A high-speed unsupervised hardware architecture for rapid diagnosis of COVID-19

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Summary
In the diagnosis of COVID-19, investigation, analysis, and automatic counting of blood cell clusters are the most essential steps. Currently employed methods for cell segmentation, identification, and counting are time-consuming and sometimes performed manually from sampled blood smears, which is hard and needs the support of an expert laboratory technician. The conventional method for the blood-count-test is by automatic hematology analyzer which is quite expensive and slow. Moreover, most of the unsupervised learning techniques currently available presume the medical practitioner to have a prior knowledge regarding the number and action of possible segments within the image before applying recognition. This assumption fails most often as the severity of the disease gets increased like the advanced stages of COVID-19, lung cancer etc. In this manuscript, a simplified automatic histopathological image analysis technique and its hardware architecture suited for blind segmentation, cell counting, and retrieving the cell parameters like radii, area, and perimeter has been identified not only to speed up but also to ease the process of diagnosis as well as prognosis of COVID-19. This is achieved by combining three algorithms: the K-means algorithm, a novel statistical analysis technique-HIST (histogram separation technique), and an islanding method an improved version of CCA algorithm/blob detection technique. The proposed method is applied to 15 chronic respiratory disease cases of COVID-19 taken from high profile hospital databases. The output in terms of quantitative parameters like PSNR, SSIM, and qualitative analysis clearly reveals the usefulness of this technique in quick cytological evaluation. The proposed high-speed and low-cost architecture gives promising results in terms of performance of 190 MHz clock frequency, which is two times faster than its software implementation.

KEYWORDS
biopsy, COVID-19, histogram analysis, histopathology, K-means clustering, lung cancer, Otsu's method, pulmonary infections
The first reports of COVID-19 infection reported in Wuhan, China, on December 28, 2019, has rapidly conquered the whole world. Moreover, the second and third waves of this catastrophe have also severely affected the population of many countries. All predictions points to a fourth wave at the end of June or mid of July 2022. As of May 1, 2022, the confirmed deaths caused due to SARS CoV-2 (severe acute respiratory syndrome coronavirus-2) are 6,260,153, and the confirmed cases worldwide are 513,193,168 (513 million) in more than 206 countries. COVID-19 has been declared as a pandemic by the WHO on March 11, 2020. The SARS CoV-2 predominantly attacks the lungs of a person leading to pneumonia.

The medical fraternity considers the diagnosis through a histopathological image as “Gold standard,” still a very few pathological research studies and tests on the basis of invasive diagnostic procedures like biopsies have been conducted on COVID-19. This is because of the lack of dedicated hardware, huge time delay, lack of specialized infrastructure, resources and the lack of technically trained health care professionals. In the last December, 2019 SARS-CoV-2 virus was identified in Wuhan, China from a group of severe pneumonia infection cases. COVID-19 has been termed as a pandemic since it has now spread globally and is responsible for hundreds of thousands of deaths. Still only a few pathological research studies on the basis of autopsies or biopsies have been conducted on COVID-19, which is a surprising truth. This lack of research not only lead to chaos and confusion in exactly pinpointing the cause of deaths, but also it ill-facilitated our understanding on the pathogenesis of the COVID-19 that in turn delayed the formulation of a timely therapeutic strategy, increasing the mortality rate and panic all over the world. Other major reasons for the lack of autopsies and biopsies included in the COVID-19 research, diagnosis, and prognosis are suddenness of the outbreak, vast patient volume in hospitals, high rate of transmission, and unavailability of pathologists and doctors.

Within the last decade, due to the exponential increase in computation power, combined with the tremendous advancement in image processing algorithms and allied techniques, powerful computer assisted analytic approaches have been introduced in the field of biomedical image analysis. This has led to the evolution of very successful CAD (computer aided diagnosis) tools. Most of the current pathological diagnosis is based on the subjective analysis and decision of pathologists. The need for quantitative image-based assessment and analysis of digital pathology-slides is very important for understanding the cause and effect relationships between the plausible reasons for the disease, its diagnosis, and possible treatments within the clinical operations. This kind of approach opens our eyes toward the biological mechanism of the disease development process. Pathological image analysis mostly focuses on the automated analysis of cytology images, which are the most commonly applied for biopsy screening mechanism. Furthermore, cytological images are easier to analyze because of the reasons like absence of complicated entities like veins, tubules, and glands and presence of isolated cells, multinucleated cells (giant cells, in the case of COVID-19 patients), and cell clusters, which often share a common chromatic flavor. A good example is segmenting the individual cells and nuclei within an image, which can be done quite easily, as most of the cells are inherently separated from one another. Certain histological structures like glands, nuclei of cancer cells, subsegmental pulmonary embolism in SARS CoV-2 infections, and other lymphocytes have to be identified as a prerequisite for clear grading and diagnosis of diseases from their histopathological images. Appearance of these kinds of structures, their size, shape, extent, and other morphological characteristics are very important clues for the presence and severity of the disease. For example, a high percentage of lymphocytes in breast cancer histopathology strongly indicate a low disease outcome. The size and extent of the glands in prostate cancer reduce with a increasing Gleason patterns indicating the severity of the disease condition. For first identifying and then classifying any disease either globally or locally, automatic identification of the structures within the histopathological image is an essential requirement. One of the upcoming challenges in the digital pathology is multimodal data fusion or registration (MDF or MDR). MDF is one of the preliminary stage of biomedical image processing. Different external conditions, varying instrument resolutions, calibrations and errors over different time, place and circumstance have to be neutralized and transformed into a common platform before giving them as input to any architecture or algorithm. But the highly dense, rapidly growing volumes of medical “Big-data” poses many problems. There are many open question like “will the advanced multimodal data classifiers be able to make recommendations of therapies concerned with specific diseases?” For this to happen, the knowledge representation for heterogeneous data fusion and data alignment techniques have to answer many questions beyond just diagnosis like “theragnosis” (prediction of therapy) and prognosis (understanding or forecasting the likely course of a medical condition).
Another important aspect that requires immediate attention is the wastage of human and machine time while processing the huge test data available, which is growing exponentially day by day. It is reported that approximately eight out of the 10 biopsies performed in the United States yearly are benign. The largest datasets that are procured on a routine basis are high resolution, high volume chest CT scans of around 134 million voxels. A digitized tissue of prostate biopsy at a nominal resolution (around 40) is approximately 225 million pixels. This adds to the fact that a single prostate biopsy comprise between 10 and 20 biopsy samples, that is, 2 to 4 billion pixels of data generated per person in the test. Moreover owing to their large content and size, these images need to be processed in a multi-resolution processing system. In the arena of radiology, computer assisted diagnosis mainly deals with grayscale images. But when it comes to histological CAD, systems need to process color images most of the time. Moreover, with the recent technological stride of hyperspectral and multispectral imaging, each pixel within an image storing histopathological data can have several hundreds of wavelengths and sub-bands.

