



REVIEW ARTICLE

**RECENT ADVANCES IN HALAL RESEARCH VIA MICROSCOPY AND IMAGING APPROACHES: OPPORTUNITIES AND CHALLENGES**

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**Abstract.** Halal authentication has garnered a lot of attention in the last few years due to improve of public awareness in ensuring the food and product are safe, good quality and permissible by syariah to muslim consumer. Traditional methods for Halal verification often rely on labor-intensive techniques, with significant challenges in detecting cross-contamination and identifying trace amounts of non-Halal substances. This review provides a comprehensive analysis of recent advances in microscopy and imaging approaches which provided new opportunities to enhance the efficiency and accuracy of Halal authentication. Techniques such as light microscopy, electron microscopy and camera imaging have been proven to be effective in identifying contaminants and verifying Halal status by detecting foreign materials and microstructures at a cellular level as well as suitable for analyzing halal-critical animal-derived materials, such as leather, bone, and bristles. Despite these advancements, current microscopy imaging technologies face limitations such as high costs, technical complexity, and the need for skilled personnel. These challenges hinder the widespread adoption of these techniques in routine Halal testing compared to other methods such as spectroscopy. Additionally, the sensitivity and specificity of imaging methods need further improvement to effectively trace the non-Halal substances. Emerging technology such as machine learning (ML) and artificial intelligence (AI) are powerful tools that can be employed in addressing these limitations. By integrating ML algorithms with imaging techniques, the speed and accuracy of Halal authentication can be significantly improved. AI-driven image analysis can provide prediction, assisting automate detection, reduce human error, and provide real-time insights, thus empowering existing technologies for better scalability. Looking forward, the convergence of advanced microscopy techniques and AI has the potential to revolutionize Halal research, enabling faster, more reliable, and cost-effective Halal authentication.

**Keywords:** Microscopy, image analysis, machine learning, artificial intelligence, halal authentication.

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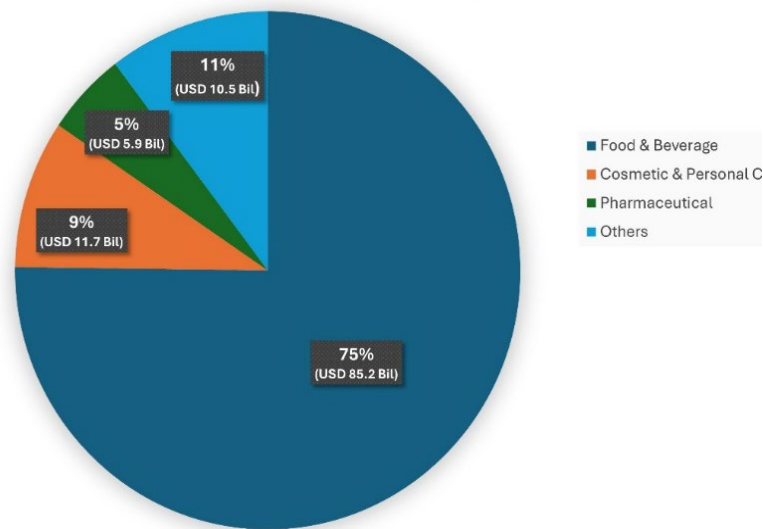
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## 1. INTRODUCTION

The halal industry has grown rapidly over the last decade due to an increase in halal awareness among the public and the market opportunities offered to industry players, even at the international level. The term 'halal' is related to the concept of Shariah (Islamic law), originally referring to what is permissible or lawful in terms of food and behavior according to Islamic faith. In terms of eating behaviors, foods such as pork, blood, alcohol and improperly slaughtered animals are forbidden for Muslims. Over the centuries, the term 'halal' has expanded beyond the regulation of food products to include social behaviors, such as tourism and hospitality, as well as financial transactions. Halal has evolved to encompass a broad array of industries, especially food and beverages, consumer goods, cosmetics, pharmaceuticals, and more. According to the State of the Global Islamic Economy Report 2023 by DinarStandard, global Muslim spending across halal-related sectors reached USD 2.29 trillion in 2022 and is projected to grow to USD 3.7 trillion by 2025. In particular, the halal food sector accounted for USD 1.27 trillion in 2022 and is expected to hit USD 1.67 trillion by 2025, highlighting robust consumer demand and growth potential [1]. Meanwhile, the growth of Malaysia’s halal market is projected to reach USD 113.2 billion by 2030, covering various sectors including food and beverage, cosmetics, pharmaceuticals and others, as shown in Figure 1 [2].



**Figure 1:** The core sector of Malaysian Halal Market by 2030 [2]

The development of halal standards is an intricate process that involves harmonizing religious interpretation with scientific assessment. Islamic scholars, through credible institutions, discuss issues related to Shariah, leading to interpretations that form the foundation of the standards. These interpretations are then incorporated into current technology and commercial aspects to produce the technical framework for the standard [3]. The concept for halal certification is *Halalan Thoyyiban*, which covers both Shariah aspects and the quality, wellness, and safety that comply with the developed standards [4]. The integration of religious principles and scientific validation across various halal certification schemes—including food premises, food products, consumer goods, pharmaceuticals, cosmetics, slaughterhouses, logistics services, OEM manufacturing, and medical devices—has significantly elevated the expectations and standards for halal products. This convergence has led to an increasing demand for the development of comprehensive standards and the implementation of robust and transparent authentication systems. However, the lack of universal standards for halal products in some countries hindered market accessibility for international trade. Furthermore, concerns over the authenticity of halal certification in some countries have led to trust issues among consumers, thereby limiting the full potential of the halal market. Emerging research and development in sensor and digital technology may be key to addressing this issue [5]. One of the key elements of Malaysia’s Halal Ecosystem, designed to boost the halal socio-economy in the Halal Industry Master Plan (HIMP) 2030, is to uphold Halal Integrity, which concerns halal traceability and authenticity.

Halal authentication is crucial in distinguishing halal-critical ingredients through laboratory analysis, which will support the raw ingredient list documentation. As for now, a lot of research has been done in developing a better and effective halal detection and authentication system. For instance, methods such as electrophoresis, dielectric, polymerase chain reaction (PCR), spectroscopy techniques and biosensors were commonly utilized to detect halal substances. These techniques have been discussed in several studies, but the focus has generally been on specific products and an overview of several authentication techniques that include meat speciation, protein, fat and oil, and alcohol content analysis [6].

On the other hand, this review offers a comprehensive and critical overview of more focused physical authentication techniques, particularly imaging, which involve the analysis of morphological and structural characteristics to determine a material's compliance with halal standards. These techniques are especially effective for detecting and differentiating animal-derived materials such as skin (leather), bone, and hair/bristles, which are commonly incorporated into consumer goods like leather products, brushes, filters, and gelatin-based items. According to MS 2200-2:2013, these components are considered halal-critical, as their permissibility depends on the species origin and whether the animal was slaughtered in accordance with Shariah requirements. Physical authentication, such as microscopy, is particularly relevant for these types of samples, where visual and structural cues can aid in distinguishing between halal and non-halal sources [7].

