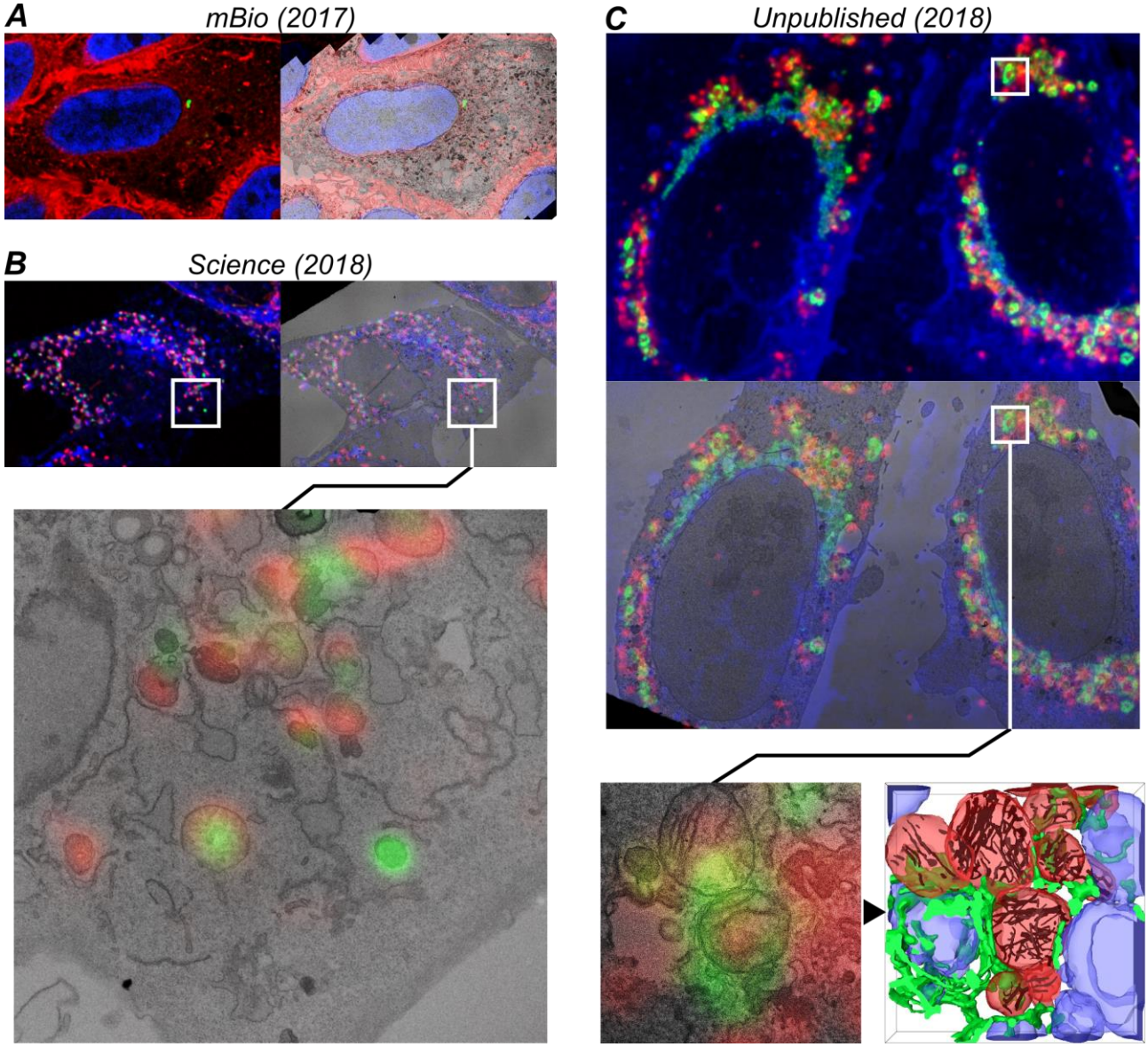
Practical considerations for the validation of spatial alignment during routine CLEM

Padman, B.[1] and Lazarou, M.[1]

[1] Monash University, Australia

Correlative light-electron microscopy (CLEM) strikes the perfect balance between electron microscopy and optical imaging by enabling ultrastructural TEM analysis of subcellular targets identified by live-cell imaging. Numerous techniques exist for overcoming the technical challenges associated with CLEM, particularly for the optimization of target relocation and improvement of sample compatibility between the imaging techniques. However, additional technical obstacles remain to be addressed, such as non-linear sample deformation, imaging artifacts, deficiencies in spatial reference systems, and potential axial/angular misalignments of Cartesian coordinates. These artifacts are subtle, but their combined effect can reduce the certainty of spatial alignment during CLEM making it difficult to determine whether the experiment was genuinely successful. My presentation will highlight the influence of these artifacts on recently published CLEM experiments analyzing bacteriophage transcytosis in mammalian cells (Figure A; *mBio*, 2017)[1] and mitochondrial DNA release (Figure B; *Science*; 2018)[2]. Unpublished examples of successful and failed CLEM experiments will also be shown (Figure C). Strategies to overcome these obstacles when navigating the 3D architecture of the cell will also be outlined.

1. Nguyen, S. *et al.* Bacteriophage Transcytosis Provides a Mechanism To Cross Epithelial Cell Layers. *mBio* **8,** e01874-17 (2017).
2. McArthur, K. *et al.* BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis. *Science* **359,** eaao6047 (2018).


A
mBio (2017)
B
Science (2018)
C
Unpublished (2018)