Feedback control promotes synchronisation of the cell-cycle across a population of yeast cells

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Abstract—The periodic process of cell replication by division, known as cell-cycle, is a natural phenomenon occurring asynchronously in any cell population. Here, we consider the problem of synchronising cell-cycles across a population of yeast cells grown in a microfluidics device. Cells were engineered to reset their cell-cycle in response to low methionine levels. Automated syringes enable changing methionine levels (control input) in the microfluidics device. However, the control input resets only those cells that are in a specific phase of the cell-cycle (G1 phase), while the others continue to cycle unperturbed. We devised a simplified dynamical model of the cell-cycle, inferred its parameters from experimental data and then designed two control strategies: (i) an open-loop controller based on the application of periodic stimuli; (ii) a closed-loop model predictive controller (MPC) that selects the sequence of control stimuli which maximises a synchronisation index. Both the proposed control strategies were validated in-silico, together with experimental validation of the open-loop strategy.

I. INTRODUCTION

Recently, applications of Control Engineering to steer biological processes in living cells, also known as Cybergenetics, have been demonstrated [1]–[7]. These applications are based on innovative technological platforms exploiting microfluidics and optogenetics to implement real-time feedback control, where the controller is implemented as a software in the computer.

Here, we investigated the possibility to synchronise a population of budding yeast by forcing cells to divide in a coordinated fashion for multiple generations. During its lifespan, the eukaryotic cell undergoes a sequence of cyclically repeated steps that lead to its division via a process known as the cell-cycle. It can be divided into four main phases as shown in Figure 1: G1 (growth phase), S (DNA synthesis starts), G2 (growth phase) and M (mitosis) that includes the actual cell division. In budding yeast, the three endogenous genes encoding the G1 cyclins (CLN1, CLN2 and CLN3) are expressed in the late G1 phase and promote the transition from G1 to the S phase. Moreover, expression of just one of these three cyclins is sufficient for the G1 to S transition to occur.

Biologists have developed different methods to force a cell population to divide synchronously. However, all of these methods do not dynamically synchronise the cell population, but essentially they just force each cell in the population to start from the same initial condition. Therefore, after a few generations, the population de-synchronises because of environmental and cell specific disturbances.

To address the cell-cycle synchronisation problem from a Control Engineering point of view, we chose a budding yeast strain where the endogenous CLN3 gene was deleted while the methionine-repressible promoter P Met3 was inserted upstream of the endogenous CLN2 gene [8]. The resulting strain will thus not express CLN2 if grown in a methionine-rich medium, but it will be able to cycle nevertheless, as the endogenous CLN1 is still present in this strain. However, by switching growth medium from methionine-rich to methionine-depleted medium, the cell can be forced to transition from the G1 phase to the S phase thanks to the resulting expression of CLN2, as represented in Figure 1. Hence the growth medium can be used as a control input.

To track the cell-cycle progression in time, a yellow fluo-
rescent protein (YFP) was fused to the endogenous CDC10 gene, which encodes a protein expressed in the M phase during cell division at the edge between the mother and daughter cell (bud neck). The intensity of the YFP represents the mass generated during the S-G2-M phases, most of which is transferred to the growing bud (daughter cell) [8]:

\[
\dot{V} = \begin{cases} 
\beta V & \text{if } \vartheta \in [0, \vartheta_c] \\
0 & \text{if } \vartheta \in [\vartheta_c, 2\pi] 
\end{cases},
\]

(4)

where \( \beta > 0 \) is the volume growth rate. Finally, we assume that at \( \vartheta = 2\pi \) cell division occurs and the bud detaches from the mother (M) cell, and thus forming a new daughter (D) cell. Let \( \vartheta_M \) be the mother cell phase, \( V_M \) its volume; \( \vartheta_D \) the daughter cell phase and \( V_D \) its volume. At each division, the birth of a daughter cell is modelled according to the following rule:

\[
\begin{align*}
\vartheta_M &\to 0, \\
\vartheta_D &\to 0, \\
V_D &\to V_M[\exp(\frac{2\pi \vartheta_c}{\vartheta_M}) \beta T - 1]
\end{align*}
\]

(5)

where at division, \( V_D \) is equal to the volume growth occurred during the S-G2-M phases at bud level.

