**Moscow Research Oncological Institute P.A. Gertzen**
**General Physics Institute A.M. Prohorov - RAS**

# FLUORESCENT DIAGNOSIS AND PHOTODYNAMIC THERAPY OF CANCER

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# Photosensitizers

**Photogem** (1992, MATChT M.V. Lomonosov)

**Photosens** (1994, «NIOPIK»)

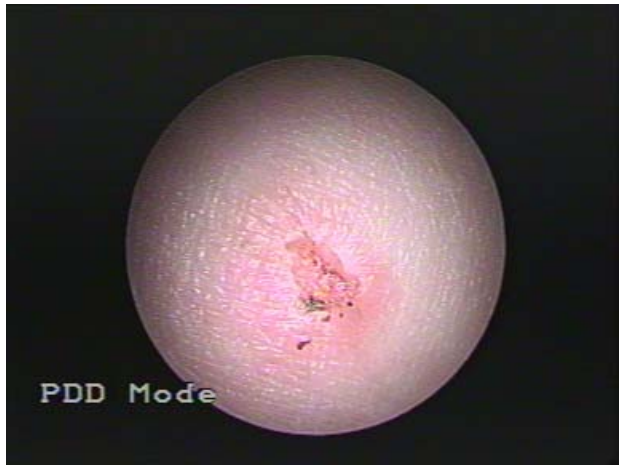
**Alasens** (1999, «NIOPIK»)

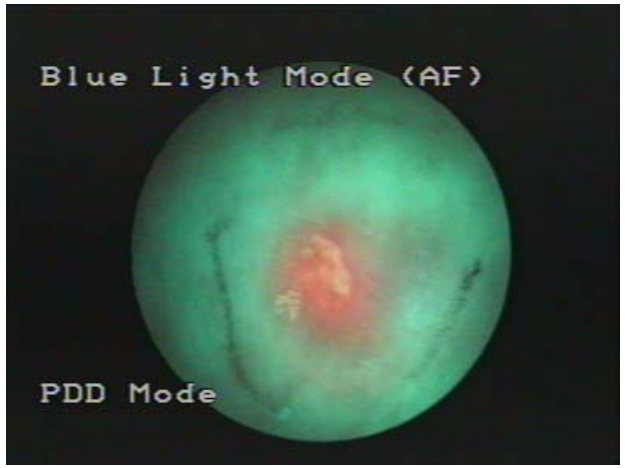
**Radachlorin** (2002, OOO «Radafarma»)

**Photoditazin** (2003, OOO «Veta-Grand»)

# Accumulation of PS in tumors:

- **Higher concentration of receptors for low-weight proteins (Jori et al, 1984),**
- **Higher level of macrophages**
- **Lower level of pH**
- **Higher interstitial volume**
- **Higher permeability of blood vessels**
- **Disturbed lymphatic drainage**
- **High level of newly synthesized collagen, attaching porphyrins (Musser , 1980)**
- **high level of lipids, with high affinity to lypophyilic dyes (Frietas, 1990).**



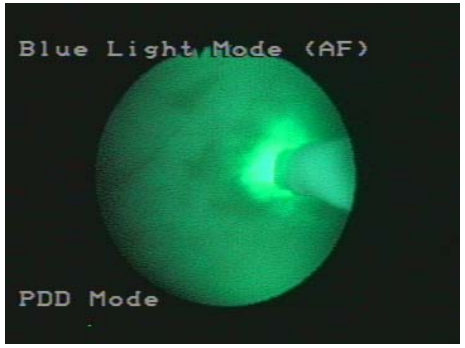




***Sokolov et al. (2005)***

# FLUORESCENCE DIAGNOSIS of PRECANCER AND EARLY STAGE CANCER

**AF IMAGING**
**460-700 nm**

**PDD IMAGING**
**630-670 nm**



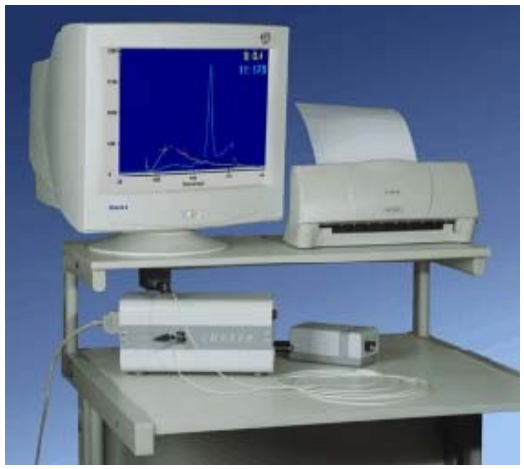

**Local Fluorescence Spectroscopy**
**400-800 nm**

normal tissue

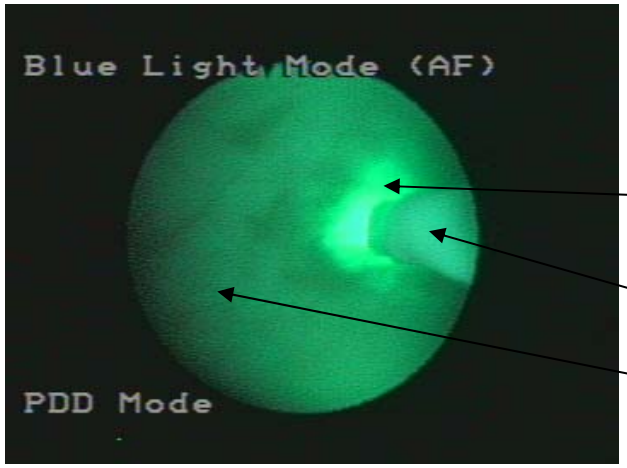
inflammation

**malignant tissue**

# Equipment for local fluorescence spectroscopy (LFS)



- Computerized spectral-fluorescence set-up “Spectr-Cluster”
- Laser excitation sources in blue and green spectral ranges (442 nm and 532 nm)
- Fiber-optical diagnostic catheter



## MAJOR ADVANTAGES

- **Multiwavelength laser excitation (408, 532 and 638 nm)**
- **Work with all photosensitizers**
- **Compatibility with any endoscopic and laparoscopic equipment**
- **Simple and convenient programme, adapted for medical users**
- **Compact and high reliability**

# Main tasks LFS in clinical investigation:

- **Autofluorescence and photodynamic diagnosis of early forms and hidden cancer sites (combined with fluorescent visualization)**
- **Border evaluation of the tumor lesions**
- **Kinetics of accumulation and reduction of the PS from the tissues**
- **Monitoring of PS in the process of PDT**

# AUTOFLUORESCENCE DIAGNOSIS OF CANCER

## ENDOGENOUS FLUOROPHORES

## 280-700 nm

| FLUOROPHORES | BIOMOLECULES / CELL LOCALISATION | EXCITATION Peak position range | EMISSION Peak position range |
|---|---|---|---|
| **Aromatic AminoAcid Residues** | Proteins<br>A= Phe+Tyr; B= Trp+Phe+Tyr | **240 – 280 nm** | **280 – 350 nm** |
| **Collagen<br>Elastin<br>Cytokeratins** | Extracellular matrix<br>Connective tissue<br>Epithelia | **330 – 340 nm<br>350, 420 nm<br>280, 325 nm** | **400 - 410 nm<br>420, 510 nm<br>495, 525 nm** |
| **Reduced Pyridine Nucleotides** | NAD(P)H<br>(Cofactors in metabolism)<br>mitochondria / cytoplasm | **330 – 380 nm** | **440 nm (bound)<br>462 nm (free)** |
| **Flavins<br>Flavin Nucleotides** | Riboflavin, FMN, FAD<br>(Coenzymes of Flavoproteins)<br>mitochondria / cytoplasm | **350 – 370 nm<br>440 – 450 nm** | **480 – 540 nm** |
| **Porphyrins (Zinc-Protoporphyrin)** | Prosthetic group of proteins<br>Hemoglobin Myoglobin<br>Cytochrome<br>Erythroid cells | **405 nm<br>500 – 600 nm** | **630, 670 nm** |
| **Lipofuscins<br>Lipopigments** | Pigments<br>(cell catabolism / cell age)<br>cytoplasm | **UV<br>400 – 500 nm** | **> 540 nm** |
| **Vitamins** | Vitamin A<br>Vitamin B6 & Precurors | **370 – 380 nm<br>290 – 310 nm / 375 – 395 nm** | **490 – 510 nm<br>375 – 395 nm / 400 – 500 nm** |
| **Lipids** | Arachidonic Acid<br>Phospholipids | **330, 350 nm<br>430 - 440 nm** | **470 – 480 nm<br>520 – 570 nm** |
| **Catecholamines<br>Serotonin** | Neurotransmitters | **280 – 290 nm<br>305, 360 (dimer), 420 (trimer) nm** | **320 – 340 nm<br>350, 440 (dimer), 520 (trimer) nm** |

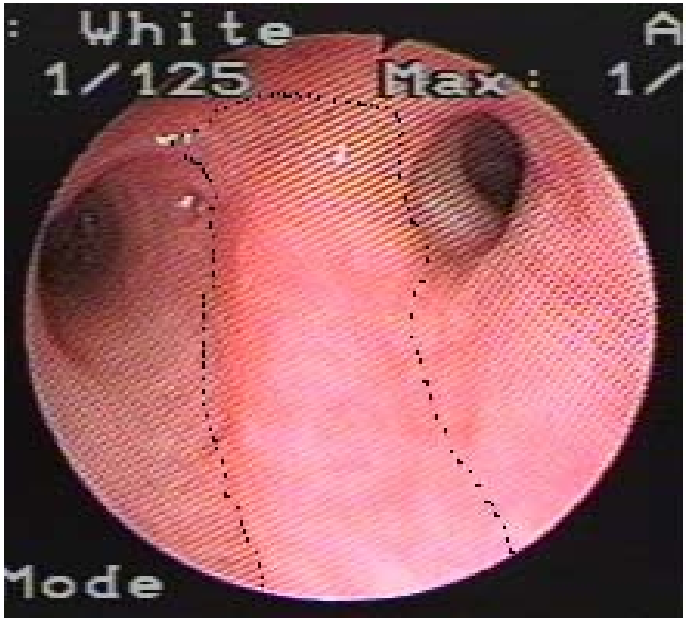
G. Bottiroli and AC. Croce. (2004)

