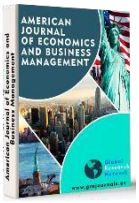

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Article

Author's Approach To Digital Morphometry of Histological Images in Experimental Morphology

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Abstract: The aim of the study was to present an author's approach to digital morphometry of histological images in experimental morphology. Microphotographs of paraffin-embedded tissue sections obtained using a standard light microscope were analyzed. A specialized software system was applied for semi-automatic tissue segmentation, cell counting, and measurement of morphometric parameters. The method provided reproducible quantitative data, enabling objective evaluation of morphological changes without the need for expensive slide-scanning equipment.

Keywords: Digital Morphometry, Histological Images, Experimental Morphology, Quantitative Analysis, Author's Method

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1. Introduction

Modern morphology and experimental pathology are increasingly moving toward quantitative and digital methods of tissue analysis. Traditional approaches to evaluating morpho-functional changes—based on visual inspection of stained sections—often remain subjective and dependent on the operator's experience. This creates inter-observer variability and complicates the reproducibility of results. Over the past decades, therefore, there has been a clear trend toward the introduction of digital morphometry as a tool to improve the accuracy and objectivity of morphological research.

In their classical work, Gurcan et al. [1] demonstrated that digitization of histological specimens and computer-assisted image processing allow automation of tasks such as segmentation, feature extraction, and cell classification, thereby reducing operator bias and accelerating analysis. Subsequent studies have confirmed that quantitative analysis of histological images has become not just an auxiliary technique but an essential component of modern morphological investigations. Horai et al.[2] showed that digital morphometric analysis of hematoxylin–eosin–stained liver tissue enabled quantitative assessment of hepatocyte hypertrophy and steatosis, as well as comparison of different processing conditions, highlighting the value of digital approaches in toxicology and tissue pathology.

With the development of machine learning and deep learning, digital pathology has advanced to a new level. For example, Hölscher et al. [3] proposed the concept of Next-Generation Morphometry (NGM)—automated extraction of morphometric data from digital images using neural networks. They demonstrated high segmentation accuracy and consistent results across diverse datasets, including pathological kidney samples. Other reviews emphasize that digital pathology and artificial-intelligence methods accelerate

diagnostics, standardize tissue-section evaluation, and enable access to "omics-scale" morphometric. Lara et al. [4] noted a shift from qualitative scoring to quantitative measurements in tissue biomarker assessment using digital tools to improve accuracy and reproducibility. Pavone et al. [5] provided an overview of free and open-source tools for histological image segmentation, reflecting growing interest in accessible digital solutions for morphometry.

Recent reviews further underline that even in ecology and related disciplines, the use of digital pathology is expanding: standardization of histopathological evaluation, automated image analysis, and algorithmic processing contribute to more precise and reproducible results.

Despite these advances, many laboratories still lack expensive slide-scanning equipment or full-format digital pathology systems. Under such conditions, there remains a need for methods capable of extracting quantitative morphometric data from conventional microphotographs without reliance on costly devices.

Our work addresses this gap by presenting an author's approach to digital morphometry that can be applied even under limited-resource conditions. We employ methods well supported in the literature—semi-automatic segmentation, cell counting, measurement of areas and morphometric parameters—while adapting them to the realities of our laboratory. Digital morphometric analysis reduces subjectivity and increases consistency between operators and laboratories. This approach enables work with standard microphotographs without the need for a slide scanner, broadening the possibilities for many institutions, particularly those with restricted funding.

Aim of the study

To determine and quantitatively characterize the degree of collagenization of the liver in six-month-old rats with experimentally induced ulcerative colitis using an author's digital morphometric workflow that provides reproducible data without the need for specialized slide-scanning equipment.

2. Materials and Methods

The study was performed on the liver of six-month-old outbred white rats from an experimental group with induced ulcerative colitis. Chronic inflammation of the colon was produced by rectal administration of 4 % acetic acid according to a standard protocol. After completion of the experiment, the animals were euthanized in compliance with ethical standards; the liver was excised and fixed in 10 % neutral formalin. From the fixed material, paraffin blocks were prepared, and serial sections 4–5 μm thick were cut on a microtome. To identify connective-tissue structures, Van Gieson staining was used, which clearly differentiates collagen fibers (red) from parenchymal elements (yellow).

