論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	COMPACT ODOR SENSING SYSTEM BASED ON INSECT OLFACTORY RECEPTORS AND FLUORESCENT INSTRUMENTATION ROBUST AGAINST DISTURBANCE
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出典(和文)	学位:博士（工学）, 学位授与機関:東京工業大学, 報告番号:甲第10321号, 授与年月日:2016年9月20日, 学位の種別:課程博士, 審査員:中本 高道,岩本 光正,山田 明,間中 孝彰,宮島 晋介,神崎 亮平
Citation(English)	Degree:, Conferring organization: Tokyo Institute of Technology, Report number:甲第10321号, Conferred date:2016/9/20, Degree Type:Course doctor, Examiner:,,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

専攻： Department of	Physical Electronics 専攻	申請学位（専攻分野）： Academic Degree Requested	博士 （ Engineering Doctor of ）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Thesis Summary

COMPACT ODOR SENSING SYSTEM BASED ON INSECT OLFACTORY RECEPTORS AND FLUORESCENT INSTRUMENTATION ROBUST AGAINST DISTURBANCE

Recently, the demands on various odor detection system in many areas of application are growing significantly. The development of odor sensor based on olfactory receptor (OR) of human or animal olfaction system is a promising approach for realizing highly selective and highly sensitive sensor since the performance of a current artificial sensor is insufficient. Among the techniques available in biosensor transducer, fluorescence microscopy offers advantages of noninvasive technique with high sensitivity. However, the main problem of this technique is the presence of ambient light when the measurement is not performed in dark condition since the intensity of fluorescence light is very weak. A lock-in measurement technique can be applied to the system to acquire a weak fluorescent signal buried under the strong ambient light and noises. Furthermore, a compact, low power, and robust measurement system is very demanding. The purpose of the research is to develop a portable fluorescent instrumentation system robust against ambient light using lock-in measurement technique.

The system consists of a cell based biological sensing element, an optical transducer, a lock-in amplifier, and a microfluidic system (MFS) chamber. Sf21 cell expressing the insect OR and calcium ion (Ca^{2+}) indicator fluorescent protein was used as sensing element. The bindings of odorant molecules by the OR triggers the increase of intracellular Ca^{2+} concentration since the OR and co-receptor forms an ion channel. The fluorescent protein is used to monitor the activity of Ca^{2+}. The higher odorant concentration is, the more Ca^{2+} influx is. Then, the increase of Ca^{2+} concentration makes the cell emit higher fluorescent intensity when it receives the excitation light. The optical system is used to provide the cell sensor with the proper blue (488nm) excitation light from laser diode and to capture the emitted green (512nm) fluorescent light on a complementary metal–oxide–semiconductor (CMOS) camera. Processing the fluorescent intensity and performing the lock-in technique were done using Matlab on PC. Cell immobilization and odorant exposure were performed in an acrylic chamber for static system and a MFS made of glass and polydimethylsiloxane

(PDMS) for flow system. The cell immobilization was done by letting the cell be adsorbed firmly at the base on the chamber.

Both static and flow systems were evaluated in this study. First, several experiments were performed on the static system. Both cells, expressing Or56a and BmOR3, responded to geosmin and bombykal with concentrations from 5μM to 100μM, respectively. It was difficult to obtain the responses to odorant with concentration smaller than 5μM in static system since it was almost the artifact level due to odorant dropping.

Then, the apparatus for flow system consists of pump, micro pipe, flow chamber, solenoid valve and sink. Two types of pumps were used, micro-pump and syringe-pump. The applied flowrate of odorant should be kept less than 400μl/min to avoid the cell removal. Typical characteristics of micro-pump are small size and low power dissipation. Thus it is suitable for realizing a portable system. However, micro-pump cannot be used for the experiment on small concentration since it produces the artifact. Experimental results show that microfluidic system using micro-pump was suitable for measuring odorant concentration between 5μM and 100μM (or above). Syringe pump has precise and stable flowrate. However, it cannot be used for portable applications because of its weight and dimension. In low flowrate (100μl/min.), almost no artifact was produced by syringe pump. Syringe-pump was used to investigate the detection limit of the cells response to the odorant. Experimental results show that the detection limit of Or56a cell lines to the geosmin odorant was 100nM while the detection limit of Or13a cell lines to the 1-octen-3-ol odorant was 50nM in the dark condition.

A lock-in measurement technique was incorporated into the system to avoid the influence of the ambient light. In the ambient light condition, the CMOS camera captures not only the fluorescent light, but also unwanted light such as background, offset, and noise. Lock-in measurement technique consists of fluorescent signal modulator, high-pass filter (HPF), phase sensitive detector (PSD), reference signal, and low-pass filter (LPF). The fluorescent signal was modulated by the laser diode system. The other blocks were implemented offline using Matlab. Experimental results in dark condition show that application of lock-in measurement technique slightly improved the detection limit down to 50 nM geosmin concentration and 25nM 1-octen-3-ol concentration for Or56a and Or13a, respectively. Compared with dark condition, applying the lock-in technique to both low (500 flux) and high (1000 flux) ambient light conditions, the limits of detections were almost the same for both cell lines with their associate odorants. The improvement of the detection limit reached approximately three orders of magnitude under the ambient light condition. Without lock-in technique, the limit of detection deteriorates much in ambient light condition.

Several improvement should be made by implementing the image and lock-in technique processing on FPGA platform to improve the speed, power dissipation, and compactness in the near future. Moreover, the cells with two or more OR types can be captured in one image to form a sensor array. Thus the system can be used to implement a compact and sensitive odor sensing system.