Microscopy and image analysis are powerful tools in identifying key challenges in halal research, such as contamination, adulteration, and product verification. Microscopy techniques, including brightfield and fluorescence, or types such as optical microscopy and electron microscopy, provide a wide range of information for studying cells and the surface morphology of products. However, each comes with drawbacks and limitations, such as sample preparation, image resolution, signal-to-noise ratio, background and contrast, speed of acquisition, and image dimension.

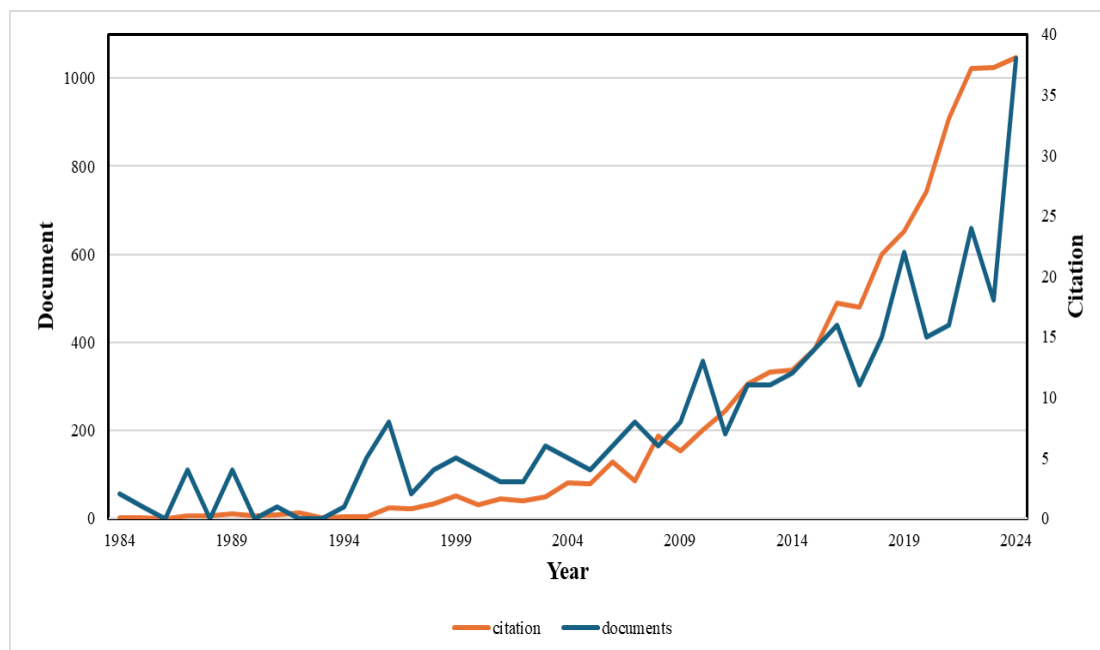
Therefore, the present review will contain four sections. The first section will provide an overview of the halal industry and its relationship with halal authentication. In section 2, we will introduce halal authentication techniques, including the utilization of microscopy and imaging, as well as the common methods used to address challenges in imaging analysis. In section 3, we discuss principles and frameworks in utilizing machine learning-based data-driven systems. Finally, we present the future perspectives in section 4, whereas a summary of various applications that showcased how the integration of data-driven microscopy opens up for new technical possibilities and opportunities for halal research.

## 2. REVIEW OF MICROSCOPY AND IMAGING TECHNIQUES

In the early stages of halal authentication, conventional methods such as electrophoresis and dielectric were commonly utilized to detect halal substances before polymerase chain reaction (PCR) techniques were invented in mid-2000s [8]. Since its introduction, PCR has become commonly employed as a more precise and reliable halal authentication technique [9]. Parallel to the development of molecular techniques, the application of various chromatographic and spectroscopic methods has also contributed to halal authentication. Methods such as liquid chromatography-tandem mass spectrometry (LC-MS/MS), which has been employed as early as 2010 [10], enable the detailed analysis of food components at the molecular level, identifying species-specific markers with high precision. In recent years, the use of high-resolution mass spectrometry such as Fourier-transform infrared (FT-IR) spectroscopy coupled with chemometrics and integration with machine learning has become increasingly common for halal authentication, allowing for the analysis of complex food matrices and the identification of trace components indicative of non-halal ingredients. Nanotechnology, particularly the use of nanocomposites in biosensors, has also contributed to the development of more sensitive and selective detection methods for halal authentication. The work by Jufri et al. (2023) on multi-walled carbon nanotubes (MWCNTs)-based sensors highlights the promise of nanomaterials in enhancing sensor performance and providing rapid halal certification in food products [11].

In recent years, the employment of high-resolution analytical techniques coupled with chemometrics and integration with machine learning also has become a trend for halal authentication. For example, a study by Syabani et al. (2023) utilized a combination of Fourier-transform infrared (FTIR), Scanning Electron Microscopy (SEM), and Differential Scanning Calorimetry (DSC) to analyze collagen fiber structures and thermal stability among leather samples. The research highlighted that pigskin exhibits a three-hole pattern on its surface morphology, contrasting with the random pores and tighter grain patterns of cowhide, while artificial leather lacks natural grain patterns and pores altogether [12]. FTIR has also been used to detect pig-based derivatives in food products, indicating its applicability in identifying non-halal components [13]. However, FTIR has limitations when used alone—it may not effectively differentiate materials with similar chemical compositions (e.g., pigskin vs. cowhide) and offers limited spatial resolution, making it less suitable for analyzing structural or surface-level differences. In contrast, imaging techniques such as SEM provide high-resolution morphological details, allowing for clearer identification of unique features associated with non-halal sources. For detecting bone and bristle materials, microscopy-based methods are particularly useful as they reveal surface characteristics and microstructures crucial for accurate halal authentication.

Microscopy and imaging also shows potential as promising tools in providing physical information for halal authenticity, particularly in food industries such as meat processing, gelatin production, and dairy manufacturing, where it helps detect microstructural differences and identify non-Halal components at cellular or subcellular levels. Figure 2 shows the research trends on image analysis in the food industry from 1984 to 2024. As we can see, the trend shows increased publications and citation rates related to imaging studies in food industries over the years. The significant increase in publications and citations started after 2010 onwards indicates the rapid application of advanced technology, especially in image analysis because of the adoption of artificial intelligence, machine learning, and computer vision.



