FUNDING PROGRAM FOR NEXT GENERATION WORLD-LEADING RESEARCHERS

Project Title: New Instrument system to evaluate mammalian embryos and stem cells based on single-cell analysis	
Name: Hitoshi SHIKU	**Institution**: Tohoku University

1. Background of research

In 2010, Dr. Robert G. Edwards received Nobel Prize for the development of the in vitro fertilization (IVF). In Japan, there are issues on aging society and decreasing birthrate. Although it has been significant progress in assisted reproductive technologies (ART), the success rate of pregnant based on in vitro culture (IVC) and IVF is still low. IVF and IVC are key technologies not only for clinical application but also for effective production of domestic animals. Many technical challenges have been carried out to improve the embryo quality that can ensure a high rate of pregnancy. Currently, however, embryo quality is judged based on morphological observation. It is required integration of data set obtained from different hierarchical layers, i. e. epigenetic, genetic, transcriptional, protein, and metabolic stages.

2. Research objectives

In this program, I will construct a new system combining multi-functional probes and probe-positioner with high spatial resolution based on scanning probe microscopy (SPM) technologies, that allows various cellular functions at single cell level. I am going to apply the developed instrument for multi-functional analysis of mammalian embryos and embryonic stem cells.

3. Research characteristics (incl. originality and creativity)

So far, we have invented a unique method to non-invasively evaluate the quality of individual mammalian embryos based on oxygen consumption. A Pt microelectrode was scanned near the single embryo sample to obtain oxygen concentration profile. Respiration activity of single embryo was estimated based on spherical diffusion theory. Further, it was found that the respiration activities of individual embryos corresponded the developmental potential of the embryos. Independently, we have developed a procedure of mRNA quantification from single-cell based on SPM featuring multi-functional probes. Next, we are going to combine the two methods mentioned above for quality control of mammalian embryos and embryonic stem cells.

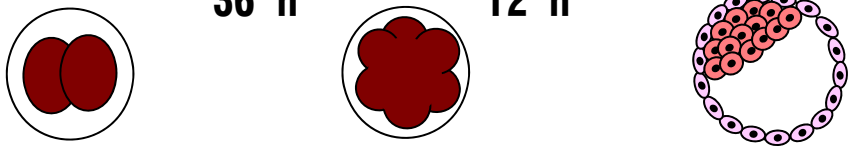
4. Anticipated effects and future applications of research

I hope our technique will contribute to the elevation of preciseness of the quality judge of embryos and embryonic stem cells.

Non-invasive Evaluation of Embryo Quality by Scanning Probe Microscopy (SPM)*

Mouse Embryo

2-cell → (36 h) → morula → (12 h) → blastocyst

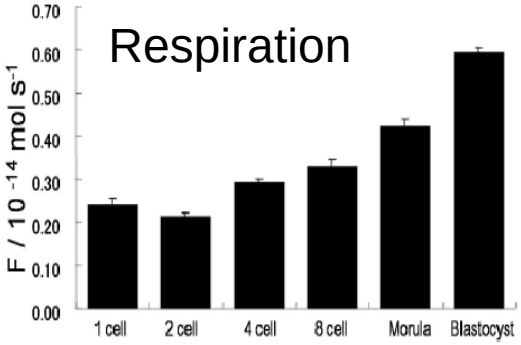


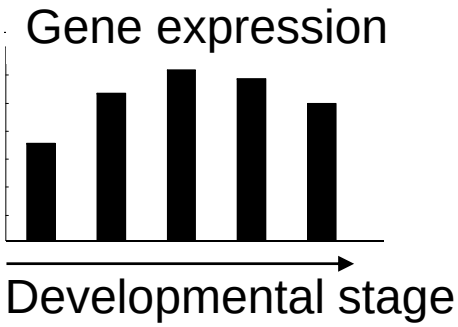
Oxygen Consumption F [mol s^{-1} embryo^{-1}] = $4\pi r_s D \Delta C$

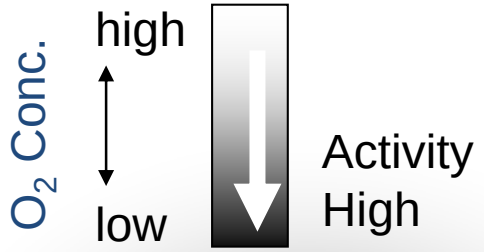
Oxygen concentration $\Delta C = C^* - C_s$

= 0.8 ~ 9.3 μM

High sensitivity





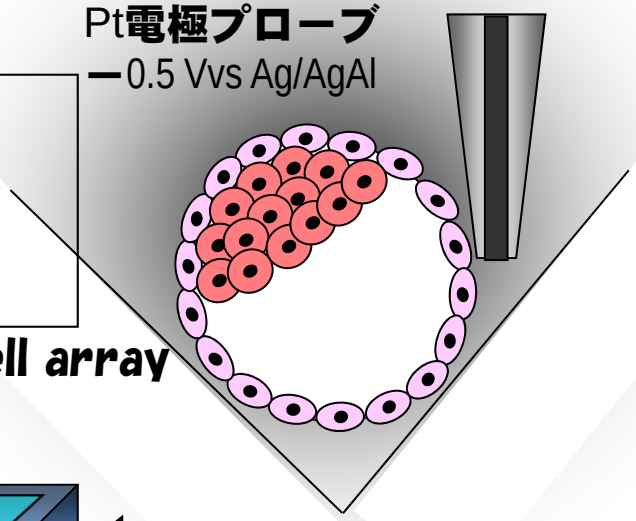


Developmental stage

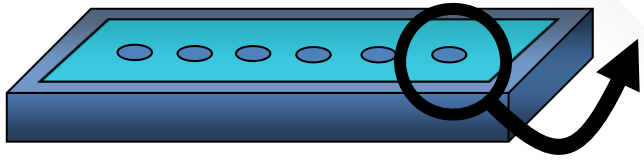
N=238

One-by-one plot of Respiration & Gene-expression activities have not yet accomplished.

