

RESEARCH ARTICLE

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Evaluation of the Diagnostic Accuracy of Cervical Cell Morphologies from Android Device-Captured Cytopathological Microscopic Images through Artificial Intelligence in Mainly Rural or Resource-Constraint Areas of India

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Abstract

Objective: This study aims at develop and evaluate an artificial intelligence programming software, an integrated system that automatically detects and classifies cells from microscopic Pap smear slide images taken on Android phones or tabs to diagnose the cervical cell morphology in a time-efficient and cost-effective manner. **Methods:** This study presents an integrated system designed to automatically detect and classify cells in Pap smear slide images, differentiating cellular morphologies. The system leverages three deep learning (DL) and one machine learning (ML) models, each tailored to specific tasks in the image analysis pipeline. The analysis of 292 hospital in-house microscopic Pap smear images was conducted from July 2023 to December 2024 at CliniMed LifeSciences, Kolkata, India. The following article describes the datasets used, the training procedures and the performance metrics for each model. **Results:** Pap smear images have been validated and standardized by using SipakMed, Herlev (public datasets) and hospital in-house data. A total of 292 in-house Pap smear images have been analysed through the newly developed AI software. Standardization and validation include an Intersection-over-Union score of cell-nuclei boundary extraction model of 71.14%, the accuracy of cell classification model and morphological feature based ML model are 99.213% and 91.23% respectively. The custom AI model could successfully classify 98.09% and 80.49% of normal and abnormal cells in hospital in-house samples respectively. Also a significant meaningful correlation is observed between biopsy (gold standard) and AI reports. **Conclusion:** AI offers a lot of promise for diagnosing cervical cancer, and its uses in cervical cytology screening are particularly well-established. Manual screening of cervical cytology smears is a time-tested method, but AI is set to revolutionize the process by improving outreach, availability, accuracy and economy. A total of 292 hospital in-house Pap smear images have been validated and examined in this study with significant accuracy percentages between AI and expert eyes.

Keywords: Cervical Cancer- Cervical Intraepithelial Neoplasia- Artificial Intelligence- Pap smear- Machine Learning

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Introduction

Cervical cancer, a serious health issue of women, is the frequently diagnosed cancer and fourth leading cause of death. The percentage of cervical cancer in developing countries is approximately 70% (Table 1). This disease is developed due to the infection of Human Papillomavirus (HPV). The main types of HPV causing cervical cancer are high-risk types 16 and 18. There are several stages of cervical cancer; Precancerous and post-cancerous form. Precancerous abnormal form is known as Cervical Intraepithelial Neoplasia (CIN) [1-7]. Cytology screening

is the effective way to screen the abnormality of cervical cells. Implementation of primary (HPV vaccine) and secondary preventive (screening) measures can reduce the mortality percentages of cervical cancer. There are mainly two types of cervical cytology techniques present; Conventional Pap smear (CPP) and Liquid Based Pap smear (LBP). A highly easy, secure, and reasonably priced diagnostic method for evaluating women's cervical health is the Pap smear, which finds aberrant, atypical cells within the cervix. In the 1940s, Georgios Papanicolaou developed the Papanicolaou test, also referred to as the Pap test or Pap smear. For over 70 years, the Papanicolaou

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Table 1. The Data of Cervical Cancer Development Rate and Mortality Rate in India from 2017 to 2022. (39)

Summary	Country Data
Population (million)	1360
Female individuals aged 20-29	137.8 (Positive)
Female individuals aged 30-59	230.5 (Positive)
HPV infection wideness percentage	2.3% - 36.9%
Cervical cancer development rate (per 100,000)	18.7
Cervical cancer mortality rate (per 100,000)	11.7
Method of screening	VIA
Target age group (Years)	35 - 55

test has been the cornerstone of cervical cancer screening. Sensitivity of cytology screening varies between 52% and 80% whereas, specificity varies between 56% to 99% [8-11]. Conventional Pap smear is a cost effective option as it requires very few equipment. In contrast, liquid based Pap smear takes more time to screen and sometimes obscuring factors make the screening difficult for the cytopathologists. Overall both smears are acceptable for cytological evaluation and takes a couple of weeks to generate reports. The VIA technique (Visual Inspection with Acetic acid) was also used in the past frequently for screening where cytology was not available. It requires low expertise but high training skills and reporting TAT of several weeks. The percentages of sensitivity and specificity of this technique are approximately 53-73% and 82-87% respectively [12-17]. HPV DNA analysis through real time PCR has high sensitivity and specificity. The percentages of sensitivity and specificity lies between 94% and 84-90% respectively. The TAT of RT PCR reporting is around 2-3 days. As cervical cancer is very common in rural or remote or low income areas, it is difficult to screen for cervical cell abnormalities on a regular basis due to the lack of facilities [18-21]. India has approximately 60-65% rural or resource constraint areas. So, execution of the advanced techniques are very difficult in these particular areas. The diagnosis process of cervical cancer is slowed down due to the limitation of tests, economy and negligence which leads to increase the morbidity and mortality. Nowadays, automated whole slide scanners with integrated image analysis system based on artificial intelligence are available which show acceptable diagnostic accuracy. This technique need not require any manual expertise or technical skills as it is designed to run in low manpower. But it cannot be implemented in low income countries like India due to the high cost of the system. The sensitivity and specificity percentages of this AI model is more than 95%. The Pap smear slides are directly evaluated by the automated slide scanner and it will deliver the accurate results.

Worldwide there are efforts to unburden the pathologists from the huge caseload, as the number of pathologists in India is low than the required number, of cervical cancer screening by the use of automated slide scanners. The challenge again is that of resources as the automated slide scanners and the composite platform equipped with

artificial intelligence are very expensive compared to the present-day requirement of a binocular microscope [9, 19, 22-24].

