Supplementary material for

Insect wing circulation: Transient perfusion through a microfluidic dragonfly forewing model

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I. Supplementary Figures

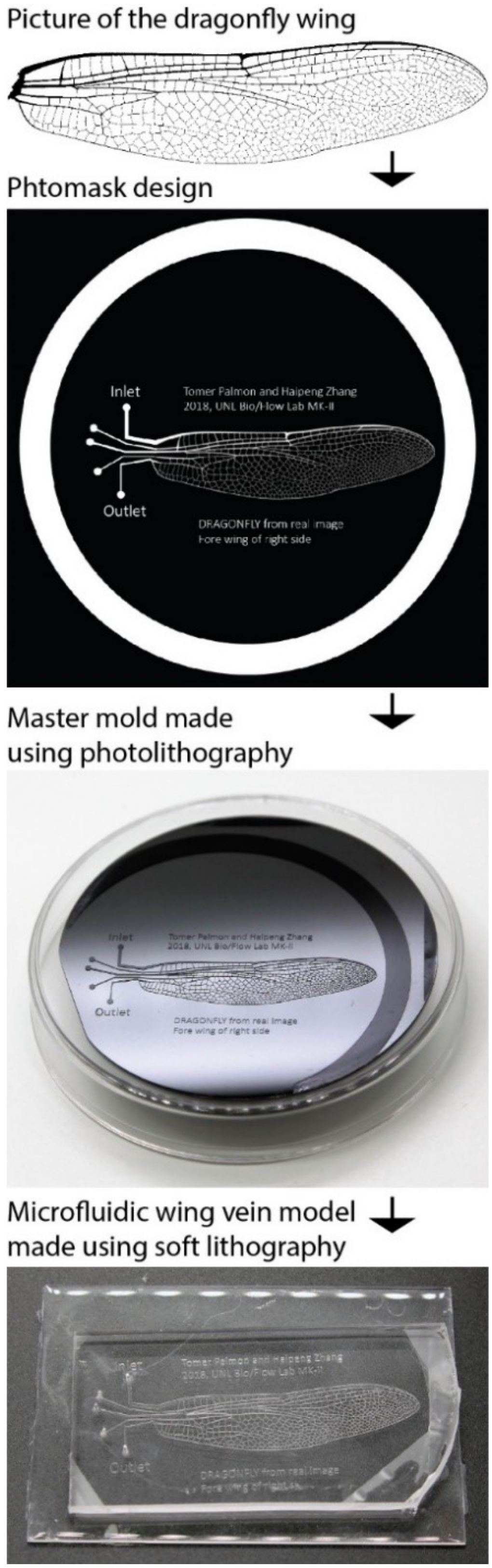

Figure S1. Fabrication procedure of the microfluidic dragonfly wing vein device.