Color image pre-processing and image normalization have received great attention these days. A robust color-based segmentation algorithm was developed by previous studies and Foran especially for histological structures that used LUV color space (image gradients) to deal with some issues of stain variability. The most important motivation for detecting and segmenting pathological image structures is to automatically count the objects generally nuclei or lymphocyte cells, which itself have good diagnostic significance for certain conditions of cancer and to detect the change in size of cell-structures like perivascular lymphocytic inflammation in case of COVID-19 patients (Table 1). Another widely used object detection and classification algorithm—YOLO—is also used for automatic counting and identification blood cells. Alam and Islam (2009) build the YOLO framework using the backbone of neural networks (NN) in order to detect an object like platelets, which has been exposed to the system during training. Prabakaran et al. (2015) used the technique of circular Hough-transform for automating the platelet count. It also detects circular objects by computing the minimum and maximum radii of cells. Dey et al. (2015) applied color segmentation technique for extracting platelets, using a color conversion block to transform the input image into “L.a.b” color space, after binarization extracted the number of platelets. Poornima and Krishnaveni (2016) used morphological operations like edge detection using Sobel filters and statistical techniques like histogram equalization. Another paper by Roy et al. (2016) introduced a telemedicine compatible android app-based segmentation and counting tool. Mahesh et al. (2017) applied K-means clustering, thresholding in-order to isolate all the platelets. Applying the grouping technique of 8-neighborhood connected pixels he counted their number as well.

| Case | Test biopsy images          | Disease               | K | PSNR   | SSIM   | Otsu_u | Otsu_v | Δk  |
|------|-----------------------------|-----------------------|---|---------|---------|--------|--------|-----|
| 1    | Hyaline membranes           | COVID-19              | 5 | 1.9678  | 0.3418  | 0.5882 | 0.4784 | 0.1098 |
| 2    | Fibroblast proliferation    | COVID-19              | 5 | 1.9712  | 0.6485  | 0.5882 | 0.4784 | 0.1098 |
| 3    | Periavascular lymphocytes   | COVID-19              | 5 | 1.643   | 0.8579  | 0.6698 | 0.634  | 0.279  |
| 4    | Multinucleation             | COVID-19              | 6 | 3.2265  | 0.818   | 0.614  | 0.5224 | 0.2311 |
| 5    | Busulfan                    | COVID-19              | 4 | 1.8044  | 0.3697  | 0.5190 | 0.6487 | 0.0961 |
| 6    | Amiodarone                  | COVID-19              | 5 | 0.8522  | 0.2846  | 0.5226 | 0.6532 | 0.0920 |
| 7    | Metaplasia                  | COVID-19              | 3 | 2.3451  | 0.2743  | 0.5678 | 0.7098 | 0.0392 |
| 8    | Eosinophils                 | COVID-19              | 3 | 4.1699  | 0.4349  | 1.0153 | 1.2691 | 0.0428 |
| 9    | Endomyocardial              | COVID-19              | 6 | 1.7179  | 0.2061  | 0.5804 | 0.549  | 0.0314 |
| 10   | lymphocytic fulminating myocarditis | COVID-19 | 4 | 1.7107  | 0.2637  | 0.5608 | 0.4627 | 0.098  |
| 11   | Renal capillary congestion | COVID-19              | 4 | 2.2016  | 0.3465  | 0.5725 | 0.4157 | 0.1569 |
| 12   | True myocarditis            | COVID-19              | 5 | 1.0568  | 0.2016  | 0.5647 | 0.4627 | 0.102  |
| 13   | Lumina ectasia              | COVID-19              | 4 | 5.2917  | 0.197   | 0.5569 | 0.5098 | 0.0471 |
| 14   | Mononuclear infiltration    | COVID-19              | 5 | 1.4162  | 0.2991  | 0.6373 | 0.7966 | 0.0917 |
| 15   | Pertibular capillaritis      | COVID-19              | 3 | 3.0514  | 0.4959  | 0.4760 | 0.5950 | 0.0873 |
Bibbo et al.\textsuperscript{22,23} found 1.1\%–4.7\% error in the Feulgen-stained prostate specimen’s cell counts as compared to manual counts. Belien et al.\textsuperscript{24,25} reported 19\%–42\% error in counting mitoses cells in Feulgen-stained sections of breast tissue. Markiewicz et al.\textsuperscript{26} found 2.8\%–10.0\% difference in counts between manual and automatic methods in immunohistochemically stained bone marrow biopsies. Around the same time, Kim et al.\textsuperscript{27} reported a correlation of 0.98 between automatic and manual counts of the immune stained slides of meningiomas. Sont et al.\textsuperscript{28} reported a 0.98 correlation between semi-automated and automated methods in counting inflammatory cells of the stained bronchial tissue.\textsuperscript{29} (Cancer image reference database, National Cancer Center Japan)

In this manuscript a novel architecture termed as HISTAK—a simplified blind or automatic segmentation technique and its high performance hardware architecture—has been identified not only to speed up but also to ease the process of diagnosis as well as prognosis of many COVID-19 cases.\textsuperscript{30} This is achieved by combining both the K-means clustering and HST—an improvement over the well-known Otsu’s thresholding method. This technique is applied on many biopsy database images of COVID-19 cases taken from open-source cancer image reference database of COVID research database.\textsuperscript{31} It can be easily perceived that this technique neither requires any user interaction nor any prior knowledge for conducting segmentation process. The results (PSNR, SSIM, and qualitative analysis) clearly reveal the usefulness of this technique in quick cytological evaluation. Moreover, the new method has been giving promising results in terms of its software execution, which is 18\% faster than existing techniques, as well as hardware implementation which is 68\% faster than its software implementation. In this work, the performance is given utmost importance keeping satisfactory accuracy in terms of PSNR and SSIM quality metrics.

The main contribution of this manuscript lies in the fact that it improves the K-means hardware architecture first proposed in 2016 by Rahul and Nanda,\textsuperscript{32} applied for real-time satellite image segmentation in 2018 by the same authors,\textsuperscript{33} to become a standalone system, to find the number of segments within an image based on a simplified technique which computes the cluster number “K” using histogram analysis. This architecture is specialized for segmenting COVID-19 biopsy images.

The organization of the manuscript is elaborated below. The fundamentals COVID-19 detection and analysis in histopathological images is explained in Section 2. Section 3 describes the proposed methodology-HST for retrieving “K” for the high speed segmentation of the biopsy images. Section 4 explains the hardware implementation of the proposed HISTAK architecture, its challenges and steps utilized for segmenting of the histopathological images. Section 5 describes the result and discussions along with their detailed validation. This section explains the quantitative and qualitative validation the resultant segmentation results. Section 6 describes the hardware implementation results and comparison with existing works, before concluding in the Section 7.

2 \mid BACKGROUND

2.1 \mid Histopathological analysis of COVID-19

The field of histopathology involves the detailed investigation of biological cells and tissues in order to examine and observe the presence of mutated, infected or diseased tissues through a microscopic arrangement. Histopathology usually constitutes a process of biopsy undertaken by a medical expert called as pathologist, involving procuring a small sample of cells from the subject for detailed observation and imaging. In order to obtain a comprehensive review of the examined tissues, image segmentation of the histopathological slides are very much effective, considered as “Gold standard” in many disease diagnosis.\textsuperscript{34} The process of histopathological image segmentation constitutes the following steps. (i) The frequency distribution of each pixel value is found and plotted as a statistical chart termed as histogram. (ii) The threshold calculation: It is a mathematical procedure where the statistical parameters relating to the pixel values like mean, median, and deviation are extracted from the image histogram. The Luv format is a three dimensional vector which gives the Luminosity vector, “u” and “v” chromatic layers of the image. The “L” Luminosity vector is discarded as it doesn’t contain any chromatic components. The histogram charts have to be plotted for both “u” and “v” dimensions. (iii) Histogram analysis (HST): The crests (maxima) and troughs (minima) can be determined from the histogram of the histopathological image, which in-turn determines the “K”-number of possible clusters or segments. Most of the gray level images are segmented in the similar manner. Any algorithm when applied on to a test image may result in the quality degradation of the image. Then one have to re-evaluate the image quality. Qualitative evaluation techniques are those methods which are based on the human judgment, so they are devoid of any mathematical intricacies. Methods using numerical parameters to compute the quality of the image are termed as quantitative evaluation methods.
2.2 Need for biopsy in diagnosing COVID-19 and other chronic diseases

A biopsy is a procedure to remove a group of sample cells or a piece of tissue from your body in order to get analyzed in a medical laboratory as shown in Figure 1. Under the consultation and prescription of a doctor, biopsy is usually done to check the presence of some medical conditions or diseases like coronavirus, cancer, and tumor. Usually, imaging tests can detect abnormality but won’t be able to confirm the presence or absence of cancerous cells. Definitive diagnosis of cancer can only be confirmed by a biopsy process. Various types of biopsy procedures include needle biopsy, skin biopsy, endoscopic biopsy, surgical biopsy, and bone marrow biopsy. In COVID-19, sample cells are taken from throat and inner nasal cavities of the patient.

