Automated Image Acquisition Process Ensures Flat Cell Culture Plates

TECHNICAL BRIEF

Microscope analysis

Cell culture plates are used for the cultivation of cells in dedicated culture media in biomedicine and for their subsequent examination. Reliable process control is essential for the production of such plates. This can be done automatically, with the help of FormFactor's FRT Micro-Prof topography measurement system.

Figure 1. Small 50 µm pads of a semiconductor IC.

In the biomedical industry, the establishment and examination of cell cultures is a key application, both in research and development and in manufacturing processes of biotechnical products. In these process chains, so-called cell culture plates are a necessary tool for growing cell cultures or performing organic tests outside of a living organism. The automated processes in the manufacturing chain result in high demands on the materials used. Cell culture plates must also meet these requirements. As a result, certain tolerances must be maintained during their manufacture.

Simple, fast and - depending on requirements - automated process control can contribute to compliance with these requirements. In this way, the entire manufacturing process is sustainably improved. In the following, such a control option is described as an example, which can be transferred to other areas with similar or deviating requirements.

The cell culture is formed on the bottom of a so-called well. Multi-well cell culture plates, with a typical plate size of approx. 80 mm x 120 mm, contain wells. This allows different cell cultures to be grown within a single well plate under the same conditions. The size of a well varies between one and several millimeters up to several centimeters, as exemplarily shown in the figure. While the body of the plates is made exclusively of suitable plastic, the bottoms of the plates on which the cell culture is placed are often covered with a thin film, the thickness of which is between 10 µm and 1 mm. Due to these thin films, bending and thus unevenness occur at the bottoms of the cell culture plates. After the cell cultures have been laid down, they are typically analyzed with the aid of automated optical microscopes. For this purpose, the well bottoms on which the cell culture lies must be at (approximately) the same height, so that the focus of the light microscope does not have to be moved in the automated image acquisition process, or only as little as possible. Therefore, the entire cell culture plate must not have too much deflection, nor must the unevenness of the individual well bottoms be too great. This results in narrow manufacturing tolerances. For example, the bending of the individual trays must not exceed a value of 10 µm.

Three typical cell culture plates with different well size and staining. (Bild: FRT)

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Flatness as a quality standard

One parameter used for the evaluation of cell culture plates is flatness. This is a measure of the maximum deviation of the surface from an ideal, mean plane and is defined by DIN 50441-5 and DIN EN ISO 12781-1. The flatness is a scalar value and is determined by the distance between two surfaces parallel to the mean plane, which include the entire topography. For a cell culture plate whose well bottoms lie as far as possible within a tolerable height range, the flatness of both the entire plate and the individual well bottoms must be as small as possible. The measuring task for a measuring system that controls the production of cell culture plates regarding these characteristics is therefore to map the surface topography as accurately and true to shape as possible. From such a surface topography measurement, the software then calculates the flatness and determines the quality level of the cell culture plates.

Automation for fast process control

Essential for metrological control is a suitable topography measurement that has no lasting, negative effects on the surface. No damage or contamination must occur as a result of the examinations on the cell culture plate, especially on the thin bottom foils. Any possible impairment of cell adhesion to the plate will have negative consequences for subsequent operations. A reliable measurement solution is also applicable to different cell culture plate types with different colors (transparent, black, white) and easily adaptable to varying geometries of the plates and individual wells. For constant process control, the measurements must also be automatable and fast. The examination of a well within a few seconds and findings over an entire plate in the range of minutes can be achieved with high-precision metrology solutions. An automatable routine is also necessary for the evaluation of the measurements in order to be as independent as possible of user influence given the tight manufacturing tolerances.

Fast profile measurements and tolerance classifications

The chromatic-confocal point topography sensor (CWL) is an optical distance sensor that can be configured for the respective application. Standard and special measuring heads with different measuring ranges are available for this purpose. The CWL's fast, non-destructive and non-contact method works equally reliably on highly and low reflective surfaces. With a measuring head that covers a vertical measuring range of 600 µm, a lateral resolution of 2 µm and a vertical resolution of < 20 nm are achieved. This makes the CWL sensor ideally suited for measuring cell culture plates.

