Supplementary Information

Supplementary Figure 1 | Ridge detection algorithm overview. (a) PCA is used to identify the major direction in the RBJH. This direction is used, along with the coordinates of the global maximum, to create the major vector (v_0). Two additional vectors (v_{-1}, v_1) are created, at ±15° of the primary directions, to ensure complete coverage of the colorspace. (b) Local maxima tangent to each vector are identified to create basis for the RBJH ridge set. (c) The local maxima identified in each direction are combined to create a map of all local maxima in the RBJH. (d) Morphological dilation and thinning is applied to the density map to create the binarized ridge set.

Supplementary Figure 2 | Ridge set registration. (a) Binarized ridge set is further registered to identify tissue components. Tissue type are determined based on the distance (lower values correspond to closer components) from each of the four pure colors (red, blue, white, and black). Nuclei are defined as being closest to blue and black, ECM and cytoplasm are closest to white, and red blood cells are closest to red. (b) Overview of ridge set registration cases for H&E and IHC images. In cases where only one component is identified for H&E images, further processing is applied. Gaussian separation creates two components by cutting according to the distance from the global maximum according to a Gaussian fit.

Supplementary Figure 3 | Distance transformation algorithm overview. (a) Watershed segmentation is applied to the binarized ridge set. (b) Three transformation functions are created: i. Tissue region function (f_{region}), ii. Absorption function ($f_{absorption}$), and iii. Ridge function (f_{ridge}). (c) The three transformation functions are combined to create an overall transformation function that can be used as a basis for image transformation.

Supplementary Figure 4 | Overview of NLTD immunohistochemistry scoring technique. . Each immunohistochemically (DAB) stained image was separated into two images using the NLTD method, a nuclei-rich image and an antigen-rich image. Pre-processing steps were performed in order to only analyze nuclei rich regions where antigen staining was present, and avoid background areas where no staining should occur. Briefly, the nuclei-rich image was segmented using Otsu's thresholding technique. Small objects were removed from the image, followed by morphological opening and closing operations and another removal of small objects. After pre-processing, a transformation score was derived based on the ratio of antigen intensity to nuclei intensity.

Supplementary Figure 5 | Qualitative comparison of NLTD and color deconvolution. Comparison of the NLTD method with a freely available color deconvolution plugin (ImageJ ''Color deconvolution'' plugin; http://www.mecourse.com/landinig/software/ cdeconv/cdeconv.html) with several built-in color deconvolution vectors. (a) Typical H&E image from breast cancer set. (b) Separation of input H&E image into nuclei (top) and non-nuclei (bottom) components. Shown are results from NLTD (left), two preset ImageJ/Fiji Color Deconvolution settings (middle-left, middle-right), and custom settings from user-input ROI using ImageJ/Fiji Color Deconvolution (right). (c) Typical immunohistochemistry image. (d)

Separation of input IHC image into nuclei (top) and antigen (bottom) components. Shown are results from NLTD (left) and preset ImageJ/Fiji Color Deconvolution settings (right). Qualitatively, the NLTD images show a smoother, better separated image for each component compared to the color deconvolution images.

