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 – Second Year

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

1. Information

I received the M.Sc. degree in Control Engineering on October 2013 from the University of Naples "Federico II". Currently, I am a Ph.D. student in Information Technology and Electrical Engineering (ITEE) – XXIX cycle – at the University of Naples 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 up to the second year:

	Credits year 1								**Credits year 2**							
		1	2	3	4	5	6			1	2	3	4	5	6	
	Estimated	bimonth	bimonth	bimonth	bimonth	bimonth	bimonth	**Summary**	**Estimated**	bimonth	bimonth	bimonth	bimonth	bimonth	bimonth	**Summary**
Modules	**20**	0	9	13	0	3	6	**31**	**10**	9	0	0	0	0	0	**9**
Seminars	**5**	1	1	2.2	1.8	0.4	1	**7.4**	**5**	1.4	2	1	1.4	1.2	0.8	**7.8**
Research	**35**	9	1	1	8	7	3	**29**	**45**	1	8	9	8.6	8.8	9.2	**44.6**
	60	10	11	16.2	9.8	10.4	10	**67.4**	**60**	11.4	10	10	10	10	10	**61.4**

2. Study and Training activities

External courses

i. **Practical Course – Part B**
Alberto Auricchio, Vincenzo Nigro, and Roman Polishchuk on March 2015
External Module at TIGEM (3 credits)

ii. **Systems Biology and Functional Genomics – Part B**
Diego di Bernardo and Sandro Banfi on March 2015
External Module at TIGEM (3 credits)

iii. **Cell Biology of Genetic Diseases – Part B**
Antonella de Matteis and Brunella Franco on March 2015
External Module at TIGEM (3 credits)

Seminars

i. **Spinal and bulbar muscular atrophy: a pure motor neuron or a metabolic disease?**
PhD Maria Pennuto on March 3rd 2015
External Seminar at TIGEM (1 hour)

ii. **SINEUPs: a new functional class of natural ad synthetic antisense long non-coding RNAs that activate translation**
Prof. Stefano Gustincich on February 26th 2015
External Seminar at TIGEM (1 hour)

iii. **Unraveling the Molecular Basis of Polycystic Kidney Disease**
Prof. Alessandra Boletta on April 14th 2015
External Seminar at TIGEM (1 hour)

iv. **Ion channel or phospholipid scramblase? The elusive function of TMEM16F**
Dr. Luis Galietta on April 10th 2015
External Seminar at TIGEM (1 hour)

v. **AAV vectors as powerful tools for in vivo investigation on gene function**
Prof. Mauro Giacca on April 21st 2015
External Seminar at TIGEM (1 hour)

vi. **Novel insights into EGFR signaling, endocytosis and its involvment in cancer**
Prof. Pier Paolo di Fiore on April 27th 2015
External Seminar at TIGEM (1 hour)

vii. **Huntington's Disease from evolution to pathology**
Prof. Elena Cattaneo on April 28th 2015
External Seminar at TIGEM (1 hour)

viii. **Pharmacological induction of autophagy for improved anticancer chemotherapy**
Prof. Guido Kroemer on May 5th 2015
External Seminar at TIGEM (1 hour)

ix. **Cellular Complexity of Alzheimer's Disease**
Prof. Lawrence Rajendran on May 12th 2015
External Seminar at TIGEM (1 hour)

x. **Investigating novel nutrient sensing functions of the lysosome**
PhD Roberto Zoncu on may 13th 2015
External Seminar at TIGEM (1 hour)

xi. **Mitochondria, endoplasmic reticulum, lysosomes and the cytoskeleton: what lies at the interface**
PhD Benoît Kornmann on May 19th 2015
External Seminar at TIGEM (1 hour)

xii. **Sphingolipids in human health and disease**
PhD Tony Futerman on may 26th 2015
External Seminar at TIGEM (1 hour)

xiii. **Gene therapy for the treatment of inherited arrhythmias**
Prof. Silvia G. Priori on June 15th 2015
External Seminar at TIGEM (1 hour)

xiv. **Viral infections and unconventional roles of the autophagy machinery**
PhD Fulvio Reggiori on June 23rd 2015
External Seminar at TIGEM (1 hour)

