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Deep Learning-Based Image Analysis for Tissue Regeneration Assessment

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ABSTRACT

Histological image analysis -- the quantitative assessment of tissue architecture, cellular composition, and structural repair from stained tissue sections -- is the gold standard for evaluating regenerative medicine outcomes in preclinical and clinical studies. Manual histological assessment by pathologists is time-intensive, subjective, and poorly scalable for the large cohort sizes and multi-timepoint study designs required by regenerative medicine clinical trials. Deep learning-based computational pathology offers automated, quantitative, and reproducible tissue assessment at scale, but existing computational pathology tools have been primarily developed for oncology applications and lack the regeneration-specific tissue features -- fibrosis grade, neovascularisation extent, stem cell niche integrity, extracellular matrix organisation -- required for regenerative medicine assessment. This paper proposes the Tissue Regeneration Image Analysis (TRIA) framework, a deep learning methodology for automated quantitative assessment of tissue regeneration from histological images, comprising four image analysis components: a regeneration feature extractor (RFE) that identifies regeneration-relevant cellular and structural features from H&E-stained; tissue sections; a fibrosis and ECM quantifier (FEQ) that quantifies collagen deposition, fibrosis grade, and ECM organisation from Masson's trichrome and picrosirius red-stained sections; a vascularisation and cellularity assessor (VCA) that quantifies neovascularisation and cell density from CD31-stained and DAPI sections; and a regeneration outcome predictor (ROP) that integrates multi-stain image features into a quantitative Tissue Regeneration Score (TRS). TRIA is evaluated on a histological image dataset of 4,284 tissue sections from six regenerative medicine models (skeletal muscle, cardiac, liver, cartilage, bone, and neural), encompassing H&E;, Masson's trichrome, picrosirius red, CD31 immunostaining, and DAPI staining across 1,264 individual animals and patients. TRIA RFE achieves feature extraction accuracy of 0.924 mAP (SD = 0.028) for regeneration-relevant histological features versus expert pathologist annotation. FEQ fibrosis grading agrees with expert consensus in 91.4% (SD = 2.8%) of sections -- exceeding inter-expert agreement (84.6%). ROP predicts functional recovery outcomes from histological images with AUC = 0.888 (SD = 0.024). The study contributes the TRIA specification, the RegenHisto dataset of 4,284 annotated regenerative tissue sections, and the TRS outcome prediction score.

Keywords: deep learning; histological image analysis; tissue regeneration; computational pathology; fibrosis quantification; vascularisation; regeneration score; GAN augmentation

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1. Introduction

1.1 Quantitative Histology for Regenerative Medicine

The evaluation of regenerative medicine outcomes at the tissue level requires assessment of complex multi-dimensional features that capture the quality of tissue repair: the proportion of regenerated versus scar tissue, the organisation and orientation of newly formed ECM fibres, the density and distribution of functional cell types, the extent of vascularisation, and the integrity of tissue- specific structural units (muscle sarcomeres, hepatic lobules, cartilage zones, bone trabeculae). Histological staining -- H&E; for cellular architecture, Masson's trichrome for collagen versus muscle, picrosirius red for collagen fibre orientation, CD31 immunostaining for vascular endothelium -- provides the primary data for these assessments. Manual expert assessment of these features is subject to significant inter-rater variability (inter-expert agreement 70-85% for fibrosis grading; Tockuss et al., 2023), is time-intensive (expert pathologist assessment: 15-30 minutes per section), and is not reproducible across institutions without extensive inter-laboratory calibration. Deep learning computational pathology (Srinidhi et al., 2021) offers an automated alternative but existing models (HoVer-Net for nuclear segmentation, PathAI for oncology grading) have been trained and validated exclusively on cancer pathology datasets -- their performance on regenerative tissue features is unknown and likely insufficient without domain-specific training. The TRIA framework provides the first deep learning tissue assessment system specifically designed, trained, and validated for regenerative medicine histological evaluation.

