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Morphologic Analysis of Glioblastoma Identifies Morphology-Driven Clusters and Molecular Correlates Associated With Patient Survival

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In Silico research using public data sources

histology
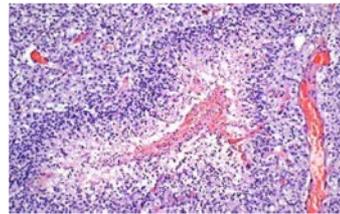
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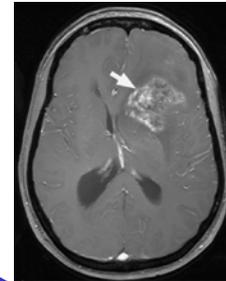
molecular
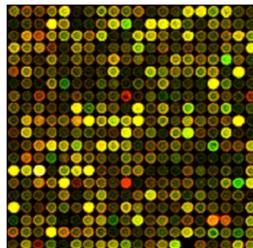
clincal\pathology

Integrated Analysis
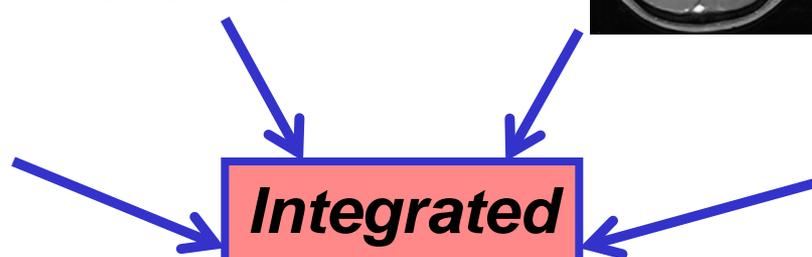
TCGA and whole slide imaging
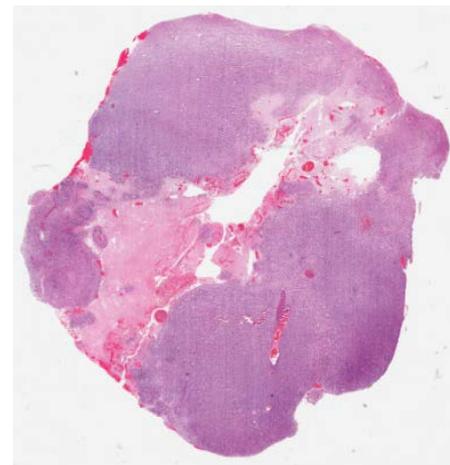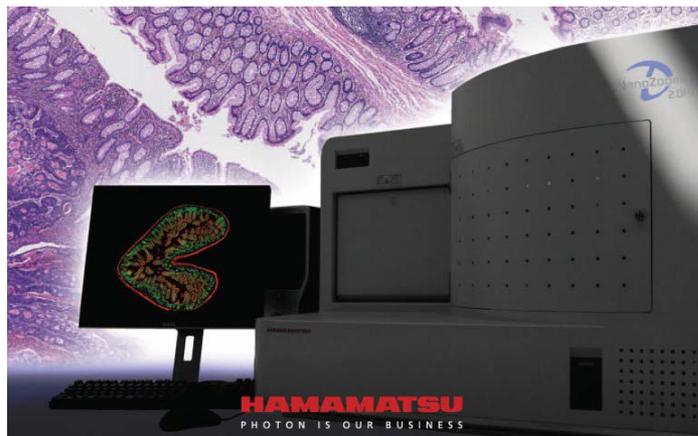
- Scans of frozen tissue associated with molecular studies

- Scans of diagnostic-block permanent sections

- 20X magnification (40X possible?)

