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In-vivo real-time control of gene expression: a comparative analysis of feedback control strategies in yeast

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Abstract

Real-time automatic regulation of gene expression is a key technology for synthetic biology enabling, for example, synthetic circuit's components to operate in an optimal range. Computer-guided control of gene expression from a variety of inducible promoters has been only recently successfully demonstrated. Here we compared, *in-silico* and *in-vivo*, three different control algorithms: the Proportional-Integral (PI) and Model Predictive Control (MPC) controllers, which have already been used to control gene expression, and the Zero Average Dynamics (ZAD), a control technique used to regulate

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10 electrical power systems. We chose as an experimental test-bed the most commonly
11 used inducible promoter in yeast: the Galactose-responsive *GAL1* promoter. We set
12 two control tasks: either force cells to express a desired constant fluorescence level of
13 a reporter protein downstream of the *GAL1* promoter (set-point), or a time-varying
14 fluorescence (tracking). Using a microfluidics-based experimental platform, in which
15 either glucose or galactose can be provided to the cells, we demonstrated that both
16 the MPC and ZAD control strategies can successfully regulate gene expression from
17 the *GAL1* promoter in living cells for thousands of minutes. The MPC controller can
18 track fast reference signals better than ZAD, but with an higher actuation effort due
19 to the large number of input switches it requires. Conversely the PI controller's perfor-
20 mance is comparable to that achieved by the MPC and the ZAD controllers only for
21 the set-point regulation.

22 Keywords

23 synthetic biology, control engineering, microfluidics, gene expression, yeast

24

25 Control Engineering aims at driving a physical system in order to attain a specific value of
26 a quantity of interest (such as a boiler that needs to warm water to a desired temperature,
27 or a car cruise-control maintaining a constant speed) despite the presence of disturbances.
28 This is achieved by appropriately varying its inputs (switch on or off a heater in the case
29 of the boiler, or accelerating or braking in the cruise-control) as a function of the difference
30 between the measured value of the output and its desired target value (control error). At
31 the core of most control schemes lies a *negative feedback* loop (1), as shown in Figure 1 -
32 A. The variable to be controlled (system output y) is measured and its value is subtracted
33 from the desired value (control reference r). The quantity that is obtained, the control error

34 e , is minimised by the controller, a set of logical and mathematical rules through which an
35 appropriate value of the input u is chosen in order to guarantee that the output y matches
36 the desired reference r .

37 Feedback control has been extensively applied to control growing conditions of cells in
38 chemostats in terms of temperature and/or CO₂ and it is a current feature of bench-top and
39 industrial chemostats (2, 3). Only recently, however, the application of Control Engineering
40 principles has been exploited to regulate molecular events in living cells, thanks to innovative
41 microfluidics and optogenetics platforms (4–8).

42 In (4, 5) we built a completely automated microfluidic platform to control in real-time
43 gene expression in yeast cells. We demonstrated the ability of the platform to reach and
44 maintain a desired value of gene expression, measured in terms of the fluorescence intensity
45 of a reporter protein expressed from the endogenous *GAL1* promoter.

46 Other successful attempts to control gene expression, or even signaling pathways, have
47 been described in the literature. They mainly differ in the control input (osmotic pressure,
48 light, small-molecules) and the control strategy adopted. Optogenetics-based light inducible
49 systems have been exploited to control gene expression in yeasts (8, 9), to regulate intracel-
50 lular signalling dynamics in mammalian cells (7), and to drive protein levels by using light-
51 switchable two-component systems in bacteria (10). Microfluidic-based devices, allowing a
52 tight control of cellular growing medium and the administration of inducer small-molecules,
53 have been successfully employed to investigate synchronisation properties of synthetic bio-
54 logical clocks in bacterial cells (11), to control the transcription from the *HOG1* promoter
55 in yeast *S. Cerevisiae* by varying the osmotic pressure(6) and, in our own work, to control
56 transcription from the *GAL1* promoter using Galactose and Glucose as input.

57 The different control strategies proposed in the literature have never been compared
58 in the same experimental model, thus making a direct comparison of their performance
59 impossible. This is extremely important for practical applications where knowing advantages
60 and limitations of each strategy can be useful, if not necessary, to select the most appropriate

61 and effective one. Here, we compared *in-silico* and *in-vivo* the performance of different
62 control algorithms when applied to the problem of controlling gene expression from the *GAL1*
63 inducible promoter. In addition to control strategies that have already been described in the
64 literature, namely the Proportional-Integral (PI) control and the Model Predictive Control
65 (MPC), we also tested a different control strategy named Zero Average Dynamics (ZAD), an
66 approach inspired by sliding control techniques (12) used to control power electronic systems,
67 but that has never been applied to biological processes. Finally, practical considerations of
68 the pros and cons of each control strategy are provided.

69 Results and Discussion

70 An experimental testbed for the assessment of control strategies.

71 The *GAL1* promoter is the most widely used inducible promoter in yeast genetics. Thousands
72 of strains, each expressing a different yeast gene, are available to the research community,
73 making this an attractive choice for practical applications of control engineering. The activity
74 of the *GAL1* promoter is governed by the presence of Galactose in the cells' growing medium.
75 This sugar is interpreted as a "switch on" signal for the expression of the *GAL1* gene; when
76 yeasts are fed with Glucose, the production of Gal1 protein is repressed (13). Yeast cells will
77 first consume all the available Glucose in the medium before starting metabolising Galactose.
78 Hence, the control input can either be Glucose (switch off signal) or Galactose (switch on
79 signal), but not an intermediate concentration of the two, because cells will not respond to
80 Galactose when Glucose is present.

81 We thus decided to use the *GAL1* promoter upstream of a reporter gene (Gfp fused with
82 the Gal1 protein) as a testbed for comparing and assessing the performance of the different
83 control strategies. When dealing with living cells, one of the major issues is represented by the
84 uncertainty affecting transcriptional and translational processes, introducing a remarkable
85 cell-to-cell variability in mRNA and protein production (14). Rather than trying to control

86 stochastic behaviour of cells, here we addressed the simpler problem of regulating the average
87 fluorescence intensity expressed by all cells as the quantity to be controlled (y), thus averaging
88 out the effects due to intrinsic and extrinsic sources of noise (15).

