What did we learn about microcirculation using lasers

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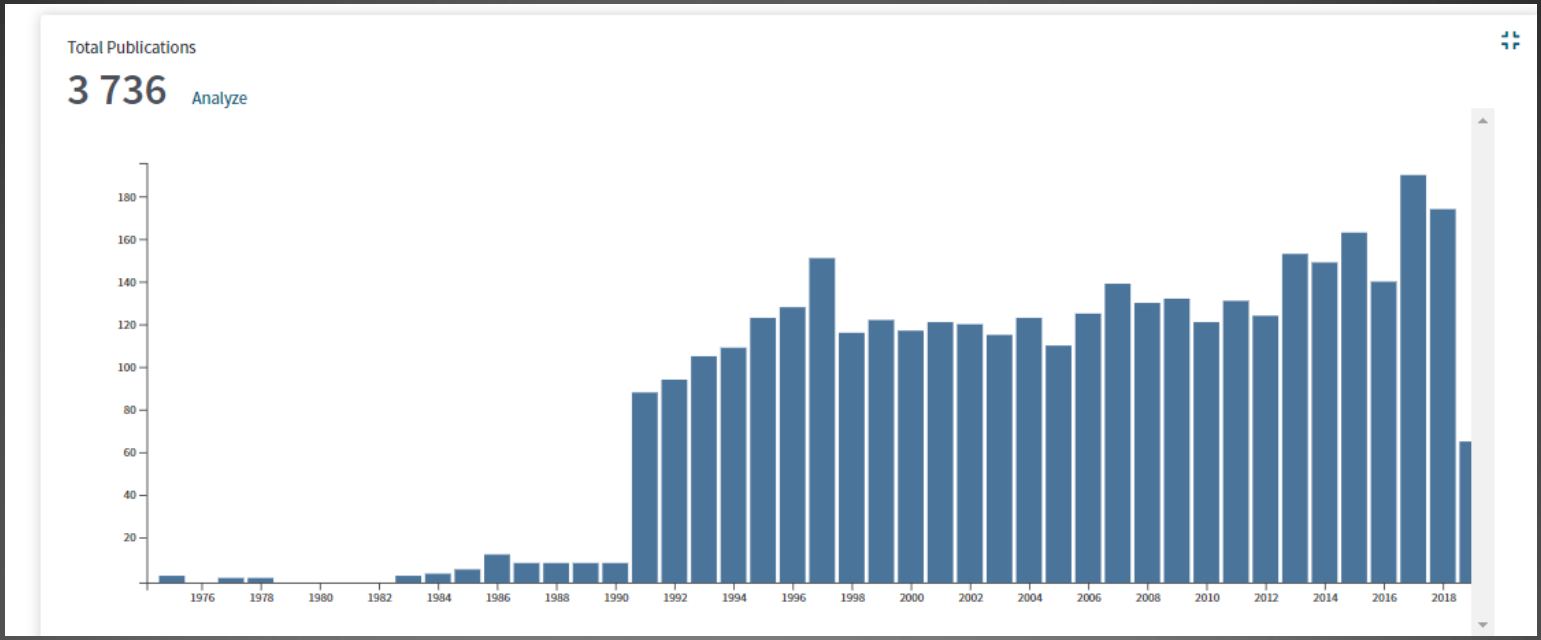
Lamelis 2019, Szeged, 4 July

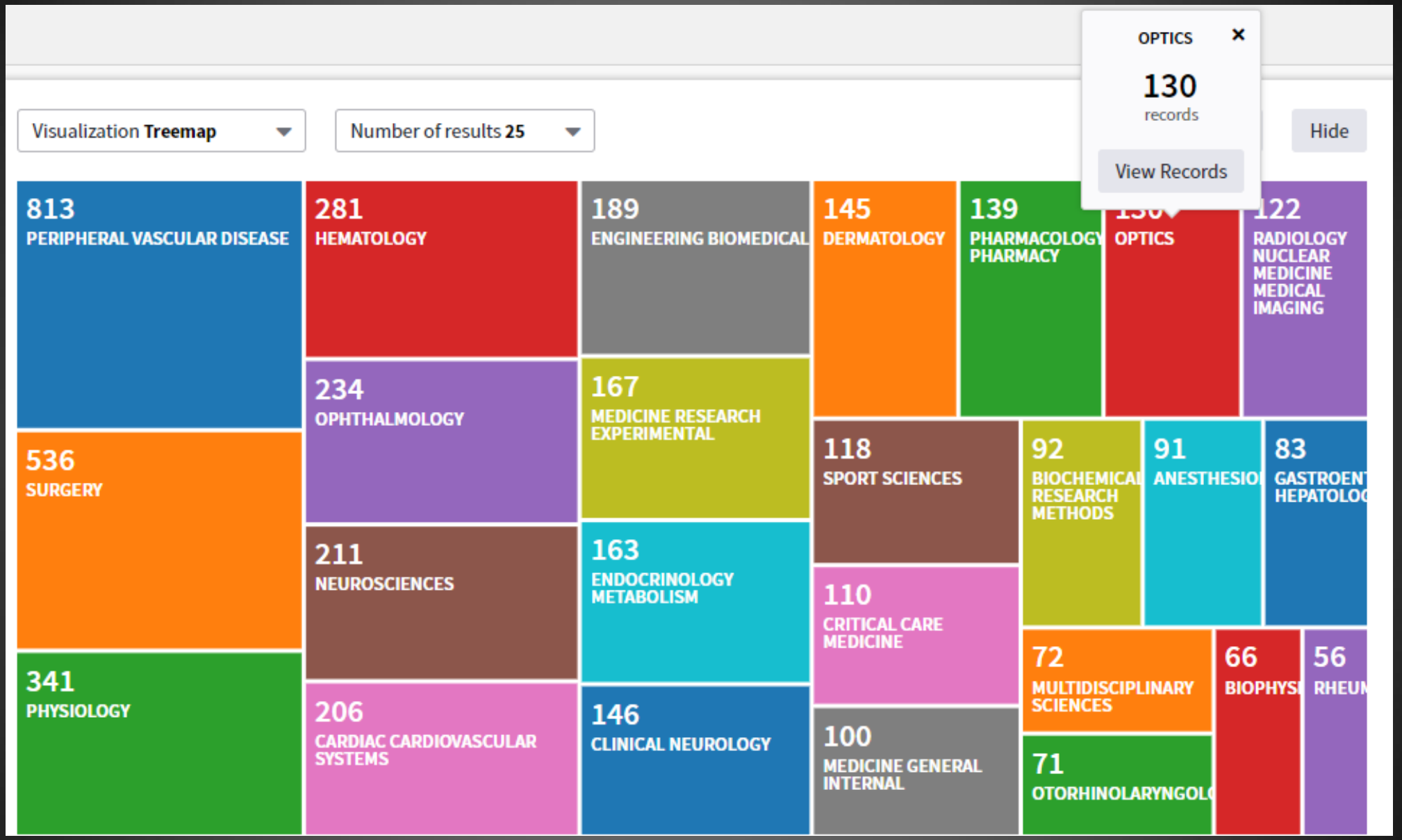
Laser AND microcirculation (3736 papers) (mainly laser Doppler)



Citation to „Laser AND microcirculation”

Sum of Times Cited:80 946

1.	**Vascular channel formation by human melanoma cells in vivo and in vitro: Vasculogenic mimicry** By: Maniotis, AJ; Folberg, R; Hess, A; et al. AMERICAN JOURNAL OF PATHOLOGY Volume: 155 Issue: 3 Pages: 739-752 Published: SEP 1999	70	78	87	79	43	1138	54.19
2.	**Biomedical photoacoustic imaging** By: Beard, Paul INTERFACE FOCUS Volume: 1 Issue: 4 Pages: 602-631 Published: AUG 6 2011	104	131	139	141	43	749	83.22
3.	**Dynamic imaging of cerebral blood flow using laser speckle** By: Dunn, AK; Bolay, T; Moskowitz, MA; et al. JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM Volume: 21 Issue: 3 Pages: 195-201 Published: MAR 2001	33	37	31	38	13	531	27.95
4.	**Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin** By: Falati, S; Liu, QD; Gross, P; et al. JOURNAL OF EXPERIMENTAL MEDICINE Volume: 197 Issue: 11 Pages: 1585-1598 Published: JUN 2 2003	22	29	31	12	7	527	31.00
5.	**Real-time in vivo imaging of platelets, tissue factor and fibrin during arterial thrombus formation in the mouse** By: Falati, S; Gross, P; Merrill-Skoloff, G; et al. NATURE MEDICINE Volume: 8 Issue: 10 Pages: 1175-1180 Published: OCT 2002	21	33	19	22	10	447	24.83


OPTICS
130
records
View Records
Visualization Treemap
Number of results 25
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813
PERIPHERAL VASCULAR DISEASE
281
HEMATOLOGY
189
ENGINEERING BIOMEDICAL
145
DERMATOLOGY
139
PHARMACOLOGY PHARMACY
OPTICS
122
RADIOLOGY NUCLEAR MEDICINE MEDICAL IMAGING
536
SURGERY
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OPHTHALMOLOGY
167
MEDICINE RESEARCH EXPERIMENTAL
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SPORT SCIENCES
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BIOCHEMICAL RESEARCH METHODS
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GASTROENT HEPATOLOG
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NEUROSCIENCES
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ENDOCRINOLOGY METABOLISM
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CRITICAL CARE MEDICINE
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PHYSIOLOGY
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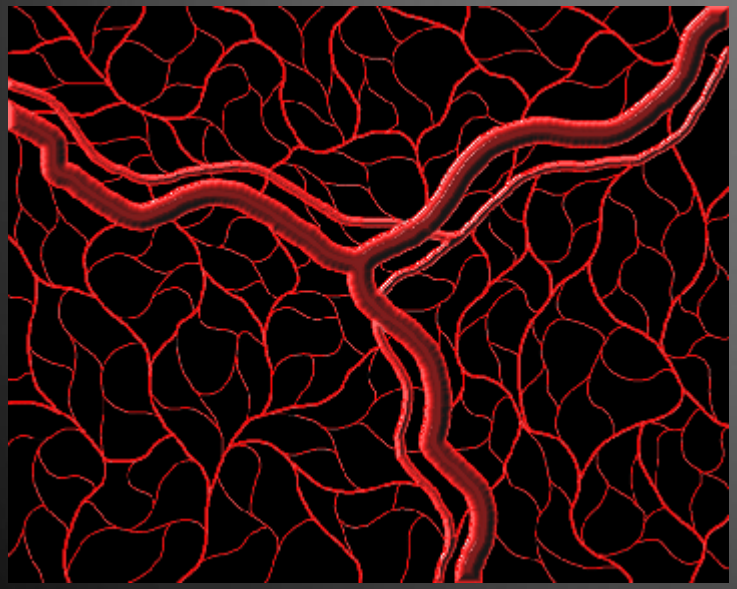
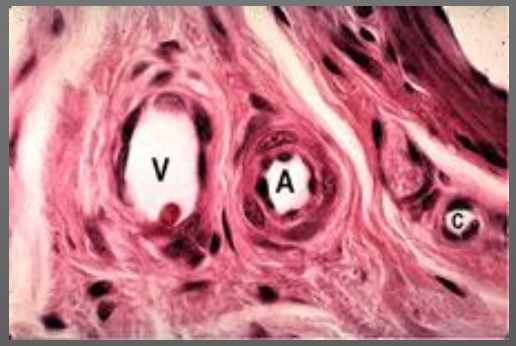
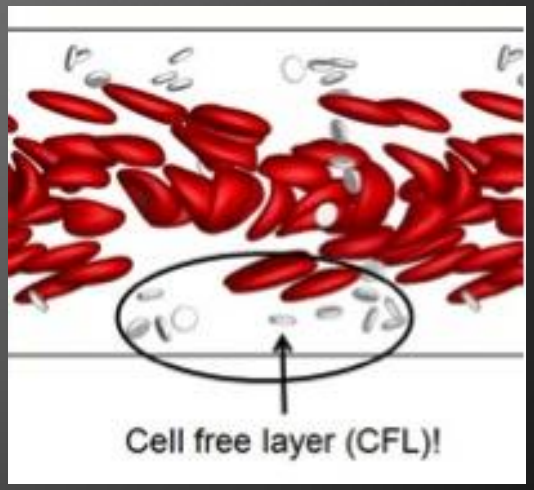
CARDIAC CARDIOVASCULAR SYSTEMS
146
CLINICAL NEUROLOGY
100
MEDICINE GENERAL INTERNAL
72
MULTIDISCIPLINARY SCIENCES
66
BIOPHYSI
56
RHEUM
71
OTORHINOLARYNGOL

The microcirculation

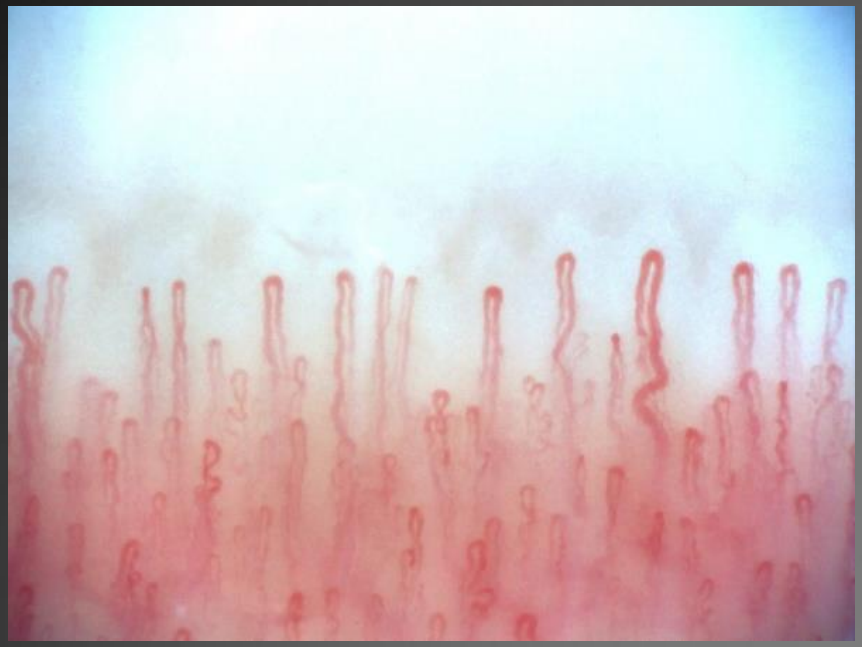
- The term microcirculation refers to the functions of the capillaries and the neighboring lymphatic vessels.
- 5 % of circulating blood volume(250 ml) is present in the capillaries at any given time.
- This takes part into the exchange of nutrients, gases and waste products between the blood & tissues.

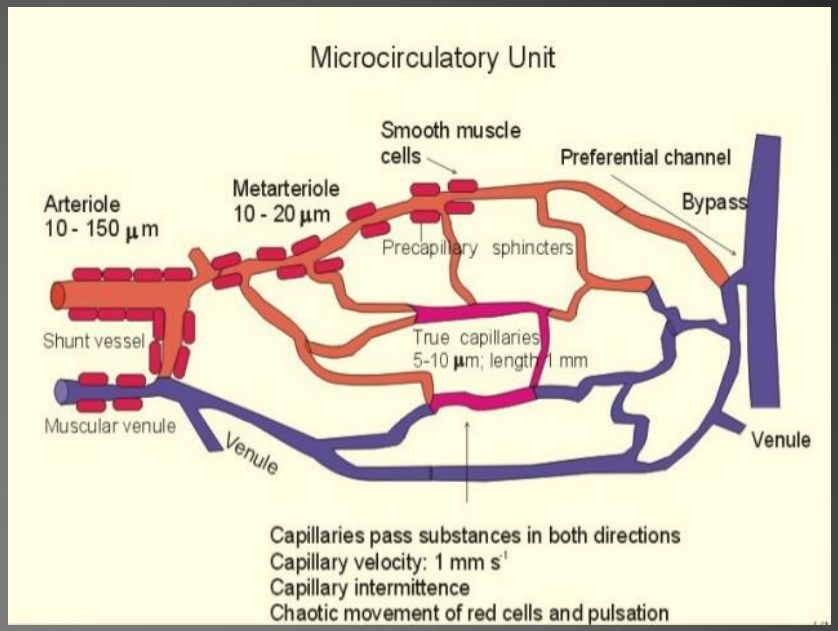


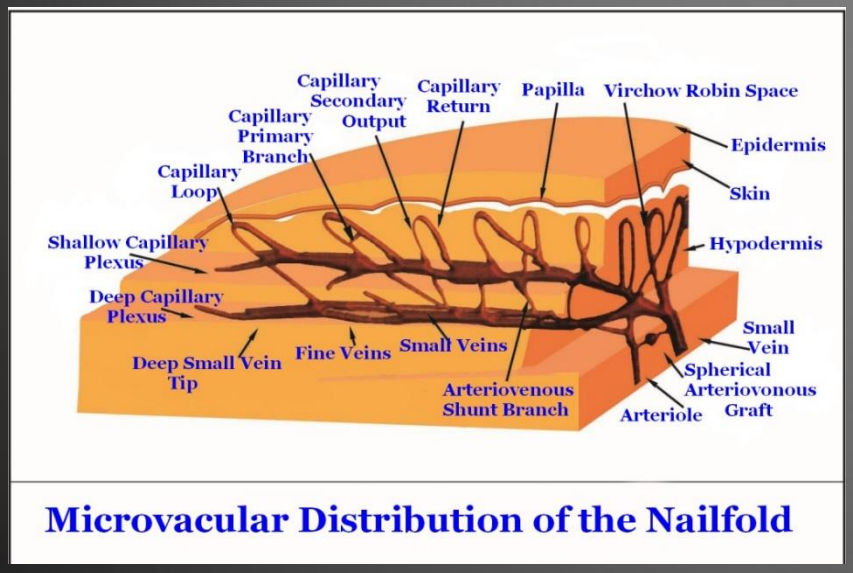




The microcirculation







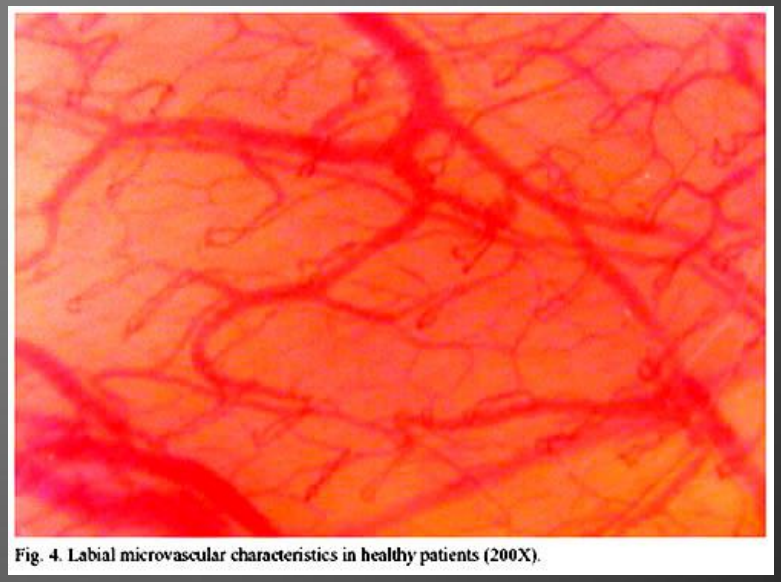


Fig. 4. Labial microvascular characteristics in healthy patients (200X).

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

Why is it important to know microvascular physiology & pathophysiology

- Almost all diseases (diabetes, cancer, hypertension, Alzheimer's disease, etc) have mircovascular components
- 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

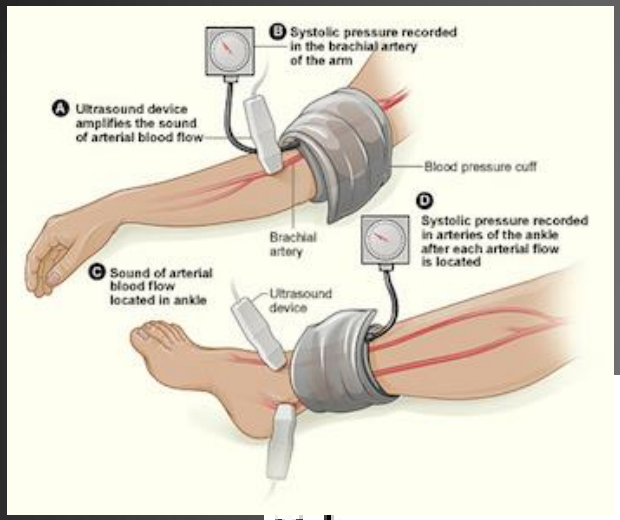
LOCAL CONTROL OF BLOOD FLOW

- **A) Autoregulation** – maintenance of constant blood flow to an organ in spite of fluctuations in BP.
  E.g. brain – auto regulation is best
  kidney – auto regulation is good
  skeletal muscle – auto regulation is poor

- **B) Active Hyperemia** - When any tissue becomes highly active [eg. Skeletal muscle during exercise]. the rate of blood flow through the tissue increases.

- **C) Reactive hyperemia**
  When blood flow to a tissue is blocked for few seconds and then is unblocked, the flow through tissue increases almost 4-7 times normal. The excess blood flow lasts long enough to repay the tissue oxygen deficit that has occurred during occlusion.

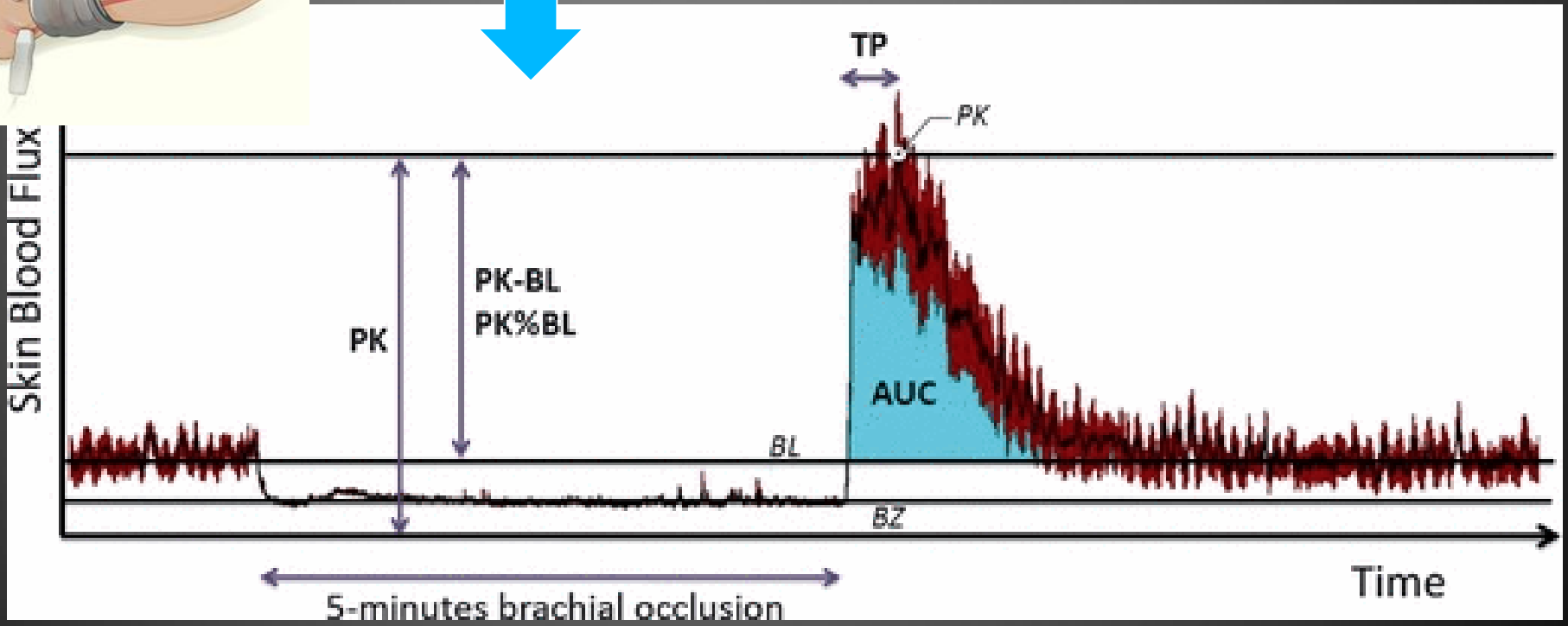
Non-invasive Assessment of Skin Microvascular Function in Humans: An Insight Into Methods



Detection by

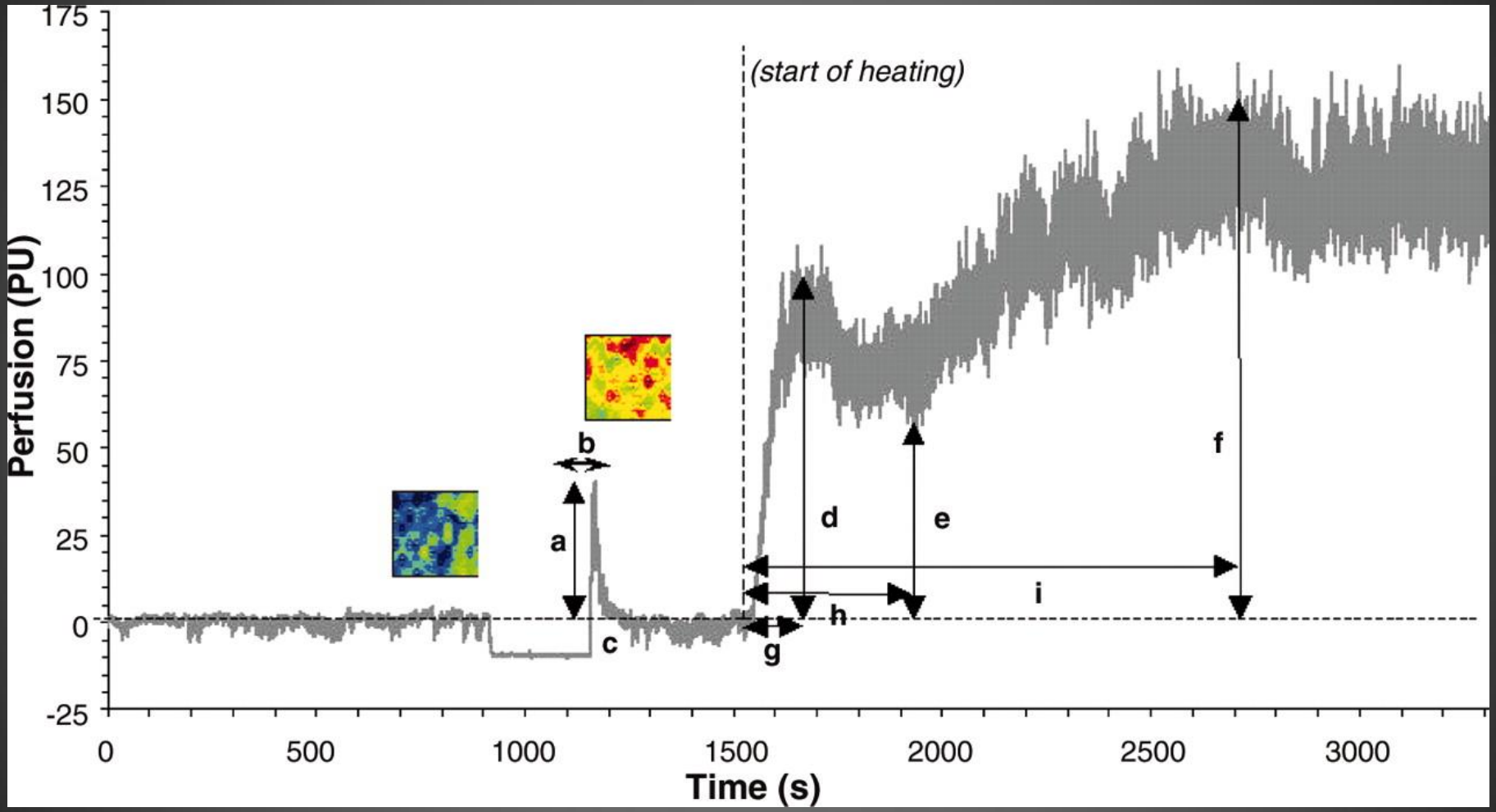
ultrasound

Laser Doppler



Microcirculation Volume 19, Issue 1, pages 47-64, 21 DEC 2011 DOI: 10.1111/j.1549-8719.2011.00129.x
http://onlinelibrary.wiley.com/doi/10.1111/j.1549-8719.2011.00129.x/full#f4

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

- **Two basic mechanisms that explain local control of blood flow**

1. Myogenic theory

Increase in blood flow

↓

Stretches the vessel

↓

Contraction of vascular smooth muscle

↓

Decrease blood flow back to normal

2. Metabolic theory

Increase in rate of metabolism

↓

Accumulation of vasodilator substances in active tissues

↓

Blood vessels dilate

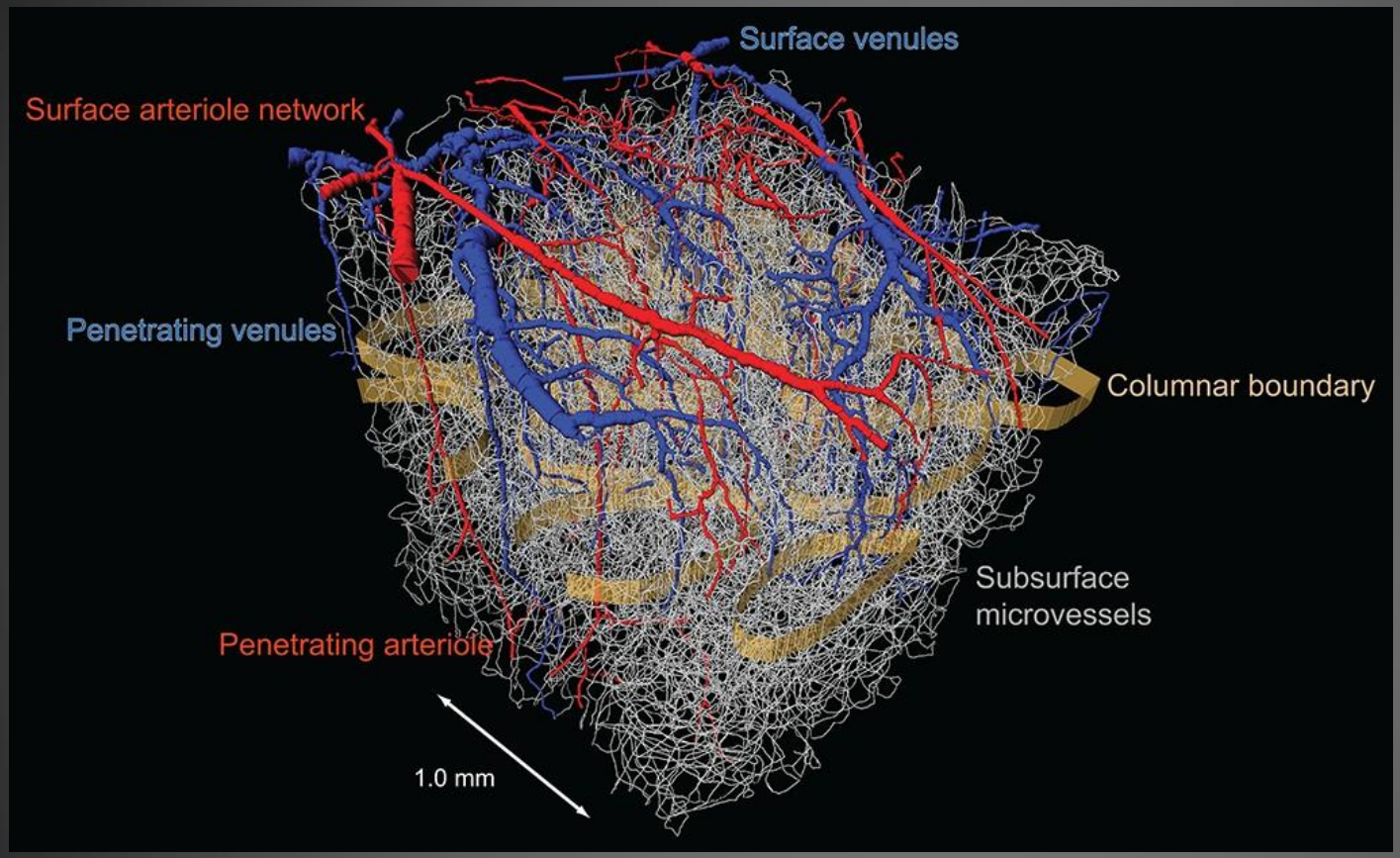
↓

Increase blood flow

Vasodilator metabolites

↓ O2 tension, ↑H, ↑ CO2 tension, ↑ Temperature, K+, lactate, Adenosine, Histamine.

