Fluorescence applications using the Typhoon Variable Mode Imager

Y. L. Han, W. Lew, and H. Butler
Amersham Biosciences Corp., Sunnyvale, CA USA

Typhoon™ Variable Mode Imagers can be used for a variety of fluorescence applications including protein and nucleic acid quantitation, immunoblotting, and microarray analysis. This article surveys the use of Typhoon in these applications. Limits of detection for common protein and nucleic acid gel stains are reported.

Introduction

Fluorescence detection offers many advantages over more traditional methods, such as colorimetry or autoradiography, and generally offers high sensitivity and a wide linear dynamic range (1). Fluorescence detection is safe and convenient, and allows multicolor analysis, which improves throughput and experimental accuracy.

Typhoon Variable Mode Imager combines multicolor fluorescence, chemiluminescence and filmless autoradiography in one system. For fluorescence applications, Typhoon employs powerful excitation sources and efficient optics to detect a wide range of fluorochromes at sub-femtomolar levels. In addition, Typhoon provides a wide linear dynamic range of five orders of magnitude. The system enables efficient imaging for a variety of sample formats, including free gels, glass gel sandwiches, blots, microplates, and slides. In combination with ImageQuant™ Solutions analysis software, Typhoon is a powerful tool for a wide range of fluorescence applications (2, 3, 4).

Typhoon fluorescence applications

This article focuses on a selection of fluorescence applications performed using Typhoon imagers. Typhoon 8600 and 9200 series have green (532 nm) and red (633 nm) lasers and the 9400 series have an additional blue laser with two excitation lines (457 nm and 488 nm). For most of the gel and blot imaging applications, pixel sizes from 100 µm and above are appropriate, which are available on all models. The higher resolution, 10-µm pixel option for microarray imaging is available on the 9210 and 9410 models.

Fluorescent protein stains

SYPRO™ protein gel stains are fluorescent stains for both standard and 2-D protein gels. When imaged with Typhoon, SYPRO stains offer a detection limit of low nanogram level plus a much wider linear dynamic range (1000 fold) than traditional silver staining (typically 10 fold). Table 1 summarizes limit of detection (LOD) and linear ranges for protein gels stained with SYPRO stains (LOD is the threshold at which the background-corrected signal-to-noise ratio is at least three).

Table 1. Summary of Typhoon limit of detection (LOD) and linear range for BSA bands stained with SYPRO Orange, Red, Ruby, and Tangerine

Gel stain	LOD (ng/band)	Linear range
SYPRO Orange	2	~ 1000 fold
SYPRO Red	2	~ 1000 fold
SYPRO Ruby	1	~ 2000 fold
SYPRO Tangerine	2	~ 1000 fold

The linear range of protein detection using SYPRO stains (Fig 1) is typically up to three orders of magnitude. The major factors limiting sensitivity and linear range are the background staining of the gel, imperfect stain/protein binding stoichiometry, and other experimental factors. This suggests that Typhoon, which offers a linear dynamic range of five orders of magnitude, has the potential of offering better imaging results when improved fluorescence staining methods become available (5).

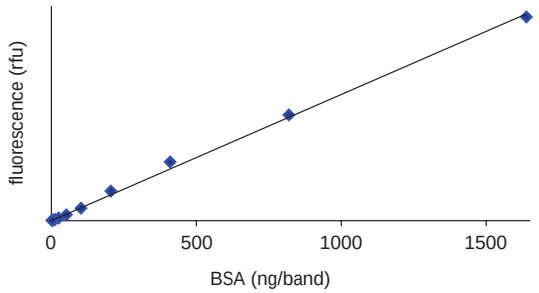

Fig 1. Linear range of protein detection using SYPRO Red. Fluorescence refers to the integrated relative fluorescence units (rfu) from ImageQuant volume analysis using rectangle objects and local median background correction. The linear fit to the data has an R^2 value of at least 0.99.

The large scan area of Typhoon (35 × 43 cm) conveniently allows imaging of large size, or multiple 2-D gels without sacrificing resolution. Furthermore, the confocal optics coupled with the moving-head mechanism offer superior image uniformity and eliminate the artifacts commonly seen with galvanometer systems. Figure 2 shows a 2-D protein gel stained with SYPRO Ruby and imaged using the Typhoon 532 nm laser line (6). The 457 nm and 488 nm laser lines were also tested for the same gel. No significant difference in the detection sensitivity was observed among laser lines.

Additionally, Typhoon can image protein solutions in microplate format with stains such as NanoOrange™ protein quantitation reagent (1).