89 To carry out *in-vivo* control experiments, we used the same integrated experimental
90 set-up presented in previous works (5, 16), comprising a microfluidic device, a time-lapse
91 microscope, and a set of automated syringes, all controlled by a computer. As depicted in
92 Figure 1 - B, the computer runs the control algorithm, which at each sampling interval: (i)
93 processes images acquired by the microscope to estimate the fluorescence y ; (ii) executes
94 the control algorithm to derive the input u for the next sampling period; (iii) controls
95 the automated syringes to provide the calculated input (i.e. Galactose or Glucose) to the
96 cells. We already demonstrated that the average fluorescence level of a yeast population
97 can be effectively regulated with this platform using a simple Proportional-Integral control
98 strategy(5).

99 **Controlling gene expression from the *GAL1* promoter: set-point and** 100 **tracking control tasks.**

101 We compared the performance of three control algorithms (PI, MPC and ZAD) when per-
102 forming two different tasks, as shown Figure 2: (i) *set-point control*, where the average Gfp
103 fluorescence must reach and maintain a desired reference level, and (ii) *signal tracking control*
104 where the average fluorescence must follow (or track) a desired time-varying signal. Specif-
105 ically, in the **set-point control** (Figure 2 - A), the desired fluorescence r was set equal to
106 50% of the initial average fluorescence expressed by the cells during the calibration phase of
107 180 min. During the calibration phase, cells are kept in Galactose, in order to let cells adapt
108 to the microfluidic environment, and to set the unit of measure of fluorescence, which may
109 vary due to technical and biological variability in each experiment. In the **signal tracking**
110 **control**, we used three different references r : (i) a descending staircase function (Figure 2
111 - B) where each step lasts 500 min, beginning at 75% of the calibration phase average flu-

112 orescence, then stepping down to 50% and then 25%, (ii) a linear descending ramp of 1500
113 min (Figure 2 - C) starting at the 100% of average fluorescence measured in the calibration
114 phase, and decreasing down to 25%, and (iii) a sinusoidal wave of period $T = 2000$ min
115 (Figure 2 - D) defined as $s(t) = 0.5 + 0.25 \sin\left(\frac{2\pi}{T}(t - 100) + \frac{\pi}{2}\right)$.

116 Control algorithms.

117 PI and MPC have been previously applied to control gene expression and protein activation.
118 Toettcher and colleagues applied a Proportional-Integral (PI) control to regulate protein
119 signaling in mammalian cells using light as control input in an optogenetics framework(7); we
120 have applied the same PI control scheme to regulate gene expression from the *GAL1* promoter
121 in yeast using galactose and glucose as control input(5); Miliadis et al (8) implemented MPC
122 to control expression from the *GAL1* promoter in yeast using light as a control input to
123 activate transcription. The same MPC strategy was also applied by Uhlenendorf et al (6) to
124 control expression from the *HOG1* promoter in yeast using osmotic pressure as the control
125 input.

126 We therefore compared the performance of PI, MPC and a new ZAD controller when
127 applied to the regulation of gene expression from the *GAL1* promoter in yeast cells.

128 We identified two major constraints affecting the control algorithms: the sampling-time
129 and the admissible values of the control input. We set the sampling time $T = 5$ min, this is
130 the time interval at which images are acquired from the microscope and it is an ideal trade
131 off to avoid photo-toxicity and capture the dynamics of the Gfp protein expression (17). The
132 control input u can assume only two values (Galactose-ON, Glucose-OFF). Thus, at each
133 sampling time kT , the control algorithms can only choose the duration of Galactose pulse
134 (ON), which can vary from 0 min to 5 min, and it is defined as the duty-cycle $d = \frac{t_{ON}}{T}$, i.e.
135 the percentage of the time interval during which Galactose is provided to the cells.

136 The Proportional-Integral (PI) control algorithm uses the *control error* $e(t) = r(t) - y(t)$
137 to choose, at each sampling time (kT), the duty-cycle value (d_k). Specifically d_k has a value

138 proportional to the weighted sum of two contributions, one proportional to the actual error
139 $e(t)$ and the other proportional to the sum of the past values of the error (the integral term).
140 The proportionality constants K_p and K_i are called respectively proportional and integral
141 gains, and their values were chosen by simple empirical rules (Methods and Supporting
142 Informations) (1).

143 The Model Predictive Control (MPC) algorithm is an optimisation-based technique which
144 uses a mathematical model of the process being controlled to predict the future values of the
145 control error and to find the best value of the duty-cycle value d_k that minimises it (Methods
146 and Supporting Informations) (18).

147 The Zero Average Dynamics (ZAD) algorithm relies on a feedback strategy devised for the
148 regulation of power converters (19, 20), it is a modified version of Sliding Mode Control (12).
149 Specifically, the ZAD calculates, at each sampling time, the best value of d_k which minimises
150 the actual control error $e(t)$ and its predicted future value (estimated by the derivative $\dot{e}(t)$)
151 over the next time interval (refer to Methods section and Supporting Informations for further
152 details).

153 **Set-point control experiments**

154 We first tested *in-silico* the PI, MPC and ZAD control strategies described above, by sim-
155 ulating the behaviour of yeast cells in a computer (Methods and Supporting Informations).
156 *In-silico* results are shown in Figure 3. PI (Figure 3 A), MPC (Figure 3 B) and ZAD (Fig-
157 ure 3 C) are able to reach and maintain the desired fluorescence value without exhibiting
158 oscillations at steady-state. Performance indexes (ISE, IAE, ITAE in Figure 3 D) are of
159 the same order of magnitude for all the control strategies; interestingly the ZAD controller
160 is able to achieve satisfying results with a reduced number of input switches (five and six
161 fold less than respectively MPC and PI). This is advantageous in the experimental setting
162 because it reduces unnecessary stress to cells.

163 *In-vivo* control experiments, shown in Figure 4, mirror *in-silico* results, showing that the
164 three strategies are indeed all able to reach and maintain the desired fluorescence level. As
165 predicted by the *in-silico* simulations, the ZAD controller employs fewer Galactose pulses
166 (Figure 4 C) and displays smaller oscillations around the set-point than the MPC feedback
167 strategy (Figure 4 B).

