

Solution of Inverse Problems for Obtaining Protein Concentrations from Fluorescent Microscopy Images

Zuyi Huang, Fatih Senocak, Arul Jayaraman, and Juergen Hahn

Abstract— The development of quantitative models of signal transduction, as well as parameter estimation to improve existing models, depends on the ability to obtain quantitative information about various proteins that are part of the signaling pathway. However, commonly-used measurement techniques such as Western blots and mobility shift assays provide only qualitative or semi-quantitative data which cannot be used for estimating parameters. This paper presents a solution of an inverse problem for quantitatively determining transcription factor profiles from green fluorescent protein (GFP) reporter data. We have used this technique to quantitatively characterize activation of the transcription factor NF- κ B by the cytokine TNF- α . The obtained results are in good agreement with qualitative descriptions of NF- κ B activation as well as semi-quantitative experimental data from the literature. While the presented approach has been applied to NF- κ B and TNF- α signaling, it can be used to determine the profile of other transcription factors with only minor modifications.

I. INTRODUCTION

SYSTEMS Biology seeks to develop models for describing cellular behavior on the basis of regulatory molecules such as transcription factors and signaling kinases. The control of gene expression by transcription factors is an integral component of cell signaling and gene expression regulation [1-2]. Different transcription factors exhibit different expression and activation dynamics, and together govern the expression of specific genes and cellular phenotypes [3]. An important requirement for the development of these signal transduction models is the ability to quantitatively describe the activation dynamics of transcriptions so that parameters can be estimated for model development. The activation of transcription factors under different conditions have been conventionally monitored using protein binding techniques such as electrophoretic mobility shift assay, chromatin immunoprecipitation [4], or fluorescent recovery after photo bleaching (FRAP) [5]. While electrophoretic mobility shift assays or chromatin immunoprecipitation provide snapshots of activation at a small set of single time points, they can yield only qualitative or semi-quantitative data at best. This approach also requires the use of multiple cell populations for each time point at which transcription factor activation is to be measured, and

often, the true dynamics of transcription factors are not captured due to limited sampling points and frequencies. Compared with the above two methods, FRAP yields the dynamic parameters for the binding interactions instead of the information about transcription factor activation. Hence, these methods are not ideal for investigating time-dependent activation of transcription factors in a quantitative manner. Other methods such as

More recently, fluorescence-based reporter systems have been developed for the continuous and non-invasive monitoring of transcription factors and the elucidation of regulatory molecule dynamics. Recent studies [6-9] have used green fluorescent protein (GFP) as a reporter molecule for continuously monitoring activation of a panel of transcription factors for 24 h. These systems involve expressing GFP under the control of a minimal promoter such that GFP expression and fluorescence is observed only when a transcription factor is activated (i.e., when the transcription factor binds to its specific DNA binding sequence and induces expression from a minimal promoter) (Fig. 1A & B). The dynamics of GFP fluorescence is used as the indicator for dynamics of the transcription factor being profiled. The primary drawback with this approach is that it does not provide direct activation rates of the transcription factors being investigated. Even though transcription factor dynamics influence GFP dynamics, the relationship between the two is non-trivial as the induction of GFP fluorescence itself involves multiple steps (i.e., transcription of GFP mRNA, GFP protein translation, and post-translational processing) [10]. The observed fluorescence dynamics in GFP reporter cell systems is the result of two different dynamics: (i) the dynamics of transcription factor activation by a stimulus-mediated signal transduction pathway and (ii) the dynamics of GFP expression, folding, and maturation. Therefore, it is necessary to uncouple the effects of these independent systems in order to quantitatively determine transcription factor activation profiles underlying cellular phenotypes.

In this work, we solve an inverse problem to derive transcription factor activation rates from GFP-based fluorescent reporter systems. We demonstrate that NF- κ B activation dynamics can be accurately determined from GFP reporter profiles, using GFP reporter data for the activation of the transcription factor NF- κ B by the cytokine TNF- α (Fig. 1C). It should be pointed out that the presented approach is not limited to NF- κ B and can be used to determine the activation profile of any transcription factor as long as GFP

Manuscript received September 15, 2008. This work was supported by the ACS Petroleum Research Fund (Grant PRF# 48144-AC9).

