

Stitching Microscopic Images for Tuberculosis Sputum Samples

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Abstract

Mycobacterium Tuberculosis is the causative agent of the transferable disease called tuberculosis (TB). Early diagnosis of TB via sputum examination is imperative in an effort to avoid transmission. Microscopic examination of sputum involves observing 100-300 fields of view (FoV) by eye, which typically takes 30-150 minutes. In practice, pathologists still manually change the FoV to cover the entire sample. This process is associated with limitations like low accuracy in identifying local features due to reduced contrast in the images, misregistration in overlapping regions, and large computation times. To correct these limitations, a digitization system in the form of whole slide imaging (WSI) is required, this method drastically minimizes examination time, with WSI bacterial detection requiring only 3-10 minutes, which is a significant improvement in diagnostic effectiveness without compromising the completeness of the analysis. In the proposed framework, invariant local features are identified using the Scale-Invariant Feature Transform (SIFT) algorithm, K-Nearest Neighbors (K-NN) and Brute Force (BF) Matcher are used in the proposed algorithm to match features precisely. Its ability to produce permanently aligned composite images is further reflected in the mosaiced result's zero-pixel measurement, which achieved at least 1,069,687 pixels.

Keywords: Brute Force Matcher, SIFT, Sputum, Stitching, Tuberculosis.

1. Introduction

Tuberculosis is an infectious disease that directly affects the human lungs and is caused by the Mycobacterium tuberculosis (MTB) bacteria. This infection mainly affects the lungs and spreads through the air when an infected person coughs or sneezes [1], [2]. TB can spread through the air from sputum splashes of TB patients who cough without covering their mouths, causing continuous transmission over a long period [3]. As a preventive measure, early diagnosis of tuberculosis by conducting sputum tests is very important to prevent transmission to others. Sputum examination is generally performed using a Fluorescence Microscope (FM) or a bright field microscope, often called a Conventional Microscope (CM). The use of CM is more common in low- and middle-income countries due to its accessibility, minimal biosafety

standard requirements, and affordable cost. It is also the primary method of detecting tuberculosis in remote areas. However, manual identification and calculation of CM results are time-consuming [4].

The World Health Organization (WHO) mandates the microscopic examination of tuberculosis sputum samples, requiring 100-300 fields of view to ensure accurate results and confirm TB-negative patients. Therefore, a system capable of combining 100-300 images from various fields of view into one whole image is required, which can be done through the Whole Slide Imaging (WSI) method. WSI microscopic images can make it easier for pathologists to count TB bacteria [5]. In addition, it is necessary to design a system to combine microscopic images of sputum samples, which is called the image stitching technique. In many biomedical studies, stitching images is needed to obtain panoramic images representing a large area of a particular structure or an entire section while maintaining the resolution of the microscopic image [6].

Previous research on stitching images was conducted by one of the pioneering researchers in this field, David G. Lowe, in 2004, who introduced the SIFT method. The SIFT method can detect important features in images automatically and is robust to changes in scale, rotation, and lighting. With this method, microscopic images from various fields of view can be automatically stitched together with a high degree of accuracy [7]. SIFT-based auto stitching starts its process by detecting specific features of each microscopic image. These features are mapped and matched between images to identify areas of overlap that allow for image merging. The algorithm then aligns similar images and integrates them into a complete image. The advantage of this method lies in its ability to reduce the potential for human error in manual image merging and speed up the overall process with more precise results [8], [9].

Thus, this study will use the SIFT-based auto-stitching method to stitch together FoV microscopic images of tuberculosis sputum samples. Implementing this method is expected to speed up and simplify the diagnosis process while increasing the accuracy of counting tuberculosis bacteria in the resulting image. With automatic image stitching, the entire microscopic FoV can be analyzed as a WSI, rather than as separate fragments. This enables the system to count the number of tuberculosis acid-fast bacilli (AFB) more comprehensively, reducing the risk of underestimation due to missed areas and minimizing repetition due to overlapping regions. The SIFT algorithm ensures that key features from each image are aligned with high precision, thus minimizing distortion during the stitching process. This ultimately leads to more accurate bacterial identification, improving diagnostic outcomes and reducing human error.

In microscopy, the main tradeoff observed is between accuracy and speed. Higher accuracy in the direct measurement of AFB directly affects the determination of TB severity, which in turn allows for a more accurate assessment of disease progression. This has a significant impact on clinical decision-making, such as adjusting the intensity of anti-TB drug regimens and monitoring therapeutic response. Therefore, in this study, the SIFT method was chosen over other methods such as ORB, SURF, and A-KAZE. The SIFT method excels in accuracy and is more resistant to rotation, translation, scaling, and lighting changes, but requires longer computation time. SURF offers higher speed and efficiency in image processing, but is less stable under extreme rotation and sensitive to lighting changes. On the other hand, ORB is very efficient and fast, suitable for real-time applications, but has lower accuracy and is more susceptible to changes in lighting conditions. In addition, SIFT can detect stable keypoints in microscope images, which often show variations in intensity. This method

is well suited for complex biological images due to its ability to extract unique features from small areas.

2. Related Work

2.1 Whole Slide Imaging (WSI)

Whole Slide Imaging is a technology that enables high-resolution digitization of whole microscopic preparations. This technology produces digital images of tissue or cell slides that can be viewed, analyzed, and shared via computer, similar to how conventional microscopes work [10]. WSI has a variety of important applications, particularly in the fields of digital pathology, medical education, research, and telepathology. This technology brings several significant advantages, including ease of remote collaboration, improved diagnosis efficiency, and the ability to perform computer-based image analysis. WSI significantly reduces the time required for diagnostic compared to traditional microscopy, with studies showing a marked decrease in the average time pathologists take [11], [12].

Image stitching is a method where multiple images are combined to create a panorama or separate photos with high resolution. The merging of two overlapping images consists of mosaicing both images into a single frame. Most approaches to combining multiple images, usually done using computer software, require precise image matching and superposition to produce a smooth image [13]. The image stitching process can be done automatically, a process referred to as auto-stitching. This auto stitching can combine overlapping fields of view automatically.

