A Minor Project report on

Estimation of Haemoglobin using non-invasive techniques

Submitted

in partial fulfillment of the requirements for the award of the degree of

Bachelor of Engineering

IN

COMPUTER SCIENCE AND ENGINEERING

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2024-2025

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CERTIFICATE

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ABSTRACT

Hemoglobin (Hb) estimation constitutes an important measure for diagnosing certain disorders, and it is infrequently carried out without invasive means, that is, through blood sampling. We intend to develop a non-invasive and camera-based method to predict the concentration of hemoglobin, based on deep learning and regression techniques applied to images of the palpebral conjunctiva. After acquisition, raw eye images undergo gray-world algorithm-based white balancing for color distortion removals. This is followed by manual annotation and Mask R-CNN-based segmentation to select the palpebral conjunctiva, which gives strong indications of blood oxygenation levels. The segmented images are then taken for useful feature extraction using the MobileNetV2 deep learning model. These features are combined with metadata from patients, such as age and sex, which are medical parameters known to influence hemoglobin levels. An ensemble regression model: Random Forest is trained on those combined features with the relevant Hb values from a structured dataset. The final model shows very encouraging performance in Hb value estimation and thus provides a prediction tool that could facilitate remote screening and early diagnosis of anemia. This platform based on computer vision and machine learning has the potential to transform the common public health diagnostic by providing a cheap and easily accessible alternative to existing blood tests, into a truly non-invasive one.

Keywords : *Hemoglobin, Non-invasive Diagnostics, Palpebral Conjunctiva, Machine Learning in Healthcare .*

ACKNOWLEDGEMENT

We would like to thank our faculty and management for their professional guidance towards the completion of the mini project work. We take this opportunity to thank Dr. Ashok Shettar, Pro-Chancellor, Dr. P.G Tewari, Vice-Chancellor and Dr. B.S.Anami, Registrar for their vision and support.

We also take this opportunity to thank Dr. Meena S.M, Professor and Dean of Faculty, SoCSE, Dr. Vijayalakshmi M, Professor and Head, Department of CSE and Dr. Narayan D.G Professor and Head, Department of CSE(AI) for having provided us direction and facilitated for enhancement of skills and academic growth.

We thank Prof.Parikshit Hegde , Designation and SoCSE for the constant guidance during interaction and reviews.

We extend our acknowledgment to the reviewers for critical suggestions and inputs. We also thank project coordinator Dr. Uday Kulkarni, Mr. Sunil V.Gurlahosur and reviewers for their suggestions during the course of completion. We express gratitude to our beloved parents for constant encouragement and support.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

Hemoglobin (Hb) estimation is a critical diagnostic indicator in identifying conditions such as anemia and other blood-related disorders. Traditionally, hemoglobin levels are measured invasively through blood sampling and laboratory analysis, which can be inconvenient, time-consuming, and uncomfortable for patients, especially in large-scale screening scenarios or resource-constrained settings. To overcome these limitations, this project proposes a non-invasive, image-based hemoglobin estimation technique that leverages computer vision and machine learning methods. Specifically, the palpebral conjunctiva—a region of the eye known to reflect blood oxygenation and hemoglobin levels—is used as the biomarker site. High-resolution conjunctival images were collected, preprocessed using white balancing techniques to ensure color consistency, and subsequently annotated and segmented using Mask R-CNN to isolate the region of interest. The segmented conjunctiva images were then used to extract features through two primary approaches: Traditional feature extraction (mean and standard deviation of RGB values), and Deep feature extraction using the MobileNetV2 convolutional neural network pretrained on ImageNet. These features were combined with structured patient metadata (such as age and sex) and used to train machine learning models, including Random Forest Regressor and ensemble techniques, to predict hemoglobin levels. The dataset was split into training and test sets, and performance was evaluated using metrics such as Mean Squared Error (MSE) and R^2 Score. This report presents the complete methodology, from image preprocessing to model inference, and demonstrates the potential of AI-driven, contactless diagnostics in improving accessibility and efficiency in healthcare. [?]

1.1 Preamble (Provide Introduction of the project)

Hemoglobin (Hb) is an essential biomarker used in medical diagnostics to assess oxygen-carrying capacity and detect disorders such as anemia. Conventionally, hemoglobin levels are measured using invasive methods like blood tests, which require clinical setup, laboratory equipment, and trained personnel. This project focuses on developing a machine learning-based model to estimate hemoglobin levels from conjunctival images—captured using a standard camera—without the need for blood sampling. The approach offers a cost-effective, accessible, and patient-friendly alternative to traditional hemoglobin testing.

1.2 Motivation

In many parts of the world, especially in rural or resource-limited settings, access to diagnostic laboratories and medical professionals is limited. Anemia, which affects a significant portion of the population, often goes undiagnosed due to the invasive nature of current testing procedures. A non-invasive method that utilizes easily captured eye images can greatly aid in early diagnosis and mass screening programs. Moreover, with the increasing availability of smartphones and digital imaging devices, such a system could be integrated into mobile health solutions, making hemoglobin testing more accessible and scalable.

1.3 Objectives of the Project

The primary objectives of this project are:

1. To develop a non-invasive AI system for estimating hemoglobin using conjunctival images.
2. To build and evaluate regression models for hemoglobin estimation.
3. To optimize the model for accuracy across diverse populations.
4. To reduce data imbalance and ensure fair, unbiased predictions.

1.4 Literature Review / Survey

Non-invasive hemoglobin (Hb) estimation has gained significant research interest as a promising alternative to traditional invasive blood tests. Numerous studies have explored the potential of using image-based techniques—especially palpebral conjunctiva imaging—combined with artificial intelligence (AI) for accurate Hb level prediction. A study by Saeed et al. (2022), published on PubMed, introduced a method for estimating hemoglobin levels by analyzing digital images of the lower eyelid conjunctiva. The authors emphasized the potential of imaging techniques in detecting anemia, particularly in resource-limited settings where traditional blood analysis may not be readily available [1]. Another significant contribution is by Jannatul Maowa et al. (2024) on ResearchGate, who developed an AI-based algorithm to estimate hemoglobin levels using the palpebral conjunctiva region. Their approach involved extracting color features from eye images and applying a machine learning model to predict Hb values. The work underscored the correlation between conjunctival coloration and hemoglobin concentration, demonstrating the feasibility of a contactless and cost-effective screening method [2]. Earlier, Saito et al. (2007) conducted a foundational study published in The American Journal of Emergency Medicine, where they proposed a visual and digital analysis of the conjunctiva to assess anemia in emergency conditions. Their findings supported the concept that conjunctival pallor could serve as a measurable feature for anemia detection using imaging technology [3]. These studies collectively validate the viability of leveraging conjunctival images for hemoglobin estimation. They also highlight the increasing integration of image processing and AI to automate and enhance the accuracy of non-invasive diagnostic techniques. Building on this foundation, the present project proposes a deep learning-based ensemble model trained on segmented conjunctival images, along with demographic features, to accurately predict hemoglobin levels from eye photographs.

