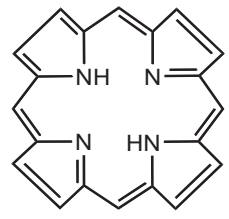
ポルフィリンの蛍光、りん光の利用
細胞内の情報を得るために

蛍光を利用するがんの検出
細胞内の酸素濃度測定

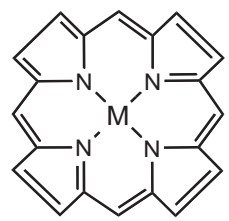
大倉一郎

Porphyrin

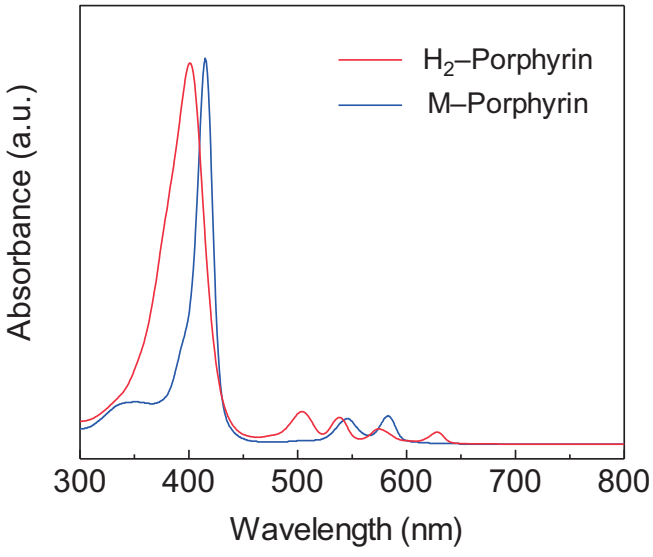
Hemoglobin	Fe-Porphyrin
Myoglobin :	Fe-Porphyrin
Vitamin B_{12} :	Co-Porphyrin
Chloroplyll :	Mg-Porphyrin



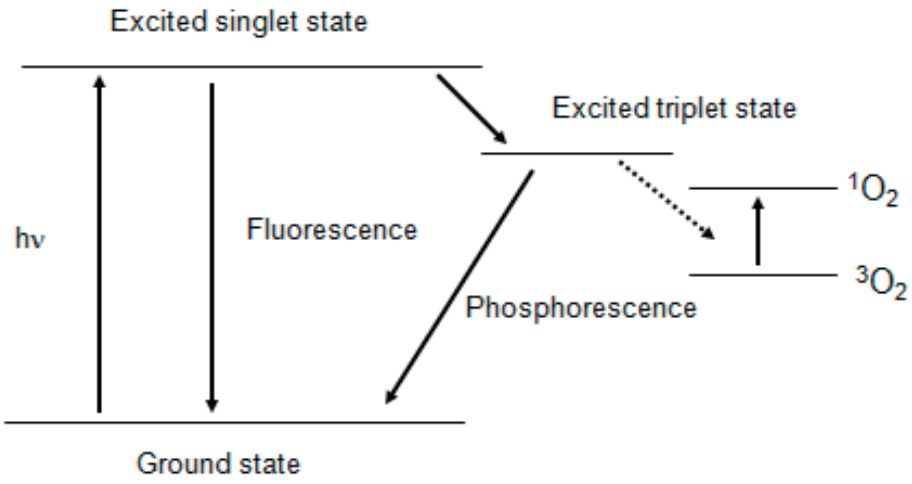
H_2 - Porphyrin



M - Porphyrin



Energy diagram



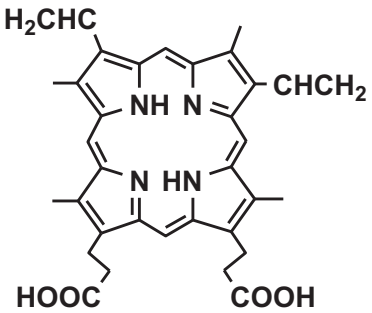
Protoporphyrin IX

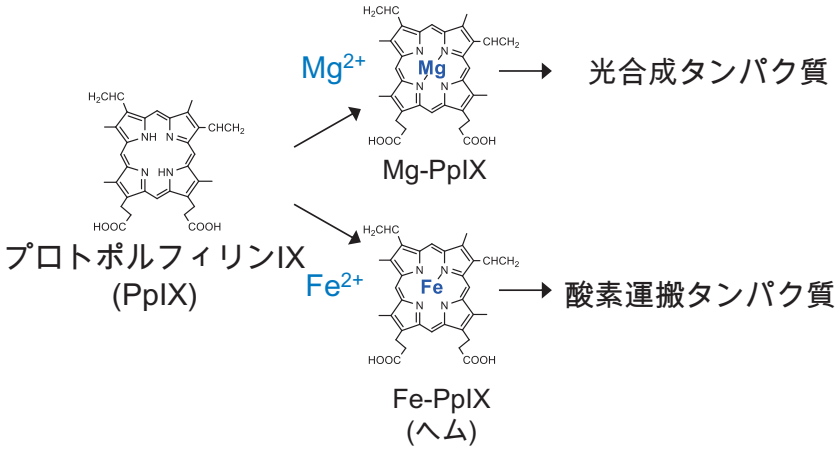
強い赤色蛍光を発する

→がんの蛍光診断・迅速診断

光照射によって活性酸素種を生成する

→がんの光線力学治療





ポルフィリンの蛍光、りん光の利用
細胞内の情報を得るために

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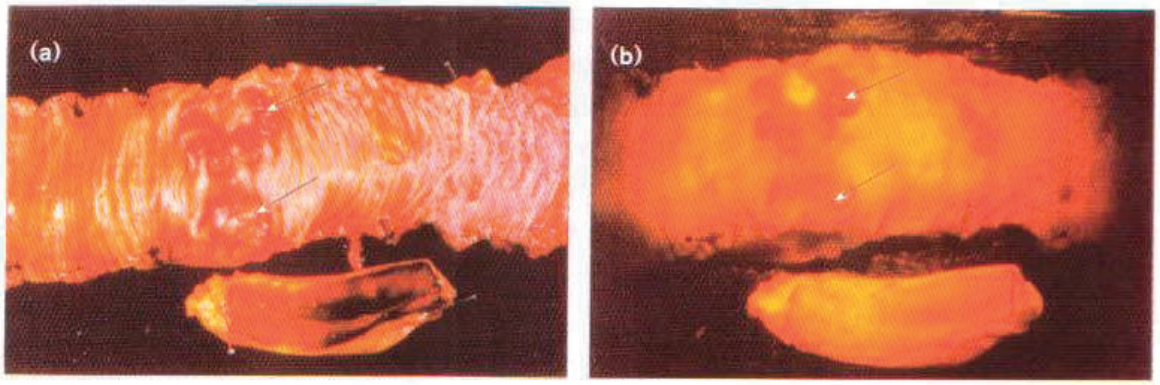
細胞内の酸素濃度測定

Human Abdominal Cancer



Methods of Extraction of Solid Tumor

Solid tumor

↓ acetic acid : ethyl acetate (4 : 1, v/v) 3 ml

Homogenized

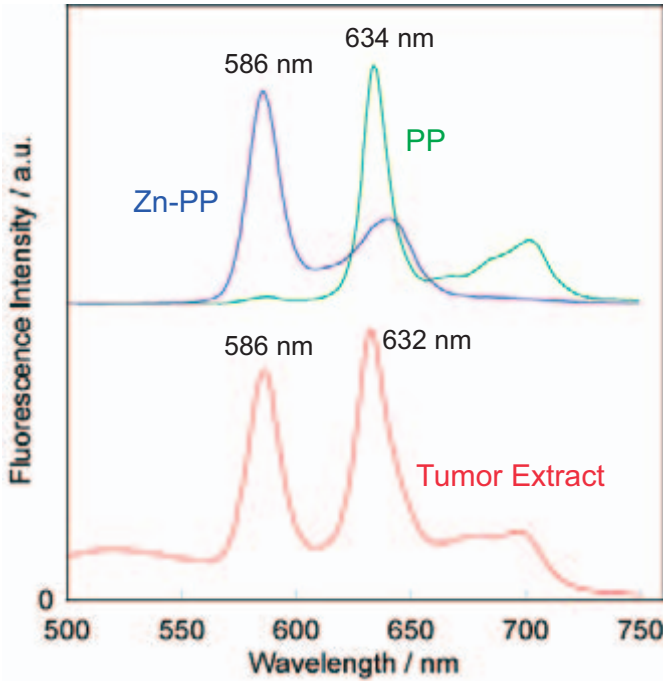
↓

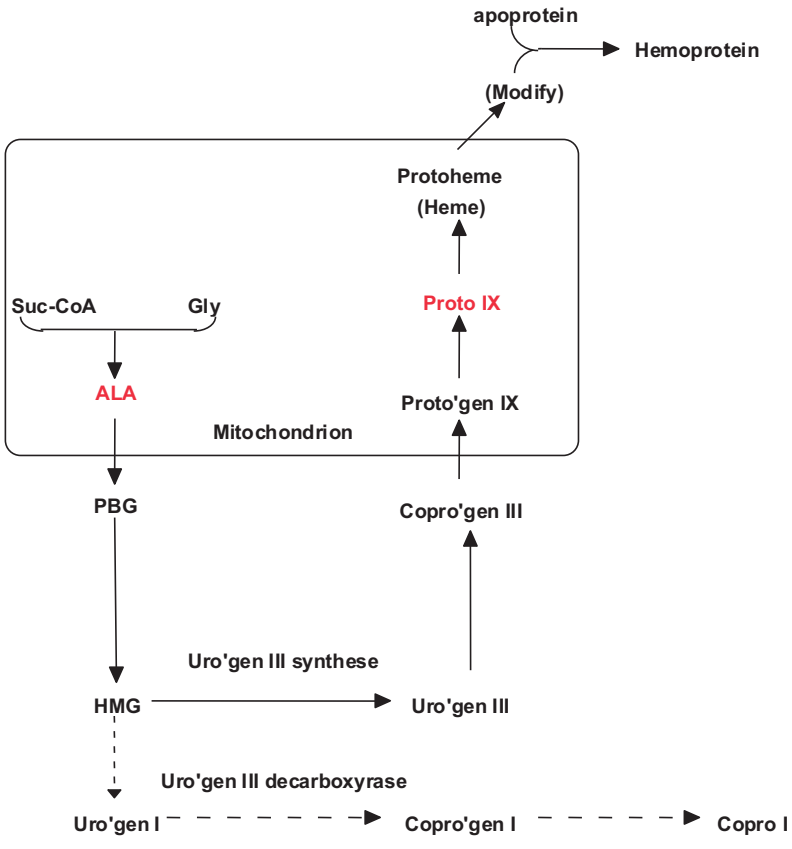
Centrifugated (2000 x g, 2 min, 4°C)