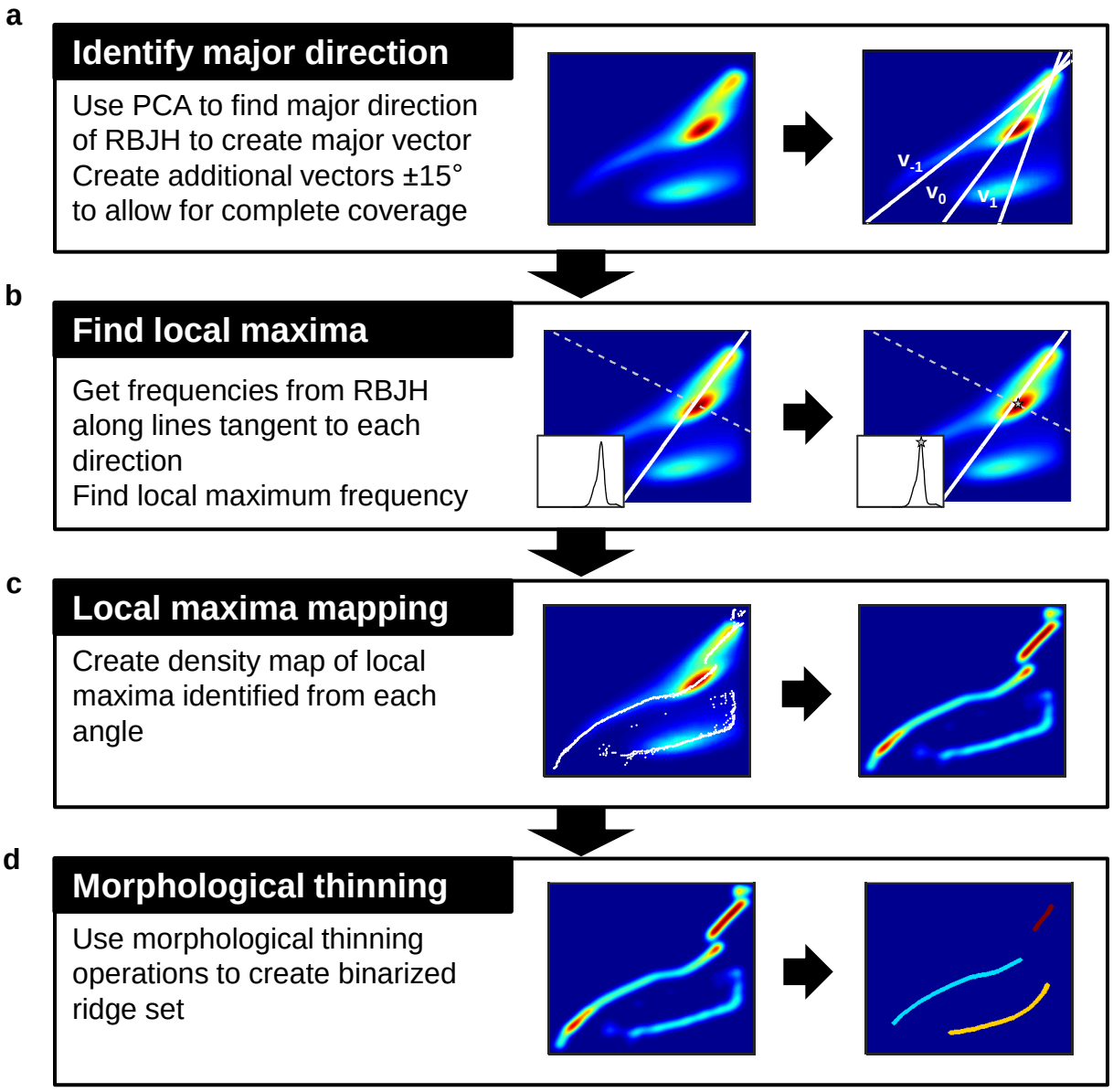
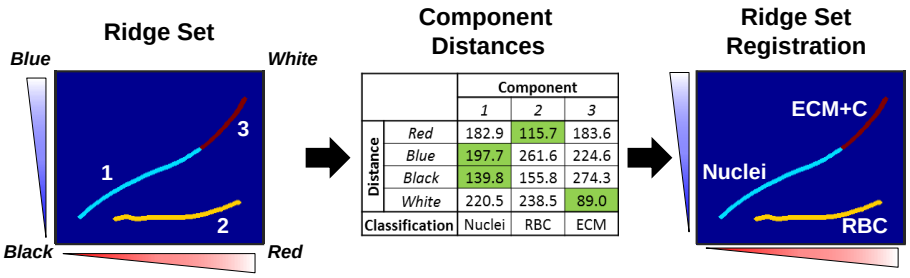

a
Identify major direction
Use PCA to find major direction of RBJH to create major vector
Create additional vectors ±15° to allow for complete coverage
v-1
v0
v1
b
Find local maxima
Get frequencies from RBJH along lines tangent to each direction
Find local maximum frequency
c
Local maxima mapping
Create density map of local maxima identified from each angle
d
Morphological thinning
Use morphological thinning operations to create binarized ridge set

