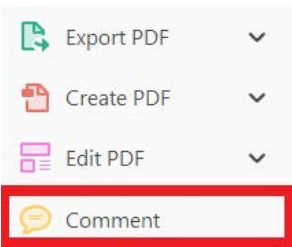


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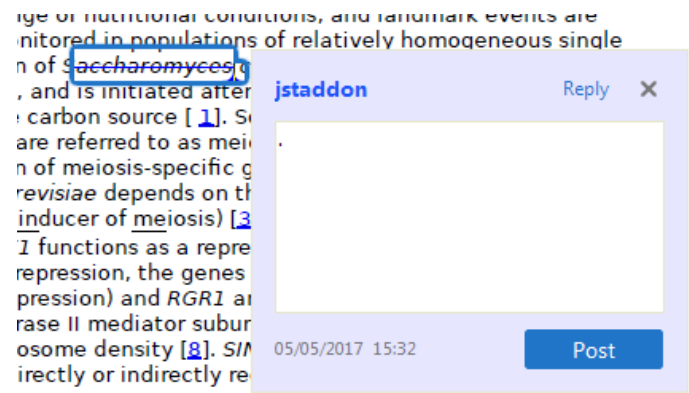


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
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experimental data if available. For ORFs to be had to meet all of the following criteria:

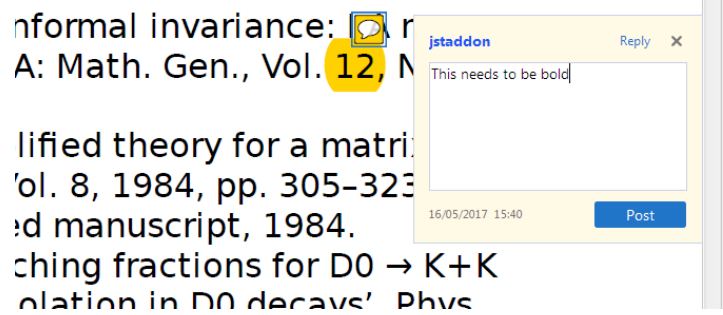
1. Small size (~~35~~-250 amino acids).
2. Absence of similarity to known proteins.
3. Absence of functional data which could not be the real overlapping gene.
4. Greater than 25% overlap at the N-terminus with another coding feature; over both ends; or ORF containing a tRNA.

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  Use these 2 tools to highlight the text where a comment is then made.

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- Click and drag over the text you need to highlight for the comment you will add.
- Click on .
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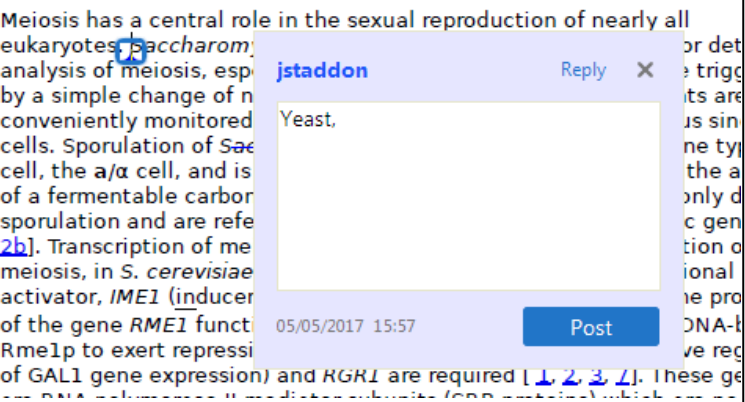


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The attachment appears in the right-hand panel.

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6. **Add stamp** Tool – for approving a proof if no corrections are required.

