

Learning from hybrid bright-dark field imaging for resolution-enhanced digital pathology

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Section 1: Programmable LED array configuration and illumination analysis

The programmable illumination source employs a dome-shaped LED array, whose meridional cross-section is shown in Fig. S1. Each LED is inserted radially along the local surface normal of the dome such that all optical axes converge toward the common geometric center O, which coincides with the sample plane. The emitters are distributed concentrically along annular rings with a uniform polar angular interval of 6.25° between neighboring LEDs. The dome has an inner curvature radius of 35 mm, defining the optical path length between each emitter and the sample plane.

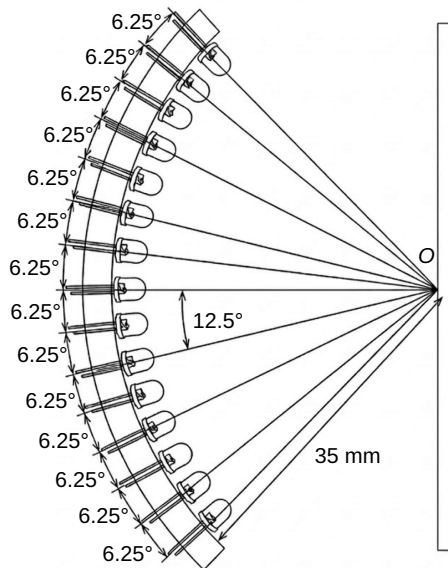


Fig. S1 | Side view of the programmable dome-shaped LED array.

To systematically access different spatial-frequency bands, four illumination modes were designed based on concentric annular activation patterns. In the bright-field mode, central illumination around the dome apex is employed, providing low-angle illumination whose wavevectors fall within the coherent transfer function passband of the objective. Three dark-field modes—P1, P2, and P3—are further implemented by selectively activating emitters located at progressively larger polar angles. Specifically, P1 corresponds to illumination from the second annular ring ($NA_{\text{ill}}^{\text{DF}} \approx 0.21$), P2 employs emitters from the fourth annular ring ($NA_{\text{ill}}^{\text{DF}} \approx 0.42$), and P3 utilizes the sixth annular ring ($NA_{\text{ill}}^{\text{DF}} \approx 0.63$). Under dark-field illumination, obliquely incident beams scatter off the specimen into spatial-frequency components beyond the bright-field OTF cutoff, thereby providing high-frequency structural priors inaccessible to bright-field imaging alone.

The spatial uniformity of illumination at the sample plane is ensured by the substantial overlap between neighboring LED illumination footprints. Each emitter possesses a half-divergence angle of $\theta_{1/2} = 30^\circ$ (corresponding to a full divergence angle of $\pm 30^\circ$ relative to the optical axis) and is positioned at a dome inner radius of 35 mm. The illumination footprint radius generated by a single emitter at the sample plane can be estimated as:

$$r = d \cdot \tan \theta_{1/2} = 35 \times \tan(30^\circ) \approx 20.21 \text{ mm} . \quad (\text{S1})$$

Corresponding to a single-emitter spot diameter of approximately 40.4 mm. The arc-length spacing between adjacent emitters along the dome surface is:

$$s = d \cdot \Delta \theta_{\text{rad}} = 35 \times \frac{6.25^\circ \times \pi}{180^\circ} \approx 3.82 \text{ mm} . \quad (\text{S2})$$

Since the single-emitter footprint radius ($\approx 20.21 \text{ mm}$) exceeds the inter-emitter arc spacing ($\approx 3.82 \text{ mm}$) by more than a factor of five, neighboring illumination footprints overlap extensively at the sample plane. Consequently, every point within the microscope field of view simultaneously receives contributions from multiple emitters, and the resulting superposition effectively averages emitter-to-emitter variability, producing a spatially homogeneous irradiance distribution across the full imaging field.

Section 2: Ablation study

To systematically investigate the contribution of each component in the proposed framework, an ablation study was conducted using the widely adopted single-image super-resolution network U-Net as the baseline model. All configurations were retrained and evaluated on the color-corrected dataset under identical experimental settings to ensure a fair comparison. Peak Signal-to-Noise Ratio (PSNR) and Structural Similarity Index Measure (SSIM) were adopted as the primary quantitative evaluation metrics. The quantitative results are summarized in Table S1.

