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Molecular Signaling Observer and Predictor: A Framework for Closed-Loop Control of Cell Behaviors Having Long Time Delay

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Abstract— **Stable *in vitro* feedback control of cell behaviors using an observer to predict a cell's future response to input cues based on real-time measurement of intracellular signaling molecules is presented. Biological cells, particularly mammalian cells, have a long latency time from receiving cues to producing output responses. Numerous steps of intracellular signal transductions are involved between cues and responses. This slow dynamics is a major obstacle for close-loop control based on measurement of the output response. This paper presents a promising approach to coping with the slow dynamics and forming a stable feedback loop. A molecular signaling observer and predictor is designed for estimating the intracellular state and predict the cell's future response so that input cues can be accommodated proactively before observing the output response. First, a brief background description on relevant cell biology and** in vitro **micro-fluidics is provided, followed by the basic concept of molecular signaling observer and predictor. Cue-signal-response processes are modeled as a time-delay system with noise dynamics. A *k*-step ahead predictor is obtained as a conditional mean, and a closed-loop control system is formed based on the future error of the predicted output response. Prediction error and its effect on control performance are analyzed. The method is applied to angiogenic endothelial cell sprouting and migration process. Numerical examples demonstrate the effectiveness of the method.**

I. Introduction

Manipulating cell behaviors via feedback control is one of the major contributions that the control community can make to the realm of biological engineering. The recent progress of *in vitro* technologies, including microfluidics, imaging, and synthetic biology, has allowed us to develop methodologies for manipulating and guiding cells under tightly regulated conditions. Endothelial cells (EC), for example, can be guided towards proper angiogenic sprouting via feedback control in microfluidic environment [Wood, et al 2009]. It is known that accommodating input cues, e.g. growth factors and physical environment, can alter EC cell's phenotype transition and migration behaviors. Coordinated control of these cues has the potential to manipulate the angiogenic sprouting process, leading to *in-vitro* culturing of blood vessels, which will be a breakthrough in tissue engineering [Das et al, 2010; Wood et al, 2010-a].

A challenge in forming a feedback loop in the proposed application is the cells' long latency time. Specifically it takes a long time for a cell to respond to input cues. EC cells, for example, has a latency time on the order of hours. Several hours after changing a growth factor concentration, EC cells just begin to sprout out and migrate towards the source of grow factor. The cell's sprouting and migration process can be monitored with an imaging system, and a feedback loop can be formed from the measurement of the sprout. Nonetheless, the long latency time hinders the feedback control based on the phenomenological cell observation. Before reaching the observable sprouting and migration, the cells make a number of intracellular state transitions along their signal transduction pathways. Changing the input cues after observing the phenomenological output response is basically too late to alter the course of cell behaviors. The cell might have made a decision to take a particular phenotype transition that would be propagated across the intracellular signaling pathway, which would appear as an output response a few hours later. Such slow internal dynamics may result in instability and unwanted oscillation, leading to failure of cell guidance and regulation.

Understanding signaling pathways is crucial in cell biology, and the control community has been making significant contribution to dynamic modeling of signal transductions [Del Vecchio, 2010; Buzi et al, 2010; Motee et al, 2010].

This paper presents feedback control of cue-signal-response processes based on real-time measurement of key signaling molecules and a predictor to predict the cell's future response from the signaling measurement. Despite slow dynamics, delay-free feedback loop will be constructed by evaluating the future error based on the prediction. The proposed method is general and is applicable to broad problems. However, the method will be presented in the context of angiogenic cell migration control for clarity and practicality.

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II. BACKGROUND

A. In Vitro Closed-Loop Control of Cell Migration

Cells migrate to create or repair a tissue construct, the process termed morphogenesis. Blood vessels, for example, are constructed as Endothelial Cells (EC) migrate to form a tubular network. In angiogenesis, Endothelial Cells sprout out from an existing blood vessel when exposed to growth factors, create a hole in the extracellular gel matrix, and extend the hole towards a higher growth factor concentration. The sprouting cell recruits other cells, guide them towards the hole, and fill the wall of the hole to make a functional lumen that transport blood. Coordinated migration must be regulated properly for successful vascular network formation.