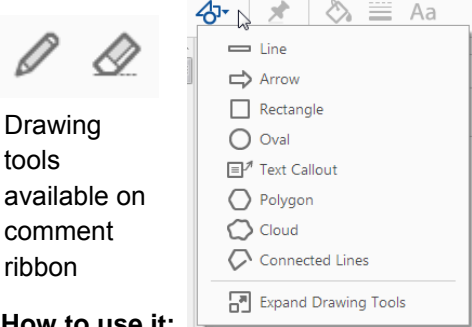
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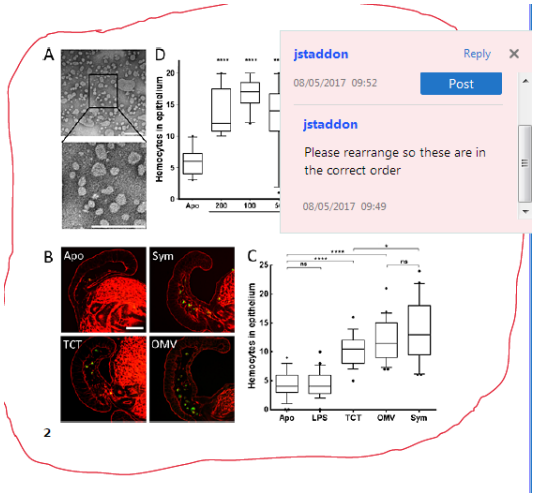
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How to use it:

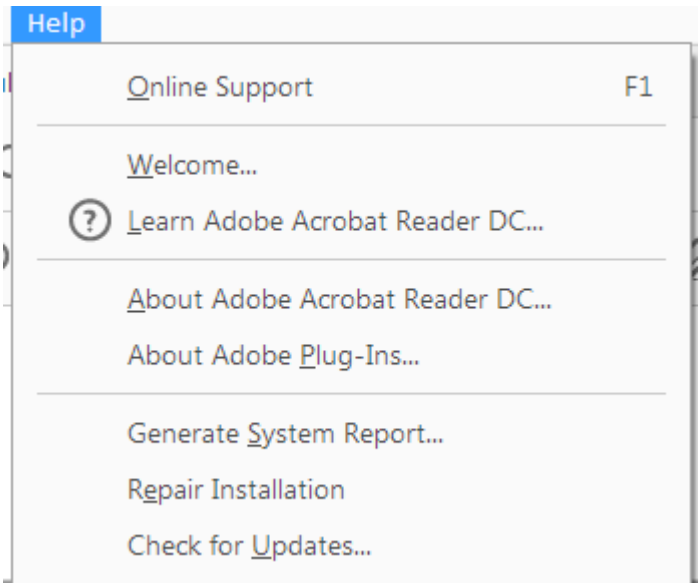
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RESEARCH ARTICLE

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A computer-based educational tool for simulating multifactorial experiments of physical processes

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Abstract

In this article, challenges in designing ^{Q2}example problems of multifactorial experiments for an academic purpose are identified, and an algorithm for simulating multifactorial experiments is presented. It is based on a proposed mathematical function, which mimics a physical system, can be employed for any number of finite factors, and contains a unique peak, with which a unique optimal solution for an experiment is guaranteed. The proposed algorithm is implemented on an application developed with JavaScript, HTML, and CSS, which has been tested in the classroom for highlighting all characteristics of experimental design.

KEYWORDS

educational tool, experimental design, generating examples, multifactorial model, problem-based learning

1 | INTRODUCTION

Almost all the fields involving experimentation use experimental ^{Q3}design [1,3,6,8]. It is part of various undergraduate and graduate curriculum, ranging from engineering to biological sciences. The objective of experimental design is to minimize cost and time of experiments and maximize its yield. As an example, it can be used to find the values of factors such as pH, oxygen concentration, sugar concentration, with which the enzyme production is maximized. Different optimization techniques can be employed to minimize number of experiments, with which the objective of experimental design is achieved [10]. On the other hand, an improper experimental design may lead to inaccurate or false conclusions, as well as a loss of money, material, and time [2].

Solving numerical examples helps students to learn statistics or mathematics [11], or to develop insight into a particular topic [9]. Moreover, students obtain good learning

experience using visual numerical examples of experiments [5]. On the other hand, finding the most accurate mathematical model for a process in experimental design involves performing various experiments with different combinations of factors. Usually, conducting experiments on a real system is not feasible due to any of the following limitations: (1) experiments on a real system can be costly; (2) a considerable amount of time may take each experiment; and (3) a great variety of experiments is difficult to be carried out. Hence, a computer program, that generates responses for some specific factors, is an excellent alternative to mimic physical systems, and to develop experimental design.

