Supporting Information to: Deconvolution of Luminescence Cross-Talk in High-Throughput Gene Expression Profiling

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Contents

1 Supporting text

1.1 Analytical expression of the bleed-through factor

Here, we work out the analytical expression Equation (2) of the main text, that expresses the bleed-through factor as a function of the distance from a single luminescence-emitting well. When the photo-detector is positioned at an offset h from a microplate well w, it still receives luminescence incoming from the emitting well $E5$ under a small angle θ, as shown in Figure S2. Calling d the distance between neighbouring wells, the detector that is positioned x wells away from $E5$ detects

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a background-subtracted intensity $O(t)$ at a time t that is inversely proportional to the square of the distance from the source $l(x)$

$$O(t,x) \propto \frac{\cos\theta(x)}{l(x)^2}. \tag{1}$$

From trigonometry considerations, the cosine of the angle θ can be expressed by

$$\cos\theta(x) = \frac{h}{\sqrt{h^2+(xd)^2}} \tag{2}$$

and the distance from the source $l(x)$ by

$$l(x) = \sqrt{h^2+(xd)^2}. \tag{3}$$

We obtain the time-averaged bleed-through factor $B(x)$ by rescaling $O(t,x)$ with respect to the reference intensity measured above the luminescent well $O(t,0) = 1/h^2$ and averaging over the measured time interval T

$$\begin{aligned} B(x) &\equiv \frac{1}{T}\sum_{t,t'=0}^{T}\frac{O(t',x)}{O(t,0)} = \\ &= \frac{1}{T}\sum_{t,t'=0}^{T}\frac{h}{\sqrt{h^2+(xd)^2}}\frac{1}{h^2+(xd)^2}h^2 = \\ &= \frac{h^3}{(h^2+(xd)^2)^{3/2}} \end{aligned} \tag{4}$$

with $l(0) = h$ and $\cos\theta(0) = 1$. If rows and columns of the microplates are identified by index m and n, respectively, where $m = 1,\ldots,8$ and $n = 1,\ldots,12$, the distance x from the emitting well $E5$ can be explicitly written as

$$x = \sqrt{(m-5)^2+(n-5)^2}. \tag{5}$$

Substituting x, we finally obtain

$$B_{m,n} = \frac{h^3}{(h^2+d^2((m-5)^2+(n-5)^2))^{3/2}}. \tag{6}$$

Equation 6 is the theory prediction of the time-averaged bleed-through factor as a function of the distance of the emitting well, as stated from Equation (2) in the main text.

1.2 Summary of the optimization algorithm

We give here a brief summary of the optimization algorithm to find the kernel D. We first measure the luminescence intensities of the calibration plate $O_{m,n}(t)$ as described in the main text and arrange them by row concatenation into a vector $\overrightarrow{O(t)}$. Then, at every iteration step k, we

1. find the bleed-through factor matrix $B(t)$, with entries $B_{m,n}(t)$ given by Equation (1) in the main text

$$B_{m,n}(t) = \frac{O_{m,n}(t)}{O_{5,5}(t)} \tag{7}$$

2. time-average $B(t)$ to obtain the time-averaged bleed-through factor matrix B (with entries $B_{m,n}$) and the corresponding matrix of standard deviations σ^B (with entries $\sigma^B_{m,n}$)

3. assemble the matrix E (with entries $E_{i,j}$) and the matrix of standard deviations σ^E (with entries $\sigma^E_{i,j}$) from B and σ^B, respectively, according to

$$E_{i,j} = \begin{cases} B_{i-3,j-7}, & \text{if } 4 \leq i \leq 11,\ 8 \leq j \leq 19 \\ \overline{B(x)}, & \text{otherwise, for all (i,j) at distance } x = \sqrt{(i-8)^2 + (j-12)^2} \text{ from E5} \\ z, & \text{otherwise,} \end{cases} \tag{8}$$

The first line represents the measured time-averaged bleed-through matrix B. $\overline{B(x)}$ in the second line represents the average of the measured values of B that are at a distance $x = \sqrt{(i-8)^2 + (j-12)^2}$ from $E5$. This result is used to fill entries at the same distance x outside the measured plate. All other entries of the extended matrix E are filled with z, computed by averaging intensities over the wells defined as background (A12-H12). A graphical explanation of the composition of the entries of E is given in Figure S5.

4. create a matrix $\widetilde{E}$ with entries given by Equation (6) in the main text

$$\widetilde{E}_{i,j} = E_{i,j} + r_{i,j}\sigma^E_{i,j} \tag{9}$$

where $r_{i,j}$ is a random number generated from a gaussian distribution with mean 0 and standard deviation 1

5. follow the prescription of Equation (3) in the main text, where E is replaced by $\widetilde{E}$, to assemble the kernel D with entries

$$D_{a,b} = \widetilde{E}_{m-l+8,n-p+12} \tag{10}$$

where $a = 12(m-1)+n$, $b = 12(l-1)+p$ with $m, l = 1, \ldots, 8$ and $n, p = 1, \ldots, 12$

6. find the vector of deconvolved intensities $\overrightarrow{R(t)}$ by applying Equation (5) in the main text

$$\overrightarrow{R(t)} = D^{-1} \cdot \overrightarrow{O(t)} \tag{11}$$

7. after transforming the vector $\overrightarrow{R(t)}$ into a matrix with entries $R_{i,j}(t)$, compute the difference between each $R_{i,j}(t)$ and the instrument sensitivity s (excluding luminescent well $E5$):

$$Q_{i,j} = R_{i,j}(t) - s \tag{12}$$

8. if every $Q_{i,j} \leq 0$, iterations stop; otherwise D is stored as $D_{\text{best}} = \prod_k D_k$, where the product is intended as matrix multiplication and for $k = 1$, D_0 is the identity matrix

9. apply the acceptance criteria:

   - if $R(t) < s$ or $R_{i,j}(t) < O_{i,j}(t)$, the bleed-through has been reduced and $R_{i,j}(t)$ is accepted
   - otherwise we set $R_{i,j}(t) = O_{i,j}(t)$

10. assume that the luminescence left-overs are the new observed intensities, by setting $O_{i,j}(t) = R_{i,j}(t)$
11. repeat from point 1).

The acceptance criteria ensure that the correction affects the wells where there is still present some residual bleed-through after the deconvolution process.

1.3 Data analysis

In this section, we explain in detail how we analyse and correct for luminescence bleed-through the microplate reader data. Although we use a 96-wells microplate, the process can be extended to any microplate. Rows and columns of the microplates are identified by index m and n, respectively, where $m = 1, \ldots, 8$ (or by using letters $m = A, \ldots, H$) and $n = 1, \ldots, 12$[1]. We prepare the calibration plate by inoculating with a luminescent bacterial strain a single well ($E5$), the other wells of the plate with a non-luminescent strain, and proceed by

(a) measuring the luminescence of the plate for 2 hours

(b) adding arabinose (at high concentration, 0.2% in our case) in $E5$ to induce maximal luminescence (at t = 0 h)

(c) measuring the luminescence of the plate for 6 hours. In general, we record the luminescence signals during the exponential growth phase.

We produce three replicates both of the calibration plate and of the experiment that needs to be bleed-through corrected. The output files of the microplate reader software are processed by our algorithm in MATLAB as described in the following and schematically summarized in Figures S20 and S21.

1.3.1 Analysis of the calibration plate

We follow the scheme of Figures S20.

(1) Raw luminescence L

We first arrange the raw luminescence data from the microplate reader files into an array L with entries $L_{m,n}(t, c)$, where the indexes m and n indicate row and column of the plate, t the time point (given as machine reading cycle) and c the experimental replicate.

(2) Luminescence background b

Next, we compute the background b of the luminescence by averaging the values of the luminescence in the wells from A12 to H12 (8 wells), before arabinose induction (between t=-2 h and t=0 h, for

[1] Notation: We use the indexes (m, n) to identify the entries of matrices which luminescence values come from direct measurement, such as the bleed-through matrix B; (i, j) to identify the entries of the matrix E, resulting from the extension of B under mathematical prescription; (a, b) to identify the entries of the matrix D, and (α, β) indicates the position on the plate of the luminescent well. Vectors are denoted by $\overrightarrow{V}$.

a total time range $T_{bi} = 2$ h), and over C-replicates (in our case three replicates):

$$b = \frac{1}{8}\frac{1}{T_{bi}}\frac{1}{C}\sum_{m=1}^{8}\sum_{t=1}^{T_{bi}}\sum_{c=1}^{C} L_{m,12}(t,c) \tag{13}$$

with variance σ_b^2

$$\sigma_b^2 = \frac{1}{8}\frac{1}{T_{bi}}\frac{1}{C}\sum_{m=1}^{8}\sum_{t=1}^{T_{bi}}\sum_{c=1}^{C}(L_{m,12}(t,c) - b)^2 \tag{14}$$

and standard deviation $\sigma^b = \sqrt{\sigma_b^2}$.

(3) Instrument sensitivity s

We also define an instrument sensitivity s as three times the standard deviation of the background

$$s = 3\sigma^b \tag{15}$$

with the corresponding variance

$$\sigma_s^2 = 9\sigma_b^2\sqrt{\frac{2}{8T_{bi}C - 1}} \tag{16}$$

where we used propagation of uncertainty and variance of variance to obtain σ_s^2. Any signal beneath s can not be significantly statistically distinguished from the background value.

(4) Background-subtracted luminescence O

The background-subtracted luminescence is obtained by subtracting the background from the raw luminescence:

$$O_{m,n}(t,c) = L_{m,n}(t,c) - b \tag{17}$$

with the corresponding variance

$$\sigma_O^2(t,c)_{m,n} = \sigma_b^2. \tag{18}$$

We arrange the standard deviations computed from the variance in the array $\sigma^O(t,c) = \sqrt{\sigma_O^2(t,c)}$.

(5) Replica average of O

Then, we weight-average the background-subtracted luminescence O over C replicates, such that O has entries

$$O_{m,n}(t) = \frac{\sum_{c=1}^{C} w_O(t,c)_{m,n} O_{m,n}(t,c)}{\sum_{c=1}^{C} w_O(t,c)_{m,n}} \tag{19}$$

with weights $w_O(t,c)_{m,n} = 1/\sigma_O^2(t,c)_{m,n}$ and variance

$$\sigma_O^2(t)_{m,n} = \frac{\sum_{c=1}^{C} w_O(t,c)_{m,n}(O_{m,n}(t,c) - O_{m,n}(t))^2}{\sum_{c=1}^{C} w_O(t,c)_{m,n}}. \tag{20}$$

We arrange the standard deviations computed from the variance in the array $\sigma^O(t) = \sqrt{\sigma_O^2(t)}$.

(6) Bleed-through factor B

The bleed-through factor B in a given well (m, n) at time t is the ratio between the observed background-subtracted luminescence intensities O in such well and in the luminescent well $E5$:

$$B_{m,n}(t) = \frac{O_{m,n}(t)}{O_{5,5}(t)} \tag{21}$$

$$\sigma_B^2(t)_{m,n} = \left(\frac{O_{m,n}(t)}{O_{5,5}(t)}\right)^2 \left[\left(\frac{\sigma^O(t)_{m,n}}{O_{m,n}(t)}\right)^2 + \left(\frac{\sigma^O(t)_{5,5}}{O_{5,5}(t)}\right)^2 - 2\frac{cov(O_{5,5}(t), O_{m,n}(t))}{O_{m,n}(t)O_{5,5}(t)}\right]. \tag{22}$$

with $\sigma^B(t) = \sqrt{\sigma_B^2(t)}$ defined as the array of the standard deviations.

(7) Selection of time interval to average B

As discussed in main text (Figure 1C), since for a specific well the bleed-through factor is almost constant at any time point after induction, we average its value over a time interval T.

(8) Time-averaged bleed-through factor B

Then, we time-average the bleed-through factor

$$B_{m,n} = \frac{\sum_T w_B(t)_{m,n} B_{m,n}(t)}{\sum_T w_B(t)_{m,n}} \tag{23}$$

with weights $w_B(t)_{m,n} = 1/\sigma_B^2(t)_{m,n}$ and weighted variance

$$\sigma_{B,m,n}^2 = \frac{\sum_T w_B(t)_{m,n}(B_{m,n}(t) - B_{m,n})^2}{\sum_T w_B(t)_{m,n}}. \tag{24}$$

We arrange the standard deviations into a matrix σ^B. If we assume for simplicity that all weights w are equal, Equation 23 becomes the analytical Equation 4.

(9) Extended matrix E

The observed luminescence intensity above a given well is given by the sum of the true intensity and several bleed-through intensities. The assumption that any luminescent well generates a light patter that resembles the one measured in the case of a single luminescent well corresponds mathematically to convolve the unknown pattern of real intensities R with the time-averaged bleed-through matrix B

$$O(t) = B * R(t). \tag{25}$$

To perform this convolution with a 96-well microplate, it is necessary to extend the matrix of the bleed-through factors B to a 15x23 matrix E, as shown in Figure S4. We extend B according to the following prescription:

$$E_{i,j} = \begin{cases} B_{i-3,j-7}, & \text{if } 4 \leq i \leq 11,\ 8 \leq j \leq 19 \\ \overline{B(x)}, & \text{otherwise, for all (i,j) at distance } x = \sqrt{(i-8)^2 + (j-12)^2} \text{ from } E5 \\ z, & \text{otherwise,} \end{cases} \tag{26}$$

where $i = 1, \ldots, 15$ and $j = 1, \ldots, 23$. The first line represents the measured time-averaged bleed-through matrix B from Equation 23 graphically represented as the coloured central matrix of Figure S5. The second line contains the average of entries of B located at the same number of well-distance $x = \sqrt{(i-8)^2 + (j-12)^2}$ from $E5$, represented as the magenta area of Figure S5. Explicitly

$$\overline{B_{i,j}} \equiv \overline{B(x)} = \frac{\sum_x w_{B(x),m,n} B_{m,n}}{\sum_x w_{B(x),m,n}} \tag{27}$$

$$w_{B(x),m,n} = 1/\sigma^2_{B,m,n} \tag{28}$$

where indexes m, n refers as before to the wells of the measured matrix. A graphical example of how such entries are computed is given in Figure S5: measured values at a fixed distance from $E5$ are averaged and the result is used to fill the entries at the same distance outside the measured plate. All other entries of the extended matrix E are filled with z, computed by averaging intensities over the wells defined as background (A12-H12). This last is the brown area of Figure S5. The final outcome of such prescription is given in Figure S5. The corresponding variance results

$$\sigma^2_{E,i,j} = \begin{cases} \sigma^2_{B,i-3,j-7}, & \text{if } 4 \leq i \leq 11,\ 8 \leq j \leq 19 \\ \sigma^2_{\overline{B(x)}}, & \text{otherwise, for all wells at distance x from E5} \\ \sigma^2_z, & \text{otherwise} \end{cases} \tag{29}$$

with

$$\sigma^2_{B(x),m,n} = \frac{\sum_x w_{B(x),m,n} (B_{m,n} - \overline{B(x)})^2}{\sum_x w_{B(x),m,n}} \tag{30}$$

$$w_{B(x),m,n} = 1/\sigma^2_{B,m,n} \tag{31}$$

where x indicates again the distance from $E5$ and

$$z = \frac{\sum_{m=1}^{8} w_{B,m,12} B_{m,12}}{\sum_{m=1}^{8} w_{B,m,12}} \tag{32}$$

$$\sigma^2_z = \frac{\sum_{m=1}^{8} w_{B,m,12} (B_{m,12} - z)^2}{\sum_{m=1}^{8} w_{B,m,12}} \tag{33}$$

$$w_{B,m,n} = 1/\sigma^2_{B,m,n}. \tag{34}$$

We arrange the standard deviations of E into a matrix σ^E with entries $\sigma^E_{i,j} = \sqrt{\sigma^2_{E,i,j}}$. The array of the observed intensities O is therefore given by the discrete convolution of E and R (Equation 25), with entries

$$O_{m,n}(t) = \sum_{l=1}^{8} \sum_{p=1}^{12} E_{m-l+8,n-p+12} R_{l,p}(t). \tag{35}$$

