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TOWARDS THE INTEGRATION OF COMPUTATIONAL  
SYSTEMS BIOLOGY AND HIGH-THROUGHPUT DATA:  
A WAY TO SUPPORT DIFFERENTIAL ANALYSIS  
OF MICROARRAY GENE EXPRESSION DATA

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# Towards the integration of computational systems biology and high-throughput data: a way to support differential analysis of microarray gene expression data

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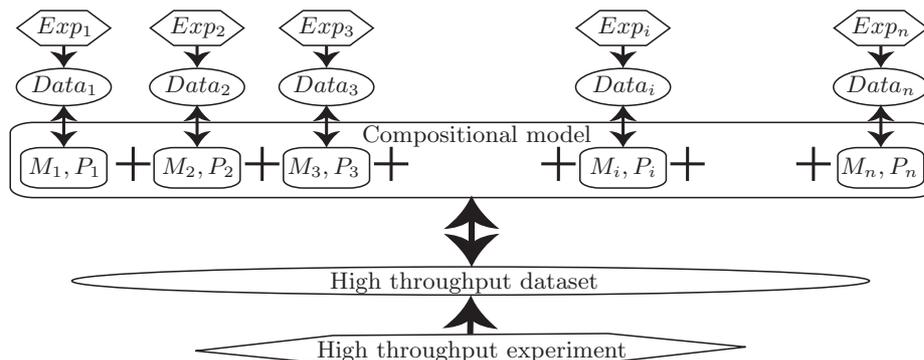
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**Abstract.** The paradigmatic shift occurred in biology that led first to high-throughput experimental techniques and later to computational systems biology must be applied also to the analysis paradigm of the relation between local models and data to obtain an effective prediction framework. In this work we show that the new relation between systems biology models and high-throughput data permits new integrations on the systemic scale like the use of *in silico* predictions to support the mining of gene expression datasets. We introduce a unifying notational framework in which we propose two applications concerning the use of system level models to support the differential analysis of microarray expression data. The approach is tested with a specific microarray experiment on the phosphate system in *Saccharomyces cerevisiae* and a computational model of the PHO pathway that supports the systems biology concepts.

## 1 Introduction

Computational modelling of biological phenomena is motivated by the need of abstract representations of complex biochemical systems with the possibility to quantitatively reproduce and predict their behaviour. The sophistication reached by computers and programming languages permits one to effectively simulate the models allowing *in silico* predictions at the level of intricate metabolic and signalling pathways like in [5][6]. Even though the complexity of these models can be very high, they still describe *local* aspects of biochemical systems. Recently, a paradigmatic shift in the dimensions and complexity of the models is occurring as consequence of the introduction of modelling paradigms that natively handle high parallelism and incremental model construction [14][12]. An example of application of these new paradigms is the PHO pathway model presented in [16] in which we model and simulate the phosphate systems in *Saccharomyces cerevisiae* with the stochastic  $\pi$ -calculus, a language that support compositionality (the ability of building models incrementally). In this scenario the models can reach the *systems biology level* (as schematized with the compositional model of Figure 1). Although the system level modelling of simple microorganisms is still more theoretical than effective, the potentialities of the *in silico* approach in this context are extremely promising for the biological community.

The systems biology concept [8] that is now becoming crucial in the computational modelling field, received the definitive incentive with the success, especially in certain areas, of the “globalists” over the “localists” [7]. The main factor for the shift from the local study of biochemical pathway to the genomic scale analysis of interaction networks was the developing of massively-parallel and high-throughput techniques [9] which made available a huge amount of unstructured gene-specific or protein-specific data. One of the more important high-throughput techniques is the microarray technology, used for example in [3] to detect the genes related to the phosphate accumulation and polyphosphate metabolism in *Saccharomyces cerevisiae*. The identification of genes that show significant changes in expression associated with experimental variables of interest on a microarray is called differential analysis of gene expression data [4][13] and it is not a trivial task because the high-throughput datasets are large, extremely noisy and subject to biological bias. The interpretation of the set of differentially expressed genes is a hot topic in research mainly because the typical high cardinality of the set prevents the functional profiling and the validation of each gene singularly. Some authors addressed the problem through literature profiling [2] or Gene Ontology-based tools [11].



**Fig. 1:** Integration between local models and data and between systemic model and high-throughput data. Each local model  $M_x$  with the corresponding parameters  $P_x$  can be integrated with classical relations of tuning and validation with specific data ( $Data_x$ ) retrieved by proper experiments ( $Exp_x$ ). The systems biology model, built composing the local sub-models, have new possibilities of integration with high-throughput data. The + symbol stands for the abstract compositional operator of the models.

Regardless to the dimensionality, a model can assume a biological valence only if there are experimental data for the same biological aspect. Typical relations between data and models are parameter estimation, model validation and output data interpretation. When the biological description is not on a system level, there is a one-to-one relation between a local model and the pos-

sibly available data that permits *in silico* simulations and thus biological predictions (schematized in Figure 1 with the double arrows between each local model and each dataset derived from specific experiments). However, data from of high-throughput techniques are not suitable for tune and validate local pathway models because of the genomic scale and the uncertainty of the datasets. With the introduction of computational systems biology, new analysis paradigms of the relation between systems biology models and high-throughput can be explored (the big double arrow in Figure 1). For example, the definition of these new paradigms can highlight the relation between the PHO pathway model [16] and the genic expression data on the phosphate metabolism in *Saccharomyces cerevisiae* [3]. Therefore, as the shift from biology to systems biology needed a paradigmatic change, also this new relation needs investigation to understand how to handle some critical aspects like the uncertainty and dimensionality of the high-throughput techniques and the computational weight of the models that makes too many replicas of *in silico* predictions heavy.

