Detection of colon cancer stages via fractal dimension analysis of optical transmission imaging of tissue microarrays (TMA)

Shiva Bhandari†, Sri Choudannavar‡, Ethan Ross Avery†, Peeyush Sahay† and Prabhakar Pradhan†

†BioNanoPhotonics Laboratory, Department of Physics and Materials Science, University of Memphis, Memphis, TN 38152, United States of America
‡CRESH Summer Student, University of Memphis, Memphis, TN 38152, United States of America

E-mail: pp838@msstate.edu

Keywords: cancer, TMA, fractal dimension, correlation length, entropy, colon cancer

Abstract

At epidemic proportions worldwide, one in four persons now has cancer, and this statistic will change to one in two persons in near future. An important step in the fight against cancer is its early and accurate detection. This calls for affordable, quick and easy diagnostic methods. Standard pathological detection of cancer involves microscopic examination of morphological changes using stained biopsy samples, but this method is prone to human error and misdiagnosis. A tissue is a spatially heterogeneous medium with fractal properties owing to self-similarity in mass distribution. With the progress of cancer, this tissue heterogeneity changes owing to more mass accumulation and rearrangement of intracellular macromolecules like DNA, RNA, and lipids. Recently, commercially available tissue microarray (TMA) samples have gained significant attention in research studies, as the array of numerous tissue samples of different cases of a disease on a glass slide allows ease of conducting comprehensive studies. The present study uses optical transmission imaging to analyze the fractal dimension of colon TMA samples of 5 μm thickness and 1.5 mm diameter to correctly distinguish different stages of colon cancer. Results of this specialized analysis are also supported by entropy and spatial correlation length analysis. The application of this method of cancer diagnostics is discussed.

1. Introduction

Cancer is characterized by unchecked cell division, proliferation, and survival of abnormal cancerous cells. Cancer begins with the genetic mutations of DNA and RNA, followed by proliferation and metastasis (Loeb et al 1974, Mantovani et al 2008, Weinberg 1996, Scholzová et al 2007). At near epidemic proportions (Siegel et al 2017), it is critical to accurately detect cancer at its earliest stages (WHO 2007, Wulfkuhle et al 2003, Lingen et al 2008). However, most early diagnostics still rely on visual examination of microscopy images of stained biopsy specimens, and direct observation of tissues under the microscope by a pathologist qualitatively looking for abnormal cellular features like high indices of mitosis or structural irregularities (Fletcher 2007). Such technique is prone to human error and, consequently, misdiagnosis. Additionally, the approach cannot be used to accurately identify different stages of cancer. Therefore, more quantitative approaches are desired in cancer diagnostics (Baish and Jain 2000).

Biological tissues are varying dielectric/refractive index media with spatial heterogeneity in their mass density distribution. It is well known that most tissues have self-similar structure and therefore can be analyzed in terms of fractal dimension (Losa and Nonnenmacher 1996), which is 'a ratio providing a statistical index of complexity comparing how detail in a pattern changes with the scale at which it is measured' (Falconer 2003; Sagan 1994, Vicsek 1992) (see Methods for detail). The progress of cancer results in more mass accumulation inside the cells, rearrangement of the DNA, RNA and heterochromatin, as well as a change in the structure of the extracellular matrix (ECM). Such changes alter the spatial structure of tissues, which may vary from ‘nano’ to ‘micron’ length scales, leading to changes in spatial heterogeneity, as...
2. Methods

2.1. Mathematical methods

2.1.1. Fractal and fractal dimension

A fractal is a structure which exhibits self-similar structural patterns at different length scales. It is often characterized in terms of a mathematical parameter called 'fractal dimension' (Mandelbrot 1983). Fractals can be deterministic or random. Deterministic fractals are obtained recursively in a deterministic way, whereas the random fractals are obtained through a stochastic process (Bunde and Havlin 2013). The fractals occurring in nature like coastlines, mountain ranges, etc are random fractals. Random fractals are used to describe many highly irregular real-world objects. It should be noted that naturally occurring biological samples are more akin to random fractal patterns, where self-similarity at different length scales can be seen upon statistical analysis of different structures of these natural biological samples. Box counting is a commonly used method to characterize such random fractal patterns by measuring their fractal dimensions (Peitgen et al. 1992, Li et al. 2009). In this method, the fractal structure is assumed to be lying on an evenly spaced grid, and the number of boxes required to cover the fractal structure are counted. The fractal dimension is then calculated by noting how this number changes as the grid is refined by applying a box counting algorithm. Thus, fractal dimension is essentially an index for characterizing fractal patterns by quantifying their complexity as a ratio of change in structural detail to the change in scale. It can also be considered as a measure of the space-filling capacity of a pattern. That is, if \( N(r) \) is the number of the boxes of side length \( r \) required to cover the fractal structure, then the fractal dimension is defined as (Li et al. 2009):

\[
D_f = \frac{\ln(N(r))}{\ln \frac{1}{r}}
\]

Considering the random nature of a structure, we take an average of the many realizations, or ensemble averaging, of the fractal structure to get the average fractal dimension \( D_f \).

