



Article

Fractal Parameters as Independent Biomarkers in the Early Diagnosis of Pediatric Onset Inflammatory Bowel Disease

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Abstract: Inflammatory bowel disease (IBD), which encompasses two different phenotypes—Crohn's disease (CD) and ulcerative colitis (UC)—consists of chronic, relapsing disorders of the gastrointestinal tract. In 20–30% of cases, the disease begins in the pediatric age. There have been just a few studies that used fractals for IBD investigation, but none of them analyzed intestinal cell chromatin. The main aim of this study was to assess whether it is possible to differentiate between the two phenotypes in pediatric patients, or either of the phenotypes versus control, using the fractal dimension and lacunarity of intestinal cell chromatin. We analyzed nuclei from at least seven different intestinal segments from each group. In the majority of colon segments, both the fractal dimension (FD) and the lacunarity significantly differed between the UC group and CD group, and the UC group and control group. In addition, the ileocecal valve and rectum were the only segments in which CD could be differentiated from the controls based on the FD. The potential of the fractal analysis of intestinal cell nuclei to serve as an observer-independent histological tool for ulcerative colitis diagnosis was identified for the first time in this study. Our results pave the way for the development of computer-aided diagnosis systems that will assist the physicians in their clinical practice.

Keywords: fractal dimension; lacunarity; medical image analysis; nucleus chromatin architecture; inflammatory bowel disease



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1. Introduction

Over the past two decades, medical image analysis has become an invaluable tool in the field of life sciences. Amongst other descriptors, fractal-based parameters have been used with success. As pointed out in [1], many natural and man-made objects can be characterized using the classical geometry and integer dimension; however, the random growth and/or branching of natural objects, at different scales, can be described in a sufficiently precise and concise way only by means of a non-integer fractal dimension. Some of the representative examples include a fern leaf, the branching in human lungs, a broccoli head, and the electric discharge in a storm [1]. In fractal objects, self-similarity at varying scales can be observed. Applications of fractal analysis to biomedical problems have been well established in ophthalmology, in exhibiting the fractal structure of vascular networks, and in the study of branching patterns of nerve dendrites, as well as in the studies of altered tissue and cell characteristics in cancers [1–6].

The dependences of the structural or functioning-related parameters of interest on the scale can be expressed by the power-law equations [1,7,8]. The fractal dimension (FD), which corresponds to the first-order mass moment of probability of finding a certain fractal measure amount at a certain scale [9,10], can be easily determined from the power-law dependence. In medical image analysis, the FD provides a statistical index that quantifies complexity and pattern reproducibility. As the biological specimens exhibit pronounced self-similarity at different scales, the FD is being increasingly used for diagnostic purposes in medicine [3]. Another parameter, which is often used alongside the FD, is the lacunarity (also sometimes referred to as “gappiness” or inhomogeneity). The lacunarity is calculated by taking into account the first-order and second-order mass moments of probability of finding a certain fractal measure amount at a certain scale [9]. It is a measure of heterogeneity and translational and rotational invariance that describes the space-filling property of fractals [9]. It is highly useful as an additional parameter to differentiate natural surfaces and textures with the same or similar fractal dimension [6,11].

In the field of medical science, the concept of fractals has been used for both image [1–6,12–14] and signal [15–18] analysis. The fractal feature-based image analysis employed so far in gastroenterology has shown promising results in cancer research [19–23] and has helped in the analysis of wireless capsule endoscopy [24] and colonoscopy images [25]. On the other hand, fractal-based signal analysis methods have been applied in gastroenterology in the study of digestion [26], colonic pressure [27], and intestinal sound analysis [28]. Although it produced very good results in the investigations of other intestinal pathologies, fractal-based methodology has rarely been used in inflammatory bowel disease (IBD) research [11,29,30].

The FD of nuclear chromatin [31,32] proved to be a sensitive parameter capable of the early detection of fine structural and textural changes in the cell nucleus. Nuclear chromatin FD changes have been found during the processes of cell differentiation, development, mitosis, apoptosis, aging, and cancer development [33,34]. Lacunarity has also been employed for chromatin structure evaluation in, among others, studies of apoptosis, postnatal development, and aging. In many of the studies, lacunarity has been inversely related to the FD, i.e., there has been a negative correlation between the FD and lacunarity, although, depending on the underlying processes, this association does not apply in general [33].

IBD, which encompasses two major phenotypes—Crohn’s disease (CD) and ulcerative colitis (UC)—consists of chronic, relapsing disorders of the gastrointestinal tract [35,36]. Continuous mucosal inflammation that starts from the rectum and proceeds to more proximal colon segments, with variability in extent, is typical for UC [37]. On the other hand, discontinuous transmural granulomatous inflammation occurring at any part of the gastrointestinal tract characterizes CD [38]. The disease etiology is still not fully elucidated, but it has become clear that genetic factors, environment, diet, and changes in the microbiome are implicated in the pathogenesis. CD can affect any part of the gastrointestinal system, although it is most often evidenced in the terminal part of small intestine (terminal ileum) and the colon. On the other hand, UC is a disease restricted to the colon and rectum [35]. Both phenotypes sometimes display extraintestinal manifestations, which might stem from factors such as chronic inflammation or nutrient malabsorption [36]. In 20–30% of cases the disease begins in the pediatric age. We are currently witnessing an increase in incidence in the pediatric population [39]. Pediatric ulcerative colitis sometimes has an atypical endoscopic presentation like rectal sparing or patchy disease, which makes the differentiation between the two IBD phenotypes far more challenging [40]. The new European Crohn’s and Colitis Organization (ECCO) guidelines [41] stress the importance of novel and possibly noninvasive biomarkers in diagnostics. The only recommended biomarker of intestinal inflammation so far is fecal calprotectin. Hence, there is a great demand for the invention of new efficient biomarkers for IBD, which could be combined with the standard medical procedures [42].

There have been just a few studies [11,29,30] which have employed fractals for IBD investigation. To the best of our knowledge, the fractals have not yet been applied in the

analysis of the chromatin structure in the intestinal cells. However, there are indications that alterations in nuclear chromatin are very likely to occur in many functional gastrointestinal and motility disorders [43]. It has been demonstrated that the gene regulation by the microbiota is linked to DNA methylation and chromatin accessibility changes [44]. The underlying epigenetic mechanisms have recently been recognized as highly important in the pathogenesis of inflammatory bowel disease (IBD). Therefore, possible alterations of the fractal parameters describing the intestinal cell chromatin structure need to be investigated. The main aim of this study was to assess whether it is possible to differentiate between children with one of the two IBD phenotypes and children in a control group and to differentiate between the two phenotypes, based on the intestinal cell chromatin fractal dimension and lacunarity. Also, we checked the potential and the methods of application of fractal analysis as an observer-independent tool for IBD diagnosis.

2. Materials and Methods

2.1. Patients

The current study was conducted on children newly diagnosed with IBD (14 cases of CD and 10 cases of UC). The control group ($N = 16$) comprised children with irritable bowel syndrome (IBS) (without intestinal inflammation) or healthy controls. Seven tissue samples per patient were obtained by colonoscopy, corresponding to seven intestinal segments. Each sample contained one to four endoscopic biopsies. The samples belonged to the terminal ileum, cecum, ascending, transversal, descending, and sigmoid colon, and rectum, respectively. In cases when the ileocecal valve appeared affected, an additional 8th sample from the valve was obtained.

2.2. Intestinal Tissue Preparation and Staining

Intestinal tissue was fixed in 4% neutral buffered formaldehyde. The tissues were dehydrated in graded ethanol, according to the routine procedure, and then embedded in paraffin blocks and sectioned. The paraffin tissue blocks were cut into 4 μm thick sections and stained with hematoxylin and eosin.

