BioTek's Counting Chamber and Adapter

Instructions for Use

Requirements

1. Counting chamber (p/n 1322131) and adapter (p/n 1320016)
2. Cytation™ 5 with a phase contrast module and 4x objective
3. Gen5™ 2.07 or higher
4. Counting chamber plate type file (**Counting chamber.xml**)
5. Sample cell counting protocol (**Cell counting sample.prt**)

Import the plate type file into Gen5

1. Save **Counting chamber.xml** on the local drive
2. Go to the Gen5 System menu. Choose Plate types > Import.
3. Browse to **Counting chamber.xml** and choose Open
4. A new plate type called **Counting Chamber Holder** will now be on the plate type list. Make sure that it is checked as a "Favorite".

Run a new experiment

1. Save **Cell counting sample.prt** on your local drive or in the Gen5 database
2. Open Gen5's Task Manager and choose Experiments > Create using an existing protocol.
3. Browse to **Cell counting sample.prt** and choose Open
4. Run the experiment and review the parameters to edit (below) if necessary.

   Best practice tip: Define a different protocol with the appropriate counting criteria for each cell type.

About the sample protocol

Principle: Cells are counted on the entire, known field of view (about 1.9 x 1.5 mm) at 4x for each image. The manual counting grids (1 x 1 mm) are not used and only imaged for reference. The grids do not need to be centered or fully imaged for an accurate cell count; although for consistency, it is recommended that at least a portion of the grid is visible in the field of view.

Procedure, Read Step: The four corner grids of the counting chamber (comprised of 16 squares) are imaged using phase contrast with a 4x objective.

Data Reduction:

1. Cellular Analysis is used to count the individual cells using threshold and object size as the criteria. A subpopulation analysis step uses circularity and phase contrast mean intensity to differentiate cells from debris.
2. Transformation: Live Cells/4 grids is used to normalize the field of view against the actual grid size to be consistent with a manual count. The ratio of the field of view with the counting grid is 2.9.
3. Transformation: Live Cells/1 grid is used to determine the average cell count of all four grids.
4. Transformation: Live Cells/ml is used to determine the number of cells/ml to provide a final cell density, taking a dilution factor into account.

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Protocol Parameters to edit

Procedure, Read Step:

1. In the Cell counting sample protocol, **"Exposure"** is currently set to "auto". In some cases, this may not show the cell counting grid. To image the grid along with the cells, set a fixed exposure that may have a slightly higher integration time than auto exposure settings.
2. **"Options"** is currently set to "Autofocus". With some cell types, the rounded cells may appear to have darker centers which will affect object counting results. To circumvent this issue, choose a fixed focal height + offset. By imaging slightly out of focus, your cells will have a more consistent appearance.
3. Horizontal and vertical offset may be changed to move all four imaging locations respective to the well.
4. Tile overlap may be changed to move all four imaging locations respective to each other.

Data Reduction, Cellular Analysis:

1. Threshold value and object size may be changed according to your cell type's characteristics. The default values work well for average-sized cells with a naturally round shape such as HeLa cells.
2. Subpopulation criteria can be edited according to your cell type's characteristics so that debris is ignored in the final cell count.

   Example:

	Threshold	Object Size Range	Subpopulation Criteria: Circularity
Typical cells (default), e.g. HeLa, Hek 293, MDA MB 231, MCF-7	10,000	10-30 µm	>0.2
Smaller cells, e.g. HCT 116	12,000	5-20 µm	>0.5
Less round cells, e.g. Fibroblasts	10,000	10-50 µm	>0.1