Medical Fluorescence Imaging
-
Tutorial

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Aim with the presentation

Motivate

– the use of in vivo fluorescence imaging for:
  • Early cancer diagnostics
  • Identification of tumour boundaries
  • Assessment of blood vessels
  • Visualisation of lymph vessels
  • Treatment response assessments

– the use of laser parameters necessary:
  • Average power required
  • Pulsed mode
  • Robustness, compactness and user friendliness
Clinical use of fluorescence imaging

In-vivo kinetics of inhaled ALA-Induced PpIX fluorescence in bronchial tissue

Fluorescence-guided resection of malignant gliomas


Fluorescence angiography

Fluorescein angiographic features before and after PDT for choroidal neovascularization (CNV)

Hikichi et al., RETINA 21 (2001)
Fluorescence detection of malignancies in the urinary bladder

The Storz D-Light system
M. Kriegmair *et al.* Munich
Fibre endoscope

Coherent fibre bundle

Object

Lens system

Image focused on fibres

Principal of light through a coherent fibre bundle

Image viewed by the observer

Coherent fibre bundle

http://www.atomic.physics.lu.se/biophotonics
Cross-section of the distal end

- Image channel
- Guided light fibre bundle
- Biopsy channel
- Guided light fibre bundle
- Air/fluid channel
Endoscopical PDT – in combination with fluorescence detection

Vocal fold carcinoma in situ

Clinical, laser-based, fluorescence diagnostics and photodynamic therapy of a malignant tumour.
Insert: Endoscopic view of the vocal cords (left) and the same view with a colour-coded fluorescence image superimposed (right). The yellow area indicates the presence of a squamous cell carcinoma.

ENT-Department with Dr. Roland Rydell
Lund University Hospital
The diagnosis

White-light mode

Fluorescence video mode

Imaging using the Storz D-light system for larynx diagnostics

Important developments: Multispectral analysis
Simultaneous white light and fluorescence

Typical fluorescence spectra

![Fluorescence spectra graph]

Intensity

400  500  600  700nm

Normal

Malignant
Multivariate analysis

Decomposition:

\[ X = t_1 + t_2 + E \]

Principal components

Residual

Model

Partial Least Squares (PLS): Principal components chosen for best correlation with y-variable (histopathology)
Diagnostic potential

To be useful for diagnostic purpose separation is often required on individual point basis

- 89% sens., 83% spec.

Predicted y value

- True neg. (19)
- False neg. (3)
- False pos. (4)
- True pos. (25)

Hyperplasia & Metaplasia

Low grade dysplasia & High grade dysplasia
Biological applications of \textit{in vivo} fluorescence imaging

- Animal models widely used in biomedical research
- More than 90\% of animals used are mice
- Non-invasive imaging studies very valuable tool
- Allow non-invasive \textit{longitudinal and dynamic studies}

GFP Mouse

Hoffman and Yang
Nature Protocols
(2006)

Sharma \textit{et al.}
Am. J. Physiol. (2007)
2008 Nobel Prize in Chemistry for the discovery and development of the green fluorescent protein, GFP

Osamu Shimomura
Japanese citizen, born 1928 in Japan. Ph.D. in organic chemistry 1960, from Nagoya University, Professor emeritus at Marine Biological Laboratory (MBL), Woods Hole, MA, USA and Boston University Medical School, MA, USA.

Martin Chalfie

Roger Y. Tsien
US citizen, born 1952 in New York, NY, USA. Ph.D. in physiology 1977 from Cambridge University, UK. Professor at University of California, San Diego, La Jolla, CA, USA since 1989.
What is GFP?

A small naturally occurring protein which is highly fluorescent. GFP consists of 238 amino acids, linked together in a long chain. This chain folds up into the shape of a beer can. Inside the beer can structure the amino acids 65, 66 and 67 form the chemical group that absorbs UV and blue light, and fluoresces green.

*Aequorea victoria – a jellyfish in the Northern Pacific Ocean*
Baby mice fathered by mice receiving a donation of spermatogonial stem cells from mice expressing green fluorescent protein. Only half the baby mice show the green color. This is because each spermatogonial stem cell has only one copy of the green fluorescent protein gene. When the spermatogonial cell divides, only half the cells that result from it have the gene for green fluorescent protein.
Fluorescence Proteins in all colours – Roger Y. Tsien

Using DNA technology, various amino acids in different parts of GFP were exchanged.
What is fluorescence?

Molecular energy

Absorption of light

Fluorescence emission
Fluorescence induced by exposure to ultraviolet light in vials containing various sized Cadmium selenide (CdSe) quantum dots. This is a file from the Wikimedia Commons, http://en.wikipedia.org/wiki/Fluorescence
Fluorescent minerals. This is a file from the [Wikimedia Commons](http://en.wikipedia.org/wiki/Fluorescence), http://en.wikipedia.org/wiki/Fluorescence
Fluorescence

Tissue autofluorescence

337 nm excitation

405 nm excitation

Fluorescence intensity [a.u.]

