Live Cell Stage Classification Using Deep Learning

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Abstract: Live cell imaging has transformed biological research, offering real-time insight into dynamic cellular processes. This project focuses on using deep learning techniques to automate the detection and classification of live cell stages, specifically distinguishing between the interphase and mitosis phases. Traditional methods, such as fluorescence microscopy and flow cytometry, are highly dependent on manual or semiautomated, time-intensive and error-prone approaches. Our proposed solution employs advanced deep learning architectures, including Sequential Convolutional Neural Network (SCNN), ResNet50, and EfficientNetB0, to overcome these limitations. The data set used comprises high-resolution images of nematode cells, preprocessed using resizing, normalization, and data augmentation techniques to ensure robust model training. The performance of each model is evaluated on the basis of metrics such as accuracy, positive predictive value (PPV), sensitivity, and the F1 score. In particular, EfficientNetB0 emerges as the model with the best performance, achieving a test accuracy of 98%, showcasing its superior ability to generalize in diverse data.

1 INTRODUCTION

Live cell imaging has transformed biological research by enabling real-time observation of cellular processes such as mitosis and signal transduction. Despite its advantages, manual analysis of live cell imaging data is time-consuming and error-prone, especially in distinguishing phases such as Interphase and Mitosis. This project addresses these challenges using deep learning methods, specifically a Sequential Convolutional Neural Network (SCNN) and pretrained models like ResNet50 and EfficientNetB0. These models leverage compound scaling and transfer learning from ImageNet to achieve high accuracy in binary classification tasks. By automating live cell stage classification, this project accelerates cellular analysis, offering a scalable and efficient solution for research and medical diagnostics, with significant implications for cancer studies and drug discovery.

1.1 Objective

The primary objective of this project is to develop an automated system for live cell stage classification, specifically distinguishing between Interphase and Mitosis phases, to reduce reliance on manual methods. State-of-the-art deep learning architectures, including SCNN, ResNet50, and EfficientNetB0, are utilized to ensure high accuracy and efficiency. Data preprocessing techniques such as resizing, normalization, and augmentation (e.g., rotations, flips, and brightness adjustments) are implemented to enhance model robustness and generalization. Model performance is evaluated using metrics like accuracy, positive predictive value (PPV), sensitivity, and F1-score, and ROC to ensure consistent and reliable results. The system aims to support biomedical research by enabling scalable applications such as cellular behavior analysis, cancer stage identification, and drug testing.

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1.2 Literature Survey

The classification of cell cycle stages using deep learning has been an active area of research. Several studies have explored various methods and datasets to enhance accuracy. Below is a summary of key related works:

Robust Classification of Cell Cycle Phase and Biological Feature Extraction by Image-Based Deep Learning: Okada et al. (2020) proposed a method using convolutional neural network (CNN) to classify fluorescence images of cells into G1/S and G2 phases without relying on specific cell cycle markers. The study achieved an accuracy of approximately 90%. Using Grad-CAM analysis, the authors identified critical subcellular features that contributed to the classification decisions. (Nagao, Sakamoto, et al. , 2020).

Cell Cycle Stage Classification Using Phase Imaging with Computational Specificity (PICS): Nguyen et al. (2022) introduced a label-free deep learning method for classifying cell cycle stages based on single-shot quantitative Live Cell Stage Classification Using Deep Learning phase imaging. Their model achieved comparable accuracy to traditional techniques, with atleast one stage in interphase classification below 95% accuracy. (He, Kandel, et al. , 2022).

Predicting Cell Cycle Stage from 3D Single-Cell Nuclear-Stained Images: Li et al. (2024) applied a CNN-based model to classify cell cycle stages using 3D nuclear-stained single-cell images. Their model achieved an accuracy of 93%, showcasing the potential of combining 3D imaging and deep learning. (Li, Nichols, et al. , 2024).

