Multiplexed Expansion Microscopy for Drug Response Prediction in MIBC

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Dedication

Abstract

Expansion microscopy (ExPath) enables nanoscale resolution of tissue architecture using conventional microscopes, offering a powerful alternative to traditional histopathology. In this thesis, we present a deep learning pipeline that leverages ExPath imaging combined with a biologically informed, four-channel multiplexed staining panel: DAPI, TelC, CENPB, and WGA to classify tissue types and predict chemotherapy response in muscle-invasive bladder cancer (MIBC). We propose that nuclear morphology, when captured at high resolution and enriched by chromatin and membrane-specific markers, contains sufficient information to compete with H&E and generalize across diagnostic and prognostic tasks. To test this hypothesis, we construct a preprocessing pipeline that transforms 16-bit 4-channel TIFF WSIs into normalized, pseudo-RGB 1024×1024 patches compatible with ImageNet-pretrained models. We evaluate multiple architectures (ResNet34, ResNet50, ViT-tiny, EfficientNet) and demonstrate that ResNet-based models trained on ExPath outperform simulated non-ExPath baselines and DAPI-only variants by a significant margin. Through controlled ablation experiments, we quantify the contribution of each channel and find that multiplexing substantially boosts classification accuracy. Our models achieve 89.52% tissue classification accuracy and 0.9 ROC-AUC for drug response prediction. Furthermore, we observe cross-cancer generalizability when applying MIBC-trained models to lung carcinoma ExPath images. This work establishes the feasibility of compact, multiplexed, ExPath-driven classification pipelines as a viable alternative to costly multi-modal diagnostics. It offers an early step toward a DAPI-first foundation model for computational pathology, with potential to scale across cancer types and tissue conditions using minimal staining and high-content imaging.

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Chapter 1

Background

1.1 Expansion Pathology

Expansion Pathology (ExPath) is a technique that leverages the principles of expansion microscopy (ExM) to physically magnify biological specimens, enabling nanoscale imaging of tissues using conventional microscopes. Originally developed to visualize fine neuronal structures, ExM has since been adapted to pathological contexts to enable detailed spatial mapping of cells and subcellular components in disease tissues [Chen et al., 2015; Zhao et al., 2017].

The core idea behind ExPath is to embed tissue sections in a swellable polymer matrix and then isotropically expand them several-fold, usually by 4× or more. This physical expansion circumvents the diffraction limit of light microscopy, allowing researchers to observe structures at an effective resolution of 70 nm with standard confocal or widefield microscopes. Notably, this allows subcellular features like chromatin structure, protein complexes, and cell–cell junctions to be visualized with high fidelity [Tillberg et al., 2016].

ExPath enables highly multiplexed and high-resolution imaging when combined with immunofluorescence staining. In our study, we use a four-channel fluorescence panel comprising DAPI (4',6-diamidino-2-phenylindole), TelC (a telomeric repeat probe), CENPB (centromere protein B antibody), and WGA (wheat germ agglutinin).

Compared to traditional hematoxylin and eosin (H&E) staining, which offers a more generalized and morphologically rich overview of tissue context, ExPath emphasizes fine-grained nuclear and subnuclear detail. While H&E is commonly used for clinical histopathology, it does not support multiplexing or nanoscale imaging. In contrast, ExPath enables both, making it especially powerful for high-content, quantitative analysis of tissue microenvironments and cellular phenotypes [Zhao et al., 2017].

This flexibility makes ExPath particularly promising for computational pathology tasks that rely on detailed nuclear morphology, such as segmentation, classification, and prognosis prediction in cancer.

1.2 Muscle-Invasive Bladder Cancer (MIBC)

Bladder cancer is the tenth most common cancer worldwide and exhibits a dichotomy in clinical behavior based on the depth of tumor invasion. Muscle-Invasive Bladder Cancer (MIBC) refers to tumors that have invaded the detrusor muscle (muscularis propria) of the bladder wall, which marks a shift toward more aggressive disease with a significantly worse prognosis than non-muscle invasive forms [Siegel et al., 2024; Babjuk et al., 2022].

MIBC accounts for approximately 25-30% of newly diagnosed bladder cancer cases and requires more intensive treatment due to its propensity for metastasis. The current standard of care involves neoadjuvant chemotherapy (typically cisplatin-based), followed by radical cystectomy and lymph node dissection [Grossman et al., 2003]. Common first-line regimens include MVAC (methotrexate, vinblastine, doxorubicin, and cisplatin) and dose-dense GC (gemcitabine and cisplatin). However, therapeutic response varies widely, and there is currently no robust imaging-based biomarker to predict patient response pre-operatively [Stein et al., 2001].

There is increasing interest in integrating histopathological and molecular data to stratify MIBC patients and guide treatment decisions. Although molecular subtyping (e.g., luminal vs. basal) has yielded predictive insights in some studies [Robertson et al., 2017], these approaches often require costly and labor-intensive sequencing workflows. A more scalable alternative would be to use computational analysis of pathology images, especially if such images could be collected with minimal stain or modality burden and yet still capture prognostically relevant phenotypes.

This sets the stage for imaging-based classifiers that can leverage the nuclear, chromosomal, and glycoprotein features revealed by multiplexed expansion microscopy to predict treatment response in MIBC. Unlike traditional approaches that depend on coarse morphological patterns or require expensive multi-modal imaging, we hypothesize that the micro- and nano-scale features captured in DAPI-based multiplexed ExPath images can serve as a rich basis for tissue- and drug-response classification in bladder cancer and beyond.

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