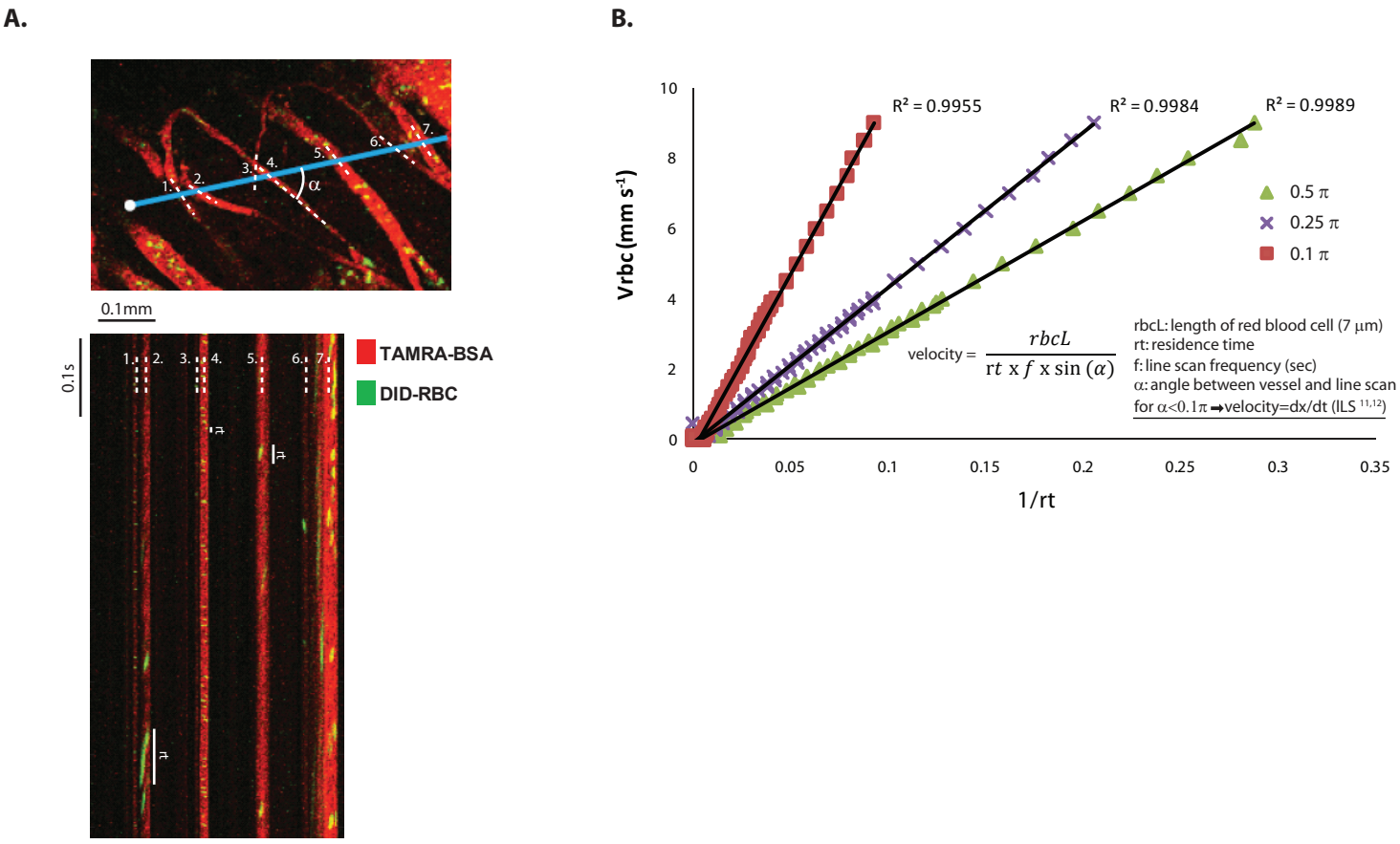
