New developments in Non-invasive Biomedical Optics

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Non-invasive
Biomedical Optics

- optical (or optically-based) techniques and instruments,
- to extract physiologically relevant information,
- from measuring physical quantities in tissue,
- in a non-invasive way, *i.e.* from the outside of the body,
- avoiding oppressing the patient;
- quantities are:

  optical or opto-acoustical characteristics of the tissue sample
  (scattering, absorption, fluorescence data, molecular composition
  (with Raman), or velocity of blood cells or temperature etc.)
Optical properties of tissue and blood

(Reduced)
Scattering coefficient:
• $\lambda = 580$ nm:
  Dermis: 3 mm$^{-1}$
  Blood: 1 …
• $\lambda = 850$ nm:
  Dermis: 1 …
  Blood: 0.5 …
In this talk:

- **oximetry**

- **optical tomographic methods:**
  1. optical coherence tomography
  2. orthogonal polarization spectral imaging
  3. transillumination tomography:
     - time-of-flight, high-frequency modulation, continuous-wave
  4. photoacoustics

- **dynamic scattering: laser-Doppler:**
  1. laser-Doppler perfusion monitoring and imaging
  2. self-mixing laser-Doppler blood flowmetry
Oximetry

Oxygen Saturation: \[ S_aO_2 = \frac{c_{HbO_2}}{c_{HbO_2} + c_{Hb}} \]

Preferred wavelengths:
- 660 nm (red) ; \( \approx \)0 absorption by HbO\(_2\)
- 940 nm (IR) ; \( \approx \) equal absorption by Hb and HbO\(_2\)
Oximetry

Pulse oximetry: measuring pulsatile and constant blood flow.

Contributions to absorption:

From pulsatile part of arterial blood

From arterial blood

From venous blood

From tissue

Theory: Lambert - Beer law:
\[ I(d) = I(0).\exp (-\mu_a d) \]
\[ \frac{\Delta I(d)}{I(d)} = \Delta \left( \frac{\ln I(d)}{I(0)} \right) = -\mu_a d \]
\[ \frac{\Delta I_R}{I_R} = \frac{\Delta I_{IR}}{I_{IR}} = \frac{\mu_{a,R}}{\mu_{a,IR}} \]

Experiment:
\[ \frac{\Delta \ln I_R}{\Delta \ln I_{IR}} = \frac{\Delta I_R / I_R}{\Delta I_{IR} / I_{IR}} = \frac{(AC / DC)_R}{(AC / DC)_{IR}} = \frac{R}{IR} \]
\[ \Rightarrow \frac{R}{IR} = \frac{\mu_{a,R}}{\mu_{a,IR}} = \frac{c_{Hb}}{c_{HbO_2} + kc_{Hb}} ; \quad k \approx 1. \]

\[ Sa_{O_2} = \frac{c_{HbO_2}}{c_{HbO_2} + c_{Hb}} \]
Oximetry

Reflection Pulse Oximetry
Dual-wavelength probe (*)

Position of the probe at fetal head

(*) Courtesy: R. Graaff, Academic Hospital Groningen, Netherlands
Oximetry

Fetal scalp calibration against blood samples

Courtesy: R. Graaff, C. Dassel, Academic Hospital Groningen, Netherlands
Oximetry

Heart-cycle fluctuations in red vs. infrared signal, measured at index finger of healthy subject. Left vs. right panel: without / with pressure on the probe. More “red” means: less saturation.

Courtesy: R. Graaff, C. Dassel, Academic Hospital Groningen, Netherlands
Imaging methods for hidden structures in turbid media (tissue)

- C(M) : (confocal) microscopy
- OCT: optical coherence tomography
- OPS: orthogonal polarization spectral imaging
- PA: photoacoustics
- TOF: time-of-flight tomography
- FM: frequency-modulated tomography

![Diagram showing depth resolution vs depth for different imaging methods: OCT/OPS, PA: NIR, PA: green, TOF/FM, (C)M.]

