

A universal intensifying screen system for enhanced detection of low- and high-energy isotopes

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A NOVEL SCREEN/FILM system has been presented to the scientific community for detection and imaging of radioisotopes. Similar systems developed in the past were optimized for medical X-ray imaging quality. Radioisotope imaging, extensively used by scientists employing autoradiographic techniques, presents substantially different constraints that demand a different screen/film configuration. The BioMax™ TranScreen™ system (Eastman Kodak Co., New Haven, CT) is designed to match BioMax MS film. The configuration transmits the light produced in the phosphor screen through a clear support to the film (*Figure 1*).

Design and description

Screen-enhanced image capture of common radioisotope emissions (primarily β particles) is achieved by converting the particle energy into light and detecting the light with film. In practice, the most common imaging system is one in which the screen and film are in intimate contact and the sample contacts the film. The screen consists of a phosphor optimized for the purpose of light production. Added detection sensitivity is conferred to the screen/film system by its exposure in a deep-freeze (about -60 to -80 °C), which increases the film speed by inhibiting latent image fading.

The image quality of the screen/film system has two major attributes: detection sensitivity and spatial resolution. Maximization of the detection sensitivity is accomplished effectively by converting the β energy into useful light to illuminate a very fast film,

sensitized to match the output spectrum of the screen. A matched phosphor and film emulsion is the basis of the BioMax MS film and screen products (a conventional film/screen system was previously developed). Maximizing the spatial resolution of the image is accomplished by producing the light in the smallest possible volume, closest to the film emulsion. The TranScreen/film system configuration places the sample in intimate contact with the screen phosphor, optimizing the β /phosphor interaction at the expense of the phosphor/film intimacy (*Figure 1*). Hence, the TranScreen configuration differs from the conventional screen/film configuration.

The TranScreen phosphor is a durable, hydrophobic, nonporous substance that is not readily contaminated with sample and can be cleaned if necessary. Since it is not necessary to overcoat the surface with a more durable layer, the phosphor is more available to low-energy radioisotope emissions (i.e., ^3H β particle) that do not efficiently penetrate protective layers such as film overcoats. Additionally, the surface is resistant to the dampness and static that often arise from the sample contacting film, a common cause of serious image artifacts. The phosphor and support are hinged

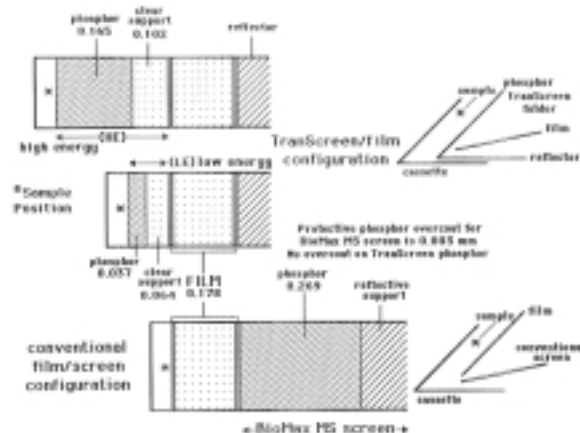


Figure 1 Screen/film system details (dimensions [in millimeters] estimated).

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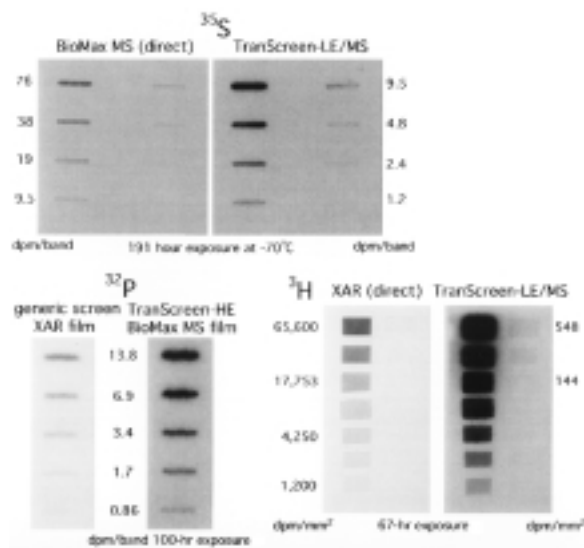


