



# **Stain Normalization in Histopathology Image Analysis Using Digital Image Processing Techniques**

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**ABSTRACT:** Histopathology designation relies on visual examination of the morphology of histological sections beneath a magnifier. We have a tendency to gift a unique approach to stain normalization in histopathology videos. Processed call support systems square measure fraught with issues that stem from colorize tissue look. we have a tendency to approach to stain normalization in histopathology videos Color de-convocation may be a technique to get stain concentration values once the stain matrix describes however the color is have an effect on. The experimental results counsel that the paradigm of color normalization.

**KEYWORDS:** Histopathology images analysis, nonlinear mapping, principal color histograms (PCH), stain color descriptor (SCD), stain estimation, stain normalization.

## **I. INTRODUCTION**

Histology deals with the study of the microscopic structure of cells and tissues of organisms. The knowledge of biological (microscopic) structures and their functions at the sub-cellular, cellular, tissue and organ levels is central to the study of disease proliferation and prognosis of disease. Also to study and analyze histological image under microscope, pathologists identify the morphological characteristics of tissue which indicates the presence of disease like cancer. The biopsy sample is processed and its sections are placed onto glass slides to observe them under microscope for analysis. To view cells, glands, nucleus, and detects the resemblance of these structures with normal vs. diseased tissue. If the disease detects the grading process is performed which deals with spreading of infected cells all over the tissue. Then for each patient the prognosis and further treatment is planned by considering grade of disease. The main theme of this paper is to investigate robust and accurate image analysis algorithms for computer-assisted interpretation of histopathology imagery. Different image processing techniques will be applied for image texture classification, gland & nuclei segmentation, cell counting, cell type identification or classification to deriving quantitative measurements of disease features from histological images and automatically determine whether a disease is present within analyzed samples or not. Also this research will help to decide the different grades or severity of disease if the disease is present in the sample. Computer aided histopathological study has been conducted for various cancer detection and grading applications, including prostate, breast, renal cell carcinoma pediatric tumor neuroblastoma and lung cancer grading. Using different segmentation, feature extraction and classification techniques the researchers analyzed histopathology images. Here in this paper the different image processing techniques on histopathological images specially breast cancer histopathology analyses are reviewed. The paper to discuss the need for, and analyze the procedure for computer aided histopathology image segmentation and classification. These analysis procedures are also applicable to all image modalities in medical image analysis like ultrasound, MRI, CT Scan etc. basic histology image analysis flow and the procedure for preparation of histopathology slides for microscopic analysis including the image magnification/resolution needed for various analyses is described. segmentation techniques for histopathological images are reviewed. It includes brief review about the feature extraction and selection for different segmented results. Finally, the overview about classification methods for histopathology image with the future analysis in the related field is discussed. Followed by, the comparison and conclusion on the study is given. In literature one can find large number of applications in microscopic image analysis feature extraction and



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selection along with many image processing methods for preprocessing, segmentation, and classification, here only some examples are presented.

## II. AN OVERVIEW OF HISTOPATHOLOGICAL IMAGE ANALYSIS

After obtaining digital histology image through biopsy sample, the manual examination of images leads to variability in diagnosis. To overcome this problem, computer assisted systems are employed which gives objective analysis of diseases. The basic steps required for implementing computer assisted analysis system are as shown in Figure 1 below. This consists of digital image processing techniques such as image segmentation, feature extraction, classification etc. They analyzed histopathology images analysis using image preprocessing, feature extraction and Classification techniques such as thresholding, morphological processing, region based, and boundary based, and supervised classification techniques. The recent advancement of 'digital histopathology' needs development of quantitative and automated computerized image analysis algorithms to assist histopathologist in interpreting the large number of digitized histopathological images. The different methods developed for histology image analysis are summarized in Table 1 given below.

