A Little Light Relief

for
Gresham College

2nd November 2016

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Past-President
Royal Society of Chemistry
Professor Emeritus
Imperial College London

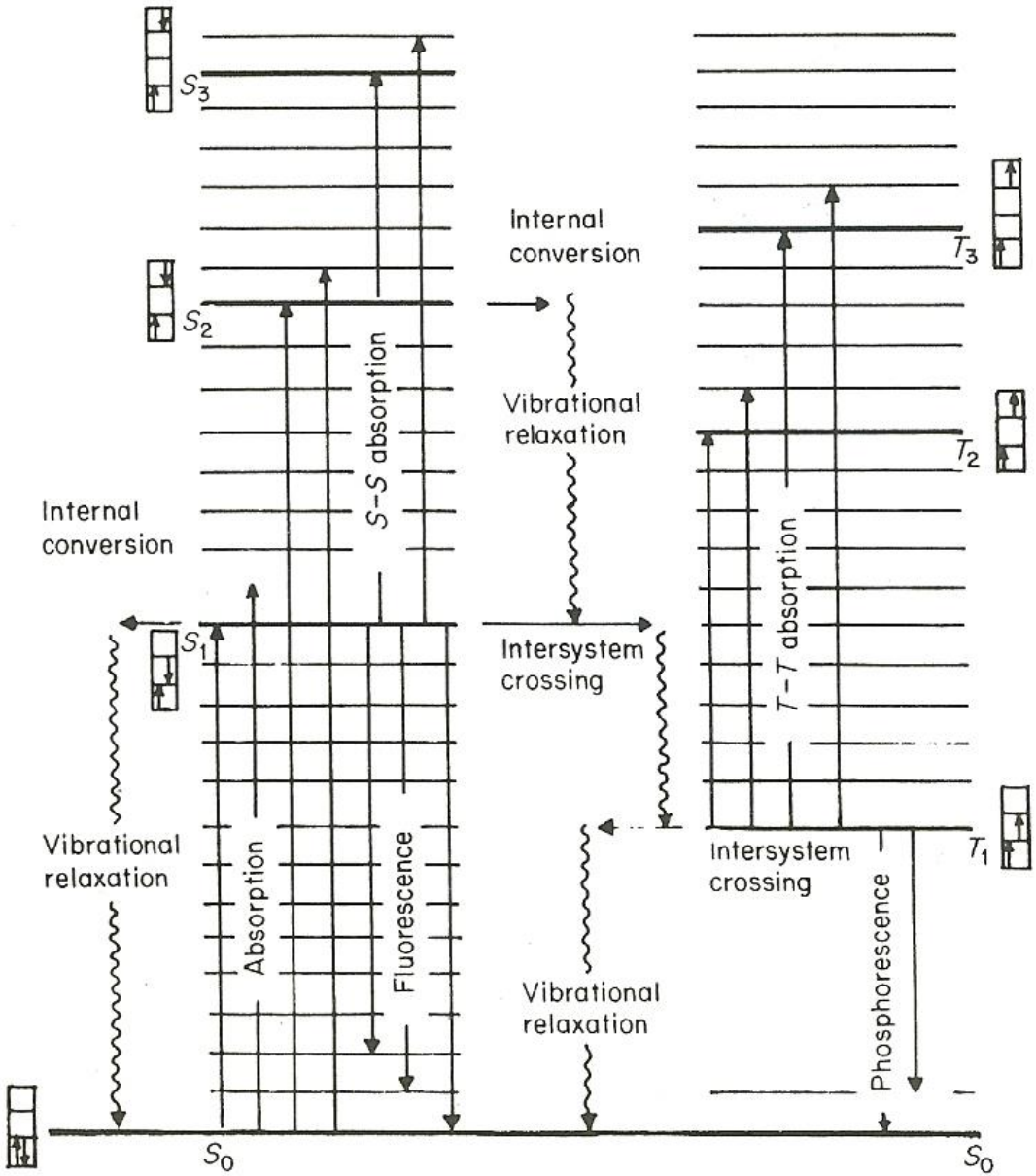
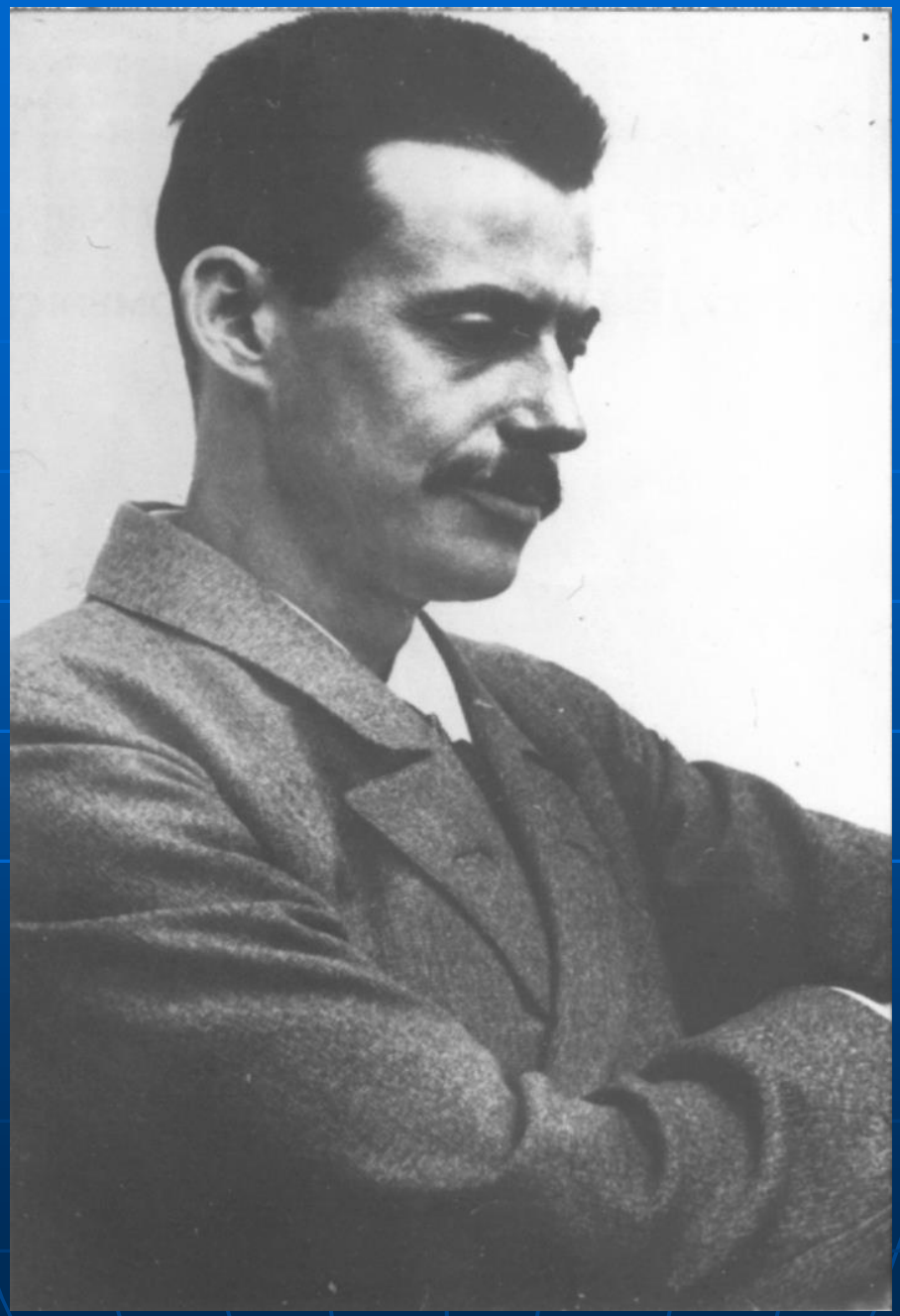
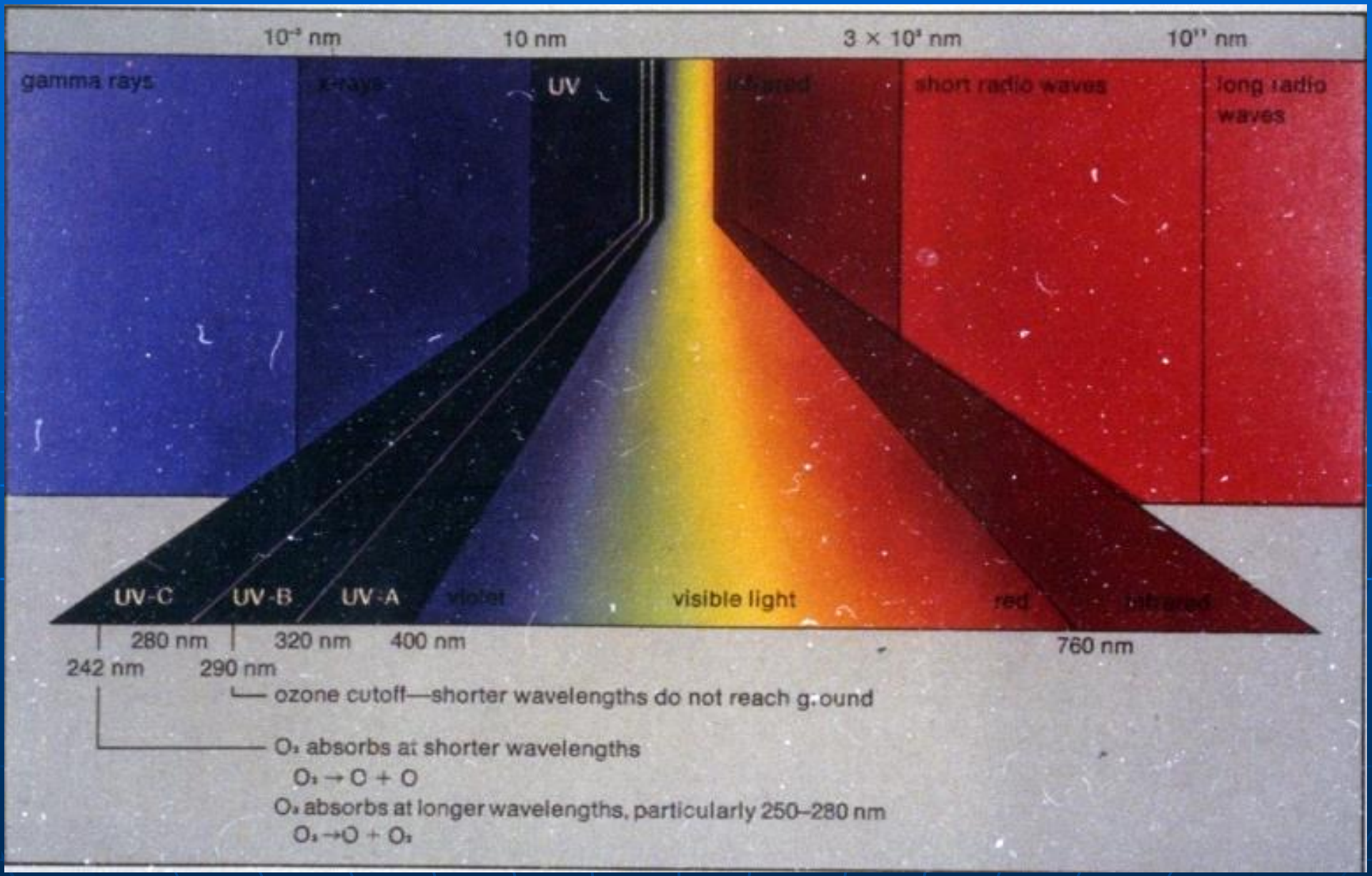
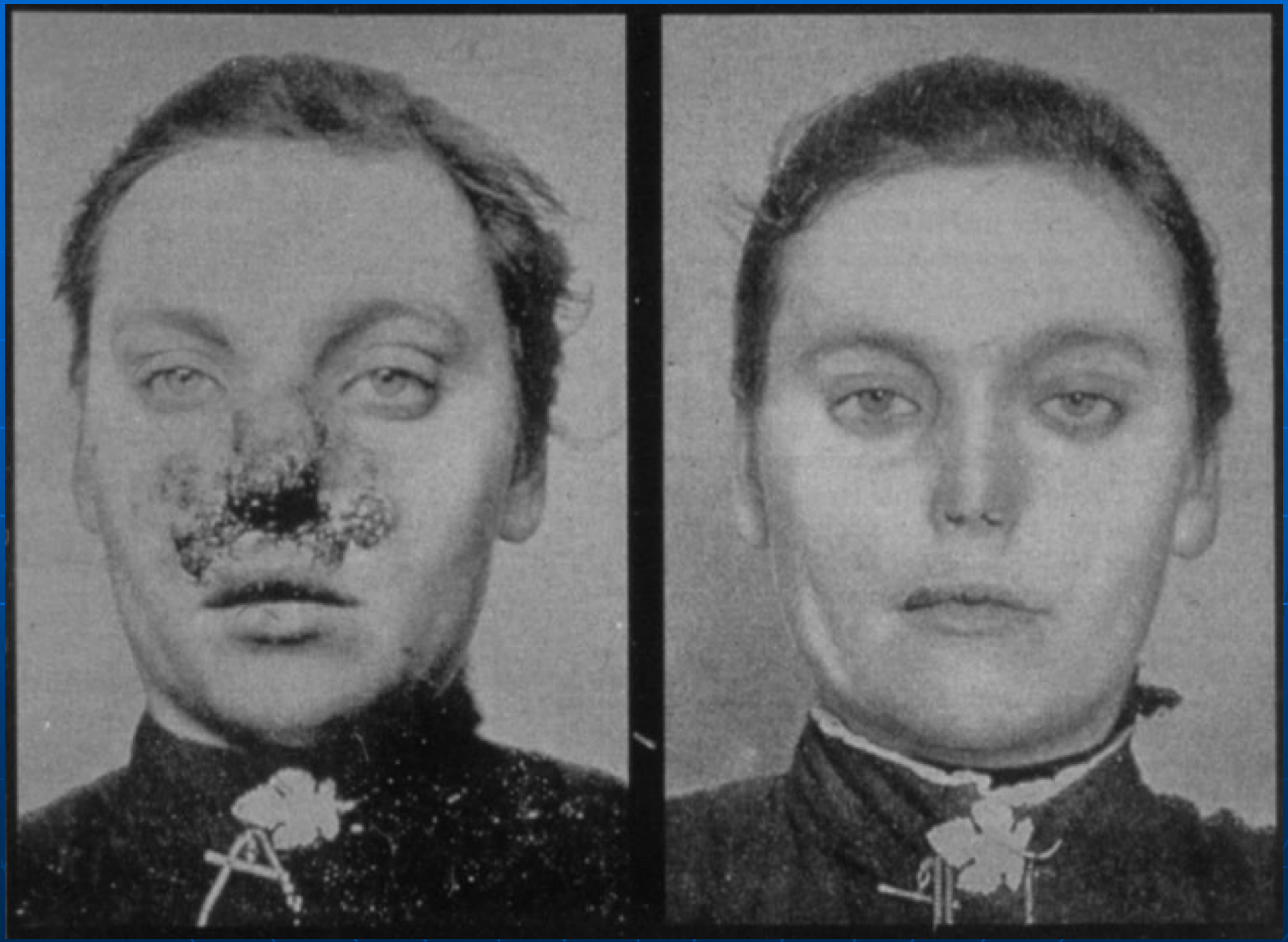


Fig. 1.1 Jablonskii diagram showing fates of polyatomic molecules upon photoexcitation.




10^{-5} nm
10 nm
3×10^{5} nm
10^{11} nm
gamma rays
x-rays
UV
infrared
short radio waves
long radio waves
UV-C
UV-B
UV-A
violet
visible light
red
infrared
280 nm
320 nm
400 nm
760 nm
242 nm
290 nm
ozone cutoff—shorter wavelengths do not reach ground
O_2 absorbs at shorter wavelengths
$O_2 \rightarrow O + O$
O_3 absorbs at longer wavelengths, particularly 250–280 nm
$O_3 \rightarrow O + O_2$



Photomedicine

- Effects of light on the skin
- Uses of light to diagnose medical conditions
- Therapeutic effects of non-laser light
- Uses of lasers

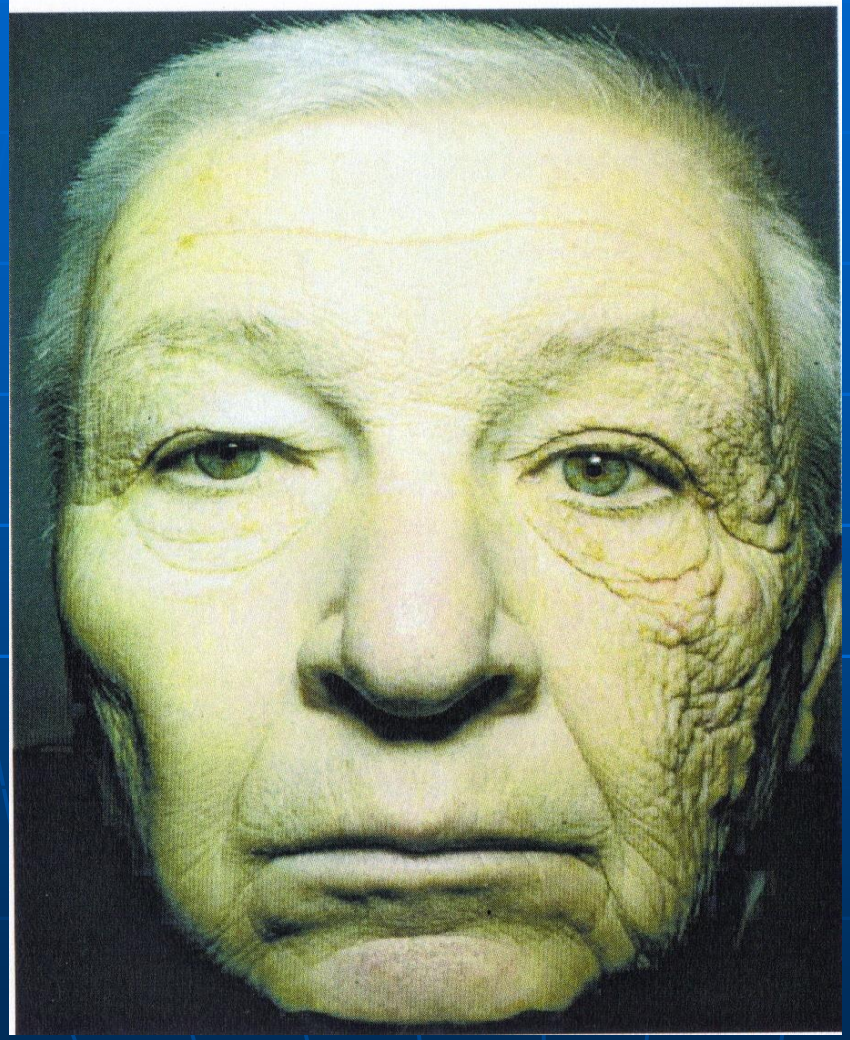
Skin

Effects of light on the skin include

- **[+] Production of vitamin D [*rickets*]**
- **[+] Tanning [*protection against uv*]**
- **[-] Ageing of skin**
- **[-] Skin cancer**

Fig. 22 Two children with rickets next to a healthy individual.

