

Deep Learning Based on DeepLabv3+ for Automated Identification, Segmentation, and Quantification of Fungi in Clinical Fluorescence Microscopy Images

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Abstract

Background

Fungal fluorescence microscopy using Calcofluor White is a rapid and sensitive diagnostic tool for fungal infections. However, manual microscopic examination is limited by high subjectivity, observer fatigue, poor reproducibility, and a heavy reliance on professional expertise—particularly in high-throughput clinical screening.

Objectives

To develop and validate a DeepLabv3+-based deep learning model for the automated identification, precise segmentation, and quantification of fungal hyphae and spores in clinical fluorescence microscopy images.

Methods

A total of 60,484 fungal fluorescence images were collected from 1,863 clinical specimens across seven medical centers. Of these, 38,494 images were used for model training, while 2,561 independent images were selected for clinical validation. Blinded manual microscopy by expert technicians served as the gold standard. The model's diagnostic performance, stability, and agreement were evaluated.

Results

The model demonstrated robust diagnostic performance, with an accuracy of 90.32%, precision of 91.58%, recall (sensitivity) of 92.42%, and specificity of 88.39%. The Youden index was 0.8081, with an area under the curve (AUC) of 0.91, indicating substantial agreement (Kappa = 0.798). The system exhibited excellent stability across repeated tests and automatically generated quantitative counts of hyphae and spores.

Conclusions

This DeepLabv3+-based deep learning system provides an accurate, stable, and high-throughput approach for clinical fungal fluorescence microscopy. It can standardize diagnostic procedures and improve efficiency for diagnosing superficial and suspected invasive fungal infections, serving as a reliable auxiliary diagnostic tool.

1. Introduction

Fungi are widely distributed eukaryotic organisms; approximately 400 species can cause human infections[1] [2] [3]. The prevalence of fungal diseases is approximately 4500 per 100,000 population, and the annual demand for medical fungal testing exceeds 400 million tests[4] [5]. Fungal fluorescence staining has become the mainstream technique for screening clinically suspected fungal infections because it is rapid, highly sensitive, simple to perform, provides clear contrast, and is subject to few interferences[6] [7] [8]. Calcofluor White (CFW) is a commonly used clinical fluorescent dye that specifically binds to β -glycosides in fungal cell walls. Upon ultraviolet excitation, it emits bright blue fluorescence, creating a striking contrast between fungal structures and the background and thereby improving detection sensitivity[9]. However, manual microscopy is influenced by specimen type and operator

experience, leading to misdiagnosis and missed detection. It cannot meet the need for standardized screening of large specimen batches[10].

In recent years, artificial intelligence has advanced rapidly in medical image analysis[11]. Convolutional neural networks (CNNs), with an end-to-end feature learning paradigm, outperform traditional algorithms in image recognition and classification tasks[12]. DeepLabv3 + is a deep learning model that integrates atrous convolution and encoder–decoder architecture, which reduces parameters and sampling operations without altering feature map size, thereby improving training efficiency and segmentation accuracy[13]. Although DeepLabv3 + performs excellently in image segmentation and recognition, no studies had applied it to the simultaneous identification, segmentation, and quantitative counting of fungal hyphae and spores in multi-type clinical fluorescence microscopy images before the initiation of this study. Therefore, we constructed a high-quality, multicenter, multi-specimen dataset of fungal fluorescence images. We applied DeepLabv3 + for the first time to simultaneously segment and quantify hyphae and spores and performed independent clinical validation. We systematically evaluated the model's diagnostic performance, stability, and agreement with manual interpretation. This work provides a basis for intelligent, standardized fungal fluorescence microscopy across a wide range of specimens, from superficial to potentially invasive infections.

2. Materials and Methods

2.1 Collection of Clinical Samples and Acquisition of Fluorescence Images

A wide variety of clinical specimen types were included to reflect real-world practice and test the model's generalizability. A total of 1,863 specimens for fungal microscopy were collected from seven medical centers in this multicenter study, including Shanghai Changzheng Hospital, The First Affiliated Hospital of Guangxi Medical University, Yan'an People's Hospital, Nanning Fourth People's Hospital, Jiangdu Maternal and Child Health Hospital of Yangzhou, Dongguan Sixth People's Hospital, and Songgang People's Hospital of Bao'an District, Shenzhen. Specimen types included 783 nail scrapings, 717 skin scrapings, 24 hair specimens, 53 pus samples, 18 oral secretions, 53 ear canal secretions, 37 genitourinary secretions, 4 conjunctival secretions, 21 nasal secretions, 67 urine samples, 37 blood samples, 26 bronchoalveolar lavage fluid samples, and 23 cerebrospinal fluid samples. All specimens were processed using fungal fluorescence staining. Three laboratory technicians (each with > 5 years of experience) performed standardized microscopy: samples were first scanned at low magnification for hyphae or spores; positive samples were then re-examined at high magnification to confirm morphology. Positive samples showed distinct hyphae or spores, whereas negative samples had no obvious fungal structures. (Keratin, collagen, plant fibers, and other substances may also fluoresce and require careful differentiation.) Images were acquired under high-power microscopy (40× objective, total magnification 400×). Five to twenty images from different fields were captured per specimen, yielding 60,484 fluorescence images (27,158 positive, 33,326 negative).

