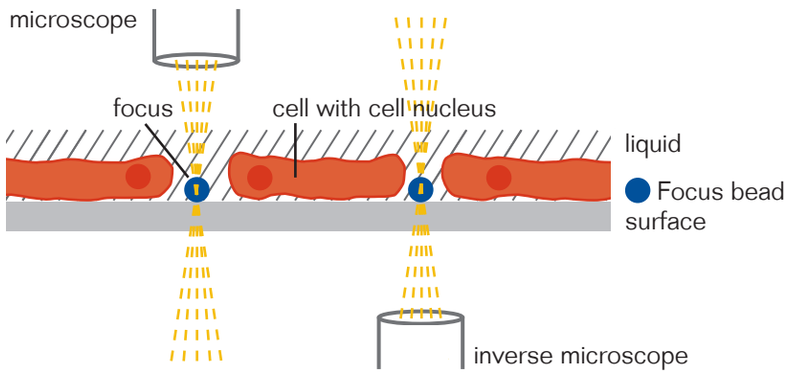
Use of focus beads for imaging of cell-based assays

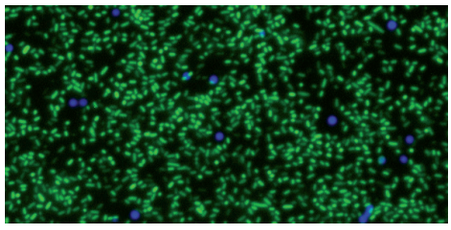
Quickly finding the correct focus level is one of the key challenges for automated, microscope based fluorescence imaging systems. In most applications DAPI-focussing on cell nuclei is used to determine the bottom of slides, plates or other carrier materials. However, this method can lead to errors when the cells detach from the bottom surface or are insufficiently stained.

PolyAn addresses this problem with our new 2 µm blue fluorescent Polymethyl-methacrylate (PMMA) beads.

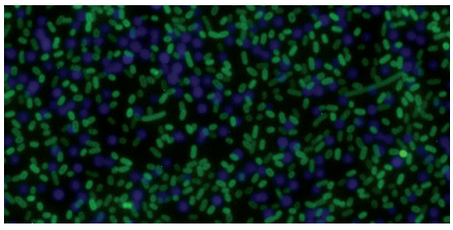
- 2 µm beads have a comparable size to cell nuclei.
- PMMA-grade is not cell-toxic and does not interfere with the cell behavior.
- Color encoded in the DAPI channel, transparent in all other channels.
- Well-defined fluorescence intensity. Problems caused by insufficient staining of the cell nuclei can thus be avoided.
- Easy handling: the beads can be easily added to the cell suspension. They quickly sink to the bottom and thus indicate the correct focus level.
- Application: standards in a range of cell assays, e.g. biofilm assays, adhesion assays or detection of bacteria.



In the examples illustrated below the read-out was done using the VideoScan HCU*, a fluorescence imaging system based on a fluorescence microscope**. The microscope focusses quickly on the focus beads independently whether bacteria adhere or not.



Focus beads (blue, DAPI channel) in combination with EPEC-bacteria on a GP2-coated plate. Only the surface of the bacteria has been stained with O26 *E.coli*-antibody sera and FITC-conjugated secondary antibody.



Focus beads (blue, DAPI channel) in combination with *E.coli*-bacteria on a GP2-coated plate. The bacteria have been stained using PI (DNA).

* Rödiger, S. et.al. Adv. Biochem. Eng. Biotechnol. 2013, 133, 35–74
** Schierack, P. et.al. Gut (BMJ Group), 2014, manuscript accepted, in press

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