Purpose: Optical-CT and optical-ECT are techniques with unique capability for high-resolution 3D imaging of structure and function in tissue samples up to 4cc. In particular, 3D vasculature structure and distribution of fluorescent bio-markers (e.g. green-fluorescent protein GFP). Quantitative imaging of these markers with optical-ECT is challenging due to attenuation within the sample which affects both emitted and excitation light. We present a novel method to implement a complete correction modeling the emission and excitation attenuation which also includes modeling the source strength variation.

Method and Materials: Corrections were implemented by modeling physical parameters in the imaging setup within the framework of an ordered subsets expectation maximum (OSEM) iterative reconstruction algorithm. Excitation source strength distribution, excitation and emission attenuation were modeled. The accuracy of the correction was investigated by imaging phantoms containing known distributions of attenuation and fluorophores. The correction was then applied to imaging a cleared mouse brain with GFP labeled vasculature and a cleared 4T1 xenograft flank tumor with constitutive RFP (red-fluorescent-protein). Reconstructions were compared to corresponding slices imaged with a fluorescent dissection microscope.

Results: Significant attenuation artifacts were observed in the uncorrected phantom images and appeared up to 80% less intense than the verification image in the central region. The corrected phantom images showed excellent agreement with the verification image with only slight variations. The corrected tissue sample reconstructions showed general agreement between the verification images.

Conclusion: Comprehensive modeling in optical-ECT imaging was successfully implemented creating quantitatively accurate 3D fluorophore distributions. This work represents the 1st successful attempt encompassing such a complete set of corrections. This method provides a means to accurately obtain 3D fluorophore distributions with the potential to better understand tumor biology and treatment responses.