Unilateral Dermatoheliosis

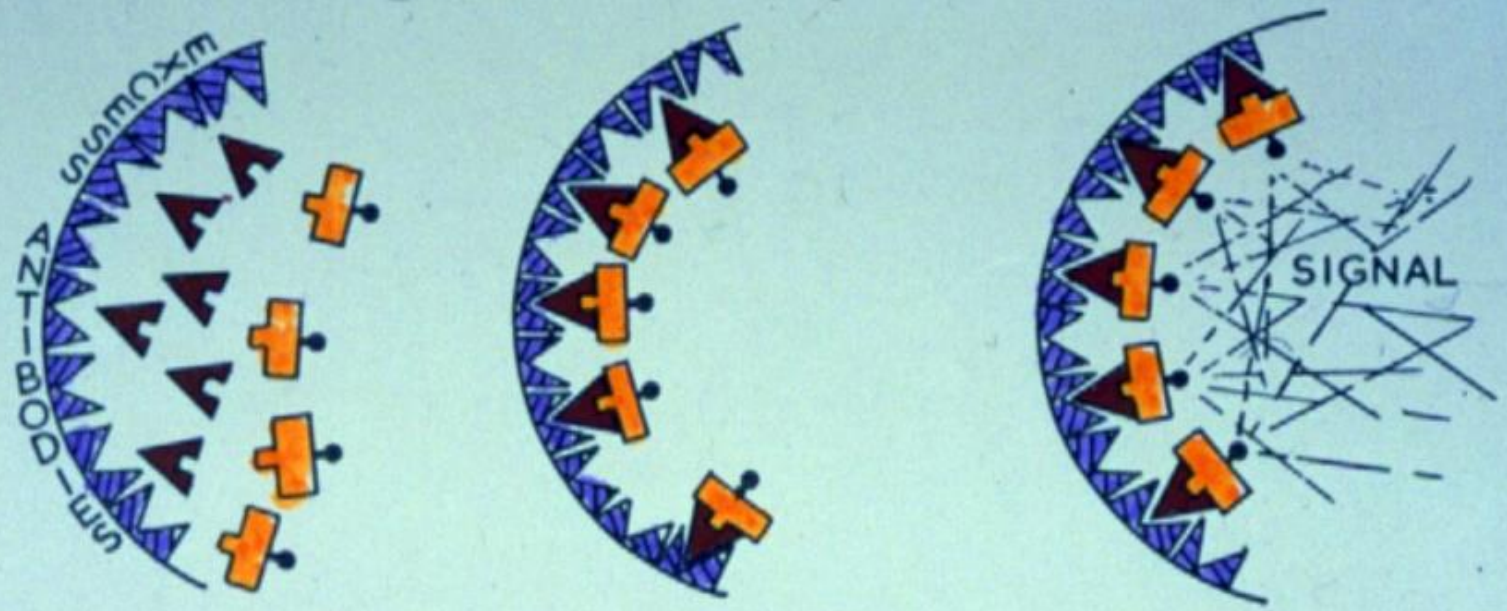


Skin

If you sunbathe

USE SUNSCREENS!

PRINCIPLE OF IMMUNOMETRIC ASSAY



A BINDING

- Sample analyte
- Second labelled antibody

B SEPARATION

Non-bound fraction removed

C MEASUREMENT

Signal directly proportional to analyte concentration

A schematic of immuno radiometric assay (IRA). The analyte binds to excess immobilised antibody. A second antibody binds to the bound analyte.

Diagnosis

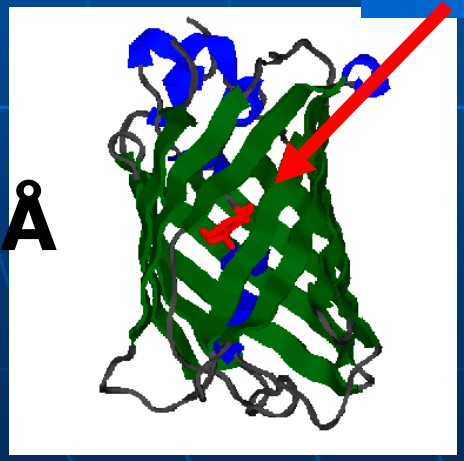
Pregnancy can be detected within the first 48 hours of it being established by immunochemical detection of the hormone

HUMAN CHORIONIC GONADOTROPHIN

The structure of Green Fluorescent Protein (GFP) and its fluorescent centre

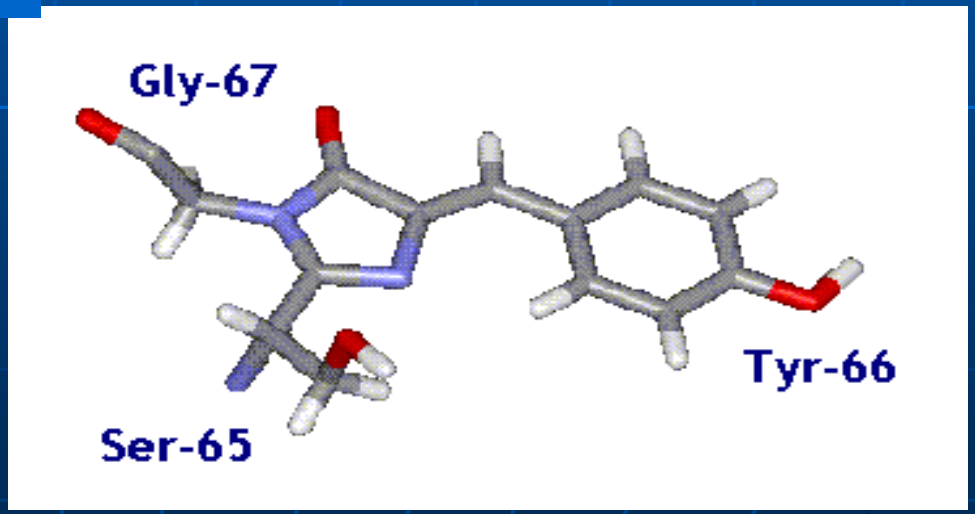
- Originally from jellyfish
- Gene can be expressed in any organism
- Fluorescently label a specific protein

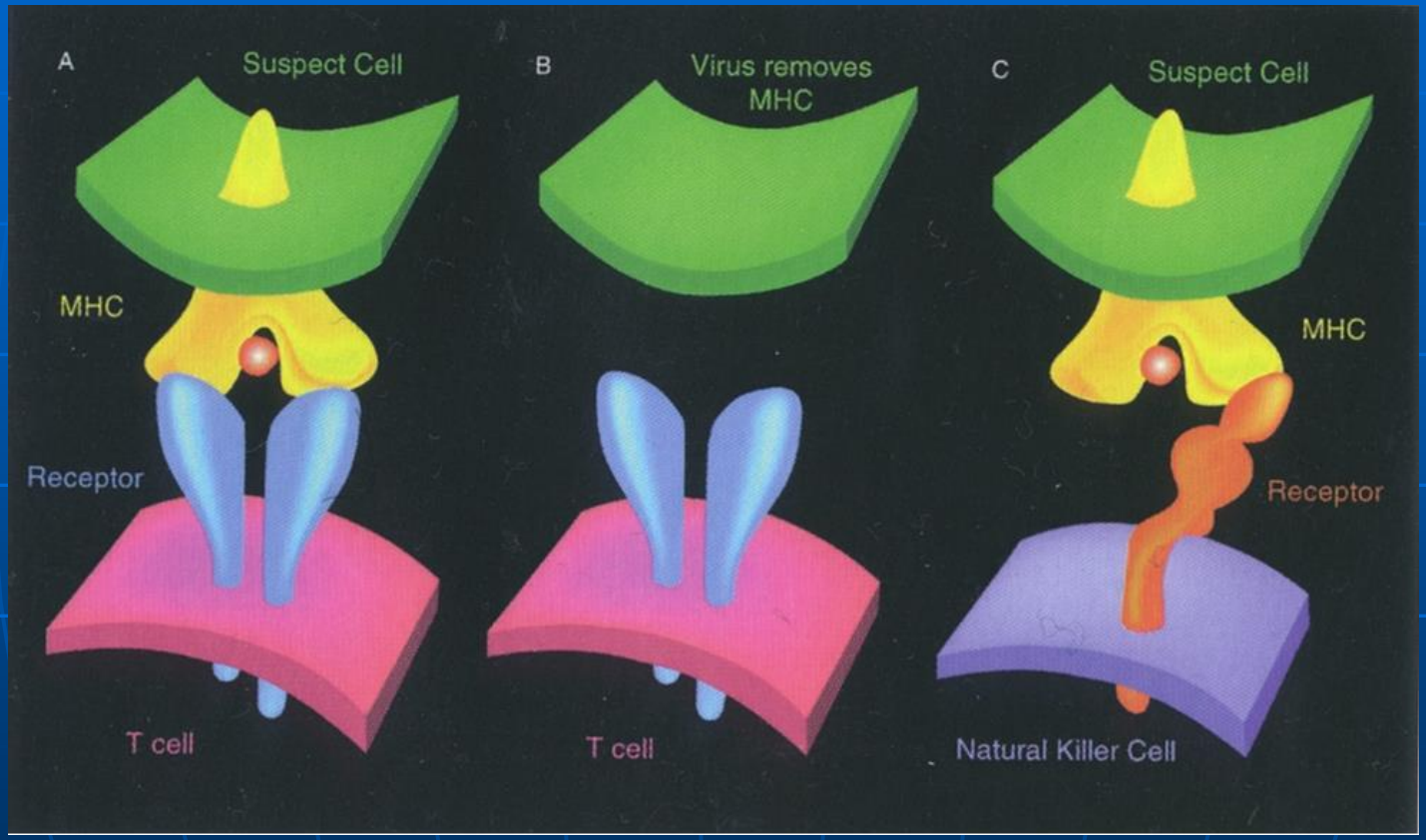
fluorophore protected by the barrel structure



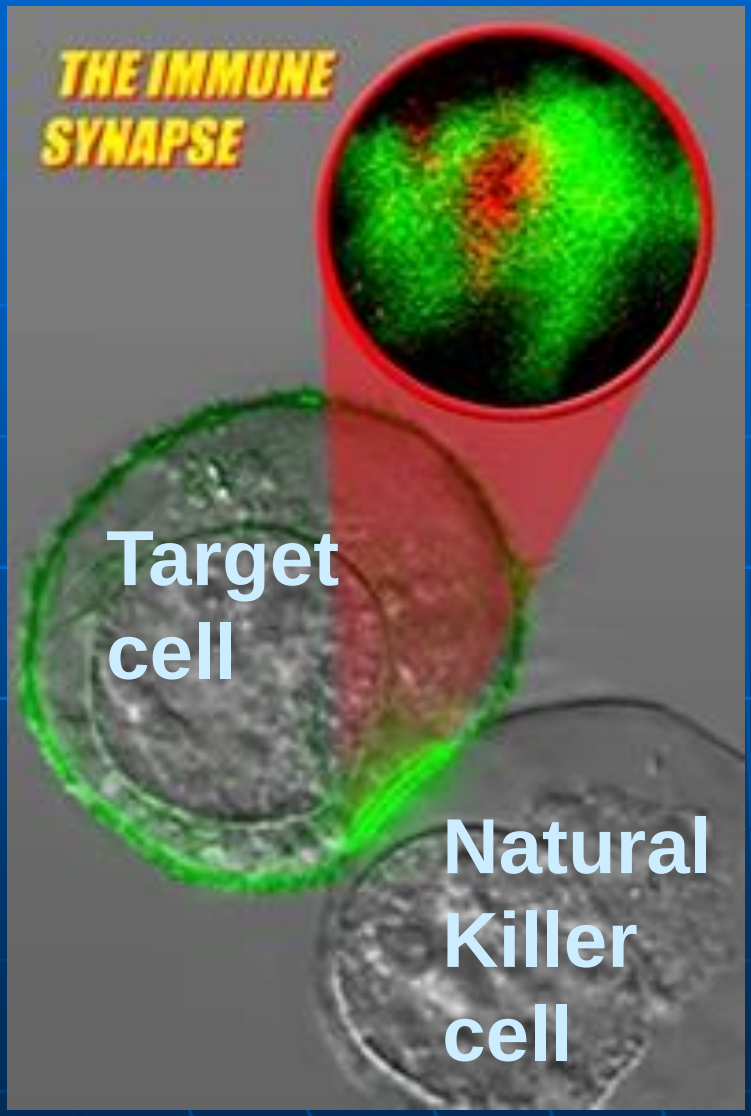
40 Å

30 Å




A
Suspect Cell
MHC
Receptor
T cell
B
Virus removes
MHC
T cell
C
Suspect Cell
MHC
Receptor
Natural Killer Cell

The Immune Synapse



- Facilitates immune surveillance

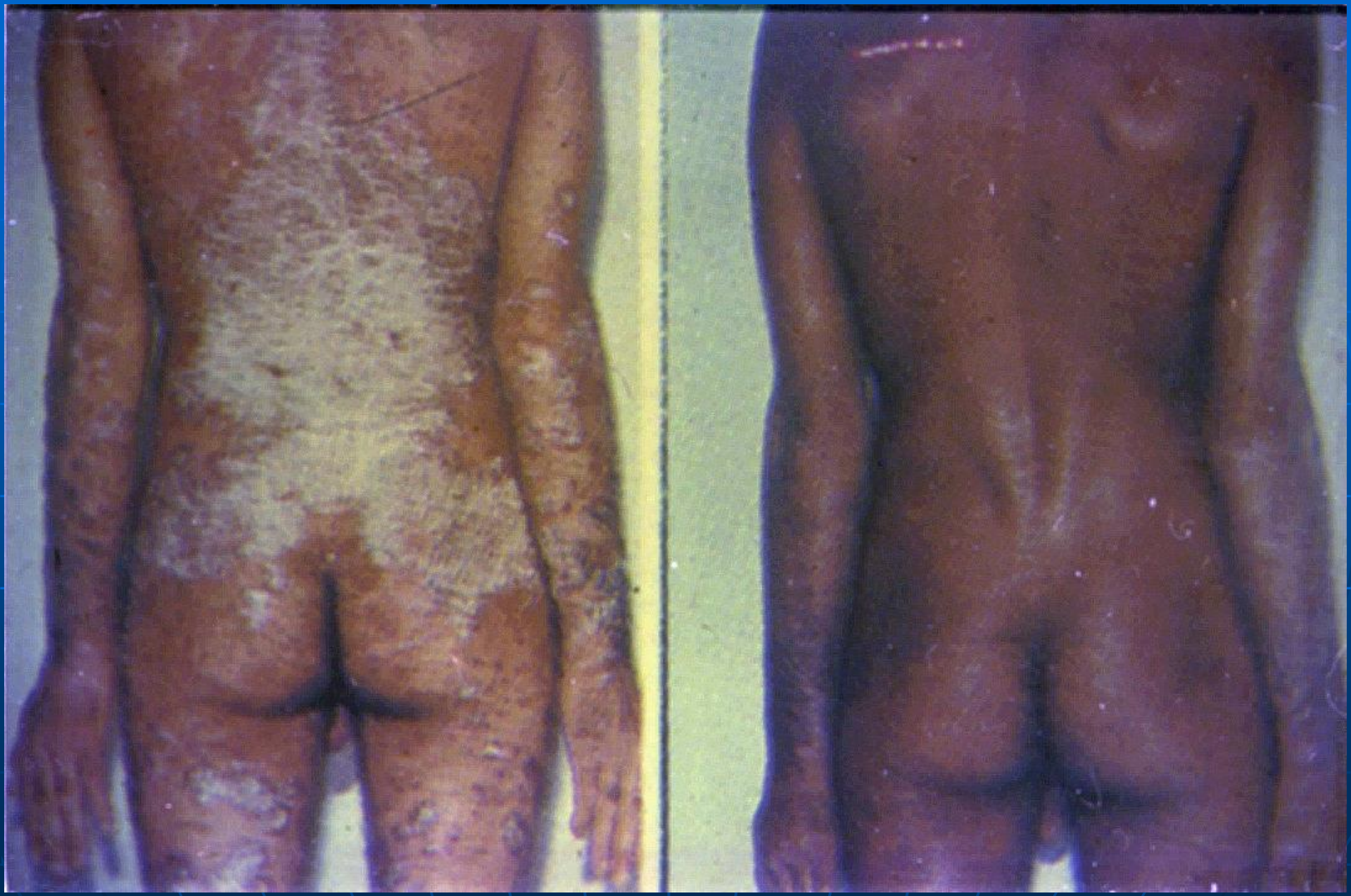
- rearrangement of proteins at intercellular contact