Pt電極プローブ
−0.5 Vvs Ag/AgAl



Cone-shaped microwell array

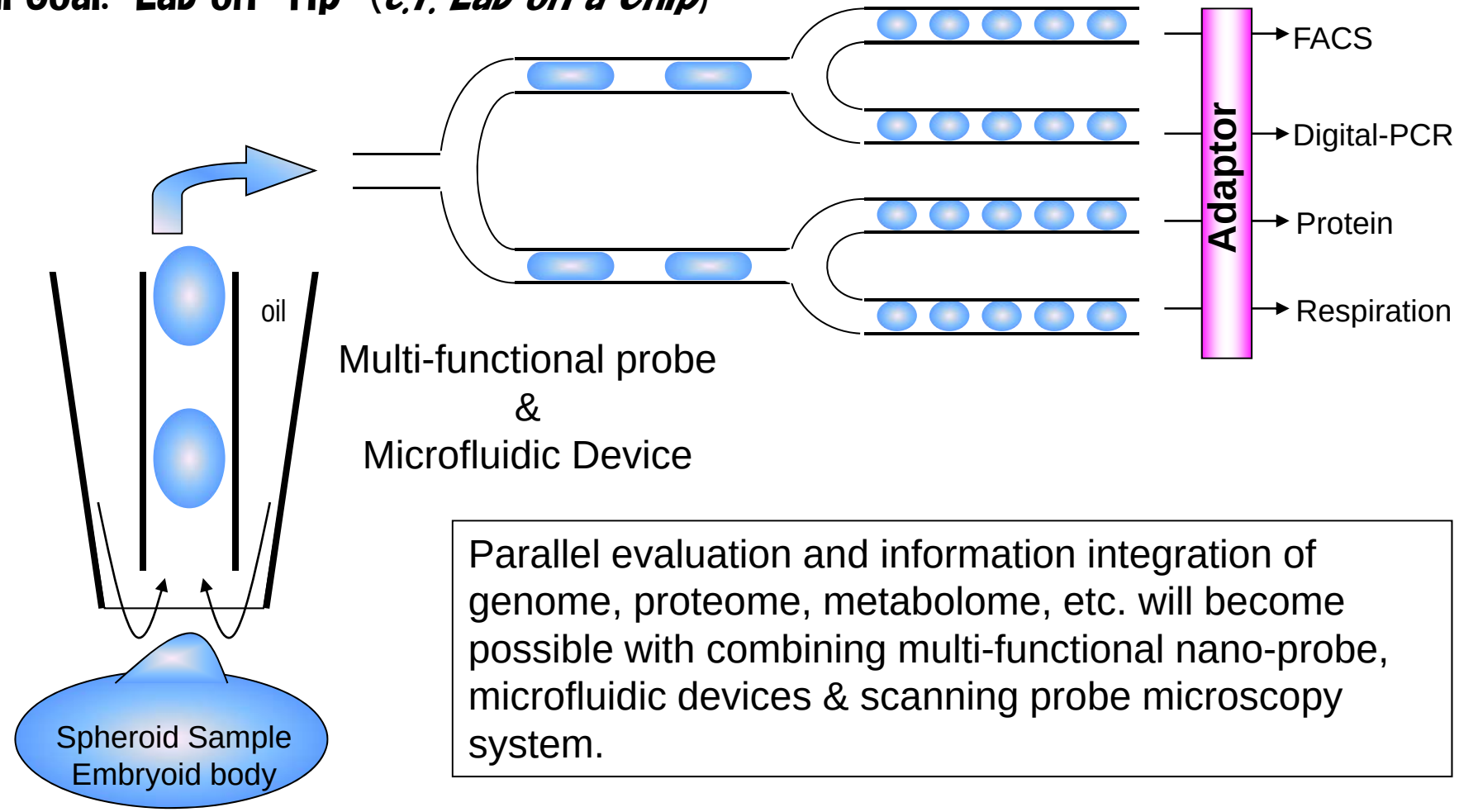


* *Young Scientist's Prize* for the Commendation of Science and Technology by the Minister of Education, Culture, Sports, Science and Technology (*MEXT*) (2009)(Shiku)

* Ichimura Academic Award (2009)(Abe&Shiku)

*Shiku et al. ***Anal.Chem*.2001,**73,3751-3757

Final Goal: Lab on "Tip" (*c.f. Lab on a Chip*)



Parallel evaluation and information integration of genome, proteome, metabolome, etc. will become possible with combining multi-functional nano-probe, microfluidic devices & scanning probe microscopy system.

Objective

Quality control of mammalian embryos (mouse, bovine etc.)

One-by-one plot of respiration & gene-expression activities