Table S1 | Ablation study on the three core components of HBDF-RE. ✓ denotes the inclusion of the corresponding component. Config. 0 corresponds to the U-Net baseline (bright-field input only, MSE loss, without attention mechanism). Config. 7 corresponds to the full HBDF-RE model.

Config.	Dark-field Input	Attention	Freq. Loss	SSIM	PSNR (dB)
0				0.831±0.034	25.312±0.174
1	✓			0.863±0.029	27.883±0.110
2		✓		0.847±0.043	26.771±0.263
3			✓	0.843±0.036	26.450±0.241
4	✓	✓		0.870±0.027	28.716±0.183
5	✓		✓	0.873±0.024	28.349±0.124
6		✓	✓	0.853±0.041	27.041±0.247
7	✓	✓	✓	0.888±0.017	28.901±0.189

Effect of dark-field reference input. Introducing the dark-field reference input alone (Config. 1) substantially improves reconstruction performance, achieving a PSNR of 27.883 dB and an SSIM of 0.863, corresponding to gains of 2.57 dB and 0.032 compared with the U-Net baseline (Config. 0: 25.312 dB / 0.831). Notably, this improvement accounts for approximately 60% of the total PSNR gain achieved by the complete HBDF-RE framework, indicating that the dark-field modality serves as the primary performance contributor. This enhancement can be attributed to the intrinsic imaging characteristics of dark-field microscopy. Compared with bright-field imaging, dark-field acquisition exhibits significantly enhanced sensitivity to scattering-related structures, edge information, and refractive index variations, thereby providing complementary contrast cues that are inaccessible to bright-field imaging alone. Such physically meaningful complementary information enables the network to more faithfully reconstruct the fine structural details present in high-numerical-aperture (NA) bright-field images, effectively alleviating the ill-posed nature of the super-resolution inverse problem. Furthermore, when the attention mechanism and frequency-domain loss are jointly introduced without dark-field guidance (Config. 6: 27.041 dB / 0.853), the performance improvement remains relatively limited, further suggesting that architectural refinement and loss-function optimization achieve maximal effectiveness only when physically grounded complementary priors are supplied by the dark-field modality.

Effect of spatial attention mechanism. Introducing the spatial attention mechanism alone (Config. 2) yields a PSNR of 26.771 dB and an SSIM of 0.847, corresponding to improvements of 1.46 dB and 0.016 over the baseline. When combined with the dark-field reference input (Config. 4), the performance further improves to 28.716 dB / 0.870, exceeding the dark-field-only configuration (Config. 1) by 0.83 dB in PSNR. This result indicates that the attention mechanism effectively exploits the complementary information introduced by the dark-field modality. Specifically, the attention module dynamically captures feature correlations between bright-field and dark-field representations through adaptive weight allocation, thereby selectively enhancing diagnostically relevant multimodal information while suppressing task-irrelevant interference. These observations demonstrate that attention-guided feature modulation consistently improves reconstruction performance and facilitates more effective multimodal feature fusion.

Effect of spatial-frequency domain joint loss function. Introducing the spatial-frequency domain joint loss function alone (Config. 3) achieves a PSNR of 26.450 dB and an SSIM of 0.843, yielding gains of 1.14 dB and 0.012 over the baseline configuration. By jointly constraining reconstruction errors in both the spatial and Fourier domains, the proposed loss function simultaneously preserves low-frequency structural consistency and high-frequency textural fidelity, thereby mitigating the tendency of conventional pixel-wise mean squared error optimization to converge toward over-smoothed solutions. Its contribution becomes more evident when combined with the dark-field reference input (Config. 5: 28.349 dB / 0.873), suggesting that frequency-domain supervision becomes particularly effective when physically meaningful high-frequency priors are already available from the dark-field modality.

