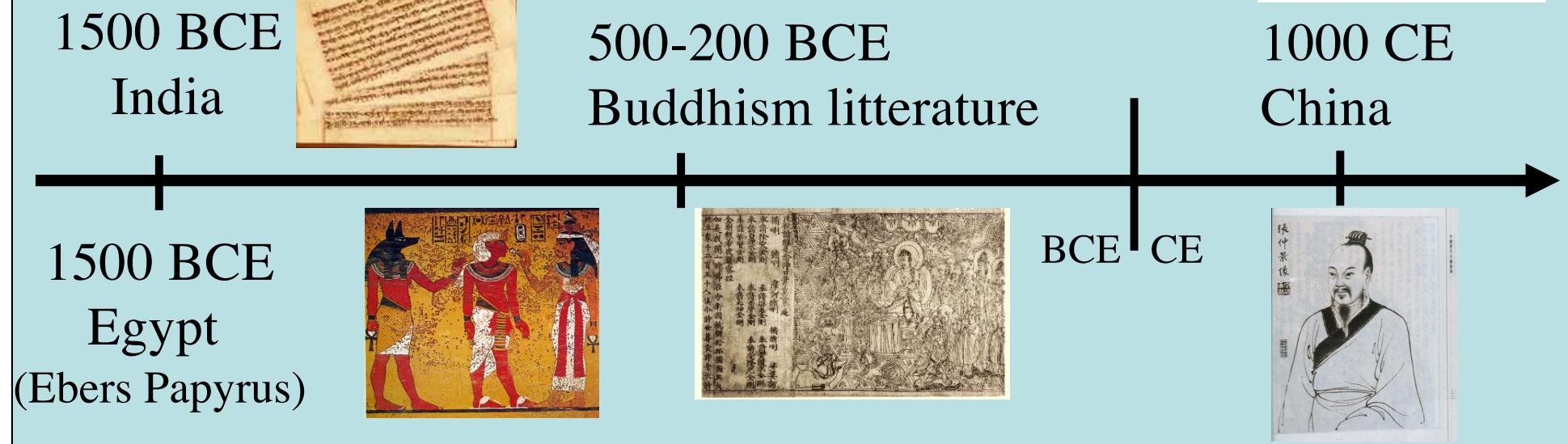


# 2. History of Photomedicine

## The antique

Man has recognized the relation between light and health for about 3000 years

Ancient medical litterature: treatment of de-pigmented skin areas (vitiligo) by application of plant seeds (psoralea) followed by the exposure to sunlight



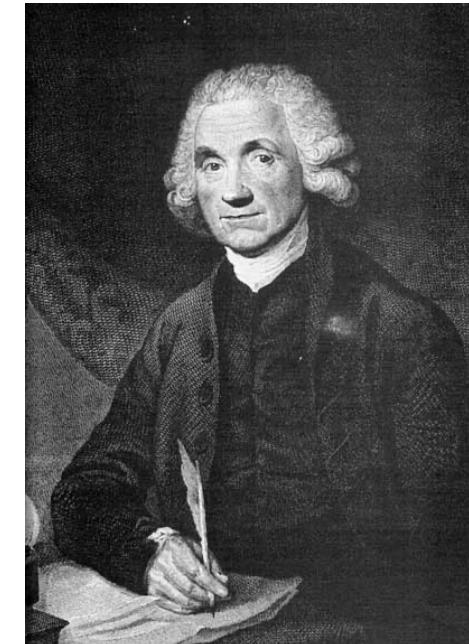
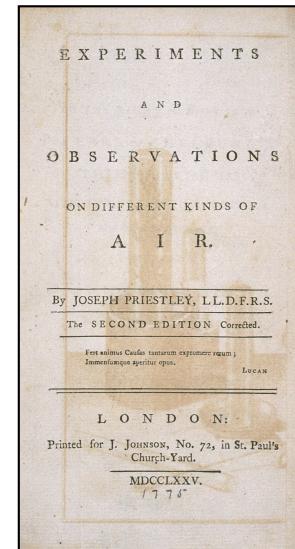
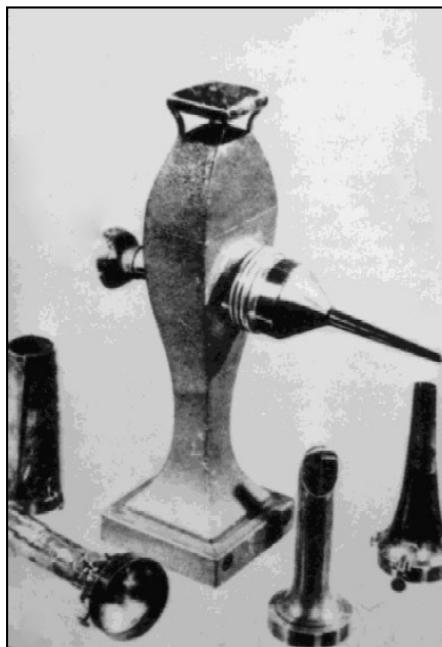
# Vitiligo



# Later and turn of the 18<sup>th</sup> century

1775

J. Priestley discovers oxygen



1806

Ph. Bozzini invents a device to "make visible the inner cavities of the body"  
=> first endoscope

# Early and middle of the 19<sup>th</sup> century

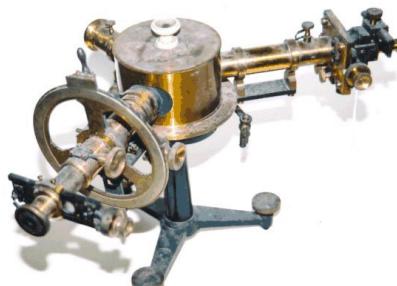
**1841**

Johann Joseph Scherer removes iron from blood with concentrated sulfuric acid => Hematoporphyrin



Pasteur

**1842** : Louis Pasteur and Paul Bert report phototoxicity in cells and germs  
Becquerel and Draper: spectral extent of UV radiation



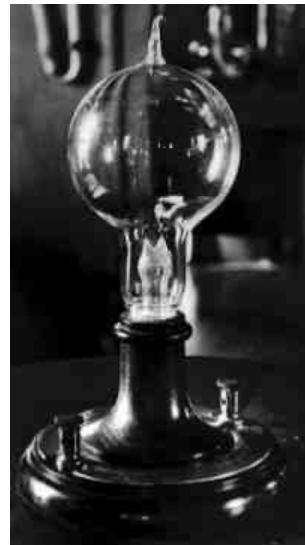
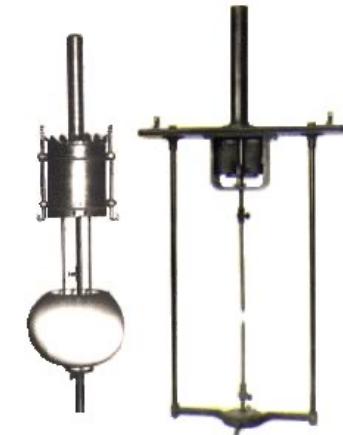
**1852** : George G. Stokes introduces the term "Fluorescence", interprets the light-emitting phenomenon, and formulates the "Stokes law"

**1859** : Kirchoff and Bunsen invent the spectroscope

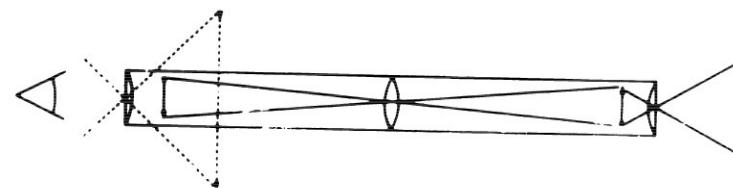
# Late 19<sup>th</sup> century

**1876/77**

Jablochkow and Brush invent the first useful carbon electrode arc lamp



**1877** : Nitze develops the first cystoscope based on a telescopic lens system

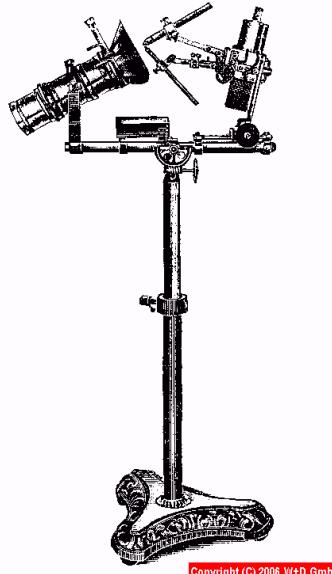


**1879** : Th. A. Edison invents the incandescent lamp

# The Beginning of modern Photomedicine

**1893-1903**

N.R. Finsen develops the phototherapy



(Finsen lamp)

**1893**

Treatment of smallpox (variole) with red light

**1895**

Treatment of lupus vulgaris (skin tuberculosis) with artificial UV light from the "Finsen lamp"



(Phototherapy for tuberculosis, Finsen Institute, ~1900)



## 2. History

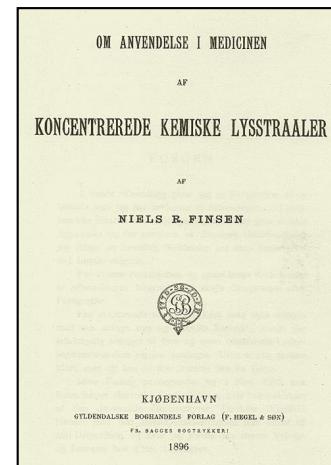
# Niels Rysberg Finsen

(\*15.12.1860, +24.9.1904)

## "Pioneer of Phototherapy"

**1893** Finsen discovered that UV light aggravated smallpox (la variole), but that red light improved healing

**1895** treatment of lupus vulgaris (dermal tuberculosis) first with sunlight and then with artificial UV light (Finsen light)



**1903** Nobel Prize *"in recognition of his contribution to the treatment of diseases, especially lupus vulgaris, with concentrated light radiation, whereby he has opened a new avenue for medical science"*

# Paul Ehrlich (\*14.3.1854, + 20.8.1915)



Paul Ehrlich "Father" of chemotherapy and immune targeting

**1897** Idea of cell-targeting therapy:



*"If we picture an organism as infected by a certain species of bacterium, it will...be easy to effect a cure if substance have been discovered which have a specific affinity for these bacteria and act...on these alone...while they possess no affinity for the normal constituents of the body...such substances would then be...magic bullets"*

**1908** Nobel Prize in Physiology and Medicine for his scientific work in immunology

# Discovery of Photosensitization

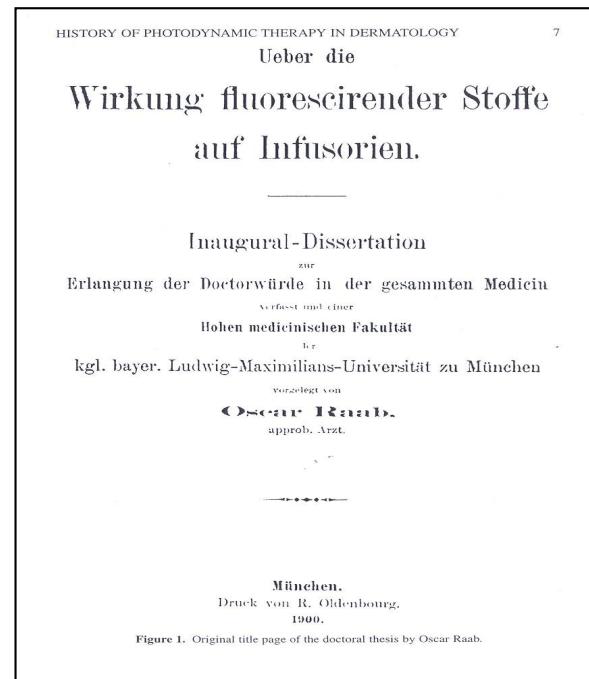
1900

J. Prime (french neurologist) used eosin orally in the treatment of epilepsy and showed that this induced dermatitis in sun-exposed areas of skin

[Prime J. (1900) "Les accidents toxiques par l'eosinate de sodium." Jouve and Boyer, Paris]

1900

Oscar Raab discovers the biological photosensitisation using chemical substance followed by exposure to light (acridine red on bacteria)

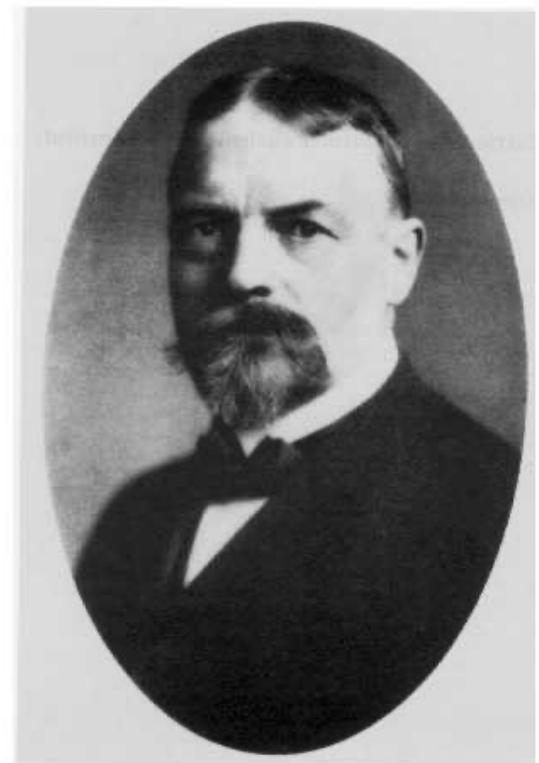


# Discovery of Photosensitization, cont.

**1901** Cooper-Hewitt produce the first commercially available mercury vapor lamp

**1903** H. von Tappeiner and A. Jesionek treat skin cancers with eosin dye followed by exposure of the lesions to sunlight

**1904** H. von Tappeiner introduces the term "photodynamic action" and conclude that the presence of oxygen is a necessary requirement to obtain photosensitization



[H. von Tappeiner, A. Jodlbauer, "Über Wirkung der photodynamischen (fluoreszierenden) Stoffe auf Protozoen und Enzyme", Dtsch Arch Klin Med 80, 427 (1904)]

## Early 20<sup>th</sup> century

1906

Coolidge invents the Tungsten lamp

1911/1913

Otto Heimstaedt and Heinrich Lehmann  
develop the first fluorescence microscope

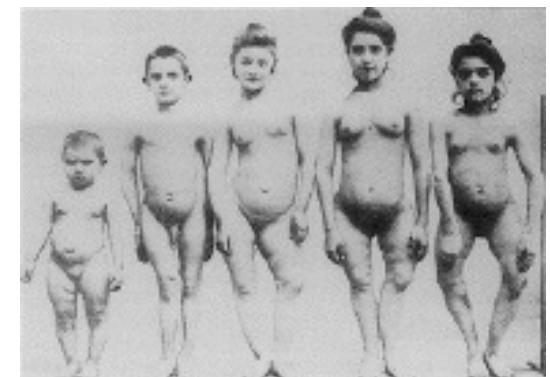
# Rickets (Rachitisme)

**1919** K. Huldschinsky first cures children of rickets using artificially-produced UV light following the observations of T. Palm (1848–1928)



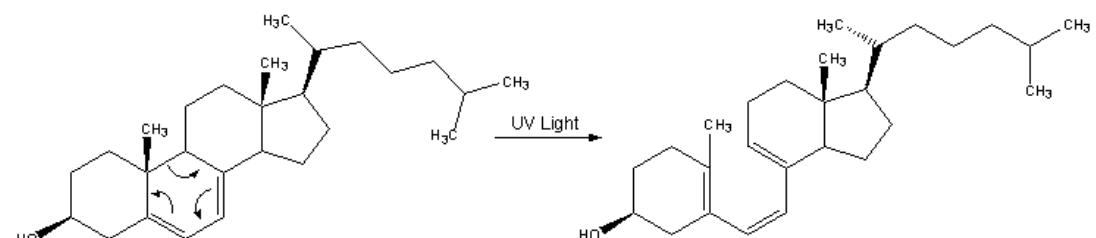
Leg deformation  
due to rickets

Rickets (osteomalacia): a frequent childhood disease in developing countries  
Origin: vitamin D (calcium) deficiency  
Symptoms: bone pain and tenderness; skeletal deformity (bowed legs, cranial, spinal, and pelvic deformities)



