NTNU

TFY4260

Cell Biology and Cellular Biophysics

Flow Cytometry

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1 Question 1

In flow cytometry, one the values measured for each cell is fluorescence. By measuring a large number of cells from the sample, a distribution N could be made, describing the number of cells N emitting light with intensity η.

As the amount of DNA in a cell changes through the cell cycle, this approach could be used to determine he distribution of cells in each phase in a sample. Using dye that binds to DNA specifically, the intensity η will be proportional to the amount of DNA in the cell.

Normally three phases are identified; G_1, S and $G_2 + M$; where the amount of DNA increases in each phase.

The fraction F of cells in a particular phase is then given by

$$F_{ph} = \frac{1}{A} \int_{\eta_1}^{\eta_2} \mathrm{d}\eta N(\eta), \tag{1}$$

where η_1 and η_2 defines the boundary intensities for the phase, and

$$A = \int_0^{\infty} \mathrm{d}\eta N(\eta). \tag{2}$$

Setting the boundaries for each phase is done by visual inspection, aided with computer filters.

As Figure 1 shows, our sample was distributed as

$$\begin{aligned} F_{G_1} &= 59.7\%, \\ F_S &= 13.1\%, \\ F_{G_2+M} &= 27.2\%. \end{aligned}$$

2 Question 2

2.1 a)

It may be interesting to know how long each part of the cell cycle last for the cells in sample. Define the normalised time τ so that a cycle starts at $\tau = 0$ and ends at $\tau = 1$. The proportion of cells n with "age" τ could be shown to be [1]

$$n(\tau) = 2\ln(2)e^{-\ln(2)\tau} \tag{3}$$

The proportion of cells in a given phase restricted by τ_1 and τ_2 is then

$$F_{ph} = \int_{\tau_1}^{\tau_2} \mathrm{d}\tau n(\tau) = \int_{\tau_1}^{\tau_2} \mathrm{d}\tau 2\ln 2 e^{-\ln(2)\tau} = 2(2^{-\tau_1} - 2^{-\tau_2}). \tag{4}$$

For F_{G_1} ($\tau_1^{G_1} = 0$) and F_{G_2+M} ($\tau_2^{G_2+M} = 1$) this simplifies to

$$\begin{aligned} F_{G_1} &= 2 - 2^{1-\tau_2^{G_1}} \\ F_{G_2+M} &= 2^{1-\tau_1^{G_2+M}} - 1 \end{aligned}$$

or, by solving for τ,

$$\tau_2^{G_1} = 1 - \log_2(2 - F_{G_1}) \tag{5a}$$
$$\tau_1^{G_2+M} = 1 - \log_2(1 + F_{G_2+M}) \tag{5b}$$

Thus, if τ_1 describes the end of phase G_1 (i.e. $\tau_1 \equiv \tau_2^{G_1}$) and τ_2 the beginning of phase $G_2 + M$ (i.e. $\tau_2 \equiv \tau_1^{G_2+M}$), we achieved in our sample

$$\tau_1 = 0.511$$
$$\tau_2 = 0.653.$$

2.2 b)

Assume now a doubling time T_d of 24 h. From 2.1 the relative length of each phase is

$$\tau_{G_1} = 51.1\%$$
$$\tau_S = 14.2\%$$
$$\tau_{G_2+M} = 34.7\%,$$

yielding absolute durations

$$t_{G_1} = T_d\tau_{G_1} = 12.3\,\text{h},$$
$$t_S = T_d\tau_S = \ 3.4\,\text{h},$$
$$t_{G_2+M} = T_d\tau_{G_2+M} = \ 8.3\,\text{h}.$$

2.3 c)

The results seem reasonable because we were supposed to assume a doubling time (meaning an entire cell cycle) of 24 hours. If we add the absolute durations of the different stages ($12.3 + 3.4 + 8.3 = 24$) we get 24 hours. Though according to some literature, the phases S and $G_2 + M$ have reversed length of duration compared to our results [2].

3 Question 3

3.1 a)

By measuring the scattering for each cell, one could say something about the cell's size. Figure 2 shows how the standardized cell sizes are distributed within the phases G_1 and $G_2 + M$.

The median value ζ for standardized cell size for the phase $G_2 + M$ is $\zeta_{G_2+M} = 492$, while the median value for G_1 is $\zeta_{G_1} = 346$. This gives the ratio of 1.42 for the relationship $\xi = \zeta_{G_2+M}/\zeta_{G_1}$.

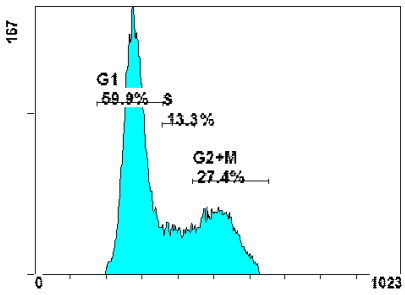

Figure 1: Histogram for fluorescence after some filtering. Visually defined phases with corresponding proportion are also shown (NB: to sum up to 100%, one has to substract 0.2% from each phase).

