細胞温度計測のための
単一蛍光マイクロセンサの細胞内導入

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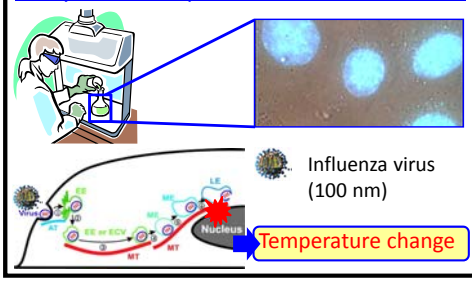
細胞核内にゲルセンサを導入して機能を探る!

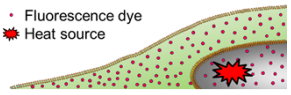
1. Background

Analysis of virus proliferation in cell nucleus



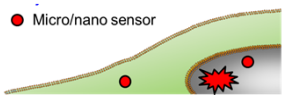
Intracellular measurement

1. Staining cell by fluorescence dye



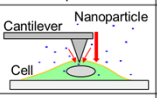
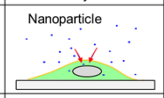
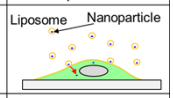
Simultaneous measurement of whole cell

2. Injection of sensor into cell



Measurement of local condition
Low stimulus

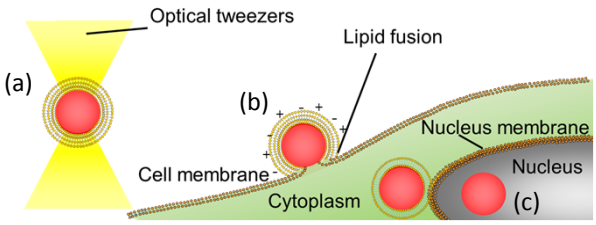
Conventional cell injection methods

Injection Method	Stanporation	Endcytosis	Lipofection
Schematic image			
Injection of um object	Yes	Yes	Yes
Damage to cell	Yes	No	No
Injection to individual cell	Yes	No	No

Selective and invasive injection is required.

2. Concept

Photo-induced selective injection process



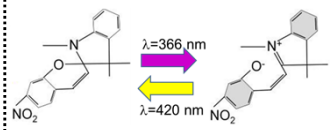
Injection process

(a) Preparation of liposome containing single sensor by spontaneous transfer method

(b) Selective adhesion of liposome to cell membrane by optical tweezers and optically-induced charge control

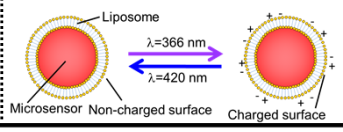
(c) Injection of sensor into nucleus by membrane fusion

Photoreaction of Spyropiran (SP)



UV illumination
→ Charged structure
VIS illumination
→ Non-charged structure

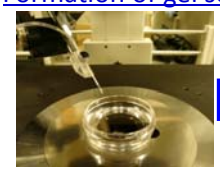
Photo-induced control of cell adhesive of liposome

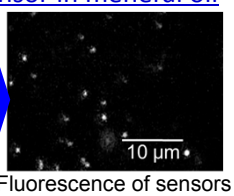


UV illumination
→ Cell-adhesive
VIS illumination
→ Non-adhesive

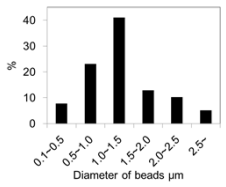
3. Methods

Formation of gel sensor in meneral oil

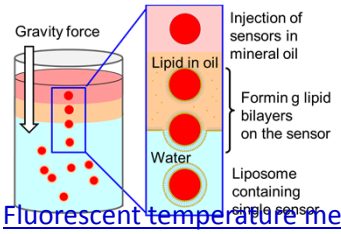




Gel generation system　Fluorescence of sensors



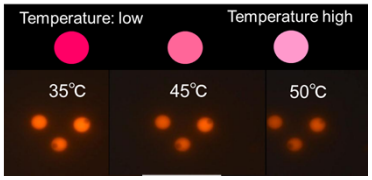
Formation of liposome containing single gel-sensor

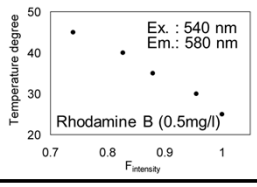


・Hydrogel contents:
Photo resin made of PEG
(ρ of PEG: 1.02 g/cm^3)
+Rhodamine B

・Liposome contents:
1mM lipid (DOPC : DOPE = 1 : 1)
+10 mM spyropiran
(ρ of mineral oil: 0.85 g/cm^3)

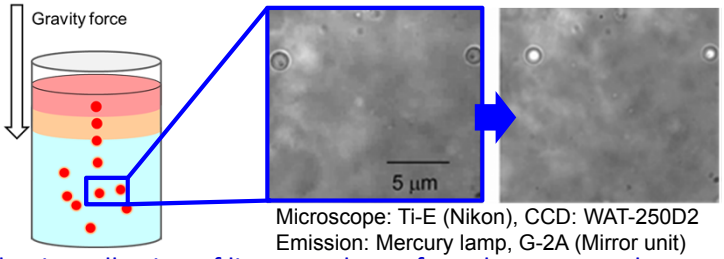
Fluorescent temperature measurement





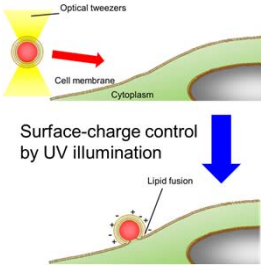
4. Experiments

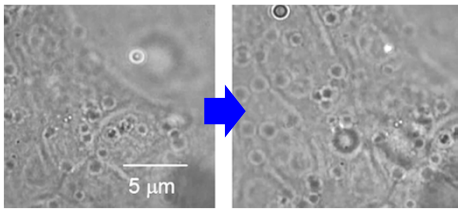
Liposome containing single sensor



Microscope: Ti-E (Nikon), CCD: WAT-250D2
Emission: Mercury lamp, G-2A (Mirror unit)

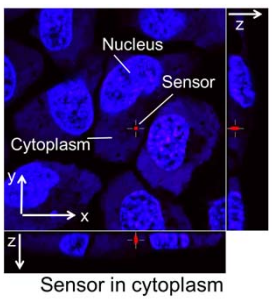
Selective adhesion of liposome by surface charge control



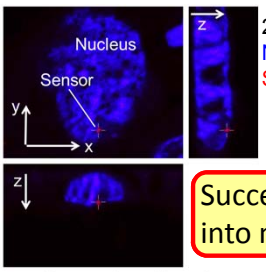


Microscope: Ti-E (Nikon), CCD: WAT-250D2
Laser: 1 W, 1064 nm, Target cell: MDCK cell

Injected sensor in cytoplasm or nucleus



Sensor in cytoplasm



Sensor in cell nucleus

20 hours after adhesion
Nucleus: Hoechst 33342,
Sensor: Rhodamine B

Success of injection into nucleus

5. Conclusions and future work

1. Preparation of liposome containing single sensor
2. Selective injection of gel-sensor into cell nucleus

6. References

H. Maruyama et al, "Functional gel-microbead manipulated by optical tweezers for local environment measurement in microchip", Microfluid Nanofluid, vol. 6, no.3, pp. 383-390, 2009..

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