Depth [µm] vs Depth [mm]

Depth resolution [µm] vs Depth [mm]
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Optical Tomographic Methods:
1. Optical Coherence Tomography

Interferometer setup:
- Detector will record only when signals from reference mirror and from sample overlap
- Scanning mirror enables depth resolution ($\approx 10 \, \mu m$)
- Maximum depth $\approx 1.0 \, mm$

Wave package of a short-coherence light source
Optical Tomographic Methods:
1. Optical Coherence Tomography

Thoracic Aorta

Width [mm]

Depth [mm]

[dB]

-90
-95
-100
-105
-110

histology

Optical Tomographic Methods:
1. Optical Coherence Tomography

Lesion in intima vessel

OCT-image

Histology

Birefringe microscopy

Optical Tomographic Methods:
1. Optical Coherence Tomography

Rat Esophagus

Depth [mm]

Width [mm]

Optical Tomographic Methods:

1. Optical Coherence Tomography

Options:

- **Color Doppler OCT**
  (measures blood velocity profiles)

- **Elastographic OCT**
  (measures blood shear rates)

- **Polarization OCT**
  (measures birefringe effects in tissue layers)
Optical Tomographic Methods: 1. Optical Coherence Tomography

Option: Color Doppler OCT measures velocity $V_s$ of blood cells, flowing under angle $\theta$ with direction of incident laser beam.

Doppler frequency $= \frac{2 V n \cos \theta}{\lambda}$

Detector Current:

Doppler frequency = frequency difference.

Optical Tomographic Methods:
1. Optical Coherence Tomography

Doppler OCT in intact *in vivo* Hamster skin tissue

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Optical Tomographic Methods: 2. Orthogonal Polarization Spectral Imaging

Analyzer and polarizer orthogonal

About 10 scattering events needed for complete de-polarization.

Green light: preferentially absorbed in blood cells (⇒ shadow view)

View field ≈ 1 mm ∅

Maximum Depth ≈ 0.5 mm
Optical Tomographic Methods:
2. Orthogonal Polarization Spectral Imaging

Capillary structure from under tongue of healthy person.

Rolling and sticking leukocytes

Mathura K and Ince C (2000)
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Optical Tomographic Methods: 3. Photon-transillumination methods

Time-of-flight: emerging photons delayed by scattering

Frequency modulation: phase lagging due to path length
Optical Tomographic Methods: 3. Photon-transillumination methods

Either the time-of-flight or the phase/modulation depth differences between the scattered signal and the reference signal are measured.

Light transport preferentially using glass fibers.
Optical Mammography using pulsed time-of-flight technique.
Left: left breast with invasive ductal carcinoma and blood vessels;
Right: healthy breast

(courtesy prof. H. Rinneberg, Physikalisch Technische Bundesanstalt Berlin)
Optical Tomographic Methods: 3. Photon-transillumination methods

Optical Mammography:
Patient · 50 years old · invasive ductal carcinoma
(pT2, G2) 4 x 4 x 2.5 cm³; cyst Ø 3 cm; breast thickness 6.2 cm / 5.7 cm

late time window

early time window

λ = 785 nm

(courtesy prof. H. Rinneberg, Physikalisch Technische Bundesanstalt Berlin)
Optical Tomographic Methods:
3. Photon-transillumination methods

Diffusion theory:
homogeneous infinite slab with spherical inhomogenity

Fit to measured distributions
(10 distributions simultaneously)

Results: in tumor: absorption $\approx 2.5$ x as high, scattering $\approx$ tissue values
(courtesy prof. H. Rinneberg, Physikalisch Technische Bundesanstalt Berlin)
Optical Tomographic Methods: 3. Photon-transillumination methods

Oxygen saturation in tissue

\[ \mu_a = \left( c_{HbO_2} \varepsilon_{HbO_2} + c_{Hb} \varepsilon_{Hb} \right) \cdot \ln 10 + \kappa_{H_2O} \cdot \mu_{a,H_2O} \]

\[ \rightarrow \geq 3 \text{ wavelengths necessary; or ...} \]

- 2 wavelengths (670 nm, 785 nm)
- assumption: \( \kappa_{H_2O} = 30\% \)
- slab with sphere \( \rightarrow \mu_{a,tumor}(\lambda), \mu_{a,normal}(\lambda) \)

\[ S = \frac{c_{HbO_2}}{c_{Hb} + c_{HbO_2}} \]

<table>
<thead>
<tr>
<th>Normal tissue</th>
<th>Tumor tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>( c_{tHb} ) (( \mu \text{mol/l} ))</td>
<td>( c_{tHb} ) (( \mu \text{mol/l} ))</td>
</tr>
<tr>
<td>S (% )</td>
<td>S (% )</td>
</tr>
<tr>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>63</td>
<td>55</td>
</tr>
</tbody>
</table>

