Stakeholders

Researchers and Scientists

- **Description:** Individuals conducting biological or medical research focusing on microglia and mitochondrial function.
- **Interests:** High accuracy in establishing the relationship between microglial morphology and mitochondrial membrane potential, detailed quantitative results for scientific analysis, and reliable tools for visualizing complex data.

Software Development Team

- **Description:** The team responsible for building the machine learning model and the application that integrates it.
- **Interests:** Clear and minimal specifications for training and running the model, scalable architecture for handling diverse datasets, and seamless collaboration with research teams.

Academic Institutions and Funding Organizations

- **Description:** Entities funding the research and development of the project.
- **Interests:** Demonstration of scientific validity, potential for broader applications in related fields, and significant contributions to the understanding of neuroinflammation and microglial function.

Functional Requirements

Machine Learning Model

Data Input:

- Support for high-resolution fluorescence microscopy images of microglial cells.
- Accept three image types per cell: non-stained, red-stained (mitochondrial membrane potential), and green-stained (mitochondrial location).
- Validate image quality and format during input.

Morphological Analysis:

- Identify and categorize the shape of microglial cells into predefined categories (e.g., round, partially activated, fully activated).
- Quantify morphological features such as cell size, shape, and branching complexity.

Mitochondrial Membrane Potential Analysis:

- Analyze the intensity of red fluorescence to quantify mitochondrial membrane potential.
- Output numerical values representing the potential for each cell.

Mitochondrial Spatial Distribution Analysis:

- Analyze green fluorescence to determine the spatial distribution of mitochondria within the cell.
- Categorize distribution as "near the nucleus" or "spread throughout the cell."

Correlation Analysis:

- Correlate microglial cell morphology with mitochondrial membrane potential and spatial distribution.
- Quantify the relationship and provide a detailed report of findings.

Visualization of Results:

- Generate graphical representations of analysis results, such as scatter plots, heatmaps, and bar charts.
- Present clear visual insights to facilitate interpretation by researchers.

Batch Processing:

- Support analysis of large datasets (e.g., thousands of images) in a single batch
- Ensure seamless and efficient processing of multiple samples.

Output Reports:

- Produce detailed reports summarizing the analysis, including numerical data, visualizations, and insights.
- Provide exportable formats (e.g., CSV, PDF) for sharing and documentation.

Application

User Authentication:

- Registration and login functionality for researchers.
- Secure password recovery process.

Image Upload and Analysis:

- Allow users to upload new datasets of fluorescence microscopy images (non-stained, red-stained, and green-stained).
- Provide real-time feedback on the dataset format and compatibility.
- Analyze the uploaded images using the trained model and display results.

Visualization of Results:

- Display results in graphs and tables for both new and past datasets.
- Enable users to filter and compare results across different datasets.
- Allow export of analysis results in common formats (e.g., CSV, PDF).

Data Management:

- Store results of analyzed datasets for future reference.
- Allow users to view, organize, and search historical data within the application.
- Enable users to delete historical datasets they no longer need.

Non-Functional Requirements

Machine Learning Model

Performance:

- Efficiently process large datasets, supporting analysis of thousands of images within reasonable timeframes (e.g., under 5 minutes per 500 images).
- Optimize for scalability, ensuring smooth operation on datasets of varying sizes without performance bottlenecks.

Portability:

- Enable deployment across diverse environments, including local workstations with GPUs and cloud-based platforms.
- Support compatibility with multiple operating systems (e.g., Linux, Windows, macOS).
- Ensure adaptability to future datasets with minimal configuration adjustments.

Interpretability:

- Provide clear and detailed outputs that align with the research goals, such as visualizations of correlations and numerical insights.
- Include an intuitive explanation of the model's analyses to assist researchers in understanding the results.

Reproducibility:

- Allow seamless re-training of the model using new datasets without requiring significant changes to the architecture or codebase.
- Maintain detailed logging of model configurations and training parameters to facilitate reproducible experiments.

Flexibility:

- Ensure support for integration with future tools and workflows, allowing researchers to extend functionality as needed.
- Provide modularity in design, enabling updates or enhancements to specific components without affecting the overall system.

Application

Usability:

- Intuitive and user-friendly interface, accessible from desktop and mobile devices.
- Provide clear guidance for uploading and analyzing datasets.

Security:

- Use HTTPS for secure communication.
- Encrypt user passwords and sensitive data.
- Ensure role-based access to sensitive functionalities.

Reliability:

- Ensure 99.9% system uptime.
- Provide automated error handling and notifications for failures.
- Automatic backup of user data every 24 hours.

Compatibility:

- Support modern browsers (Chrome, Firefox, Edge).
- Ensure compatibility with both Windows and macOS operating systems.

Use Cases

Use Case 1: User Registration and Login

- Goal: Enable users to create accounts and log into the system securely.
- Actors: Researchers.
- **Preconditions:** User accesses the registration or login page.
- Success End Condition: User gains access to the system.
- Main Flow:
 - 1. User provides registration details (email, password, affiliation).
 - 2. System validates the input and creates an account.
 - 3. User logs in with their credentials.

• Extensions:

- 1. If the email is already registered, the system prompts the user to log in or recover their password.
- 2. If the password is incorrect, the system limits login attempts.

Use Case 2: Image Upload

- Goal: Allow users to upload fluorescence microscopy images for analysis.
- Actors: Researchers.
- **Preconditions:** User is logged into the system.
- Success End Condition: Uploaded images are processed and ready for analysis.

Main Flow:

- 1. User navigates to the "Upload Images" section.
- 2. User selects one or more images for upload.
- 3. System validates the image formats and provides feedback if there are issues.
- 4. Images are stored and ready for analysis.

• Extensions:

- 1. If the image format is unsupported, the system notifies the user and suggests acceptable formats.
- 2. If the upload fails, the system retries or logs the error.

Use Case 3: Historical Data Access

- Goal: Allow users to view results of previously analyzed datasets.
- Actors: Researchers.
- **Preconditions:** User is logged into the system.
- **Success End Condition:** User views past results and optionally downloads them.

Main Flow:

- 1. User navigates to the "Historical Data" section.
- 2. System displays a list of past datasets with key metrics.
- 3. User selects a dataset to view detailed results.

• Extensions:

1. If no past data exists, the system notifies the user.

Use Case 4: Deleting Historical Data

- Goal: Allow users to delete historical datasets that are no longer needed.
- Actors: Researchers.
- **Preconditions:** User is logged into the system.
- **Success End Condition:** The selected dataset is permanently deleted from the system.

• Main Flow:

- 1. User navigates to the "Historical Data" section.
- 2. User selects a dataset they wish to delete.
- 3. System prompts for confirmation before deletion.
- 4. Dataset is deleted, and the user is notified of successful removal.

• Extensions:

1. If the dataset cannot be deleted (e.g., due to a system error), the user is notified with instructions to retry later.

Constraints

- The system will process only fluorescence microscopy images captured using predefined protocols (non-stained, red-stained, and green-stained).
- The initial training of the model requires sufficient GPU resources to handle the dataset efficiently.

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