In this article, a computer-based educational tool for simulating experiments is proposed, which takes into account factor limits selected by the user. Teachers may adopt this educational tool in order to generate numerical examples, and to highlight all characteristics of experimental design. Moreover, the proposed educational tool allows teachers to

evaluate the knowledge acquired by students during a course, and to implement problem-based learning. In this pedagogy, a student learns the topic while solving a problem given by the teacher. The core of the educational tool is a novel mathematical function that is designed to have a unique peak, thus providing a unique optimal solution of an experiment subjected to a number of finite factors. Moreover, this function has the quality that its gradient is not proportional to the distance from the optimal solution.

The paper has the following structure. Section 2 describes a multi-response model. The proposed mathematical function, employed by the computer-education tool, is presented in Section 3. The implementation of the proposed function is mentioned in Section 4. Section 5 describes the algorithm that generates an experiment of a multi-factorial process. Section 6 shows an application used to implement this algorithm. The paper ends with some concluding remarks.

2 | MULTI-RESPONSE MATHEMATICAL MODEL

A numerical example for an^{Q4} experimental design is generated through a multi-response mathematical model that represents the physical process. This model consist of a set of static functions that map the factors to the responses, and it is given^{Q5} by

$$y_j = f_j(x_1, x_2, x_3, \dots, x_n) + \xi_j \quad (1)$$

where y_j , $j \in \{1, 2, 3, \dots, m\}$ are responses, x_i , $i \in \{1, 2, 3, \dots, n\}$ are factors, f_j , $j \in \{1, 2, 3, \dots, m\}$ are nonlinear functions mapping the n factors to the m responses, and ξ_i , $i \in \{1, 2, 3, \dots, m\}$ are zero mean random noise. All factors, x_i , are constrained by upper and lower limits. Numerical examples should produce unique optimal responses, y_j^M , for the set of factors.

The algorithm developed in this paper uses a single response mathematical model, which is described by a proposed function presented in the next section. Several functions of this kind are used to mimic a multi-response system, since a set of m single response systems can represent a multi-response system.

3 | PROPOSED MATHEMATICAL FUNCTION

A static mathematical function with a single peak is achieved by any of the following functions:

$$C_1(x_1, x_2, x_3, \dots, x_n) := -\sum_{i=1}^n x_i^2 \quad (2)$$

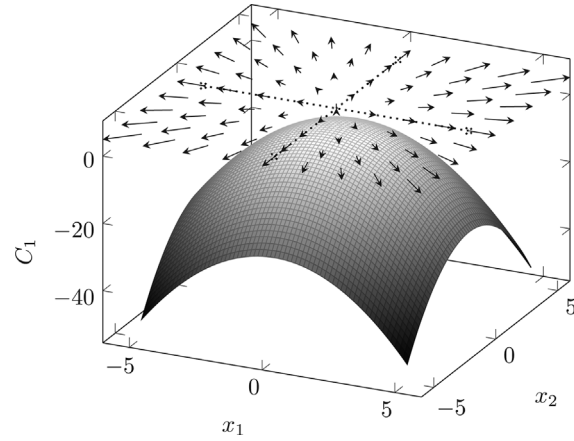


FIGURE 1 Two variable quadratic function $C_1(x_1, x_2)$

$$C_2(x_1, x_2, x_3, \dots, x_n) := \prod_{i=1}^n e^{-x_i^2} \quad (3)$$

$$C_3(x_1, x_2, x_3, \dots, x_n) := \sum_{i=1}^n e^{-x_i^2} \quad (4)$$

Functions C_1 and C_2 are a quadratic polynomial and a multivariable Gaussian function, respectively. Function C_3 is obtained by replacing the multiplication operator with a summation one. Figures 1–3 respectively represent functions C_1 , C_2 , and C_3 depending of two variables x_1 and x_2 . In addition, these figures include quiver plots, which represent the gradient's direction and amplitude. The dotted lines in these figures indicate $x_1 = 0$ and $x_2 = 0$.

