

Supplemental Digital Content

This document gives the interstitial fibrosis (IF) values of figure 2A, the interpretation of the kappa value, and describes in details the image processing techniques applied in our IF quantification method.

1. Test against staining variation

	A	B	C	D
Day1	20.93%	30.62%	39.40%	51.13%
Day2	22.02%	32.37%	41.40%	50.93%
Day3	21.96%	31.51%	38.90%	50.87%
average (+/-standard deviation)	21.02% (+/-0.006)	31.5% (+/-0.009)	39.90% (+/-0.013)	50.87% (+/-0.003)

Table 1S: Staining variation. Four biopsies were selected to assess the staining variation. IF computerized index of 3 consecutive sections of the same biopsy stained with Masson Trichrome on different days (day 1 to day 3) in figure 2A. We observe no significant variation due to the staining. The value of ICC is 0.995 with 95% CI [0.968 - 1]

2. Kappa significance

Landis and Koch [1] gave the following table for interpreting κ values.

κ	Interpretation
≤ 0.20	Poor agreement
0.21-0.40	Fair agreement
0.41-0.60	Moderate agreement
0.61-0.80	Substantial or good agreement
0.81-1.00	Excellent or very good agreement

3. Inter and intra-observer variations by ANOVA test

Three slides were randomly selected from each Banff grade (ci1, ci2 and ci3 IF) and were quantified by our system at 10 different acquisition times by the same operator (intra-operator) and by 10 different operators (inter-operators). The highest standard deviations were 3.89% (intra-) and 4.07% (inter-) variation in ci3 grade. ANOVA tests showed a p-value that was below 0.003 (Figure1S).

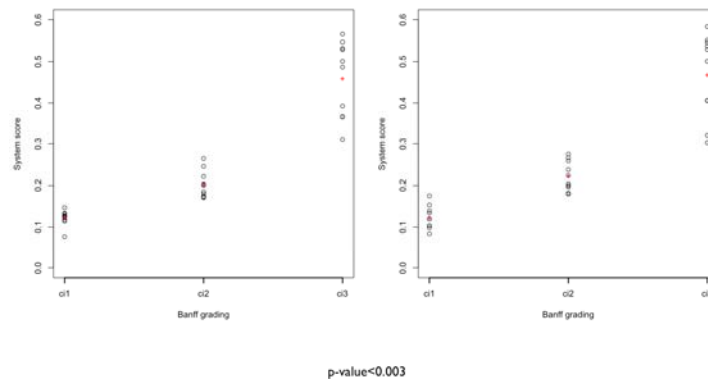


Figure 1S: Reproducibility by ANOVA test.

4. Color image analysis

The framework for quantification consists of two steps (Figure 2S): i) color segmentation that obtains the green mask (collagen pixels) and the biopsy mask; ii) post-processing step that extracts the IF mask as a result of green mask without non IF components.

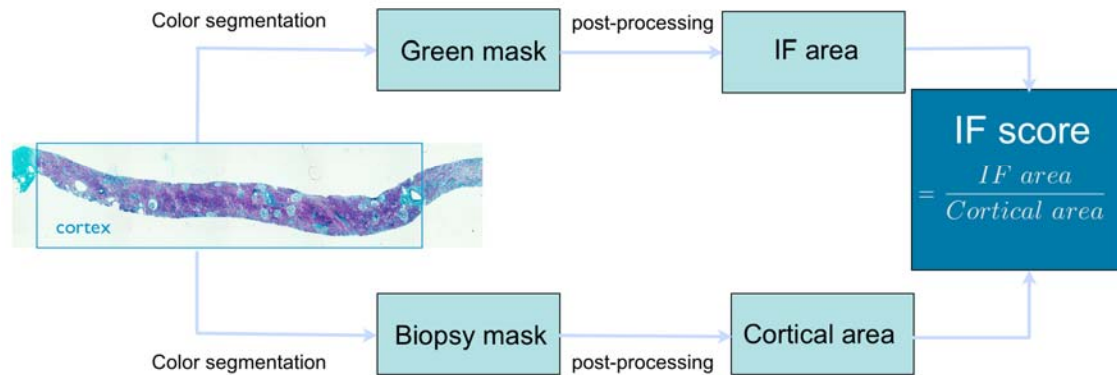


Figure 2S: From original image to IF score: The processing steps that allow computing of the IF score are schematically represented.

