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Investigating the Impact of the Bit Depth of Fluorescence-Stained Images on the Performance of Deep Learning-Based Nuclei Instance Segmentation

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Introduction

- Nuclei instance segmentation is a key step in the computer-mediated analysis of histological fluorescence-stained (FS) images
- Many semi automatic and fully automatic methods have been proposed to perform nuclei instance segmentation in FS images
- Supervised deep learning (DL)-based methods are the state-of-the-art fully automatic methods to perform this task
- FS image bit depth may affect the DL-based nuclei instance segmentation performance, but no study has been performed to investigate its impact

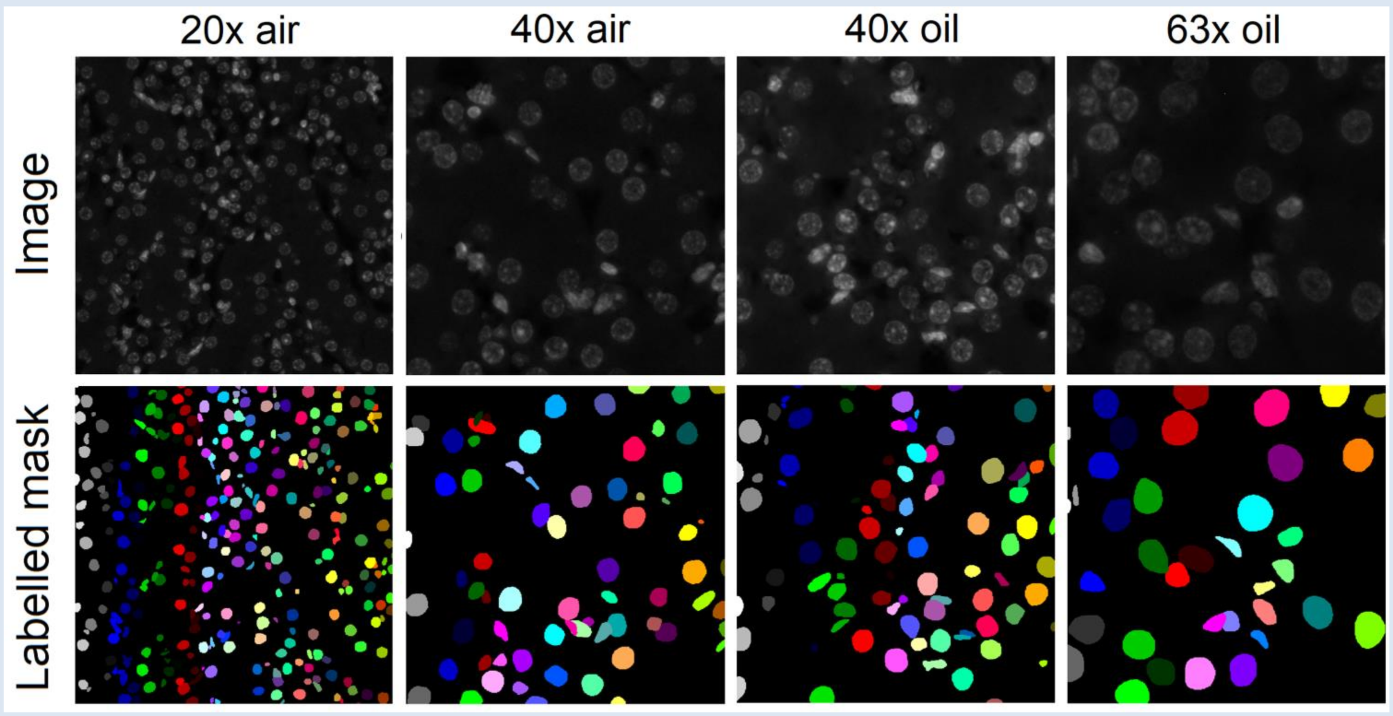
Summary

- We systematically studied the effect of image bit depth (mainly 8 bit vs. 16 bit) on the nuclei instance segmentation performance
- We also released a fully annotated FS histological image dataset of nuclei at different image magnifications (20x, 40x, and 63x) and different image bit depth (8 bits and 16 bits)
- We observed very competitive nuclei instance segmentation performances for the models trained with 8-bit and 16-bit images. This suggests that processing 8-bit images is sufficient for nuclei instance segmentation of FS images in most cases
- GitHub Link: https://github.com/masih4/BitDepth_NucSeg

