

### **Esophageal Epithelial thickness and collagen quantitation.**

Epithelial thickness is determined by staining cross-sectioned tissue samples with H&E. Quantitation of thickness is performed using ImagePro Plus imaging software by taking 3-6 lengthwise measurements per slide from the lumen to the basement membrane of each esophagus. Collagen deposition is determined by staining esophageal samples with trichrome and quantitated using ImagePro Plus imaging software. Collagen measurements are recorded as area of collagen staining per length of basement membrane.