This Week's Citation Classic®

Perry P & Wolff S. New Giemsa method for the differential staining of sister chromatids. *Nature* 251:156-8. 1974.
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If mammalian cells are cultured for two rounds of DNA replication in the presence of bromodeoxyuridine the sister chromatids stain differentially with Giemsa, or with the fluorescent dyes Hoechst 33258 or acridine orange, and sister chromatid exchanges (SCEs) can be seen. The individual use of these stains led to suboptimal preparations that suffered from many drawbacks. When, however, the cells were first stained with a fluorescent dye, allowed to fade slightly, and subsequently stained with Giemsa, permanent preparations that did not fade and could be studied at leisure through an ordinary microscope without fluorescence were produced. In such preparations, SCEs could be seen with great clarity and precision. [The *SCI*® indicates that this paper has been cited in more than 2,035 publications.]

The Production of Harlequin Chromosomes

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Until 1974, sister chromatid exchanges (SCEs) were mainly studied by the method of J.H. Taylor,[1] who grew cells for one round of replication in the presence of tritiated thymidine, followed by a second round of replication in the presence of nonradioactive thymidine. Because DNA replicates semiconservatively, this resulted in sister chromatids that were physically different from one another in that only one was radioactive, which allowed them to be distinguished from one another in autoradiograms. The autoradiographic techniques, however, had limited resolution, which was compounded by problems of image spread.

In 1974, however, it was found that if the cells were grown for two rounds of DNA replication in the presence of halogenated thymidine analogues, such as 5-bromodeoxyuridine or 5-iododeoxyuridine, and subsequently stained with Giemsa[2] or the fluorescent dye Hoechst 33258,[3] the two sister chromatids would now be chemically different, one being unifiiarly substituted with the analogue and the other being bifilarly substituted, and would stain differentially. The differential staining was not as dramatic with preparations stained with Giemsa as they were with the fluorescent dye. The latter, however, produced preparations that required the use of a fluorescence microscope for observation, and cells faded extremely rapidly, which necessitated the use of photography in an attempt to capture the fluorescence before it disappeared. All subsequent studies then had to be carried out on the photographs.

Shortly after I had carried out studies on Giemsa staining with Takaji Ikushima, I went on sabbatical leave to the Medical Research Council Clinical and Population Cytogenetics Unit in Edinburgh, where I met Paul Perry, who was just beginning his scientific career, and the two of us undertook the study of SCEs visualized without the use of autoradiography. After we were apprised of the technique with Hoechst 33258, we soon switched to its use even though the rapid fading led to many frustrations. Perry was well versed in Giemsa banding techniques for chromosomes, which actually had been pioneered in our lab in Edinburgh. In an attempt to see if the SCEs that could be seen with fluorescence occurred in the Giemsa bands or in the interbands, he subjected the fluorescent slides to a standard banding treatment wherein they were first immersed in salt sodium citrate and then stained with Giemsa. To our great delight, we found that such a combination of fluorescent staining followed by Giemsa staining led to permanently stained preparations in which SCEs could be seen with great clarity and resolution. The technique had all of the advantages of the fluorescent technique but had the additional advantages of producing nonfading, permanently stained cells that did not require fluorescence microscopy or photography for their study and that did not suffer from the lack of resolution found in either autoradiographic or simple fluorescent preparations.

I believe that this fluorescence-plus-Giemsa technique and its many minor modifications opened up the study of SCEs because it showed dramatically how easily obtained cytological preparations could be used to gather experimental data on a variety of subjects, and it even led to the use of SCE induction as a highly sensitive short-term test for the detection of many carcinogenic mutagens.[4] Because large numbers of people could now study SCEs readily, and because large numbers of papers were published as a result of the development of this technique, the paper soon became highly cited.

1. **Taylor J H.** Sister chromatid exchanges in tritium-labeled chromosomes. *Genetics* 43:515-29. 1958. (Cited 295 times.)
2. **Ikushima** T & **Wolff** S. Sister chromatid exchanges induced by light flashes to 5-bromodeoxyuridine- and 5-iododeoxyuridine-substituted Chinese hamster chromosomes. *Exp. Cell Res.* 87:15-9. 1974. (Cited 190 times.)
3. **Latt** S **A.** Microfluorometric detection of deoxyribonucleic acid replication in human metaphase chromosomes. *Proc. Nat. Acad. Sci. USA* 70:3395-9. 1973. (Cited 805 times.) (See also: **Latt** S A. Citation Classic®. *Current Contents'/Life Sciences* 31(44): 19. 31 October 1988.]
4. **Wolff** S, ed. *Sister chromatid exchange.* New York: Wiley. 1982. 306 p.

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