**Figure 2:** Research trends on Image analysis in food industry

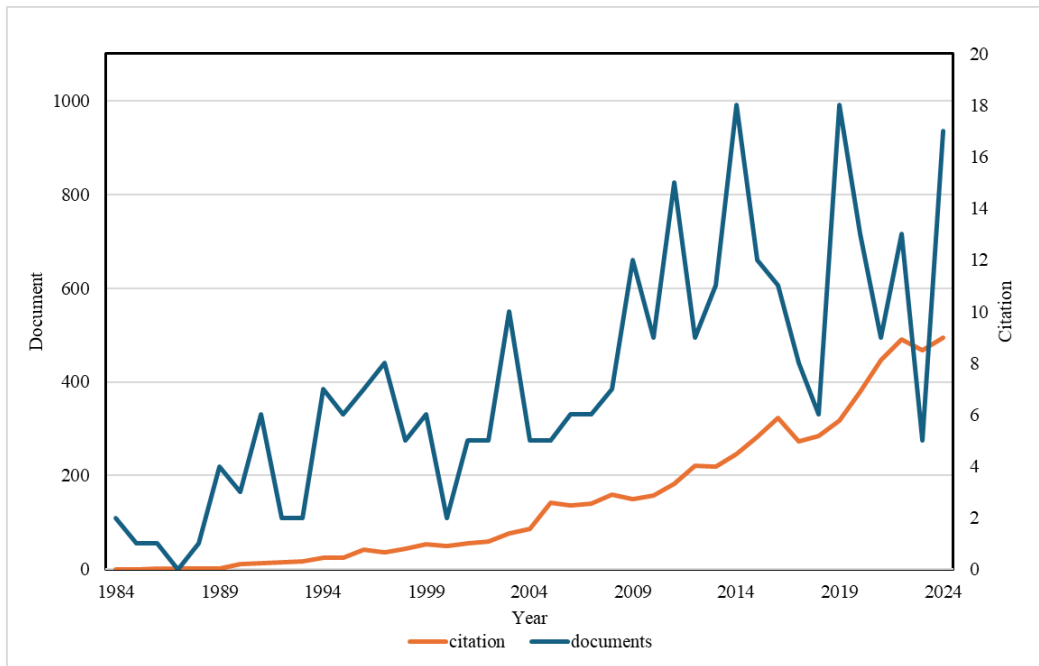
The increasing number of publications on image analysis for the food industry highlights its growing relevance as a research area, driven by demands for precise, cost-effective, and non-invasive methods to assess food quality, authenticity, and safety [14]. Other than that, the need for automation in food production lines to increase cost and production efficiency, and the increasing consumer demands for transparency in food production also contribute to the advancement and interest of imaging technology. Figure 2 shows the data obtained from Scopus databases with keywords as in Table 1. It indicates the lag between research and recognition, as the citation is lower than the research publication before 2015. The sharp increment in the citation after 2015 indicates the greater interest and applications

in the food industry aligned with emerging technologies such as smart farming and Internet of Things (IOT) applications.

**Table 1:** Image analysis and microscopy analysis keywords for Scopus search string

Figure	Analysis	Keywords
2	Image analysis	Article Title - Image* OR picture* OR visual* OR photo* AND interpret* OR process* OR analys* OR exam* OR evaluate* OR recogni* AND food* OR meat* OR fish* OR milk* OR fruit* OR vegetable* OR grain* OR cereal* OR legume* OR poultr* OR egg* OR dair AND product*
3	Microscopy analysis	Article Title - microscope* AND interpret* OR process* OR analys* OR exam* OR evaluate* OR recogni* AND food* OR meat* OR fish* OR milk* OR fruit* OR vegetable* OR grain* OR cereal* OR legume* OR poultr* OR egg* OR dair AND product*

Besides imaging, microscopy analysis is one of the oldest tools used in the food industry, providing valuable insights into the structure, composition, and quality of food products [15]. Figure 3 illustrates the research trends in microscopy analysis within the food industry from 1984 to 2024. The fluctuating trends in publications on microscopy analysis may be attributed to its integration with other advanced techniques, such as spectroscopy, imaging, and computational modeling, leading to a decline in standalone microscopy studies. In microscopy studies, there is always a lag between research and recognition over the years except in 2023. However, the graph shows that there has been significant increment in microscopy research for the last 10 years, due to the advancement of imaging technology such as the microscope and the image analysis software.



**Figure 3:** Research trend on microscopy analysis in food industry

Imaging and microscopy analysis could significantly contribute to advancements in halal science research. Imaging analysis, for instance, can play a pivotal role in applications such as halal monitoring through image recognition, evaluating food quality, and detecting non-halal adulterants. This offers direct visual evidence of structural and morphological features at micro and nano scales, enabling precise identification of non-Halal components that might be undetectable by other analytical techniques (spectroscopy). These capabilities are especially vital in ensuring compliance with Shariah laws while maintaining high standards of food safety and authenticity. In this section, several imaging techniques that have been established nor have potential for halal authentication will be discussed,

namely light microscopy for micro analysis, electron microscopy for detail analysis and digital camera imaging techniques for screening analysis.

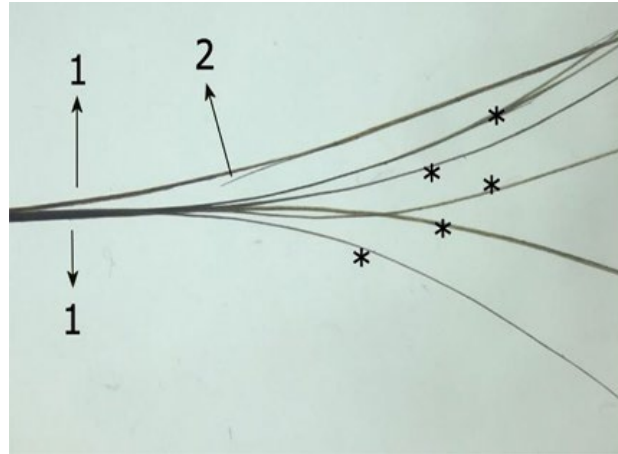
Advancements in deep learning and machine learning (ML) further enhance the potential of image analysis techniques for food products. Deep learning algorithms, such as convolutional neural networks (CNNs), can process complex visual data to accurately identify non-halal substances or contaminants in food products. ML models could also be used to develop automated systems for the classification of halal and non-halal products based on image analysis. These technologies enable faster, more accurate assessments of food quality and compliance, significantly reducing the reliance on manual or traditional methods.

## **2.1 Light Microscopy**

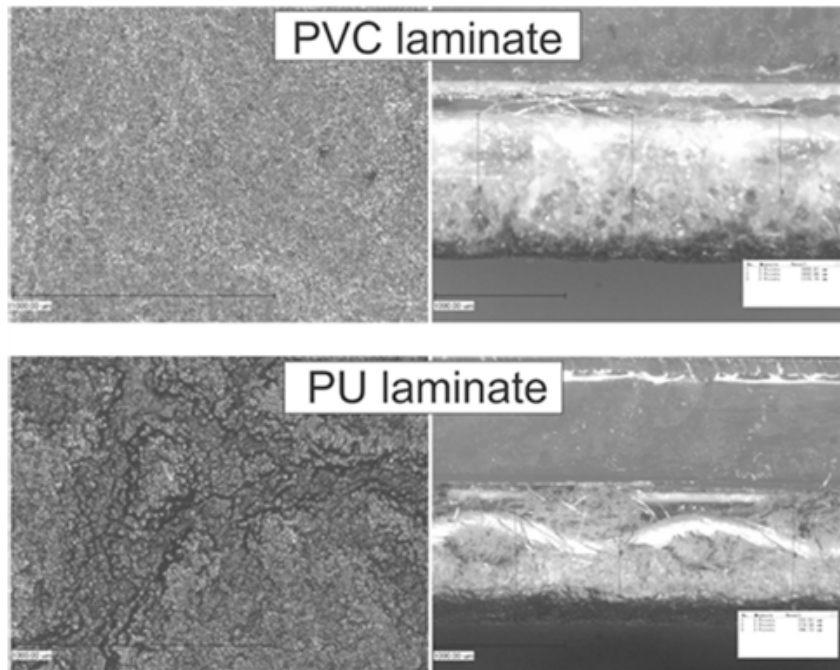
Optical microscopy, also known as light microscopy, is a widely used technique for visualizing and analyzing biological samples at the microscopic level. This technique relies on visible light and a series of lenses to magnify and resolve the details of samples. In the context of halal authentication, optical microscopes can be employed to observe the microstructure of food samples, identifying potential contamination or adulteration. The principle of optical microscopy involves passing light through a specimen, where it interacts with the sample's structure. The light is then collected by lenses to form an image, which can be viewed or captured for further analysis. This method is particularly useful for detecting physical characteristics such as cell structures, tissues, and surface morphology, which may indicate the presence of non-halal substances.