xv. **Regulation of two-fold bifurcations in planar piecewise-smoothsystems**
Prof. John Hogan on June 26th 2015
Seminar (1 hour)

xvi. **Aberrations in the endolysosomal compartment: Relevance for rare kidney disorders**
Prof. Olivier Devuyst on June 30th 2015
External Seminar at TIGEM (1 hour)

xvii. **The contribution of computational biology to biomedical problems**
PhD Anna Tramontano on July 2nd 2015
External Seminar at TIGEM (1 hour)

xviii. **Gene therapy for methylmalonic acidemia**
PhD Charles P. Venditti on July 14th 2015
External Seminar at TIGEM (1 hour)

xix. **Integrating the Myc-transctiptional regulatory network with the Wnt signaling pathway in reprogramming the epigenetic state of embryonic and cancer stem cells**
PhD Alessio Zippo on July 15th 2015
External Seminar at TIGEM (1 hour)

xx. **Why are individuals different? Genetic, environmental and stochastic influences on development**
PhD Mirko Francesconi on July 20th 2015
External Seminar at TIGEM (1 hour)

xxi. **G6PD: The implications of a world-wide human genetic polymorphism that is X-Linked**
Prof. Lucio Luzzato on July 21st 2015
External Seminar at TIGEM (1 hour)

xxii. **Directed evolution of adeno-associated virus vectors for retinal gene therapy**
Prof. John Gerard Flannery on September 8th 2015
External Seminar at TIGEM (1 hour)

xxiii. **Transcription factor control in pluripotent stem cells and preimplantation embryonic development**
Prof. Antonio Simeone on September 15th 2015
External Seminar at TIGEM (1 hour)

xxiv. **V-ATPase and SNAREs: New functions for old lysosomal proteins**
PhD Thomas Vaccari on September 22nd 2015
External Seminar at TIGEM (1 hour)

xxv. **Where we were, where we are, and where we are going in treating lysosomal storage diseases**
Prof. Mark Haskins on September 23rd 2015

External Seminar at TIGEM (1 hour)

xxvi. **Selective Autophagy in the Fight Against Aging**
MD Ana Maria Cuervo on October 6th 2015
External Seminar at TIGEM (1 hour)

xxvii. **From Cells to Mice: Unrevealing the Molecular Pathophysiology of MPS IIIC**
PhD Alexey V. Pshezhetsky on October 8th 2015
External Seminar at TIGEM (1 hour)

xxviii. **Cell and Gene Therapy for Hemophilia A**
Prof. Antonia Follenzi on October 27th 2015
External Seminar at TIGEM (1 hour)

xxix. **De novo mutations in genetic disease**
Prof. Joris Veltman on November 3rd 2015
External Seminar at TIGEM (1 hour)

xxx. **Transcription factors and miRNAs in eye development**
PhD Ruth Ashery-Padan on November 24th 2015
External Seminar at TIGEM (1 hour)

xxxi. **Single molecule approaches for studying gene expression in intact mammalian tissues**
PhD Shalev Itzkovitz on December 1st 2015
External Seminar at TIGEM (1 hour)

xxxii. **Computer simulations of molecular binding phenomena**
PhD Vittorio Limongelli on December 3rd 2015
External Seminar at TIGEM (1 hour)

xxxiii. **Role of Golgi Structure in Haemostasis**
PhD Francesco Ferraro on December 15th 2015
External Seminar at TIGEM (1 hour)

xxxiv. **Drosophila in the study of ALS pathogenesis**
PhD Giuseppa Pennetta on December 18th 2015
External Seminar at TIGEM (1 hour)

xxxv. **Non-mutational adaptive changes in melanoma driving the establishment of drug resistance**
Prof. Gennaro Ciliberto on January 12th 2016
External Seminar at TIGEM (1 hour)

xxxvi. **Who with whom? Versatile interactions by bZIP transcription factors**
PhD Matthias Wilmanns on January 18th 2016
External Seminar at TIGEM (1 hour)

xxxvii. **CLUH gives a clue about regulation of mitochondrial metabolism**

Prof. Elenar Rugarli on January 26th 2016
External Seminar at TIGEM (1 hour)

xxxviii. **ER stress and ER homeostasis**
PhD Eelco van Anken on February 9th 2016
External Seminar at TIGEM (1 hour)

3. Research activity

Identification and Control of Gene Expression in Yeast

The field of my research activity 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.