- Pathology evaluations (%necrosis, %tumor nuclei, histology 0,+1,+2)

Glioblastoma morphology

Most Oligo — Oligo-Astro Continuum — Most Astro
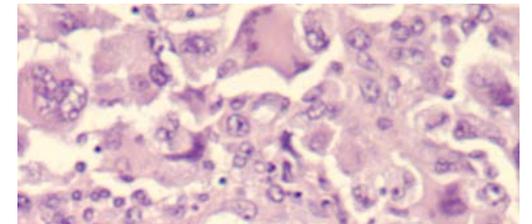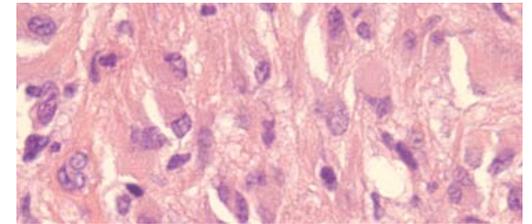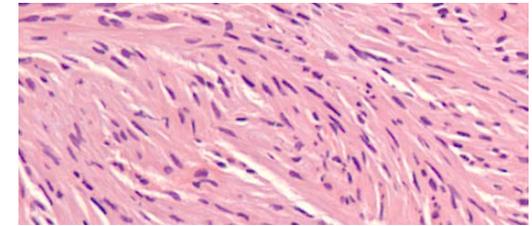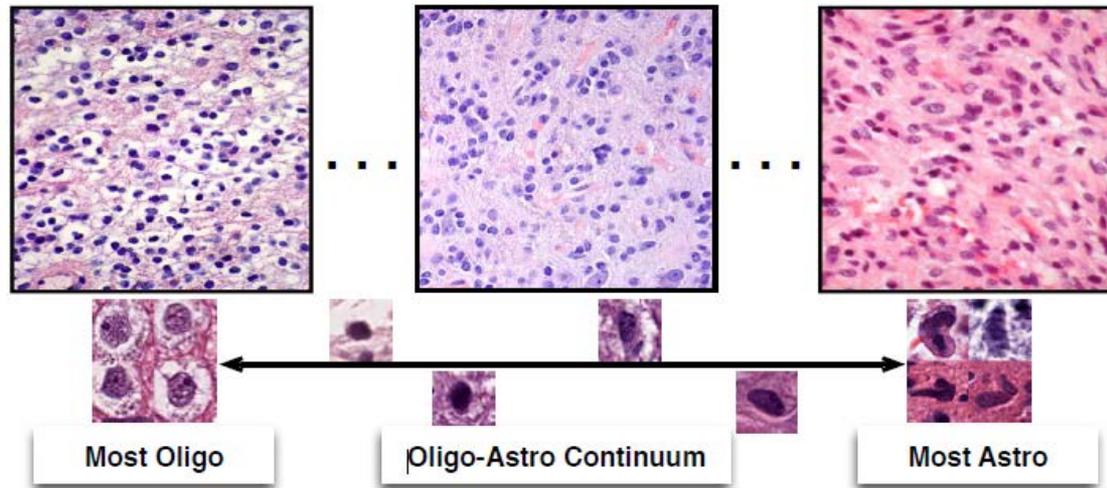
- Cell morphologies
- *Are there clusters of GBM morphology?*
- *Are there morphological links to patient outcome and molecular characteristics?*

Computational Pathology and Correlative Analysis

Morphology Engine

Segmentation · Feature Extraction · PAIS Database · Patient Modeling

Clustering Engine
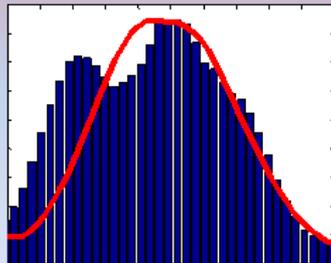
Normalization · Feature Selection · Consensus Clustering · Multidimensional Scaling
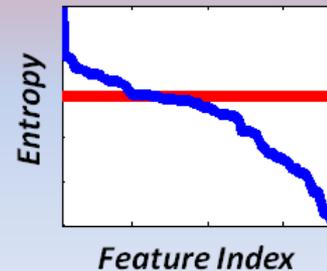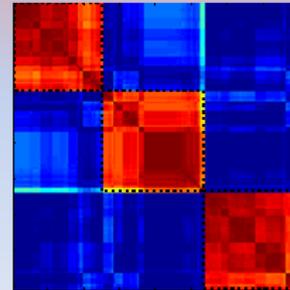
Correlative Engine
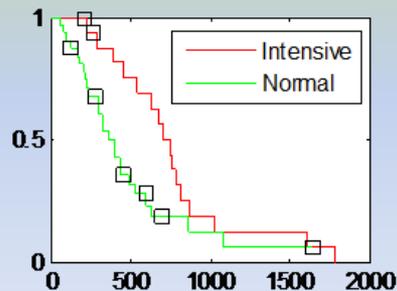
Survival Analysis · Molecular Classes · Human Pathology · Genetic Alterations

