PhD in Information Technology and Electrical Engineering

Università degli Studi di Napoli Federico II

PhD Student: Lorena Postiglione

XXIX Cycle

Training and Research Activities Report – First Year

Tutor: Dr. Diego di Bernardo

1. Information

Name: Lorena
Surname: Postiglione
Education: MS In Biomedical Engineering – Università degli Studi di Napoli Federico II
PhD course: XXIX Cycle- ITEE – Università di Napoli Federico II
Fellowship type: MIUR
Tutor: Dr. Diego di Bernardo

2. Study and Training activities

Attended Courses

Title: Theory and applications of piecewise smooth dynamical systems
Professor: Prof. John Hogan (University of Bristol)
Type: Ad Hoc Module
Place and Date: Napoli, June 16-20th 2014
Credits: 5

Title: EuroProgettazione
Professor: Dr. Gianpaolo Varchetta
Type: Ad Hoc Module
Place and Date: Napoli, October-November 2014
Credits: 3

Title: Modelli per la previsione e l'ottimizzazione
Professor: Pr. Diego di Bernardo
Type: Ms Module
Place and Date: Napoli, from October 2014 to January 2015
Credits: 6

Seminars

Title: Nano-carbon based components and materials for high frequency electronics
Professor: Prof. Sergey Maksimenko, Prof. Gregory Slepyan, Prof. Pavel Dyachkov, Prof. Alexander Lobko
Place and Date: Napoli, October 6th 2014
Credits: 0.8

Title: Differential Geometric Methods Feedback Linearization
Professor: Prof. Josep Olm
Place and Date: Napoli, April 10th 2014
Credits: 0.2

Title: Heterogeneities in temporal networks emerging from adaptive social interactions
Professor: Prof. Josep Olm
Place and Date: Napoli, April 10th 2014
Credits: 0.2

External courses (Telethon Institute of Genetics and Medicine)

Title: Systems Biology and Fuctional Genomics
Professor: Prof. Diego di Bernardo
Place and Date: Napoli, June 23-27th 2014
Credits: 3

Title: Introduction to scientific methodology and experimental design
Professor: Prof. Enrico Surace
Place and Date: Napoli, June 2014
Credits: 1

Title: Molecular Therapy
Professor: Prof. Alberto Auricchio, Prof Fulvio Mavilio, Prof Nicola Brunetti
Place and Date: Napoli, July 2014
Credits: 3

Title: Medaka fish as model system for biomedical research
Professor: Prof.Ivan Conte
Place and Date: Napoli, July2014
Credits: 1

Title: Advanced light microscopy in modern biomedical research
Professor: Prof. Roman Polishchuk
Place and Date: Napoli, July2014
Credits: 1

Title: Development and validation of cell-based high content imaging assaysProfessor: Prof. Diego Medina
Place and Date: Napoli, July2014
Credits: 1

Title: NGS technology and application
Professor: Prof. Vincenzo Nigro
Place and Date: Napoli, July2014
Credits: 1

External seminars (Telethon Institute of Genetics and Medicine)

Title and professor	Date	Credits
"From virus evolution to vector revolution. Synthetic AAV capsids for improved in vitro & in vivo gene delivery", Dr Dirk Grimm	11/03/14	0.2
"Conserved mechanisms of longevity: Regulation of lysosomal function", Dr Louis R. Lapierre	13/03/14	0.2
"Bionspired materials for advanced therapy and diagnosis", Prof. Paolo Netti	15/04/14	0.2
"Lysosomes: Small organelles with a huge impact", Dr Paul Saftig	16/04/2014	0.2
"MicroRNA regulated networks in pluripotency", Prof. Robert Blelloch	08/05/2014	0.2
"The Retinal Pigment Epithelium (RPE): a central player in retinal function and disease", Prof. Enrique Rodriguez-Boulan	16/05/2014	0.2

"A lung-on-chip to measure oxygen affinity of single red blood cells", Dr Giuseppe di Caprio	11/06/2014	0.2
"Gene therapy of genetic neuromuscular and blood diseases: the Genethon approach", Dr. Fulvio Mavilio	11/06/2014	0.2
"Propagation of synucleinopathy through lysosomal dysfunction", Prof. Seung-Jae Lee	19/06/14	0.2
"Neurodegenerative Diseases: The Dangers of Too Much Protein Stability", Prof. Huda Y. Zoghbi	09/07/2014	0.2
"Viral gene therapy approaches for human disorders of copper transport and lysosomal storage", MD Stephen G. Kaker	07/10/2014	0.2
"Wilson's disease: molecular mechanism and new approaches to treatmen", Prof. Svetlana Lutsenko	10/10/2014	0.2
"Gene Therapy of MPS I", MD James M. Wilson	22/10/2014	0.2
"Rational confederation of genes and diseases", Prof. Doron Lancet	28/10/2014	0.2
Understanding Lysosomal Storage Disorders: From tissue degeneration to phenotypic variability", PhD André Klei	16/12/2014	0.2