168 **Signal tracking control experiments**

169 *In-silico* simulation of the descending staircase reference shows that the three control strate-
170 gies have different performances. The PI is not able to properly follow the reference signal
171 (Figure 5 A). This is to be expected, since the PI controller was designed specifically to solve
172 set-point control tasks (1). The MPC control algorithm, with its intrinsic predictive abil-
173 ity, achieves a good performance, specifically noticeable in the proximity of the steps' edges
174 (Figure 5 B). Indeed, the MPC is able to foresee changes in the reference signal and to adjust
175 the control input accordingly, by starting to "switch off" the system in advance. The ZAD
176 control algorithm (Figure 5 C) achieves satisfying results, comparable to the MPC controller
177 (except in the proximity of the steps' edges), but with a smaller number of Galactose pulses.

178 *In-vivo* experiments for the descending staircase reference (Figure 6) confirm *in-silico*
179 results. The PI controller (Figure 6 A) poorly tracks the reference r , despite the high
180 number of input switches. The MPC, as already demonstrated *in-silico*, has a much better
181 performance, quantitatively confirmed by the performance indexes (Figure 6 B and D). As
182 in the case of the *in-silico* simulations, the ZAD controller (Figure 6 B and D) achieves a
183 performance comparable to that of the MPC (even if not as good in the proximity of the
184 steps' edges) employing fewer Galactose pulses than the MPC.

185 Because of the poor tracking results achieved by the PI controller, we further compared
186 only the MPC and ZAD controllers when tracking the ramp and the sinusoidal signal. Both
187 *in-silico* (Figure 7) and *in-vivo* (Figure 8) observations confirm that the ZAD controller is
188 able to guarantee a performance (Figure 7 E and Figure 8 E) similar to that of the MPC

189 strategy, but again with a reduced number of control input switches.

190 **Conclusions**

191 Precise and quantitative regulation of gene expression from inducible promoters is a key tech-
192 nology for current and future Synthetic Biology applications. It can be used to quantitatively
193 characterise biological "parts" in a single experiment by generating a desired time-varying
194 concentration of an effector protein and measuring the activity of the target. For example,
195 the level of a Transcription Factor can be controlled to follow a descending staircase ref-
196 erence while, at the same time, measuring the level of a report protein downstream of the
197 promoter to be characterised, in order to derive a quantitative dose-response curve to be
198 used for modelling. A second application of automatic control of gene expression is to ensure
199 that a synthetic circuit works in the optimal operating conditions in terms of expression of
200 its constituent proteins, similarly to what happens in a modern computer where the oper-
201 ating temperature is automatically controlled by switching on/off a fan in order to keep the
202 electronic circuits from overheating.

203 Here we provided a comparative analysis, *in-silico* and *in-vivo*, of three different strate-
204 gies to control gene expression from the *GAL1* inducible promoter, whose advantages and
205 disadvantages are summarised in Table 1.

206 To this end we implemented and compared PI and MPC controllers, which have been
207 previously reported in the literature (4–8) and proposed an additional strategy, the ZAD
208 controller (19).

209 We demonstrated that both MPC and ZAD control strategies can be successfully em-
210 ployed to control gene expression from the *GAL1* promoter to generate any desired time-
211 varying concentration of the reporter protein. These controllers require a quantitative model
212 of the system to be controlled. This is not a strong limitation, since it is possible to identify
213 a dynamical input-output model of the biological system under investigation using standard
214 system identification techniques, which work very well at least for simple inducible promoters

215 (16).

216 The PI controller, as expected from control theory (1) and from our *in-silico* predictions,
217 performs similarly to the MPC and ZAD strategies only in the set-point control task, whereas
218 it is the worst performer in the case of signal-tracking experiments.

219 The MPC and ZAD controller perform similarly well in all the control tasks. The main
220 differences are that the MPC performs slightly better than ZAD for fast switching reference
221 signals (such as the staircase signal in Figure 6), however it requires a higher number of input
222 switches when compared to the ZAD controller. The ZAD technique may be advantageous
223 in those applications in which a high cost is associated to the actuation such as when the
224 input administration can cause stress to the cells (e.g light stimuli, antibiotic, osmotic shocks
225 etc.).

226 In conclusion, automatic control of gene expression from inducible promoters is mature
227 enough to be applied routinely in synthetic biology and more generally in quantitative biology
228 applications. Although we showed the experimental application of these control strategies to
229 the *GAL1* promoter, the same techniques can be applied to other inducible promoters and
230 to different cellular models.

231 The choice of the control strategy to employ will depend on which kind of control task
232 needs to be achieved (set-point or tracking), the complexity of the synthetic circuit to be
233 controlled, the availability of a descriptive mathematical model of the circuit to be con-
234 trolled, the cost associated to the actuation effort and, whether a minimal stress to the cells
235 is required (i.e. a small number of input switches).

236

Table 1: Comparative analysis summary

Control Strategy	Model required	Pros	Cons
PI	No	<ul style="list-style-type: none"> ★Robust ★Reduced computational complexity 	–Not suitable for signal tracking control
MPC	Yes	<ul style="list-style-type: none"> ★Suitable for set-point and signal tracking control ★Best performance for fast varying references 	–High number of input switches
ZAD	Yes	<ul style="list-style-type: none"> ★Suitable for set-point and signal tracking control ★Reduced number of input switches 	–Performs slightly worse than MPC on fast varying references

237 Methods

238 Yeast strain and cell culture

239 Control experiments were performed in the yeast strain (yGIL337, Gal1-GFP::KanMX,
 240 Gal10-mCherry::NatMX) provided to us by Lang et al. (21). In this strain the Gal1 protein,
 241 expressed by the *GAL1* promoter, was fused to a green fluorescent protein (Gfp). Before
 242 each *in-vivo* control experiment started, cells were inoculated overnight in 10 mL synthetic
 243 complete medium + Galactose/raffinose (2%); the culture was then repeatedly diluted to
 244 achieve a final OD₆₀₀ of 0.01 on the day the cells were injected into the microfluidic device
 245 (Supporting Informations for further details).

