Detecting Cancer Through Fractal analysis of the Transmissivity of a Tissue Microarray

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Background/Introduction

Goal of the Study The objective is to develop new methods which enable standardized values for a tumor through analysis of fractal dimensions and the transmissivity of the sample, without the use of additional expensive stains, reagents, and invasive procedures.

Colon Cancer TMA A Tissue Microarray (TMA) contains many small representative tissue samples, (in this case, colon tissue samples) from several different cases, assembled on a single histologic slide, thus allowing thorough analysis of multiple specimens at the same time.

Fig. 1. TMA Slide

Histograms A histogram is a plot between the number of pixels and pixel intensity; it is a type of bar graph.

Fig. 2. Histogram of a benign tumor taken in ImageJ Software.
The histogram code runs by initially reading the grayscale value at the first entry and coming up with pixel intensity between 0 and 255. It will then move on to the next row or column entry until it finishes reading all the raster data.
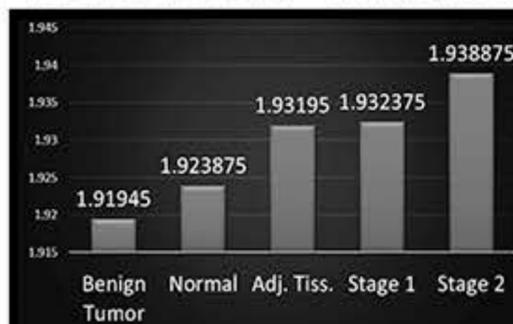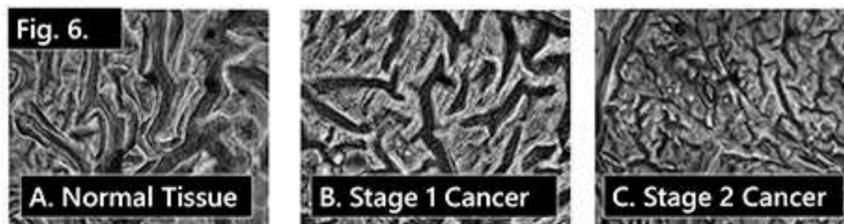
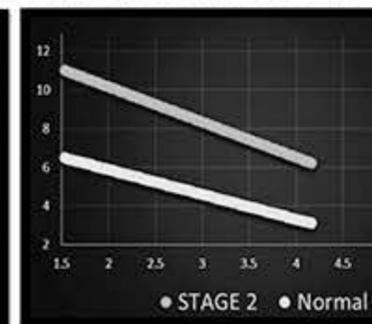Fig. 3 *Olympus BX50* and transmission diagram

What is a Fractal?

Fractals
- A natural phenomena that exhibits self-similar structural patterns at every scale.
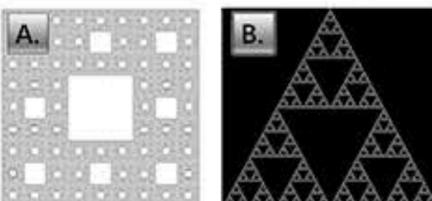- Metric properties like length, area etc. is function of scale of measurement.

Fig. 4. The Sierpinski Carpet (A) and Triangle (B) are both fractals and examples of self-similar sets.

Fractal Dimension
- Is an index for characterizing fractal patterns by quantifying their complexity as a ratio of the change in detail to the change in scale.
- It is also characterized as a measure of the space-filling capacity of a pattern.

Box Counting for Determining Fractal Dimensions
- To calculate the dimension of a fractal, imagine the fractal lying on an evenly spaced grid, and count how many boxes are required to cover the set.
- The box-counting dimension is calculated by seeing how this number changes as we make the grid finer by applying a box counting algorithm.
- Suppose that $N(\varepsilon)$ is the number of boxes of side length ε required to cover the set. Then the box-counting dimension is defined as:

$$\dim_{\text{box}}(S) := \lim_{\varepsilon \to 0} \frac{\log N(\varepsilon)}{\log(1/\varepsilon)}.$$

Method

1. Selection of Tissue Samples
- A Colon Cancer TMA was ordered from *Biomax*,
- *(CO808)*. Of 80 tissue samples found on the TMA, 40 were selected for imaging. The 40 samples ranged from normal tissue, benign tumors, Stage 1 and Stage 2 malignant tumors, and cancer adjacent tissue.