Recent progress in microfluidic device technology allows us to develop an *in vitro* experimental apparatus for culturing cells in 3-dimensional environment. See Fig.1. Unlike traditional on-the-gel 2-dimensional culturing, this new technology not only provides an *in vitro* environment that is closer to *in vivo* environment, but it also facilitates to closely observe cell behaviors, such as 3-D time-lapse images of angiogenic sprouting and migration, as well as to guide the cells by precisely delivering growth factors to the microfluidic chambers.

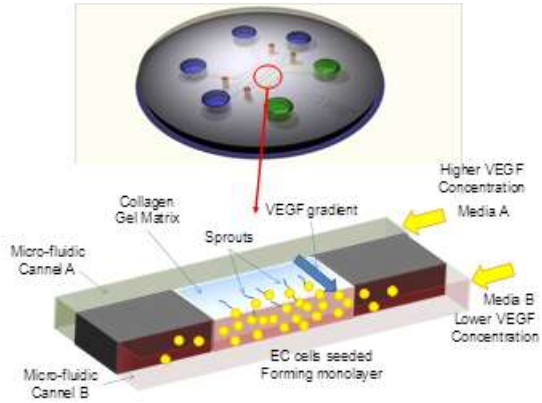

Fig.1 Microfluidic device for angiogenic sprouting experiment

Fig. 2 shows the confocal microscope image of a sprouting process of endothelial cells in a microfluidic chamber. It is observed that the extending sprout is led by a tip cell that creates a hole and recruits other cells to fill the wall. Fig.3 shows a closed-loop control system for guiding sprouting cells using the microfluidic device. Multiple growth factors are mixed and fed to the sprouting site through microfluidic channels filled with media. Vascular Endothelial Growth Factor (VEGF), the key growth factor for angiogenesis, is provided through two channels with different concentrations. VEGF diffuses to the gel between the two channels and thus creates a gradient of concentration across the gel region. Endothelial cells seeded on one side of the gel migrate towards the higher VEGF concentration. The author's group has recently found that the velocity of the tip cell, which creates a hole in the gel matrix and which recruits and guides other cells, must be regulated properly in order to construct a successful blood vessel [Wood et al, 2010-b]. Therefore, the specific objective of the feedback control system is to regulate the tip velocity by controlling the growth factor concentration. The tip cell velocity is observable in the microfluidic environment using cell tracker biomarker and confocal microscope.

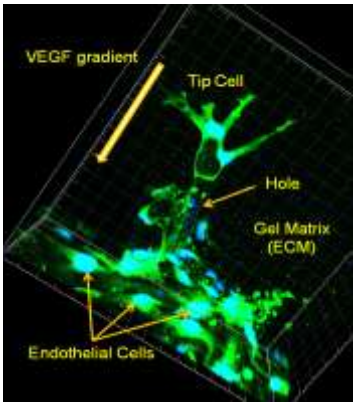

Fig.2 Confocal image of endothelial cell sprouting process

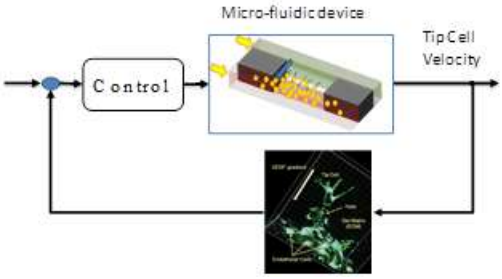

Fig.3 Feedback control of sprouting process

B. Signal Transduction Pathways and FRET Biosensors

As described previously, the challenge in forming a closed-loop control is that the cell has a significantly long time delay in responding to changes to growth factors. After growth factors bind to specific receptors on the cell membrane, numerous steps of molecular signal transductions occur before reaching specific responses, such as cell migration. Fig.4 shows a simplified version of signal transduction pathways from VEGF receptor-2 leading to a series of intracellular remodeling and interactions with the extracellular matrix, which result in cell migration. Several authors have model this cue-signaling-response process. There are a few important properties to note in modeling the cue-signaling-response process:

