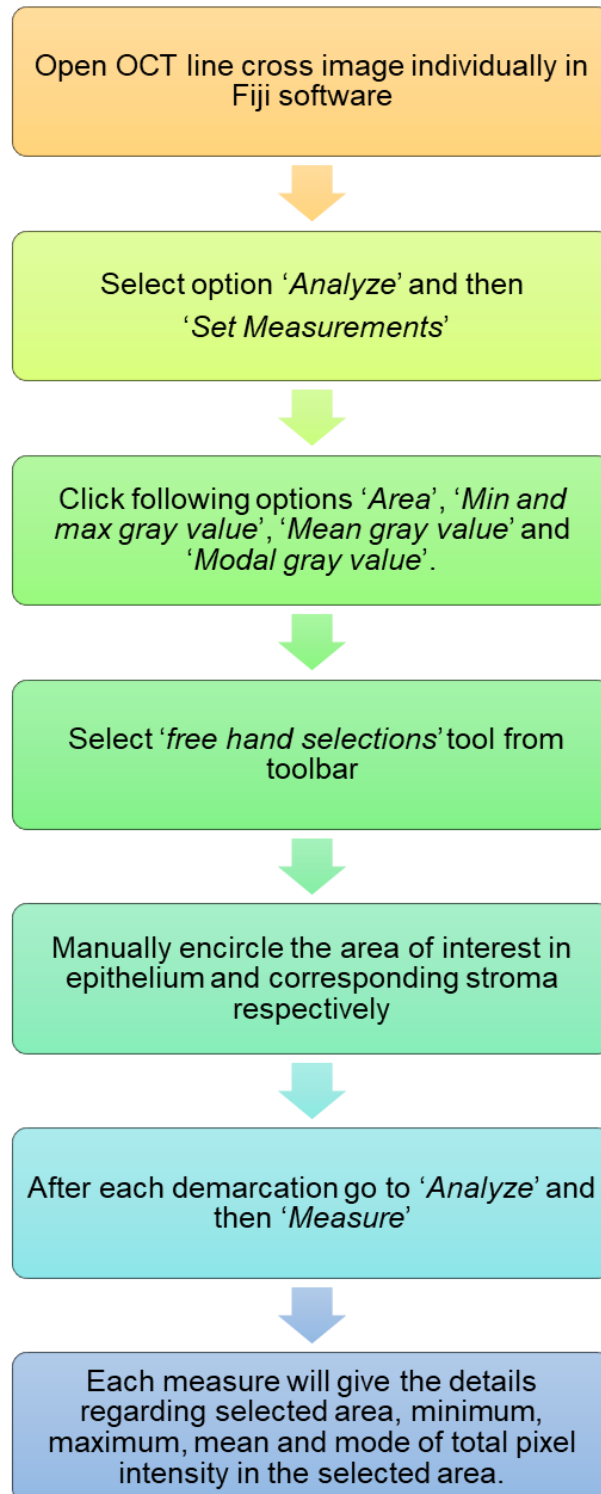
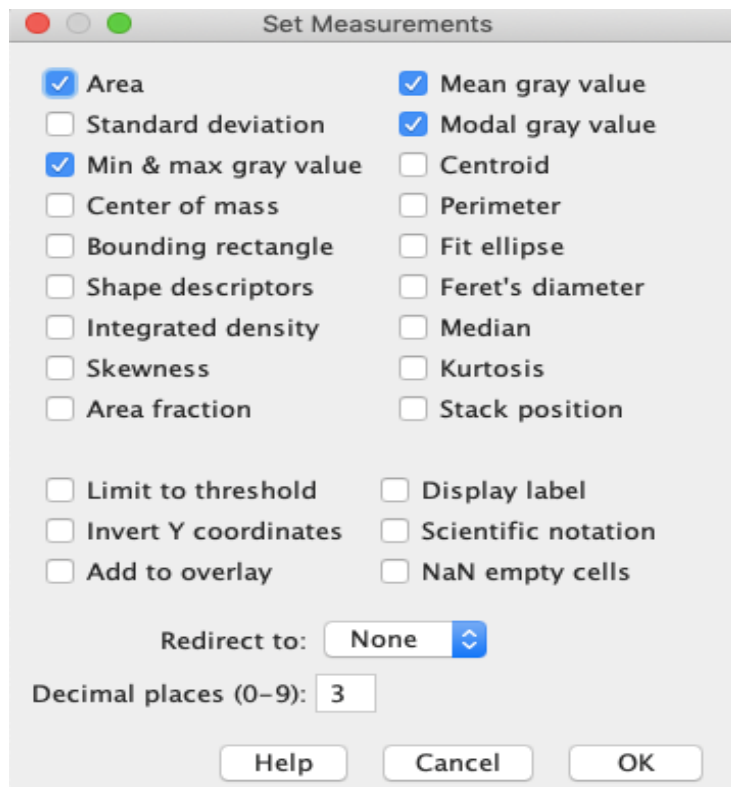


