An Automated Colony Counter Utilising A Compact Hough Transform

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Abstract: Investigating the effect of low-dose radiation exposure on cells using assays of colony-forming ability requires large cell samples to maintain statistical accuracy. Manually counting the resulting colonies is a laborious task in which consistent objectivity is hard to achieve. This is true especially with some mammalian cell lines which form poorly defined or 'fuzzy' colonies typified by glioma or fibroblast cell lines. A computer-vision-based automated colony counter is presented. It utilises novel imaging and image-processing methods involving a modified form of the Hough Transform. The automated counter is able to identify less-discrete cell colonies typical of these cell lines. The results of automated colony counting are compared with those from four manual (human) colony counts for the cell lines A172, U118 and IN1265. The results from the automated counts fall well within the distribution of the manual counts for all three cell lines with respect to surviving fraction (SF) versus dose curves, SF values at 2 Gy (SF_2) and total area under the SF curve (Dbar). An algorithm to detect the colony boundaries, and so determine their area, is also presented.

1 Introduction

The treatment of cancer by radiotherapy has been improved by the study of the response of mammalian cellular systems to radiation doses below 1 Gy [1-4]. At such low doses the survival of both cancerous and normal cells approaches 100% and specialised clonogenic assays involving large numbers of cells are required, in order to achieve acceptable statistical accuracy. One such assay involves using a fluorescence-activated cell sorter (FACS) to accurately determine the number of cells 'at risk'. After treatment the survival rate is determined by assessing the cells clone forming ability. A usual end-point is the counting of stained cell colonies by trained personnel; a procedure which is tedious and time consuming and which often results in subjective results. This is especially true with fuzzy or dispersed colonies typical of glioma and fibroblast mammalian cell lines, which tend to be indiscrete and overlap. Previous attempts to produce such a system have highlighted problems with clutter from the flask edge as well as with overlapping and dispersed colonies [5-10]

We present an automated colony counting system that utilises novel imaging and image processing to provide an accurate and objective assay end-point. The results of automated counting have been compared with those from four manual counters. We also present a processing method for determining the area of each colony since it has been shown that colony area statistics can contain important information [11].

2 Method

The automated colony counter was tested with 3 colony types of varying discreteness (A172, U118 and IN1265) and the automated results were compared with those of manual counts from four experienced biologists. The three experiments involved irradiating a large number of flasks (68 × 40 mm) containing a known number of cancer cells (utilising the FACS) and appropriate culture medium with varying X-ray doses (6 flasks at each of 14 doses, including 0 Gy). The flasks were incubated to allow colonies of cells to form which were stained and counted. The fraction of cells surviving to form colonies in each flask was calculated. These values were averaged for each dose and the results normalised with the 0 Gy result to give the final surviving fraction (SF) at each dose.

Automated counting commenced with image capture. The imaging arrangement is shown in Figure 1 where a monochrome 1/3" CCD camera, fitted with a 2.8 mm wide-angle lens, can be seen in close proximity to a culture flask. The flask was illuminated from below by an electroluminescent film (Pacel 'blue-green' type) which provided extremely uniform illumination at a wavelength of around 520 nm, appropriate for cells stained with Crystal Violet. The images were captured into the memory of a 450 MHz personal computer (PC) with a National Instruments (NI) IMAQ PCI-1408 image-capture board. The image was captured at a spatial resolution of 768 by 576 pixels and at an intensity resolution of 8 bits (256 levels), processed as a rectangular grid of square pixels. Imaging of this type allows a clear view into the corners of the flask (a region that some previous automated counters have ignored), excluding clutter from the top of the flask because of a short depth of focus.

The software for the user interface and image processing was written in the C programming language. In all cases of image capture, 10 images were stored and averaged to reduce camera random noise effects. Before the images were processed to identify the colonies, the barrel distortion introduced by the wide-angle lens was removed using geometric distortion with bilinear interpolation [12].

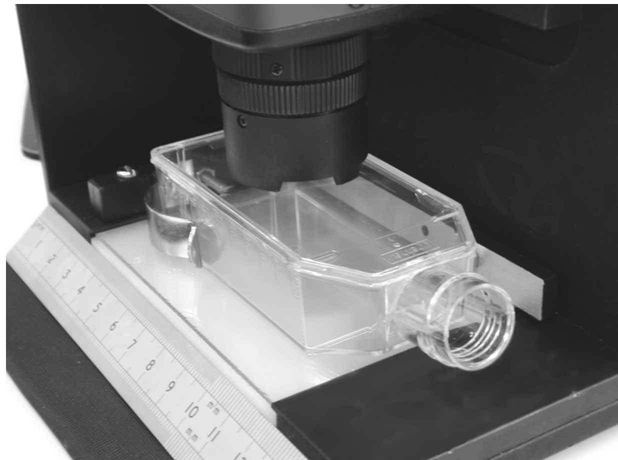

Figure 1. The imaging arrangement showing the camera lens, a flask, and the illuminating film below.