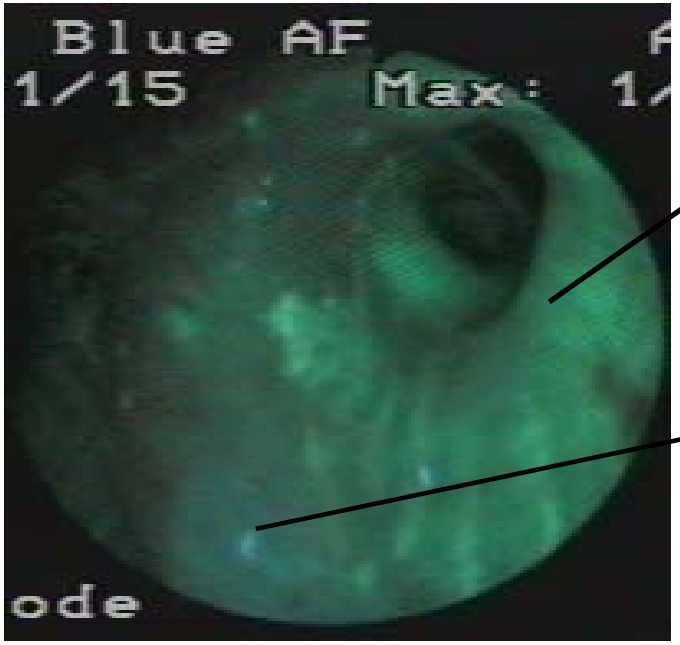
# Basics of the AFD method

- Different stages of tumor growth are related to changes in concentration, spatial distribution and metabolic activity of endogenous fluorophores
- These changes reflect on the AF spectra and could be used for diagnostic information

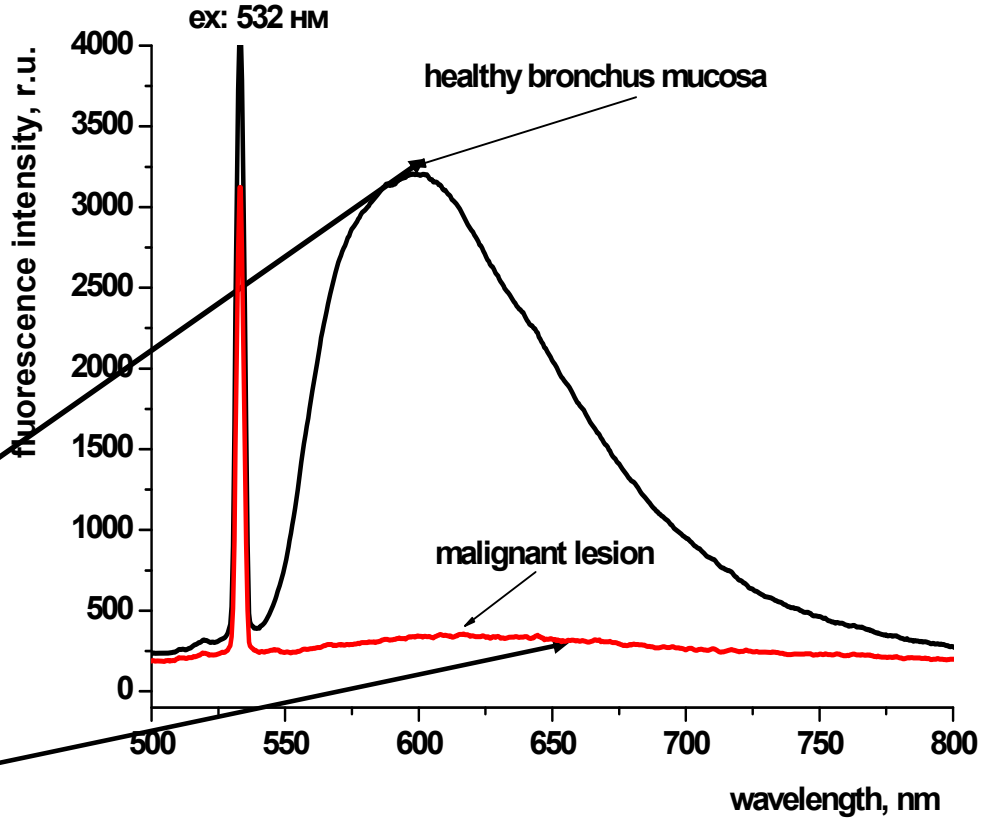
- AFD is based on the differences and spectral composition of native (endogenous, auto-) fluorescence of normal and tumor tissues

# AUTOFLUORESCENCE DETECTION OF EARLY STAGE LUNG CANCER



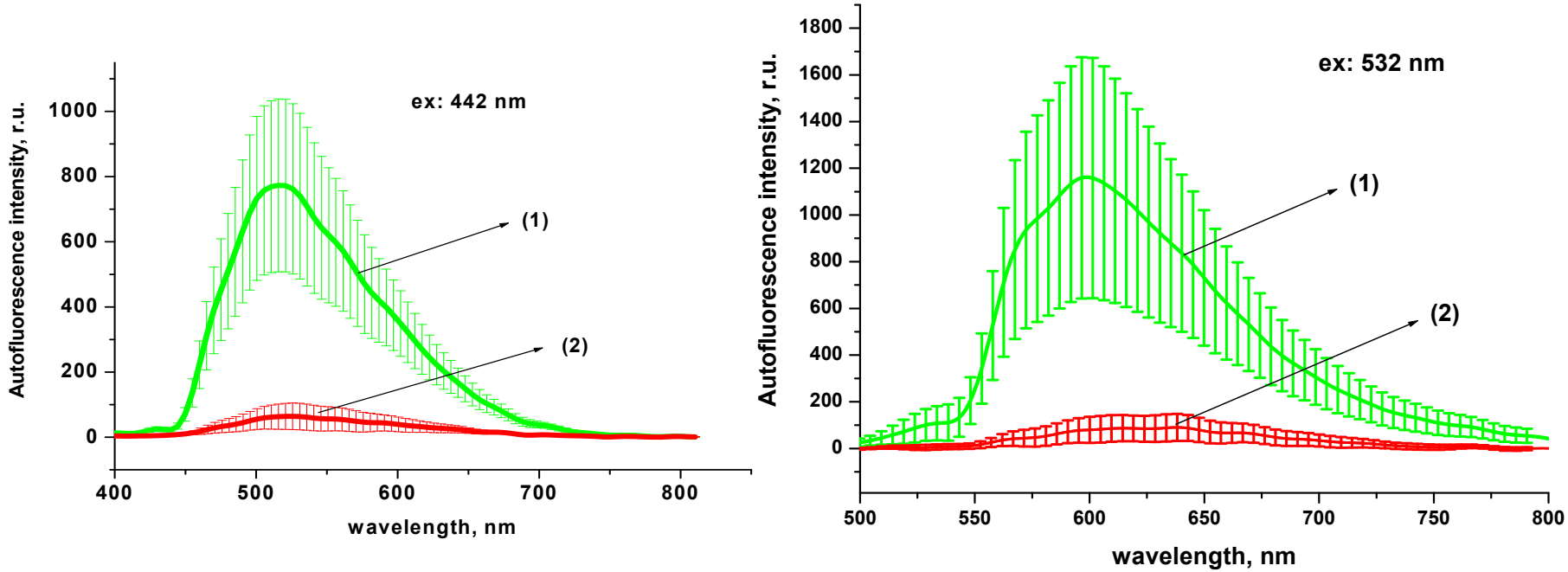




Sokolov V, Chissov V, Bulgakova N et al. Fluorescence diagnostics of early central cancer of the lung. Pulmonology 2005;1: 107-16.



# I*n vivo* AF emission spectra measured from 19 patient group under 442 nm and 532 nm laser excitation

(Bulgakova et al., MLA,2009)




***(1) - mean AF emission spectrum from normal urothelium and inflammation sites,***

***(2) - mean AF emission spectrum from malignant lesion (hard dysplasia, CIS and transitional cell carcinomas). Error bars represent standard deviations.***

***Totally 355 AF emission spectra have been recorded, including 156 spectra in unaltered one, 53 in inflammatory foci, 7 in ones with a hard dysplasia and 139 spectra in CIS and transitional cell carcinoma foci.***

## LIMITATIONS OF AFD: AUTOFLUORESCENCE BLEACHING (False-positive effect)

– Granulation
– Hypervascularization
– Preliminary biopsy
– Foregoing radiotherapy
– Foregoing laser therapy and PDT
– Foregoing chemotherapy

# PHOTODYNAMIC DIAGNOSIS (PDD)
## *EXOGENOUS AND ENDOGENOUS PHOTOSENSITIZERS*

**Photogem** (1992, MATChT M.V. Lomonosov)

**Photosens** (1994, «NIOPIK»)

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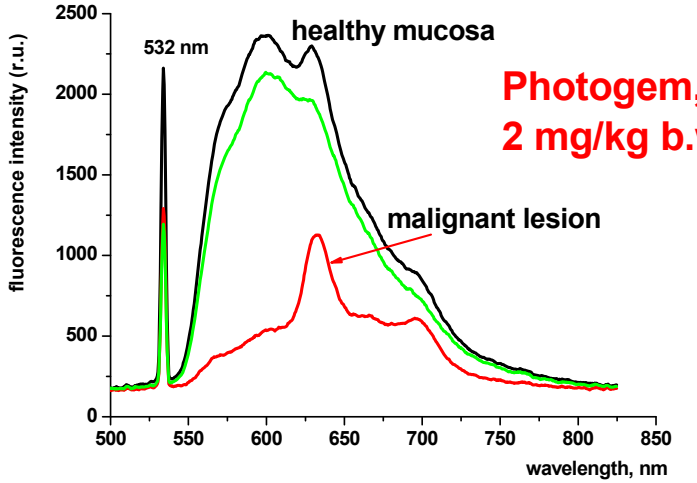
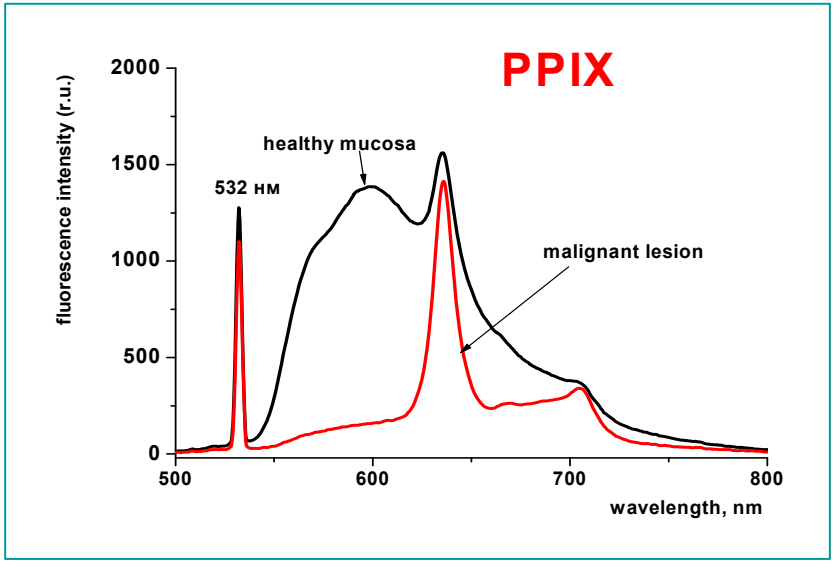
**Photoditazin** (2003, OOO «Veta-Grand»)