Gene expression is the process by which information from a gene, a discrete sequence of DNA nucleotides, is used in the synthesis of proteins. 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. In the novelty field of synthetic biology, automatic negative feedback control of gene expression is a key technology enabling, for example, synthetic circuit's components to operate in an optimal range. Additionally it can be used to attain a quantitative understanding of the dynamical behaviour of a protein.

The starting point of my research activity was a comparative analysis of the feedback control strategies used in literature to achieve regulation of gene expression in prokaryotic and eukaryotic cells. Mainly, two control strategies have been used to regulate protein expression, i.e. proportional-integral (PI) control and model predictive control (MPC). I compared these control strategies choosing as experimental test-bed the most commonly used inducible promoter in yeast: the galactose-responsive *GAL1* promoter (Figure 1). This consists of an 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).

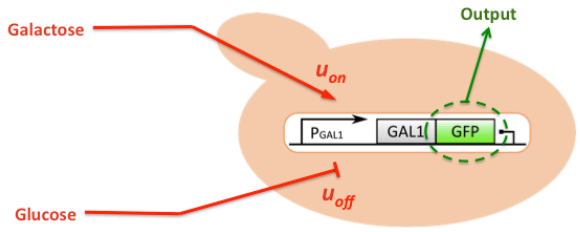


Figure 1. Biological system as test bed: *GAL1* promoter *in Saccharomyces Cerevisiae*.

Figure 1

The dynamical model describing the galactose-responsive system was identified as a linear dynamical system of order 2 with the peculiarity that the control input can only assume two discrete values.

I also investigated for new control strategies better suited to control gene expression 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.

A real-time control platform, available at the Telethon Institute of Genetics and Medicine (TIGEM), was used to assess *in vivo* the regulation of gene expression in living yeasts using the three different control strategies. 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.

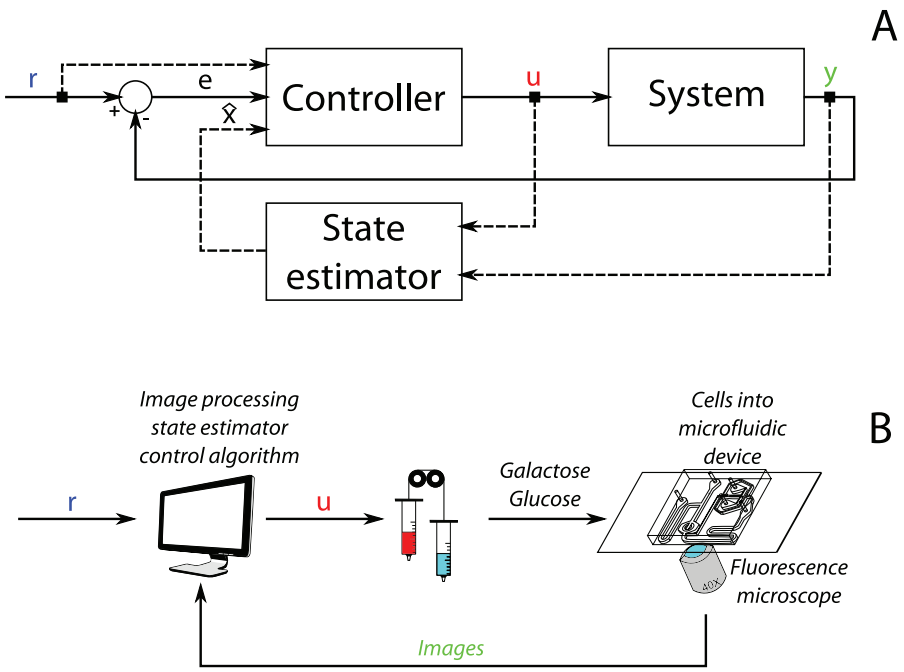
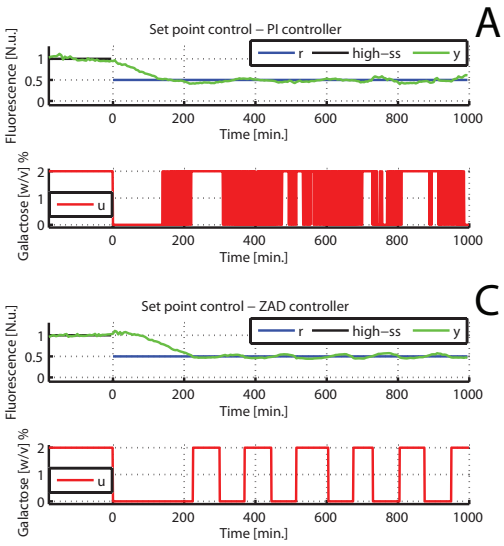


