TROUBLESHOOTING

Step	Problem	Possible reason	Solution
2	The grid number is not visible by light microscopy	Cell density is too high	Fix and image cells by light microscopy images when the cell monolayer is less than or at most 70% confluent
4	Cells do not form a compact pellet at the bottom of the tube during centrifugation	Fixation time is too long	Reduce the fixation time for cells in the dish before scraping and transferring the cell monolayer to a 1.5 mL tube
7	Can not see ribosomes on ER	rOTO procedure highlighted membrane structure which reduces the contrast of ribosomes	Change protocol to routine glutaraldehyde - osmium fixation, however, the samples are likely to have increased charging issues during SBF-SEM imaging
19	Sample can not be cut evenly	Sample has not been fully polymerized	Bake sample in a 100 °C oven for 1 h prior to loading the sample into the SEM chamber
22-24	Charging during imaging for cell pellet	Cell pellet is not grounded	Trim the blockface pillar to expose the sample at both the top and bottom of the pillar prior to mounting onto a 3View pin
29	Glass can not be separated from dish for enface embedded samples	1) Resin is left over from the final step of resin infiltration 2) Resin has overflowed from glass coverslip into the surrounding plastic region of the dish	1) Tilt the dish at a 45° angle in order to get rid of any resin that might be left over from the final resin infiltration step 2) Add 1 or 2 drops of Durcupan directly to the glass coverslip, taking care to not let it overflow into the surrounding areas of the dish
42	Charging during imaging for CLEM enface embedded sample	Too much empty resin between cell and 3View pin	Thin the non sample side of the resin piece as much as possible before gluing it to the 3View pin
51	Sample can not be cut evenly	Diamond Knife needs to be resharpened	Resharpen the Diamond knife after ~ 6000 slices
57	Sample can not be cut evenly	Accelerating voltage is too high, causing the sample surface to warp	Reduce accelerating voltage
55	Charging during imaging	Imaging parameters need optimization	Adjust imaging parameters listed in supplementary table 1