What did we learn about microcirculation using lasers

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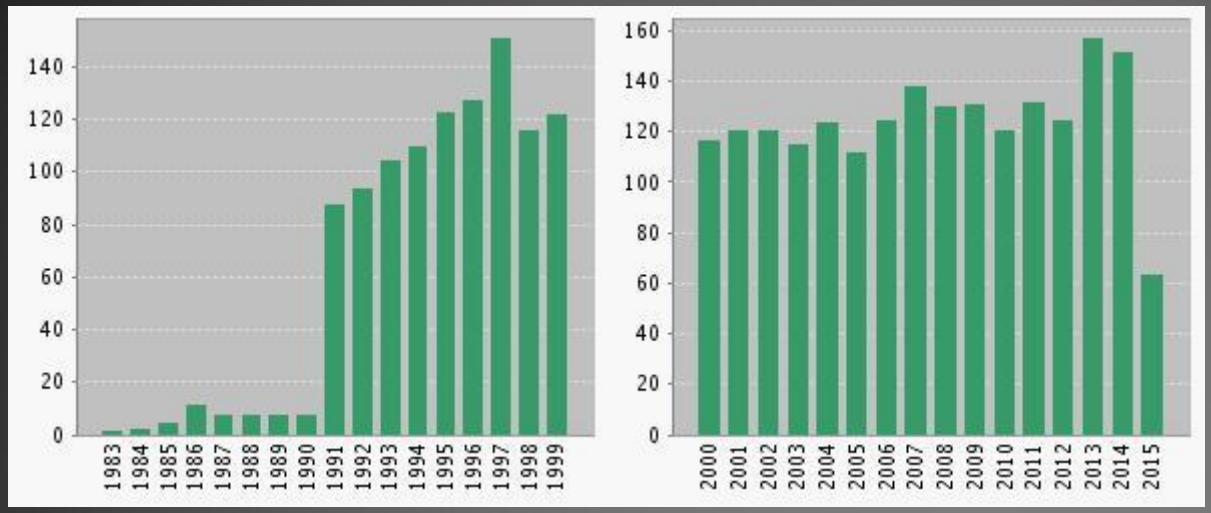
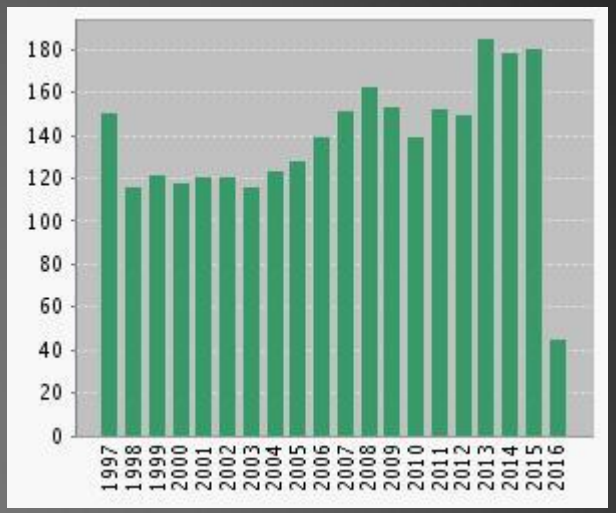
Faculty of Medicine

Department of Medical Phyics & Informatics

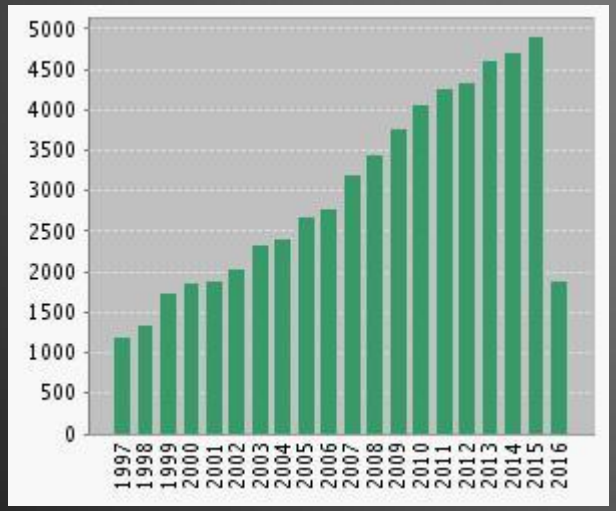
lamelis2016, Szeged, July 2

Laser AND microcirculation (mainly laser Doppler)





Citation to „Laser AND microcirculation”



Rapid Communication
Journal of Cerebral Blood Flow & Metabolism (2001) **21,** 195–201;

Dynamic Imaging of Cerebral Blood Flow Using Laser Speckle

Supported by NIH Interdepartmental Stroke Program Project, 5 P50 NS10828 (M.A.M.) and NIH 1 R29 NS38842 A 01 (D.A.B.).
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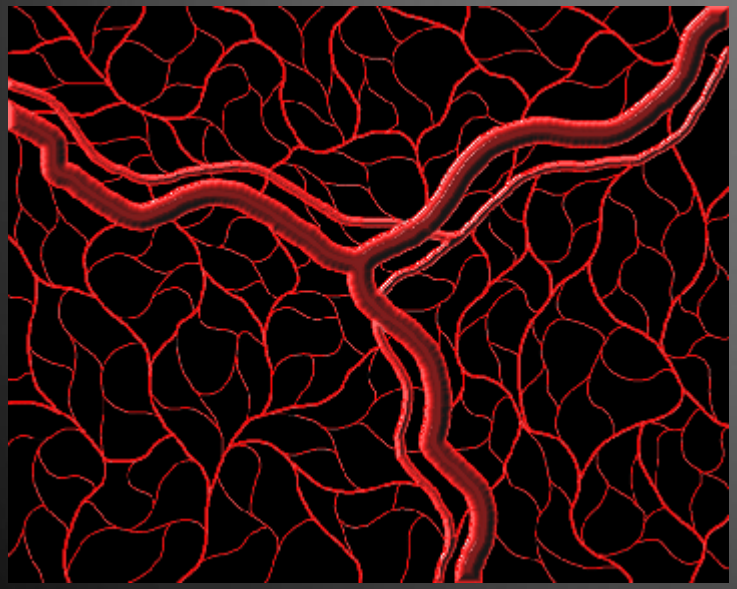
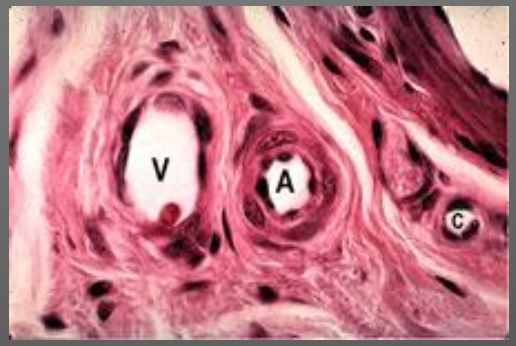
[1]A. Dunn and H. Bolay contributed equally to this work.
Received 7 November 2000; Revised 14 December 2000; Accepted 15 December 2000.

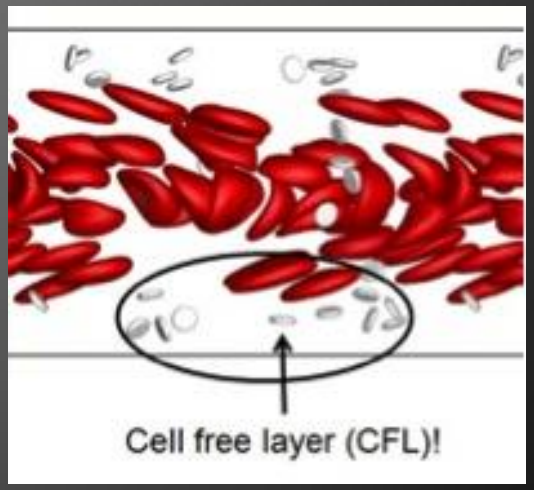
Cited:420 times

The microcirculation

- The term microcirculation refers to the functions of the capillaries and the neighboring lymphatic vessels.
- 5 % of circulating blood volume(250 mL) is present in the capillaries at any given time.
- This takes part into the exchange of nutrients, gases and waste products between the blood & tissues.

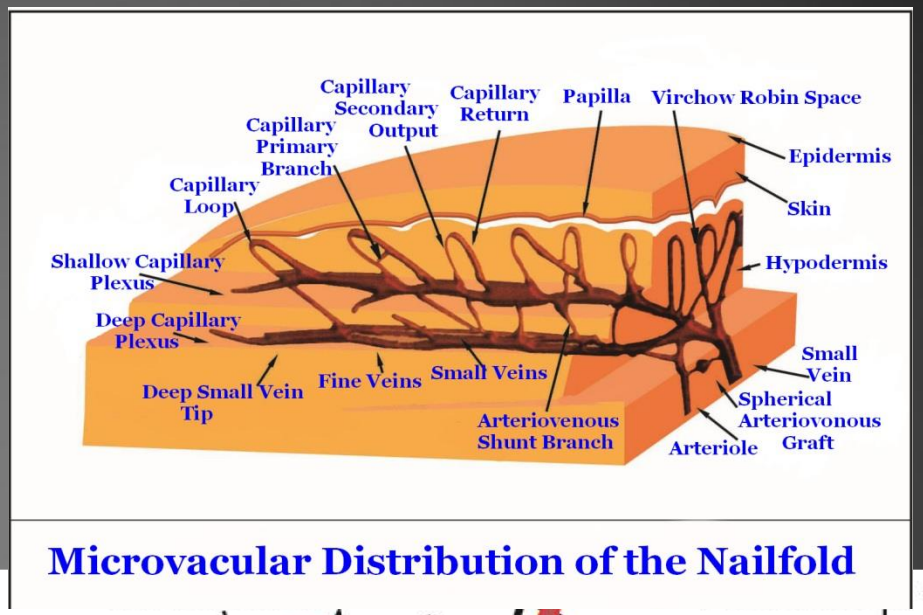




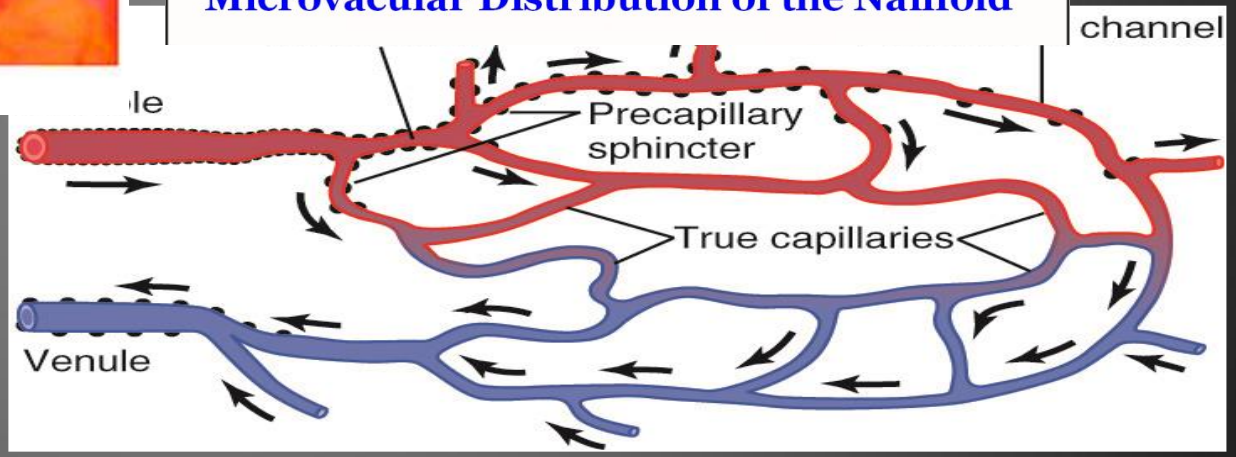


The microcirculation

Fig. 4. Labial microvascular characteristics in healthy patients (200X).



Microvacular Distribution of the Nailfold

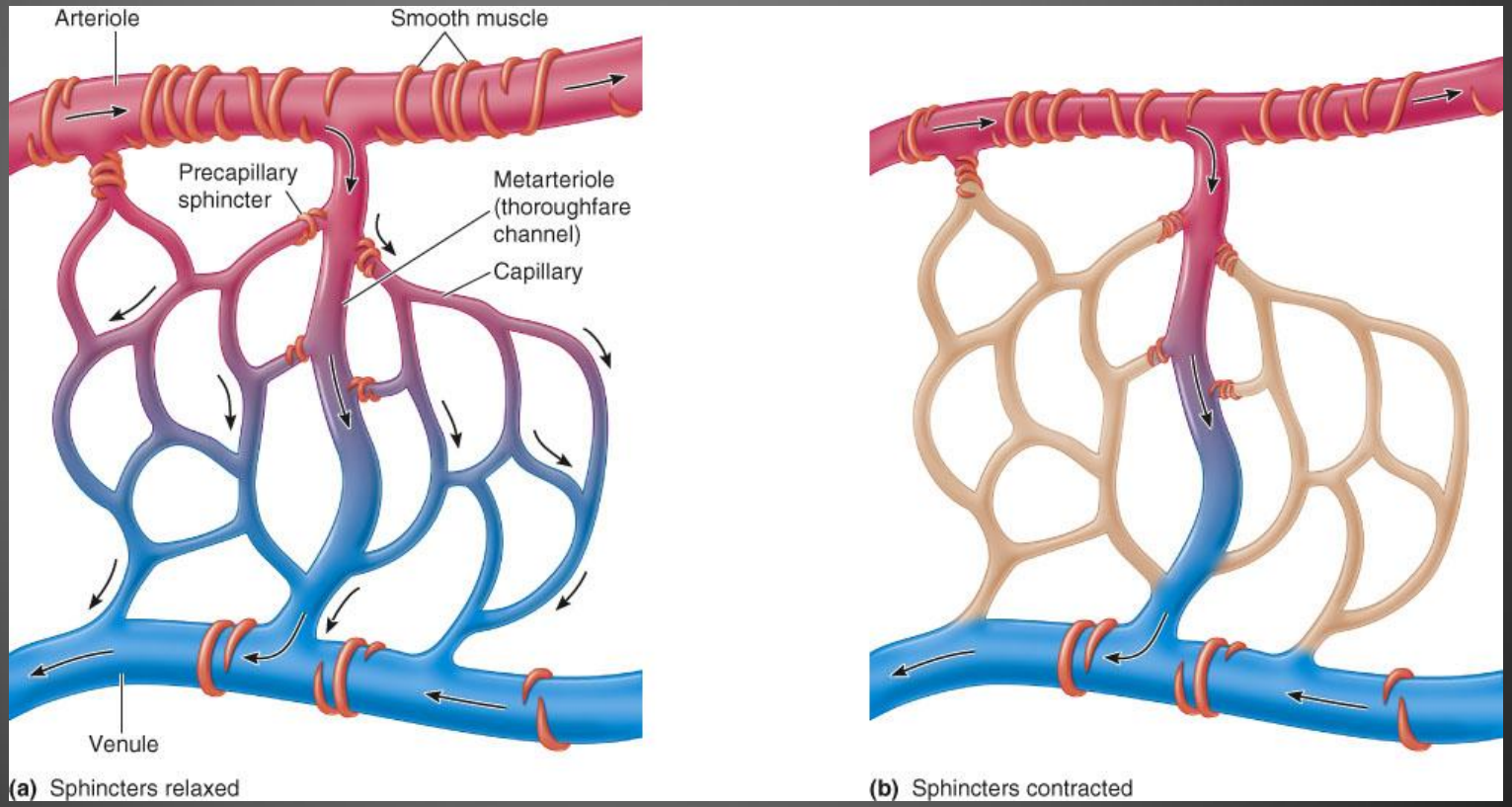


- Over 10 billion capillaries with surface area of 500-700 square meters
- Small volume of blood is exposed to larger surface area

Arteriole → Meta arteriole → Capillaries → Venules.

Pre capillary sphincter is present at the junction where the capillary arises from the Meta arteriole. This opens and closes the entrance of capillary and hence regulates the blood flow through the capillary.

The capillary wall is thin & consists of **a single layer of endothelial cells on basement membrane**. **Pores** are present between the endothelial cells that allow transport of substances including water.



Why is it important to know microvascular physiology & pathophysiology

- Almost all diseases have mircovacular components
- (diabetes, cancer, hypertension, Alzheimer's disease, etc)
- Experiences: skin, brain, nasal mucosa, inner ear…

- Brain gets ~ 750 ml/min blood, uses 20% O_2 from the body's consumption
- Brain tissue is extremely vulnerable
- Stroke is Nr. (2)-3 in respect to disabilities and death all over the world
- Dementia is linked to cererebrovascular diseases
- Perinatal asphyxia affects ~ 3-4 babies a year

Methods before the Laser Doppler

- Intravital microscopy, pletismography

- INDIFFERENT GAS METHODS
  - HYDROGEN CLEARANCE
- ISOTOPE METHODS
  - AUTORADIOGRAPHIC METHOD

    INHALATION OF O^{15} or O^{15} LABELED CO_2
  - RADIOACTIVE (LATER COLORED) MICROSPHERES
- REGIONAL CEREBRAL BLOOD-FLOW MEASUREMENTS BY XE-133-INHALATION
- LATER TRANSCRANIAL DOPPLER SONOGRAPHY
- PET

Capillaroscopy

- Find a site where there is very little scattering
- ‘Windows’ (eye, nailfold, under tongue, lower lip)
- x5/x10 microscope objective
- Polarized light capillaroscope
- Aim to detect dichroic (sickled) red blood cells in sickle cell anaemia.

Capillaroscopy (Sub-lingual)

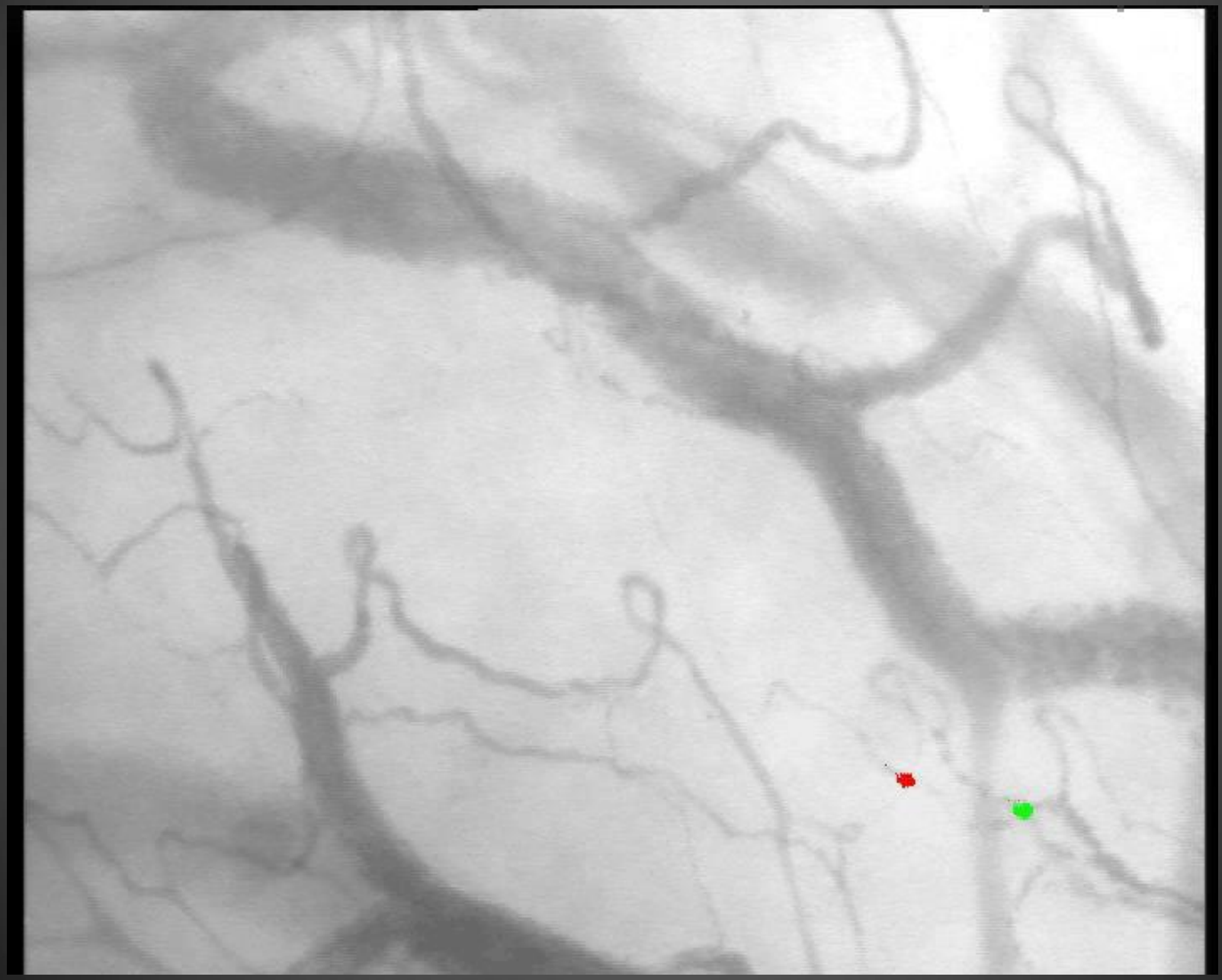
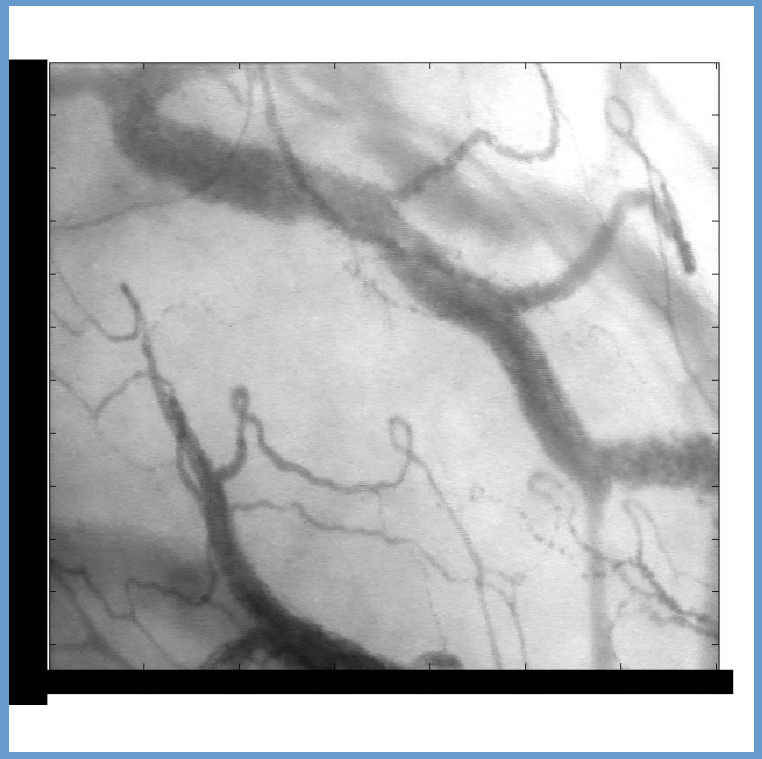


