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Computational Pathology Software for Integrative Cancer Research with 3D Digital Slides

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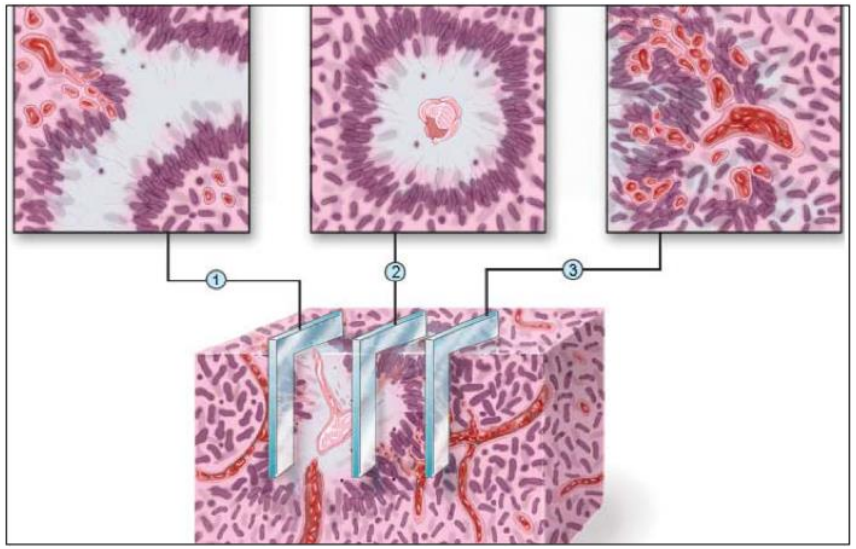
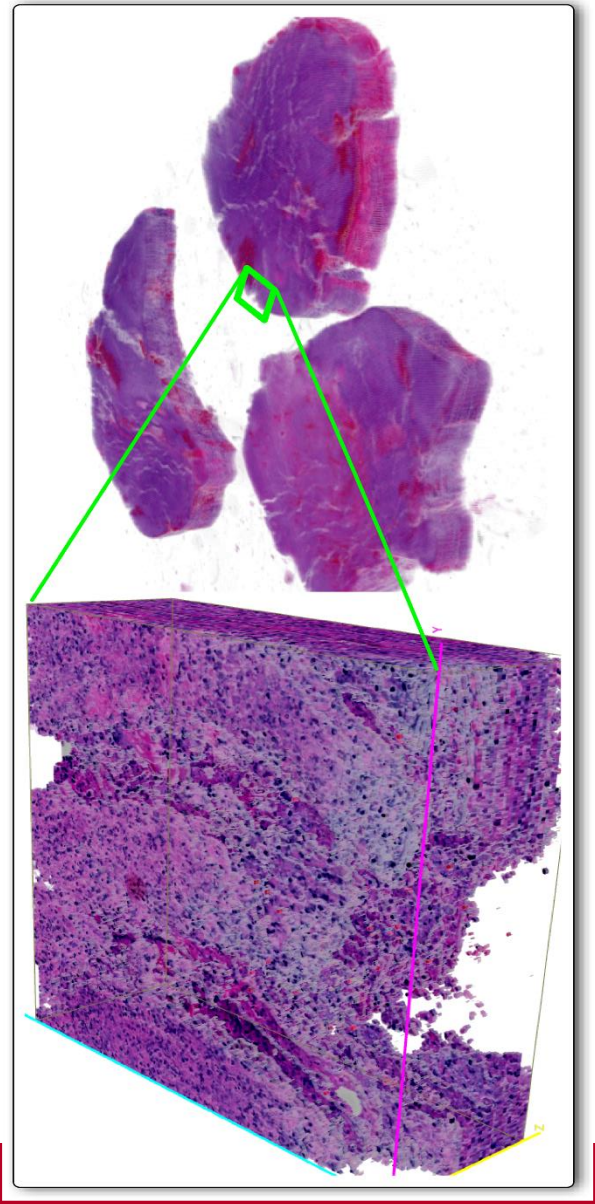
Tissue Based Cancer Research

- High-resolution tissue based pathology image analysis offers a new avenue to describe molecular, cellular and tissue-level interactions
  - Understand tumor heterogeneity and evolution
  - Predict prognosis and develop treatment strategy
- Current barriers
  - Information loss: biased information derived from 2D tissue sections
  - Lack of integration of biomarker and histology
  - TME evolution: static microscopy images will not capture cancer evolution



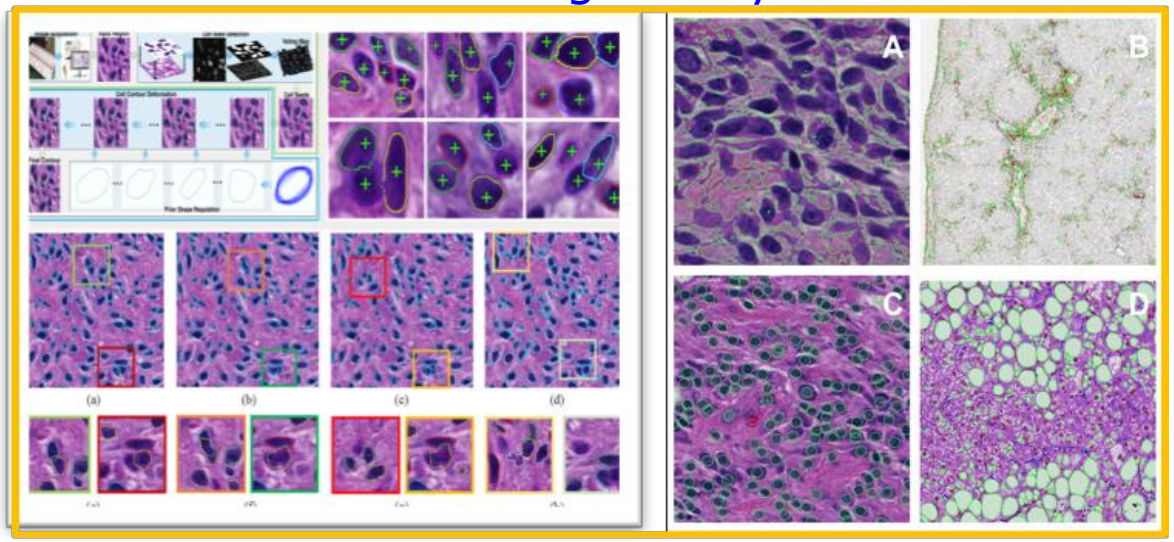
Our Project: 3D Digital Pathology Analytics Framework

- 3D digital pathology imaging data capture both histology hallmarks and molecular biomarkers from both *static* and *dynamic* environments are promising to create longitudinal human tumor atlas, necessary for in-depth TME and its progression study
  - 3D pathology image processing methods: registration segmentation, and reconstruction
  - Spatially align and analyze IHC stained biomarkers with H&E adjacent slides
  - Segment and track cellular compartments in TME using 4D (3D space + time) fluorescent microscopy images
  - Effective and scalable methods for 3D spatial data analytics to discover spatial relationships and patterns of 3D pathology objects and biomarkers