2.3 Biopsy result analysis

Once the sample is sent for analysis, it may be processed or chemically modified depending upon the type of biopsy (Figure 1). The slices of that sample are then placed on a transparent medium which are in turn stained for the need for enhancing their contrast and clarity. The final process is examining the samples under a microscope. To a doctor or an expert medical practitioner, the results of biopsy can reveal many important details like severity of tissue/organ damage/stage of COVID-19 infection, whether cancerous cells are present or not, if affirmative what kind of cancer is it, where is it originated and the level of growth and its aggressiveness (represented by a scale of 1 to 4——1 being the initial stage of Corona or low grade cancer growth and 4 being advanced stage of putting patient in ventilators like for extreme COVID-19 cases or grade-4 of cancer). Specific treatment choices have to be followed depending on this critical information. The biopsy results are available in a few days. But in exceptional cases like surgeries, the pathologist after proper examination of the samples, the result is released to the concerned surgeon in a few minutes itself.

2.4 Image segmentation as a blind-clustering problem

Image Segmentation is an important step in image-data processing in which an input image is divided either partially or completely into parts that have good correlation with objects or areas of the actual world contained in the image. Clustering is an unsupervised optimization problem where, given a set of unlabeled feature vectors, we are attempting to group them into their natural clusters. The data points within a cluster are similar and data points from different clusters are dissimilar with respect to the distance criteria, mostly Euclidean (Minkowski distance metric with $p = 2$, as given in Equation 1).

$$D(X_y, X_z) = \left( \sum_{l=1}^{d} (X_{yl} - X_{zl})^{1/p} \right)^p$$

$$Variability(i) = \left( \sum_{x_j \in i} D(\mu(i), x_j)^2 \right)^{1/2}$$

FIGURE 1 The initial stage of biopsy analysis, sample procurement, and preparation for analysis
Here, the variability is different from Variance, which can be thought of as a normalized form of variability, where it will be divided with the number of data points within a cluster. Equation (2) gives the variability of a cluster $i$, where $x_i$ and $x_j$ represents data points of cluster, whose centroid is expressed as $\mu_i$. The idea of not normalizing or preferring variability to variance is to penalize big clusters with a lot of diverse points and at the same time favoring small clusters, even though they may be having a lot of intrinsic variance. The objective of a perfect ideal clustering algorithm must be to choose a set of centroids which will minimize the variability, as given in Equation (3), where $I$ denotes the superset of all $i$.

$$\text{Minimize} \langle \text{Disimilarity}(I) = \sum_{i \in I} \text{Variability}(i) \rangle$$  \hspace{1cm} (3)

As illustrated in Equation (4), an image segmentation can be mathematically expressed as a union (U) of “k” subregions.

$$S_1 + S_2 + \ldots + S_k = \sum_{i=1}^{k} (S_i) = 1$$  \hspace{1cm} (4)

a) Within a region the set of points (pixels) are forming a close cluster with respect to their chromatic value (Equation 5).

$$\text{Variability}(x_i \in S_i, x_j \in S_j) \to 0, \ \forall i = 1, 2, \ldots, k.$$  \hspace{1cm} (5)

b) Given two adjacent regions $S_i$ and $S_j$, where the variation of the pixel intensity across their common border is lower than a finite threshold value $\gamma$. They are combined to form a bigger cluster or segment $S_p$, where $S_p$ forms the superset of regions $S_i$ and $S_j$.

$$\partial(S_i, S_j) < \gamma, \text{ then } (S_i \bigcup S_j) \subset S_p$$  \hspace{1cm} (6)

c) All the points within an individual segmented region follows the characteristic $\Omega$,

$$\Omega(S_i) = 1, \ \forall i = 1, 2, 3, \ldots n$$  \hspace{1cm} (7)

d) The $\Omega$ of the two distinct adjacent regions must vary.

$$\Omega(S_i \bigcap S_j) = 0, \ \forall \text{ adjacent regions } (S_i) \text{ and } (S_j)$$  \hspace{1cm} (8)

In general, there are two types of processing levels used in image segmentation using machine learning.

1. **Higher Level Processing** where the specialized knowledge of the task under consideration is extremely essential to achieve a perfect theoretical segmentation.

2. **Lower Level Processing** here in-depth prior knowledge is not required before executing the segmentation task. Some examples are segmentation problems consisting of bright illuminated objects contrasting from their plane backgrounds like Red Blood Corpuscles (RBCs) in blood fluid, multiple diffuse alveolar damage in patients infected by SARS CoV-2 segmented by the presence of intra-alveolar fibrin, hyaline membranes, or loosely organized connective tissue in the alveolar-septal-walls, printed alphabets or numbers, images of celestial illuminating bodies, and so on. In these examples, end results of segmentation can be easily obtained with good accuracy, without requiring much information about the possible expected output. This manuscript proposes a unique hardware architecture for automatic segmentation mechanism applying lower level processing where the input image is distributed into distinct layers based on their chromatic features. Fifteen test cases comprising COVID-19 histopathological images (biopsy) procured from open-
source image repositories and cancer image reference database of National Cancer Center, Japan and a previous study are input to the hardware architecture model and tested to access their quantitative and qualitative quality metrics for accurate diagnosis of infection spread in the case of corona or pneumonia infection, and so on.

**Algorithm 1 Proposed algorithm for biopsy analysis**

1. **for** every new Biopsy Image **do**
2.    Input-1 $I_{RGB}$: Histopathological Image to be segmented (Eq.: 6 to 8)
3.    Input-2 $i_{\text{max}}$: total number of pixels within a band.
4.    Input-3 $\text{iteration}_{\text{max}}$: maximum iterations.
5.    Input-4 $Q_{PSNR,SSIM}$: Image Quality thresholds
6.    Output: Cluster Labels $i$ to $n$ corresponding to all pixels, cell centroids, cell radii, parameter
7. **for** Pre-Process RGB to Lab $I_{\text{map}}$ **do**
8.    (i) use conversion matrix
9.    (ii) multiply with RGB matrix
10.   (iii) Discard $L$ vector.
11.   **end for**
12. **for** $\text{Quality}(PSNR,SSIM) \leq \text{Quality}_{\text{threshold}}$ **do**
13.    Statistical Analysis, compute Threshold parameter $\sigma$ (Eq.: 9 to 17)
14.    find $\Delta k$ and compare it with pixel intensity variance (Eq.: 18)
15.    Variance separable criteria is applied to find $k_{uv}$. (Eq.: 19 to 25)
16.    Perform $k$-Means clustering using number of clusters $k$.
17. **end for**
18. find the number of islands within each cluster using tracing and grouping the neighborhood of pixels.
19. cluster/segment with maximum islands is the segment of Interest- Detection of cell clusters.
20. find the centroid, radii, perimeter of each island.
21. Validate the results: Perform Quantitative and Qualitative analysis along with expert validation.
22. Output: COVID-19 Final Diagnosis, given the captured features.
23. **end for**