- Signaling path ways are highly redundant. Principal component analysis and other statistical methods have revealed that the cue-signal-response process can be approximated to a low-order model of 80~90% accuracy with just a few principal components.
- Among a number of signaling molecules involved in the intracellular signal transductions, a much fewer number

of key molecules play critical roles that determine the cell behaviors. For example, Src contributes to cell protrusion and migration in many ways. Src can phosphorylate p130cas, which recruits Crk and DOCK180 through the interaction of SH3 domain on Crk and PXXP motif on DOCK180. DOCK180 subsequently binds to ELMO and activates Rac, which leads to the activation of Wave1/Scar1(Rodriguez, Schaefer et al. 2003). Recent results indicate that Src can also directly phosphorylate Scar1(Ardern, Sandilands et al. 2006). Activated Scar1 can bind to and activate Arp2/3, which causes the branching growth of actin filaments and the formation of actin arcs adjacent to the plasma membrane (Rodriguez, Schaefer et al. 2003).

Signaling molecules represent the intracellular state. In particular, measurement of some key signaling molecules would allow us to identify the cell state and even predict a future state.

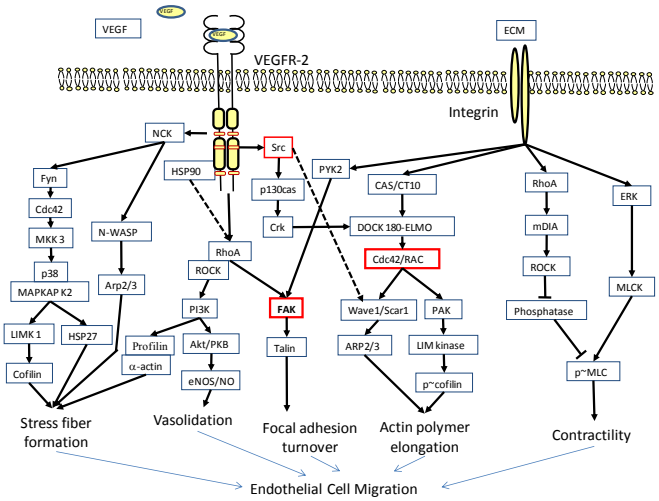

Fig.4 Simplified molecular signaling pathways

Signaling molecules are in general difficult to measure in real-time. However, a recent breakthrough has been made. Effective biosensors to detect key signaling molecules, including Src, Rac, and FAK, have been developed [Ouyang et al, 2008]. The new technology uses Fluorescence Resonance Energy Transfer (FRET) for measuring interactions between two proteins and the functional activities of different enzymes. Fig.5 shows a time-lapse experiment result of the Src signaling molecule distribution in a live mammalian cell exposed to a step change of VEGF concentration detected by the FRET biosensor. Fig.6 shows the time lapse data of the FRET intensity of Src signaling molecule. The results indicate that these improved and highly sensitive biosensors can allow the monitoring and visualization of dynamic molecular events at subcellular levels.

