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Deep-learning-enabled automatic gene abnormality detection via fluorescence in situ hybridization (FISH)

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Abstract: Fluorescence in situ hybridization (FISH) is widely used for diagnosing cancer and genetic disorders due to its high specificity and accuracy. However, traditional methods face challenges such as suboptimal focus adjustments, subjective signal counting errors, and inefficiencies in imaging, limiting their use in high-throughput screening. To address these issues, we introduce the fluorescence in situ hybridization and analysis system (FAST), an innovative solution that combines rapid filter switching, automated focusing, multi-layer fluorescence signal fusion, and the improved ResNet152 deep learning framework. Compared with clinical manual counts and analysis of case reports of 10 patients with chronic lymphocytic leukemia (CLL), the FAST system achieved an average cell segmentation accuracy of 98.28% with a 95% confidence interval (CI) of [97.43%, 99.13%]. For abnormal gene detection, the model achieved an accuracy of 97.86%, and the classification results showed a 95% CI of [97.54%, 98.17%]. Additionally, its intuitive interface allows the operator to complete the entire workflow—from scanning to report generation—within 45 minutes. FAST represents a significant advancement in cancer and genetic disorder diagnostics, offering a powerful tool for early detection.

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Key words: Fluorescence in situ hybridization (FISH); Image enhancement; Cell nucleus segmentation; Fluorescence feature fusion; Abnormal gene classification

1 Introduction

Cancer remains a leading global health challenge, with rising incidence and mortality emphasizing the urgent need for effective early detection and diagnosis (Siegel et al., 2024). To detect related chromosomal abnormalities, fluorescence in situ hybridization (FISH) has been developed and widely used due to its high specificity and sensitivity (Patino, 2024). Specifically, by using fluorescently labeled probes that hybridize with target DNA sequences, FISH enables precise visualization of genetic alterations such as the deletions in 13q14 (Cui et al., 2024), 11q (Krayem et al., 2024), 13q (Knöll et al., 2024), and 17p (Lankford et al., 2024), which are critical for cancer diagnosis, prognosis, and treatment planning (Siegel et al., 2023; Tao et al., 2023). However, as a microscopy-based technique, FISH inherently depends on imaging systems for signal detection and analysis, making its performance closely tied to the quality and efficiency of the microscopy technologies used (Li et al., 2024). For example, the commonly used wide-field fluorescence microscopy provides faster image acquisition by capturing larger sample areas simultaneously (Schermelleh et al., 2019).

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However, its susceptibility to artifacts (such as halos, low contrast, and uneven staining) can compromise the accuracy of fluorescent signal detection (Lewis et al., 2021). These imaging limitations hinder critical steps of FISH such as nuclear segmentation and abnormality identification, thereby increasing the likelihood of diagnostic errors (Scott et al., 2018; Nasir et al., 2023). Moreover, the manual and expertise-dependent nature of traditional FISH workflows increases operational complexity, making the process labor-intensive, time-consuming, and prone to subjectivity (Syrykh et al., 2025). Collectively, these challenges limit the scalability and clinical adoption of FISH in high-throughput and rapid diagnostics (Jonkman et al., 2020; Zhang et al., 2021), highlighting the urgent need for innovative solutions to enhance automation, accuracy, and efficiency.

Recent advancements in artificial intelligence (AI) offer transformative opportunities to overcome the challenges associated with traditional FISH analysis. By automating image analysis, AI significantly reduces reliance on manual expertise (Plass et al., 2023), enhances diagnostic accuracy, and enables high-throughput and standardized workflows (Perez-Lopez et al., 2024). Despite its promise, the application of AI in FISH-based genetic diagnostics still faces several critical limitations. These include the insufficient robustness of existing models when confronted with complex genomic backgrounds (Huber et al., 2018; Ordoñez et al., 2024), the need for improved accuracy and efficiency in multi-target detection, the lack of integrated platforms capable of real-time image processing and analysis, and the limited interpretability and clinical usability of current AI systems, which remain below the standards required for routine clinical adoption.

To address these issues, in this paper we introduce an innovative integrated FISH imaging and analysis system (FAST) designed to improve the diagnostic efficiency and reliability of FISH technology under complex imaging conditions. The system integrates a motorized fluorescence microscope (BX61, Olympus) with AI-enabled analytical algorithms and a grayscale camera chosen for its superior sensitivity and quantum efficiency (Otomo et al., 2024). Specifically, three key innovations underpin the FAST platform: 1) A multi-layer focal plane imaging strategy, which enables comprehensive acquisition across different focal depths to ensure complete cellular structural information, minimize information loss from single-plane imaging, and reduce subjective errors associated with manual focusing. 2) An image enhancement and fusion module, which uses multi-scale filtering and illumination compensation to automatically correct uneven lighting, enhances contrast between nuclear regions and background, and suppresses noise and artifacts. This ensures consistently high image quality even under low-light or complex imaging conditions. 3) An optimized nuclear segmentation algorithm, which combines gradient mapping, convex defect detection, and an improved watershed method to achieve highly accurate and robust segmentation, with an average segmentation accuracy of 98.28%, outperforming both traditional and deep-learning-based methods.

The system's gene abnormality detection results show high consistency with expert manual interpretation accuracy of 97.86% and demonstrate robust generalizability in real clinical validation. Moreover, the workflow is fully automated, from sample scanning to diagnostic report generation, greatly enhancing analytical efficiency and reducing reliance on operator expertise. The system features an intuitive, user-friendly interface designed for diverse user groups while maintaining professional oversight for final report verification. Collectively, the FAST platform enables scalable and automated genetic analysis across diverse cancer types and clinical applications, representing a significant advancement in precision oncology and genetic diagnostics.

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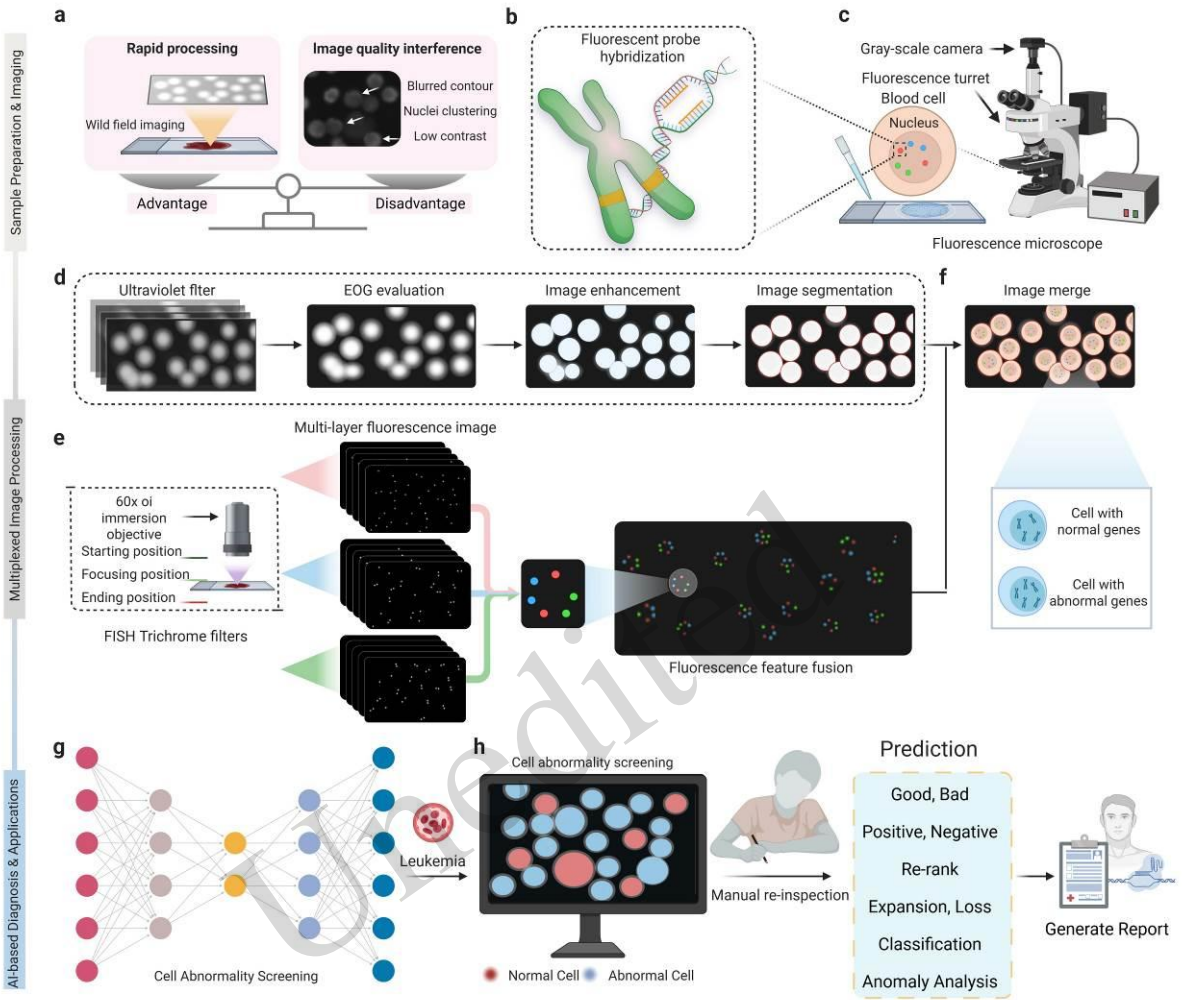