Z. Huang, F. Senocak, A. Jayaraman, and J. Hahn are with the Artie McFerrin Department of Chemical Engineering, Texas A&M University, College Station, TX 77843-3122, USA (corresponding author: J. Hahn, 979-845-3568 (JH); fax: 979 845 6446; e-mail: hahn@tamu.edu).

reporter fluorescent profiles are available and the dynamics of transcription factor can be easily mathematically described.

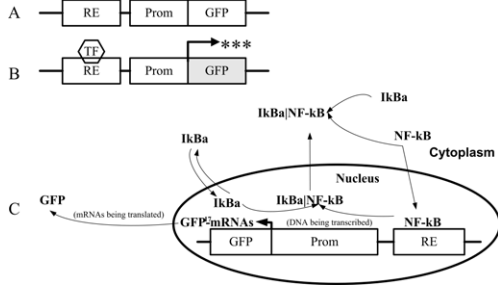


Fig. 1. GFP-based reporter systems for investigating transcription factor (TF) activation. The DNA response element (RE) to which the TF binds is upstream of a minimal promoter that controls GFP expression (A) No fluorescence is observed in the absence of TF binding, (B) Binding of TF leads to promoter activation and GFP fluorescence, (C) Dynamics of a TF (e.g., NF-kB) is influenced by activation of the TF and the dynamics of GFP expression.

II. PRELIMINARIES

A. K-means Clustering

K-means clustering is a method for identifying patterns in data and for dividing data into k disjoint clusters [11]. The principle of K-means clustering is to minimize the objective function expressed in (2) by determining centroids for each of the k clusters:

$$\min_{\mu} f = \sum_{i=1}^k \sum_{x_j \in S_i} \|x_j - \mu_i\|^2 \quad (1)$$

where S_i , $i=1, 2, \dots, k$, represents all points belonging to the i -th cluster, μ_i is the centroid of all the points $x_j \in S_i$, and μ is the collection of all the centroids. μ_i is calculated by (2).

$$\mu_i = \frac{\sum_{x_j \in S_i} x_j}{N_i} \quad (2)$$

where N_i is the total number of the data points in cluster S_i .

The procedure to perform K-means clustering consists of the following steps:

- 1) The initial centroids μ_i , $i=1, 2, \dots, k$, for the k clusters are assigned or randomly sampled from the data points;
- 2) Each data point x_j is assigned to a cluster m . This decision is made by determining the smallest value for $\|x_j - \mu_m\|^2$ among all possible ones $\|x_j - \mu_i\|^2$, $i=1, 2, \dots, k$.
- 3) The function f from (1) is computed by computing the sum of distances for all data points as well as for all clusters.
- 4) Equation (2) is used to update the centroid of each cluster by averaging the data points of the corresponding cluster;
- 5) Steps 2) through 4) are repeated iteratively until the relative change in the objective function f between iterations

is less than a certain threshold.

B. Principle Component Analysis (PCA)

Principal component analysis [12] is a well-established technique for identifying multivariable patterns in data. Using PCA a data matrix X can be composed as follows:

$$X = TP^T + E \quad (3)$$

where T is the score matrix, P is the loading matrix, and E is the residual between the actual data and the reconstruction by PCA. The columns of P represent principle components of the data matrix, while the columns of T are the projections of the data matrix onto the principle components [13].

The motivation for using PCA for image analysis in this paper is derived from the work presented in [14-15], which shows that clusters in a score plot from PCA are associated with features of an image.