In this work, we show that the expression datasets obtained with *in silico* simulations of system level models can support the differential microarray analysis. This approach is possible assuming to have a reasonable large model built in a compositional way, thus permitting the independent validation of each pathway with biological data and it is complementary to other knowledge-based mining methods. We define a unifying notational framework for the experimental high-throughput data and *in silico* high-throughput expression values obtained with a computational model. The model indirectly reflects the state of the art of the biological knowledge, so the framework allows the biological information to be included in the process of microarray expression mining with the prediction potentialities of the systems biology models. The first application we propose in the framework aims to remove from the set of regulated genes those genes that the model predicts as normal to be regulated in the specific conditions. In this way the experimenter can focus only on the genes with really new biological information. We effectively test the introduced approach and the first application to the microarray experiment of [3] using the PHO pathway model [16]. Even if this model is not on the genomic scale, it highlights the utility of the methods and confirms that, as the models scale up to the system level, our approach can give a systematic support to the the analysis of regulated genes in microarray experiments. The second application tackles the problem of the genes that are regulated by dependent and non directly controllable conditions that crowd the set of differentially expressed genes, possibly hiding genes regulated only by the direct and desired experimental conditions. This application is based on the *in silico* prediction of the effects on gene expression of the dependent variables only.

The paper is organized as follows: Section 2 introduces the unifying notational framework for microarray and *in silico* gene expression experiments. Section 3 focuses on the specialization of the framework for microarray differential analysis proposing some measures for quantifying the level and the precision of the integration. Section 4 describes the two specific application based on the framework and in Section 5 we discuss the possibility of developing general software tools

in this context. The running example based on the microarray work of [3] and the PHO pathway model [16] is discussed step by step in the sections.

## 2 A unifying notational framework

We define a framework to formally specify the microarray experiments and the model-based estimation of gene expression in order to allow integrations of the two approaches. We focus on *Affymetrix* oligonucleotide chips [10] as far as the microarray technology is concerned, even though the framework can be adapted to cDNA chips. The cDNA chips, in fact, can be modelled as the combinations of two *Affymetrix* chips reflecting the expression of Cy3- and Cy5-labeled probes, like in the case of the microarray experiment of the running example.

### 2.1 The microarray experiments

An Affymetrix microarray experiment consists in the absolute quantification of the expression profiles of a set of genes. The parameters of a microarray experiment are essentially the controlled independent variables with their levels, and the measured dependent variables. Formally, the parameters are

$$q_{\mu A} = (G_{\mu A}, C, M, P) \quad (1)$$

where:

$G_{\mu A}$  is the set of genes that are spotted on the microarray<sup>1</sup>.

$C = \{(V_1, l_1), (V_2, l_2), \dots, (V_{k-1}, l_{k-1}), (V_k, l_k)\}$  is the set of  $k$  different conditions applied on the chip. A condition  $C_i$  with  $1 \leq i \leq k$  is a pair  $(V_i, l_i)$  where  $V_i$  is an independent variable controlled by the experimenter and  $l_i \in \mathbb{R}$  is a real value assigned to the variable. Hereafter, we assume that the set of  $k$  independent variables  $V = \{V_1, V_2, \dots, V_{k-1}, V_k\}$  can be retrieved from the experiment with the function  $IV(q_{\mu A}) = V$ .

$M = \{(V_{k+1}, m_{k+1}), (V_{k+2}, m_{k+2}), \dots, (V_{n-1}, m_{n-1}), (V_n, m_n)\}$  is the set of  $n - k$  different dependent measured parameters of the chip. A dependent measured parameters  $M_i$  with  $k + 1 \leq i \leq n$  is a pair  $(V_i, m_i)$  where  $V_i$  is a dependent measured variable and  $m_i \in \mathbb{R}$  is the real value assigned to the variable. Hereafter we assume that the set of dependent variables  $V_D = \{V_{k+1}, V_{k+2}, \dots, V_{n-1}, V_n\}$  can be retrieved with the function  $DV(q_{\mu A}) = V_D$  and that  $V \cap V_D = \emptyset$ .

$P$  represents the information regarding the experiment. It should contains the parameters that are not variable and that are sufficient to reproduce the experiment in a rigorous way. For instance,  $P$  may contain the sample used, the extract preparation and labeling, the procedure and the parameters for the hybridization and the environment and instruments information. In general it can contains all the information of the MIAME exchange standard [1] not handled by the other defined parameters.

<sup>1</sup> Note that it is not always trivial to associate a gene to each spot on the microarray if an oligonucleotide microarray chip is used.

