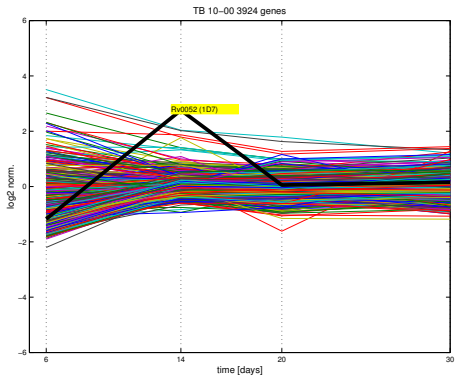
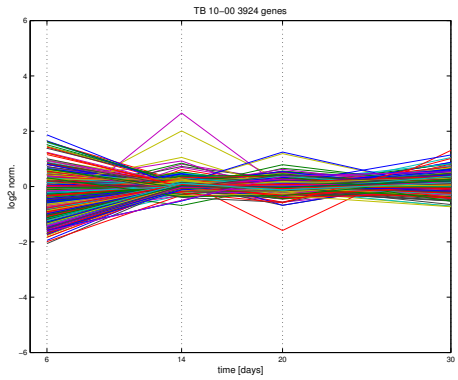
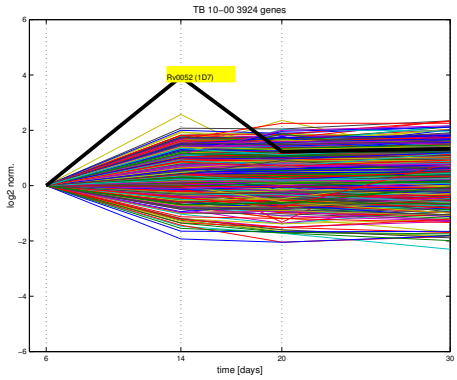


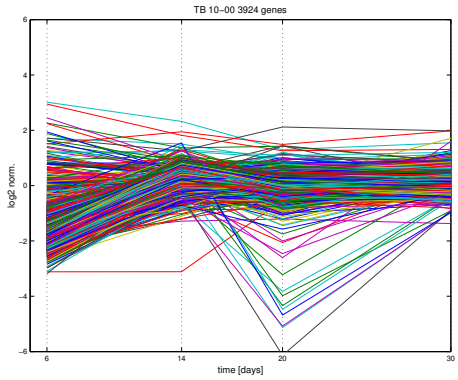
(a) Time series after dye swap normalisation.



(b) Time series after dye swap normalisation and across samples normalisation option 1.



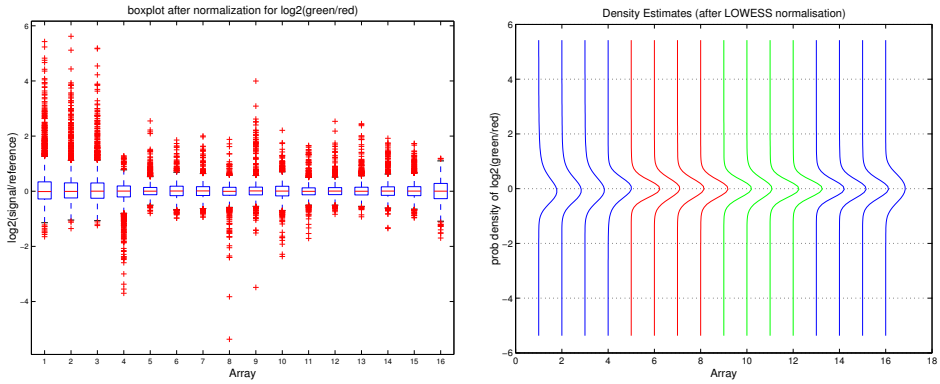
(c) Time series after dye swap normalisation and after across samples normalisation option 2.



(d) Time series after dye swap normalisation and across samples normalisation option 3.

Fig. 4.5: Time series after dye swap normalisation and different across samples options. We don't use across replicates normalisation to correct the data because this would center the median value of the ratios distributions of all the arrays around one. With the dye-swap normalisation we are trying to avoid the assumption that most of the genes in every array are equally distributed. If we do across replicates normalisation we are making at this stage of the normalisation process the same assumption.

4.4 After LOWESS normalisation



(a) Box plot all arrays after LOWESS normalisation.

(b) Distributions all arrays after LOWESS normalisation.