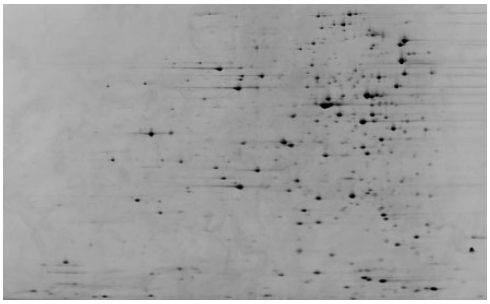

Fig 2. SYPRO Ruby staining of a 2-D gel. A total protein extract of *E. coli* (100 µg) was separated in the first-dimension using 18 cm Immobiline™ DryStrip gel (pH 4–7). The second dimenstion was separated on a 12%, 1 mm thick Laemmli gel using Ettan™ DALT*twelve* system. The gel was stained overnight with approximately five volumes of SYPRO Ruby and imaged using the 532 nm laser line of Typhoon.

Fluorescent Western blotting

Fluorescent Western detection employs either a direct or an enzyme-amplified (chemifluorescence) method, and Typhoon is compatible with both formats. Detection limits at sub- or low-nanogram level can be routinely achieved with these methods (7).

The direct fluorescence method uses antibodies labelled with fluorescent dyes, such as Cy™3 or Cy5. Multicolor imaging on Typhoon allows the detection of multiple protein targets simultaneously on the same blot, which improves throughput and accuracy. Figure 3 shows the Typhoon image of a direct fluorescent Western using Cy3 for actin and Cy5 for tubulin detection.

Typhoon also enables the detection of the fluorescent products from the chemifluorescence reactions using substrates such as ECL Plus™ and ECF™. Chemifluorescence detection of Western blots is potentially a more sensitive method than direct fluorescence due to signal amplification. Chemifluorescence can be used in place of any standard chemiluminescence method that uses alkaline phosphatase (AP)- or horseradish peroxidase (HRP)-conjugated antibodies with minimal protocol modification.

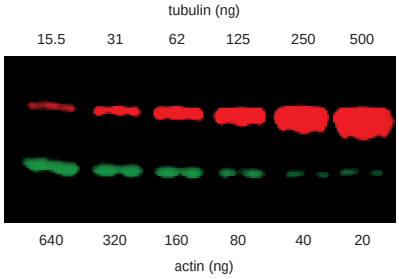

Fig 3. Typhoon image of a dual target Western blot. Serial two-fold dilutions of actin and tubulin were separated by electrophoresis and transferred to Hybond™-P blotting membrane (see reference 7 for details). Tubulin (red) was detected using anti-β-tubulin monoclonal antibody and Cy5-linked anti-mouse IgG. Actin (green) was detected with rabbit anti-actin antibody and Cy3-linked anti-rabbit IgG. Amounts are indicated above and below the gel image. Cy3 and Cy5 were imaged with 532 nm and 633 nm laser lines respectively.

Fluorescent microarray imaging

The 10-µm pixel option on Typhoon 9210 and 9410 allows high-resolution scanning suitable for microarray applications (Fig 4). Typhoon offers high sensitivity for successful detection of genes at very low relative abundance levels. While Typhoon has a wide linear dynamic range of five orders of magnitude, typically the linear range of microarray hybridization is limited to 2.5–3.5 orders of magnitude (8).

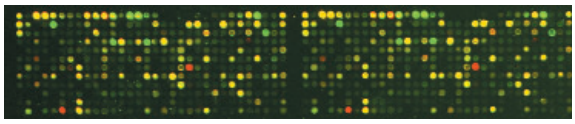

Fig 4. A section of a microarray slide imaged on Typhoon 9410. Human tissue cDNA from skeletal muscle (labelled with Cy3, shown in green) and cDNA from liver (labelled with Cy5, shown in red) were mixed and hybridized onto a slide spotted with human genes in duplicate.

Fluorescent DNA stains

Ethidium bromide is a popular choice for fluorescence detection with agarose gels. More sensitive stains, such as Vistra Green™ Nucleic Acid Stain, are available for use with both agarose and polyacrylamide gels. Table 2 summarizes the optimal laser settings, the LOD and linear dynamic ranges for gels stained with ethidium bromide and Vistra Green. Using Typhoon, the limits of detection for DNA in agarose or polyacrylamide gels are equal to or better than sensitivities obtained using standard camera systems with UV illumination.

Table 2. Typhoon detection limits for a 1.2 kb DNA band in either 1% agarose or 10% polyacrylamide gels determined using ImageQuant software

Gel stain	Laser	Agarose/Acrylamide LOD (pg/band)	Linear range
Ethidium bromide-cast with gel	Green (532)	50/ND*	~500 fold
Ethidium bromide-post-stain	Green (532)	50/6	~500/1000 fold
Vistra Green-post-stain	Blue (488)/ Green (532)	10/3	~500/1000 fold

*ND = not determined

Additionally, Typhoon can image nucleic acid solutions in microplate format with stains such as PicoGreen™ dsDNA quantitation reagent (1).

