Optical methods for exploring microcirculation: opportunities and limits

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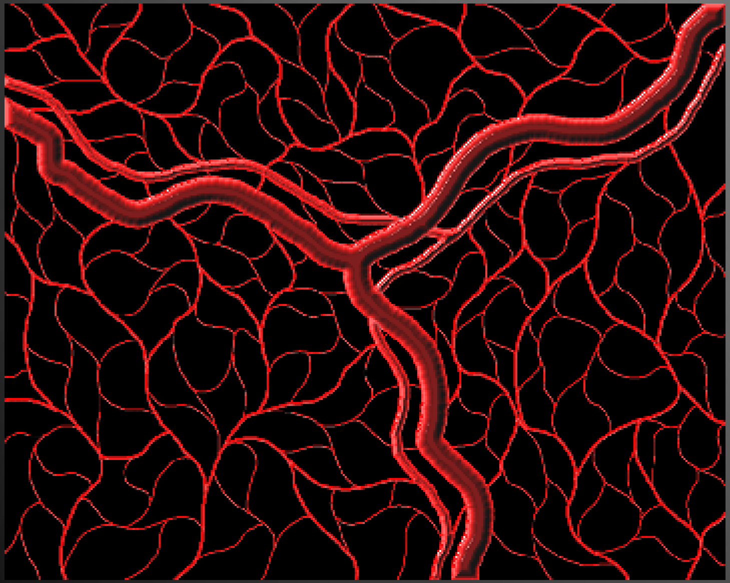
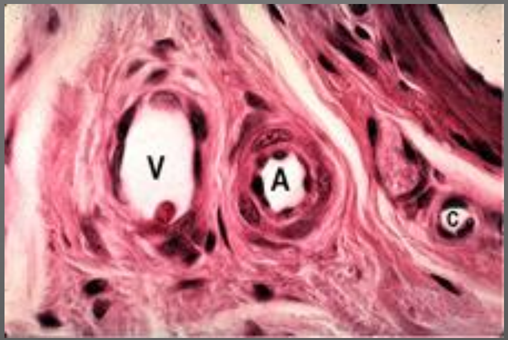
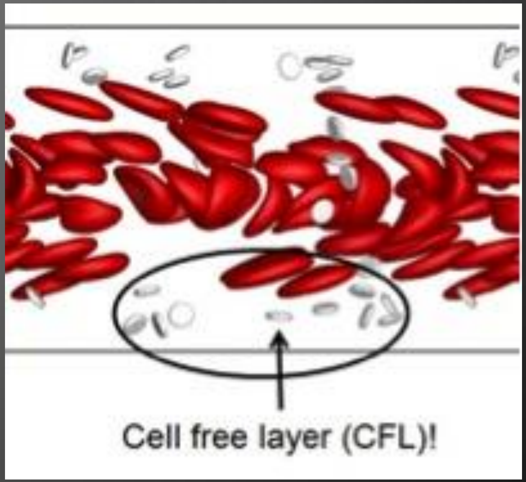
LAMELIS 2017.

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

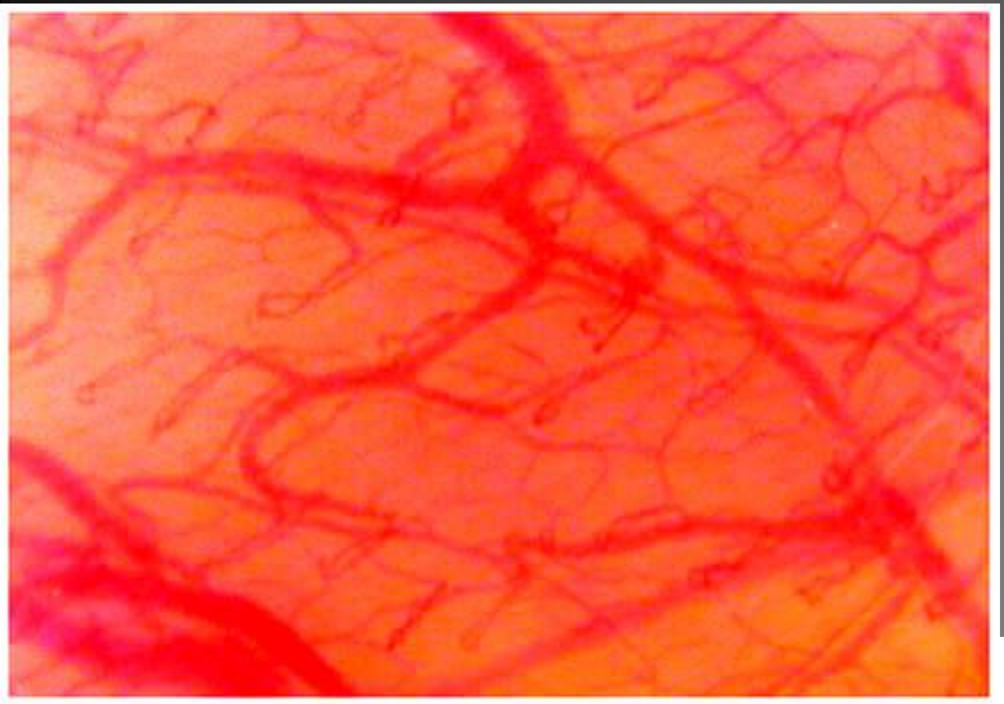
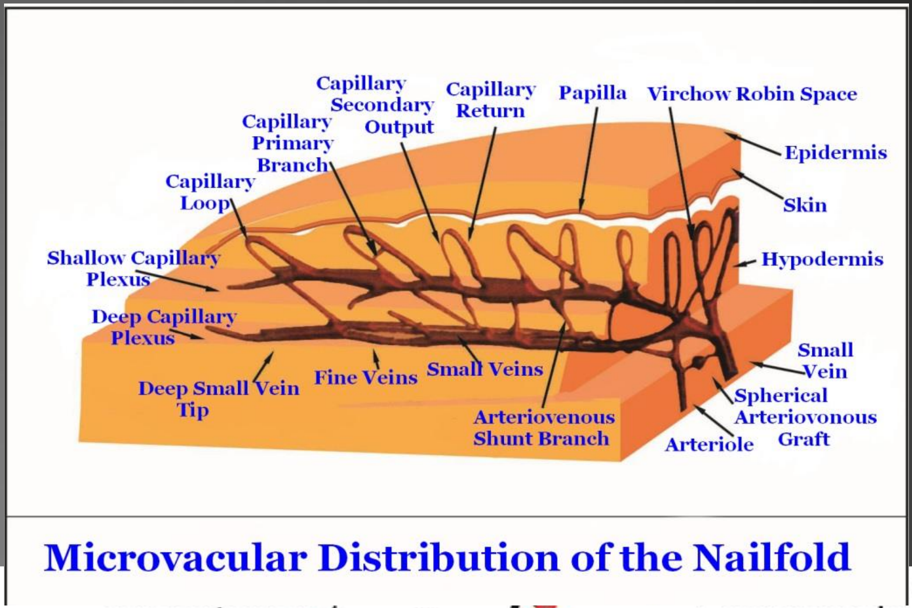
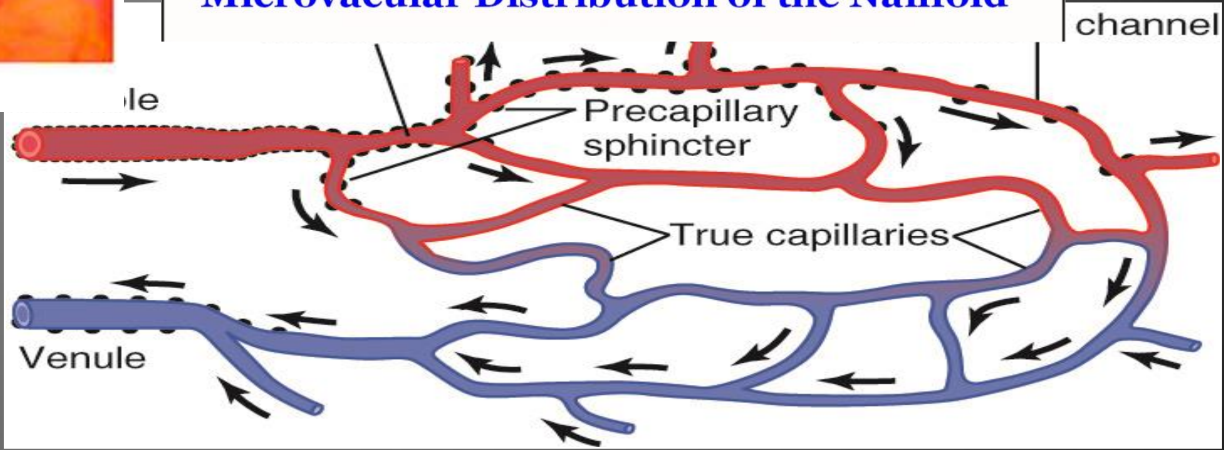


Fig. 4. Labial microvascular characteristics in healthy patients (200X).



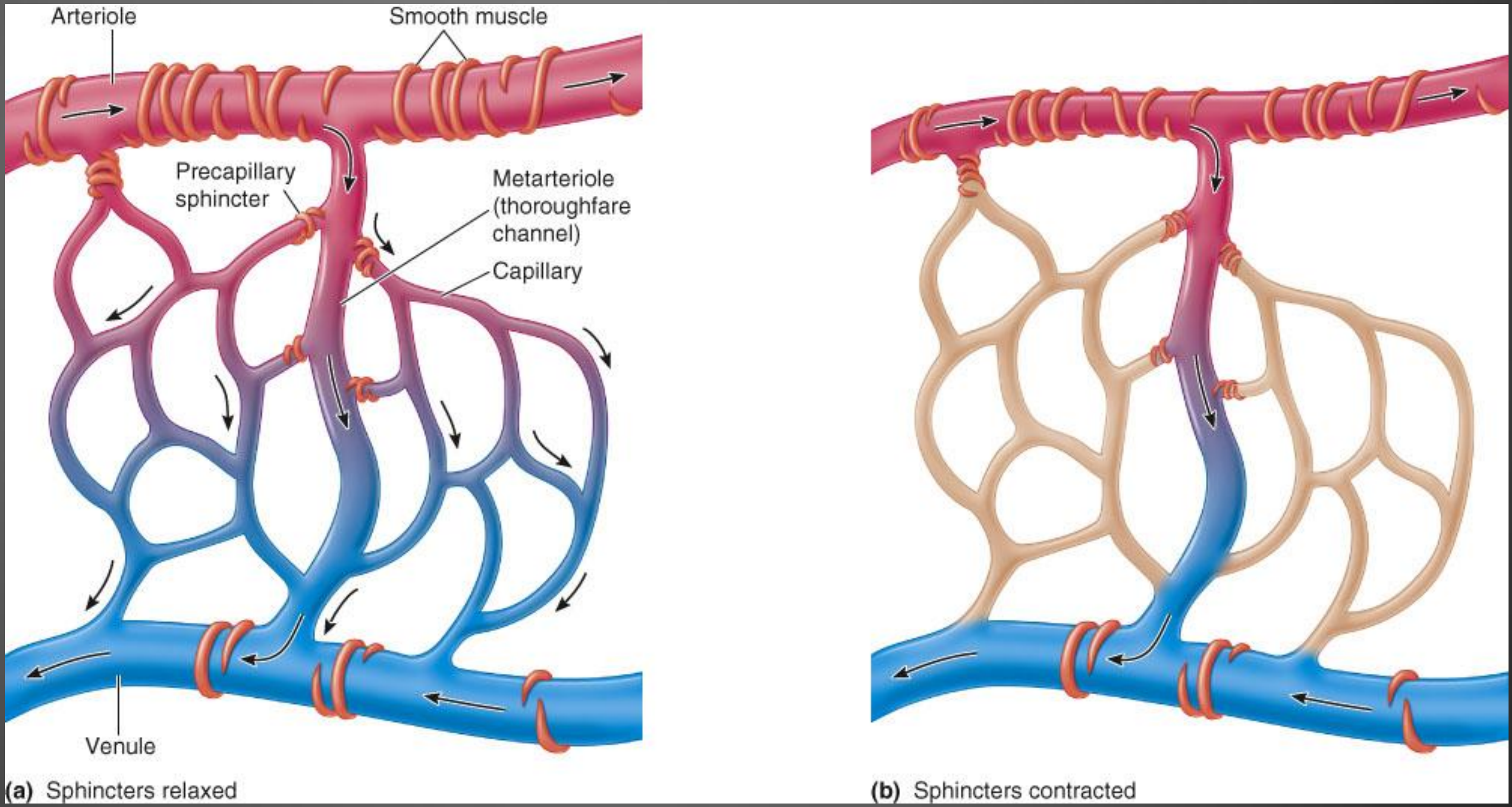


- Over 10 billion capillaries with surface area of 500-700 square meters
- Small volume of blood is exposed to larger surface area

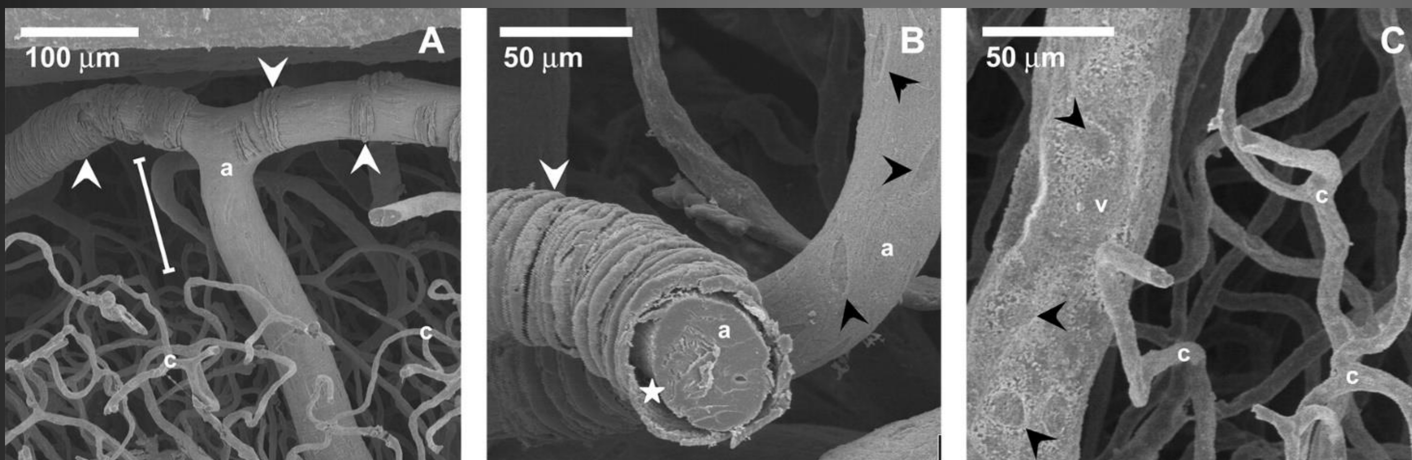
Arteriole → Meta arteriole → Capillaries → Venules.

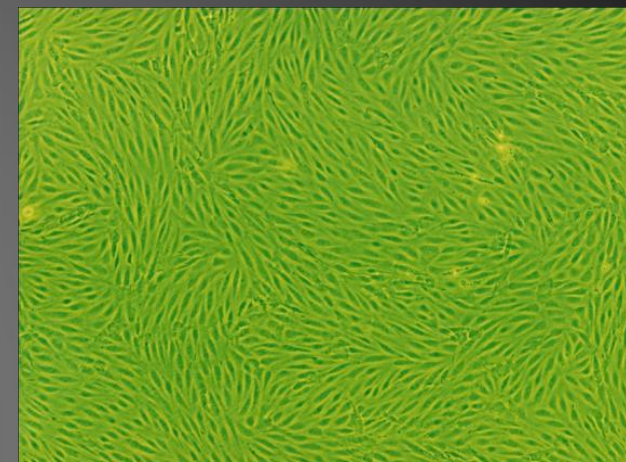
Pre capillary sphincter is present at the junction where the capillary arises from the Meta arteriole. This opens and closes the entrance of capillary and hence regulates the blood flow through the capillary.

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.



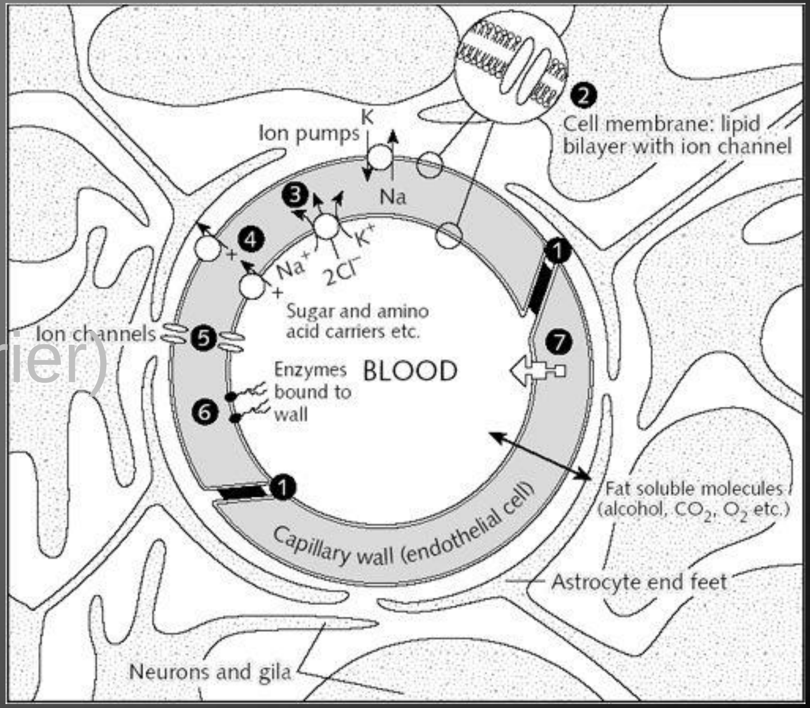
Capillaries in the brain





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

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

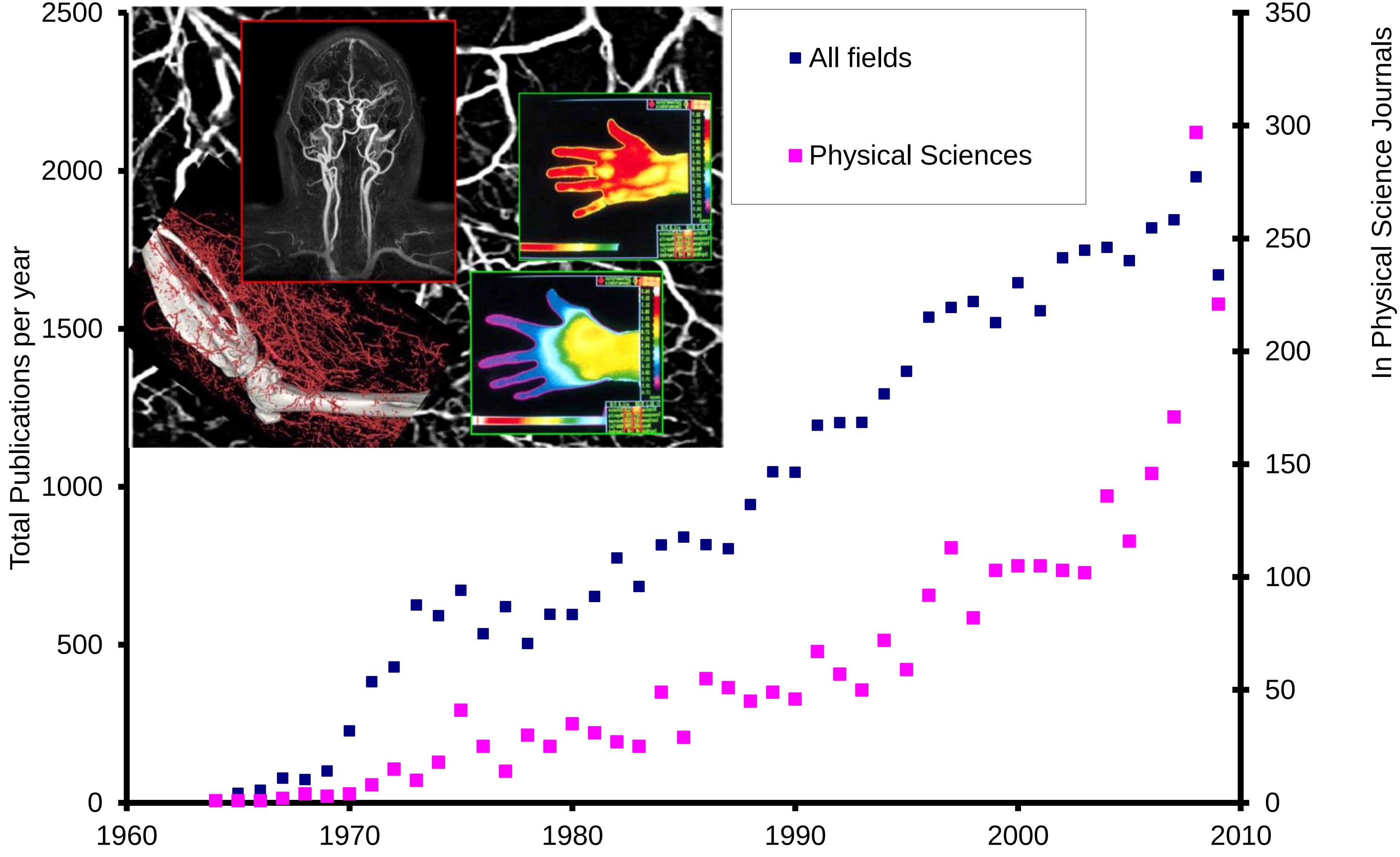
Methods before the Laser Doppler

- Intravital microscopy, pletismography

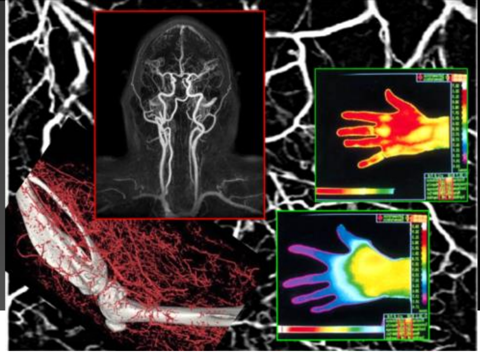
- INDIFFERENT GAS METHODS
  - HYDROGEN CLEARANCE
- ISOTOPE METHODS
  - AUTORADIOGRAPHIC METHOD

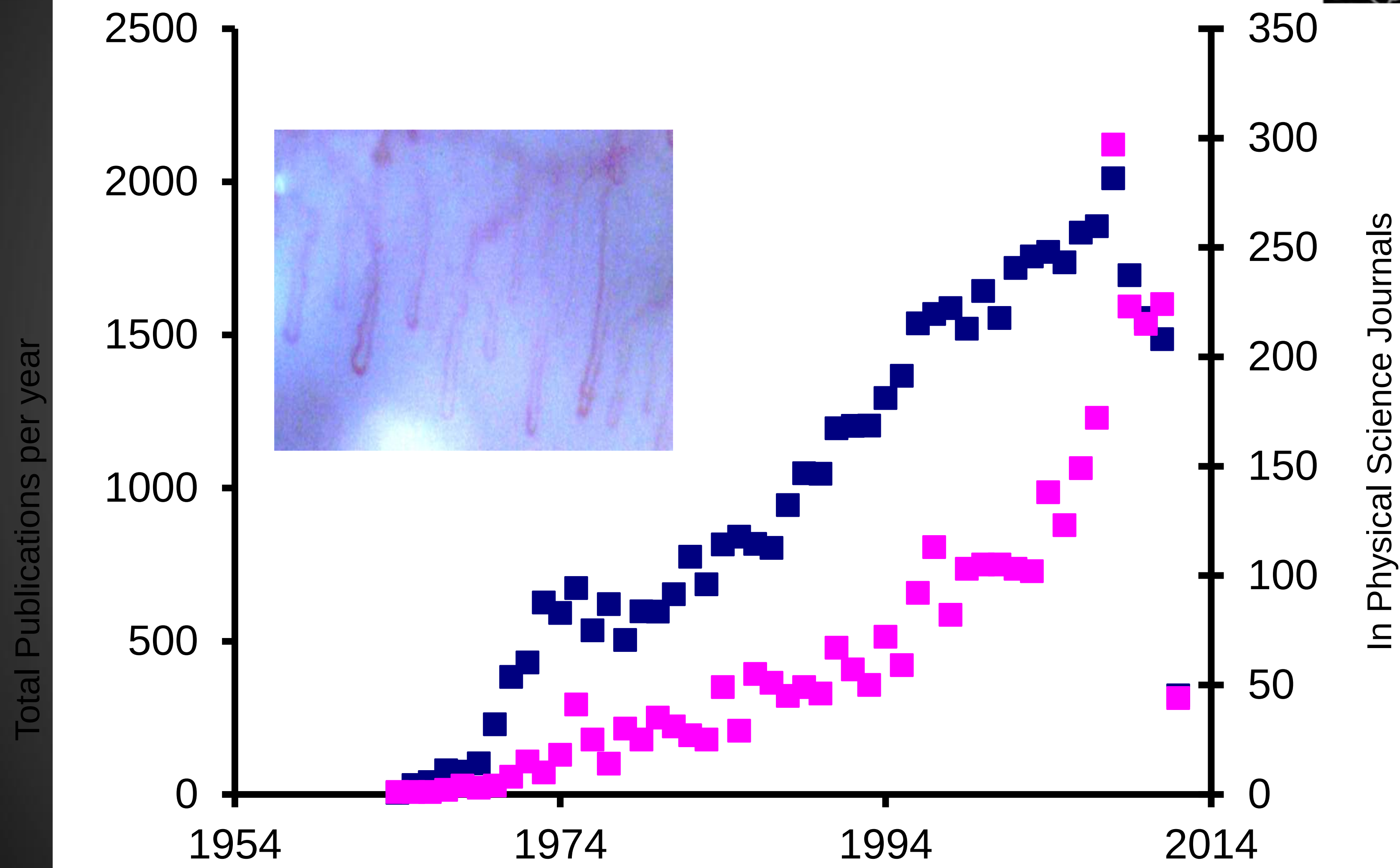
    INHALATION OF O^{15} or O^{15} LABELED CO_2
  - RADIOACTIVE (LATER COLORED) MICROSPHERES
- REGIONAL CEREBRAL BLOOD-FLOW MEASUREMENTS BY XE-133-INHALATION
- LATER TRANSCRANIAL DOPPLER SONOGRAPHY
- PET

Microcirculation



Microcirculation





Classics of Scientific Method

THE DISCOVERY OF THE CIRCULATION OF THE BLOOD

Charles Singer

1922

PLATE VIII

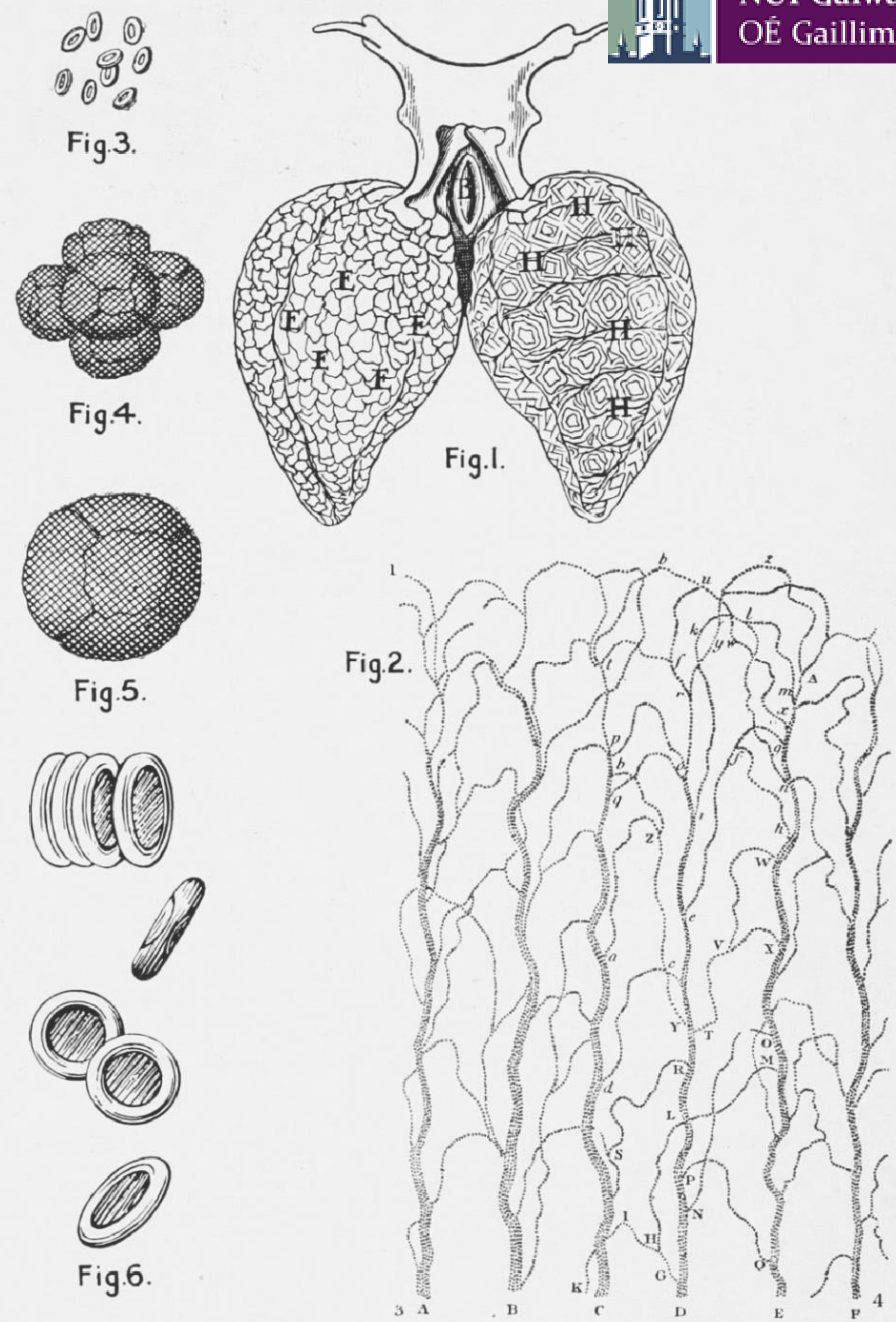


Fig. 1. Lung of Frog, magnified, showing Capillaries. From Malpighi.
Fig. 2. Capillary Network in Tail of Eel. From Leeuwenhoek. A, C, E are Veins, and B, D, F are Arteries.
Fig. 3. Red Blood Corpuscles of Salmon. From Leeuwenhoek.
Figs. 4 and 5. Human Red Blood Corpuscles. From Leeuwenhoek.
Fig. 6. Human Red Blood Corpuscles drawn from the Object under a Modern Microscope.

