

蛍光酸素センサを用いたオンチップ単一卵子酸素計測



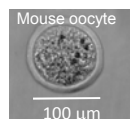
○鬼頭雅伸¹, 丸山央峰², 新井史人²
¹名古屋大学大学院工学研究科機械理工学専攻
²名古屋大学大学院工学研究科マイクロ・ナノシステム工学専攻



新方式: 一つの卵子の呼吸活性を非接触で計測する

Background

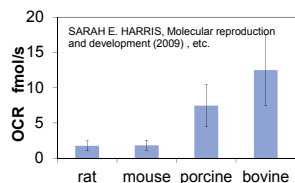
Evaluation of oocyte quality



Indicator of quality evaluation

- Morphological evaluation
- Mechanical characteristics
- **Oxygen consumption rate (OCR)**

Comparison of OCR of mammalian oocyte

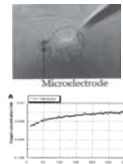


Amount of consumption depends on each mammalian (1 fmol/s ~ 20 fmol/s)

High sensitive oxygen measurement method is required!

Concept

Scanning electrochemical microscope (SECM)

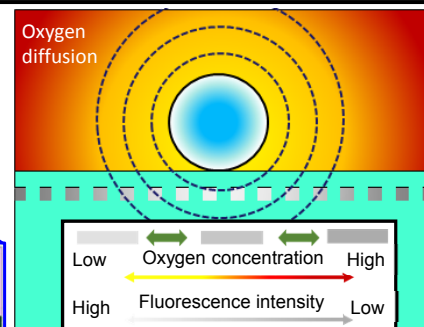
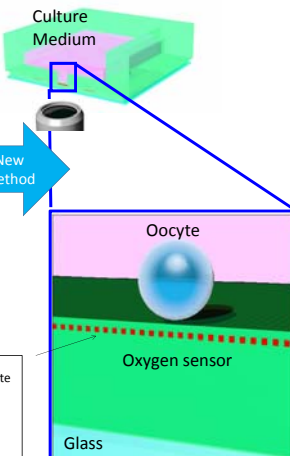


Y. Kumasako et al. Ova research. 2013

Problems:

- Damage to Oocyte.
- Probe is easily broken
- Time-Consuming (Several Minutes)

Tris(2,2'-bipyridine) dichloro-ruthenium(II) hexahydrate (Ru(bpy)₃Cl₂) + PEGDA + Pure water (ex. 561 nm, em. 620 nm) Photo-bleaching is less than 0.1% for 32s



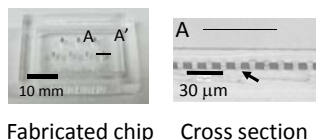
Non-contact measurement
Measurement time
 : several seconds

Stripe shaped
 → Spherical diffusion theory is applicable!

Sensor chip fabrication

Fabrication process

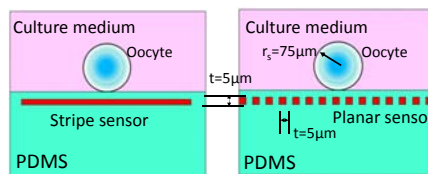
1. Make SU8 pattern
2. Put on aluminum jig on the substrate
3. Make PDMS mold
4. Inject ruthenium solution
5. Cut out PDMS
6. Bond chamber layer



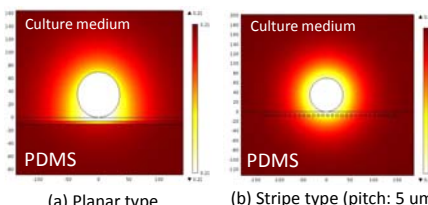
Fabricated chip Cross section

FEM analysis

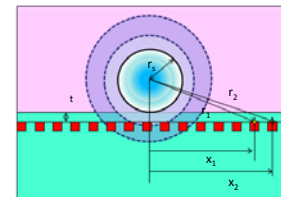
Parameters	Value
Bulk oxygen concentration	0.21 mol/m ³
Diameter of oocyte	75 μm
Oxygen consumption rate	1.0 × 10 ⁻¹⁵ mol/s
Oxygen diffusion coefficient in solution	2.18 × 10 ⁻⁹ m ² /s
Oxygen diffusion coefficient in PDMS	2.18 × 10 ⁻⁹ m ² /s
Oxygen diffusion coefficient in sensor	3.4 × 10 ⁻¹¹ m ² /s