Functions C_1 , C_2 , and C_3 have the following limitations:

1. Function C_1 has a property that its slope increases linearly as it moves far away from the optimal point, that is, its gradient at any point is directly proportional to the distance from the optimal point, which is not always true

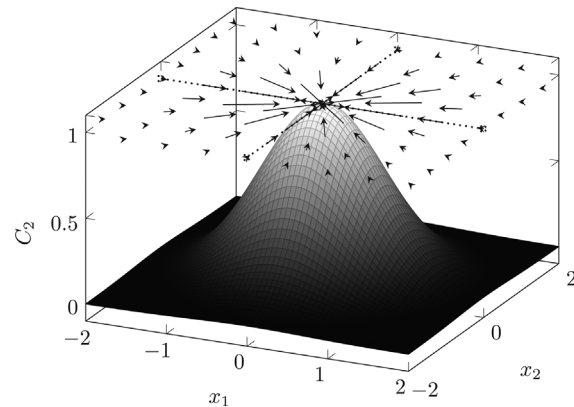


FIGURE 2 Two variable Gaussian function $C_2(x_1, x_2)$

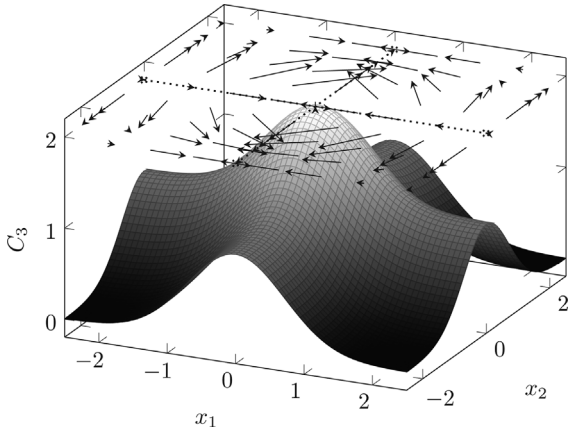


FIGURE 3 Modified version of Gaussian function $C_3(x_1, x_2)$

in physical systems. This fact is illustrated in Figure 1, where the lengths of the arrows of the quiver plot increase as a point moves far away from the intersection of the dotted lines.

2. Response surface methodology uses a second order fit algorithm. Hence, reaching an optimal solution for a system based on C_1 requires very less effort, which is not recommended as a practical problem.
3. The solutions of $\frac{\partial C_1}{\partial x_k} = 0$, $\frac{\partial C_2}{\partial x_k} = 0$, and $\frac{\partial C_3}{\partial x_k} = 0$ do not contain the terms of other factors $x_k \neq x_i$ where $i, k \in \{1, 2, 3, \dots, n\}$. Multi-factorial example problems based on these functions are easy to solve, because the optimization of a factor is independent of the optimization of the other factors. Figures 1–3 show this property, which means that some arrows of the quiver plots coincide with the dotted lines.

Multiplying function C_2 by C_3 and making a change of variable produce the following proposed

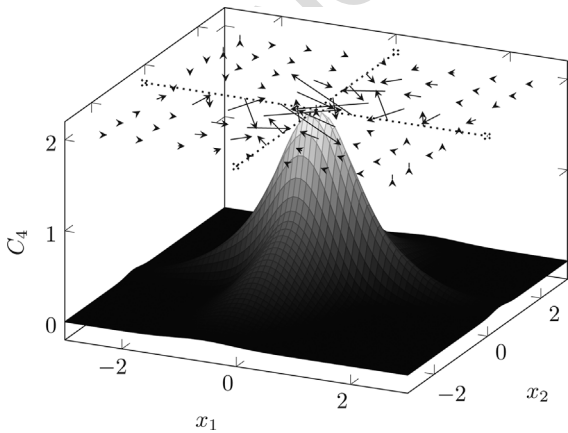


FIGURE 4 The proposed function $C_4(x_1, x_2)$ for $P = [0.448, 0.308; 0.308, 0.338]$ and $Q = [1.329, 0.493; 0.493, 2.761]$