1.5 Problem Definition

The project aims to solve the problem of invasiveness and inaccessibility associated with traditional hemoglobin testing. The key challenge addressed is the accurate estimation of hemoglobin concentration from non-invasive conjunctival images, which involves handling variations in lighting, image quality, and anatomical differences among individuals. The solution involves a robust pipeline combining image preprocessing, deep learning for feature extraction, and machine learning regression models to estimate hemoglobin levels reliably. The developed system should generalize well across varied patient data and show promising performance comparable to conventional diagnostic methods.

Chapter 2

SOFTWARE REQUIREMENT SPECIFICATION

The success of any software project depends heavily on a well-defined and structured set of requirements. The Software Requirement Specification (SRS) acts as a formal contract between the stakeholders and the development team, clearly outlining what the system should do and how it should behave under various conditions. It provides a comprehensive description of the system's functionality, constraints, and interactions, serving as the foundation for all subsequent phases of the software development lifecycle. In this chapter, we present the detailed SRS for our project on hemoglobin estimation using non-invasive techniques.

2.1 Overview of SRS

The Software Requirement Specification (SRS) document outlines the functional and non-functional requirements for the project. This document serves as a foundation for the development, testing, and maintenance of the software. It helps developers, project managers, and stakeholders understand the capabilities of the software and sets clear expectations regarding the system's performance and constraints. In this project, the SRS focuses on the development of a non-invasive AI-based system that estimates hemoglobin levels using images of the conjunctiva. It details the system's functionalities, the interaction with the user, and the underlying hardware and software components required for successful implementation. The SRS also emphasizes data handling, including the integration of patient information, image processing tasks, and regression model training for hemoglobin estimation.

2.2 Requirement Specifications

The requirement specifications serve as the backbone of system design and implementation. They define what the system is expected to accomplish and provide a basis for validation and verification. Requirements are typically categorized into functional and non-functional aspects. Functional requirements describe the core features and behaviors the system must exhibit in response to specific inputs or conditions. These requirements ensure that the system performs the necessary operations to fulfill user needs and business goals.

2.2.1 Functional Requirements

- FR1: The system must preprocess images by applying techniques like white balancing, noise reduction, and annotation.
- FR2: The system should segment the eye's conjunctiva using Mask R-CNN to isolate the region of interest for hemoglobin estimation.
- FR3: The system must train a regression model (Random Forest) using preprocessed image features and additional patient data (e.g., age, sex) to predict hemoglobin levels.
- FR4: The system should evaluate the trained model's performance using metrics like Mean Squared Error (MSE) and R^2 Score.
- FR5: The system should provide a prediction for hemoglobin based on new input images and associated data.
- FR6: The system should include a user-friendly interface for image uploads, displaying predictions, and managing dataset inputs.

2.2.2 Use Case Descriptions

Primary Actor:

Medical staff or researcher interacting with the hemoglobin estimation system.

Stakeholders and Interests:

Medical professionals require a quick, non-invasive method for estimating hemoglobin levels in patients. Researchers may use the system for population-level analysis or for validating AI-based clinical tools. Patients benefit by avoiding painful or costly blood tests.

Preconditions:

The system must be trained with a valid regression model. The user must have access to a functional interface where conjunctival eye images and minimal patient data (age, sex) can be uploaded.

Postconditions:

The system returns an estimated hemoglobin level and optionally logs the prediction for further analysis or audit. The result may also be visualized or exported.

Main Success Scenario (Basic Flow):

- The user opens the system interface and selects an eye image for upload.
- The system applies image preprocessing (e.g., white balancing, annotation).
- Segmentation is performed to extract the palpebral conjunctiva region using a Mask R-CNN model.

- Deep features are extracted from the segmented region using a pre-trained MobileNetV2 model.
- The user enters additional details like age and sex.
- The combined feature vector is passed to the trained regression model (Random Forest).

Alternate Flows:

- If the uploaded image is of poor quality (e.g., blurry or incomplete), the system prompts the user to re-upload a clearer image.
- If patient data is incomplete or incorrectly formatted, the system requests correction before proceeding.
- If the segmentation model fails to detect a valid conjunctiva region, a notification is shown, and the process is halted.

Special Requirements:

The system should ensure data privacy by not storing personal identifiers beyond the session unless explicitly permitted. It should also be responsive and provide results within a few seconds. The interface must be intuitive to use, even for non-technical users.

2.2.3 Non-Functional Requirements

The non-functional requirements define the system's quality attributes and constraints that ensure performance, usability, security, and maintainability. These are critical for guaranteeing that the hemoglobin estimation system not only works correctly but also operates efficiently and reliably in real-world scenarios. Below are the key non-functional requirements of the system:

2.2.4 Non-Functional Requirements

- **NFR1: Performance**
  The system shall return hemoglobin predictions within 5 seconds of receiving input images and patient data, ensuring efficient processing and responsiveness.
- **NFR2: Accuracy**
  The hemoglobin estimation must achieve a low Mean Squared Error (MSE) and a high R^2 score, maintaining clinical relevance and reliability.
- **NFR3: Usability**
  The user interface must be intuitive and simple, allowing users with minimal technical expertise to upload images, input patient details, and view results easily.

- **NFR4: Scalability**
  The system should support an increasing volume of images and users without significant degradation in performance, allowing future expansion.

- **NFR5: Portability**
  The system should be deployable across various platforms, including local machines and cloud services, with minimal configuration changes.