With this conventions, the cDNA chip used in [3] (FODB are the initials of the authors) for the expression profiling between low and high phosphate conditions can be represented with the two following Affymetrix microarray experiments

$$\begin{aligned} FODB_{\mu A, Cy3} &= (G_{\mu A}^{FODB}, \{P_i, 0.2mM\}, \emptyset, P^{FODB}) \\ FODB_{\mu A, Cy5} &= (G_{\mu A}^{FODB}, \{P_i, 10mM\}, \emptyset, P^{FODB}) \end{aligned}$$

Where  $G_{\mu A}^{FODB}$  is the set of genes considered in the experiment (approximately 6400 distinct DNA sequences, available in the additional materials of [3]),  $P_i$  is the phosphate concentration, and  $P^{FODB}$  contains an exhaustive qualitative and quantitative description of the experimental parameters that allows experimental reproduction. The only experimental independent variable is the phosphate concentration  $IV(FODB_{\mu A, Cy3}) = IV(FODB_{\mu A, Cy5}) = P_i$  which assumes two different values in the two chips. The work does not measure any dependent variables (so  $DV(q_{\mu A}) = \emptyset$ ).

For a specific microarray experiment we can define a function reflecting the experimental procedure (which is as standard as possible) that promotes the biochemical reactions on the chip and results in the values of absolute expression detected by the experimental instruments. This function for a microarray experiment  $q_{\mu A} = (G_{\mu A}, C, M, P)$  has the form

$$Expr_{q_{\mu A}} : G \mapsto \mathbb{R} \quad (2)$$

$Expr_{q_{\mu A}}$  associates to a gene  $g \in G_{\mu A}$  a real value reflecting its expression.

All the genes spotted on the microarray with the corresponding expression values are included in a dataset, denoted as  $E_{q_{\mu A}}$  and defined as

$$E_{q_{\mu A}} = \{(g, Expr_{q_{\mu A}}(g)) \mid g \in G_{\mu A}\} \quad (3)$$

The dataset of the microarray experiment of our example is  $E_{FODB_{\mu A}}$  and is available in the additional materials of [3] with the relative quantification between  $FODB_{\mu A, Cy3}$  and  $FODB_{\mu A, Cy5}$  since a cDNA technology is used.

## 2.2 The *in silico* model-based simulation of expression experiments

Here we propose how to simulate *in silico* an experiment to obtain a dataset of expression profiles. The model can be viewed as a set of metabolic and signalling pathways interacting each others. In particular, the prerequisites for a model to be suitable in this context are mainly three:

- The model must consider the gene transcription and allow the quantification of gene expression during the simulations.
- The model must have a genomic scale; it is not necessary to have a comprehensive model of all the genes of the cell, but the number of considered genes must be comparable to number of genes spotted on a microarray chip.
- The model must allow *in silico* experiments that accepts as inputs the environment conditions (like concentrations of the nutrients, temperature, pH, etc.) as independent variables controlled by the experimenter.

The PHO model we use to test the framework, matches the first and the last conditions. The genomic scale, instead, is not respected, and so the model is not suitable for real large-scale microarray mining, but it can still test the usefulness and quality of the approach. Moreover, the used modelling language support the incremental development, and so the model can be extended to other pathways influencing more genes.

In the definition of the microarray experiment we have the controllable conditions, the dependent variables and the parameters; the intuition is that they match the input requirements of an *in silico* simulation of a sufficient large subset of the biological network of a cell. So, similarly to the microarray experiment we can give the following definition of the parameters of a model-based *in silico* expression experiment:

$$q_m = (G_m, C, M, P) \quad (4)$$

where  $C$ ,  $M$  and  $P$  are the conditions, the measured parameters and the experimental information as defined for  $q_{\mu A}$ , while  $G_m$  is the set of genes for which the model is able to estimate the expression profile.

The simulated expression experiments described in [16] (SBP are the initials of the authors,  $lp$  and  $hp$  are the label denoting respectively low and high phosphate conditions) of the reference microarray work [3], are

$$\begin{aligned} SBP_{m,lp} &= (G_m^{SBP}, \{P_i, 0.2mM\}, \emptyset, P^{SBP}) \\ SBP_{m,hp} &= (G_m^{SBP}, \{P_i, 10mM\}, \emptyset, P^{SBP}) \end{aligned}$$

with the same definition given for  $FODB_{\mu A, Cy3}$  and  $FODB_{\mu A, Cy5}$  except for  $P^{SBP}$  which is the *in silico* correspondent of  $P^{FODB}$  and  $G_m^{SBP}$  which contains very few genes with respect to  $G_{\mu A}^{FODB}$  since the used model has not a genomic scale. In particular

$$G_m^{SBP} = \{\text{PHO2,PHO4,PHO81,PHO5}\} \quad (5)$$

The expression of a gene can be seen as the result of a particular instance of the model that is simulated with the particular inputs. So, with a conceptual analogous of the microarray expression function, for every  $q_m$  there exists an intensionally defined function that associates a real value to each gene as follows

$$Expr_{q_m} : G \mapsto \mathbb{R} \quad (6)$$

$Expr_{q_m}$  reflects the simulated biochemical reactions occurring in a living cell, while  $Expr_{q_{\mu A}}$  reflects the biochemical reactions occurring in the microarray experiment preparation.  $Expr_{q_{\mu A}}$  and  $Expr_{q_m}$  can be seen as *in silico* and high-throughput approximations of the real function that results in the gene expression regulation in a living cell. The corresponding dataset for  $Expr_{q_m}$  is

$$E_{q_m} = \{(g, Expr_{q_m}(g)) \mid g \in G_m\} \quad (7)$$

The datasets of the *in silico* PHO pathway experiment are  $E_{q_{m,lp}^{SBP}}$  and  $E_{q_{m,hp}^{SBP}}$ .