Figure 2 Comparative sensitivity of BioMax TranScreen/film systems for autoradiography. TranScreen/BioMax MS images were directly compared to commonly used autoradiography systems for ^{32}P and ^{35}S nucleic acids on membranes and ^3H on standard slides.

to a durable reflective surface, creating a folder for film insertion. The reflector material is optimized to enhance film sensitivity. The folder arrangement helps protect the film from stray light that exists in many darkrooms and from overexposure to safelights during the lengthy steps of sample handling.

Two different TranScreen systems have been developed to accommodate the wide variation of isotope energies used by scientists: a high-energy version (TranScreen-HE) for ^{32}P and ^{125}I and a low-energy version (TranScreen-LE) for ^3H , ^{14}C , ^{35}S , ^{45}Ca , and ^{33}P . Figure 1 shows the estimated dimensions of these imaging systems in cross section. The greater phosphor depth of the high-energy screen provides the increased stopping power essential to harvest the energy of radioisotope emissions having greater penetration. Since much of the thicker phosphor is far removed from the film emulsion, the TranScreen-HE (compared to the TranScreen-LE) system diminishes the spatial resolution of the image. The reduced penetration of the lower-energy emissions provides the opportunity to use the thinnest phosphor and support consistent with quality manufacturing processes. Consequently, the TranScreen-LE system provides superior spatial resolution for all isotopes while sustaining enhanced sensitivity for the lower-energy isotopes.

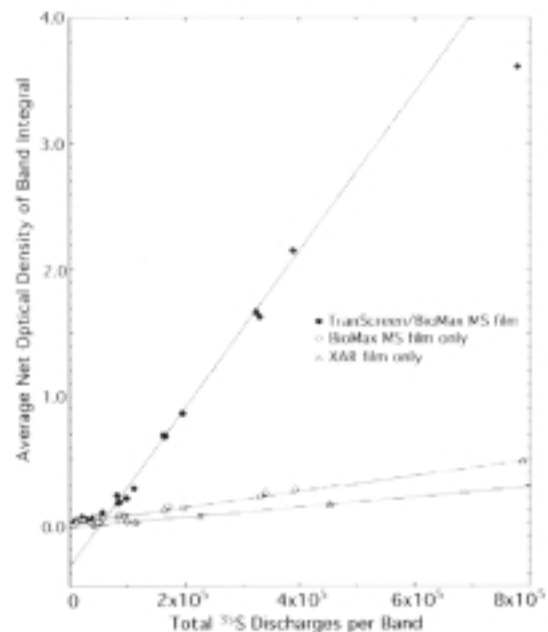
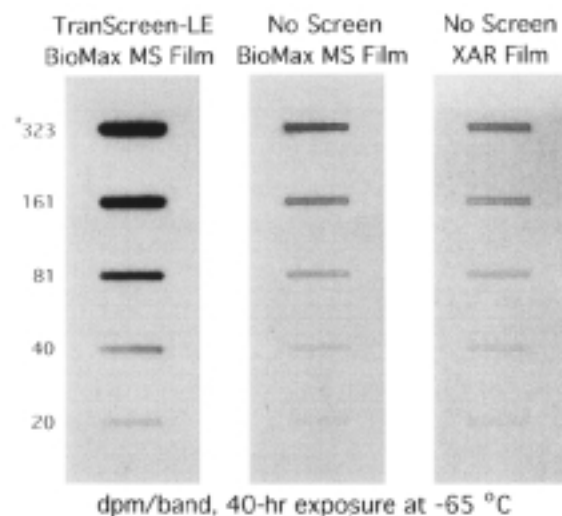


Figure 3 A comparative analysis of BioMax TranScreen-LE response to ^{35}S . Top: capture of ^{35}S slot-blot images; bottom: analysis of response to ^{35}S of TranScreen-LE and films.