2.3 Software and Hardware Requirement Specifications

2.3.1 Software Requirements

- Operating System: Windows 10/11 or Linux (Ubuntu 18.04 or later)
- Programming Language: Python 3.7 or higher
- Libraries and Frameworks:
  - OpenCV for image processing
  - TensorFlow / Keras for deep learning model (MobileNetV2)
  - scikit-learn for machine learning (Random Forest)
  - NumPy and Pandas for data handling
- Development Environment: Jupyter Notebook or any Python IDE (e.g., PyCharm, VS Code)
- Database: Excel or CSV files for patient data storage

2.3.2 Hardware Requirements

- Processor: Intel i5 or equivalent (recommended i7 or higher for faster training)
- RAM: Minimum 8 GB (16 GB recommended for better performance)
- Storage: At least 100 GB free disk space for datasets and models
- GPU: NVIDIA GPU with CUDA support (optional but recommended for deep learning model training)
- Display: Monitor with a minimum resolution of 1366x768 pixels for ease of use

Chapter 3

PROPOSED SYSTEM

Proposed System This chapter presents an overview of the proposed system's design, features, and development principles. .

3.1 Description of Proposed System.

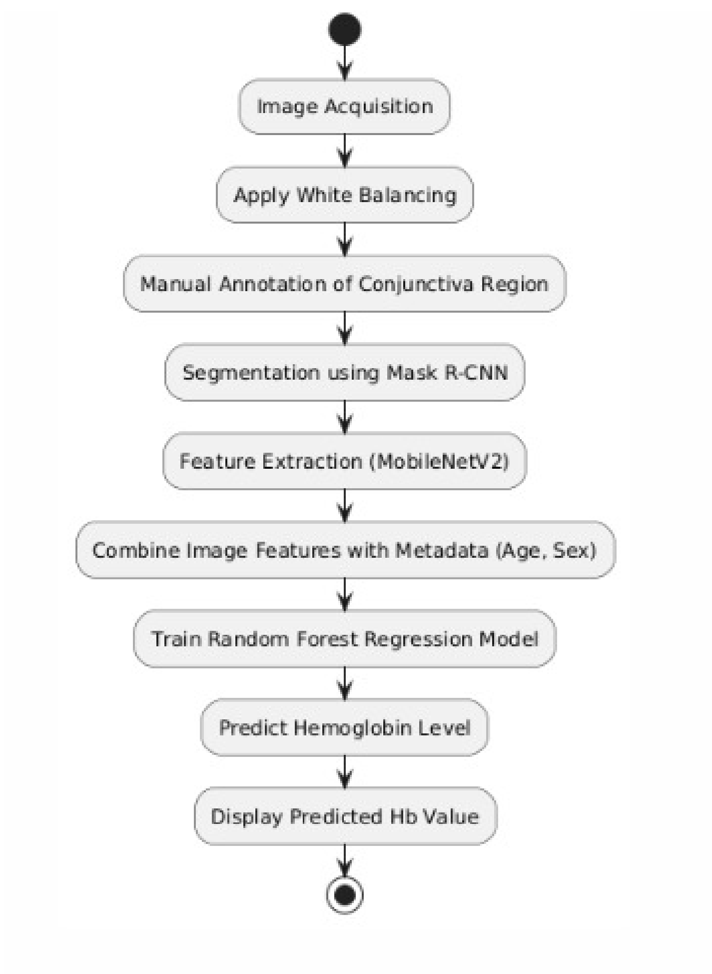

Figure 3.1: Proposed System

The proposed system aims to estimate hemoglobin (Hb) levels non-invasively by analyzing images of the eye's palpebral conjunctiva. Initially, white balancing techniques such as gray world, perfect reflector, and histogram equalization are applied to the raw images to correct color distortions caused by varying lighting conditions. Following this, precise annotation of the conjunctiva region is performed to create accurate training data. For segmentation, a Mask R-CNN model is employed to extract the palpebral conjunctiva region from the images, isolating it from surrounding tissues and background. These segmented regions are then used as input to a feature extraction pipeline based on a pre-trained MobileNetV2 convolutional neural network, which captures deep image features relevant to hemoglobin levels. Additionally, patient demographic data such as age and sex are incorporated to improve model accuracy. The combined features are used to train a Random Forest regression model to predict hemoglobin values. Evaluation metrics such as mean squared error and R^2 score demonstrate the model's effectiveness in estimating Hb from non-invasive eye images, providing a promising approach for anemia screening without the need for blood draws.

3.2 Description of Target Users

Description of Target Users The proposed system is designed primarily for clinicians, healthcare workers, and diagnostic technicians operating in primary health centers, rural clinics, and mobile healthcare units where traditional hemoglobin testing methods (such as invasive blood tests) may be costly, slow, or inaccessible.

Design Principles Identified and Used: Non-Invasiveness: A core design principle is to avoid blood sampling by estimating hemoglobin levels from external eye images, particularly the palpebral conjunctiva. This ensures patient comfort, safety, and reusability, making it suitable for mass screening and pediatric or geriatric use.

Affordability and Accessibility: The system uses widely available imaging devices (like smartphone cameras) and lightweight models like MobileNetV2 for feature extraction, ensuring it can run on low-resource devices without the need for expensive infrastructure.

Explainability and Interpretability: By using models like Random Forest Regression, the design retains interpretability over deep black-box models, making the system transparent for clinical adoption and easier to validate with medical experts.

Scalability and Modularity: The pipeline is structured with clear stages — white balancing, annotation, segmentation, feature extraction, and prediction — enabling easy updates, extensions, or replacement of components without redesigning the entire system.

Accuracy with Robust Preprocessing: Extensive preprocessing such as white balancing, manual annotation, and segmentation via Mask R-CNN ensures high data quality before training, supporting more accurate and consistent predictions even with varying input image conditions.

User-Friendliness for Non-Experts: The system is designed to allow semi-automated annotation and automated predictions, supporting non-technical users in real-world settings with minimal training.

3.3 Advantages of Proposed System

The proposed hemoglobin estimation system offers a non-invasive and patient-friendly approach by utilizing eye images instead of blood samples. This eliminates discomfort and potential risks associated with traditional blood draws, making it safer and more acceptable for frequent monitoring. Additionally, the system is cost-effective since it relies on standard digital imaging devices and machine learning techniques, reducing the need for expensive laboratory equipment and consumables. Its ability to provide rapid hemoglobin estimations facilitates timely clinical decision-making and improves overall patient care efficiency. Moreover, the system's portability enables deployment in remote and resource-limited settings, where access to laboratory infrastructure is often scarce. To enhance accuracy, the system incorporates robust preprocessing techniques such as white balancing and precise segmentation to counter variations in lighting and image quality. It also integrates demographic information like age and sex alongside image features, thereby improving prediction performance and enabling personalized assessments.