A family with rickets, France ~1900

Vitamin D can be synthetised in human skin under UV light exposure by photolysis



# Hans Fischer

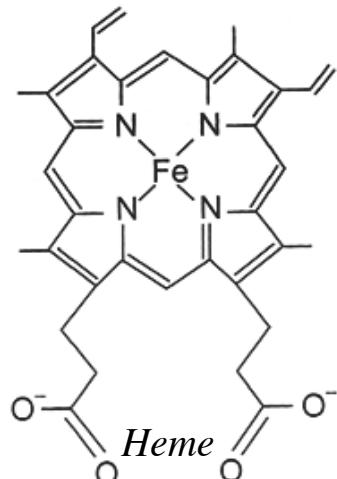
(\*27.7.1881, +31.5.1945)



German Chemist and MD

**1925** studies the structure and constituents of porphyrins

**1926** first synthesis of porphyrins



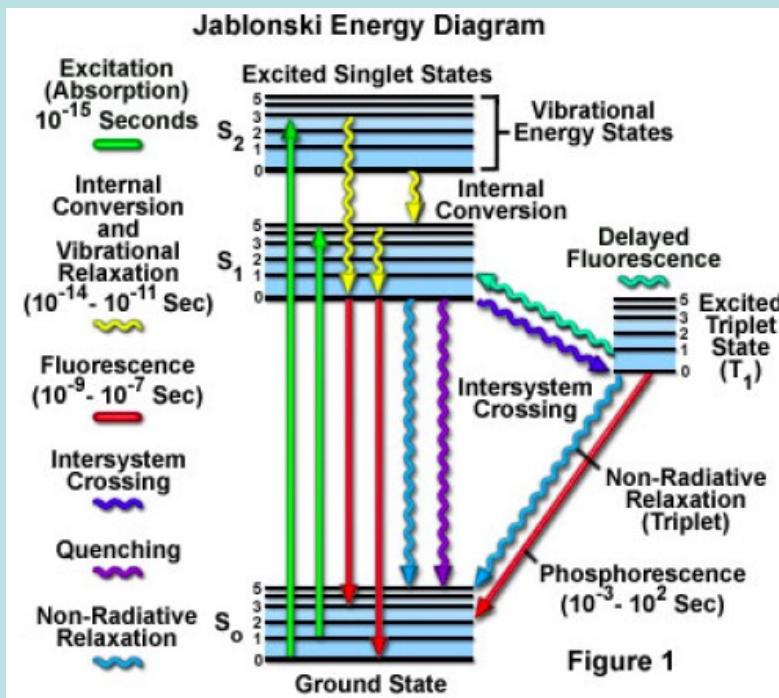
**1928** first synthesis of haemin

**1930** Nobel Prize "for his researches into the constitution of haemin and chlorophyll and especially for his synthesis of haemin".

# Fondamental and Medtech progresses

1935

Alexander Jablonski establishes his Jablonski diagram



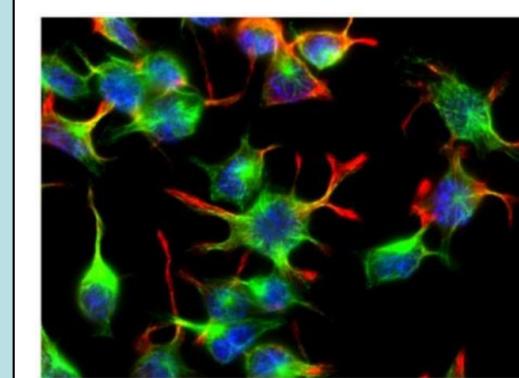
1936

Schindler and Wolf introduce a semi-flexible gastroscope

# Middle of the 20<sup>th</sup> century

1950

A. Hewett Coons and Melvin Kaplan develop the immuno-fluorescence technique in biology



Immunofluorescence:  
*Rat basophilic leukemia cells*

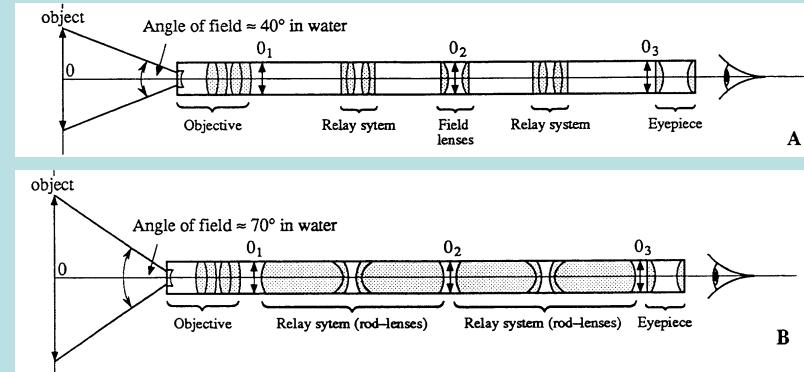
1952

Forestier uses a quartz rod inserted into a steel tube placed inside a rigid endoscope to guide light to the endoscopic site => external light source)

# Progresses in endoscopic optics

1954

H.H. Hopkins develop an improved optical system based on glass rods interspaced by air lenses, instead of standard lenses (Hopkins optics)



1954

A. van Heel and H.H. Hopkins: imaging fiber bundles

1954

A. van Heel invents the cladding of fiber-optical cables

# Endoscopy and Neonatal Jaundice

1957



Neonatal jaundice: benefit of blue light exposure on neonatal jaundice is discovered by accident

1959

Brian O'Brien and Narinder Kapany: fibroscope

# Neonatal Jaundice (Ictère "jaunisse")

Common condition in newborn (about 50%)

## Origin:

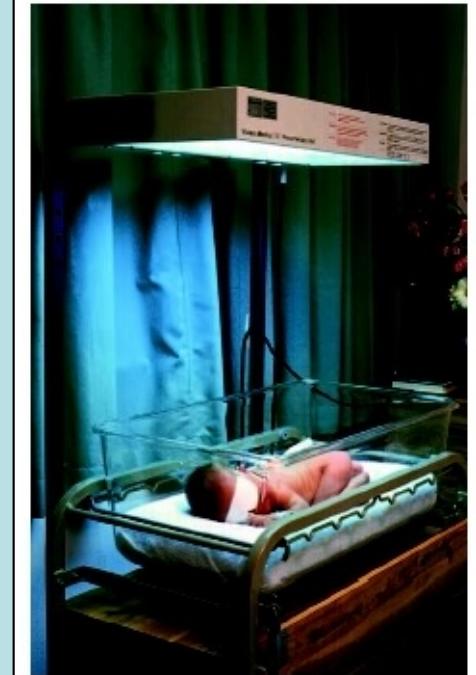
1. breakdown of fetal hemoglobin  
(replaced with adult hemoglobin)
2. immature hepatic metabolic pathways  
⇒ accumulation of unconjugated bilirubin  
⇒ neurotoxicity

## Symptoms:

yellow colour of skin and sclera

## Phototherapy:

photoisomerization and photooxidation of unconjugated bilirubin to its watersoluble, i.e. excretable, conjugated form



# The first lasers

**1960**

Theodore H. Maiman: first working ruby laser (694 nm, pulsed operation)

**1961**

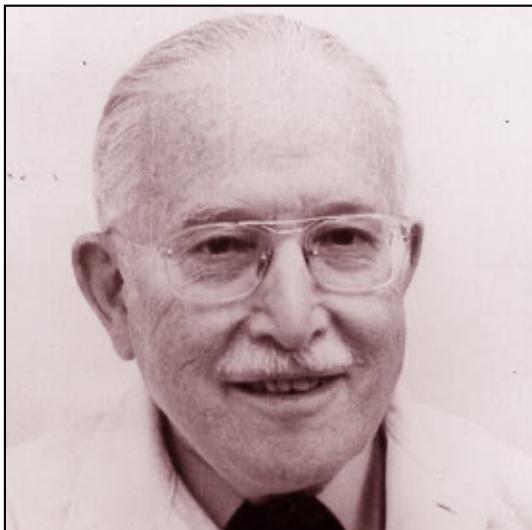
A. Javan: first HeNe laser



# Tissue imaging and lasers in medicine

**1961**

Novotny and Alvis: retina angiography with fluorescein



**1961**

L. Goldman, MD "Father of laser surgery" recognises the potential for lasers in medical applications

*"If you don't need a laser – don't use it."*

**1962**

first black and white television camera for endoscopy



# Major "Medtech" breakthrough

**1965**

first surgical CO<sub>2</sub> laser

**1968**

S. Ikeda: flexible bronchoscope



**1969**

W. Boyle and G. Smith invent the CCD (Camera)

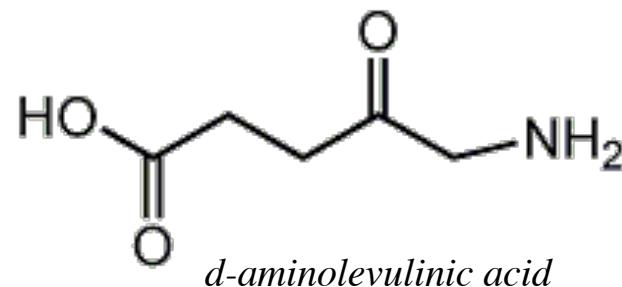
# "Medtech" and Pharma progresses

1985

First videoendoscopy system with CCD detectors at the distal tip are investigated for clinical use

1987

Malik and Lugaci use d-aminolevulinic acid (ALA) as agent for photosensitisation and cancer photodetection



# "Medtech" and Pharma progresses

**1990**

633 nm diode laser commercialised

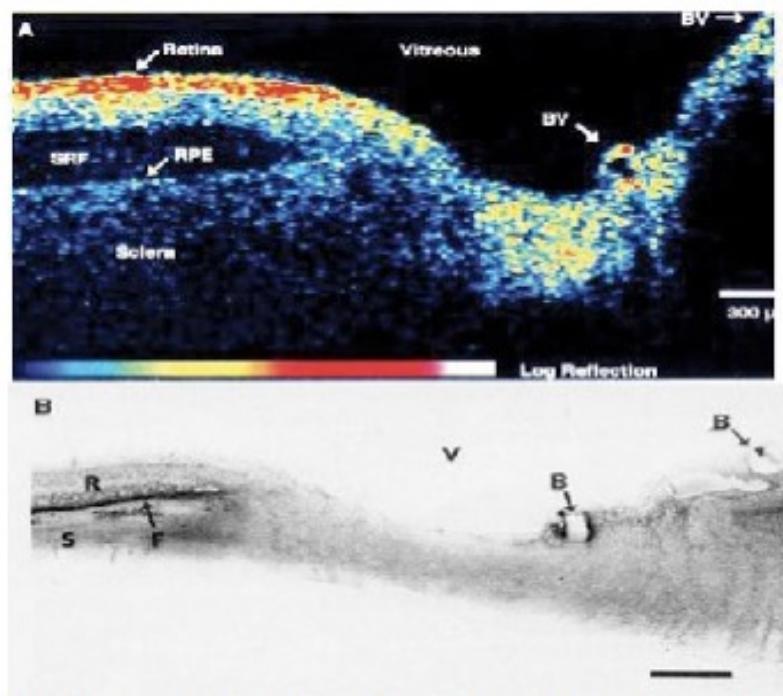
**1990/92**

Kennedy: first clinical use of topical aminolevulinic acid (ALA) for fluorescence detection and Photodynamic Therapy (PDT)

# "Medtech" progress

1990

First Optical Coherent Tomography (OCT) image



**Figure 1.** The first OCT image of the retina *ex vivo* and corresponding histology. Note the detachment of the retina, common in post-mortem eyes.

# Actinic Keratosis and basocellular carcinoma treated by PDT

2000

Levulan®  
(ALA) topically



2001

Metvix®  
(methyl-ester ALA)  
topically

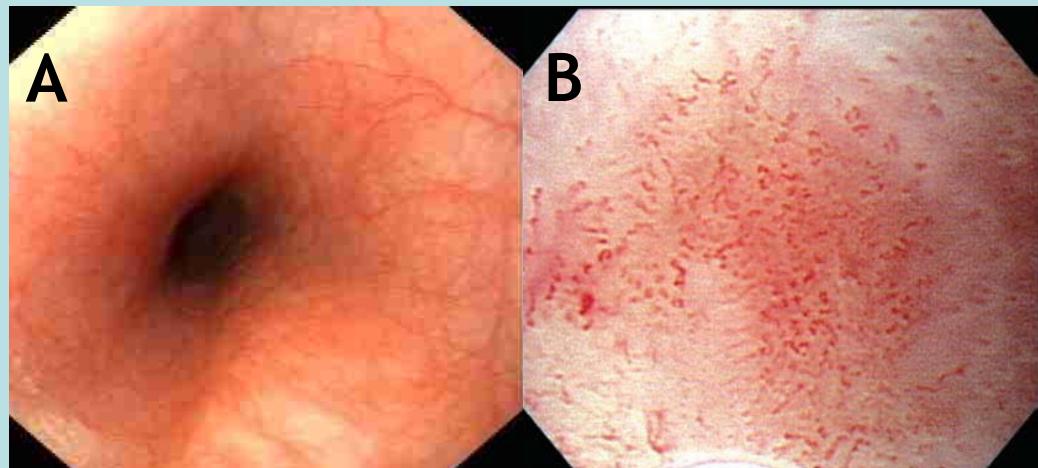


## Other present/future fields include: **High magnification endoscopy (gastroenterology)**

### - Observation of the intrapapillary loops (IPCL)

- dilatation
- weaving
- changes in caliber
- differences in shape

XEC300 & XE120, Olympus Corp., Tokyo, Japan



A) Normal view of the esophagus.

B) Magnification view of carcinoma in situ (m1; 100X).

Kumagai et al. (2006) Digestive Endoscopy 18, 165-172

## Other present/future fields include: Narrow band imaging (NBI)

**TABLE 1. Narrow band filters and display of 2-band versus 3-band NBI systems**

<b>NBI systems</b>	<b>NBI filters (center wavelength) (bandwidth)</b>	<b>Video channels used for image display</b>
2-Band RGB sequential and color CCD NBI	415 nm (30 nm)	Blue and green
	540 nm (20 nm)	Red
3-Band RGB sequential NBI*	415 nm (30 nm)	Blue
	445 nm (30 nm)	Green
	500 nm (30 nm)	Red

\*Prototype.

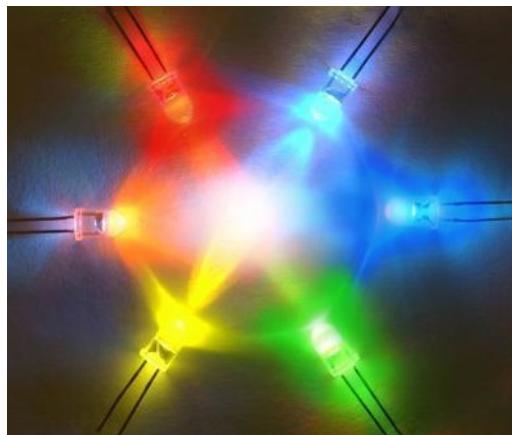
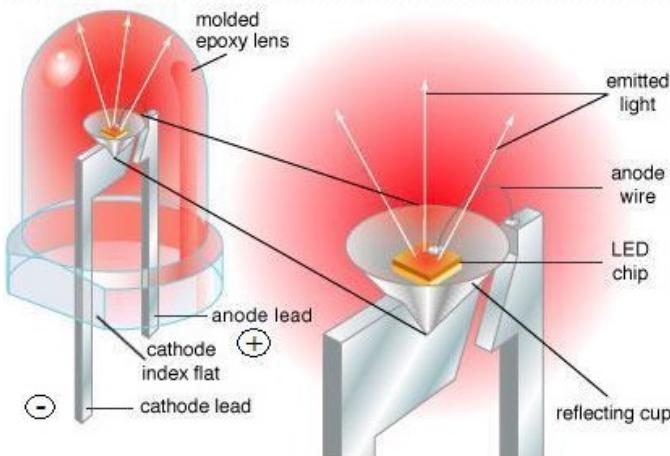
## Other present/future fields include: Narrow band imaging (NBI)

**Figure 1.** Magnified (A) white-light versus corresponding (B) NBI images of nondysplastic Barrett's mucosal vascular pattern. Under NBI, capillaries appear brown, whereas deeper vessels appear cyan.