### 3 Differential analysis of microarray gene expression data

The main objective in gene expression analysis is the detection of the genes that are differentially expressed (or regulated) between two biological samples with some experimental differences. In differential analysis the classification of the genes is made with complex statistical techniques that analyse the entire distribution of gene expressions [4][17].

We define the following microarray experiments:

$$\begin{aligned} q_{\mu A}^1 &= (G_{\mu A}, C^1, M^1, P) & \text{with } IV(q_{\mu A}^1) &= IV(q_{\mu A}^2) \text{ and} \\ q_{\mu A}^2 &= (G_{\mu A}, C^2, M^2, P) & DV(q_{\mu A}^1) &= DV(q_{\mu A}^2) \end{aligned} \quad (8)$$

$q_{\mu A}^1$  and  $q_{\mu A}^2$  can differ for definition only in the quantification of the dependent and independent measured variables.

The set  $R_{\mu A} \subseteq G_{\mu A}$  represents the genes that are regulated between the two microarray experiments  $q_{\mu A}^1$  and  $q_{\mu A}^2$  which reflect the differences between  $C^1$  and  $C^2$  and between  $M^1$  and  $M^2$ . In the formalism defined for the microarray experiments this set can be detected by a class of functions called  $\delta_{\mu A}$  that, in general, takes two datasets and returns the genes that are regulated<sup>2</sup>:

$$R_{\mu A} = \delta_{\mu A}(E_{q_{\mu A}^1}, E_{q_{\mu A}^2}) \quad (9)$$

In our microarray reference experiment [3], the  $\delta_{\mu A}$  function is based on the two-fold derepression ratio, and the resulting set of regulated genes  $R_{\mu A}^{FODDB}$ , calculated as  $\delta_{\mu A}(E_{FODDB_{\mu A, Cy3}}, E_{FODDB_{\mu A, Cy5}})$ , is

$$R_{\mu A}^{FODDB} = \{ \text{PHO5,PHO11,PHO12,PHO8,PHO84,PHO89,PHO86,PHO81,SPL2,PHM1,} \\ \text{PHM2,PHM3,PHM4,PHM5,PHM6,PHM7,PHM8,HOR2,CTF19,HIS1} \} \quad (10)$$

The same procedure can be applied to the expressions retrieved from the *in silico* simulations. The experiments are defined maintaining the same conditions and so the same dependent variables of the microarray experiment:

$$\begin{aligned} q_m^1 &= (G_m, C^1, M^1, P) & \text{with } IV(q_m^1) &= IV(q_m^2) \text{ and} \\ q_m^2 &= (G_m, C^2, M^2, P) & DV(q_m^1) &= DV(q_m^2) \end{aligned} \quad (11)$$

On  $q_m^1$  and  $q_m^2$  we can now apply the classification function  $\delta_m$  which is, in general, different from  $\delta_{\mu A}$  because the assumptions on the distribution of the expression dataset of a microarray and of *in silico* experiment can differ. The set of regulated genes with the *in silico* approach is denoted with  $R_m \subseteq G_m$  and computed with a  $\delta_m$  function :

$$R_m = \delta_m(E_{q_m^1}, E_{q_m^2}) \quad (12)$$

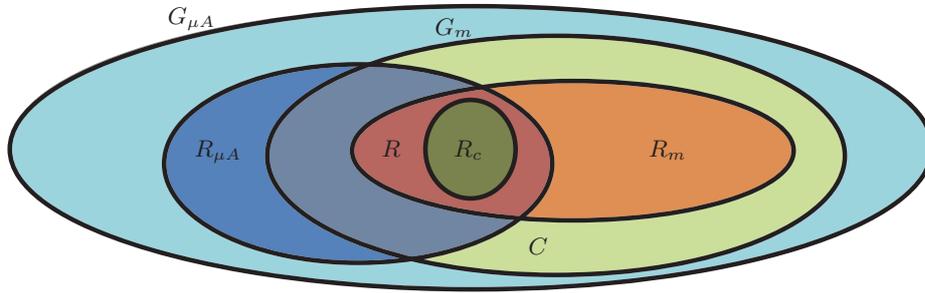
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<sup>2</sup> The  $\delta_{\mu A}$  as used here accepts two expression datasets, but it can be generalised to consider two sets of expression data in presence of experimental designs with replicas.

This formula, in the case of the PHO pathway model, is  $R_m^{SBP} = \delta_m(E_{q_{m,lp}^{SBP}}, E_{q_{m,hp}^{SBP}})$  and, via the computational simulations, gives the following set

$$R_m^{SBP} = \{\text{PHO81,PHO5}\} \quad (13)$$

In ideal conditions we should have  $R_{\mu A} = R_m$  (and  $G_m = G_{\mu A}$ ), but in a scenario where all kind of systematic and random errors can occur the  $G_{\mu A}$ ,  $G_m$ ,  $R_{\mu A}$  and  $R_m$  sets are all potentially different. We further discuss the subsets of  $R_{\mu A}$  which is the set of genes we want to “clean”, distinguishing the cases  $G_m \subseteq G_{\mu A}$ ,  $G_m \equiv G_{\mu A}$  and  $G_{\mu A} \subseteq G_m$ . Since the microarray chips can handle almost the whole genome while the computational models are still far from it (as in our case), we focus on the first case represented in Figure 2:



**Fig. 2:** Representation of the possible intersections between  $G_{\mu A}$  (the set of genes with a correspondent spot on the microarray),  $G_m$  (the set of genes considered in the model),  $R_{\mu A}$  (the set of regulated genes in the microarray) and  $R_m$  (the set of regulated genes in the *in silico* experiment) assuming  $G_m \subseteq G_{\mu A}$ .