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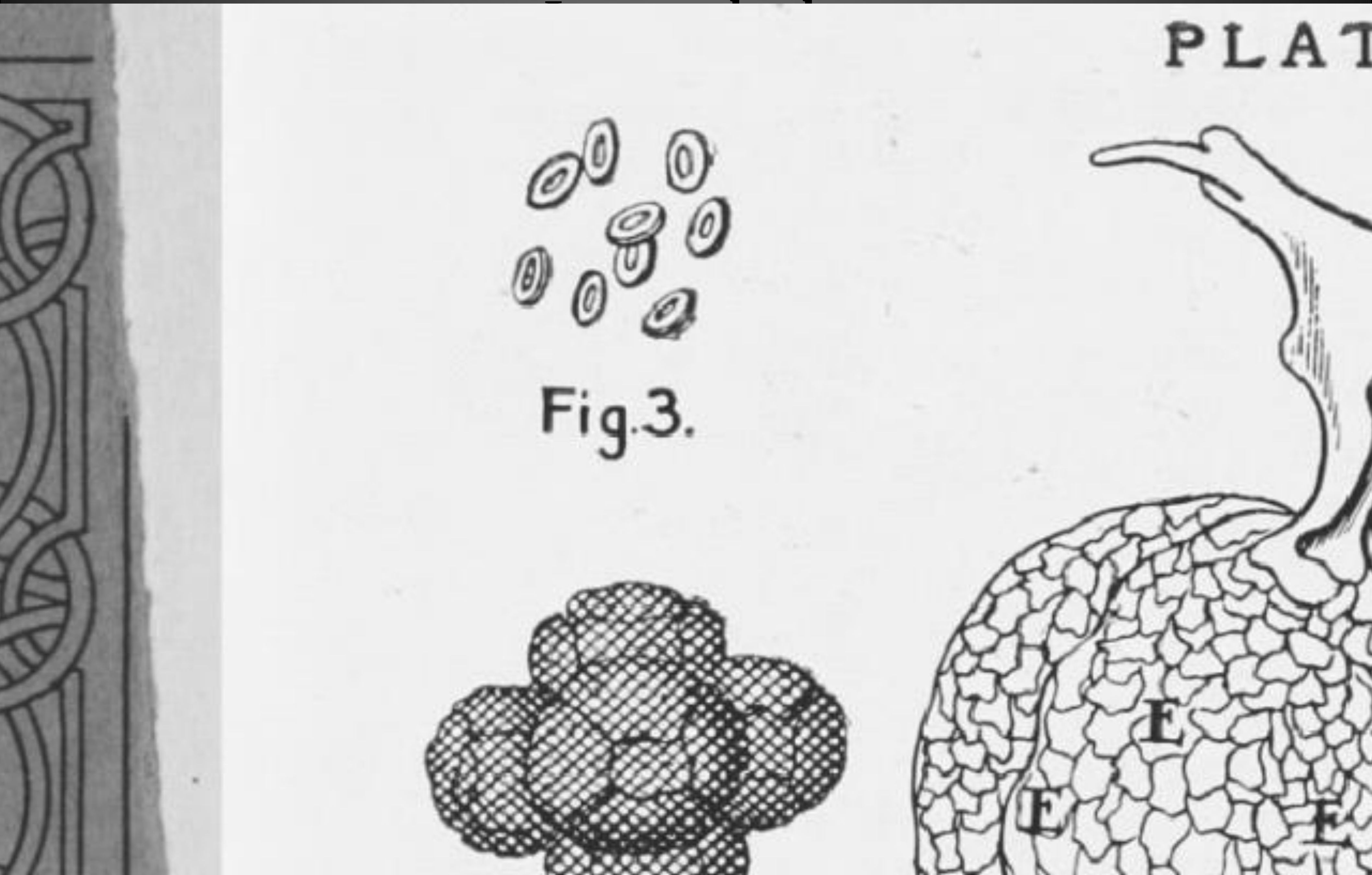
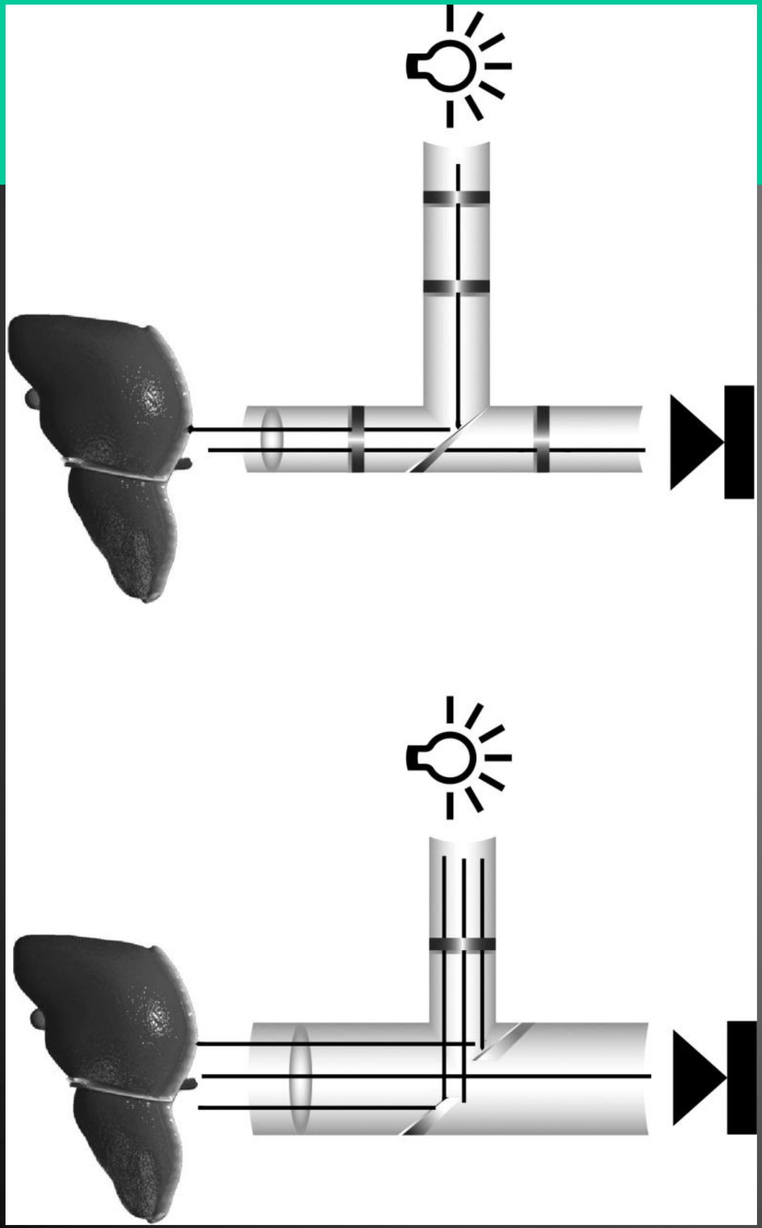
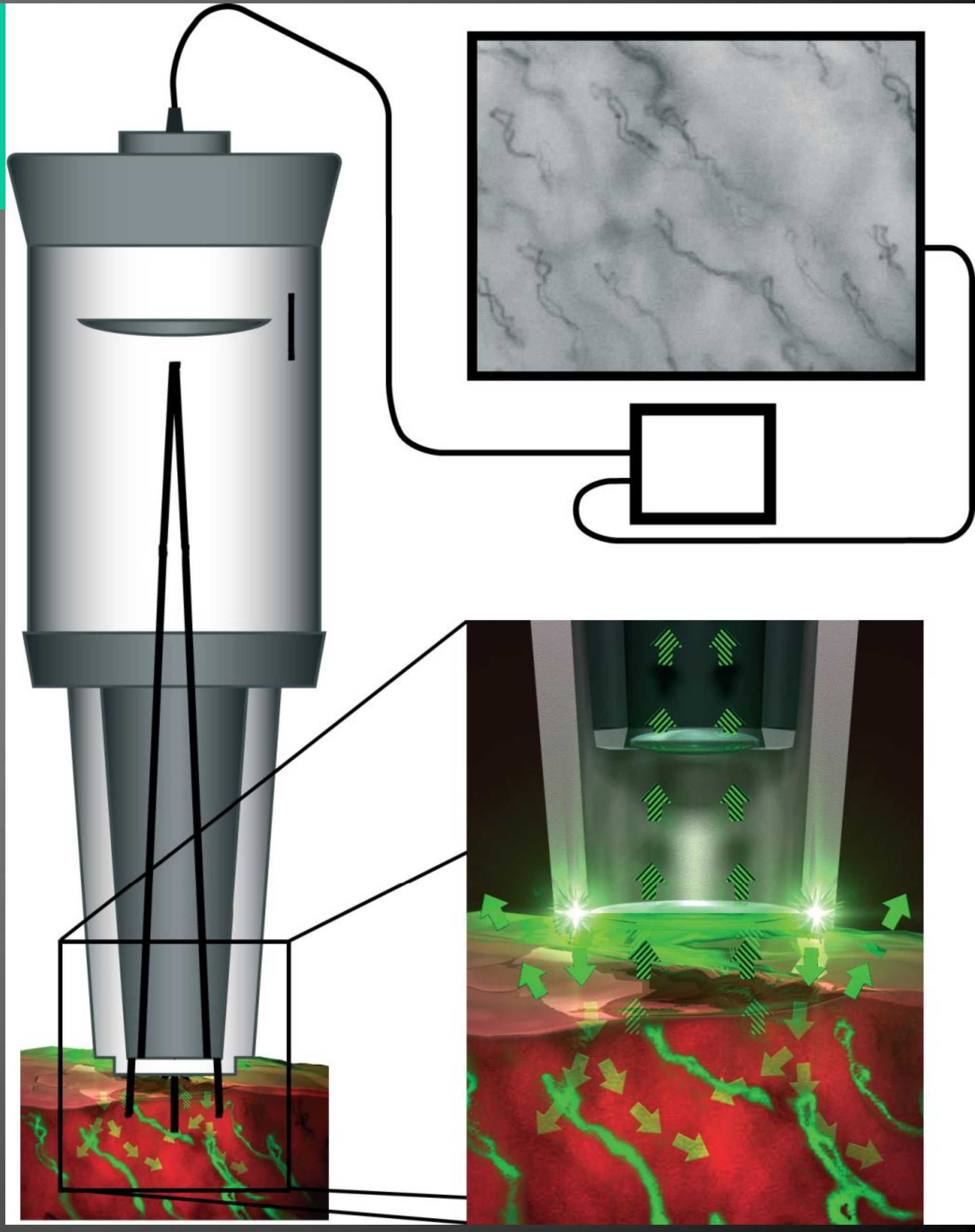


Fig.3.

Sidestream dark-field (SDF) imaging





Closed cranial window- intravital microscopy direct observation of cortical vessels

Advantages:

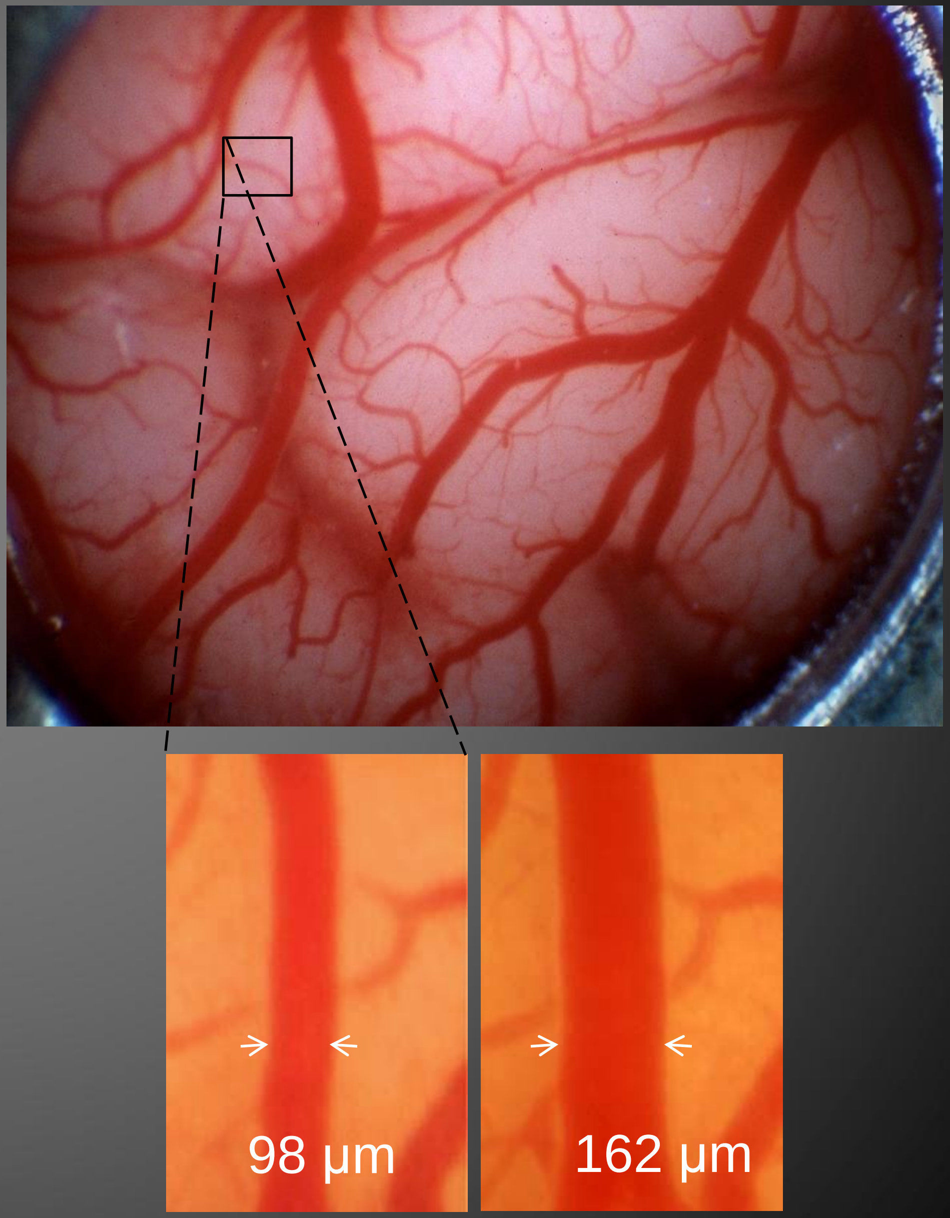
•Physiological environment

•Many kind of vessel can be studied

• Disadvantages:

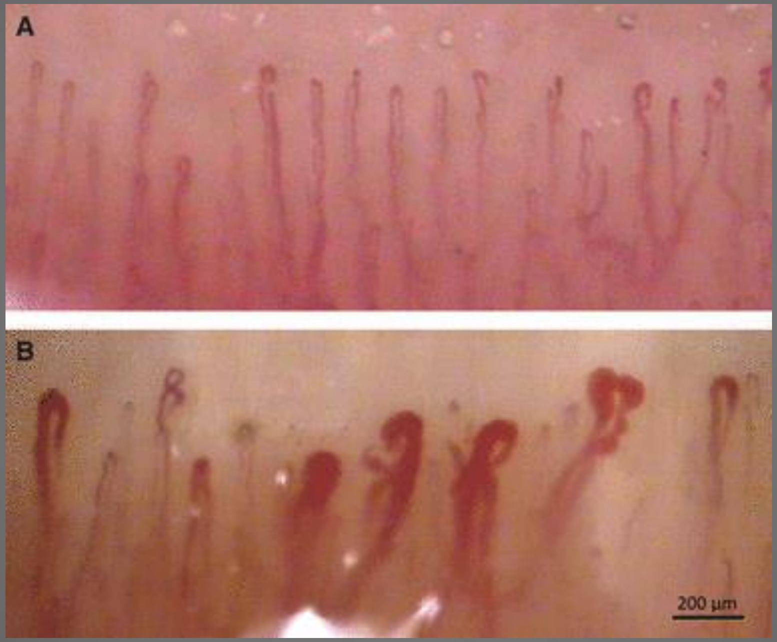
•Parenchymal circulation cannot be studied

•Limited dynamical follow-up



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

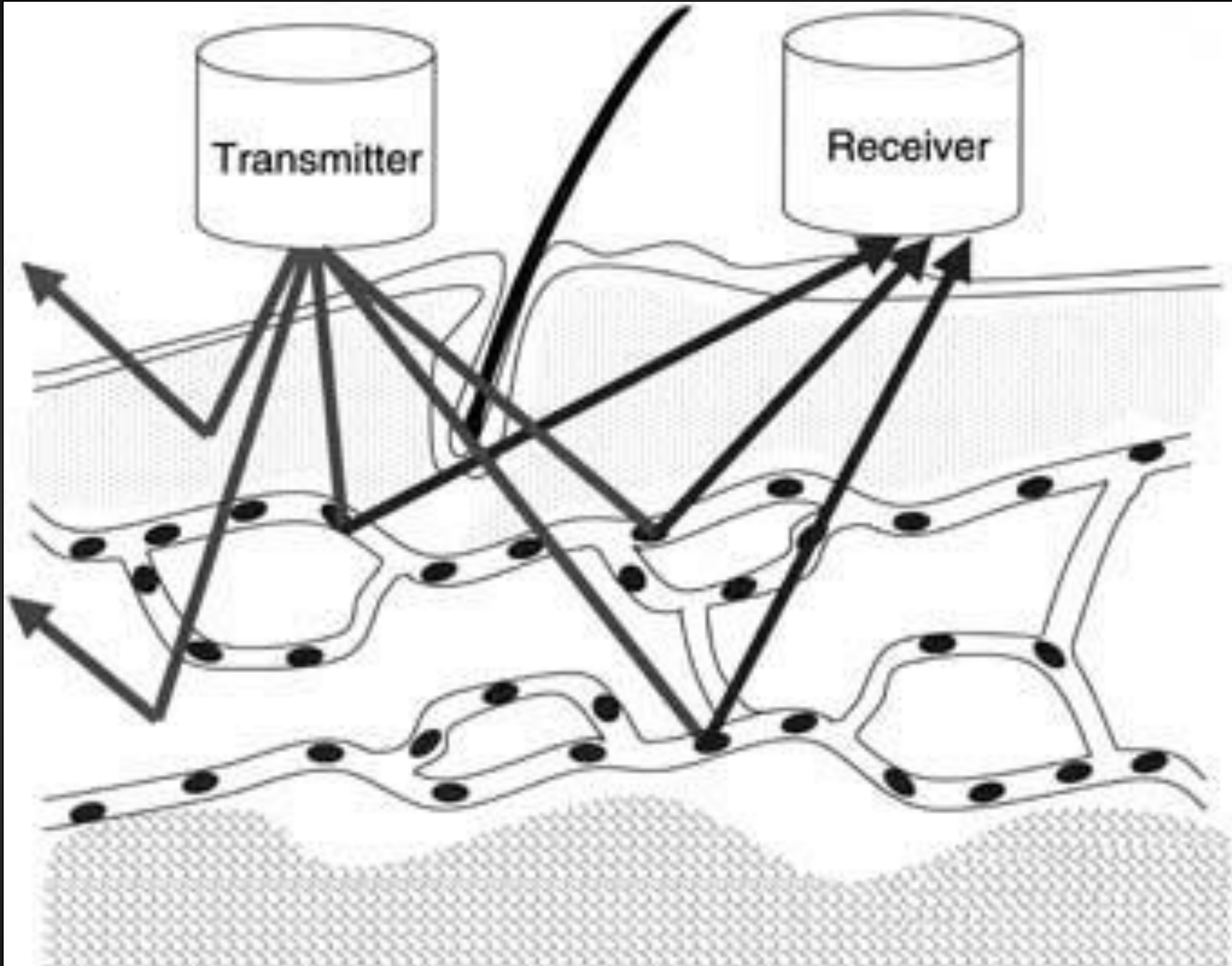
Non-invasive Assessment of Skin Microvascular Function in Humans: An Insight Into Methods

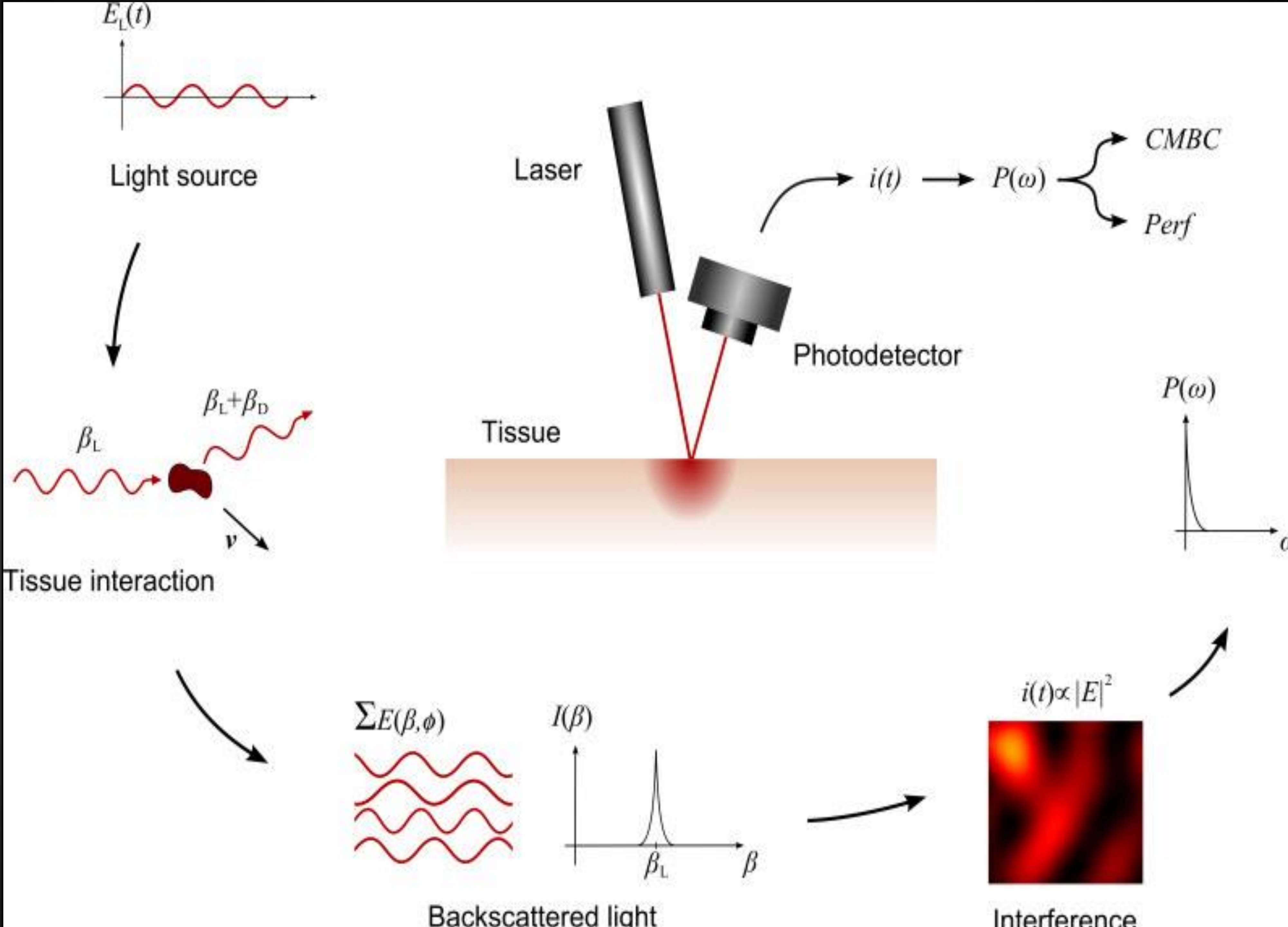


Microcirculation
Volume 19, Issue 1, pages 47-64, 21 DEC 2011 DOI: 10.1111/j.1549-8719.2011.00129.x
http://onlinelibrary.wiley.com/doi/10.1111/j.1549-8719.2011.00129.x/full#f1

Principles of Laser Doppler Flowmetry

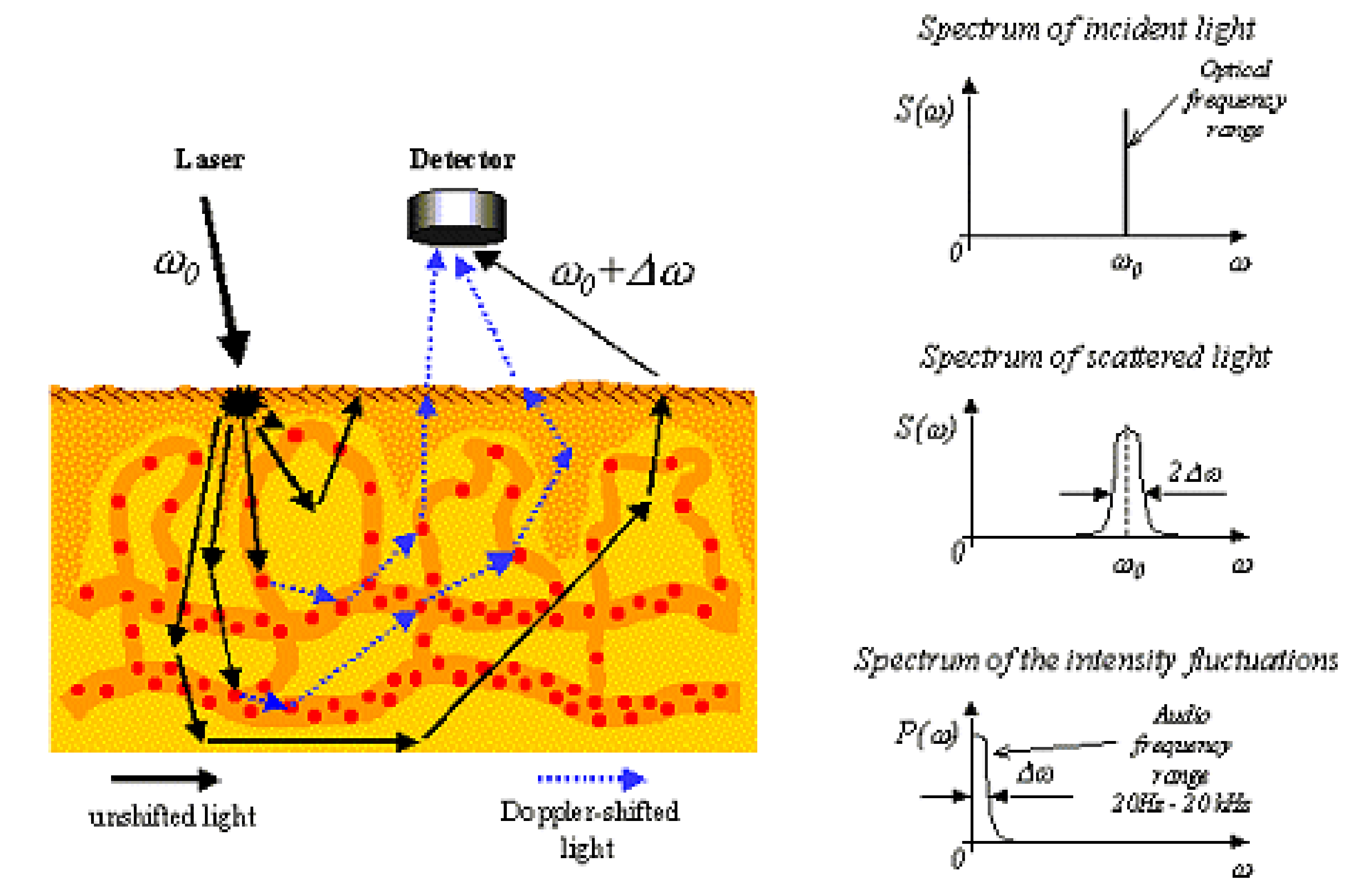
- Laser Doppler flowmetry (LDF)
  - Method to assess the tissue microvascular perfusion
  - A laser beam is directed to an area of tissue.
  - Upon contact with red blood cells in the target tissue, light waves are reflected and scattered
  - Shifts in the frequency of laser light (Doppler shift)
  -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


Transmitter
Receiver

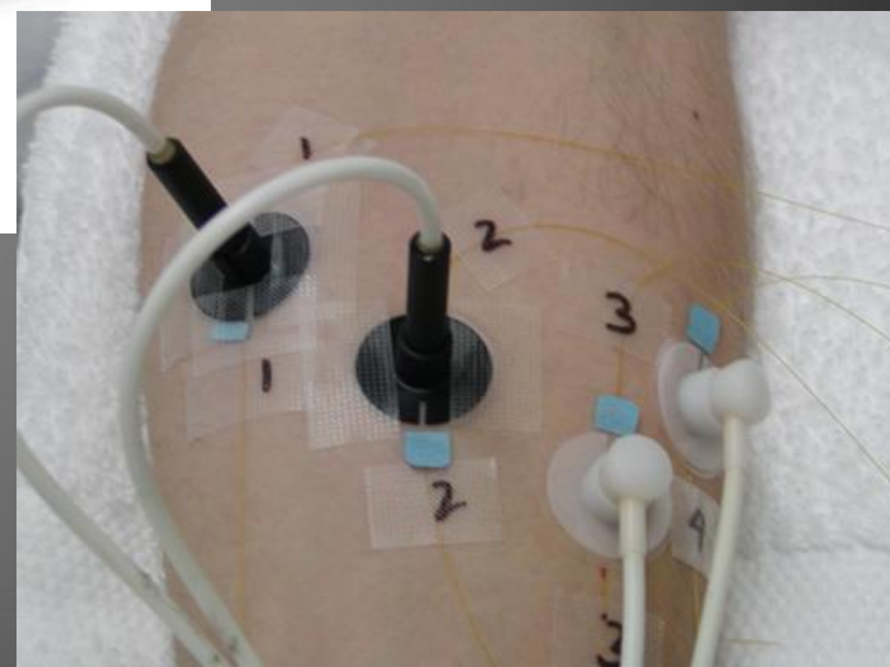


$E_L(t)$
Light source
Laser
$i(t)$
$P(\omega)$
CMBC
Perf
Photodetector
$P(\omega)$
ω
$\beta_L+\beta_D$
β_L
v
Tissue
Tissue interaction
$\Sigma E(\beta,\phi)$
$I(\beta)$
β_L
β
Backscattered light
$i(t)\propto|E|^2$
Interference

Principle of laser Doppler flowmetry



LASER DOPPLER FLOWMETRY (LDF)



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.