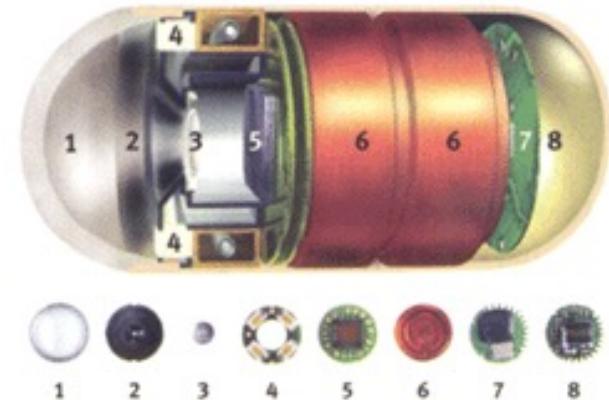


Wong Kee Song et al., GASTROINTESTINAL ENDOSCOPY, Volume 67, No. 4 : 2008

## Other present/future fields include: Light Emitting Diodes (LEDs)

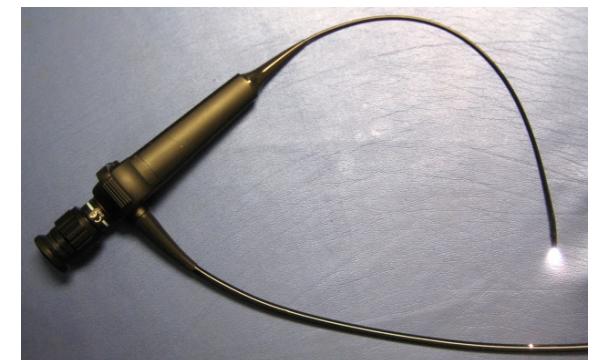


- ✓ Small
- ✓ Powerful
- ✓ High efficiency
- ✓ Cheap
- ✓ Robust
- ✓ Long lifetime
- ✓ Different wavelengths
- ✓ Relatively bright

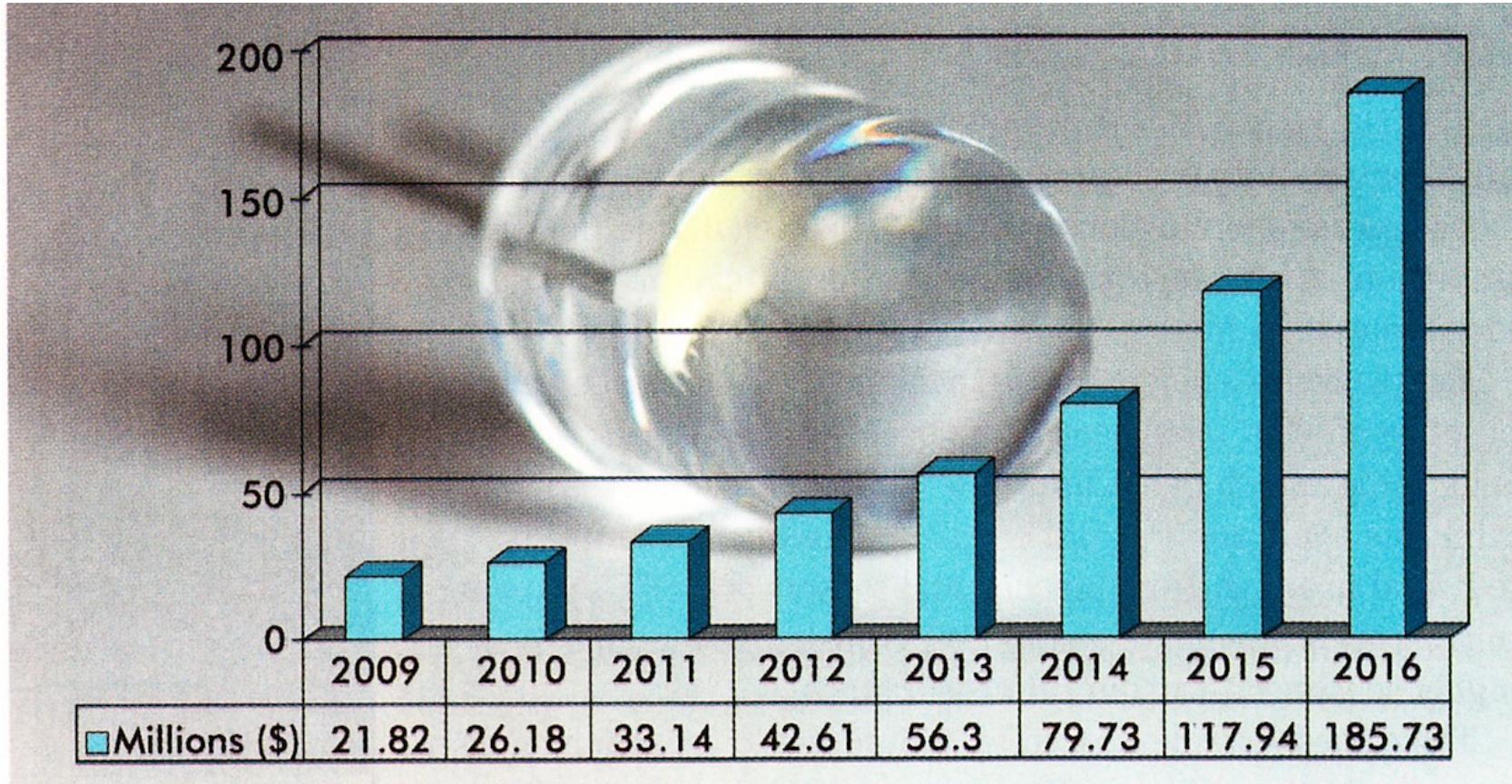


Inside the PillCam

1. Optical dome
2. Lens holder
3. Lens
4. Illuminating LEDs (light-emitting diodes)
5. CMOS (complementary metal oxide semiconductor) imager
6. Battery
7. ASIC (application-specific integrated circuit) transmitter
8. Antenna



## Other present/future fields include: Light Emitting Diodes (LEDs)



The global consumption value of LEDs in test/measurement and medical/science devices is expected to rise to \$185.73 million in 2016, with an average annual growth rate of 35.8 percent over the period from 2009. Courtesy of ElectroniCast Consultants.

# Partial list of some of the biomedical applications for laser diodes

Applications	Type	Wavelength (nm)																	
		405	450	635	652	670	689	752	766	794	810	915	940	980	1064	1210	1320	1470	1550
Aesthetics	Acne Treatment		X															X	
	Hair and Wrinkle Remover								X		X	X				X	X		
	Laser Skin Resurfacing										X	X							
	Lipolysis															X	X		
	Pigmented Lesions										X		X	X					
	Tooth Whitening										X			X					
	Varicose Vein Removal												X						
Diagnostics	MRI									X									
Photodynamic Therapy	Aged-Related Macular Degeneration							X	X										
	Cancer Treatment			X	X	X													
	Wound Healing	X												X					
Surgical Treatment	Dental Treatment														X				
	Endovenous Treatment												X						
	General Surgery													X	X		X	X	X
	Microsurgery												X	X					
	Urology												X			X	X		

..... to be continued ..... !

- Nanoparticles-based delivery of photosensitizers
- Light-induced photo-immunomodulation
- Photobiomodulation (LLLT)
- 2  $\text{hv}$  tissue characterization
- ...

The availability of lasers, sensitive detectors and/or advanced signal acquisition/processing/analysis techniques are at the origin of numerous approaches used in **microscopy** !

- Fluorescence Correlation Spectroscopy (**FCS**)
- Fluorescence Lifetime Imaging (**FLIM**)
- Fluorescence Recovery After Photobleaching (**FRAP**)
- Light sheet fluorescence microscopy (**LSFM**)
- Confocal microscopy (**CFM**)
- ...

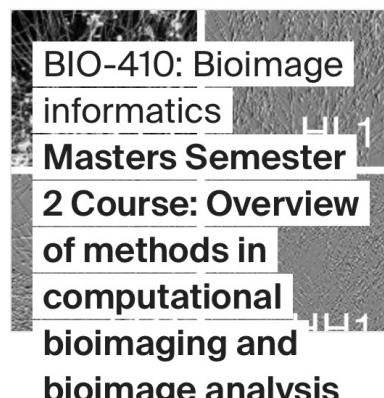
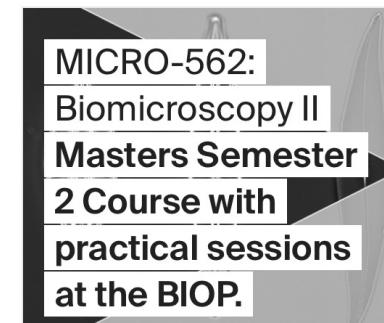
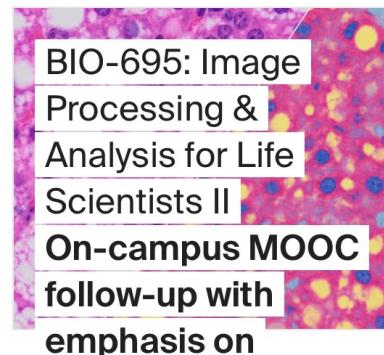
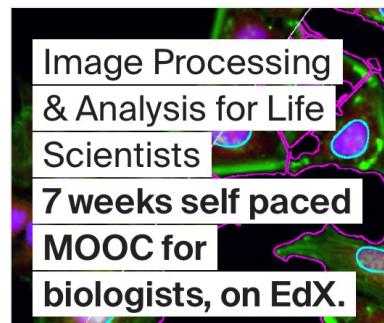
The availability of lasers, sensitive detectors and/or advanced signal acquisition/processing/analysis techniques are at the origin of numerous approaches used in **microscopy** ! (Cont.)

Super-resolution techniques have gained momentum over the last few years, pushing the limits of light microscopy beyond the classical diffraction limit.

- Structured Illumination (**SIM**)
- Stimulated Emission Depletion (**STED**)
- Stochastic Optical Reconstruction (**STORM**)
- ....

The BioImaging and Optics Core Facility (BIOP-EPFL) is active through multiple teaching programs.

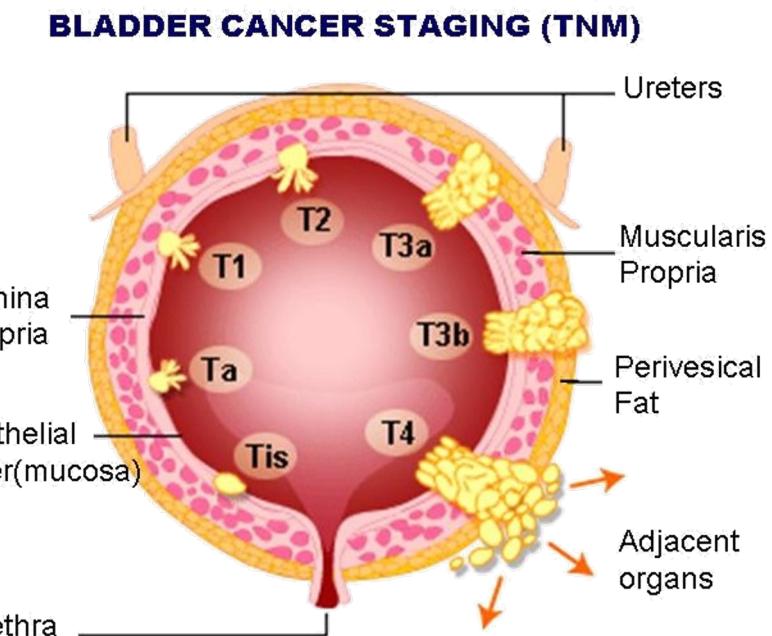
Therefore, these microscopy approaches will not be addressed in this course!



## An interesting case: (A diagnostic application of photomedicine)

- *Newly diagnosed patients, annually:  
Europe (127 000), US (67 000),  
Rest of the world (150 000)*
- *4<sup>th</sup> leading cause of cancer in men,  
8<sup>th</sup> in women*
  - *High risks associated with the presence  
of carcinoma in situ (CIS, Tis), clearly  
identified as a bad prognosis factor of  
the disease evolution*
  - *frequently superficial, flat and difficult  
to detect during standard white light  
cystoscopy.*

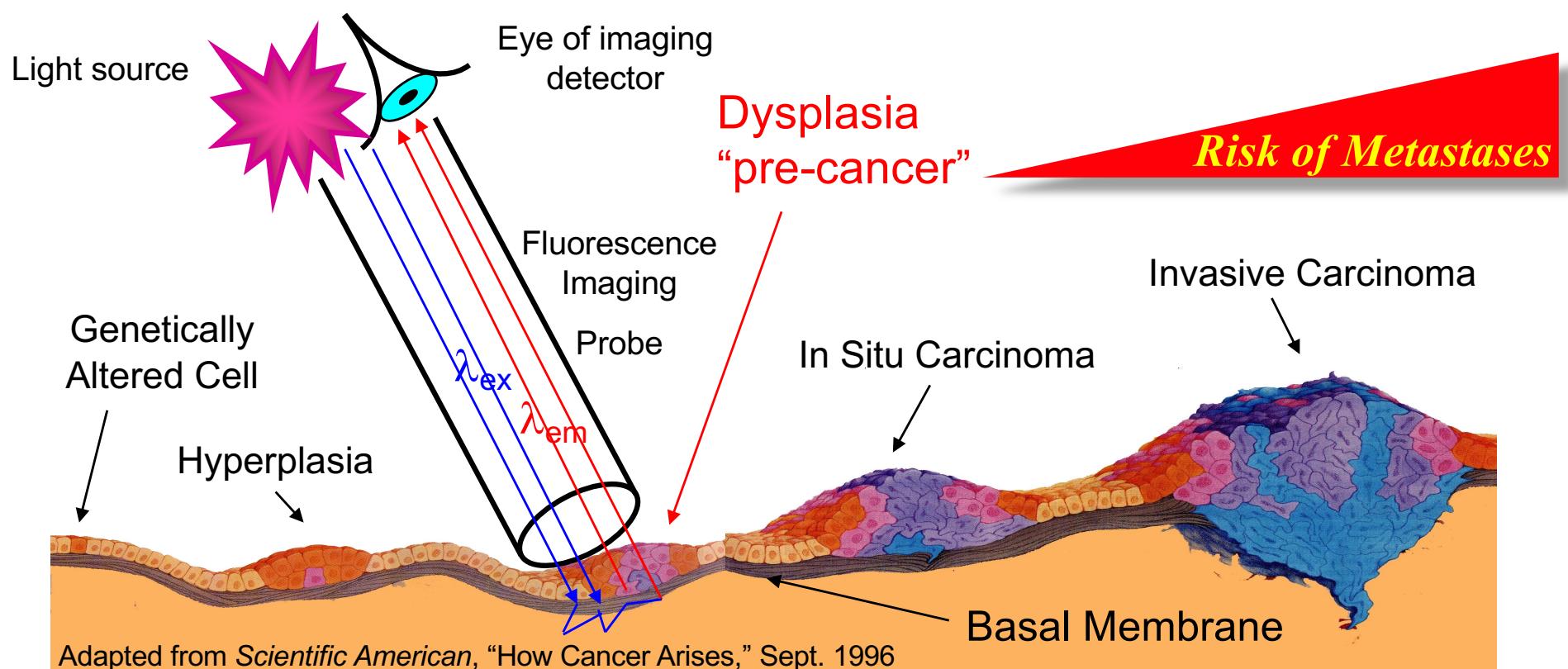
*The detection of early bladder cancer  
by fluorescence cystoscopy with Hexvix®*  
First studies: early 90's  
First approval: 1999