What can we study if examine microcirculation



Architecture

vessel morphology

vessel density

and geometry

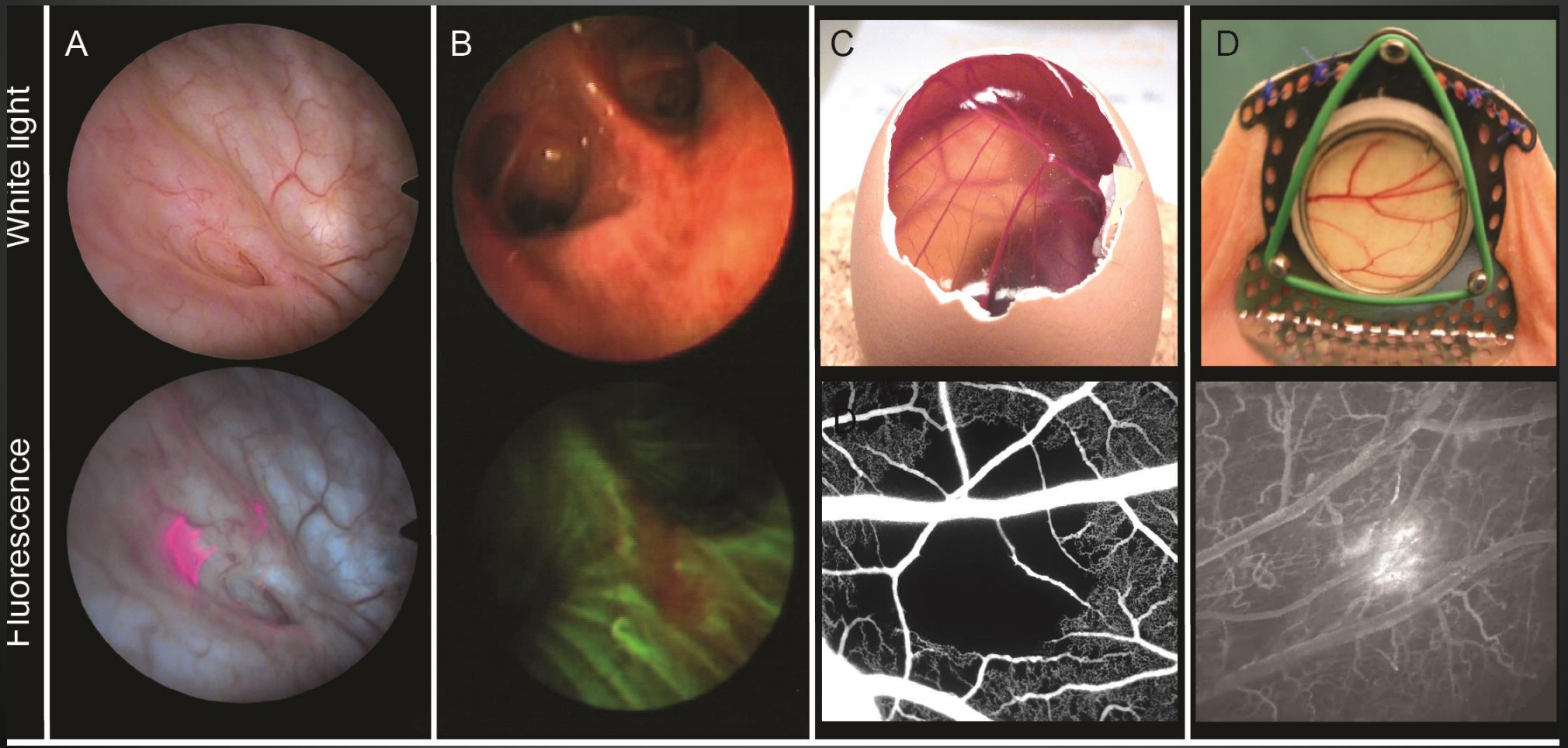
function of vessels

angiogenesis

metabolism

Methods before the Laser Doppler

Intravital microscopy



Closed cranial window- intravital microscopy direct observation of cortical vessels

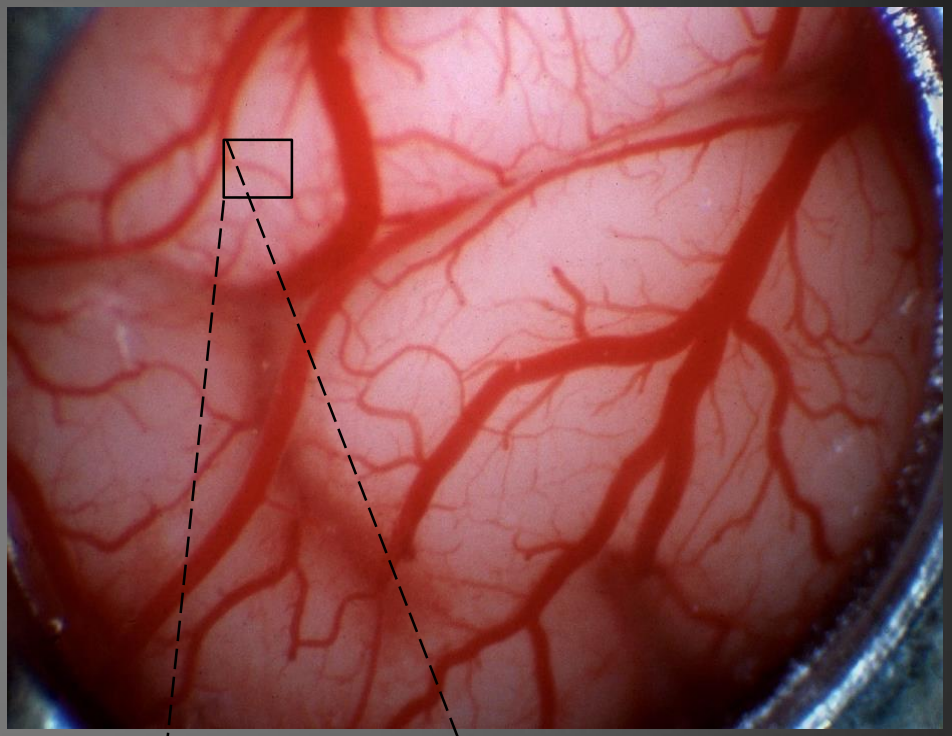
Advantages:

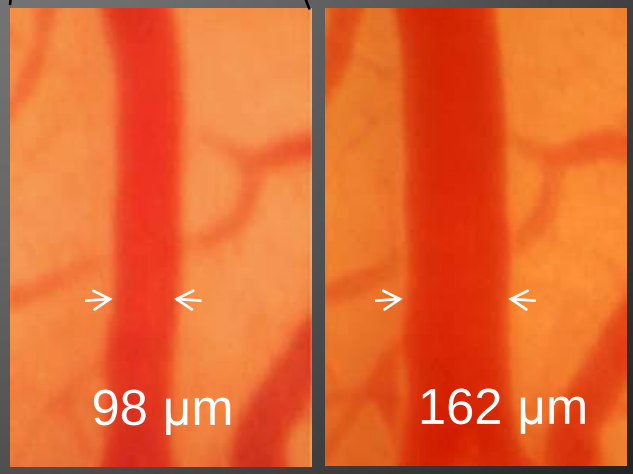
- Physiological environment
- Many kind of vessel can be studied
- Disadvantages:
- Parenchymal circulation cannot be studied
- Limited dynamical follow-up

$Q \sim \Delta P$ (change in perfusion pressure)

$Q \sim r^4$ change in diameter

$Q \sim 1/\eta$ change in viscosity



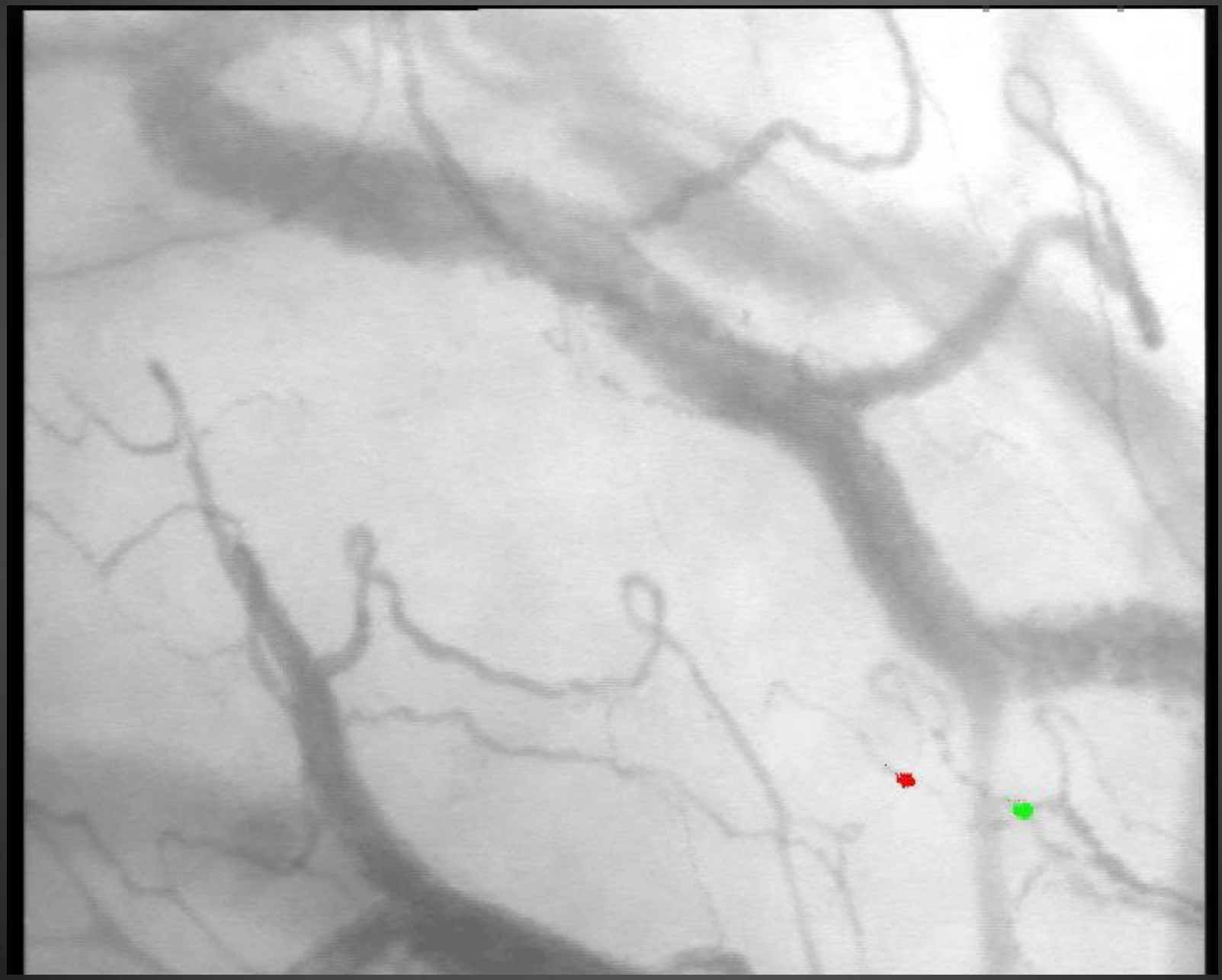


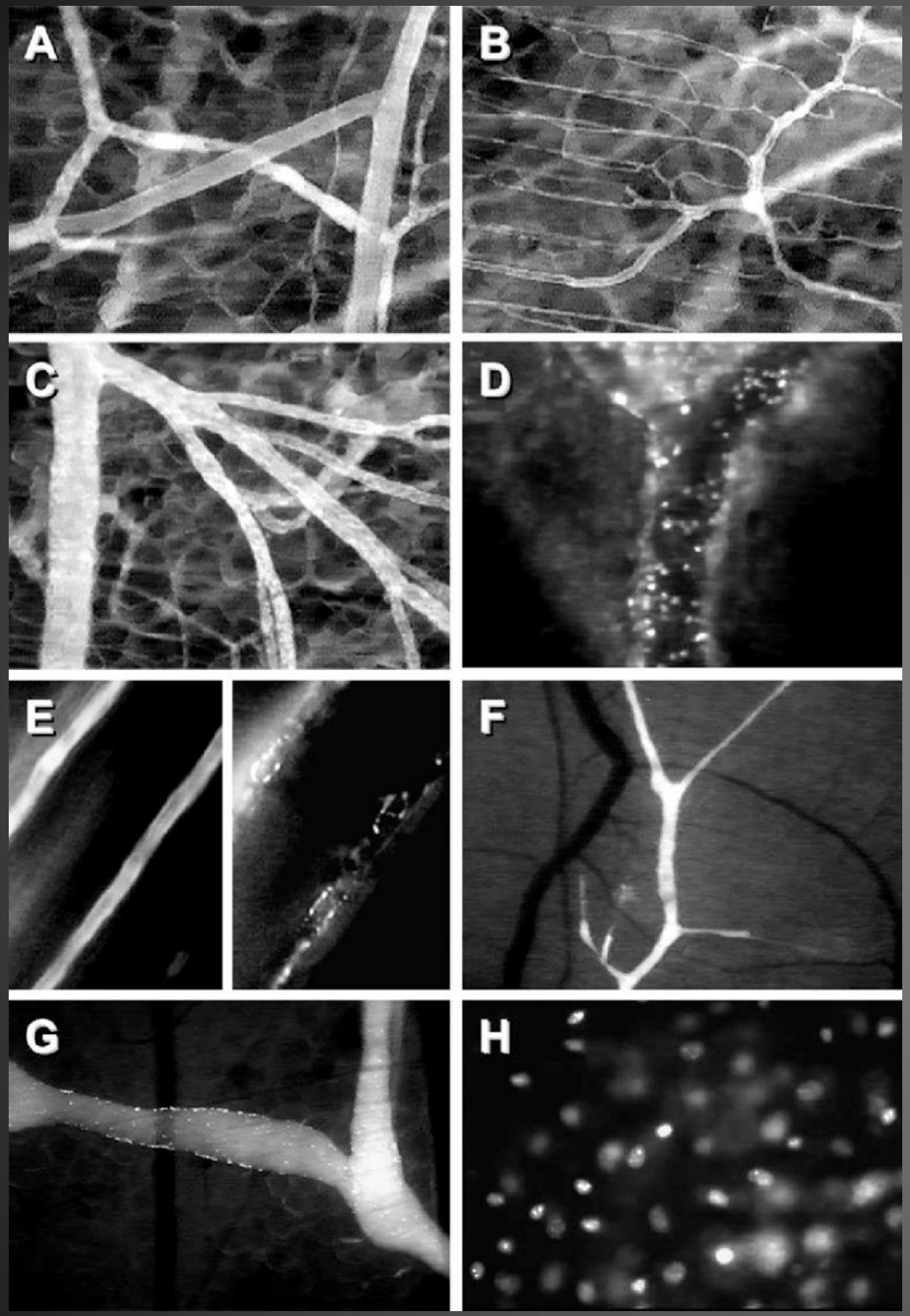
before and after NMDA (10^{-4} M)

Capillaroscopy

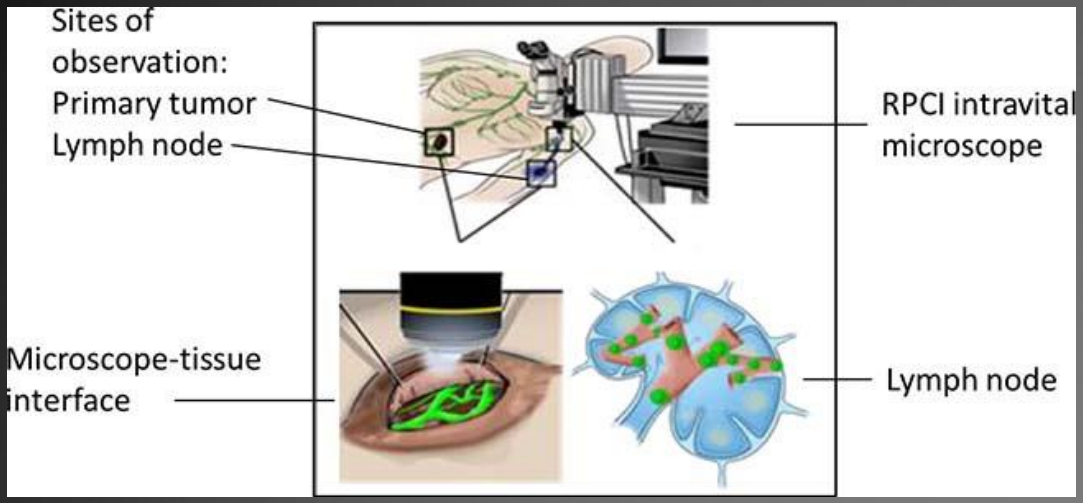
- Find a site where there is very little scattering
- ‘Windows’ (eye, nailfold, under tongue, lower lip)
- x5/x10 microscope objective
- Polarized light capillaroscope
- Aim to detect dichroic (sickled) red blood cells in sickle cell anaemia.

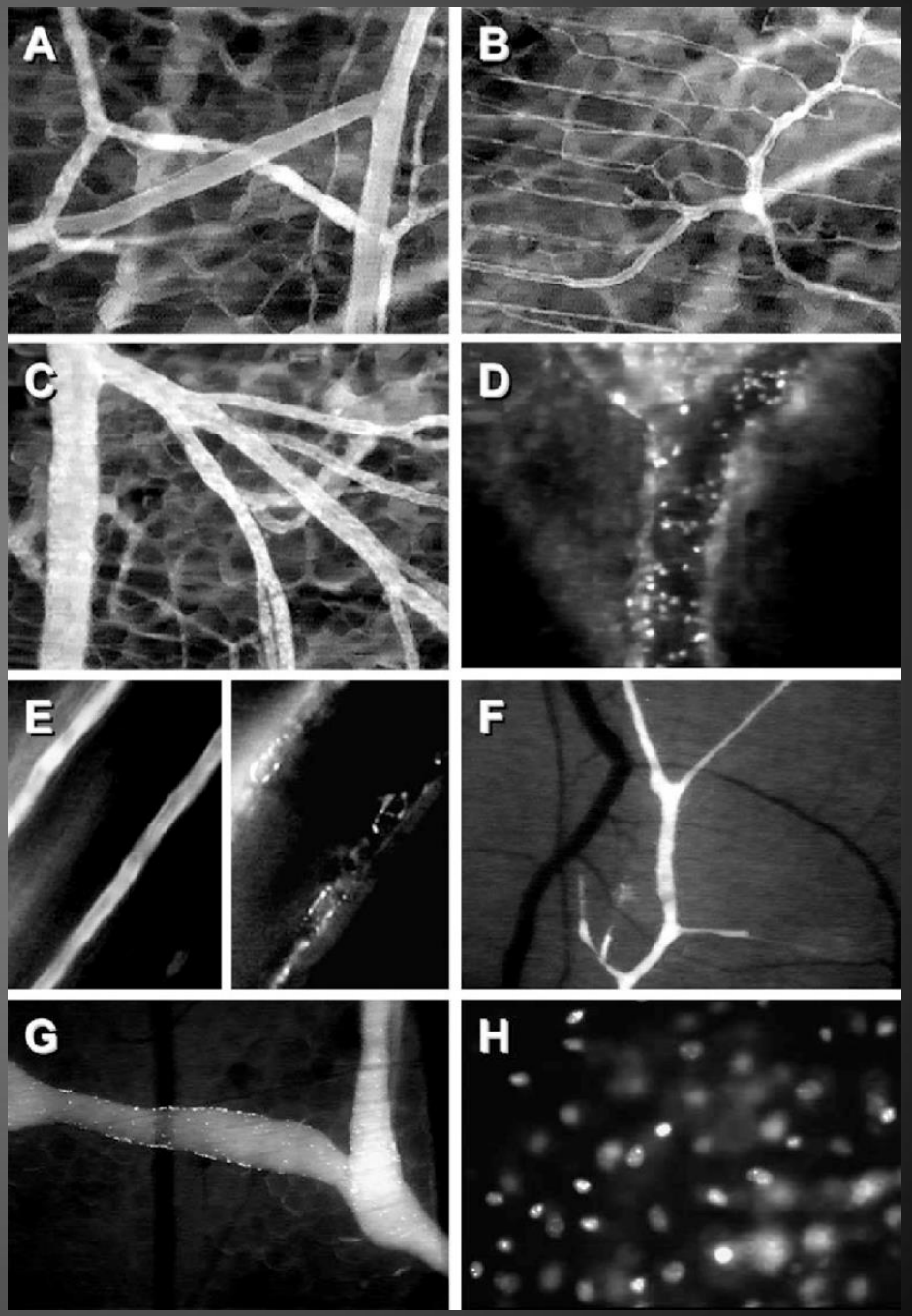
Capillaroscopy (Sub-lingual)




A
B
C
D
E
F
G
H

Advanced intravital microscopy

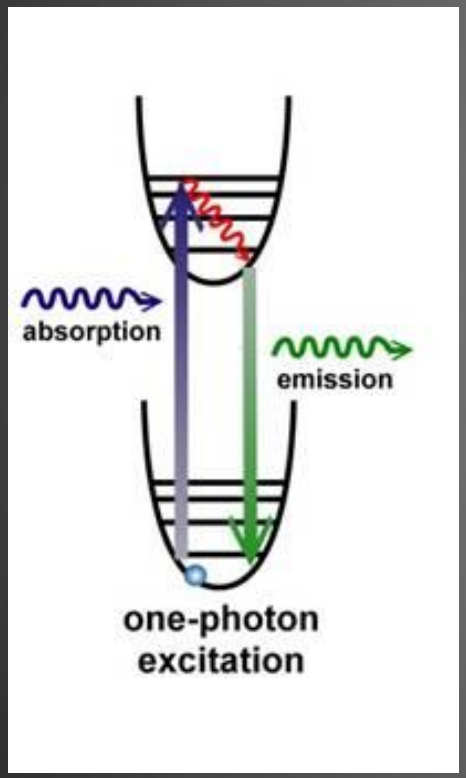


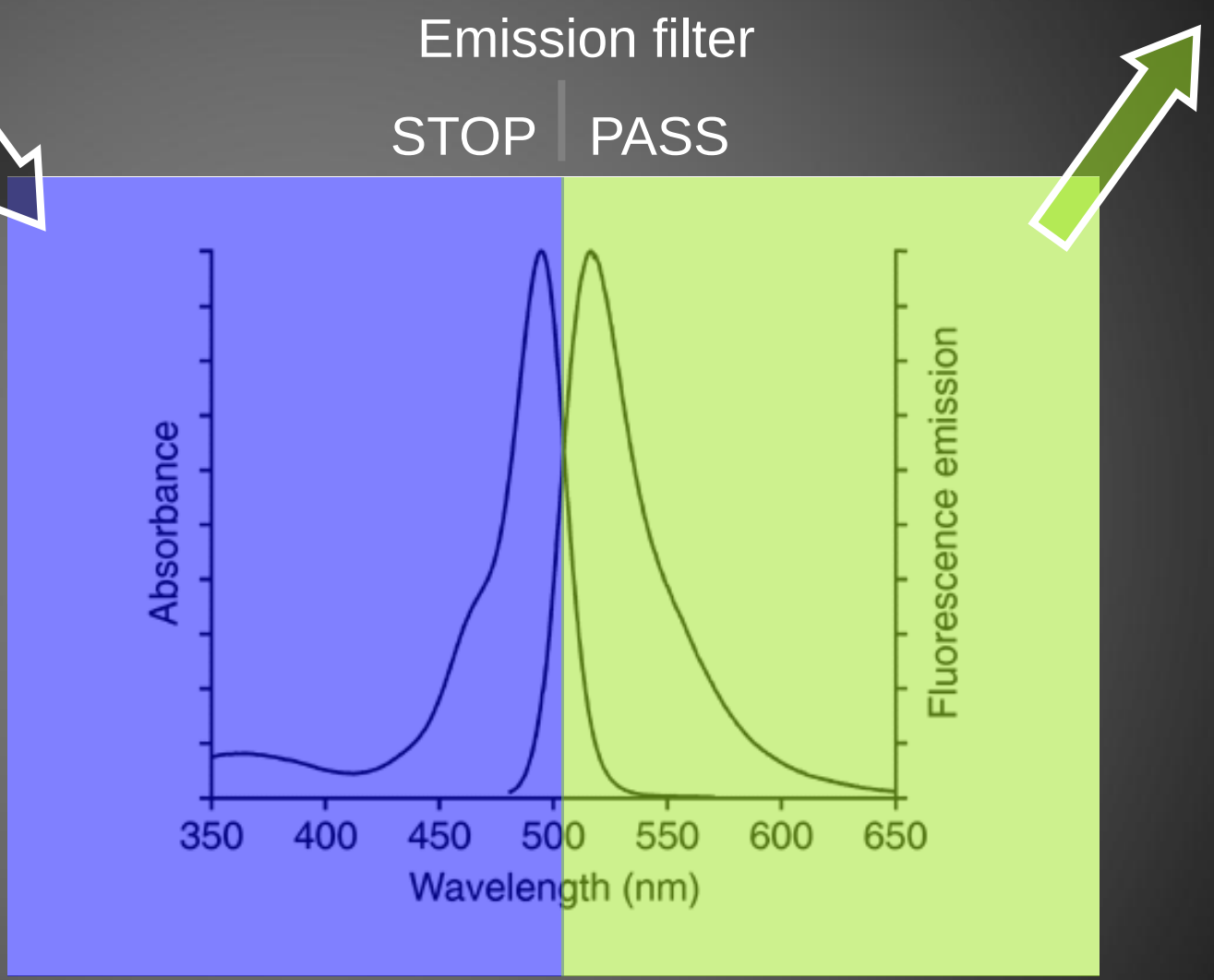


A-H Intravital fluorescence microscopy of the microcirculation of the striated muscle microcirculation in the skinfold of the Syrian golden hamster. Contrast enhancement with fluorescein-isothiocyanate (FITC)-dextran 150.000 allows for the analysis of blood perfusion in arterioles (A), capillaries (B) and venules (C). In vivo staining with rhodamine 6G allows for the detection of leukocyte (D) and platelet (E) endothelial cell interaction. Interstitial application of lowmolecular-weight FITC-dextran 4.000 (F) and fluorescently labeled latex particles (G) provides information on microlymphatic transport. Topical staining with bisbenzimide allows in vivo analysis of parenchymal cell apoptosis (H)

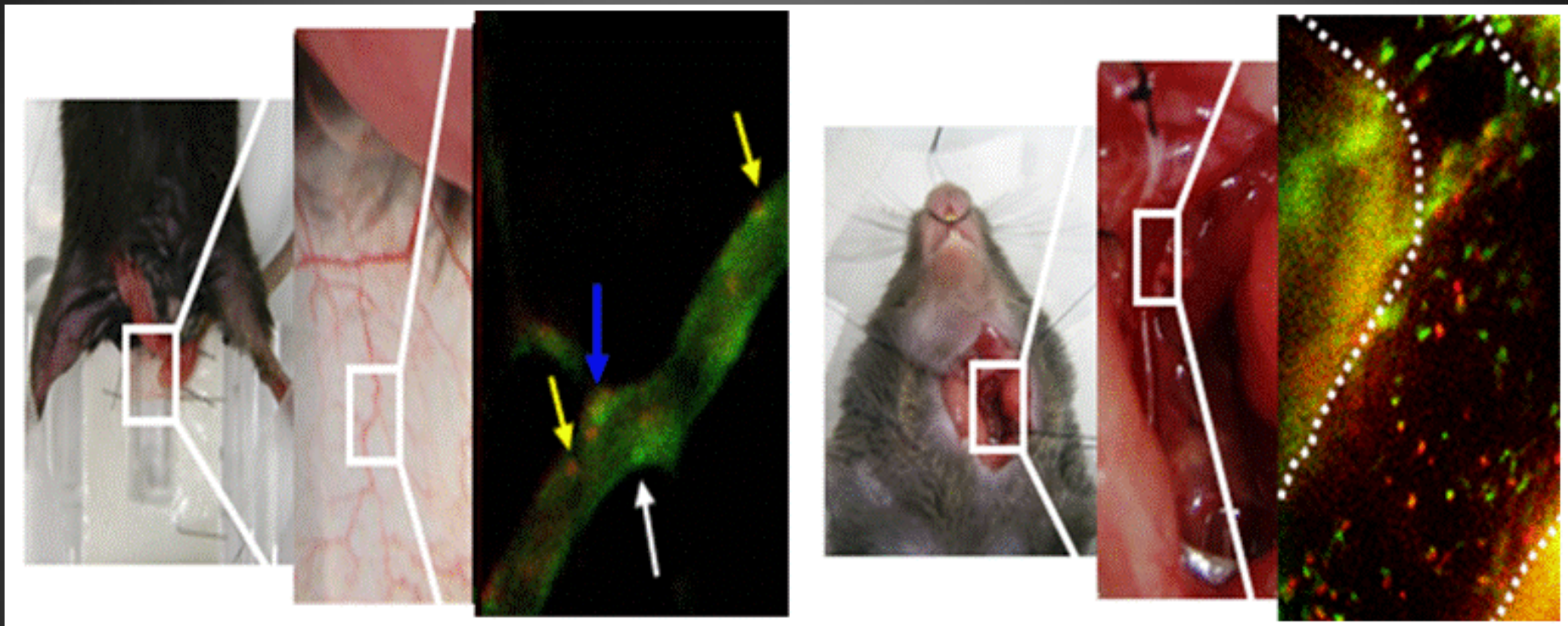
Henrik Thorlacius et al, 2003

Principle of fluorescence measurment



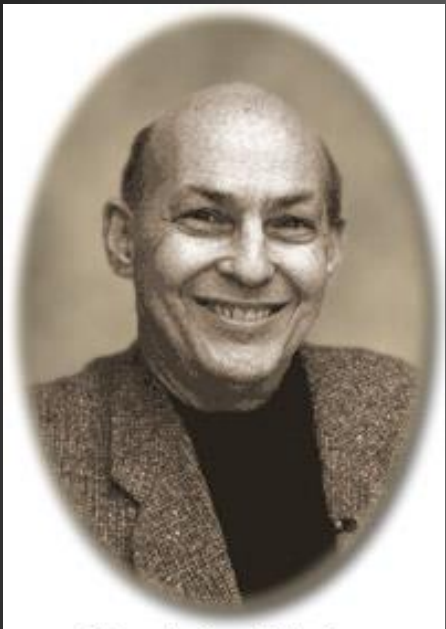


Emission-absorption spectrum of Fluo-4



Examples of fluorescence intravital microscopy in mouse tissue. Detection of neutrophils (yellow arrow), classical (blue arrow) and non-classical monocytes (white arrow) recruitment in a) exposed cremaster muscle b) exposed carotid artery bifurcation.