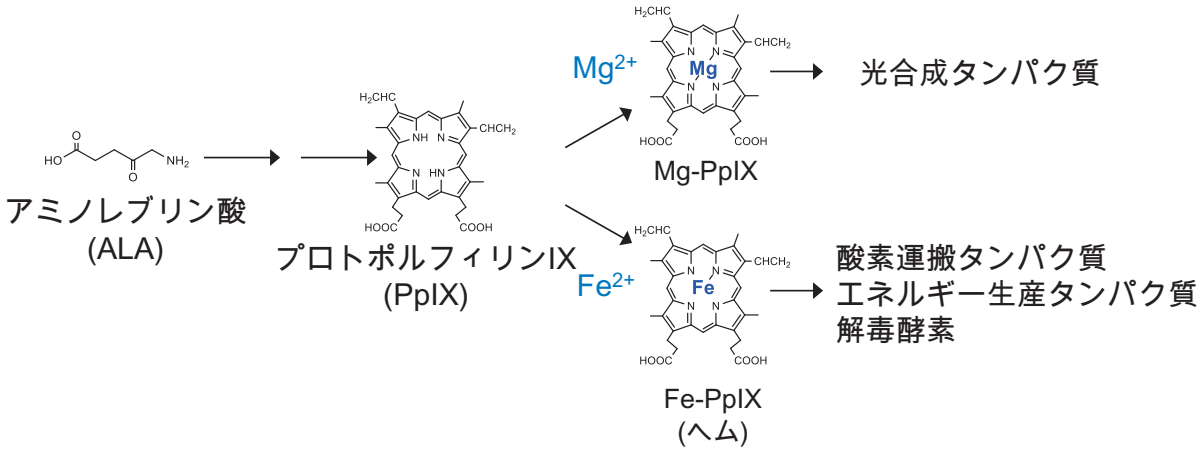
↓

HPLC, Fluorescence

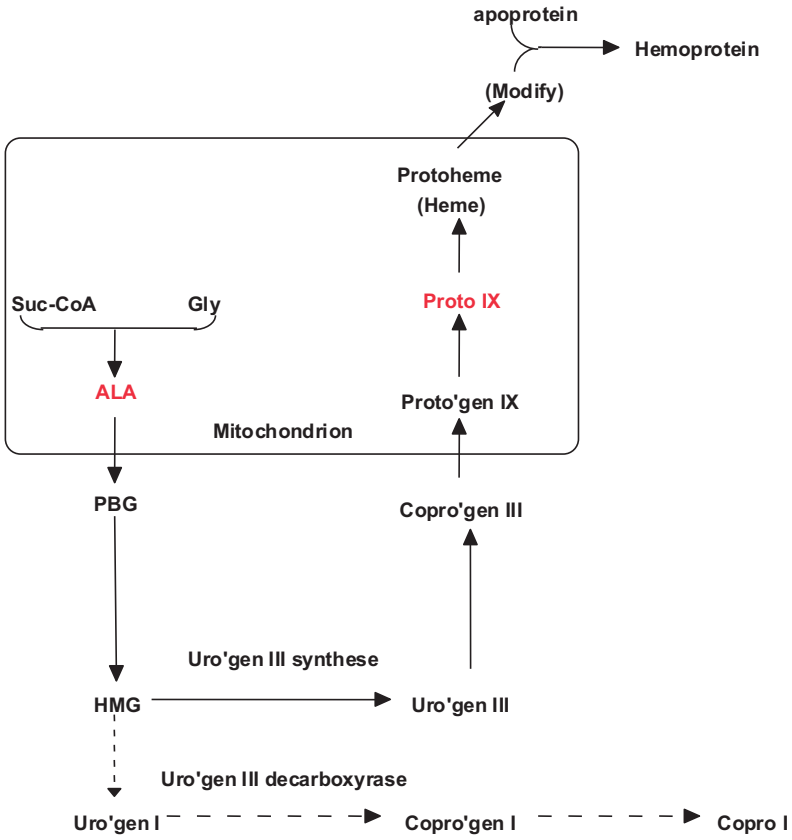
Fluorescence Spectrum from Tumor Extract



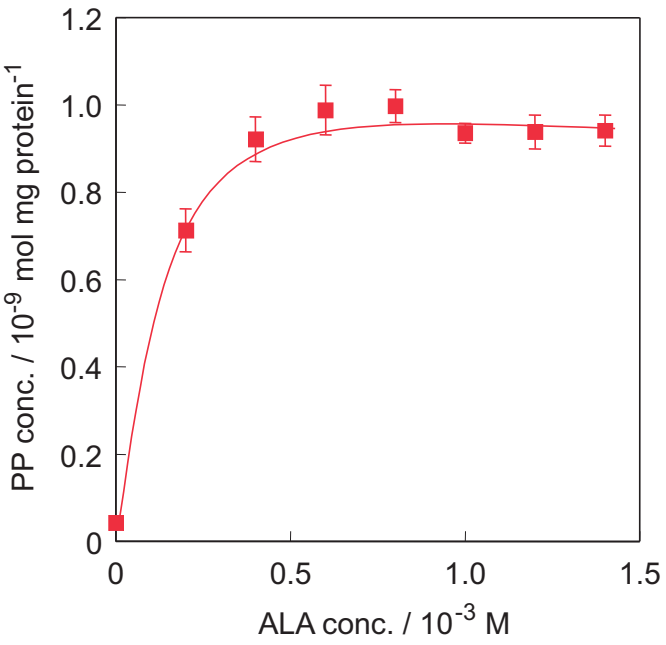




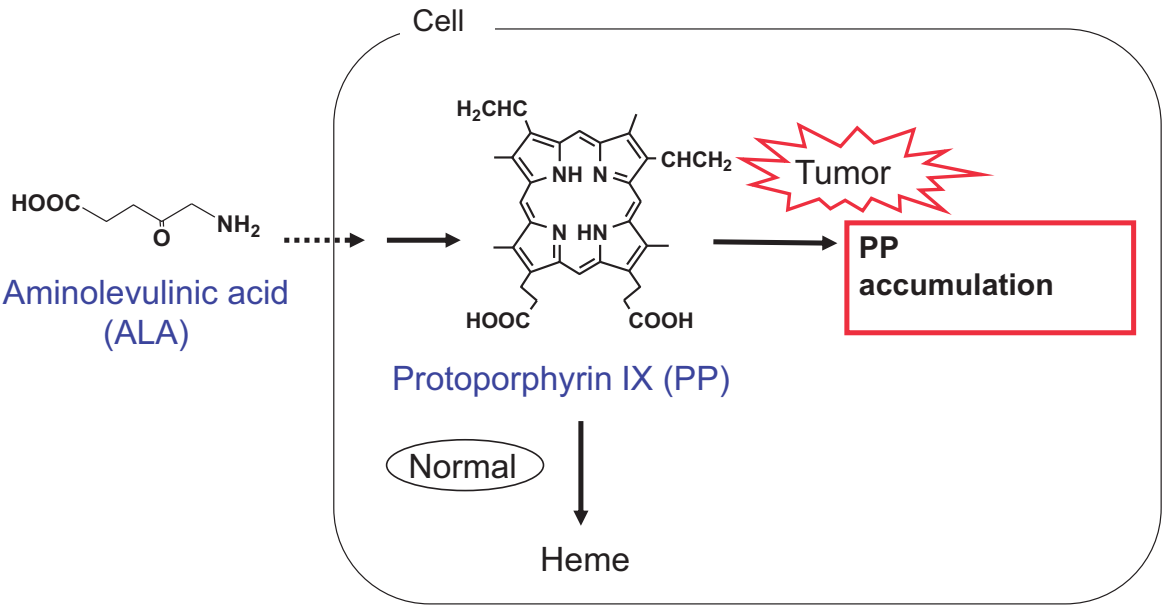
ALAを配合した肥料で栽培したサラダ菜
ALA配合肥料で生育したサラダ菜（右）およびALA非配合肥料で生育したサラダ菜（左）



