AbstractID: 8326 Title: Automated colony counting using color and image processing techniques

Purpose: To automatically and efficiently count cell-clusters of a clonogenic assay using color information and image processing techniques.

Method and Materials: A computer algorithm was designed in the Matlab (Natick, MA**)** programming environment to automatically count cell colonies. In this implementation, six-well tissue culture plates with stained colonies were scanned using a commercial flatbed scanner. These images were then read into the program as three-channel (Red, Green, Blue) color images. Color windows were applied to each channel to isolate colony clusters from the background. Region labeling techniques were then used to identify individual cell clusters. Next, individual cluster statistics (area, eccentricity, etc.) were obtained and used to determine if a cluster were a viable colony (>50 cells) and whether it was a single, double or triple colony. The number of colonies counted was compared with the number determined manually.

Results: The images obtained from the flatbed scanner were of high quality and were observed to have minimal shadowing effects. The time for the automated colony counting software to analyze and count individual wells was on the order of 7-20 sec. Comparison between manual and computer-assisted counting showed agreement of 3-5%. The Wilcoxon signed-rank test showed no statistically significant difference ($p > 0.05$) between the number of colonies counted manually or with the computer program.

Conclusion: Images from the flatbed scanner effectively represent the required colony data, and no advanced equipment is required to record the images. Color range filtering removed the need for most post-processing techniques and also is an effective method for dealing with errors from shadowing along the edges of the plates. Use of this automated technique will allow for a large quantity of clonogenic survival data to be analyzed in an efficient and consistent manner.