C. Mathematical Description of Digital Images

The tristimulus theory states that any visual color can be represented by overlaying three color information channels. For television and computer graphics, the standard colors used are red, green and blue [14]. An RGB image can be represent by a three-dimensional matrix

$$M = \begin{bmatrix} (r, g, b)_{11} & \cdots & (r, g, b)_{1j} \\ \vdots & \ddots & \vdots \\ (r, g, b)_{i1} & \cdots & (r, g, b)_{ij} \end{bmatrix} \quad (4)$$

where M is of size $i \times j \times 3$. $i \times j$ is the resolution of the image, which means that there are i rows of pixels in the images and each row has j columns of pixels. Each pixel of the image has three intensity values, i.e. one for red, green, and blue. M can be rewritten into a two-dimension matrix X with the size of $(i \times j) \times 3$ in (5) by listing the three intensity values of each pixel line by line so that each row of X represents the red, green and blue values of a pixel:

$$X = \begin{bmatrix} r_1 & g_1 & b_1 \\ r_2 & g_2 & b_2 \\ \vdots & \vdots & \vdots \\ r_{i \times j} & g_{i \times j} & b_{i \times j} \end{bmatrix} \quad (5)$$

The intensity for each pixel is defined as the sum of the red, green, and blue values.

$$I = r + g + b \quad (6)$$

Image analysis extracts information from a time-series of images, represented by M matrices recorded at different points in time. A common image analysis procedure is to (1) record images at different time points, (2) separately analyze the images, and (3) combine the image analysis results for different time points to determine dynamics of the system.

III. IMAGE ANALYSIS BASED ON PCA AND K-MEANS CLUSTERING

A series of images taken by fluorescent microscopy were

analyzed to generate a time series of data representing the average fluorescent intensity of the cells in the images. In order to compute a fluorescent intensity profile it is required to first determine the areas in the image representing cells where fluorescence can be seen. The procedure for determining these areas makes use of principal component analysis and K-means clustering. A second step involves computing the average fluorescence intensity over these areas. The detailed steps involved in these procedures are described in the following.

Principal component analysis can be performed on X to determine pixels with similar brightness in the images by calculating the distance from a pixel to the first principal component (PC 1) as illustrated in Fig. 2.

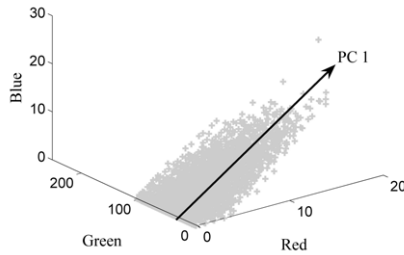


Fig. 2. Principal component analysis applied to images to determine pixels with similar features.

The projection of a point onto PC 1 can be used as a measure for clustering the pixel brightness into different sets via K-means clustering. Fig. 3 illustrates the procedure of fluorescent cell searching based on K-mean clustering and PCA. In an initial step PCA is used to divide the pixels of the image into two clusters based upon their projection onto PC1. K-means clustering iteratively updates the pixels and centroids of the two clusters until the sum of distances from all the pixels in each cluster is minimized. The cluster with the larger variation is divided in a next step. The centroids of the two new clusters, which are determined by PCA, and the centroid of the un-divided cluster are used as the initial centroids of the three clusters for K-means clustering, which then sorts the pixels of the image belonging to one of the three clusters. This procedure can be repeated until any number of desired clusters is obtained. The clusters with higher fluorescent intensity are considered to represent the cells which show a significant level of fluorescence. Once the cell region has been determined it is possible to compute the average fluorescent intensity by the following formula:

$$I = \left(\frac{\sum_{k=1}^{N_f} I_{f,k}}{N_f} - \frac{\sum_{k=1}^{N_b} I_{b,k}}{N_b} \right)_{stimulation} \quad (7)$$

where $I_{f,k}$ refers the fluorescent intensity of the k_{th} pixel in a fluorescent cell region, $I_{b,k}$ refers the fluorescent intensity of the k_{th} pixel belonging to the background, N_f is the total number of pixels in the fluorescent cell region, N_b is the total

number of pixels in the background. The reason for subtracting the intensity of the pixels representing the background is to reduce measurement noise due to brightness variations.