However, early screening prevents the development of cervical cancer from cervical cell abnormalities such as CIN. It is further categorized into two groups; High grade Squamous Intraepithelial Lesions (HSIL) and Low grade Squamous Intraepithelial Lesions (LSIL) may develop to carcinoma. The probability of developing carcinoma is high in HSIL than LSIL. Therefore, in low to medium income countries there are very few alternatives present to screen manually the Pap stained cervical smears which requires a huge share of the expert cytopathologists' time. Sometimes there is also a requirement of referral of smears for second opinion [25-31]. In an effort to unburden the cytopathologists, as the number is very less in India and give them a cost effective platform before second opinion, this study evaluates the effectiveness of an artificial intelligence-based image analysis system that can analyse images acquired by mobile android devices. The actual goal is to evaluate the cervical cells in a microscope independent way.

In this study, we have developed the artificial intelligence software with standardization and validation with public datasets and hospital in-house data. The uniqueness of the study is the cost effective pathway, low time consumption with high accuracy and easy access of the AI software to an android device which will help to diagnose cervical cancer prior taking second opinion of cytopathologists.

Materials and Methods

This is a hospital based cross-sectional study that recruited patients screened for cervical neoplastic and pre-neoplastic lesions by cervical cytology using Papanicolaou smear test. The smears were examined through two experienced cytopathologists. The smears agreed upon by the cytopathologists were included in the study. Digital images from microscopic field of the smears were taken on android mobile phone(s). Several microscopic images of normal and abnormal cases were used for training the AI module and 292 microscopic images from hospital suspected cervical cancer patient sample slides were used to test the accuracy where the Pap smear and microscopy are performed as per the standard protocols. The total test samples are collected from two reputed Govt. hospitals of eastern India. Among 292 patient samples, 37 biopsy positive patient cervical cancer samples were identified and then microscopic field images were taken as per the standard protocol and proceed for comparative study.

This study presents an integrated system designed to automatically detect and classify cells in Pap smear slide images, distinguishing normal and abnormal cases (Supplementary Figure 1). The system leverages three deep learning models (DL) and one machine learning (ML) model, each tailored to specific tasks in the image analysis pipeline (Figure 1). The following describes the datasets used, the training procedures and the performance metrics for each model.

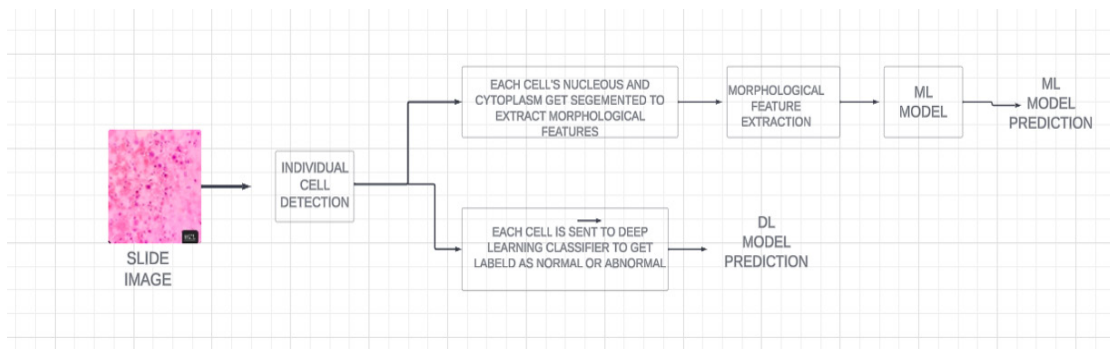


Figure 1. Architectural Model of AI Software where the detection characteristics are shown. The software is based on machine learning and deep learning model. First of all individual cervical cells have been identified and observed for extraction of morphological features and then the cells are evaluated through machine learning and deep learning models. AI: Artificial Intelligence

Methods of Pap smear and Biopsy

In cervical cancer, Pap smear is mandatory to evaluate the cervical cell morphologies in women and later on, biopsy is done for selected patients where confirmation is required. A Papanicolaou smear test is regulated by utilizing a brush or spatula to carefully drag the cellular material from the squamo-columnar junction of the cervix, which is then smeared onto a glass slide. The slides are subsequently fixed in methanol and stained with Pap stain, followed by a visual examination under a microscope by cytopathologists. The cytological interpretation of the smears is performed in accordance with the new Bethesda system for reporting cervical cytology established in 2014. According to the 2014 Bethesda system, lesions are generally categorized into negative for Intraepithelial Neoplasia (NILM) and abnormalities of epithelial cells, which include both squamous and glandular cells i.e. ASC-US, ASC-H, HSIL and LSIL. The HSIL and LSIL cases sometimes proceed further for cervical biopsy for confirmation, when Pap smear can't provide an interpretation. It involves biopsy methods i.e. punch biopsy, cone biopsy etc. where cervical tissue samples are collected and then observed under microscope. But in many cases colposcopy are also done where the magnifying view of cervix is observed through a colposcope. In this study, the conventional biopsy methods were used to evaluate the 37 hospital suspected cervical cancer patient samples where a small tissue sample was collected from cervix and then blocked in Parra film and later on fixed on slide under moist heat. After proper fixation, the slide was stained with specific biopsy stains and then examined under microscope through expert cytopathologists [11, 32-34].

