

Robust Parameter Identification for Biological Circuit Calibration

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Abstract—The aim of this work is to compare some deterministic optimization algorithms and evolutionary algorithms on parameter estimation in a biological circuit design problem: the negative feedback loop between the tumor suppressor p53 and the oncogene Mdm2. We compared deterministic optimization algorithms and evolutionary algorithms in terms of robustness of the resulting parameters including all sources of uncertainty into the statistical representation of reference data and evaluating the obtained solutions in terms of confident limits.

The experimental results obtained show as evolutionary algorithms are more robust with respect of deterministic optimization algorithms in particular the algorithm Differential Evolution (DE) showed the best performance over the minimization of the fitting function.

I. INTRODUCTION

Accurately modeling and simulating biological networks is a challenging problem, due to the complex interaction between large numbers of interacting pathways, feedback inherent to the system, and the stochastic nature of biological processes. However, recent techniques have been developed to model and simulate large-scale biological networks using analogies from electrical circuits [15], [11]. Exploiting the similarities between biological networks and electrical circuit networks is an efficient methodology that can be used to robust parameter identification of some circuit equivalents of biological processes. The aim of this work is to give a computational tool to analyze the robustness of the parameters of multivariate, multi-scale, hybrid biological networks.

The major problem is that the results of the computation, as with any complex simulation, are highly dependent not only on the numerical accuracy of the simulation technique, but also on the particular values of model parameters as well as the simulations initial conditions. Biological circuits are inherently hybrid, with both discrete and continuous components. Hybrid systems are notorious for their non-intuitive behavior, and potentially high sensitivity to variations in model parameters.

Blindly choosing unknown parameters can make it impossible to simulate the desired behavior for example, it could not be possible for a model to reach a desired equilibrium from a given initial condition, even though the mechanics of the model are correct. Additionally, biological parameters

that are not known experimentally (incomplete or noisy data), may have an unintentionally large effect on biological simulation accuracy [15].

A variety of computational approaches, based on optimization [7], evolutionary algorithms [4], [12], and other methodologies, have been used to estimate biological parameters. However, it has never been done a study on the robustness of the reached set of parameters for each tested algorithm to provide a highly accurate, though often computationally intensive, description of the systems behavior.

In this paper we present a methodology inspired by the electronic circuit design described in [1] to study the most robust optimization algorithms (less sensitive to the noise of the experimental data) for parameter identification that are critical for matching the known experimental data. In this study the biological circuits are defined by quantitative system model through systems of Ordinary Differential Equations(ODE).

In order to compare the robustness of the sets of parameters and also the computational effort we have tested classical methods such as LSQNONLIN of MATLAB, DIRECT, and a Pattern Search Algorithm and two evolutionary algorithms: CMA-ES and DE.

We tested our methodology to one of the best-studied protein circuits in human cells: the negative feedback loop between the tumor suppressor p53 and the oncogene Mdm2. In the p53 system, p53 transcriptionally activates Mdm2. Mdm2, in turn, negatively regulates p53 by both inhibiting its activity as a transcription factor and by enhancing its degradation rate. For different parameters of the feedback loop, the dynamics can show either a monotonic response, damped oscillations, or undamped (sustained) oscillations in which each peak has the same amplitude as the previous peak. The stronger the interactions between the proteins, the more oscillatory the dynamics. Other parameters, such as high basal degradation rates of the proteins, tend to damp out the oscillations.

II. METHODOLOGY

Parameter estimation problems of nonlinear dynamic systems are stated as minimizing a cost function that measures the goodness of the fit of the model with respect to a given experimental data set, subject to the dynamics of the system (acting as a set of differential equality constraints) plus possibly other algebraic constraints. Mathematically, the formulation is that of a nonlinear programming problem (NLP) with differential-algebraic constraints [12]:

Find \mathbf{p} to minimize:

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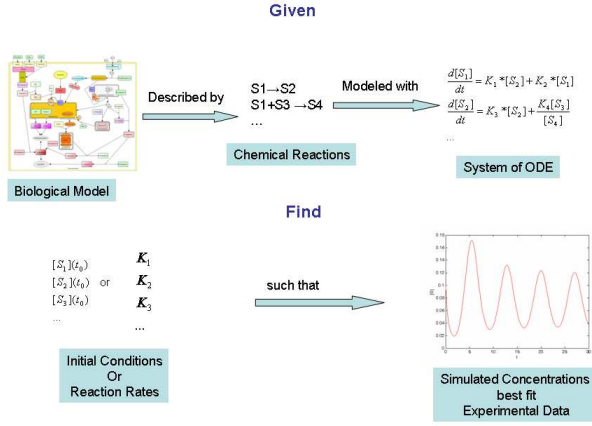


Fig. 1. Problem definition of the Optimization framework in Biological Parameter Estimation

$$Z = \int_0^{t_f} (y_{msd}(t) - y(\mathbf{p}, t))^T W(t) (y_{msd}(t) - y(\mathbf{p}, t)) dt \quad (1)$$

subject to:

$$\begin{aligned} f\left(\frac{dx}{dt}, x, y, p, v, t\right) &= 0 \\ x(t_0) &= x_0 \\ h(x, y, p, v) &= 0 \\ g(x, y, p, v) &\leq 0 \\ \mathbf{p}^L &\leq \mathbf{p} \leq \mathbf{p}^U \end{aligned}$$

where Z is the cost function to be minimized, \mathbf{p} is the vector of decision variables of the optimization problem, the set of parameters to be estimated, y_{msd} is the experimental measure of a subset of the (so-called) output state variables, $y(p, t)$ is the model prediction for those outputs, $W(t)$ is a weighting (or scaling) matrix, x is the differential state variables, v is a vector of other (usually time-invariant) parameters that are not estimated, f is the set of differential and algebraic equality constraints describing the system dynamics (i.e., the nonlinear process model), and h and g are the possible equality and inequality path and point constraints that express additional requirements for the system performance. Finally, \mathbf{p} is subject to upper and lower bounds acting as inequality constraints. The formulation above is that of a nonlinear programming problem (NLP) with differential-algebraic (DAEs) constraints. Because of the nonlinear and constrained nature of the system dynamics, these problems are very often multimodal (nonconvex).

This process is usually performed as a sequence of optimizations, usually based on the Levenberg-Marquard algorithm, which require a good initial guess and yield only local minimum (corresponding to different set of parameters). In this context two problems arise. First, get a robust estimation

for parameters when there are several measurement curves. Second, how to choose the most convenient set of parameter values to obtain the best approximation for the circuit model.

Preliminary investigations have been carried out with different optimization software and they have yielded different sets of parameters. This fitting is based on a initial estimation of the parameter. Comparison of this data shows large variance of identified parameter values [7], [12]. Causes of these behaviors could be non-homogeneous kind of variables; variables can converge with different speed rates; merit function of optimization on l^2 norm can find different balancing among errors; first and second derivative do not lead the optimization in useful regions. Previous remarks compel to consider the quality of results is sense of robustness.

In some previous works [2], [9], [3] focused on robust biological circuit design, robustness is defined as a measure of tolerance of kinetic parameter variations with the existence of the steady states of the biochemical network preserved. Sensitivity analysis are conventionally employed to assess the robustness of biochemical networks [17].

In our case the concept of uncertainty wants to summarize various problem related to degree of model approximation, imprecisions on performing calculations, statistical representation of data. In electronic circuit design problems [1], a general practice is to include all sources of uncertainty into the statistical representation of data and evaluate the robustness of solution in terms of confident limits. The term "robust" was coined in statistics by G.E.P. Box in 1953. General, referring to a parameter extraction for fitting a statistical model of data, it means "insensitive to small departures" from the idealized assumptions for which the data model is optimized. The word "small" can have two different interpretations, both important: either fractionally small departures for all data points, or else fractionally large departures for a small number of data points. It is the latter interpretation, leading to the notion of outliers, that is generally the most stressful for statistical procedures. In this work we used the M-estimate obtained by minimizing the mean square deviation.