In halal authentication, optical microscopy can be utilized to observe various aspects of food products to ensure they meet halal standards. Specifically, it allows for the examination of animal tissues and plant cells in meat and food samples to verify their halal status and safety. For instance, adulteration in meat products can be identified by the distinctive characteristics of contaminants, such as fibers and fat cells, which differ from those of the original meat.

By identifying these features, optical microscopy aids in detecting non-halal substances and ensuring the safety of the products. Microscopy is a valuable tool for assessing the hygienic quality of food, allowing the detection of biological, microbiological and physical contaminants. This method is considered low-cost compared to other authentication techniques, while its effectiveness depends on the expertise and knowledge of the analyst. For example, Oliveira et al. [16] discuss the employment of microscopy in food analysis, particularly in relation to the utilization of plant anatomy and the future perspectives of its association with other analytical methods for plant quality control. The review covers several studies on plant adulteration, such as coffee and wheat flour, as well as adulterated starches in meat [16, 17]. Other than that, optical microscopy techniques also can distinguish pig skin and fur due to the unique characteristics: (i) Hair follicles on the skin arranged in triangular group of three and (ii) split end of pig bristle (Figure 4) [18-20]. This can distinguish from other animal leather, as well as synthetic leather such as polyurethane (PU) and polyvinyl chloride (PVC) (Figure 5) [21].



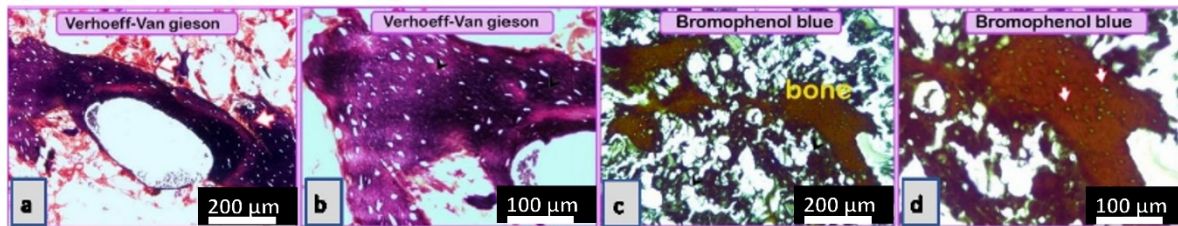
**Figure 4:** Stereomicroscopic hair image, (1) Primary branch (2) A posteriorly directed secondary branch, Asterisk: secondary branch [19]



**Figure 5:** Light microscopy image of artificial or synthetic leather [21]

Specialized techniques, such as fluorescent microscopy, can enhance the capabilities of optical microscopes in halal authentication. Fluorescent microscopy involves the use of fluorescent dyes or probes that bind specifically to certain components of a sample, emitting fluorescence when illuminated by a particular wavelength of light. This technique can be particularly useful for detecting trace amounts of specific substances, that may indicate non-halal contamination and adulteration. Sample preparation for fluorescent microscopy typically requires treating the sample with fluorescently labeled antibodies or markers that target specific components, such as pork-derived proteins. Additionally, sample fixation and staining are essential steps to preserve the structure and ensure clear visualization under the microscope. By utilizing these advanced techniques, optical microscopy becomes a powerful tool in verifying the authenticity and integrity of halal food products. For example, Abdel-Maguid et al. studied meat adulteration using several microscopy techniques, i.e., light microscopy, fluorescence microscopy, and histochemical microscopy. Several different special staining techniques such as haematoxylineosin (HE), Verhoeff's-Van Gieson and Bromophenol blue stain have been employed to differentiate between the various structures in detecting adulterated tissues in the meat such as nuchal ligament, blood vessels, muscular artery, lungs, cardiac muscle fibers, tendons, spongy bone, tissue, cartilage, and the muscle in

visceral organs [22]. Fluorescence microscopy is also considered an effective technique for detecting bone and cartilage, as shown in Figure 6.

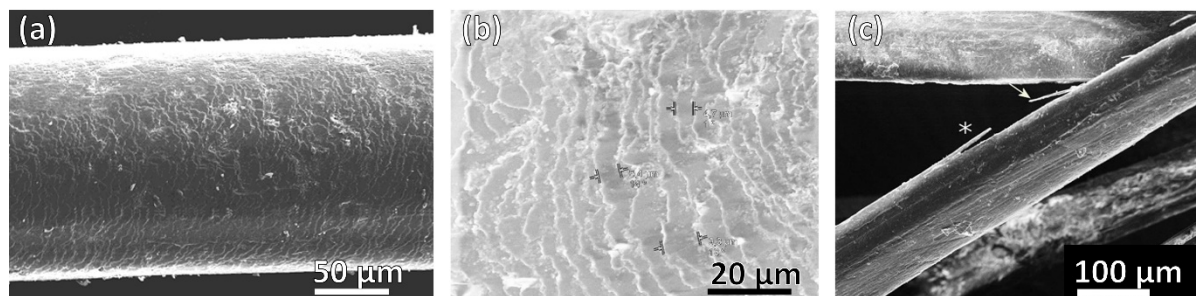


**Figure 6:** Optical micrographs of paraffin sections show adulteration on lucheon meat with different staining: (a) and (b) shows the sample appears black from the VVG stain. The presence of osteocytes located in the lacunae in (c) and (d) indicated the existence of bone tissue in the meat sample [22]

