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Stereo Investigator AI: Applying deep learning methods for automatic cell counts in a new unbiased 3D stereology software package

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Introduction

Stereology is a rigorous and unbiased methodology for quantifying features of biological tissues such as the size, shape, distribution, and quantity of objects. Although it is the gold-standard for quantification, wide-spread adoption of stereological analysis has been hindered because it is labor intensive, even with modern software tools.

Stereo Investigator AI dramatically accelerates stereological cell counting through the use of machine learning. Once the machine learning algorithms are trained, Stereo Investigator AI identifies cells in 3D volumes throughout brain regions using the same observer criteria as a human expert.

Manual and automated stereology were performed in order to assess the performance of Stereo Investigator AI across multiple labels. True positive and false positive rates were quantified and compared between cell counting methods.

Object Detection and validation using Machine Learning

Stereo Investigator AI integrates candidate object detection, machine learning, and stereology counting rules to identify, classify, and quantify cells in 3D microscopy images. The Stereo Investigator AI machine learning engine is based on a custom Convolutional Neural Network (CNN) architecture inspired from the FRCNN design [1]. This network can both detect and accurately locate multiple objects of various sizes within a field of view. The system operates on partial intensity projections in XY, YX, and XZ planes or all XY focus planes and combines results into 3D objects.

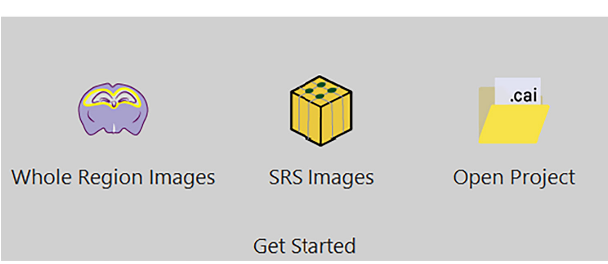
Our deep learning engine performs the task of (i) candidate region proposal detection, (ii) classification of candidate regions into object categories (cell, artifact, etc..); (iii) bounding box regression to adjust object size and position.

Our flexible deep learning engine can either operate on regions of interest (ROI) or operate on an entire 3D volume of cells of a specified range. Regions of interest can be generated from a variety of sources including Lapaclacian of Gaussian (LoG) filters, and lower resolution deep learning detection.

Stereological counting rules are applied to the updated cell models. The combination of advanced machine learning algorithms and established stereological counting rules enables accurate and unbiased quantification of cells in 3D microscopy images that permit validation.

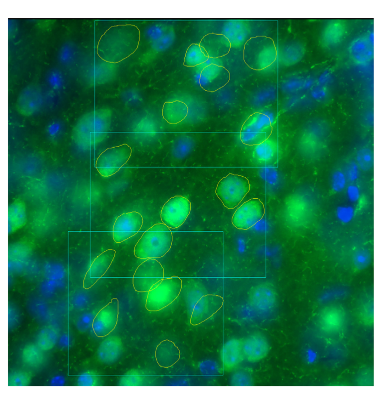
Stereo Investigator AI workflow operation

Sample requirements



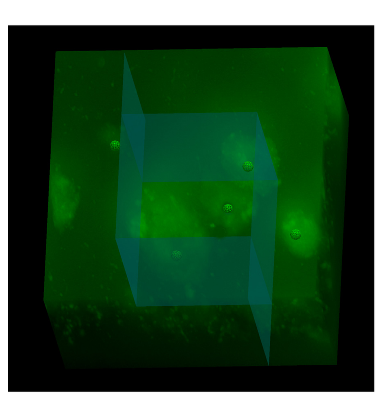
Stereo Investigator AI utilizes pre-acquired 3D whole-section images or systematic random sampling (SRS) image stacks. A workflow guides the user through the process of setting up a study; delineating contours, defining the counting frame, grid spacing, and disector parameters.