(courtesy prof. H. Rinneberg, Physikalisch Technische Bundesanstalt Berlin)
Optical Tomographic Methods: 3. Photon-transillumination methods

High-frequency modulation provides phase and path information

Differences in composition of the tissue sample causes differences in phase $\Delta \phi$ as function of frequency

Actual accuracy at 100 MHz: 1 % in scattering $\rightarrow$ 10-30 mM glucose
Expected at 1 GHz: factor 10 better
Optical Tomographic Methods: 3. Photon-transillumination methods

High-frequency modulation provides phase and path information, => Scattering and absorption data => localisation of inhomogeneities

Source fiber  Detector fibers
19-25 mm

Blood vessel: diameter 2 mm, depth 2 mm

$\mu_s' = 0.15 \text{ mm}^{-1}$

19 and 25 mm source/detector separation
High (0.5 mm$^{-1}$) and low (0.15 mm$^{-1}$) scattering of "blood" in vessel
Smaller phase measured means higher phase observed due to negativity of instrumental phase

- 25 mm distance, high scattering
- 25 mm distance, low scattering
- 19 mm distance, high scattering
- 19 mm distance, low scattering
Optical Tomographic Methods: 3. Photon-transillumination methods

Frequency modulation:
Phased Array Detection

Dashed line: null-signal plane

Homogeneous sample: detectors no difference

Inhomogeneities present: phase difference detected
Optical Tomographic Methods: 3. Photon-transillumination methods

Phased-array breasts scan, showing presence of inhomogeneities
(Courtesy: B. Chance, University of Pennsylvania, School of Medicine, Philadelphia, USA)
Optical Tomographic Methods: 3. Photon-transillumination methods

Functional Near-Infrared Imaging

Unlike with X-rays, Photon transillumination enables to measure in reflection, thus avoiding oppressing the patient.

(Courtesy: B. Chance, University of Pennsylvania, School of Medicine, Philadelphia, USA)
Optical Tomographic Methods: 3. Photon-transillumination methods

Functional Near-Infrared Imaging
7 x 7 cm maps of the left motor cortex area during 20 sec finger tapping (rate 2 Hz)

O$_2$Hb

HHb

(courtesy: W. Colier & B. Oeseburg, Physiology, Univ. Med. Centre Nijmegen)
Non-invasive Biomedical Optics

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  3. transillumination tomography:
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Optical Tomographic Methods: 4. Photoacoustic Imaging

- light pulse is absorbed in blood cell
- adiabatic heating
- pressure pulse emerging (≈ 1500 m/s)
- detection at tissue surface

Depth:
- Green light: ≈ 0 - 9 mm
- Near-infrared: ≈ 0 – 30 mm

Depth resolution: ≈ 10 μm
Optical Tomographic Methods:
4. Photoacoustic Imaging

Bipolar PA-signal generated by a spherical Gaussian Source

\[ \tau_a = \frac{R_\sigma}{\nu} \]

\[ \tau_e = \sqrt{\tau_a^2 + \tau_l^2} \]

\[ P(r,t) = -P_{\text{max}}(r)\sqrt{e} \frac{t-\tau}{\tau_e} \exp\left\{-\frac{1}{2} \frac{(t-\tau)}{\tau_e}\right\} \]

\[ P_{\text{max}}(r) = \frac{\beta E_a}{2\sqrt{e(2\pi)^{3/2} c_p \tau_e^2 r}} \]

\[ \tau = \frac{r}{\nu} \]
Optical Tomographic Methods:
4. Photoacoustic Imaging

Diameter: \( \approx 10 \, \mu m \)
(compared with a 12 \( \mu m \) blue polystyrene sphere)
detection distance: \( \approx 1.7 \, mm \) (\( = 1.15 \, \mu s \times 1500 \, m/s \))
medium: water/PBS

Two erythrocytes
Optical Tomographic Methods: 4. Photoacoustic Imaging

Double-Ring Detector
One-fiber illumination

Disk-shaped Detector
Ring illuminator
Optical Tomographic Methods:
4. Photoacoustic Imaging

Directional Sensitivity

Disk Detector:
FWHM : Depth
1 : 5

Ring Detector:
FWHM : Depth
1 : 70
Optical Tomographic Methods: 4. Photoacoustic Imaging

A human hair in chicken breast tissue.

