Breast cancer is the second leading cause of cancer death of women in the United States, so breast cancer screening for early detection is common. The purpose of this dissertation is to optimize quantitative ultrasound (QUS) methods to improve the specificity and objectivity of breast ultrasound. To pursue this goal, the dissertation is divided into two parts: 1) to optimize 2D QUS, and 2) to introduce and validate 3D QUS. Previous studies had validated these methods in phantoms. Applying our QUS analysis on subcutaneous breast fat demonstrated that QUS parameter estimates for subcutaneous fat were consistent among different human subjects. This validated our in vivo data acquisition methods and supported the use of breast fat as a clinical reference tissue for ultrasound BI-RADS® assessments. Although current QUS methods perform well for straightforward cases when assumptions of stationarity and diffuse scattering are well-founded, these conditions often are not present due to the complicated nature of in vivo breast tissue. Key improvements in QUS algorithms to address these challenges were: 1) applying a “modified least squares method (MLSM)” to account for the heterogeneous tissue path between the transducer and the region of interest, ROI; 2) detecting anisotropy in acoustic parameters; and 3) detecting and removing the echo sources that depart from diffuse and stationary scattering conditions. The results showed that a Bayesian classifier combining three QUS parameters in a biased pool of high-quality breast ultrasound data successfully differentiated all fibroadenomas from all carcinomas. Given promising initial results in 2D, extension to 3D acquisitions in QUS provided a unique capability to test QUS for the entire breast volume. QUS parameter estimates
using 3D data were consistent with those found in 2D for phantoms and *in-vivo* data. Extensions of QUS technology from 2D to 3D can improve the specificity of breast ultrasound, and thus, could lead to improved screening with this modality.