole slide imaging file formats. This enables automated sampling of image fields, stacks and matched image pairs in brightfield, widefield, and confocal fluorescence microscope.

Settings for optimal imaging and sampling can be stored in the program. newCAST™ also allows for import and analysis of series of images or image pairs acquired outside the system.

Count: In newCAST™ stereological probes may be combined to allow for simultaneous analysis of several end-points. Modern stereological methods introduce a need for efficient hand-ling of local thickness measurements and guard zones at artificial edges for calculation of exact sampling probabilities. In newCAST™ local thickness measurement and guard zone handling is seamlessly integrated in the analysis.

All system settings defined for a project, such as those related to imaging, sampling, or counting, can be stored as electronic protocols in order to increase ease of use, and serve as documentation.

Document: All relevant data about probes and analysis results are automatically stored in the integrated MS-SQL database.

It is possible to simultaneously acquire high quality photomicrographs during sampling and counting procedures, enabling generation of figures that are directly applicable for publication.

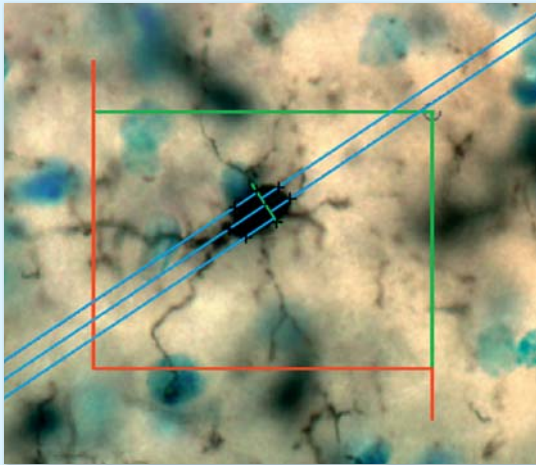
Calculate: The data collected in newCAST™ are immediately available for export to MS Excel in a format which facilitates creation of calculation templates.

The newCAST™ system also offers automated calculation for a range of widely used estimators. The availability of both tools provides maximum flexibility for users at all levels.

Publish: The results generated in newCAST™ are unbiased estimations, which meet and exceed the high level of standard that a growing number of leading scientific journals are setting forth for publication.

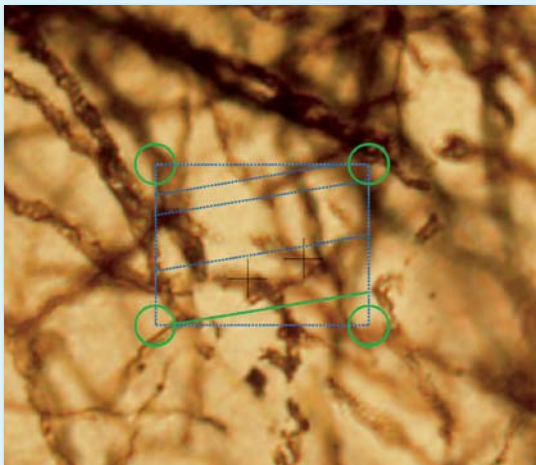


Application examples



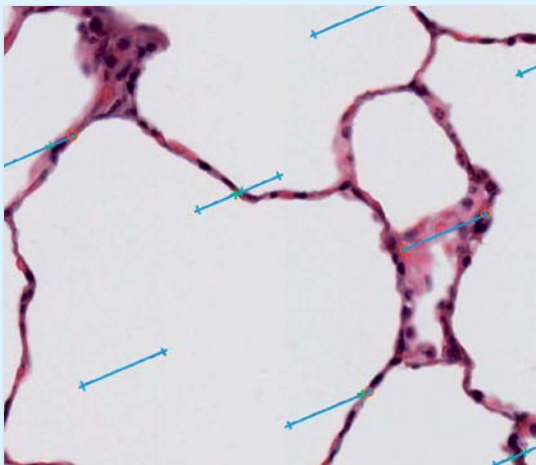
CELL NUMBER AND CELL SIZE

Analysis of the number and cell sizes of IBA-1 labeled microglial cells in the mouse hippocampus is a classical example of the optical fractionator for counting of cells, combined with the rotator probe for estimation of the individual cell size.*



