Whole Slide Digital Imaging of Normal Breast Tissue

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Susan G. Komen® has been tackling the frontier of breast cancer research since 1982. A pioneering project that Komen has funded involves the collection of normal breast tissue to further the understanding of normal breast to elucidate breast oncogenesis. This study sought to discover a way to analyze normal breast tissue through the use of whole slide digital imaging. The 350 normal breast tissue samples provided by the Susan G. Komen® Tissue Bank at IU Simon Cancer Center (KTB) were scanned using the Aperio automated whole slide scanning system. All samples were divided into four groups based on the amount of epithelial tissue visually present: Abundant, Moderate, Minimal, and None. Contrary to its name, the “None” category did contain some epithelial tissue and was included in the analysis. To analyze the normal breast biopsies, an algorithm was created to recognize epithelial tissue in normal breast tissue. All samples were analyzed using this “Purple” algorithm, revealing the ratio of epithelial tissue to other tissue types. Originally, the analysis of the samples excluded adipose tissue, resulting in the following average percent of epithelial tissue in each category: 18.12% in Abundant, 10.93% in Moderate, 9.30% in Minimal, and 7.20% in None. Additionally, the average percent of epithelial tissue for the whole sample, including adipose tissue, was calculated, resulting in the following: 11.30% in Abundant, 4.41% in Moderate, 2.73% in Minimal, and 0.80% in None. By defining the tissue content of an acquired tissue sample, the KTB can better determine how much of a given sample to release to investigators involved in breast cancer research.

The collection and analysis of normal breast tissue is a fairly novel endeavor. The Susan G. Komen® Tissue Bank at the IU Simon Cancer Center was the first to create a biorepository of annotated normal breast tissues. The KTB was formed to speed research focused on prevention of breast cancer through observation of normal breast tissue and the use of normal tissue as a control in research. This study has furthered this goal by using whole slide digital imaging to capture and analyze the distribution of epithelial tissue throughout normal breast tissue. Through this study, the Komen Tissue Bank can use the information gleaned to enhance the collection process as well as determine which samples can best be used for RNA and DNA extraction. The use of RNA and DNA from normal breast tissue could allow researchers to discover potential biomarkers of breast cancer [1].

The samples used in this study were normal breast tissue provided by the Susan G. Komen® Tissue Bank at the IU Simon Cancer. Three hundred and fifty breast tissue samples were evaluated after being mounted onto positively charged slides and stained for routine H&E. All tissue samples were collected following a detailed IRB approved protocol, informed patient consent, and HIPAA compliance protocol.

The Aperio whole slide digital imaging system (Vista, California) was used for imaging all of the breast tissue samples, which were then housed and stored in Aperio’s Spectrum software system. Computer-assisted morphometric analysis of digital images was completed using the Aperio software included with
the Aperio scanner. One hundred samples were analyzed for each of the following categories: Abundant, Moderate, and Minimal, while only 50 samples were analyzed from the None category. An altered algorithm for the positive pixel algorithm, the “Purple” algorithm, was set up for H&E staining and was used for the imaging of the breast tissue H&E slides. The positive pixel algorithm was modified to distinguish between the epithelial tissue (blue) and stroma (pink) of each sample. The “Purple” algorithm was created to recognize epithelial tissue as positive (red) and other tissues as negative (blue). The first amount of data excluded breast adipose tissue in the analysis of the sample to determine the ratio of epithelial tissue and stroma. The adipose tissue was later included in the data, through calculations, to reveal the amount of epithelial tissue in the whole sample. After analysis, five samples with the highest percent positivity and five samples with the lowest percent positivity from each category were visually observed to determine that the percent positivity was correct.

With the exclusion of adipose tissue, the average percent of epithelial tissue present in the different categories were the following: 18.12% in Abundant, 10.93% in Moderate, 9.30% in Minimal, and 7.20% in None, while the calculated percent of epithelial tissue with the adipose tissue was 11.30% in Abundant, 4.41% in Moderate, 2.73% in Minimal, and 0.80% in None. These results reveal the significant correlation between the percentage of epithelial tissue determined by the algorithm and the original category in which the biopsies were placed, providing the algorithm as a less subjective approach to determining the amount of epithelial tissue in each biopsy. Through a pathological hand-count, it was determined that the Abundant, Moderate, and Minimal categories did provide a sufficient amount of epithelial cells (>400 cells) to be used for RNA and DNA analysis. With modern techniques such as tissue enrichment and laser capture microdissection these normal biopsies can produce high quality RNA and DNA yields to be used in genomic research, allowing a further understanding of normal breast tissue. A quantitative image analysis allows the investigator to better assess individual samples and thus facilitates his or her research.

Reference:


![Figure 1](image.png)