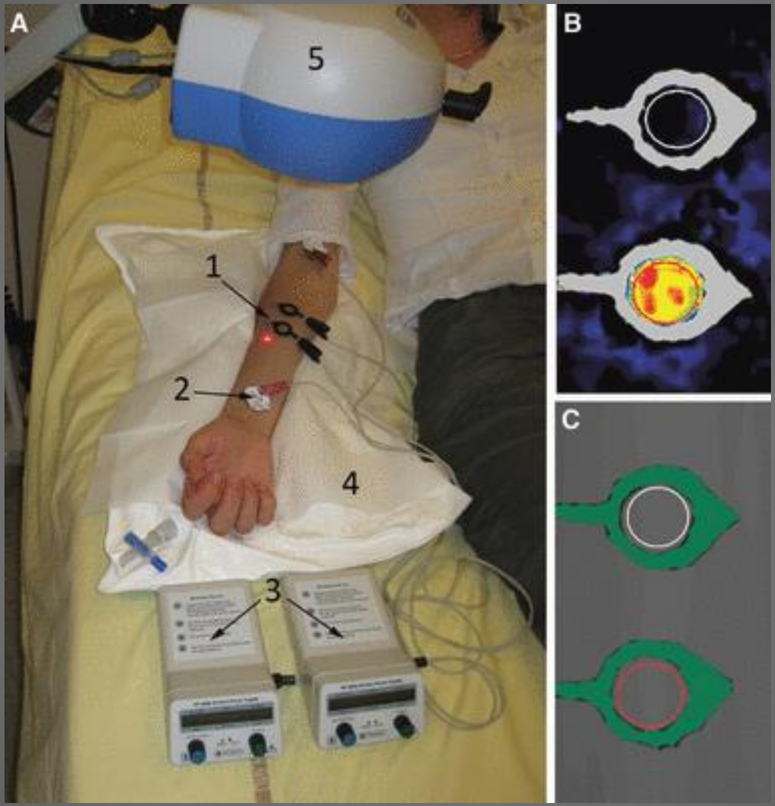
Real time measurement-
a window towards
the dynamics of microvascular regulation

Autoregulation-range and dynamics under various circumstances

Rhythmic patterns in the microcirculation-vasomotion

Neurovascular coupling

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



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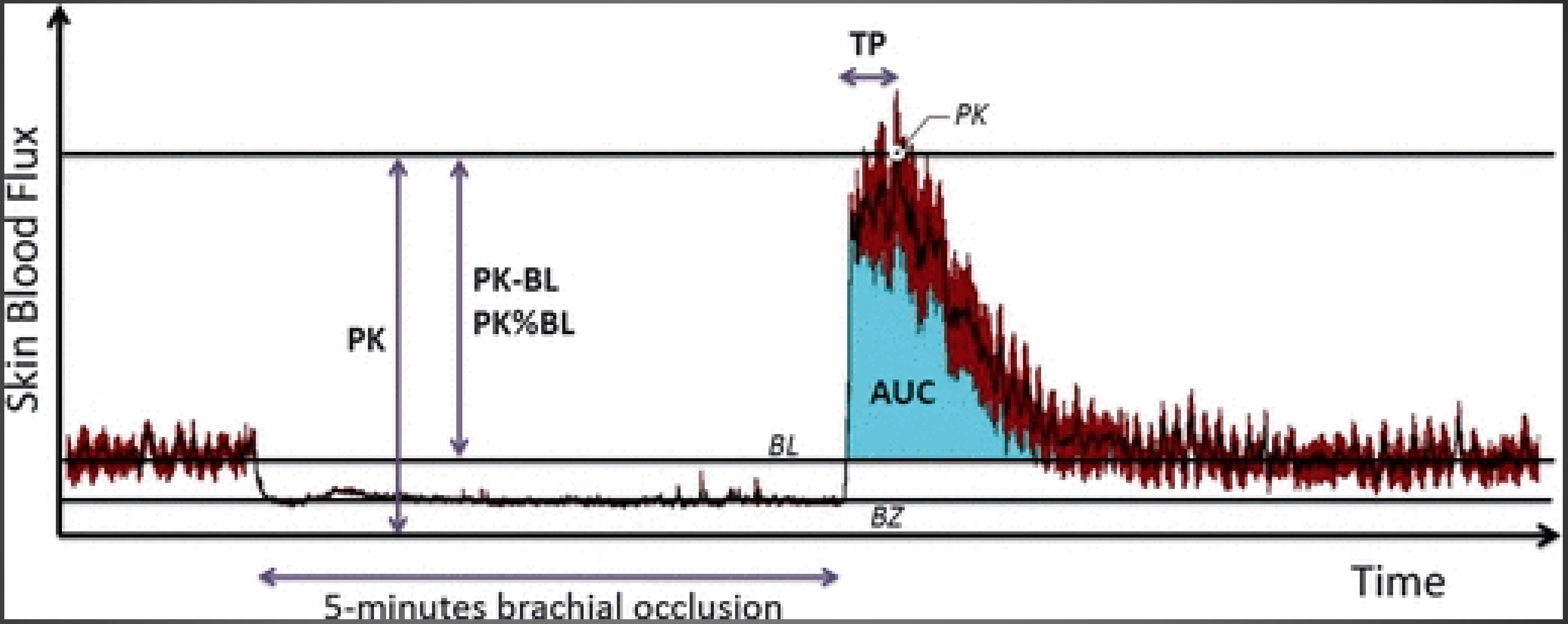

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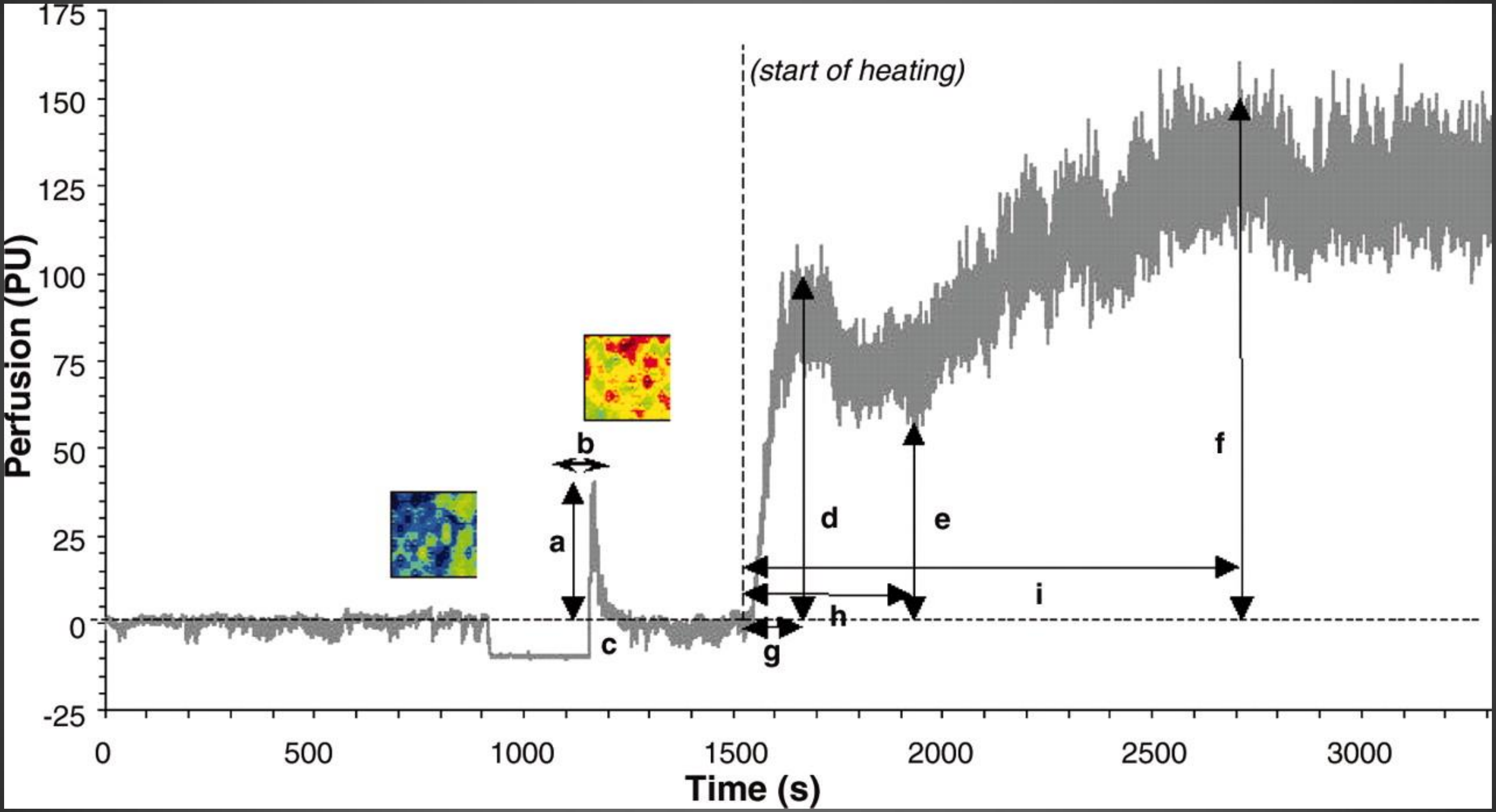
Representative tracing of control postocclusive hyperemia (PORH) and thermal hyperemia (TH).



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

AMERICAN JOURNAL OF PHYSIOLOGY
Heart and Circulatory Physiology

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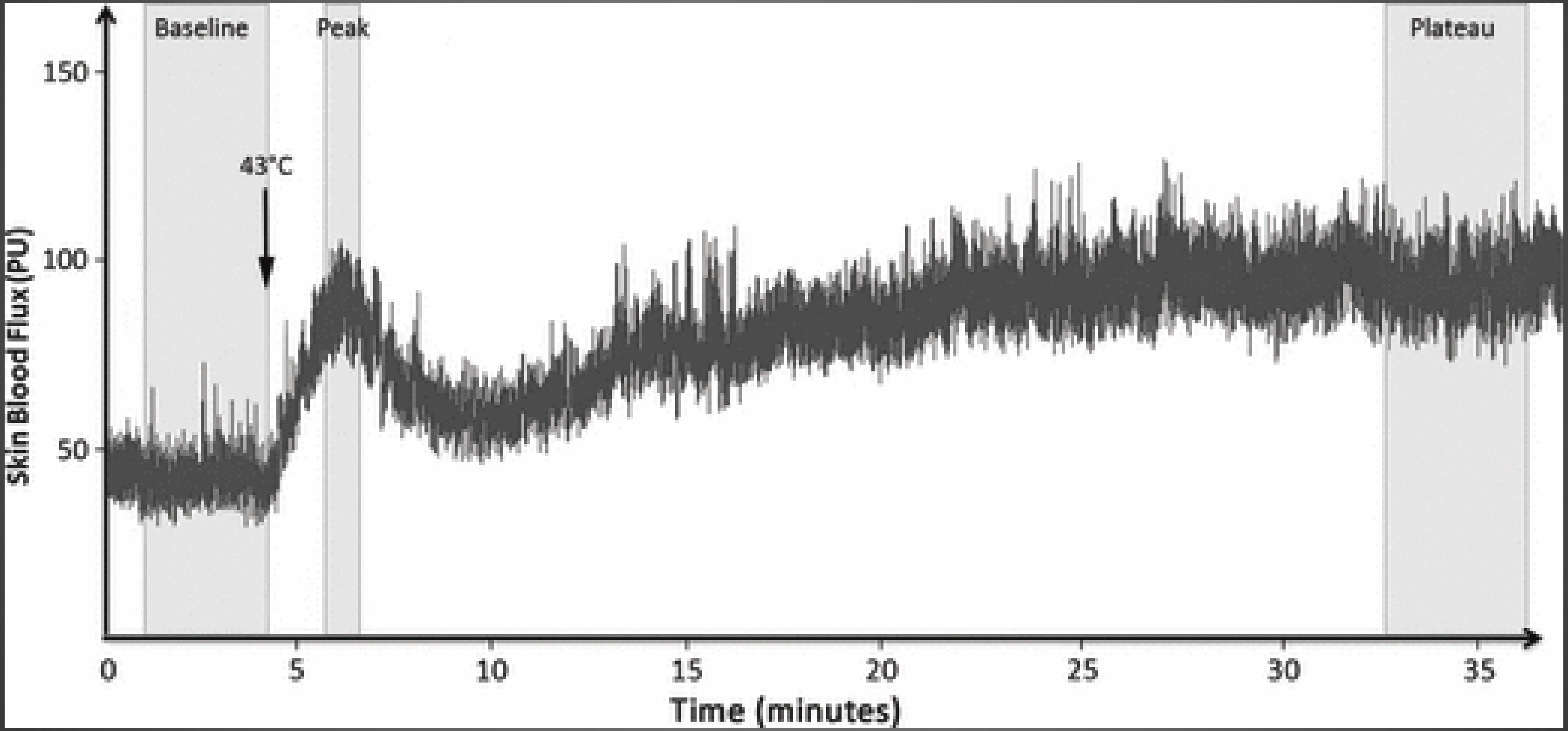

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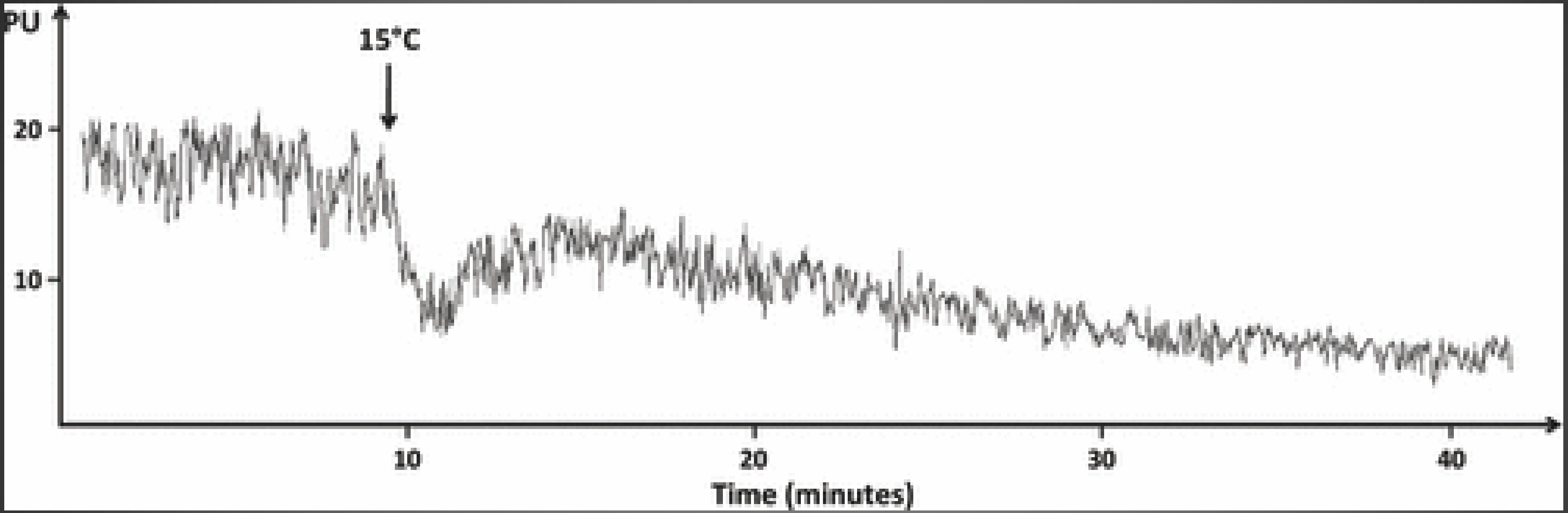

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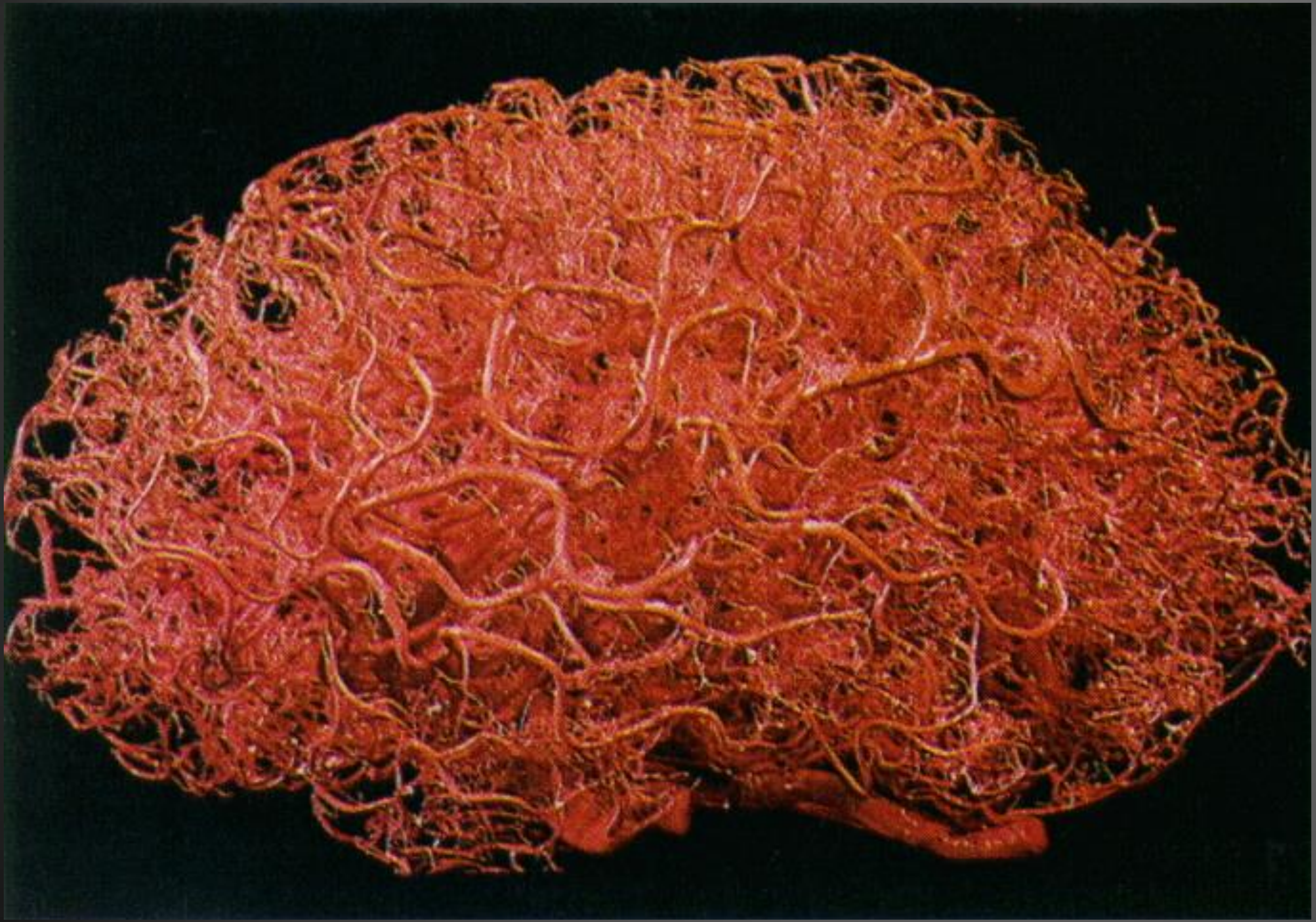
http://onlinelibrary.wiley.com/doi/10.1111/j.1549-8719.2011.00129.x/full#f6

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.

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

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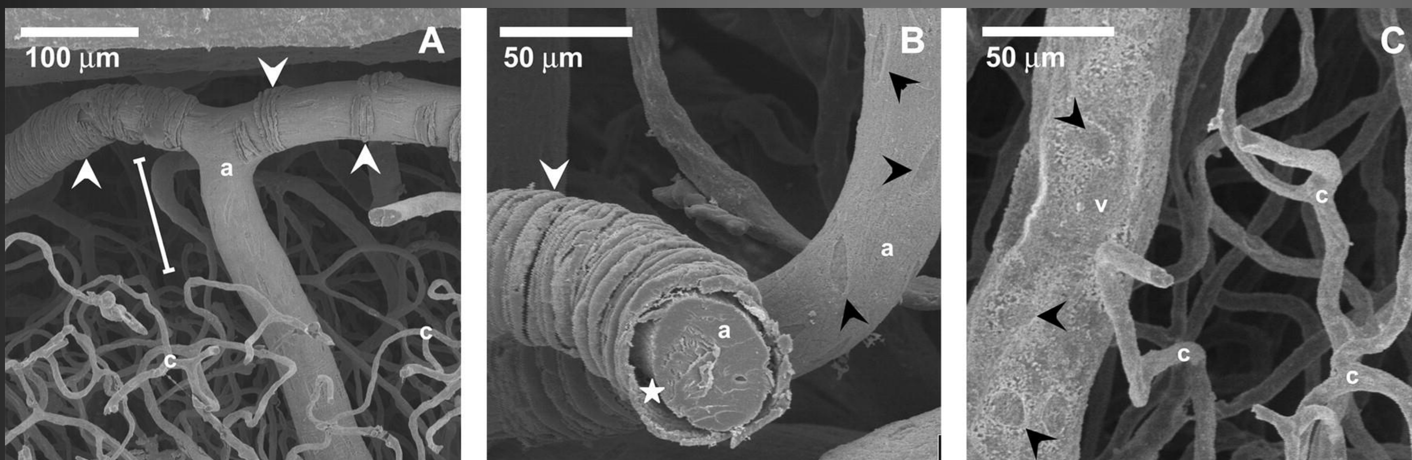
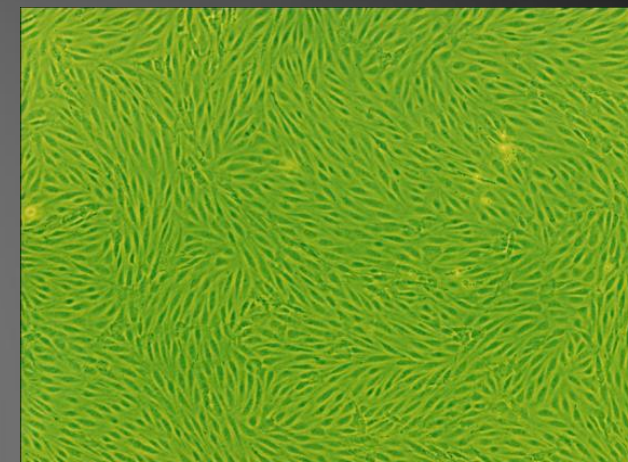
Laboratory Investigations

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

Capillaries in the brain





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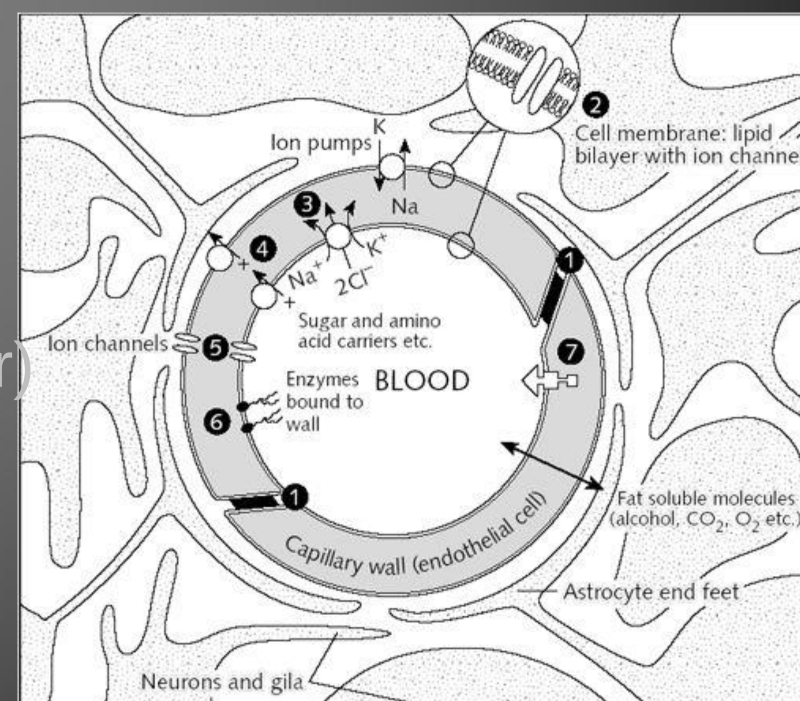
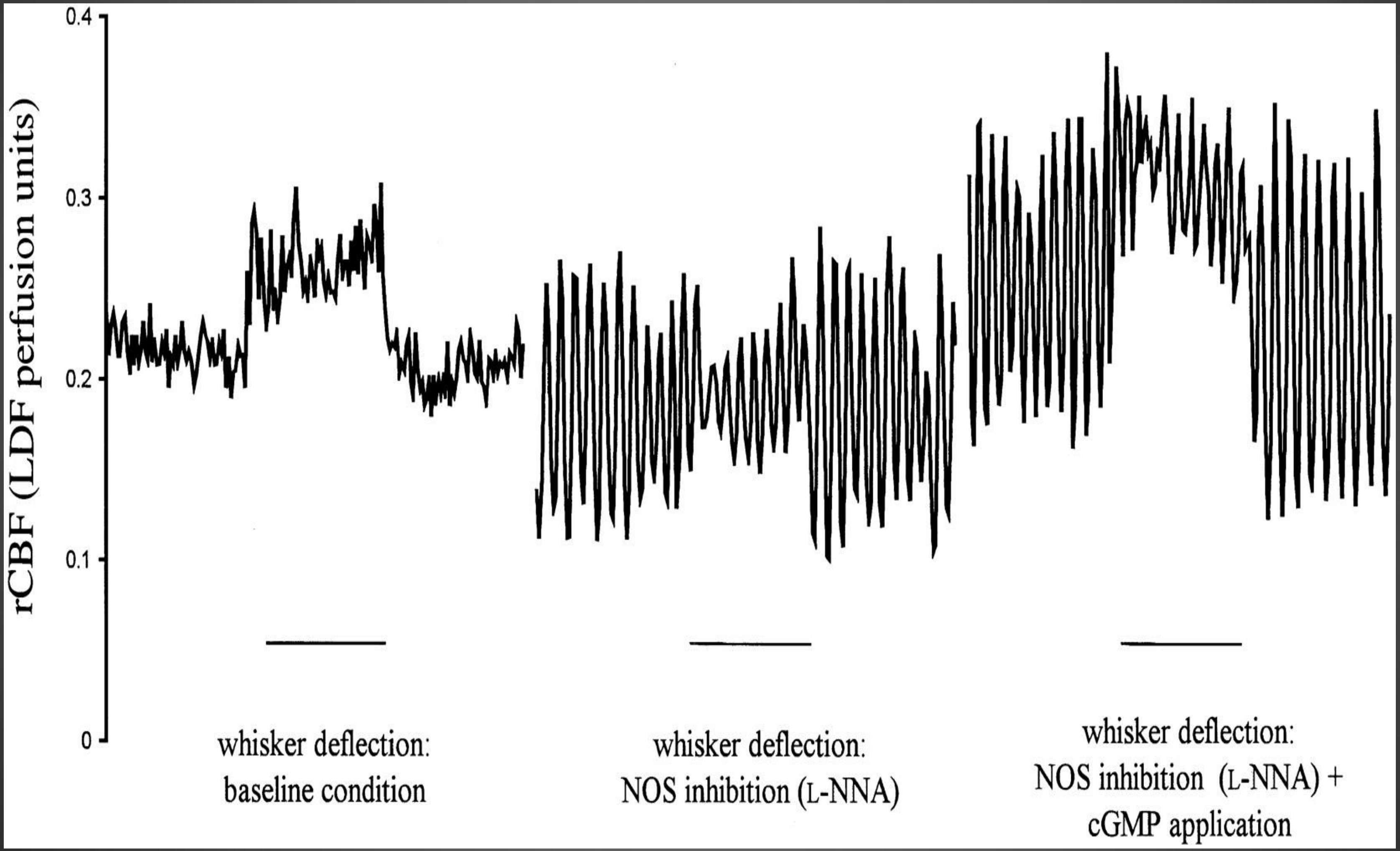


