ENHANCED AUTORADIOGRAPHIC DETECTION OF ^{32}P AND ^{125}I USING INTENSIFYING SCREENS AND HYPERSENSITIZED FILM

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1. Introduction

This paper reports that autoradiographic sensitivity can be greatly increased for isotopes which emit γ rays (e.g., ^{125}I) or high energy β particles (e.g., ^{32}P) by placing a pre-exposed film between the sample and a calcium tungstate ($CaWO_4$) X-ray intensifying screen and exposing at $-70°C$. The combination of pre-exposed Kodak X-OMAT R film with either a Fuji Mach 2 screen or a Du Pont Lightning Plus screen at $-70°C$ enhances detection efficiency 10.5-fold for ^{32}P and 16-fold for ^{125}I when compared to conventional autoradiography on Kodirex film.

The method is applicable to wet or dry gels, to chromatograms on paper or thin layer plates, or to nitrocellulose filters. Emissions from the sample pass through the film producing a direct autoradiographic image. Emissions which pass completely through and beyond the film are absorbed more efficiently by the screen where they produce multiple photons of visible light which return through the film superimposing a photographic image over the autoradiographic image.

Although intensifying screens are used routinely in medical radiography [2], their use for the much longer exposures used in biochemical autoradiography depends on by-passing the initial reversible stage of latent image formation [3]. As explained previously this problem can be overcome by lowering the temperature to $-70°C$ [1,4] and hypersensitizing the film by pre-exposing it to an instantaneous (1 ms) flash of light [1]. Neither pre-exposure nor exposure at low temperature increases the efficiency of conventional direct autoradiography. We shall refer to the method described in this paper as indirect autoradiography. It is unsuitable for isotopes which emit low-energy β particles such as ^{3}H, ^{14}C or ^{35}S.

2. Methods and results

2.1. *Description of the method*

A screen type X-ray film such as Kodak X-OMAT R (XR, formerly RP/Royal X-Omat) is pre-exposed to a single, instantaneous (approx. 1 ms) flash of light from an electronic photographic flash unit as described previously [1]. An intensity of pre-exposure is chosen to increase the absorbance of the film to 0.15 (A_{540}) above the background absorbance of unexposed film. The pre-exposed film is enclosed between the sample and a $CaWO_4$ intensifying screen (see below). These are clamped together between glass plates or in a radiographic cassette and placed at $-70°C$ for exposure. After exposure the film is developed according to the manufacturer's instructions and the screens are retained for re-use.

2.2. *Sensitivity of the method compared to autoradiography*

Table 1 demonstrates that the combination of pre-exposed Kodak X-OMAT R film with certain $CaWO_4$ screens at $-78°C$ increases detection efficiency compared to direct autoradiography on Kodirex. Enhancement is 10.5-fold for ^{32}P and 16-fold for ^{125}I. By this method 100 dpm $^{125}I/cm^2$ or 50 dpm $^{32}P/cm^2$ blacken film detectably (A 0.02)

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Table 1
Relative sensitivity of film detection methods for ^{32}P and ^{125}I

	^{32}P		^{125}I	
	+22°C	−78°C	+22°C	−78°C
Direct autoradiography on Kodirex film	1	$\leqslant 1$	1	$\leqslant 1$
Mach 2 or Lightning Plus screen + XR film unfogged	1[a]	3[a]	1[a]	5[a]
Mach 2 or Lightning Plus screen + XR film prefogged to *A* 0.15	5	10.5[b]	8	16[b]

[a] Numbers marked thus are necessarily approximations since image absorbances produced by screens are only proportional to radioactivity when pre-exposed film is used [1]
[b] Under these conditions 50 dpm $^{32}P/cm^2$ or 100 dpm $^{125}I/cm^2$ blacken film detectably (*A* 0.02) in 24 h

in 24 h. Figure 1 demonstrates that the image absorbance obtained on pre-exposed film using a screen at −78°C is proportional to the amount of radioactivity in the sample. Therefore film images can be quantitated accurately by densitometry. Kodirex is chosen as the standard because it is more sensitive than screen type films like X-OMAT R for direct autoradiography. Kodak 'No Screen' (NS) appears 1.5-fold more sensitive than Kodirex for ^{125}I and 1.4-fold for ^{32}P but this film is not available in Britain and therefore we have been able to test only a limited sample (kindly supplied by Dr J. Sambrook).

