Slide-free tissue histology using back-illumination interference tomography and virtual H&E staining

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Abstract:
Back-illumination interference tomography (BIT) is a novel epi-illumination microscopy technique that enables high-contrast, label-free 3D imaging of bulk tissues. We used deep learning to virtually stain BIT images into realistic H&E-like images.

1. Introduction

Histological staining of tissue biopsies using hematoxylin and eosin (H&E) is the gold-standard for disease diagnosis. While the interpretation of H&E-stained slides is a fundamental diagnostic tool, the staining process can be time-consuming, potentially delaying diagnoses for days or even weeks after a biopsy. Therefore, a rapid tissue assessment with H&E-like contrast could improve many medical procedures, including surgical margin assessment and cancer screening. To achieve whole-slide, rapid virtual H&E-stained (vHE) imaging of bulk tissue, we propose a simple microscope configuration that captures H&E-like contrast data using a novel technique called back-illumination interference tomography (BIT) and demonstrate the conversion of BIT images to vHE using a generative adversarial deep learning pipeline. Furthermore, using the same microscope, we acquire pixel-wise registered microscopy with ultraviolet surface excitation (MUSE) images [1]. Virtual H&E images generated from MUSE data using a physics-based staining model [3] serve as a direct analog to conventional H&E and act as ground truth for comparing histological features with BIT vHE images.

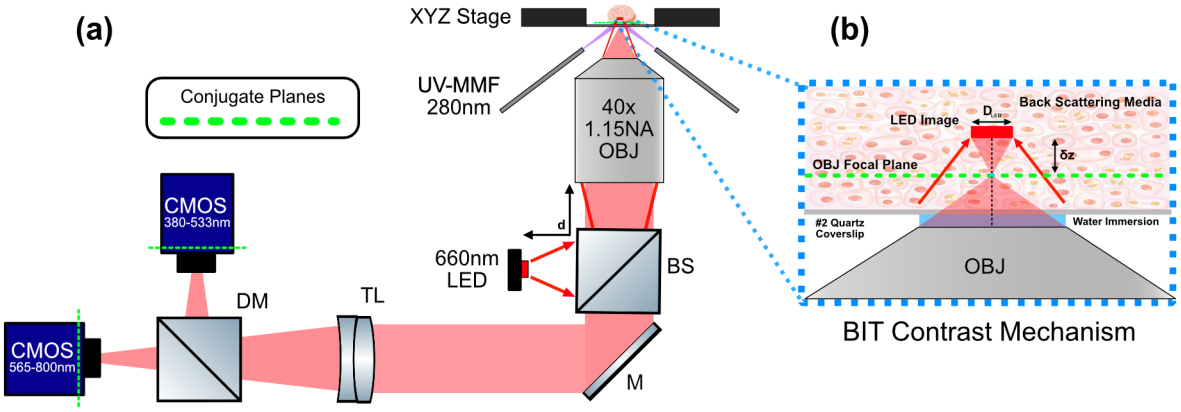

Fig. 1. Multimodal BIT-MUSE optical setup **(a)**. Enlarged region of interest demonstrating how BIT achieves contrast through backscattered illumination from a partially spatially coherent source **(b)**.

2. Multimodal Back-illumination tomography microscope

The multimodal BIT and MUSE optical system is depicted in **Fig. 1a**. BIT contrast is generated by a 1mm 660nm red LED reflected onto the back aperture of the objective lens through a beam splitter (BS) at a distance d away from the objective back aperture. The diverging light passes through an infinity-corrected objective lens (OBJ, Nikon 1.15NA APO LWD WI λS) forming an image of the LED beyond the focal plane. Backscattered light from the LED image passes through the object near the focal plane, yielding transmission-like illumination with a partially coherent source. Using the thin lens equation, at $d = 133$mm we calculated the distance of the demagnified LED to be 195μm beyond the focal plane (δz) and the diagonal (D_{LED}) to be 55μm. The BIT contrast mechanism is shown in more detail in **Fig. 1b**. Similar to [2], BIT contrast occurs when the diffracted light from the object around the focal plane interferes with semicoherent back-reflected light. The contrast changes from constructive to destructive interferences as the object moves from through the objective focal plane due to the Guoy phase shift. For MUSE imaging, Hoechst and Rhodamine were used to stain nuclei and cytoplasmic structures, respectively, and excited at 280nm by a 0.22 NA 365μm core multi-mode fibers (UV-MMF) oriented obliquely (82.5°) on opposite sides of the objective lens front aperture. Back-illuminated light or fluorescence emission is imaged through a tube lens (TL) onto CMOS sensors, separated into two color channels using a 550nm cut-on dichroic mirror.