Fig. 1 Integrated FISH imaging and analysis system (FAST) workflow for cancer diagnosis. (a) Overview of the advantages and limitations of fluorescence microscopy. (b) FISH probe hybridization targeting specific genetic sequences. (c) Image acquisition of hybridized samples using a motorized 2D fluorescence microscope with a grayscale camera, capturing multilayer images through four-color fluorescence filters. (d) Image enhancement and segmentation of DAPI-stained nuclei to extract clear nuclear contours. (e) Fusion and analysis of multilayer fluorescence signals (orange, aqua, and green) visualized through tricolor FISH channels. (f) Comprehensive cytogenetic data integration from all channels, enabling accurate identification and classification of chromosomal abnormalities. (g) A tailored CNN model in the dataset for automated cell abnormality classification, leading to (h) accurate and efficient cancer diagnosis with a user-friendly diagnostic report. The system thereby provides a promising automated solution for early cancer detection and personalized prognosis management.

2 Methods

2.1 Overview of the FAST system

Fig. 1 illustrates the overall workflow of the FAST system (Supplementary Fig. 1a), which comprises three key steps: (I) imaging performance evaluation and acquisition, (II) multiplexed and multilayer image processing, and (III) AI-driven detection of cellular abnormalities. In this study, chronic lymphocytic leukemia (CLL) was chosen as the model disease for system validation due to its relevance to various cancers and operational stability. To highlight the motivation for system development, Fig. 1a summarizes the advantages (Supplementary Table 1) and limitations (Supplementary Table 2) of conventional fluorescence microscopy.

In Step I, using blood samples collected from suspected CLL patients (Supplementary Table 3), FAST specifically targets the prevalent 13q14 deletion in this highly genetically heterogeneous

cancer (Skerget et al., 2024). To identify cytogenetic markers associated with CLL, the samples were pretreated with 4',6-diamidino-2-phenylindole (DAPI) (Xie et al., 2024) for cell interphase nucleus localization. Tricolor FISH probes XL RB1/DLEU/LAMP1 (green, orange, and aqua) were then applied to label the corresponding DNA fragments (Fig. 1b, Supplementary Fig. 1b, & Supplementary Table 4). During image acquisition, each slide was scanned across 36 distinct fields of view, with the system automatically selecting the field containing the highest cell density. The tetrachromatic optical filters switched automatically to acquire multilayer grayscale images at 11 focal depths (102.2-106.6 μm), thereby ensuring optimal axial resolution and signal clarity (Fig. 1c & Materials and Methods).

In Step II, image processing focuses on extracting nuclear contours and fluorescence probe signals. Each sample generates 44 images (4 channels × 11 scanned layers, as shown in Supplementary Fig. 1c). Among these, the image with the highest clarity is selected, and the nuclear contours are segmented (Fig. 1d & Supplementary Note 1). In parallel, fluorescence features from the orange, aqua, and green channels are processed and fused into the segmented images (Fig. 1e). Finally, the nuclei's contours and fluorescence information are merged to localize and analyze the signals (Fig. 1f).

In Step III, a convolutional neural network (CNN) model is used to detect chromosomal abnormality in individual cells for CLL diagnosis (Fig. 1g). Synergistically, this automated and multiplexed FAST system enables novice operators to rapidly, accurately, and cost-effectively visualize and evaluate cytogenetic features (including deletions and qualitative assessments). Empowered by AI-driven algorithms, FAST achieves precise and reliable detection of chromosomal abnormalities, markedly enhancing diagnostic accuracy. The system features an intuitive and user-friendly interface that streamlines conventional FISH workflows by integrating image acquisition, signal processing, and result interpretation within a unified platform. Through the incorporation of deep learning algorithms, FAST automates nuclear segmentation, fluorescence signal fusion, and abnormality detection, thereby minimizing human error and inter-operator variability. The platform also supports batch automated processing, which significantly improves analytical throughput and reproducibility. Ultimately, FAST generates a comprehensive diagnostic report that undergoes expert manual review for confirmation and quality assurance (Fig. 1h). By combining multiplexed fluorescence imaging with optimized microscopy protocols, this platform shows substantial potential for early cancer detection, prognostic evaluation, and clinical management across diverse cancer types, including cervical cancer (Zhong et al., 2023), pancreatic cancer (Zhao et al., 2023), lung cancer (Elkrief et al., 2024), liver cancer (Sun et al., 2023), breast cancer (Hu et al., 2023), and leukemia (Teierle et al., 2023). In this study, experimental validation was performed using a CLL dataset, and all results were manually verified and confirmed by certified cytogenetics experts.

2.2 Image enhancement and nuclei segmentation

Image enhancement was conducted using the multi-scale Retinex32 algorithm and an image fusion model. Each image consists of two components: irradiation and reflection (Sun et al., 2022). The irradiation component represents the low-frequency part, while the reflection component contains the high-frequency details. Based on the energy orientation gradient algorithm (EOG) (Choudhary and Sethi, 2023), the optimal gradient energy image $I(x, y)$ can be obtained as:

$$I(x,y) = L(x,y)R(x,y). \tag{1}$$

High-frequency details (**DoG**) are extracted via multi-scale Gaussian filtering (Kim et al., 2015). The globally enhanced image first undergoes Gaussian kernel transformations G_1=1.0, G_2=2.0, G_3=3.0, three blurred images are generated, and the corresponding contour details are computed as:

$$L_1' = G_i * L(x,y), D_i = L_i' - L_{i+1}', i = 1,2,3. \tag{2}$$

To prevent oversaturation while preserving image structure, a fusion model combines multi-scale details:

$$D^* = \left[1 - \omega_1 * \mathrm{sgn}(D_1)\right] * D_1 + \omega_2 * D_2 + \omega_3 * \alpha * D_3. \tag{3}$$

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Here, ω_1, ω_2 and ω_3 denote the image clarity at each scale (computed via the Laplacian operator), and sgn(·) is the sign function. D^* represents the overall contour information fused across scales. An empirical parameter α=0.1 controls the retention of original image content, achieving a balance between detail enhancement and natural appearance. This approach effectively enhances FISH and other microscopic images, yielding superior visual quality through multi-scale detail fusion.

$$R'(x,y)=I(x,y)+D^*. \tag{4}$$

Gaussian high-pass filtering is applied to the enhanced high-frequency components $R'(x, y)$ in the frequency domain, and the filter is defined as:

$$H(u,v)=1-e^{-D^2(u,v)/2D_0^2}. \tag{5}$$

To precisely adjust highlights while maintaining balanced exposure and detail, the top 0.1% of brightness values—representing key highlight regions—are selectively processed. This targeted adjustment enhances image quality and preserves essential information without overexposure.

$$H'(u,v)=C_{oe}\cdot H(u,v)+L(x,y). \tag{6}$$

A Fourier transform is applied to the processed image, and the inverse transform is combined with the incident component $H'(u, v)$. Gain adjustment using C_{oe} prevents overexposure, producing the final enhanced image (Supplementary Note 2).