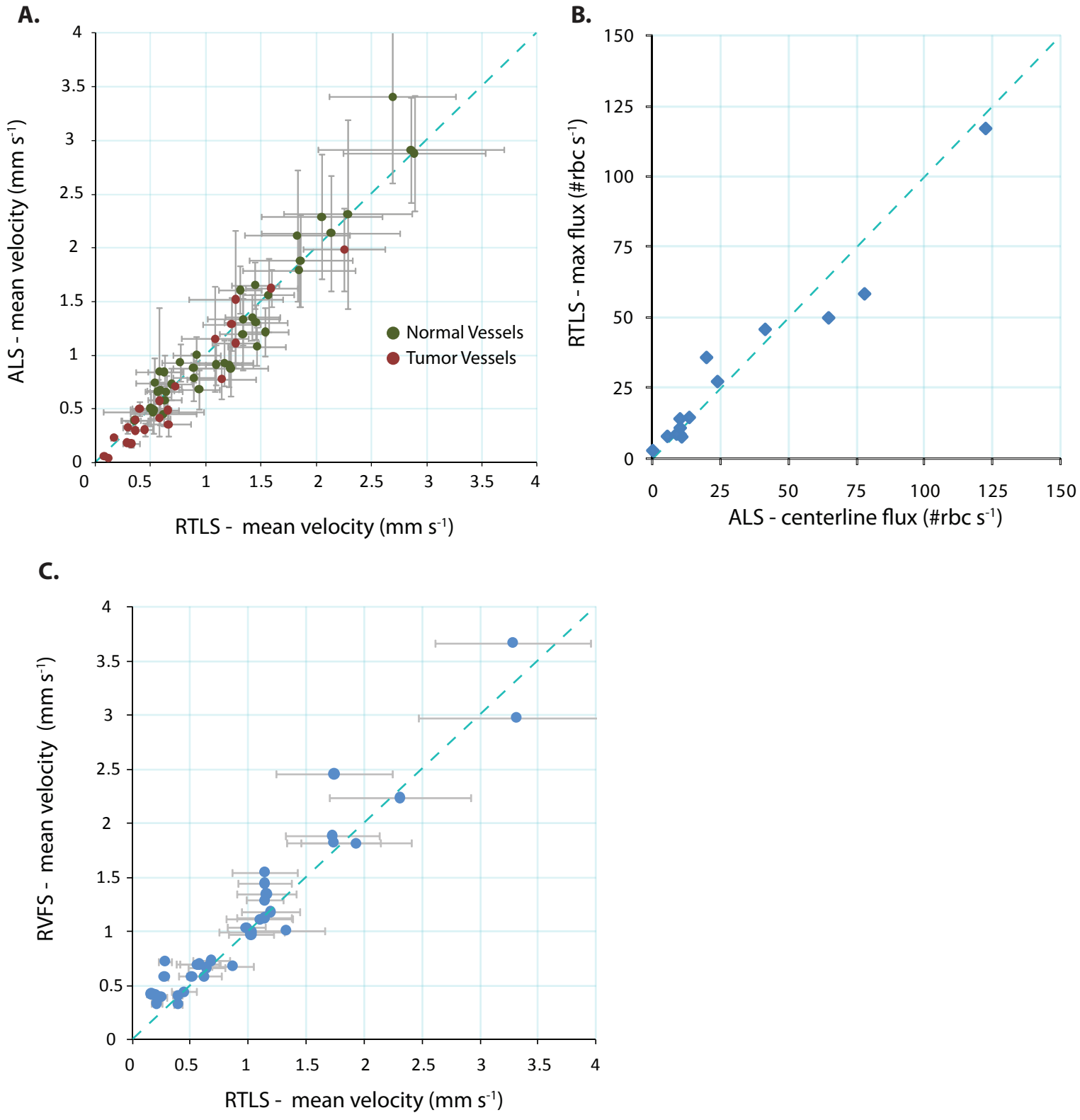
Supplementary Figure 1.