2.2 Classification and Annotation of Fluorescence Images

The polygon annotation tool of the image acquisition system was used to precisely annotate target features. Standardized manual annotation before model training can further improve learning efficiency and enrich model output [14]. A total of 2,000 negative and 2,000 positive images were randomly selected and imported in batches. Hyphae, spores, and keratin were manually annotated using the polygon tool. In positive images, isolated hyphae and spores were outlined with full contours. Densely interwoven or clustered regions were labeled as a single affected area (without individual segmentation) to ensure annotation efficiency and clinical practicality. Negative

images were primarily annotated for keratin (Fig. 1). After excluding blurred, overexposed, out-of-focus, and duplicate images, a total of 143,953 annotations were completed (119,710 positive, 24,243 negative), giving a positive-to-negative ratio of approximately 5:1.

2.3 DeepLabv3 + Algorithm

Convolutional neural networks (CNNs) reduce parameters via local connectivity and weight sharing. Convolutional layers extract features; pooling layers reduce dimensionality and help prevent overfitting[15]. DeepLabv3 + incorporates atrous convolution to preserve boundary features and combines an encoder-decoder architecture with batch normalization, improving small-object segmentation. It has been used in autonomous driving and medical image analysis but not previously for fungal fluorescence image recognition[16]. The workflow is shown in Fig. 2.

Key training parameters were set as follows. Input images were resized to 512×512 pixels. The Adam optimizer was used with an initial learning rate of 1×10^{-4} . We used a batch size of 16 and trained the model for 100 epochs with early stopping. Data augmentation (random rotation, flipping, scaling) was also performed.

2.4 Image Training, Validation, and Evaluation Metrics

The model was implemented in Python using mainstream deep learning frameworks. Hyperparameters were set to default optimal values and further optimized by grid search. We then performed basic performance testing followed by independent clinical validation.

First, an independent test set of 4,000 images (2,000 positive, 2,000 negative) was selected from the 60,484 images, with no overlap with the training set. This set was used only for baseline performance testing and overfitting detection.

Second, the training set comprised 38,494 images (16,633 positive, 21,861 negative) after excluding low-quality images. Five-fold cross-validation was applied with data augmentation and a class-weighted loss function to address class imbalance.

Finally, an independent clinical validation set of 2,561 images (1,544 positive, 1,017 negative) was obtained from 192 clinical specimens collected at Shanghai Changzheng Hospital. The reference standard was established by blinded readings from three laboratory technicians (> 5 years of experience), who were independent of the model training and annotation teams and blinded to the model's predictions. Inter-reader agreement was assessed using Fleiss' Kappa; consensus results served as the gold standard. Model predictions were compared with manual readings to calculate diagnostic performance. Recognition time (including preprocessing, inference, and output) was recorded for both the model and manual reading. Model stability was also evaluated.

2.5 Statistical Analysis

Data analysis was performed using GraphPad Prism 9 software. The Kappa test was used to evaluate diagnostic agreement between AI and manual microscopy, with Kappa > 0.75 indicating good agreement. Categorical data (positive rate, accuracy, recognition results) were analyzed using McNemar's test. Recognition time (mean ± SD) was analyzed using Welch's t-test after testing for normality and equal variance. The stability of repeated measurements was described using the coefficient of variation (CV) without significance testing. Diagnostic metrics were calculated as: precision = $TP/(TP + FP)$; recall = $TP/(TP + FN)$; accuracy = $(TP + TN)/\text{total samples}$. Specificity, Youden index, and 95% CI were also computed. Receiver operating characteristic (ROC) curves were plotted, and the area under the curve (AUC) was calculated. A two-tailed test was used, and statistical significance was set at $\alpha = 0.05$.

3.Results

3.1 Source of Fluorescence Image Samples

A total of 60,484 fluorescence images were acquired from 1,863 clinical samples, which were mainly derived from nail scrapings (783 samples, 42.1%) and skin scrapings (717 samples, 38.5%). After screening, 38,494 images were used for model training (and an independent test set of 4,000 images was also selected, as described in Methods). A total of 143,953 manual annotations were completed, including 119,710 positive annotations and 24,243 negative annotations, with a positive-to-negative ratio of approximately 5:1.

3.2 Training Results of the DeepLabv3 + Model

Baseline performance was assessed on the independent test set (2,000 positive, 2,000 negative). Of the 2,000 positive images, the model correctly identified 1,759 (misclassified 241); of the 2,000 negative images, it correctly identified 1,839 (misclassified 161). The model achieved a precision of 91.61%, a recall of 87.95%, and an accuracy of 89.95% on the test set, indicating favorable baseline recognition performance without obvious overfitting (Table 1).