2.2 SIFT (Scale Invariant Feature Transform)

The SIFT (Scale Invariant Feature Transform) algorithm is used in computer vision to detect and describe local features in images. It matches images based on keypoints, where images are converted into local feature vectors that recognize objects through those keypoints. SIFT is one of the most popular feature extraction methods, developed by David Lowe in 1999 and patented in Canada by the University of British Columbia. This algorithm is often used to detect objects at various scales and rotations [13]. There are several algorithms used in the SIFT method.

- **Keypoint Extreme Value**

The first step in keypoint detection is determining the position and scale that may change when the object is viewed from different angles. This is done using scale space, which enables keypoint detection at various scales. After that, extreme values are searched for potential keypoints. This process utilizes a Gaussian function to ensure the selected points are truly significant at each scale [14].

To find extreme values, the scale space method incrementally calculates the difference between two scale levels. The resulting values are then filtered using the Difference of Gaussian (DoG) method to eliminate insignificant points [14]

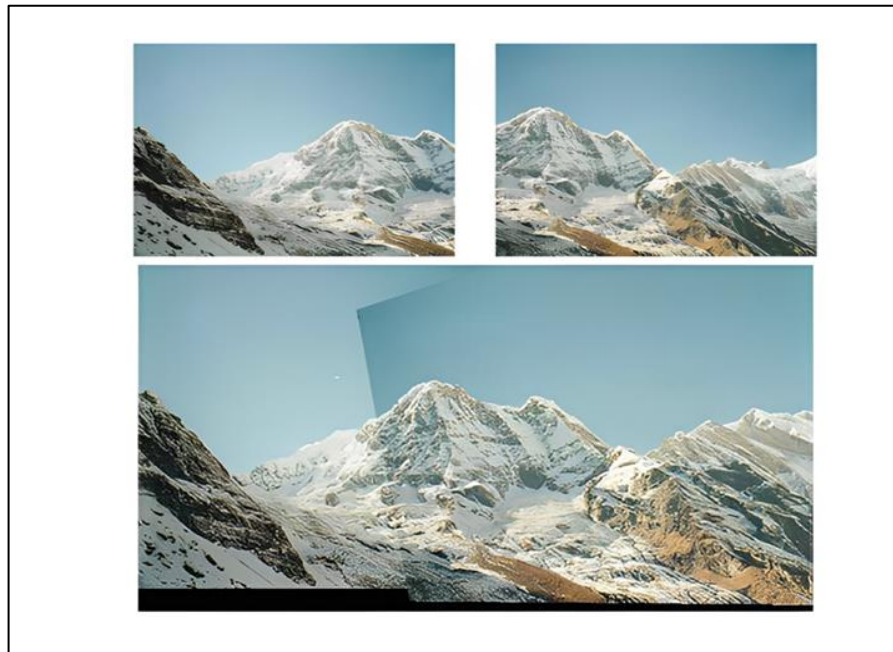


Figure 1. Sample Image Stitching Results

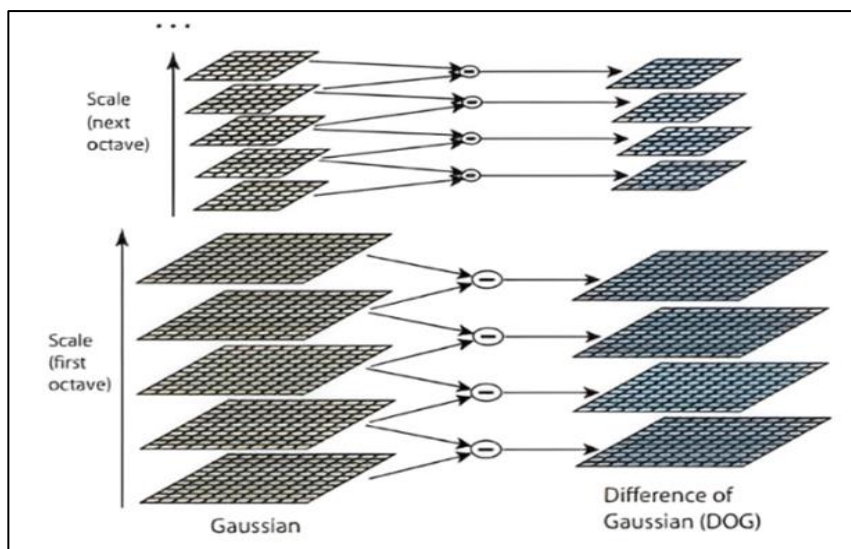


Figure 2. Elimination Using Different of Gaussian

- **Keypoint Localization**

After calculating the scale space using the Difference of Gaussian (DoG) method, the next step is calculating the Laplacian of Gaussian to determine the keypoint. This process aims to find the right location for the keypoint by filtering out points with low values. Next, a search for maxima and minima in the DoG image is performed by examining each pixel and its neighbors to ensure that the selected point is significantly extreme across the various scales available.

Maxima and minima of different Gaussians are detected by comparing a pixel marked with X, which is a keypoint with its 26 neighbors in the 3x3 region at the current and adjacent scales [7].