2.1.2. Fractal dimension by box counting method

The basic steps in calculating the fractal dimension by box counting method from the microscopic colonic image is shown in figure 1.

Research have been conducted to characterize different types of cancers such as human breast cancer, cervical cancer (Ohri et al. 2004), ovarian cancer, liver cancer (Nielsen et al. (2002, 2005)), colon cancer (Esgiar et al. 2002, Rathore et al. 2013) etc using the fractal dimension analysis method. The general aim of our...
research is to investigate the possibility of standardizing numerical index values to characterize different colon tumors, e.g., through fractal dimension analysis, without the use of additional expensive stains, reagents and invasive procedures on standard TMA sample of a particular thickness and dimension. Accordingly, colon TMA samples were used.

2.1.3. Spatial correlation of the mass density fluctuations in tissues
The spatial correlation of mass density fluctuations corresponding to refractive index fluctuations depends on various parameters. That is, if the system is fractal, power law decay correlation is expected. However, owing to finite size effect, we calculated the correlation as a weakly varying decay function and matched with an exponential decay form. In case of a finite size sample, there is one to one functional dependence between power law decay and exponential correlation decay lengths. However, we will prefer here to express in the spatial exponential correlation decay length that is more physical. The two-point exponential correlation decay length function can be written for two refractive index fluctuation points at \( r \) and \( r' \) as \( n(r) \) and \( n(r') \), respectively:

\[
\langle dn(r) \times dn(r') \rangle = \langle dn^2 \rangle \times \exp (-|r - r'|/l_e),
\]

where \( l_e \) is the spatial weak correlation decay length scale for the refractive index fluctuations, and \( \langle \rangle \) is the spatial ensemble averaged.

2.1.4. Entropy of structural disorder
Given a structure, we can measure its structural disorder by measuring the entropy (S) defined as follows:

\[
S = - \sum_r n(r) \times \log n(r),
\]

Where \( n(r) \) in equation (3) represents bins for logical arrays in the images and \( S \) represents the Shannon entropy calculated from the gray values of the digitalized images. Entropy provides a measure of randomness of the sample and thus allows quantification of a sample’s texture.

Fractal analysis performed in this work with an aim of characterizing the colon tissue samples is further supported by the correlation length and entropy measurement.

2.2. Tissue samples and image collection

2.2.1. TMA colon tissue samples
A colon cancer TMA (CO808, Biomax, USA) was used as our colon tissue samples. The tissue array consists of colon tissue samples where each sample in the array has 1.5 mm diameter and 5 \( \mu m \) thickness. Out of 80 total colon cores in the TMA, only 40 were selected for imaging that look similar quality in microscope. Selected colon tissue samples are from different patients ranged from normal to different stages of cancer as (1) normal tissue, (2) benign tumors, (3) stage 1 malignant tumors, (4) stage 2 malignant tumors, and (5) cancer adjacent tissue.

2.2.2. Transmission optical microscopy of TMA samples
Optical microscopy images were acquired using an Olympus BX50 Microscope (Olympus, USA) and an Infinity2 Microscopy Camera (Lumenera Corp., Canada) attached to the microscope head to capture the images. The glass slide containing TMA colon samples was kept on the sample holding platform of the microscope, which was operated in transmission mode, to acquire the tissue optical images. The images were collected with the Lumenera CAPTURE software. For each case (normal, benign, adjacent, stage 1 and stage 2), eight samples were considered for ensemble averaging. Images at magnification \( \times 100 \) were sampled and digitized to eight bits of gray level and stored as digital files.

For a given biological sample, it has certain refractive index (RI) properties. In turn, a small linear increase in the mass density of the sample due to cancer will also change in the refractive index that is linearly proportional (Davies and Wilkins 1952, Barer et al 1953). Here we assume that the contrast in the gray scale image obtained in the optical transmission microscopy is due to the spatial mass density variation inside the sample, which in turn provides refractive index variation. Therefore, the pixel intensity or transmission intensity in the refractive index in the image is linearly related to the mass density inside the sample at those points for small variation of the mass.