Staining protocols across data sources (Public datasets vs. in-house data)

The public and in-house both datasets have used Papanicolaou smear staining for cervical cell morphology detection. The protocol is as follows:

After collecting the sample(s) from suspected subjects, conventional Pap smear will be done by following the procedure:

Take the cell fixed slide for hydration
↓
Dip the slide in 4 liquids for 1 minute; 80% ethanol, 70% ethanol, 50% ethanol and water respectively
↓
Stain the slide with Hematoxylin Harris solution for 5 minutes.
↓
Immerse the slide for 6 times in 1 second interval.
↓
Submerge in 0.5% Hydrochloric acid for 8 times in 1 second interval.
↓
Rinse the slide with tap water for 5 minutes and pass the slide through 50%, 70%, 80% and 96% alcohol. Slide must be dipped into each alcoholic solution for 30 seconds.
↓
Stain the slide with OG 6 for 1 to 1.5 minutes.
↓
Wash the excess stain of the sample with 96% ethanol for 3-4 seconds. (repeat 2 times)
↓
Stain the sample with EA 50 for 1.5 to 2 minutes.
↓
Wash the sample with 96% ethanol in three different containers for 3-4 seconds each.
↓
Wash the sample with absolute ethanol.
↓
Transfer the slide into 1:1 xylene and incubate for 4 minutes.
↓
Rinse the slide with xylene for 3 minutes.
↓
Mount the slide with mounting medium.
↓
Examine the slides under microscope

Cell Detection Model

The first step in the analysis pipeline involves detecting individual cells within the Pap smear images. For this purpose, we employed a YOLOv8 segmentation model. This model is designed to identify and segment individual cells from complex backgrounds. The model was trained on a composite dataset, which includes images from a local hospital original data procured with required patient consent, the SipakMed dataset and the Cric dataset.

Custom annotations were created for these datasets. Three SMEs manually segmented & annotated all the local hospital-based data to ensure high-quality training data while both SipakMed & Crics were pre-annotated.

Training Dataset

The training dataset consisted of images from the Hospital data, SipakMed, and Cric public datasets, all of which were annotated with custom cell and nucleus segmentation.

Number of Validation Images

33 images are set aside for validation.

Number of Testing Images

292 images are used for testing.

Performance Metrics

mAP50: The model achieved a mean Average Precision (mAP) of 72.7% at an IoU threshold of 0.5.

mAP95: The model recorded a mean Average Precision of 33.9% at IOU thresholds ranging from 0.5 to 0.95.

Cell-Nuclei Boundary Extraction Model

Following the detection of individual cells, the next step involved extracting the boundaries of the cell nuclei and cytoplasm. This was accomplished using a UNET segmentation model with a ResNet50 backbone as the feature extractor. This model was specifically trained to segment the nuclei and cytoplasm, which are critical for subsequent morphological analysis. The training data for this model consisted of cropped cell images from the SipakMed dataset, ensuring that the model focused on the relevant cell structures.

Training Dataset

A total of 3240 cropped cell images from the SipakMed dataset were used for training.

Validation Dataset

The model was validated on 809 cropped cell images, also from the SipakMed dataset.

Performance Metric

Validation IOU Score

The Intersection over Union (IOU) score for the validation set was 71.14%, indicating the model's effectiveness in accurately segmenting the cell boundaries.

Cell Classification Model

Once the cells were detected and their boundaries delineated, the next step was to classify each cell based on its visual features. Given the diverse types of cells present in Pap smear images, it was crucial to focus the classification model on the cells of interest. To achieve this, cells were first cropped from the larger image before being passed to the classifier. This approach allowed for more precise classification, free from the influence of irrelevant surrounding tissues. The classification model was trained on a combined dataset from the SipakMed,

Herlev and hospital datasets.

Training Dataset

The model was trained on a combined dataset comprising 4049 images from SipakMed, 917 images from the Herlev dataset, and 1387 cell images from the hospital data.

Performance Metric

Validation Accuracy

The model achieved a validation accuracy of 99.213%, demonstrating its high effectiveness in accurately classifying cell types.

Morphological Feature-Based ML Model

In addition to visual classification, we developed an ML model to classify cells based on their morphological features. This step was crucial for enhancing the overall diagnostic accuracy by incorporating shape-based criteria. Features such as circularity, eccentricity, roundness, and hull convex area were extracted from the segmented nuclei and cytoplasm, resulting in a comprehensive tabular dataset. Various ML algorithms, including Support Vector Machine (SVM), XGBoost, and Random Forest were evaluated. The Random Forest classifier emerged as the most effective model for this task.

Training Dataset

The morphological features were extracted from a combined dataset of the Herlev and SipakMed datasets, which were then used to train the ML models.

Performance Metric

Validation Accuracy

The Random Forest model achieved a validation accuracy of 91.23%, indicating its robustness in distinguishing between normal and abnormal cells based on shape features.

Precision, Recall, F1-score of per class

Metrics for class: HSIL

- * Precision: 0.997
- * Recall: 0.9987
- * F1-Score: 0.99

Metrics for class: LSIL

- * Precision: 0.99
- * Recall: 0.75
- * F1-Score: 0.8571

Metrics for class: SCC

- * Precision: 0.996
- * Recall: 0.6667
- * F1-Score: 0.8

Metrics for class: NILM

- * Precision: 0.998
- * Recall: 0.9823
- * F1-Score: 0.9911

Methodological Transparency *Image Acquisition Protocol*

The study used a cross-sectional approach, recruiting patients screened for cervical lesions via the Papanicolaou smear test. Digital images from the microscopic field of the smears were captured using Android mobile phones. A total of 292 microscopic images were collected from hospital patient slides suspected of cervical cancer. The Pap smear and microscopy procedures were conducted according to standard protocols. In cases where biopsy confirmation was required, samples were also collected and processed according to conventional biopsy methods.

Preprocessing Steps

The document does not detail specific pre-processing steps like resizing, normalization, or augmentation. However, it does mention that for the Cell Classification Model, cells were cropped from the larger images before being passed to the classifier. For the Morphological Feature-Based ML Model, features such as circularity, eccentricity, roundness, and hull convex area were extracted from the segmented nuclei and cytoplasm.

Model Training Procedures

The AI software was developed using three deep learning (DL) models and one machine learning (ML) model.

Cell Detection Model

A YOLOv8 segmentation model was trained to detect and segment individual cells. The training dataset was a composite of images from a local hospital, the SipakMed dataset, and the Cric dataset. Custom annotations were created, with local hospital data manually segmented and annotated by three subject matter experts (SMEs), while the SipakMed and Cric datasets were pre-annotated.