Effect of integrating all three components. HBDF-RE, integrating all three components (Config. 7), achieves the best

overall performance across both evaluation metrics, reaching a PSNR of 28.901 dB and an SSIM of 0.888, corresponding to improvements of 3.59 dB and 0.057 over the U-Net baseline, respectively. The consistent incremental gains observed as the three components are progressively integrated demonstrate a clear synergistic relationship among the dark-field reference input, spatial attention mechanism, and spatial-frequency domain loss function. Collectively, these components provide complementary advantages that jointly enhance both global structural fidelity and fine-detail restoration, thereby validating the rationality and necessity of the overall HBDF-RE framework design.

Section 3: Color calibration algorithm

In pathological diagnosis, color accuracy and consistency are critical for clinical decision-making. Pathologists rely on tissue color characteristics to identify cellular morphology, tissue architecture, and pathological features; color inconsistencies directly compromise diagnostic accuracy and may result in misdiagnosis or missed diagnosis. However, digital pathology images are susceptible to color variations that differ from direct visual observation through eyepieces. These discrepancies arise from multiple factors inherent to digital imaging systems: aging of illumination light sources causes spectral shifts, sensor degradation over time alters spectral response characteristics, and device-specific sensor properties introduce systematic color biases. Consequently, the same tissue specimen may appear with noticeably different colors when imaged by different digital systems or even by the same system at different time points, despite appearing identical under direct microscopic observation. In the context of telepathology consultations and digital pathology archiving systems, color consistency across different acquisition devices and over time is fundamental to ensuring diagnostic quality and reproducibility.

Moreover, with the increasing adoption of artificial intelligence in pathological diagnosis, color consistency has become equally essential for AI model performance. Deep learning models inherently learn specific color distribution patterns during training^{S1–S4}; when test data exhibit color deviations, model generalization capability deteriorates substantially. This is particularly critical in supervised learning frameworks, where color discrepancies between input and label data compel the model to learn color transformation mappings rather than authentic spatial structure and texture reconstruction capabilities. Consequently, valuable network capacity is allocated to compensating for color variations instead of extracting and reconstructing high-frequency spatial information. Therefore, ensuring color consistency between training and test sets, as well as between input-output data pairs, constitutes a prerequisite for developing robust and reliable AI-assisted diagnostic systems^{S5}.

To solve this problem, a principles-based color calibration is required. Conventional approaches—including histogram equalization, color transfer, and learning-based correction—operate on image statistics without physical grounding and may propagate systematic biases. We instead propose a standard color chart-based preprocessing strategy that leverages colorimetric reference targets with known spectral properties to establish device-independent mappings. Specifically, we employ the Applied Image calibration slide with 24 colors as our reference standard. Our approach adopts a calibration framework similar to that of Ohnishi et al.^{S6}, which demonstrated effective color standardization in immunohistochemistry analysis. However, we extend this framework by incorporating higher-order nonlinearities in the color correction matrix design to better capture the complex nonlinear response characteristics of our imaging system. By resolving color inconsistencies at the acquisition source through this enhanced physically grounded calibration, networks can focus on genuine spatial detail reconstruction rather than color space adaptation.

The core procedural steps of the proposed workflow are presented in Fig. S2. Calibration effectiveness requires that pathologically stained slides and Color Calibration Slides (CCS), as shown in Fig. S2(a), be acquired simultaneously under identical imaging system configurations. We define linear RGB color space as the standard device-independent space for subsequent image analysis. As shown in Fig. S2(b), during actual imaging processes, RGB values output by the system may be device-dependent or consistent with standard color spaces such as sRGB. While some systems can directly output linear RGB values, most imaging systems' raw RGB signals typically require gamma calibration to meet display standards. Non-unity gamma values indicate that the system outputs uncorrected nonlinear responses. Therefore, the color calibration process, as shown in Fig. S2(c), must simultaneously consider both matrix-based color space transformation and gamma linearization effects. To derive gamma linearization parameters and color space transformation matrices, we utilize known standard color patches on the CCS as references, fitting and comparing the measured colors with standard colors^{S7}. As illustrated in Fig. S2(d), after obtaining gamma parameters for linearization and the matrix-based color-calibration transform, these two steps are sequentially applied to each image to be processed, ultimately yielding corrected linear RGB images in a standard device-independent color space. The linearization process can be expressed as:

$$Color^{DEV, Lin} = (Color^{DEV})^\gamma, \quad (S3)$$

where $Color^{Dev} = R^{Dev}, G^{Dev}$ or B^{Dev} represents the device-dependent RGB values before gamma calibration, $Color^{Dev, Lin} = R^{Dev, Lin}, G^{Dev, Lin}$ or $B^{Dev, Lin}$ denotes the linearized RGB values after processing, and γ is the gamma value derived by the system. The color space conversion process employs nonlinear regression to solve the calibration matrix, as formu-