History



Marvin Lee Minsky
1927 - 2016

Electrode based techniques dominate
Extracellular electrodes, patch clamp, sharp electrode

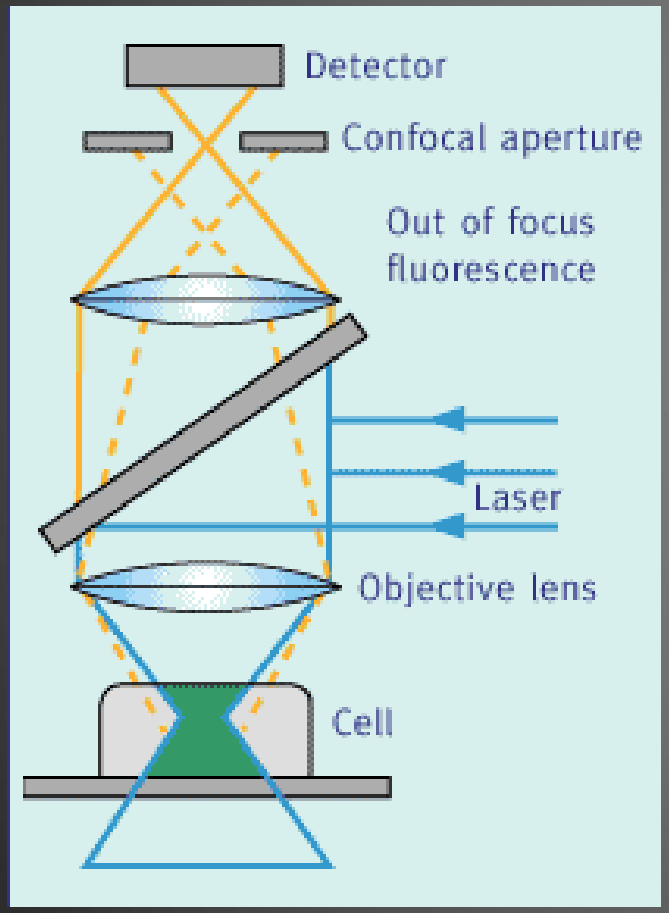
Calcium indicators developed
The principle of **confocal imaging** was patented by Marvin Minsky in 1961
- most of the excitation outside of focus
-information cut by pinhole

Winfried Denk

Two-photon excitation concept first described by *Maria Göppert-Mayer* in 1931.
Two-photon microscopy was pioneered by *Winfried Denk* in the lab of *Watt W. Webb* at Cornell University in 1990
- all light is taken: no pinhole

Laser scanning confocal microscopy

Confocal microscope



Detector: photomultiplier

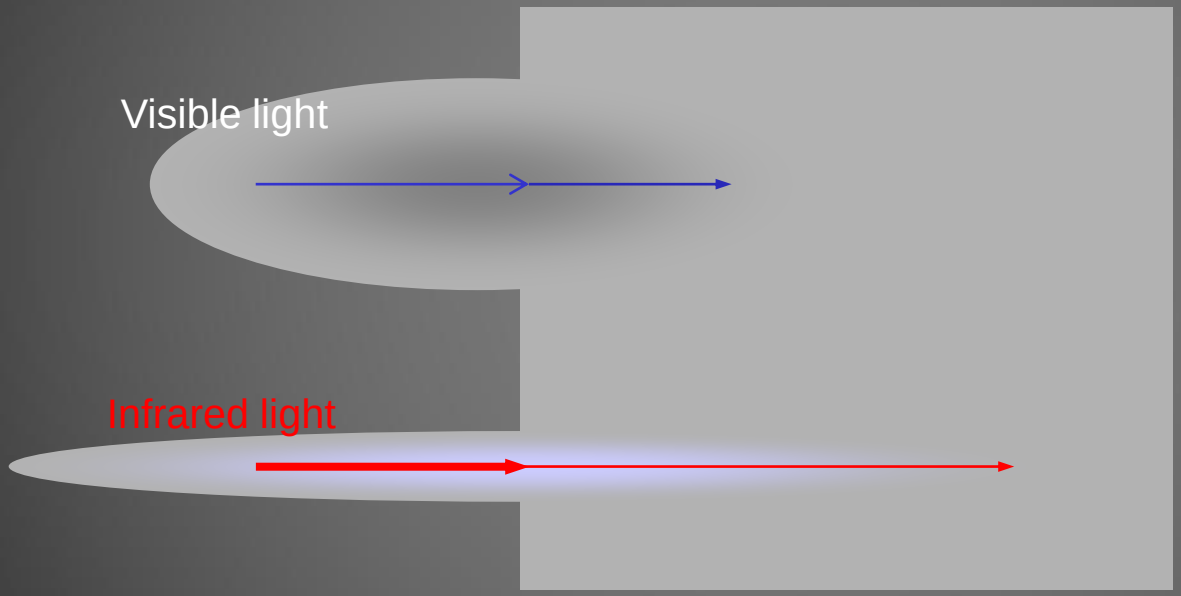
Light source: laser
Power
Wavelength

Filters

Scanner

Two-photon excitation requires IR laser

Scattering ~ (wavelength)$^{-4}$

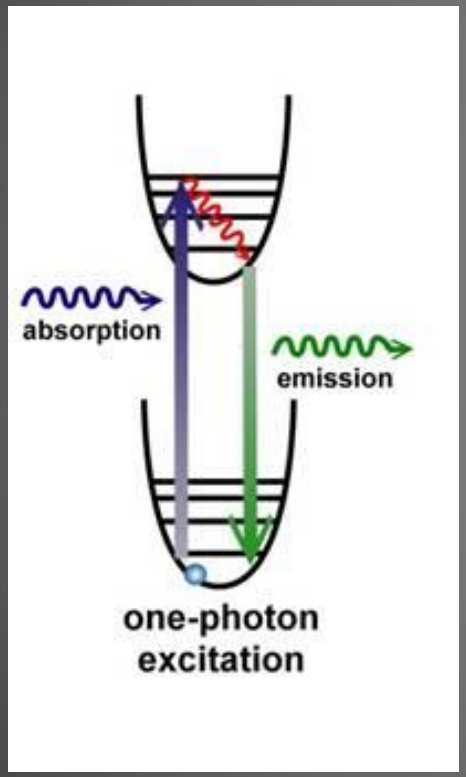


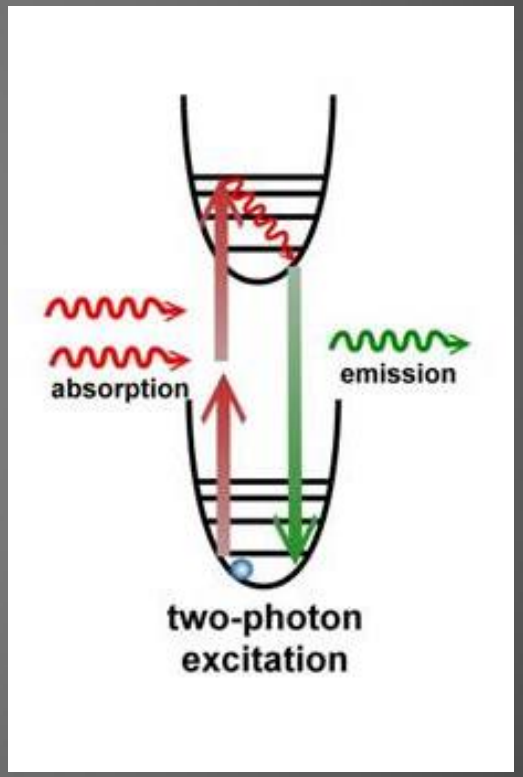
IR penetrates tissue much deeper

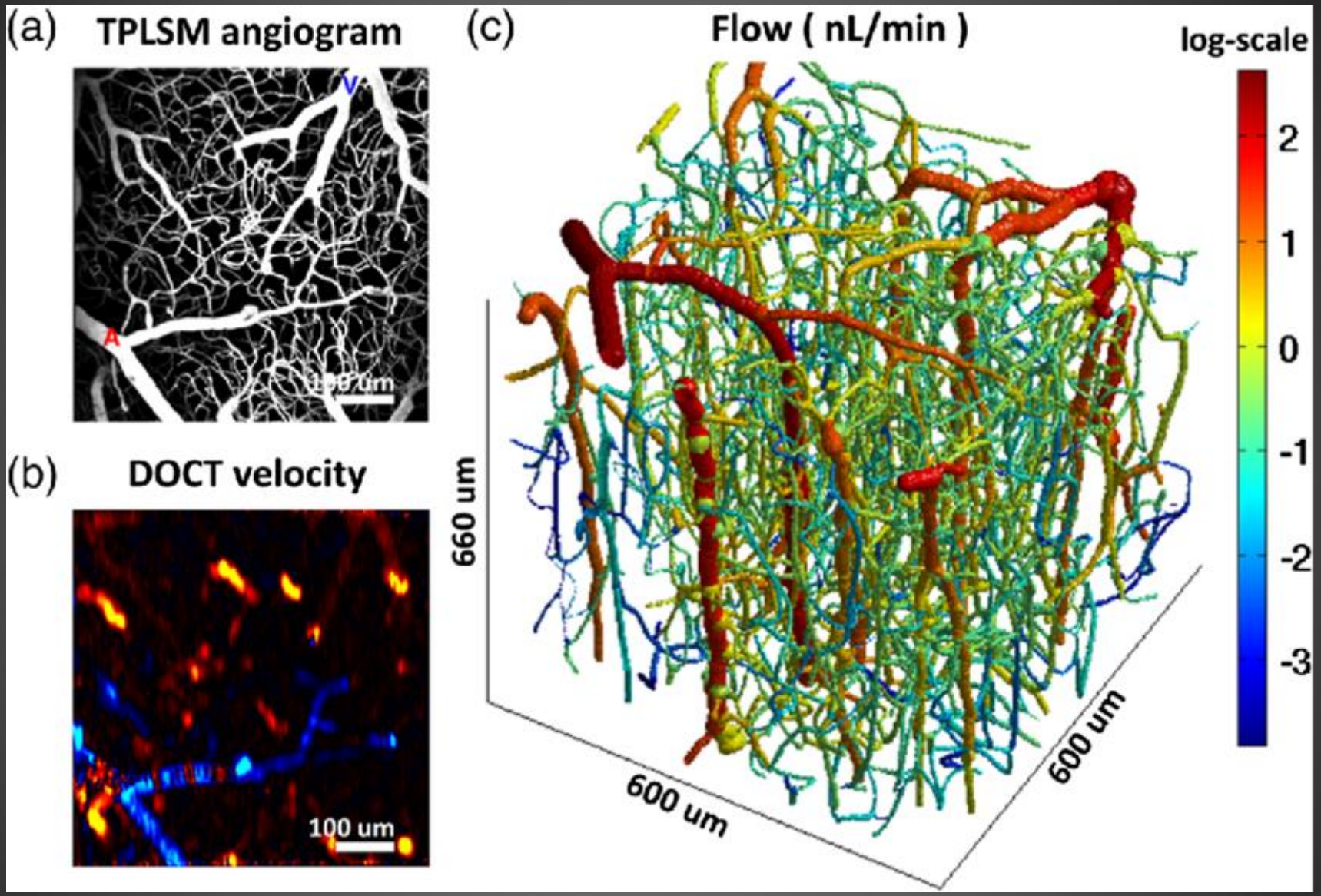
Advantages of two photon imaging

- No out-of-focus fluorescence
- Better in depth resolution
- Less photobleaching of the dye
- Less photodamage of the dye
- Less phototoxicity for the tissue

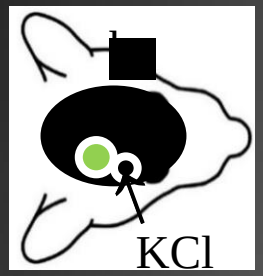
Principle of two photon excitation



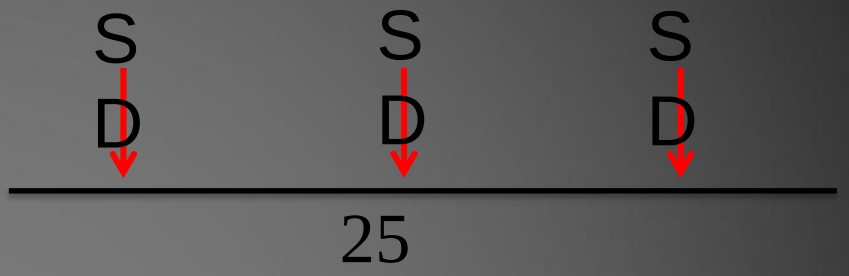


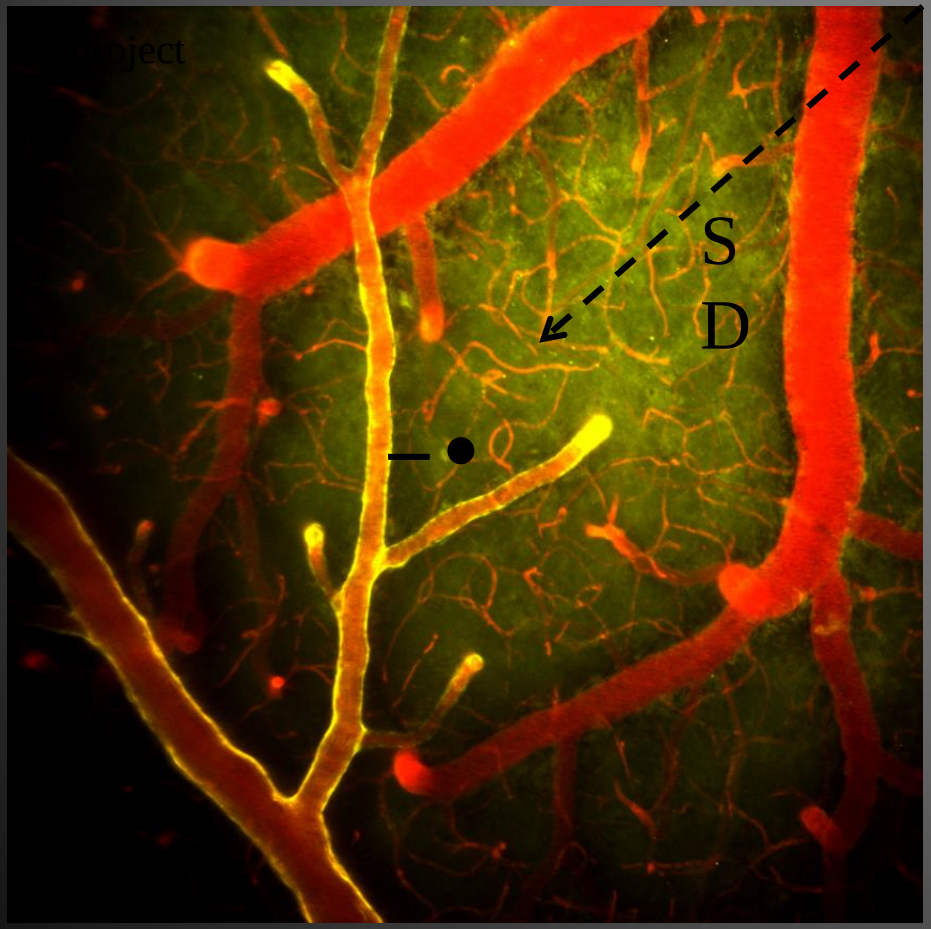

(a) TPLSM angiogram
V
A
100 um
(b) DOCT velocity
100 um
(c) Flow (nL/min)
log-scale
2
1
0
-1
-2
-3
660 um
600 um
600 um

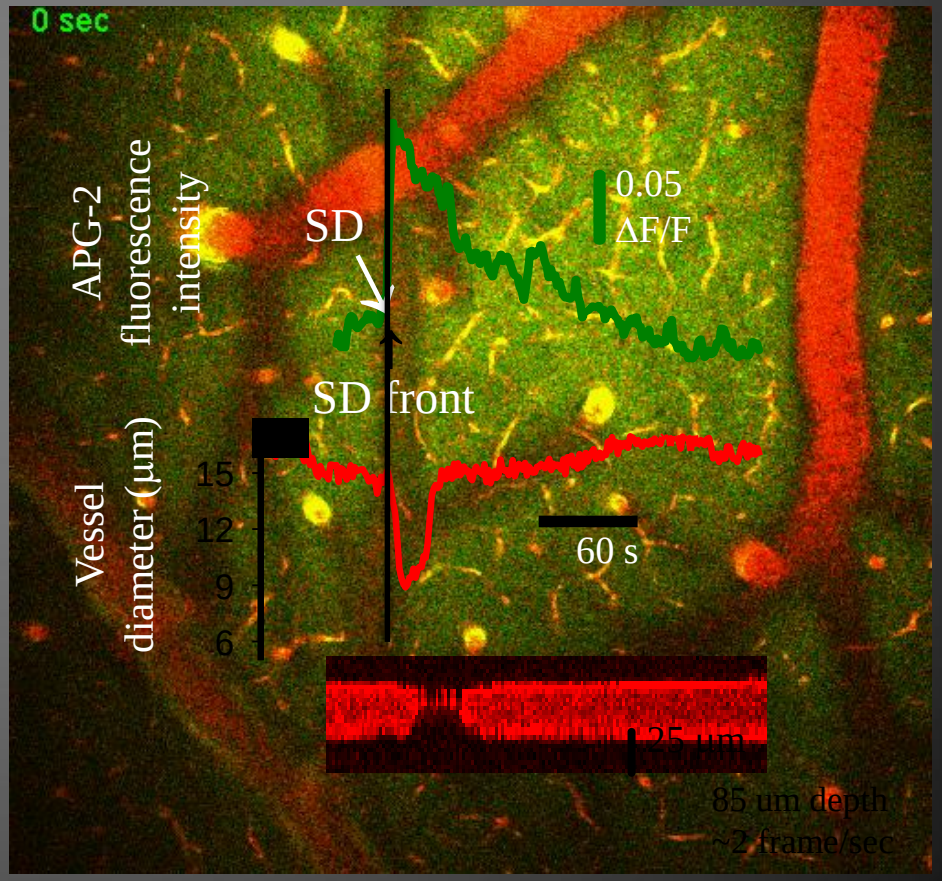
The potassium wave of SD causes vasoconstriction



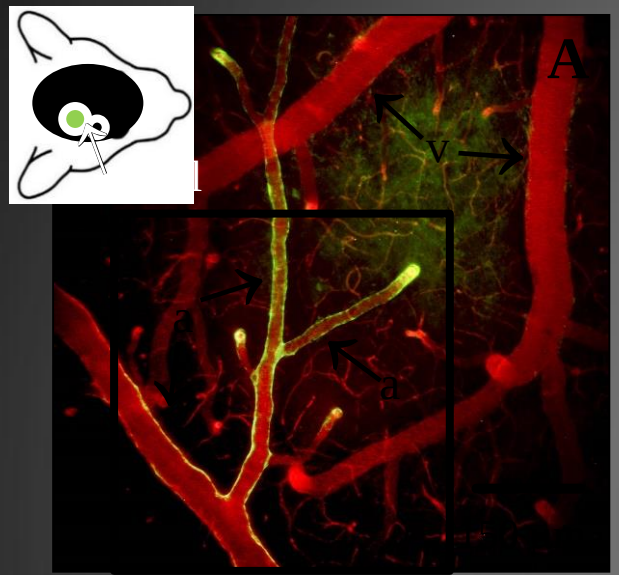
- C57BL/6 mice, n=6
- Avertin (4 mg/kg) anesthesia
- Green: Assante Potassium Green-2 (75 µM)
- Red: Rhodamine dextrane (5 mg)

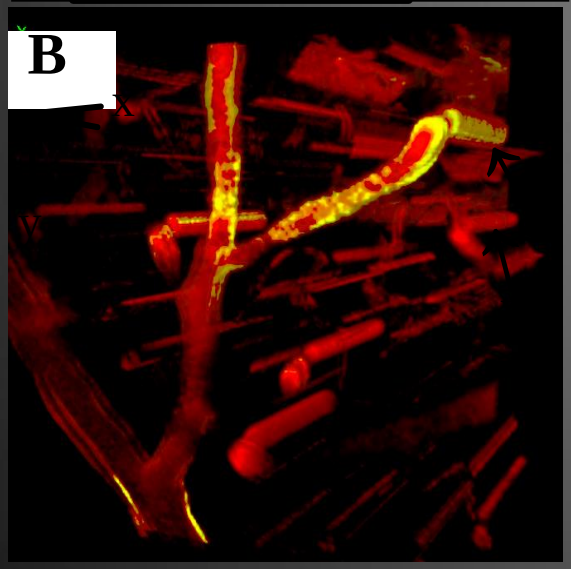


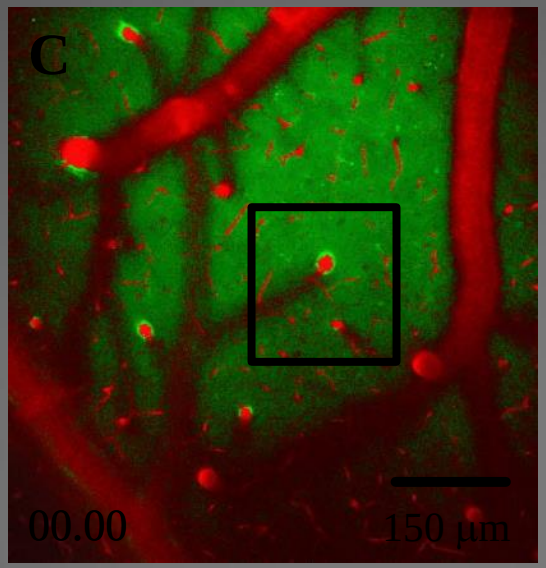


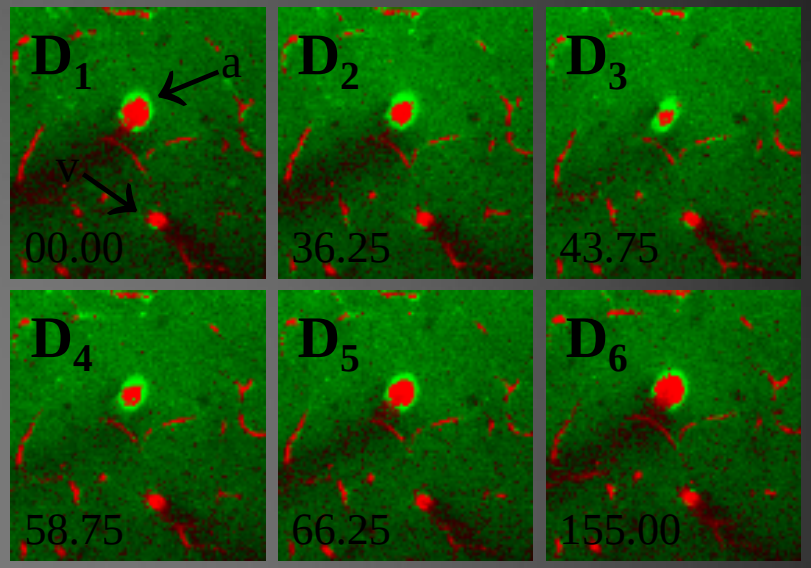


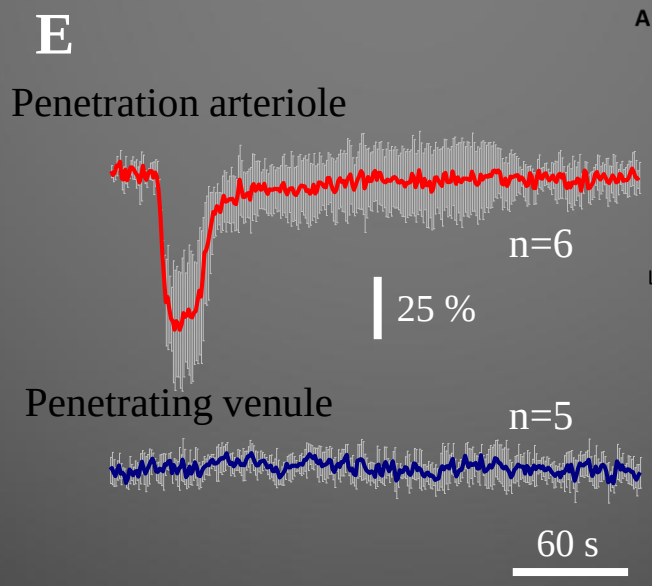
Potassium wave of SD causes arteriole constriction

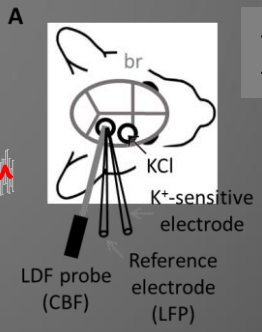




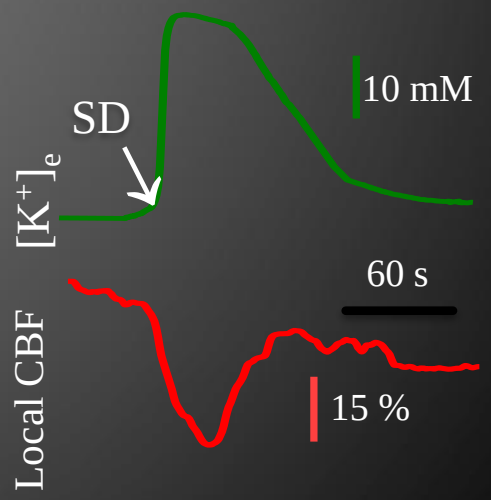








Electrophysiology



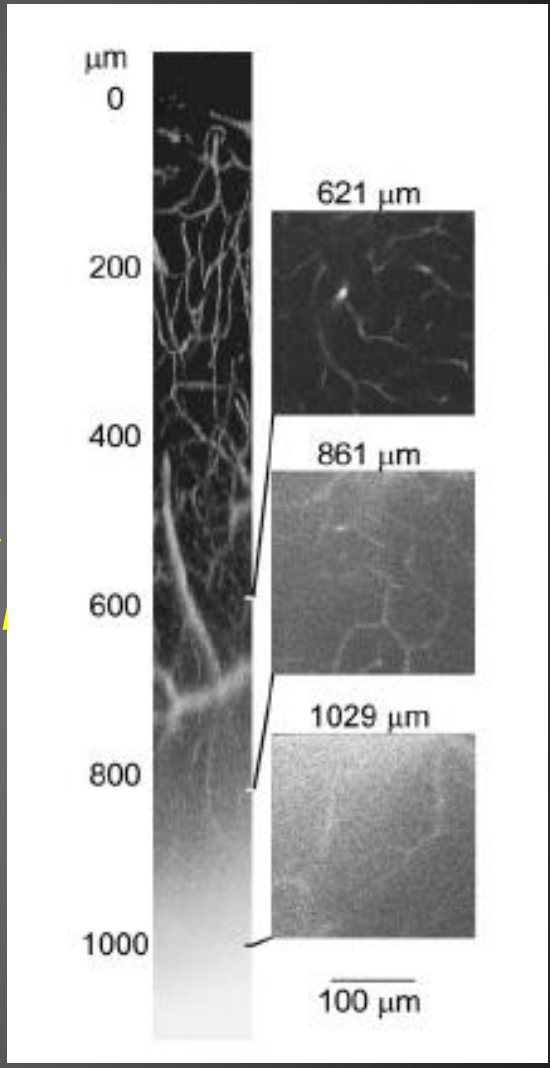
Limitations of multiphoton imaging

1. Two photon imaging has depth limit
   out of focus light (background) > 1000 mm
   Theer, Hasan, Denk. Opt Lett. 2003

2. *Scanner frame rate is relatively slow compare to open field imaging*

3. *light with wavelength over 1400 nm may be significantly absorbed by the water in living tissue – limits multiphoton excitation*

4. *IR lasers are expensive*



Methods before the Laser Doppler

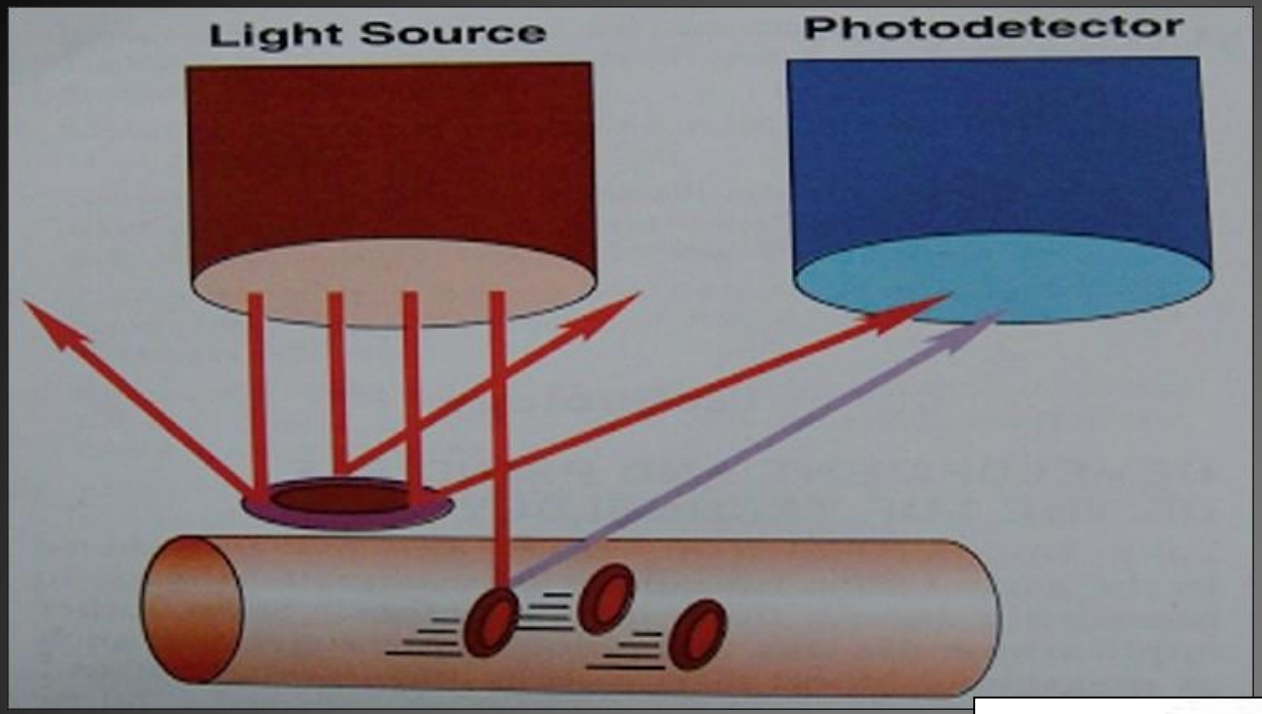
- Intravital microscopy, pletismography

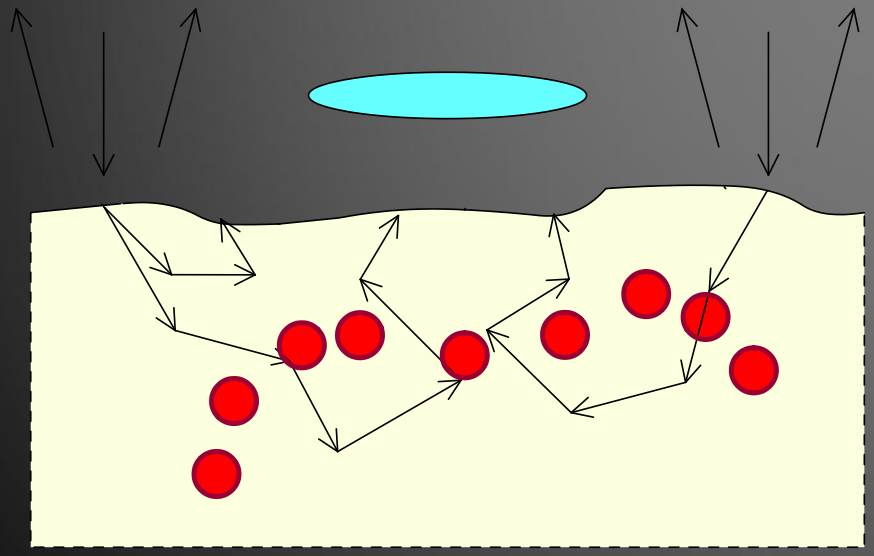
- INDIFFERENT GAS METHODS
  - HYDROGEN CLEARANCE
- ISOTOPE METHODS
  - AUTORADIOGRAPHIC METHOD