## 2.2 Electron Microscopy

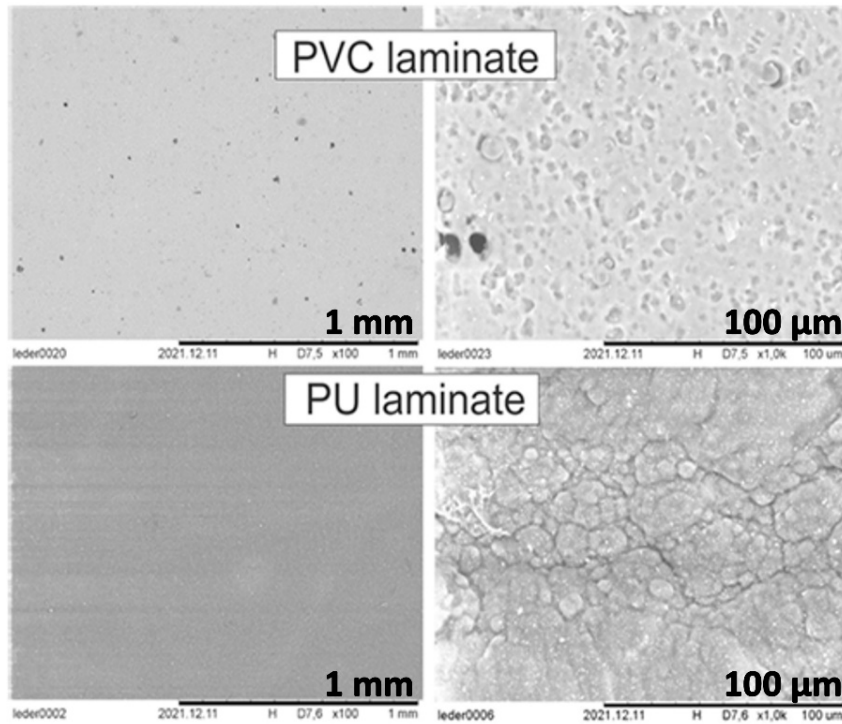
Electron microscopy (EM) is a powerful technique that uses a beam of electrons instead of visible light to examine the microstructure of samples with extremely high resolution. Unlike optical microscopy, which is limited by the wavelength of visible light, electron microscopes can resolve much finer details, enabling the observation of structures at the nanometer scale. There are 2 types of EM, i.e., scanning electron microscopy (SEM), which provides detailed 3D surface images, and transmission electron microscopy (TEM), which reveals internal structures at a higher resolution by transmitting electrons through thin samples. Sample preparation for SEM is relatively straightforward, as normally the sample for SEM only needs to be conductive. The advantages of SEM such as high magnification, greater depth of field, better resolution, and ease of sample observation resulting them as one of the most widely employed instruments by researchers.

In halal authentication, electron microscopy is particularly valuable because it provides highly detailed images of food components and contaminants, aiding in the identification of adulteration or non-halal substances. The electron beam interacts with the sample, and the resulting signals are used to create high-resolution images that reveal the fine structure of cells, tissues, and materials, enabling the detection of unwanted particles, such as pork fibers or other non-halal contaminants. Atalgın et al. shows detail characteristic of split end of pig bristle (Figure 4) with scale pattern hair and medulla structure (Figure 7) [19]. Other examples show a detailed image of synthetic leather (Figure 5) which shows detailed surface information of pores and grain that is different from animal leather (Figure 8).



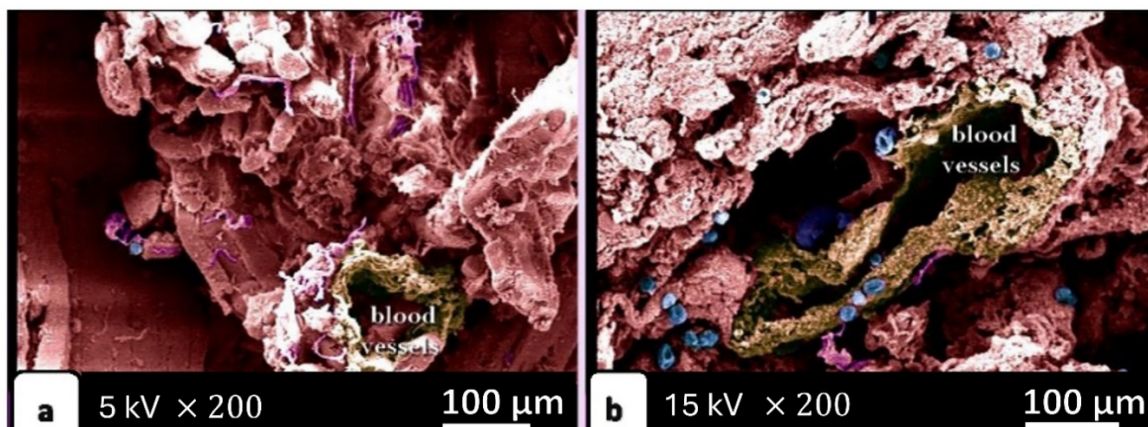
**Figure 7:** SEM micrographs of (a) pig hair image hair surface, (b) high magnification of hair surface that show scale pattern and (c) split secondary hair extending towards the hair tip [19]

Electron microscopy also can be used to observe various aspects of food samples to ensure compliance with halal standards. For example, electron microscopes can examine the tissue structure of meat products, identifying specific characteristics of halal and non-halal species at the cellular level. Pork muscle fibers, for instance, have a distinct texture and arrangement compared to those from halal animals, which can be observed using electron microscopy.



**Figure 8:** SEM micrographs of artificial or synthetic leather [21]

Additionally, micro-level contamination in processed foods also can be detected, such as traces of animal-derived gelatin, bone fragments, or other non-halal additives. For instance, Figure 9 shows SEM analysis of meat adulteration that contains blood vessels [22]. The SEM image were digitally coloured to distinguish between different tissues and organs by using Photo Filter program [23]. Moreover, a study by Kashim et al. found that blood plasma, with its ability to dissolve, emulsify, and form a strong gel, is used as a binder to improve the texture and offer a cost-effective solution for producing low-fat meat products [24]. However, blood plasma can also contain various microorganisms and potentially harmful toxins and blood proteins, which could compromise the halal status of the product. These fine details are crucial in verifying the authenticity and integrity of food products, particularly in cases of potential cross-contamination.



**Figure 9:** SEM micrographs show adulteration on lucheon meat: The unauthorised tissue found in the scanned samples of meat luncheon was nuchal ligament (Fig. 9a) and blood vessels (Figure 9(b)) [22]

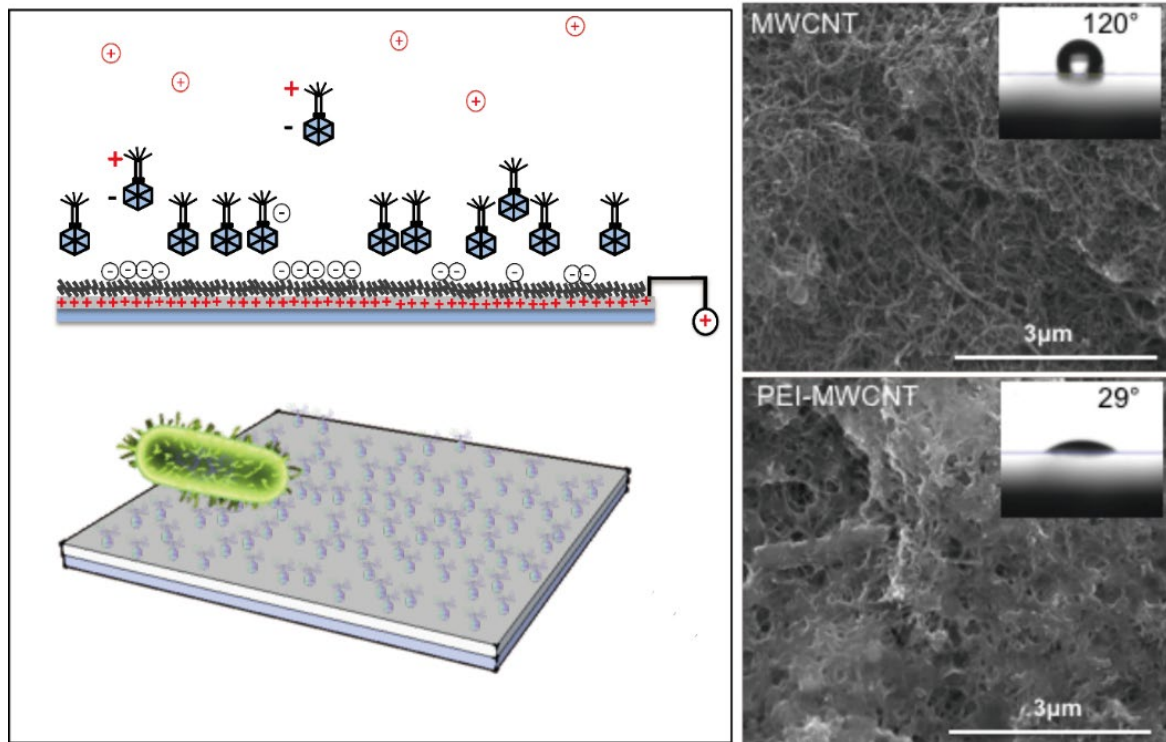
Sample preparation for electron microscopy is more complex than optical microscopy and requires specific steps to ensure that the sample is suitable for high-resolution imaging. Samples must be thinly sliced, typically less than 100 nanometers thick, to allow the electron beam to penetrate and