4.1 Color segmentation

To handle color variations, the segmentation is performed by color quantization and clustering in 3 classes in the I1H2H3 color space defined by $I1 = (R+G+B)/3$, $H2 = R - G$ and $H3 = (R+G)/2 - B$ [2], followed by a relaxation step that allows for spatial regularization [3]. Color quantization is done by using Brun's quantization method [4] that reduces the number of colors by splitting the color space uniformly into M color bins (for this application, $M=2048$) and then by merging these clusters into N classes according to the least mean square error (LMSE) criterion. N is automatically adapted to the color image based on color evaluation criteria. The final merging step is performed by grouping the N colors in the three predetermined classes of interest: background (the highest intensity value), green color of the trichrome staining (the minimum $H2$ value), and the remaining part of the tissue staining (all other values in $H2$) (Figure 4S). In order to quantify IF in a renal biopsy, we used two parameters extracted from the color image: i) the cortical biopsy area (Figure 4S upper left). ii) the green mask area (Figure 4S upper right). In order to take into account spatial features, the regions whose areas are under a defined size value and whose color mean is far from the color class were removed. With this spatial regularization, the tubular brush regions that could be stained in blue-green as mentioned in [5] are automatically removed (Figure 4S bottom).

4.2 Non interstitial fibrosis removal

The automatic quantification of IF is based on the ratio between green pixels representing fibrosis and all the pixels representing the region of interest of the biopsy (cortex part). The green mask that characterizes the collagen staining corresponds to several entities: IF, renal capsule, tubular basement membranes, vessels and glomeruli. Then, in order to keep only the IF area, the other regions are automatically detected and excluded from analysis as follows:

4.2.1 Capsule

(Figure 5S top): the capsule is (generally) located at the end axis of the biopsy and presents a deeper green color. The segmentation is performed by using k-means clustering (1967) on the $H2$ component image in 6 classes, followed by mathematical morphology operators, and localization.

4.2.2 Basement membrane

(Figure 3S bottom): the normal basement membrane is assessed as being 2 pixels thick. All membrane-like structures thinner than this value are automatically removed. For atrophic tubules, which have thickened tubular basement membranes, we consider that the thickened part is pathologic and integrated in the IF.

4.2.3 Sclerotic glomeruli

After the segmentation of green color, the regions are filtered by their shape and size. The typical size values of SG are within the range of 250 to 1570 pixels and the compacity parameter (2) characterizing the shape roundness is higher than 0.8 (Figure 6S).

4.2.4 Normal glomeruli

Their aspect can vary from one biopsy to another (Figure 7S). To handle this variability, a model is defined on each biopsy from the lumen area. The contours of the lumen regions are extracted, polygonized and a convex hull is computed (Figure 8S). Finally an ellipse fitting [6] is performed on the convex hull points. The glomeruli extraction is validated by post-processing analysis based on the ellipse axis ratio, and color mean (Figure 8S). Typical value for the ellipse axis ratio is 1.4.

4.2.5 Large or medium blood vessel detection

As in the case of normal glomeruli, the vessel is defined based on the lumen detection. The contours extracted from the lumen regions are polygonalized, and dilated with a thickness obtained from the green local region. These regions are extracted based on the observation that the vessels appear darker than the interstitial fibrosis. First, we threshold the intensity image to get the regions with the darker pixels. Then, we compute the distance map of this extracted region to compute the thickness of the local region (Figure 10S).

At the end of the process, the IF surface area is defined as the surface of green pixel minus basement membranes, capsule, glomeruli and vessels. The tissular surface is defined as the number of pixels in the original biopsy minus the capsule. The index of the IF surface is defined as the ratio between the IF surface area and the total surface of the cortex area in the biopsy (Figure 2S).

References

- [1] J. R. Landis and G. G. Koch, "The measurement of observer agreement for categorical data," *Biometrics*, vol. 33, pp. 159–174, 1977.
- [2] V. Meas-Yedid, E. Glory, E. Morelon, Ch. Pinset, G. Stamon, and J-Ch. Olivo-Marin, "Automatic color space selection for biological image segmentation," in *ICPR*, 2004, vol. 3, pp. 514–517.
- [3] M. Sonka, V. Hlavac, and R. Boyle, *Image Processing, Analysis, and Machine Vision*, PWS Publishing, 1999.
- [4] J.P. Braquelaire and L. Brun, "Comparison and optimization of methods of color image quantization," *IEEE Trans. Image Processing*, vol. 6, no. 7, pp. 1048–1052, 1997.
- [5] P. C. Grimm, P. Nickerson, J. Gough, R. McKenna, E. Stern, J. Jeffery, and D. N. Rush, "Computerized image analysis of sirius red-stained renal allograft biopsies as a surrogate marker to predict long-term allograft function," *Journal of the American Society of Nephrology*, vol. 14, no. 6, pp. 1662–1668, 2003.
- [6] A. W. Fitzgibbon, M. Pilu, and R. B. Fisher, "Direct least-squares fitting of ellipses," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, vol. 21, no. 5, pp. 476–480, 1999.

