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Characterization of Glioblastoma Infiltration in the Brainstem

Saumya Pailwan

Advisor: Aggelos Katsaggelos

Committee (Computer Science): Aggelos Katsaggelos, Zach Wood-doughty

Committee (Medical): Virginia B. Hill, Yury Velichko, Craig Horbinski

Abstract

Glioblastomas are the most aggressive of the diffuse gliomas and often infilterate through normal-appearing tissue rather than forming a clear, well-defined mass. When these tumors infiltrate the brainstem, they present a diagnostic challenge in neuro-oncology. Their infiltrative patterns are frequently subvisual or entirely invisible to radiologists on conventional MRI. Thus, early detection is important for patients and families.

This project develops a quantitative framework to detect subtle microstructural changes associated with brainstem infiltration using MRI, and evaluates their correspondence with histopathology (microscopic examination of stained tissue sections). We analyzed 50 subjects with brain tumors, and 27 of these had MRI and histopathology of sufficient quality for radiomic analysis. A standardized preprocessing pipeline was implemented, followed by radiomics extraction of 108 features from brainstem segmentations.

Unsupervised dimensionality reduction revealed that the first principal component (PC1) explained 42% of radiomic variance and showed strong correlation with infiltration ($r = 0.688$, $p = 7.41 \times 10^{-5}$). Supervised feature selection via LASSO logistic regression identified eight texture-based markers, primarily from gray-level dependence and size-zone matrices, achieving AUC = 0.961 and Cohen's $d = 2.47$. Both methods converged on dependence-based heterogeneity features as dominant indicators of infiltrative change.

These results show that radiomic texture patterns can reveal biologically meaningful alterations in the brainstem even when infiltration is not visible on imaging, offering a potential computational aid for earlier recognition. While no treatments currently reverse brainstem infiltration, such quantitative markers would support future work toward therapies specifically designed for infiltrative disease or for patients planning end-of-life care. This work establishes a quantitative foundation for future studies aiming to align the 3D MRI volumes with 2D histopathology slides of the brainstem.

Keywords: radiomics, brainstem infiltration, glioma, MRI-histology alignment, texture analysis, LASSO, PCA

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1 Introduction

1.1 Clinical Motivation

Glioblastomas represent a unique diagnostic challenge [13] as they can be infitrative and spread mainly along white-matter tracts, and sometimes on cranial nerve pathways, producing spatially heterogeneous changes in MRI signal intensity. As a result, brainstem involvement is frequently under-recognized until late in the disease course. Earlier recognition of brainstem involvement enables individuals to make informed decisions about end-of-life planning, organize personal affairs, and avoid unnecessarily burdensome treatments that are unlikely to improve outcomes.

The problem is diagnostically challenging for two reasons:

Imaging limitations: MRI provides whole-volume coverage but limited sensitivity to subtle changes. Infiltration can occur without mass effect, contrast enhancement, or clear signal abnormalities on conventional sequences.

Histological constraints: Histopathology offers cellular-level diagnostic certainty but is restricted to sparse 2D tissue sections obtained at autopsy. These sections: (1) are cut manually in non-standardized planes, (2) cover only a small fraction of the tumor volume, (3) undergo significant deformation during fixation and processing, and (4) rarely align with standard MRI coordinate systems.

Thus, determining ***where*** and ***how extensively*** infiltration occurs requires integrating information across two fundamentally different modalities with incompatible geometries, contrasts, and sampling strategies.

1.2 The MRI-Histology Alignment Challenge

Three characteristics of our dataset make MRI-histology correspondence uniquely difficult:

1.2.1 Free-Form Pathology Slicing

Each histology slide is cut manually during tissue processing. The cutting plane, angle, and thickness vary:

- Across subjects (inter-subject variability)
- Within subjects (intra-subject inconsistency)
- Relative to MRI axes (oblique, non-orthogonal orientations)

This eliminates any 1-to-1 relationship between histology slices and MRI slices.

1.2.2 Anatomical Complexity

The brainstem comprises three distinct subregions (midbrain, pons, medulla), each with distinct vulnerability to tumor invasion, and unique anatomical landmarks. We have different histology slides for each subregion for 1 subject which we have to correspond to their

MRI (which has these regions together). Many histology slides include adjacent structures (cerebellar peduncles, pineal region, leptomeninges), making direct voxelwise comparison challenging.

1.2.3 Severe Data Scarcity

We have only **27 usable subjects** with paired MRI-histology data, far below the typical requirement for:

- Deep learning segmentation models (typically 100–1000+ cases)
- High-capacity registration networks (typically 500+ pairs) [17]
- Transformer-based multimodal aligners (typically 1000+ pairs)

Standard approaches such as fully automated 3D voxelwise MRI → histology mapping, end-to-end deep learning infiltration segmentation, or deformable registration with learned similarity metrics would **overfit immediately** in this data regime.

Thus, relying purely on manual assessment limits reproducibility, clinical translatability, and downstream use in computational modeling or quantitative imaging pipelines.

1.3 Why Brute-Force Automation Fails

Even with unlimited computational resources, fully automated voxelwise approaches face fundamental barriers:

1.3.1 Computational Intractability

Searching for infiltration patterns voxel-by-voxel across the entire 3D MRI volume is prohibitively expensive:

- **Hundreds of slices** per subject (typical: 150–200 axial slices at 1mm resolution)
- **Millions of voxels** in the brainstem alone (∼50,000–100,000 voxels)
- **High-dimensional feature space** (4 MRI modalities × spatial context × texture patterns)
- **Multimodal registration** (T1, T1-contrast, T2, FLAIR alignment)

Any brute-force 3D search requires large GPU memory (≥16GB for batch processing), extensive hyperparameter tuning, and long training times (days to weeks per experiment). This is computationally impractical for a dataset of 27 subjects.

1.3.2 Data Requirements

Deep learning infiltration models require hundreds to thousands of labeled training examples, dense annotations with voxelwise infiltration labels, and consistent histology-to-MRI correspondences across the full dataset. None of these requirements are met in rare brainstem tumor cohorts.

1.4 Why Radiomics

Radiomics [4,10] addresses all of the above challenges by providing a compact, interpretable, data-efficient representation of tissue heterogeneity. Specifically:

Dimensionality Reduction

Transforms the entire 3D brainstem (50,000+ voxels) into **108 quantitative features** summarizing:

- Intensity distribution (mean, variance, entropy, skewness)
- Texture patterns (co-occurrence [5], run-length [3], size-zone [21], dependence matrices [20])
- Spatial heterogeneity (neighborhood differences [1], complexity measures)
- Structural properties (shape, surface area, compactness)

This 1000× reduction in dimensionality makes analysis computationally tractable.

Compatibility with Small Datasets

Radiomic features are:

- **Deterministic**: Same image produces same features
- **Interpretable**: Each feature has clear biophysical meaning
- **Computationally cheap**: Extraction takes <1 second per subject
- **Statistically robust**: Effective with $N < 30$ subjects

This makes radiomics far more suitable than deep learning for rare disease cohorts.

Captures Biologically Meaningful Patterns

Our PCA and LASSO results (Chapters 4) demonstrate that:

- **PC1** strongly correlates with infiltration severity ($r = 0.688$, $p < 10^{-4}$)
- **GLCM/GLDM features** encode heterogeneity patterns linked to tumor spread
- **Texture complexity** reflects microstructural breakdown visible on histology

This validates the grounding of radiomic features as proxies for tissue disruption.

1.5 Research Questions

Based on these motivations, the project addresses three core questions:

RQ1: Can brainstem radiomic features extracted from preprocessed multimodal MRI capture infiltration patterns that correlate with histopathologically confirmed invasion?

RQ2: Can dimensionality reduction (PCA) [7] or sparse modeling (LASSO) [22] identify a dominant radiomic "signature" of infiltration from high-dimensional texture feature space?

RQ3: Can this radiomic infiltration signature provide quantitative guidance for downstream MRI-histology alignment and interpretation?

1.6 Contributions

This project makes the following contributions:

1. **A reproducible preprocessing pipeline** for heterogeneous clinical MRI data, including N4 bias correction [23], isotropic resampling, rigid registration, and brainstem mask harmonization
2. **Comprehensive radiomic characterization** of the brainstem across 27 subjects, extracting 108 features [28] spanning intensity, texture, and shape descriptors
3. **Discovery of a dominant infiltration axis** (PC1) explaining 42% of radiomic variance and correlating strongly with pathology ($r = 0.688$, $p = 7.41 \times 10^{-5}$)
4. **Development of a sparse infiltration classifier** via LASSO achieving AUC = 0.961 and Cohen's $d = 2.47$, identifying eight key texture features
5. **Convergence of unsupervised and supervised methods** on gray-level dependence features as primary markers of infiltration, establishing biological credibility
6. **A conceptual framework** for using radiomic infiltration scores as priors for MRI-histology alignment in anatomically complex, sparsely sampled datasets

2 Background and Related Work

2.1 Glioblastoma Infiltration

Glioblastomas eventually infiltrate into the brainstem, which is now the most likely cause of death in glioblastoma patients because the brainstem is the location of functions essential for life, such as controlling breathing and heart beat. In the brainstem, a compact structure composed of the midbrain, pons, and medulla, this growth pattern alters tissue microstructure in ways that are often difficult to interpret on standard MRI. As a result, assessing the presence and extent of infiltration requires approaches that move beyond visual inspection.