246 Microfluidics

247 The MFD0005a device was used for all the microfluidics experiments(17). This device houses
 248 a micro-chamber (height: 3.5 μ m) which "traps" yeast cells, that can only grow in a mono-
 249 layer, thus allowing easier automated image analysis. Microfluidics devices were fabricated as
 250 described in(17). Details of the microfluidics set-up and of the galactose and glucose growing

251 medium used in the experiments can be found in (5) and Supporting Informations.

252 **Microscopy and image analysis**

253 To monitor cellular processes dynamics, as well as, to check for the right administration of
254 external inputs to trapped cells, we took advantage of an inverted fluorescence microscope
255 (Nikon Eclipse Ti) equipped with an automated and programmable stage, an incubator to
256 guarantee fixed temperature and gasses to cell environment and a high sensitivity Electron
257 Multiplying CCD (EMCCD) Camera (Andor iXON Ultra897). The microscope and the
258 camera were programmed to acquire, at 5 min intervals, two types of images: (a) a bright field
259 image (phase contrast) and (b) fluorescence images (with the appropriate filters) to monitor
260 cell fluorescence and to track the dye (sulforhodamine) added to the inducer compound in
261 order to evaluate the control input administered to the cells. Fluorescence was quantified
262 using a previously developed custom image processing algorithm (5). The algorithm is able
263 to locate cells within each Phase Contrast image thus identifying all pixels belonging to the
264 cells. This information is used to calculate the fluorescence being expressed by the entire
265 population as well as by each single cell. The actual measured fluorescence is mainly affected
266 by the efficiency of the fluorescent lamp, and by the background light in the surrounding
267 microscope environment. We observed that as the fluorescent lamp nears its life-time, its
268 brightness decreases and this affects the measured fluorescence in the cells. Indeed, the
269 measurement units for the fluorescence are considered arbitrary and, thus, a calibration
270 phase at the beginning of each experiment is needed to calculate a reference value for the
271 fluorescence.

272 **Control strategies implementation**

273 The control input is described as follows, where ON means galactose administration and
274 OFF glucose administration:

$$u(t) = \begin{cases} u_{MAX} = ON & kT \leq t < (k + d_k)T \\ u_{MIN} = OFF & (k + d_k)T \leq t < (k + 1)T \end{cases} \quad (1)$$

275 **PI:** Proportional and integral gains, K_p and K_i were calculated with the Ziegler-Nichols'
 276 open-loop tuning method (1) applied to the mathematical model of the *GAL1* promoter
 277 described in the Supporting Informations. Thus the gains were set to $K_p = 13.49$ and
 278 $K_i = 0.17$.

279 Given the constraints on the control input as well as on the sampling time described
 280 above, a modulation on the PI output was implemented to calculate the duty cycle d_k as:

$$d_k = \frac{\hat{u} - u_{MIN}}{u_{MAX} - u_{MIN}}. \quad (2)$$

281 where \hat{u} is the output of the PI regulator saturated between $u_{MIN} = 0$ and $u_{MAX} = 2$.
 282 To avoid delays and overshoots introduced by the saturation of the regulator output (1)
 283 an anti-windup block, described in the Supporting Informations, was added to the feedback
 284 loop.

285 **MPC:** The MPC strategy chooses, at each sampling time kT , the optimal control input
 286 that minimises the sum of the squared control error (SSE):

$$SSE \triangleq \sum_{i=k+1}^{k+N} (N + 1 + k - i) \epsilon_i^2 = \sum_{i=k+1}^{k+N} (N + 1 + k - i) (\hat{y}_i - r_i)^2 \quad (3)$$

287 where \hat{y} is the output provided by the dynamical model of the the *GAL1* promoter
 288 (Supplementary Information), which is computed by a Kalman state estimator, able to
 289 reconstruct system states from the measured output y , as shown Figure 1 - A. The integer
 290 $N = 12$ (corresponding to 60 min) defines the length of the prediction horizon in terms of
 291 sampling intervals. The forgetting factor $(N + 1 + k - i)$ weights the error samples more at
 292 the beginning of the prediction horizon than at the end; this guarantees faster corrections

293 of output deviations from the reference. The optimisation was carried out by adopting the
 294 Matlab implementation of the Genetic Algorithm described in (22).

295 The result of the optimisation is an array of N optimal duty cycles $d_{k+i}, i \in [1, N]$.
 296 In the absence of external disturbances and other sources of uncertainty, the optimal input
 297 computed by the MPC could, in principle, be applied to the yeast cells over the entire
 298 prediction horizon. However, in order to make the control action robust to any source of
 299 uncertainty and variability, the feedback loop is closed by applying only the first element
 300 of the calculated control input and at the next sampling time $(k + 1)T$ when the entire
 301 procedure is repeated.

302 **ZAD:** Zero Average Dynamics (ZAD) control relies on a feedback strategy devised for the
 303 regulation of power converters and allows to directly calculate the duty cycle d_k of a switching
 304 control input (19, 23). ZAD control is a practical implementation of sliding mode control
 305 (12), where the control objective consists in attracting and then maintaining onto a fixed
 306 surface $s(x) = 0$ (denoted as the sliding surface) the states of the system by appropriately
 307 switching the available inputs.

308 In the ZAD control approach, the sliding condition has to be fulfilled only on average
 309 over each sampling period kT , thus allowing to directly calculate the duty cycle d_k via the
 310 solution of the following integral equation:

$$\mathbb{E}_T[s(x(t))] = \frac{1}{T} \int_{kT}^{(k+1)T} s(x(t)) dt = 0 \quad (4)$$

311 where \mathbb{E}_T indicates the operator taking the average over the time interval T .

312 To control *GAL1* promoter dynamics onto the desired reference signal, we considered
 313 the following sliding surface, which was derived using the dynamical model of the *GAL1*
 314 promoter as described in the Supplementary Information:

$$s(x(t)) = (x_2(t) - x_{2_{ref}}(t)) + (\dot{x}_2(t) - \dot{x}_{2_{ref}}(t)) \quad (5)$$

315 where x_2 is the state variable describing the dynamics of the fluorescent reporter note
316 that $\dot{x}_{2_{ref}}(t) = 0$ in the case of set-point regulation.

