Counting cell colonies

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Now there's a really fascinating activity: looking at an endless number of flasks and counting all the faint and not-so-faint cell colonies that have been rendered visible through staining with crystal violet or some similar dye. So why not attempt to devise an imaging system that can do this automatically….Well, that's just we set out to do a few years ago and here are some details of our experiences.

Our first imaging device is shown on the right, Figure 1. It consisted of a standard video rate CCD camera with a short focal length (3.5 mm) lens imaging the flask. The idea was to 'see' a large field of view (i.e. wide-angle lens) which can focus on the lower flask surface while ensuring that the top surface is suitably out of focus: the presence of dirt/scratches etc. would thus not significantly affect the image. The other significant development was the use of an electroluminescent panel to provide even and somewhat faint transillumination of the flask. Faint illumination was a definite advantage since it allowed the lens to be fully opened and ensured that the focal plane was well-defined. The electroluminescent panel was driven at around 400 Hz, derived by dividing down the camera's line scan frequency.

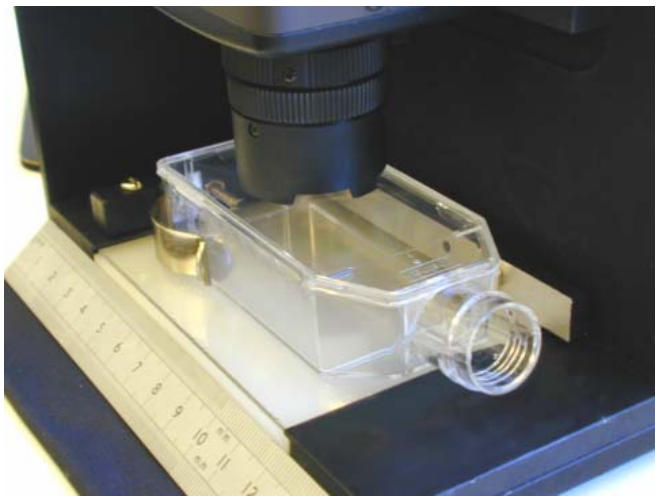

Figure 1: The 'business end' of the Mk1 cell colony counter imaging hardware; a box and curtain shielded the optics from stray room light.

Of course, lens barrel distortion was appreciable, but this was of secondary importance, since a variety of image processing steps were necessary to count the colonies anyway. Well, it all worked very well indeed, once the image processing software was worked out. Typical results can be seen in Figures 2 and 3.

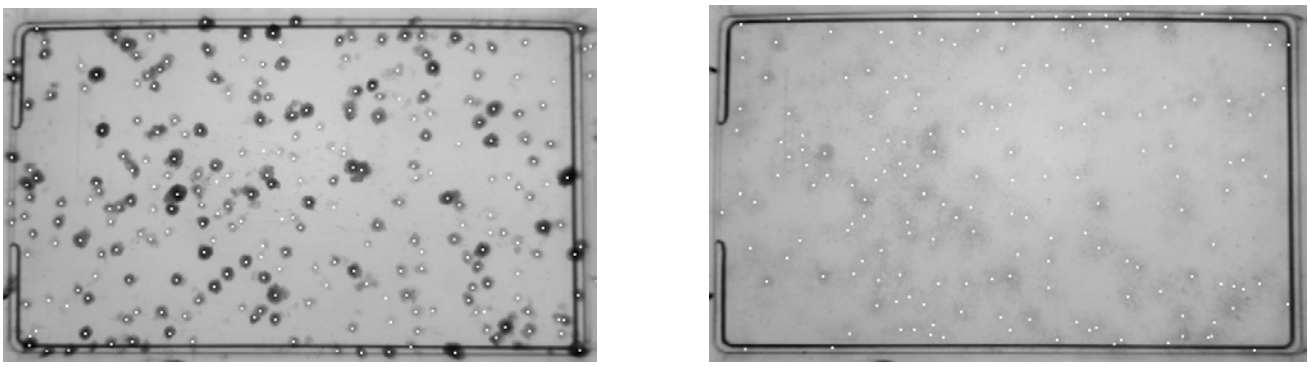

Figure 2: Processed and 'counted' images of well-defined colonies, formed from the A 172 cell line (left) and from the IN 1265 cell line right), which forms faint and indistinct colonies.

We then progressed to making a second unit which provided a larger field of view to enable larger flasks to be imaged, using a rather better lens (Schneider Cinegon 8 mm f.l. F/1.4) and a higher resolution (1024 x 1320 pixel) CMOS camera (Vitana PixeLink Imager/PLA 630 series) as well as a more convenient image acquisition interface (FireWire IEEE1394). This is shown in Figure 3. This also performed very well and is in fact still in use at GCI. So how do we process the images and discriminate between dirt, flask features and colonies, even when these are 'fuzzy', as is often the case with mammalian cell colonies?

Figure 3: The Mk2 colony counter hardware, based on the principles as the Mk1 version, but providing a larger working area.

Image Processing

Image distortion was corrected by recalculating pixel positions based on a measured centro-symmetric distortion profile. This profile was measured in the same manner as for image mosaicing (See: "Notes on image mosaics.doc"). Bilinear interpolation was used to provide a nice smooth resulting image.

The key to counting is the CHARM algorithm. CHARM stands for Compact Hough And Radial Map. A novel and compact Hough transform is performed to local the centre of any fairly circular objects (more detail can be found in: Barber, P.R., Vojnovic, B., Kelly, J., Mayes, C.R., Boulton, P., Woodcock, M. and Joiner, M.C. (2001) Automated Counting of Mammalian Cell Colonies. Physics in Medicine and Biology 46, 63-76.). From each object centre a searches are made in several (32) directions for likely edge points. When this is complete a radial map is built of each object. By comparing objects that overlap, overlaps and occlusions can be resolved and a nice segmentation of the objects can be achieved.

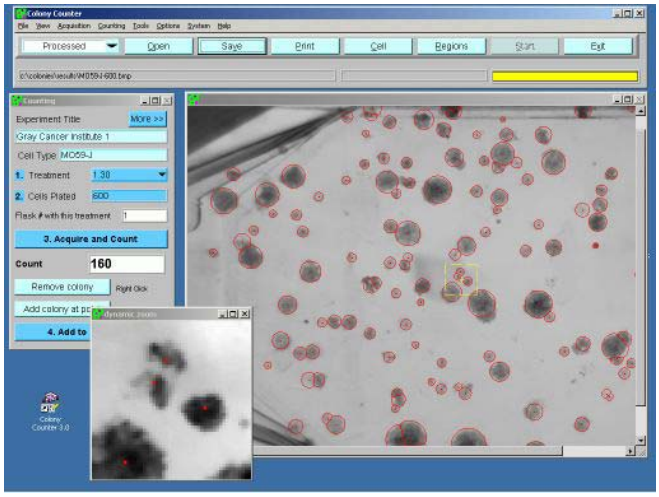

Figure 4: A screen shot of the version 3 software showing how the CHARM algorithm can resolve overlapping colonies.

Commercial Expliotation

In 2002, Oxford Optronix (www.oxfordoptronix.com) put the colony counter in their product lineup. After further development and polishing the ColCount went on sale, please visit their web site for more details.