3 | PROPOSED METHODOLOGY FOR HISTOPATHOLOGICAL IMAGE SEGMENTATION

This manuscript proposes a simple, efficient, and high speed methodology and architecture-“HISTAK” for histopathological image segmentation, which is represented in Figures 2, 3, and 4. This method neither requires any user interaction nor any prior knowledge or learning to automatically segment, count and encircle the distinct features like multinucleated-cells, cytoplasm, pulmonary embolisms, and tumors very quickly, so that the image is ready for final decision. The images procured from the Bbiopsy databases of hospitals, have to be pre-processed first. The format of the raw image is usually png or jpeg. So the first step is to convert input histopathological image to a “Luv” image format. Any input dimension which doesn’t yield some kind of chromatic information is to be discarded, as it simply constitutes noise. Hence, we discard the luminosity matrix “L” of the “luv” format image.

$$P_u(k) = \sum_{i=0}^{k} P_{ui}$$  \hspace{1cm} (9)

$$P_v(k) = \sum_{i=0}^{k} P_{vi}$$  \hspace{1cm} (10)
Then “u” and “v” dimensions of the image are utilized to extract the number of possible clusters in the image using a “histogram separation technique”—an improvement over the famous Otsu’s thresholding method, as explained in Figures 2, 3 and 4. On finding the possible number of clusters K, the image is then segmented with respect to its chromatic characteristics, explained in Section 5. After completing the clustering process using the model, we proceed with the quantitative validation. The essential step of quantitative validation involves computing the M-SSIM and PSNR of the resultant clusters with respect to the number of clusters “k.” The procedure concludes with a qualitative analysis based on the true features. Next section describes the step by step proceeding of the mathematical extraction of the statistical parameters. The input Histopathological image is converted into the “Luv” format. Discard the L-matrix which doesn’t give us any chromatic information. Both the “u” and “v” layers of the “LUV” image resemble a pair of dependent images expressed in grayscale. The histogram represented by each of the two grayscale image is plotted, from where the parameters like $p_{ui}(k)$ and $p_{vi}(k)$ are found. $p_{ui}(k)$ and $p_{vi}(k)$ denotes the histogram derived parameters of u
and v dimensions from ‘i=0 to L-1’. \( P_u(k) \) and \( P_v(k) \) denotes the cumulative sums derived from \( p_{ui}(k) \) and \( p_{vi}(k) \), for “k=0 to L-1” which is computed for both dimensions. Also find the cumulative means (average intensity), \( m_u(k) \) and \( m_v(k) \), for k=0 to L-1 for both the “u” and “v” layers of the “LUV” image.

\[
m_u(k) = \sum_{i=0}^{k} i \cdot p_{ui}
\] (11)

\[
m_v(k) = \sum_{i=0}^{k} i \cdot p_{vi}
\] (12)

Calculation of \( m_u(k) \) and \( m_v(k) \), the global intensity mean has to be carried out afterwards.

\[
m_{Gu}(k) = \sum_{i=0}^{L-1} i \cdot p_{ui}
\] (13)


\[ m_{Ci}(k) = \sum_{i=0}^{L-1} i \cdot p_{ci} \]  

(14)

Calculate the class variance for “u” and “v” images for \( k = 0 \) to \( L-1 \).

\[ \sigma_{Bu}(k) = \frac{[(m_{Bu}p_u(k) - m_u(k))^2]}{P_u(1 - p_u(k))^2} \]  

(15)

\[ \sigma_{Bv}(k) = \frac{[(m_{Bv}p_v(k) - m_v(k))^2]}{P_v(1 - p_v(k))^2} \]  

(16)

From these existing parameters extract the \( k_u \) and \( k_v \), thresholds derived from Otsu’s method, for both “u” and “v” dimensions for which \( \sigma^2_{Bu}(k) \) is highest. Finally find the variance separation index \( \Delta k = |k_u - k_v| \) and correlate the same with their PIVs (Pixel Intensity Variance) \( \sigma^2_{uv}(k) \), obtained from the combination of “u,” and “v” dimensions (u-v), where is expressed by the final equations 11 to 17. Equations (18) to (25) are a contribution of this proposed architecture derived from the indepth analysis of a large number of image data sets pertaining to biomedical and other multimedia applications. Experimental observations helped in fine turning these parameters used in these equations. It has been experimentally found that these parameters work well with satellite and multimedia images, as well.

\[ \sigma^2_{uv}(k) = \frac{1}{2MN} \sum_{t=0}^{2MN-1} (x_{uvt} - \mu_{uv})^2 \]  

(17)

\[ \Delta k = |k_u - k_v| \]  

(18)

If these thresholds (\( otsu_u \) and \( otsu_v \) in Figures 2 and 3) are widely separated (i.e., \( \Delta k > 0.2 \)), then two means between them are computed for getting two new thresholds \( k_u \) and \( k_v \) as given by Equations (19) to (21), as shown in Figure 4A, thus dividing the image into 6 clusters. If they are closely placed (i.e., \( 0.15 < \Delta k < 0.2 \)) compute their mean to obtain a single threshold, for the whole image thus dividing the image into 5 clusters, whose thresholds are found using Equations (22) and (23) as shown in Figure 4B. Else if \( 0.1 \sigma^2_{uv}(k) < \Delta k < 0.15 \sigma^2_{uv}(k) \), then use both initial thresholds \( k_u \) and \( k_v \) to divide the image into 4 clusters, with the mean threshold given by Equation (24), as shown in Figure 4C. If \( 0.05 \sigma^2_{uv}(k) < \Delta k < 0.1 \sigma^2_{uv}(k) \), then there will be 3 clusters with the same \( k_u \) and \( k_v \), as shown in Figure 4D. And finally, if \( \Delta k < 0.05 \sigma^2_{uv}(k) \) only two clusters are to be considered, as shown in Figure 4E. Finally, both the PSNR and M-SSIM quantities are tabulated to derive quantitative inference.

Calculation of the new cluster means for thresholding.

(i) When segmenting into three clusters, condition: \( \sigma^2_{uv} < \Delta k < 0.1 \sigma^2_{uv} \). Use the same \( k_u \) and \( k_v \).

(ii) When segmenting into six clusters, condition: \( \Delta k > 0.2 \sigma^2_{uv} \).

\[ k_{uv2} = \frac{k_u + k_v}{2} \]  

(19)

\[ k_{uv1} = \frac{k_u + k_{uv2}}{2} \]  

(20)

\[ k_{uv3} = \frac{k_v + k_{uv2}}{2} \]  

(21)

(iii) When segmenting into five clusters, condition: \( 0.15 \sigma^2_{uv} < \Delta k < 0.2 \sigma^2_{uv} \).
\[ k_{uv1} = \frac{k_u - k_v}{3} \]  
\[ k_{uv2} = \frac{2(k_u - k_v)}{3} \]  

(iv) When segmenting into four clusters, condition: \( \sigma_{uv}^2 < \Delta k < 0.15\sigma_{uv}^2 \),

\[ k_{uv1} = \frac{k_u + k_v}{2} \]  

(v) When segmenting into two clusters, condition: \( \Delta k < 0.05\sigma_{uv}^2 \),

\[ k_{uv} = \frac{k_u + k_v}{2} \]