Tis, Ta, T1: superficial, non-invasive  
T2, T3, T4: invasive

➤ ***A sensitive and specific detection method is needed !***

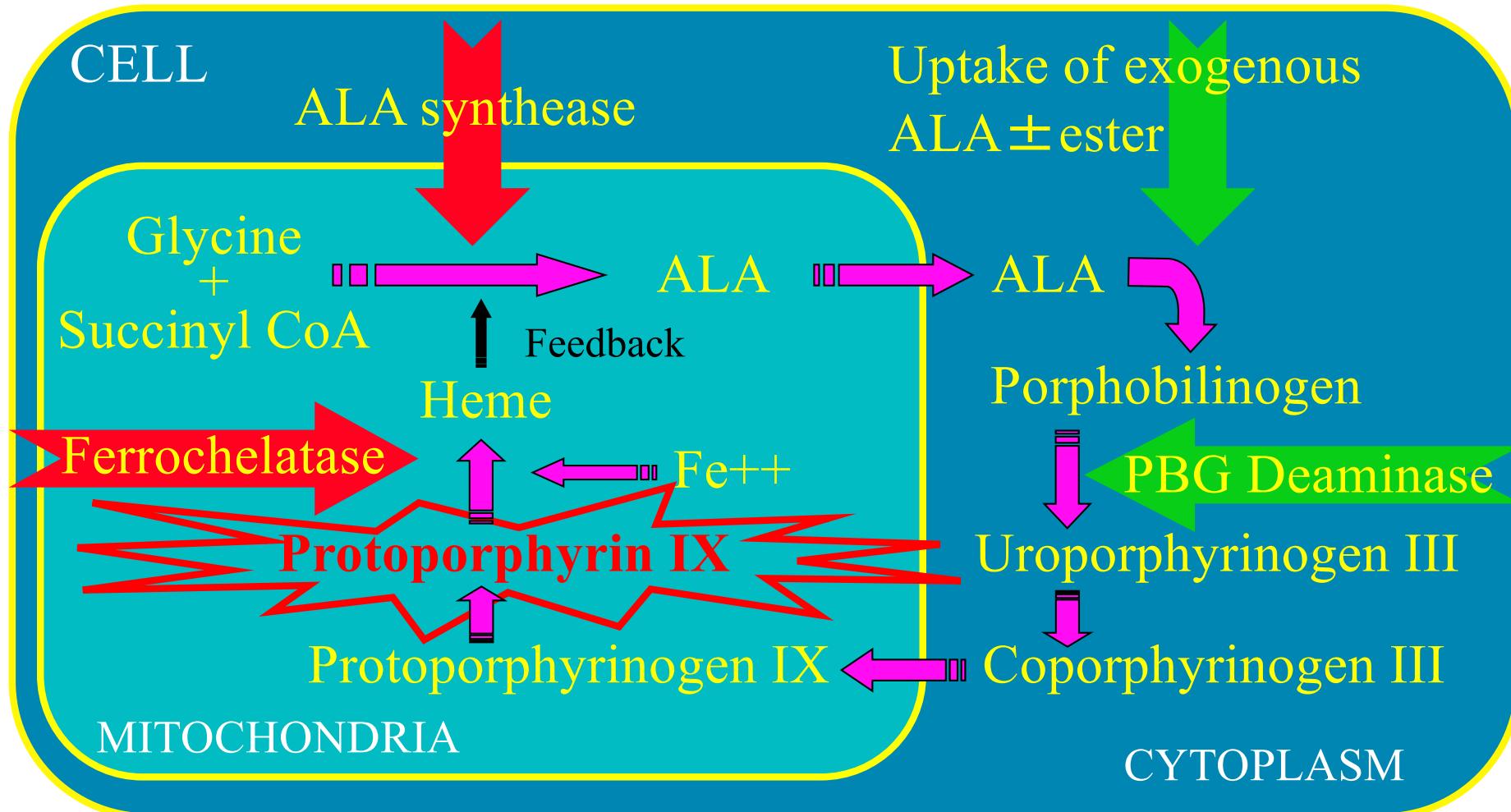
# Localization of early carcinoma by fluorescence imaging: Principle



Stages detectable by conventional methods

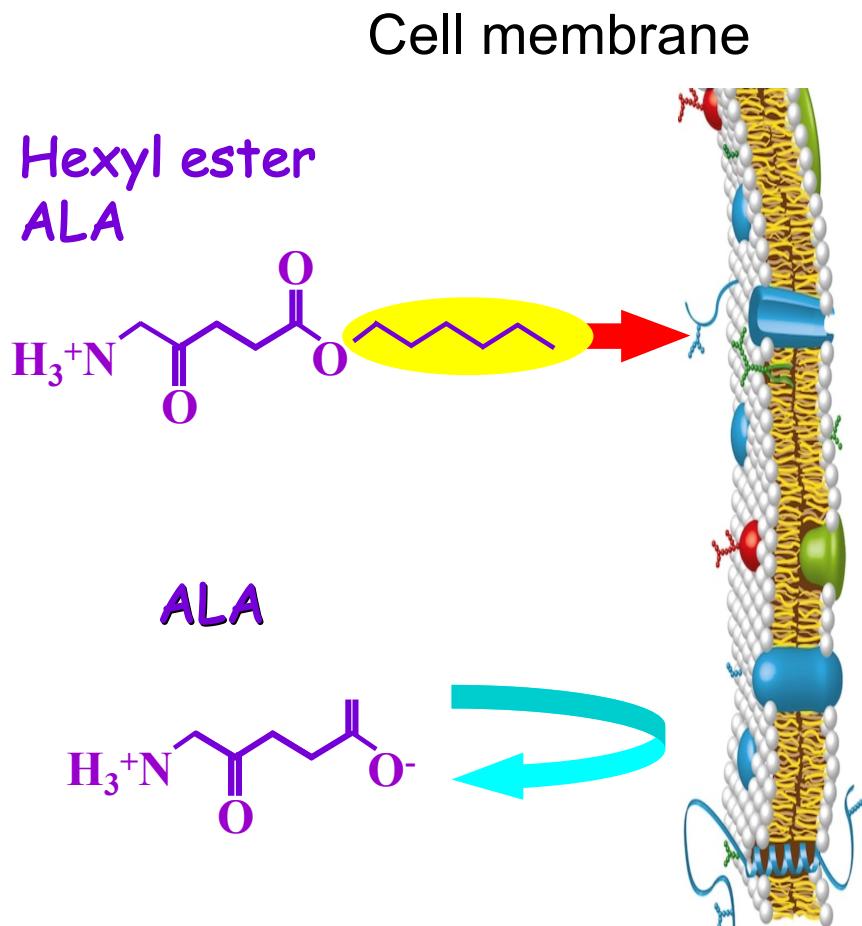
Fluorescence imaging

# Aminolevulinic acid (ALA)-induced production of protoporphyrin IX in the biosynthetic pathway of heme

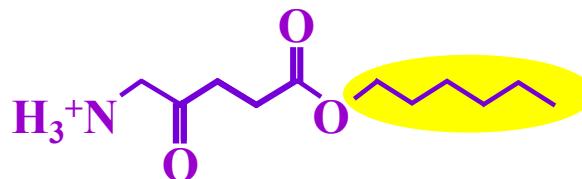


**Enhanced in certain cancerous cells**  
**Reduced in certain cancerous cells**

# Advantage of Esters of Aminolevulinic Acid



More lipophilic,  
improved membrane permeability  
Selective for certain cancer cells



Hexvix® produced by Photocure  
(Cysview® in the USA)

Too hydrophilic,  
weak membrane permeability

Lange et al., BJC, 1999

Uehlinger et al., JP&P, 2000

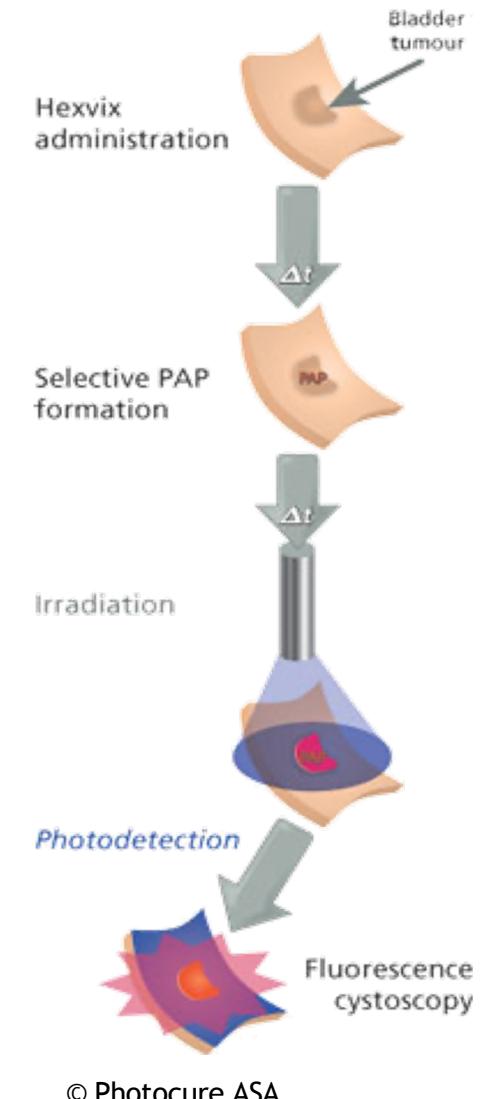
Uehlinger et al., P&P, 2006

## Fluorescence cystoscopy using Hexvix®

### Principles:

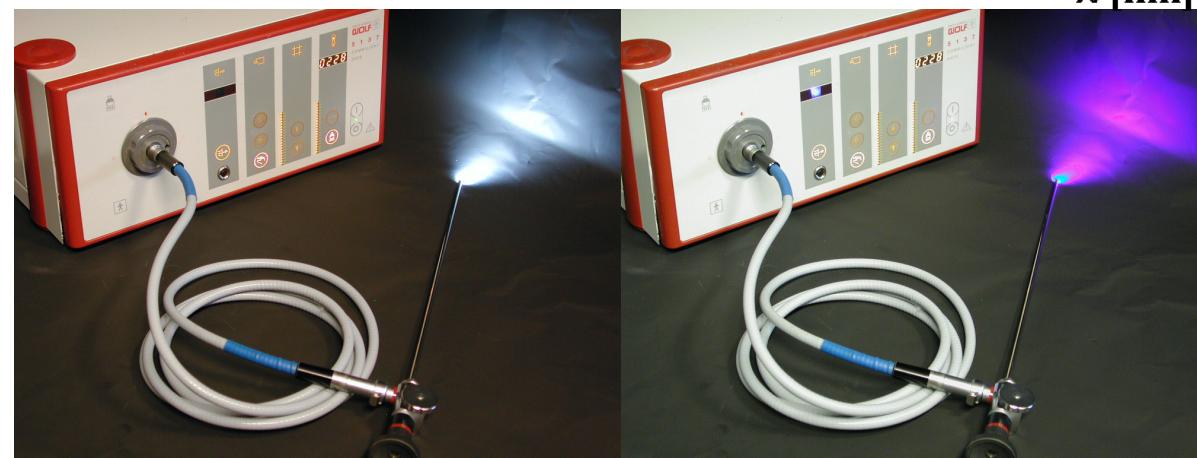
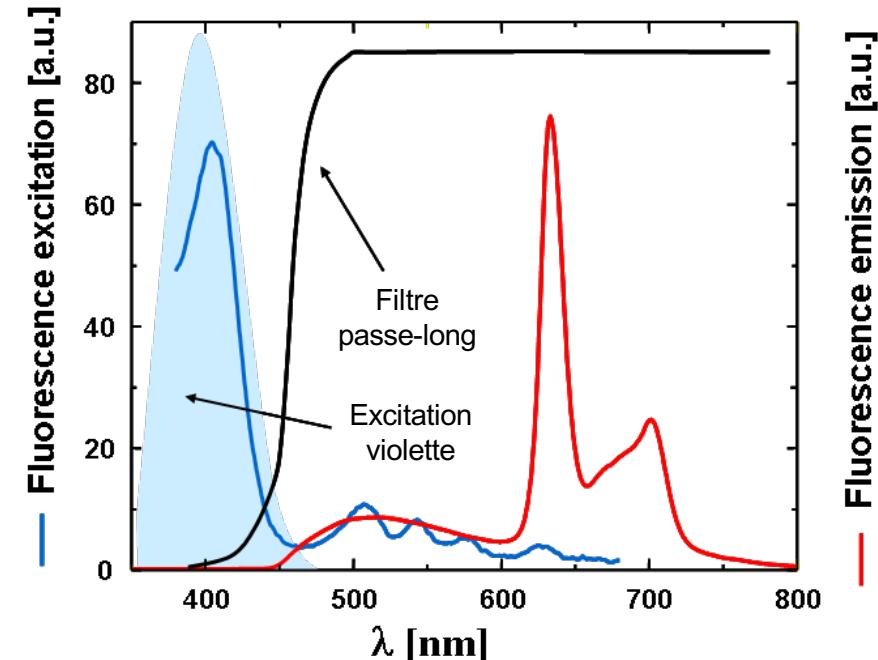
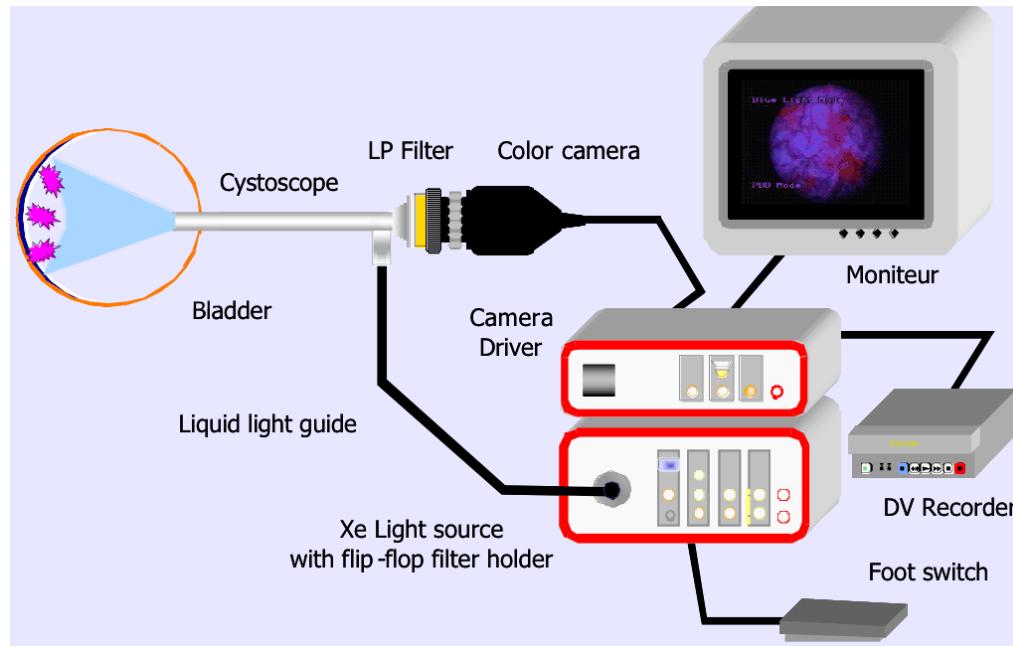
- **Instillation** (50 ml; 8 mM) of an exogenous precursor (hexylester of aminolevulinic acid, Hexvix®) of protoporphyrin IX (PpIX) during one hour prior to examination
- **Detection** of the red (peak @ 635 nm) fluorescence produced by the lesions under violet (410 nm) light excitation.

Ideal for the detection of non muscle-invasive bladder cancers !

