# Assessment of Uterine Leiomyomas in the Fibroid Growth Study (FGS) using Histological Sampling Methods

BJ Davis, KT Miner*, KE Haneke, HL Vahdat, PE Blackshear, DD Baird, and SD Peddada (NIEHS, RTP, NC)

**Objectives:** The objective of the FGS is to investigate why some fibroids grow to become health problems in women while others do not. Using semi-quantitative histological analyses, we will identify molecular, cellular, and pathological characteristics of the leiomyomas with differing growth dynamics to correlate the histopathologic characteristics of fibroid growth.

**Methods:** Histochemistry was performed on samples of leiomyoma and myometrium for Ki67, Factor VIII, and Masson's Trichrome. Random images of fibroids and myometrium sections were captured with a macro specifically designed for this study and analyzed using Image-Pro Plus image analysis software. The number of images collected varied based on tissue size with 30 images captured for tissues approximately 1.0 $cm^3$ and 15 images for tissues approximately 0.5 $cm^3$ or less. Cell proliferation was assessed by expression of the proliferative nuclear antigen marker Ki67 using colorimetric vector red and methyl green immunohistochemistry. Red cells, (Ki67 positive) and green cells (non-proliferating cells) were automatically identified and counted using the Image-Pro Plus color cube-based identification tool. Factor VIII, an endothelial cell marker, was used to determine tumor vascularity. Each vessel was manually traced using the Image-Pro Plus polygon tool, which determined the area of each vessel as well as the total number of vessels present. Masson's Trichrome stain was used to distinguish muscle from fibrous components of the tumors. The color cube-based identification tool provided by Image-Pro Plus automatically determined the percent of fibrous tissue and smooth muscle tissue.

**Results/Conclusions:** Preliminary data analysis suggests that there is no detectable difference in cell proliferation rates, but that fibrous and vascular components of tumors vary with size of the leiomyomas.