PhD in Information Technology and Electrical Engineering

Università degli Studi di Napoli Federico II

PhD Student: Giansimone Perrino

XXIX Cycle

Training and Research Activities Report – First Year

Tutor: Diego di Bernardo – co-Tutor: Mario di Bernardo

1. Information

Giansimone Perrino

MS in *Automation Engineering* – Università di Napoli Federico II

PhD Student in ITEE – XXIX Cycle – Università di Napoli Federico II

Fellowship provided by *Telethon Institute of Genetics and Medicine* (TIGEM)

Tutor: Diego di Bernardo

Co-Tutor: Mario di Bernardo

The following table reports the credits summary for first year.

	Credits year 1							
		1	2	3	4	5	6	
	Estimated	bimonth	bimonth	bimonth	bimonth	bimonth	bimonth	Summary
Modules	**20**	0	9	13	0	3	6	**31**
Seminars	**5**	1	1	2,2	1,8	0,4	1	**7,4**
Research	**35**	9	1	1	8	7	3	**29**
	60	10	11	16,2	9,8	10,4	10	**67,4**

2. Study and Training activities

Courses

i. **Theory and Applications of Piecewise Smooth Dynamical Systems**
John Hogan on June 16th - 20th 2014
Ad hoc module (5 credits)

ii. **EuroProgettazione**
October and November 2014
Ad hoc module (3 credits)

iii. **Modelli per la Previsione e l'Ottimizzazione**
Diego di Bernardo from October 2014 to January 2015
Module from MS in Biomedical Engineering (6 credits)

iv. **Systems Biology and Functional Genomics**
Diego di Bernardo and Sandro Banfi on June 2014
External Module provided by Tigem (3 credits)

v. **Introduction to scientific methodology and Experimental design**
Enrico Maria Surace on June 2014
External Module provided by Tigem (1 credit)

vi. **Molecular Therapy**
Enrico Maria Surace on July 2014

External Module provided by Tigem (3 credits)

vii. **Medaka fish as model system for biomedical research**
Ivan conte on July 2014
External Module provided by Tigem (1 credit)

viii. **Advanced light microscopy in modern biomedical research**
Roman Polishchuk on July 2014
External Module provided by Tigem (1 credit)

ix. **Development and validation of cell-based high content imaging assays**
Diego Medina on July 2014
External Module provided by Tigem (1 credit)

x. **NGS technology and application**
Vincenzo Nigro on July 2014
External Module provided by Tigem (1 credit)

xi. **The Eighth q-bio Summer School**
Jeff Hasty and Lev Tsimiring on July 27th - August 12th, 2014
External Module provided by UCSD (6 credits)

Seminars

i. **From virus evolution to vector revolution. Synthetic AAV capsids for improved in vitro & in vivo gene delivery**
Dr Dirk Grimm on March 11th 2014
External Seminar provided by Tigem (1 hour)

ii. **Conserved mechanisms of longevity: Regulation of lysosomal function**
Dr Louis R. Lapierre on March 13th 2014
External Seminar provided by Tigem (1 hour)

iii. **Bionspired materials for advanced therapy and diagnosis**
Prof. Paolo Netti on April 15th 2014
External Seminar provided by Tigem (1 hour)

iv. **Lysosomes: Small organelles with a huge impact**
Dr Paul Saftig on April 16th 2014
External Seminar provided by Tigem (1 hour)

v. **MicroRNA regulated networks in pluripotency**
Prof. Robert Blelloch on May 8th 2014
External Seminar provided by Tigem (1 hour)

vi. **The Retinal Pigment Epithelium (RPE): a central player in retinal function and disease**
Prof. Enrique Rodriguez-Boulan on May 16th 2014
External Seminar provided by Tigem (1 hour)

vii. **A lung-on-chip to measure oxygen affinity of single red blood cells**
Dr Giuseppe di Caprio on June 11th 2014
External Seminar provided by Tigem (1 hour)