Total cross sectional area ~12 m^2 f

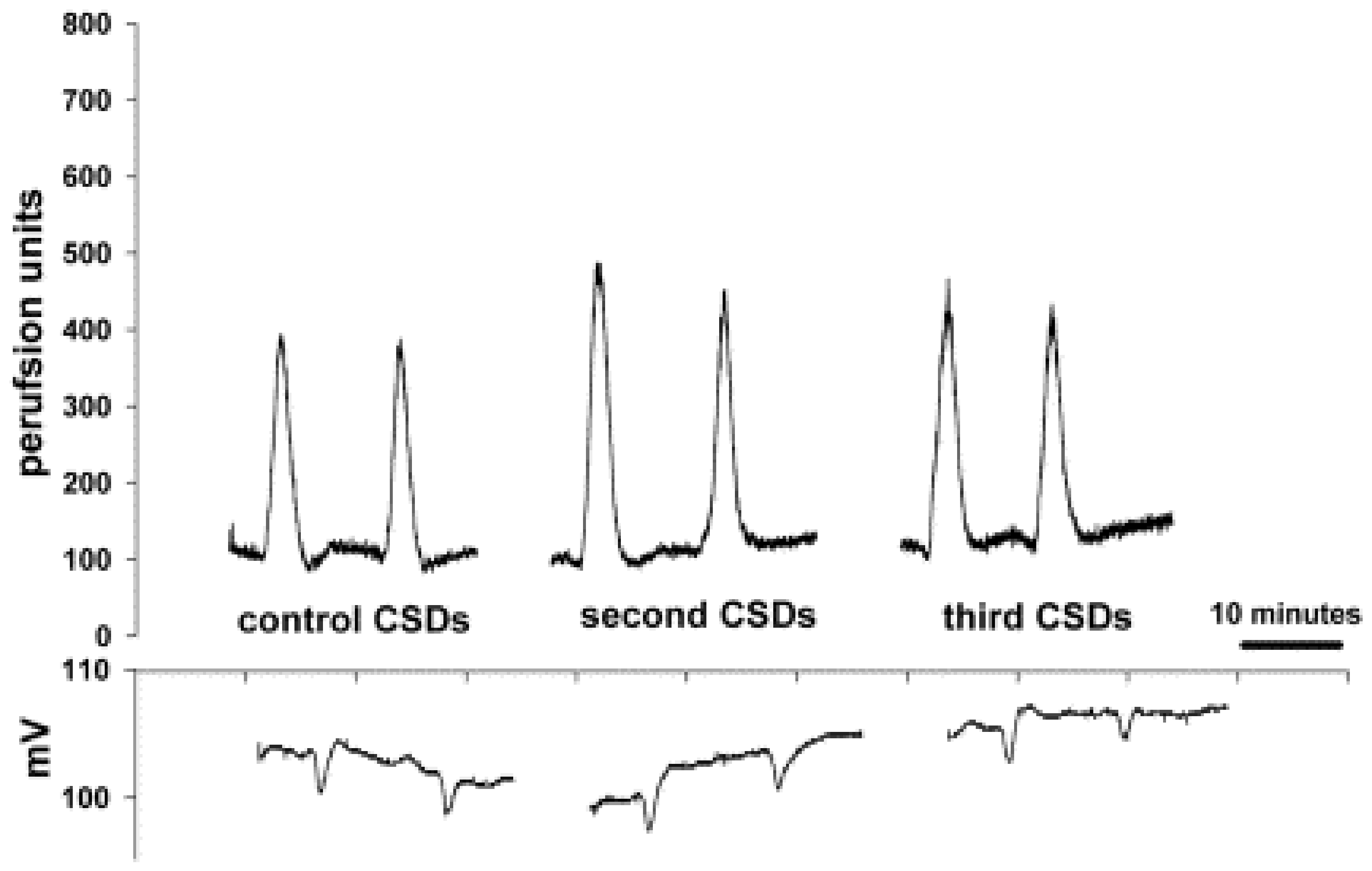
LDF provided a means to follow functional activation and to analyse rhythmic components of microcirculation



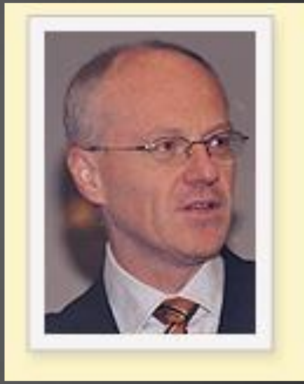
Lindauer et al, AJP, 1999

Neurovascular coupling-cortical spreading depression

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

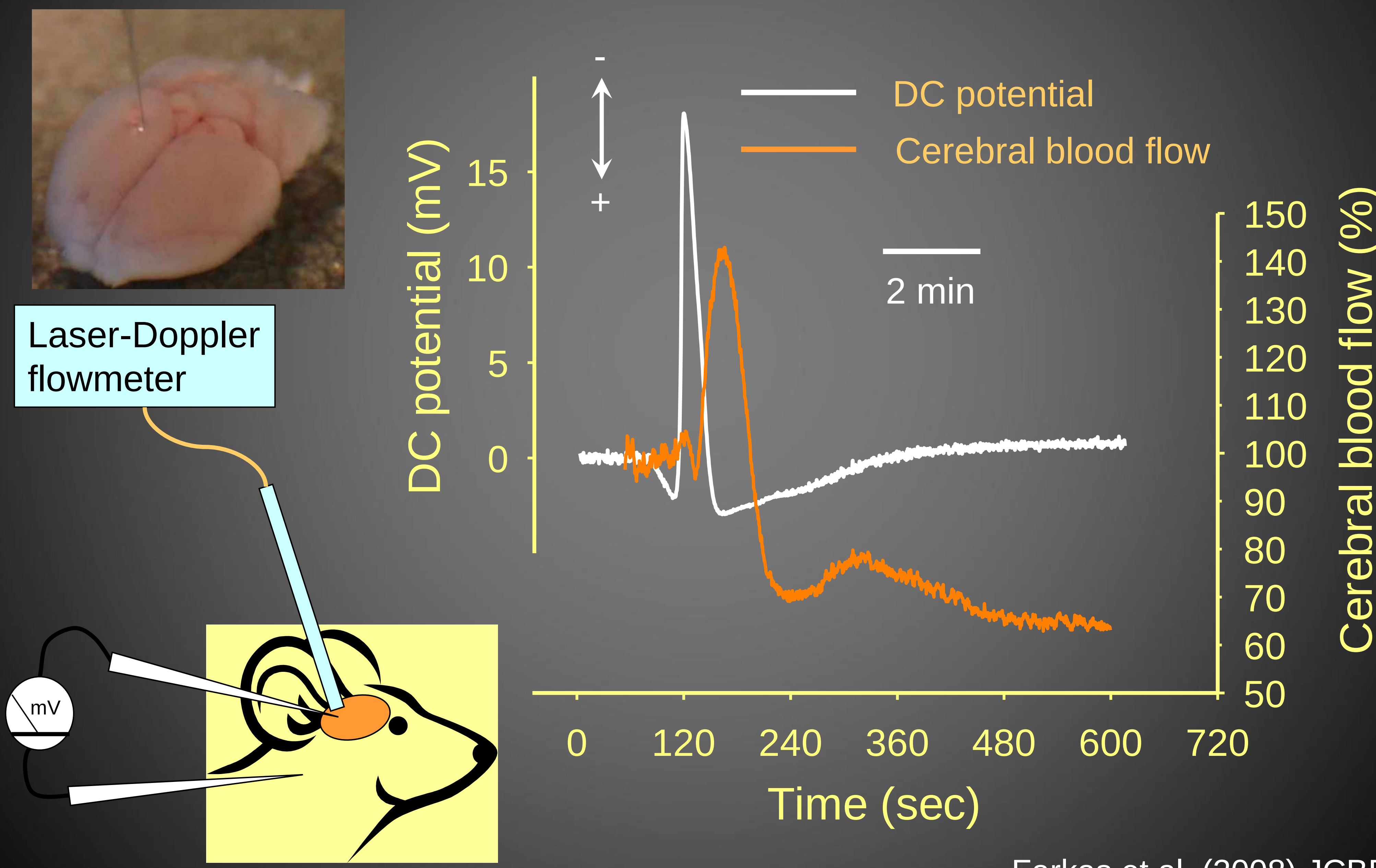


Original recordings of cerebral blood flow (CBF) responses (upper wave) and DC deflections (bottom wave) during the three series of CSD. Ten mg/kg of -NAME was given between the first and second sets of CSDs.



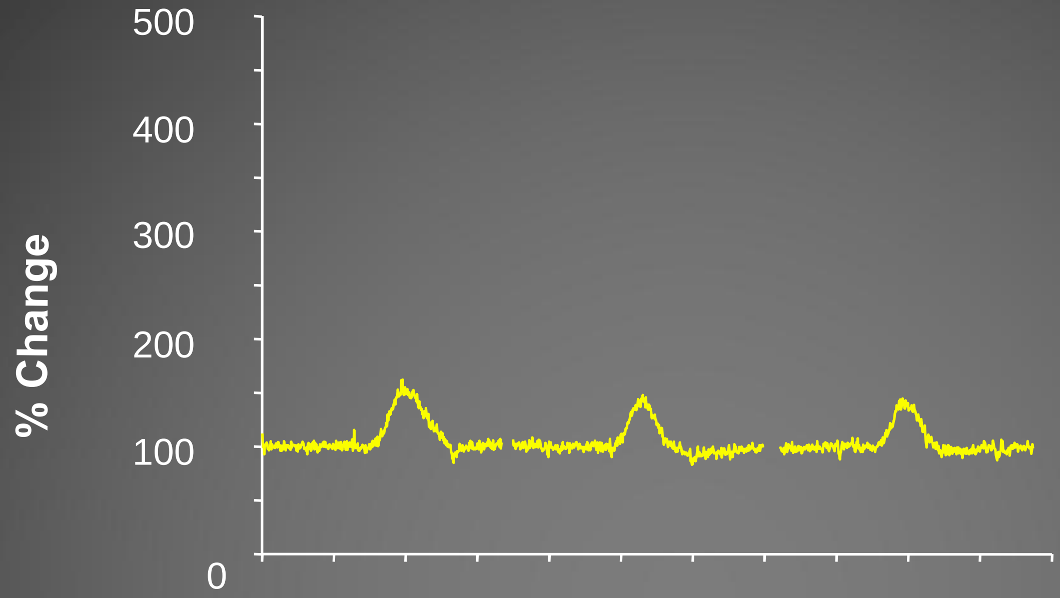
Prof. Peter Goadsby-
Pioneer in headache &
Cerebral microcirculation

Spreading depolarization



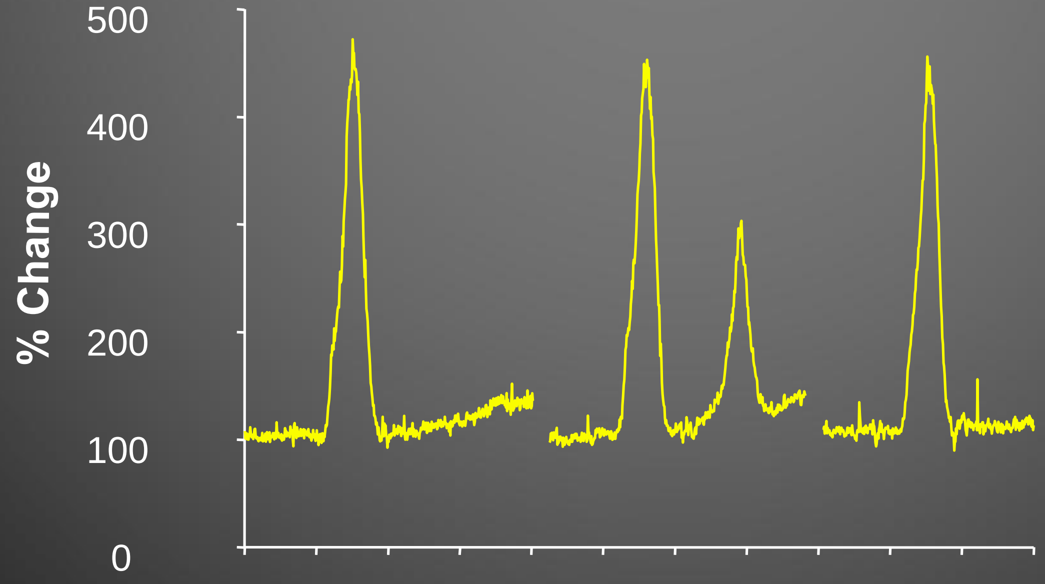
Farkas et al. (2008) JCBFM

Depressed hyperemic responses



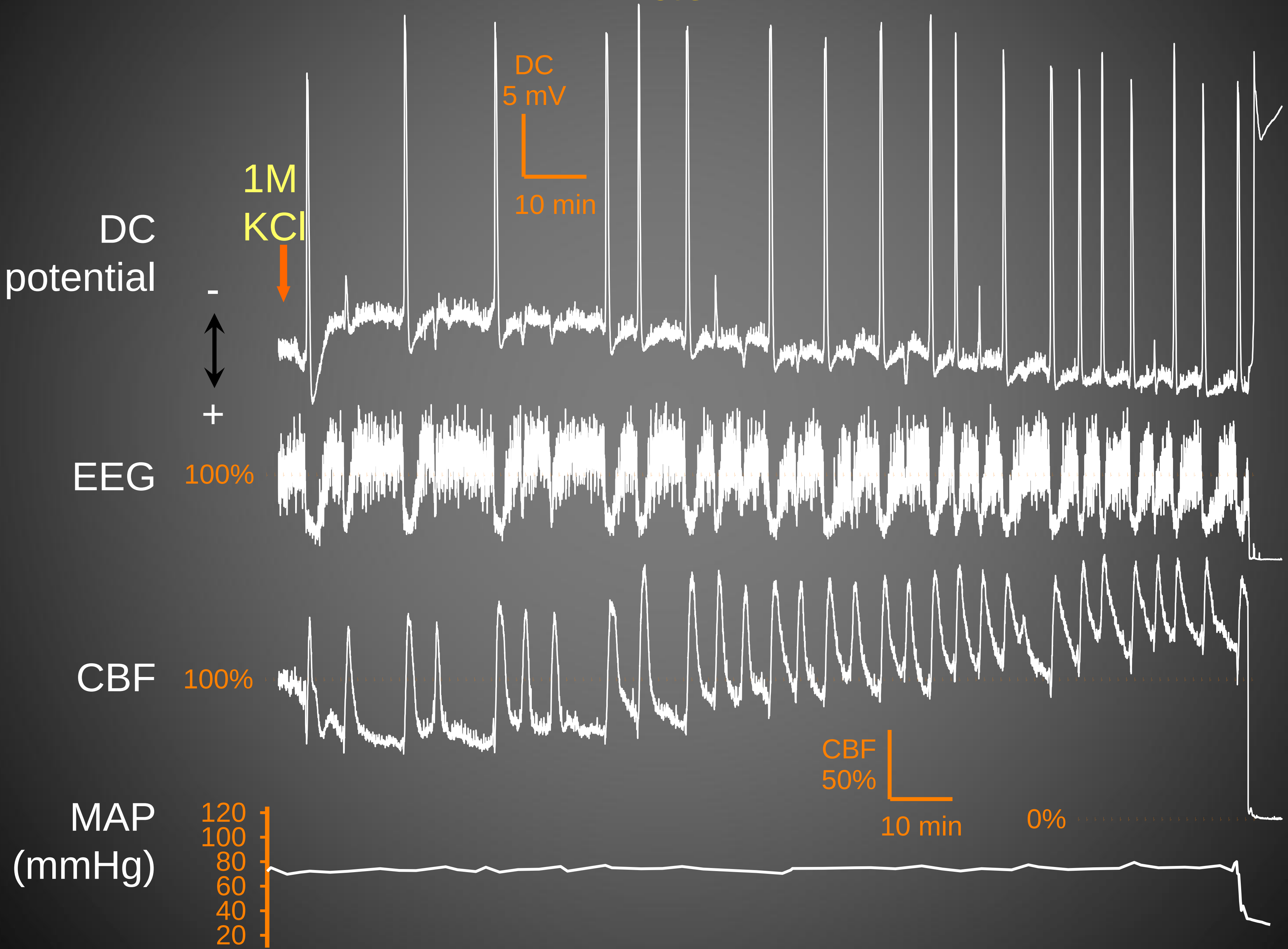
100 mg/kg - 48 hr

Enhanced hyperemic responses

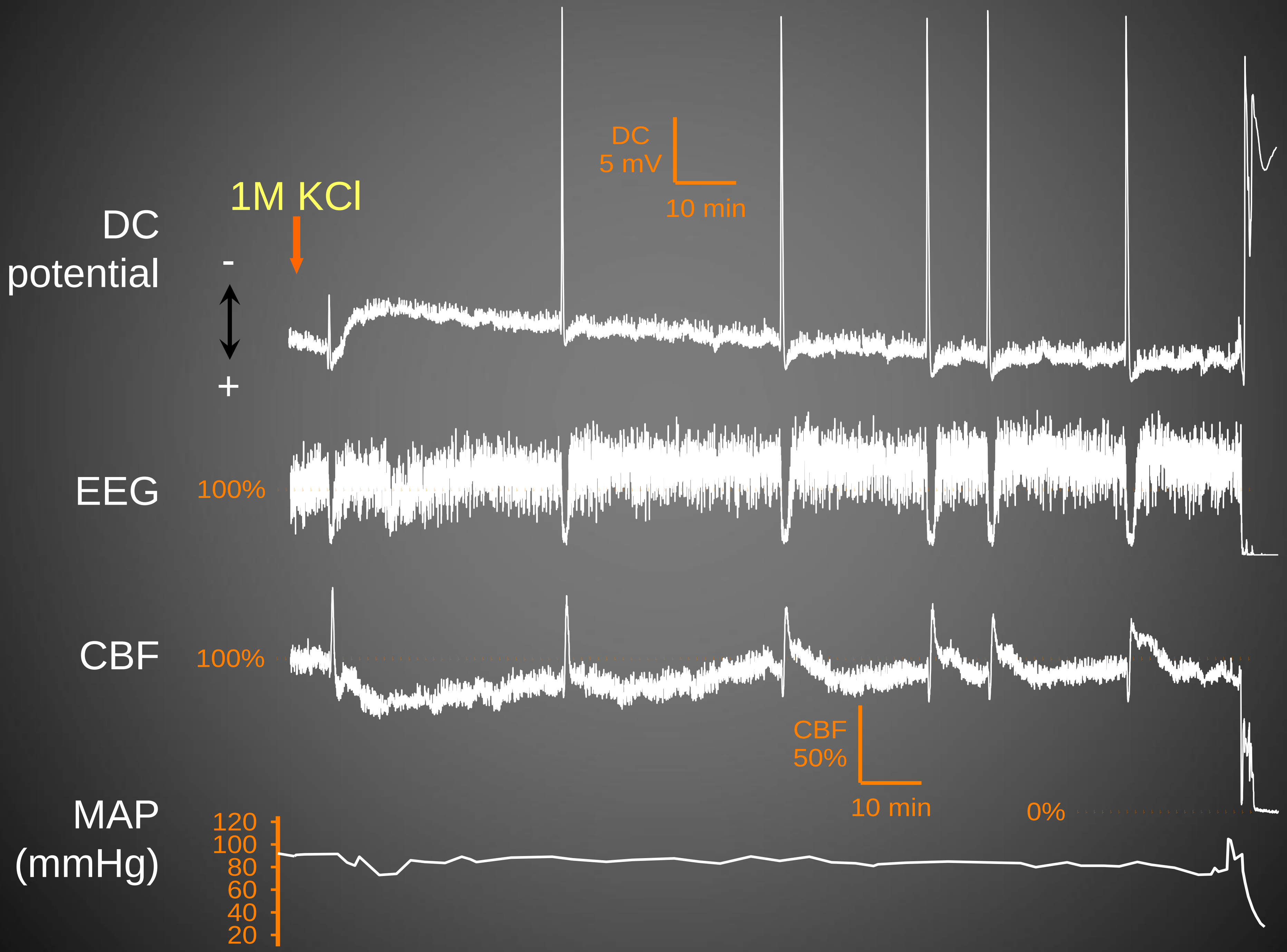


100 mg/kg - 96 hr

Representative experiment: young rat

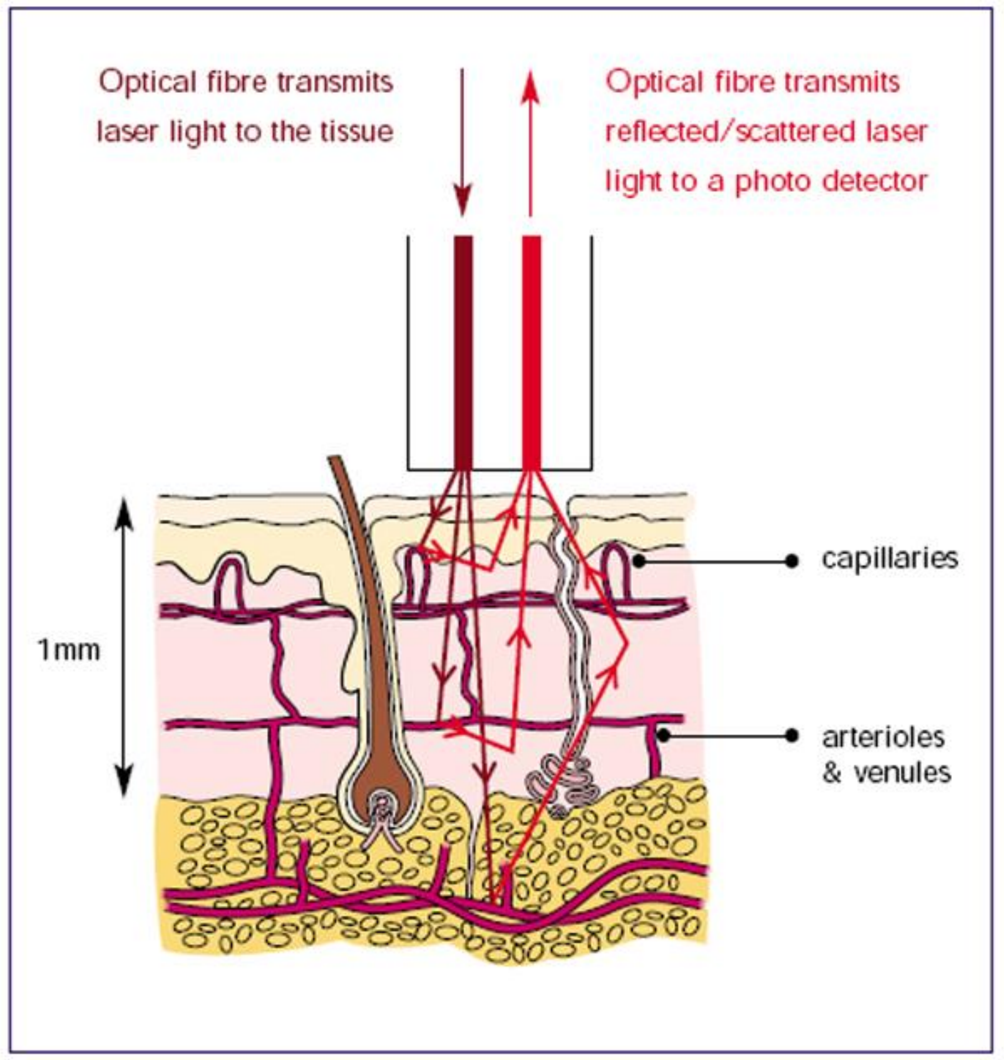


Representative experiment: old rat



Single point blood flow imaging

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



Scanning System

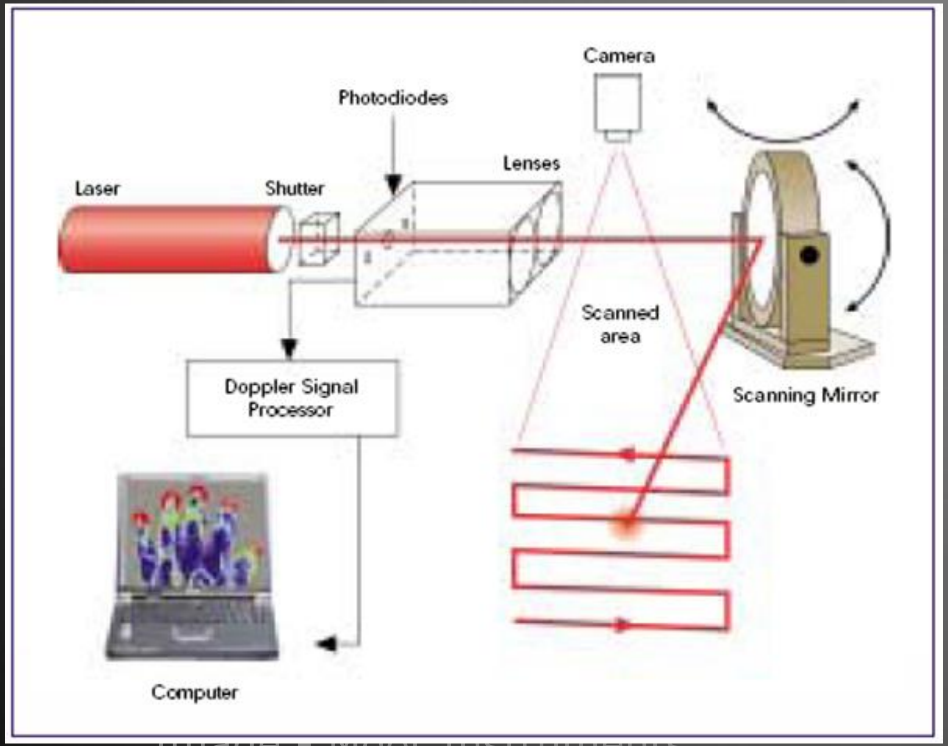
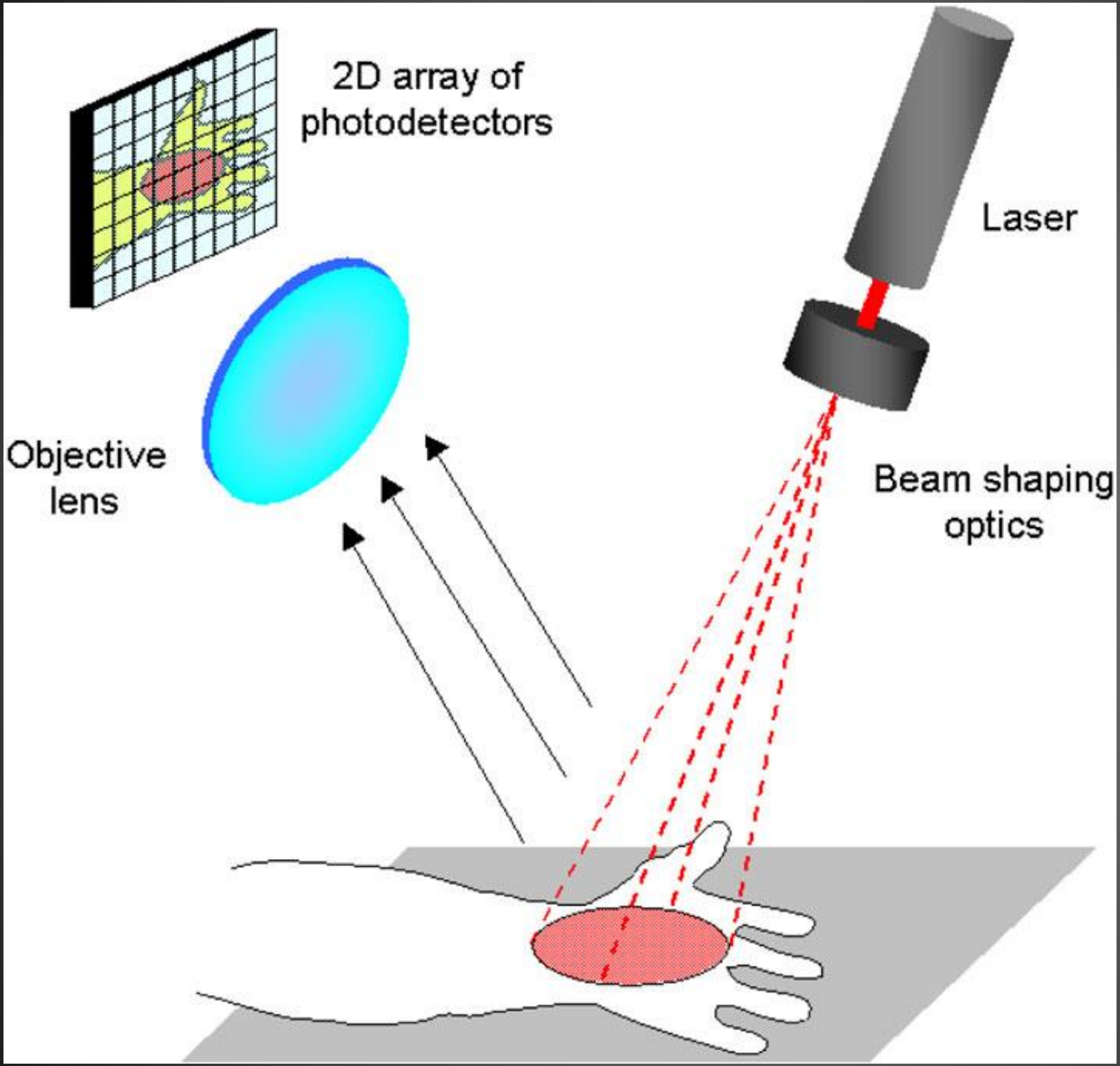
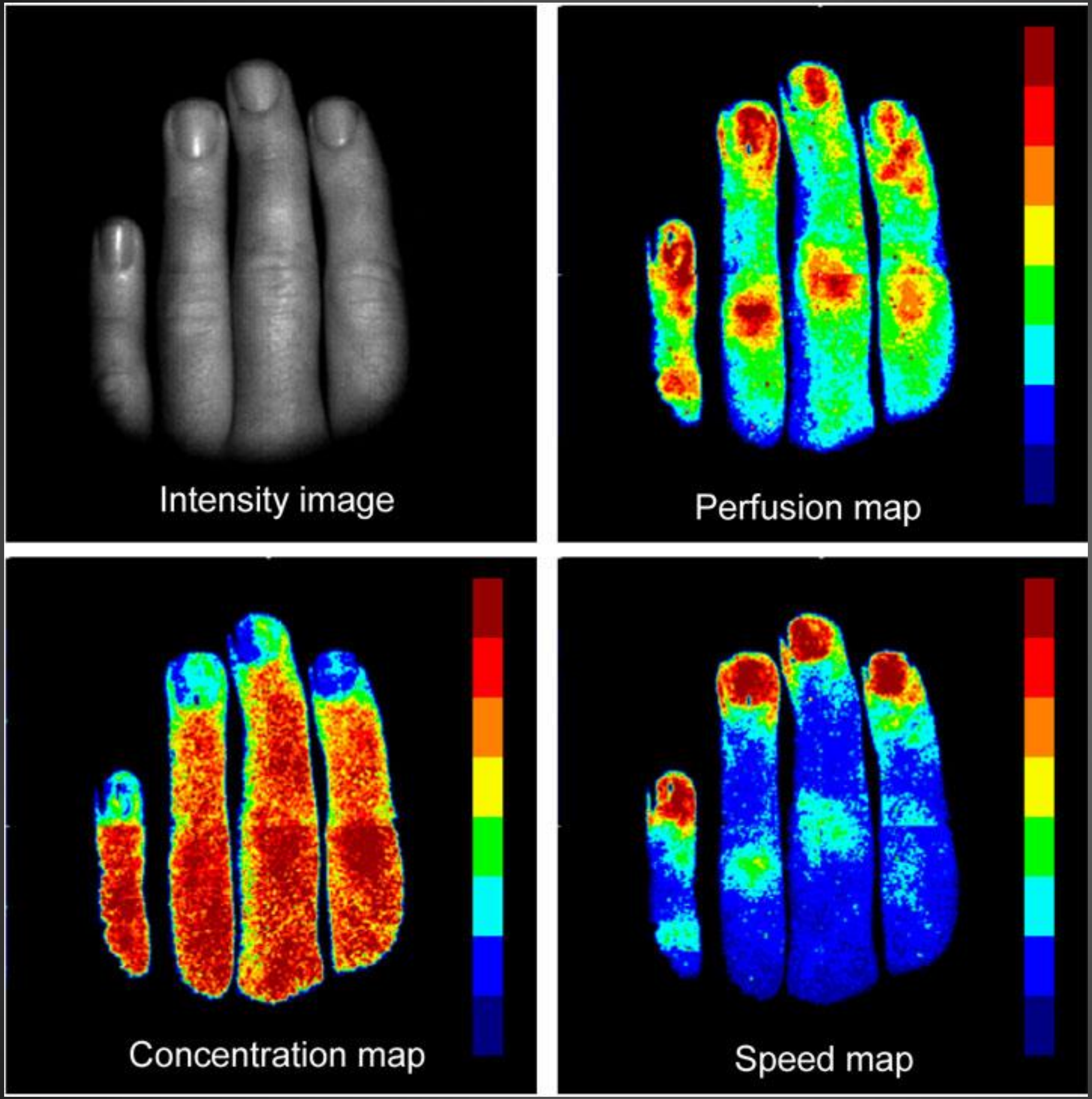