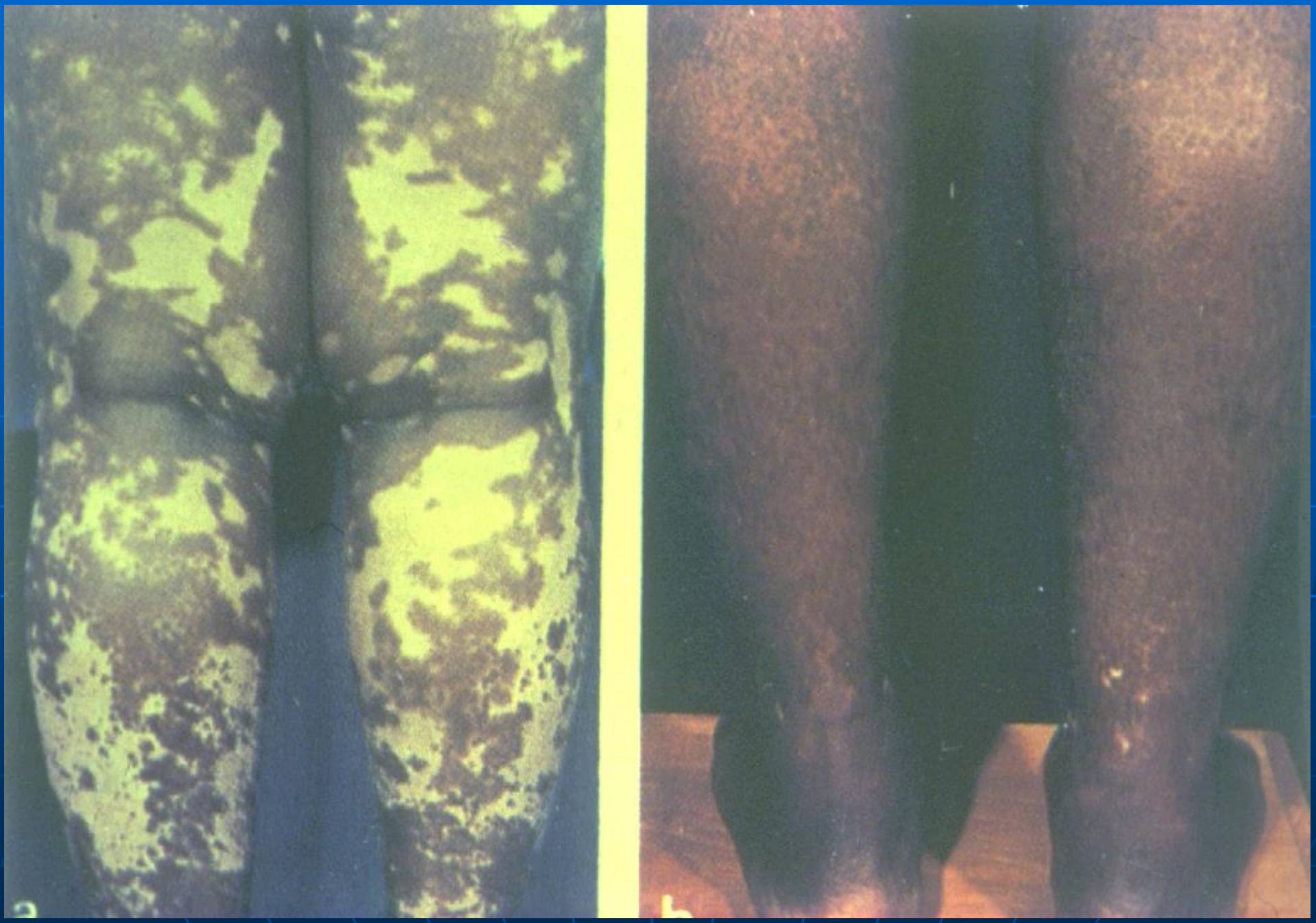
- Major Histocompatibility Complex (MHC) class I molecules play crucial role in immune recognition and target cell fate

Therapy

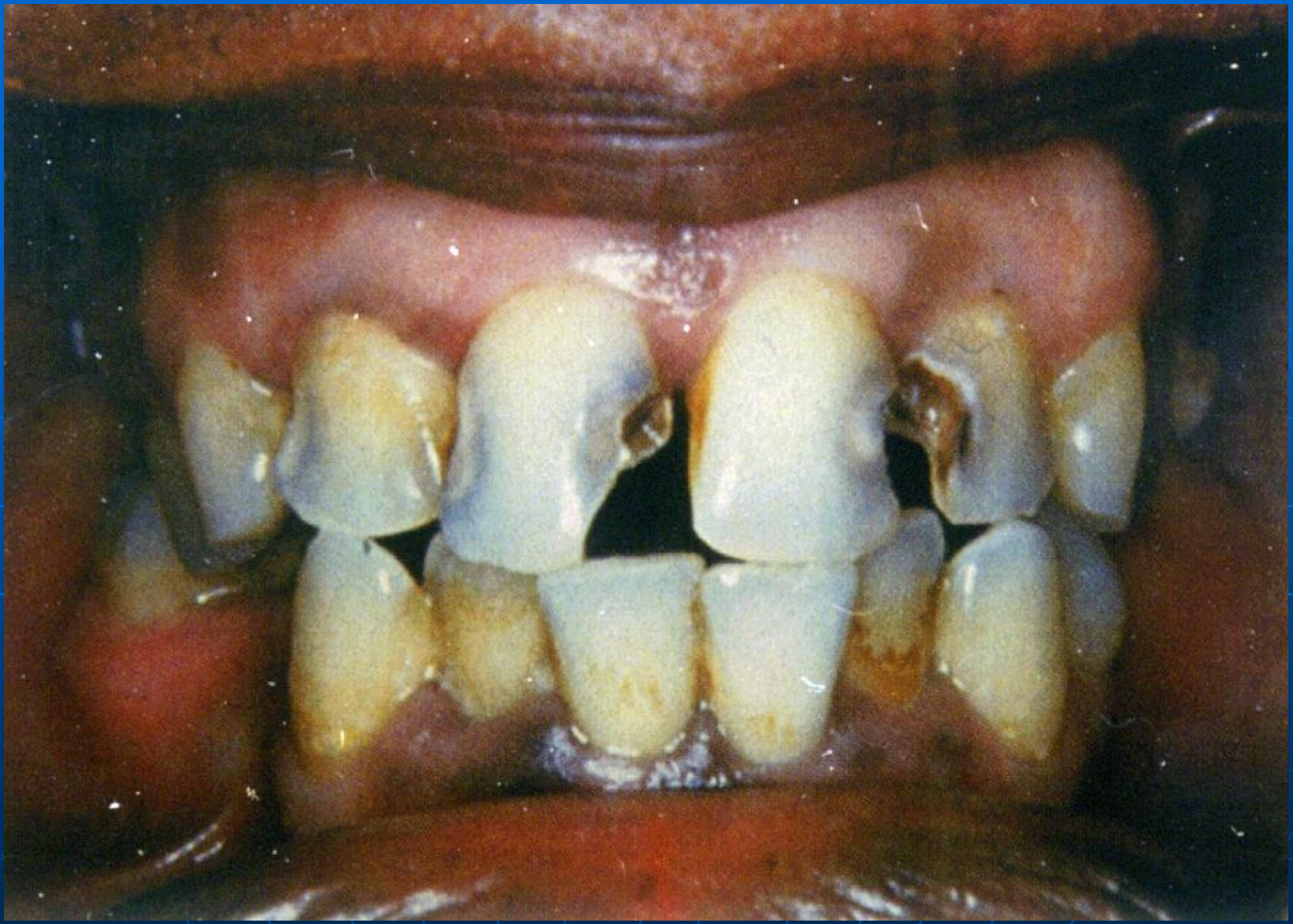
Uses of light to treat disease include

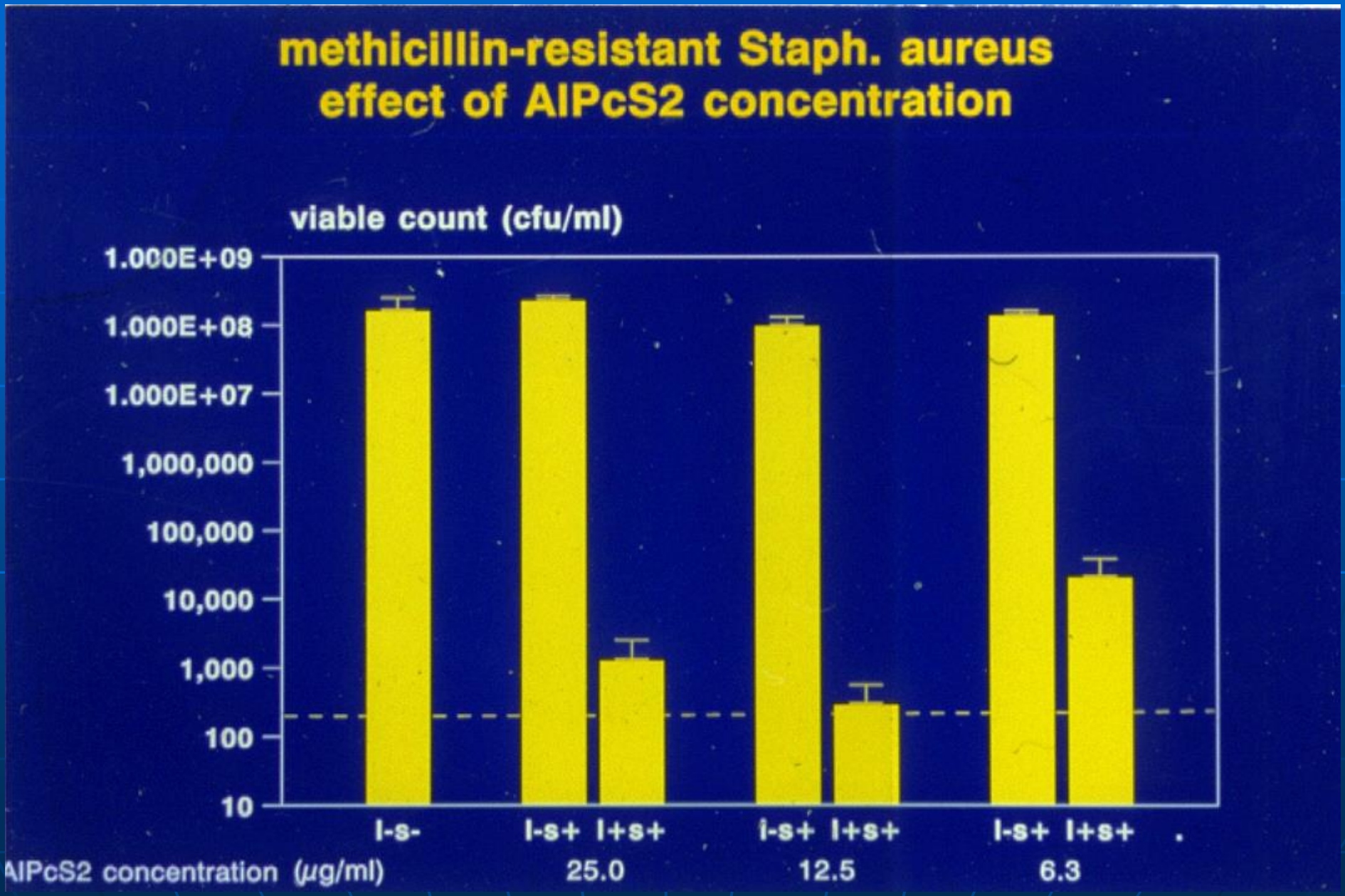
- **PUVA, psoralen + UVA** — ***Psoriasis, vitiligo***
- **PDT, sensitiser + red light** — ***Cancer, bacterial infections***
- **Jaundice, blue light** — ***Newborn babies***
- **SAD, white light** — ***Winter blues***
- **Laser surgery**





