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A Statistically Rigorous Multi-Scale Texture Analysis Framework for 3D Spheroid Characterization: Temporal Autocorrelation Correction and Molecular Validation

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INTRODUCTION & AIM

Background: Three-dimensional tumor spheroid cultures bridge traditional 2D cell cultures and in vivo tumor models, recapitulating essential features including cell-cell interactions, diffusion gradients, and drug penetration barriers. Time-lapse microscopy captures morphological dynamics continuously, but existing analysis pipelines treat observations as independent samples—violating fundamental statistical assumptions when temporal autocorrelation exists.

The Gap: No current framework combines: (1) multi-scale texture integration with variance normalization, (2) temporal autocorrelation characterization with appropriate resampling strategies, and (3) external biological validation.

Aim: To develop and validate a statistically rigorous computational framework for autocorrelation-corrected texture analysis of 3D spheroid time-series, enabling valid hypothesis testing in longitudinal imaging studies.

METHOD

Cell Lines & Treatment:
- A549 (epithelial phenotype) and H1299 (mesenchymal phenotype) NSCLC
- 50 μM capecitabine treatment vs. Control
- 48 hourly timepoints per condition (4 conditions total)

Texture Feature Extraction (37 features):
- Gray-Level Co-occurrence Matrix (GLCM): 24 features
- Discrete Wavelet Transform: 7 features
- Gabor Filters: 6 features

Statistical Framework:
- Global standardization (z-score across 192 observations)
- Autocorrelation function analysis → 4-hour decorrelation time
- Block bootstrap resampling (5-hour blocks, 10,000 iterations)
- Fisher Linear Discriminant Analysis for feature ranking
- Bonferroni-corrected significance (α = 0.001351)

External Validation:
- Cancer Dependency Map (DepMap) RNA-sequencing
- VIM/CDH1 ratio as EMT marker (1,699 cell lines)

RESULTS & DISCUSSION

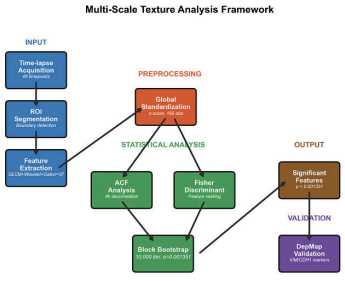
1. Baseline Comparison

Mean pixel intensity compared against multi-scale texture features:

A549 (Epithelial):
- Mean intensity: F = 0.10, Cohen's d = 0.45
- Best texture: F = 0.46 (detail_d1_variance)
- **Improvement: 4.5-fold**

H1299 (Mesenchymal):
- Mean intensity: F = 0.03, Cohen's d = 0.22
- Best texture: F = 7.72
- **Improvement: ~300-fold**

Critical: Mean intensity alone suggests A549 responds MORE (F=0.10 > 0.03), contradicting texture analysis. Intensity-only analysis yields misleading conclusions.



Pipeline Schematic

2. Discrimination Capacity

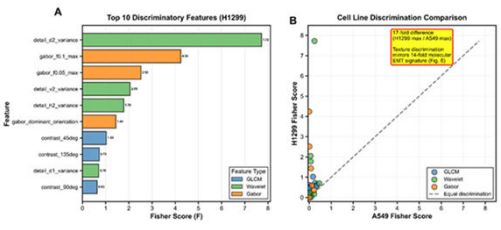
Fisher discriminant analysis quantified treatment discrimination:

H1299 (Mesenchymal):
- Maximum Fisher score: F = 7.72
- Significant features: 17/37 (46%)
- Strong discrimination capacity

A549 (Epithelial):
- Maximum Fisher score: F = 0.46
- Significant features: 6/37 (16%)
- Moderate discrimination capacity

Discrimination Ratio:
H1299/A549 = 7.72 / 0.46 = 16.8-fold

Mesenchymal cells exhibit greater morphological plasticity, consistent with known EMT-associated phenotypic flexibility.



Fisher Score Comparison

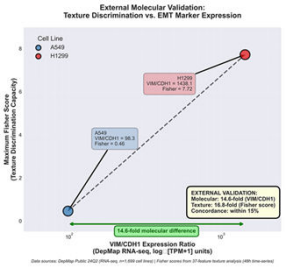
3. Molecular Validation

External validation using DepMap RNA-sequencing:

VIM/CDH1 Expression Ratios:
- H1299: 1,438 (strongly mesenchymal)
- A549: 98 (epithelial-biased)
- **Molecular ratio: 14.6-fold**

Correspondence:
- Texture discrimination ratio: 16.8×
- Molecular marker ratio: 14.6×
- **Agreement within 15%**

Validation Strength
Three forms of independence:
1. Sample independence (different passages)
2. Measurement independence (microscopy vs RNA-seq)
3. Temporal independence (2024 vs DepMap 2019)



DepMap Validation

KEY FINDINGS

- Texture analysis achieves up to 300-fold improvement in discrimination over mean intensity baseline
- Block bootstrap with 5-hour blocks accounts for 4-hour temporal autocorrelation in time-series data
- 16.8-fold texture discrimination ratio corresponds to 14.6-fold molecular marker ratio (within 15%)
- Mean intensity alone yields misleading biological conclusions; multi-scale texture is required

CONCLUSIONS

1. This framework enables statistically valid inference from temporally autocorrelated 3D culture time-series through block bootstrap resampling.

2. Texture discrimination capacity corresponds quantitatively to molecular EMT marker differences, validating biological relevance.

3. The modular design allows integration with existing feature extraction platforms (CellProfiler, Traject3D, etc.).

4. Framework is immediately applicable to high-content screening platforms and spheroid/organoid characterization.

FUTURE WORK / REFERENCES / ACKNOWLEDGMENT

Future Directions:
- Biological replication (n≥3 per condition, multiple cell lines)
- Dose-response studies across drug concentrations
- Extension to breast cancer (MCF7) and other cancer types
- Machine learning classifiers for multi-group comparisons

Key References:
1. Haralick RM et al. (1973) IEEE Trans SMC 3:610-621 2. Künsch HR (1989) Ann Stat 17:1217-1241
3. Ghaffarizadeh A et al. (2018) PLoS Comput Biol 14:e1005991 4. Barretina J et al. (2012) Nature 483:603-607 [DepMap]

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Data Availability: Code available upon publication. **DOI: https://doi.org/10.1038/s41598-026-51722-5**