Supplementary File

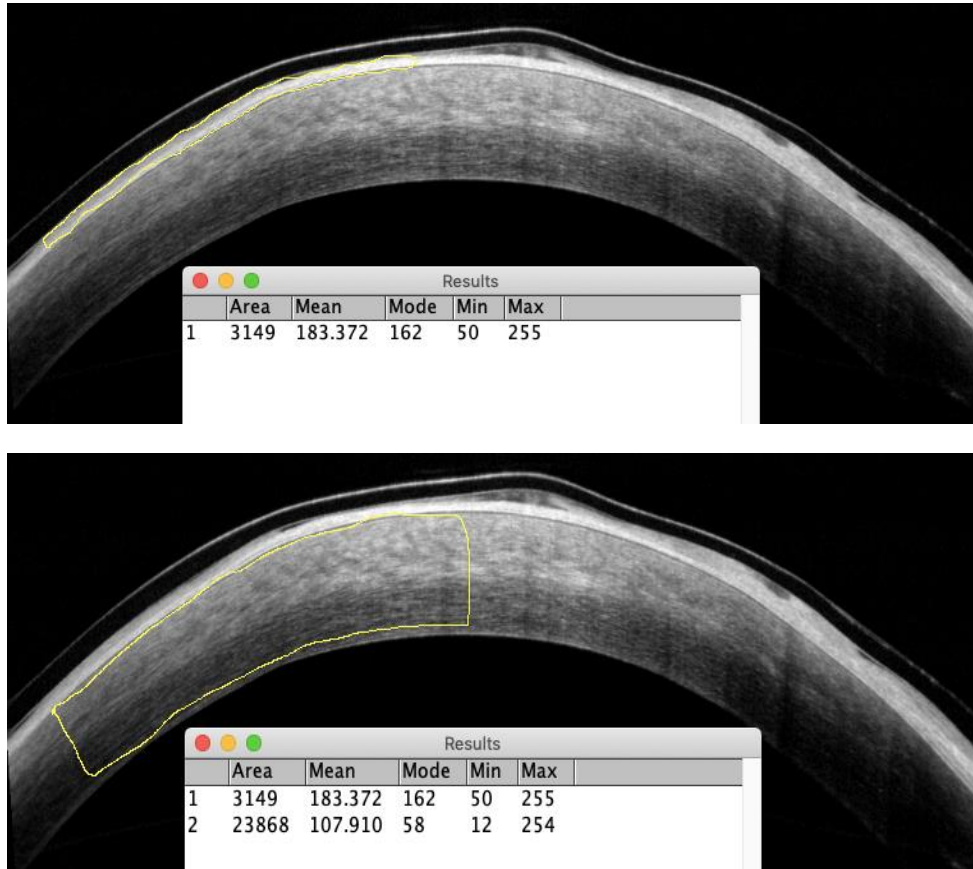
1. HR-OCT Reflectivity Analysis



'Set Measurements' option box in Fiji

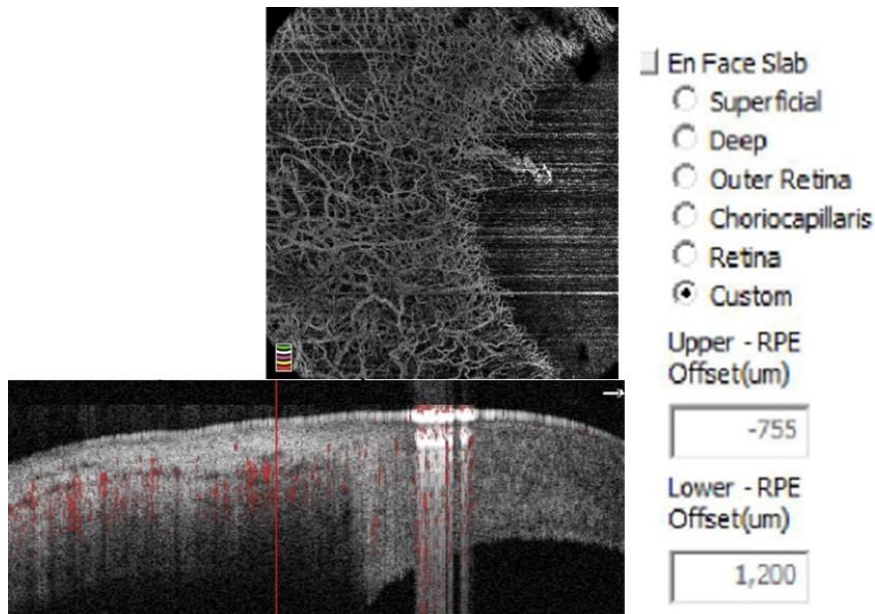


2 Epithelial and stromal reflectivity analysis:



Supplementary figure S1 : *Top* – Epithelial reflectivity analysis,
Bottom – Stromal reflectivity analysis

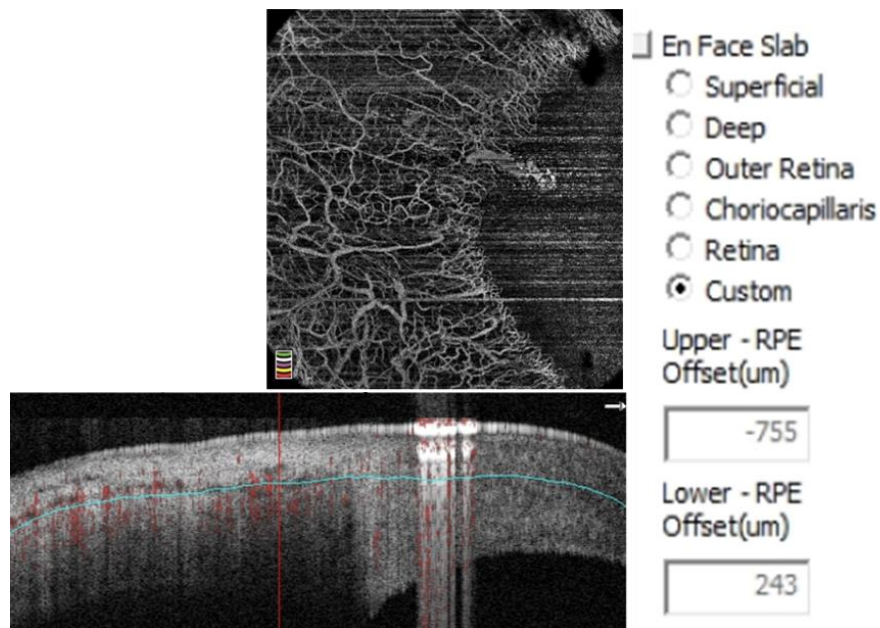
Upper RPE offset is set at -800. On setting the lower RPE offset at 1200 the corresponding scanning zone at limbus covers the full thickness of sclera which is indicated in the cross-sectional side image. (Supplementary Fig S2)