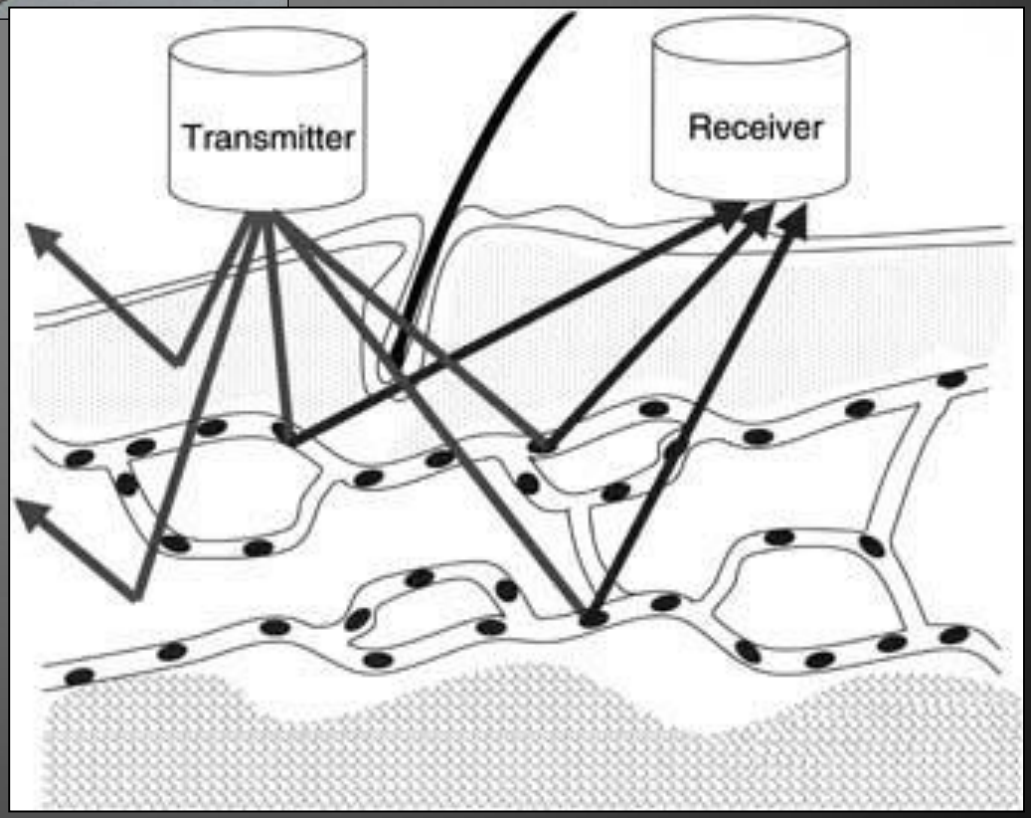
    INHALATION OF O^{15} or O^{15} LABELED CO_2
  - RADIOACTIVE (LATER COLORED) MICROSPHERES
- REGIONAL CEREBRAL BLOOD-FLOW MEASUREMENTS BY XE-133-INHALATION
- LATER TRANSCRANIAL DOPPLER SONOGRAPHY
- PET

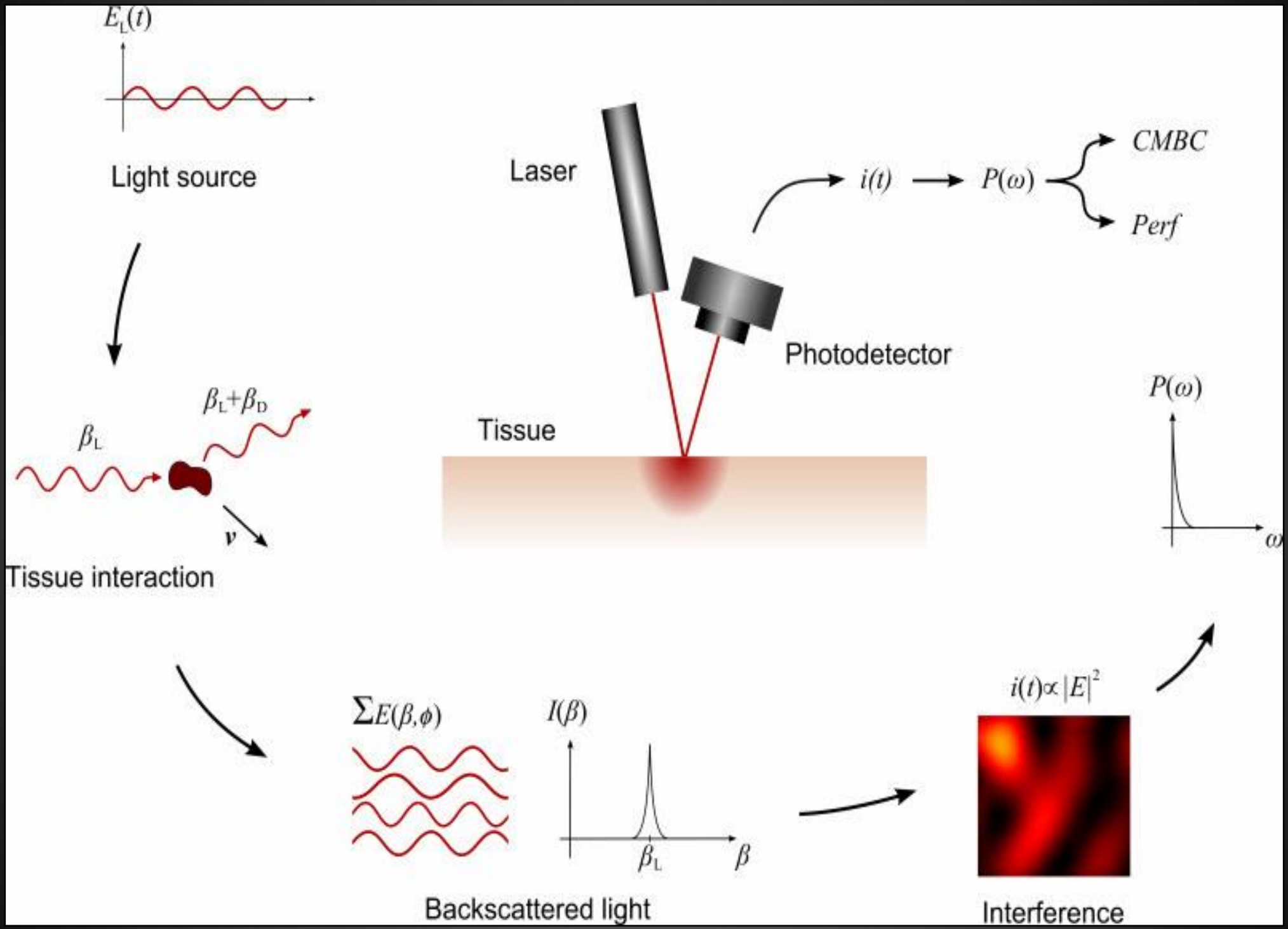
Principles of Laser Doppler Flowmetry

- 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.

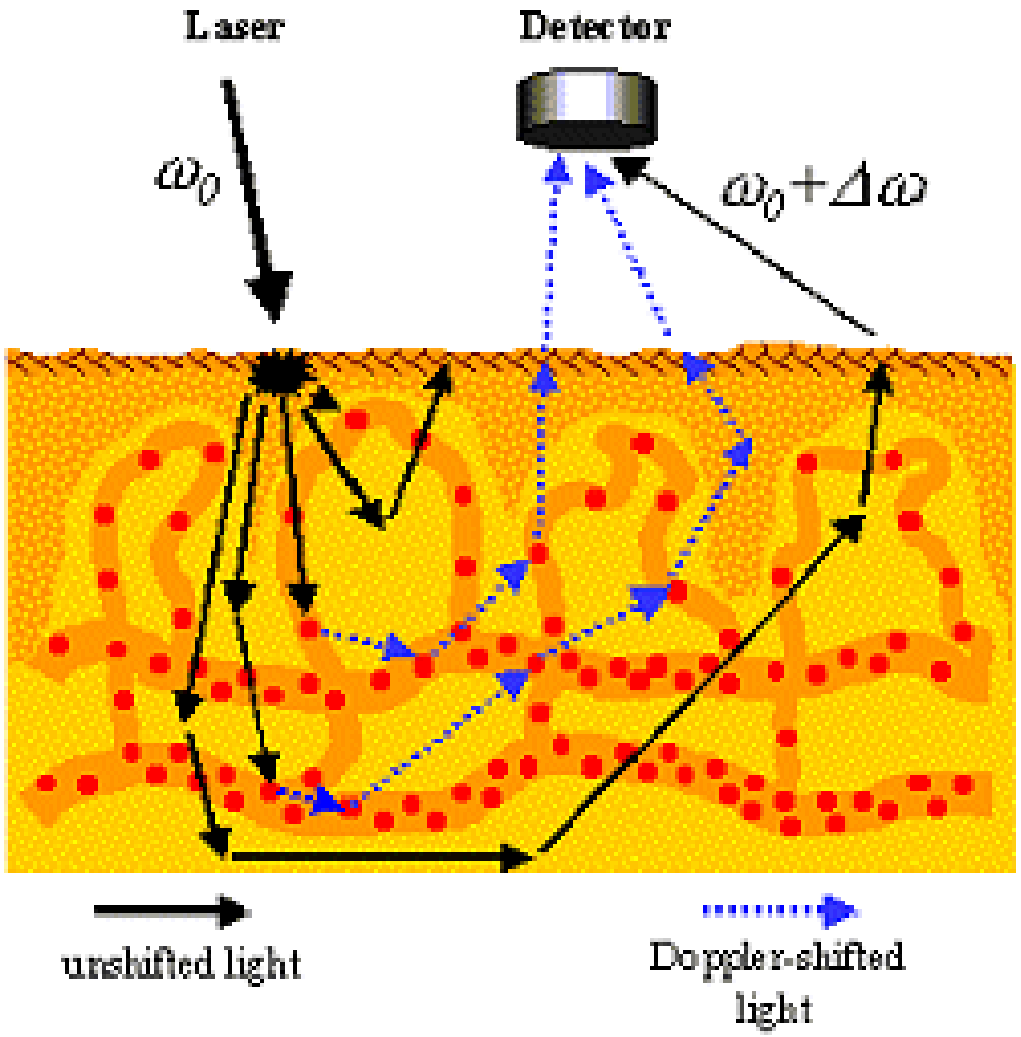

Light Source
Photodetector

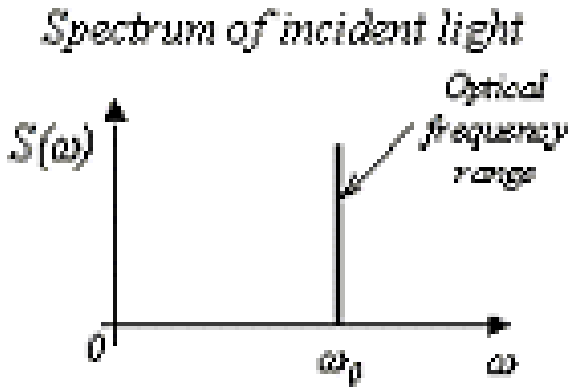


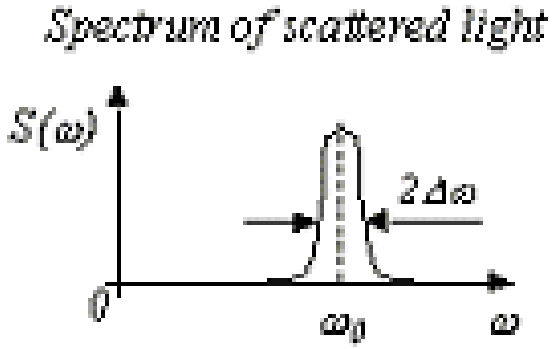

Transmitter
Receiver

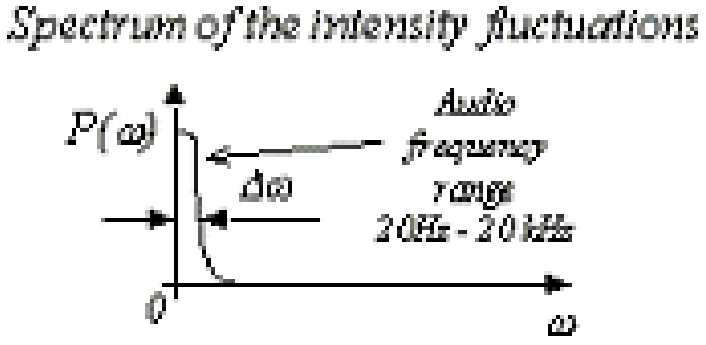

$E_L(t)$
Light source
Laser
$i(t)$
$P(\omega)$
CMBC
Perf
Photodetector
Tissue
$P(\omega)$
ω
β_L
$\beta_L+\beta_D$
v
Tissue interaction
$\Sigma E(\beta,\phi)$
$I(\beta)$
β_L
β
Backscattered light
$i(t)\propto|E|^2$
Interference

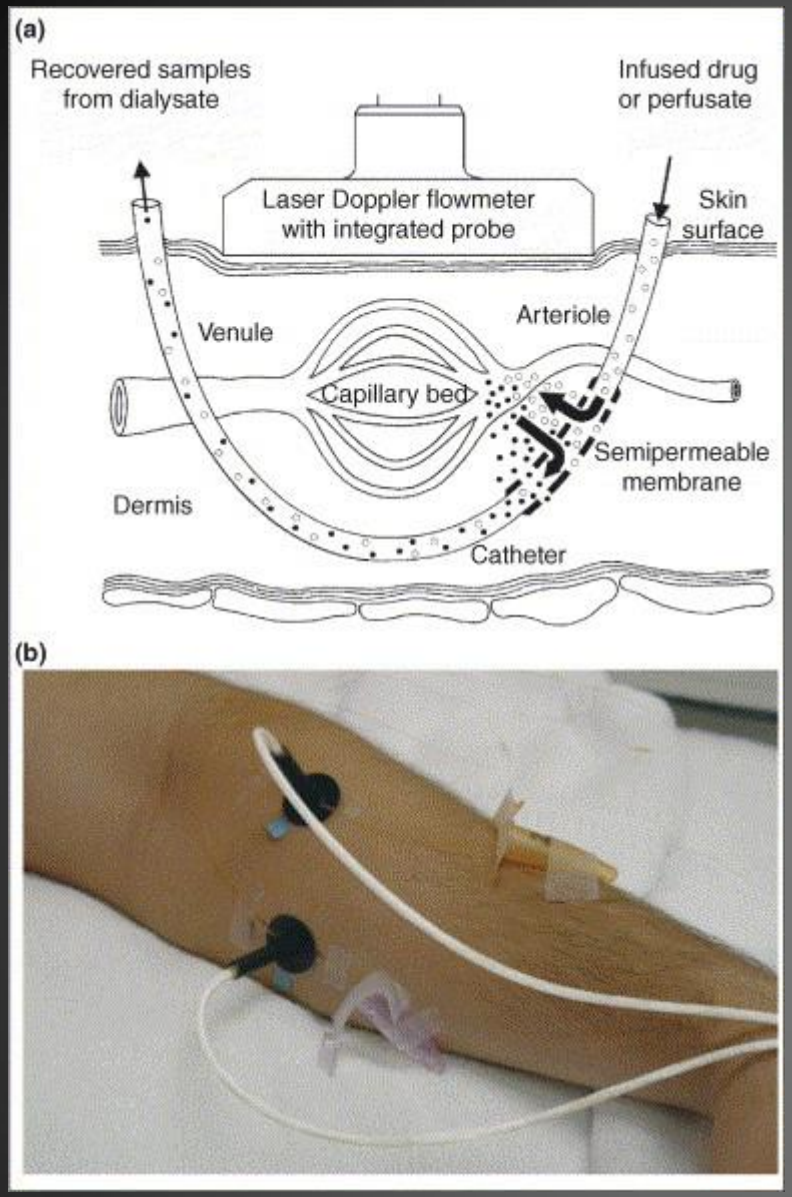
Principle of laser Doppler flowmetry

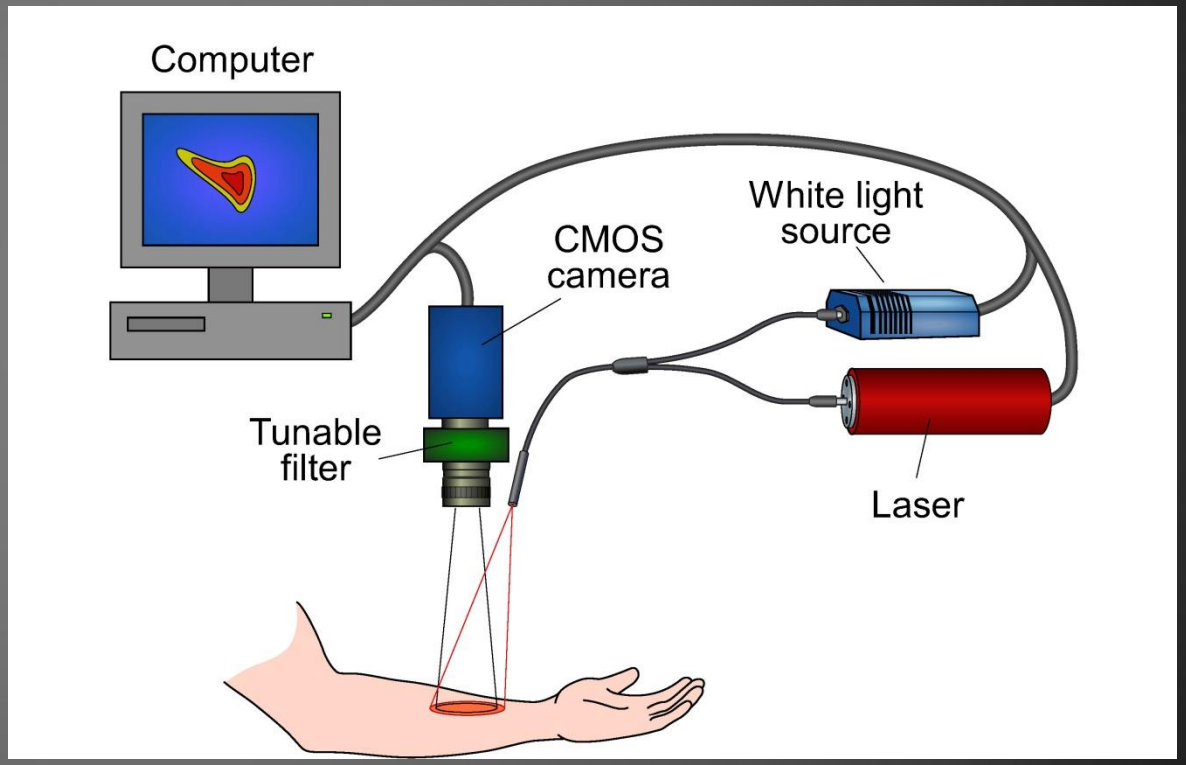




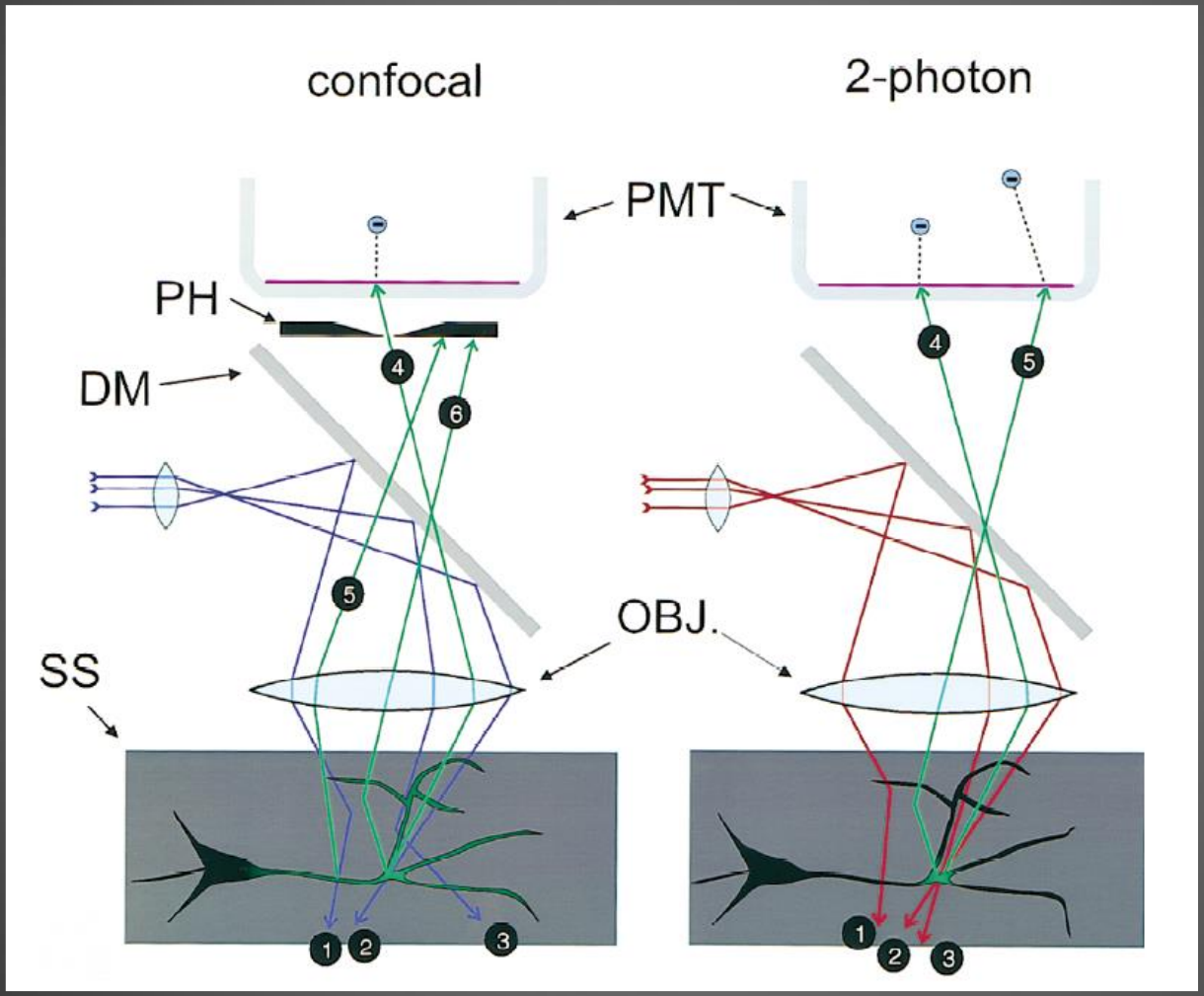





(a)
Recovered samples from dialysate
Infused drug or perfusate
Laser Doppler flowmeter with integrated probe
Skin surface
Venule
Arteriole
Capillary bed
Semipermeable membrane
Dermis
Catheter
(b)

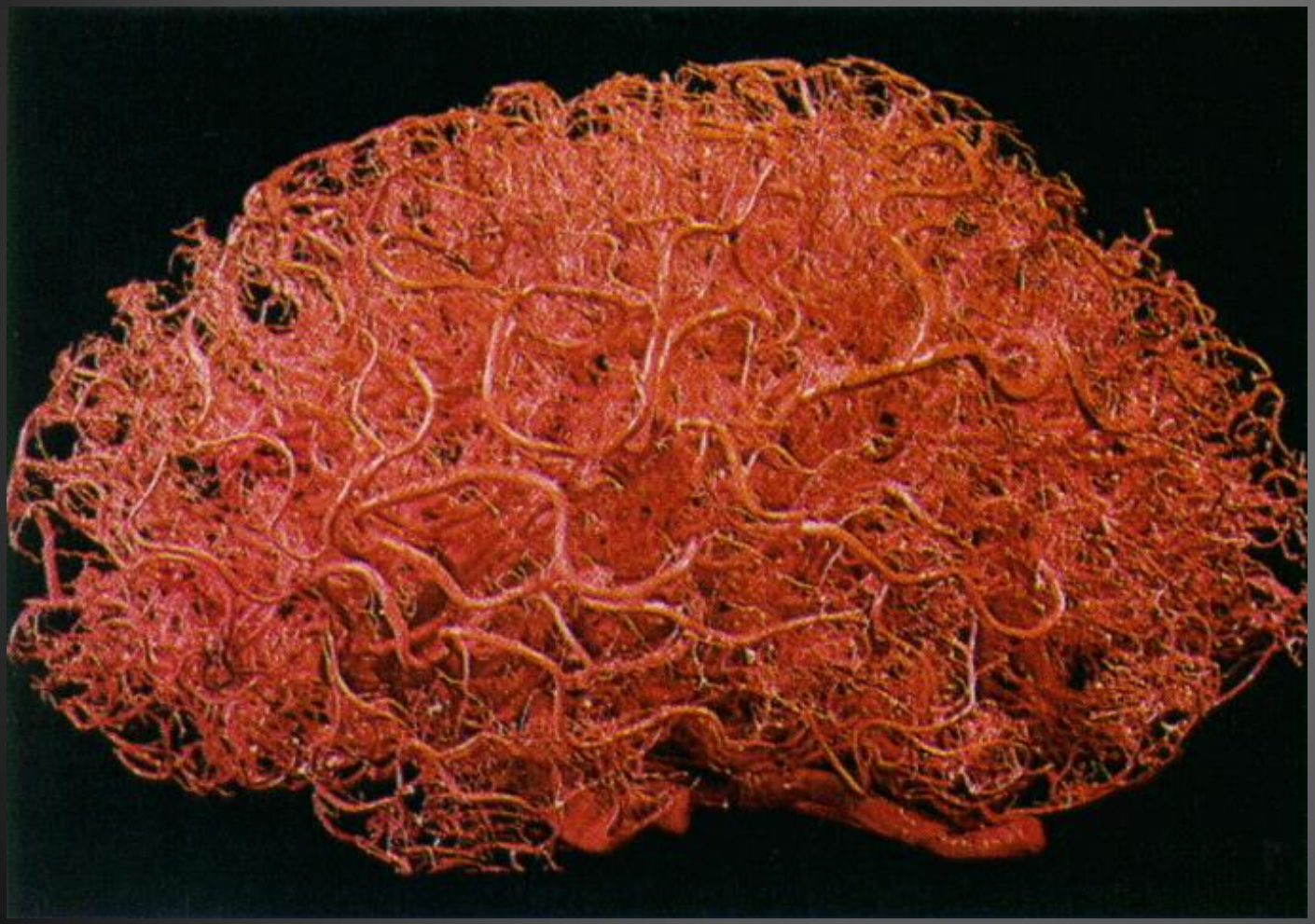

Computer
CMOS camera
White light source
Tunable filter
Laser

Difference between single photon and two photon imaging



Winfried Denk and Karel Svoboda
Neuron, Vol. 18, 351–357, March, 1997

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

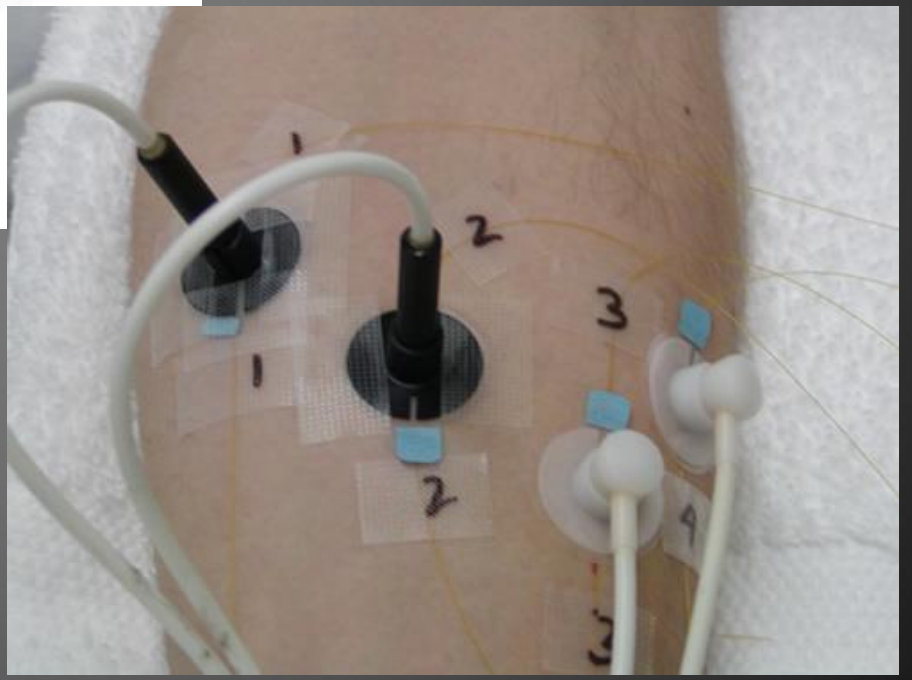
Dexmedetomidine-induced decrease in cerebral blood flow is attenuated by verapamil in rats: a laser Doppler study

