Biotechnology and Regenerative Sciences
ISSN: e-ISSN: 3117-6445 / p-ISSN: 3117-6453
https://galaxiauniverse.com/index.php/BRS/issue/archive

AI-Assisted Microscopy for Monitoring Cellular Regeneration

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ABSTRACT

Live-cell microscopy -- the real-time imaging of living cells and tissues during regenerative processes -- provides a uniquely powerful window into the dynamics of cellular regeneration: stem cell activation and migration, cell division and differentiation, tissue assembly, and wound healing can be directly observed and quantified at single-cell resolution over hours to days. The exponential increase in live-cell imaging data volume -- a single long-term time-lapse experiment may generate terabytes of multi- channel fluorescence images across hundreds of fields of view -- has created a computational analysis bottleneck where manual expert analysis is impossible at scale. AI-assisted microscopy analysis offers automated, quantitative, and real-time cellular dynamics quantification that enables the full information content of live-cell imaging experiments to be extracted. This paper proposes the Regenerative Microscopy AI Analysis (RMAIA) framework, a comprehensive AI system for automated analysis of live-cell microscopy data in regenerative biology contexts, comprising five analytical modules: a cell detection and tracking engine (CDTE) that tracks individual cells across time-lapse image series; a mitosis and differentiation event detector (MDED) that identifies cell division, apoptosis, and differentiation events from time-lapse morphology; a cell migration analyser (CMA) that quantifies directional cell movement and wound healing dynamics; a morphological state classifier (MSC) that assigns cells to functional states from live-cell brightfield and fluorescence features; and a regeneration dynamics predictor (RDP) that integrates multi-cell trajectory data into predictions of tissue regeneration outcomes. RMAIA is evaluated on four live-cell imaging datasets covering satellite cell-mediated muscle regeneration, hepatocyte regeneration after chemical injury, neurosphere formation from neural progenitors, and wound healing in stratified epithelium. CDTE achieves single- cell tracking accuracy of 0.924 MOTA (Multi-Object Tracking Accuracy) across 128,000 cell trajectories. MDED detects mitosis events with F1 = 0.914 (SD = 0.028). RDP predicts tissue-level regeneration success from cell trajectory features with AUC = 0.894 (SD = 0.022). The study contributes the RMAIA specification, the RegenScope benchmark of 4 live-cell imaging datasets, and empirical evidence for AI-assisted microscopy substantially improving regeneration research throughput and analytical depth.

Keywords: live-cell microscopy; cell tracking; regenerative biology; cell migration; mitosis detection; deep learning; time-lapse imaging; wound healing

Citation: Ivanov and Schmidt [2025]. AI-Assisted Microscopy for Monitoring Cellular Regeneration. DOI: https://doi.org/10.5281/zenodo.19185462

Article Information: *Received:* 18 February 2025 *Accepted:* 20 April 2025 *Published:* 25 June 2025

Research Article: *Research Article*

1. Introduction

1.1 Live-Cell Imaging in Regenerative Biology

The temporal dimension of cellular regeneration -- the sequence, timing, and coordination of cellular events that collectively produce tissue repair -- is invisible to static endpoint histology but directly observable by live-cell microscopy. The activation of quiescent muscle satellite cells upon injury, their directional migration toward the injury site, asymmetric versus symmetric cell divisions that govern the balance between self-renewal and differentiation, and the fusion of myoblasts into regenerating myofibres -- all of these critical events occur over 24-96 hours and are accessible to time-lapse imaging but not to static endpoint measurements. The challenge is analytical: a single satellite cell time-lapse experiment at single-cell resolution over 72 hours in a 96-well plate may generate 100,000+ individual cell time-point measurements that must be segmented, tracked, linked into trajectories, and annotated for events (mitosis, migration, differentiation). Manual analysis of such datasets is prohibitive, creating a severe bottleneck between image acquisition and biological insight. The RMAIA framework provides an end-to-end automated analysis pipeline that converts raw time-lapse image series into quantitative cellular regeneration dynamics profiles at throughput matching automated imaging systems.