$R_{\mu A} \setminus G_m$  contains the microarray regulated genes which are not considered in the computational model. The genes in this set are those whose investigation can potentially increase the biological knowledge and improve the model.  $R = R_{\mu A} \cap R_m$  represents the set of genes that are regulated in both approaches.  $R_c \subseteq R$  contains, for definition, the genes of the model that are regulated in a consistent way with respect to the regulations detected by the microarray:

$$R_c = \left\{ g \in R_{\mu A} \cap R_m \mid \frac{Expr_{q_{\mu A}^1}(g)}{Expr_{q_{\mu A}^2}(g)} \simeq \frac{Expr_{q_m^1}(g)}{Expr_{q_m^2}(g)} \right\} \quad (14)$$

The expression comparison operator ( $\simeq$ ) is not precisely defined since different levels of consistency can be considered. The most restrictive is the  $=$ , while the less restrictive is an operator that evaluates to true if the gene is over or under expressed (expression ratio greater or less than 1) in both approaches, but other intermediate operators can be defined. Also the comparison of the expression ratios is not the only comparison technique [17].

The set of the genes that are regulated consistently (with a comparison based on the over- or under-expressed condition) between  $R_{\mu A}^{FODB}$  and  $R_m^{SBP}$  of our running example is

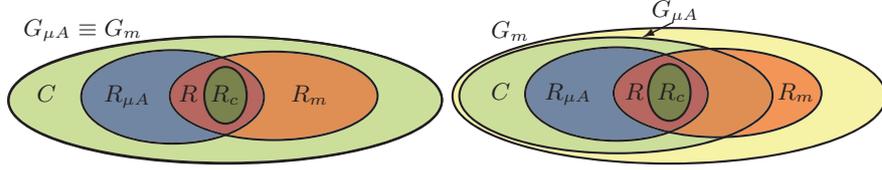
$$R_c^{PHO} = \{PHO81, PHO5\} \quad (15)$$

$(R_{\mu A} \cap G_m) \setminus R$  contains the genes regulated in the microarray but not in the *in silico* simulations. The mismatch can be due to errors, or to the lack of biological information on which the model is constructed.

Other meaningful sets are:

$C = (G_{\mu A} \cap G_m) \setminus (R_{\mu A} \cup R_m)$  the set of genes that are constitutive (i.e. not regulated) in both microarray and *in silico* experiments. In the running example we have that  $C^{PHO} = \{PHO2, PHO4\}$

$R_m \setminus R$  the set of genes that are regulated only in the *in silico* experiments.



**Fig. 3:** Representation of  $G_{\mu A}$ ,  $G_m$ ,  $R_{\mu A}$  and  $R_m$  assuming  $G_m \equiv G_{\mu A}$  and  $G_{\mu A} \subseteq G_m$ .

The cases  $G_m \equiv G_{\mu A}$  and  $G_{\mu A} \subseteq G_m$  are represented in Figure 3; the main differences with the  $G_m \subseteq G_{\mu A}$  case are that  $R_{\mu A} \setminus G_m = \emptyset$  and that in the  $G_{\mu A} \subseteq G_m$  case there is the  $R_m \setminus G_{\mu A}$  set of the genes considered in the computational model but not on the microarray chip.

### 3.1 Accuracy and coverage measures

From a methodological point of view, for the genes in  $R_c$  and in  $C$  the modelling approach is consistent with the microarray one, while the genes in  $R \setminus R_c$ ,  $(R_{\mu A} \cap G_m) \setminus R$  and  $(R_m \cap G_{\mu A}) \setminus R$  represent an incongruence between the approaches. With these sets we can formally define the accuracy of the combination of computational modelling (and indirectly of the biological knowledge) and of the microarray information in the following way:

$$A = \frac{|R_c| + |C|}{|G_{\mu A} \cap G_m|} \times 100 \quad (16)$$

Another measure of the quality of the framework applied to specific cases reflects the gene coverage level of the model with respect to the microarray chip. This measure is called chip coverage and can be defined as follows:

$$Cov = \frac{|G_{\mu A} \cap G_m|}{|G_{\mu A}|} \times 100 \quad (17)$$

It is an *a priori* quantification of the percentage of the genes in the microarray chip that are also considered in the model. However, a low chip coverage does not imply that the approach is useless because the microarray regulated genes can be highly covered by the model even if the chip is poorly covered. So another coverage measure, that we call experimental coverage ( $Cov_e$ ), is introduced:

$$Cov_e = \frac{|R_{\mu A} \cap G_m|}{|R_{\mu A}|} \times 100 \quad (18)$$

This value can be calculated only when both the microarray and the *in silico* experiment are performed and it is referred only to a single experiment, but it is a more accurate evaluation of the experimental quality. Moreover, minimizing the chip coverage after maximizing the experimental coverage, gives the same results of a complete chip coverage, but it requires a much lower computational effort to perform the *in silico* experiments. On the other hand a complete chip coverage permits to use the same computational model for all the possible experiment with that chip. In any case, the coverage and the accuracy must be considered together for evaluating the overall quality of the approach for a performed experiment.