317 For further details on the implementation of the ZAD controller refer to Supporting
318 Informations.

319 **Supporting Informations**

320 Additional text and figures referenced in this article.

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389 **Figure Legends**

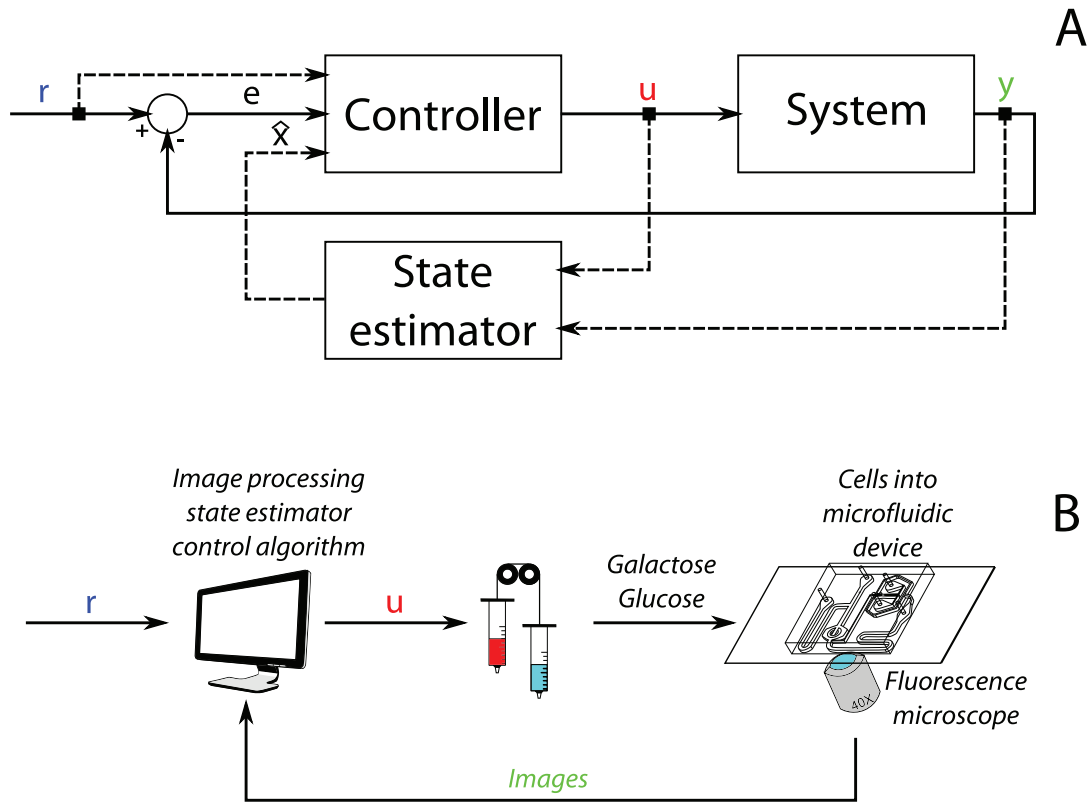


Figure 1: **Control scheme and experimental set-up.** (A) Generalised control scheme used to implement PI, MPC and ZAD regulators. In the case of model-free regulators (e.g. PI), the control error e (namely the difference in between the control reference r and the system's output y) is minimised by the controller calculating the control input u . Model-based controllers, i.e. MPC and ZAD, use not only the control error e but also the dynamical model of the *GAL1* promoter in the State estimator block. (B) Experimental set-up: a PC governs the entire platform running an algorithm that during each sampling interval: (i) processes the images acquired by the microscope to calculate the output y , (ii) runs the state estimator (when needed) and the control algorithms to calculate the input u for the next sampling interval. (iii) controls the automated syringes so as to provide the calculated input to the cells.

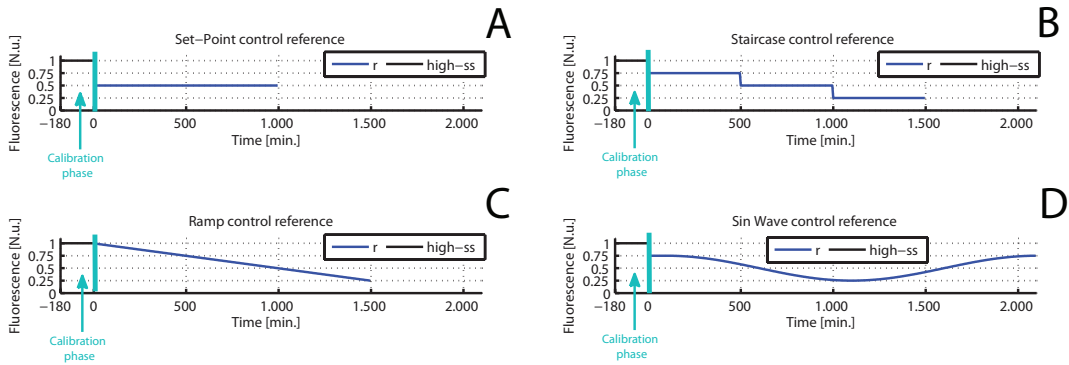


Figure 2: **Reference signals for set-point and tracking control tasks.** (A) The desired set-point r (**blue line**) is equal to 50% of the average fluorescence measured during the initial calibration phase of 180 min (**black line**) (B) The desired level of fluorescence (r) is a three-step descending staircase signal, each step is set at a given percentage (75%, 50% and 25%) of the average fluorescence measured during the initial calibration phase of 180 min. (C) The desired level of fluorescence (r) is a linear descending ramp starting at 100% of the average fluorescence measured during the initial calibration phase of 180 min and going down to 25%. (D) The desired level of fluorescence (r) is a steady state signal equal to 75% of the average fluorescence measured during the calibration phase, with a duration of 100 min; followed by a sinusoidal wave of period $T = 2000$ min defined as $s(t) = 0.5 + 0.25 \sin\left(\frac{2\pi}{T}(t - 100) + \frac{\pi}{2}\right)$

