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Glass-based BioMEMS devices for optically excited cell impedance measurement

Xiaodong Zhou [a,*], Daniel Puiu Poenar [b], Kai Yu Liu [b], Wen Li [b], Man Siu Tse [b], Hui Chen [c], Chew-Kiat Heng [c], Swee Ngin Tan [d]

[a] *Institute of Materials Research and Engineering, 3 Research Link, Singapore 117602, Singapore*
[b] *Microelectronics Centre, School of Electrical and Electronic Engineering, Nanyang Technological University, Singapore 639798, Singapore*
[c] *Department of Paediatrics, National University of Singapore, Singapore 119074, Singapore*
[d] *National Institute of Education, Nanyang Technological University, Singapore 637616, Singapore*

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Abstract

This paper presents for the first time the design and fabrication of BioMEMS devices for living cells' electrochemical impedance measurement under optical excitation. An interdigitated electrode design is adopted in order to increase the sensitivity of living cells' impedance measurement. The magnitudes of the parasitic components were calculated and their influence on the chip performance was simulated. It is shown that our glass-based realization enables a very good performance up to a measurement frequency of 10 MHz when the solution resistance is 1 MΩ. The main difficulties that had to be solved in the fabrication process were related to adhesion and patterning of the various metal films, and especially to developing the correct bonding recipe. Unlike most of the reported data, no direct bonding was employed in the realization of our devices, but rather an intermediary polymer layer (e.g. SU8 or BCB) was used. The impedance of the devices with living yeast cells are measured with and without laser excitation at 532 nm. The laser excitation also redistributes the cells, and the suspending cells in the PBS buffer settle down much quicker after the light excitation.

Keywords: BioMEMS; Electrochemical impedance; Glass wafer; Optical excitation; Living cells

1. Introduction

Electrochemical impedance spectroscopy (EIS) is a sensitive technique to measure the living cells' characteristics both quantitatively and qualitatively. Suehiro et al. reported a bacteria impedance measurement of 10^2 CFU/ml for a 15 min diagnosis time [1]. In our case, however, we are most interested to determine how an external excitation may affect the cells as measured using EIS. Light excitation is convenient to be applied without major changes in the design of the measurement device. When laser excitation is applied to the cells through a glass cover, two effects might happen. Firstly, the cells could be trapped and manipulated by the laser beam, depending on its intensity and beam intensity profile [2–4]. However, this requires a special beam shaping and adequate focusing. We are much more interested in the second possible effect: the optical excitation of the living cells might transfer energy into them and 'activate' them by altering the internal biochemistry, hence modifying their behavior and response towards impedance characterization. This may reveal more data about the cells' vitality or their intrinsic structure and properties than cells' pure impedance measurement.

For this purpose it was necessary to design and fabricate a BioMEMS chamber in which at least one of the devices' top or bottom covers is transparent. The top wafer must be transparent, while the bottom one can use any convenient substrate. This structure can be realized in two ways: for the top wafer one can either start from a Si wafer, etch through its whole depth at the central area of the chamber and employ only a thin membrane (e.g. of nitride) to seal the fluidic channel, or to use a glass wafer. The device fabricated on the Si wafer will be more fragile after through-wafer etching and releasing the membrane, and this will

* Corresponding author. Tel.: +65 68 72 7522.
E-mail address: donna-zhou@imre.a-star.edu.sg (X. Zhou).

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cause difficulties in fabrication handling and subsequent dicing, thus potentially reducing the overall yield. Therefore, employing a glass wafer for the top cover is a better choice.

In our device design, we employed 6 in. glass wafers for both the top and bottom wafers in order to optimize not only the robustness of the device but also to reduce the parasitic electrical components contributed by the bottom one. Moreover, in comparison with the case when the bottom wafer would be made of Si, the entire bonded complex can easily be diced visibly through the backside of the wafer, so as to prevent contamination with dust or particles resulted from dicing in the inlet and outlet areas of the devices which would be difficult to wash thoroughly and remove after fabrication.

Among the difficulties to be solved in the processing of glass wafers in a Si-based fabrication line, one can specify a few: the relative poor adhesion of metals unto glass; the necessity of manual loading in equipment chambers; a higher UV exposure energy than that used for a Si wafer because of the transparency-induced light loss; finding the optimal bonding recipe.

