HIGH THROUGHPUT CONTACTLESS **VOLUME VERIFICATION** FOR HIGH DENSITY MICROTITER PLATES AND MICROARRAYS

Michael Klinger[1], Martin Thoma[1], Andreas Traube[1]
[1]Fraunhofer Institute for Manufacturing Engineering and Automation IPA, Stuttgart, Germany

Almost every single biological assay in microtiter plates and microarrays is volume-dependent, thus the smallest deviations in volume can have a huge impact on an experiment's result. With an increasing demand for process monitoring and quality assurance in today's laboratory automation, the need for volume verification systems becomes greater than ever. At the same time, the trend towards assay miniaturization decreases the volume per sample, while the overall number of samples increases rapidly. Existing technologies for volume verification do not meet these growing requirements or can simple not be adapted towards modern sample carriers, such as 1536 well plates or microarrays.

System Design

We developed a contactless, optical volume verification system, which is suitable for virtually any labware, independent of well size, geometry, pitch and total well count. It is based on the optical detection of the liquids surface and the subsequent computation of the sample volume in the well. An optical sensor (1) is mounted above a kinematic assembly, comprising two orthogonally stacked linear axis (2, 3) and a carrier, suitable for the accommodation of SBS-standard compatible labware such as a microtiter plate (4) or microarrays with an adapter. The kinematic assembly, as well as the sensor are controlled via an external PC software (5), allowing the axis to move underneath the sensor, such that each well of the microtiter plate can be positioned directly below the sensor.

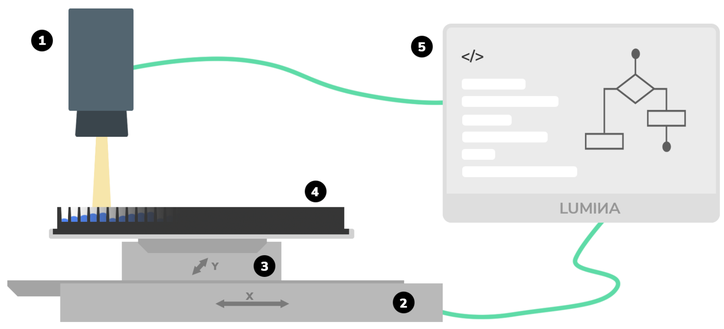

Figure 1: Overview of the system design, comprising a optical sensor (1), a kinematic axis assembly (2,3), a carrier for SBS-plates (4) and a control PC / software (5)

Methods and Material

We use two different microtiter plates for our measurement: Greiner Microplate 384 well (128/85 MM, PP, Item No. 784209) and Greiner Microplate 1536 well (PS, F-BOTTOM, HIBASE, Item No. 782076). For sample dispensation we use a Thermo Multidrop Combi in combination with a Small Tube Metal Tip Dispensing Cassette. The cassette has been used for an unknown period of time without calibration, and will thus deliver non-optimal dispensing results to challenge the volume verification system.
As a dispensing reagent, we used DMSO and created three symmetric volume patterns for the 384 well plate, starting from 25 µl to 95 µl in 10 µl steps, and six symmetrical volume patterns for the 1536 well plate, starting from 3 µl to 10 µl in 1 µl steps (see Figures 2-5). The plates were centrifuged after dispensation in order to eliminate air bubbles in the wells.

Results

The results of the plate measurements can be seen in Figures 2-5, whereas Figure 2 and Figure 3 show the absolute volume measured per well, and Figure 4 and Figure 5 show the relative error between measured volume and the respective target volume of the dispenser. All data is displayed in form of a heat map, whereas the color-coding is different for each Figure and can be derived from the legend beneath the plate.