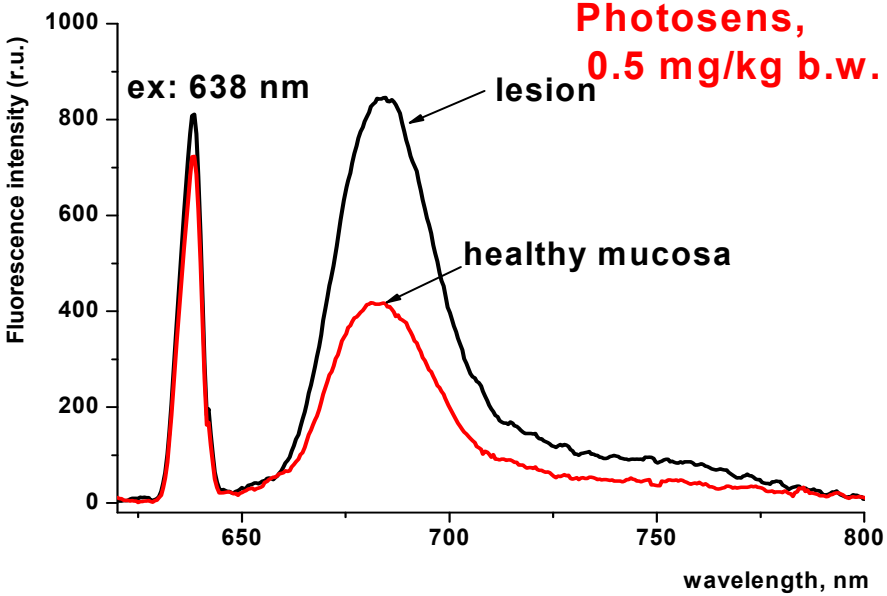
# PS first generation and PD of early cancer

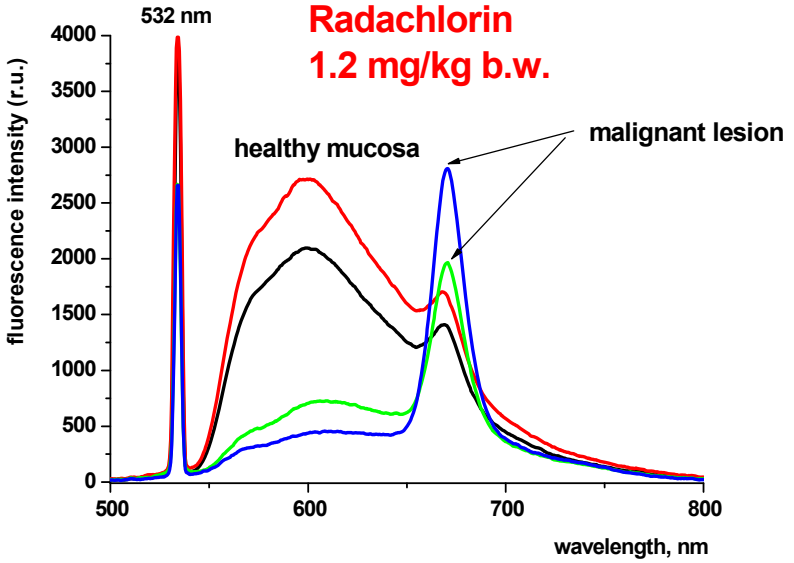
- **Complicated chemical composition**
- **Low fluorescence contrast tumor-normal (< 2)**
- **Long lifetime of the drug in the organism**
- **Necessity for special light regime for the patient**

# In vivo LFS in PDD of early stage bronchial neoplasia with clinically approved PSs (Sokolov et al, 2006)



# 5-ALA-induced PPIX guided PDD of superficial bladder cancer

- Intravesically instilled exogenous 5-ALA induces a 10 times more intense synthesis of **endogenous protoporphyrin IX(PPIX)** in cells of transitional cell bladder cancer, beside healthy urothelium *(S. Datta et al. 1998)*
- As a result of 5-ALA-induced PPIX accumulation in malignant cells, there is a possibility of revealing bladder cancer's foci by **the specific fluorescence of PPIX** in a red part of the optical spectrum. This phenomenon forms the basis for the **photodynamic diagnosis (PDD)** of bladder cancer *(H.Stepp et al., 1999)*
- The clinical study has proven **the sensitivity of PDD** of superficial bladder cancer **to exceed 90%,** which is much higher than the maximum sensitivity of a routine cystoscopy procedure (under 50%) *(D. Zaak et al. 2001)*
- But its **specificity** is notably lower **(50-65%),** since a non-specific fluorescence of 5-ALA-induced PPIX can be seen within inflammatory or metaplastic foci as well.

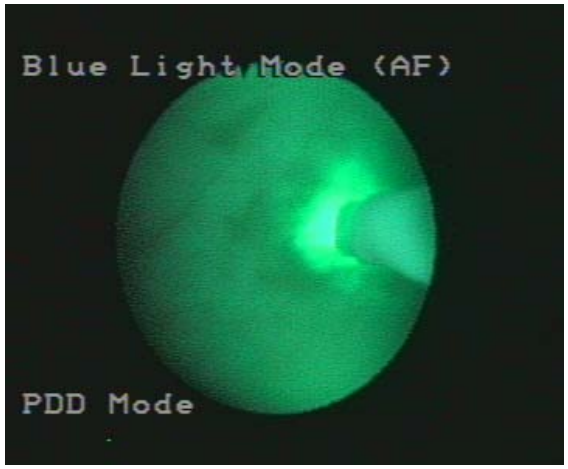
# In vivo LFS in PDD of superficial bladder cancer after Alasense installation

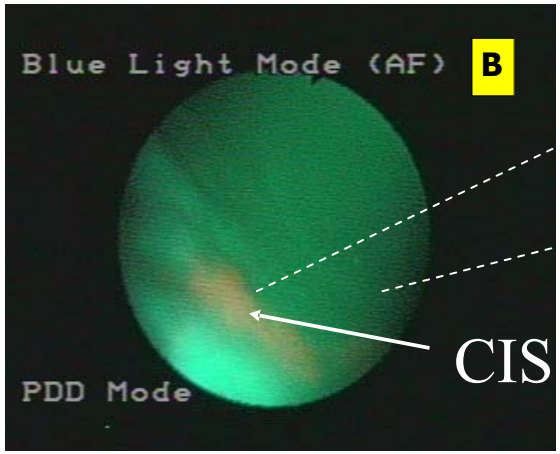
(Bulgakova et al., MLA,2009)

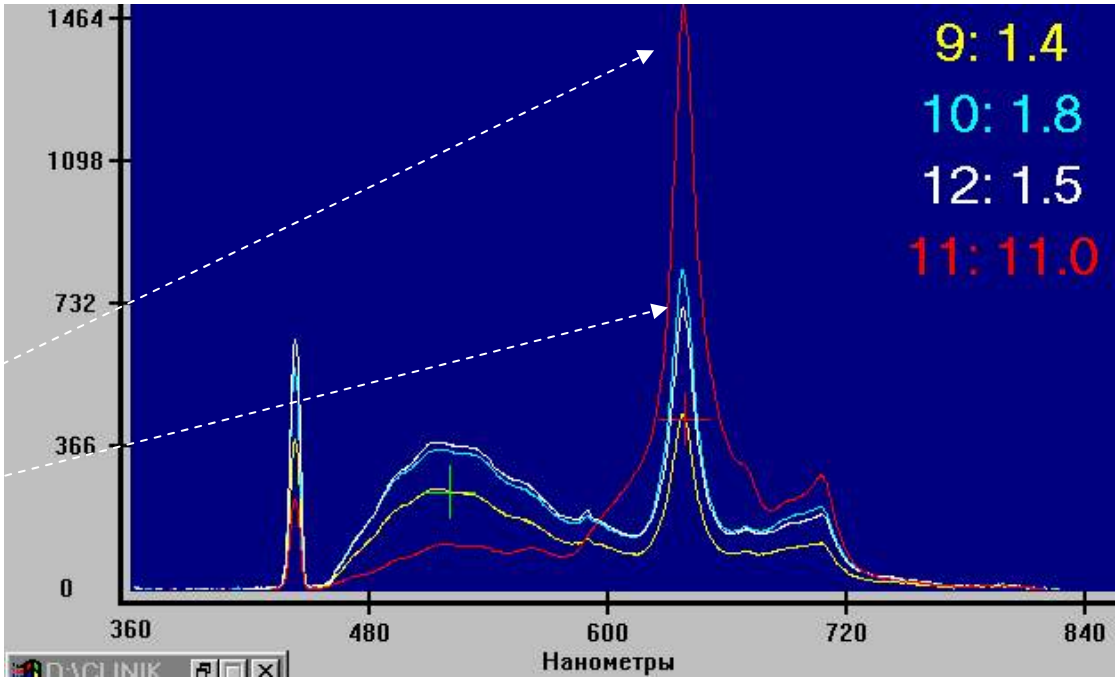
- 62 patients with superficial malignancies of urinary bladder (e.g. hard dysplasia, cancer *in situ*, primary and recurrent transitional cell carcinoma) participated in fluorescence examinations
- 44 patients underwent PDD, 19 patients underwent AF study alone
- Fluorescence examination under Blue Light
- In vivo LFS of the red fluorescence spots that were revealed in the course of fluorescence imaging. On average, 10 to 15 spectra were recorded per each examination. Adding of the spectral measurements extended the total time of endoscopy examination procedures by no longer than 5…7 minutes
- In total, 528 fluorescence spectra have been recorded in the bladder, including 278 spectra from healthy urothelium, 92 from inflammatory areas, 42 from cancer in situ (CIS) or hard dysplasia foci and 216 from superficial bladder cancer foci
- Biopsies were taken from all red fluorescence sports (3.5 per one patient)
- Spectral Data processing
- **Correlation of spectral parameters with histological diagnosis**