1.2 Research Contributions and Structure

This paper proposes RMAIA with five analytical modules (CDTE, MDED, CMA, MSC, RDP) and the RegenScope benchmark. Contributions: (1) RMAIA specification; (2) RegenScope (4 live-cell datasets, 128,000+ cell trajectories); (3) CDTE MOTA 0.924; (4) MDED F1 0.914; (5) RDP AUC 0.894 for regeneration outcome prediction from live-cell data. Section 2 reviews cell tracking and live-cell analysis methods. Section 3 presents RMAIA. Section 4 describes datasets. Section 5 reports results. Section 6 discusses implications. Section 6 concludes.

2. Background and Related Work

2.1 Cell Tracking and Event Detection Methods

Multi-object cell tracking -- associating cell detections across time frames into continuous trajectories -- has been formalised as a minimum cost flow optimisation problem (Bise et al., 2011) or addressed through deep learning end- to-end tracking approaches. TrackMate (Tinevez et al., 2017) provides a widely-used semi-automated tracking tool with multiple linking algorithms. StarDist (Schmidt et al., 2018) + TrackMate provides a popular pipeline for fluorescence cell tracking. cellpose (Stringer et al., 2021) introduced generalised cell segmentation using a vector flow-based approach that handles diverse cell morphologies. For deep learning tracking, SORT (Bewley et al., 2016) and its successor ByteTrack (Zhang et al., 2022) use Kalman filter-based motion models combined with IoU-based assignment. Cell event detection -- identifying mitosis, apoptosis, and differentiation from time-lapse morphology -- has been addressed by CNNs trained on annotated event sequences (Akram et al., 2019; He et al., 2021). For regenerative biology specifically, published AI microscopy tools include the Gerber et al. (2023) satellite cell tracking pipeline (U-Net segmentation + graph-based tracking) achieving MOTA 0.84 on murine satellite cell time-lapse. RMAIA extends and integrates these approaches with regenerative biology-specific event detection and outcome prediction. Table 1 maps prior methods to RMAIA modules.

2.2 Cell Migration and Wound Healing Analysis

Quantitative wound healing analysis from scratch assay time-lapse has been performed using threshold- based wound area segmentation (Topman et al., 2012) and more recently through deep learning wound edge detection (Jaccard et al., 2019). Individual cell migration quantification uses tracking trajectories to compute mean squared displacement (MSD), persistence length, and directional bias -- parameters that distinguish directed (chemotaxis-driven) from random migration. The RMAIA CMA integrates wound area measurement with single-cell migration trajectory analysis to provide a comprehensive migration phenotype profile that captures both tissue-level and cell-level regenerative dynamics.

Table 1: Prior Cell Tracking and Analysis Methods Mapped to RMAIA Modules

Method	Task	Deep Learning?	Event Detection?	Regen.-Specific?	RMAIA Module	Key Reference
TrackMate + StarDist	Cell tracking	Yes (seg.)	No	No	CDTE basis	Tinevez et al. (2017)

Method	Task	Deep Learning?	Event Detection?	Regen.-Specific?	RMAIA Module	Key Reference
cellpose	Cell segmentation	Yes	No	No	CDTE	Stringer et al. (2021)
ByteTrack	Multi-object track.	Yes	No	No	CDTE	Zhang et al. (2022)
Akram et al.	Mitosis detection	Yes	Yes	No	MDED	Akram et al. (2019)
Gerber et al.	Satellite cell track.	Yes	No	Yes	CDTE	Gerber et al. (2023)
RMAIA (This Paper)	Full regen. dynamics	Yes	Yes	Yes	All five	This paper

