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Cohort Method for Lymphocyte Proliferation Analysis: Theory and Applications

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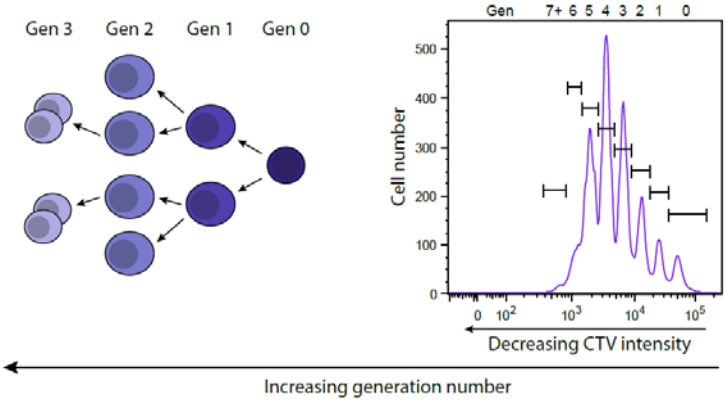
Introduction

Division tracking dyes, such as Cell Trace Violet have revolutionised quantification of lymphocyte response kinetics [1,2]. Knowing cell division history enables estimation of survival features not easily achieved with other methods. Our analysis of mouse and human T and B cell responses by multiple stimuli demonstrate a canonical pattern of response, the basic parameters of which can be estimated without complex mathematical models, requiring only a few quantitative plots and minimal analysis.



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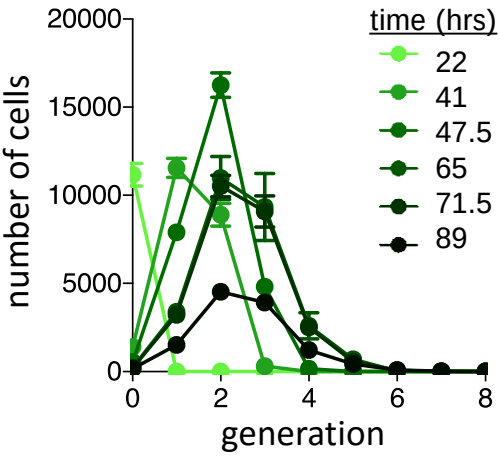
The net kinetics of the response result from the interplay between the *rates of division and death*, as well as the number of divisions cells undergo before returning to quiescence (*division destiny*). The **Cohort Method** is a computational approach that enables estimation of each of these parameters [1,2,3]. We illustrate basic principles of the method below.

Note that additional factors, such as proportion of responding cells or longer time to first division are also typical features of lymphocyte responses. Our method is capable of estimation these parameters as well (not shown).

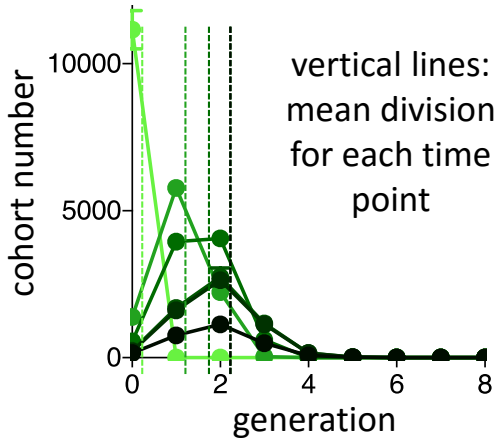


number of cells

$\mathrm{x}(k,t)$

cohort number: normalising for expansion

$$c(k,t) = \frac{x(k,t)}{2^k}$$



cohort sum

$$S(t) = \sum_{k=0}^{\infty} c(k,t)$$

mean division number

$$g(t) = \sum_{k=0}^{\infty} \frac{kc(k,t)}{S(t)}$$

Data: Marchingo J. Stimulated OTI Bcl2l-/- CD8 T cells [3]

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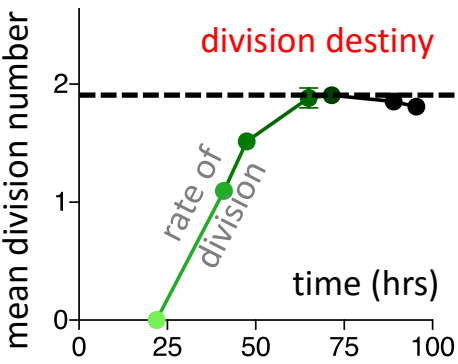
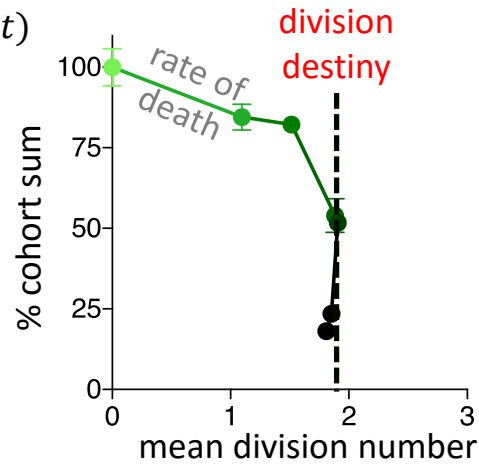
division destiny: $d(t) = \lim_{t\to\infty} g(t)$