4 | PROPOSED ARCHITECTURE FOR AUTOMATIC IMAGE SEGMENTATION

The proposed architecture shown in Figure 5, is an efficient re-configurable architecture for blind segmentation, cell counting and retrieving the cell parameters like radii, area, and perimeter has been identified not only to speed up, but also to ease the process of diagnosis as well as prognosis of COVID-19. This is achieved by combining three algorithms: the K-means algorithm, a novel statistical analysis technique-HIST (histogram separation technique) an improvement over the well-known Otsu’s method, and a islanding method an improved version for CCA algorithm (blob detection technique). The proposed method is applied on eight chronic respiratory disease cases of COVID-19 taken from high profile hospital databases. Proposed method neither requires any user interaction nor any prior knowledge or learning to automatically segment, count and encircle the distinct features like multinucleated-cells, cytoplasm, pulmonary embolisms, and tumors very quickly, so that the image is ready for final decision. The output in terms of quantitative...
parameters like PSNR, SSIM as well as qualitative analysis, clearly reveal the usefulness of this technique in quick cyto-
logical evaluation. The following are the notable features of the proposed architecture. This architecture parallelizes the
image-frame computations using re-configurable logic (Figure 6). FPGAs can be used to accelerate real-time image
segmentation problems by employing digital signal processing simplification techniques. The FSM dealing with this
architecture composed of a Moore machine with 15 stages. Here, all the data bus lines composed of data-bus with a
width of 32-bits. Initially, the histogram analyzer will accept the image data and calculates the possible number of cen-
troids or clusters for the same image. The rest of the hardware comprises that of a reconfigurable clustering machine
whose labeled output is displayed in a VDU, after proper formatting. The input to this system includes: data points,
maximum number of data points “N” and maximum number of iterations. The block diagram explains the HISTAK architecture for thresholding process, shown in Figure 7. Because the
image resolution is varying as per the biopsy image and each pixel has 8-bits for each U and V dimensions, the input
constants has to be adjusted according to the biopsy image. The histogram sub module independently analyses the
whole pixels within the image after equally dividing and grouping them into units and combines their results in the
synthesis stage. The main unit accumulates the temporary results to carry forward them into cumulative histogram and
intensity area modules as shown in Figures 7, 8, and 9.

FIGURE 6  Stage-wise execution of the histogram analyzer module

FIGURE 7  Proposed hardware architecture of thresholding process in HISTAK
**Figure 8** Detailed block diagram of FIFO memory module used within the architecture of HISTAK thresholding module

**Figure 9** Detailed architecture of the histogram analyzer of HISTAK architecture

**Figure 10** State diagram of the FSM controller of HISTAK
Through the image sensor and capture module, the biopsy image is converted into a set of arrays as per its pixel intensity and depth and stored within the memory module. The statistics module acts as the primary processor for the histogram analyzer. The output of the statistics module is passed to the variance computation module, whose output in turn is connected to the threshold processing module, as shown in Figure 9.

The hardware implementation of the architecture proposed in this manuscript—HISTAK, the implementation is shown in Figure 7 to Figure 11. FPGA is the central controller which controls and coordinates all other parts of the system. The complete initialization of the image sensor configuration through the I2C module, the frame rate selected should be optimal, parameters like exposure-time of the CMOS cameras also have to be experimentally set for better performance. After receiving the initiating signal to start, input n x m pixel frames from the CMOS image sensor will go through the capture module as shown in Figure 9. and then the signals are allowed to enter statistical module in a parallel fashion, to extract the pixel intensity distribution of the captured image after get the image intensity data before it is send to HST unit.32,33

Histogram separation unit consists of a pair of modules: Statistics module and Calculation module (shown in Figure 11). While sending intensity data into Otsu module, it has to be stored in the memory module. The statistical modules constitute of a set of parallel histogram analyzers which executes Equations (9) to (25). Implementation of calculation module is shown in Figure 6. Two dividers and 3 multipliers are designed to achieve the function of pixel computation within the HST module, which is replicated in the many parallel modules. In order to get the best performance, Xilinx library pre-configured logical blocks and IP cores (of multiplier and divider) has been tried out, which reduced the design time and has served optimally for all practical purposes.32,33,36

Memory module, shown in Figure 8, is used for reading and writing the data in memory banks made of high speed SDRAM, whose reading and writing operations can be switched alternatively. Once the effective threshold is received from HST module for the current frame, the intensity data stored in memory module, is compared the data in the previous frame inside comparison module, the result is displayed in the VGA unit.41

4.1 Blob analysis

It is a single pass eight way connected component labeling algorithm and perform blob analysis. It is nothing but a machine vision framework for detection and analysis of pixels connected together within a vicinity called blobs. In the initial phase labeling is performed, and after that more accurate labeling is done in the upcoming phases. The Blob analyzer module labels each pixel in the input pixel array, flags the inter-connected areas, and computes the centroid, radii, area, and bounding circle for all detected blobs. The final output is the labeled pixels, which are stored in RAM. The signal “dataready” is asserted indicating that the FSM has reached its final state, thus the output statistics are ready. Refer Figure 11. Our initial work was automatic image segmentation using a new method HISTAK. The importance of the work is in its simple architecture and good performance, compared to existing techniques with respect to both hardware and software implementation, tested on COVID-19, and other pulmonary infections. The delay in diagnosis of
many diseases have caused huge losses in money, time, delay in law enforcement and justice, which was very well exemplified as well as illustrated during the past COVID-19 pandemic. In order to improve the functionality of our architecture, during the revision we added another module for finding the pixel islands within the individual segmented clusters, the cluster with maximum islands always consists of cells, whose various parameters like count, radii and are stored in memory. This was implemented initially using MATLAB 2022 functions like regionprops(), blobanalysis() which worked based on CCA algorithm, whose hardware architecture we could implement using HDL generation tools (HDL coder). All other modules have been hand coded in Xilinx Vivado using Verilog HDL. Therefore, this latest islanding module (Figure 11) is not as hardware efficient as we desired it to be, making it the weakest chain in the link, as far as performance is concerned. The interfacing modules connected external to the Blob analyser module reads the statistics using input port addresses. It is a software by design, which aggregates the result and displays it on to the variable matrix. The following registers were used in MATLAB 2022 to complete the blob analyser subsystem “GradThresh,” “AreaThresh,” “CloseOp,” “VideoMode,” and “blobIndex.” The output signals are “indexo,” “numo,” “totalNumo,” and “datareadyo.” The illustration of the islanding technique has been given in Figure 12.

4.2 Control path of proposed HISTAK architecture

The control path of the proposed architecture is designed using a Finite state mealy-machine, as illustrated in Figure 10. The machine starts execution with the signal “Go.” Then the user have to enter the number of elements, that is, the number of pixel elements which have to be clustered or segmented, represented by \( N_{ld} \). Then the machine reads the 8-bit pixel elements stored in the device memory or file into its RAM module after triggered by the control signals \( hst_{ld} \) and \( med_{ld} \). From here, the machine needs an initiation by signal \( “ld_hstl” \) is initiated for matrix summation “dp_{ld}” and “hst_{ld}.” “p_{ld},” “puv_en,” “pu_pv_en” are needed for starting the calculation of \( P_u, P_v \) and \( P_{uv} \). Combinational logic for the histogram separation chart will be activated by the “\( delK_en \)” and “\( delK_en2 \).” Then there is a WAIT state for synchronization and extra delay for completing the calculation. \( disp_en \) and \( Out_en \) are required to activate or display the output. The control path (unit) initiates the start of the iteration with the “Go” signal. The detailed flow of the control path is illustrated in Figure 10 using the state diagram of the Moore machine.