Supplementary Figure 1. Residence time line scan (RTLS) method description

(a) i. Representative MPLSM angiogram of a tumor blood vessel from a glioma model imaged through the cranial window visualized by injecting Rhodamine conjugated BSA and DiD labeled red blood cells. b. x vs t images showing residence time (rt) of several vessels simultaneously analyzed. (b) Lattice Boltzmann model was used to simulate RBC flow and validate the linear relationship between rt and red blood cell velocity. A single column of red blood cells flowing in a straight channel at a predetermined velocity was modeled. Line scanning at various angles intersecting the vessel from perpendicular (0.5 π) to almost parallel to the vessel wall (0.1 π) validated the linear relationship between 1/rt and RBC velocity.

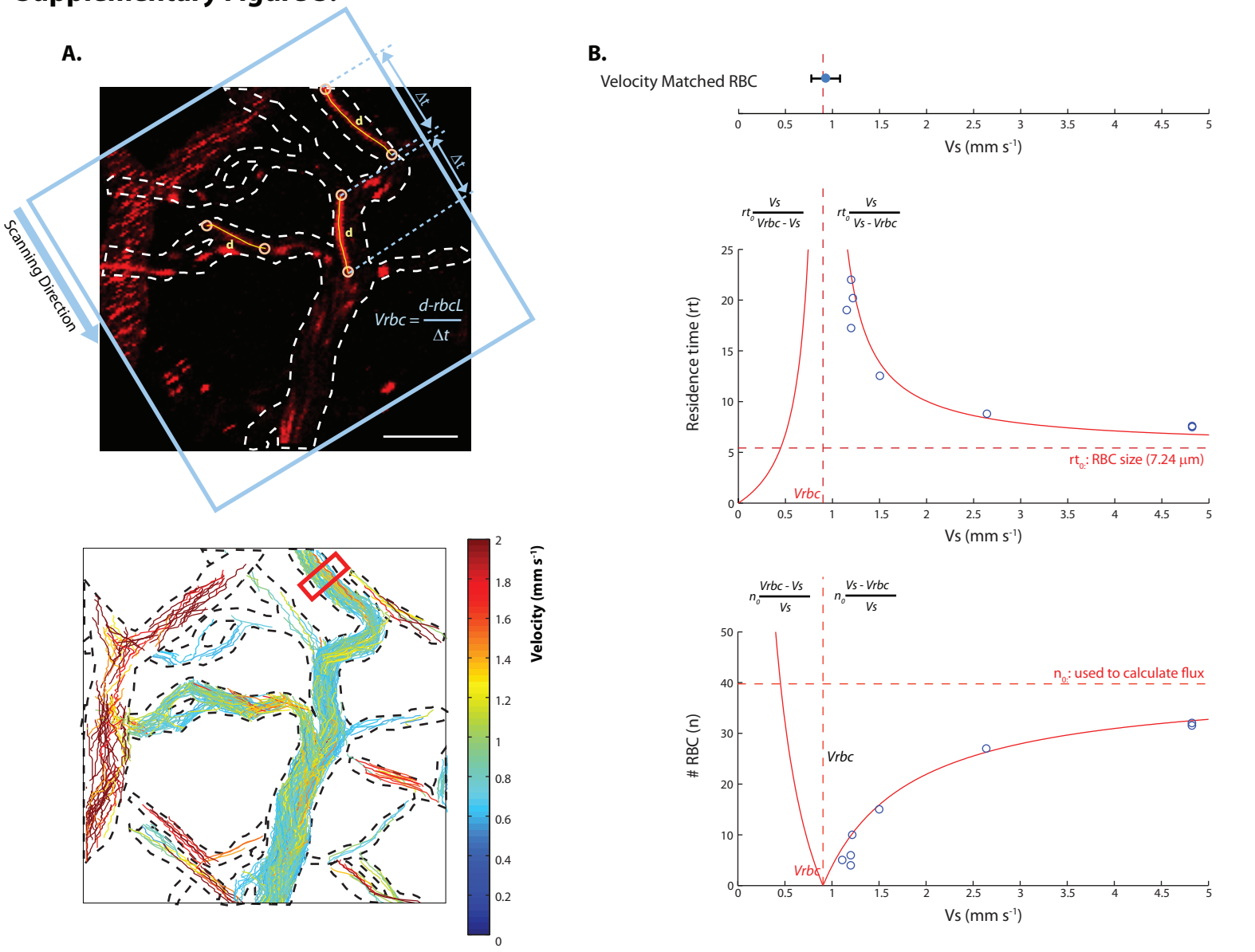
Supplementary Figure 2.



Supplementary Figure 2. RTLS & RVFS technique validation

(a) Comparison of RBC velocities (b) and fluxes measured by RTLS and ALS shows a significant correlation between both techniques ((a). mean ± s.d. (b) mean). (c) Comparison of RBC velocities measured by RTLS and RVFS shows correspondence between these techniques (for RTLS mean ± s.d.).