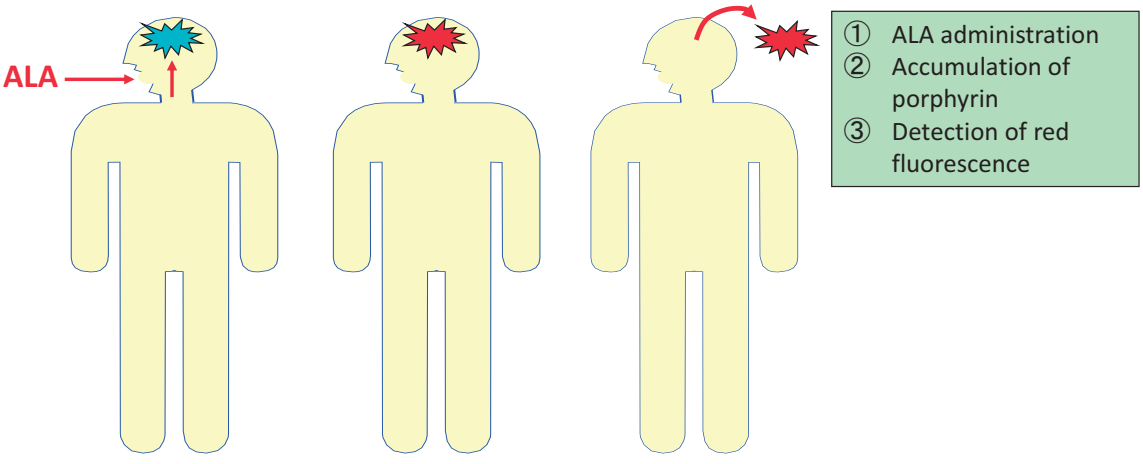
PP accumulation in HeLa cells

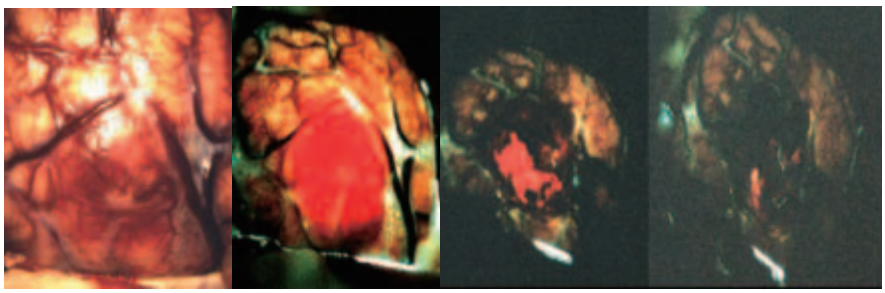


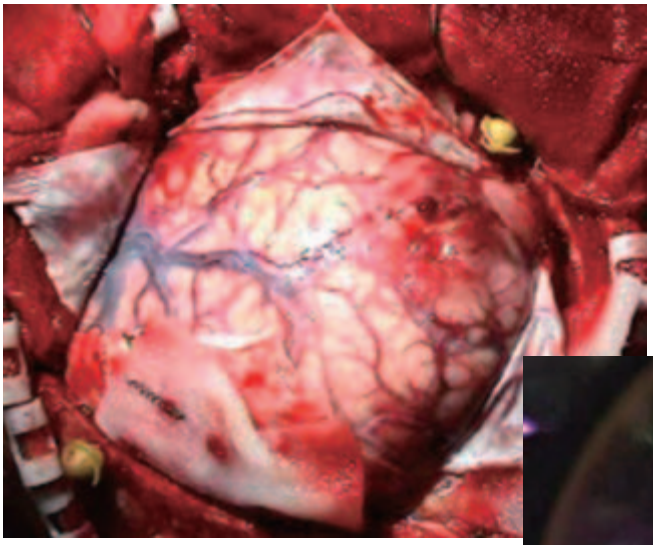
Protoporphyrin IX accumulation after ALA administration



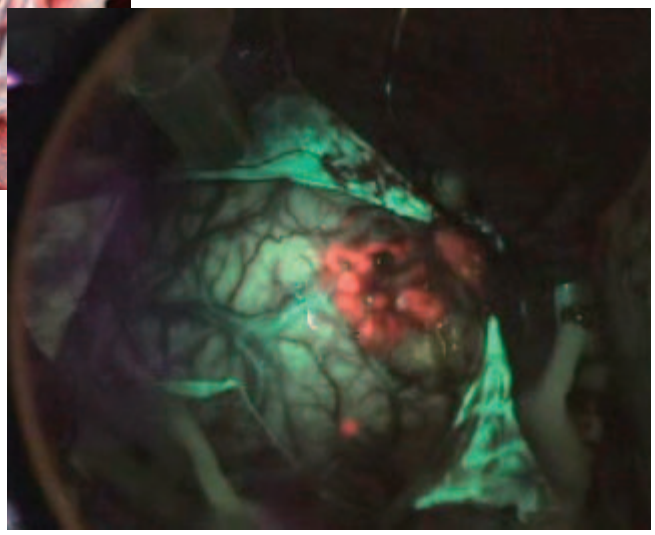
Diagnosis of brain tumor using ALA

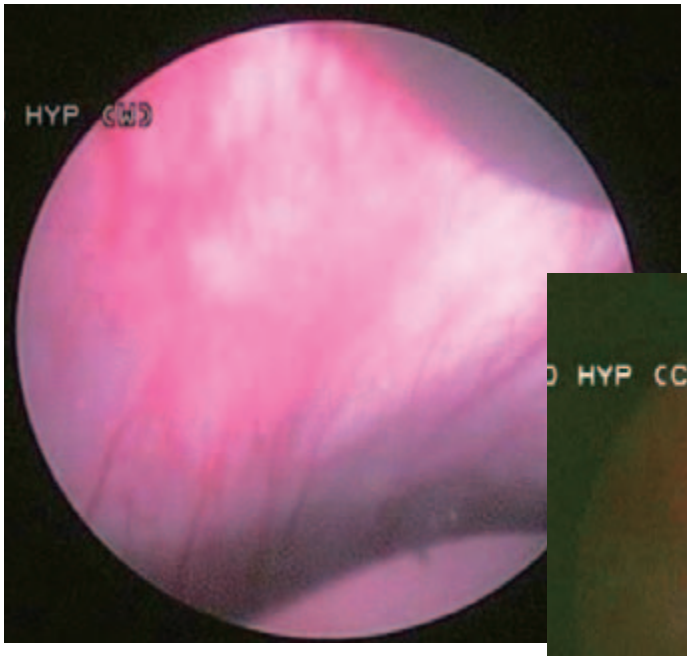




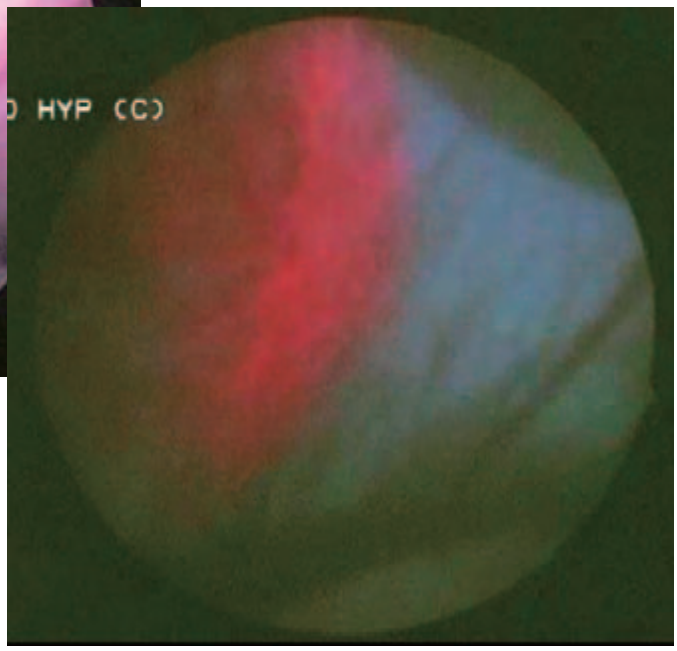


ALAを用いた
脳腫瘍の術中診断

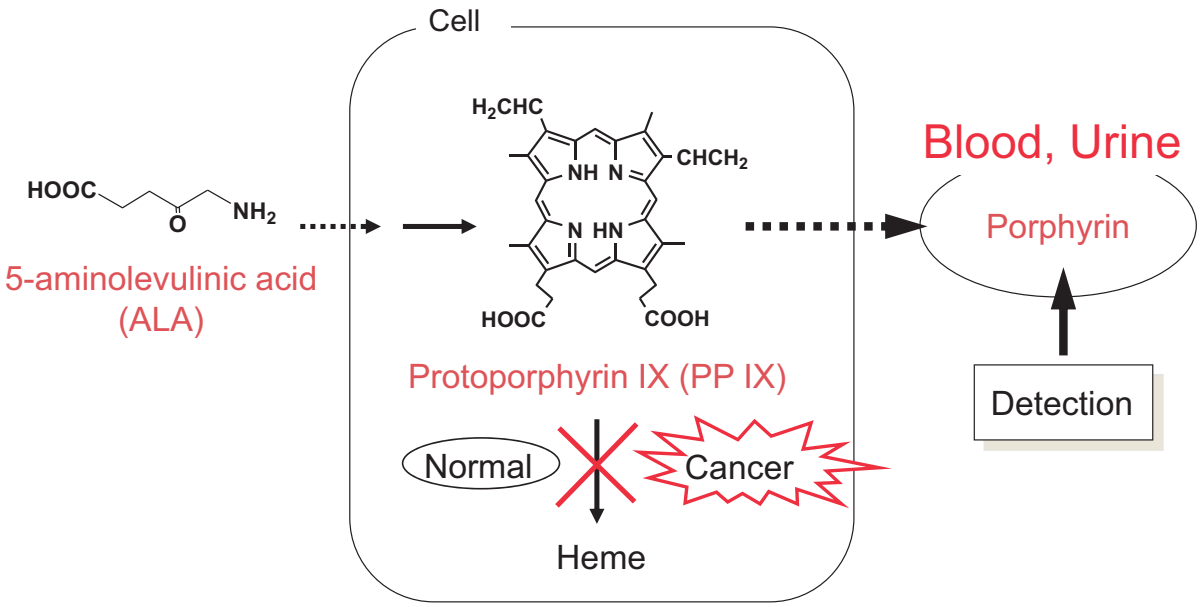




ALAを用いた
膀胱癌の術中診断



Analysis of porphyrins after administration of ALA



Fluorescence spectra of plasma

Mice (C3H/He strain, 5-weeks, female)

← MH134 cells (1 x 10^6 Cells) i.p.