Image alignment



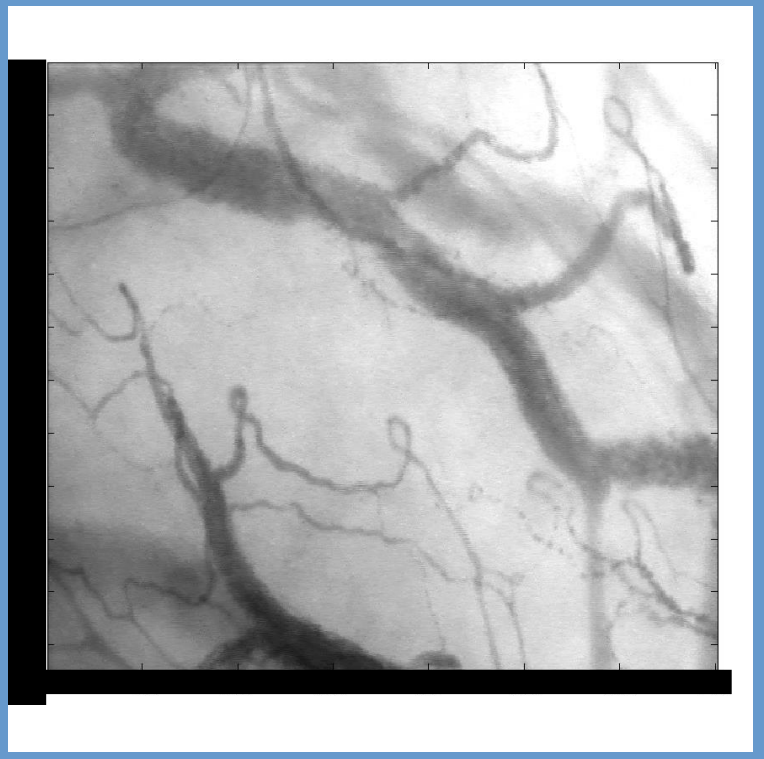
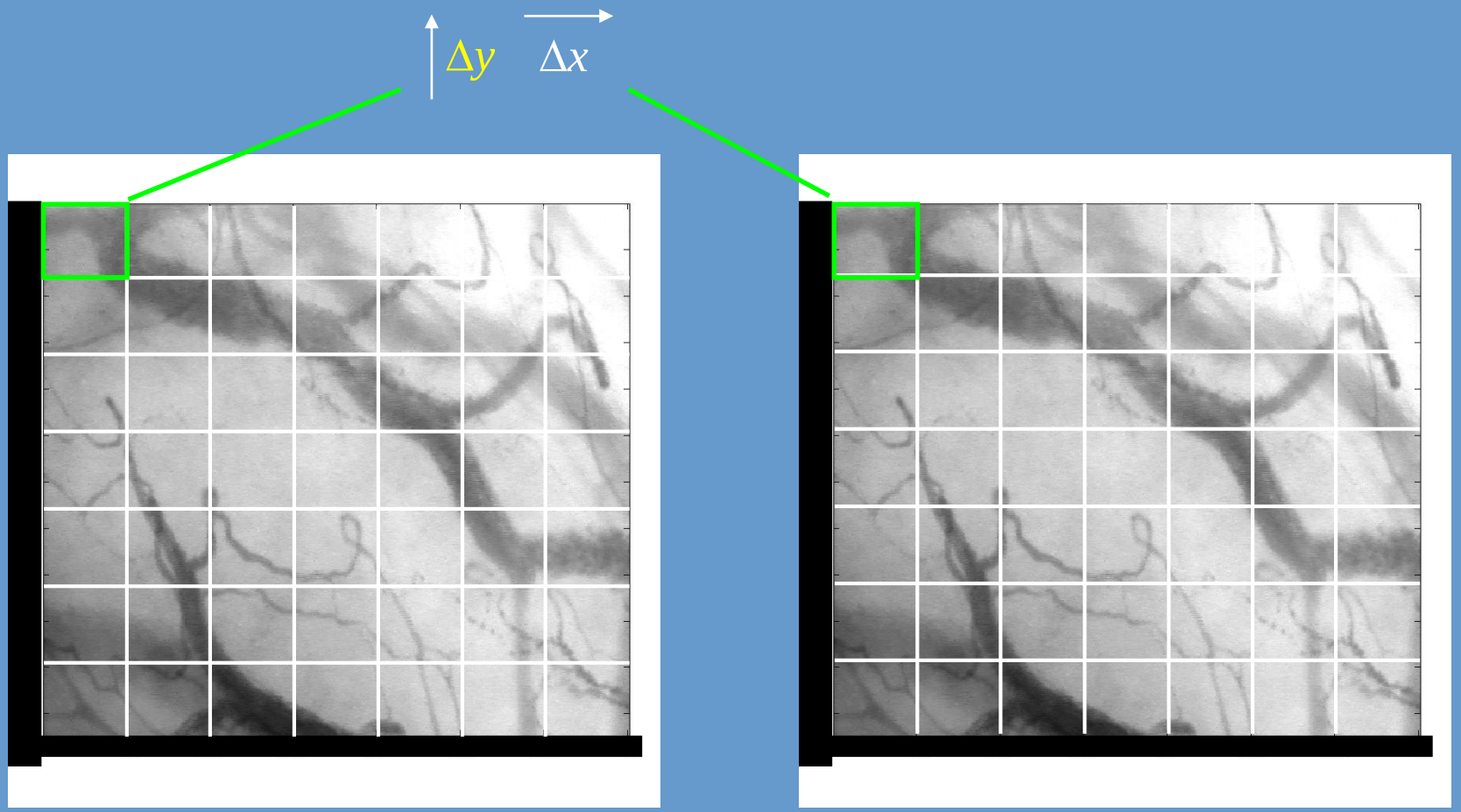


Image alignment

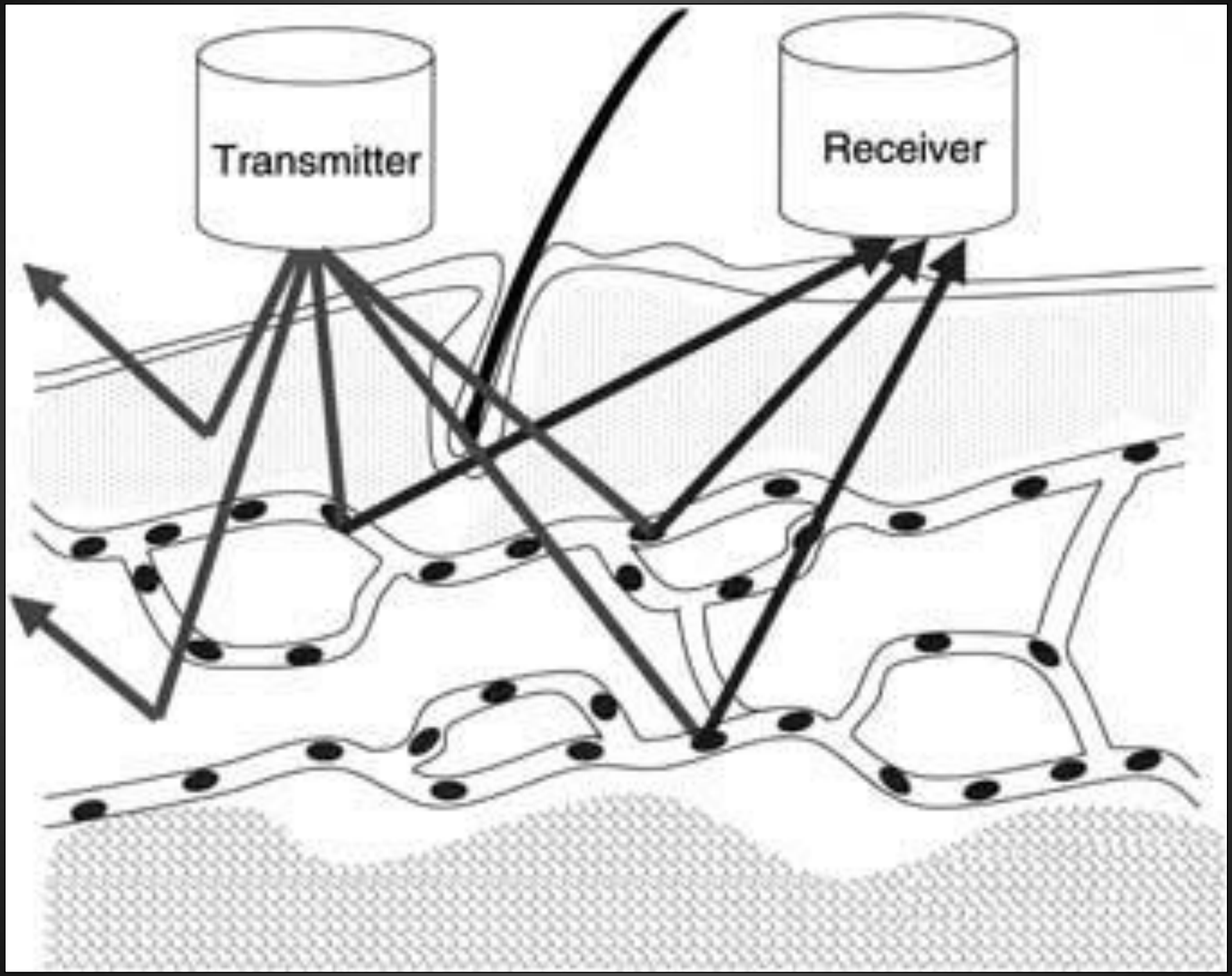


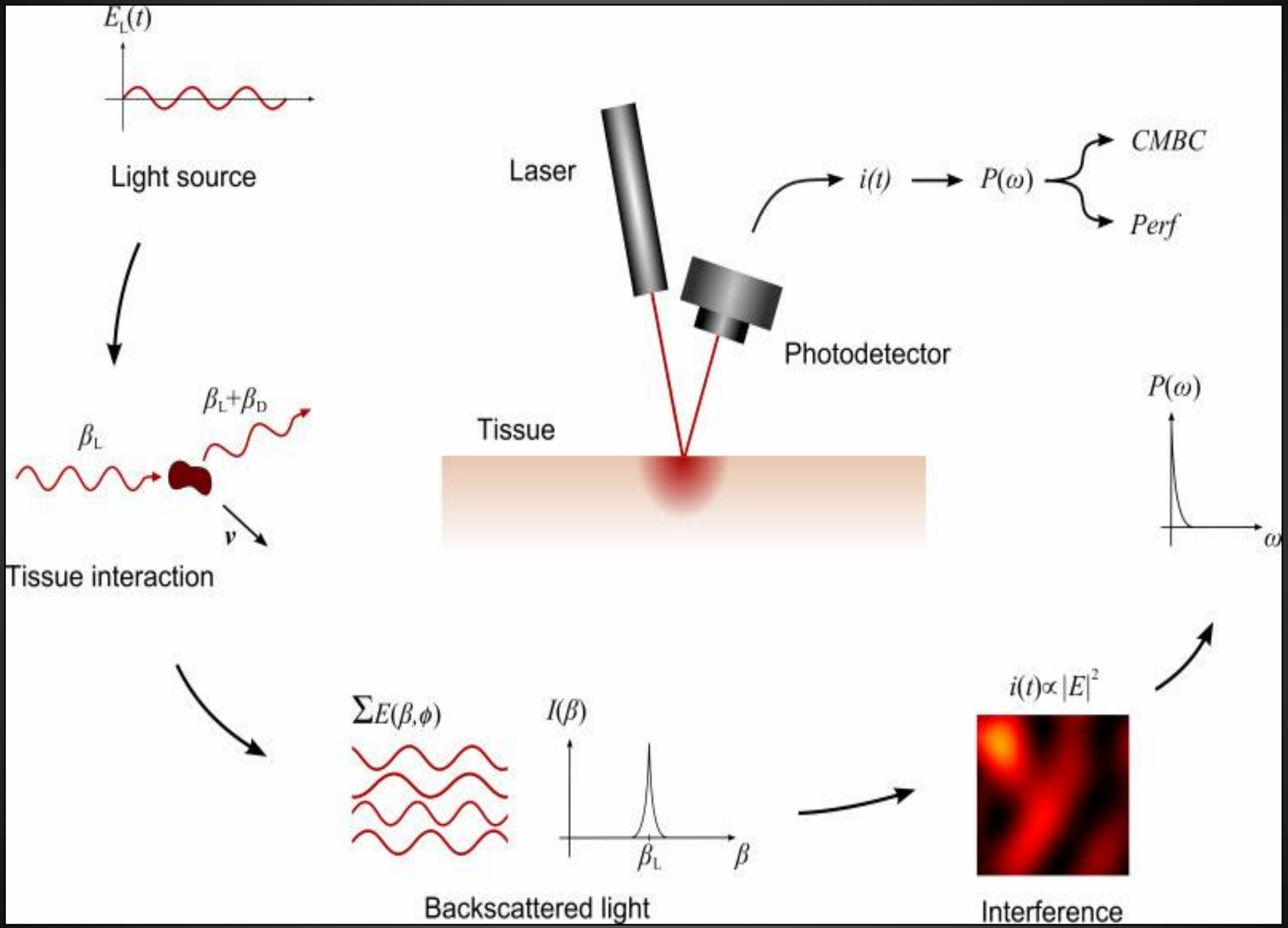
Imaging when superficial tissue is relatively thick

- skin
- indication of flow in the microcirculation
- full field laser doppler blood flowmetry
- Inflammatory responses, wounds, vein viewing

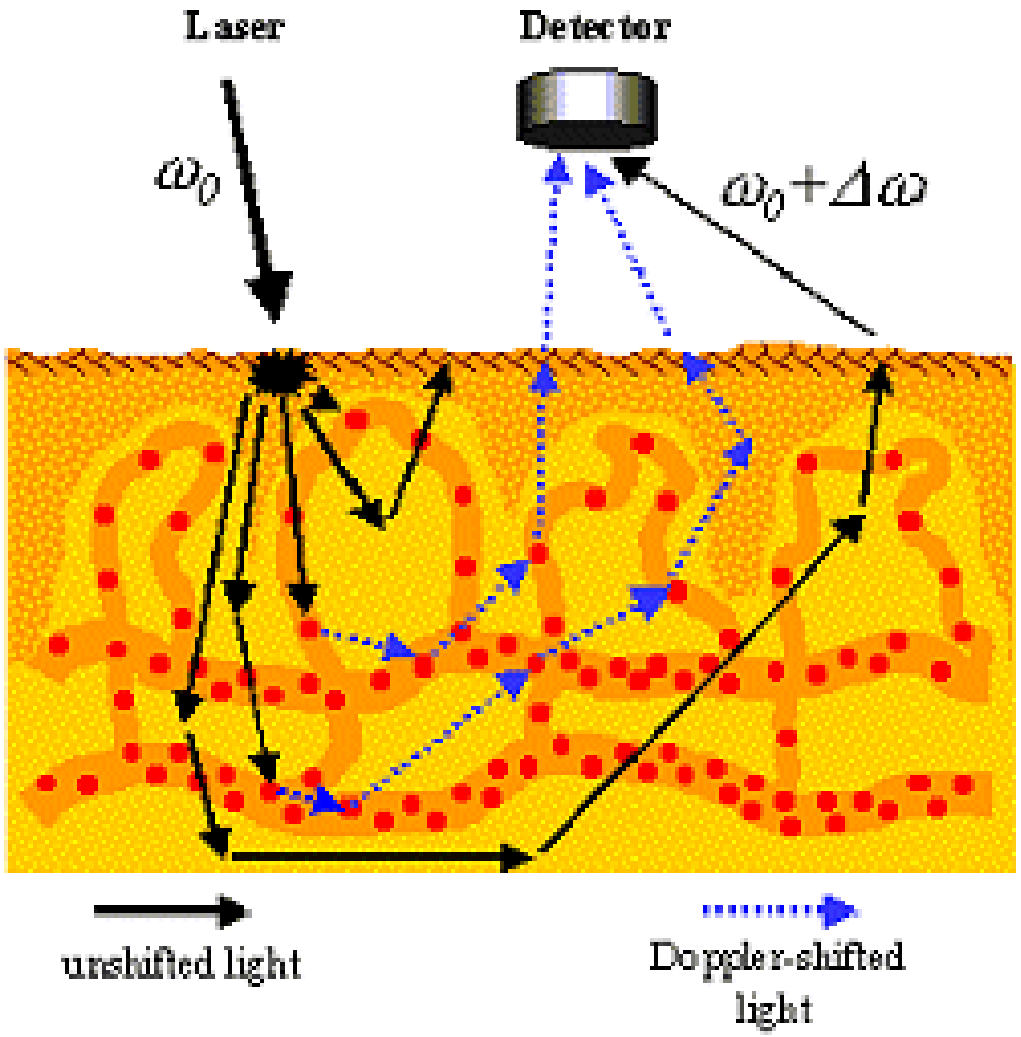
Principles of Laser Doppler Flowmetry

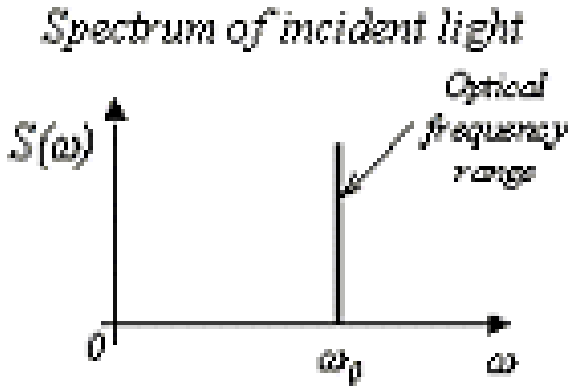
- Laser Doppler flowmetry (LDF)
  - Method to assess the tissue microvascular perfusion
  - A laser beam is directed to an area of tissue.
  - Upon contact with red blood cells in the target tissue, light waves are reflected and scattered
  - Shifts in the frequency of laser light (Doppler shift)
  - Detected and received by a photodector.

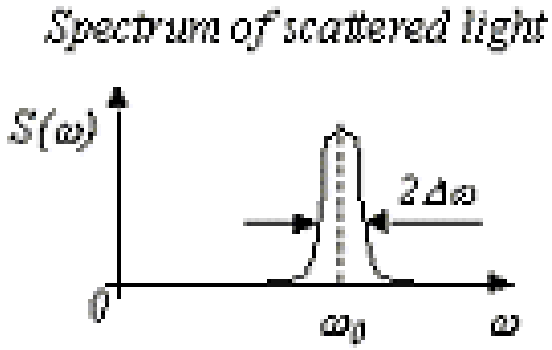

Transmitter
Receiver

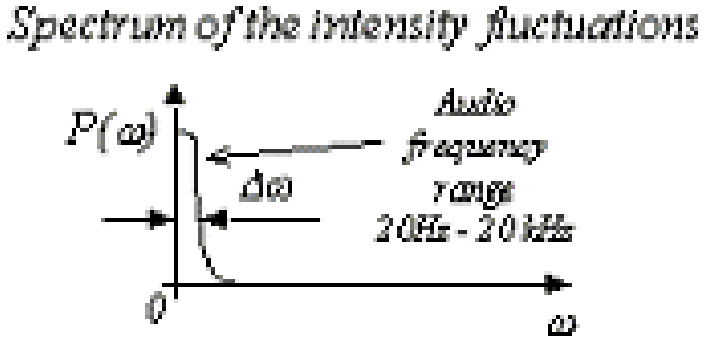

$E_L(t)$
Light source
Laser
$i(t)$
$P(\omega)$
CMBC
Perf
Photodetector
Tissue
$P(\omega)$
ω
β_L
$\beta_L+\beta_D$
v
Tissue interaction
$\Sigma E(\beta,\phi)$
$I(\beta)$
β_L
β
Backscattered light
$i(t)\propto|E|^2$
Interference

Principle of laser Doppler flowmetry

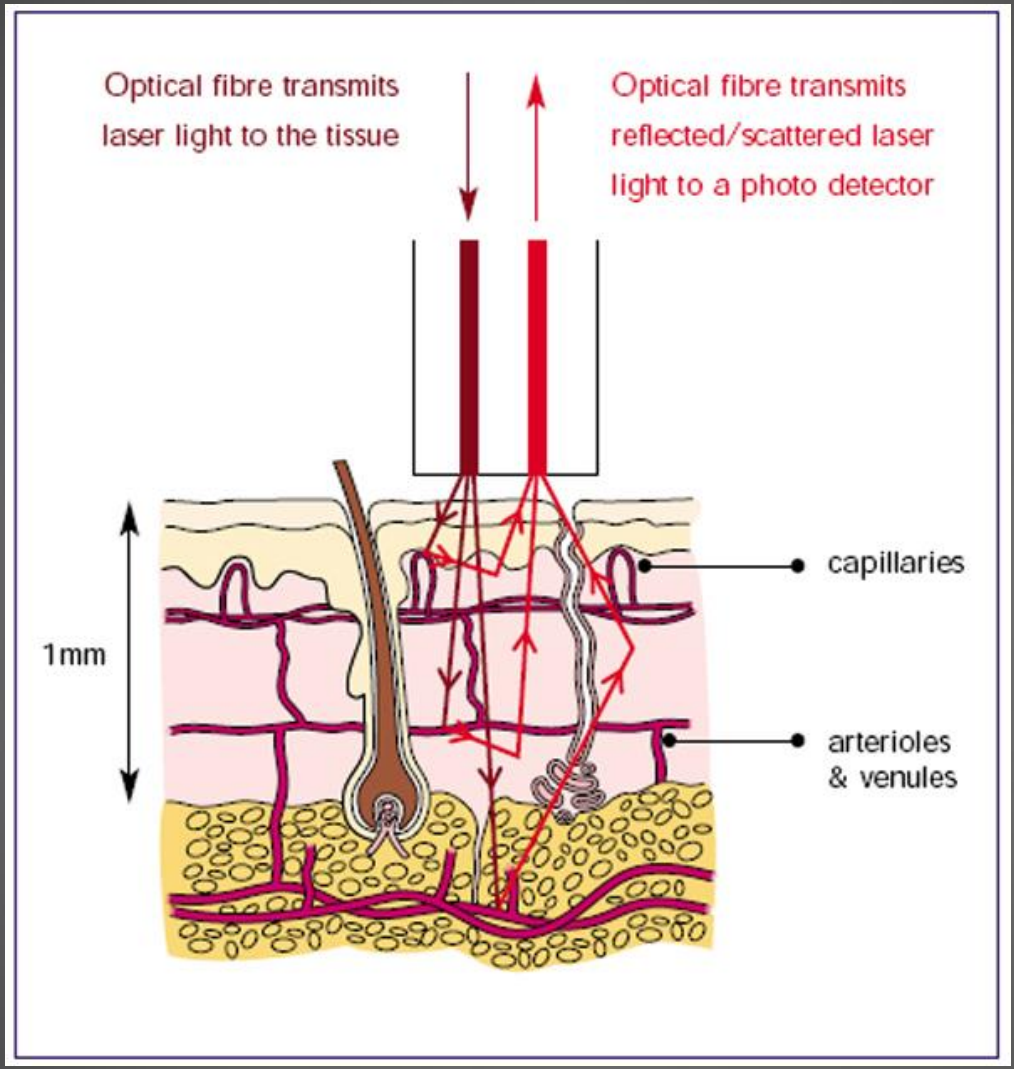








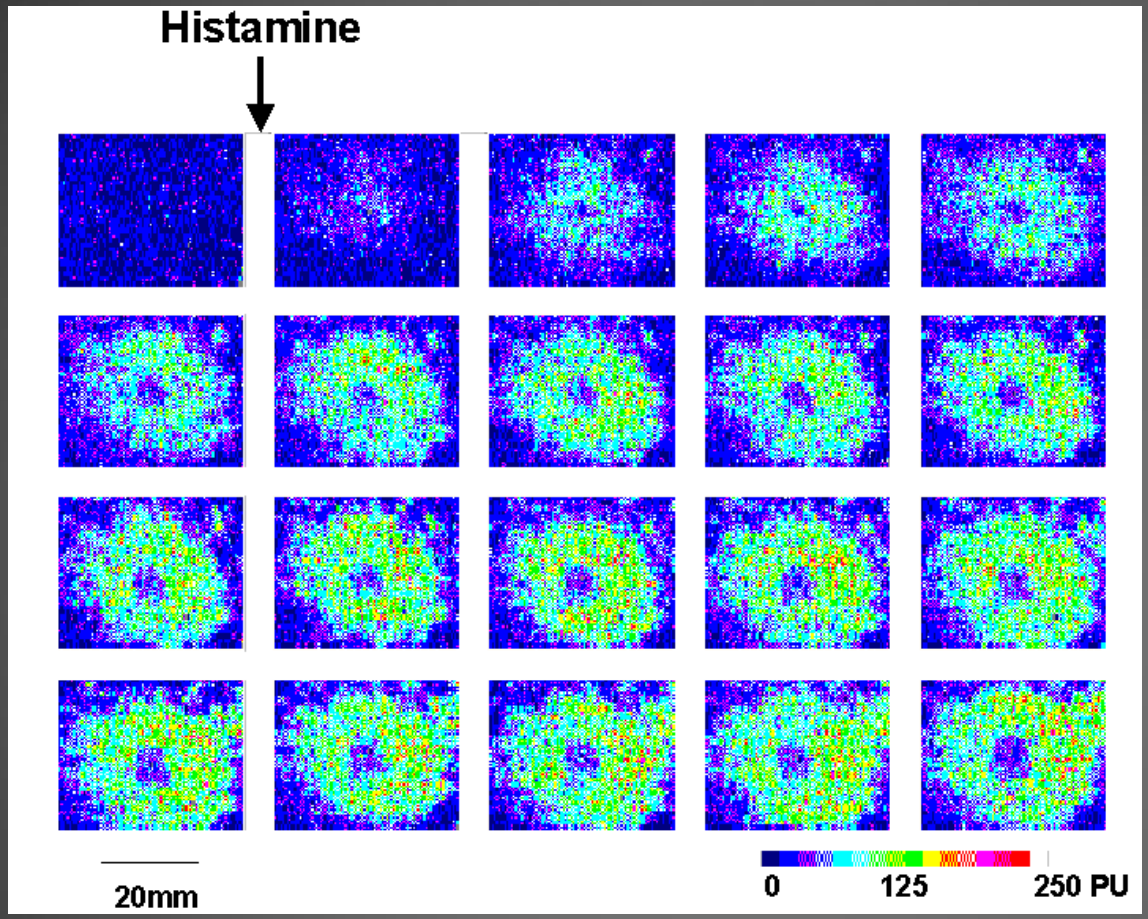
Single point blood flow imaging



Originally single point measurement system, measuring doppler shift from moving RBCs (20Hz – 20KHz)

