sciFIDUCIAL – black permanent Marker for Arrays: high contrast, easy to print and strong stability

Introduction

A broad range of detection methods for microarray-based quantitative assays are available. widespread technology used for different platforms and assay formats. Colorimetric assays are based on reagents which have a measurable color change in the presence of an analyte.
However, independently of platform and used reagents the read-out of such colorimetric microarrays is mostly based on a digital camera image acquisition including image processing to evaluate the signal intensities. SCIENIONs colorimetric sciREADER CL2 for microplates and sciREADER LF1 for lateral flow devices runs with such a software for automated signal evaluation of microarray spots. The sum of all post-data processes & algorithms delivers a quick and quantified result of a multiplex microarray, based on the analytes concentrations and the resulting degree of measurable color changes.
Nevertheless, grid finding (e.g. identifying fiducial spots) relies on the precision of the spot positions, measurable thresholds of control spots and unique array layouts, whereby the spots quality depends on the staining process performance. This may vary or even fail.

sciFIDUCIAL enables permanent and high contrast fiducial spots, independent of staining method. This results in more reliable grid finding and consequently more robust data evaluation.

Workflow

Printing on surfaces like planar microtiter plates e.g. high-binding sciPLEXPLATES type 1 and various nitrocellulose membranes was carried out with a sciFLEXARRAYER SX and a PDC80 Type 3 at 40% and 50% relative humidity. Different available sciFIDUCIAL solutions (1, 2, 3, 4, 5) were used.

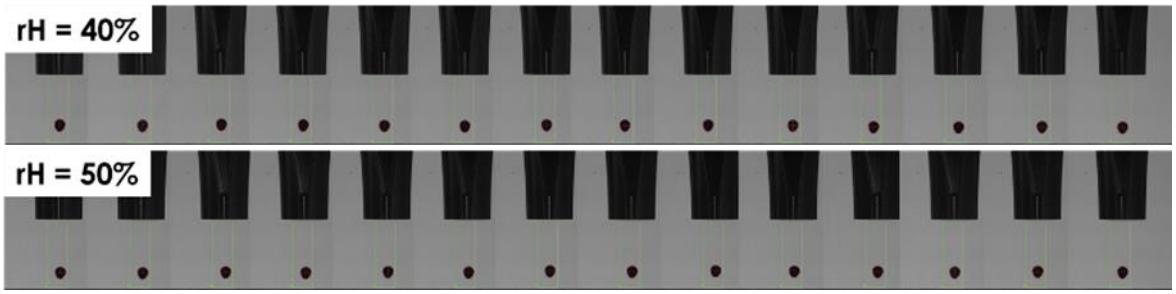

Figure 1 Performance drop stability of sciFIDUCIAL 3: printing with a PDC80 type 3 at 40% and 50% humidity; pre- and post-drop volume checks were performed before and after the print of 8 wells per microtiter plate during 1.5 h.

MTPs

Microtiter plates were washed at 37°C and 1400 rpm with 300 µl buffer 1x PBS, 1% BSA and 0.25% Tween 20 (PBS-T) for 7 hours (Figure 2 and Figure 3). Nitrocellulose coated plates were performed with PBS-T as a typical ELISA assay (Figure 4). Read-out was performed with sciREADER CL2 for plates.

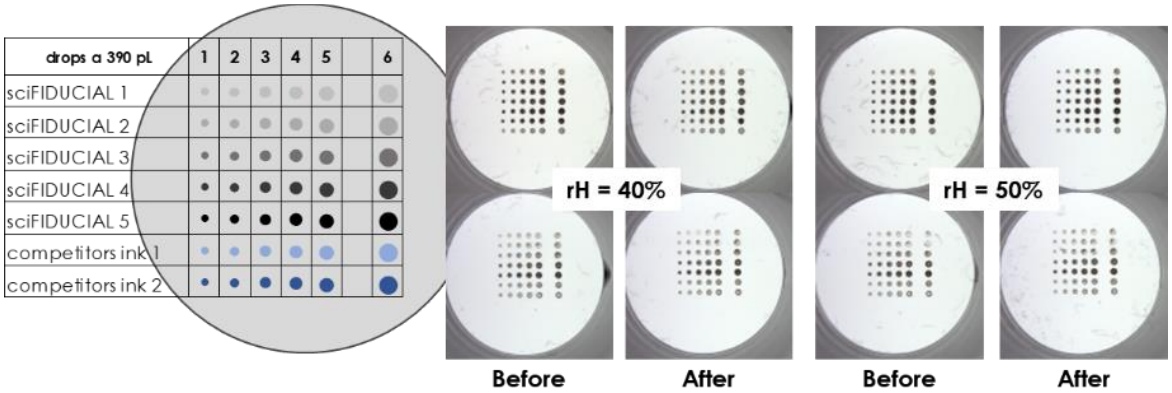

Figure 2 Array layout for different sciFIDUCIAL solutions (left), image acquisition was performed with sciREADER CL2 before and after a washing step with 300µl buffer 1x PBS, 1% BSA and 0.25% Tween 20 for 7 hours at 37°C and 1400 rpm (right).