10 days

← ALA (60 mM, 250 μl) i.p.

2 h

Blood drawing (10 ~ 20 μl)

↓

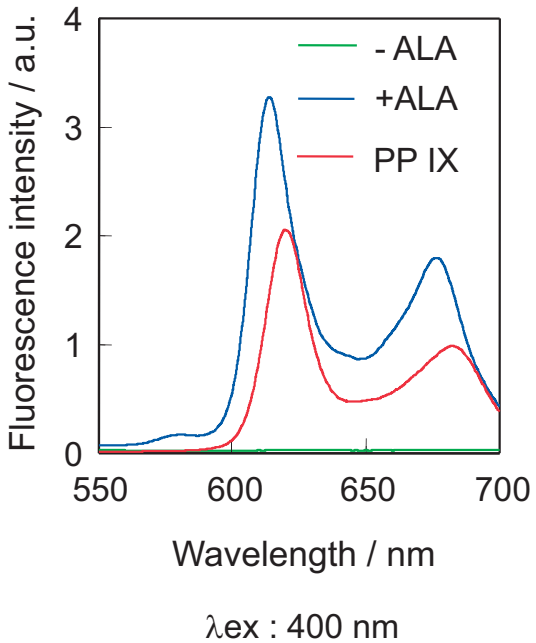
Centrifugation (2000 x *g*, 5 min)