Image - Moor Instruments

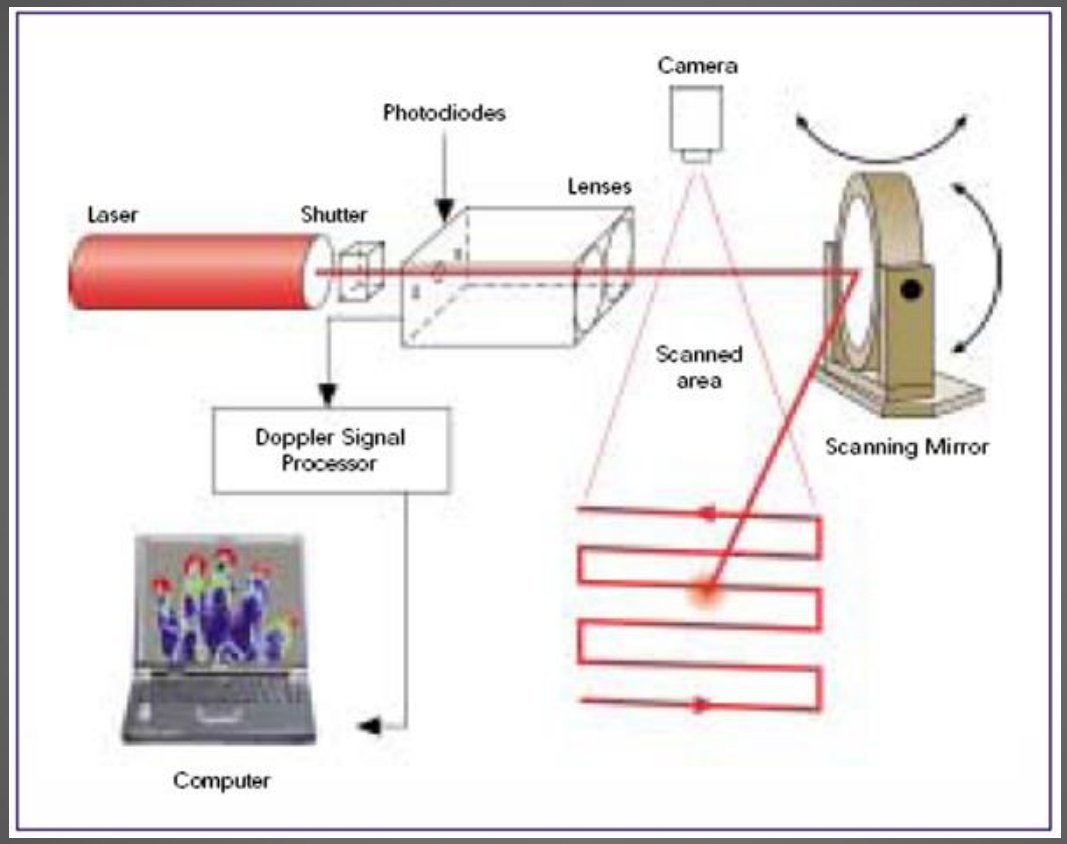
Full field laser Doppler blood flow imaging



vascular response to an intradermal injection of 20 µl of 1 µM histamine into the volar surface of the forearm of a healthy volunteer (33s intervals).

Image – GF Clough, MK Church, University of Southampton

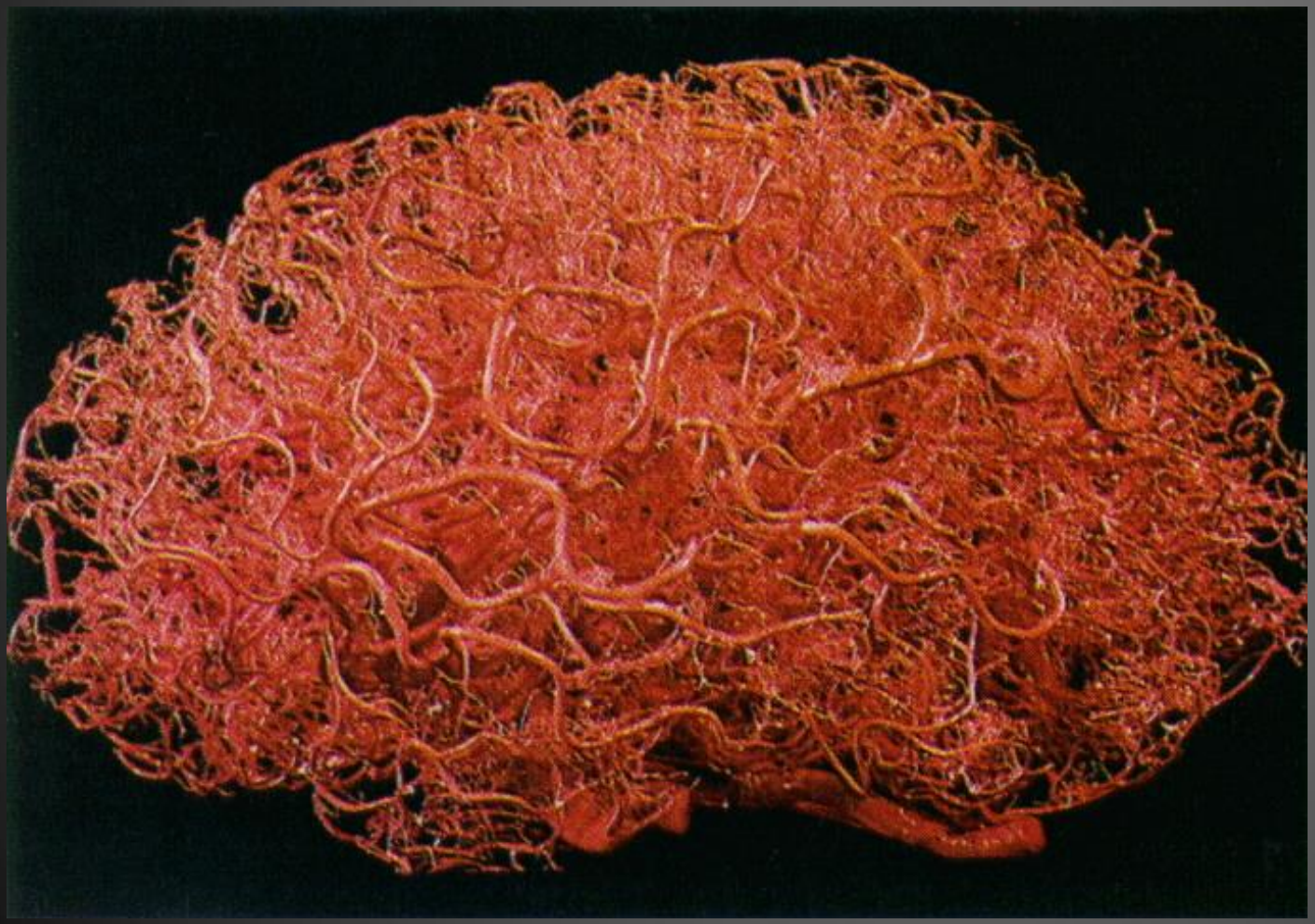
Scanning System



Builds up image point by point, slow

Image - Moor Instruments

Capillaries in the brain



Blood vessels are responsible for 25-30% of total brain volume

Capillaries:
- diameter 6-7μm
- at a distance of 40 μm
- total lenght ~ 650 km

Zlokovic and Apuzzo: Neurosurgery, 43.: 877-878 1998.

Personal history to laser Doppler

1989-90 Max Plank Institut, Bad Nauheim

Prof. K. Pireau and Prof. K. Pleschka

skin microcirculation- PF 3

1992- Albert Szent-Györgyi Medical Univ

Dept of Physiology and Dept of Neurosurgery

brain, skin, cochlea, nasal mucosa

1994- PF 4000

1995-97: Wake Forest University, Bowman Gray School of Medicine,

brain

EXPERIMENTAL STUDIES

Significance of the Rate of Systemic Change in Blood Pressure on the Short-Term Autoregulatory Response in Normotensive and Spontaneously Hypertensive Rats

Pál Barzó, M.D., Ferenc Bari, Ph.D., Tamás Dóczi, M.D., Gábor Jancsó, M.D., Mihály Bodosi, M.D.

Departments of Neurosurgery (PB, MB) and Physiology (FB, GJ), Albert Szent-Györgyi Medical University, Szeged, Hungary; and Department of Neurosurgery, University Medical School (TD), Pécs, Hungary

Laboratory Investigations

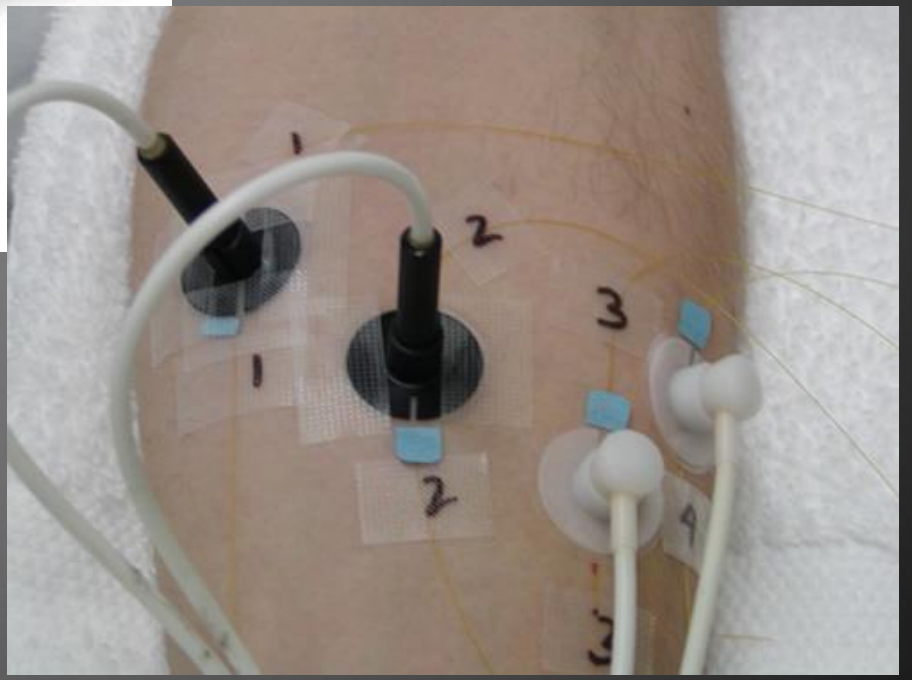
Dexmedetomidine-induced decrease in cerebral blood flow is attenuated by verapamil in rats: a laser Doppler study

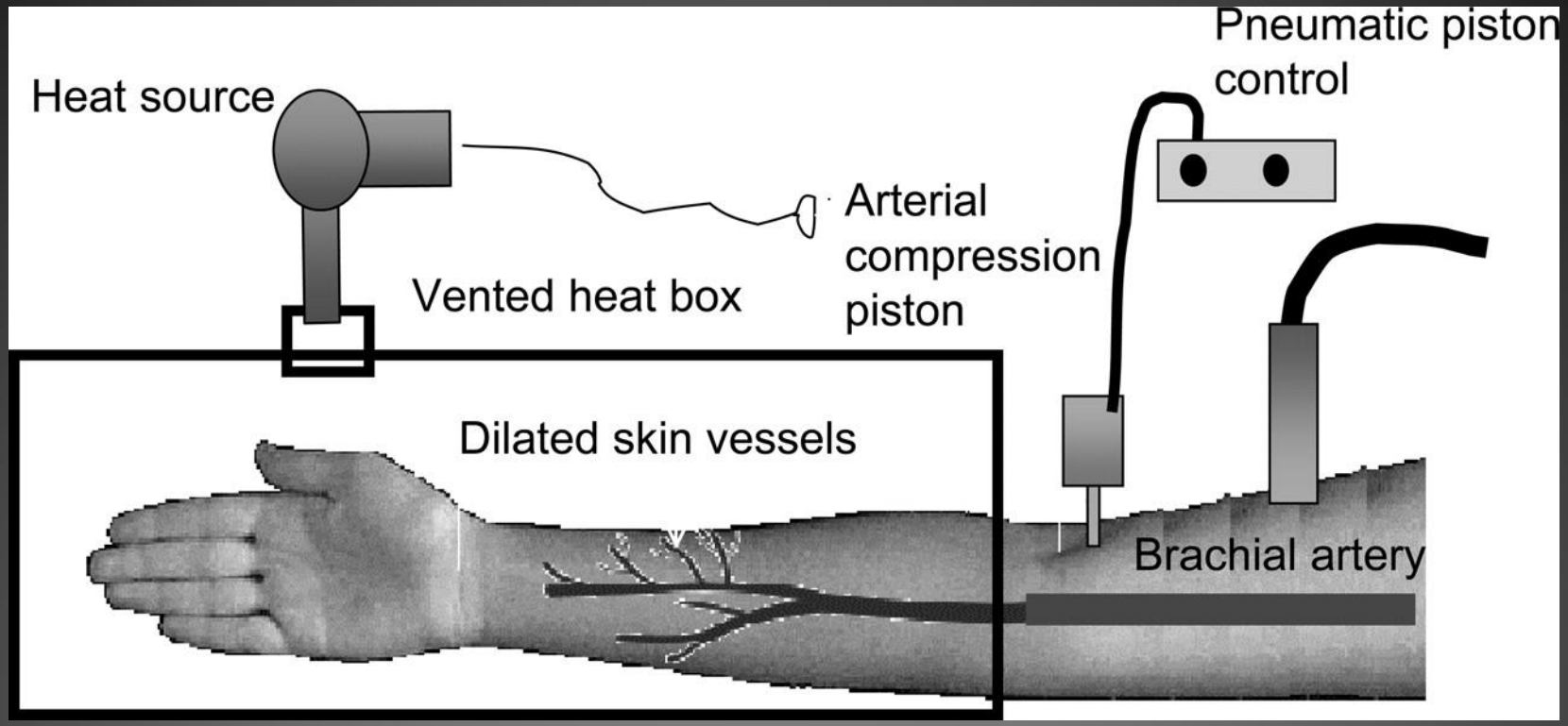
Ferenc Bari PhD, Gyöngyi Horváth MD, György Benedek MD, PhD DSc

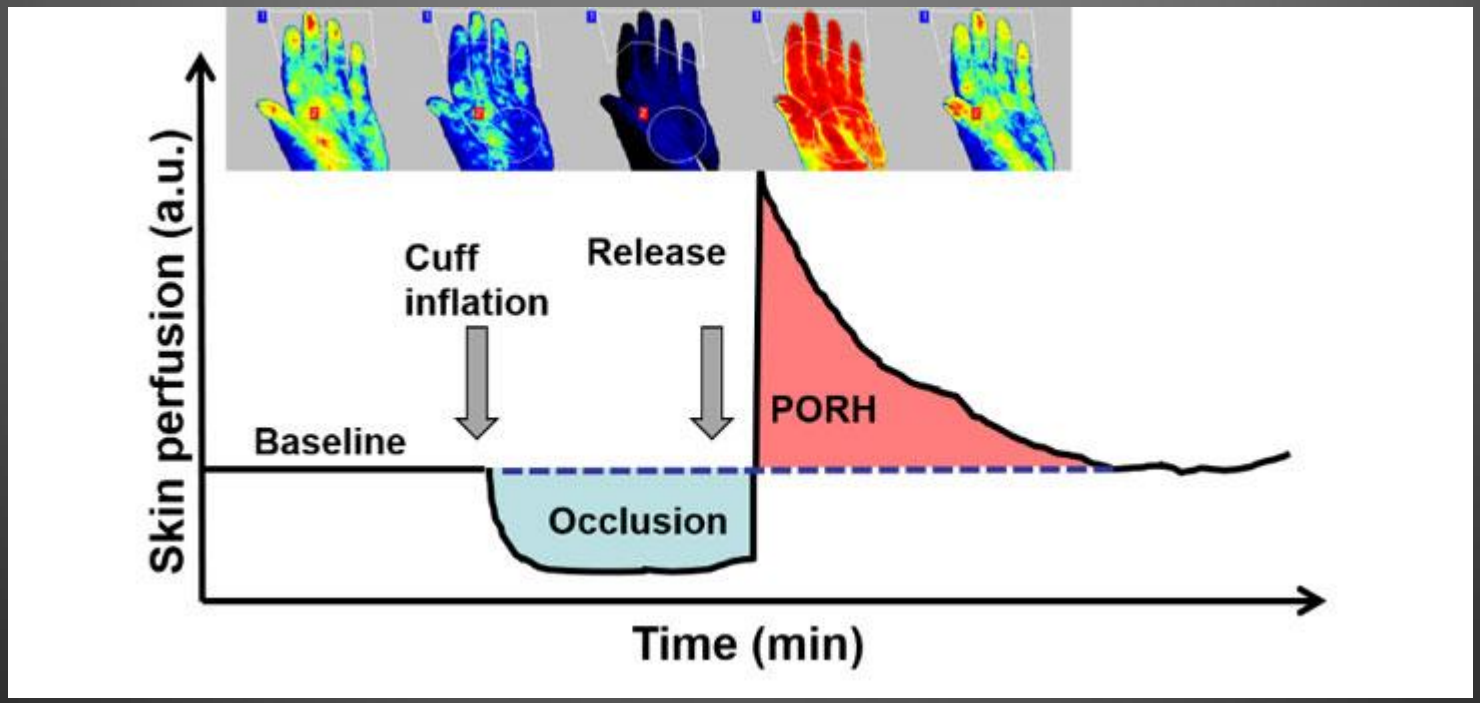
Advantages of LDF technique

- Highly sensitive
- Responsive to local blood perfusion and
- Versatile and easy to use for continuous real-time monitoring.
- Non-invasive
- Does not disturb the normal physiological state of the microcirculation
- The small dimensions of the probes have enabled it to be employed in experimental and clinical environments not readily accessible using other techniques.

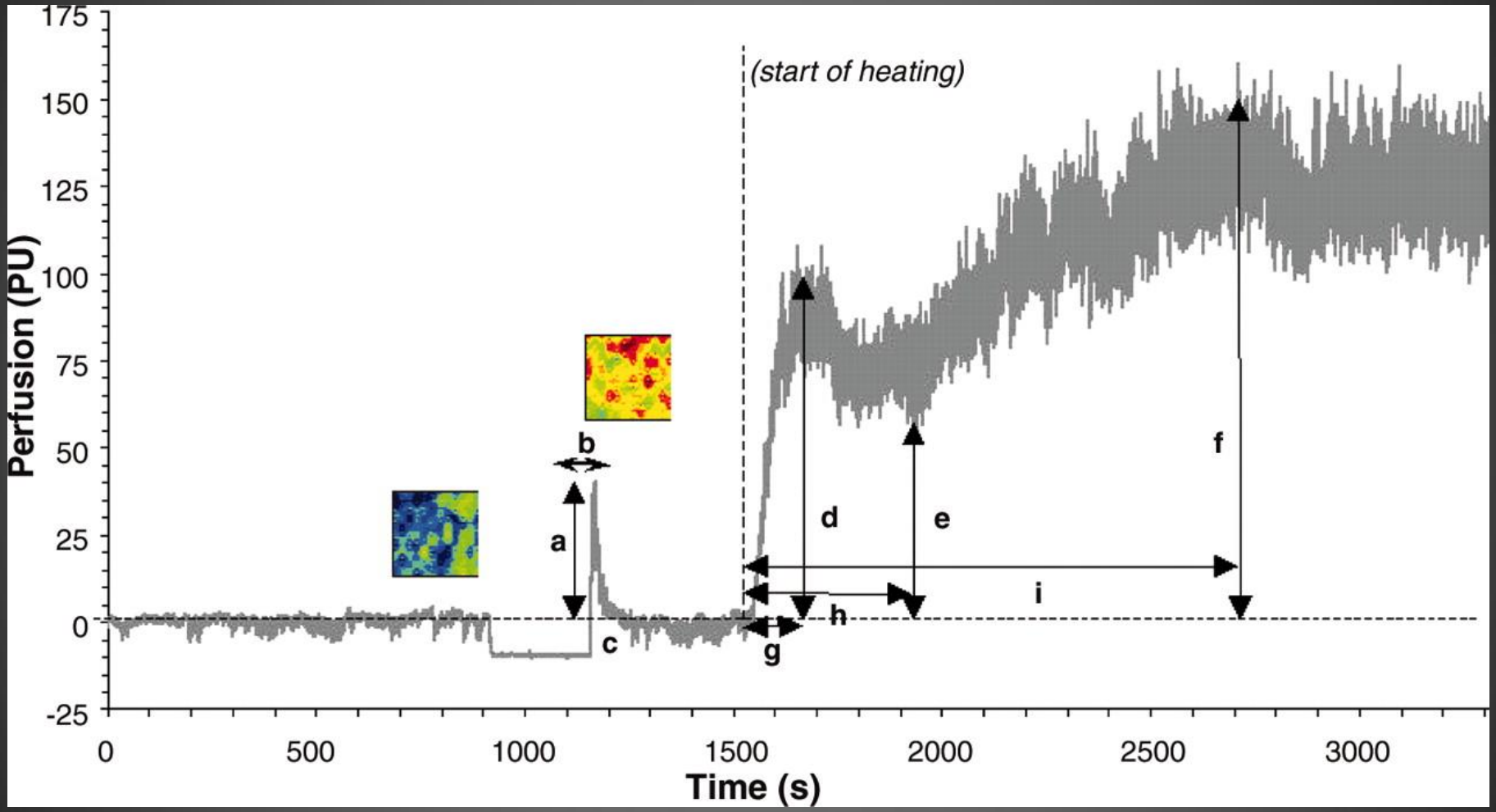
LASER DOPPLER FLOWMETRY (LDF)




Heat source
Pneumatic piston control
Arterial compression piston
Vented heat box
Dilated skin vessels
Brachial artery


Skin perfusion (a.u.)
Cuff inflation
Release
Baseline
Occlusion
PORH
Time (min)

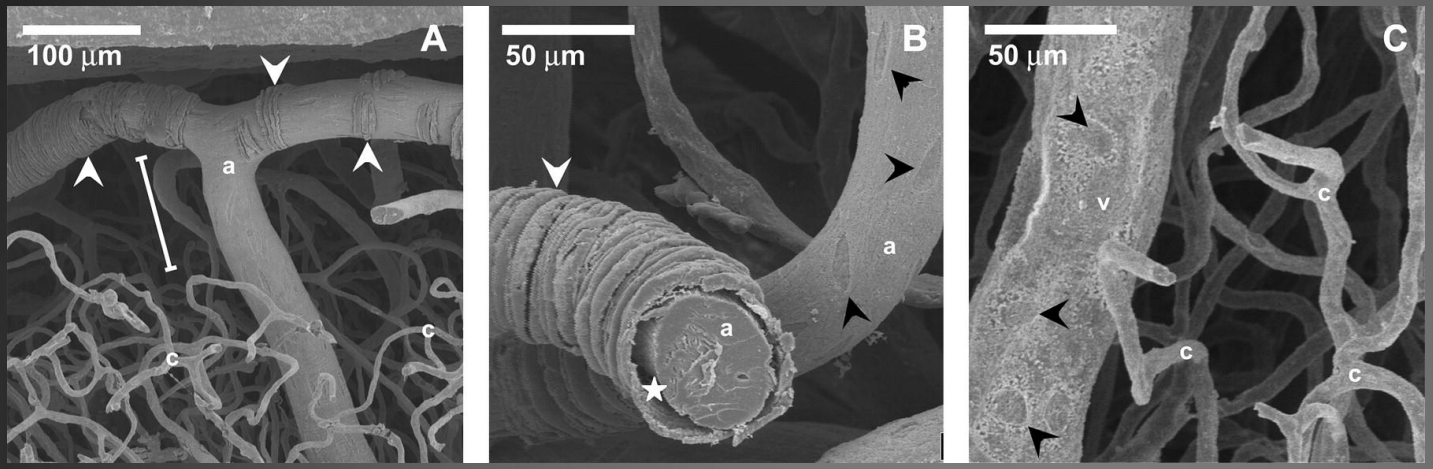
Representative tracing of control postocclusive hyperemia (PORH) and thermal hyperemia (TH).