5 RESULTS AND DISCUSSIONS

The results of this simplified automatic segmentation technique, introduced for histopathological images (RGB images) for 15 COVID-19 cases (contributed by Toru Igari, Jin Takahashi, and Shinyu Izumi31,39 as given in the Table 1. Qualitative and Quantitative validation in terms of the efficiency and speed of the proposed method are shown in the Tables 1 to 4, Table 6 and Table 7. The overall histogram analysis of the individual biopsy images (as shown in Figures 13 to 41) and processing has been identified to ease the process of diagnosis as well as prognosis of COVID-19. It can be easily perceived that this technique neither requires user interaction nor any prior knowledge to automatically segment the
cells, cytoplasm, and other features within the histopathological image in a very less time (as shown in Figures 13 to 41). After that, all the segments are ready for recognition. Quantitative results in terms of PSNR, SSIM and their histogram analysis clearly reveal the usefulness of this technique in cytological evaluation, as shown in Tables 1–7. This technique can provide a faster, efficient and an economical alternative to the prevailing costlier tests and scans for medical diagnosis and prognosis.

5.1 | COVID-19 case studies

Case 1:

Hyaline membrane deterioration is an acute lung injury resulting from COVID or other infections, a pathologic correlate of the neo-natal Respiratory affliction. Another reason can be reduction of surfactant can be a reason for
inadequate resorption of lung liquid at birth of a neo-natal leading to a dilutional deficiency. Surfactant deficiency results in an increased alveolar surface tension, with subsequent resistance to inflation and alveolar collapse at the end of expiration. Thus the alveoli get injured, because of the shear stresses on the walls of alveoli. Mechanical-ventilation pressures or huge abnormal respiratory effort can also aggravate its severity. This may also lead to DAD, similar in appearance to that observed in adult cases of SARS (severe acute respiratory syndrome) or acute respiratory distress syndrome. Figure 13A shows the lung biopsy result of a patient infected with SARS CoV-2 virus. Figure 13B–F gives the results of segmenting Figure 13A using HISTAK. Cluster-1 represents the cytoplasm and fluids, cluster-2 captures the blood fluid part (RBCs) and cluster-4 captures all cells within it, cluster-3 and 5, shows the loosely organizing connective hyaline tissues in organizing DAD. The histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 14. Clustering of the pixel distribution of the Hyaline membrane of a patient infected by SARS CoV-2 is shown in Figure 21A, the x-axis represents u-matrix and y-axis denotes the v-matrix.
Case 2:

Figure 15 shows the lung biopsy result of a patient infected with COVID-19. These histopathological descriptions of organizing pneumonia stated that the initial intra-alveolar material, initially consisted of fibrin, further colonized by fibroblasts and replaced by a fibrillated connective tissue. Intra-alveolar buds of granulation tissue consisting of intermixed myo-fibroblasts, fibroblasts, and connective matrix, especially consisting of collagens are the hallmark of organizing pneumonia resulting from the SARS CoV-2 infection.

Figure 15A shows the lung biopsy result of a patient infected with SARS CoV-2 virus. Figure 15B–F gives the results of segmenting Figure 15A using HISTAK. The Histogram plot of the u-v matrix derived from the “Luv” format of histopathological image-Case 3: Periavascular lymphocytes of a patient infected by SARS CoV-2. F is the frequency, I is the pixel intensity. (A) Histogram of u-matrix, (B) histogram of v-matrix, (C) histogram of u-v matrix, combined

Case 3:

Fibroblasts play a key role in tissue healing by producing the majority of extracellular matrix components (ECM), favoring granulation tissue formation, and stimulating re-epithelialization. Hyaluronan is a component of ECM and its
FIGURE 19  The clustering results of Case 4: Multinucleation of a patient infected by SARS CoV-2.

FIGURE 20  Histogram plot of the u-v matrix derived from the “Luv” format of histopathological image-Case 4: Multinucleation of a patient infected by SARS CoV-2. F is the frequency, I is the pixel intensity. (A) Histogram of u-matrix, (B) histogram of v-matrix, (C) histogram of u-v matrix, combined

FIGURE 21  Clustering distribution of the (A) Case 1: Hyaline membranes of a patient infected by SARS CoV-2. (B) Case 2: Fibroblast proliferation of a patient infected by SARS CoV-2
**FIGURE 22** Clustering distribution of the (A) Case 3: Periavascular lymphocytes of a patient infected by SARS CoV-2. (B) Case 4: Multinucleation of a patient infected by SARS CoV-2

**FIGURE 23** Clustering distribution of the (A) Case 5: Drug toxicity secondary to busulfan, (B) Case 6: Amiodarone toxicity

**FIGURE 24** Clustering distribution of the (A) Case 7: Bronchiolar metaplasia, (B) Case 8: Dense infiltration by eosinophils
anti-inflammatory effects and properties in enhancing wound closure have been deeply studied. Figure 17A shows the lung biopsy result of a patient infected with SARS CoV-2 virus. Figure 17A–F gives the results of segmenting Figure 17A using HISTAK. It is interesting to find that cluster-5 Figure 17F captures all the cells in “dark magenta”
color, whose surrounding fluids clustered in Cluster-4 in “rosewood” color, Figure 17E. Clusters-1 to 3, as shown in Figure 17B–D shows the boundary, inner layers and plasma respectively. The histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 18. Clustering of the pixel distribution of the Periavascular lymphocytes of a patient infected by SARS CoV-2 is shown in Figure 22A, the x-axis represents u-matrix and y-axis denotes the v-matrix.
Case 4:

Figure 19 shows the lung biopsy result of a COVID-19 patient. MGC (Multinucleated giant cells) originates from the fusion of monocyte-macrophage lineage cells. Formation of these giant cells enhances the defensive capacities of macrophages—is a type of phagocyte, which is a cell responsible for recognizing, engulfing and killing pathogens and apoptotic-cells. Macrophages are produced through the differentiation of monocytes, which turn into macrophages when they leave the blood. Morphologically, MGC are generally classified into foreign body giant cells (FBGC) and Langhans giant cells (LGC). LGCs are normally visible in immune granulomas with the epithelioid macrophages. FBGC formations can be seen in macrophage infusion as a result of a response induced by foreign bodies and biomaterials. Figure 19A shows the lung biopsy result of a patient infected with SARS CoV-2 virus. Figure 19B–G gives the results of segmenting Figure 19A using HISTAK. The cluster-4 shown in Figure 19E reveals the MGC. The RBCs and their boundary are clustered in the cluster-1, Figure 19B and cluster-6, Figure 19G, respectively. The Histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 20. Clustering of the pixel distribution of the pulmonary multinucleation happening in a patient infected by SARS CoV-2 is shown in Figure 22B, the x-axis represents u-matrix and y-axis denotes the v-matrix.

Case 5:

This is a good example of action of chemotherapeutic agents. Cytotoxic-drugs often leads to acute lung damage, as seen in Figure 25. Commonly known agents are bleomycin and busulfan, although carmustine and methotrexate also are connected with the same. Majority of the histologic findings can be characterized by nonspecific changes of Diffuse-alveolar-damage with substantial hyaline-membrane formation. Busulfan-toxicity is characterized by the combination of Diffuse-alveolar-damage with prominent hyperplasia of type-II pneumocytes displaying markedly pleomorphic and enlarged nuclei with prominent nucleoli. The Histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 26, their pixel distribution within the clusters revealed in Figure 23A.