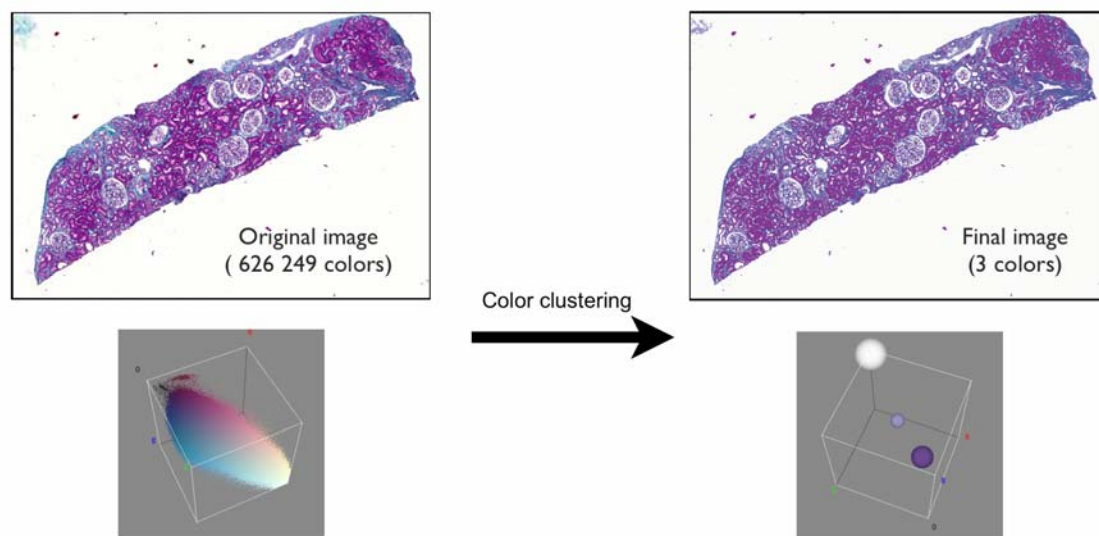


Figure 3S: Color quantization: Color clustering consists in reducing the number of colors used to encode an image. All

colors of the original image are represented in the RGB space (left bottom). After clustering, the final image is represented by only 3 colors: blue-green, purple and white (right).