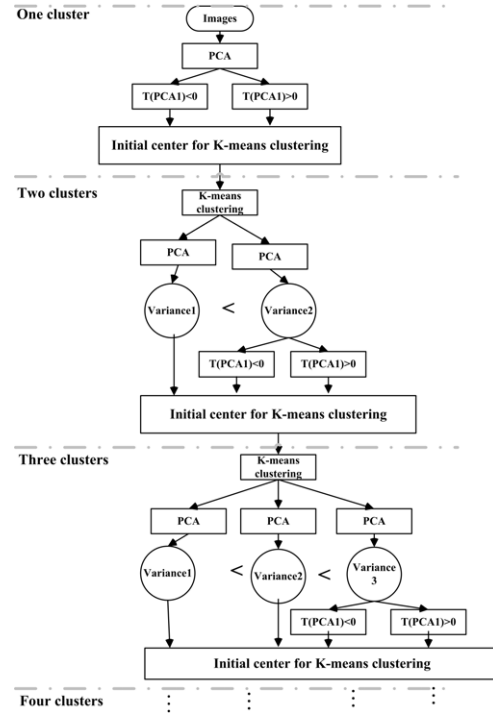


Fig. 3. K-means clustering algorithm used for identifying cell regions in fluorescent images.

This procedure has to be repeated for each image taken at different points in time to generate a time series of data for the fluorescent intensity. An example of the outcome of this procedure can be seen in Fig. 4 where the first three clusters represent fluorescent cells while the pixels included in clusters 4 and 5 correspond to the background.

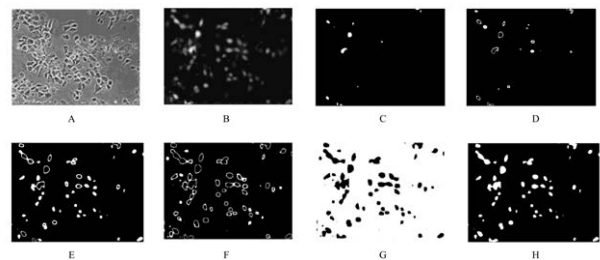


Fig. 4. Results of the image analysis algorithm. (A) Fluorescent microscopy image, (B) Fluorescent regions detected by the image analysis procedure: (C) – (G) clusters 1 through 5 detected by the algorithm; white pixels refer to pixels included in a specific cluster, (H) cumulative results of clusters 1, 2, and 3; the white region in (H) is chosen as the region representing cells with GFP while the black pixels shown in (H) represent the background.

IV. SOLUTION OF AN INVERSE PROBLEM TO OBTAIN THE PROFILE OF TRANSCRIPTION FACTORS FROM THE INTENSITY PROFILE

The structure of this section is as follows: Subsection A presents a model describing transcription, translation, and activation of GFP. The model uses the NF-kB concentration as the input and predicts the fluorescence intensity profile that can be measured. In subsection B, a solution of an inverse problem involving the model from subsection A to determine the NF-kB concentration is presented.

A. Model Describing Transcription, Translation, and Activation of GFP

Since the presence of NF-kB in the nucleus (i.e., activation of NF-kB) does not immediately lead to fluorescence seen in the images it is required to develop a model describing transcription/translation as well as activation of GFP. The equations of this model are based upon the model described by Subramanian and Srienc [10] where modifications are made to account for the constant reporter DNA levels in our experiments (i.e., due to stable integration of the reporter plasmid into the genomic DNA in our reporter cell line [8]) as well as to include the effect of transcription factor concentrations on the transcription rate. These changes result in the following model describing the measurement dynamics:

$$\begin{aligned}\frac{dm}{dt} &= S_m p \frac{C_{NF-kB}}{C + C_{NF-kB}} - D_m m \\ \frac{dn}{dt} &= S_n m - D_n n - S_f n \\ \frac{df}{dt} &= S_f n - D_n f\end{aligned}\quad (8)$$

where C_{NF-kB} is the concentration of activated NF-kB in the nucleus, m is the mRNA concentration, n is the concentration of GFP, and f corresponds to the concentration of activated GFP. The values of the parameters shown in (8) are given in Table 1. The procedure for estimation of C is described below.

