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Stereology-mediated cell count using StereoInvestigator

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ABSTRACT

Protocol for cell counting using StereoInvestigator software

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EXTERNAL LINK

https://www.mbfbioscience.com/help/stereo_investigator/Default.htm

PROTOCOL CITATION

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MATERIALS TEXT

MBF Bioscience StereoInvestigator 11 (64 bits) Software (Micro Brightfield)

1 Set slide properly on the microscope and set new reference point

2 Click on Probes → Optical Fractionator Workflow → Start a new subject →

3 Enter cut thickness (5 µm (cut with microtome, 20 µm or 30 µm (cut with cryostat))

4 Enter interval according to the thickness: 17 if 5 µm, 6 if 20 µm or 4 if µm)

5 In Select Low Mag Lens, click 4X

5.1 Click on Next Step

6 Select the Contour of Interest(s)

7 In Select High Mag Lens select 100X

7.1 Click on Next Step

8 In Manually enter the average mounted thickness enter the corresponding one

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8.1 Click on Next Step

9 In Define the Counting frame enter 50 x 50 µm

9.1 Click on Next Step

10 Enter 25 Percentage

10.1 Click on Display Changes

11 Enter 100x75 µm (if cut thickness is 5 µm) or 125x100 (if 20 µm or 30 µm)

11.1 Click on Display Changes

11.2 Click on Next Step

12 In Optical Disector Height enter 5 µm, 20 µm or 30 µm

12.1 Click on Next Step

Select your first section

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13.1 Click on Yes

14 Select the desired region

14.1 Click on start counting

15 Focus on the tissue

15.1 Click on OK

16 Select a number of markers according to the desired cell types

IMPORTANT 1: Count only the cells with visible nucleus within the square
IMPORTANT 2: 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.

17 Count every defined region from every set section

17.1 Click on Next Step

17.2 Click on Display Probe Run List

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18 Click on Section Name → Click on Counter/Marker Name → Select all sections belonging to the same region

19 Click on View Results

IMPORTANT: Verify that interval corresponds to the interval set in Step 3

20 Click on the different markers to see the number of cells counted and the estimated cell number

20.1 Click on CE Scheaffer to view the area if needed

20.2 Repeat steps 17 through 20.1 for each region

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