

# CCD Technology compared with laser-based scanning for analysing fluorescently labelled proteins

by Dr P. Oatey

The increasing number of gene expression studies has led to a greater need for analysis of proteins on 1D and 2D polyacrylamide gels. As scientists are often looking for proteins which are expressed at low levels, they have become more demanding about the sensitivity of the dyes used to stain proteins and this has spurred the development of fluorescent dyes such as Sypro Ruby, Deep Purple and the Cy dyes, all of which have greater dynamic range and sensitivity than traditional visible Coomassie Blue or silver stains [1, 2].

The use of fluorescent labelling has in turn driven the need for equipment that can rapidly detect and analyse fluorescent protein bands and spots. Currently Charged Coupling Device (CCD) image analysers and laser scanners have emerged as capable of meeting these technically challenging demands and this article details how these two technologies compare in terms of sensitivity and speed.

## PRINCIPLES OF LASER SCANNING AND CCD IMAGING

Imaging a gel with a laser scanner is a two stage process involving excitation of the fluorescently labelled protein followed by amplification and signal capture. Excitation occurs when the scanner's laser beam passes over the gel surface making fluorescent dyes, which respond to the laser's wavelength, emit photons. The photons are detected by a photomultiplier tube (PMT) which converts them to electrons and these are accelerated and focused onto a dynode to produce a number of secondary electrons, (a process known as PMT gain). PMT gain is repeated at each subsequent dynode until all the secondary electrons are collected at the anode where the electrical signal produced is recorded by a computer.

Imaging with a CCD based system is also a two stage process in which fluorescently labelled proteins are excited to emit light but here the gel is illuminated with visible light shining from a Cy dye lighting module. The fluorescent signals from the whole gel are captured simultaneously by a digital camera containing a CCD, which is an electronic grid of light-sensitive diodes that convert photons into electrons. These are altered by the camera's built-in analogue-to-digital converter into a digital value which is downloaded to a computer.

## GENERATING IMAGES USING CCD AND LASER SCANNING TECHNOLOGY

To compare the performance of fluorescent gel imaging technologies, a CCD imaging system, Dyversity, (Syngene) set at 90micron resolution and a Typhoon 9400 laser scanner (GE Healthcare) set at 100micron resolution were used to image a 1D gel containing a titration series of two proteins, carbonic anhydrase (29kDa) and bovine serum albumin (66kDa), both labelled with Cy5 dye. A 2D gel containing total soluble proteins (50 µg) from *Arabidopsis* cell suspension was also labelled with Cy5.

The CCD based system produced an image of the 1D gel in 30 seconds and detected 0.5ng of BSA [Figure 1, page 16]. The

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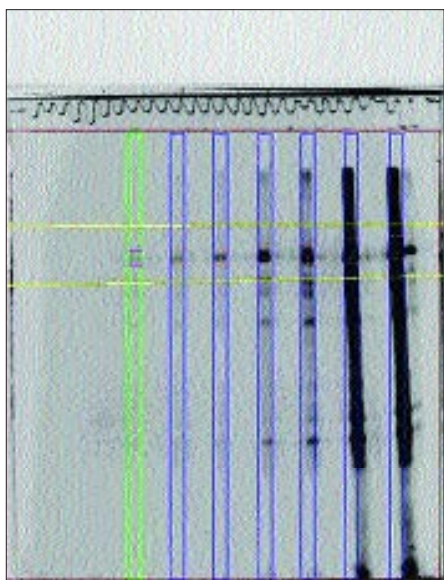


Figure 1. 1D polyacrylamide gel stained with Cy5 showing BSA (66kDa) top band from right to left in the following amounts: 0.05, 0.1, 0.5, 1, 2, 5, 10, 50 and 100ng. The gel image was captured using CCD technology.

laser based scanner also detected 0.5ng of protein in an eight minute scan [Figure 2].

Using GeneTools software (Syngene) the images were analysed and the dynamic range of each system was calculated as being 0.5-100ng for BSA; both systems showed 99 percent linearity of detection [Figures 3 and 4]. The CCD based system produced an image of the 2D gel in ten seconds and the laser scanner generated an image of the 2D gel in eight minutes. Images from each system were analysed with Dymension 3 software and the results showed a highly comparable pattern of



Figure 2. 1D polyacrylamide gel stained with Cy5 showing BSA (66kDa) top band from right to left in the following amounts: 0.05, 0.1, 0.5, 1, 2, 5, 10, 50 and 100ng. The gel image was acquired using a laser scanner.

protein peaks [Figures 5 and 6, page 18].

**SPEED OF IMAGE GENERATION**

The results show that given the correct camera, filter and illumination conditions, CCD technology can generate comparable images of fluorescently labelled proteins in 2D gels almost 50 times faster than laser scanning. The reason a CCD based system can produce images more rapidly is that the Cy dye lighting module illuminates the entire gel simultaneously while the digital camera images the whole gel in one step, so image acquisition times are not dependent on gel size but solely on the exposure time required to achieve the desired sensitivity. With a laser based system imaging time depends on the speed the laser moves across the gel (a process which scales linearly with gel size, so a 24cm x 18cm gel could take approximately three times longer to scan than an 18cm x 16cm gel) and the speed of the PMT gain. Since both processes are slower than

imaging with a CCD based system, overall image generation can take minutes rather than seconds.

**FUTURE PERSPECTIVES**

The development of CCD-based systems, which utilise a camera capable of detecting different visible emission peaks, integrated with a range of emission filters, a dual wavelength transilluminator, epi illumination and Cy dye lighting module means that the same high quality images of fluorescent proteins in 1D and 2D gels as those produced by laser scanner, are generated in a tenth to a fiftieth of the time it takes by laser scanning.

