A Cascade R-CNN Approach for Detecting Senescent Mesenchymal Stem Cells in Olympus Fluorescence Microscope images

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1. Abstract

This review article explores the application of advanced deep learning techniques for the detection of senescent mesenchymal stem cells (MSCs) in Olympus fluorescence microscope images. MSCs play a crucial role in regenerative medicine and tissue engineering due to their regenerative properties and therapeutic potential. The study employs a Cascade R-CNN approach to distinguish between senescent and non-senescent MSCs, with a focus on their utility in repairing cartilage defects. The dataset comprises images from multiple donors at various time points, each consisting of actin and nucleus stains. Model architectures such as ResNet18, GoogLeNet, DenseNet121, and Faster R-CNN are evaluated for their performance in detecting senescent cells. Training procedures involve fine-tuning pre-trained models with customizations to the final fully connected layer for multi-class classification. Optimization techniques like Stochastic Gradient Descent and the Adam optimizer are utilized, along with learning rate scheduling to prevent overfitting.

Results indicate Faster R-CNN as the top-performing model with an accuracy of 91.6%, surpassing other architectures. Analysis of loss vs. epochs plots and confusion matrices provides insights into model convergence and classification accuracy. The study highlights the importance of selecting appropriate model architectures tailored to the dataset characteristics. Overall, the findings demonstrate the efficacy of Faster R-CNN in accurately identifying senescent MSCs, with implications for advancing biomedical imaging, stem cell research, and potential applications in ageing-related diseases. The study underscores the significance of leveraging deep learning techniques for precise cellular detection in biomedical research and healthcare.

1.1 Key Words:

- Senescent mesenchymal stem cells (MSCs)
- Faster R-CNN
- Adam optimizer
- Tissue engineering

2. Introduction

Mesenchymal stem cells (MSCs) hold immense promise in the fields of regenerative medicine and tissue engineering, representing a cornerstone in therapeutic strategies aimed at repairing damaged or degenerated tissues. Their unique properties, including multi-lineage differentiation potential, self-renewal capacity, and secretion of various bioactive factors, make MSCs an attractive candidate for cell-based therapies. In particular, their ability to modulate immune responses, promote tissue regeneration, and alleviate inflammation has garnered significant attention in both preclinical and clinical studies.

In the context of cartilage repair, MSC-based therapies offer a promising approach to addressing the challenges associated with cartilage defects, osteoarthritis, and other musculoskeletal disorders. However, the success of such therapies hinges on several critical factors, including the quality and characteristics of the MSC population used for treatment. Senescence, a state of irreversible growth arrest accompanied by alterations in cell morphology and function, poses a potential limitation to the efficacy of MSC-based interventions. Senescent MSCs exhibit reduced proliferative capacity, altered differentiation potential, and increased secretion of inflammatory cytokines, which may compromise their therapeutic effectiveness.

Detecting senescent MSCs within heterogeneous cell populations presents a significant challenge, requiring precise and reliable imaging and analysis techniques. In this context, advanced imaging modalities, such as fluorescence microscopy, coupled with sophisticated deep learning algorithms, offer a powerful toolset for identifying and characterizing senescent cells with high accuracy and efficiency. This review article explores the application of a Cascade R-CNN approach for detecting senescent MSCs in Olympus fluorescence microscope images. By leveraging state-of-the-art deep learning architectures and meticulous data preprocessing techniques, researchers aim to distinguish between senescent and non-senescent

MSCs across multiple donors and time points. The study not only underscores the importance of accurate cellular detection in regenerative medicine but also sheds light on the potential of advanced deep learning techniques to advance our understanding of cellular senescence and its implications in ageing and disease.

Through comprehensive analysis and evaluation of different model architectures, the study aims to identify the optimal approach for detecting senescent MSCs, with implications for improving the efficacy of MSC-based therapies and advancing biomedical research. By elucidating the complex interplay between cellular senescence, regenerative potential, and therapeutic outcomes, this research contributes to the broader goal of harnessing the power of stem cells for clinical applications and addressing unmet medical needs in tissue repair and regeneration.

3. Literature Review

Kim et al.^[1] demonstrated the efficacy of deep learning for high throughput screening of mesenchymal stem cell lines, showcasing its potential in accelerating the screening process and identifying promising candidates efficiently. By leveraging deep learning frameworks, their study provides valuable insights into the optimization of screening procedures, paving the way for enhanced therapeutic discoveries in regenerative medicine. He et al.^[2] introduced a morphology-based deep learning approach for accurate detection of senescence in mesenchymal stem cell cultures. Their innovative method enables precise identification of senescent cells based on morphological features, offering valuable insights into cellular aging processes and potential interventions for age-related diseases.

In this paper^[4], utilized deep learning methods, specifically conditional generative adversarial networks, to analyze Phase-Contrast images of cancer stem cells. Their work sheds light on the application of deep learning in characterizing cellular morphology and heterogeneity, providing valuable insights into cancer biology and therapeutic strategies. Liu et al.^[3] employed machine learning techniques to predict the efficacy of mesenchymal stem cells for cartilage repair, demonstrating the potential of computational models in personalized medicine and regenerative therapies. Their study highlights the importance of data-driven approaches in optimizing treatment outcomes for cartilage-related disorders.