Hyperparameters

- * epochs=200,
- * patience=50,
- * batch=16,
- * imgsz=640.

Cell-Nuclei Boundary Extraction Model

A UNET segmentation model with a ResNet50 backbone was used to extract the boundaries of cell nuclei and cytoplasm. This model was trained on 3240 cropped cell images from the SipakMed dataset.

Inputshape = (256, 256, 3), learning_rate= 0.001, patience = 5, mode = 'max', verbose = 1, min_lr=0.00001, factor=0.1

Cell Classification Model

A densenet model was trained to classify each cropped cell based on its visual features. The training dataset was a combination of 4049 images from SipakMed, 917 from the Herlev dataset, and 1387 from the hospital data.

num_filter = 40, dropout_rate = 0.0, compression = 1, img_height, img_width, channel = 64,64,3

Morphological Feature-Based ML Model

A Random Forest classifier was chosen as the most effective model for classifying cells based on morphological features. The training data for this model was created by extracting morphological features from a combined dataset of the Herlev and SipakMed datasets.

'n_estimators': 200, 'max_depth': 3, 'gamma': 0, 'colsample_bytree': 0.8

Augmentation Pipeline

The document outlines several data augmentation strategies, categorized by their application:

Albumentations Cell Crop Augmentation: Applied to cropped cell images, these augmentations include

- * Horizontal Flip with a probability of p=0.5.
- * Random Contrast with a probability of p=0.9.
- * Channel Shuffle, which randomly permutes the image channels.

Albumentations Entire Slide Augmentation: These augmentations are applied to full slide images

- * ColorJitter with a probability of p=0.5 and parameters for brightness, contrast, and saturation all set to a range of (0.8, 1).
- * HueSaturationValue with a probability of p=0.5, using shift limits for hue (20), saturation (30), and value (20).

Imgaug Augmentation: The pipeline also incorporates several augmentations from the Imgaug library

- * MotionBlur (k=15).
- * GlassBlur (severity=2).
- * ZoomBlur (severity=1).
- * DefocusBlur (severity=4).
- * Jpeg Compression (severity=5).
- * Pixelate (severity=5).

Stain Mix-up

The document also highlights a specialized histopathology augmentation strategy called Stain Mix-up, which is designed to account for variations in staining protocols, labs, and scanners. This method, proposed by Chang et al., performs augmentation by mixing a source and a target dataset.

Overlapping Cell Handling

The approach uses a multi-stage process to handle cell detection and segmentation, which also addresses common issues like debris and cell overlap.

Initially, the system performs a primary cell detection step using a model like YOLOv8. This initial detection is designed to primarily identify cells, which helps to minimize the misidentification of debris.

Following this, a UNET segmentation model is used to specifically process any detected clusters of overlapped cells. This model works by delineating the boundaries of each individual cell within the cluster, effectively separating them for accurate analysis. This segmented region is then used for further classification and morphological feature extraction (Supplementary

Table 2. Accuracy Report of the Newly Developed Artificial Intelligence Software Trained and Validated with SipakMed and Herlev Datasets and Hospital in-House Data Respectively

Data	Trained Data (Cropped microscopic cell images)	Validated Data	% of Accuracy (True Negative)
Existing Dataset 1 (Herlev)	917	124	97
Existing Dataset 2 (SipakMed)	4049	809	95.6
Our Hospital in-house Data	1387	33	96

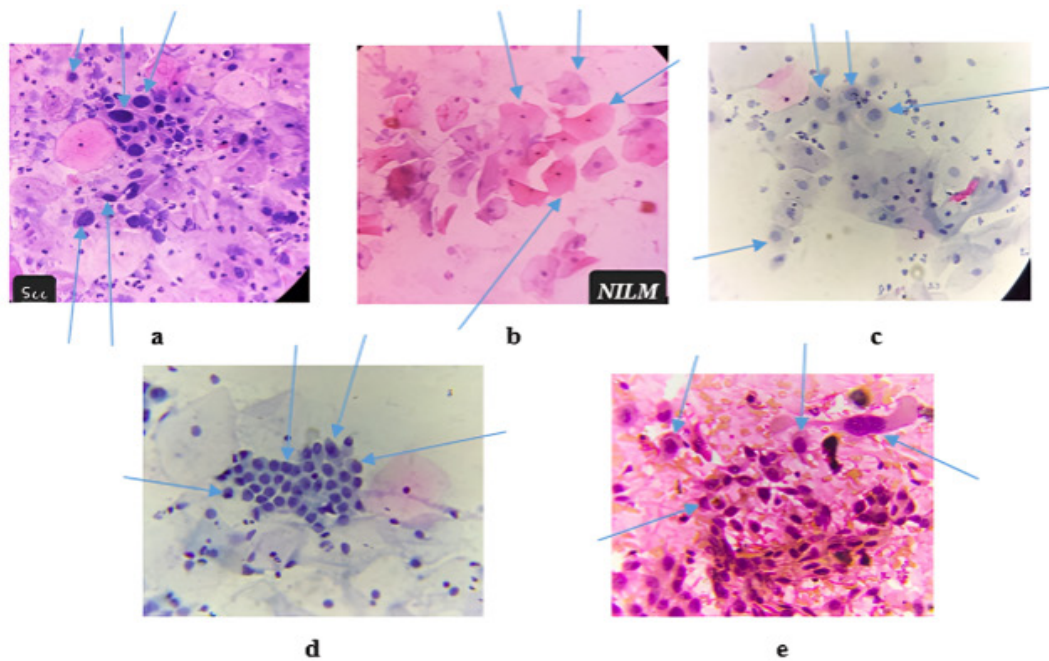


Figure 2. Hospital In-House Microscopic View of Cervical Cells. a. This is the image of squamous cell carcinoma where the cells are distorted and the nucleus to cytoplasm ratio is higher. b. This is the image of normal cervical cells and falls into the NILM (Negative for Intraepithelial Lesion or Malignancy) category where nucleus to cytoplasm ratio is low. c-e. These are the images of low and high squamous intraepithelial neoplasia and squamous cell carcinoma.