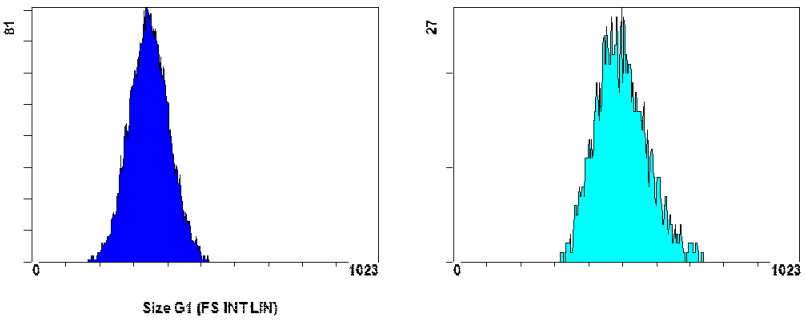

Figure 2: Histograms for standardized cell size for the phases G_1 (left) and $G_2 + M$ (right).

3.2 b)

Assume that the protein content is proportional to the surface area of the cell, and that scattering is mostly caused by surface proteins. The surface area of a sphere is given by

$$A_{sp}(r) = 4\pi r^2 \tag{6}$$

whilst the volume is given by

$$V_{sp}(r) = \frac{4\pi}{3} r^3. \tag{7}$$

Assume further that a cell immediately before mitosis has the volume of two daughter cell. That is, the volume of a cell at the end of M corresponds to two cells at the beginning of G_1,

$$V_M = 2V_{G_1}. \tag{8}$$

Solving for r yields the relationship

$$r_{G_1} = \frac{r_M}{\sqrt[3]{2}}. \tag{9}$$

Inserting this into (6) gives the ratio

$$\hat{\xi} = \frac{A_M}{A_{G_1}} = \frac{r_M^2}{r_{G_1}^2} = 2^{2/3} = 1.59. \tag{10}$$

As $\hat{\xi}$ is calculated using the extreme points of the cell cycle, it is reasonable that ξ, which is calculated from median values, is smaller than $\hat{\xi}$.

4 Question 4

Define the normalised protein content p so that $p = 1$ corresponds to the least protein content and $p = 2$ to the most. The proportion of cells n_p with protein content p could be shown to be [1]

$$n_p(p) = \frac{2}{p^2}. \tag{11}$$

As half of the cells in phase G_1 would have a protein content less than P_{G_1}, we achieve

$$\frac{1}{2} F_{G_1} = \int_1^{P_{G_1}} \mathrm{d}p\, n_p(p) = 2 \int_1^{P_{G_1}} \mathrm{d}p \frac{1}{p^2} = 2\Big(\frac{1}{1 - P_{G_1}}\Big),$$

or by solving for P_{G_1}

$$P_{G_1} = \frac{4}{4 - F_{G_1}}. \tag{12}$$

Similarly, half of the cells in phase $G_2 + M$ would have a protein content above P_{G_2+M},

$$\frac{1}{2}F_{G_2} = \int_{P_{G_2+M}}^{2} \mathrm{d}p\, n_p(p) = 2\int_{P_{G_2+M}}^{2} \mathrm{d}p \frac{1}{p^2} = 2\Big(\frac{1}{P_{G_2+M}} - \frac{1}{2}\Big).$$

Solving for P_{G_2+M} yields

$$P_{G_2+M} = \frac{4}{2 + F_{G_2+M}}. \tag{13}$$

As the fractions are known, values for (12) and (13) are, for our sample,

$$\begin{aligned} P_{G_1} &= 1.18, \\ P_{G_2+M} &= 1.76. \end{aligned}$$

The ratio P_{G_2+M}/P_{G_1} becomes, from the same equations,

$$\tilde{\xi} = \frac{P_{G_2+M}}{P_{G_1}} = \frac{4 - F_{G_1}}{2 + F_{G_2+M}} = 1.49 \tag{14}$$

This result does not deviate much from the previous found ξ, and also $\tilde{\xi} < \hat{\xi}$ which seems reasonable given $\hat{\xi}$ as an upper boundary. Note that the initial assumptions in 3.2 constitute the relation between ξ (light scattering), $\hat{\xi}$ (surface area) and $\tilde{\xi}$ (protein content).

References

[1] Introduction to lab exercise "Flow Cytometry", retrieved from itslearning, 23.03.2017.

[2] G. M. Cooper. The Cell: A Molecular Approach. Sinauer Associates, 2000. Retrived from https://www.ncbi.nlm.nih.gov/books/NBK9876/ 27.03.2017.