viii. **Gene therapy of genetic neuromuscular and blood diseases: the Genethon approach**
PhD Fulvio Mavilio on June 11th 2014
External Seminar provided by Tigem (1 hour)

ix. **Propagation of synucleinopathy through lysosomal dysfunction**
Prof. Seung-Jae Lee on June 19th 2014
External Seminar provided by Tigem (1 hour)

x. **Neurodegenerative Diseases: The Dangers of Too Much Protein Stability**
Prof. Huda Y. Zoghbi on 9th July 2014
External Seminar provided by Tigem (1 hour)

xi. **The Eighth q-bio Summer School**
Jeff Hasty and Lev Tsimiring on July 27th-August 12th 2014
External Seminar provided at UCSD (2 credits)

xii. **Systematic transcriptome/promotorome analysis based on CAGE technology**
PhD Harukazu Suzuki on September 9th 2014
External Seminar provided by Tigem (1 hour)

xiii. **Nanocarbon materials for electromagnetic applications**
Prof. Sergey Maksimenko on October 6th 2014
Seminar (1 hour)

xiv. **Method of quantum equivalent circuits in nano-electromagnetism for microwave and terahertz frequency ranges**
Prof. Gregory Slepyan on October 6th 2014
Seminar (1 hour)

xv. **Carbon nanotubes and carbines: an introduction**
Prof. Pavel Dyachkov on October 6th 2014
Seminar (1 hour)

xvi. **Nanodiamond Targets for Accelerator X-Ray Experiments**
Prof. Alexander Lobko on October 6th 2014
Seminar (1 hour)

xvii. **Viral gene therapy approaches for human disorders of copper transport and lysosomal storage**
MD Stephen G. Kaker on October 7th 2014
External Seminar provided by Tigem (1 hour)

xviii. **Wilson's disease: molecular mechanism and new approaches to treatmen**
Prof. Svetlana Lutsenko on October 10th 2014
External Seminar provided by Tigem (1 hour)

xix. **Gene Therapy of MPS I**
MD James M. Wilson on October 22nd 2014

External Seminar provided by Tigem (1 hour)

xx. **Rational confederation of genes and diseases**
Prof. Doron Lancet on October 28th 2014
External Seminar provided by Tigem (1 hour)

xxi. **Heterogeneities in temporal networks emerging from adaptive social interactions**
Prof. Takaaki Aoki on November 14th 2014
Seminar (1 hour)

xxii. **Understanding Lysosomal Storage Disorders: From tissue degeneration to phenotypic variability**
PhD André Klein on December 16th 2014
External Seminar provided by Tigem (1 hour)

xxiii. **Signaling pathways that control protein homeostasis in muscles**
MD Marco Sandri on January 20th 2015
External Seminar provided by Tigem (1 hour)

xxiv. **Role(s) of intracellular catabolism during skeletal development**
PhD Carmine Settembre on February 3rd 2015
External Seminar provided by Tigem (1 hour)

xxv. **The transparent editorial process and research integrity at EMBO Press**
PhD Roberto Buccione on February 10th 2015
External Seminar provided by Tigem (1 hour)

xxvi. **How to avoid commitment–epigenetic/post-transcriptional maintenance of pluripotency**
MD Robert Blelloch on February 12th 2015
External Seminar provided by Tigem (1 hour)

xxvii. **Regulation of self renewal in cancer stem cells**
Prof. Giuseppe Pelicci on February 17th 2015
External Seminar provided by Tigem (1 hour)

3. Research activity

Identification and Control of Gene Regulatory Networks

The field of my research activity, as PhD student, is the application of Control Engineering to biology. The concept behind such research is that a biological system can be modelled mathematically by a set of differential equations describing a dynamical system, like any other physical phenomenon. Thus, systems and control engineering methods can be applied to steer the behaviour of biological systems by first simulating its response to inputs calculated via negative feedback control schemes, and then experimentally *in-vivo,* by applying the control algorithms simulated *in-silico* to living cells.