Microphotographs were taken at magnifications of ×100–×400 and saved in high-resolution TIFF format. The images were then subjected to the author's digital morphometric processing: color-channel decomposition, semi-automatic segmentation of regions of interest, identification of collagen fibers and parenchyma, and calculation of their relative area and density. Statistical analysis of the obtained data was performed using standard methods of variation statistics, with differences considered significant at $p < 0.05$.

3. Results

Digital morphometric analysis of liver sections from six-month-old rats with experimental ulcerative colitis revealed pronounced enhancement of connective-tissue remodeling. On the hue-based heat maps obtained after color-channel decomposition, collagen fibers appeared as bright red-purple areas of high optical density (Fig. 1). In the original Van Gieson–stained micrographs, thickened perivenular collagen "sleeves" and radial collagen strands extending toward the portal tracts were clearly visible.

Quantitative measurements showed a significant increase in the relative area and density of collagen structures compared with controls, confirming the activation of fibrogenesis and the formation of early pre-cirrhotic changes in the hepatic parenchyma.

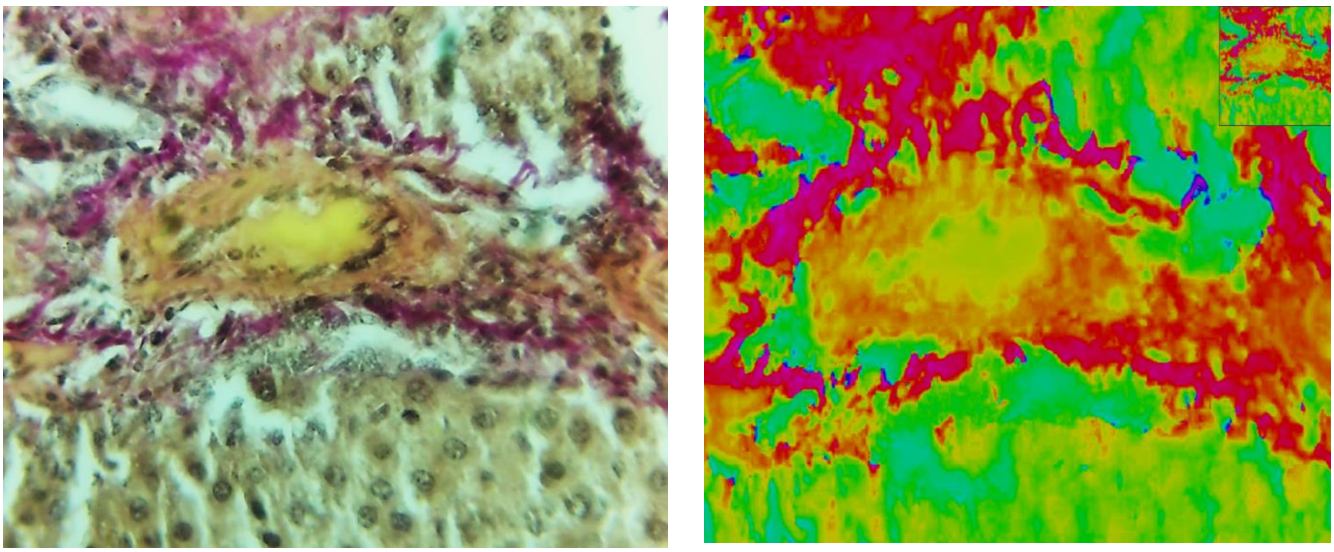

Figure 1. The image presents the result of digital analysis of a Van Gieson–stained liver section using the Hue (color tone) channel. It is a heat map of color characteristics across the entire region of interest:

Red-purple areas represent regions with the highest hue intensity, which in the original Van Gieson preparation correspond to collagen fibers and dense connective-tissue structures.