Method

Datasets

- We used two datasets:
  - **BitDepth** dataset (released in this work) [1]
    - 70 images with ~3400 manually segmented nuclei
    - 5 mouse organs (bone, heart, kidney, liver, muscle)
    - DAPI-stained nuclei
    - 20x, 40x, and 63x objectives
    - Fixed size of 512x512 pixels
    - Some examples from bone are shown below:



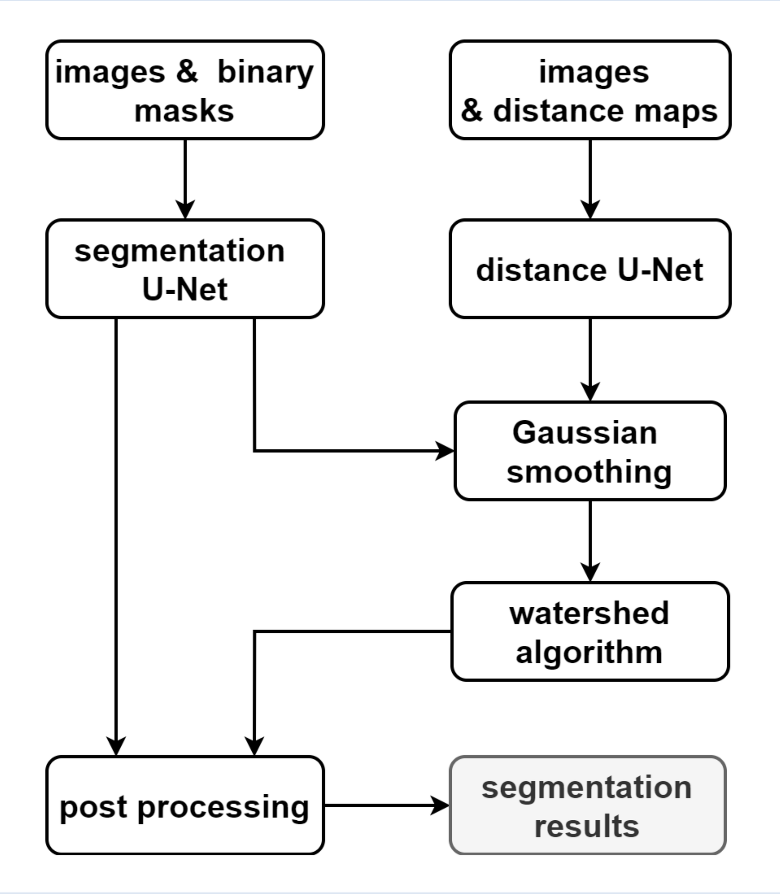
  - **Caicedo et al.** dataset [2]
    - 200 images with ~23000 manually segmented nuclei
    - U2OS cells
    - DAPI-stained nuclei
    - 20x objective
    - Fixed size of 520x696 pixels

Pre-processing

- We tested different normalization techniques and chose min-max normalization with outlier removal as the main preprocessing step (further details in [1])

DL-based nuclei segmentation model

- We employed our recently published algorithm for nuclei instance segmentation [3]
- The flowchart of the model is shown below:

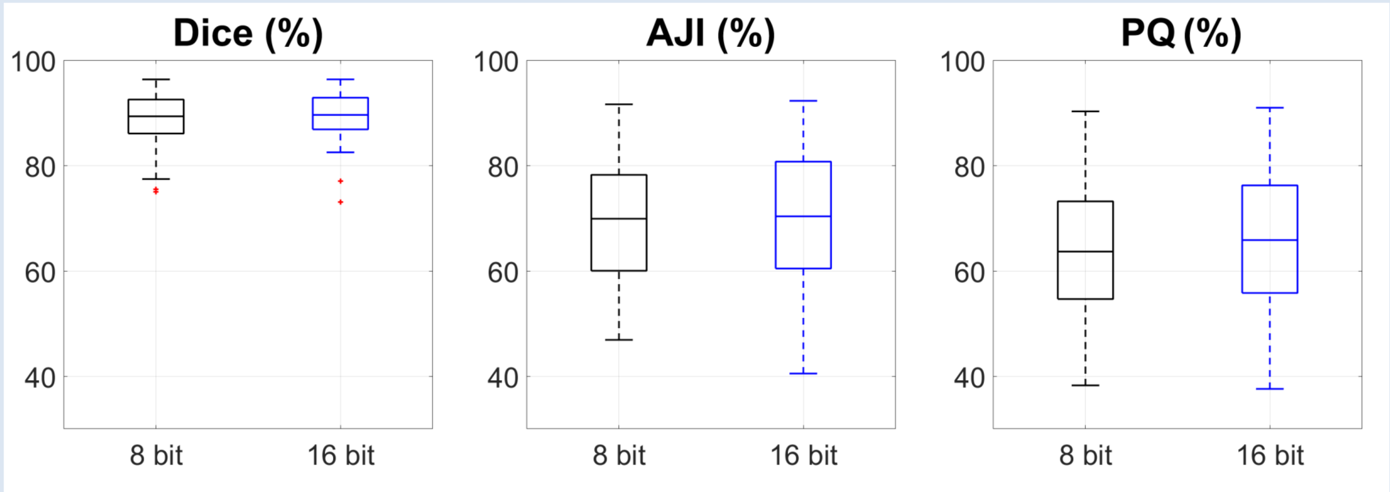


Evaluation

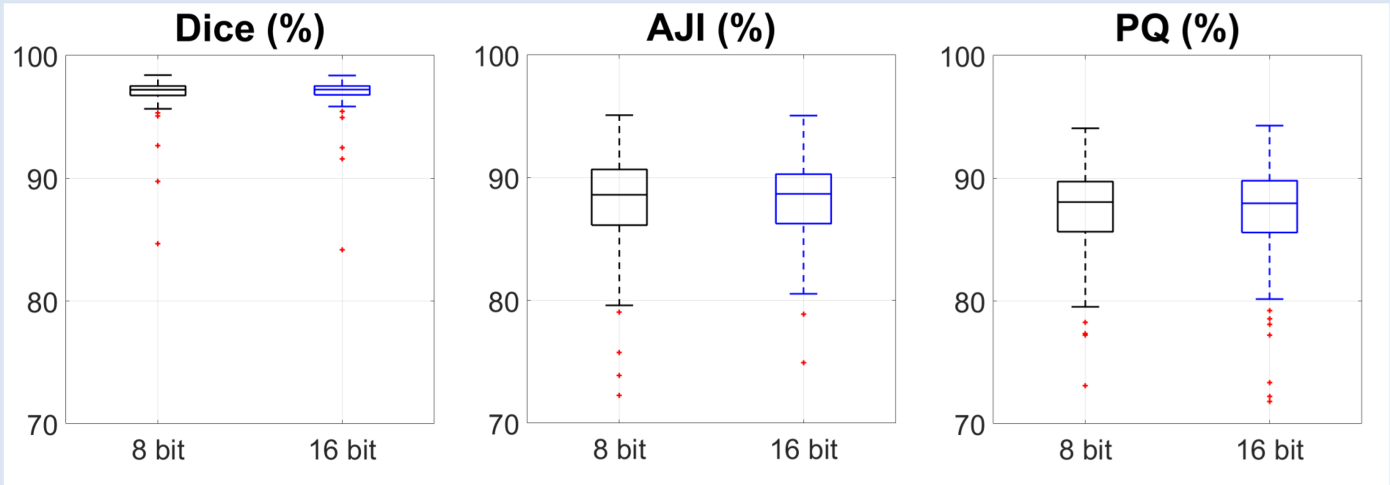
- Dice score, aggregate Jaccard index (AJI), and panoptic quality score (PQ) [4]

