Multiple sclerosis is a progressive autoimmune disease where myelin is gradually stripped from axons. Axon degeneration inevitably follows protracted myelin loss ultimately leading to irreversible neurological decline. To better understand the cellular mechanisms associated with the axon loss phase of the disease, spinal cord axons from the experimental autoimmune encephalomyelitis (EAE) animal model of multiple sclerosis were examined using correlated in vivo time-lapse microscopy and serial section transmission electron microscopic (ssTEM) reconstruction. A novel technique, termed near infrared burning (NIRB), was developed that took advantage of a femtosecond-pulsed mode locked laser’s ability to create photoconvertible fiducial markers for routine identification of previously imaged axons for ssTEM reconstruction. This combination of imaging techniques revealed the subcellular milieu that underlies axon degeneration at both the light and electron microscopic level. In particular, paranodal regions of axons in EAE animals contained a significantly higher population
of mitochondria with large rounded, electron lucid, vesiculated mitochondria with unorganized cristae compared to controls. This effect was largely restricted to the paranodal region and was not always associated with direct immune cell interaction or myelin loss. Together, these results suggest a novel mechanism for axon degeneration that is not only focal in nature, but decoupled with myelin loss in the EAE animal model of multiple sclerosis.