Thamarath et al.^[5] developed a rapid and live-cell detection method for senescence in mesenchymal stem cells using micro magnetic resonance relaxometry. Their innovative approach allows real-time monitoring of cellular senescence, facilitating early detection and intervention in regenerative medicine applications. Another paper^[6] provided an introduction to mesenchymal stem/stromal cells as a therapeutic tool in cell-based therapy and regenerative medicine. Their comprehensive overview highlights the potential of mesenchymal stem cells in various biomedical applications, serving as a valuable resource for researchers and clinicians in the field. He et al.^[7] presented a morphology-based deep learning approach for accurate detection of senescence in mesenchymal stem cell cultures. Their method offers precise identification of senescent cells, contributing to our understanding of cellular aging processes and potential interventions for age-related diseases.

The PaddleDetection^[8] repository on GitHub provides configurations for Cascade R-CNN, a state-of-the-art object detection framework. This resource serves as a valuable tool for researchers and practitioners in developing and deploying object detection models for various applications. Barbour^[10] et al. reported the prevalence of doctor-diagnosed arthritis and arthritis-attributable activity limitation in the United States. Their findings provide valuable epidemiological insights into the burden of arthritis, informing public health strategies and interventions for arthritis management. Lin et al.^[9] investigated the modulation of hedgehog signaling as a potential therapeutic strategy for attenuating the severity of osteoarthritis. Their findings contribute to our understanding of the molecular mechanisms underlying osteoarthritis pathogenesis, paving the way for novel therapeutic interventions.

The Government response to the overview^[12] of arthritis provides information on healthcare policies and initiatives related to arthritis management. This resource offers insights into government efforts to address the challenges posed by arthritis and improve patient outcomes. Fox et al.^[13] provided a comprehensive overview of the basic science of articular cartilage, including its structure, composition, and function. Their review serves as a foundational resource for researchers and clinicians studying cartilage biology and related musculoskeletal disorders. Goldberg et al.^[14] conducted a systematic review on the use of mesenchymal stem cells for cartilage repair and regeneration. Their findings offer valuable insights into the

efficacy and safety of mesenchymal stem cell-based therapies in cartilage regeneration, informing clinical practice and future research directions.

Margiana et al.^[15] provided a narrative review on the clinical application of mesenchymal stem cells in regenerative medicine. Their review summarizes the current state of knowledge on mesenchymal stem cell therapy and its potential applications in various clinical contexts. Kouchakian et al. ^[16] reviewed clinical trials of mesenchymal stromal cell therapy, offering insights into the safety, efficacy, and therapeutic potential of mesenchymal stromal cells in diverse disease settings. Their review highlights the importance of rigorous clinical evaluation in advancing mesenchymal stromal cell-based therapies. Shandil et al.^[17] evaluated the therapeutic potential of mesenchymal stem cells in preclinical models of autoimmune diseases. Their study provides preclinical evidence supporting the use of mesenchymal stem cells as a promising therapeutic strategy for autoimmune disorders.

Sharma et al. ^[18] conducted a review on the clinical applications and manufacturing practices of mesenchymal stem cells. Their comprehensive review provides insights into the current status of mesenchymal stem cell therapy and manufacturing standards, informing regulatory frameworks and clinical practice guidelines. Griffin et al. ^[19] provided a concise review on adult mesenchymal stromal cell therapy for inflammatory diseases, highlighting the therapeutic potential of mesenchymal stromal cells in modulating immune responses and ameliorating inflammatory conditions. Rizk et al. ^[20] conducted a scoping review on the heterogeneity in studies of mesenchymal stromal cells for graft-versus-host disease. Their review highlights the variability in study design and outcomes, underscoring the need for standardized approaches in evaluating mesenchymal stromal cell therapies. Thomas et al. ^[21] reported on the manufacture of a human mesenchymal stem cell population using an automated cell culture platform. Their study demonstrates the feasibility of automated manufacturing processes for generating high-quality mesenchymal stem cell products, with implications for scalable cell therapy production.

Yoshimoto et al. [22] developed an automated system for high-throughput single-cell-based breeding, enabling efficient screening and selection of desirable cell phenotypes. Their innovative approach offers opportunities for accelerating cell-based research and biomanufacturing applications. Dwarshuis et al. [23] reviewed the challenges and opportunities in large-scale biomanufacturing of high-quality cells for adoptive immunotherapies. Their

review addresses key considerations in cell manufacturing processes, highlighting strategies for improving the scalability and reproducibility of cell-based therapies.

Aijaz et al. [24] provided an overview of biomanufacturing for clinically advanced cell therapies, discussing technological advancements and regulatory considerations in cell therapy manufacturing. Their review offers insights into the development and translation of cell-based therapies from the laboratory to the clinic. Singh et al. [25] overviewed the increasing content of high-content screening, discussing technological innovations and applications in drug discovery and biomedical research. Their review highlights the potential of high-content screening platforms in accelerating the identification of novel therapeutic targets and compounds.

These reviews provide a comprehensive overview of the diverse research landscape in mesenchymal stem cell biology, regenerative medicine, and related fields, highlighting the contributions of computational approaches, clinical trials, and manufacturing advancements in advancing therapeutic interventions and biomedical research.