Case 6:

This is a best example of amiodarone, as seen in Figure 27. The β-blocker, amiodarone, used for the control of cardiac arrythmias, is another well-recognized cause of pulmonary toxicity. The pathologic findings comprise, diffuse-
alveolar-damage chronic interstitial-pneumonitis with fibrosis, and organizing pneumonia. A fairly distinctive finding in amiodarone toxicity is the presence of fine cytoplasmic-vacuolation of intra-alveolar macrophages and type-II pneumocytes, as shown in Figure 27A–F. The Histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 28 and their pixel distribution within the clusters is revealed in Figure 23B.

**Case 7:**
Systemic lupus erythematosus (SLE) is a chronic systemic auto-immune disease that can affect the lungs and which can range from acute-lupus-pneumonitis to higher stages of fibrosis, as seen in Figure 29. Pulmonary hemorrhage, Acute lung injury and vasculitis often are associated with SLE. But many patients are in advanced stages with CIP (cellular-interstitial-pneumonia) and different degrees of interstitial-fibrosis. The second is associated with a more favorable prognosis than ALP (acute-lupus-pneumonitis). The sub-acute and chronic manifestations of lupus-pneumonitis manifest with a NSIP (non-specific-interstitial-pneumonia pattern), which is mainly characterized by a CIP with variable interstitial fibrosis, as shown in Figure 29B–E. The Histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 30 and their pixel distribution within the clusters is shown in Figure 24A.

**Case 8:**
This is a case of Chronic eosinophilic pneumonia (CEP). CEP is a condition of unknown etiology characterized by a typical clinical syndrome and radiographic appearance that includes cough, fever, dyspnea and weight loss, with or without peripheral blood-eosinophilia, and bilateral sub-pleural infiltrates with poorly defined margins that can act like migratory infiltrates (disappear spontaneously to re-occur after some duration in the same locations) as seen in...
Figure 31. The radiologic appearance is often linked to photographic negative of pulmonary edema. The disease mostly affects women of 40-50 years, mostly accompanied by asthma or with nasal symptoms. The histologic features are very similar to those of the acute form but some distinction can be made based on the huge number of clinical findings. Normally the mild interstitial pneumonia with hyperplasia of type-II pneumocytes and accumulation of fibrin within alveolar spaces. Macrophages are also seen within the lumen of alveolar spaces. The cytologic atypia of the type II pneumocyte hyperplasia can often be shocking, sometimes simulating VCE (viral-cytopathic-effect). The most crucial
factor of the process, is the dense accumulation of eosinophils admixed with the macrophages within the alveolar lumens (Figure 31B–E). Discrete-eosinophilic micro-abscesses can also be present. The differential diagnosis for the condition is with the Churg-Strauss syndrome, from which it is distinguished histologically by the absence of vasculitis. The condition responds well to treatment with corticosteroids. The histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 32 and their pixel distribution within the clusters is shown in Figure 24B.

Case 9:

The case reveals the acute kidney injury (AKI) to sepsis-AKI (SAKI) due presumed coronavirus disease 2019, see Figure 33. The transcriptomic, morphological and proteomic characteristics of the resultant segmented biopsy were analyzed to find three clusters comprising different cells and cytoplasm as shown in Figure 33B–D. The Histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 40A and their pixel distribution within the clusters is shown in Figure 33I. The part of kidney (renal-cortex) on light microscopy severe tubular injury with lumina-ectasia (hematoxylin,eosin[HE] stain, with magnification-20).
**Figure 37**  Segmentation results of Case 13: Lumina ectasia. (A) Input image, (B–E) segmentation results, (F) pixel distribution after clustering.

**Figure 38**  Segmentation results of Case 14: Mononuclear infiltration. (A) Input image, (B–F) segmentation results, (G) lab-format image, (H) pixel distribution after clustering.
FIGURE 39  Segmentation results of Case 15: Pertibular capillaritis. (A) Input image, (B–D) segmentation results, (E) lab-format image, (F) pixel distribution after clustering.

FIGURE 40  Histogram plot of the u-v matrix derived from the “Luv” format of histopathological images (A) Case 9, (B) Case 10, (C) Case 11, (D) Case 12.
Case 10:
Case 10 lymphocytic fulminant myocarditis is shown in Figure 34. Figure 34B–E shows the segmentation output. The Histogram plot of the u-v matrix derived from the “Luv” format of histopathological images (A) Case 13, (B) Case 14, (C) Case 15

Case 11:
The case of renal capillary congestion is said to caused by the acute kidney injury after coronavirus 2 (SARS-CoV-2) infection. There is an exponentially rising recognition of potential risk of renal-dysfunction within the SARS CoV-2 infected patients, but this can also be caused due to excessive use of harmful sanitizers, vaccines, other chemicals, and

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**TABLE 2**  Comparison of performance of the proposed method with other existing methods

| Algorithm/method | Hand gesture identification technique | Based on Otsu and MoM | Bounding box method | Proposed method | Proposed method |
|------------------|---------------------------------------|-----------------------|---------------------|----------------|----------------|
| Based on         | Software                              | Software              | Software            | Software       | Hardware       |
| Execution Time   | 377 ms                                | 539 ms                | 377 ms             | 450.92 ms      | 232.25 ms      |

**TABLE 3**  Synthesis results: Performance and utilization

| Maximum combinational path delay:                      | 87.504 ns |
|--------------------------------------------------------|-----------|
| Device                                                 | xa7z010clg400-1i, Zinc, Xilinx |
| Slice logic utilization:                               |           |
| (i) Number of slice LUTs                               | 1828 out of 17600, that is, 10% |
| (ii) Number used as logic                              | 1828 out of 17600, that is, 10% |

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**Figure 41**  Histogram plot of the u-v matrix derived from the “Luv” format of histopathological images (A) Case 13, (B) Case 14, (C) Case 15
TABLE 4 Synthesis results: Hardware mapping

| Synthesis mapping   | Number of units |
|---------------------|-----------------|
| GND                 | 1               |
| LUT1                | 186             |
| LUT2                | 770             |
| LUT3                | 452             |
| LUT4                | 420             |
| MUXCY               | 1490            |
| VCC                 | 1               |

Arithmetic logic function mapping

| Adders/subtractors  | Number of units |
|---------------------|-----------------|
| 240                 |
| 10-bit adder        | 48              |
| 12-bit adder        | 48              |
| 13-bit adder        | 48              |
| 14-bit adder        | 48              |
| 8-bit subtractor    | 48              |
| Comparators         | 48              |

Input-output mapping

| Number of IOs       | Number of units |
|---------------------|-----------------|
| 92                  |
| Number of bonded IOBs: | 92 out of 100, that is, 92% |

TABLE 5 Derived values after blob analysis

| Case | Test Image                  | Clusters | Cell count | Area    | Feature |
|------|-----------------------------|----------|------------|---------|---------|
| 1    | Hyaline membranes           | 5        | 881        | 0.323   | —       |
| 2    | Fibroblast proliferation    | 5        | 692        | 0.6485  | —       |
| 3    | Periavascular lymphocytes   | 5        | 743        | 0.8579  | —       |
| 4    | Multinucleation             | 6        | 118        | 0.591   | —       |
| 5    | Busulfan                    | 4        | 442        | 0.196   | —       |
| 6    | Amiodarone                  | 5        | 742        | 0.746   | —       |
| 7    | Metaplasia                  | 3        | 391        | 0.822   | —       |
| 8    | Eosinophils                 | 3        | 769        | 0.269   | —       |
| 9    | Endomyocardial              | 6        | 279        | 0.576   | —       |
| 10   | lymphocytic fulminant myocarditis | 4 | 107 | 0.422 | — |
| 11   | Renal capillary congestion | 4        | 388        | 0.441   | —       |
| 12   | True myocarditis            | 5        | 502        | 0.312   | —       |
| 13   | Lumina ectasia              | 4        | 917        | 0.222   | —       |
| 14   | Mononuclear infiltration    | 5        | 816        | 0.674   | —       |
| 15   | Pertibular capillaritis      | 3        | 1047       | 0.4959  | —       |

so on. Case 11 Capillary Congestion is shown in Figure 35A. Figure 35B–E shows the segmentation output. The Histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 40C and their pixel distribution within the clusters is shown in Figure 35F.
Case 12:
This case of true myocarditis is also prevalent after serious viral infection. Till now pathologic studies have been very less, constrained to case reports, biopsy tests, and so on. Patients infected with SARS Cov-2 Virus gets a wide range of tubular and glomerular problems. Many findings give evidence against the infection of the kidneys directly by virus as the major pathogenic mechanism for coronavirus related kidney injury and implicate vaccine induced reactions, cytokine effects, enhanced adaptive responses of the immune system. Refer Figure 36A. Figure 36B–F shows the segmentation output. The Histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 40D and their pixel distribution within the clusters is shown in Figure 36H.

