Measure cell number and vitality with Neubauer chamber

Purpose:

This SOP describes how to determine the concentration of cells in a cell culture and their viability by using a Neubauer chamber/hemocytometer and Trypan blue coloring.

Safety equipment and precaution:

Wear lab coat. Trypan blue is cancerous, wear safety goggles and work under fume hood when preparing and disposing trypan blue.

1 Procedure

Step	description
1	Take 10 µL cell suspension and transfer it to a 1,5 mL reaction tube.
2	Add 10 µL trypan blue using a filter tip, to avoid contaminating the pipette with trypan blue. Mix the suspension by pipetting up and down a few times. We will have a dilution 1:1 of cells/trypanblue.
3	Breathe onto cover glass, to moisture the surface.
4	Lay cover glass onto the Neubauer chamber. Move it around a bit, until you see newton rings forming (looks like the pattern on oil film).
5	Pipette until chamber is filled but max. 15 µL of trypan blue cell suspension underneath the cover glass by holding the pipette on the edge of the cover glass in a low angle
6	Use 4x magnification of the microscope to count living (white) and dead (blue) cells in the four corner squares of the microscope.
7	Calculate the concentration of living and dead cells: $cells\ per\ mL = (counted\ cells/4) * 10^4 * 2$ The chamber factor of 10^4 is only applicable, when a Neubauer/Neubauer improved chamber and the described volumes of cell suspension and trypan blue are used.
8	Dispose trypan blue in special trypan blue waste (hazardous waste)

2. MATERIALS

- Tripanblue, eppendorfs and filter tips.
- Pipetus correctly working.
- Pipetes, tips and filter tips, falcons (daily consumables).
- Guava® Muse® Cell Analyzer, Luminex and Muse™ Count & Viability Cell kit.

- Neubauer chamber with cover glass.
- 2 timers.