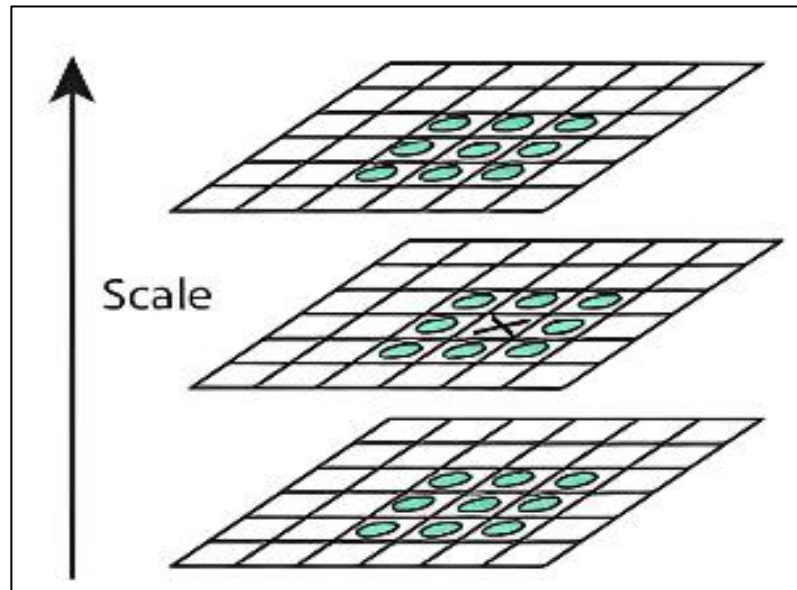


Figure 3. Determining Keypoint Maxima and Minima

The SIFT parameter configuration for this study was carefully optimized to address low-texture detection challenges. While maintaining default DoG parameters including 3 octave layers and $\sigma=1.6$, the critical contrastThreshold was reduced to 0.03 from the standard 0.04. This specific adjustment, combined with an edgeThreshold of 10, significantly enhances sensitivity to subtle contrast variations in homogeneous regions while preserving feature stability, resulting in improved keypoint density and matching performance in texture-deficient environments.

- **Orientation Assignment**

Once the key points are selected, the next step is to assign an orientation to each key point by calculating the gradient direction and magnitude around the keypoint. The dominant orientation in the area is then determined [16]. This process is important to maintain the position of the keypoints even if the image is rotated, ensuring that each keypoint remains in the same place. Thus, the local image orientation around the keypoint is set based on the direction of the gradient, ensuring the stability of the keypoint location despite changes in image orientation [7].

- **Key-point Descriptor**

Each keypoint has been given a stable position, scale, and orientation in the previous stage. A descriptor is required to manage keypoints despite viewing angles or lighting changes. This stage becomes the final step in the SIFT algorithm, where each keypoint is given a unique descriptor or fingerprint. This descriptor facilitates the identification of each keypoint. To ensure uniqueness, a window of 16 or a 4x4 area around the keypoint is taken as the basis for calculation, thus allowing for more precise analysis even if the image changes [7].

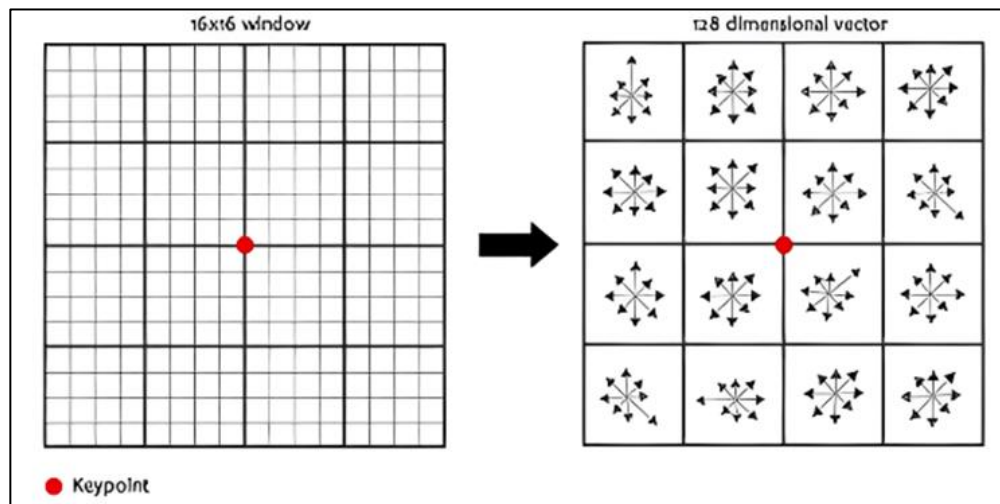


Figure 4. Keypoint Descriptor Determination

3. Proposed Work

3.1 System Design

In this research, the system design was developed through a literature study related to image stitching and image processing. Planning and analysis of image stitching and image processing were carried out using auto stitching techniques to stitch images of microscopic tuberculosis sputum samples. Auto stitching or stitching the images from the sputum examination, is performed while maintaining image quality. The stages of auto stitching can be seen in Figure 5 below.

The auto stitching technique automatically stitches two images together to form a panoramic image by utilizing the SIFT algorithm to extract features from the images. The stages in performing image stitching are as follows:

- **Input Image:** The initial step in the auto stitching process is to enter the image that has been taken from the microscope examination results of the tuberculosis sputum sample.
- **Feature Extraction:** The second step in the image stitching process is feature detection. Feature detection is the most important step, and it is important not only in stitching but also in any field of image processing. Features can be defined as elements in two or more input images that will be matched to select some homogeneous parts in the image for analysis [17].
- **Feature Matching:** In this process, feature matching is performed to compare feature descriptors or keypoints. If the keypoint is appropriate in both images, then the two features are recognized as the same point, and the images are matched [18].
- **Blending Images:** This stage is the core stage, where the corresponding images are combined at the overlapping parts. Measurement Parameter

In this process, the stitched image is tested by performing a ratio test and calculating the zero pixels in the stitched image.

- **Output:** The output or result of the auto stitching process is two input images that have been successfully combined into a panoramic image.