2.3. Medical Image Preprocessing

The following image preprocessing procedure was employed for each intestinal segment. Four different micrographs containing at least one intestinal crypt each were acquired using the Olympus DP70 camera (Olympus BX50, Tokyo, Japan) and Analysis 5.0 software (SoftImaging System, Olympus, Tokyo, Japan). The total microscope magnification was 1000 times. All the micrographs were saved as TIFF files (dimensions 4080×3072 pixels, resolution 200 DPI, bit depth 24). Using the polygon tool of the ImageJ software (NIH, Bethesda, MD, USA), twenty crypt cell nuclei per segment were acquired for every patient and transferred to the white background images (500×500 pixels). Such newly acquired micrographs of nuclei were converted to monochromatic images using the Colour Deconvolution plugin of the ImageJ software [45,46], with an option denoted as H&E 2. The hematoxylin component (R: 0.49015734, G: 0.76897085, B: 0.41040173) is dominant for nuclei; it was recorded as an 8-bit grayscale image and used in further analyses.

2.4. Niblack Thresholding

Prior to fractal analysis, all the nuclei images underwent local Niblack thresholding and conversion to binary images. The local threshold value at each pixel was calculated by taking into account the neighborhood around that pixel and summing the neighborhood mean value of the grayscale intensity with the weighted standard deviation of the grayscale intensity. Both the window radius defining a neighborhood size and the weighting coefficient have to be adjusted empirically. The default value of the weighting coefficient is 0.2 for bright objects on a dark background and -0.2 for dark objects on a light background. In our case, the default coefficient provided very good results for all the images, and it was not changed. A window of a radius of $w = 6$ px, chosen empirically, was centered around

each considered pixel. The local threshold at each pixel was then calculated according to the expression:

$$t_{Ni} = \mu(w) - 0.2 \cdot \sigma(w) \quad (1)$$

In (1), $\mu(w)$ and $\sigma(w)$ correspond to the mean and standard deviation of the grayscale intensities within a local neighborhood window of size w . Thus, a different threshold for every pixel is based on the grayscale properties of the neighboring pixels [47]; this was employed to better outline the spatial distribution of two types of chromatin—euchromatin (lighter) and heterochromatin (darker in appearance) [33]. Specifically, the altered spatial distribution of these regions seems to appear macroscopically mostly through altered nuclei texture.

2.5. Fractal Analysis

Fractal analysis was carried out on binary images using the FracLac plugin (A. Karperien, Charles Sturt University, Australia) for the ImageJ software [48]. The fractal dimension (D_b) was calculated using the box counting method as well as the cumulative mass method, with agreement of the results for the same sets of grids. We applied a scaled series 7/8, meaning that an enlargement of a consequent grid cell size was about 1/8. By using a scaled series, a sufficient number of relatively dense data points for fitting the regression lines was easily obtained. Here, a total of 18 grid cell sizes were used to obtain data points for fitting the regression curves to describe the scale–count power law dependence. Specifically, grid cells sized $\varepsilon \in \{5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 23, 27, 30, 35, 40, 46, 52\}$ px were used in both the box counting method and the cumulative mass method. Figure 1 shows an example binary image of a nucleus and also illustrates the typical nucleus size and the look of the chromatin texture, covered by grid cells of different sizes, ε . Typical grid cell arrangements are shown for four out of the eighteen utilized sizes, as denoted above each of the four plots. Twelve random grid origin positions per nucleus, per each grid cell size, were used.

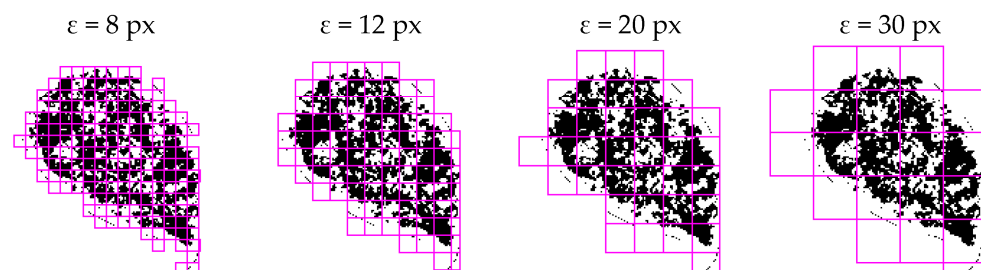


Figure 1. Grids of different grid cell sizes, ε , used to cover a binary two-dimensional (2D) object while obtaining data points for the scale–count dependence. The example nucleus shown corresponds to the 2nd patient in the CD group.

A change in the number of non-empty grid cells, $N(\varepsilon)$, with a change in the grid cell size, ε , was modeled by a best-fit regression line, whose slope served to estimate D_b .

$$D_b = -\lim_{\varepsilon \rightarrow 0} \frac{\ln N(\varepsilon)}{\ln \varepsilon} \quad (2)$$

The cumulative mass method makes an assessment of the probability of finding m pixels inside the cell of a size ε , $P(m, \varepsilon)$, by counting the pixels inside the grid cells and normalizing the obtained pixel count data for the total probability of one, $\sum_m P(m, \varepsilon) = 1$.

The first-order mass moment, $M(\varepsilon)$, and the second-order mass moment, $M^2(\varepsilon)$, were obtained as:

$$M(\varepsilon) = \sum_m m P(m, \varepsilon), \quad M^2(\varepsilon) = \sum_m m^2 P(m, \varepsilon) \quad (3)$$

The FD was then estimated from the $(\varepsilon, M(\varepsilon))$ data, as:

$$D_m = \lim_{\varepsilon \rightarrow 0} \frac{\ln M(\varepsilon)}{\ln \varepsilon} \quad (4)$$

In both cases, the adequacy of the regression line fit was estimated based on the correlation coefficient, r^2 , which was close to one in all cases, confirming almost linear data and high FD estimate accuracy. The lacunarity is calculated from the first-order and second-order mass moments as:

$$\lambda(\varepsilon) = \frac{\langle M^2(\varepsilon) \rangle - \langle M(\varepsilon) \rangle^2}{\langle M(\varepsilon) \rangle^2} \quad (5)$$

2.6. Statistical Analysis

The normality of distribution was tested with the Shapiro–Wilk test. Depending on the type of variables and the normality of the distributions, the results are presented as frequency (percent), mean ($\pm$ SD), and median (range). Statistical hypotheses were tested using the ANOVA, Kruskal–Wallis, and Mann–Whitney tests and the chi square test. The statistical hypotheses were analyzed at the significance level of 0.05. Statistical data analysis was performed using IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA).

3. Results

3.1. Demographic and Clinical Characteristics

The demographic and clinical characteristics of the study participants, i.e., the pediatric patients used in this particular study, are shown in Table 1.

Table 1. Demographic and clinical characteristics of IBD groups and control patients.

Variable	Control	UC	CD	Overall <i>p</i>
Gender				
Male	60.0%	60.0%	78.6%	0.498
Female	40.0%	40.0%	21.4%	
Age	11.3 $\pm$ 5.1	14.4 $\pm$ 3.3	12.1 $\pm$ 4.8	0.271
PUCAI	32.5 $\pm$ 19.0			
PCDAI	17.5 $\pm$ 6.3			

Abbreviations: CD—Crohn’s disease; UC—ulcerative colitis; PUCAI—Pediatric Ulcerative Colitis Activity Index; PCDAI—Pediatric Crohn’s Disease Activity Index.

3.2. Histological Activity

The histological activities of UC and CD are represented with the corresponding regional histological activity score and are presented in Table 2 and Figure 2 (Nancy score for UC) and Table 3 and Figure 3 (GHAS score for CD).

Table 2. Regional Nancy score in six different colon segments.