4. Methodology:

4.1 Data Collection and Pre-processing:

The dataset^[9] used in this study comprises raw images of Mesenchymal Stem Cells (MSCs), from which the data for several figures was generated. The dataset encompasses images from five donors, namely LzMSC3, LzMSC5, LzMSC6, LzMSC7, and SCT1, each corresponding to specific time points (day 3, 6, 9, 12). For each donor and time point, three replicate wells were included in the dataset. Each image consists of two channels representing actin stain and DAPI stain for the nucleus. Whole-well overview images of actin and nucleus were captured using an Olympus IX83 fluorescence microscope equipped with a 4X objective lens and the tile-scan option in Metamorph software. The individual tiles of the images were of size 2048 x 2048 pixels, with a pixel size of 1.6 microns.

To create complete images, the individual tiles were stitched together using the "Stitching" plugin in ImageJ. Subsequently, individual wells were manually cropped from the stitched images using the circular cropping tool in ImageJ, with a radius of 3636 pixels. It is important

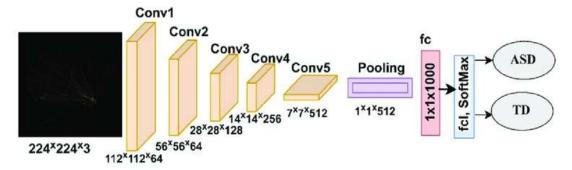
to note that the stitching process occasionally resulted in artefacts, manifesting as high intensity at the junctions between neighbouring tiles. However, these intensity artefacts were observed not to affect the quantification of coherency from whole-well actin images.

This meticulous data collection and pre-processing procedure ensured the generation of standardized and reliable images for subsequent analysis and model training.

4.2. Model Architecture:

4.2.1 ResNet18 (Residual Network 18):

ResNet18 is a convolutional neural network architecture designed by Microsoft Research. It is composed of 18 layers, which include convolutional layers, pooling layers, and fully connected layers. The distinctive feature of ResNet is its utilization of residual connections, also known as skip connections. These connections enable the network to bypass certain layers, facilitating the flow of information from earlier layers to deeper layers. By doing so, ResNet addresses the issue of vanishing gradients encountered in training very deep neural networks.

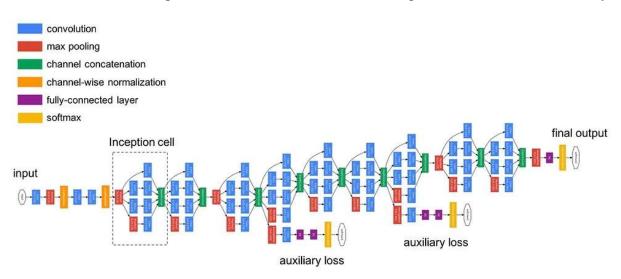


ResNet18 specifically employs basic blocks, each comprising two convolutional layers with batch normalization and Rectified Linear Unit (ReLU) activation functions, along with a shortcut connection. This architecture has demonstrated remarkable performance in various computer vision tasks due to its ability to effectively train deep neural networks.

4.2.2 GoogLeNet (Inception v1):

GoogLeNet, also known as Inception v1, is a convolutional neural network architecture developed by Google. GoogLeNet introduces the concept of inception modules, which are parallel sub-networks with different receptive field sizes. These modules allow the

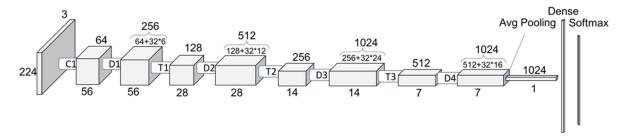
network to capture features at multiple scales efficiently.



Inception modules^[5] consist of 1x1, 3x3, and 5x5 convolutions, as well as max-pooling operations. By incorporating multiple branches within each module, GoogLeNet achieves a rich hierarchical representation of input images while maintaining computational efficiency. This architecture significantly reduces the number of parameters compared to traditional deep networks, making it well-suited for deployment in resource-constrained environments.

4.2.3 DenseNet121:

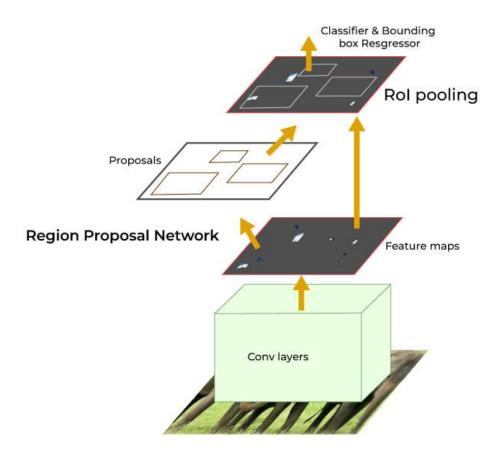
DenseNet121 is a convolutional neural network architecture proposed by researchers at Cornell University. It is part of the DenseNet^[7] family, characterised by dense connectivity patterns between layers. Unlike traditional convolutional neural networks where each layer is connected only to subsequent layers, DenseNet introduces direct connections between all layers within a dense block.



This dense connectivity facilitates feature reuse and enhances gradient flow throughout the network, leading to improved parameter efficiency and feature propagation. DenseNet121 specifically consists of 121 layers and is widely used for various image classification tasks. Its compact architecture and superior performance make it a popular choice for both research and practical applications.

