Three dimensional (3D) cell culture gels are invaluable tools for isolating complex molecular processes associated with cancer. We are developing 3D gels for studying multimodality diagnostic imaging of structural and functional features of malignant breast disease. Our first study involves EHS extracellular matrix extract (Matrigel, BD Biosciences) for viewing fibroblast cell proliferation in a controlled and well characterized microenvironment. These gels were studied by combining mechanical, optical, and magnetic resonance spectroscopic imaging (MRSI) techniques to describe stromal structure (mechanical), cell distribution and phenotype (optical), and metabolic effects (pH imaging via MRSI). After 4 days of culture in the 3D gel, a 5-fold increase in fibroblast number was observed with optical coherence tomography and histology. Using imidazole as an exogenous pH indicator, MRSI showed the cell proliferation reduced gel pH by 0.2. A concomitant increase in collagen production stiffened the gel 65%; the elastic modulus increased 268 Pa. The observed non-uniform cell migration patterns were consistent with the multimodality image data, confirming that diagnostic images can describe essential functional and structural properties. Adding normal and cancerous epithelial cells, the effects of heterotypic cell signaling essential for tumor development can be imaged. Because they create known cellular microenvironments characteristic of molecular disease, 3D gels form “living phantoms” for detailed multimodality imaging studies of cancer.