

## Review Article

# Microscopy-based Data Processing in Cell Biology

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## Abstract

By making it possible to extract intricate and significant biological information from visual imaging, data processing based on microscopy has completely changed contemporary cell biology. Researchers have overcome historical constraints by combining microscopy with sophisticated image processing technologies, opening up new possibilities for comprehending cellular architecture and functions in unprecedented detail. The goal of this study is to present a thorough examination of the methods and new developments in microscopy-driven data analysis, emphasizing both the theoretical underpinnings and real-world applications. The study starts by reviewing many microscopy techniques, including light, fluorescence, confocal, super-resolution, and electron microscopy, emphasizing their unique advantages and functions in contemporary cell biology. After that, it examines crucial picture preprocessing methods that are necessary for data dependability, such as contrast enhancement, background correction, and noise reduction. The segmentation and feature extraction techniques that allow precise cellular component detection and quantification are covered in detail. The article also describes new software and computational tools that facilitate automation and uniformity in the collection, processing, and analysis of images. The importance of quantitative analytic techniques for deciphering biological processes is also highlighted. These techniques include intensity measurement, colocalization, geographical distribution, and statistical analysis. Insights from microscope pictures are further improved by data visualization techniques like 3D rendering and machine learning software. The paper concludes by exploring emerging trends that have the potential to further change the field of microscopy in cell biology, including artificial intelligence, cloud-based platforms, multimodal imaging, and immersive technologies like augmented and virtual reality. To summarize, microscopy-based data processing is crucial to the advancement of cellular biology research, as it provides a wealth of opportunities for discovery through the integration of technology and multidisciplinary innovation.

## Keywords

Microscopy, Image Analysis, Feature Extraction, Visualization, Light Microscopy, Electron Microscopy

## 1. Introduction

Using a microscope to study and analyze objects too small to be seen with the human eye is known as microscopy. All areas of biomedical science use microscopes, which are crucial for comprehending pathological processes [1]. Compre-

hending the cellular foundations of human health and disease necessitates the high spatial resolution provided by microscopy, given that cells exist at the micrometer scale [2]. The initial microscopes were developed in the year 1600, marking

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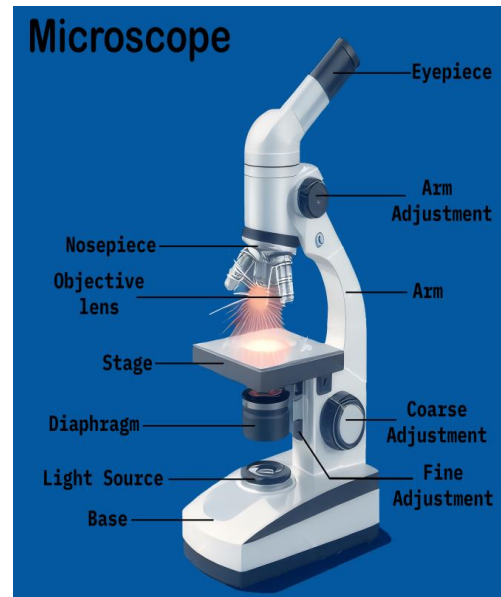
a pivotal moment in the history of scientific exploration and observation. These early instruments, although rudimentary by today's standards, allowed scientists to magnify small objects and gain insights into the previously invisible world of microorganisms and cellular structures. This technological advancement paved the way for Robert Hooke's groundbreaking observation of cells in 1665, instrumental in shifting the scientific community's understanding of the biological organization and laying the foundation for the formulation of the Cell Theory [3]. Over the past four decades, microscopy has undergone a remarkable transformation, driven by advancements in technology and a deeper understanding of cellular processes. Initially, microscopy was primarily focused on qualitative assessments of fixed cells, where samples were prepared and preserved for observation. This approach limited researchers to static images and hindered their ability to study dynamic biological processes in real time. However, with the advent of high-throughput quantitative microscopy techniques, scientists can now acquire vast amounts of data from live cells, enabling them to observe cellular behavior, interactions, and responses to various stimuli in real time [4]. The advancements in microscopy have been driven by breakthroughs like fluorescence microscopy, super-resolution imaging, and automated imaging systems. These technologies enable researchers to visualize cellular components with remarkable clarity and detail. Consequently, scientists can now investigate intricate biological systems with a precision that was once out of reach. The capability to collect quantitative data from live cells has paved the way for new research opportunities, including the exploration of cell signaling pathways, the dynamics of cellular processes, and the impact of drugs on living tissues.

## 2. Image Procurement and Preprocessing

### 2.1. Microscopy Techniques

#### 2.1.1. Light Microscopy

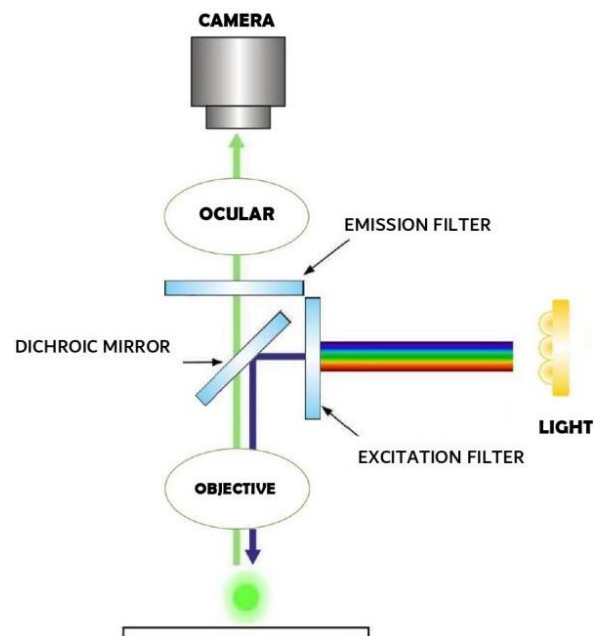
One of the most basic and popular methods in biology for seeing cells, tissues, and microbes is light microscopy. It provides a detailed view of specimens that are otherwise invisible to the human eye by magnifying objects using visible light and optical lenses. In light microscopy, an image is created by the interaction of light with the specimen. Detailed observation is made possible by the use of lenses to magnify light that either passes through or reflects off the sample. In cell biology, light microscopy is crucial for seeing colored cellular structures and live specimens. It is an essential tool for comprehending biological functions and phenomena in a variety of research fields because it enables scientists to examine cellular processes and interactions [5]. From early discoveries to sophisticated super-resolution tools, it has developed, allowing scientists to efficiently describe subcellular structures and activities in cells [6].



*Figure 1. Light Microscope.*

With technological advancements enabling the viewing of ever-increasing intricacies of cell structure, the light microscope continues to be a fundamental instrument used by cell biologists [7].

#### 2.1.2. Fluorescence Microscopy



*Figure 2. Functioning of a Fluorescence Microscope.*

The idea behind fluorescence microscopy is that fluorescently-labeled material can be illuminated at one wavelength and emit light or fluoresce at another, making it a non-invasive method that enables the dynamic recording of

molecular events in living cells, tissues, and animals [8]. Fluorophores, which are molecules that absorb light at particular wavelengths and release it at longer wavelengths—a process known as the Stokes shift—are essential to fluorescence microscopy [9]. A strong light source (such as xenon or mercury lamps), specific filters, and a dichroic mirror to separate excitation and emission light are necessary parts [10, 11].

Because it allows for the observation of cellular processes without interfering with physiological circumstances, fluorescence microscopy is essential for researching the behavior of live cells [12]. Targets at low concentrations can be seen with excellent specificity thanks to fluorescent probes' ability to bind to particular molecules.

This method improves our comprehension of intricate biological interactions by enabling the simultaneous monitoring of several targets inside a single sample [13].

The use of jellyfish green fluorescent protein (GFP) to view proteins within live cells was a ground-breaking development in fluorescence microscopy. Without fixing and staining the cell as would be necessary for the identification of proteins using antibodies, GFP may be fused to any protein of interest using ordinary recombinant DNA techniques. The GFP-tagged protein can then be produced in cells and observed by fluorescence microscopy [14].

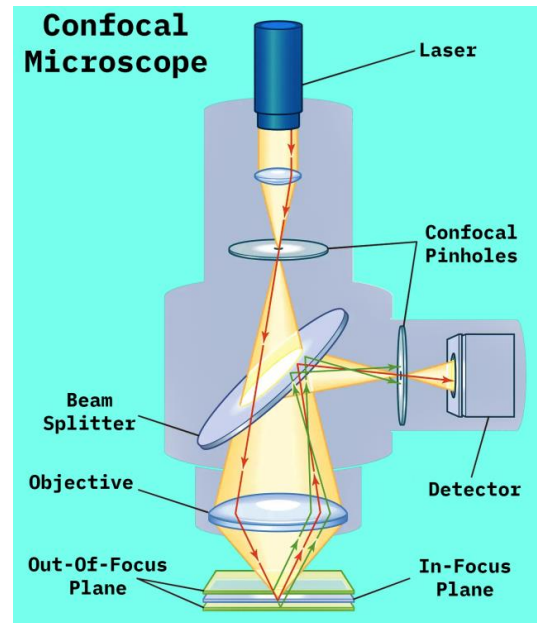
### 2.1.3. Confocal Microscopy

Confocal microscopy (CFM) is an advanced imaging method that removes out-of-focus light to improve picture quality and contrast. This technique allows for the precise viewing of tissue features, which makes it very useful in a variety of professions, including dermatology and ophthalmology. In order to create high-resolution pictures that can be converted into three-dimensional representations, CFM scans a sample point by point. A pinhole aperture is used in confocal microscopy to exclude out-of-focus light, improving picture quality and clarity. By reconstructing 3D pictures from a sequence of 2D slices, this optical sectioning capability offers comprehensive insights into sample architecture [15].

To produce photos of superior quality, sample processing must be done correctly. While fixed samples must be carefully preserved to preserve structural integrity, living samples must endure high light. Methods like fluorescent labeling are frequently used to improve contrast and make it easier to see certain cellular components [16]. Numerous disciplines, such as dermatology for in vivo skin imaging and corneal research for examining ocular microstructures, make extensive use of confocal microscopy. Its flexibility in clinical practice is demonstrated by its uses, which include tumor surveillance, presurgical assessments, and treatment response monitoring [17].

In Vivo Confocal Microscopy (IVCM) is used to diagnose illnesses of the animal eye's surface, including corneal dystrophies and keratitis. To comprehend disorders such as diabetic neuropathy and other neurodegenerative ailments,

IVCM helps measure corneal nerve density [18]. Confocal microscopy reduces the need for biopsies by providing non-invasive imaging for skin disorders. It makes it possible to examine removed tissues right away, which improves surgical results in dermatology [19].



*Figure 3. Functioning of the Confocal Microscope.*

### 2.1.4. Super-resolution Microscopy

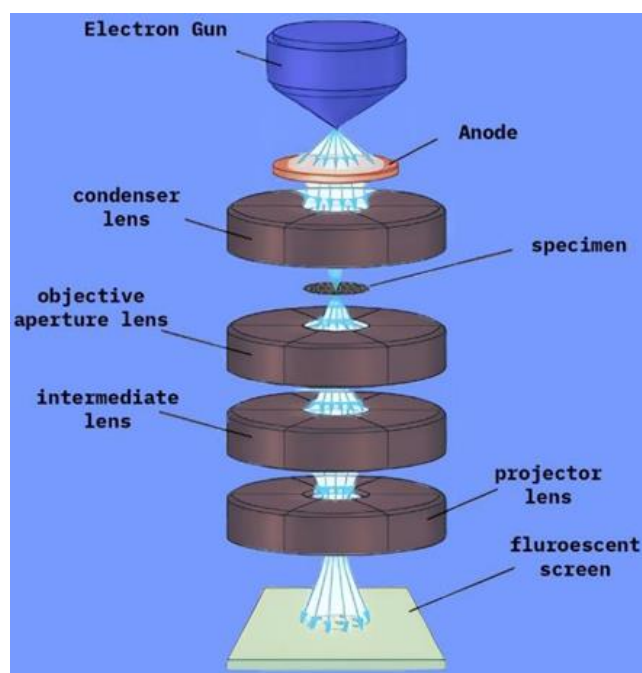
A variety of sophisticated imaging methods that go above the diffraction limit of traditional microscopy are included in super-resolution microscopy, which makes it possible to see subcellular structures in great detail. In order to enable high-resolution imaging in the biological and material sciences, this discipline has developed to encompass a variety of approaches, each with distinct benefits and uses. Advanced imaging techniques used in super-resolution microscopy provide sub-diffraction active regions for high-throughput imaging. It creates comprehensive super-resolution pictures of samples over time by using time delay integration and long-lived fluorophores with broad field and patterned illumination [20]. Super-resolution optical microscopy utilizes techniques like stimulated emission depletion (STED), photoactivated localization microscopy (PALM), Structured Illumination Microscopy (SIM), and Stepwise Optical Saturation (SOS) [21]. STED improves resolution above the diffraction limit by deactivating fluorophores outside of a core point using a depletion beam [22]. PALM achieves excellent spatial resolution by successively capturing individual fluorophores and using statistical fitting to pinpoint their exact positions [23]. To reconstruct high-resolution pictures, SIM uses structured lighting and merges numerous images, hence doubling the resolution [24]. SOS, this innovative method shows practical implementation

without complicated hardware by combining numerous raw fluorescence pictures to obtain a resolution enhancement proportional to the square root of the number of photos [25].