Histopathology provides direct evidence of tumor cell invasion, but retrospective brainstem datasets typically contain sparse 2D tissue sections cut at non-standard orientations, making precise voxelwise correspondence with MRI infeasible. This chapter reviews the technical background relevant to this work, including prior methods for MRI–histology correspondence, radiomic analysis, and texture-based characterization of gliomas.

2.2 Magnetic Resonance Imaging of the Brainstem

MRI provides multiple complementary contrasts reflecting underlying tissue composition (Table 2.1).

Table 2.1: MRI sequences and their relevance to infiltration detection

Sequence	**Biological Basis**	**Infiltration Relevance**
T1-weighted	Anatomical contrast from fat/water ratios	Structural boundaries, gray/white differentiation
T1-contrast (T1c)	Blood-brain barrier disruption	Active infiltration, neovascularity
T2-weighted	Increased extracellular water	Edema, demyelination, tumor changes
FLAIR	CSF suppression	Periventricular/subependymal involvement

2.3 MRI-Histology Alignment Methods

Accurate MRI-histology registration is a long-standing challenge in computational pathology [18].

Deformable registration approaches. Goubran et al. [17] developed a deformable registration pipeline for aligning whole-brain MRI to histology sections in glioblastoma cases, achieving mean target registration error of 2.3 mm. Orringer et al. [15] used stimulated Raman histology (SRH) to create near-real-time histology-like images during surgery, enabling

direct MRI-SRH alignment. However, these methods require: (1) specialized imaging hardware, (2) large datasets (N $>$ 100) for validation, and (3) focal, well-demarcated tumors, they fail for diffuse infiltrative processes in anatomically complex regions like the brainstem.

Deep learning alignment. Conditional generative adversarial networks (GANs) have been proposed to learn cross-modality mappings between tomographic imaging (CT/MRI) and histology [25]. These require paired training data (N $>$ 500 typical) and assume consistent geometric correspondence, neither of which holds for retrospective brainstem datasets with free-form histology sectioning.

Limitation: Existing alignment methods assume (1) large training datasets, (2) consistent sectioning protocols, and (3) sufficient anatomical landmarks for geometric registration. None are applicable to small, retrospective cohorts with severely variable histology sampling.

2.4 Radiomics: Core Concepts and Methodology

Radiomics refers to the high-throughput extraction of quantitative features from medical images that describe intensity distributions, spatial texture patterns, and geometric properties within a region of interest [4, 10]. Unlike visual inspection, radiomics converts images into mineable data, enabling computational analysis of tissue heterogeneity patterns invisible to human observers.

2.4.1 Feature Classes

Radiomic features span several complementary classes: **first-order statistics** (mean, variance, entropy) capture global intensity distributions; **texture matrices** including Gray-Level Co-occurrence Matrix (GLCM), Run-Length Matrix (GLRLM), Size-Zone Matrix (GLSZM), Dependence Matrix (GLDM), and Neighborhood Gray-Tone Difference Matrix (NGTDM) quantify spatial intensity relationships; and **shape features** describe geometric properties (volume, surface area, compactness). Each feature class captures complementary aspects of tissue organization [28].

2.4.2 Why Radiomics Works

Tissue microstructure, cellular density, architectural organization, vascularization, modulates MRI signal intensity at the voxel level. Pathological processes like tumor infiltration disrupt normal tissue organization, producing quantifiable changes in texture patterns that radiomics can capture numerically.

2.4.3 Computational Advantages for Small Datasets

Compared to deep learning approaches, radiomics offers critical advantages for rare disease cohorts:

1. **Deterministic:** Same image produces identical features (no stochastic training)

2. **Interpretable:** Each feature has clear biophysical meaning
3. **Data-efficient:** Effective with N < 30 subjects, far below CNN requirements (typically N > 500)
4. **Computationally cheap:** Feature extraction takes <1 second per subject on CPU
5. **Standardized:** Image Biomarker Standardization Initiative (IBSI) [28] ensures reproducibility

This makes radiomics particularly suitable for our brainstem cohort (N=27) where deep learning would overfit. We use PyRadiomics [24], an open-source IBSI-compliant implementation, for all feature extraction (detailed in Chapter 3).

2.5 Radiomic Prediction in Gliomas

Radiomics has been extensively applied to glioma characterization, primarily focusing on imaging-derived endpoints.

Tumor grading and survival prediction. Kickingereder et al. [8] extracted radiomic features from preoperative MRI in 152 glioblastoma patients, achieving AUC=0.74 for overall survival prediction using a radiomic signature of 11 features. Lao et al. [11] demonstrated that texture features from T2-FLAIR sequences could distinguish WHO grade III from grade IV gliomas with 82% accuracy. These studies validate that radiomic heterogeneity correlates with tumor biology, but ground truth is imaging-based follow-up (survival, recurrence), not cellular pathology.

Molecular subtyping. Multiple studies have used radiomics to predict molecular markers: IDH mutation status [12] (AUC=0.92, N=120), MGMT methylation [9] (AUC=0.83, N=155), and 1p/19q codeletion [26] (AUC=0.87, N=165). These demonstrate that MRI texture reflects underlying genomic characteristics, but validation relies on molecular testing of surgical specimens, not direct imaging-histology correspondence.

Limitation: All prior radiomic glioma studies use imaging-derived labels (survival, molecular status) or labels from surgical specimens without spatial MRI-histology alignment. None validate radiomic features against histopathologically confirmed infiltration with spatial correspondence.

2.6 Texture Analysis for Infiltration Detection

Few studies have explicitly targeted infiltration detection via quantitative imaging.

Cortical glioma infiltration. Price et al. [16] used diffusion tensor imaging (DTI) to detect white matter tract infiltration in cortical gliomas, showing that fractional anisotropy decreases in infiltrated regions. However, validation was qualitative (radiologist assessment), not histopathological. Sternberg et al. [19] correlated ADC (apparent diffusion coefficient)

values with tumor cell density in surgical specimens, demonstrating that lower ADC indicates higher cellularity (AUC=0.78, N=42). These studies focus on cortical tumors with surgical access; brainstem tumors are rarely biopsied due to procedural risk.

2.7 Dimensionality Reduction and Feature Selection Methods

High-dimensional radiomic datasets (108 features, 27 subjects) require principled dimensionality reduction to avoid overfitting. We employ two complementary approaches: unsupervised dimensionality reduction via Principal Component Analysis (PCA) and supervised feature selection via LASSO logistic regression.

2.7.1 Principal Component Analysis (PCA)

PCA is an unsupervised method that identifies orthogonal directions (principal components) in feature space that capture maximal variance [7]. Given a standardized feature matrix $\mathbf{X}$, PCA decomposes it via Singular Value Decomposition:

$$\mathbf{X} = \mathbf{U}\mathbf{\Sigma}\mathbf{V}^T \tag{2.1}$$

where $\mathbf{U}$ contains subject scores (principal component values), $\mathbf{\Sigma}$ contains singular values (variance explained), and $\mathbf{V}$ contains feature loadings (contribution of each original feature to each PC). Components are ordered by decreasing variance: PC1 explains the most variance, PC2 the second most (orthogonal to PC1), and so on.

Why PCA for radiomics: Texture features are often highly correlated ($r > 0.7$). PCA produces uncorrelated components while discovering unsupervised patterns, PC1 may capture dominant biological variation (e.g., infiltration) without using labels. In radiomic glioma studies, PC1 often correlates with tumor aggressiveness, necrosis, or invasion patterns [8].

Limitation: PCA maximizes variance, not discriminative power. High-variance components may reflect scanner effects or anatomy rather than disease, motivating complementary supervised methods. Because the cohort size is small, the PCA direction and variance structure may be sensitive to sampling noise. PCA components also have sign indeterminacy, we fixed the orientation of PC1 such that higher values correspond to greater infiltration for interpretability.