2.3 Nuclear morphology prediction and segmentation

The Euclidean distance between each object pixel $p(x_1, y_1)$ and its nearest background pixel $q(x_2, y_2)$ is as follows (Shen et al., 2019):

$$\mathrm{dis}(p,q)=\sqrt{(x_1-x_2)^2+(y_1-y_2)^2}. \tag{7}$$

Here, $p(x_1, y_1)$ and $q(x_2, y_2)$ denote the coordinates of an object pixel and its nearest background pixel, respectively. The resulting distance set D[dis(p, q)] has maximum and minimum values D_{max} and D_{min}, respectively.

The spatial distribution of nucleus pixels is converted into grayscale values to form the distance map $G(x, y)$. The converted grayscale values are calculated as follows (Sharma et al., 2022):

$$G(x,y)=255\cdot\frac{|\mathrm{dis}(p,q)-D_{min}|}{|D_{max}-D_{min}|}. \tag{8}$$

Local extrema of $G(x, y)$ are identified, their peak coordinates recorded, and the distances between adjacent peaks are computed as follows (Jardim et al., 2022):

$$dis_{min}[p(A),q(B)]=\sum_{i,j}^{m,n}\sqrt{(a_i-c_j)^2-(b_i-d_j)^2}. \tag{9}$$

where $A(a_i, b_i)$, $B(c_j, d_j)$ represent peak points extracted from the enhanced image.

The energy field gradient mapping model highlights nuclear boundaries by analyzing pixel intensity gradients, enhancing edge contrast and reducing noise-induced false detections. It effectively emphasizes regions with strong intensity variations, reflecting texture and morphological irregularities such as protrusions or indentations.

The enhanced image gradients are computed using the Sobel operator **Eq. (9)** to obtain magnitude and direction, forming an HSV-like representation (Dai et al., 2024).The mapping model accentuates nuclear boundaries, enhances regions with strong intensity variations, and accurately defines nuclear contours (Supplementary Note 3).

For the mapped grayscale image $I_{binary}(x, y)$, binarization was performed using a threshold T=10, determined through preliminary edge analysis and Otsu-based calculations. Comparative tests with T=5, 10, and 15 showed that T=10 most effectively separated cell nuclei from the background, minimizing over- and under-segmentation. Thus, T=10 was confirmed as the optimal threshold for generating the final binary image $I_{binary}(x, y)$:

$$I_{binary}(x,y)=\begin{cases}255, & if \quad I_{gray}(x,y)>T\\ 0, & \text{otherwise}\end{cases}, \tag{10}$$

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To suppress noise, erosion is first applied to I_{binary}, followed by dilation using a 3×3 structural element K with coordinate offset (Δx, Δy):

$$I_{\text{erode}} = \min_{(v,u)\in k} I_{\text{binary}}\left(x+\Delta x, y+\Delta y\right), \tag{11}$$

$$I_{\text{dilate}} = \max_{(v,u)\in k} I_{\text{binary}}\left(x+\Delta x, y+\Delta y\right). \tag{12}$$

I_{erode} and I_{dilate} denote erosion and dilation results, respectively, forming a morphological opening that removes noise and smooths boundaries.

The boundaries of all foreground objects are detected by comparing each pixel in the binary image with its 8-neighbor grayscale values. A parent-child contour hierarchy is then constructed for subsequent processing. Finally, the contours are simplified by retaining only key corner points, so the simplified contour set C' preserves the essential shape and hierarchical structure of the image.

Based on the Teh–Chin chain code contour approximation, the least squares method fits contour points to obtain the best-fit curve by minimizing the total fitting error, defined as the vertical distance between (x_i, y_j) and the fitted curve $f(x_i)$.The optimization process is expressed as:

$$\frac{\partial \text{Error}}{\partial(\alpha,\beta)} = -2\sum_{i=1}^{n}\begin{bmatrix} x_i \\ 1 \end{bmatrix}\left[y_i - \left(\alpha x_i + \beta\right)\right] = 0. \tag{13}$$

The optimal parameters α and β of the fitting line are obtained by solving the fitting equations. The resulting line is then used to approximate the original contour point set, producing a smoothed contour set C'', expressed as:

$$C'' = \left\{\left[x_i, f\left(x_i\right)\right] \;\middle|\; i = 1,2,...,n\right\}. \tag{14}$$

Fusing two contour datasets eliminates redundant points and smooths noise while preserving the hierarchical contour structure. This enhances contour detection accuracy and efficiency across images of variable complexity.

$$C_f = C' \cup C''. \tag{15}$$

Based on the fused contour dataset C_f, convex defects are identified and visualized through a two-step screening process, ensuring efficient and accurate morphological segmentation, especially for complex nuclear structures.

Step 1: Convex Defect Detection

Within the fused contour dataset C_f, regions deviating from the convex hull are identified as convex defects. Significant concave areas are detected by computing the defect depth, defined as the perpendicular distance between the convex hull and the farthest contour point.

Step 2: Labeling and Filtering Defects

Each convex defect in C_f is evaluated by comparing its defect depth with a predefined threshold. Defects exceeding this threshold are labeled as significant morphological features and visualized (e.g., using circular markers).

If the line intersects the convex hull, the intersection point $P_t(x_t, y_t)$ is obtained, and the distance to the corresponding peak point p_i is calculated as:

$$\text{dis}\left(p_i, p_t\right) = \sum_{i,j}^{m,n}\sqrt{\left(x_i - x_t\right)^2 + \left(y_i - y_t\right)^2}. \tag{16}$$

This distance is used to assess the intersection between the peak point and the convex hull.

2.4 Multi-layer fluorescence feature fusion

The EOG (edge orientation gradient) method is used to calculate the clarity of each image layer by evaluating the gradient changes in the image.

$$\text{EOG}_i = \frac{1}{M\times N}\sum_{x=1}^{M}\sum_{y=1}^{N}\left[\left|\frac{\partial I_i(x,y)}{\partial x}\right| + \left|\frac{\partial I_i(x,y)}{\partial y}\right|\right]. \tag{17}$$

where $I_i = (x, y)$ is the pixel intensity of the i-th image layer, (M, N) denote the width and height of the image, and (∂x, ∂y) correspond to the gradients in the horizontal and vertical directions.

The image with the highest EOG value is selected as the base image I_{base}, and the global threshold T is calculated to extract significant fluorescence signals.

$$T = \frac{1}{M\times N}\sum_{x=1}^{M}\sum_{y=1}^{N} I_{\text{base}}\left(x,y\right). \tag{18}$$

Using threshold T, the fluorescence signals are

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extracted from each image layer to generate binary masks $M_i = (x, y)$:

$$M_i(x,y)=\begin{Bmatrix} I_{\text{base}}(x,y), & if & I_{\text{base}}(x,y)\geqslant T \\ 0, & & \text{otherwise} \end{Bmatrix}. \quad (19)$$

Layer by layer, the extracted signals M_2, M_3… are sequentially overlaid to obtain the fused image.

$$F_n = F_{n-2} + M_{n-1} + M_n. \quad (20)$$

Through this step-by-step fusion, the signal from the 11th layer compensates for the blurred signals from the defocused layers, thereby enhancing the precision and robustness of segmentation. With the increase in n, both image clarity and clear signal ratio progressively improve until n = 9. Beyond this point, the improvement reaches a plateau, indicating that n = 9 provided an optimal balance between signal compensation and computational efficiency for FISH analysis in this study.

2.5 Deep learning-based cell abnormality detection for early diagnosis

The ResNet152 architecture (Malik et al., 2024) was selected as the base network owing to its strong generalization capability and compatibility with pre-trained models, making it particularly suitable for analyzing the diverse fluorescence patterns and complex backgrounds typical of FISH images. This architecture is especially advantageous in scenarios involving data imbalance or limited sample availability. Compared to lighter architectures, although computationally more demanding, ResNet152 achieves an optimal balance between diagnostic accuracy, robustness, and efficiency, making it the most suitable choice for high-precision FISH-based genetic abnormality detection in this study. By stacking multiple 3 × 3 convolutional kernels, the receptive field is progressively expanded layer by layer, achieving the same effective coverage as the original 7 × 7 convolutional kernel. This strategy substantially reduces the number of parameters while maintaining equivalent feature extraction performance.

$$F(i) = F(i+1) + (K_{\text{size}} - 1)\cdot \text{stride}. \quad (21)$$

where $F(i)$ denotes the receptive field of the current layer, $F(i+1)$ represents that of the previous layer, K_{size} is the size of the convolutional kernel, and stride refers to the step size.

