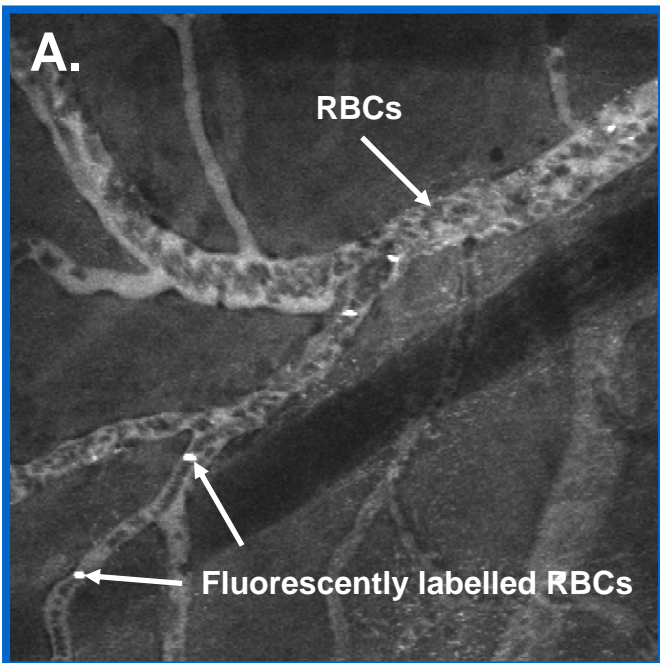
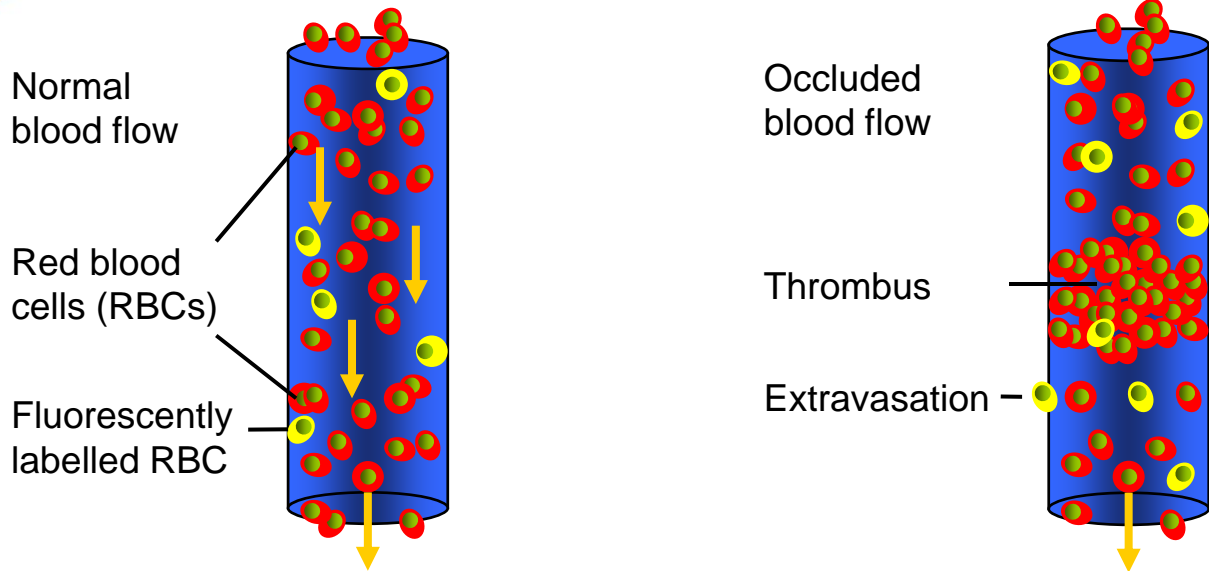
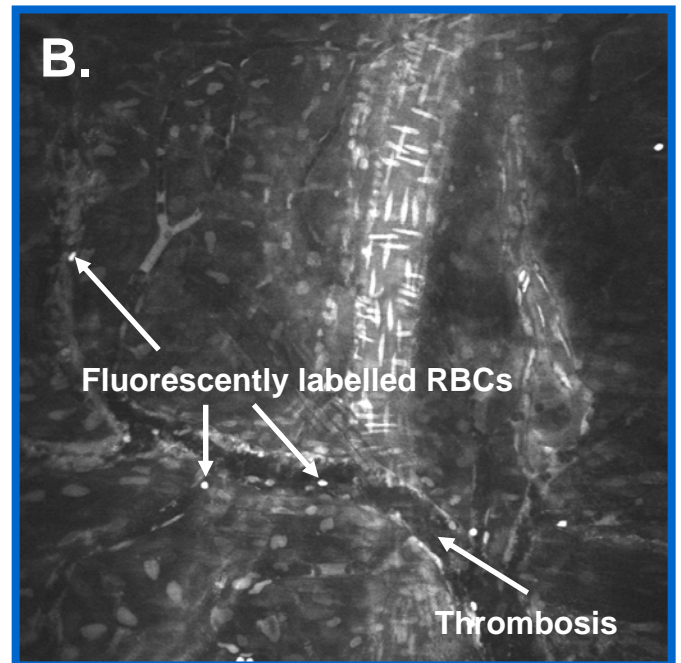


CELL TRACKING AND DETECTION OF THROMBOSIS



A tracer dye reveals the microvasculature, complete with red blood cells (RBCs) as dark circles. Fluorescently labelled cells are then administered and are seen as bright white circles moving through arterioles, capillaries and venules.



Microthrombosis observed directly in live brain. Loss of labelled plasma flow is accompanied by labelled blood cells (white) that slow or stop as they arrive at the site. Extravasation of labelled cells can also be seen in real time.

EXPERIMENTAL METHODS

Fluorescent labelling of RBCs: Fluorescein isothiocyanate (FITC)-labelling of erythrocytes was performed following blood harvesting from a sacrificed gerbil. The blood was centrifuged for 10 minutes. Buffy coat and plasma were removed and erythrocytes were washed in glucose-saline buffer. RBCs were incubated with 9 mg of FITC per ml blood for 2h and washed in buffer five times. Labelled RBCs were diluted to a hemocrit of 45% with normal saline and citrate-phosphate-dextrose for conservation.

Confocal Imaging: Animals were deeply anaesthetised by intraperitoneal injection of avertin (2.5%). The normal microvasculature (Figure A) and microthrombosis (Figure B) in a brain venule of a gerbil was examined after sequential injection of FITC-labelled erythrocytes (15 μ l of suspension injected per 10 g body weight), FITC-dextran (molecular weight 60 kDa and 150 kDa, 10 μ l/g of 25 mg/ml w/v solution; Sigma-Aldrich, Steinheim, Germany) and acriflavine hydrochloride (10 ml/g of a 0.1% solution). Confocal imaging was performed using 488 nm excitation (emission detection 505 – 550 nm). For brain imaging, the head was secured in a stereotactic frame and a cranial fenestration performed leaving the dura intact.

Unpublished image data courtesy of Martin Goetz and Ralf Kiesslich (University of Mainz, Germany).