

Re-washing microarrays to reduce background

- Although there is no standard for re-washing slides, it is possible to re-wash microarrays in an attempt to reduce background signal
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Introduction

Optimal washing conditions are essential to ensure quality microarray data. Washing parameters such as temperature, duration, and solution composition have been optimised such that complementary hybridisation is allowed to occur and non-specific hybridisation is minimised. The stringency of the washing conditions can be increased through a combination of increased temperature, decreased salt concentration, or increased detergent concentration.

The standard washing conditions described in UHNMAC protocols most often result in images with little or no background. Despite this and the best efforts of the technician performing the experiment, high background can still occur occasionally, ruining an otherwise good microarray experiment. Since repeating the experiment is not always an option (depleted RNA source, last microarray of production batch, cost issues, etc), users can re-wash the microarray in an attempt to salvage the experiment. In our hands, re-washing can reduce the “swirly” type of background but not “grainy” background, about half of the time. Unfortunately, there is no standard re-washing protocol. The severity of the background and overall level of spot intensity will dictate how stringent the re-washing protocol can and should be.

Method

First and foremost, save a high-resolution scan of the high-background image. Re-washing most often not only reduces the background, but also decreases the spot signal intensity, which on occasion will leave you with a poorer image than the initial scan.

If the background is minor (not too intense or affects 1-2 grids of a large microarray), it may be sufficient to re-wash the slide for 1-2 minutes at room temperature in 1x SSC/0.1% SDS, followed by a rinse in 0.1x SSC (2-3 sets of 5 plunges in each, room temperature) and immediately drying the array by centrifugation.

If the background is severe (intense background affecting more than half of the array), it may require a more stringent re-wash. For example, re-wash the slide for 2 sets of 2-3 minutes each in 1x SSC/0.1% SDS at 50°C, followed by a rinse in 0.1x SSC (2-3 sets of 5 plunges in each, room temperature) and immediately drying the array by centrifugation. Re-washing is most often successful if it is performed within several hours of the initial set of washes. An example of a “before and after” rewashing is shown below (Figure 1). If you find that you routinely are experiencing high background on your washes, it is recommended that you check the quality of the water that you are using as this can be a large contributor to background. In addition, you may wish to investigate the use of an automated

washing system to improve consistency.

Conclusion

Re-washing is most often successful if it is performed within several hours of the initial set of washes. The re-washing procedure varies depending on the severity of the background. Be sure to save a high-resolution image of the

initial scan (prior to re-washing) as re-washing may make the slide worse by reducing signal intensity as well as background.

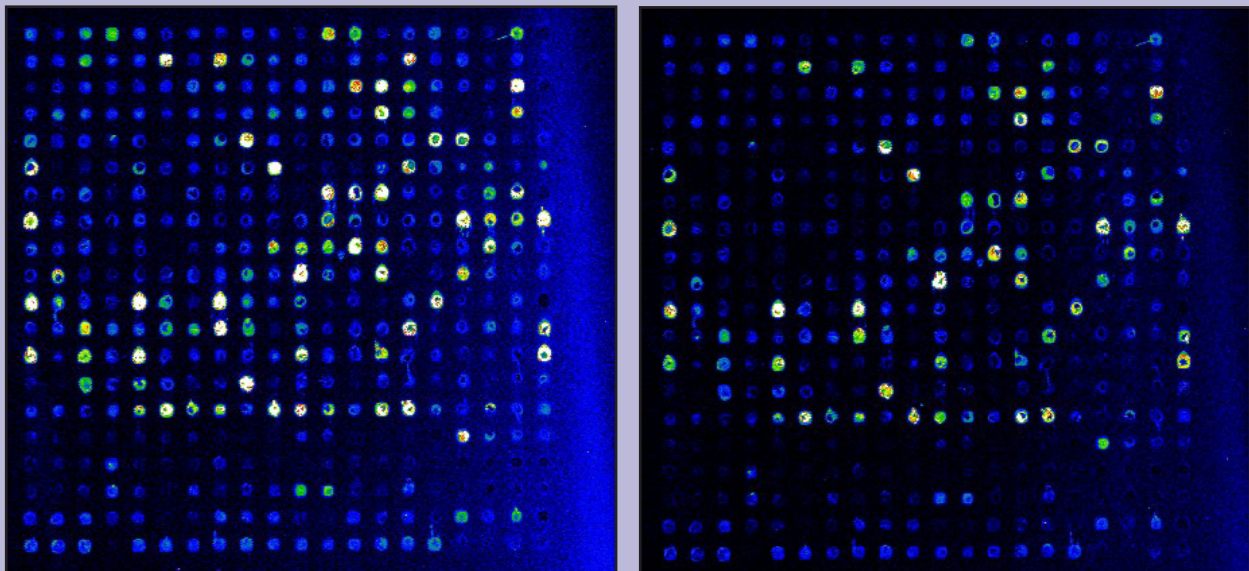


Figure 1. Left image: The slide was washed and dried using standard UHNMAC protocols, and then scanned on the ScanArray 4000 (PE). As can happen on occasion, there are regions of increased and undesirable background that will affect the use of this image. Right image: The same slide was re-washed in 1x SSC/0.1% SDS at room temperature for 5 minutes, followed by rinsing at room temperature in 1x SSC, then 0.1x SSC (twice; several plunges each time), then spun dry. While the background has decreased, the signal intensity also decreased but the overall quality and signal to noise have improved and as such, in this case, the re-washed slide was used for analysis.