Depth: \( \approx 6 \text{ mm} \)  
\( \approx 4 \mu\text{s} \times 1500 \text{ m/s} \)
Optical Tomographic Methods:
4. Photoacoustic Imaging

Sample: 5 mm thick chicken breast tissue in water
Image: 663 mm, inside sample, 35% isosurface threshold
Vessels: 3 Nylon capillaries, 0.28 - 0.40 mm diameter
Absorber: Evans Blue, flowing, $a = 300 \text{ cm}^{-1}$
Detection: at $Z = 0 \text{ mm}$, 51 x 51 points, 0.15 mm spacing
Optical Tomographic Methods: 4. Photoacoustic Imaging

Reconstruction of hidden objects

Material: carbon threads (10 μm) on transparent sheets, in 10% Intralipid-10% (resembles human tissue scattering)
Optical Tomographic Methods: 4. Photoacoustic Imaging

Depth resolution: \( \approx 10 \mu m \)
Vascular tree from a branching epigastric artery of a rat. 
Ex-vivo; medium: intralipid 1 % (≈ tissue). 
Depth (Z-coord.) ≈ 5 mm ; indicated in figure. 
Laser power 532 nm, 2mJ/pulse through fiber Ø 600 μm. 
Depth resolution / lateral resolution: 10 / 100 μm respectively.
Optical Tomographic Methods: 4. Photoacoustic Imaging

Measuring tissue thickness above bone

Photoacoustic line scan of a finger, perpendicular to the finger axis.

The surface of the tissue and the reflection from the bone can clearly be distinguished.

In between structures are seen that may be blood vessels.

- 100 scan lines; scan step 150 μm
- ~6 mJ/cm² at skin surface
Optical Tomographic Methods: 4. Photoacoustic Imaging

Line scan across finger nail

1: nail top
2: nail bottom
3: finger skin
4: reflections or blood vessels
# Optical Tomographic Methods: 4. Photoacoustic Imaging

<table>
<thead>
<tr>
<th></th>
<th>Green 550 nm</th>
<th>NIR 850 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dermis</td>
<td>blood</td>
</tr>
<tr>
<td><strong>Absorption</strong></td>
<td>0.03</td>
<td>32</td>
</tr>
<tr>
<td><strong>coefficient [1/mm]</strong></td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td><strong>Scattering coefficient (reduced) [1/mm]</strong></td>
<td>≈ 10</td>
<td>≈ 30</td>
</tr>
<tr>
<td><strong>Absorption Contrast</strong></td>
<td>1000</td>
<td>100</td>
</tr>
<tr>
<td><strong>Penetration into tissue [mm]</strong></td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td><strong>Applications</strong></td>
<td>Cutaneous perfusion, Wound healing, Diabetes, Vascular malformations, Skin tumours</td>
<td>Cerebral perfusion, Muscular perfusion, Mammography, Angiogenese</td>
</tr>
</tbody>
</table>
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  1. **laser-Doppler perfusion monitoring and imaging**
  2. self-mixing laser-Doppler blood flowmetry
Dynamic scattering:
1. Laser Doppler Perfusion

Scattering at moving cells causes Doppler frequency shift
Dynamic scattering:
1. Laser Doppler Perfusion Monitoring

LD spectra of finger tip upon occlusion of upper arm

Flux = 1\textsuperscript{st} moment of power spectrum

Concentration = 0\textsuperscript{th} moment

\langle \text{velocity} \rangle = \text{flux/concentration}
Dynamic scattering:
1. Laser Doppler Perfusion (Monitoring)

New in LD-monitoring:
- Pulsed LD-monitoring
- Depth-sensitive LD-monitor on-a-chip
- Standardization of instruments and procedures
- Low-coherent depth-sensitive LD-monitor
Dynamic scattering:
1. Laser Doppler Perfusion (Monitoring)

**Pulsed LD-monitoring**
- higher powers
- larger measuring distances
- larger depths

LD-spectrum: 0-20 kHz
Pulse frequency: 50 kHz.
Pulse width / period = 0.24
\[
\frac{4.7}{20}
\]
Dynamic scattering:
1. Laser Doppler Perfusion (Monitoring)

LD-monitor on-a-chip provides miniature depth-sensitive sensor.

