Well to well repeatability using a white 96 well microplate

Application Note

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Introduction

In order to obtain results of the highest precision, well-to-well repeatability is extremely important. Where conventional instruments have repeatability in the order of 5%, the Agilent Cary Eclipse Microplate reader has repeatability for every single well in a 96 well plate of less than 2%. Parameters that may affect repeatability are:

1. Variation in well-to-well volume

2. Variation in meniscus shape from well-to-well

3. Alignment of the microplate

Parameters 1 and 2 can be minimized by adopting correct laboratory procedures whereas parameter 3 is a function of the microplate reader alignment. The Agilent Cary Eclipse addresses the alignment issue via a sophisticated, yet easy to use software procedure which aligns a plate of specific type accurately within the accessory. This procedure accounts for any plate variations due to manufacture. Microplate variability may cause deviations from the general assumption that the wells in any 3 corners of a standard plate form a right-angle (see Figure 1). Deviations from this "right-angle assumption" can result in clipping of the sides of the well, which affects the well-to-well repeatability.

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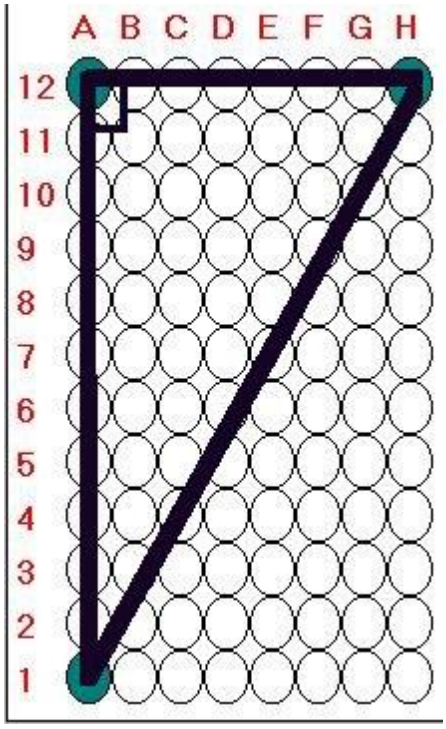

Figure 1. *Schematic of a 96 well microplate showing that Well A1, A12 and H12 form a right-angle.*

Method and Results

A volume of 300 µL of a 1 µM solution of Fluorescein was pippetted into each well of a Greiner white 96 well microplate. The plate was aligned using the Auto-align procedure in the Cary Eclipse Align application and the intensity was measured for each well using the parameters shown in Table 1. The results obtained from a typical run are shown in Table 2 (see Appendix).

Table 1. Instrument parameters used in repeatability experiment

Instrument Parameter	Setting
Excitation Wavelength	490 nm
Emission Wavelength	520 nm
Excitation Slit	10 nm
Emission Slit	10 nm
Averaging time	0.1 s
Excitation Filter	Auto
Emission Filter	Auto
PMT Voltage	400 V

Discussion

The experiment was repeated a total of 6 times and similar results were obtained. The average %RSD from the six runs was calculated to be 1.75%. The best results are obtained by ensuring that the microplate reader is correctly aligned for both the plate type being used and the volume of solution in each well.

NOTE: The volume of solution should be the same from well to well and air bubbles should be avoided. Use a multi-tip pipette for reproducible volume delivery.

Aligning the microplate reader for the plate type is a simple Auto-align software procedure. Optimizing the well volume involves adjusting the emission horizontal and vertical mirror adjustments on the accessory. However, this procedure only needs to be performed once if the total well volume remains the same between each assay and if repeatability of less than 2% is required. Otherwise, well-to-well repeatability in the order of 4% is obtained without this optimization. The Cary Eclipse Help has videos, which describe how to align your plate reader for optimum results.

Summary

The Cary Eclipse Microplate reader, by virtue of its all-reflective optical design and superior software Auto-alignment procedure, is one of the most precise microplate readers available. Well-to-well repeatability for every single well in a White 96 well microplate can be achieved to within less than 2%.

Appendix

Table 2. Results from a white 96 well plate using 1m M fluoroscein in each well

Sample name	Intensity	Sample name	Intensity	Sample name	Intensity	Sample name	Intensity
Sample A1	834.312	Sample C1	841.139	Sample E1	808.301	Sample G1	809.856
Sample A2	833.922	Sample C2	836.107	Sample E2	810.293	Sample G2	818.193
Sample A3	840.697	Sample C3	835.781	Sample E3	825.734	Sample G3	817.207
Sample A4	832.314	Sample C4	834.735	Sample E4	815.352	Sample G4	816.786
Sample A5	822.183	Sample C5	819.614	Sample E5	800.961	Sample G5	783.971
Sample A6	821.491	Sample C6	818.373	Sample E6	807.017	Sample G6	805.200
Sample A7	832.983	Sample C7	828.628	Sample E7	822.020	Sample G7	810.040
Sample A8	824.343	Sample C8	835.146	Sample E8	807.907	Sample G8	816.446
Sample A9	830.013	Sample C9	830.064	Sample E9	818.018	Sample G9	808.099
Sample A10	836.170	Sample C10	827.059	Sample E10	833.147	Sample G10	809.085
Sample A11	840.729	Sample C11	847.120	Sample E11	813.528	Sample G11	830.481
Sample A12	842.289	Sample C12	843.965	Sample E12	825.361	Sample G12	824.091
Sample B12	833.176	Sample D12	833.152	Sample F12	836.795	Sample H12	825.334
Sample B11	829.479	Sample D11	832.562	Sample F11	830.509	Sample H11	832.613
Sample B10	833.872	Sample D10	817.077	Sample F10	819.648	Sample H10	845.312
Sample B9	830.945	Sample D9	810.861	Sample F9	812.878	Sample H9	794.645
Sample B8	819.317	Sample D8	810.779	Sample F8	807.364	Sample H8	808.268
Sample B7	823.151	Sample D7	807.006	Sample F7	804.247	Sample H7	811.349
Sample B6	815.508	Sample D6	816.661	Sample F6	808.477	Sample H6	809.721
Sample B5	825.550	Sample D5	790.917	Sample F5	788.024	Sample H5	789.566
Sample B4	823.444	Sample D4	820.737	Sample F4	810.156	Sample H4	815.523
Sample B3	838.167	Sample D3	813.145	Sample F3	807.742	Sample H3	810.123
Sample B2	834.430	Sample D2	812.319	Sample F2	808.832	Sample H2	815.381
Sample B1	847.012	Sample D1	801.833	Sample F1	813.325	Sample H1	821.572
Average	820.571	**Standard deviation**	13.724	**%RSD**	1.67%		

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