



Standard Operating Procedure for **Stereology**

1.0 PURPOSE

Stereology method is used to obtain unbiased ,quantitative data of number, size ,shape , area or volume of analysed objects.

C.V.HOWARD & M.G.REED,unbiased Stereology,second Edition.garland Science/Bios Scientific Publisher 2005.

2.0 SCOPE (Should include which Cores this SOP applies to)

This procedure applies to all personnel who will test mice on the challenging beam test within the Stem Cells Engraftment and *in vivo* Analysis Core.

3.0 PROCEDURE

Multiple process of preparation material for analysis based on individual design of PI.

3.1 Apparatus

Olympus Microscope BX61 (Denmark),with motorized stage, digital camera a high precision microcator for measuring the height position of the stage, a computer and the NewCAST stereology software(Visiopharm) . Microscope has 2 options bright field or fluorescence microscopy.

3.2 General Procedures

Tissue harvesting and preparation for stereology (Brain tissue)

- 1) Whole animal fixation via transcardial perfusion
- 2) 4% Paraformaldehyde in PBS, pH 7.4 final (According to IACUC approved protocol.)
- 3) Saturation of tissue with sucrose gradient 7.5%- 30% (sucrose dissolved in PBS)
- 4) Freezing of tissue in liquid N2 and storing in -80 C

Section cutting

- 1) 40-70 um section will be cut ,either in dry mount or wet mount method.
- 2) Individual will decide on choice of appropriate sections for Systematic Uniform Random (SUI) either Isotropic Uniform Random (IUR) or Vertical Uniform Random (VUR) sections
- 3) Dry mount sections will be collected on gelatin covered microscopic slides (0.3% of gelatin,0.05% of Chromium Potassium Sulfate) stored in -80 C till processing.
- 4) Wet mount sections will be stored in refrigerator in antifreeze solution(300g sucrose,10g PVP40,300ml Ethylene Glycol/1L of PBS)

3.3 Immunostaining

Immunostaining will be performed according to standard immunohistochemical procedures and will be tailored to individual needs. Antigen/ primary antibody complex will be detected either by enzyme conjugated or fluorochrome conjugated secondary for bright field and fluorescence accordingly.

Choice of coverslipping media will be consider as part of the procedure and will be determined in pilot studies.

3.4 Stereological analysis

Manual: NewCAST TRAINING,University of Buffalo,December 15-16,2010.

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available in 430 BRB (Stereology core facilities) or here attachments.....

INDIVIDUALS USING OLYMPUS BX61/NewCAST SYSTEM MUST BE FAMILIAR WITH PRINCIPLES OF TISSUE PREPARATION,IMMUNOHISTOCHEMISTRY,CRYOSTAT/MICROTOME CUTTING PROCEDURE,MICROSCOPE OPERATION.

SPECIFIC TRAINING IN STEREOLOGICAL METHODES OF ANALYSIS WILL BE OFFERED IN FACILITIES.

Created by Ewa Stachowiak, 15 December 2011