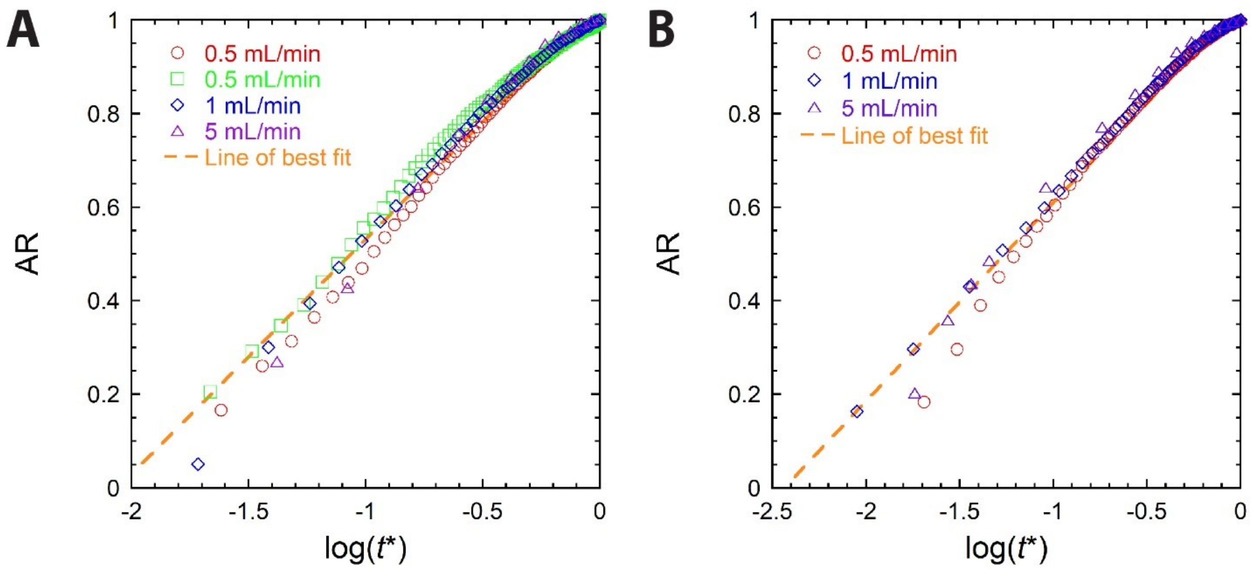

Figure S2. Line of best fit. (A) Dye injection through the inlet group. Line of best fit: $A = 1.033 + 0.502\log(t^{*})$, $R^2 = 0.983$, and $p = 1.91\times10^{-209}$. (B) Dye injection through the outlet group. Line of best fit: $A = 1.035 + 0.425\log(t^{*})$, $R^2 = 0.983$, and $p = 3.98\times10^{-150}$.

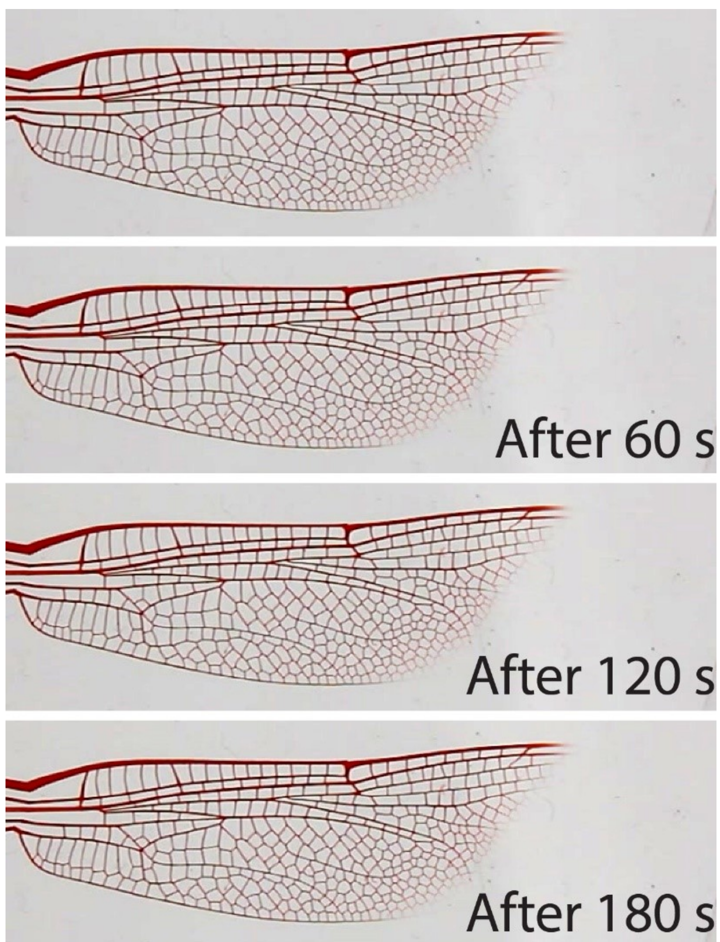

Figure S3. Diffusion of red dye into clear water at the dye-water front.

