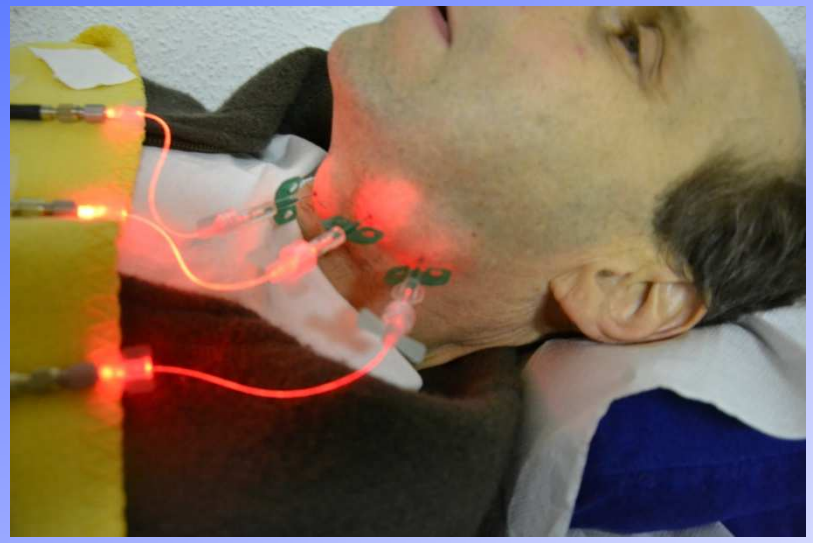
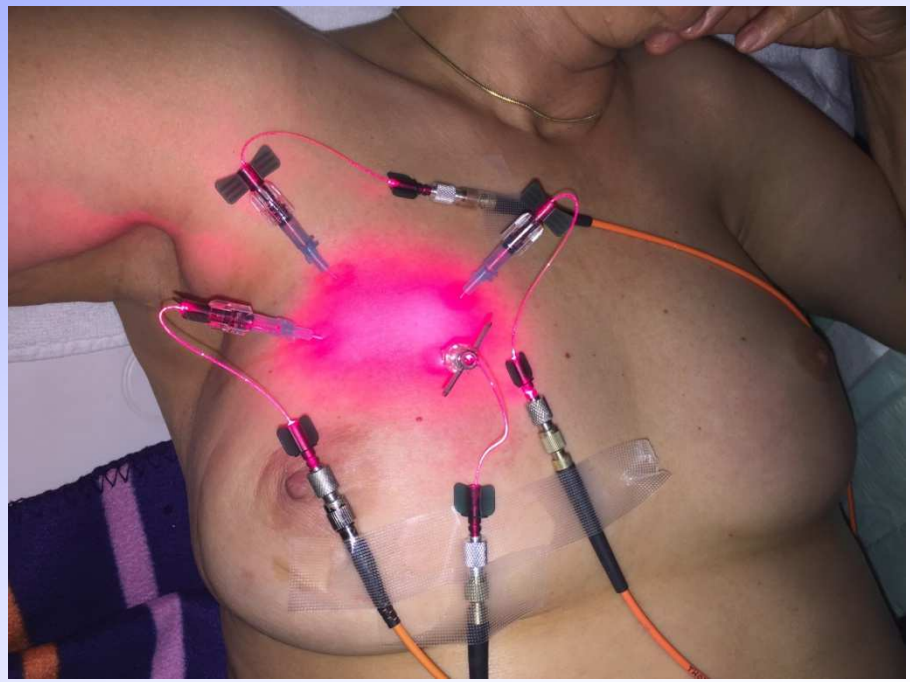
Michael H. Weber

New Methods and Laser Technology in Photodynamic Cancer Therapy





Topics

- Basics of mechanism of photodynamic therapy
- Different groups of photosensitizers
- PDT in dermatology with 5-Aminolaevulic acid
- PDT in combination with interstital and intravenous laser therapy
- Treatment examples and results
- New combinations of traditional photosensitizers with light sensitive chemodrugs and clinical results
- Immunotherapy for cancer

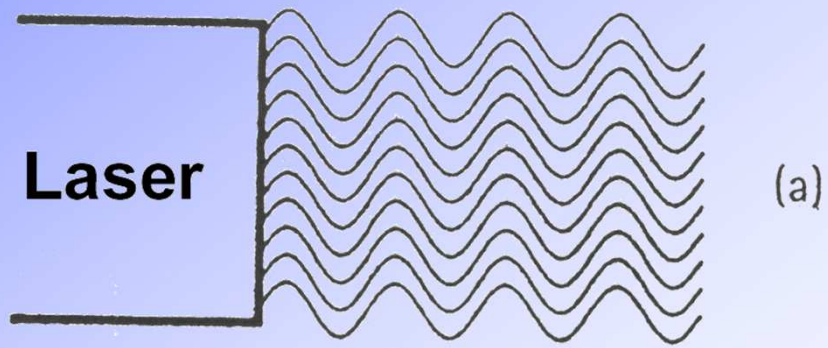
LASER
=

Light Amplification of
Stimulated Emission of
Radiation

Why Laserlight?

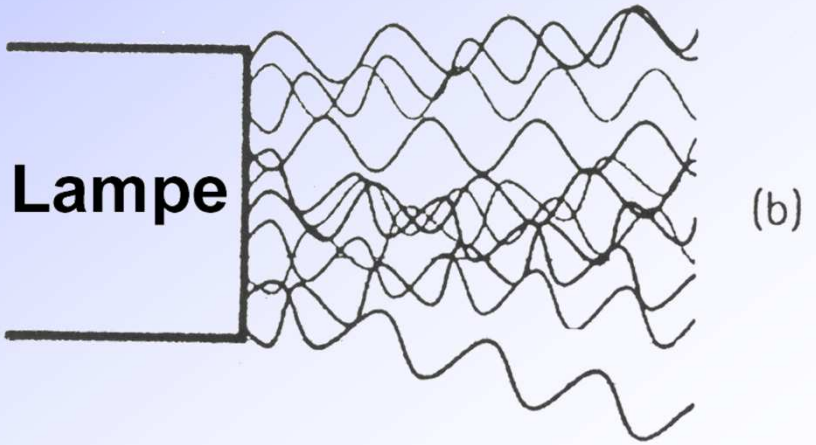
Laserlight

has a precise colour (Monochromasy) and is chracterized by an arranged photon stream (Coherence)

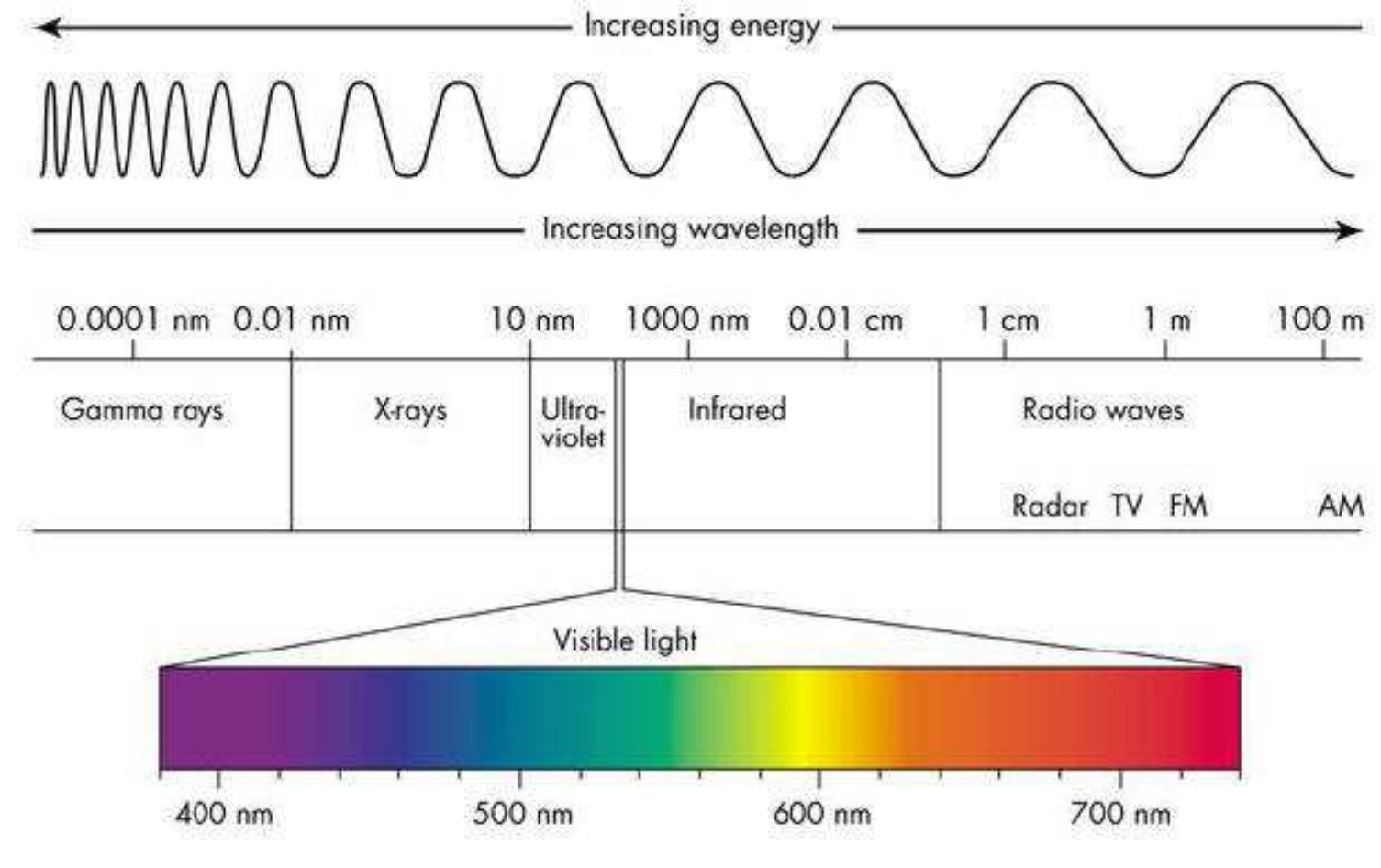


Conventional white light (bulb)

is a mixture of all colours, spreads in all directions



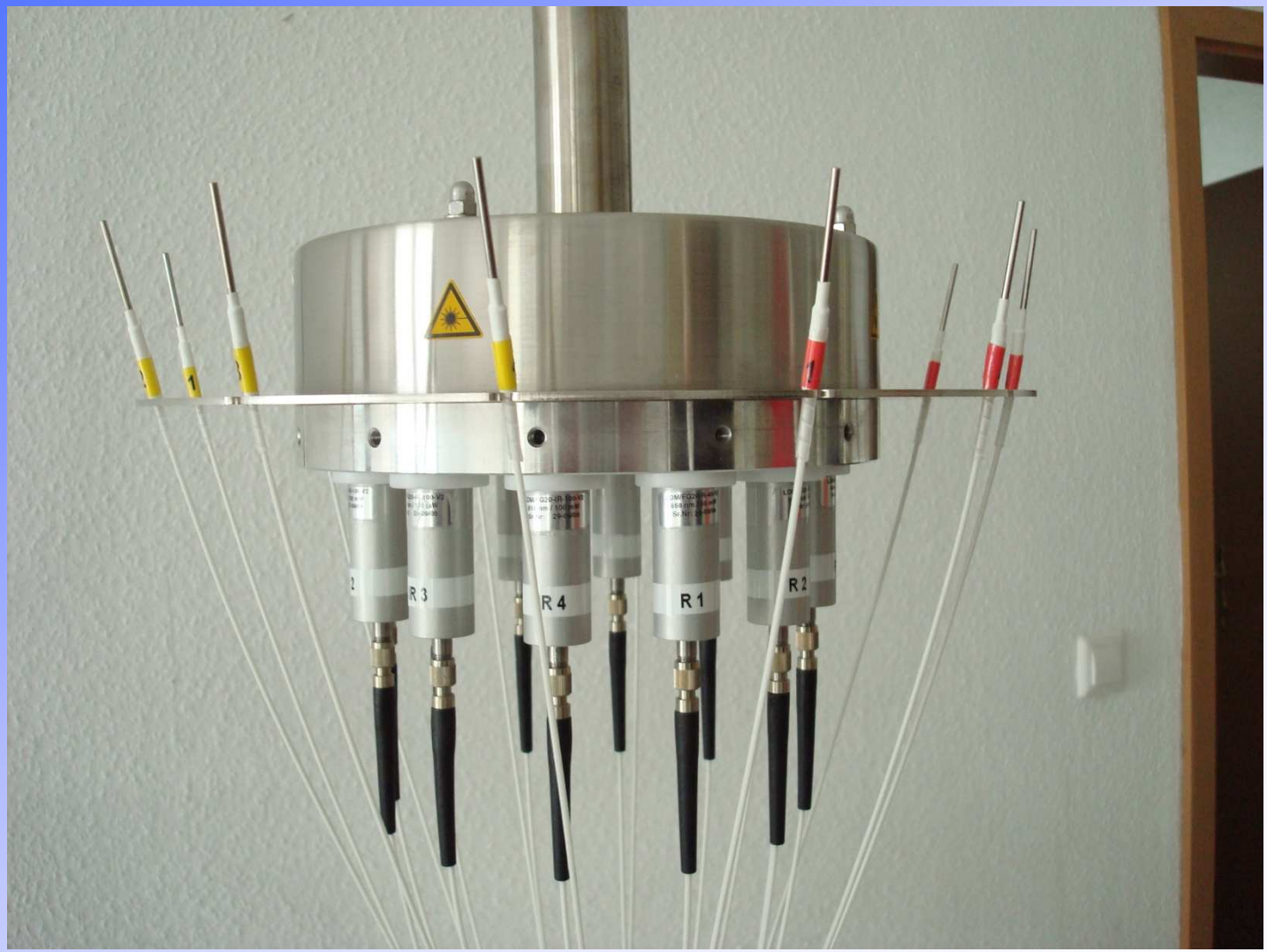
The electromagnetic spectrum



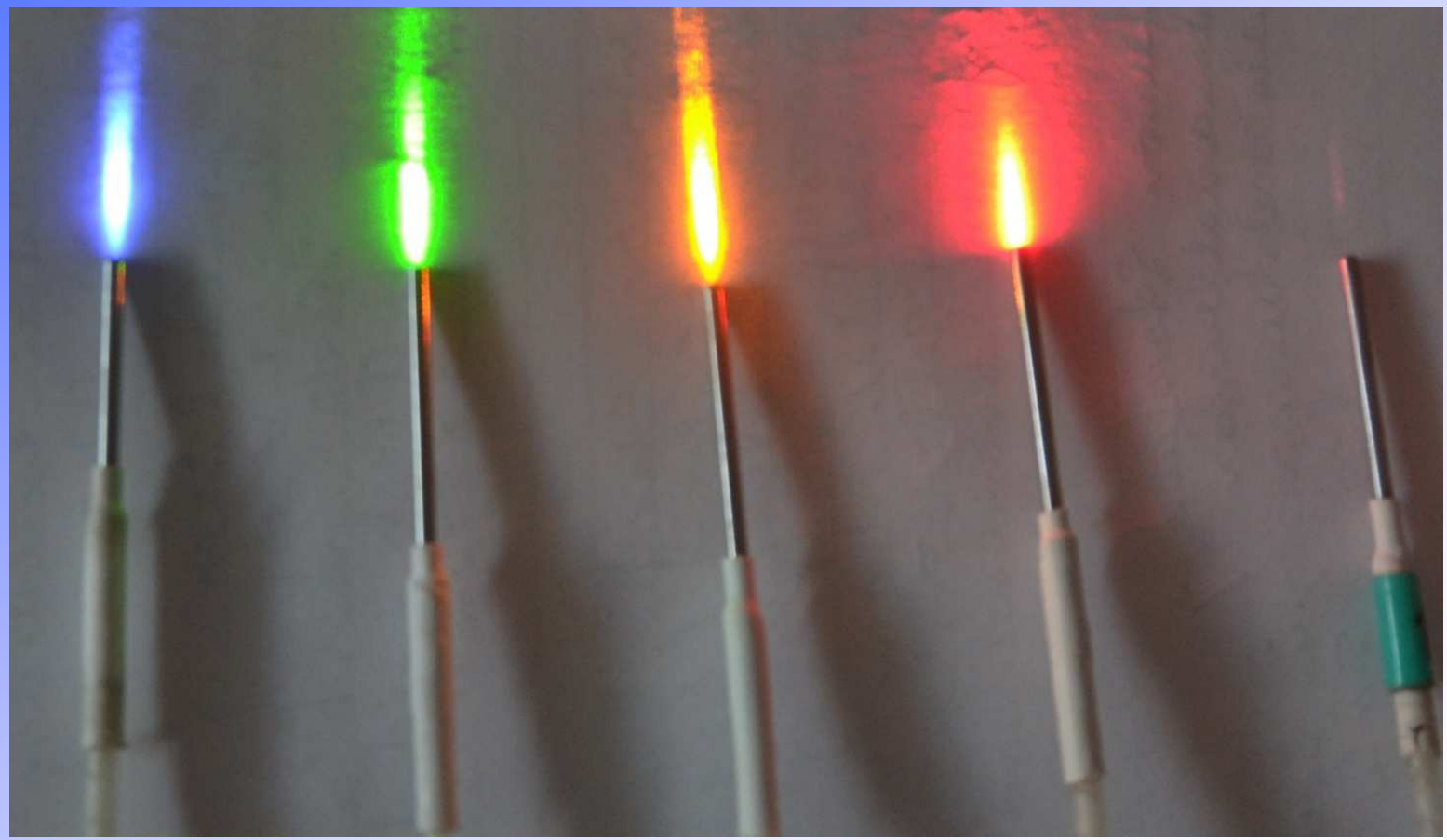
Fiberoptic laser systems and applications

Modern new fiberoptic Laser-Needle system for external laser therapy

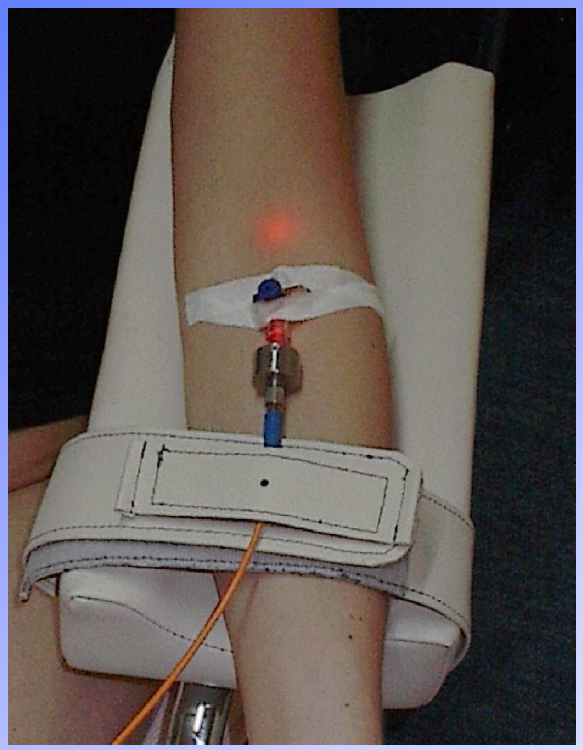
Modern new Laser-Needle system for external laser therapy

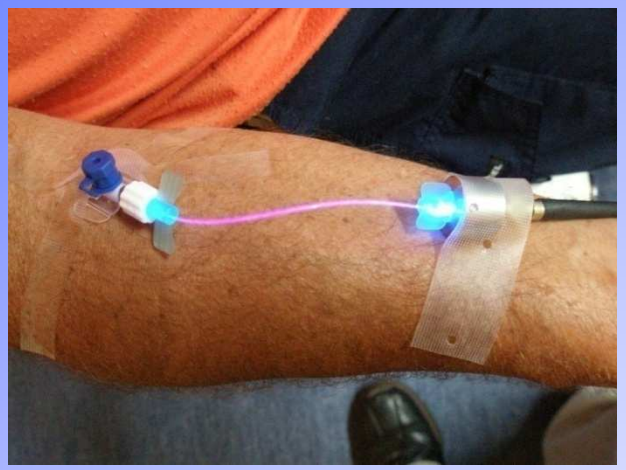


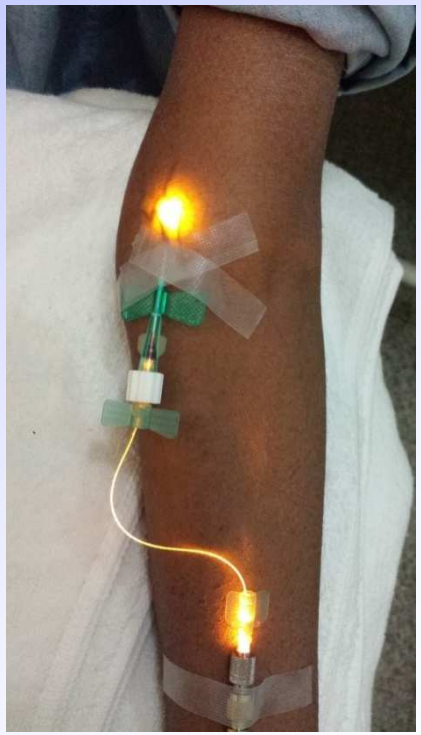
Laserneedles (non-invasive)



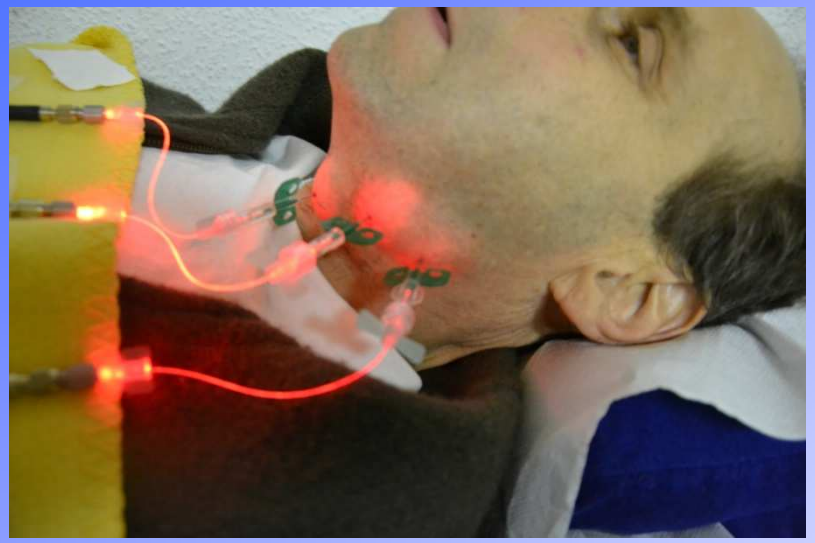
Intravenous laser therapy

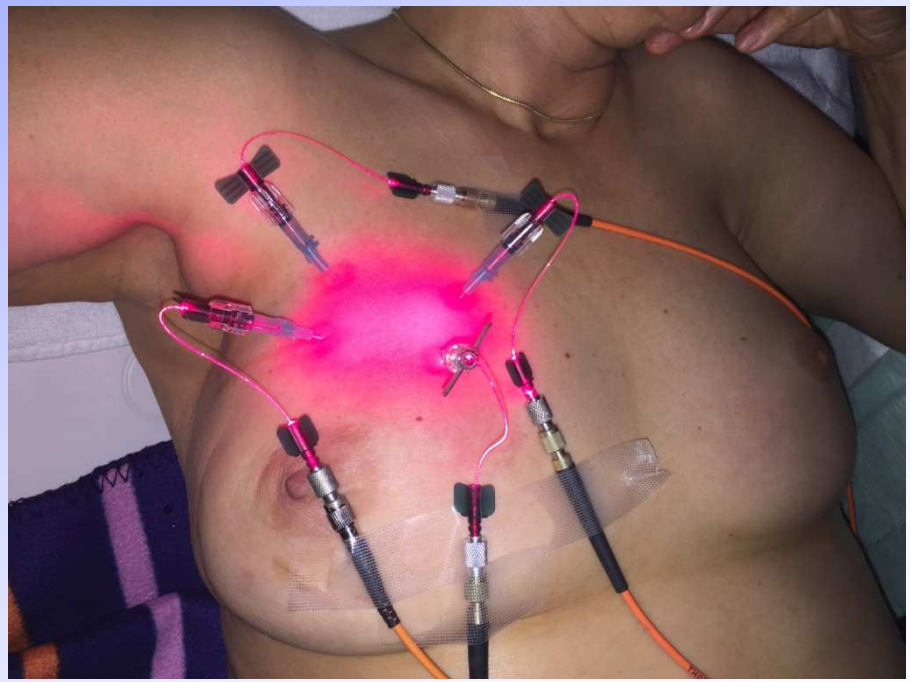




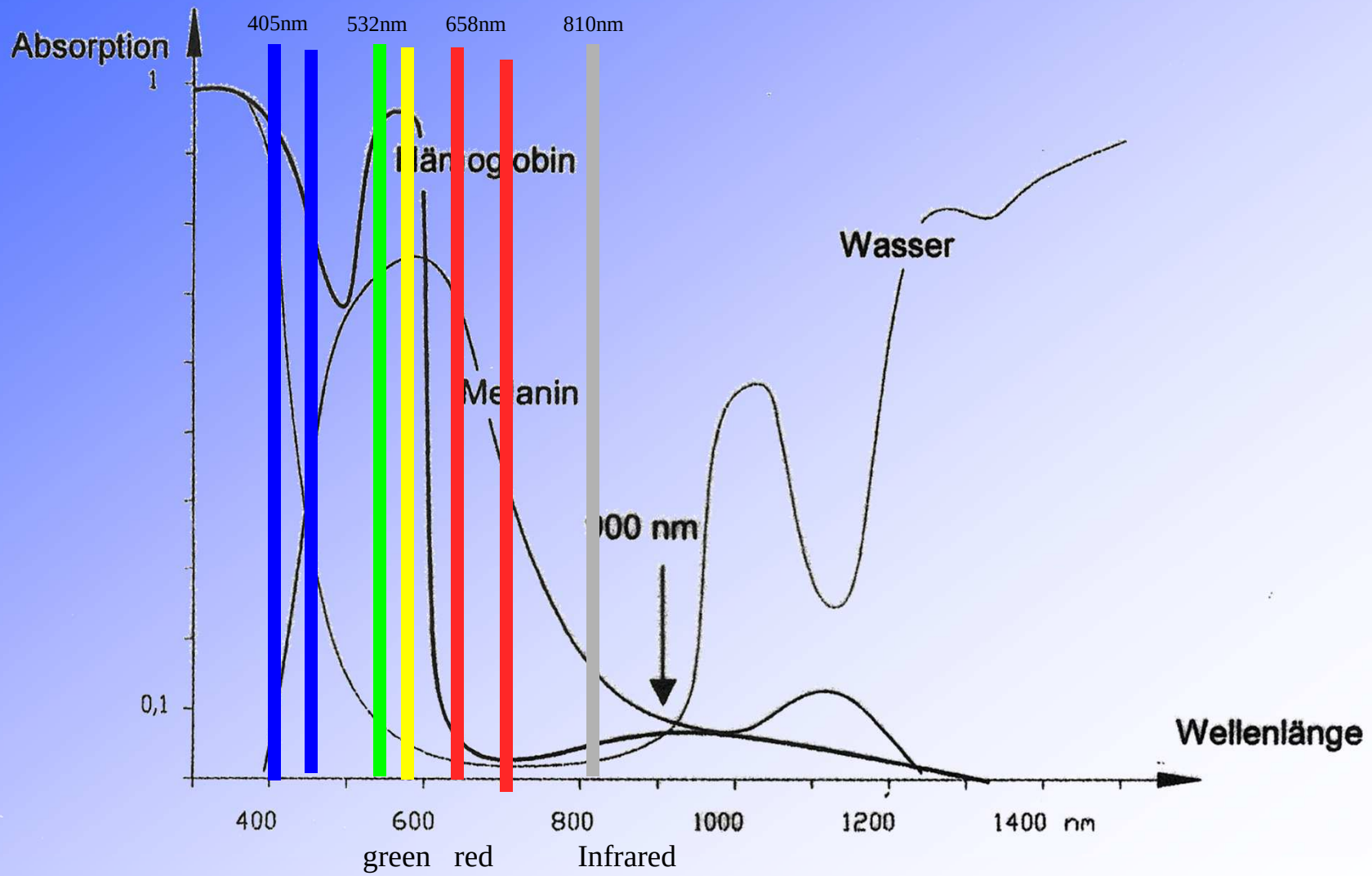


Interstitial photodynamic cancer therapy



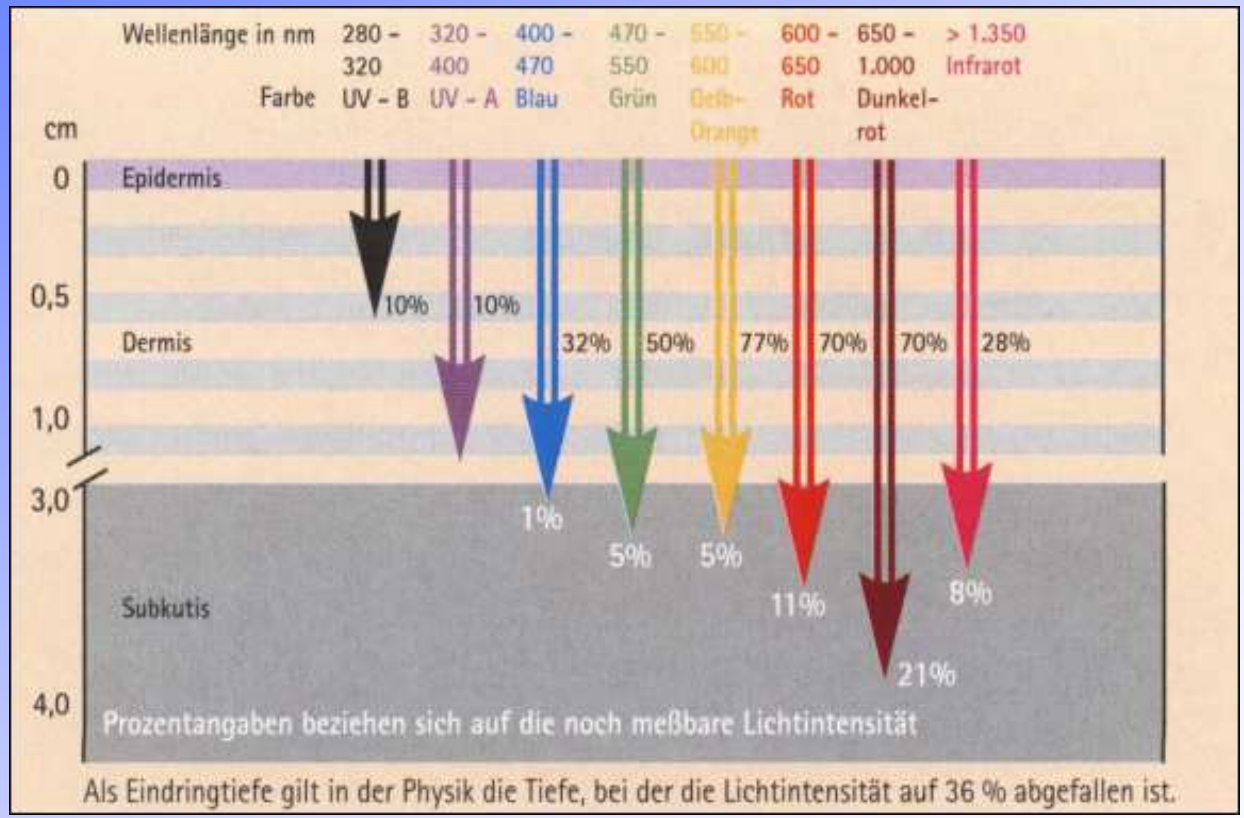


Absorption of laser light in biological tissue



Optical penetration depth of different wavelengths

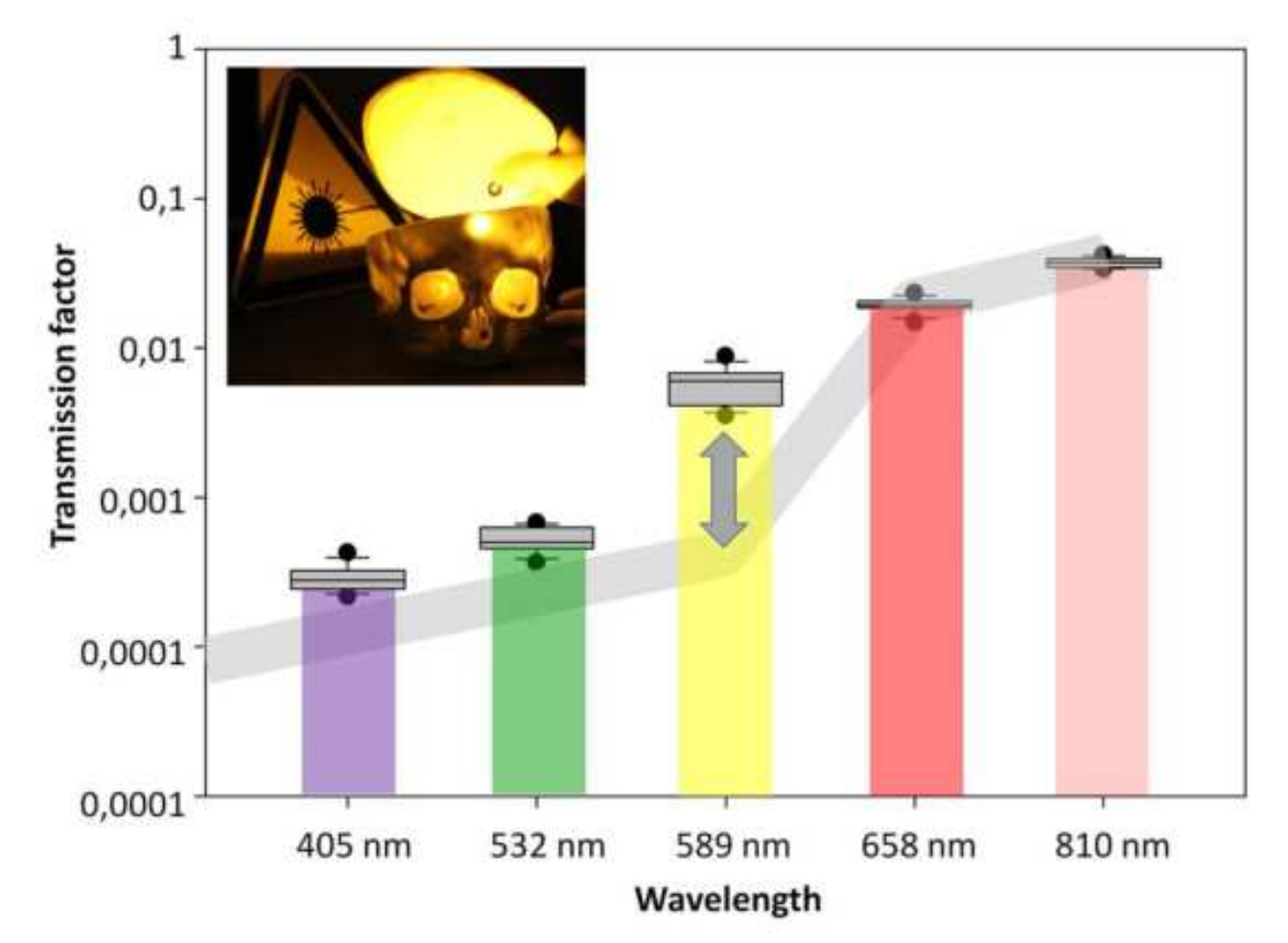
- *depends upon the wavelength*
- *Tissue penetration of blue laser very low, green laser ca. 5mm, red 3 cm, infrared 6 cm*



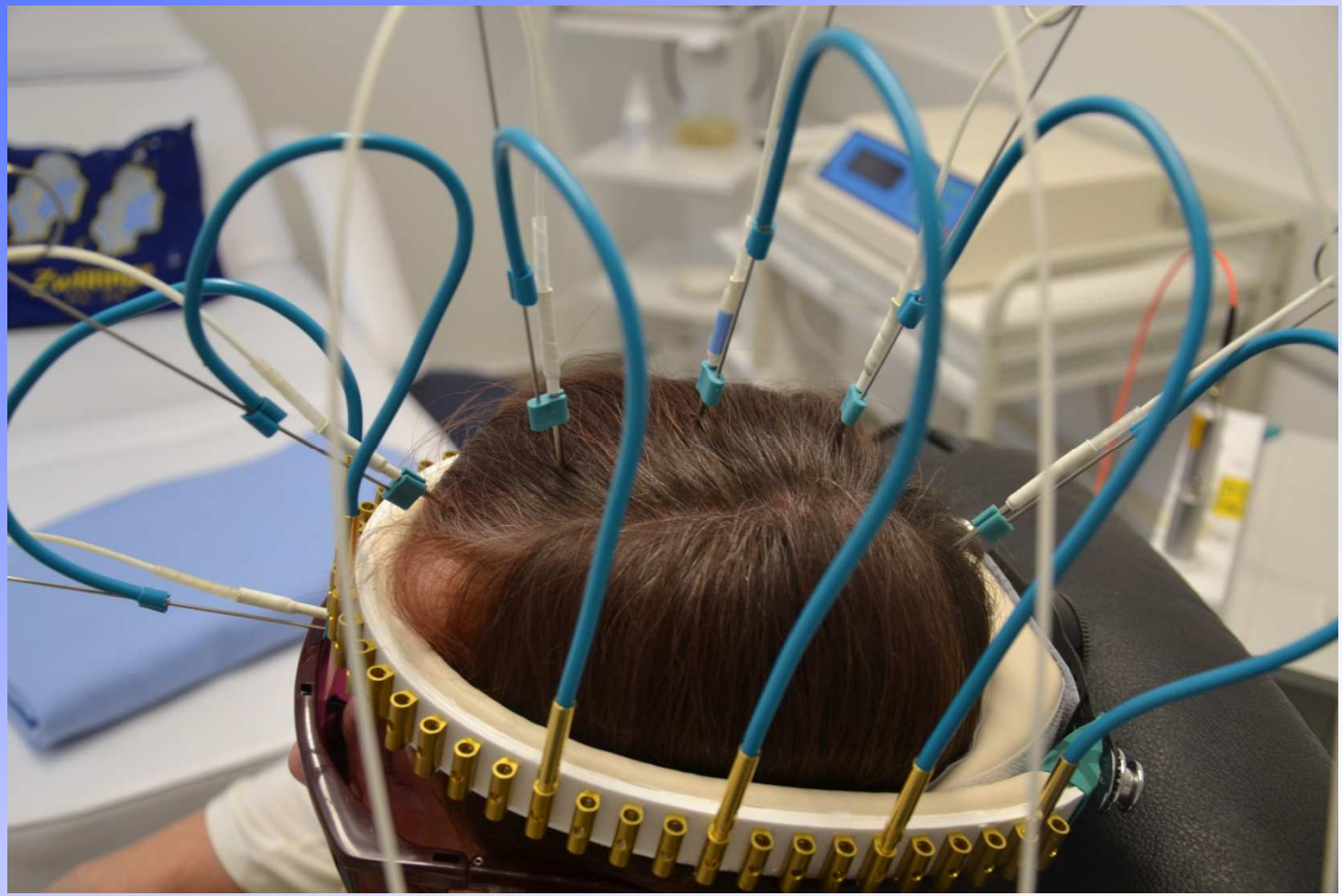
Bone penetration of infrared laser

Bild 8: Rotes Laserlicht wird im Gewebe gestreut, teilweise absorbiert, aber auch an absorbierenden Strukturen (Knochen) vorbeigeleitet. Hier wird ein Finger von einem 250 mW starken roten Laser (660 nm), und einem 400 mW starken IR Laser (830 nm) bestrahlt. In der Handfläche ist kaum rote, wohl aber etwas IR Transmission sichtbar. Mit einem empfindlichen Messgerät liegt eine jeweilig emittierte Leistung bei etwa 0,002 mW/mm² (Finger rot), 0,012 mW/mm² (Finger IR), 0,00001 mW/mm² (Hand rot) und 0,0004 mW/mm2 (Hand IR). Die IR Bilder sind mit einer Sony HDR-SR1E im (unbeleuchteten) Night-Shot Modus aufgenommen, die roten Bilder auch mit einer Sony DSC-H1. Hier erscheint elektronisch bedingt ein besonders helles rotes Licht orange bis gelblich.