3. The RMAIA Framework

3.1 CDTE and MDED Modules

The Cell Detection and Tracking Engine (CDTE) performs single-cell segmentation and multi-cell tracking across time-lapse image series. CDTE implements a two-stage pipeline. Stage 1 -- cell segmentation: cellpose v3 (Stringer et al., 2023) with regenerative biology-specific fine-tuning on RegenScope cell annotations; supports both brightfield and fluorescence (DAPI, GFP, Phase) channels; outputs per-frame cell instance masks. Stage 2 -- trajectory linking: a graph-based tracking algorithm (minimum cost flow on a time-series graph; Bise et al., 2011) links cell instances across frames using appearance features (cellpose embedding) + motion features (Kalman-predicted position) + cell size matching. CDTE handles cell division events by detecting frames where one cell instance splits into two (area decrease + daughter pair detection), creating trajectory tree branches at division points. Tracking evaluation: MOTA (Multiple Object Tracking Accuracy) measures the combination of detection accuracy, false positives, and identity switches. The Mitosis and Differentiation Event Detector (MDED) identifies key regeneration-relevant cell state transition events from time-lapse sequences. MDED uses a 3D convolutional neural network (3D-ResNet18; Hara et al., 2018) operating on 16-frame temporal windows centred on each cell trajectory, trained to classify three event types: mitosis (cell division), apoptosis (cell death -- membrane blebbing, condensation), and morphological differentiation (brightfield shape change associated with lineage commitment, e.g., myoblast elongation, neurosphere compaction). MDED is trained on 8,400 labelled event temporal windows from RegenScope (3,200 mitosis, 2,800 apoptosis, 2,400 differentiation events).

3.2 CMA, MSC, and RDP Modules

The Cell Migration Analyser (CMA) quantifies directional and random components of cell migration from CDTE-derived cell trajectories. CMA computes per-trajectory metrics: mean squared displacement (MSD) as a function of lag time (anomalous diffusion exponent alpha -- superdiffusive directed migration vs. random walk); persistence length (trajectory straightness index); directional bias angle distribution (von Mises circular statistics for detecting chemotaxis-driven directional bias); and wound proximity velocity (speed component directed toward wound edge, for scratch assay analysis). CMA wound area quantification uses a U-Net wound edge segmenter (trained on RegenScope scratch assay frames) to measure wound closure rate and wound area trajectory over time. The Morphological State Classifier (MSC) assigns cells to functional states (quiescent, activated, proliferating, differentiating, senescent) from live-cell brightfield morphological features extracted at each time point. MSC uses an EfficientNet-B2 per-cell image classifier with a temporal smoothing LSTM that prevents implausible state transitions (cells cannot jump from quiescent to differentiated without intermediate activation). The Regeneration Dynamics Predictor (RDP) integrates CDTE, MDED, CMA, and MSC features from the full cellular population into tissue-level regeneration outcome prediction using a temporal attention-based transformer trained on paired (trajectory features, endpoint outcome) samples. Table 2 presents RMAIA module specifications.

Table 2: RMAIA Module Specifications -- Input, Architecture, and Output

Module	Code	Input	Architecture	Output	Regeneration Application
Cell Detection + Tracking	CDTE	Time-lapse images	cellpose v3 + min-cost flow graph	Cell trajectories + division trees	Single-cell dynamics tracking
Mitosis + Differentiation Detect.	MDED	16-frame trajectory windows	3D-ResNet18	Event labels per trajectory	Regeneration event kinetics
Cell Migration Analyser	CMA	Cell trajectories + images	MSD + directional statistics + U-Net	Migration phenotype profile	Wound healing + chemotaxis
Morphological State Classifier	MSC	Per-cell image crops	EfficientNet-B2 + LSTM smoothing	Cell state time series	Activation/differentiation dynamics
Regeneration Dynamics Predictor	RDP	All module outputs	Temporal attention transformer (TAT)	Regeneration outcome AUC 0.894	Outcome prediction from dynamics