# In vivo LFS in 5-ALA-induced PPIX guided PDD of superficial bladder cancer

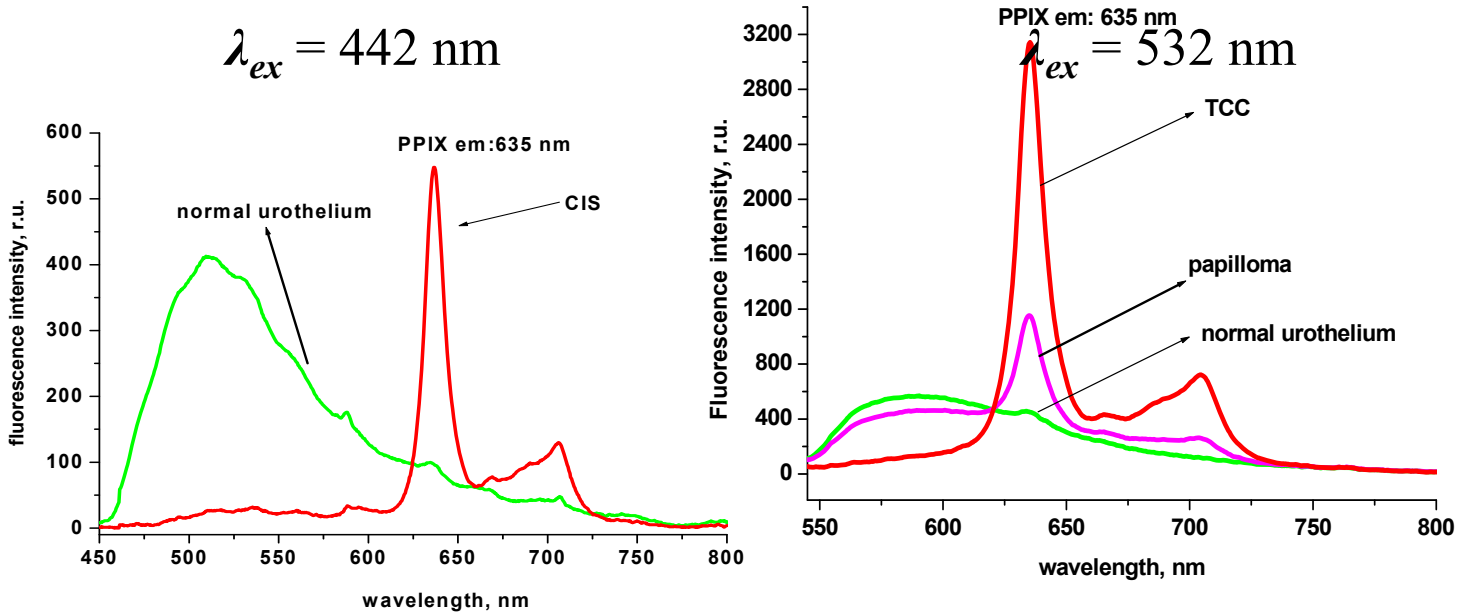
(Bulgakova et al., MLA,2009)



# Typical *in vivo* fluorescence emission spectra measured 2 hours after Alasense instillation




**λex** = 442 nm: Spectral Fluorescence Parameter $\mathbf{D_f}$ = I (620nm÷650nm) / AF (480÷520nm)

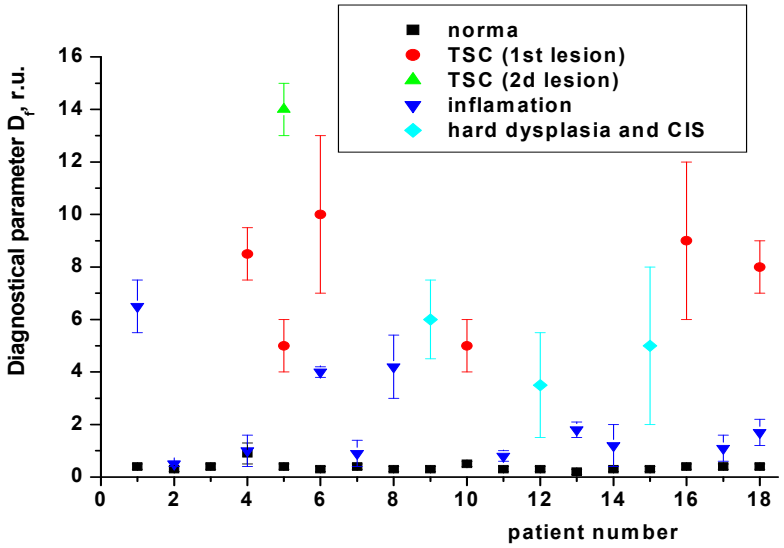
**λex** = 532 nm: Spectral Fluorescence Parameter $\mathbf{D_f}$ I (620nm÷650nm) / AF (555÷585nm)

(Bulgakova et al., MLA,2009)

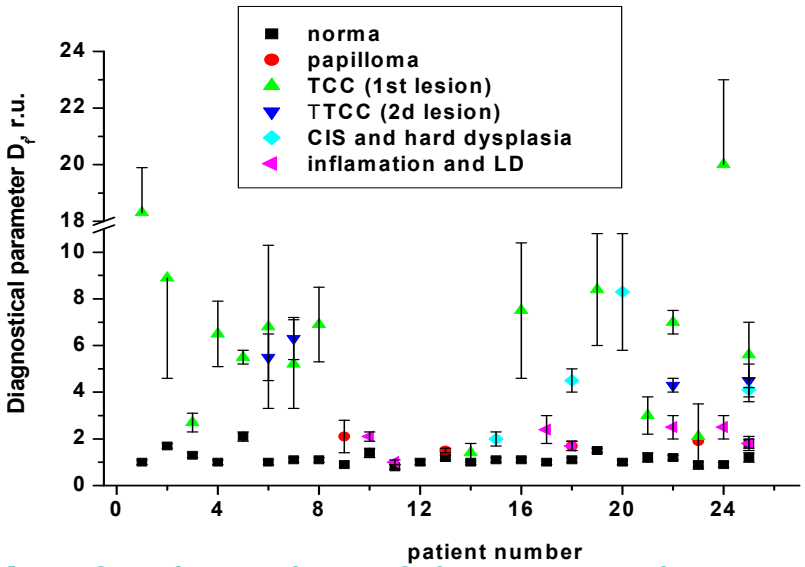
# Spectral Fluorescence Parameter $D_f$

## vs. histological status of tissue (Bulgakova et al., MLA,2009)

$\lambda_{ex}$ = 442 nm




$\lambda_{ex}$ = 532 nm

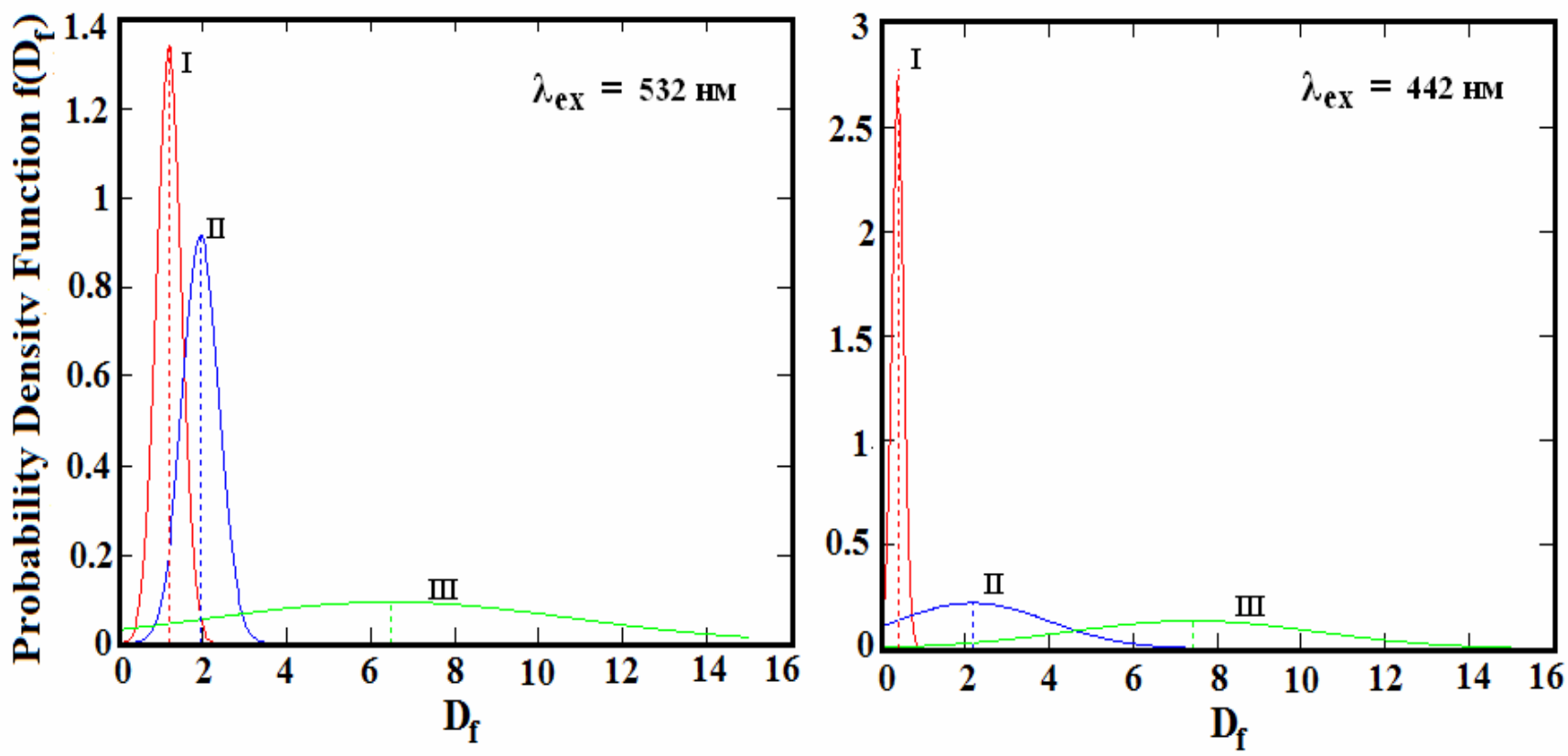



**The aim**: to find a **quantitative criterion** for the value of the spectral diagnostic parameter $D_f$ that would facilitate characterization of following tissue types:

- **Type I** - normal urothelium
- **Type II** - inflammation, papilloma, low and moderate dysplasia
- **Type III** - hard dysplasia, CIS and transitional cell carcinoma (TCC)

## Probability distribution functions (PDF) for the parameter $D_f$ that are resultant for the tissue Types

(Bulgakova et al., MLA,2009)




**Type I** - normal urothelium

**Type II** - inflammation, papilloma, low and moderate dysplasia

**Type III** - hard dysplasia, CIS and transitional cell carcinoma (TCC)

# Two approaches:

- **a posteriori estimation of the threshold values** of the spectral fluorescence parameters for inflammation and cancerous tissues

- **a posteriori probability of attributing** of an acquired emission spectrum to a histologically confirmed tissue type

# LFS in PDD of superficial bladder cancer

- Fluorescence Imaging: PPV 67%
- Fluorescence Imaging & LFS:

(1) a posteriori estimation of the threshold values:

PPV 91%

(2) a posteriori maximum probability-based approach:

PPV 91 – 100%

(Bulgakova et al., MLA,2009)

# 5-ALA-induced PPIX guided PDD of superficial bladder cancer (Bulgakova et al, MLA 2009)

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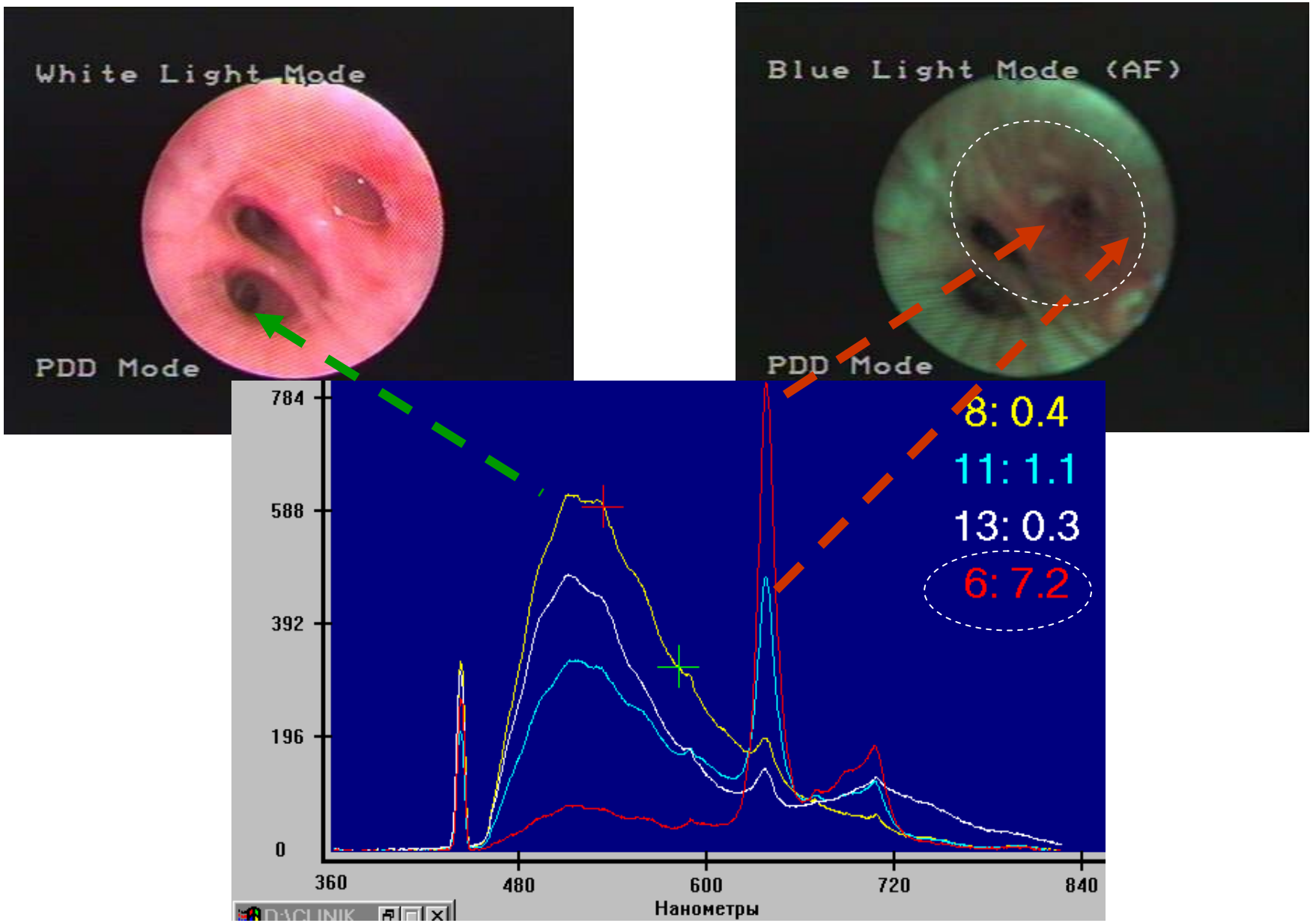
# PDD of bronchi with Alasens

- **Inhalation with solution of 5-ALA, exposition 1-2 hours**
- **White-light bronchoscopy**
- **Fluorescent bronchoscopy for suspicions areas survey**
- **LFS in suspitions areas**
- **Biopsy in the zones with higher level of protoporphyrin IX with diagnostic parameter values specific for maligned tissue**

# In vivo LFS in 5-ALA-induced PPIX guided PDD of early X-ray negative Lung Cancer (SCC RB10) (Sokolov et al, 2005)



# Fluorescence Imaging and in vivo LFS in PDD of Early Central Lung Cancer with 5-ALA

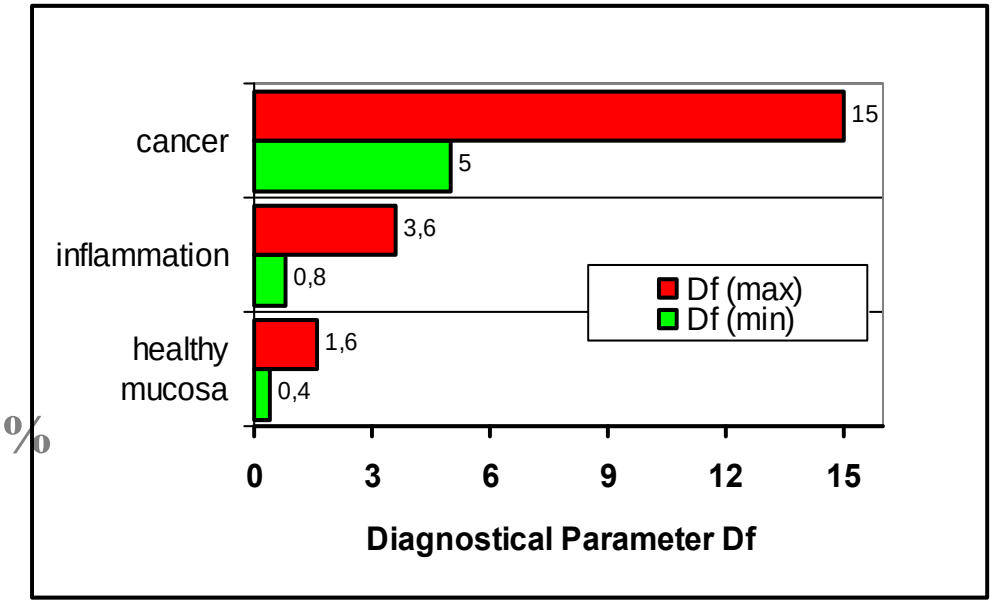
(Sokolov et al., Pulmonology, 2005)

N=35

Senstivity 96%

Specificity 89%

Positive Predictive Value = 91 %




***Occult early central lung cancer has been revealed in 12.9% of cases***

***Occult dysplasia II-III has been revealed in 6.5% of cases***

# In vivo LFS in the course of PDD allows to:

- **minimize the number of false-positive fluorescence results**
- **reduce the number of biopsy specimens**
- **increase the quality of guided biopsy**

**<u>Conclusion:</u> The method really improves the ability to localize precancerous and early cancerous lesions**

**Combination of fluorescence bronchoscopy and LFS for groups with higher onco-risk** ***(Sokolov et al, Pulmonolgy, 2005):***

- smokers;
- Workers, related to asbestos and radon;
- Chronic bronchitis, pneumonia, tuberculosis patients;
- Chronic bronchitis patients with atypical cells;
- **For detection of hidden forms of precancer and early cancer of trachea, bronchi,** for oncologic patients with diagnosis esophageal cancer, lung cancer**;**

