Letter to the Editor

Answer to the comments of K. Dobbin, J. Shih and R. Simon on the paper ‘Evaluation of the gene-specific dye-bias in cDNA microarray experiments’

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We would like to thank K. Dobbin, J. Shih and R. Simon for their comments about Martin-Magniette et al. (2005). Their remarks relate to the design of microarray experiments and notably about the use of dye-swaps. We, however, want to make it clear that our manuscript primarily focuses on the detection, quantification and correction of the gene-specific dye-bias introduced in dual-color microarray experiments.

The most important point in Martin-Magniette et al. (2005) is the following: by dint of studying technical errors, we will be able to identify and then to remove most of them. For the first time, in Martin-Magniette et al. (2005), we were able to quantify the gene-specific dye bias by calculating the Labelling Bias Index (LBI). The LBI measured on different array types shows that this artifact seems to be very low in some cases and could be thus neglected. However, it is high in other cases and it cannot thus be neglected for data issued from these array types. This measure is of crucial importance as it allows users to evaluate the impact of this bias on their data. Moreover, we think that each platform should at least know in what class it belongs for each array types. Although this artifact is not lowered nor understood, it is simply dangerous to underestimate it. The gene-specific dye bias is not an inevitability and can be well controlled, as we point out in our paper.

Recently, in another paper, Dobbin et al. (2005) studied the gene-specific dye bias. Although they reached the same conclusions, some of their remarks are explained in Martin-Magniette et al. (2005): e.g. Dobbin et al. (2005) have found that ‘(the gene-specific) dye bias appears to have masked the true differential expression’. This is explained in Martin-Magniette et al. (2005): the variance associated to a gene is overestimated by the dye bias effect in model (2) of Martin-Magniette et al.

Dye-swaps constitute a simple and effective design to remove gene-specific dye bias when it is high. Nevertheless, we agree with Dobbin et al. (2005), that a balanced block design may be better than dye-swaps in some situations. As the former designs allow the use of more biological samples, the estimation of the biological variability will be more precise. Even if balanced block designs are statistically more efficient, the following considerations should be taken into account before choosing the experimental design:

- Even with rigorous experimental procedures some sources of variability remain (quality and yield of target purification, labelling efficiency, ...). Performing dye-swaps will allow to differentiate biological from technical variability.
- It is often difficult to balance the dye for every treatment in complex designs, when samples are hardly available. For instance, such situations are encountered in sex-balanced medical studies.
- Moreover, some redundant experimental procedures (quality control of mRNA, preparation of targets for indirect dye-labelling) used in dye-swap experiments, decrease the financial cost to <2-fold the cost of a single slide hybridization, thus rendering this design much more attractive.

The experimental design must be adapted not only to the research question but also to the amount of biological material available. Finally, class prediction or differential expression, e.g. the question of interest, do not necessarily imply the same experimental design.

REFERENCES