Proneural
Classical
Mesenchymal
Proliferative
GCIMP+

TP53 +/-
EGFR Amp.
CDKN2A Del.
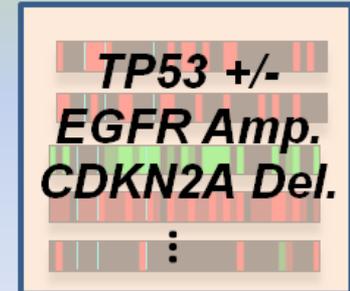
Genome Wide Analysis

Differential Expression · Copy Number · Integrate Expression, · * Gene Ontology and
DNA Methylation · Analysis · Methylation, Genetics · Pathway Analyses

Morphology engine

Morphology Engine

Segmentation   Feature Extraction   PAIS Database   Patient Modeling

f_i Texture Intensity Gradient Morphometry

f_i $E\{f_i\}$

TABLE I
NUCLEAR FEATURES

Category	Features
Morphometry	Area, Perimeter, Eccentricity, Circularity, Major Axis Length, Minor Axis Length, Extent Ratio
Intensity Statistics	Mean Intensity, Max Intensity, Min Intensity, Std. Dev. Intensity
Texture	Entropy, Energy, Skewness, Kurtosis
Gradient Statistics	Mean Grad. Magnitude, Std. Dev. Gradient Magnitude, Entropy Gradient Magnitude, Energy Gradient Magnitude, Skewness Gradient Magnitude, Kurtosis Gradient Magnitude, Sum Canny Pixels, Mean Canny Pixels

Note: Set of 23 features for characterization of nuclei fall into four broad categories.

Clustering engine

Patient Morphology Profiles

Correlative engine and genome wide analysis

Patient Cluster Labels

Clustering identifies three morphological groups

- Analyzed 200 million nuclei from 162 TCGA GBMs

- Named for functions of associated genes: Cell Cycle (CC), Chromatin Modification (CM), Protein Biosynthesis (PB)