Autophagy: The Intracellular Self-Degradation System Fighting against Diseases, Prof. Tamotsu YOSHIMORI	27/01/2015	0.2
Role(s) of intracellular catabolism during skeletal development Carmine Settembre	03/02/2015	0.2
"The transparent editorial process and research integrity at EMBO Press.", Prof. Roberto Buccione, PhD	10/02/2015	0.2
“How to avoid commitment –epigenetic/ post-transcriptional maintenance of pluripotency.”, Robert Blelloch	12/02/2015	0.2
“Regulation of self renewal in cancer stem cells.” Prof. Giuseppe Pellicci	17/02/2015	0.2

	Credits year 1							
		1	2	3	4	5	6	
	Estimated	bimonth	bimonth	bimonth	bimonth	bimonth	bimonth	Summary
Modules	18		9	7		3	6	**25**
Seminars	13	1	1	0,2	1,8	0,4	1	**5,4**
Research	34	9	1	1	8	7	4	**30**
	65	10	11	8,2	9,8	10,4	11	**60,4**

3. Research activity

Microfluidic-based automatic control of gene expression in mammalian cells.

Control Engineering can be applied to biological systems to steer cellular processes towards a desired behaviour. Application of control engineering to steer gene expression are still in the infancy. Nevertheless some interesting results have already been achieved in lesser eukaryotes ([Toettcher et al., 2011], [Uhlendorf et al., 2012], [Menolascina et al., 2014]).
My research aims at synthesing and implementing a control strategy to regulate the dynamics of gene expression in mammalian cells. This is a very challenging task, since these cells exhibit complex and adaptive behaviour and are very sensitive to external perturbations. Specifically I explore a variety of control strategies to force a population of living cells to produce a desired (fixed or time varying) amount of a fluorescent reporter protein. I will then implement and compare these strategies experimentally to pick the best performing ones.
Since control of gene expression in mammalian cells has never been attempted, during my first year, I performed a pilot study to assess whether control engineering can be indeed applied to these cells. To this end, I performed a series of control experiments using output control strategies with CHO Tet-OFF cell line (already available in the laboratory [Siciliano et al., 2011]), which expresses a destabilized fluorescent reporter (YFP) under the control of a Tetracycline-responsive promoter. Specifically in untreated medium, the cells express the reporter protein (YFP) but this expression is inhibited if Tetracycline is present in the culture medium.

The behaviour of this biological system can be described by the following differential equations:

$$\frac{dx_1}{dt} = v_1\alpha_1 + v_2\alpha_2 U - d_1 x_1$$

$$\frac{dx_2}{dt} = v_2 x_1 - d_2 x_2$$

$$\frac{dx_3}{dt} = v_3 x_2 - d_3 x_3$$

where x_1 is the production of the YFP mRNA, x_2 is the unfolded reporter protein, and x_3 is the mature, fluorescent YFP (the readout of the system). **U** represents the presence, or absence, of Tetracycline, and it can be either 0 (no Tetra, low steady state) or 1 (+ Tetra, high steady state).

Figure 1 represents the result of a numerical simulation by using the previous mathematical model. The red signal is the system input (U) and the blue

line is the system output, i.e. the fluorescent signal. As represented in Figure 1, in presence of tetracycline-treated medium (high input signal), the cell fluorescence (blue signal) decreases while it increases if the cells are fed with untreated medium (low input signal).

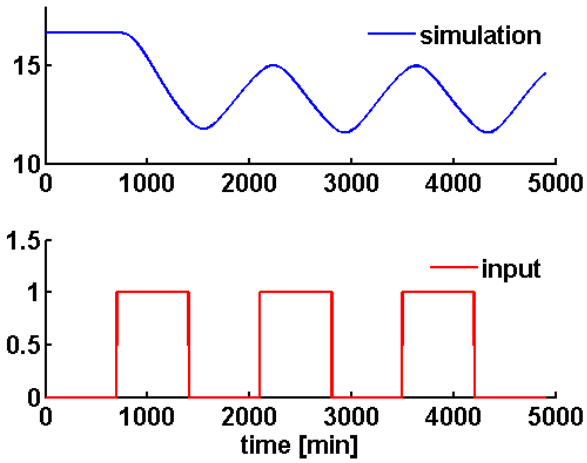

Figure 1:*In silico* set-point control of TetOFF-YFP system using a relay controller