Fig. 4.6: Distributions of the arrays after normalisation.Every four consecutive arrays represent the technical replicates of a time point.

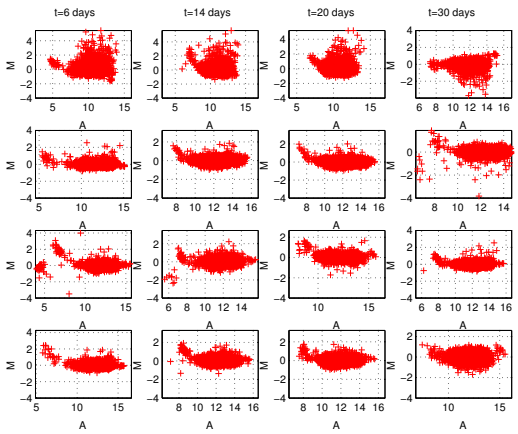
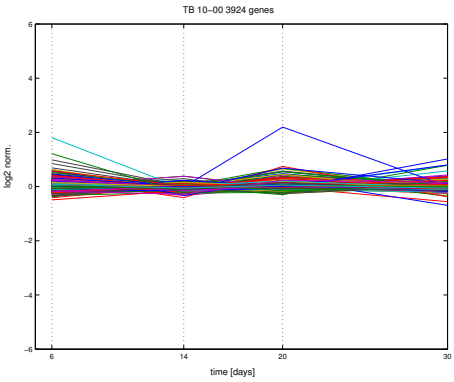
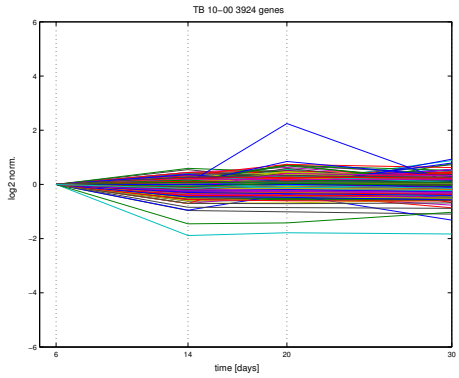


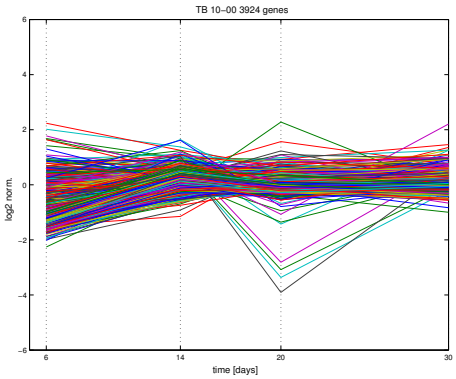
Fig. 4.7: MA plots for all the arrays after LOWESS normalisation.We can see how the very low intensity values still present a certain trend after LOWESS normalisation.



(a) Time series after LOWESS normalisation, across replicates normalisation and across samples normalisation option 1.



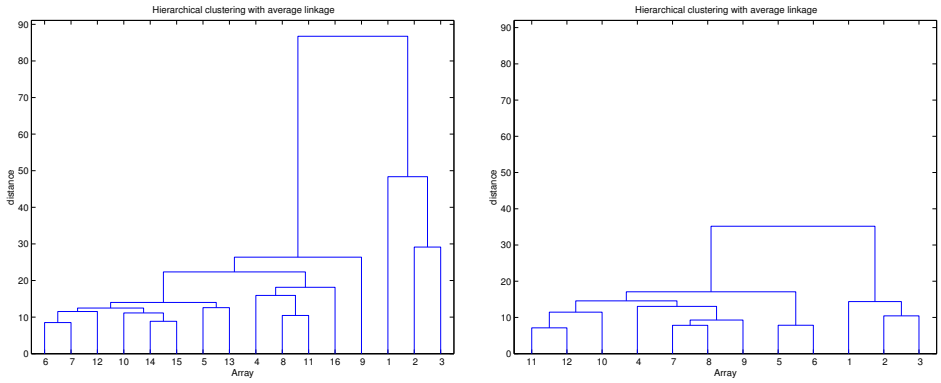
(b) Time series after LOWESS normalisation, across replicates normalisation and after across samples normalisation option 2.



(c) Time series after LOWESS normalisation, across replicates normalisation and across samples normalisation option 3.