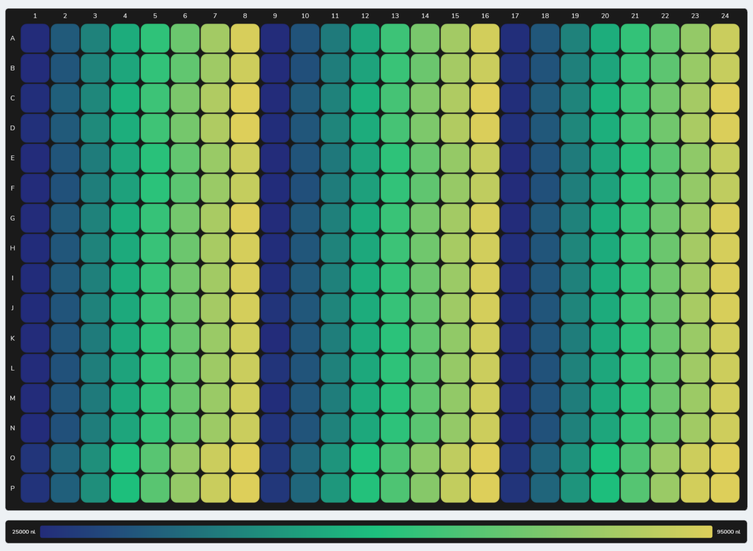

Figure 2: 384 well plate with absolute volume measurement

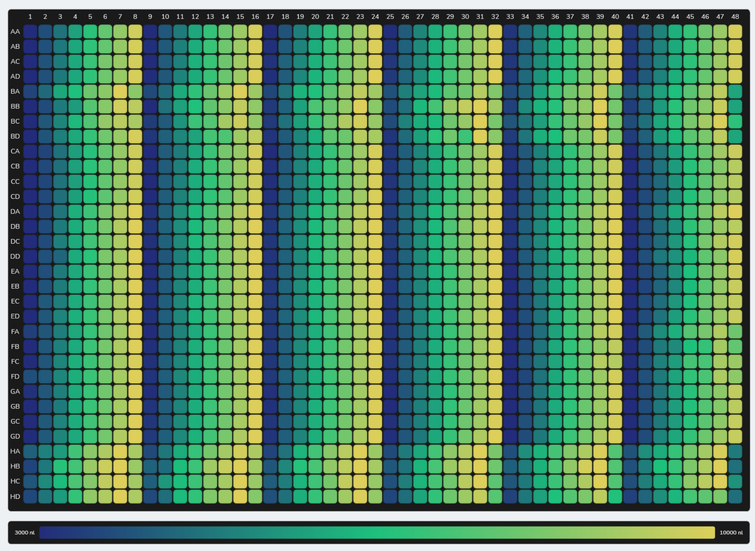

Figure 3: 1536 well plate with absolute volume measurement

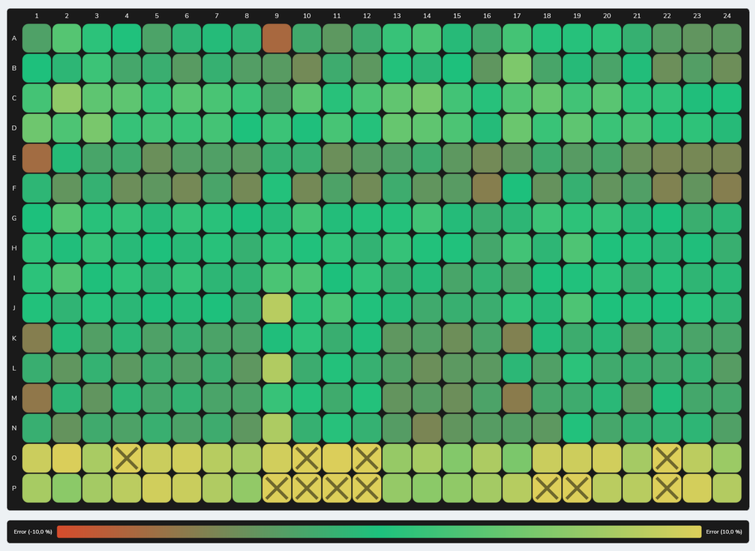

Figure 4: 384 well plate with relative volume error measurement

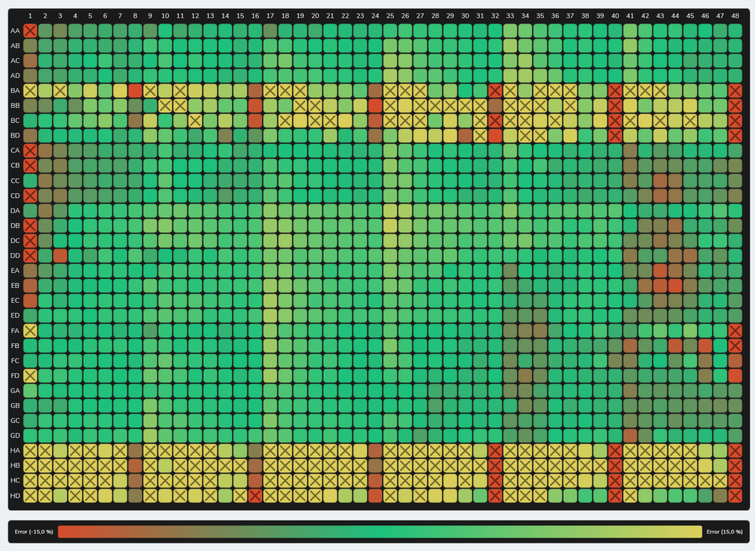

Figure 5: 1536 well plate with relative volume error measurement

Figure 2 and 3 indicate, that the scan was successful on both plate types, as a liquid level has been detected on each of the wells. The previously described dispensing pattern can also be visually retrieved from the heat map. The actual performance of the dispenser however can rather be derived from the volume error measurement displayed in Figures 4 and 5, in which green colors indicate a low volume error, red indicates a negative volume error and yellow indicates a positive volume error (measured volume > target volume).
The heat map in Figure 5 e.g. displays noticeable systematic errors, especially in the rows 5 – 8 and 29 – 32, which where filled from one specific dispensing channel of the Multidrop cassette each. In order to get a better idea of the overall dispensing performance, the data is clustered by the corresponding dispensing channels for each well and displayed in a box plot diagram (see Figure 6). It can be clearly observed that channel A and H have significant relative errors (9.1 % for A and 17.7 % for H), whereas the other channels have an average error of only 1.13 %.

