Imaging chemiluminescent blots – points to consider

Introduction

With the availability of CCD camera systems suitable for chemiluminescence detection, such as G:BOX iChemi XR, G:BOX iChemi XT and GeneGnome from Syngene, many scientists are now imaging their blots using these systems instead of film. There are many advantages in using a CCD camera for imaging: greater dynamic range, ease of analysis, no darkroom or messy developing solutions required, multiple images may be captured, image saturation can be automatically detected etc. To achieve the best results from a CCD camera, it is important to optimise capture conditions. This application note will guide the new user through the procedure, using an image capture system from Syngene.

Substrate

Which substrate is being used is an important consideration when imaging with a CCD camera. It is important to maximise the amount of light that is reaching the camera. Also, since the camera has a wide dynamic range and may collect signal over a longer period than possible with film, it is important to maintain light output.

Traditional ECL type substrates produce a flash reaction that results in a short-term emission of light. Over a period of 10-20 minutes, light output will diminish significantly. Although ECL or equivalent substrates can be used successfully with a Syngene image capture system, it is important that the blots are transferred immediately to the system after substrate addition. With blots that require longer exposure times, results from ECL may be less satisfactory.
To produce significant improvements in images collected by CCD, a change of substrate to a more modern reagent, for example, SuperSignal from Perbio, is recommended. In some countries Perbio are willing to provide free samples of reagents to customers who are having a demonstration from Syngene, or who have purchased a unit.

CDP Star or similar substrate, commonly used on Northern and Southern blots, works extremely well with a Syngene image capture system (eg G:BOX iChemi XT).

Capture

- Firstly open the GeneSnap software and enter in your username. At the top right hand side of the GeneSnap window, the configuration window is displayed. From the drop down menu, select the default configuration 'Chemi sample'. This will set up the system with the optimum parameters for chemiluminescent imaging.

  These are:
  No lighting
  No filter (allows maximum light to camera)
  Do not select EDR (Extended Dynamic Range) function. This is unsuitable for chemiluminescent imaging, in general.

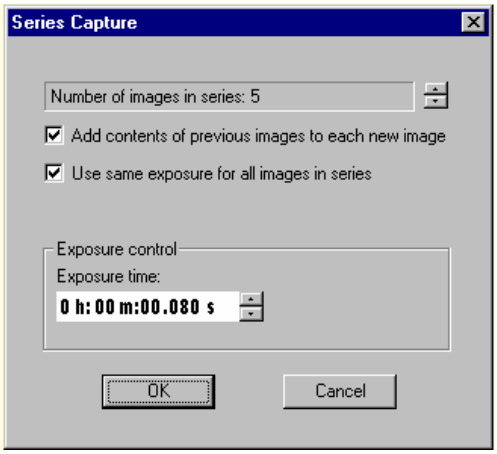
- Press the green button to view a live image and place your blot on the transilluminator. Keep the darkroom door open.

- Zoom in so that the blot fills the screen for maximum resolution, and if necessary adjust the fine focus for a crisp image.

- Open up the camera iris to maximum aperture.

- Now close the darkroom door.

- Select the series capture button to bring up the window



To decide how long to capture an image for, in the first instance, consider how long the blot would normally be put onto film. It may be necessary to expose for a little longer with a Syngene image capture system, since the dynamic range is so much greater, a lot more information can be recorded. Let us call the usual time to film X. In the first instance, we would recommend a series capture of 0.5X, X, 2X and 3X. If enough signal is collected before all of these images have been captured, it is possible to terminate the series by pressing the keyboard escape button.

Once the user is more familiar with imaging chemiluminescence using a Syngene image capture system, it is usually possible to predict more accurately how long an exposure will require. It is good practice to collect fewer, longer exposures, rather than lots of short ones. This reduces the overall background of an image and increases the signal to noise ratio.

You can watch the images being collected in GeneSnap. Once each image is downloaded, it can be examined whilst the next is collecting. If an image is to be used for quantitative analysis, it is important that the greyscale values within it are not saturated. To check this, click the saturation detection button on the right hand side of the GeneSnap window.

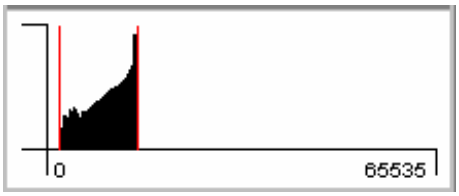
Any saturated spots or bands will appear in red. Areas appearing in blue are saturated black. Do not worry about these areas in chemiluminescent imaging, they indicate a clean background.

Optimising an image after capture

Once an image is captured, it is possible to optimise the settings within GeneSnap to obtain the best results. The Syngene image capture system camera has the ability to capture a vast amount of information. It is not possible to look at all of the information contained within the image at one time using a computer monitor.

To optimise the view it is best to adjust the histogram. View the histogram by clicking on the appropriate icon.

The histogram will dock at the base of the capture box -

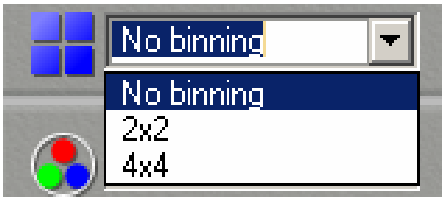


To obtain the best view of your image, adjust the red lines to alter contrast. With chemiluminescent images, the user often prefers to invert the image first, so that it appears like an autoradiogragh. Invert the image using the appropriate icon from the image toolbar.

Binning

The Syngene image capture system cameras have extremely high resolution (in the order of 1.4 million pixels depending on the system). Whilst this gives excellent spatial resolution, each pixel is relatively small, and this can have an impact upon sensitivity of the camera for chemiluminescence. The bigger the pixels, the more sensitive they are to low light levels. To allow maximum flexibility when imaging different sample types, we have incorporated a binning mode into GeneSnap.

Selecting the appropriate sensitivity level from the drop down menu will result in 2x2, 4x4 or 8x8 binning of pixels.



Each level of binning will lead to a significant decrease in exposure time required to visualise a sample. There will also be a corresponding decrease in resolution. When only looking for presence or absence of bands, the loss of resolution may not be an issue, but when looking at small differences between bands, it is best to use the highest resolution possible.

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

Syngene Europe
A Division of the Synoptics Group
Beacon House
Nuffield Road
Cambridge
CB4 1TF

Tel: +44 1223 727123
Fax: +44 1223 727101
Email: sales@syngene.com

Syngene USA
A Division of the Synoptics Group
5108 Pegasus Court, Suite M
Frederick
MD 21704

Tel: 800 686 4407/301 662 2863
Fax: 301 631 3977
Email: ussales@syngene.com