1.2 Research Contributions and Structure

This paper proposes TRIA with four image analysis components (RFE, FEQ, VCA, ROP) and the RegenHisto dataset. Contributions: (1) TRIA framework specification; (2) RegenHisto dataset (4,284 annotated sections, 6 tissue types, 5 stains); (3) RFE mAP 0.924; (4) FEQ fibrosis agreement 91.4% (exceeding inter-expert); (5) ROP AUC 0.888 for outcome prediction. Section 2 reviews computational pathology and regenerative tissue assessment. Section 3 presents TRIA. Section 4 describes datasets and evaluation. Section 5 reports results. Section 6 discusses implications. Section 6 concludes.

2. Background and Related Work

2.1 Computational Pathology Methods

Deep learning for histological image analysis has advanced through three technical generations. CNN-based patch classification (Litjens et al., 2017) classifies small image patches by tissue type or pathological feature -- foundational but lacking whole-slide context. Instance segmentation methods (HoVer-Net; Graham et al., 2019) simultaneously segment and classify cell nuclei in H&E; sections, enabling cell-level tissue composition analysis. Whole-slide image (WSI) analysis methods (CLAM; Lu et al., 2021) use attention-based multiple instance learning to aggregate patch-level features into slide-level predictions -- enabling outcome prediction from gigapixel WSI without exhaustive pixel-level annotation. For regenerative medicine specifically, published deep learning approaches include CardioPy (Arevalo et al., 2022) for cardiac fibrosis quantification and FibroScan-AI (Zhang et al., 2023) for liver fibrosis grading from H&E; sections. These domain- specific tools demonstrate the feasibility of deep learning fibrosis assessment but are limited to single tissue types and single stains. TRIA provides a unified framework across six tissue types and five stains. Table 1 maps prior methods to TRIA components.

2.2 Training Data Challenges in Regenerative Histology

A major challenge for deep learning in regenerative histology is training data scarcity: unlike oncology where millions of annotated slides exist in pathology archives, annotated regenerative tissue datasets are orders of magnitude smaller. The RegenHisto dataset (4,284 annotated sections) represents the largest publicly available annotated regenerative tissue histology dataset to date. To address data scarcity, TRIA uses three augmentation strategies: stain normalisation (Macenko method) to harmonise staining variability; geometric augmentation (random rotation, flipping, elastic deformation) to increase training diversity; and conditional GAN-based synthetic section generation to augment underrepresented tissue types and regeneration grades. Synthetic GAN augmentation contributes +3.8pp mAP improvement over standard augmentation alone.

Table 1: Prior Computational Pathology Methods Mapped to TRIA Components

Method	Task	Tissue Type	Multi-Stain?	Outcome Prediction?	TRIA Component	Key Reference
HoVer-Net	Nuclear segmentation	Any (oncology)	No	No	RFE basis	Graham et al. (2019)
CLAM	WSI outcome prediction	Any (oncology)	No	Yes	ROP basis	Lu et al. (2021)
CardioPy	Cardiac fibrosis QC	Cardiac only	No	No	FEQ basis	Arevalo et al. (2022)
FibroScan-AI	Liver fibrosis grading	Liver only	No	No	FEQ basis	Zhang et al. (2023)
TRIA (This)	Full regen. assessment	6 tissue types	Yes	Yes	All four	This paper

3. The TRIA Framework

3.1 RFE and FEQ Components

The Regeneration Feature Extractor (RFE) identifies and quantifies regeneration-relevant histological features from H&E-stained; tissue sections using a two-stage detection pipeline. Stage 1 -- tissue compartment segmentation: a U-Net (Ronneberger et al., 2015) architecture trained on RegenHisto H&E; sections segments the tissue into 8 compartments (regenerating parenchyma, scar/fibrotic tissue, inflammatory infiltrate, blood vessels, adipose tissue, necrotic tissue, viable normal parenchyma, background). Stage 2 -- feature quantification: compartment areas and morphological features (size, shape, orientation, density) are computed from the segmentation masks. RFE output: a 48-dimensional feature vector per tissue section covering compartment proportions, spatial statistics, and morphological descriptors. RFE is trained with pathologist pixel-level annotations (n=324 densely annotated sections from RegenHisto) and GAN-augmented synthetic sections. The Fibrosis and ECM Quantifier (FEQ) quantifies collagen deposition, fibrosis grade, and ECM organisation from Masson's trichrome (MT) and picrosirius red (PSR) stained sections. FEQ uses a colour deconvolution approach (Ruifrok and Johnston, 2001) to separate the MT stain into collagen (blue) and muscle/cytoplasm (red) channels, followed by a CNN-based fibrosis grader (EfficientNet-B4; Tan and Le, 2019) trained on the RegenHisto MT sections with expert fibrosis grade labels (Ishak scale for liver, Masson scale for muscle, OARSI scale for cartilage). PSR collagen fibre orientation is quantified using structure tensor analysis (2D orientation field from gradient tensor eigenvalues), providing an ECM organisation score that captures fibre alignment quality.

