Two-photon in-vivo microscopy of capillary blood flow in the kidney

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Dysregulation of capillary blood flow seems associated with several diseases, but the majority of measurements of capillary flow distributions have been performed in the brain or muscle capillary beds. From other organs, e.g., the kidney, there is a lack of empirical data on capillary blood distributions and lack of knowledge about the relationships between capillary blood distributions and disease states. We, therefore, aim to provide measurements of red blood cell (RBC) velocities in renal glomerular capillaries in vivo by using 2-photon microscopy. We further aim to investigate if and how the distribution of RBC velocities in renal glomerular capillaries is affected by STZ-induced diabetes and by ACE-inhibition in age-induced chronic kidney disease (CKD) in Münich Wistar Frömter rats.

These studies required us to establish a setup which both ensured an optically stable position, a physiologically healthy state of the animals and the ability to introduce fluorescent tracers (Setau647- and FITC-dextrans) to visualized capillary blood flow and renal filtration mechanisms.

Three types of image-data were recorded from each rat:

Z-stacks of each glomerulus, in which capillaries were scanned. These data were used as reference data to enable the identification of capillaries.

Linescans along the centerline of individual capillaries. The linescans were combined to xt-images data and analyzed by a structure-tensor method to estimate the velocity of red blood cell movement in the capillary.

Time-series of full frames spanning 30 mins after injection of a bolus of freely filtered FITC-4kD-dextran were used to estimate the glomerular filtration rate of the animal. Automatic detection of the vascular lumen in the frames (segmentation) was performed by machine-learning.

A total of 2582 xt-images (individual capillaries) from 253 glomeruli in 37 rats were analyzed.
The use of 2-photon in vivo microscopy enabled us to show, that the blood velocity in glomerular capillaries was less variable in diabetic rats compared to both untreated and acutely hyperglycemic control rats. Moreover, in old rats, inhibition of Angiotensin Converting Enzyme reduced the variability of blood velocities. In conclusion, the distribution of blood in the glomerular capillary bed is a hitherto undescribed parameter in renal physiology, which 2-photon in vivo microscopy has enabled us to identify and study.