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

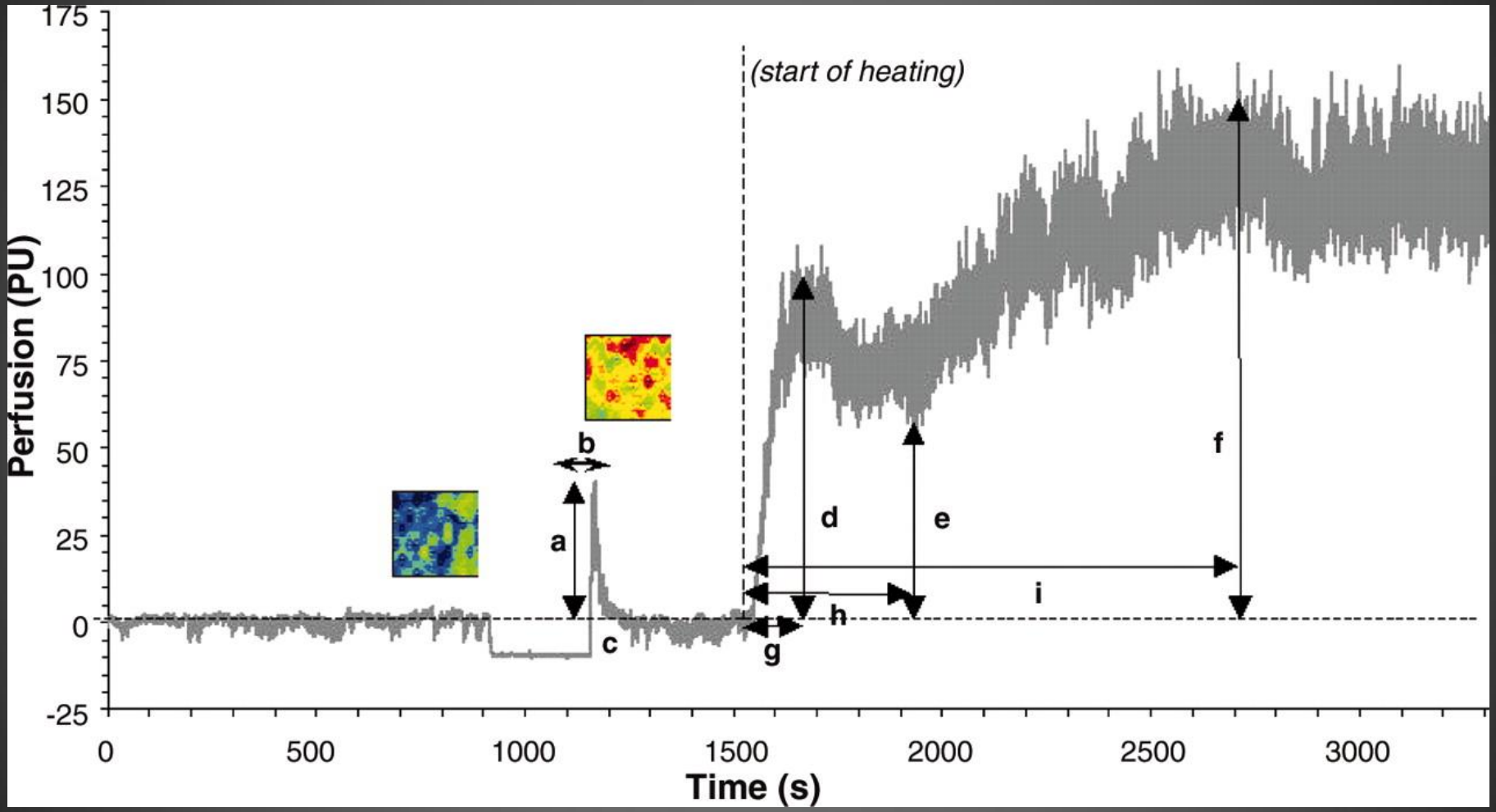
Advantages of LDF technique

- 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)



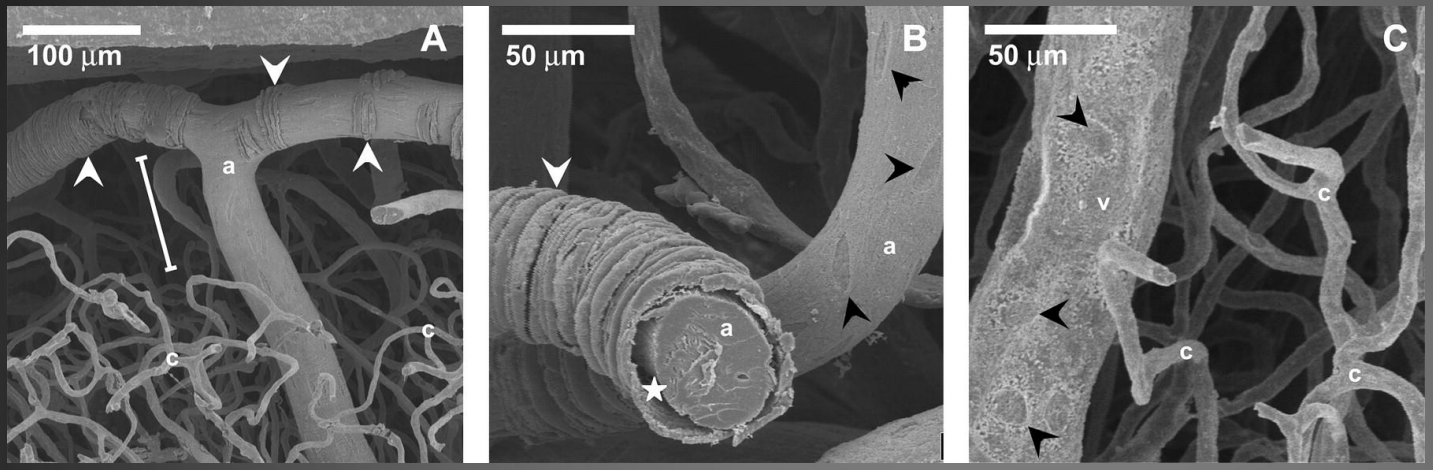
Representative tracing of control postocclusive hyperemia (PORH) and thermal hyperemia (TH).

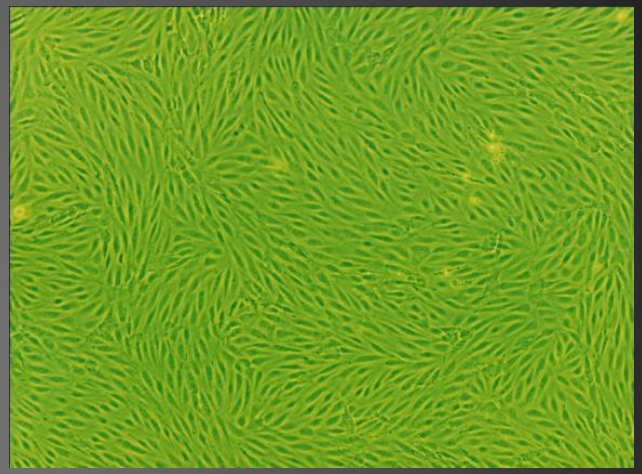


Stewart J et al. Am J Physiol Heart Circ Physiol 2004;287:H2687-H2696

AMERICAN JOURNAL OF PHYSIOLOGY
Heart and Circulatory Physiology

Capillaries in the brain

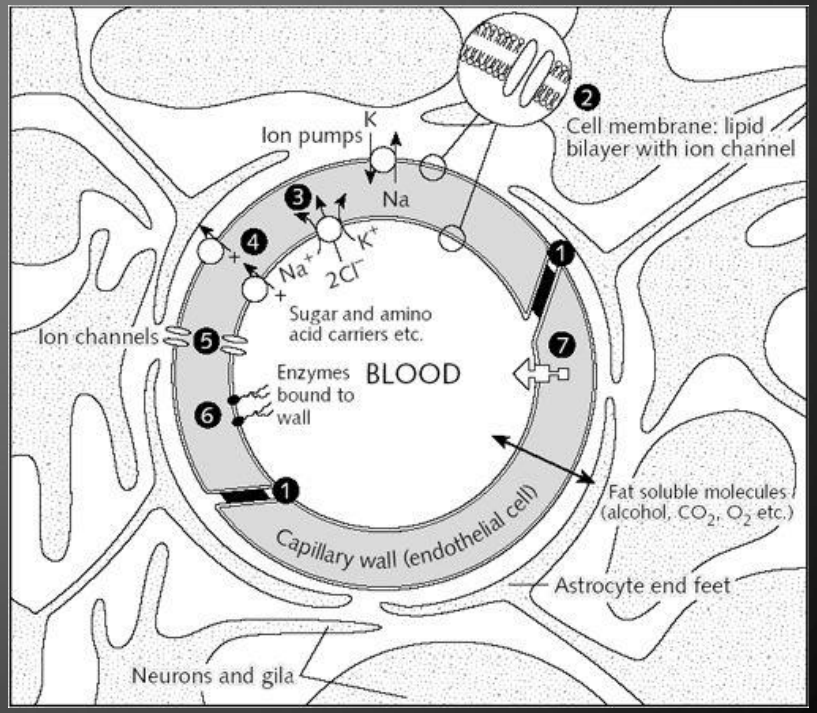




The endothelium is the thin layer of cells that lines the interior surface of blood vessels. In the brain there are highly differentiated endothelial cells to perform specialized functions;

- 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:

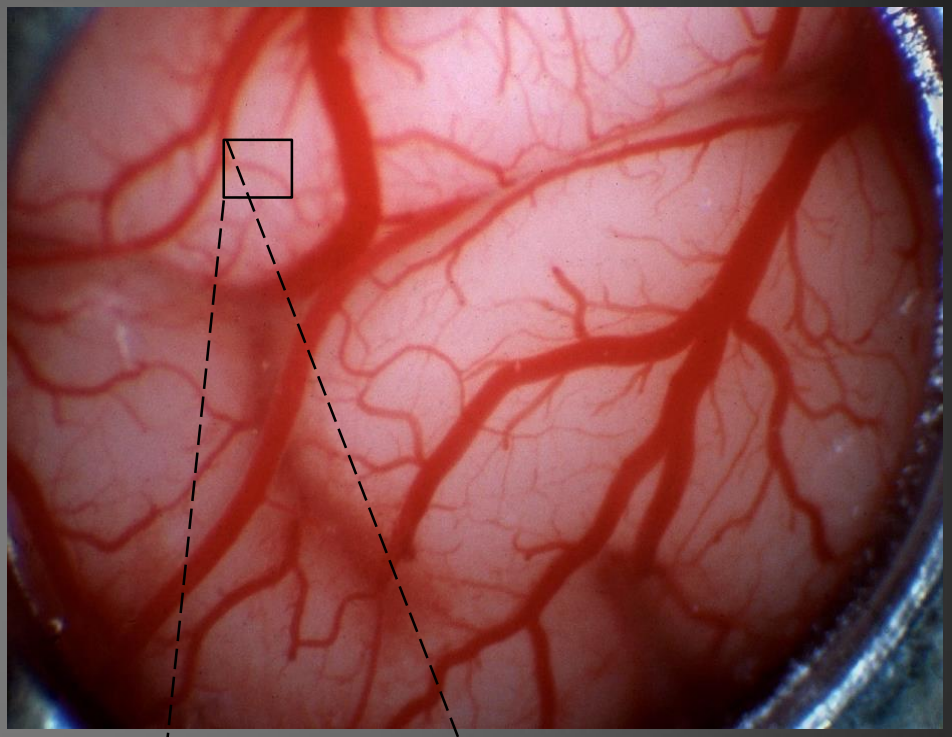
•Physiological environment

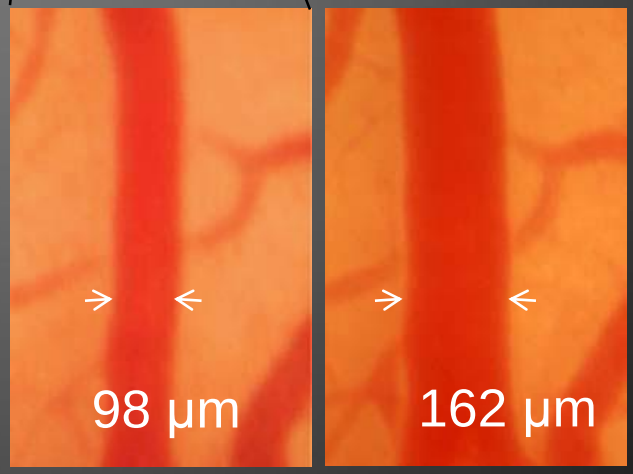
•Many kind of vessel can be studied

• Disadvantages:

•Parenchymal circulation cannot be studied

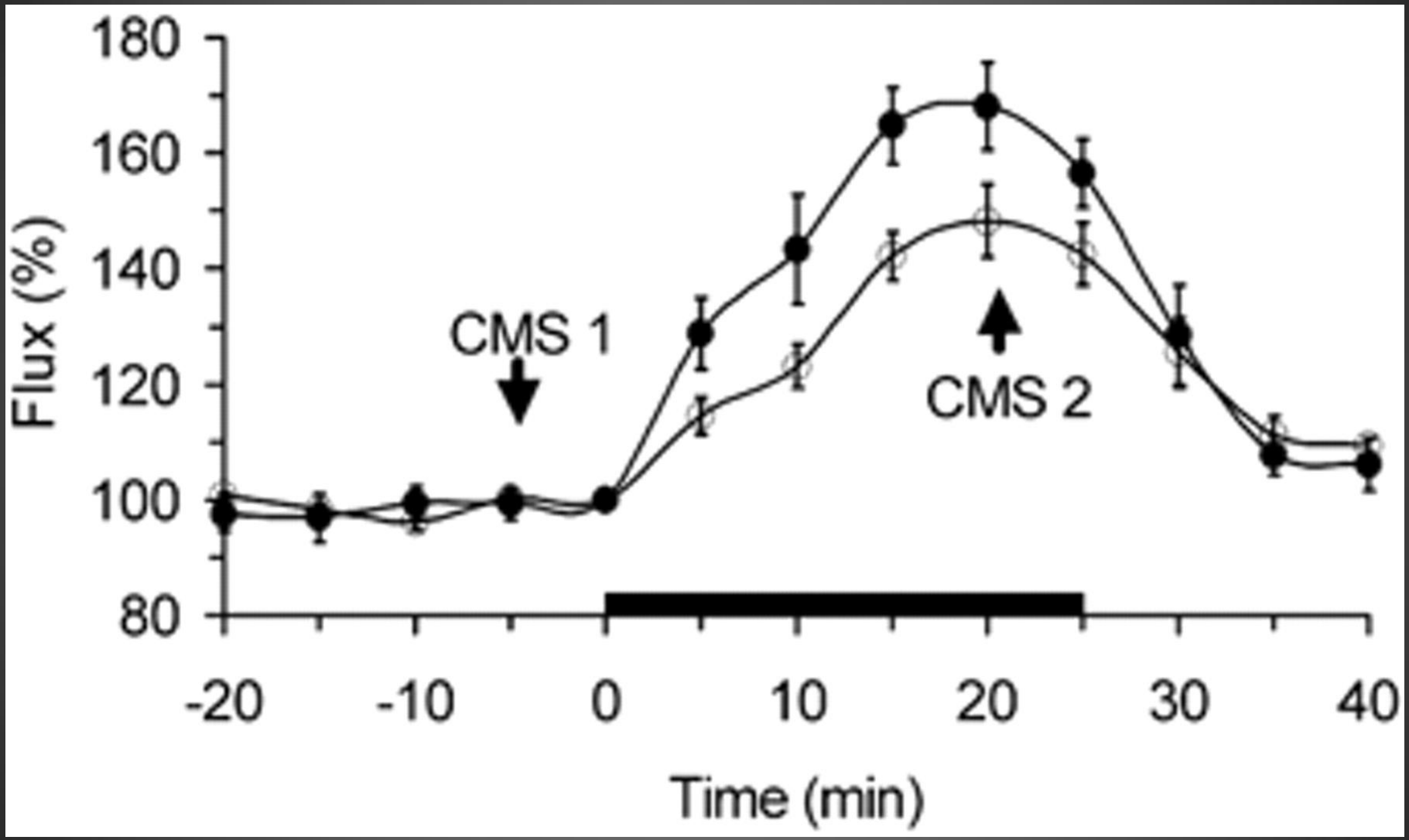
•Limited dynamical follow-up





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

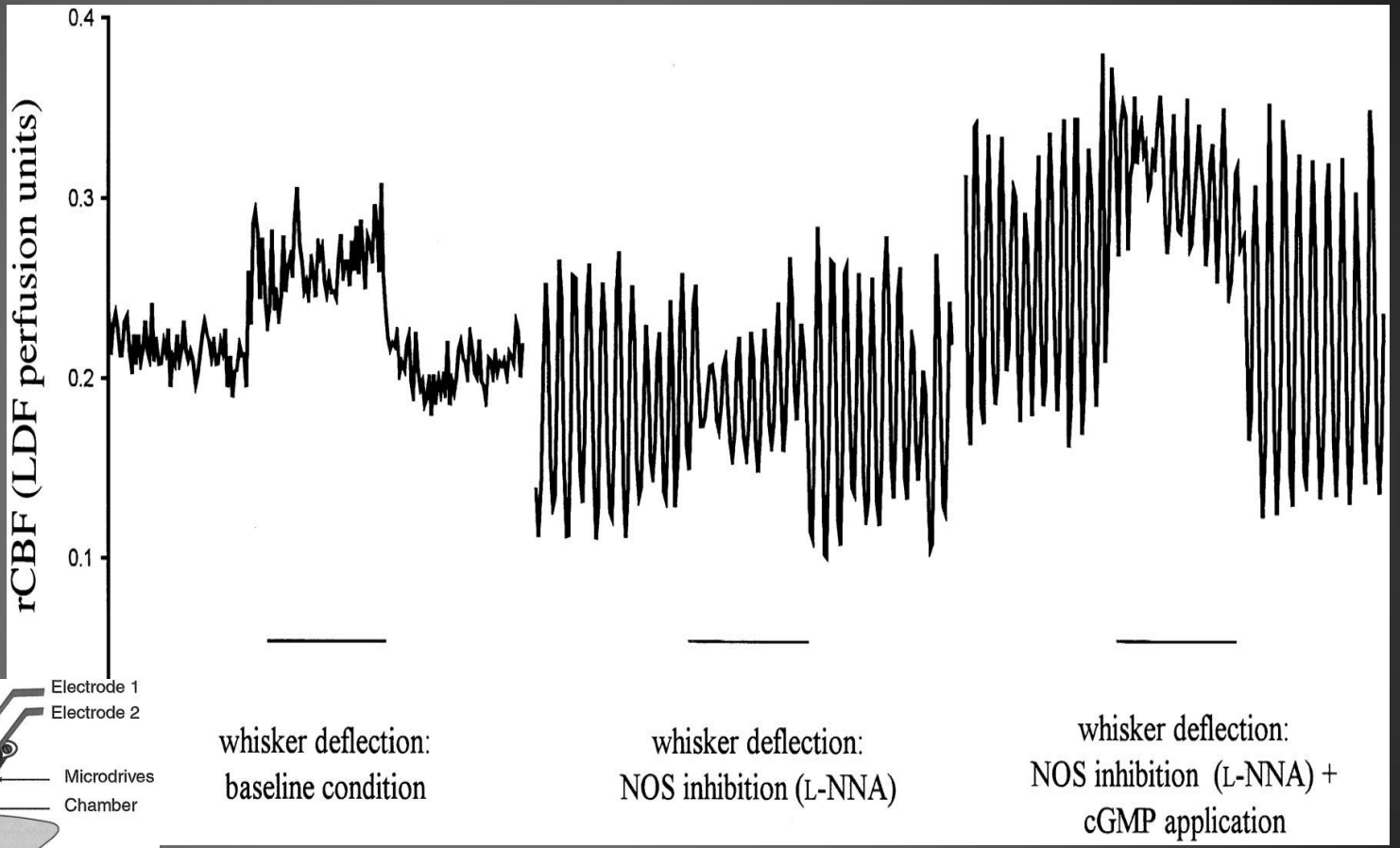
Time course of lCBF changes during maternal hypercapnia (black bar) measured by cortical surface (○, n = 6) and intracortical (•, n = 5) laser probes in fetal sheep at 110 dGAData are means ±s.e.m.

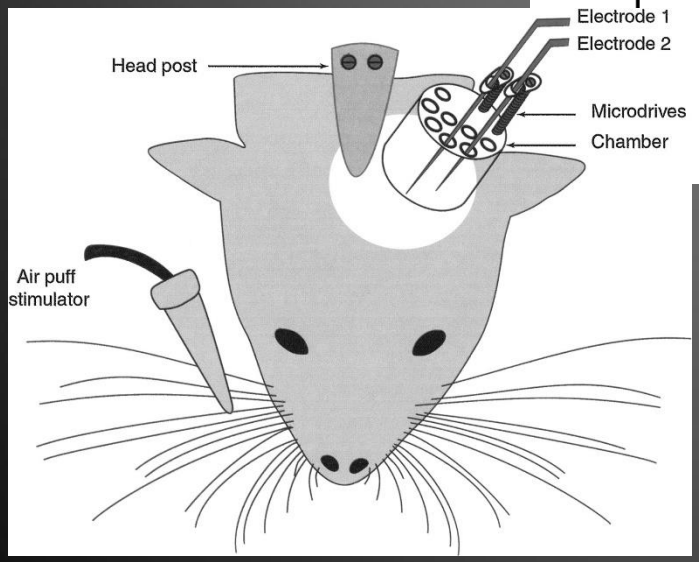


Müller T et al. J Physiol 2002;539:957-967

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

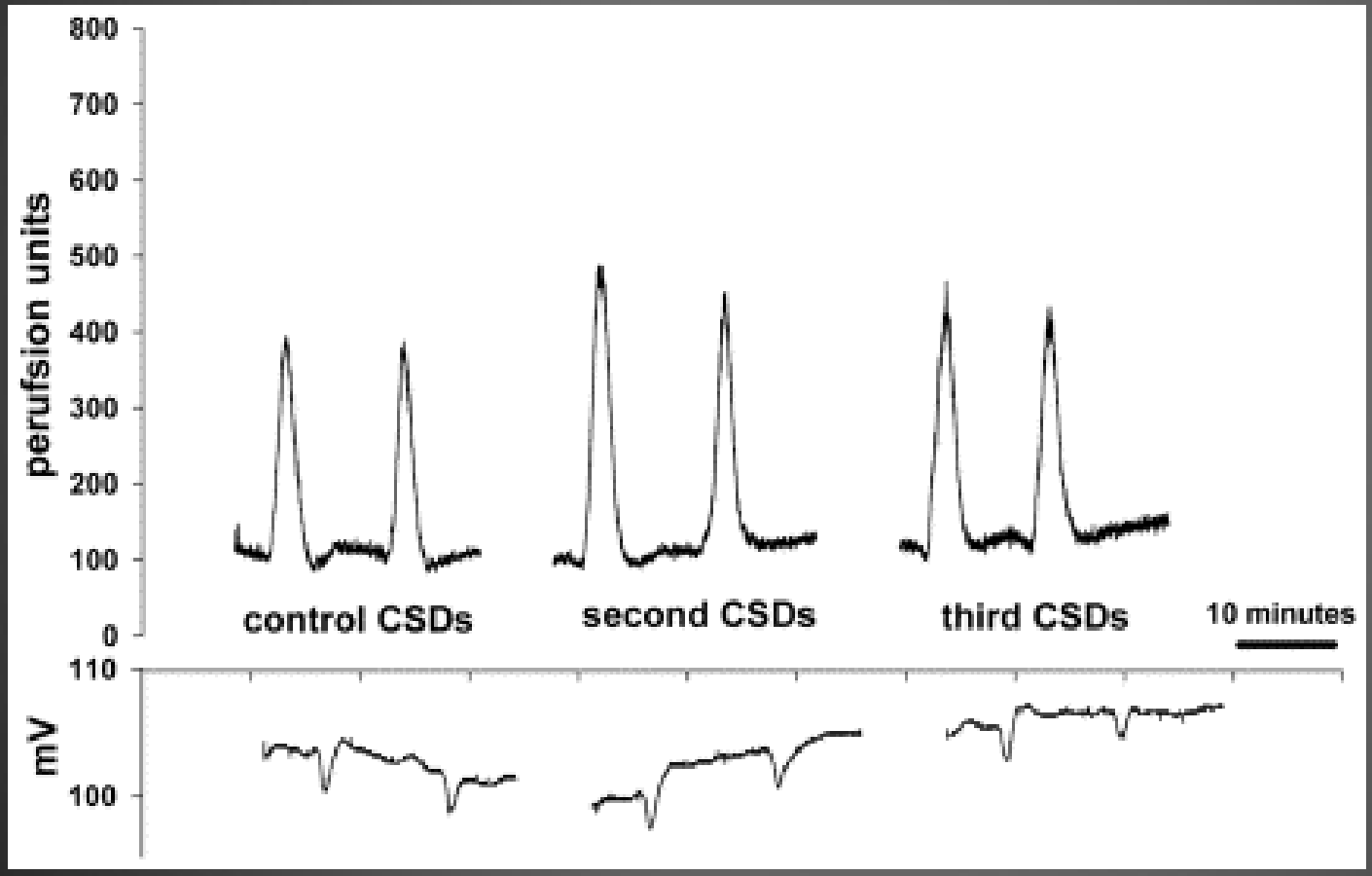


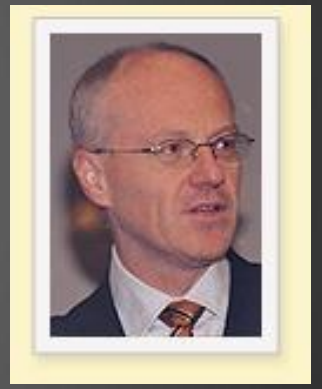


Lindauer et al, AJP, 1999

Neurovascular coupling-cortical spreading depression

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



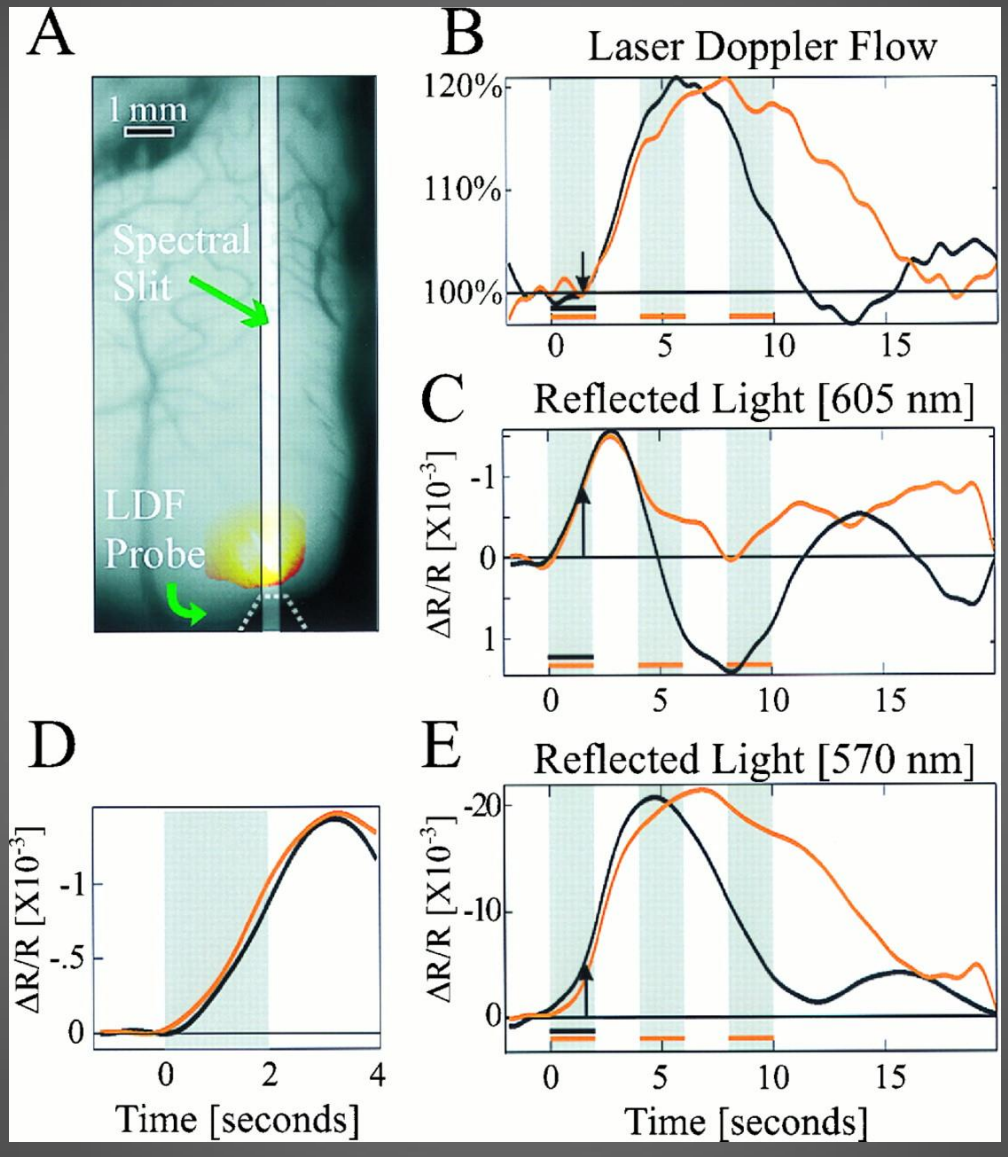


Prof. Peter Goadsby- Pioneer in headache & Cerebral microcirculation

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.