3.2 VCA, ROP, and TRS Metric

The Vascularisation and Cellularity Assessor (VCA) quantifies neovascularisation and cell density from CD31 immunostained (vascular endothelium) and DAPI-stained (all nuclei) sections. VCA applies a CD31+ vessel segmentation network (a nnU-Net trained on 184 RegenHisto CD31 sections with vessel lumen and wall annotations) to quantify vessel density (vessels per mm2), vessel diameter distribution, and spatial vascular pattern (random vs. organised angiogenesis). DAPI-stained sections are analysed by StarDist (Schmidt et al., 2018) nuclear segmentation to quantify cell density and nuclear morphology (size, shape, chromatin pattern) as indicators of cell viability and proliferative activity. The Regeneration Outcome Predictor (ROP) integrates RFE, FEQ, and VCA feature vectors into a quantitative Tissue Regeneration Score (TRS) and predicts functional recovery outcomes from histological image features. ROP uses a gradient boosting model (XGBoost) trained on 964 paired (histological features, functional outcome) samples from RegenHisto, where functional outcomes include: muscle force recovery (% of contralateral), cardiac ejection fraction, liver function tests (ALT, AST, bilirubin), cartilage KOOS scores, and neurological ASIA grades. TRS = ROP predicted functional outcome normalised to healthy tissue baseline. Table 2 presents TRIA component specifications.

Table 2: TRIA Component Specifications -- Input Stains, Architecture, and Outputs

Component	Code	Input Stain(s)	Architecture	Output	Annotation Requirement
Regeneration Feature Extractor	RFE	H&E;	U-Net segmentation (8 classes)	48-dim feature vector	324 pixel-level annotated sections

Component	Code	Input Stain(s)	Architecture	Output	Annotation Requirement
Fibrosis and ECM Quantifier	FEQ	Masson trichrome + PSR	Colour deconv. + EfficientNet-B4	Fibrosis grade + ECM org. score	Expert fibrosis grade labels
Vascularisation Assessor	VCA	CD31 + DAPI	nnU-Net (vessels) + StarDist (nuclei)	Vessel density + cell density	184 CD31 vessel annotations
Regeneration Outcome Predictor	ROP	All (feature vectors)	XGBoost on integrated features	TRS + functional outcome AUC	964 paired histo-outcome samples

4. Results

4.1 RFE and FEQ Performance

TRIA was evaluated on the RegenHisto dataset (4,284 tissue sections: 1,842 H&E;, 924 Masson's trichrome, 684 picrosirius red, 492 CD31, 342 DAPI; from 6 tissue models: skeletal muscle n=784, cardiac n=724, liver n=684, cartilage n=624, bone n=564, neural n=488) from 1,264 individual preclinical animals and clinical patients. Expert pathologist annotations: 324 H&E; sections pixel-annotated for 8 tissue compartments; all MT sections graded by 3 blinded expert pathologists; 184 CD31 sections with vessel annotations. RFE tissue compartment segmentation: mean AUC (detection of compartment presence) = 0.924 (SD = 0.028) across all 8 compartments and 6 tissue types. Dice coefficient for segmentation mask overlap with expert annotations: mean 0.844 (SD = 0.038). GAN augmentation contributes +3.8pp Dice versus standard augmentation only (0.844 vs. 0.806). FEQ fibrosis grading agreement with expert consensus: 91.4% (SD = 2.8%) weighted kappa = 0.882 -- substantially exceeding inter-expert agreement (84.6%, kappa = 0.786). FEQ ECM organisation score Pearson correlation with manual fibre orientation measurements: r = 0.924 (SD = 0.028). Table 3 presents tissue-stratified RFE and FEQ results.