Observing on the running example that  $|R_c^{PHO}| = 2$ ,  $|C^{PHO}| = 2$ ,  $|G_{\mu A}^{FODB} \cap G_m^{SBP}| = |G_m^{SBP}| = 4$ ,  $|G_{\mu A}^{FODB}| = 6400$ ,  $|R_{\mu A}^{FODB}| = 20$  and  $|R_{\mu A}^{FODB} \cap G_m^{SBP}| = 2$ , the introduced measure are:

$$A^{PHO} = 100\% \quad Cov^{PHO} = 0,0625\% \quad Cov_e^{PHO} = 10\%$$

This means that the model predicts the expression profiles consistently with the microarray experiment, but the coverage is very low as we expected since the model is local. The relatively high experimental coverage means that the model fits at least partially the pathways whose genes are interested in the regulation.

## 4 Applications in microarray differential analysis

The set of genes detected with the microarray differential analysis whose regulation is in relation with a specific condition, can be very large and so hard to analyse. The applications we propose here are based on the described framework and are intended to be applied on the set of differentially expressed genes in order to detect the most informative genes before further analysis.

### 4.1 Removing the genes regulated in the *in silico* predictions

The set of regulated genes can include many genes that are already indirectly known to be related with the conditions of the analysis. The removing of these genes allows the biologist to focus only on the really unknown genes that can thus have more new biological information. The idea is to use computational models, built on the current biological knowledge, to predict the genes that will be regulated in a specific microarray experiment and remove them from the microarray experimental results.

So, the aim of this application is to filter out from the regulated genes of the microarray experiment ( $R_{\mu A}$ ), those genes that the model suggests to be regulated ( $R_m$ ). We would remove directly the genes in  $R_m$  from the genes in  $R_{\mu A}$ . In other words the set of really interesting genes  $R_{int}$  would be:

$$R_{int} = R_{\mu A} \setminus R_m \quad (19)$$

In presence of errors and approximations, the definition of the interesting regulated genes  $R_{int}$  as  $R_{\mu A} \setminus R_m$  is no more acceptable mainly because the uncertainty on  $R_m$ . We need a more robust definition of the set of genes that can be safely removed from the microarray regulated ones. So instead of subtracting from  $R_{\mu A}$  the  $R_m$  set, we subtract the genes that are regulated in a consistent way in both approaches, i.e. the  $R_c$  set as defined in (14). So:

$$R_{int} = R_{\mu A} \setminus R_c \quad (20)$$

Returning to the running example, this definition is  $R_{int}^{PHO} = R_{\mu A}^{FODDB} \setminus R_c^{PHO}$  where the  $R_{\mu A}^{FODDB}$  and  $R_c^{PHO}$  sets are computed and shown respectively in (10) and (15). The resulting set of really interesting genes is

$$R_{int}^{PHO} = \{ \text{PHO11,PHO12,PHO8,PHO84,PHO89,PHO86,SPL2,PHM1,PHM2,PHM3,} \\ \text{PHM4,PHM5,PHM6,PHM7,PHM8,HOR2,CTF19,HIS1} \} \quad (21)$$

Our application has filtered out from the set of regulated genes of the reference microarray experiment [3] two genes (PHO5 and PHO81) predicted by the computational model [16]. So we have reduced the number of genes that represent new biological information suggesting that this application is useful.

The genes considered in the model are only 4, and the two that have been removed from  $R_{int}^{PHO}$  are those that the model predicts sensitive to different phosphate metabolism. So, as the accuracy measure ( $A^{PHO} = 100\%$ ) suggests, the model predictions are in this case the more desirable ones with respect to the very low coverage. We can conclude that this application can be a helpful tool for a biologist, especially in the cases where the microarray experiment detects an high number of regulated genes and the computational model has a reasonable good experimental coverage in addition to the accuracy.

## 4.2 Removing the genes regulated by the non controlled variables

The definition of the microarray experiment includes the notion of controlled conditions and dependent not-directly controlled variables. Obviously the effects of the dependent variables in terms of regulated genes cannot be detected in isolation or separated from the independent variable effects through the microarray experiment. Since biologists are interested in the genes regulated only by the directly controlled conditions, the idea of this application is to estimate *in silico* the genes that are regulated because of the dependent variables in order to remove them from the set of microarray regulated genes. Note that the values

of the dependent variables included in the specification of the *in silico* gene expression experiments are those measured on the microarray experiment. Since, as discussed, the microarray experiment of [3] does not provide any dependent variables, we cannot test this conceptual application with our running example.