- Prognostically-significant (logrank p=4.5e-4)
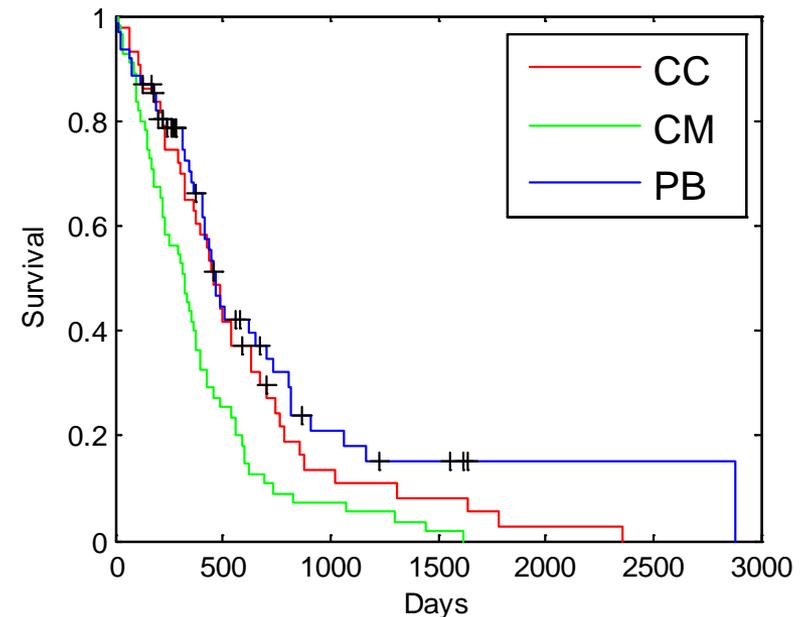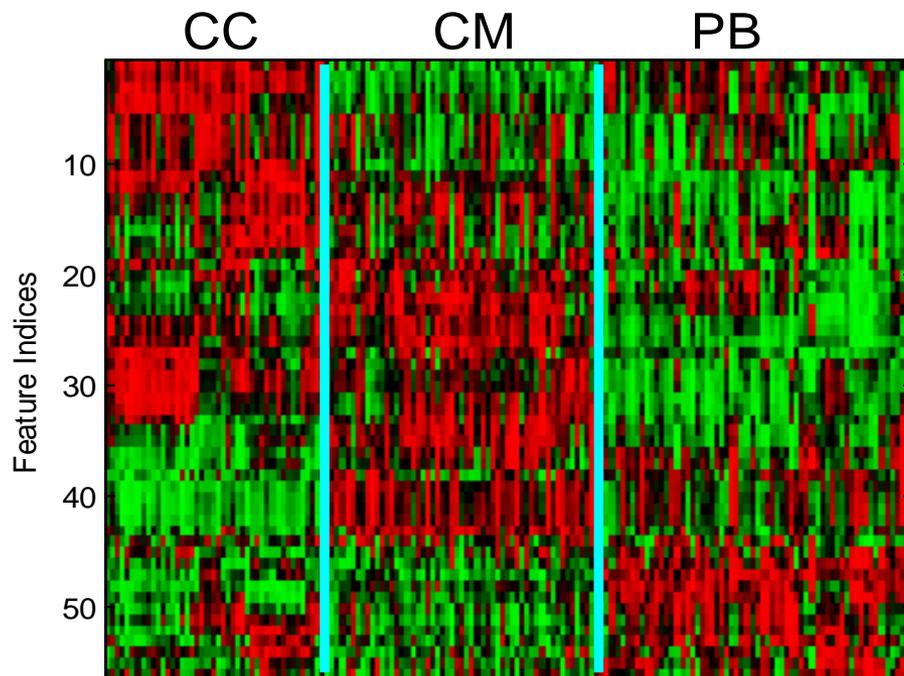
Representative nuclei