3.3 Clinical Validation Results of the DeepLabv3 + Model

We conducted independent clinical validation to evaluate recognition efficacy, stability, and real-world performance. The validation set included 2,561 images from 192 clinical specimens. Key performance metrics are presented in Table 1. The model showed excellent overall diagnostic ability. McNemar test showed no significant difference from manual microscopy ($P = 0.485$), and substantial agreement was reached ($\text{Kappa} = 0.798$). The AI model produced a fully consistent positive rate in three repeated tests ($59.82 \pm 0.00\%$). Manual microscopy varied noticeably ($59.40 \pm 1.01\%$; $P < 0.001$). This difference reflects the high stability of AI rather than a clinically relevant gap in detection. Mean recognition time per 100 images was 4.41 ± 0.81 min for manual microscopy and 4.67 ± 0.14 min for the AI model ($P > 0.05$; Table 2). The two methods had similar speed, but the AI system had lower variability. Representative segmentation results are shown in Fig. 3. The original fluorescence image (left) was processed by the DeepLabv3 + model to generate an automated output (right). Spores were marked with red circles, and hyphae were enhanced for clear visualization. ROC curve analysis confirmed strong classification performance of the model (Fig. 4).

A confusion matrix heatmap further illustrated diagnostic agreement between model predictions and the gold standard (Fig. 5). The heatmap displays counts of true-positive, true-negative, false-positive, and false-negative cases across the validation set. It confirmed high consistency between AI and manual microscopy. The model also enabled automated identification, segmentation, and accurate counting of hyphae and spores (Fig. 6). An overview of the entire study workflow and key AI-based diagnostic output is summarized in Fig. 7. This integrated figure illustrates the identification of hyphae and spores, as well as the final automated AI diagnosis, representing the core design and main findings of this work.

Table 1

Performance Evaluation of the DeepLabv3 + Model in the Test Set and Clinical Validation Set Note: Compared with the gold standard, McNemar test $P > 0.05$; Kappa > 0.75 indicates good agreement. The test set was only used for preliminary performance screening and overfitting testing, and Kappa was not calculated.

Testing Stage	Sample Size	Precision(%)	Recall(%)	Accuracy(%)	Specificity (%)	Youden Index	AUC (95%CI)	Kappa
Test set	4000	91.61	87.95	89.95	91.95	0.7990	0.90 (0.89–0.91)	-
Validation set	2561	91.58	92.42	90.32	88.39	0.8081	0.91 (0.89–0.92)	0.798

Table 2

Comparison of Positive Rate and Recognition Time Between Manual Microscopy and the AI Model

Average positive rate(%)	Manual Microscopy ($\bar{x} \pm s$)	AI Model ($\bar{x} \pm s$)	<i>P</i>
	59.40 ± 1.01	59.82 ± 0.00	< 0.001
Average recognition time (min/100 images)	4.41 ± 0.81	4.67 ± 0.14	0.627 > 0.05

Note: Average positive rate refers to the proportion of positive determinations per image; average recognition time refers to total time consumption per 100 images. Independent samples t-test was used for between-group comparison; $P > 0.05$ indicates no statistically significant difference.

4. Discussion

Fluorescence staining microscopy is a core technique for diagnosing fungal infections. However, manual microscopy is subjective, fatiguing, and poorly reproducible. Misdiagnosis and missed diagnosis are more likely to occur in non-keratin specimens such as pus and secretions, making it difficult to meet the need for rapid screening of large specimen batches. Here we show that a DeepLabv3+-based model can automatically identify, segment, and quantify hyphae and spores in fluorescence images. This offers an objective, rapid, and standardized solution for clinical fungal detection. The model was validated on diverse specimen types (nail, skin, secretions, BALF, cerebrospinal fluid), supporting its use for both superficial and suspected invasive fungal infections.

The present model achieved high diagnostic accuracy and substantial agreement with expert manual microscopy. It showed consistent performance across repeated tests, with lower variability than manual microscopy. The AI system matched manual reading in speed but offered better stability and objective quantitative output. These features support its use in routine clinical screening and longitudinal monitoring of fungal burden.

We selected DeepLabv3 + over other common models for its strength in medical image segmentation. Compared with U-Net, ResNet, and YOLO series, DeepLabv3 + better preserves fine details and boundaries of slender hyphae and small spores.[17, 18]. Its encoder–decoder structure and atrous convolution improve segmentation of dense, overlapping fungal structures[19][20]. Compared with previous studies that used YOLO v4 for hypha and spore recognition in fungal microscopy[21], this study is highly comparable in terms of task type, specimen type, and

evaluation objectives. The model achieved higher accuracy than previously reported CNN-based fungal recognition systems, confirming its suitability for clinical fungal fluorescence images.

This study has several limitations. First, densely clustered hyphae and spores were annotated as whole affected areas, not individual structures. This design may reduce pixel-level segmentation accuracy in dense regions. Second, clinical validation was performed in a single center. Multi-center external validation is needed to confirm generalization. Third, the model's performance for rare or morphologically atypical fungi requires further optimization with expanded datasets. Finally, we did not analyze model interpretability or error patterns. Visualization methods such as Grad-CAM may help identify misclassification causes and improve model reliability.