Machine learning classifier

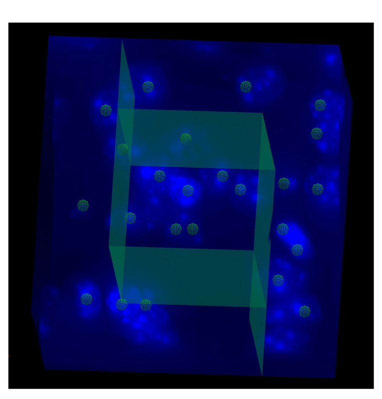


MBF Bioscience maintains and curates an extensive annotated volumetric dataset of various tissues with a variety of bio-labels and imaging modalities. This extensive data is leveraged to constantly improve a family of deep learning classification and regression networks, from the most generic cell detector to networks optimized for specific tissue preparation, labeling, and imaging modalities.

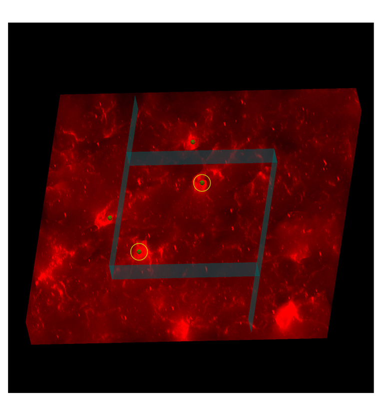
NeuN



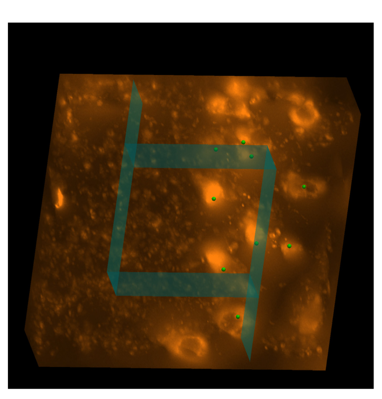
DAPI



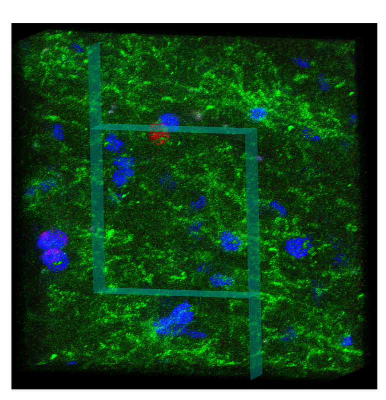
Iba1



TH



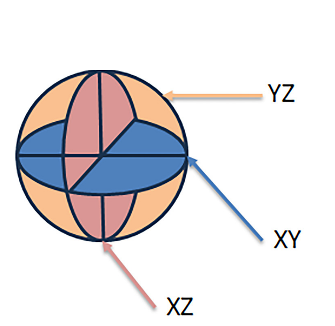
SOX10

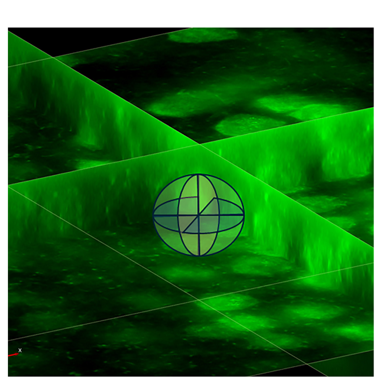


Candidate region detection

LoGSeeded - FocusXYZ

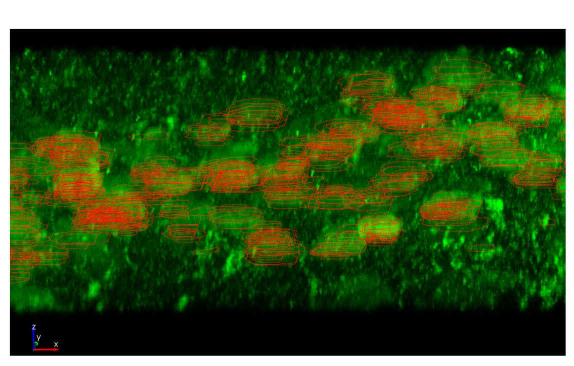
Detection in projected slices along the X, Y and Z axes are combined using a multi-view fusion algorithm to generate 3D objects