Segment	Nancy 0, 1	Nancy 2	Nancy 3	Nancy 4
S2	1 (10.0%)	3 (30.0%)	5 (50.0%)	1 (10.0%)
S3	2 (20.0%)	4 (40.0%)	2 (20.0%)	2 (20.0%)
S4	2 (22.2%)	3 (33.3%)	4 (44.4%)	
S5	2 (20.0%)	3 (30.0%)	5 (50.0%)	
S6	1 (10.0%)	3 (30.0%)	5 (50.0%)	1 (10.0%)
S7	2 (20.0%)	2 (20.0%)	5 (50.0%)	1 (10.0%)

Segment notation: S2—cecum; S3—ascending colon; S4—transversal colon; S5—descending colon; S6—sigmoid colon; S7—rectum.

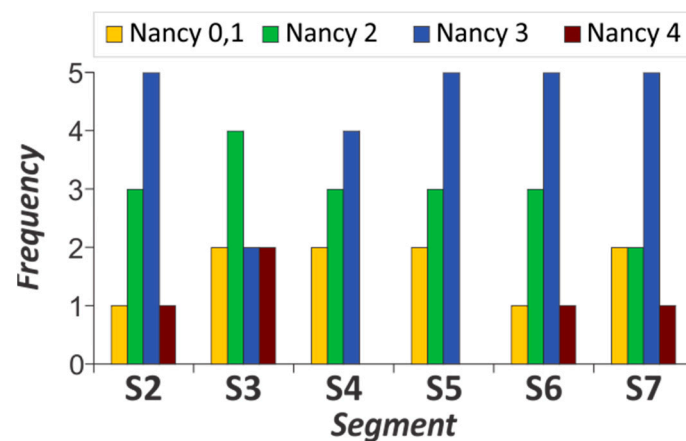


Figure 2. Regional Nancy score in six different colon segments.

Table 3. Regional GHAS score in seven different colon segments.

Segment	GHAS 1,2,3,4	GHAS 5,6,7	GHAS 8,9,10	GHAS 11–16
S1	3 (21.43%)	6 (42.86%)	4 (28.57%)	1 (7.14%)
S2	6 (42.86%)	5 (35.71%)	1 (7.14%)	2 (14.29%)
S3	6 (42.86%)	4 (28.57%)	2 (14.29%)	2 (14.29%)
S4	4 (28.57%)	4 (28.57%)	5 (35.71%)	1 (7.14%)
S5	7 (50.00%)	2 (14.29%)	2 (14.29%)	3 (21.43%)
S6	5 (35.71%)	4 (28.57%)	3 (21.43%)	2 (14.29%)
S7	7 (50.00%)	3 (21.43%)	4 (28.57%)	0

Segment notation: S1—terminal ileum; S2—cecum; S3—ascending colon; S4—transversal colon; S5—descending colon; S6—sigmoid colon; S7—rectum.

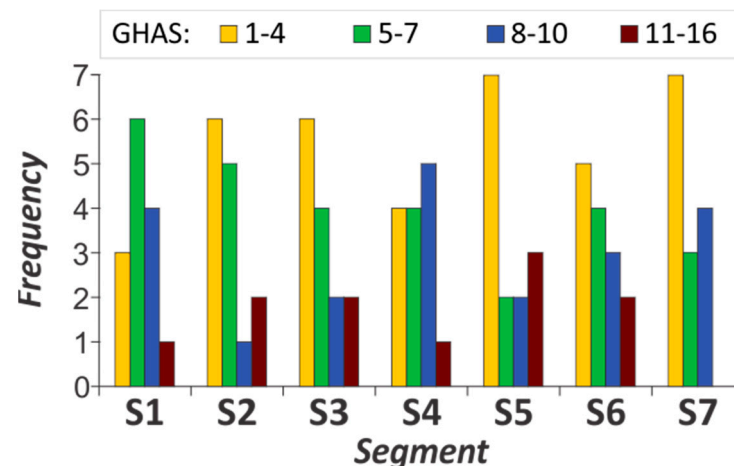


Figure 3. Regional GHAS score in seven different colon segments.

3.3. Fractal Dimension

We estimated the FD of the epithelial cell nuclei to assess potential differences in the chromatin organizational complexity between the two major IBD subtypes in the pediatric age. The same analysis was performed to assess differences in the intestinal cell chromatin complexity between either of the IBD phenotypes and the control subjects. A comparison was performed on seven different intestinal segments (terminal ileum, cecum, ascending, transversal, descending and sigmoid colon and rectum). The differences in the FDs of the investigated nuclei are shown in Figure 4 and Table 4. Figure 4 shows the mean ranks according to the nonparametric Kruskal–Wallis H test, where the mean rank is the arithmetic

average of the positions in the sorted FD list for a total of 780 analyzed epithelial cell nuclei. The blue lines show the statistically significant mean rank differences. The children with UC had a significantly higher FD of the cell nuclei chromatin texture than the controls in all colonic segments except for the rectum ($p \leq 0.035$). Similar differences were revealed between the UC and CD children. Specifically, the UC patients had significantly higher FDs in comparison with the CD patients in every colon part except for the transversal colon ($p \leq 0.010$). On the other hand, the children with Crohn's disease had FDs that were statistically different from those of the controls only in the ileocecal valve and rectum ($p = 0.005$, $p = 0.014$; respectively), with lower median FD values in the CD patients. Interestingly, the terminal ileum of the CD patients, which is one of the most frequent localizations affected by the disease, had a statistically different nuclear FD only when compared with that of the UC patients.