The comparisons in this work want to be more explicit regarding the precise meaning of these quantitative uncertainties, and to give further information about how quantitative confidence limits on fitted parameters can be estimated. Through the Montecarlo simulation it is possible to repeat virtually an experiment and to get a quality measure of fitting robustness. The simulation starts with a initial fitting in order to identify a possible set of parameters. This set of parameters is used to synthesize a new surrogated set of data which are perturbed by a white noise. In this study the noise is a gaussian error with $\mu = 0$ and $\sigma = 1/10$ of the data magnitude.

This process mimes artificially the statistical properties of real data. Then the fitting is processed on this surrogated data to get a new set of parameters. This kind of artificial process is repeated many times to get a large class of parameter. Finally, classical statistics are performed on this class of parameters and confidence limit on parameters are calculated

from these simulations.

III. ALGORITHMS

In order to compare the robustness of the sets of parameters and also the computational effort, the following three deterministic methods have been considered.

LSQ The function LSQNONLIN of Matlab solves nonlinear least-squares problems, including nonlinear data-fitting problems. In our case we use the algorithm with the default options of large scale optimization, which uses the subspace trust method based on the Levenberg-Marquardt [13] method over Gauss-Newton algorithm to compute the decreasing direction.

Direct Global search method that applies to Lipschitz continuous function and, after an initial implicit estimate of the Lipschitz constant chooses the potentially optimal rectangles and resamples them across their axis. Subsequently it divides these rectangles and proceed sampling and dividing until a stopping criteria is met [8]. This method exploits the estimation of Lipschitz constant to balance global and local search and reaches quasi-global solution in large domain.

GPS Pattern Search algorithms [10] are known as Search and Poll algorithms. In the search step, any finite set of mesh points can be evaluated. When the search step fails the algorithm calls the poll procedure that consists in evaluating the objective function at the neighboring mesh points to see if a lower function value can be found.

We compared the results testing also two evolutionary algorithms:

CMA-ES The CMA-ES (Covariance Matrix Adaptation Evolution Strategy) [6] is an evolutionary algorithm for difficult non-linear non-convex optimization problems in continuous domain. The CMA-ES is a second order approach and estimates a covariance matrix within an iterative procedure. Adaptation of the covariance matrix amounts is similar to the approximation of the inverse Hessian matrix. Restarts with increasing population size improve the global search performance.

DE Differential Evolution (DE) was introduced by Storn and Price [16]. DE works as follow: after a random initialization the objective function is evaluated and the following steps are repeated until a termination condition is satisfied. Each individual is updated using a weighted difference of a number of selected parent solutions. If the offspring replaces the parent only if it improves the fitness value, otherwise the parent is copied in the new population. The crucial idea behind DE is this new scheme for generating trial parameter vectors. DE generates new parameter vectors by adding the weighted difference vector between two

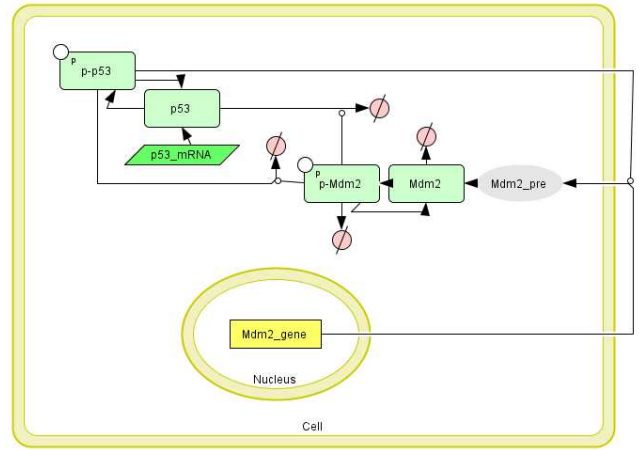


Fig. 2. Graphical model of the negative feedback loop between P53 and Mdm2.

population members to a third member. If the resulting vector yields a lower objective function value than a predetermined population member, the newly generated vector replaces the vector with which it was compared. The comparison vector can, but need not be part of the generation process mentioned above. In addition the best parameter vector is evaluated for every generation in order to keep track of the progress that is made during the minimization process. Using Storn and Price naming convention we used the classical version of DE DE/rand/1.