Results

1. Segmentation results based on 5-fold cross validation for the BitDepth dataset

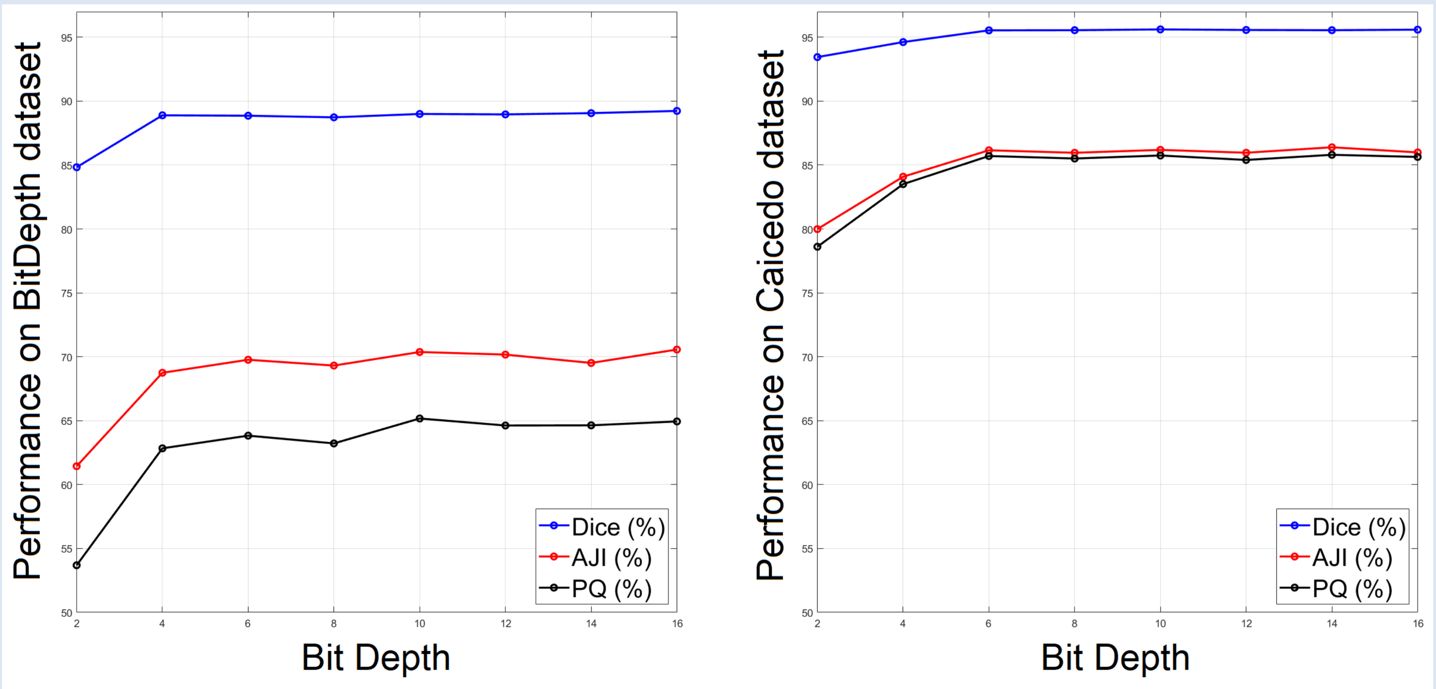


2. Segmentation results based on 5-fold cross validation for the Caicedo et al. dataset



- We observed very competitive nuclei instance segmentation performances for 8-bit and 16-bit images for both datasets.

3. Segmentation performance for various image bit depth



- The above results show that with reducing the image bit depth to two or four bits, the nuclei instance segmentation performances degraded. However, from the image bit depth of six upward, similar average results can be observed.

The competitive segmentation performance for various image bit depths suggested that the DL model relied on the nuclei morphological features rather than exact intensity values of background and foreground pixels to segment nuclei.

References

[1] Mahbod et al: Investigating the Impact of the Bit Depth of Fluorescence-Stained Images on the Performance of Deep Learning-Based Nuclei Instance Segmentation, Diagnostics, 2021
[2] Caicedo et al: Evaluation of Deep Learning Strategies for Nucleus Segmentation in Fluorescence Images, Cytom. Part A, 2019
[3] Mahbod et al: A Two-Stage U-Net Algorithm for Segmentation of Nuclei in H&E-Stained Tissues, ECDP, 2019
[4] Graham et al: Hover-Net: Simultaneous segmentation and classification of nuclei in multi-tissue histology images, Med. Image Anal, 2019

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