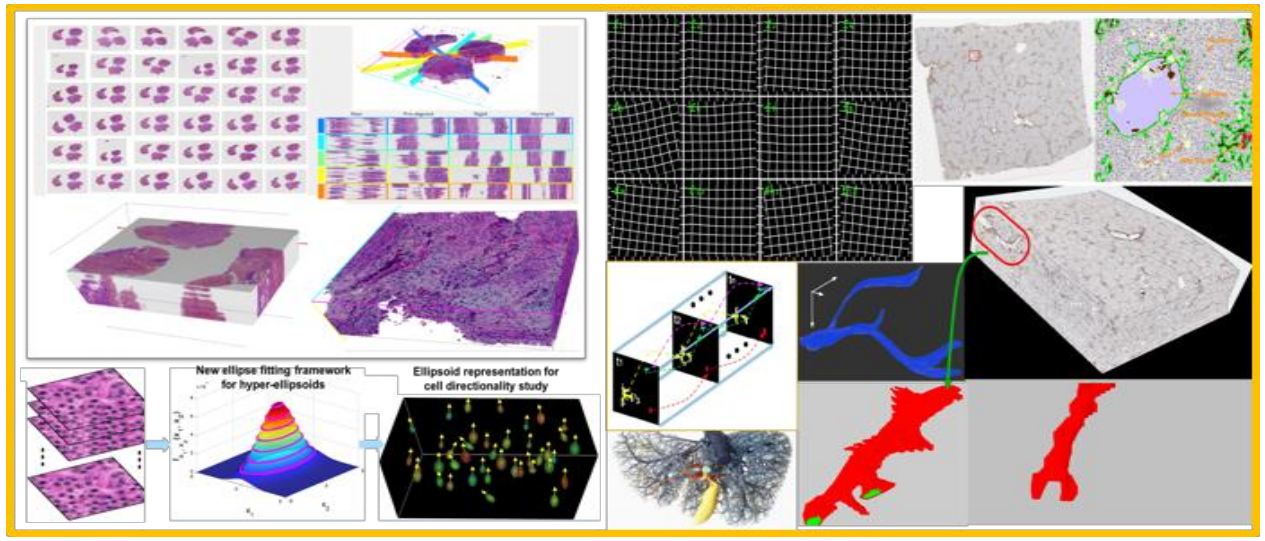


Overview of 3D Pathology Image Analysis and Spatial Analytics

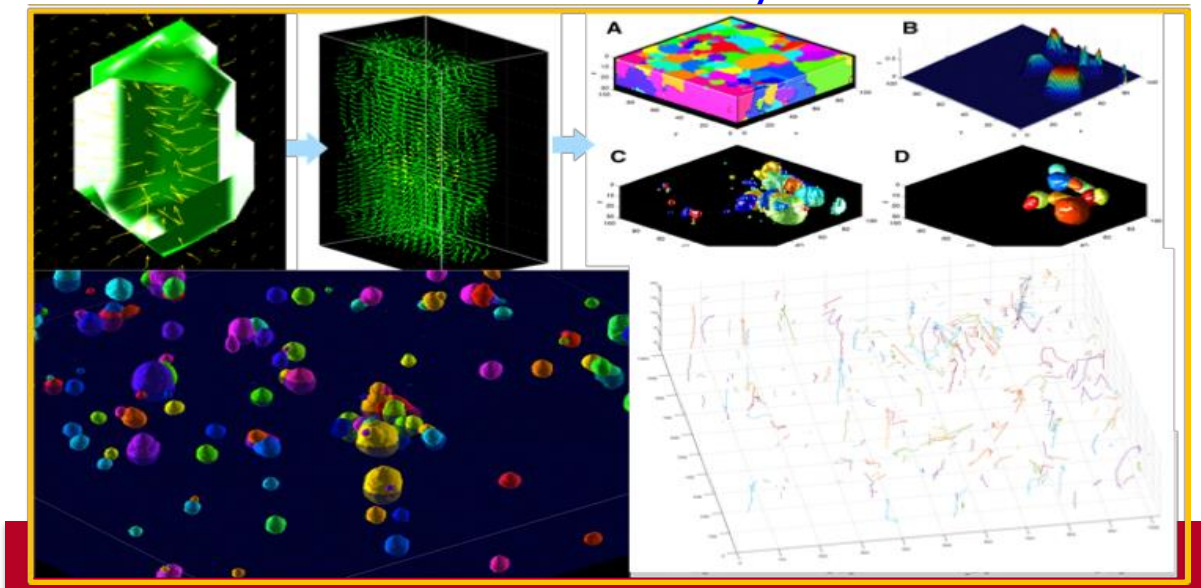
➢ 2D Tissue Slide Image Analysis



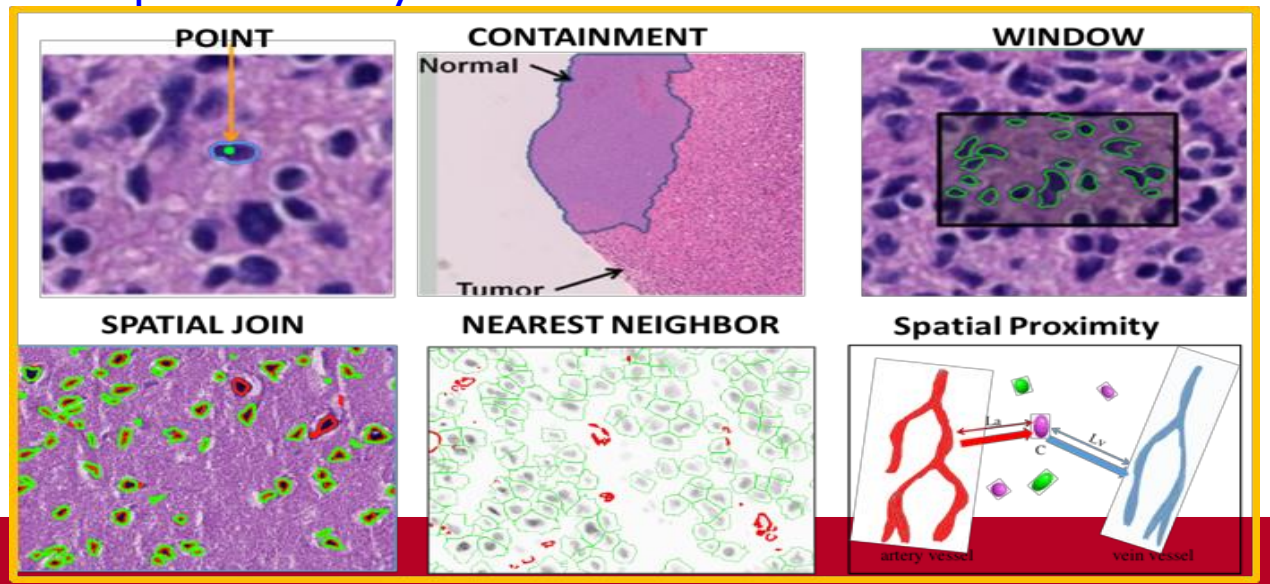
➢ 3D Tissue Slide Image Analysis



➢ Characterization of TME Dynamics

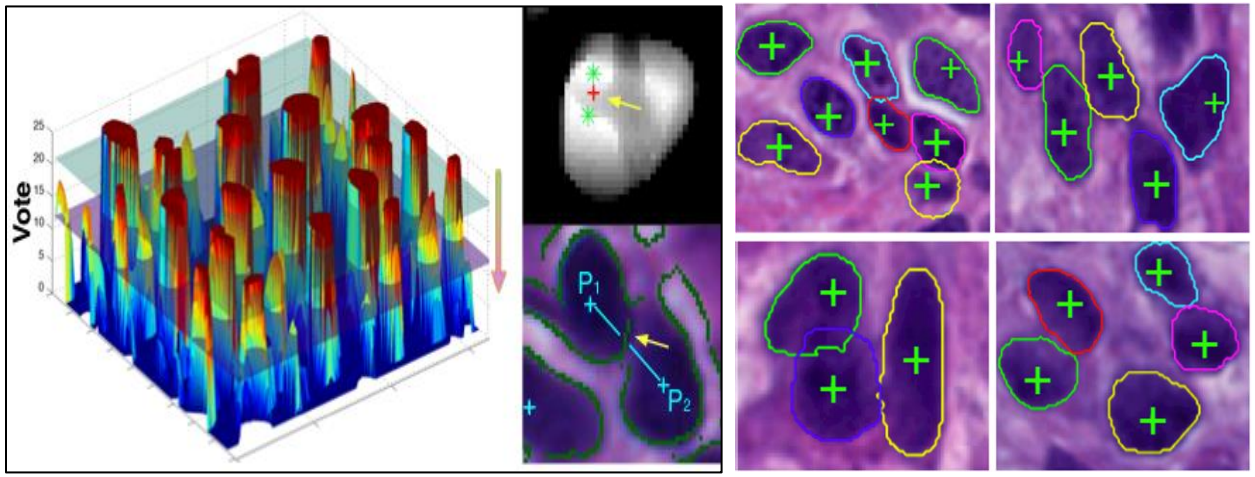


➢ Spatial Analytics

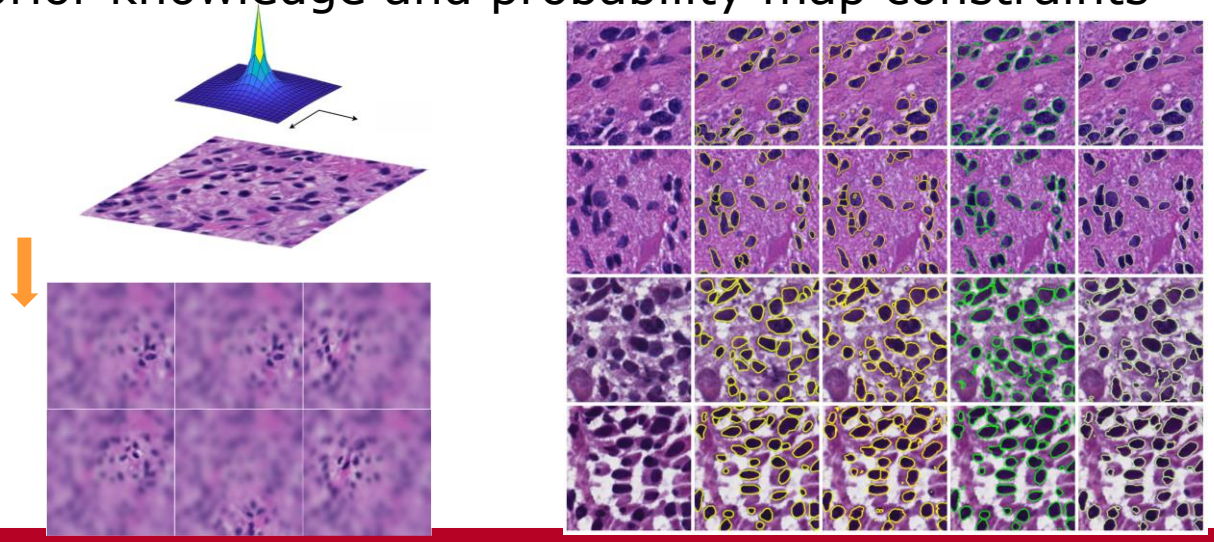


2D Tissue Slide Image Analysis

Level set based nuclei segmentation

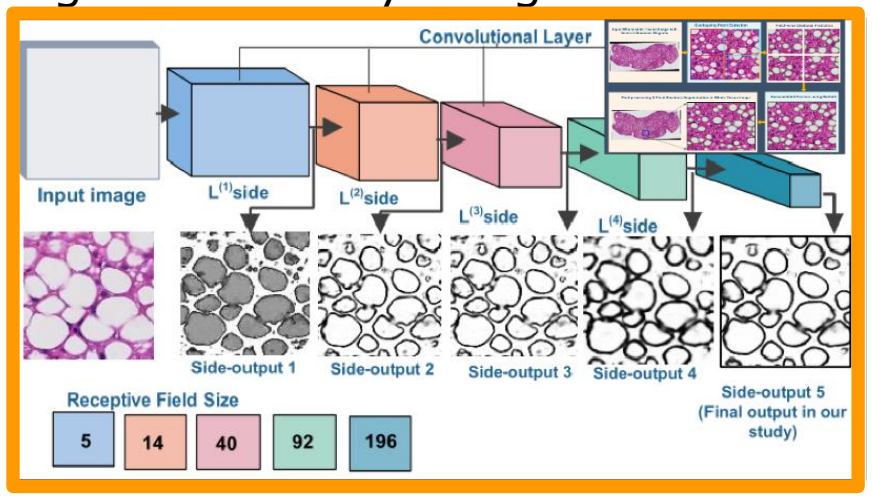


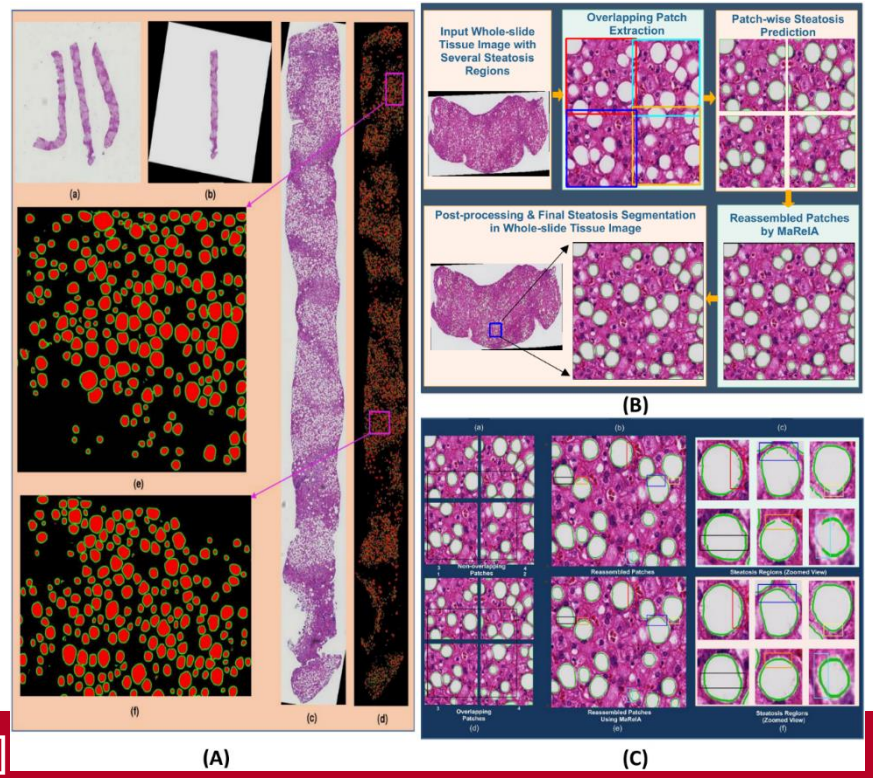
Foveal Blur boosted segmentation of nuclei with shape prior knowledge and probability map constraints



[Bioinformatics2021]

Deep learning based steatosis quantification with region-boundary integrated network

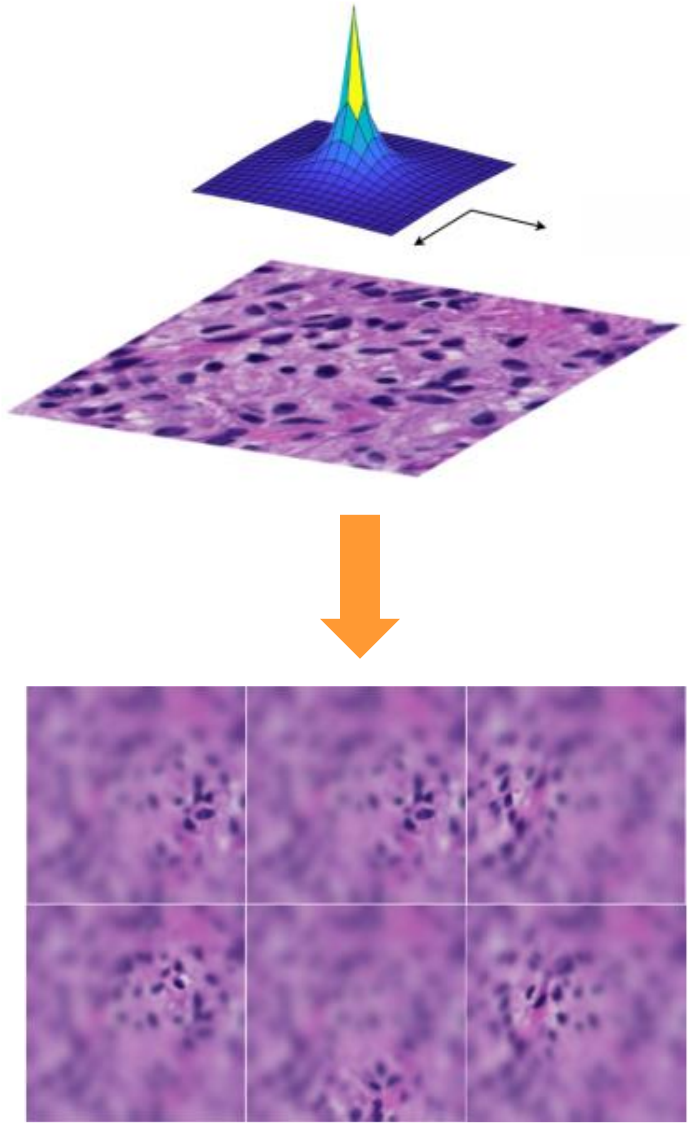




[Lab Invest 2020]

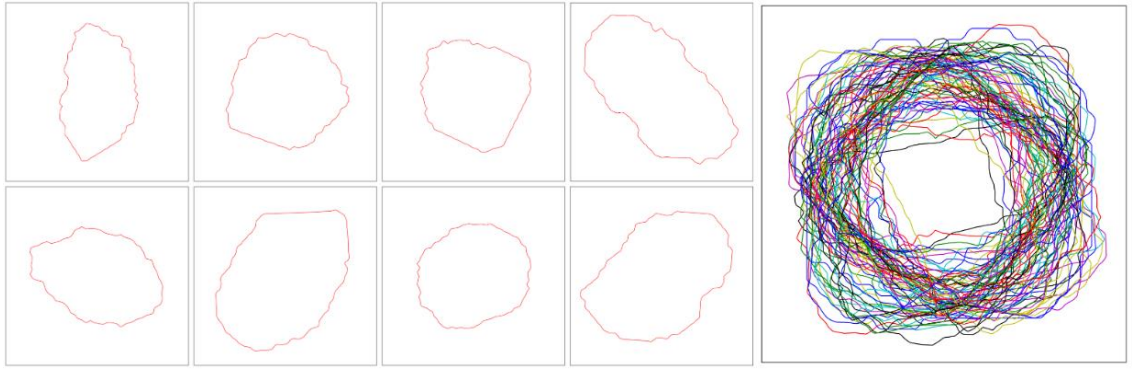
Foveal Blur-Boosted Segmentation of Nuclei in WSIs with Shape Prior Knowledge and Probability Map Constraints

- Lack of training images is a major obstacle for robust pathology image analysis, in particular for segmentation
- While pathologists have prior knowledge in nuclei's common shapes, CNN systems cannot explicitly benefit from it
- We develop a Foveal Blur based method for enriching the dataset with limited sample size, and consider shape prior knowledge and morphological constraints
- It mimicks human's vision system, and a spatial frequency map is applied to the raw pathology images with focus on each single nucleus, enriching the dataset