(10) Kernel D

In order to write Equation 35 as a matrix product, we first arrange O and R into vectors with 96 entries by row concatenation

$$\overrightarrow{O(t)} = (O_{1,1}(t), O_{1,2}(t), \ldots, O_{1,12}(t), \ldots, O_{8,1}(t), \ldots, O_{8,12}(t))^T \tag{36}$$

$$\overrightarrow{R(t)} = (R_{1,1}(t), R_{1,2}(t), \ldots, R_{1,12}(t), \ldots, R_{8,1}(t), \ldots, R_{8,12}(t))^T \tag{37}$$

and E into a 96×96 matrix that we called kernel D, with entries given by Equation 35:

$$D_{a,b} = E_{m-l+8,n-p+12}, \tag{38}$$

where $a = 12(m-1)+n$, $b = 8(l-1)+p$ with $m, l = 1, \ldots, 8$ and $n, p = 1, \ldots, 12$. A graphical example of such matrix is given in Figure S5. Equation 35 can be rewritten in matrix form as

$$\overrightarrow{O(t)} = D \cdot \overrightarrow{R(t)}. \tag{39}$$

The variance σ_D^2 follows from the same prescription used to build D. Figure S6 shows a graphical representation of the entries of the kernel D. We call the kernel assembled from Equation 26 the average kernel D, since it takes into account only the average values of the bleed-through factor B. We arrange standard deviations of the kernel into a matrix σ^D with entries $\sigma^D_{f,g} = \sqrt{\sigma^2_{D,f,g}}$ where $f = 1, \ldots, (M \cdot N)$, $g = 1, \ldots, (M \cdot N)$ and in our case $M = 8$, $N = 12$.

(10).1 Generalization of E and D

More generally, for a plate with M rows and N columns, Equation 35 becomes

$$O_{m,n}(t) = \sum_{l=1}^{M} \sum_{p=1}^{N} E_{m-l+M,n-p+N} R_{l,p}(t). \tag{40}$$

where E is a matrix with dimension $(2M-1) \times (2N-1)$. This equation can be rewritten as a matrix product. We first transform O and R into vectors with $M \cdot N$ entries by row concatenation

$$\overrightarrow{O(t)} = (O_1(t), O_2(t), \ldots, O_{M \cdot N}(t))^T \tag{41}$$

$$\overrightarrow{R(t)} = (R_1(t), R_2(t), \ldots, R_{M \cdot N}(t))^T \tag{42}$$

where we have re-labelled the entries. Then, if the calibration plate has luminescent well in position (α, β), we build E according to

$$E_{i,j} = \begin{cases} B_{i+\alpha-M,j+\beta-N}, & \text{if } M-\alpha+1 \le i \le 2M-\alpha,\ N-\beta+1 \le j \le 2N-\beta \\ \overline{B(x)}, & \text{otherwise, for all wells at distance x from } (\alpha,\beta) \\ z, & \text{otherwise,} \end{cases} \tag{43}$$

where $\overline{B(x)}$ represents the average of entries of B at the same distance $x = \sqrt{(m-\alpha)^2 + (n-\beta)^2}$ from the luminescent well (α, β) and z is computed by averaging intensities over the wells defined

in B as background. Finally, we obtain $\overrightarrow{O}$ from the matrix product

$$\overrightarrow{O_{N\cdot(m-1)+n}(t)} = \sum_{l=1}^{M}\sum_{p=1}^{N} E_{M-l+m,N-p+n}\overrightarrow{R_{N\cdot(l-1)+p}(t)} \tag{44}$$

with $m = 1,\ldots,M$ and $n = 1,\ldots,N$, or

$$\overrightarrow{O(t)} = D \cdot \overrightarrow{R(t)}, \tag{45}$$

where we have arranged the entries of E into a $(M \cdot N) \times (M \cdot N)$ matrix called kernel D with entries

$$D_{a,b} = E_{M-l+m,N-p+n} \tag{46}$$

where $a = N(m-1)+n$, $b = N(l-1)+p$ with $m,l = 1,\ldots,M$ and $n,p = 1,\ldots,N$. The process of building the kernel D from E corresponds to the creation of the associated block-Toeplitz matrix.

(10).2 A simple example of E and D

We can explicitly work out a simple example with a plate with dimension $(M,N) = (3,4)$ and the luminescent well in $(\alpha,\beta) = (2,3)$ (in red in the following). The measured time-averaged bleed-through matrix B is given by Equation 23

$$B = \begin{bmatrix} B_{1,1} & B_{1,2} & B_{1,3} & B_{1,4} \\ B_{2,1} & B_{2,2} & B_{2,3} & B_{2,4} \\ B_{3,1} & B_{3,2} & B_{3,3} & B_{3,4} \end{bmatrix}$$

and graphically represented in upper panel of Figure S7. The extended matrix E has dimensions $(2M-1)\times(2N-1) = 5\times 7$ and is given by

$$E = \left[\begin{array}{c|cccc|cc} E_{1,1} & E_{1,2} & E_{1,3} & E_{1,4} & E_{1,5} & E_{1,6} & E_{1,7} \\ \hline E_{2,1} & B_{1,1} & B_{1,2} & B_{1,3} & B_{1,4} & E_{2,6} & E_{2,7} \\ E_{3,1} & B_{2,1} & B_{2,2} & B_{2,3} & B_{2,4} & E_{3,6} & E_{3,7} \\ E_{4,1} & B_{3,1} & B_{3,2} & B_{3,3} & B_{3,4} & E_{4,6} & E_{4,7} \\ \hline E_{5,1} & E_{5,2} & E_{5,3} & E_{5,4} & E_{5,5} & E_{5,6} & E_{5,7} \end{array}\right].$$

The entries are computed according to prescription of Equation 43. Such process is explicitly shown in the second row of Figure S7. The final outcome is represented in the third row of Figure S7: the central matrix highlighted in red is the measured B, surrounded by $\overline{B}$ built by symmetry and in black the background value z. The kernel D has dimension $(M \cdot N)\times(M \cdot N) = 12\times 12$ and each

Toeplitz block has dimension $N \times N = 4 \times 4$. Applying Equation 46

$$D = \left[\begin{array}{cccc|cccc|cccc}
B_{2,3} & B_{2,2} & B_{2,1} & E_{3,1} & B_{1,3} & B_{1,2} & B_{1,1} & E_{2,1} & E_{1,4} & E_{1,3} & E_{1,2} & E_{1,1} \\
B_{2,4} & B_{2,3} & B_{2,2} & B_{2,1} & B_{1,4} & B_{1,3} & B_{1,2} & B_{1,1} & E_{1,5} & E_{1,4} & E_{1,3} & E_{1,2} \\
E_{3,6} & B_{2,4} & B_{2,3} & B_{2,2} & E_{2,6} & B_{1,4} & B_{1,3} & B_{1,2} & E_{1,6} & E_{1,5} & E_{1,4} & E_{1,3} \\
E_{3,7} & E_{3,6} & B_{2,4} & B_{2,3} & E_{2,7} & E_{2,6} & B_{1,4} & B_{1,3} & E_{1,7} & E_{1,6} & E_{1,5} & E_{1,4} \\
\hline
B_{3,3} & B_{3,2} & B_{3,1} & E_{4,1} & B_{2,3} & B_{2,2} & B_{2,1} & E_{3,1} & B_{1,3} & B_{1,2} & B_{1,1} & E_{2,1} \\
B_{3,4} & B_{3,3} & B_{3,2} & B_{3,1} & B_{2,4} & B_{2,3} & B_{2,2} & B_{2,1} & B_{1,4} & B_{1,3} & B_{1,2} & B_{1,1} \\
E_{4,6} & B_{3,4} & E_{4,6} & B_{3,2} & E_{3,6} & B_{2,4} & B_{2,3} & B_{2,2} & E_{2,6} & B_{1,4} & B_{1,3} & B_{1,2} \\
E_{4,7} & E_{4,6} & B_{3,4} & B_{3,3} & E_{3,7} & E_{3,6} & B_{2,4} & B_{2,3} & E_{2,7} & E_{2,6} & B_{1,4} & B_{1,3} \\
\hline
E_{5,4} & E_{5,3} & E_{5,2} & E_{5,1} & B_{3,3} & B_{3,2} & B_{3,1} & E_{4,1} & B_{2,3} & B_{2,2} & B_{2,1} & E_{3,1} \\
E_{5,5} & E_{5,4} & E_{5,3} & E_{5,2} & B_{3,4} & B_{3,3} & B_{3,2} & B_{3,1} & B_{2,4} & B_{2,3} & B_{2,2} & B_{2,1} \\
E_{5,6} & E_{5,5} & E_{5,6} & E_{5,3} & E_{4,6} & B_{3,4} & B_{3,3} & B_{3,2} & E_{3,6} & B_{2,4} & B_{2,3} & B_{2,2} \\
E_{5,7} & E_{5,6} & E_{5,5} & E_{5,4} & E_{4,7} & E_{4,6} & B_{3,4} & B_{3,3} & E_{3,7} & E_{3,6} & B_{2,4} & B_{2,3}
\end{array}\right]$$

that is graphically represented in last row of Figure S7. The vector of the observed intensities $\overrightarrow{O(t)}$ and expected corrected intensities $\overrightarrow{R(t)}$ are given by row concatenation

$$\overrightarrow{O(t)} = (O_{1,1}, O_{1,2}, O_{1,3}, O_{1,4}, O_{2,1}, O_{2,2}, O_{2,3}, O_{2,4}, O_{3,1}, O_{3,2}, O_{3,3}, O_{3,4})(t)^T$$
$$\overrightarrow{R(t)} = (R_{1,1}, R_{1,2}, R_{1,3}, R_{1,4}, R_{2,1}, R_{2,2}, R_{2,3}, R_{2,4}, R_{3,1}, R_{3,2}, R_{3,3}, R_{3,4})(t)^T.$$

The final convolution therefore results $\overrightarrow{O(t)} = D \cdot \overrightarrow{R(t)}$.

10.3 A general example of E and D

Given a B matrix with dimension $M \times N$ and luminescent well in (α, β)

$$B = \begin{bmatrix}
B_{1,1} & B_{1,2} & \dots & \dots & \dots & B_{1,N} \\
\dots & \dots & \dots & \dots & \dots & \dots \\
B_{\alpha,1} & B_{\alpha,2} & \dots & B_{\alpha,\beta} & \dots & B_{\alpha,N} \\
\dots & \dots & \dots & \dots & \dots & \dots \\
B_{M,1} & B_{M,2} & \dots & \dots & \dots & B_{M,N}
\end{bmatrix}$$

the extended matrix E with dimension $(2M-1) \times (2N-1)$ reads

$$E = \left[\begin{array}{ccc|ccc|ccc}
E_{1,1} & \dots & \dots & \dots & \dots & \dots & \dots & \dots & E_{1,2N-1} \\
\vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\
\hline
E_{M-\alpha+1,1} & \dots & E_{M-\alpha+1,n-\beta} & B_{1,1} & \dots & B_{1,N} & E_{M-\alpha+1,2N-\beta+1} & \dots & E_{M-\alpha+1,2N-1} \\
\vdots & \vdots & \vdots & \vdots & E_{M,N} & \vdots & \vdots & \vdots & \vdots \\
E_{2M-\alpha,1} & \dots & E_{2M-\alpha,n-\beta} & B_{M,1} & \dots & B_{M,N} & E_{2M-\alpha,2N-\beta+1} & \dots & E_{2M-\alpha,2N-1} \\
\hline
\vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\
E_{2M-1,1} & \dots & \dots & \dots & \dots & \dots & \dots & \dots & E_{2M-1,2N-1}
\end{array}\right]$$

where $E_{M,N} \equiv B_{\alpha,\beta}$. D is then a block-Toeplitz matrix with dimension $(M \cdot N) \times (M \cdot N)$

$$D = \left[\begin{array}{c|c|c|c|c} T(E_{M,N}) & T(E_{M-1,N}) & \dots & \dots & T(E_{1,N}) \\ \hline T(E_{M+1,N}) & T(E_{M,N}) & T(E_{M-1,N}) & \ddots & \ddots \\ \hline T(E_{M+2,N}) & T(E_{M+1,N}) & T(E_{M,N}) & \ddots & \ddots \\ \hline \vdots & \ddots & \ddots & \ddots & \ddots \\ \hline T(E_{2M-1,N}) & \ddots & \ddots & \ddots & T(E_{M,N}) \end{array}\right]$$

where each block $T(E_{x,y})$ is again a Toeplitz matrix

$$T(E_{x,y}) = \begin{bmatrix} E_{x,y} & E_{x,y-1} & \dots & \dots & E_{x,1} \\ E_{x,y+1} & E_{x,y} & E_{x,y-1} & \ddots & \ddots \\ E_{x,y+2} & E_{x,y+1} & E_{x,y} & \ddots & \ddots \\ \vdots & \ddots & \ddots & \ddots & \ddots \\ E_{x,2N-1} & \ddots & \ddots & \ddots & E_{x,y} \end{bmatrix}.$$

(11) Best kernel D by iteration

To take into account uncertainties in the estimation of the bleed-through factors and time fluctuations of luminescence, we replace the matrix E of the average bleed-through factors introduced by Equation 26 by a matrix with entries

$$\widetilde{E_{i,j}} = E_{i,j} + r_{i,j}\sigma^E_{i,j} \tag{47}$$

where σ^E and r are matrices which contain the standard deviations of the time-averaged bleed-through factors (obtained by taking the square root of the entries of Equation 29) and the number of standard deviations added to this last, respectively. The variance of $\widetilde{E}$ is given by

$$\sigma^2_{\widetilde{E}} = \sigma^2_E + r^2\sigma^2_E\sqrt{\frac{2}{T \cdot C - 1}} + 2r \cdot cov(E, \sigma^E), \tag{48}$$

where the second term is the standard deviation of the standard deviation of E, with C the number of replicates and T the time range over which the bleed-through factor B has been averaged. From $\widetilde{E}$, we can compute the kernel D via Equation 38. We have to look for that r that minimizes the difference between the spurious luminescence signal R after correction by deconvolution and the instrument sensitivity value s. Since such minimization is not computationally feasible in a single step, we approach the solution by iteration. The idea is to generate at each iteration a random kernel D and accept or reject it according to minimization criteria. The deconvolved plate is then used as luminescence input for the following iteration. The process is repeated till when there is no luminescence residual above the instrument sensitivity.