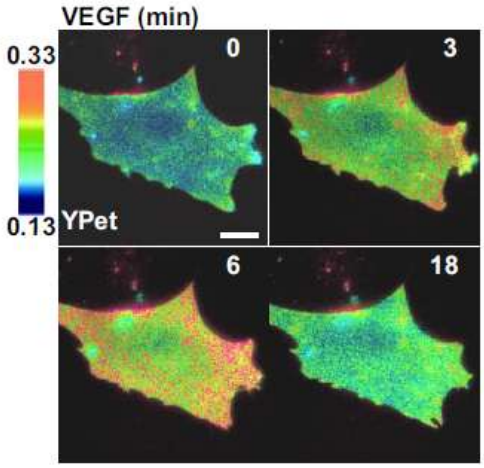

Fig. 5 FRET biosensor

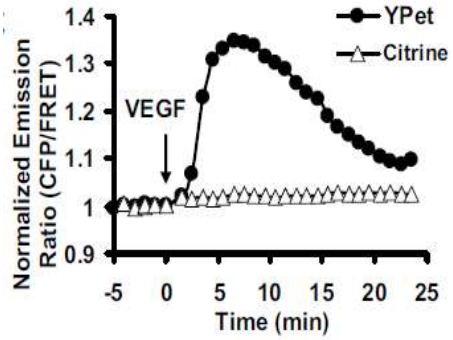

Fig.6 Step response of cell to VEGF

III. MODELING

Fig.7 shows a simple cue-signal-response model considered in this paper. Let $u(t)$ be an input cue, such as VEGF concentration, that can be regulated with the microfluidic device as described previously. Let $y(t)$ be a scalar output response, such as tip cell velocity, to be controlled so as to follow a reference input $r(t)$. Between the cue and the response there is a molecular signaling $s(t)$ that is measurable in real-time. As described above, various species of signaling molecules have been measured with FRET biosensors. Although diverse FRET biosensors are usable for off-line system identification, use of multiple biosensors concurrently is not allowed. For real-time control we have to target one molecular signaling and use a specific biosensor to detect it in real-time. Therefore, the model in the figure consists of only one signaling variable $s(t)$.

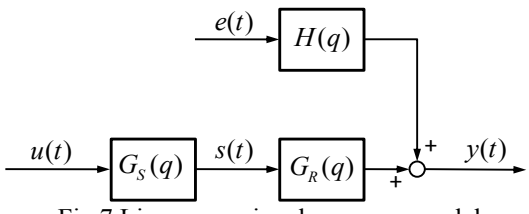

Fig.7 Linear cue-signal-response model

Experimental data are available for identifying the signaling dynamics in response to an input cue. Fig.6 shows a transient response of the Src biosensor to a step input cue of VEGF concentration. The data show that the transient response can be modeled as a linear system with a stable discrete time transfer function $G_S(q)$: $s(t) = G_S(q) \cdot u(t)$, where q is time shift operator: $u(t+1) = qu(t)$.

The dynamics of output response $y(t)$ to input signaling $s(t)$ has not yet been identified. However, references show that the response can be approximated to a linear system within a limited range of operation [Asada, 2010; Janes et al 2004]. In this paper we assume a linear transfer function $G_R(q)$ for the signal-response process. Characteristic to the signal-response process is a significantly long time delay. The transfer function $G_R(q)$ contains k-time steps of time delay between input and output:

$$G_R(q) = q^{-k} \cdot G'_R(q) \qquad (1)$$

where $G'_R(q)$ is a time-delay free transfer function.

The main objective of this paper is to overcome the long time delay associated with the signal-response process. A prediction-based method will be presented in the following section to virtually eliminate the effect of time delay in closing the loop. Prediction will be made based on signaling measurement $s(t)$ and the signal-response model subject to noise dynamics. As addressed in the literature [Das et al, 2009; Wood et at, 2009], cell behaviors are stochastic. Even under tightly controlled conditions of in vitro microfluidic experiments, endothelial cell sprouting and migration exhibit significant diversity. Stochasticity is central to modeling and control of cell behaviors. Therefore, we include noise dynamics in the signaling-response process, as shown in Fig.7.

Let $e(t)$ be an uncorrelated random process and $H(q)$ be a monic, inversely stable transfer function representing the noise dynamics:

$$H(q) = 1 + \sum_{\ell}^{\infty} h(l) q^{-l}$$

The observed output response $y(t)$ is then given by

$$y(t) = G_R(q)s(t) + H(q)e(t)$$

IV. Delay-Free Closed Loop Control Based on Prediction

Direct output feedback from observed $y(t)$ to input cue $u(t)$ is likely to cause instability and oscillation due to the long time delay q^{-k}. The objective of this section is to develop an effective control algorithm to virtually eliminate the time delay based on k-time step ahead prediction. Prediction inevitably incurs some error. It is important to examine how such prediction error may degrade control performance. The following control law and its analysis will provide a time-delay free feedback control with limited prediction error, which can be attenuated with a proper predictor and feedback mechanism.

Let $\hat{y}(t+k \mid t)$ be k-step ahead prediction of output response based on output observation $y(\tau)$ and signaling observation $s(\tau)$ for $1 \le \tau \le t$, and known input cues $u(\tau)$ for $1 \le \tau \le t-1$;

$$\hat{y}(t+k \mid t) \triangleq k) \mid u(\tau), 1 \le \tau \le t-1; y(\tau), s(\tau), 1 \le \tau \le t] \qquad (4)$$