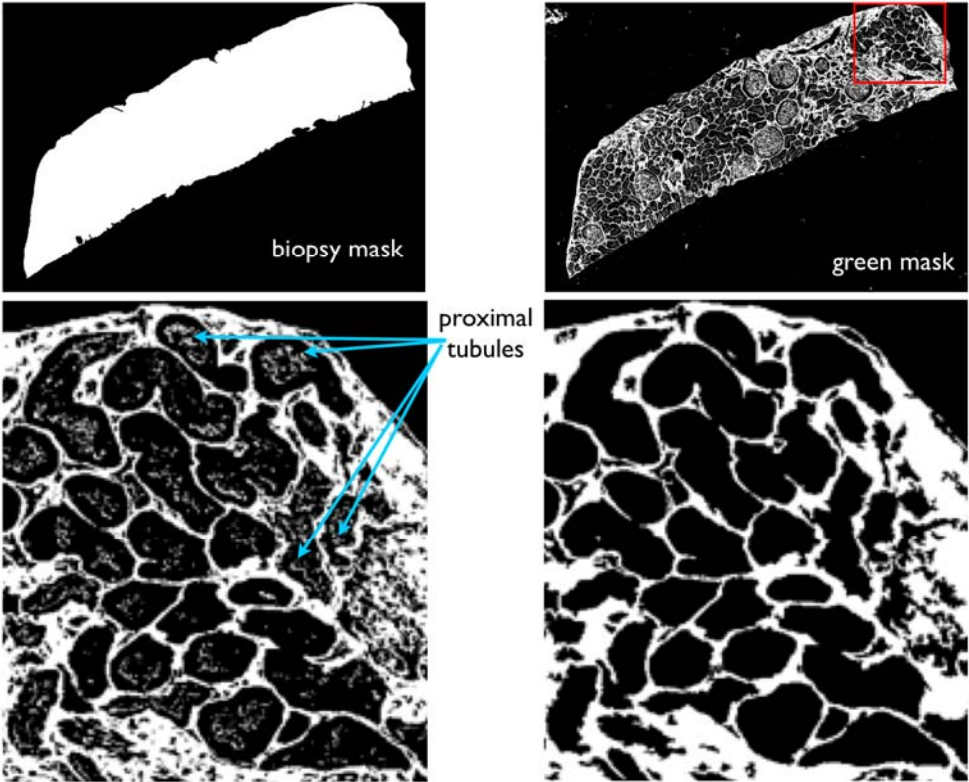


Figure 4S: Feature extraction: From the 3 color classes we are able to extract the mask biopsy and the green mask (upper left and right). Proximal tubular brush borders are stained in green by MT (bottom left, arrows) The tubular-brush pixels that are misclassified as interstitial fibrosis by the color clustering process are automatically removed (bottom right).

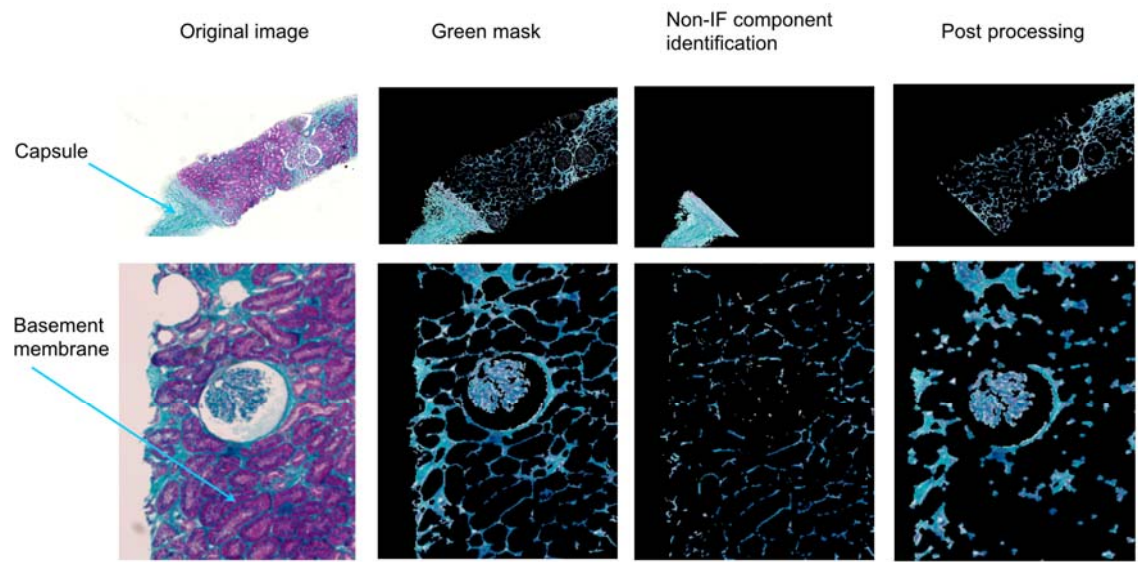


Figure 5S: Removal of non IF-components: capsules and normal basement membrane suppression. The system automatically removes the green pixels that are not IF regions, as follows: i) the capsule is automatically recognized by its location and deeper green color (upper panel). ii) normal basement membrane is defined as being two-pixel large and eliminated (bottom panel).