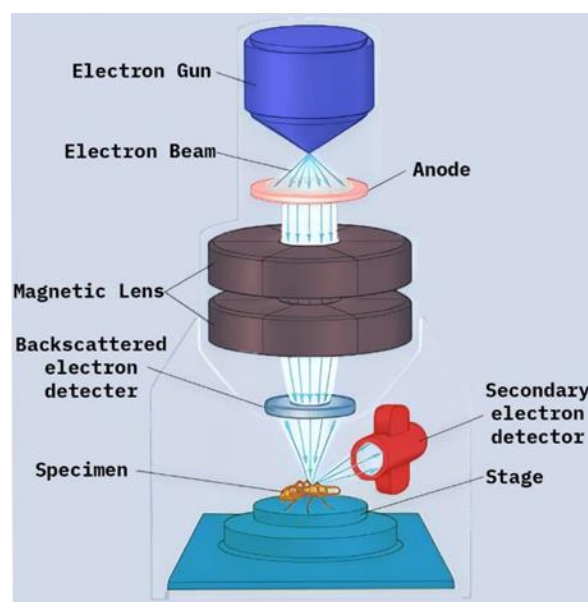
### 2.1.5. Electron Microscopy

In contrast to optical techniques, electron microscopy offers improved magnification and resolution by interacting with a sample using electron beams produced by an electron cannon. Usually, a heated filament emits electrons by thermionic emission, which are accelerated to create a beam [26]. The term "electron microscopy" refers to a group of methods that use electron beams to image and analyze materials at the nanoscale with great resolution.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are the two main techniques, and each has special benefits for characterizing materials. While TEM transfers electrons through thin samples to enable the study of interior structures, SEM scans the surface of specimens to produce pictures with an impressive depth of field [27]. Because it offers in-depth knowledge of material compositions and structures, electron microscopy is essential in many scientific and technological domains. Its uses, which range from medical research to nanotechnology, demonstrate its adaptability and significance in promoting knowledge and creativity [28]. To study the morphology and crystal structure of two-dimensional nanomaterials and promote nanotechnology breakthroughs, scanning electron microscopy (or SEM) is crucial [29]. By identifying microbes and examining ultrastructural variations in cells, electron microscopy helps diagnose gastrointestinal disorders. It improves the quality of pharmaceutical goods by verifying product formation and detecting flaws in medication formulations [30].



**Figure 4.** Transmission electron microscopy.



**Figure 5.** Scanning electron microscopy.

## 2.2. Image Acquisition and Metadata

The necessity for standardized image data formats to make data sharing and analysis easier has been acknowledged by the microscopy community. In order to overcome the difficulties caused by proprietary file formats, an open standard format for microscopy image data was proposed [31]. The plethora of image file types brought forth by digital microscopy has made it difficult for researchers to manage and analyze data. JPEG, TIFF, BMP, and raw data are examples of common formats; each has unique features and restrictions [32]. Some suggest solutions based on open-source formats like TIF and XMP in response to the growing demand for quantitative picture formats that can manage calibration parameters and formulae [33]. Common raster graphics formats used in microscopy images include TIFF, DICOM, JPEG, GIF, and BMP. Because TIFF supports uncompressed data, it is the format of choice for high-quality photographs. These formats store images as grids of pixels [34]. When moving data between software programs or making hard copies, converting photos between formats is frequently required, although it can occasionally be challenging. Selecting the right file formats is becoming more and more vital for effective data storage and analysis as digital imaging continues to play a significant role in microscopy [35].

### Understanding File Formats

The choice of image formats for microscopy, including PNG, JPEG, TIFF, BMP, PTU, DICOM, and GIF, significantly impacts data management, analysis, and interoperability in scientific research. Each format has unique characteristics that cater to different imaging needs and applications, and JPEG. These formats are widely used for their compression capabilities. While JPEG is lossy and better for smaller file sizes, meaning some image data is discarded during the compression process, it allows for significant file size reduction, making it ideal for transmitting and storing large images. PNG, on the other hand, is lossless and suitable for



high-quality images, ensuring that no image quality is lost during compression, making it ideal for preserving fine details [36]. TIFF: High-quality photographs can be stored in TIFF (Tag Image File Format), a flexible image file format. The main compression method used by TIFF is lossless compression, which ensures that no picture data is lost in the process. This guarantees that even after several adjustments and saves, the image quality will be flawless. TIFF is frequently utilized in digital pathology and microscopy because of its versatility and support for huge images, as well as its capacity to contain many layers and metadata [37]. BMP: One of the earliest and most basic picture file formats is BMP, or Bitmap Picture File. It is distinguished by its broad compatibility and simple construction. BMP is appropriate for a variety of picture formats since it usually employs lossless compression and provides a large color gamut [38]. PTU: PTU files, which are specifically made for fluorescent lifetime imaging microscopy, include intricate data structures, such as 3D stacks, which are necessary for sophisticated imaging methods [39]. DICOM: A worldwide standard called DICOM (Digital Imaging and Communications in Medicine) governs how medical pictures and related data are stored, transmitted, queried, retrieved, and displayed. X-rays, CT scans, MRIs, and ultrasounds are just a few of the medical images that are often managed and shared in healthcare settings. DICOM guarantees compatibility between various software programs and medical imaging equipment. To balance file size and picture quality, DICOM allows several compression methods, including both lossless and lossy compression. Rich metadata, including patient details, picture acquisition settings, and diagnostic results, is included in DICOM files [40].

On the other hand, certain formats' proprietary nature might make it difficult to share and interoperate data, which highlights the necessity of open standards like OME-TIFF to promote cooperation across various imaging systems [41].

For imaging data to be reproducible and usable, information extraction from microscope pictures is essential. To help with

this process, a number of frameworks and tools have been created to meet the requirements for thorough documentation and uniformity in microscopy procedures [42].

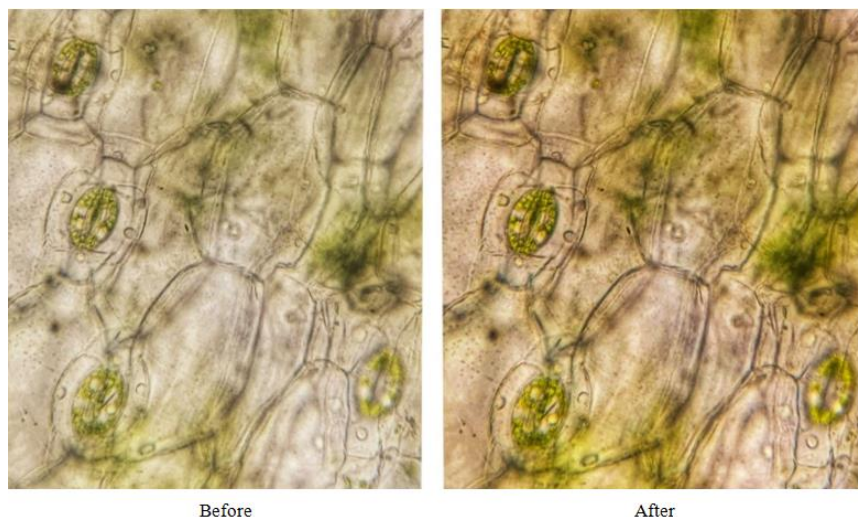
#### 1. Micro-Meta App

One well-known tool for this purpose is the Micro-Meta App, which complies with community-driven guidelines for microscopy metadata. This program improves user training in microscopy techniques while also making imaging experiment documentation easier. An open-source, user-friendly program called the Micro-Meta App was created to make gathering and maintaining microscopy information easier. For microscopy investigations to be both reproducible and interpretable, this instrument is essential. It was created as part of the 4D Nuclome collaboration and is intended to be highly interoperable and user-friendly. It automatically retrieves essential metadata from picture files, such as camera settings and device setups [43]. By offering a methodical approach to metadata gathering, it lessens the workload associated with quality assurance. Training individuals with little to no familiarity with microscopy benefits greatly from its visual interface [44].

#### 2. OMERO.mde

Integrated into the OMERO database, OMERO.mde is a tool for maintaining and changing the metadata of microscopic imaging data. Through effective metadata annotation and transmission, it supports the FAIR principles by making it easier to organize, visualize, and link imaging data. Because OMERO.mde is linked to the OMERO platform, researchers may effectively change and manage the metadata related to microscopy pictures. By ensuring that data is findable, accessible, interoperable, and reusable, it facilitates the harmonization of metadata and makes it simpler to adhere to FAIR principles. By thoroughly documenting imaging circumstances, it increases repeatability and data quality. It also makes it easier for researchers to collaborate and share data, which raises the total value of microscopy data [45].

### 2.3. Image Preprocessing



**Figure 6.** Before and after an image of stomata during image preprocessing.

Preprocessing microscopy images is crucial for improving picture quality and guaranteeing precise analysis in biological research. Numerous methods have been created to deal with typical problems, including blur, noise, and inconsistent lighting.

### 2.3.1. Noise Reduction

1. **Gaussian Filtering:** By using a Gaussian kernel to average pixel values, Gaussian filtering reduces high-frequency noise and smoothens images. Comparative tests have shown that it is very good against Gaussian noise, outperforming conventional filters in terms of maintaining image attributes. Despite its effectiveness, Gaussian filtering can cause edge blurring, which reduces information in important parts of the image [46].
2. **Wavelet Denoising:** By breaking down images into distinct frequency components, wavelet denoising enables targeted noise reduction without sacrificing a lot of detail [47]. Image quality is much enhanced and artifacts are greatly reduced when denoising models, like the physics-informed denoising diffusion probabilistic model (PI-DDPM), incorporate the physical principles of microscopy [48]. In recent years, wavelet transforms have been used with better threshold functions to reduce distortions such as the pseudo-Gibbs effect [49]. In medical imaging, where maintaining detail is crucial, this technique has demonstrated exceptional performance [50].

### 2.3.2. Background Correction

By precisely separating foreground and background elements, background correction in picture preprocessing is crucial for improving image quality. Numerous approaches have been created to deal with the difficulties caused by background noise and errors, especially in applications like scientific imaging, photography, and surveillance.

1. **Entropy Approximation:** A post-processing technique that enhances the F-score evaluation of Tensor MOG models by completing missing information in background modeling [51].
2. **Morphological Operations:** This technique achieves seamless background correction with low error by successfully reducing background underestimate in energy-dispersive x-ray fluorescence spectra [52].
3. **Mobile Applications:** Advanced techniques like color-based thresholding and semantic segmentation are used by tools like the Image Background Remover to enable accurate background removal, enabling real-time processing and user involvement [53].

### 2.3.3. Contrast Enhancement

For a variety of applications, picture preprocessing meth-

ods like contrast enhancement are essential for enhancing image quality. Among these methods, Contrast Stretching, Contrast Limited Adaptive Histogram Equalization (CLAHE), and Histogram Equalization (HE) are frequently used. To create a consistent histogram and improve overall contrast, HE redistributes an image's intensity values [54]. Images may appear odd because of over-enhancement and distortions caused by traditional HE [55]. By extending the range of intensity levels, this approach improves contrast and makes features in a picture more visible. Because it is less complicated than HE, it is frequently utilized in applications that call for rapid enhancement without requiring intricate calculations. AHE reduces distortions and preserves features by dividing the image into segments and applying HE locally [56]. By using local contrast enhancement, which helps maintain details in various areas of the image, CLAHE enhances standard HE. It may be applied to different kinds of images because it controls the improvement using factors like tile size and clip limit [57]. In medical imaging, CLAHE has demonstrated notable advantages by improving the visibility of important features without enhancing noise [58].

## 3. Image Analysis and Feature Extraction

### 3.1. Image Segmentation

By identifying anatomical features and areas of interest, image segmentation is frequently utilized in medical imaging applications. The viability of medical picture segmentation has been shown by comparing these methods with simulated findings. Making an accurate diagnosis depends on the segmentation process, which is one of the most critical stages of the automatic medical diagnosis based on the examination of the microscopic pictures. Due to intrinsic optical imperfections, image segmentation in microscopy is notoriously difficult, particularly in interference-based optical microscopy modalities [59]. Simulated differential interference (DIC) microscopy images and difficult genuine DIC images of cell populations were used to thoroughly validate the methods [60].

