UNIVERSITÉ DE GENÈVE

Photomedicine in Gynecology

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Training Course for Advanced Oncologic Laparoscopy

St. Petersburg - February 17, 2006

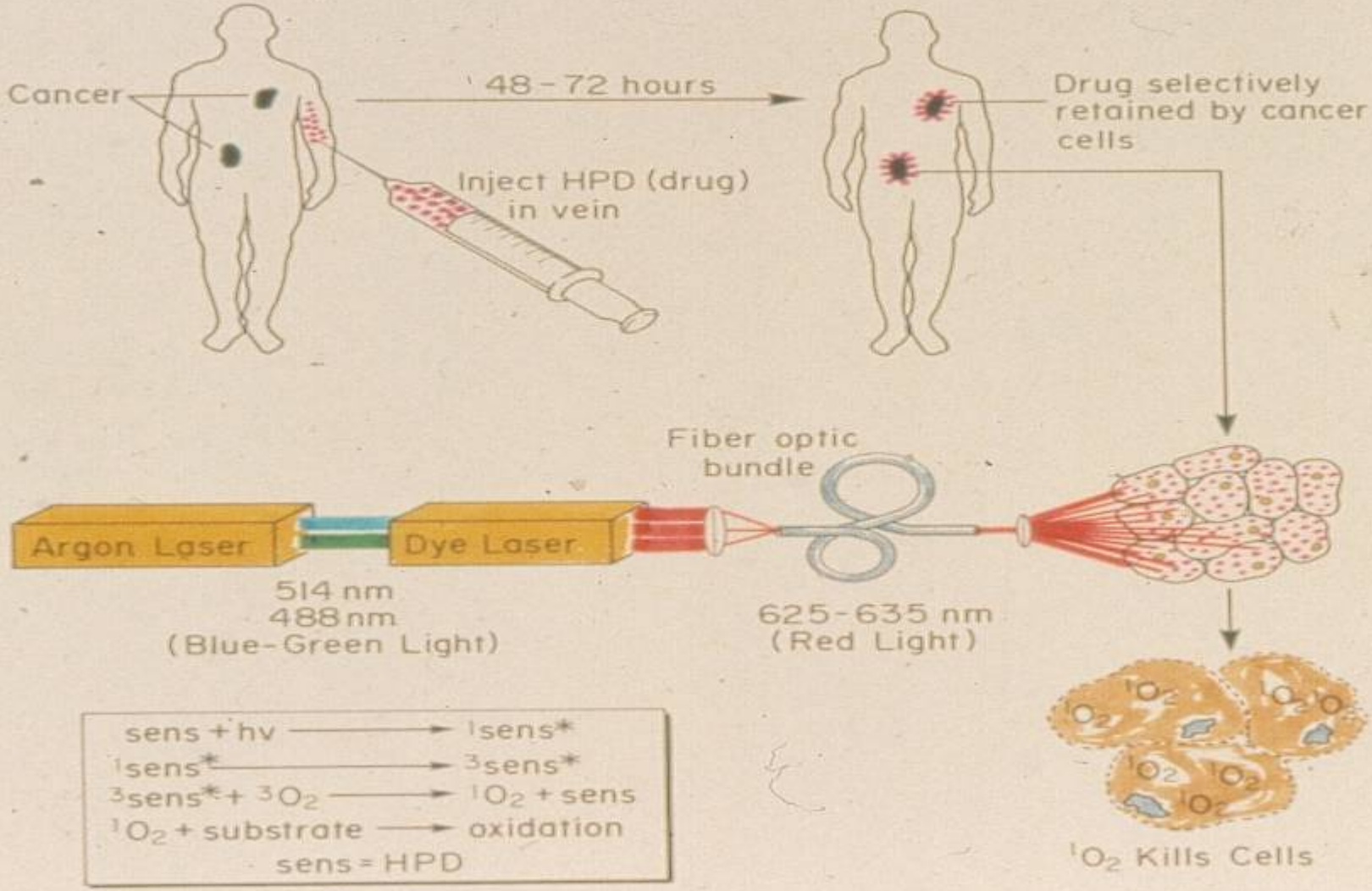
Photodynamic Principle

- Use of a photo-enhancing or photo-sensitizing chemical to aid in the diagnosis or treatment of a target cell

Clinical Studies in Gynecology

- Endometrial Ablation Wyss et al, Int J Gyn & Obst 1998, Major et al, J Gynecol Surg 1999
- Condyloma Fehr et al, Am J Obst Gyn 1998
- Cervical and vulvar dysplasia Hillemanns et al, Cancer 2000
- Ovarian cancer Major et al, Gynecol Oncol 1997, Hornung et al, J Am Assoc Gynecol Laparosc, 1998, Major et al, Laser Med Sci 2002

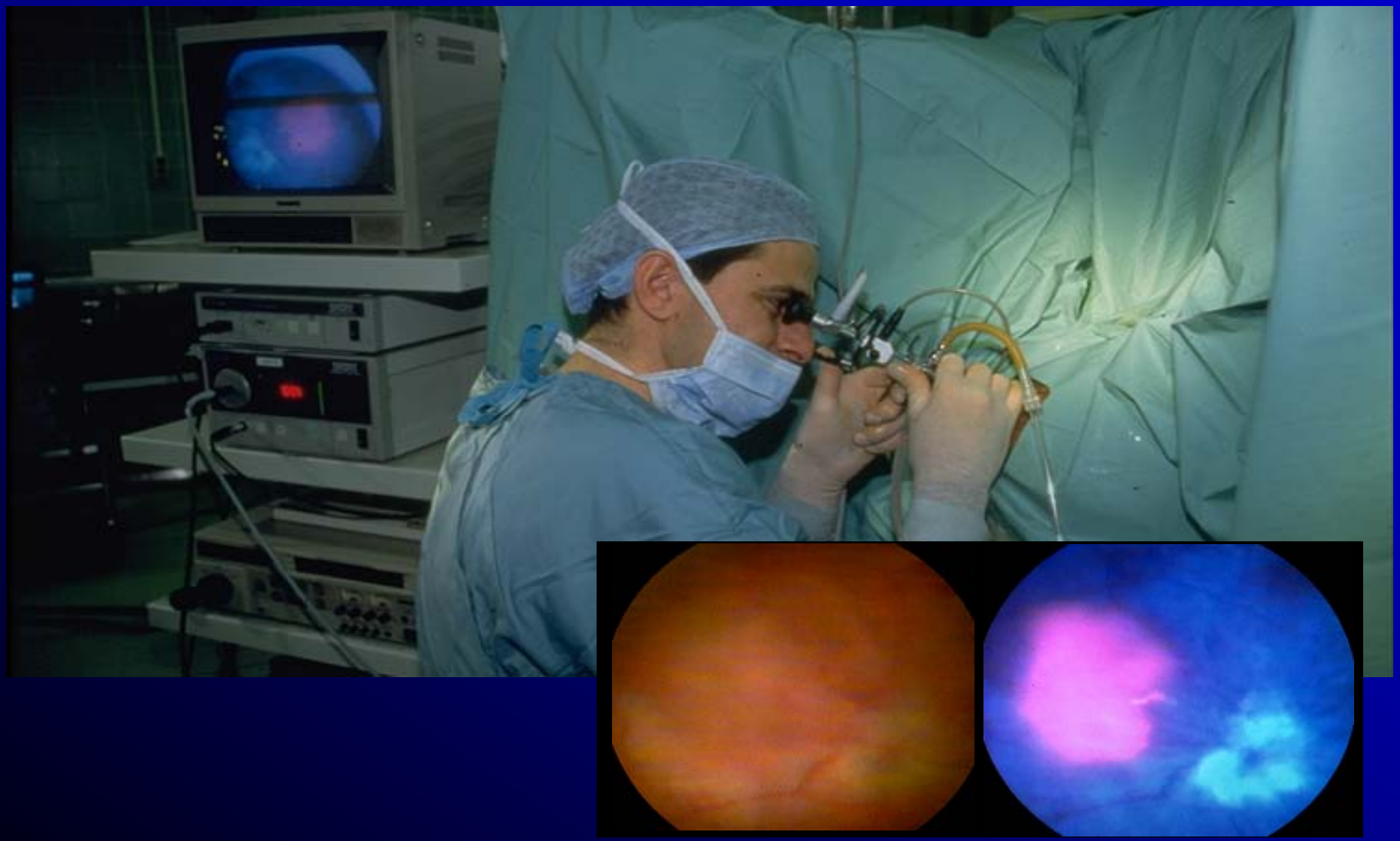
PHOTORADIATION THERAPY OF CANCER
(Laser–Hematoporphyrin Derivative)



$$\text{sens} + h\nu \longrightarrow {}^1\text{sens}^*$$
$${}^1\text{sens}^* \longrightarrow {}^3\text{sens}^*$$
$${}^3\text{sens}^* + {}^3O_2 \longrightarrow {}^1O_2 + \text{sens}$$
$${}^1O_2 + \text{substrate} \longrightarrow \text{oxidation}$$

sens = HPD

PHOTODETECTION



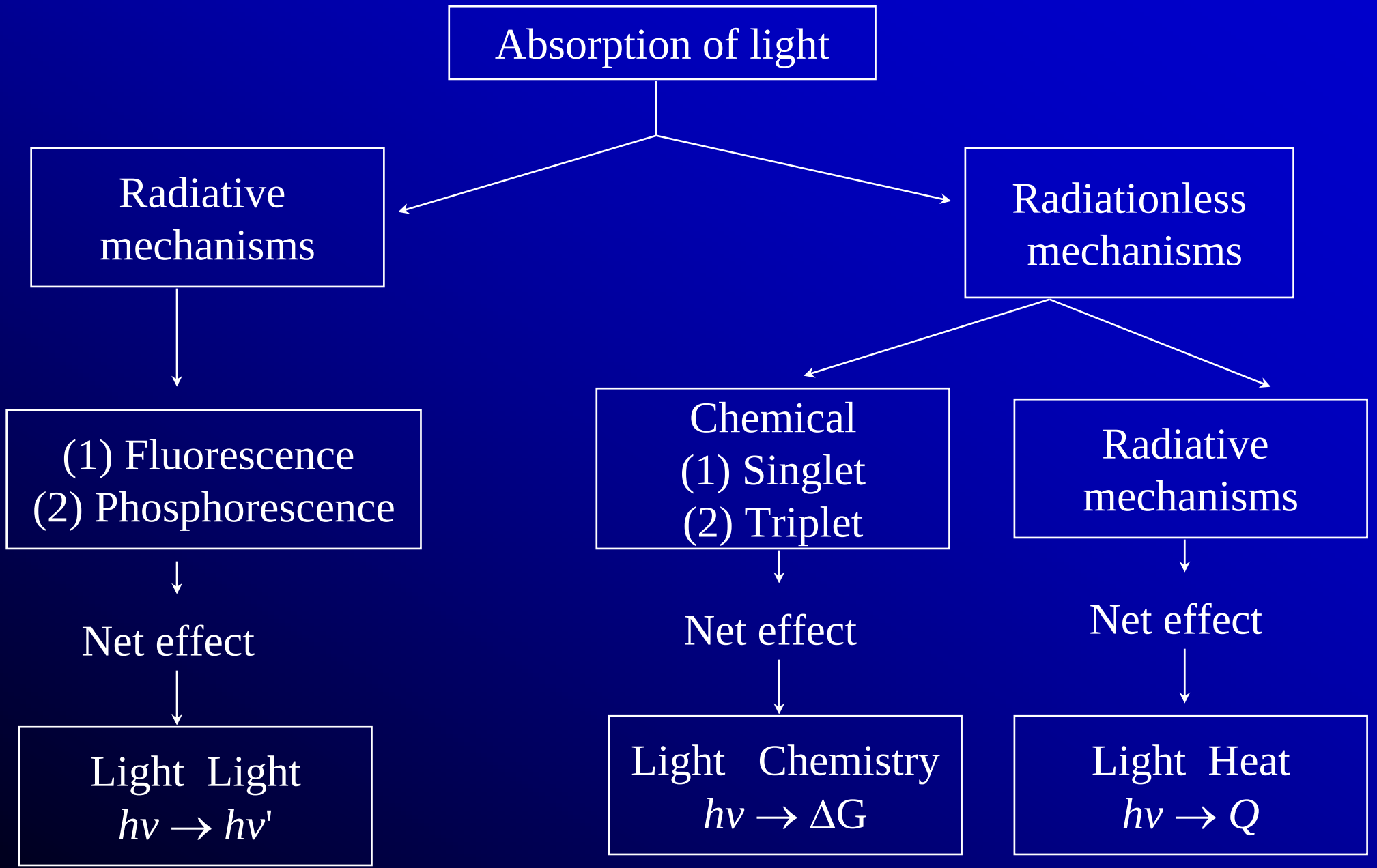
Historical

1976 J. F. KELLY + M. E. SNELL - First clinical PDT of a bladder carcinoma with HPD. (J. Urol., 115, 150, 1976).

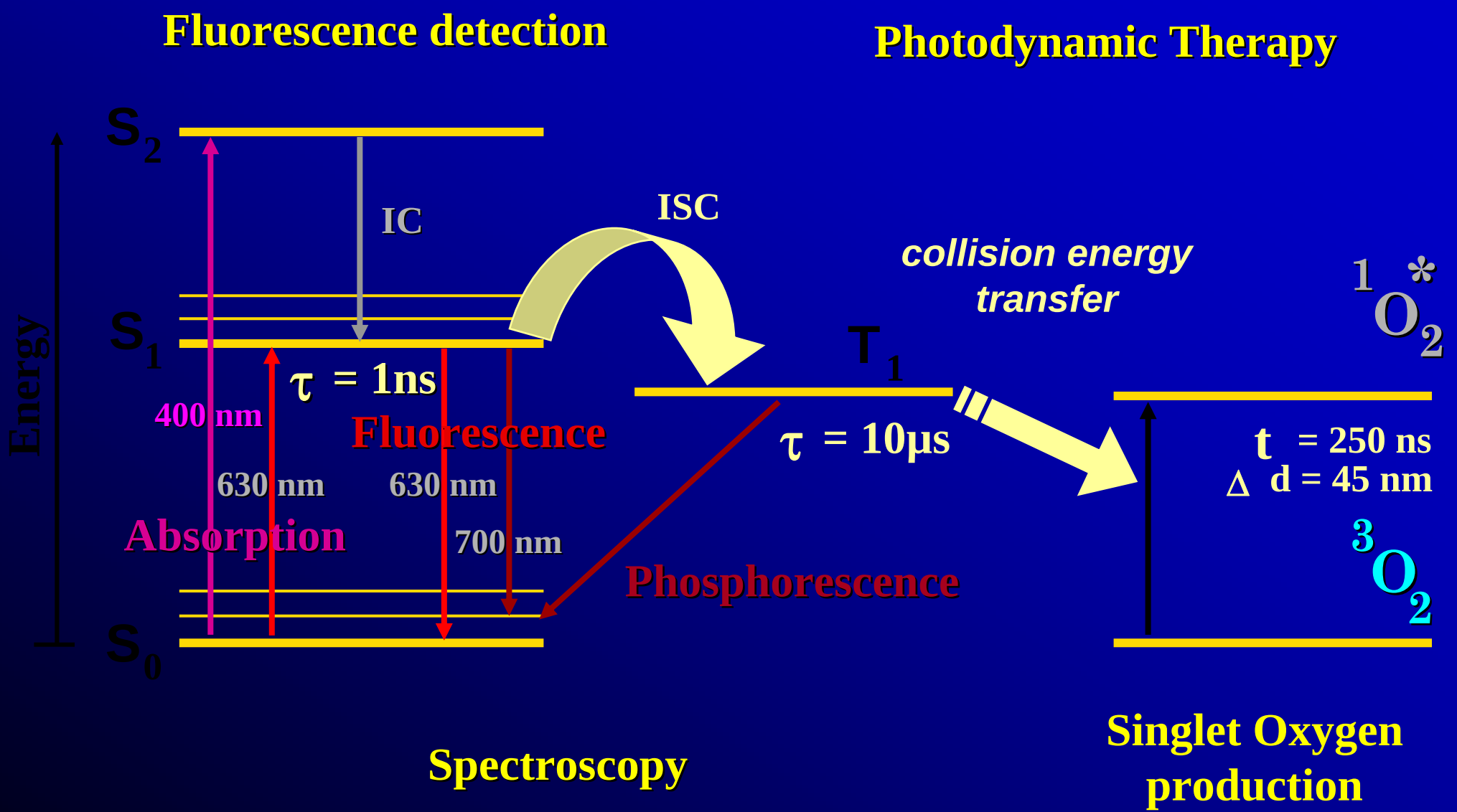
1978 T. J. DOUGHERTY et al.- Clinical assessment of PDT (Cancer Res., 38, 2628, 1978).

