

# This Week's Citation Classic™

Rothfels K H & Siminovitch L. An air-drying technique for flattening chromosomes in mammalian cells grown *in vitro*. *Stain Technol.* 33:73-7, 1958.

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The technique facilitates karyotype analysis primarily of cells grown *in vitro*. Following standard preliminary treatments, the crucial step is the complete air-drying of cells on slides directly after fixation. This produces a more complete flattening of intact chromosome complements than is usually achieved manually [The SCI® indicates that this paper has been cited in over 510 publications since 1958]

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"Louis Siminovitch, coauthor of the paper, at the time was working in the laboratory of Raymond Parker (Connaught Laboratories) on cell-cycle aspects of monkey kidney cells. These were being isolated in quantity as substrate for polio vaccine production.

"In propagating these cells in tissue culture, Parker noted that such cultures tended to decline in proliferative capacity after serial transfers and many were lost. A few revived and formed distinct colonies, capable of indefinite *in vitro* proliferation and differing from the cells of origin in various morphological and biochemical traits.<sup>1</sup> Parker termed these cells 'altered' or 'transformed.'

"Siminovitch was interested in a karyotypic comparison of these cells and fresh monkey kidney cells. He invited me to collaborate, since I had a background in chromosome cytology —specifically, of grasshoppers, though sitting in a botany department. Initially, Siminovitch's responsibility was the cultivation of cells directly on slides while I did the chromosome work. Eventually, both of us became deeply involved in this aspect, as did A.A. Axelrad and E.A. McCulloch.

"Discovery of the crucial step in the method was quite accidental. Preliminary treatments such as hypotonic extension and mitotic inhibition (colchicine) were well established at the time. While making squash preparations, using acetoorcein, we noted that, in contrast to metaphases in the middle

of slides, those at the edge frequently were absolutely flat with a completely two-dimensional display of chromosomes. We guessed that this was so because those cells had dried on removal of the slides from the fixative and before application of the stain solution. This interpretation was readily tested and proved to be correct. What resulted was a very simple technique, 'air drying,' which was described fully in the publication. The technique is simpler and more reproducible than later more elaborate ones and particularly valuable for people with an 'uneducated thumb.' The frequent citation of the paper is simply a consequence of the enormous amount of work being done that requires karyotypic analysis, particularly of human cells. Air drying presumably had been used prior to 1958, but we did not really search the literature for this, and the indication that the method might be useful came directly from our studies.

"In our own laboratory, the method proved immediately valuable, for it made possible the proof that most of the transformations noted by Parker in monkey kidney cell cultures were due to contamination with L cells.<sup>2</sup> It subsequently became apparent that transformations observed in other laboratories had a similar origin, HeLa cells being a frequent culprit.

"The contamination interpretation was supported by other studies, notably those of Axelrad, who demonstrated that the 'altered' cells behaved oncogenically like L-cells and not like monkey kidney cells.<sup>2</sup>

"Subsequent studies in our laboratory — primarily on mouse cells —showed that uncontaminated cultures can undergo karyotypic as well as other changes, but this is a gradual and progressive process.<sup>3,4</sup>

"In summary, the frequency of citation is clearly a result of the simplicity of the method and the very large number of studies, particularly of human cells, that require karyotypic analysis. The paper has no particular theoretical or philosophical merit nor an elaborate experimental basis.

"My own later interests centered largely on comparative banding-pattern studies in the polytene salivary-gland chromosomes of blackfly (simuliid) larvae. It is in this area that I have perhaps made some contributions (other than just letting slides dry out), and some of these are conceptual."<sup>5</sup>

- 1 Parker R C. Alterations in clonal populations of monkey kidney cells. *Poliomyelitis: papers and discussions presented at the fourth International Poliomyelitis Conference*. Philadelphia: Lippincott. 1958. p. 257-67.
- 2 Rothfels K H, Axelrad A A, Siminovitch L, McCulloch E A & Parker R C. The origin of altered cell lines from mouse, monkey, and man, as indicated by chromosome and transplantation studies. *Can. Cancer Conf* 3:189-214. 1959. V Rothfels K H & Parker R C. The karyotypes of cell lines recently established from normal mouse tissues. *J Exp Zool*. 142:507-20. 1959.
- 4 Rothfels K H, Kupelwieser F. B & Parker R C. Effects of X-irradiated feeder layers on mitotic activity and development of aneuploidy in mouse-embryo cells *in vitro*. *Can. Cancer Conf* 5:191-223. 1963.
5. Rothfels K H. Cytotaxonomy of blackflies (Simuliidae). *Annu. Rev. Entomol.* 24:507-39. 1979.