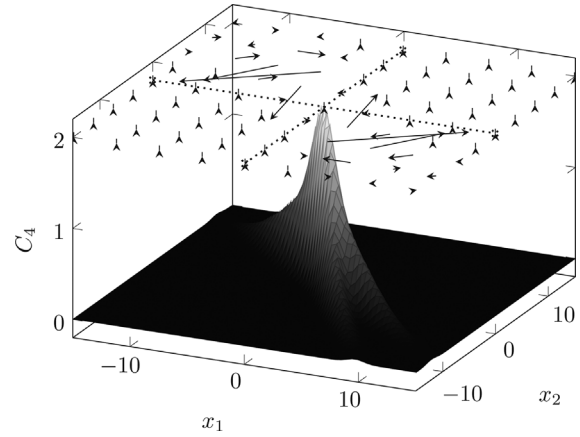


FIGURE 5 The proposed function $C_4(x_1, x_2)$ for $P = [0.1, 0; 0, 0.1]$ and $Q = [0.979, 0.636; 0.636, 0.773]$

mathematical function:

$$C_4(x_1, x_2, x_3, \dots, x_n) := \left[\prod_{i=1}^n e^{-u_i^2} \right] \left[\sum_{j=1}^n e^{-v_j^2} \right] \quad (5)$$

where $u_i := P_i x$, $v_i := Q_i x$, P_i , and Q_i are, respectively, the rows of some matrices P and Q such that $P, Q \in \mathbb{R}^{n \times n}$. Moreover, $i \in \{1, 2, 3, \dots, n\}$ and $x = [x_1 \ x_2 \ x_3 \ \dots \ x_n]^T$.

Figures 4 and 5 depict C_4 with $P = \begin{bmatrix} 0.448 & 0.308 \\ 0.308 & 0.338 \end{bmatrix}$, $Q = \begin{bmatrix} 1.329 & -0.493 \\ -0.493 & 2.761 \end{bmatrix}$ and $P = \begin{bmatrix} 0.1 & 0 \\ 0 & 0.1 \end{bmatrix}$, $Q = \begin{bmatrix} 0.979 & 0.636 \\ 0.636 & 0.773 \end{bmatrix}$, respectively. The transpose of the gradient of C_4 is given by

$$\Delta C_4^T = \left[\frac{\partial C_4}{\partial x} \right]^T = \left[-2 \prod_{i=1}^n e^{-u_i^2} \right] \left(Q^T T Q + \left[\sum_{j=1}^n e^{-v_j^2} \right] P^T P \right) x = -Mx \quad (6)$$

where $T = \begin{bmatrix} e^{-v_1^2} & 0 & \dots & 0 \\ 0 & e^{-v_2^2} & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \dots & e^{-v_n^2} \end{bmatrix}$ is a positive definite

matrix. If Q and P are non-singular, then matrices $Q^T T Q$ and $P^T P$ are positive definite, respectively [7]. Moreover, $Q^T T Q$ and $P^T P$ are positive semidefinite when Q and P are singular. Therefore, if either Q , P , or both are non-singular, then matrix M in Equation (6) is positive definite, and as a consequence a unique peak at $x_i = 0$, $i \in \{1, 2, 3, \dots, n\}$ is guaranteed.

Unlike function C_1 , the gradient of function C_4 in Equation (5) is not directly proportional to the distance from its optimum point. Moreover, in contrast to functions C_1 , C_2 , and C_3 , the solution $\frac{\partial C_4}{\partial x_k} = 0$ contains terms of other factors $x_k \neq x_i$, where $i, k \in \{1, 2, 3, \dots, n\}$. These two facts can be observed by comparing Figures 1–3 with Figures 4 and 5. Unlike Figures 1–3, the arrows of the quiver plots of Figures 4 and 5 do not coincide with the dotted lines.

In the next section, a method is presented to adapt the function C_4 , defined at Equation (5), in order to generate random experiments.