3. Virtual H&E staining:

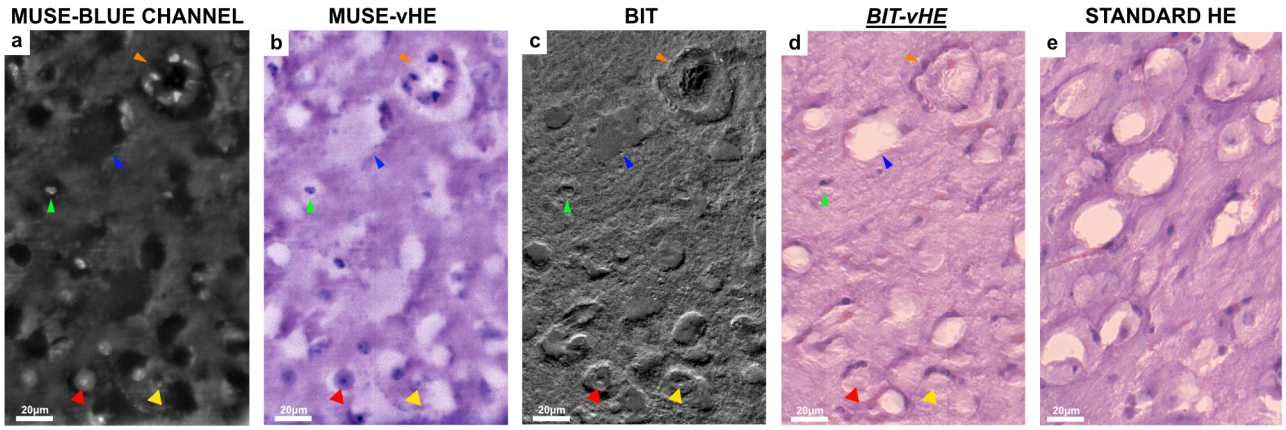

Fig. 2. MUSE blue channel **(a)**, physics-based virtually stained H&E from MUSE **(b)**, BIT **(c)**, data-driven virtually stained H&E from BIT **(d)**, and standard brightfield H&E **(e)** images of mouse brain tissue. Direct comparisons of nuclear and cytoplasmic features are highlighted by the green, blue, and orange arrows. Red and yellow arrows show regions where our *BIT-vHE* staining fails.

We obtained pixel-wise registered MUSE and BIT images of the coronal section of a mouse brain (**Fig. 2(a/c)**). We trained an unsupervised, image-to-image translation deep learning model (CycleGAN) to virtually stain BIT images to H&E. CycleGAN was trained for 86 epochs trained using 512×512 image patches, consisting of 1949 H&E patches and 3597 BIT patches. BIT images were inverted [4] and normalized to improve the training process. Visually, our virtually stained BIT images (*BIT-vHE*) shows excellent style transfer compared to standard brightfield H&E of the same tissue. MUSE images were virtually stained (MUSE-vHE) using [3], enabling quantitative ground truth comparisons. We validated the histological feature preservation of our CycleGAN model by comparing MUSE and MUSE-vHE to BIT and *BIT-vHE*. Green, blue, and orange arrows in **Fig. 2(a-d)** indicate matching histological features in both the MUSE and BIT domains. However, we also see failure cases where the CycleGAN model does not correctly learn the nuclear transformation (red and yellow arrows, **Fig. 2(a-d)**).

References

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