#### 3.1.1. Thresholding

In many applications, such as object recognition and illness detection, image segmentation using thresholding techniques—specifically, Otsu's method and adaptive thresholding—is essential. Otsu's approach is preferred because it is straightforward and efficient at differentiating objects from backgrounds, particularly in intricate situations. By maximizing inter-class variance, Otsu's method—a well-known thresholding technique used in picture segmentation—is especially good at differentiating objects from backgrounds. Numerous modifications have been made to this approach to

increase its performance in challenging situations like poor contrast and noise. It determines thresholds that optimize class variance, resulting in efficient segmentation in a variety of situations [61]. Otsu's method has demonstrated enhanced accuracy and robustness against noise when combined with other methods, such as the Chan-Vese level set methodology [62]. Otsu's approach in conjunction with the Modified Snake Optimizer (MSO) has shown improved segmentation outcomes and decreased processing expenses, especially in the study of satellite imagery [63].

Otsu's approach produced lower RMSE and higher PSNR, making it a better option for segmentation tasks, according to research comparing it with multilayer thresholding for flower photos [64]. When paired with mean denoising techniques, Otsu's method has been successfully applied to the identification of coffee plant diseases, attaining an accuracy level of 88% [65].

### 3.1.2. Region-based Segmentation

In microscopy image analysis, region-based segmentation is a crucial approach that divides images into discrete parts that correspond to biological features. Finding connected pixel sets is the main goal of this strategy, which can be accomplished in a number of ways, such as thresholding, region expanding, and morphological processing [66].

1. Watershed Method: Efficiently identifies areas based on local minima by using mathematical morphology to segment pictures by considering the intensity of pixels as a topographic surface.
2. Mean-Shift Method: A clustering method that groups pixels into clusters according to their intensity values and finds local density maxima [67].

### 3.1.3. Edge-based Segmentation

In microscopy image analysis, edge-based segmentation is an essential approach, especially for improving the precision of identifying particular features in pictures. This technique focuses on determining an object's borders by detecting variations in intensity, which is crucial for applications like biological research and medical diagnostics [68].

1. Canny Edge Detection: A popular method in picture segmentation, Canny edge detection is especially prized for its ability to detect edges while reducing noise. This technique is well known for performing better than other edge detection algorithms, which makes it the go-to option for a number of applications, such as facial recognition and the analysis of plant root images. The Canny algorithm, which is known for its exceptional performance, is frequently improved with methods like noise reduction and histogram equalization to increase edge identification and noise resistance [69].
2. Edge Information Block (EIB): By incorporating edge information into pre-existing segmentation frameworks, this technique dramatically increases accuracy

and real-time performance by up to 23.5% in some datasets [70].

## 3.2. Feature Extraction

In order to identify and quantify different cell structures, microscopy feature extraction is a crucial step in the analysis of complicated biological images. The effectiveness and precision of feature extraction in microscopy have been greatly improved by recent developments in automated systems and machine learning methodologies.

### 3.2.1. Morphological Feature Extraction Techniques

In many domains, but especially in biological research and medical imaging, the extraction of morphological traits, including size, shape, and texture, is essential. The precision and effectiveness of feature extraction procedures have been greatly increased by recent methodological developments, such as deep learning and unsupervised approaches. Tumor segmentation accuracy is increased to 97.8% by using methods like the Gray-Level Co-occurrence Matrix (GLCM) and Local Binary Patterns (LBP) to capture complex textures in medical photos [71]. By including geometric characteristics like eccentricity and circularity into segmentation frameworks, tumor borders may be more clearly distinguished [72].

Landmark-free shape analysis is made possible by the Morpho-VAE framework, which focuses on morphological characteristics that differentiate various biological forms, including the mandibles of primates [73].

### 3.2.2. Intensity-based Feature Extraction Techniques

A key method in microscopy is intensity-based feature extraction, which uses pixel intensity values to characterize picture features. This approach uses statistical metrics and sophisticated algorithms to improve the examination of a variety of materials, such as biological specimens and transformer oil [74].

Gray-Level Co-Occurrence Matrix (GLCM): This technique extracts statistical variables that describe textural aspects across time by analyzing the spatial connection between pixels in transformer oil photographs [75].

Modified Sigmoid Function and DCT: By increasing contrast and obtaining characteristics through singular value decomposition, this method improves electron microscopic pictures and reveals minute details of microscopic structures [76].

### 3.2.3. Texture Feature Extraction Technique

Medical image analysis and interpretation heavily rely on texture feature extraction in microscopy, especially using Haralick features and Gabor filters. While Gabor filters improve texture representation by capturing frequency and orientation information, Haralick features measure spatial



changes in pixel intensity, offering insights into tissue properties [77].

#### 1. Haralick Features

In brachytherapy, 3D dose distributions are characterized using Haralick properties such as homogeneity, contrast, and correlation, which show spatial variations in absorbed doses [78]. Haralick measurements in microdosimetry successfully distinguish particular energy distributions according to absorbed dosage and voxel size, suggesting its potential in radiation therapy [79]. Haralick characteristics have proven their dependability in clinical diagnostics by being used to differentiate malignant tumors from healthy tissue in MRI [80].

#### 2. Gabor Filters

Gabor filters are well known for improving image classification tasks by capturing texture information at different scales and orientations. Gabor filters and Haralick descriptors demonstrated competitive precision and accuracy in texture extraction technique evaluations for medical image retrieval systems [81].

### 3.3. Object Tracking

In biological and materials science research, microscopy object tracking is an essential technique that makes it possible to analyze dynamic cellular interactions and processes. This process is now much more accurate and efficient thanks to recent developments in automated tracking systems [82].

#### 3.3.1. Tracking Algorithms

1. **Kalman Filters:** By using motion models to predict the new position of tracked objects, Kalman filters make it easier to match new detections to tracks that already exist [83]. When estimating the trajectory of moving objects, Kalman filters work well, especially when there is noise and measurement error. To improve tracking accuracy, they employ a recursive predict-update approach [84]. By modifying parameters in response to initial tracking results, a two-stage adaptive Kalman filter enhances accuracy in cell tracking, attaining a high segmentation accuracy of 94.37% [85].
2. **Particle Filters:** Using a lower-dimensional state embedding, particle filters are excellent at tracking deformable linear objects (DLOs), enabling precise tracking even in the presence of severe occlusions. Particle filters are appropriate for real-time applications because they can preserve state variation while lowering computational complexity by utilizing auto-encoders [86].

#### 3.3.2. Cell Tracking in Time-lapse Microscopy

One of the most important methods for comprehending dynamic biological processes in time-lapse microscopy is cell tracking. Cell tracking across multiple frames is now much more accurate and efficient thanks to recent develop-

ments in computational techniques. Numerous strategies, such as deep learning and automated software tools, have been developed to deal with the difficulties in tracking cells, such as cell division and changes in appearance [87].

#### Automated Software Solutions

1. **Cell Tracker:** A user-friendly interface for managing and analyzing time-lapse microscopy data is provided by an open-source toolbox that automates cell segmentation and tracking [88].
2. **Random Sampling Algorithms:** These algorithms efficiently manage identity in transient signals by combining registration and tracking tasks; they are especially helpful in complex biological environments [89].

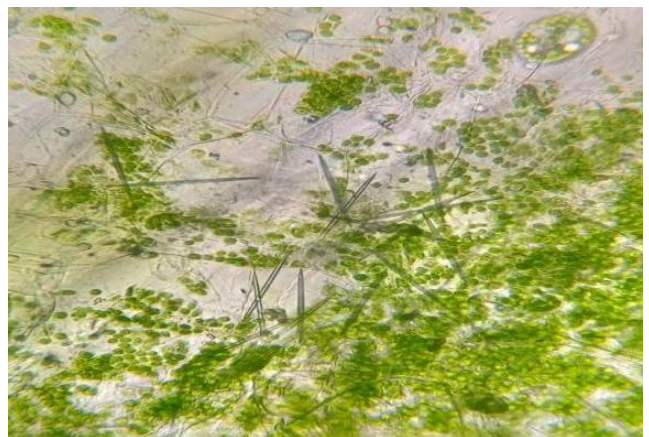
## 4. Quantitative Analysis and Data Visualization

### 4.1. Quantitative Analysis

#### 4.1.1. Cell Size and Shape Analysis



**Figure 7.** Cell shape and size help identify these cells as zebra fish gill cells.



**Figure 8.** Cell shape and size help identify these cells as chlorophyll



Microscopy-based quantitative assessments of cell size and shape are now crucial in many biological domains, especially developmental biology and ecology. By analyzing cellular structures and dynamics, these measurements allow researchers to gain a better understanding of ecological interactions and developmental processes [90].

#### Techniques for Cell Analysis

1. Image Analysis: Applied to measure cell morphology and microbial biomass, enabling comparisons between various ecosystems [91].
2. Intravital Multi-Photon Microscopy: Enables the study of cells in their natural habitats by capturing intricate cell behaviors and shapes.
3. Discrete Fourier Transforms (DFT): An innovative method for categorizing cell shapes that efficiently analyzes complex 3D shapes by converting them to 2D representations [92].

### 4.1.2. Intensity Measurement

Extracting useful biological data from microscopy requires quantitative measurements, especially intensity measurements like fluorescence intensity and optical density. These measurements can reveal information about cellular structures, biophysical characteristics, and molecular interactions [93].

#### 1. Fluorescence Intensity Measurements

The quantity of light released by fluorescently labeled molecules is measured by fluorescence intensity, which offers information on the abundance and location of proteins. It helps comprehend cellular dynamics and processes by counting biomolecules in cellular compartments [94]. The precision of measurements of fluorescence intensity can be greatly impacted by variations in illumination sources, detector technologies, and specimen positioning [95].

#### 2. Optical Density Measurements

The concentration of molecules in a specimen can be linked to the optical density, which quantifies the amount of light absorbed by the sample. Noninterferometric quantitative phase microscopy (NIQPM) is one technique that preserves sample integrity by measuring mass and density without staining [96]. Light scattering and sample thickness can affect optical density measurements, so careful experimental design is required [97].

### 4.1.3. Colocalization Analysis

In microscopy, colocalization analysis is essential for comprehending the spatial relationships among various molecular entities. These analyses are now more accurate and efficient thanks to recent methodological developments, especially in super-resolution microscopy [98].

Pearson's Correlation Coefficient (PCC): A popular statistical metric in microscopy for determining the strength of the connection between two fluorescent signals is the Pearson's Correlation Coefficient (PCC). Because it aids in determining if two biomolecules occupy the same spatial area, its use

in colocalization investigations is very important. However, depending on the geometry of the samples being examined and the pixel selection parameters, PCC's efficacy may differ [99]. It is used to measure how A- and B-type nuclear lamins interact, exposing segregation patterns in patients with laminopathy [100].

Maximum Entropy Copula (MEC): An important development in the analysis of fluorescence microscopy, especially for co-localization investigations, is the Maximum Entropy Copula (MEC). This technique quantifies spatial and nonlinear correlations between fluorescent markers by reliably estimating bivariate distributions using the Gaussian Copula in conjunction with the Maximum Entropy Method (MEM). This reliable technique increases the dependability of colocalization metrics by quantifying co- and anti-colocalization even in high background settings [101].

### 4.1.4. Spatial Distribution Analysis

Quantitative measurements in spatial distribution analysis cover a wide range of approaches and applications in diverse domains, such as histopathology, urban studies, and nuclear safety. These methods evaluate and interpret spatial patterns using statistical frameworks and computational techniques, offering insights into the underlying phenomena [102]. The capacity to extract useful spatial information from biological pictures has been greatly improved by recent developments in automated microscopy and computer image processing. In addition to helping to quantify biological variation, this study identifies hierarchical mechanisms that link specific cellular characteristics to more general biological activities. A strong foundation for examining cellular interactions in tissue samples is offered by spatial statistics, which makes it possible to quantify biological processes and neighbor relationships. These methods can account for tissue heterogeneity, improving the accuracy of statistical measures and enhancing the understanding of spatial processes in biological systems [103].

1. Dispersion Indices: A flexible method for measuring the spatial distribution of subcellular features, such as autophagic puncta and mitochondrial clustering, is the introduction of dispersion indices. This method makes it possible to analyze distribution patterns in a variety of biological situations, which helps to shed light on conditions like cancer and metabolic illnesses [104].
2. Probabilistic Spatial Analysis: Deep Bayesian learning is integrated into microscopy to provide probabilistic predictions that improve the accuracy of spatial studies and allow for uncertainty-aware cell identification. This technique enhances the understanding of biological data by enabling researchers to see geographical patterns that conventional deterministic methods could miss [105].
3. Density Distribution Maps: Density distribution maps (DDMs) are a new method for examining subcellular distributions, improving spatial mapping precision, and

lowering location uncertainty. This technique may be adjusted to different imaging resolutions, which makes it suitable for everyday laboratory usage and enables extensive research [106].