Cell Cycle Classification Using Imaging Flow Cytometry and Deep Learning: Zhang et al. (2022) developed deep learning models, including a 2-layer fully connected neural network, to classify cell cycle stages from imaging flow cytometry data. Despite exploring various architectures, the best-balanced accuracy achieved was below 95%. This study indicated room for improvement in both model design and preprocessing techniques when using imaging flow cytometry data for cell stage classification (Rade, Zhang, et al. , 2022).

Deep Learning-Based Reconstruction of Embryonic Cell-Division Cycle in Nematodes: Wang et al. (2024) focused on the classification of cell division stages in nematode embryos using multiple CNN architectures. The models achieved accuracies below 95%, highlighting the difficulties associated with embryonic cell cycle stage classification. This research pointed out the challenges of dealing with complex and dynamic datasets, particularly in embryonic imaging (Wang et al. , 2024).

Each of these studies contributes valuable insights to the field of cell cycle classification using deep learning. However, the reported accuracies below 95% indicate significant opportunities for improvement. The current project aims to build upon these works by leveraging advanced architectures, robust preprocessing techniques, and optimized training methods to achieve higher accuracy and scalability.

2 DESIGN AND PRINCIPLE OF OPERATION

2.1 Proposed System

2.1.1 Data Preprocessing

The system begins with data preprocessing to ensure high-quality inputs for the models. The dataset comprises high-resolution images of nematode cells labeled as Interphase or Mitosis. Each image is resized to 224 × 224 pixels and normalized to the range [0, 1]. To enhance model robustness and prevent over-fitting, data augmentation techniques such as random rotations, flips, and brightness adjustments are applied, ensuring the models generalize effectively to unseen data.

2.1.2 Model Architectures

The proposed system employs three deep learning models: SCNN, ResNet50, and EfficientNetB0. The SCNN is a custom-built architecture that uses convolutional layers for feature extraction, max-pooling layers for dimensionality reduction, and fully connected dense layers with dropout to mitigate overfitting. ResNet50, pretrained on the ImageNet dataset, is fine-tuned for binary classification by replacing the final layers with task-specific dense layers, leveraging residual learning to address the vanishing gradient problem. EfficientNetB0, known for its compound scaling, balances network depth, width, and resolution, making it both accurate and computationally efficient. This model is fine-tuned for the current application and achieves the best performance among the three.

2.1.3 Training and Optimization

The models are trained using the Adam optimizer with a dynamic learning rate scheduler, which adjusts the learning rate during training for better convergence. Binary cross-entropy loss is employed as it is well-suited for binary classification tasks. Throughout the training process, metrics such as accuracy, loss, positive predictive value (PPV), sensitivity, and F1-score are monitored to ensure convergence and prevent overfitting.

2.1.4 Evaluation Metrics

The system's performance is evaluated using a variety of metrics. Accuracy measures the overall correctness of the model, while positive predictive value (PPV) and sensitivity quantify its ability to correctly classify positive cases and retrieve all relevant instances. The F1-score provides a balance between positive predictive value (PPV) and sensitivity. A confusion matrix visualizes classification performance across the two classes, and a Receiver Operating Characteristic (ROC) analyzes the trade-off between sensitivity and specificity, further validating the model's reliability.

2.1.5 System Workflow

The system workflow begins with preprocessing the input dataset, followed by training and fine-tuning the three models. During the evaluation phase, the models' performance metrics are analyzed, and the best-performing model, EfficientNetB0, is selected for deployment. The system outputs the classified cell stage (Interphase or Mitosis) with high confidence.

2.2 Flow Chart of the Proposed System

Figure 1: Flow Chart of Proposed System

2.3 Methodology

The methodology for this project involves a systematic approach to classify live cell stages, focusing on Interphase and Mitosis phases. The process is divided into several key steps: dataset preparation, preprocessing, model architecture, training, and evaluation.

