Dyversity and Typhoon 9400 imaging of Cy™ dye 1D and 2D gels

Aim

To compare the dynamic range, linearity, sensitivity, and image quality of the Dyversity imaging system (Syngene, UK) with the Typhoon™ 9400 laser scanner (GE Healthcare, UK).

Introduction

The Cyanine (Cy) fluorescent dye family are produced by GE Healthcare. This family of fluorophores are a popular choice for labelling proteins in both 1D and 2D gel applications.

The Typhoon 9400 imaging system can be used to image both 1D and 2D gels. This system can also be used to image DIGE (Differential In-Gel Electrophoresis) applications which use Cy dyes to label proteins for differential quantification on a single gel.

The Syngene Dyversity Image capture system is also designed for the rapid generation of 2D protein gel images including DIGE.

Materials and Methods

1D gel

A 12% acrylamide gel with 5% arcrylamide for the stacking gel, 18cm x 16cm. Both carbonic anhydrase (29KDa) (Sigma-aldrich, UK) and bovine serum albumin (BSA) (66KDa) (Sigma-aldrich, UK) were loaded on to gel. The loading buffer contains 300mM of DTT and 3x50 microgram stock solution of each protein labeled with Cy2™, Cy3™ or Cy5™. From the Cy dyes stock solutions a dilution series was used (0.05, 0.1, 0.5, 1, 2, 5, 10, 50 and 100ng). The same dilution series was also used for the each protein loaded on to the gel.

2D gel

3x50 microgram total soluble proteins from Arabidopsis cell suspension were labeled with Cy2™, Cy3™ or Cy5™ dyes and loaded in the first dimension gel (non-linear pH 3-10 IPG strip). The IPG strip was then applied to the second dimension (12% acrylamide gel 18cm x16cm).

Image Capture Systems

Typhoon 9400 laser scanner: lasers 488, 532 and 633; emission filters 520BP40, 580BP30, 670BP30.
Dyversity imaging system: 6.3M pixel camera KAF6303E with 50mm f1.4 lens; Cy dye lighting module and band pass emission filters, sample end mirror.
GeneSnap and GeneTools software for image acquisition and 1D gel analysis respectively
Dymension 3 software – 2D gel software analysis

Imaging Exposure Times

From the user's perspective the most significant difference between using Dyversity and Typhoon is in the imaging times. The scan times on Typhoon are solely determined by the scanning resolution, and the sensitivity is determined by the PMT (photo multiplier tube) gain. On Dyversity the scan times are only dependant on the sensitivity.

Typhoon

For an 18cm x 16cm gel at 100 micron resolution the scan time is 8 minutes per channel. Between scans the system takes around 1 minute to stabilize, so a 100 micron scan for all 3 dyes takes 27 minutes. To avoid saturating the images it is often necessary to take some "pre-scan" images at a lower resolution. At 1000 micron resolution for an 18cm x 16cm gel this takes 2 minutes per channel plus the 1 minute stabilization times before each scan, - a total of 9 minutes. These scan times will scale linearly with gel size, so a 24cm x 18cm gel would take approx 3.3 times as long, i.e. 26 minutes per channel at 100 microns, and 7 minutes for a "pre-scan" per channel at 1000 microns. During the scanning the computer is essentially 'locked' i.e. no other software can be used at the same time.

Dyversity

As Dyversity images the whole gel at once and the image times are not dependant on sample size but solely on the exposure time required to achieve the desired sensitivity. 1x1 binning gives a resolution of 90 microns per pixel. However, using 8x8 binning it is possible to pre-scan the gels checking for saturation with exposure times of 1 or 2 seconds. This gives a "live" saturation preview mode. Note that the exposure times used during the Dynamic Range Assessment, especially for the saturated images, longer exposures were used than those used on typical 2D gels, as the limits of the system's sensitivity was being tested. On "normal" 2D Cy dye gels the imaging times are typically under 60 seconds per channel.

Results

Dynamic Range Assessment

A 1D gel (12% acrylamide; 18cm x16cm) loaded with Cy2™, Cy3™ or Cy5™ labelled carbonic anhydrase and BSA protein was imaged using non saturating imaging conditions on both systems. In order to assess the data quantitatively images were compared in Dymension software with background correction and noise filtering applied to both Typhoon and Dyversity images (**Figure 1**).

