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Quantitative assessment of the conjunctival microcirculation using a smartphone and slit-lamp biomicroscope

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Manuscript title Quantitative assessment of the conjunctival microcirculation using a smartphone and slit-lamp biomicroscope

Highlights

- The conjunctiva has a readily-accessible microcirculation for haemodynamic analysis
- Smartphone-assisted haemodynamic assessment of the conjunctiva is feasible
- Increasing conjunctival vessel diameter results in increased blood volume flow
- Wall shear rate decreases as conjunctival vessel diameter increases

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Title Page

1
2 **Manuscript title** Quantitative assessment of the conjunctival microcirculation
3 using a smartphone and slit-lamp biomicroscope

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37 Belfast, United Kingdom.

39 **Keywords** Conjunctival circulation; microcirculation; smartphone;
40 haemodynamic assessment; endothelial dysfunction

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120
121 **Abstract**
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123 **Purpose** The conjunctival microcirculation is a readily-accessible vascular bed
124
125 for quantitative haemodynamic assessment and has been studied previously using a
126
127 digital charge-coupled device (CCD). Smartphone video imaging of the conjunctiva,
128
129 and haemodynamic parameter quantification, represents a novel approach. We
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131 report the feasibility of smartphone video acquisition and subsequent haemodynamic
132
133 measure quantification via semi-automated means.
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136 **Methods** Using an Apple iPhone 6s and a Topcon SL-D4 slit-lamp
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138 biomicroscope, we obtained videos of the conjunctival microcirculation in 4 fields of
139
140 view per patient, for 17 low cardiovascular risk patients. After image registration and
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142 processing, we quantified the diameter, mean axial velocity, mean blood volume
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144 flow, and wall shear rate for each vessel studied. Vessels were grouped into
145
146 quartiles based on their diameter i.e. group 1 ($<11\mu\text{m}$), 2 ($11\sim 16\mu\text{m}$), 3 ($16\sim 22\mu\text{m}$)
147
148 and 4 ($>22\mu\text{m}$).
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150

151 **Results** From the 17 healthy controls (mean QRISK3 6.6%), we obtained
152
153 quantifiable haemodynamics from 623 vessel segments. The mean diameter of
154
155 microvessels, across all sites, was $18.23\mu\text{m}$ (range $6.6\text{-}39.2\mu\text{m}$). Mean axial velocity
156
157 was 0.49mm/s (range $0.12\text{-}0.79\text{mm/s}$) and there was a modestly positive correlation
158
159 ($r\ 0.404$) seen with increasing diameter, best appreciated when comparing group 4
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161 to the remaining groups ($p<0.0001$). Blood volume flow (mean 109.718pl/s , range
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163 $11.28\text{-}502.19\text{pl/s}$) was strongly correlated with increasing diameter ($r\ 0.967$,
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165 $p<0.0001$) and wall shear rate (mean 182.81s^{-1} , range $55.11\text{-}546.69\text{s}^{-1}$) negatively
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167 correlated with increasing diameter ($r\ -0.823$, $p<0.0001$).
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180 64 **Conclusions** We, for the first time, report the successful assessment and
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182 65 quantification of the conjunctival microcirculatory haemodynamics using a
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184 66 smartphone-based system.
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188 68 **Manuscript**

190 69 **I. Introduction**

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193 70 Cardiovascular disease (CVD) is a leading cause, globally, of mortality and morbidity
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195 71 while also being associated with a significant economic burden on health services¹.
196
197 72 CVD is caused by physiological changes and endothelial dysfunction, resulting in
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199 73 atherosclerosis, and it is accepted that these changes manifest earliest in the
200
201 74 microcirculatory networks within the body². Microcirculatory disease typically
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203 75 commences with endothelial dysfunction which may be clinically silent and, thus,
204
205 76 precede the onset of symptoms³ or the occurrence of a major adverse
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207 77 cardiovascular event (MACE) e.g. myocardial infarction (MI) or cerebrovascular
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209 78 accident (CVA). Microvascular dysfunction is associated with increased mortality⁴
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211 79 and thus the study of microcirculations may provide a potential tool in disease
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213 80 screening, staging and management. Imaging of systemic microcirculations has
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215 81 been applied to and, in certain disease subsets, is used in every day current practice
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217 82 in assessing disease progression e.g. the retinal microcirculation in the assessment
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219 83 of diabetes mellitus, systemic hypertension, and sickle cell disease^{5,6,7,8}. The
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221 84 sublingual mucosa and the skin also represent accessible sites in which the
222
223 85 microcirculation has been studied by videomicroscopy⁹.
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225 86 The anterior segment of the eye contains the conjunctival microvasculature, a
226
227 87 readily-accessible heterogeneous network of arterioles and venules adjacent to the
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229 88 limbal microcirculation, which gains its supply from the anterior ciliary branch of the
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89 ophthalmic artery¹⁰. The conjunctival microvasculature allows for both non-invasive
90 assessment of erythrocyte movement, and quantification of key vascular
91 physiological parameters e.g. vessel width, blood flow axial velocity and blood flow
92 rate¹¹.

93 The objective of this study was to evaluate the feasibility of assessing the
94 conjunctival microcirculation using our novel combination of a smartphone and slit-
95 lamp biomicroscope. We aimed to develop an operator-friendly, pragmatic, safe and
96 effective means of assessing this heterogeneous circulation, in addition to the
97 quantification of the haemodynamic physiological parameters seen within a
98 microcirculation.

99 A few groups have reported semi-automated or automated image analysis
100 algorithms to assess the conjunctival microcirculation, using a slit lamp
101 biomicroscope and a digital charge-coupled device (CCD) camera for image
102 acquisition^{12,13,14,15,16,17}. Using such systems, the conjunctival microcirculation has
103 been studied in patients with hypertension, diabetic retinopathy, and patients after
104 ischaemic stroke^{18, 19, 20}. In addition, one group has reported the application of such
105 methods in patients of varying predictive cardiovascular risk, assessed by the
106 Framingham risk score²¹.

107 Smartphone technology allows for remote monitoring and screening of many
108 prevalent cardiovascular conditions, for example atrial fibrillation, and represents an
109 important component of future healthcare and cardiovascular practice²². The
110 literature is scarce regarding smartphone use to assess microcirculatory
111 haemodynamics but the application of smartphone photography of the fundus has
112 been reported in diabetic and hypertensive patients^{23, 24, 25}. There are some studies
113 describing smartphone-led image analysis of the conjunctiva in the assessment of

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298 114 patients with anaemia^{26,27} and, also, quantification of conjunctival “redness” i.e.
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300 115 hyperaemia²⁸. In addition, the smartphone-based biometric has been studied on the
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302 116 visible vascular patterns on whites of the eye²⁹ but, at this time, there are no studies
303
304 117 that describe the assessment or quantification of conjunctival haemodynamics using
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306 118 a smartphone and slit-lamp combination.
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311 120 **II. Materials and Methods**

313 121 **A. Subjects**

315 122 This research study was approved by the Research and Development review boards
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317 123 of the Ulster University (UU) and the Belfast Health and Social Care Trust (BHSCT).
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319 124 All subjects were provided with verbal and written information, prior to study
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321 125 enrolment, in accordance with the Declaration of Helsinki. Exclusion criteria included
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323 126 inability to consent, prior myocardial infarction (MI), uncontrolled systemic
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325 127 hypertension, recent history of conjunctival inflammation, prior refractive surgery,
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327 128 used ocular medications (other than artificial tears) and current use of contact
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329 129 lenses.
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331 130 We recruited 17 healthy volunteers to this feasibility study. The mean age for the
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333 131 population studied was 52.5 ±10.3years, IQR 15 years. Sex distribution was roughly
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335 132 equal with 9 (53%) males and 8 females (47%). No patients had a history of prior MI,
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337 133 cerebrovascular accident (CVA), or diabetes mellitus. The QRISK 3
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339 134 (<https://qrisk.org/three/>) score algorithm was used to estimate each volunteer’s 10-
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341 135 year risk of future heart attack or stroke. The QRISK 3 algorithm is based on the
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343 136 presence/lack of specific risk factors for CVD e.g. smoking, diabetes mellitus,
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345 137 hypertension, family history angina, chronic kidney disease, age, sex, body mass
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347 138 index, history of atrial fibrillation, use of regular steroid tablets, presence of chronic
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139 inflammatory disease, and cholesterol profile. It has been well-validated in our
 140 population³⁰. The mean QRISK 3 score was 6.6 ±9%, IQR 6.9%, which correlates
 141 with a “low-risk” population (<10%). Table 1 is a summary of the baseline
 142 demographics and clinical observations for the study group.

