



## 1.0 PURPOSE

The purpose of this procedure is to establish a protocol for the uniform annotation and analysis of digitally scanned stained microscope slide specimens accessioned to the caHUB. It encompasses both normal tissues and neoplasms.

## 2.0 SCOPE

This standard operating procedure (SOP) is applicable to caHUB Pathology Resource Center (PRC), Comprehensive Data Resource (CDR) and Comprehensive Biospecimen Resource (CBR).

## 3.0 RESPONSIBILITY

- 3.1 caHUB PRC pathologists are responsible for examining representative tissues on H&E stained microscope slides or their digitally scanned whole slide images and reporting the findings, e.g., on the PRC Case Summary Report. The results are: qualitative data consisting of tissue identification, degree of autolysis, and presence of pathological features; and quantitative data consisting of sample size measurements and proportions of altered or extraneous tissues. These can be annotated on the digital image. Photomicrographs made by the caHUB pathologist can be uploaded onto the electronic report.
- 3.2 The CDR staff is responsible for maintaining the databases that store the data recorded by the caHUB pathologist.
- 3.3 The glass microscope slides from which the scanned images were made and copies of the scanned images on DVD will be stored in a secured storage area at the caHUB NCI site.

## 4.0 DEFINITIONS

- 4.1 H&E – hematoxylin and eosin
- 4.2 ImageScope™ - Aperio's viewing software for digital pathology
- 4.3 PRC – caHUB Pathology Resource Center
- 4.4 CDR - Comprehensive Data Resource
- 4.5 CBR – Comprehensive Biospecimen Resource
- 4.6 GTEx – Genotype-Tissue Expression Project
- 4.7 PA –Paraffin embedded specimen
- 4.8 CAP – College of American Pathologists
- 4.9 Digitally scanned image – a digital image of a whole microscope slide tissue section routinely stained with hematoxylin and eosin or other designated stain
- 4.10 Tumor site - the topographic site of the primary tumor
- 4.11 Tumor size - largest overall dimensions in millimeters or centimeters



### 5.0 ENVIRONMENTAL HEALTH & SAFETY

N/A

### 6.0 MATERIALS/EQUIPMENT

- 6.1 Computer with Aperio ImageScope or related software (the procedure below is applicable to the Aperio system)
- 6.2 Scanned microscope slide image on DVD or accessed from a web portal
- 6.3 Microscope with camera

### 7.0 PROCEDURE

- 7.1 Digital image of prepared slides will be made available to the PRC.
  - 7.1.1 The digitally scanned slides of paraffin embedded (PA) tissue are derived from imaging carried out by the CBR using a commercial whole slide digital scanning system, e.g., Aperio.
- 7.2 Pathology evaluation will occur as soon as possible after access to the digital images, but optimally within five working days.
  - 7.2.1 The digital images are accessed by the PRC pathologist from either the CBR server or through a web browser. Images on DVDs prepared by the CBR serve as duplicates for observation. They cannot be annotated.
  - 7.2.2 The time limit is chosen to facilitate case processing, and to determine if caHUB tissue requirements for acceptance or rejection are met.
  - 7.2.3 The review procedure will be documented using a Microscopic Slide Analysis form based on standard protocols published by the College of American Pathologists (CAP) [[www.cap.org](http://www.cap.org)] for tumor specimens or on a caHUB PRC Case Summary Report form for normal tissues, see PR-0002-F1.
- 7.3 Examination, Photography and Annotation
  - 7.3.1 Open the image from the CBR web site. The image is movable with the mouse and the magnification can be changed using either the scroll wheel on the mouse or the magnification inset on the screen.
  - 7.3.2 Click the 'Snapshot' icon to create digital photographs and save the image as a JPEG image to the computer. Label appropriately.
  - 7.3.3 Photographs can be taken from the digital images for areas of particular interest with or without annotations (See Figure 1).
  - 7.3.4 Annotate tissue PA section and record values on reporting form:
    - 7.3.4.1 Linear measurements. Activate the 'Ruler tool' icon and click the 'star' at one edge of the specimen. Drag it to the opposite edge. When the mouse



is released, the length of the specimen will appear in mm; repeat the procedure to obtain a perpendicular dimension. Record the measurements on the reporting form. These measurements will appear on the Annotation screen described below as 'Layers'.

- 7.3.4.2 Annotation. Activate the 'Annotations' icon or find Annotations in the 'View' drop-down menu. The Annotations screen allows you to add or delete an annotation layer using the '+' or 'x' symbols, respectively, and to select different colors for each layer. The 'xx' deletes all layers. You can also label measurements (e.g., 'area'), by typing in the text box. The typed words will appear on the image screen, which can be photographed to record these data. (Note that um are changed to mm by moving the decimal point 3 spaces to the left; sq um are changed to sq mm by moving decimal 6 spaces to the left.)
- 7.3.4.3 Area measurement of entire tissue section. Activate the 'Pen tool' with a left click. Left click and hold to use the pen to trace the perimeter of the specimen with an unbroken line until the beginning of the line is reached. You must touch or cross the starting point, then release the left mouse button. The Annotations screen will show the length of the perimeter in um and the area within the perimeter in sq um. Record data in mm and sq mm.
- 7.3.4.4 Area of tumor. This can be annotated by outlining the perimeter on a separate layer with a separate color and recording the area in sq mm as in 7.3.4.3)
- 7.3.4.5 Calculate percentage of tumor in relation to total section area: 7.3.4.4 divided by 7.3.4.3 x100. Make special note if viable tumor is < 70% of tissue specimen.
- 7.3.4.6 Eliminating clear areas from computations. If the specimen contains a large amount of clear or empty space, e.g., cysts or fragmentation artifact, and one wishes to measure only the solid tissue, either: 1) outline the clear space perimeter to obtain its area and subtract it from the total area; or 2) use the Analysis computation (7.3.4.7) to measure only non-clear tissue by pixel count.
- 7.3.4.7 Analysis computation is carried out after the area is determined by first outlining the perimeter using the Pen tool (7.3.4.3). Then activate the Analysis computation screen from the 'View' drop-down menu and an Algorithm box with 'Positive pixel count' will appear in the right upper corner of the screen. Choose 'Selected annotation layer' then select 'Run'. (Make sure that the layer selected in the Annotation box is the one where the area was calculated). When the 'Run' (Pixel count) is complete, the