Such a flexible point sensor - equipped with a lateral scanning device - performs both a 3D measurement and a 2D line measurement with variable lateral point spacing. Both high-resolution, detailed 3D topography images and very fast 2D/3D measurements can be implemented with it. The lateral measuring range can also be adjusted very flexibly for a point sensor. This makes it possible to measure very small wells in the millimeter range, but also very large wells in the centimeter range.

For this purpose, the FRT Micro-Prof platform provides the ideal basis for integrating the CWL sensors into a stand-alone topography measurement system. With the Micro Prof systems, a wide range of measurement tasks can be performed quickly, efficiently and intuitively. In particular, the compact Micro Prof 100 as a "table-top" solution is ideal for measuring cell culture plates. With an xy traverse stage, the instrument achieves a traverse range of 150 mm × 100 mm (x × y), which is completely sufficient for cell culture plates. Together with a CWL sensor and a CWL measuring head with 600 µm measuring range, all necessary flatness measurements on cell culture plates can be realized without contact. By extending the FRT Micro Prof 100 with the in-house automation software Acquire Automation XT, both the measurements, evaluations and subsequent reporting can be fully automated. The systematic classification of results into predefined categories (e.g., "pass/fail") is particularly helpful. In addition, a sample holder dedicated to cell culture plates can further round off the system configuration, making the Micro Prof 100 the ideal system for process control in cell culture plate manufacturing.

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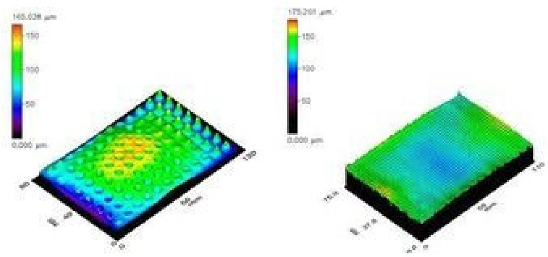
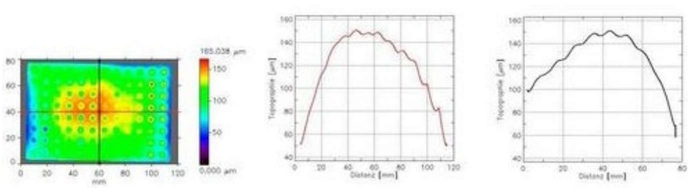
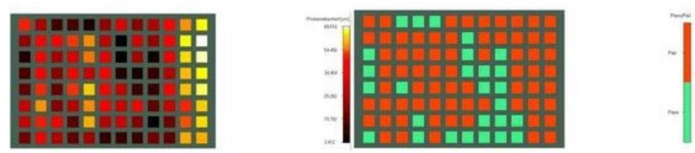


The figure shows two 3D topography images of the cell culture plates from Figure 1, which were created with such a measurement system. In the measurement for the black cell culture plate, the unevenness of the entire plate (approx. 89.3 µm) as well as the individual wells can be seen. The latter can be curved both concavely and convexly, so that the total flatness over all wells of 144.5 µm can be observed. The fact that the two values do not add up to the total flatness of 165.0 µm is due to the fact that both flatnesses partially cancel each other out. Overall, the topography measurement provides a very good representation of the extent to which the cell culture plate is bent. In the measurement of the white cell culture plate, the significantly smaller wells can be seen. Here, as well, the total flatness of 151.2 µm is comparable to the black cell culture plate. Due to the smaller wells, however, the base plate is significantly more stable and has a smaller flatness of 54.5 µm than the black cell culture plate. On the other hand, all wells have a convex shape and the flatness of all wells is 101.4 µm.



