Correction of surface tension induced refraction artefacts at the meniscus interface in multiwell plates

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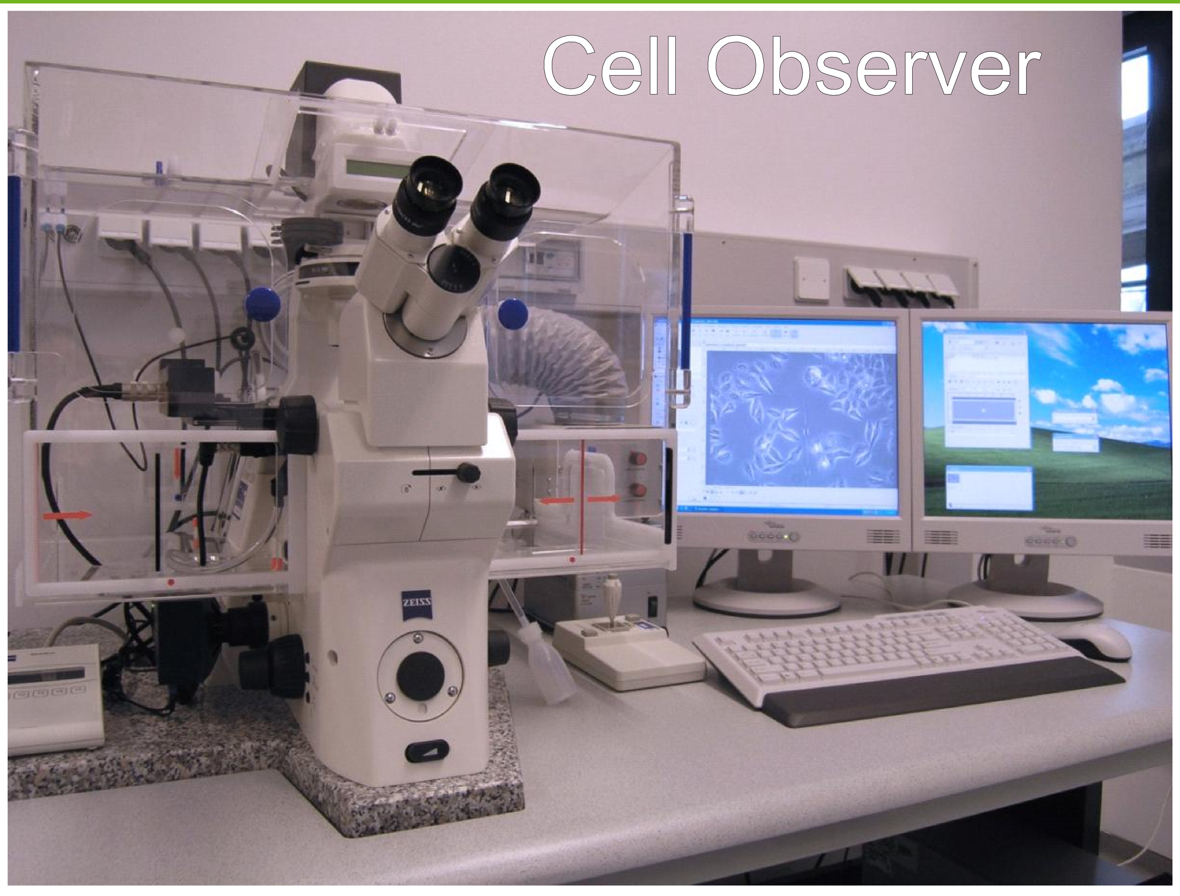
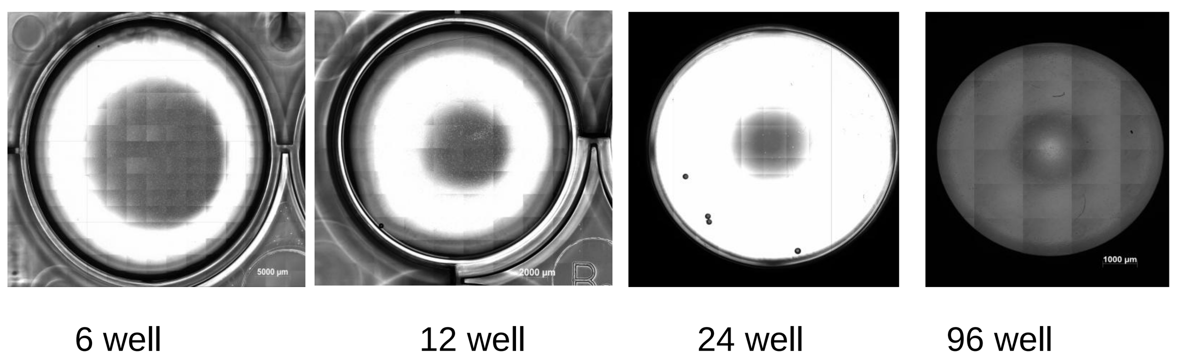
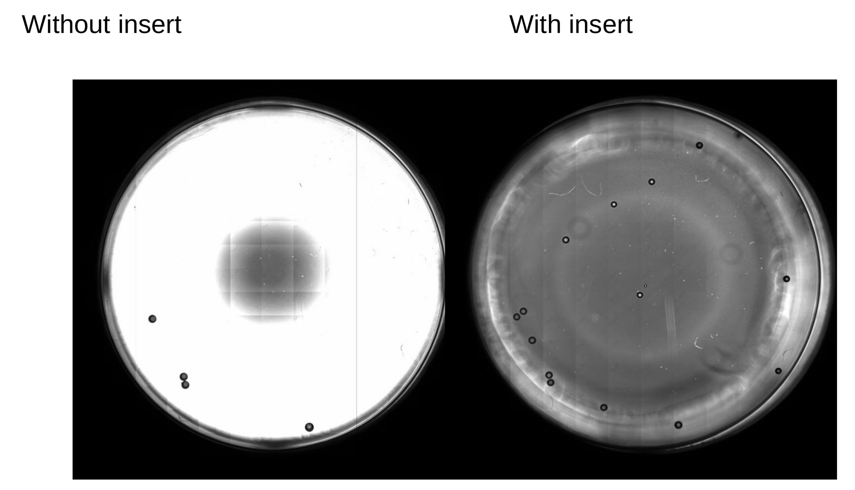
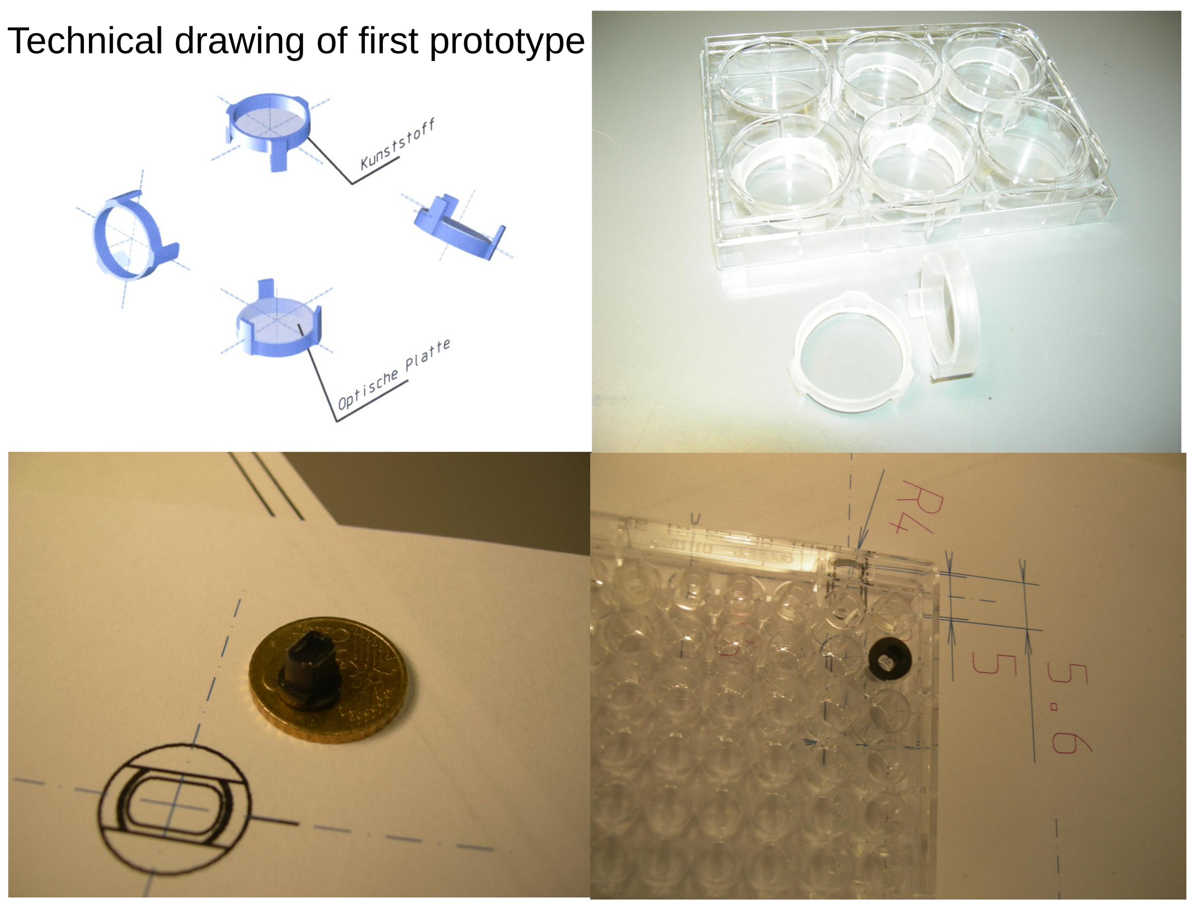