4 | IMPLEMENTATION OF THE PROPOSED FUNCTION

A scaled version of the proposed function C_4 in Equation (5) is given by

$$f(x) = F(r, s) := F_1 + \frac{F_2}{n} \left[\left[\prod_{i=1}^n e^{-r_i^2} \right] \left[\sum_{j=1}^n e^{-s_j^2} \right] + K_R \xi \right] \quad (7)$$

$$F_1 := F_L + \alpha F_I \Xi \quad (8)$$

$$F_2 := \frac{(1 - 2\alpha)F_I(1 + \Xi)}{2} \quad (9)$$

$$F_I := (F_U - F_L) \quad (10)$$

$$\Xi := 0.5(\xi + 1) \quad (11)$$

$$r := Pz \quad (12)$$

$$s := Qz \quad (13)$$

$$z_i := \frac{K_D(x_i - x_i^M)}{x_i^I} \quad (14)$$

$$x_i^I := x_i^U - x_i^L \quad (15)$$

$$x_i^M := x_i^L + \beta x_i^I + (1 - 2\beta)x_i^I \Xi \quad (16)$$

$$0 < \alpha < 0.5 \quad (17)$$

$$0 < \beta < 0.5 \quad (18)$$

where $i \in \{1, 2, 3, \dots, n\}$, $x, r, s \in \mathbb{R}^n$, ξ is a random variable such that $E(\xi) = 0$ and $|\xi| \leq 1$, with $E(\cdot)$ the expected value notation. Moreover, Ξ is a random variable with the properties $E(\Xi) = 0.5$ and $0 \leq \Xi \leq 1$. On the other hand $[F_L, F_U]$ is the function range, and K_R is a factor that introduces noise into

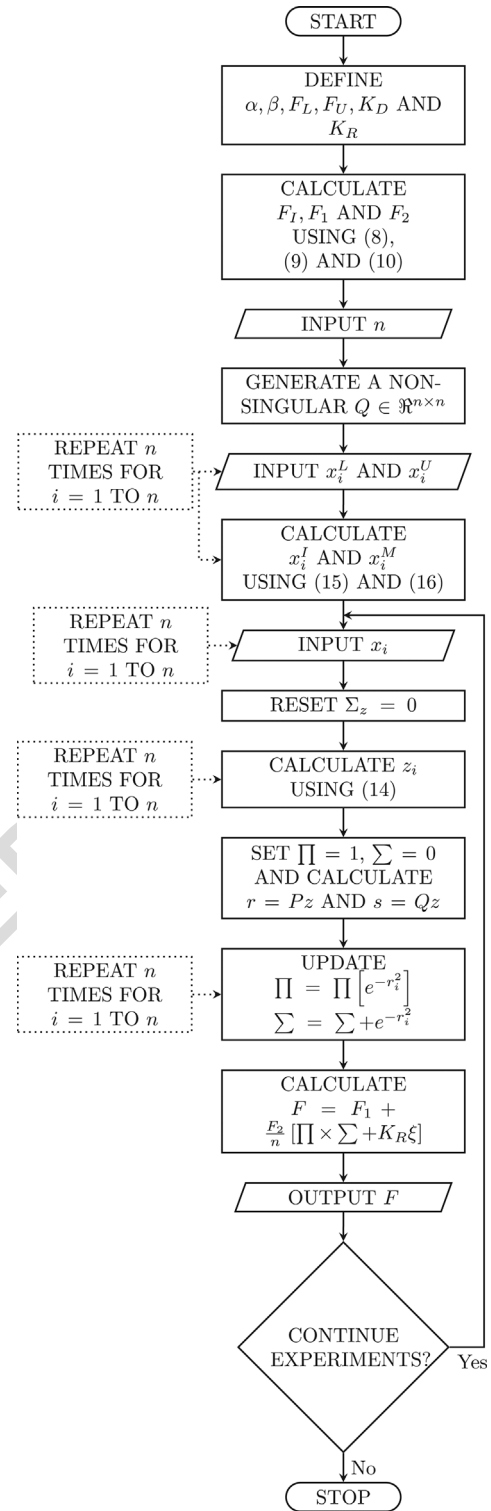


FIGURE 6 Flowchart of the proposed algorithm

the system. Parameter K_D is a difficulty factor; the higher its value, the harder it is to reach the optimum value. In addition, x_i^M are the optimal factors where function f reaches its maximum value, x_i^L and x_i^U are lower and upper limits of the i th factor respectively, α and β are padding constants that limit the maximum value of the function f within the desired region.