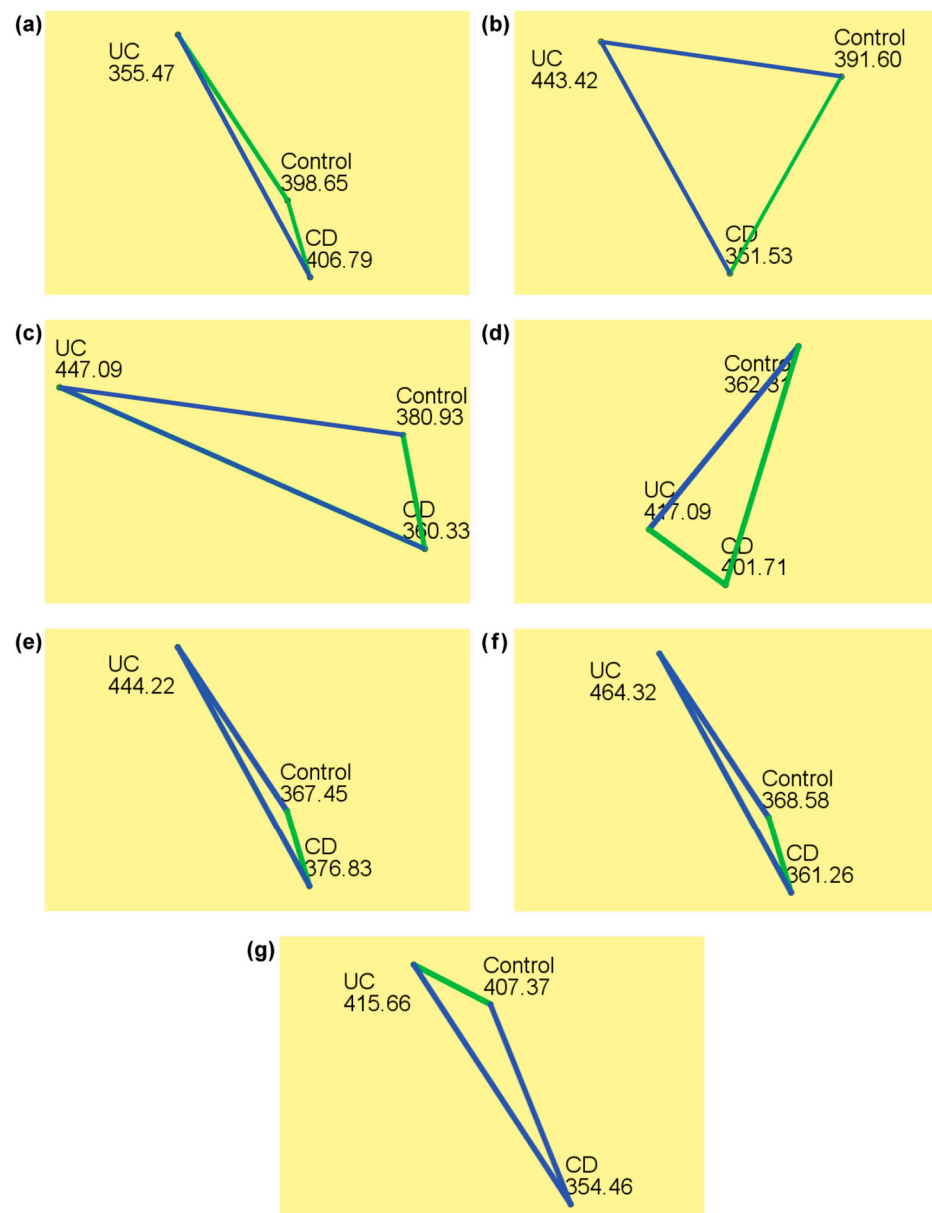


Figure 4. Fractal dimension mean rank comparison between CD, UC, and control group, for 7 different intestinal segments: (a) terminal ileum, (b) cecum, (c) ascending colon, (d) transversal colon, (e) descending colon, (f) sigmoid colon, (g) rectum.

Table 4. Fractal dimension comparison between CD, UC, and control group.

Intestinal Segment	Fractal Dimension (FD)			Statistical Signif. (<i>p</i> Values)
	Crohn's Disease (CD)	Ulcerative Colitis (UC)	Control Group	
S1	1.728(1.586–1.831)	1.722 (1.593–1.802)	1.728 (1.531–1.819)	CD–UC (0.042)
				CD–Control (1.000)
				UC–Control (0.107)
S2	1.732 (1.523–1.842)	1.755 (1.559–1.834)	1.741 (1.599–1.818)	CD–UC (<0.0001)
				CD–Control (0.097)
				UC–Control (0.035)
S3	1.732 (1.529–1.829)	1.752 (1.135–1.840)	1.736 (1.547–1.835)	CD–UC (<0.0001)
				CD–Control (0.814)
				UC–Control (0.004)
S4	1.736 (1.566–1.831)	1.738 (1.048–1.843)	1.728 (1.538–1.812)	CD–UC (1.000)
				CD–Control (0.106)
				UC–Control (0.023)
S5	1.734 (1.593–1.816)	1.747 (1.118–1.948)	1.734 (1.555–1.810)	CD–UC (0.004)
				CD–Control (1.000)
				UC–Control (0.001)
S6	1.722 (1.521–1.940)	1.749 (1.601–1.947)	1.727 (1.585–1.826)	CD–UC (<0.0001)
				CD–Control (1.000)
				UC–Control (<0.0001)
S7	1.719 (1.524–1.944)	1.733 (1.511–1.824)	1.730 (1.536–1.818)	CD–UC (0.010)
				CD–Control (0.014)
				UC–Control (1.000)
S8	1.731 (1.617–1.788)		1.749 (1.605–1.834)	CD–Control (0.005)

The results are presented as median (range). Segment notation: S1—terminal ileum; S2—cecum; S3—ascending colon; S4—transversal colon; S5—descending colon; S6—sigmoid colon; S7—rectum; S8—ileoceleal valve.

3.4. Lacunarity

The size and spatial distribution of the gaps in the cell nuclei textures, their spatial diversity, and the level of the image deviation from the rotational and translational invariance was measured by determining the texture lacunarity. Lacunarity analysis revealed additional significant differences between the Crohn's disease and ulcerative colitis ($p \leq 0.001$), as well as between the ulcerative colitis and the control ($p \leq 0.002$) (Figure 5, Table 5). In Figure 5, the blue lines show the statistically significant mean rank differences for lacunarity. A statistically significant difference was present throughout the colon with the exception of the same segments as those in the FD comparison (the transversal part for the CD–UC and the rectum for the UC–control comparison). The difference in the terminal ileum nuclear lacunarity was not statistically significant among the compared groups. The colon enterocyte nuclear lacunarity was the lowest in the UC group.

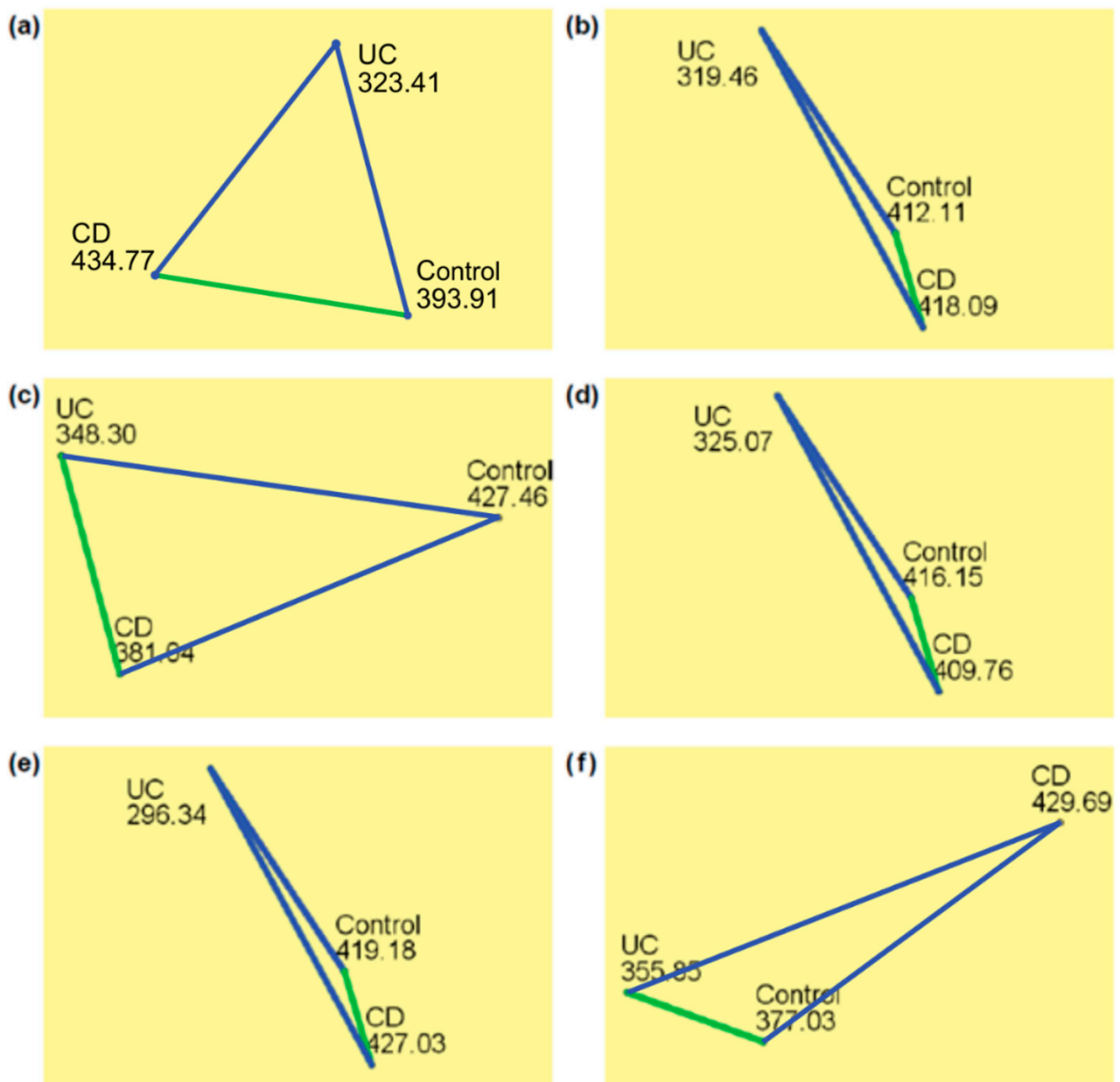


Figure 5. Lacunarity mean rank comparison between CD, UC, and control group for intestine segments: (a) cecum, (b) ascending colon, (c) transversal colon, (d) descending colon, (e) sigmoid colon, (f) rectum.