(11).1 Random kernel D

We first generate a random r with entries according to a normal distribution $\mathcal{N}(0,1)$, with average 0 and standard deviation 1. The kernel obtain in such a way, is called random kernel D^{rnd}. An equivalent solution is obtained by directly generating a random kernel D^{rnd} according to a normal distribution $\mathcal{N}$ with mean the corresponding entries of the average kernel D and standard deviation σ^D:

$$D^{\mathrm{rnd}} = \mathcal{N}(D, \sigma^D) \tag{49}$$

and use such matrix to deconvolve O and find R. The matrix of standard deviation $\sigma^{D\mathrm{rnd}}$ is obtained by uncertainty propagation. To keep track of the iteration cycle, we add an iteration index (k) to the notation ($D^{\mathrm{rnd}}_{(k)}$ for the kernel array and $R(t)_{(k)}$ for the real intensities array).

(12) Self-correction of the calibration plate

At each iteration, we substitute the array of real intensities R containing the bleed-through left-overs to the array of observed intensities: $\overrightarrow{O(t)} \equiv \overrightarrow{R(t)_{(k)}}$. Thus, by inverting Equation 39

$$\overrightarrow{R(t)_{(k+1)}} = (D^{\mathrm{rnd}}_{(k)})^{-1} \cdot \overrightarrow{R(t)_{(k)}}. \tag{50}$$

The propagation of uncertainties during matrix inversion process is discussed in Lefebvre *et al.*[1] and gives a variance

$$\sigma^2_{D^{-1}} = (D^{-1})^2 \cdot \sigma^2_D \cdot (D^{-1})^2 \tag{51}$$

that leads to a variance for R:

$$\sigma^2_{\overrightarrow{R}}(t) = \sigma^2_{D^{-1}} \cdot \overrightarrow{O(t)}^2 + (D^{-1})^2 \cdot \sigma^2_{\overrightarrow{O}} \tag{52}$$

where we assumed the covariance between O and D to be negligible. Square operation is here intended as square of each matrix entry (in MATLAB notation *x.^ 2*). We can re-arrange $\overrightarrow{R}$ into an array R by inverting the row concatenation process.

(13) Bleed-through left-over Q

After deconvolution, a perfect self-correction of the calibration plate should produce a plate that at any time point has only a single luminescent signal in $E5$ and all other wells have luminescence value under the instrument sensitivity s. We call I the array resulting from such ideal correction. I has entries

$$I_{m,n}(t) = \begin{cases} O_{5,5}(t), & m = 5,\ n = 5 \\ s, & \text{otherwise,} \end{cases}. \tag{53}$$

The best correction is achieved after (K) iterations, when there are no more Q left-overs:

$$Q = \sum_{m=1}^{M} \sum_{N=1}^{N} \sum_{t=1}^{T} (R_{m,n}(t)_{(k+1)} - I_{m,n}(t)) \equiv 0. \tag{54}$$

On the contrary, if after an iteration we still have some left-overs ($Q > 0$), we proceed by applying the acceptance criteria to the left-overs intensity array $R_{(k+1)}$.

(14) Acceptance criteria

We apply the acceptance criteria to each entry of the deconvolved $R_{(k+1)}$ independently. If a single entry of $R_{(k+1)}$ has value that is

a) smaller than the previous iteration $R_{m,n,(k+1)}(t) \leq R_{m,n,(k)}(t)$, we accept and store the new value since the bleed-through has been reduced

b) larger than the previous iteration $R_{m,n,(k+1)}(t) > R_{m,n,(k)}(t)$, we reject the move by setting back $R_{m,n,(k+1)}(t) = R_{m,n,(k)}(t)$.

Standard deviation follows the fate of the corresponding entry of R. Theoretically, at any time point the bleed-through pattern depends simultaneously on the luminescence of all wells, but here, to guide the convergence of the minimization algorithm, we accept or reject each entry of R separately. This is allowed only if each random iteration perturbs minimally the mean value of the bleed-through factors. We ensure this condition by using instead of r in Equation 47 a much smaller value $a \cdot r$, with $a \ll 1$ (in our case $a = 1/10$).

(15) Update and iterate

If after (k) iterations there still is some bleed-through ($Q > 0$), we imagine the pattern of deconvolved luminescence $R_{(k+1)}$ as the new observed luminescence O ($O \equiv R_{(k+1)}$), update the iteration index (k) and repeat the procedure from point 6.

(16) Best kernel D

The best correction is achieved at iteration (K) when, after deconvolution, all left-overs are zero at any time point ($Q = 0$). From Equation 50, it follows that over the iterations

$$\overrightarrow{O(t)} = D^{\mathrm{rnd}}_{(1)} \overrightarrow{R(t)_{(2)}(t)} \qquad \text{for k = 1} \tag{55}$$

$$\overrightarrow{R_{(2)}(t)} = D^{\mathrm{rnd}}_{(2)} \overrightarrow{R_{(3)}(t)} \qquad \text{for k = 2} \tag{56}$$

$$\vdots \tag{57}$$

$$\overrightarrow{R_{(K)}(t)} = D^{\mathrm{rnd}}_{(K)} \overrightarrow{I(t)} \qquad \text{for k = K} \tag{58}$$

where $\overrightarrow{I(t)}$ is the identity matrix transformed into vector by row concatenation. Therefore

$$\overrightarrow{O(t)} = \prod_{k=1}^{K} D^{\mathrm{rnd}}_{(k)} \overrightarrow{I(t)} \equiv D^{\mathrm{best}} \overrightarrow{I(t)} \tag{59}$$

where we define the best kernel via the product of the iteration kernels

$$D_{\mathrm{best}} \equiv \prod_{k=1}^{K} D^{\mathrm{rnd}}_{(k)}. \tag{60}$$

The matrices $\sigma^{R}_{(k)}(t)$ and $\sigma^{D}_{\mathrm{best}}$ containing the standard deviations are obtained from Equation 52 and Equation 48, respectively.

1.3.2 Analysis of the experiment plate

We follow the scheme of Figures S21.

(17) Raw luminescence L of the experiment

Next, we measure the luminescence on the plate that needs to be bleed-through corrected. For simplicity we refer to it as the test plate. We arrange the raw luminescence data into an array L^{exp} with entries $L^{\text{exp}}_{m,n}(t,c)$, where m and n indicate row and column of the plate, t the time point and c the experiment replica.

(18) Background-subtracted luminescence O of the experiment

Due to the luminescence bleed-through, the background can not be obtained from the test plate. Instead, we use the cross-talk-free background b from the calibration plate, given by Equation 13. For such reason, it is better if the two measurements are performed under the same conditions and with the same machine setup. The variance of the background is obtained from Equation 14. After subtracting the background from the raw luminescence, we obtain the array of background-subtracted luminescence $O^{\text{exp}}(t,c)$ with corresponding array of standard deviations $\sigma^{O\text{exp}}(t,c)$.

(19) Bleed-through correction R of the experiment

The array of observed luminescence is given by the product of the kernel D_{best} (Equation 60) and the array of unknown "real" intensities R:

$$\overrightarrow{O^{\text{exp}}(t,c)} = D_{\text{best}} \cdot \overrightarrow{R^{\text{exp}}(t,c)}, \tag{61}$$

where O and R are transformed into vector by row concatenation. We achieve the most probable arrangement of real intensities by inverting the previous equation

$$\overrightarrow{R^{\text{exp}}(t,c)} = (D_{\text{best}})^{-1} \cdot \overrightarrow{O^{\text{exp}}(t,c)}. \tag{62}$$

Then, we transform back $\overrightarrow{R^{\text{exp}}(t,c)}$ and $\overrightarrow{O^{\text{exp}}(t,c)}$ into arrays $R^{\text{exp}}_{m,n}(t,c)$ and $O^{\text{exp}}_{m,n}(t,c)$, respectively by inverting the row-concatenation process. The array of standard deviation is obtained again by uncertainty propagation as described by Equation 52.

(20) Replica average of the experiment

Finally, we average the luminescence over several replicates. The replica-averaged $R^{\text{exp}}(t)$ has entries

$$R^{\text{exp}}_{m,n}(t) = \frac{\sum_{c=1}^{C} w_R^{\text{exp}}(t,c)_{m,n} R^{\text{exp}}_{m,n}(t,c)}{\sum_{c=1}^{C} w_R^{\text{exp}}(t,c)_{m,n}} \tag{63}$$

with weights $w_R^{\text{exp}}(t,c)_{m,n} = 1/\sigma_R^{\text{exp}\,2}(t,c)_{m,n}$ and variance

$$\sigma_R^{\text{exp}\,2}(t)_{m,n} = \frac{\sum_{c=1}^{C} w_R^{\text{exp}}(t,c)_{m,n} (R^{\text{exp}}_{m,n}(t,c) - R^{\text{exp}}_{m,n}(t))^2}{\sum_{c=1}^{C} w_R^{\text{exp}}(t,c)_{m,n}}. \tag{64}$$

where $m = 1, \ldots, 8$, $n = 1, \ldots, 12$ and $t = 1, \ldots, T$. Standard deviations are arranged in an array $\sigma^{R\text{exp}}(t)$, with entries $\sigma^{R\text{exp}}_{m,n}(t)$.

2 Supporting reference

[1] Lefebvre M, Keeler RK, Sobie R, and White J. Propagation of errors for matrix inversion. *arXiv*, 1999.

3 Supporting figures

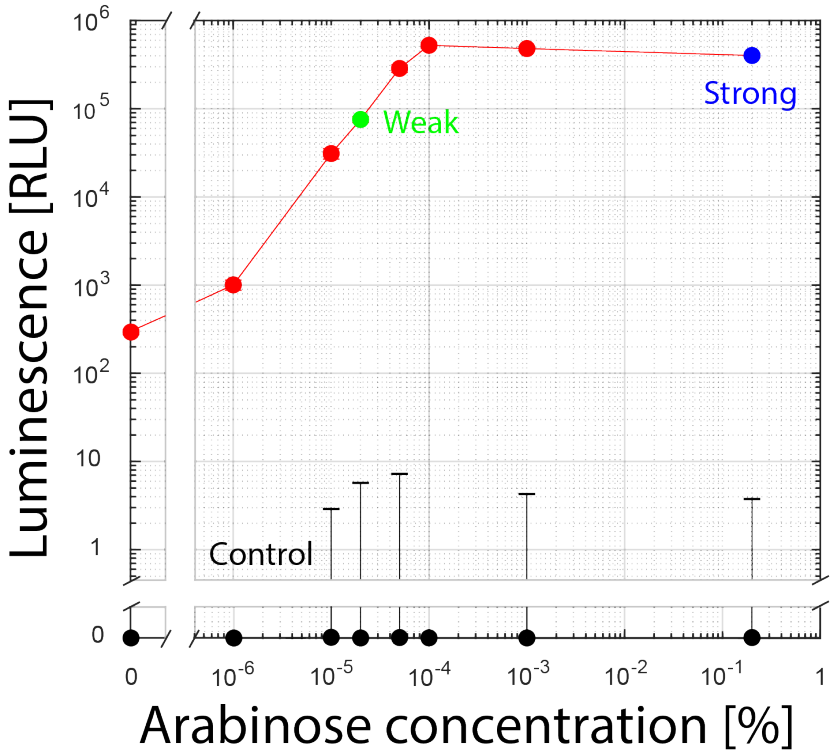

Figure S1: Dose-response plot for the *E. coli* strain GFC0153: luminescence intensity as function of arabinose concentration for three independent replicates. Luminescence intensities used to assemble the calibration plates with high and low luminescence are indicated as blue and green points, respectively. The plot is obtained from the data of Figure 3E, 3F in the main text at 270 minutes after arabinose induction. In black, the intensities of the non-luminescent *E. coli* strain SV01, used as control, with which is filled the calibration plates (excluding well $E5$).

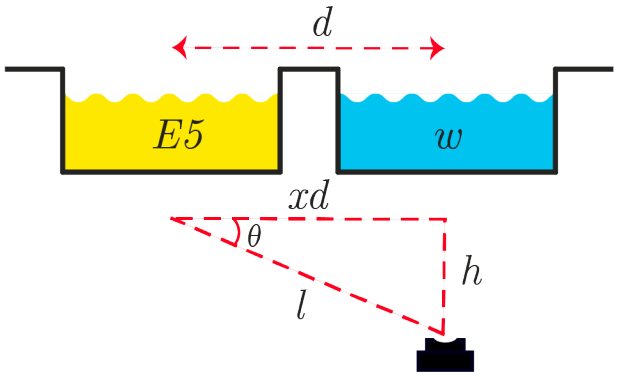

Figure S2: Representation of the microplate reader wells and of the detector. A photo-detector positioned at an offset h from a microplate well w receives luminescence incoming from the emitting well $E5$ under an angle θ. If d is the distance between neighbouring wells, xd and l are the distances between $E5$ and w and $E5$ and the detector, respectively.