Let \( u \in \{0, 1\} \) be the external trigger input to the system with \( u = 1 \) corresponding to methionine-depleted medium and \( u = 0 \) to methionine-rich medium. The effect of the input can be modelled with the following reset rule:

\[
\text{if } \vartheta \in [0, \vartheta_c] \land V \geq V_c \land u = 1 \implies \vartheta \to \vartheta_c .
\]

(6)

According to this rule, the control input \( u \) will force the G1 to S transition only in those cells that are in the G1 phase and have a volume \( V > V_c \), while the other cells will continue to cycle unperturbed. This constraint is a major limitation to the performance of any controller.

The numerical values of the parameters of this cell-cycle model were inferred from experimental data presented in Charvin et al. [8]. Thus, the cell-cycle period has been set to \( T = \frac{1}{71} \) min, the volume growth rate to \( \beta = 0.0083 \) [a.u.] min\(^{-1}\), and the critical volume to \( V_c = 1 \) [a.u.]. Moreover, the cell-cycle phase at budding has been set to \( \vartheta_c = \frac{\pi}{2} \).

### III. Problem statement

Let \( N_t \equiv \{1, \ldots, N(t)\} \) be the finite set of all cells in the population at time \( t \), where \( N(t) = |N_t| \) is the number of cells in the population at time \( t \). Note that the number of cells may vary in time as a consequence of cell births and deaths. However, here we consider only cell birth events, therefore \( N(t) : t \to \mathbb{N} \) will be at most a nondecreasing function.

Consider each cell \( i \in N_t \) as an individual agent whose cell-cycle progression is mathematically described by the model composed by (3) and (4), together with the reset rule (6) and the division rule (5).

The control objective is to synchronise the cell-cycle across the cell population. Synchronisation in a population of oscillators can be quantified by means of the Kuramoto order parameter \( R \) [12] defined as the magnitude of the complex number:

\[
Z(t) := \frac{1}{N(t)} \sum_{i=1}^{N(t)} e^{i\vartheta_i(t)} = R(t) e^{i\Theta(t)},
\]

(7)
where \( \vartheta_i \) is the phase of cell \( i \in \mathcal{N}_f \). \( R \in [0, 1] \) represents the mean phase coherence, an index to evaluate the synchronisation among a population of oscillators. When \( R \) is equal to 1, all cells are synchronised onto the same phase.

To evaluate the performance of the control algorithms in synchronising the cell population, we introduce the cost function:

\[
J = \frac{1}{t_f} \int_0^{t_f} R^2(\tau) d\tau.
\]

(8)

Thus, when \( J \) equals one then all cells are synchronised over the whole time interval.

As newborn cells may have an initial volume smaller than \( V_c \), it follows from (3) that their phases will remain equal to 0 for a certain time interval. Moreover, at this stage, these cells will not respond to the control input, hence taking into account their phases in (7) may result in misleading values of the control performance. To overcome this problem, we redefine \( Z(t) \) and \( J(t) \) by omitting non-cycling cells in their evaluation. Let \( \mathcal{K}_t = \{ i \in \mathcal{N}_t : V_i(t) \geq V_c \} \) be the subset of cells whose volume is greater than the critical one at time \( t \). Then, the Kuramoto order parameter associated to the cycling cells is defined as:

\[
Z_{\text{cyc}}(t) := \frac{1}{K(t)} \sum_{i \in \mathcal{K}_t} e^{i \vartheta_i(t)} = R_{\text{cyc}}(t) e^{i \Theta_{\text{cyc}}(t)},
\]

(9)

where \( K(t) \) is the cardinality of the set \( \mathcal{K}_t \) at time \( t \). \( R_{\text{cyc}} \in [0, 1] \) is the mean phase coherence of the cycling cells. In the same way, we introduce the cost function associated to the synchronisation of the cycling cells, defined as:

\[
J_{\text{cyc}} = \frac{1}{t_f} \int_0^{t_f} R_{\text{cyc}}^2(\tau) d\tau.
\]

(10)

We will test control strategies in two different scenarios:

1. **constant population** where all cells are mother cells (i.e. \( V_i(0) \geq V_c \)) and daughter cells are not considered so that the number of the cells is constant for all \( t \). Obviously, in this case, since every cell is a cycling mother we have that \( \mathcal{K}_t \equiv \mathcal{N}_t \) for all \( t \) and therefore \( J_{\text{cyc}} = J \);

2. **time-varying population** in which we also consider birth events of daughter cells. Thus two control problems can be formulated:

**Problem 1:** Given a constant population of \( N \) cells, whose dynamics is described by (3)-(4) with the phase reset law (6), compute the control input \( u \) that maximises \( J_{\text{cyc}} \).