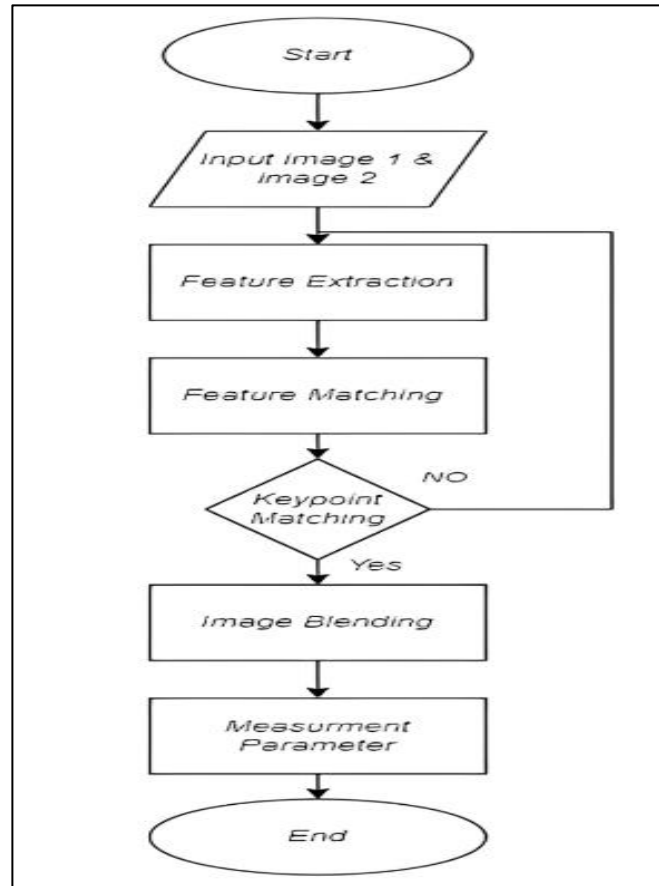


Figure 5. Technical Workflow of Microscopy Image Stitching

3.2 Dataset Retrieval Process

This study utilized a comprehensive microscopic imaging setup consisting of an Olympus CX-31 microscope equipped with a 100× oil immersion objective lens (numerical aperture 1.25). Image acquisition was performed using a DSLR 700D camera (18-megapixel resolution) capable of capturing detailed morphological features of Tb AFB. In addition, this study used an Asus Vivobook M1403QA laptop, which offers balanced performance for image processing tasks. Computational costs were optimized by utilizing the laptop's processing power (AMD Ryzen 5 5500U processor, 8GB RAM, and integrated AMD Radeon graphics), ensuring efficient handling of high-resolution image datasets during the acquisition and analysis stages.

The image dataset is used as testing material for this research. The image taken results from a tuberculosis sputum examination under a microscope that shows that the sputum sample is negative. The size of the sputum sample is 2 x 1 cm, and the preparation size is 7.6 x 2.5 cm. The sputum sample preparation can be seen in Figure 6 below.

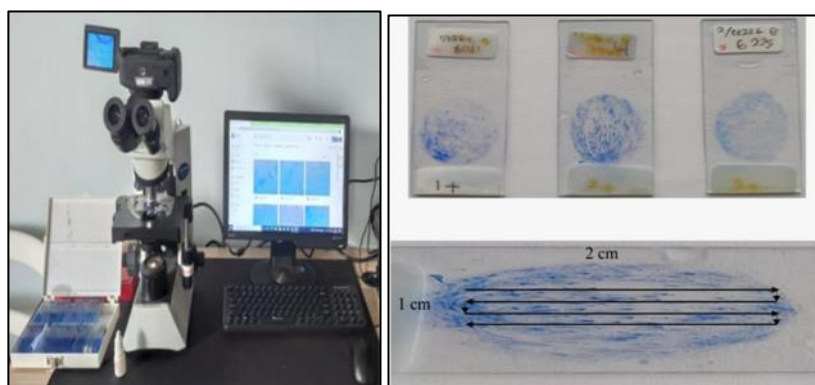


Figure 6. Microscope and Sputum Sample Preparation

The results of the microscope examination of the samples were captured using a digital camera in JPG image format. For this dataset, 100 overlapping images were taken, with 20 images used for testing. The test was conducted using two methods: a ratio test to measure the accuracy of feature matching and a zero-pixel calculation on the stitching result to evaluate the quality of image merging [19], [20]. Sputum samples from the microscope were photographed, as shown in Figure 7.

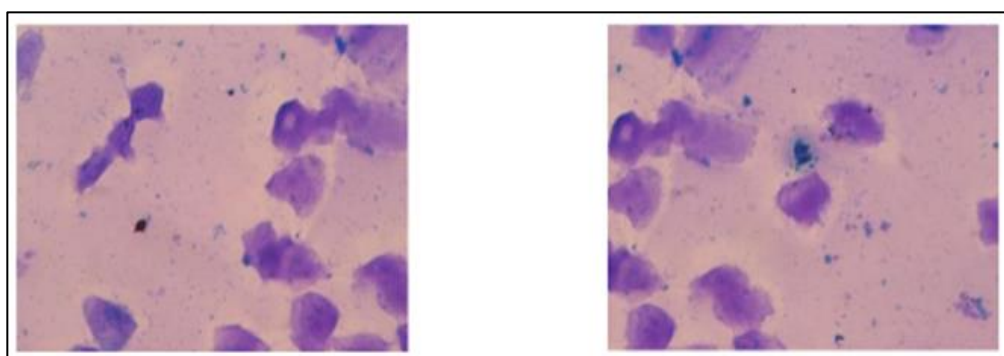


Figure 7. Dataset of TB Sputum Samples

3.3 Data Training and Testing

In this study, microscopic image quality is critical to ensuring the success of the registration process. Based on recommendations from previous studies that tested CBCT registration with SIFT at various overlap levels, we found that a 30% overlap resulted in a registration success rate of nearly 100%. However, when the overlap was reduced below 30%, the matching quality decreased significantly, indicating that an overlap of less than 2 cm can cause a drastic decline in quality and require manual intervention [21], [22]. Therefore, we applied a minimum overlap level of 30% for optimal results in our application, with the training and testing data in this study consisting of microscopic images of tuberculosis sputum samples processed by the system, total of 20 images or 10 pairs of images. Each pair of images has a 30% overlap and was taken directly using a digital camera. The data have been adjusted for the stitching process so that the system can be accurately tested according to the analysis requirements.