! LASERS + OPTICAL FIBERS !

1993 First approval (by the canadian health agency) of PDT with Photofrin® for the prophylactic treatment of bladder cancer.


Absorption of light
Radiative mechanisms
Radiationless mechanisms
(1) Fluorescence
(2) Phosphorescence
Chemical
(1) Singlet
(2) Triplet
Radiative mechanisms
Net effect
Net effect
Net effect
Light Light
hν → hν'
Light Chemistry
hν → ΔG
Light Heat
hν → Q

Photophysical Processes



Photosensitizers

- Porphyrins
  - Photofrin (PF)
  - "Aminolevulinic acid (ALA)", Protoporphyrin IX (PpIX)
- Chlorins
  - m-Tetrahydroxyphenyl chlorin (mTHPC)
  - Benzoporphyrin derivative mono-acid (BPD)
  - Tin ethyl etiopurpurin (SnET2)
- Phtalocyanines

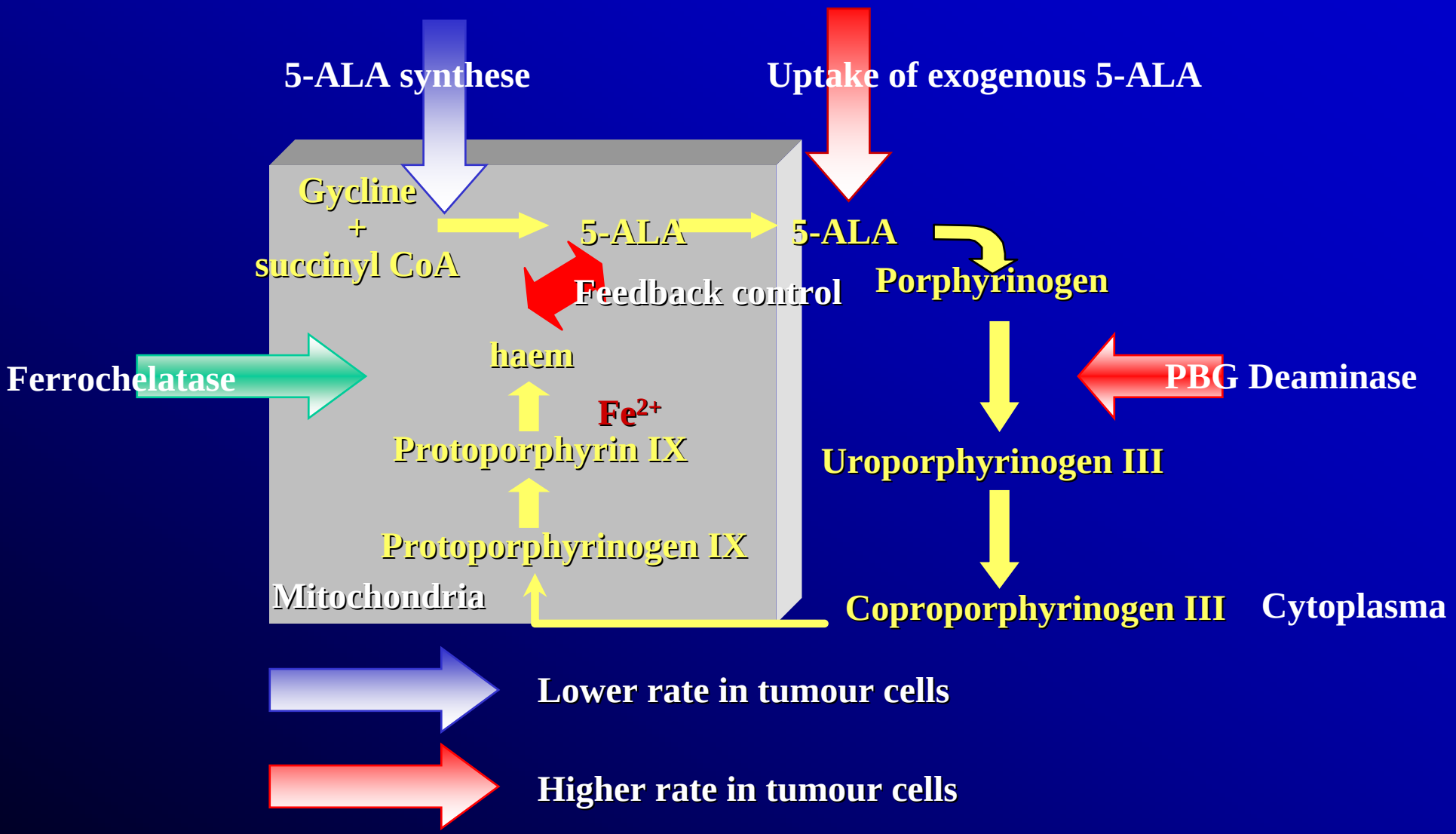
PDT with second generation PS

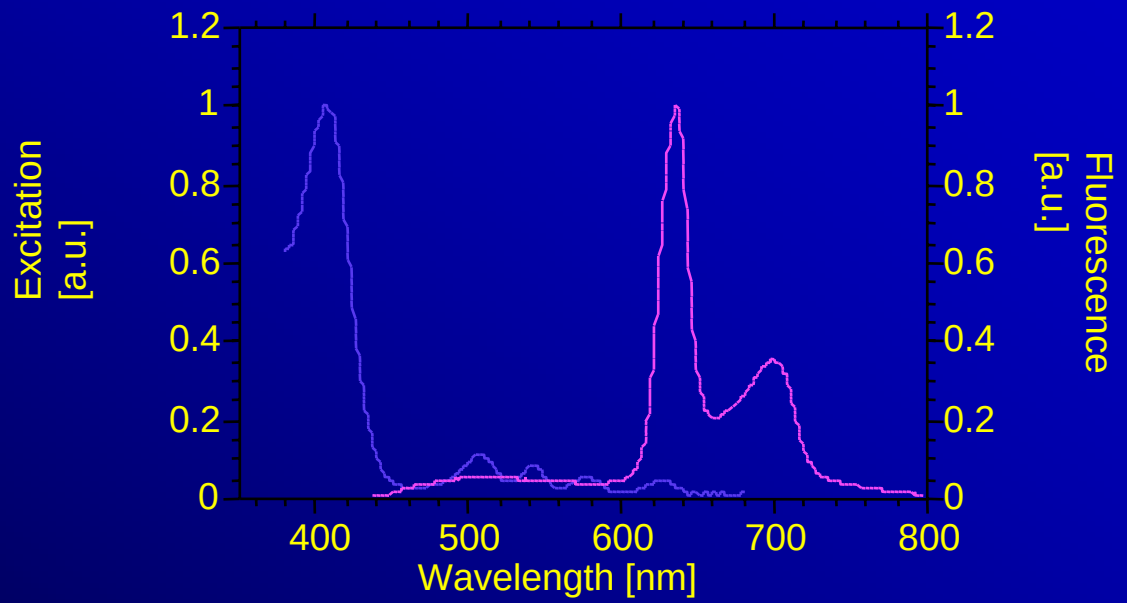
PS	Dose (mg/kg)	D / L (hours)	WL (nm)	Light dose (J/cm^2)
mTHPC	0.075 - 0.15	96	652 (red) 514 (green)	5 - 20 75 - 120
ALA-PpIX	40 - 60	4 - 6	635 and 405	10 - 200
BPD-MA	Topical 20% 0.3	0.4 - 2	(red and blue) 690 (red)	50 - 150
NPe6	0.5 - 1	4 - 8	664 (red)	50 - 100
Lu-Tex	0.6 - 7	3	732 (red)	150
SnET2	1.2	24	660 (red)	200

Photofrin Approval

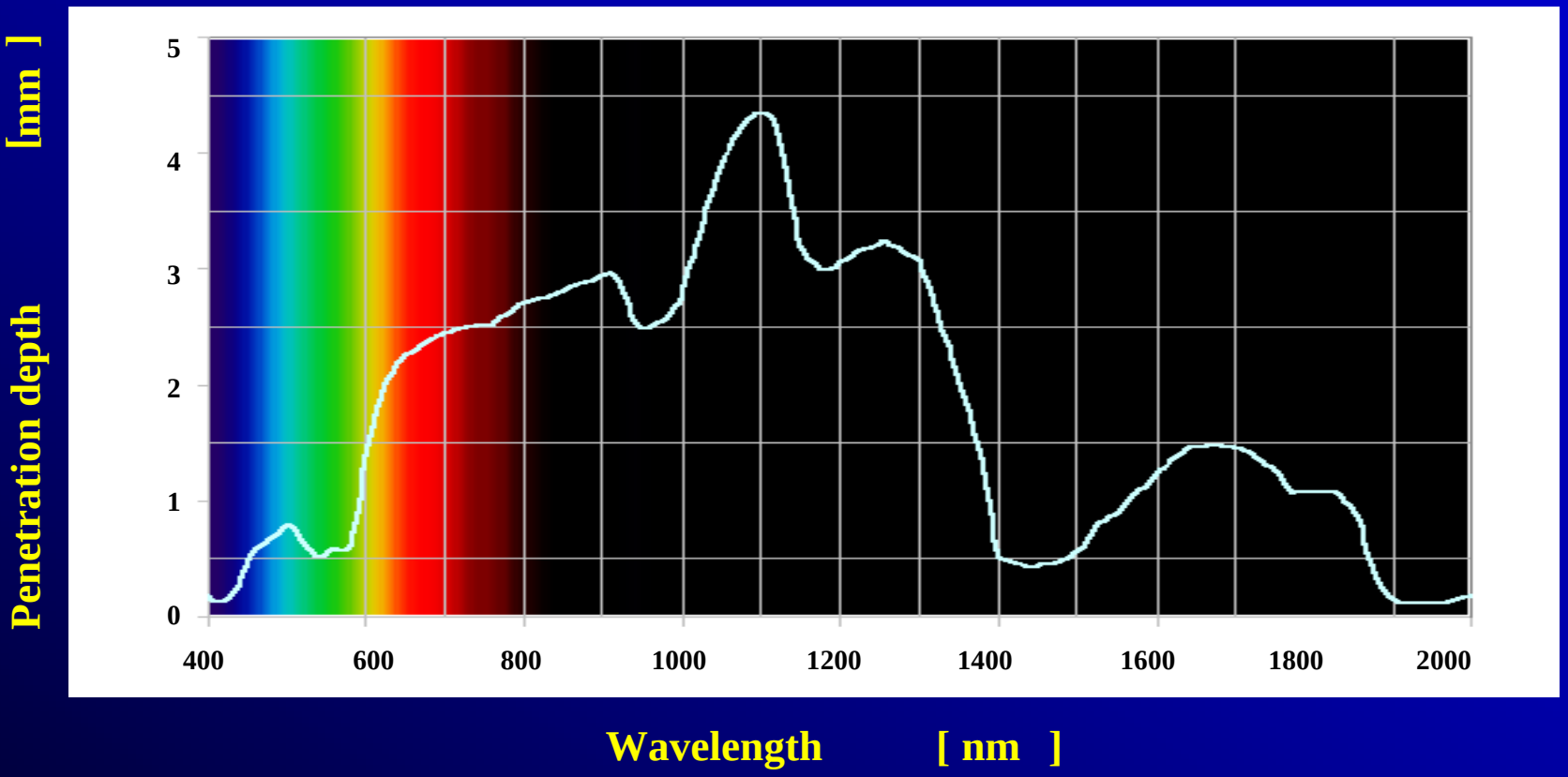
- Superficial bladder cancer (Canada 1993)
- Early and late oesophageal and lung ca (Netherlands 1994)
- Advanced oesophageal ca (USA 1995)
- Early ca of stomach, oesophagus, lung, cervix and cervical dysplasia (Japan 1994)