TABLE I
PARAMETERS FOR THE MODEL SHOWN IN EQUATION (8)

Parameter	Value	Parameter	Value
S_m	373 1/hr	S_f	0.347 1/hr
D_m	0.45 1/hr	C	108 nM
S_n	780 1/hr	p	5 nM
D_n	0.5 1/hr	$m(0), n(0), f(0)$	0 nM

The experimental measurements consist of the fluorescence intensity, I , as seen on the images which is directly proportional to the concentration of activated green fluorescent protein:

$$f = \Delta I \quad (9)$$

where Δ is the ratio between activated GFP and computed fluorescence intensity.

As I can be obtained from the fluorescence images that have been processed by the procedures described in the image analysis section, the dynamics of NF-kB can be computed by solving an inverse problem involving (8).

B. Inverse Problem Formulation for Obtaining Transcription Factor Profile from Fluorescent Intensity Data

In this subsection we develop a procedure that computes the NF-kB concentration profile from the experimental data by solving an inverse problem using the model from equations (8) and (9). While it is possible to formulate this inverse problem and solve it numerically, it is actually possible to derive an analytical solution for this specific problem which computes C_{NF-kB} from the fluorescence intensity profile I . This analytical solution treats (8) as a static nonlinearity

$$u = \frac{C_{NF-kB}}{C + C_{NF-kB}} \quad (10)$$

which is followed by a system of linear differential equations:

$$\begin{aligned}dm/dt &= S_m p u - D_m m \\ dn/dt &= S_n m - D_n n - S_f n \\ df/dt &= S_f n - D_n f\end{aligned}\quad (11)$$

Taking a Laplace transform of (11) results in $f(s)$ as a function of $u(s)$:

$$f(s) = \frac{S_f}{s + D_n} \cdot \frac{S_n}{s + D_n + S_f} \cdot \frac{S_m p}{s + D_m} u(s) \quad (12)$$

While it is possible to choose any function to describe $u(s)$, we opted for

$$u(s) = \frac{\omega_n^2}{s^2 + 2\varepsilon\omega_n s + \omega_n^2} \cdot \frac{T_\alpha}{s} \quad (13)$$

as $u(s)$ represents a concentration profile of C_{NF-kB} that shows damped oscillatory behavior for continuous stimulation as has been reported in the literature [16]. Substituting (13) into (12) and performing an inverse Laplace transform results in:

$$\begin{aligned}f(t) &= A_1 + A_2 e^{-D_n t} + A_3 e^{-(D_n + S_f)t} \\ &+ A_4 e^{-D_m t} + A_7 e^{-\varepsilon\omega_n t} \sin(\omega_n \sqrt{1 - \varepsilon^2} t + \varphi)\end{aligned}\quad (14)$$

where A_1, A_2, A_3, A_4, A_7 , and φ are constants with the values given in Appendix.

The values of the parameters ε , ω_n and T_α are estimated by fitting $f(t)$ to the experimental data for each experiment. The concentration of NF-kB is then given by:

$$C_{\text{NF-kB}} = \frac{CT_\alpha \sqrt{1-\varepsilon^2} - CT_\alpha e^{-\varepsilon\omega_n t} \sin(\omega_n \sqrt{1-\varepsilon^2} t + \phi)}{(1-T_\alpha)\sqrt{1-\varepsilon^2} + T_\alpha e^{-\varepsilon\omega_n t} \sin(\omega_n \sqrt{1-\varepsilon^2} t + \phi)} \quad (15)$$