In conclusion, the DeepLabv3+-based deep learning system enables automated identification, segmentation, and quantification of fungi in clinical fluorescence microscopy images. It shows high accuracy, good agreement with manual microscopy, and excellent stability. This system can standardize diagnostic procedures and improve efficiency for superficial and suspected invasive fungal infections. Future studies should include multi-center validation, rare-fungus datasets, and lightweight deployment for wider clinical use.

Declarations

Conflict of interest

The authors declare no competing interests.

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Author Contribution

Baiyinzi Du, Wenjie Fang and Fen Shu wrote the original paper and prepared figures and tables; Tiancheng Chen, Weihua Pan and Wanqing Liao conceived and designed the study; Mingwei Du developed the analytical pipe-line and performed the analyses; Kaisu Pan, Yanan Xu, Yanqing Zheng, Yuan Gao, Kai Ma, Wanwan Pan, Yingping Lin, and Haibin Wu performed the lab experiments, including sample collection; Weihua Pan and Wenjie Fung acquired funding for the project; Wanqing Liao supervised the project. All authors reviewed and edited the manuscript.

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Figures

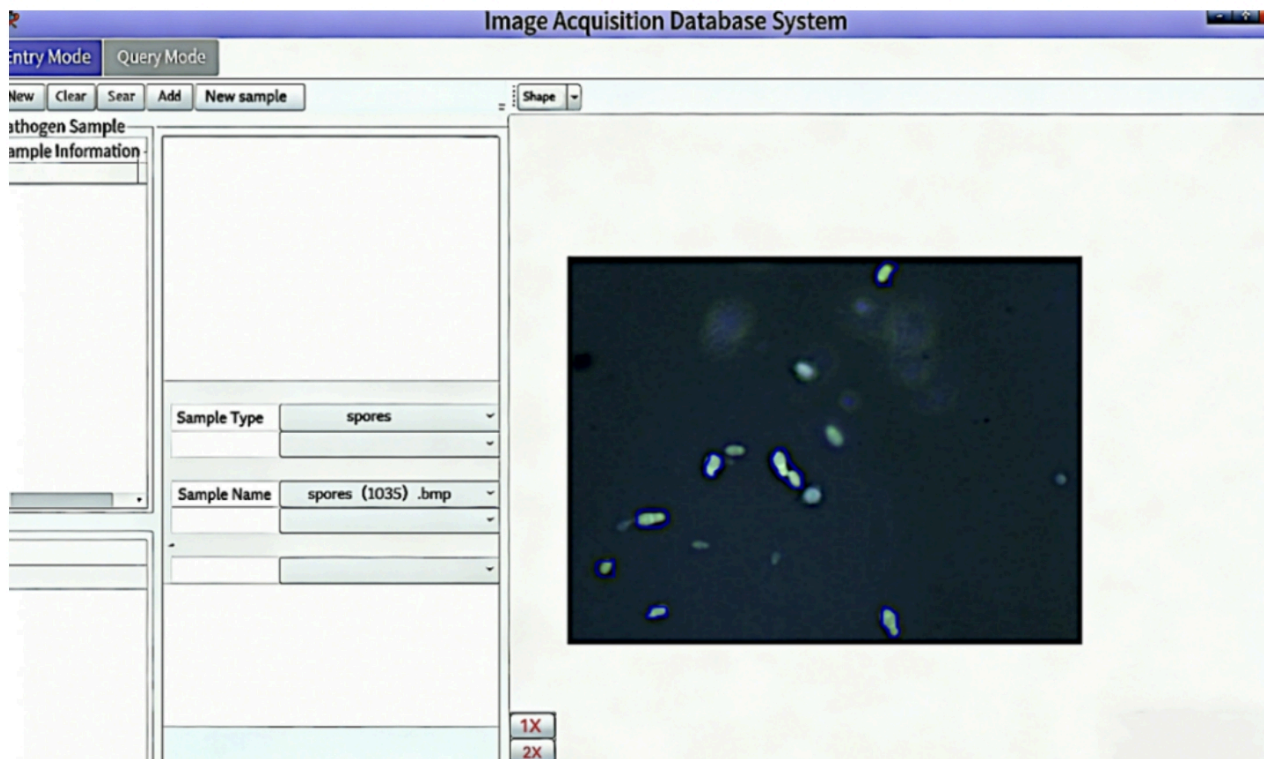


Figure 1

Example of manual annotation of fungal fluorescence images.

Note: Left: Specimen information panel; Right: 400× fluorescence image with spore contours labeled by blue polygons.