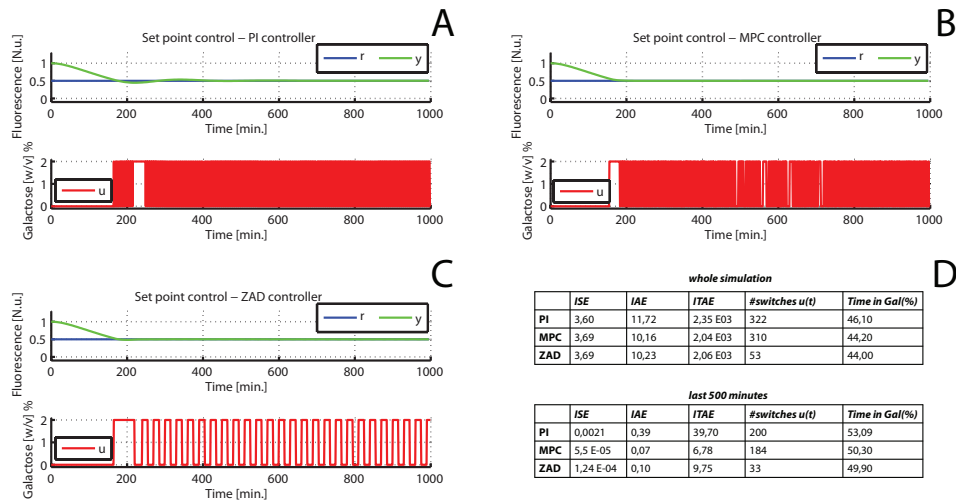


Figure 3: ***In-silico* set-point control task.** The blue line is the reference signal (r). The green line is the simulated fluorescence level (y). The red line is the control input (u). (A-C) Three *in-silico* set-point control experiments performed on the *GAL1* promoter mathematical model by the means of the PI (A), MPC (B) and ZAD (C) controllers. The initial level of fluorescence is assumed to be equal to 1. The control action starts at time $t = 0$ min and ends at $t = 1000$ min. (D) Performance indices: Integral Square Error (ISE), Integral Absolute Error (IAE), Integral Time Absolute Error (ITAE), number of switches of the control input, and the percentage of time during which the model is provided with the 'ON' input.

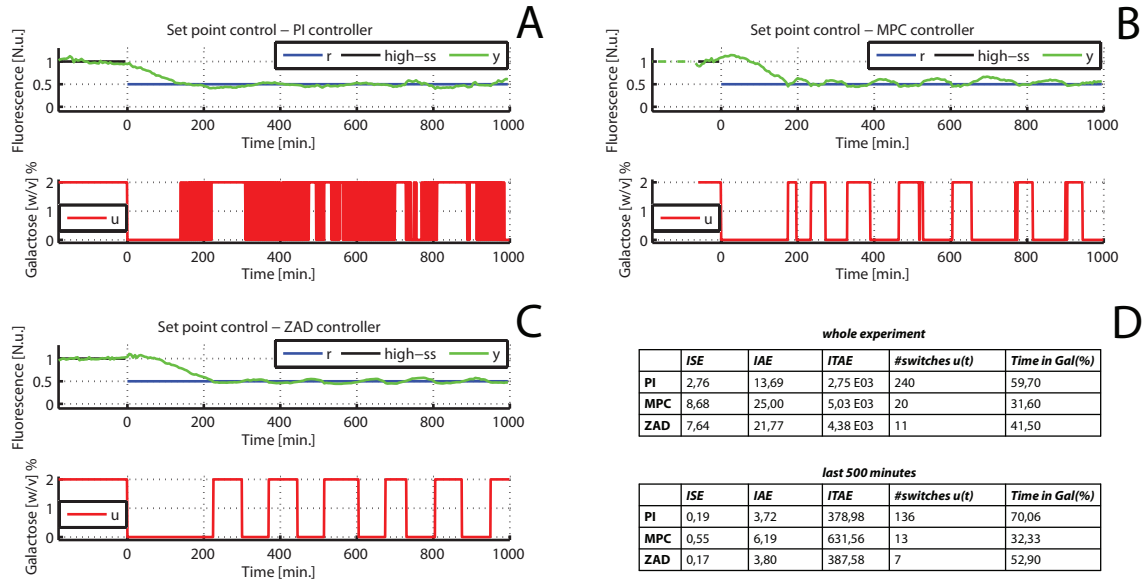


Figure 4: *In-vivo* set-point control task. The black line is the average fluorescence intensity during the calibration phase of 180 min. The blue line is the reference signal (r). The green line is the measured fluorescence level (y) across the yeast population. The red line is the control input (u). (A-C) Three *in-vivo* set-point control experiments by the means of the PI (A), MPC (B) and ZAD (C) controllers. The control action starts at time $t = 0$ min and ends at $t = 1000$ min. (D) Performance indices: Integral Square Error (ISE), Integral Absolute Error (IAE), Integral Time Absolute Error (ITAE), number of switches of the control input, and the percentage of time during which the model is provided with the 'ON' input.