Haem Biosynthesis

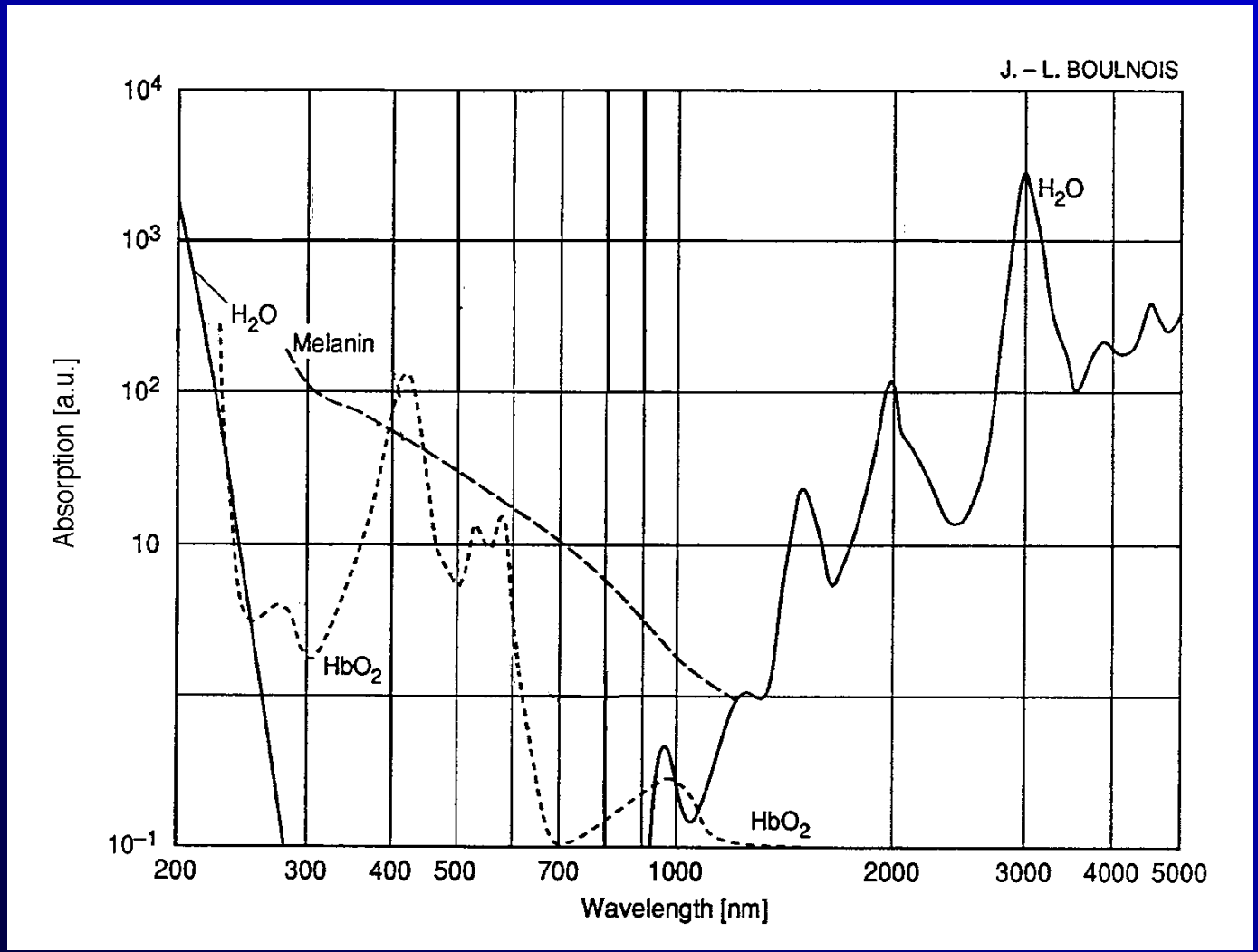




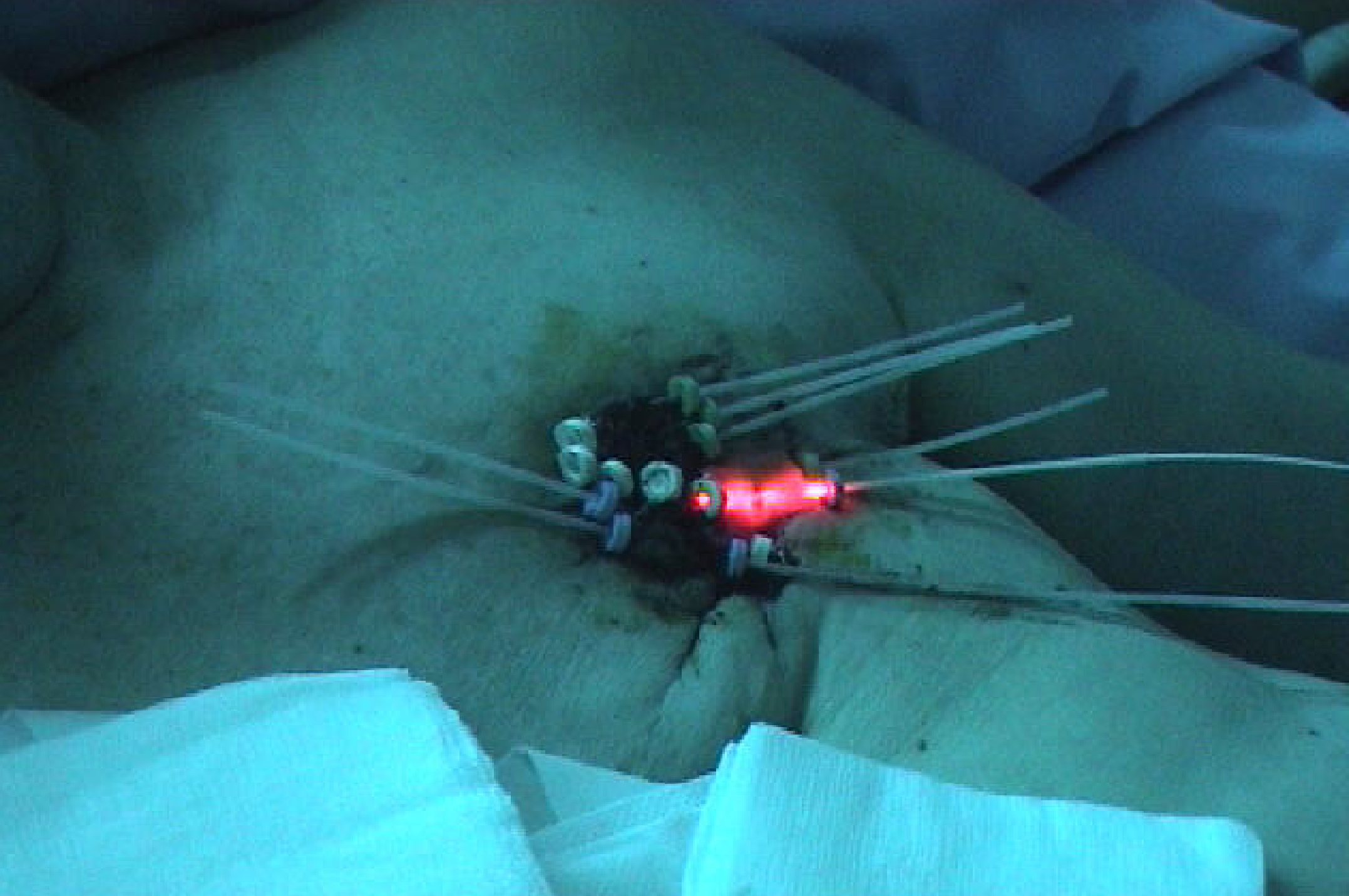
Absorption (dark line) and fluorescence (light line) spectrum of PpIX solved in DMSO. Values of absorption and fluorescence do not correspond to each other



Penetration depth of light in tissue in relation to the wavelength



Absorption of water, melanin (broken line) and oxyhemoglobin (HbO2) (dotted line)



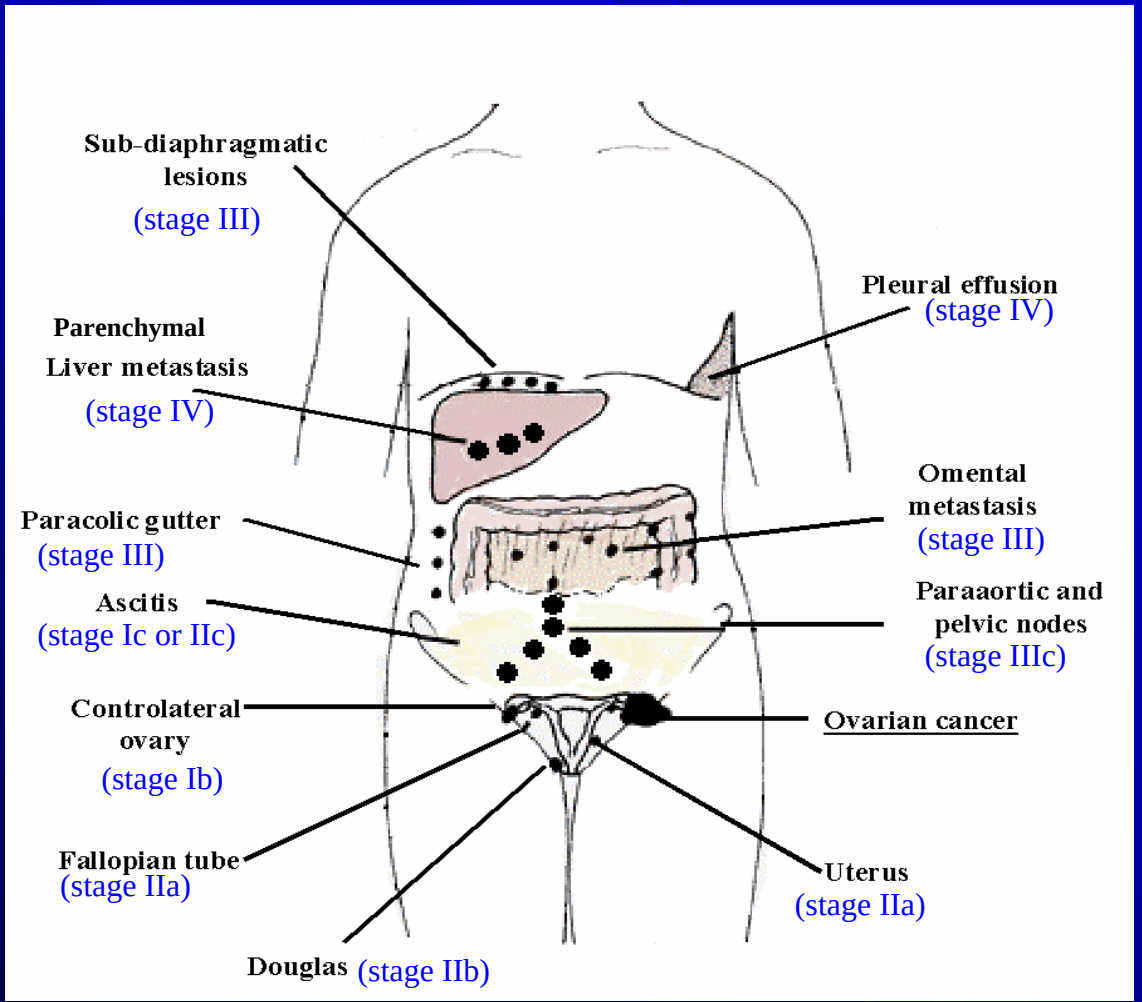
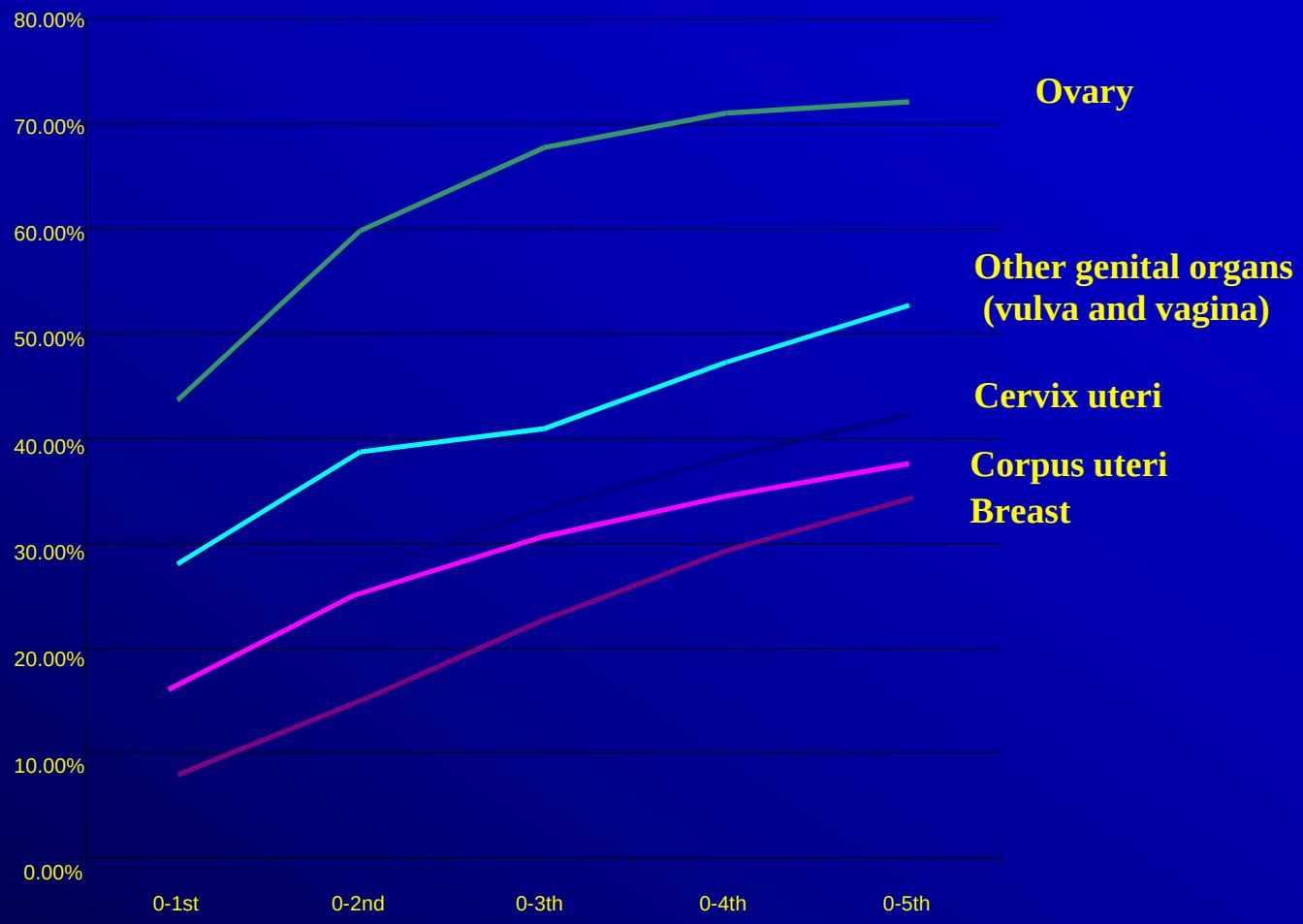


Figure 1

Common sites of ovarian cancer metastases.

Ovarian cancer spreads fast to the whole abdominal cavity by exfoliation



5-Year cumulative lethality of gynecologic malignancies in Geneva

Epithelial Ovarian Cancer

- Fourth most frequent cause of “cancer-related” death
- 65% diagnosed with stage III-IV disease
- Initial response: 80% platinum sensitive
- 5 year survival rate: 15-20%
- Second look laparotomy
  - Historically: no effect on survival
    - 1/3 macroscopic
    - 1/3 microscopic
    - 1/3 negative
  - 50% of patients with a negative second look laparotomy will recur

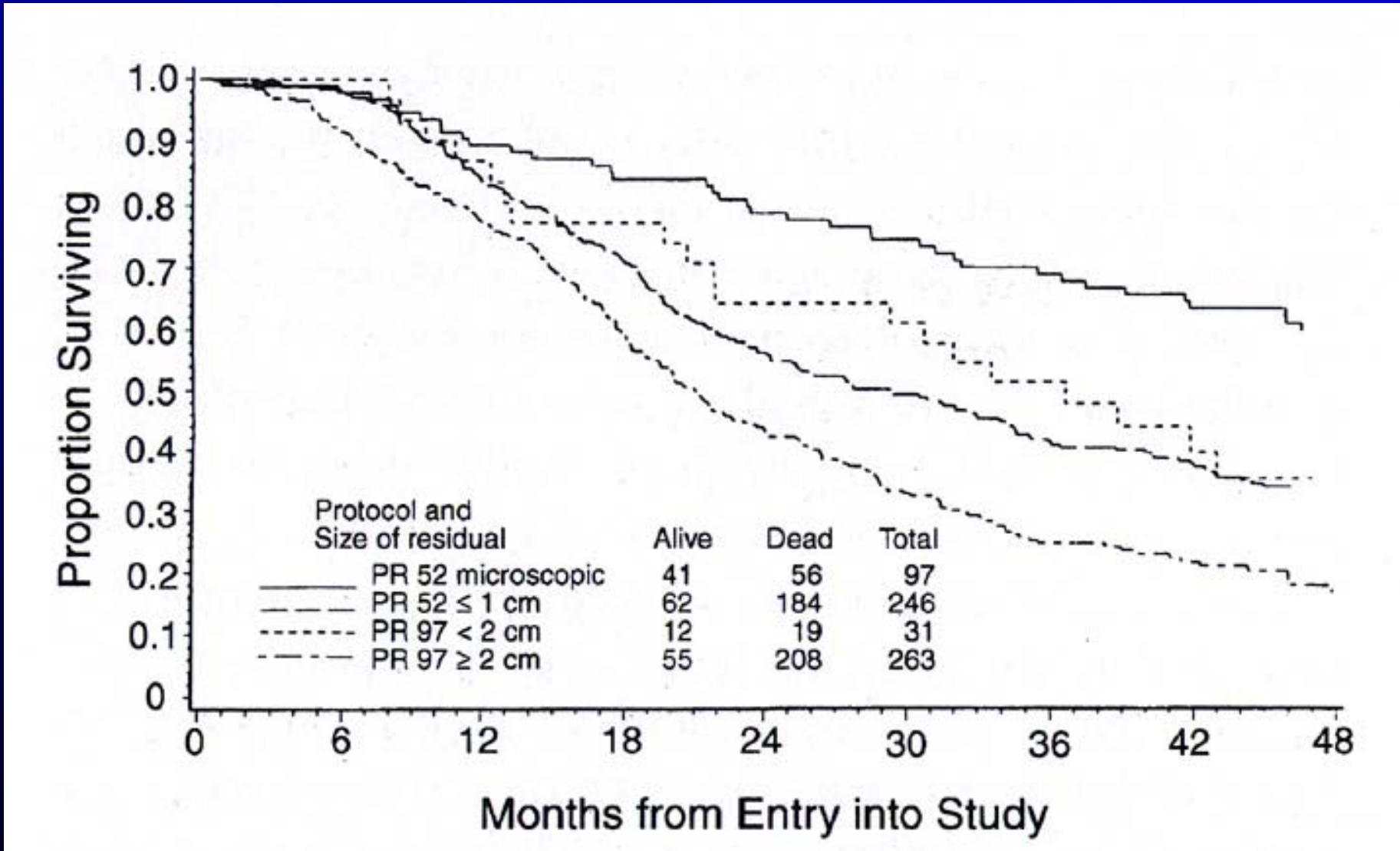
Recommended surgical staging procedures

- Peritoneal washings
- Total abdominal hysterectomy and bilateral salpingo-oophorectomy (Unilateral salpingo-oophorectomy may be appropriate for selected patients with Stage IA disease who desire to defer definitive surgery until completion of childbearing.)
- Infracolic omentectomy
- Pelvic and para-aortic lymph-node sampling
- Peritoneal biopsies from:
  - cul-de-sac
  - rectal and bladder serosa
  - right and left pelvic sidewalls
  - right and left paracolic gutters
  - right and left diaphragms
  - any adhesions

Results of restaging laparotomies in women with apparent early stage ovarian carcinoma