3.5. Intersegmental Comparison

Intersegmental comparisons of the fractal dimension and lacunarity of the cell nuclei texture are presented in Tables 6 and 7, respectively. One of the prominent results is a statistically lower rectal nuclear FD in comparison with the other segments (except for the sigmoid colon) in Crohn's disease patients. When the UC pediatric patients were considered, the nuclear FD proved highly useful in differentiating the colon (except for the rectum) and terminal ileum, the bowel part not affected by disease ($p < 0.002$). On the other hand, the FD across the control intestinal segments tended to be more uniform. As for the nuclear lacunarity, statistically significant differences were, with few exceptions, detected between the same segments as those in the case of the FD segment intercomparison.

Table 5. Lacunarity comparison between CD, UC, and control group.

Intestinal Segment	Lacunarity (Lac)			Statistical Signif. (<i>p</i> Values)
	Crohn's Disease (CD)	Ulcerative Colitis (UC)	Control Group	
S1	0.279 (0.172–0.430)	0.285 (0.209–0.455)	0.279 (0.197–0.517)	All groups (0.074)
S2	0.282 (0.163–0.470)	0.253 (0.167–0.422)	0.266 (0.178–0.407)	CD–UC (<0.0001)
				CD–Control (0.087)
				UC–Control (0.002)
S3	0.279 (0.174–0.460)	0.260 (0.175–0.404)	0.274 (0.192–0.481)	CD–UC (<0.0001)
				CD–Control (1.000)
				UC–Control (<0.0001)
S4	0.271 (0.184–0.442)	0.269 (0.181–0.554)	0.280 (0.198–0.482)	CD–UC (0.350)
				CD–Control (0.039)
				UC–Control (<0.0001)
S5	0.273 (0.273–0.430)	0.258 (0.037–0.365)	0.275 (0.191–0.475)	CD–UC (<0.0001)
				CD–Control (1.000)
				UC–Control (<0.0001)
S6	0.287 (0.042–0.510)	0.257 (0.038–0.378)	0.280 (0.190–0.467)	CD–UC (<0.0001)
				CD–Control (1.000)
				UC–Control (<0.0001)
S7	0.289 (0.040–0.480)	0.272 (0.183–0.450)	0.276 (0.182–0.504)	CD–UC (0.001)
				CD–Control (0.015)
				UC–Control (0.909)
S8	0.276 (0.221–0.396)		0.263 (0.199–0.418)	CD–Control (0.126)

The results are presented as median (range). Segment notation: S1—terminal ileum; S2—cecum; S3—ascending colon; S4—transversal colon; S5—descending colon; S6—sigmoid colon; S7—rectum; S8—ileocecal valve.

Table 6. Intersegmental comparison of nuclear fractal dimension.

Intestinal Segment	Fractal Dimension (FD)		
	Crohn's Disease (CD)	Ulcerative Colitis (UC)	Control Group
S1	1.728 (1.586–1.832)	1.723 (1.593–1.802)	1.728 (1.531–1.819)
S2	1.732 (1.524–1.843)	1.755 (1.559–1.834)	1.741 (1.600–1.819)
S3	1.732 (1.529–1.829)	1.752 (1.135–1.840)	1.736 (1.547–1.835)
S4	1.736 (1.567–1.831)	1.738 (1.048–1.843)	1.728 (1.538–1.812)
S5	1.734 (1.593–1.816)	1.747 (1.118–1.948)	1.734 (1.555–1.811)
S6	1.722 (1.521–1.940)	1.749 (1.601–1.948)	1.727 (1.585–1.826)
S7	1.719 (1.524–1.944)	1.733 (1.511–1.824)	1.729 (1.536–1.818)
Stat.sign. (<i>p</i> values)	S7–S1, S7–S3, S7–S2, S7–S5, S7–S4 (<i>p</i> < 0.025)	S1–S4, S1–S6, S1–S5, S1–S3, S1–S2 (<i>p</i> < 0.002) S7–S6, S7–S5, S7–S3, S7–S2 (<i>p</i> < 0.013)	S1–S2, S4–S2, S7–S2 (<i>p</i> < 0.090)

The results are presented as median (range). Segment notation: S1—terminal ileum; S2—cecum; S3—ascending colon; S4—transversal colon; S5—descending colon; S6—sigmoid colon; S7—rectum.

Table 7. Intersegmental comparison of cell nuclei texture lacunarity.

Intestinal Segment	Lacunarity (Lac)		
	Crohn's Disease (CD)	Ulcerative Colitis (UC)	Control Group
S1	0.279 (0.173–0.430)	0.285 (0.209–0.455)	0.279 (0.197–0.517)
S2	0.279 (0.163–0.470)	0.253 (0.167–0.422)	0.266 (0.178–0.407)
S3	0.279 (0.174–0.460)	0.259 (0.175–0.404)	0.274 (0.192–0.482)
S4	0.271 (0.184–0.442)	0.269 (0.181–0.555)	0.280 (0.198–0.482)
S5	0.273 (0.184–0.430)	0.258 (0.037–0.365)	0.275 (0.191–0.475)
S6	0.287 (0.0420–0.510)	0.257 (0.038–0.378)	0.280 (0.190–0.467)
S7	0.289 (0.040–0.480)	0.272 (0.183–0.450)	0.276 (0.182–0.504)
Stat.sign. (<i>p</i> values)	S2-S7, S3-S7, S4-S7, S5-S7 (<i>p</i> < 0.022)	S2-S1, S3-S1, S4-S1, S6-S1 (<i>p</i> < 0.0001), S2-S7, S3-S7, S6-S7 (<i>p</i> < 0.011) S2-S4 (<i>p</i> < 0.025)	S2-S1, S2-S4, S2-S6 (<i>p</i> < 0.040)

The results are presented as median (range). Segment notation: S1—terminal ileum; S2—cecum; S3—ascending colon; S4—transversal colon; S5—descending colon; S6—sigmoid colon; S7—rectum.

4. Discussion

Our results indicate that pediatric UC patients have a significantly increased nuclear structure and texture complexity, as measured with the FD, and decreased lacunarity compared to the children suffering from CD and the controls. This is a very important finding, given that the two IBD phenotypes are sometimes hard to distinguish. A significant difference was confirmed in most of the colon parts. In addition, the only intestinal segment with a significantly different nuclear fractal dimension and lacunarity between the CD patients and the controls was the rectum. However, the ileocecal valve of the CD children had a significantly decreased nuclear FD while the colon transversum had a statistically decreased lacunarity compared to the controls.

To the best of our knowledge, our study is the first which employed the calculations of the fractal dimension and lacunarity of intestinal cell nuclear chromatin in the IBD investigation. However, fractal analysis has previously been applied in the study of other intestinal pathologies. This methodology proved to be a promising aid in diagnosis [19,20] and therapy response [22,23] in intestinal carcinomas. Furthermore, a methodology based on fractal analysis was very accurate in abnormality detection in wireless capsule endoscopy (WCE) images [24]. Moreover, the differentiation between healthy and pathologic rectal mucosal vasculature can be accomplished with the fractal analysis of endoscopy images. The same analysis also proved to be useful for distinguishing between different rectal pathologies, e.g., colitis and vascular malformations [25]. Regarding IBD, fractals have been successful in ulcer identification from WCE images (caused by CD and UC, among other causes) [11] and bowel sounds detection in different intestinal pathologies, e.g., UC [30]. Finally, promising results were obtained in estimating the severity of intestinal fibrosis in a study conducted on histological slides stained with Masson's trichrome stain, which were derived from surgical specimens of CD patients. Specifically, an extracellular matrix (ECM) FD showed a significant correlation with a histological fibrosis score, and sections with different histological fibrosis scores had significantly different FDs. Thus, this investigation indicates that fibrosis progression in CD is more than a pure ECM accumulation and that it also includes structural ECM changes [29].