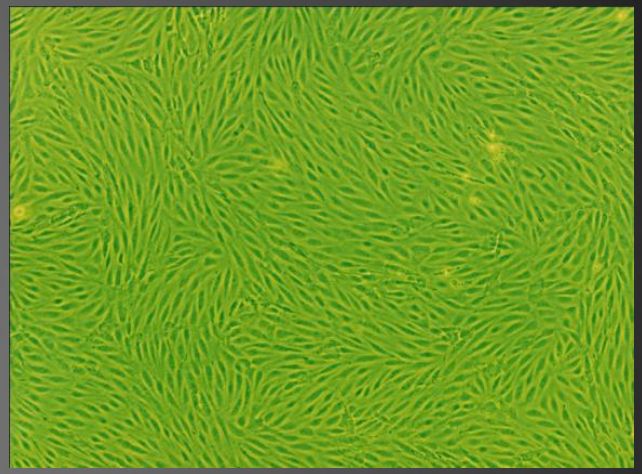


Stewart J et al. Am J Physiol Heart Circ Physiol 2004;287:H2687-H2696

AMERICAN JOURNAL OF PHYSIOLOGY
Heart and Circulatory Physiology

Capillaries in the brain

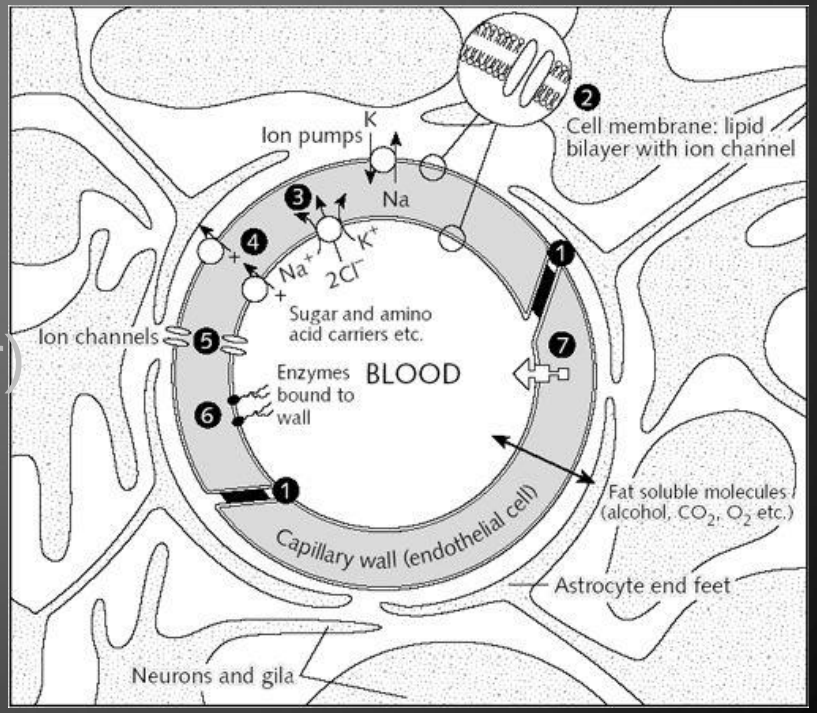




The endothelium is the thin layer of cells that lines the interior surface of blood vessels. In the brain there are highly differentiated endothelial cells to perform specialized functions;

- Protection (blood-brain barrier)
- Selective permeability
- Regulation of transport

Total cross sectional area ~12 m^2 f



Closed cranial window- intravital microscopy direct observation of cortical vessels

Advantages:

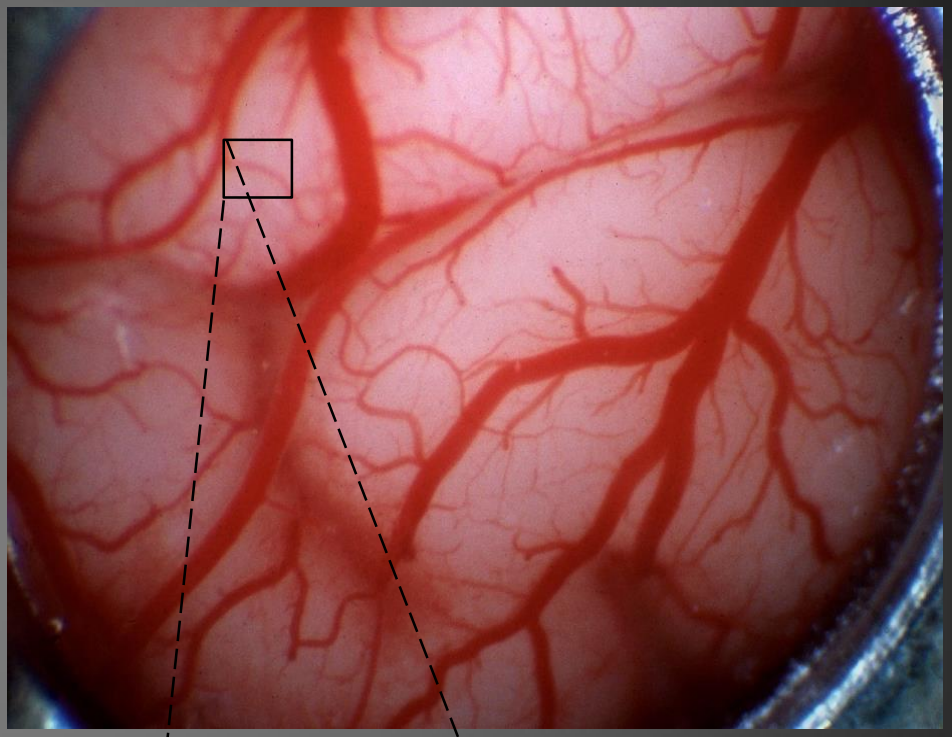
•Physiological environment

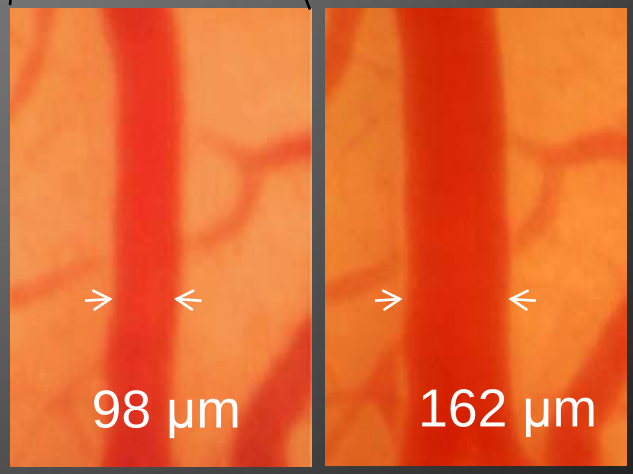
•Many kind of vessel can be studied

• Disadvantages:

•Parenchymal circulation cannot be studied

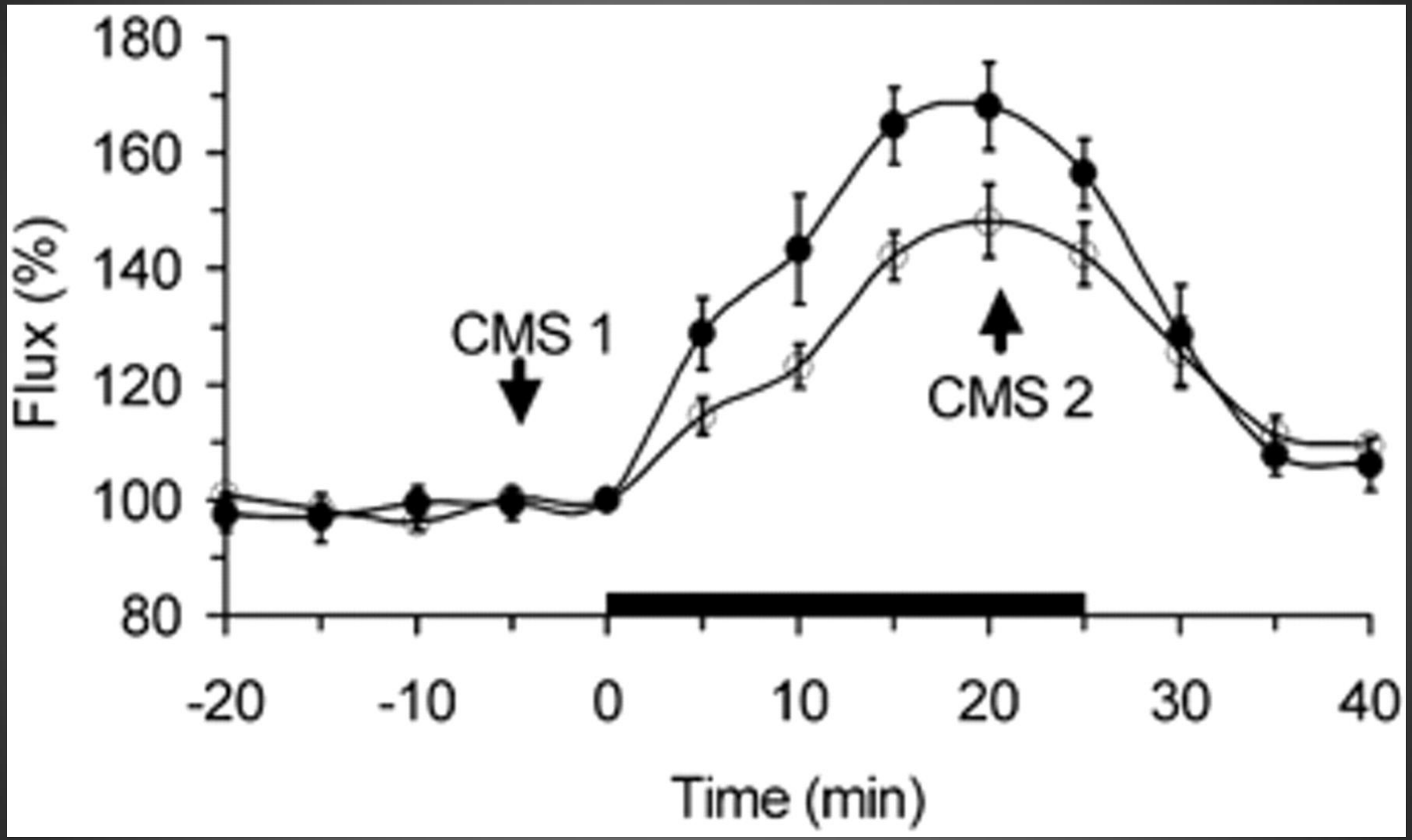
•Limited dynamical follow-up





before and after NMDA (10^{-4} M)

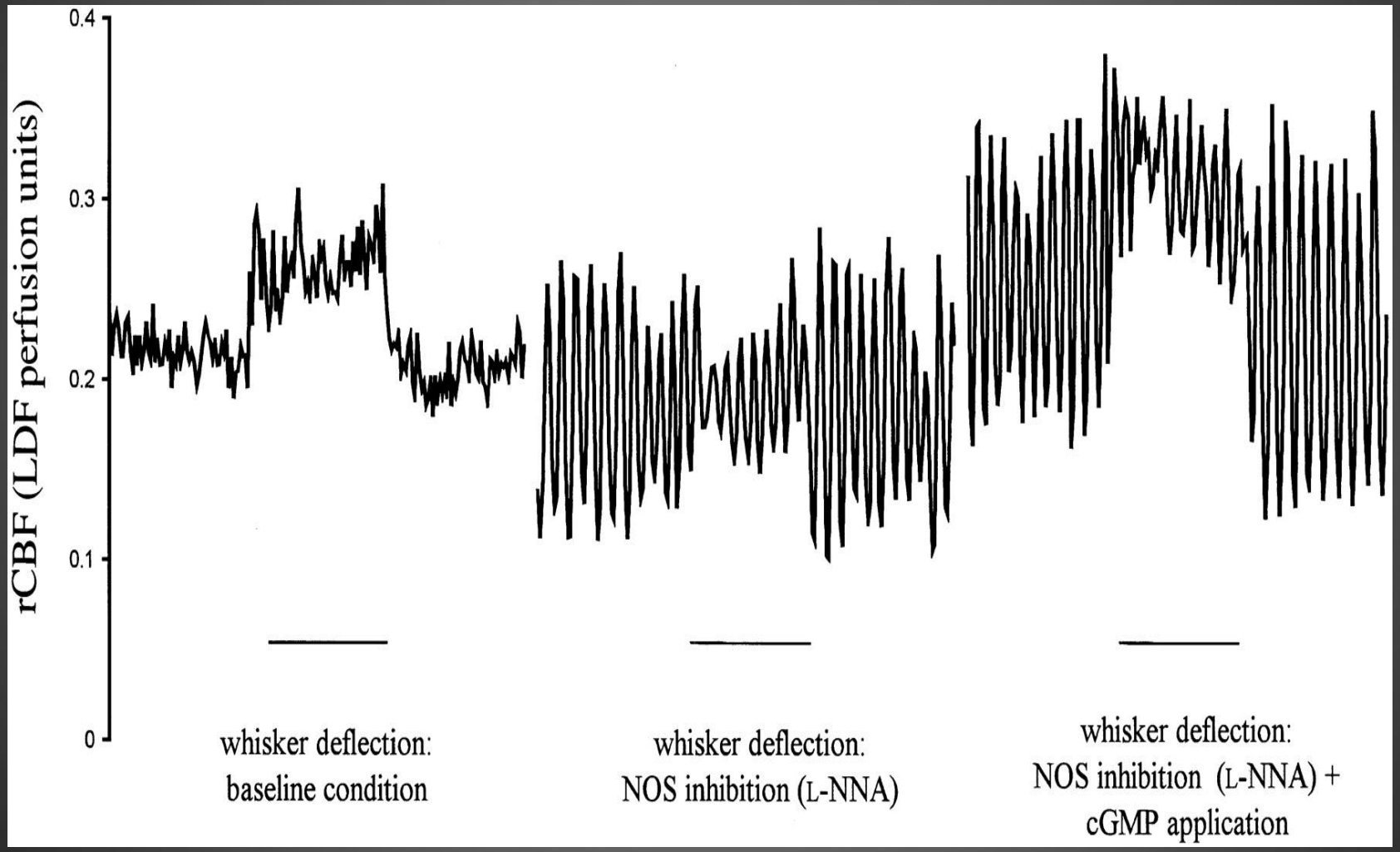
Time course of lCBF changes during maternal hypercapnia (black bar) measured by cortical surface (○, n = 6) and intracortical (•, n = 5) laser probes in fetal sheep at 110 dGAData are means ±s.e.m.



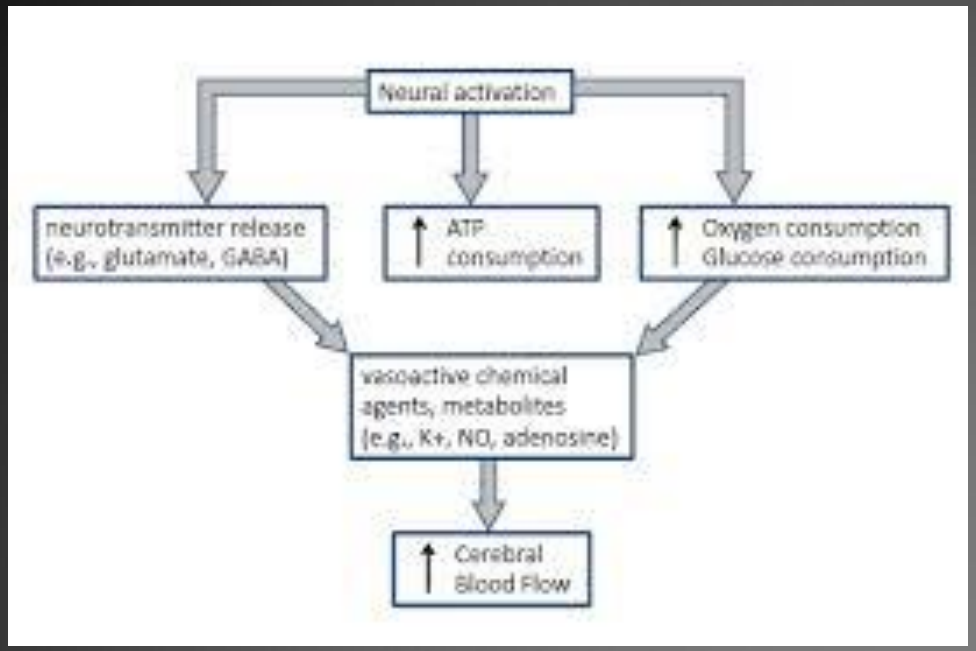
Müller T et al. J Physiol 2002;539:957-967

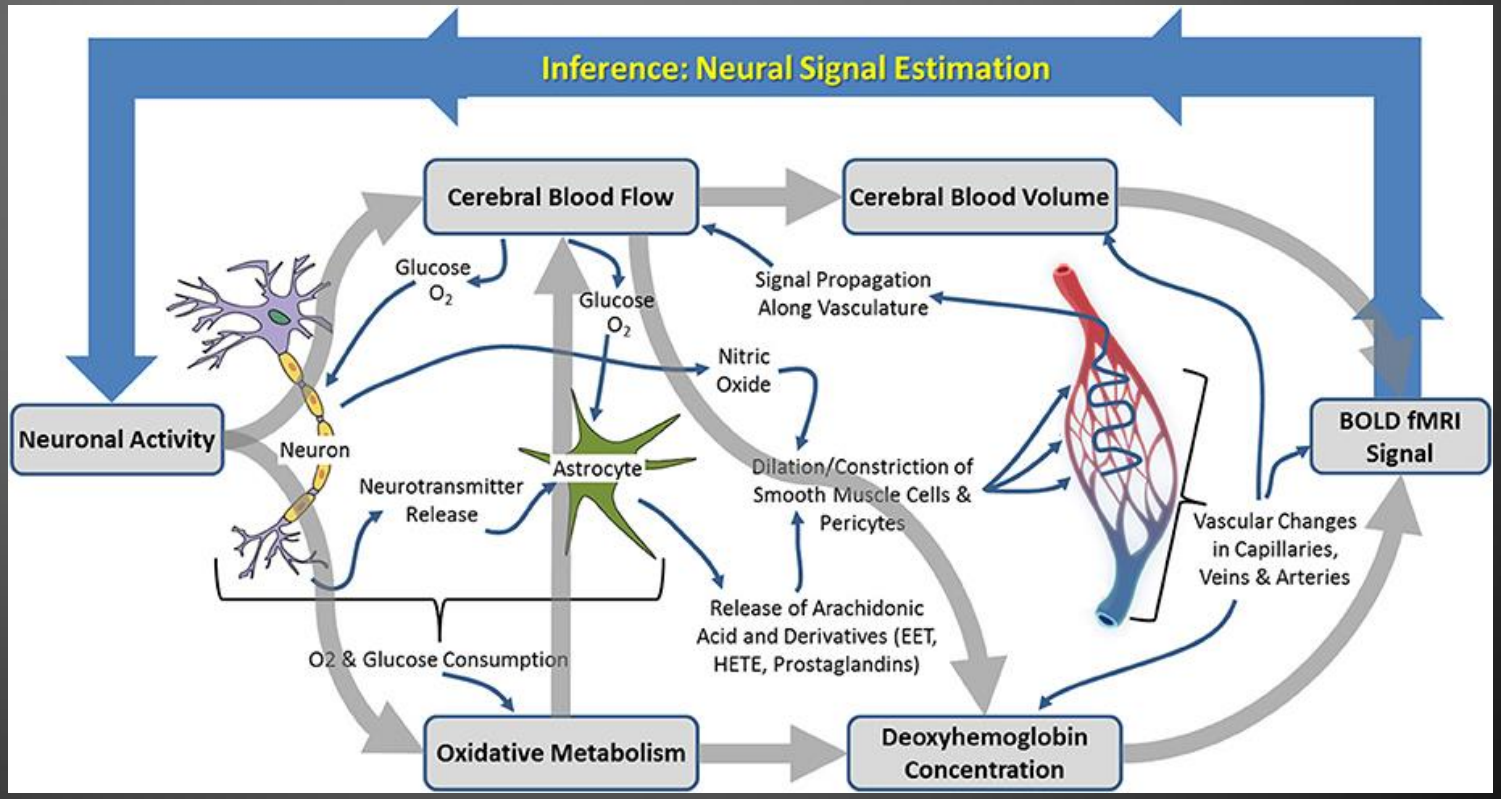
The Journal of Physiology
A publication of The Physiological Society

LDF provided a means to follow functional activation and to analyse rhythmic components of microcirculation



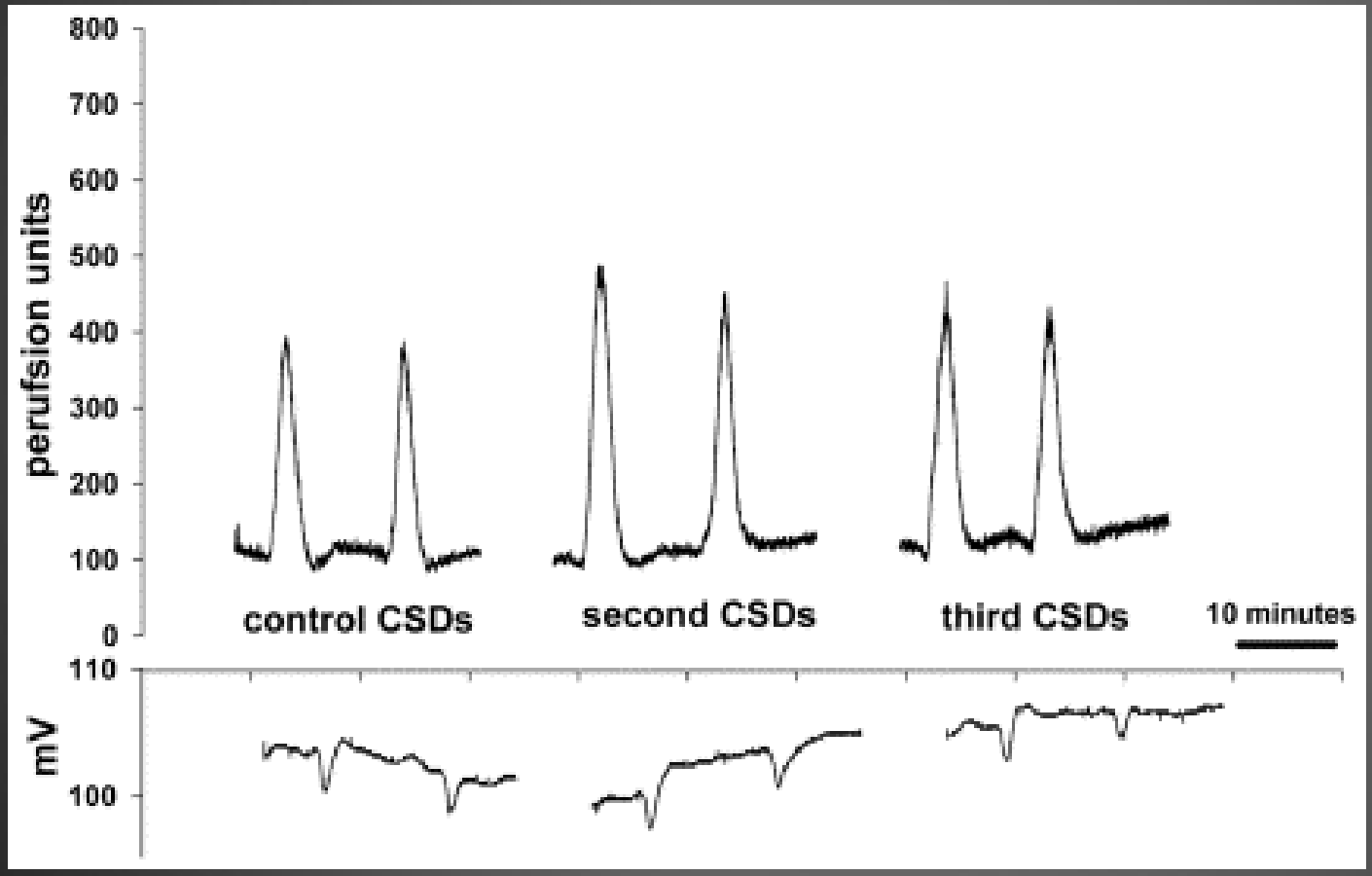
Lindauer et al, AJP, 1999


Neural activation
neurotransmitter release (e.g., glutamate, GABA)
ATP consumption
Oxygen consumption Glucose consumption
vasoactive chemical agents, metabolites (e.g., K+, NO, adenosine)
Cerebral Blood Flow


