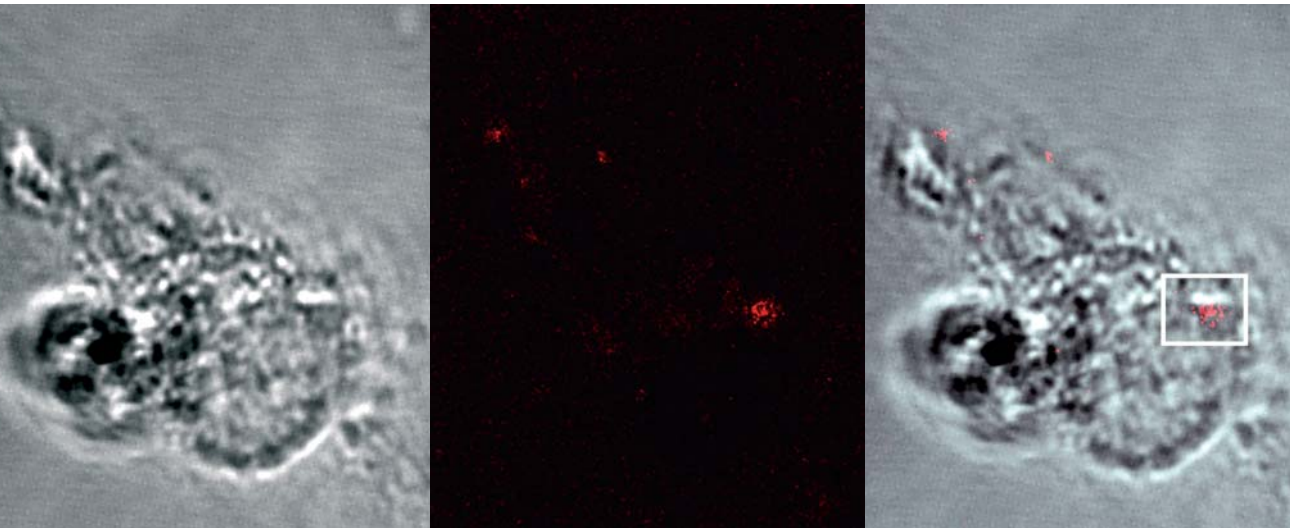
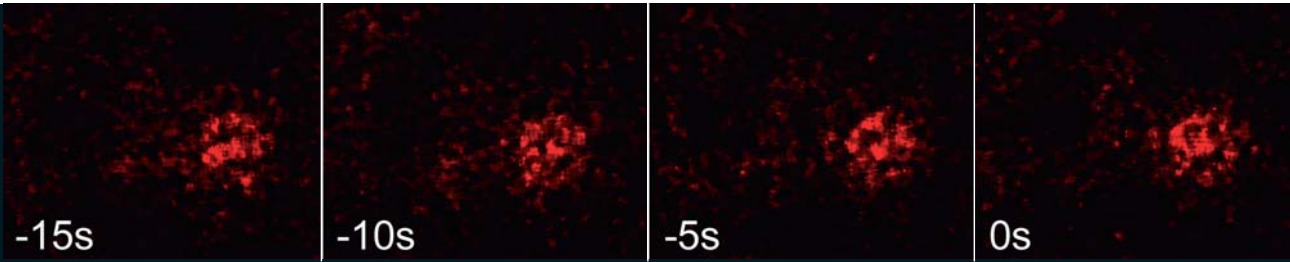


PtK2 cells were grown on sapphire discs. They were incubated with EGF-biotin for 1h on ice, and subsequently incubated with streptavidin coupled to Quantum Dots (QD655), also for 1h on ice. The cells were incubated for 30 min at 37°C to internalize the EGF-QD655 complex. A sandwich containing the live cell carrier, the sapphire disc with cells, and a finder grid was made. The sample was then imaged live under the light microscope (Figure 1–3) and subsequently high pressure frozen with the EMPACT2 & EM RTS. The sample was freeze-substituted to Epon and ultrathin sections were made and analyzed (Figure 4).

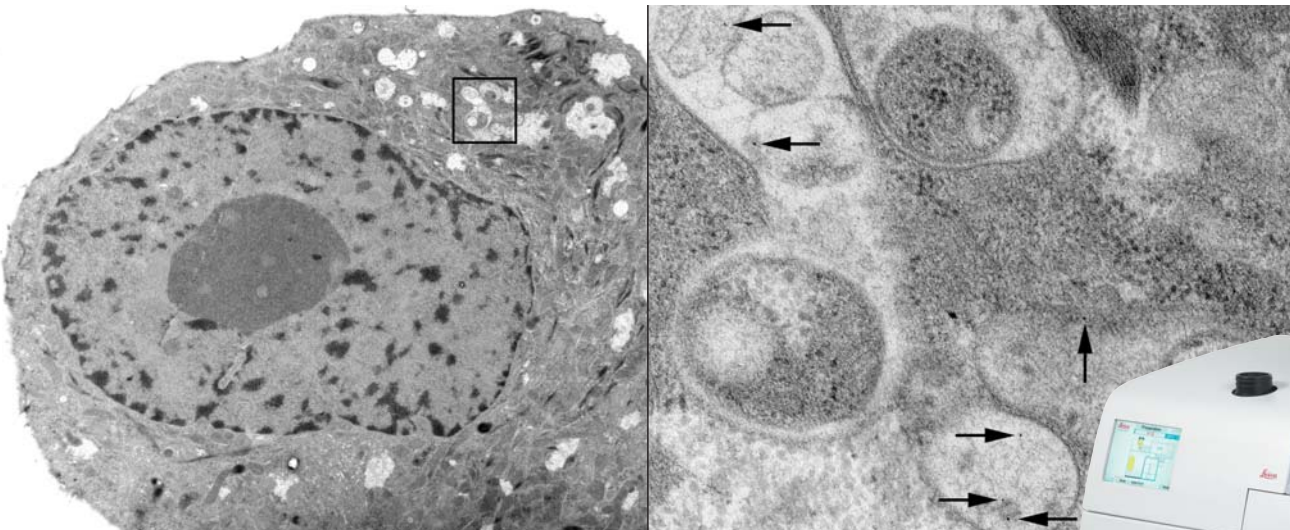
**Figure 1:** Overview images of the cells grown on the sapphire disc with the finder grid located close above the cells for retracing the sample.



**Figure 2:** When a cell of interest was located, DIC and fluorescent images (internalized EGF-QD655) were made. The overlay (right image) shows the location of the EGF-QD655 containing structures in the cell. The boxed area is the area of interest within the cell.



**Figure 3:** The structures containing the fluorescent quantum dots inside the cell of interest were followed live, making a movie sequence with images taken every second. Still images, showing images 5 seconds apart, from this movie sequence are shown. The last image was the last image of the sequence (hence time = 0s). At this moment the rapid loader was transferred from the stage insert into the RTS and the sample was frozen.



**Figure 4:** Electron micrograph of the cell of interest (left). The boxed area is the area that contains the structure of interest and a zoom into the structure of interest is shown on the right. Arrows indicate quantum dots that can be identified inside the structure. Compare the C-shape of this structure with the last image of Figure 3, and a comparable C-shape can be observed. Note that the electron micrograph is from a 70 nm section while the fluorescent image is approximately 1 µm thick.

Courtesy of Paul Verkade, MPI-CBG, Dresden, Germany



# Correlative Light Electron Microscopy with the EM PACT2 & EM RTS