Laser and brain (Litscher 2013)

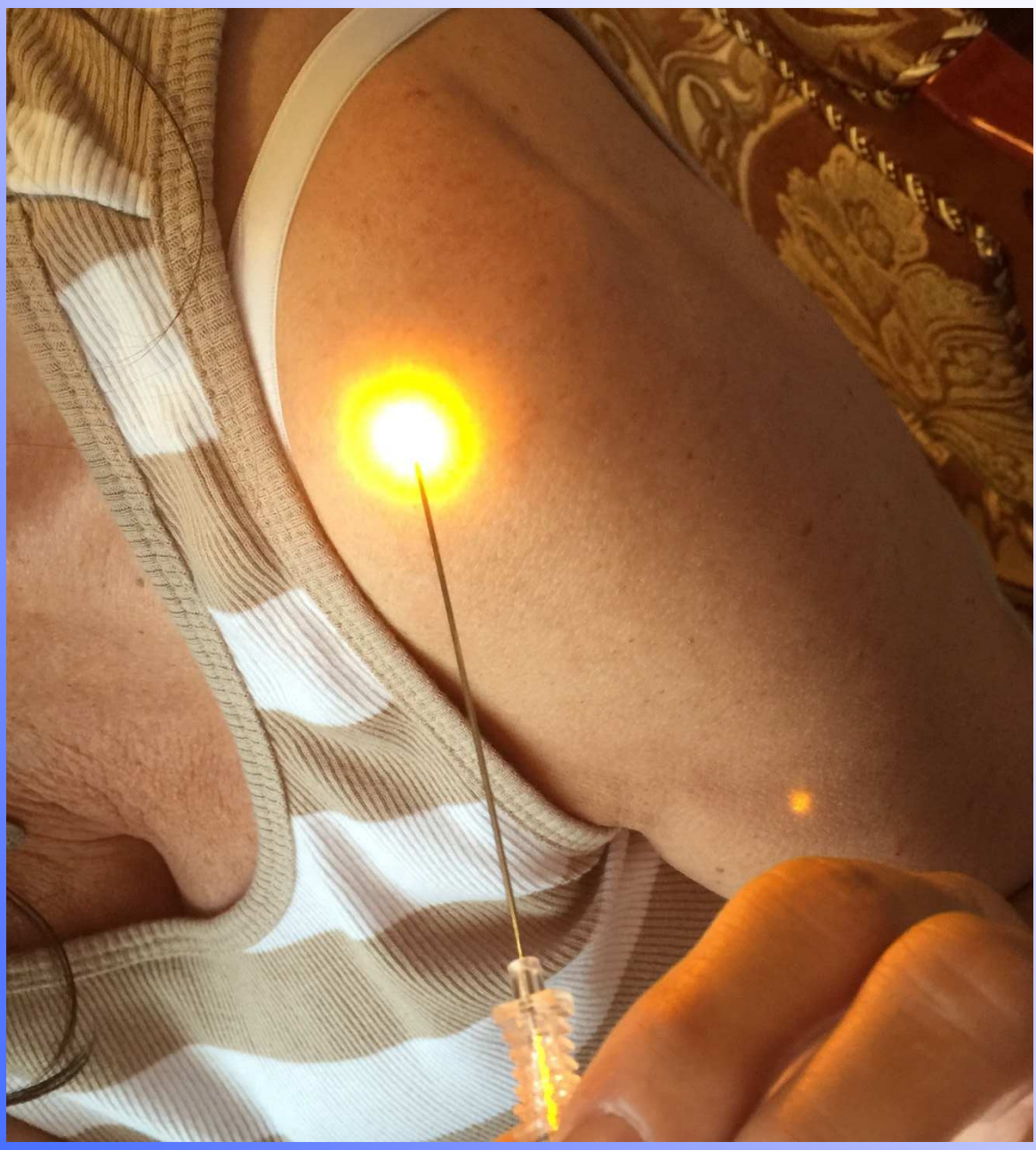


Transcranial infrared laser therapy

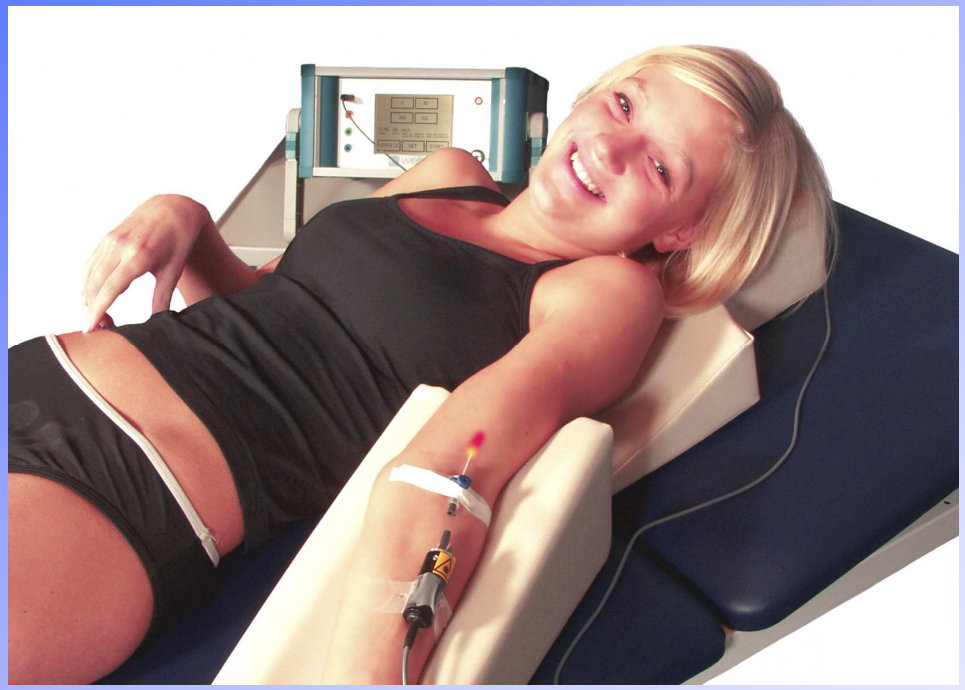


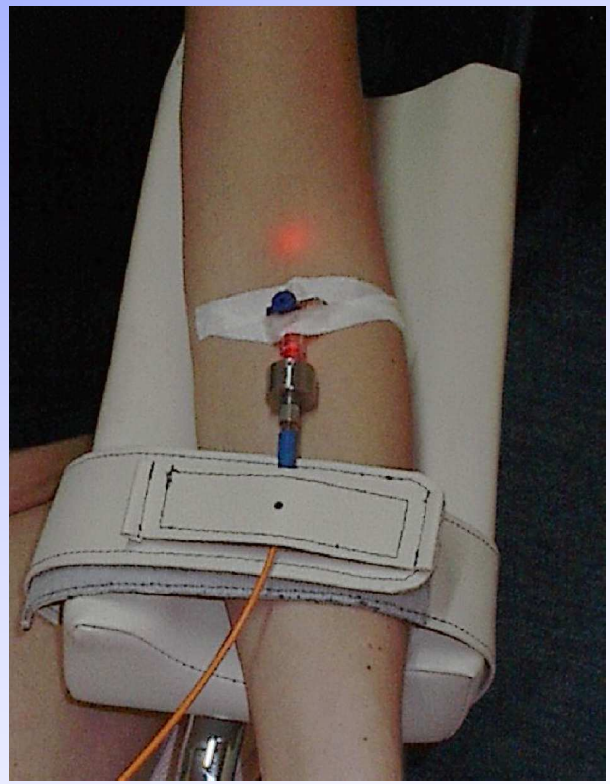
Interstitial fiberoptic laser therapy

Fiber-optic interstitial needle



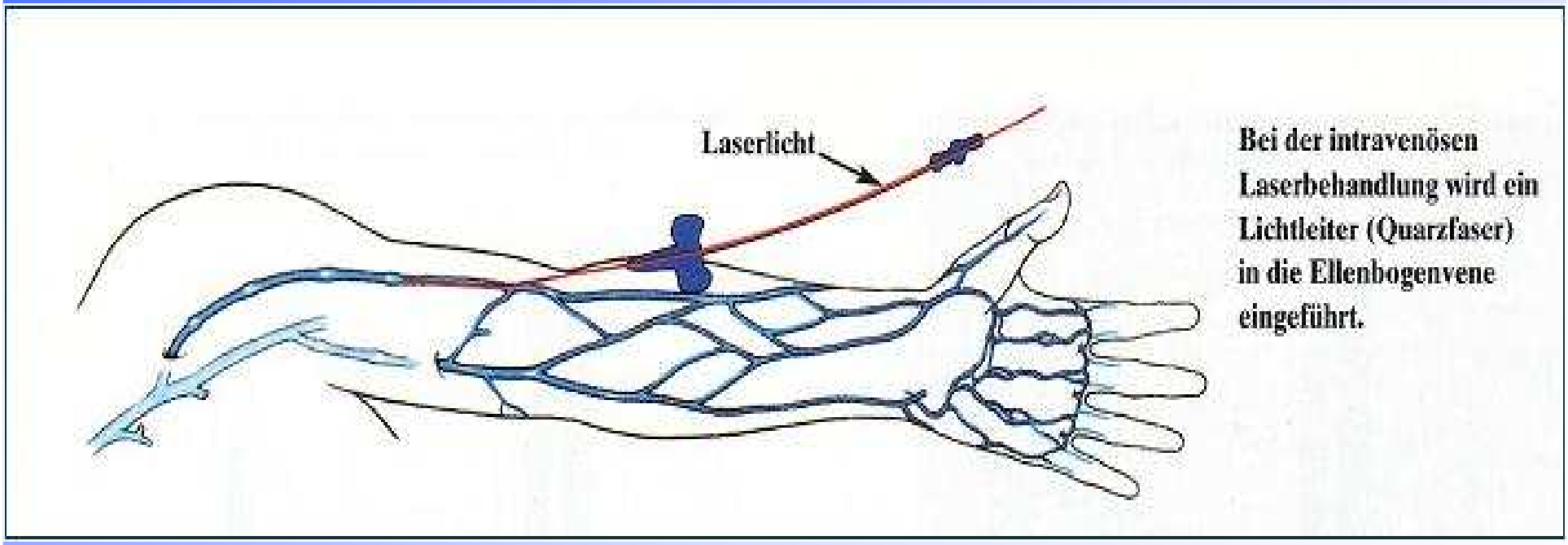
Intravenous Laser Blood Irradiation



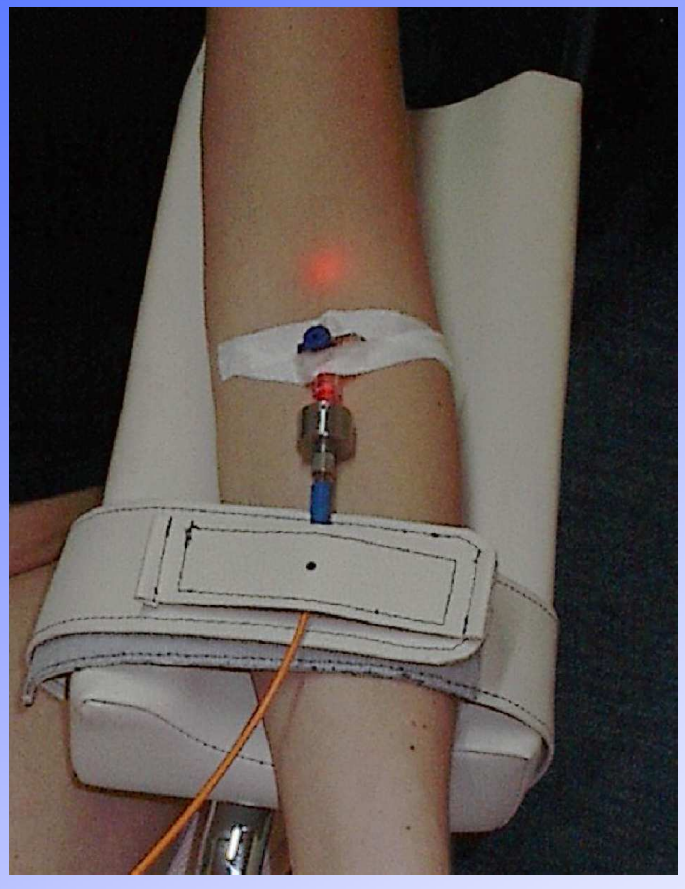


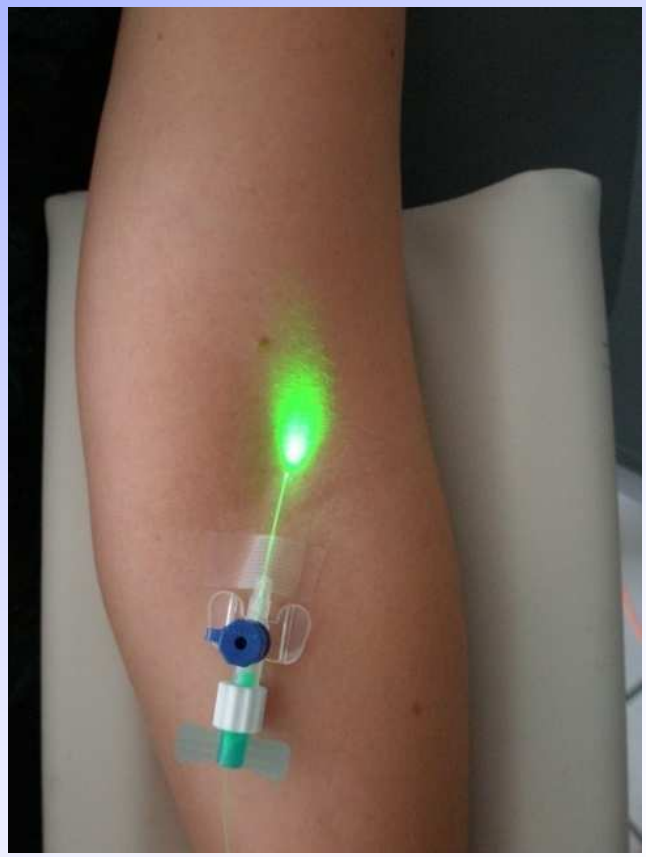
Intravenous laser therapy

-

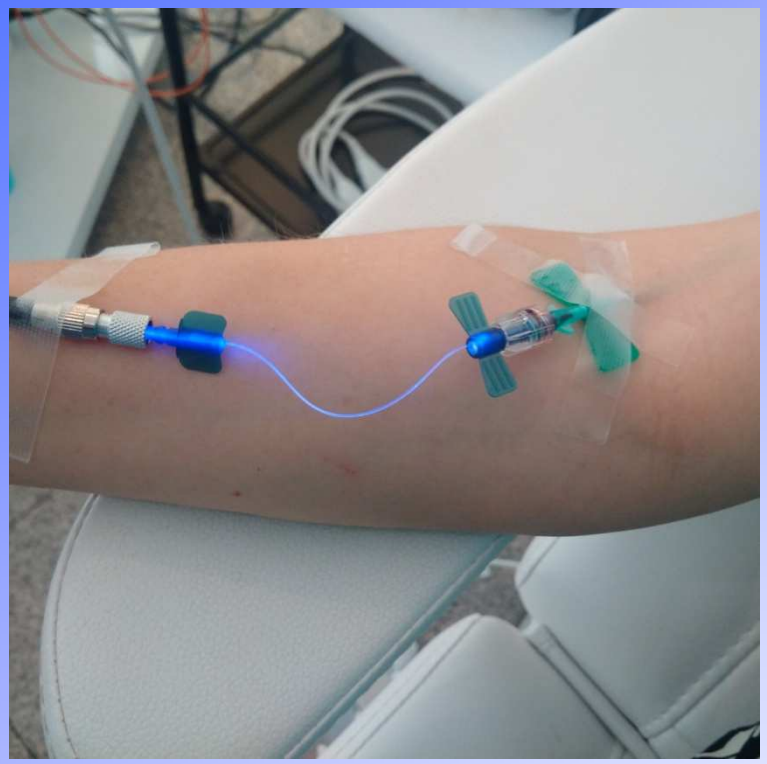


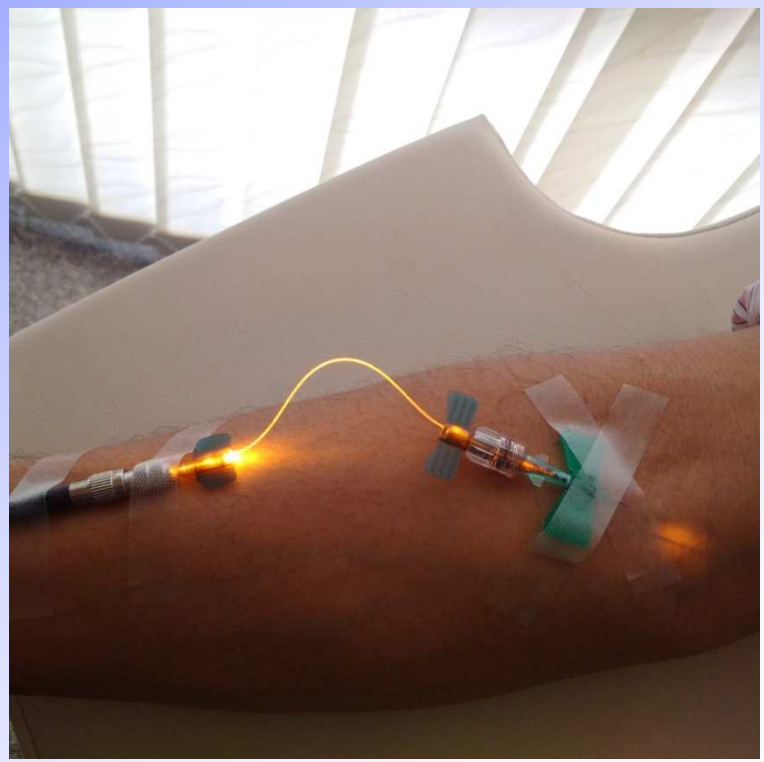
Intravenous laser therapy with red and green laser



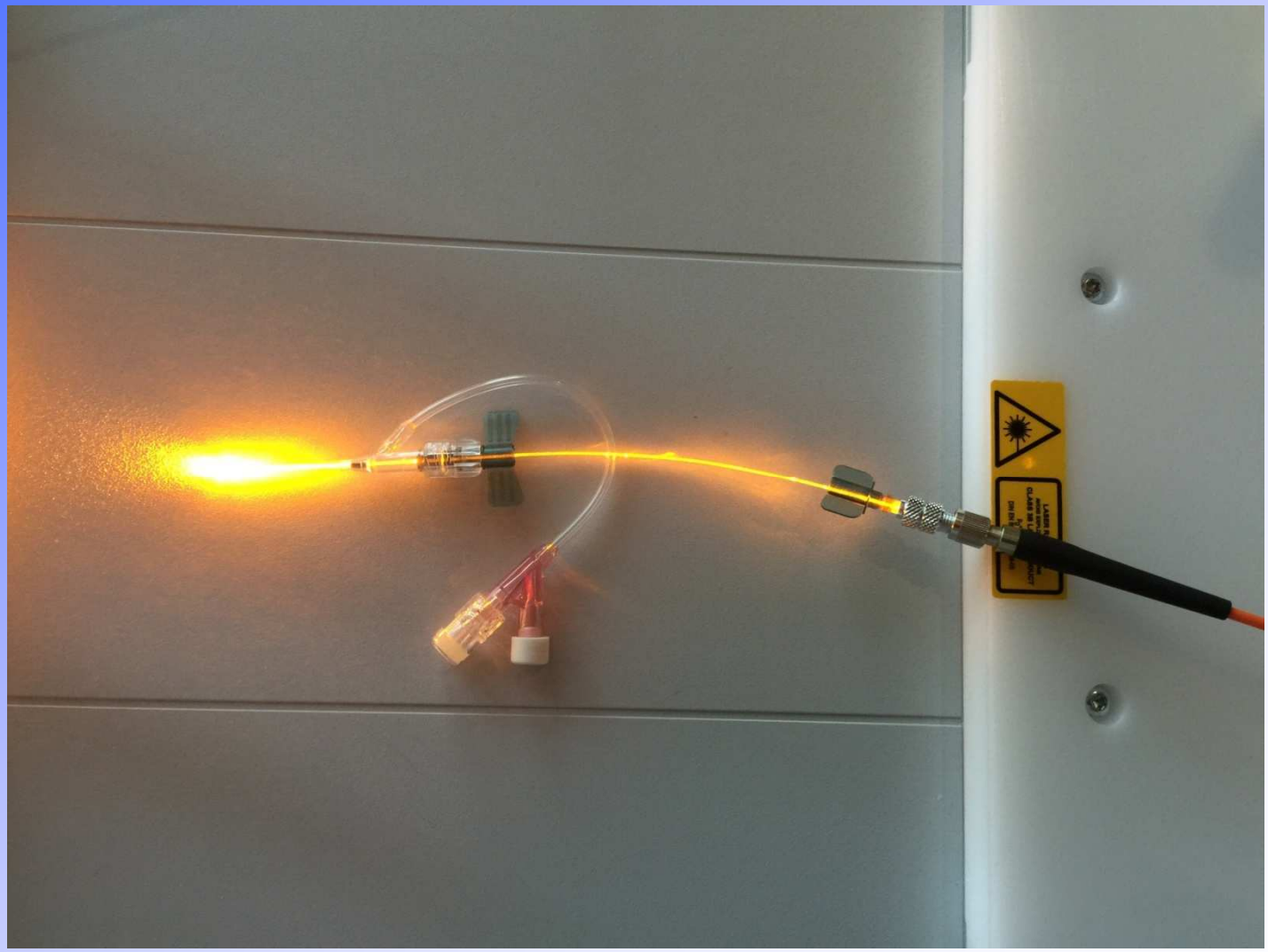


Intravenous laser therapy with blue and yellow laser

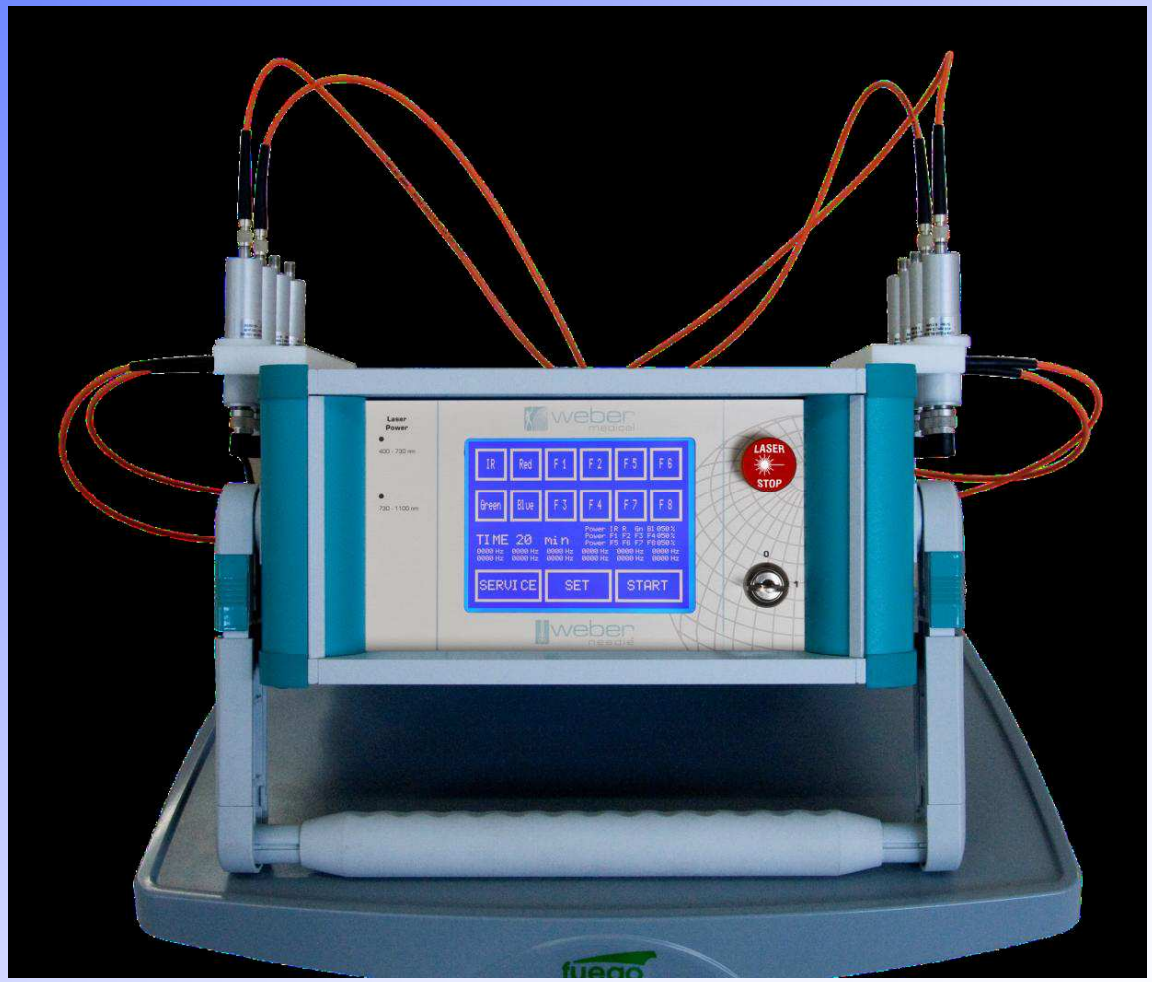




New Y-cannula for intravenous laser therapy



Weberneedle 12-channel modular **Endolaser system**



Effects of intravenous laser blood irradiation on mitochondria

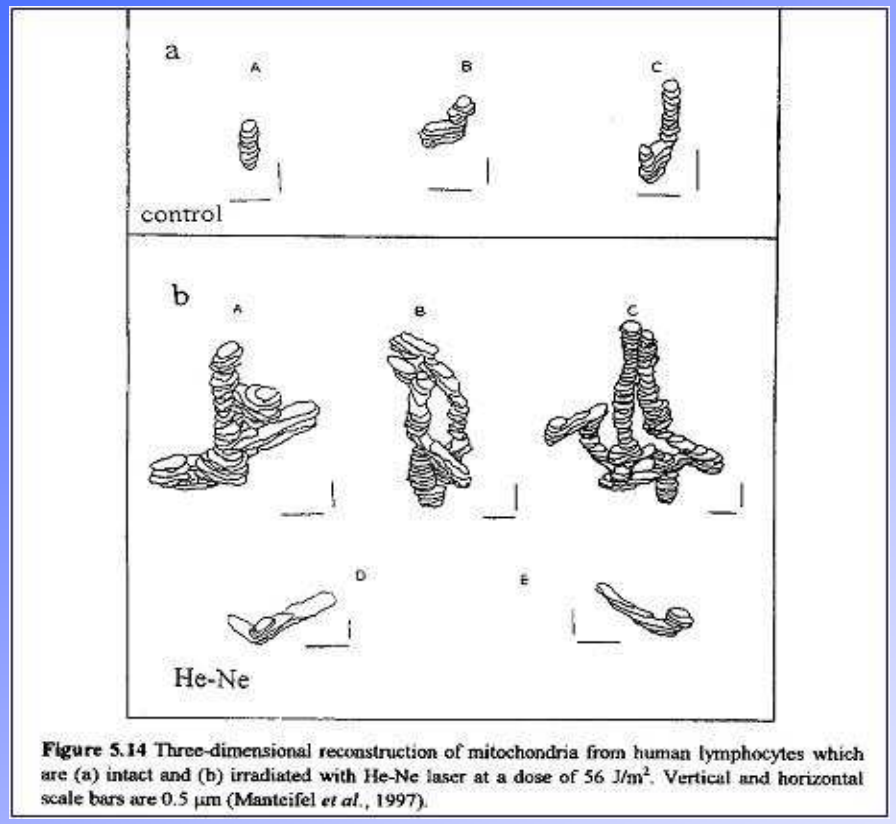


Figure 5.14 Three-dimensional reconstruction of mitochondria from human lymphocytes which are (a) intact and (b) irradiated with He-Ne laser at a dose of 56 J/m^2. Vertical and horizontal scale bars are 0.5 µm (Manteifel *et al.*, 1997).

„Giant-mitochondria“ in human lymphocytes after laser irradiation (632 nm)

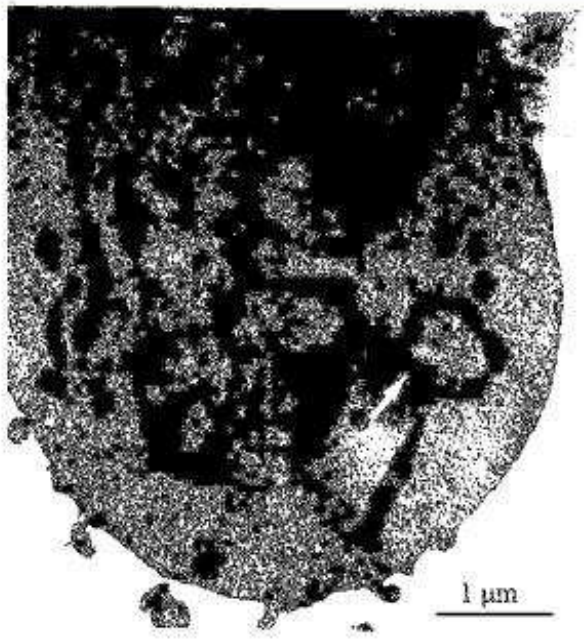


Figure 5.15 Electron micrograph of a section through an irradiated (632.8 nm, 56 J/m^2) human lymphocyte. The experimental details are described by Manteifel *et al.*, 1997. The arrow points to the ring-shaped mitochondrial profile that belongs to the giant mitochondrion (B) presented in Fig. 5.14b.

Ring-shaped mitochondria in human lymphocytes after laser irradiation (632nm)

Increased ATP production

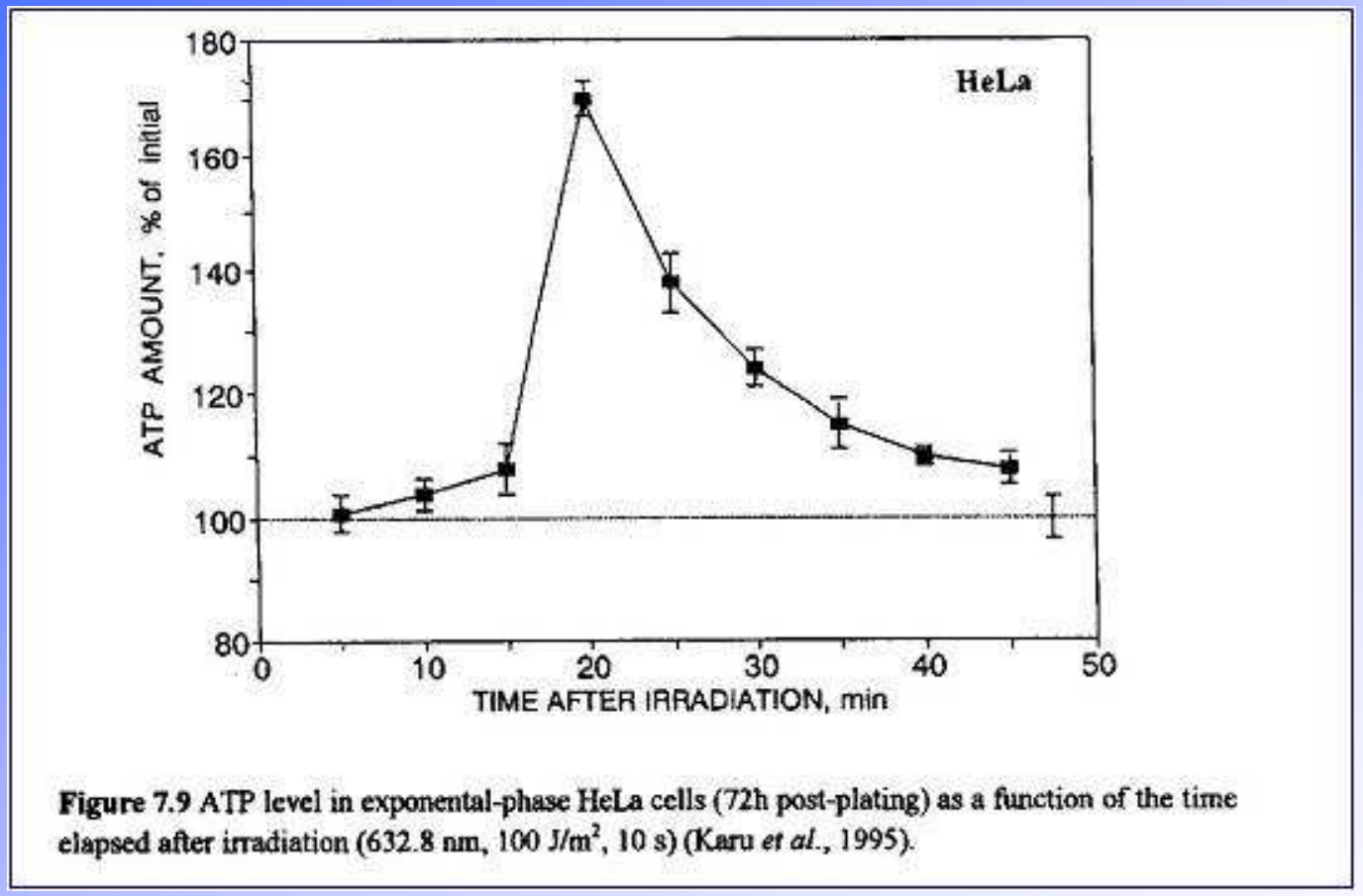
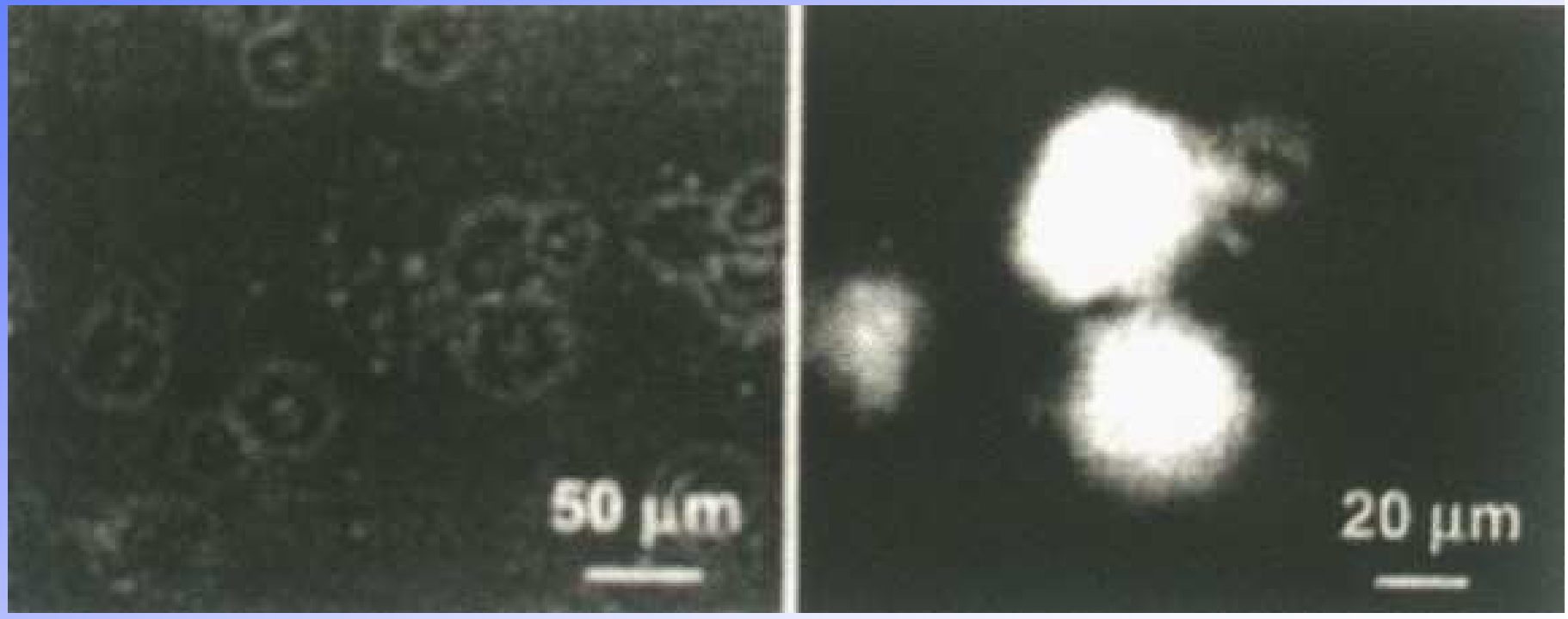


Figure 7.9 ATP level in exponental-phase HeLa cells (72h post-plating) as a function of the time elapsed after irradiation (632.8 nm, 100 J/m^2, 10 s) (Karu *et al.*, 1995).

ATP-Increase under laser irradiation (632 nm, red light) of a HeLa cell-culture

Immunological effects of iv-Laser

Activation of macrophages in fluorescent light



Photodynamic therapy: new ways of cancer with lasers and photosensitizers

Photodynamic therapy (PDT)

- Photodynamic therapy is one of the most interesting and promising approaches in the treatment of various cancers.
- The principle is the stimulation of a light sensitive drug which is injected into the blood and accumulates in cancer cells
- Tumor tissue is subsequently destroyed by irradiation with light of appropriate wavelength according to the absorption spectra of the various photosensitizers
- The basic principle behind this mechanism is the development of radical oxygen species.

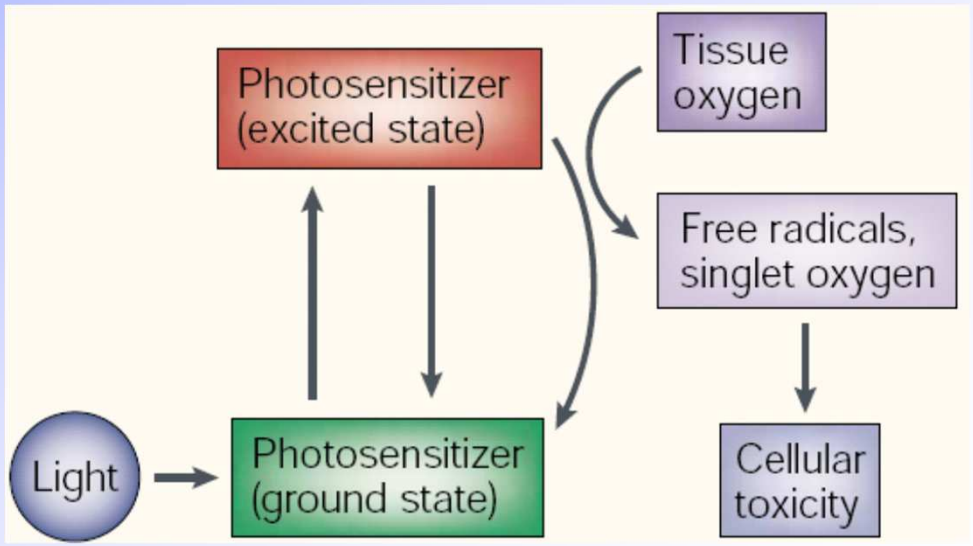
Photodynamic therapy (PDT)

- However up to today PDT was limited to cancer treatment of superficial tumors
- Because we are not able to bring the laser beam in a sufficiant concentration deeper into the body.