The colony finding algorithm has been aimed at fainter and ill-defined colonies, which have a tendency to overlap on the surface of the culture flask. It uses only the edge information of the image and a Compact Hough Transform approach to highlight the centres of circular objects [13]. Our particular implementation of a compact Hough Transform allows us to process images extremely quickly.

The first step is to find edges using 2 perpendicular Sobel operators [14]. A binary image of edge pixels (edgels) is produced by thresholding the edge magnitude. The binary image is raster scanned from top-left to bottom-right and each edgel encountered is used to increment a line of values in accumulator space along a radius towards the dark side of the edge, as shown in Figure 2. In the figure R_{min} and R_{max} specify the range of allowable colony radii and only a portion of the accumulator space is shown (the full accumulator space has the dimensions of the binary image). The values on the dark side of the edge (the opposite to the edge direction calculated from the Sobel components) are incremented because we have prior knowledge that the colonies must appear dark on a bright background. Peaks in the resultant accumulator space correspond to the centres of circular objects since each edgel contributes to the accumulator value at the centre of its colony. The accumulator space is smoothed to remove small local maxima.

Any unwanted structure detected, for example at the rounded corners of the flasks, can be excluded by taking the transform of an image of an empty flask and subtracting it from the transformed image of the cell-containing flask. This allows the detection of colonies that overlap the edges of the flask. A jig ensures reproducible flask placement and the alignment of the two transforms.

Portion of Accumulator Space

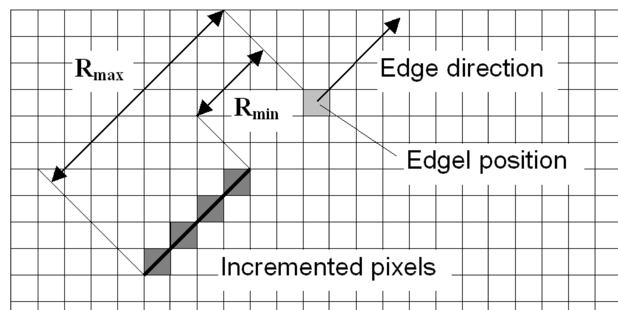

Figure 2. How the accumulator space is incremented by one edge pixel in this implementation of the Compact Hough Transform.

This fast compact Hough Transform runs in approximately 1 second on a 450 MHz PC, when processing 100,000 edgels, typically present in our colony images, from an image of 768 by 576 pixels.

Continuing from the known approximate position of the colony centres the local area can be processed to determine a colony boundary and so calculate the colony area. This processing is based on searching radially from the colony centre for a likely boundary position based on the grey level changes along each radial spoke, in a manner similar to that described in Reference [13]. In this implementation a circular boundary is fitted to each colony. Logical processing can then determine overlapping colonies and adjust boundaries as appropriate. Multiple detections are also eliminated because two detections of the same colony will clearly produce greatly overlapping areas.

3 Results and Discussion

Comparisons have been made between colony counts obtained from the automated colony counter and 4 experienced manual observers. Three experiments were performed to validate the automated counts using cell lines of different colony morphology. Example results of automated colony counting are shown in Figure 3 for