It should be mentioned, however, that our FD results, although they were consistent through the colon segments, still had some exceptions. Specifically, the nuclear chromatin FD of the transversal colon epithelial cells was similar in the CD and UC patients. In the CD patients, the median FD values were the highest in this segment. To better understand these intersegmental differences, we calculated regional scores that represented the histological disease activity for every segment. It appeared, in our group of patients with Crohn's disease, that in general its symptoms less often skipped the transversal segment than the other segments (fewer patients had GHAS < 4, indicating remission). Furthermore, in this

segment more Crohn's disease patients had moderate active disease (GHAS 8–10) than in the others. So, it appears that CD was more active in this part of colon. This finding strongly suggests the possibility that differences in the pathophysiological disease activity might have some influence on the FD results. However, this does not make fractal analysis less attractive for future diagnostic applications since in routine pathohistological diagnostics all seven different intestinal segments are being evaluated. Looking at the results of all the segments comparatively, it was confirmed without any doubt that the UC fractal dimension differed in a statistically significant way from the controls, as well as from the CD patient group.

UC is known as a disease exclusive to the colon. Therefore, as expected, in these patients the nuclear chromatin FDs in the terminal ileum were significantly lower than those of the rest of the colon (the rectum was an exception). This finding proves once more that the FD could serve as a very good discriminator of the UC-affected tissue.

In our study, the nuclear FD in the rectum was higher in the UC patients than in the other groups; however, in this case the difference was not sufficiently pronounced to provide a statistically significant result. Furthermore, in the UC patients, the nuclear FD in this segment was statistically different from those of the other colon segments (apart from the transversal colon). In general, the rectum is affected in a large majority of UC patients, but in the pediatric population, rectal sparing is not unusual. Histological rectal sparing absolute (normal rectum) or relative (less severe inflammation than in other colon parts) rectal sparing is present in 30% of pediatric patients [49]. Among our patients, 20% had absolute rectal sparing (Nancy 0 or 1), which probably mitigated the results. Therefore, rectal biopsies, although the easiest to obtain, will not be sufficient for UC diagnostics based on the FD, at least not in the pediatric population.

The rectum was also an interesting segment for the CD patients. This was the only segment in which the nuclear FDs of these patients statistically differed from those of the controls. The Crohn FDs generally tended to be lower than in the other groups, indicating decreased complexity of textures for those nuclei. However, the FD in the rectum was the lowest of all. In the intersegmental comparison among the CD patients, the rectal FD was statistically lower than in any other segment except for the sigmoid colon ($p < 0.025$). This makes the rectal nuclear chromatin FD a feature with a potential to be exploited in CD diagnosis.

One possible explanation for the increased FD in most of the colon parts in the UC patients might be an increase in euchromatin ("open" chromatin) versus the heterochromatin ratio. "Open" DNA is less densely packed, resulting in less intense staining and lighter color. In a study exploring the effects of the chromatin physical structure on the transcription of genes [31], both the mathematical predictions and the experimental evidence pointed to a link between the increased heterogeneity of the chromatin structure (increased fractal dimension) and the increased variation of the gene expression for most biological processes. The chromatin heterogeneity is considered a ubiquitous hallmark of cancer aggressiveness in tumor research [2,3,31,33]. However, changes in protein binding and chromatin structure have also been shown to play a role in gastrointestinal diseases and disorders, including the pathogenesis of inflammatory bowel disease (IBD) [44]. It might be challenging to recognize the chromatin structural changes on standard HE-dyed tissue slides [2]; this is of primary interest given that only such slides are available for diagnostic purposes. On the other hand, open chromatin can be distinguished by applying fractal analysis as it gives higher nuclear FD values [2,50]. The results of our study suggest that UC patients have a predominantly increased euchromatin/heterochromatin ratio in enterocyte nuclei compared to the CD patients and controls. One of the epigenetic mechanisms causing chromatin to be in a more open state is histone acetylation. In this process, the enhanced unwrapping ("opening") of DNA allows transcription [51]. On the other hand, the opposite process, histone deacetylation, enables histone and DNA binding, limiting access to DNA again [52]. It has been demonstrated that certain enzymes responsible for histone deacetylation—Sirtuin (SIRT)1, SIRT6 and histone deacetylase (HDAC)9—are

decreased in the inflamed colon tissue of UC patients compared to the same tissue of CD patients [53]. Decreased HDAC activity in UC patients might lead to enhanced histone acetylation and an increased amount of euchromatin, which in turn leads to a significantly higher FD compared to that of the CD patient group. Interestingly, HDAC inhibitors are seen as possible new drugs for UC considering their ability to decrease inflammation in colonic epithelial cells and dextran sulfate sodium-induced colitis [54].

According to the literature, the increase in chromatin lacunarity is often followed by the decrease in chromatin FD [33]. In this regard, our study is in line with the others. In colon tissue, nuclear lacunarity revealed the same statistically significant differences as the FD. However, lacunarity proved to be an even better colon CD discriminator than the FD since it was able to differentiate CD from the controls in more intestinal segments than the FD.

5. Conclusions

We conducted a detailed study of the fractal parameters corresponding to the altered textures of intestinal cell nuclei in pediatric patients as the prospective biomarkers for IBD. Although the changes in cell nuclei chromatin structure have been observed in cancers and have been observed to also coincide with the altered nuclei textures seen in histological samples [2,4,33], here, for the first time, a similar methodology was applied to IBD. The changes occurring in the protein binding and chromatin structure in gastrointestinal diseases motivated us to investigate the cell nuclei textures in IBD. We demonstrated that the intestinal nuclei of children suffering from UC compared with children with CD and a control group statistically increased the nuclear FD and decreased nuclear lacunarity. Furthermore, the current study provided evidence that in CD pediatric patients the rectum had a decreased nuclear FD and an increased lacunarity compared to the control group. Additionally, in the comparison between the segments, the rectum was different from almost all the other segments in terms of the nuclear FD and lacunarity. Therefore, the fractal analysis of intestinal cell nuclei was proven to have the potential to be an observer-independent histological tool for ulcerative colitis diagnosis. In addition, the rectum of CD pediatric patients also seems to be a very good candidate for the development of fractal-based diagnostics. Further studies are necessary to confirm our findings and to check on the consistency of the results in repeated trials. Furthermore, the inconsistency of the nuclear FD and lacunarity between the rectum and rest of the colon in children with CD is interesting, and it should be more thoroughly investigated. Our results pave the way for the development of computer-aided diagnosis systems that will assist in the clinical practice in gastroenterology.