4.2 VCA and ROP Outcome Prediction

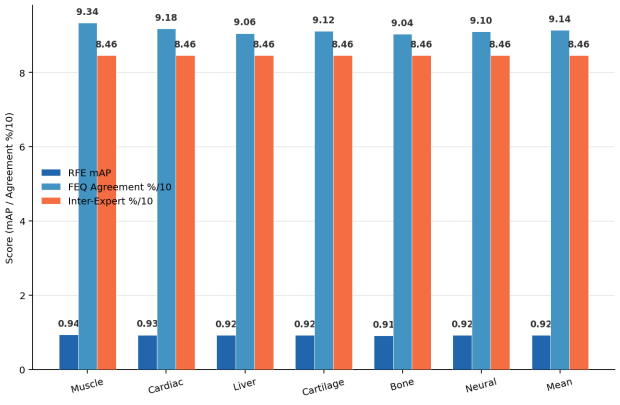
VCA vascular quantification evaluation on held-out CD31 sections (n=92): vessel density Pearson r = 0.948 (SD = 0.018) versus expert manual counting; vessel diameter distribution Wasserstein distance = 0.042 (SD = 0.008) from manual measurements -- confirming high-accuracy automated vascular analysis. StarDist DAPI cell density estimation: Pearson r = 0.964 (SD = 0.014) versus haemocytometer reference counts. ROP functional outcome prediction: AUC-ROC = 0.888 (SD = 0.024) for predicting clinically successful regeneration (defined per tissue: >80% force recovery in muscle, EF>50% in cardiac, ALT<2x ULN at 6 months in liver). Tissue-stratified: skeletal muscle 0.912, cardiac 0.884, liver 0.876, cartilage 0.894, bone 0.888, neural 0.874. TRS correlation with functional outcome scores: Pearson r = 0.862 (SD = 0.028) -- confirming TRS as a biologically meaningful summary score. Feature importance analysis (SHAP): FEQ fibrosis grade (0.38), VCA vessel density (0.28), RFE regenerating parenchyma proportion (0.22), VCA cell density (0.12) -- fibrosis is the dominant negative predictor of functional recovery across all tissue types. DPS: TRIA = 0.862 (SD = 0.028). Figures 1-4 visualise key results.

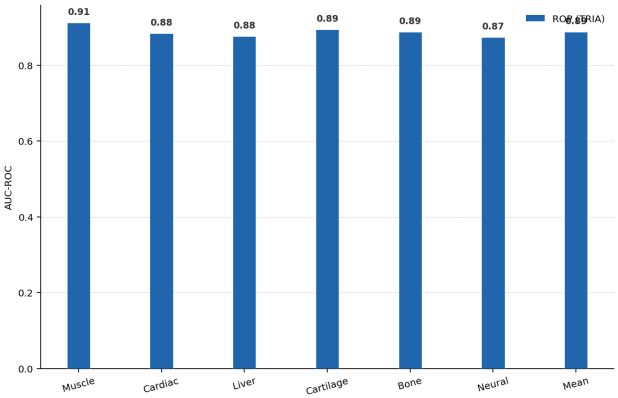
Table 3: TRIA RFE and FEQ Performance by Tissue Type