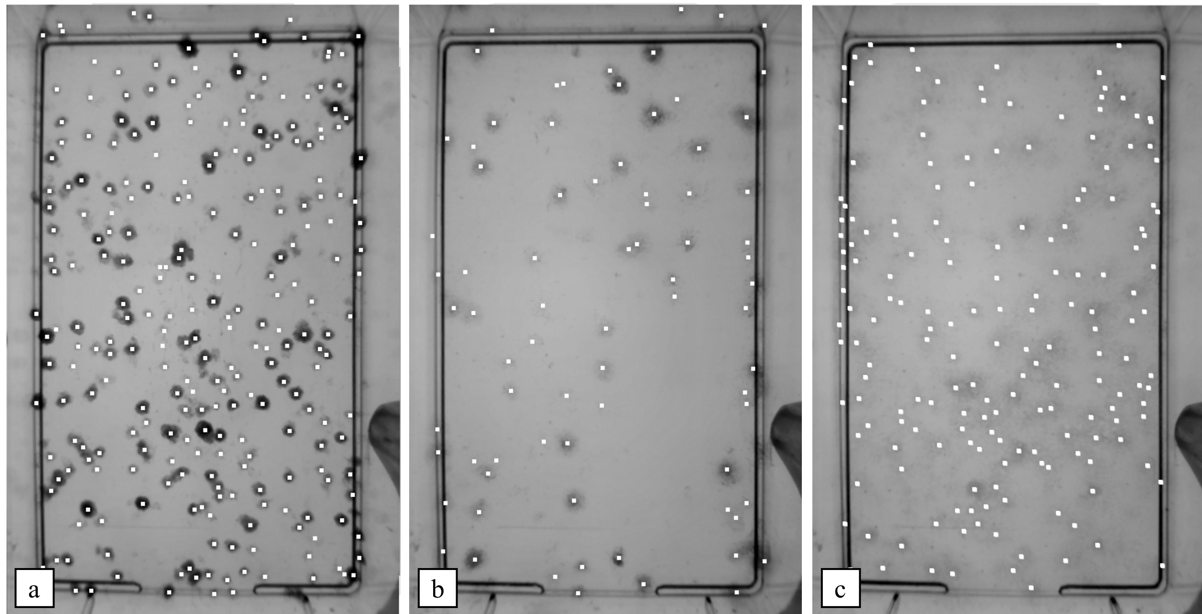

Figure 3. Example results of automated counting on cell lines A172 (a), U118 (b) and IN1265 (c).

the cell-lines A172, U118 and IN1265 (3 flasks were chosen at random) where each dot shows a colony counted overlaid onto the processed image. Individual survival curves, for the 3 experiments, were plotted from the 5 sets of counts obtained for each of the 3 cell lines in Figure 4.

At higher radiation exposures (3-5 Gy), some cell lines exhibit significant changes in their colony morphology. This can cause variations in colony counting. As a result, significant differences in SF values were observed at 2 Gy and 5 Gy when p values were calculated between the automated and the 4 manual counts. However, the differences between individual counters, either automatic or manual, were small when the SF_2 (surviving fraction at 2 Gy) and Dbar (area under the curve) values were calculated.

Analysis of variance was used to determine the overall coefficient of variation (CV) of the SF values for the 5 counts made on all 3 cell lines at 4 doses. In 2 of the 3 cell lines, the CV of the automated results was ranked second best and in the other cell line, ranked third. This indicated that as well as being able to count the correct number of colonies, the automated counter appeared to be objective and consistent in colony counting ability.

The results show that the automated colony counter is able to produce SF measurements consistent with manual

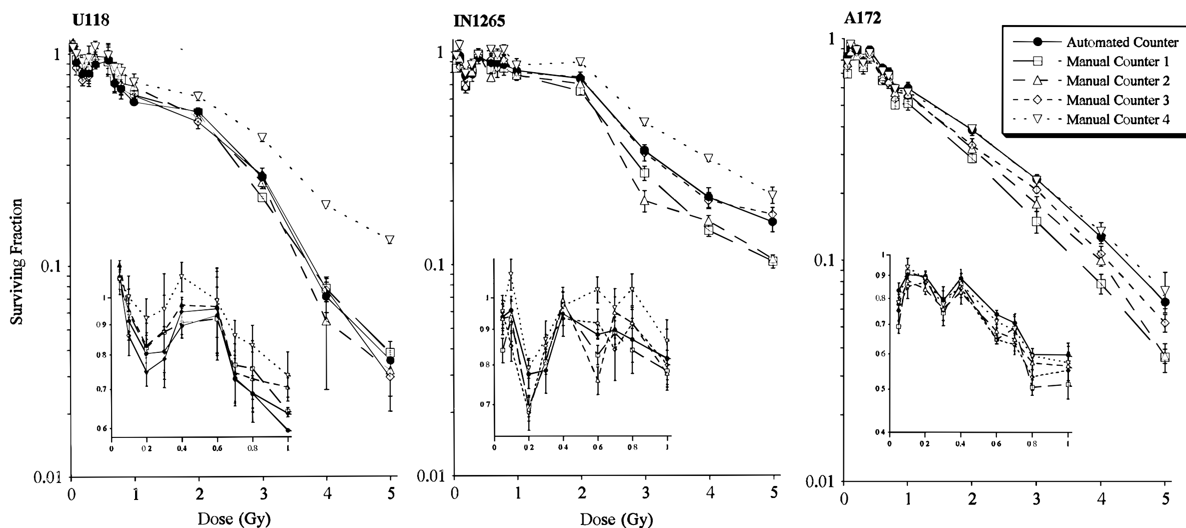

Figure 4. Survival curves of U118, IN1265 and A172 cell lines. Each graph shows 5 curves, one for each 'counter' with the automated results shown as black filled circles.

counters for cell lines with colonies as ill-defined as those for IN1265. The average time to process flasks using the automated colony counter was approximately 30 seconds per flask, including the time taken to manually load and unload the flask from the unit.