Wavelength (nm)

Collagen
Elastin
NADH
Carotene

Protoporphyrin IX

Protoporphyrin IX

af Klinteberg et al. (1999)
Fluorescence diagnostics of skin tumour following ALA administration

1. Administration of ALA
   - ALA

2. Production of PpIX
   - Protoporphyrin IX

3. Diagnostics
   - Haem
   - Iron
   - ALA
   - Protoporphyrin IX
   - 635 nm
   - Blue laser

4. Treatment
   - Tumour
   - Red laser
## Tumour localising agents

### Photosensitisers (PDT)

<table>
<thead>
<tr>
<th>Photosensitisers (PDT)</th>
<th>RED Absorption Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematoporphyrin derivative (HpD), (Photofrin)</td>
<td>630 nm</td>
</tr>
<tr>
<td>δ-aminolevulinic acid (ALA)</td>
<td>635 nm</td>
</tr>
<tr>
<td>Mesotetrahydroxyphenylenchlorin (mTHPC), (Foscan)</td>
<td>652 nm</td>
</tr>
<tr>
<td>Tin Etiopurpurin (Pyrlytin)</td>
<td>660 nm</td>
</tr>
<tr>
<td>Benzoporphyrin, (Verteporfin)</td>
<td>690 nm</td>
</tr>
<tr>
<td>Phthalocyanins</td>
<td>720 nm</td>
</tr>
<tr>
<td>Lutetium texaphyrin (Lutrin)</td>
<td>732 nm</td>
</tr>
<tr>
<td>Bacteriochlorophyll (Tookad)</td>
<td>760 nm</td>
</tr>
</tbody>
</table>
Background light interference

Without funnel

With funnel

Haj-Hossini et al. unpublished (2009)
Skin measurements

Haj-Hossini et al. unpublished (2009)
Ambient light shielding

Rigid metal tube
Non-transparent plastic funnel

Haj-Hossini et al. unpublished (2009)
Brain measurement

Healthy tissue

GBM

Astrocytoma

PpIX fluorescence

5 mm

15 mm

35 mm

Autofluorescence

5 mm

15 mm

35 mm

Anonymous12845, F
anon12845

US Linkoping
MR GEMSOW

2001.06.18
Acq: 0, Image: 28

SMEDBY

Pålsson et al. unpublished (2009)
MR and fluorescence spectra

Pålsson et al. unpublished (2009)
Fluorescence diagnostics

Point monitoring:
Whole spectrum in one small tissue site

Imaging:
Less spectral information but in larger area

http://www.atomic.physics.lu.se/biophotonics
Fluorescence spectrum

Intensity vs. Wavelength (nm)

Ratio = \frac{A}{B}

- Fitted curve
- PpIX peak
- Autofluorescence
Dermatological Multicolour Fluorescence imaging

Intensified CCD camera

White-light CCD camera

TAE
nBCC
sBCC

Fluorescence Intensity (a.u.)
Wavelength (nm)

Fluorescence image contrast

http://www.atomic.physics.lu.se/biophotonics
Multicolour Fluorescence Imaging

White light image

Digitally processed image
Spectraphos multispectral fluorescence imaging system

Multicolour fluorescence imaging

Rodent brain fluorescence following i.v. administration of ALA

Hyper Spectral Diagnostic Imager

Combines:
• video image
• reflectance scan
• fluorescence scan

Aim:
• interactive diagnostics
• integrated colposcope

Science & Technology Inc, USA
Halo naevus

16 year old male
Located at left waist
Pigmentented BC or malignant melanoma

83 years old female
located next to the eye
went for surgery
Diffuse Optical Tomography

Linear image reconstruction

Non-linear image reconstruction

X-Ray source → Image plate → Object

Light source → Detector → Object

Rotation → Rotation
Absorption and scattering
Absorption spectra of important tissue chromophores

"Therapeutic and diagnostic window"
Fluorescence-mediated tomography

Diagram:
- Light source
- Object
- Detectors
  - Rotation

http://www.atomic.physics.lu.se/biophotonics
Instrumentation from Lund Medical Laser Centre on its way to National Technical University in Athens

Collaboration with BioLitec, Bonn, Germany
Joint WP6 campaign
Instrumentation

During the last period we have spent much effort in evaluating the fluorescence imaging data from the measurement campaign in Jena.

http://www.atomic.physics.lu.se/biophotonics
Tomographic Reconstruction

Extinction coefficient

Fluorescence Spectra

Measure
Excitation light, 652 nm
Fluorescence light, 720 nm

Svenmarker et al. unpublished (2009)
In vivo non-invasive FosPeg Images

2D View

3D View

Svenmarker et al. unpublished (2009)
Novel nanoparticles as fluorescence markers

Fluorescence imaging of a rat leg

IPDT during radiation

Soto Thompson et al. JEPTO (2006)
Johansson et al. JBO (2007)
Measurements of light fluence

Interactive dosimetry – eliminates treatment failure

Soto Thompson et al. JEPTO (2006)
Johansson et al. JBO (2007)
Measurements of Sensitizer concentration

Assess sensitizer level using fluorescence
Fluorescence tomography of mTHPC concentration during prostate cancer PDT

Assessment of distribution of the photosensitiser mTHPC during photodynamic therapy of prostate cancer

Monitoring of PDT progress – prostate cancer

The Group

http://www.atomic.physics.lu.se/biophotonics

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