Fluorescent multiplex PCR and in-lane fragment analysis

Multicolor fluorescence imaging of PCR◊ products enables in-lane fragment analysis where standard and unknown fragments are resolved in the same lane. This time- and cost-effective approach eliminates lane-to-lane variation in electrophoretic migration.

Results from a demonstration of this method are shown in Figure 5. A Cy5-labelled size standard and various Cy3-labelled fragments were combined and separated by electrophoresis. Fluorescent signals from Cy3 and Cy5 labels were resolved using two-channel imaging on Typhoon. The lengths of five different Cy3-labelled fragments were determined using the Cy5 size standards resolved in the same lane (Fig 5, lane 3).

Using Fragment Analysis software (part of ImageQuant Solutions software), the mobility of the Cy5 fragments was used as a reference to determine the sizes of the Cy3 fragments. The Cy3 fragment sizes were calculated accurately to within 2.5% of their known values (9).

Conclusions

Typhoon Variable Mode Imagers provide high sensitivity, signal uniformity, and wide linear dynamic range across fluorescence applications. Typhoon fluorescence imaging can be used for a wide range of nucleic acid and protein applications and with a variety of sample formats including gels, blots, slides and microplates. The multicolor capability allows improved throughput as well as the flexibility of imaging a variety of fluorochromes.

References

1. *Fluorescence Imaging: Principles and Methods*, Amersham Biosciences, 63-0035-28, (2000).

◊ See licensing information.

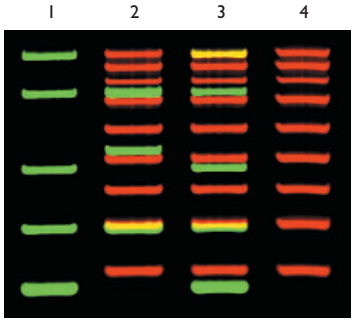

Fig 5. Cy3- and Cy5-labelled DNA fragments. Lane 1, Cy3-labelled (green) fragments (500 bp, 365 bp, 230 bp, 150 bp, 88 bp); lane 2, Cy5-labelled (red) size ladder (500 bp, 450 bp, 400 bp, 350 bp, 300 bp, 250 bp, 200 bp, 150 bp, 100 bp) with Cy3-labelled fragments (365 bp, 268 bp, 150 bp); lane 3, Cy3-labelled fragments with Cy5-labelled size ladder; lane 4, Cy5-labelled size ladder. The presence of both Cy3 and Cy5 signal in the same region of the gel is displayed as yellow by ImageQuant software (lanes 2 and 3).

2. Qi, X. *et al.* L-RCA (ligation-rolling circle amplification): a general method for genotyping of single nucleotide polymorphisms (SNPs). *Nucleic Acids Res.* **29(22)**, e116 (2001).
3. Greenbaum, D. *et al.* Chemical approaches for functionally probing the proteome. *Mol Cell Proteomic* **1**, 60–68 (2002).
4. Man, T-K. and Stormo, G. D. Non-independence of Mnt repressor-operator interaction determined by a new quantitative multiple fluorescence relative affinity (QuMFRA) assay. *Nucleic Acids Res.* **29**, 2471–2478 (2001).
5. Application Note #66: Fluorescent protein gels stains, Amersham Biosciences, 63-0031-04 (2000).
6. Application Note #69: Imaging two-dimensional protein gels stained with SYPRO Ruby, Amersham Biosciences, 63-0043-06 (2001).
7. Application Note #68: Fluorescent Western blotting, Amersham Biosciences, 63-0043-05 (2001).
8. Application Note #70: Fluorescent microarray imaging and analysis, Amersham Biosciences, 63-0043-07 (2001).
9. Application Note #67: Fluorescent multiplex PCR and in-lane fragment analysis, Amersham Biosciences, 63-0031-84 (2001).

ORDERING INFORMATION

Typhoon 9200 and ImageQuant Solutions for Windows 2000	1	63-0038-49
Typhoon 9200 and PC Workstation	1	63-0038-50
Typhoon 9210 and ImageQuant Solutions for Windows 2000	1	63-0038-51
Typhoon 9210 and PC Workstation	1	63-0038-52
Typhoon 9400 and ImageQuant Solutions for Windows 2000	1	63-0038-53
Typhoon 9400 and PC Workstation	1	63-0038-54
Typhoon 9410 and ImageQuant Solutions for Windows 2000	1	63-0038-55
Typhoon 9410 and PC Workstation	1	63-0038-56