	Number n=17
<i>Male sex, n (%)</i>	9 (53.0)
<i>Age, years ±SD</i>	52.5 ±10.3
<i>QRISK 3 score, % ±SD</i>	6.6 ±9
<i>Systolic blood pressure, mmHg ±SD</i>	125 ±22
<i>Diastolic blood pressure, mmHg ±SD</i>	77 ±12
<i>Heart rate, bpm ±SD</i>	70 ±9
<i>Prior MI/CVA/Diabetes mellitus</i>	0

143 Table 1. Baseline characteristics of the study group (n=17) with continuous variables
 144 expressed using their mean and standard deviation. Categorical variables have been
 145 expressed as a number and percentage of the total within that variable.

146 **B. Image Acquisition**

147 Image acquisition was achieved via two main hardware components. Firstly, primary
 148 illumination and magnification of the ocular vascular structure was achieved using a
 149 conventional slit lamp biomicroscope, Topcon SL-D4 (Topcon Medical Systems Inc.,
 150 USA), capable of providing a maximum magnification of 40x. Secondly, images
 151 provided by the slit lamp biomicroscope were further magnified and stored using a
 152 smartphone camera. The smartphone used in the system is an Apple iPhone 6s
 153 (Apple, Inc., USA). A number of video record settings were tested and the optimal
 154 configuration set at a resolution of 1920 x 1080 pixels, captured at 60 frames per
 155 second. The iPhone video recorder is capable of providing a further magnification of

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415
416 156 3x. Coupling of the smartphone to the eyepiece of the slit lamp biomicroscope was
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418 157 achieved using a bespoke adapter developed by Zarf Enterprises (Zarf Enterprises.,
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420 158 USA). Smartphone cameras typically give very little control over camera properties
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423 159 (focus, ISO, shutter speed, aperture) due to an emphasis on ease-of-use for
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425 160 everyday consumers, while also generating compressed video files (h.264
426
427 161 compression in the case of the iPhone 6s). To help overcome these issues we
428
429 162 captured our data using a third-party application "ProMovie Recorder"
430
431 163 (www.promovieapp.com). We used constant settings for all images (iso/shutter
432
433 164 speed/ focus/ exposure) and used the maximum compression bit-rate available to
434
435 165 reduce compression artefacts. The video zoom setting was locked at 2x, providing a
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437 166 1:1-pixel mapping of the camera sensor at 1080p resolution and thus avoiding
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439 167 interpolation artefacts. To obtain an accurate pixel to mm conversion factor we
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441 168 calibrated the system using a digital caliper and 1mm microscope calibration reticle,
442
443 169 deriving a conversion factor of 552 ± 22.6 pixels/mm. We obtained one video (5-15s)
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445 170 from 4 distinct field of views i.e. medial and temporal conjunctiva in both eyes. Fig.1.
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447 171 To reduce eye motion and blinking we used an external fixation target as a focal
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449 172 point for each patient. We acquired only 4 videos (5-15s) per patient to minimise the
450
451 173 risk of potential adverse effects, e.g. slit-lamp light exposure. There were no reported
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453 174 adverse effects at the time of, or after, image acquisition. Patients were imaged in
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455 175 the same clinical room under constant temperature and lighting settings.
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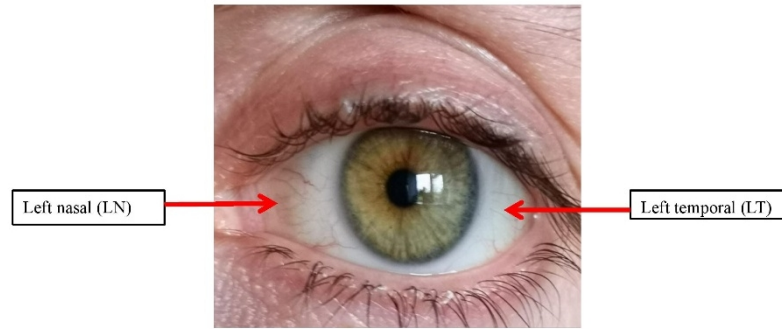


Fig. 1. Two fields of view (FOV) for the left eye of a healthy subject, with the medial and lateral FOV being labelled (red arrows) the left nasal (LN) and left temporal (LT) respectively.

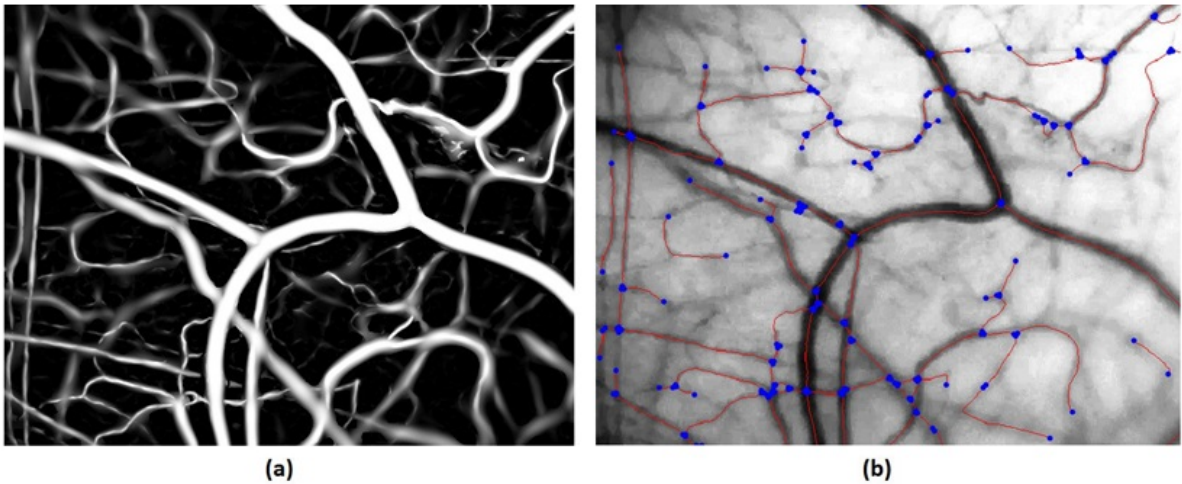
C. Image Processing

1. Pre-Processing and Vessel Segmentation

An initial pre-processing procedure was carried out for each video file. Firstly, the longest stable sequence of frames was manually selected on the basis of the vasculature being in focus, there being no blinking or large sudden movements of the eye, and the FOV not drifting by more than ~25% of the width of the frame. Next the green channel, which gave the highest vessel contrast, was extracted and information from the red channel used to correct for uneven illumination through subtraction. The sharpest frame in the sequence was then selected as a reference frame and all other frames registered to it through an affine registration procedure³¹, with a single composite image generated by averaging all registered frames. After applying a “vessel enhancement filter”³² (Fig.2 (a)), a binary map of the conjunctival

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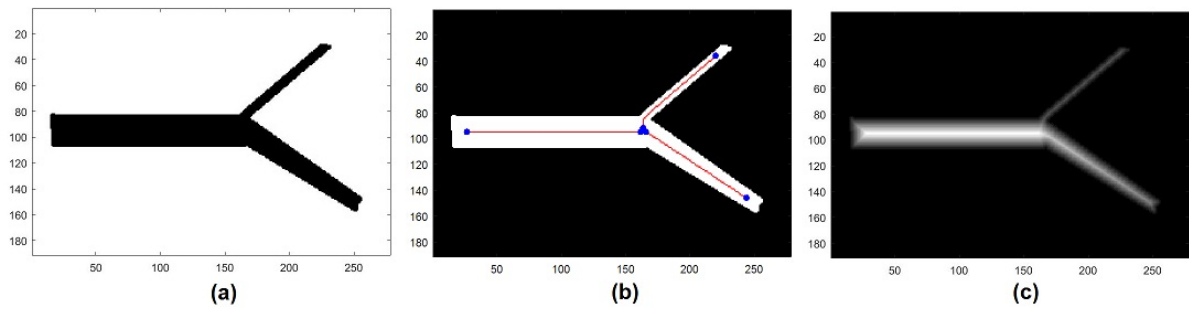
194 vasculature and corresponding centrelines were obtained via standard skeletisation
195 techniques. Finally, the connected vessel network was broken into individual vessel
196 segments (Fig.2 (b)) by setting the branch points' neighbouring pixels to zero, and
197 centreline segments, containing more than 30 pixels, selected for further
198 assessment.



199
200 Fig. 2. Microvascular network after image processing: (a) the vessel network after
201 filtering; (b) the vessel centreline (in red) and intersection points (in blue) overlaid on
202 the mean of vessel images.

2. Vessel Diameter (D)

204 The Euclidean Distance Transform (EDT) was proposed for vessel diameter
205 estimation, which is easier to implement in comparison to the commonly used
206 method via full width at half maximum (FWHM). The value at each pixel of EDT was
207 calculated based on the Euclidean distance between the pixel and its nearest
208 nonzero pixel in the binary vessel image. The centreline of the vessel was used to
209 obtain the central EDT values and thus the radius along the vessel axis. The
210 average of diameters along the vessel length provided the final vessel width
211 estimation. An example based on simulation is illustrated in Fig.3.