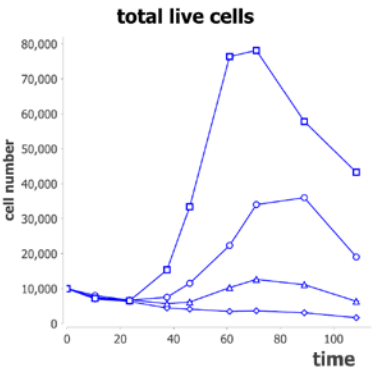


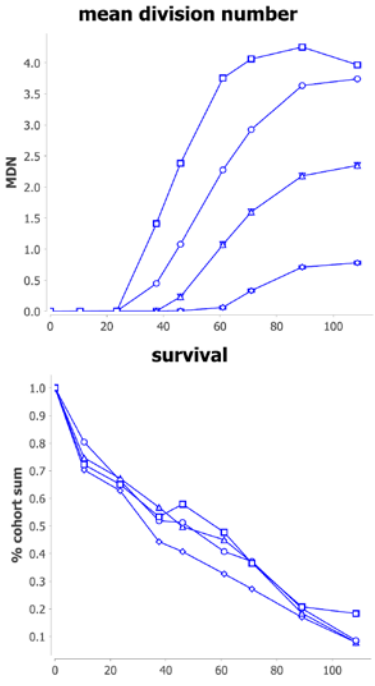


Applications

We have developed a custom **dedicated software** implementing the method [4]. The Cohort method is suited to enumerate independent influences by cytokines, signals, drugs and genetic alterations on division and/or survival, as illustrated by two examples below.

Example 1 OTI Bim-/- CD8+ T cells stimulated with N4 and aCD28 and Mycophenolic Acid (MPA) Data: Lye B. and Heinzel S.





Cohort analysis reveals that the difference in total cell numbers can be attributed exclusively to changed division kinetics. MPA does not affect death parameters. See more in-depth analysis and other drugs in the poster of Bryan Lye.

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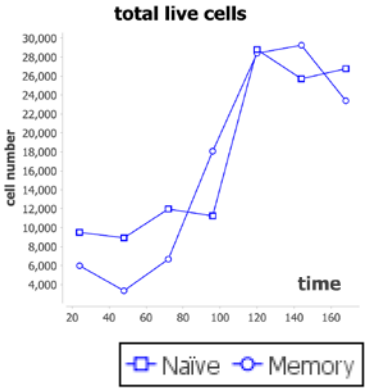
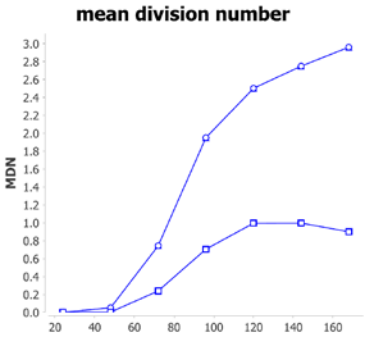
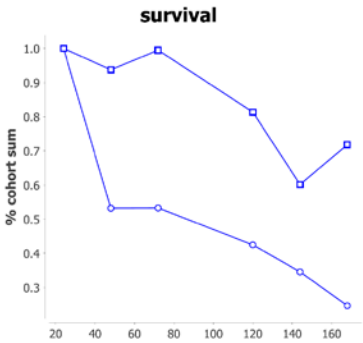
The second example demonstrates that the method can be informative not only for murine cells, but also for human cells.

Example 2
Stimulated human naïve vs memory B cells (sort-purified from healthy donor PBMCs)







Proliferation kinetics appear to be similar from total cell counts. However, cohort analysis reveals differences in both division and survival parameters

Data: Tempany J. and Bryant V.

Summary

We present a mathematical definition of the Cohort method and demonstrate the utility of the method using murine and human lymphocyte response data. To facilitate the research in the field, we have developed a free tool that implements Cohort analysis [4].

References

[1] Gett and Hodgkin, A cellular calculus for signal integration by T cells, *Nat Immunol*, 2000

[2] Hawkins et al., Quantal and graded stimulation of B lymphocytes as alternative strategies for regulating adaptive immune responses, *Nat Commun*, 2013

[3] Marchingo et al., Antigen affinity, costimulation, and cytokine inputs sum linearly to amplify T cell expansion, *Science*, 2014

[4] https://github.com/hodgkinlab/cohort-method

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