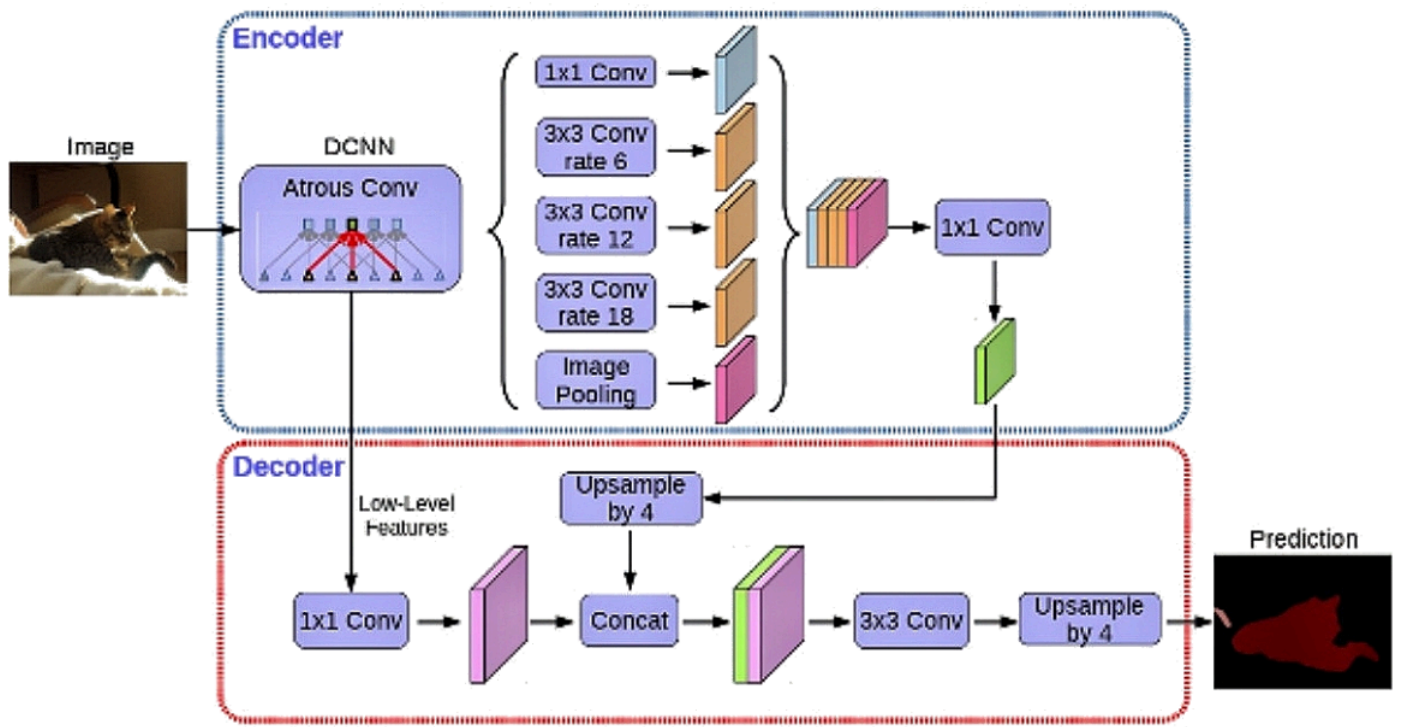


Figure 2

Schematic diagram of the DeepLabv3+ algorithm architecture.

Note: The model adopts an encoder–decoder structure with atrous convolution to achieve multi-scale feature extraction and precise image segmentation.☒

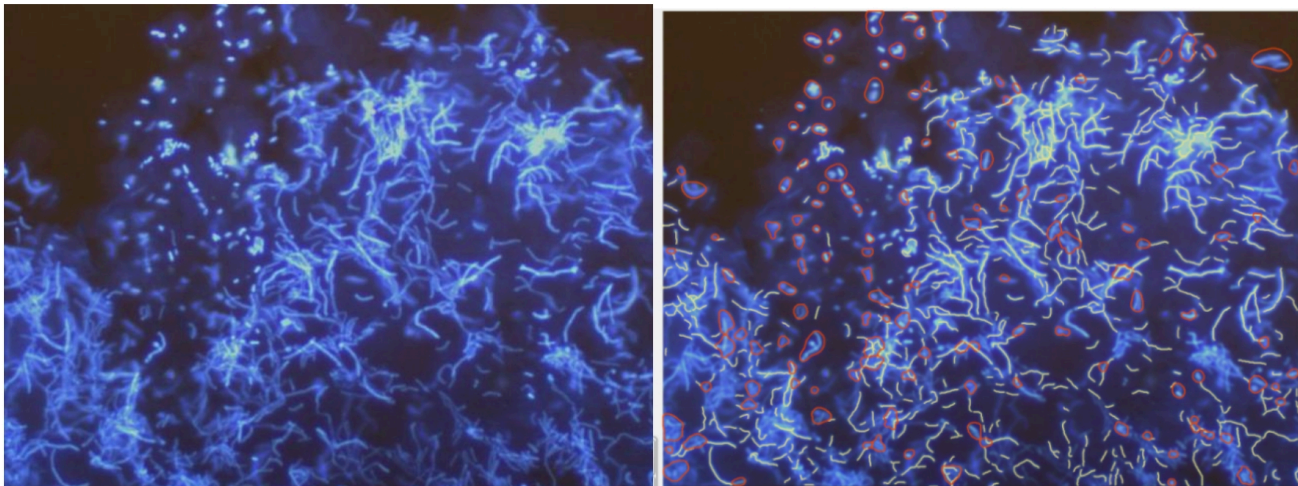


Figure 3

Comparison of original fungal fluorescence microscopy image and AI-based segmentation output (400× magnification).