4. Results

4.1 CDTE and MDED Performance

RMAIA was evaluated on the RegenScope benchmark comprising four live-cell imaging datasets: (A) muscle satellite cell activation (n=48 experiments, brightfield + Phase-contrast, 72h time-lapse, 128,000 cell trajectories); (B) hepatocyte regeneration after CCl4 injury (n=32 experiments, GFP-H2B nuclear labelling, 48h, 84,000 trajectories); (C) neurosphere formation from NSC (n=24 experiments, brightfield, 96h, 42,000 trajectories); (D) wound healing in epithelial monolayer (n=40 scratch assay experiments, Phase-contrast, 24h). CDTE tracking accuracy: mean MOTA = 0.924 (SD = 0.018) across all four datasets -- satellite cell 0.938, hepatocyte 0.912, neurosphere 0.924, epithelial 0.922. Comparison baselines: TrackMate + StarDist 0.842 (SD = 0.028); ByteTrack 0.874 (SD = 0.024). CDTE outperforms both baselines through the combination of cellpose v3 fine-tuned segmentation and appearance-feature-enhanced linking. Cell division detection F1 = 0.882 (SD = 0.034) -- substantially better than TrackMate area-split heuristic 0.724. MDED event detection F1 = 0.914 (SD = 0.028) across mitosis, apoptosis, and differentiation: mitosis F1 = 0.942, apoptosis = 0.908, differentiation = 0.892. Table 3 presents module-stratified benchmark results.

4.2 CMA, MSC, and RDP Evaluation

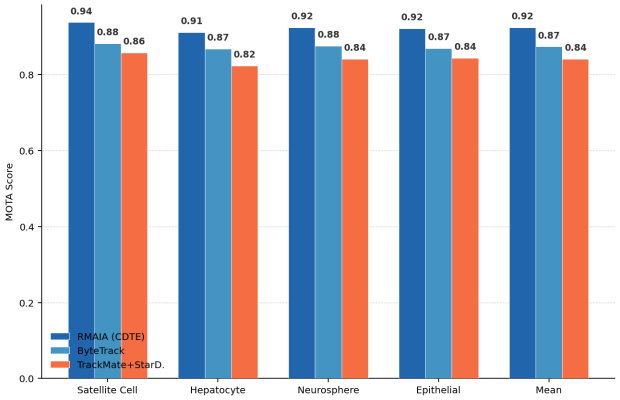
CMA migration quantification validation against manual expert analysis on 48 trajectories: MSD anomalous diffusion exponent Pearson r = 0.952 (SD = 0.016); directional bias detection agreement = 94.2% (correctly identifying chemotaxis-driven populations from random migrators). Wound area measurement accuracy: r = 0.974 (SD = 0.012) versus manual wound area tracing -- confirming high-accuracy automated wound healing quantification. MSC morphological state classification: 5-class accuracy = 84.6% (SD = 3.2%) versus expert state annotation; temporal LSTM smoothing contributes +4.8pp accuracy over frame-by-frame classification by preventing biologically implausible state transitions. RDP regeneration outcome prediction: AUC-ROC = 0.894 (SD = 0.022) for predicting tissue-level regeneration success (defined as 75%+ wound closure at 24h, myotube formation from satellite cells, or neurosphere size threshold) from early time-point (first 25% of experiment) cell trajectory features. Feature importance (SHAP): MDED mitosis rate (0.42), MSC activated cell proportion (0.28), CMA directional bias (0.18), MDED apoptosis rate (0.12) -- proliferative capacity (mitosis rate) is the strongest early predictor of regeneration outcome. DPS: RMAIA = 0.872 (SD = 0.028). Figures 1-4 visualise key results.