Steady state analysis



(a) Planar type (b) Stripe type (pitch: 5 μm)



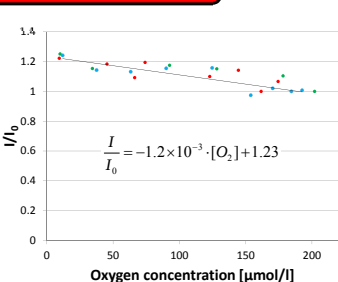
Oxygen Consumption Rate

$$OCR = F = 4\pi r_s^2 \cdot D \frac{dC(r_s)}{dr}$$

$$r = \sqrt{(r_s + t)^2 + (x)^2}$$

C: Oxygen concentration [mol/m³]
 r: Distance from center of cytoplasm [m]
 r_s: Radius of cytoplasm [m]
 t: Distance between sensor and cytoplasm [m]
 x: Distance from center of cytoplasm on image [m]

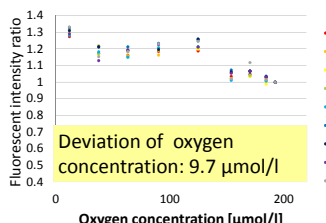
Calibration



I: Fluorescent intensity (measured value)
 I₀: Fluorescent intensity (saturated value)

Comparison of sensor sensitivity

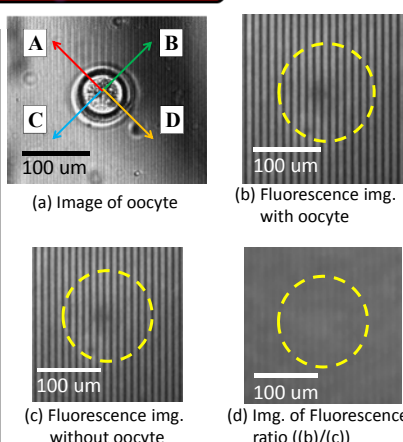
SECM (radius of electrode=5 μm)	Our sensor (by using 16 bit CCD)
0.01 μmol/l	0.016 μmol/l



Experimental condition:

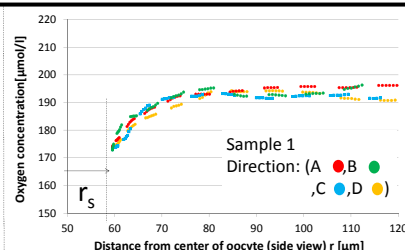
Temp.: 37 °C, CO₂: 5 %
 Laser: 561nm
 Regulating concentration of saturated solution : Na₂SO₃

Experiments



Conclusions

1. OCR can be calculated based on spherical diffusion equation
2. This method achieve measurement of two-dimensional distribution of OCR (sensitivity : 0.016 μmol/l) in culture by acquiring the fluorescent image (Average OCR: 0.39 ± 0.20 fmol/s, Divergence of OCR: 0.1 ± 0.08 fmol/s)



Profile of oxygen concentration at each direction
 OCR distribution of each oocyte

Sample	OCR at each direction fmol/s				Average OCR fmol/s	Divergence of OCR fmol/s
	A	B	C	D		
1	0.58	0.56	0.60	0.64	0.59	0.03
2	0.53	0.23	0.66	0.48	0.47	0.18
3	0.21	0.34	0.31	0.39	0.31	0.07
4	0.07	0.29	0.22	0.11	0.19	0.10