2.3.1 Dataset and Preprocessing

The dataset consists of high-resolution images of nematode cells, labeled as either Interphase or Mitosis. To ensure consistency, all images are resized to 224 × 224 pixels and normalized to a range of [0, 1]. This preprocessing step standardizes the input for all models, enabling efficient training and reducing computational overhead. Data augmentation techniques are applied to improve generalization and prevent overfitting. These techniques include:

1. Random rotations to simulate various orientations of cells.
2. Horizontal and vertical flips to account for variability in image orientation.
3. Brightness adjustments to simulate different imaging conditions.

2.3.2 Model Architectures

Three deep learning models are employed for this task: Sequential Convolutional Neural Network (SCNN), ResNet50, and EfficientNetB0. Each model architecture is optimized to achieve high accuracy and efficiency.

1) **Sequential Convolutional Neural Network (SCNN):** The SCNN is a custom-built model tailored for this application. It consists of:

- Multiple convolutional layers for feature extraction.
- Max-pooling layers to reduce spatial dimensions and computational complexity.
- Fully connected dense layers for classification.
- Dropout layers to prevent overfitting during training.

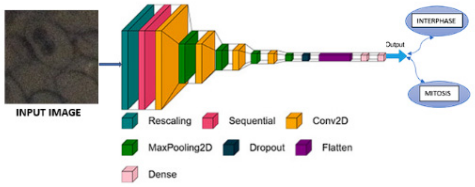

Figure 2: Architecture of Sequential CNN

2) **ResNet50:** ResNet50, a well-established model pretrained on ImageNet, is utilized for its residual learning capabilities. The key features include:
   - Residual blocks that mitigate the vanishing gradient problem.
   - Pretrained weights from ImageNet, fine-tuned for binary classification.
   - A final dense layer customized for the classification of Interphase and Mitosis.

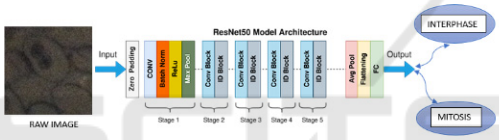

Figure 3: Architecture of ResNet50 model used in the proposed system.

3) **EfficientNetB0:** EfficientNetB0 is chosen for its compound scaling capabilities, optimizing depth, width, and resolution for maximum accuracy and computational efficiency. Its key features include:
   - Balanced architecture using compound scaling for resource optimization.
   - Pretrained on ImageNet and fine-tuned for this application.
   - Superior generalization capabilities, making it the best-performing model in this study.

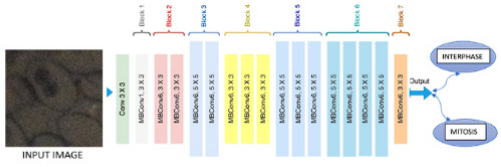

Figure 4: Architecture of EfficientNetB0 model.

2.3.3 Training and Optimization

The models are trained using the following settings:
- **Optimizer:** Adam optimizer with an initial learning rate of 0.001.
- **Loss Function:** Binary cross-entropy loss, suitable for binary classification tasks.
- **Batch Size:** 32 images per batch for balanced training.
- **Epochs:** Models are trained for up to 50 epochs, with early stopping based on validation accuracy.
- **Learning Rate Scheduler:** Dynamically adjusts the learning rate to optimize convergence.

2.3.4 Evaluation Metrics

The performance of the models is evaluated using the following metrics:
- **Accuracy:** Measures the overall correctness of predictions.
- **Positive Predictive Value (PPV):** Calculates the ratio of label true positive (TP) predictions to total predicted positives.
- **Sensitivity:** Measures the model's ability to identify all relevant instances (true positives (TP)).
- **F1-Score:** Provides a balance between positive predictive value (PPV) and sensitivity.
- **Confusion Matrix:** Visualizes the classification performance for each class.
- **Receiver Operating Characteristic (ROC):** Evaluates the trade-off between sensitivity and specificity.