Supplementary Figure S2. Settings for the parameter – Total vascular density (total limbal vasculature)

B) Superficial vascular density:

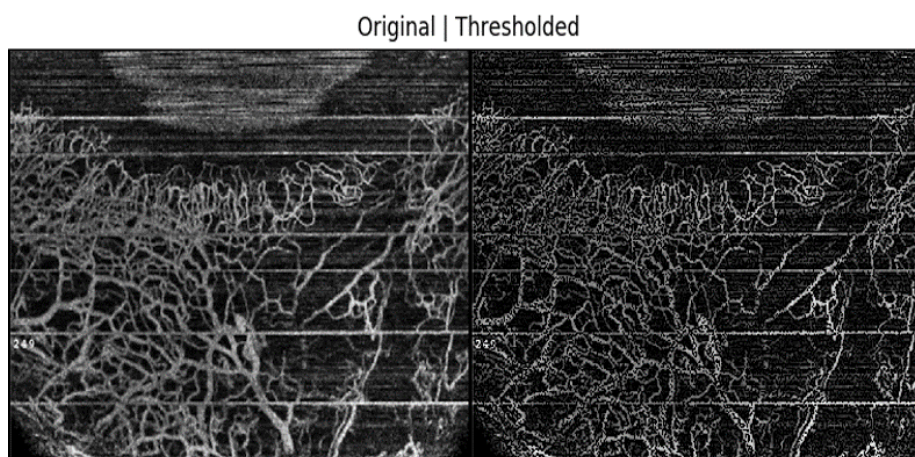
Similar to description above the upper RPE offset is set at -800. While the lower RPE is set at 243. At this setting the scan zone covers scleral thickness till superficial stroma of cornea thus giving the limbal vasculature till the level of superficial limbus. (Supplementary Fig S3)