Introduction:
Process of Photodynamic Therapy

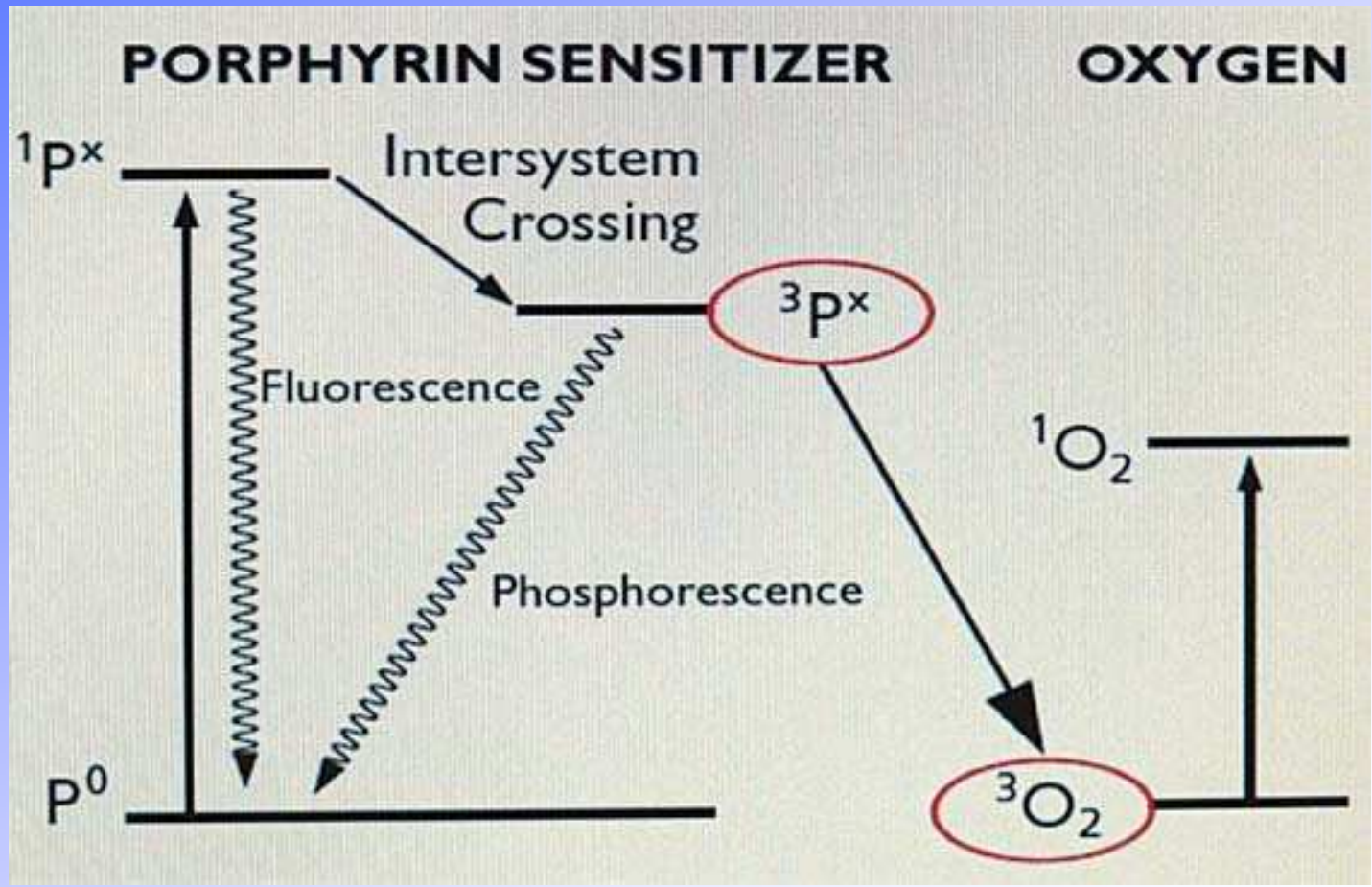
- 2 individually non-toxic components brought together to cause harmful effects on cells and tissues

  - Photosensitizing agent
  - Light of specific wavelength

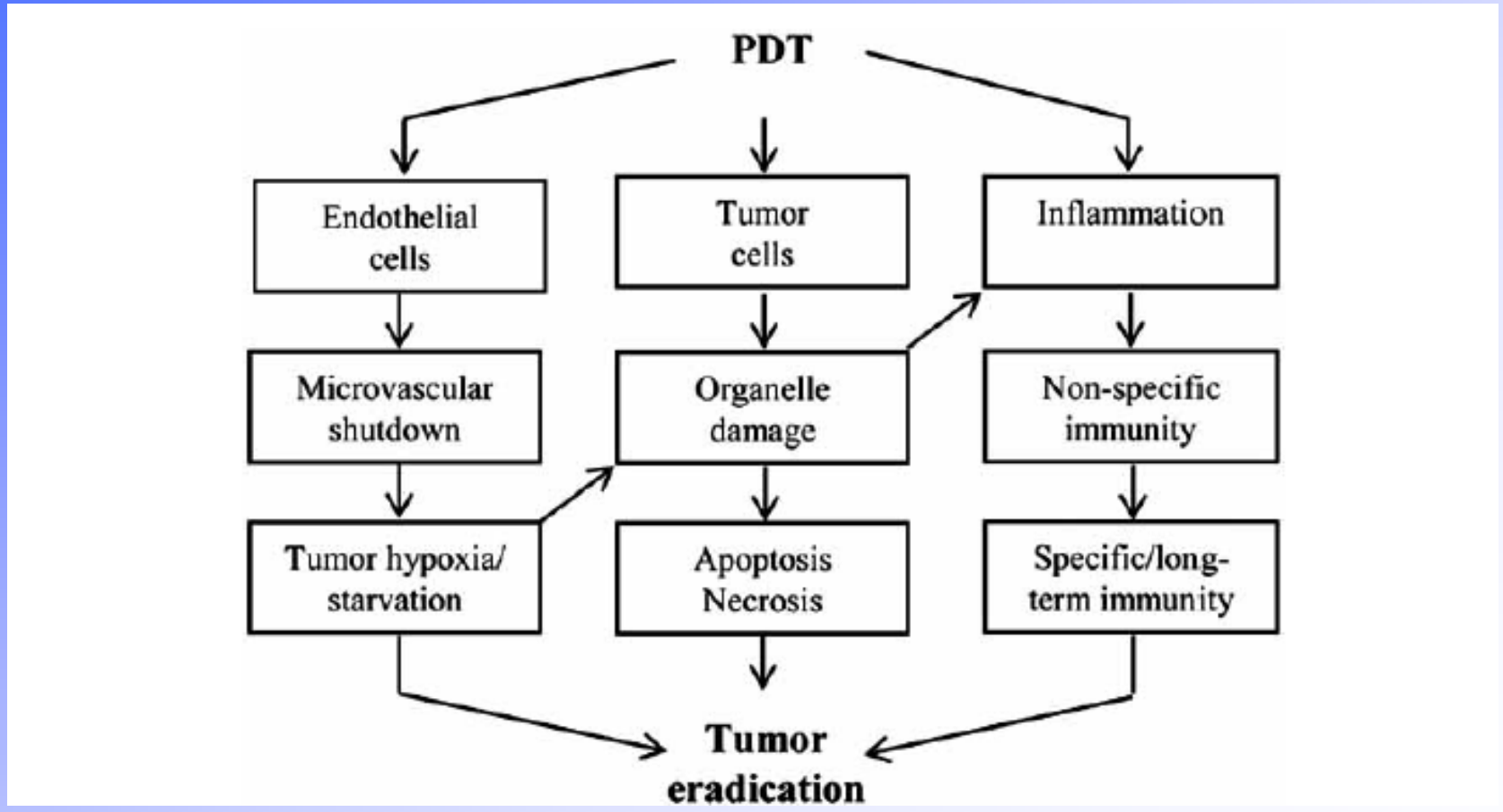


Nature **2003**, *3*, 380.

Photodynamic Therapy



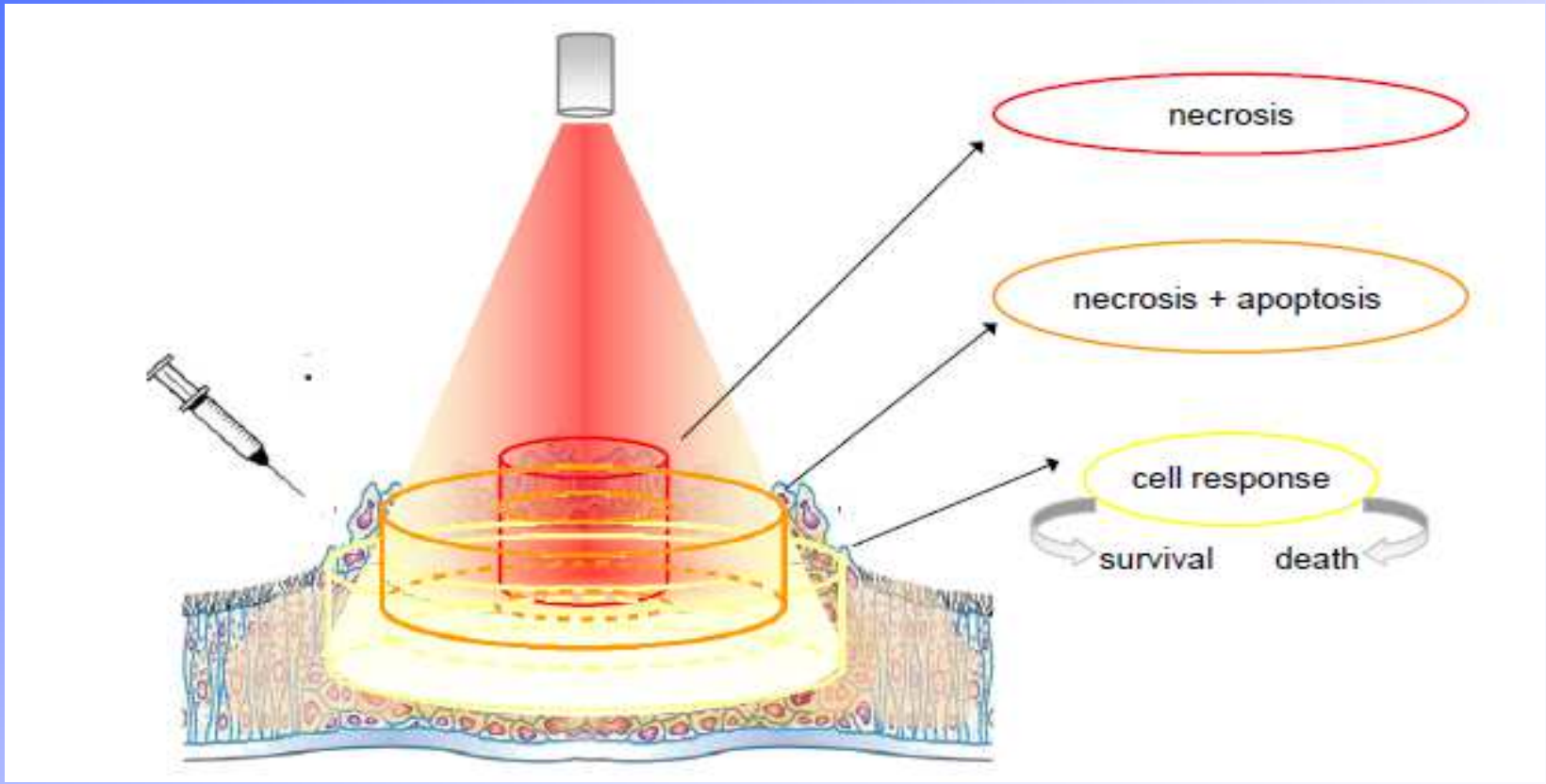
Mechanisms of PDT



Mechanisms of PDT

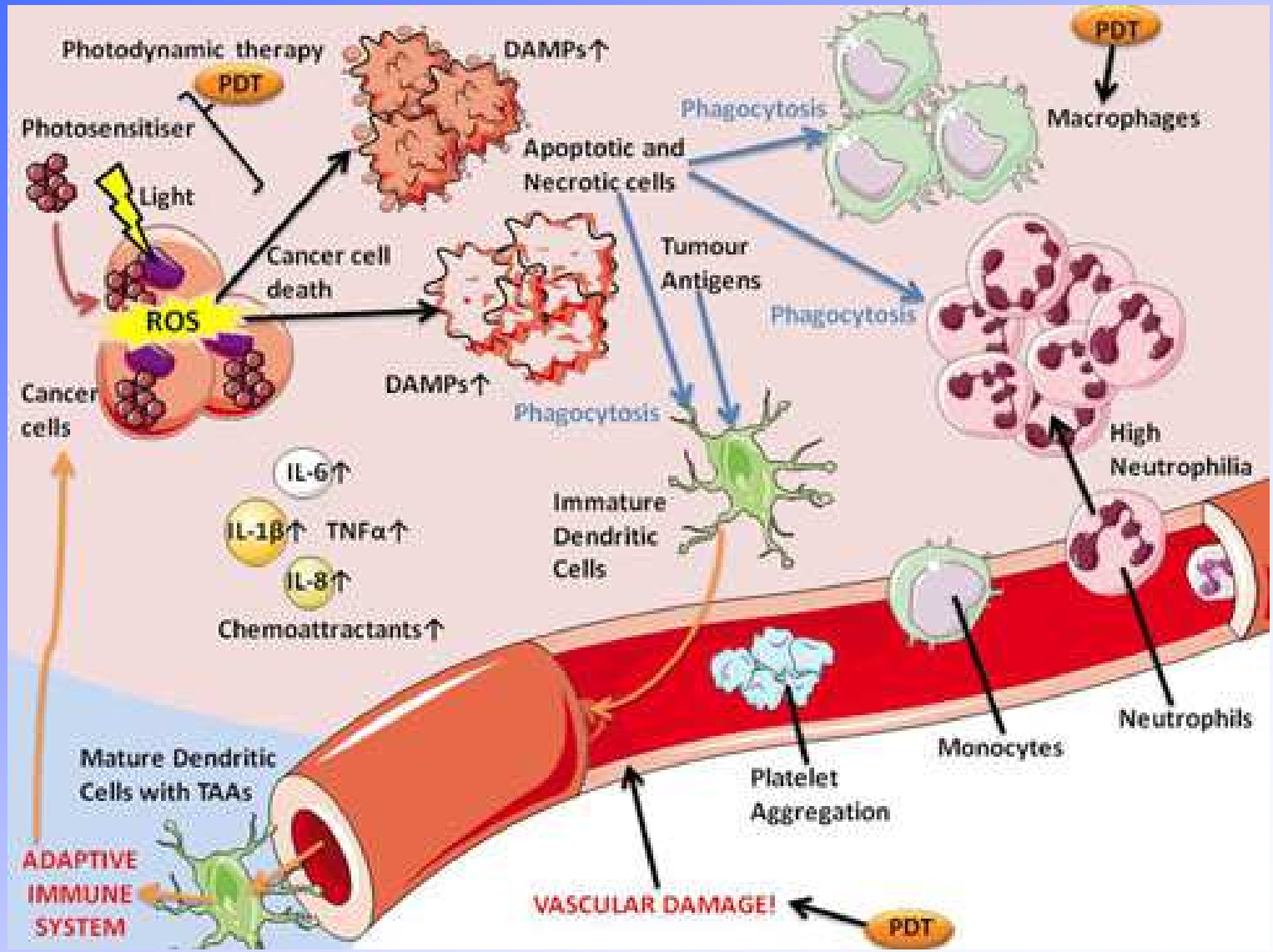
- Selective targeting of tumor cells
- Minimal side effects
- No resistance after repeated treatments
- Tumor vascular shutdown by thrombosis and haemorrhages
- Induction of local inflammation
- Immune activation

The photodymamic reaction



Light distribution and cellular response during PDT

Immunological effects of PDT



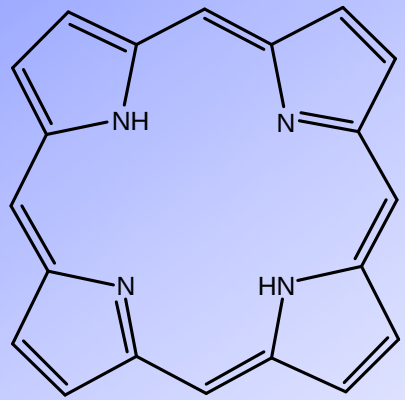
Photosensitizers

Traditional Photosensitizers
(porphyrin derived)

- Haematoporphyrins, HpD

  - Derivatives of Haem

  (Photofrine and others)



- Chlorines
  - Derivatives of Chlorophyll

- Porphycenes
  - Synthetic Porphyrines

Photodynamic Therapy traditional Photosensitizers

Table 1 Currently available photosensitizers.

Platform	Drug	Substance	Manufacturer	Web site
Porphyrin	Photofrin®	HpD	Axcan Pharma, Inc.	www.axcan.com
Porphyrin	Levulan®	ALA	DUSA Pharmaceuticals, Inc.	www.dusapharma.com
Porphyrin	Metvix®	M-ALA	PhotoCure ASA	www.photocure.com
Porphyrin	Visudyne®	Vertiporfin	Novartis Pharmaceuticals	www.visudyne.com
Texaphyrin	Antrin®	Lutexaphyrin	Pharmacylics	www.pharmacyclics.com
Chlorin	Foscan®	Temoporfin	Biolitec Pharma Ltd.	www.bioletcpharma.com
Chlorin	LS11	Talaporfin	Light Science	www.lightsciences.com
Chlorin	Photochlor	HPPH	RPCI	www.roswellpark.org
Dye	Photosens®	Phthalocyanine	General Physics Institute	www.gpi.ru

Photodynamic Therapy
Treatment indications (all superficial)

Photosensitizer	Type of diseases	Country
(*5-ALA*) 5-aminolevulinate	Actinic keratosis, Basal cell carcinoma	U.S., EU
Photofrin	Barrett's displasia	U.S., Canada, EU, UK
Photofrin	Cervical cancer	Japan
Photofrin	Endobronchial cancer	Canada, Most EU Countries, Japan, U.S.
Photofrin	Esophageal cancer	Canada, Most EU Countries, Japan, U.S.
Photofrin	Gastric cancer	Japan
Photofrin	Papillary bladder cancer	Canada
Foscan	Head and neck cancer	EU, Norway, Iceland
Verteporfin	Age-related Macular Degeneration	Canada, Most EU Countries, Japan, U.S.

Photosensitizers approved for therapy

Photodynamic Therapy
New natural derived Photosensitizers

- Chlorin E6 (Red 660 nm)
- Indocyaninegreen (Infrared 810 nm)
- Hypericin (Yellow 589 nm)
- Curcumin (Blue 447 nm)
- Riboflavin (Blue 447 nm)

Photodynamic Therapy:
new chemodrug derived Photosensitizers

- Doxorubicin, liposomal (447 nm, blue)
- Mitoxantron, (yellow 589nm, red 632nm)
- Paclitaxel, (ultraviolett, 345 nm)
- Cisplatin, (ultraviolett, 345 nm)
- 5-FU, (ultraviolett, 345nm)

Topical photosensitizer (Creme)

5-Aminolevulinic acid, 5-ALA, (Hematoporphyrin derivative)

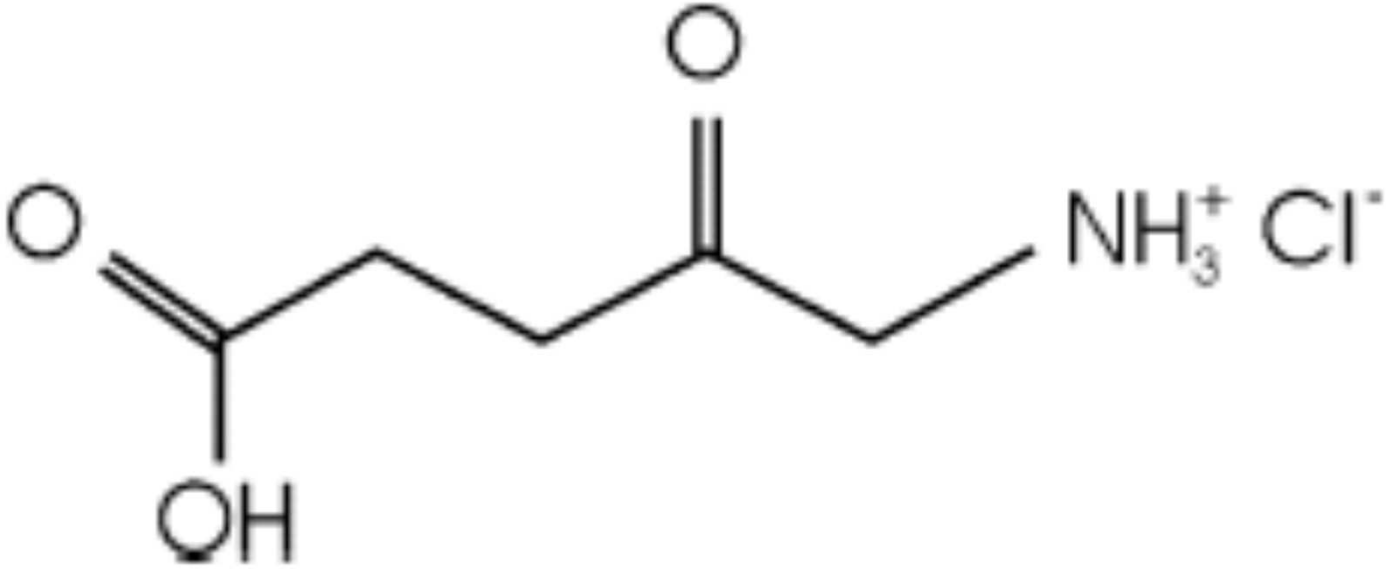
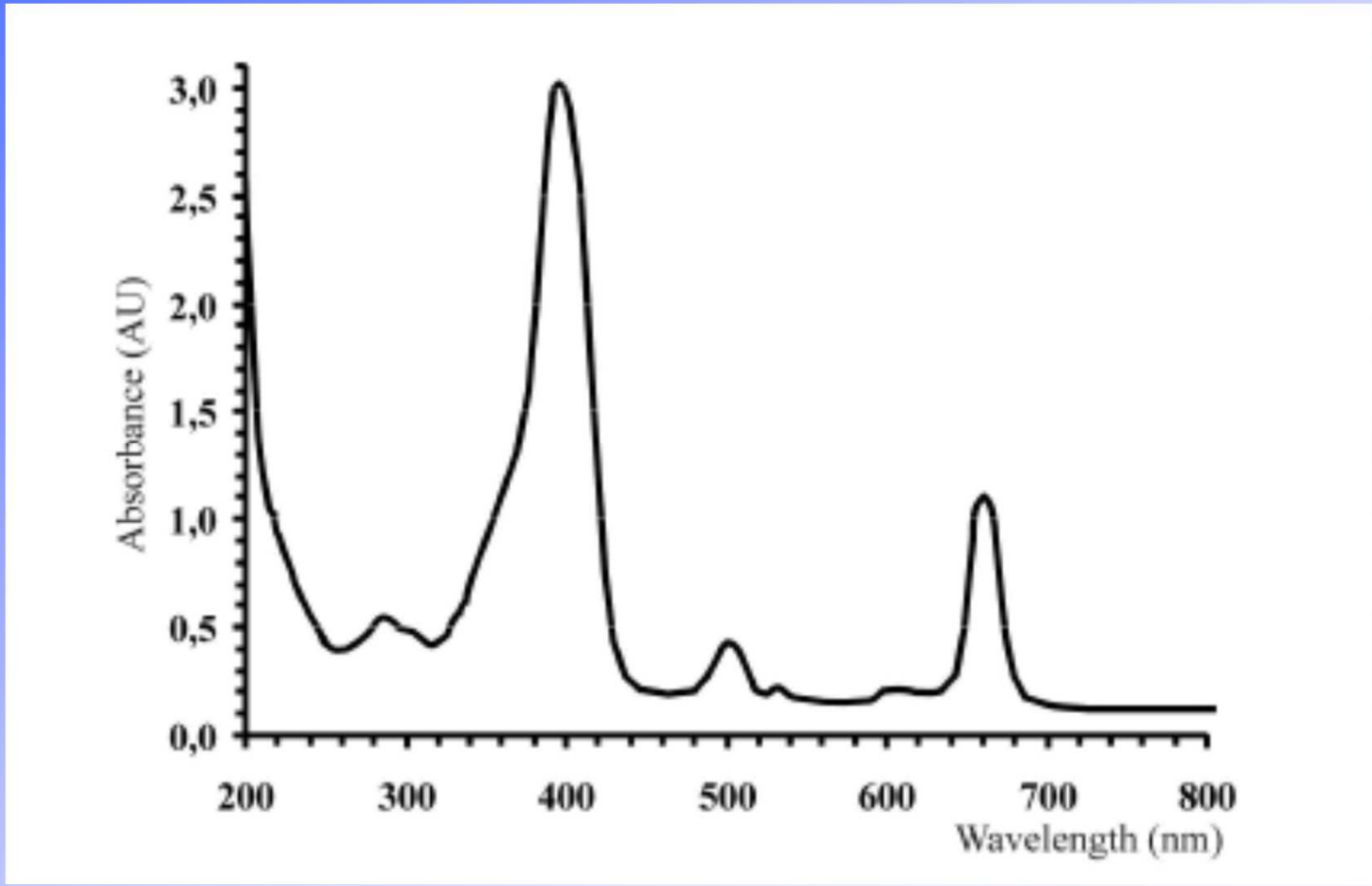


Figure 2 Molecular structure for ALA.

Photodynamic Therapy

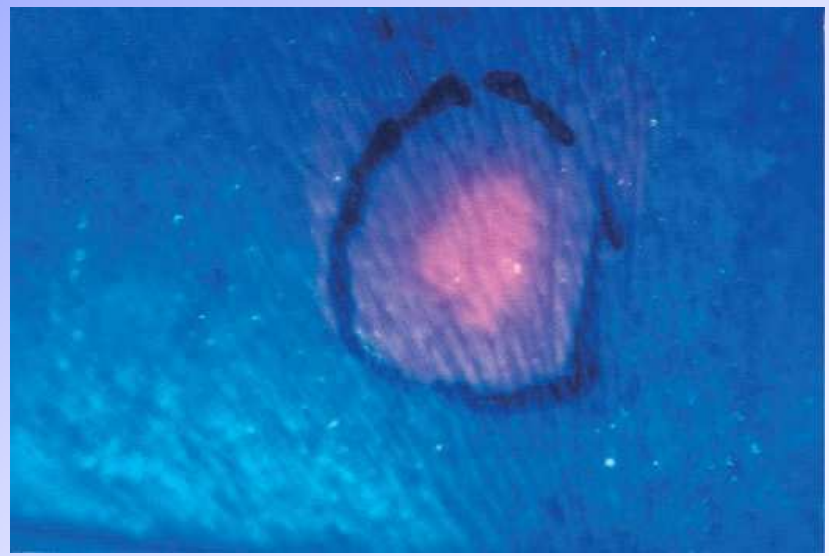
Absorption spectrum of 5-ALA



Photodynamic diagnostics PDD

(Fluorescense diagnostic with blue laser)

Fuselage skin basal cell carcinoma in daylight

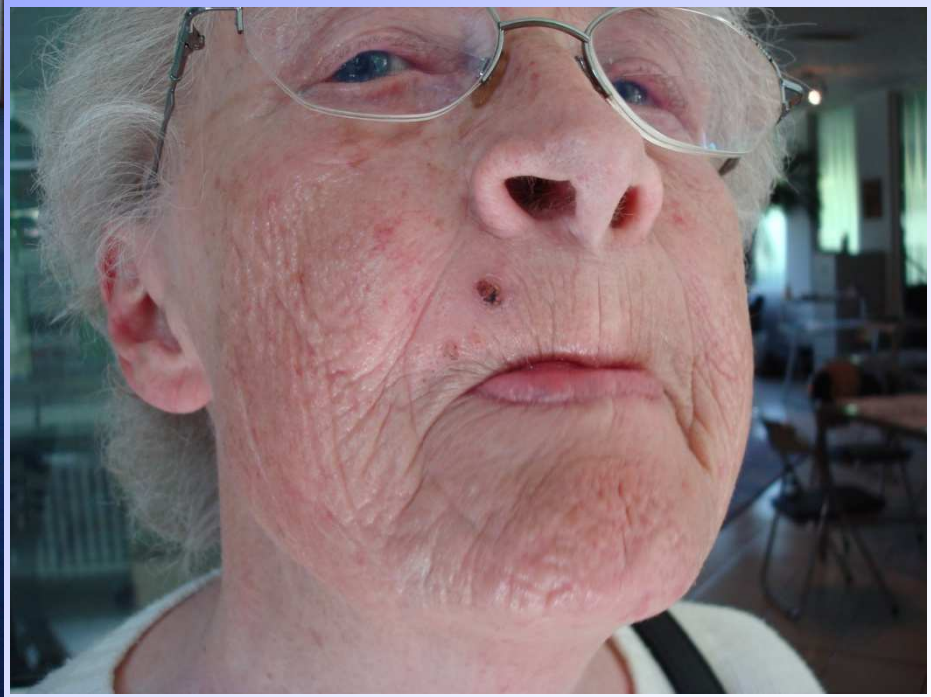


Fuselage skin basal cell carcinoma under wood light

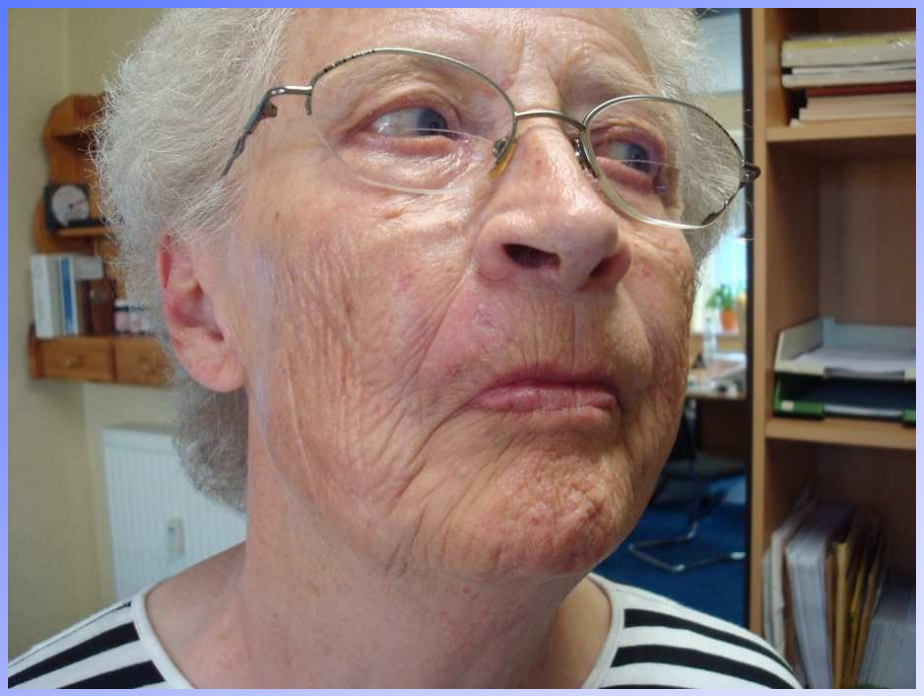
Photodynamic therapy of actinic keratosis

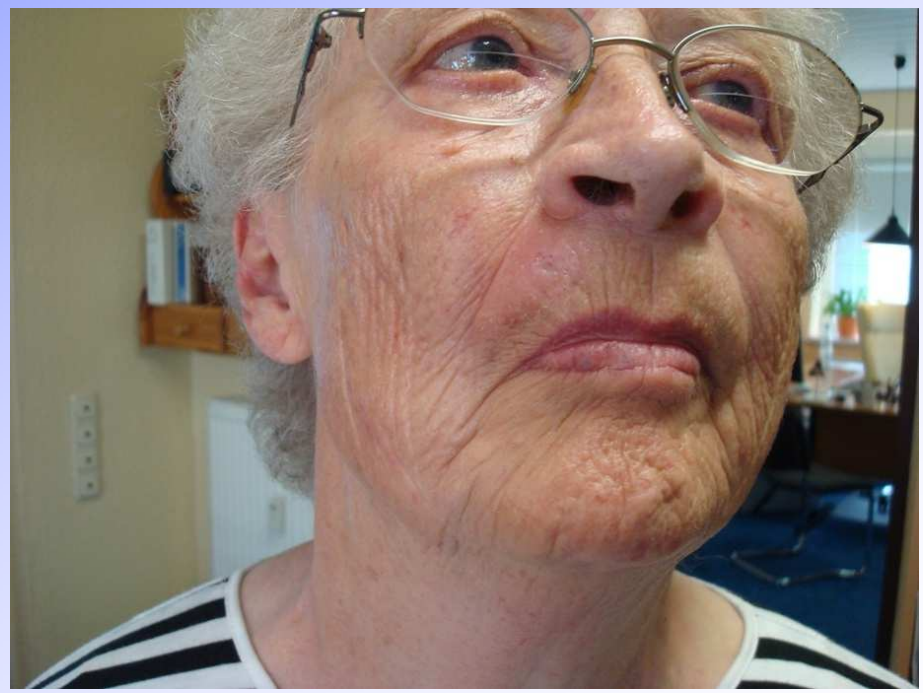
Photodynamische Therapie von Basaliomen und aktinischen Keratosen

Photodynamic therapy of basal cell carcinoma



Photodynamic therapy of basal cell carcinoma





Photodynamic therapy of basal cell carcinoma

Ulcerated basal cell carcinoma before treatment

Findings after 1 treatment PDT

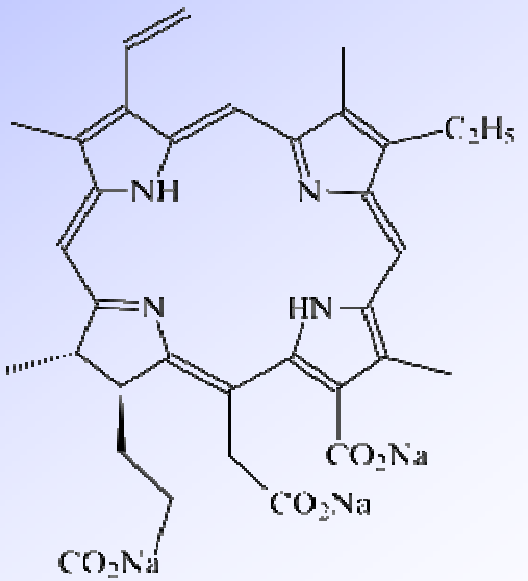
Systemic photodynamic therapy

Fotolone (Chlorin E6)

- Chlorin e6 as photosensitizer
- Indications
- current development status

Chlorin E6
(chemical properties)

- trisodium salt of the „green“ porphyrin
- high solubility in water
- Molecular formula: $C_{34}H_{33}N_4Na_3O$
- High stability of the lyophilized API



APOCARE PHARMA GMBH

Production of Chlorin E6

Natural sources (algae, grass, lucerne etc.)

FDA approved, GAP

inexhaustible availability (different sources/world-market)

