Reprinted from *American Biotechnology Laboratory* October 1999

Using intensifying screens in autoradiography to improve results

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AUTORADIOGRAPHY IS the most widely used method of visually detecting radioisotope-labeled products in life science research. Autoradiography may be divided into two categories: macroautoradiography and microautoradiography. Microautoradiography involves the use of liquid emulsions into which a sample is immersed (e.g., NTB-2 and NTB-3, **Eastman Kodak Co., Scientific Imaging Systems,** New Haven, CT) to expose individual silver grains and then visualize them under a light microscope. Macroautoradiography, the subject of this applications note, involves the use of radiography films (such as X-Omat AR [XAR] film, **Eastman Kodak Co., Scientific Imaging Systems**) to create density patterns that can be examined visually. A flat format of radioisotopes, usually the result of a chromatographic or electrophoretic separation, intimately contacts and directly exposes a radiographic film. Faster film exposure may be attained using indirect autoradiography, which converts the emitted energy to light by means of a scintillator (a fluorescent medium or intensifying screen), simultaneously exposing the radiography film. A third category of exposing a radiography film with a luminescent object (such as chemiluminescence) is outside the realm of this paper.

Intensifying screens have two general purposes: 1) to reduce the exposure time, and 2) to increase the sensitivity of a radiography film upon exposure to ionizing radiation. Intensifying screens generate many hundreds of photons upon absorbing the energy of a single isotope's decay. To generate a stable latent image, a silver halide crystal in the film requires multiple photons (*Figure 1*), but can be stabilized by the massive excitation from a single ionizing event occurring through radioactive decay. Reducing the temperature of a film during indirect exposure stabilizes the number of excited crystals, thereby enhancing the accumulation of a stable latent image and increasing the response of the film. (Note: Reducing the temperature of film in direct exposures does not increase the sensitivity of the film.) The optimal temperature for stable latent image formation with a film/screen system is about –70 °C.

Most intensifying screens used in life science research today contain $CaWO_4$ phosphor, which emits photons in the blue region of the spectrum (wavelength). The performance of these screens varies significantly with the grade of phosphor used, the size of the grains, and the coverage (consistency and thickness) of the $CaWO_4$. A wide variation in performance of $CaWO_4$ intensifying screens can be anticipated. These screens were developed for the health-care industry, where they were used to enhance film sensitivity to X-rays. To detect X-rays, a sheet of film is sandwiched between two intensifying screens to maximize sensitivity. In autoradiographic applications, a double screen format rarely

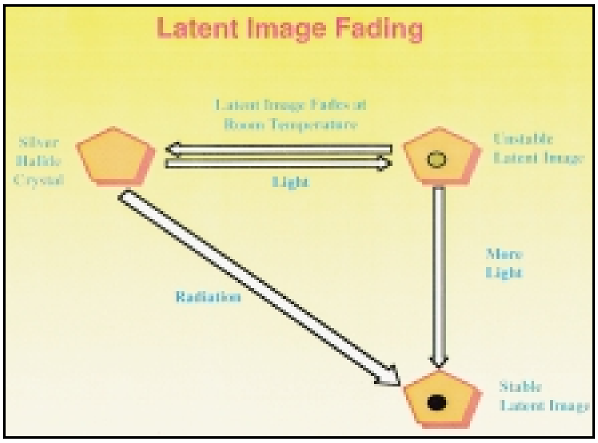


Figure 1 *Formation of a stable latent image.*

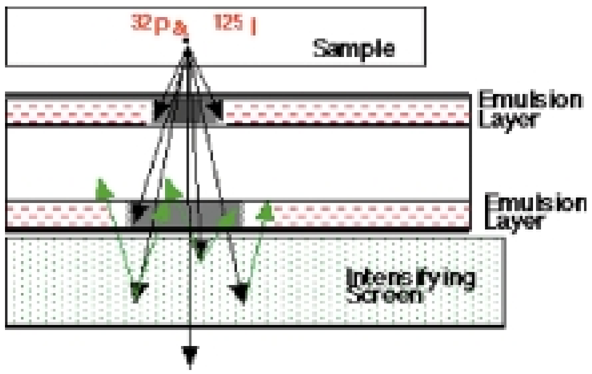


Figure 2 *Conventional screen arrangement.*

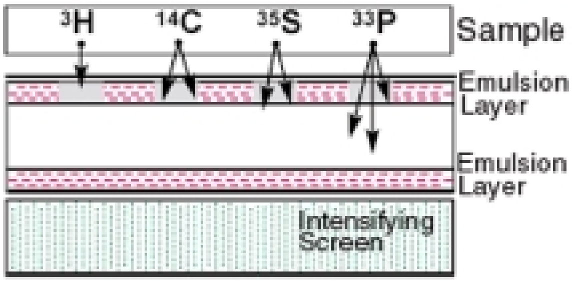


Figure 3 *Interaction of low- and moderate-energy radioisotopes with a conventional intensifying screen.*