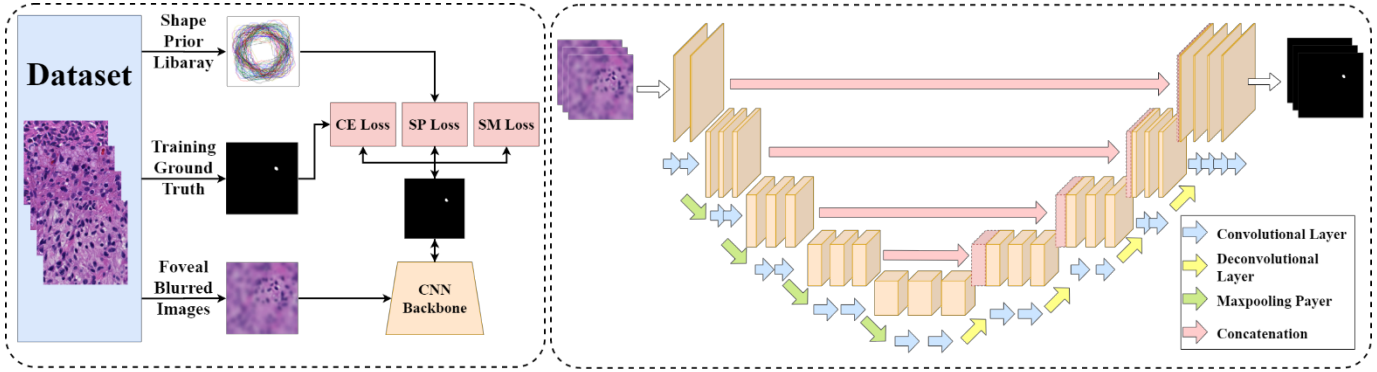


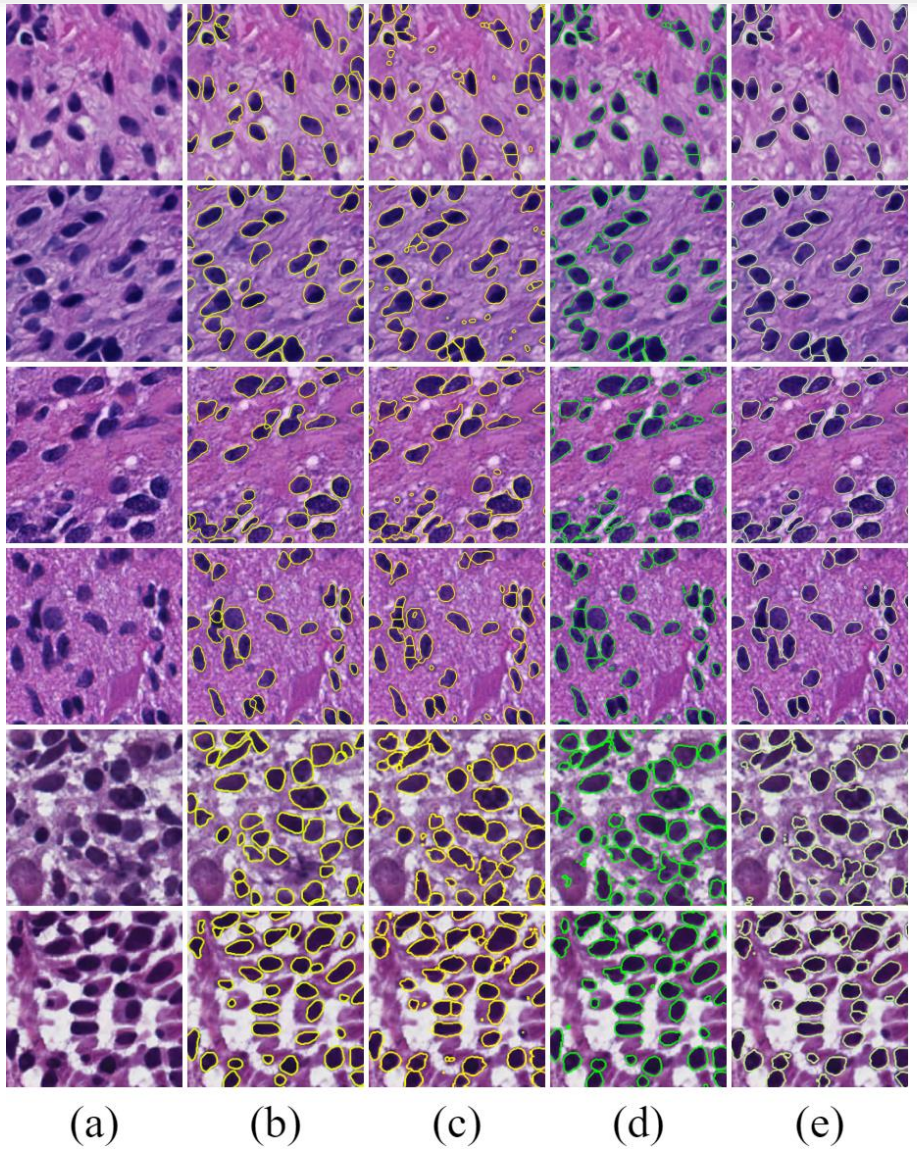
Methods and Results

Representative nuclei and the shape prior library:



- Shape Prior term is designed to penalize nuclei contour predicted with wired shapes
- Smoothness term is introduced to constrain the morphological smoothness with the first and second-order gradient of the predicted probability maps



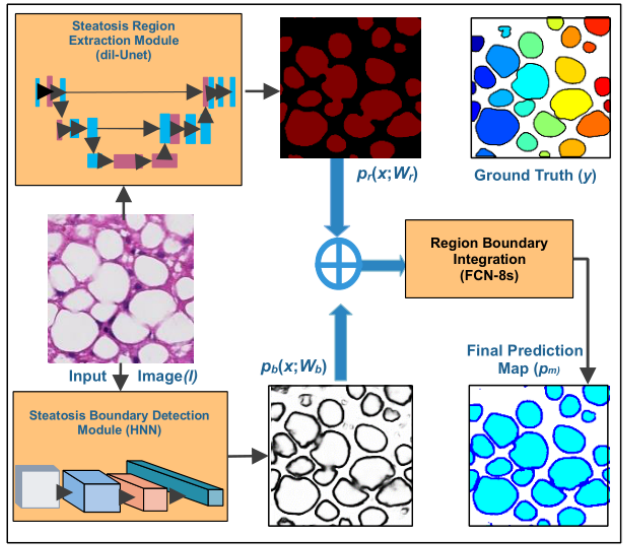


(a) Original pathology images, (b) ground truth, and results from (c) FB-Net, (d) FB+SP, and (e) FB+SP+SM; The first, the second, and the last two rows present images from GBM, TCGA, and MoNuSeg datasets, respectively.

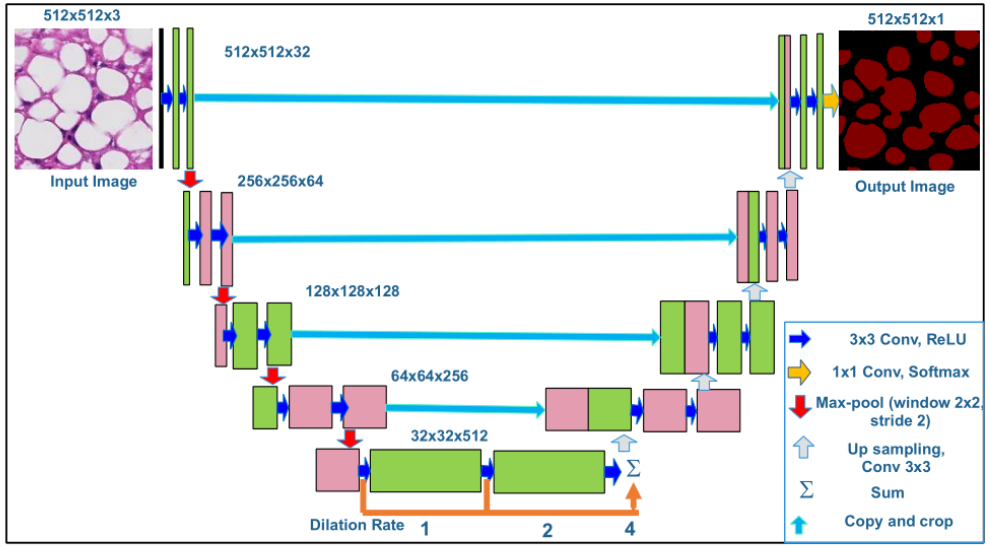
Deep Learning Based Accurate Hepatic Steatosis Quantification for Histological Assessment of Liver Biopsies

- Hepatic steatosis droplet quantification with histology biopsies has high clinical significance for risk stratification and management of patients with fatty liver diseases
- This process is challenging as there is a large number of overlapped steatosis droplets with either missing or weak boundaries
- We propose a deep learning-based region-boundary integrated network for precise steatosis quantification with whole slide liver histopathology images
- The proposed model consists of two sequential steps: a region extraction and a boundary prediction module for foreground regions and steatosis boundary prediction, followed by an integrated prediction map generation
- Missing steatosis boundaries are recovered from the predicted map and assembled from adjacent image patches to generate results for the whole slide histopathology image
- The resulting steatosis measures both at the pixel level and steatosis object level present strong correlation with pathologist annotations, radiology readouts and clinical data

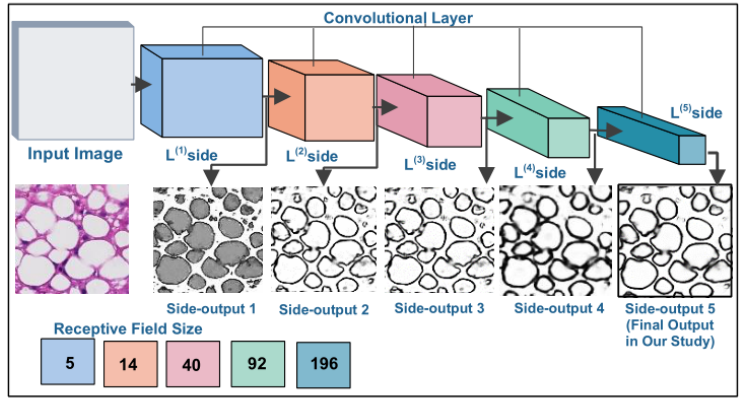
DELINEATE Model



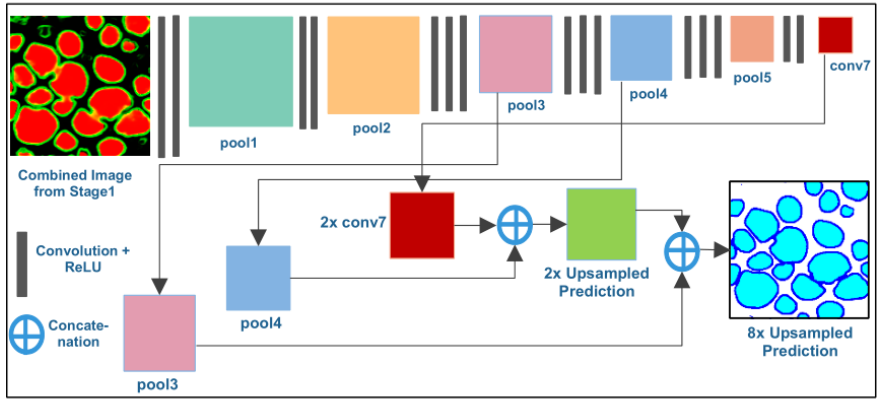
(A) Overview of DELINEATE Architecture



(B) Region Extraction Module: dil-Unet



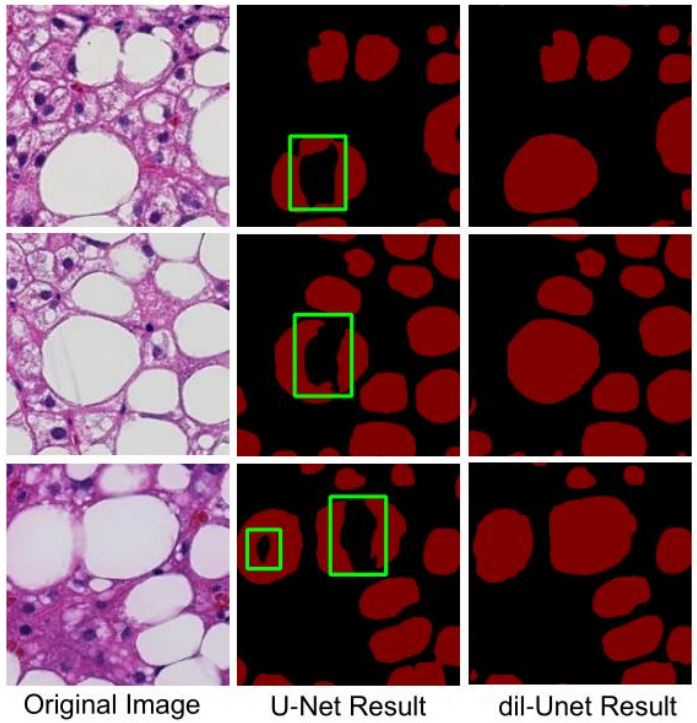
(C) Boundary Detection Module: HNN



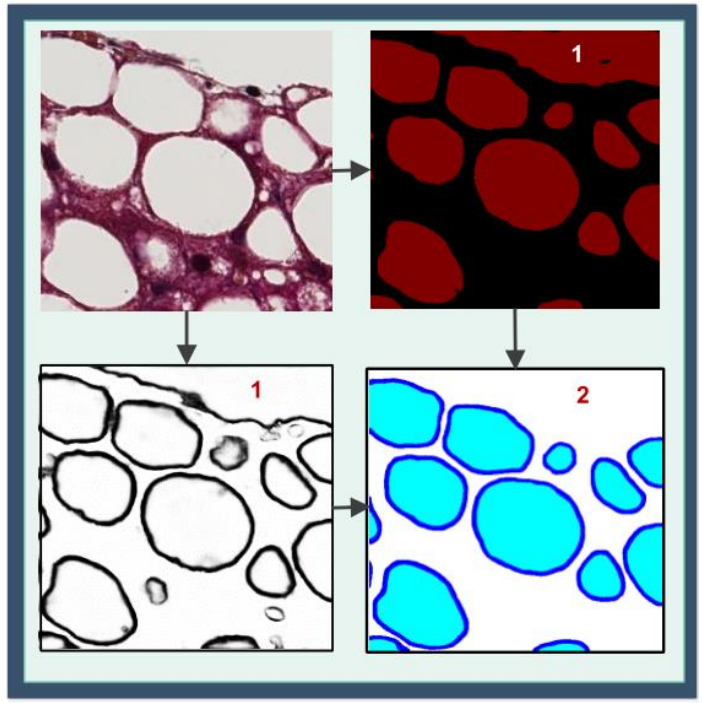
(D) Region Boundary Integration Module: FCN-8s

- The DELINEATE model first identifies regions and boundaries of steatosis droplets individually (A).
- The resulting two output predictions are combined for generating an integrated prediction map where the clumped steatosis regions are separated.
- The region extraction module detects steatosis regions with a dil-Unet module (B).
- The steatosis boundary detection module is based on a Holistically-Nested Network (HNN) (C).
- By detecting the boundary in an additional module, we can delineate the hidden boundaries of overlapped steatosis regions, and therefore, improve steatosis segmentation accuracy
- The region-boundary integration network generates the final prediction output from the integrated region and boundary information (D).