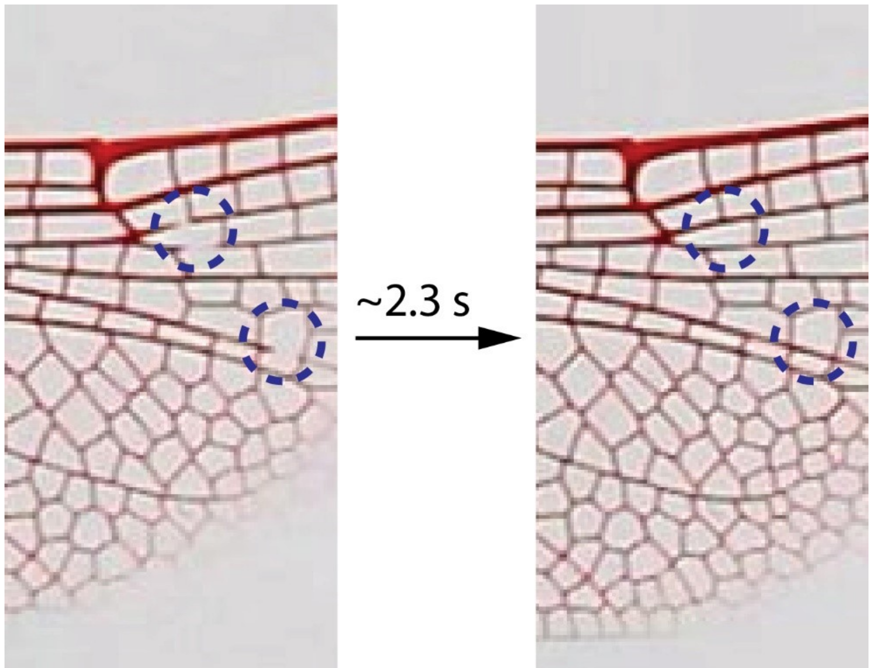

Figure S4. Disappearing water trapped by the dye.

II. Supplementary Videos

Video S1. Single port injection of red dye into Port 1: Q = 5 mL/min
Video S2. Single port injection of red dye into Port 2: Q = 5 mL/min
Video S3. Single port injection of red dye into Port 3: Q = 5 mL/min
Video S4. Single port injection of red dye into Port 4: Q = 5 mL/min
Video S5. Single port injection of red dye into Port 5: Q = 5 mL/min

Video S6. Grouped port injection of red dye into the inlet port group (Ports 1-3): Q = 0.5 mL/min
Video S7. Grouped port injection of red dye into the inlet port group (Ports 1-3): Q = 1 mL/min
Video S8. Grouped port injection of red dye into the inlet port group (Ports 1-3): Q = 5 mL/min

Video S9. Grouped port injection of red dye into the outlet port group (Ports 4-5): Q = 0.5 mL/min
Video S10. Grouped port injection of red dye into the outlet port group (Ports 4-5): Q = 1 mL/min
Video S11. Grouped port injection of red dye into the outlet port group (Ports 4-5): Q = 5 mL/min

Video S12. Grouped port injection of water into the inlet port group (Ports 1-3): Q = 1 mL/min

III. Supplementary Text

Theoretical model of dragonfly wing venation

A simple theoretical model for the wing venation of a dragonfly wing is shown in Figure S5(A). It consists of two horizontal veins, which represent the anterior and posterior veins, and two cross-veins connecting the horizontal veins. The length of these vein segments is *L* while the length of the inlet and outlet veins is *L*/2. The cross-sectional area of the vein is *A*, and hemolymph flows into the vein network through the inlet vein at a speed of *V*.