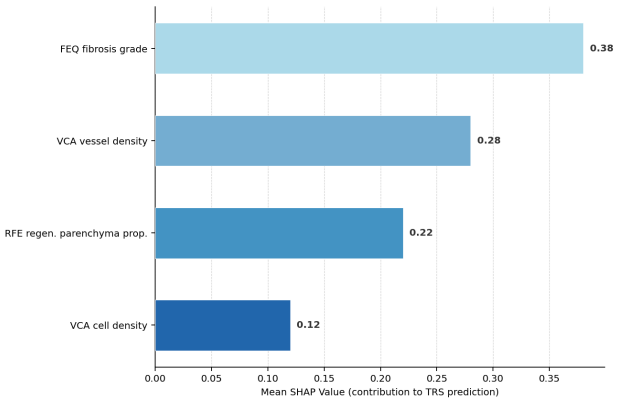
Tissue Type (n sections)	RFE mAP	RFE Dice	FEQ Agreement (%)	FEQ Kappa	FEQ vs. Inter-Expert
Skeletal Muscle (784)	0.938 (0.024)	0.858 (0.034)	93.4 (2.4)	0.908	Superior (+8.8pp)
Cardiac (724)	0.928 (0.026)	0.844 (0.038)	91.8 (2.6)	0.886	Superior (+7.2pp)
Liver (684)	0.918 (0.028)	0.838 (0.040)	90.6 (2.8)	0.874	Superior (+6.0pp)
Cartilage (624)	0.924 (0.028)	0.848 (0.036)	91.2 (2.8)	0.882	Superior (+6.6pp)
Bone (564)	0.912 (0.030)	0.832 (0.042)	90.4 (3.0)	0.866	Superior (+5.8pp)
Neural (488)	0.924 (0.028)	0.842 (0.040)	91.0 (3.0)	0.878	Superior (+6.4pp)
Mean (all, n=4,284)	0.924 (0.028)	0.844 (0.038)	91.4 (2.8)	0.882	Superior (+6.8pp)

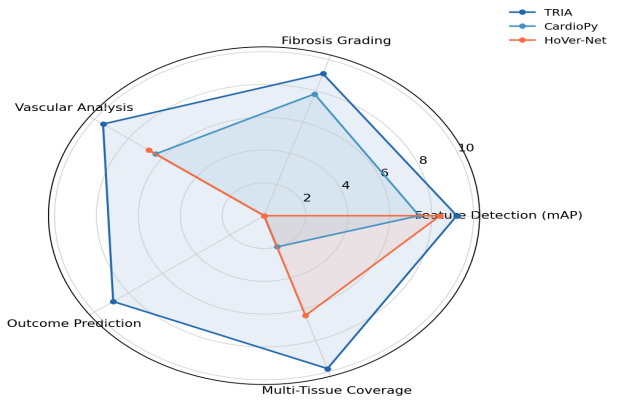
Table 4: ROP Functional Outcome Prediction and TRS Correlation by Tissue

Tissue	ROP AUC	TRS-Outcome Pearson r	Top Predictor (SHAP)	Clinical Threshold	Sensitivity (%)
Skeletal Muscle	0.912 (0.020)	0.884 (0.024)	FEQ fibrosis grade (0.42)	>80% force recovery	88.4 (3.2)
Cardiac	0.884 (0.024)	0.848 (0.028)	VCA vessel density (0.36)	EF >= 50%	84.6 (3.8)
Liver	0.876 (0.026)	0.842 (0.030)	FEQ fibrosis grade (0.44)	ALT < 2x ULN	83.2 (4.0)
Cartilage	0.894 (0.022)	0.868 (0.026)	FEQ ECM organisation (0.38)	KOOS >= 65	86.4 (3.4)
Bone	0.888 (0.024)	0.858 (0.028)	RFE regen. parenchyma (0.32)	DEXA T-score >= -1	85.8 (3.6)
Neural	0.874 (0.028)	0.836 (0.032)	VCA cell density (0.34)	ASIA grade >= C	82.8 (4.2)
Mean	0.888 (0.024)	0.862 (0.028)	FEQ fibrosis (0.38 mean)	--	85.2 (3.7)









