

細胞凝集体の培養環境の三次元酸素モニタリング -光環境センシング-



NAGOYA UNIVERSITY

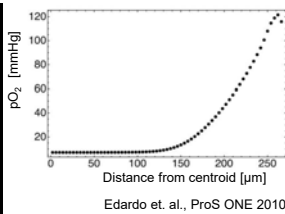
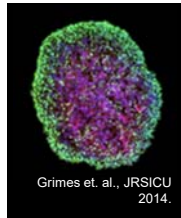
○秋田 祐甫, 丸山 央峰, 益田 泰輔, 小俣 誠二, 新井 史人
名古屋大学大学院工学研究科マイクロ・ナノ機械理工学専攻



色素拡散により, 長時間・安定に酸素濃度を計測する!

Background : Needs for fluorescent sensor

Necrosis inside spheroid

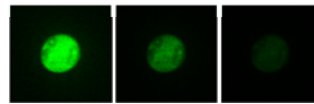


Cells undergo necrosis inside large sized spheroid, because of the lack of O_2 and metabolism.

To avoid necrosis, some design concepts are proposed such as vascularized spheroid and toroidal spheroid.

To determine whether **oxygen** and metabolism are sufficient inside spheroid. Measurement method is needed.

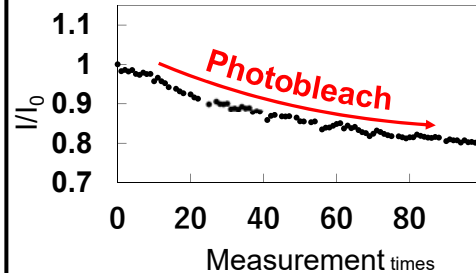
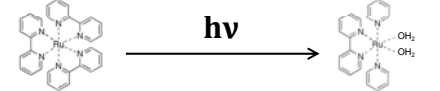
Fluorescent sensor



O_2 concentration

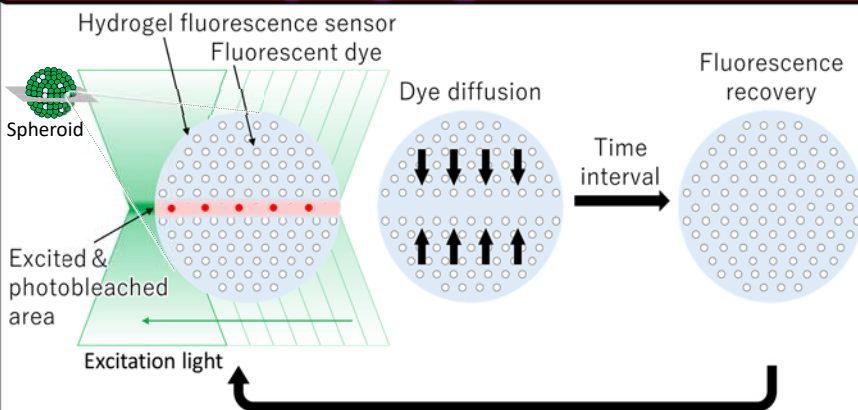
Fluorescent sensor contains fluorescent dye of which the fluorescence change according to the environment such as O_2 concentration, pH, etc.

Problem : photobleach



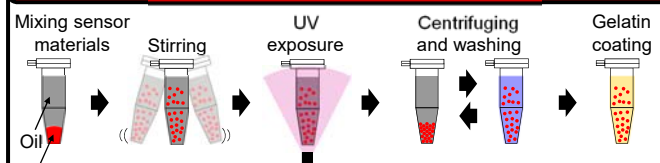
Fluorescent intensity of decreased 20% after 100 times of measurement. (time interval 10 min)

Solution concept : hydrogel fluorescent sensor



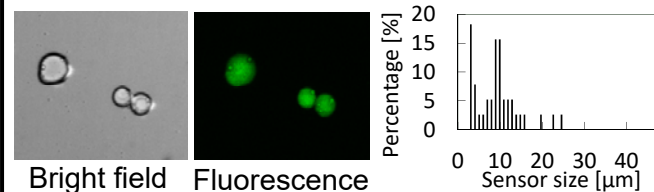
During the measurement, fluorescence dye around focal plane of excitation light is degraded (photobleach). After the measurement, by stopping the light exposure and having time interval, non-degraded dye around photobleached area diffuse to the area. After the time interval, the fluorescence of the sensor is recovered.

Sensor fabrication

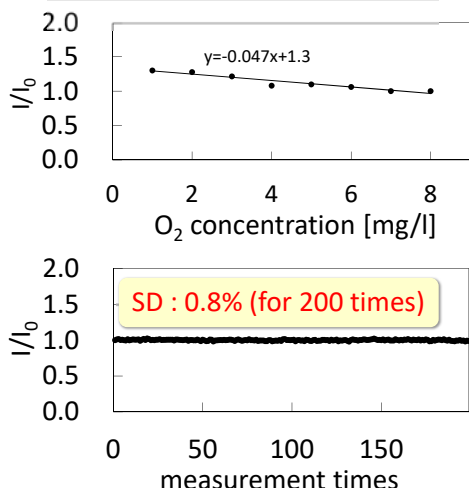


Sensor materials

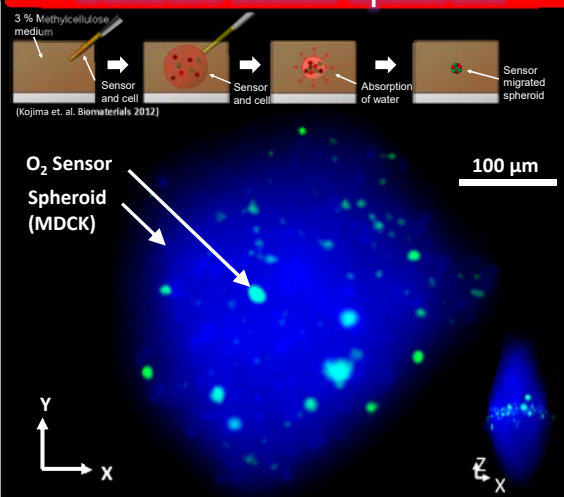
- Polyethyleneglycol diacrylate : Biocompatible and hydrophilic polymer
- $Ru(bpy)_3Cl_2$: fluorescent dye which is sensitive to oxygen (Ex. light 480 nm, Em. light 620 nm)



Sensor evaluation



Sensors inside spheroid



Conclusion and future work

Conclusion

Fabrication of hydrogel fluorescent sensor that can recover fluorescence after photobleach.
(S.D. = 0.8 % for 200 times of measurement)
(0.17 mg/l in O_2 concentration)

Future work

1. Measurement of inside spheroid
2. Homogenization of sensor size
3. Multi-parameter measurement