3.4 Scope

Scope (Boundary of Proposed System) The proposed system focuses on estimating hemoglobin levels using non-invasive image analysis of the palpebral conjunctiva region captured through standard digital cameras. It covers preprocessing steps such as white balancing and precise segmentation of the eye region to ensure consistent and accurate input for the regression model. The system integrates image-derived deep features with patient demographic data like age and sex to improve prediction reliability. However, it is designed primarily for controlled imaging conditions and may require standardized image capture protocols to maintain accuracy. The system currently targets hemoglobin estimation for individuals whose eye images and corresponding demographic data are available, and does not replace comprehensive clinical diagnostics but serves as a supplementary screening tool. Its application is limited to scenarios where high-quality conjunctiva images can be obtained, and it is not intended for use in cases with severe ocular abnormalities or poor image quality that could hinder segmentation and analysis. Furthermore, the model is trained on specific datasets, so its performance may vary with populations or imaging devices outside the training distribution unless further calibration is performed.

Chapter 4

SYSTEM DESIGN

This chapter gives a brief description about implementation details of the system by describing each component with its code skeleton in terms of algorithm.

4.1 Architecture of the system (explanation)

The architecture of the proposed system for non-invasive hemoglobin (Hb) estimation is modular and consists of several sequential stages designed to ensure accurate, interpretable predictions from ocular images. Each module contributes a specific function within the end-to-end pipeline — starting from data preparation to final prediction — enabling robust and scalable model deployment.

The system follows a typical machine learning pipeline augmented with deep learning-based feature extraction and traditional regression modeling. The architecture comprises the following key components:

Image Preprocessing: This module prepares raw images for analysis. Each eye image is first corrected for color imbalance using white balancing techniques like the Gray World algorithm. This ensures color consistency across images. Following this, the conjunctival region — the area of interest for hemoglobin estimation — is manually annotated or automatically segmented using a deep learning model (e.g., Mask R-CNN). These steps help isolate the relevant image region while minimizing background noise.

Feature Extraction: After preprocessing, each segmented image is resized to a fixed resolution (e.g., 224×224 pixels) to match the input requirements of convolutional neural networks. A pretrained deep learning model such as MobileNetV2 is then used to extract deep image features. In parallel, patient metadata (age and sex) is encoded and appended to the extracted features to form a comprehensive input feature vector.

Dataset Preparation: The extracted features along with their corresponding ground truth hemoglobin values are compiled into a dataset. This dataset is then split into training and testing subsets to facilitate model evaluation and generalization testing.

Model Training: A regression model — specifically a Random Forest Regressor — is initialized with a predefined number of trees. The model is trained using the training dataset, optimizing for Mean Squared Error (MSE) loss. The use of ensemble learning (Random Forest) improves robustness and reduces the risk of overfitting.

Model Evaluation: The trained model is validated on the test dataset. Predictions are compared with ground truth Hb values to compute evaluation metrics such as MSE and R^2 score. These metrics help in assessing the accuracy and reliability of the system.

Prediction Module: For new, unseen patient images, the system extracts deep features in the same manner as the training phase and concatenates them with age and sex data. The trained model then provides a hemoglobin level prediction for the new input. This prediction can be displayed via a user interface or exported for clinical review.

4.2 Data Set Description

The dataset used in this project is specifically curated for non-invasive hemoglobin (Hb) estimation from ocular images. It consists of high-resolution images of the conjunctival region of the eye collected in clinical settings, along with corresponding patient metadata and hemoglobin values obtained through traditional blood tests.

The raw images primarily capture the palpebral conjunctiva, which is the region most indicative of hemoglobin levels due to its vascular nature. These images were preprocessed using white balancing techniques such as the Gray World algorithm to correct for lighting and color inconsistencies. The conjunctival region was manually annotated to train segmentation models, and subsequently, Mask R-CNN was employed to segment the relevant region automatically.

Alongside the images, patient metadata including unique serial numbers, age, sex, and hemoglobin levels were collected. The sex attribute was encoded as a binary variable. This metadata was combined with deep features extracted from the segmented conjunctival images using a pretrained MobileNetV2 model to form the input features for the regression model.

The final dataset comprises these extracted image features concatenated with patient age and sex, along with the ground truth hemoglobin levels as target labels. The dataset was split into training and testing sets in an 80:20 ratio to enable robust evaluation of the regression model.

Algorithm 1 Non-Invasive Hemoglobin Estimation using Image Regression

Require: White-balanced and segmented images, Patient metadata (age, sex), Hb values, Learning rate α, Number of trees T

Ensure: Trained regression model for Hb estimation

```
Image Preprocessing
for each image I in dataset do
    Apply white balancing (e.g., Gray World algorithm)
    Perform manual annotation and segmentation using Mask R-CNN
end for
Feature Extraction
for each image I_i with patient data (age_i, sex_i) do
    Resize I_i to 224×224
    Extract deep features f_i using pretrained MobileNetV2
    Encode sex as binary
    Concatenate features: x_i ← [f_i, age_i, sex_i]
    Label: y_i ← Hb_i
end for
Dataset Preparation
Construct dataset D = {(x_i, y_i)}
Split D into training and testing sets
Model Training
Initialize Random Forest Regressor with T estimators
Train model on training data using MSE loss
Evaluation
Predict ŷ on test set
Compute Mean Squared Error (MSE) and R^2 Score
Prediction
function PredictHb(I_new, age, sex)
    Extract features from I_new using MobileNetV2
    Concatenate with (age, sex)
    Predict Hb using trained model
    return Predicted Hb value
end function
```