Layer 1: The input receptive field parameter is F_{i+1}=1, K_{size}=3, stride=1, $F(1)$=1+2·1=3.

Layer 2: The input receptive field parameter is F_{i+1}=3, K_{size}=3, stride=1, $F(2)$=3+2·1=5.

Layer 3: The input receptive field parameter is F_{i+1}=5, K_{size}=3, stride=1, $F(3)$=5+2·1=7.

The introduction of a multi-branch design replaces the traditional single 7 × 7 convolutional kernel, enabling each branch to use a 3 × 3 convolutional kernel. This architecture effectively reduces both the computational load and the number of parameters. By optimizing the network structure, the multi-branch design preserves the receptive field while simultaneously enhancing computational efficiency and reducing the risk of overfitting, resulting in improved model performance and generalization capability.

For the multi-branch design, where each branch uses a 3×3 convolutional kernel, the number of parameters can be calculated using the formula.

$$Parameter_{3\times3} = 3\cdot(C_{\text{in}}\cdot C_{\text{out}})\cdot 3\cdot 3. \quad (22)$$

where C_{in} is the number of input channels, C_{out} is the number of output channels, and 3×3 represents the size of the convolution kernel (3×3).

The parameter reduction rate for the multi-branch design is given by the formula:

$$Reduce = 1 - \frac{Parameter_{3\times3}}{Parameter_{7\times7}}. \quad (23)$$

Global average pooling (GAP) is used instead of the traditional fully connected layer, reducing the number of model parameters and preventing potential information loss introduced by the flattening operation. GAP computes the global average of each feature channel, directly mapping the 2D feature maps to corresponding global feature values for each class. This design further enhances the computational efficiency, stability, and generalization capability of

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the model.

In this study, we used an optimized cell nucleus segmentation method to extract cell nuclei from multi-layer fluorescence feature fusion images. The segmentation results were reviewed and approved by pathology experts, and about 4,500 high-quality nucleus images were screened as the initial sample set. Based on this dataset, and incorporating data augmentation strategies such as image translation, rotation, mirroring, and scaling, a representative and diverse extended dataset comprising 26,733 images was constructed. The dataset was then divided into a training set (18,713 images) and a validation set (8,020 images) using a 7:3 ratio, and the division of homologous images was strictly controlled to avoid information leakage.

For data annotation, images were classified according to the characteristics of the fluorescence signals. Images containing a single fluorescent spot or no detectable fluorescent signal were annotated as abnormal samples (i.e., gene abnormality representation), while images showing two fluorescent spots were annotated as normal samples. All annotation results were reviewed and verified in multiple rounds to ensure label accuracy and consistency. Based on this dataset, a classification model was trained and the model performance was comprehensively evaluated using multi-dimensional indicators such as confusion matrix, accuracy, sensitivity, and specificity to verify the effectiveness and reliability of the proposed method in abnormal gene classification.

Abnormal cells were detected on ten sets of trichrome multilayer fluorescence feature-fused images according to the cell morphological integrity and absence threshold relationship criteria. Through the manual operation, 200 cells in each image were selected for detection with a missing proportion threshold of 5%, i.e., 10 abnormal cells per 200 total cells. In cases where the system detects fewer than 200 cells, it calculates the threshold for an abnormal cell count by multiplying the actual cell count by the specified missing proportion threshold. The quantification of fluorescent genetic signals within individual cells using three monomeric fluorescence probes enables the identification and characterization of genetic abnormalities at the cellular level separately, and the counted number of abnormal cells is compared with the abnormal cell number threshold.

The precision, recall, and F1-score of the cell abnormality analysis results can be calculated as follows:

$$Accuracy = \frac{TP + TN}{TP + TN + FN + FP}, \tag{24}$$

$$Precision = \frac{TP}{TP + FP}, \tag{25}$$

$$Recall = \frac{TP}{TP + FN}, \tag{26}$$

$$\mathrm{F1}(score) = 2 * \frac{(Recall * Percision)}{(Recall + Percision)}. \tag{27}$$

where *TP* is true positive, *TN* is true negative, *FP* is false positive, and *FN* is false negative.

The training dataset was imported into different classification models for automated learning and training, using default parameters with a weight decay value of 0.1 and an initial learning rate of 0.001. The model was trained for 20 epochs, and to prevent overfitting, individual neurons in training were selectively ignored using a random discard technique after each fully connected layer. The classification results were output through the classifier. Upon completion of training, the final trained model, including its network structure and optimized parameters, was retrieved. The 9-layer fluorescence fusion images were then imported into different classification models as a dataset. The models were further fine-tuned and optimized using this dataset. Following this, 30% of the images were randomly selected as a test set to evaluate the model's predictive performance, yielding prediction results.

3 Results

3.1 Performance evaluation and comparison in cell nucleus segmentation

Image enhancement in the FAST system begins by selecting the optimal EOG image from the DAPI channel (Supplementary Note 1, Supplementary Fig. 2, & Supplementary Table 5). The selected image is then decomposed into low- and high-frequency components (Fig. 2a). Using a multi-scale difference of Gaussians (**DoGs**) algorithm (Li et al., 2022b), the high-frequency details are isolated to enhance nuclear

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edges and textural information. The cutoff frequency is optimized to maximize the signal-to-noise ratio (SNR) for minimized noise. In addition, local contrast factors are extracted from the brightest 0.1% of pixels to enhance low-contrast areas. The final enhanced image simultaneously amplifies high-frequency structural details while preserving low-frequency information, effectively resolving issues such as blurred edges, uneven brightness, and background noise (Supplementary Fig. 3). Compared with conventional image enhancement methods like CLAHE (Hu et al., 2024), SSR (Li et al., 2021), and HF (Ramos-Soto et al., 2021), the proposed approach shows superior contrast enhancement and noise reduction (Supplementary Note 3 & Supplementary Fig. 4).