Supplementary Figure S3. Scan settings for superficial limbal vasculature

3. Post-Processing of HR-OCT Angiography Images

The first image processing step involved identification and isolation of vasculature from the background tissue. This was accomplished by a simple operation of thresholding using a noise floor of ~14% of full signal range (Supplementary Fig S4). To ensure that density is computed at every region only in the peripheral zone outward from the eye centre at the beginning of the vascular margin, a region of interest was demarcated isolating this region.



Supplementary Figure S4. Image thresholding for analysis

On every quadrant of the Image, the inner arc of the limbus area was marked which is an input to the software. The inner arc was determined manually for each image as automated arc recognition was not possible in LSCD cases with marked distortion of limbal vasculature.

Three points along the limbus arc were determined. X and Y coordinates of each point were noted and with the help of a custom based three points calculator, the final arc coordinates of the arc were calculated. This was entered in the program before each image processing. The arc coordinates were same for analysis of superficial and total vascular density of a same quadrant of a particular patient.

The region containing the vasculature was then used to compute a vascular density map. The eye is divided into small tiles of 3 x 3 pixels and each tile is visited one-by-one. A region-of-interest (ROI) is selected around the centre of a tile by extracting a small angular sector of ± 3 degrees and radial distance of ± 20 pixels. This region-of-interest has an area say 'A' pixels.

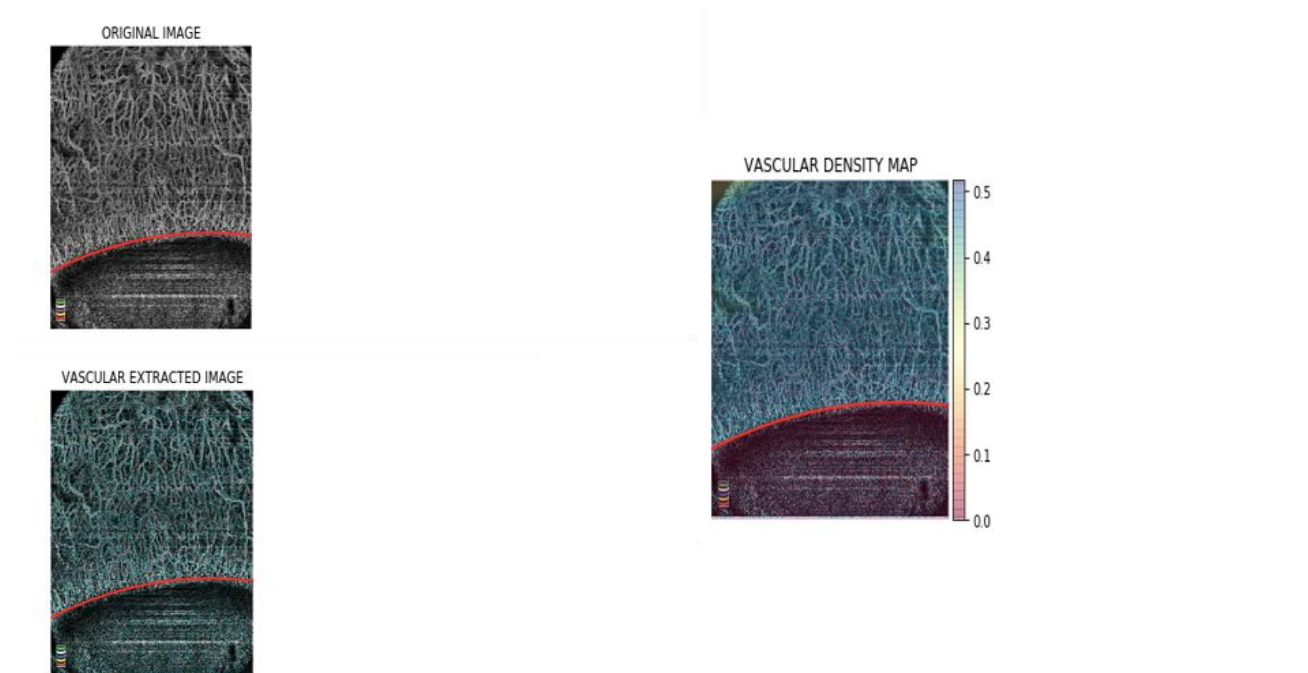
The total vascular pixels within this ROI are then counted as those pixels where the signal value is greater than a minimum threshold corresponding to a noise floor of ~14% (intensity value=36, over a full range value of 255).