Note: Left: Original CFW-stained clinical fluorescence image; Right: Automated recognition result by the DeepLabv3+ model. Spores are labeled with red circles; hyphae are highlighted with enhanced fluorescence intensity for clear visualization.

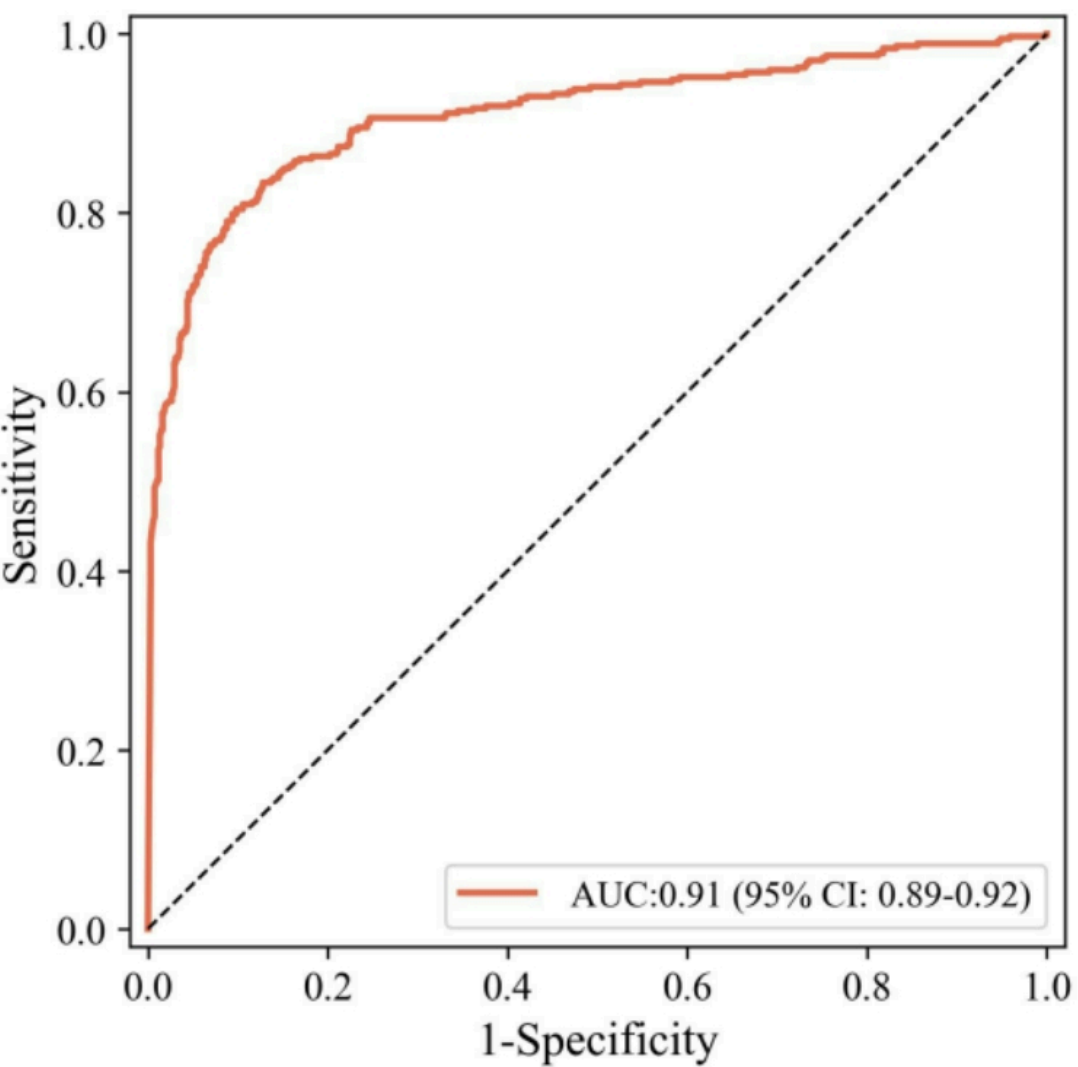


Figure 4
ROC Curve for Evaluating the Diagnostic Performance of the DeepLabv3+ Model in Fungal Fluorescence Images