Multifactorial experiment simulator

✎ Select number of factors

2

Reset

✎ Select the upper and lower limits

Factor	Lower limit	Upper limit
pH	1	13
Temperature	0	100

Modify factors/limits

✎ Perform individual experiment/measure distance from optimum value (validate optimum value)

Factor	Value
pH	7.036116534
Temperature	39.66737

Calculate response Is optimum

Response= 257.4002153

✎ Perform bulk experiments

7.036116534	39.66737
10.03611653	14.66737
10.03611653	64.66737
7.036116534	39.66737

Save results to clipboard Save results to a file

FIGURE 7 Screenshot of the application

It is worth mentioning that function $F(r, s)$, shown in Equation (7), preserves all the mathematical properties of function C_4 . Moreover, multiplying function F_2 in Equation (9) by a negative number inverts the peak of function $F(r, s)$.

Next section presents an algorithm that implements the proposed procedure.

5 | ALGORITHM

The objective of the algorithm is to generate experimental results by simulating a multi-factorial process. Teacher gives the parameters required by the multi-factorial process such as upper and lower limits of the factors, and students perform experiments at different factors. The algorithm generates responses for the given x_i , $i \in \{1, 2, 3, \dots, n\}$ factors using the equations and inequalities shown in Equations (7) to (18).

Figure 6 shows the flowchart of the proposed algorithm. The teacher should define the values of $\alpha, \beta, F_L, F_U, K_D$ and K_R , where α and β should be less than 0.5. Based on our experience, It is recommended to use $\alpha = \beta = 0.2$, and $K_D = 5$. It is important to mention that this algorithm can be implemented in any programming language.

6 | APPLICATION

The triad of JavaScript (JS), Hypertext markup language (HTML) and Cascading Style Sheets (CSS) is used to create the application presented at the web page [4]. JS is used to implement the algorithm discussed in the previous section.

Master students of the Biological Sciences Faculty, Universidad Autónoma de Coahuila, are instructed to use this application. Figure 7 shows the screenshot of the application. It is employed by students in the classroom, at homework, and in projects related to the learning of Response

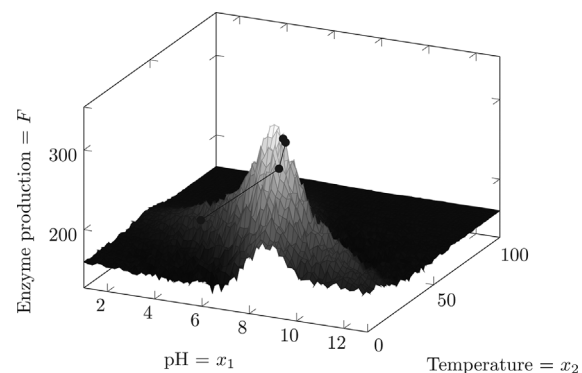


FIGURE 8 Surface plot of F obtained with the constants given in section 6. The RSM results are superimposed on this plot

Surface Methodology (RSM). Students are asked to perform experiments on this application by opening it in the web browser with the objective of maximizing the response of a biological process with n factors. The graphic user interface allows performing multiple experiments at once, where a student can paste factorial information and copy the model response to the clipboard. The factors computed by the students are compared with the optimal ones in order to evaluate the student's performance. It is worth mentioning that the optimal values are not shown in the graphic user interface. The application contains a distance tool, which measures the distance between the estimated values to the optimal ones, thus students can use this tool in order to verify if their computed values are correct or not.

