Exploration of Eukaryotic Nuclei *In-Situ* with Cryo-ET

Dr. Lu GAN
Centre for BioImaging Sciences and Department of Biological Sciences, National University of Singapore, Singapore

The eukaryotic cell nucleus is a poorly understood organelle, even though it hosts essential processes like transcription and replication. Electron cryotomography (cryo-ET) is a form of cryo-EM that can achieve ~ 4 nm resolution for "unique" entities such as cells and organelles. Cryo-ET has been in use for 20 years, but it has only recently been applied to the study of the nucleus, by our group and by others. We and our collaborators have taken advantage of sample preparation technologies such as cryomicrotomy and cryo-FIB to prepare cells thin enough to generate high-contrast cryotomograms. We have also devised image-processing workflows to analyze macromolecular complexes within the cell nuclei -- chief among them the nucleosomes. We have shown that nucleosomes do not pack into periodic helical fibers. Instead, nucleosomes form small irregular clusters that condense as larger clusters. Sequential nucleosomes follow irregular zigzag paths because individual nucleosomes have asymmetric linker DNA. These findings are consistent with other groups' conclusions that chromatin is highly dynamic and that cells do not use densely packed ordered nucleosome arrays to limit access to regulatory sequences. In addition to chromatin, we have also exploited cryo-ET to reveal the in situ structures of the yeast outer kinetochore and the synaptonemal complex. As with chromatin, we have found that these large nuclear assemblies have features both familiar and unexpected, requiring some changes in our current understanding of chromosome-segregation mechanisms. Cryo-ET of thinned eukaryotic cells will be the central tool to solve the many mysteries of nuclear function.