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種別(和文)	論文要旨
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（博士課程）
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論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	Information and Communications Engineering	系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of	(Philosophy)
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

The atmosphere is full of various odor molecules that not only influence the behavior of animals but also transmit essential information. Hence, the sensors that detect various odorants are extremely important. The creatures' olfaction has even better sensitivity, selectivity, and shorter response time than conventional gas sensors. Thus, researchers intended to create multiple biosensors to fully utilize the advantages of animals' olfactory system. Among them, the biosensors based on cell expressing OR not only have good sensitivity and selectivity but also are suitable for imitating the real olfactory system. The biosensors based on cell expressing OR (Olfactory Receptor) were first explored in liquid phase twenty years ago and liquid phase biosensors have been well developed. Considering that most of odorants exist in the atmosphere and the gas-phase odorant detection is more similar to the olfaction in the majority of animals, the gas-phase odor biosensors based on cell expressing OR have larger application scenarios and are more meaningful. However, there are many problems that need to be settled urgently at the current stage. In this study, we aimed to fabricate a gas-phase odor biosensor based on cell expressing OR with good sensitivity, simple operation procedures, and long lifetime.

A liquid phase odorant detection system has been established in our laboratory. The optical module and FPGA in that system were used in later experiment. A new chamber, gas channel and modified control part were required to extend the liquid phase into gas phase. The new chamber allowed OR response under less than 1 ml target odorant as well as simplified the experiment process. A new gas-phase odor biosensor was ready for tests.

We extracted the cell region from the raw image with CHT (Circle Hough Transform) to avoid the noise from the background area. Then noise level was decreased by freezing the cell radius. To evaluate the property of our biosensor, we applied reference gas and target odorant to the cells. It demonstrated mechanical stimulation would not influence the magnitude of fluorescence and Or13a could respond to target odorant stimulation. We evaluated the stimulation duration dependency, liquid layer thickness dependency, detection limit, selectivity, and odor concentration dependency of our biosensor. We raised a hypothesis towards the cell inhibition phenomenon that emerged under high concentration of 1-octen-3-ol headspace vapor stimulation. In addition, a human sensory test was conducted.

Its results demonstrated our gas-phase odor biosensor has better sensitivity than human olfaction in detection of 1-octen-3-ol when the time for gas exposure is short.

To extend the biosensor lifetime, we first maintained the liquid thickness. To fulfill this, the feedforward control was easy to implement but the optimal liquid supply speed was influenced by many environmental factors thus liable to fail. Feedback control could adjust the liquid compensation speed according to the real-time liquid thickness thus more stable and precise liquid thickness control could be realized. The EO (Electro-Osmotic) pump was small and friendly for control. Unfortunately, it only can work with non-conductive liquid, i.e., pure water here. Infusing pure water as the compensation liquid gave rise to fluctuation in the fluorescent curve. On the other hand, the syringe pump was able to inject conductive or non-conductive liquid. When using the syringe pump to add Ringer's solution into the cell area, the cell state remained stable for a long period despite fluorescent brightness decrease owing to fluorescence protein photobleaching. However, the biosensor lifetime had no significant difference with or without liquid thickness control.

The reason for no extension in biosensor lifespan was odorant molecules accumulation in assay buffer media. Thus, we introduced liquid exchange formed by two pumps. Finally, the biosensor lifetime could be prolonged from 2500 s to 11500 s. When different liquid exchange speeds were applied, larger liquid exchange speed always brought better OR response. Furthermore, we employed intermittent liquid exchange to enhance the biosensor sensitivity. The odor concentration dependency curve was better than no liquid thickness control.

To further improve our biosensor system. We projected to maintain the limit liquid thickness. According to the impedance variation curve, controlling limit liquid thickness at limited thickness was more difficult than thicker level. From the OR response under various impedance set points, we knew that a thinner liquid layer benefited not only magnitude of response but also the response time. This experiment results agreed with our conclusion in liquid layer thickness dependency part. To reach thin liquid film, increasing from low to high impedance step by step was better than directly setting a high impedance. The odor concentration dependency result obtained in this condition was even better than previous one under liquid thickness control and liquid exchange condition. However, the cell inhibition appeared again because of thin liquid film. Also, the long term experiment could not be executed under limit liquid thickness.

In this thesis, a gas-phase odor biosensor was manufactured. It was easy to operate, only required a tiny amount of target odorant to trigger OR response, had long lifetime and could respond to ligand stimulation for multiple times. The fundamental method to extend lifetime of odor biosensor in the gas phase was established.