Authors (year)	Number of patients	FIGO stage at initial surgery	% upstaged
Bagley 1973	5	I-II	60%
Young 1983	100	IA-IIB	31%
Helewa 1986	25	I	20-25%
Buchsbaum 1989	140	I-II	22,4%
Archer 1991	24	I-II	20,8%
Soper 1992	30	I-II	30%
Stier 1998	45	IA-IIB	16%
Leblanc 2000	28	I	21%

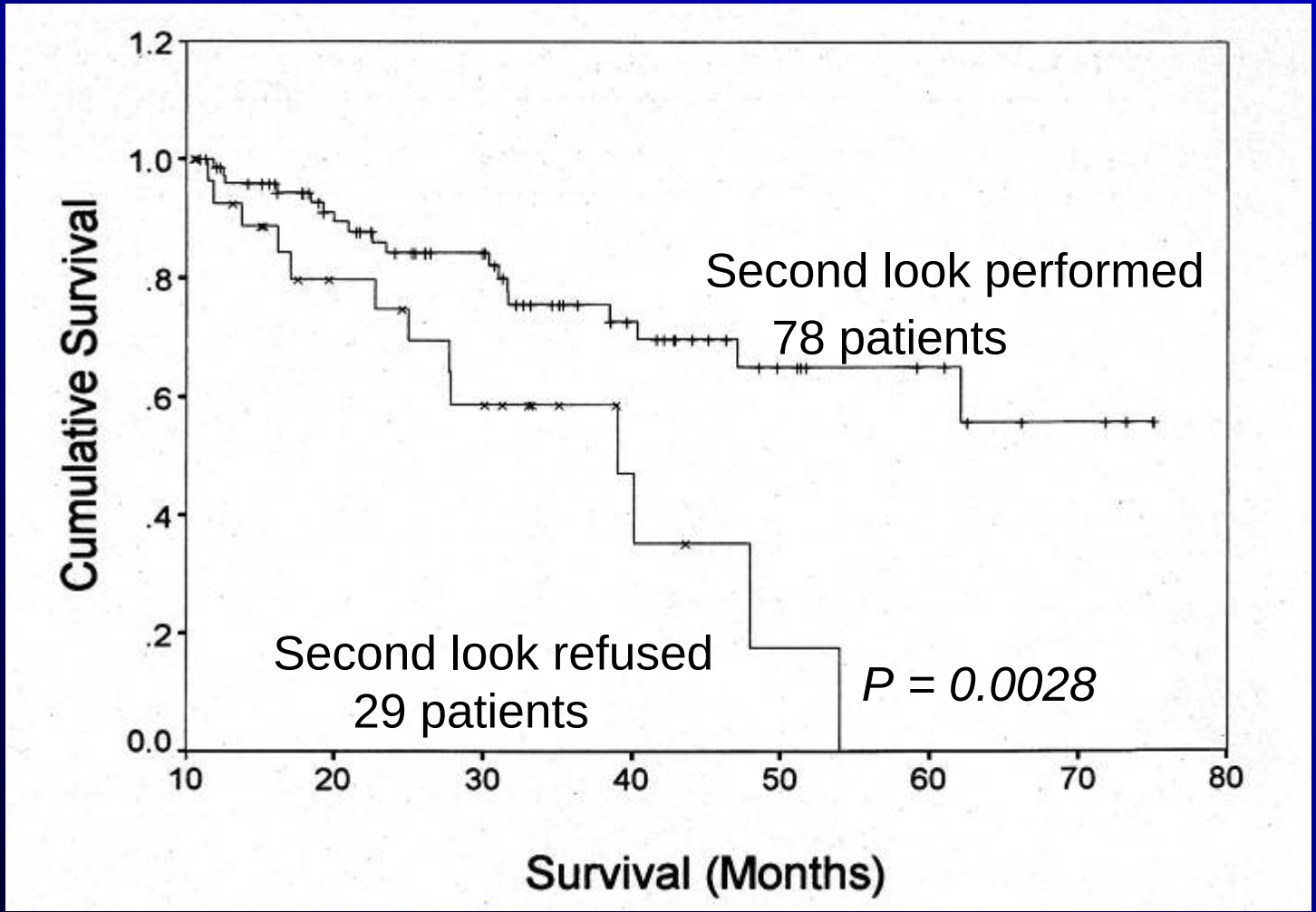
Survival by initial tumor size



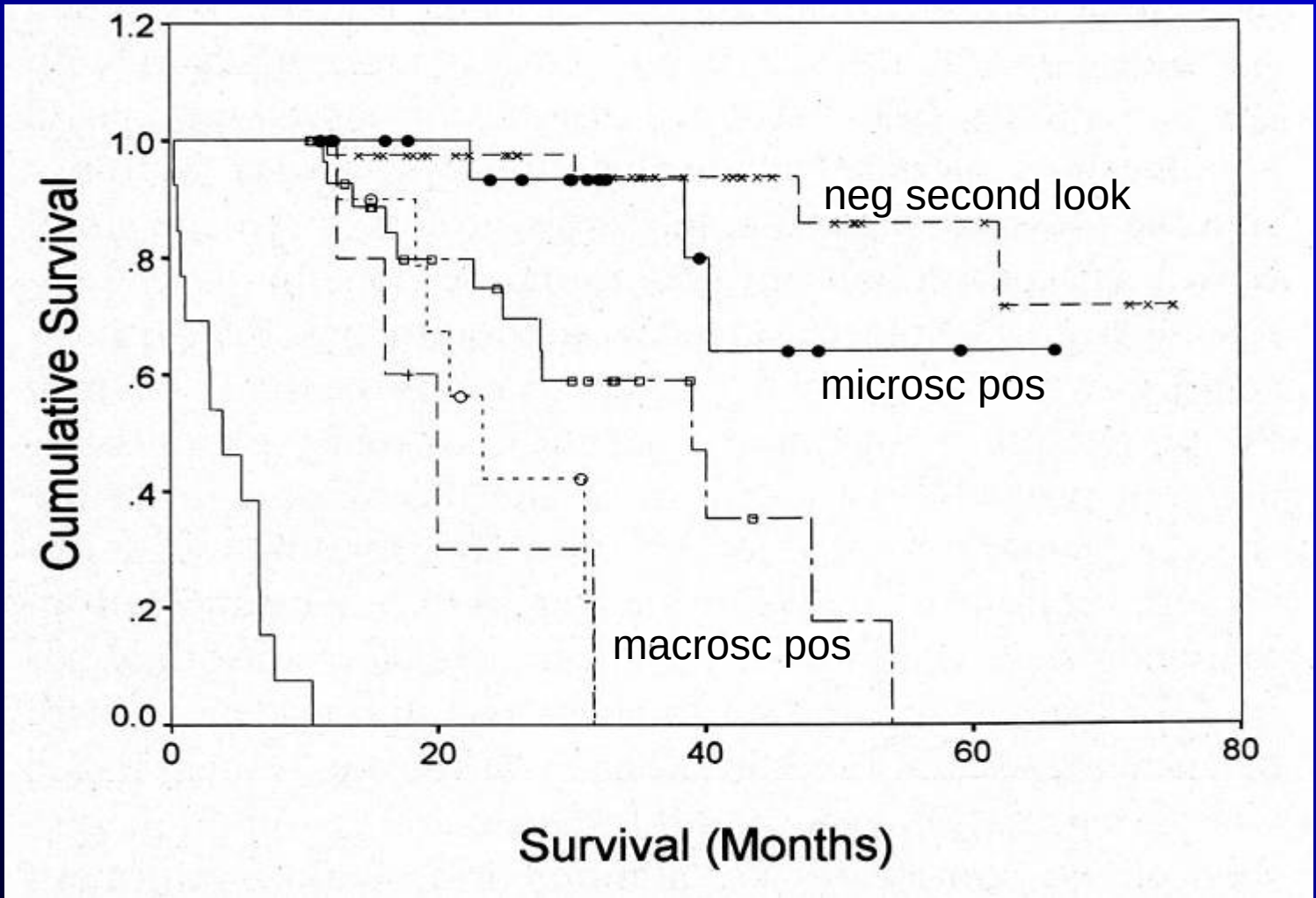
Second look surgery: Why perform it?

- **Contra**
  - Recurrence rates of 50 % after negative second look surgery
  - Absence of proven salvage therapy
  - Lack of demonstrable survival benefit
- **Pro**
  - No proven alternative surveillance techniques (CT, Ca125, etc.)
  - Possible survival benefit of secondary cytoreduction
  - Possible long term survival benefit for patients undergoing second line chemotherapy with minimal residual disease.

Survival by performance of second look



Survival by outcome of second look



Potential of *In Vivo* Fluorescence

- Staging laparotomy
  - 30% upstaged (Young RC, JAMA, 1983; Zanetta G, Ann Oncol, 1998)
- Second Look
  - 50% recurrence of negative second-look after combination chemotherapy (DiSaia PJ, Mosby-Year Book, 1997)

AIMS

- To evaluate *photodetection* of ovarian cancer peritoneal implants in the animal model
- To study pharmacokinetics of the photosensitizer precursor aminolevulinic acid (ALA)
- To evaluate *photodetection* of ovarian cancer peritoneal implants in patients
- To analyse toxicity of ALA *photodynamic therapy* (PDT) in the animal model

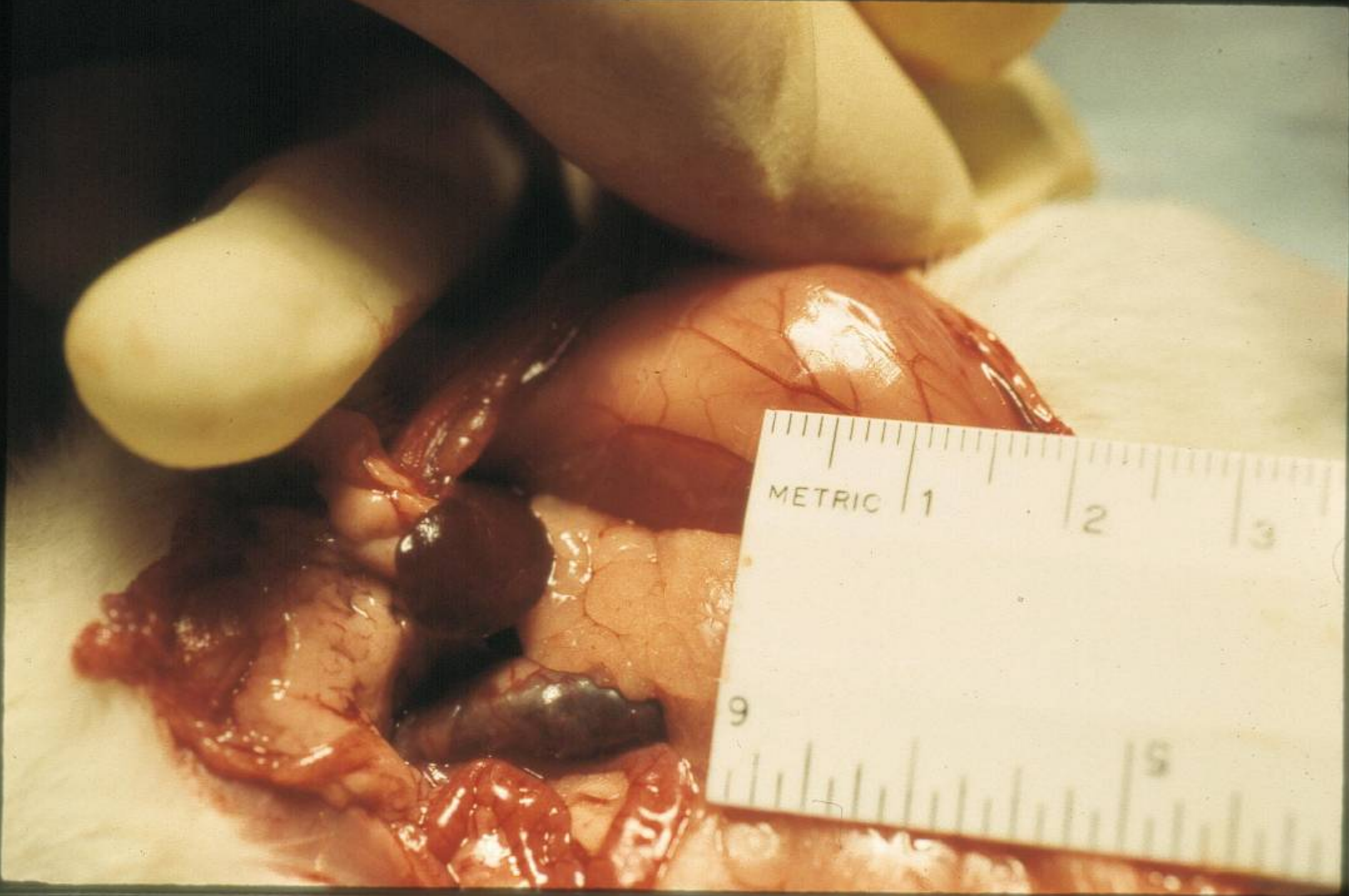
Enhanced diagnosis through photodetection

- *Photodetection* of ovarian cancer peritoneal implants in the animal model
- Determination of the best Photosensitizer
- *Photodetection* of ovarian cancer peritoneal implants in ovarian cancer patients

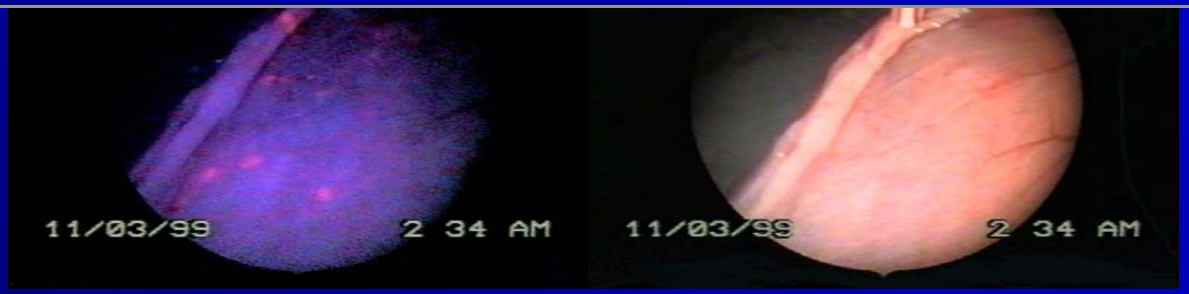
NuTu-19 Ovarian Cancer Animal Model

- **Cell line - NuTu-19 - Spontaneous mutation**
- **Histology - Poorly differentiated ovarian adenocarcinoma with papillary features**
- **Growth pattern - I.P. serosal nodules with local tissue invasion (omentum, diaphragm, liver, peritoneum)**
- **Malignant ascites - average vol. 50-70ml in 6 weeks**
- **Survival - 10^6 cells I.P are 100% fatal, mean survival of 50 days**
- **Non-immunogenic tumor developed in an immunocompetent host**