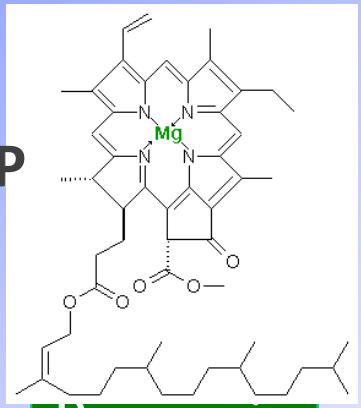
APOCARE PHARMA GMBH

Production of Chlorin E6

GAP

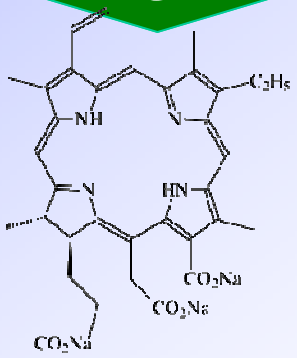
Extraction

In accordance to GMP



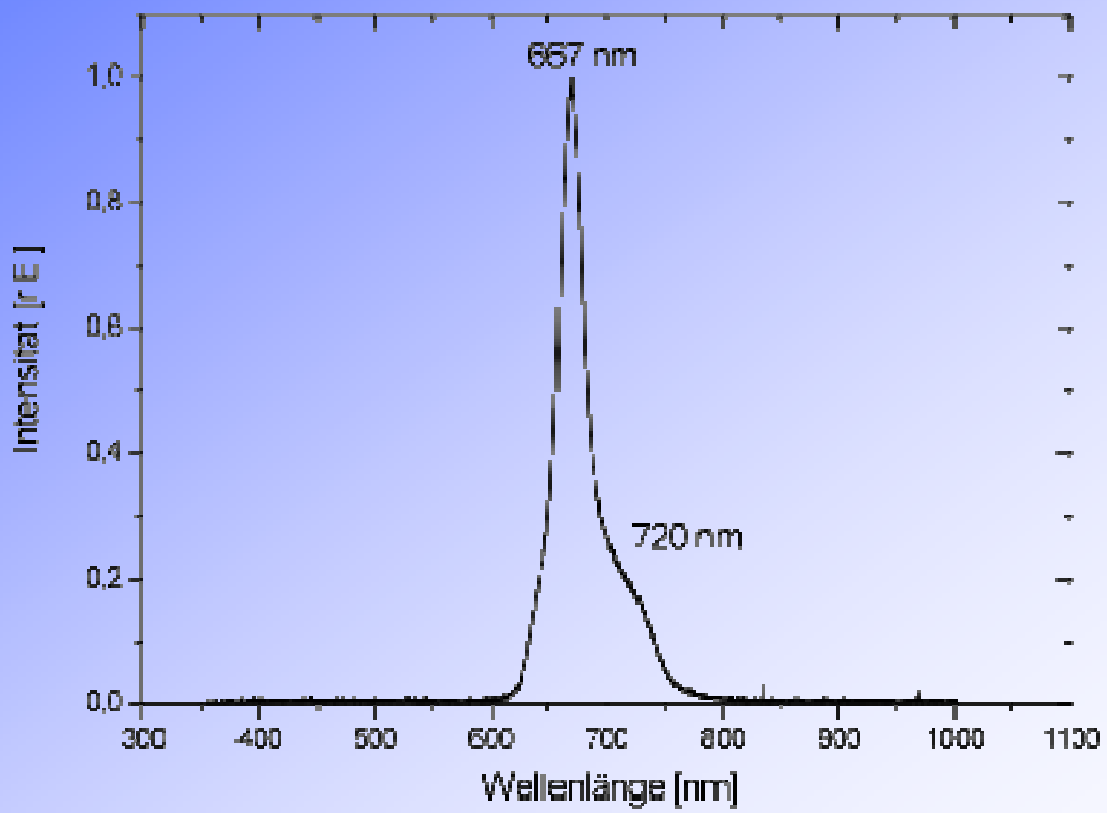
Processing

GMP process:
- unique
-Efficient
- highes purity

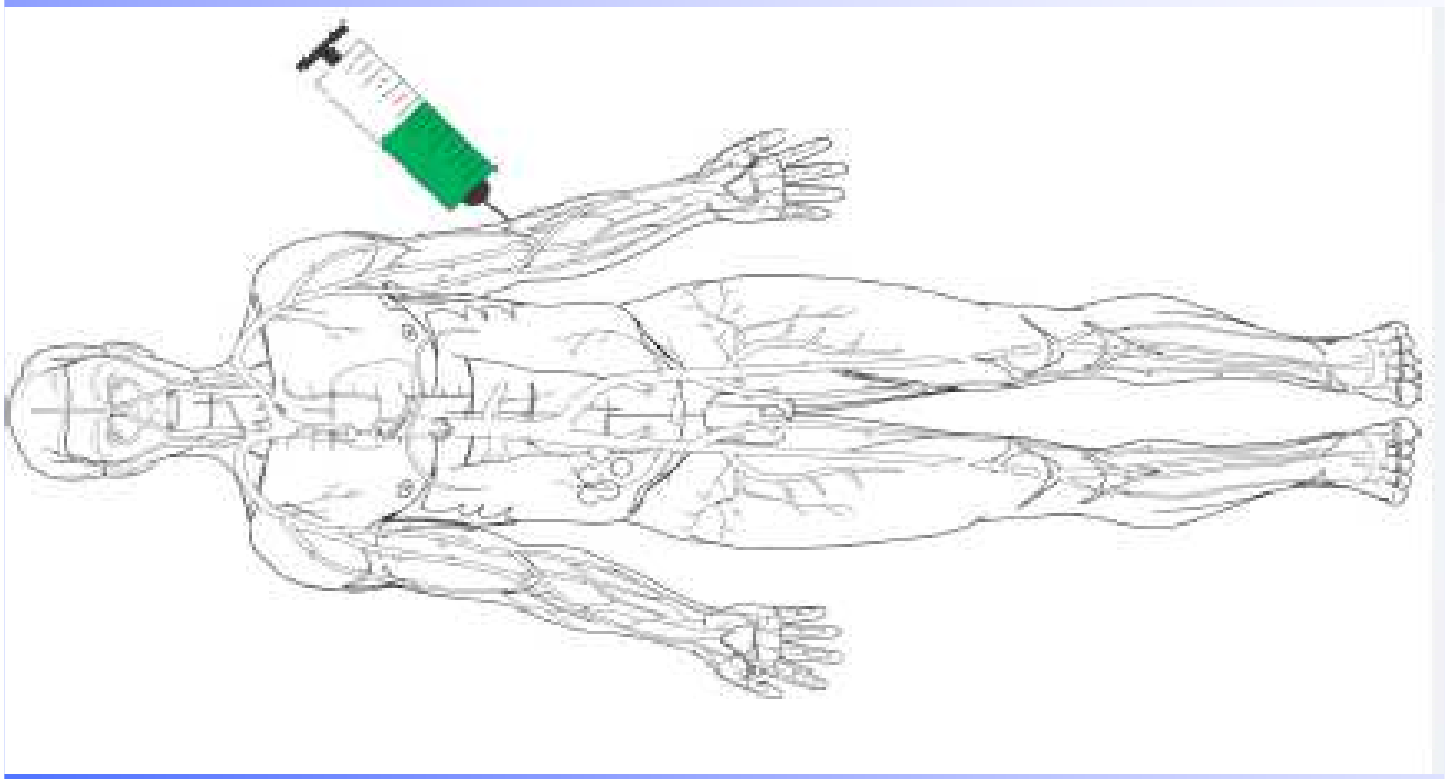


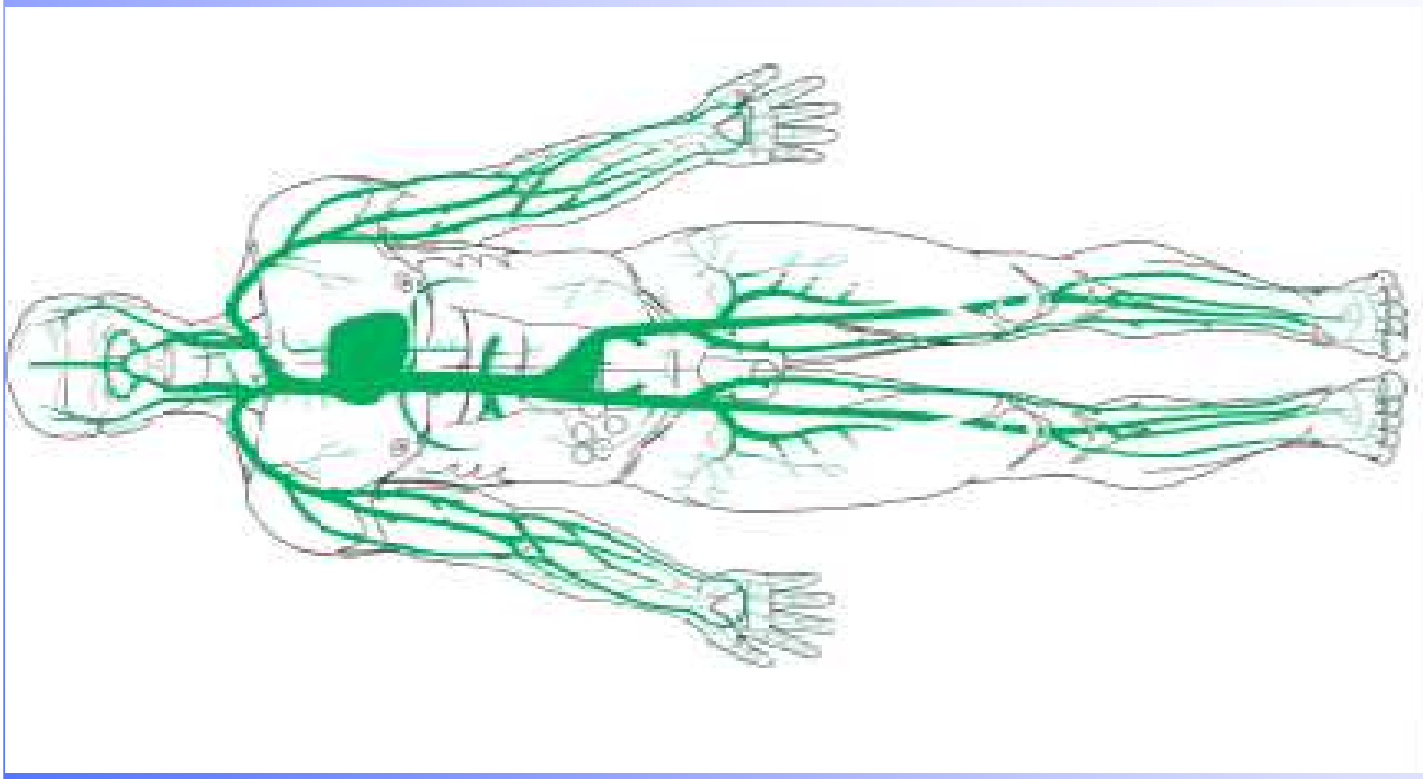
APOCARE PHARMA GMBH

Absorption spectrum of Chlorin E6

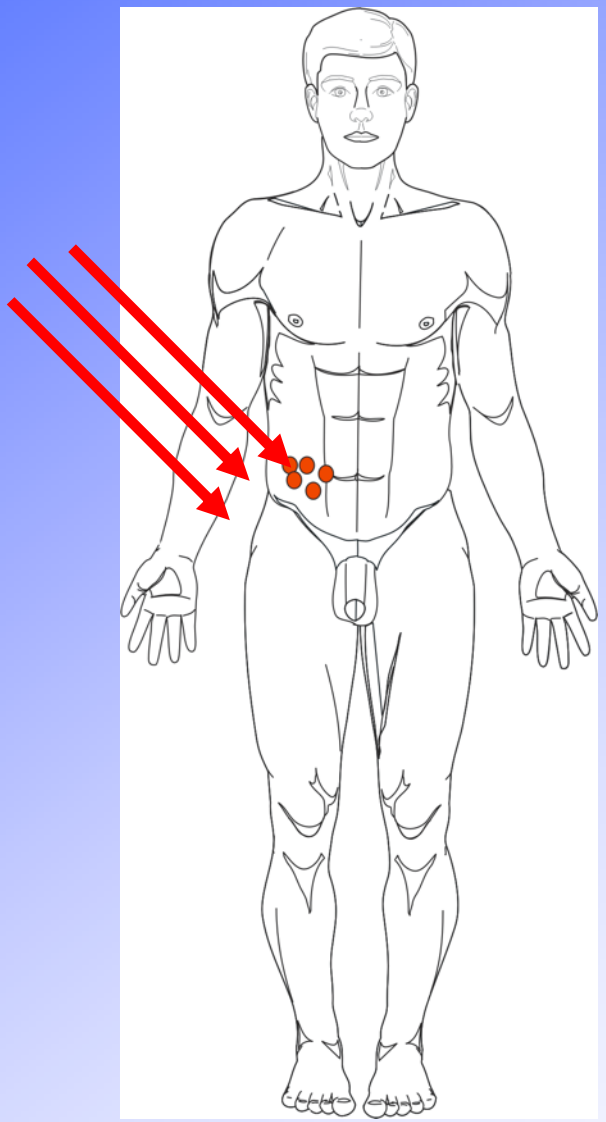


APOCARE PHARMA GMBH





1 – 15 min



PDT

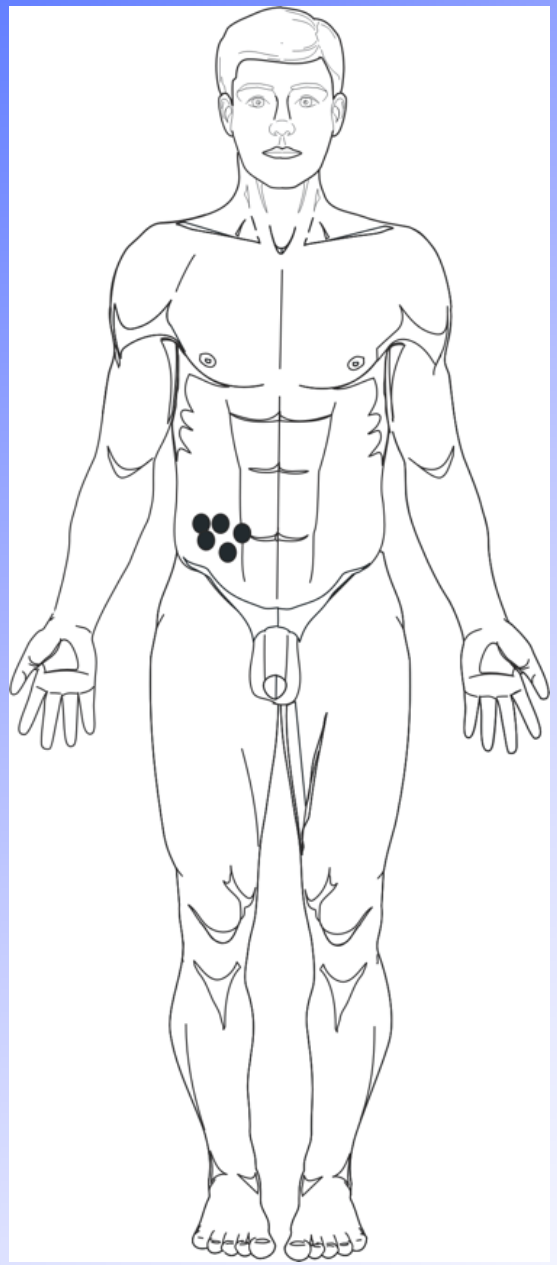
Generation of singlet oxygen

APOCARE PHARMA GMBH

24 – 48 h

- Apoptosis/ Necrosis

- Elimination of Ce6 from blood

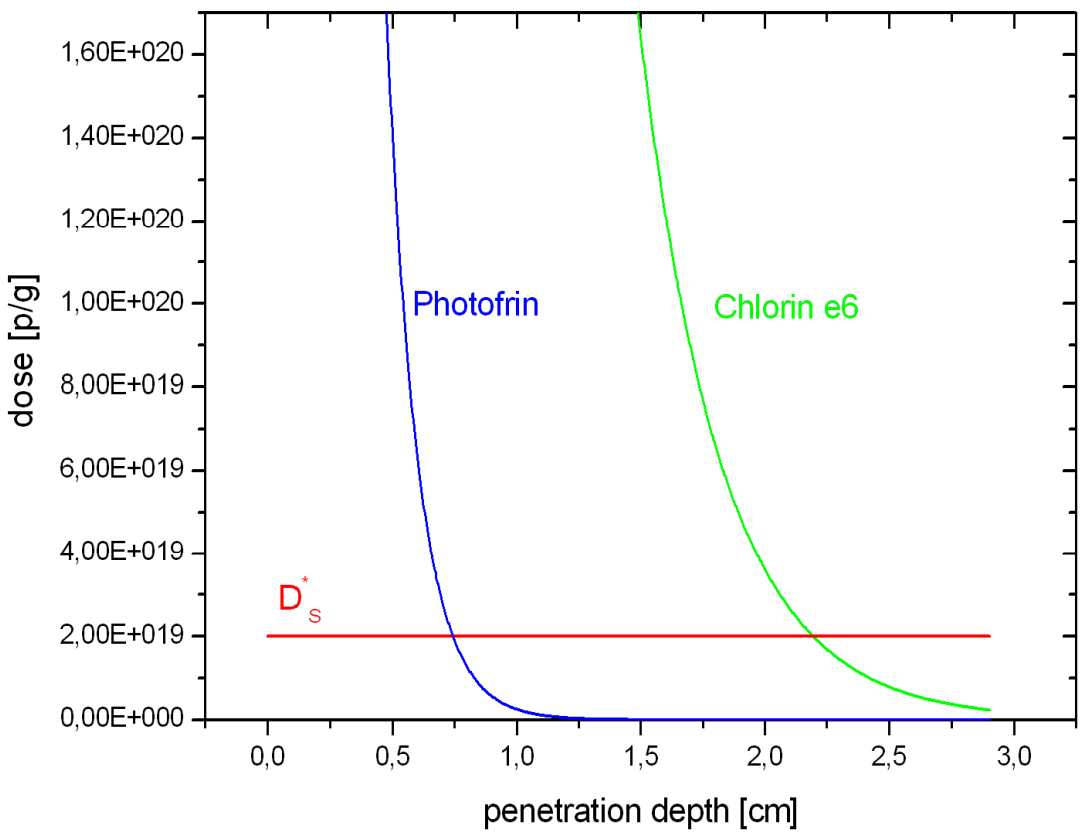


PDT

APOCARE PHARMA GMBH

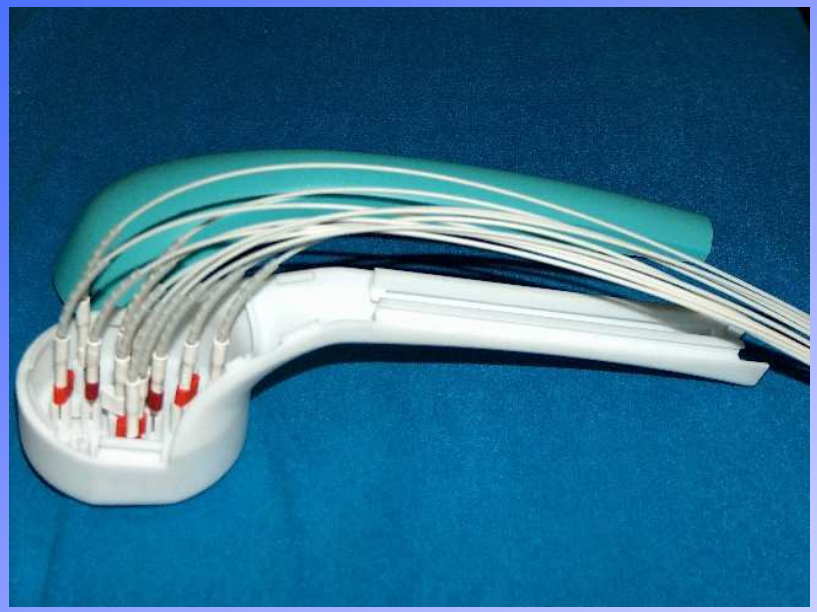
Problem of all porhyrin derived photosensitizers:

limited penetration depth with red laser and tumor size



APOCARE PHARMA GMBH

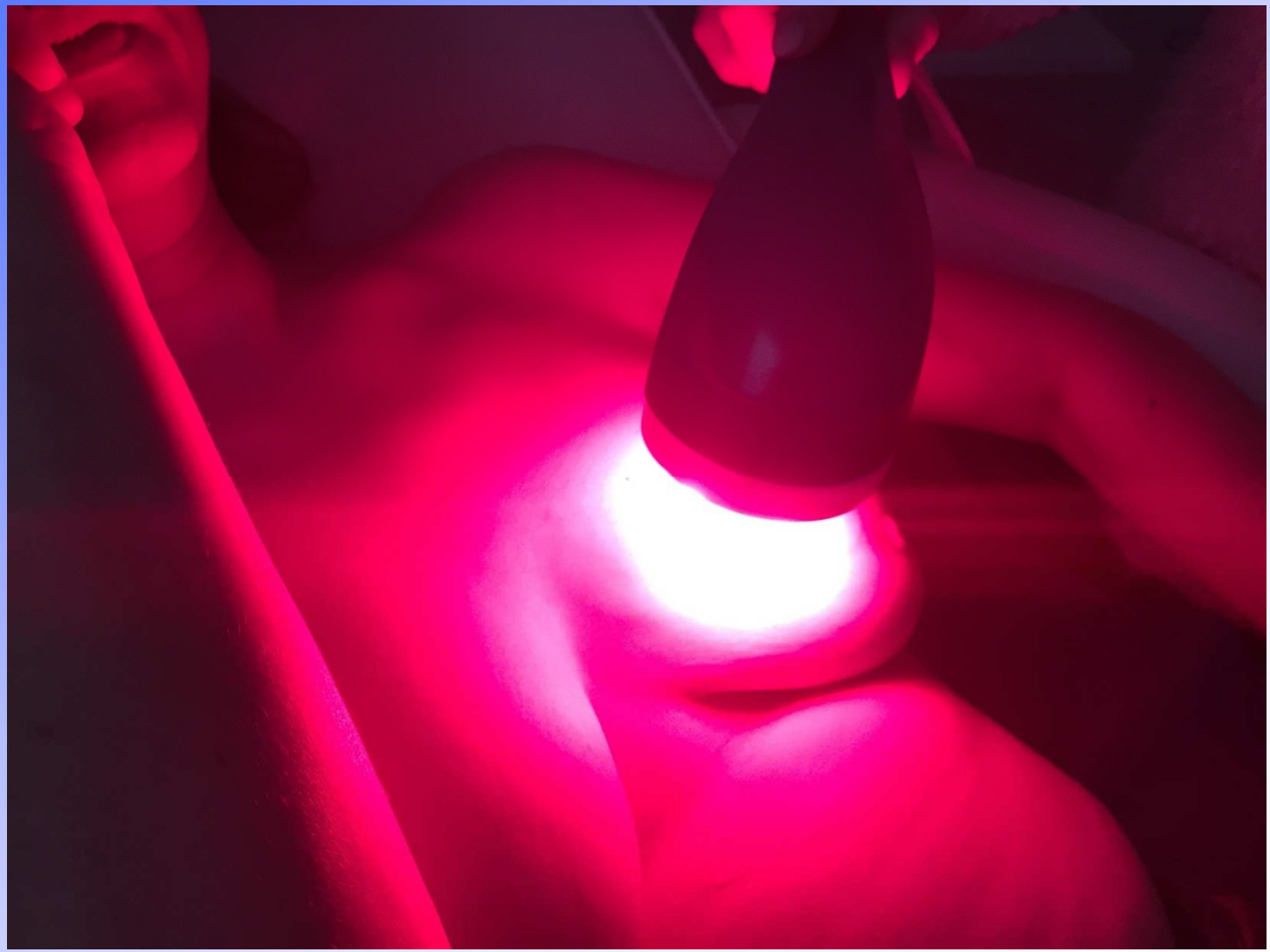
The body shower for superficial tumors with external irradiation



Insertion of laser-needles with different wavelengths into a special shower head

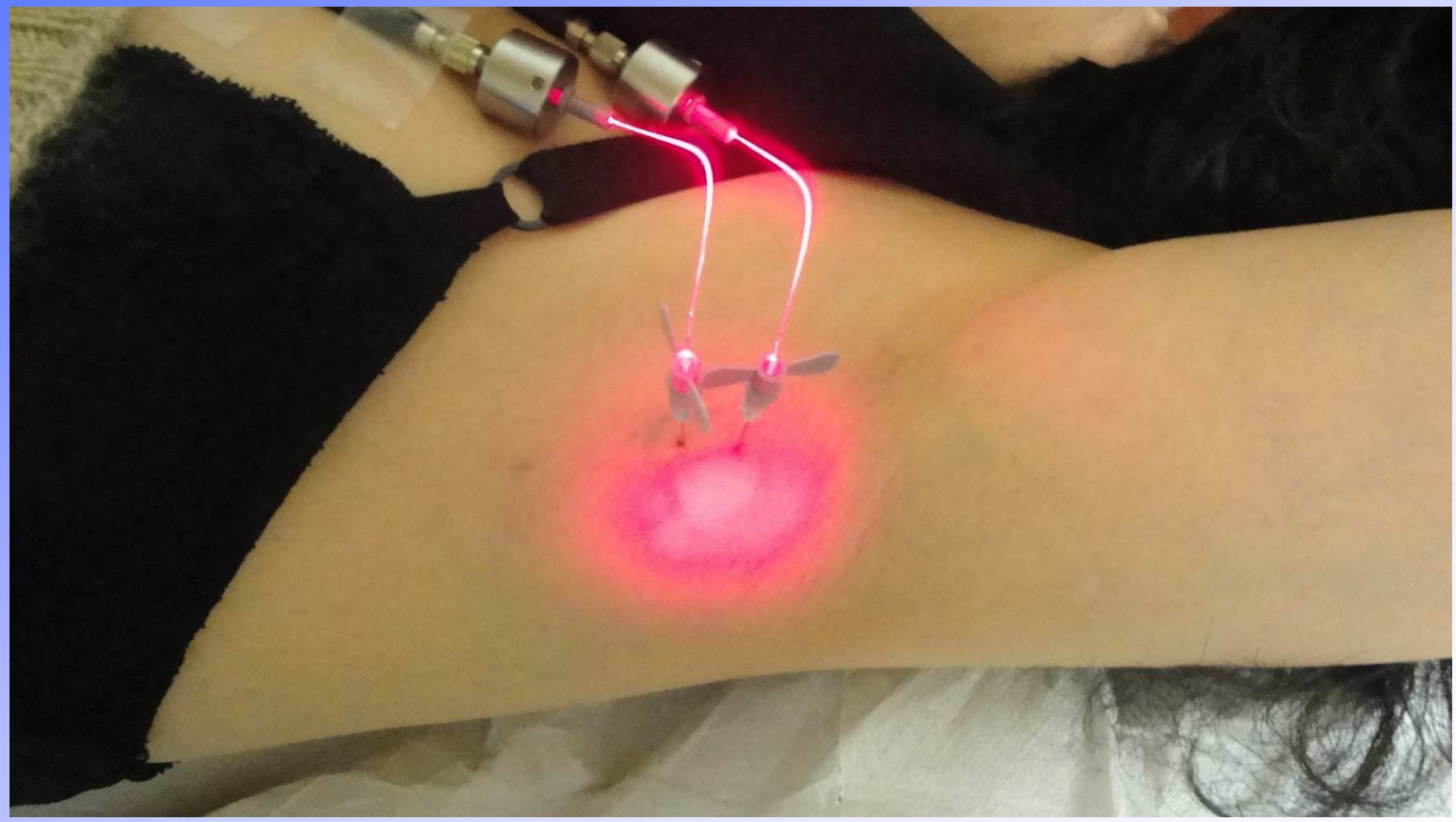
External PDT of lymph metastases

Potential overdosing with skin burn

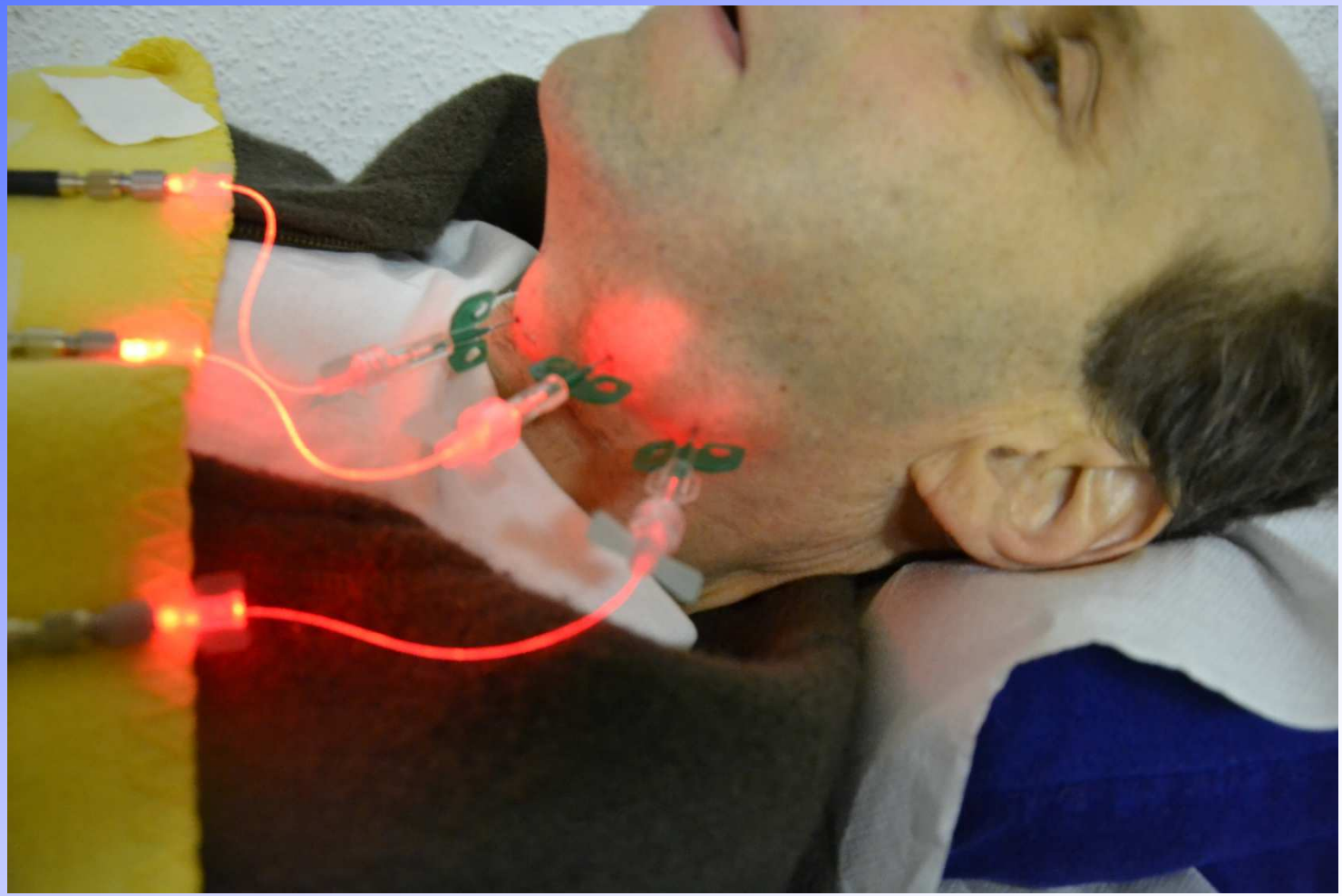


Interstitial PDT of lymph metastases

Interstitial PDT of lymph metastases

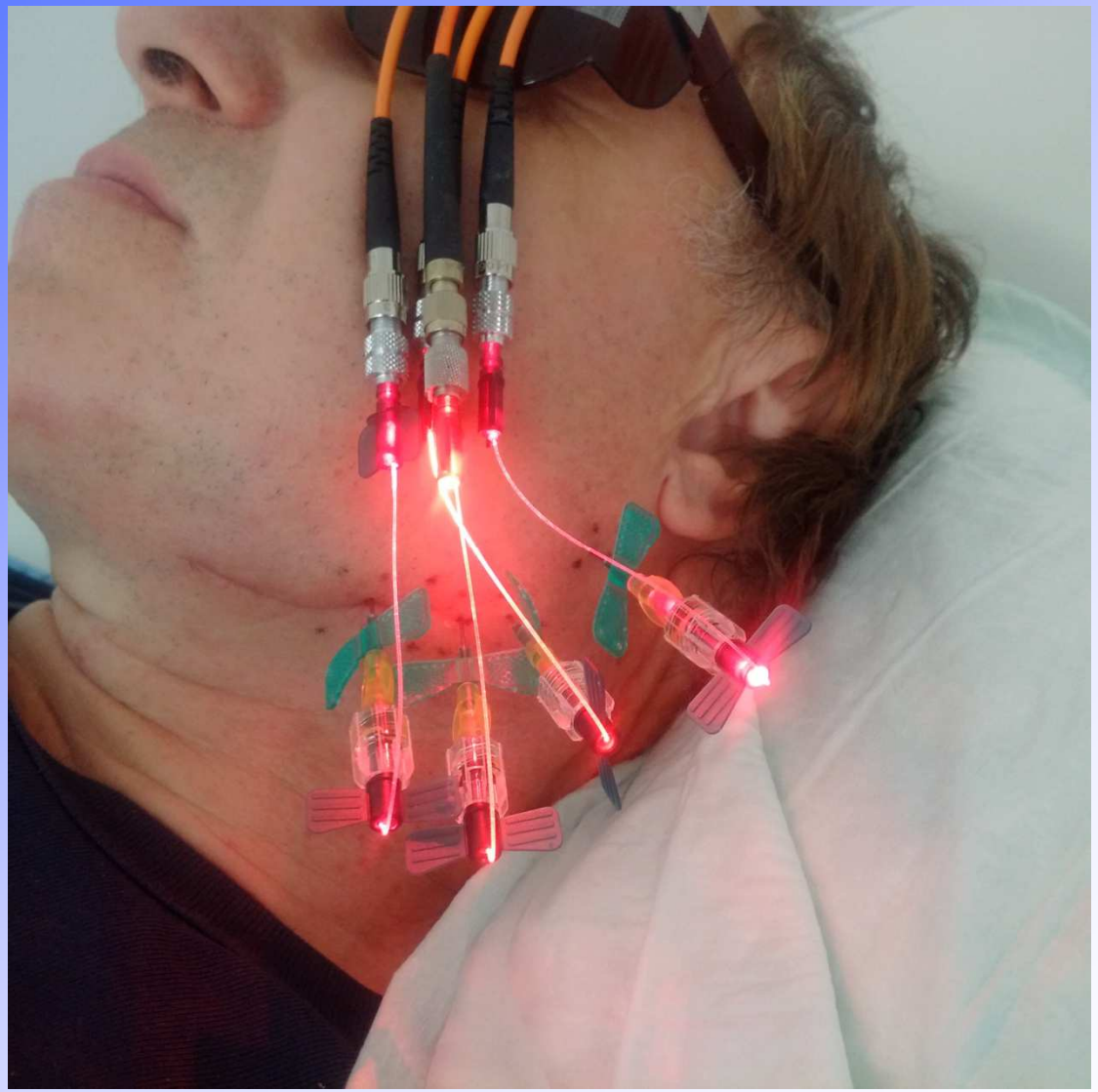


Interstitial PDT of squamous cell carcinoma



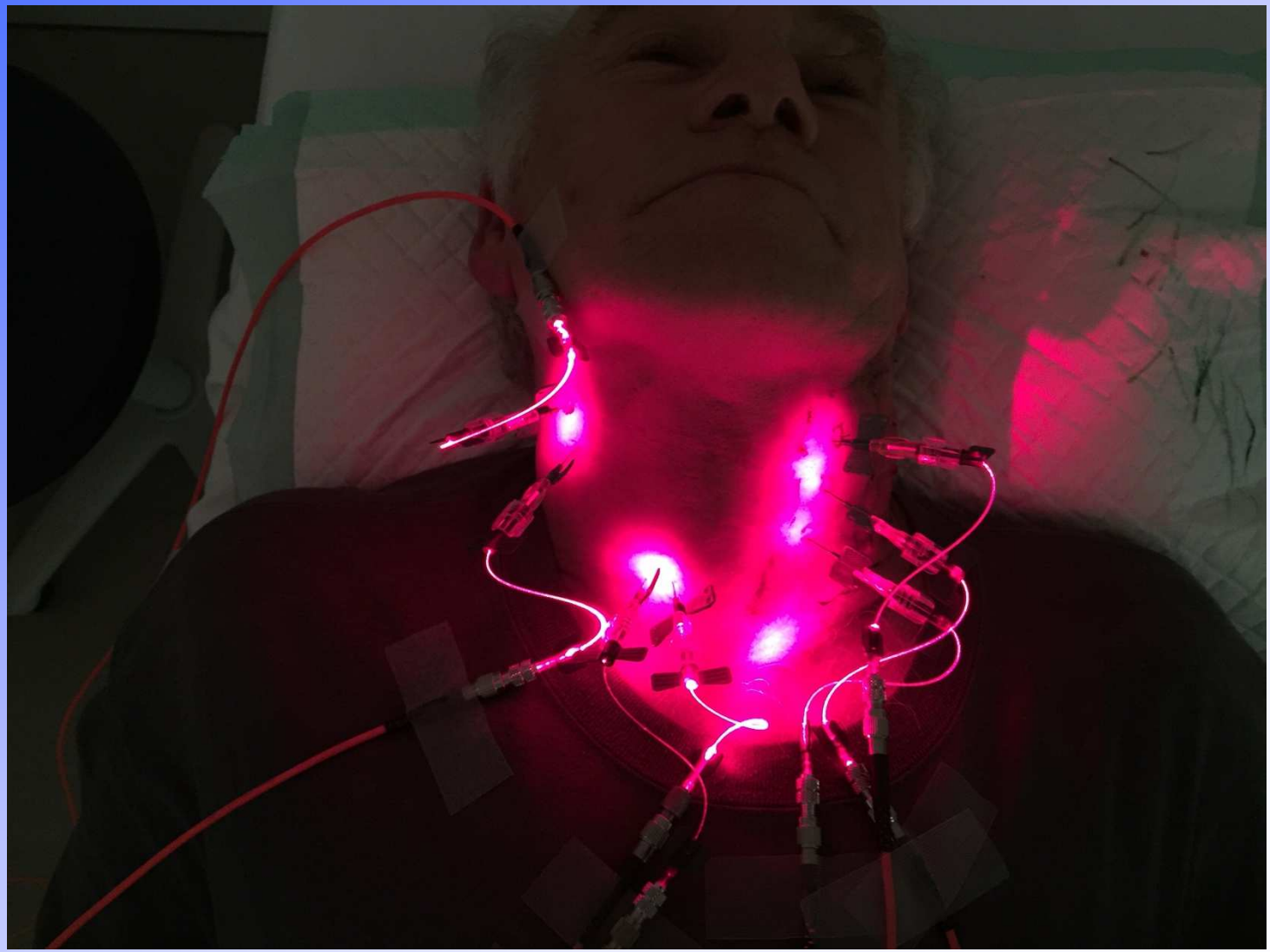
Mouth bottom cancer with lymph nodes

Interstitial laser therapy of neck lymph nodes

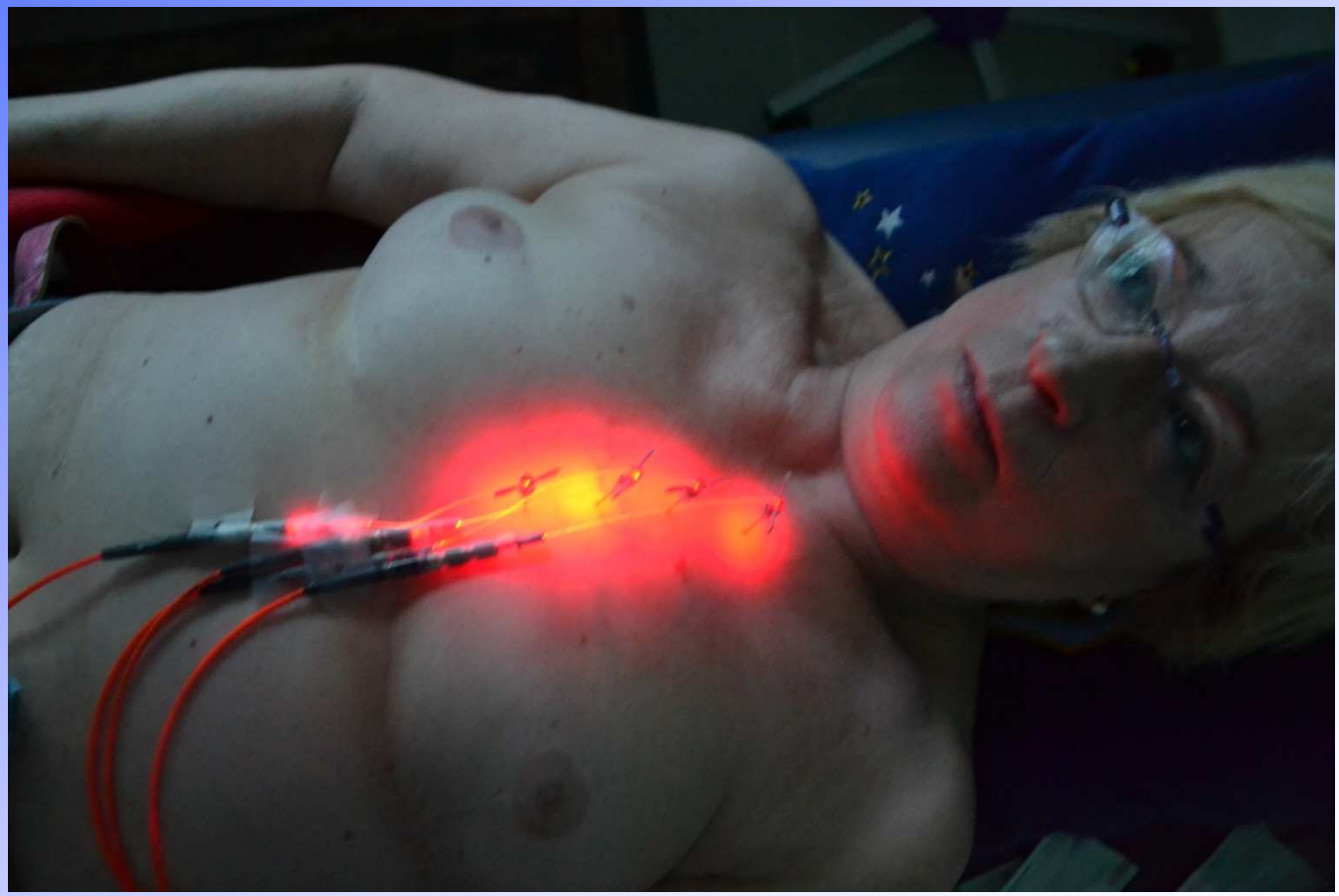


Interstitial PDT for neck lymph nodes

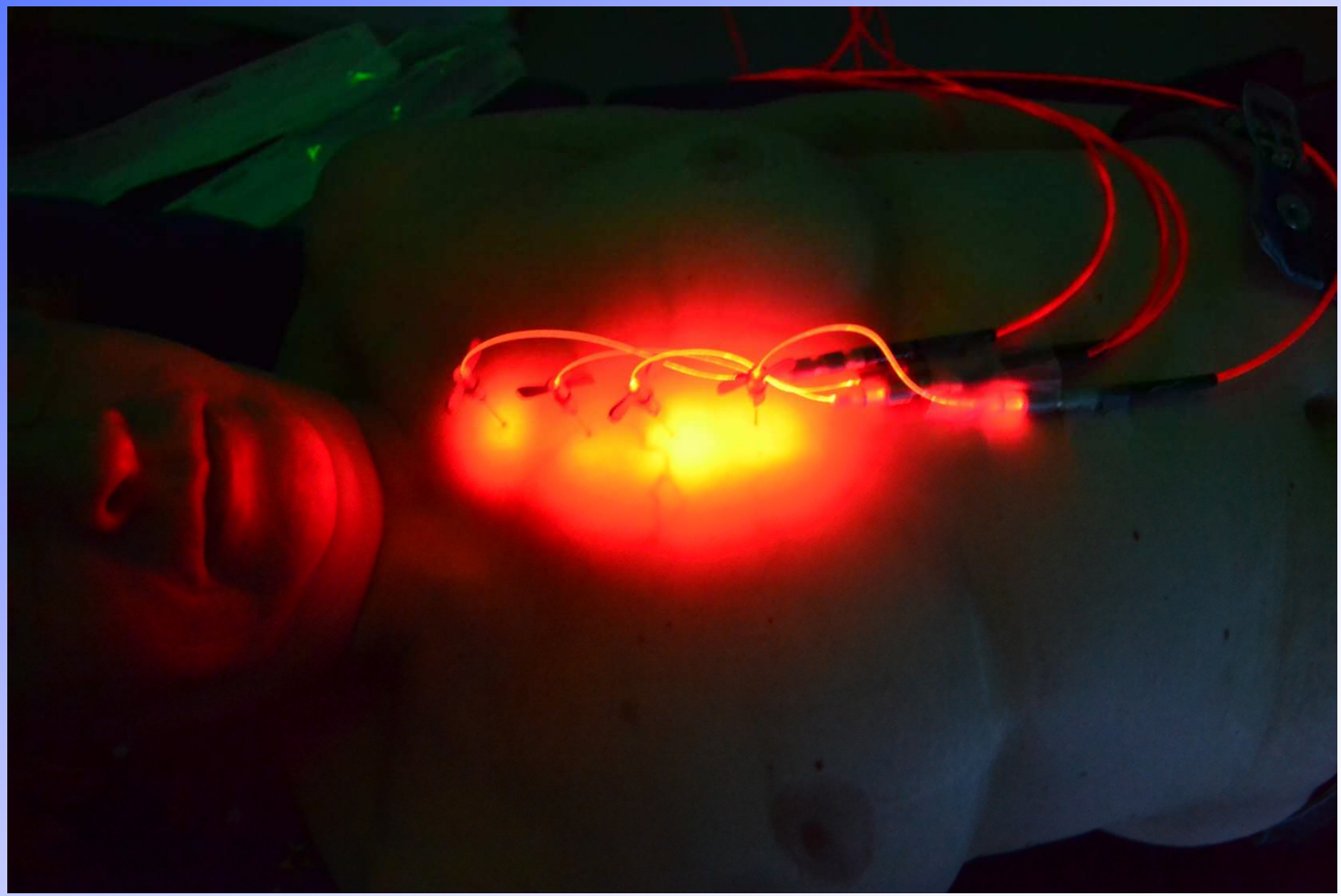
Interstitial PDT for thyroid cancer



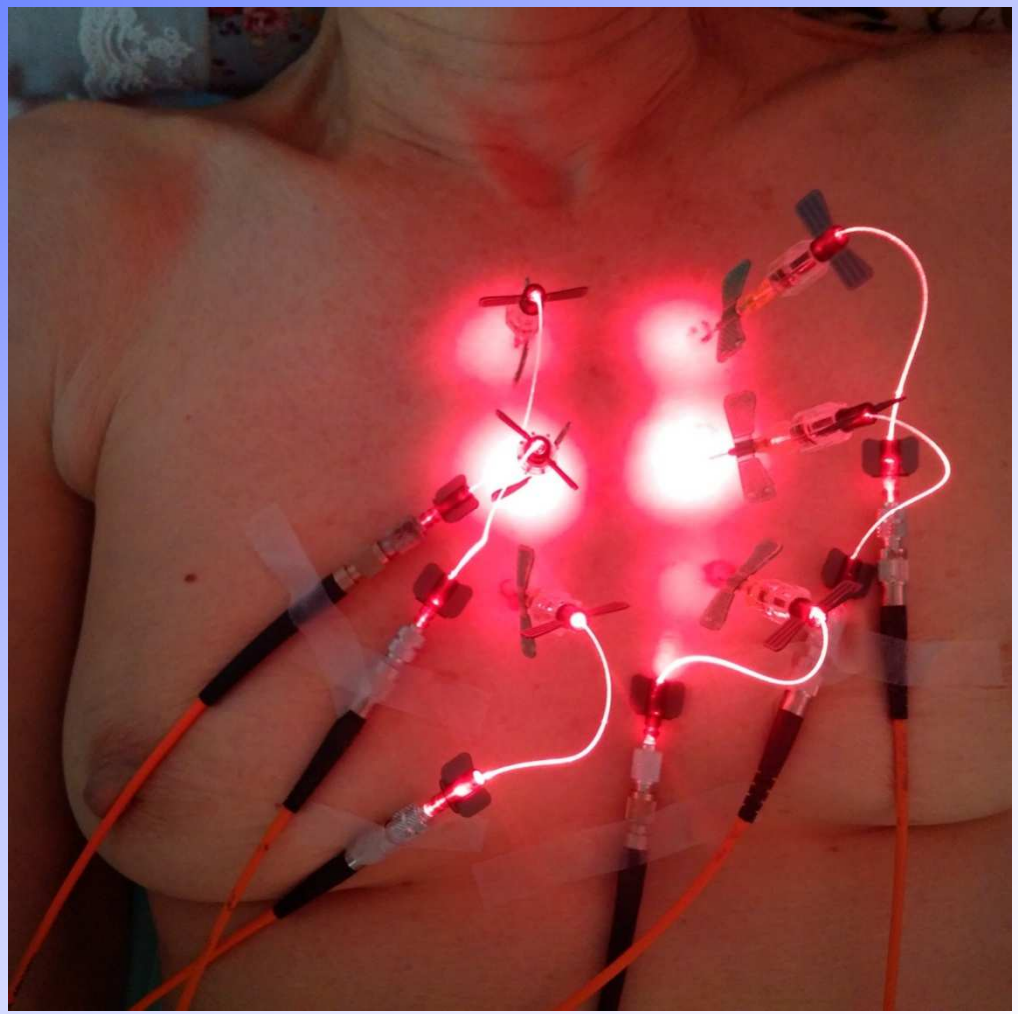
Interstitial PDT of breast cancer with mediastinal lymph metastases



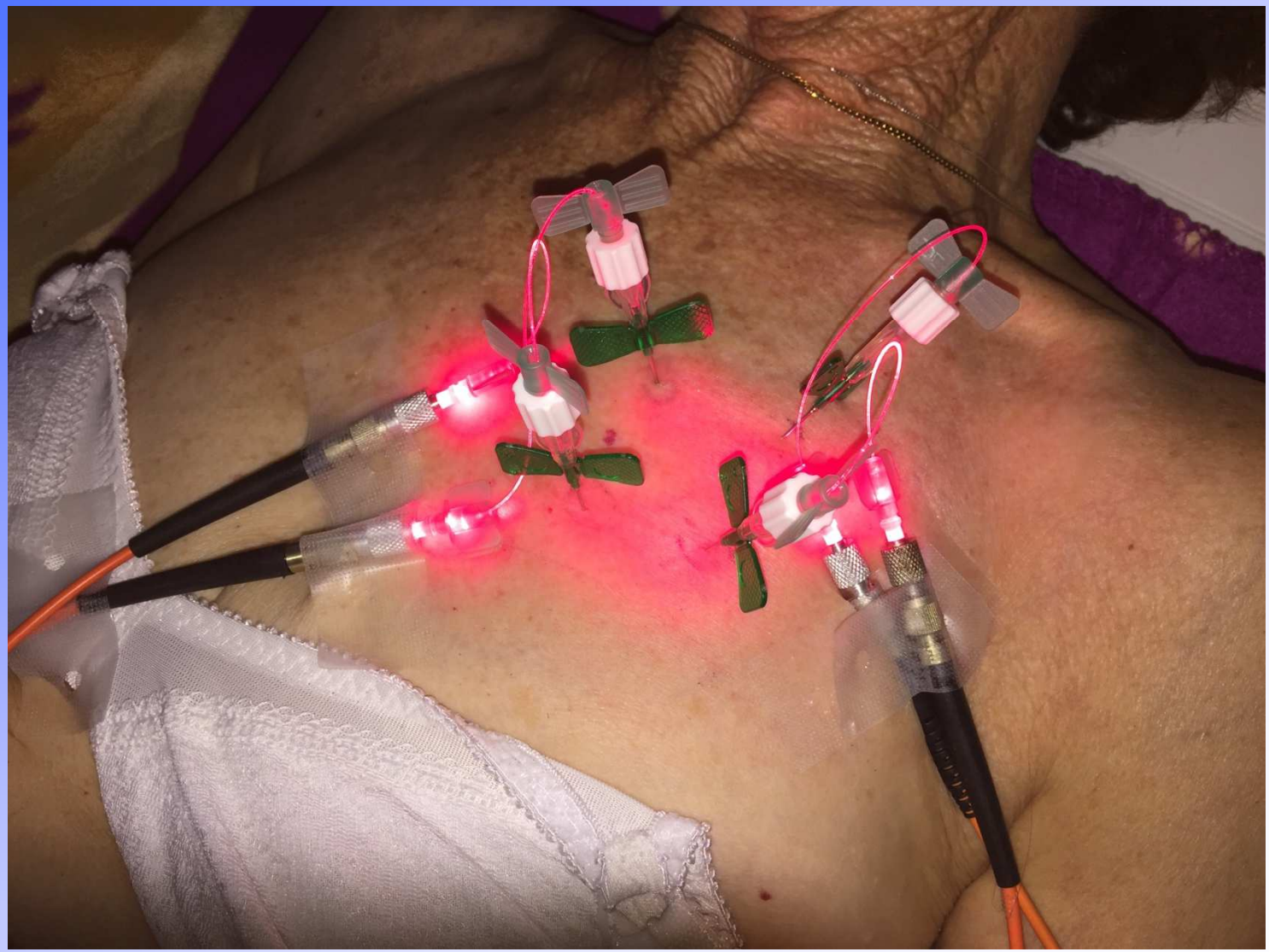
Interstitial PDT of breast cancer with mediastinal lymph metastases