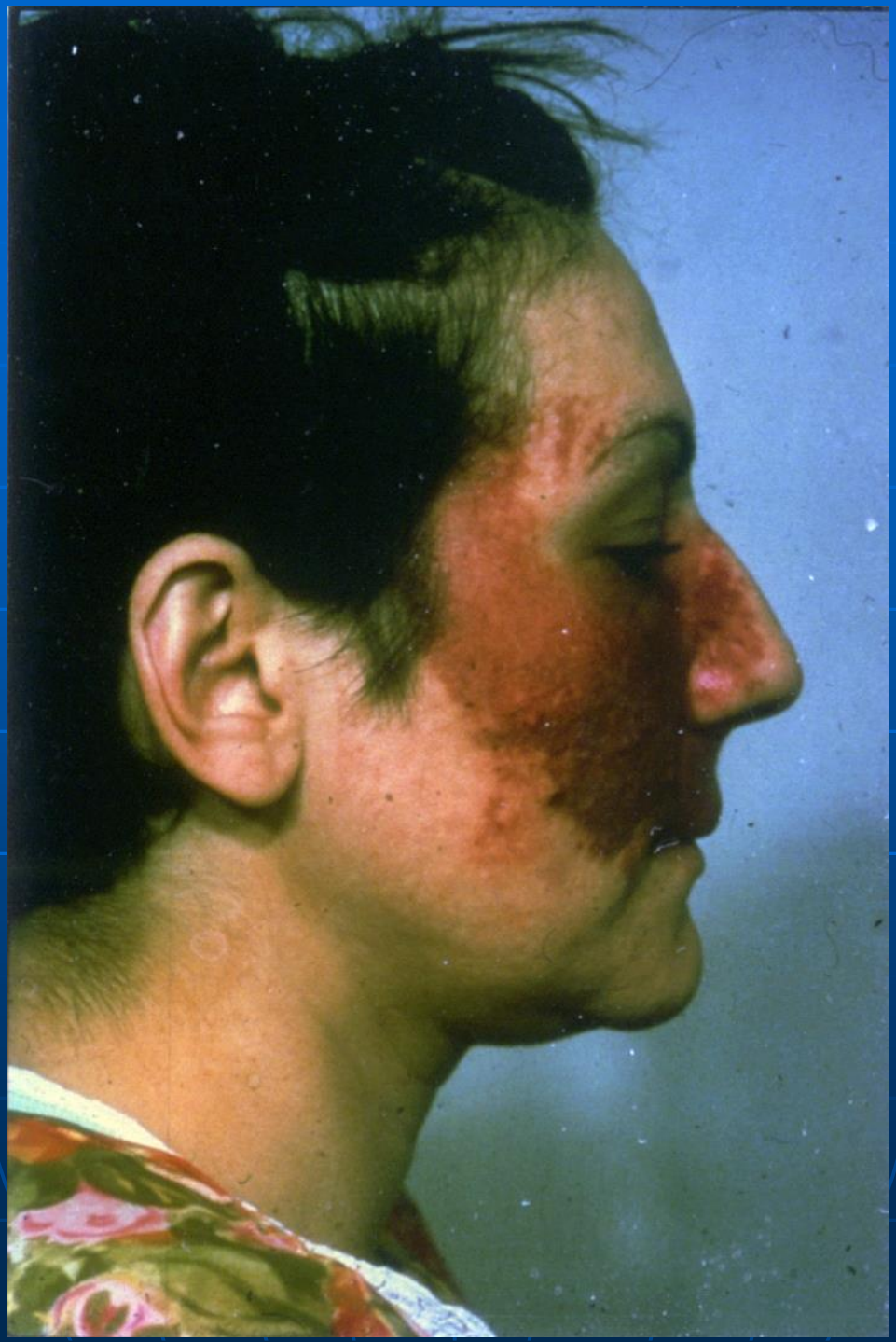
Plaque attack: danger for teeth

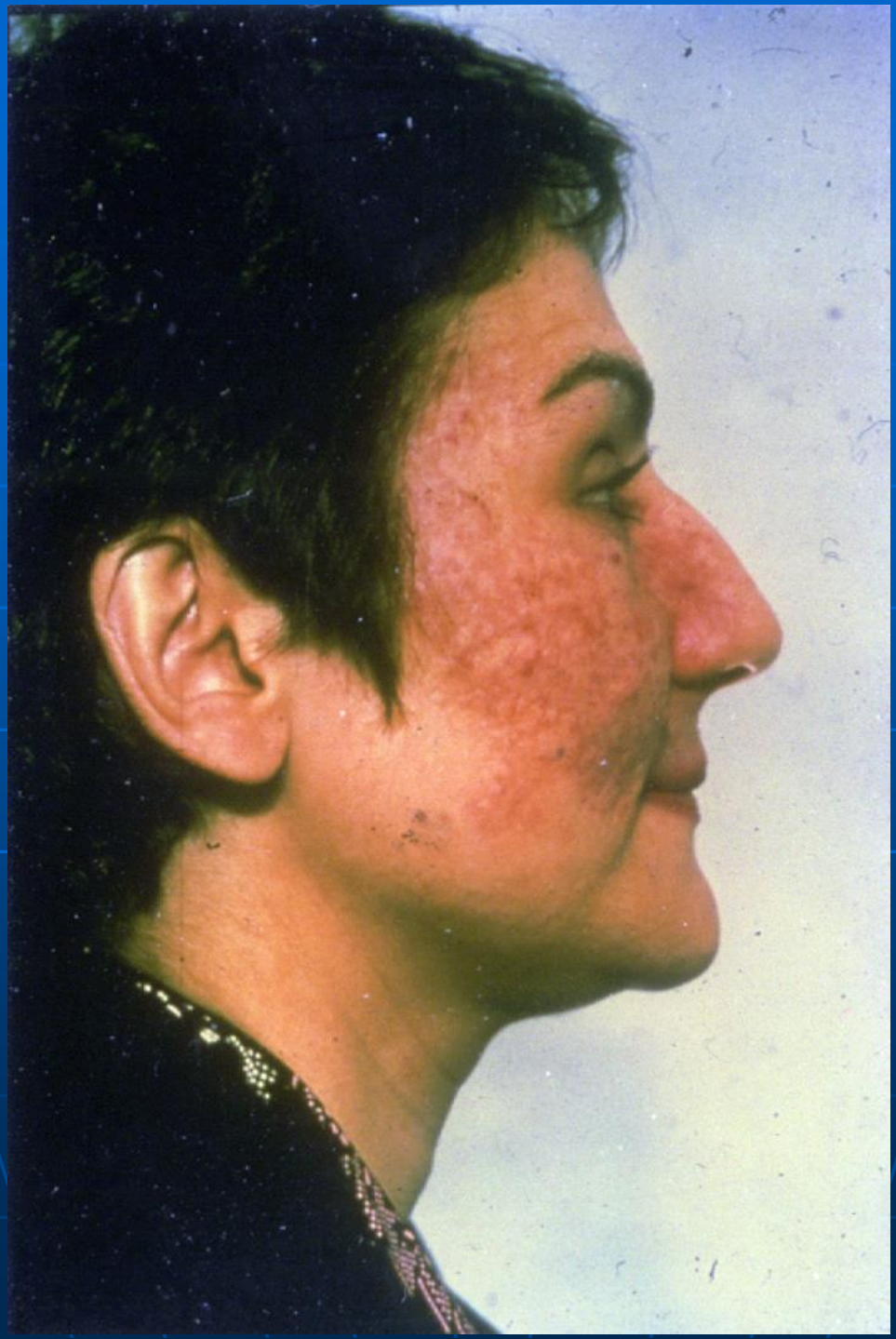


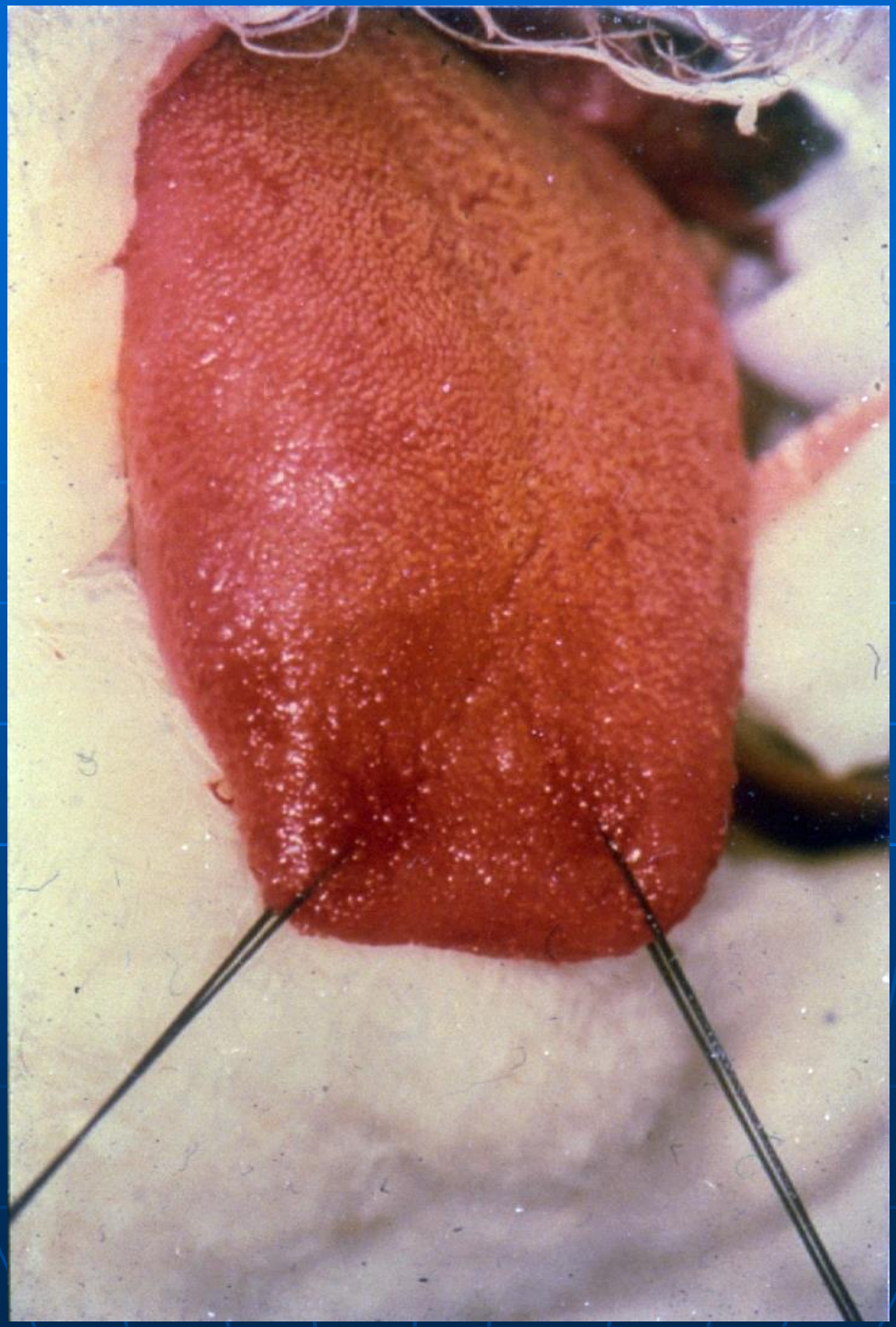

methicillin-resistant Staph. aureus
effect of AlPcS2 concentration
viable count (cfu/ml)
1.000E+09
1.000E+08
1.000E+07
1,000,000
100,000
10,000
1,000
100
10
I-s-
I-s+ I+s+
i-s+ I+s+
I-s+ I+s+
AlPcS2 concentration (μg/ml)
25.0
12.5
6.3

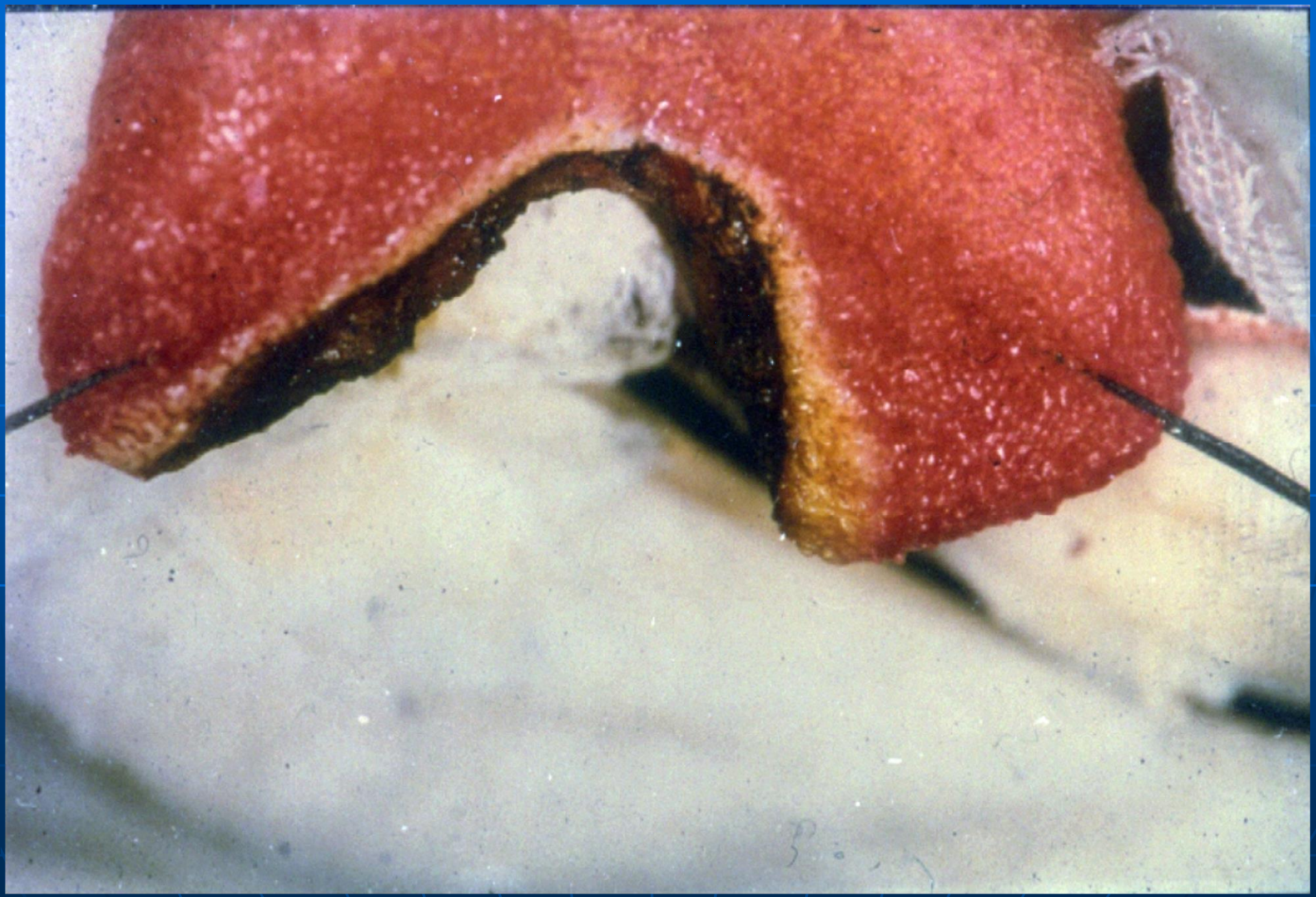
Lasers in medical use

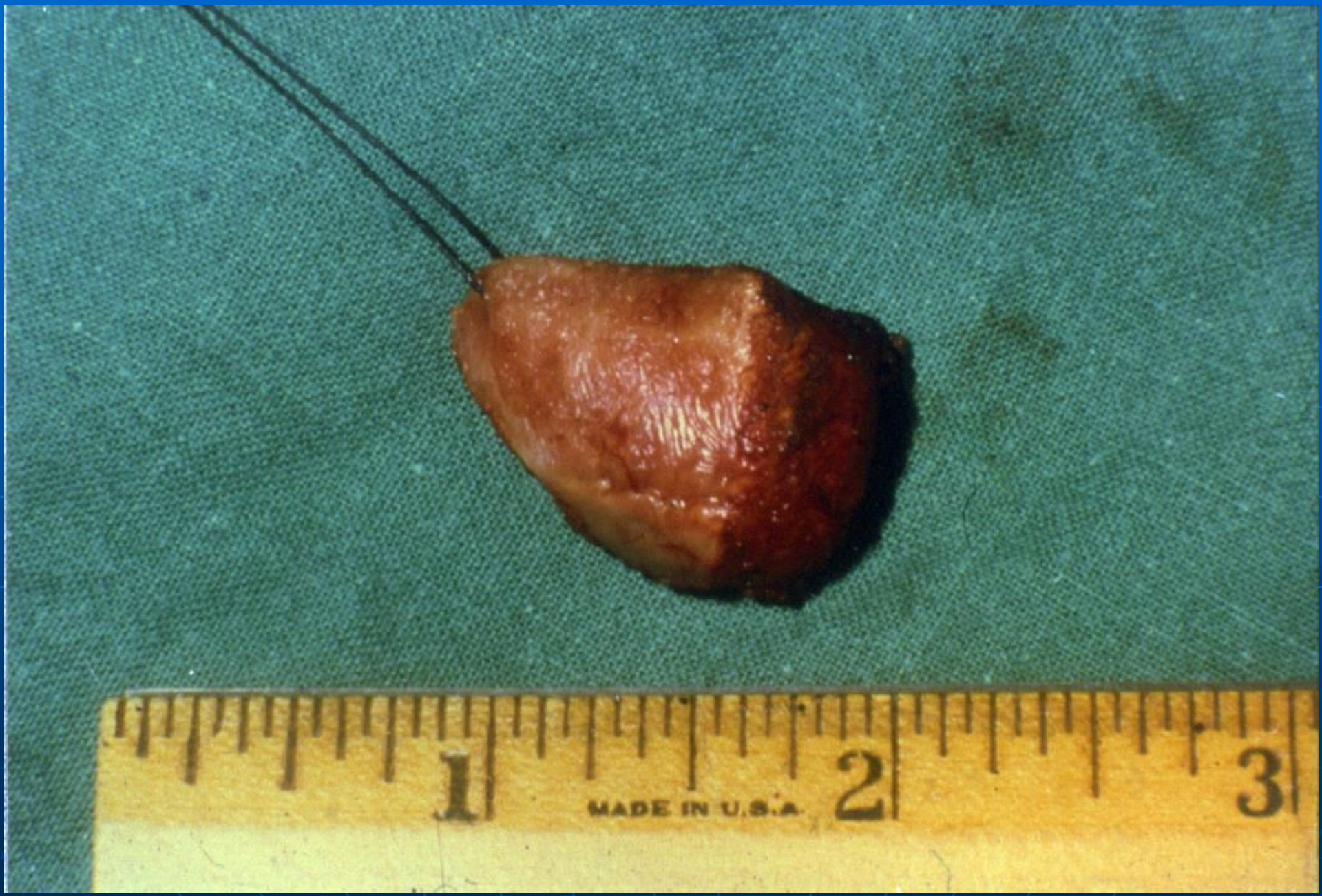
- Infra-red [Carbon dioxide[10600nm] Er:YAG [3000nm];Nd:YAG [1060nm]Soft tissue laser surgery]
- Ultra-violet [excimer lasers XeCl, XeF, ArF etc] Tissue ablation,corrective eye surgery.
- Diode lasers [red, infra-red] Photodynamic Therapy
- Femtosecond lasers [two-photon excitation, micro-laser scalpel]
- Cosmetic. Various lasers,including those above, plus ruby, alexandrite, used for hair removal, teeth whitening, acne, tattoo removal, stretch mark removal, pigmentation removal, treatment for ageing skin.