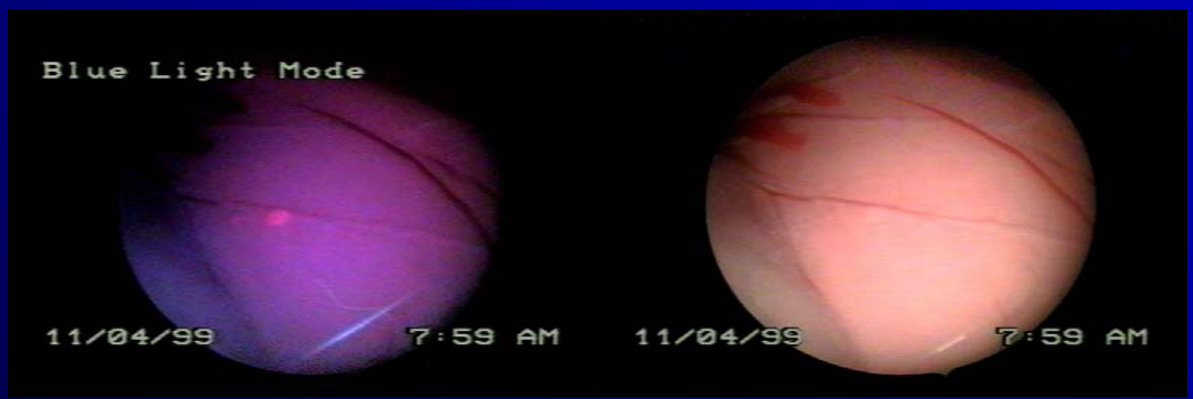
Rose et al AJOG 9/96


METRIC
1
2
3
9

Epithelial ovarian cancer PDD in NuTu-19 rat model



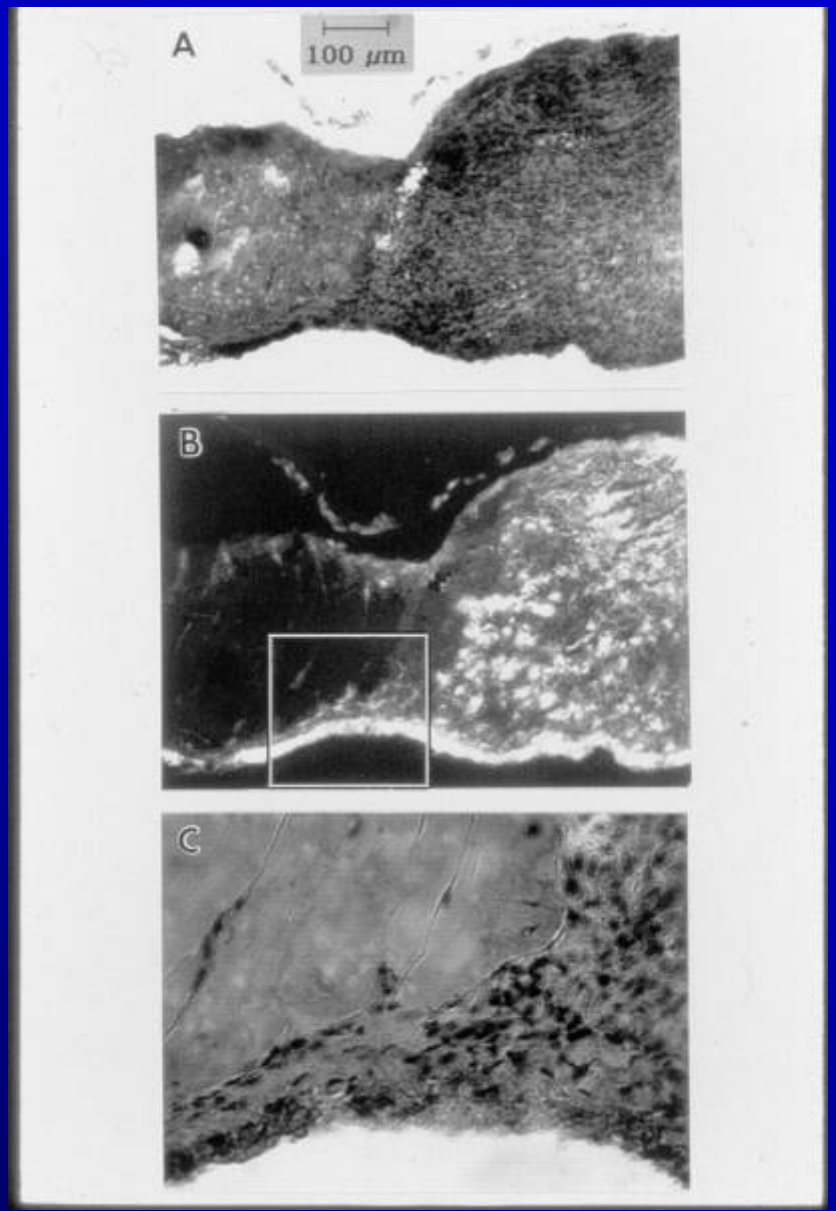
A



B

8mM h-ALA IV prior to photodetection 2 hours later

Light micrographs (A) and fluorescence (B) of a peritoneal nodule (size < 0.5 mm) 6 hr after ip ALA administration. Magnification (C) of the peritoneal serosa (boxed area in B) showing a thin layer of tumor matching with the fluorescence



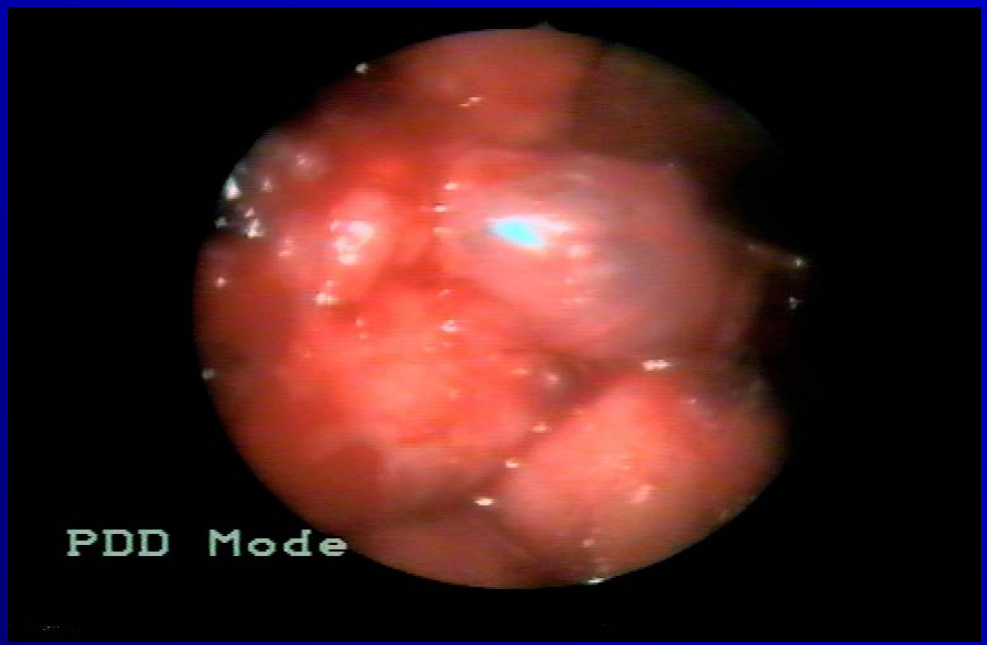
Major A. et al Gynecol Oncol 1996, 66 : 122-32.

Numbers of metastases detected with white and blue light detection for different concentrations of h-ALA and ALA

Concentration [mM]	Time after inst.	White light	Bluelight	Ratio
4	2.5	9	19	2.1
4	2.5	0	4	8
8	2.0	21	37	1.8
8	2.0	36	57	1.6
8	2.0	13	29	2.2
8	2.0	4	24	6
12	2.0	3	8	2.7
20	2.0	9	25	2.8
8 (ALA)	2.0	10	16	1.6