Let's say this count is 'V' pixels. The ratio of V/A is defined as vascular density (VD).

$$\text{Vascular Density (VD)} = \frac{V}{A} = \frac{\sum (\text{Vasculature pixels in ROI})}{\sum (\text{All pixels in ROI})}$$

This is a normalized value bounded between zero and one.

This value is computed at all points in the image and stored as a vascular density map (Supplementary Fig S5). After the generation of the Vascular Density Map (VDM), a histogram is computed to generate summary description statistics of vascular density distribution across the whole eye.



Supplementary Figure S5. Colour coded vascular density map during analysis

Tables

Table S1: Overall Model Fit

Null model -2 Log Likelihood	169.360
Full model -2 Log Likelihood	23.927
Chi-squared	145.433
DF	3
Significance level	P < 0.0001
Cox and Snell R ²	0.6790
Nagelkerke R ²	0.9254

Table S2: Coefficients and Standard Errors

Variable	Coefficient	Std. Error	Wald	P
Mean epithelial reflectivity	0.12156	0.031020	15.3572	0.0001
Mean stromal reflectivity	-0.083103	0.027061	9.4310	0.0021
Mean Superficial Vascular density	51.27159	17.44725	8.6357	0.0033
Constant	-28.14779	8.37562	11.2942	0.0008

Table S3: Hosmer and Lemeshow test

Chi-squared	4.8937
DF	8
Significance level	P = 0.7689

Table S4: Contingency table for Hosmer and Lemeshow test

Group	Y=0		Y=1		Total
	Observed	Expected	Observed	Expected	
1	13	12.999	0	0.000669	13
2	13	12.994	0	0.00558	13
3	13	12.980	0	0.0201	13
4	13	12.938	0	0.0622	13
5	12	12.789	1	0.211	13
6	13	11.643	0	1.357	13
7	3	3.461	10	9.539	13
8	0	0.165	13	12.835	13
9	0	0.0295	13	12.970	13
10	0	0.00136	11	10.999	11

Table S5: Classification table (cut-off value p=0.5) Intervals

Actual group	Predicted group		Percent correct
	0	1	
Y = 0	78	2	97.50%
Y = 1	3	45	93.75%
Percent of cases correctly classified			96.09%

Table S6: ROC curve analysis

Area under the ROC curve (AUC)	0.992
Standard Error	0.00701
95% Confidence interval	0.957 to 1.000

First parameter found to be significant - Mean epithelial reflectivity

On ROC analysis, the area under the curve (AUC) was 0.903 (95% CI=0.83 to 0.94) with a z statistic of 13.54 ($p<0.0001$). A score of >142.862 corresponded with the highest Youden index. The sensitivity at this threshold was 83.33 (95% CI: 69.8 to 92.5) and specificity of 90.00 (81.2 to 95.6). The positive likelihood ratio was 8.33 (95% CI=4.3 to 16.3), meaning a score of >142.862 was 8.33 times more likely in eyes with LSCD, than in eyes with non-LSCD. The negative likelihood ratio was 0.19 (95% CI=0.10 to 0.4), meaning a score of ≤ 142.862 was 19 times more likely in eyes with non-LSCD than in eyes with LSCD.