Comparison of Segmentation Results



(A)



(B)

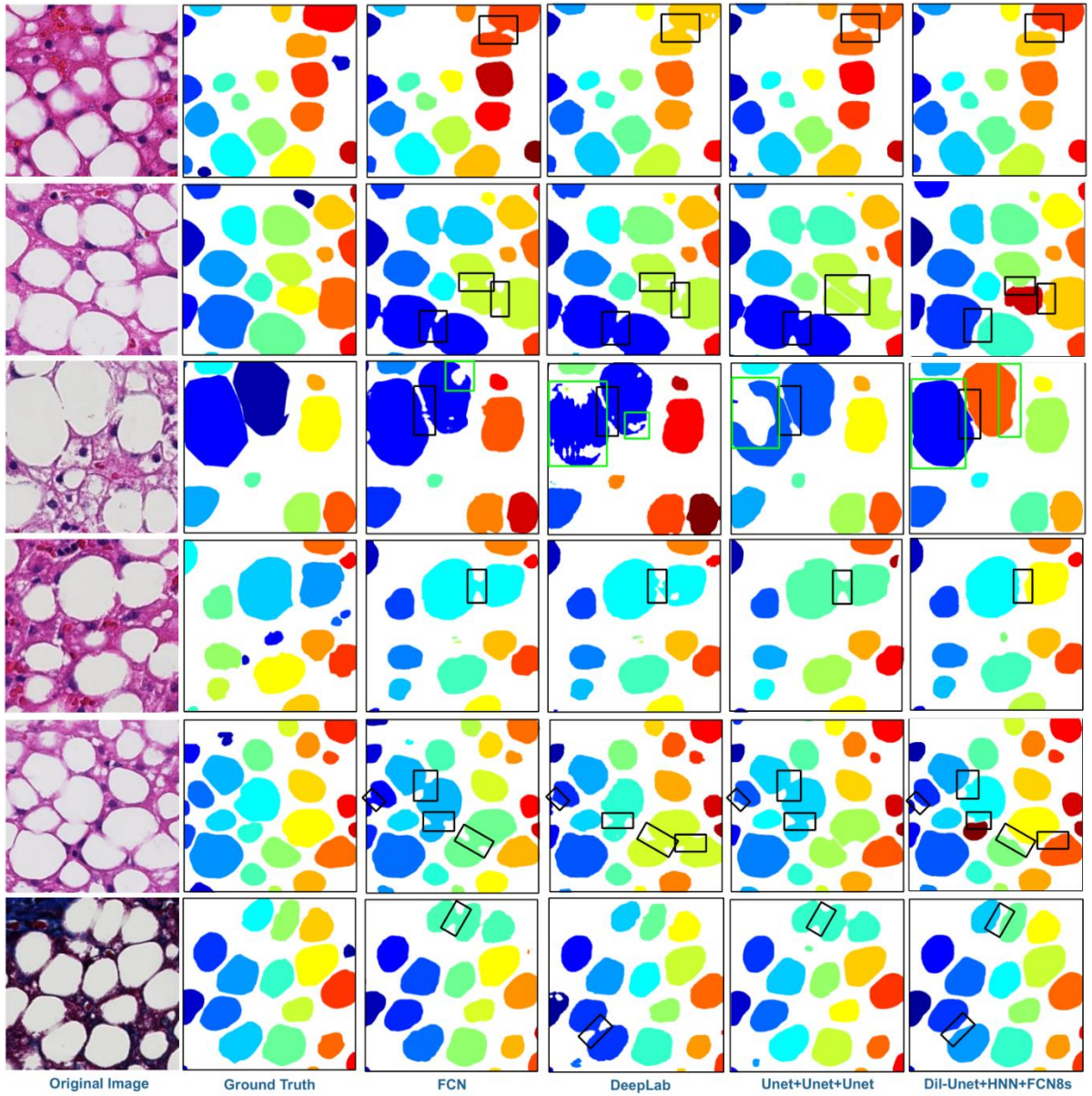
- Comparison of segmentation results between dil-Unet and standard U-Net (A)
- Left: original images; Middle: steatosis segmentation by U-Net; Right: steatosis segmentation by the proposed dil-Unet
- dil-Unet can recover steatosis regions with a substantially improved accuracy
- Comparison of results from the DELINEATE model (B)
- Top-Left: input image; Top-Right: output from the region extraction module; Bottom-Left: output from the boundary detection module; and Bottom-Right: final output of the integration module.
- “1” labels the false positive steatosis region captured by the region prediction module, and “2” labels the corrected steatosis regions by the final integration module

Comprehensive Performance Comparison of Steatosis Segmentation Methods

Models	Approach	Precision	Recall	F1-Score	Object wise Dice Index	Object wise Hausdorff Distance
Standard Models	FCN	0.99 0.01	0.86 0.06	0.92 0.04	0.8338	3.8521
	DeepLab V2	0.99 0.01	0.83 0.08	0.90 0.05	0.9083	5.3179
Variations of Our Models	dil-Unet + HNN + FCN-8s	0.98 0.01	0.91 0.06	0.94 0.03	0.9492	3.4591
	dil-Unet + HNN + FCN-4s	0.97 0.01	0.91 0.06	0.94 0.03	0.9480	3.5753
	Unet + HNN + FCN-4s	0.97 0.01	0.91 0.06	0.94 0.03	0.9489	3.4685
	dil-Unet + HNN + dil-FCN	0.97 0.01	0.91 0.06	0.94 0.03	0.9459	3.6658
	Unet + Unet + Unet	0.97 0.04	0.83 0.07	0.90 0.05	0.9247	5.8289
	Unet + Unet + FCN-8s	0.96 0.03	0.90 0.06	0.93 0.04	0.9458	3.8773

- By contrast to other methods, DELINEATE dil-Unet+HED+FCN-8s achieves the best overall performance, as indicated by F1-score, Recall, object-wise Dice index and object-wise Hausdorff distance
- DELINEATE model substantially outperforms state-of-the-art FCN and DeepLab models and achieves better performance on delineating overlapped steatosis droplets

Evaluation of DELINEATE Segmentation Accuracy



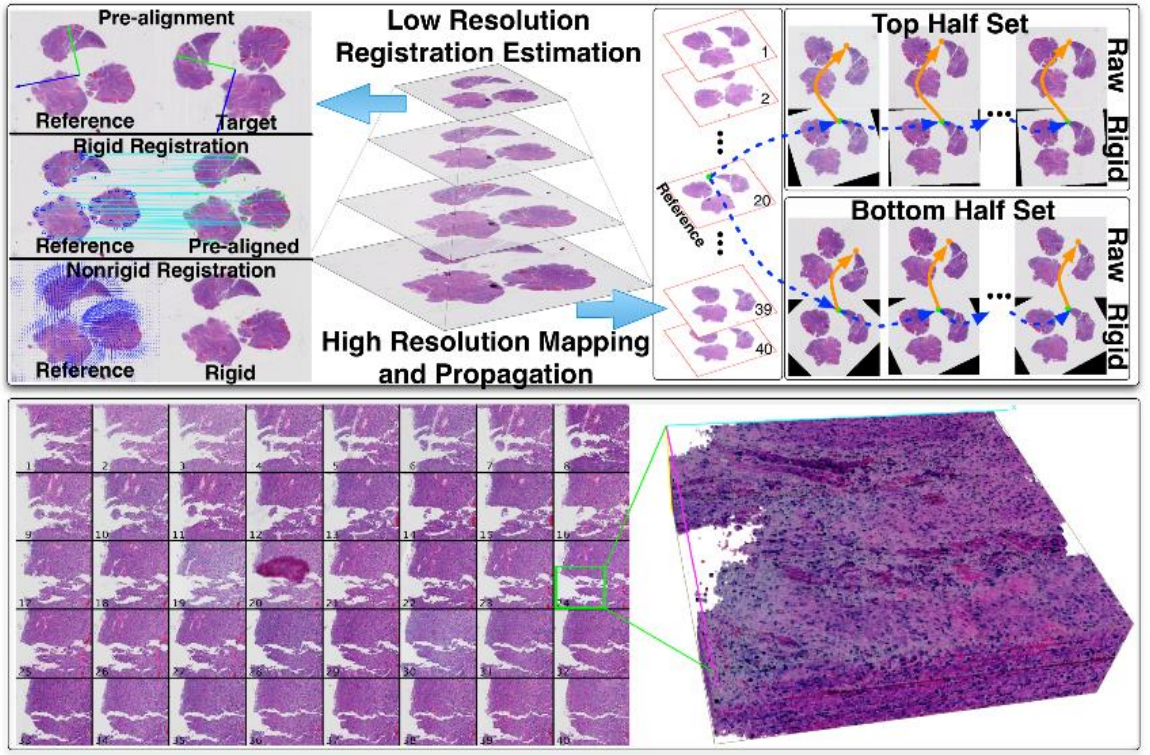
- The clumped steatosis regions indicated by black boxes in all images are well separated by DELINEATE model but failed by other methods in the comparison study. Problematic regions in green boxes are only fully recovered by DELINEATE model.
- Touching regions highlighted by black boxes are well separated by DELINEATE model, whereas they are incorrectly segmented by other methods for comparison
- Neither FCN nor DeepLab can process touching steatosis droplets accurately with missing region-boundary integrative information, resulting in lower performance scores

DELINEATE Correlation with Pathological Grading, Radiology and Clinical Data

- The results produced by the DELINEATE model present strong correlations with liver tissue pathological grading, fat quantity from MRI data, and patient clinical information
- Spearman's correlation was used to analyze the correlation between two variables
- Mann-Whitney test was used to compare the difference between two groups
- For comparisons across diverse steatosis groups, DELINEATE was logarithmically transformed before analysis
- Analysis of Variance (ANOVA) was used to study the difference among four histological steatosis grading groups
- This was followed by Tukey's multiple comparison post-test
- We assessed the difference of steatosis measurements across four histological steatosis percentage grades by DSP%, DSC%, and ASP%
- Both DELINEATE steatosis measures demonstrate statistically significant difference across grades, with the proposed steatosis count measure DSC% presenting the least p-value

3D WSI Registration

- We developed dynamic whole-slide image registration algorithm that employs an iterative transformation propagation method to align reference and non-reference images
- This dynamic multi-resolution registration method can efficiently scrutinize volumes of interest on-demand at the full image resolution without expensive computation over the entire whole-slide image domain



Histopathology Image Registration by Integrated Texture and Spatial Proximity Based Landmark Selection and Modification

- we propose a histopathology image registration fine tuning method with integrated landmark evaluations by texture and spatial proximity measures
- Representative anatomical structures and image corner features are first detected as landmark candidates
- Next, we identify strong and modify weak matched landmarks by leveraging image texture features and landmark spatial proximity measures

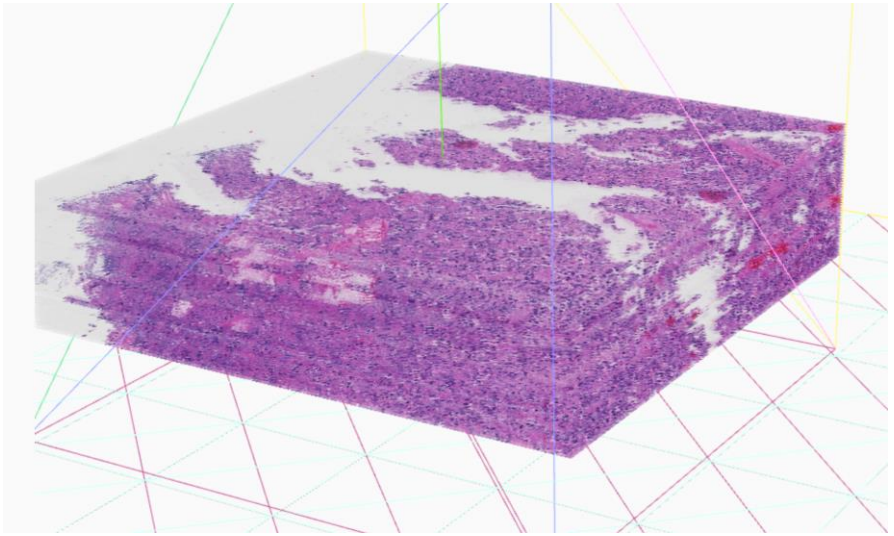


Fig 2: A 3D tissue volume composed with serial pathology image regions registered by our method.

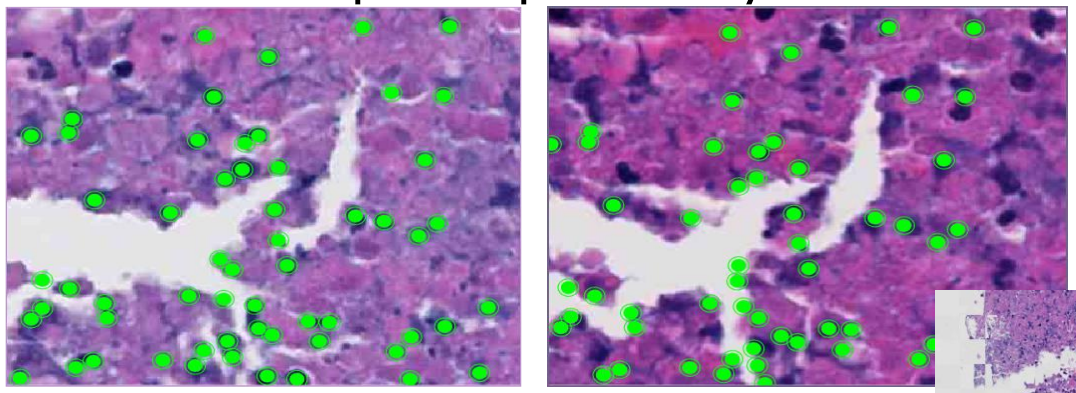


Fig 1: Representative (Left) reference, and (Right) target image with selected landmark pairs.