FIBER LENGTH

Estimation of the length of silver stained nerve fibers is performed using the virtual planes probe. The method allows for use of thick sections oriented in the way that the researcher prefers for identification of specific brain regions, i.e. the hippocampus.**



SURFACE AREA

In pulmonary research, analysis of alveolar tissue breakdown is pivotal to the evaluation of pathology. newCAST™ enables efficient estimation of the total surface area of the alveolar compartment – essential in the evaluation of COPD or emphysema. newCAST also allows for easy estimation of the volume of alveolar tissue or analysis of the alveolar number based on the Conn-Euler principle.*

*: Image courtesy of D. Hyde, University of California, Davis, USA

** : Images courtesy of B. Finsen, University of Southern Denmark

New tools for innovative stereology

newCAST™ provides the basis for working with novel principles that increase efficiency, such as the Proportionator™ sampling principle and the Autodisector™.

The Proportionator™ is a revolutionary principle introduced by Prof. Gundersen's group in Aarhus, which employs digital image analysis to guide the sampling of image fields towards regions containing the feature of interest. Bias is avoided by keeping track of the exact empirical sampling probability of each sampled field. With the Proportionator™, stereological sampling efficiency has been demonstrated to improve up to 25 times compared to systematic uniform random sampling in practical applications.

The physical fractionator principle effectively eliminates or reduces practical problems relating to shrinkage, staining penetration, limitations in size of objects, tissue opacity, and the ability to standardize staining procedures. The Autodisector™ dramatically increases the efficiency of the

physical disector principle, by automatically aligning paired sections for sampling of matched fields at high magnification for cell counting, thus making this principle feasible for virtually all applications. The Autodisector™ belongs to a family of Visiopharm® software modules designed for automated image acquisition and alignment of serial tissue sections, allowing for unsupervised sampling in a large study.

with the combination of Proportionator™, and Autodisector™ on a traditional microscope platform (or whole slide scanning devices), it is now possible to perform stereological quantification with high efficiency.



Sophisticated stereology software

newCAST™ delivers efficient and flexible solutions for sampling at microscopical level, the application of geometrical probes, data collection and documentation of results. The scientists at Visiopharm® can, in addition, provide assistance in the design of studies.

With newCAST™, it is possible to design the optimal setup for research projects, combining well-documented sam-

pling strategies and stereological probes with an increased flexibility. All custom settings for sampling, probes and counting tools are easily stored, and later retrieved for replication of the settings in future applications.

newCAST™ seamlessly records all sampling parameters, probe data, and analysis results for calculation of the final results.

¹ 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. *Journal of Microscopy* 222: 242 – 250.

TRAINING AND SUPPORT

Besides on-site installation and training, Visiopharm offers on-line support, allowing our experts to quickly assist in real time in developing Standard Operating Procedures and analysis protocols, guiding users every step of the way

HARDWARE COMPATIBILITY

Visiopharm provides systems fully configured with all hardware components, but we can also help you to retrofit to your existing system.



Visiopharm technology is designed to facilitate every step in the work-flow from prepared tissue sections to scientifically valid quantitative end-points reflecting important tissue properties.

Fully compatible with all the leading hardware brands.

Microscopes:	Leica, Nikon, Olympus (incl. DSU), Zeiss
Whole Slide file formats:	Aperio (Scanscope), Hamamatsu (Nanozoomer), 3DHISTECH (Pannoramic), Zeiss (Mirax), Leica (SCN400), Olympus (dotSlide and VS110)
Motorized stages:	Ludl, Märzhäuser, and Prior
Slide loaders:	Ludl
Camera:	Olympus, Hamamatsu, Basler, Leica, QImaging
PC:	Microsoft Windows XP™, Vista™ and 7™

Please see our latest hardware integration at:
<http://www.visiopharm.com/page-04-00.shtml>

MORE INFORMATION

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