

**Kopriwa B M & Leblond C P.** Improvements in the coating technique of radioautography. *J. Histochem. Cytochem.* 10:269-84, 1962.  
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The initial technique of radioautography did not reproduce well so we decided to analyze each step in a systematic manner. As a result it was possible to design an improved technique, which the article describes in detail. This technique is well standardized and allows for the preparation of large numbers of radioautographs, which can be assessed quantitatively by silver grain counting. [The SC1® indicates that this paper has been cited in over 1,010 publications, making it the most-cited paper published in this journal.]

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The radioautographic procedure known as "coating technique with liquid emulsion" was devised in 1946 in the Department of Anatomy at McGill University.<sup>1</sup> Its development is described in a recent volume on the history of American anatomists.<sup>2</sup> This method provided intimate contact between the specimen and the emulsion for the first time and thus allowed a precise localization of radioactivity in specimens. When I joined C.P. Leblond in 1956, the Ilford Company provided a bulk emulsion, which was melted in the dark and painted with a brush onto the section-bearing slides. Since this procedure was tedious and not controllable, we started dipping slides directly into melted liquid emulsion. Shortly thereafter, Ilford and Eastman Kodak Company produced special sensitized nuclear bulk emulsions with small silver bromide crystals. Simultaneously, an increasing number of radioactive precursors became available for biological research, and radioautography rapidly developed into a useful research tool.

Initial radiographic results, however, were not as regularly reproducible as desired; nu-

merous artifacts occurred that could not be controlled, and emulsion properties were not predictable. Related methods, such as the "mounting technique" and "stripping-film technique,"<sup>3</sup> were even less reliable. Yet comparison of the procedures indicated that the coating technique was superior to the others. With this in mind we started to systematically investigate the procedure step by step. We began with the suitability of various histological procedures and examined the effect of fixatives, stains, and section quality on the radioautographic response. The detail of emulsion coating, atmospheric conditions for drying of the coated preparation,<sup>4</sup> exposure conditions, and photographic processing were also methodically investigated. In addition, we worked in cooperation with the Eastman Kodak Company to test NTB2 and NTB3 emulsions that were still in the experimental stage. For the evaluation of emulsion background fog, we established criteria that are still in use today.

Perhaps the most important conclusion that arose from all these investigations was that the improved coating technique could be made quantitative by counting silver grains. This was first demonstrated by showing that the reaction intensity was proportional to exposure duration for periods varying from one month to one year.

Following the publication of this article, several modifications of the coating technique were devised for use with particular specimens, such as whole-mount preparations.<sup>5</sup> In 1967 we designed the first semiautomatic coating device,<sup>6</sup> which has become standard equipment for quantitative electron microscopic radioautography<sup>7</sup> and has been copied by several commercial companies.

This article is probably widely cited because we presented an improved, standardized, reproducible method for radioautography that can be used with a minimum of equipment in an ordinary darkroom. The method permits quantitative evaluation of results and has served as a basis for recent techniques of electron microscopic radioautography.

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