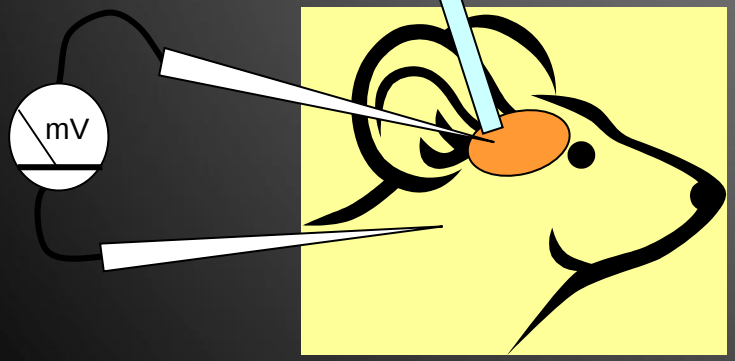
Visual stimulation
(2 sec)- black
(3x2 sec)- red

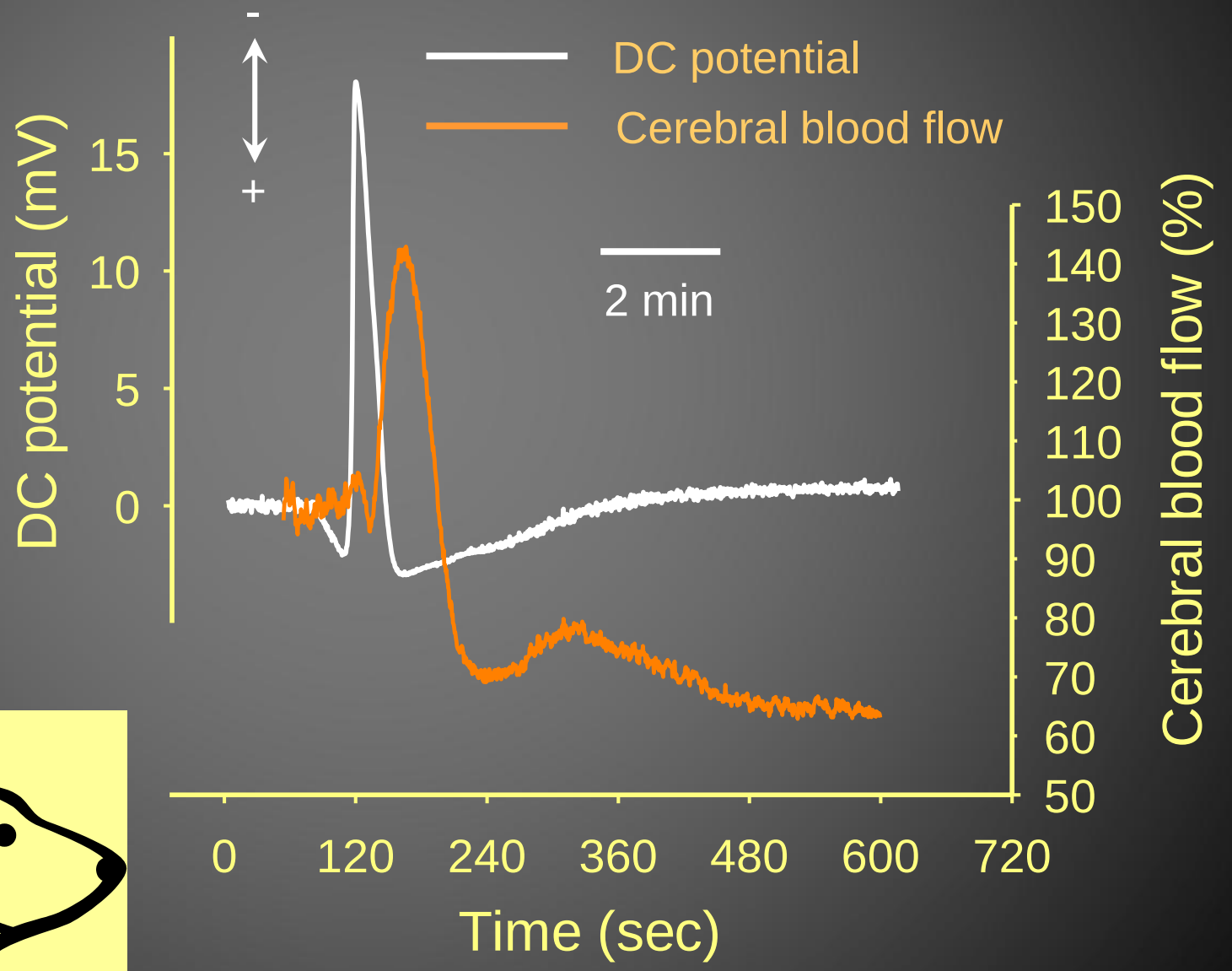


Dov Malonek et al. PNAS 1997;94:14826-14831

Spreading depolarization

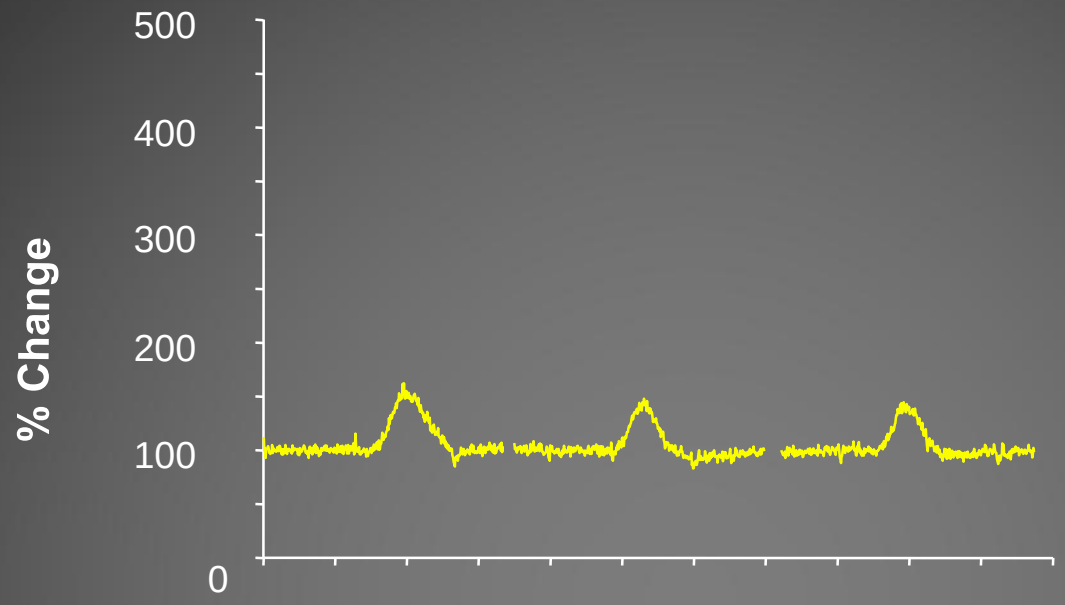
Laser-Doppler flowmeter





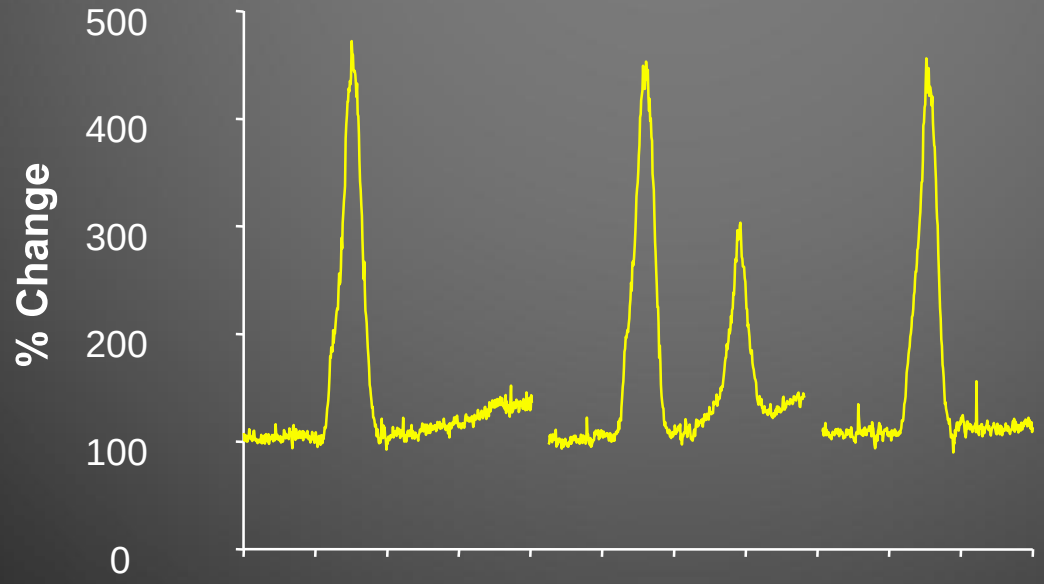
Farkas et al. (2008) JCBFM

Depressed hyperemic responses



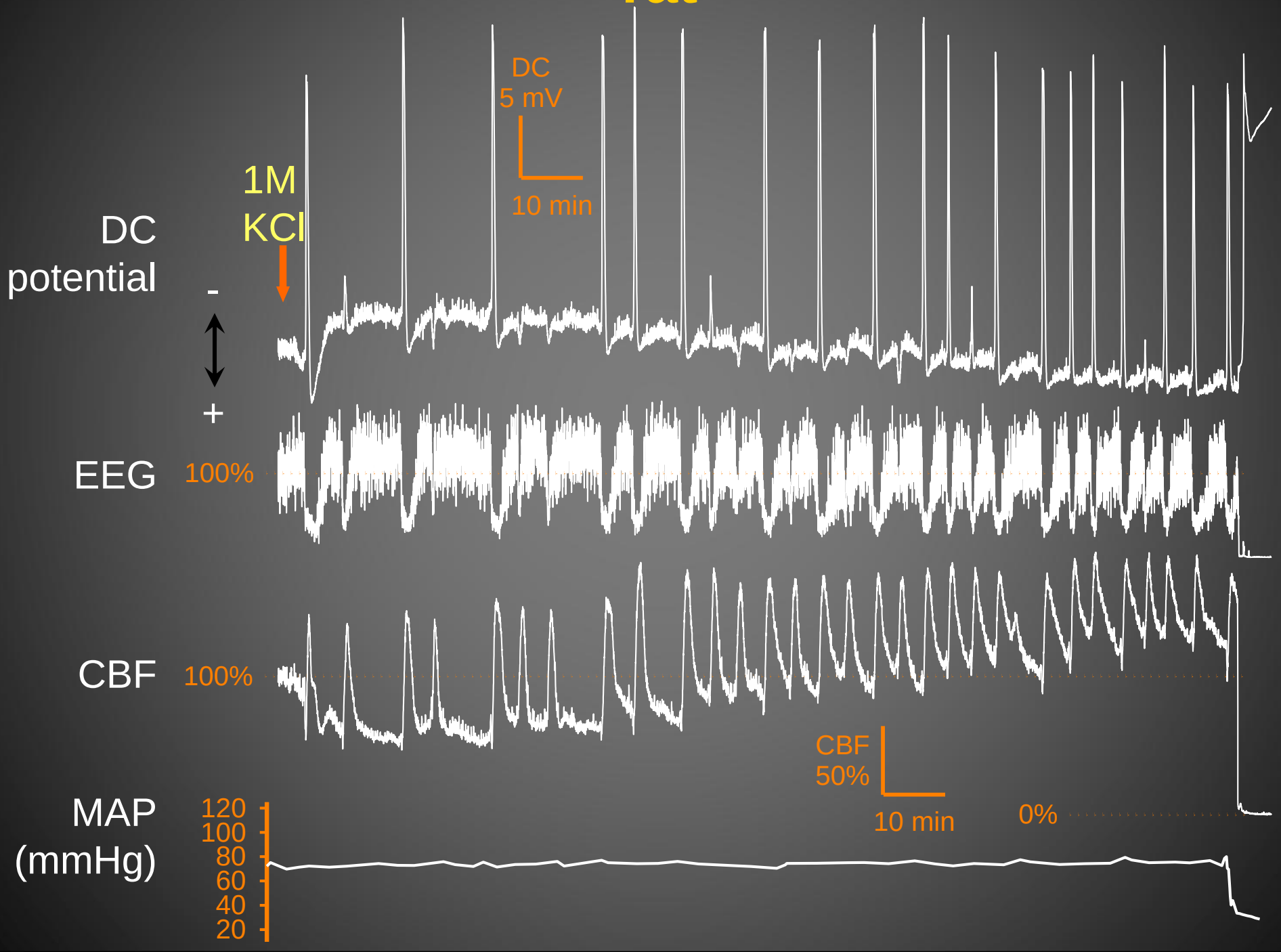
100 mg/kg - 48 hr

Enhanced hyperemic responses

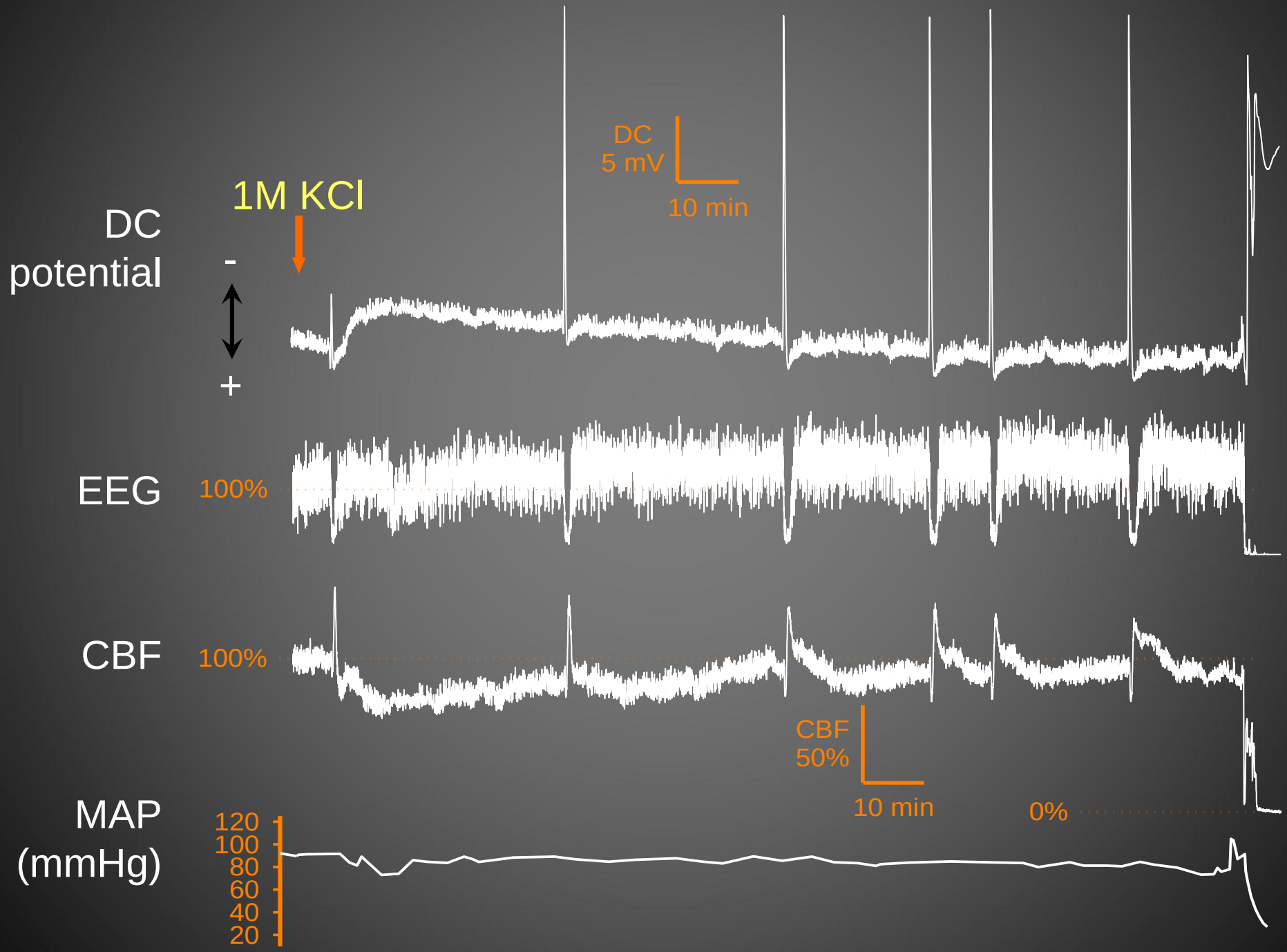


100 mg/kg - 96 hr

Representative experiment: young rat



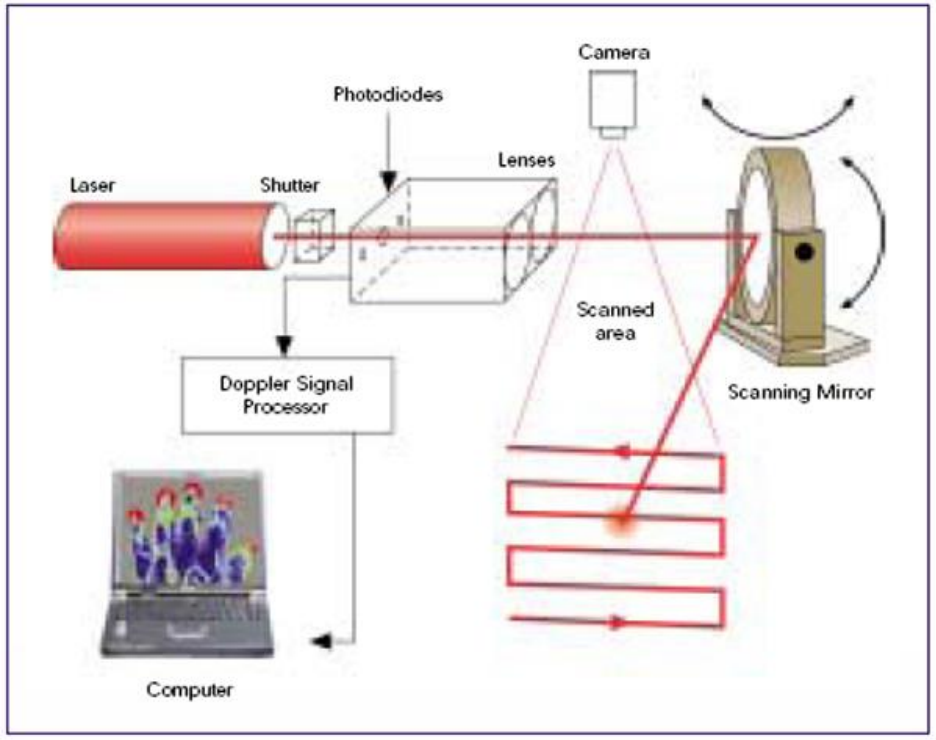
Representative experiment: old rat

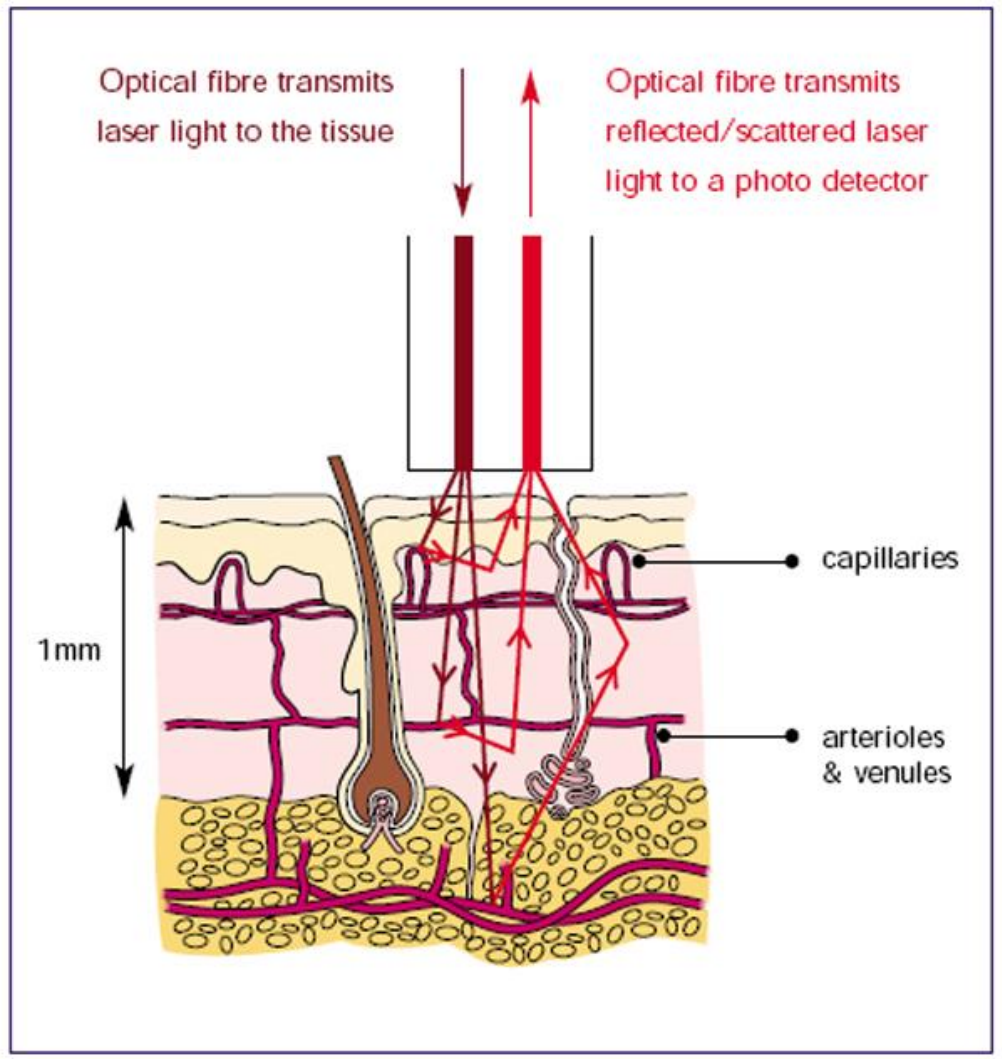


Single point blood flow imaging

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

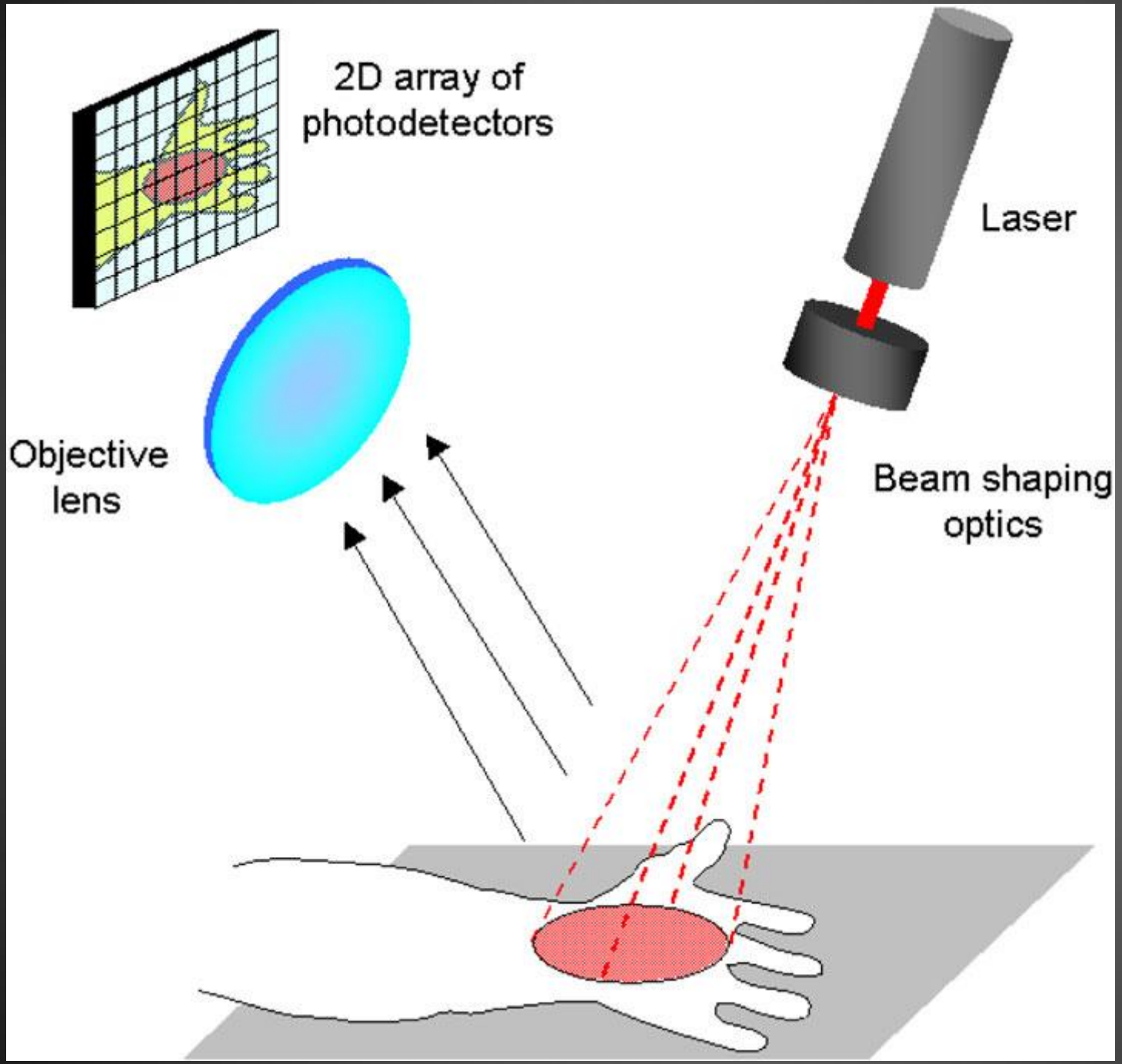
Scanning System

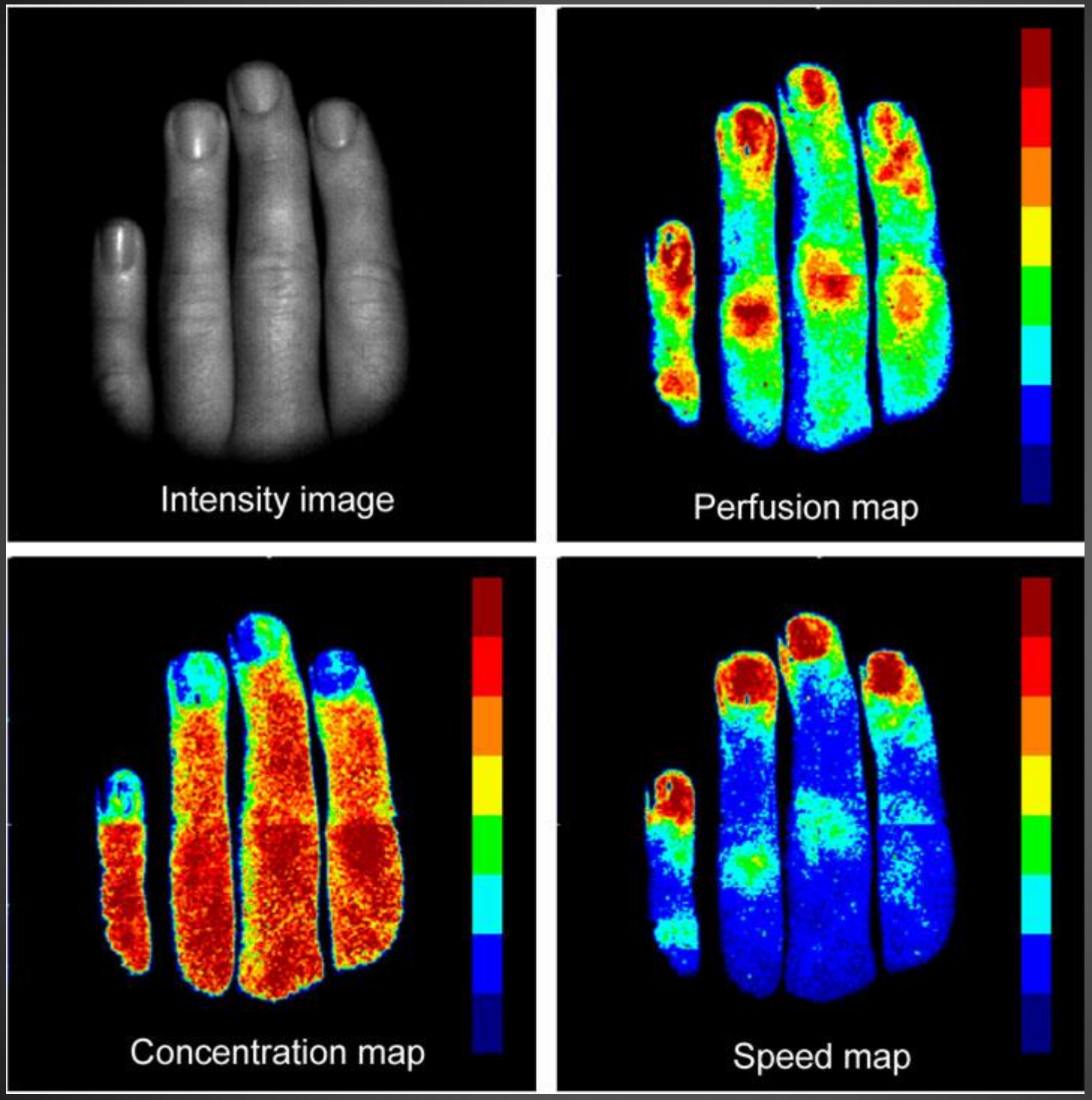




Builds up image point by point, slow

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

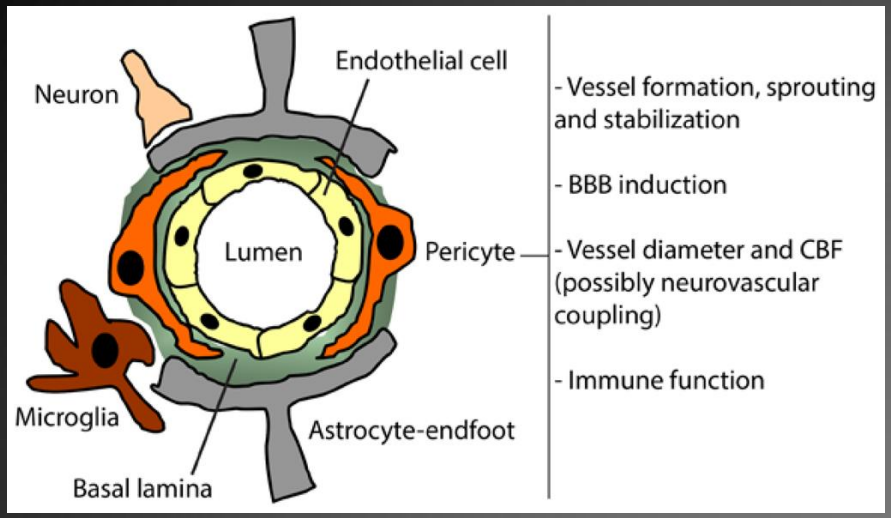
- To assess the blood flow pulsation in the pulp capillaries