Figure 2).

Timeline of data collection: July 2023 to December 2024

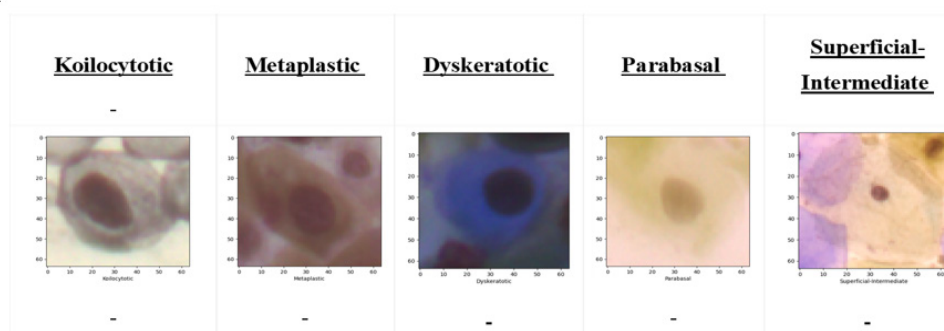
Classification of cervical cell abnormalities are demonstrated in the Figures 2 and 3, where Figure 2 represents the classification of microscopic field images of Pap smear slides and Figure 3 represents the classification of cervical cells from publicly available datasets. The AI software is standardized with the publicly available microscopic cervical cell images, classified as koilocytotic, dyskeratotic, metaplastic, parabasal and superficial-intermediate cells where koilocytotic and dyskeratotic cells are the abnormal conditions and metaplastic and parabasal cells can be normal or abnormal depends on the morphology of cells and superficial-intermediate cells lie in the normal condition. Abnormal condition includes HSIL, LSIL and SCC etc. and normal condition includes NILM. These cells are cropped from 40X microscopic field for representation. Then the hospital in-house microscopic field images of Pap smear samples are used to validate the AI software which is represented in the Figure 2 with arrow marking.

Results

The result of the newly developed AI software model is contributing valuable efforts while diagnosing cervical cancer. The custom AI model is trained and validated through SipakMed, Herlev [35, 36] and hospital in-house data and then performed the first run (Table 2). We identified 292 hospital in-house microscopic Pap smear images from July 2023 to December 2024 by following the inclusion and exclusion criteria. This study discloses the actual accuracy, efficacy and sensitivity of the custom AI model. It could successfully classify 98.09% and 80.49% normal and abnormal cells respectively. The diagnostic performance of the AI software is represented through statistical graphs. Pictorial representation of the statistical findings are given in the Figures 4, 5 and Supplementary Figure 3.

Figure 4 and Supplementary Figure 3 represents the confusion matrix of SipakMed and Herlev datasets and hospital in-house data in two repeated sets. Confusion matrix generally describes the classification of a model through considering the prediction level with its actual level. This presents the matrix of true positive, true negative, false positive and false negative data with

SipakMed



Herlev

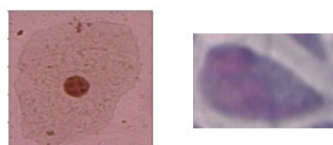


Figure 3. Different Categories of Cervical Cell Abnormalities in AI Model. These are the different categories of cervical cells identified from Sipakmed and Herlev dataset where koilocytotic, parabasal, metaplastic and dyskeratotic fall into the abnormal category and superficial-intermediate falls into the normal category. (Classification model, Performance using Vgg-16).

Table 3. Comparison of the Accuracy Percentages of AI Software with Biopsy and Pap Smear between Selected Biopsy Positive Samples

Procedure	CIN Detected	CIN Not detected	Percentage of accuracy
Pap smear	34	3	91.89
Biopsy	37	0	100
Artificial Intelligence software	33	4	89.18

accuracy percentages. At first, in set 1, the accuracy values of confusion matrix are 0.8533 for Herlev dataset, 0.9543 for SipakMed dataset and 0.9623 for hospital in-house data, where the true positive are 10, false positive are 6, false negative are 5 and true negative are 271 samples out of 292 samples respectively. So the accuracy percentage is 96.23% which is a very good indication of sensitivity and specificity of the software. In another repeated set of confusion matrix with same number of images, it was observed that the accuracy values are 0.8913 for

Herlev dataset, 0.9938 for SipakMed dataset and 0.9692 for hospital in-house data, where the true positive are 11, true negative are 272, false positive are 5 and false negative are 4 out of 292 samples respectively. There is an improvement happened for the second set.

The statistical analysis of the two confusion matrices are also represented on the Figure 5 and Supplementary Figure 3. ROC curve is generated in case of two sets of standardized data matrices for SipakMed and Herlev datasets and hospital in-house data. In set 1, the ROC area