I study biological systems called **gene regulatory networks** and, in particular, the aim of my activity is to control the amount of a specific protein to a desired value. The expression

of such protein can be activated or repressed by medium in which cells are fed, and this entire process is known as regulation of gene expression.

I started my research activity by analysing literature for the proposed control strategies used to achieve regulation of gene expression in prokaryotic and eukaryotic cells. Mainly, two control strategies have been used to regulate gene networks, i.e. proportional-integral (PI) control and model predictive control (MPC). In order to compare and analyse the performance of these different strategies, I implemented these control strategies in the *MATLAB*© environment and simulated the control on dynamical models of the biological system I will use as a test-bed in *in-vivo* experiments. Figure 1 shows the biological system used as a test-bed. This consists of a inducible promoter which drives expression of a fluorescent reporter protein in yeast Saccharomyces cerevisiae, also known as baker's yeast. When cells are fed with galactose, in the absence of glucose, expression from this reporter is activated. Vice-versa, when cells are fed with glucose, expression stops. Therefore the control input can be thought of as a discrete signal with only two values (glucose and galactose).

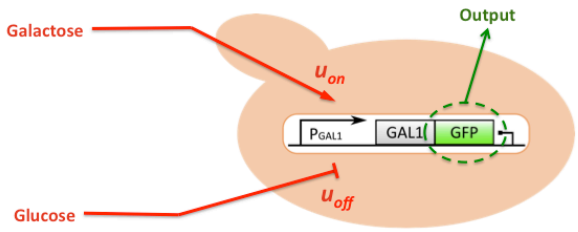


Figure 1. Biological system as test bed: *GAL1* promoter *in Saccharomyces Cerevisiae*.

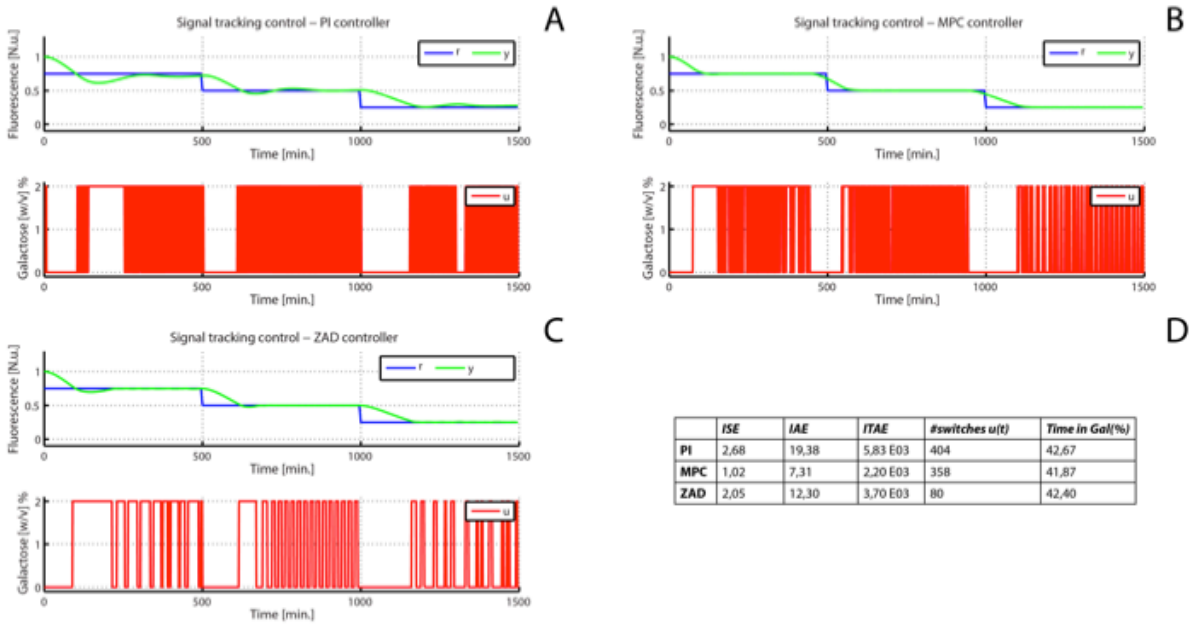
The dynamical model describing this network is a linear dynamical system of order 2 with the peculiarity that the control input can only assume two discrete values.