↓

Collecting plasma

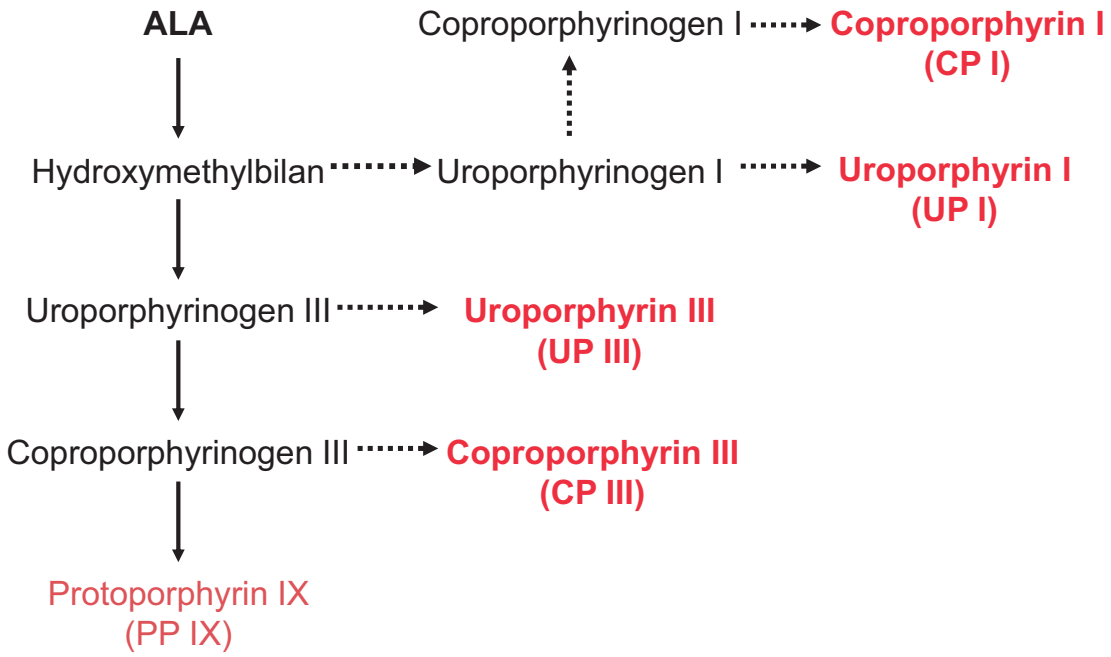
↓

Measurement or Analysis

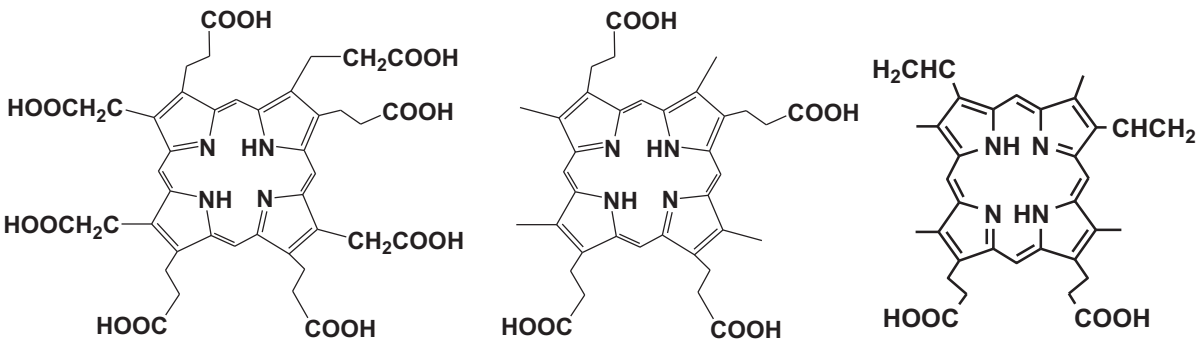


λex : 400 nm

Formation of porphyrins



Formation of porphyrins



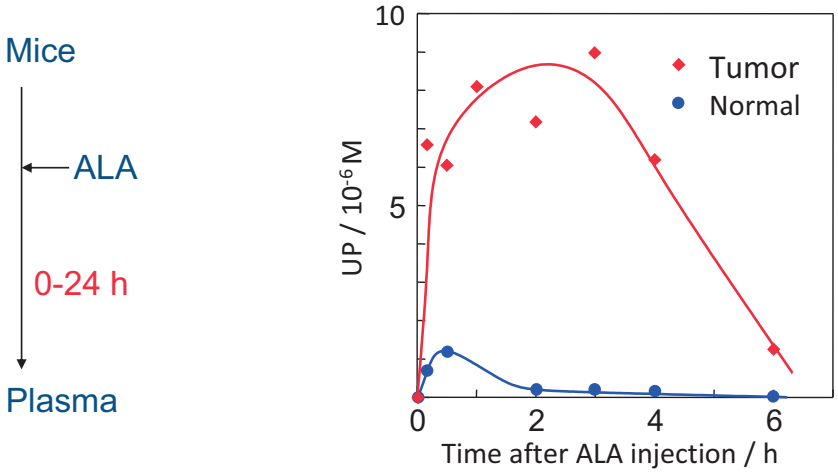
UP III CP III PP IX

Carboxyl group

8 4 2

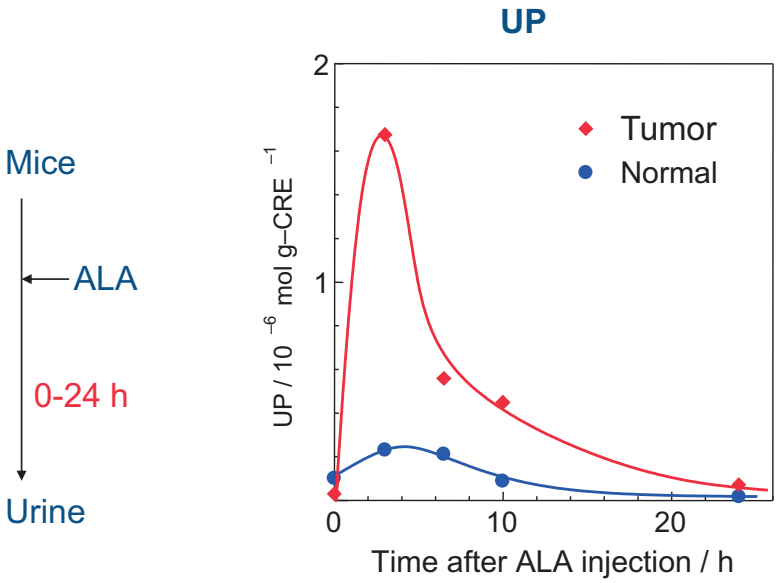
Hydrophobic

Time course of uroporphyrin in mouse plasma



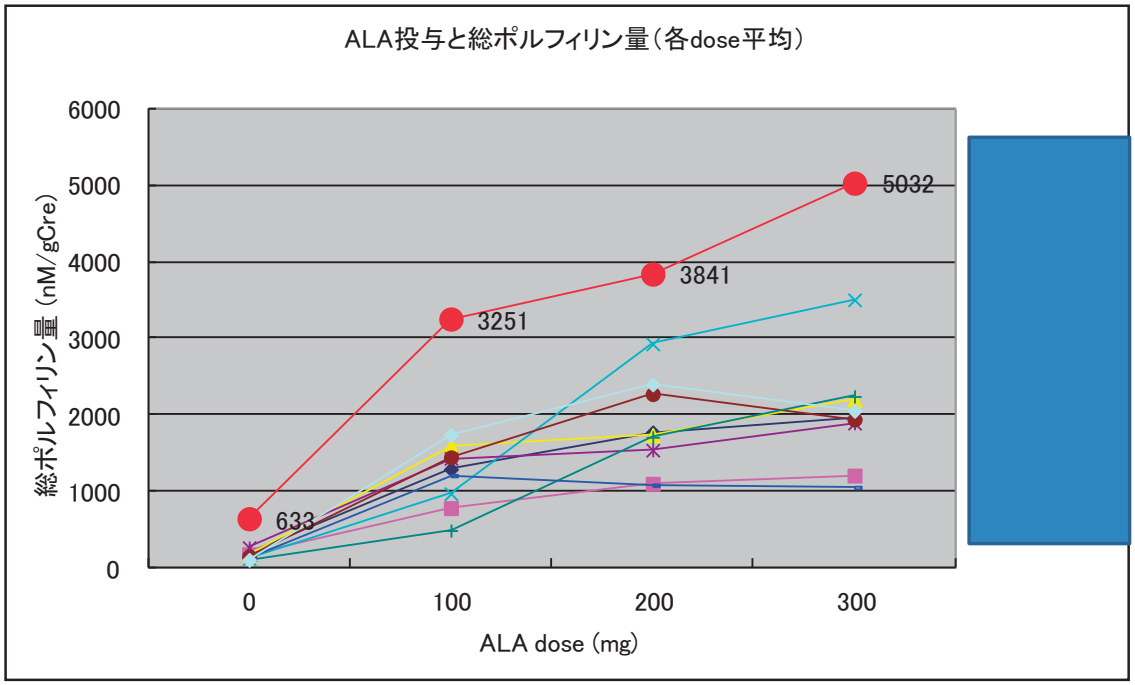
UP in plasma after administration of ALA have a possibility to be tumor marker.

Time course of uroporphyrin in mouse urine



（λex : 395 nm, λem : 617 nm）

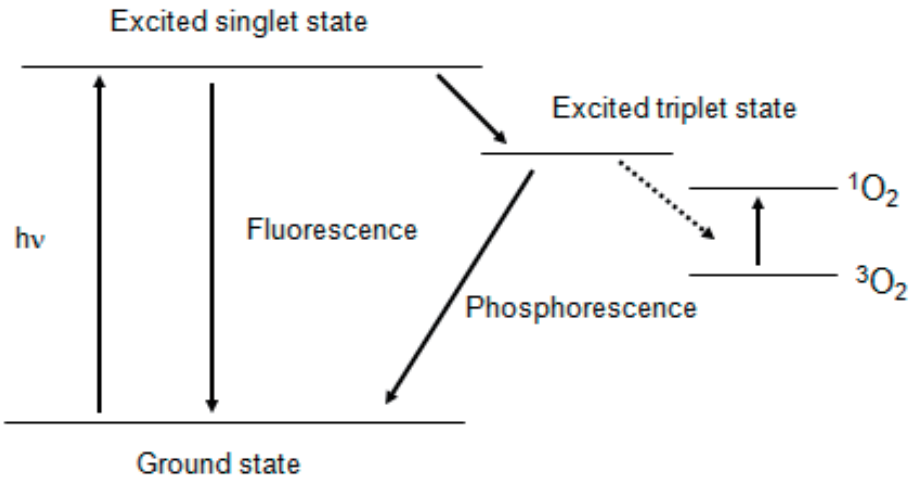
UP in urine after administration of ALA have a possibility to be tumor marker.



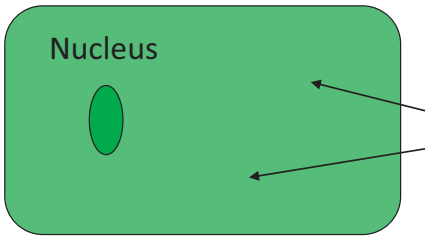
ポルフィリンの蛍光、りん光の利用
細胞内の情報を得るために

蛍光を利用するがんの検出
細胞内の酸素濃度測定

Energy diagram



Measurement of oxygen concentration distribution in a living cell



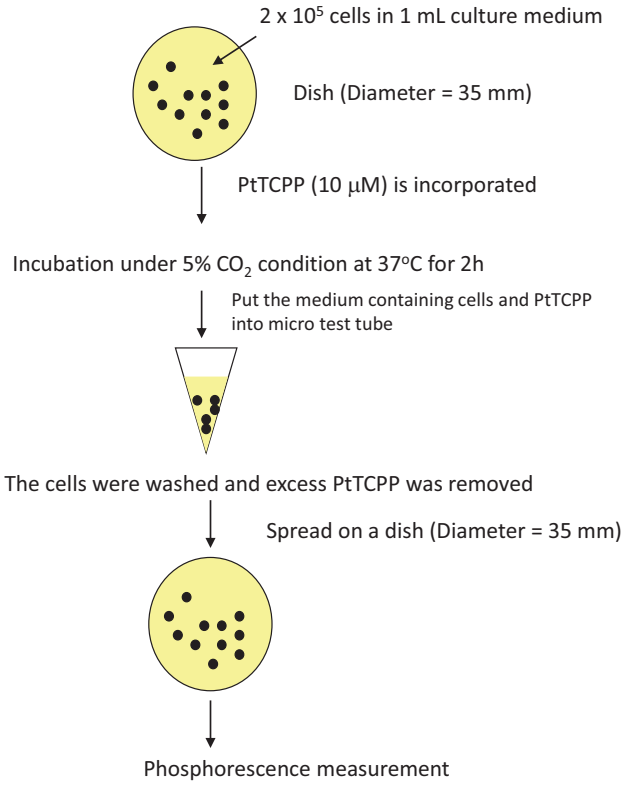
Cell

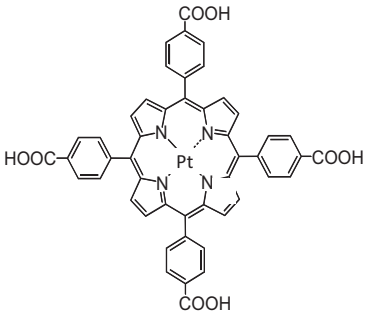
Oxygen concentration may be different at cytoplasm, cell membrane, nucleus.

Oxygen concentration imaging system for single cell is developed.

Pt-porphyrin is incorporated in living cell
→ optical oxygen sensing technique is developed

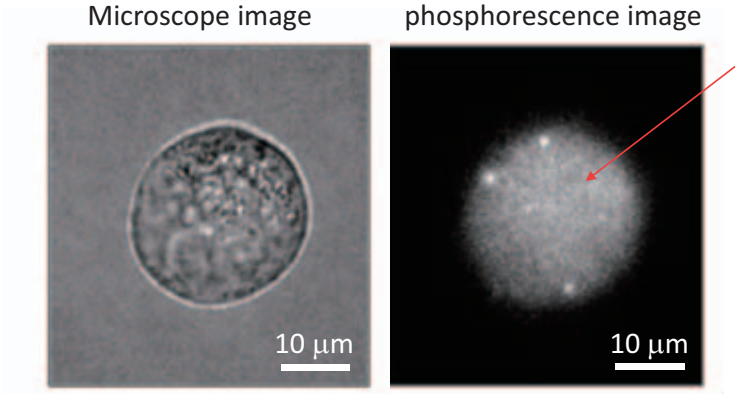
Uptake of Pt-porphyrin by MH134 cell





Pt(II)-tetra-(carboxyphenyl)-porphyrin (Pt-TCPP)

Application of oxygen sensing in a living cell



(MH134 cell in which Pt-porphyrin was taken)

Problems on phosphorescence Intensity measurement

Intensity depends on concentration of porphyrin as well as oxygen concentration

Lifetime is independent of concentration of porphyrin

Lifetime measurement is suitable for oxygen sensing inside cell

Comparison of phosphorescence intensity and lifetime measurement

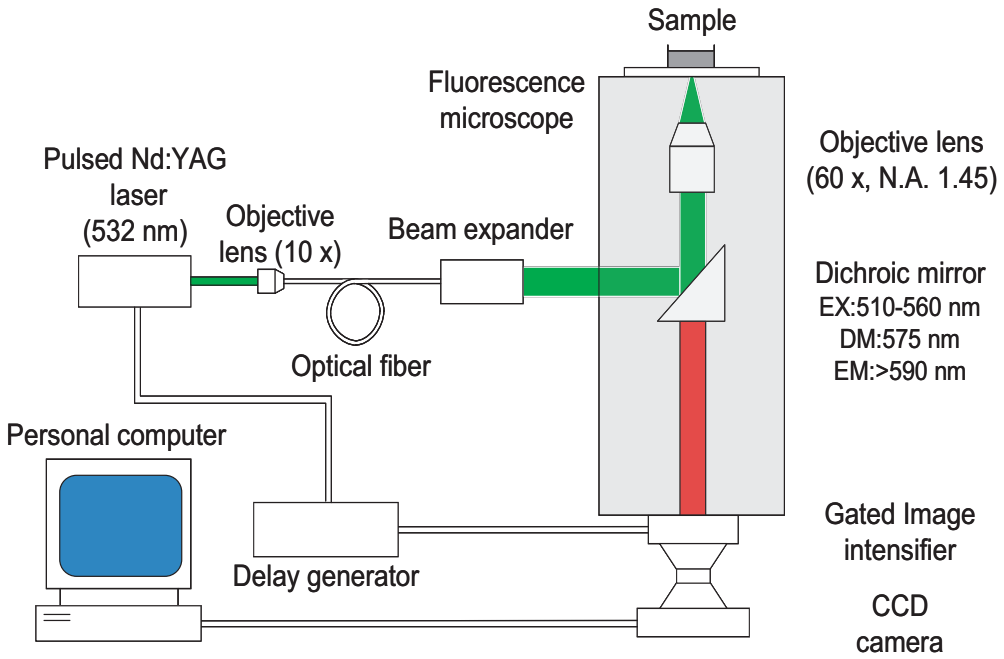
Intensity measurement

- Advantage
  - Easy to measure
- Disadvantage
  - Depend on the concentration of luminophore
  - Depend on the excitation source intensity
- Application
  - Homogeneous surface such as wind tunnel model

