Relation between the models for the real expression values and the image response

C_j^{tot} Total amount of exons in the cell at timepoint j

C^{exp} Total amount of exons applied to each array (constant)

C_{ij} Amount of exon i in the cell at timepoint j (real response)

$\bar{C}_{ij}$ Amount of exon i isolated at timepoint j that is applied to the array (image response)

x_j Time at timepoint j

c_{ij} Logarithm of C_{ij}

$\bar{c}_{ij}$ Logarithm of $\bar{C}_{ij}$

s_j Logarithm of (C^{exp}/C_j^{tot})

a_i Slope of the linear model for c_{ij} of exon i (real slope)

$\bar{a}_i$ Slope of the linear model for $\bar{c}_{ij}$ of exon i (image slope)

n Number of timepoints

In the course of the experiment the real response C_{ij} is transformed into the image response, $\bar{C}_{ij}$. For each exon i holds:

$$\bar{C}_{ij} = \frac{C^{exp}}{C_j^{tot}} C_{ij} \quad \text{or} \tag{1}$$

$$\bar{c}_{ij} = s_j + c_{ij} \tag{2}$$

The image slope, $\bar{a}_i$, of exon i is given by:

$$\bar{a}_i = \frac{\sum_{j=1}^n x_j \bar{c}_{ij} - \sum_{j=1}^n x_j \sum_{j=1}^n \bar{c}_{ij}}{\sum_{j=1}^n x_j^2 - \left(\sum_{j=1}^n x_j\right)^2} \tag{3}$$

Using equation (2) yields:

$$\bar{a}_i = \frac{\sum_{j=1}^n x_j (c_{ij} + s_j) - \sum_{j=1}^n x_j \sum_{j=1}^n (c_{ij} + s_j)}{\sum_{j=1}^n x_j^2 - \left(\sum_{j=1}^n x_j\right)^2} \tag{4}$$

$$= \frac{\left(\sum_{j=1}^n x_j c_{ij} - \sum_{j=1}^n x_j \sum_{j=1}^n c_{ij}\right) + \left(\sum_{j=1}^n x_j s_j - \sum_{j=1}^n x_j \sum_{j=1}^n s_j\right)}{\sum_{j=1}^n x_j^2 - \left(\sum_{j=1}^n x_j\right)^2} \tag{5}$$

$$\bar{a}_i = a_i + \frac{\sum_{j=1}^n x_j s_j - \sum_{j=1}^n x_j \sum_{j=1}^n s_j}{\sum_{j=1}^n x_j^2 - \left(\sum_{j=1}^n x_j\right)^2} \tag{6}$$

That means: the image slope equals the real slope plus a term that does not depend on any particular exon but on the sum of the total amounts of exons over all timepoints. This additional term is for all exons the same. If it can be estimated then the real slope can be calculated for all exons.

If we assume that some exons are stable in the considered period of time (480 min) then the image slope of these exons can be used to correct all other slopes. Another possibility is to measure the real slope for some exons by another method – such as qPCR – in order to calculate the correction term.