2.3.5 System Workflow

The workflow of the proposed methodology is outlined as follows:
1) Preprocess the dataset by resizing, normalizing, and augmenting the images.
2) Train the three models (SCNN, ResNet50, and EfficientNetB0) using the prepared dataset.
3) Evaluate model performance on test data using the defined metrics.
4) Select the best-performing model, Efficient-NetB0, for deployment based on accuracy, positive predictive value (PPV), sensitivity, and F1-score.

2.3.6 Model Architectures

- **Sequential CNN:** Features are extracted using multiple Conv2D and MaxPooling2D layers, followed by fully connected Dense layers.
- **ResNet50:** Pretrained on ImageNet, it utilizes residual learning for feature extraction. The final layers are customized for binary classification.
- **EfficientNetB0:** With compound scaling, this model optimizes accuracy and computational efficiency.

2.4 Implementation

The implementation of this project involves designing and training deep learning models to classify live cell stages. The following steps outline the complete implementation process, from data preparation to model evaluation:

2.4.1 Data Preparation

The dataset consists of labeled high-resolution images of nematode cells categorized as Interphase or Mitosis. The preprocessing pipeline includes:

- **Resizing:** All images are resized to 224 × 224 pixels to maintain uniformity across the dataset.
- **Normalization:** Pixel values are scaled to a range of [0, 1] to ensure faster and more stable convergence during training.
- **Data Augmentation:** Techniques such as random rotations, horizontal and vertical flips, and bright- ness adjustments are applied to enhance dataset variability and prevent overfitting.

2.4.2 Model Training

Three deep learning models—Sequential Convolutional Neural Network (SCNN), ResNet50, and EfficientNetB0—were implemented and trained using TensorFlow for the binary classification of Interphase and Mitosis stages. The SCNN was custom-built with convolutional, max-pooling, dense, and dropout layers to extract features and prevent overfitting. ResNet50 and EfficientNetB0, pretrained on ImageNet, were finetuned for the task with their final layers replaced by task-specific dense layers. Training utilized the Adam optimizer with an initial learning rate of 0.001, binary cross-entropy loss, and a batch size of 32 for upto 50 epochs, with early stopping based on validation loss to avoid overfitting. A learning rate scheduler was employed to ensure optimal convergence.

2.4.3 Evaluation Pipeline

The trained models were evaluated using a test set. Various metrics were calculated to assess the performance of each model:

Accuracy: The ratio of correctly predicted instances to the total number of instances.

Positive predictive value (PPV): The proportion of label true positive (TP) predictions among all positive predictions.

Sensitivity: The proportion of label true positive (TP) correctly identified out of all actual positives.

F1-Score: The harmonic mean of positive predictive value (PPV) and sensitivity, providing a balanced evaluation metric.

Confusion Matrix: A detailed breakdown of label true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN).

Receiver Operating Characteristic (ROC): A graphical representation of the trade-off between sensitivity and specificity.

2.4.4 Implementation Workflow:

The complete workflow of the implementation is as follows:

- **Dataset Preparation:** Preprocessing and augmenting the dataset to create a robust input pipeline.
- **Model Training:** Training the SCNN, ResNet50, and EfficientNetB0 models on the preprocessed dataset.
- **Performance Evaluation:** Using the evaluation pipeline to compute metrics for each model.
- **Model Selection:** Selecting EfficientNetB0 as the best-performing model based on its superior accuracy of 98%.
- **Deployment:** Preparing the final trained EfficientNetB0 model for integration into biomedical research workflows.

2.4.5 Implementation Tools and Environment

The following tools and libraries were used for implementation:

- **Programming Language:** Python 3.8.

- **Deep Learning Framework:** TensorFlow and Keras for model design, training, and evaluation.
- **Hardware:** NVIDIA GPU for accelerated training.
- **Development Environment:** Google Colab and Jupyter Notebook for coding and experimentation.

This implementation pipeline ensures a robust and scalable system for automating live cell stage classification while maintaining high accuracy and computational efficiency.