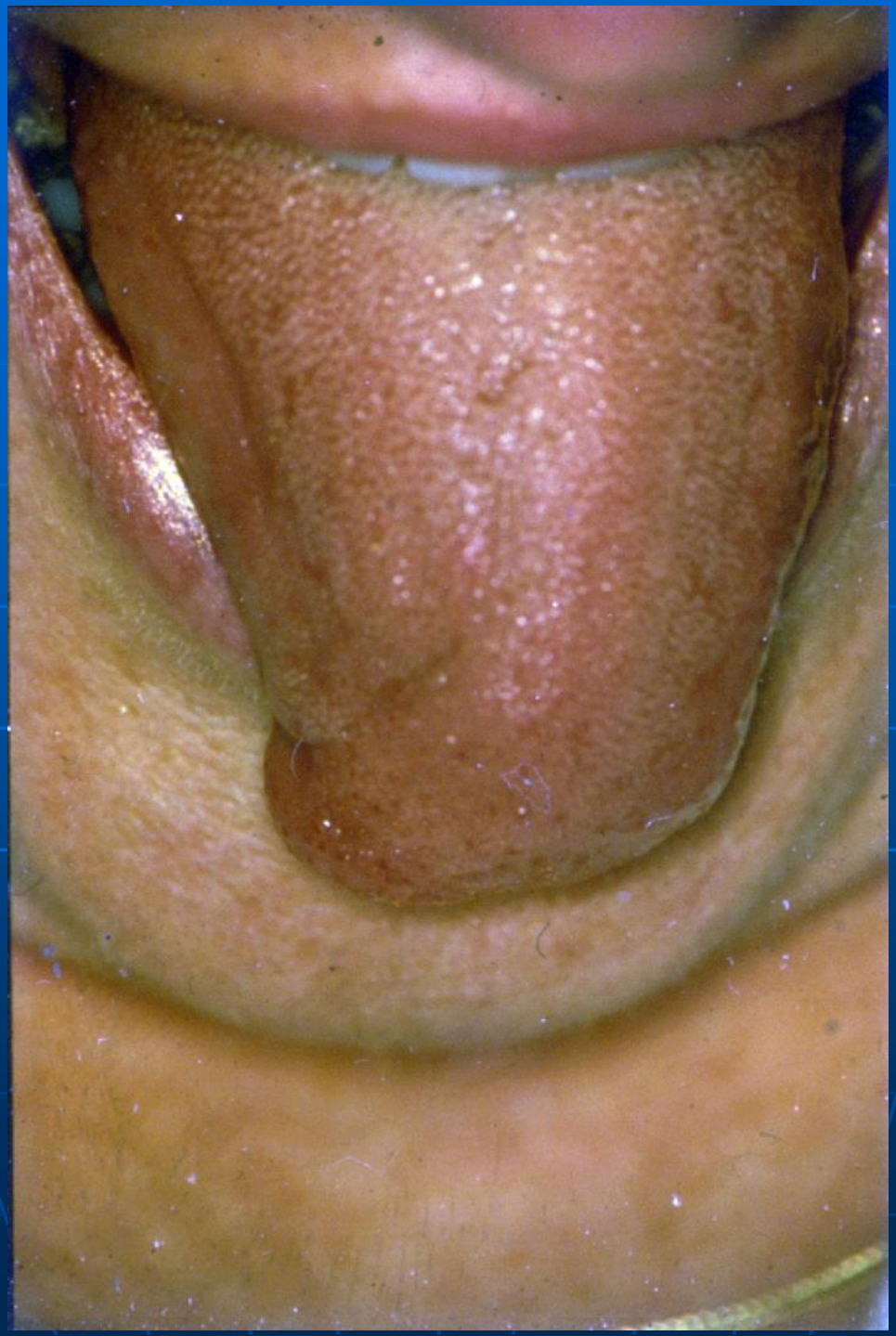


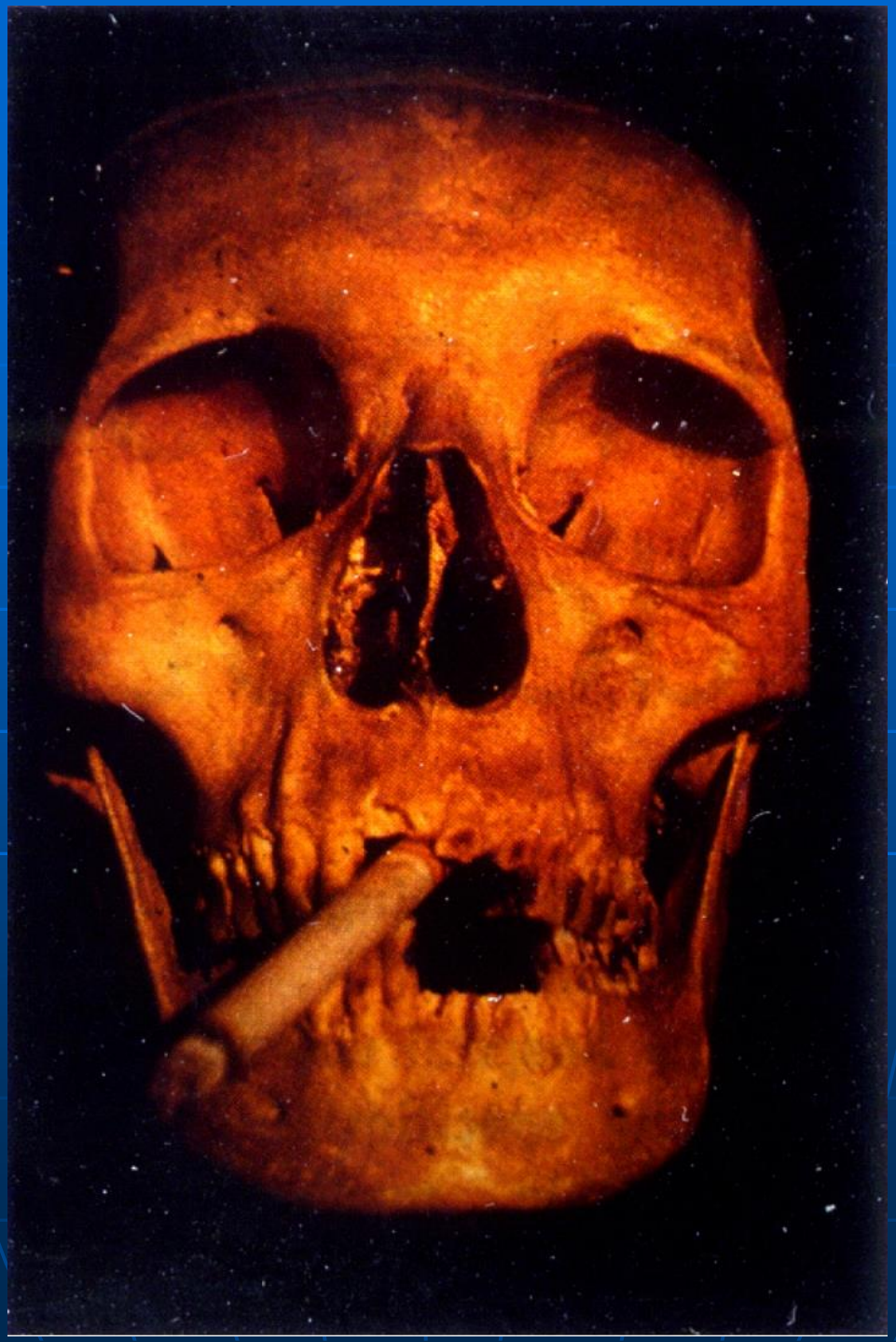


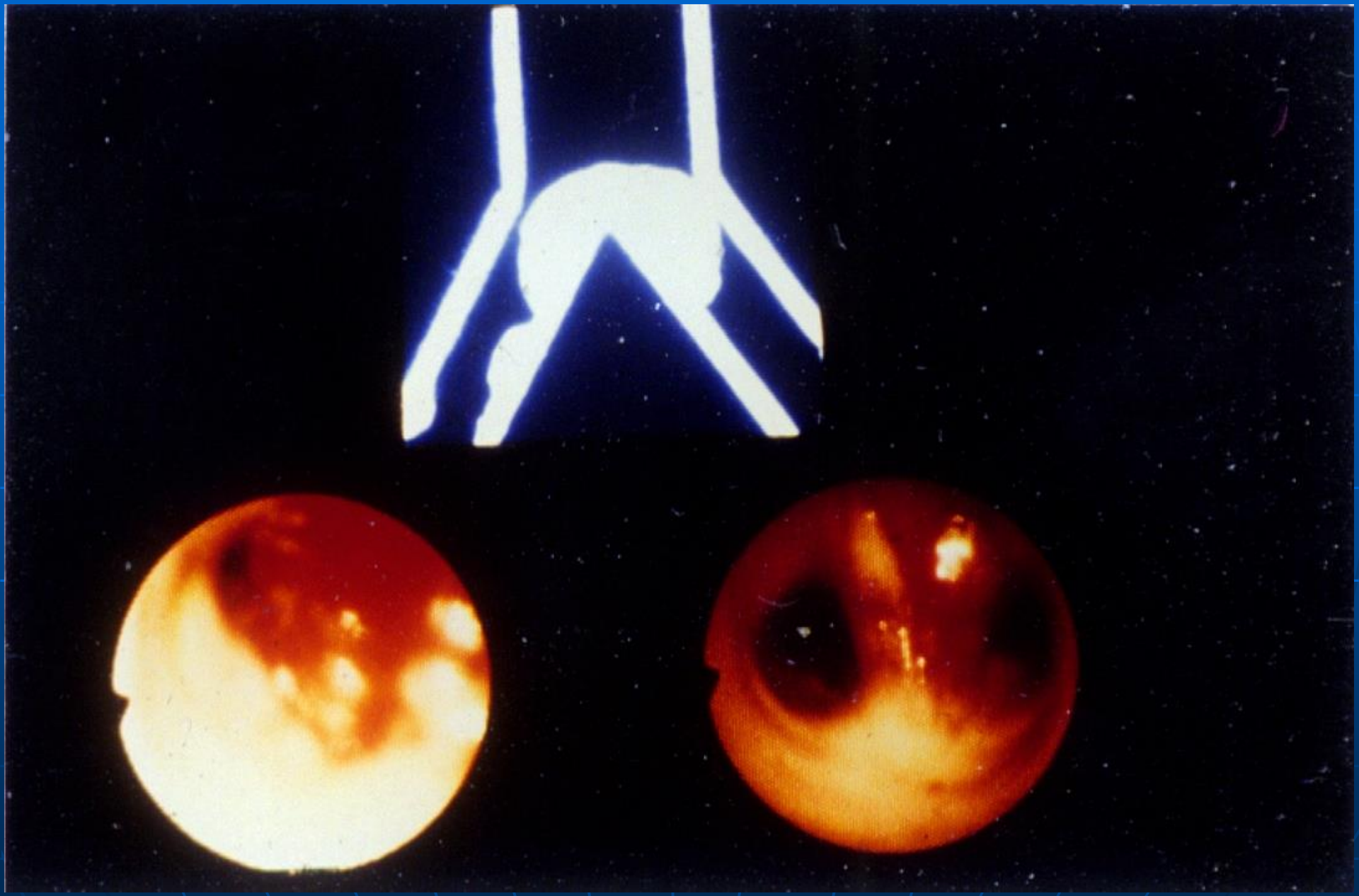





1
MADE IN U.S.A.
2
3







PDT

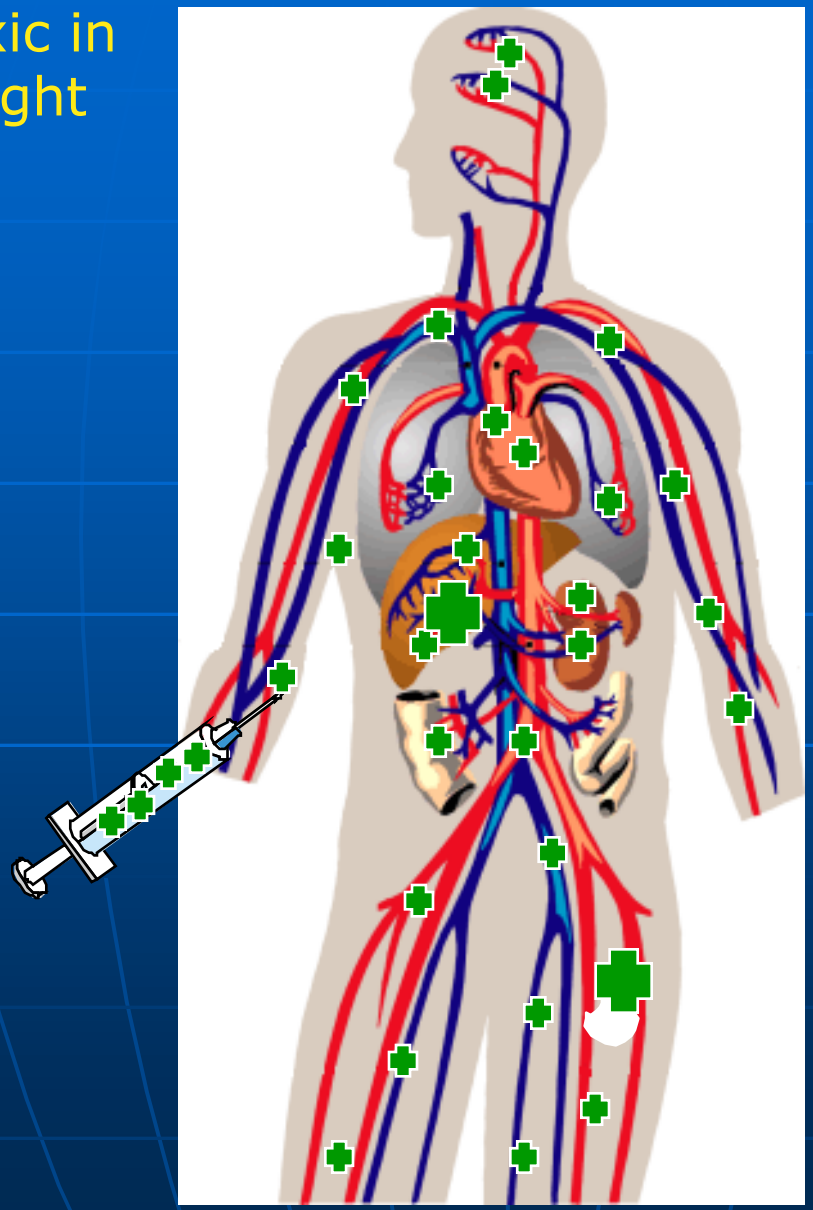
PHOTODYNAMIC THERAPY

is the use of a dye sensitiser plus red light to treat cancer

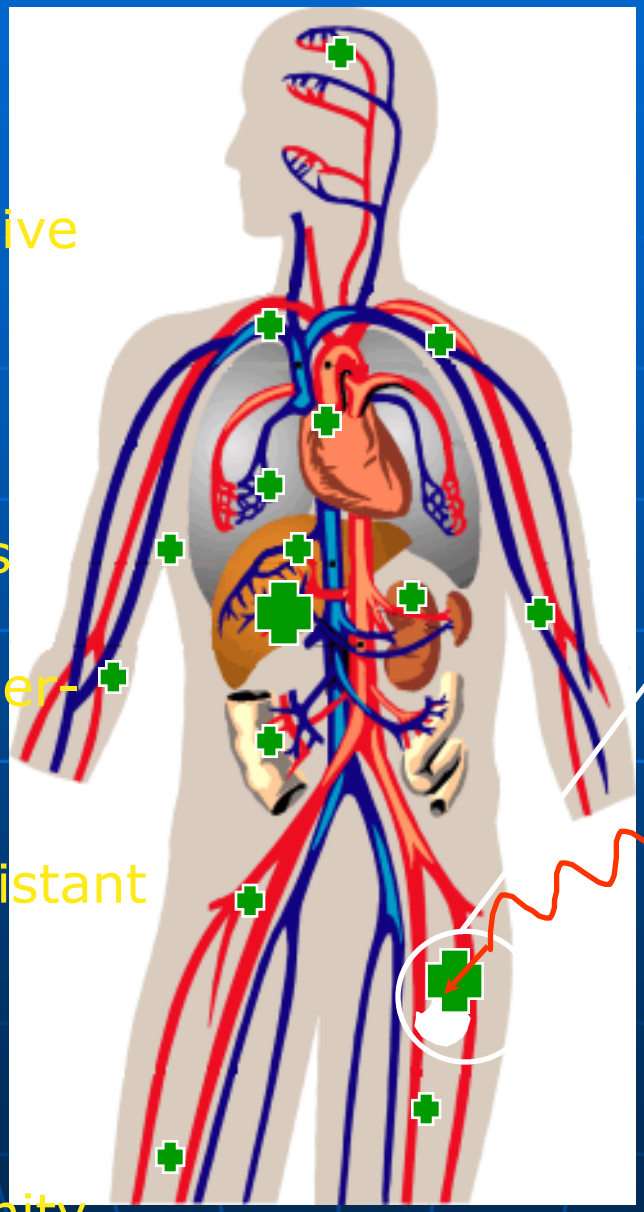
It has other uses, since bacteria and fungi can be destroyed by the same method, and some viruses can be inactivated the same way

