Supporting Information De Boer and Van den Berg et al. (2012). Appendix S1

Mathematics of labelling indices

When the dividing cells can be assumed to be in a random phase of the cell cycle, the cell cycle length is constant and the population does not increase in size during the exposure time, the fraction labelled cells ($F$) is determined by the exposure time and the unknown lengths of the S-phase and the cell cycle [1,2]. When two different exposure times are used, the unknown lengths can be derived.

Derivation of the equation for $T_C$

According to the equation given by Sanders and co-workers [1] the labelling fraction after CldU exposure is given by:

$$F_{ci} = \frac{T_s + T_{ci}}{T_c} \quad [1]$$

which can be solved for $T_s$:

$$T_s = F_{ci}T_c - T_{ci} \quad [2]$$

Similarly, the exposure to IdU results in the labelling fraction of:

$$F_i = \frac{T_s + T_i}{T_c} \quad [3]$$

Substitution of Eq. [2] into Eq. [3] gives:

$$F_i = \frac{F_{ci}T_G - T_{ci} + T_i}{T_c} \quad [4]$$

which can be solved for $T_C$:

$$T_C = \frac{T_i - T_{ci}}{F_i - F_{ci}} \quad [5]$$

With Eq. [6] it is easy to see that an incorporation lag ($T_L$) has no effect on observed $T_C$, because such a lag affects both exposure times in the same way:

$$T_C = \frac{(T_i - T_L) - (T_{ci} - T_L)}{F_i - F_{ci}} = \frac{T_L - T_{ci}}{F_i - F_{ci}} \quad [6]$$
The labelling fraction after exposure to CldU for a population with a growth fraction (FD) is given by Equation 1a [1,2]. This equation is used to derive the equation for TC, including the growth fraction (the equations are in the same order as above):

\[ F_{ci} = \frac{T_S + T_{ci}}{T_C} \cdot F_D \]  

[1a]

which can be solved for T_S \cdot F_D:

\[ T_S \cdot F_D = F_{ci} \cdot T_C - T_{ci} \cdot F_D \]  

[2a]

The similar exposure to IdU results in the labelling fraction of:

\[ F_i = \frac{(T_S + T_I) \cdot F_D}{T_C} = \frac{T_S \cdot F_D + T_I \cdot F_D}{T_C} \]  

[3a]

Substitution of Eq. [2a] into Eq. [3a] results in:

\[ F_i = \frac{F_{ci} \cdot T_G - T_{ci} \cdot F_D + T_I \cdot F_D}{T_C} \]  

[4a]

which can be simplified to:

\[ T_C = \frac{T_I - T_{ci}}{F_i - F_{ci}} \cdot F_D \]  

[5a]

Equation 5a shows that the observed TC of a population, i.e. the population doubling time, is the cell cycle length of the dividing cells multiplied by the growth fraction of the population.

**Derivation of the equation for Ts**

The equation given by Sanders and co-workers [1] states that the labelling index after exposure to CldU is given by:

\[ F_{ci} = \frac{T_S + T_{ci}}{T_C} \]  

[7]

which, solved for T_C reads like:

\[ T_C = \frac{T_S + T_{ci}}{F_{ci}} \]  

[8]

Similarly the exposure to IdU results in the labelling fraction of:

\[ F_i = \frac{F_{ci} \cdot T_G - T_{ci} \cdot F_D + T_I \cdot F_D}{T_C} \]  

[9]

Substitution of Eq. [8] in Eq. [9] then gives
This can be rearranged to give the following equation for $T_S$:

$$T_S = \frac{F_{cl} \cdot T_I - F_I \cdot T_{cl}}{(F_I - F_{cl})} \quad [11]$$

Equation 11 can be simplified to:

$$T_S = F_{cl} \cdot T_c - T_{cl} \quad [11a]$$

as was derived in Figure 1.

When the growth fraction ($F_D$), which is the fraction of cells that is dividing is constant, $T_S$ is derived as follows (the equations are in the same order as above):

$$F_{cl} = \frac{(T_S + T_{cl}) \cdot F_D}{T_c} \quad [7a]$$

$$T_c = \frac{(T_S + T_{cl}) \cdot F_D}{F_{cl}} \quad [8a]$$

$$F_I = \frac{(T_S + T_I) \cdot F_D}{T_c} \quad [9a]$$

$$F_I = \frac{(T_S + T_I) \cdot F_D}{(T_S + T_{cl}) \cdot F_D} \quad [10a]$$

In Eq. [10a] it is clear that $F_D$ disappears from the equation. Therefore, the growth fraction has no influence on the calculation of the S-phase length.

The bias due to the insertion a lag time ($T_L$) between injection and incorporation, on determined S-phase is equal to the incorporation lag. With the definition of $T_S$ in Eq. 11 and a lag phase $T_L$, the real $T_S$ can be defined as

$$T_S \text{ Real} = \frac{F_{cl} \cdot (T_I - T_L) - F_I \cdot (T_{cl} - T_I)}{(F_I - F_{cl})} \quad [12]$$

which after re-arranged reads as:

$$T_S \text{ Real} = \frac{F_{cl} \cdot T_I - F_I \cdot T_{cl}}{(F_I - F_{cl})} + \frac{F_I \cdot T_L - F_{cl} \cdot T_I}{(F_I - F_{cl})} \quad [13]$$

The first part on the right is the $T_S$ that will be observed because the presence of a lag phase is unknown, the second part simplifies to $T_L$: 3
Equation 14 shows that the observed S-phase length is too short when an incorporation lag is present. The length of this lag has to be added to obtain the real S-phase length. 

The actual underestimation of the observed S-phase length would thus be equal to such an incorporation lag. The effect of this lag phase explains the discrepancy between our S-phase equation (11a) and those previously published [3,4]: by assuming a lag phase that is as long as the exposure time to the second label, these authors ignore the second exposure time and the S-phase length is thus overestimated by the length of this exposure time.

**Division of labelled cells**

When the exposure time to the first label (IdU) is longer than \( T_{G2} + T_M \), cells that were labelled during \( T_S \) reach the end of \( T_M \) and will divide. In that case, the fraction of labelled cells at the moment of fixation that was defined as \( F_I \), is also equal to the fraction of cells in \( S \), \( G2 \) and \( M \) plus a fraction of cells that results from the cell division:

\[
F_I = F_S + F_{G2} + F_M + F_{\text{division}} \tag{15}
\]

or

\[
F_{\text{division}} = F_I - (F_S + F_{G2} + F_M) \tag{16}
\]

With

\[
F_S + F_{G2} + F_M = \frac{T_S + T_{G2} + T_M}{T_C} \tag{17}
\]

and Eq. 3 for \( F_I \), \( F_{\text{division}} \) (Eq. 16) can be re-written as

\[
F_{\text{division}} = \frac{T_I}{T_C} - \frac{T_S + T_{G2} + T_M}{T_C} \tag{18}
\]

which simplifies to

\[
F_{\text{division}} = \frac{T_I - (T_{G2} + T_M)}{T_C} \tag{19}
\]

Equation [19] shows that for exposure times (\( T_I \)) longer than the sum of \( T_{G2} \) and \( T_M \), an extra group of dividing cells is counted and added to \( F_I \). This will increase the
denominator in Eq. 5 and the observed $T_C$ will thus be underestimated. Therefore, the exposure time should be kept shorter than the sum of $T_{G2}$ and $T_M$.

References

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2. Nowakowski RS, Lewin SB, Miller MW (1989) Bromodeoxyuridine immunohistochemical determination of the lengths of the cell cycle and the DNA-synthetic phase for an anatomically defined population. J Neurocytol 18: 311-318.

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