



Figure 2 Scheme for EM specimen preparation of mammalian cells cultured on sapphire discs
(a) The 3 x 0.16 mm sapphire discs labelled with solvent resistant pen, sterilized with UV light both sides for 30 min, placed on the bottom of a 35mm culture dish with the marked side facing the bottom;
(b) Capping the sapphire disc on the 0.025mm side of aluminum carrier (with marked side facing outside of the carrier) ;
(c) Sketch showing the sapphire disc capped on the aluminum carrier;
(d) Customized 3.05x0.66 mm specimen holder with a step for loading the capped specimen on Leica HPM 100 or ICE high pressure freezing machine;
(e) Customized 3.05x0.66 mm specimen holder with a step for loading the capped specimen on Wohlwend HPF compact 01/02/03 high pressure freezing machine;
(f) Sapphire disc with cells embedded in BEEM embedding capsule, trimmed to exposure the disc, plunge into liquid nitrogen for 10 seconds for detaching the cells, then trim the specimen to trapeoidal shape for thin sectioning.