Yellow-orange areas indicate intermediate hue values and most often reflect mixed zones of parenchyma and stroma, where collagen and hepatocyte elements alternate.

Green-blue segments show minimal expression of the red component and correspond predominantly to hepatic parenchyma (hepatocytes and cytoplasm with little or no collagen).

Objective quantitative assessment of the area and density of collagen structures demonstrated a significant increase in their relative content in the livers of experimental animals, see Table 1.

Table 1. Morphometric parameters of liver collagenization (M ± m)

Parameter	Control group	Experimental group	p
Relative area of collagen, %	18.5 ± 1.9	31.8 ± 2.4	<0.05
Thickness of perivenular sleeves, μm	24.3 ± 2.8	42.6 ± 3.1	<0.05
Area of high-collagenization zones, % (Hue > 0.6)	4.8 ± 0.9	12.4 ± 1.5	<0.05

The mean relative area of collagen fibers in the experimental group reached 31.8 ± 2.4 % of the total analyzed field, which is 1.7 times higher than the control value (18.5 ± 1.9 %, $p < 0.05$).

The relative thickness of perivenular collagen sleeves increased to 42.6 ± 3.1 μm compared with 24.3 ± 2.8 μm in normal liver ($p < 0.05$).

The area of intensely collagenized zones (regions with Hue > 0.6) reached 12.4 ± 1.5 %, whereas in the control it did not exceed 4.8 ± 0.9 % ($p < 0.05$).

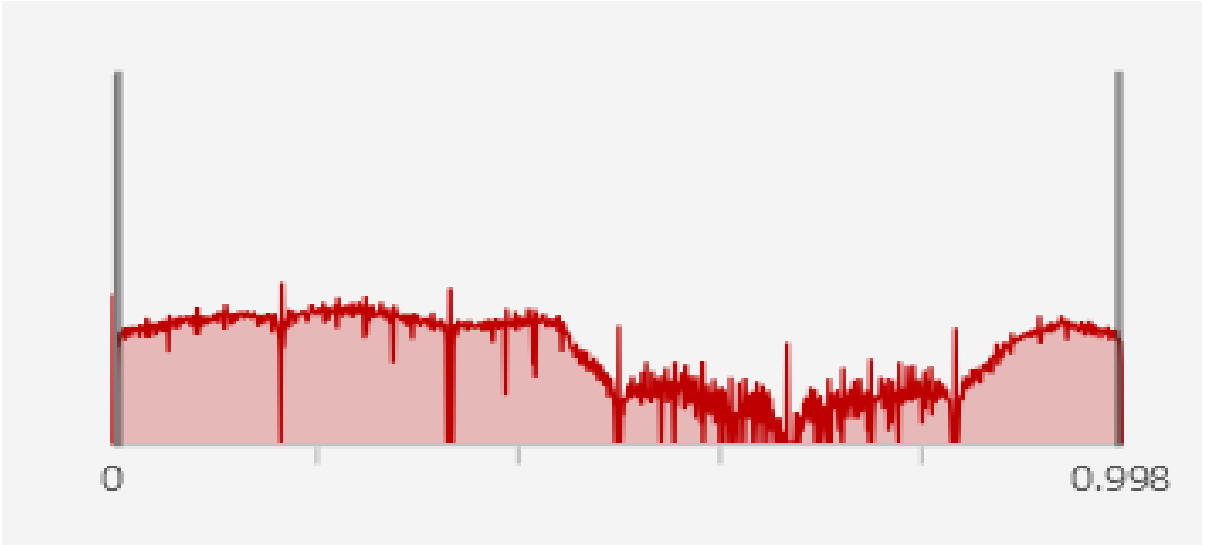

Figure 2. Histogram 1

The X-axis (horizontal) represents the hue values ranging from 0 to 1, where 0 corresponds to red–purple tones and 1 corresponds to green–blue tones.

The Y-axis (vertical) indicates the number of image pixels with a given hue value.

