Limited systemic delivery in solid tumors has greatly reduced the efficacy of most therapeutic options in pancreatic ductal adenocarcinoma (PDAC). The excess of desmoplasia in PDAC tumor microenvironment elevates total tissue pressure, which is often measured via solid stress and interstitial fluid pressure. While these two factors are well-studied to characterize their influence on limited diffusion and convection transport in solid tumors, the nature of point-probe based measurements makes it difficult to assess the heterogeneous profile of tissue pressure. Therefore, this study aimed to explore tissue stiffness as an alternative biological parameter that has the potential of being a non-invasive measurement as well as having the resolution to detect tissue heterogeneity in PDAC. An ex vivo stiffness mapping system was developed which included a motorized xyz table and a fiber optic pressure sensor operated by a micro-opto-mechanical system. The motorized table tracked the spatial coordinates of the sensor as it moved along the tissue surface to independently map out stiffness at 300 micron resolution. At each location, the sensor measured pressure based on a 3-step loading test to determine stiffness. This stiffness mapping system was calibrated with ultrasound elastography to produce absolute Young’s modulus values. The study has showed that stiffness heterogeneity in two orthotopic xenograft models, AsPC-1 and BxPC-3, was strongly correlated with collagen content in the tumors. The inverse correlation between stiffness and vascular patency highlighted the feasibility of using stiffness to predict drug penetration in PDAC tumors. Texture analysis to evaluate collagen thickness and structure were performed to further explore the cause of heterogeneity in pancreatic tumor microenvironment across different tumor lines.