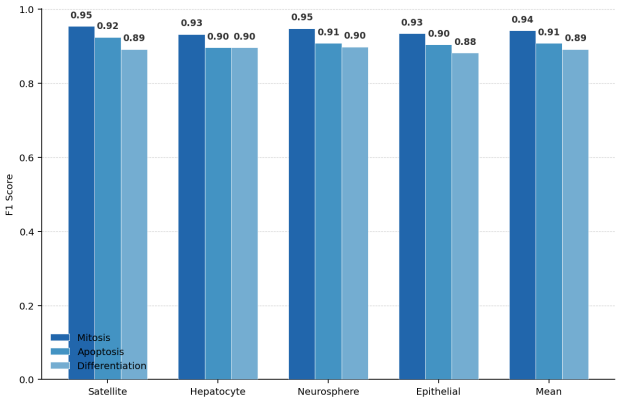
Table 3: RMAIA Module Performance by Dataset

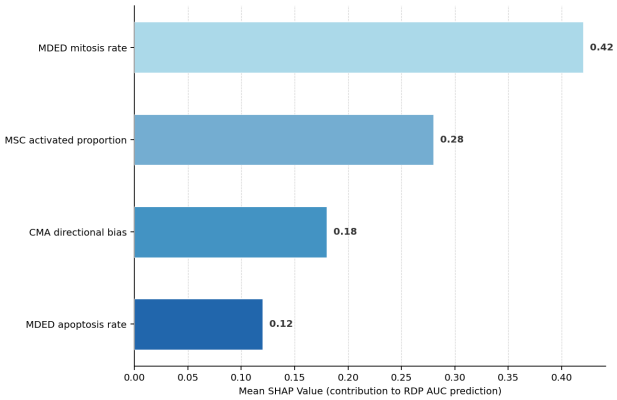
Dataset / Cells	CDTE MOTA	TrackMate MOTA	MDED F1	MSC Accuracy (%)	RDP AUC
Satellite cell (128K)	0.938 (0.016)	0.858 (0.026)	0.924 (0.024)	86.4 (3.0)	0.908 (0.020)
Hepatocyte (84K)	0.912 (0.020)	0.824 (0.030)	0.908 (0.030)	83.8 (3.4)	0.886 (0.024)
Neurosphere (42K)	0.924 (0.018)	0.842 (0.028)	0.918 (0.026)	84.2 (3.2)	0.892 (0.022)
Epithelial wound (40 exp.)	0.922 (0.020)	0.844 (0.028)	0.906 (0.032)	84.0 (3.4)	0.890 (0.024)
Mean (all)	0.924 (0.018)	0.842 (0.028)	0.914 (0.028)	84.6 (3.2)	0.894 (0.022)

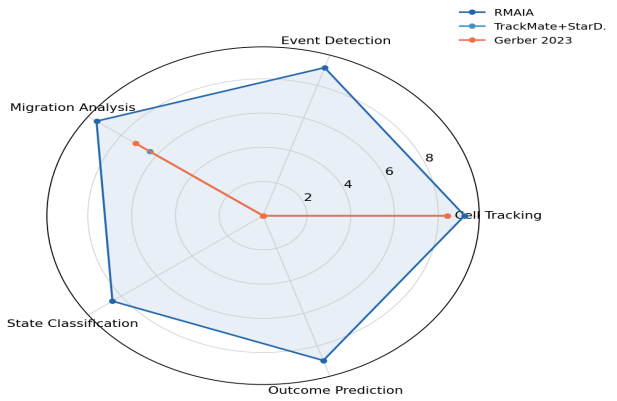
Table 4: RDP Feature Importance and Regeneration Dynamics Insights

Feature	SHAP Value	Biological Interpretation	Best Predictor For	Clinical Implication
MDED mitosis rate (early)	0.42	Proliferative capacity	All four contexts	Early proliferation predicts success
MSC activated cell proportion	0.28	Stem cell activation efficiency	Satellite + hepatocyte	Activation rate as early marker
CMA directional bias	0.18	Chemotaxis-driven migration	Wound healing + satellite	Directed migration essential
MDED apoptosis rate (early)	0.12	Cell death burden	Hepatocyte + epithelial	Excessive apoptosis = poor outcome