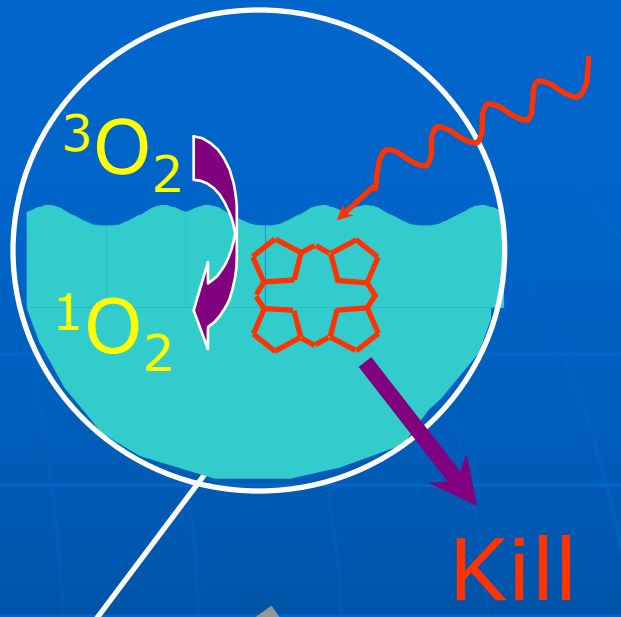
PDT treatment advantages

- Drug non toxic in absence of light

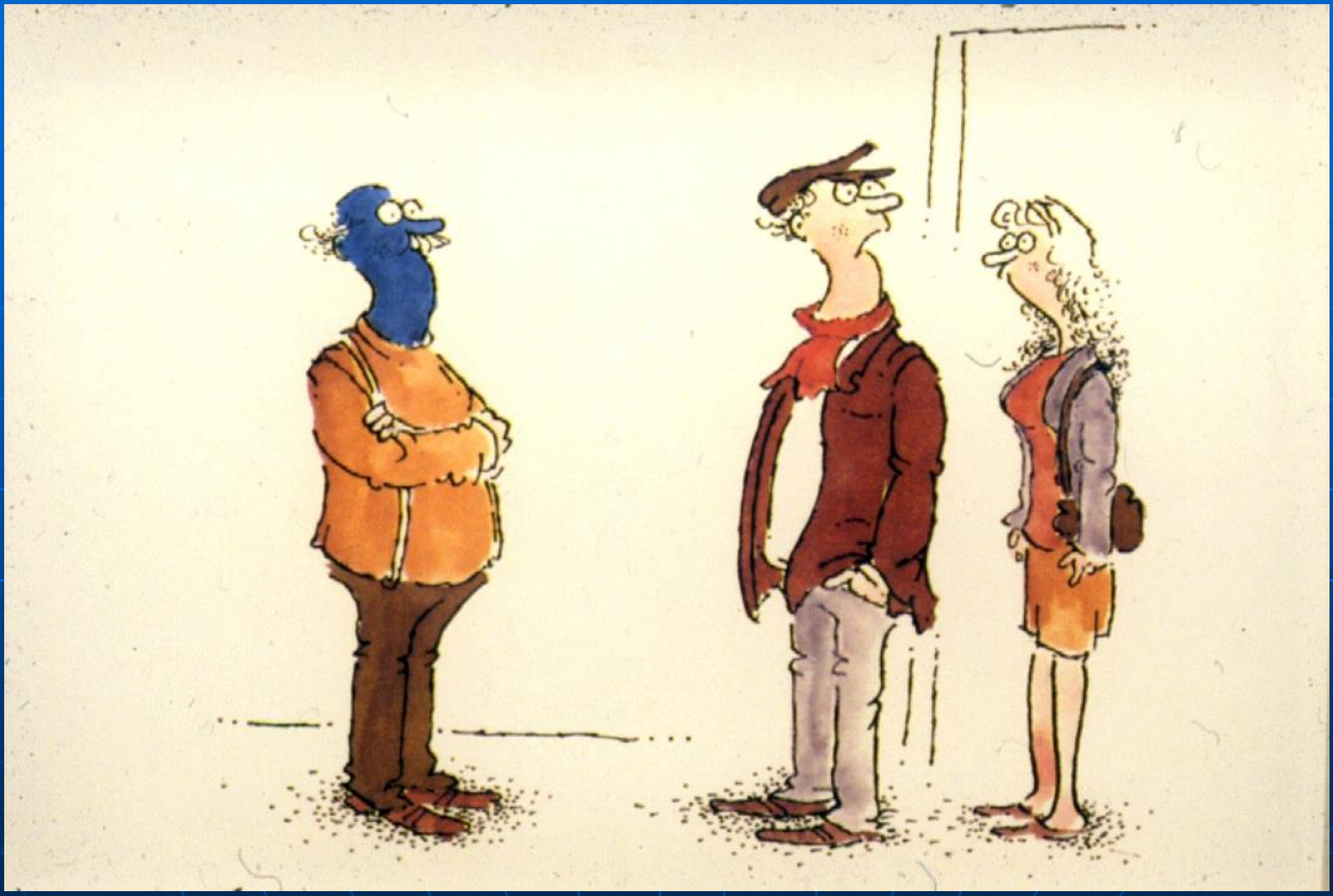


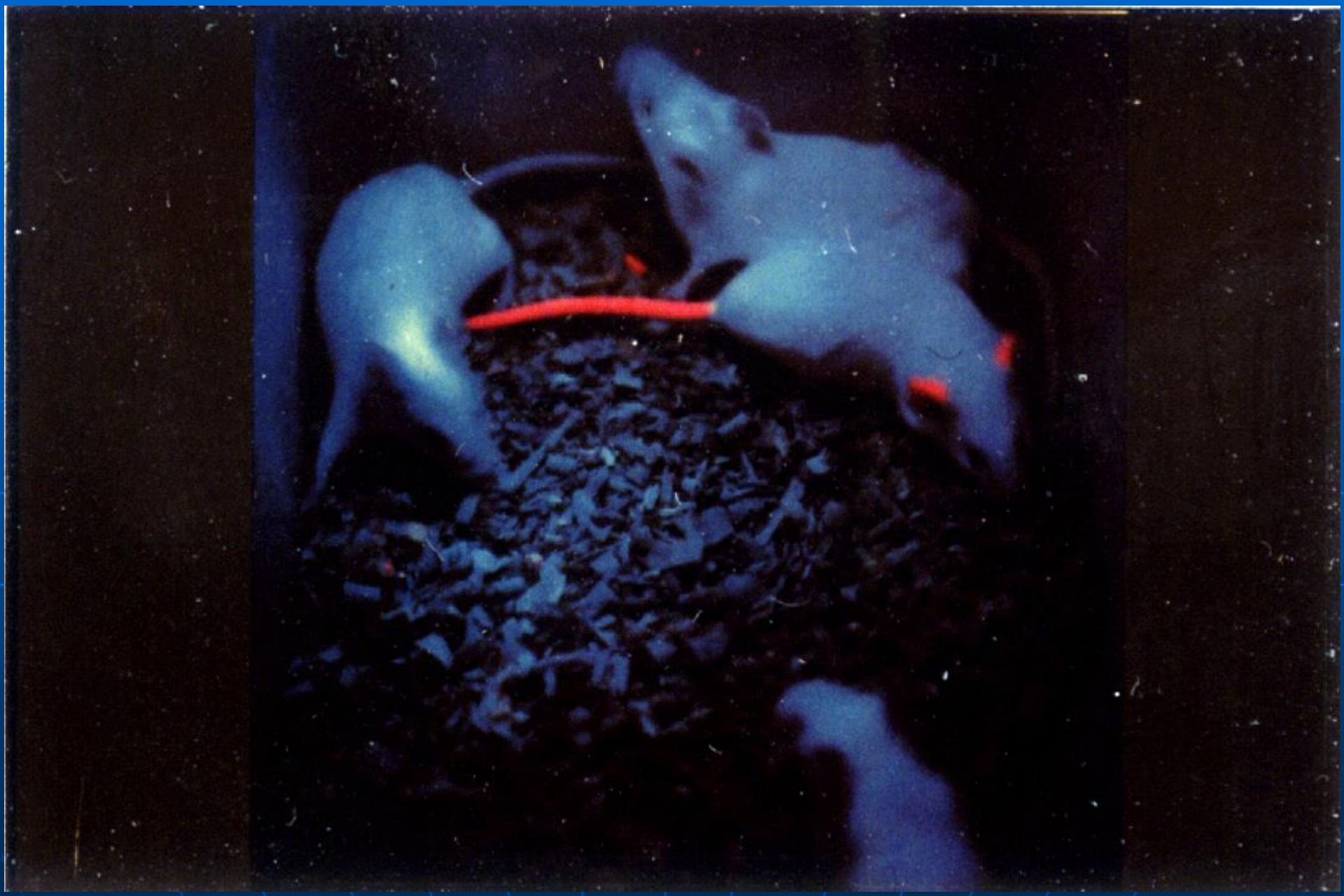
- Drug non toxic in absence of light
- Drug slightly selective for target cells
- Safe, non-invasive treatment for superficial diseases
- Singlet oxygen generated at rapid rate
- Cells cannot be resistant to singlet oxygen
- Tumour 'suicide'
- Longer term immunity

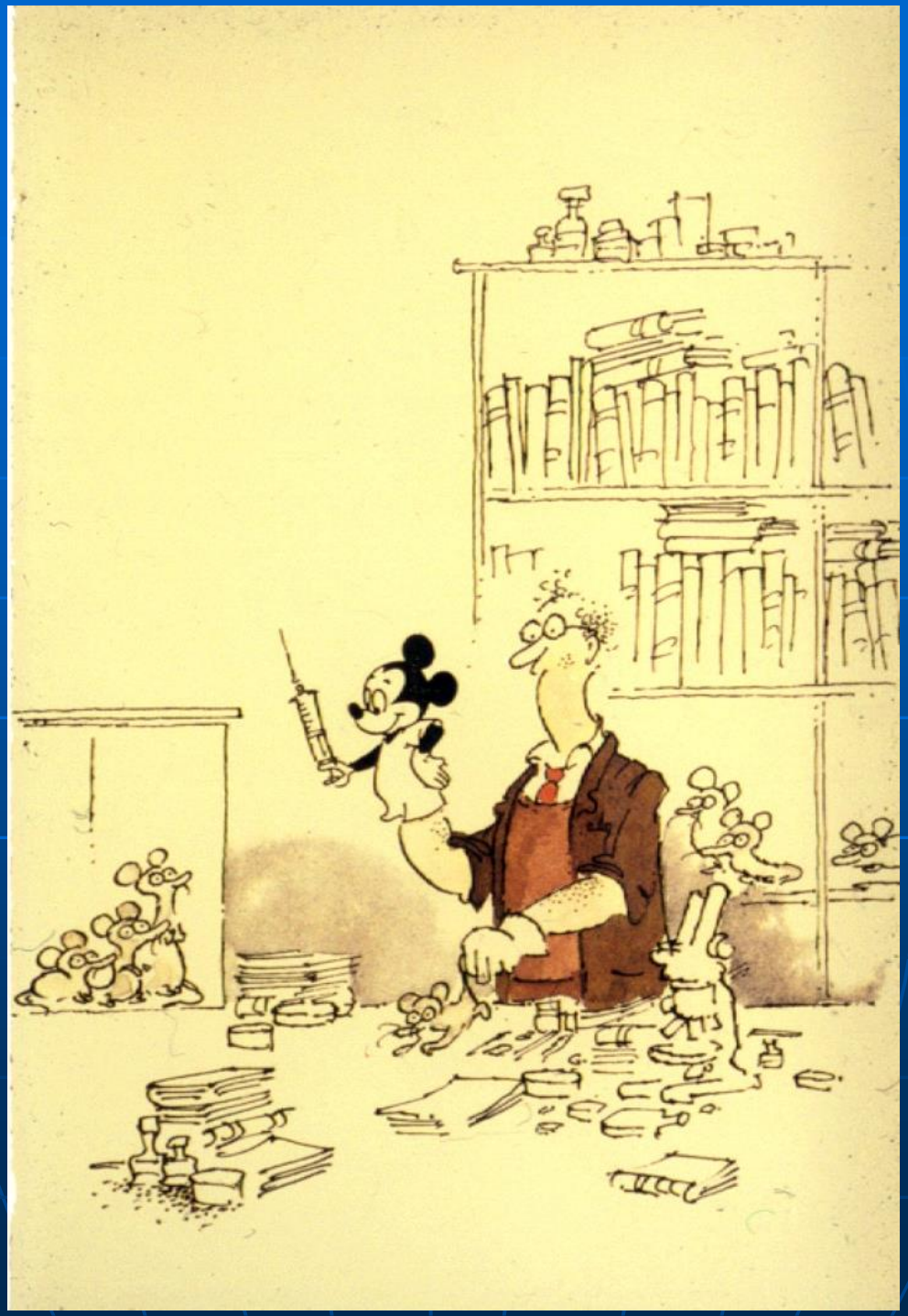




Laser light source directed at target tissue







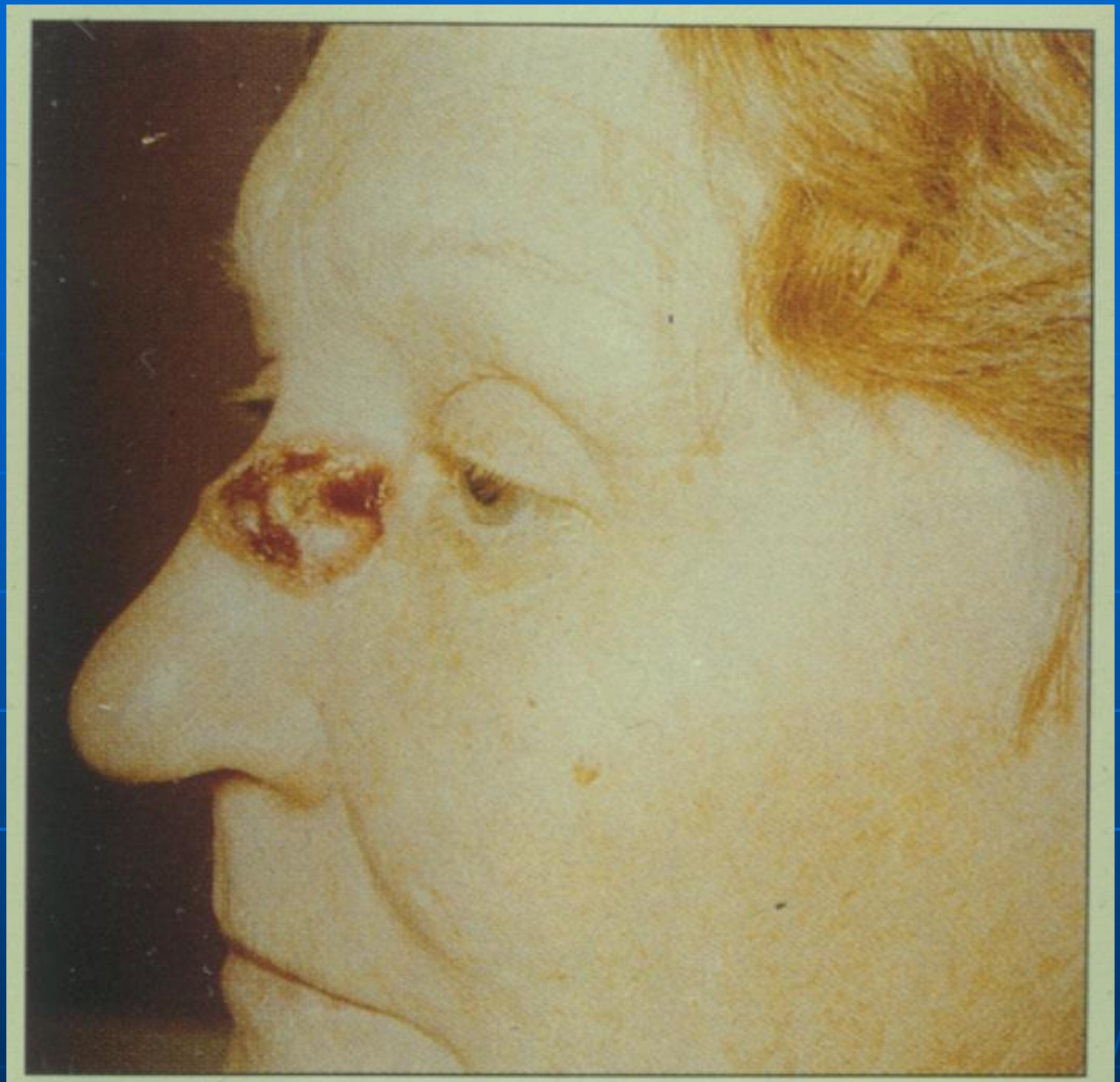


Figure 1: Basal-cell carcinoma located on the skin of nose, 2.5cm diameter, 56 year-old female.

