Transcranial optical vascular imaging (TOVI) of cortical hemodynamics in mouse brain

Vyacheslav Kalchenko[1†], David Israeli[2†], Yuri Kuznetsov[1] and Alon Harmelin[1]*

[1]Department of Veterinary Resources, Weizmann Institute of Science, Rehovot, Israel

[2]Department of Veterinary Resources, Weizmann Institute of Science, Rehovot, Israel and Jerusalem Center for Mental Health, affiliated with the Hebrew University, Jerusalem, Israel

Supplementary Figure Legends

Supplementary Figure 1. Examples of raw and enhanced laser speckle images. (a,b) Raw LS images of the brain cortex of an untreated control mouse (a) and of a mouse with occlusion of the middle cerebral artery (MCAO). **(c,d)** Enhanced LS images of control (c) and MCAO (d) mice. Scale bars, 1 mm.

Supplementary Figure 2. The generation of FMIP images. Flowchart of the computation of filtered MIP image from a stack of raw DF images.

Supplementary Figure 3. The generation of HUE maps. Flowchart of the computation of HUE map from filtered subtracted DF images.

Supplementary Figure 4. The generation of IHS and RGB images. Flowchart of the computation of IHS and RGB images from FMIP and HUE map images.

Supplementary Figure 5. The generation of IHS and RGB images. Flowchart of the computation of IHS and RGB images from LS and FMIP images.

Supplementary Figure 6. The pathology seen in the mice following the induced “acute ischemic event " by carotid ligation, (In serial sections of the brains): There is only one area, in the subarachnoid space with notable pathology. As shown in the figure there is a fibrin thrombus within the cerebral vein at the base of the frontal lobe. There is some hemorrhage at the base of the brain which is most pronounced in the area with the fibrin clump.