4.2. Statistical Analysis

In the field of cell biology microscopy, statistical analysis has improved dramatically, utilizing sophisticated computer methods to glean valuable insights from intricate imaging data. By using statistical techniques, cellular dynamics, morphology, and interactions are better understood, which eventually aids in biological research and medication creation [107].

4.2.1. Hypothesis Testing

In cell biology, hypothesis testing is essential, especially when examining data obtained through microscopy methods. It enables scientists to distinguish between random fluctuations in cellular behavior and different biological phenomena. It entails developing two opposing hypotheses: the research hypothesis, which proposes a particular effect or difference, and the null hypothesis, which asserts no effect or difference. The procedure usually produces a p-value, which shows the likelihood of seeing the data if the null hypothesis is correct. Typically, significance thresholds are set at  $p < 0.05$  [108].

NULL HYPOTHESIS - The new algorithm does not significantly reduce noise compared to the existing algorithm.			
	Image	Noise_Before	Noise_After
	Image_1	32.5	25.3
	Image_2	30.1	24.8
	Image_3	28.7	23.1
	Image_4	35.2	27.4
	Image_5	29.8	24.9
	T-STATISTICS =	5.6712	
	P-VALUE =	0.0054	
	The p-value (0.0054) is less than $\alpha$ .		
we reject the null hypothesis and conclude that the new algorithm significantly reduces noise compared to the existing method.			

Figure 9. Shows a sample hypothetical testing on images before and after noise reduction done by 2 different algorithms.

To improve drug screening procedures, sophisticated workflows, like those created for Cell Painting, compare the morphological profiles of treated and untreated cells using hypothesis testing [109]. Hypothesis testing is used by methods such as single-molecule localization microscopy (SMLM) to obtain quantitative information about cellular structures, which helps inform biological interpretations [110].

4.2.2. Correlation Analysis

In cell biology, correlation analysis is an essential tool for comprehending intricate biological systems, especially when it comes to gene regulation and cellular behavior. The construction of gene regulatory networks and the interpretation of cellular dynamics can both benefit from this analysis,

which can highlight connections between different cellular components [111].

Distance histograms are used by a correlation analysis framework for super resolution microscopy to quantify cellular structures and interactions, outperforming conventional techniques in situations such as image alignment and colocalization [112].

To overcome zero-value biases in single-cell RNA-sequencing data, a novel graph-based k-partitioning technique has been created, improving the identification of correlated gene pairs crucial for network building [113].

Cell clustering is quantitatively evaluated by pair-correlation functions, which provide information about collective behaviors. The results of these analyses are greatly impacted by the bandwidth selection, so it must be done carefully to capture pertinent features [114].

### 4.2.3. Principal Component Analysis

A strong statistical method for examining complicated data, especially in microscopy, is Principal Component Analysis (PCA), which is used extensively in cell biology. It is extremely useful for interpreting high-dimensional data from a variety of imaging modalities, including Brillouin microscopy and structured illumination microscopy (SIM), due to its capacity to reduce dimensionality while maintaining variance [115].

1. Brillouin Microscopy: By processing spectral data, PCA improves comprehension of the micromechanical characteristics of biological materials. It efficiently manages the intricacy of spectral overlaps and variations, enabling data reconstruction and sample composition insights [116].
2. Structured Illumination Microscopy (SIM): Real-time analysis with minimal photobleaching and phototoxicity is made possible by PCA-SIM's improved parameter estimation for super-resolution imaging. Because of its high accuracy and resilience to noise, this technique is appropriate for live-cell imaging [117].

By extracting essential features that clarify macromolecular dynamics, PCA makes it easier to analyze high-dimensional datasets, like those produced by protein molecular simulations [118]. By adding Bayesian variable selection, methods such as SuSiE PCA improve on traditional PCA, increasing computational speed and signal detection in the analysis of large biological datasets [119].

### 4.2.4. Cluster Analysis

Understanding molecular interactions and spatial organization in cell biology requires cluster analysis, especially when using single-molecule localization microscopy (SMLM). To examine clustering in SMLM data, several algorithms have been created; each has advantages and disadvantages of its own [120]. SMLM data is frequently clustered using algorithms like DBSCAN, ToMATo, and KDE; the performance of each algorithm varies depending on the underlying data distribution.<sup>[120]</sup> Machine-learning techniques that effectively classify large datasets, differentiate clustered from non-clustered points, and refine clusters for additional analysis are examples of recent developments [121]. The effectiveness of clustering algorithms has been evaluated systematically using metrics such as the Adjusted Rand Index (ARI) and Intersection over Union (IoU). By addressing issues like background noise and localization uncertainty, Bayesian approaches offer a reliable model for clustering analysis [122].

## 4.3. Data Visualization

The need for efficient data interpretation and improvements in imaging techniques has led to a significant evolution in the visualization of microscopy data in cell biology. This development includes genetically encoded imaging tools, interactive analytics, and high-throughput techniques,

all of which are intended to improve our comprehension of cellular dynamics.

### 3d Visualization

A deeper understanding of cellular architecture and dynamics is now possible thanks to the development of microscopy techniques, which have greatly improved the 3D visualization of cellular structures in cell biology. Numerous techniques have been developed, such as electron microscopy and super-resolution microscopy, each with special benefits for researching intricate cellular environments [123]. With a lateral resolution of less than 30 nm, the introduction of 4x and 12x 3D-ExM enables reliable super-resolution imaging. This technique makes it possible to visualize nanoscale structures such as nuclear pore complexes and chromosomes, which makes it suitable for a variety of cell biology studies [124].

Despite its high sensitivity, fluorescence microscopy has drawbacks such as photobleaching. By predicting organelle locations in three dimensions, the 3DCNAS method improves cell dynamics analysis [125]. Organelles and their spatial distribution within cells can be thoroughly analyzed structurally thanks to methods like electron tomography and 3D scanning electron microscopy. High-resolution insights are provided by these techniques, but frequently at the expense of the volume examined [126]. Confocal microscopy data can be better interpreted on standard PCs thanks to new mathematical techniques for 3D reconstruction that enhance the visualization of intricate cell structures [127].

## 5. Computational Tools and Software

The analysis and interpretation of cellular data has been completely transformed by the incorporation of computational tools and software in cell biology. These developments make it easier to study intricate biological processes, improve image analysis, and comprehend how cells interact [128].

### 5.1. Image Processing Software

Software for processing images from microscopy is essential to cell biology because it makes it easier to analyze intricate cellular structures and behaviors. By automating and streamlining the processing of microscopy images, these software programs help researchers glean valuable insights from sizable datasets [129].

#### 5.1.1. ImageJ/Fiji

Through a variety of plugins and macros, ImageJ/Fiji is a potent tool in cell biology that makes it easier to analyze intricate cellular behaviors and structures [130]. By improving the classification and quantification of cellular components, these tools help researchers extract valuable information from microscopy images. Protein puncta in linear cellular structures can be reliably identified and quantified thanks to the Fiji macro created by Hulsey-Vincent et al. It provides reproducible data analysis in an open-source format by measuring pa-



rameters like intensity, density, and full-width at half-maximum (FWHM) [131]. Another tool from Fiji, Lusca, uses machine learning to automatically segment and analyze cellular structures. Compared to conventional techniques, it provides 29 morphometric parameters, greatly increasing the analysis's speed and accuracy [132]. Without the need for programming knowledge, the Cell-Type Analyzer plugin simplifies the classification of cells according to user-specified criteria, enabling effective analysis of numerous images. The precise identification of different cell types is facilitated by this modular approach [133].

### 5.1.2. Matlab

Many scientific and engineering fields use MATLAB, a potent computational tool, for data analysis, modeling, and visualization. It is essential for researchers and practitioners because of its user-friendly environment, which makes complex numerical computations easier [134]. MATLAB is excellent at statistical analysis, curve fitting, spectral analysis, and managing complicated datasets [135]. The presentation of experimental data and simulation results is improved by

MATLAB's simple commands for making two- and three-dimensional plots [136]. Through techniques like noise reduction, filtering, and histogram manipulation, MATLAB offers tools for improving image quality [137]. Numerous filters, including Gaussian low pass and Butterworth filters, have been used; the Butterworth filter performs better in terms of execution time and output [138]. Real-time image processing made possible by MATLAB's integration with industrial robotics enhances the precision of dynamic material tracking on conveyor belts [139]. MATLAB-based tools facilitate quantitative analysis of tissue properties in biomedical research, improving our comprehension of biological processes [140]. In order to ensure privacy when sharing images, MATLAB also facilitates the creation of applications that integrate data security features like image encryption and steganography [141].

### 5.1.3. Python

In the area of image processing for microscopy, Python has become a potent tool that improves data collection, analysis, and visualization.

```

1  import cv2
2  import numpy as np
3  from matplotlib import pyplot as plt
4
5  def process_microscopy_image(image_path):
6      # Load the image
7      image = cv2.imread(image_path)
8      if image is None:
9          print("Error: Image not found!")
10         return
11     # Resize for easier processing (optional)
12     resized_image = cv2.resize(image, (512, 512))
13     # Convert to grayscale
14     gray_image = cv2.cvtColor(resized_image, cv2.COLOR_BGR2GRAY)
15     # Enhance contrast using histogram equalization
16     enhanced_image = cv2.equalizeHist(gray_image)
17     # Apply Gaussian blur to reduce noise
18     blurred_image = cv2.GaussianBlur(enhanced_image, (5, 5), sigmaX=0)
19     # Thresholding for segmentation
20     _, binary_image = cv2.threshold(blurred_image, thresh=128, maxval=255, cv2.THRESH_BINARY | cv2.THRESH_OTSU)
21     # Edge detection using Canny
22     edges = cv2.Canny(binary_image, 50, 150)
23     # Display the images
24     titles = ['Original', 'Grayscale', 'Enhanced', 'Blurred', 'Binary', 'Edges']
25     images = [resized_image, gray_image, enhanced_image, blurred_image, binary_image, edges]
26     plt.figure(figsize=(12, 8))
27     for i in range(len(images)):
28         plt.subplot(2, 3, i + 1)
29         if len(images[i].shape) == 2: # Grayscale
30             plt.imshow(images[i], cmap='gray')
31         else: # Color
32             plt.imshow(cv2.cvtColor(images[i], cv2.COLOR_BGR2RGB))
33         plt.title(titles[i])
34         plt.axis('off')
35     plt.tight_layout()
36     plt.show()
37
38     # Path to the microscopy image
39     image_path = "microscopy_image.jpg"
40     process_microscopy_image(image_path)

```

**Figure 10.** A Python application that uses the well-known OpenCV and NumPy modules to prepare a picture for microscopy. The application may segment regions of interest, apply denoising filters, and improve contrast [142].

Six processed variants of the input image will be shown by the program: Original image, Grayscale image, Contrast-enhanced image, Denoised (blurred) image, Thresholded binary image, Edges detected using the Canny algorithm [143].

Its incorporation into a number of microscopy software programs and libraries enables researchers to take advantage of sophisticated processing methods and optimize workflows. The integration of Python scripting into Gatan Microscopy Suite (GMS) has improved live processing capabilities by allowing users to manipulate data and images in real-time. Python's versatility in various microscopy contexts is demonstrated by libraries like the one for digital holographic microscopy, which offer crucial computational tools for reconstructing holograms [144]. Effective image processing is made possible by the Montage toolkit, which also enables the creation of mosaics that preserve calibration fidelity—a critical aspect of microscopy. Quantization is used in cutting-edge charged particle microscopy techniques to reduce image acquisition errors, demonstrating Python's contribution to the creation of complex algorithms [145]. Python makes it possible to retrieve and alter image sub-regions according to resolution metrics, facilitating sophisticated image processing techniques in digital pathology [146].