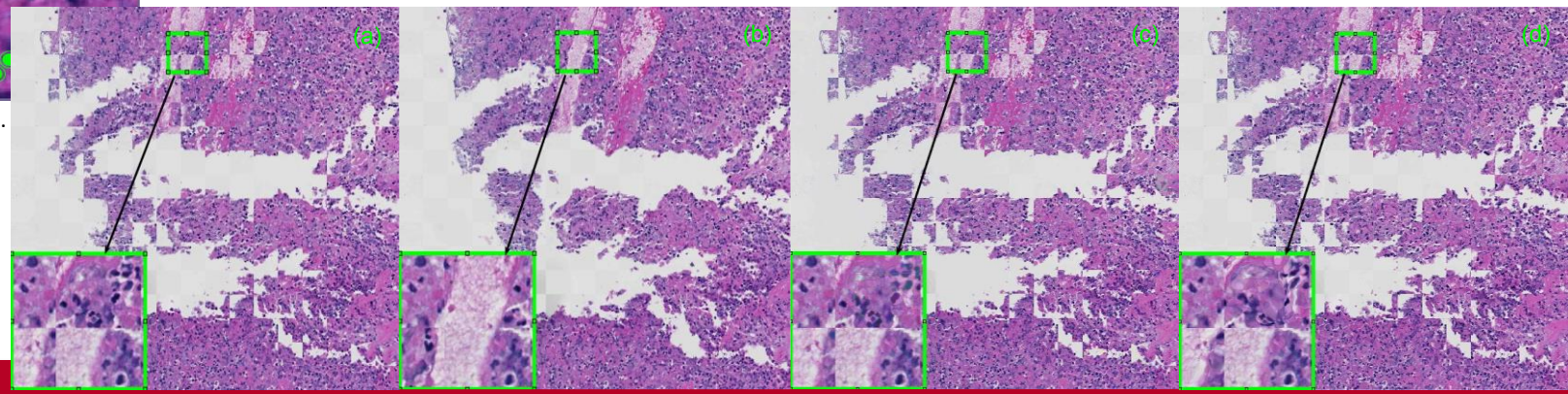


Fig 3: We present checkerboard views of representative registration results (a) before, after (b) our registration method, (c) pTV, and (d) Demons method.

HistoRegNet: Deep Learning based Registration for IHC Images

- We developed HistoRegNet, an end-to-end unsupervised patch-based deep learning registration model to spatially align IHC histopathology images
- HistoRegNet consists of an affine module, a deformable component, and a spatial transformer network
- The affine and a deformable module learns the Displacement Vector Field (DVF) by both affine and deformable transformation optimization
- A new deformable component uses a Histo-incept module (similar to Google's incept module) for capturing and comparing information from multiple spatial resolutions
- The learned DVF is provided to a spatial transformer network that generates registered images
- We further develop multiple regression layers that produce multiple deformation fields appropriate for WSI registration
- The proposed method outperforms the state-of-the-art methods consistently by multiple performance metrics

Registration Performance : Quantitative

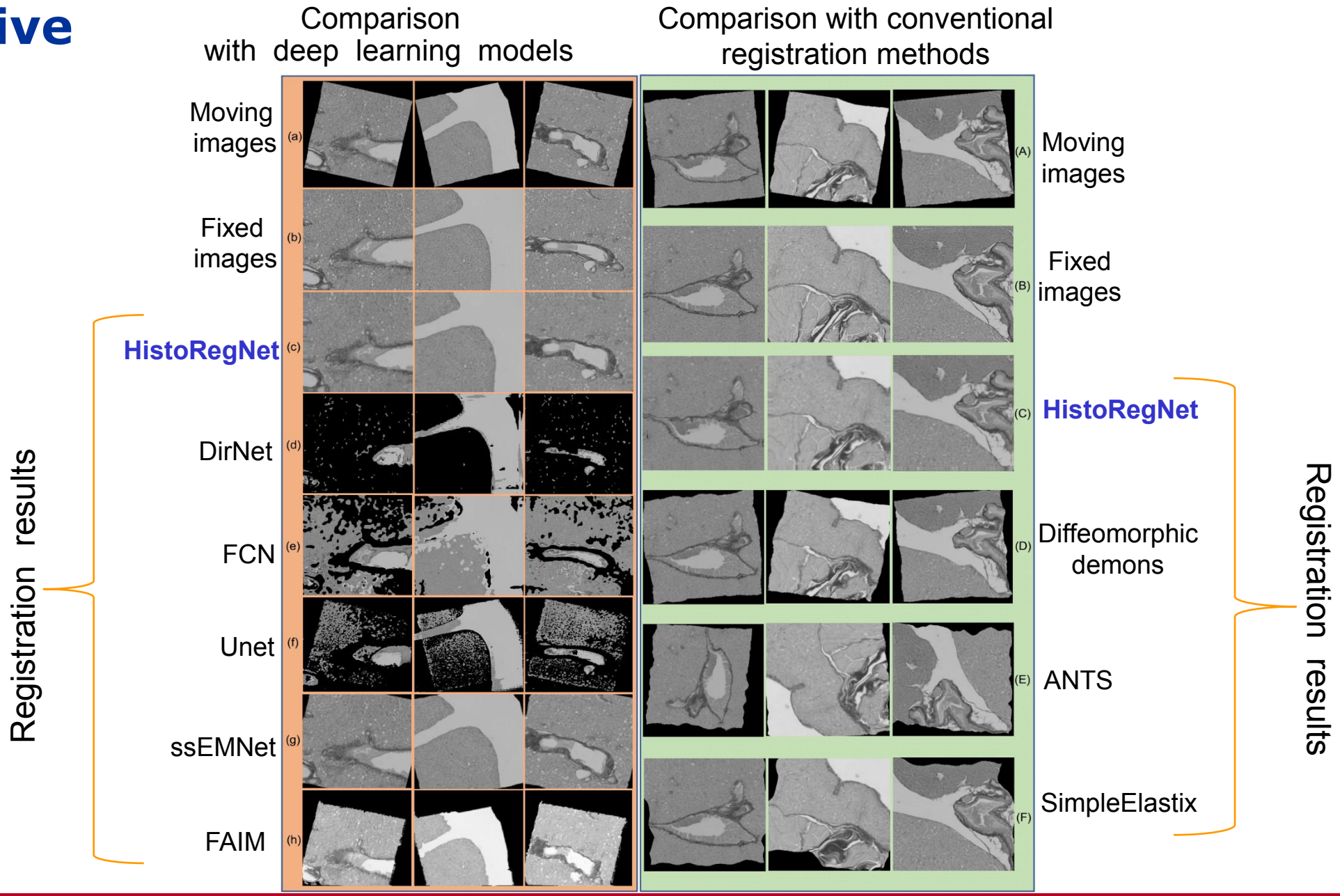
Metric name	Method Name								
	HistoReg -Net	DirNet	FCN	UNet	FAIM	ssEMNet	Diffeomorphic demons	Elastix	Ants
NCC	0.337	-0.423	0.314	0.410	0.001	0.168	0.152	0.203	0.191
SSIM	0.279	0.459	0.310	0.385	0.362	0.297	0.405	0.463	0.279
MSE	0.003	0.289	0.015	0.117	0.108	0.004	3583.55	2813.45	2942.82
NMI	0.173	0.058	0.171	0.184	0.008	0.013	0.131	0.044	0.029

Quantitative evaluations of registration results from HistoRegNet, state-of-the-art deep learning models, and conventional registration methods

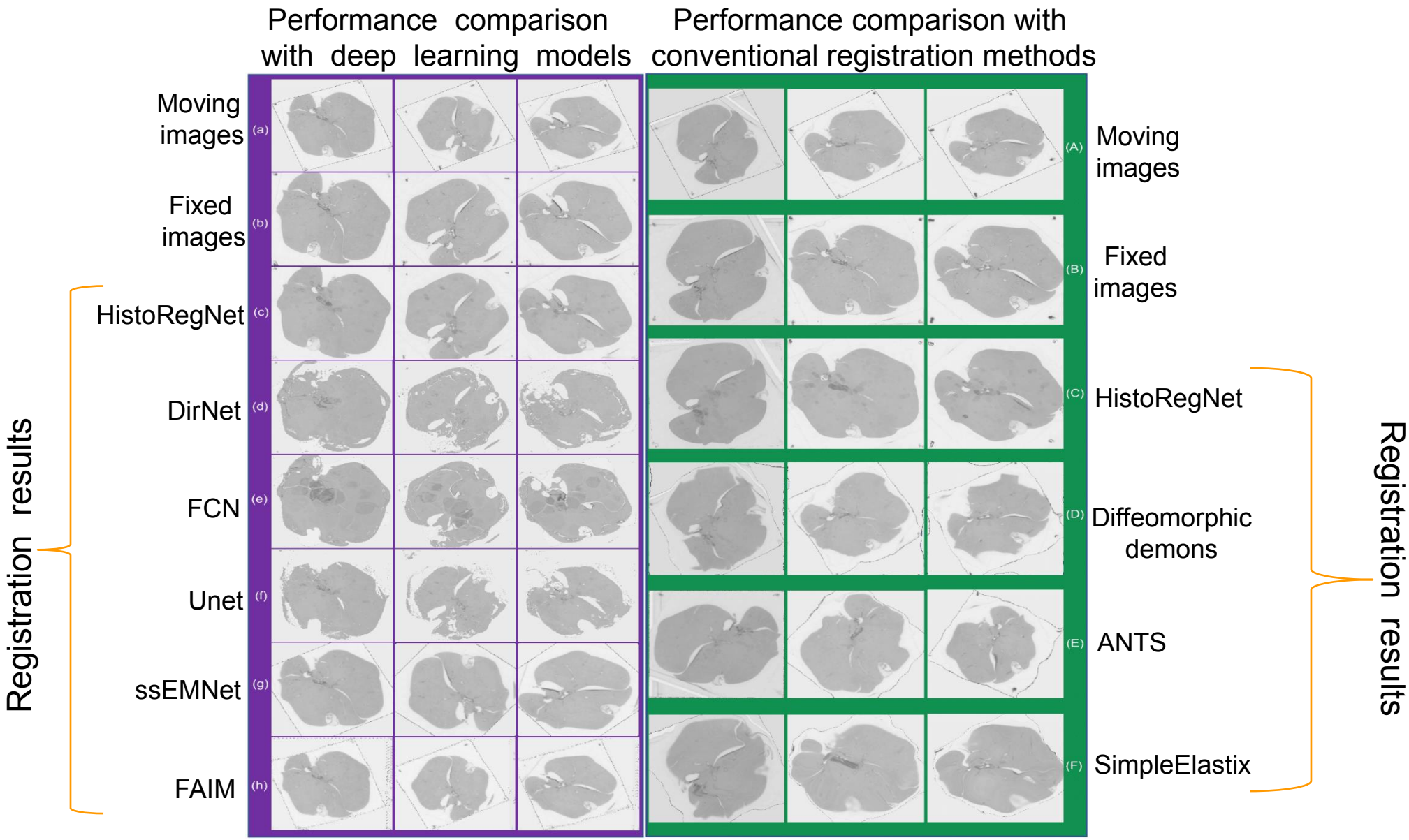
Metric Name	Method Name							
	Histo-RegNet	DirNet	FCN	UNet	ssEMNet	Diffeomorphic Demons	Elastix	Ants
Dice	0.823	0.342	0.7435	0.46	0.807	0.722	0.893	0.443

Comparison of Dice co-efficient from HistoRegNet, state-of-the-art deep learning models, and conventional registration methods