Supplementary Movies

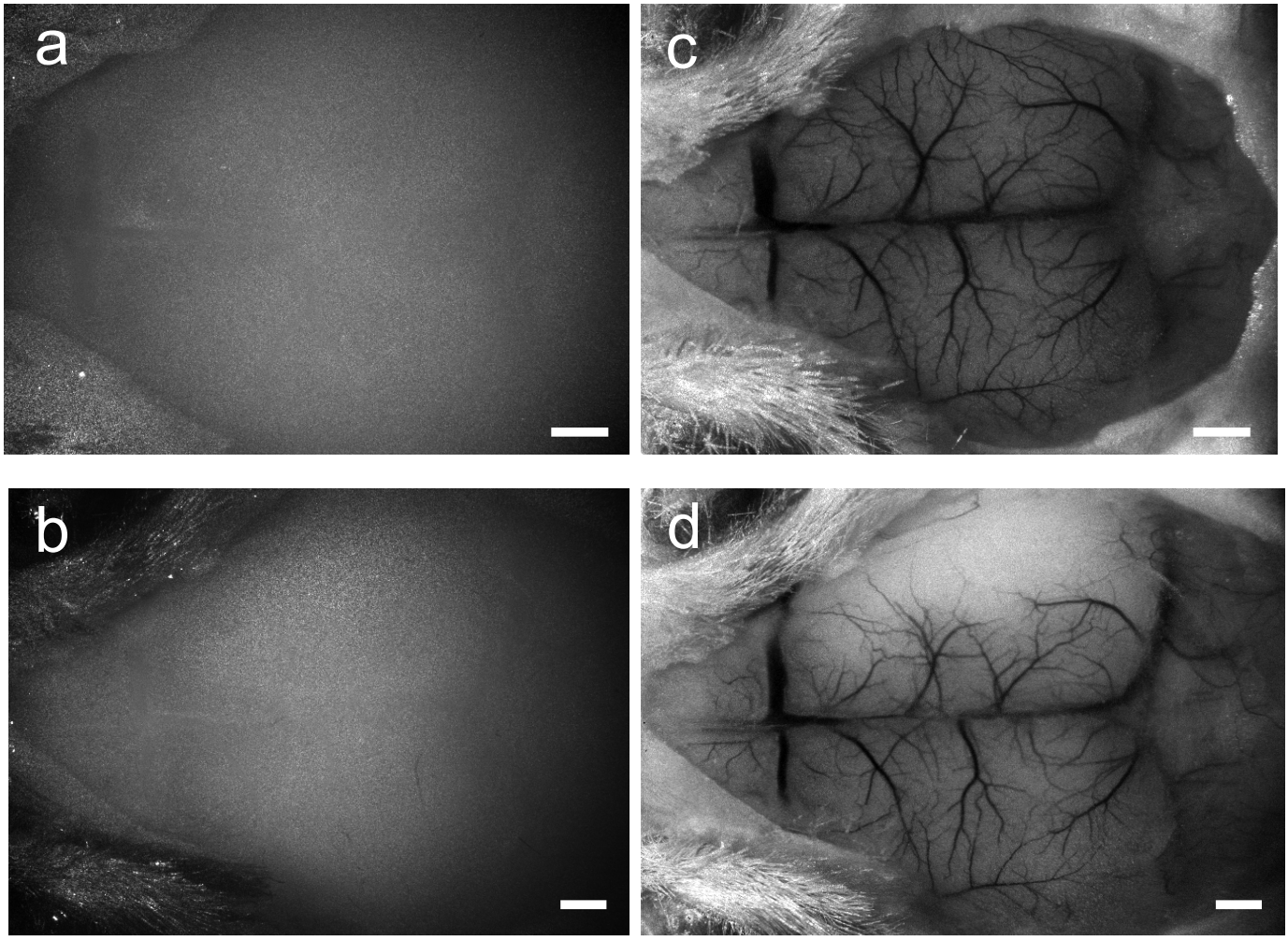
Supplementary Movie 1

Real-time video sequence of dynamic fluorescence images showing cortical blood flow in an untreated control mouse. The images were taken during a 20 seconds interval after fluorescein administration into the tail vein. The movie is played back at the same frame rate at which the images were taken.

Supplementary Movie 2

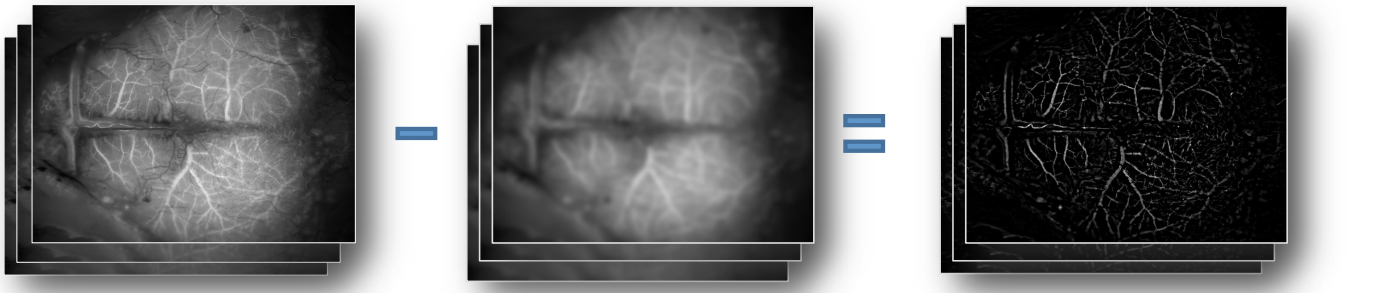
Real-time video sequence of dynamic fluorescence images showing cortical blood flow following occlusion of the middle cerebral artery (MCAO). The images were taken during a 20 seconds interval after fluorescein administration into the tail vein. The movie is played back at the same frame rate at which the images were taken.