CC
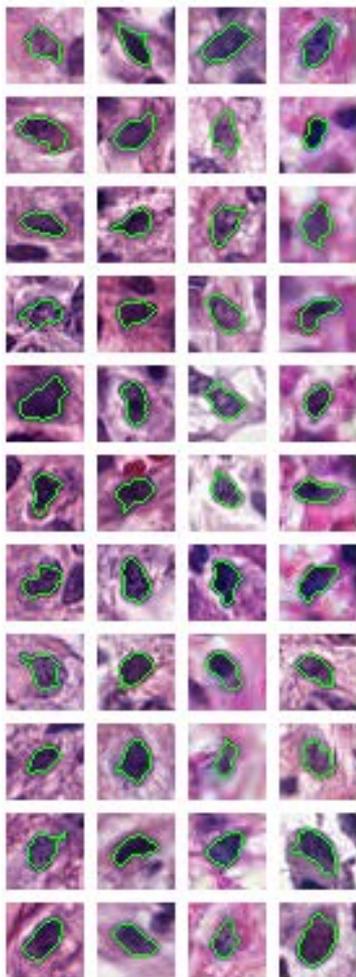
CM
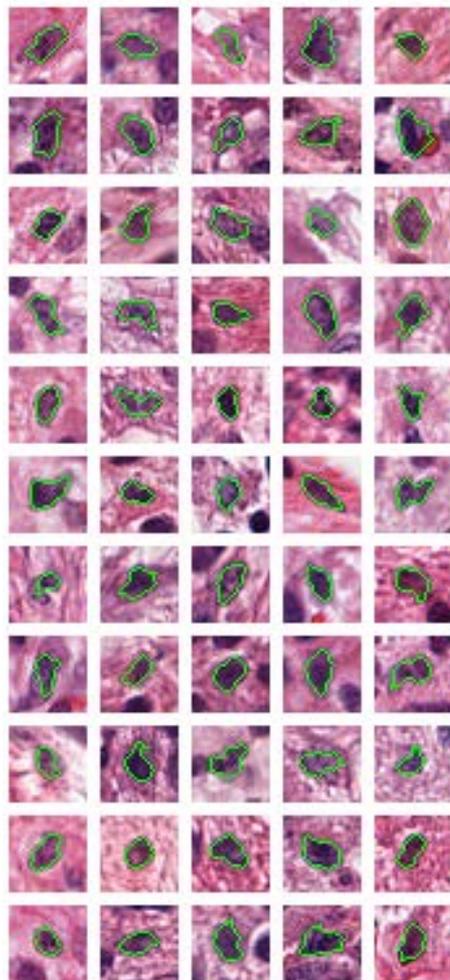
PB
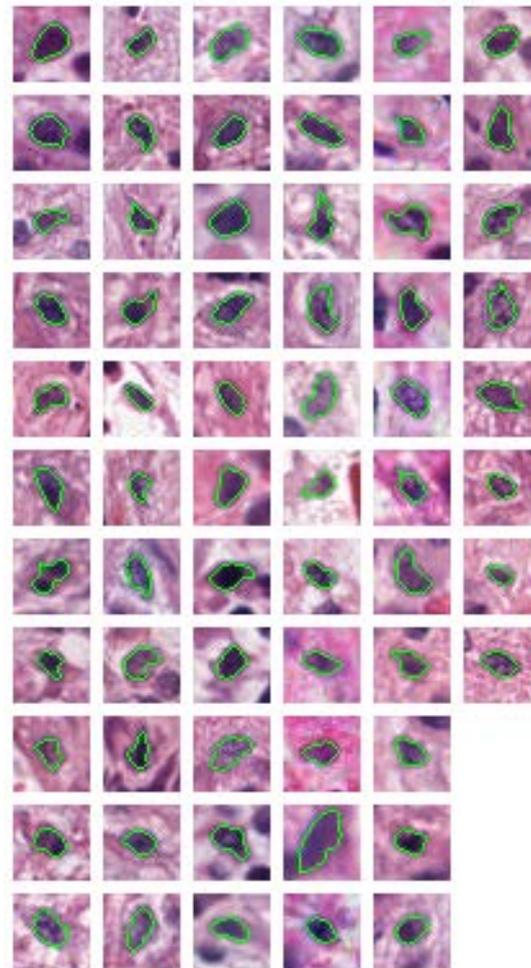
Validation