Image - Moor Instruments

Builds up image point by point, slow



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

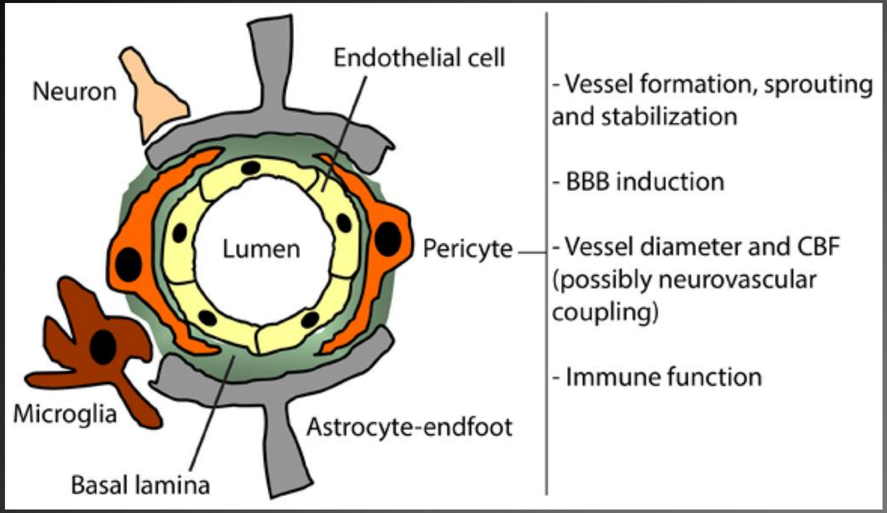
- To assess the blood flow pulsation in the pulp capillaries

7. Laboratory animal studies

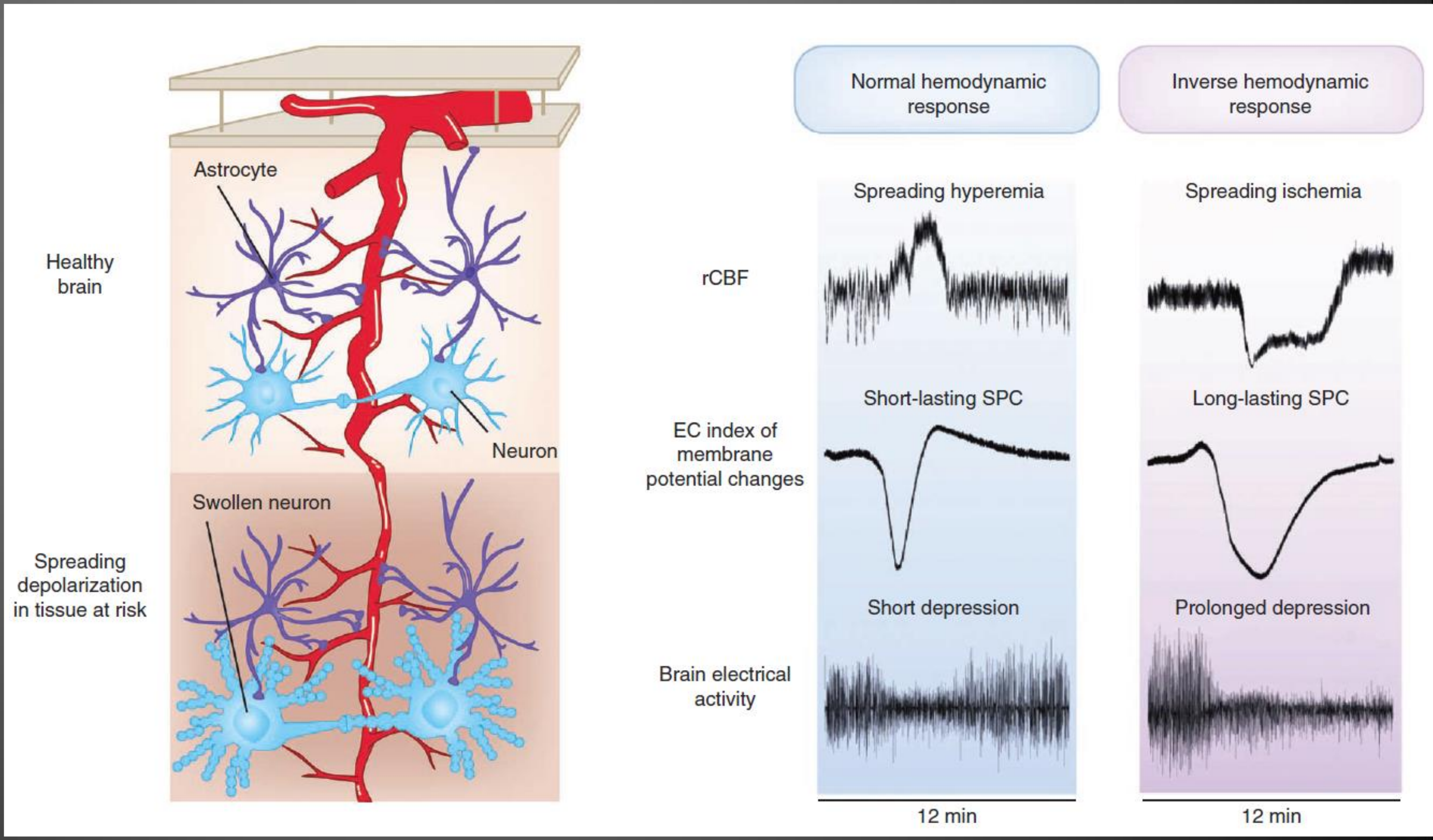
- For ocular, cerebral, cutaneous, auricular, splanchnic, and renal blood flow

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

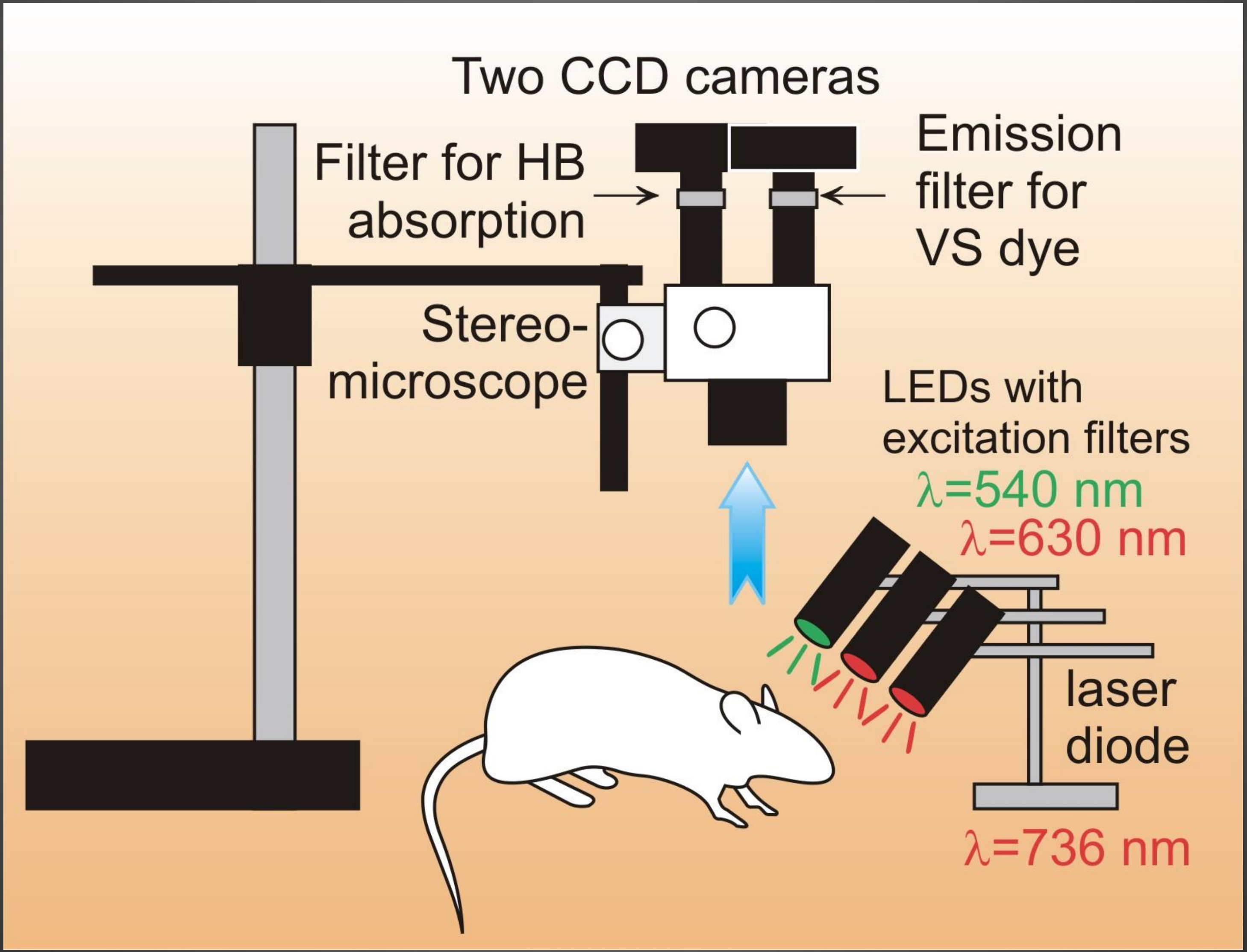


Neurovascular coupling and spreading depolarization in the injured brain

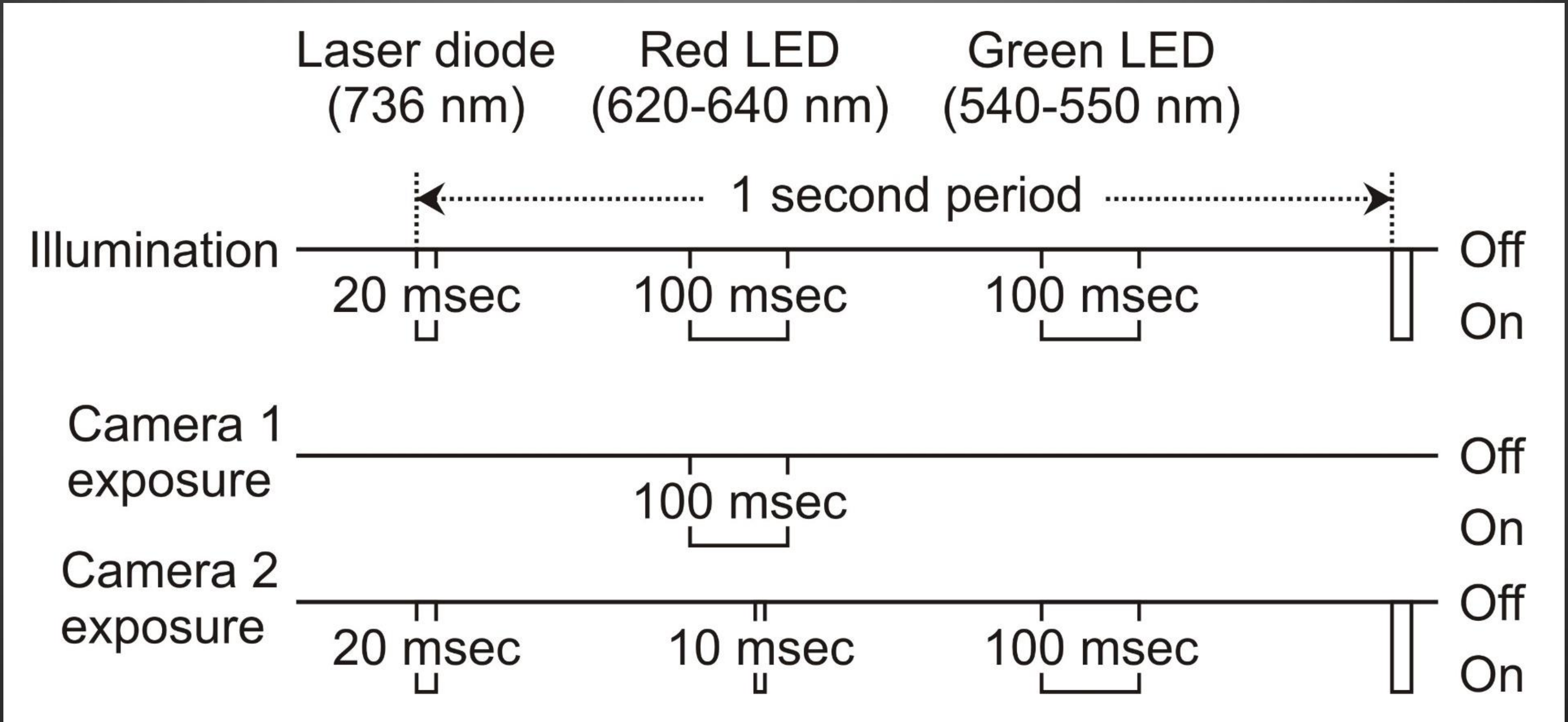


Dreier (2011) Nat. Med.

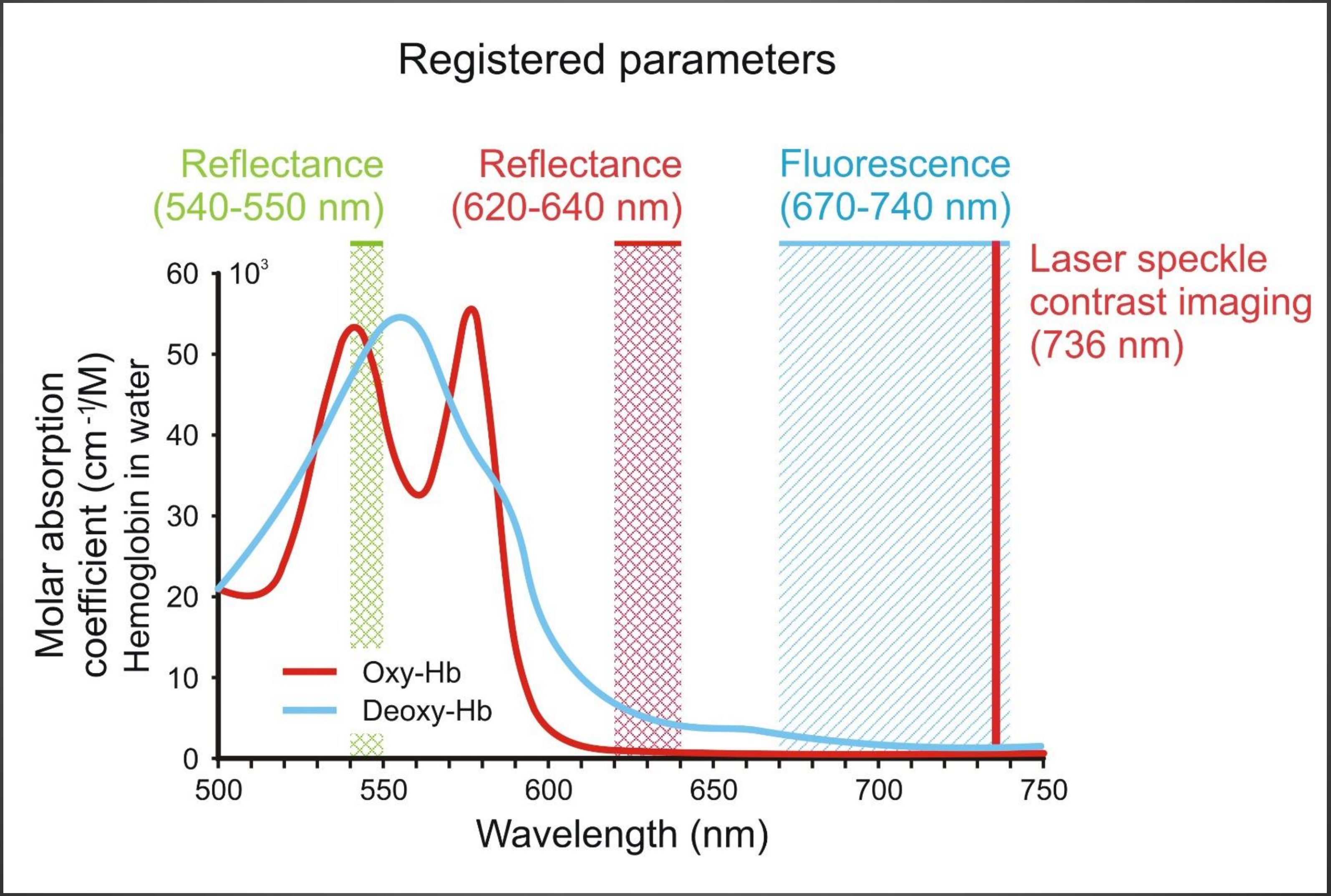
Draft of the arrangement of the setup



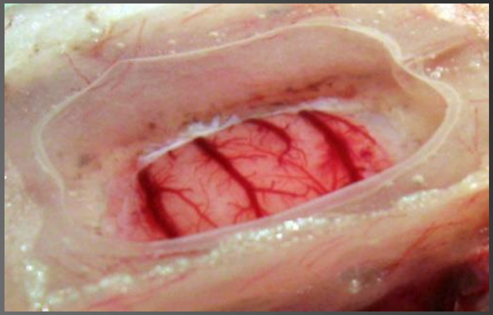
Synchronization of the respective illumination/image capture

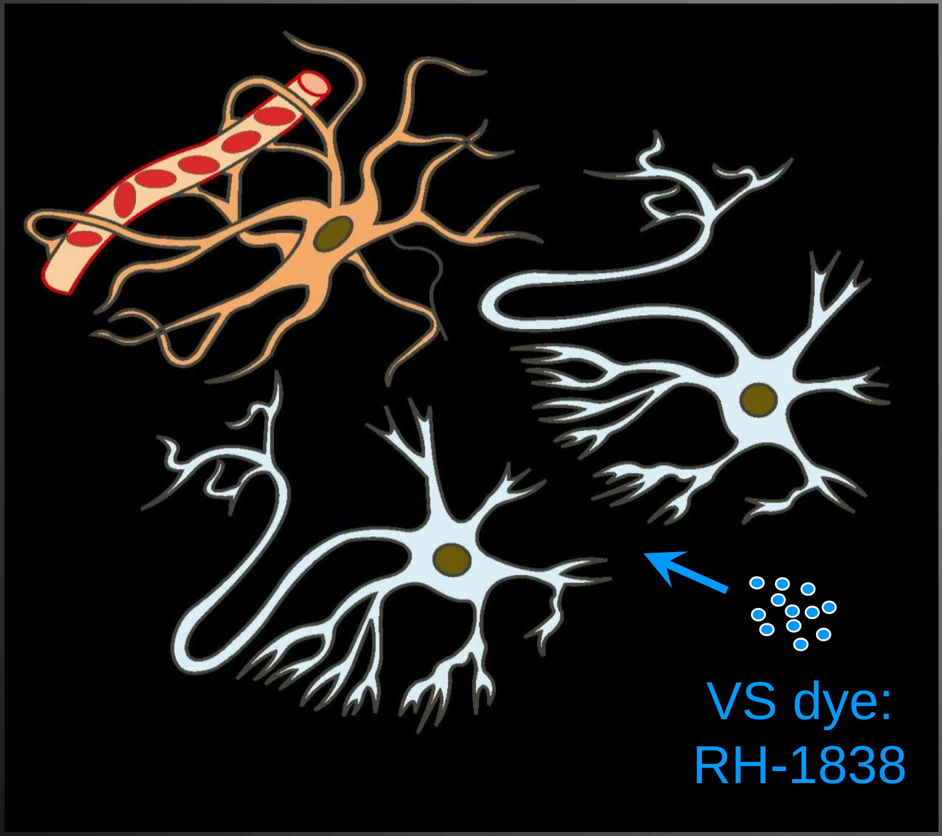


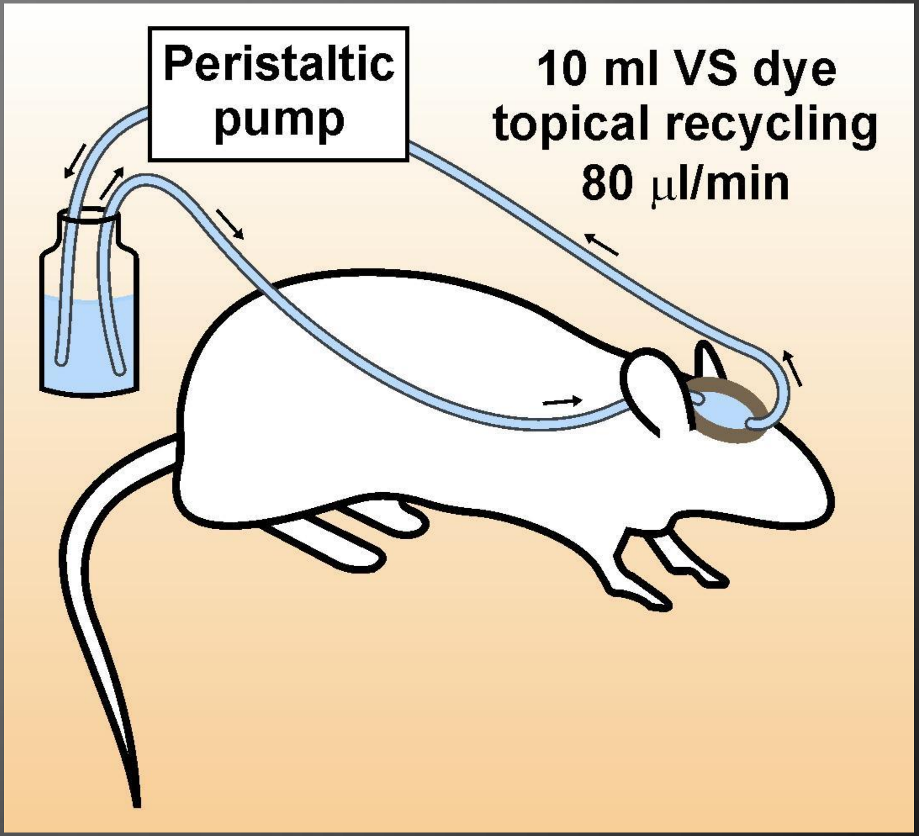
Optical principles for multimodal imaging



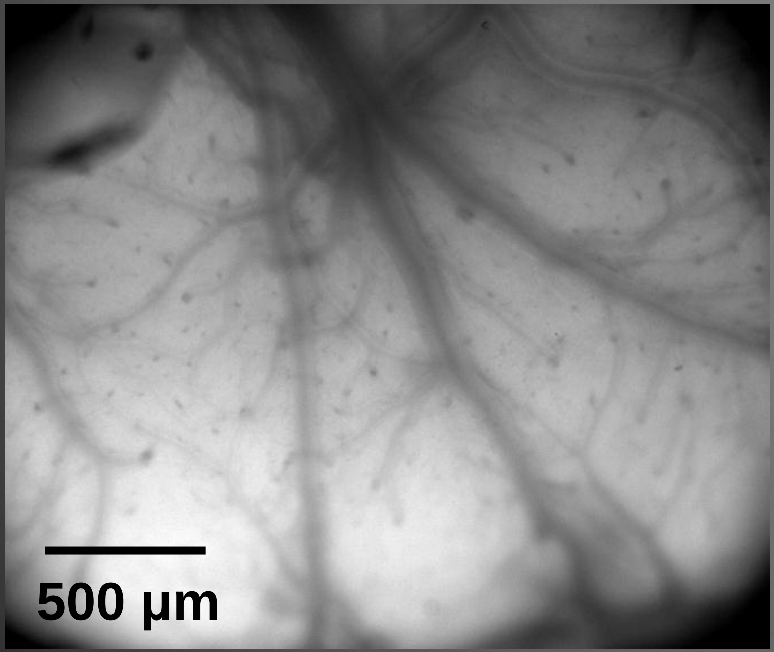
Voltage sensitive dye loaded in a closed cranial window

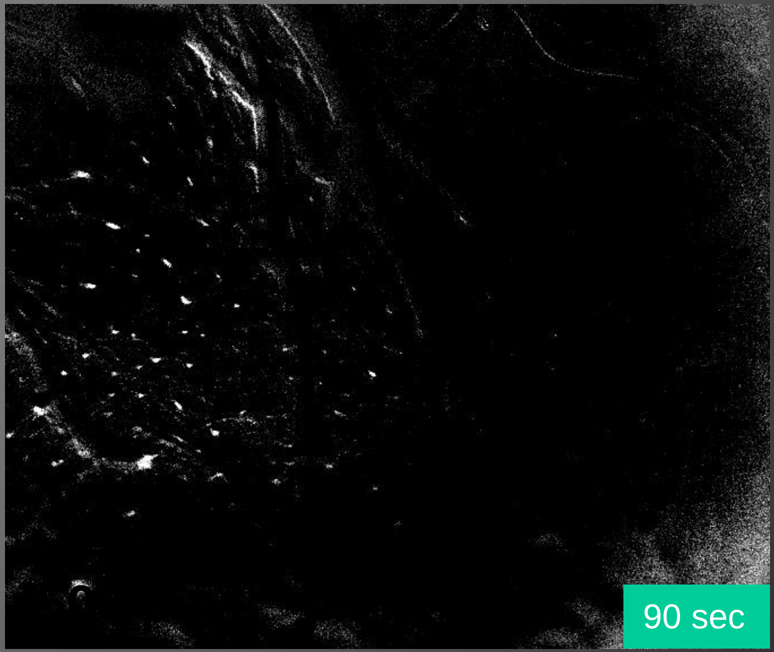






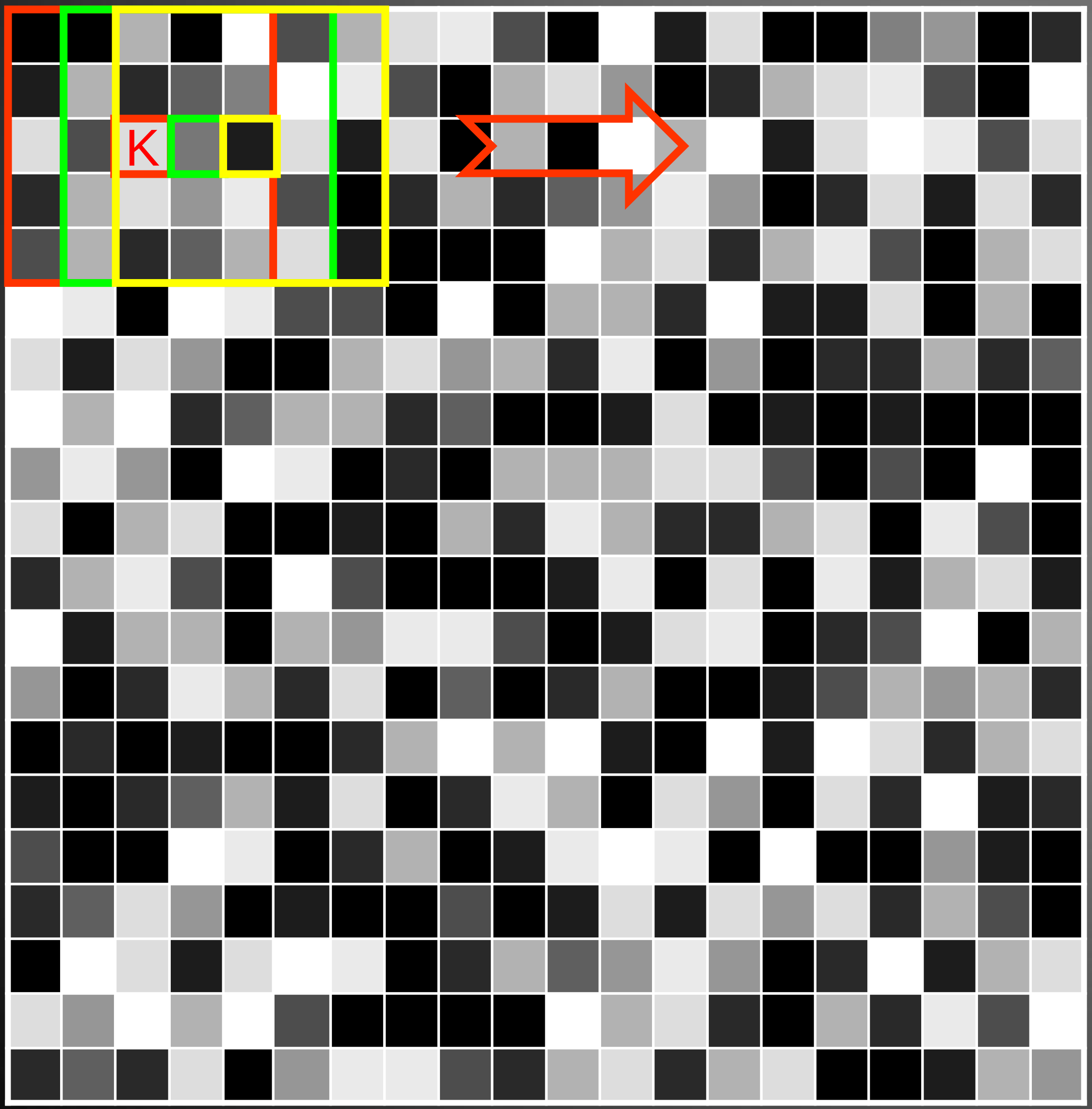
Representative video for SD-related changes in VS dye fluorescence





