Development of Partially-thinned LAPS Structures and Their Applications to Chemical Imaging and Analysis of Multiple Samples

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論 文 内 容 の 要 旨

Chapter 1: Introduction

The chemical imaging sensor based on the principle of the light-addressable potentiometric sensor (LAPS) has been a key technique for detecting the distribution of target ions in the sample on its sensing surface for some decades, which is expected to be an effective detection tool for chemical and biochemical analytes. In addition, it is considered as a highly potential multi-analyte, multi-sample sensor due to its possibility of spatially resolved detection of ion concentration on its sensing surface addressed by a focused light beam. The development of LAPS-based chemical imaging sensor responds to the demand of biochemical analysis in various fields such as environmental monitoring, clinical diagnosis and drug screening.

The spatial resolution and the measurement speed are both very important to the performance of the chemical imaging sensor, and much effort has been focused on improving them. It has been demonstrated that a thin sensor plate could achieve a higher resolution because of the restriction of carrier diffusion. It has been also demonstrated that a thin sensor plate could improve the signal-to-noise ratio at higher frequencies, which would be advantageous for high-speed measurements. However, the fragileness of a thin sensor plate has been a problem for practical applications.

In this thesis, partially-thinned structures of LAPS, which are advantageous in the spatial resolution, the measurement speed as well as the mechanical strength, are investigated. Furthermore, their applications and benefits in measuring a plurality of samples on a single sensor plate without the need of additional multi-chamber structures are studied.

Chapter 2: Partially-etched structure on the backside of LAPS sensor plate

A partially-etched structure of LAPS sensor plate was fabricated by optimization of an anisotropic etching process based on tetramethylammonium hydroxide (TMAH). Partially-etched structures were fabricated on n-type Si (100) substrates with a thickness of 200 μm, coated with insulating layers of 50 nm silicon dioxide (SiO_2) and 50 nm silicon nitride (Si_3N_4), respectively. The frame region with a larger thickness supports the

etched region with a smaller thickness.

In this study, the characteristics of the etched region with a thickness of about 50 μm and the frame region with a thickness of 150 μm on the same sensor plate were compared to guarantee that measurement parameters were identical except for the thickness. The maximum photocurrent of the *I-V* curve for a pH 7 buffer solution in the etched region was 2.6 times that obtained in the frame region. Furthermore, the etched region could visualize smaller structures on the sensing surface. The frequency bandwidth was enlarged by a factor of more than 10 and 12-fold-enhancement of photocurrent amplitude at 120 kHz was also achieved.

These results suggest enhancement of the photocurrent signal and therefore the signal-to-noise ratio in the etched region, while the mechanical strength of the sensor plate is reinforced with the frame structure. The availability of a wider frequency bandwidth can allow frequency-domain multiplexed measurement with a plurality of light sources for movie recording at a high frame rate.

Chapter 3: Multi-well sensor based on partially-etched structure on the frontside of LAPS sensor plate

Partially-etched structures or multi-wells were formed on the front side of the sensor plate rather than on the back side. Compared to the proposed structure in Chapter 2, the multi-wells on the sensing surface can directly accommodate a plurality of liquid samples to be analyzed independently, without the need of additional multi-chamber structures. At the same time, an enhancement of the sensor performance is expected because of the reduced recombination of photocarriers in the thinned active layer.

A multi-well LAPS plate is developed for measuring a plurality of sample solutions on a single sensor plate. This sensor utilized the partially-etched structures in Chapter 2 as multiple sensors with the SiO_2 sensing surface having an area of 14×14 mm^2 including the wells. The capacity of each well was about 0.35 μL. At the bottom of the well structure, a test pattern with a minimum width of 19.9 μm was resolved. A wider frequency bandwidth of up to 200 kHz for the sensor signal was obtained in the well structure.

As a demonstration of multi-well measurement, a plurality of different pH buffer solutions were measured in different wells on the same sensor plate. Nine wells were filled with pH buffer solutions of pH 1 to 9 and the shift of the bias voltage at the inflection point of the *I-V* curve with respect to its value at pH 7 was calculated for each well. A good linearity was obtained between the shift and the pH value, which suggested the validity of the calibration procedure.

These results suggest that the multi-well LAPS can be used as an array of independent sensors for analyzing a plurality of samples placed on a single sensor plate, which is capable of high-resolution chemical imaging inside the well structure and is suitable for high-speed measurement and recording chemical images at high frame rates. This structure can be applied, for example, for quantification of metabolic activities and imaging of biological samples incubated in the well structure.