## 5.2. Machine Learning and Deep Learning

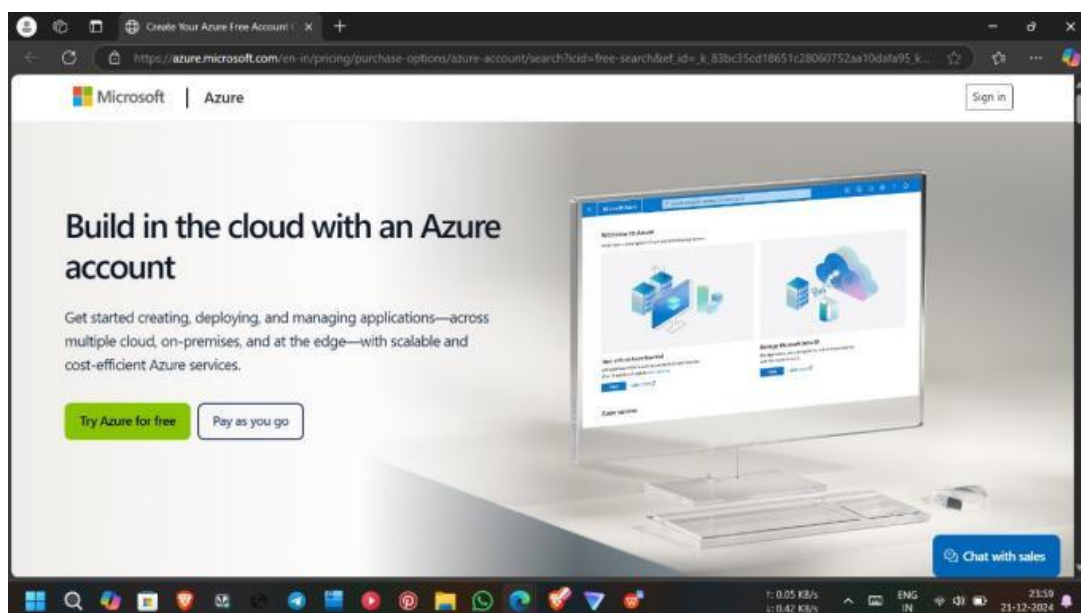
Microscopy image processing is being revolutionized by machine learning (ML), which improves data analysis efficiency, accuracy, and automation [147]. This change is especially noteworthy in the life sciences, where machine learning techniques enhance image interpretation quality and make it easier to explore intricate visual patterns [148]. By automating time-consuming procedures, machine learning algorithms free up researchers to concentrate on analysis rather than tedious

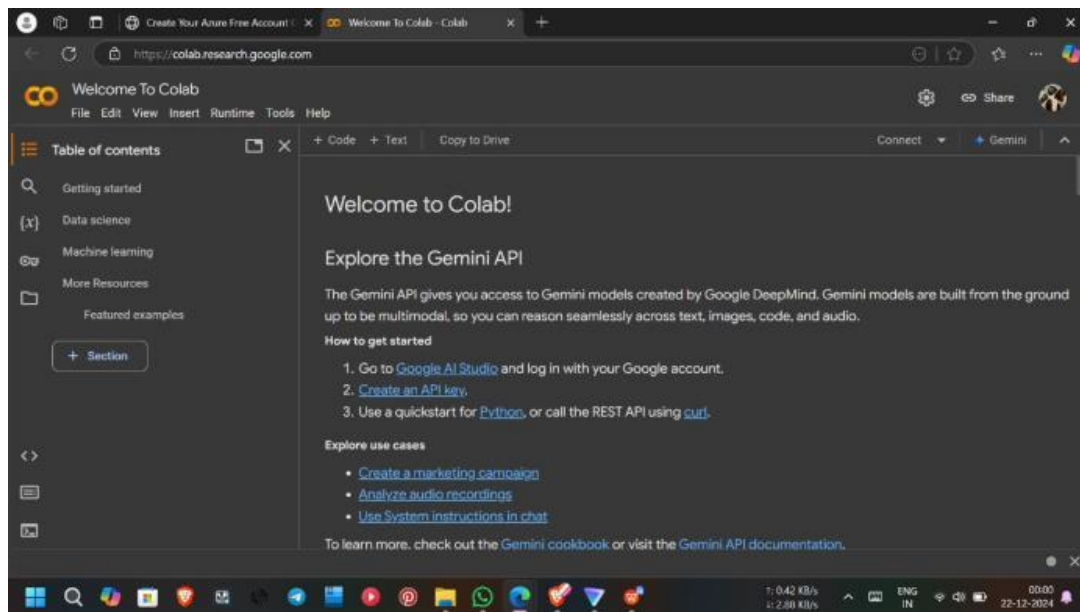
image processing. ML is integrated into data-driven microscopy to dynamically optimize imaging parameters, improving speed and resolution while reducing phototoxicity [149]. Code-free machine learning platforms increase accessibility by allowing biologists to use sophisticated algorithms without the need for programming knowledge [150].

The qualities of the input data have a significant impact on the efficacy of ML models, so careful dataset selection and curation are required [151]. Significant computational resources are needed for high-performance machine learning models, which can present memory and processing time issues [152]. Unseen data may cause problems for models trained on particular datasets, underscoring the necessity of reliable training techniques [153].

## 5.3. Cloud-based Platform

Because cloud-based platforms offer scalable, effective, and affordable solutions, they have drastically changed image processing in microscopy. Large datasets and intricate calculations can be handled with these platforms, which is crucial for applications involving microscopy [154]. For example, Google Collaboratory, Amazon Web Services (AWS), Microsoft Azure. Large volumes of microscopy images can be processed using cloud platforms' on-demand resources, which eliminates the need for substantial local infrastructure. Techniques like GPU computing speed up processing, detecting image forgeries up to 6.5 times faster [155]. This can be used for quick analysis in microscopy. Strong security protocols, such as secure multi-party computation and homomorphic cryptosystems, are essential for safeguarding private microscopy data in cloud settings [156]. Fuzzy C-means Clustering and other machine learning techniques are integrated to improve data classification while preserving security [157].





**Figure 11.** Cloud-based systems have fundamentally altered image processing in microscopy by providing scalable, efficient, and reasonably priced solutions. For instance, Microsoft Azure (L) and Google Collaboratory R [158].

Applications like illness diagnosis and treatment planning are supported by cloud-based image processing, which is used in a variety of domains, including medical imaging [159].

## 6. Emerging Trends and Future Directions

### 6.1. Artificial Intelligence and Machine Learning

By improving image analysis and providing fresh perspectives on cellular dynamics, artificial intelligence (AI) and machine learning (ML) are transforming microscopy in cell biology [161]. By making it easier to process large volumes of data, these technologies enhance the precision and effectiveness of microscopy methods [162]. Large microscopy volumes can be processed in real time using AI techniques like Adaptive Particle Representation, which gets around the drawbacks of traditional pixel grids. Cellular structure segmentation, classification, and tracking are accomplished through the use of deep learning models, which greatly enhance quantitative analysis of dynamic processes. Automation powered by AI expedites the analysis of microscopy images, enabling advancements in precision medicine and drug development. Atomic force microscopy (AFM) analysis is improved by machine learning, which opens up the technique for clinical use in cancer diagnosis [163].

### 6.2. Multimodal Imaging

Through the integration of many imaging techniques, mul-

Multiple image processing algorithms can be integrated thanks to cloud services' flexibility, which raises the general caliber and dependability of microscopy analyses [160]. timodal imaging in microscopy greatly improves the study of cellular biology by offering thorough insights into cellular structures and activities. A greater comprehension of biological processes and disease causes is made possible by this method, which enables researchers to extract several kinds of information from a single sample [164].

By combining optical fluorescence microscopy (OFM) with X-ray fluorescence microscopy (XFM), specificity and sensitivity may be increased while studying molecular interactions and the impact of native components on cells. Label-free quantitative phase microscopy and fluorescence imaging are combined in a multimodal platform to enable dynamic and highly selective cellular investigation.<sup>[164]</sup> The combination of digital holographic microscopy with flow cytometry enables high-throughput investigation of living cells, enhancing the detection of cellular structures and recording biophysical properties.

Accurate analysis requires that pictures from many modalities be aligned. Multimodal microscopy image registration is improved by a new branch-and-bound method, which also increases matching quality and throughput.<sup>[166]</sup> A comprehensive picture of molecular processes is offered by multimodal mass spectrometry imaging, which is essential for researching intricate biological models such as three-dimensional cell cultures [165].

### 6.3. Quantitative Biology

Through the use of quantitative biology in microscopy, our knowledge of cellular structures and functions has greatly ex-



panded. This method improves the investigation of cellular dynamics and morphology by using a variety of imaging techniques to derive exact data from biological materials [166].

#### Quantitative Microscopy Techniques

1. By measuring phase shifts in light as it passes through cells using optical interference, quantitative phase microscopy (QPM) enables label-free imaging of a variety of cell types, including immune and cancerous cells [167].
2. Real-time monitoring of cellular functions is made possible by live cell imaging, which offers insights into gene expression and the behavior of cells over time.

In order to get a better knowledge of biological systems, quantitative microscopy has proven essential in the study of cellular responses to stimuli and the evolution of illness. It is anticipated that integrating AI and machine learning into imaging methods would improve the capacity for data interpretation and analysis [168].

## 6.4. Virtual And Augmented Reality

Cell biology microscopy is being transformed by virtual and augmented reality (VR/AR) technologies, which improve visibility, engagement, and learning results. These immersive technologies overcome the drawbacks of conventional 2D imaging and provide a more thorough knowledge of cellular dynamics and architecture [169]. Through real-time interaction with volumetric data, VR and AR make it easier to explore intricate 3D cellular structures. Different cell types may be manipulated in immersive situations, which improves learning and fosters deeper comprehension [170]. Through captivating, game-like experiences, virtual reality (VR)-based learning resources have been created to educate cellular operations, including the cell membrane [171]. Because AR technologies offer accessible and interactive content, they have demonstrated notable increases in learning outcomes, especially in STEM education [172].

## 7. Conclusion

A fundamental component of contemporary cell biology, microscopy-based data processing offers crucial instruments for deriving meaningful conclusions from intricate biological images. The most recent techniques, such as picture acquisition, preprocessing, analysis, feature extraction, and data visualization, are thoroughly reviewed in this chapter. It highlights the special powers of light and electron microscopy and stresses how crucial preprocessing methods like background correction and noise reduction are to the clarity of images. Extensive segmentation and feature extraction techniques are described, demonstrating how well they can identify cellular structures. The chapter also discusses computer technologies that automate these operations and emphasizes the need for quantitative analysis and data visualization in making complex data interpretable. With regard to the future, it examines cutting-edge developments like artificial intelligence, multimodal

imaging, and immersive visualization technologies, highlighting their revolutionary potential. In summary, this chapter provides a thorough review of current approaches and potential future directions in microscopy-based data processing, making it a vital resource for cell biologists. It seeks to stimulate more creativity and cross-disciplinary cooperation, which will ultimately improve our knowledge of cellular biology and spur scientific advancement in this quickly developing subject. The future of cell biology has the prospect of revealing new levels of understanding and knowledge via persistent research and technological development.

## Abbreviations

CLSM	Confocal Laser Scanning Microscopy
TIRF	Total Internal Reflection Fluorescence Microscopy
STED	Stimulated Emission Depletion Microscopy
STORM	Stochastic Optical Reconstruction Microscopy
PALM	PhotoActivated Localization Microscopy
FRET	Förster Resonance Energy Transfer
FRAP	Fluorescence Recovery After Photobleaching
ROI	Region of Interest
PSF	Point Spread Function
FIJI	Fiji Is Just ImageJ

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## Conflicts of Interest

The authors declare no conflicts of interest.

## References

- [1] T., A., Sims., Q., Wang. (2022). Microscopy. <https://orcid.org/10.1093/hesc/9780198831228.003.0007>

