Investigating Fibroblast Migration in a Wound Healing Assay Using AI-Based Image Segmentation and Deep Learning

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Introduction

- Wound healing is a vital process that restores tissue after injury. Fibroblasts are essential for this, as they migrate into the wound area and generate extracellular matrix (ECM), helping close the wound.
- Manual segmentation of wound healing images is slow, subjective, and inefficient.
- This project aims to build an AI-powered image segmentation pipeline using U-Net and CNNs to automate this process.
- By analyzing bright-field microscopy images of fibroblast migration, we seek insights into healing dynamics and possible clinical applications, including rare connective tissue disorders like classical Ehlers-Danlos Syndrome (cEDS).

Literature Background

- Traditional tools like ImageJ and TScratch are commonly used for wound closure analysis but are error-prone. Reported errors: ImageJ (22.59%), TScratch (33.88%).
- Deep learning models, particularly U-Net, U-Net++, and Attention U-Net, are more accurate and adaptable.
- Preprocessing steps like CLAHE and data augmentation (flips, rotation, zoom) have been shown to significantly enhance model performance.
- Our approach builds on Doğru et al. (2024), applying U-Net and modern enhancements to bright-field wound assay data.

Strategy Preprocessing Pipeline

- Initially, we planned to use a custom GUI tool for manual mask labeling.
- This approach was later dropped due to time constraints and limited scalability.
- Instead, we automated mask generation for all images using Python and OpenCV.
- Full preprocessing pipeline:
  - Convert 16-bit TIFF → 8-bit PNG
  - CLAHE to enhance contrast
  - Gaussian blur to reduce noise
  - Otsu's thresholding to extract wound area
  - Morphological operations (open/close) to clean noise
  - Remove small blobs using contour area thresholding
- Result: Binary masks created for **all 145 frames**, not just a subset.

Dataset Summary

- Dataset includes ~2,000 bright-field microscopy images over a 40-hour time lapse.
- Images represent fibroblast migration and wound closure.
- Manual quality checks performed to remove blurry or low-contrast frames.
- 145 frames were preprocessed and had masks automatically generated.
- Data split: 80% for training, 20% for validation.
- Data augmentation applied:
  - Horizontal/vertical flips
  - ±10° rotations
  - Random zoom and translations

U-Net Architecture

- U-Net is designed for pixel-level biomedical image segmentation.
- Input: 256x256 grayscale image | Output: 256x256 binary mask
- Architecture includes:
  - Encoder path: convolution + max pooling
  - Bottleneck layer for deeper context
  - Decoder path: upsampling + skip connections
- Training details:
  - Optimizer: Adam
  - Loss: Binary Cross-Entropy
  - Platform: CPU (resource-constrained)

CNN Architecture

- A basic CNN model was implemented as a comparison benchmark.
- Simpler architecture with convolution + pooling + fully connected layers.
- Trained using categorical cross-entropy with SGD optimizer.
- Surprisingly, the CNN achieved comparable Dice scores, but U-Net was more consistent and robust.

Results and Performance

- **U-Net Dice Scores:**
  - Epoch 1: Train Dice = 0.2383, Val Dice = 0.2505
  - Epoch 3: Train Dice = 0.9095, Val Dice = 0.9122
  - Epoch 10: Train Dice = 0.9129, Val Dice = 0.9144
- **CNN Dice Scores:**
  - Epoch 3: Val Dice = 0.8838
  - Epoch 5: Val Dice = 0.9050
  - Epoch 10: Train Dice = 0.9065, Val Dice = 0.9075
- Final U-Net model saved as small_unet_cpu.pth
- Dice scores plotted to visualize convergence.

Visual Output & Interpretation

- Predicted binary masks were post-processed using Gaussian filters.
- Wound contours were extracted and overlaid on original grayscale images.
- Results outline fibroblast monolayer borders and wound fronts.
- Visual comparison between raw-input and segmentation output confirms model accuracy.

Conclusion & Future Work

- We built an end-to-end AI pipeline for fibroblast wound segmentation.
- Automated mask generation replaced manual GUI-based approach.
- Achieved >90% Dice score using both U-Net and CNN.
- **Next Steps:**
  - Implement flat-field correction for better lighting consistency
  - Explore semi-supervised learning on unlabeled images
  - Quantify wound closure rates and cell migration speed over time
  - Package the tool into a user-friendly interface for researchers

Thank you!