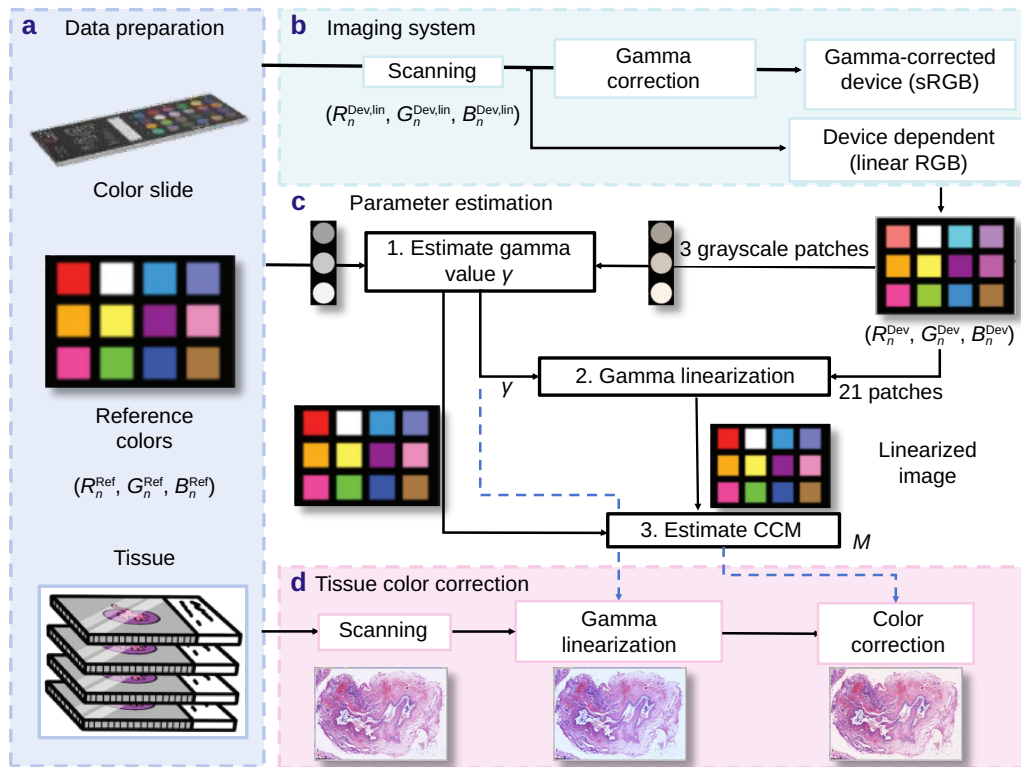


Fig. S2 | Overview of the proposed color calibration workflow for pathological images. (a) Data preparation. (b) Imaging system. (c) Parameter estimation for gamma and color correction matrix. (d) Tissue color correction.

lated in the following equation:

$$C_r = M(C_s), \quad (S4)$$

where

$$C_s = \begin{pmatrix} (R^{DEV}, G^{DEV}, B^{DEV}, \sqrt{R^{DEV}G^{DEV}}, \sqrt{R^{DEV}B^{DEV}}, \sqrt{G^{DEV}B^{DEV}}, 1) \\ (R^{DEV}, G^{DEV}, B^{DEV}, \sqrt{R^{DEV}G^{DEV}}, \sqrt{R^{DEV}B^{DEV}}, \sqrt{G^{DEV}B^{DEV}}, 1) \\ (R^{DEV}, G^{DEV}, B^{DEV}, \sqrt{R^{DEV}G^{DEV}}, \sqrt{R^{DEV}B^{DEV}}, \sqrt{G^{DEV}B^{DEV}}, 1) \end{pmatrix}^T, \quad (S5)$$