Figure S1

a


Ridge Set
Blue
White
Black
Red
1
2
3
Component Distances
Component
1 2 3
Distance
Red 182.9 115.7 183.6
Blue 197.7 261.6 224.6
Black 139.8 155.8 274.3
White 220.5 238.5 89.0
Classification Nuclei RBC ECM
Ridge Set Registration
ECM+C
Nuclei
RBC

b

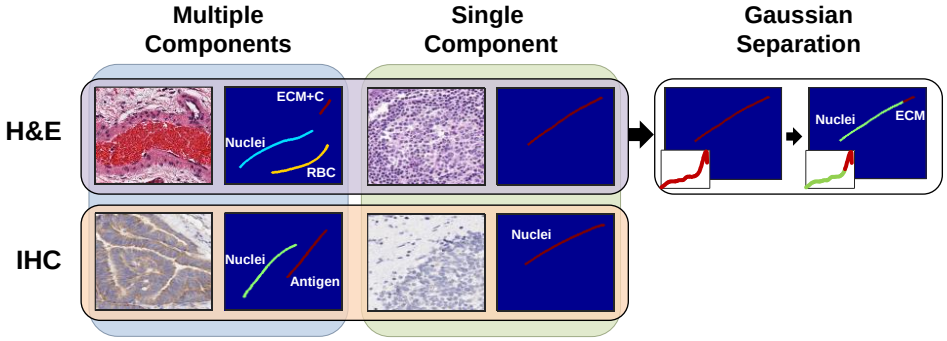

Multiple Components
Single Component
Gaussian Separation
H&E
IHC
ECM+C
Nuclei
RBC
Antigen
ECM