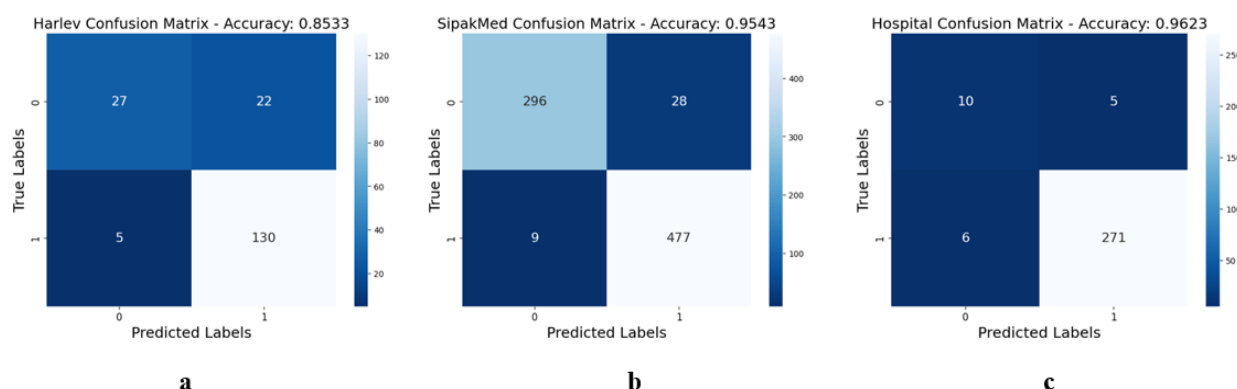


Figure 4. Set 1-Confusion Matrix. These are the percentages of accuracy (Confusion matrix) of public datasets and hospital in-house data. a. It indicates the accuracy of AI model for Herlev dataset i.e. 85.33%, b. It indicates the accuracy of AI model for SipakMed dataset i.e. 95.43% and c. It indicates the accuracy of AI model for in-house data i.e. 96.23%. AI: Artificial Intelligence

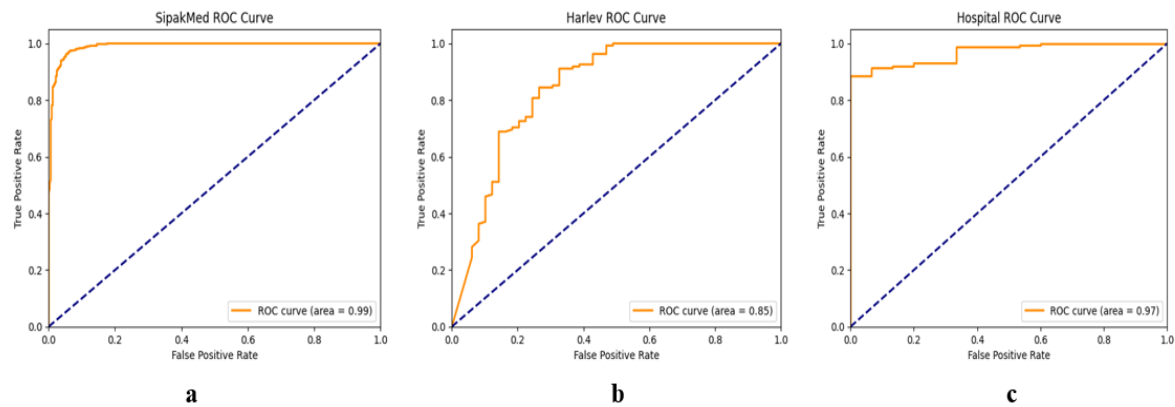


Figure 5. Set 1-Statistical Analysis of Standardized data. a. ROC curve of SipakMed dataset, b. ROC curve of Herlev dataset and c. ROC curve of in-house hospital data. ROC: Receiver Operating Characteristic

values for the identification of cervical cells of Herlev and SipakMed datasets and hospital in-house patient data are 0.85, 0.99 and 0.97 respectively. While in set 2, the area values of the same datasets are 0.95, 1.00 and 0.98 respectively which represents a significant improvement of the custom AI model for the identification of cervical cells. A fair number of the cases in which biopsy was available, showed good agreement between biopsy reports from expert eye of the pathologists and cytology reports produced by Artificial Intelligence. Total 37 biopsy positive cervical cancer slides from 292 patient samples were taken where the Pap smear was already done before biopsy. These 37 samples were further proceed to the comparative study with AI software which is represented in Table 3. As biopsy is the gold standard and 37 samples were already interpreted as biopsy positive, so the accuracy for biopsy was 100% and interestingly, Pap smear showed 34 positive samples out of 37 with accuracy of 91.89%. The custom AI software performed good in a first chance where 33 positive samples were perfectly identified out of 37 with accuracy of 89.18% which are represented in Supplementary Figure 4b. Some of the microscopic field images of biopsy slides are represented in Supplementary Figure 4a and also the detection of normal and abnormal cervical cells through AI software are represented in Supplementary Figure 1. In addition, Supplementary Figure 1 also represents the methodology of detecting cervical cell morphologies through the AI software where the green boxes represent normal cases and the red boxes represent the abnormal one. The overall analysis of the results are showing a significant potentiality of the AI software which will gift a quality life to many

of the women cervical cancer patients in low cost and time as well.

Discussion

Cervical cancer is the most dangerous life threatening cancer for women worldwide. Diagnosis of cervical cancer is not new in case of manual procedures. But diagnosis through machine learning especially in low cost and time with mobility is a new concept. India has approximately 60% of rural areas across the country with low percentages of hospital facilities and expert cytopathologists. In addition, the turnaround time of different confirmatory tests take much more time to interpret cervical cancer which is responsible for the delay of the initiation of treatment. Real time PCR takes minimum 2 days to interpret but expensive, conventional Pap smear takes several weeks to interpret, also liquid based Pap smear takes several weeks to interpret. In 2024, India diagnosed around 1.27 lakhs new cervical cancer cases in women. Cervical cancer ranks second out of all types of cancers in India. These active cases depend on the time of diagnosis, time of HPV vaccination, initiation of treatment etc. factors. The manual diagnosis of cervical cancer involves time taking costly methods like CPP, LBP, VIA, biopsy etc. The minimum cost required is 600 rupees to maximum cost of 3800 rupees approximately for these conventional methods. The problem is that these all methods require expert pathologists to interpret the final results. But India lacks the sufficient number of pathologists required to treat more than 1 billion people. Also one very crucial thing is that from clinician analysis to report interpretation