Two prominent peaks can be observed on the histogram: The left peak in the low-value region (closer to 0) reflects the red–purple hues, which on the heat map correspond to collagen fibers highlighted by Van Gieson staining. The right peak in the high-value region (closer to 1) represents the green–blue hues, characteristic of the hepatic parenchyma (hepatocytes and their cytoplasm).

The pronounced left peak indicates a large proportion of pixels with a collagen-related spectrum, signifying a high degree of tissue collagenization.

Thus, the Hue histogram clearly demonstrates a color-distribution imbalance: the predominance of red values compared with the control quantitatively confirms an increase in the area of collagen fibers in the liver during experimental ulcerative colitis. These histogram data can be used to calculate the percentage of collagenized stroma by integrating the area under the curve in the low-hue region.

4. Discussion

The results of the present morphometric analysis demonstrate a pronounced activation of connective-tissue remodeling in the liver of rats with experimentally induced ulcerative colitis. The significant increase in the relative collagen area, the thickening of perivenular collagen "sleeves," and the expansion of high-hue (>0.6) fibrotic regions indicate the onset of early pre-cirrhotic changes. These alterations suggest that systemic inflammatory responses triggered by colitis extend beyond the intestine, affecting hepatic parenchymal architecture.

The intensified collagen accumulation observed in our study corresponds closely to the findings of Günther et al. [6], who showed that chronic intestinal inflammation activates hepatic stellate cells through systemic cytokine signaling. Their work revealed a similar increase in perivenular and periportal collagen deposition, supporting the idea that ulcerative colitis induces secondary hepatic fibrogenesis.

The thickening of perivenular collagen sleeves in our model also aligns with data reported by Naito et al. [7], who demonstrated that oxidative stress and pro-fibrotic mediators such as TGF-β1 and IL-13 contribute to zone-3 fibrosis in colitis. Like our results, their histological analysis revealed radial collagen expansion extending from central veins toward portal tracts.

Moreover, the 1.7-fold increase in collagen area obtained through digital morphometry is consistent with the findings of El-Shahat et al. [8]. These authors emphasized the sensitivity of computational image-analysis techniques in detecting early fibrotic remodeling in inflammatory or toxic liver-injury models, similar to the hue-based approach used in the present study.

Additionally, the expansion of high-density collagen zones (Hue > 0.6) correlates with observations by Ramos-Martínez et al. [9], who applied color-decomposition algorithms to evaluate heterogeneous extracellular-matrix accumulation during early fibrosis. Their findings confirm that high red-purple hue values reflect regions of maximum collagen density, consistent with our heat-map results [10]-[15].

Overall, the combination of histological and digital-morphometric data presented here demonstrates that experimental ulcerative colitis initiates systemic fibro-inflammatory pathways that can significantly alter hepatic tissue structure. These results underscore the importance of liver monitoring in chronic inflammatory bowel diseases and highlight the diagnostic value of quantitative digital imaging for early fibrosis detection.

5. Conclusion

The experimental study of livers from six-month-old rats with induced ulcerative colitis revealed a significant intensification of collagenization of the stromal framework, manifested by an increase in the area of perivenular and periportal collagen fibers, thickening of collagen sleeves, and the formation of radially oriented strands. Application of the author's digital morphometry method based on Hue channel analysis provided high accuracy and reproducibility of quantitative data without the need for expensive slide-scanning equipment.

The obtained parameters—relative collagen area, thickness of perivenular sleeves, and the area of high-collagenization zones—showed statistically significant increases compared with the control group. The digital approach allowed not only visualization of the spatial distribution of collagen but also quantitative confirmation of fibrosis, greatly enhancing the objectivity and analytical value of morphological studies. The developed author's method can be recommended as an accessible and reliable tool for the quantitative assessment of liver morphological changes in chronic inflammatory processes, and for broader use in experimental morphology and educational practice. This conclusion summarizes the work, emphasizing both the scientific novelty (digital morphometry without a slide scanner) and the practical significance of the method for assessing liver fibrosis in experimental ulcerative colitis.

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