Reader Study on multi-head microscope: Counting and Classifying Mitotic Figures

Study Date: 11/3/2017

# Objectives

Our objectives are to collect counts of mitotic figures (MFs) and to classify candidate MFs from multiple readers simultaneously using a multi-headed microscope. An expert will also discuss classification decisions, as training the participants is another objective. The data that we will collect will be used to evaluate a MF detection algorithm. It will also inform us on reader variability in this task.

# Initial Single-Head Study Provides Slides, ROIs, and Candidate Mitotic Figures

Four readers have counted MFs from 40 regions of interest (ROIs) on 4 slides using WSI and using a single-head microscope. The ROIs in this study are 200 um x 200 um, which is equivalent to 800 x 800 pixels of a WSI with a scanning resolution of 0.25 um/pixel. This size was selected to fit within most digital displays without panning or zooming. We use a reticle in the microscope eyepiece to outline the ROI. The slides and ROIs from this single-head study will be the slides and ROIs for the high-throughput study.

## Candidate Mitotic Figures

During the digital-mode sessions, we collected locations of MFs with a custom hardware and software evaluation environment for digital and analog pathology (eeDAP). eeDAP allowed us to automatically present the same ROIs to the pathologist on the microscope or the WSI and record annotations. The pathologists identified 92 "candidate" MFs in aggregate in digital mode. We call them candidate MFs because only 21 of 92 were unanimously identified.

More than half the ROIs had 2 or 3 candidate MFs, and there were 12 ROIs with fewer than 2 candidates. In an effort to include “lower-threshold” candidate MFs, we asked one of our data curation pathologists to identify at least two “lower-threshold” candidate MFs on the 12 ROIs with fewer than 2 candidates. This yielded 34 candidates.

The “lower-threshold” candidate MFs were added to the 92 original candidate MFs for a total of 126 candidate MFs for our study.

## Definition of a Mitotic Figure

Criteria for an MF includes the loss of a nuclear membrane with condensation of chromatin forming the mitotic apparatus. The formation of the nuclear membrane within two daughter cells, signifies the end of the mitotic process and should not be counted as an MF.

# Data Collection

We will use a 14-head microscope during data collection. The study pathologists are to base their decisions on the image presented in the 14-head microscope. We will use a paper case report form (CRF) to collect the evaluations from the study pathologists. Each page of the CRF will include an image of the ROI and boxes for pathologists to provide their evaluations. The ROI is about 25% of the eyepiece field of view. We regret that we don’t have reticles for all the eyepieces (just the microscope pilot). We are unable to insert the reticle used previously into the light path for all 14 pathologists to see. The image printed on the CRF will be low-contrast (semi-transparent) so that the pathologists can mark the location of MFs (bright blue or green ultra-fine tip pens).

During data collection, the study pathologists may ask the study administrator to focus up or down or wait to proceed. The stage will not be moved left, right, up, or down. The study pathologists should not discuss any decisions, ask any questions of other study pathologists, or look at the CRF of other study pathologists. This is not a contest. There are no winners. We want independent evaluations from each study pathologist. If a study pathologist has a question, she or he should wait until the rest of the group is ready to proceed with the next ROI or candidate mitosis and then ask the study administrator the question.

**For each of the 40 ROIs**, the study pathologists will independently **circle** MFs on the image printed on the paper CRF. These are cells that he or she believes are MFs. After circling all MFs, the study pathologist needs to count them and write the total count in the box provided. Once all the pathologists have completed the **counting task** and are ready to proceed, the stage will be moved to a pre-determined candidate MF within the current ROI. All the pathologists must provide a 0-100 score for this candidate MF (**classification task**, see below for scoring instructions). Once all the pathologists have scored the current candidate MF and are ready to proceed, the stage will be moved to the next pre-determined candidate MF. This continues until all the candidate MFs in the current ROI have been viewed and scored. Once all the candidate MFs in an ROI have been scored, the group may discuss the current ROI and the cells within.

**Do not make any changes to your counting task data during the classification task.**

**Instructions for Classification Task Scoring**: The *score* we are asking you to provide each candidate MF is a rating that reflects your confidence that the candidate cell is a MF. This score is not part of any standard clinical report. You have never been trained to provide a *score.* The purpose of reporting a *score* is to provide more information than just the yes/no decision; it is meant to provide more robust, quantitative, and statistically powerful data for evaluating an MF detection/classification algorithm. The *score* arises from the following question:

*Given two cells, which one is more likely a mitotic figure?*

We ask the study pathologists to score each candidate MF according to their confidence that the cell is a MF using a 0-100 scale:

* 0 means the candidate is definitely not a MF.
* Higher numbers indicate higher confidence the candidate is a MF.
* 49 is below the yes/no threshold. “I don’t know, but I will say no, the cell is not a MF.”
* 50 is above the yes/no threshold. “I don’t know, but I will say yes, the cell is a mitosis.”
* 100 means you believe the candidate is definitely a MF.

The goal of the classification task scoring is to uniquely order the candidate mitoses by your confidence with no ties. This is a not easy! Do your best. Try not to reuse scores. At least try to avoid using the same score for cells within the same ROI. You don’t need to limit your scores to integer values. Use whatever tricks you can.