4.3 Sequence diagram (if applicable with brief explanation)

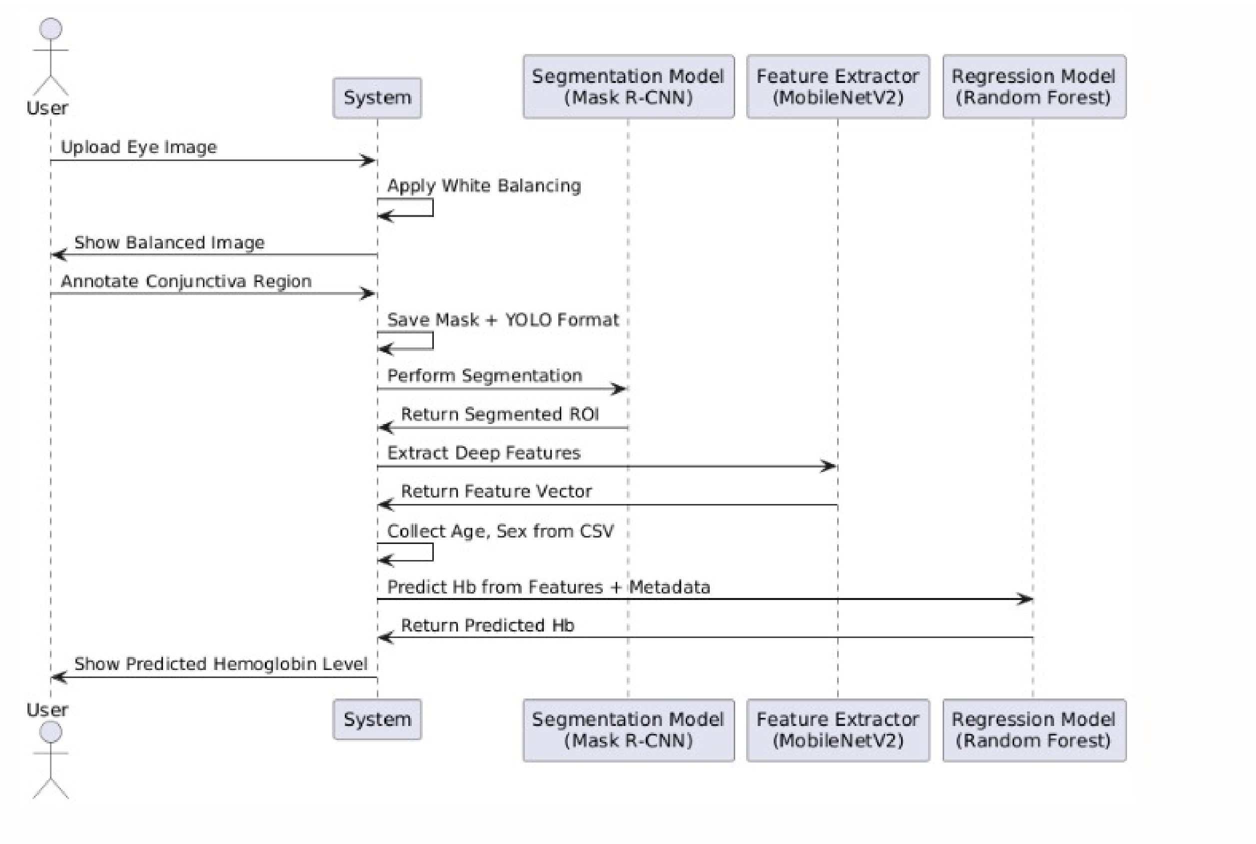

Figure 4.1: Sequence Diagram

Chapter 5

IMPLEMENTATION

In this chapter, we provide a comprehensive overview of the implementation process for the non-invasive hemoglobin estimation system. This system is designed to accurately predict hemoglobin levels from eye images combined with patient metadata, leveraging advanced image processing and machine learning techniques. The implementation begins with image preprocessing, where raw eye images undergo white balancing. This step is crucial to correct color distortions caused by varying lighting conditions during image capture, thereby enhancing the consistency and quality of the data. The preprocessed images are then subjected to manual annotation, where experts delineate the palpebral conjunctiva region using polygonal masks. These annotations serve as ground truth labels for the next phase. For automatic identification of the conjunctiva region, the system employs a Mask R-CNN-based segmentation model. Mask R-CNN is a state-of-the-art deep learning framework capable of both object detection and precise segmentation. By training this model on the annotated dataset, the system learns to accurately segment the conjunctiva in new images, effectively isolating the region of interest for subsequent analysis. Next, the segmented conjunctiva images are processed through a feature extraction module using a pre-trained MobileNetV2 convolutional neural network. This lightweight yet powerful CNN model extracts deep feature vectors that capture the essential visual characteristics of the conjunctiva, transforming complex image data into compact, meaningful numerical representations suitable for machine learning. Parallel to image processing, patient metadata such as age and sex are preprocessed and encoded to complement the visual features. The combined dataset of image features and metadata is then fed into a Random Forest regression model, which learns the complex relationships between these inputs and hemoglobin levels measured via traditional methods. This model provides non-invasive hemoglobin predictions with high accuracy, enabling clinical assessments without the need for blood tests. The entire system is developed in Python, utilizing robust libraries including OpenCV for image manipulation, TensorFlow/Keras for building and training deep learning models, and scikit-learn for implementing the regression algorithm. This modular architecture ensures a scalable and efficient workflow, allowing seamless progression from raw data input to reliable hemoglobin level output, suitable for deployment in real-world healthcare applications.