where M represents the nonlinear regression mapping function. Unlike conventional approaches that employ simple polynomial transforms, we adopt a 2nd-order root-polynomial model that incorporates cross-channel interaction terms (e.g., $\sqrt{R \cdot G}$, $\sqrt{R \cdot B}$, $\sqrt{G \cdot B}$) to better capture the nonlinear color response characteristics of the imaging system. The regression parameters are derived by fitting the measured color matrix C_s from the CCS to the reference color matrix C_r . This enhanced nonlinear mapping enables the generation of optimal color calibration matrices tailored to different objective lens magnifications, thereby effectively mitigating color deviations induced by multi-magnification imaging and enhancing the consistency and accuracy of multi-source pathological image analysis.

Section 4: Quantitative resolution evaluation using USAF 1951 target

To quantitatively assess the spatial resolution boundaries and structural fidelity afforded by the proposed HBDF-RE framework, benchmarking experiments were performed employing a standard USAF 1951 resolution target. This target provides a calibrated metric to identify the minimum resolvable feature size and evaluate the modulation transfer characteristics of the reconstruction model across diverse spatial frequency domains. The synthesized full field-of-view (FOV) results, magnified sub-regions, and corresponding cross-sectional intensity profiles are illustrated in Fig. S3.

As characterized in Fig. S3(b), the raw low-resolution image acquired via a $4\times/0.16$ -NA objective exhibits severe blur, primarily arising from the limited resolving capability imposed by the diffraction-limited optics. In the mid-frequency regime (the upper row of Fig. S3(b)), the $4\times$ baseline suffers from isotropic edge degradation. Conversely, the HBDF-RE framework effectively sharpens the structural boundaries and preserves the pristine gap topology, yielding an isotropic resolution

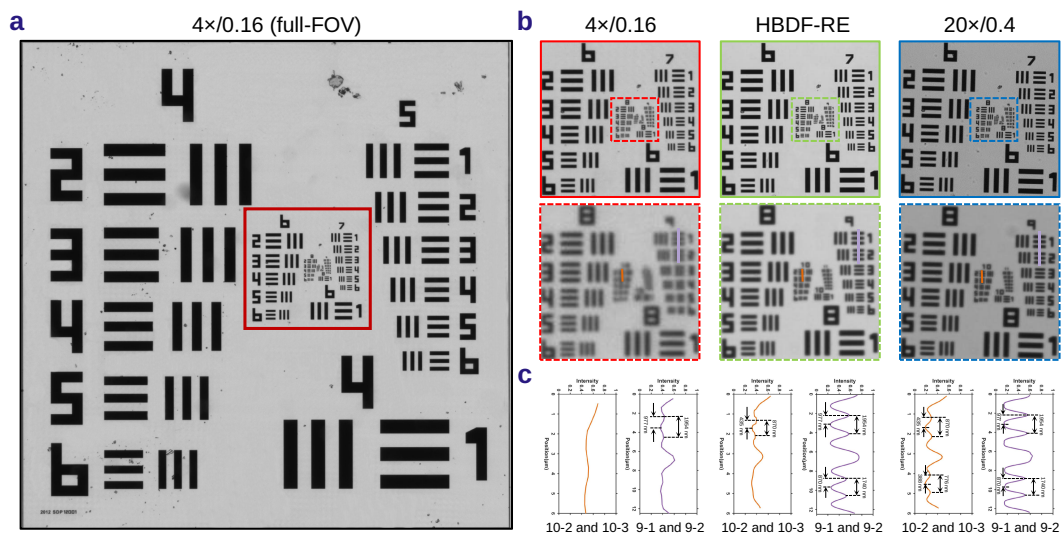


Fig. S3 | Quantitative resolution assessment using the USAF 1951 target. (a) Full field-of-view (FOV) image captured under the low-resolution network input. (b) Magnified views of the target elements for performance comparison. (c) Normalized intensity profiles along the purple lines for Elements 9-1 and 9-2, and along the orange lines for Elements 10-2 and 10-3.

enhancement that matches the geometric fidelity of the $20\times$ /0.4-NA high-resolution reference. This demonstrates the model's capacity for high-fidelity spatial-frequency spectrum restoration without generating hallucinatory artifacts.