Chapter 4: Measurement of *Escherichia coli* metabolism with multi-well LAPS

The developed multi-well LAPS sensor plate was applied to measure the metabolic activity of *Escherichia coli* (*E. coli*) bacteria. Nine samples were measured on a 9-well sensor plate to measure the extracellular acidification of *E. coli* from their potential shifts. Three of these samples were pH buffer solutions of pH 4, 6 and 8, and the other six samples were LB media with different *E. coli* concentrations of 0, 1.25×10^6, 2.5×10^6, 5×10^6, 1×10^7 and 2×10^7 CFU/ml. The pH values of samples with *E. coli* started to decrease after 1-3 hours and became

stable at about ΔpH = -1.5. Based on the relation between the initial cell concentration and the time until a certain pH shift of -0.8 was achieved, the doubling time was calculated to be about 24 min.

By using the multi-well LAPS sensor plate combined with the setup for long-term measurement, it is able to measure a plurality of samples with small volumes under well-defined and controllable conditions, which contributes to the measurement of metabolic activities of bacteria and the determination of the doubling time.

Chapter 5: Microfluidic channel for dispensing samples into multiple wells

A multi-well sensor plate was combined with a microfluidic channel. The microfluidic channel was designed and cut from an 80-μm-thick double-side tape, one side of which was bonded with the sensor surface and the other side of which was bonded with an ITO glass for electrical contact. The channel dispenses and separates a solution into different wells on the multi-well sensor. As a result, it was possible to dispense and separate the solution into the wells. With electrical contact of the solution to the ITO surface, it was possible to obtain the photocurrent image.

By increasing the number of wells connected with a microfluidic channel, this structure is expected to be applicable to cell counting. This structure can be applied in biochemical analysis with advantages of real-time, accurate, compact and disposable chip.

Chapter 6: Conclusions

This thesis discussed the performance improvements of LAPS for chemical imaging and applications for multi-well sensor arrays, especially in relation to the spatial resolution and the measurement speed. A partially-etched structure of LAPS was fabricated using anisotropic wet etching in TMAH solution for high-spatial-resolution and high-speed imaging of chemical species. Its applications to measuring multiple biological samples on a same sensor plate was also proposed. The enhanced characteristics of the sensor plates would be advantageous in quantitative analysis in different applications of environmental detection, clinical diagnosis and drug screening platforms.

論文審査結果の要旨

半導体化学・バイオセンサの一種である light-addressable potentiometric sensor (LAPS) は，センサ面上のイオン濃度分布を光走査により位置分解的に測定できることから，化学・バイオイメージングへの応用が期待されている。本論文は，異方性エッチングプロセスを用いて LAPS を部分的に薄層化することで，センサの機械的強度を維持しつつ，フォトキャリアの面内拡散と再結合を抑制してセンサ性能の向上を図った研究の成果をまとめたものであり，全文 6 章よりなる。

第 1 章は序論であり，研究の背景と目的，LAPS の概要と課題ならびに水酸化テトラメチルアンモニウム (TMAH) 溶液によるシリコンの異方性エッチングプロセスについて述べている。

第 2 章では，まず TMAH 溶液によるエッチングプロセスの最適化を行い，次にこれを用いてセンサ裏面を部分的にエッチングした構造を作製し，センサ性能を評価している。約 47 □mに薄層化した領域では，薄層化していない領域と比べて約 2.6 倍の信号電流が得られること，幅 20 □mのパターンを解像できること，光走査に用いるレーザ光の変調周波数を 10 倍以上高くできることを示している。こ

れらは化学・バイオイメージングの高分解能化・高速化に資する結果である。

第 3 章では，部分的にエッチングしたシリコン表面上に感応面を形成することにより，マルチウェル構造を有する LAPS を開発している。このデバイスは各ウェル中に試料液滴を保持することができ，ウェル内では前章と同様の性能向上が確認された．これは，多数の試料を同一条件下で培養・測定する必要がある生物試料への応用において特に有益である。

第 4 章では，前章で開発したマルチウェル構造を有する LAPS を用いて，大腸菌を含む複数の試料液滴の培養と代謝活動の定量を同一センサ上で行い，大腸菌の倍加時間を測定している。これは，マルチウェル構造を有する LAPS の有用性を示す結果である。

第5章では, マルチウェル構造を有する LAPS とマイクロ流体チャネルを組み合わせることにより，各ウェルへの試料液滴の分注を簡便に行う方法を提案している。

第 6 章は結論であり，得られた成果をまとめている。

以上要するに本論文は，LAPS の部分薄層化による性能向上を実現し，多数の試料液滴を保持できる構造が微生物代謝測定に有用であることを実証したものであり，化学・バイオセンサ工学の発展に寄与するところが少なくない。

よって，本論文は博士(医工学)の学位論文として合格と認める。