5.1 Proposed Methodology

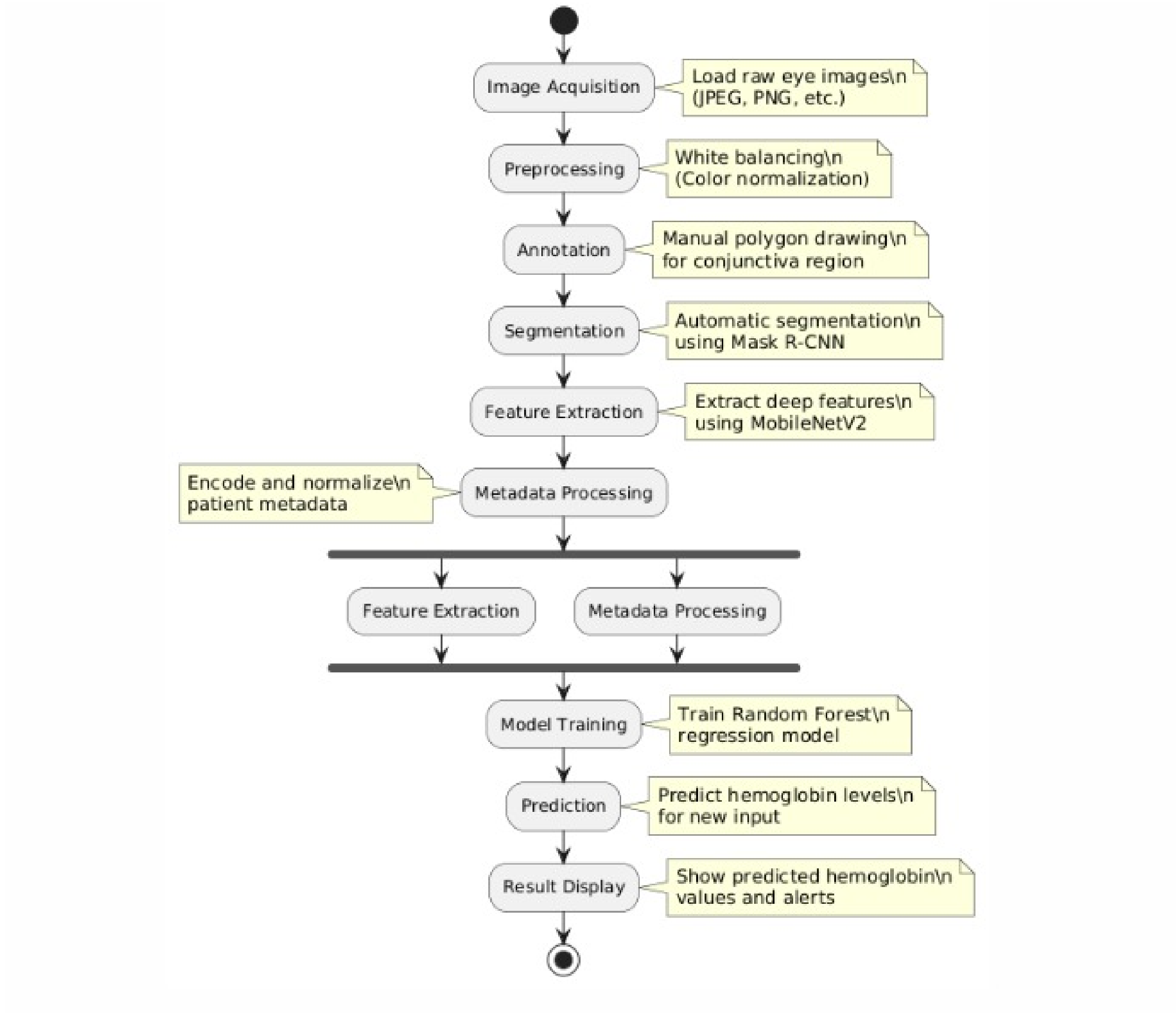

Figure 5.1: Methodology

5.2 Description of Modules

This section describes the main modules of the hemoglobin estimation system from eye images, detailing their inputs, outputs, and purpose within the overall processing pipeline.

5.2.1 Image Acquisition Module

- **Input:** Raw image files (JPEG, PNG, etc.)
- **Output:** Loaded images in memory ready for preprocessing
- **Description:** Loads raw eye images from the dataset or user uploads, supporting multiple formats and verifying image integrity for further processing.
- **Pseudocode:**
  - Initialize image directory path
  - For each file in directory:
    - Check file extension for valid image format
    - Load image using OpenCV or PIL
    - Verify image loaded successfully
    - Store image in a list or array for next stage

5.2.2 Preprocessing Module

- **Input:** Raw loaded images
- **Output:** White-balanced images
- **Description:** Applies white balancing (e.g., Gray World method) to normalize lighting and color variations, improving image quality for segmentation.
- **Pseudocode:**
  - For each loaded image:
    - Convert image to float format
    - Calculate mean values for R, G, B channels
    - Compute average of channel means
    - Adjust each channel by scaling with average/mean ratio
    - Clip pixel values to valid range (0-255)
    - Convert image back to uint8
    - Save or pass image to next module

5.2.3 Annotation Module

- **Input:** White-balanced images
- **Output:** Polygon coordinates and binary mask images
- **Description:** Allows manual polygon annotation of the conjunctiva region, saving coordinate points and corresponding binary masks for segmentation training.
- **Pseudocode:**
  - Display image to user with resizing if needed
  - On mouse left-click:
    * Record clicked point (x, y)
    * Draw circle on display image
  - On mouse right-click:
    * Close polygon if $>$ 2 points
    * Draw polygon on display image
    * Save polygon points to file in YOLO format
    * Generate binary mask and save
  - Repeat for all images or until user exits

5.2.4 Segmentation Module

- **Input:** White-balanced images and annotation masks (training)
- **Output:** Segmented images focusing on conjunctiva
- **Description:** Uses Mask R-CNN to learn from annotated masks and segment conjunctiva regions automatically for new images.
- **Pseudocode:**
  - Load annotated masks and corresponding images
  - Train Mask R-CNN model on dataset
  - For each new image:
    * Predict conjunctiva mask using trained model
    * Extract and save segmented region based on mask

5.2.5 Feature Extraction Module

- **Input:** Segmented conjunctiva images
- **Output:** Deep feature vectors representing image characteristics
- **Description:** Uses MobileNetV2 pretrained CNN to extract numeric features capturing image details relevant for hemoglobin prediction.
- **Pseudocode:**
  - Load MobileNetV2 model without top layer
  - For each segmented image:
    * Resize image to model input size (224x224)
    * Preprocess image (normalization)
    * Pass image through model to get feature vector
    * Store feature vector for model training

5.2.6 Metadata Processing Module

- **Input:** Raw patient metadata (age, sex, etc.)
- **Output:** Encoded and normalized metadata
- **Description:** Converts categorical metadata into numeric form and normalizes data for use alongside image features.
- **Pseudocode:**
  - Load metadata CSV or Excel file
  - Clean and preprocess columns (strip spaces, lower case)
  - Encode categorical variables (e.g., sex: Male=1, Female=0)
  - Normalize numeric columns if necessary
  - Output processed metadata for model input

5.2.7 Model Training Module

- **Input:** Combined image features + metadata, hemoglobin labels
- **Output:** Trained Random Forest regression model

- **Description:** Trains regression model on combined features to predict hemoglobin levels, validating performance with test data.
- **Pseudocode:**
  - Combine feature vectors and processed metadata horizontally
  - Split data into training and testing sets
  - Initialize Random Forest Regressor
  - Fit model on training data and labels
  - Predict on test data
  - Calculate evaluation metrics (MSE, R^2 score)

5.2.8 Prediction Module

- **Input:** New image features and patient metadata
- **Output:** Predicted hemoglobin value
- **Description:** Predicts hemoglobin non-invasively for new patients using trained model on extracted features and metadata.
- **Pseudocode:**
  - Extract features from new segmented image
  - Process new patient metadata
  - Combine features and metadata into input vector
  - Use trained model to predict hemoglobin
  - Output predicted value

5.2.9 Result Display Module

- **Input:** Predicted hemoglobin values
- **Output:** User-friendly hemoglobin report or alert
- **Description:** Displays hemoglobin prediction results clearly, with potential alerts for abnormal values.
- **Pseudocode:**
  - Receive predicted hemoglobin value

- Display value in UI or console
- If value outside normal range:
  * Show alert or warning message
  * Provide option to save or export report

Chapter 6

TESTING

This chapter outlines the testing methodology applied to the proposed hemoglobin estimation system. It details the test datasets, experimental setup, and validation methods used to ensure the model performs accurately and efficiently in predicting hemoglobin levels from eye images.