2.3. Image analysis
The acquired microscopy images were analyzed by ImageJ (NIH, USA) software. The fractal dimension of each image was calculated via the box counting method described in the flowchart, and then ensemble averaging was performed for each sample type (normal to stage 2). Fractal results are supported by two-point correlation function and entropy calculations.

3. Results
Representative microscopic images of normal, stage 1 and stage 2 tissue types are shown in figure 2. These types of images were then further processed by ImageJ statistical analyses software to find the fractal dimension, using box counting method described in the Method Section.

3.1. Fractal dimension result
Fractal dimension was calculated for each of five different cases of colon cancer tissue types: normal, adjacent, benign, stage 1, and stage 2 categories. The results are shown in figure 3. The bar graph result
shows that the value of the fractal dimension increases with the increase in the cancer stage.

As it can be seen in figure 3, the normal tissue sample had the lowest fractal dimension, followed by the tissue sample adjacent to tumor. Subsequently, benign tumor, stage 1, and stage 2 cancer tissue samples had fractal dimensions in increasing order of magnitude. The actual or absolute fractal dimension values calculated for the samples were: 1.7192 for normal tissues, 1.7560 for adjacent tissues, 1.7720 for benign tumors, 1.8820 for stage 1 cancer tissues, and 1.9336 for stage 2 cancer tissues. Student’s t-test for each pair of measurements obtained p-value <0.05, suggesting that the fractal dimension values were different for different tissue samples of colon cancer. This is a logical finding in that the progress of cancer results in more mass accumulation with more space filling in tissue, as noted above, as well as in ECM (Dang 2012). This leads to expansion and accumulation of mass in the tissue sample, and also irregularity in the shape of the outer cell linings and, hence, an increase in fractal dimension. Also, fractality can be regarded as a quantitative measure of the space filling/irregularity of the cell structure (Bizzarri et al 2011). With the progress of cancer, the regular structure of the cell is disturbed, and the number of cancerous cells is increased (Baba and Cătoi 2007). The increase in irregularity of the cell increases the fractal dimension in tissues due to the increase in the number of cancerous cell filling the intra tissue space.

3.2. Spatial correlation length result
To support the results obtained by fractal dimension analysis, we further calculated the spatial correlation length of the mass density fluctuation and, thus of refractive index fluctuation of the sample. As pointed out above, intensity fluctuations in a transmission mode microscopy image can be attributed to the refractive index fluctuations at those points inside the sample. Since we have assumed that the small change in local refractive index inside a tissue sample is directly proportional to the change in local mass density inside the tissue, the change in intensity variation map in the 2D (2-dimension) image of the sample can be considered as a representative of change in mass density variation map of the sample (Pradhan et al 2011). Thus, by analyzing variation of intensity correlation in the sample image, the spatial correlation of mass density/refractive index variation in the sample can be obtained. We calculated the spatial
correlation decay lengths of intensity variations in the 2D sample images, with exponential correlation between the two points, as described in section 2 under Spatial Correlation of the Mass Density Fluctuations in Tissues. The results, as shown in figure 4, show that correlation length increases with the progress of cancer. With the progression of cancer, there is space filling in the cancer tissue. The space filling makes the sample more solid (i.e. less porous) and in turn the spatial correlation length increases. This result is consistent with fractal dimension analysis. Again, this increase in correlation length with the progress of cancer can be attributed to increasing mass density in tissue as the cancer/tumor grows, i.e., in an equal volume of space the cancerous tissue will have more mass compared to that in normal tissue.

Correlation length ($l_c$) indicates the nature of fluctuations, and it influences structural disorder; however, in this work, we only matched correlation length with weak exponential decay, as discussed in Method Section. To account for this, we next calculated the entropy to evaluate the randomness of the sample. To accomplish this, we used the intensity map of the tissue samples and performed the entropy calculation as described in methods above.

### 3.3. Entropy result

Finally, we calculated Shanon entropy of microscopic images of TMA samples using the method described above and using equation (3). Figure 5 shows the bar graphs of ensemble averaged entropy results for five different tissue sample cases. As randomness increases, we see more mass density accumulation and this bring more numbers of mass density fluctuations. As the number of mass density fluctuations increases, the structural disorder amount in tissue increases, in turn entropy of the tissue increases. During the carcinogenesis process, in cellular level also, there occurs mutation of the genome and epigenetics alterations which in turn reduce internal cellular information content by increasing the spatial randomness, and hence increasing the spatial entropy inside the nucleus. This results in the increase of entropy in nuclear chromatin with the increase in malignancy of the tumor (Metze et al 2010). According to equation (3), Shanon entropy depends mainly on the number of spatial fluctuations only, it does not have the information about the spatial correlation. Therefore, increased in mass density, that is also associated with the increased in number of mass density fluctuation points, also increases the Shanon entropy. Increase in mass density fluctuation (due to the
accumulation of more mass), also increases the space filling, this may also increase the correlation length.