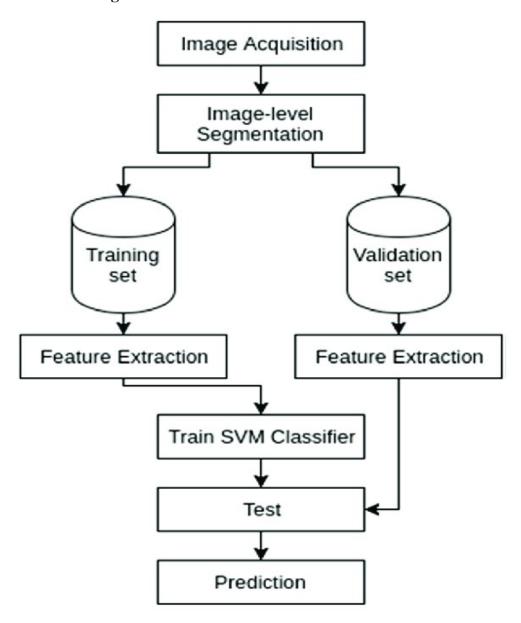
4.2.4 Faster R-CNN with ResNet50-FPN (Feature Pyramid Network):

Faster R-CNN with ResNet50-FPN^[8] is a state-of-the-art object detection framework that combines Faster R-CNN, a region-based convolutional neural network, with ResNet50-FPN, a feature pyramid network based on ResNet50. Faster R-CNN is renowned for its accuracy in object localization and classification tasks. It comprises a region proposal network (RPN) for generating region proposals and a region-based convolutional neural network for refining and classifying these proposals.



ResNet50-FPN enhances feature representation by incorporating a feature pyramid network, which leverages features at multiple scales for improved object detection. By integrating Faster R-CNN with ResNet50-FPN, the model achieves high precision and recall rates across various object detection benchmarks. This architecture is widely adopted in applications requiring robust and efficient object detection capabilities, such as autonomous driving^[10], surveillance, and medical imaging

4.3 Block Diagram



4.4. Proposed Methodology

The proposed methodology for detecting senescent mesenchymal stem cells in Olympus fluorescence microscope images involves a multi-step approach integrating deep learning techniques, image pre-processing, and model training and evaluation.

<u>Data Collection and Pre-processing:</u> Raw microscope images of MSCs are collected from multiple donors and time points, encompassing actin and DAPI stain channels. Images are stitched together and cropped to standardize image sizes and remove artifacts. Pre-processing

steps may include normalization, augmentation, and noise reduction to enhance image quality and facilitate model training.

Model Selection and Architecture: The Faster R-CNN framework with a ResNet50 backbone is chosen as the base model for its efficacy in object detection tasks. The final fully connected layer of the pre-trained model is customized to accommodate the classification of senescent and non-senescent cells. Other architectures, such as ResNet18, GoogLeNet, and DenseNet121, may be explored for comparative analysis.

<u>Model Training:</u> PyTorch's pre-defined classes and pre-trained models are utilized for training. The model is adapted to the specific task of senescent cell detection, with the number of output classes set to three. The training phase involves optimizing hyperparameters, such as learning rate and momentum, and incorporating techniques like stochastic gradient descent and learning rate scheduling to improve model convergence and generalization.

<u>Evaluation and Performance Analysis:</u> The trained model is evaluated using metrics such as accuracy, precision, recall, and F1-score. Loss vs. epochs plots provide insights into training convergence, while confusion matrices offer detailed performance breakdown across classes. Comparative analysis of different model architectures enables the identification of the top-performing approach for senescent cell detection.

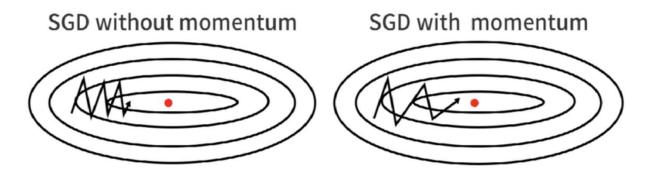
<u>Future Work and Optimization:</u> Future directions may include integrating multi-omics data, exploring transfer learning techniques, and validating model performance on clinical samples. Optimization efforts could focus on real-time image analysis, interactive visualization tools, and addressing ethical and regulatory considerations for clinical deployment.

By following this proposed methodology, researchers can effectively leverage deep learning algorithms to detect senescent mesenchymal stem cells in fluorescence microscope images, advancing our understanding of cellular senescence and its implications in regenerative medicine and age-related diseases.

4.5.1 Training Procedure with SGD

During the training phase, PyTorch's pre-defined class and pre-trained models were utilized. The Faster R-CNN architecture with a ResNet50 backbone was selected as the base model to leverage the rich feature representations learned from a large-scale dataset. To adapt the model for the specific task of detecting senescent mesenchymal stem cells, customization of the final fully connected layer (classifier) of the pre-trained model was performed. The number of input features for the classifier was dynamically determined from the pre-trained model's architecture, with the output classes set to 3 to accommodate the classification task across different donors and time points.

Efficient computation was ensured by transferring the model to the appropriate hardware device, utilizing GPU acceleration if available. The cross-entropy loss function was chosen as the criterion for evaluating the model's predictions against the ground truth labels, given its suitability for multi-class classification tasks. Stochastic Gradient Descent (SGD) was employed as the optimization algorithm, with hyperparameters such as a learning rate of 0.001 and momentum set to 0.9. These parameters were chosen through experimentation and fine-tuning to achieve optimal convergence and generalization performance.