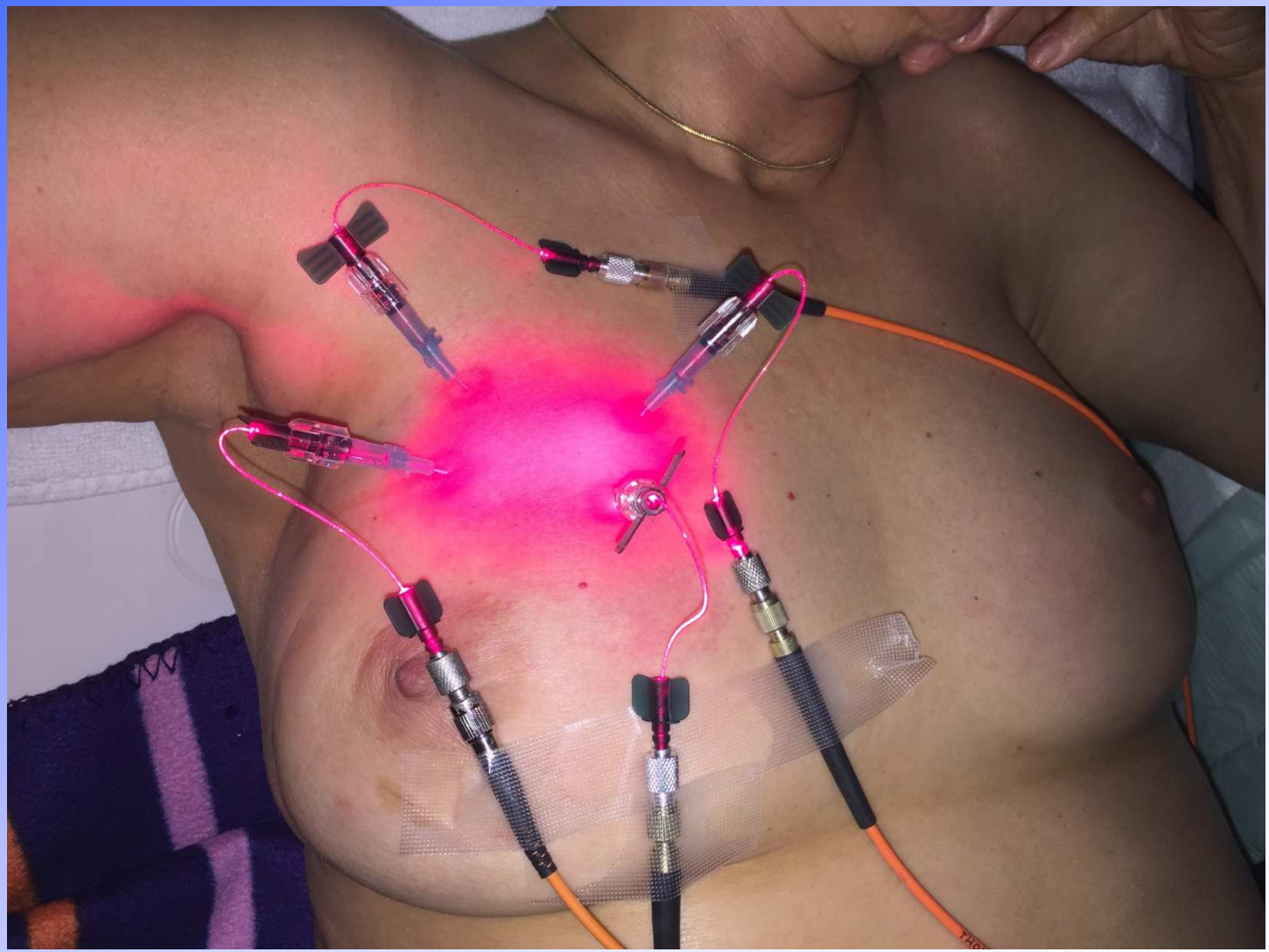
Interstital therapy for mediatinal metastases



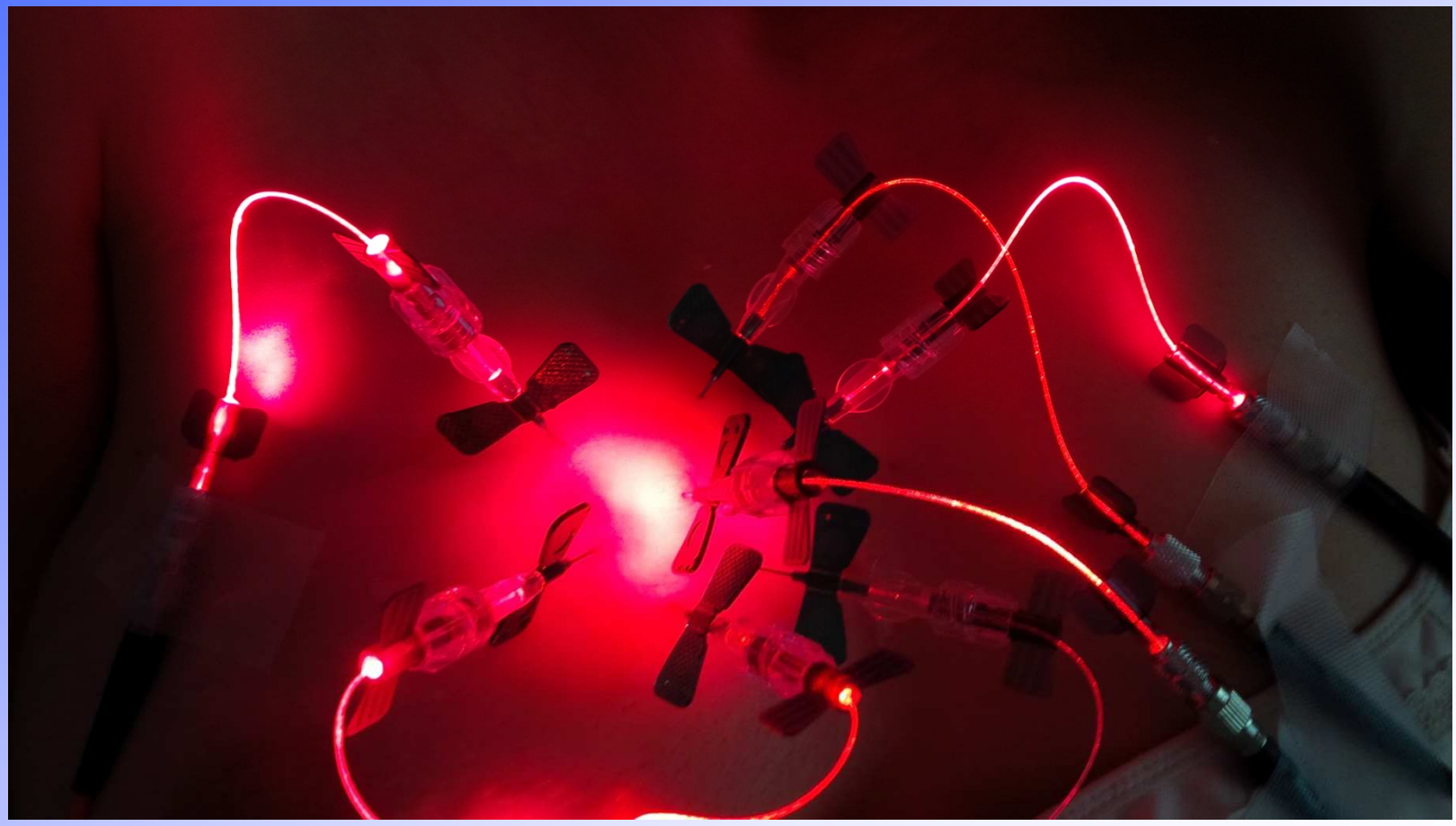
Lung cancer (needles on pleura)



Interstitial PDT of breast cancer

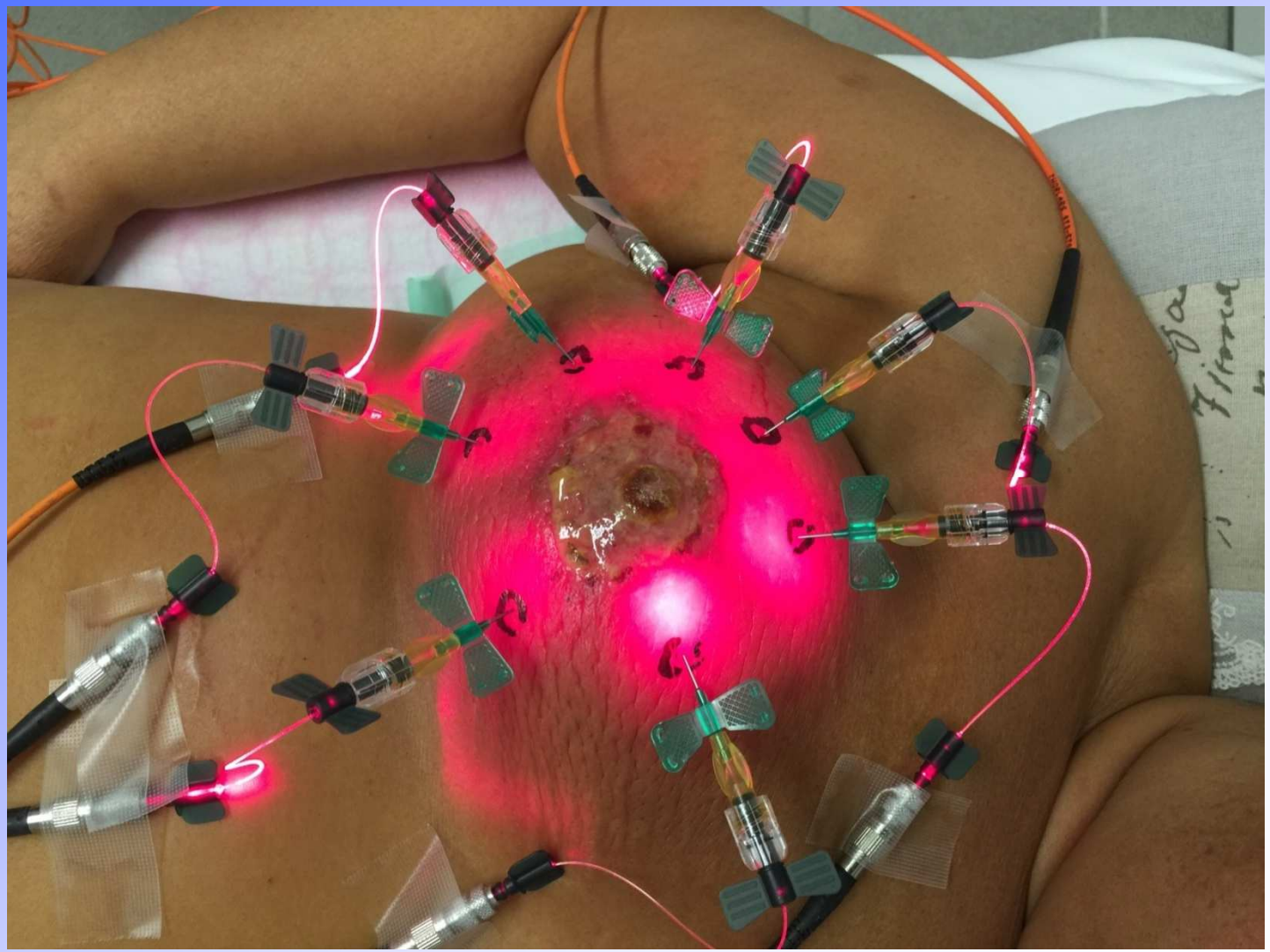


Interstitial breast cancer treatment

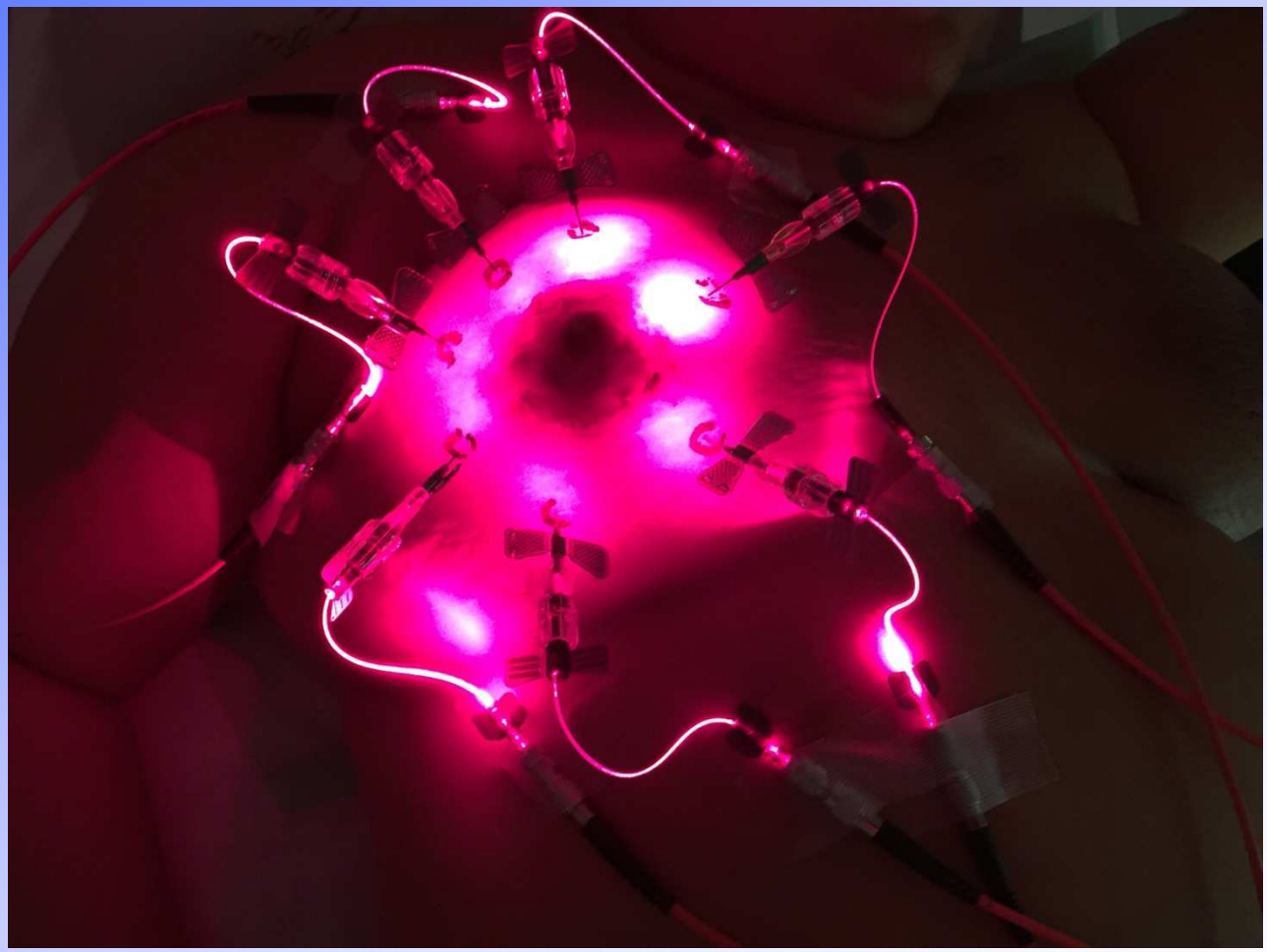


Interstital PDT of breast cancer

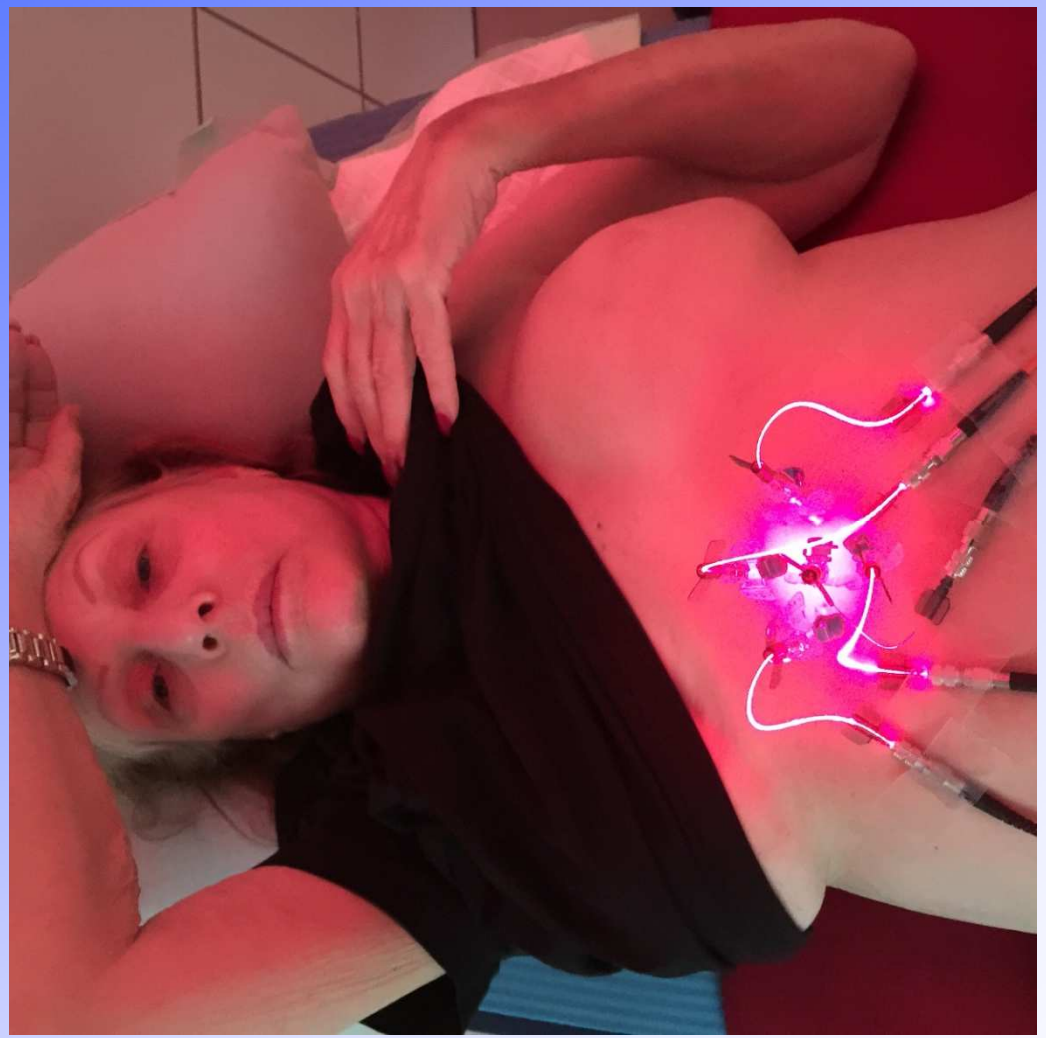
Interstital PDT of breast cancer



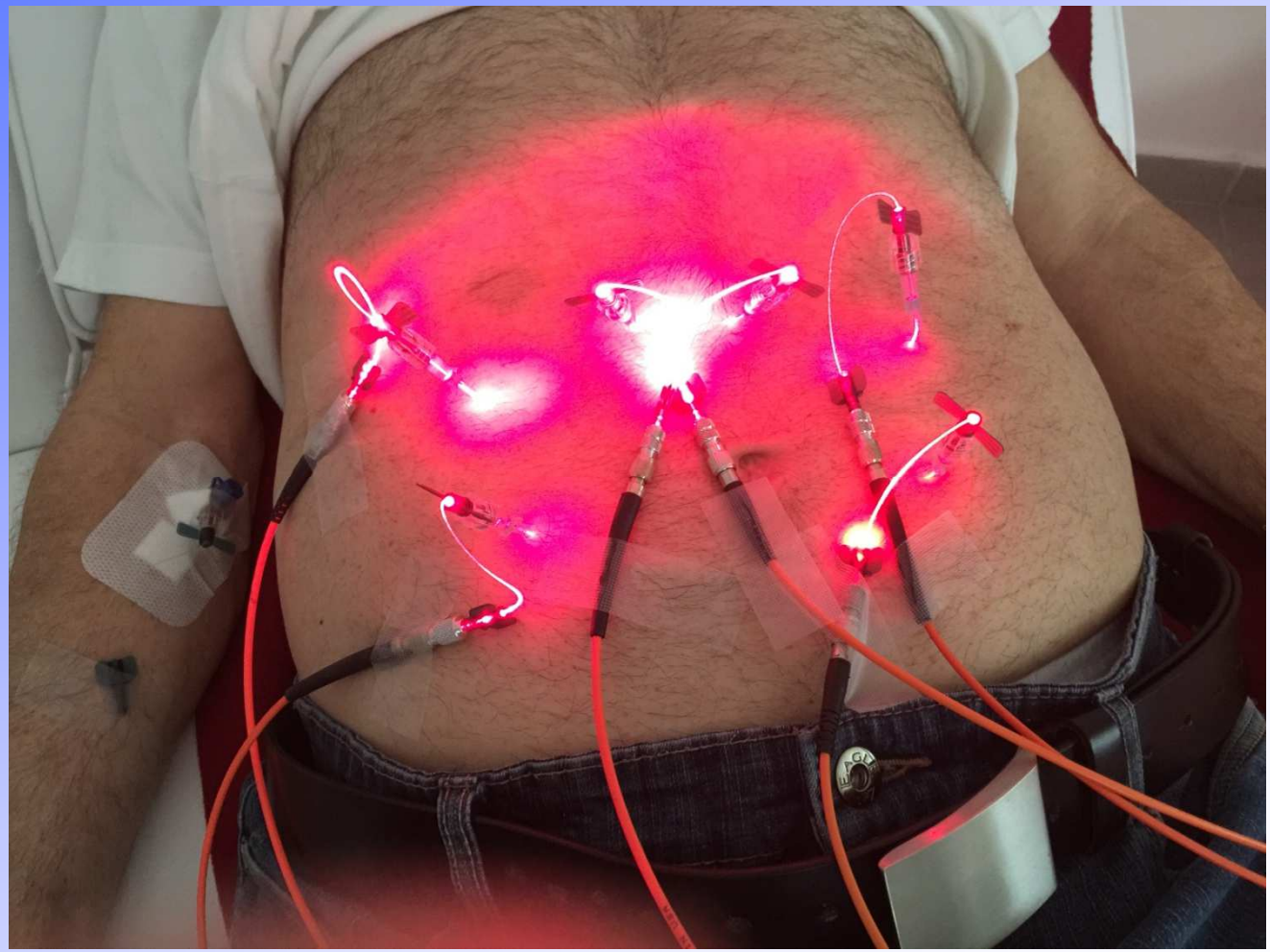
Interstital PDT of breast cancer



Interstitial PDT for pancreatic cancer

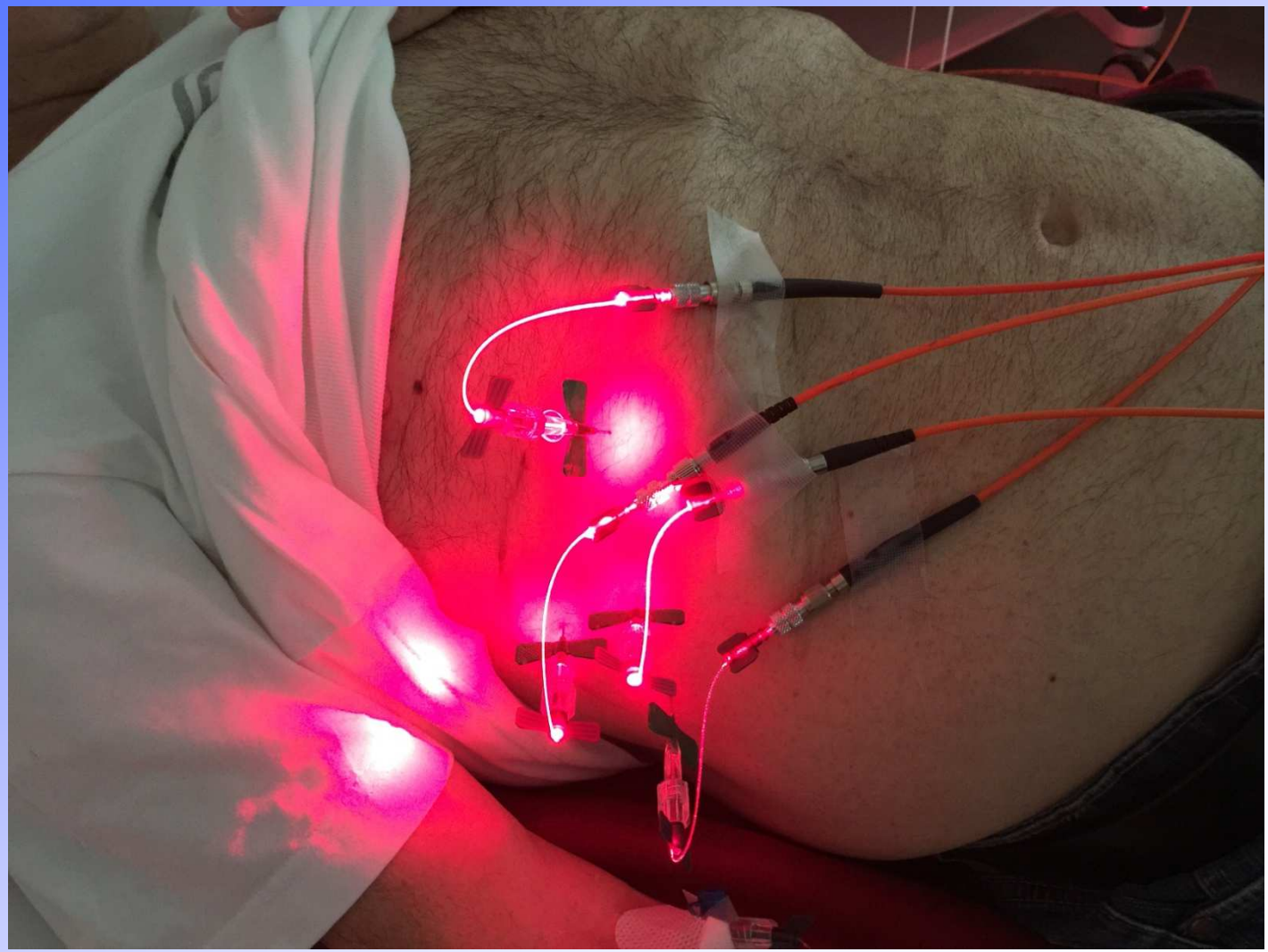


Peritoneal carcinosis



Case report: ovarian cancer with peritoneal carcinosis

Liver metastases

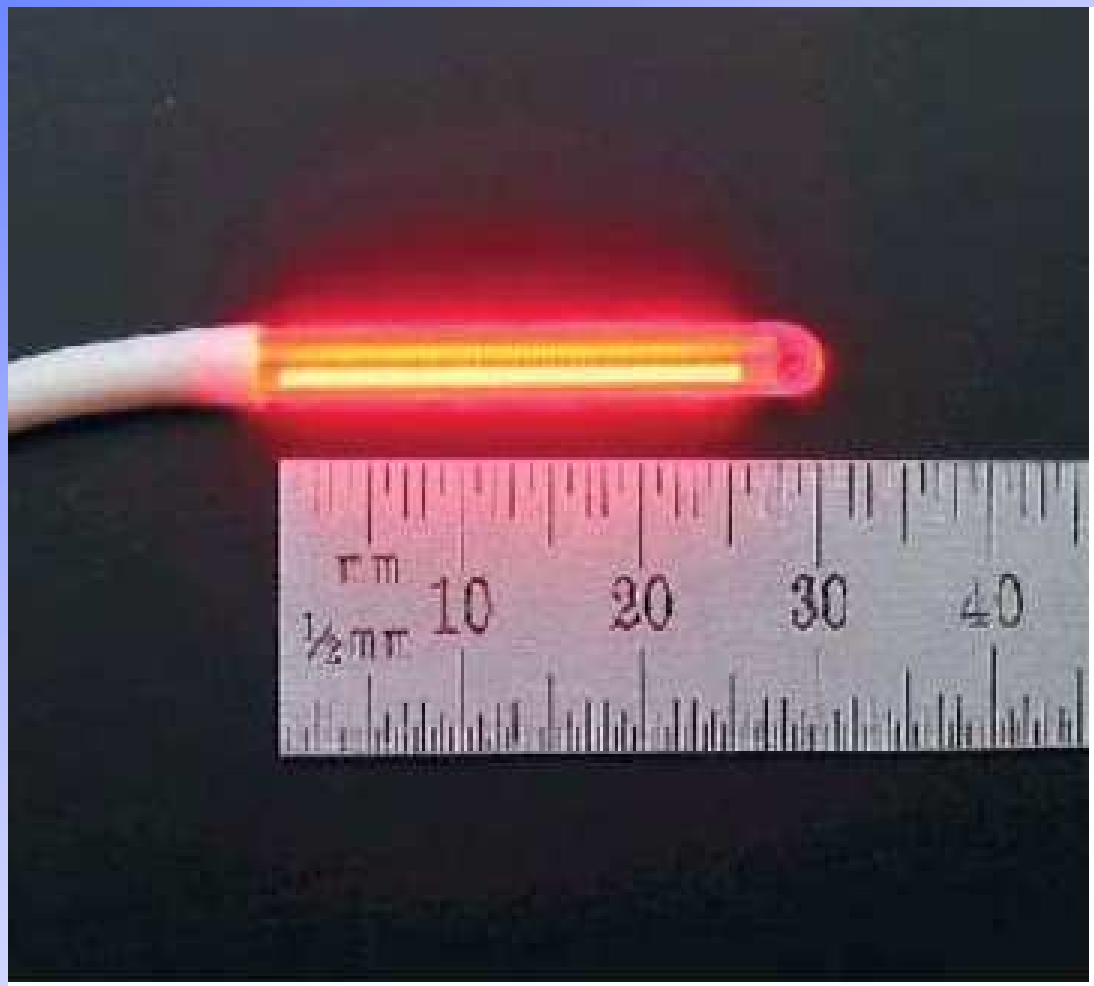


PDT in Urology

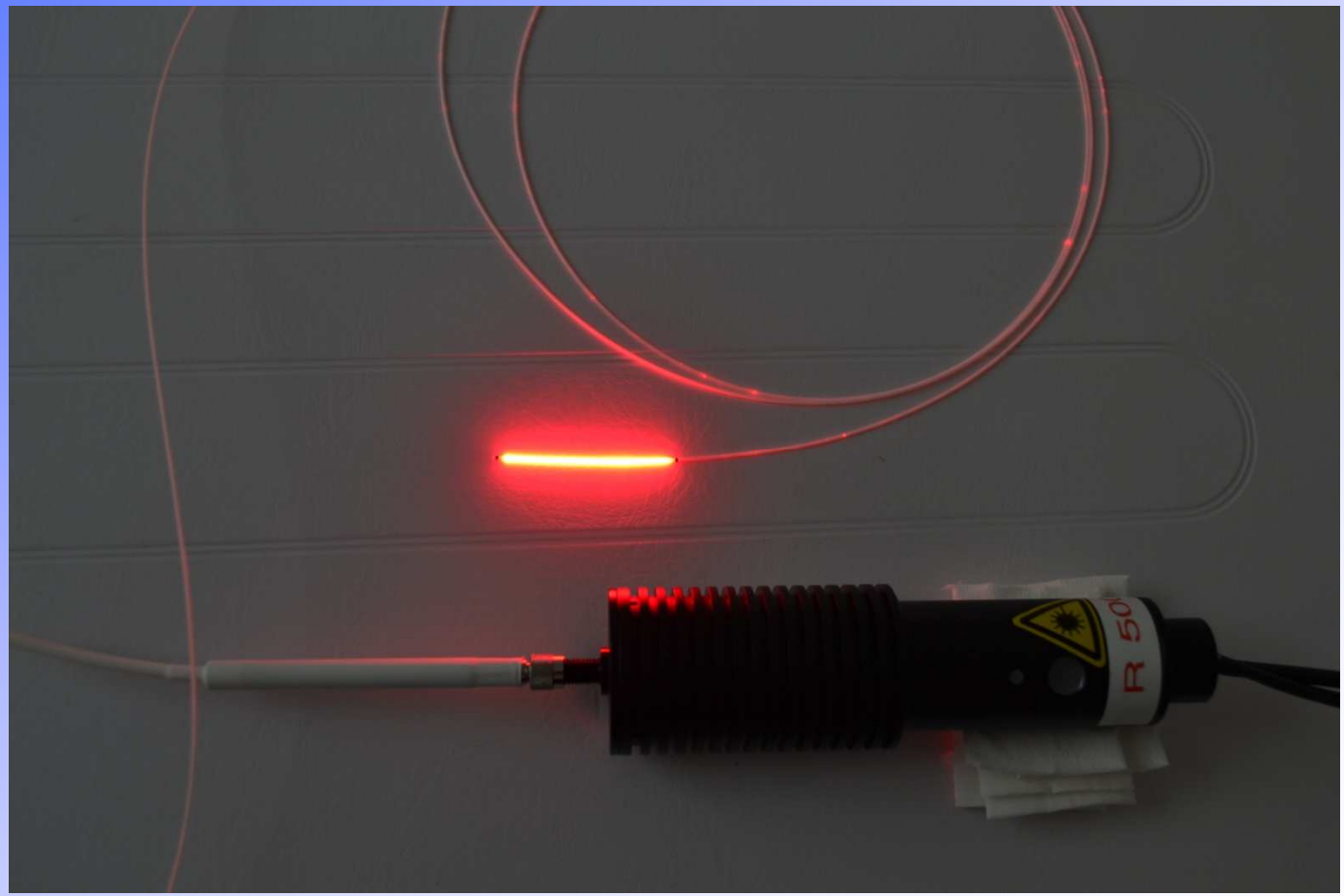
PDT in urology

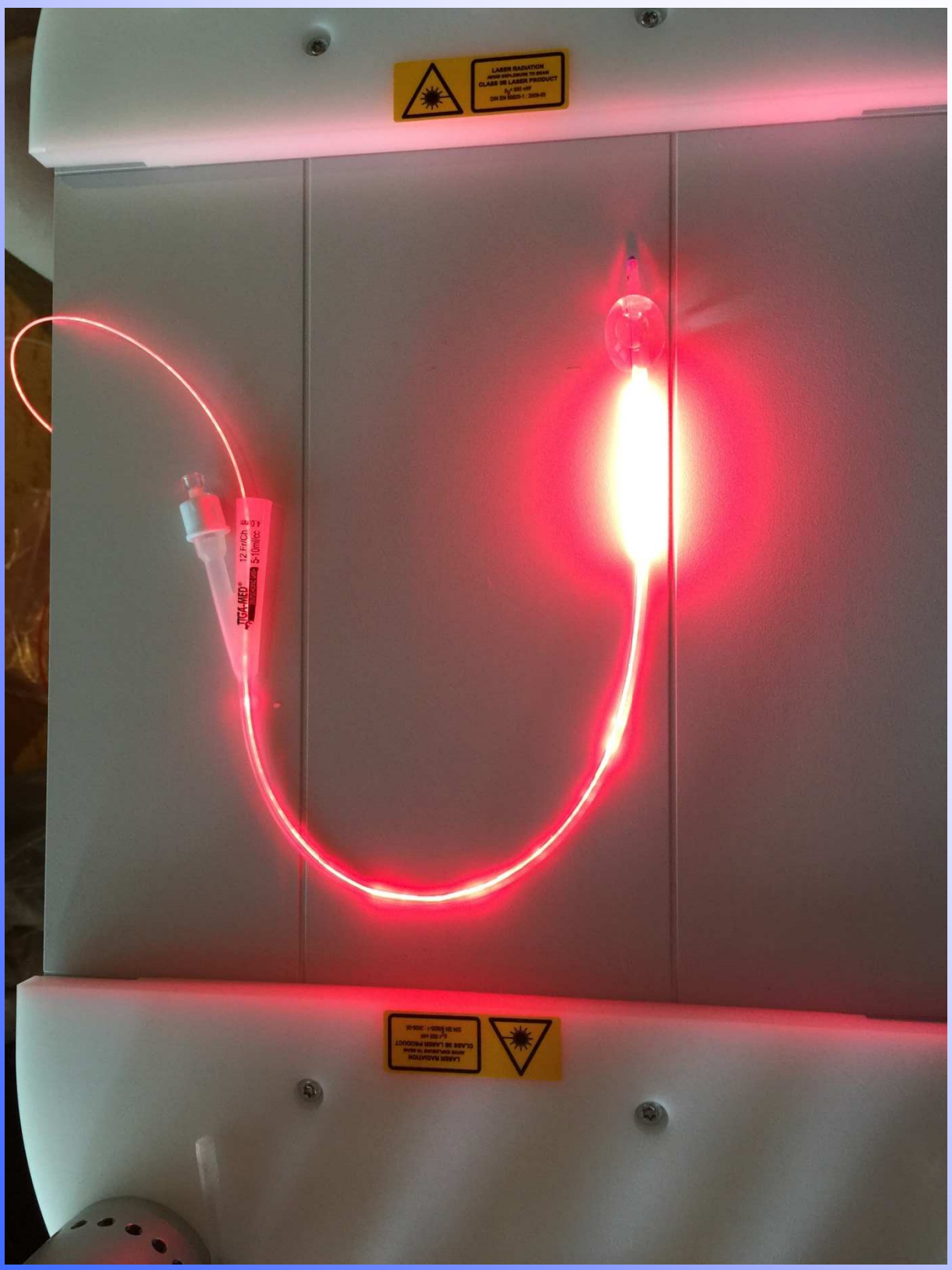
Fiberoptic catheter with circular irradiation (for prostate cancer)