Performance Comparison with High Resolution Image Patches: Qualitative

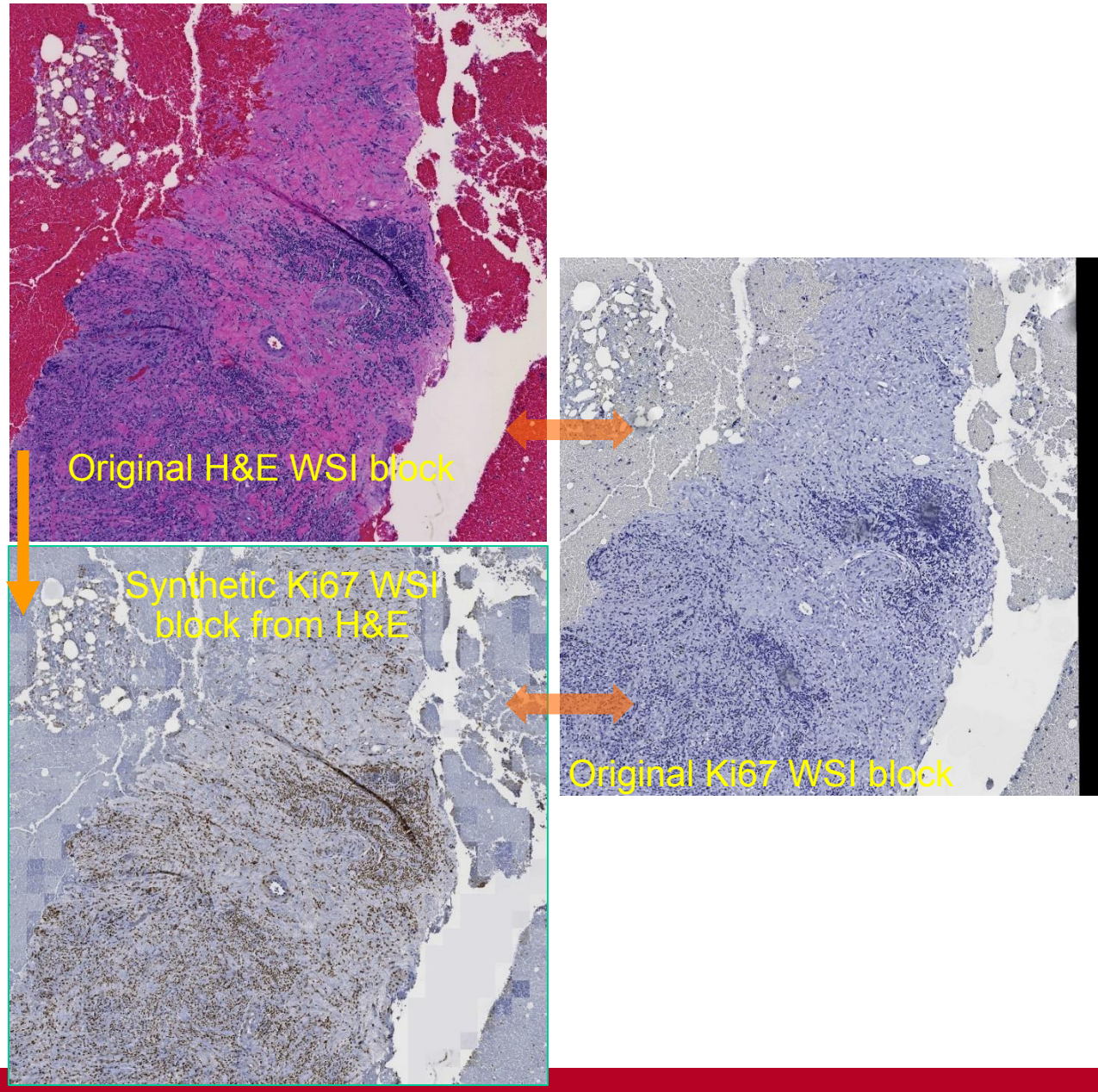


WSI Registration Performance Comparison: Qualitative

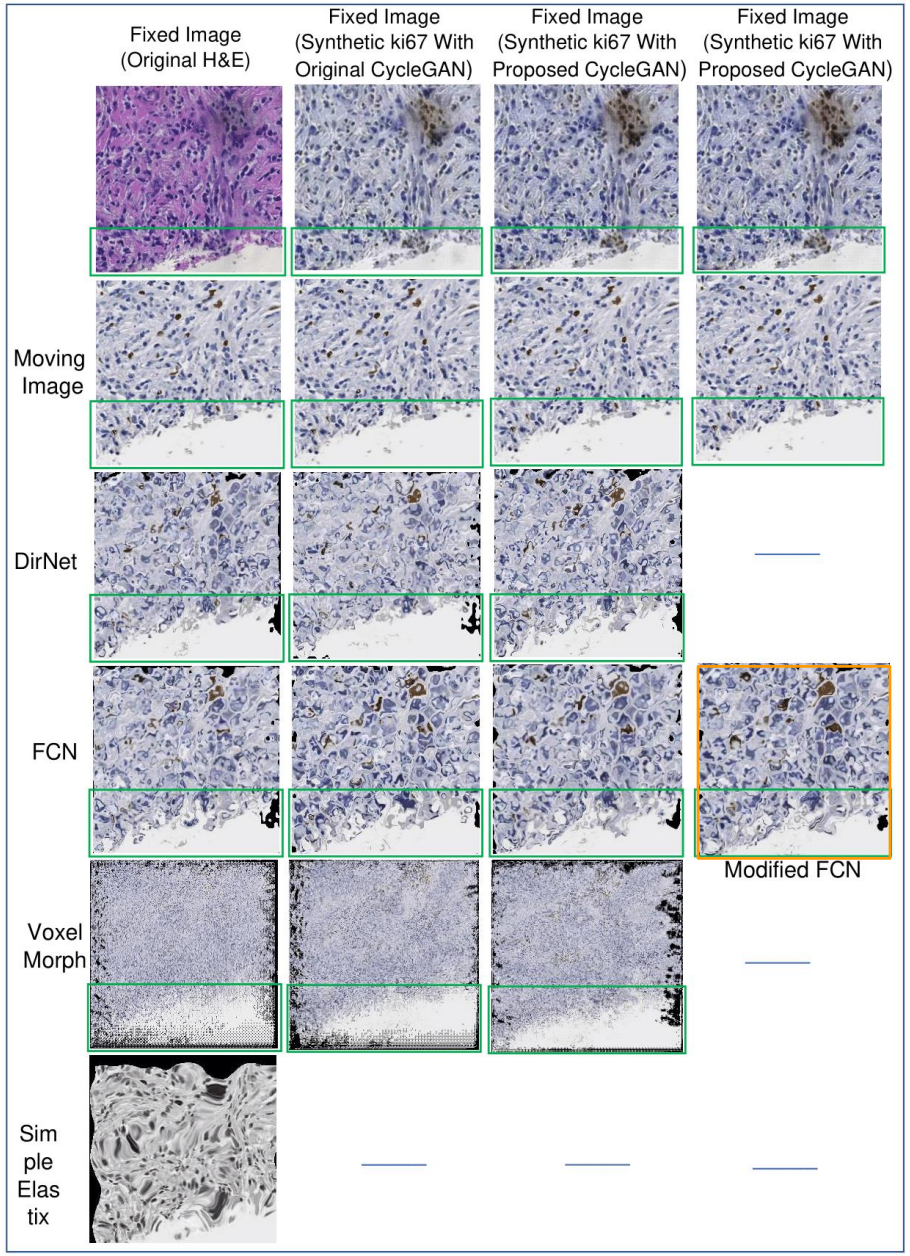


Multi-modal H&E & IHC Image Registration through Adversarial Network

- Traditional deep learning-based registration methods are not ideal for multi-modal registration
- Generative Adversarial Networks have used in various registration work, mainly for radiology images, and not applicable to WSI images
- Our approach: convert multi-modality images into one modality through GAN and register them
  - Translated H&E to synthetic IHC image for registration
  - Improved registration performance
  - Extended FCN registration model with multi-scale features recovering better IHC registered images

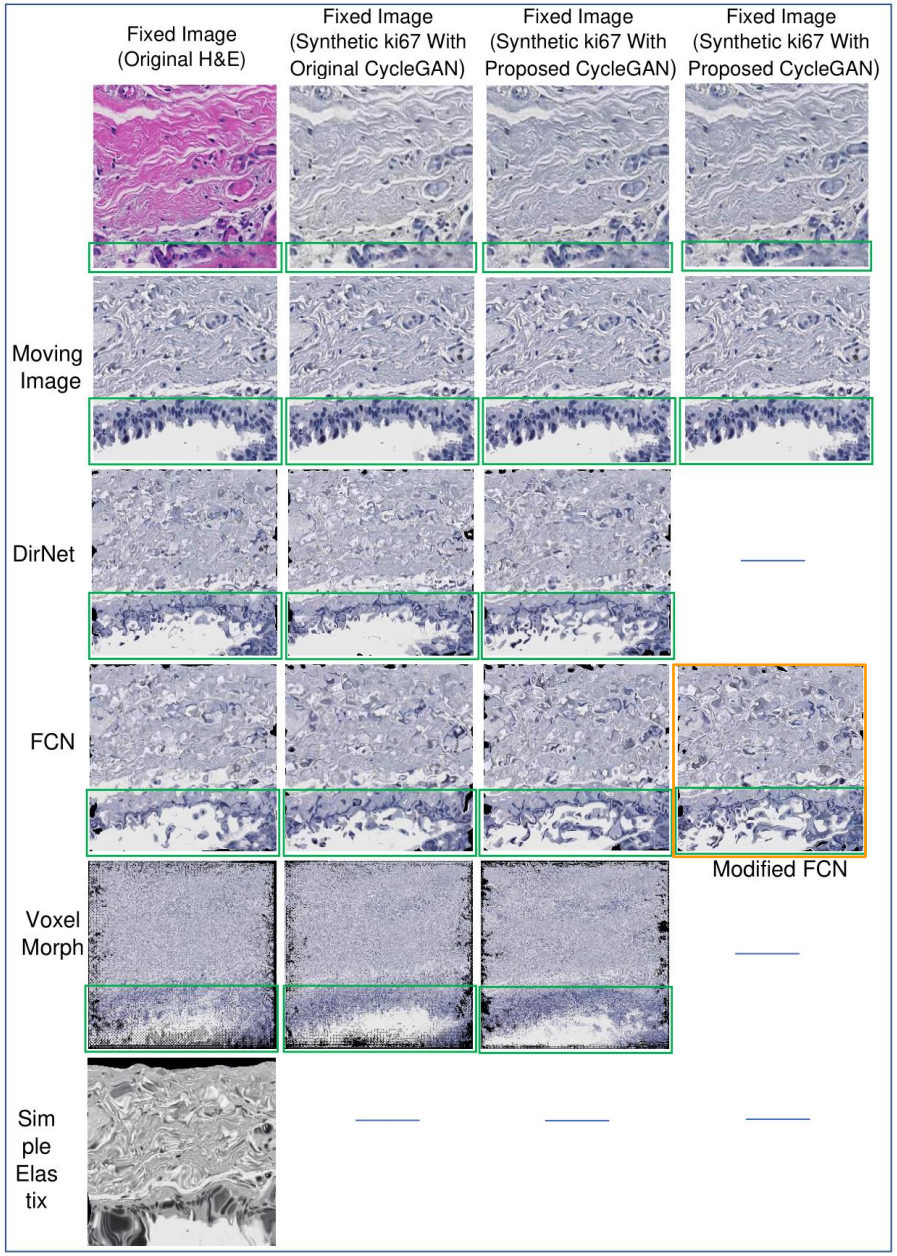


Preliminary Results



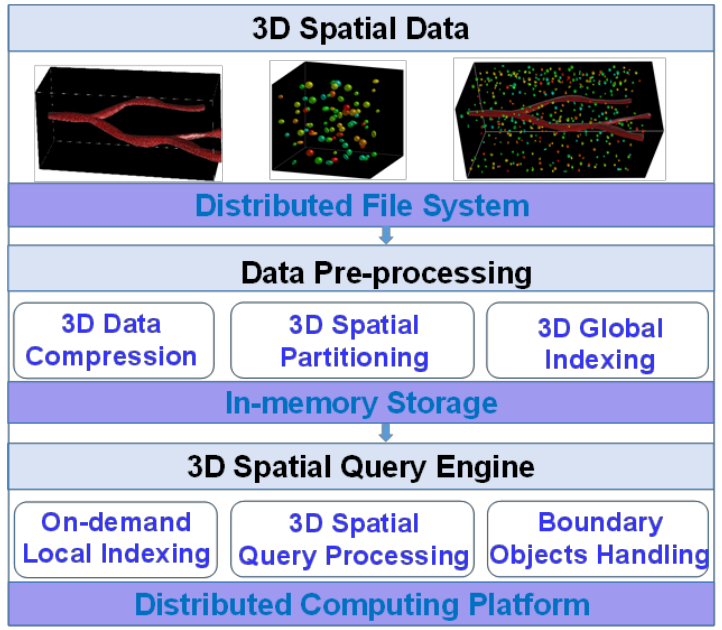
H&E and its IHC synthetic one

IHC Image



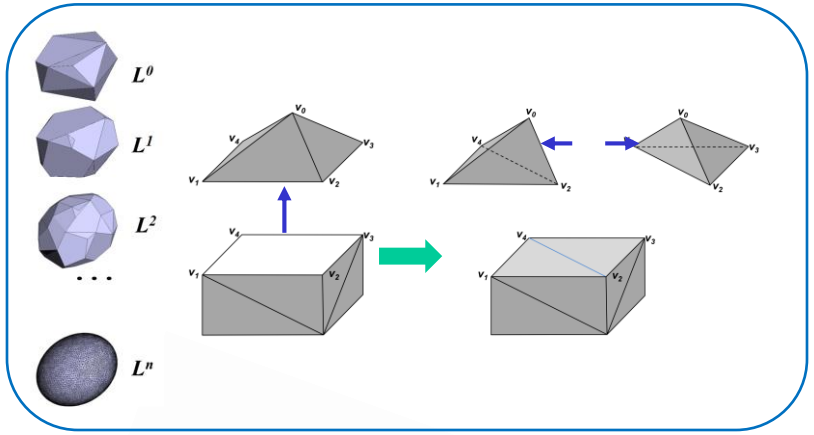
Scalable 3D Data Management and Queries for 3D Tissue Maps at Extreme Scale

- High resolution, high throughput imaging technologies are producing unprecedented amount of information at cellular and subcellular level from human tissues
- The explosion of 3D spatial objects such as nuclei, cells and vessels pose significant challenges for spatial data management
  - Explosion of data: millions of objects per image, tens of millions of 3D objects per volume: demanding for high throughput
  - High computational complexity: demanding high performance
- We develop a highly effective 3D data management and querying system 3DPro for 3D digital pathology

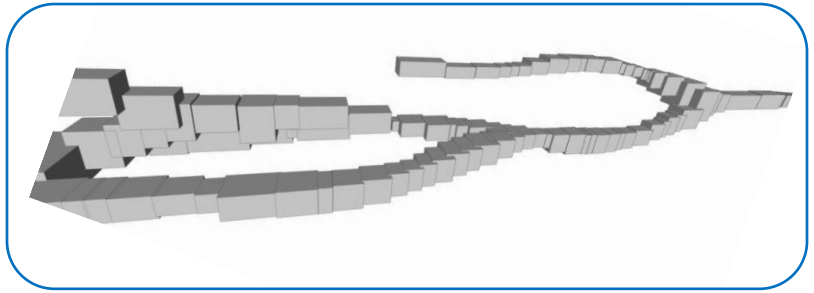


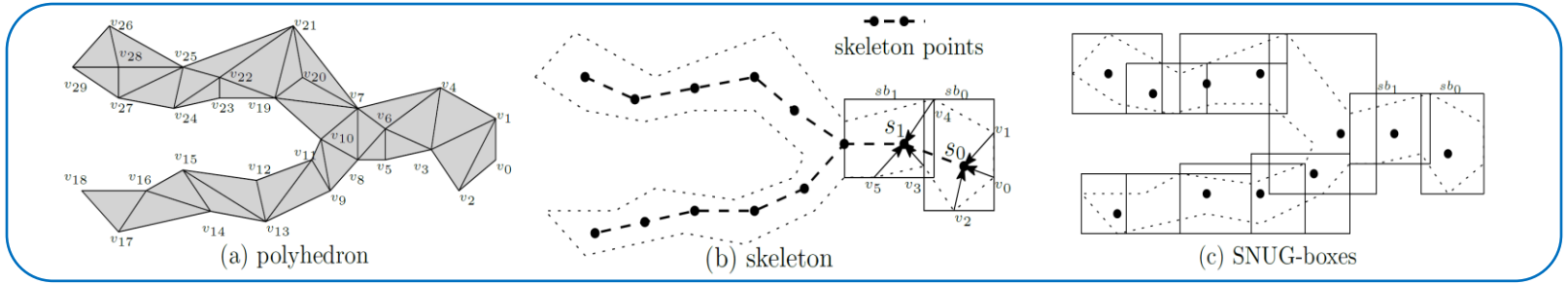
3DPro: Querying 3D Complex Objects with Progressive Refinement