$$\text{where } \phi = \arctan \frac{\sqrt{1-\varepsilon^2}}{\varepsilon}$$

The values of C from (10) and Δ from (9) only need to be estimated once and can be assumed to be constant for all future experiments. We have chosen the concentration profile for NF-kB as reported in the paper by Hoffman et al. [16], which corresponds to a stimulation with 10 ng/ml of TNF- α , as the input, and have estimated C and Δ from experimental data that we have collected for stimulation with 10 ng/ml of TNF- α . The value of C was determined to be 108 nM and Δ was found to be equal to 2.5562×10^4 . It should be noted that some of the data derived from a stimulation with 10 ng/ml of TNF- α was used for determining these parameter values, while other data points will be used for testing model. Fig. 5 shows the fit of (14) to the data generated by this experiment.

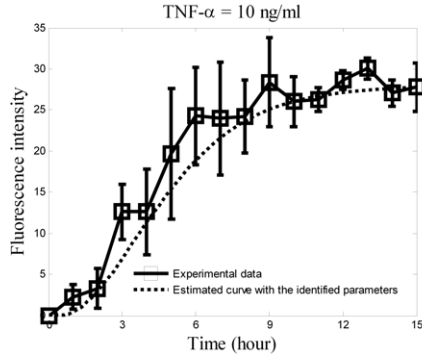


Fig. 5. Comparison of experimental data and the model predictions for f/Δ where the NF-kB concentration serves as the input to the model and is taken from Hoffman et al.'s paper [16].

V. APPLICATION OF THE PROCEDURE TO ADDITIONAL EXPERIMENTAL DATA

The activation of NF-kB in H35 reporter cells was investigated by stimulating with different TNF- α concentrations (6ng/ml, 10ng/ml, 13ng/ml, and 19 ng/ml). The data was analyzed using the described image analysis procedure, resulting in the fluorescence intensity profiles shown (Solid line) in Fig. 6. The error bars indicated \pm one standard deviation from the mean of the measurements taken for each time point. Fig. 6. also depicts the results of the estimated fluorescence intensity given by estimating model parameters for equation (14) for each case. The values for C and Δ are constant for these experiments, however, the values for ε , ω_n and T_α , as shown in Table 2, are estimated separately for each data set. The corresponding concentration profiles for NF-kB, as computed by (15) are shown in Fig. 7. It can be seen that stimulation with higher concentrations of TNF- α results in larger long-term concentrations of NF-kB as well as in higher peak concentrations. One important aspect of this procedure is that the data obtained is quantitative (i.e., numerical values of the NF-kB profile at each time point are obtained) and not merely qualitative or semi-quantitative.

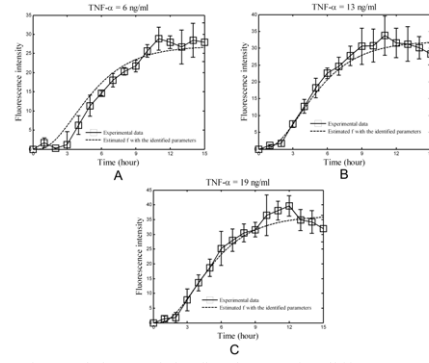


Fig. 6. Experimental data and the fitted curve for different TNF- α

TABLE 2
ESTIMATED VALUES FOR ε , ω_n AND T_α

TNF- α Concentration	ε	ω_n	T_α
6 ng/ml	0.2	4.52	0.26
13 ng/ml	0.2	4.52	0.31
19 ng/ml	0.28	4.61	0.35

concentrations.

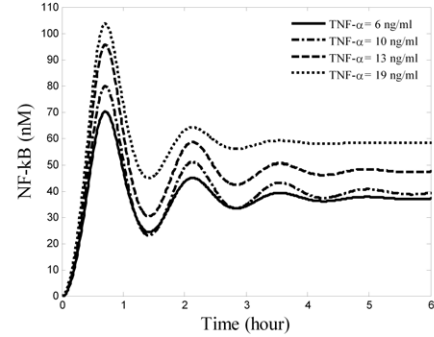


Fig. 7. NF-kB profiles computed via solution of the inverse problem for TNF- α concentrations of 6 ng/ml, 10 ng/ml, 13 ng/ml, and 19 ng/ml.