Consider the following three microarray experiments<sup>3</sup>:

$$q_{\mu A} = (G, C, M, P) \quad q_{\mu A}^{ctr} = (G, C^{ctr}, M^{ctr}, P) \quad q_{\mu A}^h = (G, C, M^{ctr}, P)$$

with  $IV(q_{\mu A}) = IV(q_{\mu A}^{ctr}) = IV(q_{\mu A}^h)$  and  $DV(q_{\mu A}) = DV(q_{\mu A}^{ctr}) = DV(q_{\mu A}^h)$ . The first two chips are a microarray experiment ( $q_{\mu A}$ ) and the relative control chip ( $q_{\mu A}^{ctr}$ ), while the third ( $q_{\mu A}^h$ ) is a hypothetical variation of the microarray chips in which the actual values of dependent variable are replaced with the control ones. Notice that the  $q_{\mu A}^h$  experiment cannot be really performed since the values of the independent variables  $C$  force the values of the dependent controlled variables to be  $M$  and not  $M^{ctr}$ . Suppose to apply a  $\delta_{\mu A}$  function in the following way:

$$R_{\mu A} = \delta_{\mu A}(E_{q_{\mu A}}, E_{q_{\mu A}^{ctr}}) \quad R_{\mu A}^V = \delta_{\mu A}(E_{q_{\mu A}^h}, E_{q_{\mu A}^{ctr}}) \quad R_{\mu A}^{VD} = \delta_{\mu A}(E_{q_{\mu A}}, E_{q_{\mu A}^h})$$

$R_{\mu A}$  is the standard set of genes that are regulated because of the differences between levels of the independent a dependent variables between the two chips,  $R_{\mu A}^V$  contains the genes that are regulated only by the independent variables (since  $q_{\mu A}^h$  and  $q_{\mu A}^{ctr}$  have the same values of dependent variables  $M^{ctr}$ ), and  $R_{\mu A}^{VD}$  represents the genes that are regulated only by the dependent variables (since  $q_{\mu A}$  and  $q_{\mu A}^h$  have the same values of the independent variables  $C$ ).  $R_{\mu A}^V$  is the set of genes that the experimenter would have because it is not influenced by the dependent variables, but it is not possible to obtain because the  $q_{\mu A}^h$  chip is only hypothetical.

Under the assumption that the sets of genes regulated by the dependent and independent variables are disjoint,  $R_{\mu A}^V$  could be estimated subtracting from  $R_{\mu A}$  the genes that are regulated because of the dependent variable  $R_{\mu A}^{VD}$ . So

$$R_{\mu A}^V = R_{\mu A} \setminus R_{\mu A}^{VD} \quad (22)$$

but also  $R_{\mu A}^{VD}$  is not possible to obtain, since it needs the hypothetical  $q_{\mu A}^h$  chip.

However, in this framework we have the possibility to estimate expression experiments with the computational model. In particular, all the following experiments can be performed *in silico*:

$$q_m = (G, C, M, P) \quad q_m^{ctr} = (G, C^{ctr}, M^{ctr}, P) \quad q_m^h = (G, C, M^{ctr}, P)$$

With these *in silico* experiments it is possible to detect the gene regulated only by the independent ( $R_m^V$ ) and only by the dependent variables ( $R_m^{VD}$ ):

$$R_m^V = \delta_m(E_{q_m^h}, E_{q_m^{ctr}}) \quad R_m^{VD} = \delta_m(E_{q_m}, E_{q_m^h})$$

<sup>3</sup> In this subsection we assume that the set of genes spotted on the microarray and the set of genes considered in the model are identical, without losing generality. Formally:  $G = G_{\mu A} \equiv G_m$

The direct approximation of  $R_{\mu A}^V$  with  $R_m^V$  is useless because in this way we rely only on the model, losing the information of the microarray experiment.

The idea for integrate the microarray and the *in silico* data consists in filtering out from the set of microarray regulated genes those genes that are regulated because of the dependent variables, substituting  $R_{\mu A}^{V_D}$  with  $R_m^{V_D}$  in (22)

$$R_{\mu A}^V \simeq R_{\mu A} \setminus R_m^{V_D} \quad (23)$$

If a gene is regulated both because of the independent and because of the dependent variables, the estimation of  $R_{\mu A}^V$  will not include that gene. For this reason is necessary to assure that  $V$  and  $V_D$  regulate two different set of genes meaning that the influence on gene regulation of the two set of variables is disjoint.

**Definition 1.**  $V$  and  $V_D$  are disjoint with respect to the gene regulation, or simply, ge-disjoint, if and only if for every possible values associated to the variables the following holds:  $R_{\mu A}^V \cap R_{\mu A}^{V_D} = \emptyset \wedge R_m^V \cap R_m^{V_D} = \emptyset$

This definition is too restrictive because it is impossible to directly obtain  $R_{\mu A}^V$  and  $R_{\mu A}^{V_D}$ . However, the theoretical definition can be made less strict for an effective application if we have an high accuracy and a good confidence in the quantitative estimation of gene expression of the model.

**Definition 2.**  $V$  and  $V_D$  are ge-disjoint, if and only if for every possible values associated to them the following holds:  $R_m^V \cap R_m^{V_D} = \emptyset$

The complete definition for removing from the set of regulated genes of a microarray, the genes that are regulated only by the dependent variables, is