Exp. code: imag55, SD1

Laser speckle contrast analysis



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

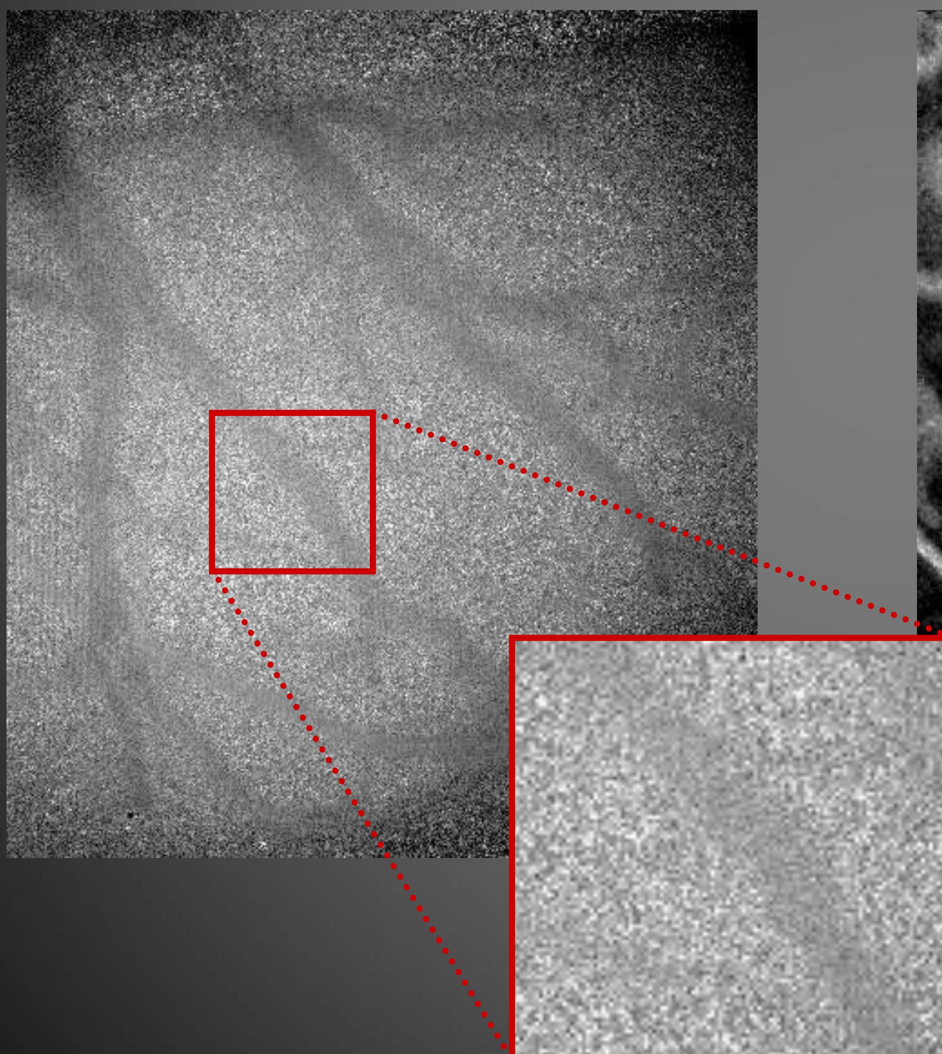
$$K = \frac{\sigma}{\langle I \rangle}$$

⇨ $1/K^2$

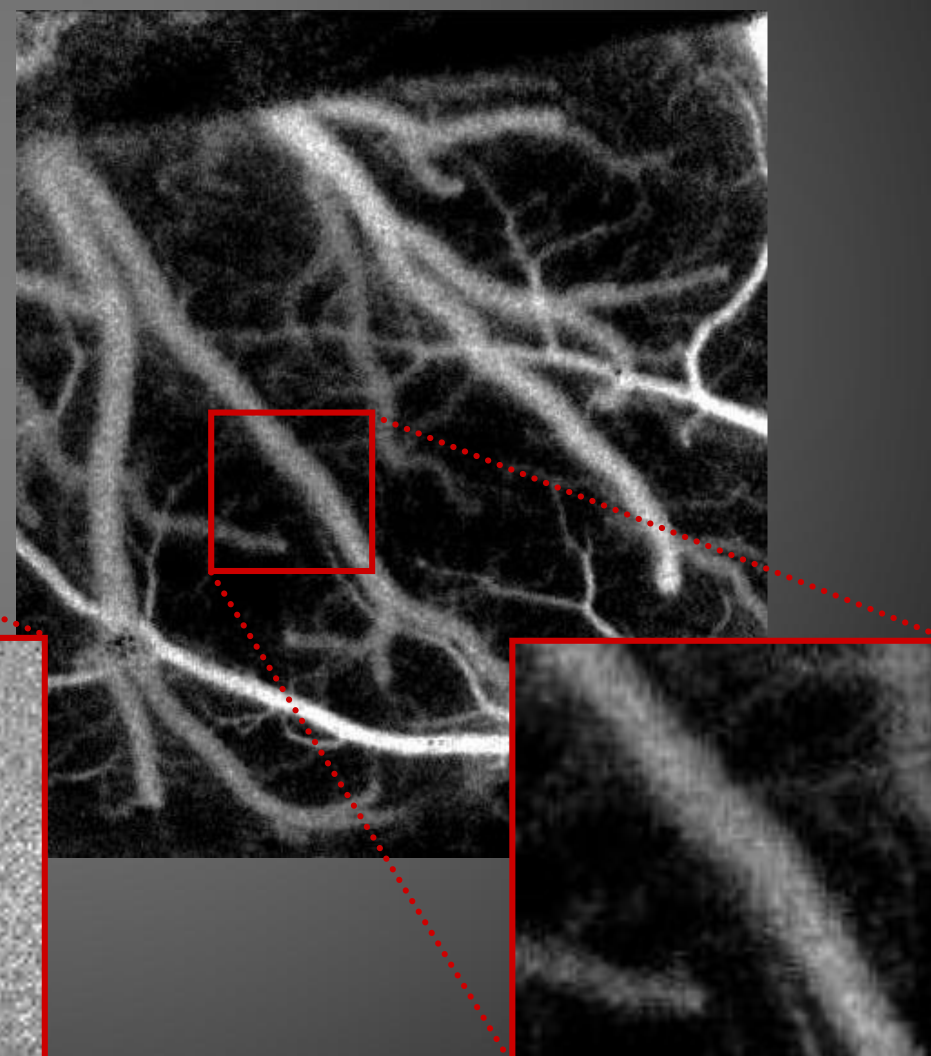
Particles with low motility → high contrast
Particles with high velocity → low contrast
⇨ The velocity of particles is proportional with:
- the decrease in speckle contrast
- (time of exposure)

Acquired images

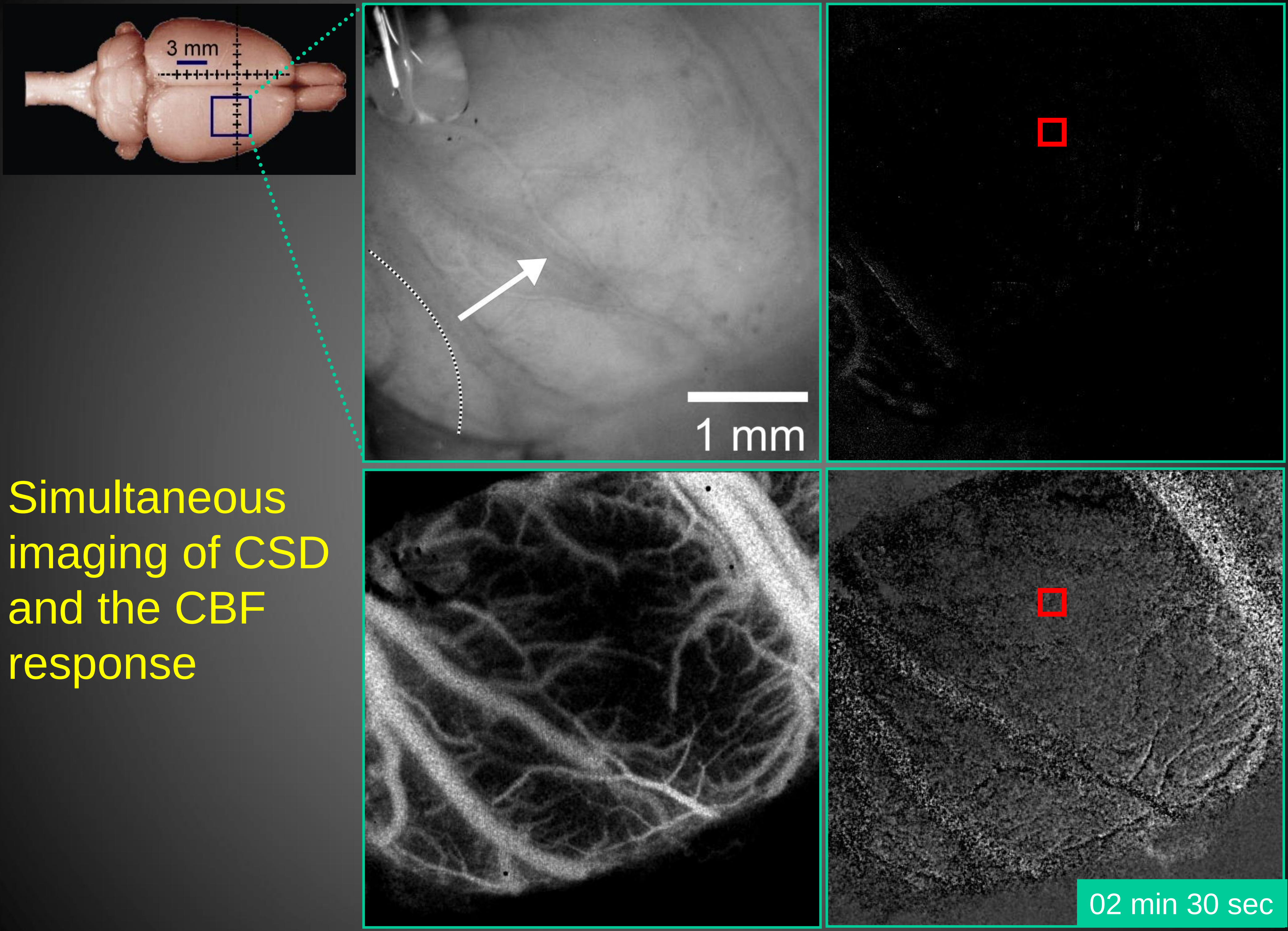
raw speckle image



flow map



Simultaneous imaging of CSD and the CBF response



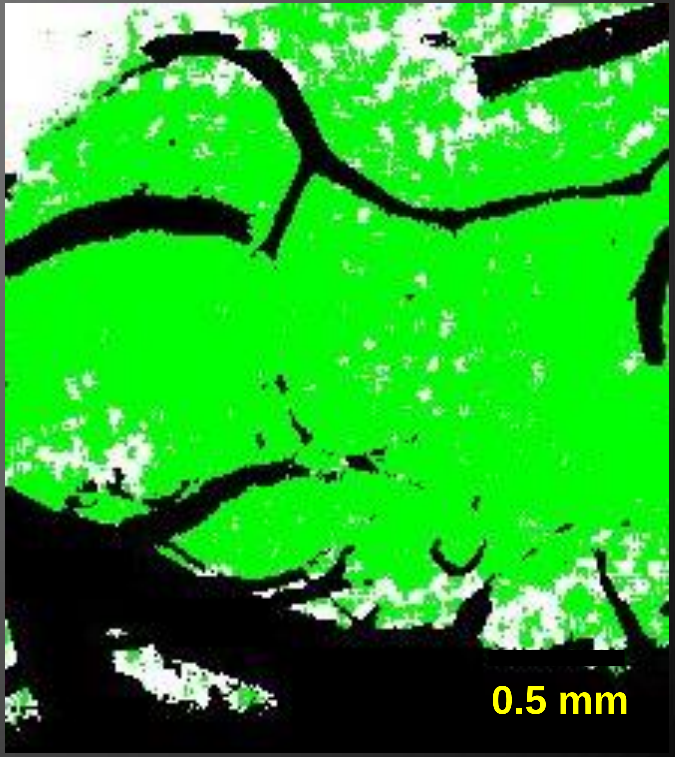
Exp. code: VSLS07 CSD1

Obrenovitch et al (2009)

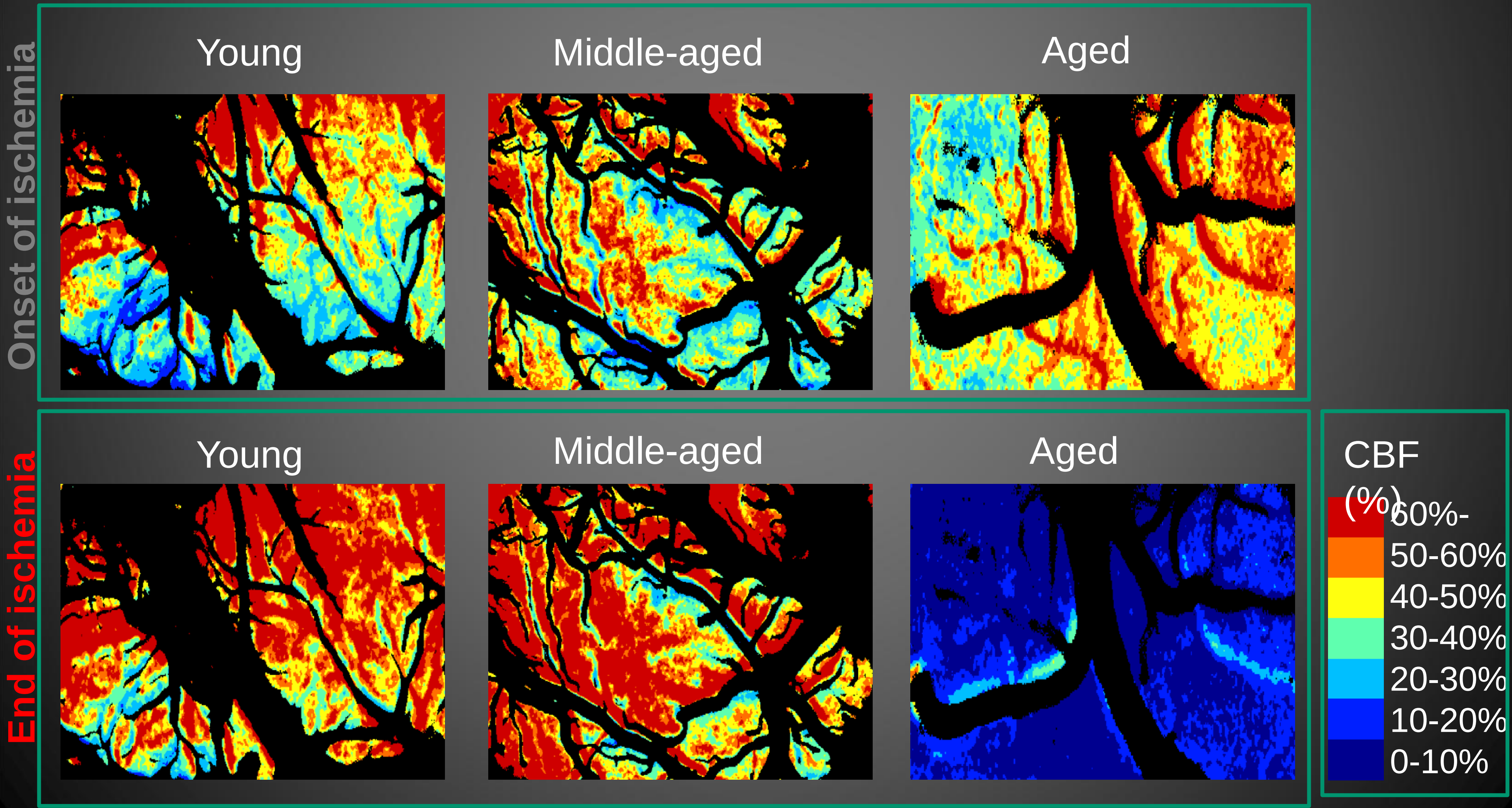
Whole field analysis of the VS dye signal

Area terminally depolarized in various age groups

Young | Middle-aged | Aged



Whole field analysis of cerebral blood flow maps



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National Development Agency
www.ujszechenyiterv.gov.hu
06 40 638 638

HUNGARY'S RENEWAL

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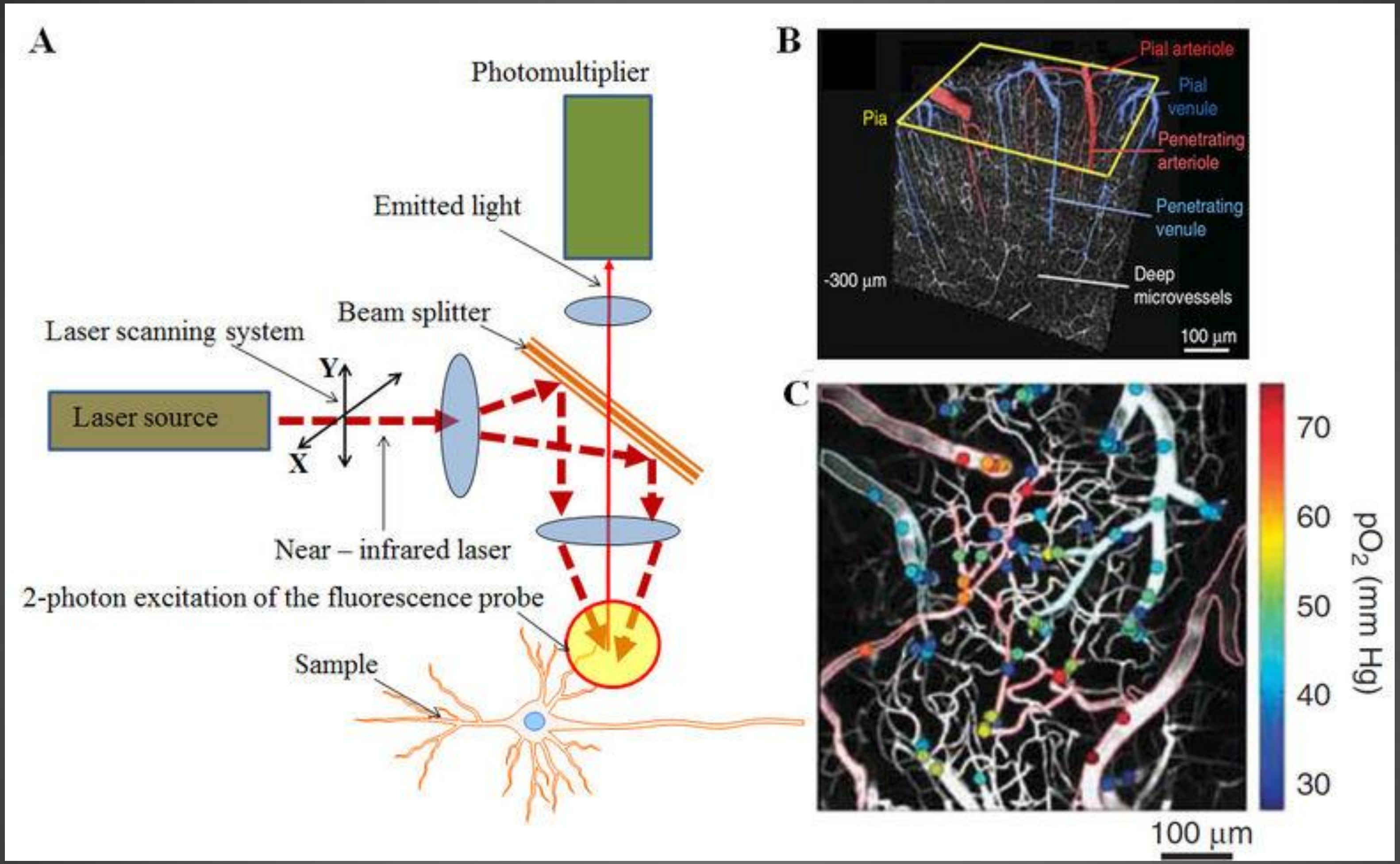
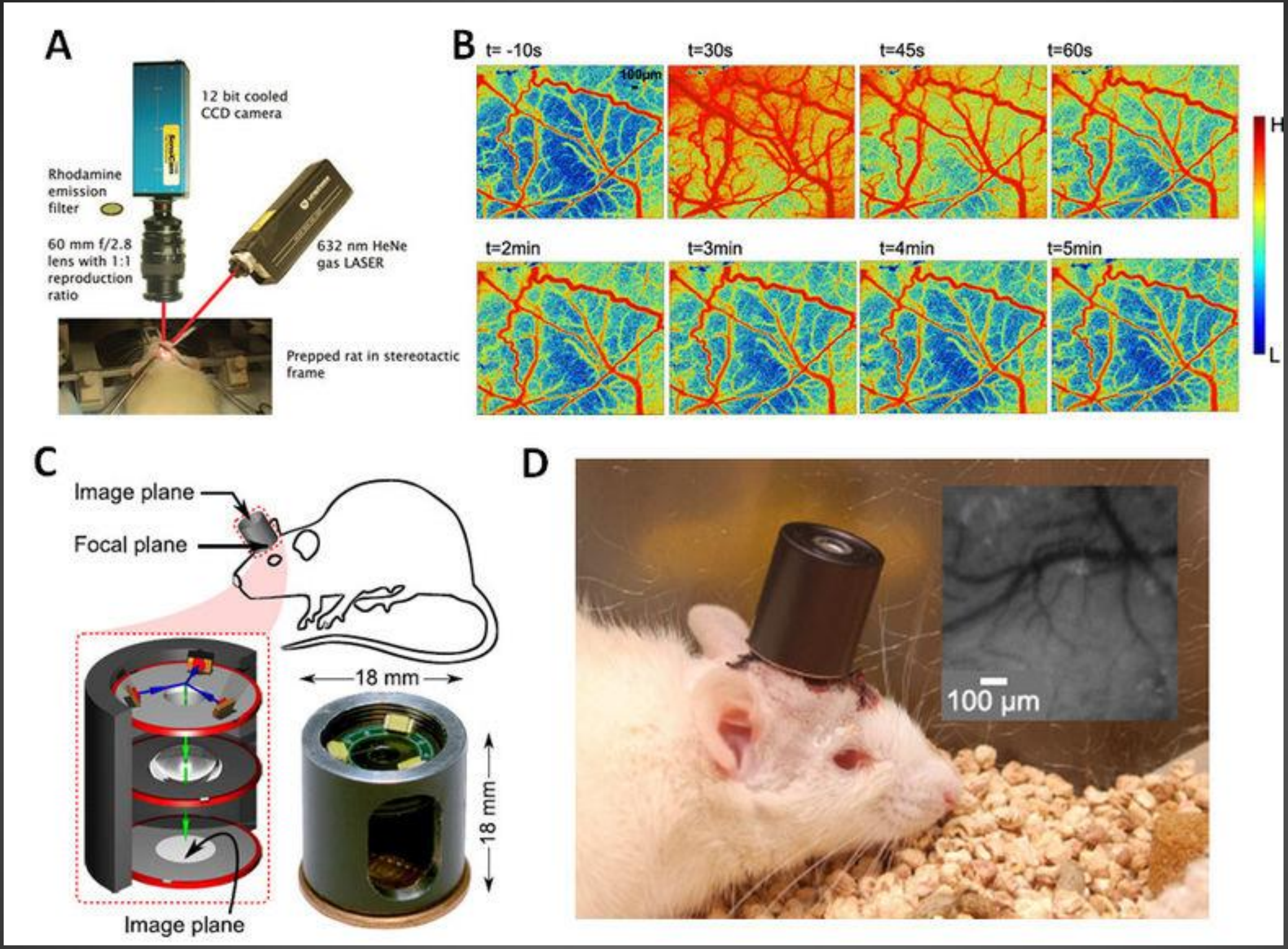
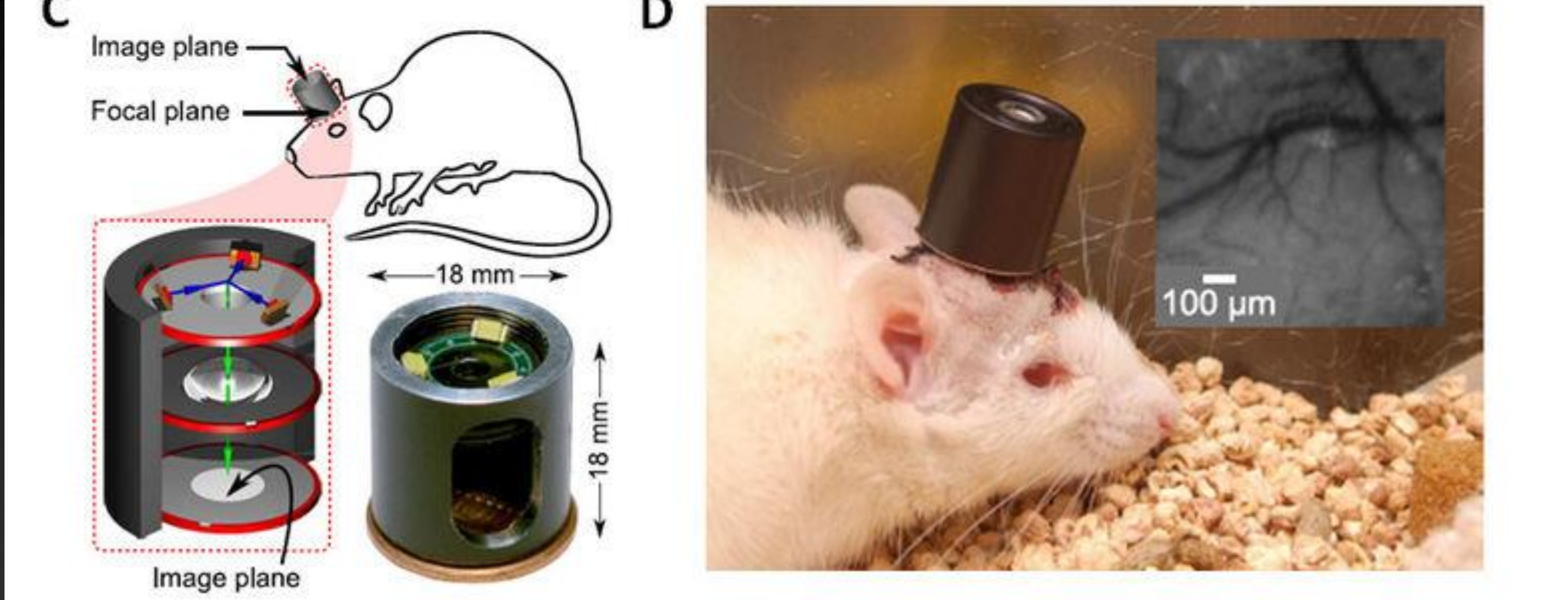
2-photon laser scanning microscopy

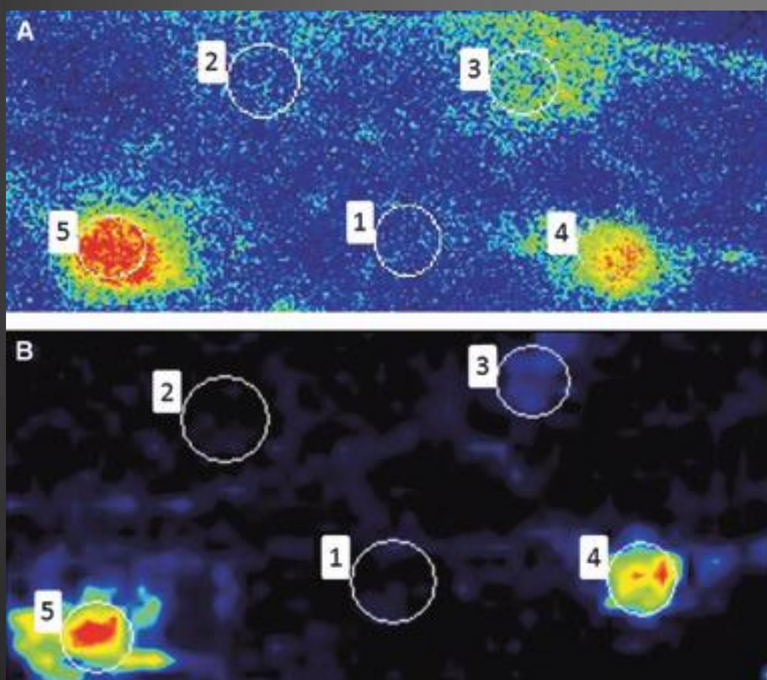


2-photon excitation can have advantages for 3D imaging of the relatively thick, up to 1 mm and more objects in vitro as well as in vivo. (**B**) Three-dimensional TPLSM high-resolution image [25]. (**C**) Intravascular oxygen could be measured over various depths of cortex by TPLSM. The color bar shows the calculated partial pressure of oxygen at the measured location [25].

