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Laskey R A & Mills A D. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. *Eur. J. Biochem.* **56**:335-41, 1975.
[Medical Research Council Lab. Molecular Biology, Univ. Postgrad. Med. Sch., Cambridge, England]

Pre-exposure of X-ray film to a flash of light was found to increase fluorographic sensitivity of X-ray film, improving isotope detection efficiency. It also allowed quantitative interpretation of film images. These findings subsequently allowed us to apply intensifying screens to detection of ^{32}P and ^{125}I.[1] [The *SCI*® indicates that this paper has been cited in over 2,110 publications since 1975.]

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"In 1974, William Bonner and I showed that tritium could be detected in polyacrylamide gels using a solution of the scintillator PPO in dimethyl sulphoxide.[2] The scintillator converts β particles from the sample to blue light which is recorded by X-ray film at -70°C. In order to use this method quantitatively, Tony Mills and I tested the relationships between image absorbance and amount of radioactivity or exposure time. Neither was linear; small amounts of radioactivity were seriously underrepresented. While preparing a statement to warn other users of the method, we sought a procedure for overcoming the non-linearity. An anxious reading of photographic physics immediately identified the problem as 'low intensity reciprocity failure,' arising from subdivision of each β particle to multiple smaller quanta of light. The single silver atom produced by a single photon is unstable and reverts in approximately 1 s at ambient temperature. However, once two silver atoms are formed in a grain they remain stable and all subsequent photons have an equally high chance of contributing to the image. This explained why exposure at -70°C is necessary, because it would increase the half-life of the first atom, increasing the chance of forming a stable pair. In addition, it should then be possible to bypass the troublesome reversible phase of image formation by pre-exposing the film to an instantaneous flash of light so that each silver halide grain acquires a stable pair of silver atoms and can then accumulate further atoms linearly in response to each new photon from the sample.

"We consulted a major film manufacturer who assured us that this would not work. Nevertheless, we tried pre-exposure and found that it did overcome the nonlinearity of the film response and thereby it greatly increased sensitivity for small amounts of radioactivity. Two weeks later, the film manufacturer phoned to say that it might be worth trying We assured them it was. Ironically, we found later that a similar technique of pre-exposure had been used to sensitize film in the cinema industry and astronomy for many years.

"We also considered another possible method of pre-exposure, namely, exposing films to tritiated luminous paint at -70°C. However, the small container of paint we received contained 0.25 curies suggesting that this might never become a highly cited method. The reasons why our published procedure has been cited frequently appear to be that it provides a simple method for extending the limits of sensitivity of radioisotope detection and it allows accurate quantitation of the isotope distribution.

"A by-product of this work was the method of enhancing detection of ^{32}P and ^{125}I using intensifying screens at -70°C since it was clear that low temperature and preexposure should also allow the blue light produced by intensifying screens to be recorded efficiently at low intensities.[1]

"Mills has since left his technician post to become a schoolteacher."

1. **Laskey R A & Mills A D.** Enhanced autoradiographic detection of ^{32}P and ^{125}I using intensifying screens and hypersensitized film. *FEBS Lett.* **82**:314-16, 1977.
2. **Bonner W M & Laskey R A.** A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46**:83-8, 1974. [Citation Classic. *Current Contents/Life Sciences* **26**(1):16. 3 January 1983.]