

Two-photon fluorescence microscopy of collateral blood flow following photothrombotic stroke in rat neocortex

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Optical excitation of the dye rose bengal by tightly-focused 532-nm laser light induces clot formation in individual blood vessels in rat cortex. Changes in blood flow during and after clot formation are observed using two-photon microscopy. We find that flow reverses direction downstream from clotted arterioles, thereby maintaining tissue perfusion. ©2003 Optical Society of America

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Despite years of basic and clinical research, there are few effective treatments for stroke. Most research conducted to date has focused on physiological events that occur hours to days after an ischemic event [1]. Here we present a technique for real-time observation of changes in blood flow in rat cortex following localized clot formation in near-surface vessels. Using this technique, we demonstrate that downstream flow in arterioles does not stop after clot formation. Rather, flow is reestablished at the first downstream branch, albeit with reduced speed, with the flow direction reversed in one of the branches.

Craniotomies over parietal cortex were performed on 200 – 300-g animals anesthetized using urethane (1.5 mg/kg animal weight). The exposed cortex was covered with a thin glass window, allowing optical access to the cortical vasculature [2]. We induce clot formation by optical excitation of an intravenously injected photosensitizer, the fluorescein derivative rose bengal (4 mg/injection). Photoexcited rose bengal interacts with molecular oxygen to form singlet oxygen, which is known to damage fatty acids and proteins in the vessel endothelium, thereby triggering the natural clotting cascade and leading to vessel obstruction [3]. In this study, we excite rose bengal using 1-s duration, 0.5-Hz repetition-rate pulses of 100- μ W, 532-nm laser light, focused to a 10- μ m diameter spot on the vessel wall. Images are taken between pulses of 532-nm irradiation.

We use two-photon laser-scanning microscopy (TPLSM) of intravenously injected fluorescein to visualize blood flow during and after clot formation [2]. The fluorescein labels only the blood serum, so red blood cells and other solid blood constituents appear as dark objects in the fluorescence image. Red blood cell motion between the successive line scans that form the image leads to streaks that can be used to quantify the velocity. Clots are characterized by a non-fluorescent region of densely packed cells, frequently accompanied by a brightly fluorescent region of stalled flow upstream from the clot.

Figure 1 shows changes in blood flow during and after clot formation in an arteriole. The arrows in the figure indicate the direction of flow and the circle in Fig. 1a indicates the region irradiated with 532-nm laser light, beginning 30 s after the start of observation. At 300 s a clot begins to form (vessel region near * in Fig. 1b), and at 455 s the clot is complete, obstructing both the targeted vessel and the downstream branches (Xs in Fig. 1c). Later, and after the 532-nm irradiation is turned off, flow is restored in the downstream branches, with a reversed flow direction in the branch on the right (Fig. 1d). This flow pattern remained stable for over four hours. In all cases studied (13 clots in 6 rats, vessel diameters 20 – 50 μ m), downstream flow continues due to flow reversal at a downstream branch as shown in Fig. 1d. We find that the speed of red blood cells in the branches is roughly 45% (n = 4) of the speed of the original, unperturbed flow.

In conclusion, we have demonstrated a powerful model for the study of real-time changes in blood flow following the formation of a localized photothrombotic clot. Data obtained with this model has revealed the persistent nature of downstream tissue perfusion following clot formation in an arteriole.

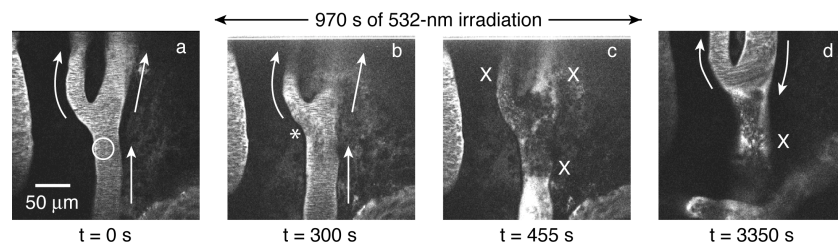


Fig. 1. Photothrombotic clotting dynamics in an arteriole observed by TPLSM. **a)** Image prior to rose bengal excitation. The circle indicates the region of the vessel that will be irradiated with 532-nm laser light and arrows indicate the direction of blood flow. **b)** A clot begins to form near the region indicated by *. **c)** The vessel is completely occluded (stalled flow indicated by X). **d)** Flow spontaneously restarts in branches downstream from the clot. Flow direction has reversed in the branch on the right.

1. P. Lipton, "Ischemic cell death in brain neurons," *Physiol Rev* **79**(4), 1431-1568 (1999).
2. D. Kleinfeld, P. P. Mitra, F. Helmchen, and W. Denk, "Fluctuations and stimulus-induced changes in blood flow observed in individual capillaries in layers 2 through 4 of rat neocortex," *Proc of the Nat Acad of Sci USA* **95**(26), 15741-15746 (1998). Errata: *PNAS* **96**, 8307 (1999).
3. B. D. Watson, W. D. Dietrich, R. Busto, M. S. Wachtel, and M. D. Ginsberg, "Induction of reproducible brain infarction by photochemically initiated thrombosis," *Ann Neurol* **17**(5), 497-504. (1985).