offers any benefit because the sample usually obscures one of the screens. The evolution of phosphor technology has led to the introduction of gadolinium oxysulfide:terbium (GOS), a very efficient phosphor that emits in the green region of the spectrum. The BioMax MS intensifying screen (**Eastman Kodak Co., Scientific Imaging Systems**) was the autoradiographer's first exposure to GOS phosphor. Use of the high-performance GOS phosphor screens is steadily increasing.

In autoradiographic applications, conventional intensifying screens are applied by placing a sample onto a sheet of radiography film, which is subsequently placed onto a screen (*Figure 2*). Radiation emanating from a sample must first pass through the film to reach the intensifying screen. Having penetrated the film, the radiation interacts with the screen, resulting in the emission of photons that then interact with the emulsion layer of the film. The most common radioisotopes used in autoradiography are ^{32}P, ^{125}I, ^{14}C, ^{35}S, ^{33}P, and ^{3}H. For an intensifying screen to be of benefit under the conventional format, the radiation emanating from the radioisotope must have sufficient energy (an approximate beta energy of greater than 0.4 MeV) to reach the intensifying screen. Therefore, conventional intensifying screens are limited to use with high-energy radioisotopes (i.e., ^{32}P and ^{125}I) only. Low- and moderate-energy radioisotopes (i.e., ^{14}C, ^{35}S, ^{33}P, and ^{3}H) lack sufficient energy to penetrate the plastic substrate of the film. The radiation emanating from low- and moderate-energy radioisotopes is attenuated or blocked by the film prior to reaching the second emulsion layer, let alone the phosphor layer of the screen (*Figure 3*).

This limitation of conventional screens can be frustrating to the autoradiographer, because it is these lower-energy radioisotopes that require the greatest boost for detection by film. The BioMax TranScreen system (**Eastman Kodak Co., Scientific Imaging Systems**) is an intensifying screen designed specifically for scientific applications. The system incorporates GOS phosphor with a means for improved detection of low-

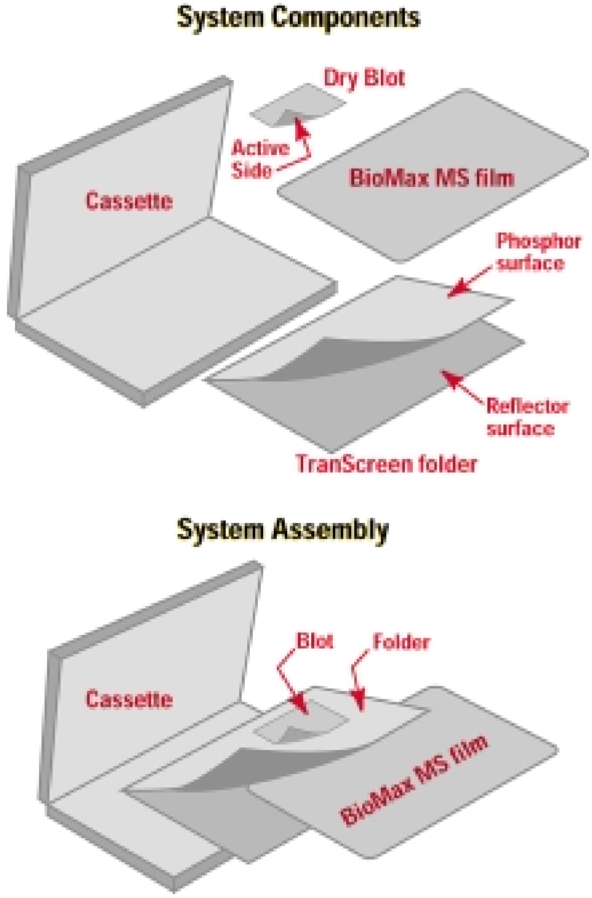
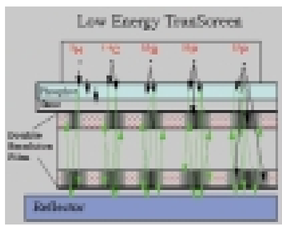


Figure 4 *BioMax TranScreen assembly.*



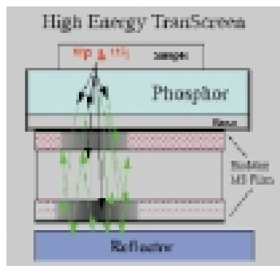


Figure 5 *BioMax TranScreen low- and high-energy interaction with isotopes.*

and moderate-energy radioisotopes as well as improved detection of high-energy radioisotopes (*Figure 4*). Patented TranScreen technology permits the radiated particles to interact with the phosphor of the intensifying screen prior to interacting with the film (*Figure 5*).