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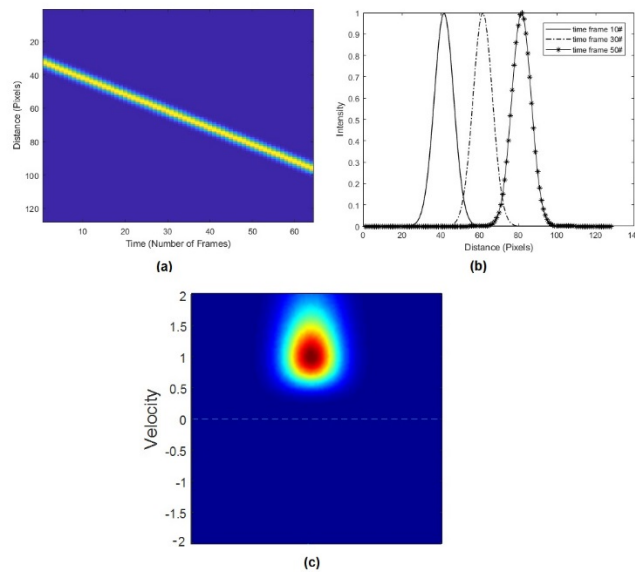
Fig. 3. Simulation for vessel diameter estimation: (a) three vessels are generated with mean diameter 25.3 pixels, 16.5 pixels, and 8.3 pixels, respectively; (b) the vessel centreline, end points and branch points overlaid on the binary vessel image; (c) EDT of the binary vessel image. The mean of estimated diameters via EDT are 25.9 pixels, 16.6 pixels, and 8.6 pixels, respectively.

Given the complex and heterogeneous distribution of conjunctival microvessels, we applied a grouping classification to our results, described in previous work, based on vessel D i.e. group 1 ($<11\mu\text{m}$), group 2 ($11\text{-}16\mu\text{m}$), group 3 ($16\text{-}22\mu\text{m}$) and group 4 ($>22\mu\text{m}$)¹¹.

3. Axial velocity (V_a)

The blood flow V_a in a single vessel segment was estimated based on the spatial-temporal image (STI), with the change in intensity in STI reflecting erythrocyte movement through the vessel. Since STI signal is the one dimension of space plus time, i.e., a 1D+T signal, a novel approach based on spatial temporal 1D+T continuous wavelet transform (1DTCWT) is proposed for V_a estimation. The CWT method has been used previously as a spatiotemporal filter for motion capture of 1D+T signals for moving target tracking and parameter calculation³³, but not yet exploited in microvascular blood flow velocity estimation. Firstly, 2D fast Fourier transform (FFT) is performed for STI. The velocity vector space is defined and

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 651
 652 232 1DTCWT is then run at each time interval. The energy is subsequently calculated
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 654 233 based on the 1DTCWT output. The velocity is obtained by searching the maximum
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 656 234 energy point as shown in Fig.4. The average of the absolute velocity across all
 657
 658 235 frames was used as the final estimation of V_a . The method was programmed in
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 660 236 MATLAB2017 together with an open source implementation of CWT ³⁴.



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 683 238 Fig.4. Simulation for velocity estimation based on 1DTCWT. (a) synthetic STI
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 685 239 generated by shifting Gaussian signal with speed of 1 pixel/frame; (2) plot of
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 687 240 signals at the 10th, 30th and 50th frames, which shows the Gaussian signal
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 689 241 shifting in distance; (c) a colour spectrum map via 1DTCWT shows the velocity is
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 691 242 corresponding to the maximum of the energy (at 1 pixel/frame).

693 243 4. Blood flow (Q) and wall shear rate (WSR)

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 697 244 Using the measurements for D and V_a , we calculated Q and WSR using previously
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 699 245 described methods ^{11,12}. Q provides key information regarding the architecture and
 700
 701 246 function of the vascular system, whereas WSR is the blood velocity at a specific wall
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711 247 position, within a vessel, and represents a surrogate for the pressure exerted by
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713 248 blood within its' respective transport vessel ^{35, 36, 37}.

715 716 249 717 718 719 250 5. Statistical analysis

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721 251 For statistical analysis SPSS for Apple iOS version 25 (property of IBM) and R
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723 252 version 3.5.3 (www.r-project.org) were used. Continuous variables were described
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725 253 using the mean, standard deviation of the mean and interquartile range (IQR) for the
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727 254 variable. Categorical variables were described as a number and percentage of the
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729 255 total category number to which the variable belonged. Sample origin, distribution and
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731 256 variance were assessed by non-parametric ANOVA (Kruskal-Wallis test). Correlation
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733 257 analysis (Spearman rank), with a Loess regression fit, was applied to assess
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735 258 relationships between D and independent variables, principally Va, Q and WSR.
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737 259 Non-parametric ANOVA (Kruskal-Wallis) with or without Dunn's post-hoc tests was
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739 260 used to compare D, Va, W, and WSR by vessel width group, with the tests being
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741 261 conducted separately across site, i.e., left/right nasal and temporal, or for all sites
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743 262 merged.

744 745 746 747 263 **III. Results**

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749 264 For the 17 healthy patients studied, using our semi-automated approach, we were
750
751 265 able to obtain repeated measurements in 623 vessel segments (mean 37 segments
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753 266 per patient), hereafter referred to as "microvessels", which exhibited observable flow.
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755 267 The mean diameter (D) of microvessels, across all sites, was 18.2 μ m (range 6.6-
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757 268 39.2 μ m). Group 4 (>22 μ m) microvessels were measured most frequently, with group
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759 269 1 (<11 μ m) being the least commonly encountered i.e. 295 vs 64 microvessels
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761 270 respectively. Mean Va was 0.49mm/s (range 0.12-0.79mm/s), Q 109.72pl/s (range
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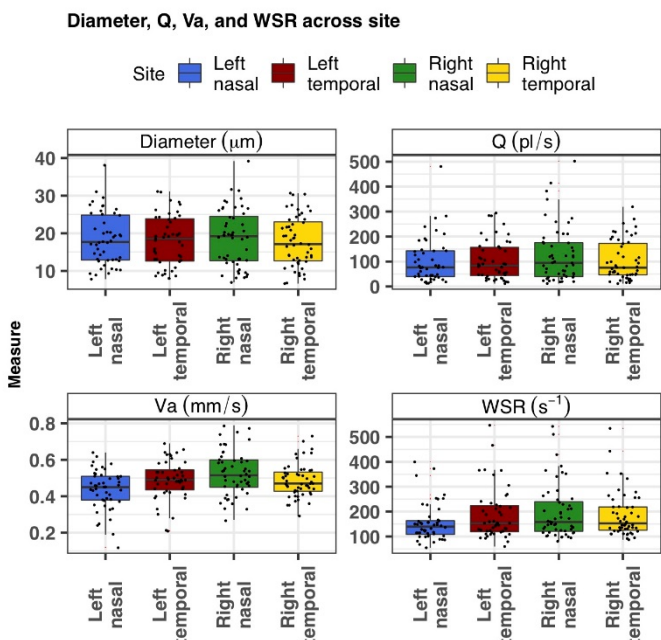
271 11.28-502.19pl/s) and WSR ranged between 55.11-546.69s⁻¹, with a mean WSR of
 272 182.81s⁻¹. The mean and SD of all microvessel conjunctival haemodynamic
 273 parameters are illustrated in Table 2. Statistical comparisons for Va, Q and WSR
 274 were made within the vessel groups. There was a statistically significant increase in
 275 Q for increasing diameter size (p<0.0001), with a statistically significant inverse
 276 correlation between WSR and increasing diameter size (p<0.0001). Va tended to
 277 increase with increasing microvessel diameter and was significantly elevated in
 278 group 4 (>22µm) vessels, compared to the remaining three groups (p<0.0001).

Group D µm	No. vessels N=623	D (µm)	Va (mm/s)	Q (pl/s)	WSR (s⁻¹)
<11 Group 1	64	9.1 ±2.8	0.45 ±0.05	23.65 ±2.96	332.75 ±60.75
11~16 Group 2	113	13.44±3.7	0.44 ±0.06	46.81 ±8.02	200.19 ±32.89
16~22 Group 3	151	19.2 ±3.5	0.47 ±0.06	97.13 ±17.21	136.67 ±20.35
>22 Group 4	295	26.9 ±2.7	0.56 ±0.09	224.45 ±66.35	115.27 ±17.7
			p<0.0001	p<0.0001	p<0.0001
	Mean	18.2	0.485	109.718	182.81
	Range	6.6-39.2	0.12-0.79	11.28-502.19	55.11-546.69
	Interquartile range (IQR)	12.74-24	0.42-0.55	39.86-161	116.33- 221.72

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280 Table 2. Summary of haemodynamic measures D, Va, Q and WSR based on the
281 vessel diameter groups (1-4).