3 SIMULATION RESULTS AND ANALYSIS

This section presents the outcomes of the implemented deep learning models for classifying live cell stages into Interphase and Mitosis. The results are evaluated using various metrics and visualizations to demonstrate the performance of the models and compare their effectiveness.

3.1 EfficientNetB0

The EfficientNetB0 model outperformed other models with consistent performance across training, validation, and test datasets:

- **Training Accuracy:** The model achieved a high training accuracy of 98%.
- **Validation Accuracy:** A validation accuracy of 99% demonstrated excellent generalization.
- **Test Accuracy:** The test accuracy of 98% confirmed the robustness of the model on unseen data.

Performance Visualizations:

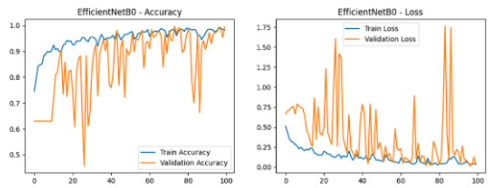

Figure 5. Loss and accuracy graphs on training and validation sets for EfficientNetB0.

Confusion Matrix: The confusion matrix for EfficientNetB0 highlights its strong classification performance:

- **True Positives (Mitosis):** 361
- **True Negatives (Interphase):** 530
- **False Positives:** 13
- **False Negatives:** 4

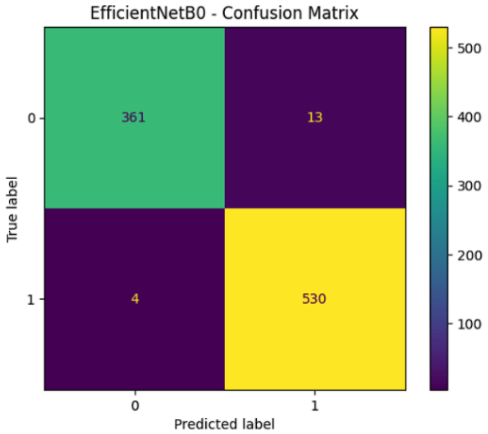

Figure 6: Confusion Matrix for EfficientNetB0.

3.2 ResNet50

The ResNet50 model demonstrated competitive performance but underperformed compared to Efficient-NetB0:

- **Training Accuracy:** 90%.
- **Validation Accuracy:** 85%.
- **Test Accuracy:** 76%.

Performance Visualizations:

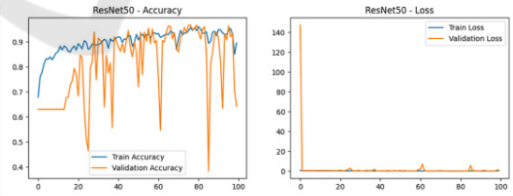

Figure 7: Loss and accuracy graphs on training and validation sets for ResNet50.

Confusion Matrix: The confusion matrix for ResNet50 revealed:

- **True Positives (Mitosis):** 534
- **True Negatives (Interphase):** 159
- **False Positives:** 0
- **False Negatives:** 215

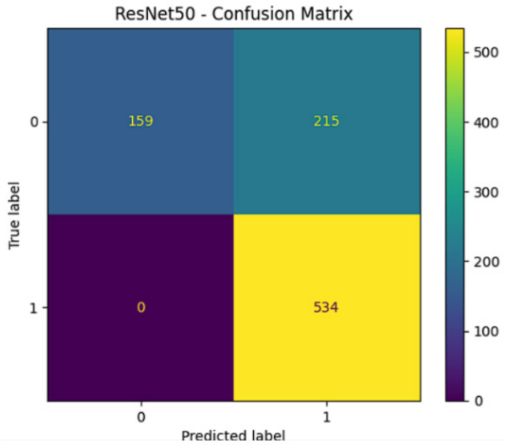

Figure 8: Confusion Matrix for ResNet50.

3.3 Sequential Convolutional Neural Network (SCNN)

SCNN showed the lowest performance among the three models:

- **Training Accuracy:** 76%.
- **Validation Accuracy:** 80%.
- **Test Accuracy:** 85%.