Lüdicke F et al, Britsh J Cancer

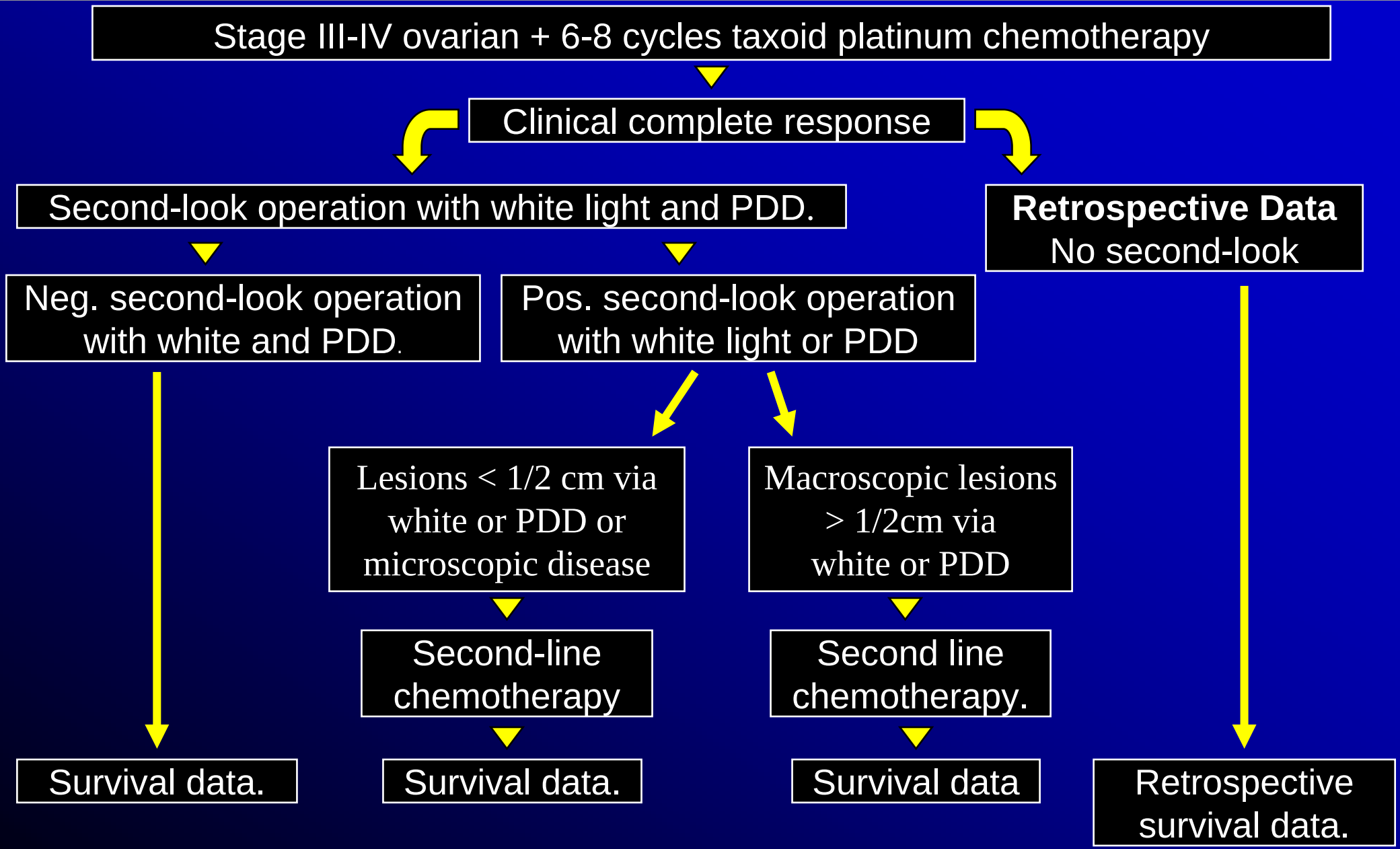
Human Epithelial Ovarian cancer PDD



10mg/ml ALA applied topically prior to photodetection

Major AL et al, Laser Med Sci 2002

Ovarian cancer PDD second-look feasability Study

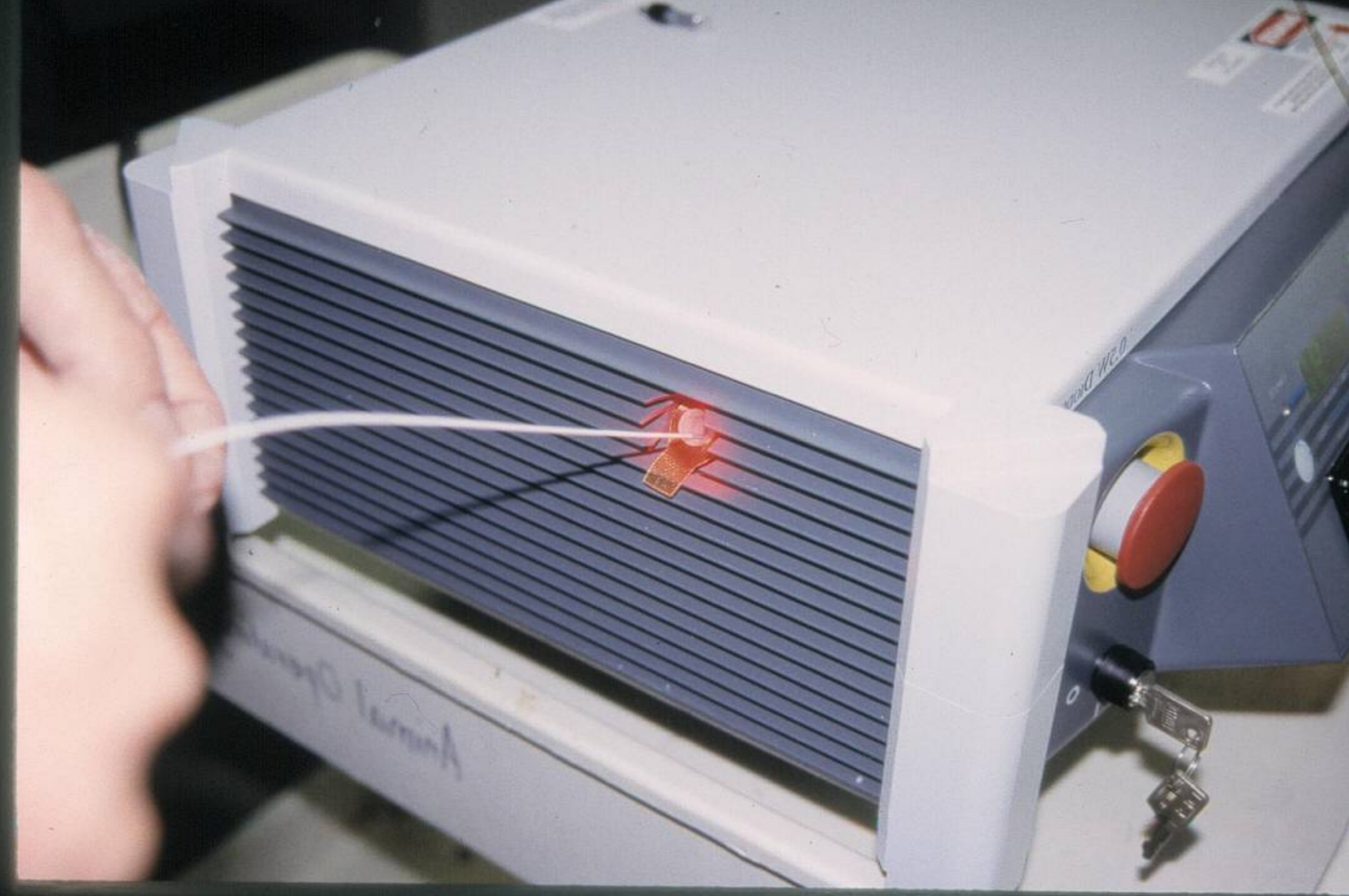


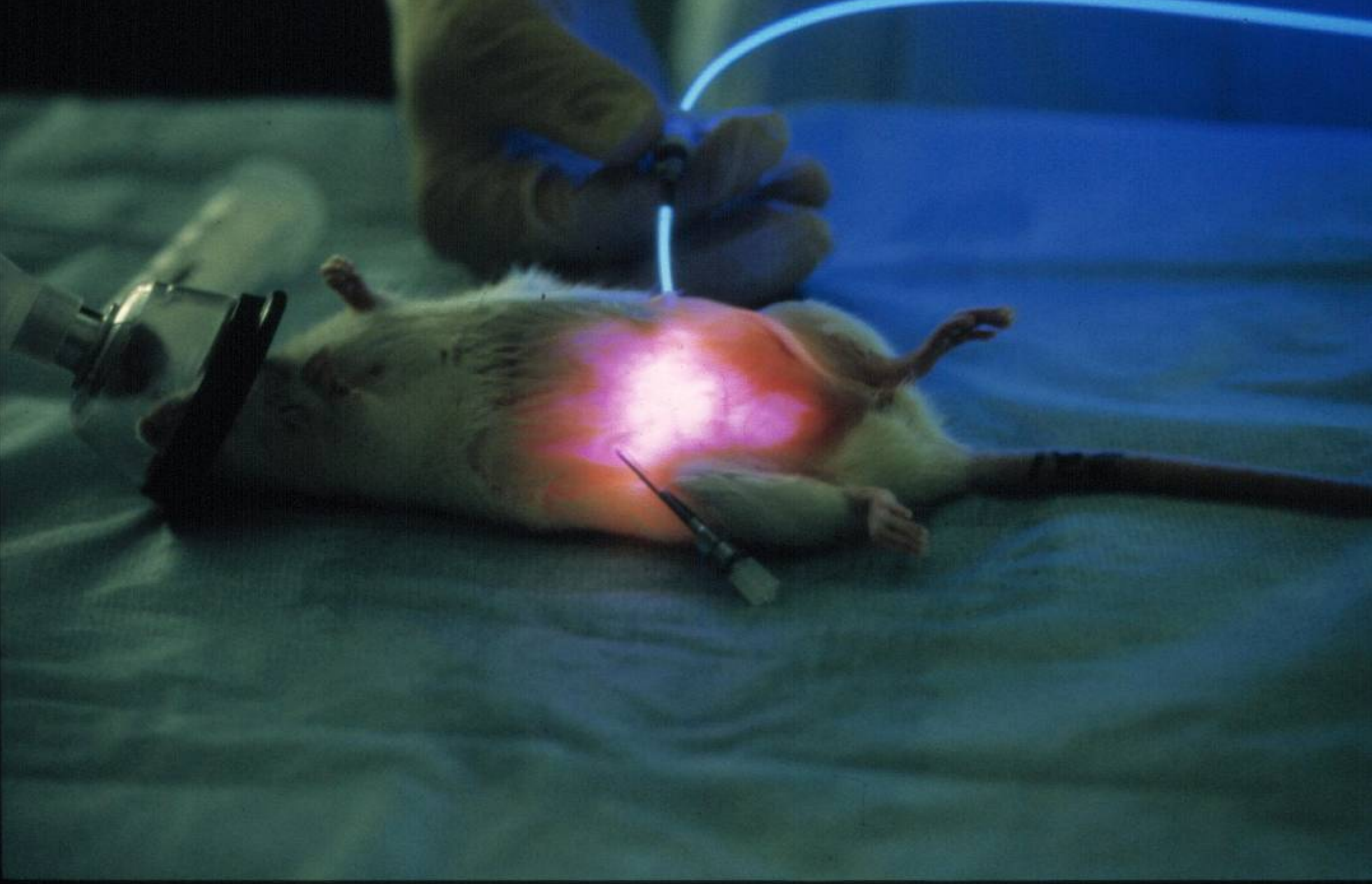
CONCLUSIONS

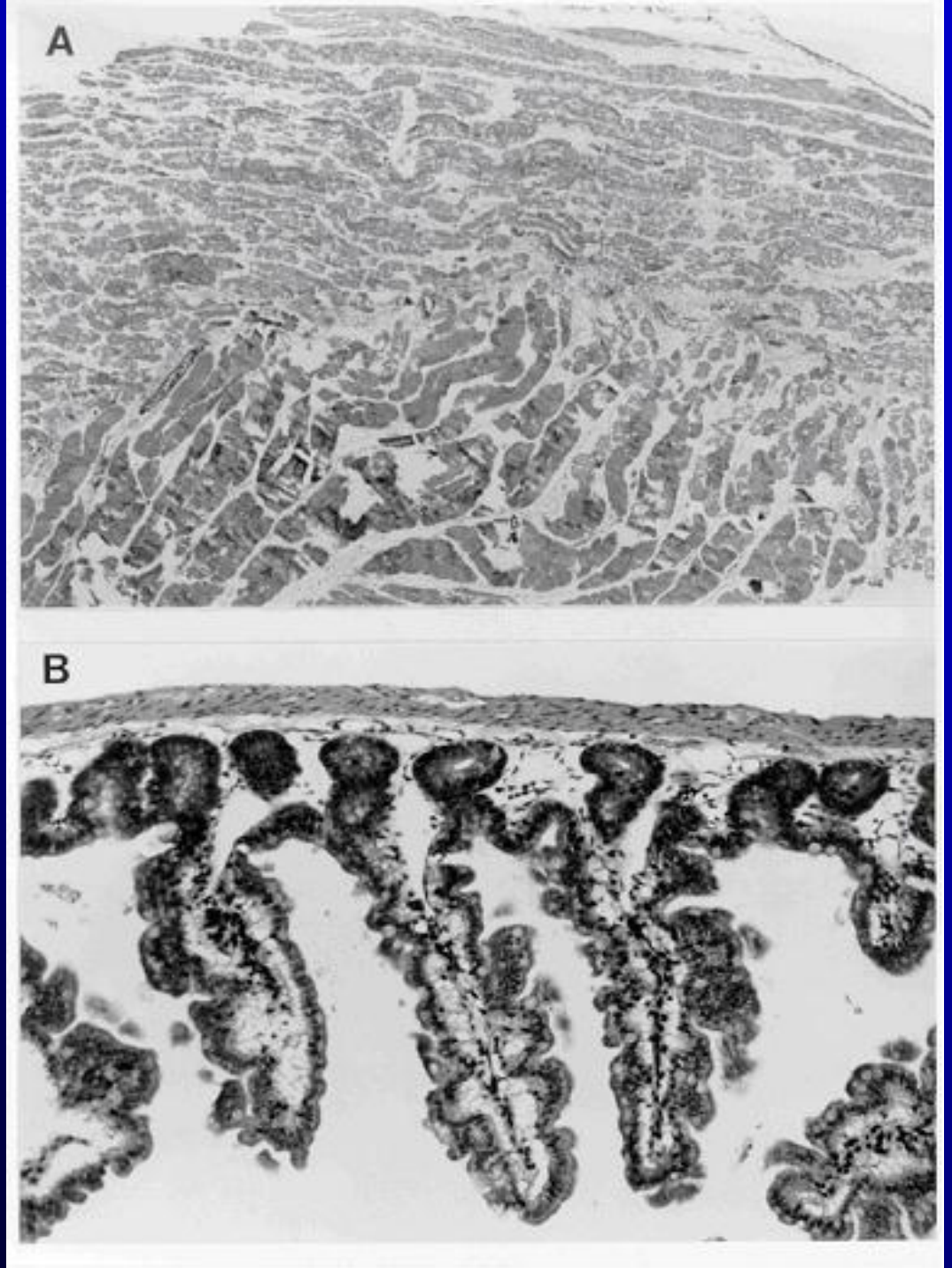
- Photodetection has been shown to be efficient in the animal model and feasible in patients
- Photodetection of ovarian cancer peritoneal implants, not visible by other methods, is a conceivable goal for the future
- The impact on survival has to be demonstrated in further studies

“The facts remains that a large number of patients are being treated almost to the point of “cure” and an additional stroke of some sort is needed.”

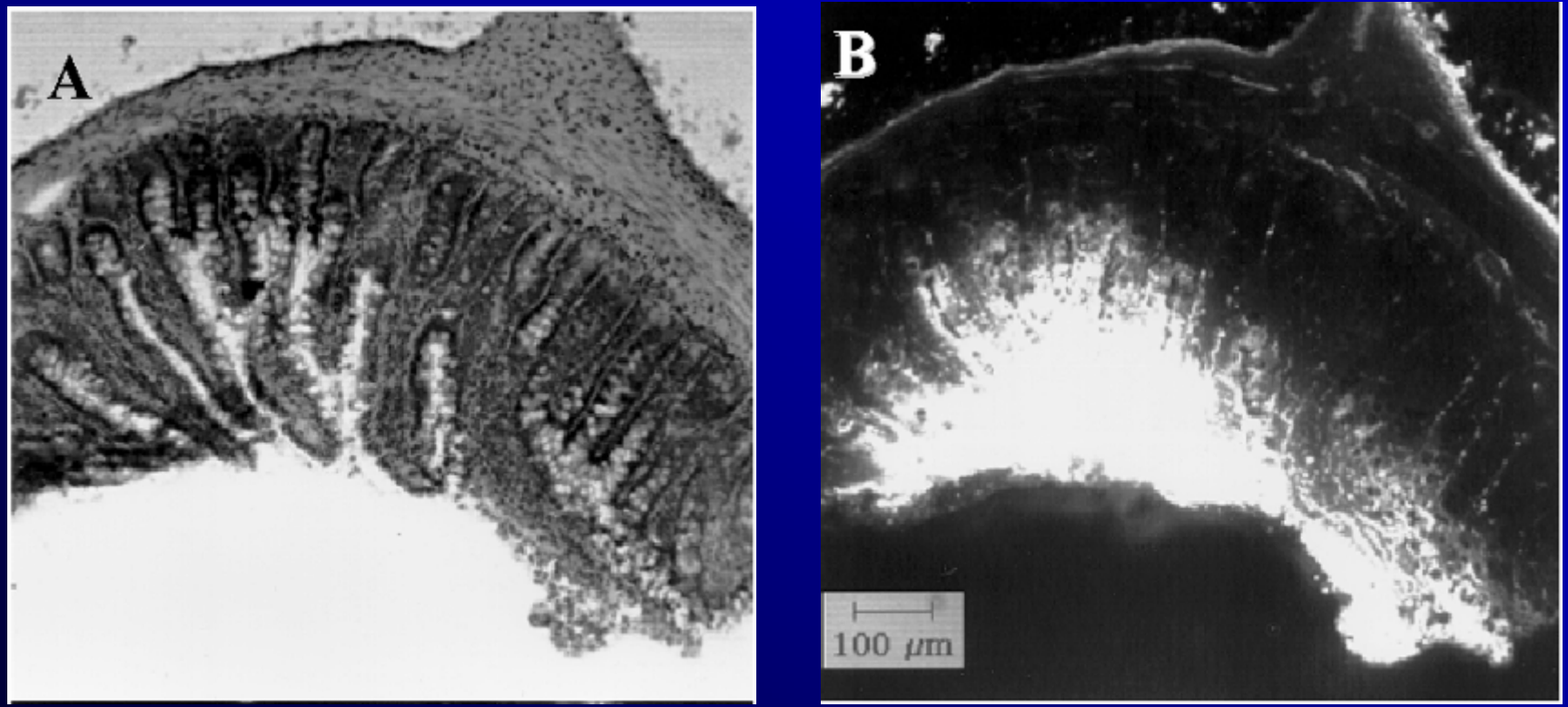
(DiSaia, Clinical Gynecological Oncology, Mosby-Year Book, 1997)






A
B

Light micrographs (A) and fluorescence (B) of small intestine 6 hr after ip ALA



Major A. et al Gynecol Oncol 1996, 66 : 122-32.

AIMS

- Proof of principle of gene based photodynamic therapy of the peritoneal cavity after IP administration of ALA-S virus (establishment of a stable NuTu 19 ALA-S mutant cell line)

Problems in gene therapy

- Transfection, transduction rate
- Side effects
- Tissue penetration
- Immune reaction
- Specificity

Results

- Pp IX production in the NuTu-19 ovarian cancer cell line after ALA and ALA-S mutant adenovirus application
- Toxicity (cell killing) of ALA, ALA-S virus and LacZ adenovirus in NuTu-19 cells
- Transduction rate of GFP adenovirus (CMV) in NuTu cells and in control cells (293T)

Perspective

- Proof of efficient photodynamic therapy in the animal model after I.P ALA-S virus administration, impact on survival
- Increase transduction rate
- Achieve cancer specific expression of the transgene

PDT of cervical intraepithelial neoplasia

- Rationale
- Introduction
- Study design
- Material and Methods
- Results

Aim

- **Determine if h-ALA is selectively absorbed by dysplastic cells at various times after topical application (5min to 7 hours)**

Rationale

- Increasing incidence of cervical precancerous lesions in younger women
- Treatment pf precancer of the cervix (conization) is an invasive procedure with its peri-operative risk and potential long term risk for fertility

Treatment of CIN: Excisional methods

- Cold knife conisation
- Loop electrocurgical excision procedure (LEEP)

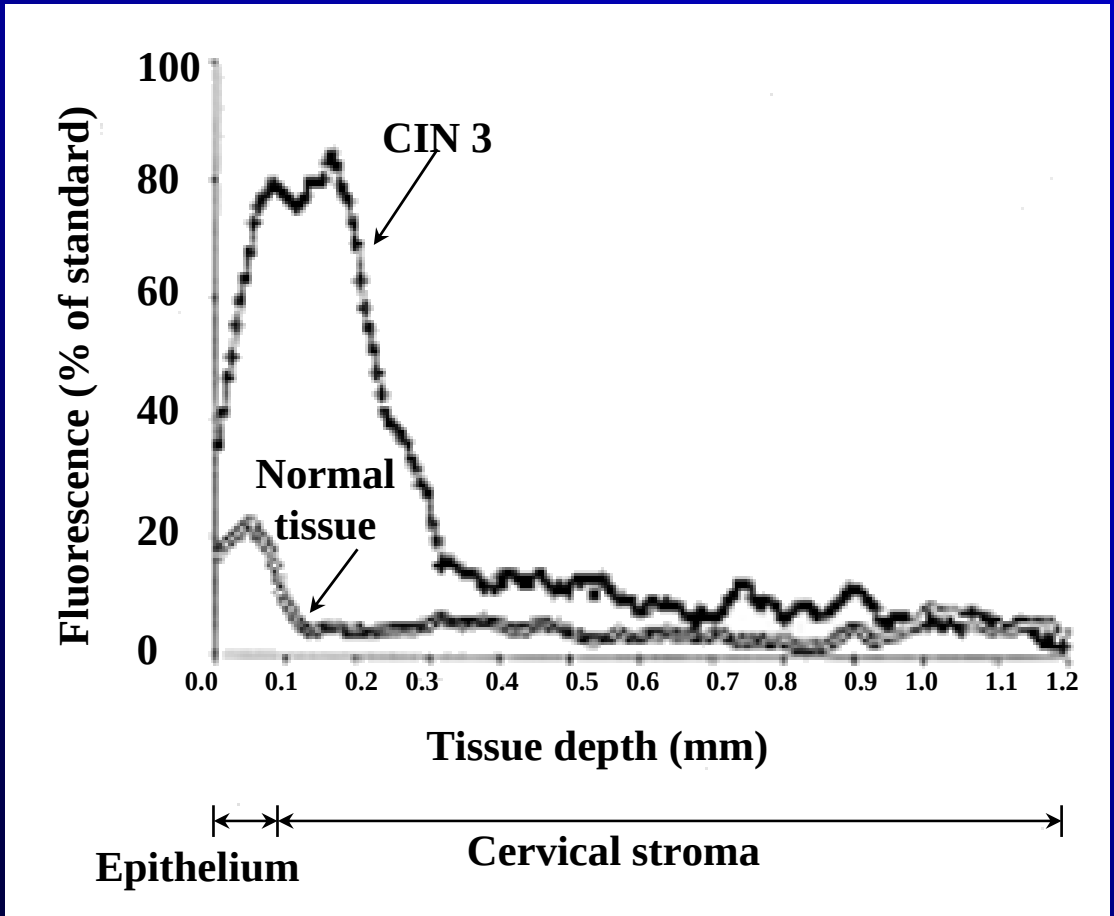
Treatment of CIN: Ablative methods

- Cryotherapy
- Laser vaporisation
- Photodynamic therapy

Advantages to treat CIN with PDT

- Outpatient clinic
- Specificity (drug, light)
- Tailored to the shape of the cervix
- No stromal destruction (stenosis, cervix insufficiency)
- Cell death by apoptosis (no inflammation, no scaring)
- Specific HPV destruction (tetrapyrrol)
- Repeatable

Representative spatial distribution of 5-ALA induced porphyrin fluorescence related tissue type

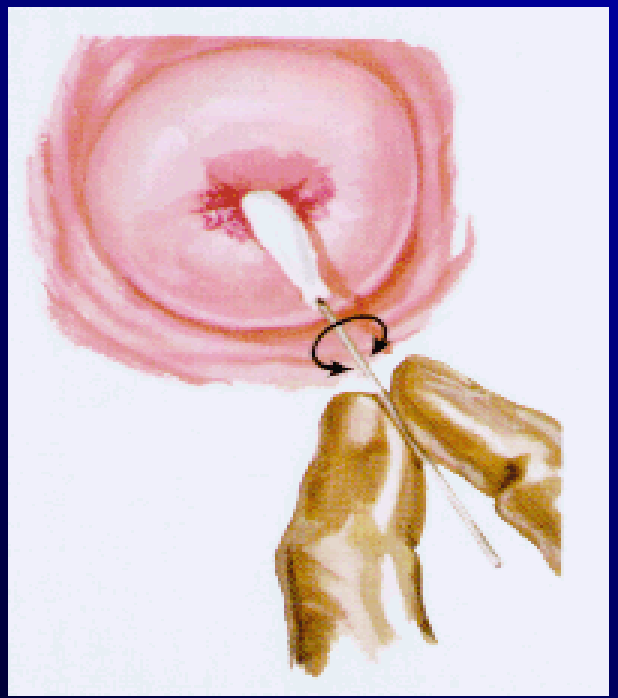


Pahernik et al Int J Cancer 78 : 310-314, 1998

Material and methods

- Phase I clinical trial involving 30 non-pregnant women with already biopsy-proven CIN 1-3
- Application of a 0.5 % h-ALA
- Random biopsies at time points ranging from 5min to 7 hours
- Image analysis on frozen tissue sections using Zeiss Axiophot image analysis system

