



Correlation Microscopy 3View Application Note

HeLa cells, stably expressing LC-GFP, were grown on gridded glass-bottom coverslip dishes (MatTek, Co.) and starved for 2 h in serum-free medium. The cells of interest were then identified by confocal microscopy. The cells were processed *in-situ* for electron microscopy (EM) and the coverslips dissolved from the epoxy resin with hydrofluoric acid. The cells were again identified in the resin block and subsequent serial images.



Figure 1. (A) 10x GFP image. (B) 10x DIC (C) GFP-DIC image. (D) Block-face of the same area of interest, H2 image with a stereoscope.



Figure 2. (A) The block-face imaged at low magnification in the SEM with a backscatter detector; the left-hand side of the image has been cut *in-situ* with the 3View[®] system while the right-hand side has yet to be cut. (B) Higher magnification image of the cell of interest that has yet to be cut. 63x GFP-DIC merge image of the cell of interest.



Figure 3. (A) 10x GFP image of the cell of interest. (B) SBFSEM image acquired after the block-face has been cut *in-situ* with the 3View system. Imaged every 50 nm, the data set contained approximately 150 1k x 1k images acquired in about 1 h. (C) GFP-SBFSEM merge.



Figure 4. Future directions include merging 3D volumes of confocal (A) and SBFSEM (B) data.

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