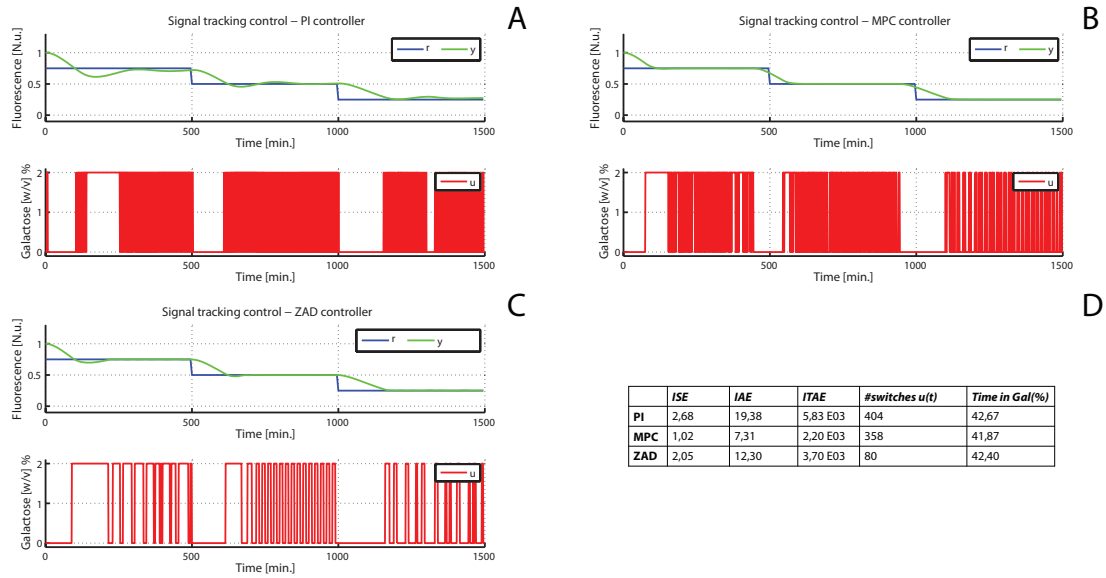


Figure 5: *In-silico* staircase tracking control task. The blue line is the reference signal (r). The green line is the simulated fluorescence level (y). The red line is the control input (u). (A-C) Three *in-silico* staircase tracking control experiments performed on the *GAL1* promoter mathematical model by the means of the PI (A), MPC (B) and ZAD (C) controllers. The initial level of fluorescence is assumed to be equal to 1. The control action starts at time $t = 0$ min and ends at $t = 1000$ min. (D) Performance indices: Integral Square Error (ISE), Integral Absolute Error (IAE), Integral Time Absolute Error (ITAE), number of switches of the control input, and the percentage of time during which the model is provided with the 'ON' input.

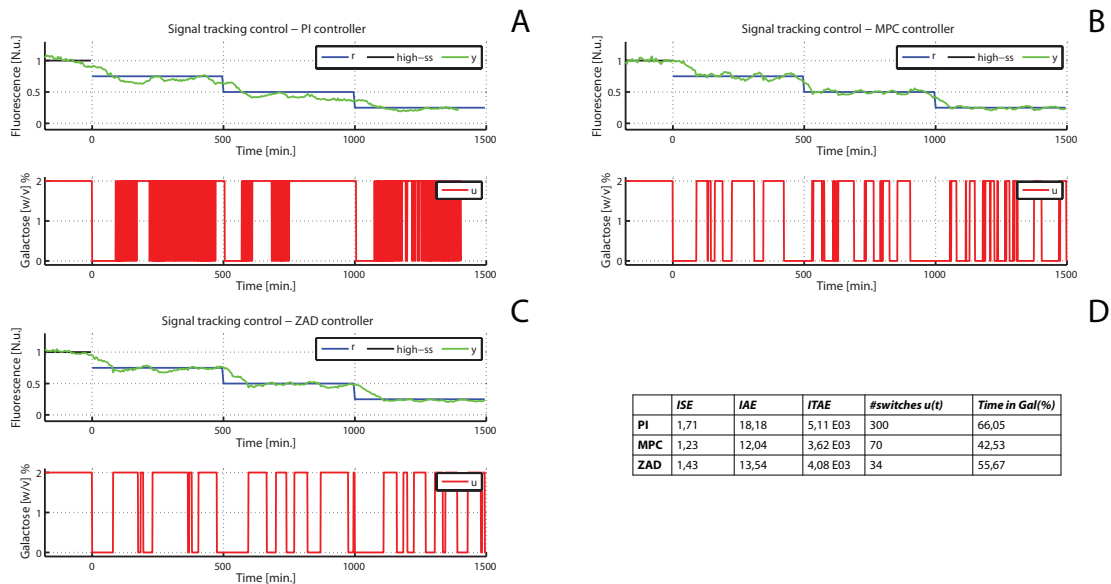


Figure 6: *In-vivo* staircase tracking control task. The black line is the average fluorescence intensity during the calibration phase of 180 min. The blue line is the reference signal (r). The green line is the measured fluorescence level (y) across the yeast population. The red line is the control input (u). (A-C) Three *in-vivo* staircase tracking control experiments by the means of the PI (A), MPC (B) and ZAD (C) controllers. The control action starts at time $t = 0$ min and ends at $t = 1000$ min. (D) Performance indices: Integral Square Error (ISE), Integral Absolute Error (IAE), Integral Time Absolute Error (ITAE), number of switches of the control input, and the percentage of time during which the model is provided with the 'ON' input.

