Correlative Microscopy Cover Slips

Product No. 260500, 260501, 260502

HOW TO USE

1. Sterilize the coverslip with alcohol, then dry and add the culture.
2. Ensure that the grid is positioned correctly so that the text is readable.
3. Observe your cell culture using light microscopy (transmitted and / or fluorescence) and identify the area of interest *(Fig. 1 and 2)*.
4. Record the images needed, noting the coordinates of the squares where there are cells of interest *(Fig. 1 and 2 show coordinate 8C)*.
5. Fix, dehydrate and embed with resin for examination by transmission electron microscopy.
6. At the end of the embedding procedure, invert a BEEM type capsule filled with resin onto the coverslip covering the selected cells of interest *(Fig. 3)*.
7. Cure and detach the coverslip *(Fig. 4)*, the footprint of the grid *(Fig. 5)* allows location of the position. Trim the block *(Fig. 6)* in the selected area then make cuts using an ultramicrotome.

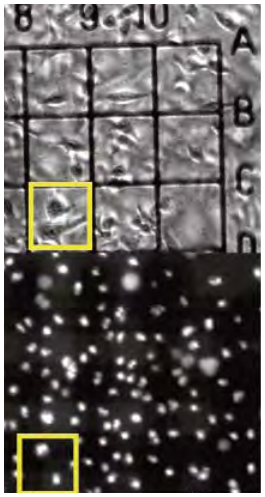


Figure 1 & 2


Figure 3

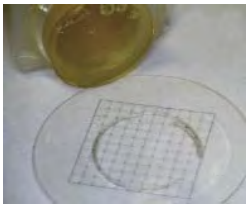


Figure 4

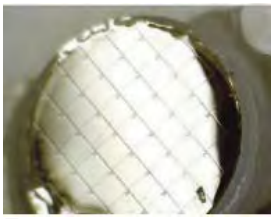


Figure 5

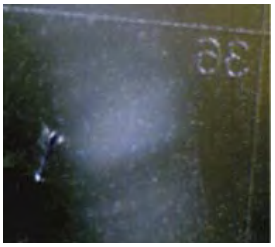
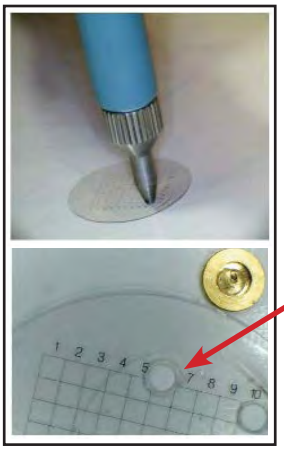


Figure 6



Punch area of interest for cryo-fixation

Figure 7

HINTS AND TIPS ON USING CORRELATIVE COVER SLIPS

1. Use with Fluorescent Microscopes. These new Correlative Microscopy Coverslips (CMCs) are not designed to replace glass or quartz coverslips, which have superior optical properties, and so are far better for fluorescent microscopy. The CMCs are designed to satisfy the needs of correlative microscopy where an initial, general analysis is performed using light or fluorescent microscopy and then the specimen is further processed for analysis by SEM, TEM or cryofixation.

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If you need to perform detailed analysis using fluorescent microscopy then the CMCs are not the solution. If however, you want to perform a comprehensive analysis using different analytical techniques, then the CMCs offer an excellent solution *(Fig. 7).* The key advantage of the CMCs is that they are made on special film that can be cut or punched (for cryo applications), something that is not possible with glass coverslips. The grid image is also transferred to any embedded specimen making cell location far easier.

2. Best Types of Resin to Use. Because this is new technology it often requires a change in your preparation method. The method that you have traditionally used for glass coverslips may not be suitable for these film ones.

The key to successful use of these CMCs seems to be the type of resin and the preparation. We have already mentioned the need to ensure a hermetic seal between the BEEM capsule and the CMC film. The preparation works perfectly with low viscosity epoxy resins (e.g. Spurr, EPON). Sufficient polymerization is obtained in 24 hours at 60°C; it does not normally require 48 hours.

We are gathering information all the time from users as we refine the preparation methods, so all feedback is very useful.

3. Film Type. The CMCs are made on a special polyester base film which is 0.18mm thick. There is no absolute tolerance specified on the thickness of the film.