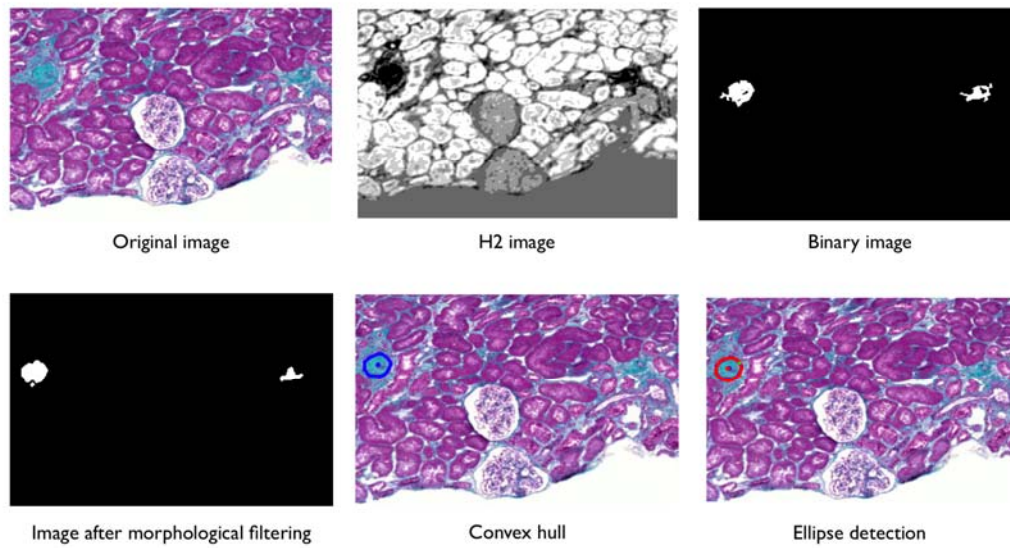


Figure 6S: Sclerotic glomeruli are identified by the combination of their shape and color.

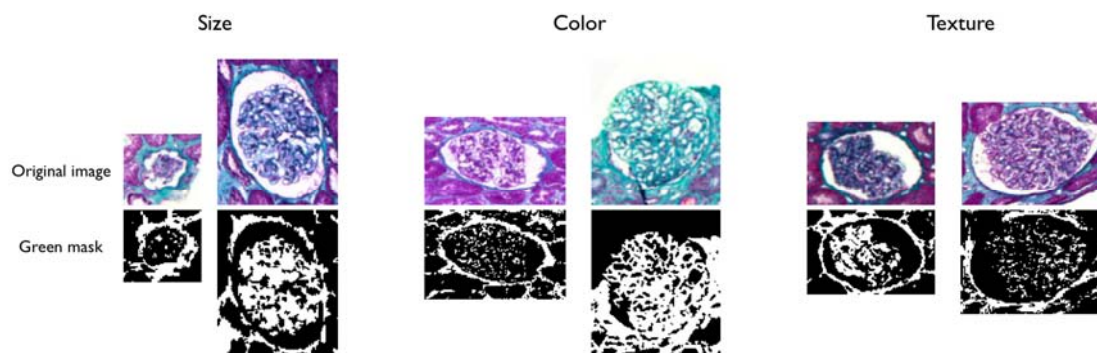


Figure 7S: Variations of normal glomeruli in size, color and texture

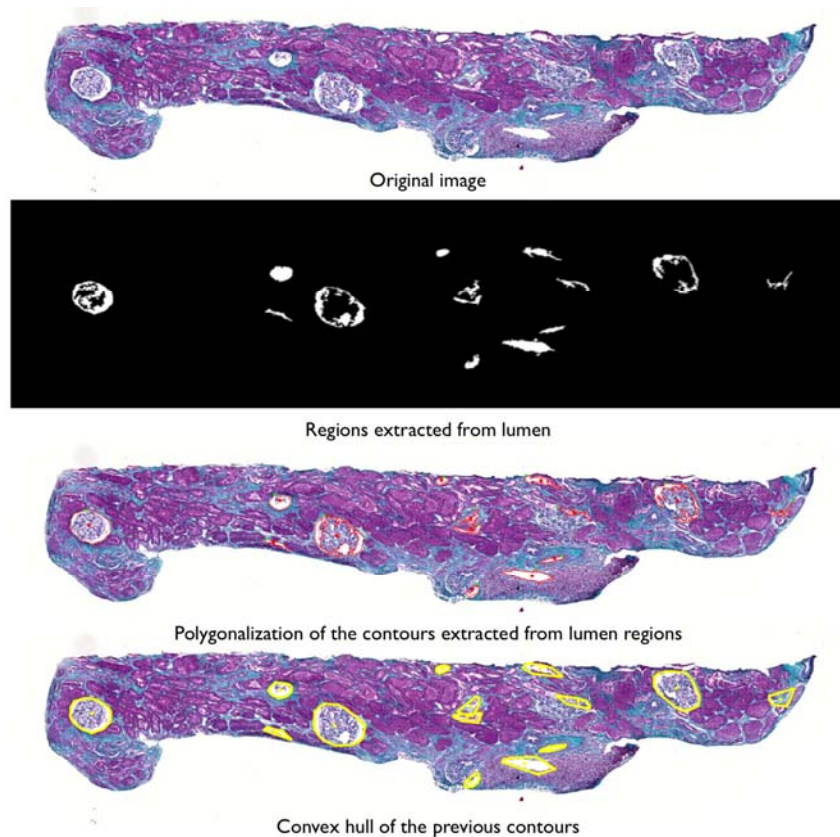


Figure 8S: Normal glomeruli detection based on lumen detection. Normal glomeruli are recognized based on the lumen area (second panel). The contours of the lumen regions are extracted, polygonized (third panel) and a convex hull is computed (fourth panel).

