Registration accuracy between Whole Slide Images and Glass Slides in eeDAP workflow

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250 Word Abstract

We have previously developed the Evaluation Environment of Digital and Analog Pathology (eeDAP) framework for evaluating Whole Slide Imaging (WSI). eeDAP includes hardware components (computer, microscope, eyepiece camera, microscope step motor stage) and software to control the stage, perform registration and collect data. The purpose of eeDAP is to help conduct studies in which pathologists view and evaluate the same fields of view (FOVs) in a glass slide on a microscope and in a whole slide image (WSI) on a digital display by registering the two. Registration happens at the beginning of a study (global registration) and during a study (local re-registrations). Global registration is interactive and defines the relationship between the WSI and stage coordinates. Local re-registrations are automatic and ensure the pathologist evaluates the correct FOVs. All registrations are based on image-based cross correlation. This study evaluates the registration accuracy achieved throughout a study. To measure the accuracy, we used an eyepiece ruler reticle to measure the shift distance between the center of eyepiece and a target feature expected in the center. Two readers independently registered 60 FOVs from 6 glass slides. The glass slides included canine oral melanoma, human lymph node and human breast tissues with H&E, pHH3, ER and HER2 stains. Preliminary result show that registration was within 2.5 micrometers in more than 90% of the FOVs. Registration did not appear to depend on tissue type, stain, or operator. The accuracy error was mainly dependent on the image quality and properties of the FOV such as homogeneity and contrast.

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We evaluated the accuracy of a tool that registers glass slides on a microscope to corresponding regions in whole slide images. The study investigated the registration performed by two readers for different types of tissues and stains, across different fields of view (FOV). Preliminary result show that registration was within 2.5 micrometers in more than 90% of the FOVs. Registration did not appear to depend on tissue type or stain. The accuracy was mainly dependent on the image quality and properties of the FOV such as homogeneity and contrast.

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Purpose

The Evaluation Environment of Digital and Analog Pathology (eeDAP) was developed to conduct studies in which pathologists can evaluate the same fields of view (FOVs) in a glass slide on a microscope and in a whole slide image (WSI) on a digital display [1]. This can be achieved by glass slide to WSI registration enabled by eeDAP. In this study, we examine the registration accuracy of this system.

Methods

eeDAP includes hardware components (computer, microscope, eyepiece camera, microscope step motor stage) and software to control the stage, perform registration and collect data. Registration is performed at the beginning of a study (global registration) and during a study (local re-registrations). Global registration is interactive and defines the

relationship between the WSI and stage coordinates. Local re-registrations are automatic and ensure the pathologist evaluates the correct FOVs.

All image registrations are based on normalized 2D cross correlation [2] and use a patch of the WSI and a camera image of the FOV seen through the microscope. The camera image is rescaled to the scale of the WSI FOV. Registration by cross correlation convolves the two images. The convolution result is high where the images are similar. Thus, the direction and distance from the peak of the convolved matrix to its center is the shift between camera image and WSI FOV.

There are two modes of registration: Padding mode and Non-Padding mode. In Padding mode, the larger of the two images being compared (the base image) is padded with zeros. This mode can find the target (the smaller of the two images being compared) when it is on the boundary of the base image, but the padding can lead to registration errors (finding the wrong location). In Non-Padding mode, the target image must be completely contained within the base image for it to be found. This mode has higher registration accuracy for the center image area, but it cannot find the target if it is located on the boundary of the base image.

In our current study, two readers performed independent evaluations for 6 glass slides. The study covers different tissue types, stains, and scanners in order to understand how robust our registration methods are. eeDAP may be affected by all of these factors. One of the slides was purposefully selected to stress-test the system; it was previously observed that it resulted in a poorly focused WSI, probably due to uneven tissue.10 FOVs were randomly chosen per slide. All FOVs had a small identifiable target in the center. A virtual reticle was used to locate the target in WSI (Figure 1 A). After registration, the readers looked through the microscope and used a rotatable ruler eyepiece reticle (figure 1 B) to measure the distance between the target and the center of the eyepiece view. The ruler has 100 divisions. Each division is 2.5 micrometers at 40X (5.0 micrometers at 20X).

There were two parts in this study: Global Registration Accuracy Study and Whole System Registration Accuracy Study, the former evaluating the global registration and stage motion; the latter evaluating the accuracy of the whole system (Global Registration + Local Re-Registrations).

In the Global Registration Accuracy Study, the reader performed global registration and measured the errors for each FOV. During the study, the reader did not perform any local re-registrations to refine registration.

In the Whole System Registration Accuracy Study, the reader began with global registration. During the study, for each FOV, the reader focused the microscope, used Padding and Non-Padding modes to refine the registration, and then measured the errors. The two modes are measured independently.
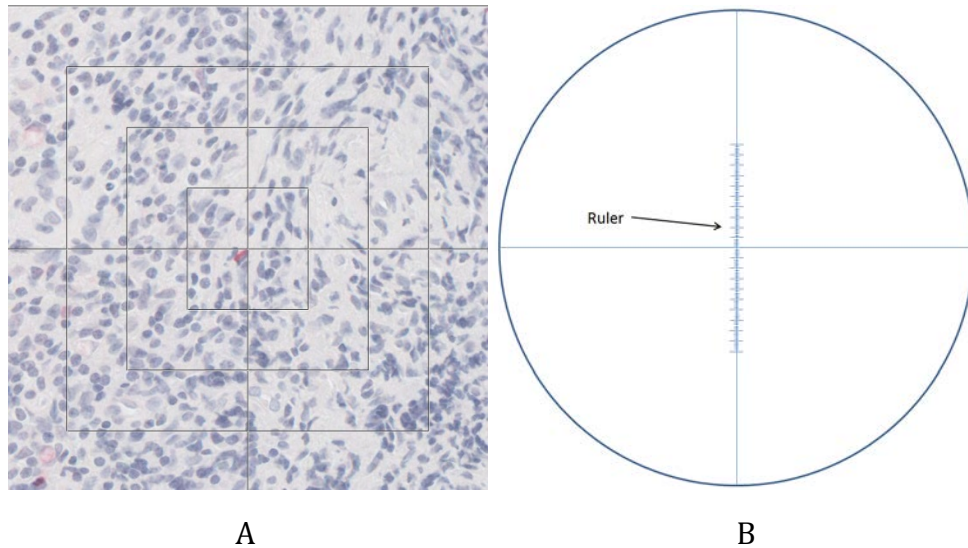
Figure 1: A) Virtual reticle indicates target (red cell) in WSI. B) Eyepiece ruler reticle.