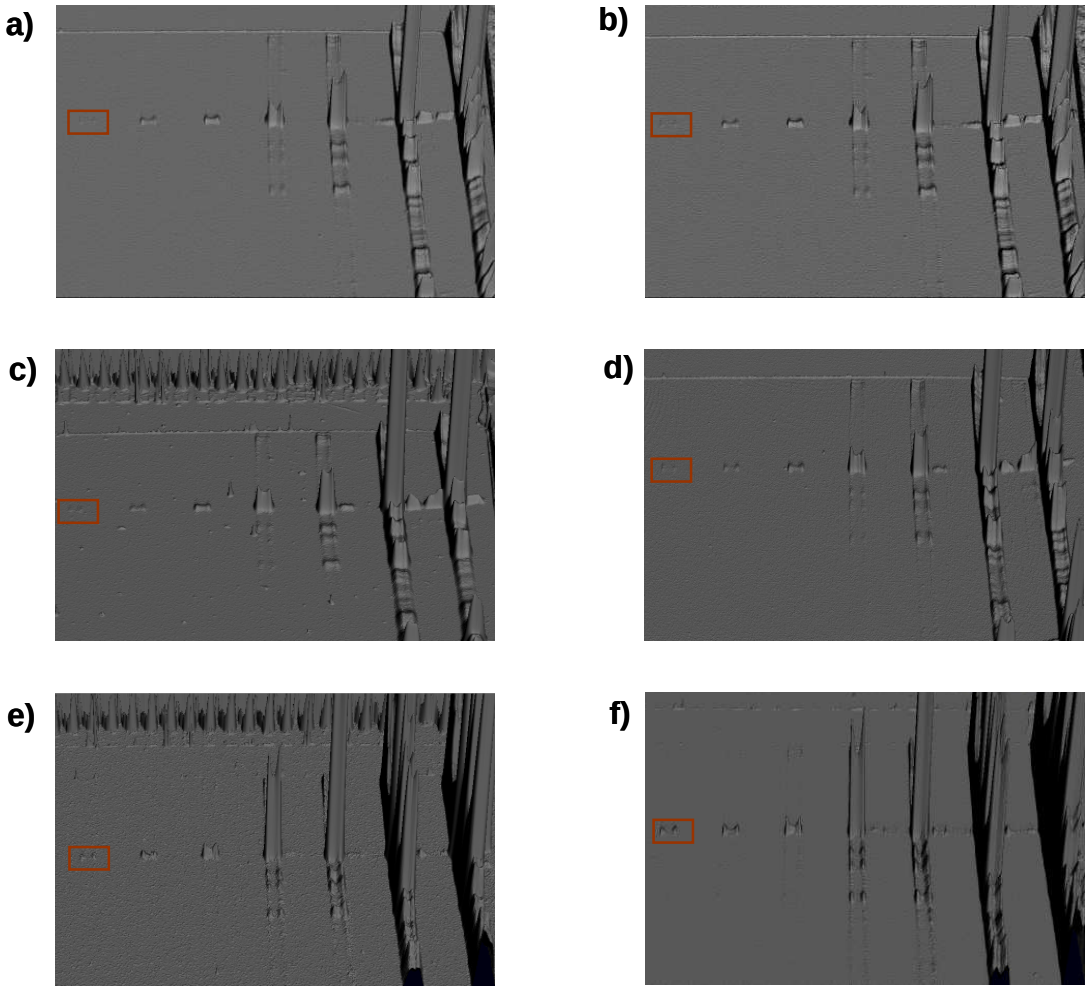


Figure 1- Dynamic range assessment of the Dyversity image capture system and the Typhoon 9400 scanner.

A 1D gel was imaged using the following parameters: For the Typhoon 9400 system (100 micron resolution) Cy2 PMT 525V, Cy3 PMT 550V and Cy5 PMT 530V each image was scanned for 8 minutes. For the Dyversity system (90 micron resolution) Cy2 and Cy3 imaged for a 2 minute exposure. Cy5 image was obtained from a 30 second exposure.

0.5ng of BSA protein was loaded on to the gel these protein peaks are highlighted by the red box.

a), c) and **e)** are Cy2, Cy3 and Cy5 dye gel images respectively using the Syngene Dyversity image capture system. **b), d)** and **f)** are **Cy2, Cy3** and **Cy5 dye** gel images respectively using the Typhoon 9400 scanner.

In order to examine the linearity of the signal a GeneTools analysis was performed for the Cy5 images using automatic track location, rolling disk correction (radius 30). All the bands were assigned quantities (for visible bands only) *i.e.*0.5,1,2,5,10,50 and 100ng and a quantity calibration curve was generated. A R^2 value close to 1 indicates the data is more linear. The dynamic range was also calculated.