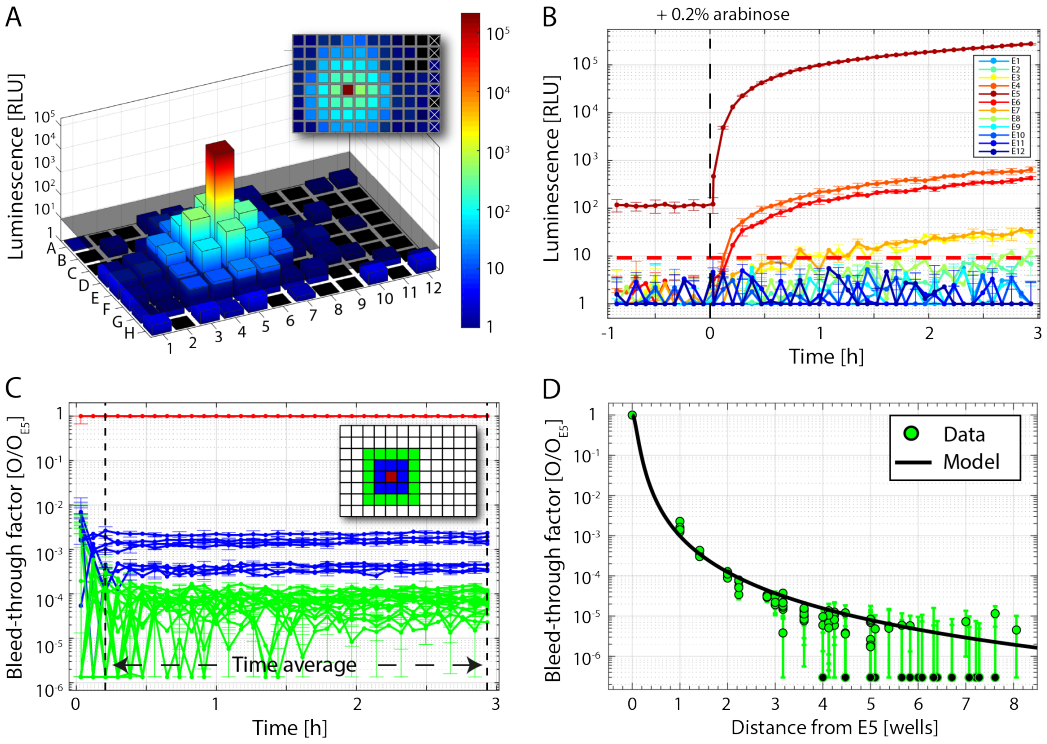

Figure S3: Quantification of luminescence bleed-through in the VICTOR plate reader. (A) The calibration plate was prepared with a single highly luminescent spot in the well $E5$. All data are background-subtracted and averaged over three experimental replicates. The background was obtained by averaging the signal of the wells indicated by the white crosses in the inset over the time before addition of arabinose (induction time). The 3D-plot shows the observed luminescence signal at 140 minutes after the induction. (B) Time evolution of the observed luminescence signal of the E-row. The red line represents the instrument sensitivity value, defined as three times the standard deviation of the background value. (C) Luminescence bleed-through factor of the two shells closest to $E5$, in blue and green. Values were obtained dividing the observed signal from a specific well by the signal recorded in $E5$. The dashed lines indicate the time range over which the bleed-through was averaged to obtain in (D) the bleed-through factor as a function of the distance from $E5$ (green dots). The black solid line is the parameter free prediction of the bleed-through given by Equation 6.

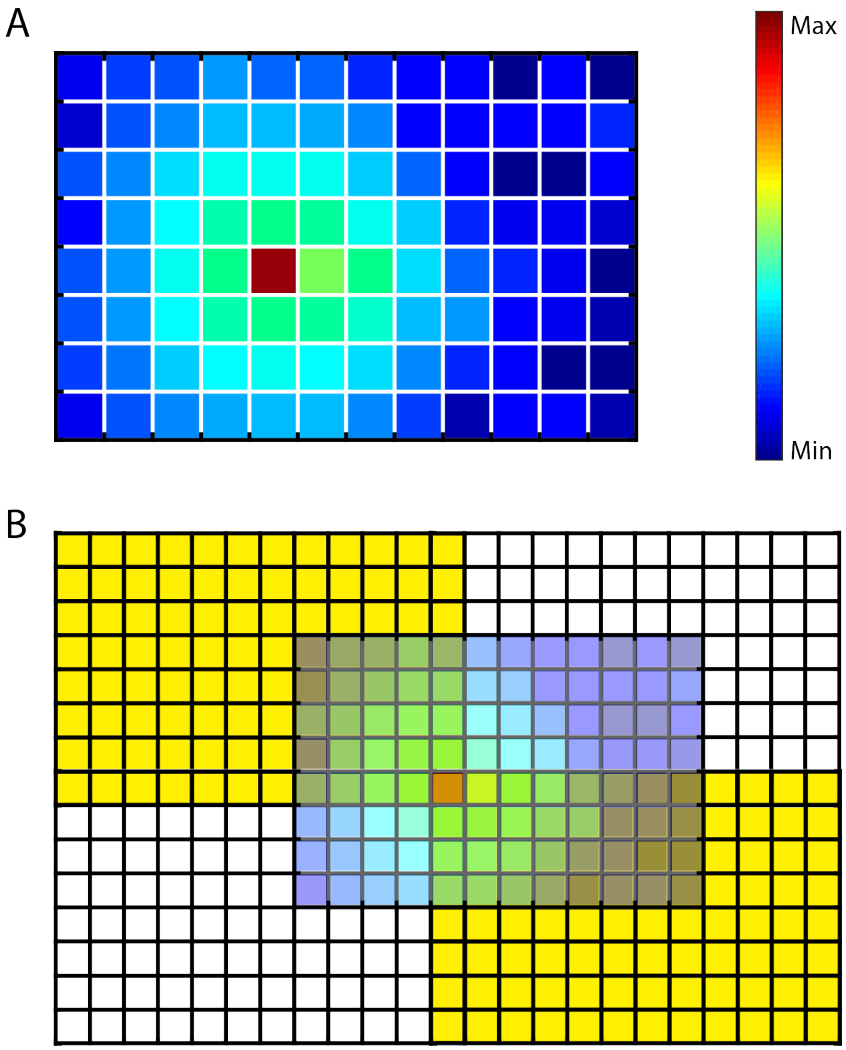

Figure S4: (A) Representation of a bleed-through matrix B generated by measuring the bleed-through pattern produced in a plate with a single luminescent well $E5$. (B) To convolve the bleed-through matrix B with the array of real intensities R (in yellow), we enlarge B (in color) to an extended bleed-through matrix E (black grid).

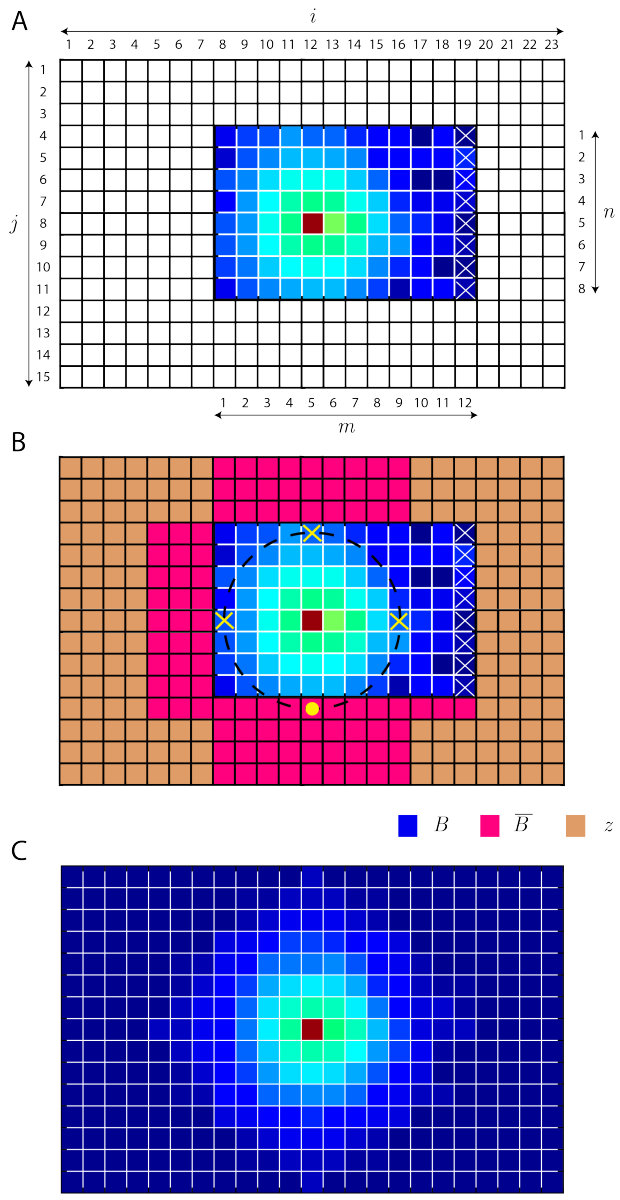

Figure S5: Graphical representation of the entries of an extended matrix E generated by a calibration plate with luminescent well in $E5$. The central part is occupied by the measured bleed-through factor B (in colors, panel A), the magenta area is filled by symmetry with $\overline{B(x)}$ and the brown area with the value z (in panel B). The entries of E that we called $\overline{B}$ fall in the magenta area. They are computed by averaging the measured values of B at a same distance x from the luminescent well $E5$ (see Equation 26). For example, when $x = 4$, we average the three measured values in the wells with yellow crosses to obtain the value represented by the yellow dot, that lies outside the measured matrix. The entries of E where such symmetry construction is not possible fall in the brown area. They are given by the value z as indicated in the third line of Equation 26 and computed by averaging the values of B in the wells indicated by the white crosses. Panel C shows the final result.

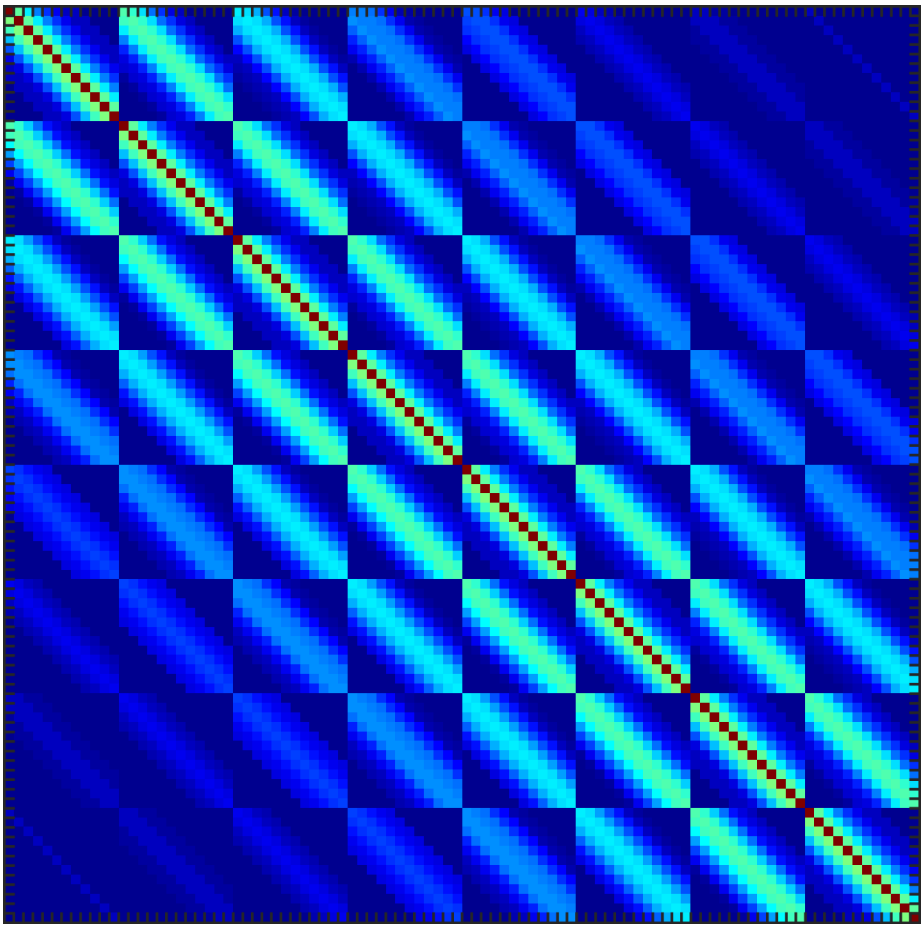

Figure S6: Graphical representation of the entries of the kernel D generated by the extended matrix E of Figure S5. Each pixel corresponds to an entry.

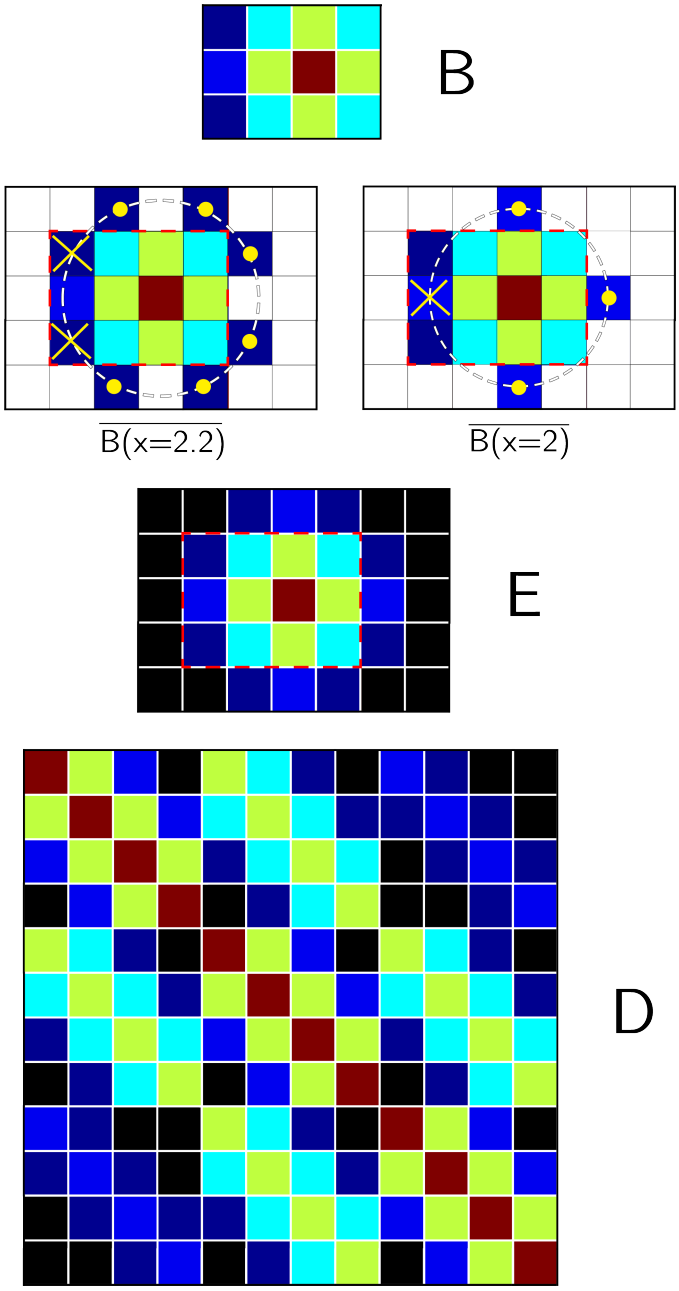

Figure S7: Graphical representation of the example given in section 10.2. The first row shows a measured time-averaged bleed-through matrix B. The following rows show step-by-step how we extended the matrix to obtain E: we averaged the measured values of B at a same distance x from the luminescent well, first for $x = \sqrt{5} \simeq 2.2$ and then for $x = 2$. Yellow crosses show the measured values. The results are used to fill the entries of E for that specific distance x outside the measured matrix, indicated by yellow dots. We highlight in red the measured values of B. The remaining entries of E are filled with the background value z, in black. The last row shows the corresponding kernel D, computed following Equation 46.