**Problem 2:** Given a time-varying population, with an initial number of \( N(0) = N_0 \) cells, whose dynamics is described by equations (3)-(5) with the phase reset law (6), compute the control input \( u \) that maximises \( J_{\text{cyc}} \).

A. Numerical simulations

All in-silico simulations were performed using the MATLAB ode45 solver with event detection routines to accurately detect cell division at \( \vartheta_i = 2\pi \) and the critical volume \( V_l = V_c \) for cells with initial volume \( V_l < V_c \).

For the constant population (Problem 1), we considered \( N = 100 \) mother cells, with an initial volume \( V_i(0) \) for all the cells equals to the critical value \( V_c \). Instead, the initial phases \( \vartheta_i(0) \) of individual cells were uniformly spaced in the interval \([0, 2\pi]\).

For the time-varying population (Problem 2), we considered an initial number of \( N_0 = 1 \) cell having initial volume \( V_l(0) = V_c \) and phase \( \vartheta(0) = \vartheta_c \).

All simulations were run with the numerical parameters reported in Section II, for a time interval lasting \( t_f = 15T \).

Since we considered the same numerical parameters for each cell agent, the cell-to-cell variability across the cell population inherently arises from the cell division events.

IV. OPEN-LOOP CONTROL

We designed a simple open-loop control strategy which consists in forcing the cells with a periodic control input of constant period \( \tau \). This strategy relies on the phase-locking phenomenon observed to occur in yeast when forced with a periodic external stimulus [8]. Numerical simulations for the control problems 1 and 2 (refer to Section III-A) were performed for different values of forcing period \( \tau \) from 1 min to 142 min (that is two times the nominal period \( T \)) with increments of 1 min. As an example, we report in Figure 2 the response of the time-varying cell population, with initial number \( N_0 = 1 \), to a control input of period \( \tau = 64 \) min. In this case, the population exhibits an intermediate level of synchronisation as indicated by the evolution of the performance index \( R_{\text{cyc}}(t) \). In Figure 5, we report the value of the cost indices \( J \) and \( J_{\text{cyc}} \) as a function of the forcing period \( \tau \) for both control problems.

It is apparent that the open-loop control strategy has a satisfactory performance for the constant population but clearly fails in the case of the time-varying population, as shown in Figure 3(b).

V. FEEDBACK CONTROL

Although being very simple, the previous open-loop control strategy requires precise tuning of the control parameter \( \tau \) and has the drawback of not being robust to uncertainties and variation of the parameters of the cell-cycle model. To overcome these limitations, we devised a model predictive control algorithm, that is a feedback control strategy that can guarantee better performances in synchronising cell-cycle phases [9].

A. Model Predictive Control

The MPC algorithm consists in solving an open-loop optimal control problem repeatedly over a receding horizon [13]. This means that at each iteration of the algorithm, the solution to the optimal open-loop control problem gives an optimal control input that minimises (or eventually maximises) a cost function over a finite prediction horizon \( T_p \).

The optimal control input is applied only over a finite control horizon \( T_c \leq T_p \). Then, the optimisation is repeated again.

To address the problem of synchronising the cell-cycle phases, we chose as cost function the performance index \( J_{\text{cyc}} \), by setting \( t_f = T_p \) in equation (10). Since cell-cycle phases are synchronised when \( J_{\text{cyc}} \) is equal to one,
in this case we have to solve a maximisation optimal control problem.

To reduce the computational complexity of the optimal control problem, the control input \( u \in U \) is assumed to be a finite sequence of triggers. Defining \( P \in \mathbb{N} \) as the maximum number of triggers that may be applied in a finite prediction horizon \((t, t + T_p]\), then the time interval occurring between two consecutive triggers is \( \frac{T_p}{P} \). The feasible set \( U \) is defined as the set of all admissible control sequences. Considering only the finite prediction horizon \( T_p \), the set \( U \) is composed by \( 2^P \) possible combinations of triggers. Thus, the optimal control problem is solved by maximising the performance index \( J_{\text{cyc}} \) over the prediction horizon \( T_p \). The optimisation is achieved by exploring all the possible combinations of sequence of triggers.

For the numerical analysis, we set the length of both the prediction horizon \( T_p \) and the control horizon \( T_c \) equal to the nominal cell-cycle duration \( T_c \), and the number of triggers \( P \) spanning in the range \([1, 6]\). Figure 4 shows the response of the time-varying cell population, with an initial cell number \( N_0 = 1 \), to the control input generated by the MPC algorithm with control parameter \( P = 5 \).