- [2] Morris JD, Payne CK. Microscopy and Cell Biology: New Methods and New Questions. *Annu Rev Phys Chem.* 2019 Jun 14; 70: 199-218.  
<https://orcid.org/10.1146/annurev-physchem-042018-052527>
- [3] Hooke R. Micrographia: or, some physiological descriptions of minute bodies made by magnifying glasses. 1st ed. London: J. Martyn and J. Allestry; 1665.
- [4] Mattiazzi Usaj M, Styles EB, Verster AJ, Friesen H, Boone C, Andrews BJ. High-Content Screening for Quantitative Cell Biology. *Trends Cell Biol.* 2016 Aug; 26(8): 598-611.  
<https://orcid.org/10.1016/j.tcb.2016.03.008>
- [5] Brian, J., Ford, Hon, FRMS, Hon, FLS. (2023). Discovery with the Light Microscope.  
<https://orcid.org/10.1002/9781119788232.ch1>
- [6] Timothy, J., Hawkins. (2023). Light Microscopy Technologies and the Plant Cytoskeleton.  
[https://orcid.org/10.1007/978-1-0716-2867-6\\_28](https://orcid.org/10.1007/978-1-0716-2867-6_28)
- [7] Anatoly, K., Khitrin., Jonathan, C., Petruccielli., Michael, A., Model. (2017). Bright-Field Microscopy of Transparent Objects: A Ray Tracing Approach.. *Microscopy and Microanalysis*,  
<https://orcid.org/10.1017/S1431927617012624>
- [8] Shuai, Gao., Jianxuan, Xiong., Ali, K., Yetisen., Félix, Salazar-Bloise., Alexander, Koch., Xinghua, Yang., Shengjia, Wang. (2023). Vector Differential Interference Contrast Microscopy Based on a 3-in-1 Phase Mask through a Dynamic Diffractive Optical Element. *ACS Photonics*,  
<https://orcid.org/10.1021/acsp Photonics.3c01459>
- [9] Catherine, H., Kaschula., Dirk, M., Lang., M., Iqbal, Parker. (2012). Live In-Cell Visualization of Proteins Using Super Resolution Imaging. <https://orcid.org/10.5772/37948>
- [10] Ghiran I. C. (2011). Introduction to fluorescence microscopy. *Methods in molecular biology* (Clifton, N. J.), 689, 93–136.
- [11] Brian, Herman. (1998). 3. Fluorescence Microscopy. *Current protocols in cell biology*,  
<https://orcid.org/10.1002/0471143030.cb0402s13>
- [12] Renz M. (2013). Fluorescence microscopy-a historical and technical perspective. *Cytometry. Part A: the journal of the International Society for Analytical Cytology*, 83(9), 767–779.
- [13] Jiayu, Li. (2023). Principles and applications of fluorescent probe imaging technology.  
<https://orcid.org/10.54254/2753-8818/6/20230139>
- [14] Coling, D., & Kachar, B. (2001). Principles and application of fluorescence microscopy. *Current protocols in molecular biology*, Chapter 14.
- [15] Sanderson, M. J., Smith, I., Parker, I., & Bootman, M. D. (2014). Fluorescence microscopy. *Cold Spring Harbor protocols*, 2014(10), pdb. top071795.
- [16] Zimmer M. (2009). GFP: from jellyfish to the Nobel prize and beyond. *Chemical Society reviews*, 38(10), 2823–2832.
- [17] Elliott A. D. (2020). Confocal Microscopy: Principles and Modern Practices. *Current protocols in cytometry*, 92(1), e68.
- [18] Nwaneshiudu, A., Kuschal, C., Sakamoto, F. H., Anderson, R. R., Schwarzenberger, K., & Young, R. C. (2012). Introduction to confocal microscopy. *The Journal of investigative dermatology*, 132(12), e3.
- [19] Shahriari, N., Grant-Kels, J. M., Rabinovitz, H., Oliviero, M., & Scope, A. (2021). Reflectance confocal microscopy: Principles, basic terminology, clinical indications, limitations, and practical considerations. *Journal of the American Academy of Dermatology*, 84(1), 1–14.
- [20] Chiang, J. C. B., Roy, M., Kim, J., Markoulli, M., & Krishnan, A. V. (2023). In-vivo corneal confocal microscopy: Imaging analysis, biological insights and future directions. *Communications biology*, 6(1), 652.
- [21] Rakhe, Jayamohan. (2022). Confocal microscopy – Working principle and applications in dermatology. *Journal of Skin and Sexually Transmitted Diseases*,  
[https://orcid.org/10.25259/jsstd\\_23\\_2022](https://orcid.org/10.25259/jsstd_23_2022)
- [22] Sigal, Y. M., Zhou, R., & Zhuang, X. (2018). Visualizing and discovering cellular structures with super-resolution microscopy. *Science* (New York, N. Y.), 361(6405), 880–887.
- [23] Yang, Z., Samanta, S., Yan, W., Yu, B., & Qu, J. (2021). Super-resolution Microscopy for Biological Imaging. *Advances in experimental medicine and biology*, 3233, 23–43.
- [24] Otomo, K., Hibi, T., Kozawa, Y., & Nemoto, T. (2015). STED microscopy--super-resolution bio-imaging utilizing a stimulated emission depletion. *Microscopy* (Oxford, England), 64(4), 227–236.
- [25] Owen, D. M., Magenau, A., Williamson, D. J., & Gaus, K. (2013). Super-resolution imaging by localization microscopy. *Methods in molecular biology* (Clifton, N. J.), 950, 81–93.
- [26] Amjadian, M., Mostafavi, S. M., Chen, J., Kavehvash, Z., Zhu, J., & Wang, L. (2021). Super-Resolution Photoacoustic Microscopy Using Structured-Illumination. *IEEE transactions on medical imaging*, 40(9), 2197–2207.
- [27] Zhang, Y., Nallathamby, P. D., Vigil, G. D., Khan, A. A., Mason, D. E., Boerckel, J. D., Roeder, R. K., & Howard, S. S. (2018). Super-resolution fluorescence microscopy by stepwise optical saturation. *Biomedical optics express*, 9(4), 1613–1629.
- [28] Volkmann, N., & Hanein, D. (2003). Electron microscopy. *Methods of biochemical analysis*, 44, 115–133.
- [29] Zhao, J., Yu, X., Shentu, X., & Li, D. (2024). The application and development of electron microscopy for three-dimensional reconstruction in life science: a review. *Cell and tissue research*, 396(1), 1–18.
- [30] Treder, K. P., Huang, C., Kim, J. S., & Kirkland, A. I. (2022). Applications of deep learning in electron microscopy. *Microscopy* (Oxford, England), 71(Supplement\_1), i 100–i 115.
- [31] Lametschwandtner, A., Lametschwandtner, U., & Weiger, T. (1984). Scanning electron microscopy of vascular corrosion casts--technique and applications. *Scanning electron microscopy*, (Pt 2), 663–695.

- [32] Sorzano, C. O., Jonic, S., Cotteville, M., Larquet, E., Boisset, N., & Marco, S. (2007). 3D electron microscopy of biological nanomachines: principles and applications. *European biophysics journal: EBJ*, 36(8), 995–1013.
- [33] Linkert, M., Rueden, C. T., Allan, C., Burel, J. M., Moore, W., Patterson, A., Loranger, B., Moore, J., Neves, C., Macdonald, D., Tarkowska, A., Sticco, C., Hill, E., Rossner, M., Eliceiri, K. W., & Swedlow, J. R. (2010). Metadata matters: access to image data in the real world. *The Journal of cell biology*, 189(5), 777–782.
- [34] Bode, M. (2004). A Few Thoughts About Image File Storage. *Microscopy Today*, 12(1), 26-29.
- [35] Moldovan, G., & Zabel, M. (2020). Quantitative Image Format for Electron Microscopy. *Microscopy and Microanalysis*, 26(S2), 1176-1178.
- [36] Shaw, S. L., & Hinchcliffe, E. H. (2013). 65,000 shades of grey: use of digital image files in light microscopy. *Methods in cell biology*, 114, 317–336.
- [37] Bailey, J. (1994). Converting Microscopy Images Into Other Formats. *Microscopy Today*, 2(5), 6-7.
- [38] Carson, G. S. (1997). Standards pipeline PNG, VRML 97, BIFF, imaging standards. *ACM SIGGRAPH Computer Graphics*, 31(3), 18-20.
- [39] Swedlow, J. R. (2007). The Open Microscopy Environment: A collaborative data modeling and software development project for biological image informatics. In *Imaging cellular and molecular biological functions* (pp. 71-92). Berlin, Heidelberg: Springer Berlin Heidelberg.
- [40] Bourke, P. (1998). Bmp image format. *BMP Files*. July, 8.
- [41] Cornelia, Wetzker. (2023). Example of Fluorescence Lifetime Imaging Microscopy (FLIM) image stack in. ptu format. <https://orcid.org/10.5281/zenodo.7656539>
- [42] Clunie, D. A. (2021). DICOM format and protocol standardization—a core requirement for digital pathology success. *Toxicologic Pathology*, 49(4), 738-749.
- [43] Blackburn, C., Allan, C., Besson, S., Burel, J. M., Carroll, M., Ferguson, R. K., & Swedlow, J. R. (2016, July). The Open Microscopy Environment: open image informatics for the biological sciences. In *Software and Cyberinfrastructure for Astronomy IV* (Vol. 9913, pp. 823-830). SPIE.
- [44] Rigano, A., Ehmsen, S., Ozturk, S. U., Ryan, J., Balashov, A., Hammer, M., & Strambio-De-Castillia, C. (2021). Micro-Meta App: an interactive software tool to facilitate the collection of microscopy metadata based on community-driven specifications. *BioRxiv*, 2021-05.
- [45] Mukaddem, K. T., Beard, E. J., Yildirim, B., & Cole, J. M. (2019). ImageDataExtractor: a tool to extract and quantify data from microscopy images. *Journal of chemical information and modeling*, 60(5), 2492-2509.
- [46] Kunis, S., Hänsch, S., Schmidt, C., Wong, F., Strambio-De-Castillia, C., & Weidtkamp-Peters, S. (2021). MDEmic in a use case for microscopy metadata harmonization: facilitating FAIR principles in practical application with metadata annotation tools. *arXiv preprint arXiv: 2103.02942*.
- [47] Ryan, J., Pengo, T., Rigano, A., Llopis, P. M., Itano, M. S., Cameron, L., & Brown, C. M. (2021). MethodsJ2: a software tool to improve microscopy methods reporting. *BioRxiv*, 2021-06.
- [48] Pepper, J., Senin, A., Jebbia, D., Breen, D., & Greenberg, J. (2022, December). Metadata verification: A workflow for computational archival science. In *2022 IEEE International Conference on Big Data (Big Data)* (pp. 2565-2571). IEEE.
- [49] Weli, M. M., & Abdullah, O. M. (2024). Digital Image Noise Reduction Based on Proposed Smoothing and Sharpening Filters. *The Indonesian Journal of Computer Science*, 13(4).
- [50] Deng, J., Yan, M., Wang, X., & Bao, J. (2024, June). Image Denoising Algorithm Based on Gaussian-Pepper Noise. In *2024 4th International Conference on Machine Learning and Intelligent Systems Engineering (MLISE)* (pp. 16-19). IEEE.
- [51] Li, R., della Maggiora, G., Andriasyan, V., Petkidis, A., Yushkevich, A., Kudryashev, M., & Yakimovich, A. (2023). Microscopy image reconstruction with physics-informed denoising diffusion probabilistic model. *arXiv preprint arXiv: 2306.02929*.
- [52] Li, B., Cong, Y., & Mo, H. (2024). Image denoising method integrating ridgelet transform and improved wavelet threshold. *PLoS One*, 19(9), e0306706.
- [53] Taassori, M. (2024). Enhanced Wavelet-Based Medical Image Denoising with Bayesian-Optimized Bilateral Filtering. *Sensors*, 24(21), 6849.
- [54] Trung, T. T., & Ha, S. V. U. (2022, December). Post processing algorithm for background subtraction model based on entropy approximation and style transfer neural network. In *2022 RIVF International Conference on Computing and Communication Technologies (RIVF)* (pp. 422-427). IEEE.
- [55] Zhao, W., Ai, X., Xiao, X., Xiao, W., Qi, S., Li, J., & Lei, W. (2024). A background correction method for energy-dispersive x-ray fluorescence spectra based on morphological operation. *X-Ray Spectrometry*, 53(1), 27-37.
- [56] SenthilPandi, S., Paulraj, D., & Kumar, N. (2023, November). A Novel Approach for Image Background Elimination. In *2023 International Conference on Research Methodologies in Knowledge Management, Artificial Intelligence and Telecommunication Engineering (RMKMATE)* (pp. 1-6). IEEE.
- [57] Jiang, C., Chen, T., Lu, C., Wu, Z., Liu, C., Shao, M., & Cao, J. (2023, April). Automatic Inhomogeneous Background Correction for Spatial Target Detection Image Based on Partition Processing. In *Photonics* (Vol. 10, No. 4, p. 433). MDPI.
- [58] Bataineh, B. (2023). Image contrast enhancement for preserving entropy and image visual features. *International Journal of Advances in Intelligent Informatics*, 9(2).
- [59] Manjunath, A., Yatnalli, V., & Bhusare, S. S. (2023). Performance analysis of graph theory-based Contrast limited adaptive histogram equalization for image enhancement. *WSEAS Transactions on Systems*, 22, 219-230.