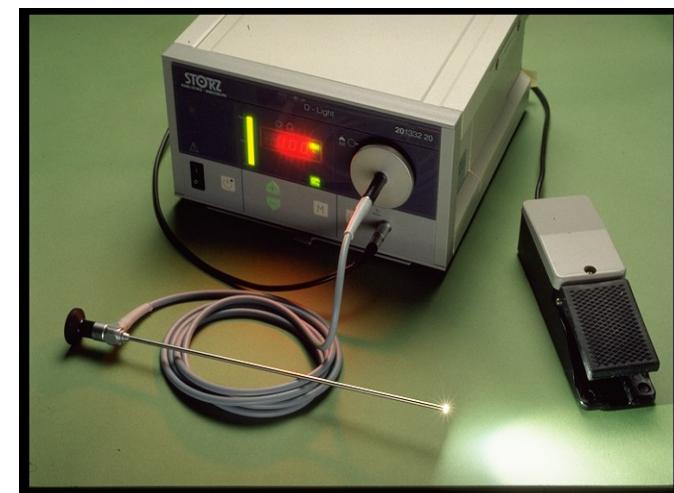
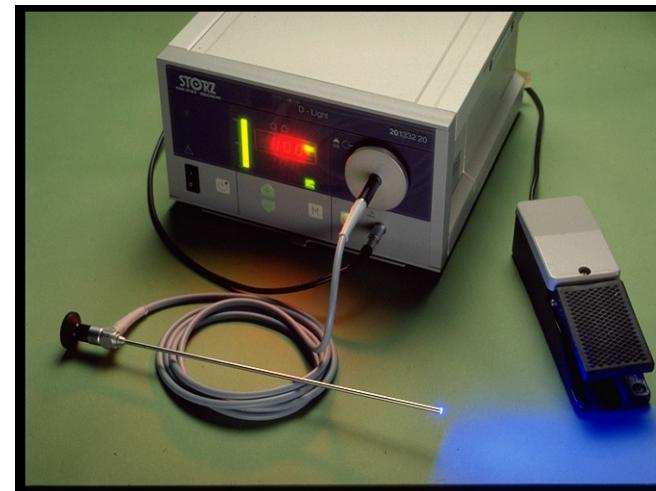


## 2. History

# Setup to detect bladder cancers by PpIX-based fluorescence cystoscopy



# Combined development of Medtech and Pharma technologies



# Photodetection of flat lesions (Carcinoma in situ) in the bladder

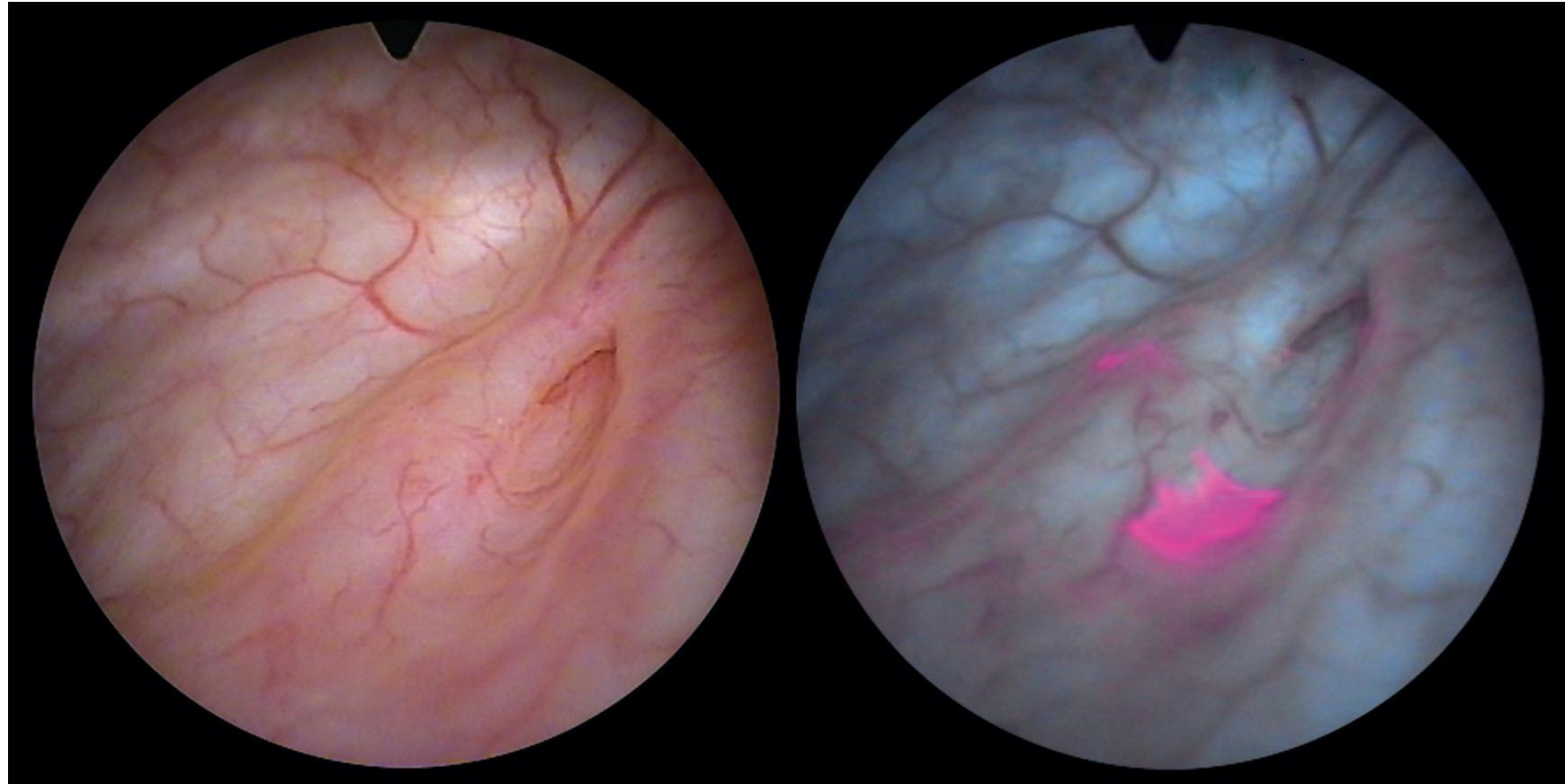


White light  
Cystoscopy

2. History

# Photodetection of flat lesions (Carcinoma in situ) in the bladder

---



White light  
Cystoscopy

ALA-Hexylester  
Cystoscopy

# Hexvix® Fluorescence vs White Light Cystoscopy: Key Clinical Benefits



- **Very good sensitivity** (93%) vs. WL (73%)

Indeed, fluorescence cystoscopy (FC) detects :

At least one more CIS detected in 41.5% of the patients.

*Fradet et al., J. Urol 2007*

One more pTa or pT1 in ~30% of the patients

*Grossman et al., J. Urol 2007*

- **Limited specificity** (61%) vs. WL (65%)

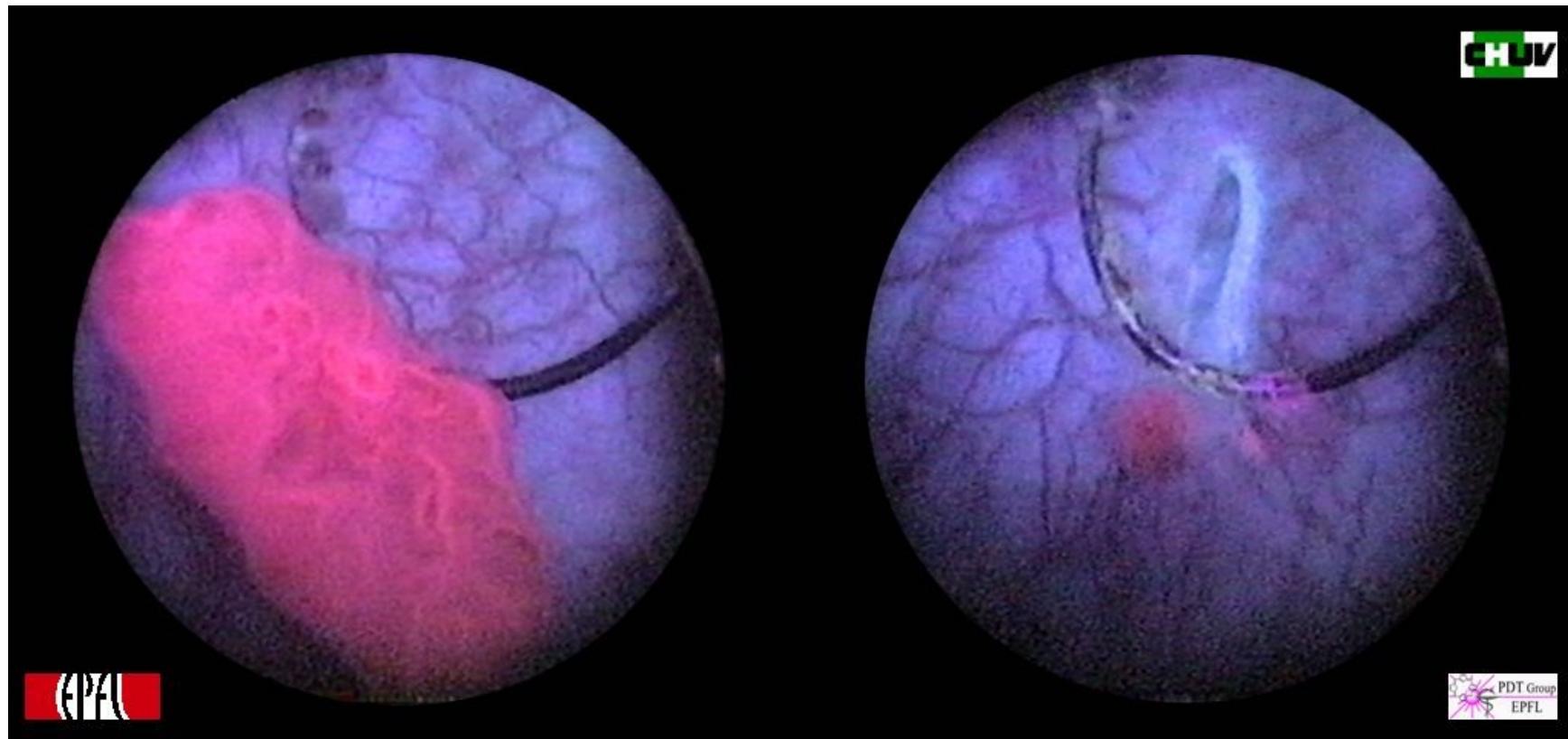
mainly due to high rate of false positive :

**inflammation**, hyperplasia, metaplasia are often cause of false-positive intravesical therapy (BCG), geometrical aspects.

*Draga et al. Eur Urol 2009; Jichlinski et al. J.Urol 2003;*

*Lerner et al., Urologic Oncology 2011.*

# Fluorescence-guided electroresection

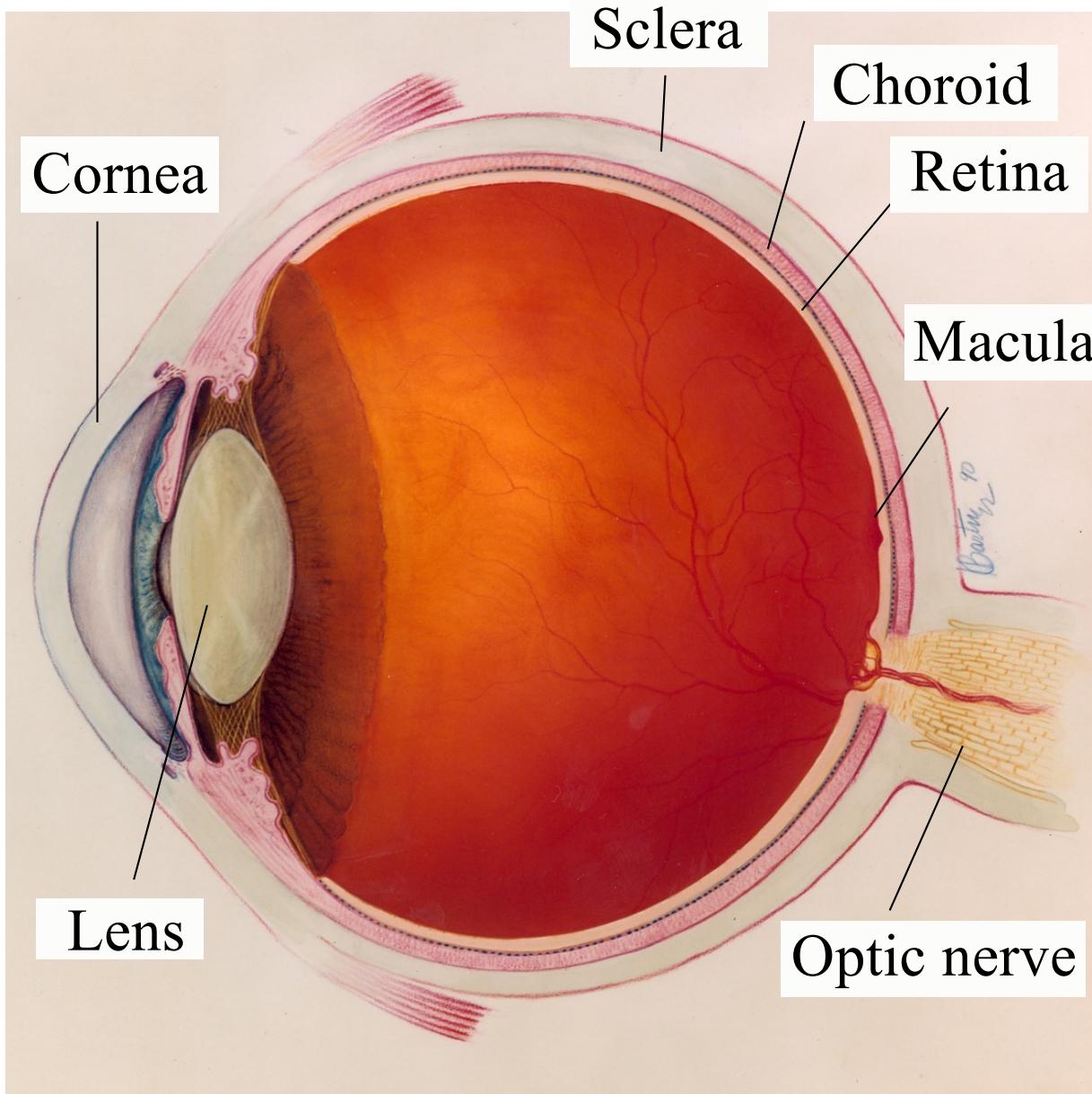


## Hexvix® and Cysview® are now commercialized by Photocure and Ipsen

Between 50'000 and 100'000 procedures / year at present

- Approved in all EU/EEA countries in 2005.
- Recommended in 2005 as standard method by the European Association of Urology
- Reimbursement obtained in many European countries since 2007
- Approval of Cysview® by the US FDA in May 2010
- Approval of the fluorescence cystoscopy system from Karl Storz by the US FDA in May 2012
- Approval of the extension of Cysview® to include Flexible Cystoscopes, which are used in the ongoing surveillance of patients with bladder cancer in 2018