2. Imaging of Colon TMA
Images of Colon tissue on a TMA Slide were captured utilizing the following tools:
- *Olympus BX50 Microscope*
- *Infinity2 Microscopy Camera*
- *Lumenera CAPTURE Software*

3 images were taken of each sample to achieve an accurate representation of the tissue. Imaging techniques found in FIG.3.

3. Image Analysis and Processing
Using *ImageJ Software* collected images were processed by:
A. Converting images to 8-bit format
B. Converting images to grayscale
C. Converting image to binary
Processed images were then analyzed through use of Histograms and via code written to calculate Fractal Dimensions in *ImageJ*.

4. Data Analyses
- The Fractal dimensions of each image were calculated via the box counting method to measure porosity and density. Therefore, fractal theory gives a favorable layer of structures of different models that will address the complexity of the disordered, heterogeneous, and porous media of cells [2]
- Histogram data for each image including the mean and standard deviation of the sample transmissivity were recorded.
- Each tissue sample received an average value for each of the parameters in the three images taken, respectively. Values were then plotted in *Excel* to better analyze trends in the data.

Fig. 5. Processed image of a stage 2 cancerous colon tissue sample.

Results

Fig. 6.

A. Normal Tissue B. Stage 1 Cancer C. Stage 2 Cancer

Fig. 7. Average Fractal Dimension
Benign Tumor 1.91945; Normal 1.923875; Adj. Tiss. 1.93195; Stage 1 1.932375; Stage 2 1.938875

Fig. 8. Fractal Dim. Plot of Normal v S2 tissue
● STAGE 2 ● Normal

Fig. 9. STD of STD in Histogram Vals.
Normal 0.850231; Adj. Tiss. 1.249458; Benign Tumor 4.529642; Stage 1 5.235945; Stage 2 5.979283

Fig. 10. Avg. of Mean Histogram Vals.
Normal 89.42575; Benign Tumor 90.67375; Stage 1 91.74975; Adj. Tiss. 94.7525; Stage 2 102.138

Conclusion and Future Applications

Conclusion
- The study conveys that cancerous colon tissue exhibits a higher degree of fractality when compared to parameters such as normal tissue and benign tumors (Fig. 7), which leads to the conclusion that the statistical distribution of irregularities on cell contours and intracellular medium are larger and more numerous in cancerous tissue than normal tissue.
- Grayscale averages collected from the histograms (Fig. 10), convey that the degree of disorganization of the tissue reduces the light transmissivity of the tissue, which directly relates to the stage of cancer/aggressiveness of the colon tissue or the potential of tissue to become cancerous.
- According to the findings from Fig. 9, it is clear that malignant tumors display chaotic phenomena by developing less uniformity to surrounding tissue. The normal tissue data conveys how deviations in the grayscale values are miniscule, which further supports the unorganized and unregulated behavior of tumor growth. Even from the microscope images, it is visible that stage 2 cancer tissue (Fig.6C) appears much less organized and differentiated as compared to the normal tissue (Fig.6A).

Future Applications
- Today, the diagnosis of cancer is an invasive and complicated procedure. Tissue samples must be collected via biopsy, stained using specific antibodies and biomarkers and then studied for specific markers. This particular method is time intensive, expensive, and only correctly diagnoses about 85% of the time.
- Using fractal geometry, medical professionals may be able to identify cancer cells more reliably and much faster due to the study of the tissue under a microscope, negating the need for special preparations.
- Fractal data from cancer research can be compiled into a database for doctors to compare patient sample data and to diagnose cancers precisely and efficiently.

References
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2. P. F. F. de Arruda, M. Gatti, F. N. F. Junior, J. G. F. de Arruda, R. D. Moreira, L. O. Murta, L. F. de Arruda, and M.F. de Godoy, " Quantification of fractal dimension and Shannon's entropy in histological diagnosis of prostate cancer," BMC Clin. Pathol. (2013)