

**[0012]** The following presents a summary of the present disclosure in order to provide a basic understanding of some of the aspects of the present disclosure. This summary is not an extensive overview of the present disclosure. It is not intended to identify key/critical elements of the present disclosure or to delineate the scope of the present disclosure. Its sole purpose is to present some of the concepts of the present disclosure in a simplified form as a prelude to the more detailed description that is presented later.

**[0013]** The present disclosure provides systems and methods for tissue classification. An image of a tissue sample (*e.g.*, from a subject) on a substrate is obtained as a plurality of pixels in electronic form (*e.g.*, using transmission light microscopy). In some embodiment the plurality of pixels comprises at least 1000, 10,000, 100,000,  $1 \times 10^6$ ,  $2 \times 10^6$ ,  $3 \times 10^6$ ,  $4 \times 10^6$ ,  $5 \times 10^6$ ,  $8 \times 10^6$ , or  $15 \times 10^6$  pixels. The image includes fiducial markers on the substrate boundary. Pixels are assigned to a first class, indicating tissue sample, or a second class, indicating background. The assigning uses the fiducial markers to define a bounding box within the image and removes pixels outside the box. Then, heuristic classifiers are applied to the pixels: for each respective pixel in the plurality of pixels, each heuristic classifier votes for the respective pixel between the first and second class, thereby forming an aggregated score for each pixel that is one of first class, likely first class, likely second class, and obvious second class. *E.g.*, each pixel receives an aggregate score from the heuristic classifiers indicating obvious tissue, likely tissue, likely background, or obvious background. The aggregated score and intensity of each pixel is applied to a segmentation algorithm such as a graph cut algorithm (*e.g.*, a GrabCut segmentation algorithm) to assign a probability to each pixel of being tissue sample or background.

**[0013a]** According to an aspect, the present disclosure provides a tissue classification method. The method comprises: A) obtaining an image of a tissue sample on a substrate, as a plurality of pixels in electronic form, wherein the image includes a plurality of fiducial markers on an outer boundary of the substrate; and B) assigning each respective pixel in the plurality of pixels to a first class or a second class, wherein the first class indicates overlay of the tissue sample on the substrate and the second class indicates background, and wherein the plurality of pixels comprises at least 100,000 pixels, by a procedure that comprises: (i) using the plurality of fiducial markers to define a bounding box within the image, (ii) removing respective pixels falling outside the bounding box from the plurality of pixels, (iii) running, after the removing (ii), a plurality of heuristic classifiers on the plurality of pixels, wherein, for each respective pixel in the plurality of pixels, each respective

heuristic classifier in the plurality of heuristic classifiers casts a vote for the respective pixel between the first class and the second class, thereby forming a corresponding aggregated score for each respective pixel in the plurality of pixels, wherein each corresponding aggregated score is a class in a set of classes comprising obvious first class, likely first class, likely second class, and obvious second class, and (iv) applying the aggregated score and intensity of each respective pixel in the plurality of pixels to a segmentation algorithm to independently assign a probability to each respective pixel in the plurality of pixels of being tissue sample or background.

**[0014]** One aspect-embodiment of the present disclosure provides a tissue classification method. The method comprises obtaining an image of a sectioned tissue sample on a substrate (*e.g.*, from a subject) as a plurality of pixels in electronic form. The image includes a plurality of fiducial markers (*e.g.*, on an outer boundary of the substrate). In some embodiments the plurality of fiducial markers comprises more than 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 500, 1000, or 5000 markers. In some embodiments, the plurality of fiducial markers consists of less than 1000, 2000, 3000, 4000, or 5000 fiducial markers. The image is acquired using transmission light microscopy. Each respective pixel in the plurality of pixels is assigned a class. For instance, in some embodiments, each pixel is assigned to a first class or a second class. In some embodiments, each pixel is assigned to any one of a plurality of classes, where the plurality of classes consists of N