Fig. 4.8: Time series after LOWESS normalisation, across replicates normalisation and different across samples options. Not to perform across replicates normalisation results in a much bigger spreadability of the data.

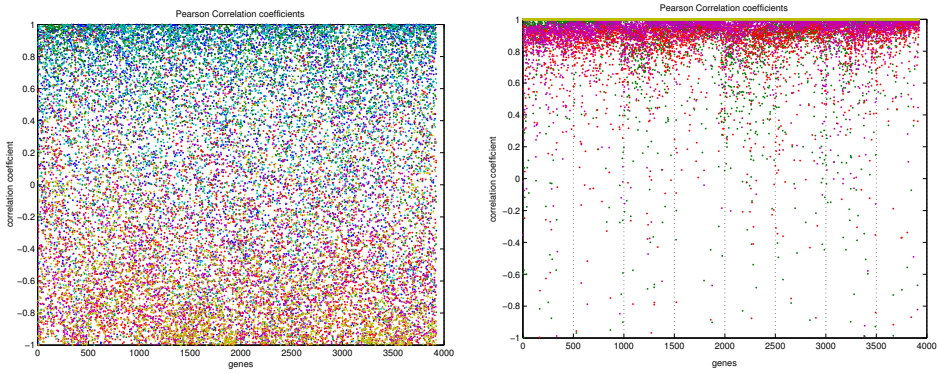
4.5 Replicates after normalisation



(a) Hierarchical clustering in the replicates after LOWESS normalisation.

(b) Hierarchical clustering in the replicates after dye swap normalisation.

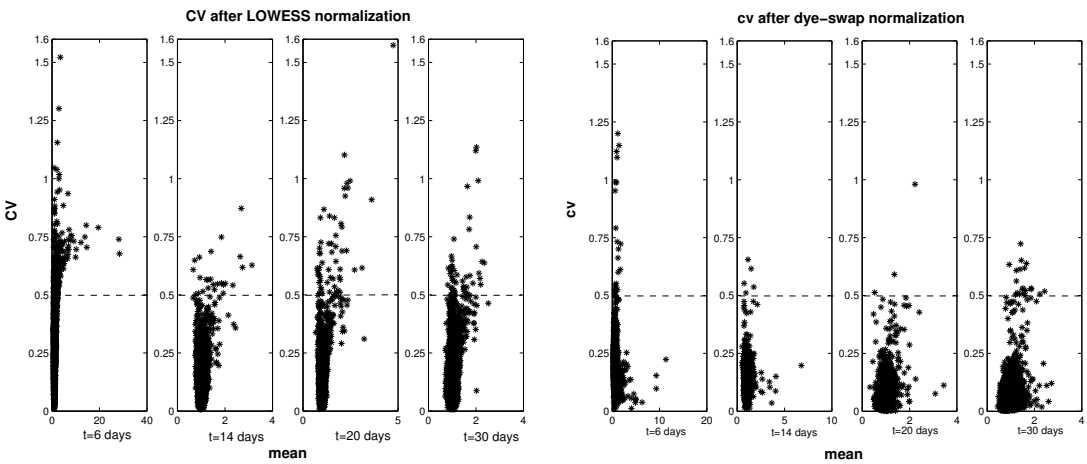
Fig. 4.9: After dye swap normalisation, the replicates corresponding to the same time point are close together. However, after LOWESS normalisation, the replicated arrays of the different time points are mixed and the distances among them are much bigger than after dye swap within array normalisation. A good normalisation method should increase the overall R^2.



(a) Curtain plot for the replicates after LOWESS normalisation.

(b) Curtain plot for the replicates after dye swap normalisation.

Fig. 4.10: After dye swap normalisation, the curtain plot shows how the correlation among the replicates is very good (correlation coefficient around one for most of the genes and combination of replicates). However, after LOWESS within array normalisation, the curtain plot shows how the correlation coefficient is smaller than 0.5 for a great number of genes.



(a) CV after *LOWESS* normalisation.

(b) CV after dye-swap normalisation.

Fig. 4.11: Because the use of replicated profiles does not reflect the conditions of the experimental design, we propose also this scatterplot of mean-CV for each one of the four time points. The mean and standard deviation of the four replicated ratios at every time point were calculated after the two normalisation methods. It can be seen how after dye-swap normalisation the number of genes for which the CV is greater than 0.5 is much smaller than after LOWESS normalisation.