



NanoString GeoMX slide scan of lymphatic malformation stained with morphology markers Alexa Fluor® 647 CD31/PECAM-1 (general endothelial cell marker, red), Alexa Fluor® 594 Podoplanin (specific lymphatic endothelial cell marker, green), and Alexa Fluor® 488 Syto13 (nuclear stain, blue).

Lymphatic malformations (LM) are caused by defective morphogenesis of lymphatic vessels and surrounding tissue overgrowth due to post-zygotic activating mutations in the oncogene *PIK3CA*. Interestingly, less than 10% of cells in LM are mutant. Data on how such a small number of mutant cells produce bulky, complex LM are lacking, but localized, intralesional paracrine signaling must play a role. Techniques that combine single cell mutation detection with gene expression profiles are both novel and essential to understand the pathophysiology of LM, including which cell types drive the formation of LM and how signaling is aberrant.

The overall goal of this investigation is to improve our understanding of LM pathophysiology and unlock answers to two central questions: how does such a small population of mutant cells produce these complex LM phenotypes (Aim 1), and what cell type(s) possess the driving mutation (Aim 2)?

To address Aim 1, we will compare gene expression data between lymphatic endothelial cells (LECs) and non-LECs within LM using spatial transcriptomics (NanoString GeoMX). This image shows a NanoString GeoMX slide scan of a lymphatic malformation stained with morphology markers Alexa Fluor® 647 CD31/PECAM-1 (general endothelial cell marker, red), Alexa Fluor® 594 Podoplanin (specific lymphatic endothelial cell marker, green), and Alexa Fluor® 488 Syto13 (nuclear stain, blue). These morphology markers guide selection of specific regions of interest, and subsequent measurement and analysis of RNA and protein expression from those regions allows for precise examination of intralesional heterogeneity - for example, comparing the expression programs of one cyst vs. another cyst within the LM, along with LEC-adjacent cells vs. LEC-distant cells. Since LECs are known to possess the driving mutation, this may provide the first direct evidence of a paracrine signaling mechanism in LM.