# PDD of early cancer (V.Sokolov et al, 2007)

Fluorescent diagnosis of early lung cancer ***(2mm x 200microns)***

1. **Early central lung cancer**

***sensitivity-96,5%; specificity- 89,7% ; hidden + 20%***

2. **Early esophageal and stomach cancer**

***sensitivity - 88,9%; specificity- 70,2% ----------***

3. **Early bladder cancer**

***sensitivity - 86 %; specificity- 67 %; hidden + 28%***

4. **Early cervix cancer**

***sensitivity - 98 %; specificity- 54%; hidden + 34%***

5. **Basal cell carcinoma of skin**

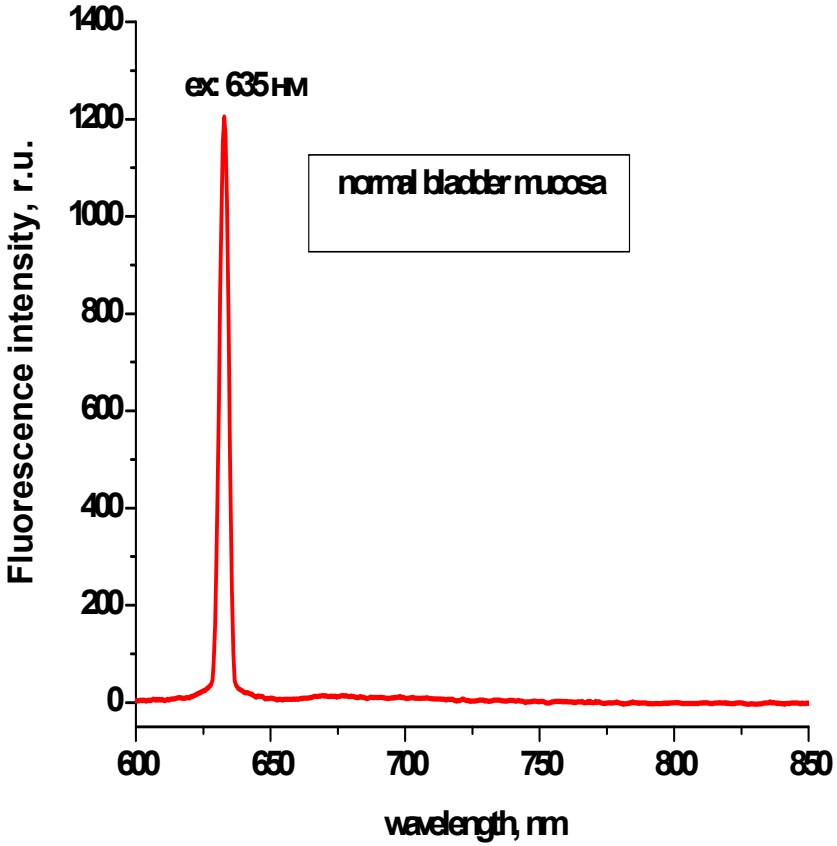
***sensitivity -100%; specificity- 59%; hidden + 26%***

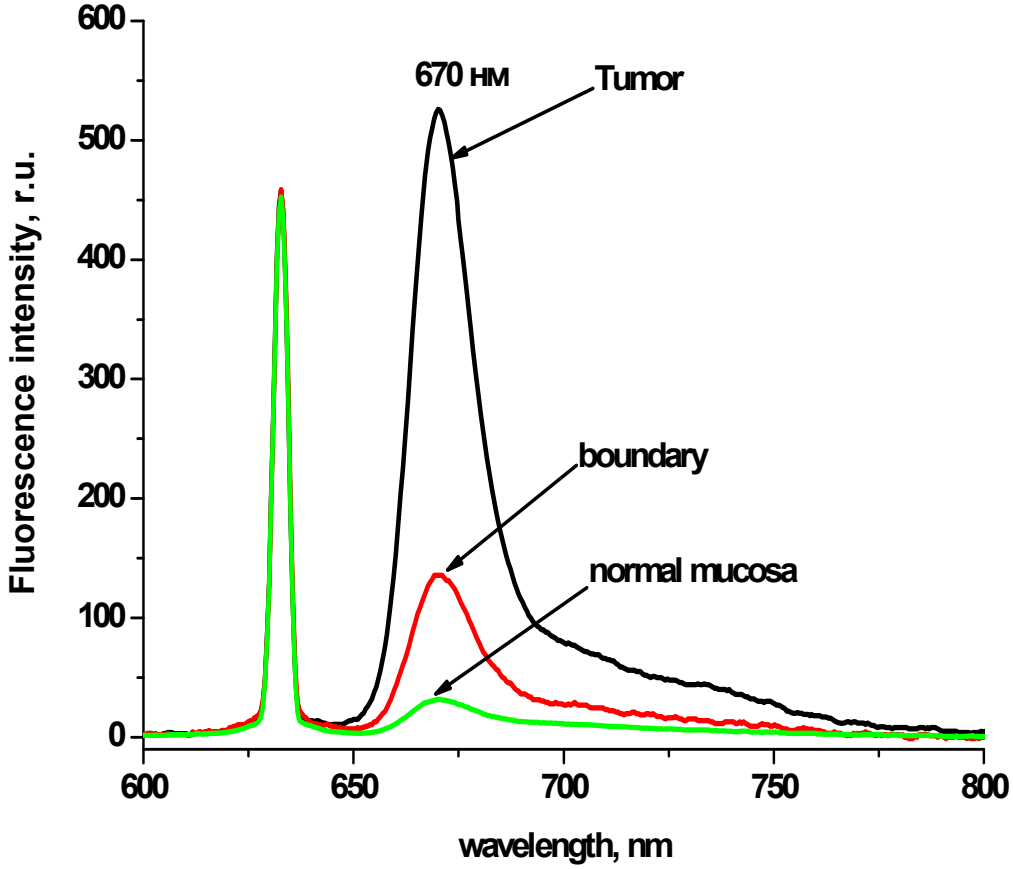
6. **Skin melanoma (ZOOM –dermatoscopy)**

***sensitivity - 80%; specificity- 70% ----------***

# In vivo LFS under 633 nm excitation in fluorescence detection of bladder cancer after i.v. injection of chlorine-based photosensitizer

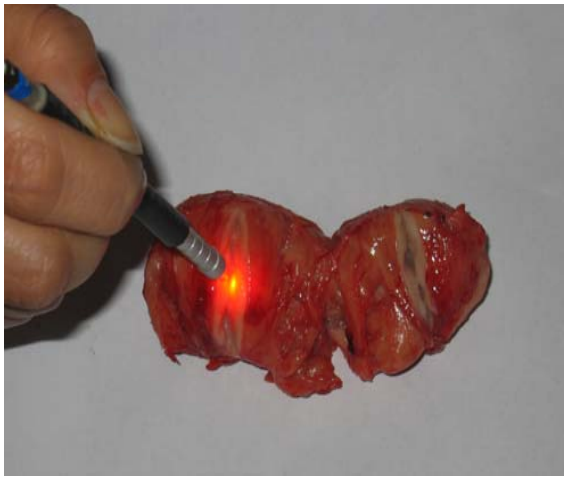
**(D. Jagudaev, N.Bulgakova et al. Laser medicine 2007)**

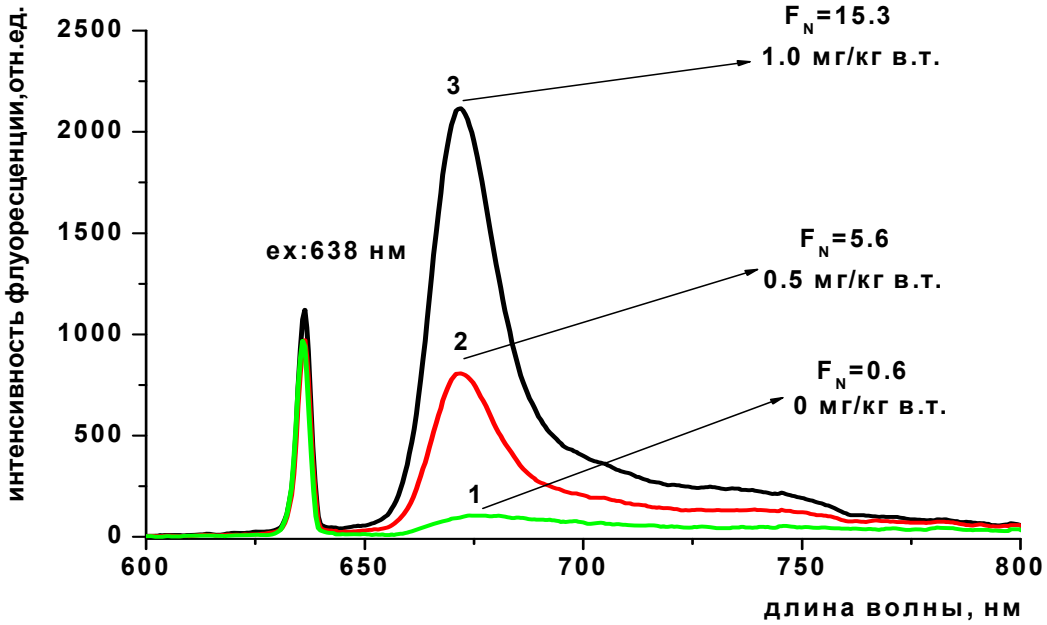


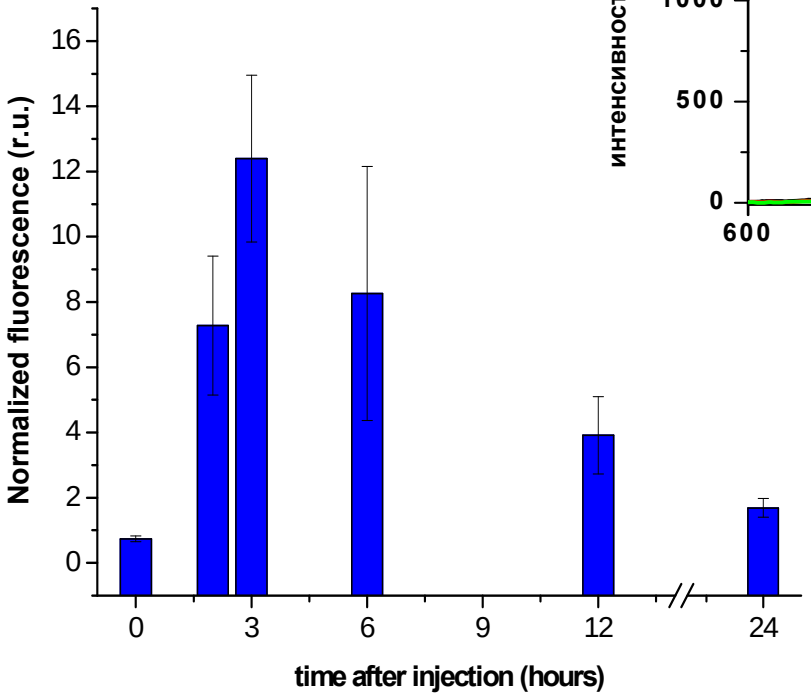



# Ex vivo studying of chlorine-based photosensitizer accumulation in adenoma of prostate after intravenous injection

## (0,6 and 1,0 mg/kg b.w.)






D.Jagudaev, N. Bulgakova et al
Urology, 2006

# Advantages

- Very high sensitivity!
- Possibility of **noninvasive real-time *in vivo*** retrieving of quantitative data on tissue fluorescence emission spectra
- The single LFS-exposed sampling volume of the tissue depends on its optical properties and **may amount to a 1…4 mm$^3$** on the average, which is comparable to the volume of biopsy specimens taken by forceps.
- **The area of the tissue subject to LFS, can be much wider** than in biopsy case, since fluorescence spectra can be easily recorded at various places, whereas the biopsies number is limited.
- **Recording a single fluorescence spectrum takes much shorter time than one biopsy** so, that **many spectra** can be acquired at many places of the tissue while one biopsy is being taken.