4. Conclusions and discussions

In this paper, we studied structural properties of colon cancer TMA samples. From a tissue microarray (TMA), containing several cases of colon cancer from different patients, we took five different cases and calculated their fractal dimension using ImageJ statistical analysis software. Fractal dimension analysis was demonstrated as an efficient way to distinguish between normal and cancerous tissues at different stages. Our results show that cancerous colon tissues are more fractal in nature compared to normal and benign tissues. The hierarchy of fractal dimension is as follows: normal < adjacent to the cancer tissue < benign < stage 1 < stage 2. The gray scale averages obtained from histograms indicate that the degree of tissue disorganization reduces its light transmissivity, which directly relates to the stage, or aggressiveness, of colon cancer tissue, or the potential of colon tissue to become cancerous. Normal tissue data show how deviations in the grayscale values are miniscule, whereas microscopic images show that stage 2 cancer tissue has higher fractal dimension, with more accumulation of mass density, compared to normal tissue. Importantly, we were able to distinguish different stages of colon cancer cases via fractal dimension analysis of colon TMA samples using optical transmission imaging. The increase in the fractal dimension with the progress of cancer also increases the spatial correlation length and entropy in the tissue, due to the more mass accumulation, and increase in number of mass density fluctuation peaks.

Conventionally, cancer diagnosis is performed by pathologists using qualitative analysis of microscopic tissue samples. These tissue samples are collected via biopsy and are viewed under the microscope to detect any structural irregularities in the sample in comparison to a normal healthy sample. However, such approach is highly prone to human error and misdiagnosis. Similarly, in chemical analysis, the biopsy samples are stained using specific antibodies and biomarkers and then studied for specific markers. This method is time-consuming and expensive. Additionally, both methods lack quantitative accuracy and, as such, cannot discriminate among cancer stages. In the alternative, using fractal analyses technique, as shown in this work with colon TMA, it may be possible to analyze colon cancer stages with more reliability and speed by avoiding the need for special preparation or reagents. Moreover, since fractal analysis is based on tried-and-true mathematical equations, human error can be avoided. Finally, fractal data from cancer research can easily be compiled into a database so that physicians can compare patient samples and then make more precise and efficient diagnoses. Using emerging TMA samples, in this work further paves the way toward performing comprehensive studies on different cancer types that will lead to the more accurate detection of cancer at different stages using a simple mathematical tool which might be effective than the existing pathological methods. There might exists a connection between the change in shape of a cell and metastasis, so we can find fractal dimension of the cancer cell and find the level of the cancer basis on this value which is a simple way to detect cancer. This approach with further investigation can improve the detection of cancer.

Acknowledgments

National Institutes of Health (NIH) grants (Nos. R01EB003682 and R01EB016983) and FedEx Institute of Technology grant, for Pradhan. Pradhan also acknowledges the University of Memphis where partial work was performed.