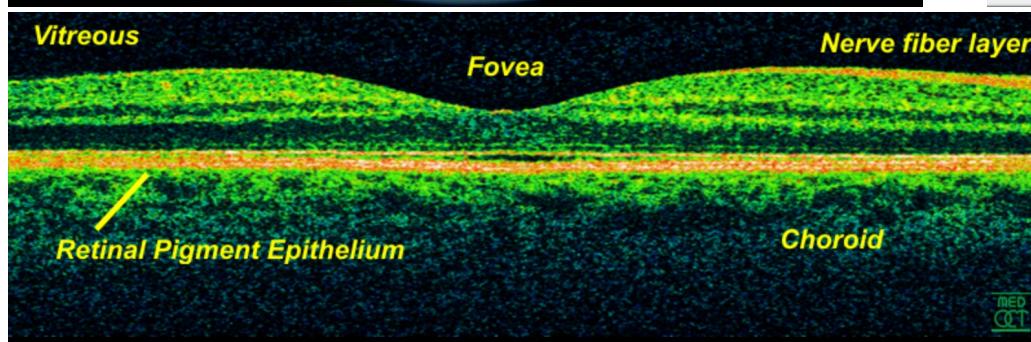
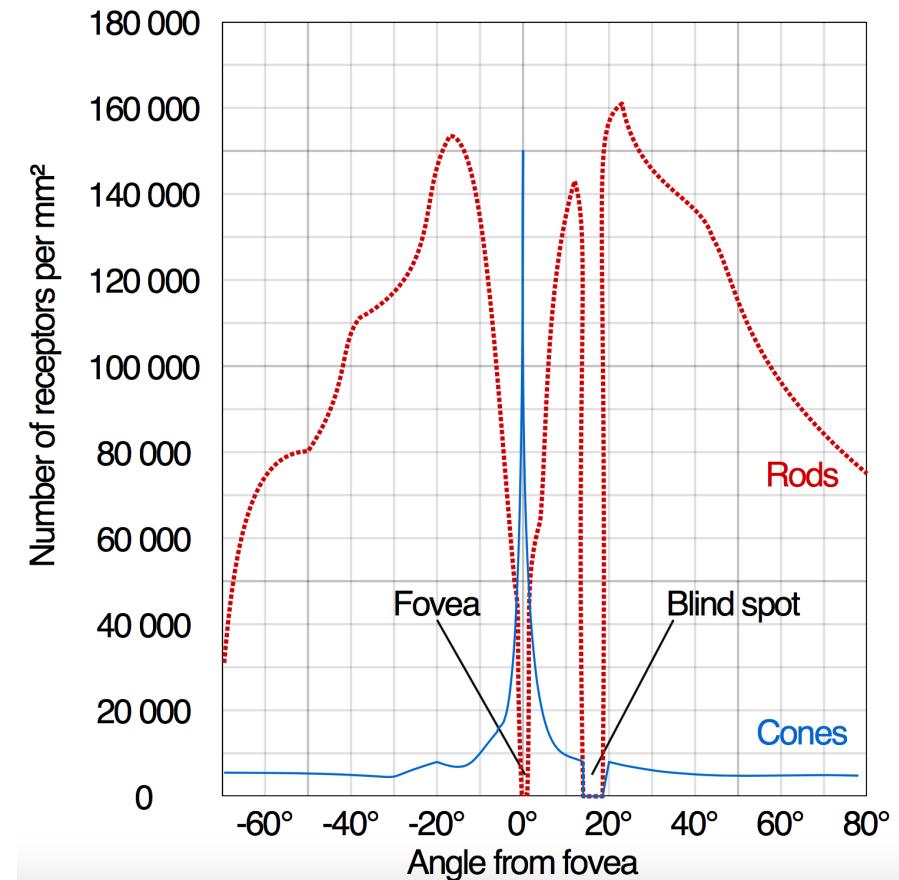
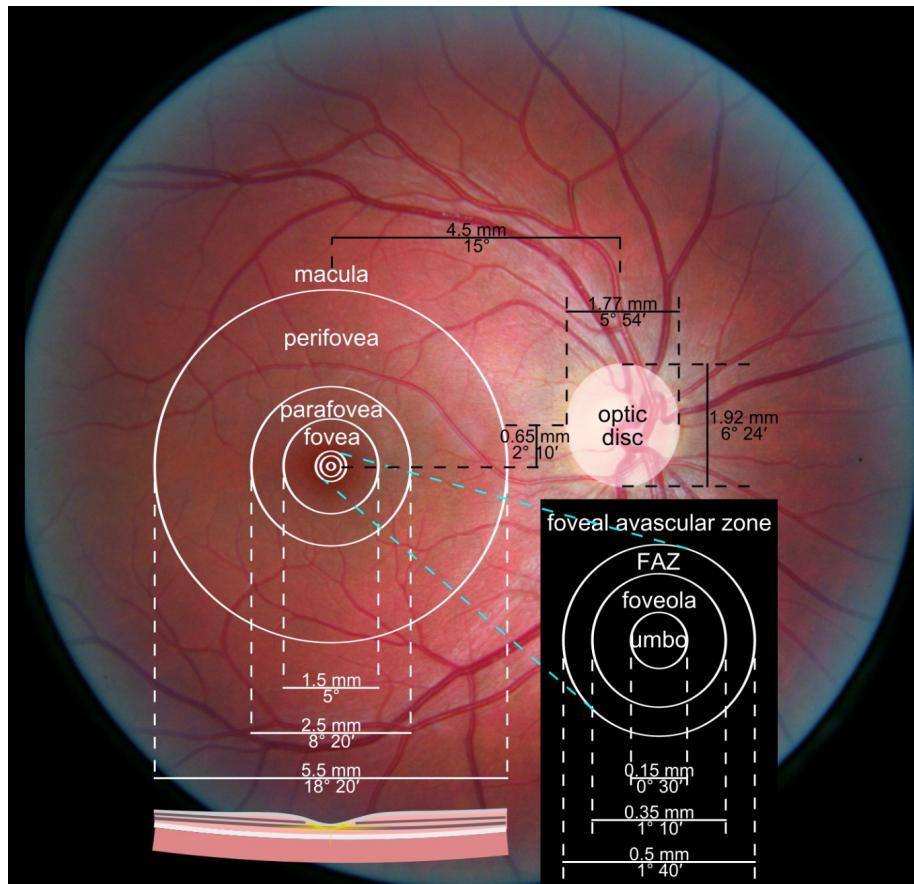
**An interesting case:**  
**(A therapeutic application  
of photomedicine)**

**Treatment of age-related  
Macular degeneration by  
photodynamic therapy  
With Visudyne®**

**First studies: early 90's  
First approval: 1999**

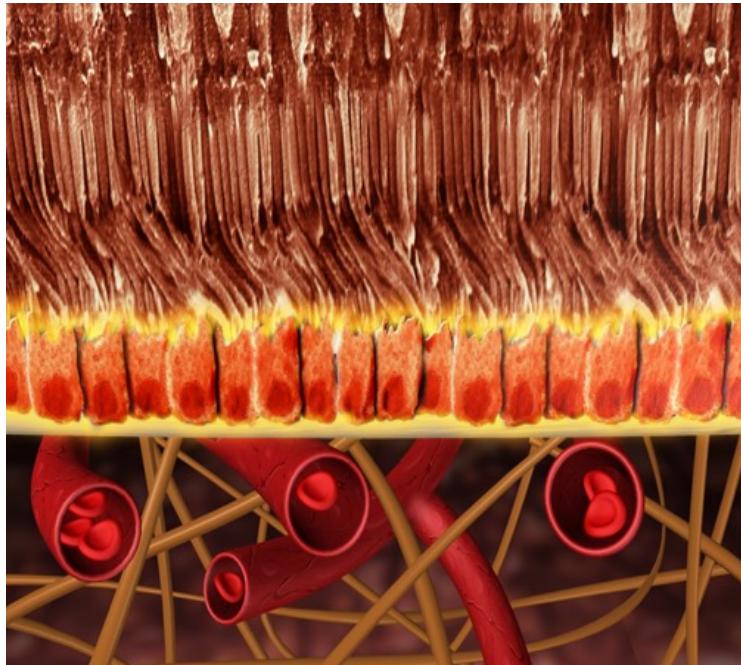
***Anatomy of the  
human eye***

# Location of the macula



OCT cross-section image at 800 nm

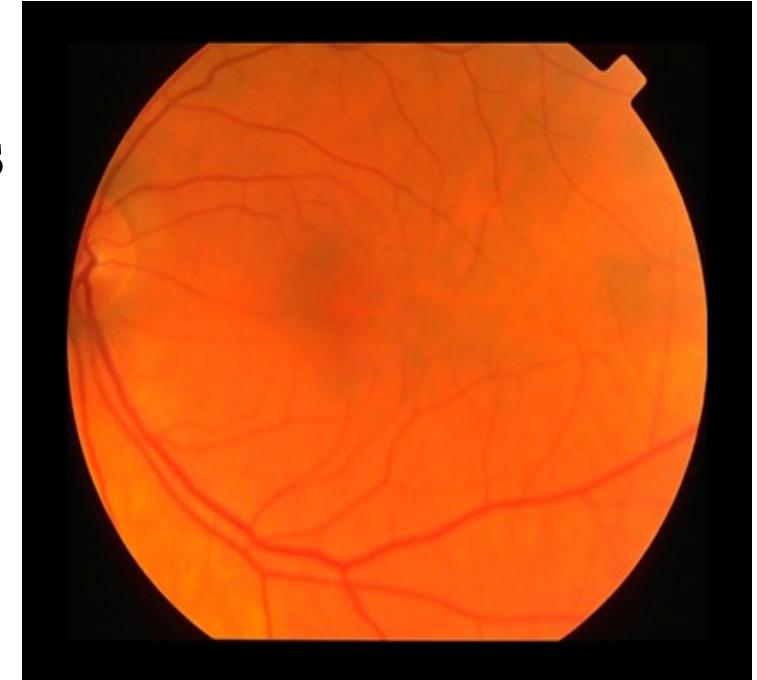
# Normal retina



Photoreceptors

RPE

Choroid



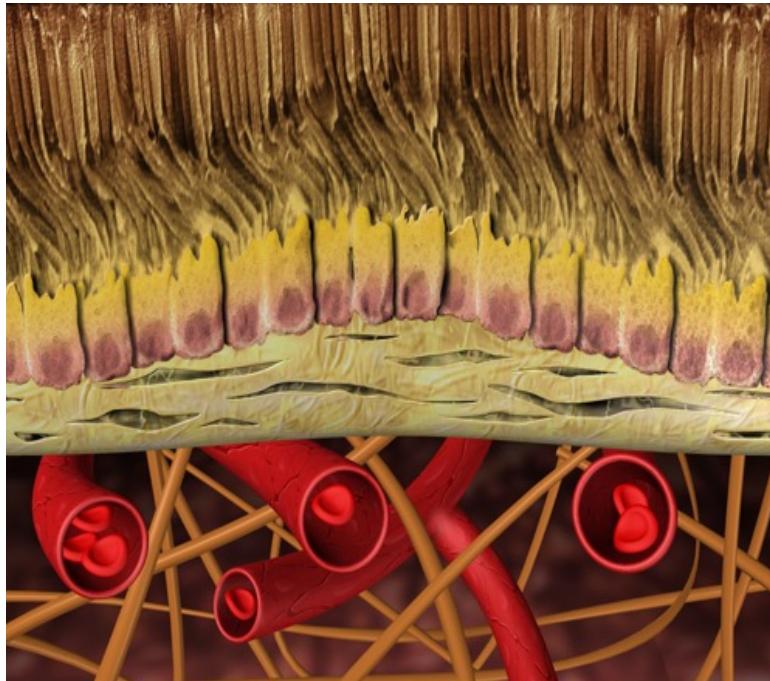
fundus photograph

**Vision = high metabolic activity**

**Retinal Pigmented Epithelium (RPE): major role in nutrition + protection**

Source: Novartis Ophthalmics

# Aging of macula



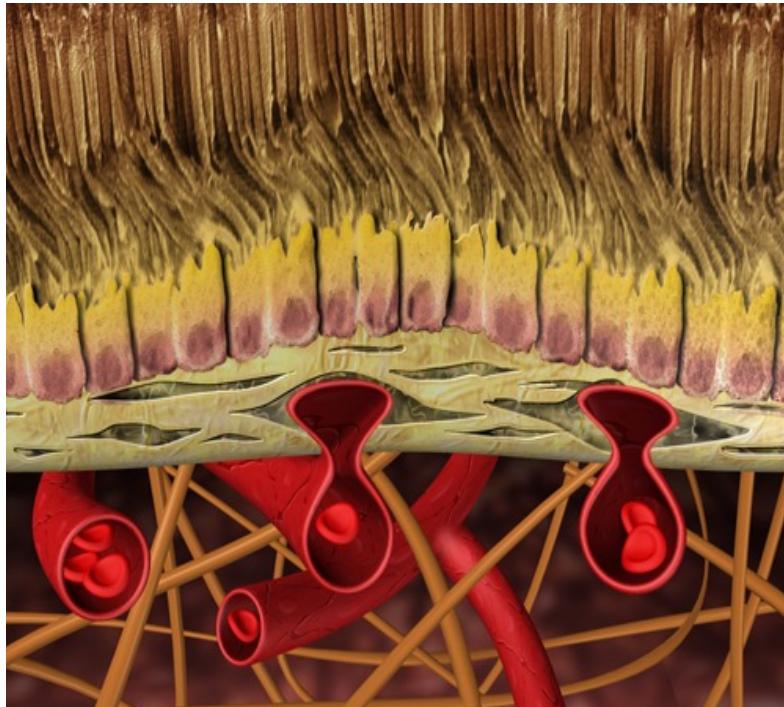
fundus photograph

**Drusen: accumulation of lipofuscin**  
**Bruch's membrane thickens + calcification**  
**Less choriocapillaris**  
**Rupture of blood-retina barrier**

Source: Novartis Ophthalmics

# Aging of macula

## One form of AMD: Bruch's membrane penetration by choroidal neovessels (CNV)

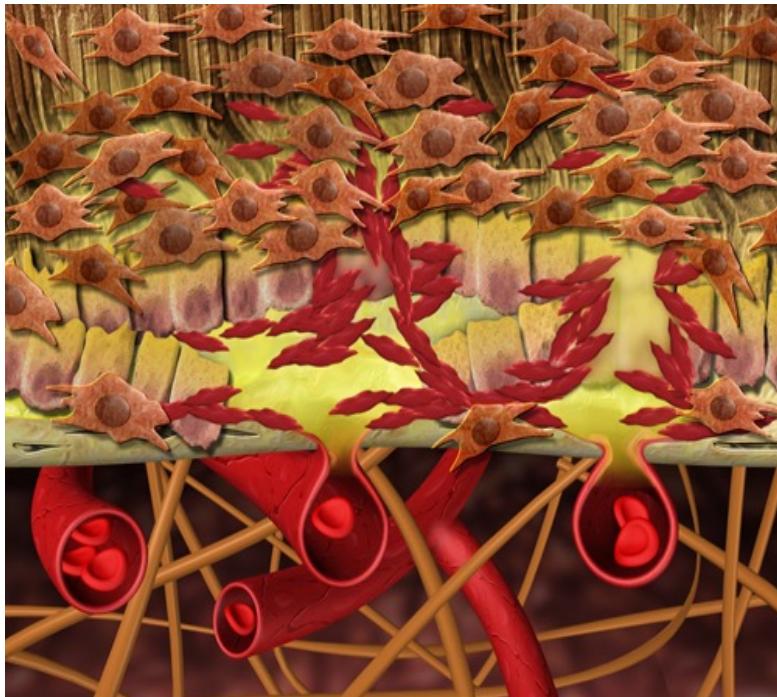


**fundus photograph**

Source: Novartis Ophthalmics

# Formation of disciform scar

→ **Vision loss**



**Fibroblasts**  
+  
**Neovascular**  
**Endothelial**  
**cells**



**fundus photograph**

Source: Novartis Ophthalmics

## 2. History

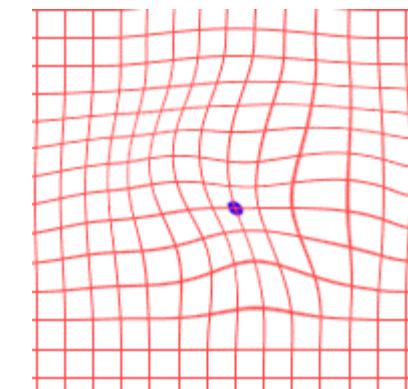
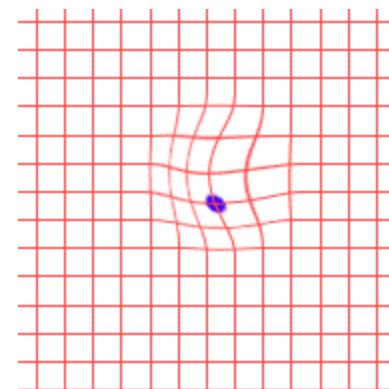
### Normal vision



### Vision with (AMD)



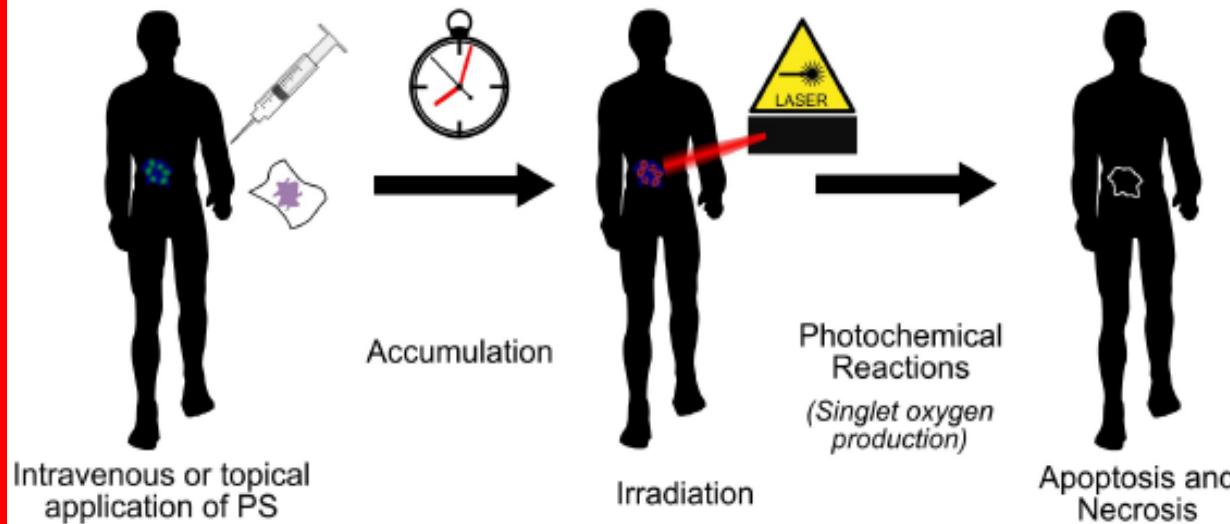
With macular degeneration, print may appear distorted, and parts of words may be missing.