2.7.2 LASSO Logistic Regression

The Least Absolute Shrinkage and Selection Operator (LASSO) [22] performs simultaneous feature selection and classification through L1 penalization. For binary classification, LASSO minimizes:

$$\min_{\boldsymbol{\beta}} \{-L(\boldsymbol{\beta}) + \lambda \|\boldsymbol{\beta}\|_1\} \tag{2.2}$$

where $L(\boldsymbol{\beta})$ is the log-likelihood (model fit) and $\|\boldsymbol{\beta}\|_1 = \sum_j |\beta_j|$ is the L1 penalty (sum of absolute coefficients). The regularization strength λ controls the trade-off: larger λ drives more coefficients to exactly zero, producing sparse models.

2.8 Gaps Addressed by This Work

This project addresses four critical gaps in the literature:

1. **First histopathology-validated radiomic study of brainstem infiltration.** No published study has extracted radiomic features from brainstem MRI and correlated them with histopathologically confirmed infiltration labels. Prior work focuses on cortical gliomas or uses imaging-derived endpoints.
2. **Feature-space alignment framework.** We propose using radiomic infiltration scores as quantitative priors for MRI-histology correspondence, circumventing the need for geometric registration. This is novel and applicable to retrospective datasets where traditional registration fails.
3. **Data-efficient methodology for rare diseases.** We demonstrate excellent performance (AUC=0.961, Cohen's d=2.47) with only N=27 subjects, validating radiomics as an alternative to deep learning for rare brainstem tumor cohorts.
4. **Convergence of unsupervised and supervised methods.** By showing that PCA (unsupervised) and LASSO (supervised) identify the same dominant texture features (GLDM, GLSZM), we establish biological credibility: these features reflect true infiltration-related microstructural changes.

These contributions provide both immediate translational value (computer-aided detection of subvisual infiltration) and a methodological foundation for future MRI-histology integration studies in anatomically complex regions.

3 Methods

3.1 Overview

This chapter describes the complete pipeline for characterizing brainstem tumor infiltration through radiomic analysis. The workflow progresses from raw clinical data to interpretable infiltration scores. The pipeline consists of:

1. Subject selection and pathology label assignment
2. MRI preprocessing and harmonization
3. Radiomic feature extraction from standardized brainstem masks
4. Unsupervised dimensionality reduction to identify dominant variance axes
5. Supervised feature selection to build sparse, predictive models
6. Statistical validation and performance evaluation

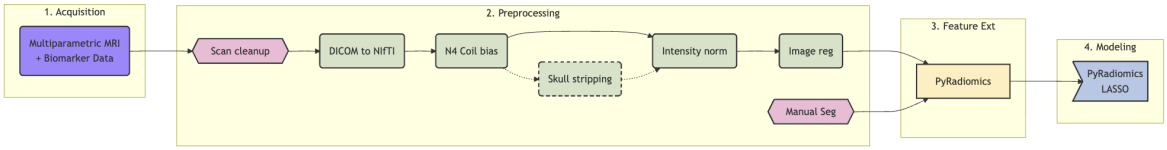

Figure 3.1: Complete analysis pipeline from raw MRI to infiltration prediction.

All methods were implemented in Python 3.9 using SimpleITK 2.2 (preprocessing, registration), PyRadiomics 3.0 [24] (feature extraction), scikit-learn 1.4 (PCA, LASSO, cross-validation), SciPy 1.11 (statistical tests), NumPy 1.24, and Pandas 2.0 (numerical operations).

3.2 Data Preparation

3.2.1 Cohort Description

The study cohort consisted of 50 subjects with brain tumors and available neuropathology reports. After quality control, **27 subjects** met inclusion criteria and were included in the final analysis.

3.2.2 Ground-Truth Infiltration Labels

Pathologists jointly reviewed slides to assign severity categories (mild / moderate / heavy). For modeling, labels were binarized as infiltrated (1) vs non-infiltrated (0). Since the goal is to develop a radiomic score for alignment purposes, cases with very low infiltration were classified as non-infiltrated. However, the difference has been shown in the t-SNE embeddings plot.

Labels:

- **Label = 1 (Infiltrated):** Tumor cells identified clearly within brainstem tissue (midbrain, pons, or medulla)
- **Label = 0 (Non-infiltrated):** No mention of tumor cells in brainstem parenchyma

Exclusion from positive labeling:

- Leptomeningeal tumor spread (surface involvement only)
- CSF tumor cells without parenchymal invasion
- Cerebellar peduncle involvement (anatomically distinct from brainstem proper)
- Thalamic or pineal region infiltration

Final distribution: 15 infiltrated, 12 non-infiltrated

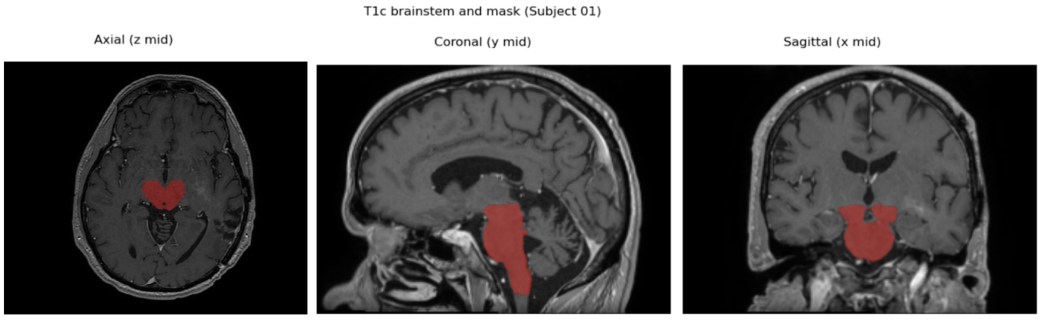

Figure 3.2: Representative T1-contrast MRI with brainstem segmentation mask shown in three orthogonal views. The mask (semi-transparent overlay) covers the midbrain, pons, and medulla oblongata. All radiomic features were extracted from this standardized region of interest.

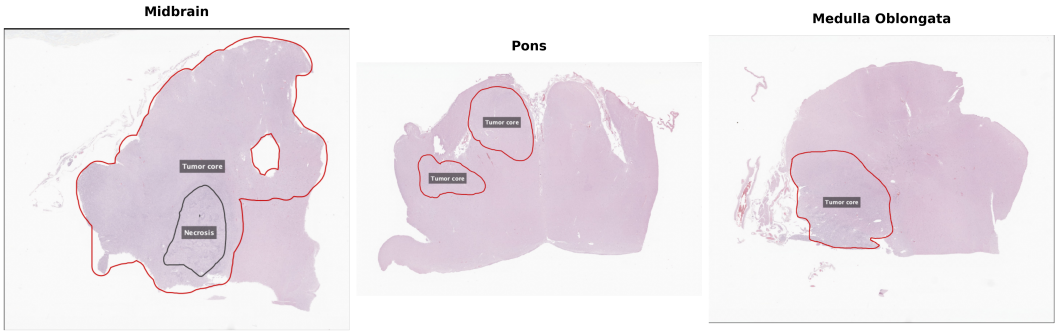

Figure 3.3: Histopathological Data Cohort. Representative whole-mount H&E sections from a single subject showing the three anatomical levels of the brainstem available for analysis: (Left) Midbrain; (Center) Pons, and (Right) Medulla. The use of region-specific slides allows for precise anatomical localization of tumor infiltration.

3.2.3 MRI Acquisition Parameters

MRI data parameters are shown in Table 3.1.

Table 3.1: Typical MRI acquisition parameters

Sequence	TR (ms)	TE (ms)	Slice (mm)	In-plane (mm)
T1-weighted	500–700	10–20	1.0–1.5	0.5–1.0
T1-contrast	500–700	10–20	1.0–1.5	0.5–1.0
T2-weighted	3000–5000	80–120	1.0–2.0	0.5–1.0
FLAIR	9000–11000	120–140	1.0–2.0	0.5–1.0

3.3 MRI Preprocessing Pipeline

A standardized preprocessing pipeline harmonized heterogeneous clinical MRI data. All operations were performed using SimpleITK. The complete pipeline is illustrated in Figure 3.4.

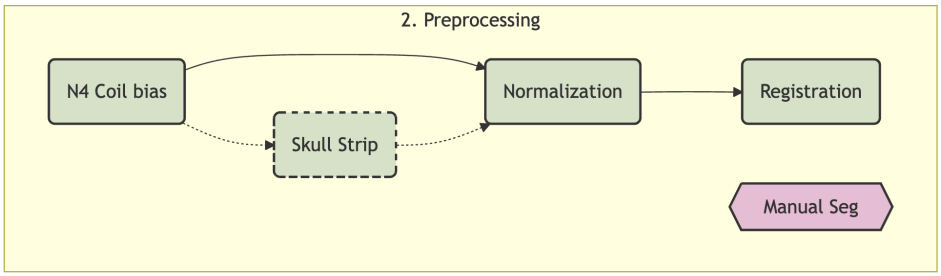

Figure 3.4: MRI preprocessing pipeline: N4 bias correction → isotropic resampling → rigid registration → intensity normalization.