7. Laboratory animal studies

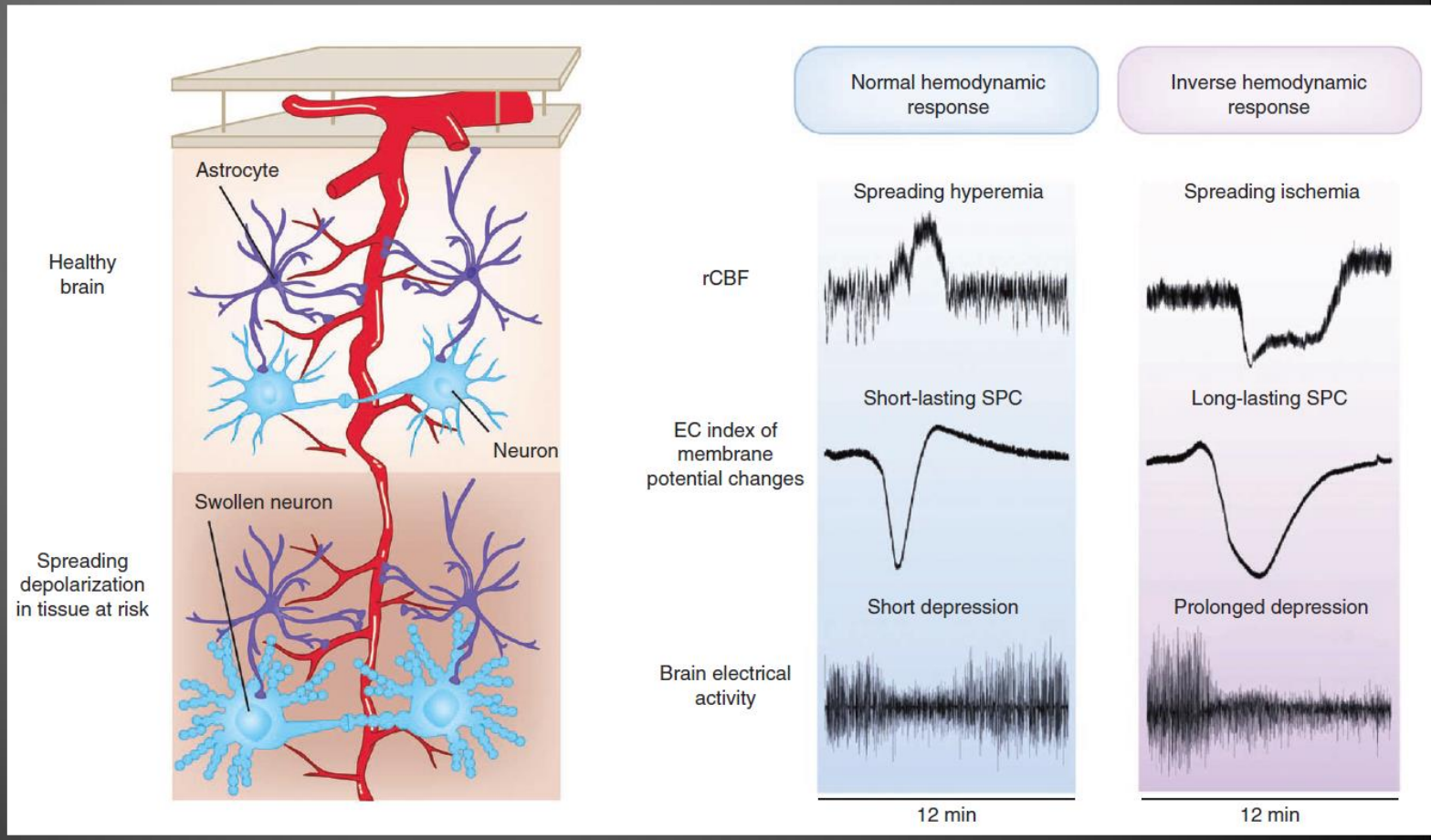
- For ocular, cerebral, cutaneous, auricular, splanchnic, and renal blood flow

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

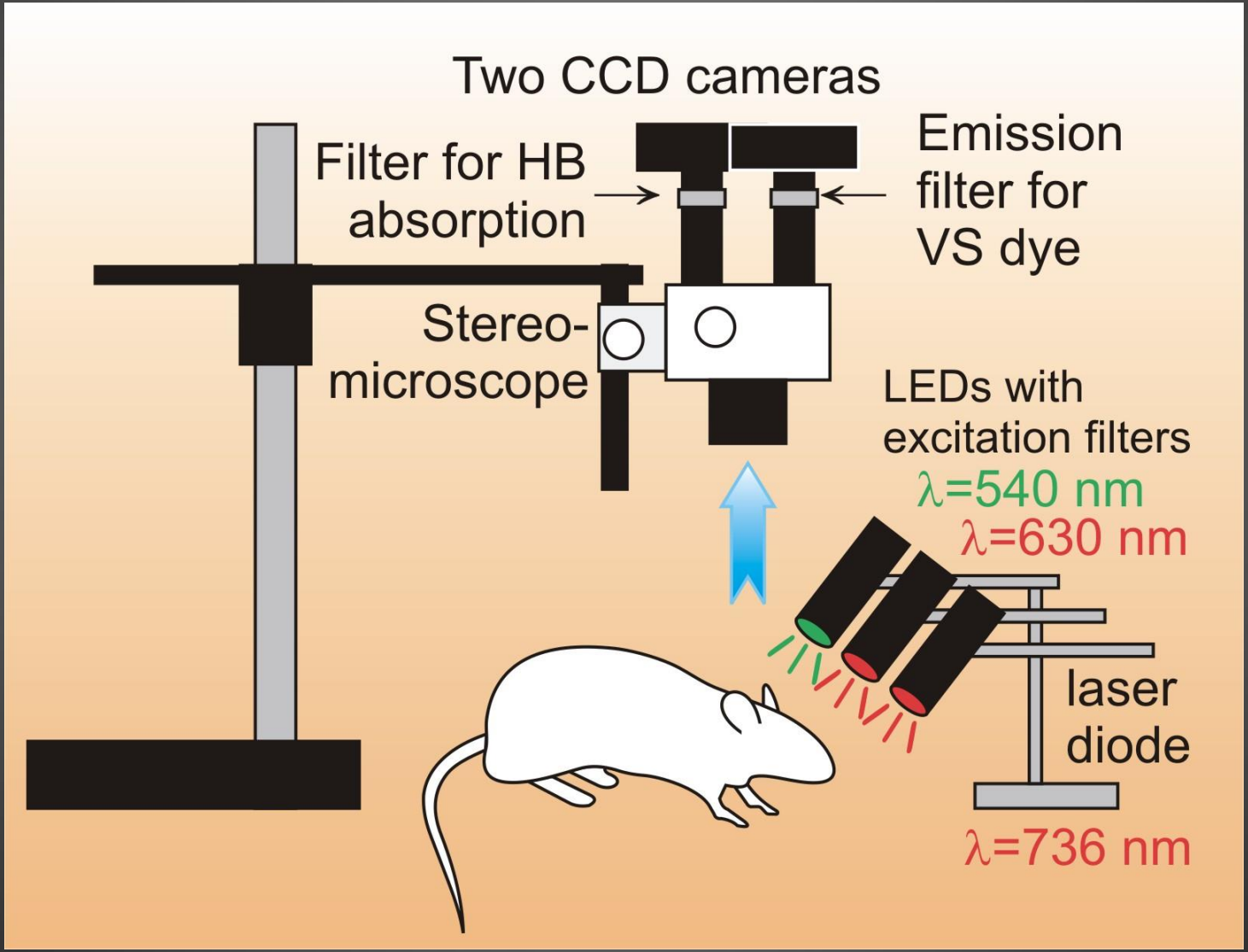


Neurovascular coupling and spreading depolarization in the injured brain

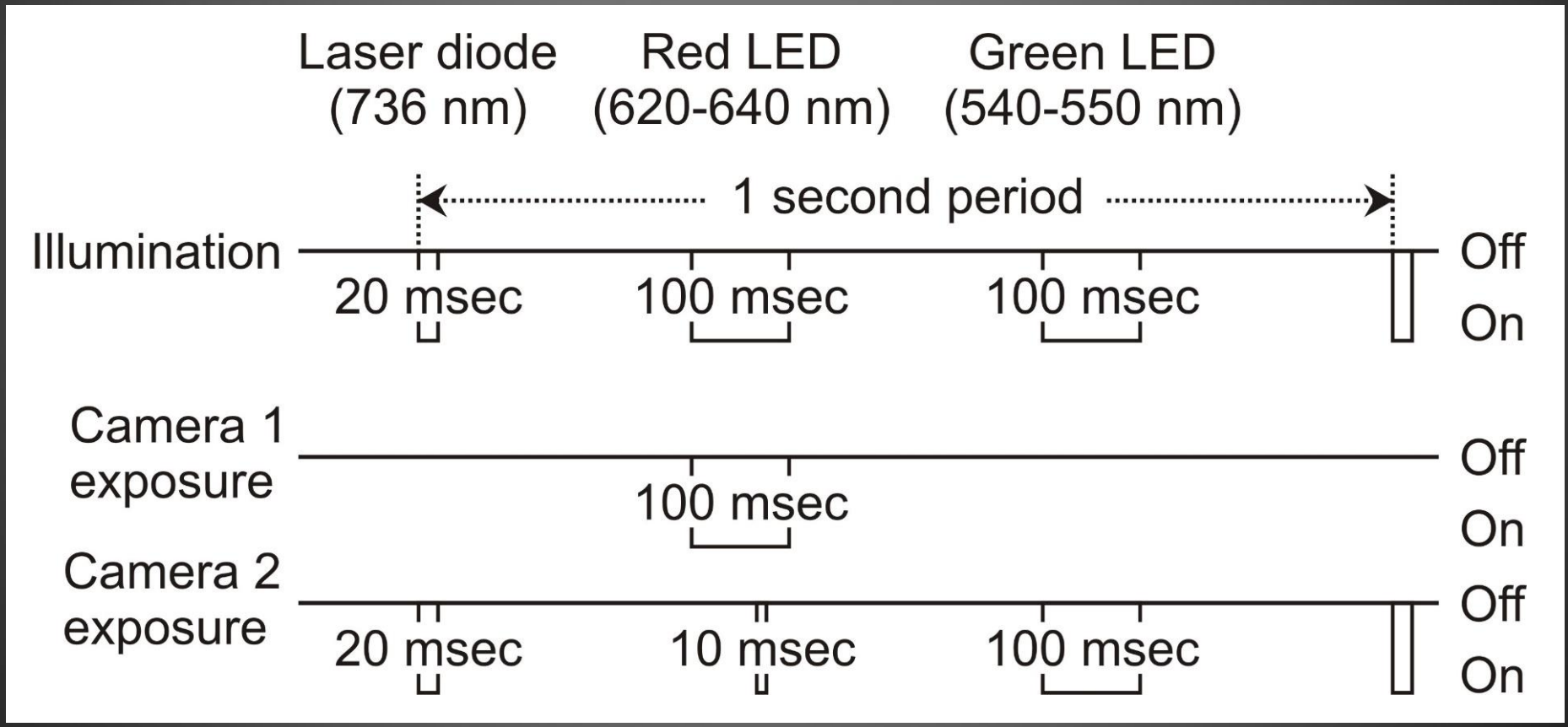


Dreier (2011) Nat. Med.

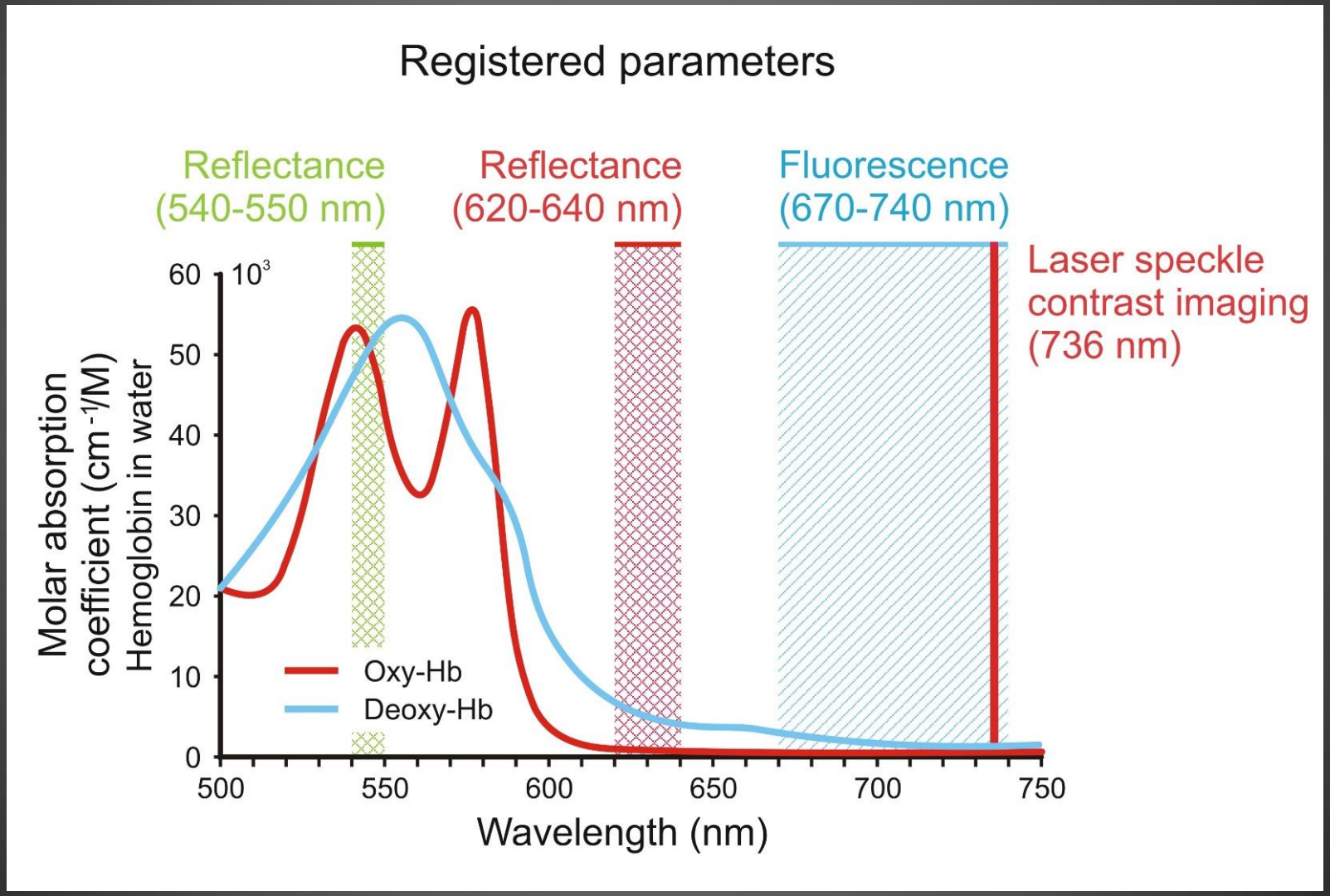
Draft of the arrangement of the setup



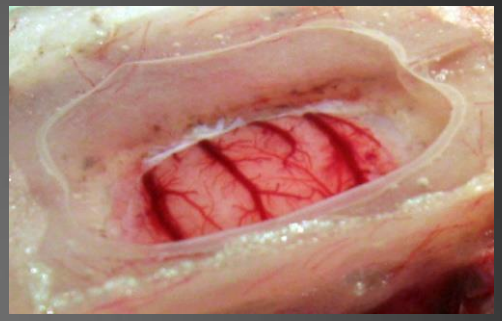
Synchronization of the respective illumination/image capture

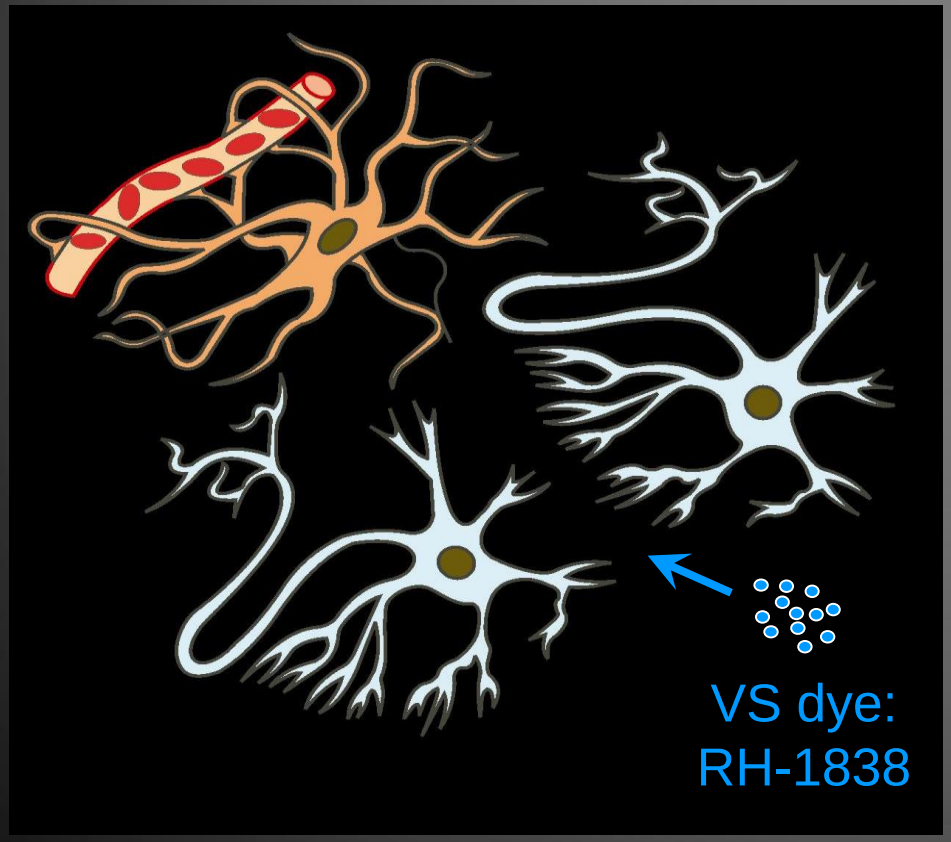


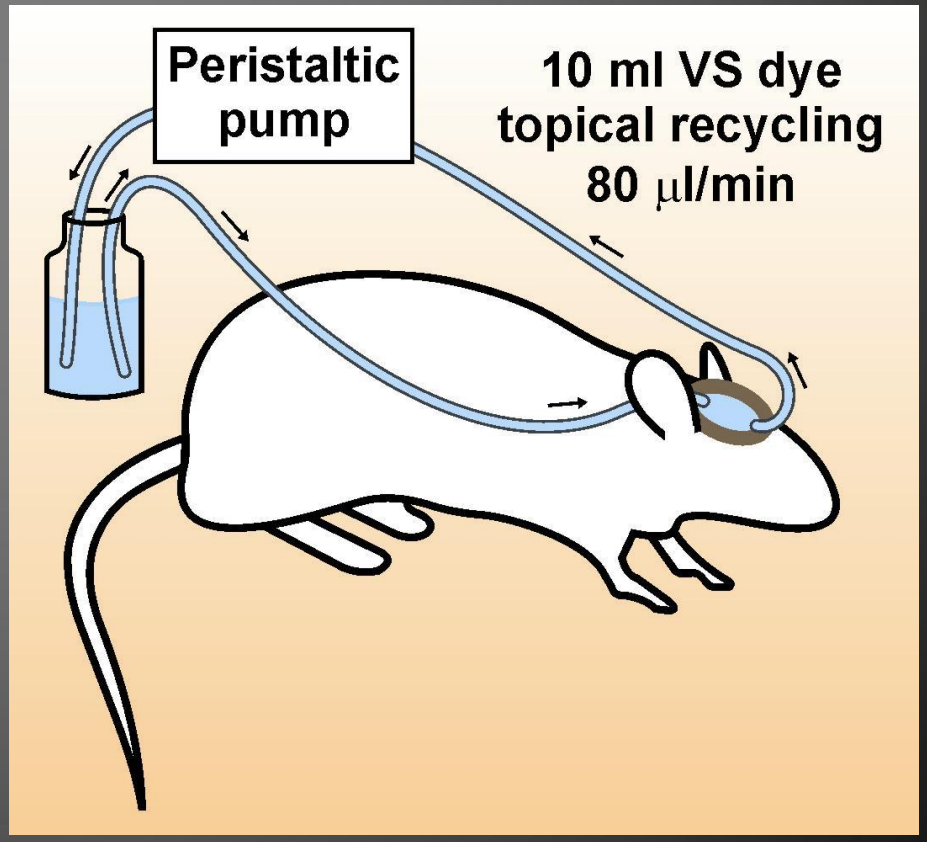
Optical principles for multimodal imaging



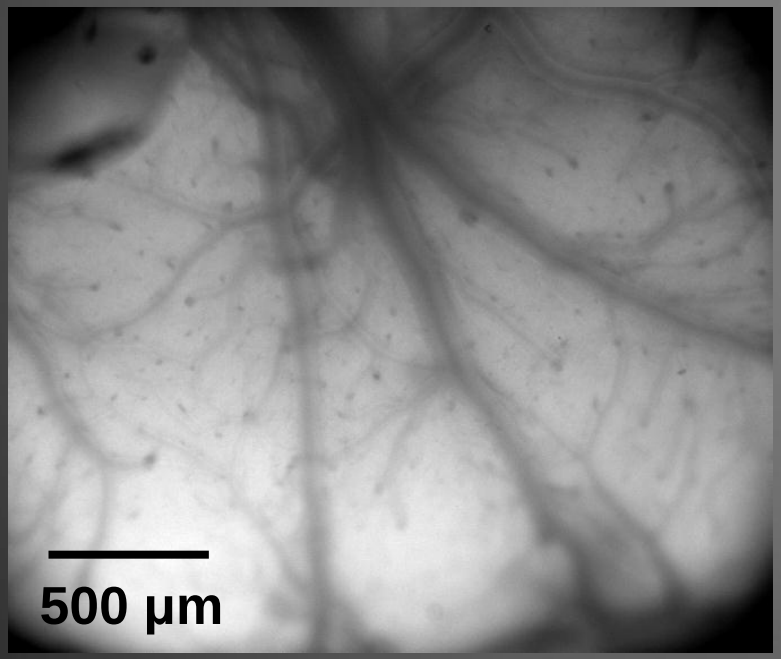
Voltage sensitive dye loaded in a closed cranial window

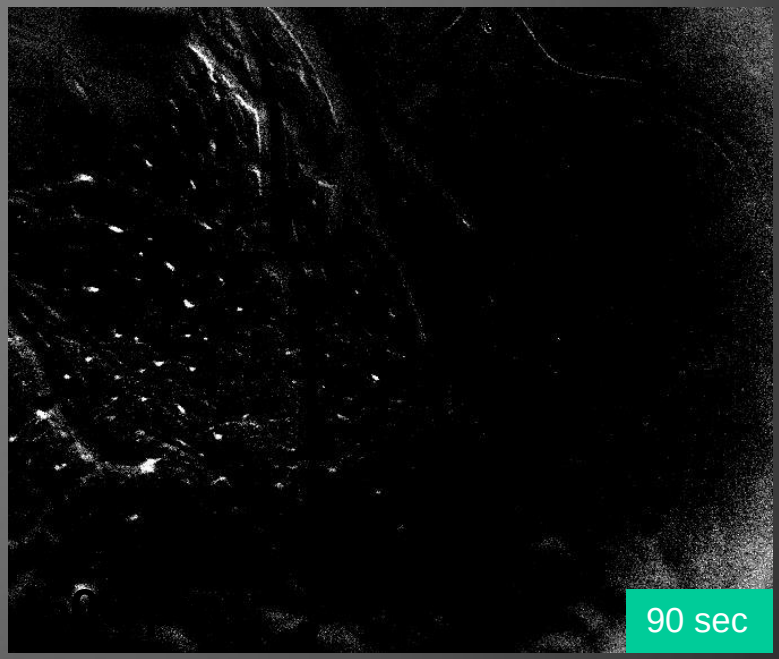






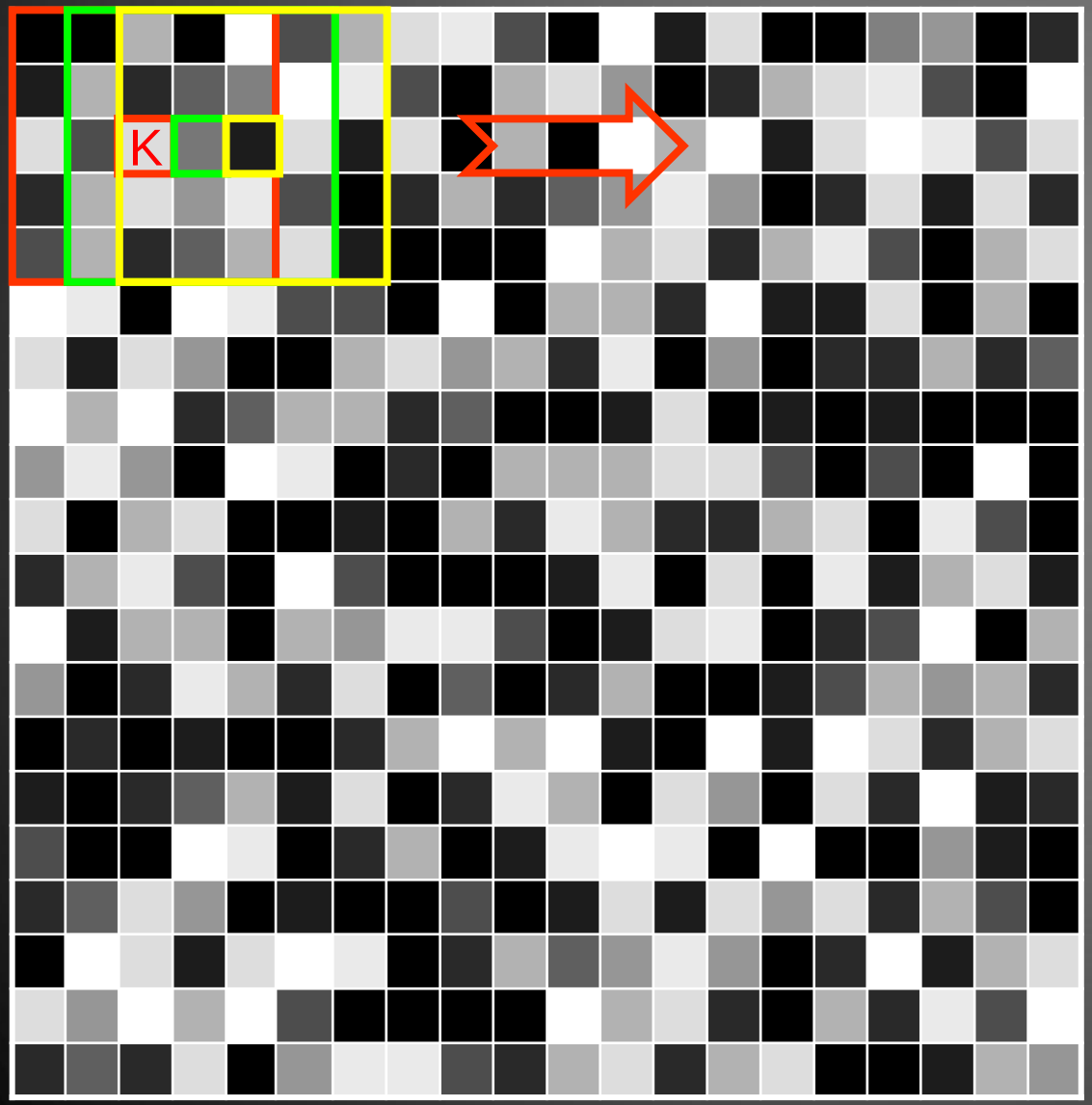
Representative video for SD-related changes in VS dye fluorescence





Exp. code: imag55, SD1

Laser speckle contrast analysis



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

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

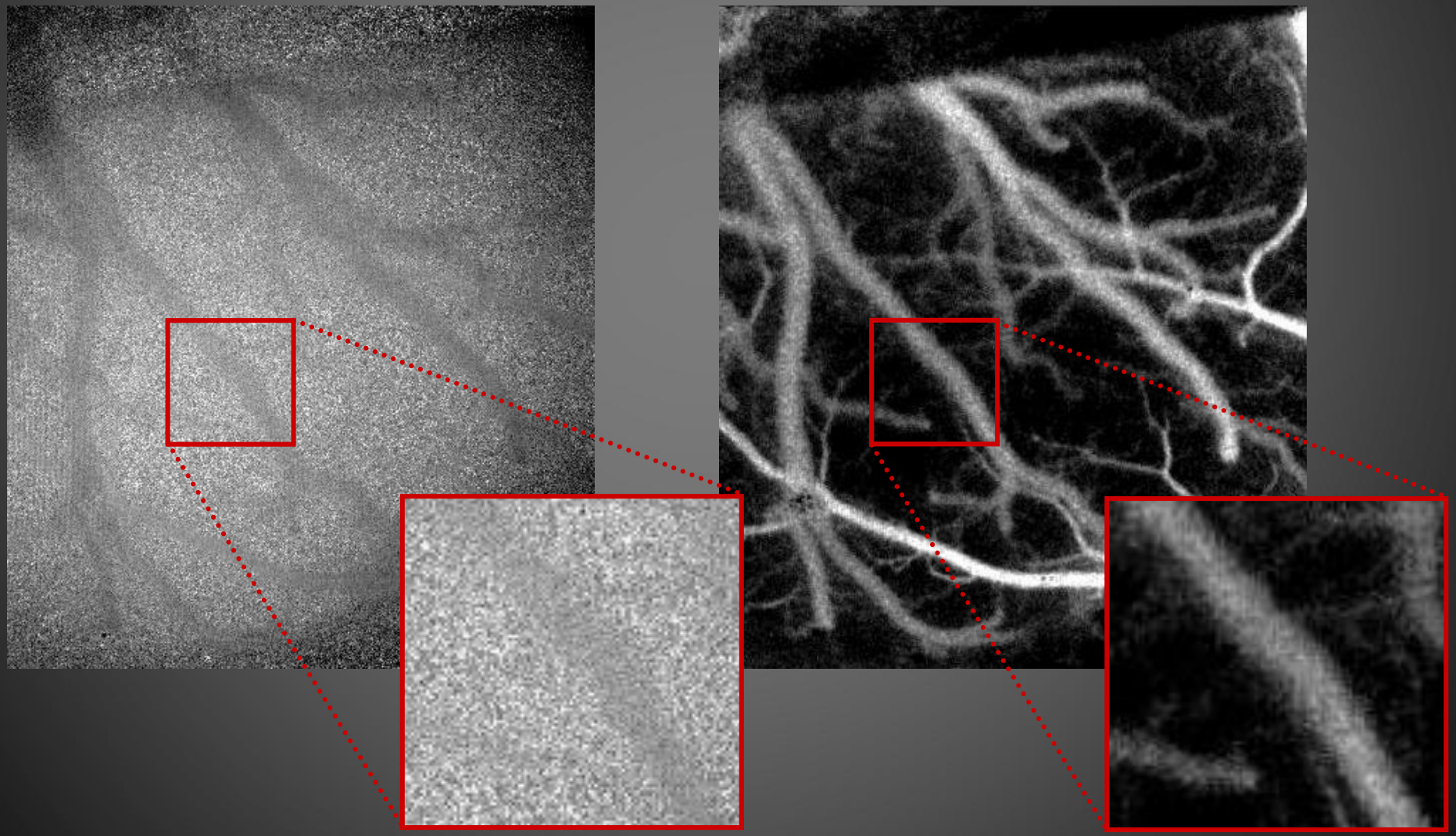
⇨ $1/K^2$

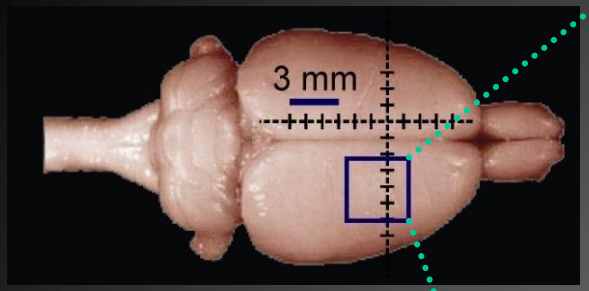
Particles with low motility➙ high contrast
Particles with high velocity ➙ low contrast
⇨ The velocity of particles is proportional with:
- the decrease in speckle contrast
- (time of exposure)