interact with the material. This process often involves fixing the sample using chemical solutions, followed by dehydration and embedding it in resin for stability. Additionally, some samples may need to be coated with a thin layer of metal, such as gold or platinum, to enhance contrast and conductivity when examined under the microscope. Specialized techniques, such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM), can be applied depending on the type of analysis required. SEM provides surface details of the sample, while TEM allows for the examination of internal structures, both of which are instrumental in detecting adulterants or non-halal substances within food products. Mahmoud Elghoul et al. reported that distinctive features of piglet skin can be identified through light and transmission electron microscopy [25]. Notable features include hair follicles among epidermal cells and melanin granules scattered around flattened keratinocytes. Their novel TEM imaging also revealed the presence of Merkel cells with neuroendocrine granules and Langerhans-like cells observed between the keratinocytes of the stratum spinosum, which can be significant to identify pig skin. Table 2 summarizes the techniques used to identify pig skin and fur using several microscopy techniques. From the table, it can be understood that there are advantages and disadvantages in using different types of imaging techniques for skin and fur. While advances technique (EM) managed to obtain details in Halal authentication, it requires high cost of instrumentation, complex sample preparation, time consuming and expert personal. On the other hand, light microscope (including fluorescent microscope) offered moderate complexity and cost in halal detection. While Digital imaging offered more economic approach for rapid screening, but there were lacked in accuracy.

**Table 2:** Types of imaging techniques for pig skin & hair samples

Type of samples	Analytical methods	Information	Reference
Leather & Leather Products	Light and Scanning Electron Microscopy, FTIR	Leather pigskin characteristic vs cow, goat, sheep and synthetic(polyurethane) leather	[18]
Pig hair	Scanning Electron Microscopy	Split hair, scale pattern	[19]
Wild boar dorsal guard hairs	Light and Transmission Electron Microscopy	Brown coat with tinged grey colour hairs, the profile is straight, thicker in diameter ( $109.4 \pm 41.1 \mu\text{m}$ ) and have distinct microscopic characters	[20]
Pig Skin	Digital camera: texture images using Local-Global Block analysis	Homomorphic filtering technique shows that the illumination given is more balanced	[22]
Piglet Skin	Light and Transmission Electron Microscopy	Ultrastructure of the skin of two-month-old piglets' integument with Langerhans-like cells seen in between keratinocytes of stratum spinosum cells	[25]

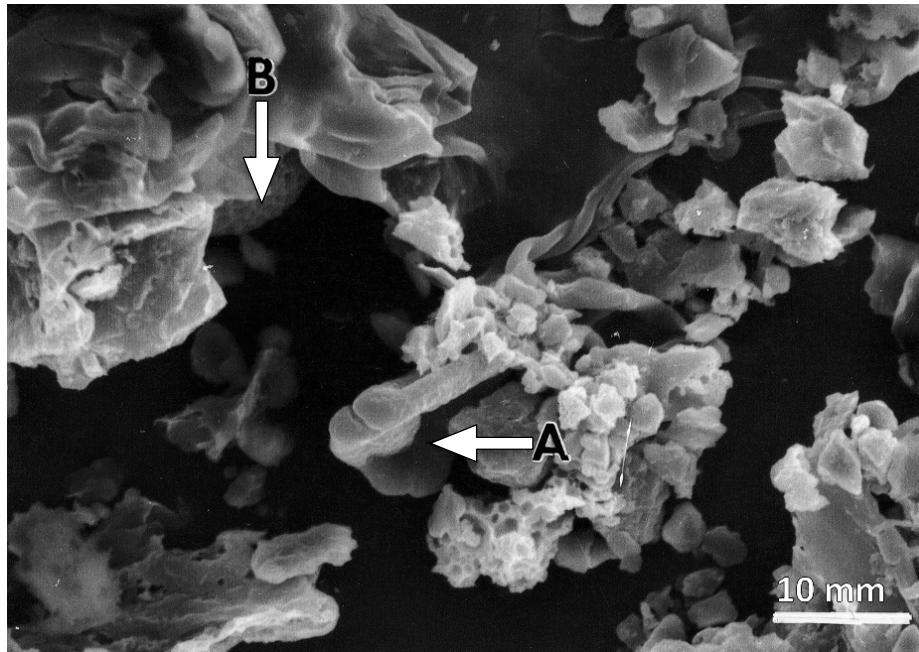
Other than that, SEM also is an important equipment that contributed in the development of biosensors for halal authentication. The use of nanomaterials, such as gold nanoparticles and carbon nanotubes, has been widely explored in the development of highly sensitive biosensors (Figure 10) [26]. These nanomaterials are chosen for their unique properties, such as high surface area, biocompatibility, and conductivity, which make them ideal for detecting specific molecules or contaminants. For example, gold nanoparticles can be functionalized with specific antibodies or biomarkers that bind to non-halal substances, such as non-halal proteins or gelatin, allowing for rapid detection. SEM plays a crucial role in characterizing the structure of these nanomaterials, ensuring their uniformity and stability, which are essential for the reliability of the biosensor. This utilization of SEM in altering nanomaterials enhances the sensitivity and accuracy of halal authentication, offering a promising approach for detecting even trace amounts of non-halal contaminants in food products. Subara et al. has reported the use of gold nanoparticles to identify 11 types of meat compounds, such as pork-shad, pork venison, and shad-venison [27]. Das et al. also have utilized Au-MWCNTs as components in developing biosensors for alcohol detection [28].