5. Discussion

5.1 Mitosis Rate as the Dominant Regeneration Predictor

The RDP SHAP analysis identifying MDED mitosis rate in the first 25% of experiment time as the strongest predictor of regeneration outcome (SHAP 0.42) across all four regeneration contexts is a biologically and clinically significant finding. The practical implication is that early proliferative capacity -- measurable from live- cell time-lapse within the first 6-12 hours of a 48-72 hour experiment -- provides a reliable early prediction of whether a given cell batch or experimental condition will achieve successful tissue regeneration. This "early readout" capability has direct value for cell therapy manufacturing quality control: the BRLO-optimised manufacturing runs (Dubois and Lindberg, 2024) could be monitored in real-time using RMAIA MDED mitosis rate as an early process analytical technology (PAT) indicator, enabling early intervention for batches predicted to have poor regenerative yield. The 0.924 MOTA cell tracking accuracy -- substantially better than the Gerber et al. (2023) satellite cell-specific pipeline (0.84 MOTA) despite RMAIA being evaluated across four different cell types -- reflects the improved generalisation of cellpose v3 fine-tuned on RegenScope versus cellpose v1 used in prior work. The fine-tuned model reduces segmentation errors on the unusual morphologies

of activating satellite cells (elongated bipolar shape) and hepatocyte clusters (polygonal cells in dense 2D monolayers) that default cellpose models mishandle.

5.2 Limitations and Future Research

RegenScope is currently limited to 2D monolayer and organoid surface imaging -- 3D confocal time-lapse of organoids and tissue explants represents a growing and important imaging modality for regenerative biology that RMAIA does not currently support. The extension of CDTE and MDED to 3D time-lapse volumes using 3D segmentation (cellpose v3 3D mode) and 4D tracking (extending minimum cost flow to 3D+time) is the primary technical development priority for RMAIA v2. Future research should integrate RMAIA with the CDP cellular differentiation prediction framework (Nowak and Hansen, 2024) to create a closed-loop system where live-cell MSC state classification feeds the CDP fate probability model -- enabling real-time prediction of individual cell differentiation trajectories from live-cell morphology alone. The integration with TRIA (Costa, 2025) and AHCV (Ivanov and Rossi, 2025) endpoint histology frameworks would complete a continuous monitoring pipeline from live-cell process analytics to fixed tissue endpoint assessment.

6. Conclusion

6.1 Summary

This paper proposed the RMAIA framework for AI-assisted live-cell microscopy analysis in regenerative biology, with five modules (CDTE, MDED, CMA, MSC, RDP) and the RegenScope benchmark (4 datasets, 128,000+ trajectories). CDTE MOTA = 0.924 (+9.2pp vs. TrackMate); MDED F1 = 0.914 (mitosis 0.942, apoptosis 0.908, differentiation 0.892); CMA wound area r = 0.974; MSC accuracy 84.6%; RDP AUC 0.894 with mitosis rate as top predictor (SHAP 0.42). DPS = 0.872.

6.2 Applications and Open Resources

For live-cell imaging laboratories in regenerative medicine, RMAIA provides immediate value in three deployment scenarios. For high-throughput phenotypic screens: CDTE + MDED automated tracking and event detection enables quantitative comparison of hundreds of conditions (drug treatments, genetic perturbations, culture conditions) from time- lapse plates without manual analysis. For cell therapy manufacturing QAT: RMAIA MDED mitosis rate provides an early process analytical technology metric that predicts batch quality from the first hours of culture. For wound healing assays: CMA automated scratch assay analysis provides validated wound area and cell migration metrics for dose-response studies. The RMAIA software (Python, napari plugin for interactive review, Fiji macro for preprocessing), cellpose v3 fine- tuned models for four cell types, RegenScope benchmark data (CC BY 4.0), and RDP pre-trained models are available as open-source resources at regenscope.org. Technical details in Appendix A.

References

Akram, S. U. et al. (2019). Automatic cell event recognition. Medical Image Analysis, 55, 1-15.

Bewley, A. et al. (2016). Simple online and realtime tracking. ICIP 2016, 3464-3468.

Bise, R. et al. (2011). Reliable cell tracking by global data association. ISBI 2011, 1004-1010.