Figure 2

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 demonstrated, performing the *in vivo* experiments, that both the MPC and ZAD control strategies can successfully regulate gene expression from the *GAL1* promoter in living cells for thousands of minutes. The MPC controller can track fast reference signals better than ZAD but with a higher actuation effort due to the large number of input switches it requires (Figure 4 and 5). Conversely, the PI controller's performance is comparable to that achieved by the MPC and the ZAD controllers only for the set-point regulation (Figure 3).



D

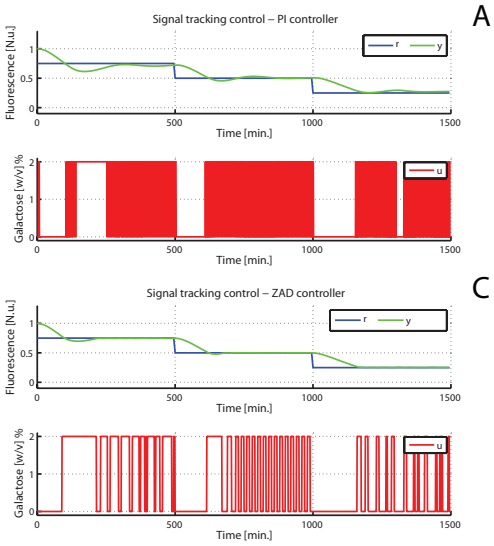
whole experiment

	ISE	*IAE*	*ITAE*	*#switches u(t)*	*Time in Gal(%)*
PI	2,76	13,69	2,75 E03	240	59,70
MPC	8,68	25,00	5,03 E03	20	31,60
ZAD	7,64	21,77	4,38 E03	11	41,50

last 500 minutes

	ISE	*IAE*	*ITAE*	*#switches u(t)*	*Time in Gal(%)*
PI	0,19	3,72	378,98	136	70,06
MPC	0,55	6,19	631,56	13	32,33
ZAD	0,17	3,80	387,58	7	52,90

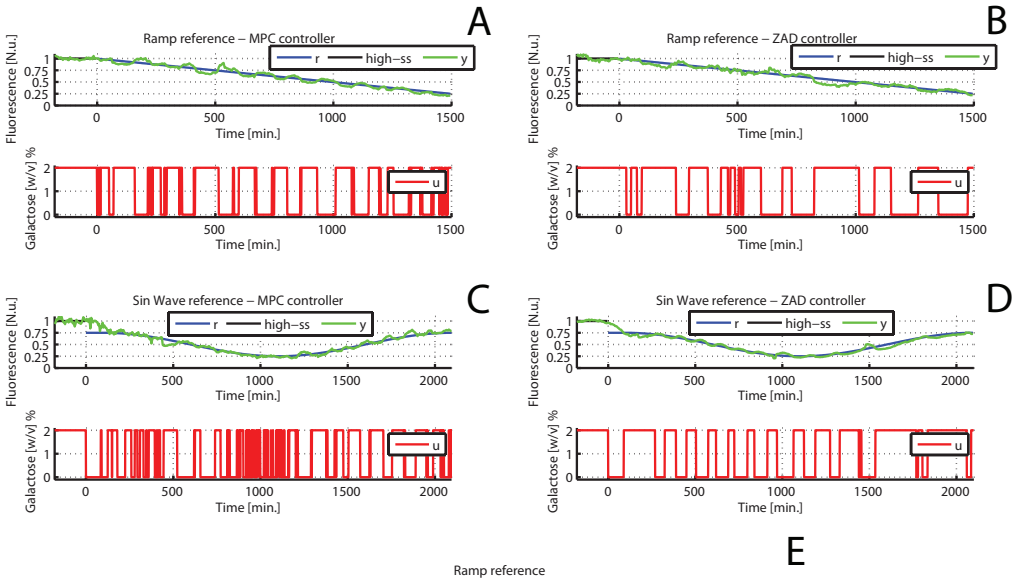
Figure 3



D

	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 4



E

Ramp reference

	ISE	*IAE*	*ITAE*	*#switches u(t)*	*Time in Gal(%)*
MPC	0,75	12,76	3,84 E03	71	54,27
ZAD	0,78	12,19	3,67 E03	24	55,00