Table S7: Area under ROC curve for mean epithelial reflectivity

Area under the ROC curve (AUC)	0.903
Standard Error ^a	0.0297
95% Confidence interval ^b	0.838 to 0.948
z statistic	13.541
Significance level P (Area=0.5)	<0.0001

^a DeLong et al., 1988

^b Binomial exact

Table S8: Youden index for mean epithelial reflectivity

Youden index J	0.7333
Associated criterion	>142.862
Sensitivity	83.33
Specificity	90.00

Table S9: Criterion values and coordinates of the ROC curve

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
>130	91.67	80.0 - 97.7	71.25	60.0 - 80.8	3.19	2.2 - 4.5	0.12	0.05 - 0.3
>130.798	89.58	77.3 - 96.5	71.25	60.0 - 80.8	3.12	2.2 - 4.5	0.15	0.06 - 0.3
>130.975	89.58	77.3 - 96.5	72.50	61.4 - 81.9	3.26	2.3 - 4.7	0.14	0.06 - 0.3
>131.027	87.50	74.8 - 95.3	72.50	61.4 - 81.9	3.18	2.2 - 4.6	0.17	0.08 - 0.4
>132.456	87.50	74.8 - 95.3	75.00	64.1 - 84.0	3.50	2.4 - 5.2	0.17	0.08 - 0.4

>132.638	85.42	72.2 - 93.9	75.00	64.1 - 84.0	3.42	2.3 - 5.1	0.19	0.10 - 0.4
>140.032	85.42	72.2 - 93.9	87.50	78.2 - 93.8	6.83	3.8 - 12.3	0.17	0.08 - 0.3
>141.93	83.33	69.8 - 92.5	87.50	78.2 - 93.8	6.67	3.7 - 12.1	0.19	0.1 - 0.4
>142.862	83.33	69.8 - 92.5	90.00	81.2 - 95.6	8.33	4.3 - 16.3	0.19	0.10 - 0.4
>152.93	70.83	55.9 - 83.0	90.00	81.2 - 95.6	7.08	3.6 - 14.0	0.32	0.2 - 0.5
>152.991	70.83	55.9 - 83.0	91.25	82.8 - 96.4	8.10	3.9 - 16.8	0.32	0.2 - 0.5
>153.78	66.67	51.6 - 79.6	91.25	82.8 - 96.4	7.62	3.7 - 15.9	0.37	0.2 - 0.5
>153.891	66.67	51.6 - 79.6	92.50	84.4 - 97.2	8.89	4.0 - 19.7	0.36	0.2 - 0.5

Second parameter found to be significant – Mean stromal reflectivity

On ROC analysis, the area under the curve (AUC) was 0.678 (95% CI=0.590 to 0.758) with a z statistic of 3.741 (p = 0.0002). A score of ≤151.8 corresponded with the highest Youden index. The sensitivity at this threshold was 100 (95% CI: 92.6 to 100) and specificity of 28.75 (19.2 to 40). The positive likelihood ratio was 1.40 (95% CI=1.2 to 1.6), meaning a score of ≤151.8 was 1.4 times more likely in eyes with LSCD, than in eyes with non-LSCD. The negative likelihood ratio was 0.

Table S10: Area under ROC curve for mean stromal reflectivity

Area under the ROC curve (AUC)	0.678
Standard Error ^a	0.0476
95% Confidence interval ^b	0.590 to 0.758
z statistic	3.741
Significance level P (Area=0.5)	0.0002

^a DeLong et al., 1988. ^b Binomial exact

Table S11: Youden index for mean stromal reflectivity

Youden index J	0.2875
Associated criterion	≤151.8
Sensitivity	100.00
Specificity	28.75

Table S12: Criterion values and coordinates of the ROC curve

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
≤120.555	75.00	60.4 - 86.4	43.75	32.7 - 55.3	1.33	1.0 - 1.7	0.57	0.3 - 1.0
≤123.89	75.00	60.4 - 86.4	41.25	30.4 - 52.8	1.28	1.0 - 1.6	0.61	0.3 - 1.1
≤124.8	79.17	65.0 - 89.5	41.25	30.4 - 52.8	1.35	1.1 - 1.7	0.51	0.3 - 0.9
≤126.242	79.17	65.0 - 89.5	38.75	28.1 - 50.3	1.29	1.0 - 1.6	0.54	0.3 - 1.0
≤129.591	85.42	72.2 - 93.9	38.75	28.1 - 50.3	1.39	1.1 - 1.7	0.38	0.2 - 0.8
≤130.743	85.42	72.2 - 93.9	37.50	26.9 - 49.0	1.37	1.1 - 1.7	0.39	0.2 - 0.8