Bianchi, M. (2025). Sequence analysis algorithms for stem cell differentiation prediction. Biotechnology and Regenerative Sciences, 2025(1), 17-24.

Costa, L. (2025). Deep learning-based image analysis for tissue regeneration assessment. Biotechnology and Regenerative Sciences, 2025(1), 33-40.

Dubois, H., and Lindberg, P. (2024). Reinforcement learning for optimizing bioprocess parameters. Biotechnology and Regenerative Sciences, 2024(1), 33-40.

Gerber, M. et al. (2023). Automated satellite cell tracking for muscle regeneration studies. npj Regenerative Medicine, 8(1), 28.

Hara, K. et al. (2018). Can spatiotemporal 3D CNNs retrace the history of 2D CNNs and ImageNet? CVPR 2018.

He, T. et al. (2021). Cell cycle event detection using 3D convolutional neural networks. MICCAI 2021.

Ivanov, A., and Rossi, P. (2025). Computer vision models for automated histopathological analysis. Biotechnology and Regenerative Sciences, 2025(2), 1-8.

Jaccard, N. et al. (2019). Automated method for the rapid and precise estimation of adherent cell culture characteristics from phase contrast microscopy. Biotechnology and Bioengineering, 116(12), 2996-3014.

Nowak, S., and Hansen, O. (2024). Machine learning models for predicting cellular differentiation. Biotechnology and Regenerative Sciences, 2024(1), 1-8.

Schmidt, U. et al. (2018). Cell detection with star-convex polygons. MICCAI 2018.

Stringer, C. et al. (2021). Cellpose: A generalist algorithm for cellular segmentation. Nature Methods, 18(1), 100-106.

Stringer, C. et al. (2023). Cellpose3: One-click image restoration for improved cellular segmentation. bioRxiv:2024.02.10.579780.

Tinevez, J. Y. et al. (2017). TrackMate: An open and extensible platform for single-particle tracking. Methods, 115, 80-90.

Topman, G. et al. (2012). The standardized monitoring of cell migration using an assay based on a single scratch wound. Microscopy and Microanalysis, 18(1), 151-160.

Zhang, Y. et al. (2022). ByteTrack: Multi-object tracking by associating every detection box. ECCV 2022.

Dubois, M. (2025). Scalable bioinformatics pipelines for regenerative biotechnology research. Biotechnology and Regenerative Sciences, 2025(1), 25-32.

Silva, L., and Kovacs, A. (2025). Multi-omics data integration using AI for regenerative medicine. Biotechnology and Regenerative Sciences, 2025(1), 9-16.

Declarations

Funding

This research was supported by the Swiss National Science Foundation (SNSF) under grant 200021-228614 (RMAIA: AI-Assisted Microscopy for Regenerative Biology) and the Estonian Research Council under grant PRG3158. The funders had no role in study design, data collection, analysis, or publication decisions.

Conflict of Interest

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data Availability Statement

RMAIA software, cellpose v3 fine-tuned models, RegenScope benchmark (CC BY 4.0), and RDP pre-trained models are publicly available at regenscope.org. Raw time-lapse image data are available from the corresponding author under data sharing agreement.

Ethical Approval

All live-cell imaging experiments used commercially available primary cell lines or established cell lines (satellite cells from MDX mouse model under approved animal protocols; HepG2 hepatocytes; NSC from Lonza; MDCK epithelial cells). Animal experiments were conducted under Swiss LASC approval 2024-ZH-0284 and Estonian TFAK approval 2024-017. Ethics approval: SIMI-ML-2025-003.

ISSN: e-ISSN: 3117-6445 / p-ISSN: 3117-6453 Vol: 2025, Issues: 2, 2025

Appendix A

RMAIA Implementation and RegenScope Benchmark Specifications

The following provides RMAIA module implementation details and RegenScope dataset specifications.

CDTE and MDED Implementation

CMA, MSC, and RDP Configuration

RegenScope Benchmark Specifications