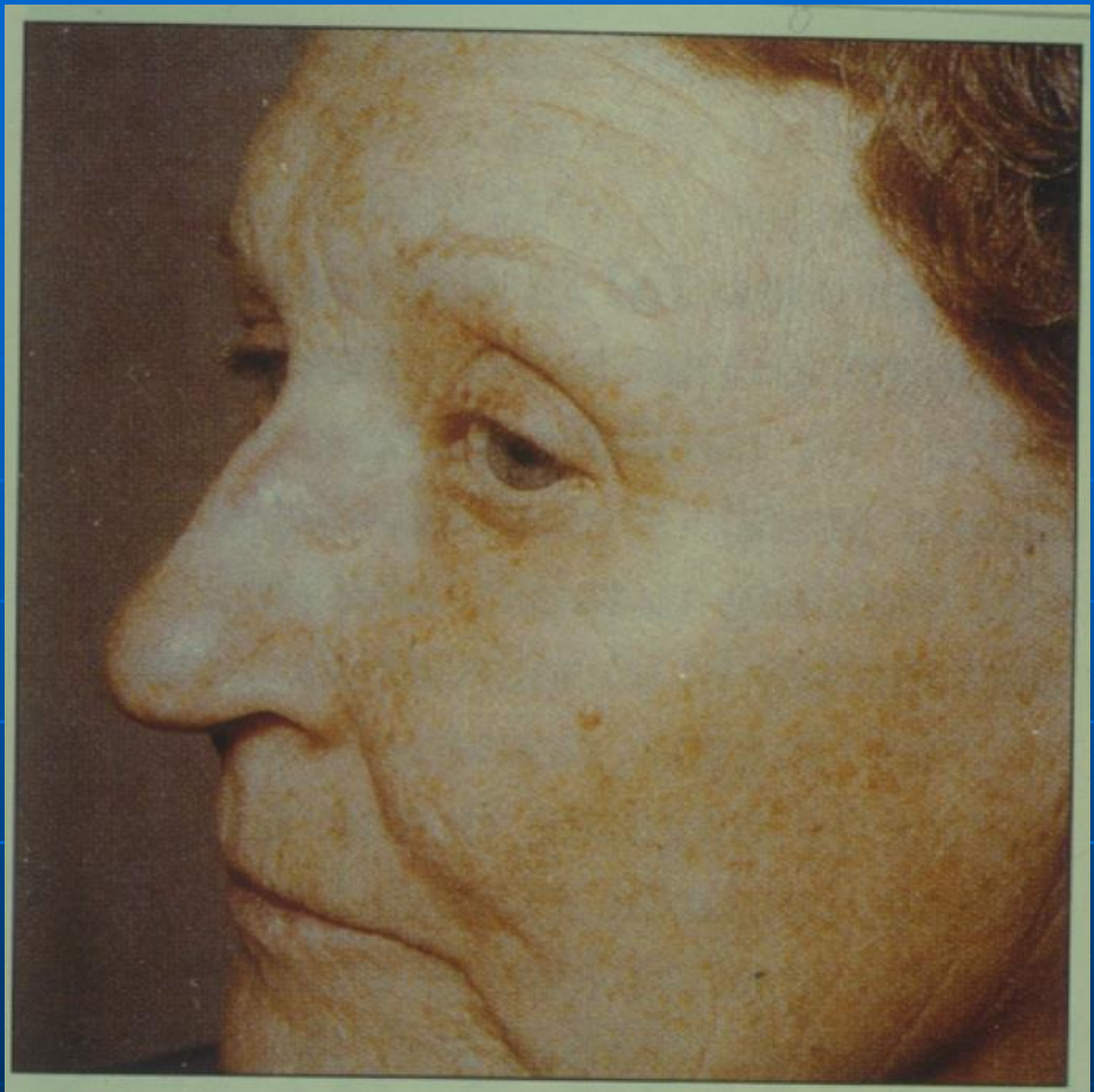
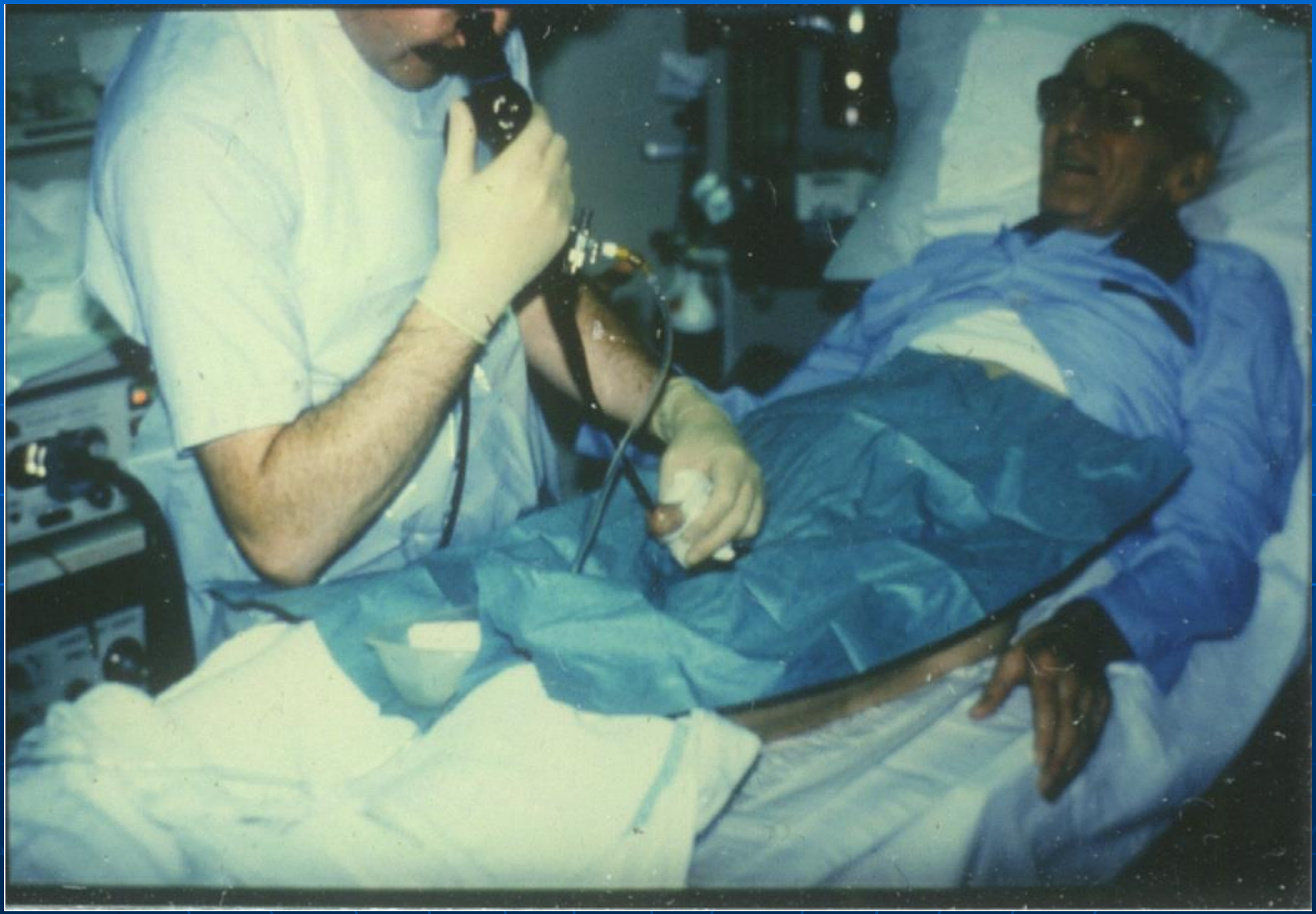
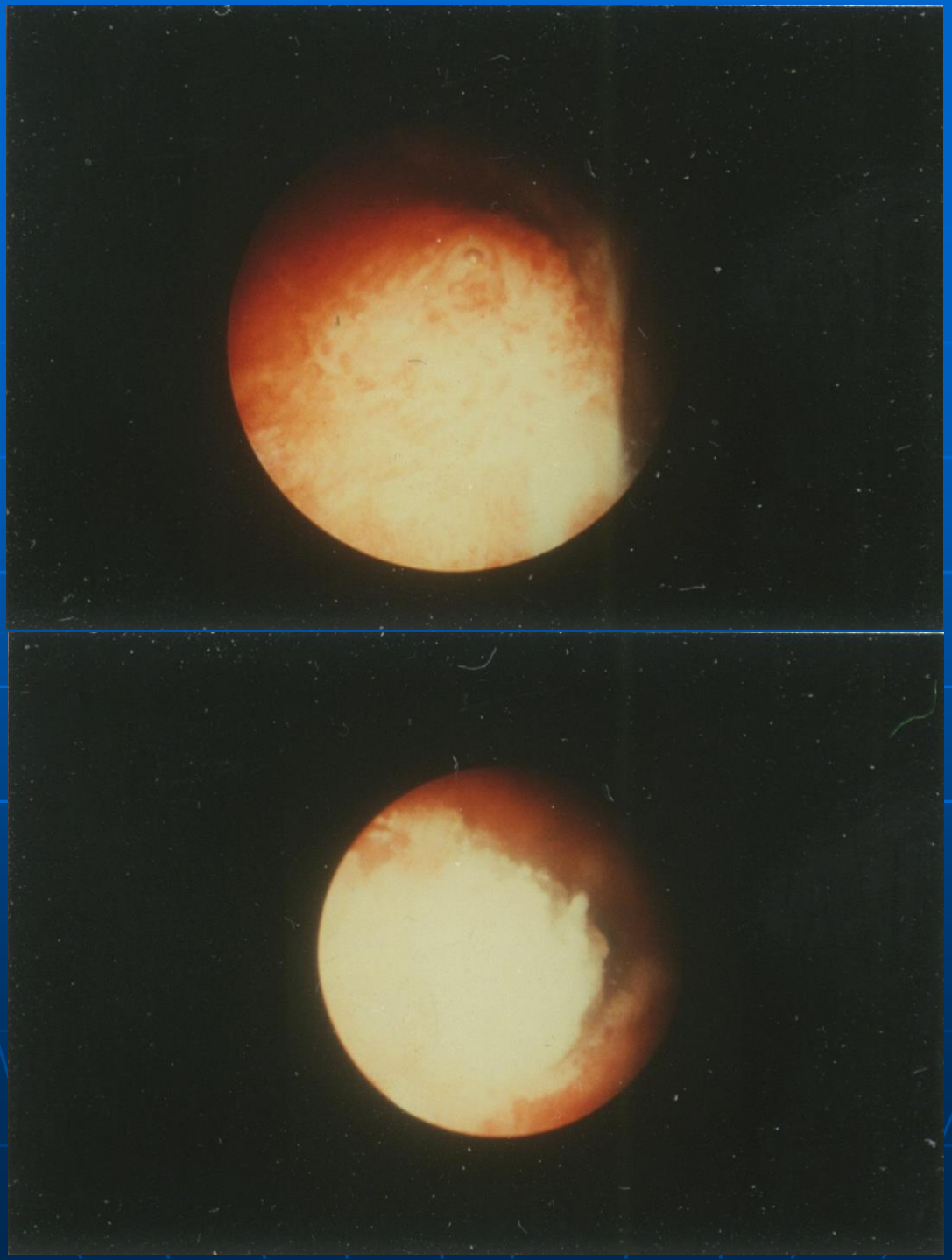
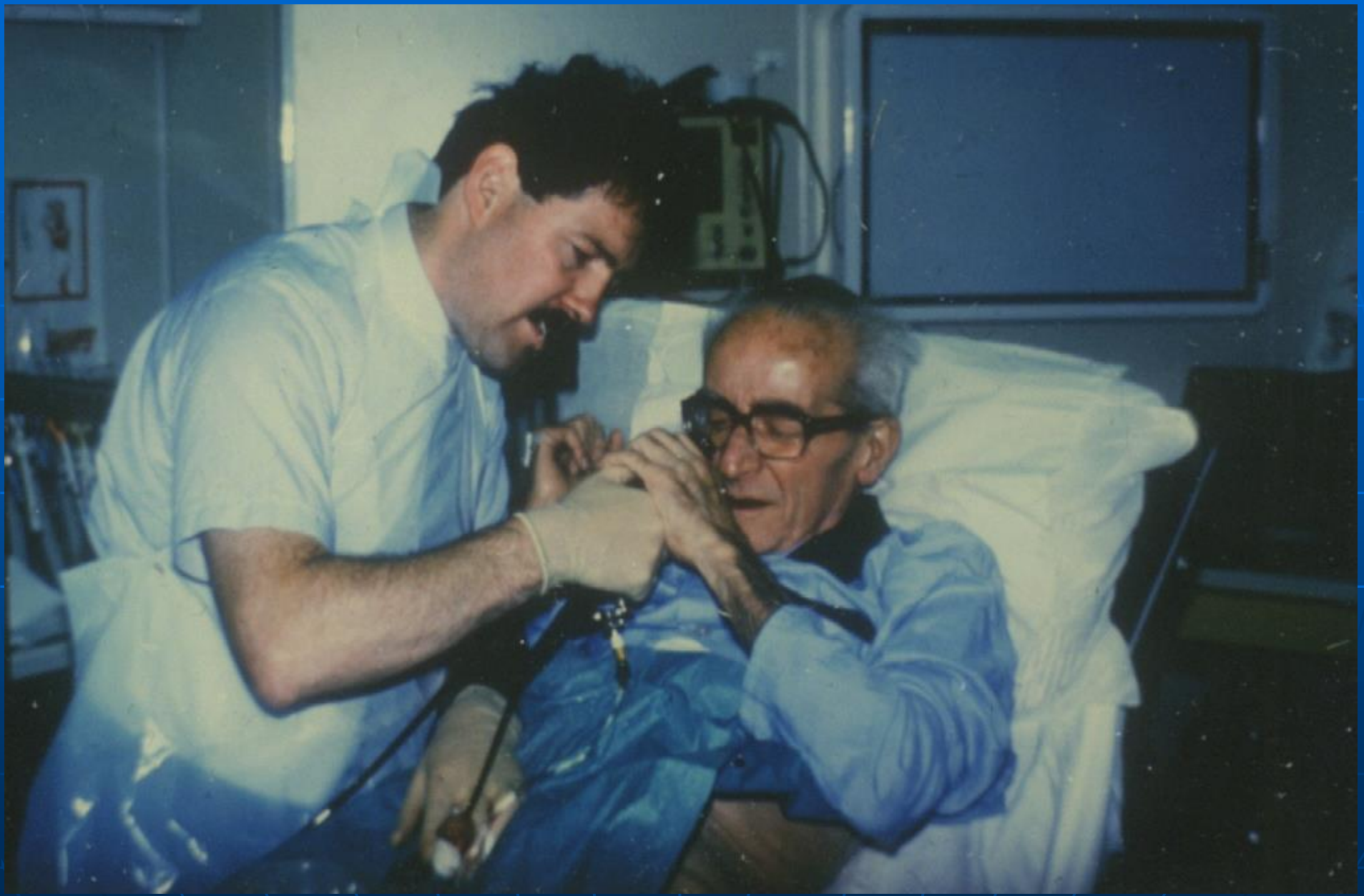


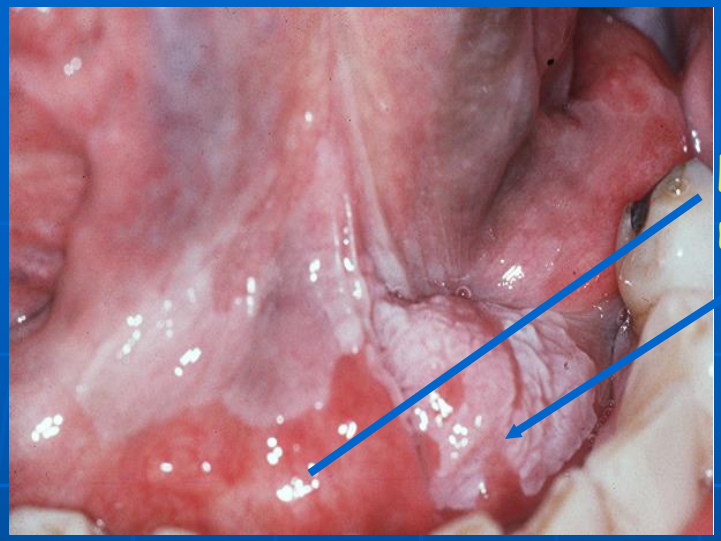
Figure 2: The same patient 6 months later after PDT. A complete response of the tumour.





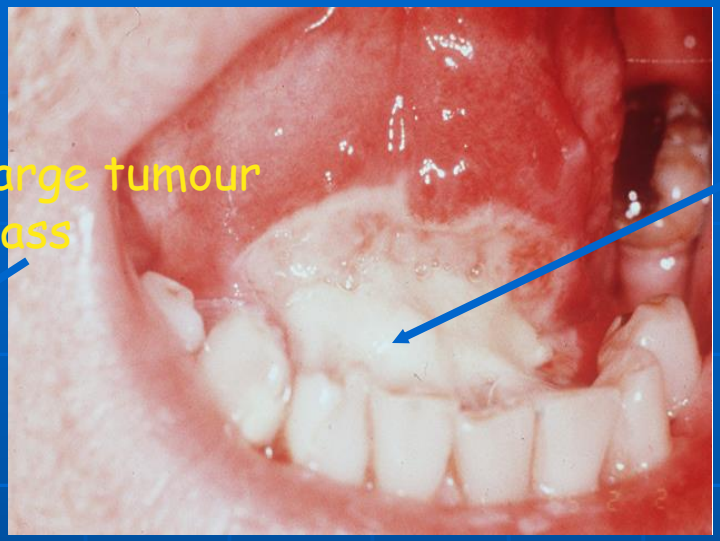


Current PDT: safe, non-scarring treatment



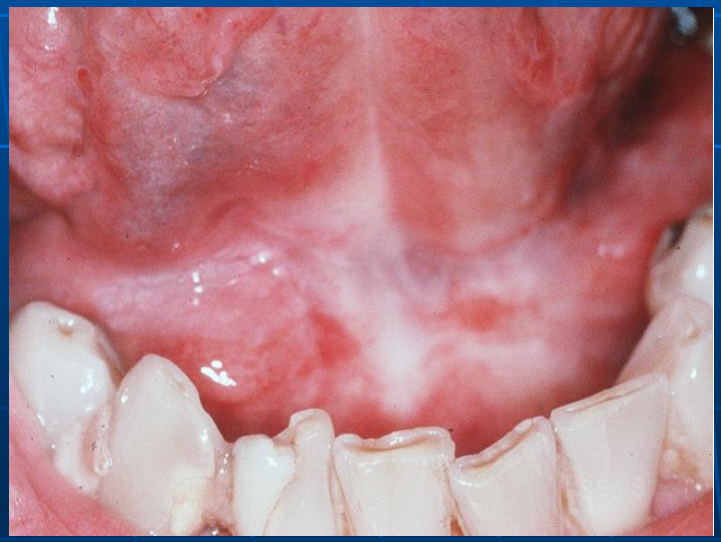
Large tumour mass

Before PDT

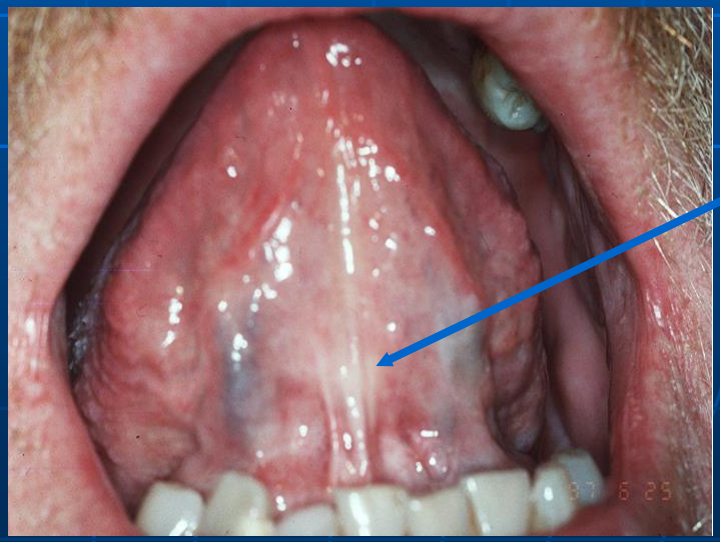


Treated/ necrotic area

1 week after



3 weeks after



Completely healed

6 months after

Pictures courtesy of Dr C.Hopper & Dr A. Lou. National Medical Laser Centre University College London

Photochemistry of sensitiser S for PDT

$S + h\nu \rightarrow {}^1S^*$	Absorption to give singlet state
${}^1S^* \rightarrow S + h\nu_F$	Fluorescence
${}^1S^* \rightarrow {}^3S^*$	Cross over to give long-lived triplet state
${}^3S^* + O_2 \rightarrow S + {}^1O_2{}^*$	Energy transfer to give singlet oxygen

${}^1O_2{}^* + \text{tissue} \rightarrow \text{necrosis}$

Approaches to achieving targeting

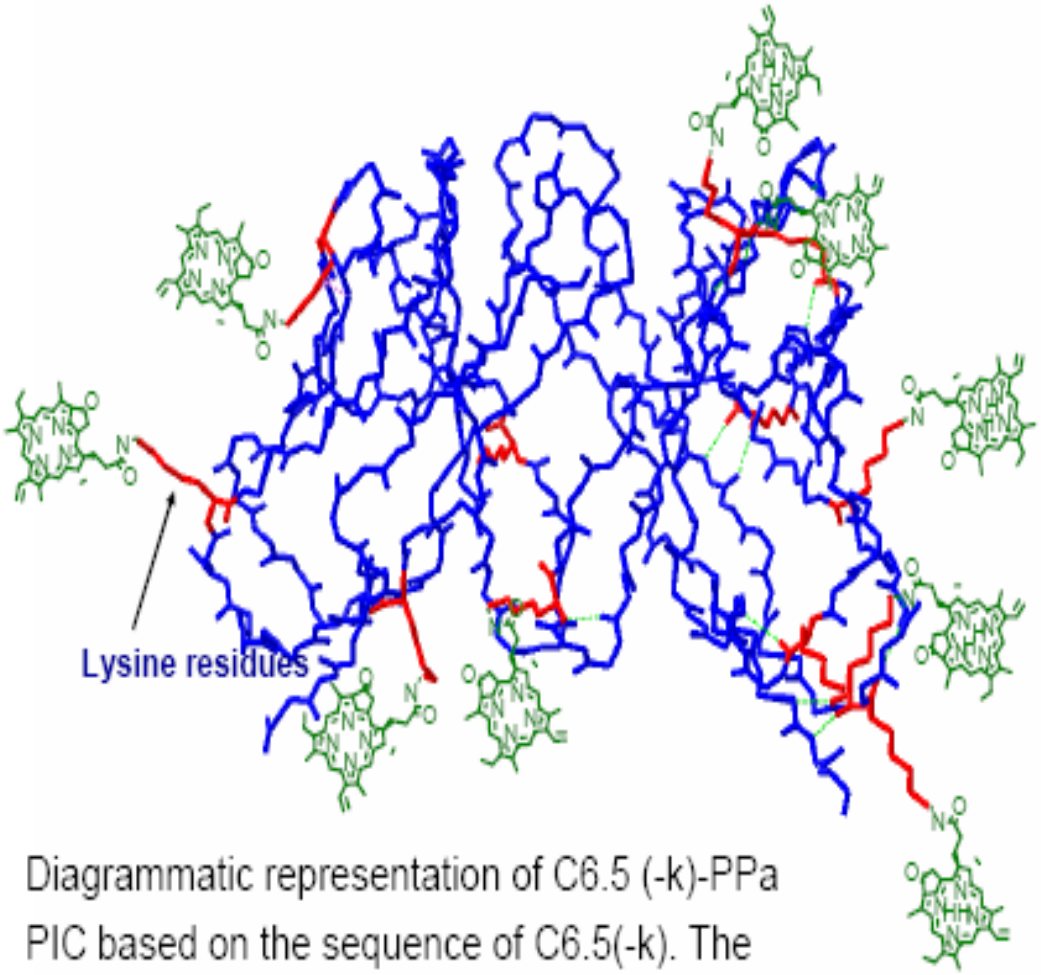
- Monoclonal Antibodies
- Monoclonal antibody fragments
- Spatial control through two-photon excitation
- Proteins, folic acid, nanoparticles