The Cy5 gel images from both systems were analysed using GeneTools (**Figure 2**). The Cy5 image using the Dyversity image capture system had a linear regression of R^2= 0.996 and the Cy5 image using the Typhoon system had a linear regression of R^2=0.998.

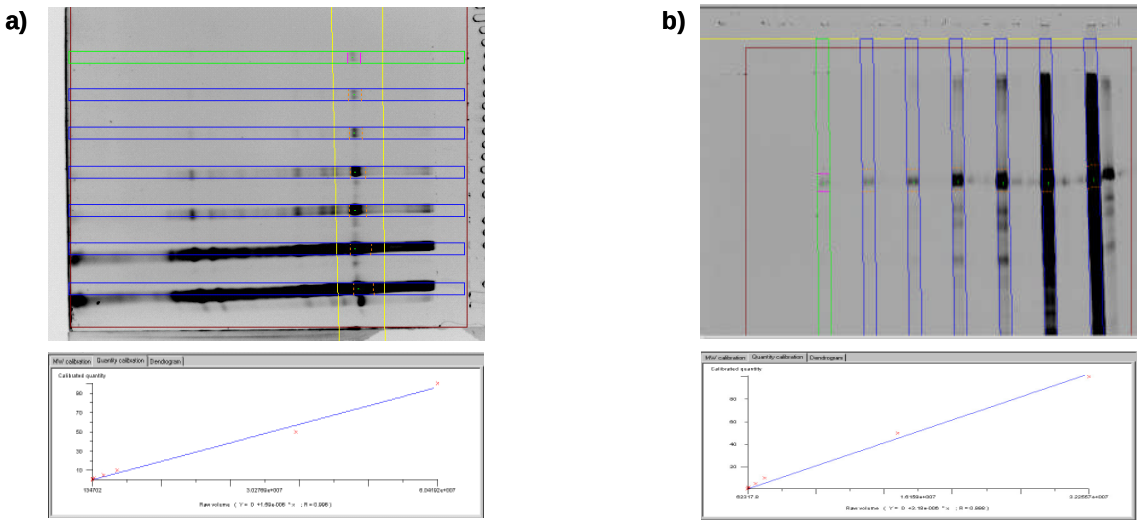


Figure 2- GeneTools analysis of Cy5 dye gel image fro both systems to determine signal linearity

a) Cy5 dye image using the Dyversity system. From the GeneTools analysis a R^2 value of 0.996 was determined. **b)** Cy5 dye image using the Typhoon scanner which had a R^2 value of 0.998.

The dynamic range of BSA protein labelled with Cy5 dye for concentrations ranging from 100 to 0.5 ng and imaged using the Dyversity or the Typhoon system was 2.3 orders of magnitude.

Sensitivity and Image Quality Assessment

The same ID gel was imaged using saturating imaging conditions and the following parameters were used: for the Dyversity image capture system (90 micron resolution) Cy2, Cy3 and Cy5 dye were imaged for 8 minutes, 4 minutes and 2 minutes respectively. For the Typhoon 9400 laser scanner (100 micron resolution) Cy2 PMT 735V, Cy3 PMT 750V and Cy5 PMT 775V were all scanned for 8 minutes.

To assess the sensitivity of each imaging system the Cy2 dye gel image from each imaging system was compared by observing the level of saturation present using GeneSnap software (**Figure 3**). When an image is saturated the peaks appear truncated.

To determine the quality of the images obtained from both imaging systems a 1D Cy3 dye gel image and a 2D Cy5 gel image were used (**Figure 4)**. The Cy5 2D gel was imaged using non saturating conditions using the following parameters: for the Dyversity Image capture system (90 micron resolution) Cy2 and Cy3 were imaged for 45 seconds and Cy5 dye was imaged for 10 seconds. For the Typhoon 9400 laser scanner Cy2 PMT 500V, Cy3 PMT 525V and Cy5 PMT 500V were scanned for 8 minutes each. For all of the images the background was examined using Dymension software. For 1D gel images the background correction and noise filtering was turned on and for 2D gels this was turned off.

Conclusions

For the BSA protein, the dynamic range, linearity and sensitivity are identical for both imaging systems (**Figures 1, 2** and **3**). The image quality was determined by examining the background of the 1D gel images (background corrected and noise filtered) and the signal for the raw/uncorrected 2D gel images in 3D mode revealed the presence of background artefacts for the Typhoon image only (**Figure 4b**). For the raw 2D gel images there is no apparent difference in the image quality indicating comparable performance.