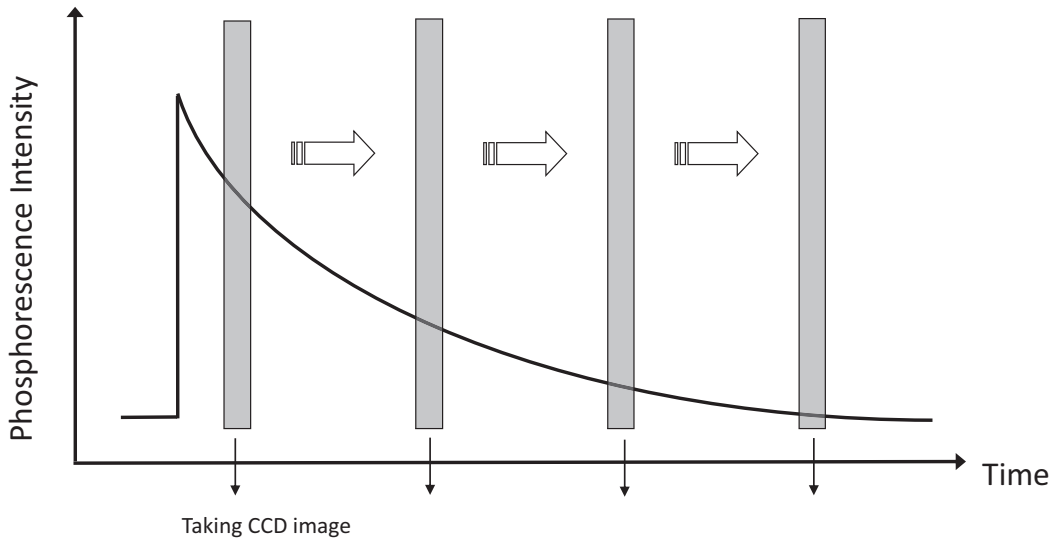
Lifetime measurement

- Advantage
  - Independent of the concentration of luminophore
  - Independent to the excitation source intensity
- Disadvantage
  - Rather complex measurement system
- Application
  - Suitable for intracellular oxygen imaging

Lifetime measurement system combined with microscope and laser flash

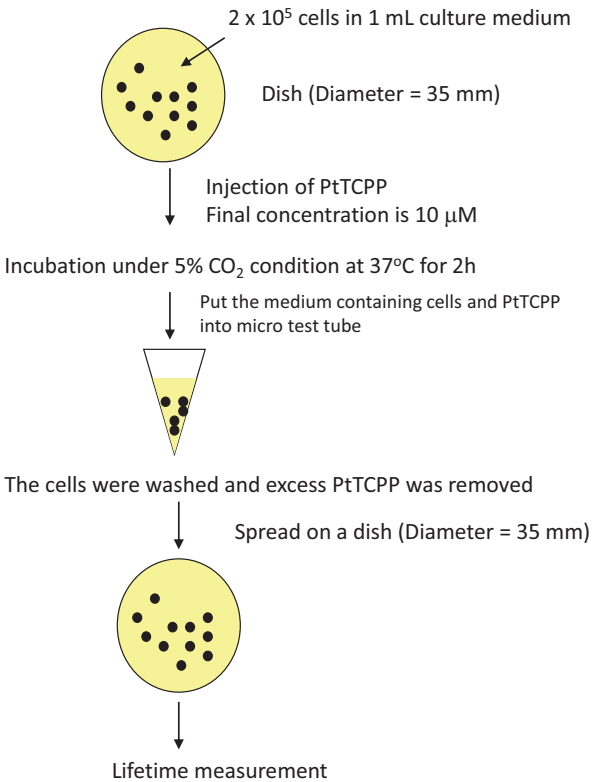


Measurement of phosphorescence lifetime



Time resolved phosphorescence intensity ⇒ Time dependence of the intensity ⇒ Calculation of Lifetime

Lifetime measurement



Pulse laser wavelength : 532 nm

Pulse laser frequency: 6 kHz

Pulse laser power: 6.75 mW

Pulse width: 10 ns

Detected wavelength: >590 nm

Exposure time of CCD camera : 6.08 s

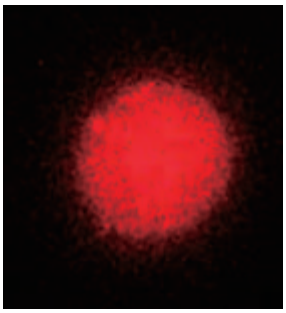
Gating periods of Imaging intensifier: 1 μs

Delay time : 0.5 μs (the first sampling data is 0.5 μs after laser pulse irradiation)

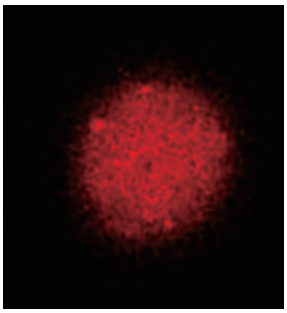
Sampling intervals : 2 μs (0.5 ～28.5 μs)

Time resolved phosphorescence image

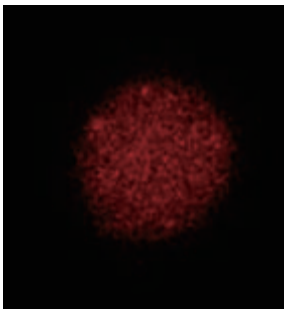
0.5 μs
(after pulse laser irradiation)



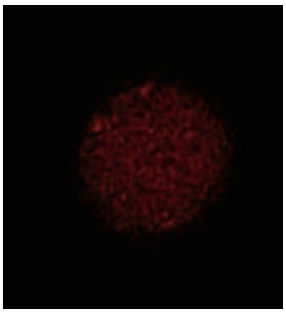
4.5 μs



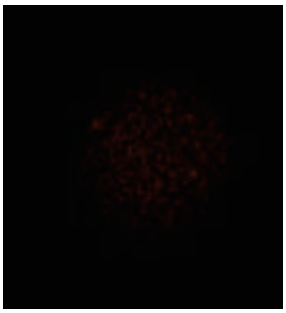
10.5 μs



14.5 μs

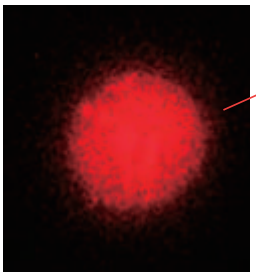


22.5 μs

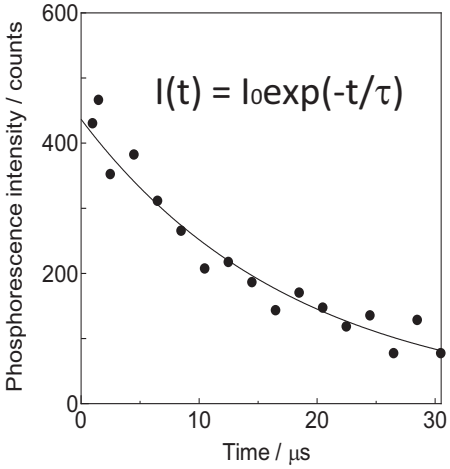


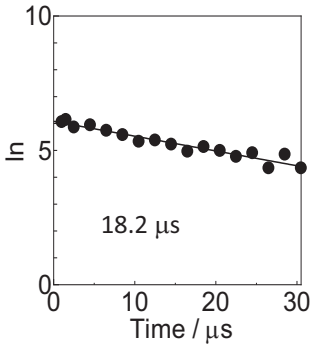
Calculation of phosphorescence lifetime

This picture consists of 1024 x 1024 pixels



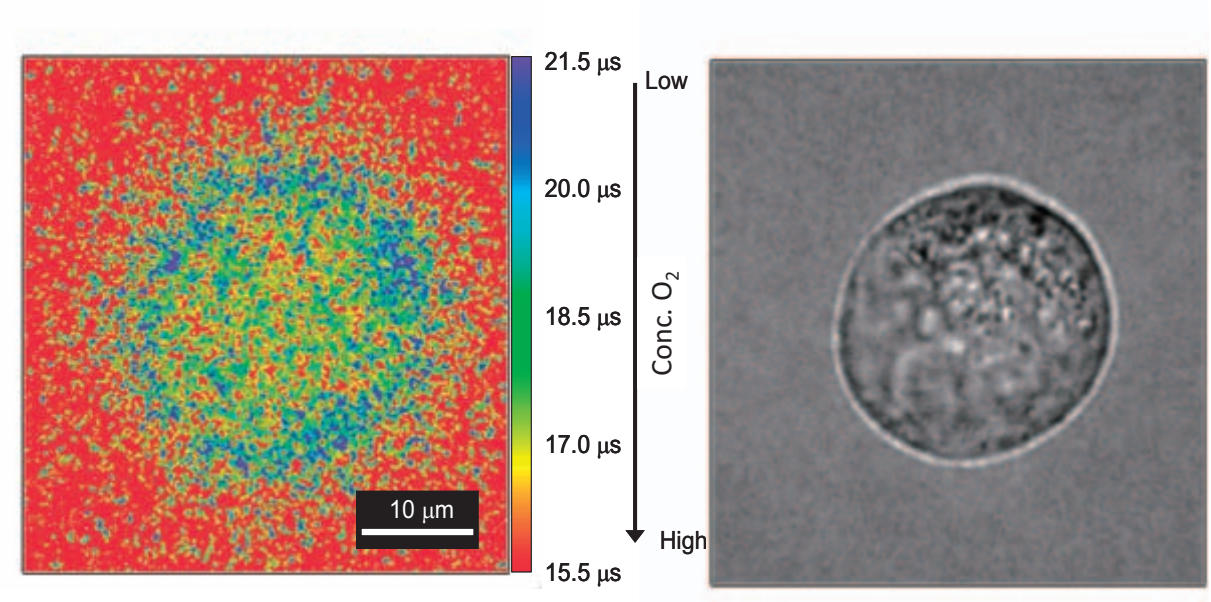
Time dependence of phosphorescence intensity at one pixel.