### Amsler grid

# Photodynamic Therapy (PDT)

*PDT is a well established technique that is effective in treating several non malignant and malignant conditions*



## Three main components:

1. **Drug:** photosensitizer (PS)
2. **Light:** energy source that activates the drug
3. **Oxygen:** interacting with the excited PS, induce cell death

## *Advantages:*

- *Good selectivity in certain cases*
- *Can be repeated*
- *Minimally invasive*
- *...*

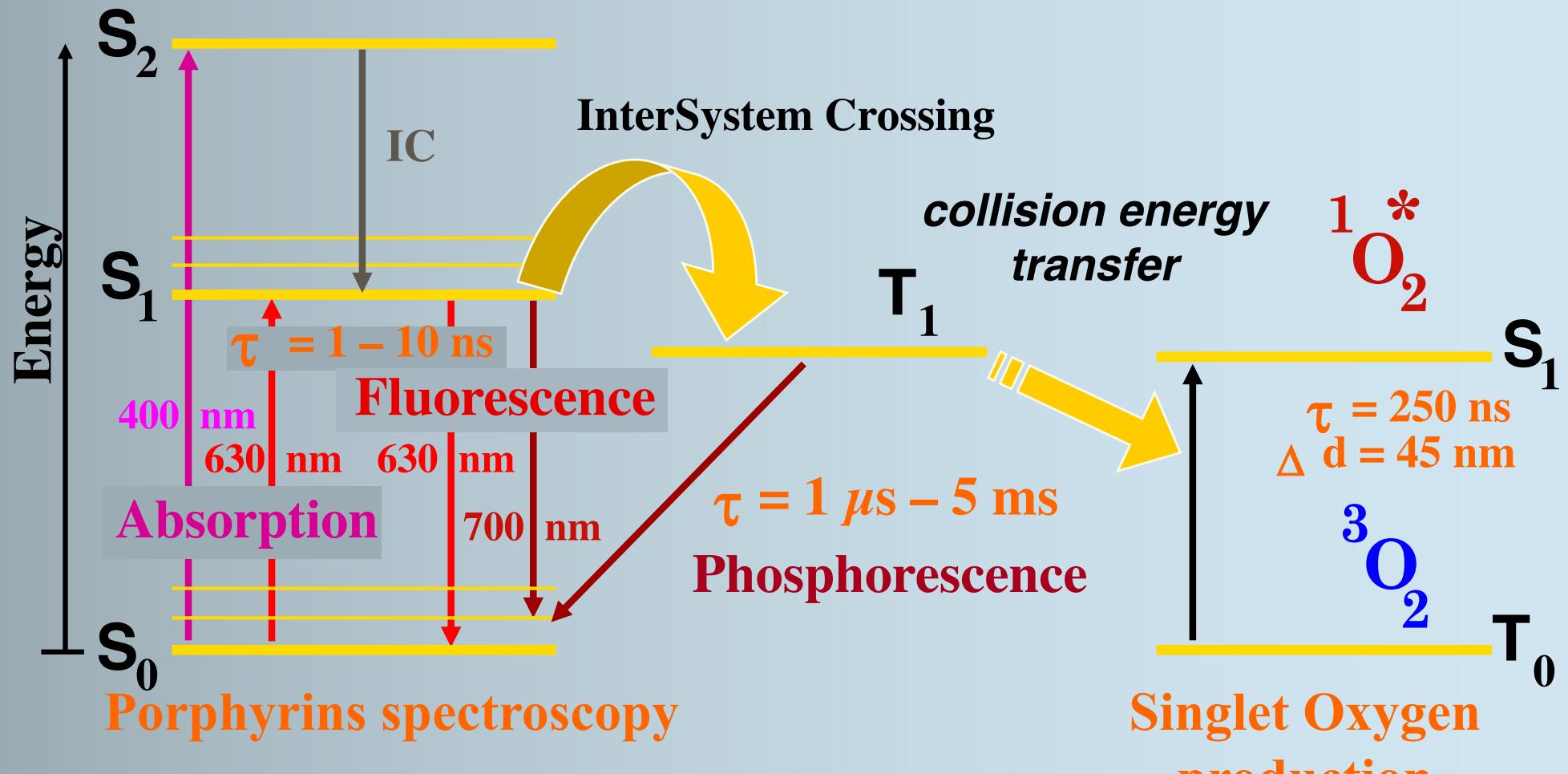
## *Drawbacks:*

- Delayed response
- Skin photosensitization
- Only small/superficial volumes can be treated
- Inter and intra patient fluctuation
- *...*

# Photophysical Processes in:

Fluorescence detection

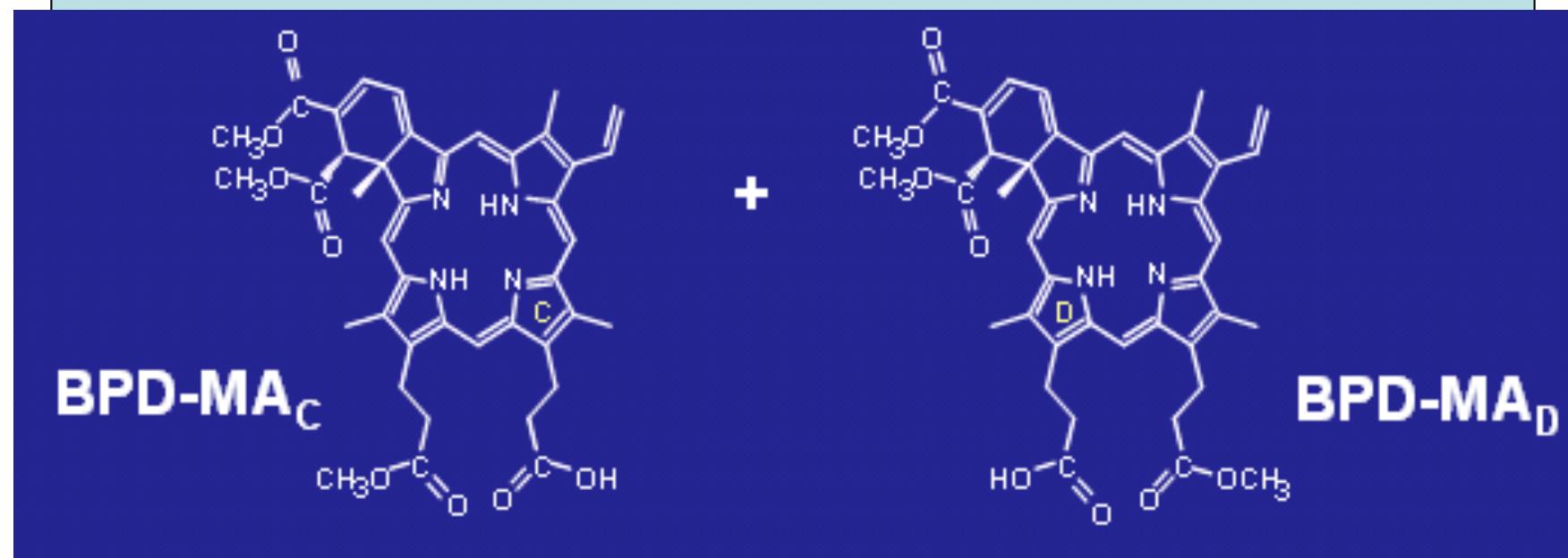
Photodynamic Therapy



Collisions with  $O_2$  shorten the PS's triplet state lifetime  $\tau$

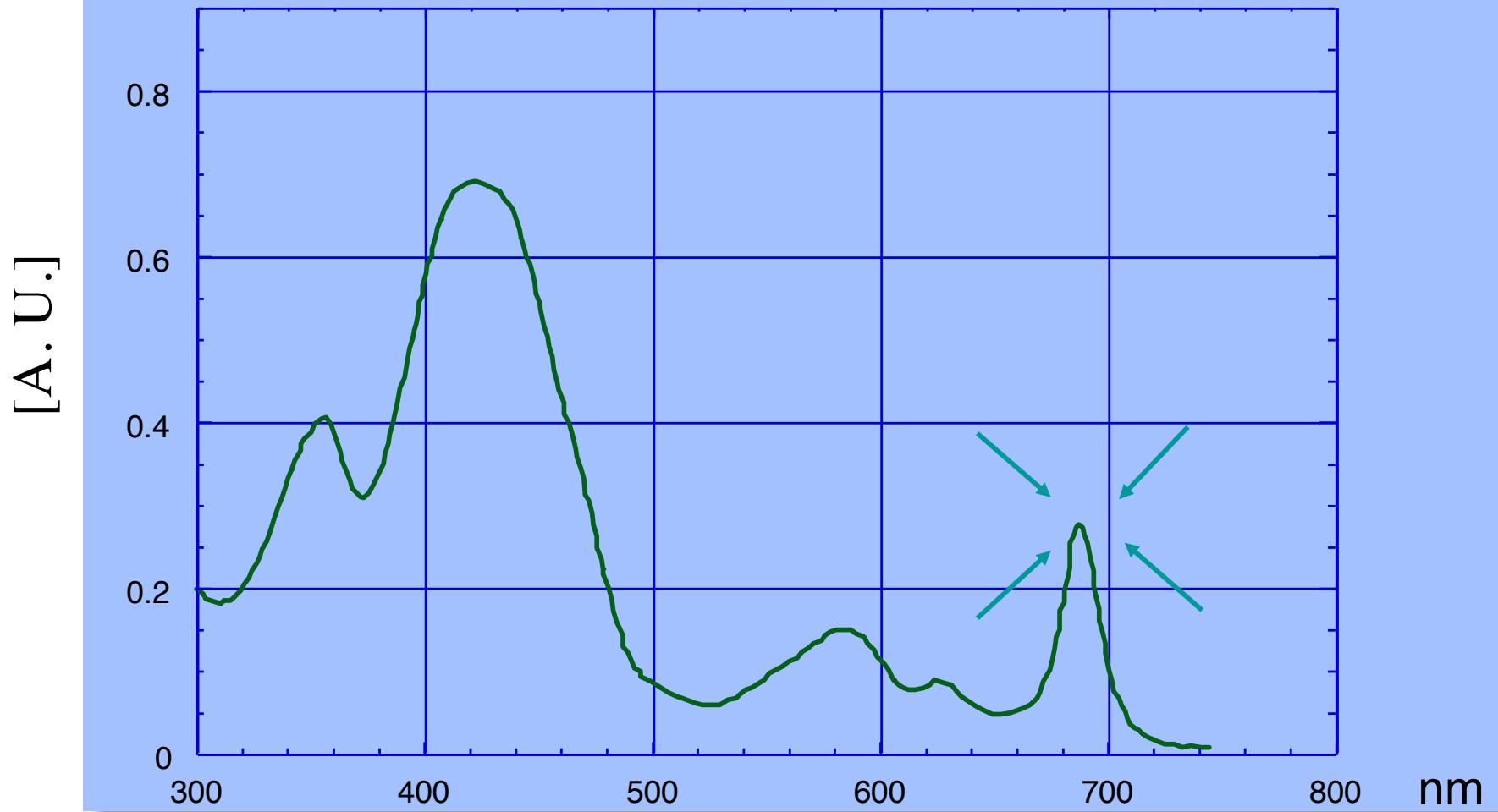
# Visudyne® is an angio-occlusive photosensitizer

Visudyne (verteporfin) exists in an equal mixture of two pharmacologically active benzoporphyrin-derivative monoacid (BPD-MA) regioisomers

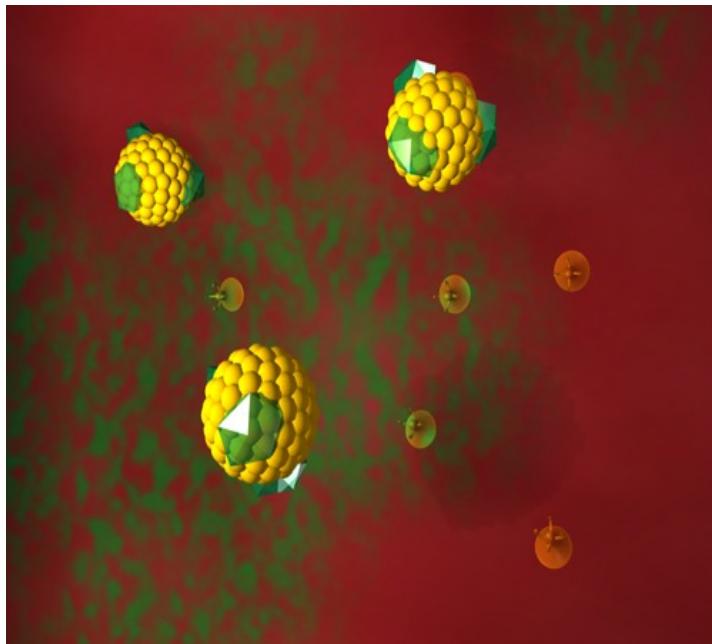


Houle JM, Strong A. *J Clin Pharmacol.* 2002;42:547–557.