IV. CASE STUDY: A NEGATIVE FEEDBACK LOOP

The considered test case is the negative feedback loop between the tumor suppressor p53 and the oncogene Mdm2 [14] which is one of the best-studied protein circuits in human cells. In the p53 system, p53 transcriptionally activates Mdm2. Mdm2, in turn, negatively regulates p53 by both inhibiting its activity as a transcription factor and by enhancing its degradation rate. For different parameters of the feedback loop, the dynamics can show either a monotonic response, damped oscillations, or undamped (sustained) oscillations in which each peak has the same amplitude as the previous peak. The stronger the interactions between the proteins, the more oscillatory the dynamics. Other parameters, such as high basal degradation rates of the proteins, tend to damp out the oscillations.

Figure 2 shows the graphical model of the negative feedback loop between p53 and Mdm2.

The mathematical formulation of the nonlinear dynamic model consists of the system of 5 ODEs shown in equations 2 that describes the variation of the proteins concentrations with time.

$$\begin{aligned}
\frac{d[p53]}{dt} &= -\frac{V1 \cdot [p53]}{[p53] + kp1} + kdp1 \cdot [p - p53] + kf1 + \\
&\quad + \frac{kd1 \cdot [p53] \cdot [p - Mdm2]}{[p53] + kdeg1} \\
\frac{d[p-p53]}{dt} &= \frac{V1 \cdot [p53]}{[p53] + kp1} - kdp1 \cdot [p - p53] + \\
&\quad - \frac{kd2 \cdot [p-p53] \cdot p - Mdm2}{[p-p53] + kdeg2} \\
\frac{d[Mdm2]}{dt} &= -\frac{V2 \cdot [Mdm2]}{[Mdm2] + kp2} + kdp2 \cdot [p - Mdm2] + \\
&\quad + kf3 \cdot [Mdm2_pre] - kd3 \cdot [Mdm2] \\
\frac{d[p-Mdm2]}{dt} &= -kd4 \cdot [p - Mdm2] + \frac{V2 \cdot [Mdm2]}{[Mdm2] + kp2} + \\
&\quad - kdp2 \cdot [p - Mdm2] \\
\frac{d[Mdm2_pre]}{dt} &= kf2 \cdot [p - p53] - kf3 \cdot [Mdm2_pre]
\end{aligned} \tag{2}$$

The parameters involved in the mathematical formulation of the problem are the following:

- kf1 p53 translation rate
- V1 Enzyme reaction rate for p53 phosphorylation
- Kp1 Michaelis constant for p53 phosphorylation
- kdp1 p-p53 dephosphorylation rate
- kd1 p-Mdm2 enzyme reaction rate for p53 degradation
- Kdeg1 Michaelis constant for p53 degradation
- kd2 p-Mdm2 enzyme reaction rate for p-p53 degradation
- Kdeg2 Michaelis constant for p-p53 degradation
- kf2 Mdm2 transcription and translation rate
- kf3 Mdm2 post-translational modification rate
- V2 Enzyme reaction rate for Mdm2 phosphorylation
- Kp2 Michaelis constant for Mdm2 phosphorylation
- kdp2 p-Mdm2 dephosphorylation rate
- kd3 Mdm2 degradation rate
- kd4 p-Mdm2 degradation rate

In our study the global optimization problem was stated as the minimization of the following quadratic objective function

$$Z = \sum_{i=1}^n \sum_{j=1}^m ([y_{pred}(i) - y_{exp}(i)]_j)^2 \tag{3}$$

where n is the number of data for each specie, m is the number of species, y_{exp} represents the known experimental data, and y_{pred} is the vector of states that corresponds to the predicted theoretical evolution using the model with a given set of the parameters.

To better assess the performance of the optimization algorithms for the solution of the inverse problem, pseudo-experimental data were generated by simulation from a set of chosen parameters (to be considered as the true, or nominal, values) shown in Table I. Thus, pseudo-measurements of the concentrations of the species were the result of different experiments (simulations) in which their concentrations were varied. These simulated data represent results of experiments with the additional measurement noise.

In bold are showed the five parameters that were chosen for the optimization because are critical in the calculation of the error between the simulated data and the known experimental data. This subset of parameters was identified through Latin Hypercube sampling and calculating the correlation coefficients with respect to the error residue.