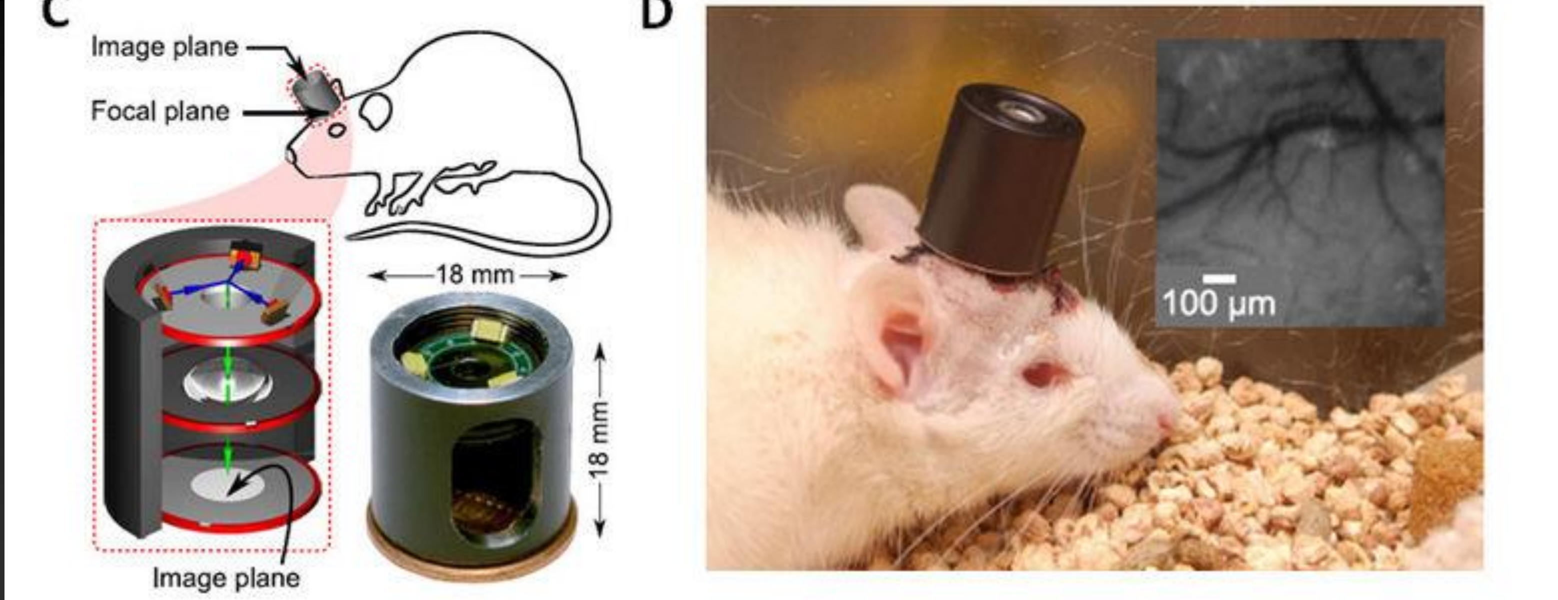

A
12 bit cooled CCD camera
Rhodamine emission filter
60 mm f/2.8 lens with 1:1 reproduction ratio
632 nm HeNe gas LASER
Prepped rat in stereotactic frame
B
t= -10s
t=30s
t=45s
t=60s
t=2min
t=3min
t=4min
t=5min
H
L
C
Image plane
Focal plane
18 mm
18 mm
Image plane
D
100 µm



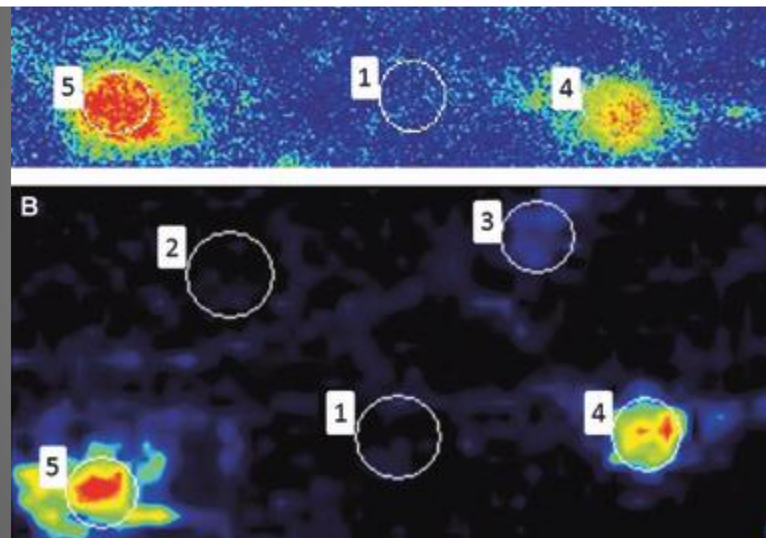
ght Into Methods



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#f7



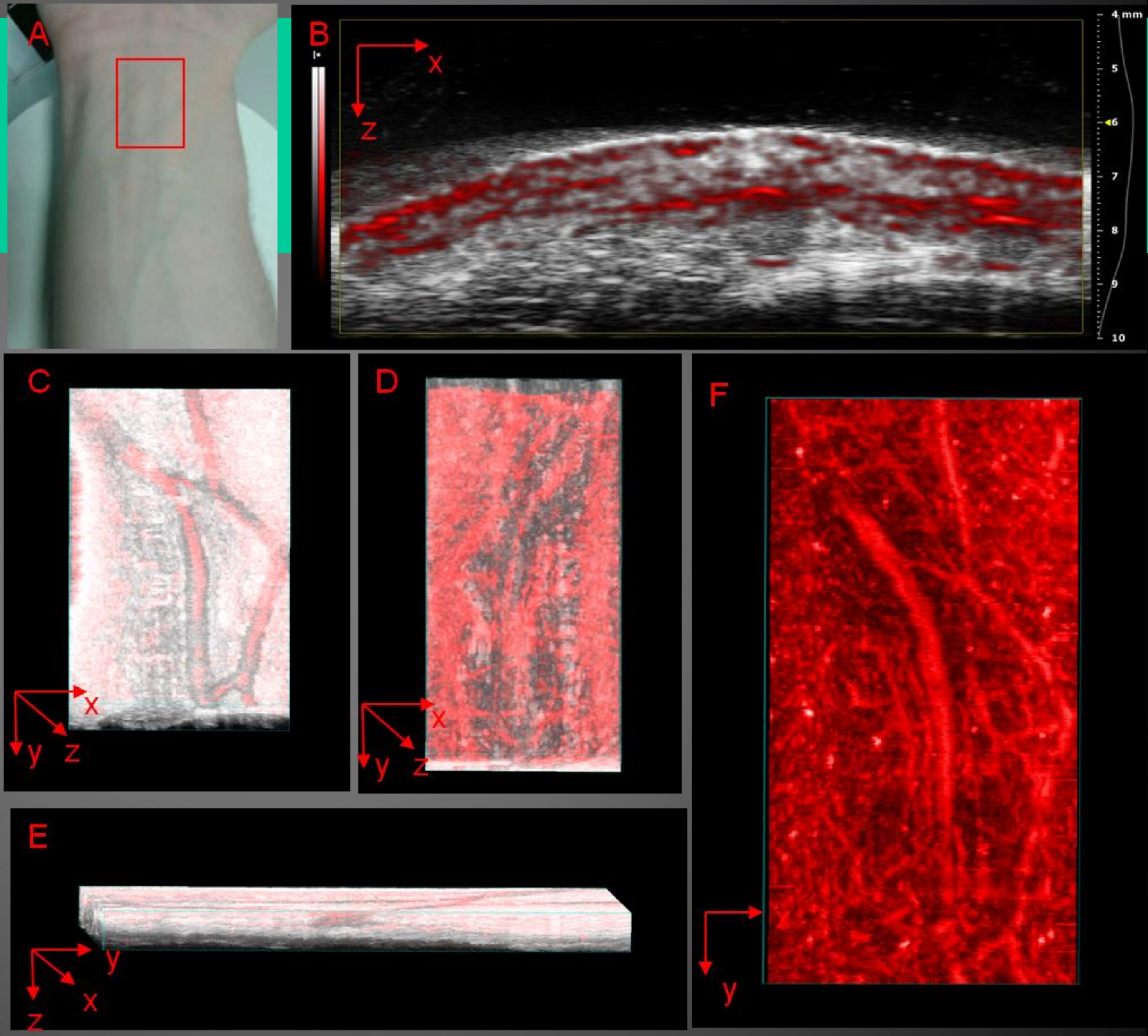
ght Into Methods



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#f7

In vivo imaging of human forearm using 40 MHz transducer

• In vivo PA and high frequency ultrasound images of the human forearm for a 30.5 (length) x 14.1 (width) x 10 (depth) region using 40 MHz probe at 860 nm.

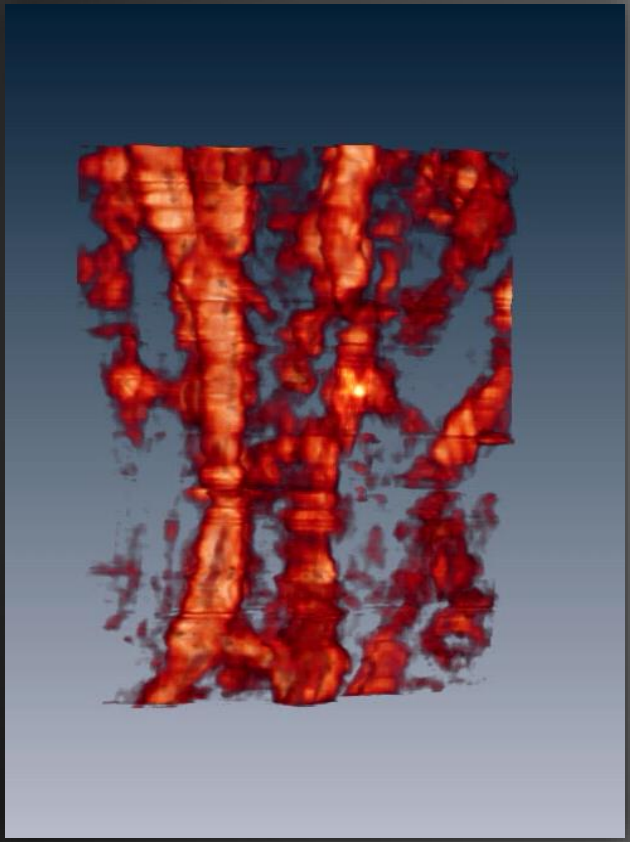


submitted to Journal of Investigative Dermatology, May 2014.

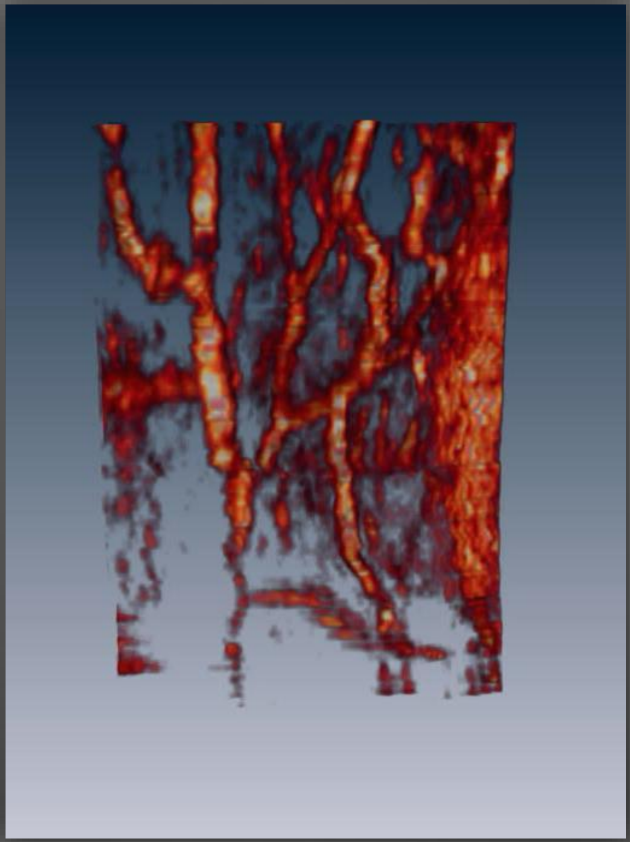
Comparison of 15, 21 & 40 MHz transducers

- Comparison of *in vivo* images of the human forearm acquired at the same location using 15 MHz, 21 MHz and 40 MHz transducer probes at 1064 nm.

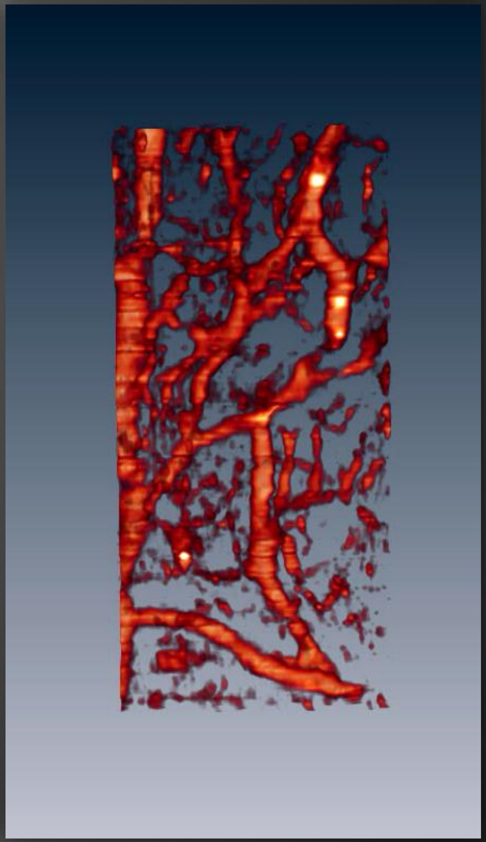
15 MHz (rendered)
30.5 mm x 23 mm (l x w).



21 MHz (rendered)
30.5 mm x 23 mm (l x w).



40 MHz (rendered)
30.5 mm x 14 mm (l x w).

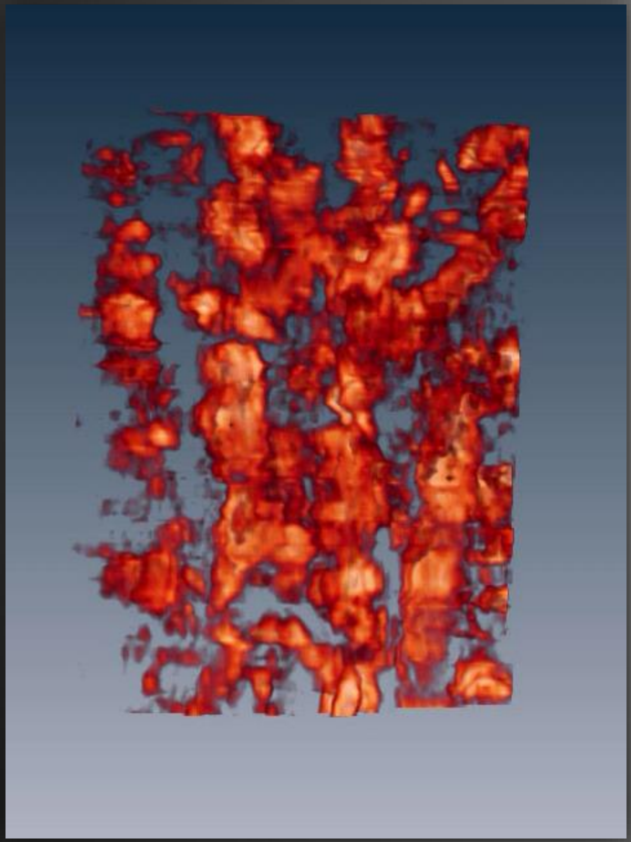


Leahy et al., submitted to Journal of Investigative Dermatology, May 2014.

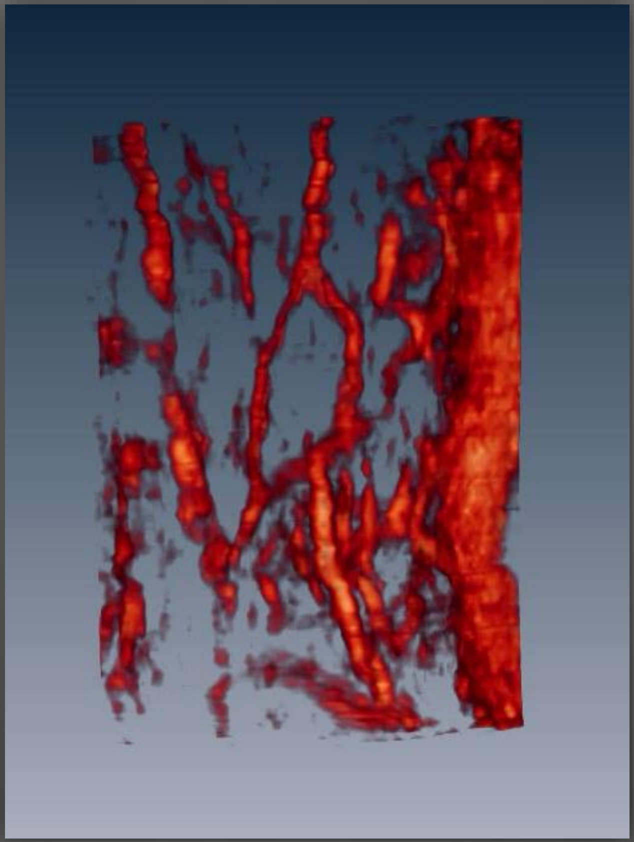
Comparison of 15, 21 & 40 MHz transducers

• Comparison of *in vivo* images of the human forearm acquired at the same location using 15 MHz, 21 MHz and 40 MHz transducer probes at 800 nm.

15 MHz (rendered)
30.5 mm x 23 mm (l x w).



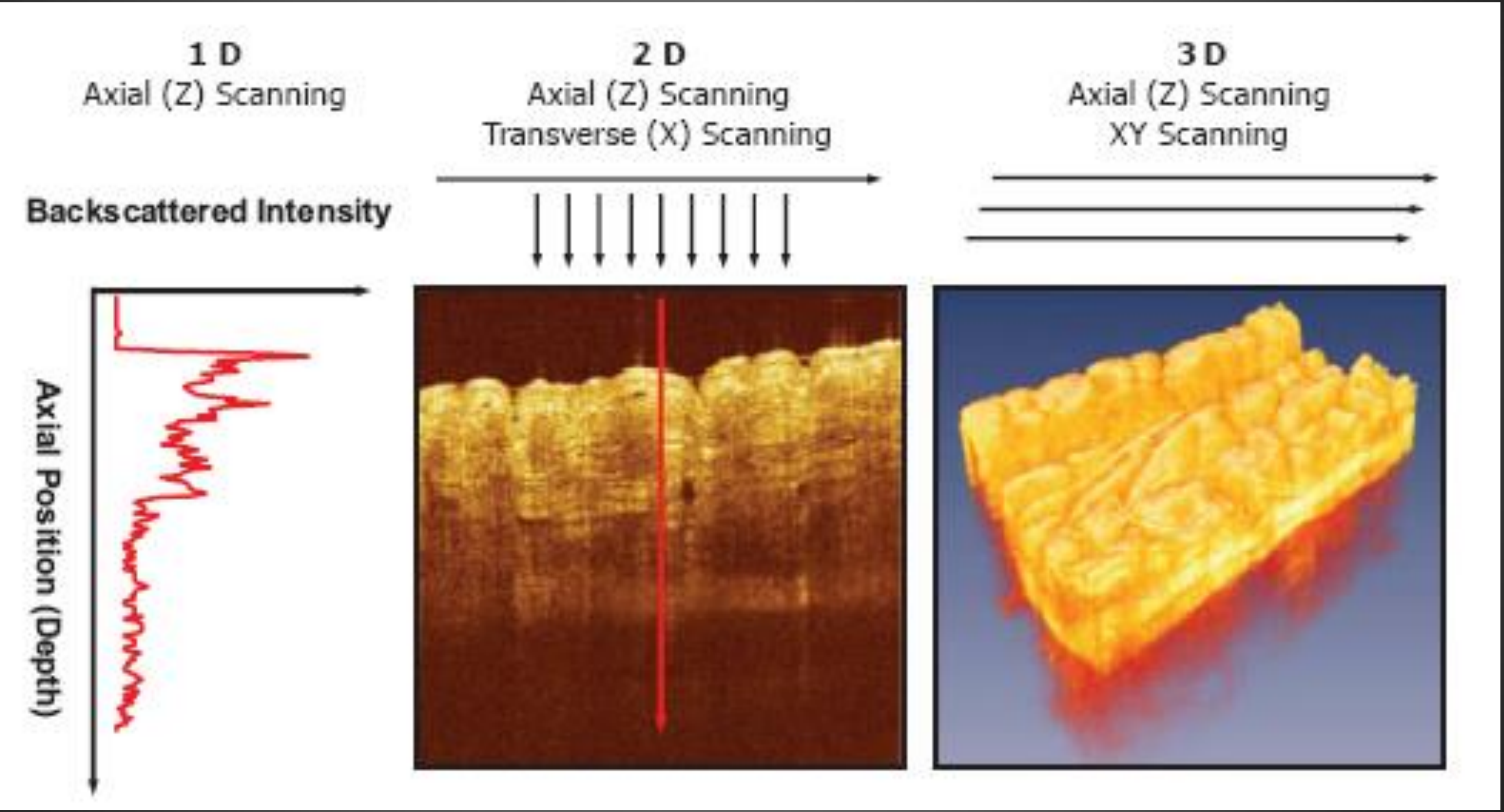
21 MHz (rendered)
30.5 mm x 23 mm (l x w).



40 MHz (rendered)
30.5 mm x 14 mm (l x w).

submitted to Journal of Investigative Dermatology, May 2014.

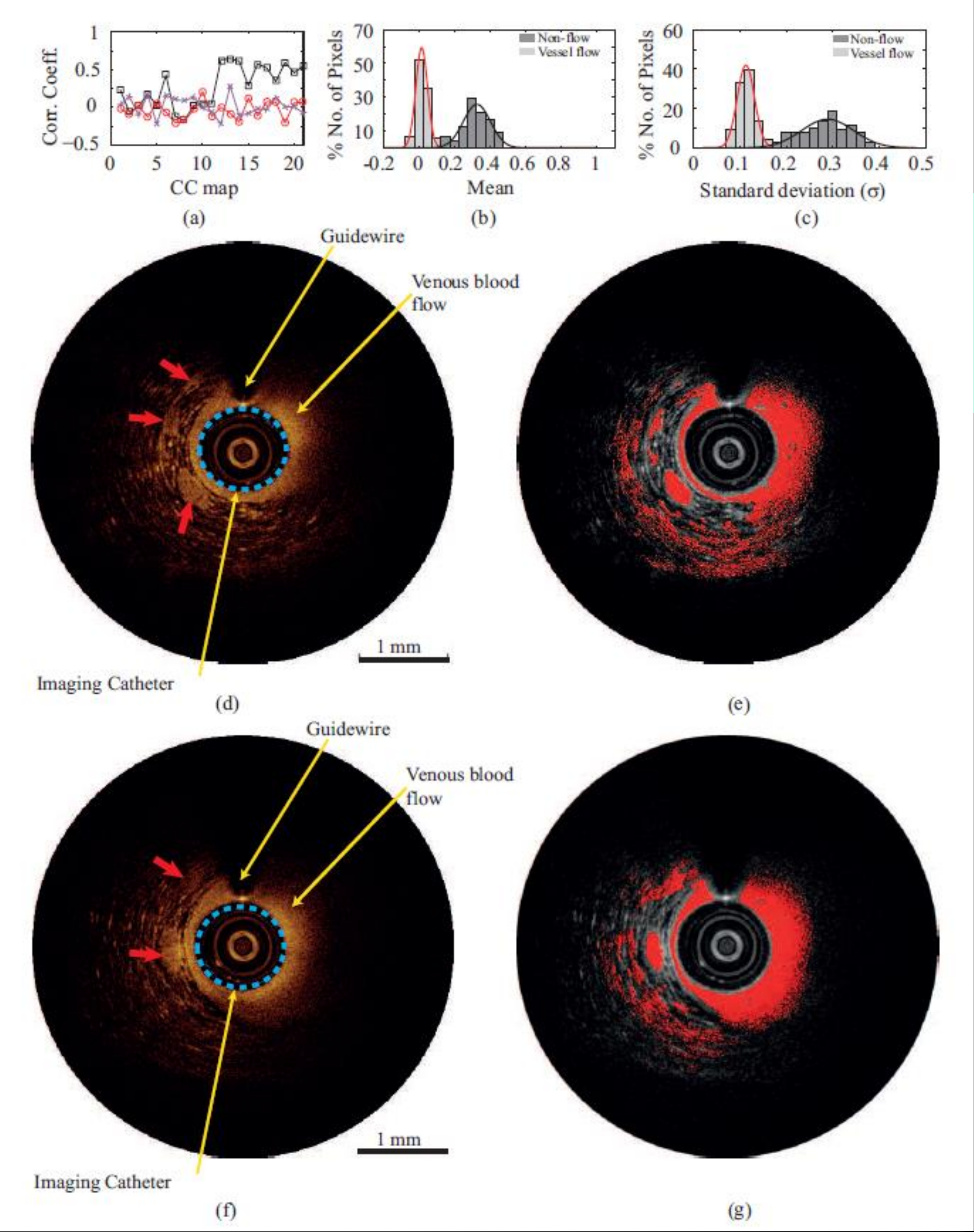
OCT: optical analogue of pulsed-wave ultrasound



J. Fujimoto, 2008

Human Coronary Sinus using the every frame CC mapping method.

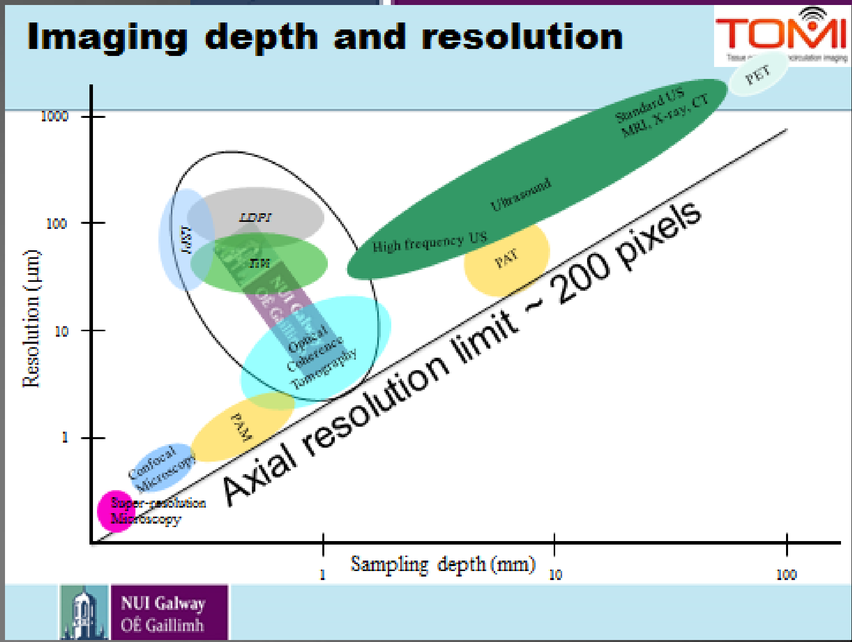
(d and f) Cross-sectional OCT images obtained with zero pullback. Bold red arrows indicate the vessels.
(e and g) Flow maps corresponding to (d) and (f) superimposed onto the respective OCT images.
Flow regions are marked red.