As an example of an experiment, consider a biological process with two factors, which are pH and temperature that takes values between 1 to 13 and 0 to 100°C, respectively. Let x_1 and x_2 the factors pH and temperature, respectively, and let $f(x_1, x_2)$ the enzyme production that will be maximized. The lower and upper limits of these factors are $x_1^L = 1$, $x_1^U = 13$, $x_2^L = 0$, and $x_2^U = 100$ as shown in Figure 7. The scaled version of function C_4 , described in Equations (7) to (18), uses the parameters: $n = 2$, $\alpha = \beta = 0.2$, $F_L = 100$, $F_U = 600$, $K_D = 5$, $K_R = 0.1$, $x_1^L = 1$, $x_1^U = 13$, $x_2^L = 0$, and $x_2^U = 100$, $P = \begin{bmatrix} 0.5 & 0 \\ 0 & 0.5 \end{bmatrix}$, $Q = \begin{bmatrix} 2.128 & 0.675 \\ 0.675 & 1.452 \end{bmatrix}$, $F_1 = 160.498$, $F_2 = 167.908$, $x_1^I = 12$, $x_2^I = 100$, $x_1^M = 7.784$, and $x_2^M = 23.606$. Figure 8 shows the surface plot of $f(x_1, x_2)$. Moreover, Figures 9 and 10 show, respectively, the contour and surface plots of the function $f(x_1, x_2)$ when it is not corrupted by the noise term ξ , i.e., $K_R = 0$. In this experiment, the Response Surface Methodology estimates the optimal values of x_1 and x_2 , and the results of each iteration are superimposed over these plots.

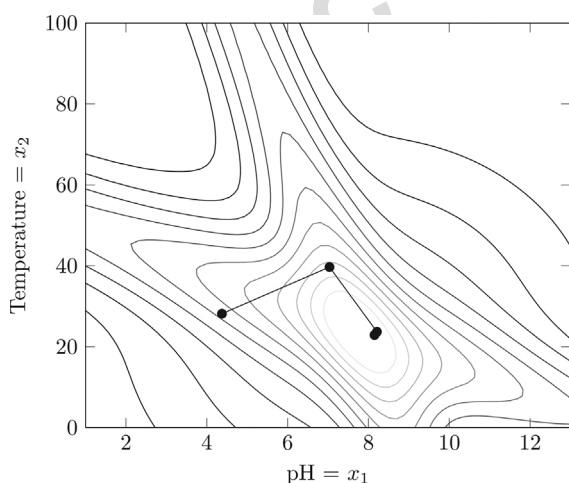


FIGURE 9 Contour plot of F obtained with $K_R = 0$ and the other constants given in section 6. The RMS results are superimposed on this plot

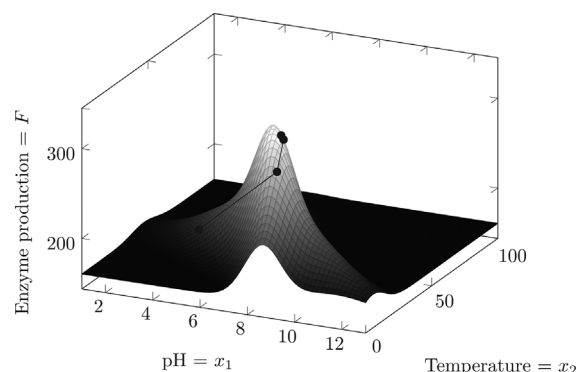


FIGURE 10 Surface plot of F obtained with $K_R = 0$ and the other constants given in section 6. The RMS results are superimposed in this plot

7 | CONCLUSIONS

This article presented a computed educational tool, designed in HTML, CSS, and JavaScript, in order to generate random processes with factors provided by a teacher. It is based on a novel single response, unique peak multivariable mathematical function, denoted as C_4 . This function mimics a multi-response physical process designed for experiments, it is adapted to generate experimental data for a selected range of factors, and its gradient is not proportional to the distance from the optimal point. Students that learn the topic of Response Surface Methodology use the proposed education tool in order to perform experiments with the objective of maximizing the response of a biological process with factors. This educational tool can be used for showing the performance of other optimization algorithms that can maximize the response of a process, such as the Taguchi methods.

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