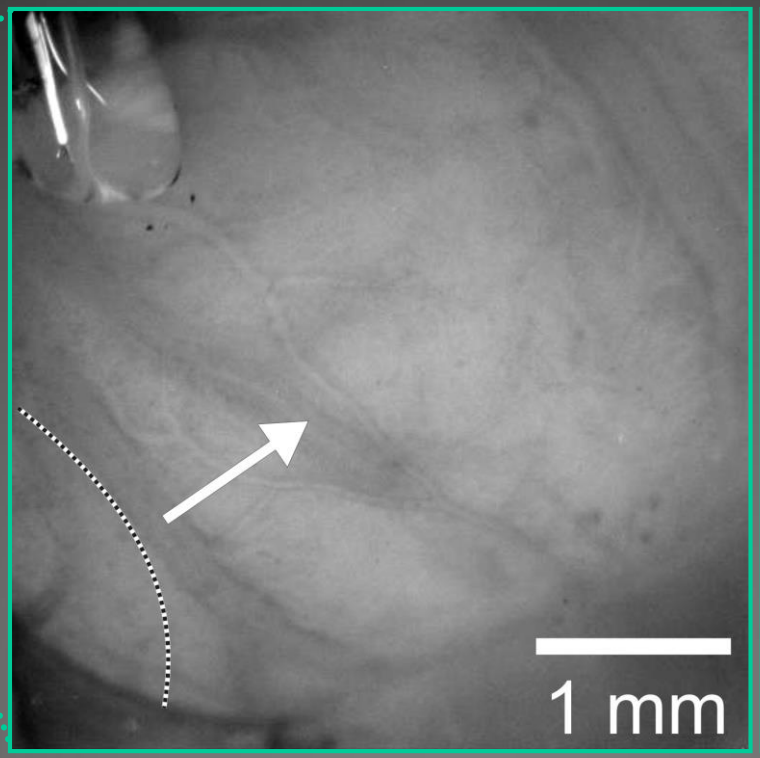
Acquired images

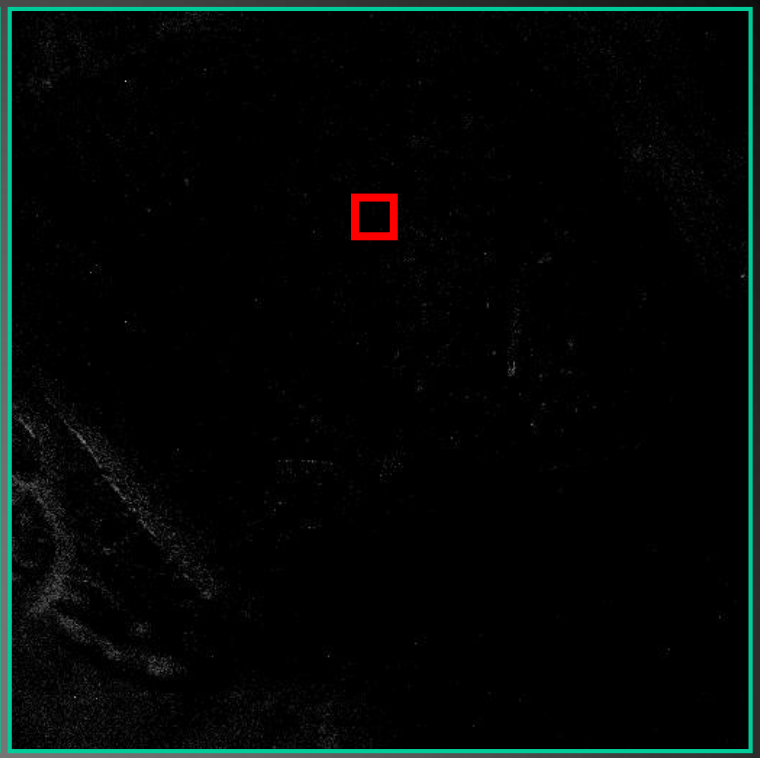
raw speckle image

flow map

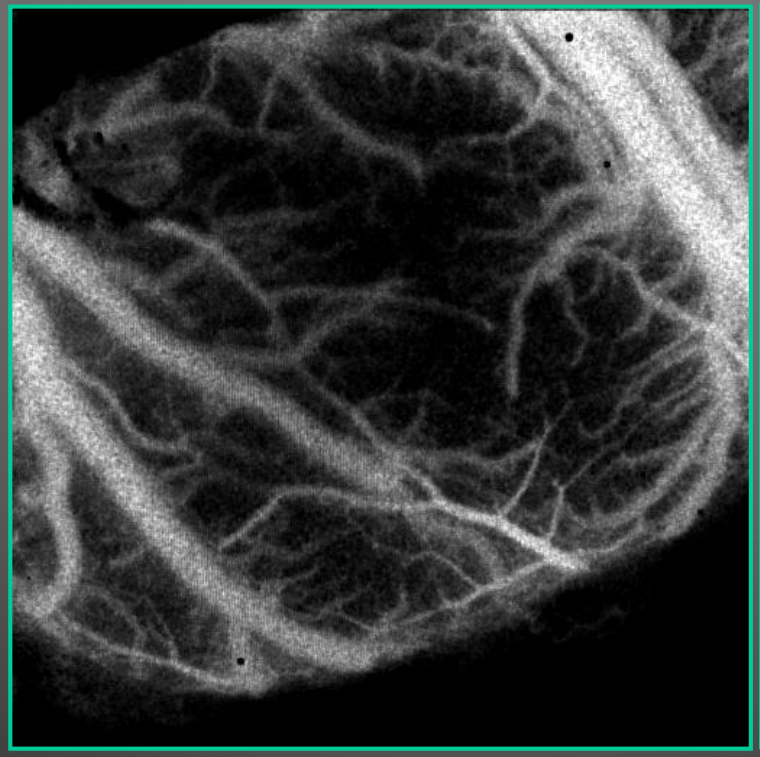


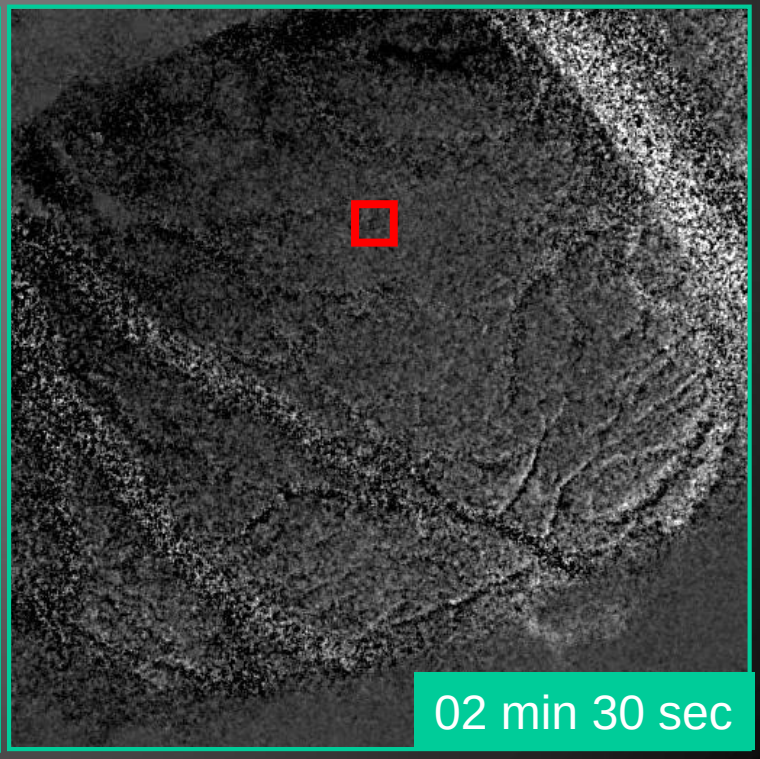






Simultaneous imaging of CSD and the CBF response





Exp. code: VSLS07 CSD1

Obrenovitch et al (2009)

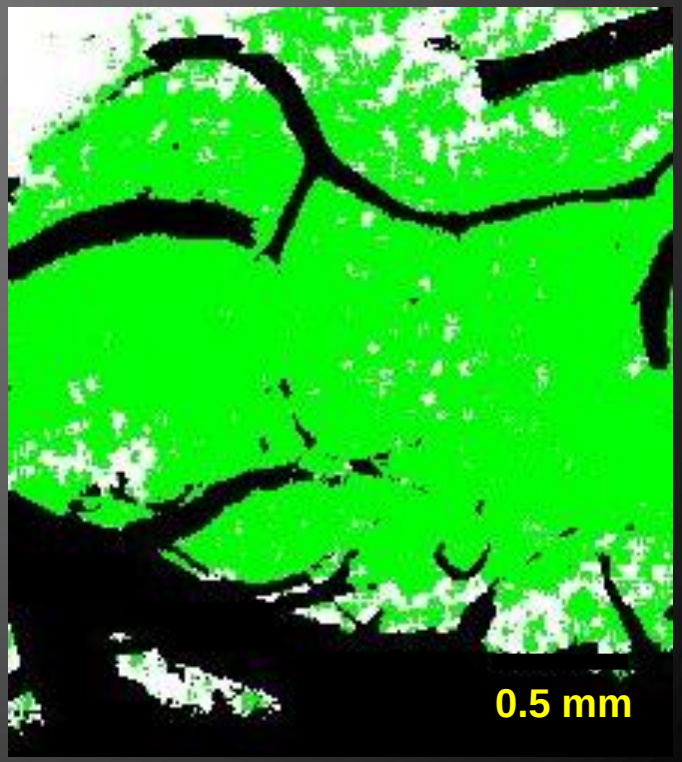
Whole field analysis of the VS dye signal

Area terminally depolarized in various age groups

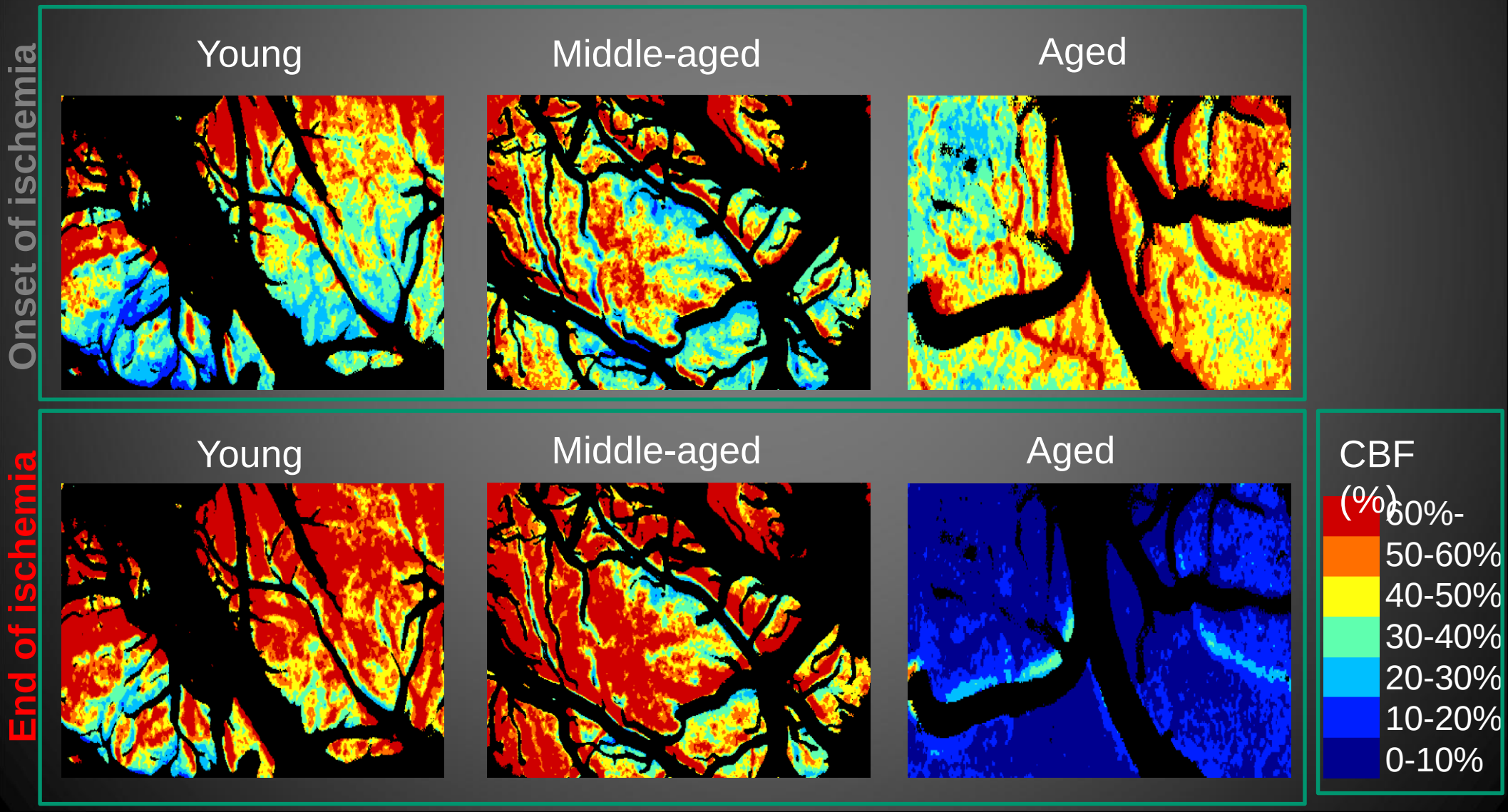
Young

Middle-aged

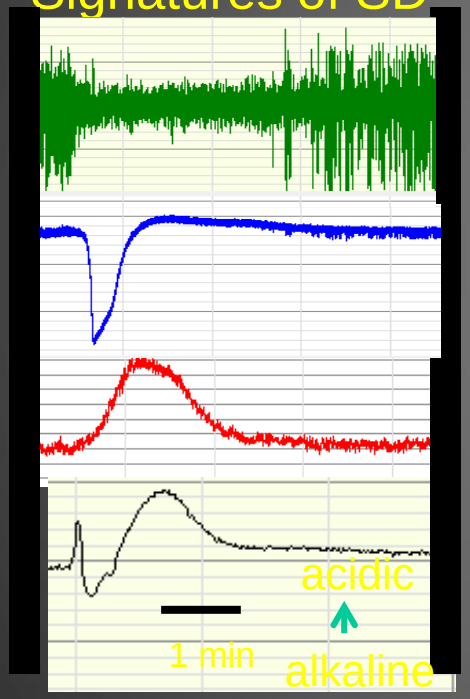
Aged



Whole field analysis of cerebral blood flow maps



Spreading depolarization

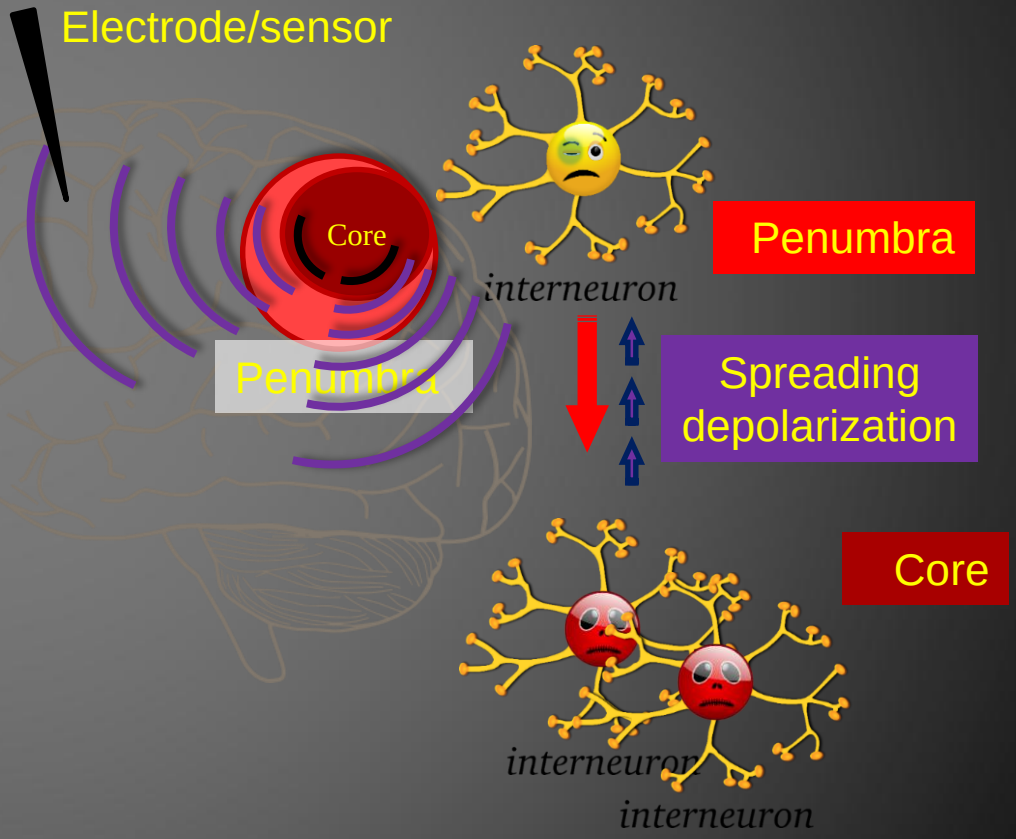


Spreading depression of activity

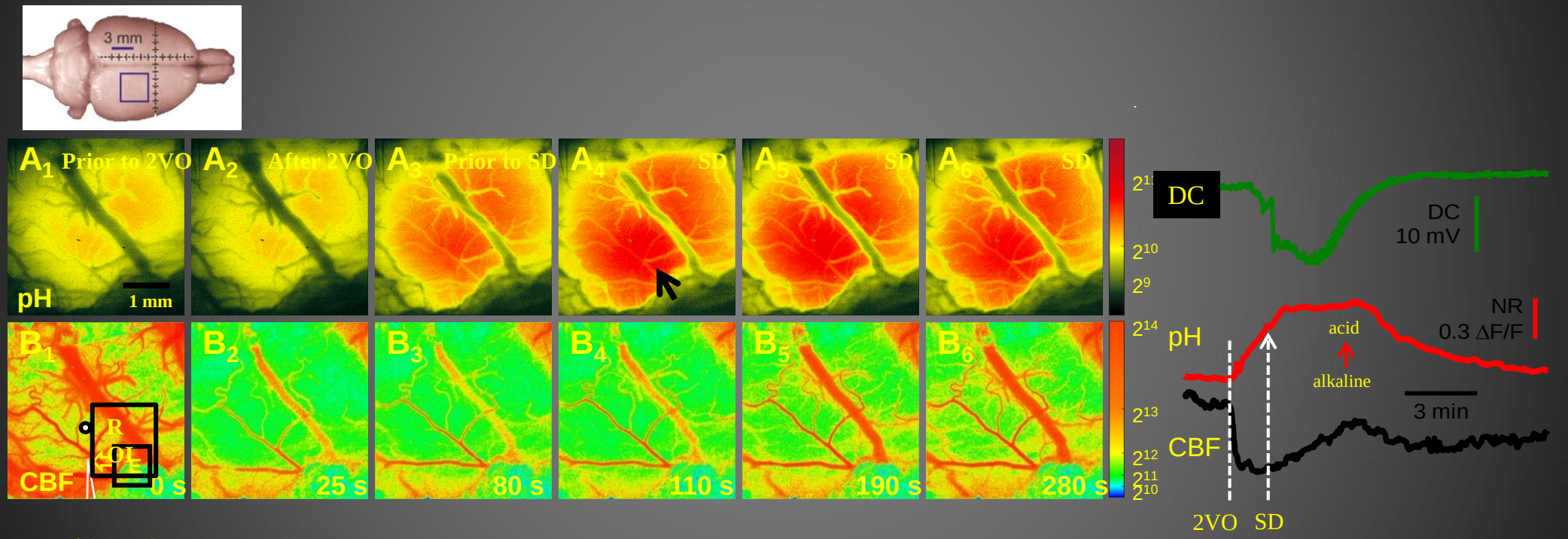
Spreading depolarization

Spreading hyperemia

Spreading acidosis



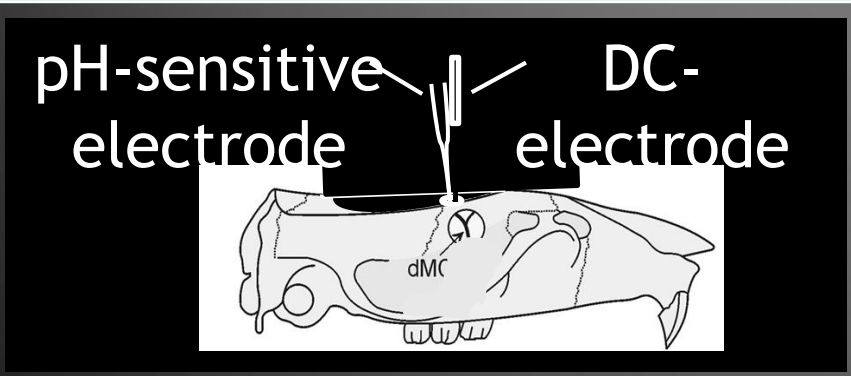
Spreading depolarization and tissue acidosis

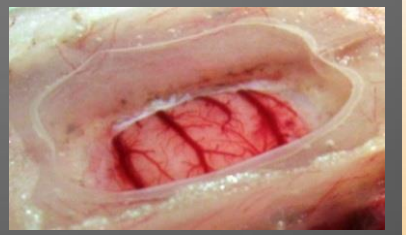


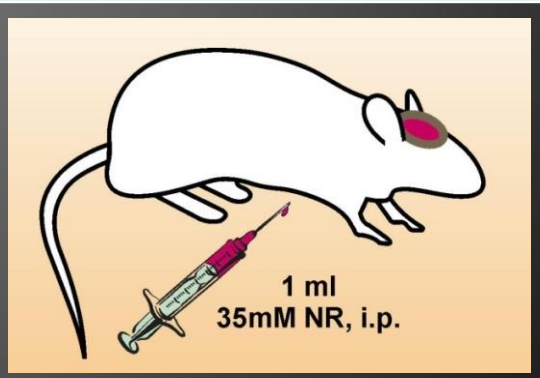
Menyhárt et al., Sci. Rep., 2017

Methods

Method ❶ Traditional pH measurement	Method ❷ pH imaging
Small, open craniotomy	Closed cranial window (4 x 4 mm)
Implantation of pH-sensitive electrode	Loading with pH-sensitive dye
Laser-Doppler flowmetry	Laser-speckle contrast analysis



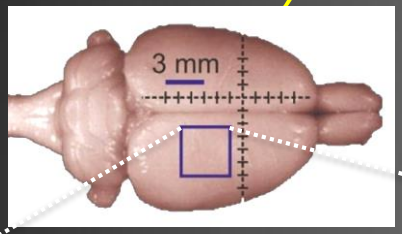




Intracellular pH imaging

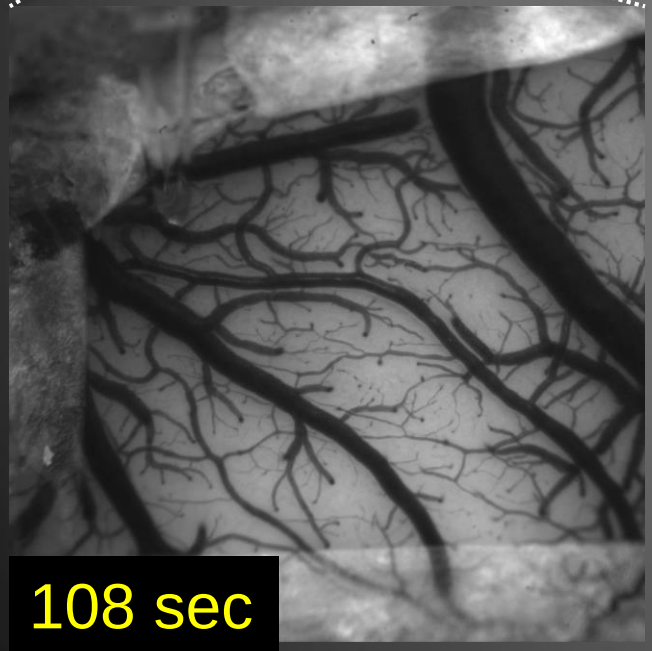
Representative image sequences (SD elicited during baseline)

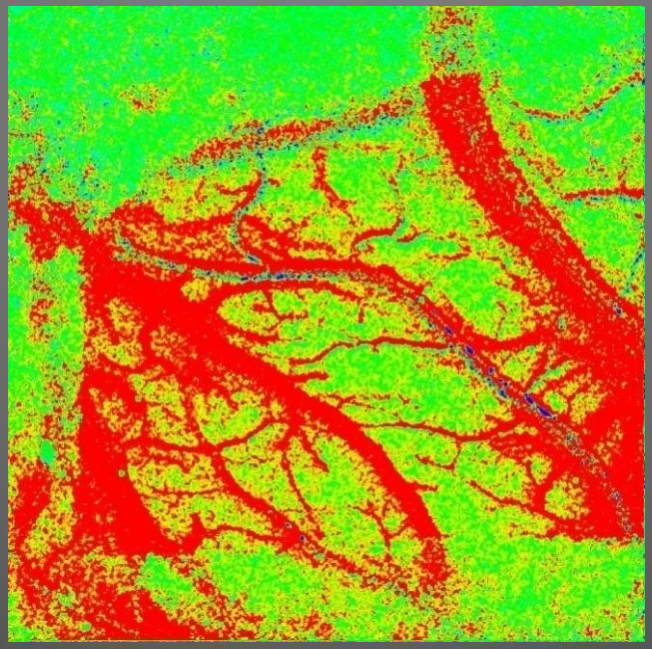
Field of view: green reflectance)

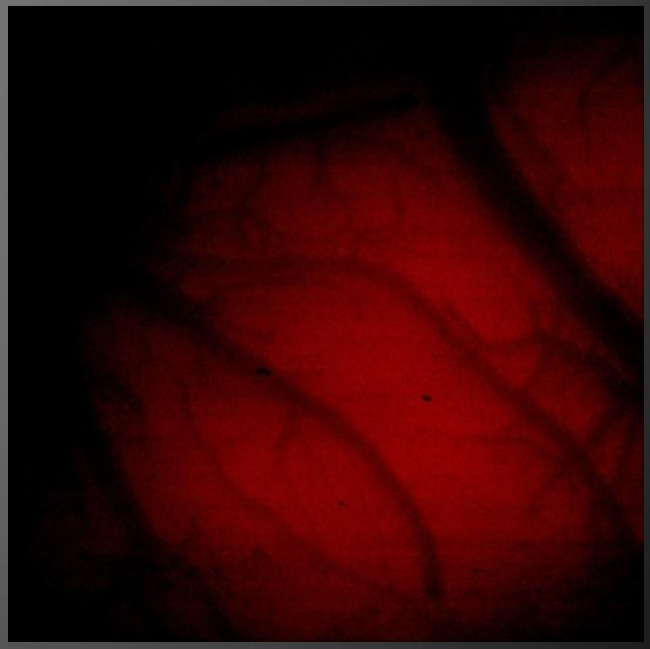


Laser-speckle contrast analysis:
Cerebral blood flow (CBF)

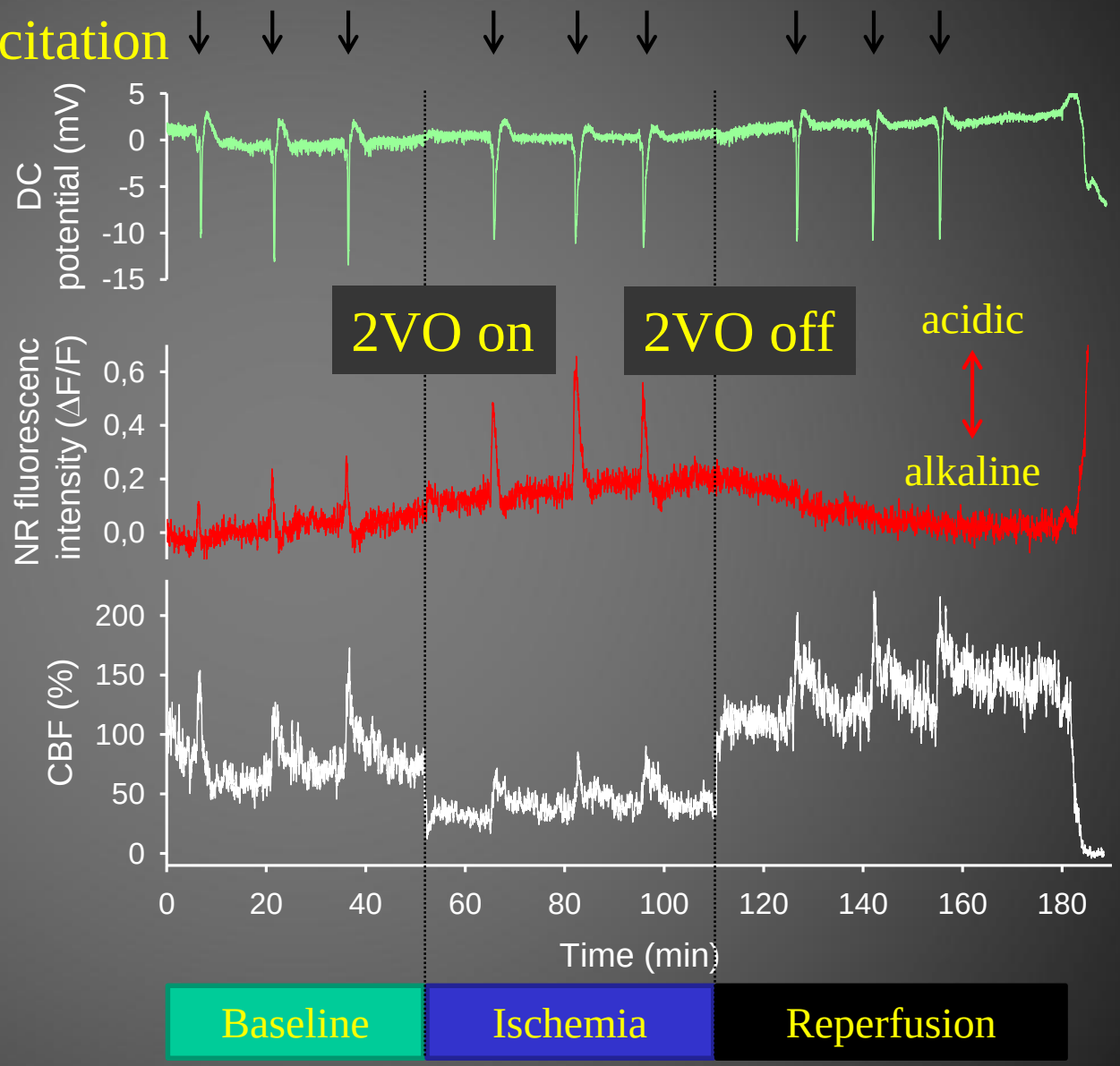
Neutral red fluorescence:
Intracellular pH







Representative recording:
- **DC potential**
- **tissue pH**
- **CBF**



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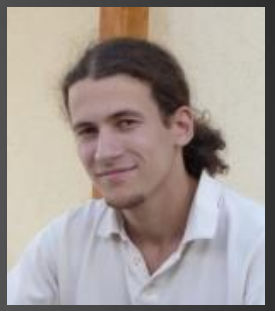

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Real time measurement-
a window towards
the dynamics of cerebrovascular regulation

Autoregulation-range and dynamics under various circumstances

Rhythmic patterns in the microcirculation-vasomotion

Neurovascular coupling