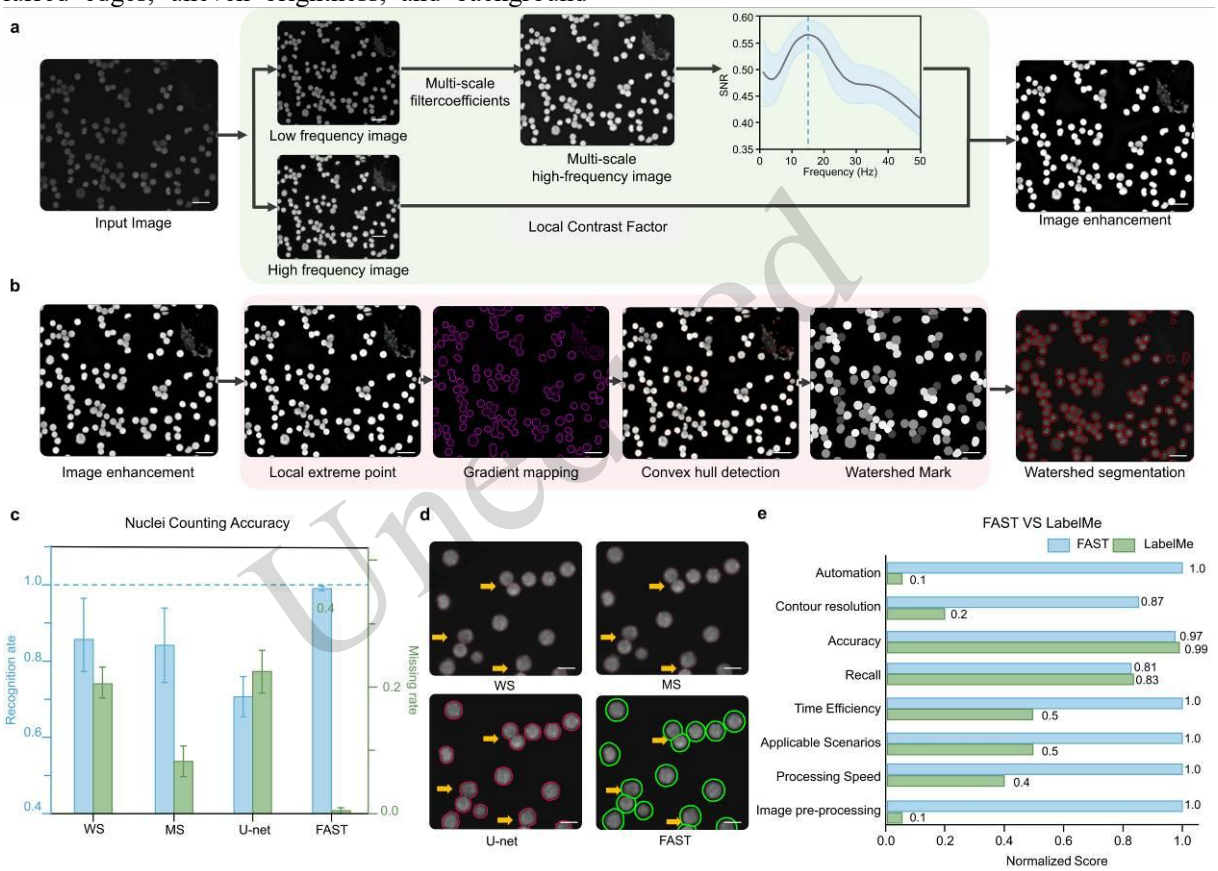

Fig. 2 Image enhancement and nuclei segmentation for accurate nuclear contour identification. (a) Workflow of the image enhancement process. The tailored multiscale DoGs algorithm and image fusion model improve edge sharpness and contrast, enhancing nuclear contours and details. (b) Accurate segmentation of nuclear regions. Representative raw images with complex patterns are processed through local maxima identification, gradient mapping for feature extraction, convex defect analysis for edge detection, and iterative refinement of seed points. (c) Performance comparison of nuclei counting accuracy across different segmentation methods. Error bars represent ± s.d. (d) Nuclei segmentation results. Comparative analysis of clustered nuclei segmentation by our method and established methods, with orange arrows highlighting segmentation performance on clustered nuclei. (e) Comprehensive performance comparison between FAST and LabelMe across key metrics, including accuracy, recall, efficiency, automation, and processing speed. Scale bars: 10 μm in (a) and (b), 5 μm in (d).

To achieve accurate and efficient nuclear segmentation, an optimized watershed-based algorithm was developed (Fig. 2b). This process begins with identifying local extrema in the enhanced images as seed points, which are refined using Sobel gradients (Wang et al., 2024) and flow field information. The images are then transformed into HSV color space (Chyad et al., 2025) to improve edge visibility and reduce misidentification (Supplementary Fig. 5). Further refinement involves a

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two-pass convex hull detection to address over- and under-segmentation. Moreover, iterative adjustments to watershed mark parameters based on peak distances ensure accurate delineation of individual nuclei, even when there are overlapping and complex backgrounds (Supplementary Fig. 6). Comparative analysis with traditional methods like marker-based watershed (Abrol et al., 2023), morphological segmentation (Vincent and Dougherty, 2020), and deep learning-based methods (U-Net) (Punn and Agarwal, 2022) shows that our proposed approach achieves superior segmentation performance (Supplementary Fig. 7). With an average segmentation accuracy of 98.28% and a missing rate (i.e., the ratio of undetected nuclei to total nuclei) below 2%, the algorithm outperformed other methods (accuracy range: 54.8%–82.7%) in counting nuclei (Fig. 2c, & Supplementary Note 5). In scenarios involving tightly packed nuclei, our proposed approach produced clear segmentation results, avoiding over-segmentation and nucleus omission commonly observed in other approaches (Fig. 2d & Supplementary Note 6).

FAST also exhibits significant advantages in scalability and efficiency compared with commercial solutions like LabelMe. Although both FAST and LabelMe achieved comparable accuracy (0.97 vs. 0.99) and recall (0.81 vs. 0.83), FAST showed superior efficiency and required fewer computational resources (Fig. 2e & Supplementary Note 7). Moreover, in contrast to traditional deep learning methods like U-Net (Soltanian-Zadeh et al., 2019; Minaee et al., 2022), FAST's lightweight design reduces dependency on large-scale datasets and high-performance hardware, enabling rapid processing of extensive imaging datasets. This advantage is particularly beneficial for complex nucleus recognition tasks, where FAST efficiently completes the entire workflow from image preprocessing to segmentation.

3.2 Evaluation of multi-layer fluorescence feature fusion indicators

In this study, CLL detection focused on key gene regions on chromosome 13 (RB1, DLEU1/MIR15A/MIR16-1, and LAMP1) (Durak Aras et al., 2021). To visualize genetic abnormalities, FISH probes labeled with green, orange, and blue fluorescence targeted distinct chromosomal regions, enabling direct microscopic observation (Fig. 3a & Supplementary Fig. 8). To enhance detection accuracy, a multi-layer fluorescence feature fusion strategy was applied to improve fluorescence characteristics within the nuclei (Caicedo et al., 2017). For each fluorescence signal, 11 layers with increasing focal distances were captured and ranked according to their EOG values (Fig. 3b). The layer with the highest EOG value was selected as the base layer, and fluorescence features from the top n layers (n = 1, 3, 5, 7, 9, 11) were progressively fused into this base, resulting in a pseudo-colored composite image (Fig. 3c & Supplementary Fig. 9). This multi-layer fusion process enhances fluorescence signals and addresses common issues such as information loss and blurriness (Fig. 3d & Supplementary Fig. 10) (Xu et al., 2023).

To assess image quality, the average accumulated EOG for each signal and the ratio of clear signals were calculated (Supplementary Note 8). Results show that image quality plateaued after n = 9 (Fig. 3e and Fig. 3f), indicating that n = 9 provided an optimal balance between signal enhancement and processing cost for FISH analysis in this study (Supplementary Fig. 10). In the fused microscopy images, the fluorescent patterns convey diagnostic information, enabling the distinction of aberrant cells (Fig. 3g). As shown in Fig. 3h, normal cells exhibit two green (2G), two orange (2O), and two blue (2B) signals, whereas aberrant cells show reduced signal patterns, such as 1O, 1G, or 1B. In CLL (Zhang et al., 2024). More than 50% of patients had deletions in the 13q14 region (Saeed et al., 2024).

To statistically determine the optimal fusion depth for tricolor FISH image analysis, six fusion layers (n = 1, 3, 5, 7, 9, and 11) were compared using two quantitative indicators: accumulated EOG (signal intensity) and clear signal count (signal quality). Ten independent samples were analyzed under identical imaging conditions, and the results were assessed by 95% confidence intervals (CIs) and one-way ANOVA with Tukey's HSD post-hoc tests (α = 0.05). Both metrics stabilized as the fusion depth increased. The accumulated EOG increased from 8.23 (n = 1) to 18.51 (n = 9) with narrowing CIs, while n = 11 showed no further improvement (18.48, CI = [16.42, 20.54]), indicating signal saturation. Meanwhile, the

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clear signal count decreased from 25.67 (n = 1) to 5.60 (n = 9) as noise was suppressed, but slightly dropped at n = 11, suggesting minor signal loss. ANOVA confirmed a significant effect of fusion depth on both metrics (EOG: F = 21.87, p < 0.001; clear signal count: F = 18.42, p < 0.001). Post-hoc tests showed that low-depth fusions ($n \leqslant 3$) differed markedly from higher-depth ones (p < 0.001), while n = 9 and n = 11 did not differ in EOG (p = 0.982) but differed in signal count (p = 0.043). Overall, n = 9 was the optimal number of image layers, focal depths, or segmentation model hyperparameters across different processing stages. Repeated experimental validation confirmed that n = 9 provides the most effective trade-off among accuracy, detection capability, and computational efficiency, particularly under diverse and noise-prone imaging conditions.