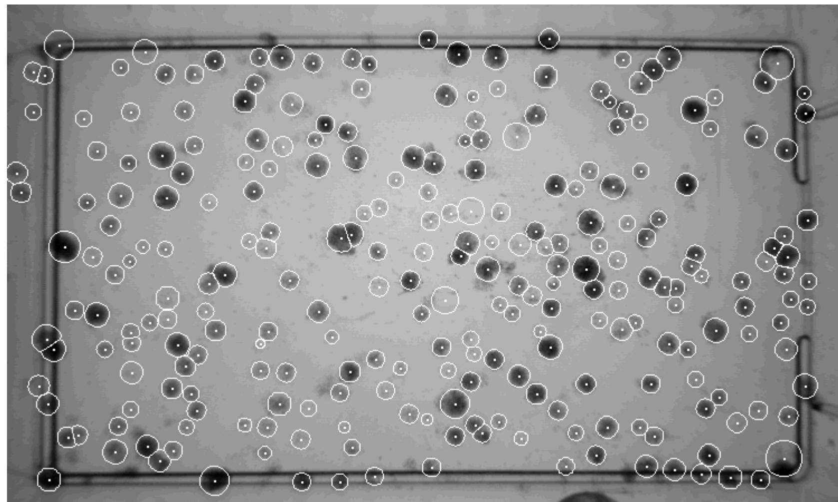

Figure 5. An example of local processing to determine the area of each colony.

An example of colony boundary determination is shown in Figure 5. Initial experiments on the statistics of colony areas show an initial dip in area from the 0 Gy value, rising to a peak near 1 Gy and then a gradual decrease in area with increasing dose. These observations are consistent with previous experiments [11]. The extra processing of colony areas takes less than 1 second per flask to complete.

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References

1. B.G. Wouters, and L. D. Skarsgard "The response of a human tumour cell line to low radiation doses: evidence of enhanced sensitivity." *Radiation Research* **138:** S76-S80, 1994.
2. S.C. Short and M. C. Joiner "Cellular response to low-dose irradiation." *Clinical Oncology* **10:** 73-77, 1998.
3. S.C. Short, S.A. Mitchell, P. Boulton, M. Woodcock and M. C. Joiner "The response of human glioma cell lines to low-dose radiation exposure." *International Journal of Radiation* **75:** 1341-1348, 1999.
4. S.C. Short, C. Mayes, M. Woodcock, H. Johns and M. C. Joiner "Low dose hypersensitivity in the T98G human glioblastoma cell line." *International Journal of Radiation* **75,** 847-855, 1999.
5. H. W. Thielmann and R. Hagedorn "Evaluation of colony-forming ability experiments using normal and DNA repair-deficient human fibroblast strains and an automatic colony counter." *Cytometry* **6**: 130-136, 1985.
6. R. L. Parry, T. W. Chin, K. Donahoe "Computer-aided cell colony counting." *BioTechniques* **10**(6): 772-774, 1991.
7. I. G. Wilson "Use of the IUL Countermat automatic colony counter for spiral plated total viable counts." *Applied and Environmental Microbiology* **61**(8): 3158-3160, 1995.
8. D. P. Mukherjee, A. Pal, S. Eswara and D. D. Majumder "Bacterial colony counting using distance transform." *Int. J. Bio-Medical Computing* **38**: 131-140, 1995.
9. S. J. Hoekstra, D. K. Tarka, R. O. Kringle and J. R. Hincks "Development of an automated bone marrow colony counting system." *In Vitro & Molecular Toxicology* **11**(2): 207-213, 1998.
10. K. Dobson, L. Reading and A. Scutt "A cost-effective method for the automatic quantitative analysis of fibroblastic colony-forming units." *Calcified Tissue Int.* **65**: 166-172, 1999.
11. I. Spadinger, B. Marples, et al. "Can colony size be used to detect low-dose effects?" *Radiation Research* 138: S21-S24, 1994.
12. Gonzalez and Woods "Digital Image Processing." Addison Wesley, 1993, Ch. 5.
13. T. Mouroutis, S. J. Roberts and A. A. Bharath. "Robust cell nuclei segmentation using statistical modelling." *Bioimaging* **6**: 79-91, 1998.
14. I. Sobel "An isotropic 3 × 3 image gradient operator." In H. Freeman, editor, *Machine Vision for Three-Dimensional Scenes*. Academic Press, 1990.