- [60] Janani, V., & Shanthi, C. (2023, December). Infrared Image Enhancement Using Contrast Limited Adaptive Histogram Equalization and Denoising Convolution Neural Network. In 2023 12th International Conference on System Modeling & Advancement in Research Trends (SMART) (pp. 3-6). IEEE.
- [61] Borra, S. R., Tejaswini, N. P., Malathy, V., Kumar, B. M., & Habelalmateen, M. I. (2023, November). Contrast Limited Adaptive Histogram Equalization based Multi-Objective Improved Cat Swarm Optimization for Image Contrast Enhancement. In 2023 International Conference on Integrated Intelligence and Communication Systems (ICIICS) (pp. 1-5). IEEE.
- [62] Haj-Hassan, H., Chaddad, A., Tanougast, C., & Harkouss, Y. (2015, April). Comparison of segmentation techniques for histopathological images. In 2015 Fifth International Conference on Digital Information and Communication Technology and its Applications (DICTAP) (pp. 80-85). IEEE.
- [63] Obuchowicz, A., Hrebien, M., Nieczkowski, T., & Marciniak, A. (2008). Computational intelligence techniques in image segmentation for cytopathology. *Computational intelligence in biomedicine and bioinformatics: current trends and applications*, 169-199.
- [64] Li, K., & Kanade, T. (2009, July). Nonnegative mixed-norm preconditioning for microscopy image segmentation. In International Conference on Information Processing in Medical Imaging (pp. 362-373). Berlin, Heidelberg: Springer Berlin Heidelberg.
- [65] Chiranjeevi, K., Naidu, M. S. R., Mohan, G. K., Manohar, V., Gottapu, S. K., & Indugupalli, A. K. (2024). A Novel Optimization Algorithm for Otsu's Entropy-Based Multi-Level Thresholding for Image Segmentation. *Research Reports on Computer Science*, 1-24.
- [66] Jing, Z., & Tang, B. (2024, August). Improved image segmentation method based on Otsu thresholding and level set techniques. In *Journal of Physics: Conference Series* (Vol. 2813, No. 1, p. 012017). IOP Publishing.
- [67] Fu, J., & Seththawong, R. (2023, December). A Modified Snake Optimizer Algorithm with Otsu-based Method for Satellite Image Segmentation. In *Proceedings of the 13th International Conference on Advances in Information Technology* (pp. 1-7).
- [68] Hadiq, H., Solehatin, S., Djuniarto, D., Muslim, M. A., & Salahudin, S. N. (2023). Comparison of the suitability of the otsu method thresholding and multilevel thresholding for flower image segmentation. *Journal of Soft Computing Exploration*, 4(4), 242-249.
- [69] Rahmawati, A., Yulianti, I., & Nurajizah, S. (2023). Image Segmentation Analysis Using Otsu Thresholding and Mean Denoising for the Identification Coffee Plant Diseases. *Jurnal Riset Informatika*, 6(1), 7-14.
- [70] Gould, S., Gao, T., & Koller, D. (2009). Region-based segmentation and object detection. *Advances in neural information processing systems*, 22.
- [71] Raja, S. K., ABDUL KHADIR, A. S., & Ahamed, S. R. (2009). MOVING TOWARD REGION-BASED IMAGE SEGMENTATION TECHNIQUES: A STUDY. *Journal of Theoretical & Applied Information Technology*, 5(1).
- [72] Farag, A. A. (1992). Edge-based image segmentation. *Remote sensing reviews*, 6(1), 95-121.
- [73] Lourdu, Jennifer, J. R., Joy, Vasantha, Rani, S. P. (2024). Enhanced Edge Detection for Image Segmentation and its Real-Time Implementation. 1-6.
- [74] Wang-Su, Jeon., Aram, Kim., Hojin, Jang., Sang-Yong, Rhee. (2024). Real-Time Image Segmentation using Edge Information. *Journal of Korea Multimedia Society*, 27(6): 675-684.
- [75] Shambhu, S., Koundal, D., & Das, P. (2023, April). Edge-based segmentation for accurate detection of malaria parasites in microscopic blood smear images: a novel approach using FCM and MPP algorithms. In 2023 2nd International Conference on Smart Technologies and Systems for Next Generation Computing (ICSTSN) (pp. 1-6). IEEE.
- [76] Sahayam, S., & Jayaraman, U. (2024). Integrating Edges into U-Net Models with Explainable Activation Maps for Brain Tumor Segmentation using MR Images. *arXiv preprint arXiv: 2401.01303*.
- [77] Santhoshi, A., & Muthukumaravel, A. (2024, March). Texture and Shape-Based Feature Extraction for Colorectal Tumor Segmentation. In 2024 10th International Conference on Advanced Computing and Communication Systems (ICACCS) (Vol. 1, pp. 315-320). IEEE.
- [78] Kumar, S., Pradhan, J., & Pal, A. K. (2021). Adaptive tetrolet based color, texture and shape feature extraction for content based image retrieval application. *Multimedia Tools and Applications*, 80(19), 29017-29049.
- [79] Tsutsumi, M., Saito, N., Koyabu, D., & Furusawa, C. (2022). A method for morphological feature extraction based on variational auto-encoder: an application to mandible shape. *bioRxiv*, 2022-05.
- [80] Li, L., Feng, L., Liu, S. L., Sun, M. X., Wu, J., & Wang, H. B. (2018). Intensity-based co-occurrence local ternary patterns for image retrieval. *J. Comput.*, 29(4), 12-30.
- [81] Maheshan, C. M., & Prasanna Kumar, H. (2021). Intensity-Based Feature Extraction of Real-Time Transformer Oil Images. In *Advances in VLSI, Signal Processing, Power Electronics, IoT, Communication and Embedded Systems: Select Proceedings of VSPICE 2020* (pp. 379-396). Springer Singapore.
- [82] Zhu, D., Semba, S., & Yang, H. (2021). Matching intensity for image visibility graphs: a new method to extract image features. *IEEE Access*, 9, 12611-12621.
- [83] Kurz, D., & Meier, P. (2017). U.S. Patent No. 9,679,384. Washington, DC: U.S. Patent and Trademark Office.
- [84] Kusi-Duah, S., Appiah, O., & Appiahene, P. (2022). Performance Evaluation of State-of-the-Art Texture Feature Extraction Techniques on Medical Imagery Tasks. Available at SSRN 4315803.

- [85] Mansour, I. R., Miksys, N., Beaulieu, L., Vigneault, É., & Thomson, R. M. (2024). Haralick texture feature analysis for Monte Carlo dose distributions of permanent implant prostate brachytherapy. *Brachytherapy*.
- [86] Mansour, I. R., & Thomson, R. M. (2023). Haralick texture analysis for microdosimetry: characterization of Monte Carlo generated 3D specific energy distributions. *Physics in Medicine & Biology*, 68(18), 185003.
- [87] Le, D. B. T., Narayanan, R., Sadinski, M., Nicholas, K., Nacev, A., Kumar, D., & Venkataraman, S. Application of Haralick Texture Analysis to Differentiate Suspicious Prostate Lesions from Normative Tissue on Low-field MRI.
- [88] Shotton, D. M., Rodriguez, A., Guil, N., & Trelles, O. (2000, September). Object tracking and event recognition in biological microscopy videos. In *Proceedings 15th International Conference on Pattern Recognition. ICPR-2000 (Vol. 4, pp. 226-229)*. IEEE.
- [89] McAfee, L., Heath, Z., Anderson, W., Hozi, M., Orr, J. W., & Kang, Y. The development of an automated microscope image tracking and analysis system. *Biotechnology Progress*, e3490.
- [90] Augenreich, J., Poddar, A., Belew, A. T., El-Sayed, N. M., & Briken, V. (2024). da\_Tracker: Automated workflow for high throughput single cell and single phagosome tracking in infected cells. *bioRxiv*.
- [91] Tyson, C., Gaire, S., Pegg, I., & Sarkar, A. (2023). Video-Microscopy-Based Automated Trajectory Determination for High-Velocity, Densely Clustered, Indistinguishable Objects Moving in A Directed Force Field. *bioRxiv*, 2023-10.
- [92] Zhao, Z., Wang, J., Horn, M., Ding, Y., He, T., Bai, Z., & Xiao, T. (2023). Object-centric multiple object tracking. In *Proceedings of the IEEE/CVF International Conference on Computer Vision (pp. 16601-16611)*.
- [93] Jeong, J. M., Yoon, T. S., & Park, J. B. (2014, September). Kalman filter based multiple objects detection-tracking algorithm robust to occlusion. In *2014 Proceedings of the SICE Annual Conference (SICE) (pp. 941-946)*. IEEE.
- [94] PALE-RAMON, E. L. I. G., Shmaliy, Y. S., Morales-Mendoza, L. J., & González-Lee, M. (2022). Bounding box stabilization for visual object tracking using Kalman and FIR filters. *WSEAS Transactions on Signal Processing*, 18, 11-20.
- [95] Naghshbandi, H., & Damavandi, Y. B. (2022, February). Automated cell tracking using adaptive multi-stage kalman filter in time-laps images. In *2022 International Conference on Machine Vision and Image Processing (MVIP) (pp. 1-8)*. IEEE.
- [96] Yang, Y., Stork, J. A., & Stoyanov, T. (2022). Particle filters in latent space for robust deformable linear object tracking. *IEEE Robotics and Automation Letters*, 7(4), 12577-12584.
- [97] Chen, M. (2021). Cell tracking in time-lapse microscopy image sequences. In *Computer Vision for Microscopy Image Analysis (pp. 101-129)*. Academic Press.
- [98] Li, R., Gao, Q., & Rohr, K. (2021, April). Multi-object dynamic memory network for cell tracking in time-lapse microscopy images. In *2021 IEEE 18th International Symposium on Biomedical Imaging (ISBI) (pp. 1029-1032)*. IEEE.
- [99] Zhang, T., & Sun, K. (2021). Deep Semantic edge for cell counting and localization in time-lapse microscopy images. In *Pattern Recognition and Computer Vision: 4th Chinese Conference, PRCV 2021, Beijing, China, October 29–November 1, 2021, Proceedings, Part III 4 (pp. 337-349)*. Springer International Publishing.
- [100] Hu, T., Xu, S., Wei, L., Zhang, X., & Wang, X. (2021). CellTracker: an automated toolbox for single-cell segmentation and tracking of time-lapse microscopy images. *Bioinformatics*, 37(2), 285-287.
- [101] Amarteifio, S., Fallesen, T., Pruessner, G., & Sena, G. (2021). A random-sampling approach to track cell divisions in time-lapse fluorescence microscopy. *Plant Methods*, 17, 1-12.
- [102] Thomson, E. (1930). Quantitative microscopic analysis. *The Journal of Geology*, 38(3), 193-222.
- [103] La Ferla, R., Maimone, G., Caruso, G., Azzaro, F., Azzaro, M., Decembrini, F., & Paranhos, R. (2014). Are prokaryotic cell shape and size suitable to ecosystem characterization?. *Hydrobiologia*, 726, 65-80.
- [104] Kriegel, F. L., Köhler, R., Bayat-Sarmadi, J., Bayerl, S., Hauser, A. E., Niesner, R., & Cséresnyes, Z. (2018). Cell shape characterization and classification with discrete Fourier transforms and self-organizing maps. *Cytometry Part A*, 93(3), 323-333.
- [105] Yevick, H. G., & Martin, A. C. (2018). Quantitative analysis of cell shape and the cytoskeleton in developmental biology. *Wiley Interdisciplinary Reviews: Developmental Biology*, 7(6), e333.
- [106] Ryabov, A., Kerimoglu, O., Litchman, E., Olenina, I., Roselli, L., Basset, A., & Blasius, B. (2020). Shape matters: cell geometry determines phytoplankton diversity. *bioRxiv*, 2020-02.
- [107] Culley, S., Caballero, A. C., Burden, J. J., & Uhlmann, V. (2023). Made to measure: an introduction to quantification in microscopy data. *arXiv preprint arXiv: 2302.01657*.
- [108] Jung, S. R., Fujimoto, B. S., & Chiu, D. T. (2017). Quantitative microscopy based on single-molecule fluorescence. *Current opinion in chemical biology*, 39, 64-73.
- [109] Garsha, K. (2008). Quantitative fluorescence microscopy: Considerations and controls. *Standardization and Quality Assurance in Fluorescence Measurements II: Bioanalytical and Biomedical Applications*, 55-88.
- [110] Phillips, K. G., Baker-Groberg, S. M., & McCarty, O. J. (2014). Quantitative optical microscopy: measurement of cellular biophysical features with a standard optical microscope. *Journal of visualized experiments: JoVE*, (86), 50988.
- [111] Palima, D., Villangca, M. J., Bañas, A. R., Kopylov, O., & Glückstad, J. (2015). Quantitative phase in microscopy: back-to-basics measurements. In *Focus on Microscopy 2015*.
- [112] Vega-Lugo, J., da Rocha-Azevedo, B., Dasgupta, A., Touret, N., & Jaqaman, K. (2022). Analysis of conditional colocalization relationships and hierarchies from three-color microscopy images. *Biophysical Journal*, 121(3), 530a.

