Deconvolution of Cell Cycle Time-series Data
Reveals Gene Expression Details at High Resolution
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Supplementary Text S1

Quadratic form of the cost function $C(\lambda)$

The predicted value $\hat{G}(t_m)$ of the $m$th measurement $G(t_m)$ may be approximated by an inner product of sampled functions

$$\hat{G}(t_m) = \int Q(\phi, t_m)f(\phi)d\phi \approx \delta q^T(t_m)f = \delta q^T(t_m)\Psi \alpha , \quad (S1)$$

where $q(t_m) = [Q(\phi_1, t_m) ... Q(\phi_{N_k}, t_m)]^T$ and $\delta = 1/N_k$ is the knot spacing.

The cost function $C(\lambda)$, given in Models as:

$$C(\lambda) = \frac{1}{N_m} \sum_{m=1}^{N_m} \frac{(G(t_m) - \hat{G}(t_m))^2}{\sigma^2_m} + \lambda \int \{f''(\phi)^2\}d\phi , \quad (S2)$$

with $\hat{G}(t_m) = \int Q(\phi, t_m)f(\phi)d\phi$ may be written as a quadratic form in $\alpha$ as follows: define $Q$ as the $N_m \times N_k$ measurement matrix $Q = [q(t_1) ... q(t_{N_m})]^T$ that maps the sampled expression function $f$ to the predicted measurements $\hat{g} = [\hat{G}(t_1) ... \hat{G}(t_{N_m})]^T$

$$\hat{g} = Qf = Q\Psi \alpha . \quad (S3)$$

In Eq. (S3) and what follows, we absorb the height scaling constant $\delta$ into the expression function $f$ that is to be estimated. With $f(\phi) = \sum_{i=1}^{N_k} \alpha_i \psi_i(\phi)$, the second term of Eq. (S2) may be written

$$\lambda \alpha^T \Omega \alpha , \quad (S4)$$

where $\Omega = \{\Omega_{ij}\}$, $\Omega_{ij} = \int \psi'_i(\phi)\psi'_j(\phi)d\phi$. Letting $R$ be a diagonal matrix of measurement variances $\sigma^2_1 ... \sigma^2_{N_m}$, the cost function (Eq. (S2)) may be written

$$C(\lambda) = (g - Q\Psi \alpha)^TR^{-1}(g - Q\Psi \alpha) + \lambda \alpha^T \Omega \alpha , \quad (S5)$$

which is a quadratic form in $\alpha$.

Justification for use of the synchronous average expression function $f(\phi)$

It has been shown that as a result of noise in gene expression, the levels of expression (measured with fluorescent reporters) are normally-distributed about the population mean [1]. Letting $f(\phi) =$
(1/N) \sum_k f_k(\phi) denote the average population expression, we may then write the single-cell expression function \( f_k(\phi) \) for cell \( k \) as the product of a scaling factor \( s_k \) with the population average

\[
f_k(\phi) = s_k f(\phi),
\]

where \( s_k \) is independent of all other cell parameters \( \theta, \phi \) and is drawn from a normal distribution with mean 1. For a given species of RNA, the total number of transcripts in the population at time \( t \) is then given by:

\[
R(t) = \sum_{k=1}^{N(t)} f_k(\phi)v_k(\phi)
\]

\[
= \sum_{k=1}^{N(t)} (s_k \times f(\phi))v_k(\phi)
\]

\[
\approx N(t)E_{\theta,\phi,s}[sf(\phi)v_\theta(\phi)]
\]

\[
= N(t)E_s[sE_{\theta,\phi}[f(\phi)v_\theta(\phi)]]
\]

\[
= N(t)\int f(\phi)\tilde{Q}(\phi, t) d\phi,
\]

where \( E_X[...] \) denotes statistical expectation over the random variable \( X \). As \( s \) is random and independent of \( \theta \) and \( \phi \), the expectation of the product of \( s \) and \( f(\phi)v_\theta(\phi) \) is equal to the product of their expected values (1 and \( \int f(\phi)\tilde{Q}(\phi, t) d\phi \), respectively).