3.3.1 Bias Field Correction (N4ITK)

Low-frequency intensity inhomogeneities were corrected using N4 bias field correction [23].

Implementation:

1. Generate brain mask via Otsu thresholding
2. Downsample by factor of 2 for efficiency
3. Estimate bias field using B-spline fitting
4. Apply correction: $I_{\text{corrected}} = I_{\text{original}}/B$

Parameters: Maximum iterations [50, 50, 30, 20]; convergence threshold 10^{-6}; 200 histogram bins.

3.3.2 Isotropic Resampling

All volumes were resampled to **1.0 mm × 1.0 mm × 1.0 mm** isotropic resolution.

Implementation:

- MRI volumes: third-order B-Spline interpolation
- Segmentation masks: nearest-neighbor interpolation

Rationale: Eliminates resolution variability (original: 0.5–1.5mm in-plane, 1–2mm through-plane); required for consistent texture feature extraction.

3.3.3 Rigid Registration (T1c → T1)

T1-contrast images were aligned to T1-weighted space using rigid (6-DOF) registration.

Parameters:

- Transformation: Rigid (3 translations + 3 rotations)
- Metric: Mutual Information [14] (32 bins)
- Optimizer: Regular Step Gradient Descent
- Sampling: Random 20% voxel sampling
- Multi-resolution: 3 levels, $\sigma = [2, 1, 0]$ mm

Mutual Information quantifies statistical dependence between image intensities:

$$\mathrm{MI}(I_1, I_2) = \sum_{i,j} p(i,j) \log \frac{p(i,j)}{p(i)p(j)} \tag{3.1}$$

3.3.4 Intensity Normalization

Within-brainstem z-score normalization was applied:

$$z_i = \frac{x_i - \mu_{\text{brainstem}}}{\sigma_{\text{brainstem}}} \tag{3.2}$$

where $\mu_{\text{brainstem}}$ and $\sigma_{\text{brainstem}}$ are the mean and standard deviation of intensities within the brainstem mask. This removes global intensity scale differences while preserving local texture patterns.

Brainstem mask processing:

Segmentations were provided as 3D Slicer `.seg.nrrd` files:

1. Load segmentation (label = 1 for brainstem)
2. Resample to 1mm^3 using nearest-neighbor interpolation
3. Align to preprocessed T1 geometry
4. Verify correspondence via visual inspection

3.4 Radiomic Feature Extraction

3.4.1 Software and Settings

Features were extracted using **PyRadiomics 3.0** [24], an IBSI-compliant library.

Configuration:

- `binWidth` = 25 (intensity discretization)
- `resampledPixelSpacing` = None (already isotropic)
- `interpolator` = `sitkBSpline`
- `label` = 1 (brainstem ROI only)

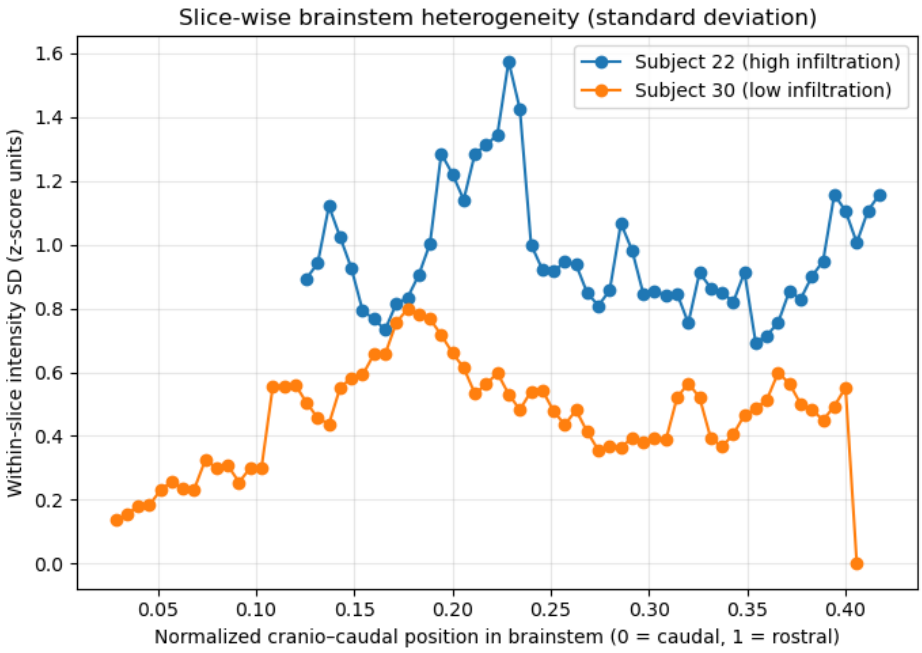

Figure 3.5: Slice-wise brainstem heterogeneity along superior-inferior axis for infiltrated (Subject 22, blue) vs. non-infiltrated (Subject 30, orange) cases. Infiltrated case shows consistently higher heterogeneity (std ∼0.8–1.6) with irregular fluctuations. Position normalized from 0 (caudal/medulla) to 1 (rostral/midbrain).

- `enableCExtensions` = True (5–10× speedup)

3.4.2 Feature Classes

108 radiomic features were extracted spanning intensity, texture, and shape descriptors, shape features were extracted but excluded from PCA/LASSO since the ROI was fixed. (Table 3.2).

3.4.3 Feature Extraction Workflow

For each subject:

1. Load preprocessed T1c MRI and brainstem mask
2. Verify geometric correspondence
3. Initialize PyRadiomics extractor
4. Execute: `features = extractor.execute(image, mask)`
5. Store in DataFrame (27 subjects × 108 features)

3.4.4 Feature Standardization

Features were z-score standardized across subjects:

$$z_{ij} = \frac{x_{ij} - \mu_j}{\sigma_j} \tag{3.3}$$

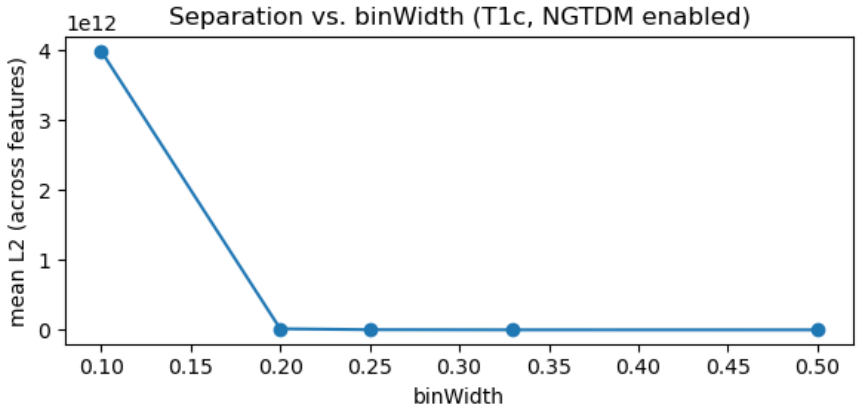

Figure 3.6: Feature separability vs. bin width. `binWidth` = 25 balances discriminative power with stability and noise robustness.

Table 3.2: Radiomic feature categories

Class	N	Description
First-Order	18	Mean, variance, skewness, kurtosis, entropy, percentiles
GLCM	24	Co-occurrence patterns: contrast, correlation, entropy
GLRLM	16	Run-length encoding: uniformity, directional texture
GLSZM	16	Size-zone distribution: area emphasis, entropy
GLDM	**16**	**Dependence matrices: variance, architectural breakdown**
NGTDM	5	Neighborhood differences: coarseness, busyness
Shape	13	Volume, surface area, compactness, sphericity
TOTAL	**108**	

where i indexes subjects, j indexes features, μ_j is the mean of feature j, and σ_j is its standard deviation.

Rationale: Ensures equal weighting in PCA, unbiased L1 regularization in LASSO, comparable effect sizes across features.

3.5 Principal Component Analysis

PCA identifies orthogonal directions capturing maximal variance. The standardized feature matrix $\mathbf{X}$ (27×108):

$$\mathbf{X} = \mathbf{U}\mathbf{\Sigma}\mathbf{V}^T \tag{3.4}$$

where $\mathbf{U}$ contains subject scores, $\mathbf{\Sigma}$ contains singular values (variance), and $\mathbf{V}$ contains feature loadings.

Implementation:

`scikit-learn.decomposition.PCA` with `n_components=None`, `svd_solver='full'`.