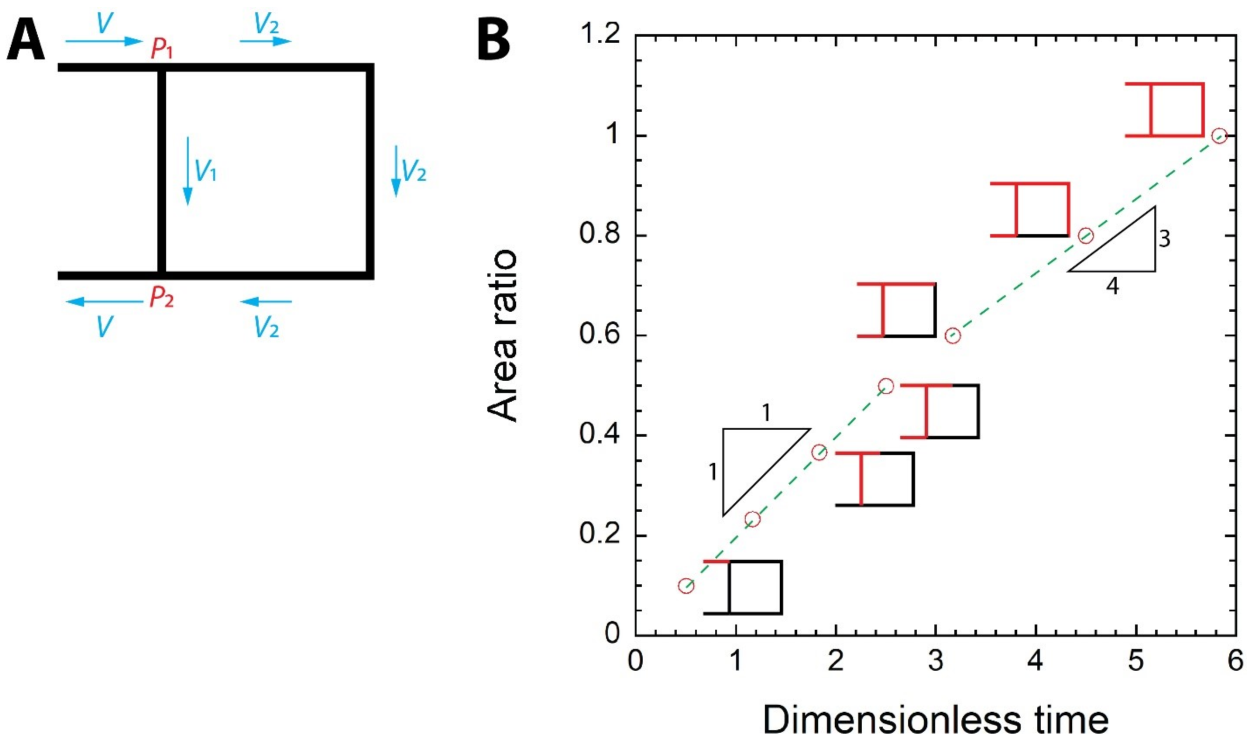

Figure S5. Theoretical models for a simple dragonfly wing vein network. (A) Model with two cross-veins. (B) Temporal increase in perfusion area ratio. Red indicates perfused venation.

Because the inner diameter of insect wing veins is small and hemolymph flows quite slowly through veins, laminar flow (i.e., the Hagen-Poiseuille flow) is assumed (see main text). The flow velocity through each vein segment is found as follows. From the conservation of mass, the following two relations are found:

$$V = V_1 + V_2 \tag{S1}$$

From the Hagen-Poiseuille flow, the following relations between pressure drop and flow speed are found:

$$P_1 - P_2 = \frac{8\pi\mu L V_1}{A} = \frac{8\pi\mu(3L)V_2}{A} \tag{S2}$$

From these equations, the flow speeds through veins are found to be:

$$V_1 = \frac{3}{4}V,\ V_2 = \frac{1}{4}V \tag{S3}$$

Assume that a red dye is introduced to the vein model while hemolymph is flowing through the vein network. The dye perfuses through the model as shown in Figure S5(B). Based on the found flow speeds, the total length of the perfused vein (l) is found as shown in Table S1. In this theoretical model, the area ratio of the perfused vein is found by dividing l with the total length of veins (i.e., $5L$), and time t can be normalized by being divided with L/V ($t^* = tV/L$). Figure S5(B) shows that area ratio (AR) increases roughly linearly proportional to dimensionless time. However, the slope (= dAR/dt) changes at $t^* = 5/2$ from 1 to 0.75 (=3/4) as shown by the green dashed lines. This slope change happened when the dye has perfused the inlet and outlet veins and the first vertical vein and then it begins to perfuse the rest of the vein network slowly.