New catheter for bladder and prostate cancer



500 mW Red laser 658 nm

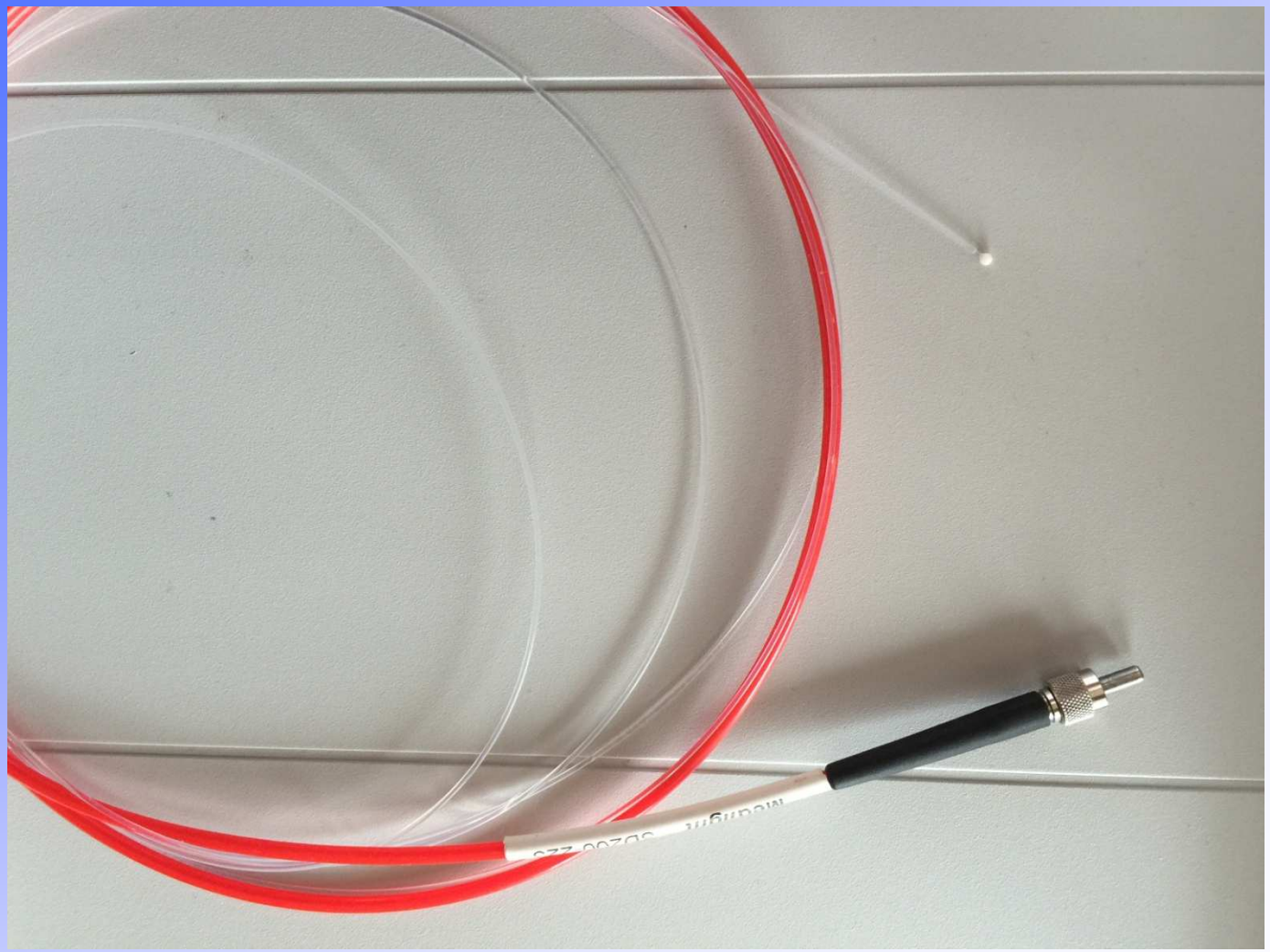


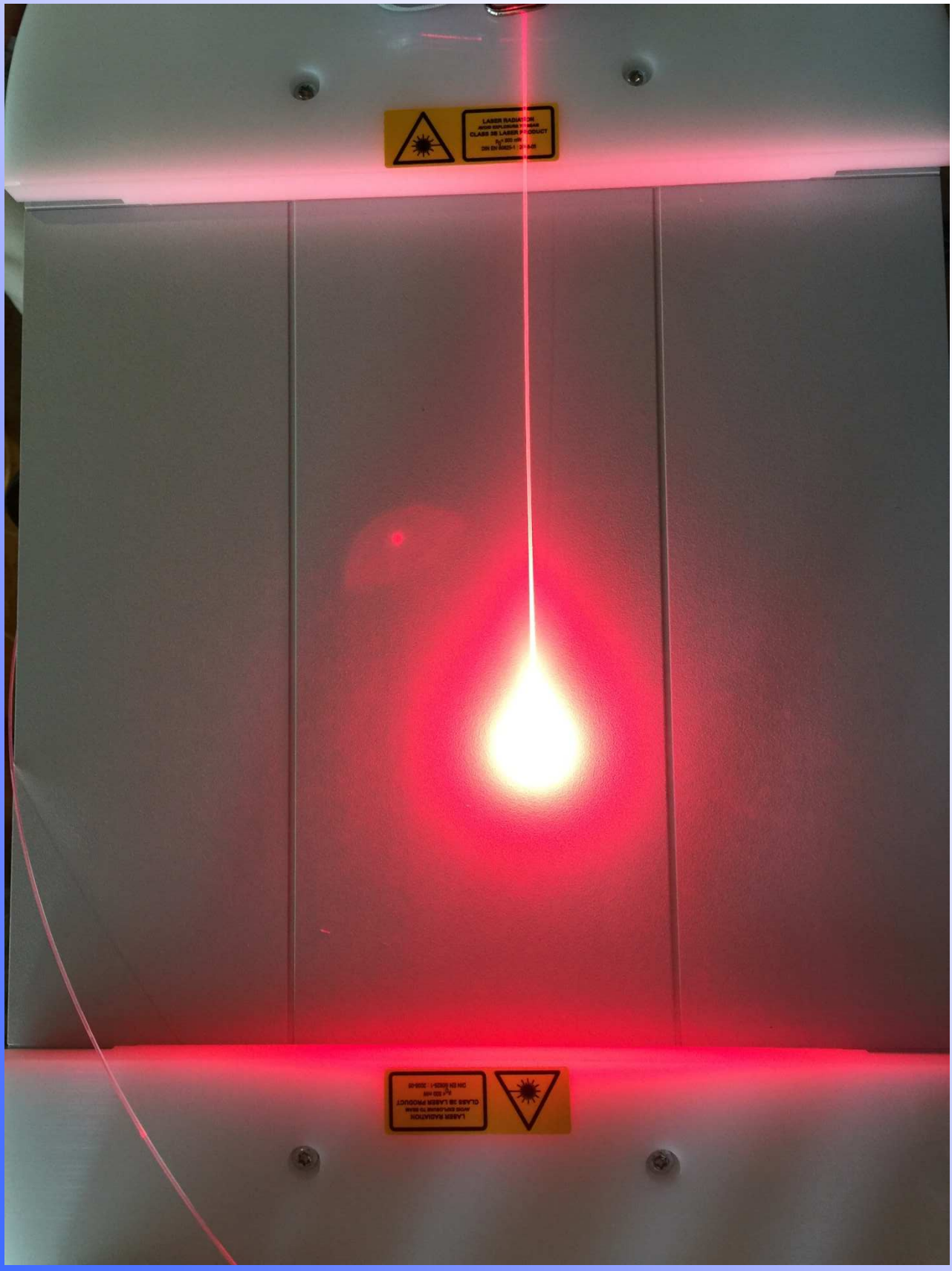

LASER RADIATION
CLASS 3B LASER PRODUCT
12 Fr/Ch
5-10ml/cc
TOTAL MED

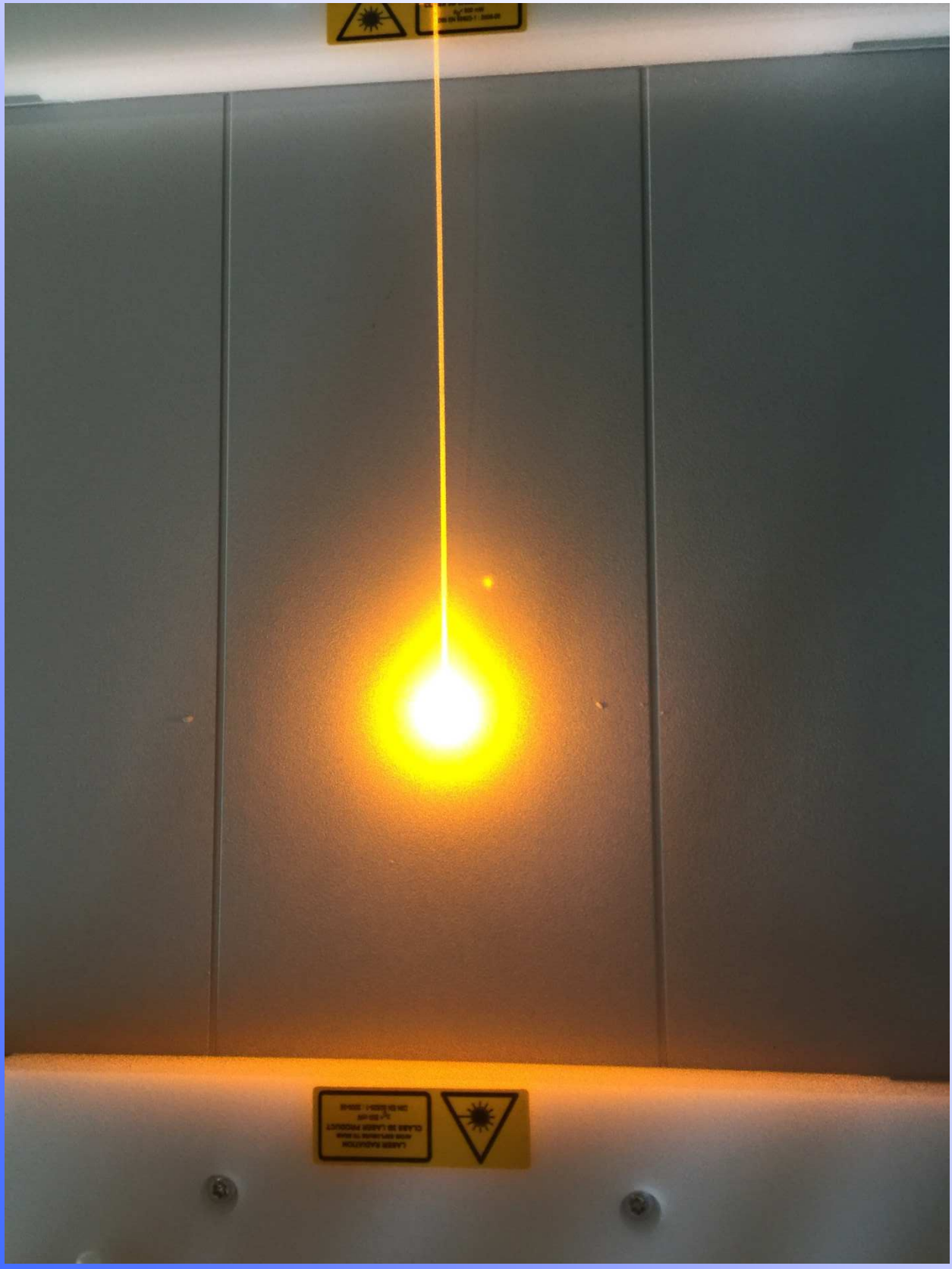

12 Fr/Ch
4,0 mm
5-10ml/cc
TIGA-MED

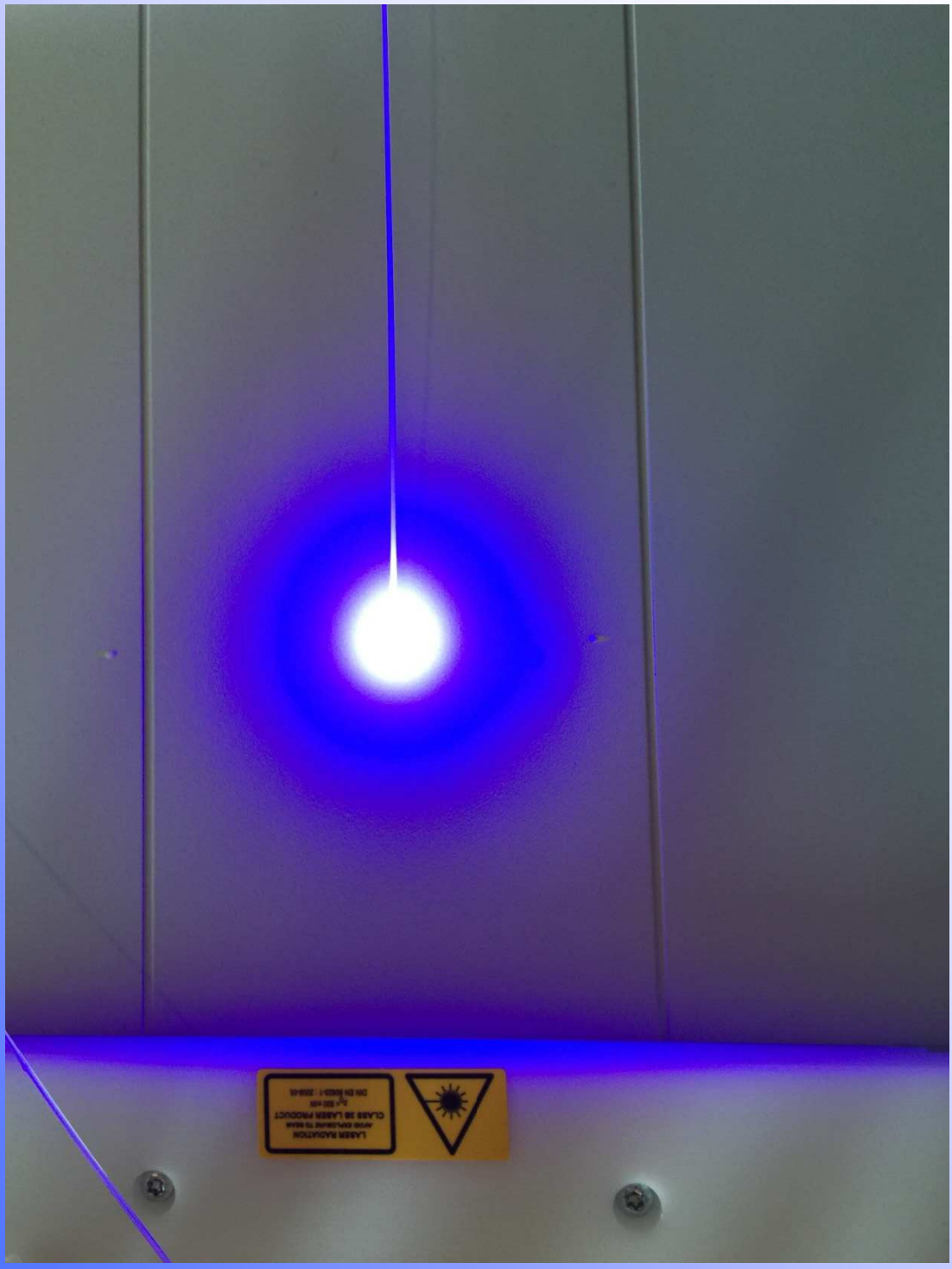

LASER RADIATION
CLASS 3B LASER PRODUCT

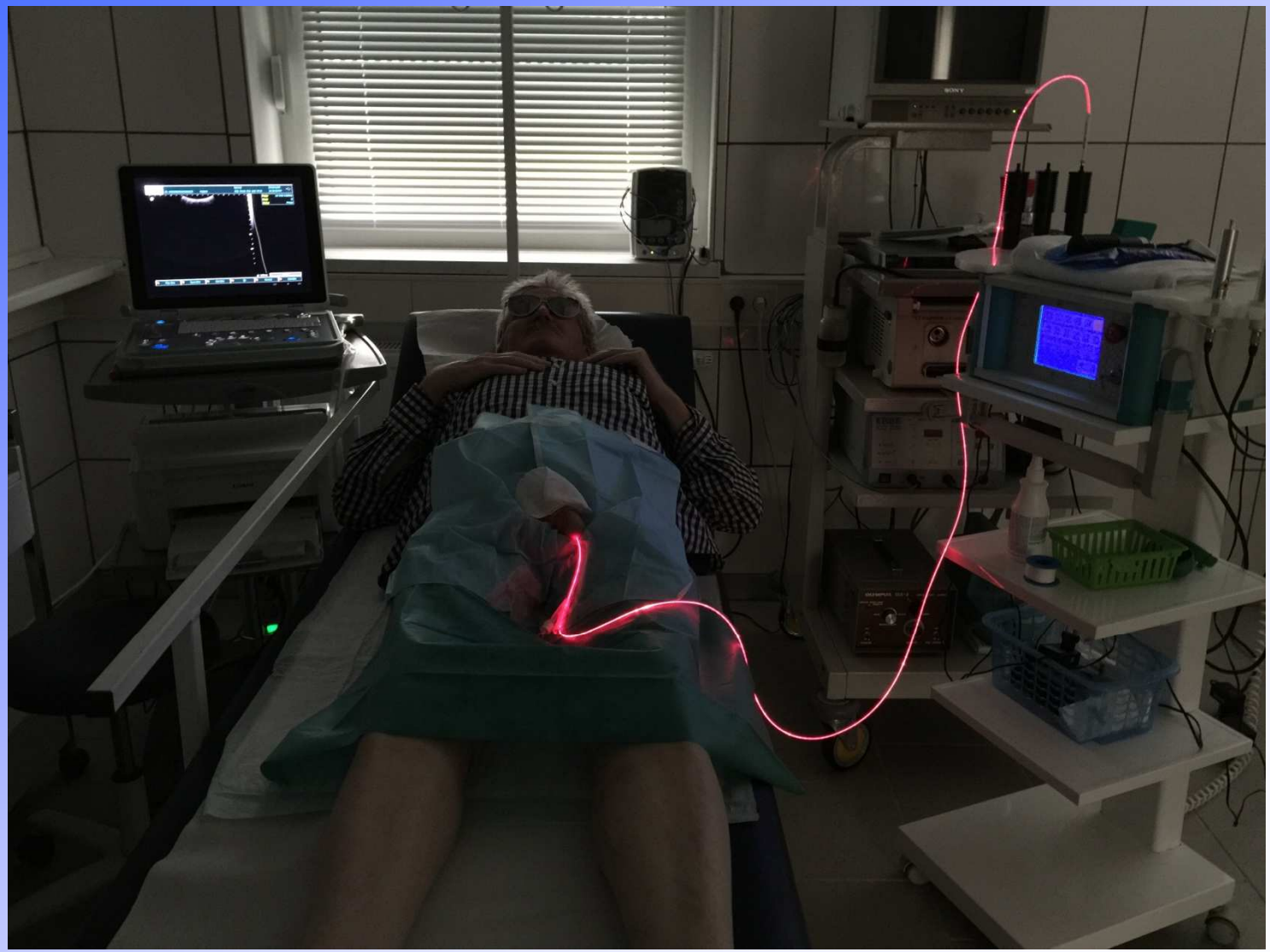
Fiberoptic catheter with spheric irradiation for bladder cancer



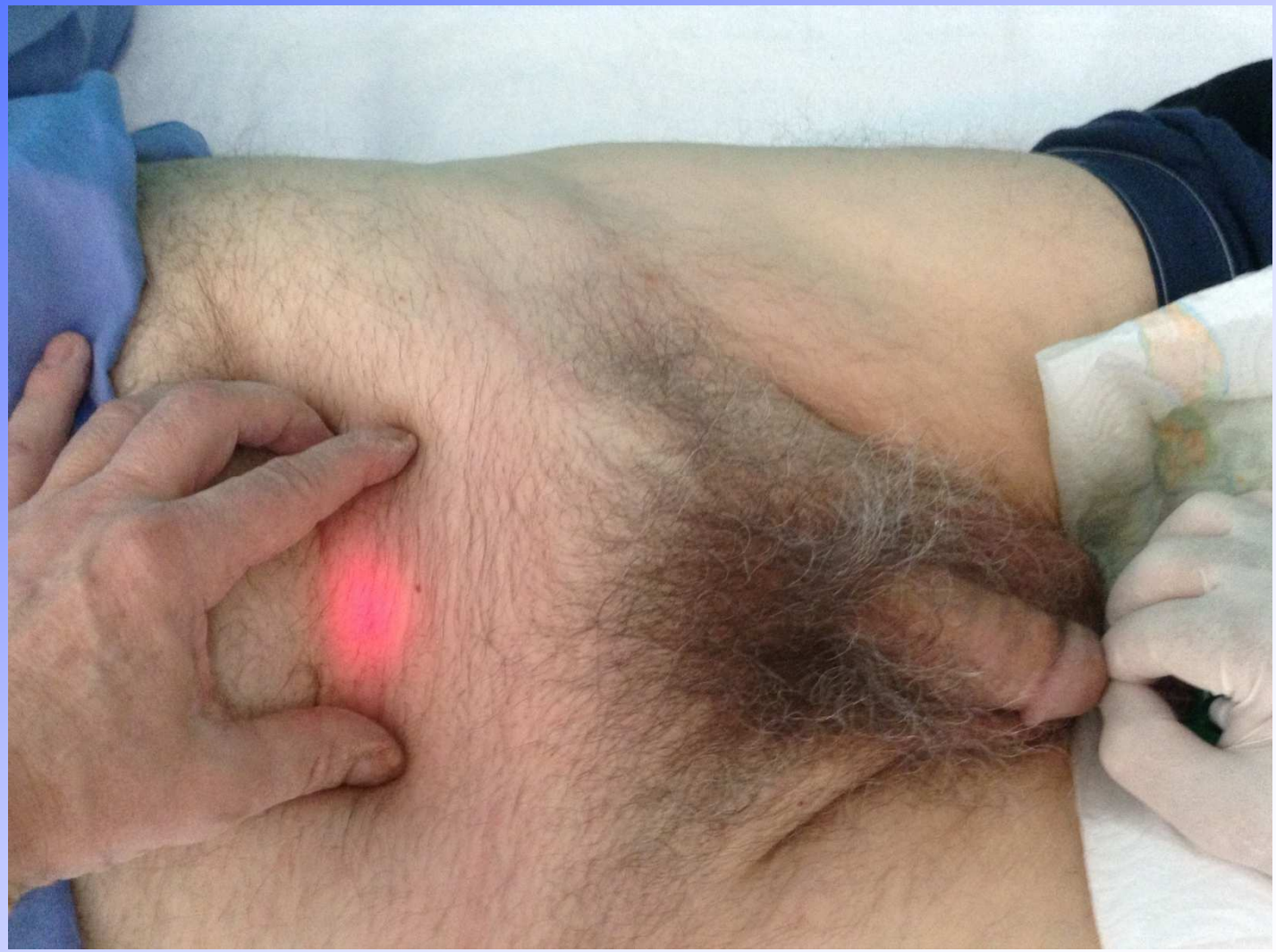




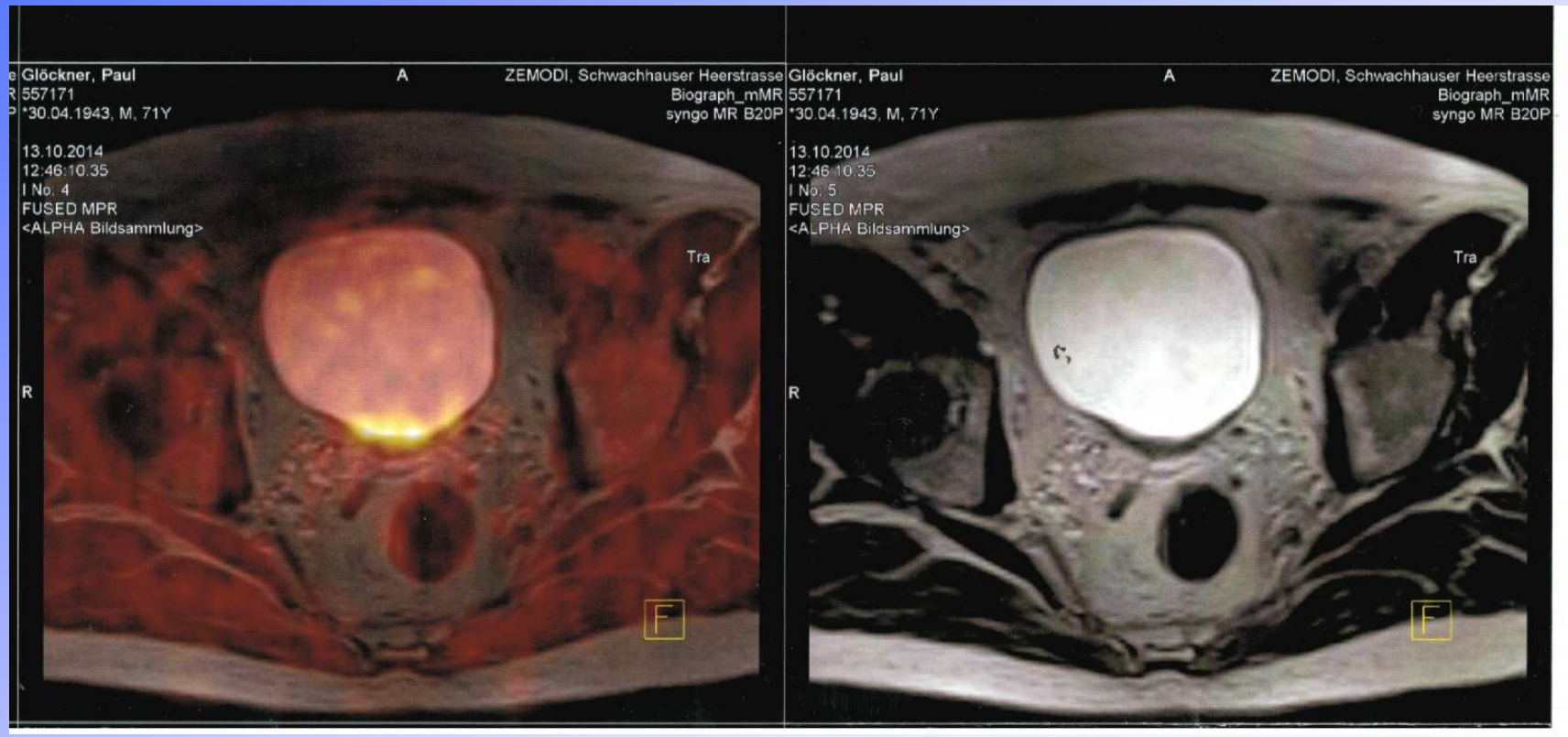

LASER RADIATION
CLASS 3R LASER PRODUCT



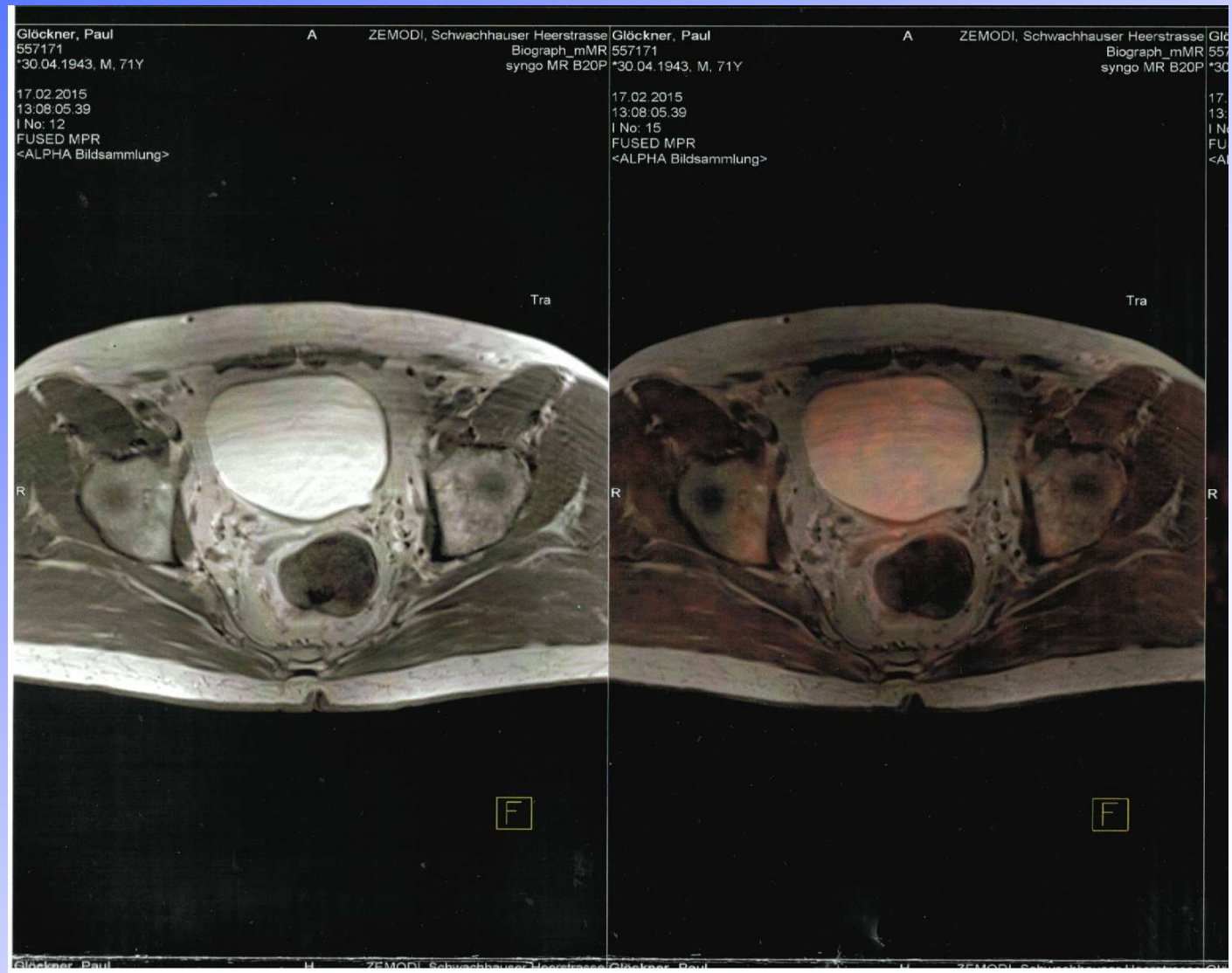
Bladder Cancer



Bladder cancer PET 10/2014 before treatment

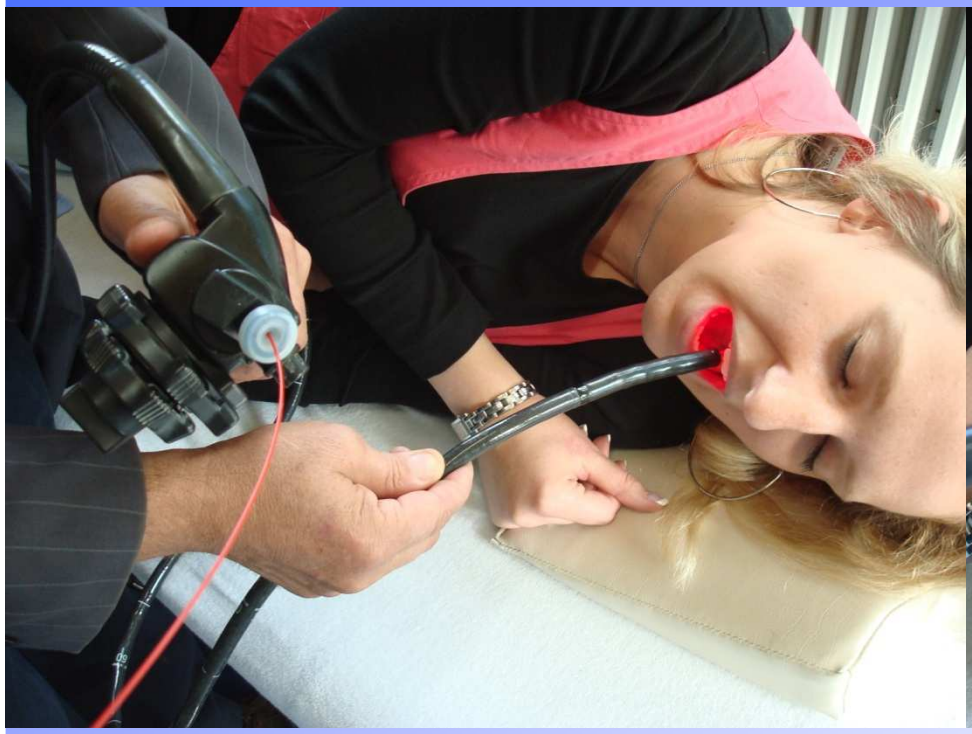


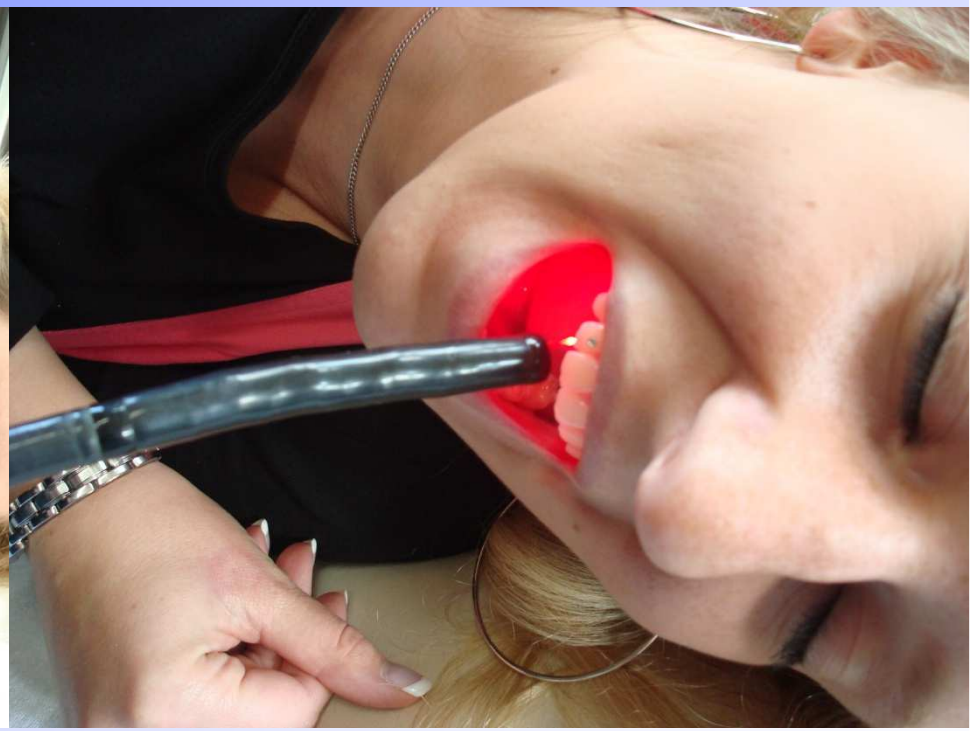
Bladder cancer 2/2015 after PDT



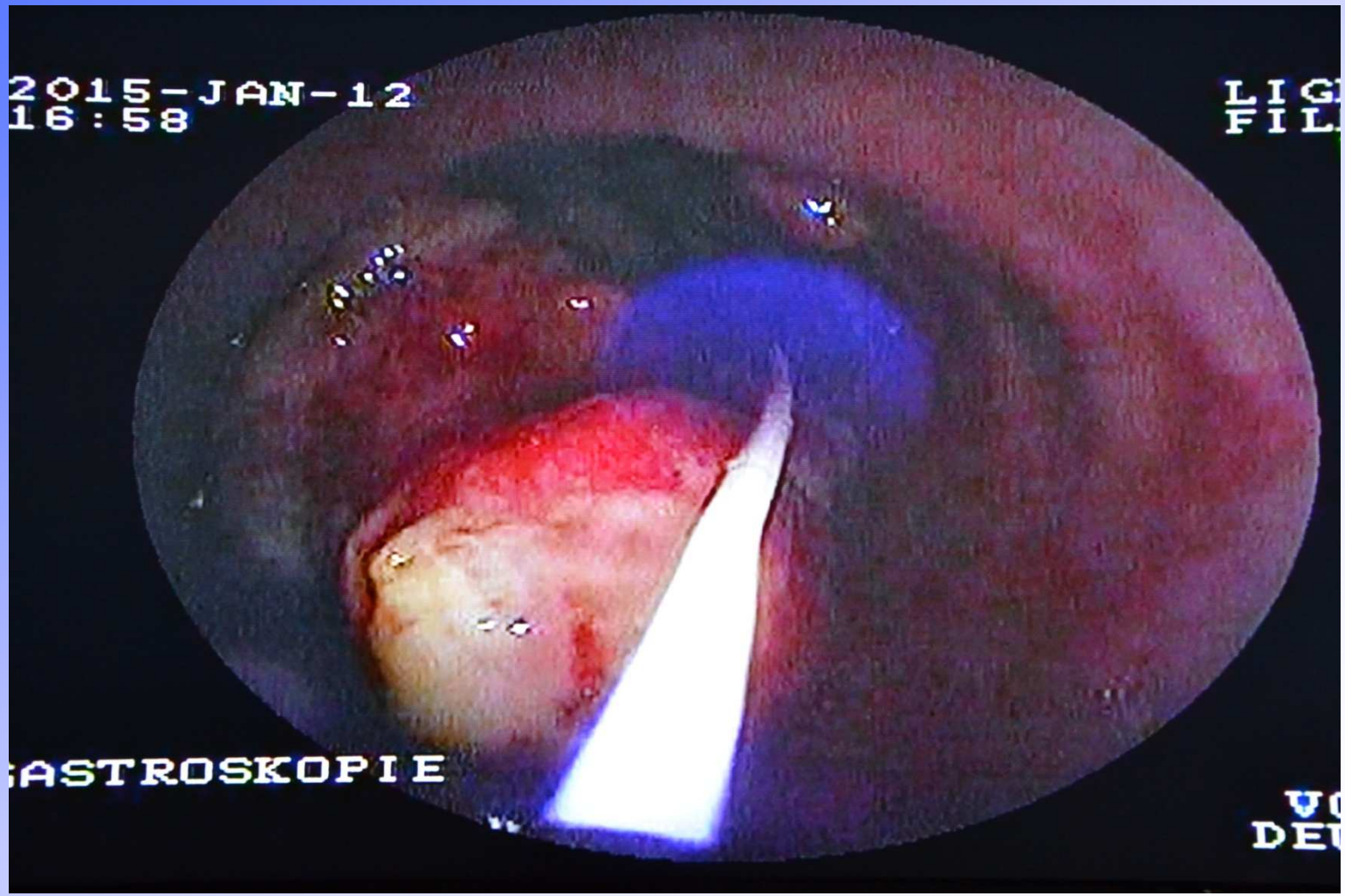
Endoscopic PDT

Endoscopic PDT

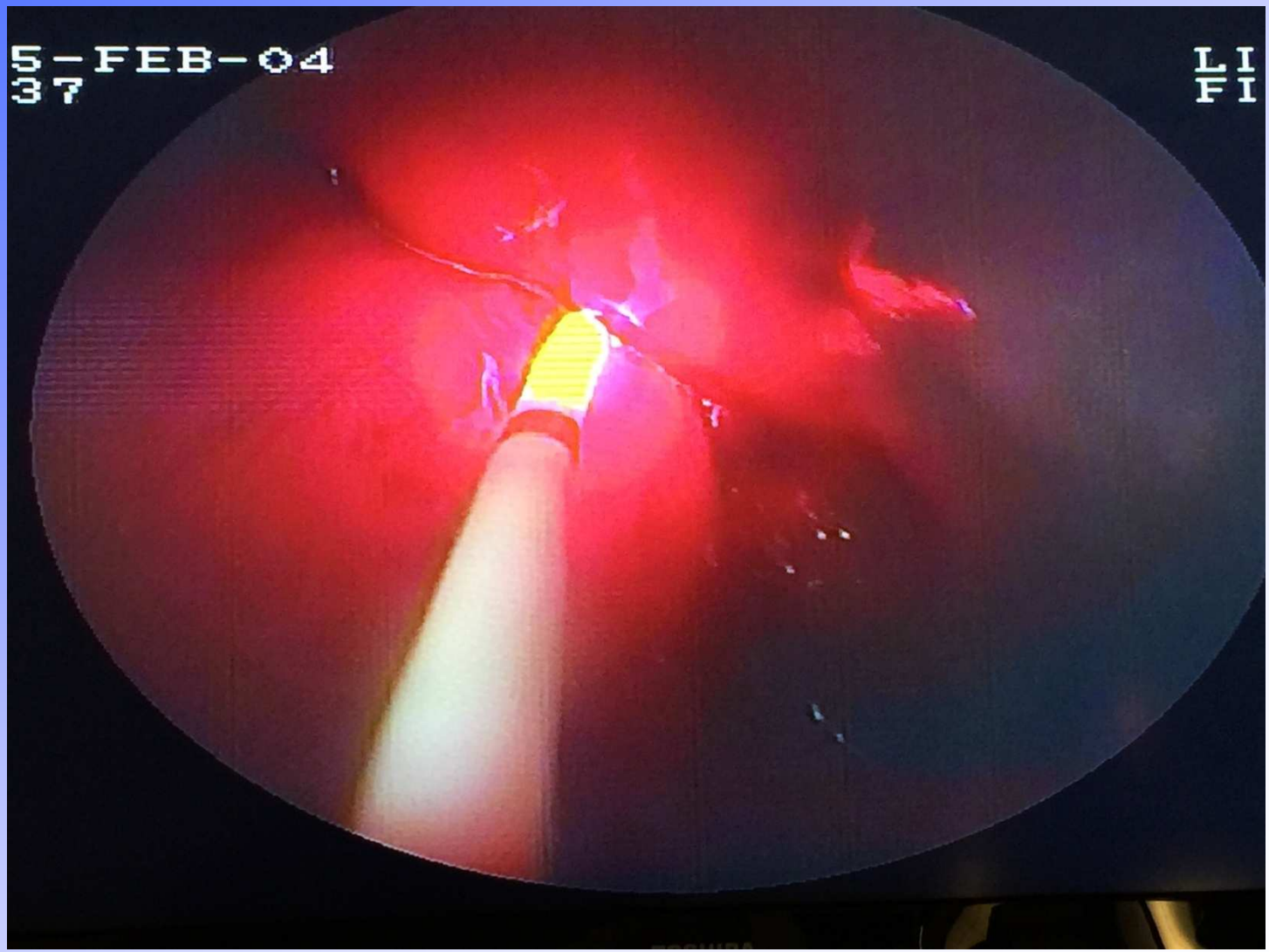




Endoscopic PDT



Endoscopic PDT



The big problems still remain:

- Limited succes by using red laser only
- Limited penetration depth (max. 2,5 cm)
- Limited tumor size: max 2,5 cm
- Burning and ulceration with overdosage
- Light sensitivity
- No good success with liver metastases
- Limited success for bone metastases
- No success in treatment of brain tumors