This screen configuration eliminates any attenuation by the film support. The system is offered in two formats: The TranScreen HE intensifying screen is designed for high-energy radioisotopes (^{32}P and ^{125}I), and the TranScreen LE intensifying screen is an all-purpose intensifying screen. Both use GOS phosphor technol-

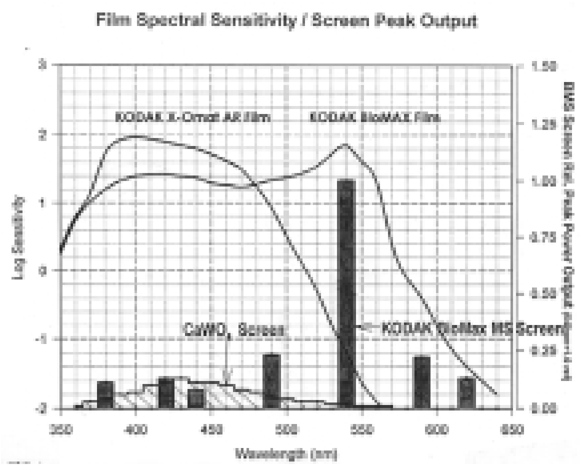


Figure 6 *Intensifying screen emission output compared with film spectral sensitivity.*

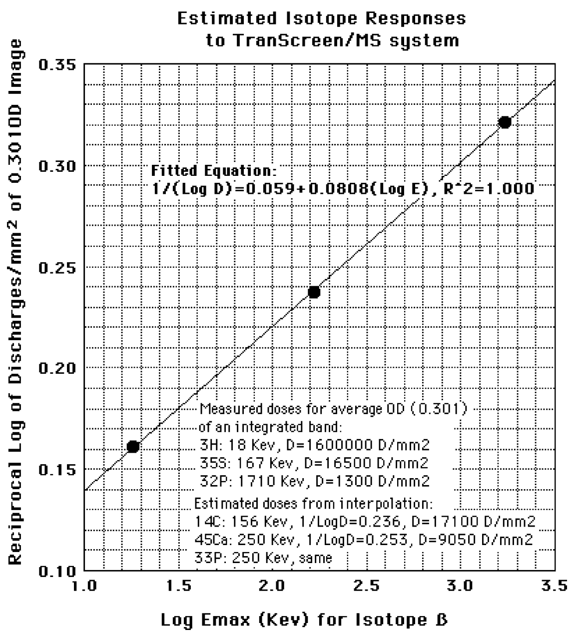


Figure 7 *BioMax TranScreen/BioMax MS film response curve.*

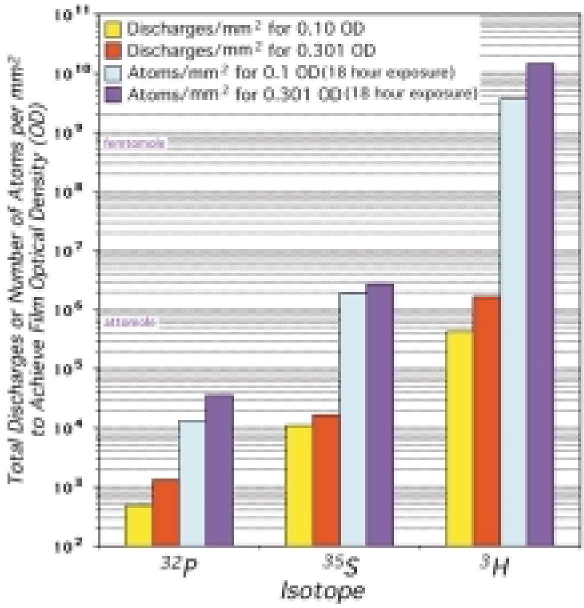


Figure 8 *BioMax TranScreen/BioMax MS film sensitivity table.*

ogy, which emits photons at a wavelength that matches the peak response of the BioMax MS film, to form an optimal match (*Figure 6*). Furthermore, the phosphor grain sizes, purity, and coverage are carefully controlled to provide consistent performance.