282 Across site (field of view) comparisons were made with the haemodynamic
283 measures. Q and WSR did not statistically differ between the 4 image fields. There
284 was a statistically higher Va noted in the right nasal (RN) hemisphere compared to
285 the left nasal (LN, (p = 0.0003)), for which the clinical significance is unknown and
286 may require further exploration. The relationship between the haemodynamic
287 measures and similarities for each field of view is shown in Fig.5. Note the elevated
288 Va in the RN FOV, compared to the other FOVs, as before.



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291 Fig.5. Summary of diameter D (μm), Va (mm/s), Q (pl/s) and WSR (s^{-1}), for each field
292 of view i.e. left nasal (LN), left temporal (LT), right nasal (RN) and right temporal
293 (RT).

294 The correlation, expressed via the correlation coefficient (r) and the best fit trend line,
295 between increasing microvessel diameter and the haemodynamic measures Va, Q
296 and WSR were consistent across the 4 fields of view, which are individually

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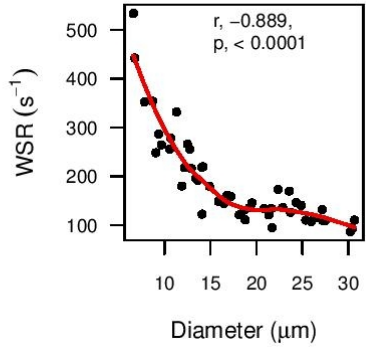
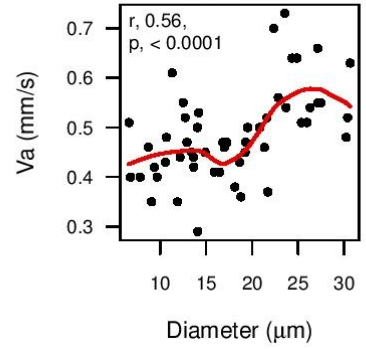
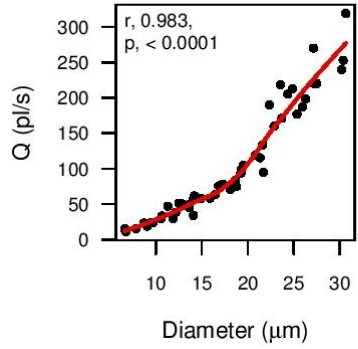
297 illustrated in Fig.6a-d.

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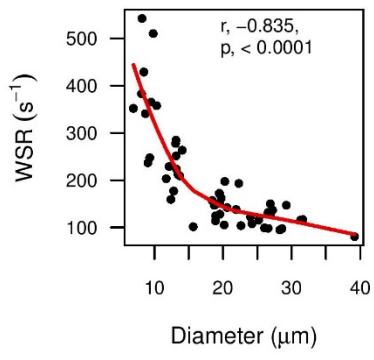
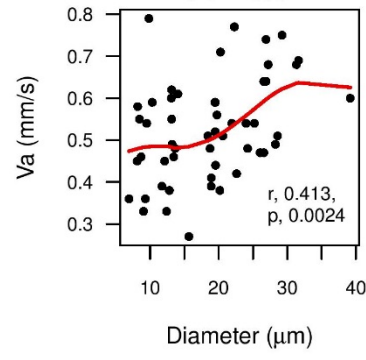
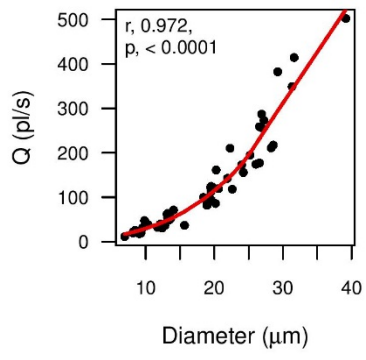
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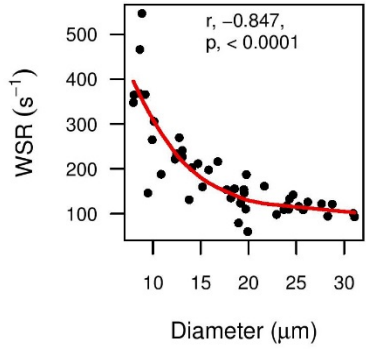
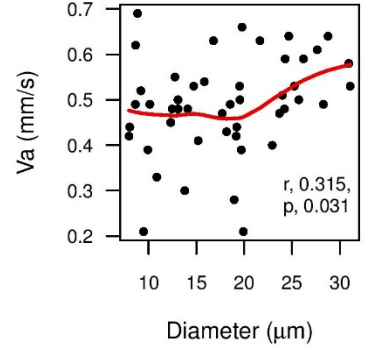
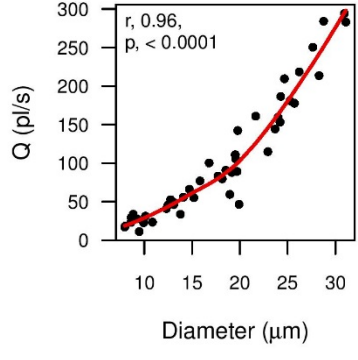
**Right temporal,
correlations to vessel
diameter**

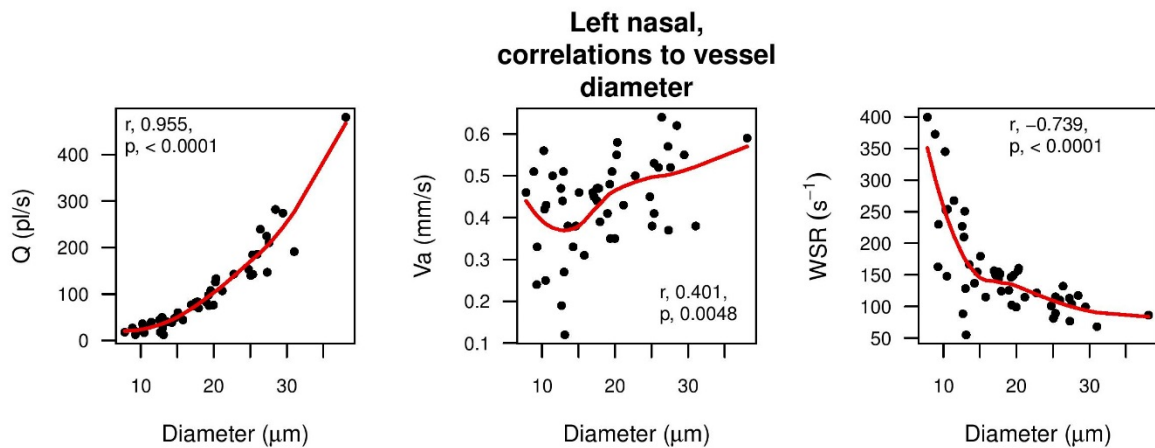


**Right nasal,
correlations to vessel
diameter**



**Left temporal,
correlations to vessel
diameter**



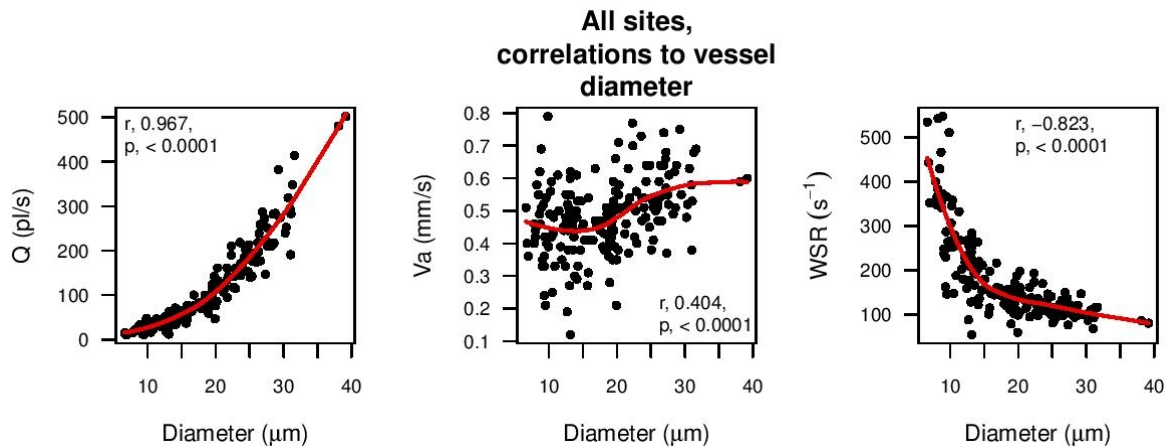


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302 Fig.6. Correlation plots between microvessel diameter D (μm) vs V_a (mm/s), Q (pl/s)
 303 and WSR (s^{-1}) for each field of view ((a) RT, (b) RN, (c) LT, (d)LN).