≤133.8	87.50	74.8 - 95.3	37.50	26.9 - 49.0	1.40	1.1 - 1.7	0.33	0.1 - 0.7
≤134.966	87.50	74.8 - 95.3	33.75	23.6 - 45.2	1.32	1.1 - 1.6	0.37	0.2 - 0.8
≤135.133	89.58	77.3 - 96.5	33.75	23.6 - 45.2	1.35	1.1 - 1.6	0.31	0.1 - 0.7
≤135.442	89.58	77.3 - 96.5	32.50	22.4 - 43.9	1.33	1.1 - 1.6	0.32	0.1 - 0.8
≤135.721	91.67	80.0 - 97.7	32.50	22.4 - 43.9	1.36	1.1 - 1.6	0.26	0.10 - 0.7
≤137.653	91.67	80.0 - 97.7	31.25	21.3 - 42.6	1.33	1.1 - 1.6	0.27	0.10 - 0.7
≤137.714	93.75	82.8 - 98.7	31.25	21.3 - 42.6	1.36	1.2 - 1.6	0.20	0.06 - 0.6
≤138.154	93.75	82.8 - 98.7	30.00	20.3 - 41.3	1.34	1.1 - 1.6	0.21	0.07 - 0.7
≤144.169	97.92	88.9 - 99.9	30.00	20.3 - 41.3	1.40	1.2 - 1.6	0.069	0.010 - 0.5
≤149.201	97.92	88.9 - 99.9	28.75	19.2 - 40.0	1.37	1.2 - 1.6	0.072	0.01 - 0.5
≤151.8	100.00	92.6 - 100.0	28.75	19.2 - 40.0	1.40	1.2 - 1.6	0.00	
≤232.513	100.00	92.6 - 100.0	0.00	0.0 - 4.5	1.00	1.0 - 1.0		

Ratio of mean epithelial reflectivity to stromal reflectivity

On ROC analysis, the area under the curve (AUC) was 0.970 (95% CI=0.92 to 0.99) with a z statistic of 26.34 (p < 0.0001). A score of >1.29 corresponded with the highest Youden index. The sensitivity at this threshold was 91.67 (95% CI: 80 to 97.7) and specificity of 98.75 (93.2 to 100). The positive likelihood ratio was 73.3 (95% CI=10.4 to 515.2), meaning a score of >1.29 was 73.3 times more likely in eyes with LSCD, than in eyes with non-LSCD. The negative likelihood ratio was 0.084 (95% CI=0.02 to 0.2), meaning a score of <1.29 was 8 times more likely in eyes with non-LSCD than in LSCD.

Table S13: Area under ROC curve for Ratio of Mean epithelial reflectivity to stromal reflectivity

Area under the ROC curve (AUC)	0.970
Standard Error ^a	0.0178
95% Confidence interval ^b	0.924 to 0.992
z statistic	26.346
Significance level P (Area=0.5)	<0.0001

^a DeLong et al., 1988

^b Binomial exact

Table S14: Youden index for Ratio of Mean epithelial reflectivity to stromal reflectivity

Youden index J	0.9042
Associated criterion	>1.290909091
Sensitivity	91.67
Specificity	98.75

Table S15: Criterion values and coordinates of the ROC curve

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
≥0.557082371	100.00	92.6 - 100.0	0.00	0.0 - 4.5	1.00	1.0 - 1.0		
>0.833247789	100.00	92.6 - 100.0	31.25	21.3 - 42.6	1.45	1.3 - 1.7	0.00	