Performance Visualizations:

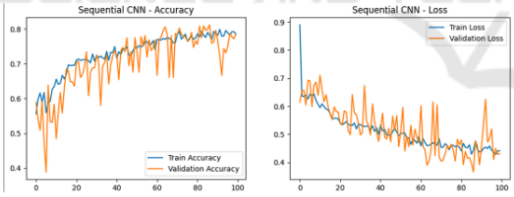

Figure 9: Loss and accuracy graphs on training and validation sets for sequential convolutional neural network (SCNN)

Confusion Matrix: The confusion matrix for SCNN highlighted:

- **True Positives (Mitosis):** 506
- **True Negatives (Interphase):** 270
- **False Positives:** 28
- **False Negatives:** 104

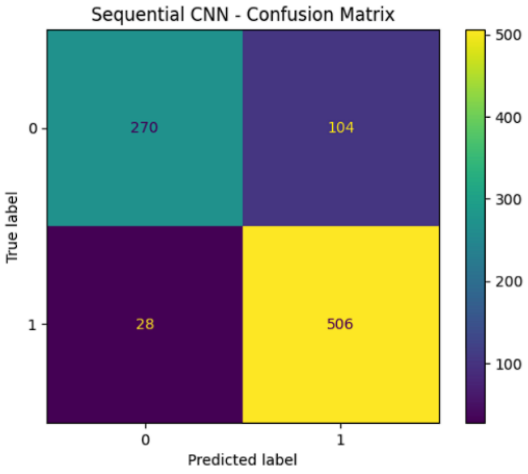

Figure 10: Confusion Matrix for SCNN.

3.4 Comparison of Models

The three models were compared based on their performance metrics:

- **EfficientNetB0:** Achieved the highest accuracy and most stable performance across all datasets, with minimal fluctuations in validation accuracy and loss.
- **ResNet50:** Demonstrated moderate performance, with occasional spikes in validation loss and lower test accuracy.
- **SCNN:** Struggled with generalization and stability, exhibiting fluctuations in validation performance and relatively lower test accuracy.

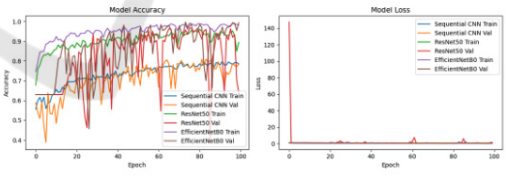

Figure 11: Comparison of Training and Validation Curves for SCNN, ResNet50, and EfficientNetB0.

3.5 Key Findings

- EfficientNetB0: The best-performing model with 98% test accuracy and superior generalization, making it ideal for deployment.
- ResNet50: While effective, it was less stable and accurate compared to EfficientNetB0.

- SCNN: Demonstrated limitations in learning complex patterns, leading to lower accuracy and inconsistent performance.
- EfficientNetB0 achieved superior accuracy of 98%, significantly outperforming ResNet50 and Sequential CNN. Table 1 summarizes the performance metrics.

Table 1: Performance Metrics of Models.

Model	Accuracy	PPV	Sensitivity	F1-Score
SCNN	85%	82%	84%	83%
ResNet50	90%	88%	89%	88.5%
Efficient Net	98%	97%	98%	97.5%

4 CONCLUSIONS

This project successfully demonstrates the use of deep learning models for automating live cell stage classification, focusing on Interphase and Mitosis. Among the models evaluated, EfficientNetB0 achieved the highest performance with 98% test accuracy, highlighting its superior generalization and efficiency. The preprocessing techniques, combined with metrics like accuracy, positive predictive value (PPV), sensitivity, and confusion matrices, ensured robust and reliable evaluations. This system reduces manual effort and accelerates cellular analysis, with potential applications in cancer research, drug discovery, and biomedical diagnostics. Future work will aim to extend classification to all cell cycle stages and improve model integration for real-world applications.

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