Case 13:
The case of lumina ectasia reveals the acute kidney injury (AKI) to sepsis-AKI (SAKI) due presumed coronavirus disease 2019. The transcriptomic, morphological and proteomic characteristics of the resultant segmented biopsy were analyzed to find three clusters comprising different cells and cytoplasm. The part of kidney (renal-cortex) on light microscopy severe tubular injury with lumina-ectasia (hematoxylin,eosin[HE] stain, with magnification-20). See Figure 37. Figure 37B–E shows the segmentation output. The Histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 41A and their pixel distribution within the clusters is shown in Figure 37F.

Case 14:
The case reveals the acute kidney injury due to COVID-19, mild mononuclear-interstitial infiltration along with the peritubular capillary-congestion(HE stain, with magnification-40). Refer Figure 38. Figure 38B–F shows the segmentation output. The Histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 41B and their pixel distribution within the clusters is shown in Figure 38H.

Case 15:
The case of reveals the acute kidney injury due to COVID-19- Peritubular capillaritis, congested capillaries is the root cause. The micro-vascular inflammation is grouped of CD-68 positive macrophages, CD-3 positive T-cells.Refer Figure 39. Figure 39B–D shows the segmentation output. The Histogram plot of the u-v matrix derived from the “Luv” format of this histopathological image is given in Figure 41C and their pixel distribution within the clusters is shown in Figure 39F.

5.2 Results of blob analysis on a few cases

Figure 42, Figure 43, Figure 44, and Figure 45 show the blob analysis of biopsy cases 1,2,3 and 4 respectively, pertaining to SARS CoV-2 affect on Lungs. Figure 46, Figure 47 (CD68-positive macrophage), Figure 48 and Figure 49 show the cell centroid and physical parameter identification of kidney tissue after COVID-19. The cells are individually detected and labeled at the same time computing their area, perimeter, diameter, and so on as shown in Table 5.

6 HARDWARE IMPLEMENTATION AND COMPARISON WITH THE LITERATURE

Previous research work on the implementation of image segmentation designs were based on some traditional mathematical algorithms or based on some parallel or pipelined network of switching modules, utilizing many complex
TABLE 7  Comparison of the existing implementation of segmentation in the literature

| Work | 1-33 | 2-47 | 3-47 | 4-48 | 5-36 | 6-39 | 7-49 | 8-50 | 9-46 |
|------|------|------|------|------|------|------|------|------|------|
| Type | K-Means | Genetic | Genetic | Genetic | Genetic | Roller Dung Beetle | Quadrant | Fuzzy-C-Means | Proposed work |
| Family | XC7K series | XC4000 series | Virtex II | Virtex (no data) | Kintex 7 | Virtex 6 | Microsemi | Virtex-7 | Zynq Ultrascale |
| Device | XC7K70T-3FBG676 | XC4010D /BORG | Xc2v1000 | V1000FG680 | XC7K70T-3FBG676 | XC6VCX75T-2-FF484 | RTAX2000S | Xa7a10ctc1 gs324-2 | ZCU104 |
| Slice-flops | 259 | 689 | 712 | 615 | 1223 | 33712 | 33%t | 19% | – |
| Total slices | 310 | 403 of 6192 | 310 of 5120 | 813 of 12288 | 1132 of 10,250 | 41487 of 46560 | – | 20990 | – |
| Gate-count | – | 17469 | 18732 | 15210 | 23,191 | 15990 | – | – | – |
| Max Clock | 162 MHz | 25.03 MHz | 87.187 MHz | 23.572 MHz | 14.32 MHz | 230.52 MHz | 50 MHz | 254 MHz | 190 MHz |
| Max net delay | – | 11.49 ns | 13.312 ns | 10.537 ns | 69.8 ns | 4.34 ns | – | 10 ms | 232.2 ms overall |
computations hungry for large power and adding more and more logical delays (Table 2). Moreover, they demanded high-speed data-transfer capability needing redundant copies of FDE (fetch, decode, execute) cycles and large bandwidth (in terms of bit width) as well high speed data buses, needing intricately interlaced data processing and control logic, essentially making the hardware more complex and less efficient. The resultant high dense computational structures occupies a larger area, increasing the cost and time to market as well, illustrated in the logic utilization row of Table 3. Existing designs had another problem of inability to scale as per demands, hence full redesign for various data
FIGURE 45  The cell centroid and physical parameter identification of multinucleation, Case 4

FIGURE 46  The cell centroid and physical parameter identification of kidney tissue due to SARS CoV-2 action on functional receptor ACE-2

FIGURE 47  The cell centroid and physical parameter identification of kidney tissue after COVID-19: CD68-positive macrophage
lengths, along with increased coupling effects and fan-outs. Another problem is due to the jitter, timing constraints, clock skewness, and so on. The multicore processor with their pipelined datapath and large bus widths can also increase the complexity and power consumption. Memory coherency issues can be the next level challenge where individual data in no time needs to match with the results emerging out from local modules must match the global data. These concerns were overcome by this new architecture and algorithm for image segmentation, targeted for reconfigurable designs as well as custom/semicustom ICs focused on real-time power aware processing. Some of the example applications are IOT processors, communication/Networking processors on UAV or Autonomous vehicles, video processors, other data mining applications. The proposed automatic segmentation (clustering) algorithm for image segmentation have the following features:

1. Supports fine grained parallelism.
2. The easiness to scale, simplicity in the design.
3. The complexity $O(n^2)$ which in turn improves the performance with the help of pipelining and parallel processing (refer Tables 3 and 4).
4. Reduced delay time.

7 | CONCLUSION

Because of the want of huge investment in infrastructure, time and expert human personals, nowadays critical invasive diagnostic procedures are given a less of a clinical priority. This delays the timely formulation of effective therapeutic
strategies to control diseases like lung cancer, tuberculosis and pandemic infections like COVID-19. The “HISTAK” architecture proposed in this manuscript employs a novel method named “HST” based on histogram analysis to determine the effective number of chromatic segments within the input histopathological image.

The proposed method has been tested on 15 biopsy images procured from standard hospital databases. The results in terms of its performance on software (450.92 ms as well as on hardware implementation (232.25 ms, 2 times faster than software implementation) shows its application in rapid diagnosis of chronic diseases. Moreover, this technique neither requires user interaction nor any prior knowledge about the medical history to automatically segment the tumors, cells, cytoplasm, and other features within the histopathological image in a very less time.

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DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**SUPPORTING INFORMATION**
Additional supporting information can be found online in the Supporting Information section at the end of this article.

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