The numerical analysis was performed on this linear model with both control strategies (PI and MPC). However, since the control input is discrete the PI controller requires an extra block to modulate the control input which is a continuous signal. To this end I used a Pulse-Width-Modulation scheme. The results of the simulations I performed proved that both PI and model-predictive control strategies can achieve successfully *in-silico* the regulation of gene expression in cells' population. However, MPC has a much better performance, but it requires considerably more computational power and it relies heavily on the model of the dynamical system, which in the case of biological systems, are usually far from optimal.

Therefore, I investigated for new control strategies better suited to control biological systems with the requirement that the control input had to be a discrete signal. I thus found a strategy named zero average dynamics (ZAD) control, a *quasi-sliding* model-based control strategy, which has been extensively used in electrical power converters that require

a discrete input, as the biological system under investigation. I therefore implemented in MATLAB this control strategy and compared it to PI and MPC controller, and demonstrated its superiority compared to the PI controller and a similar performance to MPC.

Results from the numerical analysis are shown in Figure 2.



	ISE	IAE	ITAE	#switches u(t)	Time in Gal(%)
PI	2,68	19,38	5,83 E03	404	42,67
MPC	1,02	7,31	2,20 E03	358	41,87
ZAD	2,05	12,30	3,70 E03	80	42,40

Figure 2.

I then validated these control strategies in yeast cells *in-vivo* on a real-time control platform available at TIGEM (Telethon Institute of Genetics and Medicine). The platform is based on a microfluidic device to trap cells and provide the control input, a time-lapse microscopy apparatus, and a set of actuated syringes, as shown in Figure 2.

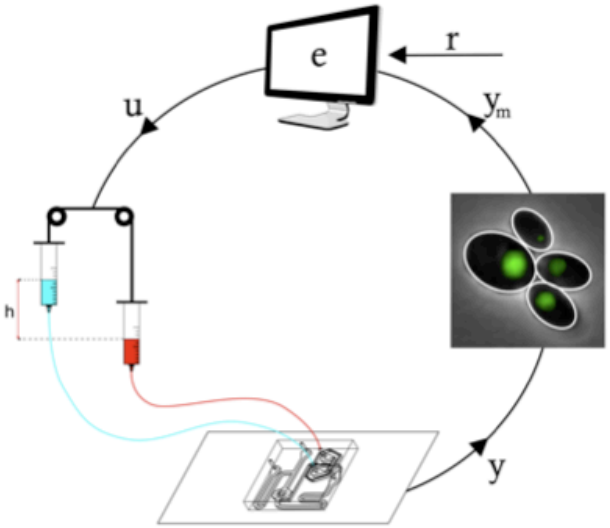
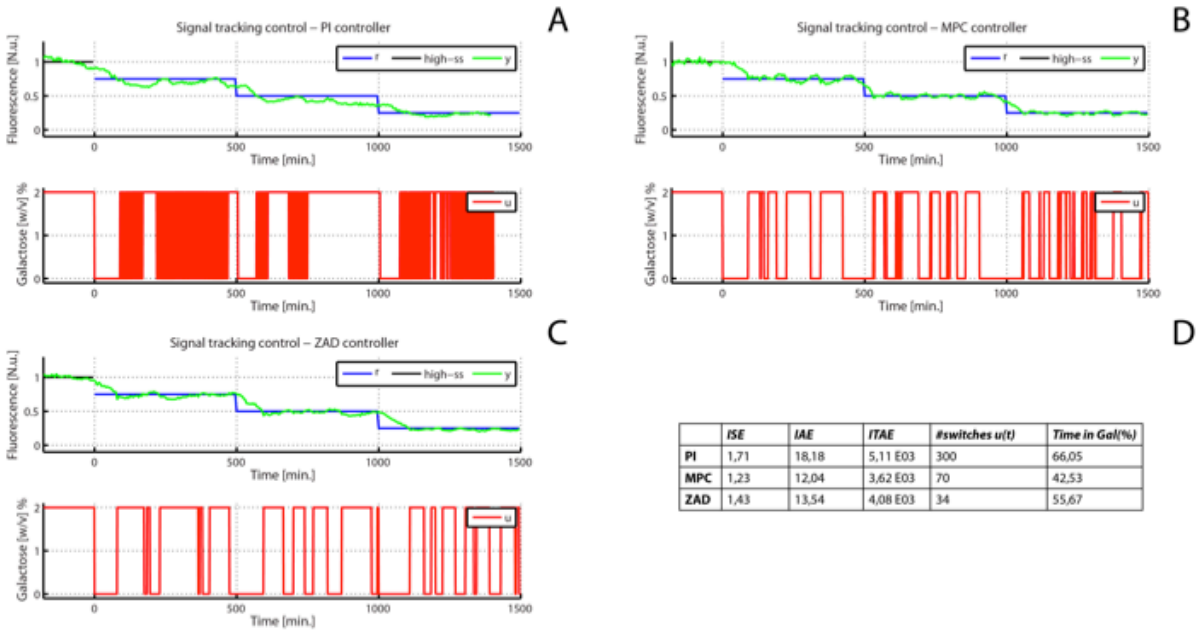