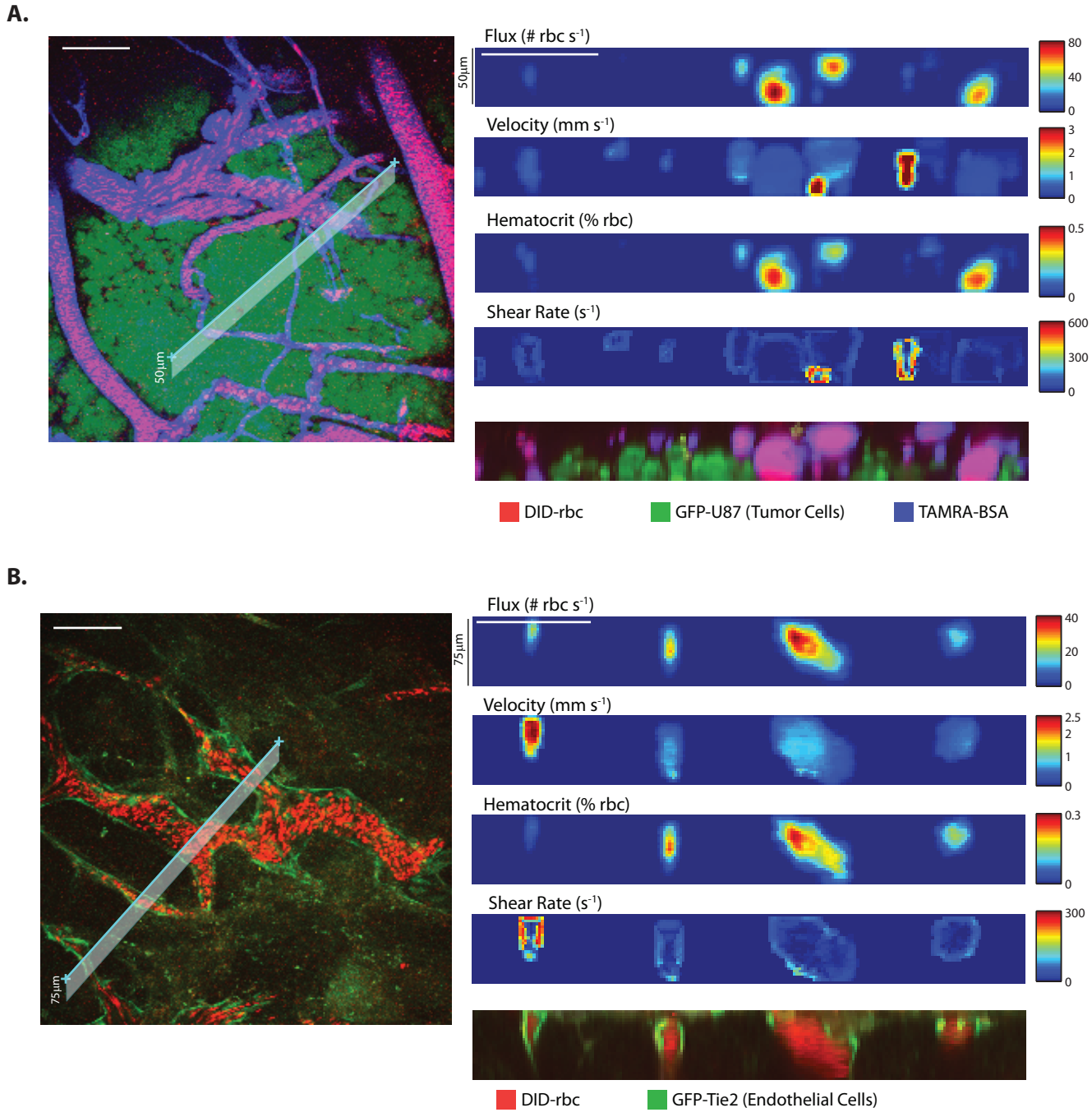
Supplementary Figure 3.



Supplementary Figure 3. Relative velocity field scanning (RVFS) method description

(a) Representative MPLSM image of brain vasculature imaged through the cranial window visualized by injecting DiD labeled red blood cells. Field was scanned at a 30 angle (Cyan arrow and field). "Velocity -matched RBC" (vmRBC) are shown, and traveled distance (d) and time (Δt) are highlighted (yellow line and cyan arrow). Velocity map generated by analyzing vmRBC tracks (color mapped lines). (b) Fitting the number of RBCs and residence times to scanning velocities for scans in the same direction as the flow allows computation of velocity (Vrbc) and number of RBC (n0) used to calculate flux (Equation derived from the Doppler effect). Vrbc computed from #RBC and residence times correlate with Vrbc computed from vmRBC. Scale bars, 50 μm (a)

Supplementary Figure 4.