Inference: Neural Signal Estimation
Cerebral Blood Flow
Cerebral Blood Volume
Glucose
O2
Glucose
O2
Signal Propagation Along Vasculature
Nitric Oxide
Neuronal Activity
Neuron
Astrocyte
Neurotransmitter Release
Dilation/Constriction of Smooth Muscle Cells & Pericytes
BOLD fMRI Signal
Vascular Changes in Capillaries, Veins & Arteries
Release of Arachidonic Acid and Derivatives (EET, HETE, Prostaglandins)
O2 & Glucose Consumption
Oxidative Metabolism
Deoxyhemoglobin Concentration

Neurovascular coupling-cortical spreading depression

endothelium-derived dilator factors are unlikely to mediate CSD-induced hyperemia in the brain

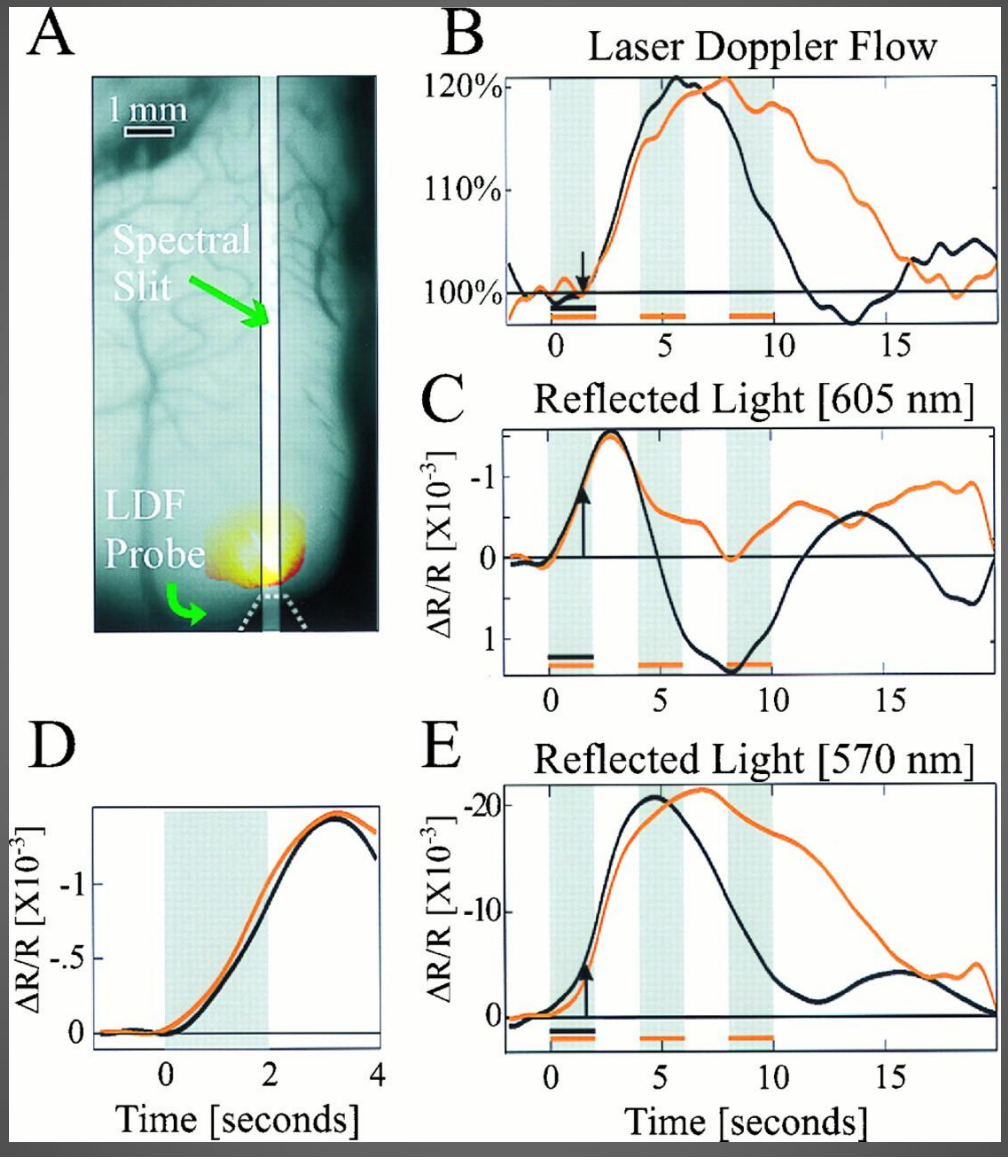


Prof. Peter Goadsby- Pioneer in headache & Cerebral microcirculation

Original recordings of cerebral blood flow (CBF) responses (upper wave) and DC deflections (bottom wave) during the three series of CSD. Ten mg/kg of -NAME was given between the first and second sets of CSDs.

Simultaneous measurement of cortical reflection and CBF. (A) An image of the cortical surface, the location of slit used for imaging spectroscopy, the tip of LDF probe, and the reflection of its beam from the cortex.

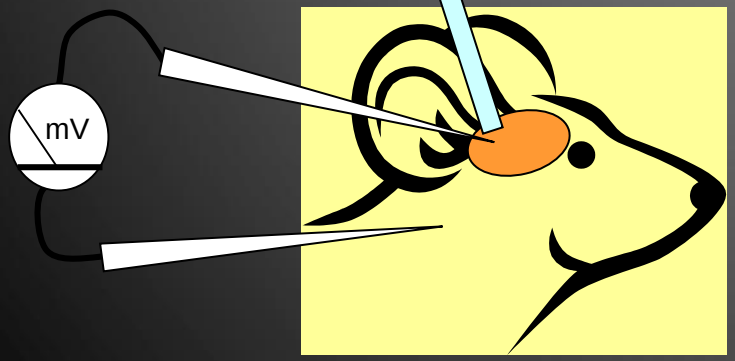
Visual stimulation
(2 sec)- black
(3x2 sec)- red