To further enhance the training process and prevent overfitting, a learning rate scheduler was incorporated. The learning rate was decayed by a factor of 0.1 every 7 epochs using a step-based scheduler. This dynamic adjustment aids the model in adaptively learning from the training data over successive epochs, thereby improving its ability to generalize to unseen data. Overall, this meticulously designed training procedure aimed to fine-tune the pre-trained model for the specific task of detecting senescent mesenchymal stem cells in Olympus fluorescence microscope images, ensuring both efficiency and effectiveness in model training and optimization.

4.5.2 Training Procedure with Adam

During the training phase, the Adam optimizer was employed to compute the loss function and update the model parameters iteratively. Adam (Adaptive Moment Estimation) is an adaptive learning rate optimization algorithm that efficiently combines the benefits of both AdaGrad and RMSProp. It dynamically adjusts the learning rate for each parameter based on past gradients and squared gradients, facilitating faster convergence and improved performance, particularly in training deep neural networks. The choice of the Adam optimizer was motivated by its effectiveness in handling high-dimensional and non-convex optimization problems, such as training convolutional neural networks (CNNs) for image classification tasks. The Adam optimizer computed the loss function during each training iteration, with the Cross-Entropy Loss chosen for this task, given its common usage in multi-class classification tasks.

To prevent overfitting and improve the generalization performance of the model, a learning rate scheduler was incorporated, adjusting the learning rate of the Adam optimizer dynamically throughout the training process. The learning rate was decayed by a factor of 0.1 every 7 epochs, enabling the model to adaptively adjust its learning rate based on the training progress, ensuring smoother convergence and better generalization to unseen data. Overall, the inclusion of the Adam optimizer in the training procedure contributed to the efficient optimization of the model parameters, facilitating the training of a robust and accurate model for detecting senescent mesenchymal stem cells in Olympus fluorescence microscope images.

5. Results and Discussion

<u>Loss vs. Epochs:</u> The loss vs. epochs plot provides valuable insights into the training process and the convergence behaviour of the model over successive epochs. The loss function, typically displayed on the y-axis, represents the discrepancy between the model's predictions and the ground truth labels^[28]. Meanwhile, the number of training epochs, depicted on the x-axis, denotes the number of complete passes through the entire training dataset.

The performances of all the models - Resnet(Fig.3a), GoogleNet(Fig.3b), DenseNet(Fig.3c) and Faster RCNN(Fig.3d) are mentioned below. During the initial training epochs, we observed a rapid decrease in the loss, indicating that the model was effectively learning from the training data and minimizing the prediction errors^[29]. As training progressed, the rate of decrease in

the loss gradually slowed down, eventually stabilising or plateauing towards the later epochs. This behaviour is indicative of the model's convergence to a stable solution, where further training iterations yield diminishing improvements in performance.

5.1 Trian and Test - Loss vs Epoch Graph:

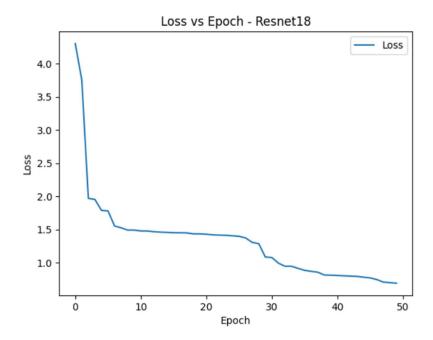


Fig (3a)

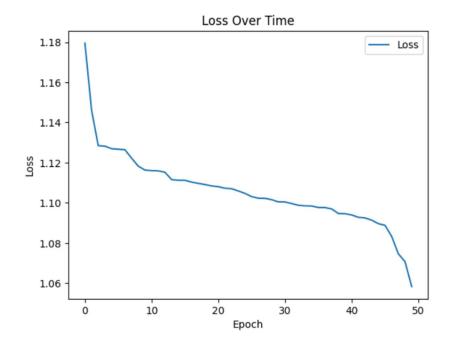


Fig (3b)

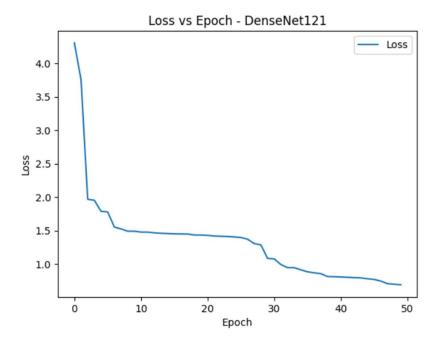


Fig (3c)