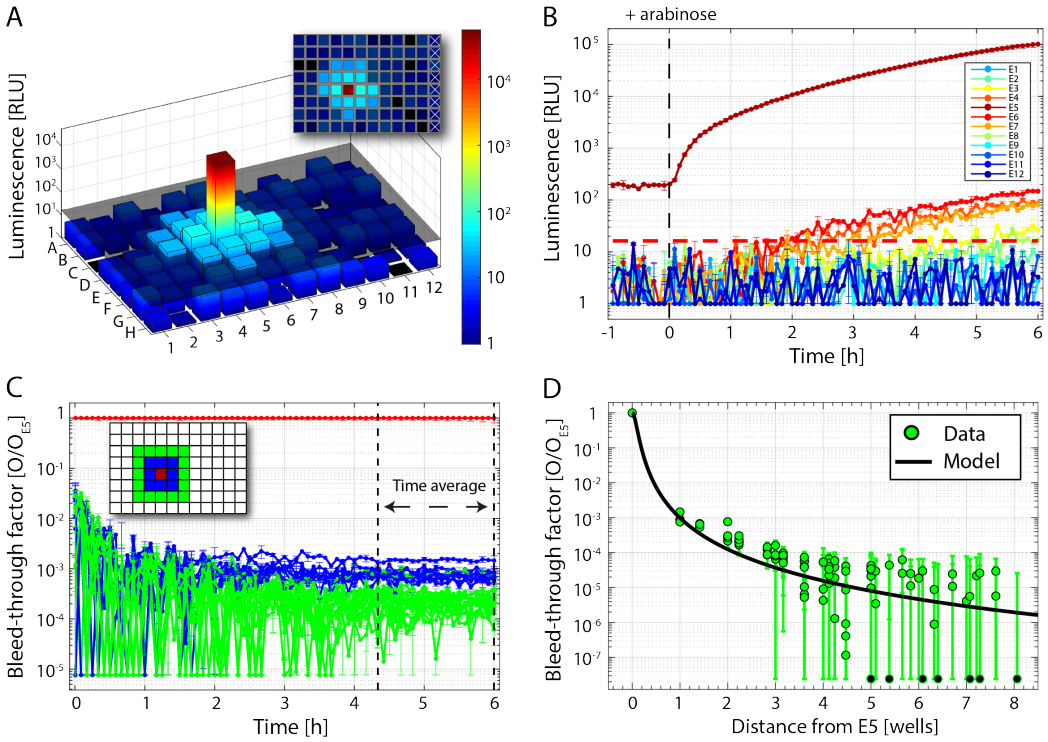

Figure S8: Quantification of luminescence bleed-through in the black calibration microplate for a calibration plate with low luminescence intensity. (A) The black calibration plate was prepared with a single mildly luminescent spot in the well $E5$. All data are background-subtracted and averaged over two experimental replicates. The background was obtained by averaging the signal of the wells indicated by the white crosses in the inset over the time before addition of arabinose (induction time). The 3D-plot shows the observed luminescence signal at 270 minutes after the induction with $2 \cdot 10^{-5}$% arabinose. (B) Time evolution of the observed luminescence signal of the E-row. The red line represents the instrument sensitivity value, defined as three times the standard deviation of the background value. (C) Luminescence bleed-through factor of the two shells closest to $E5$, in blue and green. Values were obtained dividing the observed signal from a specific well by the signal recorded in $E5$. The dashed lines indicate the time range over which the bleed-through was averaged to obtain in (D) the bleed-through factor as a function of the distance from $E5$ (green dots). The black solid line is the parameter free prediction of the bleed-through given by Equation 6.

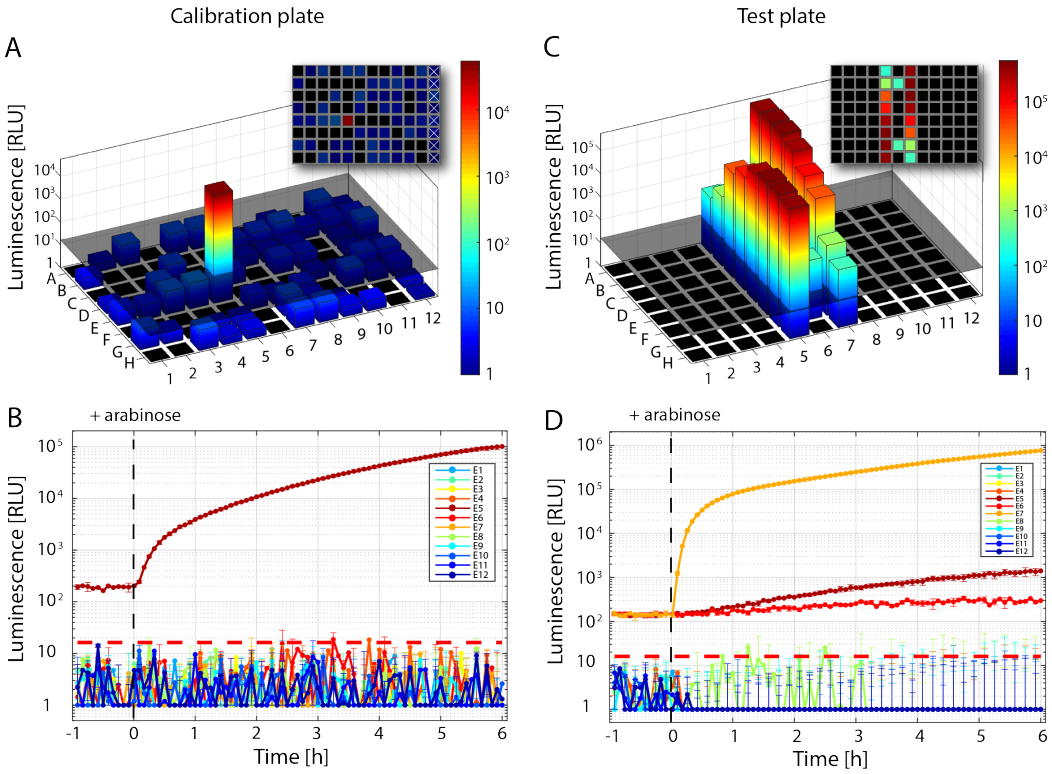

Figure S9: (A, B) Deconvolution of luminescence signals in the calibration microplate with low luminescence intensity. Luminescence values on the calibration plate 270 minutes after the induction of *E. coli* strain GFC0153 (in well *E*5) with with $2 \cdot 10^{-5}\%$ (A) and its time evolution in the wells of row E after deconvolving by using the best kernel D^{best} (B). (C, D) Deconvolution of luminescence signals in the test microplate by using the calibration plate with low luminescence intensity. Luminescence values on the test plate 270 minutes after the induction of *E. coli* strain GFC0153 with varying concentrations of arabinose (in wells A5-H5: 0%, $10^{-6}\%$, $10^{-5}\%$, $2 \cdot 10^{-5}\%$, $5 \cdot 10^{-5}\%$, $10^{-4}\%$, $10^{-3}\%$, $2 \cdot 10^{-1}\%$; in wells A7-H7: $2 \cdot 10^{-1}\%$, $10^{-3}\%$, $10^{-4}\%$, $5 \cdot 10^{-5}\%$, $2 \cdot 10^{-5}\%$, $10^{-5}\%$, $10^{-6}\%$, 0%; in wells B6 and G6: 0%) in C. The time evolution of luminescence values is shown in the wells of row B after deconvolving by using either the best kernel D^{best} (in D).

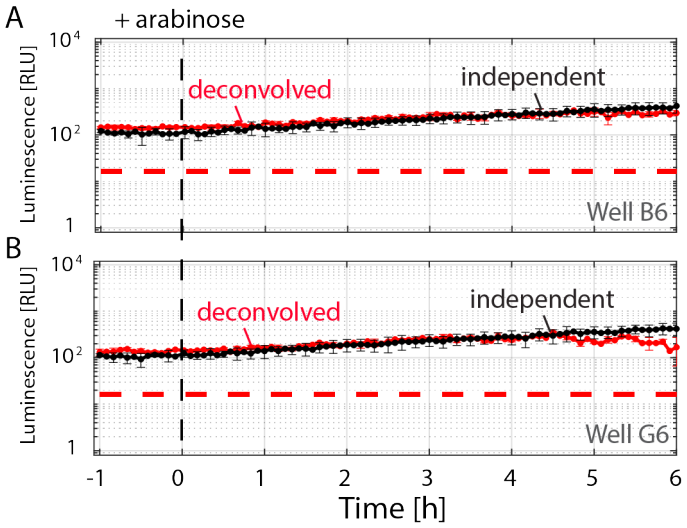

Figure S10: Comparison between deconvolution results and independent measurement. The luminescence values of wells B6 (panel A) and G6 (panel B) of the deconvolved test plate as compared to independent measurements in the absence of bleed-through, for which *E. coli* strain GFC0153 in wells B6 and G6 was not induced with arabinose, as described in the main text Figure 4. Black and red lines represent the independently measured and the deconvolved signal, respectively.

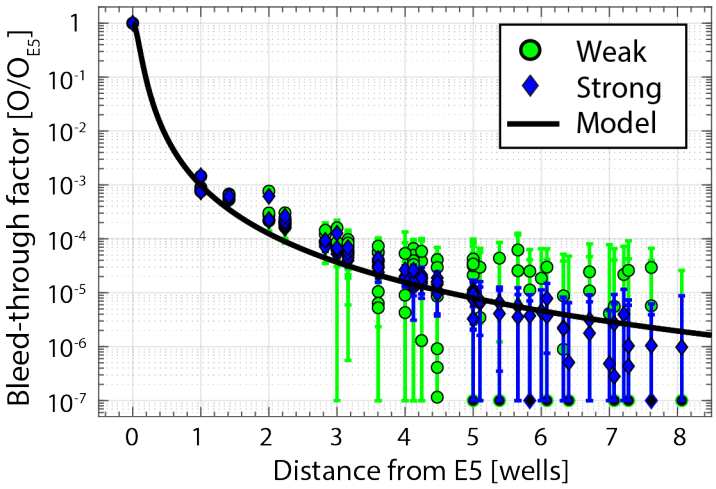

Figure S11: Comparison between bleed-through factor generated from the strongly induced luminescent strain (induced with 0.2% arabinose, from Figure 1 of the main text) and the weakly induced luminescent strain (induced with $2 \cdot 10^{-5}$% arabinose, from Figure S8) as a function of the distance from $E5$ as blue diamonds and green points, respectively. The figure shows that the bleed-through factor is independent of the luminescence signal strength, for values of the signal above the background.

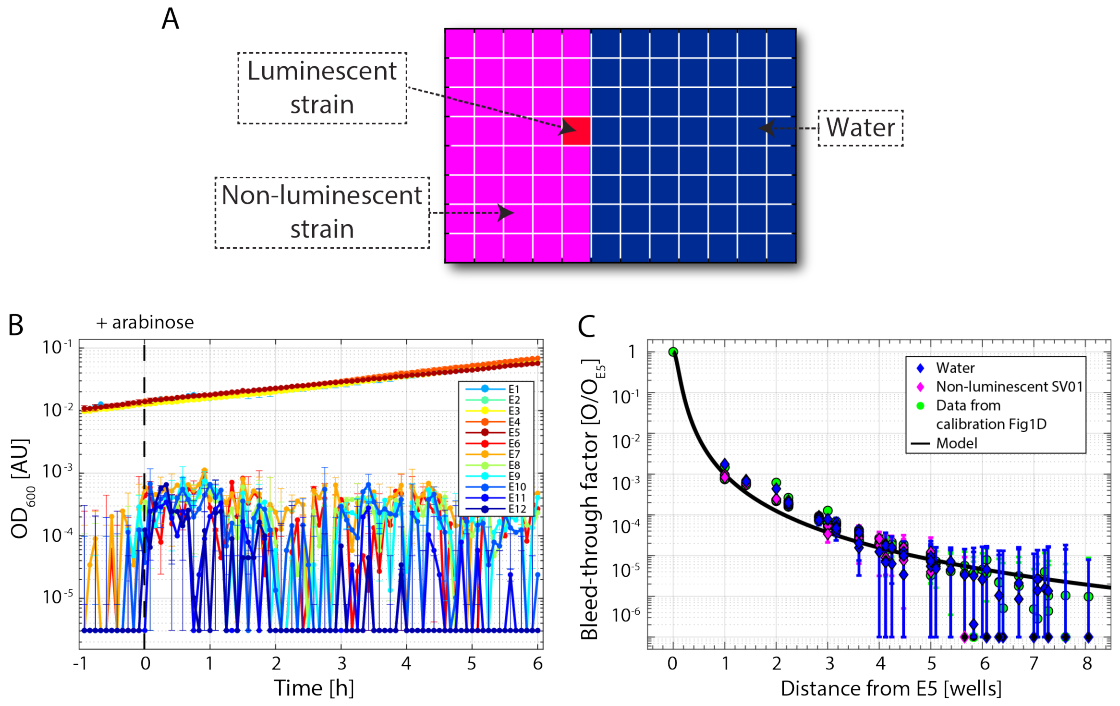

Figure S12: Panel A shows the arrangement of the calibration plate: left half (columns 1 to 5) was filled with the non-luminescent *E. coli* strain SV01 and right half (columns 6 to 12) with water. Water and bacterial strain SV01 have two order of magnitude difference in the measured OD (10^{-4} and 10^{-2}, respectively), as shown from the OD measured along row E of the plate, in panel B. Panel C demonstrates that bleed-through factors of water (blue diamonds) and SV01 (magenta diamonds) have same values of the calibration plate of Figure 1D in the main text (green points).