304

305 A summary of the correlations between microvessel D and the quantified
 306 haemodynamic measures are illustrated in Fig.7. demonstrating the strong overall
 307 linear correlation with Q and WSR (r 0.967, r -0.823 respectively). A modest
 correlation was seen for V_a (r 0.404).



308

309 Fig.7. Correlation plots between microvessel diameter D (μm) vs V_a (mm/s), Q (pl/s)
 310 and WSR (s^{-1}) across all sites.

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312 The correlations between increasing vessel diameter and V_a , Q , and WSR are in
 313 keeping with that reported in previous work^{11,12}, whereby similar fluid dynamics and
 microvascular relationships have been observed.

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1008 316 **IV. Discussion**
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1010 317 The conjunctival microcirculation represents a readily-accessible vascular network
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1012 318 for non-invasive assessment. Physiological measures in the conjunctival
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1015 319 microcirculation display the same trends and correlations as they do elsewhere in
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1017 320 the circulation and, based on this rationale, may represent a key microcirculation that
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1019 321 could be assessed in the evaluation of circulatory health and, if so, correlated with
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1021 322 risk. Correlations between cardiovascular risk estimation and quantitative
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1023 323 conjunctival haemodynamic measures, namely velocity and blood flow, were
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1025 324 demonstrated in previous work²¹.
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1027 325 In recent years, there have been several reports regarding the clinical utility of
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1029 326 conjunctival microcirculatory study. Conjunctival haemodynamic assessment has
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1031 327 extended to patients with diabetes mellitus, in correlation with diabetic retinopathy
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1033 328 status, with differences between Va, Q and WSR being observed for differing grades
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1035 329 of retinopathy¹⁹. Quantitative assessment of the conjunctival haemodynamics was,
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1037 330 also, evaluated in patients with ischaemic unilateral stroke and Va was found to be
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1039 331 significantly lower in the ipsilateral eye to the stroke compared to the contralateral
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1041 332 eye, demonstrating the physiological relationship shared by the internal carotid
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1043 333 arterial system and the conjunctival microcirculation²⁰.
1044

1045 334 We have described the application of smartphone technology, combined with a slit-
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1047 335 lamp, in the quantitative assessment of conjunctival haemodynamics, namely D, Va,
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1049 336 Q and WSR. With our approach, we have demonstrated the feasibility of obtaining
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1051 337 haemodynamic results, similar to the correlations and trends described elsewhere by
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1053 338 other groups using a digital charged coupled camera. We have done so, though,
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1055 339 using a smartphone which served as an efficient, pragmatic and reliable means of
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340 acquiring the conjunctival images for subsequent analysis. Our system performed as
341 well as the more complex and time-consuming CCD devices and represents a
342 potential major advancement within the scope of conjunctival microcirculation
343 assessment. Our biomicroscope/smartphone apparatus and post-capture analysis is
344 validated by comparison to results obtained previously. We obtained a mean
345 diameter of 18.2 μ m (range 6.6-39.2 μ m) in 623 microvessels, selected manually
346 according to the quality of STI, on post-processed images and these results are
347 similar to, and within range, of that reported by other groups¹¹. The strong
348 positive/negative correlation between microvessel diameter (D) and blood flow (Q)/
349 wall shear rate (WSR), reported in the present work, is reflective of the dependence
350 of Q/WSR with increasing D, as represented in fluid dynamics formulae and
351 observations reported in other studies^{11, 12, 13}. We did not find as strong a correlation
352 for axial velocity (Va) and D ($r = 0.404$), but it is important to note that the calculation
353 of both D and Va, using our previously described methods, are entirely independent
354 of each other and that similar relationships between D and Va have been reported
355 previously^{12, 16}. Statistical significance, though, was observed for group-4 vessels
356 and their associated Va, compared to groups 1-3 ($p < 0.0001$).

357 Combined smartphone and slit-lamp based quantitative assessment has been
358 demonstrated in this present work and it is feasible that it could be of potential future
359 application in the assessment of cardiovascular health. We studied a “low-
360 cardiovascular risk” patient group, as evidenced by a mean QRISK 3 score of 6.6%.
361 QRISK 3 is a well-validated 10-year cardiovascular risk assessment, with the largest
362 sample size of contemporary cardiovascular estimation systems, implemented within
363 major European guidelines³⁰.

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364 We acknowledge certain limitations of our study. We, similar to other feasibility
365 studies¹³, have reported results for all visible microvessels without separating
366 arterioles and venules. The feasibility of artery-vein classification, using our
367 approach, in the conjunctiva requires further exploration, which we intend to pursue.
368 In addition, cardiac-gated haemodynamic measures, primarily end-systolic and end-
369 diastolic measures using conjunctival vessel pulse waveform characteristics, have
370 been reported previously and could be of potential use in future clinical application
371 with certain cardiovascular disease subsets³⁵. A key aim of our future work is to
372 implement and validate a fully automated smartphone-based approach to remove
373 potential human error, promote consistency, and improve the efficiency of the
374 examination. By quantifying the conjunctival haemodynamics our method potentially
375 allows the inexpensive assessment of patients with established cardiovascular and
376 systemic disease, with promise for improving the diagnosis, risk stratification and,
377 potentially, evaluating disease status and treatment modification of cardiovascular
378 disease(s). The addition of smartphone technology, with its application (APP)
379 versatility, wealth of data management, and computerised machine learning
380 algorithms, modernises the slit-lamp biomicroscope assessment of the conjunctival
381 microcirculation.

382 **V. Conclusion**

383
384 We have described, for the first time, the successful measurement of dynamic
385 microcirculatory haemodynamic measures using smartphone technology combined
386 with a slit-lamp biomicroscope. Our semi-automated method found a positive linear
387 relationship between increasing microvessel diameter (D) and blood flow (Q). An
388 inverse relationship was observed for wall shear rate WSR, a direct surrogate of

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389 WSS. These findings corroborate prior ones, for the same haemodynamic measures,
390 reported by groups using a CCD camera for image acquisition, and support the
391 feasibility of our smartphone-derived approach. Image acquisition was performed
392 without clinical complication in a group of patients with low cardiovascular risk. The
393 ease and speed with which images were reliably acquired holds promise for the
394 future clinical application of this smartphone-based conjunctival microcirculatory
395 assessment model.

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VII. Disclosure/Conflict of interests

403 The authors, collectively, have no conflicts of interest or anything to disclose with
404 respect to this original research manuscript.