The implementation of real-time control on cells requires a control platform that couples a control algorithm, an actuator and a sensor to cells. This was achieved by making use of a microfluidic device coupled to a time-lapse fluorescence microscope. The control platform is schematised in the following figure:

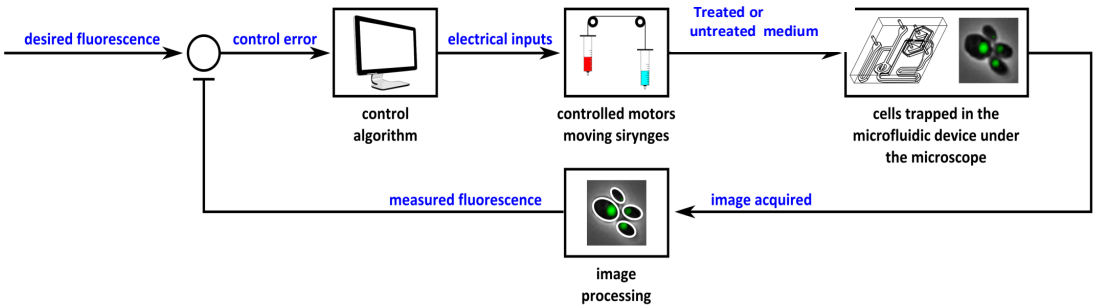

Figure 2: Control Platform

Cells grow in the microfluidic device [Kolnik et al., 2012] in a CO2 - and temperature-controlled environment under an inverted fluorescence microscope. Images, acquired every 15 minutes, are elaborated by a segmentation algorithm for quantification of fluorescence. The actual level of fluorescence is compared to the desired value and the control error is computed. As Figure 1 shows, the control algorithm uses the error to instruct the actuator how to change the height of two syringes containing either untreated medium or Tetracycline-treated medium, as to close the loop in a feedback mechanism. The cells in the microfluidic device are fed

with the medium that is in the highest syringe and their fluorescence decreases if they are receiving treated medium, while increases if they are fed with untreated medium.

This platform is simple and is highly customizable. The only constraints that apply to it are the time required for image processing and actuation, which is anyway in the order of a couple of minutes (therefore it does not pose a problem given the sampling time of 15 minutes), and the fact that the actuator is able to give cells only a binary input (either +Tetracycline or no Tetracycline).

First I learned how to build microfluidics devices from scratch. To this aim, a master mold has been produced using a silicon wafer as substrate. Once the mold was ready I used (Tridecafluoro-1,1,2,2-Tetrahydrooctyl)- 1-Trichlorosilane to prevent polymer from sticking to microstructures. I then prepared a 10:1 mixture of PDMS prepolymer and curing agent (Sylgard 184, Dow Corning) and degassed it under vacuum for 1 hour. Then the mixture is poured on the mold, and to facilitate the polymerization and the cross-linking, it is cured on a hot plate at 75◦C for 3h. After this step the PDMS layer, containing the microfluidic channels, is peeled from the master and it is cut with a scalpel to separate the single devices; holes are bored through them with a 20-gauge blunt needle in order to create fluidic ports for the access of cells and liquid substances. For each PDMS piece containing microchannels a thin glass slide (150um) is exposed to oxygen plasma and brought into contact to form a strong irreversible bond between two surfaces.

I then performed *in vivo* set-point control experiments. The first control strategy I implemented was the simplest one I could think of, that is the Relay controller with 5% hysteresis. Figure 3 shows the result of a set-point control experiment in which the task is to maintain the value of cell fluorescence (green signal) as close as possible to 50% of the initial value (blue line) for 3500 minutes. The input signal (red line) is high when Tetracycline-treated medium is given to the cells and it is low when the cells are fed with untreated medium.

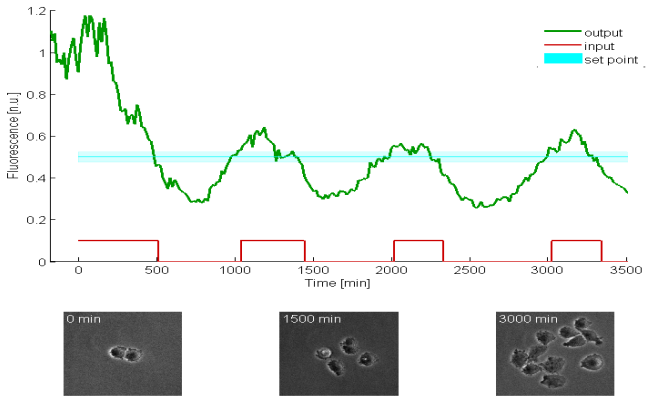

Figure 3: *In vivo* set-point control of TetOFF-YFP system using a relay controller

My results show for the first time that is possible to apply Control Engineering to regulate at will protein expression in mammalian cells but it is evident, as expected, that the relay controller is not able to achieve an optimal performance (the fluorescence never stabilizes on the set-point value) due to both the oscillations caused by relay itself and the low speed of the transcriptional system response.