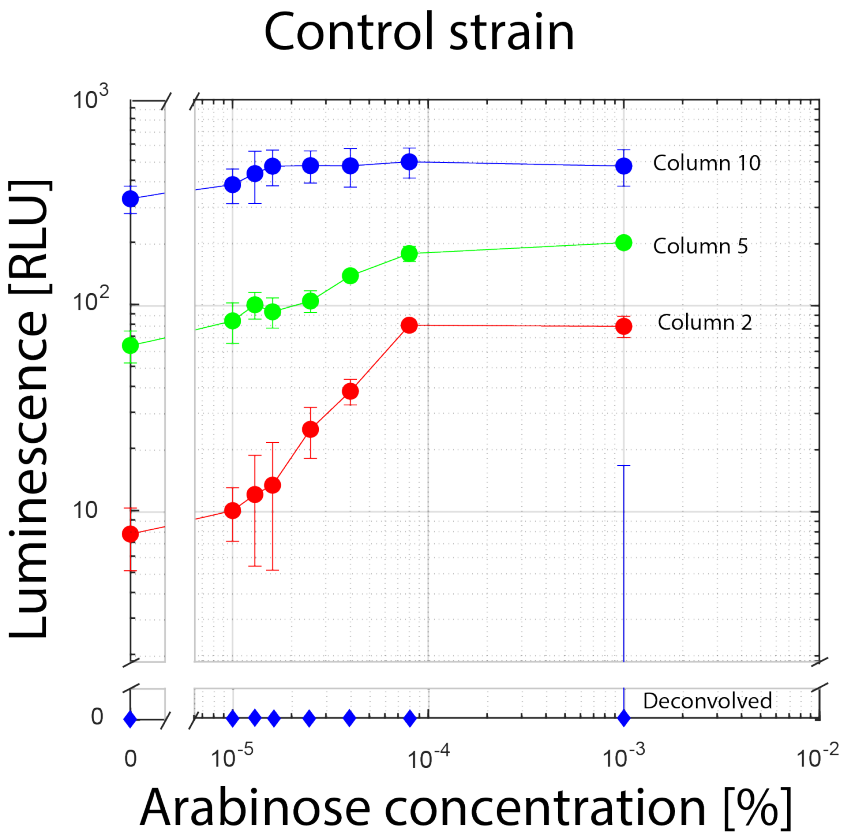

Figure S13: Dose-response of the bleed-through signals of the non-luminescent control strains in column 2, 5 and 10 of the plate in Figure 5 before (points) and after (diamonds) deconvolution process at 270 minutes after adding arabinose.

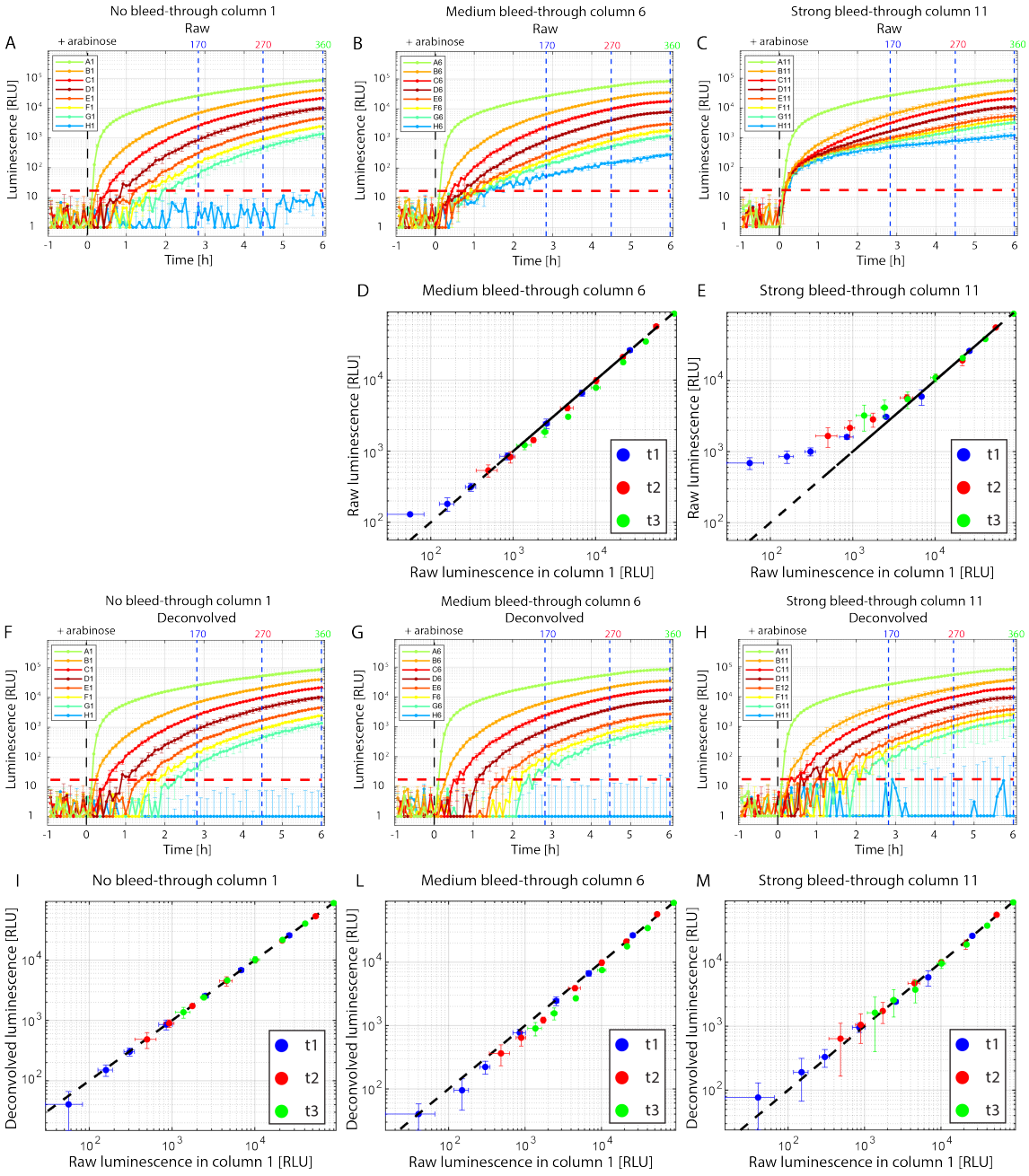

Figure S14: Time evolution of luminescence values of column 1 (no bleed-through, panel A raw and F deconvolved), of column 6 (medium bleed-through, panel B raw and G deconvolved) and of column 11 (strong bleed-through, panel C raw and H deconvolved) of Figure 5 in main text. Panels D, E are the scatter plot of raw luminescence of column 6 and 11 (medium and strong bleed-through, respectively) as function of raw luminescence of column 1. Panels I, L and M show the scatter plots of deconvolved luminescence of column 1 (no bleed-through, G), 6 (medium bleed-through, H) and 11 (strong bleed-through, I) as function of luminescence of column 1. Scatter plots are represented at three time points: 170 minutes in blue, 270 minutes in red and 360 minutes in green. The black dashed line has slope one.

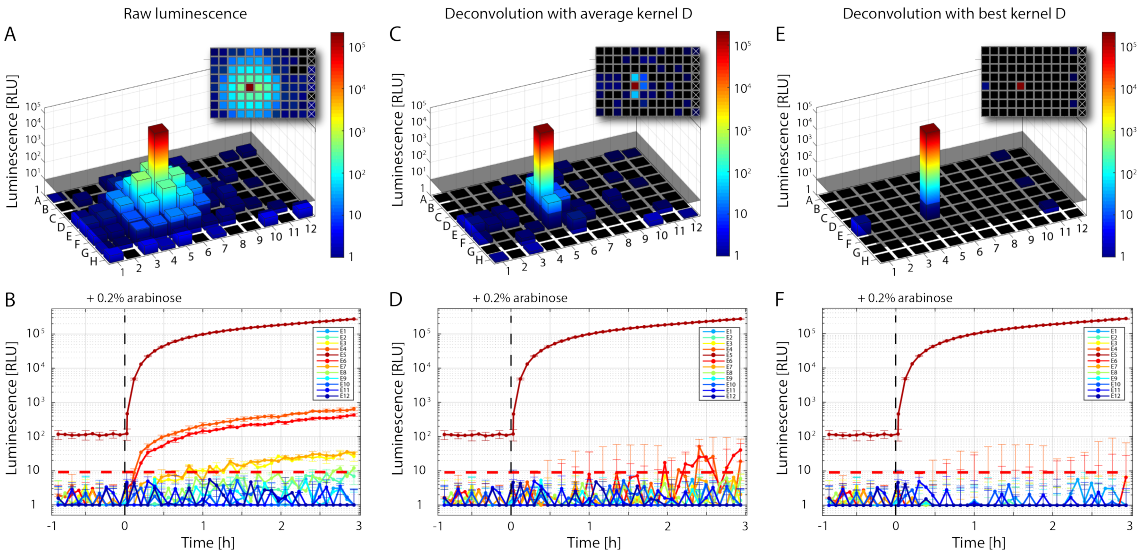

Figure S15: Calibration microplate in the VICTOR plate reader. Luminescence values on the calibration plate at 140 minutes after the induction (A, C and E) and its time evolution on the E-row (B, D and F) for raw signal (A, B), after deconvolving by using either the average kernel D (C, D) or the best kernel D_{best} (E and F).

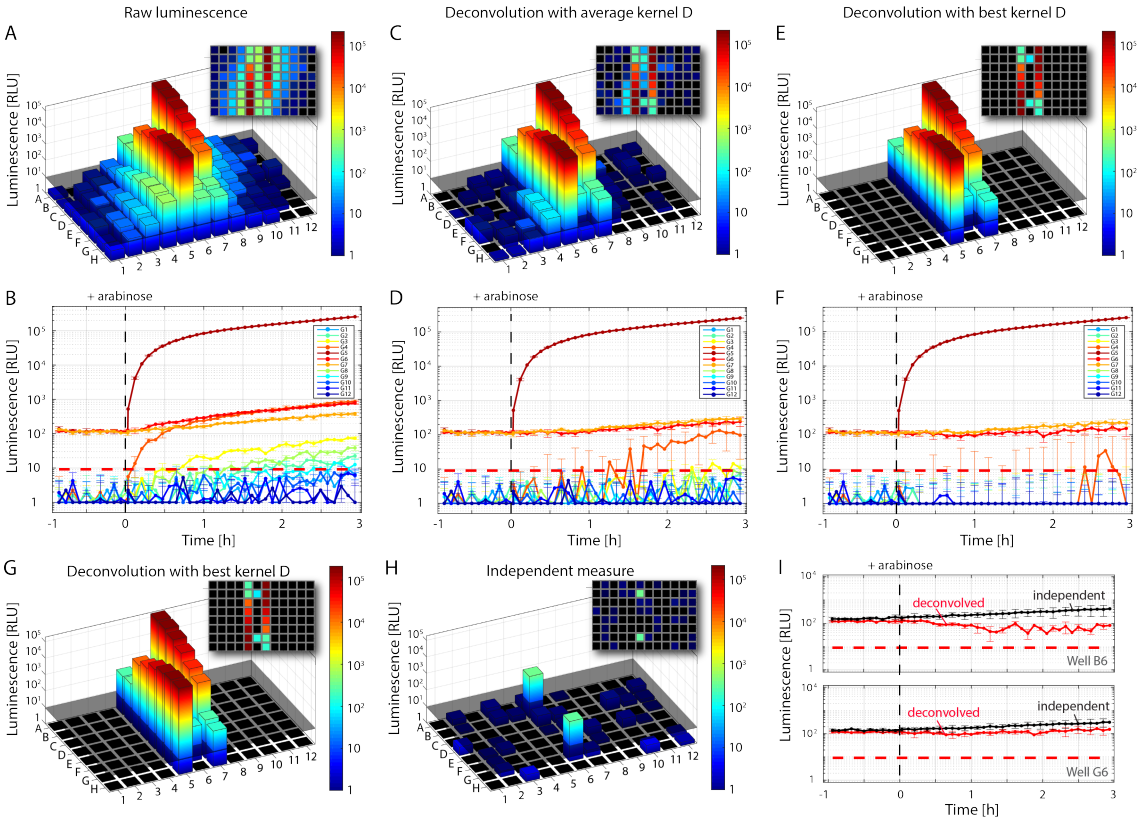

Figure S16: Test microplate in the VICTOR plate reader: luminescence values on the test plate at 270 minutes after the induction (A, C and E) and its time evolution on the G-row (B, D and F) for raw signal (A, B), after deconvolving by using either the average kernel D (C, D) or the best kernel D_{best} (E and F). Panels G H and I show the comparison between deconvolved test plate and independent measure. The luminescence values of wells B6 and G6 of the deconvolved test plate (G) were compared to independent measure in the absence of bleed-through (H). Black and red lines in (I) represent the independently measured and the deconvolved signal, respectively.

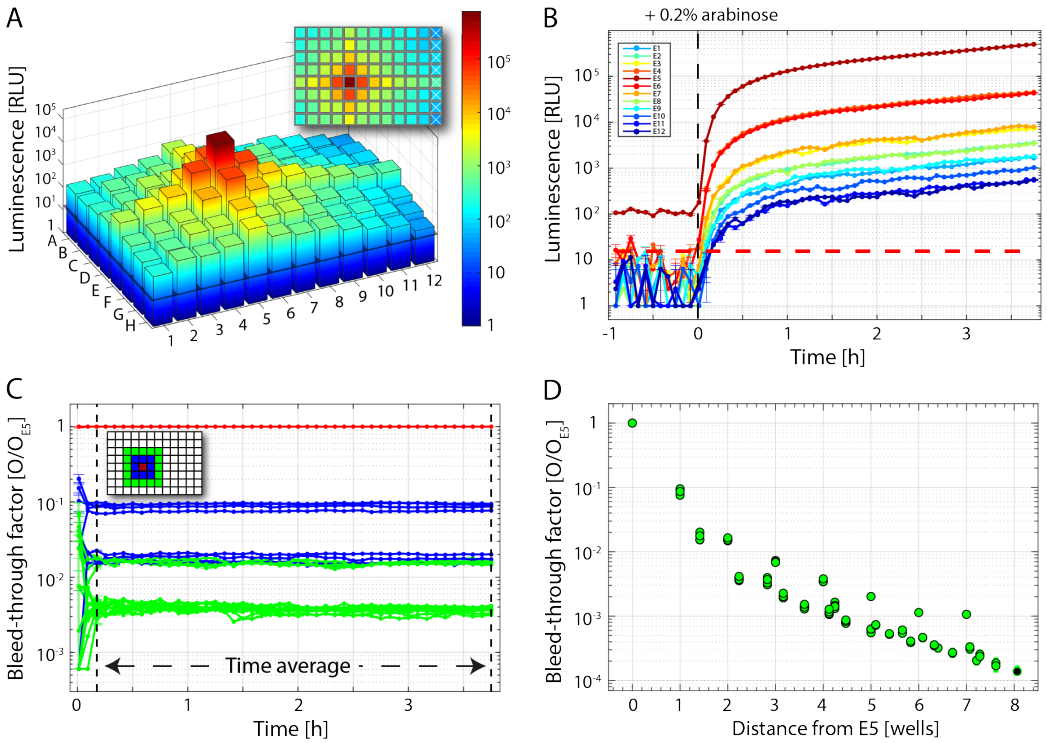

Figure S17: Quantification of luminescence bleed-through in the Tecan plate reader using a transparent microplate. (A) The calibration plate was prepared with a single highly luminescent spot in the well $E5$. All data are background-subtracted and averaged over three experimental replicates. The background was obtained by averaging the signal of the wells indicated by the white crosses in the inset over the time before addition of arabinose (induction time). The 3D-plot shows the observed luminescence signal at 135 minutes after the induction. (B) Time evolution of the observed luminescence signal of the E-row. The red line represents the instrument sensitivity value, defined as three times the standard deviation of the background value. (C) Luminescence bleed-through factor of the two shells closest to $E5$, in blue and green. Values were obtained dividing the observed signal from a specific well by the signal recorded in $E5$. The dashed lines indicate the time range over which the bleed-through was averaged to obtain in (D) the bleed-through factor as a function of the distance from $E5$ (green dots).