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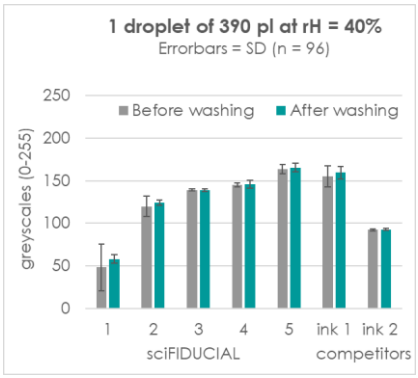


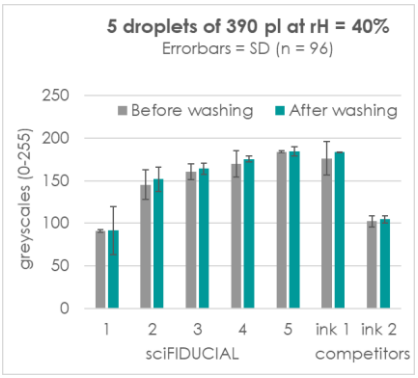
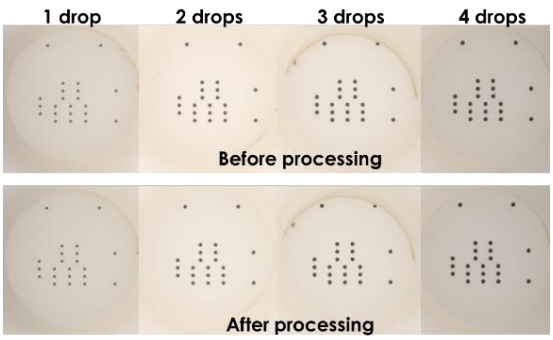


Figure 3 Selected graphical correlations of greyscale intensities (median) with different sciFIDUCIAL solutions of 1 and 5 droplets at rH = 50% before and after a strong washing step.



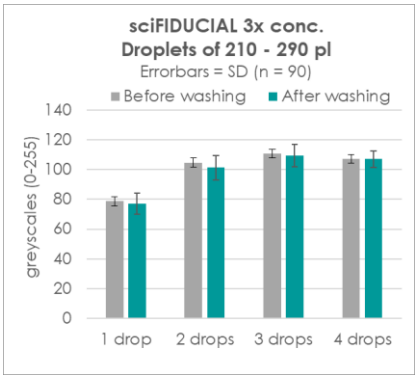


Figure 4 Selected arrays of sciFIDUCIAL 3 in 0.22 µm nitrocellulose coated wells at different spot volumes and the graphical correlations of greyscale intensities (median) before and after performing, a typical sandwich assay procedure.

Strips

After drying and assembling the membrane, wicking- and conjugate pad, all 5.0 mm width strips were processed with 100 µl sciLFA buffer as dip-stick (Figure 5). Read-out was performed with sciREADER LF1 for stripes.

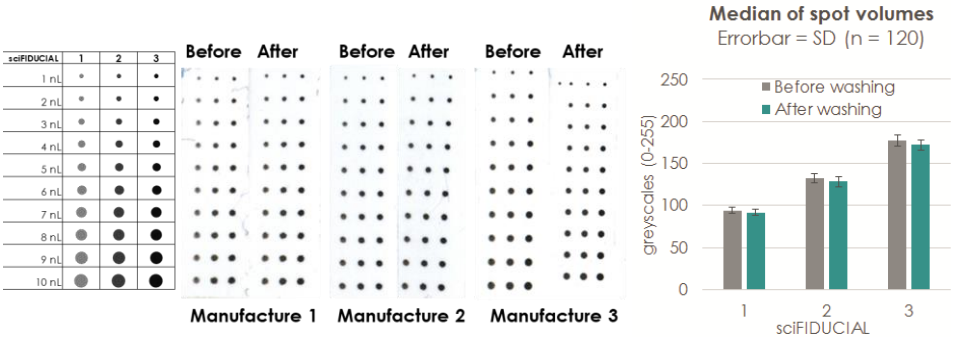


Figure 5 Selected arrays of sciFIDUCIAL 1 to 3 on different nitrocellulose membranes and the graphical correlations of greyscale intensities (median) before and after running as dip-stick on membrane 1.

Summary & Conclusion

There is no significant difference in greyscale intensities of sciFIDUCIAL permanent spots before and after an intensive strong washing process on sciPLEXPLATE type 1 and various nitrocellulose substrates. CVs of all sciFIDUCIAL spot intensities for one batch were below 5% after washing processes.
There are various potential applications for sciFIDUCIAL. It can be used as permanent marker for pre- and post-fiducials, as dye for QR coding inside an array, as normalization spots and/or as reader calibration spots for one batch of e.g. diagnostic tests.

Acknowledgement

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