Imaging with the Dyversity imaging system is faster compared to the Typhoon laser scanner for similar resolution and this has been demonstrated for 1D and 2D gels (e.g. Cy3 1D gel imaging is 4X faster and for Cy3 2D gel imaging is 10X faster).

The main advantage of the Dyversity imaging system compared to the Typhoon laser scanner is that the Dyversity image capture system provides fast acquisition times of 1D and 2D Cy Dye gels.

a) b)

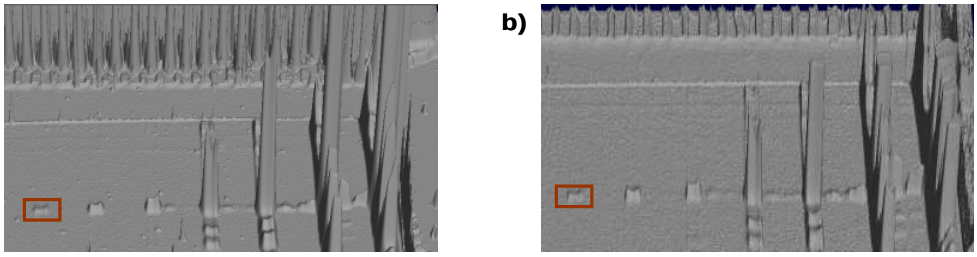
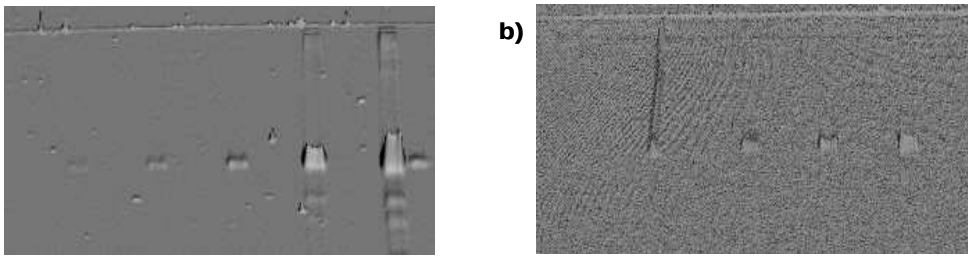


Figure 3- Sensitivity assessment comparing Cy2 dye gel images from the Dyversity and Typhoon image systems

a) and **b)** Cy2 dye gel imaged using the Dyversity image capture system and theTyphoon 9400 imager respectively.
The BSA 0.5ng peak is present on both gels (highlighted by the red box). The Typhoon image has truncated peaks showing that the peaks are more saturated compared to the Dyversity image capture system.

a) b)



c) d)

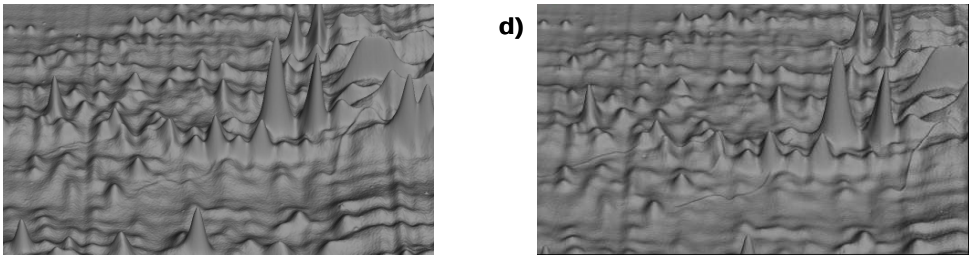


Figure 4- Image quality assessment comparing Cy dye gel images from the Dyversity and Typhoon image systems

a) and **b)** Cy3 dye gel imaged using the Dyversity image capture system and the Typhoon 9400 imager respectively. **c)** and **d)** Zoomed in gel image of the Cy5 dye imaged using the Dyversity system and Typhoon 9400 imager respectively.
The background is even and flat for the Dyversity (image a)) whereas the Typhoon image has swirls present on the background (image b)). There is no observed difference in image quality for 2D gels imaged on both systems.

Syngene reserves the right to amend or change specifications without prior notice. This Application Note supersedes all earlier versions.

May 2010

UK tel: +44 (0)1223 727123
Email: sales@syngene.com

USA tel: 800 686 4407/301 662 2863
Email: ussales@syngene.com

www.syngene.com