>0.8411319 72	97.92	88.9 - 99.9	31.25	21.3 - 42.6	1.42	1.2 - 1.7	0.06 7	0.009 - 0. 5
>0.9767441 86	97.92	88.9 - 99.9	52.50	41.0 - 63.8	2.06	1.6 - 2.6	0.04 0	0.006 - 0. 3
>1.0110810 16	95.83	85.7 - 99.5	52.50	41.0 - 63.8	2.02	1.6 - 2.6	0.07 9	0.02 - 0.3
>1.1875035 18	95.83	85.7 - 99.5	93.75	86.0 - 97.9	15.3 3	6.5 - 35.9	0.04 4	0.01 - 0.2
>1.1916541 42	93.75	82.8 - 98.7	93.75	86.0 - 97.9	15.0 0	6.4 - 35.2	0.06 7	0.02 - 0.2
>1.2130406	93.75	82.8 - 98.7	96.25	89.4 - 99.2	25.0 0	8.2 - 76.1	0.06 5	0.02 - 0.2
>1.2632938 08	91.67	80.0 - 97.7	96.25	89.4 - 99.2	24.4 4	8.0 - 74.4	0.08 7	0.03 - 0.2
>1.2909090 91	91.67	80.0 - 97.7	98.75	93.2 - 100. 0	73.3 3	10.4 - 515. 2	0.08 4	0.03 - 0.2
>1.4790996 78	62.50	47.4 - 76.0	98.75	93.2 - 100. 0	50.0 0	7.0 - 355.0	0.38	0.3 - 0.5
>1.4857142 86	62.50	47.4 - 76.0	100.00	95.5 - 100. 0			0.38	0.3 - 0.5
>4.4642314 25	0.00	0.0 - 7.4	100.00	95.5 - 100. 0			1.00	1.0 - 1.0

Third parameter found to be significant – Mean superficial vascular density

On ROC analysis the area under the curve (AUC) was 0.914 (95% CI=0.85 to 0.95) with a z statistic of 17.03 (p < 0.0001). A score of >0.38 corresponded with the highest Youden index. The sensitivity at this threshold was 97.9 (95% CI: 88.9 to 99.9) and specificity of 73.75 (62.7 to 83). The positive likelihood ratio was 3.73 (95% CI=2.6 to 5.4), meaning a score of >0.38 was 3.73 times more likely in eyes with LSCD, than with in eyes with non-LSCD. While, the negative likelihood ratio was 0.028 (95% CI=0.02 to 0.2), meaning a score of <0.38 was 2.8 more likely in eyes with non-LSCD than in eyes with LSCD.

Table S16: Area under ROC curve for mean superficial vascular density

Area under the ROC curve (AUC)	0.914
Standard Error ^a	0.0243
95% Confidence interval ^b	0.852 to 0.956
z statistic	17.030
Significance level P (Area=0.5)	<0.0001

^a DeLong et al., 1988

^b Binomial exact

Table S17: Youden index for mean superficial vascular density

Youden index J	0.7167
Associated criterion	>0.38
Sensitivity	97.92
Specificity	73.75

Table S18: Criterion values and coordinates of the ROC curve

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
≥0.28	100.00	92.6 - 100.0	0.00	0.0 - 4.5	1.00	1.0 - 1.0		
>0.35	100.00	92.6 - 100.0	38.75	28.1 - 50.3	1.63	1.4 - 1.9	0.00	
>0.36	97.92	88.9 - 99.9	53.75	42.2 - 65.0	2.12	1.7 - 2.7	0.039	0.006 - 0.3
>0.38	97.92	88.9 - 99.9	73.75	62.7 - 83.0	3.73	2.6 - 5.4	0.028	0.004 - 0.2
>0.39	83.33	69.8 - 92.5	82.50	72.4 - 90.1	4.76	2.9 - 7.8	0.20	0.1 - 0.4
>0.4	77.08	62.7 - 88.0	86.25	76.7 - 92.9	5.61	3.2 - 9.9	0.27	0.2 - 0.4
>0.41	68.75	53.7 - 81.3	90.00	81.2 - 95.6	6.88	3.5 - 13.6	0.35	0.2 - 0.5
>0.42	58.33	43.2 - 72.4	93.75	86.0 - 97.9	9.33	3.9 - 22.5	0.44	0.3 - 0.6
>0.43	43.75	29.5 - 58.8	96.25	89.4 - 99.2	11.67	3.7 - 37.1	0.58	0.5 - 0.8
>0.44	35.42	22.2 - 50.5	97.50	91.3 - 99.7	14.17	3.4 - 58.7	0.66	0.5 - 0.8
>0.45	18.75	8.9 - 32.6	97.50	91.3 - 99.7	7.50	1.7 - 33.3	0.83	0.7 - 1.0
>0.46	14.58	6.1 - 27.8	100.00	95.5 - 100.0			0.85	0.8 - 1.0
>0.48	0.00	0.0 - 7.4	100.00	95.5 - 100.0			1.00	1.0 - 1.0