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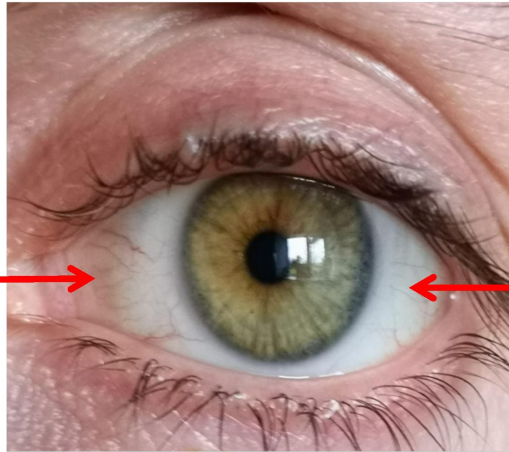
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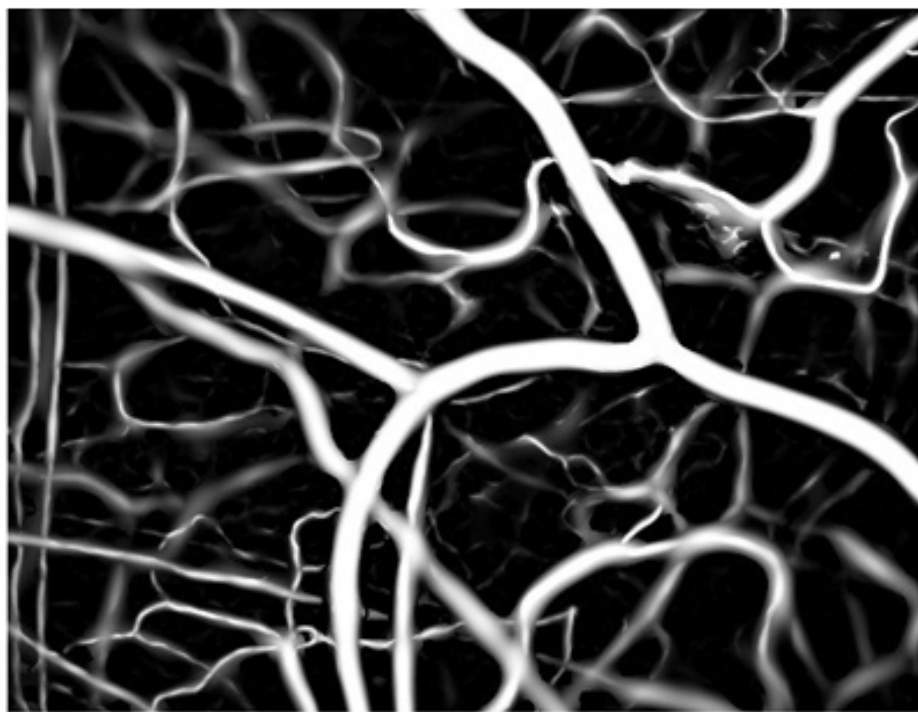
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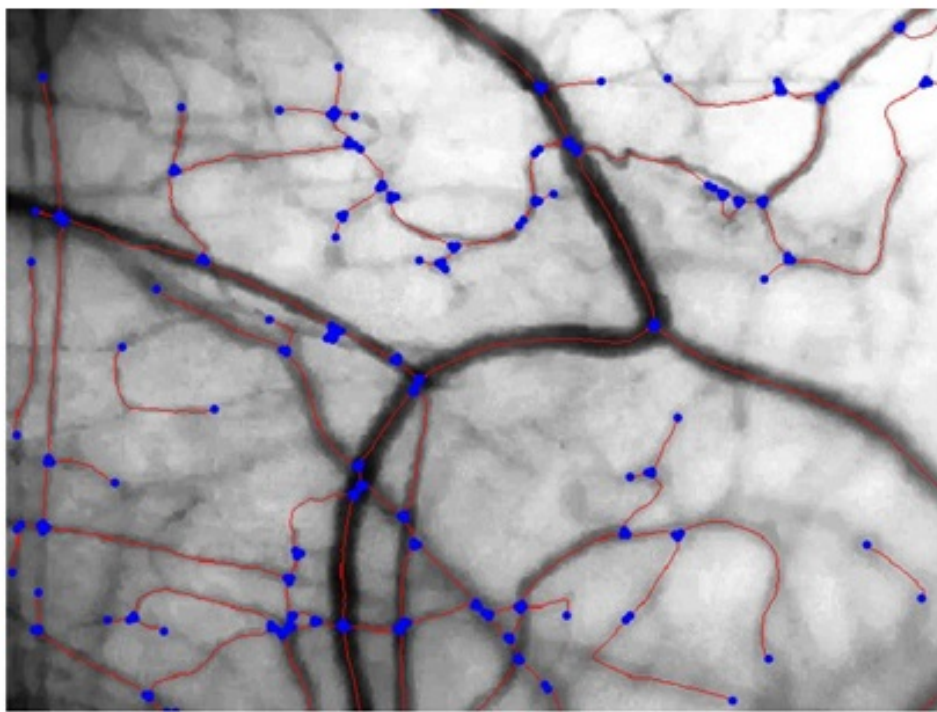
Left nasal (LN)



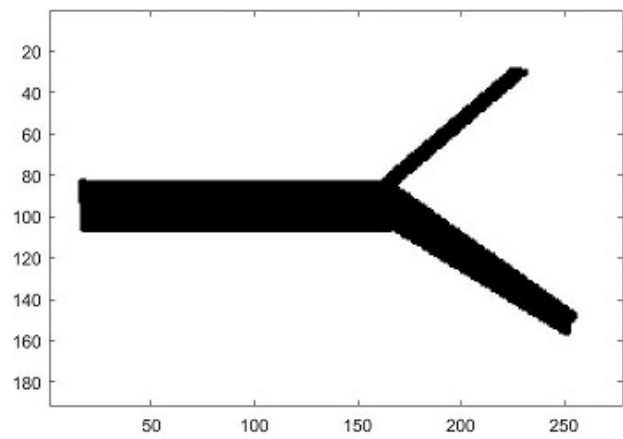
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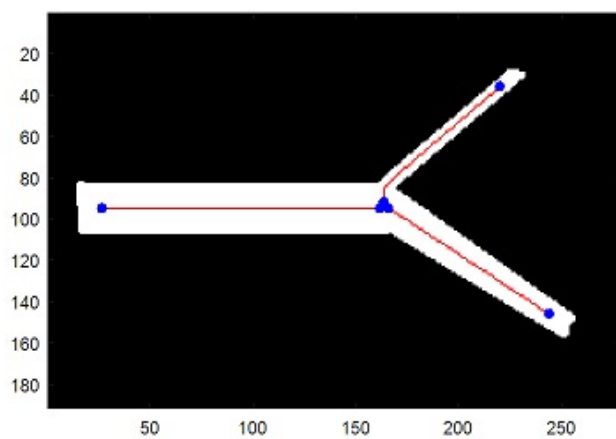
(a)



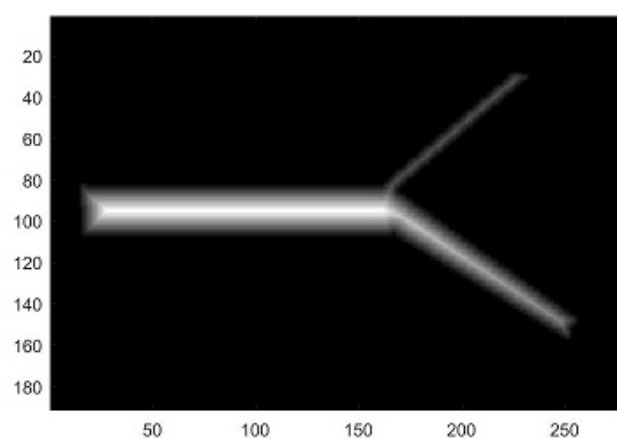
(b)



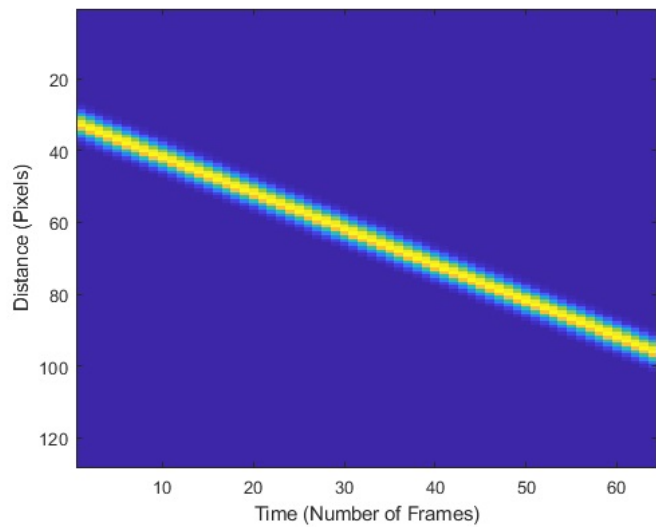
(a)



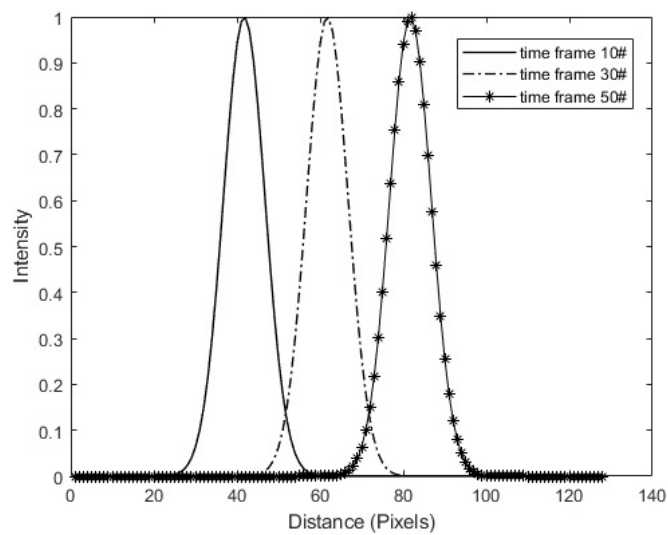
(b)