In Fig.7 the output response consists of deterministic component $G_R(q)G_S(q)u(t)$ and a random process:

$$v(t) = H(q)e(t) = y(t) - G_R(q)s(t) \qquad (5)$$

which is a correlated (colored), zero-mean random process. Let $F(q)$ be a stable prediction filter to predict the k-step ahead value of $v(t)$:

$$\hat{v}(t+k \mid t) = F(q)[y(t) - G_R(q)s(t)] \qquad (6)$$

The output prediction is then given by

$$\hat{y}(t+k \mid t) \triangleq {}_S(q)u(t+k) + F(q)[y(t) - G_R(q)s(t)] \qquad (7)$$

where the first term $G_S(q)G_R(q)u(t+k)$ includes only the input sequence at most up to time t-1 because of time delays involved in $G_R(q) = q^{-k} \cdot G'_R(q)$ as well as in $G_S(q)$,

$$G_R(q)G_S(q)u(t+k) = G'_R(q)G_S(q)u(t). \qquad (8)$$

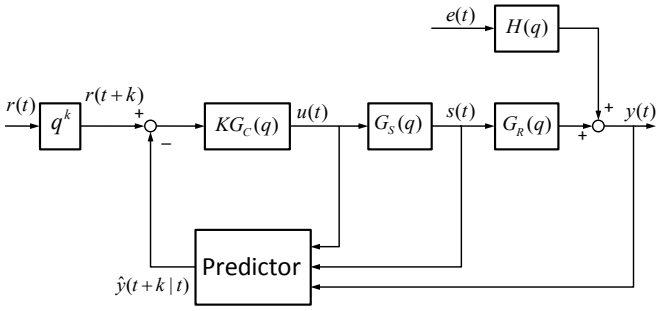

Fig.8 Prediction based delay-free feedback control

We construct the current input $u(t)$ based on the discrepancy between the k-step ahead reference $r(t+k)$ and the predicted output response $\hat{y}(t+k \mid t)$.

$$u(t) = G_C(q)[r(t+k) - \hat{y}(t+k \mid t)] \qquad (9)$$

where $G_C(q)$ is a proper dynamic compensator. From (7) and (9),

$$s(t) = \frac{q^k G_C(q) G_S(q)}{1 + [q^k G_R(q) - F(q)G_R(q)]G_s(q)G_C(q)} r(t) - \frac{G_s(q)G_C(q)F(q)}{1 + [q^k G_R(q) - F(q)G_R(q)]G_s(q)G_C(q)} y(t) \qquad (10)$$

From (1), the term $q^k G_R(q)$ becomes a delay-free transfer function: $G'(q)$. Substituting (10) into (3) yields

$$y(t)=\frac{G_S(q)G_C(q)G'_R(q)}{1+G_S(q)G_C(q)G'_R(q)}r(t)+\left[1-\frac{G_S(q)G_C(q)F(q)G_R(q)}{1+G_S(q)G_C(q)G'_R(q)}\right]\cdot H(q)e(t) \quad (11)$$

Proposition Consider a cue-signal-response process with *k*-step time-delay and correlated, zero-mean noise dynamics given by (3). The feedback control (9) based on the *k*-step ahead output prediction (7) has the following properties:

a) The output response to reference input $r(t)$ is free of time delay with a transfer function given by:

$$R(q)=\frac{G_S(q)G_C(q)G'_R(q)}{1+G_S(q)G_C(q)G'_R(q)} \quad (12)$$

where $G'_R(q)=q^k\cdot G_R(q)$ has no time delay. $R(q)$ does not depend on the prediction filter $F(q)$.

b) If no prediction filter is used; $F(q)=0$, the output response is perturbed 100% by the noise $H(q)e(t)$ added to the output. With the prediction filter the output response error is attenuated to

$$W(q)=1-\frac{G_S(q)G_C(q)F(q)G_R(q)}{1+G_S(q)G_C(q)G'_R(q)} \quad (13)$$

□

The prediction filter is to be designed to suppress the noise. If the noise dynamic $H(q)$ is available, the filter can be optimized to minimize the conditional mean of prediction error [Ljung, 1999]. From (2), the k-step ahead correlated noise $v(t+k\,|\,t)$ can be expressed by two terms: one depending on future random noise, $e(t+1),\cdots$), and the other determined by already observed noise, $e(t),e(t-1),\cdots$. Therefore,