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References

- Husain, A.; Nanda, M.N.; Chowdary, M.S.; Sajid, M. Fractals: An eclectic survey, part II. *Fractal Fract.* **2022**, *6*, 379. [\[CrossRef\]](#)
- de Mattos, A.C.; Florindo, J.B.; Adam, R.L.; Lorand-Metze, I.; Metze, K. The fractal dimension suggests two chromatin configurations in small cell neuroendocrine lung cancer and is an independent unfavorable prognostic factor for overall survival. *Microsc. Microanal.* **2022**, *28*, 522–526. [\[CrossRef\]](#)
- Karri, S.; Aviel-Ronen, S.; Firer, M.A. Fractal and textural imaging identify new subgroups of patients with colorectal cancer based on biophysical properties of the cancer cells. *Pathol. Res. Pract.* **2022**, *238*, 154040. [\[CrossRef\]](#)
- Dinčić, M.; Todorović, J.; Nešović-Ostojić, J.; Kovačević, S.; Dunđerović, D.; Lopičić, S.; Spasić, S.; Radojević-Škodrić, S.; Stanisavljević, D.; Ilić, A.Ž. The fractal and GLCM textural parameters of chromatin may be potential biomarkers of papillary thyroid carcinoma in Hashimoto's thyroiditis specimens. *Microsc. Microanal.* **2020**, *26*, 717–730. [\[CrossRef\]](#)
- Dinčić, M.; Popović, T.B.; Kojadinović, M.; Trbovich, A.M.; Ilić, A.Ž. Morphological, fractal, and textural features for the blood cell classification: The case of acute myeloid leukemia. *Eur. Biophys. J.* **2021**, *50*, 1111–1127. [\[CrossRef\]](#) [\[PubMed\]](#)
- Einstein, A.J.; Wu, H.-S.; Gil, J. Self-affinity and lacunarity of chromatin texture in benign and malignant breast epithelial cell nuclei. *Phys. Rev. Lett.* **1998**, *80*, 397–400. [\[CrossRef\]](#)
- Ioelovich, M. Fractal dimensions of cell wall in growing cotton fibers. *Fractal Fract.* **2020**, *4*, 6. [\[CrossRef\]](#)
- Arsac, L.M.; Weissland, T. Multifractality in the movement system when adapting to arm cranking in wheelchair athletes, able-bodied athletes, and untrained people. *Fractal Fract.* **2022**, *6*, 176. [\[CrossRef\]](#)
- Smith, T.G., Jr.; Lange, G.D.; Marks, W.B. Fractal methods and results in cellular morphology—Dimensions, lacunarity and multifractals. *J. Neurosci. Methods* **1996**, *69*, 123–136. [\[CrossRef\]](#) [\[PubMed\]](#)
- Tél, T. Fractals, multifractals, and thermodynamics: An introductory review. *Z. Naturforschung A* **1988**, *43*, 1154–1174. [\[CrossRef\]](#)
- Charisis, V.S.; Hadjileontiadis, L.J.; Liatsos, C.N.; Mavrogiannis, C.C.; Sergiadis, G.D. Capsule endoscopy image analysis using texture information from various colour models. *Comput. Methods Programs Biomed.* **2012**, *107*, 61–74. [\[CrossRef\]](#)
- Oprić, D.; Stankovich, A.D.; Nenadović, A.; Kovačević, S.; Obradović, D.D.; De Luka, S.; Nešović-Ostojić, J.; Milašin, J.; Ilić, A.Ž.; Trbovich, A.M. Fractal analysis tools for early assessment of liver inflammation induced by chronic consumption of linseed, palm and sunflower oils. *Biomed. Sign. Process. Control* **2020**, *61*, 101959. [\[CrossRef\]](#)
- Stojić, D.; Radošević, D.; Rajković, N.; Marić, D.L.; Milošević, N.T. Classification by morphology of multipolar neurons of the human principal olivary nucleus. *Neurosci. Res.* **2021**, *170*, 66–75. [\[CrossRef\]](#)
- Lyu, X.; Jajal, P.; Tahir, M.Z.; Zhang, S. Fractal dimension of retinal vasculature as an image quality metric for automated fundus image analysis systems. *Sci. Rep.* **2022**, *12*, 11868. [\[CrossRef\]](#)
- Freeborn, T.J.; Fu, B. Fatigue-induced Cole electrical impedance model changes of biceps tissue bioimpedance. *Fractal Fract.* **2018**, *2*, 27. [\[CrossRef\]](#)
- Gladun, K.V. Higuchi fractal dimension as a method for assessing response to sound stimuli in patients with diffuse axonal brain injury. *Sovrem. Tehnologii Med.* **2020**, *12*, 63. [\[CrossRef\]](#)
- Naik, G.R.; Arjunan, S.; Kumar, D. Applications of ICA and fractal dimension in sEMG signal processing for subtle movement analysis: A review. *Australas. Phys. Eng. Sci. Med.* **2011**, *34*, 179–193. [\[CrossRef\]](#) [\[PubMed\]](#)
- Stylianou, O.; Kaposzta, Z.; Czoch, A.; Stefanovski, L.; Yabluchanskiy, A.; Racz, F.S.; Ritter, P.; Eke, A.; Mukli, P. Scale-free functional brain networks exhibit increased connectivity, are more integrated and less segregated in patients with Parkinson's disease following dopaminergic treatment. *Fractal Fract.* **2022**, *6*, 737. [\[CrossRef\]](#)
- Goh, V.; Sanghera, B.; Wellsted, D.M.; Sundin, J.; Halligan, S. Assessment of the spatial pattern of colorectal tumour perfusion estimated at perfusion CT using two-dimensional fractal analysis. *Eur. Radiol.* **2009**, *19*, 1358–1365. [\[CrossRef\]](#)
- Streba, L.; Forțofoiu, M.C.; Popa, C.; Ciobanu, D.; Gruia, C.L.; Mogoantă, S.; Streba, C.T. A pilot study on the role of fractal analysis in the microscopic evaluation of colorectal cancers. *Rom. J. Morphol. Embryol.* **2015**, *56*, 191–196. [\[PubMed\]](#)
- Watanabe, H.; Hayano, K.; Ohira, G.; Imanishi, S.; Hanaoka, T.; Hirata, A.; Kano, M.; Matsubara, H. Quantification of structural heterogeneity using fractal analysis of contrast-enhanced CT image to predict survival in gastric cancer patients. *Dig Dis Sci.* **2021**, *66*, 2069–2074. [\[CrossRef\]](#) [\[PubMed\]](#)
- Dzik-Jurasz, A.; Walker-Samuel, S.; Leach, M.O.; Brown, G.; Padhani, A.; George, M.; Collins, D.J. Fractal parameters derived from analysis of DCE-MRI data correlates with response to therapy in rectal carcinoma. In Proceedings of the International Society for Magnetic Resonance in Medicine 11, ISMRM 12th Scientific Meeting, Kyoto, Japan, 15–21 May 2004; p. 2503.
- Tochigi, T.; Kamran, S.C.; Parakh, A.; Noda, Y.; Ganeshan, B.; Blaszkowsky, L.S.; Ryan, D.P.; Allen, J.N.; Berger, D.L.; Wo, J.Y.; et al. Response prediction of neoadjuvant chemoradiation therapy in locally advanced rectal cancer using CT-based fractal dimension analysis. *Eur. Radiol.* **2022**, *32*, 2426–2436. [\[CrossRef\]](#)
- Jain, S.; Seal, A.; Ojha, A.; Krejcar, O.; Bureš, J.; Tachecí, I.; Yazidi, A. Detection of abnormality in wireless capsule endoscopy images using fractal features. *Comput. Biol. Med.* **2020**, *127*, 104094. [\[CrossRef\]](#) [\[PubMed\]](#)

