Top 5 Questions (and answers) about the optical fractionator probe for cell counting

Q: What should the thickness of my sections be after accounting for tissue shrinkage?
A: We recommend cutting the thickest sections you can while still achieving complete stain penetration. The sections must be thick enough so that you can have guard zones that prevent counting in damaged surfaces of the section and also allow for a disector that will give you about 10 focal planes. Therefore, the recommendation is that the sections should be at least 20 microns thick after shrinkage.

Q: Does the orientation of sectioning matter when you are counting the number of cells?
A: No. Since we are counting a unique feature of the cell that approximates a point (called the unique point, e.g. a cell top), that “point” will look the same no matter what direction you approach it from. With the Optical Fractionator, you can section your tissue preferentially (e.g., coronal, sagittal) so you know where rostral, medial, and dorsal are.

Q: How should I count cells in very densely populated regions such as the cell body layer of the CA1 or the dentate gyrus of the rat hippocampus? Is there a different protocol I should follow for such areas?
A: In order to count particles you have to be able to see them and find their tops. Make sure that you are using an objective that gives you a thin depth of field (60X or 100X and 1.3 or 1.4 numerical aperture). If you are using brightfield illumination, ensure that your optics are adjusted for the thinnest z-plane (Koehler Illumination). Many people use confocal imaging in the dentate gyrus because the cells are very tightly packed. To get a better idea of best practices, read published papers that have counted cells in the region of interest that you are examining.

Q: How is the grid size determined? Is it based on the distribution of cells in my tissue?
A: Yes, it is based on the distribution of cells within and among sections. In addition to the heterogeneity of the distribution of cells, sampling density is also based on how far apart the control group is from the experimental group—which you may or may not have an understanding about a priori. Sometimes you just need to pick a starting point for your grid size and see how the Coefficient of Error (CE) comes out. Another method is to perform a comprehensive pilot study that will permit resampling the data to identify efficient parameters using Stereo Investigator.

What Coefficient of Error (CE) should we aim for? Less than 1?
A: The higher a CE is, the less precise the method of cell counting. A CE of 1 is the highest you can get. Published CE results are generally less than 0.1, but you should use whatever precision you need that suits the needs of your experiment. This is based on the distribution of the particles and what effect you need to show. This is a good paper to read on the subject:


Still have questions? Watch a webinar on the optical fractionator: mbfbioscience.com/webinars