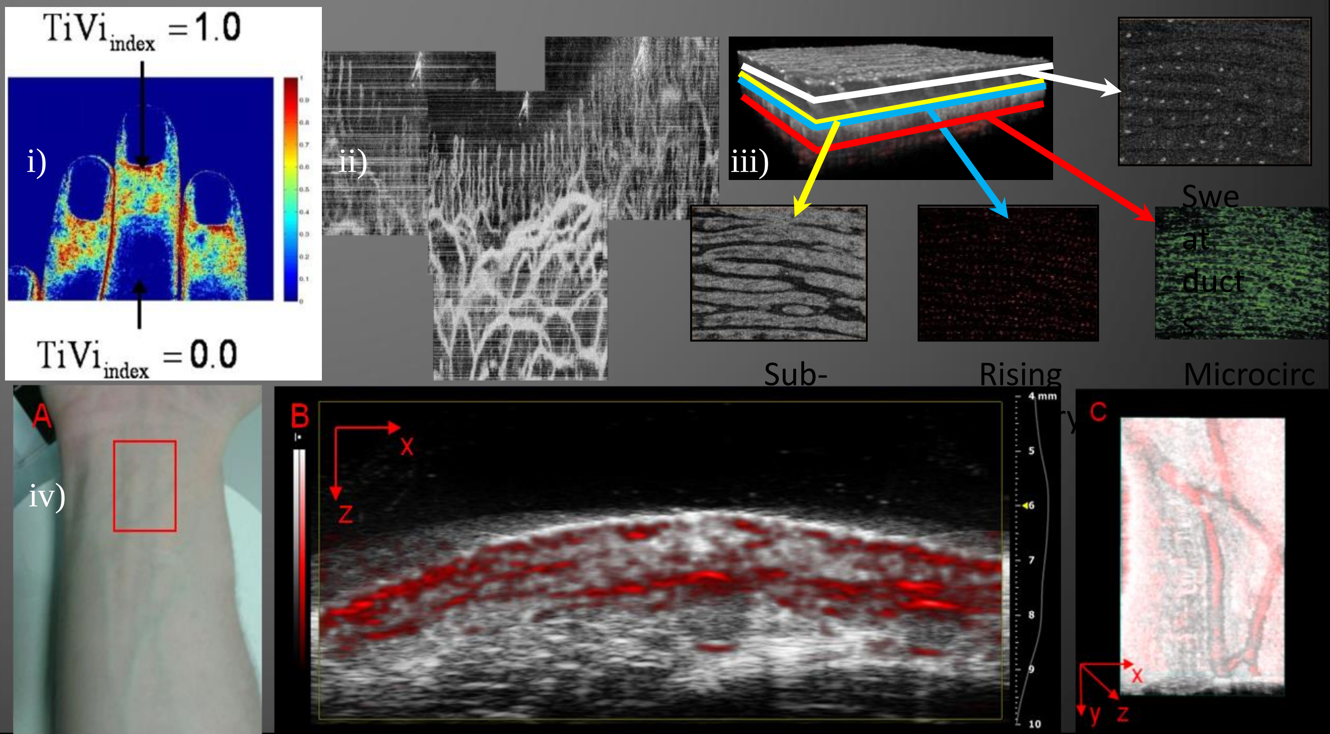


- In vivo / Ex vivo
- Scattering or non-scattering tissue?
- Depth versus resolution
- Speed – frames per second – motion?
- Functional – flow, oxygenation, molecular sensitivity
- Sub-resolution

content/activity

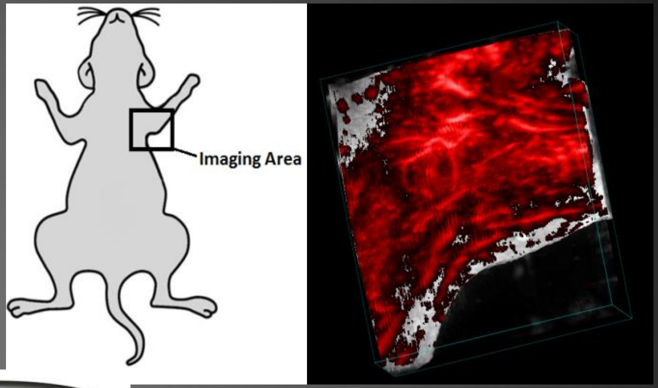
- Fit for purpose

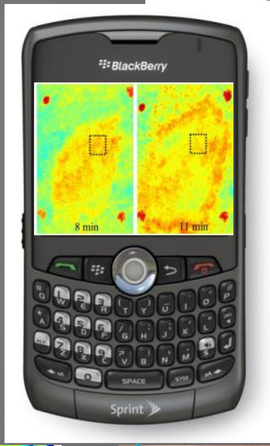


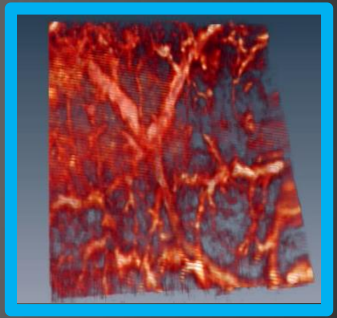


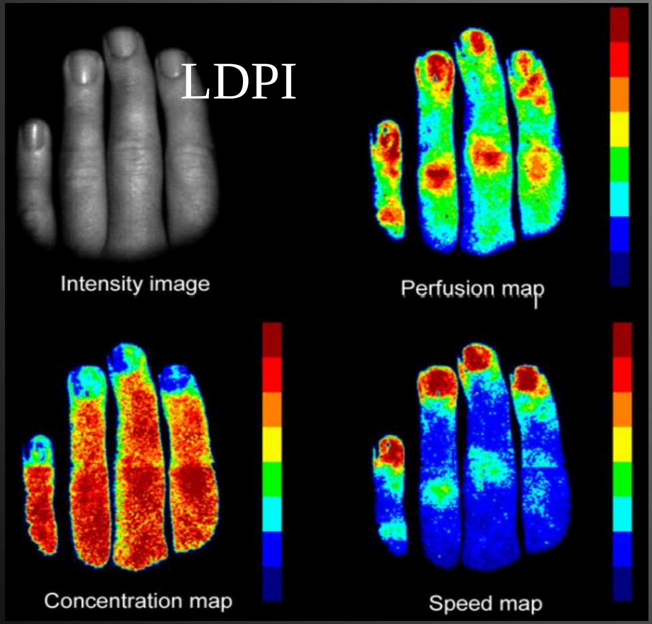
Microcirculation Imaging Techniques – TOMI lab

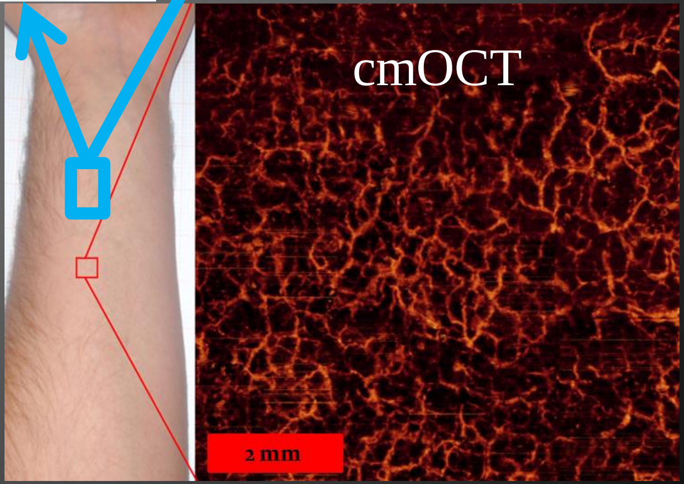
- Laser Doppler perfusion imaging (LDPI)
- Laser speckle contrast imaging (LSCI)
- Tissue viability imaging (TiVi)
- Photoacoustic Imaging (PAI)
- Optical coherence tomography (OCT)











Light penetration

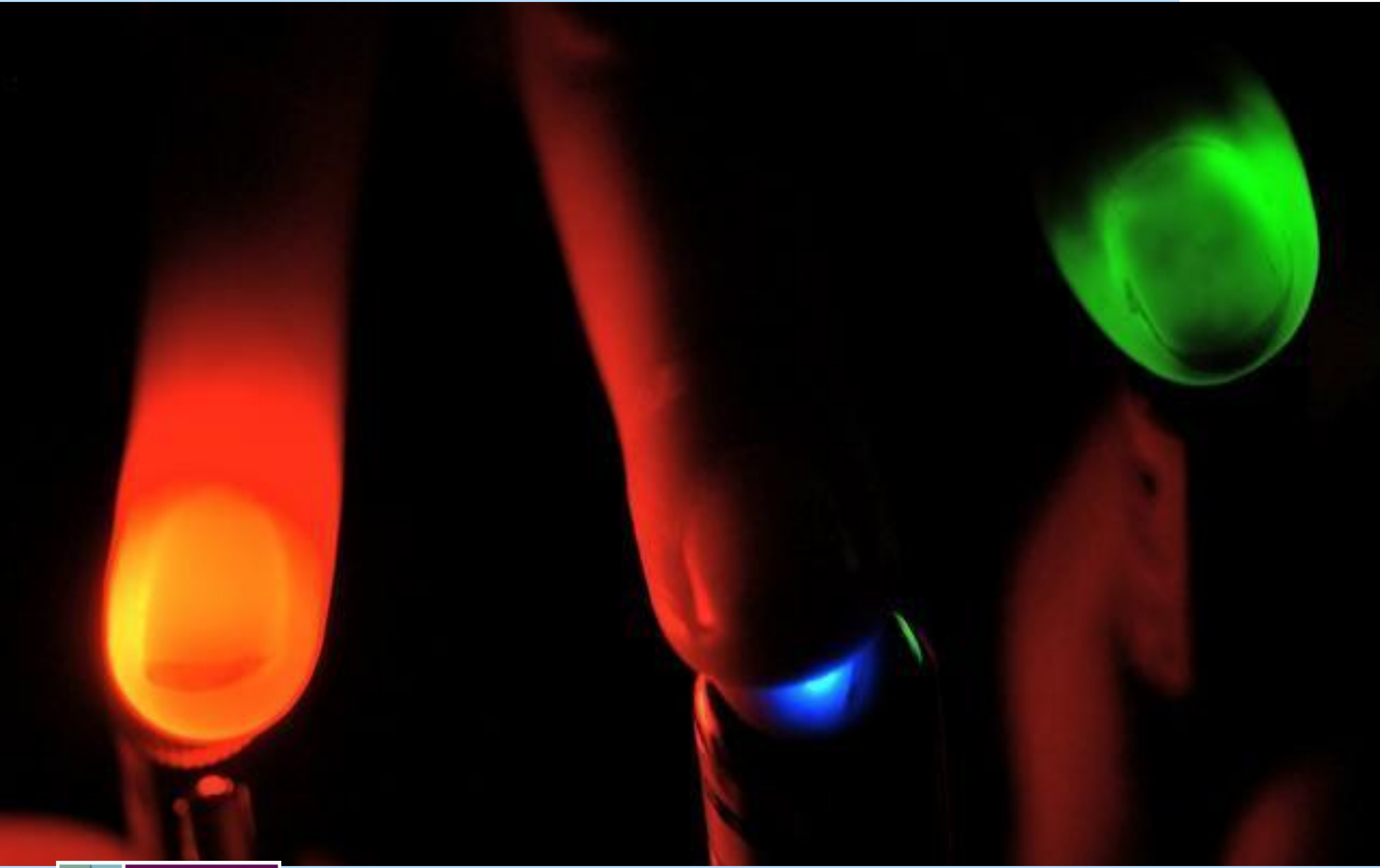
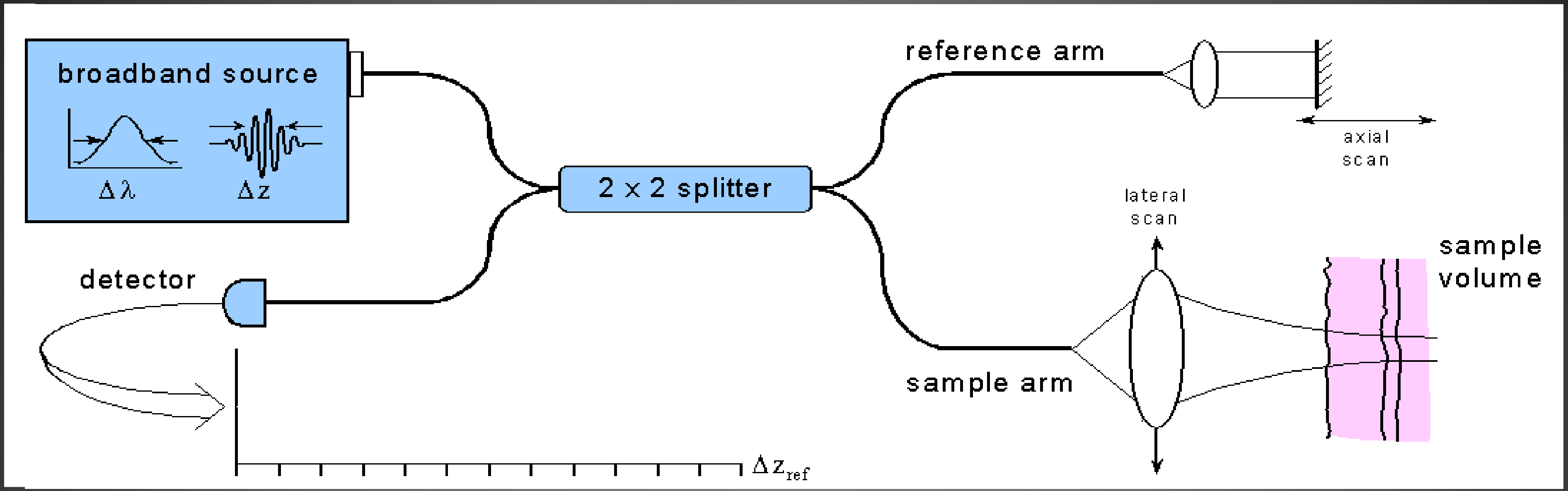
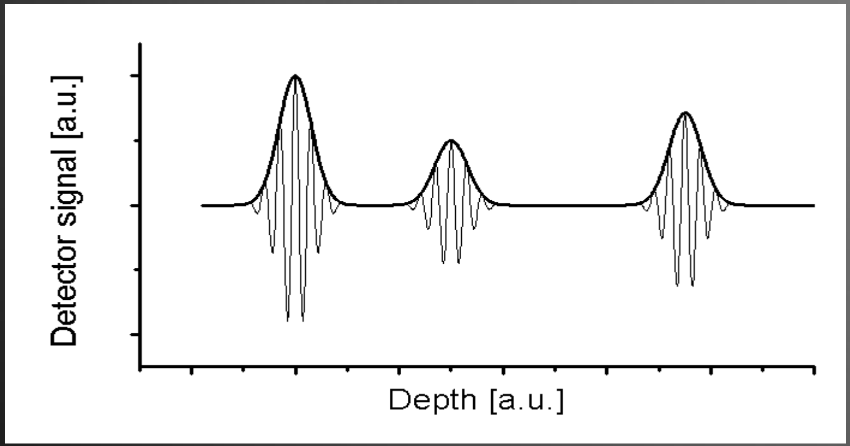
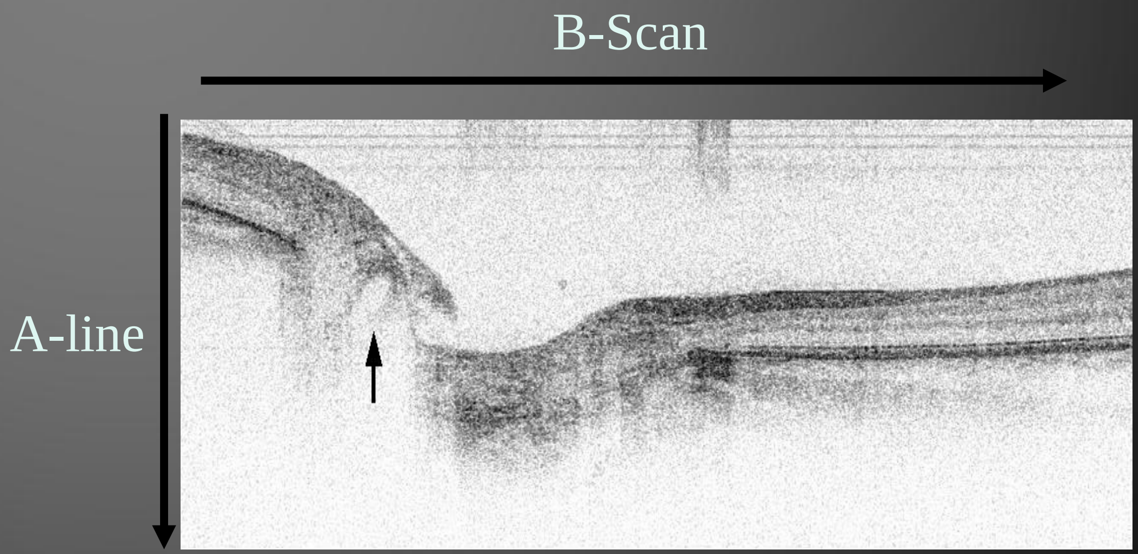


Image from www.biophonticsWorld.com

Optical Coherence Tomography

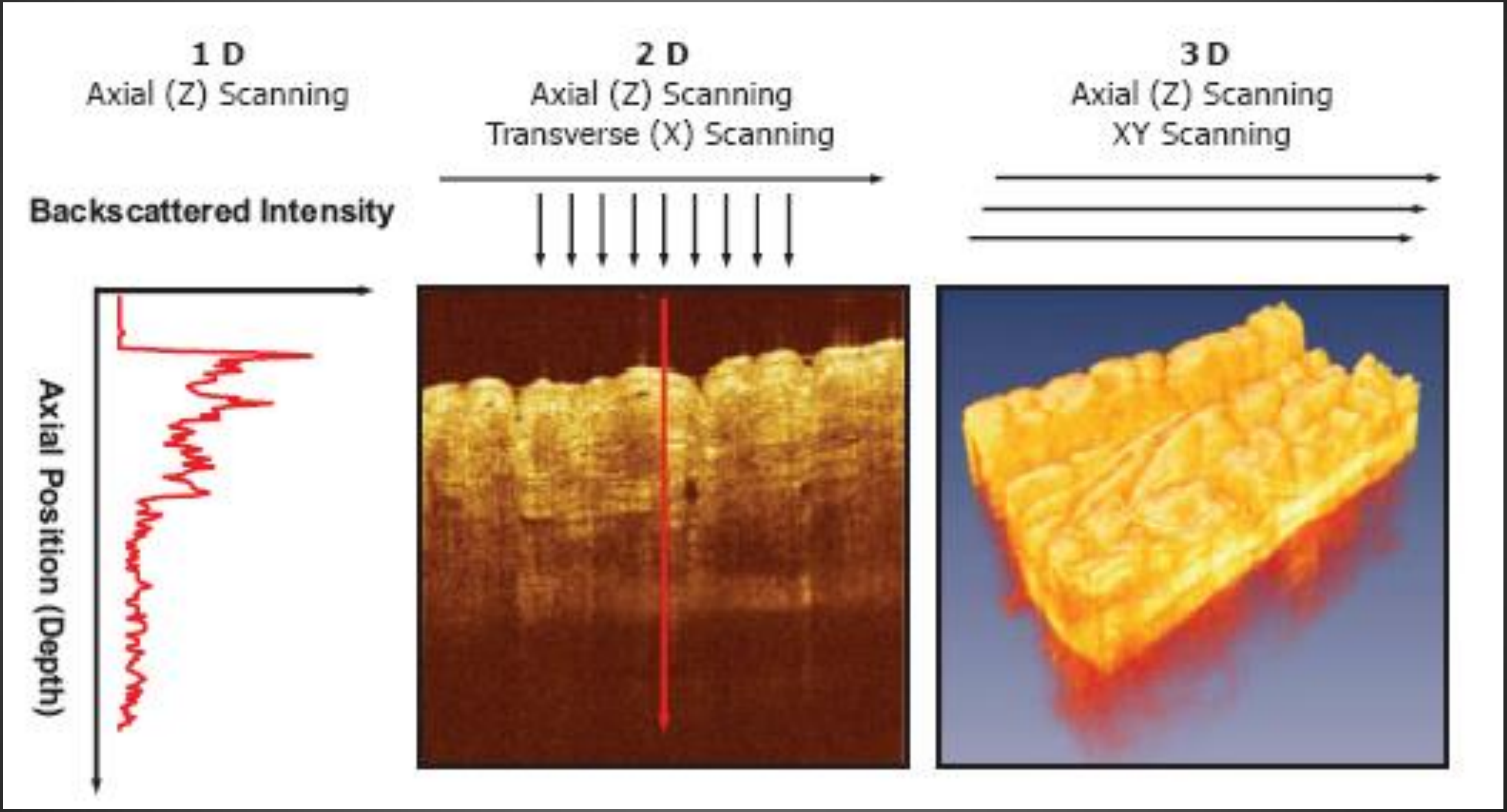






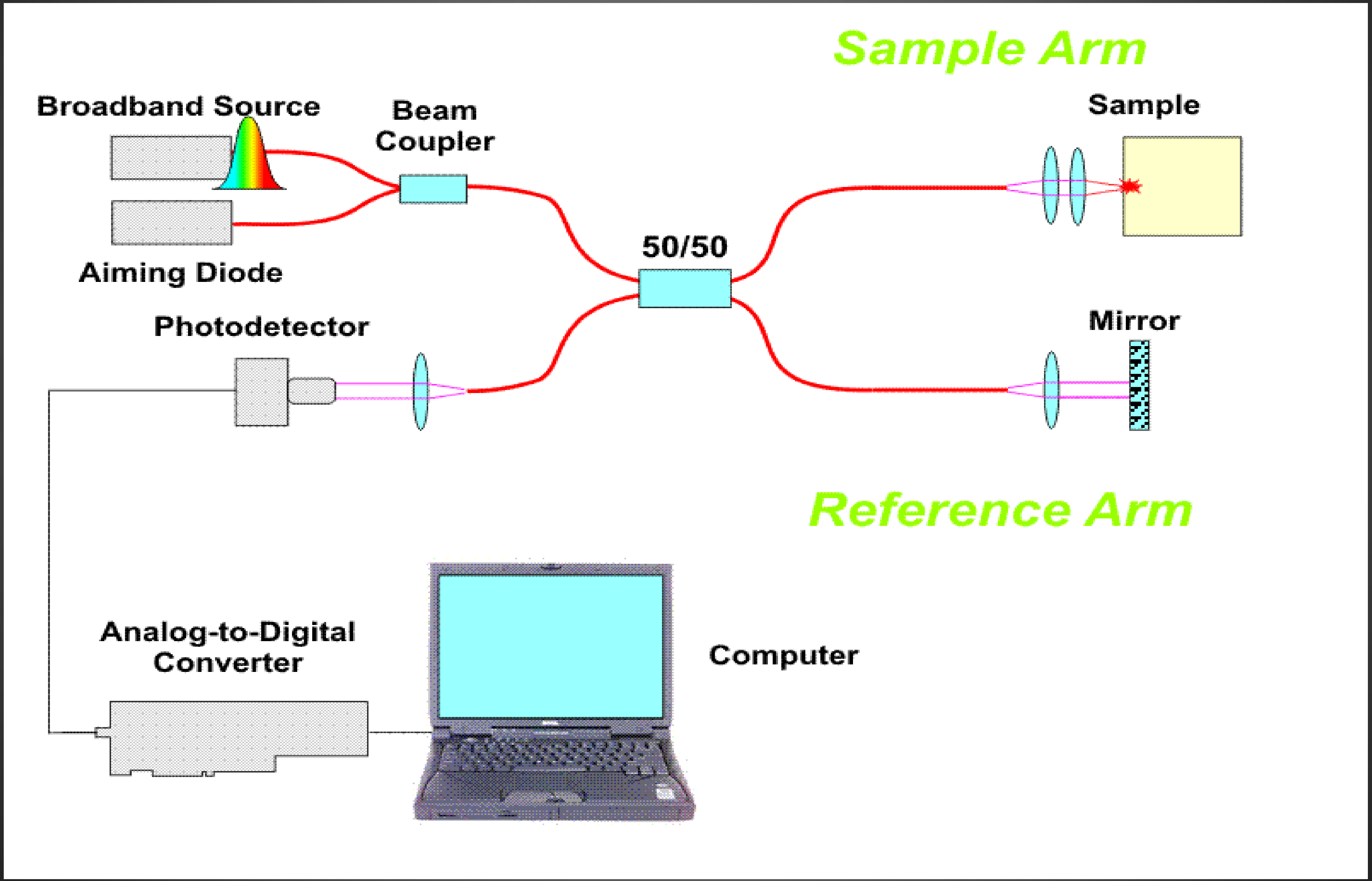
Courtesy of Johannes de Boer

OCT: optical analogue of pulsed-wave ultrasound



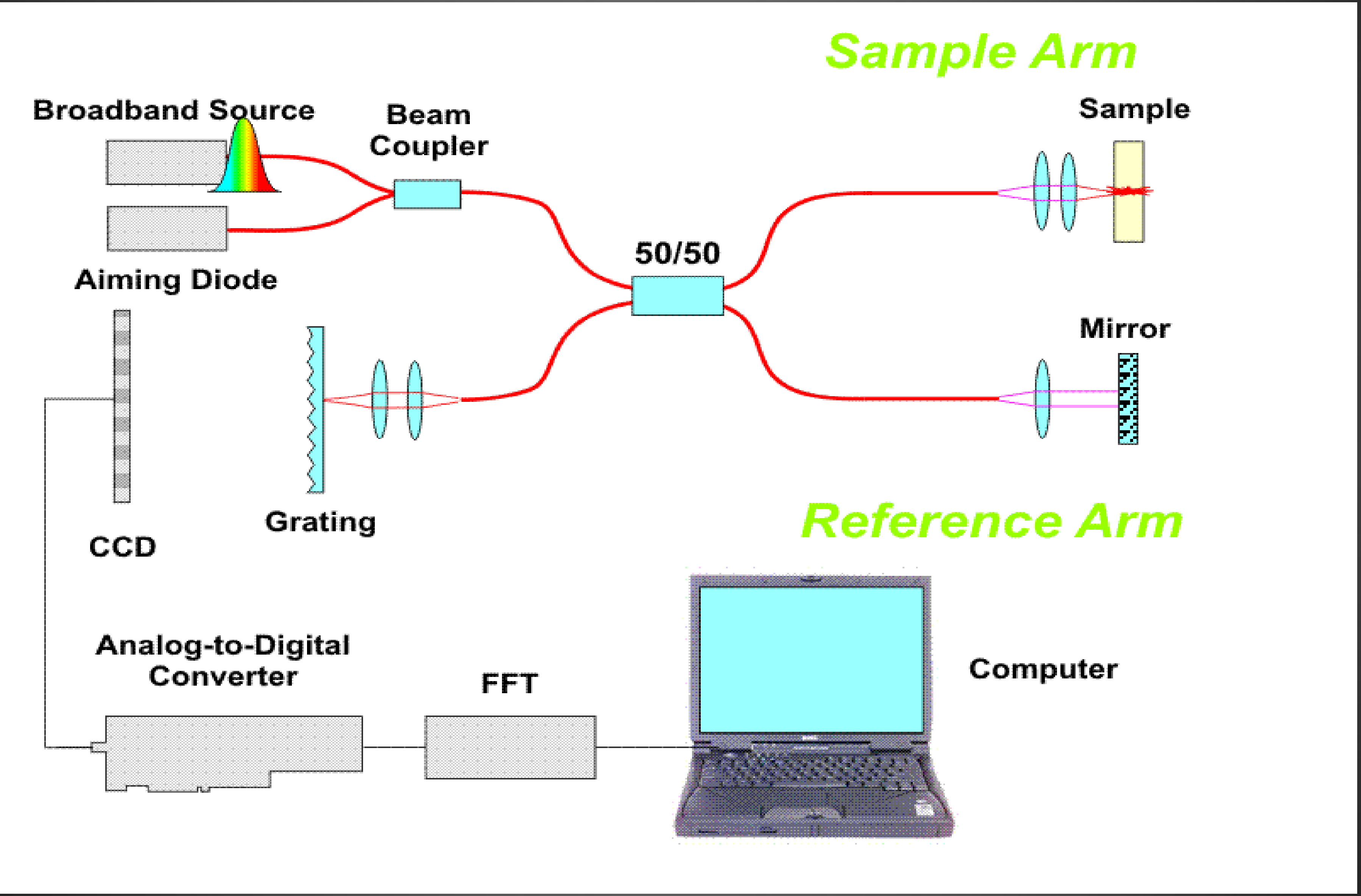
J. Fujimoto, 2008

Time-Domain OCT



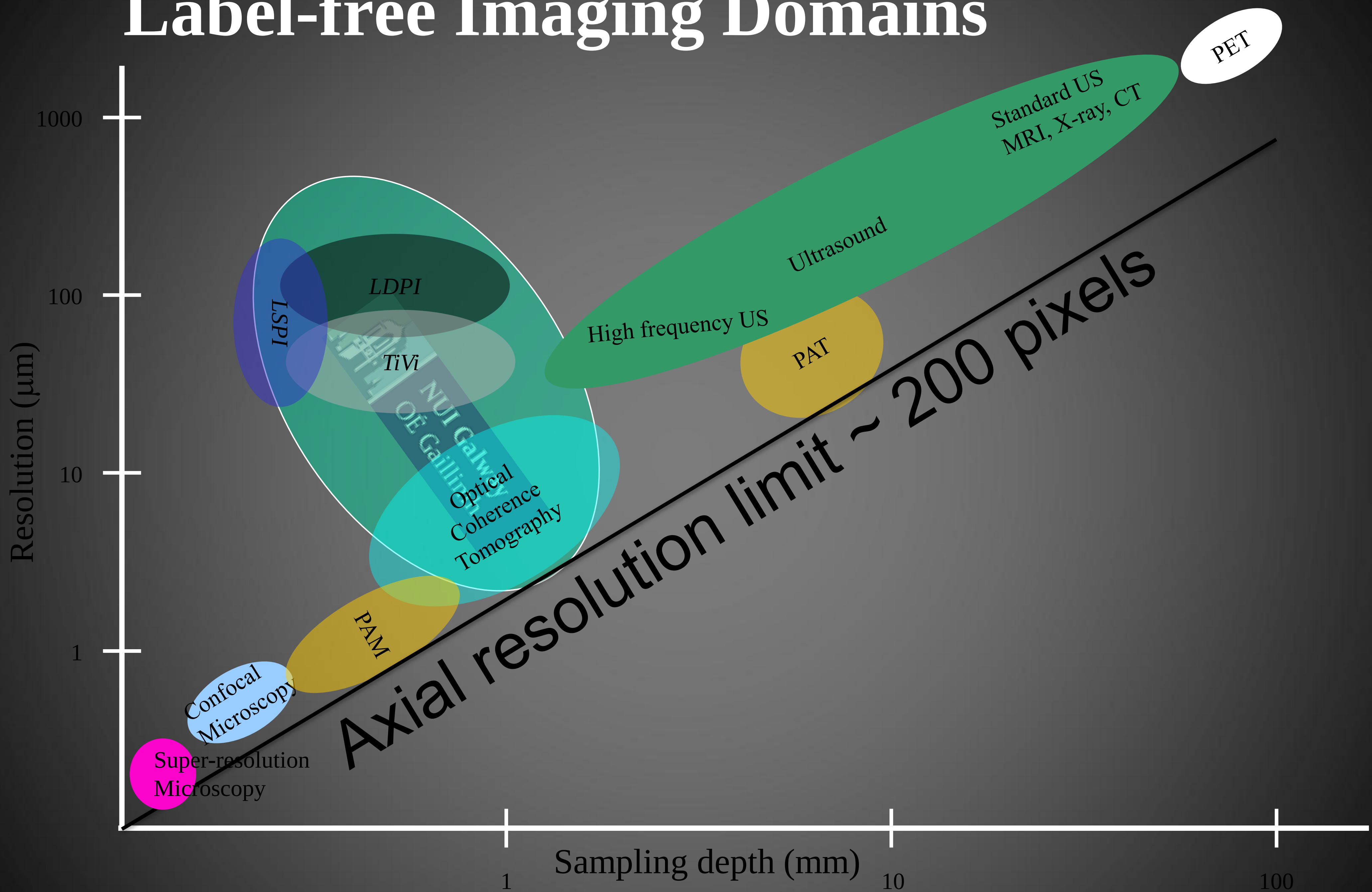
Slides from de Boer and Larin

Fourier-Domain OCT



Slides from de Boer and Larin

Label-free Imaging Domains



Optical Coherence Tomography

- OCT uses low coherence interferometry to produce a two or three dimensional image of optical scattering from internal tissue microstructures.
- OCT can provide both micro structural and functional information with high resolution and sensitivity
  - High resolution (2-15 μm)
  - 3D imaging in scattering tissue (2-3 mm)
  - Non invasive – “Optical Biopsy”