Supplementary Figure S1



Supplementary Figure S2

1. Fiji: Process/Filters/ Gaussian Blur/Sigma (Radius) 5
2. Fiji: Process/Image Calculator/(Raw DF) subtract (Blurred DF)



Stack of raw DF images

Stack of blurred DF images (σ=5)

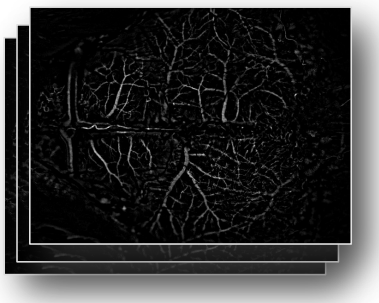
Stack of subtracted DF images

3. Fiji: Image/Stacks/Z Project/ Max Intensity

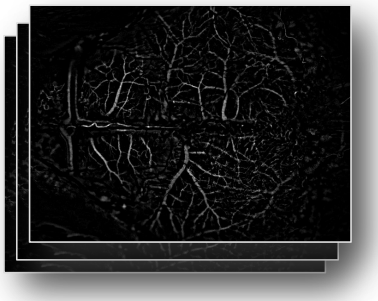
FMIP

Supplementary Figure S3

Fiji: Process/Filters/ Gaussian Blur 3D/X,Y sigma (0), Z sigma (3)



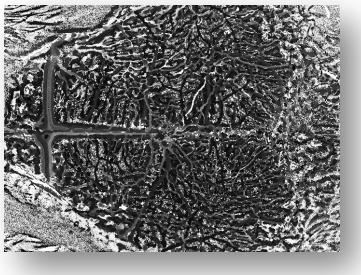
Temporal Gaussian blurring



Subtracted DF images

Temporal filtered (blurred) subtracted DF images

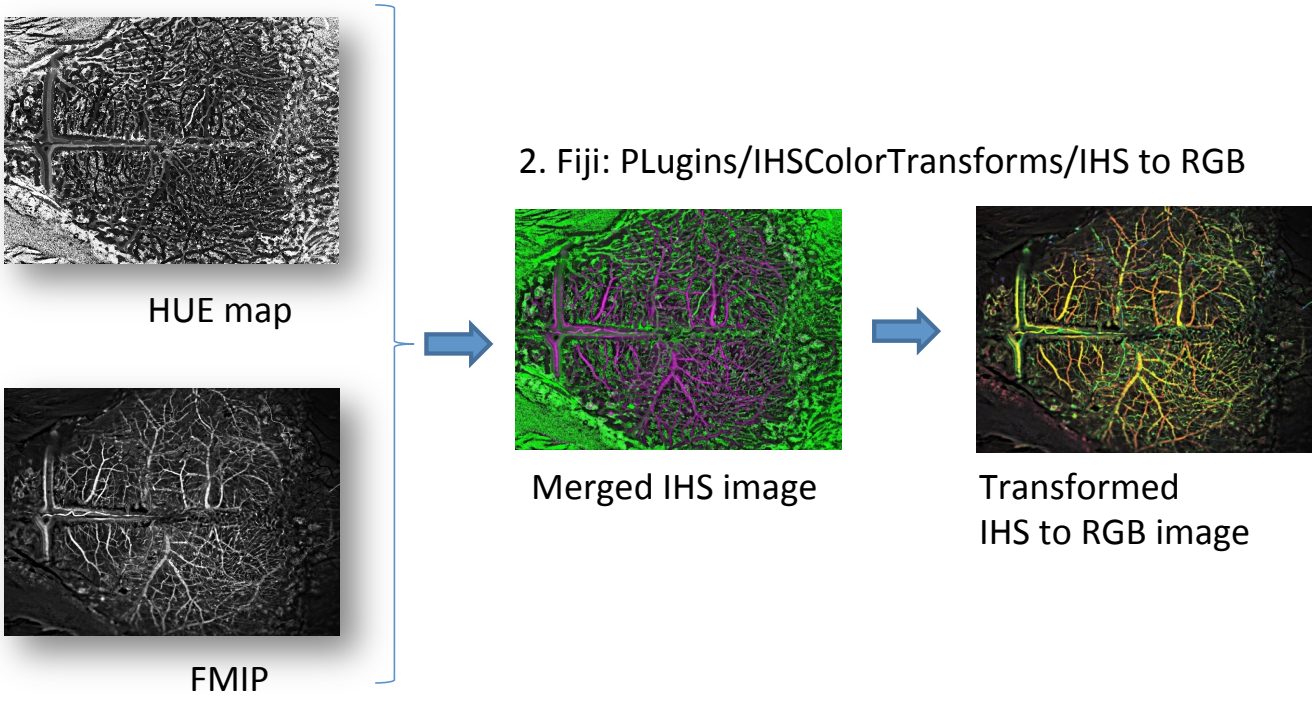
Fiji: Plugins/DF/open file (Filtered subtracted DF images)