**Figure 10.** Example of SEM application in developing biosensors using nanomaterials such as MWCNTs. The left image shows schematic diagram of charge-directed orientation and bacteriophages immobilization on to PEI modified by functionalized-CNT electrode surface. The right images are SEM observation of the CNT before and after PEI modification [26]

### 2.3 Challenges in imaging

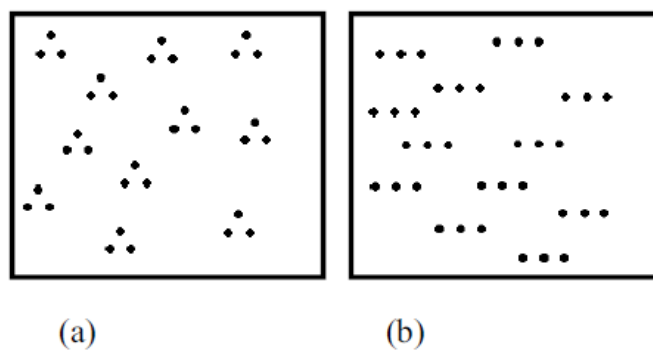
One of the key challenges in imaging for halal authentication is detecting trace amounts of non-halal contaminants, especially when they are integrated into processed food products. Contaminants such as pork-derived gelatin or small bone fragments can be physically indistinguishable from halal ingredients at the macroscopic level, requiring high-resolution techniques for identification. Additionally, sample preparation for imaging can be complex, as food products often contain diverse materials that must be carefully processed to maintain their integrity during examination. Another challenge lies in ensuring the reliability and reproducibility of results, as imaging techniques such as digital cameras, electron microscopy, or fluorescence microscopy require skilled operators to accurately interpret the data. Moreover, the presence of overlapping signals from different components in a sample can complicate the identification of non-halal substances. For example, Figure 11 shows areas that are difficult or even impossible to identify, even in high-resolution images of honey grains [29]. This work by Jones et al. compares sample extraction, processing time, and micrograph data obtained from light and scanning electron microscopy. Ultimately, the choice of imaging technique will depend on the balance between convenience and resolution. Overcoming these challenges requires continuous advancements in imaging technology and more refined sample preparation methods to improve the sensitivity, specificity, and accuracy of halal authentication.



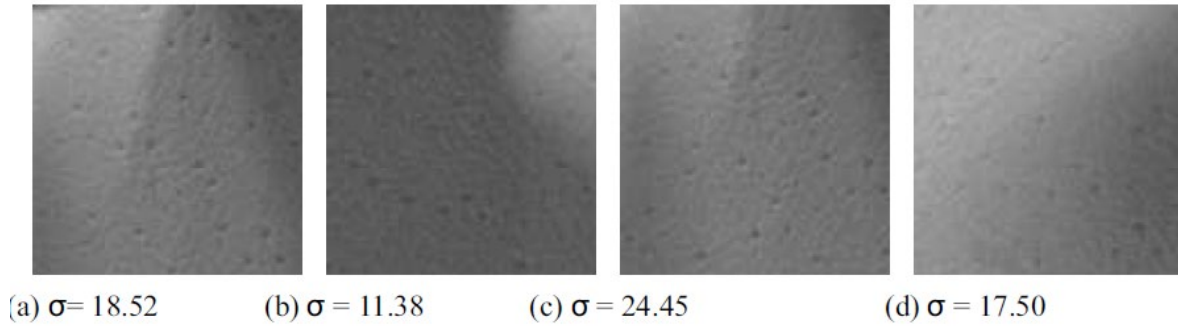
**Figure 11.** SEM micrograph of pollen grains. (A) shows SEM image of pollen grain “obscured” by a poor orientation for identification, while (B) shows pollen grain that obscured by debris, resulting extremely difficult to identify and visualize [29]

### 3. MACHINE LEARNING INTEGRATION IN HALAL AUTHENTICATION

To address the challenges, integration of technology such as statistical, spectral analysis and artificial intelligence (AI) can be significant to enhanced sensitivity, specificity, and accuracy of halal authentication, such as food or meat adulteration [30]. For example, Shamsul et al. proposed illumination compensation in pig skin texture by local-global block analysis methods to make the pattern clearer to ideal dots pattern as shown in Figures 12 and 13 [31]. Meanwhile, different approaches have been demonstrated by Ropodi et al. that shows meat adulteration detection using multispectral and hyperspectral imaging analysis [32].

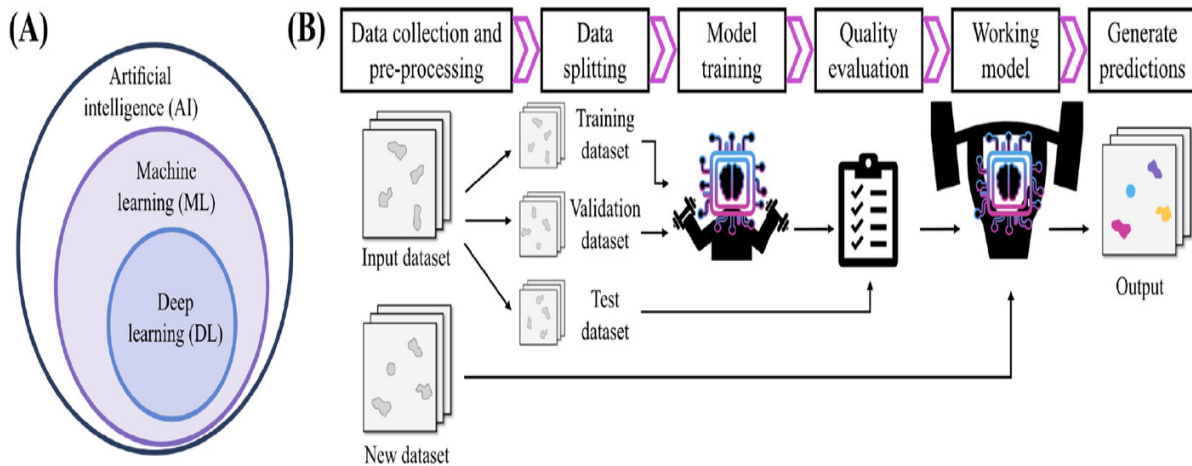


**Figure 12.** An ideal pattern for pig skin leather that consists of 3 dots (a) triangle shape and (b) line shape [31]



**Figure 13.** The images of variable illumination of pig skin texture. The value of the standard deviation indicates the level of fluctuations of the intensity in an image [31]

