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Unbiased Stereology (MBF Bioscience, StereoInvestigator Software)

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ASAP Collaborative Res...

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Protocol status: Working
We use this protocol and it's working

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Keywords: ASAPCRN, unbiased stereo logical assessment of neuron, stereoinvestigator software, using stereoinvestigator software, unbiased stereo logical assessment, unbiased stereology, mbf bioscience, specific brain region, other cells in specific brain region, neuron

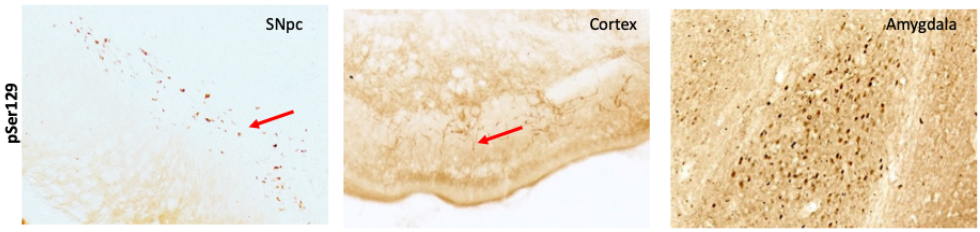
Abstract

This protocol allows for unbiased stereo logical assessment of neurons and other cells in specific brain regions using StereoInvestigator Software.

Guidelines

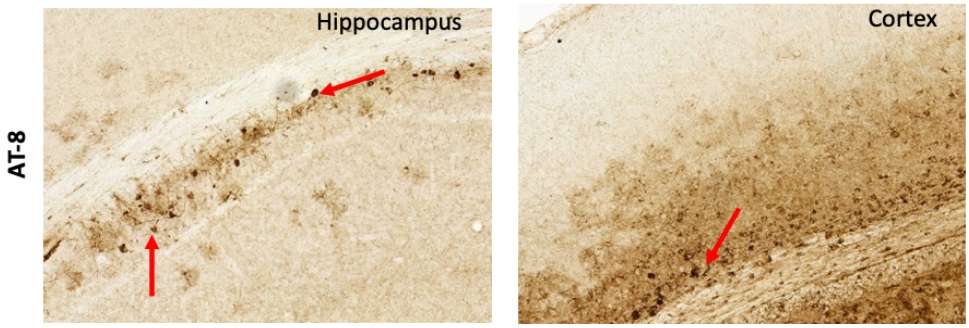
	A	B	C	D
	REGION	**COUNTING FRAME**	**GRID SIZE**	**SECTION #**
	Striatum	40µmx40µm	300×300	6
	Hippocampus	30µmx30µm	130×130	5-7
	SNpc	50µmx50µm	100×100	5-7

This table describes the set parameters for counting cells within different brain regions. Note that evaluation interval should be based on how tissue was sectioned. For whole brain collected in 12-well plate: interval = 12, for brain sections collected in 24-well plate: interval = 6. Confidence interval should be less than 0.1.



Stereology on sections stained with pSer 129 Antibody:

- Follow all the steps as mentioned in the master protocol for Stereology.
- The important thing is locating the pSer-DAB signal, as this may look slightly different depending on the region of interest in the brain section. For example, pSer staining in the cortex may look a bit different than SNPc or Amygdala.



Stereology on sections stained with AT8 antibody:

- Follow all the steps as mentioned in the master protocol for Stereology.
- AT8 antibody recognizes the phosphorylated tau protein, so you will see this staining in the hippocampus and in some cortical regions near the hippocampus.

WORKFLOW

1 Create a map of your slide using the stereology worksheet and make note of the estimated population and total markers counted

2 Set slide properly on the microscope.

3 Click on Probes > Optical Fractionator Workflow > Start a new subject
- Label subject/study
- Existing sample in configuration: no
- Number of sections to count:__*see table
- Enter cut thickness: 40µm
- Section Evaluation Interval: __*see table
- Start with number one
- z-value of first section = 0

4 In Select Low Mag Lens, click 5X > Next Step

5 Trace the Contours of Interest for each section on your slide. If you need more contours for other brain regions, label them in preferences.

6 Select High Mag Lens select 40X (use light focuser on) > Next Step

7 Select 'manually enter the average mounted thickness'. Average Mounted thickness is 25µm

8 Define the counting frame (see the guidelines for more specs): your goal is to have 5-10 cells for each counting frame and about 20-25 sites/region

9 Manually enter grid size (see the guidelines for more specs): Enter 15 percentage. Approximate sites = 1 > Display changes.

10 Optical Director Height > program will calculate this based on settings > Manual Focus > Next Step

11 Count objects: Select your first section > Select the desired region > Start counting

12 Focus on the top of the tissue > OK (if you struggle with this, find the bottom of the section and then count up 20 units from there)

13 Select your markers according to the desired cell types (these can also be customized)
- For each site, scroll up and down throughout the z plane and adjust brightness
- Count cells with visible nucleus in the square. If a cell touches a line of the counting frame: if it touches the green line, count the cell; if it touches a red line, do NOT count it.

14 Once finished with counting: Select "Completed Counting" To view results and export data to excel: View Results > Export to Excel

15 To go to the new reference icon in the top left corner and repeat steps.