A New Spline-Based Image Processing Technique with Applications to Whole-Slide Imaging of Plant Roots

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***Abstract*—A new spline-based method was developed to transform over 100,000 multi-gigapixel plant root whole-slide scanning images into deep learning-ready image patches. We validated the robustness of our approach by analyzing eight bootstrap samples of a thousand images each classified as excellent, moderate, or bad quality by four independent experts. The goodness of fit, roughness, and irregularity of the splines were uniform across all quality levels, confirming our method's reliability for generating patches from gigapixel plant root images. These patches will be used as an input to deep learning based algorithms capable of detecting and classifying mycorrhized root segments and types of fungal structures presented.**

***Keywords*—cubic spline, deep learning, image processing, whole-slide imaging, arbuscular mycorrhizal fungi**

I. INTRODUCTION

The interaction between plants and Arbuscular Mycorrhizal Fungi (AMF) significantly influences disease resistance, drought tolerance, and biomass [1]. Recent studies on the genomic regulation of plants on AMF symbiosis require increasing number of root samples to be screened [2, 3]. To facilitate the design of large-scale genomic studies, whole-slide scanning proves to be valuable in this context [4]. Traditionally, trained operators spent extensive hours manually screening and annotating whole-slide images (WSIs). To speed up the WSI data analysis and improve its effectives in operational, high throughput conditions, deep learning (DL) algorithms can be used as a promising technology [5, 6].

Despite the potential of DL algorithms, the challenge in this field of work still lies in the sheer size of WSIs, often reaching multi-gigapixel dimensions, making them unsuitable as direct inputs for DL algorithms [7]. Our contribution to addressing this limitation is a spline-based algorithm tracing the object contours during patch extraction that serves a dual purpose. Firstly, it efficiently removes the background, eliminating the need for trained operators or algorithms to spend time and computational power to processing redundant white backgrounds. Secondly, the technique extracts patches tailored to the unique shape of plant roots, ensuring that our approach is applied effectively to the processed WSIs. Our innovative approach is the first step in the optimization of DL application in the analysis of large-scale genomic studies involving mycorrhizal fungi and plant roots.

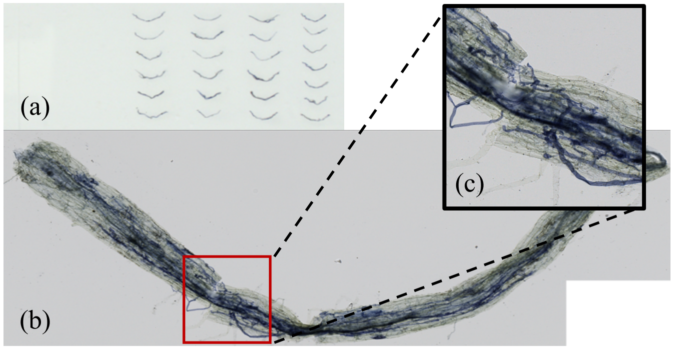

Fig. 1. (a) Root segments mounted on a slide; (b) a root segment with mycorrhizal fungi stained in blue; (c) fungal structures were captured in high resolution as shown by the zoom-in image.

II. RESULTS

Approximately 101K WSIs of fine root filaments were captured from 4,044 sorghum plants, representing 337 genotypes. These samples were stained with a modified ink and vinegar protocol [8]. For each plant, 20-30 root segments of 1 cm were prepared on slides, uniquely labeled with genotype and block information (Fig. 1a). The ZEISS Axioscan 7 microscope equipped with an Axiocam 705 captured up to 100 slides at 10× magnification at a time, achieving 3.45 μm/pixel resolution (72 PPI). WSIs were saved in Zeiss CZI format ranging from 1.06

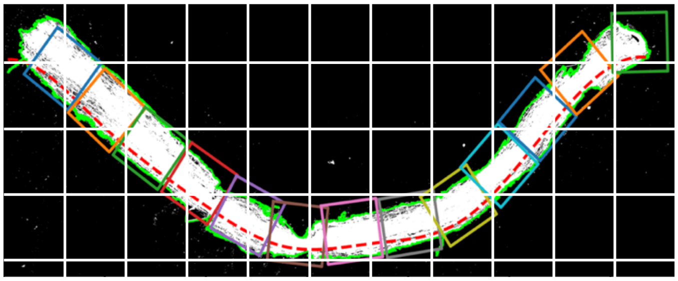

Fig. 2. Thirteen spline guided patches shown in random color were automatically generated to cover the root region, providing a more efficient solution than standard sliding window method shown with white gridlines.