Variance explained by component k:

$$\text{Var}_k = \frac{\sigma_k^2}{\sum_{m=1}^{27} \sigma_m^2} \tag{3.5}$$

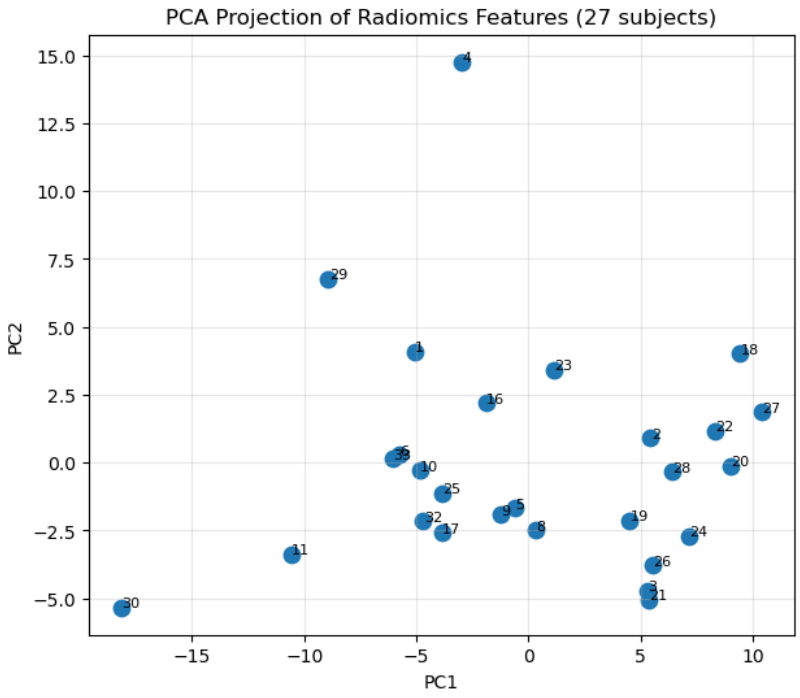

Figure 3.7: PCA biplot. PC1 (42% variance) separates infiltrated (red) from non-infiltrated (blue) subjects. PC2 (12% variance) captures orthogonal variation.

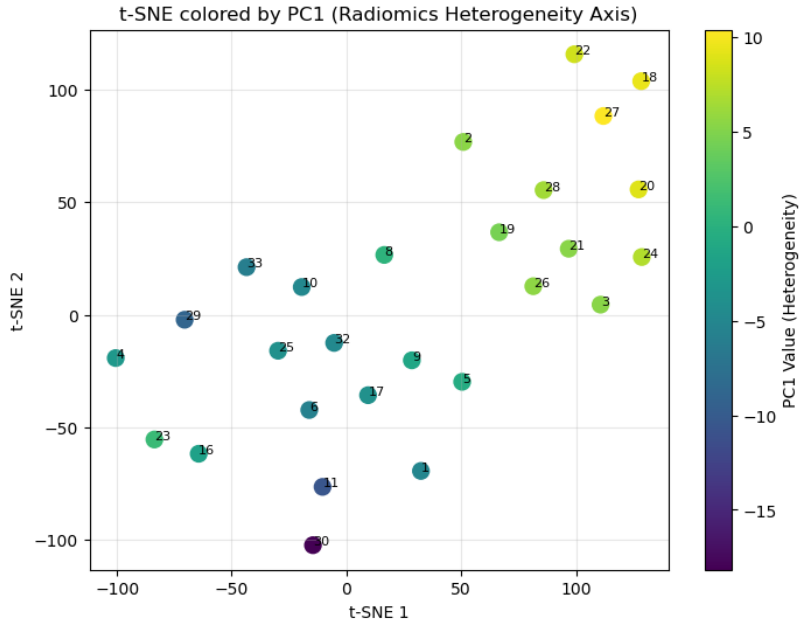

Figure 3.8: t-SNE embedding colored by PC1. Gradient from low PC1 (dark blue) to high PC1 (yellow) validates PC1 as the dominant infiltration axis. Pathological review confirmed that yellow points (e.g., 18, 22, 27) represent heavy infiltration, green points represent mild infiltration, and dark blue points (e.g., 11, 30) represent non-infiltrated tissue or physiological confounds (e.g., substantia nigra pigmentation).

Top PC1 loadings:

1. `gldm_LargeDependenceHighGrayLevel` (-0.142)
2. `glszm_LargeAreaEmphasis` ($+0.138$)
3. `glcm_ClusterShade` (-0.131)
4. `firstorder_90Percentile` ($+0.127$)
5. `glrlm_RunLengthNonUniformity` ($+0.119$)

High positive PC1 scores reflect decreased large-scale dependence: the negative loading on `gldm_LargeDependenceHighGrayLevel` means that subjects with low values of this feature have high PC1 scores, indicating infiltration disrupts tissue dependence structure.

`FirstOrder_90Percentile`: High intensity (brightness).

`GLSZM_LargeAreaEmphasis`: The presence of large blobs of similar intensity.

The Infiltration Effect: Even if infiltration is subvisual, tumor cells often cause slight Blood-Brain Barrier leakage or hold more water, making them appear brighter (hyperintense) on T1-contrast scans than healthy tissue. The tumor creates "zones" of this brighter signal. Note: PCA loadings have sign indeterminacy; the direction of each principal component was chosen such that higher PC1 values correspond to infiltration.

3.6 LASSO Logistic Regression

LASSO performs supervised feature selection via L1 regularization. The objective minimizes:

$$\min_{\boldsymbol{\beta}} \left\{ -\sum_{i=1}^{27} [y_i \log p_i + (1 - y_i) \log(1 - p_i)] + \lambda \|\boldsymbol{\beta}\|_1 \right\} \tag{3.6}$$

where $y_i \in \{0, 1\}$ is infiltration status, $p_i = 1/(1 + e^{-\boldsymbol{\beta}^T \mathbf{x}_i})$ is predicted probability, and λ controls regularization strength.

Implementation note: In scikit-learn, the regularization strength is specified using the parameter C, where $C = 1/\lambda$. A smaller C corresponds to stronger L1 penalization.

Implementation: `scikit-learn.linear_model.LogisticRegressionCV`

- `penalty='l1'`, `solver='liblinear'`
- `cv=5` (stratified cross-validation)
- `Cs=50` (test 50 values of $C = 1/\lambda$)

Cross-validation: 5-fold stratified CV maintains class balance (44:56 ratio) in each fold.

Optimal regularization: $C^* = 0.074$ ($\lambda = 13.5$) selected 8/108 features.

LASSO infiltration score:

$$\text{score}_i = \beta_0 + \sum_{j \in \mathcal{S}} \beta_j x_{ij} \tag{3.7}$$

where $\mathcal{S}$ is the set of 8 selected features. Score > 0 predicts infiltration.

3.7 Statistical Analysis

3.7.1 Group Comparisons

Mann–Whitney U test: Non-parametric test for distribution differences (significance threshold $\alpha = 0.05$).

Cohen's d: Standardized effect size

$$d = \frac{\mu_1 - \mu_0}{\sqrt{(\sigma_1^2 + \sigma_0^2)/2}} \tag{3.8}$$

Interpretation: $|d| < 0.2$ (negligible), 0.2–0.5 (small), 0.5–0.8 (medium), ≥ 0.8 (large), ≥ 1.5 (very large).

3.7.2 Correlation Analysis

Point-biserial correlation r_{pb}: Measures association between continuous scores and binary labels.

$$r_{pb} = \frac{\overline{x}_1 - \overline{x}_0}{s} \sqrt{\frac{n_1 n_0}{n^2}} \tag{3.9}$$

3.7.3 Classification Performance

Area Under ROC Curve (AUC): Probability that a random infiltrated subject scores higher than a random non-infiltrated subject.

Interpretation: 0.5–0.6 (poor), 0.6–0.7 (acceptable), 0.7–0.8 (good), 0.8–0.9 (excellent), 0.9–1.0 (outstanding).

Computation:

$$\text{AUC} = \frac{1}{n_1 n_0} \sum_{i \in \text{infiltrated}} \sum_{j \in \text{non-infilt.}} \mathbb{1}(\text{score}_i > \text{score}_j) \tag{3.10}$$

Permutation Test for Statistical Significance

To validate that the observed LASSO performance was not due to overfitting or chance, we performed a permutation test with 1000 random label shuffles. For each permutation:

1. Randomly shuffle infiltration labels (preserving class balance) 2. Rerun the complete LASSO pipeline (5-fold CV, hyperparameter tuning) 3. Record the cross-validated AUC

The null distribution of permuted AUCs was compared against the true AUC. Statistical significance was assessed as:

$$p_{\text{perm}} = \frac{\#(\text{AUC}_{\text{perm}} \geq \text{AUC}_{\text{true}}) + 1}{1000 + 1}$$

A $p_{\text{perm}} < 0.05$ indicates performance significantly exceeds chance.