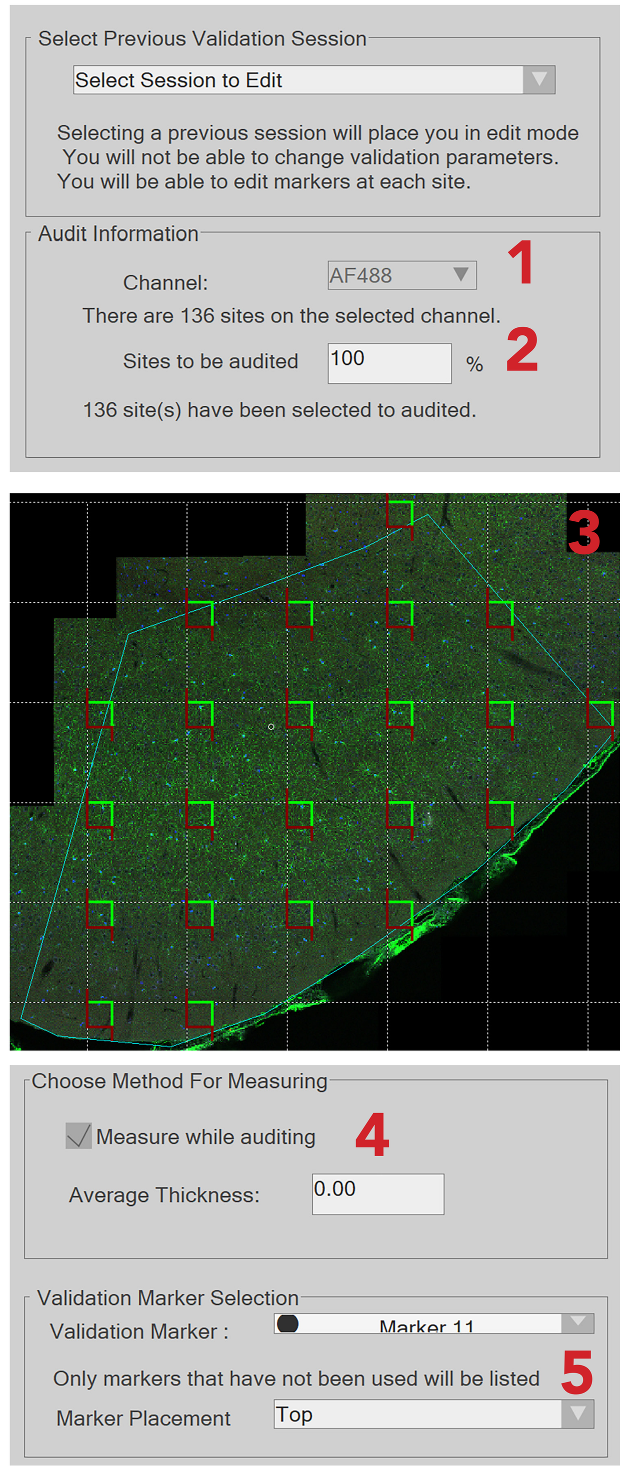


XY- Scan

Individual detection on XY focus planes are combined using clustering techniques into 3D objects