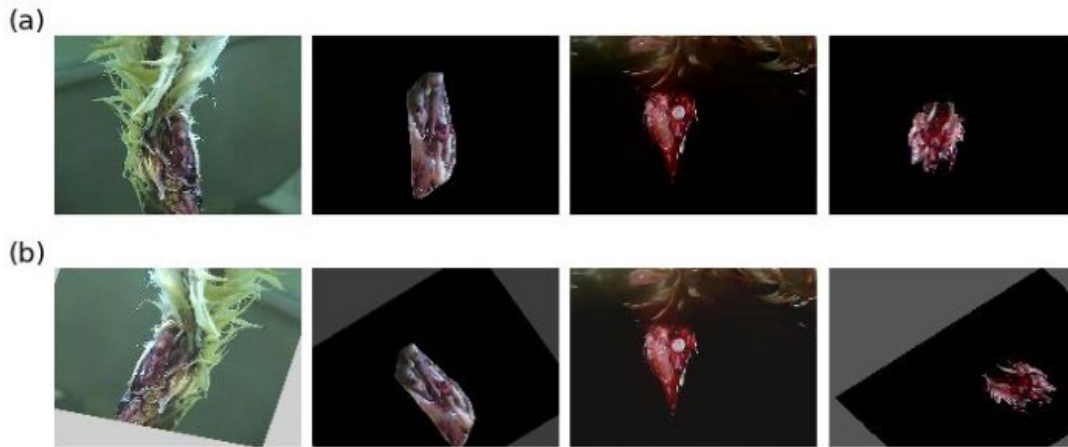
Machine learning is the subset of AI that enables the systems to learn patterns from imaging data and provide predictions nor decision without being programmed beforehand. By integrating them into microscopy or any imaging analysis, it will involve using algorithms to automatically detect, classify, and quantify features in images. Machine learning can also be integrated into microscopy image acquisition workflows to optimize various aspects, such as illumination, modality switching, and acquisition rates. This integration enhances critical parameters, including resolution, acquisition speed, signal-to-noise ratio, multiplexing, and image dimensions, resulting enhanced accuracy and efficiency in interpreting complex biological or physical structures. Figure 14 illustrates the concept of integrating machine learning with imaging. First, data is collected, preprocessed, and divided into training, validation, and test datasets [33]. The model is trained using the training dataset, and its performance is evaluated with the validation set to prevent overfitting. After training, the model undergoes quality control with an independent test dataset. If the model performs well, it can then be used to make predictions on new, unseen data. This approach has demonstrated significant improvements in optimizing illumination, modality switching, acquisition rates, and event-triggered imaging. As a result, it increases the efficiency and information content of image acquisition, enabling more effective studies of dynamic biological processes across different scales.



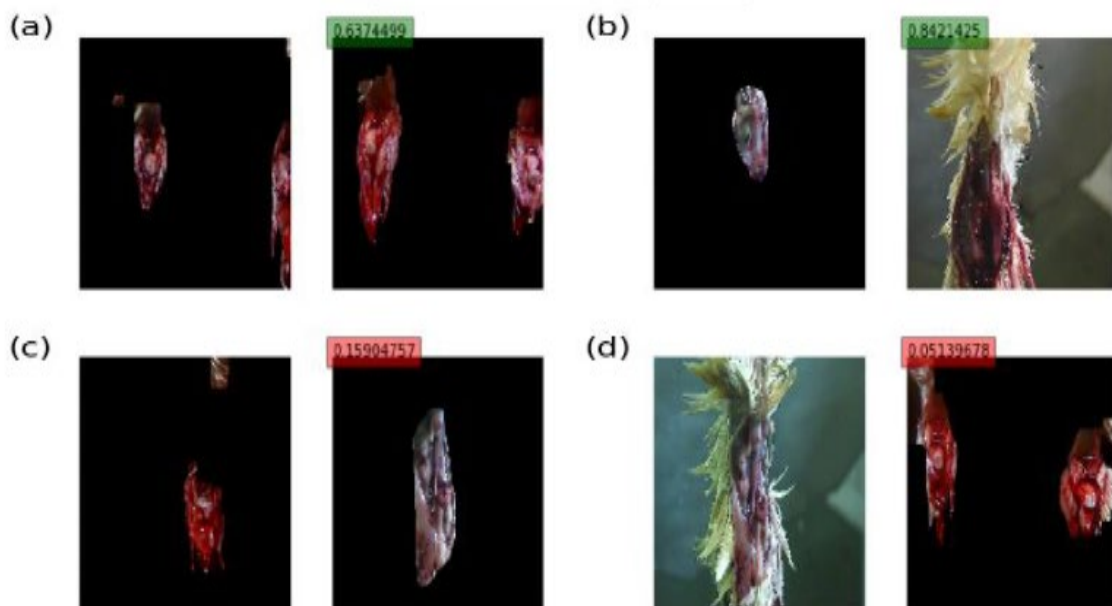
**Figure 14:** Overview of machine learning concepts for microscopy image analysis. Schematic shows that (A) deep learning is a subset of machine learning that part of artificial intelligence. (B) major steps on training of the data set and employed to machine learning model [33].

For example, Elfakharany used a deep neural network to classify halal-slaughtered chicken by augmenting images of the slaughter cut, as shown in Figure 15, with the network’s predictions displayed in Figure 16 [34]. The results show promising enhancement in prediction accuracy of the cut for halal slaughtering by applying the machine learning concept into halal authentication. This finding shows significant potential in complementing Halal Checkers in industry which expose to human error. The

developed techniques and algorithms also have potential to be develop as a guideline for imaging-based Halal testing that regulatory bodies might adopt in the future. As there is already an effort for development of Malaysian Standard (MS) on Halal for skin and fur imaging and chemometric analysis in halal authentication, these efforts can be compliment for future MS development.



**Figure 15.** The images of the chicken slaughter cut. (a) before image augmentation and (b) after image augmentation [34]



**Figure 16.** The images of the chicken slaughter cut that shows network predictions were correct. (a) both halal images that the network predicted reveal that the probability of being from the same class is 0.637. (b) both non-halal images with predicted probability of 0.8421. (c) and (d) are pair of halal and non halal images with the predicted probabilities were 0.159 and 0.051, respectively [34]

However, there is still room of improvements for these studies, as they are limited to controlled areas (lab-scale / small-scale). The sample sizes are still considered small to cater for the halal authenticity in the industrial scale. Therefore, there is still a lack of large-scale validation in developing a bigger database for respective subjects. Other than that, another challenge will be the limited user-friendly AI integration, cost constraints and shortage of trained personnel which hindered the progress of this field. Therefore, joint development of technology such as open-source AI models and data sharing for standardized databases from all the stakeholders (halal institutions, agencies and authorities) are very important to ensure the advancement of these efforts.

#### 4. CONCLUSIONS AND FUTURE PERSPECTIVE

Halal authentication has become more important due to increasing public awareness of the need for food and products to be safe, high-quality, and compliant with Syariah law for Muslim consumers. Traditional methods of Halal verification are often labor-intensive and struggle with detecting cross-contamination and trace amounts of non-Halal substances. Recent advancements in microscopy and imaging technologies, like scanning electron microscopy (SEM), fluorescence microscopy, and hyperspectral imaging, offer new ways to improve the accuracy and efficiency of Halal authentication by identifying contaminants and verifying Halal status at the cellular level. However, challenges remain, such as high costs, technical complexity, and the need for skilled personnel, which limit the widespread use of these methods. Additionally, the sensitivity and specificity of these techniques need improvement to detect small amounts of non-Halal ingredients. Combining these imaging technologies with machine learning (ML) and artificial intelligence (AI) could greatly enhance the speed, accuracy, and scalability of Halal testing, making it more reliable and cost-effective. To fully realize this potential, the support from various stakeholders such as research institutions, agencies and regulatory bodies is very important in the development and sharing of standardized image datasets that reflect diverse sample types and conditions, towards the development of one large Halal database. Additionally, the creation and deployment of open-source AI models tailored to Halal criteria can foster transparency, reproducibility, and broader collaboration across the communities. These advancements will not only improve analytical performance but also support global trust and compliance in Halal certification processes. These advancements could benefit industries beyond food, including consumer goods, pharmaceuticals, cosmetics, and tourism, which provide better consumer protection. Further research is needed to refine these techniques and make Halal authentication more accessible, particularly for small producers and markets.

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#### Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

#### Disclosure of Conflict of Interest

The authors have no disclosures to declare.

#### Compliance with Ethical Standards

The work is compliant with ethical standards.

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