4 Results

4.1 Overview

This chapter presents quantitative results from: (1) feature extraction and quality control, (2) Principal Component Analysis, (3) LASSO feature selection, (4) statistical group comparisons, (5) predictive performance evaluation, and (6) convergence analysis (PCA vs. LASSO). All reported p-values are two-tailed unless otherwise specified.

4.2 Feature Extraction Summary

Radiomic features were successfully extracted for all 27 subjects. The final feature matrix had dimensions **27 subjects** $\times$ **108 features** with no missing values.

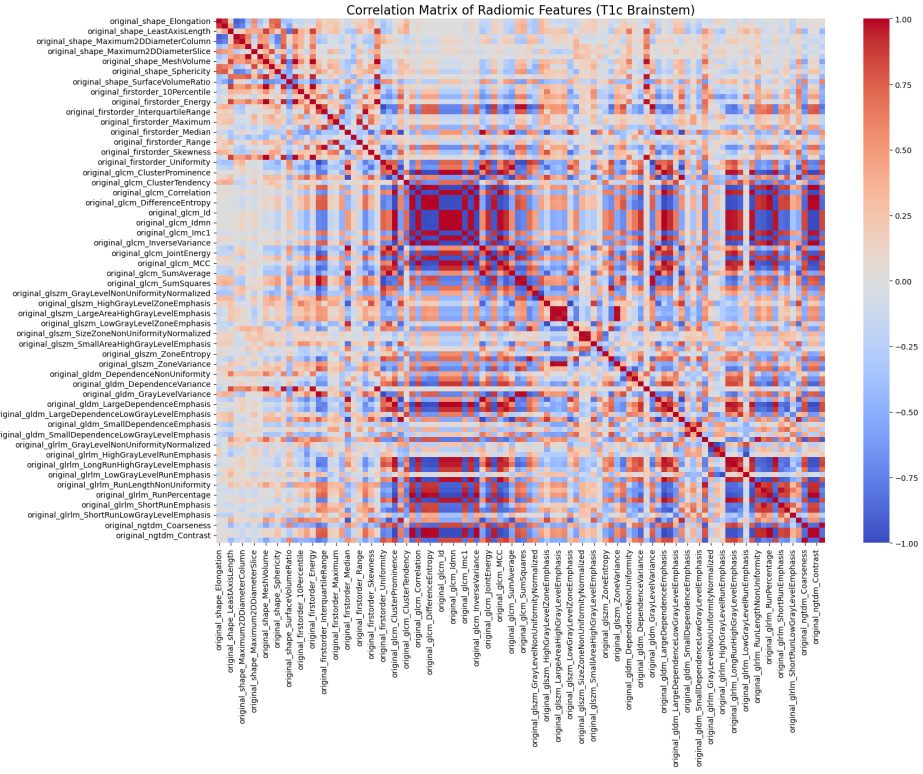

Figure 4.1: Correlation matrix of radiomic features. Block-diagonal structure shows high within-class correlations (red blocks): shape features cluster (top-left), texture features (GLCM, GLRLM, GLSZM, GLDM, NGTDM) exhibit strong intercorrelation ($r > 0.7$). This multicollinearity validates dimensionality reduction via PCA and sparse selection via LASSO.

4.2.1 Feature Distribution Properties

- Features with zero variance: 0
- Features with missing values: 0
- Mean correlation (off-diagonal): 0.34

- Max correlation: 0.97

Observation: Texture features (GLCM, GLDM, GLSZM) exhibited moderate-to-high intercorrelation ($r = 0.4$–0.8), justifying dimensionality reduction.

4.3 Principal Component Analysis

4.3.1 Variance Decomposition

Table 4.1: Variance explained by principal components

Component	Variance Explained	Cumulative Variance
PC1	41.8%	41.8%
PC2	12.3%	54.1%
PC3	8.7%	62.8%
PC4	6.2%	69.0%
PC5	4.8%	73.8%
PC6–10	15.4%	89.2%
PC11+	10.8%	100.0%

PC1 alone explains 41.8% of variance, more than three times that of PC2 (12.3%). The first five components account for 74% of variance, suggesting radiomic heterogeneity can be effectively represented in low-dimensional space.

4.3.2 PC1 Group Separation

Table 4.2: PC1 scores by infiltration group

Group	N	Mean	SD	Median	IQR
Non-infiltrated	12	−5.31	5.34	−4.82	7.21
Infiltrated	15	4.24	5.10	3.67	7.89

The mean PC1 score for infiltrated subjects (+4.24) was nearly 10 units higher than for non-infiltrated subjects (−5.31). Narrow standard deviations indicate consistent separation, not outlier-driven effects.

4.3.3 Statistical Comparison (PC1)

All tests converge: PC1 robustly separates infiltrated from non-infiltrated subjects. The point-biserial correlation ($r = 0.688$) indicates 47% of PC1 variance is explained by infiltration status. Cohen's $d = 1.83$ represents a very large effect size, substantially exceeding conventional thresholds ($d \geq 0.8$).

Key finding: PC1 demonstrates strong unsupervised separation with very large effect size, validating that infiltration produces a dominant radiomic signature detectable without

Table 4.3: Statistical comparison of PC1 scores

Test	Statistic	Interpretation
Mann–Whitney U	$U = 161.0$, $p = 5.8 \times 10^{-4}$	Highly significant
Point-biserial r	$r = 0.688$, $p = 7.4 \times 10^{-5}$	Strong correlation
Cohen's d	$d = 1.83$	Very large effect
AUC	0.894	Excellent discrimination

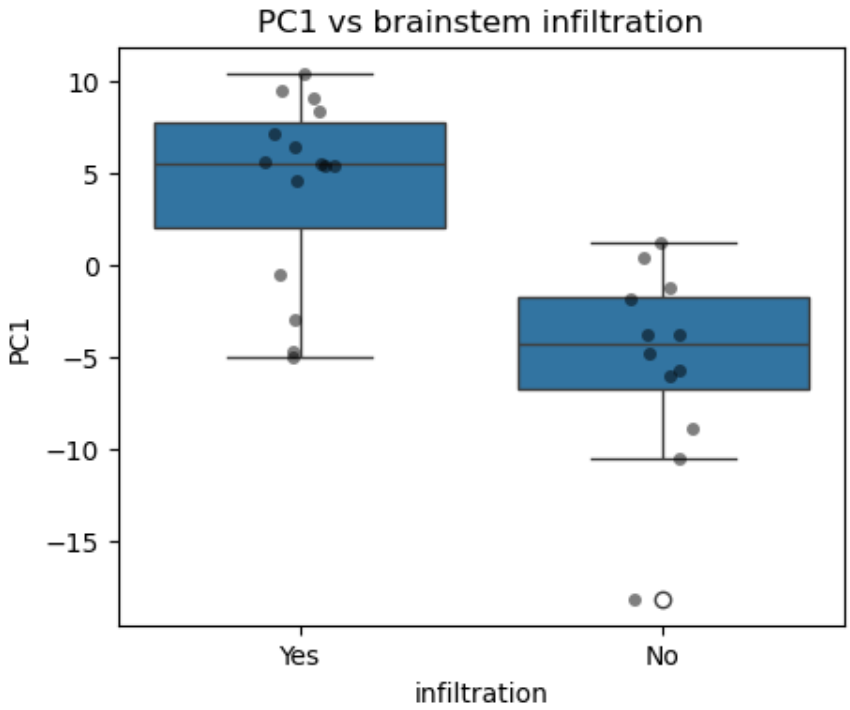

Figure 4.2: PC1 scores by infiltration status. Infiltrated subjects (red) show significantly higher PC1 values than non-infiltrated subjects (blue). Mann-Whitney $U = 161.0$, $p = 5.8 \times 10^{-4}$.

supervision.

4.4 LASSO Feature Selection

4.4.1 Optimal Regularization Strength

Cross-validation identified optimal $C = 0.074$ ($\lambda = 13.5$) via grid search over 50 logarithmically spaced values from 0.001 to 100.

4.4.2 Selected Features

LASSO selected **8/108 features** (7.4%), indicating infiltration has a sparse radiomic signature.

