Development of an automatic counting system for cell spheroids in suspension

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Introduction

To understand the radiosensitivity of cancer stem(-like) cells or tumor initiating cells is of particular interest for applications in radiotherapy. However, in general it is challenging to determine radiosensitivity of these cells since they are often cultured in so-called self-renewal conditions in serum-free culture medium where they grow as spheroids. These cell spheroids do not attach to the culture flask and thus cannot be fixed and stained, so that manual counting of the spheroids gets extremely tedious. It is thus highly desirable to have an automated analysis system that is capable to detect unstained cell spheroids and to automatically scan complete culture vessels to determine the total number of spheroids.

Methods

The CARL (*Clonogenic Assay Recognition System*) system has been developed, implemented and tested using standardized commercially available components, like a 3840x2748 pixel CMOS camera (UI_1490SE from IDS), LED ring illumination (LDR-176LA-1 from CCS Inc.), and a motorized scanning stage (VT-80 2SM MLS from PImicos). The control software has been developed in Python language using the openCV image processing library on a Linux platform.

Analysis of a culture flask comprises the following steps:

- Image Acquisition: the required resolution of 10μm/pixel does not allow to cover the whole flask in a single field of view; thus, multiple images obtained by shift of the scanning stage have to be combined.
- Image Fusion: the special optical features of the culture vessels required combination of multiple images with different exposure settings and subsequent fusion to obtain a single image in HDR mode.
- Object recognition: a tophat filter is used for binarization and the watershed transformation for object separation.
- Object classification: several criteria like e.g. size and roundness are used to distinguish the cell spheroids from particles of artificial background.

A graphical user interface was implemented to control the general settings, the image acquisition and the analysis process. Figure 1 shows the setup of the automatic spheroid detection system.

Results

After extensive testing and calibration procedures the apparatus is now used in routine experiments. It could be demonstrated that the automatic counting is in good agreement with the results from manual counting; Figure 2 compares the results from manual scoring with the results obtained with the CARL system for U87 glioblastoma cells. Similar results have been obtained also for other cell lines (see e.g. Hartel et al., this report). An adapation of the system to facilitate also the analysis of cell colonies attached to the culture vessel seems feasible.

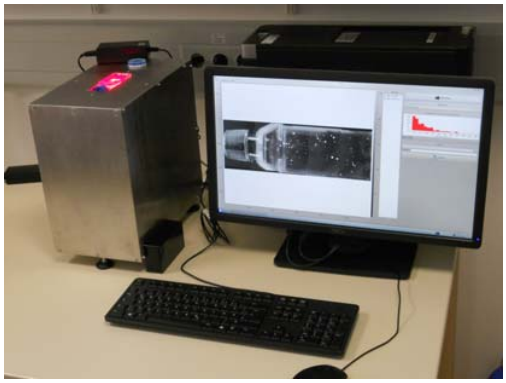

Figure 1: Setup of the CARL automatic cell spheroid detection system

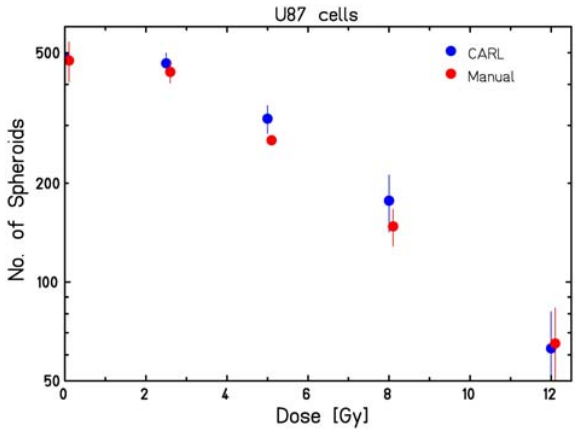

Figure 2: Comparison of manually counted and automatically analyzed spheroid numbers