- **Preprocessing**

Library installation is the first step required to run the designed system at this stage. After that, modules are imported from the installed library to support the process. Next, the image to be processed is input. The image will be converted into grayscale format to have only gray color levels, which aims to simplify visual analysis. This process is part of the stage, as seen in the flow chart in Figure 8 below.

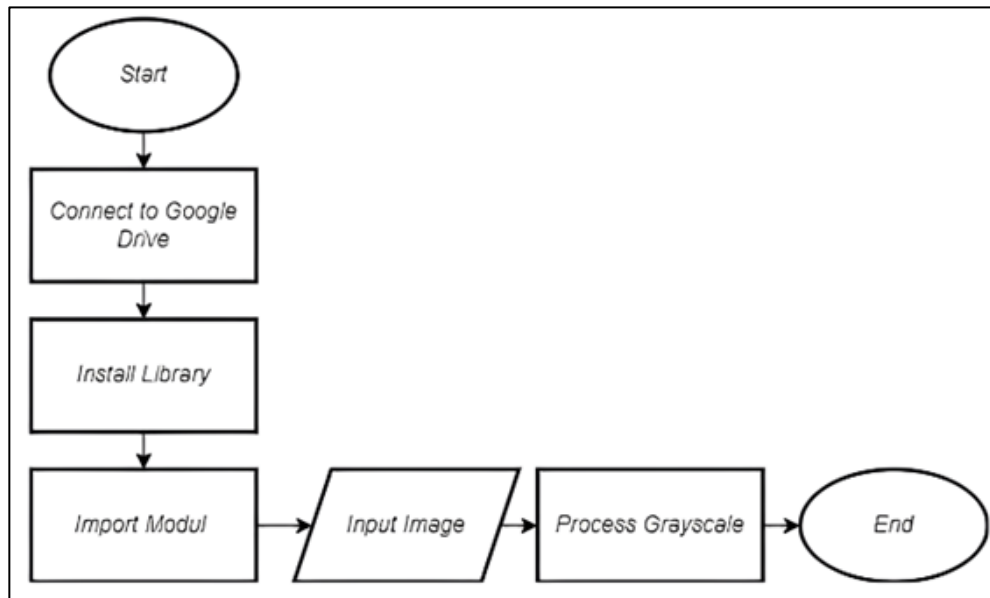


Figure 8. Workflow Diagram

- **Feature Extraction**

Feature extraction is the process of identifying local features present in an image. In this context, the SIFT method detects and describes these local features. SIFT functions to identify important points contained in the image and produces descriptors that can be used for various analysis purposes, such as object matching. The feature extraction process in programming can be seen in the flowchart below Figure 9.

- **Feature Matching**

At this stage, a matching process is carried out to identify similarities between image 1 and image 2 that have been inputted. The feature matching process uses several algorithms, namely BF Matcher, KNN, FLANN, and Lowe's Ratio Test, to efficiently match the keypoints in both images. The stages of this process can be seen in the figure. The flowchart describes the process after the image features are found, where the two images are compared to measure the match level based on the detected keypoints and features.

The algorithm used will search for key matches by calculating the closest distance. If the two images produce a match, the system will display a connecting line between the corresponding points. However, the connecting line will not be drawn if the images are different.

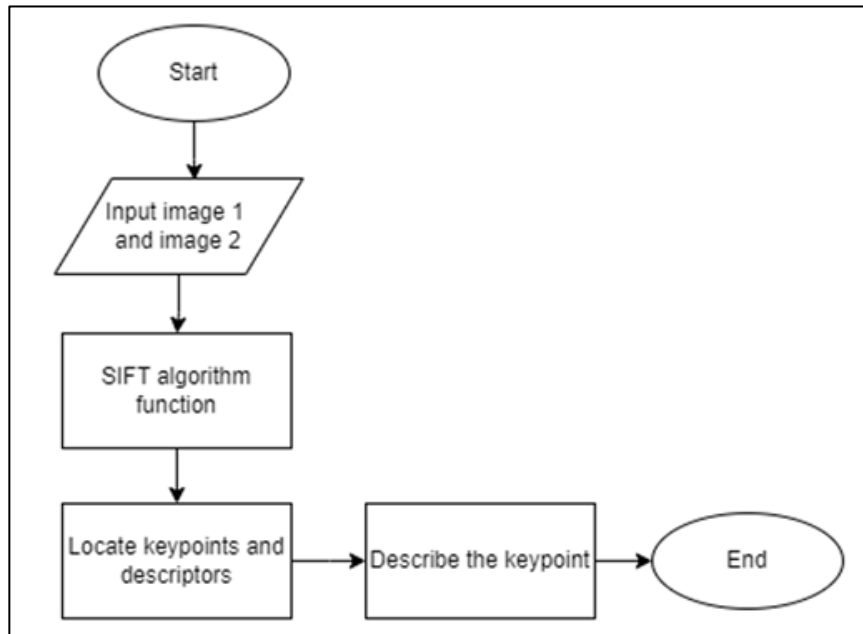


Figure 9. Flowchart of SIFT Algorithm



Figure 10. Feature Matching Validation Process

- **Image Blending**

The image blending process merges the first image and the second matched image. During this process, separation between inliers and outliers is also performed to distinguish valid matches from invalid matches using the RANSAC method. The image blending procedure can be seen in Figure 11.