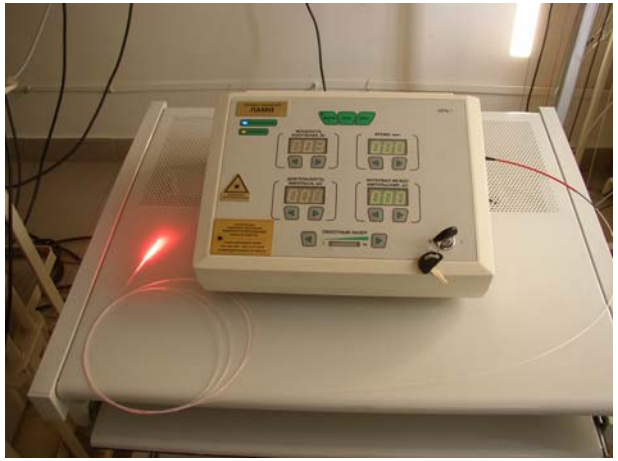
# Equipment for PDT

1. Light sources:
   - Lamps
   - Lasers
   - LEDs

2. Fiber systems for irradiation delivery to biological tissue

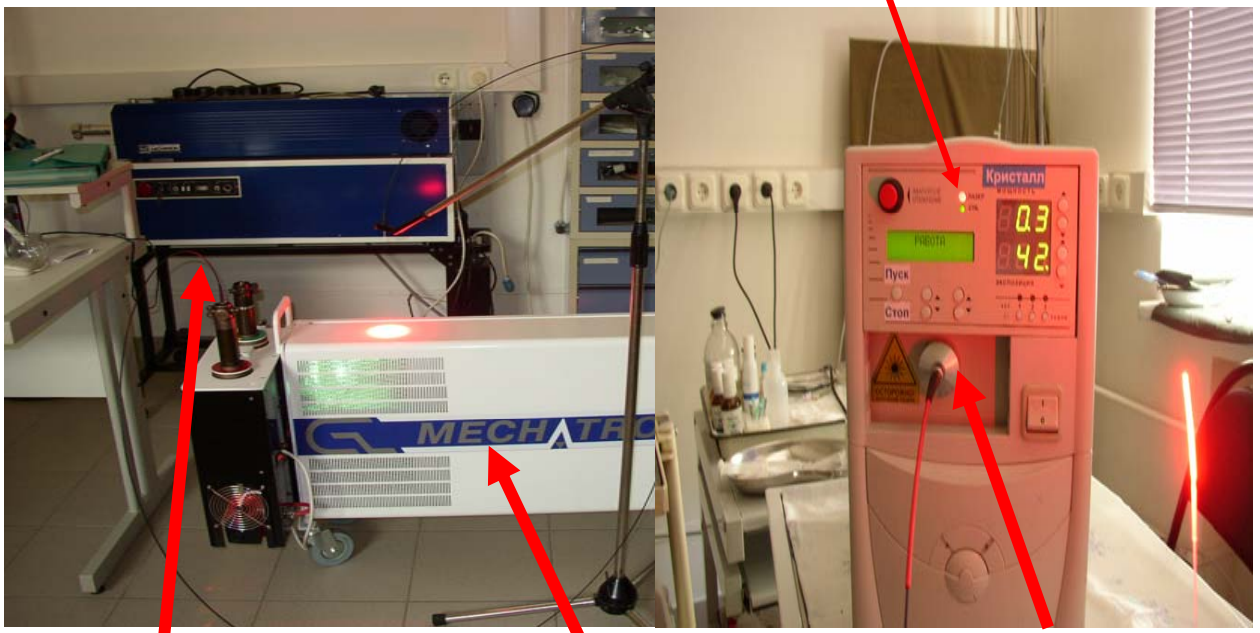
# Laser sources

Diode laser (670 nm)

Diode laser (662 nm)

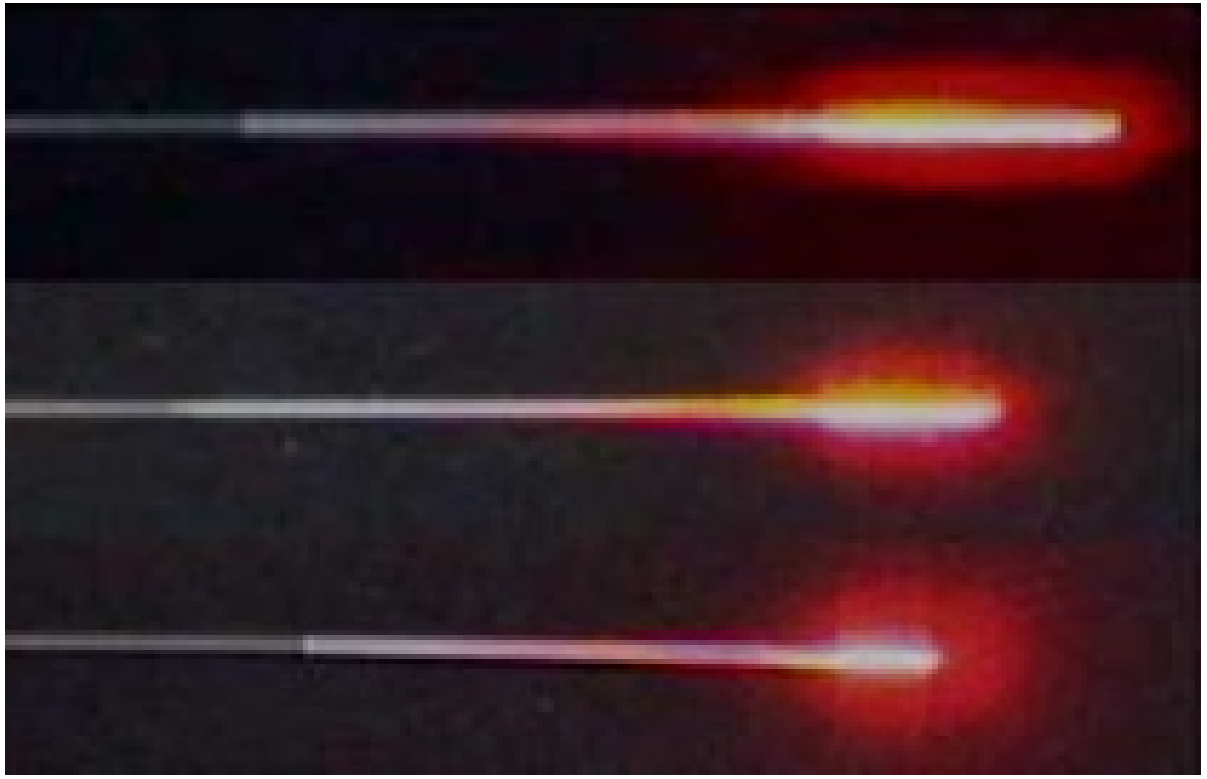
Diode laser (670 nm)




Au-vapour laser (628 nm)