VI. DISCUSSION AND CONCLUSION

We have demonstrated in this work that transcription factor activation profiles can be quantitatively extracted from fluorescent reporter data. The presented approach was effective in deriving transcription factor activation rates from GFP profiles generated from NF-kB reporter cells stimulated with 6–19 ng/mL of TNF- α , a concentration range that is within the range of those commonly used in cell culture experiments [6, 17] and reported to result in strong activation of NF-kB [9]. However, predicting NF-kB activation at lower concentrations further improvement (e.g., in the image analysis methods) is needed to increase the GFP signal/noise ratio for effectively predicting profiles of low abundance transcription factors.

Another discrepancy between the model and experimental data is predicting long-term NF-kB activation profiles. The

data in Fig. 6 shows that fluorescence decreases after ~ 11 h even though the stimulus (TNF- α) is continually present, with the decrease being more pronounced at the higher concentrations. However, this decrease is not reflected in Fig. 6 which shows NF- κ B levels being constant beyond 11 h as the assumed model structure from (13) cannot represent this decrease. It is possible to postulate a different profile for the transcription factor, resulting in differences in (13), e.g., one that can reflect such a decrease. However, it is not clear if the decrease in fluorescence observed after ~ 11 h of stimulation results from experimental artifacts (i.e., fluorescent photobleaching and cell death arising from cells being repeatedly exposed to UV light for imaging) or is a real biological phenomenon (i.e., consequence of change in gene expression arising due to constant stimulation with TNF- α). A better understanding of long-term activation is needed to evaluate this behavior.

In summary we have developed a methodology for quantitatively determining transcription factor profiles. This technique makes use of fluorescence microscopy images from a GFP reporter system for transcription factor activation and involves solving an inverse problem to determine the transcription factor profile from the fluorescence intensity dynamics. Data generated by this method can then be used to estimate parameters for signal transduction pathway models. This technique was applied to the activation of NF- κ B by TNF- α , however, it can be used for other transcription factors with only minor modifications.

APPENDIX

Equations for computing the values of the constants found in (14)

$$\begin{aligned}
 A_1 &= S_f S_n S_m p T_a / D_n (D_n + S_f) / D_m \\
 A_2 &= -S_n S_m p \omega_n^2 T_a / (D_m - D_n) / (D_n^2 - 2\epsilon \omega_n D_n + \omega_n^2) / D_n \\
 A_3 &= S_n S_m p \omega_n^2 T_a / (D_m - D_n - S_f) / ((D_n + S_f)^2 - 2\epsilon \omega_n (D_n + S_f) + \omega_n^2) / (D_n + S_f) \\
 A_4 &= S_f S_n S_m p \omega_n^2 T_a / (D_n - D_m) / (D_m - D_n - S_f) / (D_m^2 - 2\epsilon \omega_n D_m + \omega_n^2) / D_m \\
 A_5 &= -C_0 d_1 / (b d_1^2 + b d_0^2) \\
 A_6 &= C_0 (a d_1 + b d_0) / (b d_1^2 + b d_0^2) \\
 A_7 &= \sqrt{A_5^2 + \left(\frac{A_6 - A_5 \epsilon \omega_n}{\omega_n \sqrt{1 - \epsilon^2}} \right)^2} \\
 a &= -\epsilon \omega_n \\
 b &= \omega_n \sqrt{1 - \epsilon^2} \\
 d_1 &= -(a_3 + 4a)b^3 + (3a_3 a^2 + 2a_2 a + 4a^3 + a_1)b \\
 d_0 &= b^4 + a_3 a^3 - (3a_3 a + a_2 + 6a^2)b^2 + a_2 a^2 + a^4 + a_1 a \\
 a_1 &= (D_n^2 + D_n S_f) D_m \\
 a_2 &= D_n^2 + D_n S_f + 2D_n D_m + D_m S_f
 \end{aligned}$$

REFERENCES

[1] S. Bandyopadhyay, N. Soto-Nieves, and F. Macián, "Transcriptional regulation of T-cell tolerance," *Semin. Immunol.*, vol.19, pp.180-187, 2007.