Autoradiographic performance

Comparative images for ^3H , ^{35}S , and ^{32}P (Figure 2) demonstrate the enhanced detection sensitivity of the TranScreen/BioMax MS film systems. Clearly, the extensive range of TranScreen/film applicability is suggested. Note that, in the case of the TranScreen-LE system images (for example, ^{35}S images in Figure 2 and Figure 3), the sharpness of a screen-generated band image is substantially similar to a band of similar gray level captured by film alone; maintaining a sharp image implies that the Tran-

Screen-LE system conserves much of the spatial resolution inherent to the sample.

A more complete analysis of ^{35}S detection sensitivity is shown in Figure 3, in which differing films and the TranScreen-LE/BioMax MS film systems are compared and analyzed. The experimental design comprises a cellular protein labeled with ^{35}S -methionine, slot-blotted in a band format onto a membrane. The experimental samples are confined to the membrane surface (confirmed by other experiments). The experimental configuration closely approximates many experimental applications, except that the sample deposition is controlled in the present case, providing accurate estimates of the number of radioactive emissions per area of format. The analysis is performed in terms of film optical density (OD) versus the total number of ^{35}S discharges or total discharges per area (mm^2). The film is digitized on a flat-bed scanner, and the net integrated OD of any band is readily calculated and reduced to average pixel OD (Figure 3). The data, reduced and analyzed in this manner, are substantially linear over a range from minimally perceptible band images to those "black" bands with ODs that significantly exceed instrument sensitivity. Note that the film response depends on the number of total discharges occurring during exposure and is not significantly influenced by rate of discharge.

The graphical analysis shows a much higher slope (sensitivity, speed, and contrast) for the TranScreen/MS system than that of film alone (direct β exposure). Also apparent is that the sensitivity to direct ^{35}S exposure of BioMax MS film is greater than that of X-Omat AR™ (XAR) film (Eastman Kodak Co.), which is expected from the tabular grain performance of the Bio-

Max films.¹ The TranScreen/film and film responses extrapolate to a point of approximately equal sensitivity, but the band images at that point are below the level of reasonable perception or measurement. Hence, the TranScreen/film combination is generally more sensitive and is much more sensitive if darker images are desired. While the increased sensitivity or speed may be desirable or necessitated by many experimental designs, the attending higher contrast produced by any screen-enhanced system does compromise the extended dynamic range of measure that is inherent to direct film responses to β emissions. On the basis of these data, about a threefold loss of dynamic range can

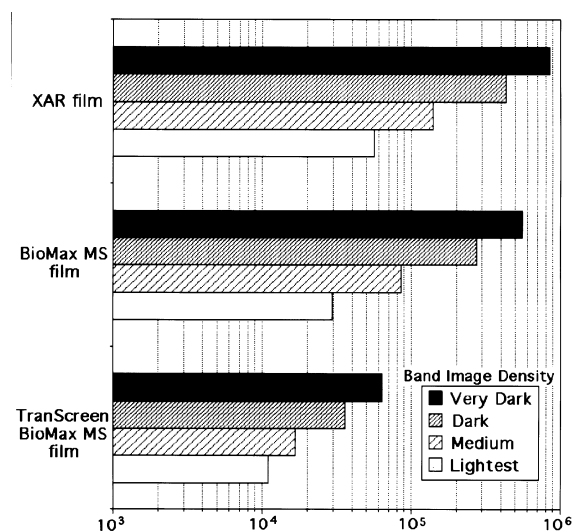


Figure 4 Sensitivity and range of measurement of ^{35}S for BioMax TranScreen-LE and film systems. (Total discharges/ mm^2 of ^{35}S required for image capture.)