Figure 2. Technological platform enabling in-vivo control experiments.

Varying the height of each syringe, I can change the medium inside the microfluidic device in which the cells are trapped, activating or repressing the expression of *GAL1* gene. The amount of the protein expressed by *GAL1* gene is measured indirectly by the fluorescence emitted by a fluorescent protein called *GFP*, which is fused to *GAL1* gene and so directly proportional to its level of expression.

I first learned to produce microfluidics chips from a silicon mold available at TIGEM specifically designed for yeast cells. The procedure is quite laborious and requires several steps from silicon mold preparation to PDMS polymer based molding and bonding to a glass slide.

I then designed several control experiments to carry out validation of control strategies I considered during my numerical analysis, namely PI, MPC and ZAD controllers, considering both set point and time-varying references. The analysis proved that MPC and ZAD strategies can achieve successfully the regulation of gene expression on living cells as shown in Figure 3, whereas the PI control strategy has a worse performance, at least for time-varying references, confirming the numerical simulations in Figure 2.



	ISE	IAE	ITAE	#switches u(t)	Time in Gal(%)
PI	1,71	18,18	5,11 E03	300	66,05
MPC	1,23	12,04	3,62 E03	70	42,53
ZAD	1,43	13,54	4,08 E03	34	55,67

Figure 4.

Now, I am planning to apply these control strategies to regulate the amount of a protein that can cause a genetic disease if it is expressed at abnormal levels. This protein is named *Alpha-synuclein*, and it is responsible for Parkinson's disease when not rightly expressed.

Simultaneously, as side project, I'm studying new approaches to model biological processes in cell populations. For this aim, I collected several data by running a single-cell segmentation and tracking algorithm on experiments that I made for regulation of gene expression. Starting from these datasets, I'm trying to identify stochastic mathematical models that can better describe the behaviour of biological processes. The work on this part is still at the beginning, so I have not yet significant results on this topic.

4. Products

Until now, I have not yet published any results, but I'm going to submit the following paper to an *international peer-reviewed journal*:

i. **A comparative analysis of strategies for *in-vivo* real-time control of protein expression from endogenous and synthetic gene networks**
Gianfranco Fiore, Giansimone Perrino, Mario di Bernardo, Diego di Bernardo
ACS Synthetic Biology **In Preparation**

5. Conferences and Seminars

I have not attended any conference during this year.

6. Activity abroad

   I attended “The Eighth q-bio Summer School” in experimental synthetic biology (July 27th-August 12th, 2014) at UCSD (University of California, San Diego).

7. Tutorship

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