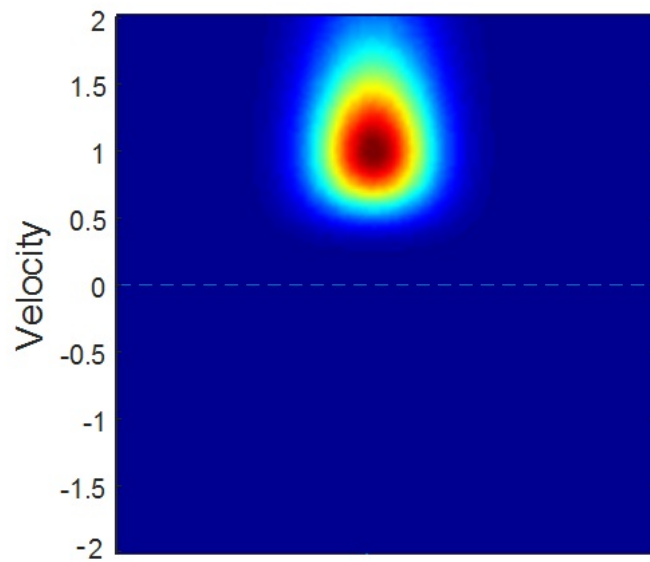
(c)



(a)



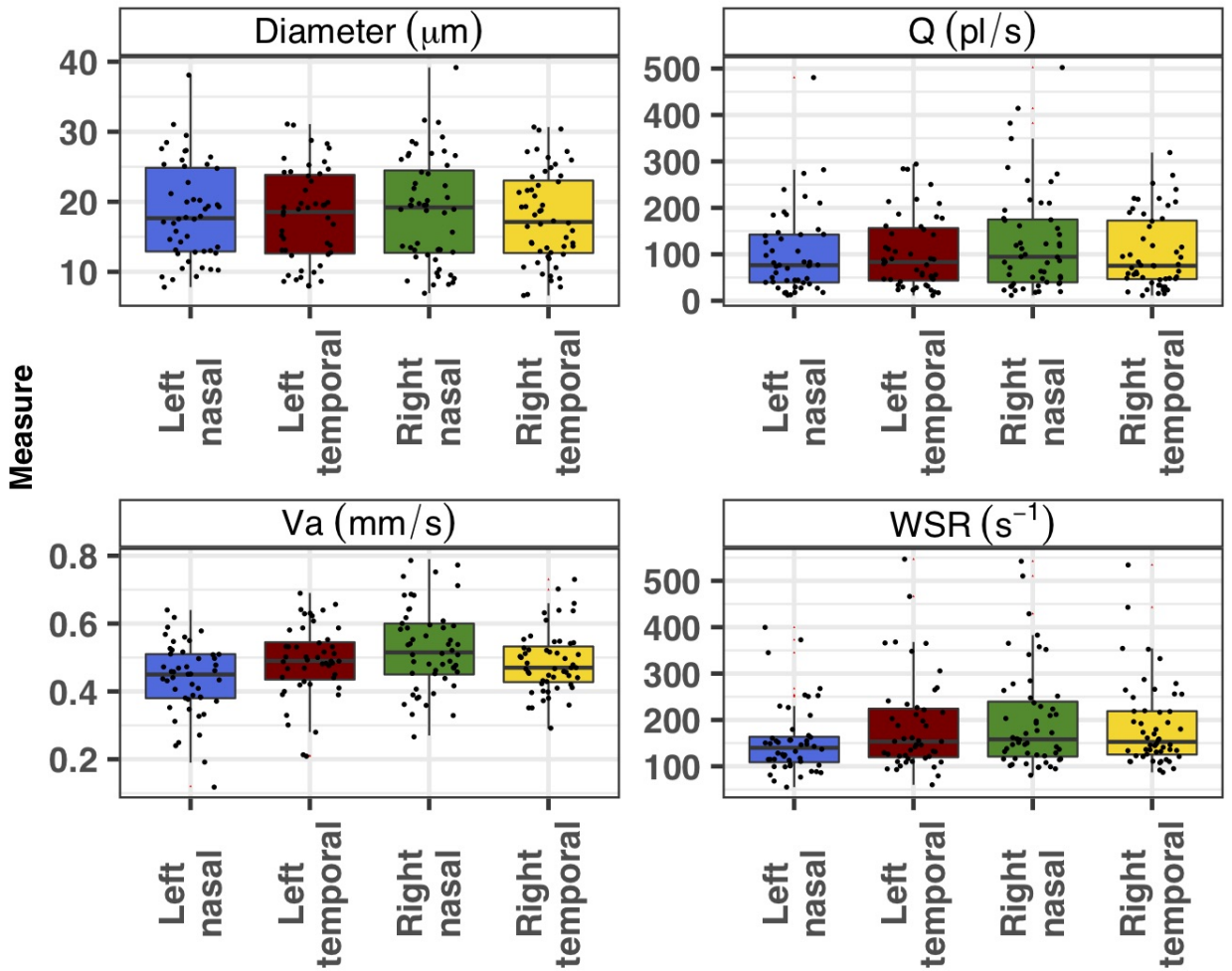
(b)



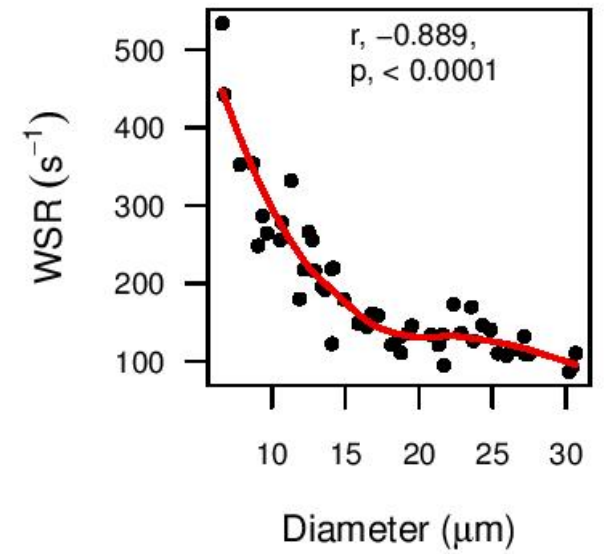
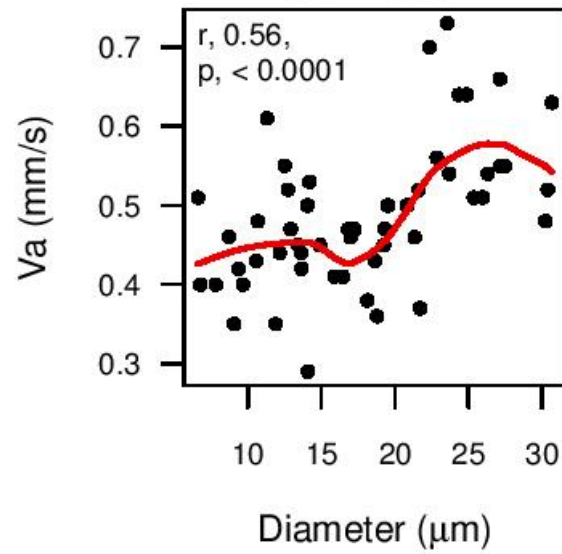
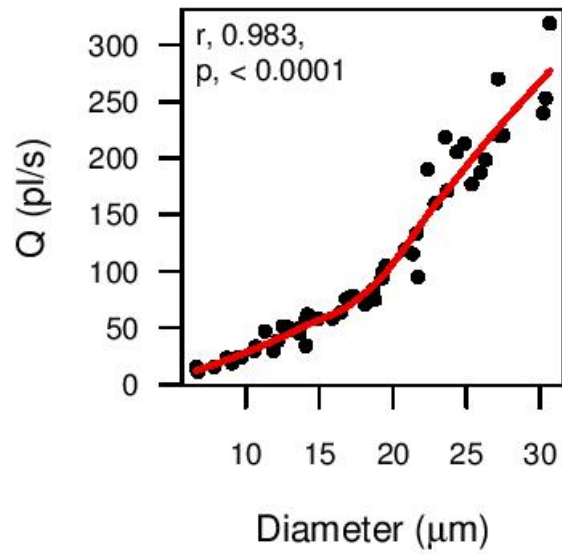
(c)