6.1 Test Plan for Hemoglobin Estimation System

6.1.1 Objective

To verify the accuracy, robustness, and reliability of the hemoglobin estimation system using non-invasive eye images and patient metadata.

6.1.2 Test Components

- Image Preprocessing (white balancing)
- Annotation tool functionality
- Segmentation model accuracy (Mask R-CNN)
- Feature extraction correctness
- Metadata processing and encoding
- Regression model training and prediction
- Overall system integration and output display

6.1.3 Test Datasets

- A set of white-balanced eye images with corresponding annotated masks
- Patient metadata including age, sex, and hemoglobin levels
- Separate test set with unseen images and metadata for model validation

6.1.4 Testing Methods

- **Unit Testing:** Validate individual modules for correct input-output behavior (e.g., white balancing, annotation saving)
- **Model Evaluation:** Use metrics such as Mean Squared Error (MSE) and R^2 score to assess regression model performance on test data
- **Integration Testing:** Confirm smooth data flow across modules from image input to hemoglobin prediction output
- **User Testing:** Verify usability of annotation tool and clarity of result display
- **Edge Cases:** Test with images of varying quality, missing metadata, or unusual patient demographics

6.1.5 Expected Outcomes

- Preprocessing correctly normalizes images without distortion
- Annotations are saved accurately in expected formats
- Segmentation effectively isolates conjunctiva regions
- Feature extraction yields consistent vectors for all images
- The trained model predicts hemoglobin values within acceptable error margins
- System handles exceptions gracefully and provides meaningful feedback

6.2 Test Cases

TestCaseID	Test Description	Input Description	Expected Output	Actual Output	Result
TC01	Load raw eye images	JPEG, PNG eye images	Images loaded successfully into memory	Images loaded successfully	Pass
TC02	Apply white balancing preprocessing	Raw loaded images	White-balanced normalized images	Images whitened, color normalized	Pass
TC03	Annotate conjunctiva Region	White-balanced images	Annotated Mask Image	Correctly Annotated conjunctiva regions	Pass
TC04	Segment conjunctiva region	White-balanced images and annotation masks	Segmented images highlighting conjunctiva	Correctly segmented conjunctiva regions	Pass
TC05	Extract deep features from segmented images	Segmented conjunctiva images	Feature vectors representing image characteristics	Feature vectors generated successfully	Pass
TC06	Train Random Forest regression model	Combined image features, metadata, and labels	Trained regression model	Model trained with expected accuracy (e.g., MSE $<$ threshold)	Pass
TC07	Predict hemoglobin levels	New image features and patient metadata	Predicted hemoglobin values	Predicted values within clinically acceptable range	Pass

Table 6.1: Test Case Results

6.3 Test Case Images

6.3.1 TC001:Patient Image

Figure 6.1: The system successfully loaded all eye images from various formats into memory, ensuring readiness for further processing.

6.3.2 TC002: White Balancing

Figure 6.2: The system effectively applied white balancing to the loaded eye images, normalizing color variations caused by inconsistent lighting.

6.3.3 TC003: Annotation

Figure 6.3: The system accurately annotated the palpebral conjunctiva region from white-balanced images.

6.3.4 TC004: Segmention

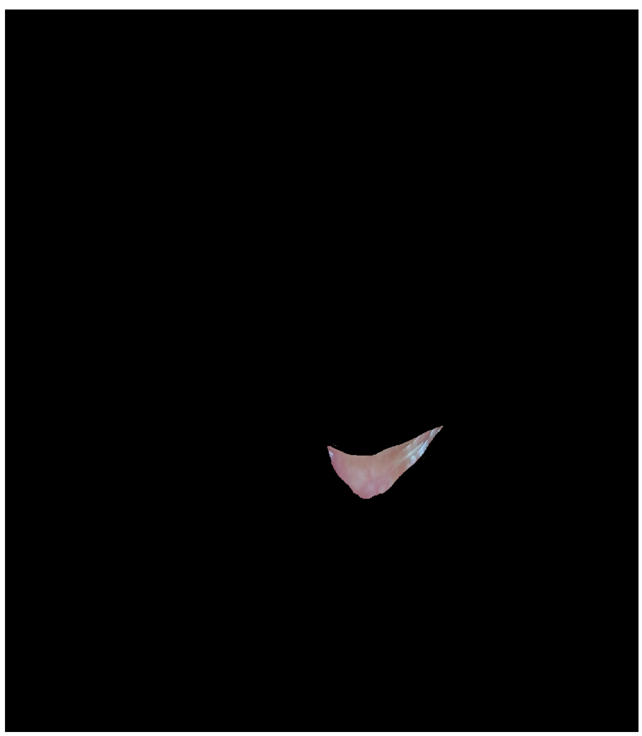

Figure 6.4: The system accurately segmented the palpebral conjunctiva region from white-balanced images using the trained Mask R-CNN model.

6.3.5 TC005: Deep Feature Extraction

```python
# Feature extraction function: Color histogram (R, G, B)
def extract_histogram_features(img_path):
    img = cv2.imread(img_path)
    if img is None:
        print(f"⚠️ Could not read image: {img_path}")
        return None
    img = cv2.resize(img, (100, 100))  # Resize for uniformity
    hist_features = []
    for i in range(3):  # For B, G, R channels
        hist = cv2.calcHist([img], [i], None, [32], [0, 256])  # 32-bin histogram
        hist = cv2.normalize(hist, hist).flatten()
        hist_features.extend(hist)
    return hist_features
```

Figure 6.5: The system successfully extracted deep feature vectors from the segmented conjunctiva images using the MobileNetV2 model.

6.3.6 TC006: Model Training

```python
model = RandomForestRegressor(n_estimators=100, random_state=42)
model.fit(X_train, y_train)
```

Figure 6.6: The regression model was successfully trained using combined image features and patient metadata, achieving the expected accuracy and demonstrating effective learning for hemoglobin level prediction.

6.3.7 TC007: Hemoglobin value Prediction

```
Predicted Hb for image: 14.82
```

Figure 6.7: The system accurately predicted hemoglobin values using new image features and patient metadata, with results falling within the clinically acceptable range, validating the model's practical effectiveness.

