



Tech Report 206:

Viewing Cells on Flexcell®'s Culture Plates

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Culturing Cells in a Mechanically Active Environment™
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VIEWING CELLS ON FLEX I® AND FLEX II® SERIES CULTURE PLATES

Cells growing on top of the silastic membranes in the Flex I® culture plates can be difficult to see through the standard inverted cell culture microscope unless the refractive index of air/rubber interface is modified. The simplest method to change the refractive index is to set the Flex I® plate into a shallow dish filled with enough water to contact the bottom of the silastic membrane. Once the air/rubber interface is changed to air/water/rubber, cells on the growth surface are easily visualized with phase contrast or bright field and photographed.

A lid from a 6-well, 35 mm culture dish will hold both the Flex I® plate and more than enough water. Do not over fill with water, add enough to bring the level in contact with the underside of the rubber membrane. Immerse one end of the Flex I® plate in the water initially so that air underneath the plate will be forced out. Be sure no air is trapped under the membrane.

Water vapor condensing on the lid of the Flex I® plate can lessen the contrast, so keep the plate bottom and lid warm. If the lid is not removed or lifted, then sterility of the well will remain intact. The bottom of the Flex I® plate can be wiped with 70% EtOH before replacing the plate into an incubator.

Flex II® plates can be viewed as above but usually do not require the water tray. The plastic bottom of these plates will change the refractive index, and cells can be viewed without the additional setup.

VIEWING CELLS ON BIOFLEX® CULTURE PLATES

An inverted or upright (standard) microscope can be used to view cells on the BioFlex® plate. Instructions for using each are as follows:

Inverted Microscope

An inverted microscope allows viewing of the cells from the underside of the membrane. The BioFlex® plate is slightly higher and thus farther away from the microscope objective. To view cells on the growth surface of the BioFlex® membrane, the mechanism that stops the travel of the objective must be removed or adjusted so that the objective can be raised to a position close enough to the membrane to view cells. A spacer ring can also be placed between the microscope objective turret and the objective. This spacer ring is usually a screw-in device. Contact your microscope manufacturer to determine the best method and to inquire about a spacer ring. Before viewing cells, remove the silicone lubricant from the bottom of the rubber membrane. See instructions below for removing lubricant.

Upright (Standard) Microscope

A standard microscope allows viewing of the cells directly from above. A 40X water-immersion objective should be used to view the cells. The objective should be inserted into the BioFlex® well from the top. (**NOTE:** *the walls of the well will somewhat limit the viewable area of the membrane.*) Before viewing cells, remove the silicone lubricant from the bottom of the rubber membrane. See instructions below for removing lubricant.



**REMOVING LOCTITE® SILICONE
LUBRICANT FROM BIOFLEX® MEMBRANES**

***NOTE:** Perform this procedure after cells are fixed.*

1. Using a dry cotton swab, wipe off as much of the excess lubricant as possible. This may require a couple of swabs. Only a thin film of lubricant should remain on the membrane surface.
2. Wet the tip of a new cotton swab with a mild cleaning solution such as Formula 409®, window cleaner, or hand soap. Rub the wet swab gently over the entire surface of the well. Repeat with a new cotton swab and fresh cleaning solution as necessary to remove all residual lubricant from the membrane surface.
3. Rinse the cleaned surface with deionized water using a spray, wash, or dispensing bottle.
4. Blot the surface dry with a Kimwipe or other lint-free wipe