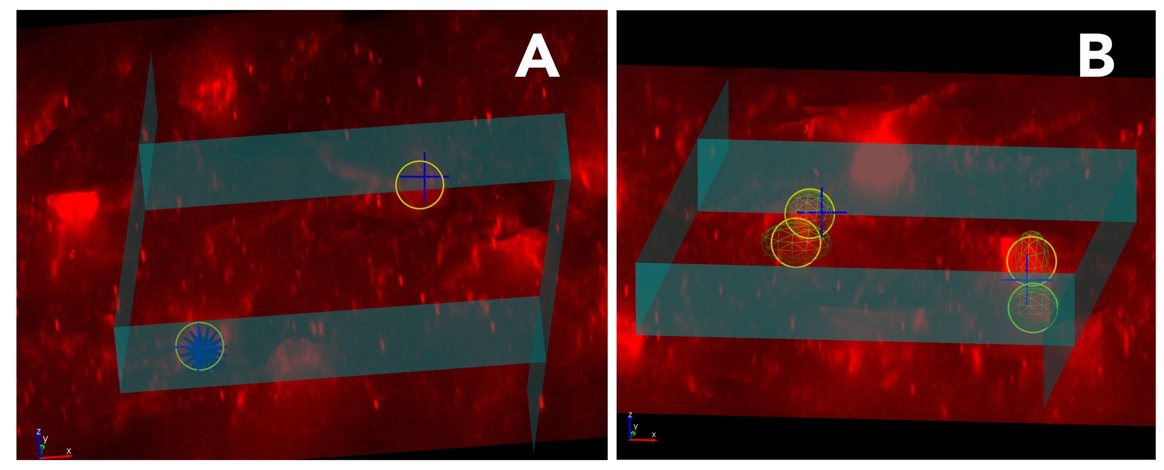


Validation workflow



The user is able to validate results blindly. Once the channel (1) and percentage of the sampling sites to be validated are selected (2), the software will randomize the selection of the sampling sites (3) for a complete blind assessment of cell counts and sample thickness (4). The user can select the criteria for unique point placement (5). Finally, Stereo Investigator AI tabulates results that include False Positives, False Negatives and calculations such as Precision and Recall.

After the validation assessment, a 3D visualization that includes the disector, interactively-identified cells, and computer-detected objects enables the user to review results and identify inconsistencies in the detection.



Example of false positive counts due to wrong cell volume segmentation. (A) Side view (see rotation axes at bottom left of the picture) of a counting site showing Iba1 positive cells counted by a human annotator (blue crosses) and correctly detected with Stereo Investigator AI (yellow circles). (B) Side view of a different sampling site showing duplicated cell counts by partitioning one cell into two due to elongated Z stretch. The ellipsoidal mesh is the cell volume representation made by Stereo Investigator AI

Materials and Results

Imaging fluorescent mouse brain specimens

Tissue images were acquired in a Zeiss Axioscan system using a 20X/0.8 NA PlanApo objective and AxioCam 705m camera (lateral resolution 0.17µm/pixel). Section thickness ranged between 35-15 µm with Z-steps between 0.5-1 µm.

Results

Cell validation results				
Classifier	Training iteration	Precision %	Recall %	F1 (Harmonic mean)
Iba 1	v9	74	99	85
TH	v1	70	84	76

Thickness validation results		
Classifier	Expert human annotator (µm+SD)	Stereo Investigator AI (µm+SD)
Iba 1	13.90+/-1.57	13.80+/-1.33
TH	25.88+/-1.09	31.25+/-1.65

Discussion and future directions

- Our cell-type specific classifiers and image modality candidate region detection have performed well, with very high Recall percentages. Precision values were affected by double counts of the same cells due to improper cell volume assessment in the Z-axis
- Validation performance differences between the two tested classifiers can be attributed to training load. Despite Iba1 more complex shape, this classifier has been subject to more training iterations. We expect an improvement in TH classifier detection with additional training
- Algorithms for determining section thickness align well with manual counters on thin sections but further refinement is necessary for an accurate determination of the height measurement in thick sections, specially on widefield images where initial top and bottom are more diffuse
- The use of stereology ensures that machine learning results can be validated simply and robustly. This is beneficial for testing new classifiers and running large studies alike

Acknowledgments and references

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- Conflict of Interest: Content reflects use of products made by commercial employers of authors.

[1] Faster R-CNN: Towards Real-Time Object Detection with Region Proposal Networks; Ren, S., He, K., Girshick R., Sun, J. (2016) arXiv (ePrint 1506.01497)