Green: photodiode rows

Blue/red: electronics: amplifiers/multiplexers

Red dot in yellow area: VCSEL- laser diode
Dynamic scattering:
1. Laser Doppler Perfusion (Monitoring)

Low-coherent depth-sensitive LD-Monitoring

The reference mirror selects the depth in the sample from which a coherent Doppler-shift signal will be measured.
Dynamic scattering:
1. Laser Doppler Perfusion (Monitoring)

Monte-Carlo photon transport simulations

Tissue considered as
- Layered structure
- Including blocks, spheres, tubes, cones, mirror planes
- Varying scattering and absorption coefficients
- Varying scattering functions (Mie, Rayleigh,…)
- Reflection and refraction
- Rectangular or ring-shaped detectors
- Scattered, transmitted or absorbed photons detected
- Doppler spectra: varying velocity profiles
Dynamic scattering:
1. Laser Doppler Perfusion (Imaging)

Superficial perfusion of the dorsal side of the hand, characters UT written using muscular balm.

Upper left: perfusion, not normalized;
Upper right: DC-reflection from tissue;
Lower left: perfusion, normalized with DC;
Lower right: perfusion, normalized with $\text{DC}^2$. 
Dynamic scattering:  
1. Laser Doppler Perfusion (Imaging)

Typically the highest perfusion is in the boundary around the ulcer, in inflammatory skin and in granulating tissue inside the ulcer area.

Dynamic scattering:
1. Laser Doppler Perfusion (Imaging)

The effect of micro-trauma

The dialysis fibre probe tip causes hyperperfusion

Insertion of a micro-dialysis fibre into the skin.

No hyperperfusion at the point of introduction because the skin is anesthetized.

After 30 minutes the hyperperfusion is reduced.

(Courtesy: Lisca Sweden)
Dynamic scattering:
1. Laser Doppler Perfusion (Imaging)

Basal cell carcinoma

Before treatment

Neo-vascularisation in tumour area.

After treatment

Immediately

Inflammatory response.

One week

Inflammatory response with excessive perfusion.

8.5 months later

Back to normal.

(Courtesy: Lisca Sweden)
Dynamic scattering:
1. Laser Doppler Perfusion (Imaging)

Day 1: wound creation

Day 4

Day 7

Day 10

Day 13

Black inc marker on skin

Crust formation in centre of wound

Crust off

Perfusion returning to normal

Flow-Maps of a Healing Wound

(Courtesy: Lisca Sweden)
Dynamic scattering:
1. Laser Doppler Perfusion (Imaging)

The healing process of a burn wound

Day 2
- Reduced perfusion in burnt areas.
- Increased perfusion in surrounding skin.

Day 13

Day 28
- Towards normalisation.

(Courtesy: Lisca Sweden)
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Dynamic scattering:
2. Self-mixing Laser-Doppler Flowmetry

Principle:

- laser light reflected/scattered by moving blood cells,
- partly back-reflected into laser cavity,
- with Doppler-shifted frequency,
- in cavity: mixing with “original” light,
- Doppler signal results,
- can be measured with photodiode
Dynamic scattering:
2. Self-mixing Laser-Doppler Flowmetry

Branching in iliac artery of healthy pig

Cut-off frequency at 400 kHz corresponds with a velocity of 16 cm/s.
(Independent measurement using an electromagnetic probe: 14.5 ± 1.0 cm/s)
(L. Scalise & F.F.M. de Mul).
Imaging methods for hidden structures in turbid media (tissue)

C(M) : (confocal) microscopy

OCT: optical coherence tomography

OPS: orthogonal polarization spectral imaging

PA: photoacoustics

TOF: time-of-flight tomography

FM: frequency-modulated tomography
Non-invasive Biomedical Optics

Conclusions:

• several techniques available, at various depths,
  to 1 mm : OCT, OPS
  to 10 mm : PA – green
  to 50 mm : TOF, FM, PA-infrared.

• resolution / depth ≈ 1 / 10 (with OCT, OPS, PA: 1/100)
Non-invasive Biomedical Optics

In our lab (UT – Applied Physics – Biomedical Optics)

• Laser-Doppler Monitoring / Imaging
  (chip design, calibration, speckle optics, low-coherence)
• Photon transillumination  (…1.8 Ghz frequency modulation)
• Photo-acoustic imaging  (blood in tissue -> mammography)
• Compound concentration determination by light scattering
• Monte-Carlo light transport simulations
Non-invasive Biomedical Optics

the end