Diameter, Q, Va, and WSR across site

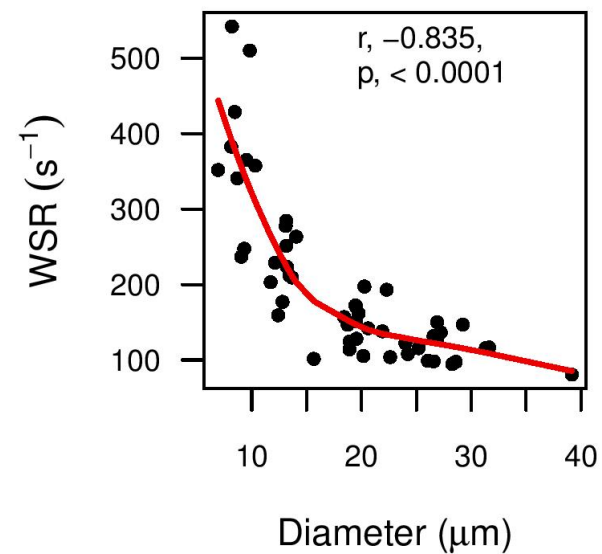
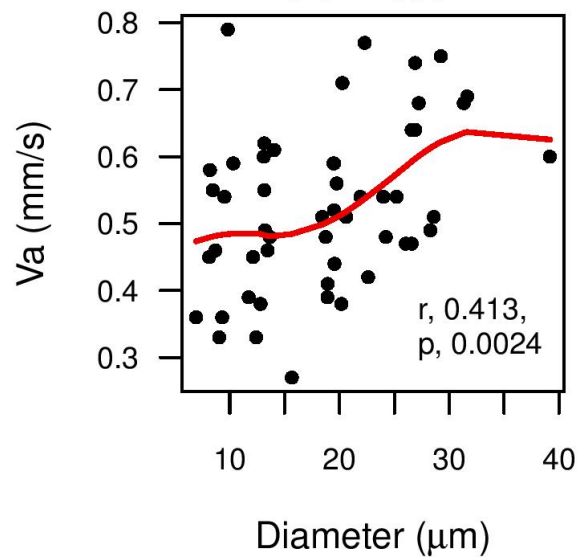
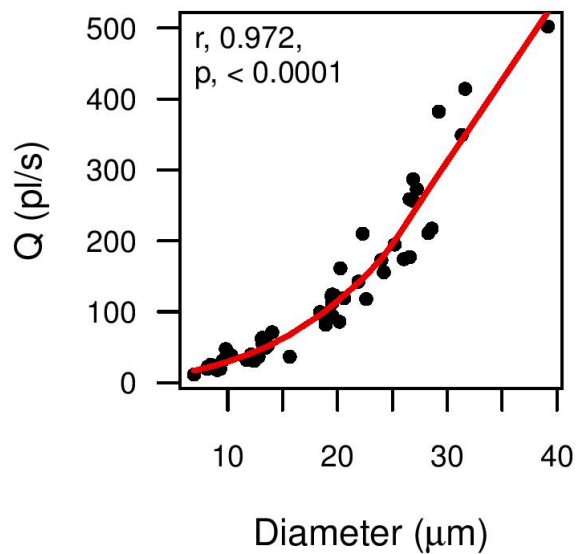
Site ■ Left nasal ■ Left temporal ■ Right nasal ■ Right temporal



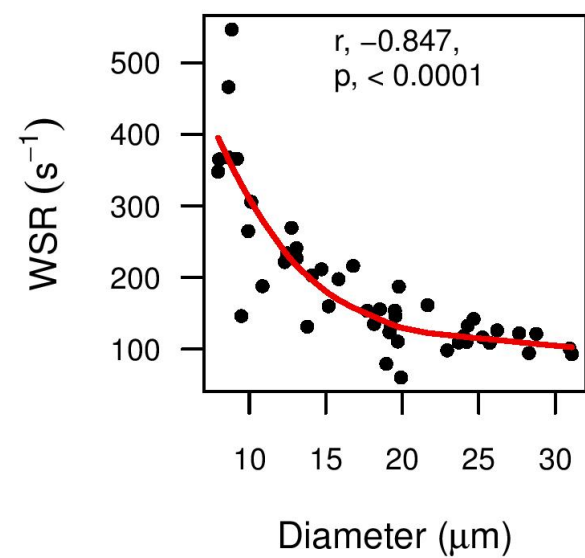
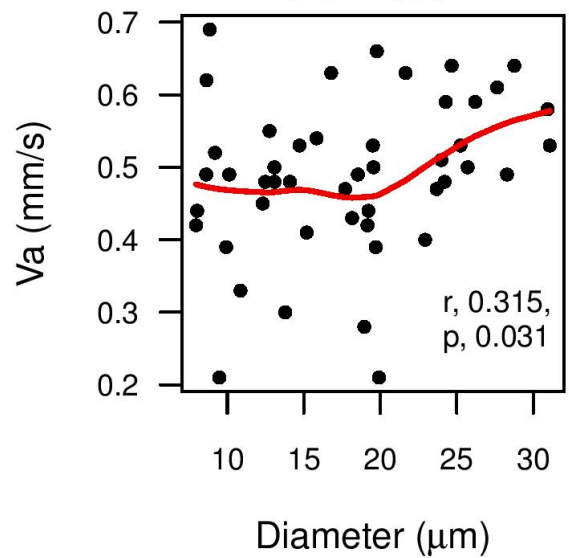
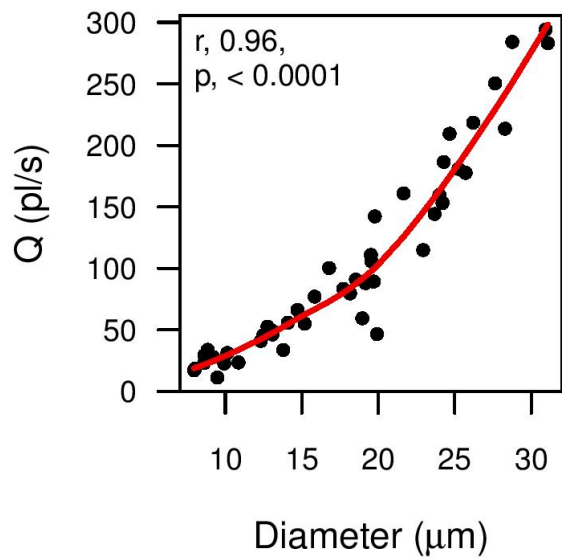
Right temporal, correlations to vessel diameter



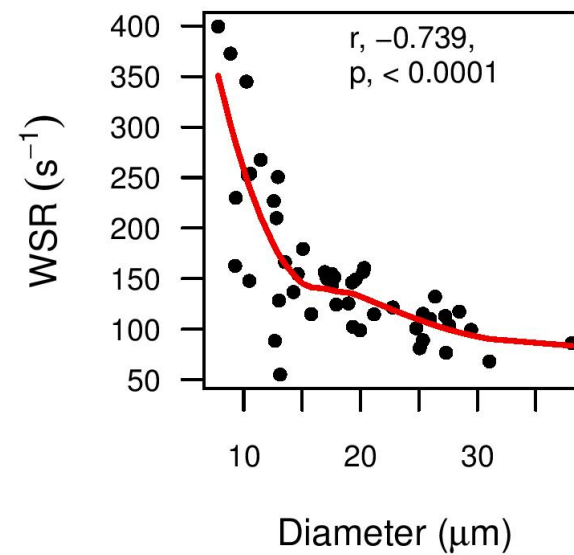
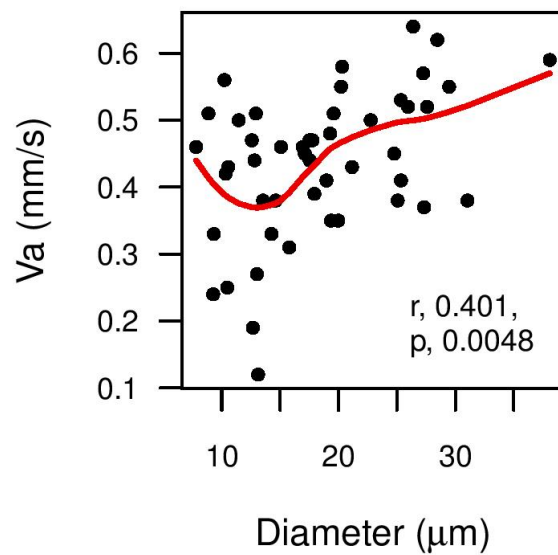
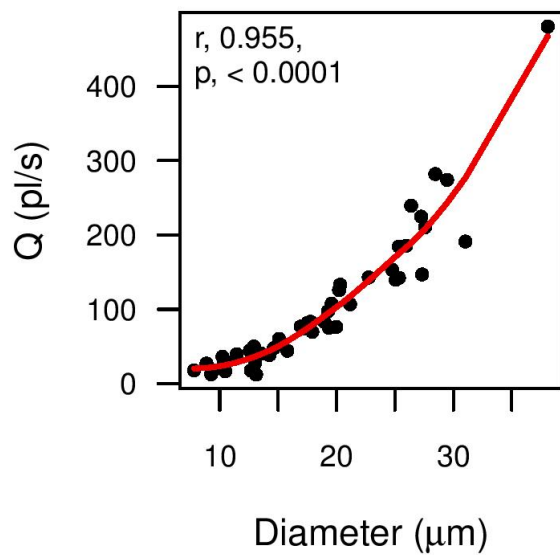
Right nasal, correlations to vessel diameter



Left temporal, correlations to vessel diameter



Left nasal, correlations to vessel diameter



**All sites,
correlations to vessel
diameter**