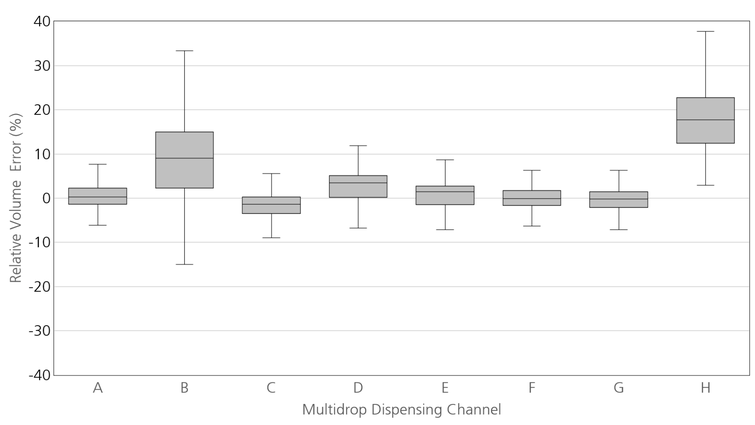

Figure 6: Relative volume errors for the 1536 well plate, clustered into the respective dispensing channels of the Multidrop cassette. Horizontal line shows the median, the box represents the 50 percentile and error bars show the 95 percentile.

The processing time for the plates were 2 minutes for the 384 well plate and 4 minutes for the 1536 well plate.

Conclusion

The presented optical volume verification system is capable of measuring and verifying sample volumes in microtiter plates with up to 1536 wells and small pitched micro arrays. The system delivers instant qualitative and quantitative feedback on a dispensing process, in order to detect errors early in the process.
For an improved absolute accuracy, a quantitative analysis of the measurement results must be performed. This can most likely be achieved when the system is benchmarked against a gravimetric setup, which is the state-of-the art for total assay volume analysis.
Overall, the system offers great potential for many applications in todays laboratory environment.

Acknowledgments

We would like to thank our partners from Merck, Darmstadt, Germany for their support and feedback on the volume verification system.

Contact

Martin Thoma
martin.thoma@ipa.fraunhofer.de

Michael Klinger
michael.klinger@ipa.fraunhofer.de

Fraunhofer IPA
Nobelstraße 12
70569 Stuttgart
Germany
www.fraunhofer.de