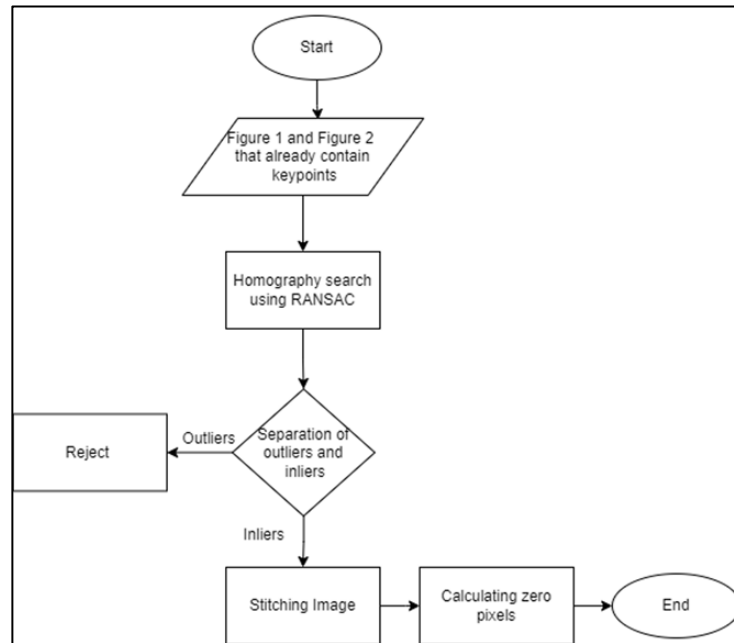


Figure 11. Flowchart of the Image Registration and Blending Process

After separating outliers and inliers, the image stitching process follows. In the stitching results, black regions often appear. These black areas are assumed to be zero pixels, with a zero-value indicating a black color. The number of zero pixels in the stitching result will be counted to determine the total number of zero-valued pixels in the image. As an illustration, the complete proposed method, which is part of the process for examining a sputum sample for TB diagnosis based on the IUATLD scale, can be seen in Figure 12.

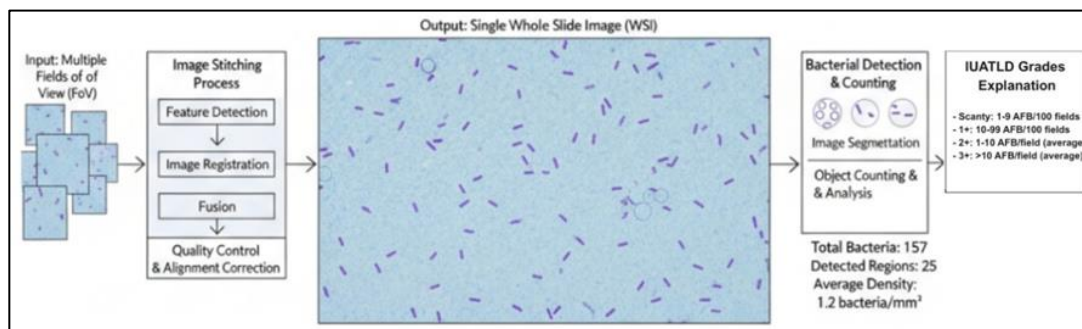


Figure 12. Overall Process of Sputum Sample Examination for TB Diagnosis based on the IUATLD Scale

3.5 Image Stitching Process

The process of image processing with the auto stitching technique on microscopic images of tuberculosis sputum samples can be seen as follows:

- **Image Input**

In this section, the input image that will be used in the stitching process is introduced, where the images used overlap each other. One of the sample images used for the stitching process can be seen in Figure 13 below.

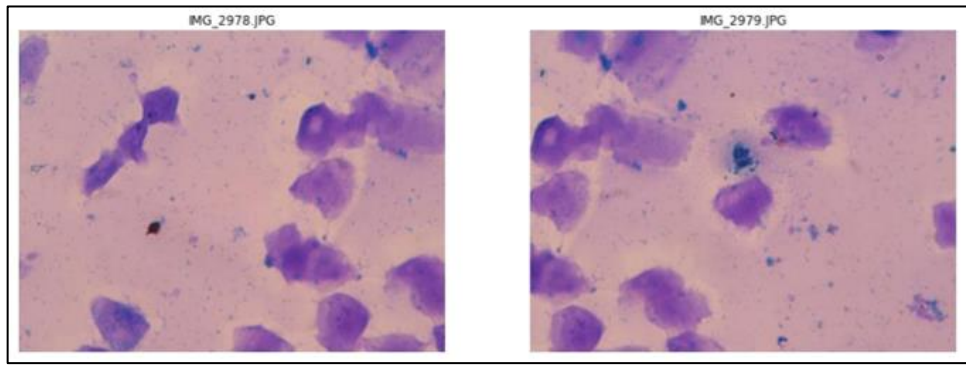


Figure 13. Sample of Sputum Sample Images

- **Matching Keypoint**

After entering the image or input image, the image will be processed to find keypoints on each image entered. The first stage will be to search for keypoints and then look for local features or feature extraction in the image using SIFT. After that, the descriptors of both images will be matched using BF Matcher and KNN. The process can be seen in the figure 14 below:

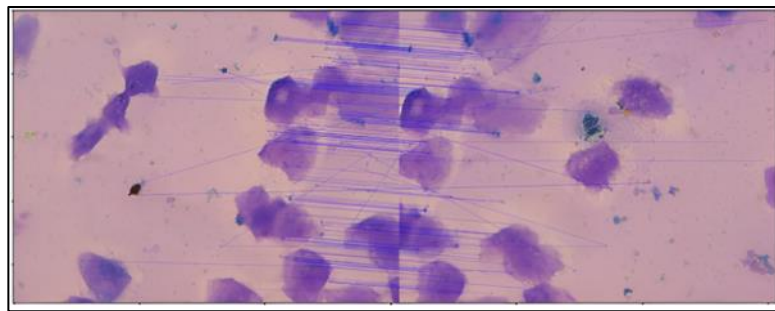


Figure 14. Matching Keypoint between Two Images of Sputum Sample

- **Stitched Image**

In this process, after matching keypoints on both images, the next step is to stitch the images or merge the two matched images. This is also the final step of stitching images, where the stitched image results will emerge after the stitching process. Here is an example of the resulting stitched image in Figure 15.