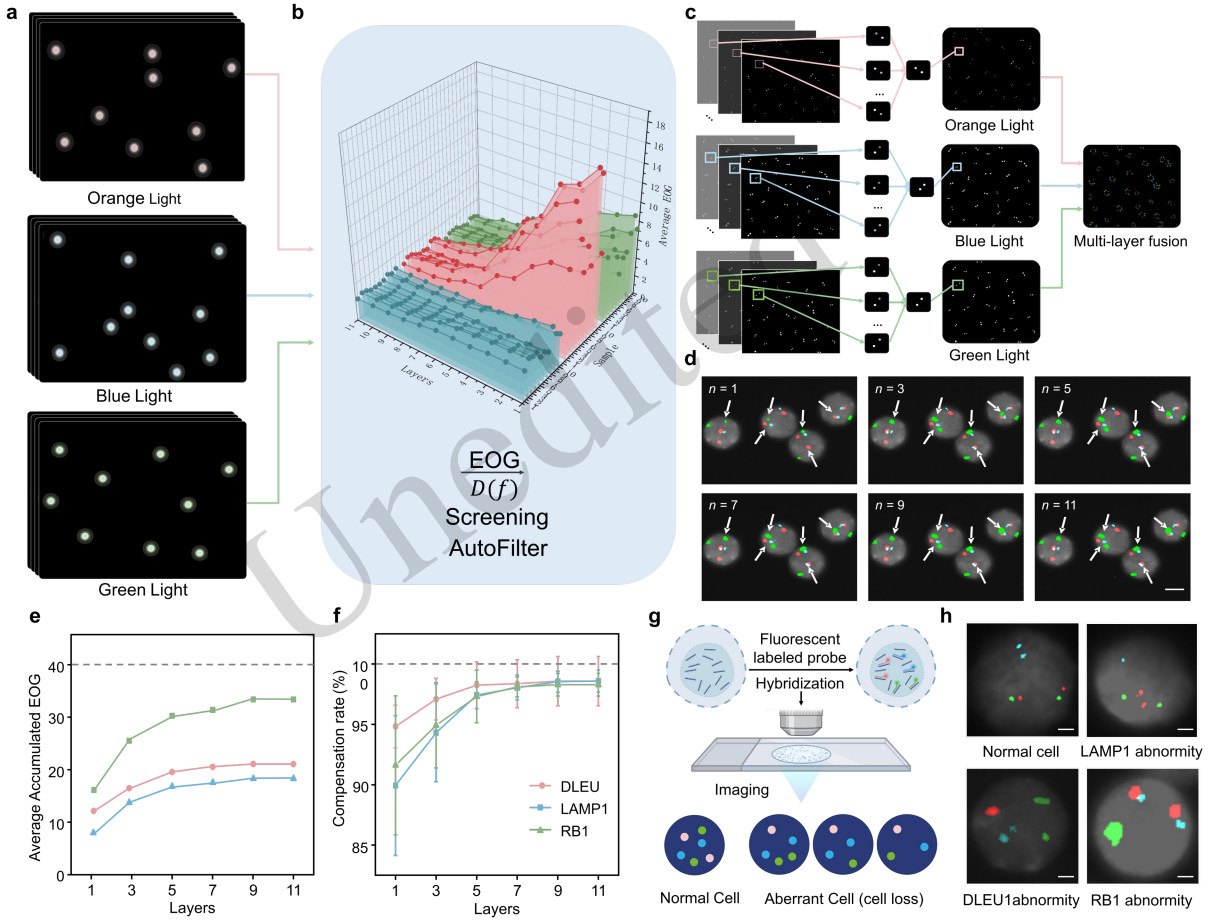

Fig. 3 Multilayer fluorescence feature fusion for chromosomal abnormality screening. (a) FISH images from different channels show specific fluorescent signals. (b) Ranking of fluorescence images based on the energy of gradient (EOG) for signal clarity. (c) Automated filtering and multilayer fusion of tricolor fluorescence images. (d) Comparison of fluorescence signal restoration through n-layer fluorescence feature fusion (n = 1, 3, 5, 7, 9, 11), showing a progressive enhancement of fluorescence signals with increasing n. (e) Average accumulated EOG values and (f) average clear signal ratio across n-layer feature fusion (n = 1 to 11). (g) Workflow of FISH technology for detecting chromosomal abnormalities in CLL. (h) FISH patterns showing gene deletions in the 13q14 region in CLL patients. Scale bar: 10 μm in (d) and 5 μm in (h).

This method allows accurate detection of chromosomal abnormalities in CLL, providing a crucial foundation for clinical prognosis evaluation. Using the proposed multi-layer fluorescence feature fusion approach, an in-depth assessment of chromosomal abnormalities was performed. The accumulated EOG values and chromosomal abnormality ratios (*Ral*) for each signal were analyzed. As the number of fused layers (n) increased from 1 to 11, the abnormality rates stabilized,

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reflecting a gradual enhancement of fluorescence signals (Supplementary Table 6). To classify cell abnormalities and diagnose cancer, a 5% threshold across the three FISH channels was set (Supplementary Table 7). Specifically, samples were considered negative if *Ral* values in all channels were below this threshold, and positive otherwise. By applying the multilayer fluorescence feature fusion method, chromosomal abnormalities in ten independent samples with different n values were further evaluated based on accumulated EOG measurements (Figs. 4a-j & Supplementary Figs. 11-14). The results show that abnormality classification accuracy improved consistently as n increased, which further strengthened the reliability of FISH analysis. These findings indicate that the proposed method effectively reduces issues like halo, blurring, and signal loss, providing a more accurate representation of fluorescence intensity within the nuclei.

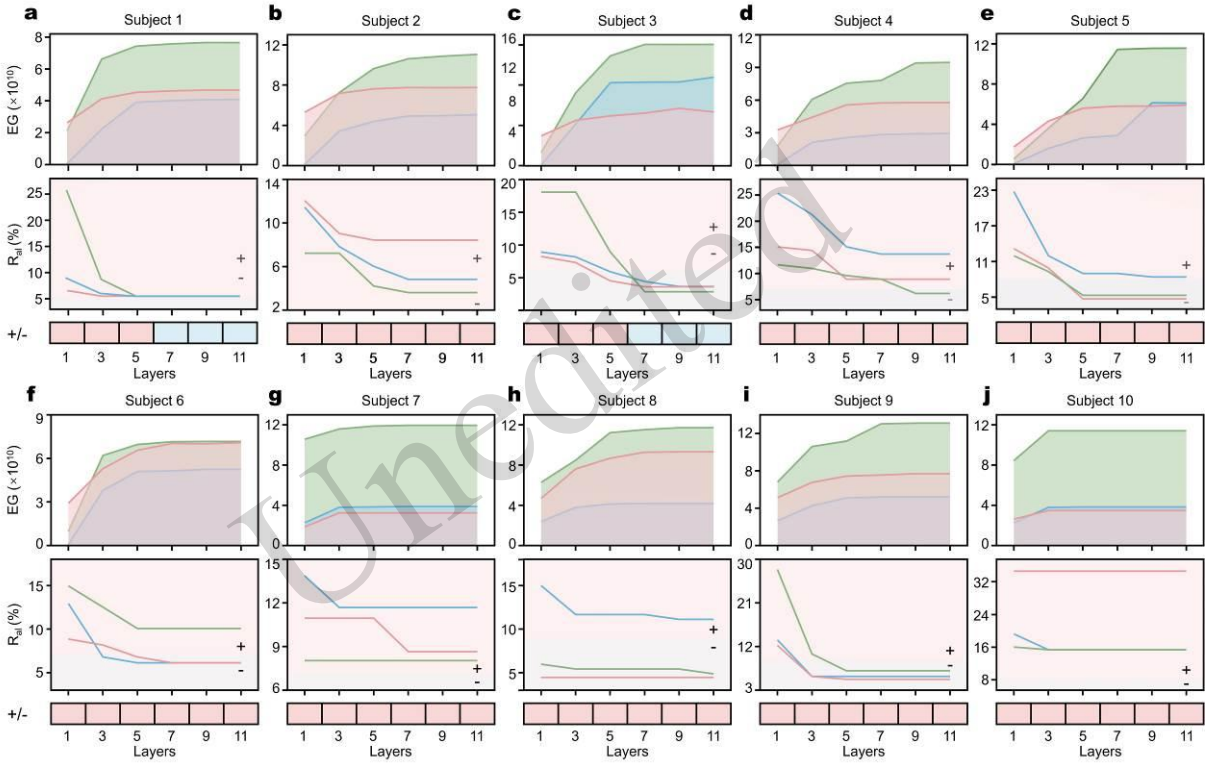

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Fig. 4 Evaluation of the specificity of multi-layer fluorescence feature chromosome signals. (a)–(j) Individual performance metrics across ten samples, illustrating the change in accumulated EOG and rate of chromosomal abnormality involving tricolor fluorescence signals against the increase of n. areas in the Ral–Layer plots and shaded cells denote manual classification of chromosomal abnormality and corresponding cell abnormality, respectively, with red denoting abnormality and blue denoting normality. R_{al}, rate of chromosomal abnormality; + and -, positive and negative, respectively.