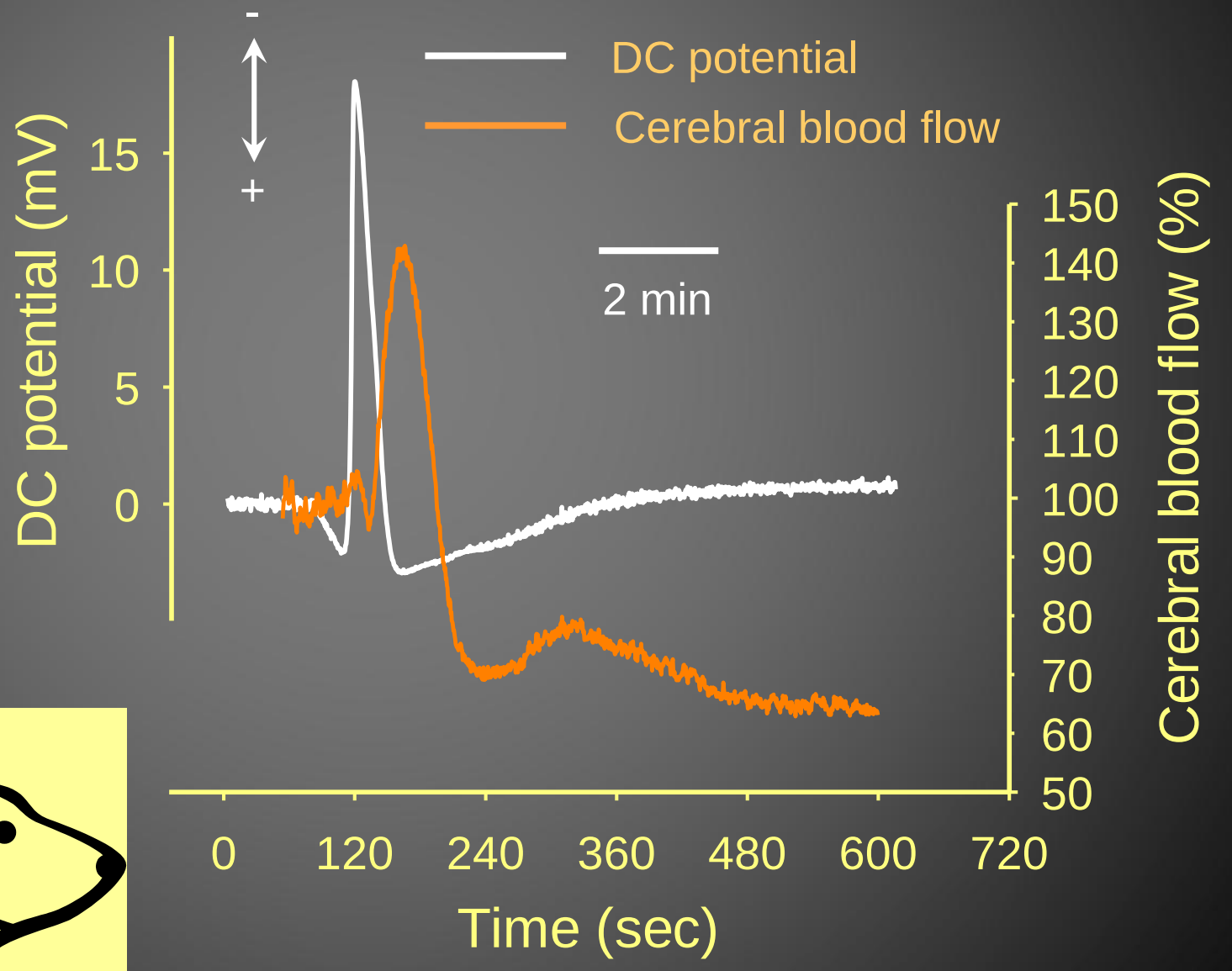


Dov Malonek et al. PNAS 1997;94:14826-14831

Spreading depolarization

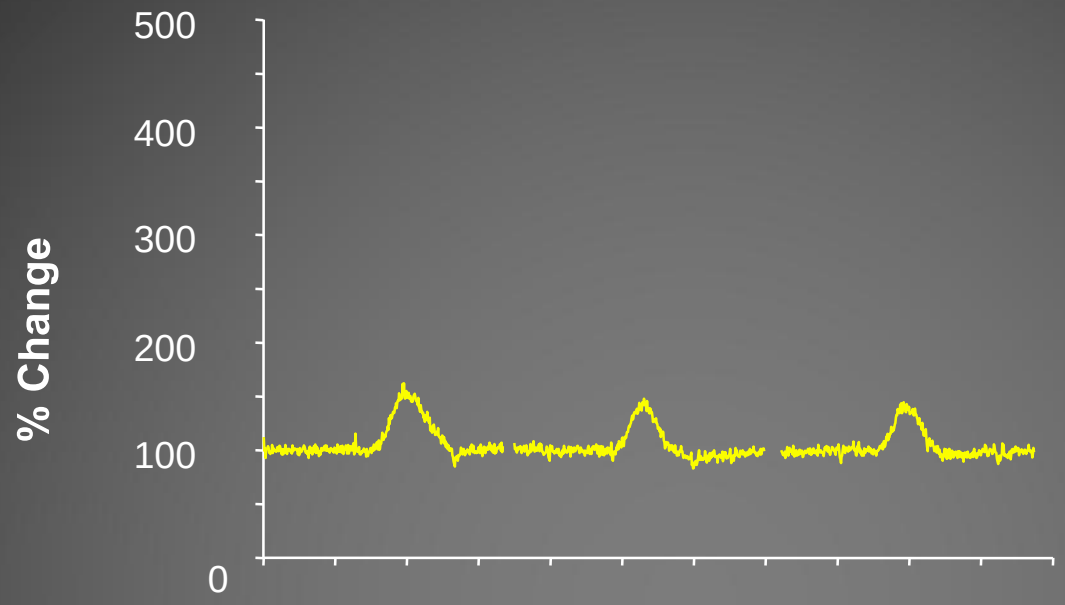
Laser-Doppler flowmeter





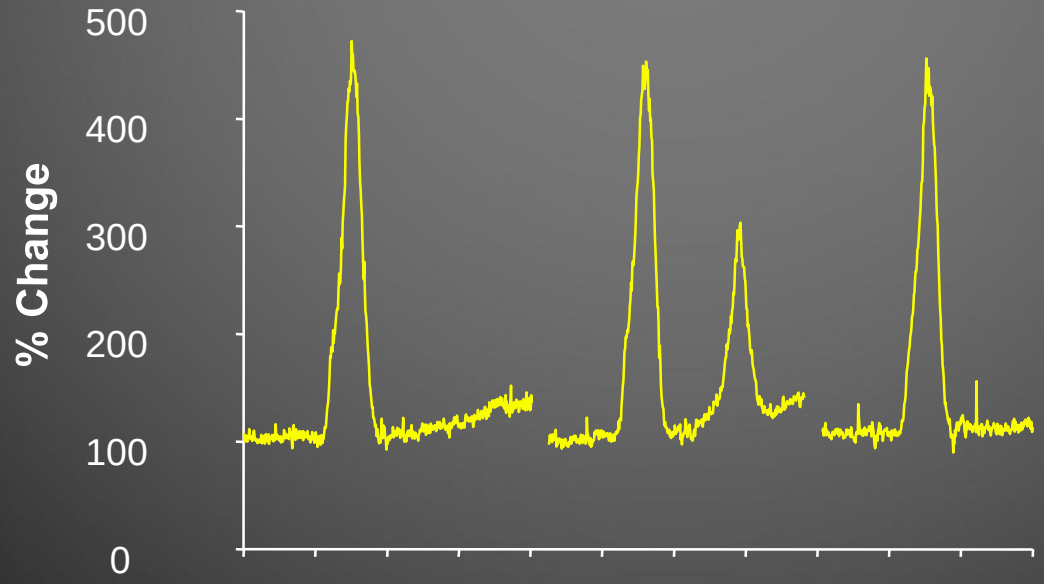
Farkas et al. (2008) JCBFM

Depressed hyperemic responses



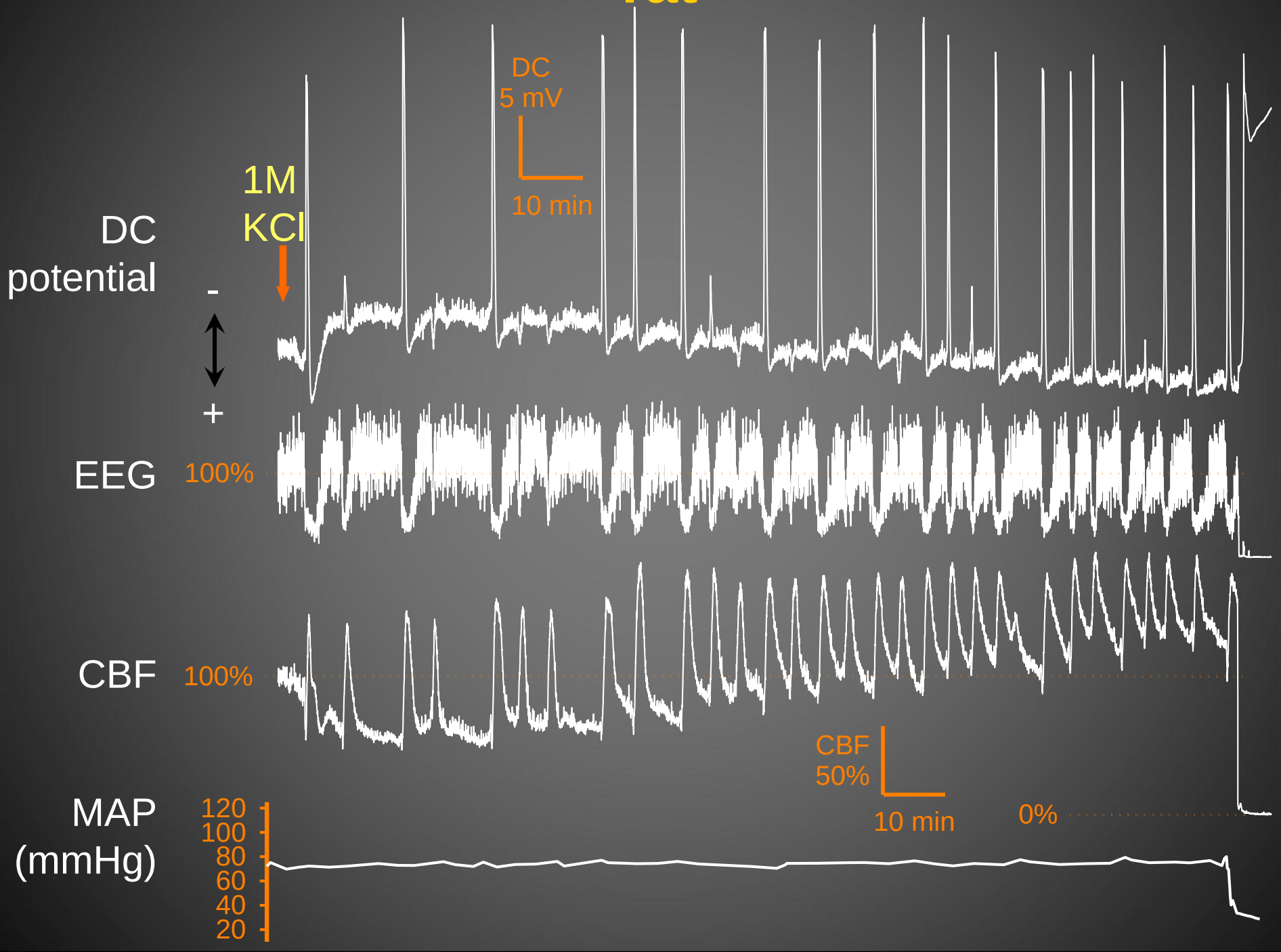
100 mg/kg - 48 hr

Enhanced hyperemic responses

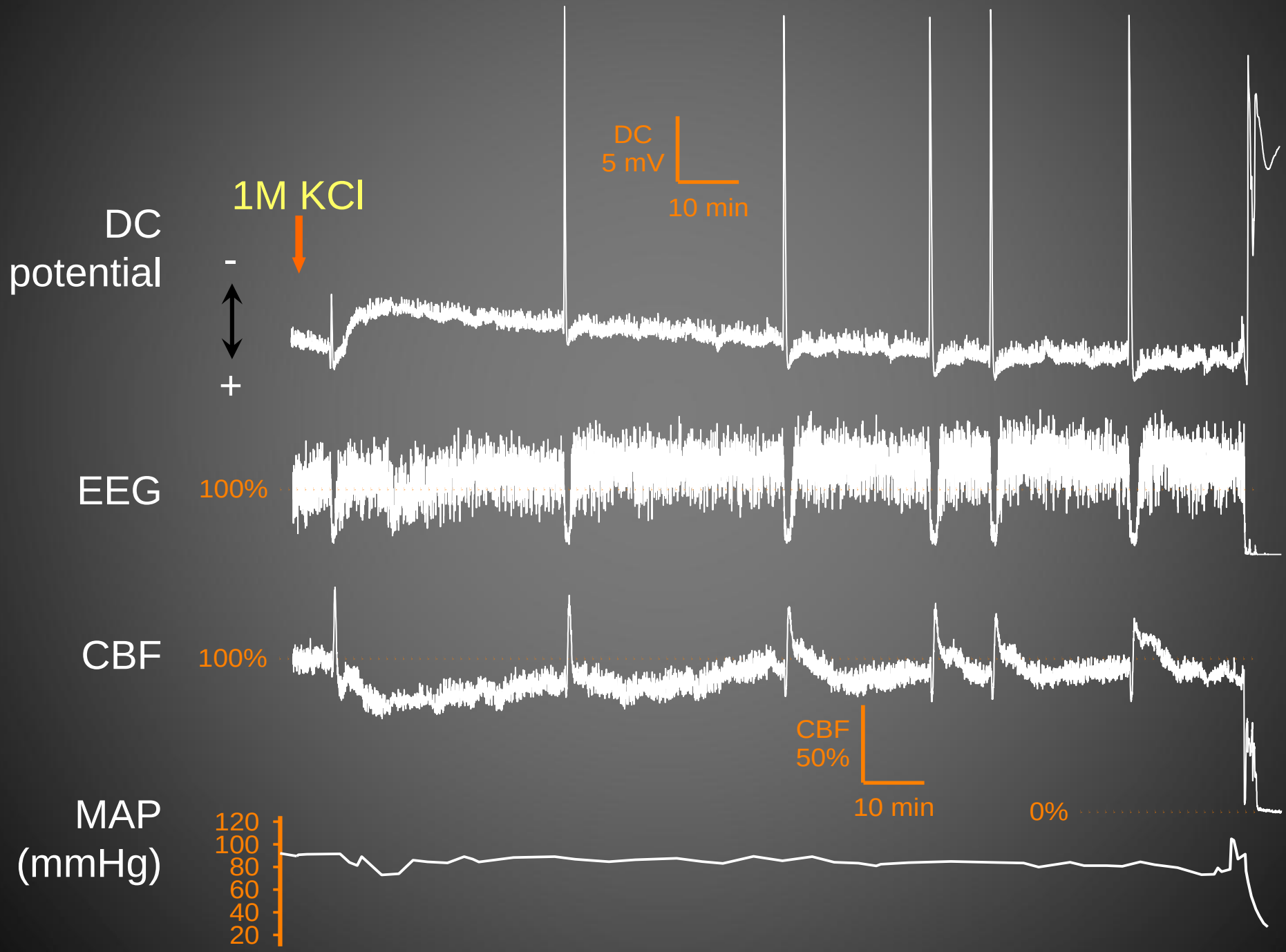


100 mg/kg - 96 hr

Representative experiment: young rat



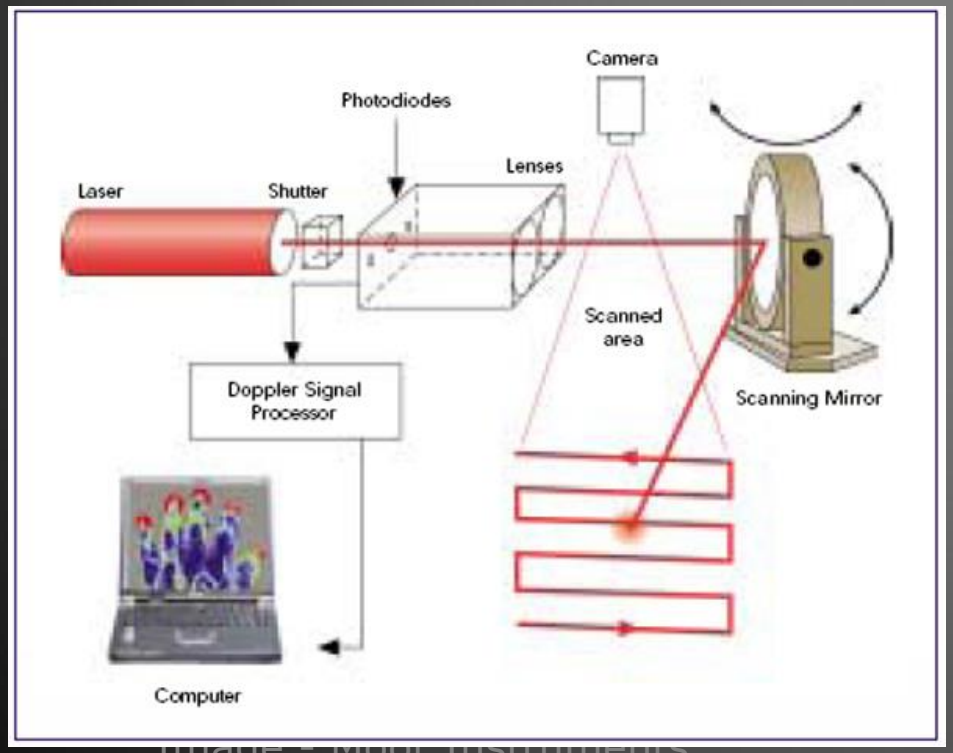
Representative experiment: old rat

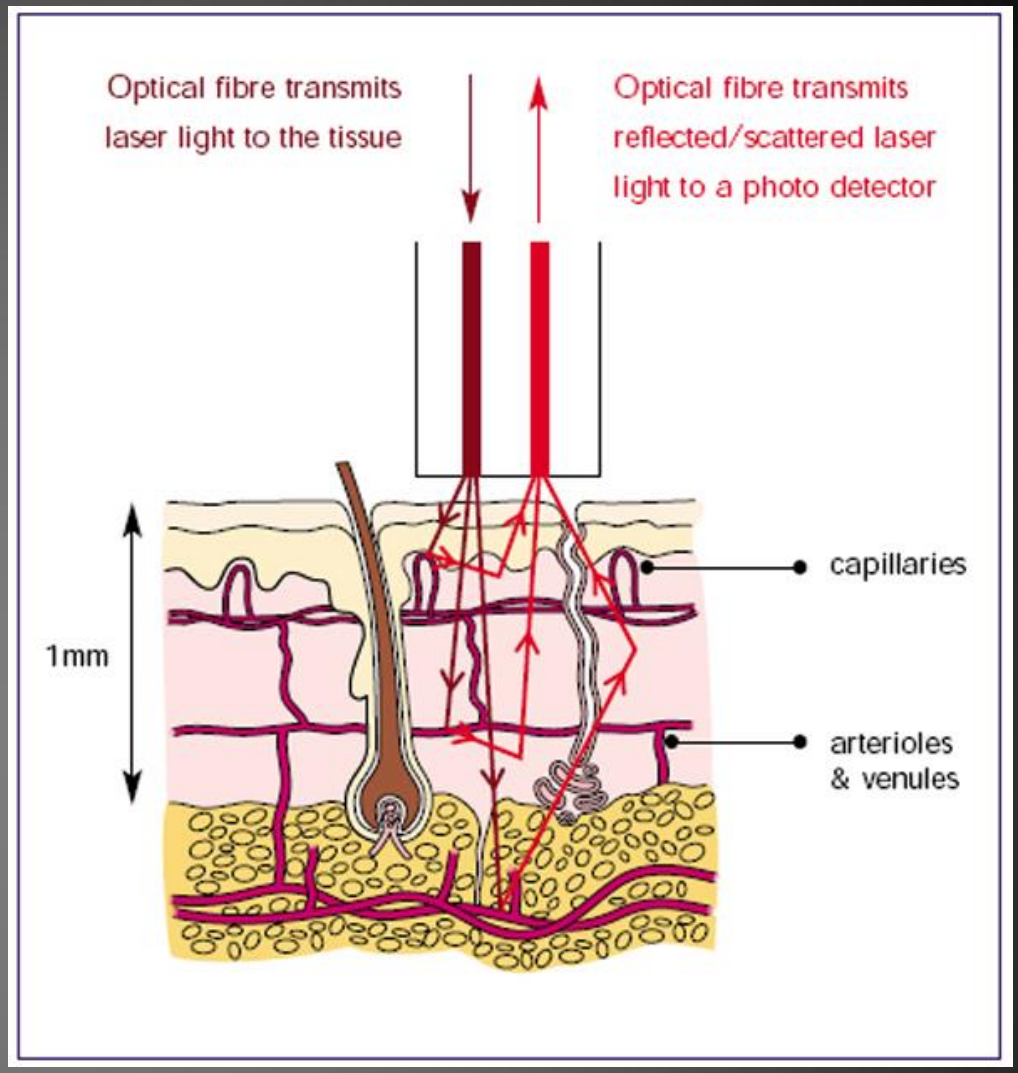


Single point blood flow imaging

Originally single point measurement system, measuring doppler shift from moving RBCs (20Hz – 20KHz)

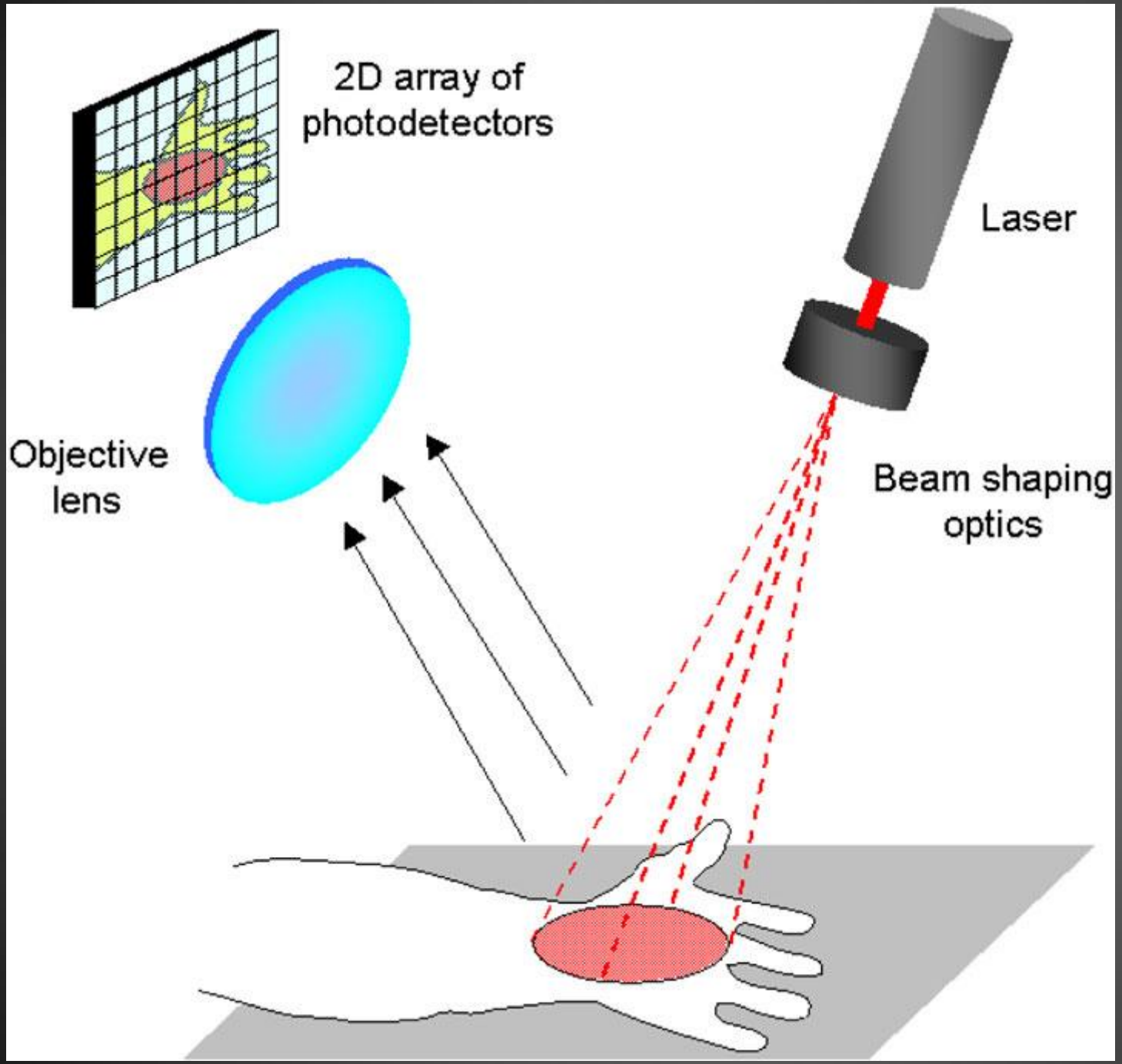
Scanning System

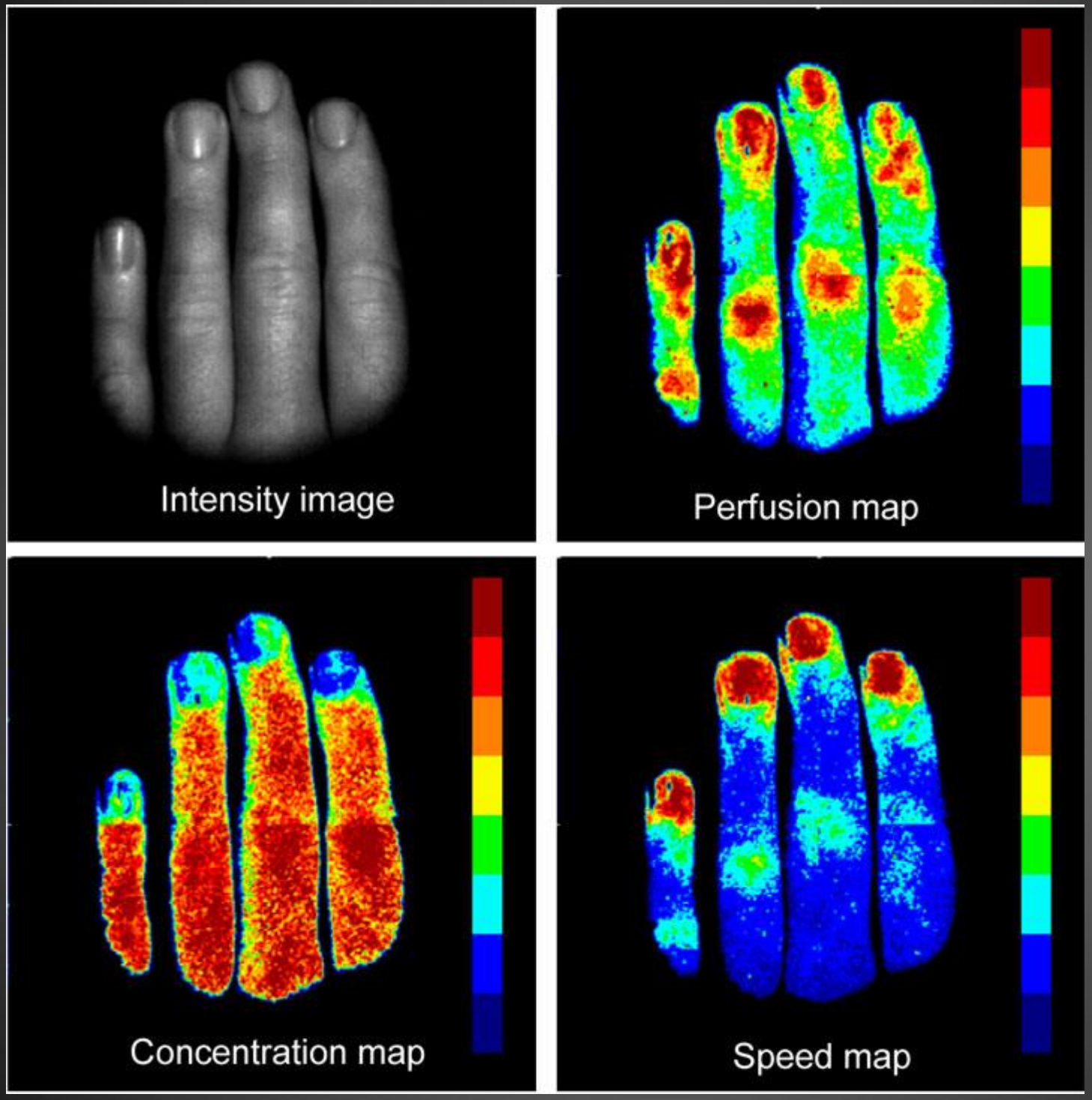




Builds up image point by point, slow

Image - Moor Instruments


2D array of
photodetectors
Laser
Objective
lens
Beam shaping
optics


Intensity image
Perfusion map
Concentration map
Speed map

Applications of LDF

1. **Post-operative monitoring of free tissue transfer**
   - Monitoring and quick recognition of disruption of flap perfusion reduces the flap failure.
   - (Burn depth assessment)
2. **Allergy patch testing, skin diseases research**
3. **Gastroenterology**
   - To assess blood flow of the gastric mucosa and disorders or to measure the effect of treatment intervention

4. Cerebral Blood Flow

- To assess of cerebral blood in head injury patients

5. Pharmacology Trials

- To assess the effects of topical or systemic vasoactive drugs on tissue blood flow

6. Tooth Vitality Testing

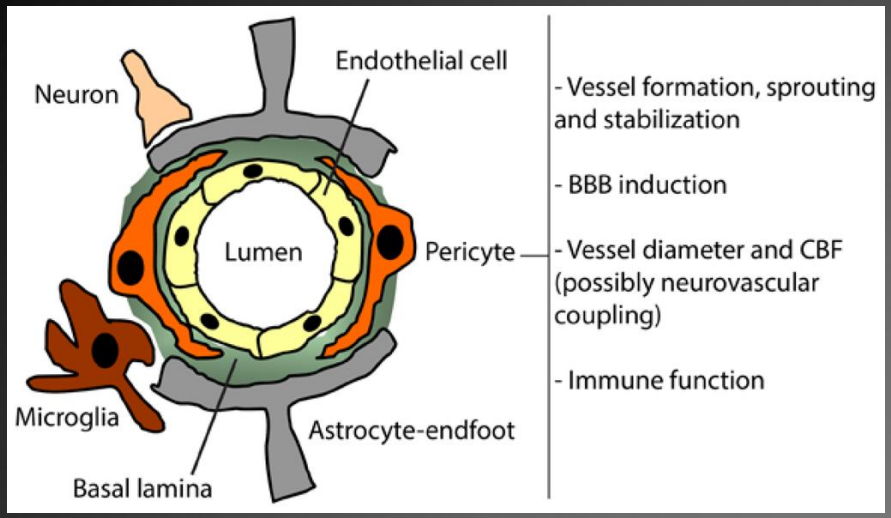
- To assess the blood flow pulsation in the pulp capillaries

7. Laboratory animal studies

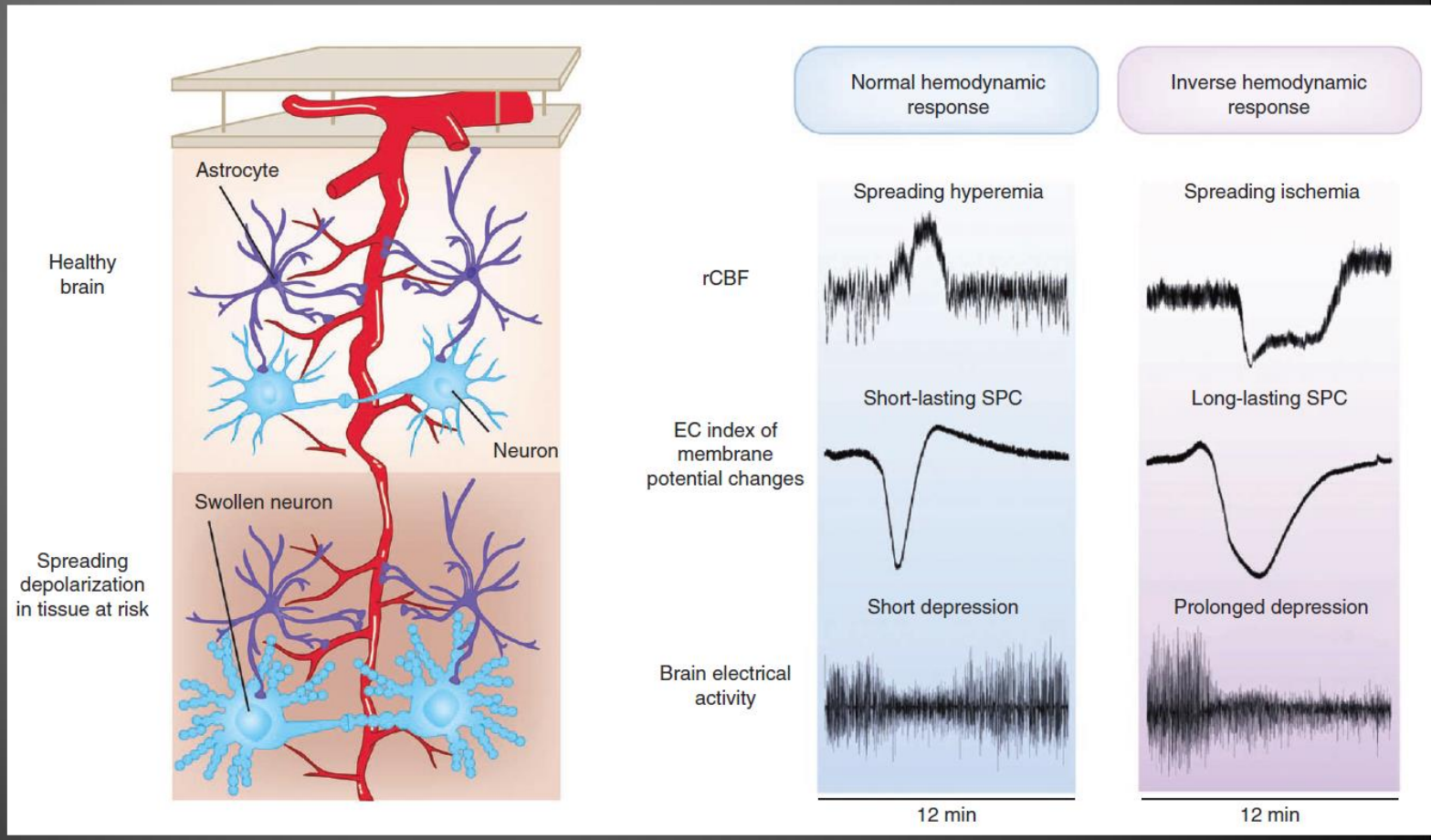
- For ocular, cerebral, cutaneous, auricular, splanchnic, and renal blood flow

Limitation of current LDF

- Currently LDF does not give an absolute measure of blood perfusion
  - Limiting factor in clinical setting
  - Not routinely used in health care

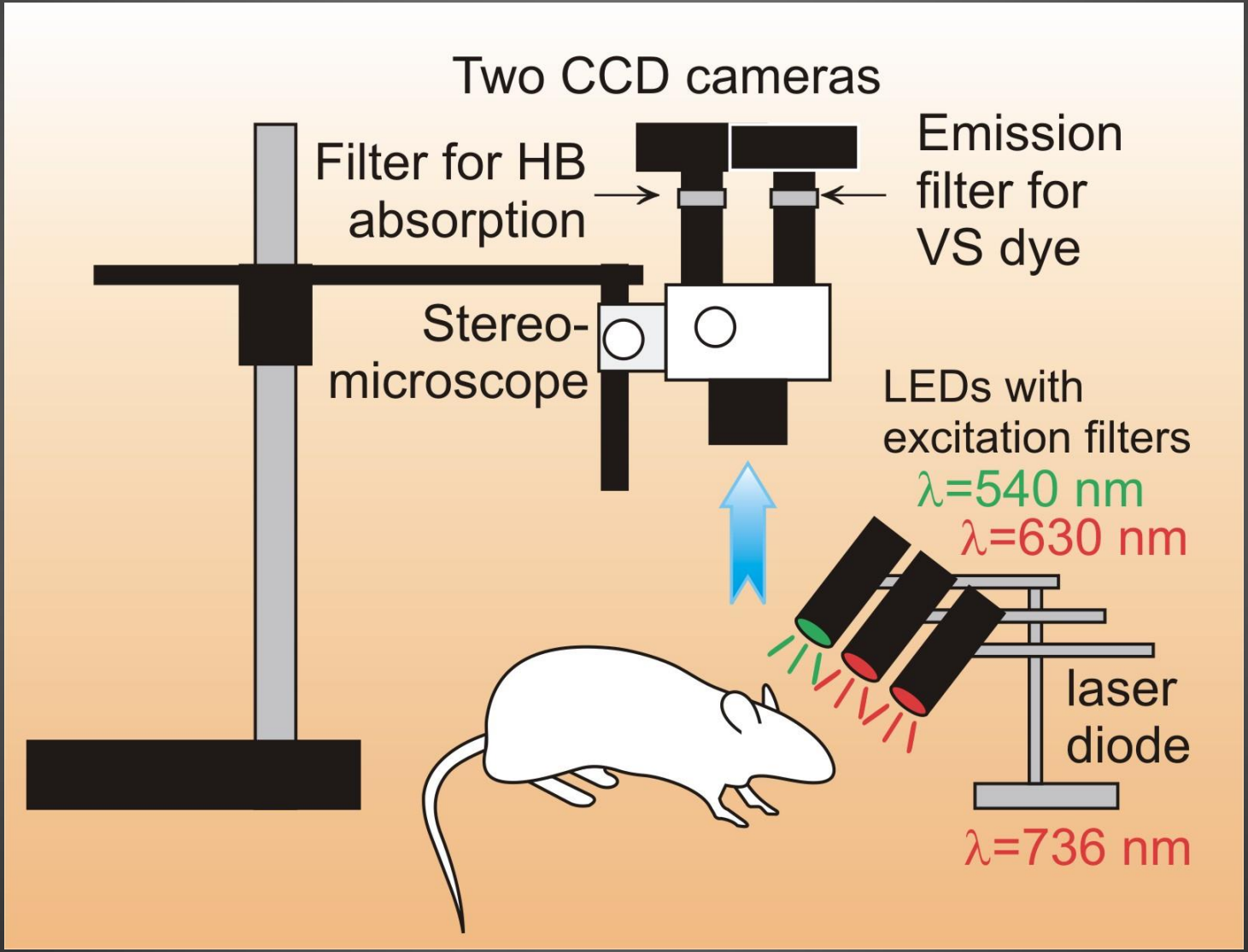


Neurovascular coupling and spreading depolarization in the injured brain

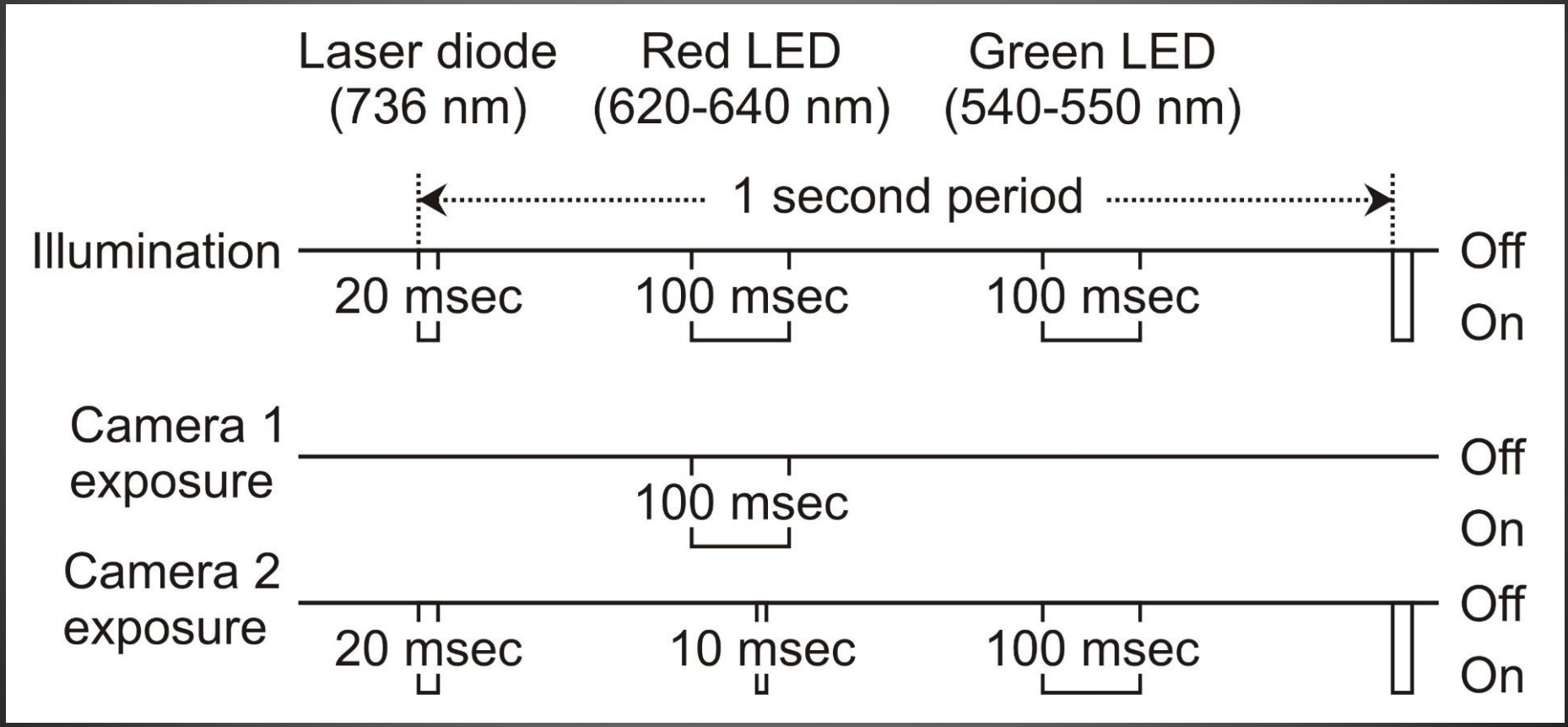


Dreier (2011) Nat. Med.