Furthermore, the lower row of Fig. S3(b) illustrates the reconstruction performance at the extreme high-frequency limit. Due to the physical optical cutoff frequency of the $4\times$ objective, the micro-grating arrays in Group 9 and Group 10 are entirely obscured, degenerating into a homogeneous background. The HBDF-RE framework successfully circumvents this hardware-bound resolution barrier, resolving distinct three-bar patterns down to Element 10-2.

To validate this resolution breakthrough numerically, the normalized intensity profiles extracted along the purple trajectories for Elements 9-1 and 9-2 and the orange trajectories for Elements 10-2 and 10-3 were analyzed, as presented in Fig. S3(c). The raw low-resolution baseline ($4\times$ /0.16-NA) exhibits severe structural distortion and modulation degradation. For the mid-high frequency regime (Elements 9-1 and 9-2), the baseline profile manifests unresolvable blur with isotropic broadening, whereas for the extreme high-frequency regime (Elements 10-2 and 10-3), the periodic peaks and valleys are completely merged into a flat line due to the physical optical cutoff. In stark contrast, the HBDF-RE framework successfully reconstructs well-defined periodic modulations with steep gradient transitions. For Elements 9-1 and 9-2, HBDF-RE accurately restores the sharp three-peak topology, nearly replicating the characteristic periods of 1954 nm and 1740 nm, as well as the 977 nm half-pitch resolution observed in the $20\times$ /0.4-NA high-resolution reference. More importantly, at the hardware-bound resolution barrier, the HBDF-RE profile resolves distinct periodic peaks for Group 10, demonstrating an ultimate experimental resolution cutoff at Element 10-2. Based on the restored spatial modulation, this cutoff directly corresponds to an optimal full-pitch resolution of 870 nm and a half-pitch resolution of 435 nm. The framework successfully extracts these sub-diffraction fine structures from the homogeneous background with prominent modulation depth, tightly matching the $20\times$ higher-NA bright-field reference and highly approaching the theoretical synthetic aperture limit of 431 nm.

This robust quantitative enhancement solidifies that the HBDF-RE framework significantly expands the synthetic numerical aperture and optimizes the system's optical transfer function. Consequently, it achieves the dual efficacy of maintaining a wide macroscopic FOV while simultaneously attaining a microscopic 435 nm half-pitch resolution, presenting an ideal solution for high-throughput quantitative imaging.

Section 5: Gland segmentation task performance

To validate the practical value of the proposed method in downstream tasks, we designed gland segmentation experiments to evaluate the quality of images reconstructed by different super-resolution methods. The experiments employed a pre-trained DeepLabv3 model as the segmentation network^{S8}, which was fine-tuned on the GlaS (Gland Segmentation) dataset^{S9}. The test set comprised 18,486 low-resolution glandular tissue images, which were reconstructed to high-resolution using different super-resolution methods (U-Net, SRGAN, HBDF-RE) and compared against high-resolution ($20\times$) images through the segmentation model. All segmentation masks (higher-NA bright-field reference) were manually annotated by three experi-

enced pathologists using Labelme software and cross-validated.

High reliability in reconstruction and the suppression of hallucinations are important, as clearly demonstrated in the representative segmentation visualization results presented in Fig. S4(a). U-Net reconstruction suffers from residual over-smoothing artifacts that compromise boundary precision, while SRGAN introduces hallucination artifacts that cause false gland detections and boundary distortions (highlighted in yellow boxes). In contrast, our method effectively suppresses artifact generation (up to 84% reduction compared to conventional methods), maintaining superior gland boundary clarity and structural integrity in reconstructed images with up to 6.5% improvement in segmentation performance, thereby substantially improving segmentation outcomes with performance approaching that of 20× /0.4-NA images.