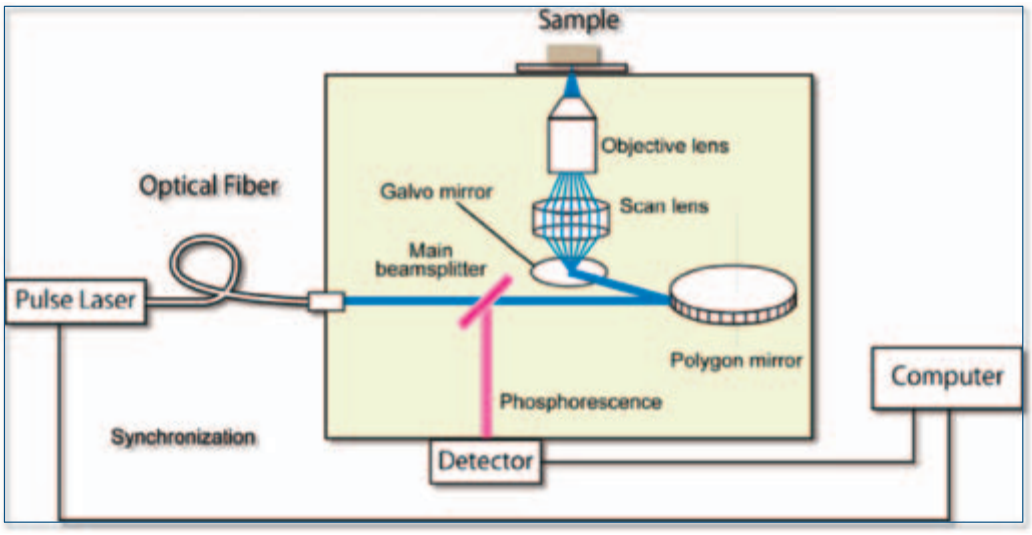
Lifetime imaging is obtained from the lifetime calculation at each pixel (1024 x1024)

Oxygen concentration imaging in a single cell



Phosphorescence lifetime imaging microscope (PLIM)

- *Phosphorescence lifetime is measured under conforcal microscope*

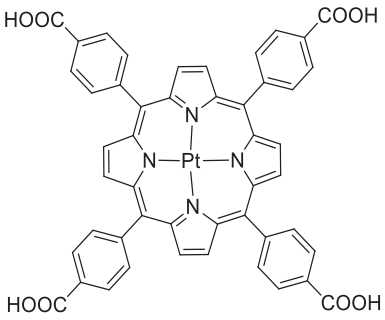


O_2 concentration response of the cancer cell

Colon-26 (Mouse rectal cancer)
- Incubated with 50 μM PtTCPP for 6 h.
- Wash with PBS(-) twice
- RPMI 1640 media

$[O_2]$ imaging was obtained under different O_2 concentration

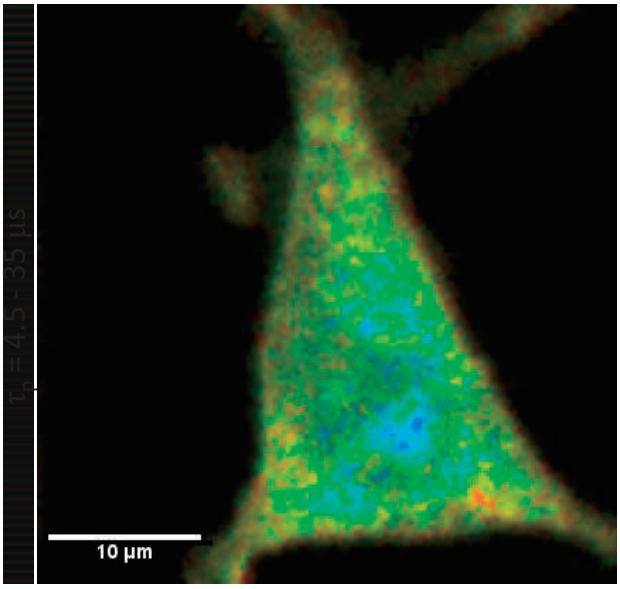
Microscope environmental chamber



PtTCPP

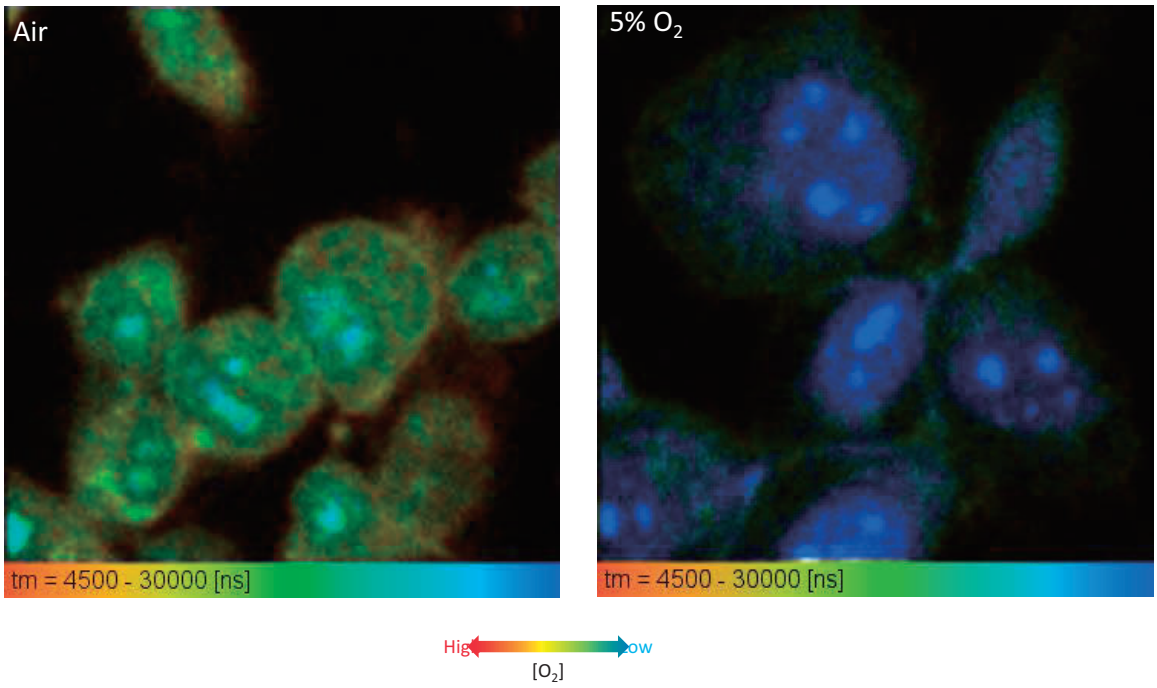
Phosphorescence lifetime imaging microscope (PLIM)