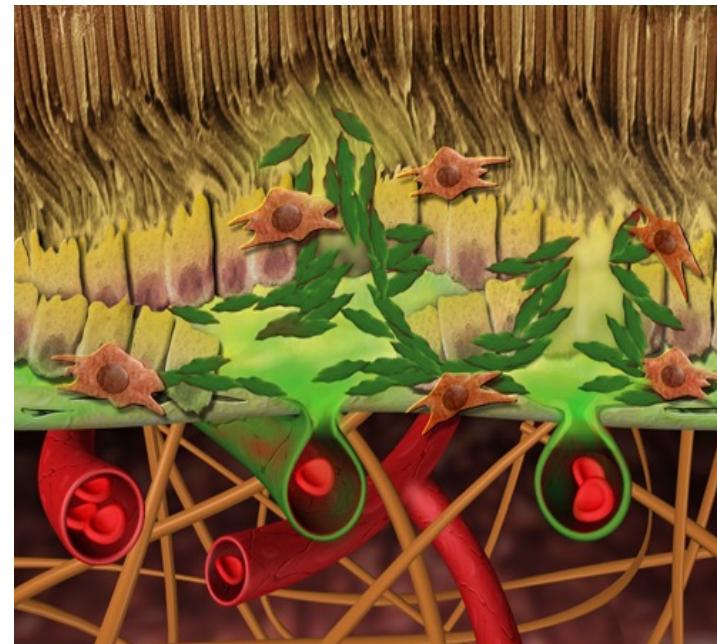
## Absorption spectrum of Visudyne®



## Verteporfin-based photodynamic therapy (PDT): Mechanism of action



Verteporfin complexes with LDL

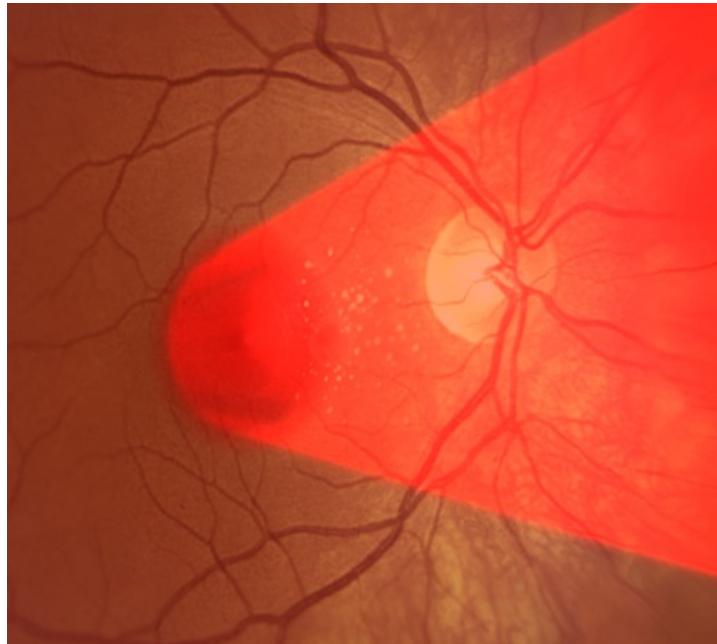


Dye accumulates in neovascular tissue

Source: Novartis Ophthalmics

# PDT with Visudyne™

## Treatment procedure

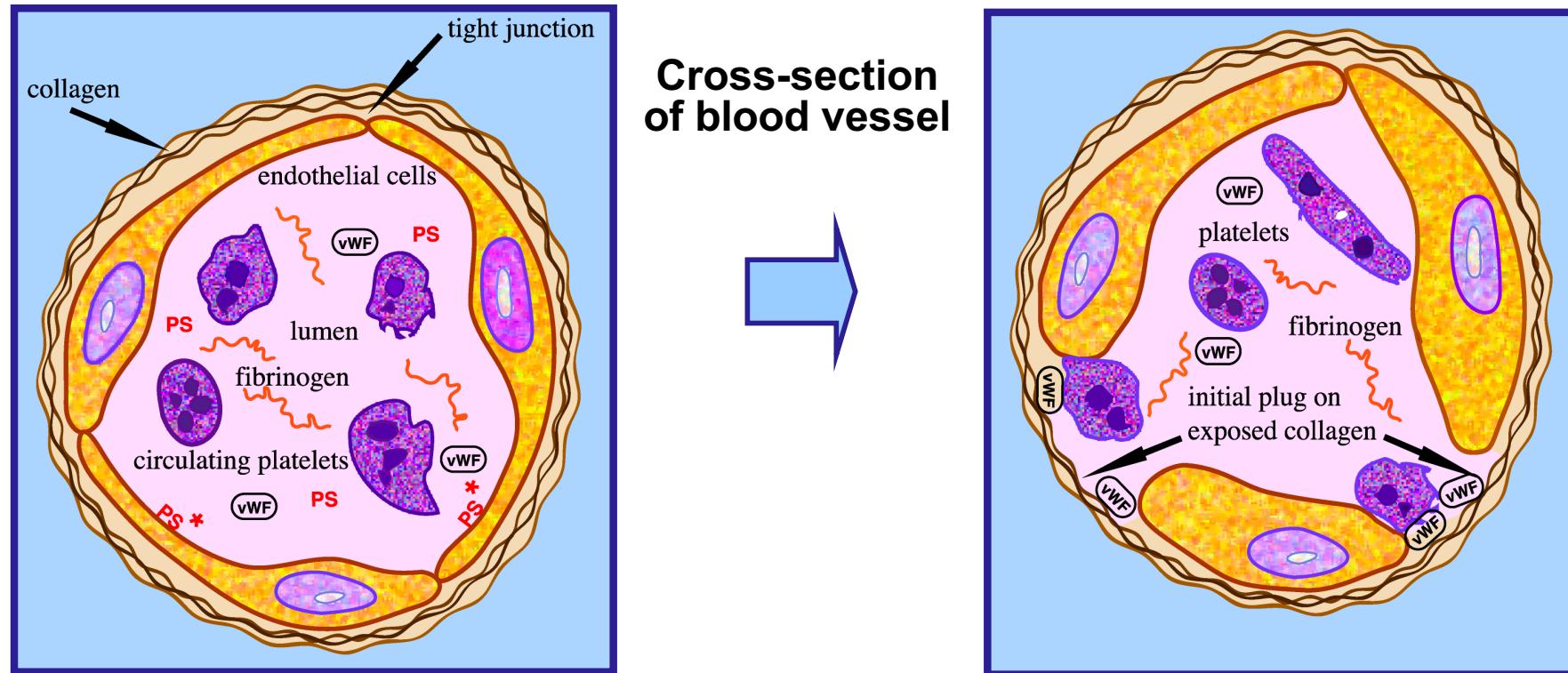


- Drug dose:  $6 \text{ mg/m}^2$
- Light dose:  $50 \text{ J/cm}^2 @ 690 \text{ nm}$
- Light dose rate:  $600 \text{ mW/cm}^2$
- Outpatient procedure

Laser started 15 min  
after infusion begins

Source: Novartis Ophthalmics

# The short-lived reactive oxygen species cause endothelial cell changes



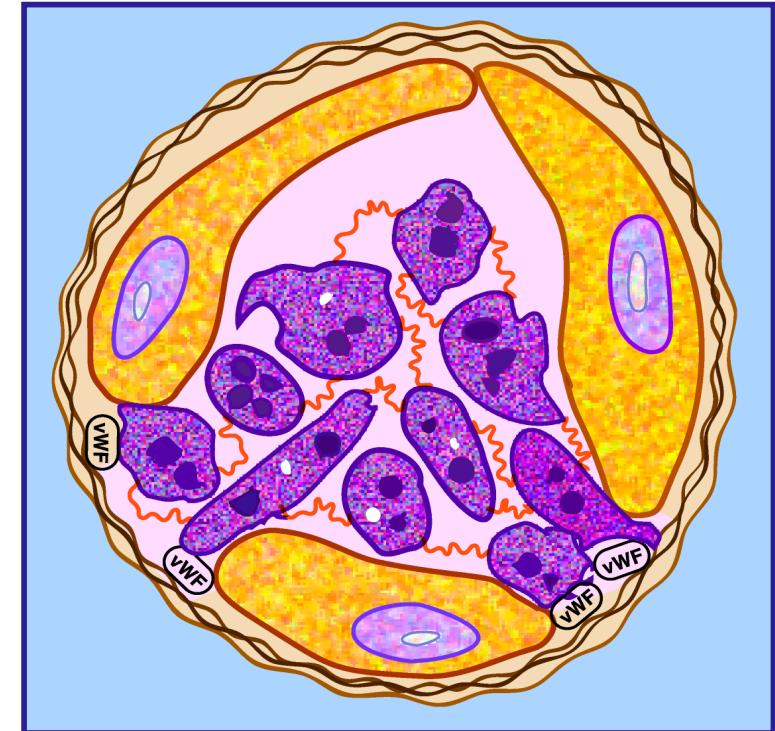
- Cytoskeleton changes cause rounding and contraction of the vascular endothelial cells.
- Exposure of the subendothelial basement membrane triggers platelet binding and aggregation.

Beaumont P et al. *Arch Ophthalmol.* 2004;122:1546–1547.  
 Schmidt-Erfurth U, Hasan T. *Surv Ophthalmol.* 2000;45:195–214.

# Verteporfin therapy triggers a process leading to neovascular angioocclusion

- The activated platelets adhere to the collagen of the exposed basement membrane
- A fibrin-stabilized plug is formed as the blood clots
- Complete closure of the choroidal neovasculature occurs within 1 day

Cross-section of blood vessel



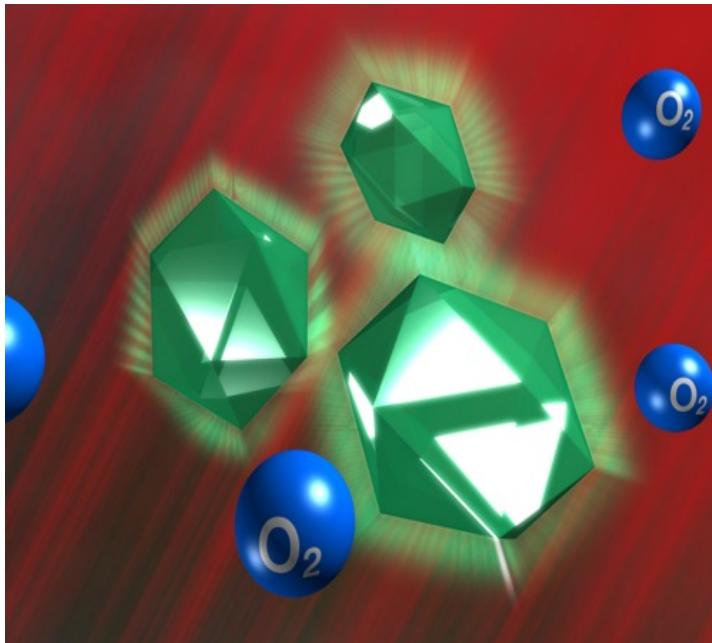
Beaumont P et al. *Arch Ophthalmol.* 2004;122:1546–1547.

Michels S, Schmidt-Erfurth U. *Invest Ophthalmol Vis Sci.* 2003;44:2147–2154.

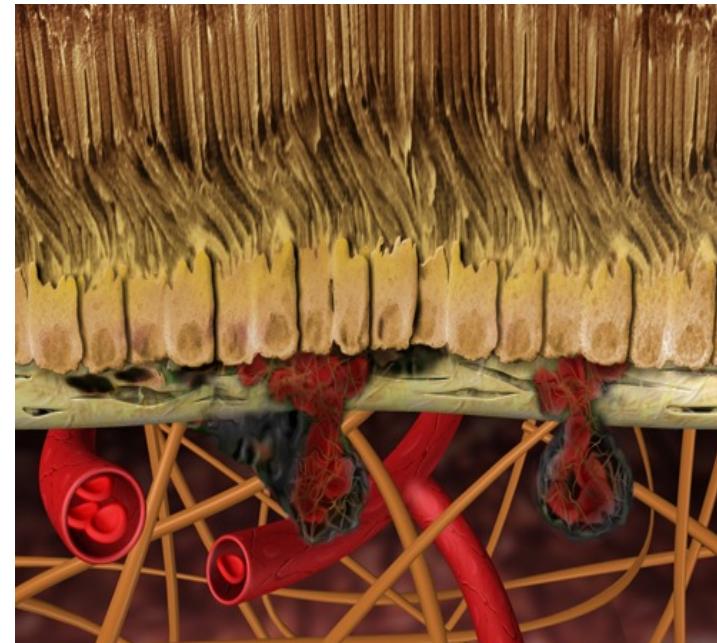
Visudyne US Package Insert. 2005.

Michels S et al. *Invest Ophthalmol Vis Sci.* 2006;47:371–376.

## Verteporfin: Mechanism of action



**Verteporfin reacts  
with oxygen after  
absorption of light**

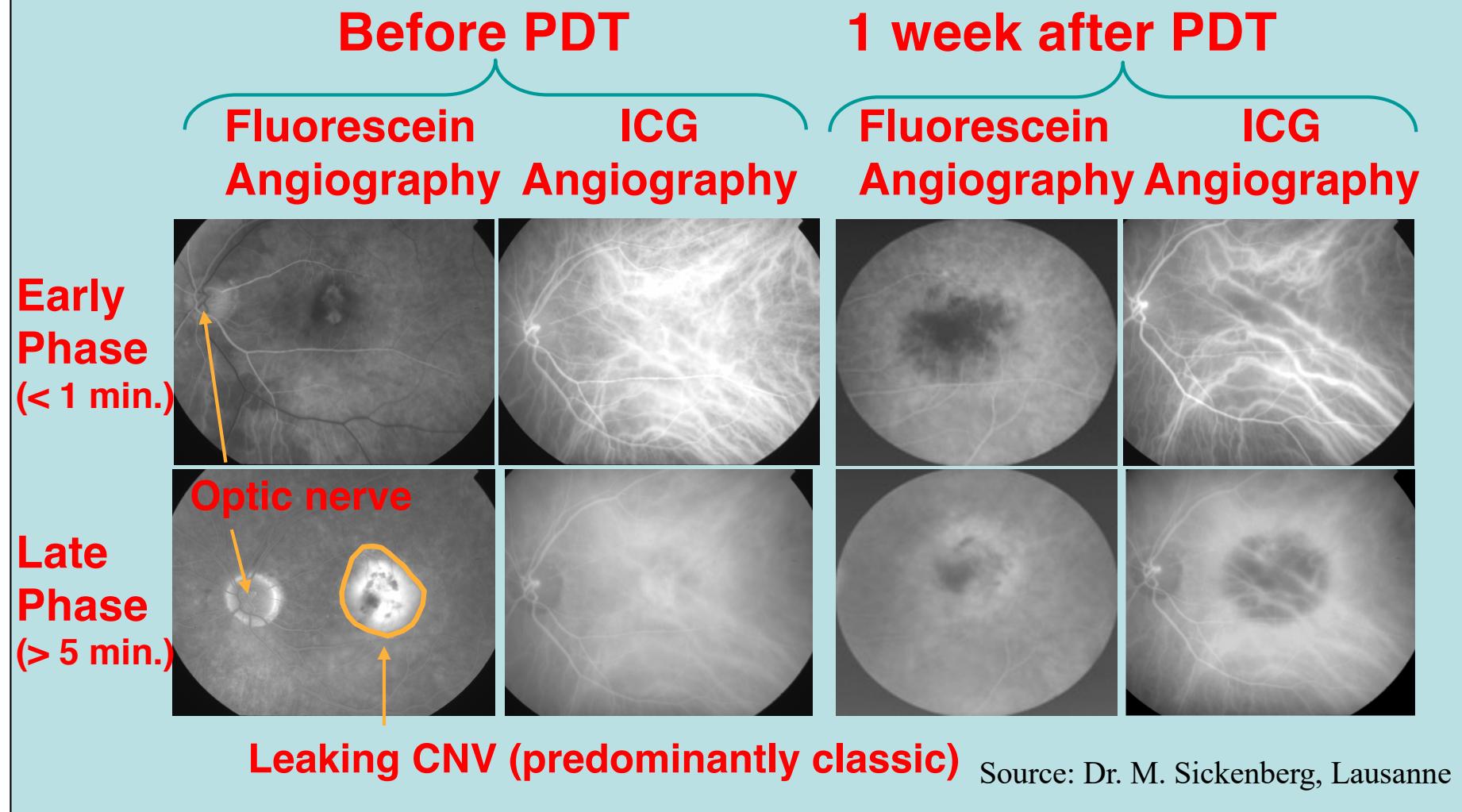


**Damaged Choroidal  
neo-vessels  
(thrombosis)**

Source: Novartis Ophthalmics

## Fluorescence angiographies showing the PDT effects

Swissmedic **1999** and FDA **2000** approvals of Visudyne™ for the treatment of Age-related Macular Degeneration (AMD)



## 2. History

# Visudyne® 5 years Follow up

Résultats à 5 ans de l'étude TAP sur l'acuité visuelle.<sup>1,9,10,11</sup>