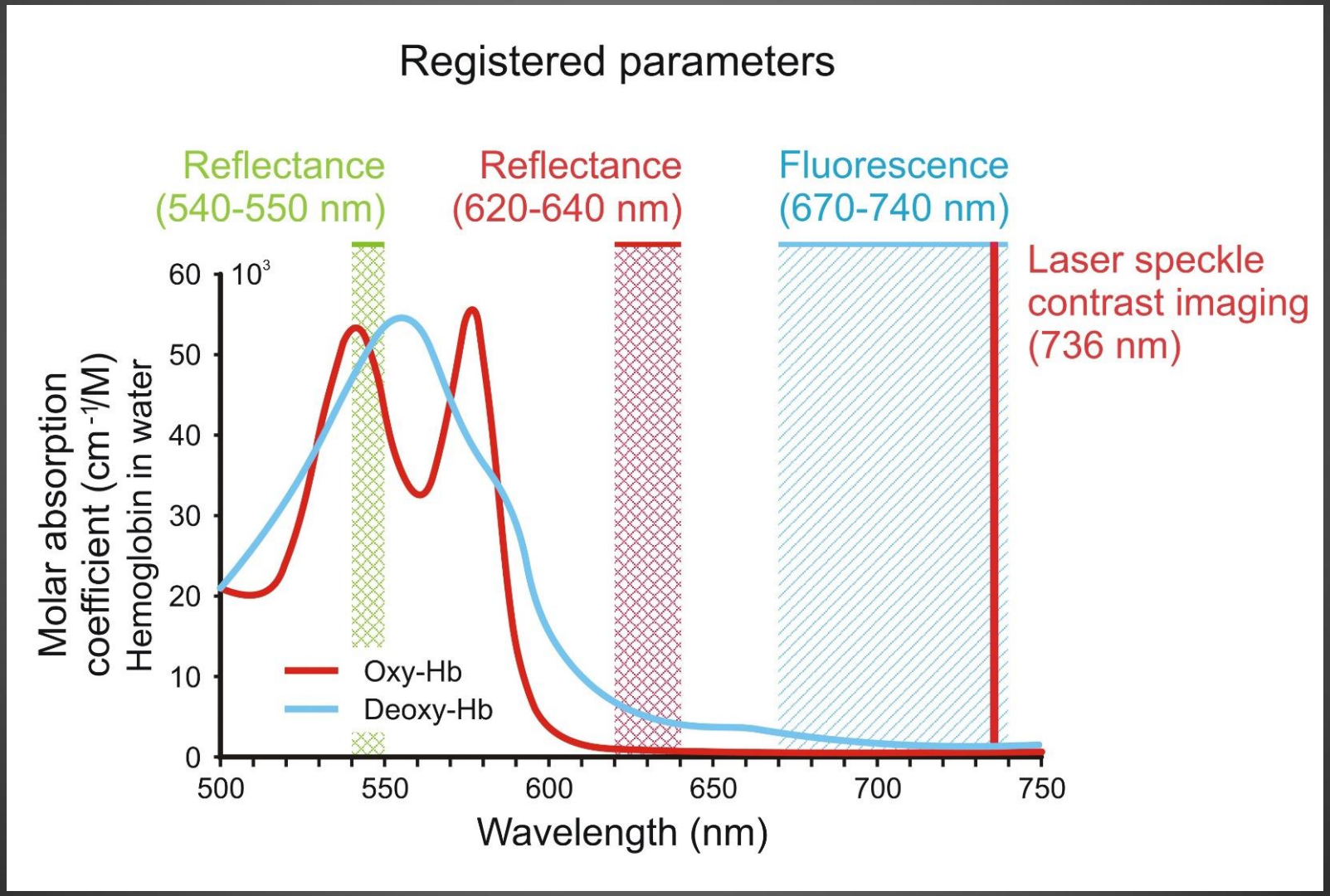
Draft of the arrangement of the setup



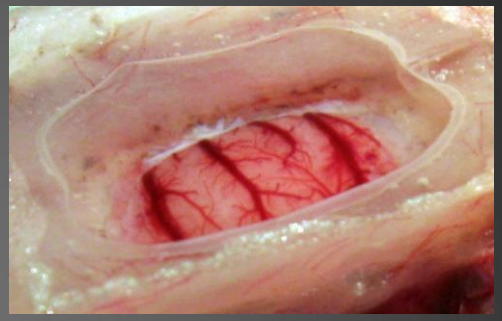
Synchronization of the respective illumination/image capture

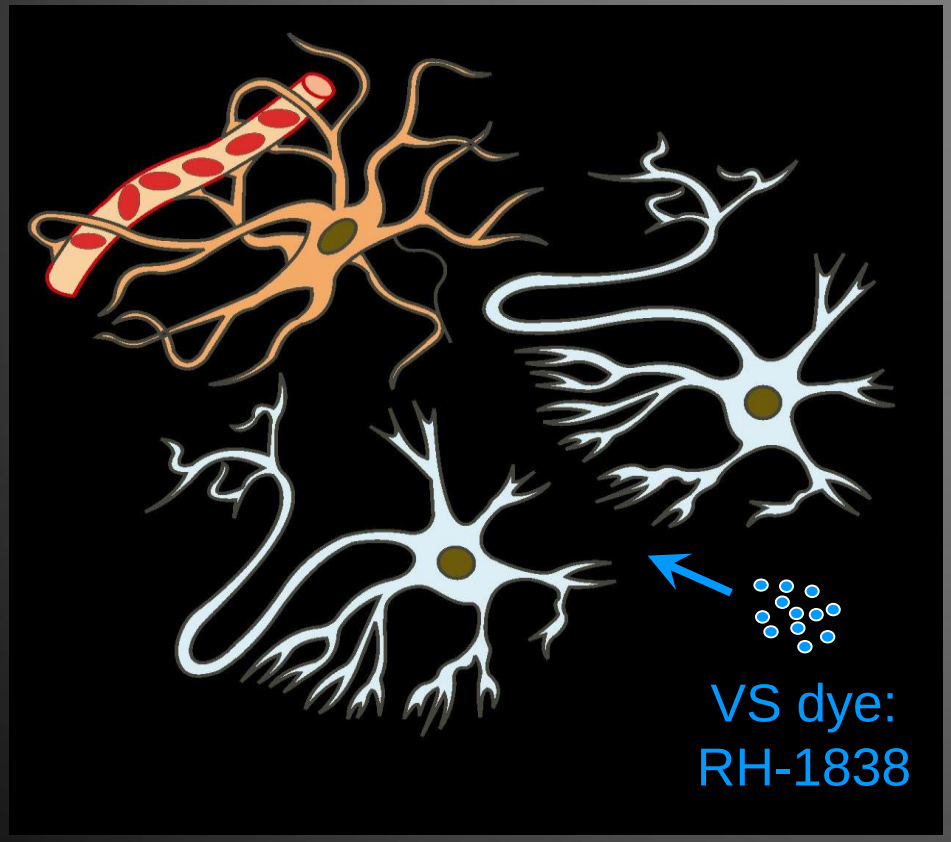


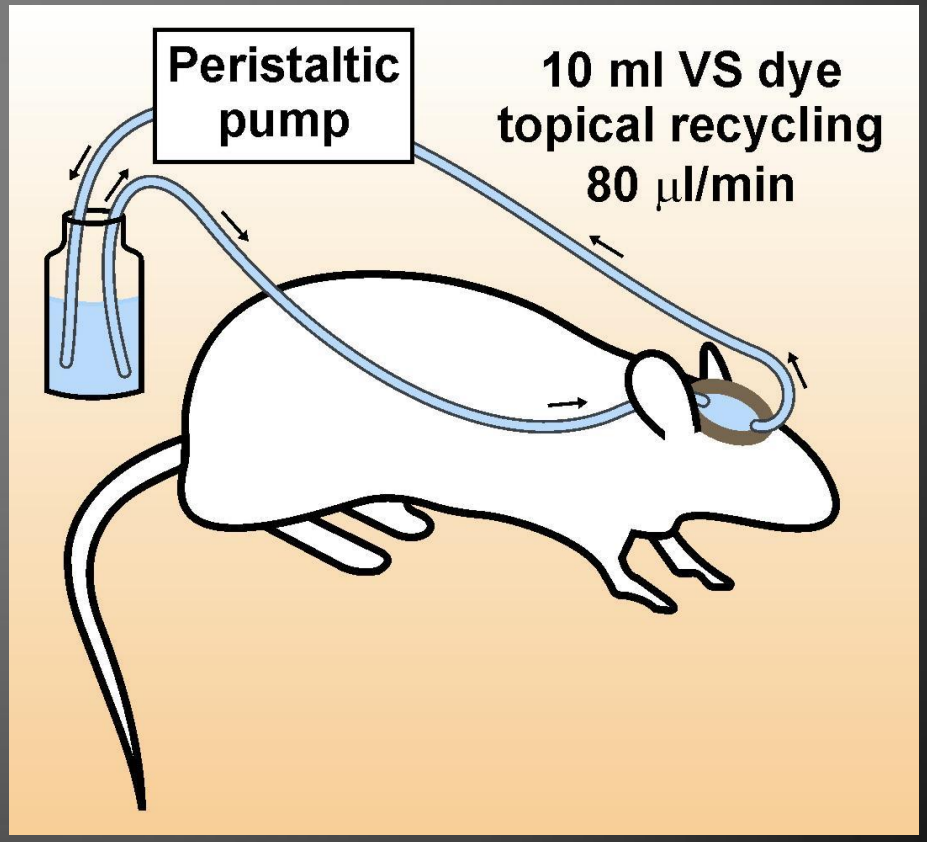
Optical principles for multimodal imaging



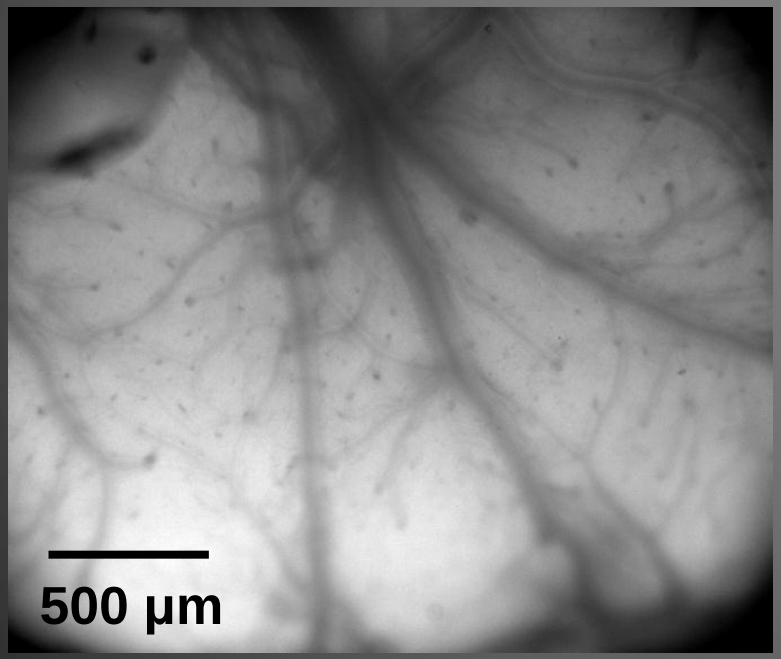
Voltage sensitive dye loaded in a closed cranial window

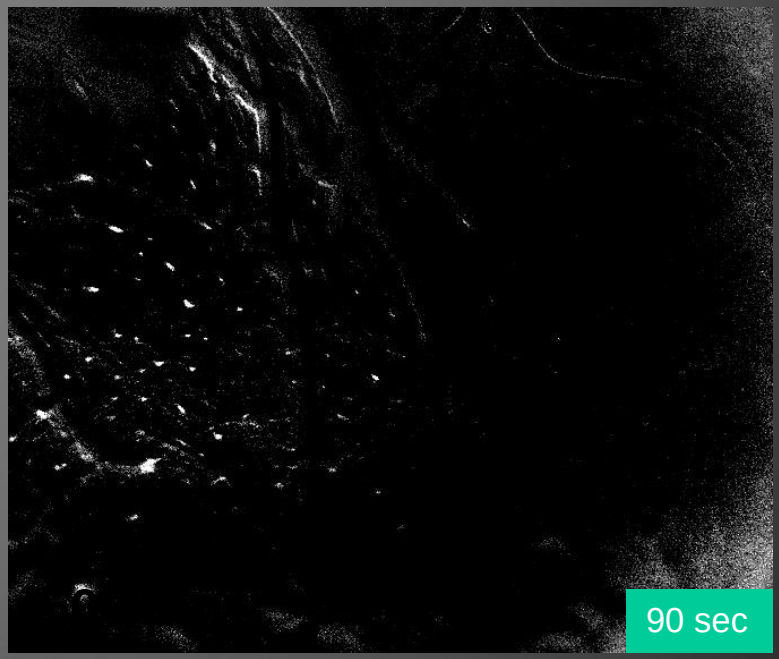






Representative video for SD-related changes in VS dye fluorescence





Exp. code: imag55, SD1

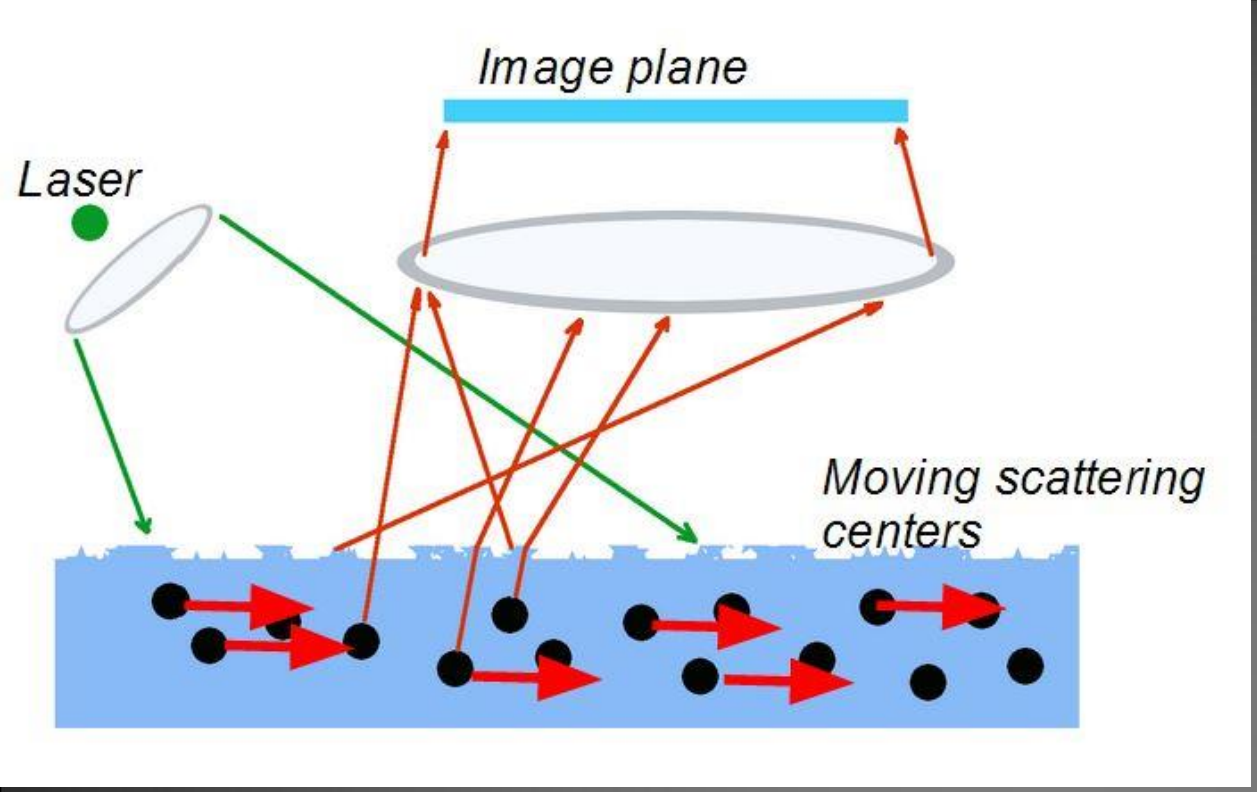
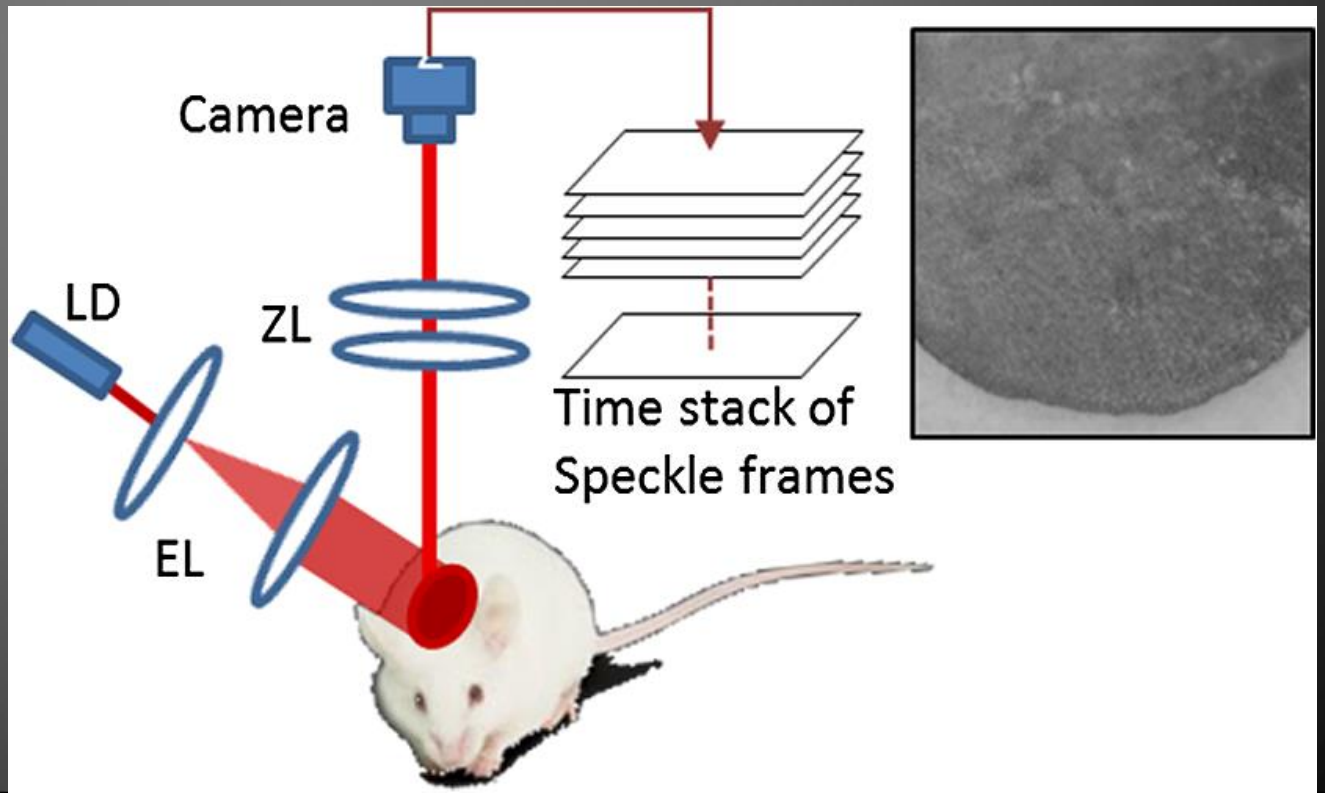
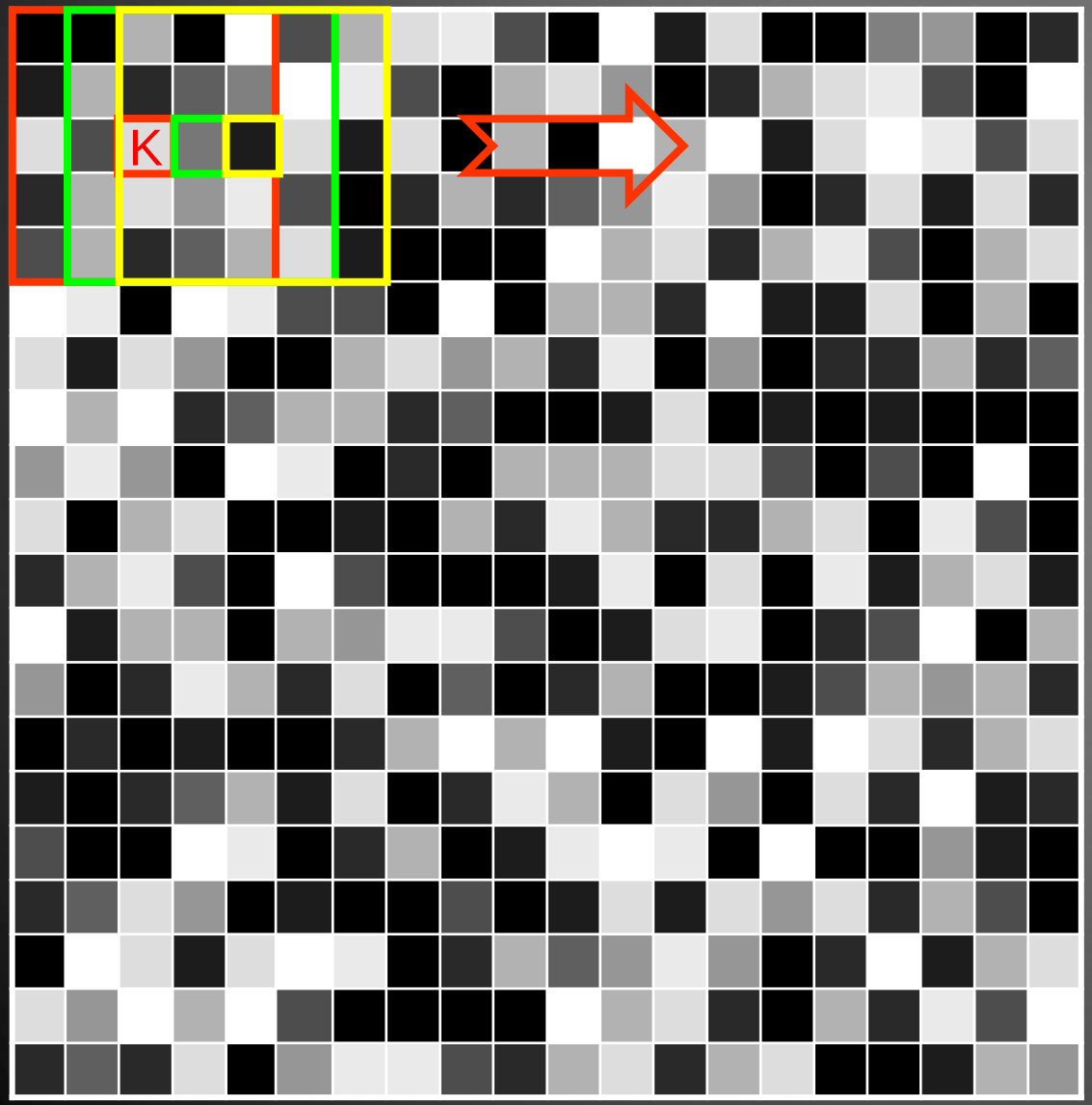