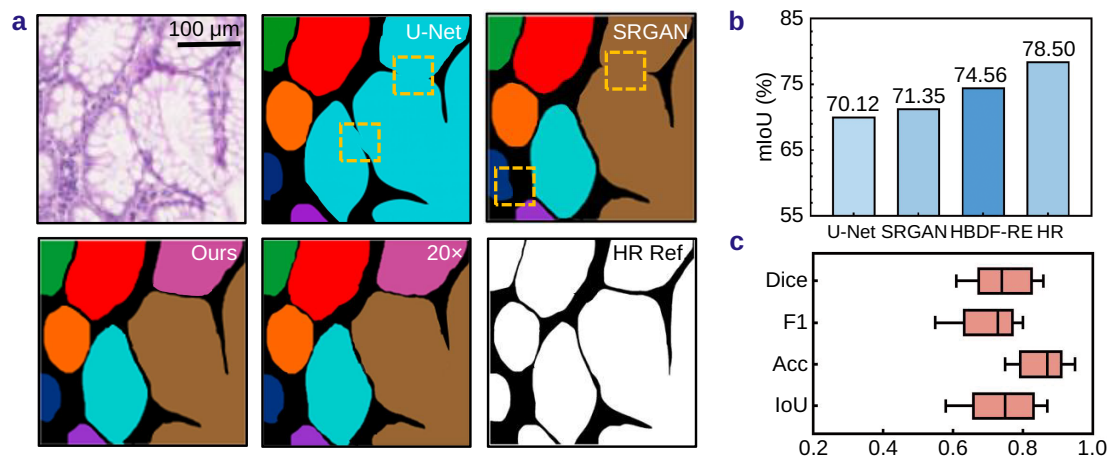


Fig. S4 | HBDF-RE model for gland segmentation performance. (a) Illustrations of four experiment setups for testing DeepLabv3 on reconstructed images. (b) The segmentation performance of different networks. (c) Four medical indicators of HBDF-RE segmentation effect.

Furthermore, Fig. S4(b) and S4(c) summarize key segmentation metrics for our model. Our method achieves optimal performance across all metrics: Dice coefficient of 0.735, mean IoU of 0.745, mean accuracy of 0.853, and F1 score of 0.724. In comparison, the U-Net method achieves a mean IoU of 0.701, and SRGAN achieves 0.713, both significantly lower than our method. As an upper-bound performance reference, direct segmentation using 20× /0.4-NA images yields a mean IoU of 0.785, indicating that our method achieved 94.8% of its performance. The experimental results indicate that HBDF-RE reconstructed images are suitable for direct pathological diagnosis, faithfully representing true tissue morphology rather than algorithmic artifacts while eliminating the need for time-consuming high-magnification scanning—both representing key clinical advantages of the proposed method.

Section 6: Generalization across staining protocols

6.1 Generalization to immunohistochemistry-stained tissue sections

To further substantiate the generalization capability of HBDF-RE beyond the primary training distribution, we conducted additional reconstruction experiments on tissue sections stained with Immunohistochemistry (IHC)—a modality entirely absent from the training cohort. Compared with the Hematoxylin and Eosin (H&E) staining protocol employed during training, IHC imaging presents fundamentally distinct visual characteristics, including a characteristic brown diaminobenzidine (DAB) and blue hematoxylin dual-channel contrast that encodes protein expression information at the subcellular level. This modality shift therefore constitutes a rigorous out-of-distribution evaluation of cross-staining robustness. Representative reconstruction results are presented in Fig. S5.

As shown in Fig. S5, HBDF-RE achieves a PSNR of 28.101 dB and an SSIM of 0.879 on IHC-stained specimens, substantially outperforming both U-Net (25.554 dB / 0.841) and SRGAN (25.213 dB / 0.839). As indicated by the yellow arrowheads, HBDF-RE effectively resolves DAB-positive membrane-localized staining boundaries that are critical for biomarker quantification, whereas U-Net and SRGAN exhibit notable contrast degradation and boundary ambiguity in these diagnostically sensitive regions. Similarly, the blue arrowheads highlight hematoxylin-stained nuclear regions in which conventional approaches produce over-smoothed reconstructions that obscure chromatin-level detail, while HBDF-RE faithfully restores nuclear morphology and cytoplasmic texture closely approaching the 20×/0.4-NA high-resolution reference. These results