Scans of multiwell plates demonstrating the loss of phase contrast



24 well plate with and without the insert. This shows a significant increase in the phase contrast over the whole of the well.



Phase contrast microscopy, first described in 1934 by Dutch physicist Frits Zernike, is a contrast-enhancing optical technique that can be utilized to produce high-contrast images of transparent specimens, such as living cells. Anomalies can be seen when using phase contrast microscopy in an environment where the meniscus at the air to medium surface interface results in a curved surface. The refraction at this interface results in a displacement of the surround wave light path away from the annulus ring. This leads to a loss in contrast and even loss of the image. It's correction requires changing the interface so that it is parallel to the multiwellplate bottom. An insert carrying a coverslip parallel to that of the multiwellplate bottom, allows observation of the specimen at large distances from the centre of the well without incurring distortion or loss of the image. The insert can be adapted for use in all sizes of multiwellplates where a correction for the reduction of refraction anomalies is necessary. In order to attain this, the insert, consists of a plastic tube with an optical disc sealing one end. The tube stands on three legs to maintain a parallel orientation of the disc to the observation surface. The insert is smaller than the well in which it is placed to enable gas exchange between the fluid medium and the surrounding gas atmosphere for the cells growing below it. In Fig. 1, a demonstration of the effectiveness can be seen. The images on the left show the results without the insert, and on the right with it. As one ascends the columns the displacement from the centre line of the well, can be seen and in the associated sketches next to each of the images. The insert can and does significantly increase the number of images obtainable from a single well. This then requires less medium, fewer cells and fewer reagents for the tests. This simple cheap and easily modifiable construct could simplify robot controlled analyses as the meniscus refraction problem is instantly solved without the use of complex optics.

