

# Achieving Targeted Granulocyte Differentiation Through The Use Of Interpolation and Optimization Techniques

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**Abstract**—Cellular differentiation is a complex process for which systematic design of control strategies has not been widely investigated. As a first step towards this aim, a control strategy for achieving a desired percentage of differentiating cells is proposed. A population balance model structure parallels the known granulocyte/monocyte differentiation pathway. Transition rate functions that characterize the movement of cells from one differentiation state to the next were identified from experimental data obtained via flow cytometry. An additional experiment demonstrates the efficacy of the proposed model and control strategy.

## I. INTRODUCTION

Cellular processes involve complex interactions which present unique challenges for the modeling and control of such systems. Cellular differentiation is a process that has largely been researched experimentally with some modeling efforts, but a systematic approach to control such processes has not been researched [1-3]. A model for the differentiation process of human promyelocytic leukemia (HL60) cells into mature granulocytes and monocytes is presented here.

HL60 cells can naturally differentiate into monocytes and granulocytes in small numbers; however, in an effort to minimize the heterogeneity of a cell population, full-scale differentiation is desired and must be induced using chemical agents. Dimethyl sulfoxide (DMSO) initiates differentiation into granulocytes [4]. The dynamics of this process is largely dependent on the concentration of DMSO. Using this model, it is possible to make predictions of the DMSO concentration necessary to achieve a target level of maturing cells. Future work will design controllers to rationally manipulate the composition of the differentiation population throughout a given time period.

## II. MODEL DEVELOPMENT

An age-structured population-balance model (PBM) naturally accommodates this process within its structure. A PBM describes how the population distribution progresses through discrete stages over time. For an age-structured PBM, the transition between these discrete stages is a

function of the time since its last state transition, the “age” of the cell [5]. This initial age-structured PBM uses the continuous cell-age variable to account for the variable time period in which the intracellular signal transduction and regulatory gene networks direct the differentiation process.

The PBM describes how a population of HL60 cells moves through discrete differentiation stages towards mature granulocytes and monocytes as shown in Fig 1. The discrete differentiation phases were defined based on experimentally distinguishable benchmarks (via flow cytometry). The first indication of differentiation is the expression of the cell surface marker CD-11b. A maturing granulocyte will then express the marker CD-16, and a maturing monocyte will express the marker CD-14 [6].

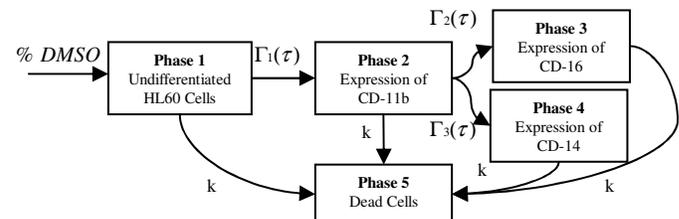


Fig 1. Phases of the HL60 differentiation model. Cells transition out of phases according to an age-dependent transition rate ( $\Gamma(\tau)$ ). Cells may die in any phase according to a constant, phase-independent death rate,  $k$ . A constant growth rate associated with each phase,  $b_i$ , which is due to cell division (not shown).

The number density of cells in the  $i^{\text{th}}$  phase is  $n_i(\tau, t)$ , where  $\tau$  is age and  $t$  is time. The population balance equation is written as 
$$\frac{\partial \mathbf{n}(\tau, t)}{\partial t} + \frac{\partial \mathbf{n}(\tau, t)}{\partial \tau} = -\mathbf{D}_{\text{out}}(\tau) \mathbf{n}(\tau, t),$$

where  $\mathbf{n}(\tau, t) = [n_1(\tau, t) \ n_2(\tau, t) \ n_3(\tau, t) \ n_4(\tau, t) \ n_5(\tau, t)]^T$

$$\text{and } \mathbf{D}_{\text{out}}(\tau) = \begin{bmatrix} \Gamma_1(\tau) + k - b_1 & 0 & 0 & 0 & 0 \\ 0 & \Gamma_2(\tau) + \Gamma_3(\tau) + k - b_2 & 0 & 0 & 0 \\ 0 & 0 & k - b_3 & 0 & 0 \\ 0 & 0 & 0 & k - b_4 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}.$$

As most cells begin as undifferentiated HL60 cells, the initial age distribution in each phase is  $\mathbf{n}(\tau, 0) = 0$ . A boundary condition describes the cells that have transitioned out of one stage and have become new cells in another stage:

$$\mathbf{n}(0, t) = \int_0^{\infty} \begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ \Gamma_1(\tau) & 0 & 0 & 0 & 0 \\ 0 & \Gamma_2(\tau) & 0 & 0 & 0 \\ 0 & \Gamma_3(\tau) & 0 & 0 & 0 \\ k & k & k & k & 0 \end{bmatrix} \mathbf{n}(\tau, t) d\tau.$$

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The method of characteristics was employed to simulate the model [5]. A Nelder-Mead simplex optimization function was used to find the state transition rate functions that best fit the experimental data. The transition functions were assumed to be lognormal and could be parameterized by two unknowns (mean and standard deviation).