Dye-laser

# Cylindrical diffusers for PDT



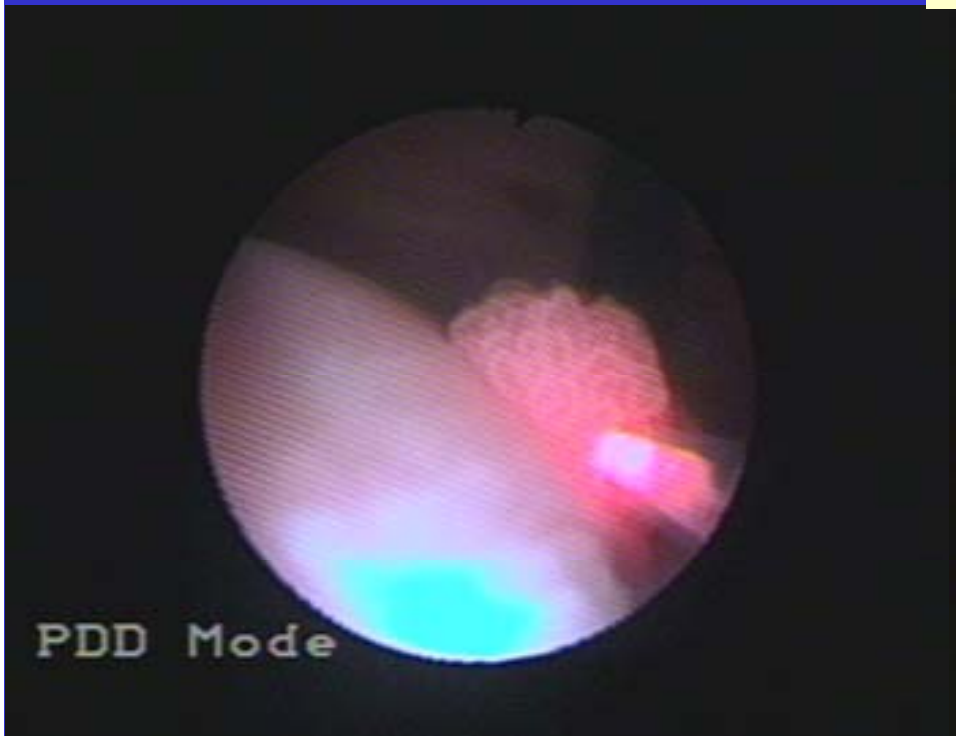
# PDT irradiation variants

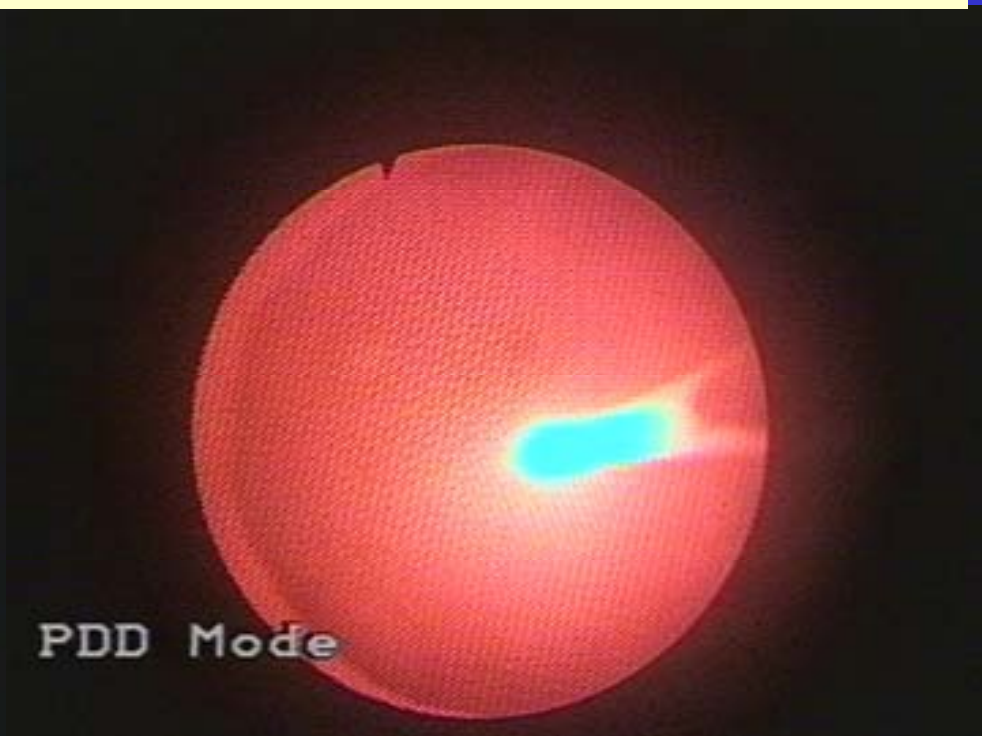
(Uljanov et al, 2007)

Treatment

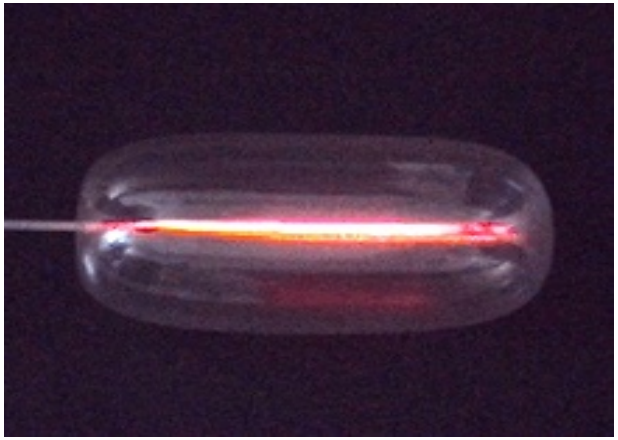
Prophylactic

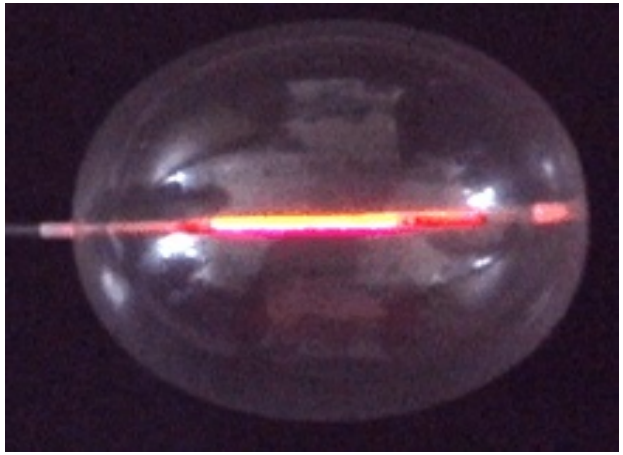
**Spherical tip of the fiber is applied**

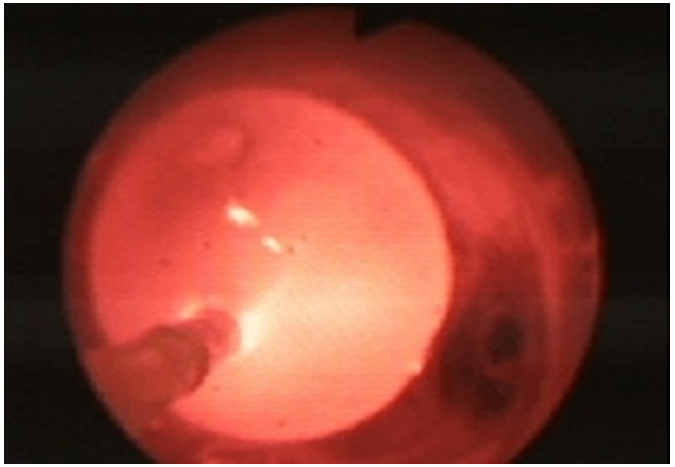


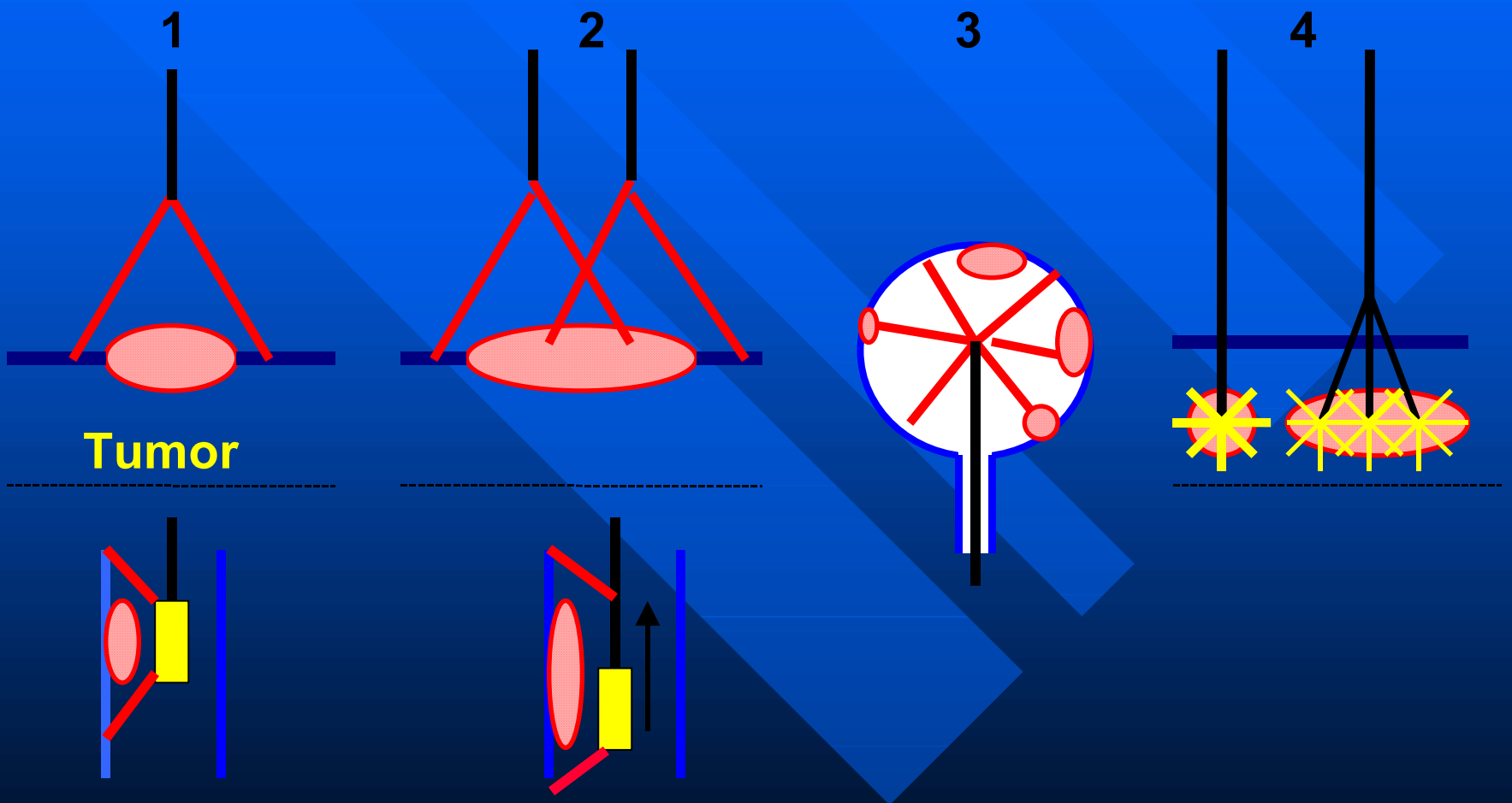
# Diffusers for PDT
# balloon type







# PDT of malignant tumors
# The ways of laser light delivery to tumors



PDT early cancer in FSU ROI P.A. Gertzen

1. **Early central lung cancer**

   full regression 89 –100 %;  recurrence - - - - 15%

2. **Early esophageal cancer**

   full regression 87 –  91%; recurrence - - - -  14%

3. **Early stomach cancer**

   full regression 60 – 88%; recurrence - - - - -  5%

4. **Basal cell carcinoma of skin**

   full regression 86 -  93%; recurrence - - - - - - 8%

## Possible applications of PDT in oncology

**(Cissov et al. 2006)**

1. **Single-course PDT**
2. **Multiple-course PDT**
3. **Multiple-course PDT + surgery** ***(pre-,intra-, post-operative)***
4. **Prolonged PDT**
5. **Prolonged PDT + chemotherapy**
6. **Prolonged PDT + laser-induced**

## Indications for prolonged PDT
## (V. Sokolov et al 1999)

- Aggressive primary and recurrent tumors
- Metastases of breast cancer under skin
- Intra- and under cutaneous melanoma metastases (multiple small lesions)

# MAIN ADVANTAGES OF PDT

- ✓ Selectivity of the light interaction – minimized damages of normal tissues
- ✓ Possibility for repeatable application – multiple PDT(Sokolov et al. 1999)
- ✓ Combined application of PDT and LT or PT

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