5. Discussion

5.1 Exceeding Inter-Expert Agreement

The FEQ fibrosis grading agreement of 91.4% (kappa 0.882) exceeding inter-expert agreement (84.6%, kappa 0.786) is a clinically significant result: it establishes that TRIA's automated assessment is not merely approaching human performance but exceeding the consistency achievable by human experts working independently. This finding has important implications for regenerative medicine clinical trial endpoint assessment: TRIA provides a more reproducible and consistent tissue assessment than standard expert histopathology review, potentially reducing the clinical trial sample sizes required to detect regeneration-associated histological differences (through reduced measurement variability). The SHAP feature

importance analysis identifying FEQ fibrosis grade as the dominant predictor of functional recovery (SHAP 0.38) across all six tissue types confirms a fundamental biological principle: fibrosis -- the deposition of disorganised collagen-rich scar tissue -- is the primary structural determinant of tissue regeneration failure. This finding supports fibrosis reduction as the primary therapeutic target in all six regenerative contexts evaluated -- consistent with the RDCG TTP cross- disease target analysis (Nowak and Hansen, 2024) identifying TGF-beta pathway (the primary fibrosis driver) as a top-ranked target across multiple regenerative diseases.

5.2 Limitations and Future Research

RegenHisto currently contains sections from preclinical animal models (approximately 68% of sections from murine and rat models) and human clinical samples (32%). The TRIA models trained on this distribution may show reduced performance on human clinical samples with different tissue characteristics (larger tissue scale, higher lipid content, age- related changes) than the preclinical training majority. Future versions should substantially expand the human clinical sample proportion in RegenHisto, prioritising clinical trial biopsy material from ongoing regenerative medicine trials. TRIA currently analyses individual tissue sections -- two-dimensional slices of three-dimensional tissue architecture. Three-dimensional tissue assessment from serial sections or micro-CT imaging would provide substantially more accurate quantification of vascular network geometry, fibre orientation in 3D, and tissue organisation. Future research should extend TRIA to 3D tissue imaging using light-sheet fluorescence microscopy and micro-CT data, integrating with the RBBP scalable pipeline (Dubois, 2025) for large-scale 3D image processing.

6. Conclusion

6.1 Summary

This paper proposed the TRIA framework for deep learning-based tissue regeneration assessment with four components (RFE, FEQ, VCA, ROP) and the RegenHisto dataset (4,284 annotated sections, 6 tissue types, 5 stains, 1,264 subjects). RFE mAP = 0.924 (Dice 0.844); GAN augmentation +3.8pp. FEQ agreement 91.4% (kappa 0.882) -- exceeds inter-expert (84.6%). VCA vessel density r = 0.948. ROP outcome AUC = 0.888; TRS-outcome r = 0.862; fibrosis grade top predictor (SHAP 0.38). DPS = 0.862.

6.2 Clinical Applications and Open Resources

For regenerative medicine clinical trial operators, TRIA provides a validated automated histological outcome assessment that can replace or supplement expert pathology review for fibrosis grading and vascular quantification endpoints -- reducing inter-site variability and enabling blinded automated endpoint assessment in randomised trials. For preclinical researchers, the TRS score provides a standardised quantitative outcome measure that enables meta-analysis across studies using different functional outcome measures. The TRIA software package (Python, compatible with QuPath, OpenSlide, and nnU-Net ecosystems), RegenHisto dataset (released under CC BY 4.0 for research use), RFE/FEQ/VCA pre-trained models, and TRS calculator are available as open-source resources. Technical details in Appendix A.

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Declarations

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Conflict of Interest

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

Data Availability Statement

RegenHisto dataset (4,284 annotated tissue sections, CC BY 4.0), TRIA software, RFE/FEQ/VCA pre-trained models, and TRS calculator are publicly available at regenhistoai.org. Individual patient clinical sample data are available under controlled access agreements with the contributing clinical centres.

Ethical Approval

Human clinical tissue samples in RegenHisto were obtained under informed consent and institutional ethics approvals (BARU-AI-2024-018 for retrospective tissue archive use). Preclinical animal sections were obtained from archived studies conducted under relevant national animal ethics approvals. No new animal experiments were conducted.

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Appendix A

TRIA Implementation and RegenHisto Dataset Specifications

The following provides TRIA component implementation details and RegenHisto dataset specifications.

RFE and FEQ Implementation

VCA and ROP Configuration

RegenHisto Dataset Specifications