ORCID iDs

Shiva Bhandari © https://orcid.org/0000-0002-6365-9131

References

American Cancer Society 2017 Colorectal Cancer Facts and Figures 2017–2019 (Atlanta : American Cancer Society)
Baba A I and Câtoi C 2007 Tumor Cell Morphology (Bucharest: The Publishing House of the Romanian Academy)
Baish J W and Jain R K 2000 Fractals and cancer Cancer research 60 3683–8
Barer R, Ross K F A and Tkaczyk S 1953 Refractometry of living cells Nature 171 720
Bizzarri M, Giuliani A, Cucina A, D’Anselmi F, Soto A M and Sommenschin C 2011 Fractal analysis in a systems biology approach to cancer Seminars in Cancer Biology Vol 21 175–82
Bunde A and Havlin S (ed) 2013 Fractals in Science (Berlin: Springer)
Dang C V 2012 Links between metabolism and cancer Genes & Development 26 877–90
Davies H G and Wilkins M H F 1952 Interference microscopy and mass determination Nature 169 541
Einstein A J, Wu H S, Sanchez M and Gil J 1998 Fractal characterization of chromatin appearance for diagnosis in breast cytology The Journal of Pathology 185 366–81
Esgiar A N, Naguib R N, Sharif B S, Bennett M K and Murray A 2002 Fractal analysis in the detection of colonic cancer images IEEE transactions on information technology in biomedicine 6 54–8
Falconer K 2003 Fractal Geometry (New York: Wiley) pp 308
Fletcher C D 2007 Diagnostic Histopathology of Tumors: 2-Volume Set With CD–Roms (UK: Elsiever Health Sciences)
González-García I, Solé R V and Costa J 2002 Metapopulation dynamics and spatial heterogeneity in cancer Proc. Natl Acad. Sci. 99 11085–9
Jawhar N M 2009 Tissue microarray: a rapidly evolving diagnostic and research tool Annals of Saudi Medicine 29 123
Li J, Du Q and Sun C 2009 An improved box–counting method for image fractal dimension estimation Pattern Recognit. 42 2460–9
Lingen M W, Kalmar J R, Karrison T and Speight P M 2008 Critical evaluation of diagnostic aids for the detection of oral cancer Oral oncology 44 19–22
Loeb L A, Springgate cf and Battula N 1974 Errors in DNA replication as a basis of malignant changes Cancer research 34 2311–21

Losa G A and Nonnenmacher T F 1996 Self-similarity and fractal irregularity in pathologic tissues Modern pathology: An Official Journal of the United States and Canadian Academy of Pathology, Inc 9 174–82

Mandelbrot B B 1983 The Fractal Geometry of Nature Vol 173 (New York: WH freeman)

Mantovani A, Allavena P, Sica A and Balkwill F 2008 Cancer-related inflammation Nature 454 436

Metze K, Adam R L, Kayser G and Kayser K 2010 Pathophysiology of cancer and the entropy concept Model-based Reasoning in Science and Technology 199–206(Berlin, Heidelberg: Springer)

Nielsen B, Albregtsen F and Danielsen H E 2002 Fractal signature vectors and lacunarity class distance matrices to extract new adaptive texture features from cell nuclei Fractals in Biology and Medicine (Basel: Birkhäuser ) 55–65

Nielsen B, Albregtsen F and Danielsen H E 2005 Fractal analysis of monolayer cell nuclei from two different prognostic classes of early ovarian cancer Fractals in Biology and Medicine (Basel: Birkhäuser ) 175–86

Ohri S, Dey P and Nijhawan R 2004 Fractal dimension in aspiration cytology smears of breast and cervical lesions Analytical and quantitative cytology and histology 26 109–12

Peitgen H O, Jurgens H and Saupe D 1992 Part One Introduction to Fractals and Chaos Fractals for the Classroom 1 (New York: Springer-Verlag)

Pradhan P, Damania D, Joshi H M, Turzhitsky V, Subramanian H, Roy H K, Tatlove A, Dravid V P and Backman V 2011 Quantification of nanoscale density fluctuations by electron microscopy: probing cellular alterations in early carcinogenesis Phys. Biol. 8 026012

Rathore S, Hussain M, Ali A and Khan A 2013 A recent survey on colon cancer detection techniques IEEE/ACM Transactions on Computational Biology and Bioinformatics (TCBB) 10 545–65

Sagan H 1994 Space-Filling Curves (Berlin: Springer) pp 156

Scholzová E, Malík R, Ševčik J and Kleibl Z 2007 RNA regulation and cancer development Cancer Letters 246 pp 12–23

Shergill I S, Shergill N K, Arya M and Patel H R H 2004 Tissue microarrays: a current medical research tool Current Medical Research and Opinion 20 707–12

Siegel R L, Miller K D, Ahnen D J, Meester R G, Barzi A and Jemal A 2017 Colorectal cancer statistics, 2017 CA: A Cancer Journal for Clinicians 67 177–93

Tambasco M, Eliasziw M and Magliocco A M 2010 Morphologic complexity of epithelial architecture for predicting invasive breast cancer survival Journal of Translational Medicine 8 140

Vicsek T 1992 Fractal Growth Phenomena (Singapore New Jersey: World Scientific) p 10

Weinberg R A 1996 How cancer arises Sci. Am. 275 62–70

WHO 2007 Cancer Control: Knowledge Into Action: WHO Guide for Effective Programmes, Volume 2 (WHO Guide for Effective Programmes) (Switzerland: World Health Organization)

Wulfkuhle J D, Liotta L A and Petricoin E F 2003 Early detection: proteomic applications for the early detection of cancer Nat. Rev. Cancer 3 267