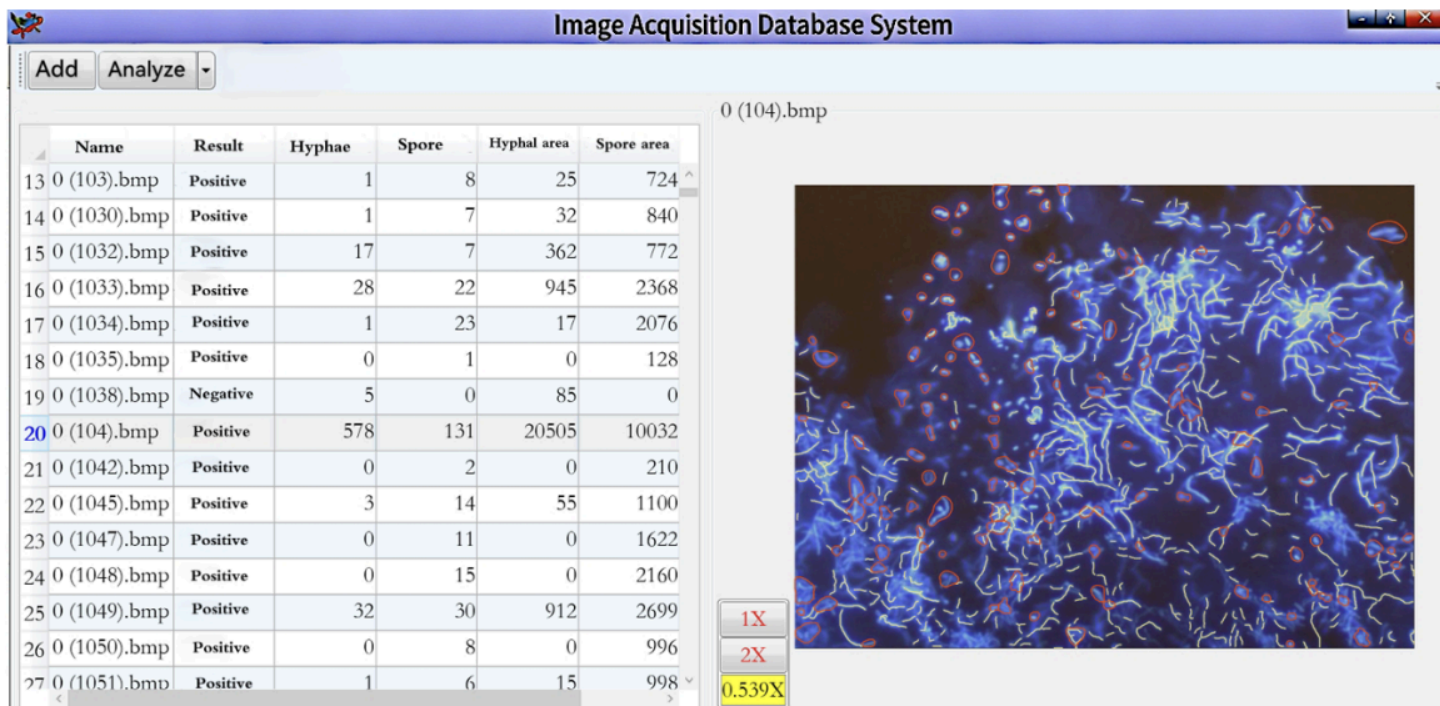


Figure 5

Confusion matrix heatmap of the DeepLabv3+ model on the independent clinical validation set (n=2,561 images).

Note: Columns represent the gold standard of manual microscopy; rows represent model predictions. Color intensity indicates the count of correctly/incorrectly classified samples, reflecting diagnostic agreement.