The solution: liposmal Indocyanine Green

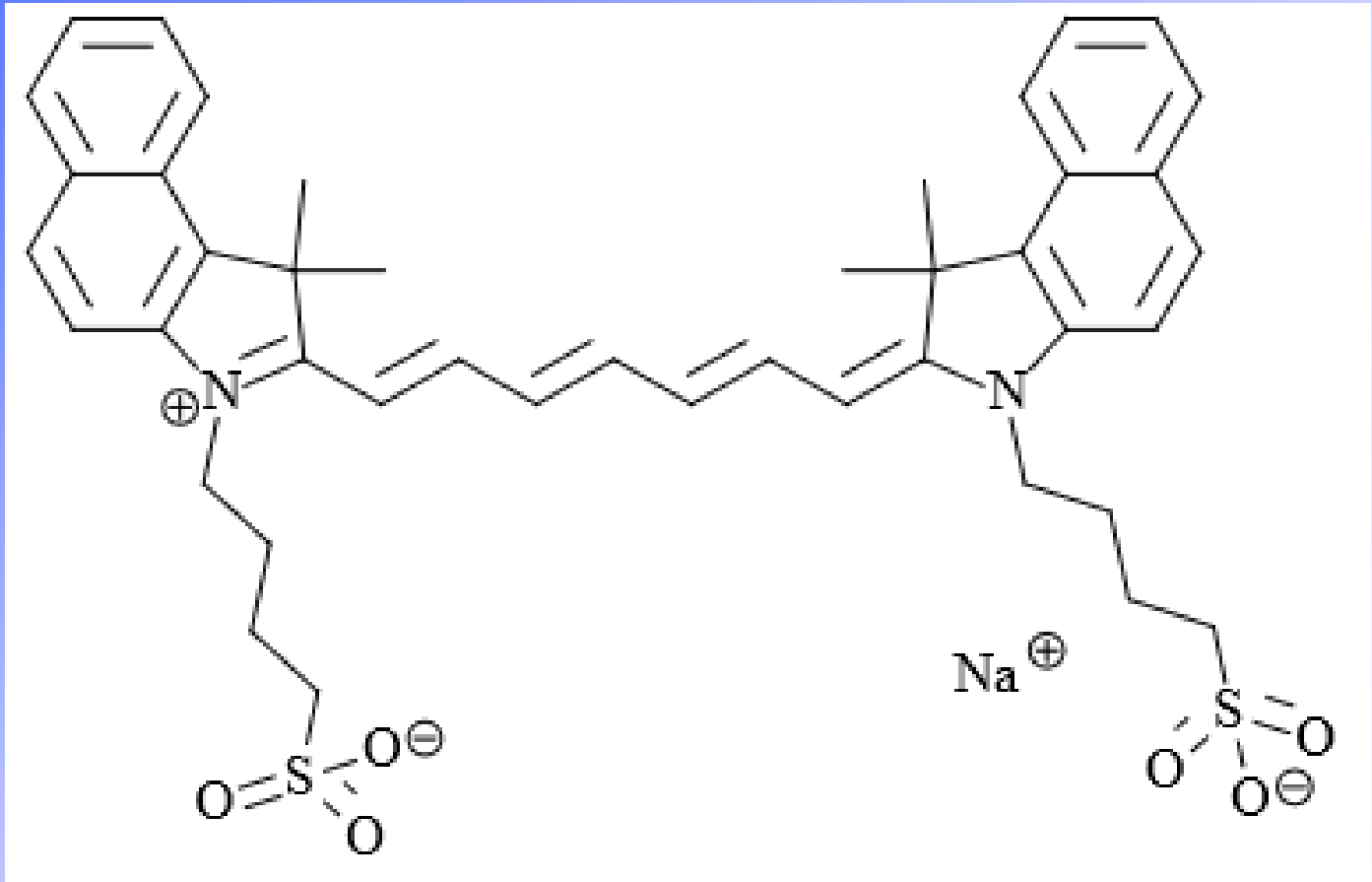
- **Indocyanine Green** is a fluorecent green dye and absorbs light in the infrared range (810 nm)
- It is applied intravenously
- Indocyanine Green is an approved drug used for fluorescense diagnostics (blood flow in eyes, liver heart) even FDA approved in the USA

Indocyanine Green liposomal as a new photosensitizer

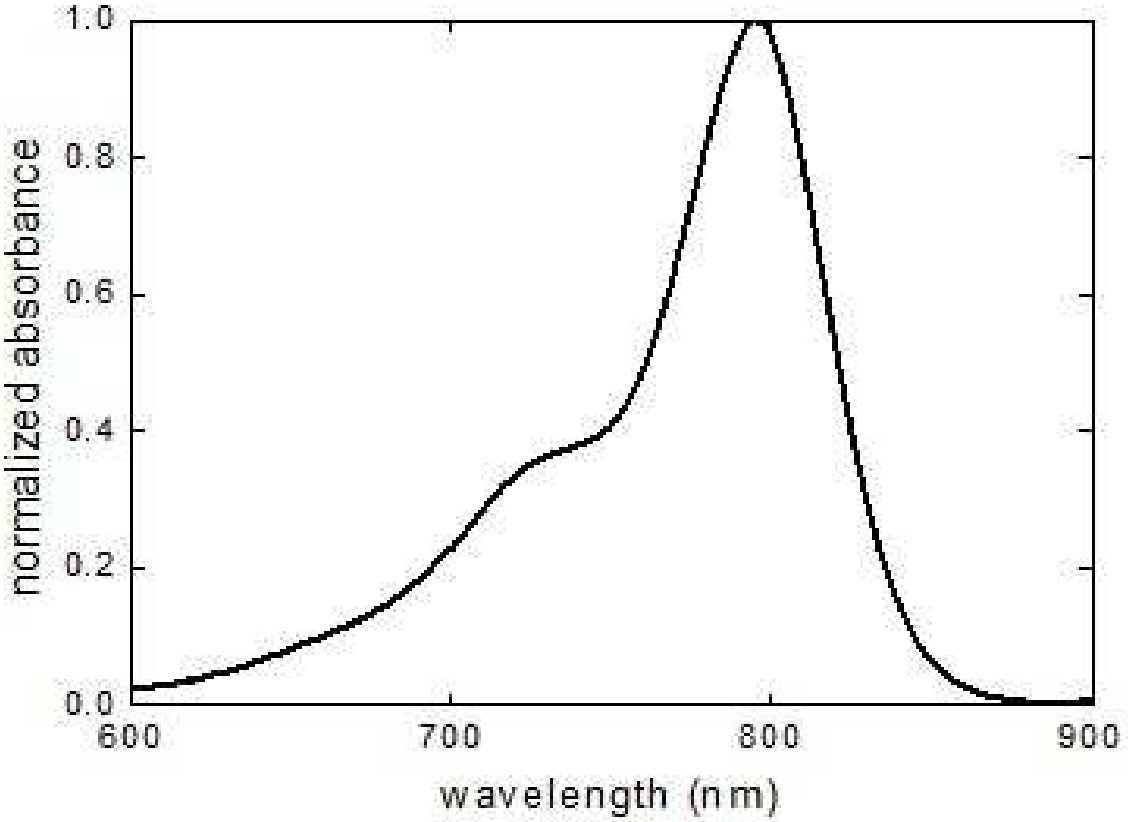
- Pure Indocyanine Green binds to plasma proteins and is removed from the body in about 30 minutes and cannot be used as photosensitizer

- In liposomale form however it will be in integrated in tumor cells and can so be used for PDT with infrared laser

Indocyanine Green, chemical structure



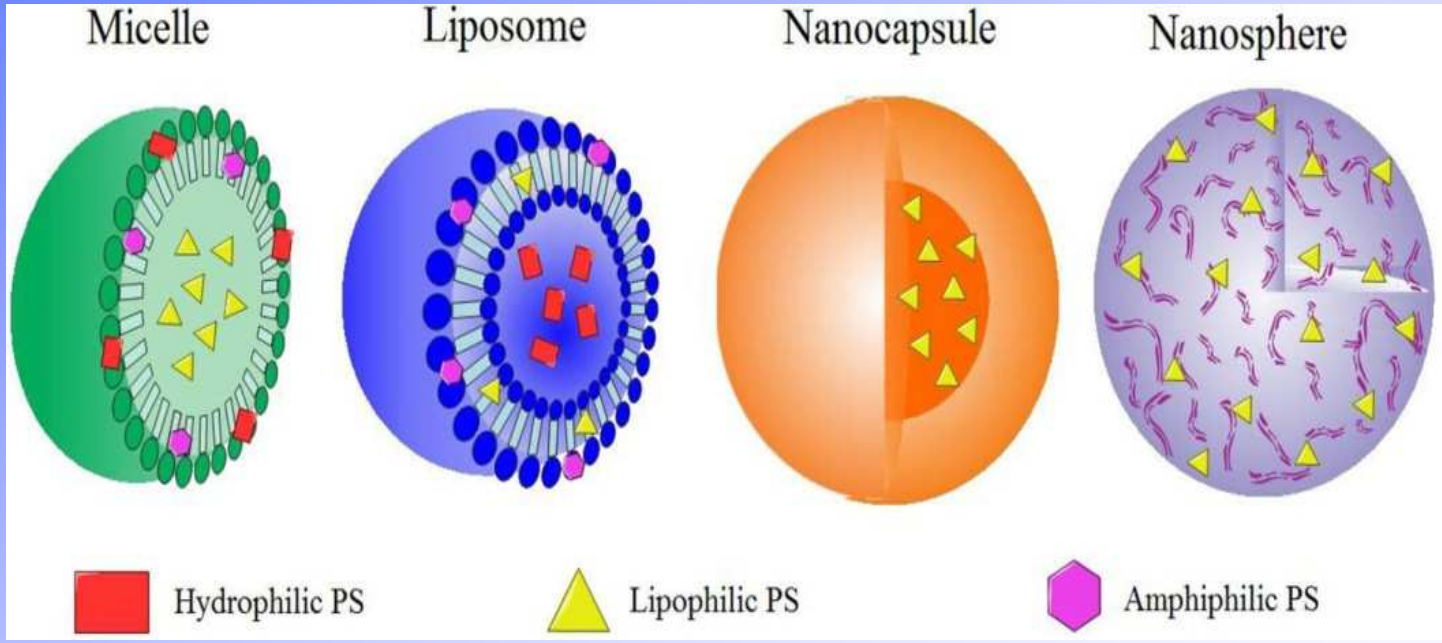
Indocyanine Green, absorption spectrum



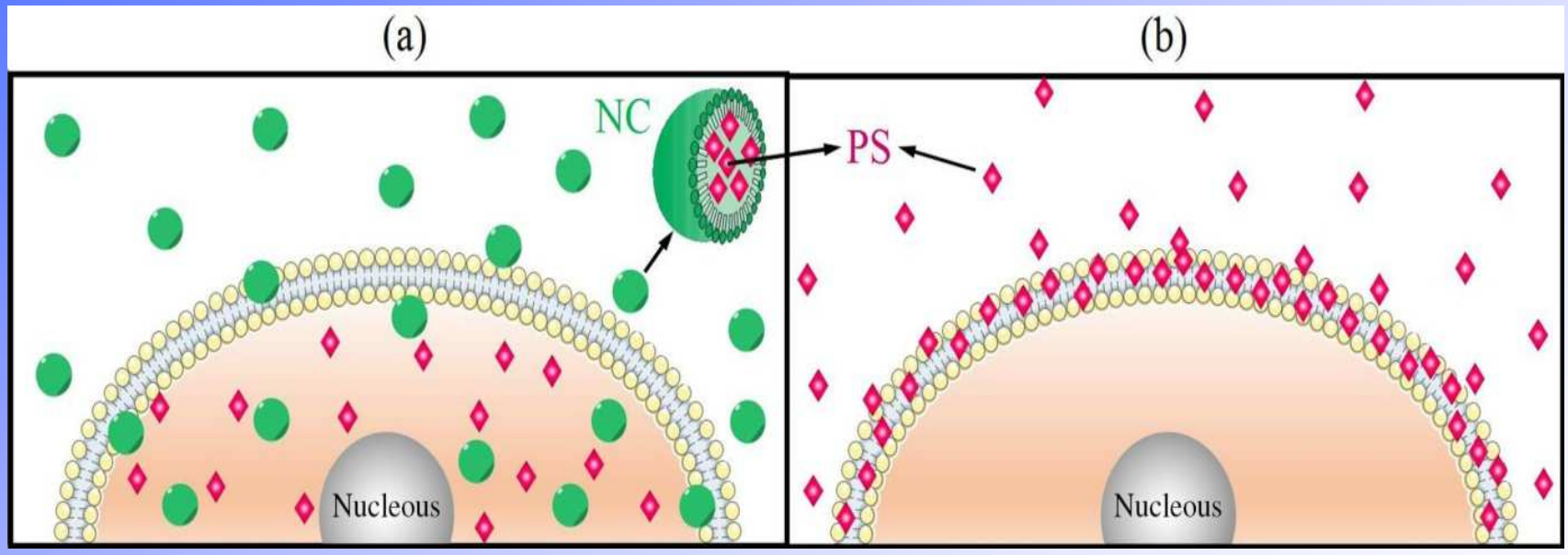
Indocyanine Green as photosensitizer

A new option for improved tumor targeting and uptake is the formulation of ICG in nanopartikels like liposomes .

Nanoparticles for transport of photosensitizers



Cellular integration of a lipophile photosensitizer



Selektive "Over-heating" of tumor tissue by infrared stimulated Indocyanine Green

- ICG absorbs infrared light 810 nm.
- Infrared light has the highest penetration depth in the tissue. Besides activation of the ICG with production of **singlet oxygen** tumor tissue will be warmed up

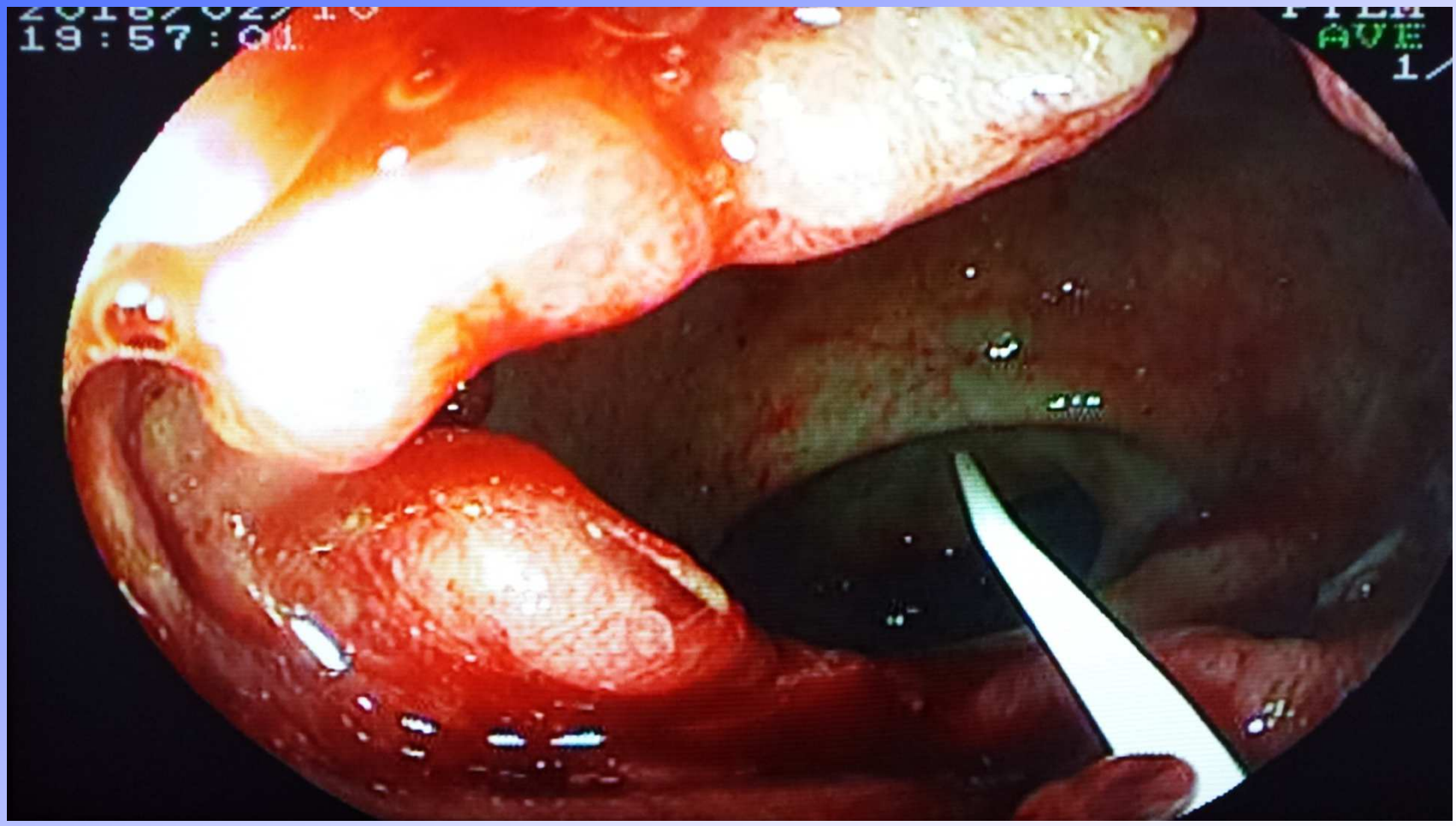
  (**overheating effect**) and so supports the photodynamic reaction without damage of surrounding healthy tissue.
- The combination of overheating and PDT leads to an improved reaction with „tumor melting",
- We can call it **„Photothermodynamic therapy (PTDT)"** or **„Photothermoablation"** of tumor tissue**.** [21]

