Automated Morphological Subtyping of B-Lineage Acute Lymphoblastic Leukemia Using Deep Residual Learning and Stain Normalization

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Abstract

The differentiation of B-lineage Acute Lymphoblastic Leukemia (B-ALL) subtypes—Pro-B, Pre-B, and Early Pre-B—is a critical diagnostic task that traditionally relies on flow cytometry and immunophenotyping. This study investigates a computational approach to morphological subtyping using deep residual learning applied to digital peripheral blood film images. To address inter-laboratory staining variability, Reinhard stain normalization was implemented to standardize color characteristics across samples. A ResNet50 convolutional neural network was trained on 3,256 labeled micrographs, achieving a peak classification accuracy of 98.35%. The model demonstrated strong performance in distinguishing malignant lymphoblasts from benign hematogones, a known diagnostic challenge in hematopathology. These findings highlight the potential of deep learning–based morphological analysis as a decision-support approach in digital hematopathology.

Background and Rationale

In clinical hematopathology, the distinction between malignant B-ALL lymphoblasts and benign reactive B-cell precursors (hematogones) remains a frequent source of diagnostic ambiguity, particularly during bone marrow regeneration. While flow cytometry, cytogenetics, and molecular assays serve as diagnostic gold standards, they are resource-intensive and not universally accessible.

Morphological assessment remains a cornerstone of hematologic evaluation, yet many diagnostically relevant features—such as nuclear-to-cytoplasmic (N:C) ratio, chromatin density, and nucleolar prominence—are challenging to quantify consistently by visual inspection alone. Advances in deep learning have demonstrated the capacity of convolutional neural networks to extract subtle, high-dimensional features from medical images. This project explores whether deep residual learning can capture morphology-driven patterns in leukemic cells to support automated subtyping of B-lineage ALL from digital microscopy.

Methods

The study utilized a dataset of 3,256 high-resolution clinical micrographs representing four classes: Pro-B ALL, Pre-B ALL, Early Pre-B ALL, and benign hematogones. To mitigate staining variability across samples, Reinhard stain normalization was applied as a preprocessing step, transforming images into the LAB color space and aligning color statistics to a reference template.

A ResNet50 convolutional neural network architecture was employed, leveraging residual connections to facilitate deep feature learning. The dataset was partitioned into training, validation, and test sets using an 80/10/10 split. Model training was performed using the Adam optimizer with

categorical cross-entropy loss. Performance was evaluated using accuracy metrics and confusion matrix analysis to assess subtype-specific classification behavior.

Key Findings and Significance

The trained model achieved a final classification accuracy of 98.35% across all classes. Confusion matrix analysis revealed particularly strong performance in identifying Pro-B ALL, the most immature and clinically aggressive subtype. Importantly, the model demonstrated effective separation between Early Pre-B lymphoblasts and benign hematogones, addressing a common source of morphological misclassification.

Although exploratory in nature, this study demonstrates the feasibility of applying deep learning to digital hematopathology for morphology-based leukemia subtyping. The findings support further investigation into AI-assisted decision-support systems that integrate image-based analysis with established diagnostic workflows, particularly in settings where advanced immunophenotyping resources are limited.