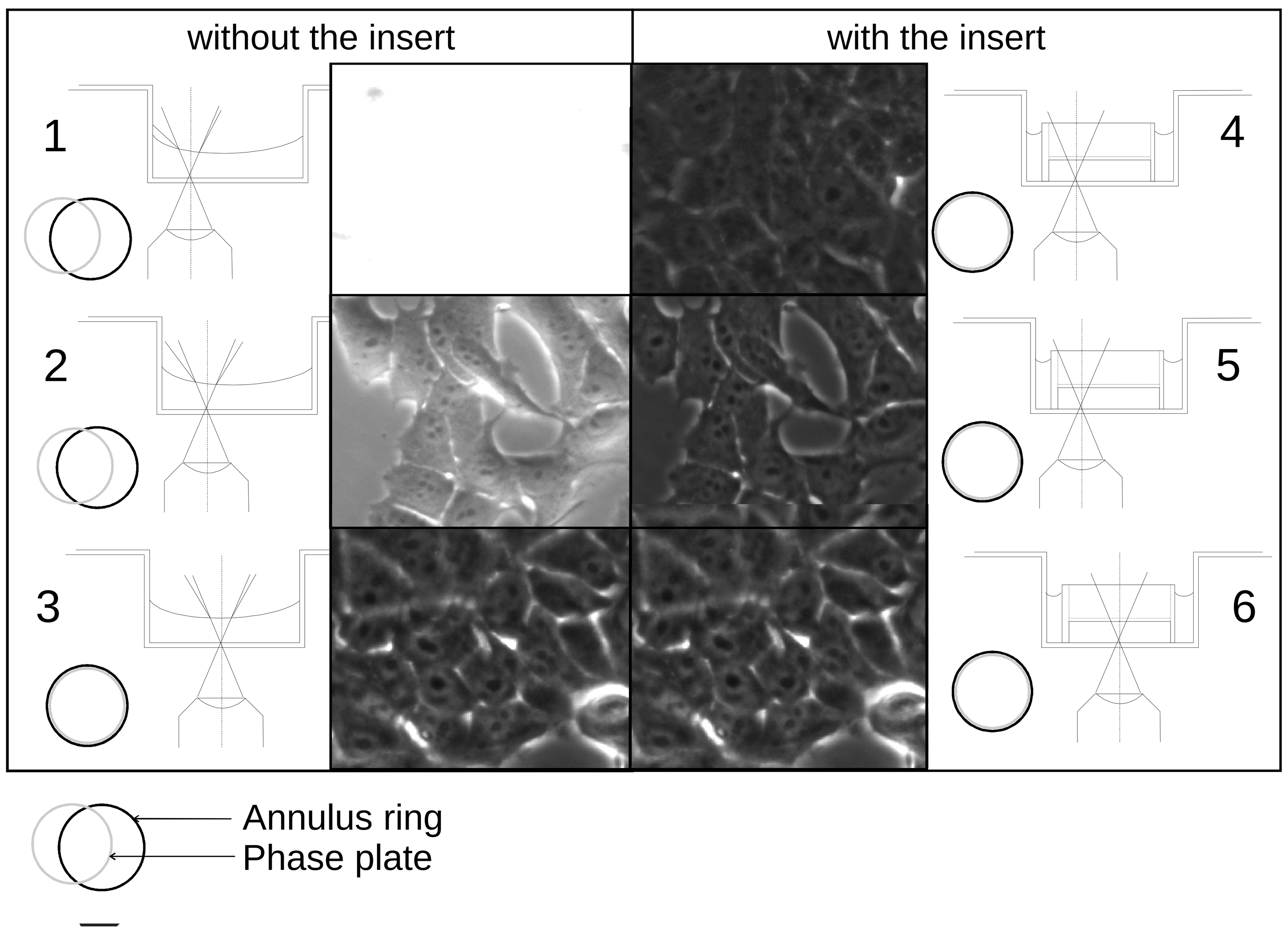


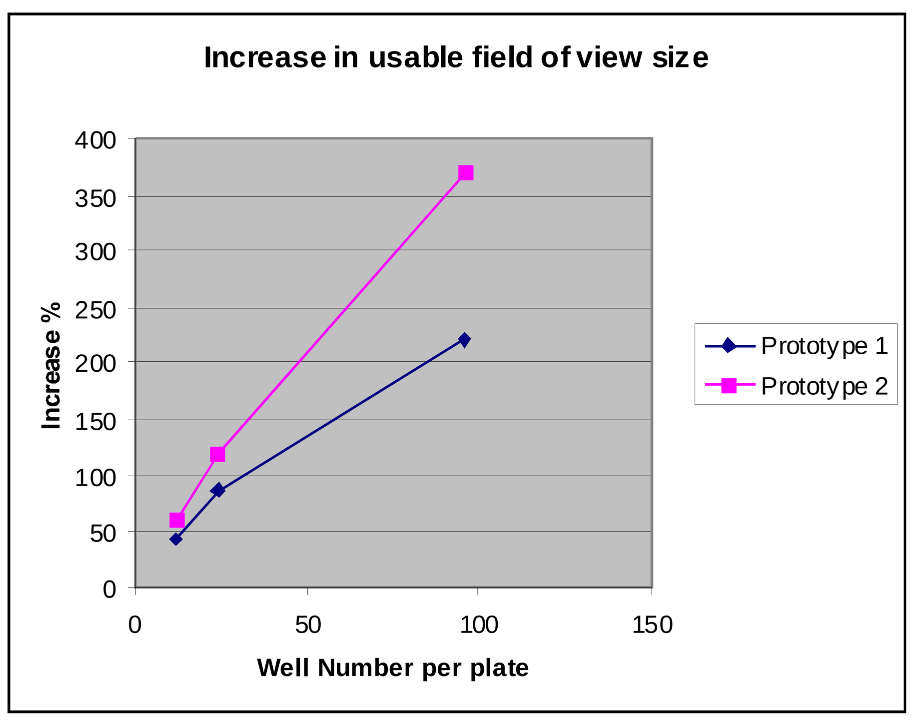
Fig. 1 Phase contrast images in a 6 well multiwell plate with and without the insert. The dislocation of the annulus ring to the phase plate is caused by the meniscus. Images 1 to 3 are images at various distances from the centre of the well without the insert and images 4 to 6 at the same positions with the insert.

Multiwell plate adapter for increasing usable the field of view.

Multi wells plate have various well sizes e.g. 6 12, 24, 96, etc. Due to meniscus formation at the fluid surface a reduction in the usable normal field of view for e.g. phase contrast observation is restricted. In Table 1 the well size and the measured maximum usable diameter of the field of view is shown. Two prototype constructions and the increase in the field of view for various well sizes is shown whereby prototype 2 makes the available FOV substantially bigger and is able at the size of a 24 well plate able to double the size of the FOV. Both prototypes allow for gas exchange with the culture medium.

Wells	Diam. Of Well	Normal FOV	Prototype 1 FOV	Increase %	Prototype 2 FOV	Increase %
12 well	**22mm**	12,6	18	43	20	59
24 well	**15,6mm**	6,2	11,6	87	13,6	119
96 well	**6.0mm**	?	2,2	220	3,7	370

Table 1 Increase in the usable Field of view at various well sizes.



Advantages of use of the insert

- Positive displacement meniscus correction enables the use of96 well plates for phase contrast observation.
- Reduction of the medium and cell quatities for observation
- Increased throughput for cell migration studies
- Form allows gas exchange for long term studies up to at least5 days without medium change
- Reduced evaporation
- Cheap and easily modifiable as reusable or single use versions
- The optical disc can be replaced with alternative discs for use under various optical conditions (e.g. Pol filter)
- No training required
- No special equipment
- Fits all standard multiwellplates
- Digital image analysis is made easier.