Table 1

Isotope and exposure	TranScreen/BioMax MS film detection sensitivities (estimated for average density of film within an integrated band image)			Amount of isotope (mol/ mm^2) for medium image in 18-hr exposure	Suggested TranScreen application notes
	Total discharges/ mm^2 Light image 0.1 OD	Medium image 0.301 OD	Dark image 1.0 OD		
^{32}P					
Film only	1.0×10^4	2.6×10^4	8.0×10^4	1.2×10^{-18}	Southern: 1-hr exposure using a 500-base unique sequence probe used at 1% efficiency
TranScreen (HE)	4.8×10^2	1.3×10^3	4.2×10^3	6.0×10^{-20}	
^{35}S					
Film only	2.9×10^4	8.5×10^4	2.8×10^5	2.4×10^{-17}	Thin section (or in situ hybrid): approx. 3×10^4 ^{35}S atoms per 100- μm -diam area
TranScreen (LE)	9.5×10^3	1.7×10^4	3.6×10^4	4.6×10^{-18}	
^3H					
Film only	7.1×10^6	2.7×10^7	9.7×10^7	4.0×10^{-13}	Western blot: 10^{-4} cell fraction of a 10-atom label per protein of 30-kD mass, 10- μg gel load
TranScreen (LE)	4.2×10^5	1.6×10^6	5.8×10^6	2.3×10^{-14}	

be anticipated, but a threefold to tenfold gain in speed is appreciated with the TranScreen/film system compared to direct film exposure.

Further reduction of the data allows for more simplified comparisons of the imaging systems, which may be used as a practical guide. The bar graph in *Figure 4* segregates band images captured using film or screen/film systems into light images (barely perceptible, 79% net transmission, 0.1 OD); medium images (50% net transmission, 0.301 OD); dark images (10% net transmission, 1.0 OD); and very dark images (1% net transmissions, 2.0 OD).

These image characteristics summarize the response of the associated systems to the total ^{35}S discharges/ mm^2 and can be used to estimate the system response to objects of arbitrary shape. As a practical example, *Figure 4* implies that a medium band image (of publication quality) can be captured with the TranScreen/film system about nine times faster than a light image (0.1 OD) can be captured directly on XAR film. Finally, as a comparative summary of isotopes, the TranScreen-HE (for ^{32}P), TranScreen-LE (for other isotopes), and BioMax MS film responses are shown in *Table 1*, using the descriptive image criteria (total discharges/ mm^2 required to elicit a light, medium, or dark image). The absolute number of atoms (moles per mm^2) is also noted. Perusal of this table provides a renewed appreciation of the extreme sensitivity of isotope detection, and a few relevant application notes suggest experimental details that can contribute to existing laboratory practice.

Regarding the universal nature of the BioMax TranScreen/film system application, a single methodology and experimental design successfully detects a very wide range of β energies. With appropriate calibration, it is very possible that the captured images may serve as estimates or reasonable measures of the amount of isotope in a sample, especially if the amount is very low. As an example, a fairly high-precision measurement of 6000 ^{32}P atoms/ mm^2 could be accomplished with the TranScreen-HE system in about 100 hr. Attempting to measure the same (0.02 dpm/ mm^2) by other methods may be difficult if any amount of confidence is required.

Summary

As a film capture methodology, the TranScreen/BioMax MS film system is a sensitive method of image capture. By comparison, it was clearly shown by Quemeneur and Simonet² that direct β detection by film of membrane-bound isotopes blotted from separatory gels far exceeded the detection sensitivity of the fluorographic methods as applied to gels. It is shown here that the TranScreen/film system offers substantially more sensitivity than direct β detection by film. The compound sensitivity increase implies a tenfold to hundredfold increase over the levels of sensitivity that many investigators commonly use, with no increase in background and a certain increase in spatial resolution when compared to fluorography of gels.

Enhanced sensitivity for such a large range of β energies implies that the TranScreen/film system is universally applicable to almost any experimental methodology involving a flat format that can be intimately presented to the phosphor surface. Numerous autoradiographic examples such as DNA sequencing gels, Southern and Western blots, thin protein gels, thin-layer chromatography (TLC) plates, *in vivo* sections, and *in situ* hybridizations have already emerged from application testing. It is entirely likely that the TranScreen application may extend to other methodologies in the biological and material sciences, such as alpha-particle detection and the imaging of multiple labels. The application of the transmission screen configuration to other light-sensitive media is anticipated, and an initial attempt using an analogous system has been reported.³

References

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