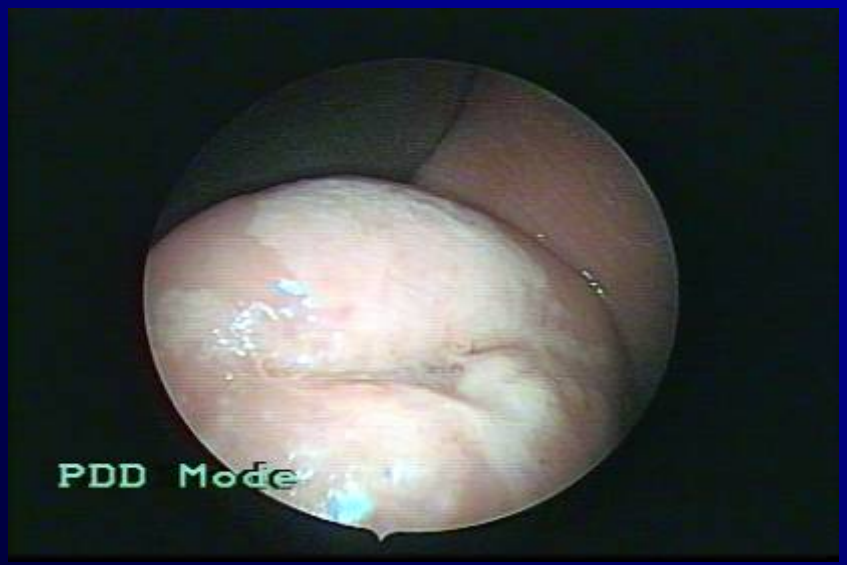
Topical application of h-ALA



- Rinsing the cervix with physiological NaCl
- A solution of h-ALA of 0.5% is applied topically on the cervix with help of a gauze sponge and cervical cup
- 5 biopsies are taken in dimmed light before performing conization

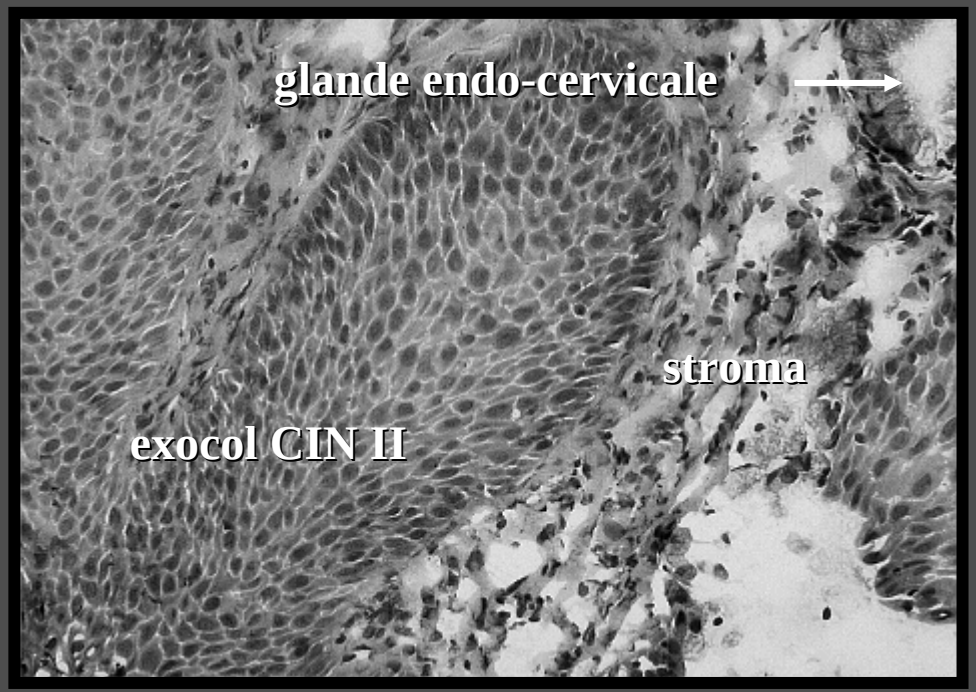
Fluorescence image of the cervix after h-ALA application

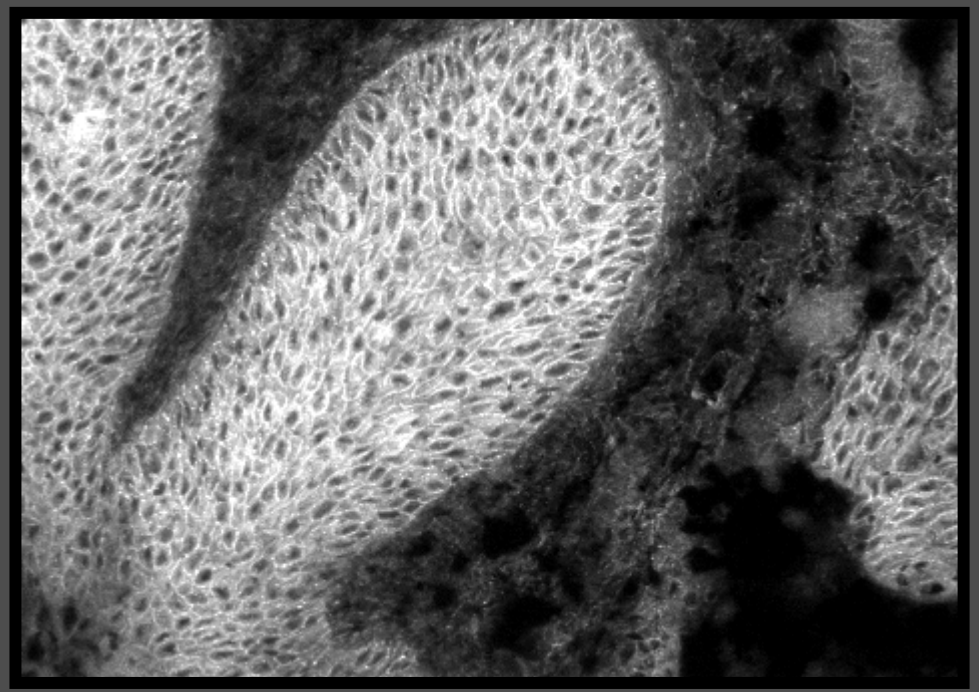
White light



Fluorescence

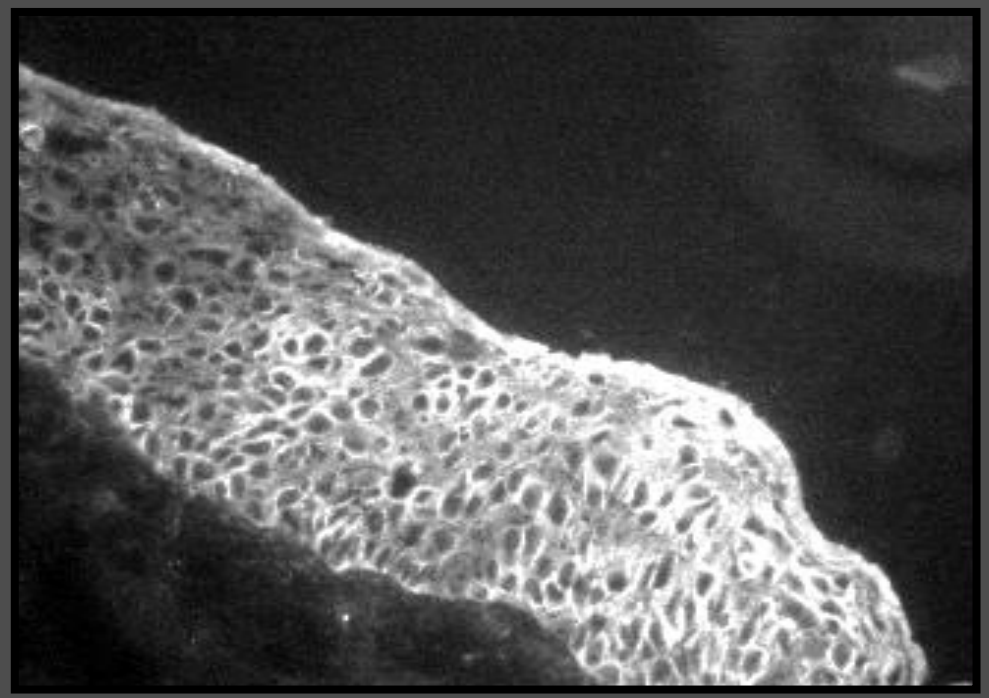
Fluorescence image and white light image of the cervix uteri after the application of 3% acetic acid. Application of 10mg h-ALA in 10ml 0.9% NaCl solution on the cervix during 3 hrs.

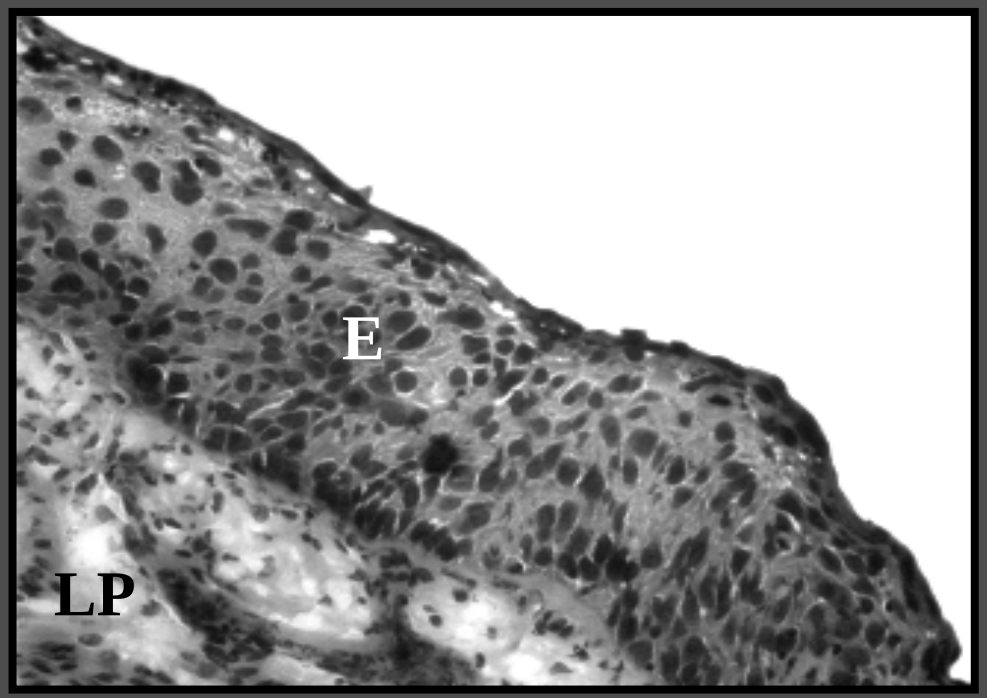




Départements de Pathologie Clinique et de Gynécologie et Obstétrique HUG & EPFL, mai 2002

Cin III 20x (exocol), h-ALA 0,5%, 75 min

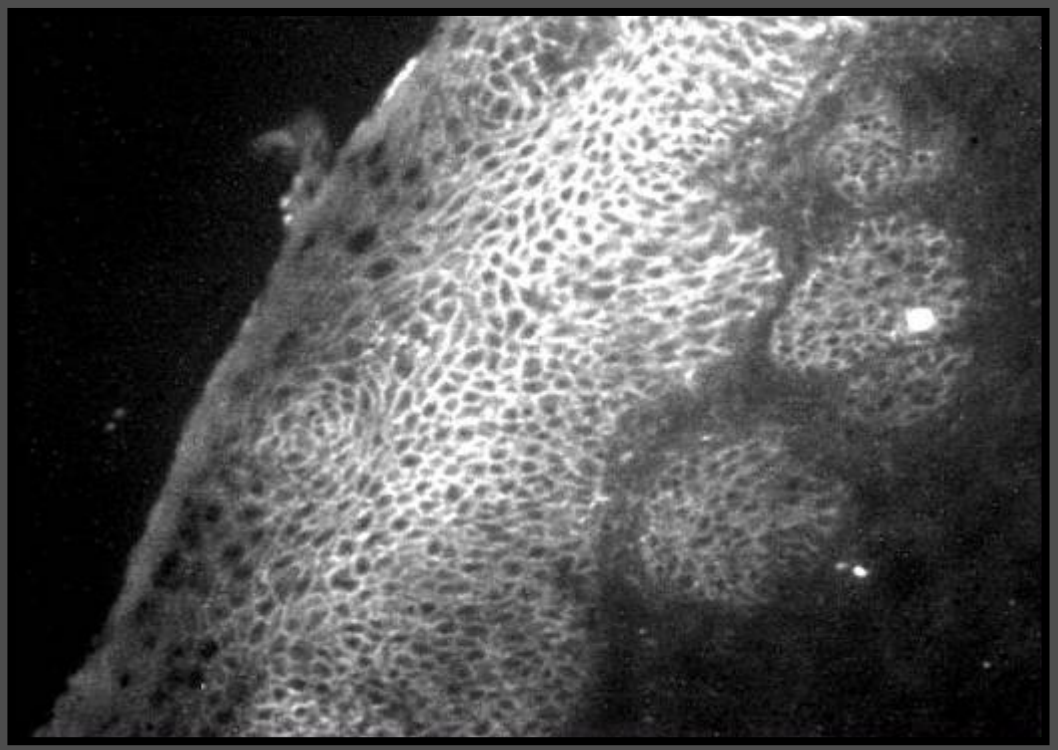


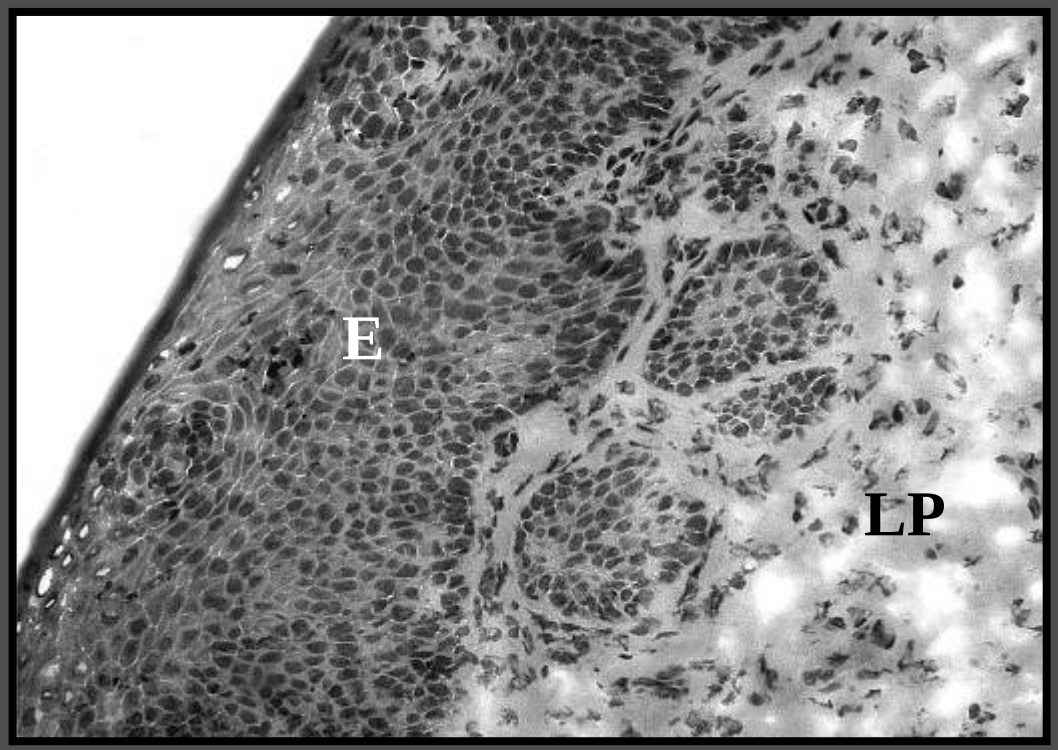


E- epithelium, LP-lamina propria

Départements de Pathologie Clinique et de Gynécologie et Obstétrique HUG & EPFL, mai 2002