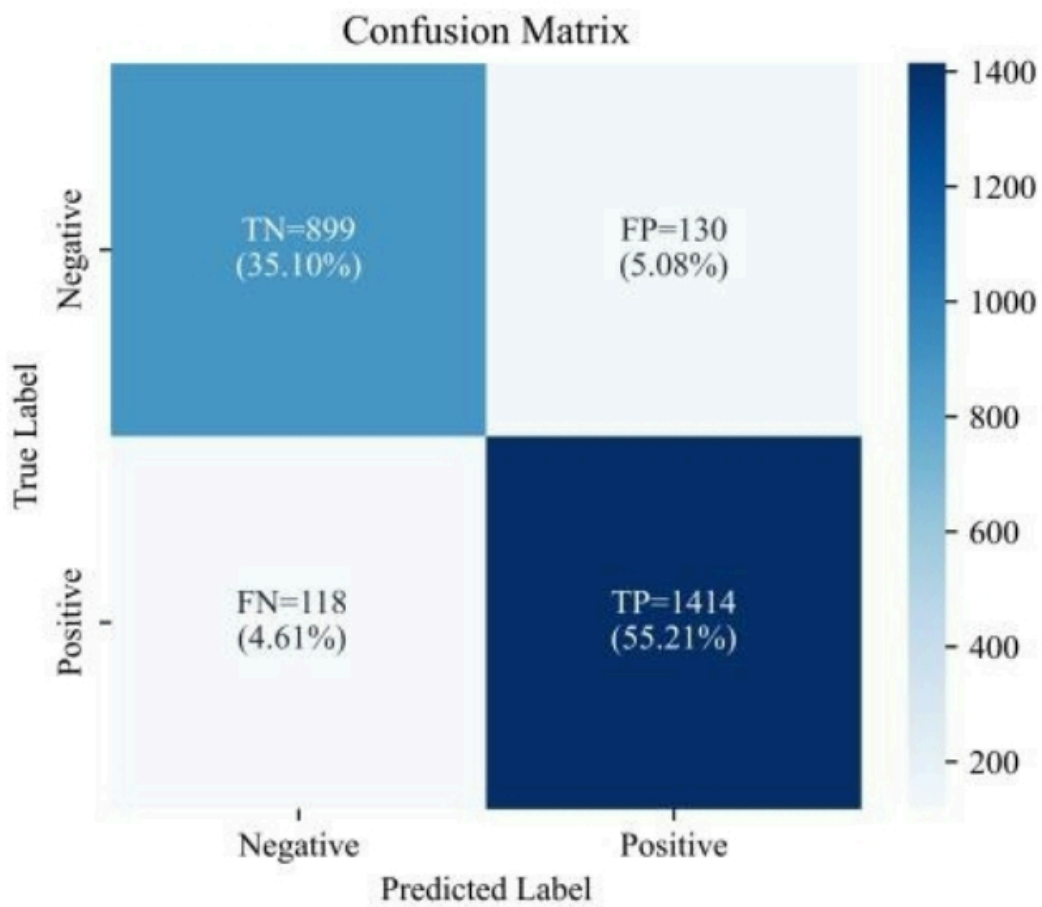


Figure 6

Example of Quantitative Output by the Model

Note: The selected region in the table represents a positive image, with hyphal count = 578 and spore count = 131.

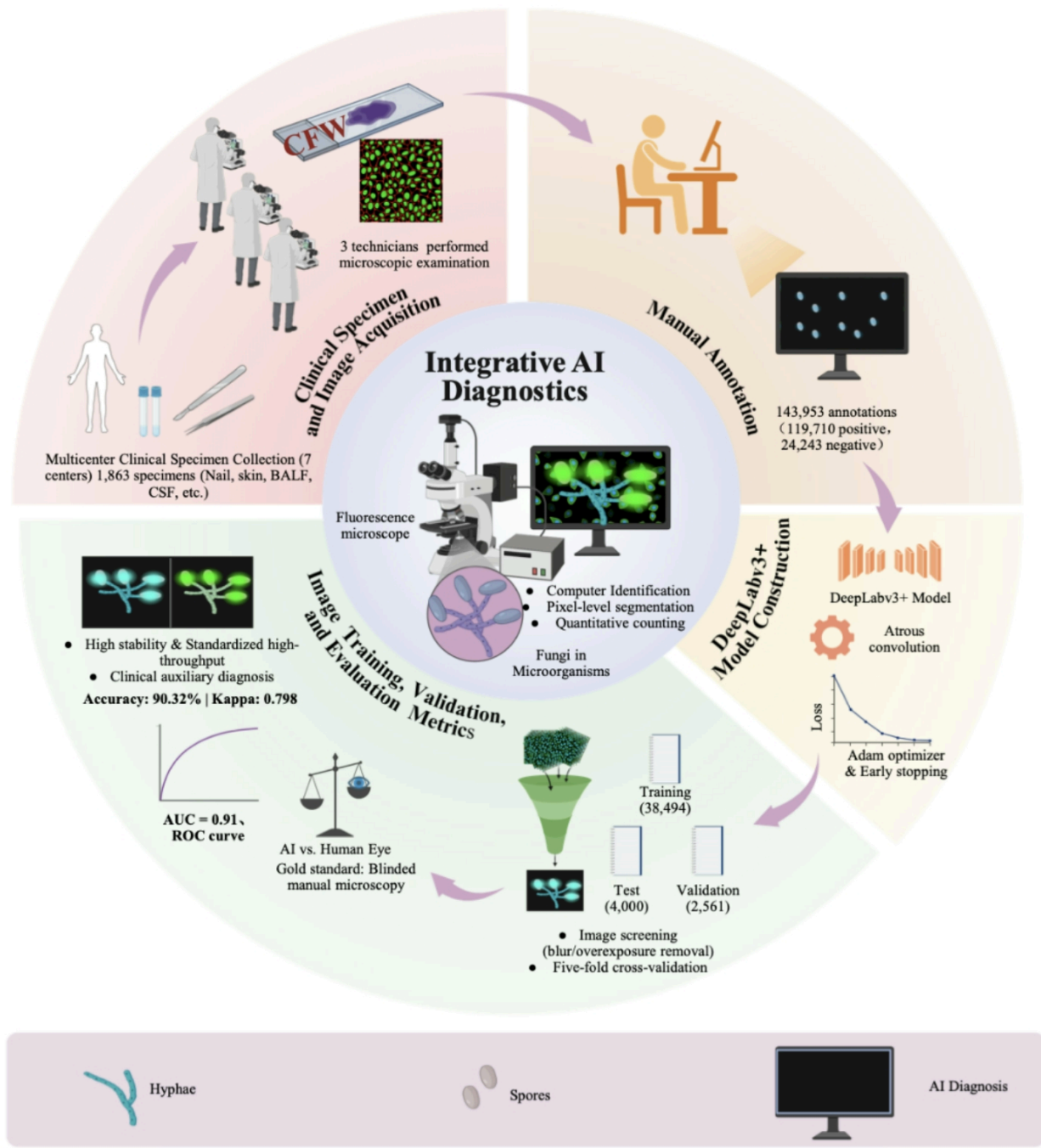


Figure 7

Overview of the study workflow and automated AI-based fungal diagnosis.

Note: The figure summarizes the full experimental design and key results, including automated recognition of hyphae, spores, and the final integrated AI diagnosis output for clinical application.