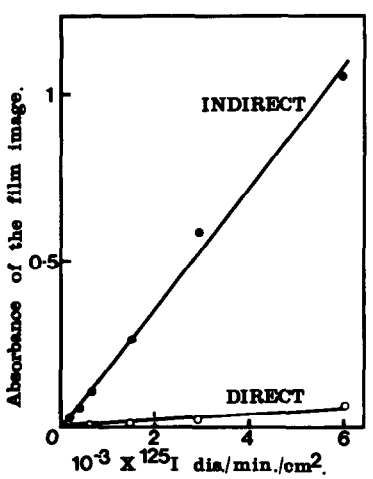

Fig.1. Relationship of image absorbance to ^{125}I radioactivity for direct autoradiographic exposure on Kodirex (o——o) and indirect autoradiographic exposure using a Fuji Mach 2 screen with pre-exposed Kodak X-OMAT R film at −70°C (•——•). Samples containing serial dilutions of ^{125}I were exposed for 24 h. Image absorbance was measured on a Joyce Loebl microdensitometer using a wedge absorbance range of 0–2.25 and a cylindrical condenser.

Table 1 shows that some enhancement is obtained at +22°C by indirect autoradiography on pre-exposed film, whereas indirect autoradiography at +22°C with untreated film is no better than direct autoradiography, and at −70°C with untreated film it gives limited enhancement which varies with the amount of radioactivity. Therefore omission of the pre-exposure step is not recommended. Exposure at +4°C or −20°C gives only marginal advantage over exposure at +22°C.

Although the three following procedures are not recommended for routine use, they increase sensitivity further. Firstly pre-exposure of film to a brighter flash of light, increasing the absorbance to 0.4 above that of unexposed film increases detection efficiency as described previously [1]. However it gives a non-linear relationship of radioactivity to image absorbance and increases blackening of the film by background environmental radiation. Secondly enclosure of the film between two screens (sample: screen A: film: screen B) also increases efficiency for ^{32}P, but causes loss of resolution through scattering by the screen which lies between the film and the sample. Efficiency of detection of ^{125}I is not significantly increased by

Table 2
Relative performance of intensifying screens and films for indirect autoradiography of ^{125}I and ^{32}P

Intensifying screen	Manufacturer	Relative efficiency[b]
Mach II	Fuji	1
Cronex Lightning Plus	Du Pont	1
Fast Tungstate	Ilford	0.66
X-Omatic	Kodak	0.6
Lanex	Kodak	approx. 1.25
Quanta II	Du Pont	but see text
Film		**Relative sensitivity[b]**
X. OMAT R	Kodak	1
RX[a]	Fuji	0.75
X-OMAT H	Kodak	0.6
Curix RP 1[a]	Agfa-Gevaert	0.55
Cronex 4[a]	Du Pont	0.6 (see text)

[a] Cronex 5, Curix RP 2 and Fuji RX-S should be more sensitive than their listed counterparts, but they were not available in Britain and were not tested

[b] These figures apply only to performance for isotope detection at −70°C after pre-exposure of the film

use of 2 screens, because a single screen absorbs most of the emissions from this isotope. Thirdly impregnating gels with PPO [5] increases sensitivity of pre-exposed film to ^{125}I approx. 2-fold.

2.3. *Relative merits of alternative screens and films*

Table 2 demonstrates that 2 types of $CaWO_4$ screen were substantially more sensitive than the other $CaWO_4$ screens tested for detection of ^{32}P and ^{125}I. Screens consisting of BaFCl : Eu^{2+} (DuPont Quanta II) or rare earth oxysulphides (Kodak Lanex used with Kodak Ortho G film) were more efficient than $CaWO_4$ for detection of ^{32}P, but they were found unsuitable for long exposures to hypersensitized film at −70°C since they blackened the film spontaneously, increasing its absorbance by approx. 0.2 per week, compared to < 0.1 per week using $CaWO_4$.

Table 2 demonstrates that Kodak X-OMAT R film was the most sensitive of those tested, though its limited availability ($\geqslant 50$ m^2 minimum order in Britain), shelf life and image quality partially offset its sensitivity advantage over Fuji RX. Cronex 4 (Du Pont) appeared almost as sensitive at −70°C without pre-exposure as after pre-exposure but only for large amounts of radioactivity. The relative merits of films listed here apply equally to their use for fluorographic detection of ^{3}H, ^{14}C or ^{35}S by organic scintillators. 'Direct' films such as 'Kodirex' and 'No Screen' are inefficient at recording the visible light produced by intensifying screens or organic scintillators.

3. Discussion

Indirect autoradiography offers marginally less resolution than direct autoradiography but in most cases the loss is insignificant. However direct or indirect autoradiography of hydrated gels at −70°C causes a considerable loss of resolution. Gels made according to formula I of Blattler et al. [6] can be dried before exposure, but if this is inconvenient, resolution can be maintained in hydrated gels by indirect autoradiography at room temperature.

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