The measuring times for such complete 3D measurements range from a few minutes to about an hour, depending on the selected point spacing. In the context of process control, however, it is important to be able to determine a reliable quantity as quickly as possible. Especially in the case of smaller wells, the point spacing cannot be chosen too large to determine the well flatness unambiguously. Therefore, for a measurement of the flatness of a cell culture plate (plate flatness and well flatness), it is more convenient to perform it by means of profile measurements. For this purpose, the measurement procedure is divided into two steps. First, the flatness of the base plate is determined in the x- and y-directions using two cross profiles. The figure shows an example of this for the black cell culture plate. If necessary, these two profiles can be extended by further profiles, e.g., measurements across the diagonal. Since such a profile measurement takes only a few seconds, the flatness of the base plate can be determined in less than 30 seconds.

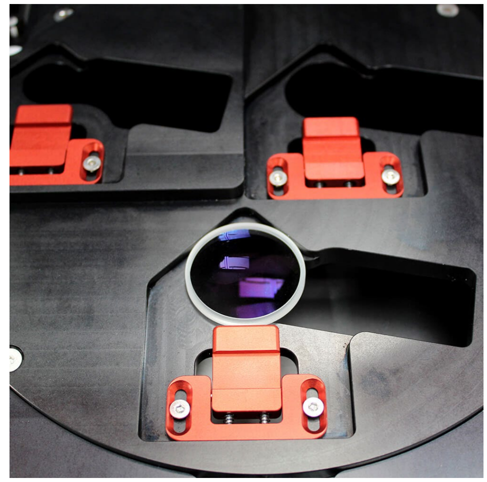


For the flatness measurement of the individual wells, a star profile is measured on each well in a second step, as shown in the figure. From these four profiles, an overall flatness is determined for each well, and thus a flatness value is assigned to each. To ensure that the profiles are centered on each well, as shown, the position of the cell culture plate is determined automatically in advance by means of a camera and the measurement positions are aligned based on this. The figure above shows the result of this automated measurement with the false color representation of the flatness per well. Here it can be seen that the flatness is particularly high at the right edge of the cell culture plate. In addition, the highest bending of 89.5 can be seen there. The measurement time for such a star profile is also only < 2 seconds per well. The total measurement time per well plate essentially depends on the number of wells or their size. Since a low flatness per well is required for the quality of a cell culture plate and there are typically specified tolerance values here, it makes sense for the measuring system to compare the results directly with these tolerances.

The figure also shows the classification of the determined flatnesses into "pass/fail" classes. A well fails as soon as the flatness exceeds the tolerance of 20 µm. This is illustrated accordingly in red. Based on this classification of the wells, the cell culture plate as such can then also be assigned to a class, considering the previously determined total plate bending. The system can also do this independently. At the end, the user receives a report in the form of a pdf, a csv or an xml file for documentation.

Optionally, the results can even be uploaded to a SEGS/GEM environment.

This allows the Micro Prof 100 with FRT-CWL sensor to inspect cell culture plates in a fully automated way, i.e., measurement of topography, evaluation of flatness and generation of the corresponding reports including classification. In addition, the FRT-Micro-Prof platform is modularly expandable with further sensors and software, giving the user access to additional measurement parameters. For example, the CWL sensor can also be used to determine the roughness of surfaces and supplementing the CWL sensor with a layer thickness sensor allows the film thickness of cell culture plates to be measured. Thus, a variety of measurement tasks can be realized on the same system, not only on cell culture plates.



Wide range of optimization options thanks to optical metrology

Essential for metrological control is a suitable topography measurement that has no lasting, negative effects on the surface. No damage or contamination must occur as a result of the examinations on the cell culture plate, especially on the thin bottom foils. Any possible impairment of cell adhesion to the plate will have negative consequences for subsequent operations. A reliable measurement solution is also applicable to different cell culture plate types with different colors (transparent, black, white) and easily adaptable to varying geometries of the plates and individual wells. For constant process control, the measurements must also be automatable and fast. The examination of a well within a few seconds and findings over an entire plate in the range of minutes can be achieved with high-precision metrology solutions. An automatable routine is also necessary for the evaluation of the measurements in order to be as independent as possible of user influence given the tight manufacturing tolerances.

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