**Supplementary Data**

**Algorithm for segmenting the vasculature**

**Sup. Figure 1** shows the flowchart for generating the vessel masks from spectral images. Red (R), green (G), and blue (B) channel images were collected for each site. The B channel of the 440 nm image, the G channel of the 540 and 580 nm images, and the R channel of the 600 image from the same site were used as inputs for generating the vessel mask and were aligned to the G channel of the 540 nm image. After the images were aligned, ratiometric images were computed (B/R for 440/600, and G/R for 540/600 and 580/600). Adaptive histogram equalization was applied to the ratiometric images to enhance contrast.

Multiscale Gabor filtering (σx=10, σy=10, frequency=0.03, orientations = 0, 22, 45, 67, 90, 112, 135 and 157 degrees) was then applied to each contrast-enhanced ratiometric image to generate a Gabor response. The Gabor response was then normalized by subtracting the Gabor response from the maximum Gabor response value. The 3 normalized Gabor responses (from the 3 ratiometric images (440/600, 540/600, and 580/600)) were combined into a single image. Specifically for each pixel location, the lowest value from the 3 normalized Gabor responses was assigned to the final image. The vessel mask (Mask 1) was created from the final combined Gabor image using the Dijkstra forest vessel segmentation algorithm. If no vessel in Mask 1 had a diameter greater than 55 µm, Mask 1 was the final vessel mask.

For vessels >55 µm, the frequency of the Gabor filter was reduced to generate a new mask (Mask 2). Specifically, the parameters of the multiscale Gabor filter were updated (σx=5, σy=5, frequency=0.02, orientations=0, 22, 45, 67, 90, 112, 135, and 157 degrees) and applied to the 580/600 nm enhanced image (enhanced by the adaptive histogram equalization algorithm) and the derived Gabor response was used to segment the vessels (Mask 2). The wavelength pair, 580/600 nm was selected because this combination has the greatest contribution pf large vessels in comparison to the other ratiometric images.

All vessels with a diameter larger than 55 µm were removed in Mask 1 to obtained Mask S. All vessels in Mask 2 with a diameter smaller than 55 µm were removed to obtain Mask B. The final mask was the sum of Mask B and Mask S. The parameters used for multiscale Gabor filtering and threshold for the diameter (55 m) were determined empirically by comparing the final masks to the gold-standard (hand-traced) masks for 12 sites (3 normal, 3 hyperplasia, 3 dysplasia and 3 SCC).

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**Sup. Figure 1. Flowchart for vessel segmentation.** After the spectral images were obtained, the images from the same site were aligned to 540 nm and ratiometric images (440 nm/600 nm, 540 nm/600 nm and 580 nm/600 nm) were computed. Next, an adaptive histogram equalization algorithm was applied to the ratiometric images to enhance the vascular contrast. A multi-scale Gabor-based algorithm was used to segment the vessels in the images (Mask 1). Due to the large variation in vascular diameter, diameters in Mask 1 were measured to determine if a lower Gabor filter frequency should be used to segment the large vessels. If a large vessel (>55 µm) was detected in Mask 1, the frequency of the Gabor filter was reduced to generate a new mask (Mask 2). The final mask was the sum of the small vessels in Mask 1 (Mask S) and the large vessels in Mask 2 (Mask B).

**Algorithm for splitting the vessel masks by diameter**

**Sup. Figure 2** shows the steps for splitting the original representative vessel mask into a large and a small vessel mask. First, isotropic erosion was applied to the original vessel mask until all vessels were eroded to assign diameter values for vessels in different regions in the mask. Next, the mask with diameter values for each pixel in the vascular area was threshed using 14 µm as a cutoff. This resulted in two masks – a preliminary small vessel mask and a preliminary large vessel mask. If a vessel in the preliminary small vessel mask had two or more contacts with the vessels in the preliminary large vessel mask, it was classified as a connecting vessel. The connecting vessels were removed from the preliminary small vessel mask and added to the preliminary large vessel mask to obtain the final small and large vessel masks.

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**Sup. Figure 2. Flowchart for vessel splitting.** Diameter was first measured from the original mask. 14 µm was used as a threshold to separate the original mask into two masks. The connecting vessels between two or more larger vessels were identified and were classified as parts of the large vessel masks. The algorithm was able to split each original mask into a large vessel mask and a small vessel mask.

**Breakdown of AUC data using individual parameters and their combinations**

Supplemental Table I: AUC data generated using various vascular parameters to classify low risk lesions vs. high risk lesions.

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Summary of the area under the curve data from classifying low risk lesions (normal and hyperplasia) versus high risk lesions (low grade dysplasia, high grade dysplasia and/or squamous cell carcinoma) using vascular features of large vessel and small vessel masks and large over small mask ratios, as well as a combination of large vessel tortuosity, small vessel diameter, and large over small mask length ratio. N: normal, H: hyperplasia, LD: low grade dysplasia, HD: high grade dysplasia, SCC: squamous cell carcinoma, AUC: area under the curve, L/S: large over small.

**Quantification of additional vascular parameters**

**Sup. Figure 3** shows two additional vascular features that were collected for each group but did not typically display significant changes between normal and abnormal groups. Distance indicates the nearest neighbor distance between vascular and non-vascular pixels, and the mean values for normal, hyperplasia, dysplasia, and SCC were comparable in each mask group. However, some significant trends were observed in the standard deviation of the diameter, decreasing significantly in cancer compared to normal in the original and large vessel masks. In small vessel masks, standard deviation of diameter increased significantly in cancer compared to normal.

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**Sup. Figure 3. Quantification of additional vascular features.** Generally, no significant trends were observed for distance between vascular and non-vascular pixels. However, significant decreases in standard deviation of the diameter were observed in all and large vessels, while increases were seen in small vessels. \*\*: p <0.01, \*: p <0.05. N: normal, H: hyperplasia, LD: low grade dysplasia, HD: high grade dysplasia, SCC: squamous cell carcinoma.

**Future applications using Pocket microscope**

**Sup. Figure 4** displays a comparison between the dark field system used in our study and the Pocket microscope, also developed by our group. **(A)** and **(B)** are schematics of the dark field system and the Pocket microscope, respectively. The two representative images shown in **(C)** illustrate comparable vascular contrast and resolution obtained by the two systems in a hamster cheek pouch. Because the Pocket microscope is well-suited to image tissues in the oral cavity, future studies will likely focus on adapting our ratiometric approach and segmentation algorithms for use with this technology.



**Sup. Figure 4. Dark field microscope compared to Pocket microscope.** (A) shows the dark field microscope used in this study. (B) shows an image of the Pocket microscope that can be translated for use in a clinical setting. (C) shows representative images in the same region of a hamster cheek pouch acquired with our dark field system and Pocket microscope, respectively.