- [113] McCall, A. D. (2024). Colocalization by cross-correlation, a new method of colocalization suited for super-resolution microscopy. *BMC bioinformatics*, 25(1), 55.
- [114] Seefelder, M., Kochanek, S., & Klein, F. A. (2024). Protein-CoLoc streamlines Bayesian analysis of colocalization in microscopic images. *Scientific Reports*, 14(1), 13277.
- [115] Lopez, S. G., Samwald, S., Jones, S., & Faulkner, C. (2023). On the pixel selection criterion for the calculation of the Pearson's correlation coefficient in fluorescence microscopy. *Journal of Microscopy*.
- [116] Stiekema, M., Gibson, O. N., Veltrop, R. J., Ramaekers, F. C., Broers, J. L., & Zandvoort, M. A. V. (2024). Detailed Colocalization Analysis of A-and B-type Nuclear Lamins: a Workflow Using Super-Resolution STED Microscopy and Deconvolution. *bioRxiv*, 2024-09.
- [117] Farsani, Z. A., & Schmid, V. J. (2021). Co-localization analysis in fluorescence microscopy via maximum entropy copula. *The International Journal of Biostatistics*, 17(1), 165-175.
- [118] Shakhov, A. S., Kovaleva, P. A., Churkina, A. S., Kireev, I. I., & Alieva, I. B. (2022). Colocalization Analysis of Cytoplasmic Actin Isoforms Distribution in Endothelial Cells. *Bio-medicines*, 10(12), 3194.
- [119] Summers, H. D., Wills, J. W., & Rees, P. (2022). Spatial statistics is a comprehensive tool for quantifying cell neighbor relationships and biological processes via tissue image analysis. *Cell Reports Methods*, 2(11).
- [120] Parra, E. R. (2021). Methods to determine and analyze the cellular spatial distribution extracted from multiplex immunofluorescence data to understand the tumor microenvironment. *Frontiers in Molecular Biosciences*, 8, 668340.
- [121] Martin, A., Zhang, S., Williamson, A., Tingley, B., Pickus, M., Zurakowski, D., & Grinstaff, M. W. Dispersion indices for universal quantification of fluorescently-labelled subcellular structure spatial distributions. *bioRxiv*.
- [122] Gomariz, A., Portenier, T., Nombela-Arrieta, C., & Goksel, O. (2022). Probabilistic spatial analysis in quantitative microscopy with uncertainty-aware cell detection using deep Bayesian regression. *Science Advances*, 8(5), eabi8295.
- [123] De Santis, I., Zanoni, M., Arienti, C., Bevilacqua, A., & Tesei, A. (2021). Density distribution maps: a novel tool for subcellular distribution analysis and quantitative biomedical imaging. *Sensors*, 21(3), 1009.
- [124] Kermany, D., Ahn, J. Y., Vasquez, M., Zhang, W., Wang, L., Liu, K., Xu, Z., Cho, M. S., Carlos-Alcalde, W., Lee, H., Raghunathan, R., Sheng, J., Hao, X., Zhao, H., Afshar-Kharghan, V., Zhang, X., & Wong, S. T. C. (2025). Multiscale 3D spatial analysis of the tumor microenvironment using whole-tissue digital histopathology. *Cancer Communications*. <https://doi.org/10.1002/cac2.12655>
- [125] Johansson, R. (2017). Model-based hypothesis testing in biomedicine: how systems biology can drive the growth of scientific knowledge (Vol. 1877). Linköping University Electronic Press.
- [126] Forsgren, E., Cloarec, O., Jonsson, P., Lovell, G., & Trygg, J. (2024). A scalable, data analytics workflow for image-based morphological profiles. *Chemometrics and Intelligent Laboratory Systems*, 254, 105232.
- [127] Wu, Y. L., Tschanz, A., Krupnik, L., & Ries, J. (2020). Quantitative data analysis in single-molecule localization microscopy. *Trends in Cell Biology*, 30(11), 837-851.
- [128] Ryabukha, O. I., & Dronyuk, I. M. (2019). Application of correlation analysis in cytology: Opportunities to study specific activity of follicular thyrocytes. *Regulatory Mechanisms in Biosystems*, 10(3).
- [129] Schnitzbauer, J., Wang, Y., Zhao, S., Bakalar, M., Nuwal, T., Chen, B., & Huang, B. (2018). Correlation analysis framework for localization-based superresolution microscopy. *Proceedings of the National Academy of Sciences*, 115(13), 3219-3224.
- [130] Xu, H., Hu, Y., Zhang, X., Aouizerat, B. E., Yan, C., & Xu, K. (2021). scCorr: A graph-based k-partitioning approach for single-cell gene-gene correlation analysis. *bioRxiv*, 2021-03.
- [131] Binder, B. J., & Simpson, M. J. (2015). Spectral analysis of pair-correlation bandwidth: application to cell biology images. *Royal Society Open Science*, 2(2), 140494.
- [132] Qian, J., Cao, Y., Bi, Y., Wu, H., Liu, Y., Chen, Q., & Zuo, C. (2023). Structured illumination microscopy based on principal component analysis. *ELight*, 3(1), 4.
- [133] Mahmodi, H., Poulton, C. G., Leslie, M. N., Oldham, G., Ong, H. X., Langford, S. J., & Kabakova, I. V. (2024). Principal component analysis in application to Brillouin microscopy data. *Journal of Physics: Photonics*, 6(2), 025009.
- [134] Qian, J., Cao, Y., Bi, Y., Wu, H., Liu, Y., Chen, Q., & Zuo, C. (2023, January). Illumination parameter estimation of structured illumination microscopy based on principal component analysis. In *International Conference on Optical and Photonic Engineering (icOPEN 2022)* (Vol. 12550, pp. 185-189). SPIE.
- [135] Kitao, A. (2022). Principal component analysis and related methods for investigating the dynamics of biological macromolecules. *J*, 5(2), 298-317.
- [136] Yuan, D., & Mancuso, N. (2023). SuSiE PCA: A scalable Bayesian variable selection technique for principal component analysis. *Iscience*, 26(11).
- [137] Nieves, D. J., Pike, J. A., Levet, F., Williamson, D. J., Baragilly, M., Oloketuyi, S., & Owen, D. M. (2023). A framework for evaluating the performance of SMLM cluster analysis algorithms. *Nature methods*, 20(2), 259-267.
- [138] Williamson, D. J., Burn, G. L., Simoncelli, S., Griffi é J., Peters, R., Davis, D. M., & Owen, D. M. (2020). Machine learning for cluster analysis of localization microscopy data. *Nature communications*, 11(1), 1493.
- [139] Griffi é J., Shannon, M., Bromley, C. L., Boelen, L., Burn, G. L., Williamson, D. J., & Rubin-Delanchy, P. (2016). A Bayesian cluster analysis method for single-molecule localization microscopy data. *Nature Protocols*, 11(12), 2499-2514.



- [140] Fishman, E. K., Magid, D., Ney, D. R., Chaney, E. L., Pizer, S. M., Rosenman, J. G., & Robertson, D. D. (1991). Three-dimensional imaging. *Radiology*, 181(2), 321-337.
- [141] Norman, R. X., Chen, Y. C., Recchia, E. E., Loi, J., Rosemarie, Q., Lesko, S. L., & Suzuki, A. (2024). One step 4x and 12x 3D-ExM: robust super-resolution microscopy in cell biology. *bioRxiv*.
- [142] Sun, G., Liu, S., Shi, C., Liu, X., & Guo, Q. (2023). 3DCNAS: A universal method for predicting the location of fluorescent organelles in living cells in three-dimensional space. *Experimental Cell Research*, 433(2), 113807.
- [143] Radulović, S., Sunkara, S., Rachel, R., & Leitingner, G. (2022). Three-dimensional SEM, TEM, and STEM for analysis of large-scale biological systems. *Histochemistry and Cell Biology*, 158(3), 203-211.
- [144] Martišek, D. (2022, December). Mathematical Methods for 3D Reconstruction of Cell Structures. In *MENDEL* (Vol. 28, No. 2, pp. 83-92).
- [145] Georg, M., Fernandez-Cabada, T., Bourguignon, N., Karp, P., Peñaherrera, A. B., Helguera, G., & Mertelsmann, R. (2018). Development of image analysis software for quantification of viable cells in microchips. *PloS one*, 13(3), e0193605.
- [146] Merchant, F., & Castleman, K. (Eds.). (2022). *Microscope image processing*. Academic press.
- [147] Vosatka, K. W., Lavenus, S. B., & Logue, J. S. (2022). A novel Fiji/ImageJ plugin for the rapid analysis of blebbing cells. *PloS one*, 17(4), e0267740.
- [148] Hulsey-Vincent, H., Alvinex, N., Witus, S., Kowalski, J. R., & Dahlberg, C. (2023). A Fiji process for quantifying fluorescent puncta in linear cellular structures. *Micropublication Biology*, 2023.
- [149] Šimunić, I., Jagečić, D., Isaković, J., Dobrivojević Radmilović, M., & Mitrečić, D. (2024). Lusca: FIJI (ImageJ) based tool for automated morphological analysis of cellular and subcellular structures. *Scientific Reports*, 14(1).
- [150] López, A. C., Gómez-Pedrero, J. A., Blanco, A. M., & Sorzano, C. O. S. (2022). Cell-TypeAnalyzer: A flexible Fiji/ImageJ plugin to classify cells according to user-defined criteria. *Biological Imaging*, 2, e5.
- [151] Niazaei, S., Rahimzai, A. A., & Atifnigar, H. (2023). Applications of MATLAB in Natural Sciences: A Comprehensive Review. *European Journal of Theoretical and Applied Sciences*, 1(5), 1006-1015.
- [152] Palm, W. J. (2011). *Introduction to MATLAB for Engineers*. New York: McGraw-Hill.
- [153] Hodneland, E., Kögel, T., Frei, D. M., Gerdes, H. H., & Lundervold, A. (2013). CellSegm-a MATLAB toolbox for high-throughput 3D cell segmentation. *Source code for biology and medicine*, 8, 1-24.
- [154] Karmakar, S., Mandal, D., Pratihari, M., Chakraborty, A., Biswas, A., & Talukdar, S. (2023, December). A MATLAB Expedition Into Image Processing. In *2023 7th International Conference on Electronics, Materials Engineering & Nano-Technology (IEMENTech)* (pp. 1-6). IEEE.
- [155] Sharma, G. (2017). Performance analysis of image processing algorithms using matlab for biomedical applications. *Int. J. Eng. Manuf.(IJEM)*, 7(3), 8-19.
- [156] Youan, T., Lingyan, Z., & Gandong, C. (2023, November). Image Processing Method Based on MATLAB in the Application of Belt Tracking with Industrial Robot. In *International Conference on Computer Engineering and Networks* (pp. 515-526). Singapore: Springer Nature Singapore.
- [157] Landau, S., Shor, E., Radisic, M., & Levenberg, S. (2024). Quantitative image analysis of tissue properties: a MATLAB tool for measuring morphology and co-localization in 2D images. *bioRxiv*, 2024-04.
- [158] Singh, P., & Singh, K. (2013). Image encryption and decryption using blowfish algorithm in MATLAB. *International Journal of Scientific & Engineering Research*, 4(7), 150-154.
- [159] Umesh, P. (2012). Image processing in python. *CSI Communications*, 23(2), 23-24.
- [160] Miller, B., & Mick, S. (2020). Data Processing Using Python in DigitalMicrograph. *Microscopy and Microanalysis*, 26(S2), 1172-1174.
- [161] Castaneda, R., Trujillo, C., & Doblas, A. (2022, July). An Open-Source Python library for Digital Holographic Microscopy Imaging. In *Computational Optical Sensing and Imaging* (pp. JTh2A-1). Optica Publishing Group.
- [162] Good, J., & Berriman, G. B. (2019). Image Processing in Python With Montage. *arXiv preprint arXiv: 1908.09753*.
- [163] Janssen, B. J., van der Heide, A., Roeven, H. G., & Thomasen, J. A. M. (2018). U.S. Patent No. 10,014,158. Washington, DC: U.S. Patent and Trademark Office.
- [164] Saxton, W. O. (2013). *Computer techniques for image processing in electron microscopy* (Vol. 10). Academic Press.
- [165] Morgado, L., Gómez-de-Mariscal, E., Heil, H. S., & Henriques, R. (2024). The rise of data-driven microscopy powered by machine learning. *Journal of Microscopy*.
- [166] Cunha, I., Latron, E., Bauer, S., Sage, D., & Griffi é J. (2024). Machine learning in microscopy—insights, opportunities and challenges. *Journal of Cell Science*, 137(20).
- [167] Chechekhina, E., Voloshin, N., Kulebyakin, K., & Tyurin-Kuzmin, P. (2024). Code-Free Machine Learning Solutions for Microscopy Image Processing: Deep Learning. *Tissue Engineering Part A*.
- [168] Day, A. L., Wahl, C. B., Gupta, V., Dos Reis, R., Liao, W. K., Mirkin, C. A., & Agrawal, A. (2024). Machine Learning-Enabled Image Classification for Automated Electron Microscopy. *Microscopy and Microanalysis*, ozae042.
- [169] Altarawneh, M. O. K. H. L. E. D., Al-qaisi, A., & SALAMAH, J. B. (2019). Evaluation of cloud computing platform for image processing algorithms. *J. Eng. Sci. Technol*, 14, 2345-2358.

- [170] Mathivanan, B., Nandhashree, K. R., Radhakrishnan, C., Ananthi, S., Suganthi, P., & Asokan, R. (2022, November). Deployment of Scalable Cloud Based Image Processing Application Using Fuzzy Coupled Learning Technique. In 2022 1st International Conference on Computational Science and Technology (ICCST) (pp. 1-5). IEEE.
- [171] Walia, S., & Kumar, K. (2019). Digital image forgery detection: a systematic scrutiny. Australian Journal of Forensic Sciences, 51(5), 488-526.
- [172] Pandey, N. K., & Diwakar, M. (2020, March). A review on cloud based image processing services. In 2020 7th International Conference on Computing for Sustainable Global Development (INDIACom) (pp. 108-112). IEEE.