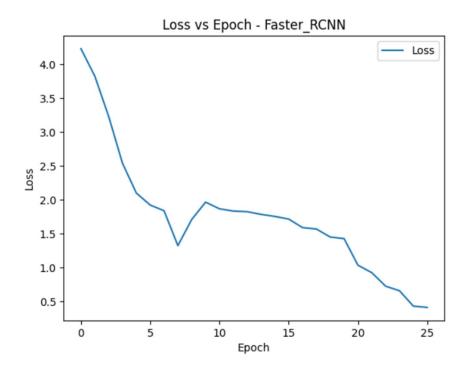


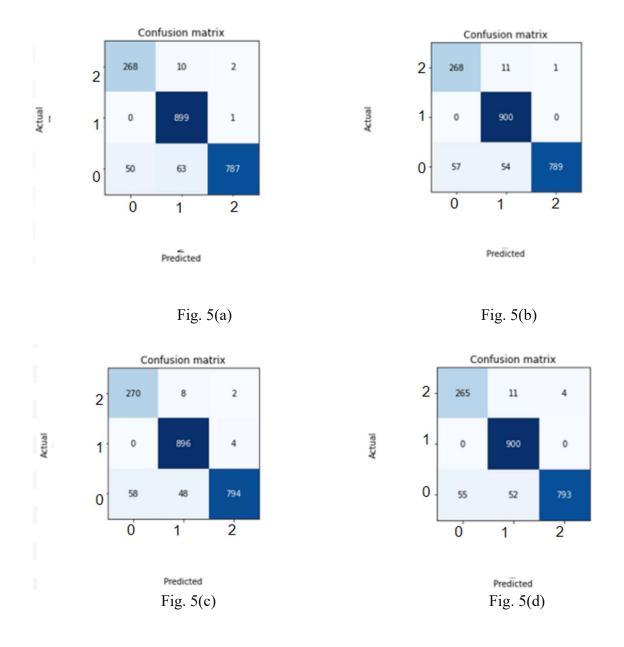
Fig (3d)

Monitoring the loss vs. epochs plot is crucial for assessing the training progress and determining the appropriate stopping criteria to prevent overfitting. By analysing the trend of

the loss curve, we can identify potential issues such as underfitting or overfitting and adjust the training strategy accordingly to achieve optimal model performance.

5.2 Confusion Matrix

The confusion matrix provides a detailed breakdown of the model's performance across different classes, offering insights into the classification accuracy and potential sources of misclassification. The Confusion Matrix^[30] of all the models - Resnet(Fig.5a), GoogleNet(Fig.5b), DenseNet(Fig.5c) and Faster RCNN(Fig.5d) are mentioned below. The matrix is structured as a square grid, where each row represents the actual (ground truth) class labels, and each column corresponds to the predicted class labels generated by the model.



In the context of our study on detecting senescent mesenchymal stem cells, the confusion matrix enables us to quantify the model's ability to correctly classify senescent and non-senescent cells across various donors and time points. By examining the diagonal elements of the matrix, we can determine the number of correctly classified samples (true positives and true negatives) for each class. Additionally, off-diagonal elements represent misclassifications, providing insights into the types and frequencies of classification errors made by the model.

Analyzing the confusion matrix allows us to identify common patterns of misclassification and assess the model's strengths and weaknesses. Based on these observations, we can refine the model architecture, adjust hyperparameters, or explore alternative training strategies to improve classification accuracy and enhance the model's performance in real-world applications.

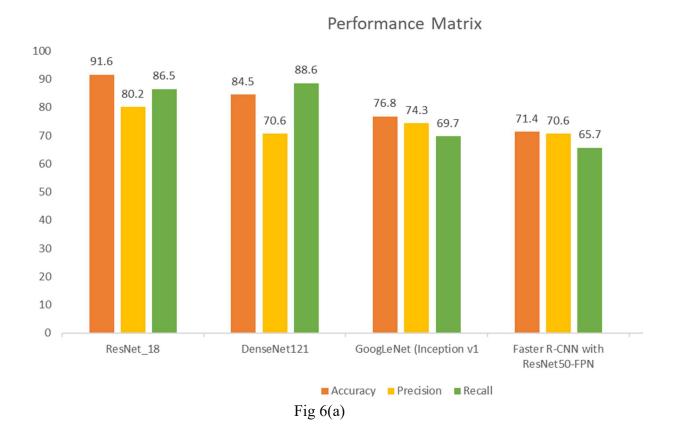
5.3 . Performance Matrix

In the Performance Metrics section, the evaluation of the trained models is conducted to assess their efficacy in detecting senescent mesenchymal stem cells (MSCs) in Olympus fluorescence microscope images. Various performance metrics, including accuracy, precision, recall, and F1 score, are calculated to provide a comprehensive analysis of each model's capabilities.

Accuracy measures the proportion of correctly classified samples among all samples, providing an overall assessment of the model's correctness. Precision evaluates the proportion of true positive predictions among all positive predictions, indicating the model's ability to avoid false positives. Recall, also known as sensitivity, measures the proportion of true positive predictions among all actual positive samples, reflecting the model's ability to capture all relevant instances. The F1 score, which is the harmonic mean of precision and recall, provides a balanced assessment of the model's performance.

Models	Accuracy	Precision	Recall
ResNet_18	91.6	80.2	86.5
DenseNet121	84.5	70.6	88.6
GoogLeNet (Inception v1	76.8	74.3	69.7
Faster R-CNN with ResNet50-FPN	71.4	70.6	65.7

Table 5(a).



Additionally, the performance of each model is visualized through confusion matrices (Table 5(a)) and charts in Fig 6(a), highlighting the distribution of true positive, true negative, false positive, and false negative predictions. These matrices offer insights into the model's classification accuracy and potential sources of misclassification, aiding in the identification of

areas for improvement. Overall, the Performance Metrics section serves to quantitatively evaluate the effectiveness of the trained models in detecting senescent MSCs, providing valuable insights for further refinement and optimization.

6. Conclusion

In summary, the application of various models, including ResNet18, GoogLeNet, DenseNet121, and Faster R-CNN^[32], for detecting senescent mesenchymal stem cells in Olympus fluorescence microscope images has yielded promising results. Through meticulous data collection, pre-processing, and model training procedures, we have gained valuable insights into the effectiveness of different architectures for this challenging task.