HUE map

Supplementary Figure S4

1. Fiji: Image/Color/Merge channels/
   C1(red) – FMIP, C2(green) – HUE map, C3(blue) – FMIP



Supplementary Figure S5

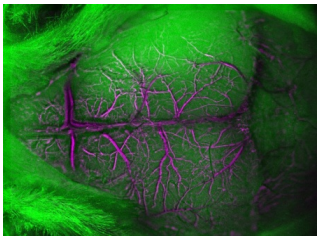
1. Fiji: Image/Color/Merge channels/
   C1(red) – FMIP, C2(green) – LS, C3(blue) – FMIP

LS

FMIP

2. Fiji: Plugins/Align RGB Planes

3. Fiji: PLugins/IHSColorTransforms/IHS to RGB

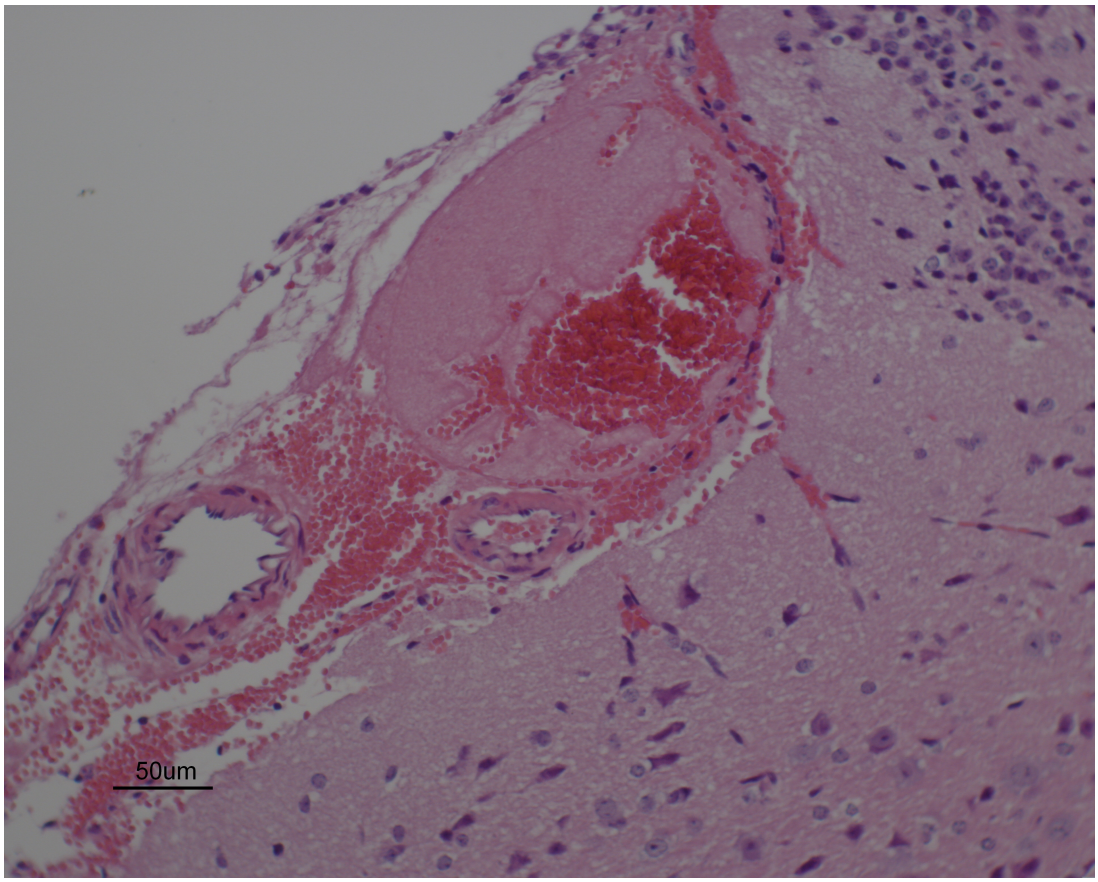


Merged IHS image

Transformed
IHS to RGB image

Supplementary Figure S6

Subarachnoid space fibrin Thrombus with hemorrhage within a vein at the base of the frontal lobe.



Supplementary Note 1

Macro for Fiji for computation of LS image from raw LS image stack.

```
//*********************************************
// LS Plugin v1.04 Feb 2014
// Laser Speckle Imaging
// Integration = 10 frames
// Vyacheslav (Slava) Kalchenko, MD, PhD, FRMS
// In Vivo Optical Imaging Unit
// Department of Veterinary Resources
// Weizmann Institute of Science
// email: a.kalchenko@weizmann.ac.il
//*********************************************

name = getTitle;
Raw=getImageID();

setBatchMode(true);

run("Grouped Z Project...", "projection=[Standard Deviation] group=10");
Sd=getImageID();

selectImage(Raw);
run("Grouped Z Project...", "projection=[Average Intensity] group=10");
Mean=getImageID();
selectImage(Raw);
close();

imageCalculator("Divide create 32-bit stack", Sd, Mean);//
Rstack=getImageID();
selectImage(Rstack);
run("Z Project...", "start=1 stop=1000 projection=[Average Intensity]");
Result1=getImageID();
selectImage(Rstack);
close();
