FLOW CYTOMETRIC ANALYSIS OF THE CELL CYCLE: MATHEMATICAL MODELING AND BIOLOGICAL INTERPRETATION

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ABSTRACT

Estimation of the repartition of asynchronous cells in the cell cycle can be explained by two hypotheses:

- the cells are supposed to be distributed into three groups: cells with a 2c DNA content (G0/1 phase), cells with a 4c DNA content (G2+M phase) and cells with a DNA content ranging from 2c to 4c (S phase);

- there is a linear relationship between the amount of fluorescence emitted by the fluorescent probe which reveals the DNA and the DNA content.

According to these hypotheses, the cell cycle can be represented by the following equation:

\[ \text{DNA}_g(y) = \int_0^\infty \text{DNA}_s(x) \cdot P(x,y) \, dx \]

All the solutions for this equation are approximations. Non parametric methods (or graphical methods: rectangle, peak reflect) only use one or two phase(s) of the cell cycle, the remaining phase(s) being estimated by exclusion. In parametric methods (Dean & Jett, Baish II, Fried), the DNA_g(x) distribution is supposed to be known and is composed of two gaussians (representative of G0/1 and G2+M) and a P(x,y) function representative of S phase. Despite the generality, these models are not applicable to all sample types, particularly heterogeneous cell populations with various DNA content. In addition, the cell cycle is dependent on several regulation points (transition from quiescence to proliferation, DNA synthesis initiation, mitosis induction) and biological perturbations can also lead to cytokinesis perturbations.

Before the emergence of flow cytometry, the current view of cell cycle resided in the assessment of cell proliferation (increase in cell number) or the kinetic of molecules incorporation (DNA precursors). The widespread development of flow cytometry has revealed the concept of cell cycle distribution and this snapshot distribution required methods to analyze this new information; this aspect led to the notion of cell cycle Mathematical Modeling.
1. MATHEMATICAL MODELING OF THE CELL CYCLE

1.1. The basic equation (Pierrez & Guerci, 1988)

The equation which supports the cell cycle can be established from common hypotheses. These are:

1) The cells are supposed to be distributed into three groups: cells with a 2c DNA content (GO/1 phase), cells with a 4c DNA content (G2+M phase) and cells with a DNA content ranging from 2c to 4c (S phase). This hypothesis leads to the theoretical distribution as shown in figure 1A.

2) The fluorescence of the cells stained for DNA content, using a fluorescent probe, is directly proportional to their DNA content.

The variabilities due to the cytometer and to the staining techniques are responsible of a dispersion of the measured fluorescence compared to the theoretical fluorescence. The variabilities include especially: 1) the cell position in the exciting beam, ii) the instability related to the fluorochrome/staining used (fixation, temperature, ...) and iii) the instability of the detectors, of electronics and of the light source. This induces a spreading of the theoretical distribution, leading to the typical and well known cell cycle curve (figure 1B).

For a cell with an x DNA content, the detected fluorescence in relation with the fluorochrome bound to the DNA will be y. Due to the previously described instabilities, the y value cannot be predicted. Only the probability to detect a y fluorescence intensity, P(y) for a given x value, can be anticipated. For a current x value, the probability for a cell with an x DNA content to be detected as any fluorescence intensity is P(x,y). If this concept is applied to all the cells whose DNA distribution is DNA_T(x), the observed fluorescence will be DNA_E(y) calculated according to the following equation:

\[ DNA_E(y) = \int_0^\infty DNA_T(x) \cdot P(x,y) \cdot dx \]

**Remarks on P(x,y):**

The information concerning P(x,y) can be obtained by analyzing cells with known