Parameters	Nominal Value
kf1	0.9
V1	4
Kp1	2
kdp1	1
kd1	8.5
Kdeg1	0.1
kd2	0.85
Kdeg2	0.01
kf2	1.1
kf3	0.8
V2	0.8
Kp2	0.2
kdp2	0.4
kd3	0.08
kd4	0.8

TABLE I

THE PARAMETERS OF THE MODEL AND THEIR NOMINAL VALUES.

From the biological point of view the above selected parameters directly/indirectly control the steady state level of p53, suggesting that, to maintain a stable p53 concentration in the system, they are critical for proper cell response.

A. Results

These results show the best values obtained after performing 60 independent runs for each algorithm while the Montecarlo simulation used synthetic data set created adding a gaussian error with $\mu = 0$ and $\sigma = 1/10$ of data magnitude in the initial data set. All methods tackled use as termination condition the maximum number of objective function evaluations. In particular in this test case, the maximum number of function evaluations has been fixed to 1000. The bound of each parameter was set to the same order of magnitude that contains the reported values showed in Table I.

The tested algorithms demonstrated different degrees of reliability in reaching the solutions over the all independent runs. For each method, after the Montecarlo simulation, we calculated the percentage of success. Each success implied an identification of a set of parameters which characterizes the behavior of the curves (the variation of the concentrations over the time) accurately with respect to the experimental data. Direct and DE showed 100% of success in the identification of the parameters, PSearch and CMA had a 90% of success while LSQ only a 60%.

The LSQ method showed larger confidence limits in the Montecarlo simulation for the parameters estimated in Table II as we can see through the standard deviation of the parameters. The most robust parameter are found by DE algorithm (first best algorithm) and Direct algorithm even if Direct reaches a smaller value of the minimized function slower than the other algorithms (see Figure 4). The confidence interval of the estimated parameters reached by DE and Direct algorithms are shown in Figure 3 in which the minimum, mean and maximum reached values are shown while the stars represent the nominal values.

In Table III are showed comparison among the tested optimization algorithms on the mean value and standard deviation of the minimized objective function Z during the

	Kf1	Kdp1	Kd1	V2	Kdp2
Direct	0.93±0.01	1.08±0.05	8.56±0.25	0.81±0.07	0.46±0.15
LSQ	0.67±0.25	1.34±0.47	6.46±2.11	0.73±0.16	0.54±0.18
GPS	0.86±0.14	1.06±0.11	8.04±0.96	0.87±0.12	0.55±0.18
CMA-ES	0.88±0.06	1.04±0.15	8.18±0.86	0.82±0.12	0.5±0.2
DE	0.9±0.02	1±0.01	8.52±0.25	0.8±0.03	0.41±0.07

TABLE II

COMPARISON AMONG THE TESTED OPTIMIZATION ALGORITHMS ON THE MEAN VALUE AND STANDARD DEVIATION OF EXTRACTED PARAMETERS AFTER THE MONTECARLO SIMULATION.

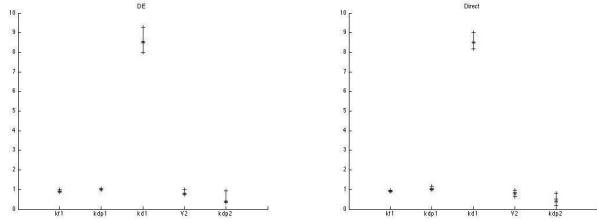


Fig. 3. Minimum, maximum and mean value for each parameter estimated by DE and Direct during the Montecarlo simulation

Montecarlo simulation. The best results over the objective function minimization were reached by DE, while the second best method was Direct.

	mean(Z)	std(Z)
Direct	0.004	0.005
LSQ	0.16	0.2
GPS	0.035	1.14
CMA-ES	0.06	0.32
DE	2.02e⁻⁴	6.36e⁻⁴

TABLE III

COMPARISON AMONG THE TESTED OPTIMIZATION ALGORITHMS ON THE MEAN VALUE AND STANDARD DEVIATION OF THE MINIMIZED OBJECTIVE FUNCTION Z DURING THE MONTECARLO SIMULATION.