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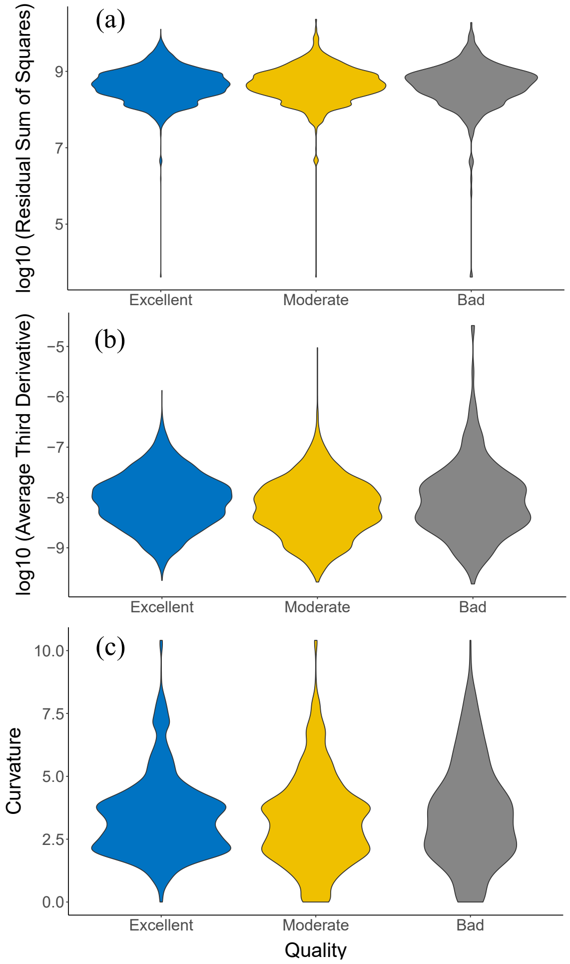

Fig. 3. Violin plots are used to show the distribution of three spline interpolation quality metrics by groups of image quality: (a) Residual sum of square of spline on log10 scale; (b) Average third derivatives of spline on log10 scale; (c) Curvature of spline.

to 6.00 Gigabytes in size, with individual root segments identifiable for image processing (Fig. 1b).

We utilized the Otsu's thresholding [9] to isolate the significant root contours, selecting the largest to convert into a list of points. A quadratic polynomial was fitted to the points. The second derivative of the polynomial was computed to determine the concavity of the root segment. WSIs were rotated so that all root segments were convex. To satiate the injective condition of spline interpolation on a fixed coordinate frame, the smallest y value was selected for a multivalued x. Then, the unique shapes of contours were represented by a piecewise cubic function with six evenly distributed internal knots [10]. We ensured a smooth interpolation by applying two continuous derivatives at each knot. Our algorithm computes uniformly spaced anchor points along the cubic spline, based on estimated arc length using the composite trapezoidal rule. The orientation and vertices of the patches were computed using the tangent and normal vectors at the anchor points, to position patches along the spline's curvature (Fig. 2.).

The robustness of the algorithm was tested with 1000 WSIs bootstrapped 8 times. Four operators independently categorized WSIs into Excellent, Moderate, or Bad based on U-shape integrity, orientation, root branching, and clarity. Excellent images exhibited a complete, correctly oriented U-shape; Moderate images allowed for broken U-shapes and minor imperfections; Bad images included misshapen or highly branched roots and empty or out-of-focus images. Across bootstraps, the image quality distribution averaged 44.91 (±5.55)%, 50.09 (±5.35)% and 5.80 (±1.25)% for Excellent, Moderate, and Bad, respectively. Our performance metrics for fit, roughness, and irregularity—residual sum of squares, third derivative average, and curvature— were stable across quality categories (Fig. 3).

III. CONCLUSIONS

We present a robust algorithm for generating patches as DL inputs. To showcase its efficiency, we aim to compare it to the standard sliding-window method in producing informative patches from WSIs of plant roots. Enhancements to patch quality may involve optimizing the area of overlap with root segments and using local histogram equalization to improve patch image quality. We anticipate the broad applicability of our algorithm, extending to roots taken under contexts beyond mycorrhizal colonization research and analogous structures, such as roads, cracks, and blood vessels, in DL research.

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