High-frequency (>15 MHz, i.e., wavelength <100 μm) ultrasound has the potential to characterize biological tissues quantitatively at the microscopic level. In this study, quantitative ultrasound (QUS) methods were developed to evaluate freshly excised human lymph nodes from patients with possible metastatic cancer. The objectives were to improve detection of small but clinically significant lymph-node metastases that often are missed during routine histological evaluation. Three-dimensional (3D) ultrasound data acquisition was conducted on freshly excised nodes using a spherically-focused transducer with a center frequency of 21 MHz. Average scatterer sizes and acoustic concentrations were estimated using QUS methods with a Gaussian scattering model over a bandwidth extending from 11 to 27 MHz. Strategies were developed to recover lymph-node orientation and size after histological sectioning to allow for spatially matching QUS estimates to histology. The signal- and image-processing aspects of QUS estimation are presented and the preliminary results are shown that demonstrate our ability to acquire 3D ultrasound and histological data, to co-register them spatially, and to obtain scatterer-size and acoustic-concentration estimates for specific tissue types (cancer versus non-cancer) within the nodes. In future studies, these QUS estimates will be used to identify histologically difficult-to-detect micrometastases in lymph-nodes. [Supported by NIH R01CA100183]