Figure S2

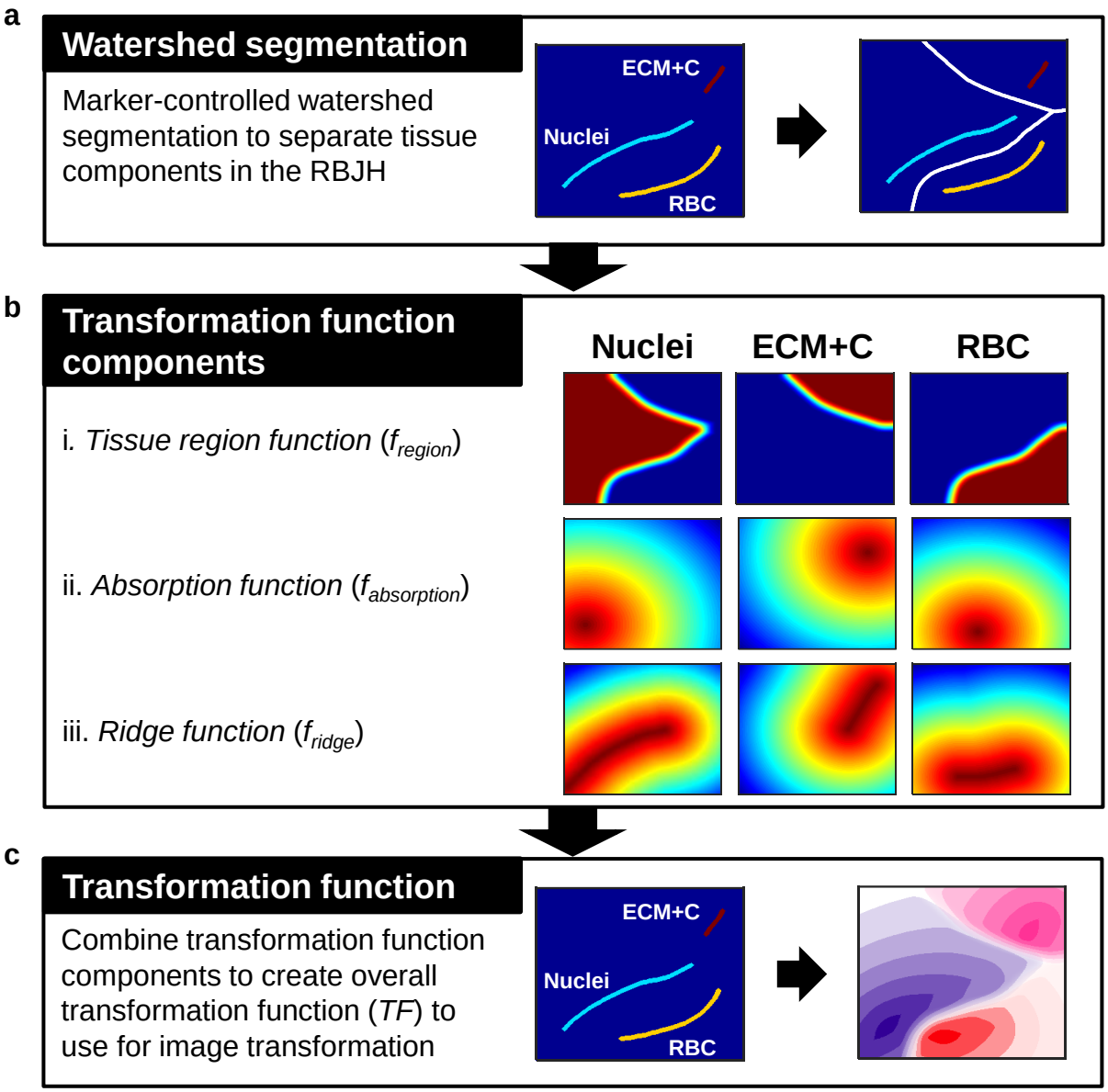

a
Watershed segmentation
Marker-controlled watershed segmentation to separate tissue components in the RBJH
ECM+C
Nuclei
RBC
b
Transformation function components
i. Tissue region function (f_{region})
ii. Absorption function ($f_{absorption}$)
iii. Ridge function (f_{ridge})
Nuclei
ECM+C
RBC
c
Transformation function
Combine transformation function components to create overall transformation function (TF) to use for image transformation
ECM+C
Nuclei
RBC