For future applications, CCD-based camera systems such as Diversity will become the method of choice in order to produce accurate images of fluorescently labelled proteins in large gels or to rapidly detect low abundance differential protein expres-

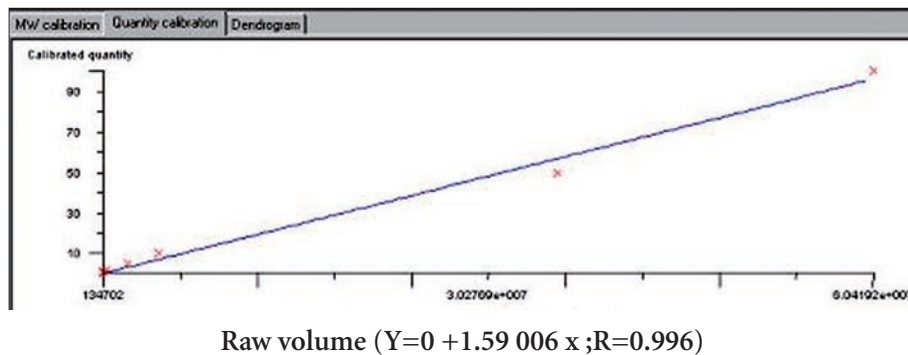


Figure 3. Dynamic range of a CCD based system imaging a titration of Cy5 labelled BSA.

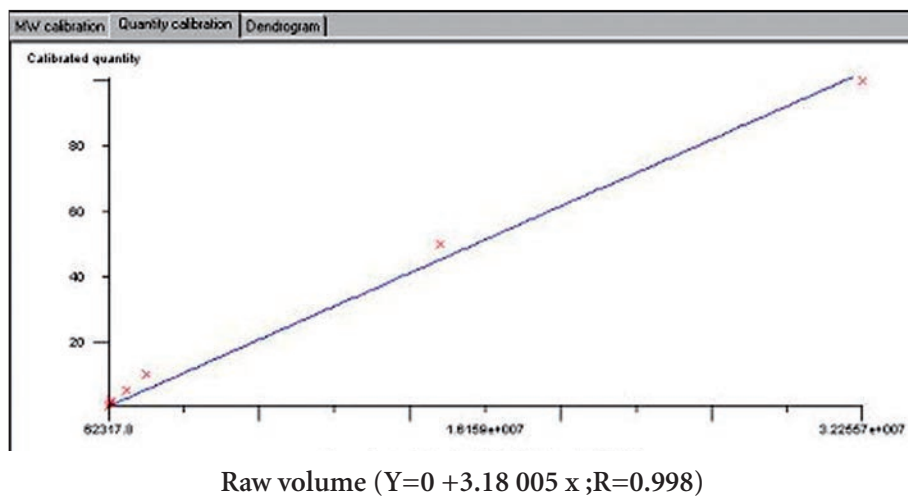


Figure 4. Dynamic range of a laser scanner imaging a titration of BSA labelled with Cy5.

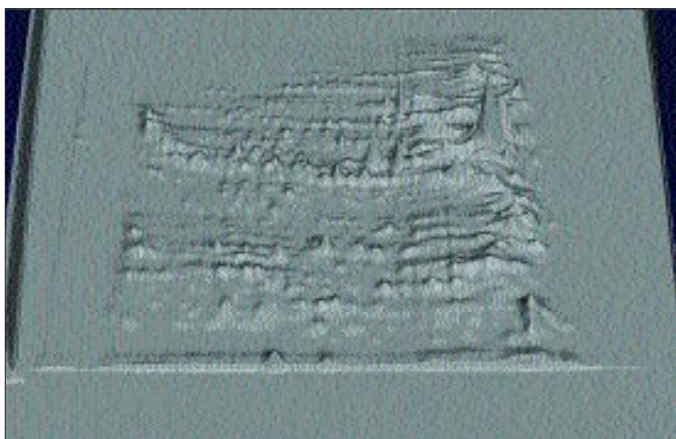


Figure 5. CCD generated image of 2D gel of Cy5 labelled Arabidopsis proteins.

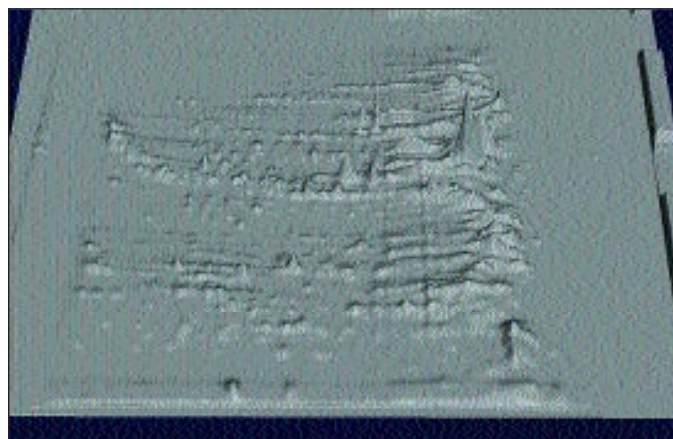


Figure 6. Image of 2D gel of Cy5 labelled Arabidopsis proteins generated by a laser scanner.

sion in DIGE gel images where the proteins may be labelled with up to three different Cy dyes.

**REFERENCES**

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2. Nishihara JC, Champion KM. *Electrophoresis* 2002; 23(14): 2203-15.

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