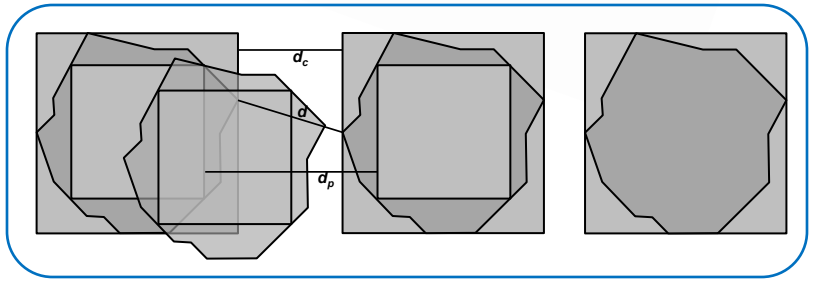
3DPro

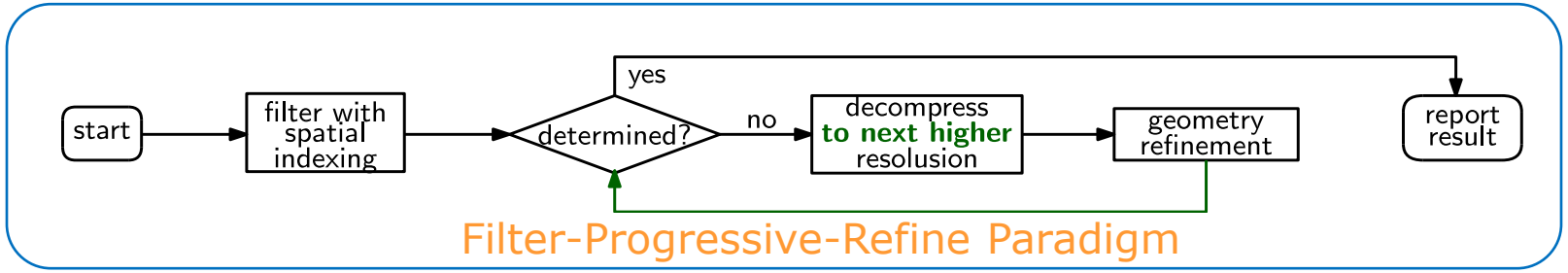


- A progressive protruding-vertex pruning mesh compression method reducing object complexity progressively to create multi-resolutions of 3D representations
- A Filter-Progressive-Refine paradigm to minimize geometric computation, which can provide early returns of accurate results from lower resolution whenever possible
- Memory centered approach for data management and querying processing to mitigate I/O cost





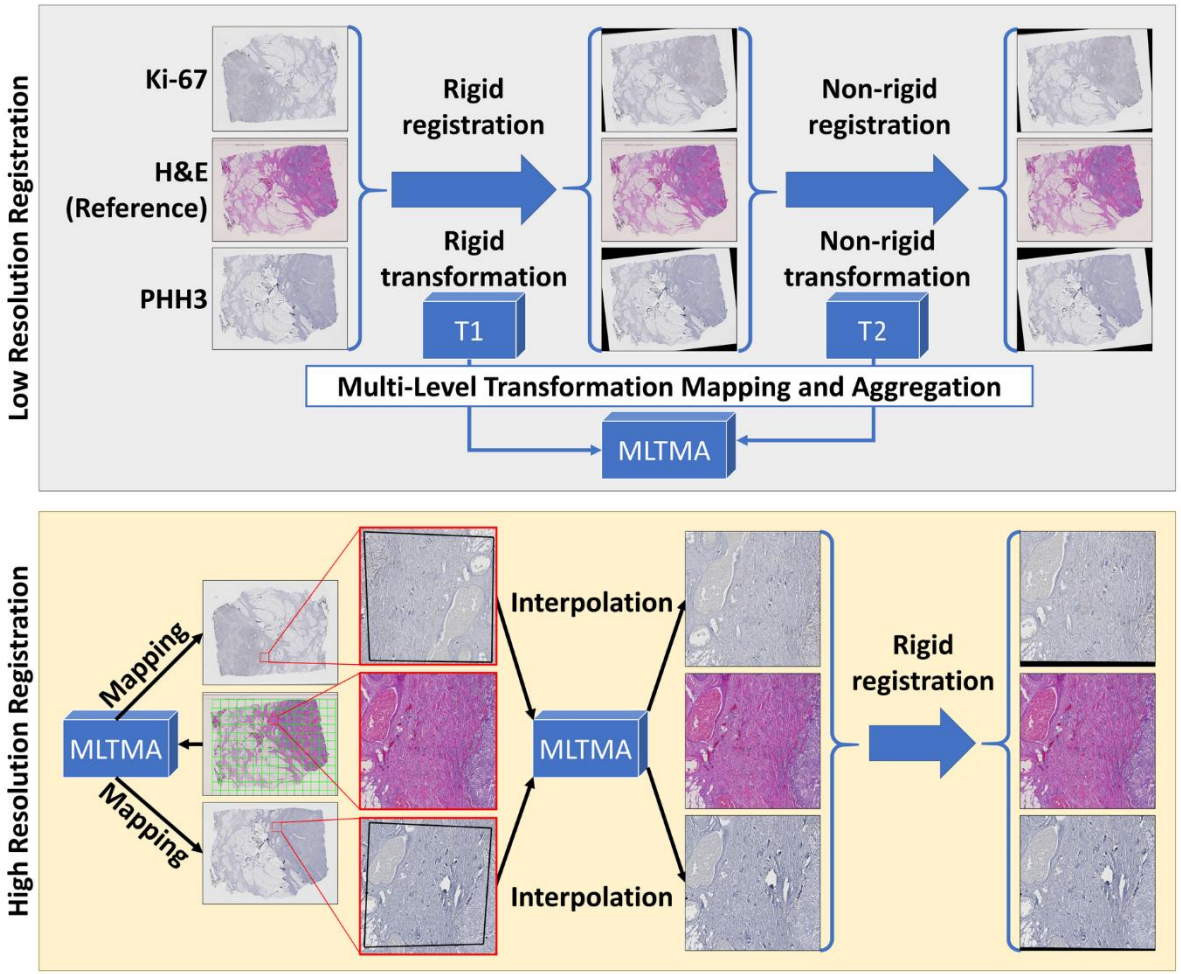




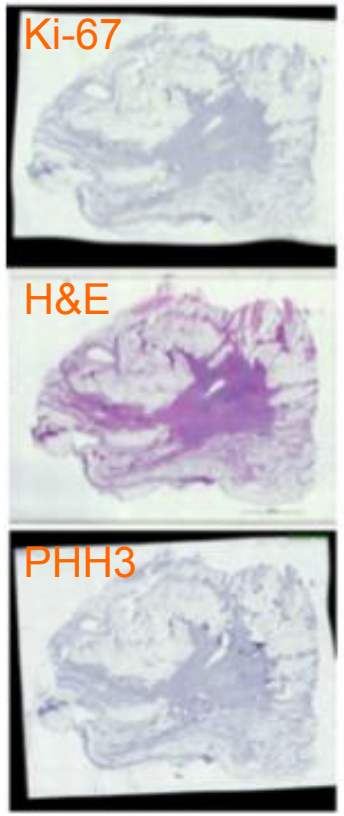
Spatial Attention-based Deep Learning System for Breast Cancer Pathological Complete Response Prediction with Multi-Modal WSIs

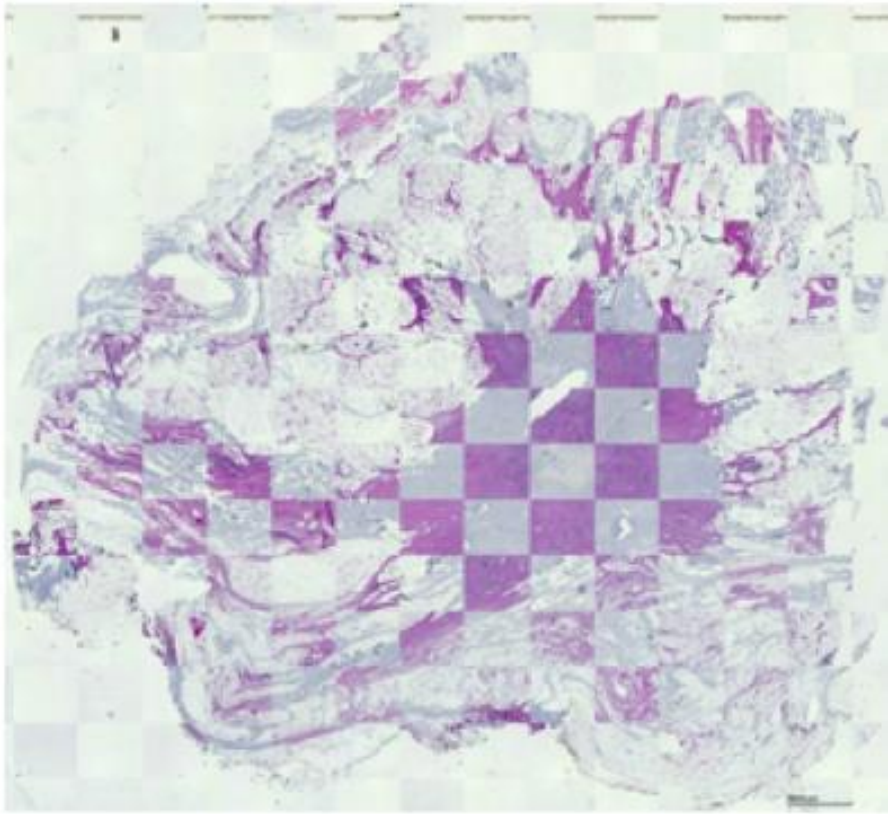
- In early prediction of PCR to Neoadjuvant chemotherapy, except for H&E images explored before, IHC images stained with KI-67 and PHH3 biomarkers can provide decisive information as well
- Biomarkers and pathology features integration with 3D registration: H&E, Ki-67, and PHH3, are jointly utilized
- Multi-task: Our proposed system detects and classifies cells before PCR prediction. Key information, such as cell type, shape, spatial organization, and the cell proliferation cycle status is provided to the PCR prediction module
- Spatial attention mechanism: We created a novel spatial attention module that informs the PCR prediction module of tissue spatial importance map (tumor cells and TILs)
  - Make the methods more interpretable for pathologists

Multi-Scale Registration of Multi-Modal WSI Images



Schema of registration working pipeline



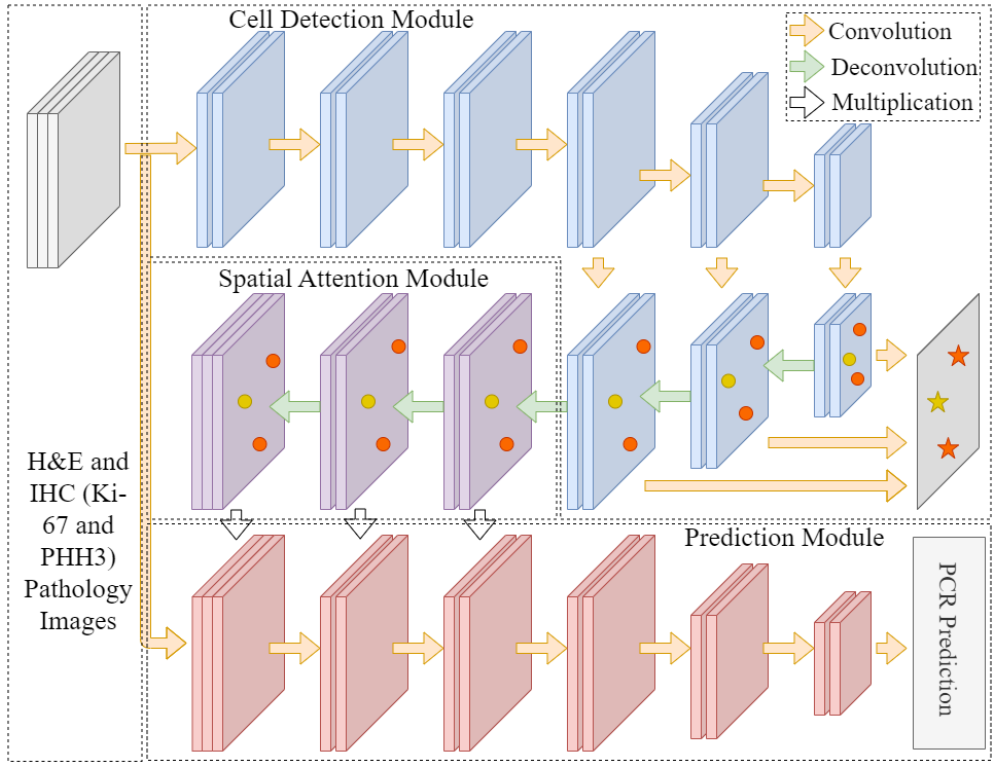


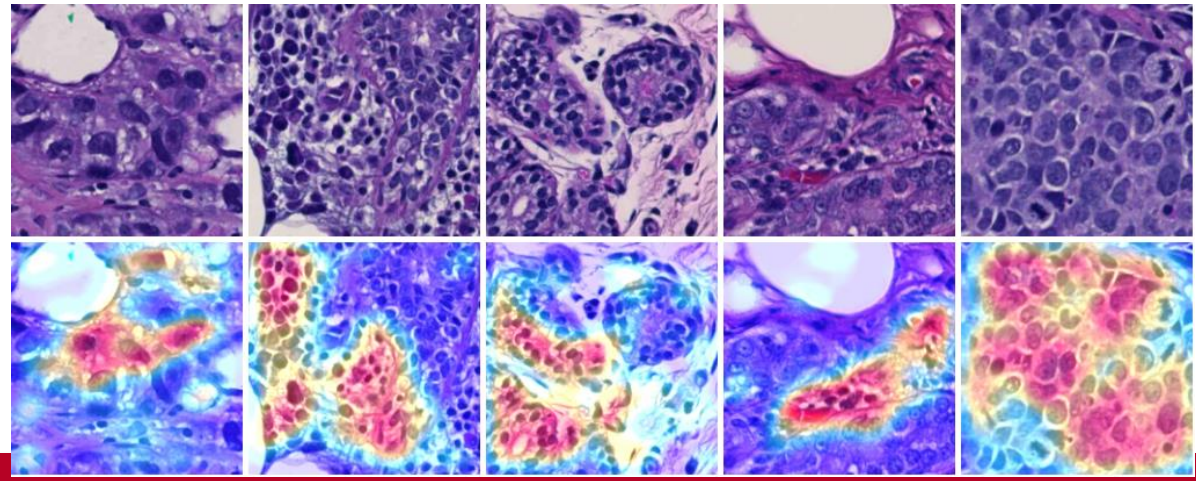
A check board view of registered Ki67 and H&E tissue slides