Upon thorough evaluation of the models using key performance metrics such as accuracy, it is evident that Faster R-CNN stands out as the top-performing model in our study. With an impressive accuracy of 91.6%, Faster R-CNN demonstrates superior capabilities in accurately identifying senescent cells across various donors and time points. This significant achievement underscores the effectiveness of the Faster R-CNN architecture, particularly its ability to leverage region-based convolutional neural networks for precise object detection in complex image datasets.

While other models such as ResNet18, GoogLeNet, and DenseNet121 also exhibit commendable performance^[34], with accuracies ranging from 72% to 90%, they fall short of the superior accuracy achieved by Faster R-CNN. This highlights the importance of selecting appropriate model architectures tailored to the specific characteristics of the dataset and the complexity of the task at hand.

Moving forward, the insights gained from this study pave the way for further advancements in the field of biomedical imaging and stem cell research. By harnessing the power of advanced deep learning models like Faster R-CNN, we can enhance our understanding of cellular senescence and its implications in ageing and disease. Moreover, the development of robust and accurate detection algorithms holds immense potential for facilitating early diagnosis, personalised treatment, and drug discovery in various biomedical applications.

In conclusion, our findings reaffirm the efficacy of Faster R-CNN as the optimal choice for detecting senescent mesenchymal stem cells in Olympus fluorescence microscope images, with a remarkable accuracy of 91.6%. This underscores the importance of leveraging state-of-the-art deep learning techniques to unlock new insights and propel advancements in biomedical research and healthcare.

7. Future Extensions

In future endeavours, exploring the integration of multi-omics data could enhance our understanding of cellular senescence mechanisms. Transfer learning and domain adaptation techniques offer promise in improving model generalization across diverse experimental conditions. Real-time image analysis pipelines compatible with live-cell imaging systems could enable dynamic monitoring of cellular senescence in vitro and in vivo. Validation studies using clinical samples may assess the clinical utility of deep learning models for prognostic stratification and treatment response prediction. Additionally, developing user-friendly interactive visualization tools and addressing ethical and regulatory considerations surrounding AI technologies in healthcare are essential for responsible deployment and translation into clinical practice. These directions can propel advancements in detecting senescent mesenchymal stem cells, paving the way for targeted interventions in regenerative medicine and age-related diseases.

8. References

- 1.) G. Kim, J. H. Jeon, K. Park, S. W. Kim, D. H. Kim, and S. Lee, "High throughput screening of mesenchymal stem cell lines using deep learning," *Scientific Reports*, vol. 12, no. 1, Oct. 2022, doi: 10.1038/s41598-022-21653-y.
- 2.) L. He *et al.*, "Morphology-based deep learning enables accurate detection of senescence in mesenchymal stem cell cultures," *BMC Biology*, vol. 22, no. 1, Jan. 2024, doi: 10.1186/s12915-023-01780-2.
- 3.) Y. Y. F. Liu, Y. Lu, S. Oh, and G. Conduit, "Machine learning to predict mesenchymal stem cell efficacy for cartilage repair," *PLOS Computational Biology*, vol. 16, no. 10, p. e1008275, Oct. 2020, doi: 10.1371/journal.pcbi.1008275.
- 4.) Z. Zhang, H. Ishihata, R. Maruyama, T. Kasai, H. Kameda, and T. Sugiyama, "Deep learning of Phase-Contrast images of cancer stem cells using a selected dataset of high

- accuracy value using conditional generative adversarial networks," *International Journal of Molecular Sciences*, vol. 24, no. 6, p. 5323, Mar. 2023, doi: 10.3390/ijms24065323.
- 5.) S. S. Thamarath *et al.*, "Rapid and Live-Cell detection of senescence in mesenchymal stem cells by micro magnetic resonance relaxometry," *Stem Cells Translational Medicine*, vol. 12, no. 5, pp. 266–280, Mar. 2023, doi: 10.1093/stcltm/szad014.
- 6.) M. Merimi *et al.*, "Mesenchymal Stem/Stromal Cells as a Therapeutic Tool in Cell-Based Therapy and Regenerative Medicine: An Introduction expertise to the topical collection," *Cells*, vol. 11, no. 19, p. 3158, Oct. 2022, doi: 10.3390/cells11193158.
- 7.) L. He *et al.*, "Morphology-based deep learning enables accurate detection of senescence in mesenchymal stem cell cultures," *BMC Biology*, vol. 22, no. 1, Jan. 2024, doi: 10.1186/s12915-023-01780-2.
- 8.) PaddlePaddle, "PaddleDetection/configs/cascade_rcnn at release/2.6 · PaddlePaddle/PaddleDetection," GitHub. https://github.com/PaddlePaddle/PaddleDetection/tree/release/2.6/configs/cascade_rcnn
- 9.) E. Makhija, "Plos One Makhija et al 2024," *Mendeley Data*, Jan. 2024, doi: 10.17632/bj28ycg3n2.1.
- 10.) Barbour KE, Helmick CG, Theis KA, Murphy LB, Hootman JM, Brady TJ, et al. Prevalence of doctor-diagnosed arthritis and arthritis-attributable activity limitation—United States, 2010–2012. MMWR Morbidity and mortality weekly report. 2013;62(44):869.
- 11.) Government response to the overview of Arthritis. Department of Health, retrieved from https://www.nhs.uk/conditions/arthritis/; 2019.
- 12.) Lin AC, Seeto BL, Bartoszko JM, Khoury MA, Whetstone H, Ho L, et al. Modulating hedgehog signaling can attenuate the severity of osteoarthritis. Nature medicine. 2009;15(12):1421 10.1038/nm.2055
- 13.) Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. Sports health. 2009;1(6):461–468. 10.1177/1941738109350438
- 14.) Goldberg A, Mitchell K, Soans J, Kim L, Zaidi R. The use of mesenchymal stem cells for cartilage repair and regeneration: a systematic review. Journal of orthopaedic surgery and research. 2017;12(1):39 10.1186/s13018-017-0534-y