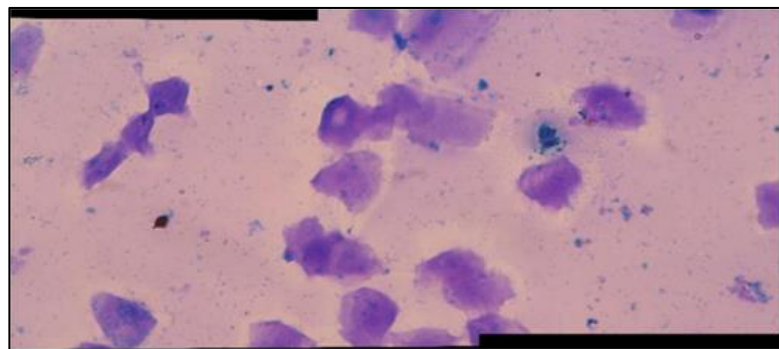


Figure 15. Result Shows a Black Region

4. Results and Discussion

This section will discuss the results of the simulation analysis carried out in the previous chapter. The simulation was run using Google Colab to execute a program developed with Python. Two algorithms were used in this simulation to compare the stitching results, namely the SIFT algorithm. Testing was conducted through two scenarios to evaluate the performance of each algorithm in the stitching process, namely:

- Scenario 1 was tested by conducting a ratio test using several different ratio values.
- Scenario 2 involved counting the number of zero pixels or black pixels in the stitched image.

In both scenarios, 20 images were selected from 100 images taken, with overlapping images. From these 20 images, ten pairs of images were created that were used in the image stitching process. Tests were conducted using two algorithms, including the SIFT algorithm, to compare the results between the two test scenarios. This research aims to evaluate the performance of stitching with a limited number of images under various overlapping conditions.

4.1 1st Scenario Testing

This first test performs a ratio test based on the distance ratio between the two closest points. For example, d_1 and d_2 ($d_1 < d_2$) are the distances from an end to the nearest points that match best. This test is performed using various ratio values between 0.3 and 0.7. In this process, keypoint matching in stitching is performed using the SIFT algorithm to optimize matching accuracy based on predetermined ratio values.

4.2 Ratio Test Results on SIFT

Testing using the ratio test on the SIFT algorithm is done with ten pairs of images that have overlapping areas. After the test is performed, the stitching results are analyzed based on the application of the ratio test, with ratio values ranging from 0.3 to 0.7. This SIFT method is used to evaluate the quality of feature matching between images, and the results can be seen in Figure 16, which shows how the ratio value affects the stitching quality of the tested images.

Based on the graph above, image stitching is maximally successful on ten pairs of image samples with ratio values of 0.5 and 0.6. Examples of image stitching success and failure are shown in the figure below 17.

In this test scenario, the results show that the success rate of image stitching reaches 92% overall, with an average processing time of about 13 seconds. Based on the results shown in Table 1, the highest percentage of success is achieved at ratio values of 0.5 and 0.6, with the success rate reaching 100%. This indicates that these ratios provide optimal performance in image stitching for this test

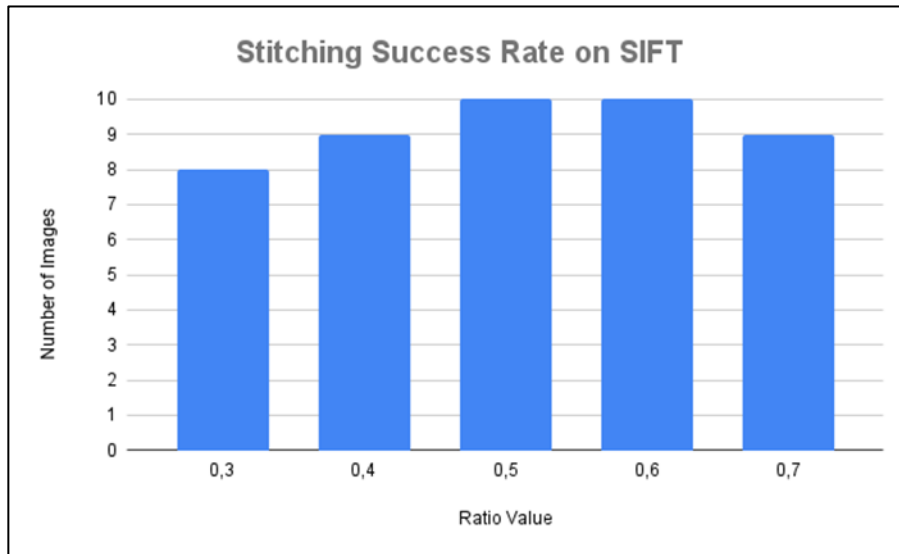


Figure 16. Ratio Test Result Graph on SIFT

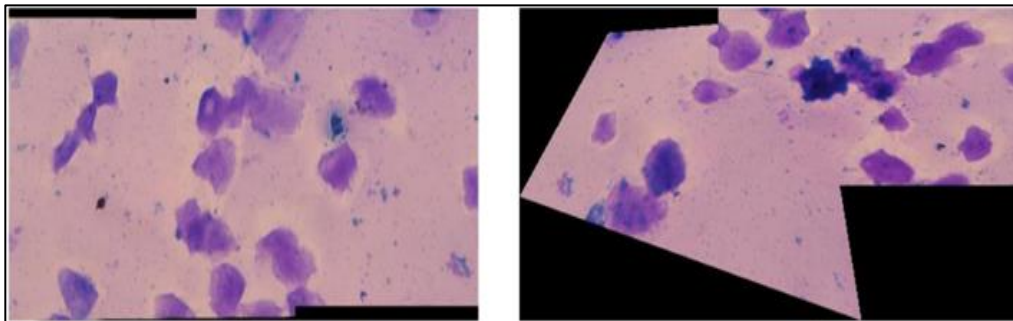


Figure 17. a). Successful Stitching Example, (b). Example of Failed Stitching

Table 1. Percentage Ratio Test on SIFT

No	Sample	Ratio Value				
		0.3	0.4	0.5	0.6	0.7
1	IMG_1278 & IMG_1279	✓	✓	✓	✓	✓
2	IMG_1280 & IMG_1281	✗	✗	✓	✓	✓
3	IMG_1282 & IMG_1283	✓	✓	✓	✓	✓
4	IMG_1296 & IMG_1297	✗	✓	✓	✓	✓
5	IMG_1302 & IMG_1303	✓	✓	✓	✓	✓
6	IMG_1314 & IMG_1315	✓	✓	✓	✓	✓
7	IMG_1316 & IMG_1317	✓	✓	✓	✓	✓
8	IMG_1318 & IMG_1319	✓	✓	✓	✓	✓
9	IMG_1320 & IMG_1321	✓	✓	✓	✓	✓
10	IMG_1334 & IMG_1335	✓	✓	✓	✓	✓
Total		80%	90%	100%	100%	100%
Total Success		94%				