25. Gryglewski, A.; Henry, B.M.; Mrozek, M.; Żelawski, M.; Piech, K.; Tomaszewski, K.A. Sensitivity and specificity of fractal analysis to distinguish between healthy and pathologic rectal mucosa microvasculature seen during colonoscopy. *Surg. Laparosc. Endosc. Percutan. Tech.* **2016**, *26*, 358–363. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Paramasivam, A.; Kamalanand, K.; Emmanuel, C.; Mahadevan, B.; Sundravadivelu, K.; Raman, J.; Jawahar, P.M. Influence of electrode surface area on the fractal dimensions of electrogastrograms and fractal analysis of normal and abnormal digestion process. In Proceedings of the 2018 International Conference on Recent Trends in Electrical, Control and Communication (RTECC 2018), Malaysia, Malaysia, 20–22 March 2018; pp. 245–250. [\[CrossRef\]](#)
27. Yan, R.; Guo, X. Nonlinear fractal dynamics of human colonic pressure activity based upon the box-counting method. *Comput. Methods Biomech. Biomed. Eng.* **2013**, *16*, 660–668. [\[CrossRef\]](#)
28. Dimoulas, C.; Kalliris, G.; Papanikolaou, G.; Kalampakas, A. Long-term signal detection, segmentation and summarization using wavelets and fractal dimension: A bioacoustics application in gastrointestinal-motility monitoring. *Comput. Biol. Med.* **2007**, *37*, 438–462. [\[CrossRef\]](#)
29. Weber, M.C.; Schmidt, K.; Buck, A.; Kasajima, A.; Becker, S.; Wilhelm, D.; Friess, H.; Neumann, P.A. P067 Fractal analysis of extracellular matrix as a new histological method for observer-independent quantification of intestinal fibrosis in Crohn's disease. *J. Crohn's Colitis* **2023**, *17* (Suppl. 1), i234. [\[CrossRef\]](#)
30. Hadjileontiadis, L.J.; Rekanos, I.T. Detection of explosive lung and bowel sounds by means of fractal dimension. *IEEE Signal Process. Lett.* **2003**, *10*, 311–314. [\[CrossRef\]](#)
31. Almossalha, L.; Tiwari, A.; Ruhoff, P.; Stypula-Cyrus, Y.; Cherkezyan, L.; Matsuda, H.; Dela Cruz, M.A.; Chandler, J.E.; White, C.; Maneval, C.; et al. The global relationship between chromatin physical topology, fractal structure, and gene expression. *Sci. Rep.* **2017**, *7*, 41061. [\[CrossRef\]](#)
32. Bancaud, A.; Lavelle, C.; Huet, S.; Ellenberg, J. A fractal model for nuclear organization: Current evidence and biological implications. *Nucl. Acids Res.* **2012**, *40*, 8783–8792. [\[CrossRef\]](#)
33. Metze, K.; Adam, R.; Florindo, J.B. The fractal dimension of chromatin—A potential molecular marker for carcinogenesis, tumor progression and prognosis. *Expert Rev. Mol. Diagn.* **2019**, *19*, 299–312. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Vesković, M.; Labudović-Borović, M.; Zaletel, I.; Rakočević, J.; Mladenović, D.; Jorgačević, B.; Vučević, D.; Radosavljević, T. The effects of betaine on the nuclear fractal dimension, chromatin texture, and proliferative activity in hepatocytes in mouse model of nonalcoholic fatty liver disease. *Microsc. Microanal.* **2018**, *24*, 132–138. [\[CrossRef\]](#)
35. Ray, G.; Longworth, M.S. Epigenetics, DNA organization, and inflammatory bowel disease. *Inflamm. Bowel Dis.* **2019**, *25*, 235–247. [\[CrossRef\]](#)
36. Fedor, I.; Zold, E.; Barta, Z. Temporal relationship of extraintestinal manifestations in inflammatory bowel disease. *J. Clin. Med.* **2021**, *10*, 5984. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Gajendran, M.; Loganathan, P.; Jimenez, G.; Catinella, A.P.; Ng, N.; Umapathy, C.; Ziade, N.; Hashash, J.G. A comprehensive review and update on ulcerative colitis. *Dis. Mon.* **2019**, *65*, 100851. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Sathiyasekaran, M.; Shivbalan, S. Crohn's disease. *Indian J. Pediatr.* **2006**, *73*, 723–729. [\[CrossRef\]](#)
39. Fuller, M.K. Pediatric inflammatory bowel disease: Special considerations. *Surg. Clin. N. Am.* **2019**, *99*, 1177–1183. [\[CrossRef\]](#)
40. De Roche, T.C.; Xiao, S.Y.; Liu, X. Histological evaluation in ulcerative colitis. *Gastroenterol. Rep.* **2014**, *2*, 178–192. [\[CrossRef\]](#)
41. Torres, J.; Bonovas, S.; Doherty, G.; Kucharzik, T.; Gisbert, J.P.; Raine, T.; Adamina, M.; Armuzzi, A.; Bachmann, O.; Bager, P.; et al. ECCO Working Group. ECCO Guidelines on Therapeutics in Crohn's Disease: Medical Treatment. *J. Crohn's Colitis* **2020**, *14*, 4–22. [\[CrossRef\]](#)
42. Szymanska, E.; Wierzbicka, A.; Dadalski, M.; Kierkus, J. Fecal zonulin as a noninvasive biomarker of intestinal permeability in pediatric patients with inflammatory bowel diseases—Correlation with disease activity and fecal calprotectin. *J. Clin. Med.* **2021**, *10*, 3905. [\[CrossRef\]](#)
43. Ordog, T.; Syed, S.; Hayashi, Y.; Asuzu, D.T. Epigenetics and chromatin dynamics: A review and a paradigm for functional disorders. *Neurogastroenterol. Motil.* **2012**, *24*, 1054–1068. [\[CrossRef\]](#)
44. Ansari, I.; Raddatz, G.; Gutekunst, J.; Ridnik, M.; Cohen, D.; Abu-Remaileh, M.; Tuganbaev, T.; Shapiro, H.; Pikarsky, E.; Elinav, E.; et al. The microbiota programs DNA methylation to control intestinal homeostasis and inflammation. *Nat. Microbiol.* **2020**, *5*, 610–619. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Ruifrok, A.C.; Johnston, D.A. Quantification of histochemical staining by color deconvolution. *Anal. Quant. Cytol. Histol.* **2001**, *23*, 291–299.
46. Landini, G.; Martinelli, G.; Piccinini, F. Colour deconvolution: Stain unmixing in histological imaging. *Bioinformatics* **2021**, *37*, 1485–1487. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Singh, T.R.; Roy, S.; Singh, O.I.; Sinam, T.; Singh, K.M. A new local adaptive thresholding technique in binarization. *IJCSI Int. J. Comp. Sci.* **2011**, *8*, 271–277. [\[CrossRef\]](#)
48. Karperien, A.L.; Jelinek, H.F. Box-counting fractal analysis: A primer for the clinician. In *The Fractal Geometry of the Brain*; Di Ieva, A., Ed.; Springer: New York, NY, USA, 2016; pp. 91–108. [\[CrossRef\]](#)
49. Glickman, J.N.; Bousvaros, A.; Farraye, F.A.; Zholudev, A.; Friedman, S.; Wang, H.H.; Leichtner, A.M.; Odze, R.D. Pediatric patients with untreated ulcerative colitis may present initially with unusual morphologic findings. *Am. J. Surg. Pathol.* **2004**, *28*, 190–197. [\[CrossRef\]](#)

50. Bancaud, A.; Huet, S.; Daigle, N.; Mozziconacci, J.; Beaudouin, J.; Ellenberg, J. Molecular crowding affects diffusion and binding of nuclear proteins in heterochromatin and reveals the fractal organization of chromatin. *EMBO J.* **2009**, *28*, 3785–3798. [[CrossRef](#)] [[PubMed](#)]
51. Mobley, A.S. Chapter 4—Induced pluripotent stem cells. In *Neural Stem Cells and Adult Neurogenesis*; Mobley, A.S., Ed.; Academic Press: London, UK, 2019; ISBN 9780128110140. [[CrossRef](#)]
52. Chessum, N.; Jones, K.; Pasqua, E.; Tucker, M. Recent advances in cancer therapeutics. *Prog. Med. Chem.* **2015**, *54*, 1–63. [[CrossRef](#)]
53. De Bruyn, J.; Wichers, C.; Radstake, T.; Broen, J.; D’Haens, G. Histone deacetylases in inflammatory mucosa distinguish Crohn’s disease from ulcerative colitis. *J. Crohn’s Colitis* **2015**, *9* (Suppl. 1), S87–S88. [[CrossRef](#)]
54. Blanchard, F.; Chipoy, C. Histone deacetylase inhibitors: New drugs for the treatment of inflammatory diseases? *Drug. Discov. Today* **2005**, *10*, 197–204. [[CrossRef](#)] [[PubMed](#)]

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