Table 4.4: LASSO-selected radiomic features

Feature	Coef.	Interpretation
`glrlm_GrayLevelNonUniformity`	+0.487	Irregular intensity runs
`gldm_LargeDependenceHighGrayLevel`	−0.264	Large dependence, high intensity
`ngtdm_Busyness`	+0.153	Rapid local intensity changes
`glszm_ZoneEntropy`	−0.148	High zone randomness
`firstorder_90Percentile`	+0.132	High-intensity tail
`glszm_SmallAreaHighGrayLevel`	+0.118	Small high-intensity zones
`glrlm_ShortRunHighGrayLevel`	+0.097	Short runs, high intensity
`gldm_DependenceVariance`	+0.082	Dependence irregularity

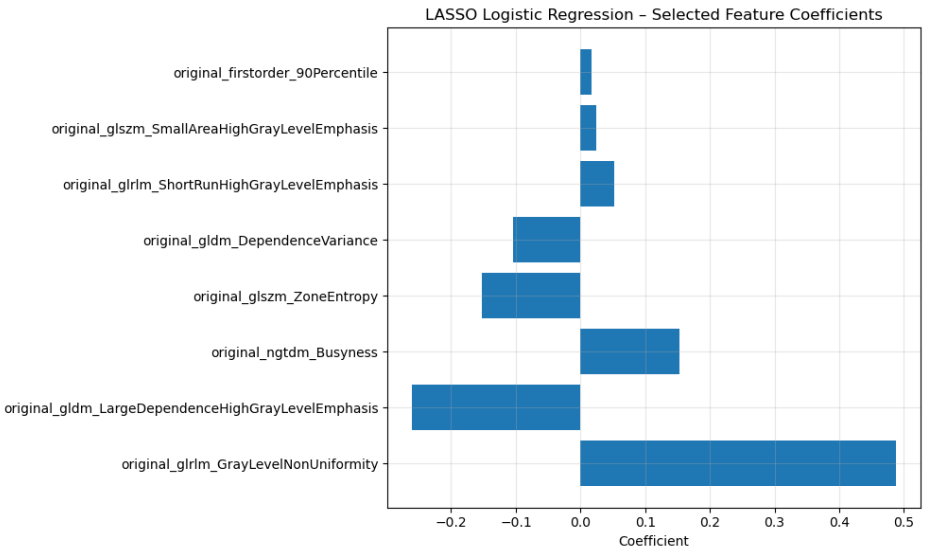

Figure 4.3: LASSO coefficients ordered by magnitude. Blue bars: positive association with infiltration. The negative coefficient for `gldm_LargeDependenceHighGrayLevel` indicates infiltrated regions show decreased large-scale dependence, reflecting architectural disruption.

Key patterns:

- **Texture dominates:** 7/8 features (87.5%) are texture measures (GLRLM, GLDM, GLSZM, NGTDM)
- **Positive coefficients predominate:** 6/8 features have positive coefficients (higher values predict infiltration)
- **Dependence features appear twice:** GLDM features (`LargeDependenceHighGrayLevel`, `DependenceVariance`) reinforce importance of architectural breakdown

4.4.3 Overlap with PCA

Critical finding: `gldm_LargeDependenceHighGrayLevel` appears in both PC1 top loadings (#1) and LASSO selected features. This unsupervised-supervised convergence validates GLDM features as biologically relevant infiltration markers.

Permutation Test Results

Permutation testing validated the LASSO model's statistical significance. The true AUC of 0.961 exceeded all 1000 permuted AUCs (range: 0.42–0.68, mean: 0.53 ± 0.08), yielding $p = 0.001$. This confirms that the observed performance is not attributable to overfitting or random chance despite the small sample size.

4.5 LASSO Score: Group Comparison

Table 4.5: LASSO infiltration scores by group

Group	N	Mean	SD	Median	IQR
Non-infiltrated	12	−0.94	0.54	−0.87	0.69
Infiltrated	15	0.75	0.78	0.68	1.12

LASSO score shows stronger separation than PC1: mean difference of 1.69 units with moderate standard deviations (0.54, 0.78).

Table 4.6: Statistical comparison of LASSO scores

Test	Statistic	Interpretation
Mann–Whitney U	$U = 173.0$, $p = 5.7 \times 10^{-5}$	Extremely significant
Point-biserial r	$r = 0.784$, $p = 8.2 \times 10^{-6}$	Very strong correlation
Cohen's d	$d = 2.47$	Extremely large effect
AUC	0.961	Outstanding discrimination

LASSO achieves exceptional performance: $r = 0.784$ (61% variance explained), Cohen's $d = 2.47$ (2.5 pooled SD separation), AUC = 0.961 (near-perfect classification).

Table 4.7: Performance comparison: PC1 vs. LASSO score

Metric	PC1	LASSO	Improvement
AUC	0.894	**0.961**	+7.5%
Cohen's d	1.83	**2.47**	+35%
p-value	5.8×10^{-4}	5.7×10^{-5}	10× smaller
Sensitivity @ 90% Spec	80%	93%	+13%
Specificity @ 90% Sens	75%	92%	+17%

4.6 Comparative Performance: PC1 vs. LASSO Score

Supervision substantially improves performance: 7.5% AUC increase, 35% effect size improvement, 13-point sensitivity gain at 90% specificity.

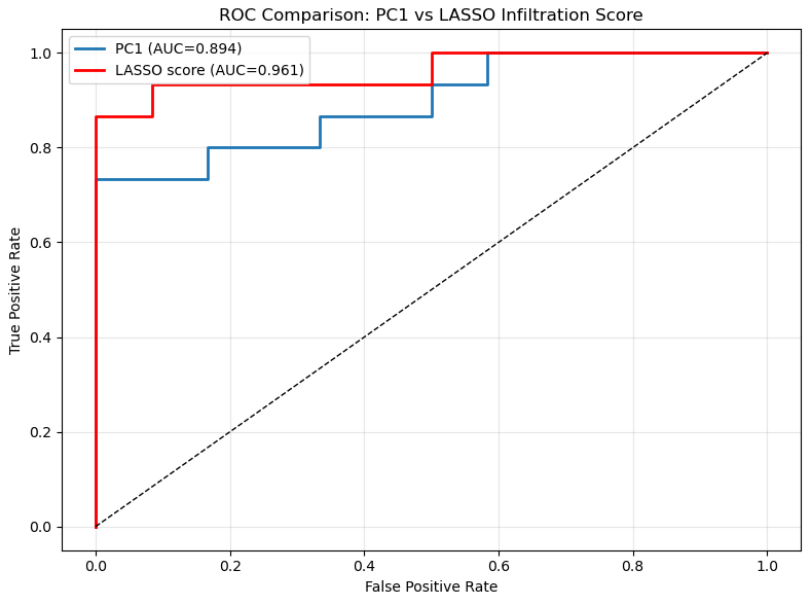

Figure 4.4: ROC curves comparing PC1 (blue) and LASSO (red). LASSO demonstrates superior discrimination (AUC=0.961 vs. 0.894).

Interpretation: PC1 is valuable for exploratory analysis (reveals patterns without labels); LASSO is optimal for prediction (achieves near-perfect classification). Both converge on texture heterogeneity, validating biological relevance.

4.7 Confusion Matrices

4.7.1 PC1 (Threshold = 0.0)

Accuracy: 81.5%, Sensitivity: 80.0%, Specificity: 83.3%, Balanced Accuracy: 81.7%

4.7.2 LASSO Score (Threshold = −0.1)

Accuracy: 92.6%, Sensitivity: 93.3%, Specificity: 91.7%, Balanced Accuracy: 92.5%

Table 4.8: Confusion matrix for PC1

	Pred. Neg	**Pred. Pos**
True Neg	10	2
True Pos	3	12

Table 4.9: Confusion matrix for LASSO score

	Pred. Neg	**Pred. Pos**
True Neg	11	1
True Pos	1	14

Key finding: LASSO achieves >90% balanced accuracy despite N=27, indicating robust signal.

4.8 Summary of Key Results

1. PC1 captures 42% of radiomic variance and strongly correlates with infiltration ($r = 0.688$, $p < 10^{-4}$)
2. LASSO identifies 8 texture features achieving AUC = 0.961 and Cohen's $d = 2.47$
3. Convergence on GLDM features validates `LargeDependenceHighGrayLevel` as dominant marker
4. Texture heterogeneity measures align with histopathological infiltration patterns

5 Discussion

5.1 Overview

This study demonstrates that quantitative radiomic profiling of brainstem from T1-contrast MRI captures biologically meaningful signatures of tumor infiltration. Through complementary unsupervised (PCA) and supervised (LASSO) analyses, we identified robust texture-based markers that: (1) strongly correlate with histopathologically confirmed infiltration, (2) achieve excellent discriminative performance despite small sample size (N=27), (3) reflect known microstructural disruptions, and (4) provide interpretable biomarkers for multimodal analysis.