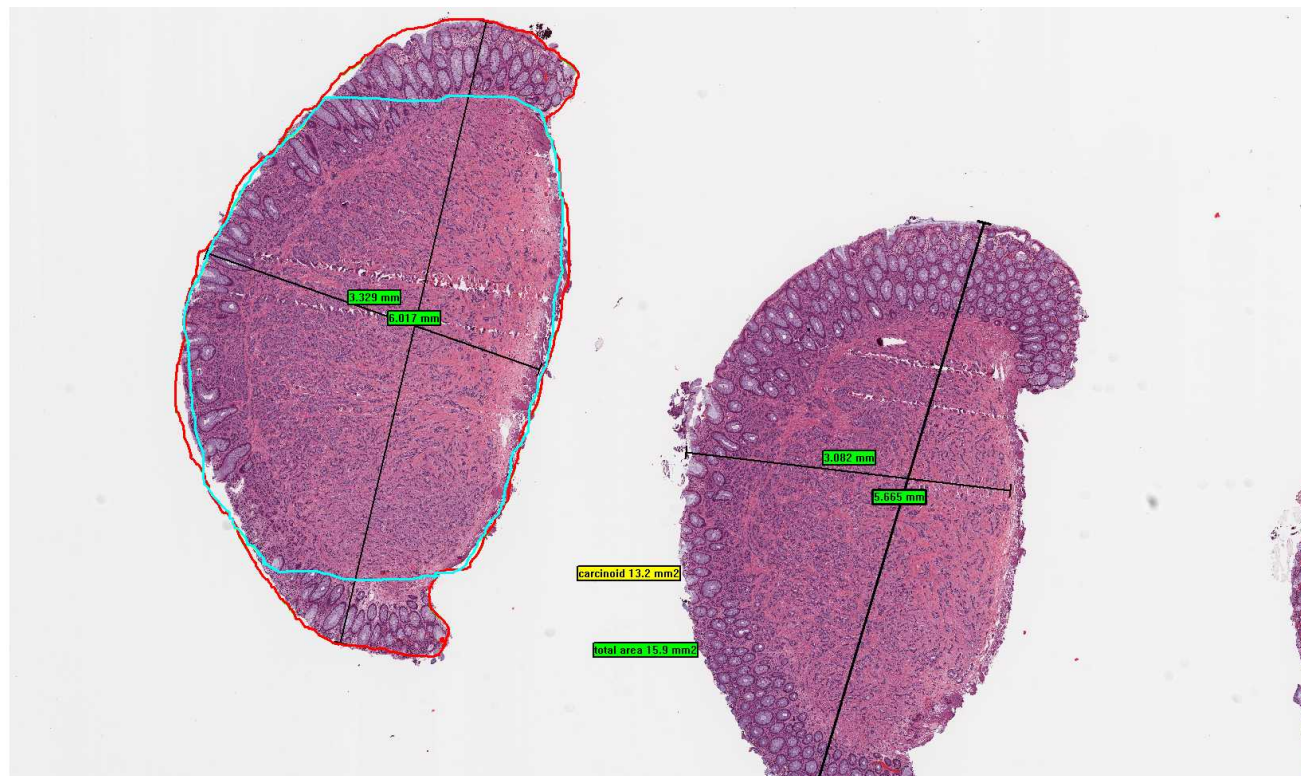


'Total area' in sq mm will be in the 'Layer Attributes' column of the Annotation box. The area measured will appear in another color.

- 7.3.4.8 Note presence of necrosis, inflammation, tumor-free fibrosis, autolysis, cysts, etc. on Tissue Reporting Form. Annotate and measure areas in sq mm and percentages by perimeter drawing, if any are over 10% of specimen
- 7.3.4.9 Estimate tumor density, (e.g., as densely concentrated tumor cells vs. widely dispersed tumor cells, as in desmoplasia).
- 7.3.4.10 Indicate, measure, and annotate, character of adjacent tissue, (e.g., mucosa, peritumoral fibrosis, extensive hemorrhage, etc.).
- 7.3.5 Completed caHUB tumor or normal tissue reporting form is transmitted to CDR.
- 7.4 Ten percent of cases will be selected at random for review by a second PRC pathologist to confirm assessment and annotations.



FIGURE 1 – ANNOTATED TISSUE PHOTOMICROGRAPH



## 8.0 REFERENCES

8.1 Aperio website: [www.aperio.com](http://www.aperio.com)

## 9.0 ATTACHMENTS

None



# Annotating Digitally Scanned Microscope Slides

PR-0002-W1

VER. 1.0.0

Effective Date: 03/07/2012

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## APPROVALS

NAME / TITLE	SIGNATURE	DATE

## INITIATION/REVISION HISTORY

REV #	DESCRIPTION OF CHANGE	AUTHOR	EFFECTIVE DATE