Monoclonal antibody targets and fragments

- HER2 is an 185kDa glycoprotein over-expressed in 25-30% of invasive breast tumours, and in many epithelial cancers
- MFE targets HER2
- MFE is a single-chain scFv derived by phage display
- 10 lysine residues

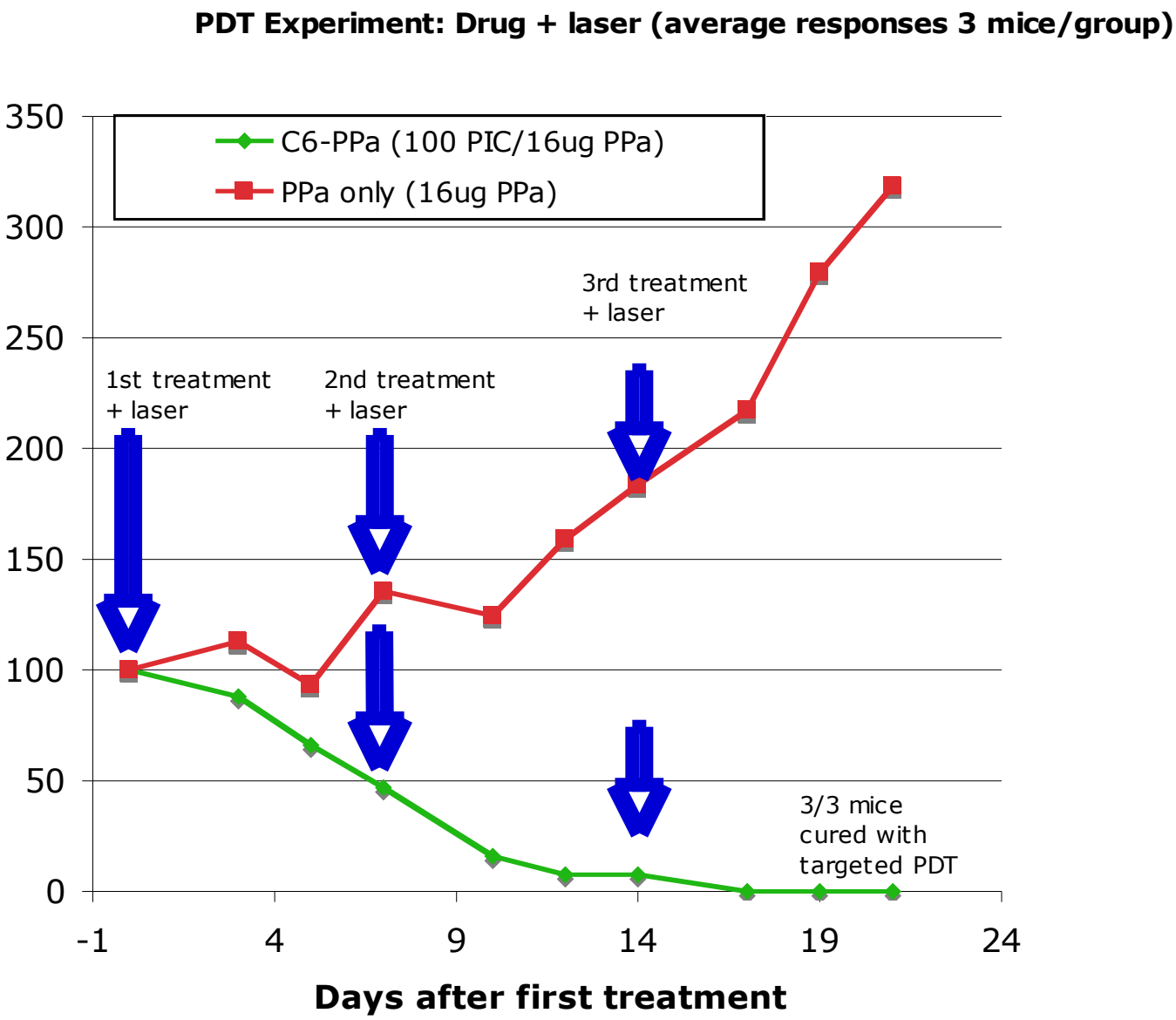
Monoclonal antibody fragments

- C6.5 scFv
- 13 lysines, but is engineered to remove lysine 100 in the antibody binding site to eliminate coupling of sensitiser at this site, and thus compromising immuno-reactivity
- Targets CEA+ and HER2



Diagrammatic representation of C6.5 (-k)-PPa PIC based on the sequence of C6.5(-k). The potential sites for conjugation and the spatial separation of the conjugated photosensitisers are shown.

Figure 4.1 Three-dimensional representation of the structure of C6.5(-k)-PP*a* PIC pointing out the spatial separation of the lysines. The sequence of the scFv was used to obtain the model on Swiss PDB. The lysines are shown in red and the PS depicted in green.


PDT Experiment: Drug + laser (average responses 3 mice/group)
C6-PPa (100 PIC/16ug PPa)
PPa only (16ug PPa)
1st treatment + laser
2nd treatment + laser
3rd treatment + laser
3/3 mice cured with targeted PDT
350
300
250
200
150
100
50
0
-1
4
9
14
19
24
Days after first treatment

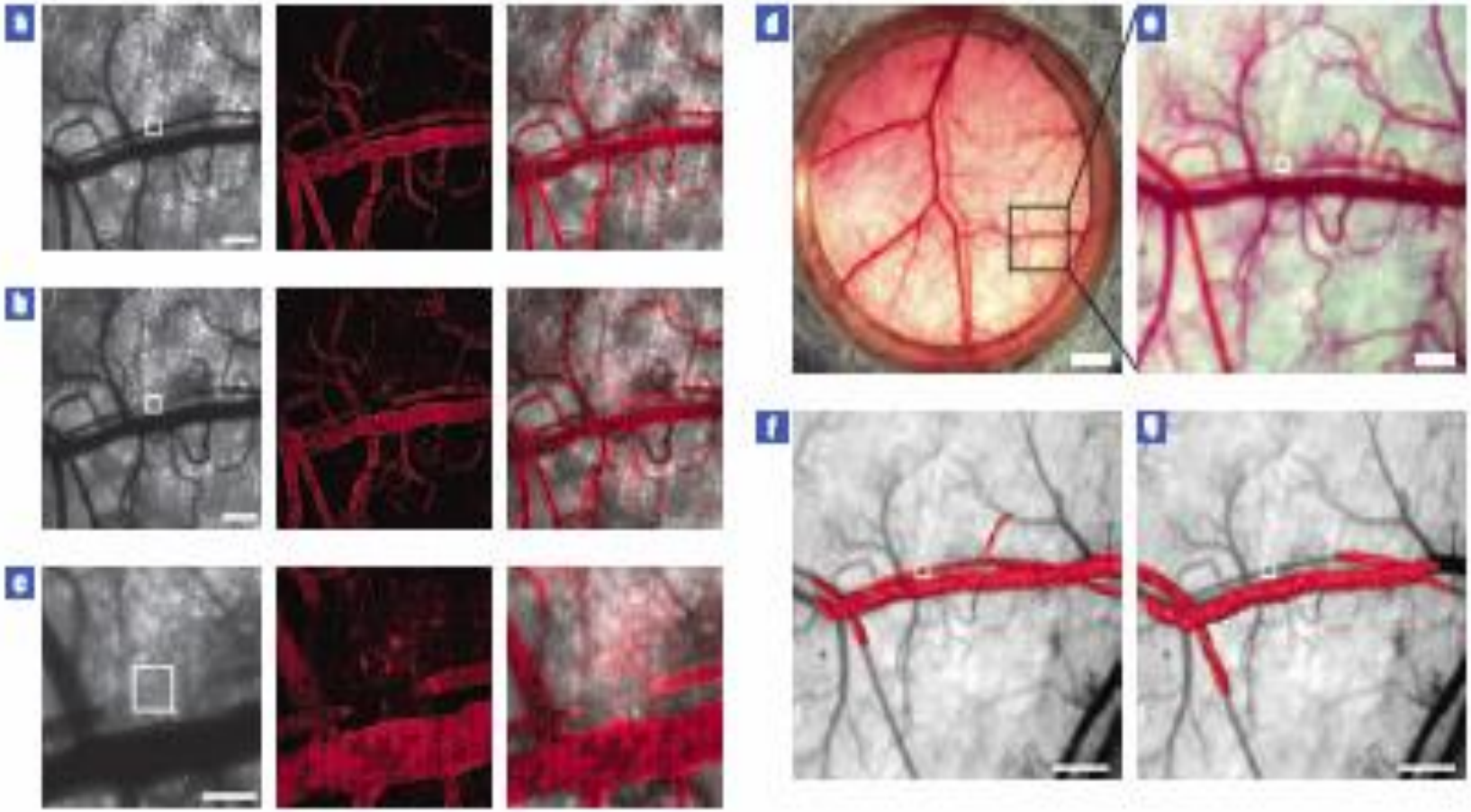
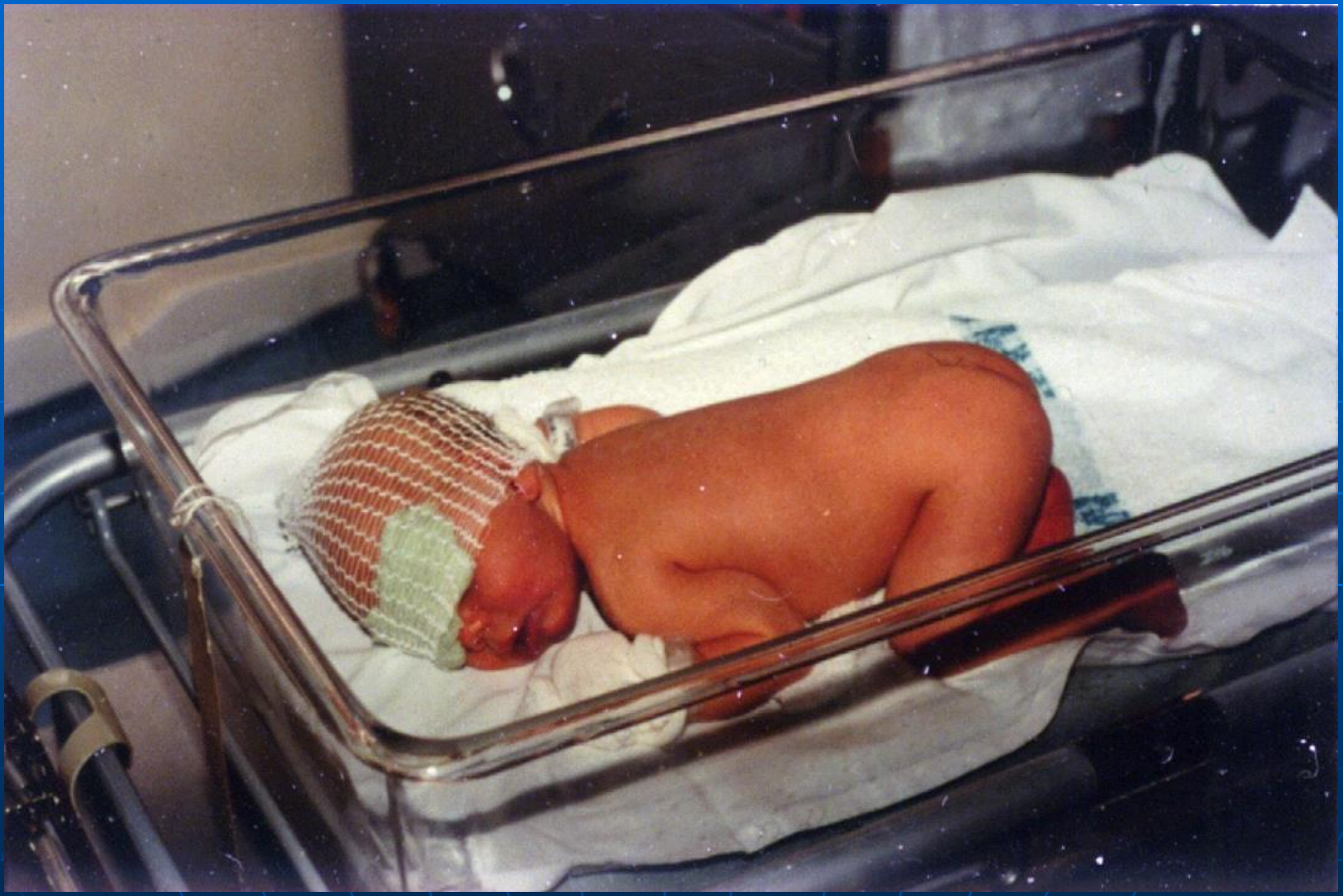
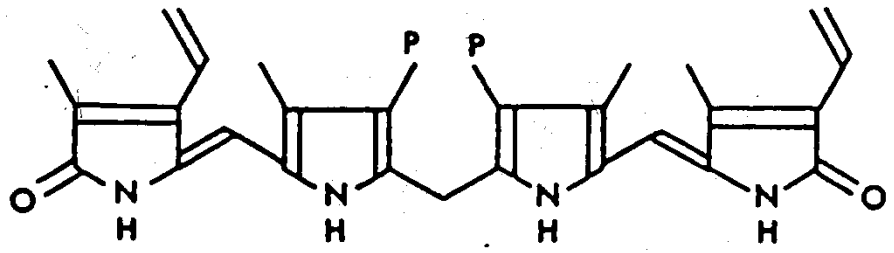
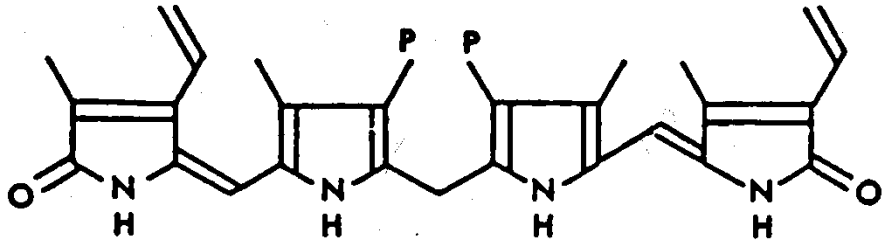


Figure 4 *In vivo* two-photon blood-vessel closure with photosensitizer 1. Mice bearing dorsal window chambers were administered 10 mg kg^{-1} of 1 diluted from a 10 mM stock in DMSO. A selected artery was targeted with 920-nm light (<3 mW) guided by the striations caused by blood flow visible in the transmission image (see Supplementary Information, Fig. S7). An 83 × 83 × 40 μm volume was irradiated four times (39 mW) as a stack of five planes, 10 μm apart, each plane consisting of 512 × 512 pixels, with a dwell time of 0.8 μs per pixel. a, Pretreatment confocal (×5, 0.25 NA) transmission (left panel, λ_{ex} = 543 nm), TRITC–dextran fluorescence (middle panel, λ_{ex} = 543 nm, λ_{em} = 565–615 nm) and superimposed (right panel) images (scale bar, 200 μm). b, Images immediately after TPE-PDT with 1 (scale bar, 200 μm). c, Expanded image of irradiated area (scale bar, 100 μm). d, Pretreatment stereomicroscope image of the entire dorsal window chamber (scale bar, 1.0 mm). e, Post-treatment image (scale bar, 200 μm). f,g, 3D-rendered images of blood flow produced by Doppler OCT imaging pretreatment (f) and post-treatment (g) (scale bar, 400 μm); the images are overlaid on the pretreatment stereomicroscope image. The blood flow in the targeted artery is from left to right. The white boxes indicate the irradiated region.

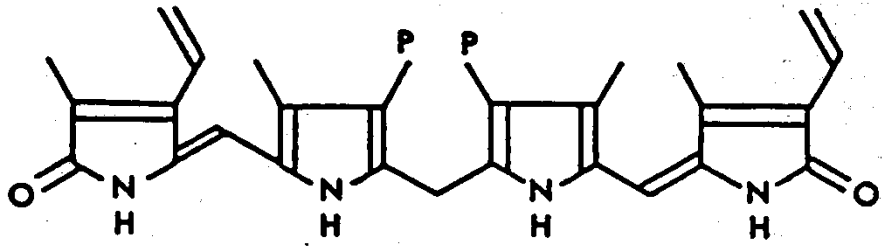




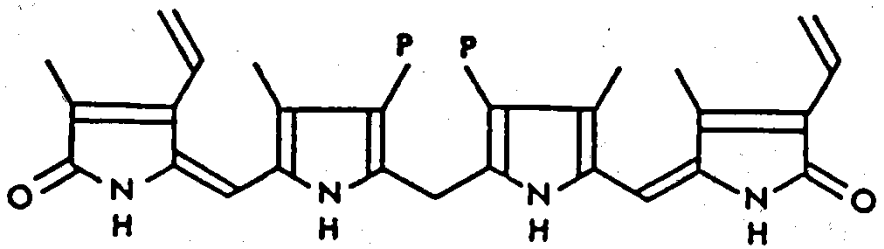
5*Z*, 15*Z*



5*E*, 15*Z*



5*Z*, 15*E*



5*E*, 15*E*