**Microarray data noise model**

It has been previously shown that the dominant source of noise in experiments using Affymetrix microarrays is hybridization noise [2]. This leads to a signal variance on each gene expression value that is, above a certain threshold, proportional to the value itself,

\[
\sigma^2_{G>G_0} \simeq \beta \times G,
\]

where \( G \) is the level of gene expression, \( G_0 \) is the threshold, and \( \beta \) is a constant. Although the experiments in [2] use mRNA from a human Burkitts lymphoma cell line, it was suggested that the hybridization noise component proportional to signal intensity does not depend on the type of genechip and the sample being used. Consistent with that work, we used a noise model with \( \beta=5 \). Also, the threshold is the 10th percentile of all expression values, which, for the entire Caulobacter data set analyzed here, is 0.188. Far below this threshold, i.e., at low levels of expression, we assumed that the variance is constant. To combine these two limits into a single noise model, we assumed that at the threshold intensity the variance diverges from proportionality by 5%, so that

\[
\sigma^2 = 5 \times G + 0.05 \times (5 \times 0.188)
\]

\[
= 5 \times G + 0.047.
\]

**Constraints on the average single-cell expression functions**

There are typically multiple solutions to inversion problems of the kind used here. We therefore constrained the single-cell expression profiles by excluding non-physical solutions for which RNA concentration became negative. We also applied a continuity constraint, since for every cell \( k \) the concentration of
Division time assays

To determine the division times for *Caulobacter* SW and ST cells, we used a simple microfluidic apparatus and followed the microfluidic protocol described previously [4, 5]. ST cells attach to the glass surface of the microfluidic via the adhesive holdfast and are oriented along the direction of the flow of growth medium. Following cell division, ST cells remain attached to the surface while the majority of daughter SW cells are flushed out of the channel. ST cells continue to grow and divide for the duration of the experiment. The time between division events is then the ST cell division time. Following division events, SW cells do occasionally attach to the glass cover slip prior to separation, and remain attached downstream of the ST cells when division is complete. The cells then stay attached to the surface as they transition from the SW to the ST phase, and the stalk and holdfast develop. The time between the first attachment of a SW and the first division of that cell is then the full cell cycle time.

Cell division took place in microfluidic channels measuring 200 μm wide by 50 μm deep by 2 cm long and made with polydimethylsiloxane (PDMS, Sylgard Brand 184 Silicone Elastomer Kit). The PDMS
and glass cover slip were cleaned and sealed using a Plasma Prep II plasma cleaner (SPI Supplies). Sodium hydroxide (2M solution), ethanol, and water were sequentially flowed into the channels to clean the interior before cell loading.

Individual colonies of wild-type *Caulobacter crescentus* strain CB15 [6] were taken from a peptone/yeast extract (PYE)-agar plate and grown overnight in 5 ml PYE medium at 30°C, diluted to 0.1 optical density at 660 nm (OD$_{660}$) and regrown for 2 hours. Cells were then loaded into the microfluidic chamber and incubated for an additional hour prior to imaging. A Harvard Apparatus PHD2000 infuser was used to induce a constant flow of PYE medium at a rate of 12 µl/min for the duration of the experiment.

Cells were imaged with a Leica DM5000 at 630x magnification in phase contrast mode. Images were collected at 2 minute intervals on a Hamamatsu Orca-ER digital camera, and the light dosage was limited to 200 msec exposure and ~5 second manual focus time per exposure. The temperature in the room was maintained at 30°C. Cell growth and division were monitored for 12-14 hours during each of four independent experimental runs.

Images were imported into ImageJ (National Institutes of Health, Bethesda, MD) for processing. The images were converted into binary stacks by subtracting the image backgrounds and adjusting the threshold pixel intensity. Cell areas were calculated in ImageJ, and data were further analyzed using Mathematica (Wolfram Research, Inc., Champaign, IL).

**References**

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**Supplementary Table S1**

| Gene name | $\lambda_{0.25}$ | $\lambda_{0.15}$ |
|-----------|------------------|------------------|
| $ctrA$    | $2.73 \times 10^{-7}$ | $2.89 \times 10^{-6}$ |
| $dnaA$    | $7.02 \times 10^{-7}$ | $1.80 \times 10^{-6}$ |
| $ccrM$    | $1.13 \times 10^{-6}$ | $7.44 \times 10^{-6}$ |
| $gcrA$    | $7.02 \times 10^{-7}$ | $4.64 \times 10^{-6}$ |
| $cckA$    | $1.06 \times 10^{-7}$ | $1.80 \times 10^{-6}$ |
| $chpT$    | $3.07 \times 10^{-5}$ | $7.90 \times 10^{-5}$ |
| $pleC$    | $1.19 \times 10^{-5}$ | $4.92 \times 10^{-5}$ |
| $divJ$    | $1.91 \times 10^{-5}$ | $1.19 \times 10^{-5}$ |
| $divK$    | $2.89 \times 10^{-6}$ | $1.19 \times 10^{-5}$ |
| $ftsZ$    | $1.19 \times 10^{-6}$ | $1.80 \times 10^{-6}$ |

Regularization parameters determined by cross validation for $\mu_{sst} = 0.25$ ($\lambda_{0.25}$) and $\mu_{sst} = 0.15$ ($\lambda_{0.15}$).