- Imaging of a single cell



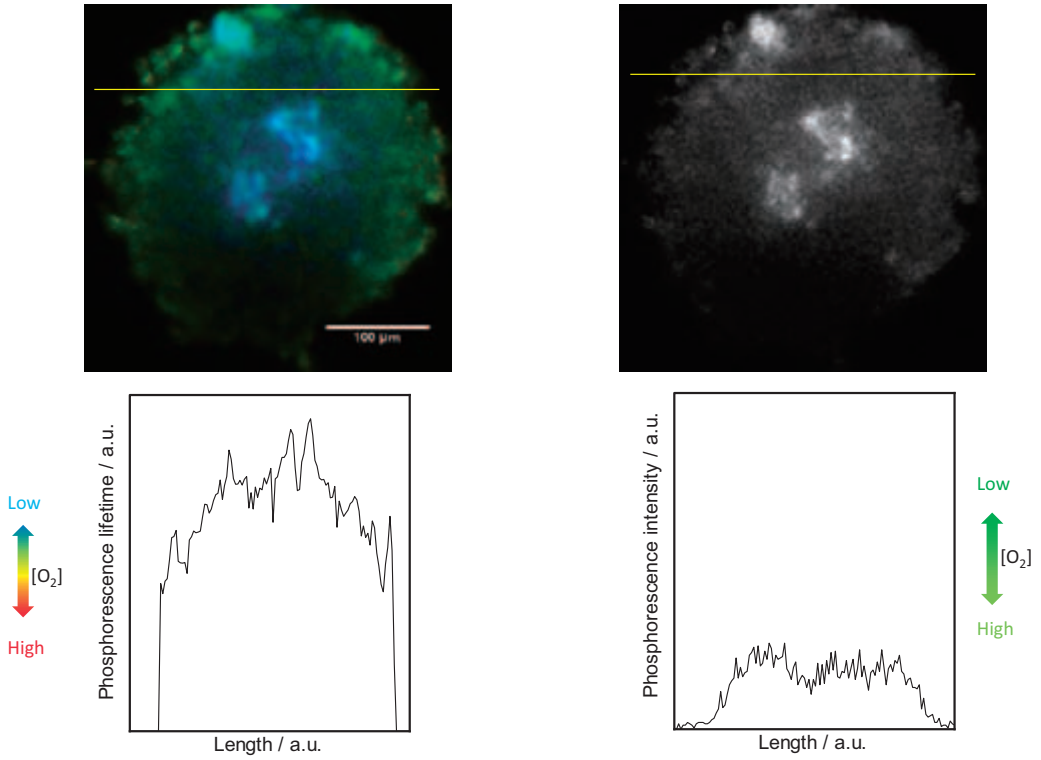
High ⟷ Low
O_2 concentration

O_2 concentration response of the cancer cell

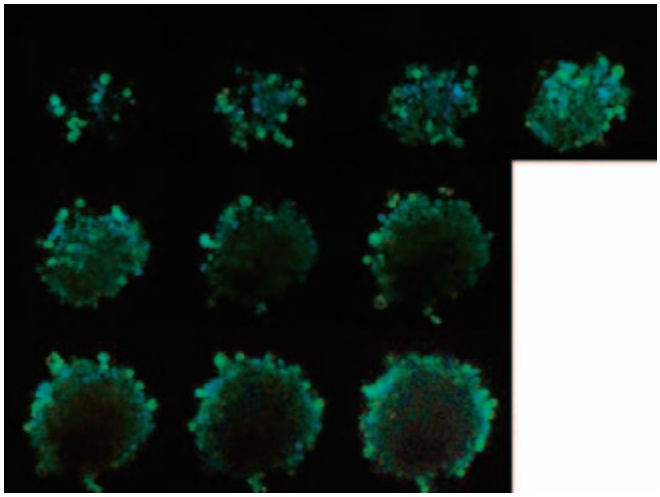


Comparison of phosphorescence intensity and lifetime measurement

Phosphorescence Lifetime **Phosphorescence Intensity**

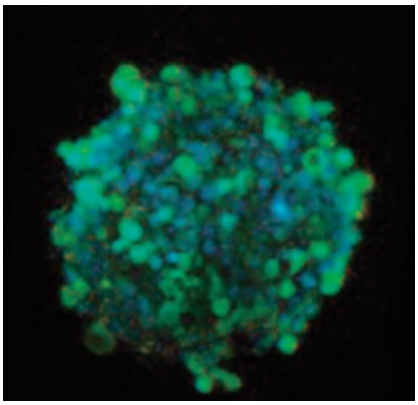


Z stack images for spheroid of Colon-26



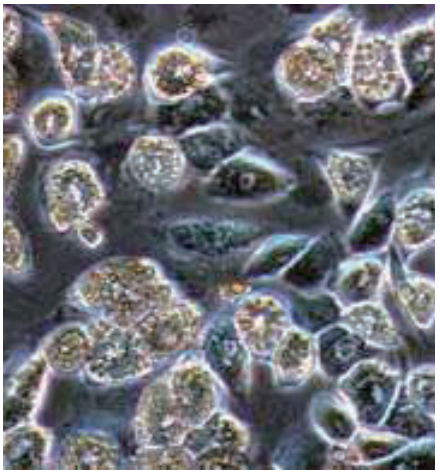
Z stack images were collected at 10 μm intervals from bottom to top of spheroid.

Colon-26 was incubated with RPMI1640 + 2% B27 supplement in a ultra low attachment plate (Corning Costar)

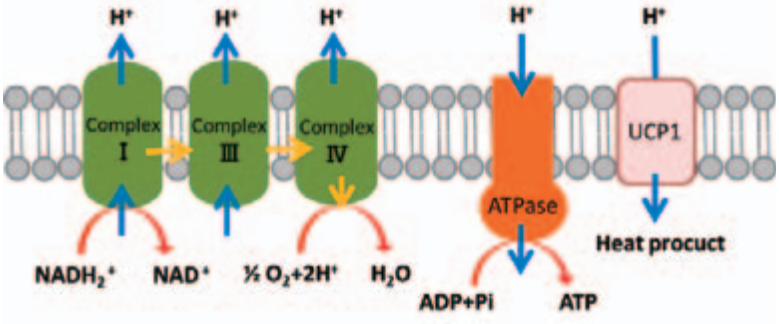


A merged z stack image of Colon-26 spheroid

- Brown adipose tissue (BAT)
  - one of two types of fat or adipose tissue
  - primary function is to generate body heat
  - contain numerous smaller lipid droplets and a much higher number mitochondria



Brown



Mitochondrial inner membrane

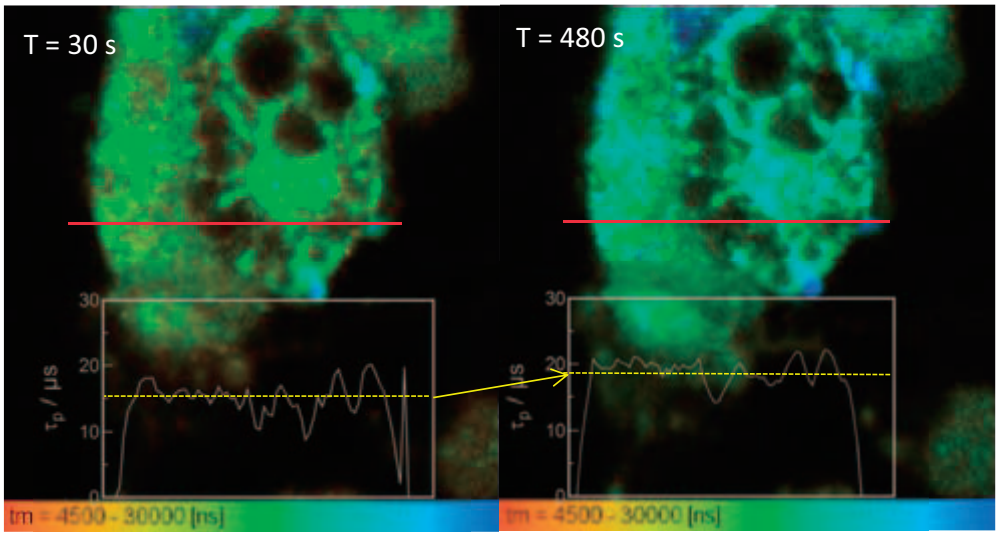
  - UCP1 plays a role in generation of heat

$[O_2]$ consumption of using brown adipocytes

Differentiated adiocyte

- Incubate with 10 μM PtTCPP, 4h
- Wash with PBS(-) twice
- Wash with PBS(-), addition of 5 μM CCCP

$[O_2]$ imaging was obtained



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Preparation of oxygen sensing beads

Solvent evaporation method

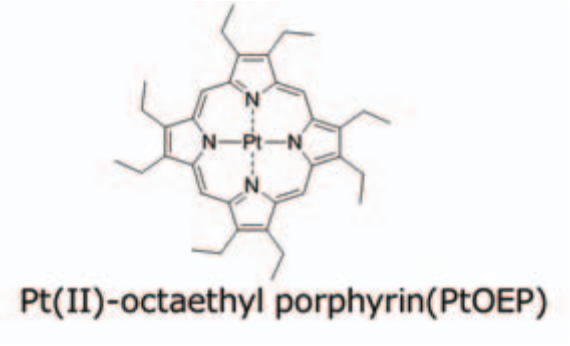
5 g/L PVA aq. 700 mL

CH_2Cl_2 130 mL

← 6.5 μM PtOEP, PS in CH_2Cl_2 10 mL

Vigorous stirring for 20 h, in dark

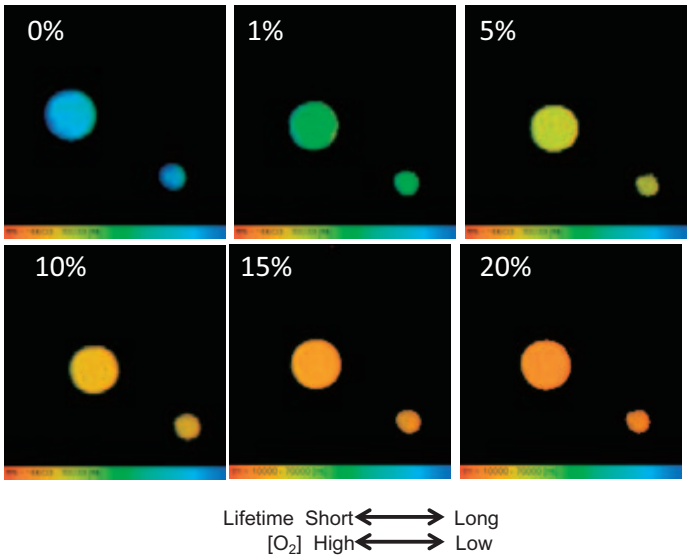
Washed by centrifugation x 3 (3500 x g, 5 min)



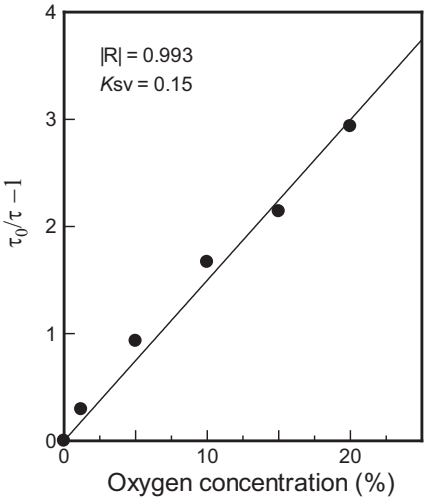
Pt(II)-octaethyl porphyrin(PtOEP)

Oxygen sensing beads

Oxygen response



Stern-Volmer Plot



Simultaneous measurement of intracellular and extracellular oxygen concentration