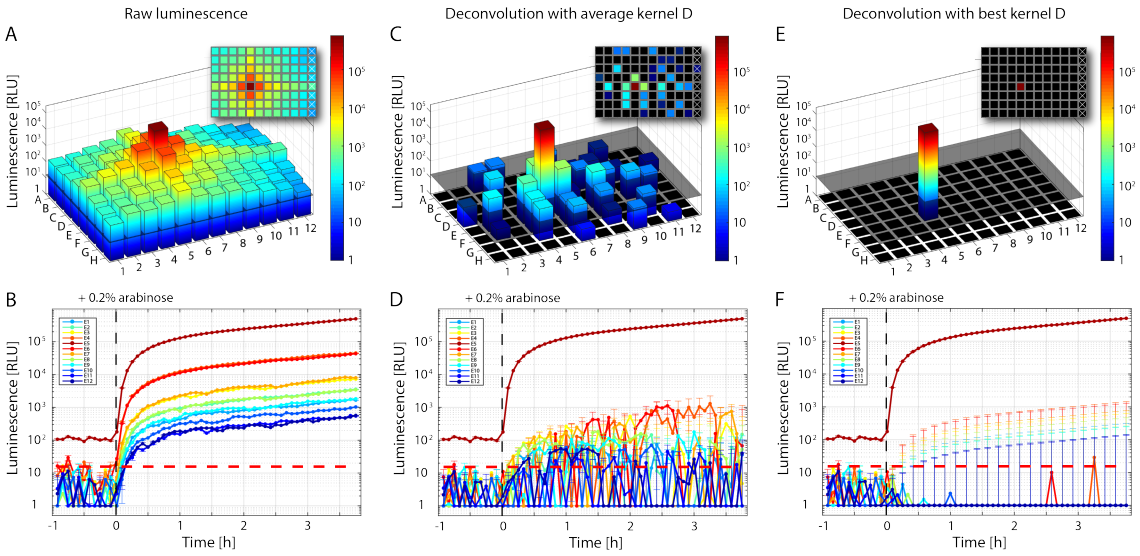

Figure S18: Calibration microplate in the Tecan plate reader with a transparent microplate. Luminescence values on the calibration plate at 135 minutes after the induction (A, C and E) and its time evolution on the E-row (B, D and F) for raw signal (A, B), after deconvolving by using either the average kernel D (C, D) or the best kernel D_{best} (E and F).

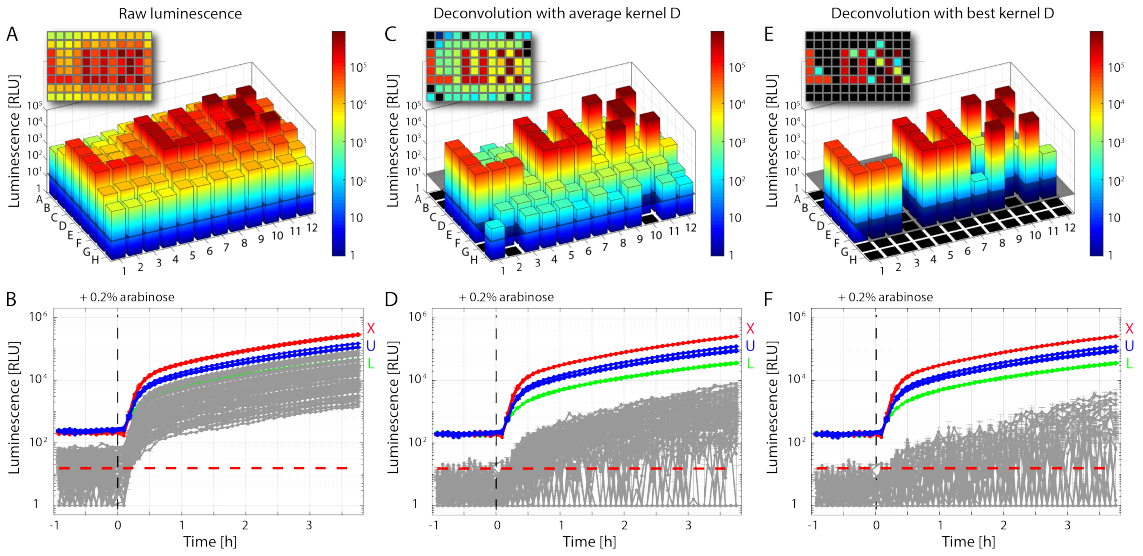

Figure S19: Test microplate in the Tecan plate reader with a transparent microplate. Luminescence values on the test plate at 135 minutes after the induction (A, C and E) and its time evolution on the E-row (B, D and F) for raw signal (A, B), after deconvolving by using either the average kernel D (C, D) or the best kernel D_{best} (E and F). In the test plate we arranged luminescent wells to compose the word LUX. In B, D and F the time evolution of the luminescence of L, U, X and remaining wells are represented in green, blue, red and gray, respectively.

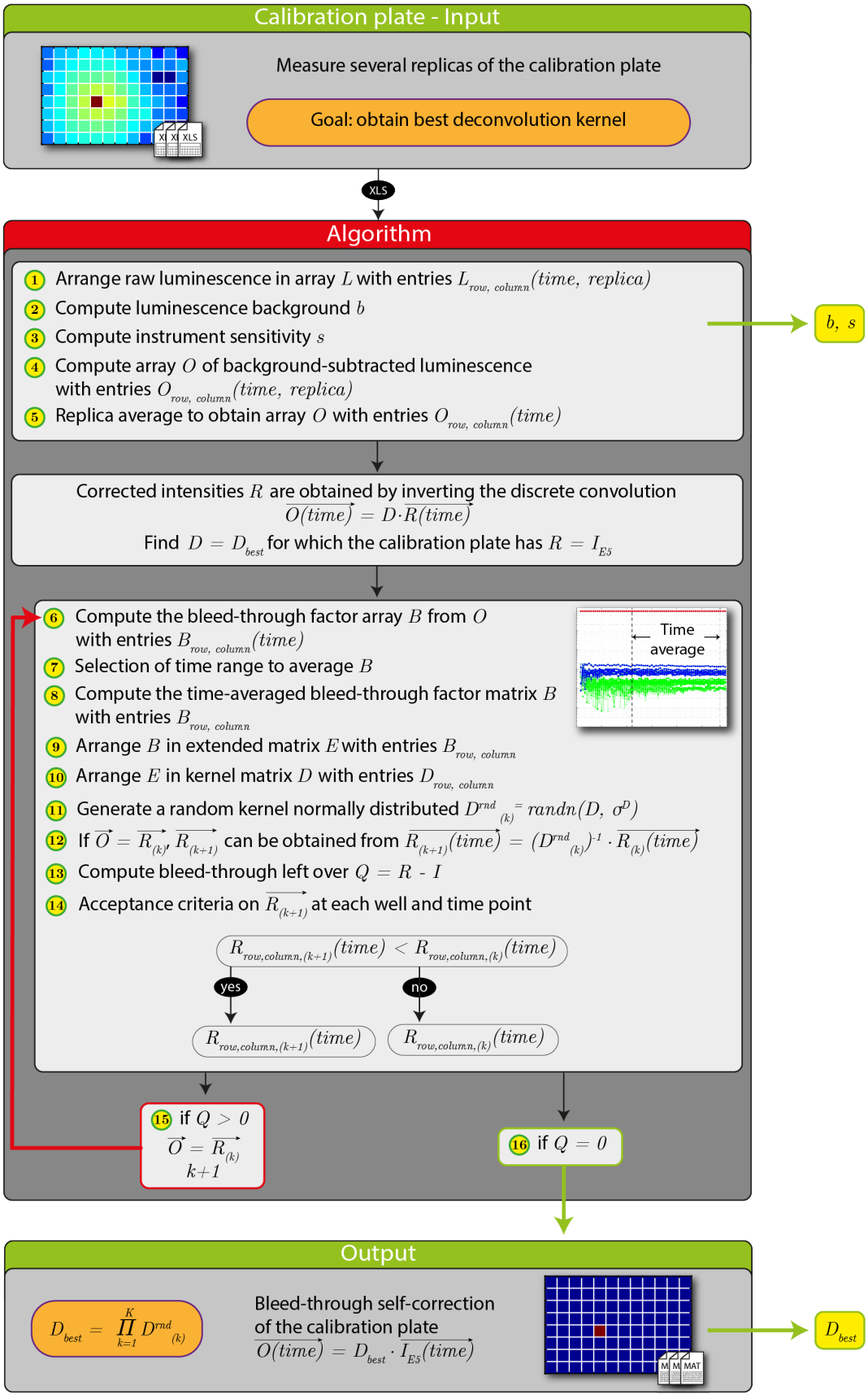

Figure S20: Workflow to find the best kernel D_{best} from the calibration plate measurement.

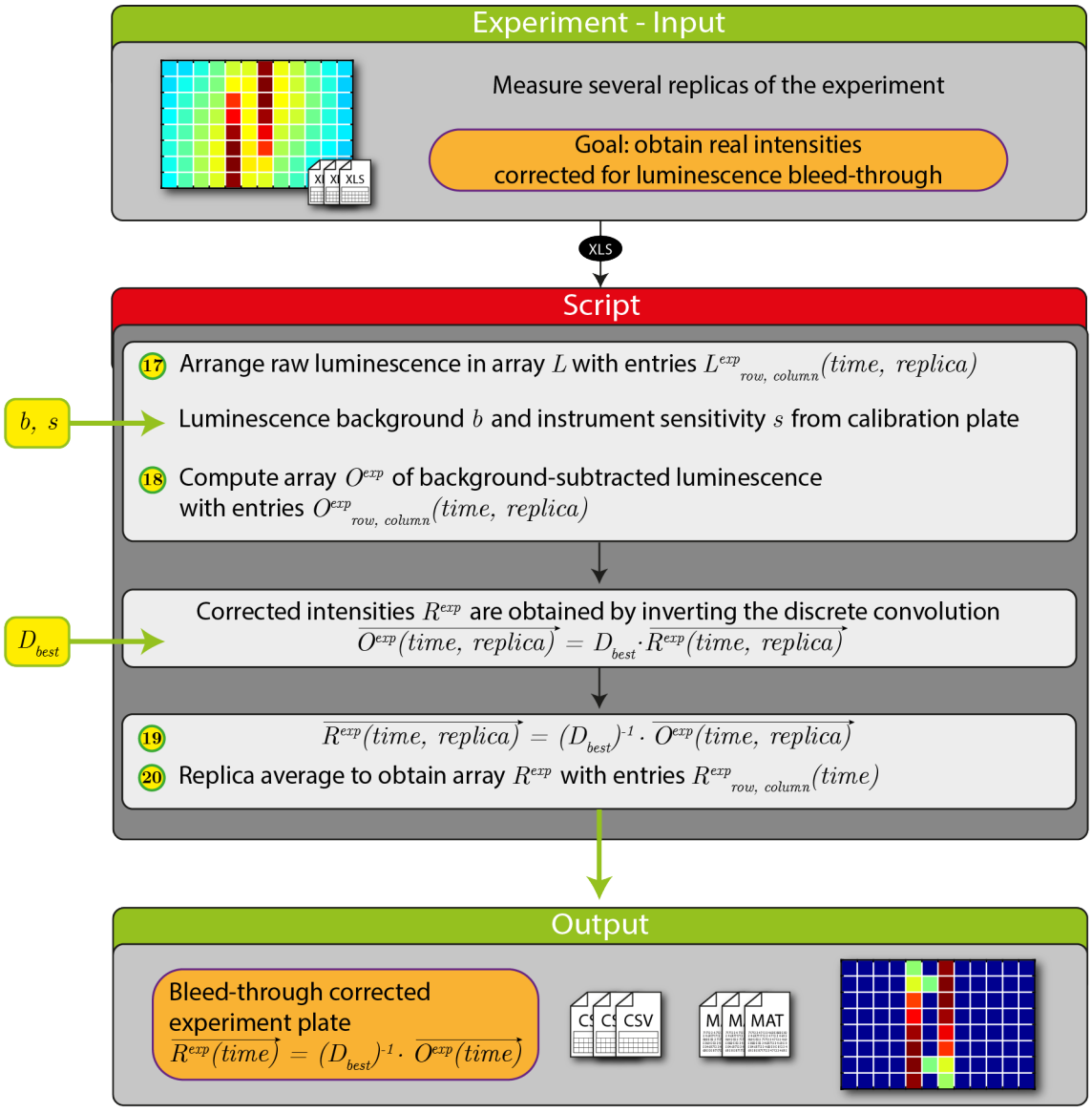

Figure S21: Workflow of the luminescence bleed-through correction of the experiment.