Application: Breast Cancer pCR Prediction

Architecture of our Multi-Task Deep Learning System

- Cell Detection Module detect all tumor cells and TILs, generate feature maps with spatial information on tumor cells and TILs
- Spatial Attention Module resizes the feature maps to the original images, creating distribution map overlaying original images
- The original images with importance distribution maps will be fed into Prediction Module for the final PCR prediction





Performance

Comparison of PCR prediction performance of progressively improved deep learning models at the image patch level

	Single Stain + Prediction Only	Multi-stain + Prediction Only	Single Stain + SAM Prediction	Multi-stain + SAM Prediction
Accuracy	0.507	0.635	0.709	0.783
AUC	0.622	0.644	0.762	0.803
Sensitivity	0.726	0.625	0.765	0.701
Specificity	0.467	0.612	0.664	0.829
BA	0.596	0.619	0.715	0.765

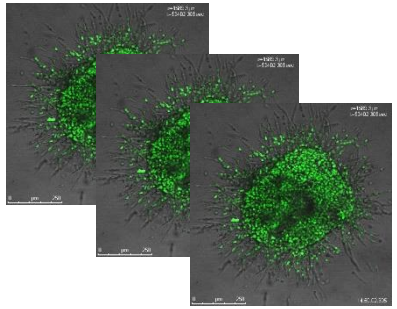
Prediction performance comparison at patch, region, and patient-level with the number of corrected predictions, total cases, and accuracy:

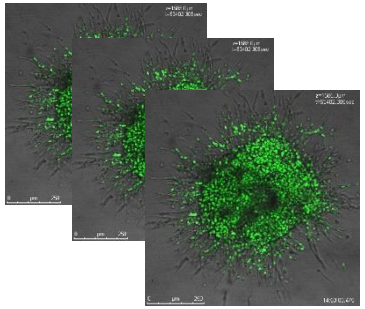
	Single Stain + Prediction Only	Multi-stain + Prediction Only	Single Stain + SAM Prediction	Multi-stain + SAM Prediction
Patient Level	18/40 (45.0%)	27/40 (67.5%)	32/40 (80.0%)	35/40 (87.5%)
Region Level	289/583 (49.6%)	387/583 (66.4%)	446/583 (76.5%)	488/583 (83.7%)
Patch Level	23325/46029 (50.7%)	29248/46029 (63.5%)	32674/46029 (70.9%)	36059/46029 (78.3%)

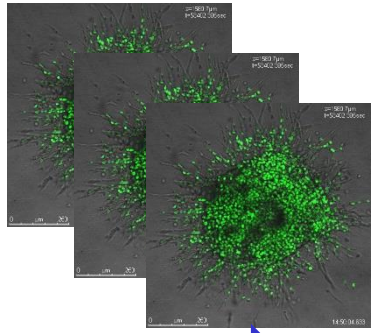
Lung Cancer Cell Migration Dynamics with Time-lapse Microscope

- Numerous studies show that it is not a single metastatic cell but rather collective packs of invasive cells that are observed histologically
- These collective invasion packs and streams are in patient tumors, mouse models, and 3D cultures
- In many cases, a hierarchical group of leader and follower cells is observed, where the invasive leader cell at the tip of the invading pack helps propel the proliferative follower cells forward
- For lung cancer, however, no computational method, especially for super- resolution, has been developed to facilitate a better understanding of how leader and follower cells interact with each other spatio-temporally in 3D spheroid cultures, if there is any significant differences in leader and follower cell spatial migration patterns in 3D spheroid cultures under different experimental conditions, and what spatial dynamics they follow to reverse their complementary phenotypic states during migration process in invasion packs
- We hypothesize that a robust 3D cell segmentation and tracking method for super-resolution imaging will provide unprecedented cell morphological and spatial dynamic information to unveil spatio- temporal interaction mechanisms by leader and follower cells during lung cancer cell invasion
- Our approach can effectively segment and track the 3D spatial movement of cells that traditional particle filtering approaches fail to reasonably describe with the Gaussian model. The quantitative tracking results can help researchers learn more about the mechanisms of cell invasion activities

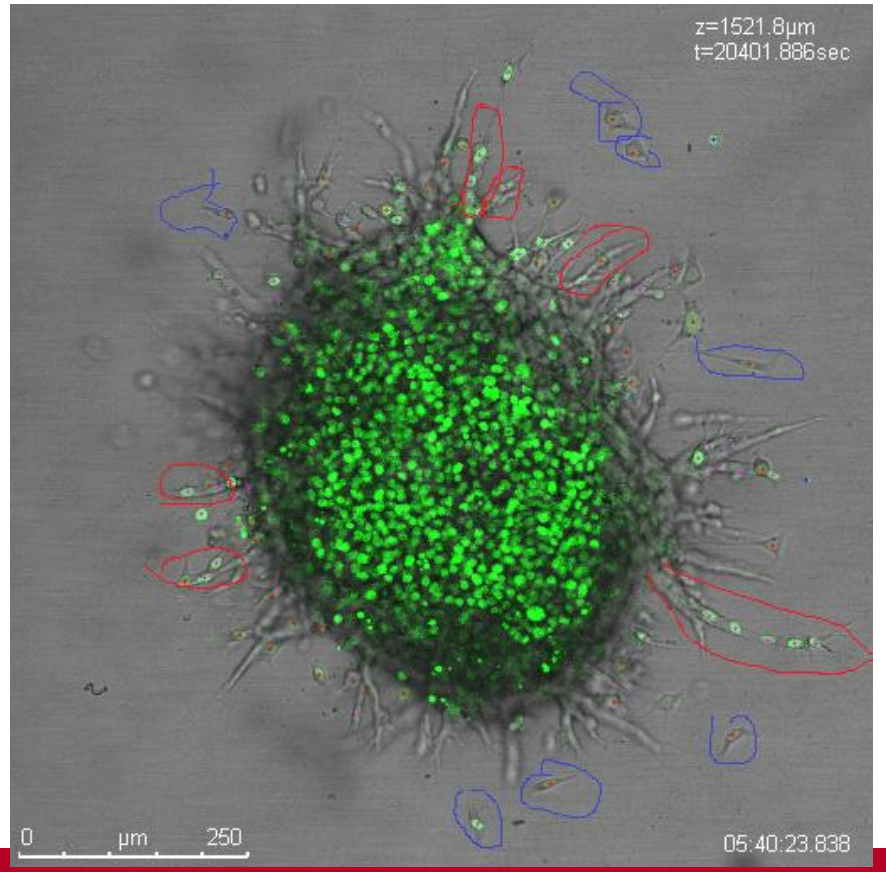
3D Lung Cancer Cell Data



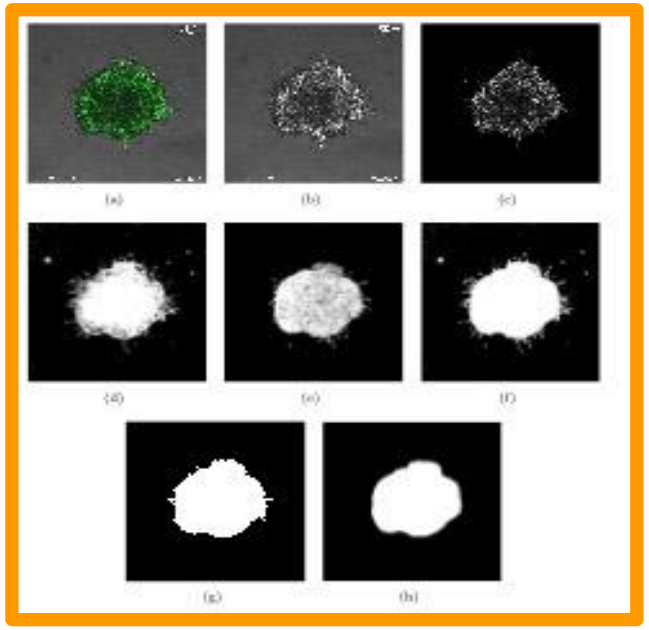




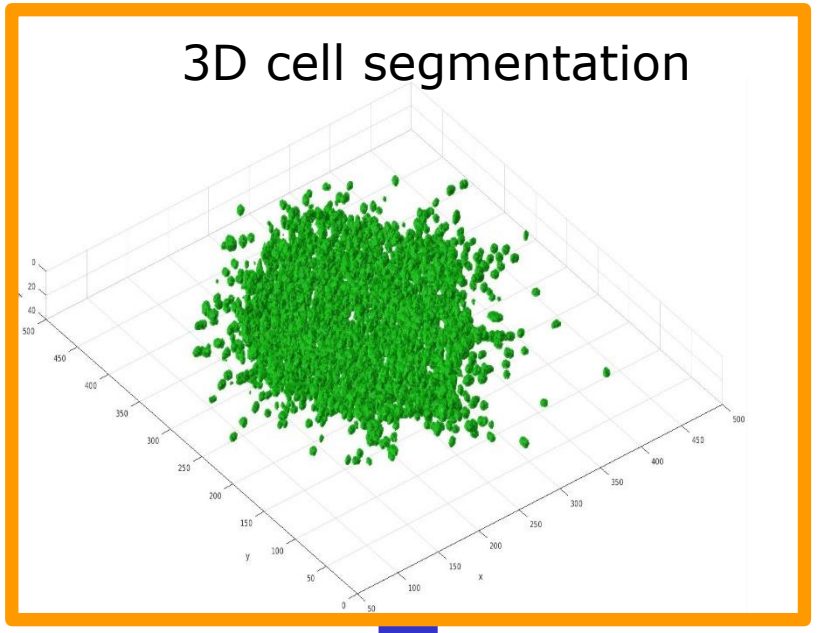
Time

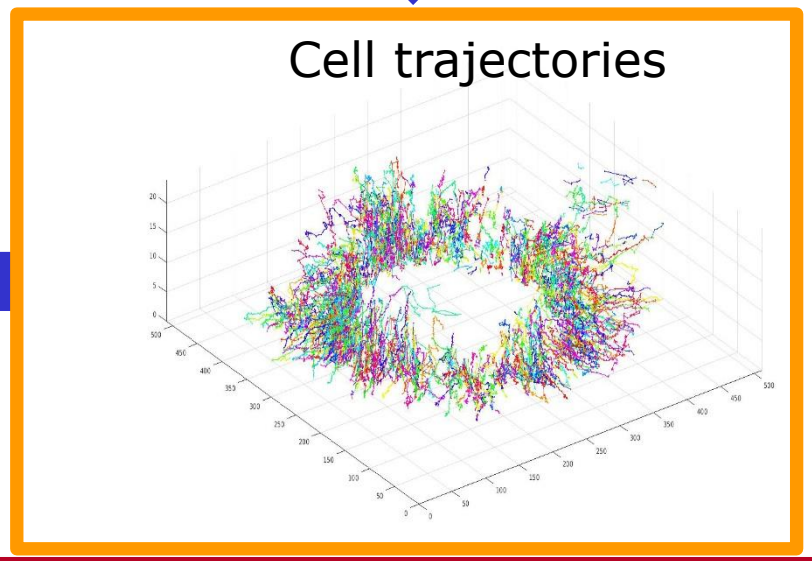


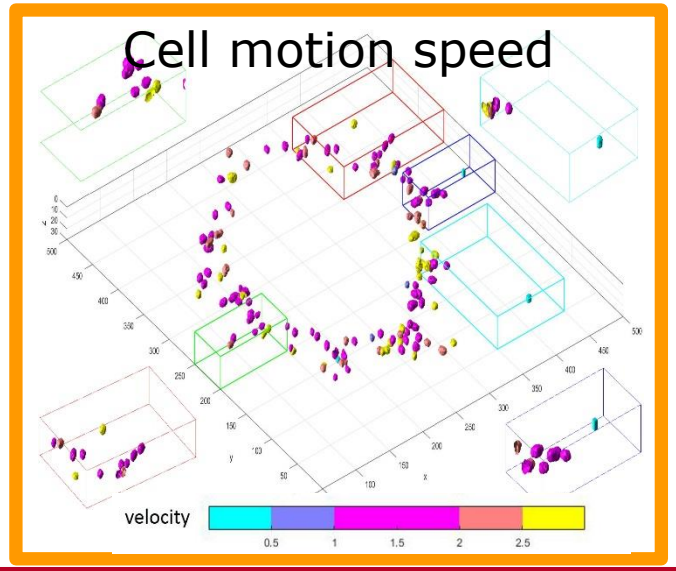
3D Lung Cancer Cell Analysis



Lung Spheroid Detection







Web-Based Digital Pathology Workbench