## COULTER COUNTER

Switch settings:

- lower threshold = 10
- upper threshold = 100
- aperture current = 2
- amplification = 8
- matching switch = 20 K
- gain trim = 10
- selection of sample = 0.5

Open current switch. Warm up 5 min before using.

Wash the camera with isotonic solution (IS). Exchange the detergent solution with IS both in vials and reservoir; open both the upper and lower stopcock to wash the electrode, then reclose both the valves and leave the electrode in saline solution.

Trypsinize cells (ex. for 100 mm plate 1.5 ml of trypsin + 5 ml media). Transfer 0.5 ml of sample to vial and bring up to 20 ml with IS (1:40 dilution).

Put vial in the coulter counter and open upper stopcock. When light in sizing window is visible, reclose the stopcock and wait until the counting is ended. The number obtained must be multiplied by 40 (dilution factor) x 2 x 1.27 (correction factor) to obtain the number of the cells/ml present in the sample. During counting, check the sizing and the debris windows. If all the vertical lines are over the middle of the window, or if waves are designed in the sizing window, and/or if debris is passing through the hole, rewash the electrode and recount.

The coulter counter should be left open all day. At the end of the day wash the camera with detergent solution and replace IS with detergent in both reservoir and vials; open both stopcocks and reclose. Leave electrode in detergent and close the current power.