4.3 2nd Scenario Testing

The second test analyzed the number of zero or black pixels appearing in the stitched image. The presence of zero pixels is caused by the size difference between the canvas and the stitching result, where the canvas has larger dimensions than the resulting image. This condition results in empty areas (zero-pixels) that need to be considered when evaluating the quality of the stitching result.

4.4 Zero-pixels Calculation Result on SIFT

The result of image stitching using the SIFT algorithm shows black regions, which are also called zero-pixels. The number of zero pixels is calculated to determine the minimum and maximum values in the stitched image result. The zero-pixel calculation results are presented in Table 2, which shows the best simulation results using the ratio test with ratio values of 0.5 and 0.6. This calculation is important for evaluating stitching quality and ensuring minimal unfilled areas in the stitched image.

From the test results, the maximum and minimum values are obtained for ratios 0.5, 0.6, and 0.7. At a ratio of 0.5, the minimum value achieved was 1,088,877 on sample images IMG 1316 & IMG 1317, while the maximum value was 1,958,070 on samples IMG 1314 & IMG 1315. For a ratio of 0.6, the minimum value is recorded as 1,069,687 in samples IMG 1316 & IMG 1317, and the maximum value reaches 2,010,333 in samples IMG 1314 & IMG 1315. This data shows the variation of feature match values based on the ratio used.

Table 2. Zero-pixel Calculation Result on SIFT

No	Sample	Zero pixel	
		0.5	0.6
1	IMG_1278 & IMG_1279	1.333.845	1.361.982
2	IMG_1280 & IMG_1281	1.524.240	1.319.892
3	IMG_1282 & IMG_1283	1.638.594	1.640.241
4	IMG_1296 & IMG_1297	1.902.180	1.666.532
5	IMG_1302 & IMG_1303	1.446.852	1.332.459
6	IMG_1314 & IMG_1315	1.958.070	2.010.333
7	IMG_1316 & IMG_1317	1.088.877	1.069.687
8	IMG_1318 & IMG_1319	1.390.564	1.340.980
9	IMG_1320 & IMG_1321	1.262.871	1.071.947
10	IMG_1334 & IMG_1335	1.779.337	1.713.616
Max Zero pixel		1.958.070	2.010.333
Min Zero pixel		1.088.077	1.069.687

4.5 Discussion

Image stitching performance can be compromised by poor image quality, insufficient overlap (<30%), and noise interference, which collectively impair keypoint detection and feature matching. Low-resolution images with weak contrast reduce detectable features, while limited overlap restricts matchable regions. Although techniques like image enhancement and parameter optimization can mitigate these issues, processing speed remains challenging for large datasets. This study utilized conventional SIFT with Euclidean distance (L2)

normalization and Lowe's ratio test (0.7 threshold), providing reliable baseline performance but limiting adaptability to complex conditions.

The reliance on full homography estimation without fallback to affine or similarity models resulted in failures when inlier counts dropped below four points. Additionally, the absence of exposure compensation led to visible seams and contrast variations between tiles due to vignetting and illumination differences. These limitations are particularly problematic in sputum microscopy, where sample thickness, mucus deformation, and tile capture inconsistencies produce ghosting artifacts and misalignments that affect diagnostic accuracy.

Future work should incorporate adaptive methods such as Root-SIFT normalization for improved lighting invariance, dynamic threshold adjustment for low-overlap cases, and exposure compensation to minimize tile blending artifacts. Implementing fallback transformation models and advanced RANSAC refinements would enhance robustness in challenging scenarios. These improvements could address current limitations in handling complex biological samples and increase the reliability of whole-slide image analysis for clinical applications.

5. Conclusion

In this research, a system has been designed to perform stitching automatically on tuberculosis sputum samples, also called auto-stitching. The method uses SIFT as the keypoint detector, with keypoint matching performed using BF Matcher and the FLANN algorithm. The matching results are then evaluated using Lowe's Ratio Test and the KNN algorithm. The dataset contains 20 images (10 pairs) in JPG format with an overlap area of 30%. Experiments demonstrated that SIFT identified at least 1,069,687 zero-pixels. Additionally, post-stitching ratio testing demonstrated 100% success with a time expense of 13 seconds. This document presents an auto-stitching pipeline from SIFT to tuberculosis sputum microscopy to stitch images from multiple fields of view into one high-resolution composite image.

This approach circumvents the limited visual field characteristic of conventional microscopy by considering more general AFB distribution. The technique has some positive practical benefits for clinical laboratories, including reduced repetitive hand scanning, removal of human counting error, and optimal diagnostic efficiency. The technique has potential extensions to other microbiological imaging requirements in scanning large surface areas. Some of the disadvantages noted in the article include reliance on proper image overlap (20-30%), stable lighting conditions, and visible features between tiles of images. Secondly, computationally costly SIFT becomes unaffordable for real-time use in resource-constrained situations. Future studies will compare other detectors such as ORB or AKAZE, deep learning, and computer-based AFB detection software applications to obtain greater scalability and clinical utility. They can also bypass inherent pitfalls of the method and maximize utility for other diagnostic imaging specialties.

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