selectImage(Mean);
close();
selectImage(Sd);
close();
selectImage(Result1);
rename ("LSI" + "_" + name);
setBatchMode(false);
```

Supplementary Note 2

Macro for Fiji for computation of HUE map image from enhanced subtracted DF image stack.

```
//------ Prototype--------
//TCM Time Color Maping - FAST 11.11.13
//Part of "TOVI Project"
//Currently this programm produces HUE map for IHS or HSV color space
//hue = (index of the image of the maximum value for the pixel)/(images per hue cycle)
//Author: Vyacheslav (Slava) Kalchenko, MD, PhD, FRMS
// In Vivo Optical Imaging Unit
// Department of Veterinary Resources
// Weizmann Institute of Science
// email: a.kalchenko@weizmann.ac.il
setBatchMode(true);
path = File.openDialog("Select a File");
open(path);
scr=getImageID();
Stack.getStatistics(voxelCount, mean, min, max, stdDev);
run("32-bit");
run("Divide...", "value="+max);
y=getHeight();
x=getWidth();
newImage("hue", "32-bit black", x, y, 1);
hue=getImageID();//New image for Hue
progress=0;
```

```
step=1/(x*y);
for(j=0;j<y;j++) {
	for (i=0;i<x;i++) {
		progress=progress+step;
		showProgress(progress);
//_________________ find HUE value <h>_________
map=0;
h=0; //first Hue parameter
selectImage(scr);
for(s=1;s<nSlices;s++){
	setSlice(s);
	ps=getPixel(i,j); //_____current pixel
	if (map==ps){h=s;}   // index of maximal value <<h>>
		if (map<ps){map=ps;}// find maximum

}
//_________________end find HUE value <h>_________

hn=(h/nSlices); //----< hn >----- normalisation on number of frames
selectImage(hue);
setPixel(i,j,hn);
	}
}
setBatchMode(false);
```