Cin II (exocol) 10X, h-ALA 0,5%, 30 min

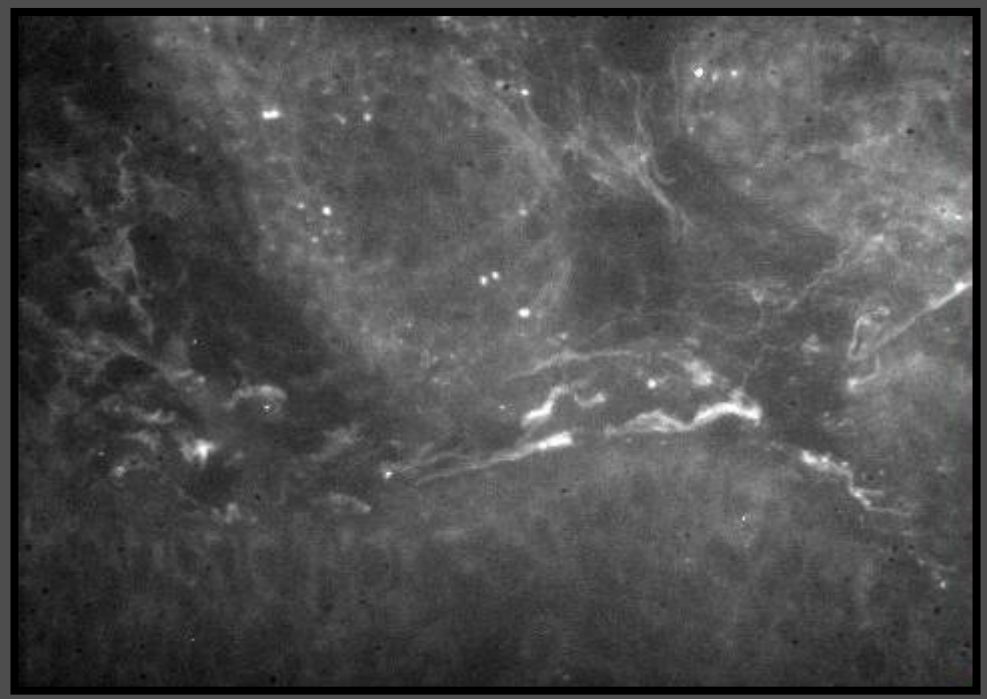


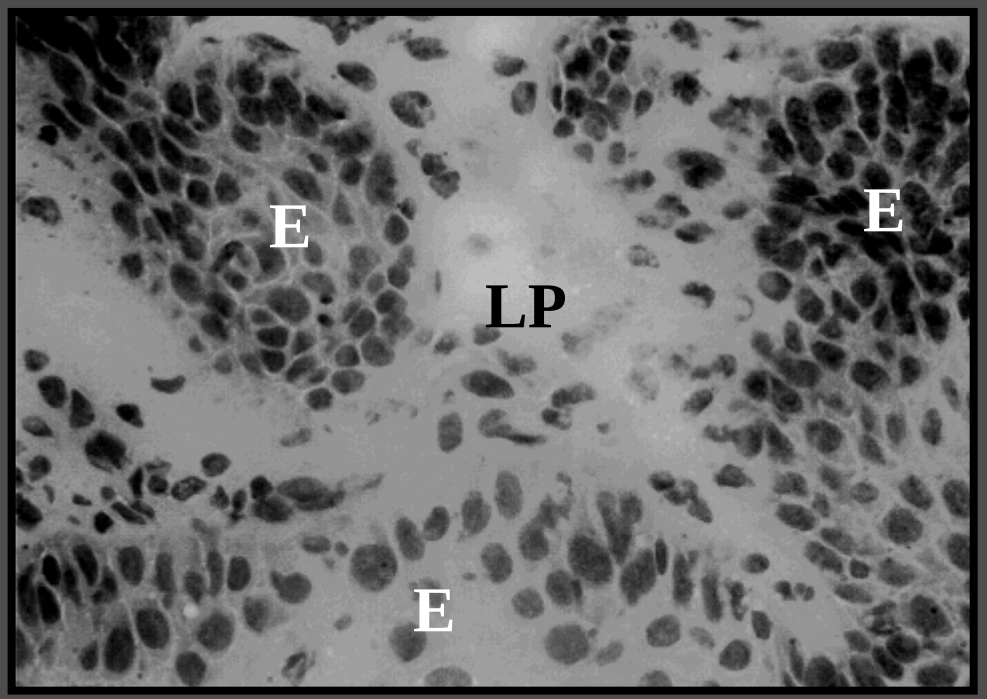


E- epithelium, LP-lamina propria

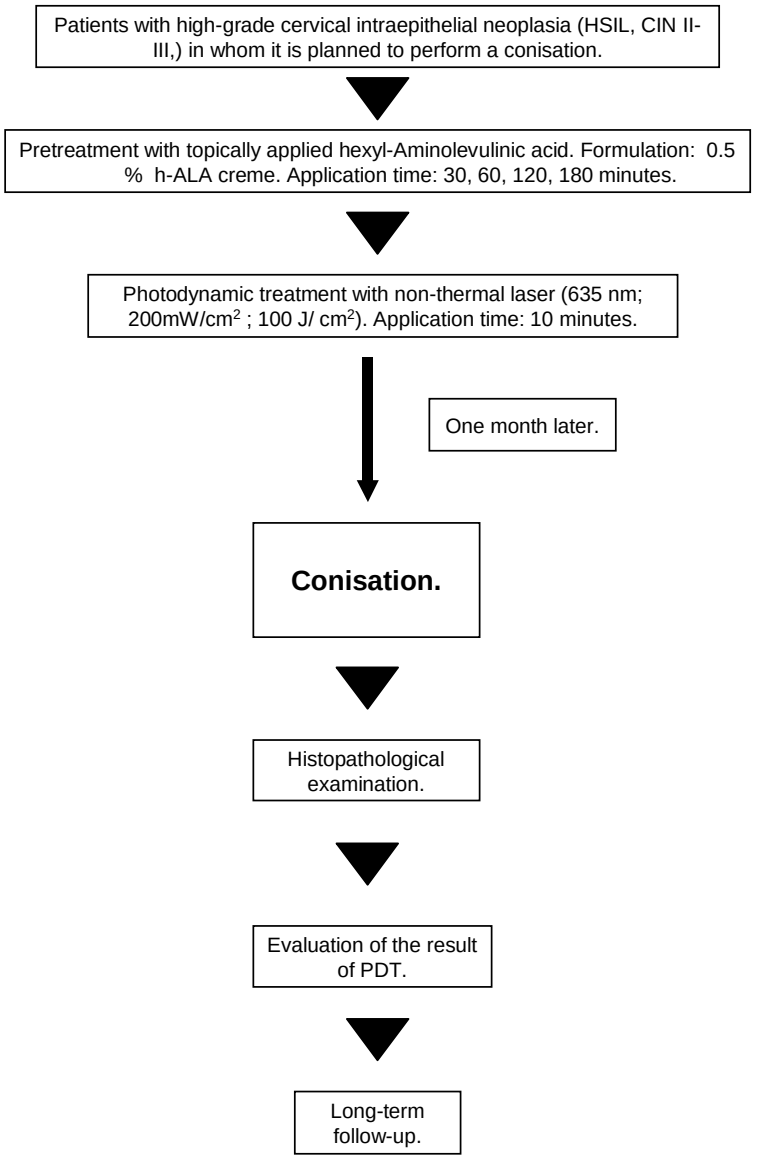
Départements de Pathologie Clinique et de Gynécologie et Obstétrique HUG & EPFL, mai 2002

Cin II 20X (exocol), h-ALA 0,5%, 5min.



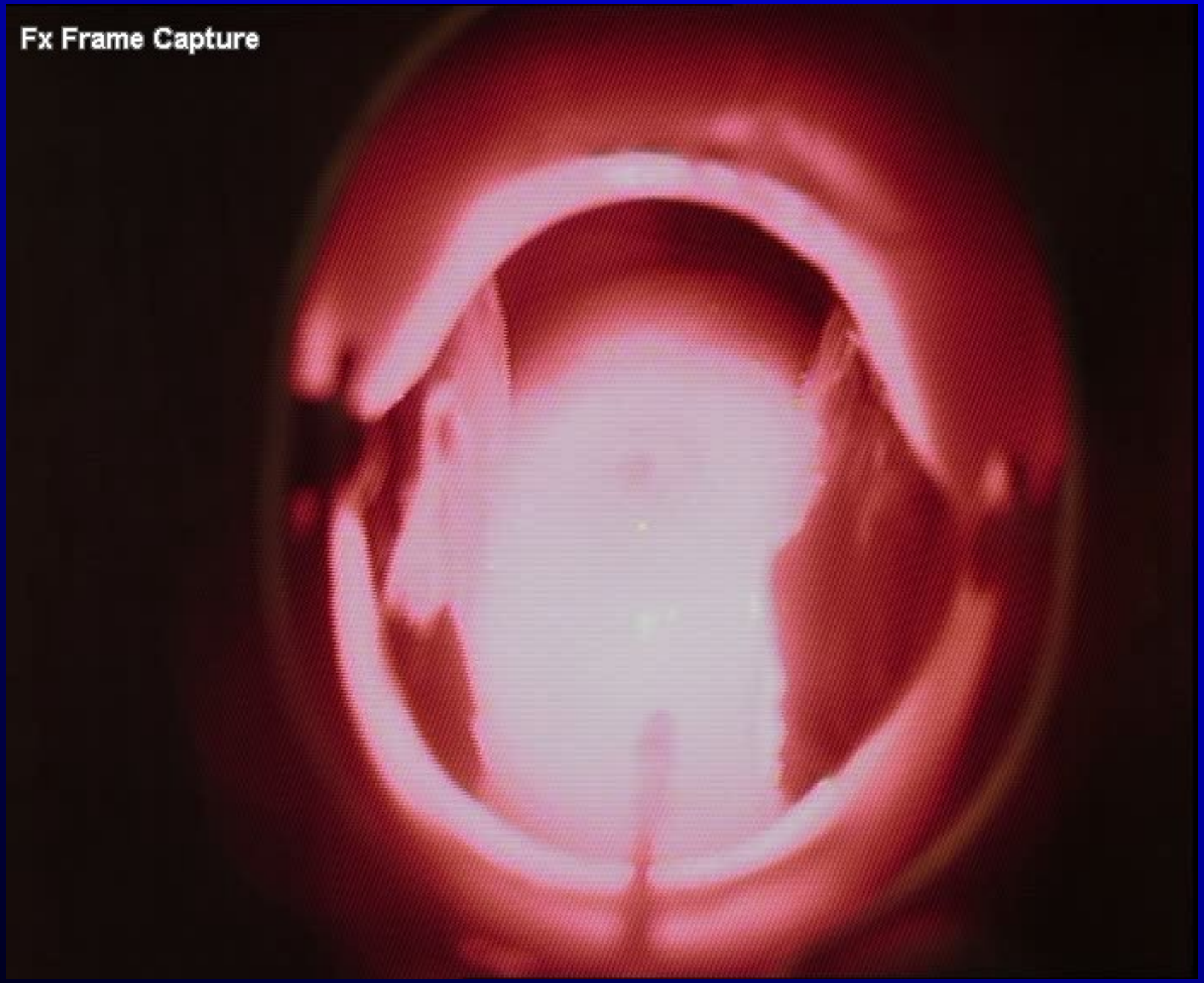


E- epithelium, LP-lamina propria


Patients with high-grade cervical intraepithelial neoplasia (HSIL, CIN II-III,) in whom it is planned to perform a conisation.
Pretreatment with topically applied hexyl-Aminolevulinic acid. Formulation: 0.5 % h-ALA creme. Application time: 30, 60, 120, 180 minutes.
Photodynamic treatment with non-thermal laser (635 nm; 200mW/cm2 ; 100 J/ cm2). Application time: 10 minutes.
One month later.
Conisation.
Histopathological examination.
Evaluation of the result of PDT.
Long-term follow-up.


Fx Frame Capture


Fx Frame Capture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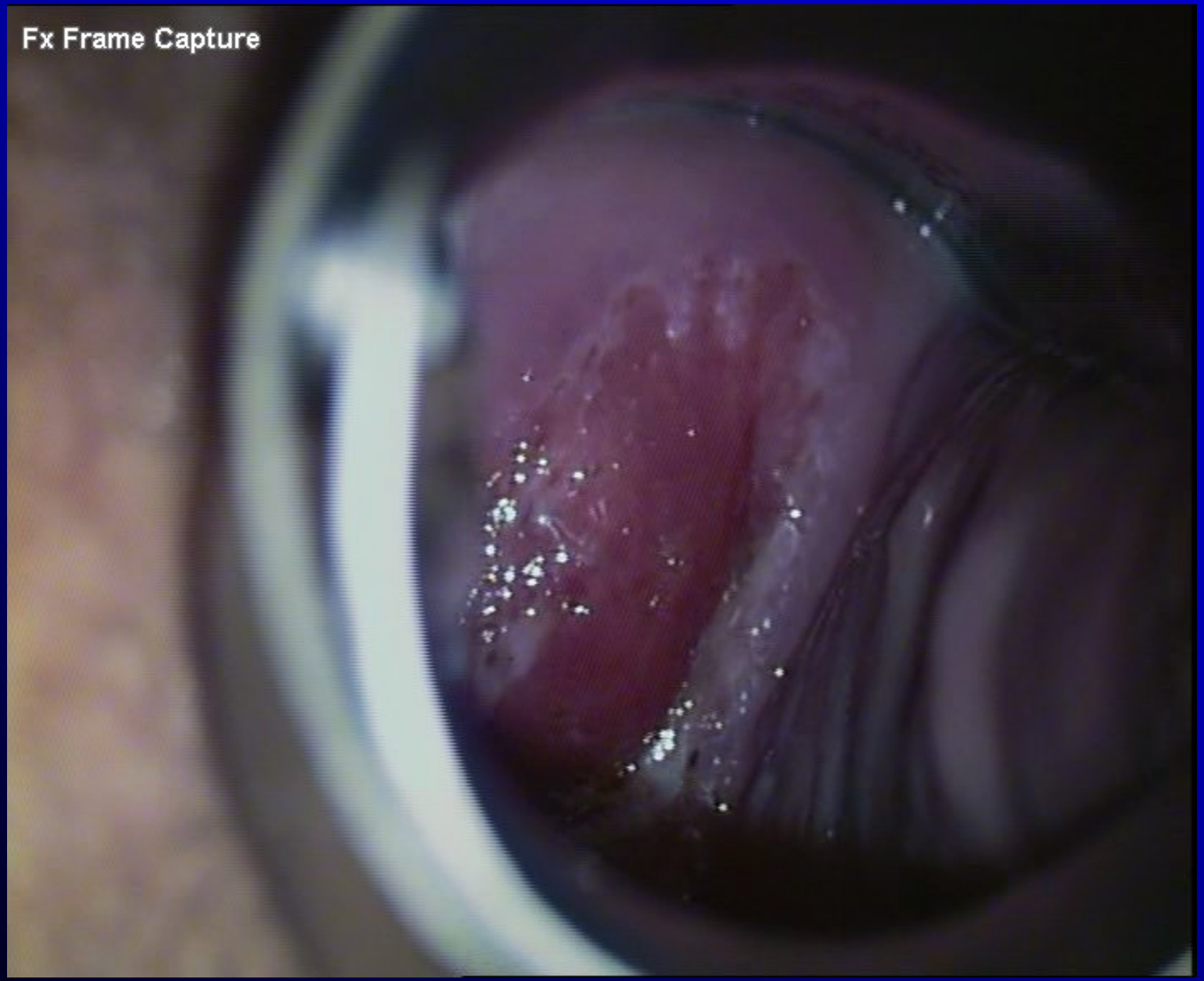
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