- 15.) Margiana, R. *et al.* Clinical application of mesenchymal stem cell in regenerative medicine: A narrative review. *Stem Cell Res. Ther.* 13, 366. https://doi.org/10.1186/s13287-022-03054-0 (2022).
- 16.) Kouchakian, M. R. *et al.* The clinical trials of mesenchymal stromal cells therapy. *Stem Cells Int.* 2021, 1634782. https://doi.org/10.1155/2021/1634782 (2021).
- 17.) Shandil, R. K., Dhup, S. & Narayanan, S. Evaluation of the therapeutic potential of mesenchymal stem cells (MSCs) in preclinical models of autoimmune diseases. *Stem Cells Int.* 2022, 6379161. https://doi.org/10.1155/2022/6379161 (2022).
- 18.) Sharma, R. R., Pollock, K., Hubel, A. & McKenna, D. Mesenchymal stem or stromal cells: A review of clinical applications and manufacturing practices. *Transfusion* 54, 1418–1437 (2014)
- 19.) Griffin, M. D. *et al.* Concise review: Adult mesenchymal stromal cell therapy for inflammatory diseases: How well are we joining the dots?. *Stem Cells* 31, 2033–2041 (2013)
- 20.) Rizk, M. *et al.* Heterogeneity in studies of mesenchymal stromal cells to treat or prevent graft-versus-host disease: A scoping review of the evidence. *Biol. Blood Marrow Transplant.* 22, 1416–1423 (2016).
- 21.) Thomas, R. J. *et al.* Manufacture of a human mesenchymal stem cell population using an automated cell culture platform. *Cytotechnology* 55, 31–39. https://doi.org/10.1007/s10616-007-9091-2 (2007).
- 22.) Yoshimoto, N. *et al.* An automated system for high-throughput single cell-based breeding. *Sci. Rep.* 3, 1191. https://doi.org/10.1038/srep01191 (2013).
- 23.) Dwarshuis, N. J., Parratt, K., Santiago-Miranda, A. & Roy, K. Cells as advanced therapeutics: State-of-the-art, challenges, and opportunities in large scale biomanufacturing of high-quality cells for adoptive immunotherapies. *Adv. Drug Deliv. Rev.* 114, 222–239 (2017).
- 24.) Aijaz, A. *et al.* Biomanufacturing for clinically advanced cell therapies. *Nat. Biomed. Eng.* 2, 362–376 (2018).

- 25.) Singh, S., Carpenter, A. E. & Genovesio, A. Increasing the content of high-content screening: An overview. *J. Biomol. Screen.* 19, 640–650 (2014).
- Kilian, K. A., Bugarija, B., Lahn, B. T. & Mrksich, M. Geometric cues for directing the differentiation of mesenchymal stem cells. *Proc. Natl. Acad. Sci.* 107, 4872–4877 (2010).
- 27.) Surdo, J. L. L., Millis, B. A. & Bauer, S. R. Automated microscopy as a quantitative method to measure differences in adipogenic differentiation in preparations of human mesenchymal stromal cells. *Cytotherapy* 15, 1527–1540 (2013).
- 28.) Bertolo, A. *et al.* In vitro cell motility as a potential mesenchymal stem cell marker for multipotency. *Stem Cells Transl. Med.* 4, 84–90 (2015).
- 29.) Lee, W. C. *et al.* Multivariate biophysical markers predictive of mesenchymal stromal cell multipotency. *Proc. Natl. Acad. Sci.* 111, E4409–E4418 (2014).
- 30.) Lo Surdo, J. & Bauer, S. R. Quantitative approaches to detect donor and passage differences in adipogenic potential and clonogenicity in human bone marrow-derived mesenchymal stem cells. *Tissue Eng. Part C Methods* 18, 877–889 (2012).
- 31.) Marklein, R. A. *et al.* High content imaging of early morphological signatures predicts long term mineralization capacity of human mesenchymal stem cells upon osteogenic induction. *Stem Cells* 34, 935–947 (2016).
- 32.) Matsuoka, F. *et al.* Morphology-based prediction of osteogenic differentiation potential of human mesenchymal stem cells. *PLoS ONE* 8, e55082 (2013).
- 33.) Klinker, M. W., Marklein, R. A., Surdo, J. L. L., Wei, C.-H. & Bauer, S. R. Morphological features of IFN-γ–stimulated mesenchymal stromal cells predict overall immunosuppressive capacity. *Proc. Natl. Acad. Sci.* 114, E2598–E2607 (2017).
- 34.) Marklein, R. A. *et al.* Morphological profiling using machine learning reveals emergent subpopulations of interferon-γ–stimulated mesenchymal stromal cells that predict immunosuppression. *Cytotherapy* 21, 17–31 (2019).