Today, a variety of probes are available for assessing cell viability (*e.g.*, see Section 15.2 'Viability and Cytotoxicity Assay Reagents' in *Molecular Probes Handbook*[86]), but the results obtained with different probes unfortunately differ from each other. This is not surprising because the rationales for using certain probes are based on different concepts of what exactly is the main criterion of cell viability (the integrity of the plasmalemma, the ability to synthesize ATP, the level of dehydrogenase activity, cell respiration rate, *etc.*). In other words, this is a fairly common situation when a given cell is classified as live in one test and as dead in another.

In our experiments, we repeatedly determined the proportion of dead cells in the same "stationary aged" culture (not subcultured for 2–3 weeks) by directly examining the cells under a microscope and by taking digital images of the culture and taking cell counts on a computer display. In both cases, the cells were examined either "as is" (without any special treatment) or after adding dyes/probes commonly used for differential staining of live and dead cells (in particular, trypan blue, methylene blue, neutral red, and MTT). In many cases, the dead cell ratio detected by these methods proved to differ significantly, which casts doubt on the efficiency of such an approach to cell viability assessment in cytogerontological research. It should also be noted that some popular dyes have a number of side effects, which researchers often fail to mention. In particular, this concerns tetrazolium salts (MTT, XTT, *etc.*), which are inexpensive and can be used in experiments with cells of different origin, from bacteria to mammalian cells. However, some specialists consider that these probes are not optimal for assessing cell viability, even though they allow correct estimation of metabolic activity.[87] First, cell metabolic activity may change due to a variety of factors, even when the number of live cells in the population remains unchanged;[88] second, formazan crystals formed in the cells can damage the plasma membrane, thereby contributing to cell mortality.[87] The accuracy of analysis may be improved by using standard reagent kits containing several molecular probes each,[89] but this does not solve the problem in general.

Three groups of approaches to assessing the viability of cultured cells (Table 4.1) were diagrammatically represented in a paper of ours.[90] Table 4.1 does not cover all possible variants of live/dead cell tests but provide an idea of how broad the spectrum of such approaches can be. All methods have certain advantages and drawbacks. In particular, the occurrence of holes in the plasma membrane is not necessarily fatal for the cell, since sometimes the membrane can be repaired.[91]

Meanwhile, there is one method that usually gives a correct answer to the question about the proportion of dead cells in the test culture under study, in which the viability of cells is estimated from their colony-forming efficiency (CFE).[92–94] This method was widely introduced in cytogerontological experimentation in the 1970s, with the development of studies on the Hayflick phenomenon, *i.e.*, aging *in vitro*.[95,96] In particular, this was due to the fact that the proportion of colonies consisting of at least 64 cells (in some studies, at least 16 cells) proved to be a good indicator of the "biological age" of normal cell culture, well correlated