3.3 Abnormal gene screening and application based on deep learning

Building upon the multilayer fluorescence feature detection results, a deep learning model based on the ResNet152 backbone was developed to enable automated cell abnormality detection and facilitate early cancer diagnosis (Fig. 5a). In this model, the CNN leverages the robust feature-extraction capability of ResNet152 and is trained using uniformly resized input images (224 × 224 pixels) with a segmented learning-rate decay strategy (initial rate = 0.001). To determine the optimal fusion level, datasets with n-layer fusion (n = 1, 3, 5, 7, 9) were evaluated. Among these, the 9-layer fusion dataset showed the fastest convergence and highest predictive accuracy, striking a balance between information density and task complexity (Fig. 5b). Consequently, the 9-layer fusion dataset was selected for chromosomal and cellular abnormality

classification, achieving high accuracy in detecting abnormalities at both levels (Fig. 5c & Supplementary Fig. 15, Tables 8-11). To enhance usability and interpretability, the system overlays chromosomal pattern masks onto segmented nuclei boundaries, with red and blue indicating positive and negative cases, respectively (Supplementary Fig. 16). The precision rate generally remains between 0.95 and 1.0, indicating that the model has a low false-positive rate strong classification ability. It showed significant accuracy and robustness in detecting cell abnormalities and CLL-positive diagnoses across diverse patient datasets, providing a robust solution to mitigate errors associated with manual detection. The model robustness was further validated through benchmarking against eight state-of-the-art deep learning algorithms using identical training and validation datasets: AlexNet (Battleday et al., 2020), VGG16 (Li et al., 2020), VGG19 (Le Goallec et al., 2022), ResNet50 (Zheng et al., 2022), ResNet101 (Ismael and Şengür, 2021), DenseNet121 (Singh et al., 2025), DenseNet169 (Biancalani et al., 2021), and InceptionV4 (Li et al., 2022a). Our ResNet152-based model achieved the highest cancer-detection accuracy of 97.86% (Supplementary Fig. 17), outperforming all benchmarked methods and demonstrating strong clinical diagnostic potential. It also surpassed commercial AI-based systems across multiple metrics, including the Dice coefficient, Jaccard index, Recall, and Accuracy (Fig. 5d). Unlike end-to-end commercial solutions, the proposed system uses a two-step framework: first, a refined segmentation algorithm extracts nuclei boundaries, addressing challenges like fuzzy contours and overlapping nuclei; second, a classification algorithm detects chromosomal abnormalities within segmented regions. This design reduces false-positive rates (FP) and enhances sensitivity, ensuring reliable performance across complex datasets.

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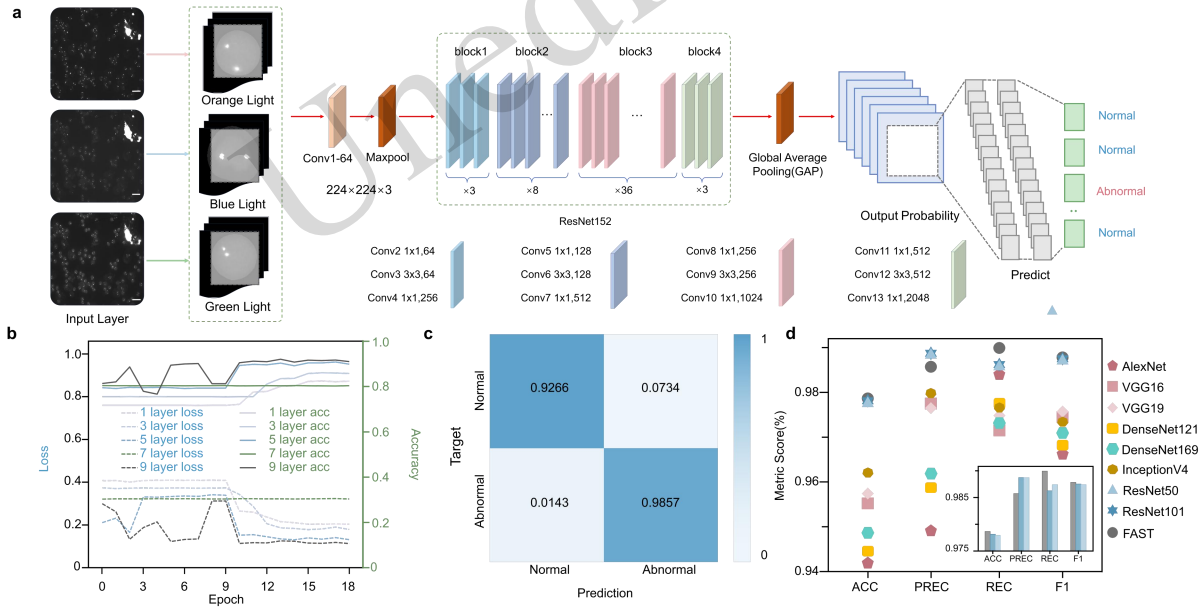

Fig. 5 Deep learning-based cell abnormality detection and cancer diagnosis. (a) the CNN ResNet152 network model in the dataset for automated cell abnormality classification. (b) Learning curves of the ResNet152 network trained on datasets from n-layer fusion (n = 1, 3, 5, 7, 9). (c) Confusion matrix of ResNet152-based prediction versus manual screening across ten samples. (d) Performance comparison between our method and commercial deep learning models. Metrics include Jaccard score (Jaccard), recall (REC), and accuracy (ACC). Scale bar: 50 μm in (a).

To efficiently identify and visualize cell abnormalities, the system locates the nuclei with assigned chromosomal patterns and superimposes corresponding nucleus masks over the nucleus boundaries (insets in Fig. 6a & Supplementary Table 8-10). To further reduce the time and cost required for personnel training and operation, these functionalities are fully integrated into a comprehensive multiplexed FAST designed for early cancer diagnosis (Fig. 6b & Supplementary Note 9). The platform features a

user-friendly graphical user interface (GUI) that simplifies operation by supporting real-time monitoring of image acquisition and processing, adjustable imaging parameters, and flexible program execution. This enables users to automate key tasks, including image acquisition, nuclei and fluorescence signal processing, AI-driven abnormality analysis, and diagnostic report generation (Supplementary Table 11). Further, the system supports both automated and manual segmentation adjustments to meet diverse analytical requirements. The entire workflow, from image acquisition to report generation, can be completed within 45 minutes (Supplementary Table 12) on a common computer. By integrating cost-effective microscopy, targeted fluorescent probes, and optimized FISH protocols, the FAST platform provides a reliable, rapid, and cost-effective solution for cellular abnormality detection, advancing early cancer diagnostics.