Table S1. Total length of the perfused vein of the model with two cross-veins [Figure S5(A)]

t	$\frac{1}{2}\frac{L}{V}$	$\frac{7}{6}\frac{L}{V}$	$\frac{11}{6}\frac{L}{V}$	$\frac{5}{2}\frac{L}{V}$	$\frac{19}{6}\frac{L}{V}$	$\frac{9}{2}\frac{L}{V}$	$\frac{35}{6}\frac{L}{V}$
l	$\frac{1}{2}L$	$\frac{7}{6}L$	$\frac{11}{6}L$	$\frac{5}{2}L$	$3L$	$4L$	$5L$

After having noticed that perfused area begins to grow more slowly when the dye begins to fill the vein on the wing tip, we added one more cross-vein as shown in Figure S6.

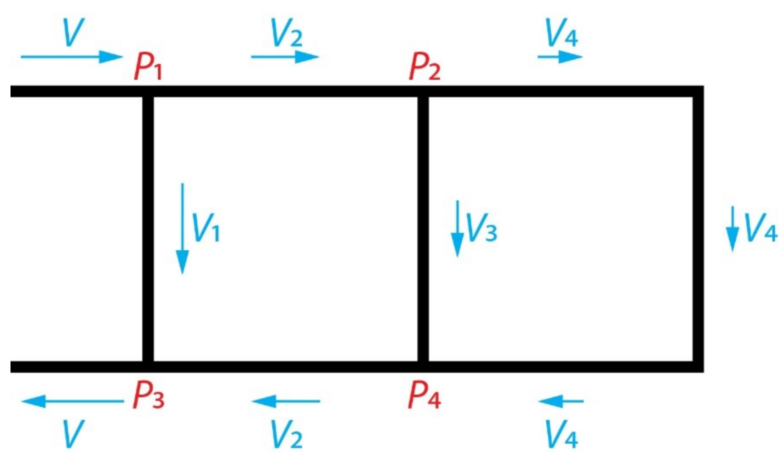

Figure S6. Theoretical models for dragonfly wing vein network with three cross-veins.

Thus, the theoretical model now consists of two horizontal veins and three cross-veins. The flow speed through each vein segment is found as follows. From the conservation of mass, the following two relations are found:

$$V = V_1 + V_2$$
$$V_2 = V_3 + V_4 \tag{S4}$$

Assuming Hagen-Poiseuille flow, the following relations between pressure drop and flow speed are found:

$$
\begin{aligned}
P_1 - P_3 &= \frac{8\pi\mu L V_1}{A} \\
P_1 - P_2 = P_4 - P_3 &= \frac{8\pi\mu L V_2}{A} \\
P_2 - P_4 &= \frac{8\pi\mu L V_3}{A} = \frac{8\pi\mu (3L) V_4}{A}
\end{aligned}
\tag{S5}
$$

From these equations, the flow speed through veins is found to be:

$$
V_1 = \frac{11}{15}V,\ V_2 = \frac{4}{15}V,\ V_3 = \frac{3}{15}V,\ V_4 = \frac{1}{15}V \tag{S6}
$$

Based on the found flow velocity, the total length of the perfused vein (l) is found as shown in Table S2. In the model with three cross-veins, AR increases proportional to log(t^*) as shown in Figure 6B.

Table S2. Total length of the perfused vein of the model with three cross-veins (Figure S6)

t	$\frac{1}{2}\frac{L}{V}$	$\frac{41}{22}\frac{L}{V}$	$\frac{26}{11}\frac{L}{V}$	$\frac{187}{44}\frac{L}{V}$	$\frac{407}{44}\frac{L}{V}$	$\frac{572}{44}\frac{L}{V}$	$\frac{2541}{132}\frac{L}{V}$	$\frac{4521}{132}\frac{L}{V}$	$\frac{6501}{132}\frac{L}{V}$
l	$\frac{1}{2}L$	$\frac{41}{22}L$	$\frac{802}{330}L$	$3L$	$\frac{13}{3}L$	$\frac{67}{12}L$	$6L$	$7L$	$8L$