- Separate set of 84 GBMs from Henry Ford Hospital
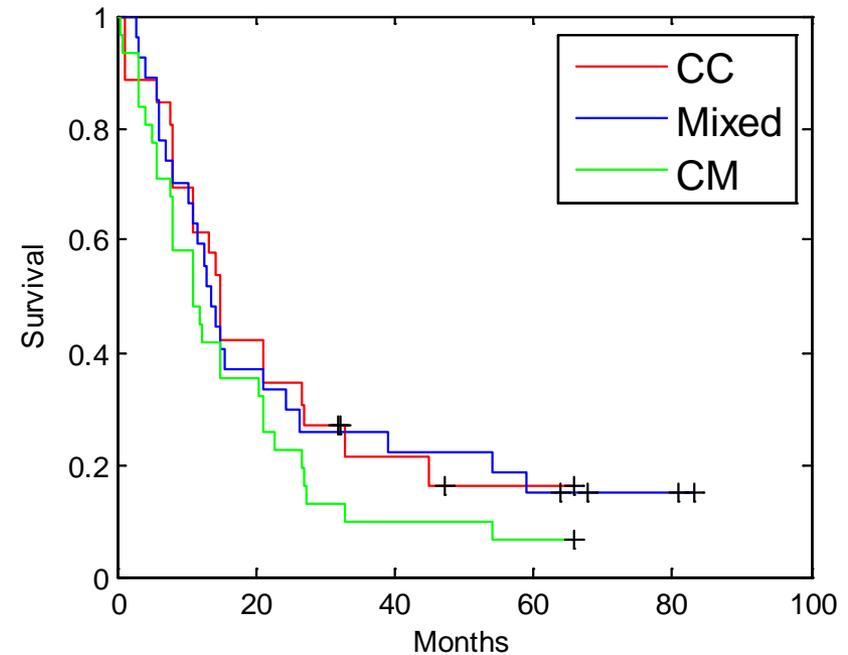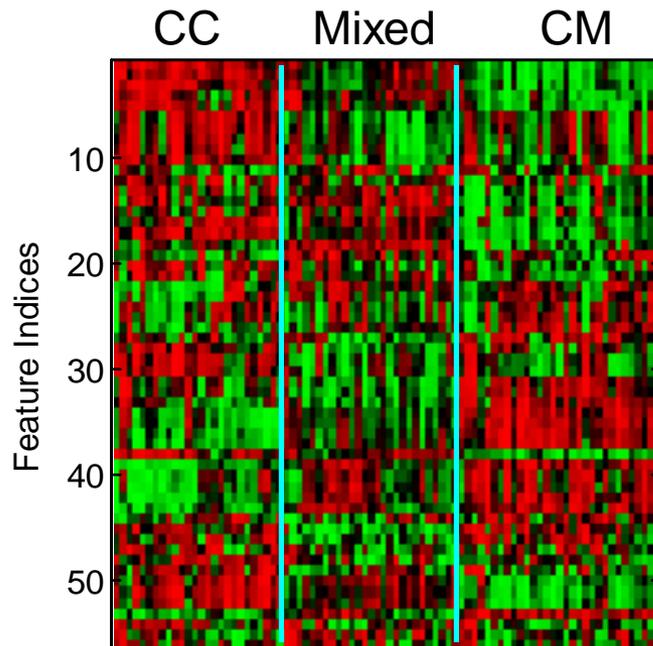
Associations

	CC Cluster	CM Cluster	PB Cluster
Prognosis	Average	Poor	Better
Subtype Associations	Neural Depleted	Neural enriched Proneural Depleted	None
Pathology	Small cells enriched	Lymphocytes enriched	Inflammation depleted
Genetics	*NF1* mutant depleted *TP53* mutant depleted	None	None