$$\begin{aligned}\hat{v}(t+k\,|\,t)&=E\left[v(t+k\,|\,t)\right]\\&=\sum_{\ell}^{k-1}h(l)E[e(t+k-l)]+\sum_{\ell}^{\infty}h(l)q^k e(t-l)\\&=\bar{H}_k(q)e(t)=\bar{H}_k(q)H^{-1}(q)\left[y(t)-G_R(q)s(t)\right]\end{aligned} \quad (14)$$

where

$$\bar{H}_k(q)=\sum_{\ell}^{\infty}h(l+k)q^{-l} \quad (15)$$

Therefore, the prediction filter is given by

$$F(q)=\bar{H}_k(q)H^{-1}(q) \quad (16)$$

where $H(q)$ is inversely stable.

In case the noise dynamic model is not available, a standard state observer using $y(t)$ and $s(t)$ can be built to estimate the state associated with the signal-to-response dynamics. Let *A* and *C* are an observable pair of state transition and observation matrices associated with the signal-to-response process, and *L* be an observer gain. The prediction filter is then given by

$$F(q)=CA^k(qI-A)^{-1}L \quad . \quad (17)$$

V. Simulation Experiment

The dynamics of the cue-signal process of an EC cell has been identified based on experimental data of the FRET biosensor [Ouyang et al, 2008]. The step response data of Fig.6 was used for identifying the transfer function $G_S(q)$. It has been found that a second-order model can approximate the response with an excellent goodness of fit, $r^2=0.985$:

$$G_S(q)=\frac{0.08603q^{-1}-0.0786q^{-2}}{1-1.798q^{-1}+0.8476q^{-2}} \quad (18)$$

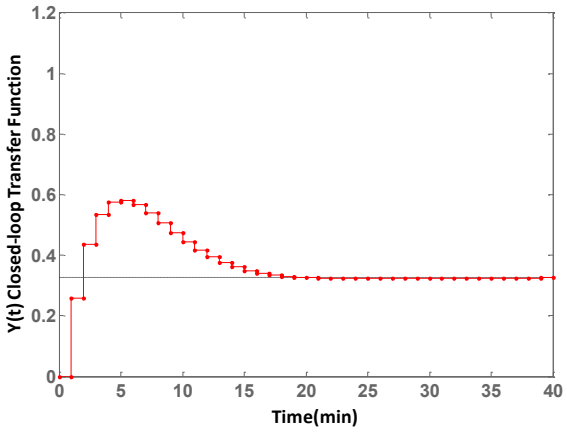

Fig. 9 Step response of delay-free feedback control based on *k*-step ahead predictor, $a=0.063$, $K=3$

In this stage the signal-response process has not yet been identified. Instead it is assumed that it is given by the following form:

$$G_R(q)=\frac{q^{-k}}{1-aq^{-1}} \quad (19)$$

where $0<a<1$. Simulation was performed for this delayed first order system.

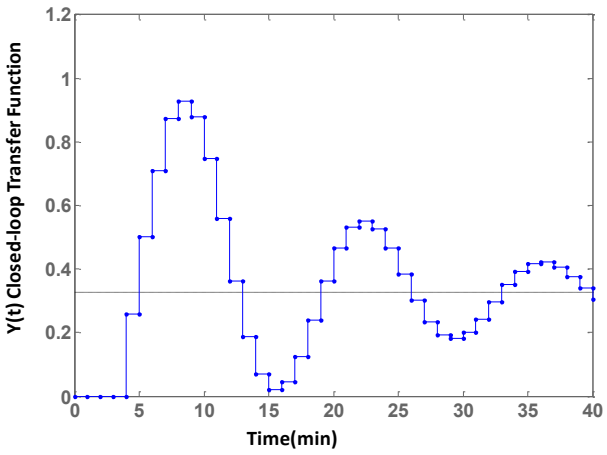

Fig.10 Step response without prediction, $a = 0.063, K = 3, k = 3$

Fig.9 shows the step response of the prediction-based delay-free feedback control system. For simplicity, only a simple proportional control is used. The system is stable and converges quickly. In contrast, Fig. 10 shows the case without prediction-based control. Even with a short time delay, the system becomes oscillatory, and it becomes unstable as the feedback gain K and the delay time k increase.