[2] A. Hoffmann, G. Natoli, and G. Ghosh, "Transcriptional regulation via the NF- κ B signaling module," *Oncogene*, vol.25, pp. 6706-6716, 2007.

[3] C.A. Grove, and A.J.M. Walhout, "Transcription factor functionality and transcription regulatory networks," *Mol. Biosyst.*, vol.4, pp. 309-314, 2008.

[4] L. Elmitski, V.X. Jin, P.J. Farnham, and S.J. Jones, "Locating mammalian transcription factor binding sites: a survey of computational and experimental techniques," *Genome Res.*, vol.16, pp. 1455-1464, 2006.

[5] B. L. Sprague, and J. G. McNally, "FRAP analysis of binding: properand fitting," *TRENDS in Cell Biology*, vol.15, no.2, pp. 84-91, 2005.

[6] K.R. King, S. Wang, A. Jayaraman, M. Toner, and M.L. Yarmush, "A High-throughput Microfluidic Real-time Gene Expression Living Cell Array," *Lab-on-Chip*, vol.7, pp. 77-85, 2007.

[7] K.R. King, S. Wang, A. Jayaraman, M.L. Yarmush, and M. Toner, "Microfluidic flow-encoded switching for parallel control of dynamic cellular microenvironments," *Lab-on-Chip*, vol.8, pp. 107-116, 2008.

[8] D.M. Thompson, K.R. King, K.J. Wieder, M. Toner, M.L. Yarmush, and A. Jayaraman, "Dynamic gene expression profiling using a microfabricated living cell array," *Anal. Chem.*, vol.76, pp. 4098-4103, 2004.

[9] K.J. Wieder, K.R. King, D.M. Thompson, C. Zia, M.L. Yarmush, and A. Jayaraman, "Optimization of reporter cells for expression profiling in a microfluidic device," *Biomed Microdevices*, vol.7, pp.213-222, 2005.

[10] S. Subramanian, and F. Srieenc, "Quantitative analysis of transient gene expression in mammalian cells using the green fluorescent protein," *J. Biotechnol.*, vol.49, pp. 137-151, 1996.

[11] L. Kaufman, and P. J. Rousseeuw, *Finding groups in data: an introduction to cluster analysis*, John Wiley & Sons Press, 1990.

[12] H. Hotelling, "Analysis of a complex of statistical variables into principal components," *Journal of Educational Psychology*, vol. 24, pp. 417-441, 1933.

[13] J. E. Jackson, *A user's guide to principal components*, Hoboken, N.J: John Wiley & Sons Press, 2003.

[14] P. Geladi, and H. Grahn, *Multivariate image analysis*, Chichester, U.K: John Wiley & Sons Press, 1996.

[15] M. H. M. Bharati, and J. F. Macgregor, "Multivariate image analysis for real-time process monitoring and control," *Industrial and Engineering Chemistry Research*, vol.37, pp. 4715-4724, 1998.

[16] Hoffmann A, Levchenko A, Scott ML, Baltimore D: The I κ B-NF- κ B signaling module: temporal control and selective gene activation. *Science* 2002, 298:1241-1245.

[17] Damelin LH, Coward S, Kirwan M, Collins P, Selden C, Hodgson HJF: Fat-loaded HepG2 spheroids exhibit enhanced protection from Pro-oxidant and cytokine induced damage. *J Cell Biochem* 2007, 101:723-734.

of TNF- α (< 10 ng/mL) was not as effective due to low levels of GFP signal. This is evident from Fig. 6 which shows a better correlation between the model and experimental data at higher (13 and 19 ng/mL) than at lower (6 ng/mL) TNF- α concentrations. Therefore, while our method is effective for moderate-to-high levels of activation,