Indozyanine green liposomal

Indozyanine green liposomal

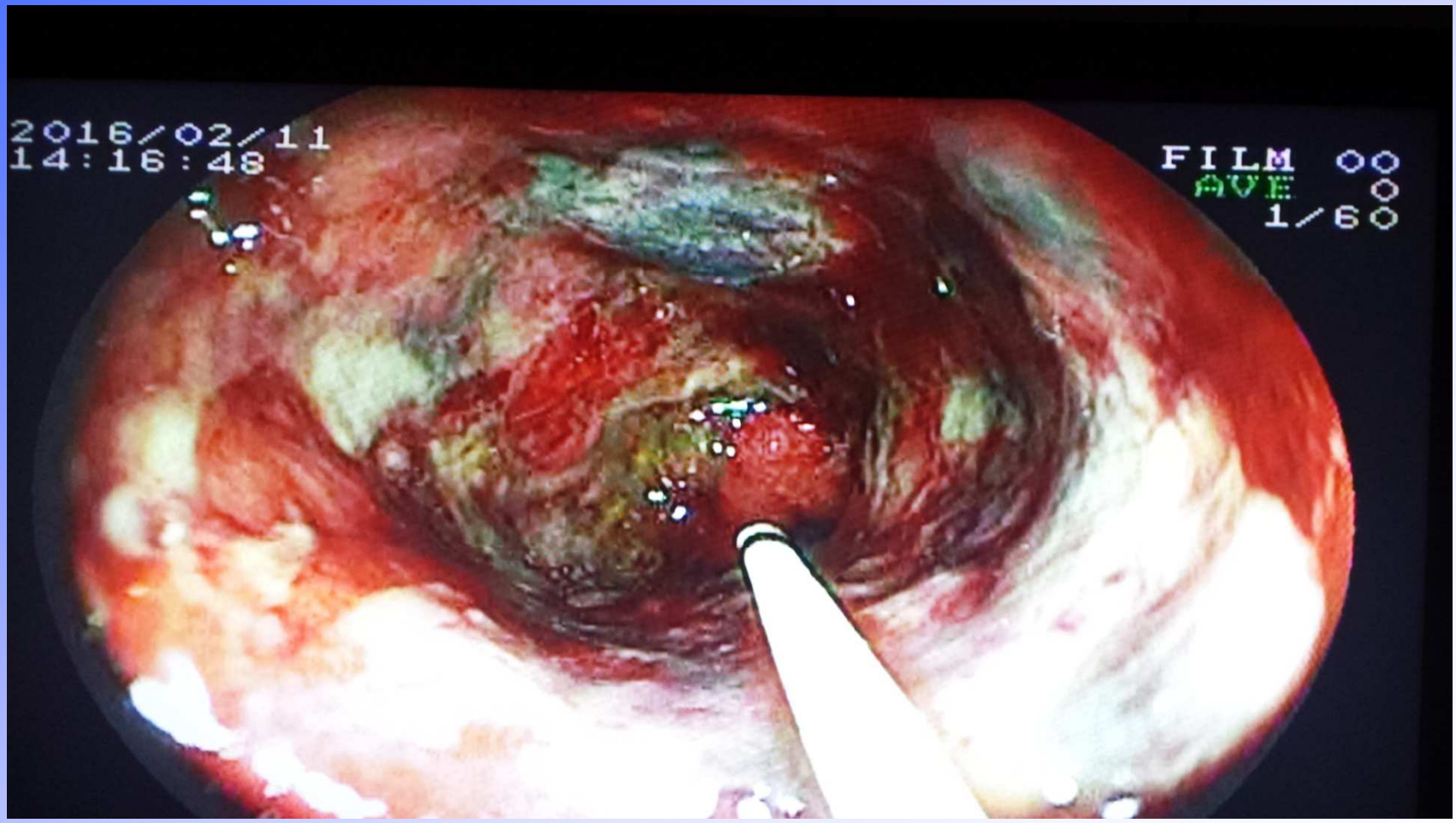
Lip-ICG-PDT: Rectal Cancer

Lip-ICG-PDT: Rectal Cancer

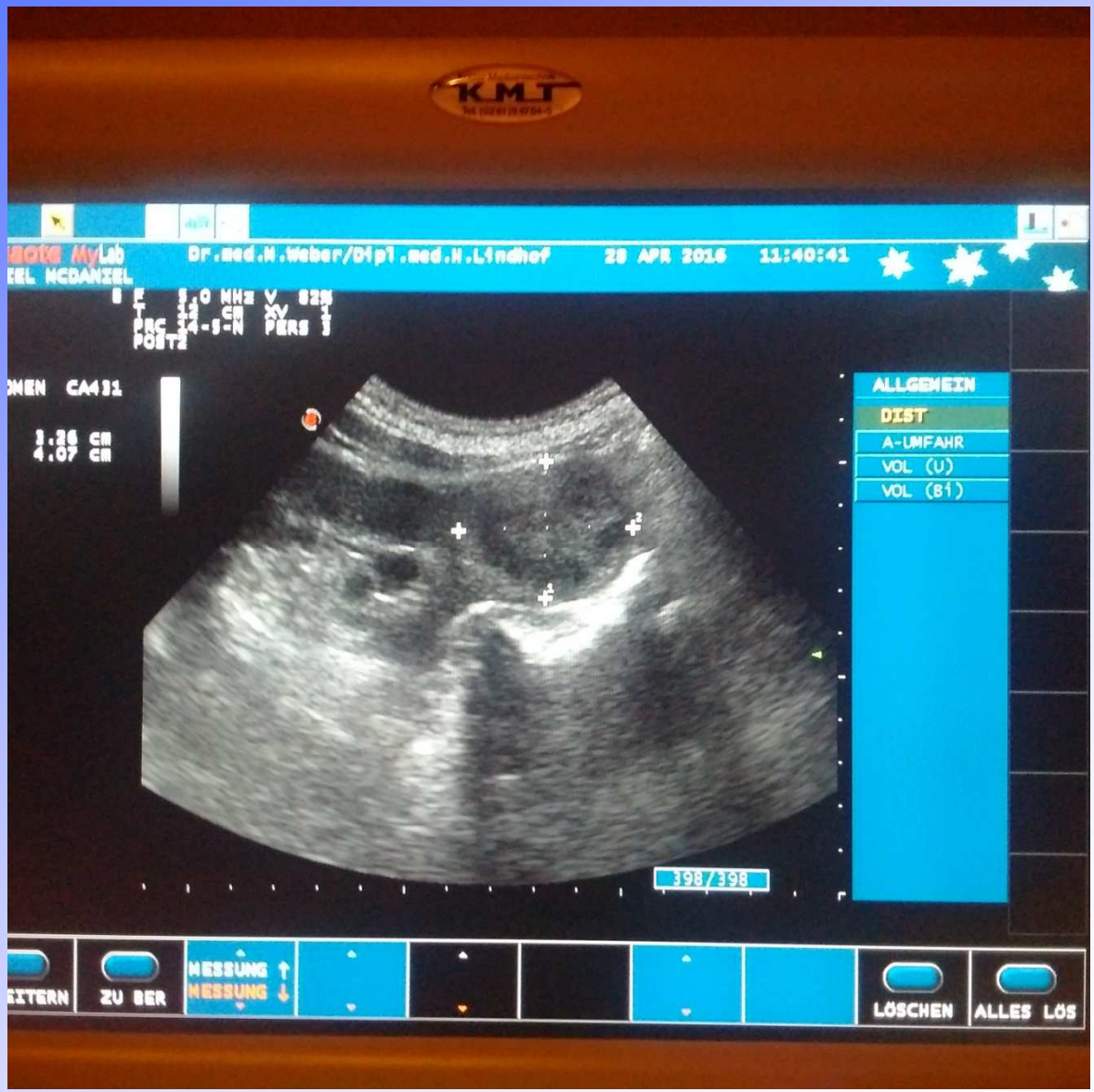


Lip-ICG-PDT, Rectal Cancer

Lip-ICG-PDT: Rectal Cancer

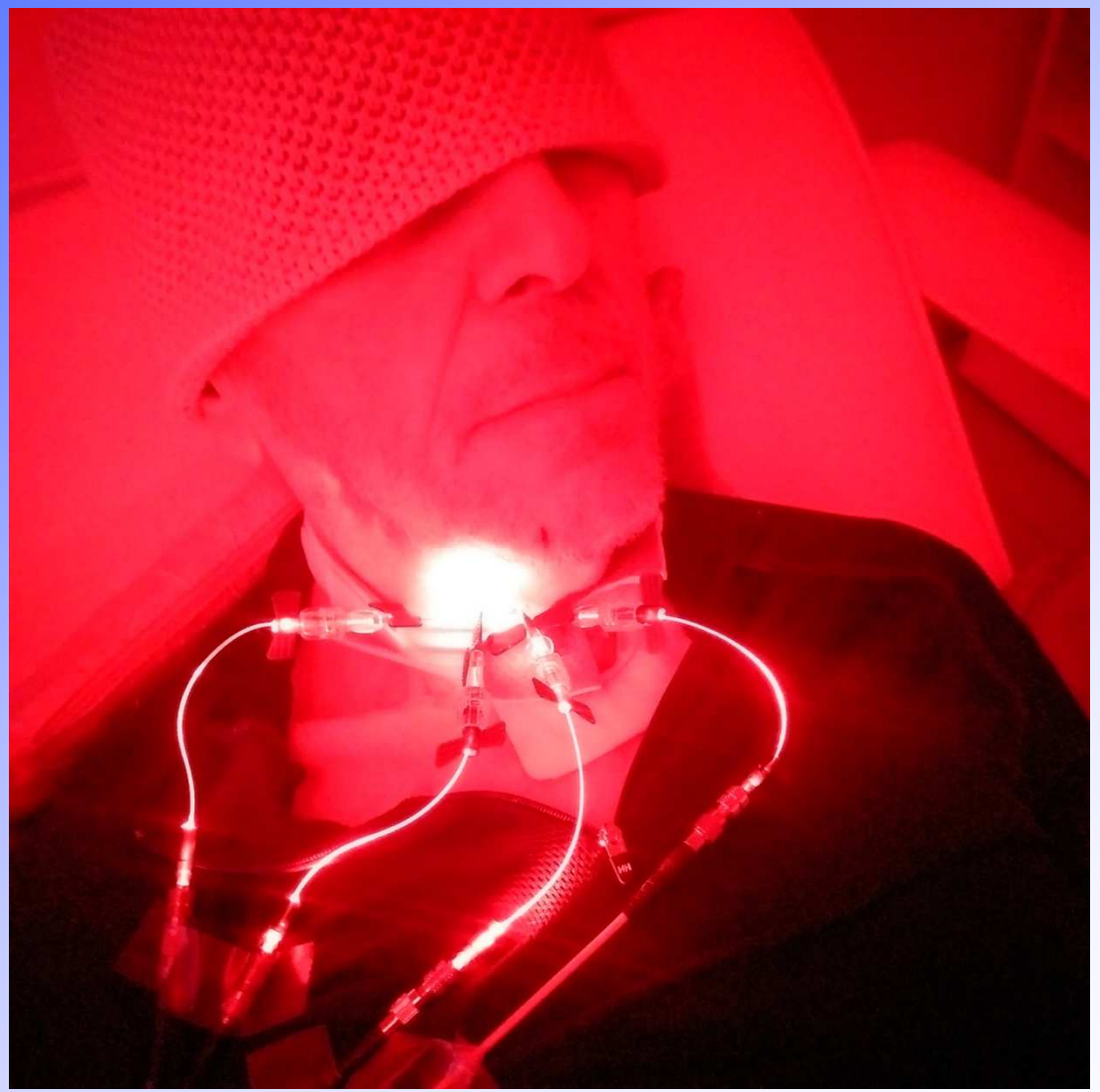


Larynx cancer, spreading in the neck



Larynx cancer

Larynx cancer

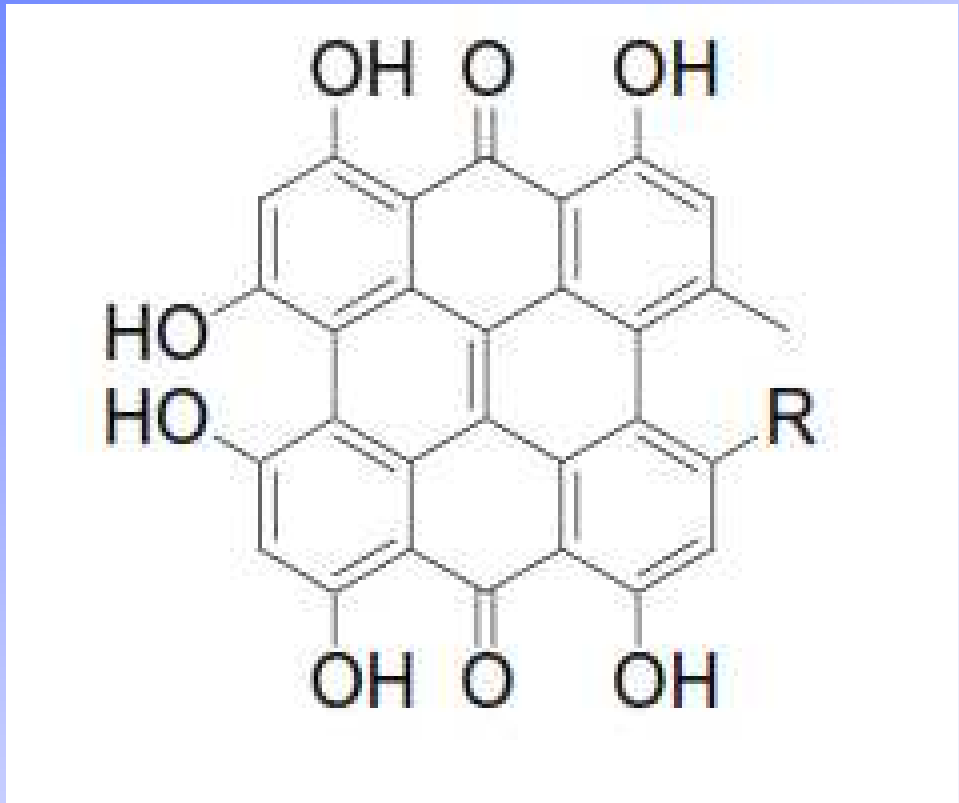


Other natural photosensitizers

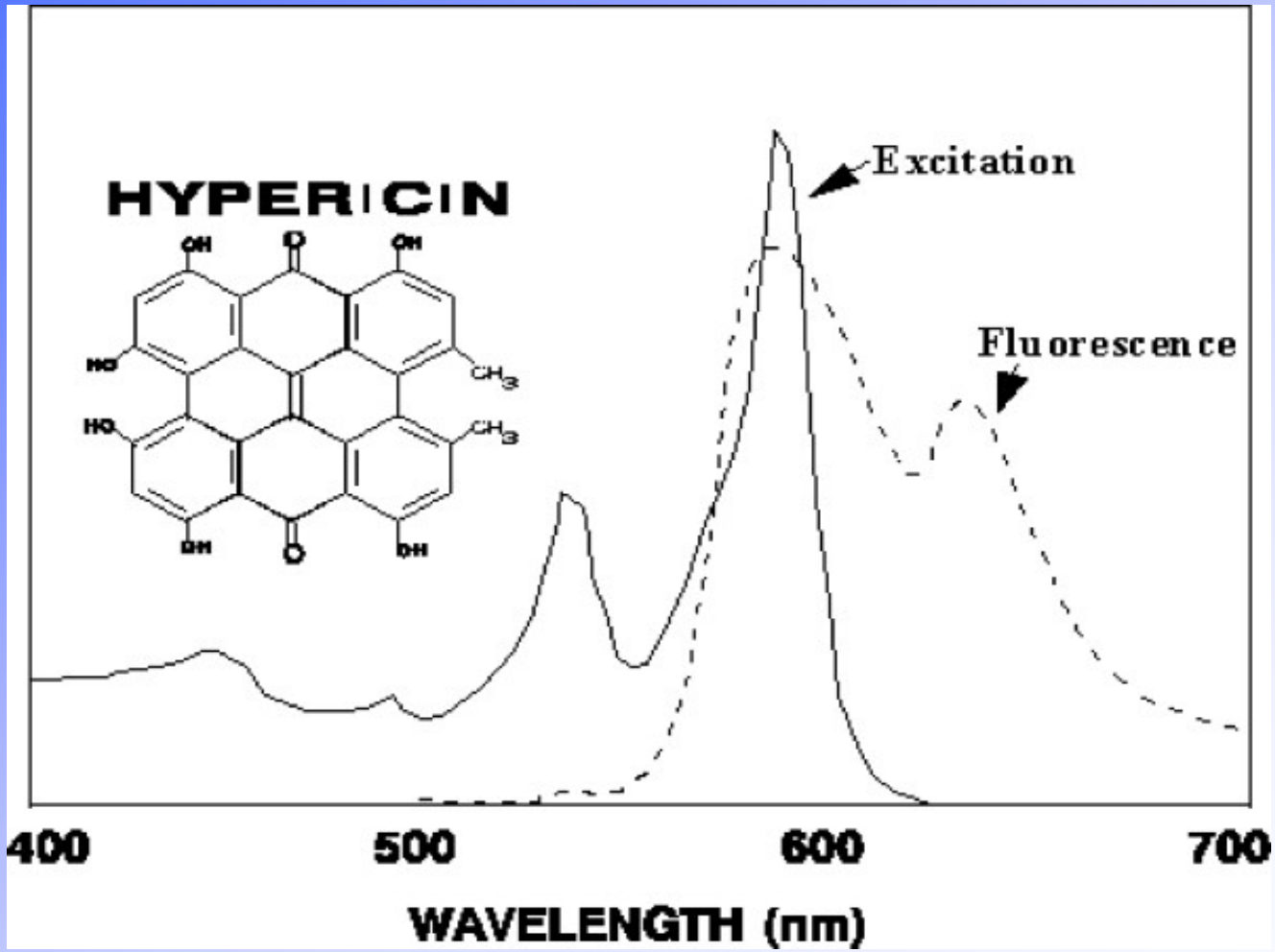
Hypericin as photosensitizer

St. John‘s wart plant

Hypericin as photosensitizer

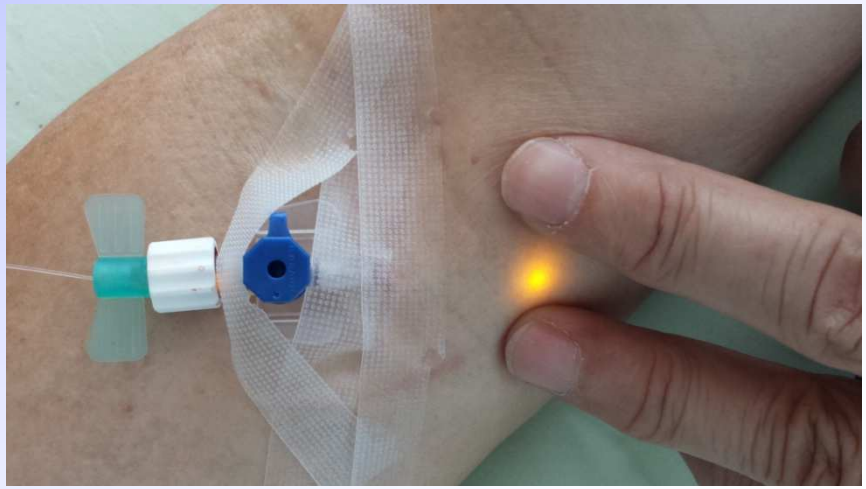


Hypericin as photosensitizer



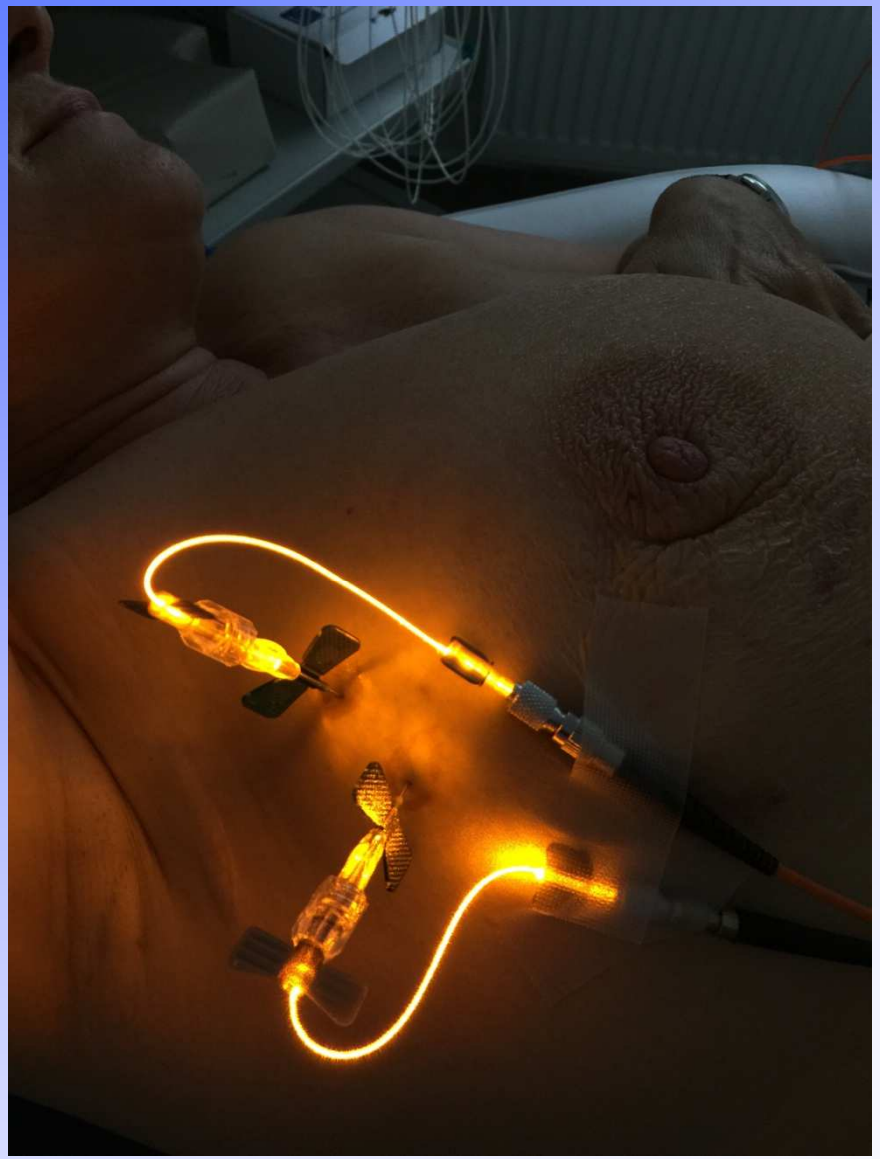

14442411
G-5%
250 ml
B|BRAUN

Hypericin as photosensitizer in combination with yellow laser therapy



Hypericin as photosensitizer

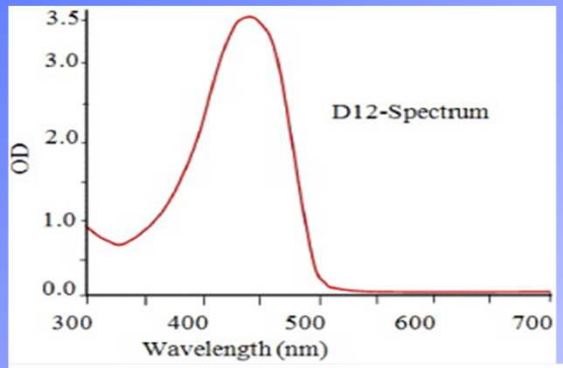
Interstitial PDT of breast cancer

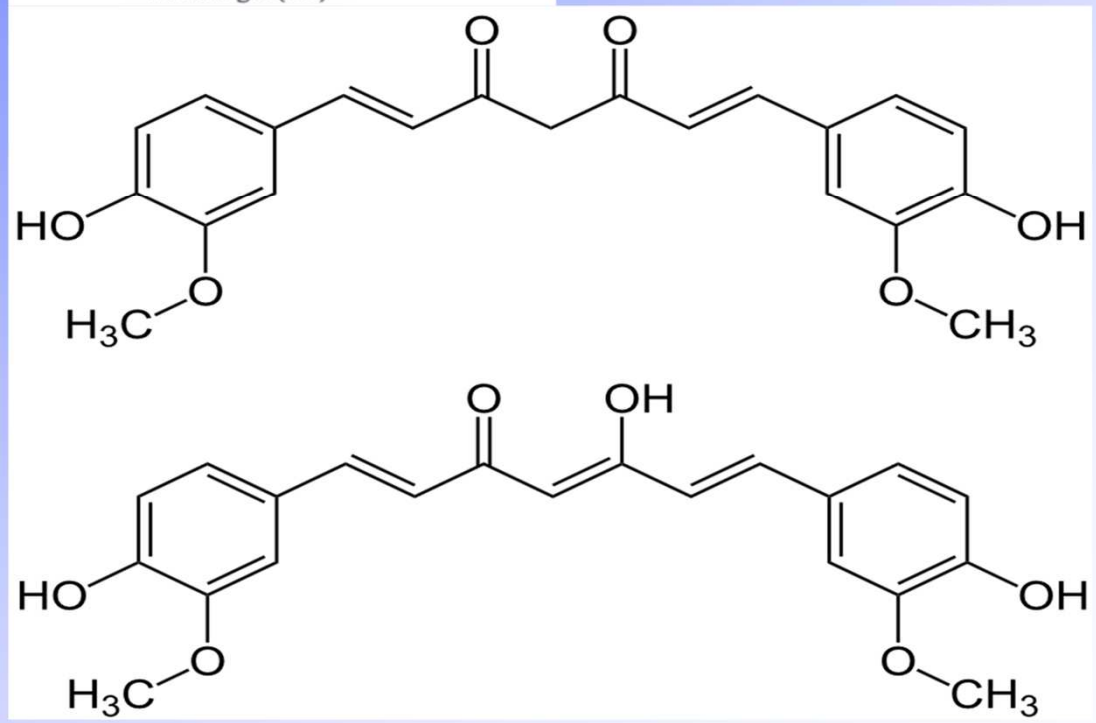


Curcumin as photosensitizer

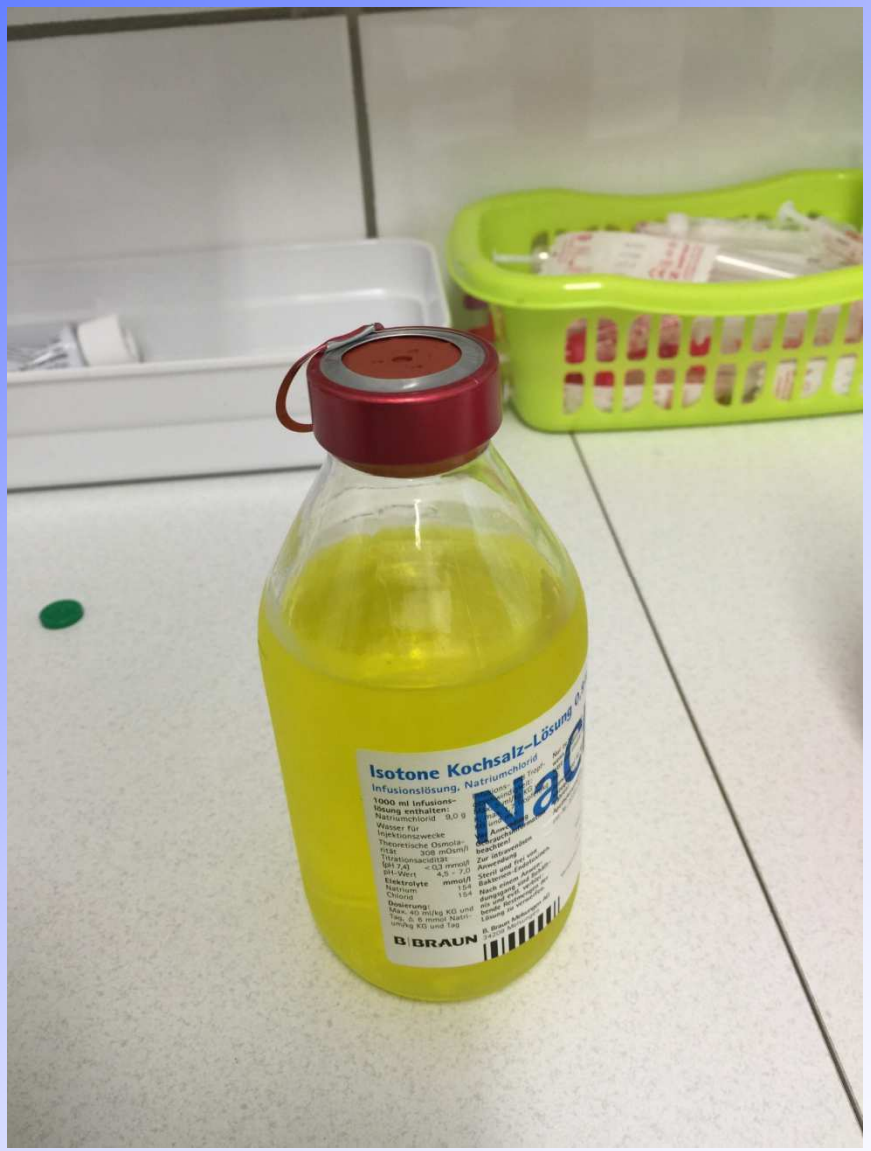
Curcuma powder

Curcumin

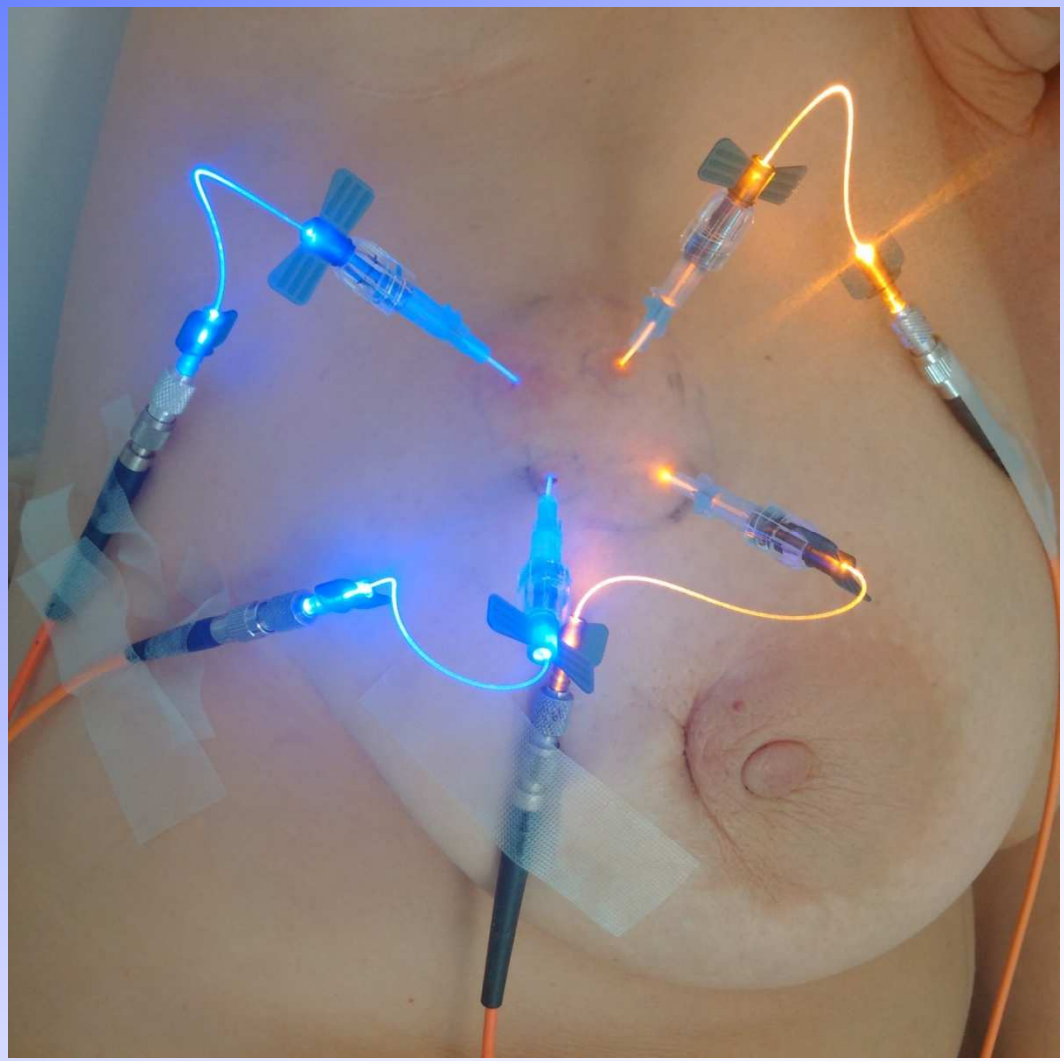

D12-Spectrum
OD
3.5
3.0
2.0
1.0
0.0
300
400
500
600
700
Wavelength (nm)


O
O
HO
OH
O
O
H3C
CH3
O
OH
HO
OH
O
O
H3C
CH3

Curcumin


Isotone Kochsalz-Lösung
Infusionslösung, Natriumchlorid
B|BRAUN

Interstitial PDT combination after Hypericin and Curcumin



Absorption spectra of different phtotosensitizers

- Chlorin E 6 absorbs 660 nm red laser
- Indocyanine Green absorbs 810 nm infrared laser
- Hypericin absorbs 589 nm yellow laser
- Curcurmin absorbs 447 nm blue laser
- Riboflavin absorbs 447 nm blue laser

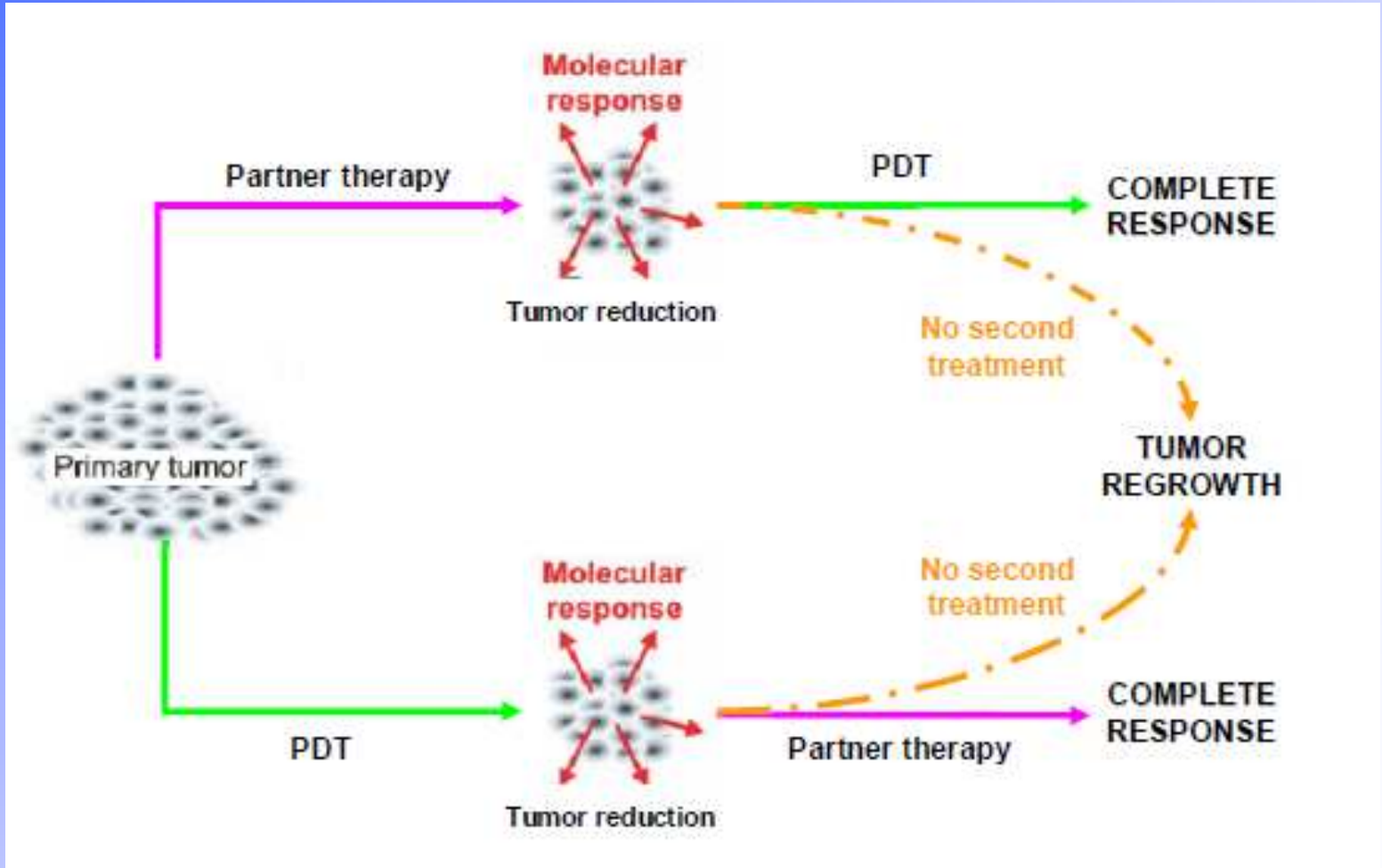
Cancer combination therapy

- Small single tumors are ideal for PDT treatment alone
- **PDT alone is not effective in**

- big tumors
- widely spreading tumors
- multiple metastases

Here we need combination of PDT with other anticancer drugs and methods.

Cancer combination therapy



Cancer combination therapy

1. Combination with traditional chemotherapy
2. Combination with light sensitive chemodrugs **(using chemodrugs as photosensitizers)**
3. Combination with antioxidants
4. Combination with antiangionesis inhibitors
5. Combination with Cox-2 inhibitors
6. Combination with antibodies
7. Combination with different natural compounds
8. Combination with immunotherapy

5-Fluorouracil as a Phosensitiser

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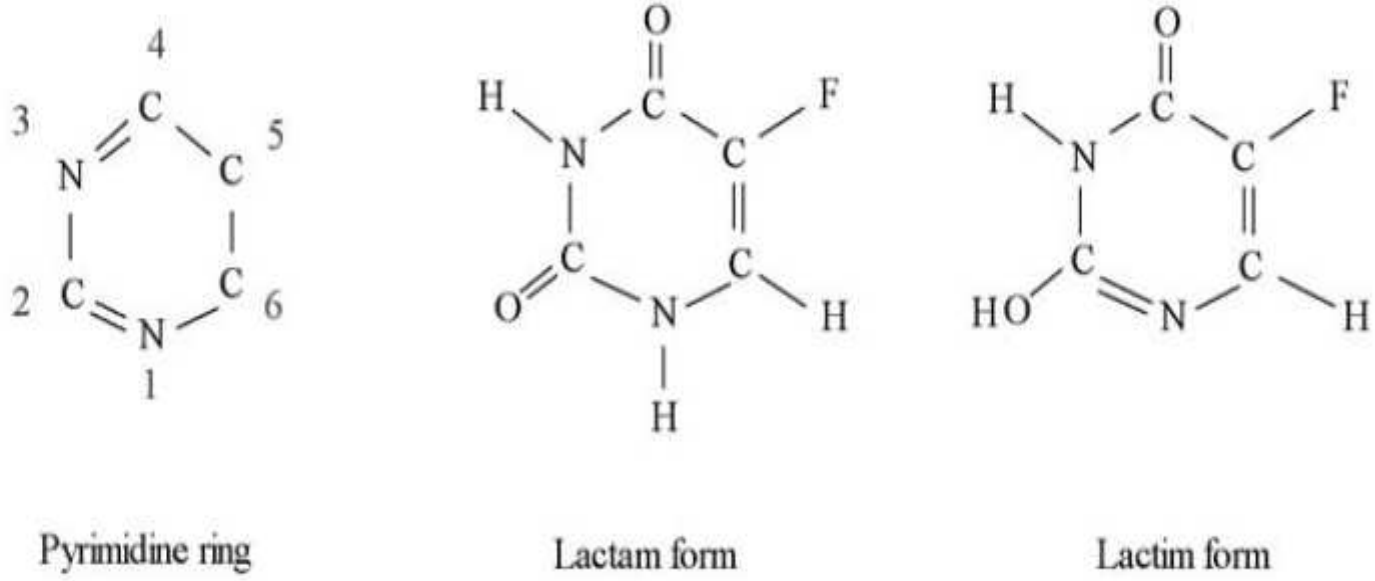
Abstract

5-FU exhibits a high fluorescence after irradiation with UV-vis light. An enhancement of the cytostatic activity of 5-FU under UV-vis irradiation was observed on an in vivo experimental model.

The tautomeric forms of 5-FU



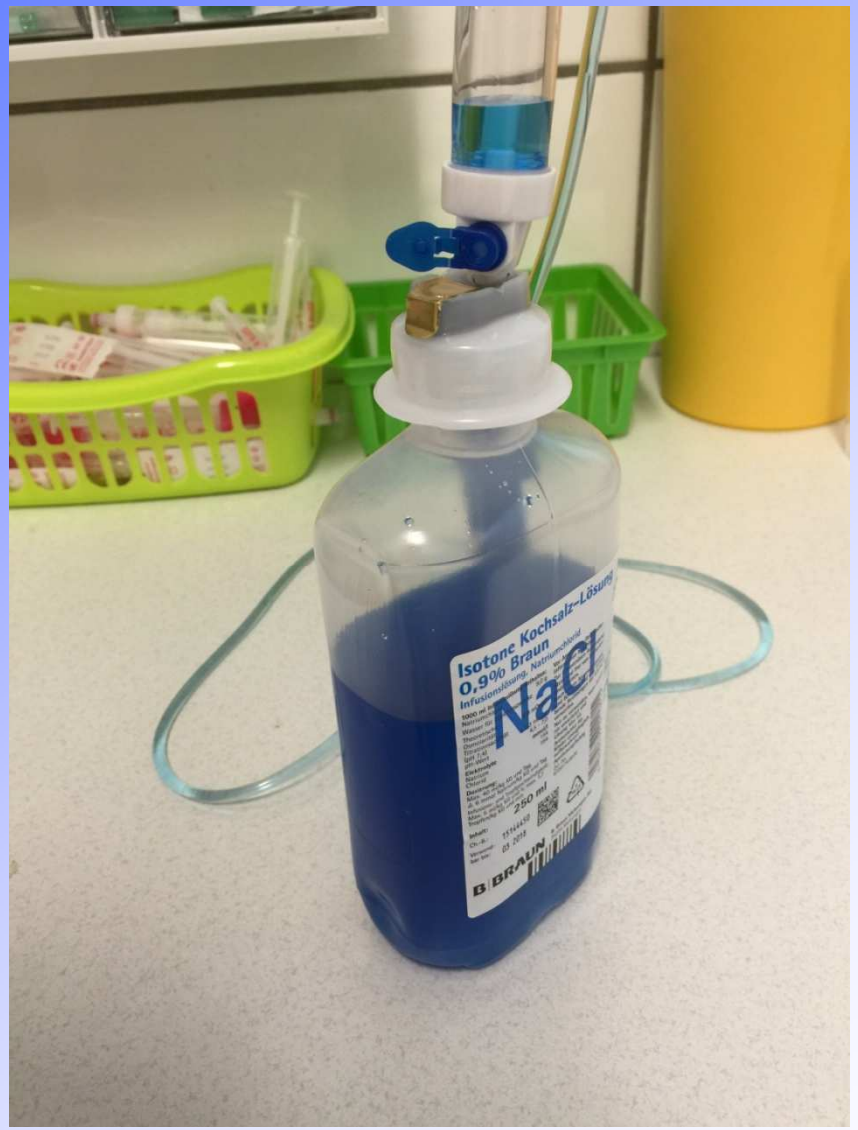
Pyrimidine ring Lactam form Lactim form

Figure 1. *The pyrimidine ring and the two 5-FU tautomers: lactam and lactim forms.*

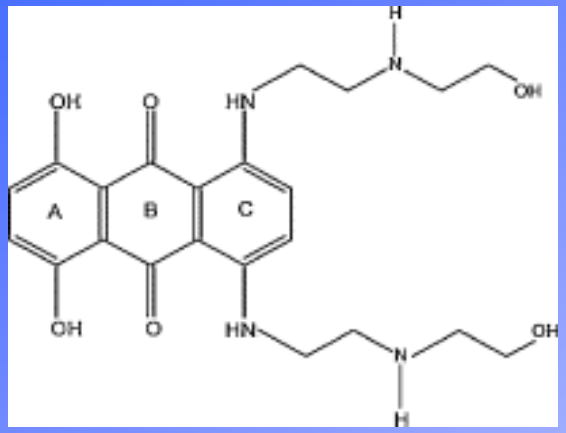
Mitoxantron as photosensitizer

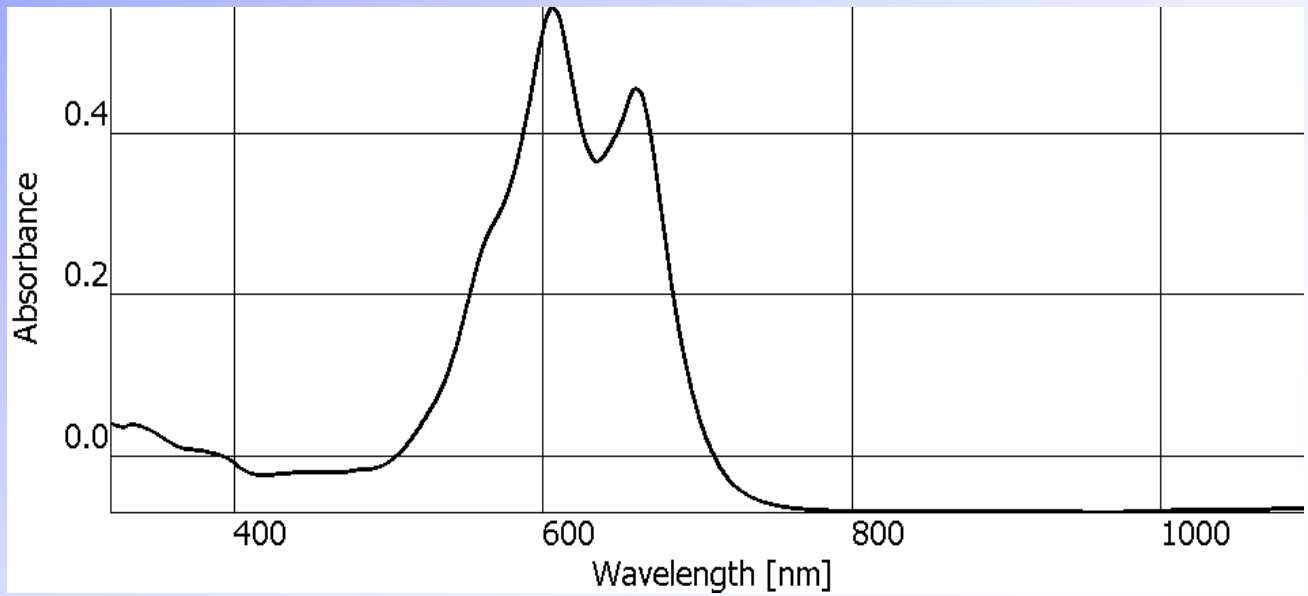
- Mitoxantron is a blue substance
- Mitxantron is activated by yellow and red light
- Mitoxantron is a strong chemo-photosensitizer
- Is effective in multiple cancer varieties

Mitoxantron

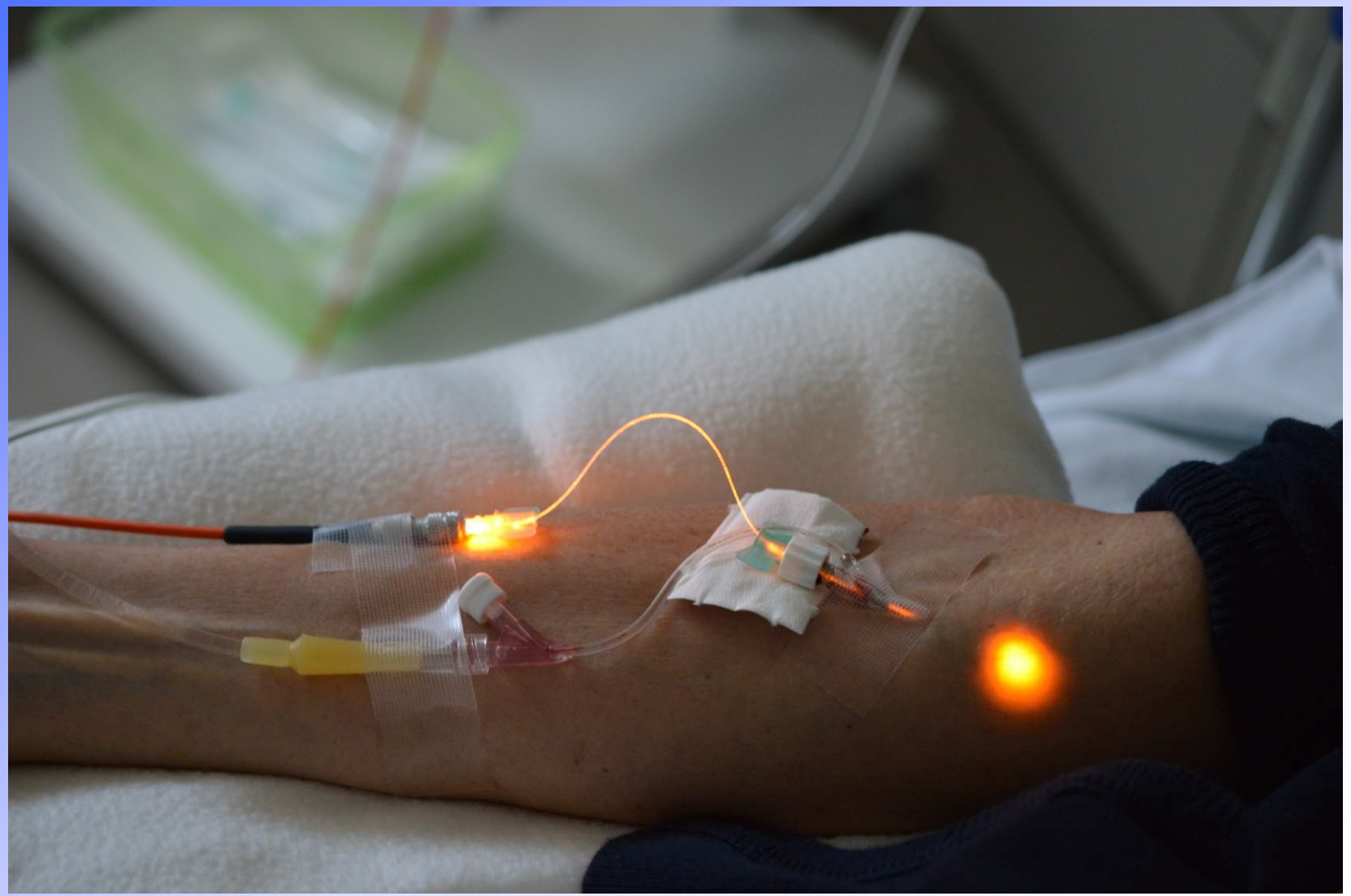


Mitoxantron as photosensitizer





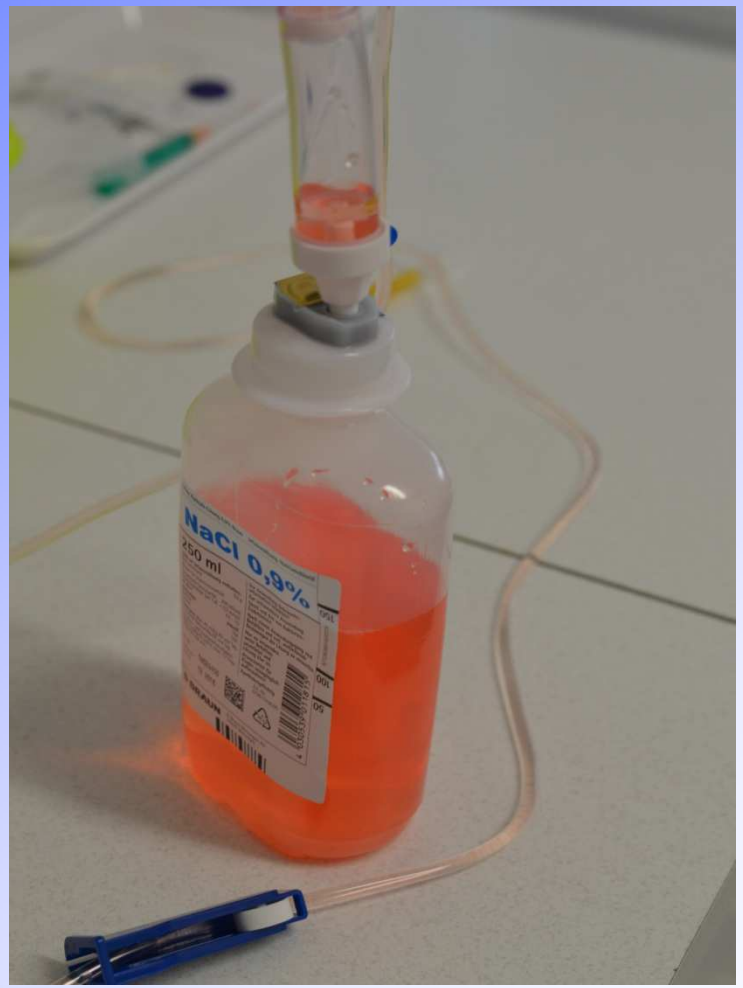
Mitoxantron stimulation (Y-cannula)



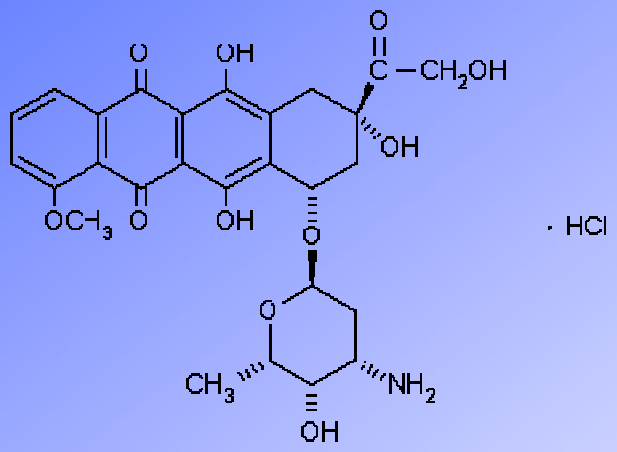
Doxorubicin (liposomal) as photosensitier

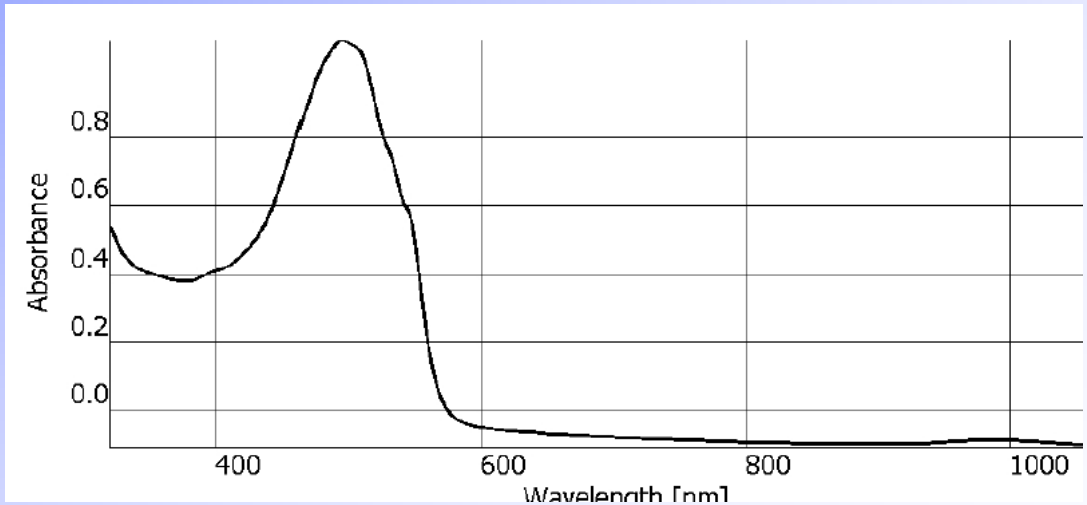
- Is widely used for many different cancers (Anthracyclin antibiotics)
- Is an orange solution and is stimulated by visible laser light
- Can be enhanced by liposomal delivery (Doxil)
- Stimulation by blue-green light

Doxorubicin liposomal

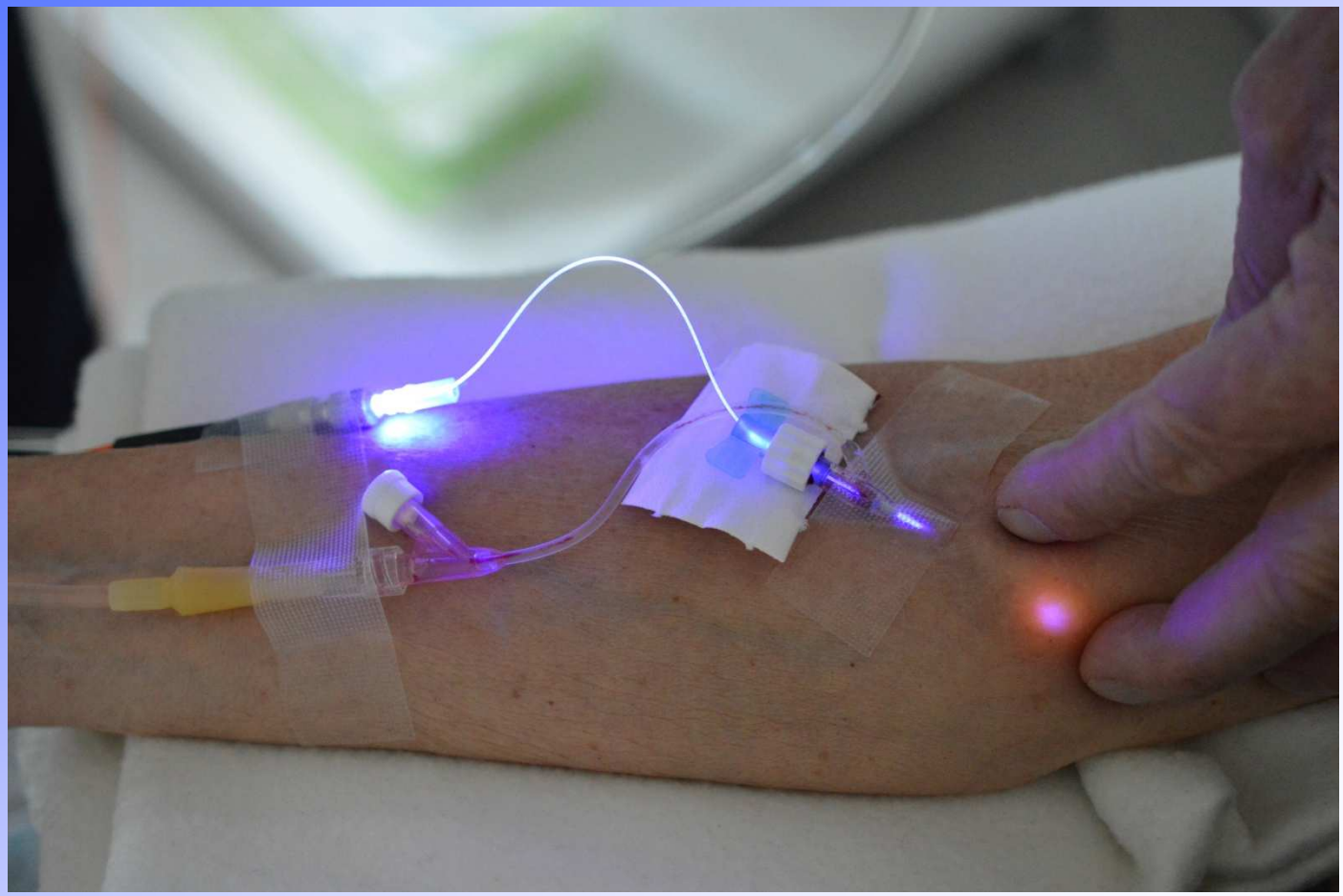


Doxorubicin (liposomal) as photosensitizer

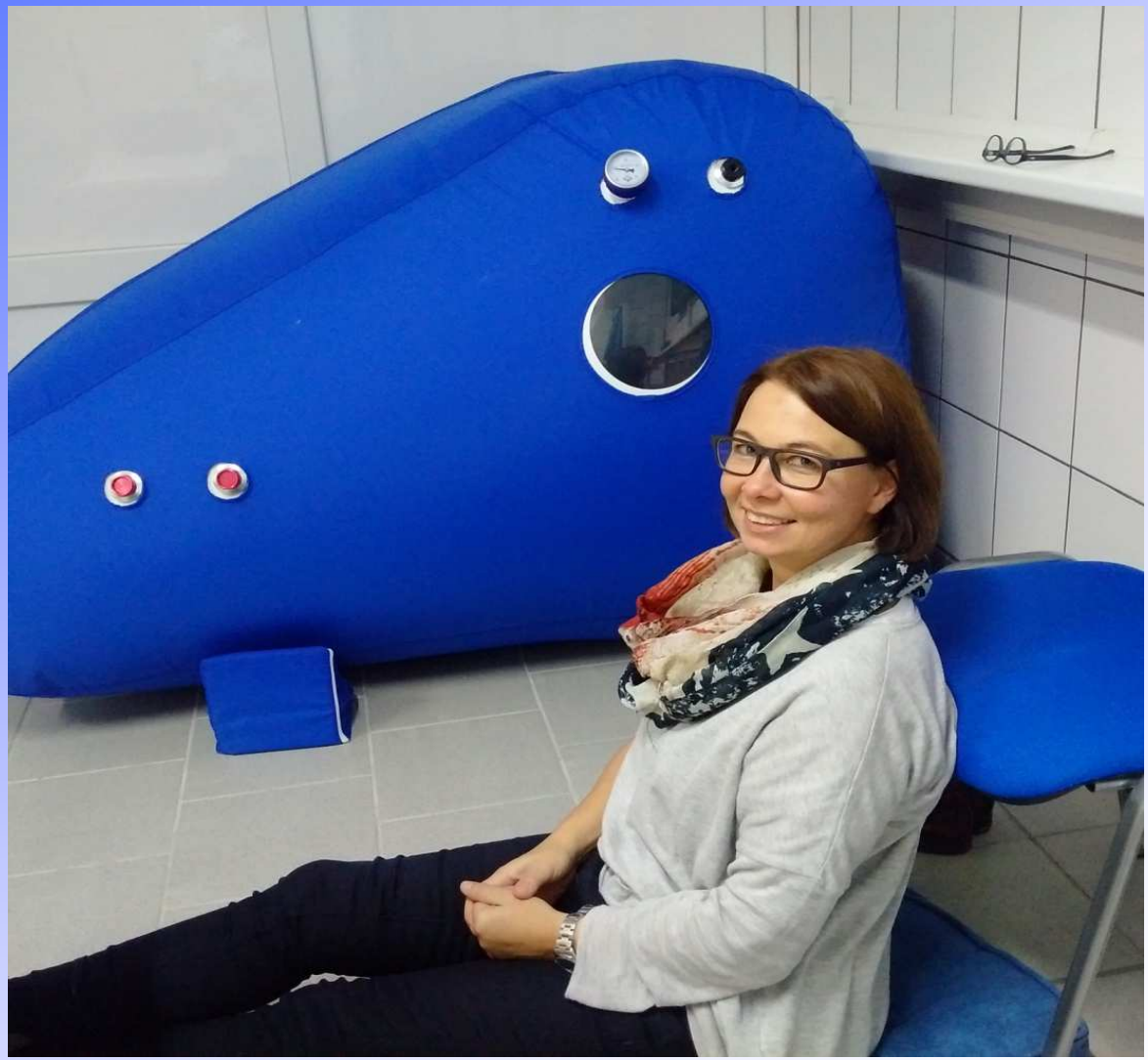




Doxorubicin stimulation



Hyperbaric oxygen chamber



New therapeutic strategies for cancer therapy

- Photodynamic therapy with liposomal ICG, Chlorin E6, Hypericin and Curcumin

  (external, interstitial, intratumoral irradiation)
- Hyperbaric oxygene therapy
- Low dose chemotherapy using chemodrugs as photosensitizers
- Immunotherapy with intravenous laser blood irradiation
- Immunotherapy with GcMAF and others

GcMAF

- Vitamin D binding protein is known as Gc Protein.
- This protein binds in the body to 25-hydroxy vitamin D
- Macrophage activating factors (MAF) are glycoproteins that increase macrophage activity and transform them in natural killer cells.
- Vitamin DBP (Gc-protein) is the primary MAF.
- The glycosylated Gc protein is the best MAF.

GcMAF

- NaGalaser is an enzyme produced in small amount by liver cells
- But is produced in large amounts by cancer cells.
- NaGaLase deglycosylates Gc protein and so has an immunsuppressive effect.
- NaGaLase is also produced by different viruses, bacteria and fungi

GcMAF

Gc-MAF macrophage activation therapy is useful in the treatment of many diseases, such as cancer, HIV AIDS, Hepatitis B virus (HBV), Hepatitis C virus (HCV), Herpes Simplex virus (HSV), Tuberculosis, Pneumonia infection, Epstein-Barr virus (EBV), cystitis/urinary tract infection

TBL-12 from sea Curcumber

- Works against neoangiogenesis by inhibiting VEGF
- Inhibits proliferation of cancer cells by apoptosis
- Promising effects on melanoma and leukemia
- Taken orally
- No side effects

Suppression of Human Multiple Myeloma Cell Growth by TBL-12 in Combination with low doses of Velcade: Insight in to the modulation of IL-6/STAT-3 mechanisms

Bhagavathi A. Narayanan, PhD1, Caroline Cunnigham1 and Amitabha Mazumder, MD2
1Departments of Environmental Medicine, 2Clinical Cancer Center New York University School of Medicine, NY

TBL-12 from sea Curcumber

Results:
We observed cell survival rate reduced from 100 % to 30% at 48h and significantly reduced to 20% at 72h ($p<0.001$) in both MM1 and U266 cells. These findings suggest low dose effect of Velcade in combination with TBL-12 in a time dependent manner.

Conclusion:
Overall findings from this study suggest the potential use of TBL-12 in combination with Velcade against MM. Ongoing trials with TBL-12 at NYUCI and this correlative data could support future clinical trials.

You are Invited!

ISLA Annual Laser Conference

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International Society for Medical Laser Applications

June, 10th/11th 2016

Thank you

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