In Figure 5 we report the value of the cost functions \( J \) and \( J_{\text{cyc}} \) for the parameter \( P \) ranging from 1 to 6. The best performance are obtained for \( P \) equal to 5 and 6 in both fixed and time-varying population scenarios.

With respect to the synchronisation indices obtained with the open-loop control reported in Figure 3, the MPC has clearly better performances in both constant population scenario and especially in the time-varying population scenarios. Moreover, the MPC is obviously more robust to uncertainties in the model parameters since it does not require any tuning of parameters as in the open-loop controller. However, the performance of the MPC is strongly dependent on the particular choice of admissible control sequences \( U \) that have to be carefully selected.

VI. PRELIMINARY IN VIVO VALIDATION

To investigate the cell-cycle synchronisation problem experimentally, we performed a preliminary validation of the open-loop control strategy described in Section IV. To this end, we took advantage of a microfluidics-based experimental platform that was already used to control gene expression levels on living yeast cells [7], [10], [14]. We performed two experiments: (i) a calibration experiment where yeast cells were grown in methionine-rich growth medium (control input \( u = 0 \)); (ii) an open-loop control experiment in which the growth medium is periodically switched between methionine-rich and methionine-poor with a period \( \tau = 60 \text{ min} \) (control input), i.e. close to the optimal 54 min period.
found with the numerical simulations (Figure 3). In this latter experiment, differently from the numerical simulations where the control input consisted of instantaneous pulses, the control input \( u(t) \) is a square wave with a period \( \tau = 60 \text{ min} \) and a duty cycle \( D = 33\% \), that is methionine-poor medium was applied to the cell population for a duration of 20 min, that is one-third of the forcing period \( \tau \) used in this experiment. This is necessary, as a shorter stimulus will not be sensed by the cell [8]. Both experiments lasted 14 hours, that are almost 12 times the nominal period of the cell-cycle of our yeast strain. The results of these two experiments are reported in Figure 6, where the output of each cell, i.e. the yellow fluorescence signal, is shown. Cells in the unforced experiment (Figure 6(a)) appear to be poorly synchronised when compared to those subjected to periodic stimuli (Figure 6(b)). Indeed, in the open-loop control experiment cells appear to be phase-locked with the external periodic stimulus (ratio 2:1). Nevertheless, not all of the cells appear to respond to every external stimuli. Further experiments, including closed-loop feedback control will need to be performed to validate the numerical simulations.

**VII. Conclusions**

In this work, we have addressed the cell-cycle synchronisation problem in budding yeast considering a real biological scenario. We considered a budding yeast strain whose cell-cycle can be reset from the G1 phase to the S phase in the presence of a proper external stimulus, that is the absence of methionine in the growth medium supplied to the cells. We characterised the biological system deriving a phase-oscillator model to describe the cell-cycle phase and volume growth dynamics. Building on the experimental work of Charvin et al., we devised an open-loop control strategy that relies on the concept of phase-locking and, supported by our preliminary experiments, demonstrated its efficacy in synchronising the cell-cycle across a population of cells. However, open-loop control strategy faces several hurdles in a practical setting and relies on the proper choice of the forcing period. To overcome such limitations, we devised a feedback control strategy based on Model Predictive Control, which has been already successfully applied to biological control problems. Through numerical analysis, we demonstrated the feasibility of using MPC to synchronise the cell-cycle phases, obtaining better performance when compared to the open-loop strategy. Nevertheless, several issues remain open both in terms of control performance and experimental implementation. Indeed, model uncertainty and parametric variability could affect the control performance, thus adaptive and robust control strategies may be required.

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**Fig. 4.** Example of a feedback control simulation using MPC. Upper panel, time evolution of \( \theta_c(t) \), as well as their average value over the whole population, are reported respectively in orange and purple colours. Middle panel, time evolution of \( R(t) \) and \( R_{\text{cyc}}(t) \) are reported respectively in yellow and green colours. Bottom panel, the corresponding control signal \( u(t) \), generated by the MPC with control parameter \( P = 5 \), is reported in red colour.

**Fig. 5.** Model Predictive Control: synchronisation index as a function of the maximum number of triggers \( P \) that may be applied in a finite prediction horizon \( T_p \). \( J \) is the synchronisation index associated to the whole population, whereas \( J_{\text{cyc}} \) is the synchronisation index computed considering only the cycling cells.
Moreover, estimation of the cell-cycle phase from the YFP fluorescence signal is non-trivial and requires improvement in image segmentation algorithms.

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