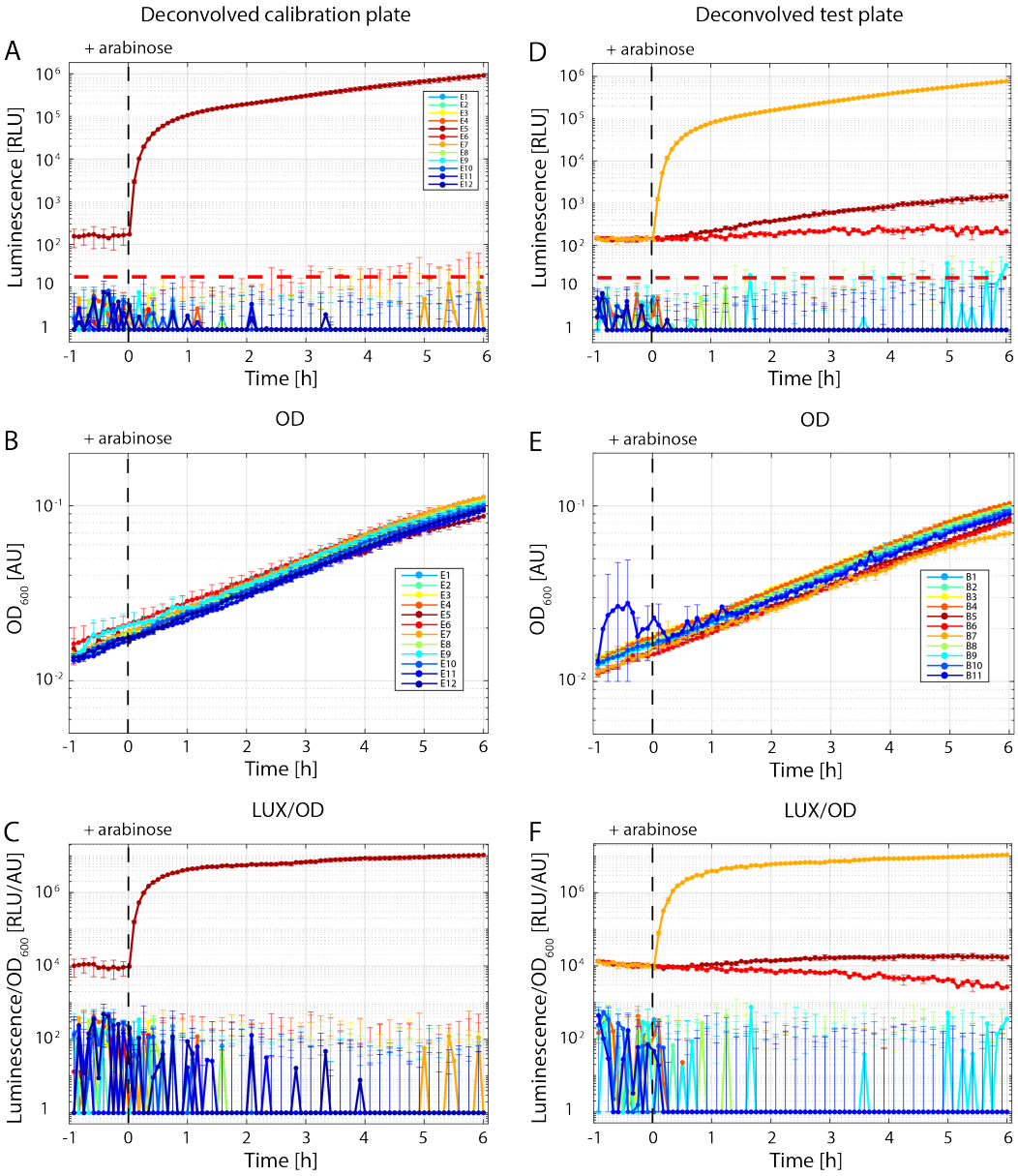

Figure S22: Deconvolved luminescence intensities (A and D), optical density (B and E) and deconvolved luminescence per optical density (C and F) of the row E of the calibration plate of Figure 2 in the main text and of the row B of the test plate of Figure 3 in the main text (A, B, C and D, E, F, respectively).

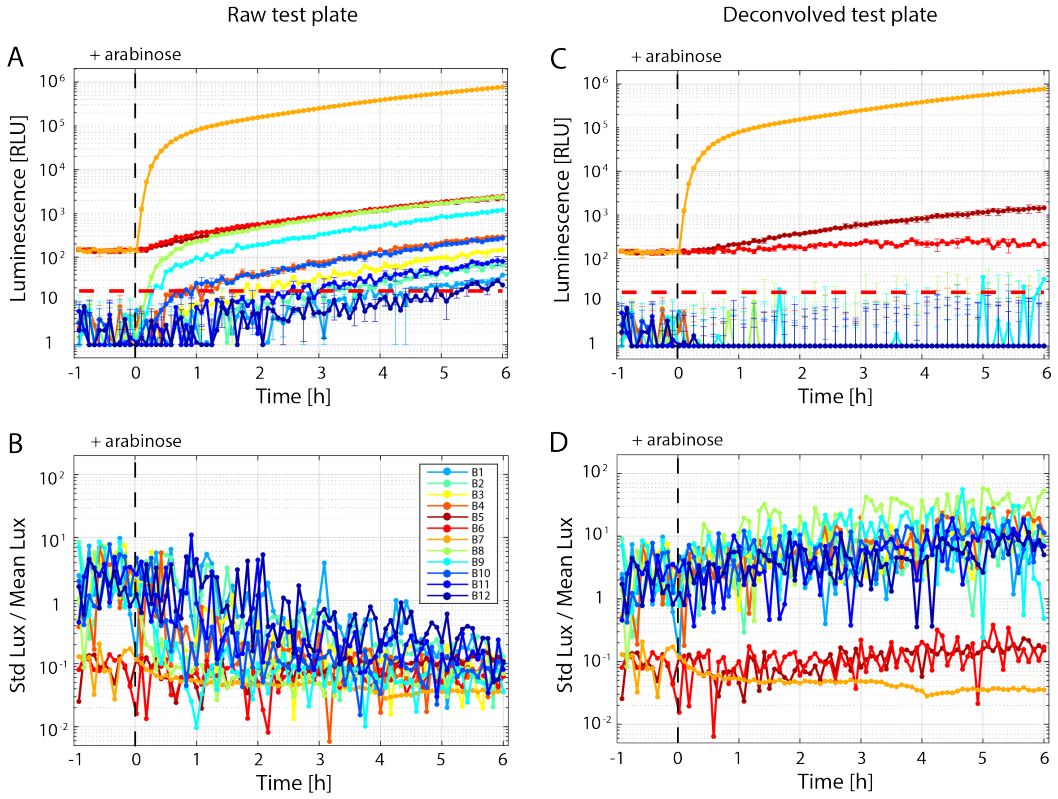

Figure S23: Raw (A) and deconvolved luminescence (C) of the row B of the test plate of Figure 3B and 3F in the main text. Panels B and D show the coefficient of variations (standard deviation over mean) corresponding to the data of A and C, respectively.

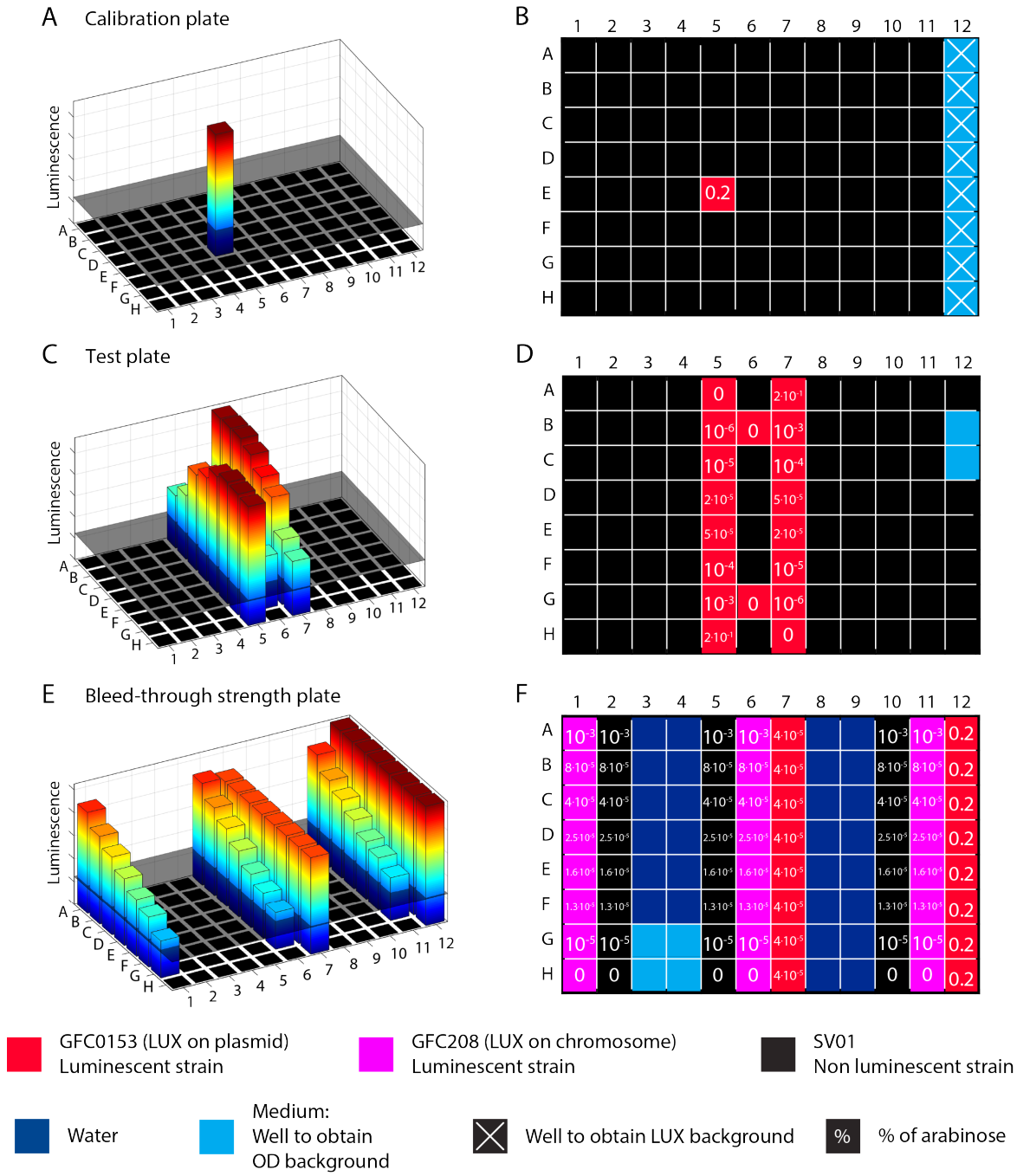

Figure S24: Summary of the plate arrangements used in the experiments.

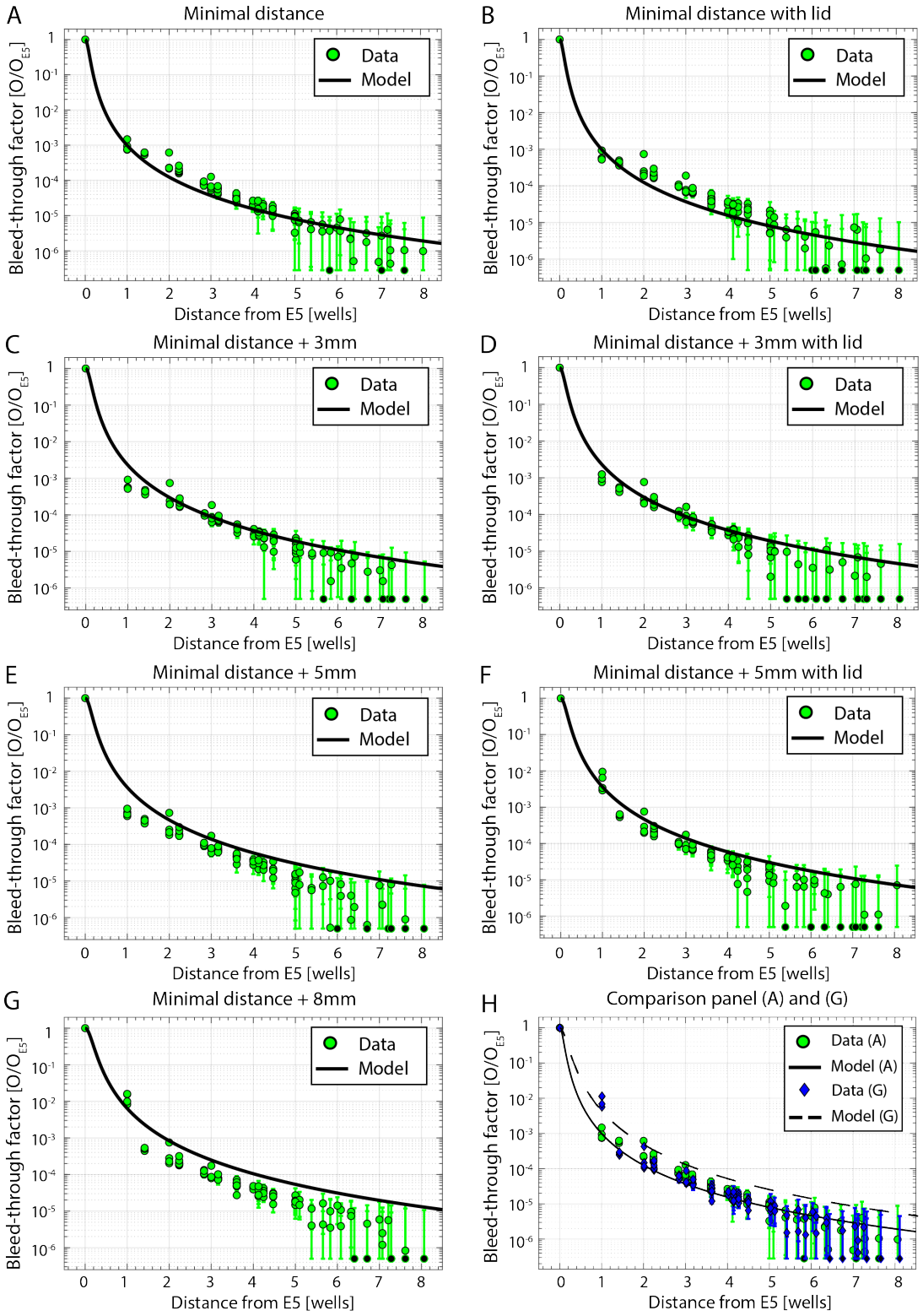

Figure S25: Bleed-through factor as a function of the distance from E5 in the case when the distance between the detector and the plate is minimal ($h = 0.9$ mm, A), is minimal but the plate mounts a lid (B), is 3 mm above minimal distance without and with lid (C and D, respectively), is 5 mm above minimal distance without and with lid (E and F, respectively), is 8 mm above minimal distance (G). Data points and error bars represent mean and standard deviations from either two or three experimental replicates. The black solid line are the parameter free predictions of the bleed-through given by Equation (2) in the main text drawn by modifying the parameter h representing the distance between the plate and detector. (H) Comparison between the minimal distance (green points) and 8 mm above the minimal distance (blue diamonds), as represented in (A) and in (G), respectively.

4 DNA sequence of pSVM-MC_038

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5 DNA sequence of pSV012

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