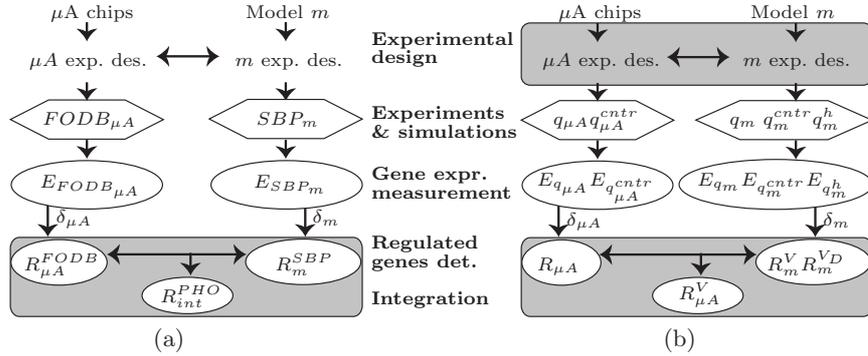
$$R_{\mu A}^V \simeq R_{\mu A} \setminus R_m^{V_D} \quad \text{if } R_m^V \cap R_m^{V_D} = \emptyset \quad (24)$$

If  $V$  and  $V_D$  are not ge-disjoint (or if it is not possible to show it), we can partition  $V_D$  in two subsets  $V_D', V_D'' \subset V_D$  with  $V_D' \cap V_D'' = \emptyset$  and  $V_D' \cup V_D'' = V_D$  such that  $V_D'$  and  $V$  are ge-disjoint. At this point we can still remove some genes from  $R_{\mu A}$ , and precisely the genes that are regulated because of  $V_D'$ .

So in the cases where it is possible to show that the set of independent variables (or a subset) are ge-disjoint from the set (or from a subset) of the dependent measured variables, we can remove all (or some of) the genes that are not directly regulated by the experimental conditions.

## 5 From a conceptual to a software framework

Our notational framework can be a guide for the development of software tools for supporting analyses that combine the two fields of the microarray technology and the system level modelling and simulation of biological networks. While for the first a lot of bioinformatics tools have been developed for every aspect of the technology, the second still needs research in order to make the development and the simulation of systems biology models effective.



**Fig. 4:** These schemes highlight the modules interested by the application for removing the genes regulated in the *in silico* predictions (a) and by the application for removing the genes regulated by the non controlled variables (b).

Our conceptual framework assumes to have a computational model and microarray chips and can actively act on different phases: the experimental conditions and design, the expression profiling, the regulated genes detection and the integration of regulated genes belonging to the two approaches. The first application (Figure 4.a) concerns only the manipulation of the regulated genes belonging to microarray and *in silico* experiments designed with the same conditions, while the second (Figure 4.b) interests also the design, requiring some experiments with particular settings of the dependent and independent conditions. Other applications can regards also the other phases. Regardless of the number of phases interested in the specific case, each phase requires formal and software tools to allow real implementations of applications in the framework. We discuss the availability of implemented software or formal specification for each phase:

**Experimental design** Appfondite studies have been done to make microarray experiments maximally informative, given the effort and the resources [18] [19]. In this work we assume that an *in silico* simulated microarray experiment has the same abstract behaviour of the real one; for this reason all the designs reported in literature can be applied also to the simulated case. However, some improvements can be done relying on the fact that once the model is developed the simulation cost is much lower than the microarray experimental one. Moreover some hybrid designs are possible integrating in the same design simulated and real microarray experiments.

**Experiments and simulations** The microarray experimental procedure has reached a good level of standardization [1]. Instead, the possible sources of variability in the computational simulation rely on model errors and approximations in the simulation algorithm; however, both problems regard in general the computational modelling of biological systems, while the simulation procedure is intrinsically standard.

**Gene expression measurement** The output of a microarray experiment is obtained with the optical scan of the array and the analysis of the resulting image. Software tools for this operation are available. Both microarray output after scanning and *in silico* expression results, needs normalization procedures to make meaningful comparisons of expression levels. For microarray experiments, statistical methods and software are available [15], while for the *in silico* simulations specific normalization procedures must be developed because the distribution of the expression can *a priori* be different from the microarray ones. Since *in silico* microarray outputs are not available, more precise discussions cannot be done, but it is reasonable to adapt some microarray techniques with specific parameters and thresholds.

**Regulated genes detection** Statistical techniques for the  $\delta_{\mu A}$  like [17] are available and implemented.  $\delta_m$ , instead, was never developed, but the tuning of some  $\delta_{\mu A}$  functions is reasonable, considering also that the *in silico* simulations are not affected by the random experimental error and by approximation in the optical scanning of the chip and so they are less noisy.

**Integration of regulated gene sets** The integration of the set of regulated genes are normal operations on sets and can thus be easily implemented.

## 6 Conclusions

The traditional use of biological models concerns the parameters estimation and the qualitative and quantitative description of not directly observable and high level behaviours. In this work we propose to use systems biology models to support analysis of high-throughput data; it can be seen as a way to compare the current biological knowledge with new genomic experiments. The comparison aims to discover unknown aspects of complex and wide biological networks allowing to focus further investigations only on that very specific subnetworks. The overall procedure is somehow recursive since the new discoveries reached starting from the model suggestions, permits improvements of the biological knowledge from which it is possible to construct more precise models.

The notation we introduced for the microarray experiments and the *in silico* simulations of gene expression was specialized in the context of differential analysis. Then, we proposed two real applications of our conceptual framework with the purpose of supporting the mining of regulated genes detected with microarray expression data. In the example on which we tested the work, we were able to remove two genes from the regulated genes of the microarray experiment, thus obtaining encouraging results and highlighting the utility of the approach, even if we used a local model. For this reason, as the model size grows reaching the systems biology level the impact of our approach on the high-throughput data interpretation can be very important.

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