Image plane
Laser
Moving scattering centers


Camera
LD
ZL
EL
Time stack of
Speckle frames

Laser speckle contrast analysis

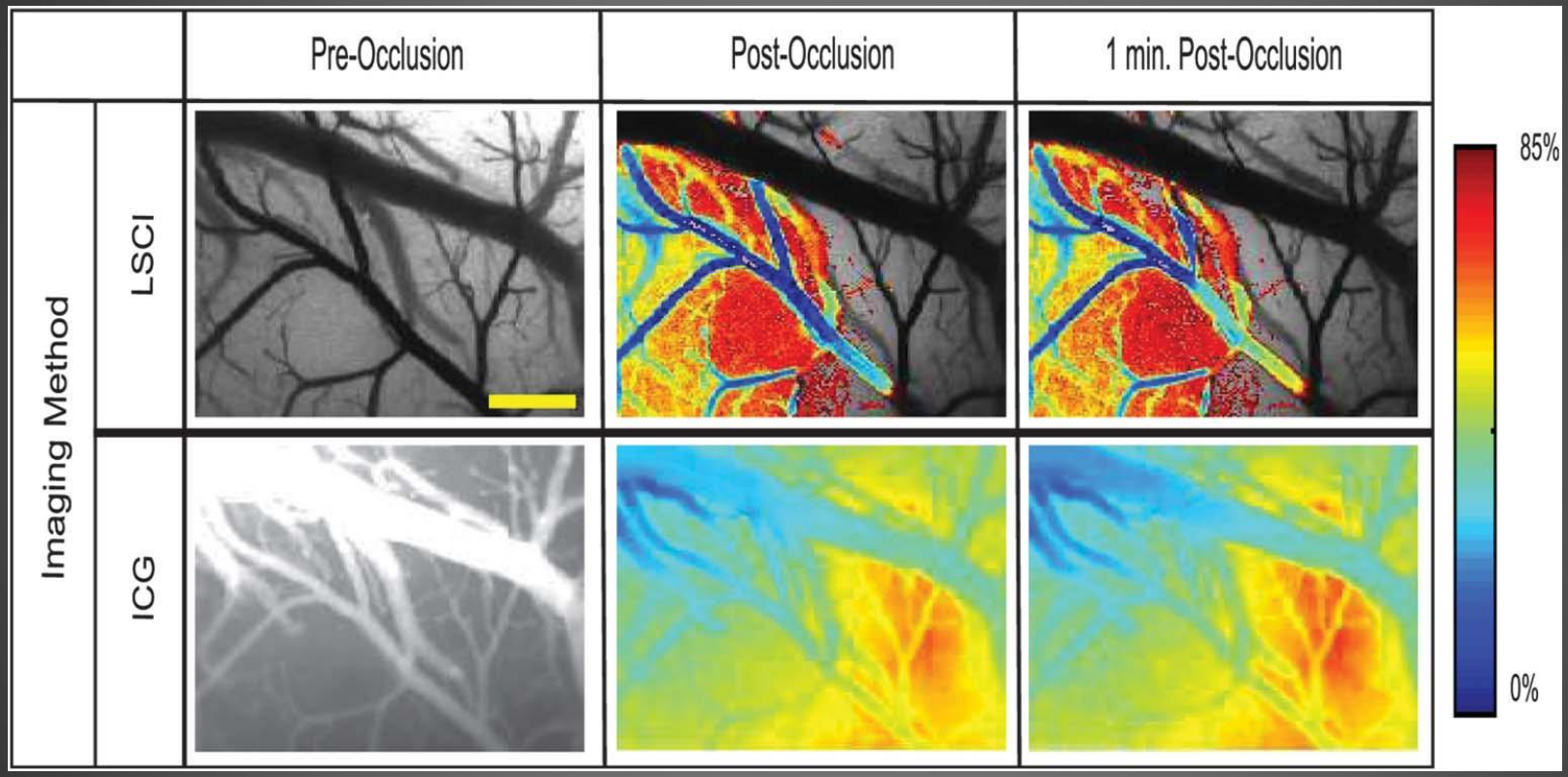


Average gray level (5x5 matrix): (I)
Standard deviation: σ
Speckle contrast: K

$$K = \frac{\sigma}{\langle I \rangle}$$

⇨ $1/K^2$

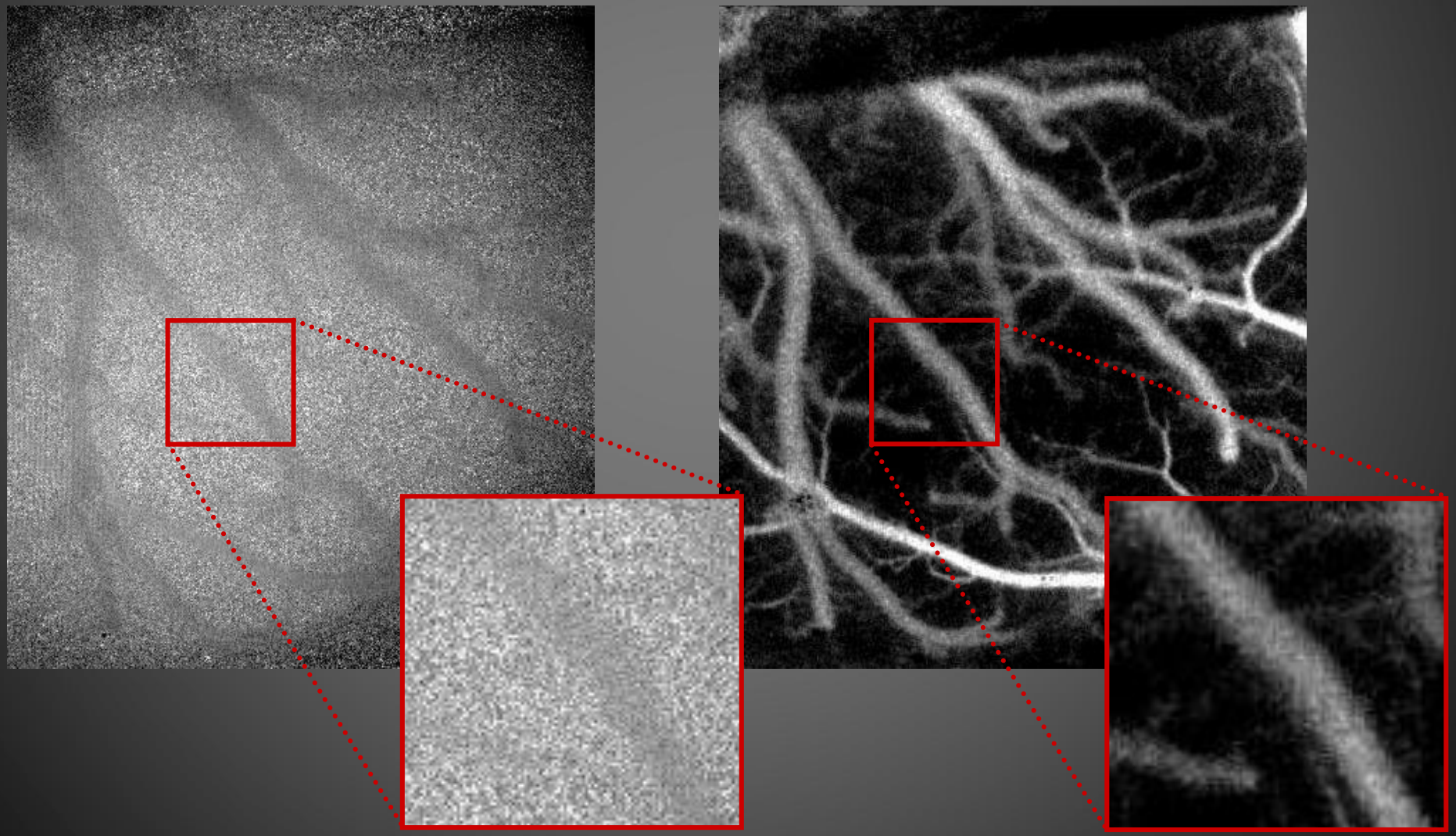
Particles with low motility➙ high contrast
Particles with high velocity ➙ low contrast
⇨ The velocity of particles is proportional with:
- the decrease in speckle contrast
- (time of exposure)

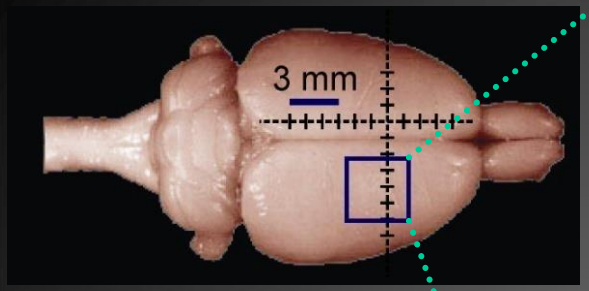

Pre-Occlusion
Post-Occlusion
1 min. Post-Occlusion
Imaging Method
LSCI
ICG
85%
0%

Acquired images

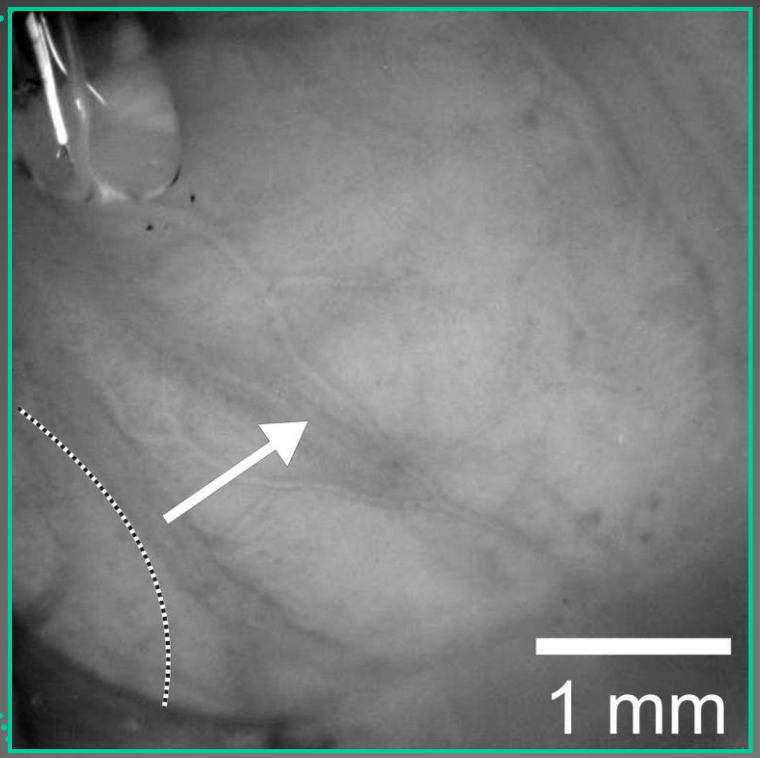
raw speckle image

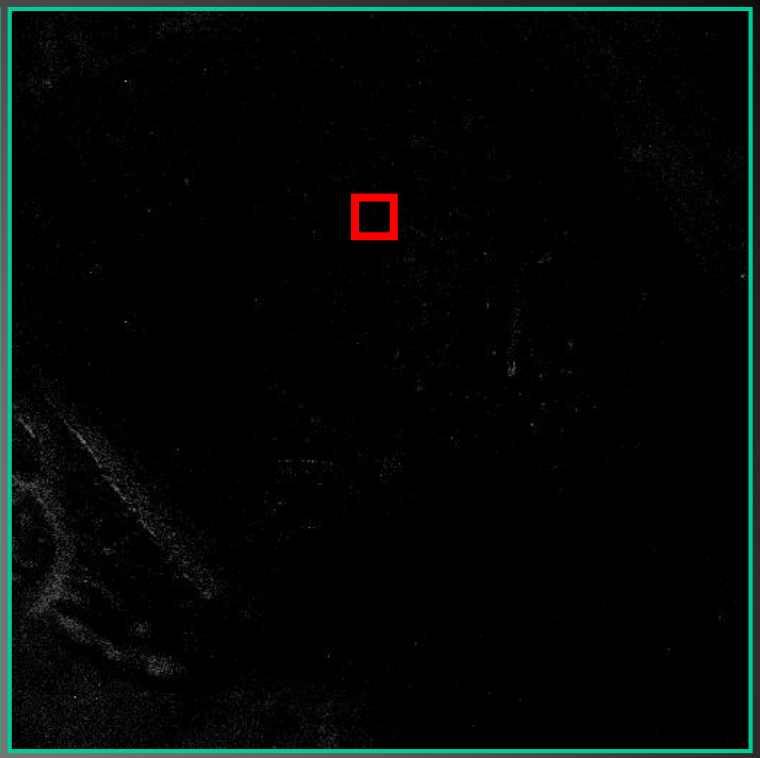
flow map

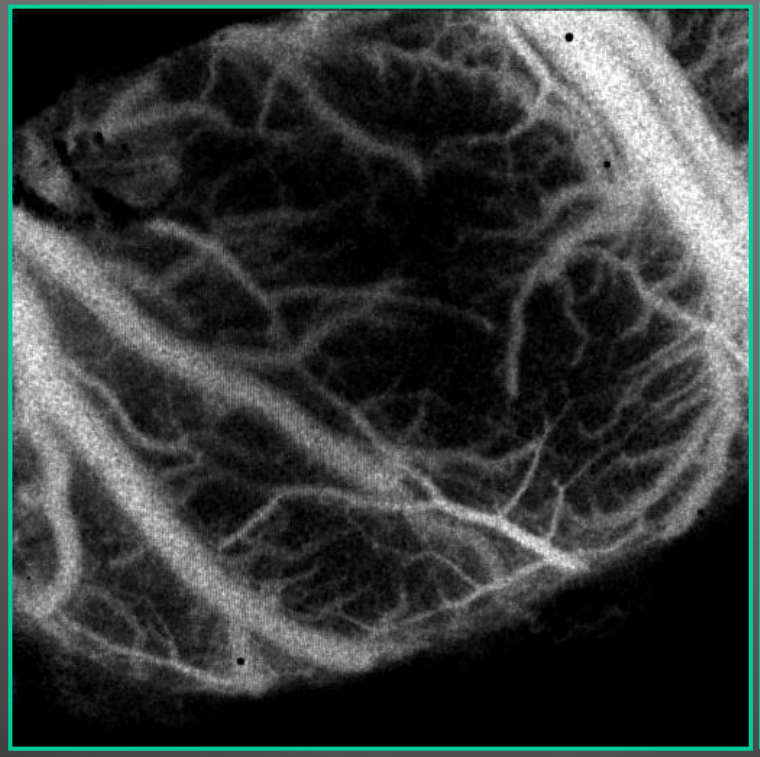


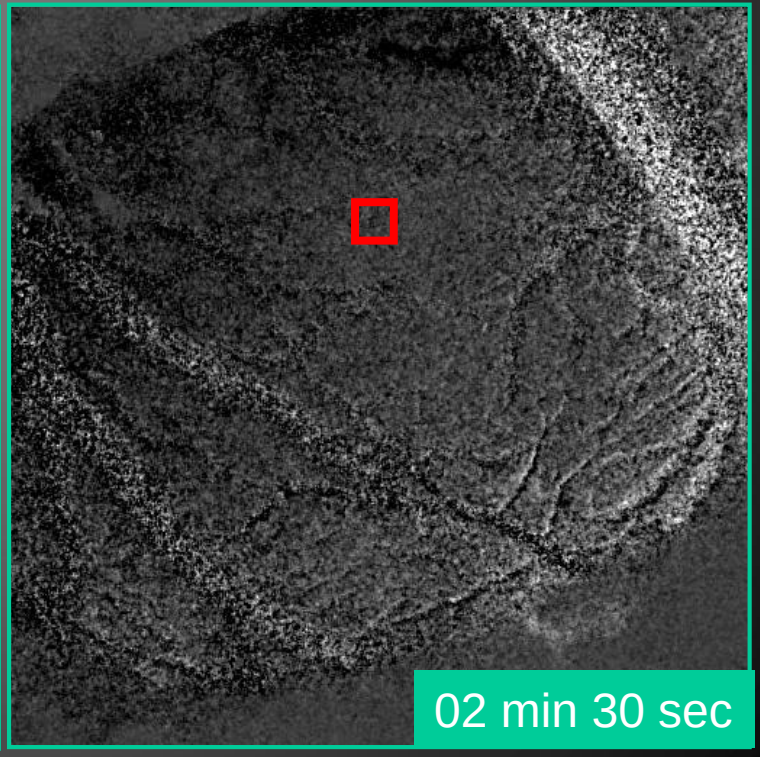


Simultaneous imaging of CSD and the CBF response









Exp. code: VSLS07 CSD1

Obrenovitch et al (2009)

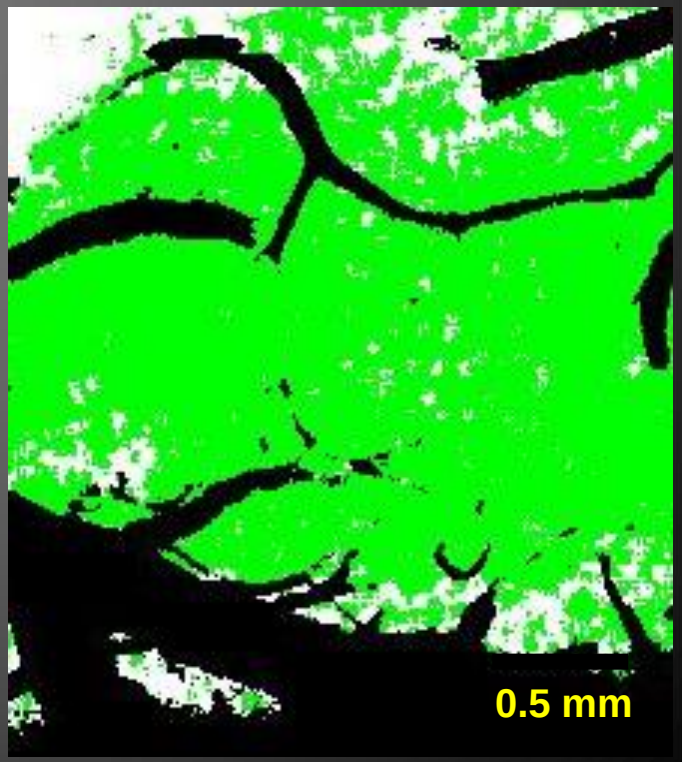
Whole field analysis of the VS dye signal

Area terminally depolarized in various age groups

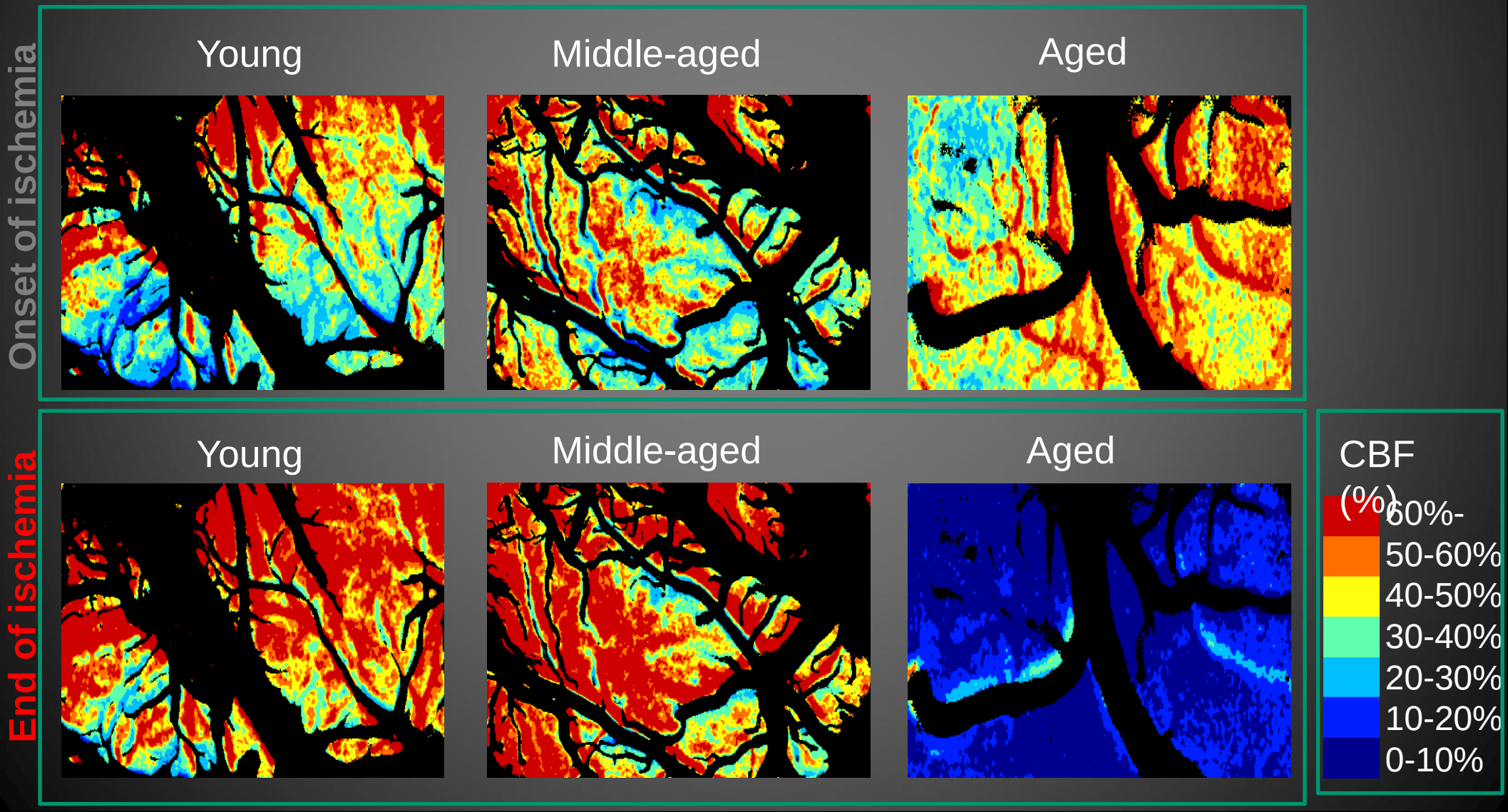
Young

Middle-aged

Aged



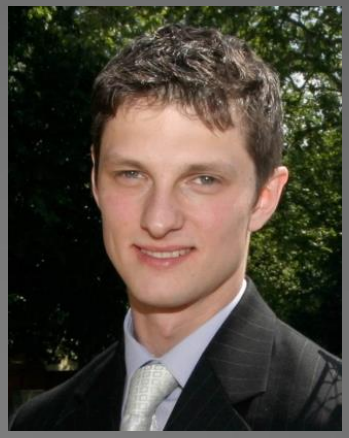
Whole field analysis of cerebral blood flow maps



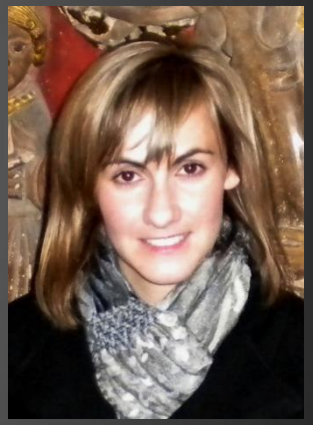
Acknowledgements

Prof. Tihomir Obrenovitch

University of Bradford U.K.



Ferenc Domoki, MD, PhD



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Eszter Farkas PhD

Adam Institoris MD, PhD

Zsófia Bere MD

Gabor Kozák MSc, MD

Real time measurement-
a window towards
the dynamics of cerebrovascular regulation

Autoregulation-range and dynamics under various circumstances

Rhythmic patterns in the microcirculation-vasomotion

Neurovascular coupling