S.No	Title of Paper and Author Name	Methods used for HIA
1.	Automated classification of cells in sub-epithelial connective tissue of oral sub-mucous fibrosis – An SVM based approach, Muthu Rama krishnan et.al.	SVM, Classifier [1]
2	Image Analysis for Neuroblastoma Classification: Segmentation of Cell Nuclei, Metin N. Gurcan, Tony Pan, Hiro Shimada, and Joel Saltz	Hierarchical Normalized cut, Color Gradient Active Contour [2]
3	Texture measures combination for improved meningioma classification of histopathological images- Omar S. Al-Kadi,	Texture Classification using fractal features [3]
4	Partitioning Histopathological Images: An Integrated Framework for Supervised Color-Texture Segmentation and Cell Splitting- Hui Kong, Metin Gurcan	Color Texture cell Segmentation [6]
5	Computerized Classification of Intraductal Breast Lesions Using Histopathological Images- M. Murat Dundar, Sunil Badve	Gaussian Mixture Model based segmentation [4]
6	Graph Run-length Matrices For Histopathological Image Segmentation- Akif Burak Tosun, Cigdem Gunduz-demir	Graph Run length matrices for image segmentation [5]

**Table.1. Different methods developed for Histology Image Analysis**

The five basic steps for preparation of histopathology samples. and it includes:

1. **Fixing:** Samples of biological tissue/ gross are "fixed" with chemical fixation to preserve the cells/tissue.
2. **Processing:** Tissue processing is essential to remove water from the gross tissue (dehydration) and replace it with a medium which solidifies it. This helps to cut thin sections of sample
3. **Embedding sample in wax:** The result of embedding hardened wax blocks contains the original biological samples together with other substances in complete preparation process.
4. **Sectioning:** Sectioning an embedded tissue sample is the step necessary to produce sufficiently thin slices of sample that the detail of the microstructure of the cells/tissue can be clearly observed using microscopy techniques. Then transfer the thin cut of sample on to a clean glass slide.



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5. **Staining:** Finally, the mounted sections are treated with an appropriate histology stain. Staining biological tissues is done to both increase the contrast of the tissue and also highlight some specific features of interest - depending on the type of tissue and the stain used.

### III. EXISTING SYSTEM

An existing system considering the variation in colors within/across histopathology sections, color texture features may be highly sensitive to staining/scanner variations and thus may significantly affect the Performance of an automated system. In order to overcome these limitations a different approach and normalize color distributions of source image to those of a target image before performing.

#### Disadvantages:

- Complete standardization is not possible to achieve with the current technology.
- Doesn't improving color constancy in images formed via Lambert ion (reflective) model of image formation, these methods are not applicable to color images formed via light transmission through a tissue specimen, and thus are inappropriate for histopathology image analysis.
- A large number of methods presented in the area of automatic image analysis of color histopathology images bypass the problem of color constancy by transforming the images to grayscale.
- A texture analysis for tissue type classification has been performed on grayscale image.
- The results in less image artifacts than existing approaches due to its robustness we use the term "stain or color normalization" to refer to the process of adjusting the color values of an image on a pixel-by-pixel basis so as to match the color distribution of the source image to that of a target image.

### IV. PROPOSED SYSTEM

Histopathology diagnosis is based on visual examination of the morphology of histological sections under a microscope. Color variation is a problem in histopathology based on light microscopy due to a range of factors such as the use of different scanners, the advent of digital imaging and automatic image analysis, color variation in histopathology has become more of an issue Typically, two or three different colored stains are used to highlight cellular and ubcellular target components. The intensity of each color is related to the concentration of the orresponding component. Considering the variation in colors within/across histopathology sections, color texture features may be highly sensitive to staining/scanner variations and thus may significantly affect the performance of an automated system.