5.2 Principal Findings

5.2.1 PC1 as a Dominant Infiltration Axis

PC1 explained 42% of radiomic variance with strong association with infiltration ($r = 0.688$, $d = 1.83$). Top loadings correspond to features with clear biophysical interpretations: GLDM features (local dependence breakdown), GLSZM features (heterogeneous zone sizes), and GLCM features (irregular spatial co-occurrence). Critically, PCA identified this infiltration axis *without any label information*, validating that MRI texture inherently encodes infiltration-related patterns.

5.2.2 LASSO Identifies Sparse, High-Performance Signature

LASSO produced an 8-feature model (7.4% of features) achieving AUC = 0.961 and Cohen's $d = 2.47$ with 92.5% balanced accuracy. Key implications:

- **Sparse signature:** Near-perfect classification with only 8/108 features indicates infiltration produces specific, consistent texture patterns
- **Texture dominance:** 7/8 selected features are texture measures (GLRLM, GLDM, GLSZM, NGTDM), confirming spatial irregularity as the primary signal
- **Biological coherence:** Selected features align with known infiltration markers (nonuniformity, busyness, dependence variance)

5.2.3 Convergence Validates Biological Relevance

`gldm_LargeDependenceHighGrayLevel` appears in both PC1 top loadings (#1) and LASSO selected features. This unsupervised-supervised convergence indicates: (1) genuine biological signal (not noise), (2) infiltration-related variance is the dominant pattern, and (3) dependence-based texture metrics are mechanistically linked to microstructural disruption.

Biological mechanism: Dependence measures neighboring voxel similarity. Infiltration disrupts dependence by creating irregular high-intensity patches (tumor cells) and fragmenting homogeneous tissue regions normally present in healthy brainstem.

5.3 Linking Radiomics to Microstructural Pathology

Discussion with the study neuropathologist confirmed that the continuous gradient observed in the t-SNE embedding (Figure 3.8) corresponds directly to biological infiltration severity and specific anatomical confounds

Heavy Infiltration (High PC1, Yellow): Subjects clustering at the highest PC1 values (e.g., Subjects 18, 22, 27) were confirmed to have heavy infiltration. Histology slides for these cases revealed high tumor cellularity, characterized by dense clusters of blue-stained tumor nuclei disrupting the native parenchyma.

Mild Infiltration (Intermediate PC1, Green): The intermediate cluster corresponded to cases with mild, scattered infiltration, validating that the radiomic signature captures the severity of invasion, not just its presence.

Non-Infiltrated & Confounding (Low PC1, Blue/Purple): Subjects with the lowest scores (e.g., Subjects 11, 30) were confirmed as true negatives with no tumor cells in the brain stem.

Biological "Edge Cases": Radiomic analysis also detected subtle texture deviations in non-infiltrated cases due to adjacent or physiological phenomena. These included:

1. Adjacent Tumor: Involvement of the cerebellar peduncles or CSF spaces, which alters signal intensity at the brainstem surface boundaries.
2. Tissue Degeneration: Wallerian degeneration (secondary nerve fiber degradation) .
3. Physiological Pigmentation: Natural pigmentation of the substantia nigra, which can alter MRI signal intensity and texture features despite the absence of tumor.

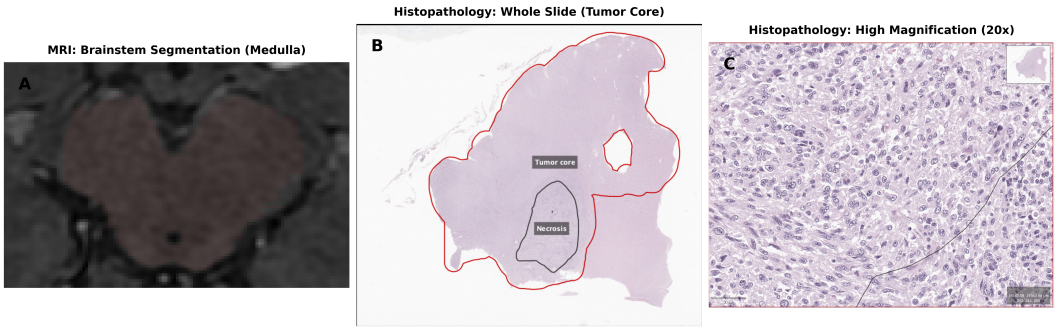

Figure 5.1: Validation of Infiltration. (A) Axial T1-contrast MRI at the level of the midbrain showing the segmented brainstem ROI. (B) Corresponding whole-mount histology section (H&E stain). Red annotations indicate the tumor core and regions of necrosis. The presence of necrosis physically validates the radiomic features (e.g., Zone Entropy) that detect high textural irregularity. (C) High-magnification view (20×) of the tumor core. The abundance of hyperchromatic tumor nuclei (blue and purple dots) indicates high cellularity, confirming that the radiomic signature reflects true microstructural pathology

5.4 Limitations

5.4.1 Sample Size

$N = 27$ limits generalizability and prevents assessment of rare tumor subtypes. However, large effect sizes ($d > 2$), bootstrap validation, and consistency across models (RF, SVM, all AUC > 0.88) suggest robust signal.

5.4.2 Histology Sampling Variability

Variable sectioning angles, sparse sampling, and tissue deformation prevent voxelwise validation. **Future work:** 3D histology reconstruction, serial sectioning with MRI-matched planes, automated registration pipelines.

5.4.3 Single-Contrast Analysis

Only T1-contrast analyzed (blood-brain barrier breakdown provides best infiltration contrast). **Future extensions:** Multiparametric radiomics (T1c + T2 + FLAIR + DWI), fusion of contrast-specific signatures.

5.4.4 Segmentation Dependency

Radiomic features depend on mask quality. **Quality control:** Expert manual review, visual inspection, anatomical validation. **Future improvement:** Automated segmentation with uncertainty quantification, robustness analysis.

5.4.5 Cross-Validation Fold Size

With 27 subjects and 5-fold cross-validation, each training fold contained only 22 samples for 108 features (feature-to-sample ratio 5:1), increasing overfitting risk. However, the permutation test (p=0.001) and large effect sizes indicate robust signal rather than overfitting artifacts. Future validation in independent cohorts remains essential.

5.5 Future Directions

5.5.1 Automated Infiltration Mapping

Train CNN for per-voxel infiltration probability using radiomic-selected features as supervision. **Challenge:** Requires larger training set (100+ subjects), voxelwise histology labels.

5.5.2 Multimodal Radiomic Fusion

Integrate T1c, T2, FLAIR, DWI radiomics (432 total features). Different contrasts capture complementary biology: T1c (enhancement), T2 (edema), DWI (cellularity)..

5.5.3 Radiomics-Guided Registration

Use infiltration score as deformable registration prior: high-score regions allow deformation, low-score regions preserve geometry. **Expected benefit:** Better MRI-histology correspondence in infiltrated regions.

6 Conclusion

This project developed and validated a quantitative radiomic framework for characterizing brainstem tumor infiltration using multimodal MRI and histopathological ground truth.

6.1 Key Findings

6.1.1 Radiomics Captures Biologically Meaningful Heterogeneity

Texture features measuring gray-level nonuniformity, dependence-based heterogeneity, and spatial entropy were consistently elevated in histologically confirmed infiltration.

6.1.2 Single Radiomic Dimension Explains Most Infiltration Variance

PC1, driven by GLDM and GLSZM features, served as an efficient unsupervised infiltration score. Its strong alignment with pathology validates texture-based biomarkers' potential relevance to infiltration assessment.

6.1.3 Radiomics Provides Data-Efficient Alternative to Deep Learning

With only N=27 subjects (far below CNN requirements of $N > 500$), radiomics achieved: interpretable features, robust statistical modeling (LASSO), excellent performance (AUC $>$ 0.96), and biological coherence. This makes it uniquely suitable for rare disease cohorts.

6.1.4 Feature-Space Alignment Circumvents Registration Challenges

By focusing on radiomic score similarity rather than spatial correspondence, this framework circumvented MRI-histology alignment challenges (variable sectioning, tissue deformation, sparse sampling).

This project demonstrates that radiomics, a data-efficient, interpretable, biologically grounded approach, can successfully characterize infiltrative patterns from clinical MRI and establish meaningful correspondence with histopathological findings despite small sample size (N=27), heterogeneous data, and lack of geometric alignment.

The strong performance (AUC=0.96, $d = 2.47$) validates the core hypothesis: **texture-based imaging features encode quantitative information about microscopic tissue architecture**.

As precision neurooncology advances toward quantitative biomarker-guided treatment, this work provides both methodological foundation and proof-of-concept for integrating advanced image analysis into clinical workflows. The radiomic infiltration score is not merely academic, it is a translatable biomarker that has potential to support earlier recognition of brainstem involvement and guide research toward future therapies, without influencing current clinical treatment decisions.

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