I then implemented also a Proportional-Integral (PI) controller with a Pulse Width Modulator (PWM) to discretise the continuous PI output into a binary signal to give to the cells that respond only to the absence or presence of a stimulus (discrete signal). Figure 4 shows the result of a preliminary *in vivo* control experiment by using PI-PWM control strategy.

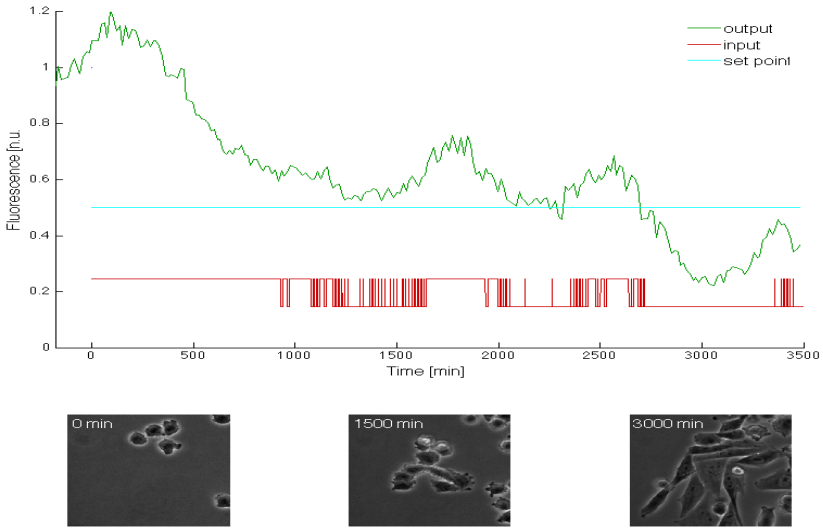

Figure 4: *In vivo* set-point control of TetOFF-YFP system using a PI-PWM controller

These preliminary experiments are pivotal in directing the future efforts in the field of automatic control of a transcriptional system in mammalian cells, highlighting the principal challenges that a successful approach has to overcome. The fact, for example, that transcription occurs at very large timescales in mammalian cells is responsible for the low performance of an approach that is not based on a mathematical model of the system, such as the relay controller; the only way of counteracting the inherent inertia of the system would be to apply more advanced control strategies. For this reason my next work will be directed to develop a model-based control, e.g. Model Predictive Control-MPC and to perform a tracking control to some time-variant reference value. Furthermore, in the next future a novel challenge will be to develop a single cell tracking to control gene expression in single cell.

[Kolnik et al., 2012] Kolnik, M., Tsimring, L. S., and Hasty, J. (2012). Vacuum-assisted cell loading enables shear-free mammalian microfluidic culture. *Lab on a chip.*

[Menolascina et al., 2014] Menolascina, F., Fiore, G., Orabona, E., De Stefano, L., Ferry, M., Hasty, J., di Bernardo, M., and di Bernardo, D. (2014). In-vivo real-time control of protein expression from endogenous and synthetic gene networks. *PLoS computational biology*, 10(5):e1003625.

[Siciliano et al., 2011] Siciliano, V., Menolascina, F., Marucci, L., Fracassi, C., Garzilli, I., Moretti, M. N., and di Bernardo, D. (2011). Construction and Modelling of an Inducible Positive Feedback Loop Stably Integrated in a Mammalian Cell-Line. *PLoS Computational Biology*, 7(6):e1002074.

[Toettcher et al., 2011] Toettcher, J. E., Gong, D., Lim, W. a., and Weiner, O. D. (2011). Light-based feedback for controlling intracellular signalling dynamics. *Nature methods*, 8(10):837-9.

[Uhlendorf et al., 2012] Uhlendorf, J., Miermont, A., Delaveau, T., Charvin, G., Fages, F., Bottani, S., Batt, G., and Hersen, P. (2012). Long-term model predictive control of gene expression at the population and single cell levels. *Proceedings of the National Academy of Sciences of the United States of America,* 109(35):14271-6.

4. Products

Automatic control of gene expression in a mammalian cell line
Chiara Fracassi, Lorena Postiglione, Gianfranco Fiore, and Diego di Bernardo, ACS Synthetic Biology (in preparation)

The Second International Mammalian Synthetic Biology Workshop,
April 2015,Massachusetts Institute of Technology Cambridge, MA, USA