Table 4. Age Distribution and HPV Status of 292 Hospital In-House Patients

Total number of hospital in-house Patient(s)	Total Negative as per AI software	Total Positive as per AI software	Patient Age	Cervical cancer negative cases	HPV status (Positive cases)	Type (Positive cases)
292	277	15	18-22	180	Positive	LSIL
			23-30	60	Positive	LSIL, ASCUS
			31-45	7	Positive	HSIL
			46-55	10	Positive	HSIL, SCC
			56-70	20	Positive	SCC

Table 5. Time and Cost Comparison of AI Model with Other Conventional Methods

Test / Component	Per-Test (INR)	Total for 100 patients (INR)	Reporting TAT	Cost per correct result (INR)
Conventional Pap Smear	600-800	60,000-80,000	A few days to 1 week	Approx. 632-842
Liquid-based Pap Smear	1,350 – 1,450	1,35,000 – 1,45,000	A few days to 1 week	Approx. 1,421 – 1,526
VIA	1,597	159,700	Several Weeks	1,681
HPV RT PCR	1,450 – 3,800	1,45,000 – 3,80,000	2-3 days	1,526 – 4,000
AI Model	2.63 / patient	263	30s / patient (50 min approx.)	2.76

takes minimum one month to initiate the treatment of a cervical cancer suspected individual. This lagging period can be a logging period of cervical cancer to spread. In addition, upgrading of existing diagnostic parameters are the runway of scientists to achieve more accuracy and perfection. As a result, artificial intelligence gets the entry in manual world. Carolyn Nakisige et. al., 2023, observed that an AI decision support system has been developed through Manipal School of Information Sciences, Manipal Academy of Higher Secondary Education of India. The system has been trained through 100 images after acetic acid application collected from VIA clinics. The algorithm will generate a map immediately after the image is captured and will be able to distinguish between a normal cervix (negative) and an abnormal cervix (positive) that requires further evaluation. Sensitivity and specificity of medical personnel were determined as 80.4%, 80.5%, sensitivity and specificity of experts as 81.6% and 93.5%, respectively. The sensitivity and accuracy of the algorithm were 80.0% and 83.3%, respectively. The AUC values of 0.80 (95% CI 0.70 – 0.90) for medical personnel, 0.93 (95% CI 0.87 – 1.00) for professionals and 0.84 (95% CI 0.75 – 0.93) for Artificial Intelligence [29, 37, 38]. Another study by Jue Wang et.al., 2024, aimed to develop and validate the AICCS system for cervical cytology with cervical WSI analysis. The AICCS (Artificial Intelligence Cervical Cancer Screening) system was studied and tested on a different dataset consisting of 16,056 participants. It uses two AI models: one for cell detection and the other for WSI classification. This study used multicentre, retrospective and prospective population-based data as well as a randomized controlled trial. The proposed method achieved an AUC (Area under Curve) of 94.7%, sensitivity of 94.6% and specificity of 89.0% and an accuracy of 89.2% respectively [20, 39, 40]. Automated slide scanner is a fully automated AI instrument where Pap smear slides can be evaluated without any pathologists with around 95% accuracy. But in a middle income country like India, it is difficult to implement the system in every laboratory due to high cost. Several methods of many studies have been observed to diagnose cervical cancer through artificial intelligence.

In our study, the focus is based on the diagnosis of cervical cancer through AI but in low cost and time. Total 292 Pap smear microscopic images of hospital in-house patient samples have been validated and examined in our present study through a custom AI software with statistical

analysis (Table 4). The custom AI software is developed by using three deep learning and one machine learning models. The deep learning model learns the software to label a cell that is normal or abnormal. While machine learning model learns to extract the morphological features of a cell for categorisation and segmentation. The DL and ML models are trained and validated with more than 6000 cropped cell images obtained from the SipakMed and Herlev datasets and hospital in-house data and then 292 in-house microscopic field images are sent to the first run. The identification of normal and abnormal cells has reached to the efficient level of the AI software. The Confusion matrix presents the matrix containing true positive, true negative, false positive, and false negative data along with their corresponding accuracy percentages. Initially, in set 1, the accuracy values of the confusion matrix are recorded as 0.8533 for the Herlev dataset, 0.9543 for the SipakMed dataset, and 0.9623 for the hospital in-house data. In this set, the true positives are 10, false positives are 6, false negatives are 5, and true negatives are 271 (compared to gold standard method) samples out of a total of 292 samples. Consequently, the accuracy percentage is calculated to be 96.23%, which serves as a strong indicator of the software's sensitivity and specificity (Figure 4). In a subsequent repetition of the confusion metrics with the same number of images, it was noted that the accuracy values improved to 0.8913 for the Herlev dataset, 0.9938 for the SipakMed dataset, and 0.9692 for the hospital in-house data. In this instance, the true positives are 11, true negatives are 272, false positives are 5, and false negatives are 4 (compared to gold standard method) out of 292 samples. This indicates a notable enhancement in the second set (Supplementary Figure 3).

The statistical analysis of the two confusion matrices are also illustrated. The ROC curve is generated for the two sets of standardized data matrices pertaining to the SipakMed and Herlev datasets and hospital in-house data. In set 1, the ROC area values for identifying cervical cells in the Herlev and SipakMed datasets and hospital in-house data are 0.85, 0.99, and 0.97, respectively (Figure 5). In contrast, in set 2, the area values for the same datasets are 0.95, 1.00 and 0.98 respectively, indicating a significant improvement in the custom AI model's ability to identify cervical cells (Supplementary Figure 3). The average sensitivity, specificity and accuracy remain at around 85-90%, which is a good indication to achieve higher level of accuracy. To confirm the accuracy percentage,

37 biopsy positive samples were taken from 292 hospital patient samples and proceed for the comparative study. Table 3 represents the accuracy percentages of Pap smear with normal manual microscopy, biopsy and Pap smear with AI software analysis. Out of 37 biopsy positive samples, 34 samples are detected through normal manual microscopy and 33 samples are detected through AI software. The accuracy percentages with respect to biopsy (Gold standard) are 91.89% and 89.18% respectively. Again the accuracy percentage of AI software is proved near about 90%. The promise of our AI model was to screen the cervical cell morphologies in a cost-effective and time-efficient manner which we have achieved to diagnose it within 30 seconds per sample in very low cost of around 2.7 rupees per sample approximately (Table 5) which will contribute a milestone in diagnosis era and also boost the follow-up and screening percentage in the rural population. But there is room for improvement in the following areas: processing of non-target cells and debris, segmentation of overlapping nuclei, cluster of cells and proper control of slide staining discrepancies etc. Although our model shows promise, but it will be improved for much more accuracy as we continue to extract more information from single images and incorporate them in further refining the classification skills of the AI model. The future implementation will be the installation of the AI software in an android device and perform in a microscope independent way especially where the number of pathologists and microscope both are restricted.

