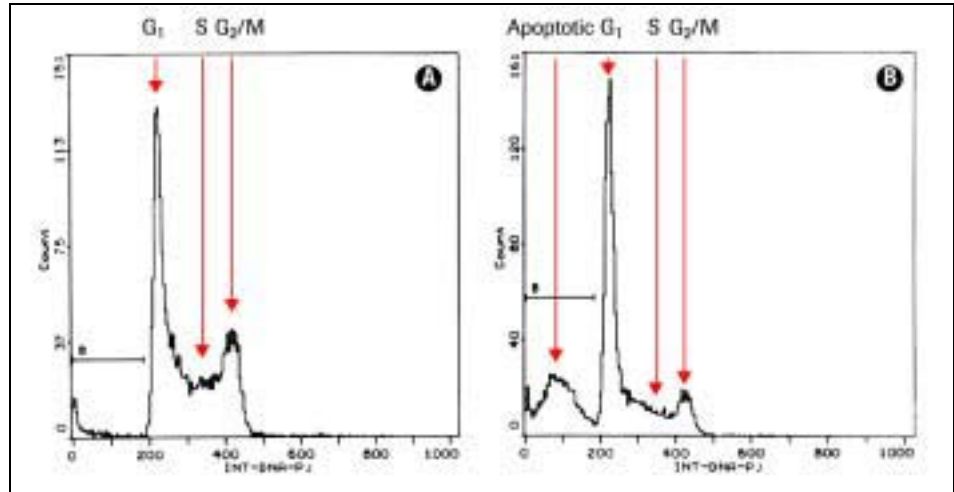


# A



▲ Figure 37: Typical flow cytometric profile of the DNA content in normal (A) and apoptotic cells (B), stained with PI.

Result: A prominent “sub-G<sub>1</sub>” peak (earliest peak) appears in apoptotic cells, but not in normal cells.

### Morphological changes

On the other hand, the bisbenzimidazole dye, Hoechst 33342 (and also acridine orange), penetrates the plasma membrane and stains DNA in cells; without permeabilization. In contrast to normal cells, the nuclei of apoptotic cells have highly condensed chromatin that is uniformly stained by Hoechst 33342. This can take the form of crescents around the periphery of the nucleus, or the entire nucleus can appear to be one or a group of featureless, bright spherical beads. These morphological changes in the nuclei of apoptotic cells may be visualized by fluorescence microscopy. They are also visible in permeabilized apoptotic cells stained with other DNA binding dyes like DAPI (Figure 38).

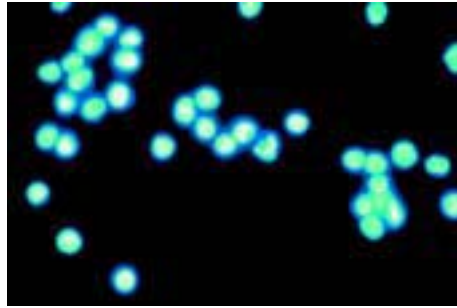
Dive et al.<sup>30</sup> have reported that during a short exposure to Hoechst 33342, apoptotic cells have stronger blue fluorescence compared to non-apoptotic cells. Co-staining of the cells with propidium iodide (PI) allows the discrimination of dead cells from apoptotic cells. If 7-amino-actinomycin is used instead of PI, cell surface antigens immunostained with fluorescein and phycoerythrin may be quantitated simultaneously<sup>31</sup>.

One drawback of using any vital staining method for measuring apoptosis is the variability of active dye uptake in different cells and its possible change during certain treatments. Therefore, the ability of Hoechst 33342 to discriminate apoptotic cells from normal cells by increased uptake of dye has to be tested for each new cell system<sup>31</sup>.

Reagent	Cat. No.	Pack size	Fluorescence	Typical results
Propidium iodide*	11 348 639 001	20 ml	red orange	See Table 9, Figure 33 and 34
DAPI 4',6-Diamidino-2'-phenylindole dihydrochloride	10 236 276 001	10 mg	blue	See Table 9 and Figure 38

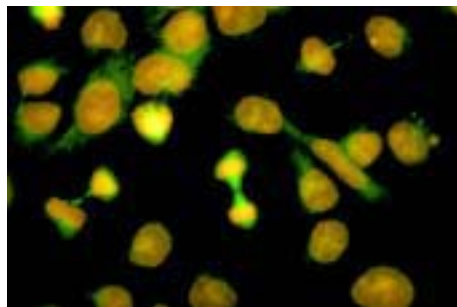
▲ Table 10: Fluorescent dyes that stain double-stranded DNA

\*Only sold in the US



▲ **Figure 38: Fluorescent microscopic analysis of apoptotic cells stained with DAPI.** DAPI stains the nucleic of all cells (blue).

**Result:** The characteristic condensed nuclei of apoptotic cells are clearly visible here.



▲ **Figure 39: Fluorescent microscopic analysis of mitotic cells stained with ethidium bromide.** DNA was stained with ethidium bromide (orange). Mitotic spindles were stained with anti-tubulin antibody (green).

**Result:** Mitotic cells (with condensed DNA) are brightly stained. Without the double stain, mitotic cells could be mistaken for apoptotic cells, since both have condensed DNA.