VI. Conclusion

A new approach to *in vitro* closed-loop control of cell behaviors based on key signaling molecule measurement has been presented. A prediction-based control is developed to overcome a cell's long time delay in signal-to-response dynamics. The effect of prediction error on the output response is analyzed based on noise dynamic model. An optimal prediction filter is also obtained from the noise dynamic model.

The author's group is currently working on the identification of the cue-signaling-response process by using the FRET biosensors with a long term goal of closed loop control of angiogenic sprouting processes.

References

[Ardern st al, 2006]
Ardern, H., E. Sandilands, et al. (2006). "Src-dependent phosphorylation of Scar1 promotes its association with the Arp2/3 complex." Cell Motil Cytoskeleton 63(1): 6-13.

[Asada, 2010]
Asada, H., "Reduced-Order Cue-Signal-Response Modeling for Angiogenic Cell Migration Control: A Principal Signal Approach", Proceedings of 2010 ASME Dynamic Systems and Control Conference, Cambridge MA, September 2010.

[Buzi et al, 2010]
Buzi, G, Topcu, U, Doyle, J., "Quantitative Nonlinear Analysis of Autocatalytic Networks with Applications to Glycolysis", Proceedings to 2010 American Control Conference, pp.3592-3597, July 2010.

[Das et al, 2009]
Das, A., Lauffenburger, D., Asada, H., and Kamm, R., "A Hybrid Continuum–Discrete Modeling Approach to Predict and Control Angiogenesis: Analysis of Combinatorial Growth Factor and Matrix Effects on Vessel-Sprouting Morphology", Philosophical Transactions of the Royal Society A: Mathematical, Physical, and Engineering Sciences, vol. 368, no. 1921, pp. 2937-2960, June 2010

[Del Vecchio, 2010]
Del Vecchio, D., "The Impact of Retroactivity on the Input/Output Static Characteristics of a Signaling Components", Proceedings of 2010 ASME Dynamic Systems and Control Conference, September 2010.

[Janes et al, 2004]
Janes, K., Kelly, J.R., Gaudet, S., Albeck, J.G., Sorger, P.K., and Lauffenburger, D., 2004. "Cue-Signal-Response Analysis of TNF-Induced Apoptosis by Partial Least Square Regression of Dynamic Multivariate Data", Journal of Computational Biology, 11(4), pp.544-561.

[Ljung, 1999]
Ljung, Lennart, "System Identification: Theory for the User, Second Edition", Prentice-Hall, 1999.

[Motee, et al, 2010]
Motee, N, Bamieh, B., Khammash, M., "Stability Analysis of a Class of Biologica Network Models", Proceedings of 2010 American Control Conference, pp.5939-5941, July 2010.

[Ouyang et al, 2008]
ouyang, M., Sun, J., Chien, S., and Wang, Y., "Determination of Hierarchical relationship of Src and Rac at Subsellular Locations with FRET Biosensors", Proceedings of the National Academy of Sciences (PNAS), Vol.105, No.38, pp.14353 – 14358, 2008.

[Rodriguez et al, 2003]
Rodriguez, O. C., A. W. Schaefer, et al. (2003). "Conserved microtubule-actin interactions in cell movement and morphogenesis." Nat Cell Biol 5(7): 599-609.

[Wood et al, 2009]
Wood, L., Das, A., Kamm, R. and Asada, H., "A Stochastic Broadcast Feedback Approach to Regulating Cell Population Morphology for Microfluidic Angiogenesis Platforms", IEEE Transactions on Biomedical Engineering, Vol.56, Issue 9, Part 2, pp.2299-2303, September, 2009

[Wood et al, 2010-a]
Wood, L., Kamm, R., and Asada, H., "Stochastic Modeling and Identification of Emergent Behaviors of an Endothelial Cell Population in Angiogenic Pattern Formation", accepted for publication in the International Journal of Robotics Research, Special Issue on Stochasticity in Robotics and Biological Systems, September 2010

[Wood et al, 2010-b]
Wood, L., Kamm, R., and Asada, H., "Time Lapse Observation-Based Modeling and Identification of Cell Behaviors in Angiogenic Sprout Development", Proceedings of 2010 ASME Dynamic Systems and Control Conference, Cambridge MA, September 2010.