Results

Table 1 shows preliminary results. Overall, 87% of the Padding mode local re-registrations and 97% of the Non-Padding mode local re-registrations had errors less than 2.5 micrometers (diameter of cells in the study was about 8 micrometers). However, for the Padding mode, one FOV on the Canine oral slide had an error of 125 micrometers and three FOVs on the Human lymph node slide received an error of 150 micrometers. The Non-Padding mode decreased 3 of these large FOVs errors with the final FOV having an error of 75 micrometers. There was 100% agreement between the two readers.

Table : Number of FOVs with registration error less than 2.5 micrometers

	Padding mode	Non-Padding mode
Canine oral (H&E)	10/10	10/10
Canine oral (pHH3)	9/10	10/10
Human lymph node (H&E)	7/10	9/10

Figure 2 shows one FOV which was found to have large error in both Padding and Non-Padding mode. We used a circle to identify the target, a triangle to identify the Padding mode registration result, and a star to identify the Non-Padding mode registration result. Figure 3A shows the WSI FOV. Figure 3B shows the camera image before Local Re-registration. From these two figures we can see the center of the FOV was sparse and uniform. Figure 3c shows the result of the Padding mode registration. The peak of the convolution result occurred near the boundary, the area where the padding can lead to registration errors (finding the wrong location). In Figure 3D, the Non-Padding mode found a feature similar as the target (the star), but was not the actual target.

In the future, we plan to collect more registration data. We also plan to quantify WSI and slide properties that correlate with registration accuracy error (contrast and homogeneity, and slide preparation issues like tissue thickness).
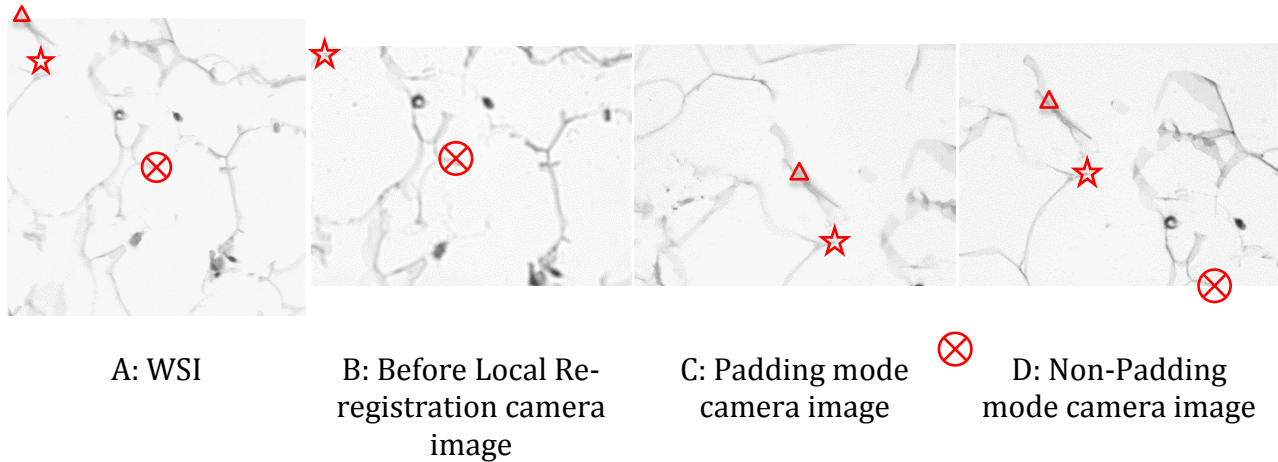
Figure 2: One FOV registration result. The circular fiducial marks the center feature (target). The triangle and star mark the center of registrations.

New or Breakthrough Work

eeDAP is a tool for conducting correlated studies between WSI and microscopy. Registration accuracy between the microscope and WSI FOVs is a critical feature of eeDAP and has not been assessed systematically. This work quantifies the registration accuracy when using this tool.

Conclusions

This work shows that the registration accuracy of Evaluation Environment of Digital and Analog Pathology is high and stable. The accuracy is mainly based on the image quality and content properties of the FOV, such as contrast and homogeneity. We recommend pre-study examination of the registration accuracy of any study to be conducted prior to a pivotal study. This examination can allow the study designer to change the study FOVs before starting the study.

This work has not been submitted anywhere else.

References

1. Gallas, Brandon D. et al. "Evaluation Environment for Digital and Analog Pathology: A Platform for Validation Studies." *Journal of Medical Imaging* 1.3 (2014): 037501. *PMC*. Web. 1 Aug. 2017.
2. Lewis, J. P. "Fast Normalized Cross-Correlation." (1995).