Strength and Limitations

This study has validated 292 in-house Pap smear images, with remarkable outcomes. The ability to distinguish between normal and abnormal cells has advanced to an impressive degree. In order to attain maximum accuracy, the software's sensitivity, specificity, and accuracy stay at or above 85%. Nevertheless, there are still a few minor problems with segmentation technology, which is used for automatic classification. Furthermore, as previously mentioned, some classifications are not based on segmentation technology. This could be the path of future progress and will avoid many of the earlier steps. In addition, if an individual needs to test primarily to check the status of the cervical cell morphologies, our model is quite capable but in case of MRD (Minimum Residual Disease), an individual must proceed for gold standard methods i.e. Real time PCR, Biopsy etc. because these conventional molecular methods are more reliable for MRD checking.

Further Possible Result Improvement

Although we have achieved some remarkable results in identifying and labelling cells, there are specific limitations that present opportunities for further enhancement. Currently, our system struggles in segmentation of cells where the cell boundaries are not vivid or where overlapping occurs. Additionally, for a more confident labelling process, it is imperative to analyse cells in the context of the entire slide image rather than in isolation.

At this stage, our approach focuses on labelling a cell as either normal or abnormal. Even though our model

is promising, it can still be improved as we continue to extract more information from individual images and use it to further evaluation of the AI model's classification abilities. Moving forward, we aim to extend this classification to identify the specific type of abnormality a cell belongs to. This advancement will not only improve the diagnostic precision but also provide deeper insights into the cellular characteristics of specific abnormalities.

Conclusion

In this study, the microscopic images of cervical cells were evaluated through customized artificial intelligence based software where three deep learning and one machine learning models were used. The AI software is trained through the upgraded YOLOv8 segmentation model for the identification of cervical cells in a complex background. Several experimental steps were followed to standardize and validate the AI software where significant accuracy percentages were observed. The software were trained with both normal and abnormal cervical cell morphologies with the classification of NILM, HSIL, LSIL, SCC etc. Different statistical analysis were done (Supplementary Figure 5) for the hospital in-house patient samples where the overall accuracy percentage lies between 85-90%. A comparative study was also conducted with biopsy positive samples where the performance of AI software with conventional methods was notable. So it is concluded that, the custom AI software for detection of cervical cell morphologies is set a milestone in the world of pathology. Also the installation of the software in mobile android devices are an achievement for the cervical cancer screening. It may still be improved as we continue to extract additional information from individual photographs and use it to better evaluation of the AI model's categorisation abilities. But the AI software is set to revolutionize the process by improving outreach, availability, accuracy and economy. In future it will demonstrate the ability of the AI software to evaluate cervical cells in women through a mobile android device except conventional binocular microscope.

Author Contribution Statement

Arindam Ray – Data validation, Administration, Investigation, Supervision, Methodology and development guidance of AI software. Arindam Karmakar – Writing – Original draft, review & editing, Validation, Administration, Methodology, Investigation, Supervision, Conceptualization of AI software, provision of hospital Pap smear images and evaluation of hospital in-house data. Moumita Maiti – Administration, Investigation and provision of hospital Pap smear images. Soumyabroto Banerjee – Writing – Original draft, Validation, Methodology, Development of AI software, Statistical result generation. Swati Dasgupta – Validation, Administration, Investigation, Supervision and guidance in the evaluation of hospital in-house data. Sayan Dey – Writing – Original draft, review & editing, Methodology and Administration. Umar Faruk – Writing – Original draft, review & editing. Priyabrata Das – Administration, Investigation and Supervision. Prosenjit

Saha – Conceptualization, Investigation and Supervision.

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Data Availability

The SipakMed dataset is publicly available on universe.roboflow platform (<https://universe.roboflow.com/>). The Herlev dataset is also publicly available on kaggle platform (<https://www.kaggle.com/datasets/>). The hospital in-house data is governed by agreements or restrictions. That's why, it is not publicly available.

Ethical Statement

All the data were collected after the approval of Institutional Ethics Committee of Calcutta National Medical College and Hospital and Nil Ratan Sircar Medical College and Hospital.

Funding Statement

This research study is solely funded by the R&D division of CliniMed LifeSciences. There is no other funding applied to conduct the study.

If any scientific body approved it / if it is part of an approved student thesis

All the data were collected after the approval of Scientific Committee and Institutional Ethics Committee of Calcutta National Medical College and Hospital and Nil Ratan Sircar Medical College and Hospital. It is not the part of any student thesis. We have attached the approval letter of both the ethics committee as supplementary file named Supplementary file_AI paper revision.

How the ethical issue was handled

The ethical issue of the research study was handled through the approval of Institutional Ethics Committee of Calcutta National Medical College & Hospital and Nil Ratan Sircar Medical College & Hospital before initiating the study. We have attached the approval letter of both the ethics committee as supplementary file named Supplementary file_AI paper revision.

Conflict of Interest

The authors declare that there is no financial and personal relationships with other people or organizations that could inappropriately influence or bias the work. No conflict of interest is present.

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