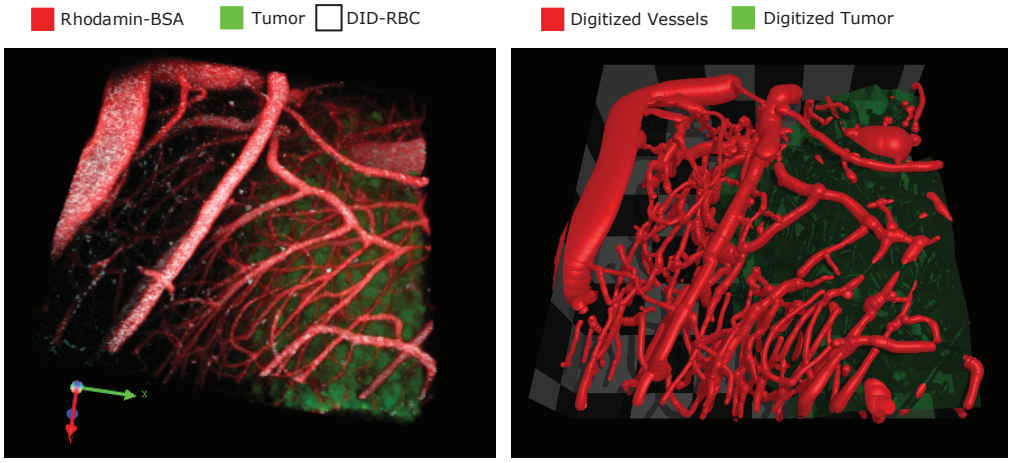


Supplementary Figure 4. Analysis of cross-sectional flow profiles within tumor vessels

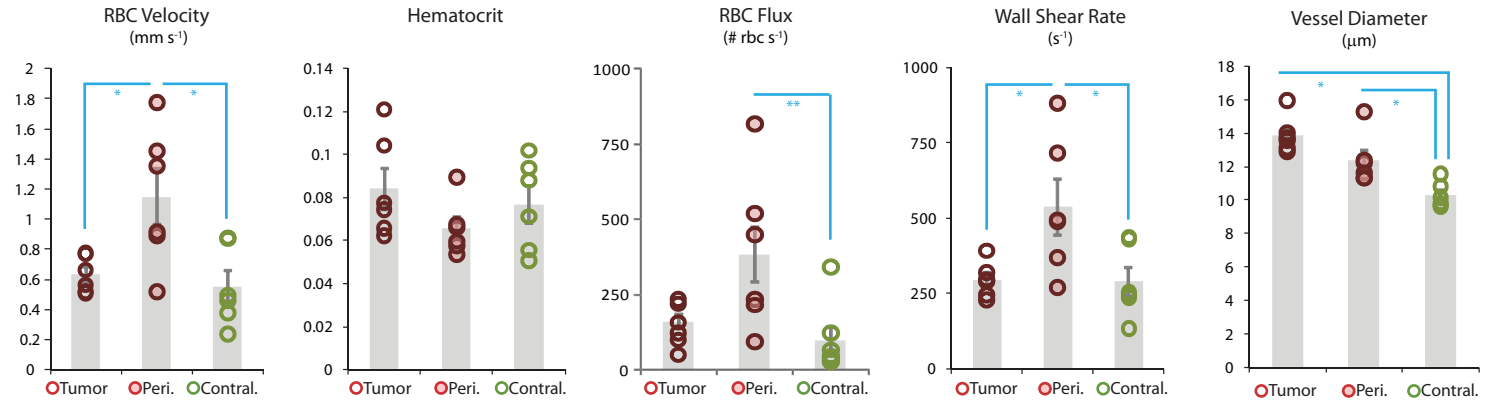
(a) Representative MPLSM angiogram of a glioma in which cross-sectional analysis of 3D flow profiles was performed along a specific plane (Light blue). Cross-sectional velocity, flux, hematocrit and raw data maps can be generated using RTLS. The location of each vessel (i.e., peritumor and tumor) is determined relative to eGFP expressing tumor cells. (b) MPLSM angiogram of a mammary carcinoma implanted in the mammary fat pad of a Tie2-GFP mouse. Cross-sectional velocity, flux, hematocrit and raw data maps generated using RTLS. Scale bars, 100 μm (a), 100 μm (b).

Supplementary Figure 5.

A.



B.



Supplementary Figure 5. Analysis of mean flow parameters to compare vessels within tumor, peritumor and contralateral brain regions in a glioma model

(a) MPLSM micrographs collected in a glioma model. Glioma cells expressed GFP (GFP-U87), allowing accurate localization of the tumor and determination of the tumor edge. (b) mean ± s.e.m. per animal (circles) and per experimental group (bar graph) of RBC Velocity, hematocrit, RBC flux, wall shear rate and vessel diameter in each region (* $P < 0.05$, $n = 6$).