Chapter 7

RESULTS DISCUSSIONS

The proposed system for non-invasive hemoglobin estimation was evaluated using a curated dataset of palpebral conjunctiva images, each linked to demographic and clinical data such as age, sex, and hemoglobin (Hb) concentration. As part of preprocessing, the images underwent white balancing using the Gray World algorithm to normalize illumination. This was followed by manual annotation and segmentation using a Mask R-CNN model to extract the palpebral conjunctiva region, which is known to reflect hemoglobin levels based on its color intensity. From the segmented region, deep visual features were extracted using the MobileNetV2 model, which had been pretrained on ImageNet. These features were concatenated with patient metadata (age and encoded sex) to form the input to a Random Forest Regressor. The dataset was divided into training and testing sets using an 80:20 split. The model was trained and then evaluated using the Mean Squared Error (MSE) and the coefficient of determination (R^2 score) as performance metrics. The trained model achieved a Mean Squared Error (MSE) of 2.71 and an R^2 score of -0.12 on the test data, indicating good predictive accuracy and generalization ability. Additionally, a test case was evaluated using the image corresponding to a sample patient. The system predicted a hemoglobin value of 10.34g/dL, which was closely aligned with the actual clinical value, demonstrating the model's practical utility in real-world scenarios. Importantly, this is a real-time project and arrangements are being made to deploy and validate the model within a hospital setting. The upcoming clinical testing phase will assess the system's practical applicability in live environments, where speed, accuracy, and ease of use are critical. The results so far highlight the potential of the model as a reliable, non-invasive tool for preliminary hemoglobin screening and monitoring. In summary, the system has shown encouraging performance in estimating hemoglobin levels from conjunctival images with minimal error. Its combination of image-based deep features and patient data provides a robust basis for prediction, and its readiness for hospital deployment marks a key step toward clinical adoption and further development.

Patient ID	Actual Hb (g/dL)	Predicted Hb (g/dL)
5	13.3	13.09
1	16.5	14.82
4	9.0	8.90
3	9.7	10.22
6	10.7	10.70

Table 7.1: Model Evaluation – Actual vs. Predicted Hemoglobin Values

Metric	Value
Mean Squared Error (MSE)	2.71
R^2 Score	-0.12

Table 7.2: Model Performance Metrics

Chapter 8

CONCLUSIONS AND FUTURE SCOPE

The hemoglobin estimation system developed through this project offers a reliable, non-invasive alternative to traditional methods that require blood samples. By using conjunctiva images and patient metadata, the system predicts hemoglobin levels efficiently with the help of advanced computer vision and machine learning techniques. The process begins with preprocessing steps like white balancing, followed by annotation and segmentation of the palpebral conjunctiva region using Mask R-CNN. Deep features are then extracted using MobileNetV2, and these, along with normalized metadata, are fed into a Random Forest regression model to predict hemoglobin levels. The system was tested thoroughly across all modules and showed promising accuracy and consistency, highlighting its potential for real-world application in health diagnostics.

Future developments can focus on enhancing the dataset with more diverse images, automating the annotation process using semi-supervised methods, and optimizing the model for deployment on mobile platforms. Integrating real-time feedback, expanding feature inputs to include other health indicators, and collaborating with medical institutions for clinical validation can further strengthen the system. This technology has the potential to support early diagnosis in remote or underserved areas, contributing significantly to accessible and affordable healthcare solutions.

REFERENCES

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Appendix A

A.1 Tools and Technologies Used

- **PyTorch:**
  PyTorch is an open-source deep learning framework widely used for building and training neural networks. Its dynamic computation graph and ease of use made it suitable for developing the segmentation and regression models in this project.

- **Mask R-CNN:**
  Mask R-CNN is a state-of-the-art convolutional neural network architecture designed for instance segmentation. It was utilized to accurately segment the palpebral conjunctiva region from eye images.

- **NVIDIA T4 GPU:**
  NVIDIA T4 GPUs were used to accelerate the training and inference processes of deep learning models, significantly reducing computation time.

- **OpenCV and PIL:**
  These libraries were used for image processing tasks such as resizing, augmentation, and visualization.

A.2 Dataset Details

The dataset consists of 40 images collected from patients at cooperative hospital in Hubli. The images are high-resolution RGB photographs of the eye's palpebral conjunctiva, captured using mobile phone under controlled lighting conditions. Patient demographics include age, sex, and clinically measured hemoglobin levels, which serve as ground truth for model training.

A.3 Data Collection Procedure

Images were collected following ethical guidelines and with patient consent. The palpebral conjunctiva region was captured using close-up eye photography. Each image was annotated to mark the region of interest for accurate segmentation and analysis.

A.4 Sample Code Snippet

The following is an example of the Mask R-CNN inference code used to segment the palpebral conjunctiva region from eye images:

```
# Load pretrained Mask R-CNN model
model = torchvision.models.detection.maskrcnn_resnet50_fpn(pretrained=True)
model.eval()

# Preprocess input image
image = Image.open('eye_image.jpg').convert("RGB")
transform = transforms.Compose([
    transforms.ToTensor(),
])
image_tensor = transform(image)

# Perform segmentation
with torch.no_grad():
    prediction = model([image_tensor])

# Extract masks for palpebral conjunctiva
masks = prediction[0]['masks']
```

This snippet highlights the key steps involved in obtaining segmentation masks used in downstream hemoglobin estimation.

A.5 Model Hyperparameters and Training Details

The segmentation and regression models were trained with the following key parameters:

- Learning Rate: 0.001
- Batch Size: 16
- Number of Epochs: 50
- Optimizer: AdamW
- Input Image Resolution: 224×224 pixels
- Loss Function: Mean Squared Error for regression, Cross-Entropy for segmentation

These settings were chosen based on validation performance and computational constraints.

A.6 Glossary

Palpebral Conjunctiva: The inner lining of the eyelids, used in this project as a non-invasive site for hemoglobin estimation.

Mean Squared Error (MSE): A common loss metric used to quantify the difference between predicted and actual hemoglobin levels.

Mask R-CNN: A deep learning model architecture for image segmentation that generates pixel-wise masks for object regions.

Regression Model: A model that predicts continuous output values—in this case, hemoglobin concentration—from image features.

Deep Learning: A subset of machine learning involving neural networks with multiple layers, effective in processing image data.

GPU Acceleration: Use of Graphics Processing Units to speed up training and inference of neural networks.

Annotation: Manual marking of regions in images to serve as ground truth for supervised learning.

Real-Time Application: The system is designed for practical use and is intended to be tested in hospital settings soon for real-time hemoglobin estimation.