The TranScreen LE intensifying screen (the only intensifying screen for use with low- and moderate-energy radioisotopes), when combined with BioMax MS film, decreases the exposure times of low- and moderate-energy isotopes by 5× in comparison to X-Omat AR film. For penetrating radiation, such as ^{32}P and ^{125}I, the BioMax TranScreen LE/BioMax MS film confers significantly higher image resolution while exceeding the sensitivity of generic screen/X-Omat AR film. The thicker phosphor provided by the TranScreen HE intensifying screen converts more of the penetrating radiation (i.e., ^{32}P and ^{125}I) energy to photons. When combined with BioMax MS film, the TranScreen HE offers a greater than 4× improvement in sensitivity for the detection of high-energy radioisotopes compared to XAR film with a conventional screen.

BioMax TranScreen intensifying screens can be used with any film. However, BioMax MS film and BioMax TranScreen intensifying screen combinations offer optimal performance because the photons emitted from the screen are spectrally matched to BioMax MS film. A common issue in autoradiography is determining the length of an exposure. A helpful guide for exposure is summarized in *Figure 7*, which indicates the amount of isotope required to obtain a 0.301 o.d. image (50% transmission).

Optimal screen/film combination

Table 1 lists film and intensifying screen combinations for achieving improved performance. Most users seek improvements in two major areas of analysis: sensitivity and resolution. Increasing the sensitivity of a particular analysis will allow one of two improvements:

Table 1

Recommended screen/film combination

Applications	Screen/film combination
Maximum sensitivity for ^{32}P and ^{125}I with improved image quality	TranScreen HE Screen/BioMax MS film
High sensitivity with high resolution for ^{32}P and ^{125}I (greater sensitivity than XAR film with a generic screen—requires no change in protocol)	TranScreen LE Screen/BioMax MS film
Substitute for fluorography—improved sensitivity and resolution (i.e., ^{14}C, ^{3}H, and ^{35}S)	TranScreen LE Screen/BioMax MS film
Shortest exposure times for low- and medium-energy beta-emitting isotopes (i.e., ^{3}H, ^{14}C, ^{35}S, ^{45}Ca, ^{33}P, and ^{59}Fe)	TranScreen LE Screen/BioMax MS film

Table 2

BioMax TranScreen intensifying screen/film detection sensitivities*

Isotope	Screen	Total discharges/mm^2 required to create a medium image (50% net transmission) (not dpm)	Number of mol/mm^2 required to achieve a medium film response (18-hr exposure @ –70 °C)
^{32}P	BioMax TranScreen HE intensifying screen	1.3×10^3	6.0×10^{-20}
^{35}S	BioMax TranScreen LE intensifying screen	1.7×10^4	4.6×10^{-18}
^{14}C	BioMax TranScreen LE intensifying screen	1.7×10^4**	4.6×10^{-13}**
^{45}Ca	BioMax TranScreen LE intensifying screen	9.1×10^3**	4.7×10^{-18}**
^{33}P	BioMax TranScreen LE intensifying screen	9.1×10^3**	7.2×10^{-19}**
^{3}H	BioMax TranScreen LE intensifying screen	1.6×10^6	2.3×10^{-14}

*The measures cited above are average band densities for isotopes deposited on a white backing. Total doses of one-third to four times these values are recommended to take advantage of film contrast. Note that these estimates are given as total discharges; hence, a typical small target on a Southern blot may be 100 dpm of ^{32}P confined to a 10-mm^2 band, yielding a perceptible image in only 100 min of film exposure. Very low energy isotopes, especially ^{3}H, are subject to self-attenuation; the estimates derive from infinitely thick acrylic samples. Surface deposits of ^{3}H may yield a much higher response per decay.

**Estimated values.

1) shortening the exposure time for the same amount of isotope and thereby decreasing the total protocol time, or 2) keeping the exposure protocol the same but reducing isotope consumption, improving the cost per analysis.

Table 2 and *Figure 8* demonstrate the high sensitivity of the BioMax TranScreen system by listing the minimum levels of isotope required to achieve a film response (0.301 or 0.1 o.d.) for various beta emitters. The BioMax TranScreen HE is used to determine the minimum level for ^{32}P, while the BioMax TranScreen LE is used for ^{3}H, ^{14}C, ^{35}S, ^{45}Ca, and ^{33}P.

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