A novel method developed by Carbonari et al. makes it possible to perform four-color immunophenotyping in conjunction with DNA labeling. The ability to extract information from multiple cell subsets simultaneously, and then to discover how each subset influences the different phases of the DNA cell cycle, is useful to many scientists.

This new method has been termed integral hot staining (IHS). It subjects cells to a mild permeabilization and fixation treatment at room temperature followed by labeling with fluorochrome-conjugated monoclonal antibodies and with the DNA dye 7-aminoactinomycin D (7-AAD) at 56°C.

Current protocols involving immunophenotyping and simultaneous DNA labeling significantly modify differential light scattering (DLS). This new procedure maintains good DLS emissions. DLS is useful in the identification of morphologically different cell sub-populations. The quality of immunostaining in conjunction with DNA-labeling showed improvement over past methods, as well. Past methods utilized the DNA dye propidium iodide (PI) which had an emission wavelength too close to other fluorochrome conjugates such as phycoerithrin (PE).

Finally, by combining the labeling of fluorochrome-conjugated monoclonal antibodies and 7-AAD dye in a single step, this new method demonstrated good fluorochrome expression and a well-resolved DNA peak width measurement for viewing DNA content and lymphocyte sub-populations. Overall this new labeling method provides enhanced sensitivity as well as good recovery of input cells.