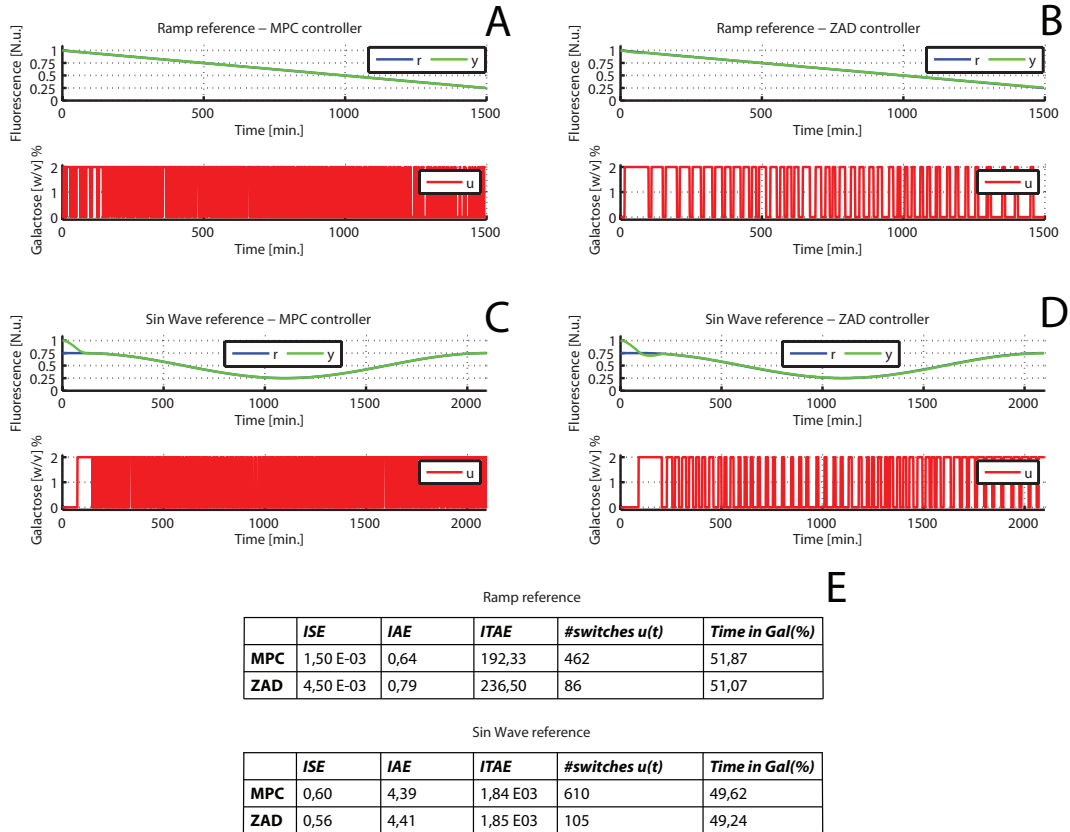


Figure 7: *In-silico* ramp and sin wave tracking control tasks. The blue line is the reference signal (r). The green line is the simulated fluorescence level (y). The red line is the control input (u). (A-B) Two *in-silico* ramp tracking control experiments performed on the *GAL1* promoter mathematical model by the means of the MPC (A) and ZAD (B) controllers. The initial level of fluorescence is assumed to be equal to 1. The control action starts at time $t = 0$ min and ends at $t = 1500$ min. (C-D) Two *in-silico* sin wave tracking control experiments performed on the *GAL1* promoter mathematical model by the means of the MPC (C) and ZAD (D) controllers. The initial level of fluorescence is assumed to be equal to 1. The control action starts at time $t = 0$ min and ends at $t = 2100$ min. (E) Performance indices: Integral Square Error (ISE), Integral Absolute Error (IAE), Integral Time Absolute Error (ITAE), number of switches of the control input, and the percentage of time during which the model is provided with the 'ON' input.

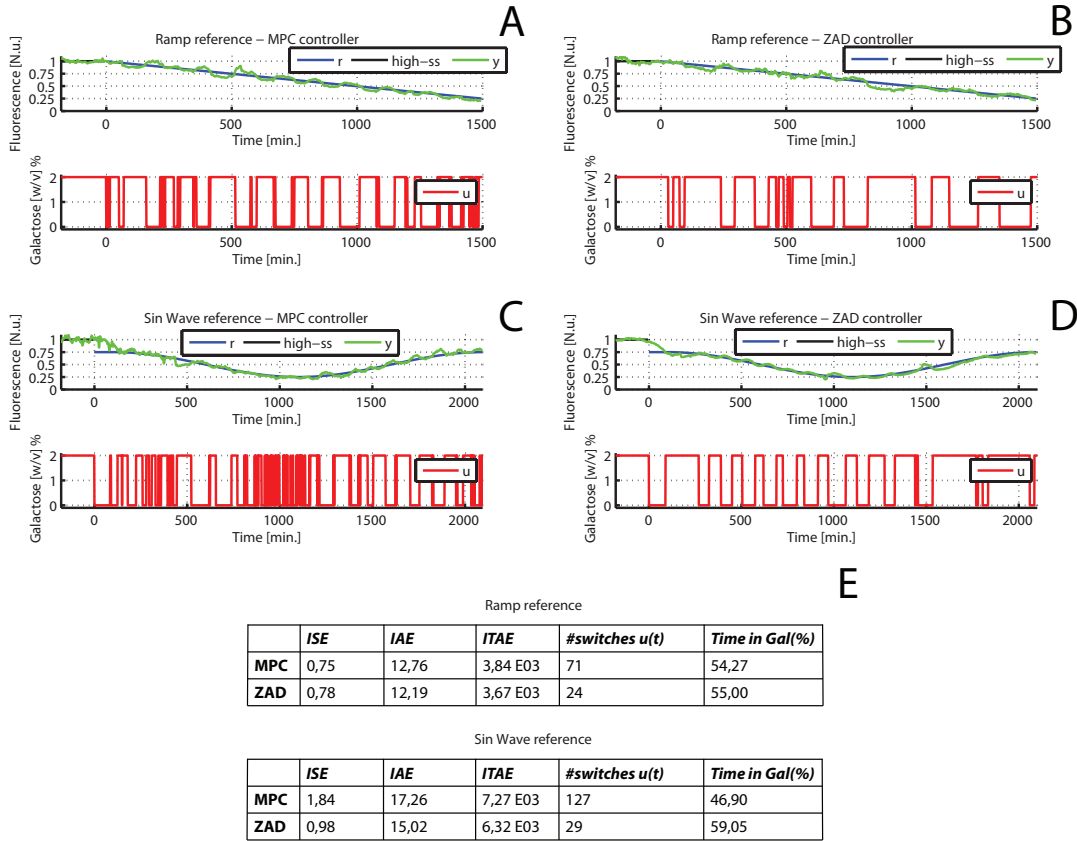


Figure 8: ***In-vivo* ramp and sin wave tracking control tasks.** The black line is the average fluorescence intensity during the calibration phase of 180 min. The blue line is the reference signal (r). The green line is the measured fluorescence level (y) across the yeast population. The red line is the control input (u). (A-B) Two *in-vivo* ramp tracking control experiments by the means of the MPC (A) and ZAD (B) controllers. The control action starts at time $t = 0$ min and ends at $t = 1500$ min. (C-D) Two *in-vivo* sin wave tracking control experiments by the means of the MPC (C) and ZAD (D) controllers. The control action starts at time $t = 0$ min and ends at $t = 2100$ min. (E) Performance indices: Integral Square Error (ISE), Integral Absolute Error (IAE), Integral Time Absolute Error (ITAE), number of switches of the control input, and the percentage of time during which the model is provided with the 'ON' input.