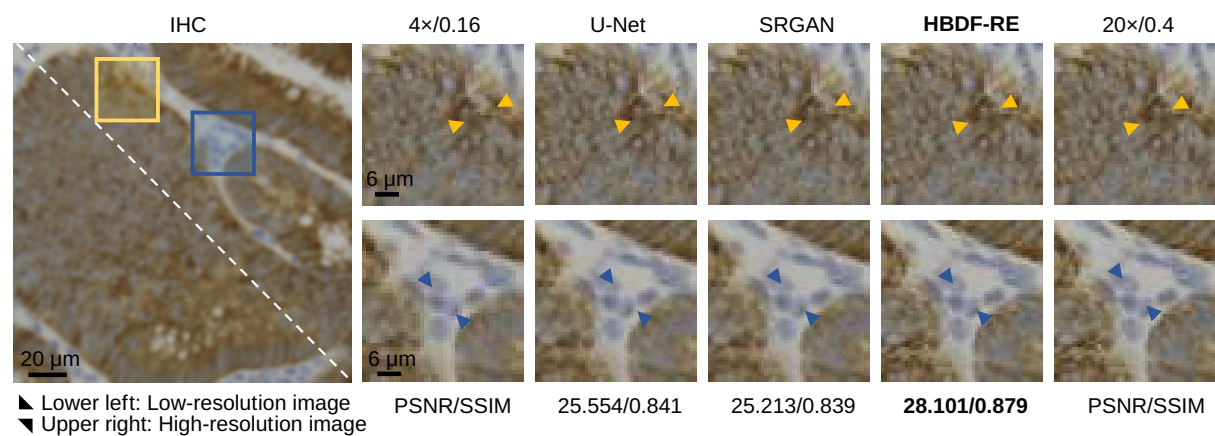


Fig. S5 | Super-resolution reconstruction results on IHC-stained tissue sections

confirm that the resolution enhancement achieved by HBDF-RE generalizes robustly to previously unseen tissue types and staining modalities, further validating the clinical applicability and broad generalizability of the proposed framework.

6.2 Robustness of illumination mode selection across staining protocols

To systematically validate whether the optimal illumination selection criterion established in the main text generalizes beyond a single staining modality, we evaluated the three dark-field illumination modes-P1 ($NA_{\text{ill}}^{\text{DF}} \approx 0.21$), P2 ($NA_{\text{ill}}^{\text{DF}} \approx 0.42$), and P3 ($NA_{\text{ill}}^{\text{DF}} \approx 0.63$)-across three representative histological staining protocols: Hematoxylin and Eosin (H&E), Papanicolaou (Pap), and Immunohistochemistry (IHC) staining. Quantitative results are summarized in Table S2.

As shown in Table S2, the P2 illumination mode consistently achieves the highest PSNR and SSIM values across all three staining protocols, reaching 28.347 dB / 0.874 (H&E), 27.991 dB / 0.881 (IHC), and 28.591 dB / 0.876 (Pap), with performance margins over P1 and P3 that are both statistically meaningful and directionally consistent with the findings reported in the main text. These results demonstrate that the superiority of the P2 mode is not an artifact of any particular staining characteristic or tissue preparation method, but rather reflects its physically principled advantage in complementary contrast provision. Therefore, the consistent cross-staining advantage of P2 confirms that the illumination selection criterion established in the main text exhibits robust generalizability across heterogeneous clinical staining conditions, which can further support its adoption as a principled design choice for dark-field-assisted super-resolution in digital pathology.

Table S2 | Reconstruction performance (PSNR dB / SSIM) of three dark-field illumination modes across different histological staining protocols. The best result in each row is highlighted in bold.

Staining protocol	P1 (PSNR dB / SSIM)	P2 (PSNR dB / SSIM)	P3 (PSNR dB / SSIM)
H&E	26.076 / 0.863	28.347 / 0.874	26.968 / 0.868
IHC	26.147 / 0.857	27.991 / 0.881	26.591 / 0.867
Pap	26.932 / 0.860	28.591 / 0.876	27.466 / 0.863

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