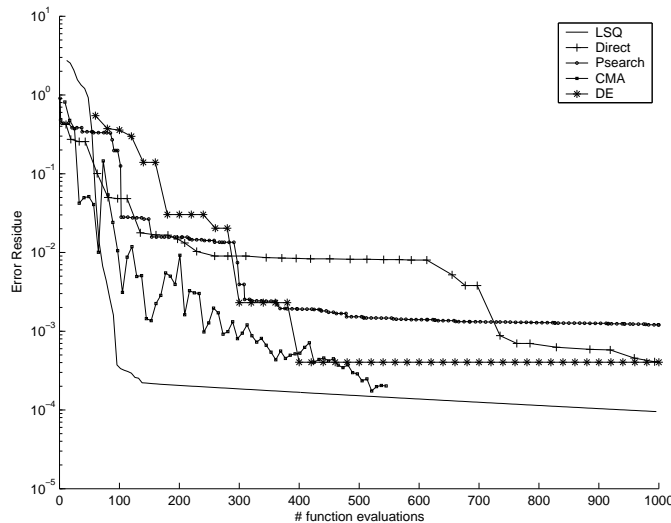


Fig. 4. Objective Function value during one run of each tested algorithm

As a preliminary testing we focused on the parameter estimation problem without the analysis of the robustness taking into account all the 15 parameters of the model. The numerical simulations showed that only LSQ, DE and CMA-ES reached a reasonable fitting of the data while both Direct and GPS got stuck into an unfeasible point. Table IV shows the comparison among the optimization algorithms LSQ, CMA-ES and DE on the parameter estimation problem in terms of reached object function value and number on function evaluations. Each algorithm made use of the stopping criterium of the minimum tolerance of the evaluated objective function of 10^{-3} .

	Z	# func eval
LSQ	0.0008	432
CMA-ES	0.0003	4573
DE	0.0006	75000
Direct	N.A.	N.A.
GPS	N.A.	N.A.

TABLE IV

COMPARISON AMONG THE OPTIMIZATION ALGORITHMS LSQ, CMA-ES AND DE ON THE PARAMETER ESTIMATION PROBLEM IN TERMS OF REACHED OBJECT FUNCTION VALUE AND NUMBER ON FUNCTION EVALUATIONS.

Table V shows the search parameters utilized by the evolutionary algorithms CMA-ES and DE on the parameter estimation problem that take into account all the 15 parameters of the model.

CMA-ES	N=15; $\lambda=12$; $\mu=6$; MaxFunEval=10000; TolFun= $10e-4$
DE	VTR= $10e-4$; st=1; D=15; NP=200; IterMax=500; F=0.85; CR=1

TABLE V

SEARCH PARAMETERS UTILIZED BY THE EVOLUTIONARY ALGORITHMS

V. CONCLUSIONS

In this article we compared evolutionary algorithms and deterministic optimization methods facing an optimization problem on a biological circuit design test case. The optimization problem concerned parameter identification of the negative feedback loop between the tumor suppressor p53 and the oncogene Mdm2. Based on this biological circuit design problem and on the above reported statistical analysis, we can make the following statements. For the parameter identification problem of the biological circuit model, using a Montecarlo simulation, the evolutionary strategy DE and the deterministic method Direct are the most robust in the sense that they are less sensitive to the noise of the experimental data. Both Direct and DE showed 100% of success in the identification of the parameters that characterize the curves of the variation of the concentrations over the time accurately with respect to the experimental data. In terms of the data

fitting (value of the objective function) the evolutionary algorithm DE is more effective than the other tested optimization methods.

Considering the complete problem of identification of the whole set of parameters LSQ showed the best results in terms of reached object function value and the number on function evaluations even if it is more dependent to the chosen initial search point.

It is recommendable to perform a first global search on the complete parameter set using LSQ algorithm which require less computational effort in terms of CPU time and number of function evaluations but using a multi-start strategy [5] using the method repeatedly, starting from a number of different initial points to avoid to stuck in a local minimum.

After defining the value of the whole parameters of the set, identify the parameters which are more critical for matching the known experimental data (e.g. through Latin Hypercube sampling and calculation of correlation coefficients), and perform the robustness of that parameters using DE.

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