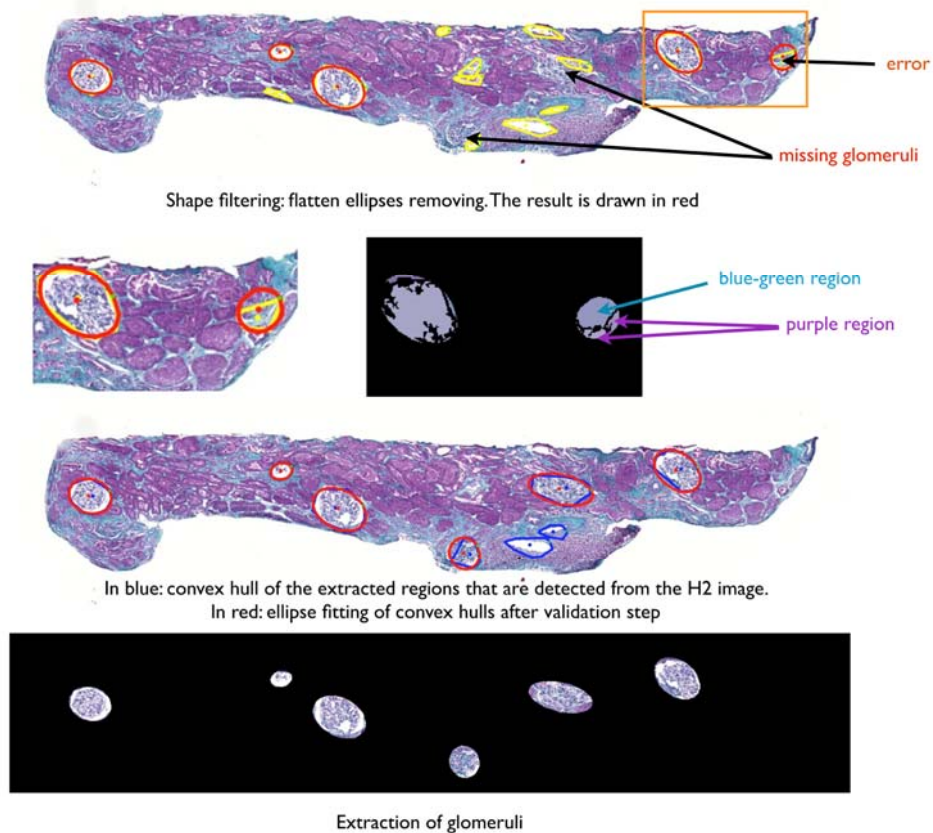


Figure 9S: Normal glomeruli detection based on lumen detection: shape and color filtering. After an ellipse fitting on the convex hull points (top panel), the glomeruli extraction is validated by post-processing analysis based on the ellipse axis ratio, and color mean. The bottom panel shows the final glomeruli extraction.

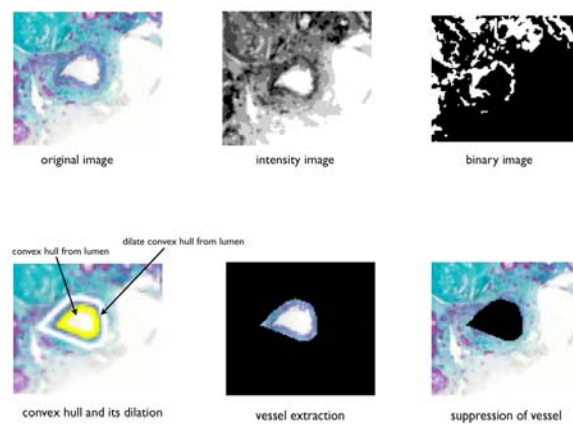


Figure 10S: Removal of non IF-components: vessel extraction. Vessels are recognized based on the lumen area and contours are dilated with a value adapted to the green local thickness region.