*Overview of the Algorithm*

Earlier we have described the fundamental issues in counting cells, specifically bovine endothelial cells, to this end we have developed a methodology that will be capable of addressing these issues and able to count cells. The cell-counting algorithm we have developed is described by the cascaded architecture displayed in figure (XX). The algorithm implements multiple image-processing techniques along with statistical analysis to accurately count cultured cells in an image.

Firstly an image must be acquired using a specific objective under a light microscope, the image is first prepared by removing all unnecessary noise and speckling that would cause difficulties in resolving individual cells.  A median filter, a nonlinear digital filtering technique, was used to remove random high intensity pixels that cause distortion and maintain cell membrane edges.  Optimizing the reduction of speckling and preservation of cell resolution was a necessary component during the initial processing step.  Once the median filter was applied, histogram equalization was used to enhance the image contrast and provide better cell membrane resolution.  Thresholding was then used as a means by which defined borders could be established based on pixel intensity values above and below a specific optimal range.  Once all image processing was conducted to enhance the distinct cell borders, the Moore neighborhood tracing algorithm was used to count the individual cells.  Every iteration of the Moore neighborhood algorithm traced the cell boundary and produced unique cell outlines that were then accumulated in a data structure. The final step of the algorithm analyzes the statistics of the cells accumulated in the data structure and is able to dynamically adjust its count for errors in the Moore neighbor-tracing algorithm.

In conjunction with a detailed discussion of the algorithm we trace through the algorithm with the use of a representative BECS images. The use of the representative image will illuminate and exemplify the properties of the methodology we have developed. Figure XX shows this representative image of bovine endothileial cells. This image is of interest to us because it displays both confluent cells and non-confluent cells. Furthermore, we notice that cells are more or less randomly oriented and there is a reasonable variability in cell sizes. And as we discussed earlier these cells are littered with organelles another characteristic problem in cell counting.

*Initial Filtering*

As discussed previously the properties of cell images are not congruent with the requirements of the simple Moore-Neighbor tracing algorithm. In order to meet these needs we apply both a median filter and adaptive histogram equalization filter to the image multiple times. Appendix XX verifies that there is no discernable difference in images that are filtered by the adaptive histogram filter multiple times followed in series with several median filtration cycles and those images that are filtered in multiple cycles of adaptive histogram equalization and median filtration one after another.

The need for adaptive histogram equalization arises from the lack of contrast in the initial images. Adaptive histogram equalization is the approach of choice to increase the contrast because it changes the contrast of the image in a section-wise fashion. Due to the non-homogenous lighting conditions these images are subjected to it becomes favorable to use adaptive histogram equalization. Figure XX compares the original grayscale image with the same image histogram equalization. From this we can see qualitatively that the contrast has increased, if we take a look at the intensity histogram of the filtered image we will find that the contrast has increased quantitatively as well. After applying this filtering technique we have achieved more defined borders in the image; this comes at the cost of increased borders around speckles, non-cell objects and organelles.

The adaptive histogram equalization method leaves much to be desired for the simple contour tracing we will use to count the number of cells in the image. The next step in the filtration process, median filtration, morphs the image into something that can be binary-thresholded and finally counted by the Moore-Neighbor function. Qualitatively, the median filtration has the effect of blurring the image; the traditional use of this filter is to remove salt and pepper noise in an image. This is highly desirable because in context of a BECS image this blends organelles into cell bodies and deteriorates the impact of speckling outside of cell boundaries. Figure XX shows how the original image becomes smoothed and the obvious reduction of speckling and degradation of organelles. Analytically the median filter selects a pixel and assigns to it the median pixel value from a specified neighborhood around the image. The effects of neighborhood size are explored in detail in our discussion of further research.

*Thresholding*

After undergoing the filtration process the grayscale image is ready to be thresholded and turned into a black and white image. <Daniel>

*Cell Counting*

Initial research and the problems