Transcriptional class associations
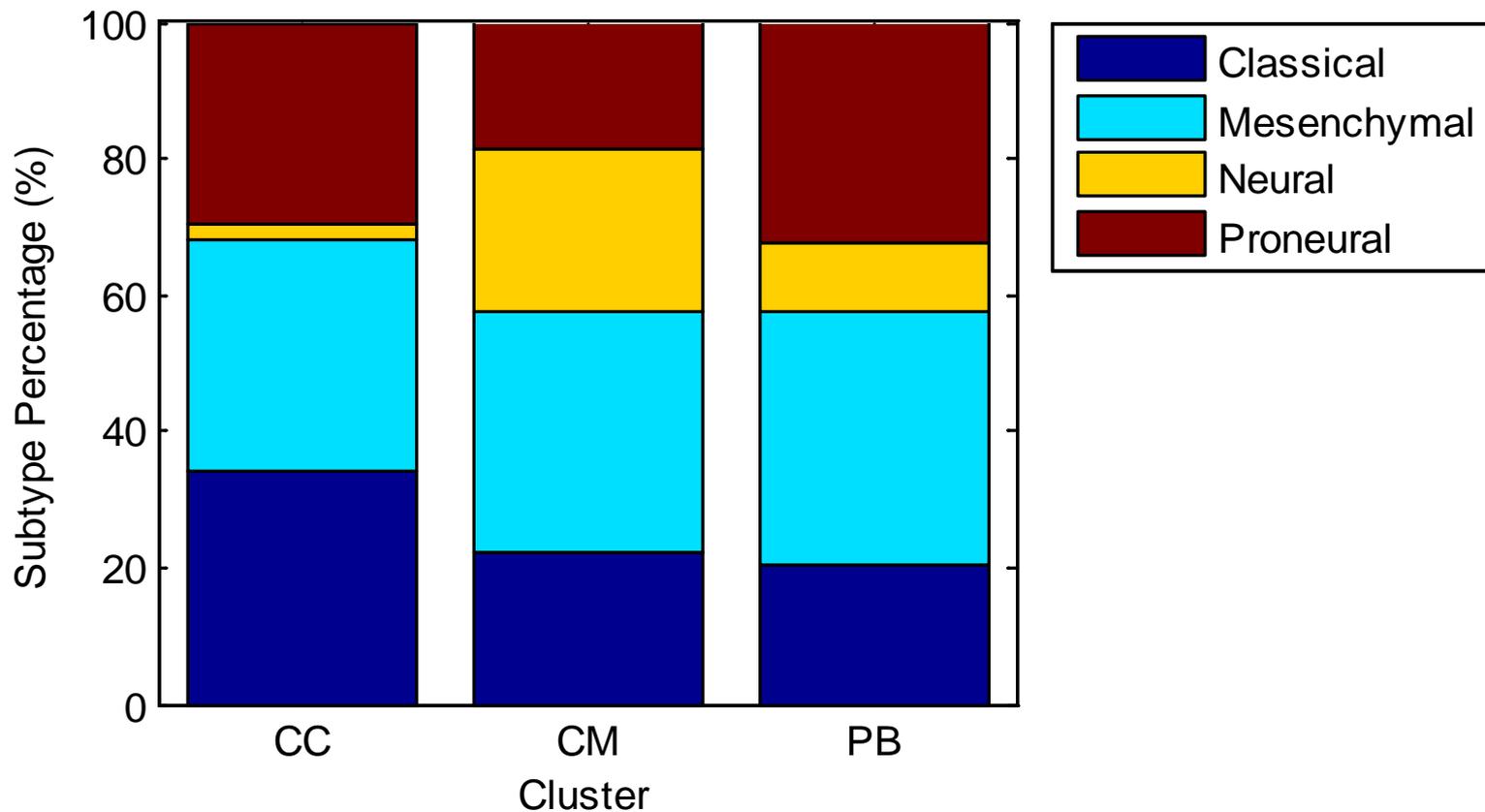
Molecular associations

	CC Cluster	CM Cluster	PB Cluster
Prognosis	Average	Poor	Better
Differential Expression	2740 / 663 Genes up/down 97 / 100 miRNAs up/down	200 / 463 Genes up/down 121 / 81 miRNAs up/down	0 / 188 Genes up/down 15 / 5 miRNAs up/down
Differential Methylation	69 Genes hypermethylated	244 Genes hypermethylated	45 Genes hypomethylated
Copy Number	1068 Deletions 38 Amplifications	301 Deletions 5 Amplifications	399 Deletions 7 Amplifications
Expression Mapping	23 mapped to methylated sites 595 mapped to CNV sites	8 mapped to methylated sites 27 mapped to CNV sites	1 mapped to methylated sites 19 mapped to CNV sites

Gene Ontology and Pathway Analysis

- Nuclear lumen localization most highly enriched in cluster associated genes

  (CC p=2.8e-36, CM p=2.17e-19, PB p=1.08e-15)

- Other enriched GO terms: DNA repair, *cell cycle*, *protein biosynthesis*, *chromatin modification*, m-phase

- Differences in activation of cancer-related pathways including *ATM* and *TP53* DNA damage checkpoints, *NFκB* pathway, *Wnt* signaling and *PTEN/AKT* pathways

Conclusion

- Whole-slide images contain signal

- Image analysis can provide scalable, quantitative measurements of cellular morphology

- Datasets like TCGA present a unique opportunity to correlate morphology with genomics and patient outcome

- Need more complex models to account for heterogeneity

Thank You

- Emory In Silico Brain Tumor Research Center
  NCI Contract HHSN261200800001E

  Joel Saltz, MD, PhD (Director)

  Dan Brat, MD, PhD (Scientific PI)

  David Gutman, MD, PhD

  Jun Kong, PhD

  Lisa Scarpace (Henry Ford Hospital)

  Tom Mikkelsen, MD (Henry Ford Hospital)