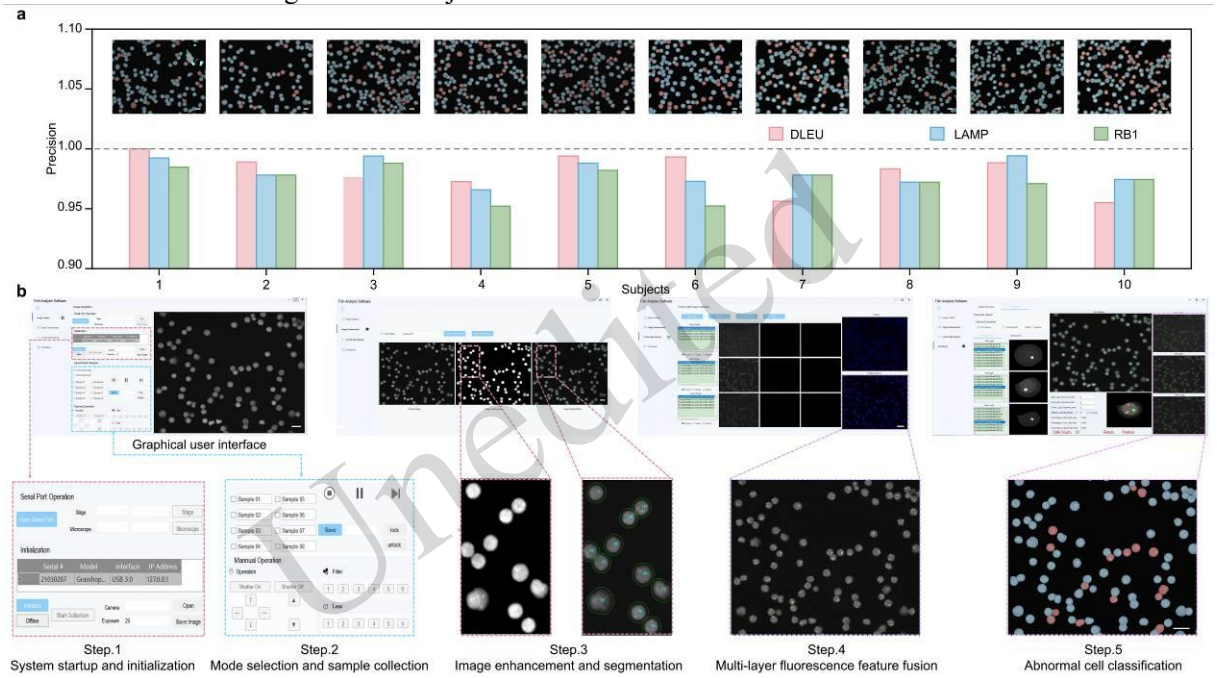

Fig. 6 Abnormality analysis results and system graphical user interface (GUI). (a) Classification accuracy of chromosomal abnormality involving tricolor fluorescence probes using the ResNet152CNN network. Insets show microscopic sample images with color masks where red and blue indicate abnormality and normality, respectively. (b) Visualization of user-friendly interface and diagnostic report of the integrated FISH imaging and analysis system, tailored for non-experts and facilitating early cancer diagnosis and prognostic evaluation. Scale bar: 50 μm in (a) and (b).

4 Conclusions

In this paper, we have introduced an automated, multiplexed FAST system tailored for diagnosing CLL. The system comprises four core functionalities: automated image acquisition, image enhancement and nuclei segmentation, multilayer fluorescence feature fusion, and AI-driven chromosomal and cell abnormality detection. During image acquisition, FAST automatically selects the clearest image layers, effectively minimizing imaging artifacts. Nuclei contours are refined through imaging enhancement and segmentation, while multilayer fluorescence fusion enhances the cytogenetic information necessary for detecting cell abnormalities. Our segmentation and abnormality detection models outperform traditional methods, even in challenging regions with overlapping or densely packed nuclei. In terms of efficiency, FAST achieves accuracy and data richness comparable to commercial software while requiring fewer computational resources and smaller sample sizes. The entire process, from image capture to analysis, can be completed in under 45 minutes, significantly improving the speed, accuracy, and reliability of genetic analysis.

A standout feature of the FAST platform lies in its ability to identify abnormalities in densely packed

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nuclei, overcoming challenges commonly encountered by commercial methods. The seamless integration of automated segmentation and fluorescence feature fusion enables precise detection of chromosomal and cellular abnormalities, which is critical for early cancer detection. Its user-friendly graphical interface further enhances accessibility for non-expert users, facilitating intuitive image processing, analysis, and interpretation. Collectively, FAST provides a transformative and scalable solution for early cancer detection and personalized healthcare. We plan to further refine the deep learning models and extend the platform's capabilities to accommodate larger and more complex datasets, thereby broadening its applicability to other diseases and biological scales, ranging from single cells to tissues (Ueda et al., 2020). Ultimately, the FAST system aims to support personalized treatment strategies, enhance prognostic evaluations, and improve patient outcomes through fast, accurate, and automated genetic analysis.

Despite leveraging deep learning techniques for accurate detection, several limitations remain in the current study:

1) Model interpretability: Although the ResNet152-based model exhibits excellent diagnostic accuracy, its interpretability remains limited. Clinicians may require clearer visualization and explanation of how the model arrives at diagnostic conclusions, which is essential for building trust and integrating AI tools into clinical workflows.

2) Multi-target detection and complex genomic contexts: The current implementation focuses mainly on the 13q14 deletion associated with CLL. Expanding the system to accurately detect multiple genetic abnormalities simultaneously remains a challenge, particularly in complex genomic contexts where overlapping signals or structural variants may interfere with probe interpretation.

3) Dataset diversity and scale: The model was validated on 100 clinical CLL cases. Although results were consistent with expert interpretations, larger and more diverse datasets covering different cancer types, imaging modalities, and staining conditions are necessary to further improve model robustness and generalizability.

4) Hardware and imaging constraints: While FAST is designed to operate with standard wide-field fluorescence microscopy, imaging performance may vary with hardware configurations, optical alignment, or sample preparation quality, which could affect reproducibility across institutions.

Building on these findings, our future efforts will focus on several key directions:

I) Enhancing model explainability: Integrating attention heatmaps, gradient-weighted class activation mapping (Grad-CAM), or SHAP-based visualization to provide interpretable diagnostic reasoning and strengthen clinician confidence.

II) Extending multi-target and multi-cancer detection: Developing adaptive probe designs and multi-class classification frameworks to enable simultaneous detection of multiple chromosomal abnormalities across diverse cancer types such as breast, cervical, and pancreatic cancers.

III) Scaling clinical validation: Expanding clinical trials with large multi-center datasets to validate system reliability under diverse imaging and genetic conditions, ensuring the system's translational applicability in real-world hospital environments.

IV) Optimizing real-time processing: Incorporating GPU-accelerated inference and cloud-based computation to achieve near real-time diagnostic feedback, enhancing throughput and integration with digital pathology systems.

V) Integrating multi-modal data: Combining FISH imaging data with next-generation sequencing (NGS) and histopathological features to build a hybrid diagnostic model that supports comprehensive precision oncology.

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Author contributions

Author contributions Conceptualization, L.S., Y.Z. and X.F.; methodology, L.S., C.L., and X.F.; software, L.S., H.Q. and D.S.; validation, L.S., Y.Z.; formal analysis, P.G. and C.L.; investigation, M.H., M.L., and H.Z.; resources, P.G., and X.F.; data curation, H.Z., and S.J.; writing—original draft preparation, L.S., S.J., and Y.Z; writing—review and editing,

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L.S., X.F, and S.J; visualization, L.S.; supervision, X.F. and S.J.; project administration, L.S.,Y.Z., and H.Z.; funding acquisition, P.G., X.F. and S.J. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Electronic Supplementary materials

Section S1-S9, Figs. S1–S17, Table S1-S12.

中文概要

题　目： 基于深度学习的荧光原位杂交 (FISH) 自动基因异常检测

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目　的： 开发一种基于深度学习的基因异常检测自动化系统 (FAST)，以突破传统 FISH 技术在癌症和遗传疾病诊断中的局限性。

创新点： 1. 多层焦平面成像策略：增强不同焦深的成像，实现精确的细胞分析。2. 图像增强与融合：结合多尺度滤波技术，提升信号清晰度。3. 深度学习集成：优化 ResNet152 深度学习框架进行高效的基因异常分类，分割与分类的准确率分别为：细胞分割准确率：98.28%（95%置信区间：[97.43%，99.13%]），基因异常检测准确率：97.86%（95%置信区间：[97.54%，98.17%]）。

方　法： FISH 成像：利用三色 FISH 探针和电动荧光显微镜进行多层成像。图像处理：多层荧光信号融合、图像增强，以及细胞核和荧光信号的自动分割。AI 模型：基于 ResNet152 的 CNN 模型，经过训练用于检测染色体异常（例如慢性淋巴细胞白血病中的 13q14 缺失）。

结　论： FAST 为早期癌症检测提供了一种高精度、自动化的解决方案，显著提高了诊断速度并降低了对操作员的依赖。与传统方法和其他 AI 解决方案相比，该系统在检测染色体异常方面表现出卓越的性能。系统能够在 45 分钟内生成诊断报告，具有很高的临床应用潜力。

关键词： 荧光原位杂交 (FISH)；图像增强；细胞核分割；荧光特征融合；异常基因分类；癌症诊断。

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