### III. PROBLEM FORMULATION

The objective of this study was to determine the concentration of the inducing agent (DMSO) that would produce a population of HL60 cells in which 65% expressed CD-11b seven days after induction. Using an interpolation method, the DMSO concentration and transition rate parameters necessary to achieve this differentiation level were found. These results were verified using experimental data gathered at the target DMSO concentration.

### IV. RESULTS

The number of cells expressing CD-11b, CD14, and CD16 over seven days of incubation in 1.2% DMSO was found experimentally and is shown in Fig 2. The percentage of cells beginning to differentiate (expressing CD-11b) after seven days of incubation was 74%. Similarly, in 0.6% DMSO, 40% of the population expressed CD-11b. Using a spline interpolation, a concentration of 1% DMSO would yield a population in which 64% expressed CD-11b.

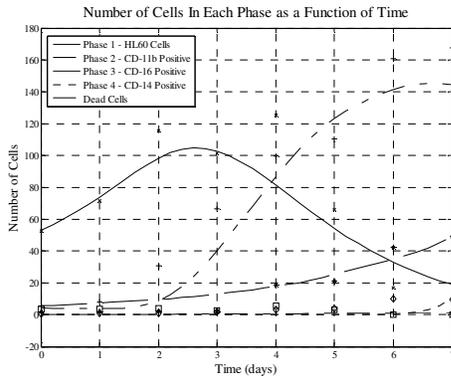


Fig 2. To find the parameter set, the model was fit to experimental data taken at 1.2% DMSO. One sample was taken every day for seven days. For each sample, the cells were labeled with fluorescent antibodies to the CD-11b, CD16, and CD14 surface markers. Samples were analyzed using flow cytometry techniques. The process was similar to [7].

Using the model and optimization technique described above, the means and standard deviations of the lognormal transition functions were found for each of the two experimental DMSO concentrations. This data is summarized in Table I. The mean and standard deviation for each of the three transition rates was determined for a concentration of 1% DMSO using six individual spline

TABLE I

DMSO Concentration	$\Gamma_1(\tau)$		$\Gamma_2(\tau)$		$\Gamma_3(\tau)$	
	$\mu_1$	$\sigma_1$	$\mu_2$	$\sigma_2$	$\mu_3$	$\sigma_3$
1.2%	3.93	1.45	5.08	0.28	70.00	49.02
0.6%	5.02	1.64	10.54	10.17	52.08	34.12

Transition-rates parameters for two levels of DMSO (values in days)

interpolations and are shown in Table II.

As a verification of the linearity assumptions utilized in this interpolation strategy design, experimental data was collected for a population incubated in 1% DMSO and was analyzed to determine the phase percentages. The percentage of HL60 cells expressing CD-11b was 64.5%, less than 1% error from the target value.

From the experimental time course data at 1% DMSO, the transition rate parameters were found, fitting the model to the experimental data with the Nelder-Mead simplex optimization function. A comparison of the parameters obtained via interpolation and optimization can be seen in Table II.

TABLE II

DMSO Concentration	$\Gamma_1(\tau)$		$\Gamma_2(\tau)$		$\Gamma_3(\tau)$	
	$\mu_1$	$\sigma_1$	$\mu_2$	$\sigma_2$	$\mu_3$	$\sigma_3$
1% (Interpolation)	4.29	1.51	6.90	3.58	64.03	44.05
1% (Model)	4.53	1.30	5.24	4.30	61.34	39.84

Transition-rate parameters for 1% DMSO (values in days)

The interpolated transition-rate parameters provide close approximations for those obtained via optimization techniques on the differentiation model. The transition rate parameter identification process is a computationally expensive and time consuming process; it may be advantageous to utilize interpolations to identify initial estimates which can be refined using local optimization.

### V. SUMMARY AND FUTURE WORK

The proposed model identifies three distinct transition rates governing the differentiation dynamics of HL60 cells. The quantitative description of this process provided by the developed PBM predicts the movement of cells through the differentiation phases and thus provides a structure for systematically designing controllers to manipulate the outcome in a desired manner. As a first step towards achieving this aim, this work determined a strategy to achieve a target percentage at a given time point. Anticipated future work will utilize a model predictive control strategy to systematically direct the time-course differentiation dynamics.

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