#### Advantage

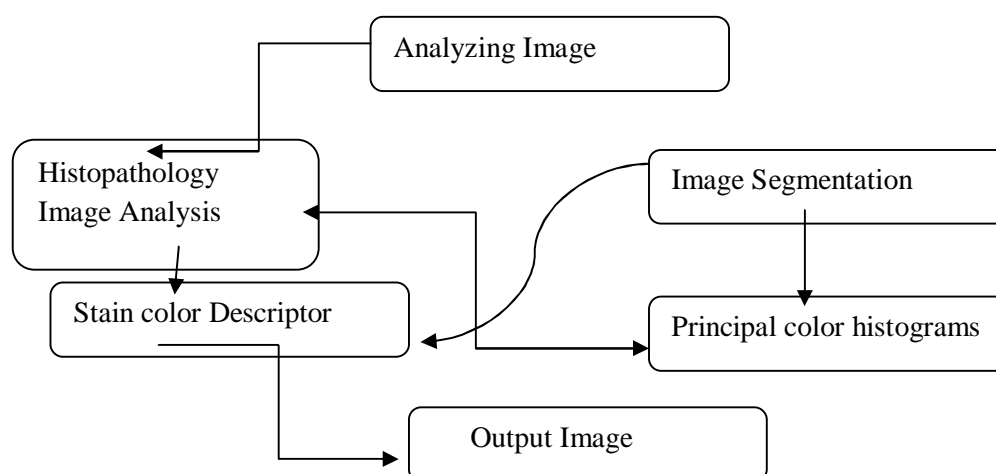
- The advantage of digital imaging and automatic image analysis,
- Color variation in histopathology has become more of an issue.
- For example, many commercial image analysis algorithms require parameters defining the expected color of anatomy of interest and fail if these parameters are incorrect.

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## Architectural diagram



There are different components Histopathology namely as follows;

**Histopathology images analysis:** With the advent of digital imaging and automatic image analysis, color variation in histopathology has become more of an issue. For example, many commercial image analysis algorithms require parameters defining the expected color of anatomy of interest and fail if these parameters are incorrect. For example, texture analysis for tissue type classification has been performed on grayscale images using features based on grayscale concurrence matrices nonlinear mapping. For each channel of deconvolved target and source images, we calculate a set of statistics and smoothly map the statistics of each source image channel to those of the statistics of corresponding target image channel using a spline-based nonlinear mapping.

**Stain Color Estimation:** Tissue sections are prepared using colored his to -chemical stains that bind selectively to cellular components. Color variation is a problem in histopathology based on light microscopy due to a range of factors such as the use of different scanners, variable chemical coloring/reactivity from different manufacturers batches of stains, coloring being dependent on staining procedure.

**Stain Normalization:** The proposed methods apply the stain matrix estimation, CD, nonlinear mapping of channel statistics, and reconstruction. We apply a nonlinear correction to normalize each channel of  $X^{\wedge}$  separately (based on the statistics calculated from the corresponding channel. drawback of histogram-based approaches is that they introduce considerable visual artifacts in images. This is due to the implicit assumption that the proportion of pixels of each stain type is same in the target and source images. This is clearly not always correct. before performing color-based segmentation. In the remainder of this paper, we use the term “stain or color normalization”

**Color Mismatch Analysis:** In this color are mismatched with original images and then that can be analyzed and mismatched with the colors. This is due to the implicit assumption that the proportion of pixels of each stain type is same in the target and source images. This is clearly not always correct. Some authors have included color information within texture based image classification in digital histopathology image analysis. major limitation of this approach is that it introduces artifacts near pixels that lie on the class boundary.

## V. CONCLUSION

we studied different steps to automatically analyze histopathological images for objective diagnosis. This automated analysis assists pathologist in diagnosis and lessen their time for reviewing large number of tissue slide per day. The digital histopathological images are acquired through computerized electron microscope after tissue slide preparation.



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For computer aided analysis there are mainly three steps: Segmentation, Feature Extraction and Classification. The algorithms developed for automated analysis and evaluation of histology images assists the pathologists in disease diagnosis and also reduces human error.

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