Figure S3

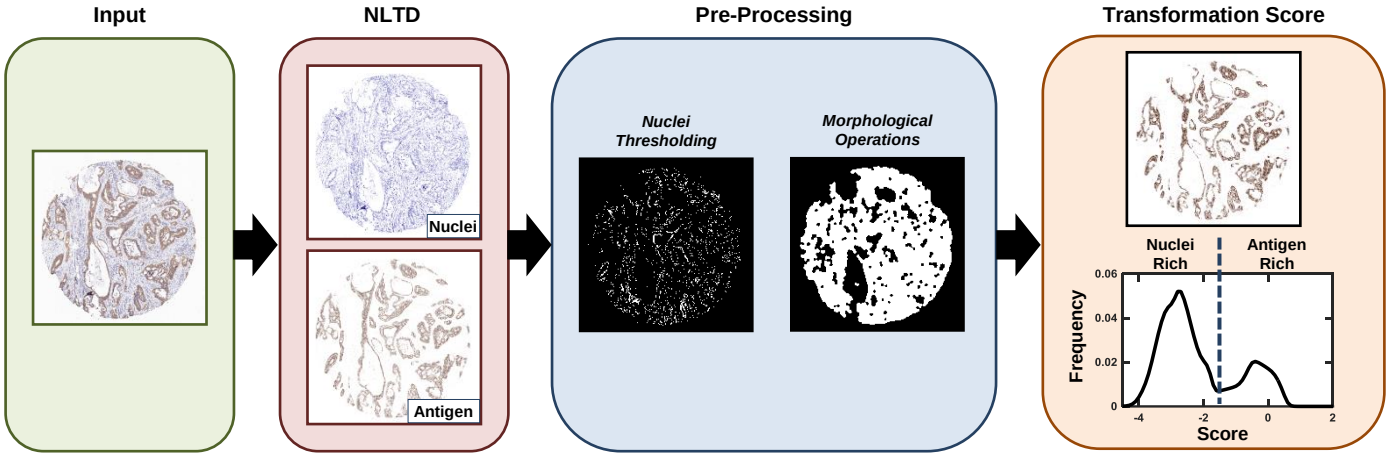

Input
NLTD
Pre-Processing
Transformation Score
Nuclei
Antigen
Nuclei Thresholding
Morphological Operations
Nuclei Rich
Antigen Rich
Frequency
0.06
0.04
0.02
0
-4
-2
0
2
Score

Figure S4

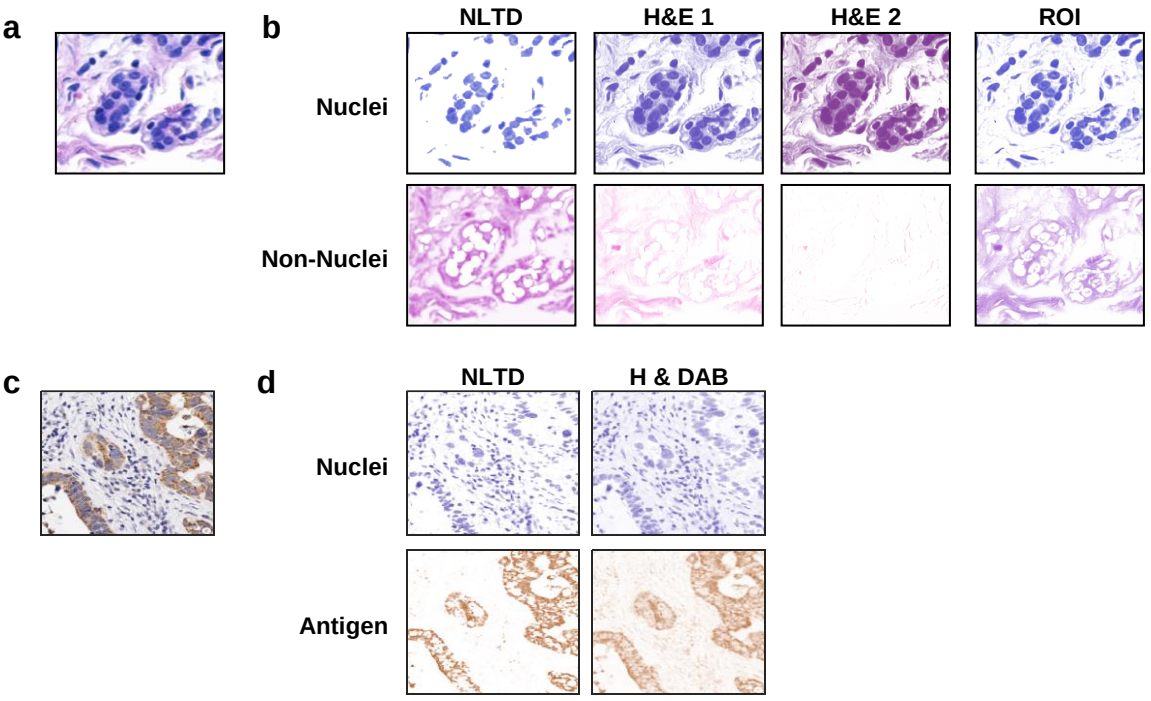

a
b
NLTD
H&E 1
H&E 2
ROI
Nuclei
Non-Nuclei
c
d
NLTD
H & DAB
Nuclei
Antigen

Figure S5