Sin Wave reference

	ISE	*IAE*	*ITAE*	*#switches u(t)*	*Time in Gal(%)*
MPC	1,84	17,26	7,27 E03	127	46,90
ZAD	0,98	15,02	6,32 E03	29	59,05

Figure 5

As next step, I am applying the methodology described above for the study of neurodegenerative disorders.

Neurodegenerative disorders are associated to the formation of abnormal protein aggregates that interfere with the normal functions of neurons, causing the progressive disruption of the neuronal population. For example, the dysfunction of α-synuclein (α-syn) protein is involved in Parkinson's disease (PD) and related neurodegenerative disorders. A yeast model expressing wild-type and mutant human α-syn protein has been used to qualitatively study its aggregation properties. However, α-synuclein overexpression is toxic also for yeast cells, thus making it difficult to study, if its expression is not carefully controlled. Indeed, a quantitative study of the aggregation dynamics of the α-syn protein is still lacking.

So, using the proposed methodology, I am trying to attain a quantitative understanding of the dynamics of α-syn protein's aggregation, by carefully regulating its expression and following its dynamics in real-time. Driving the expression of α-syn protein at different values, I can assess quantitatively the dynamics that lead to the formation of protein aggregates (Figure 6).

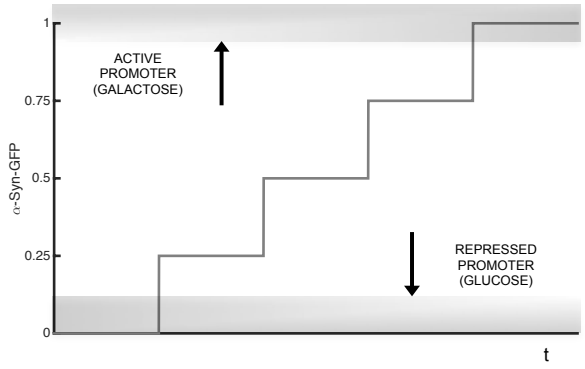


Figure 6

I'm also 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 already made for the regulation of gene expression. Starting from these datasets, I identified a linear mixed-effects model that describes better the behaviour of the population of yeasts at the single-cell level. Now, I am developing a simulator to reproduce *in silico* the behaviour of the entire population of cells during an *in vivo* experiment.

Lastly, I am beginning to work on the development of stochastic control strategies to control the gene expression of a population of cells at the single-cell level. The mixed-effects approach describes above can help me to assess the goodness of the stochastic controllers, since I can simulate with it an entire heterogeneity population of cells.

4. Products

Peer Reviewed Journals

i. **In Vivo Real-Time Control of Gene Expression: A Comparative Analysis of Feedback Control Strategies in Yeast.** Gianfranco Fiore*, Giansimone Perrino*, Mario di Bernardo, and Diego di Bernardo. *ACS Synthetic Biology* **2016** *5* (2), 154-162

5. Conferences and Seminars

i. "*How to best control gene expression in cell populations in real-time?".* Poster presentation held at the **2015 Synthetic Biology: Engineering, Evolutions & Design** conference on June 2015 in Boston, Massachusetts, USA.

ii. *"In-vivo real-time control of gene expression: a comparative analysis of feedback control strategies in yeast".* Poster presentation held at the **Design, optimization and control in systems and synthetic biology (DOC'15)** workshop on November 2015 in Paris, France.

6. Activity abroad

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7. Tutorship

   Assistant of the course "*Modelli per la Previsione e l'Ottimizzazione"* held by Prof. Diego di Bernardo for Laurea Magistrale in Ingegneria Biomedica (2 hours)