Only a few successful glass-based devices for impedance measurement are reported, most probably because of the difficulties of direct glass-to-glass bonding, especially if thick electrodes are also presented on the glass surface. Hong et al. [5] reported a successful glass device with microelectrodes, but the Pt film used for his electrodes is about 220 nm thick. In contrast, our device has metal lines five times thicker (about 1.2 μm, as is detailed in the subsequent sections), which caused more difficulties at the bonding stage, and no direct glass- or silicon-to-glass bonding was used but rather a polymer intermediary layer.

With the interdigitated electrodes device we designed and fabricated, some interesting experiments on living yeast cells' impedance measurements were conducted combined with laser excitation. It is found that the 532 nm laser light projected on the yeast cells does stimulate the living cells in some way, cells congregate at the laser spot and its nearby areas during the light excitation, which is a similar effect of laser tweezers [2–4], and settle down quicker to the bottom of the chamber after the laser turned off.

2. Device design

Fig. 1a shows the simplified device layout. The device structure patterns layers of Au and Al to define the measurement electrodes and the metallization connections to bonding pads, respectively. In our case the thin (1000 Å) gold layer is used as-is only for the interdigitated electrodes in the central measurement chamber (in the centre of both Fig. 1a and b). The rest of the Au pattern is covered with a thick Al layer (1 μm) in order to minimize the parasitic serial resistance of the metallization lines to the bonding pads.

Moreover, for a good quality device and a high measurement sensitivity to cells' impedance parameters, the parasitic coupling capacitance due to the substrate should also be negligible in the frequency range of interest. This was the reason which compelled us to use a glass substrate for the bottom wafer as well. The schematic equivalent circuit of the device is drawn in Fig. 2.

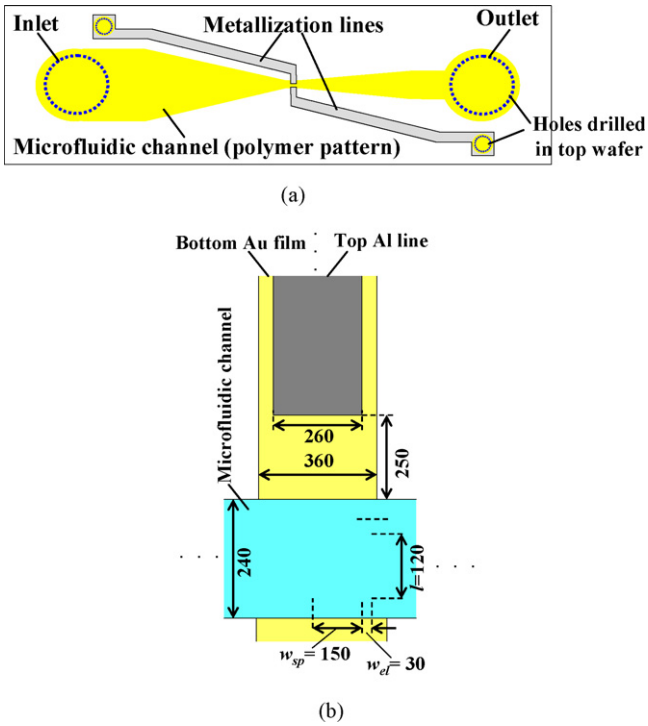

Fig. 1. Simplified representation of the fabricated BioMEMS device: (a) general layout and (b) central area with the measurement chamber where the interdigitated electrodes are placed. All dimensions are in micrometers.

The most important component in the circuit of Fig. 2 is the interdigitated electrode's parasitic capacitance through the substrate C_{pg}. Since its reactance $X_{C_{pg}} = 1/(\omega C_{pg})$, if C_{pg} is too large, the operation of the entire device is compromised at high frequency. According to Gerwen et al. [6], the parasitic capacitance through the glass substrate is given by

$$C_{pg} = n \cdot l \cdot \varepsilon \cdot \frac{K(\cos(\pi w_{sp}/2(w_{sp} + w_{el})))}{2K(\sin(\pi w_{sp}/2(w_{sp} + w_{el})))} \quad (1)$$

where n is the number of fingers, l the length of the fingers for the interdigitated electrodes, ε the permittivity of the glass substrate, $K(x)$ the complete elliptic integral of the first kind with variable x, w_{sp} the spacing between the electrodes fingers and w_{el} is the electrode finger width.

For our device, $n = 4$, $l = 120$ μm, $\varepsilon = \varepsilon_r \varepsilon_0 = 5.181 \times 8.8542 \times 10^{-12}$ F/m for the Corning Eagle 2000 glass substrate, $w_{sp} =$

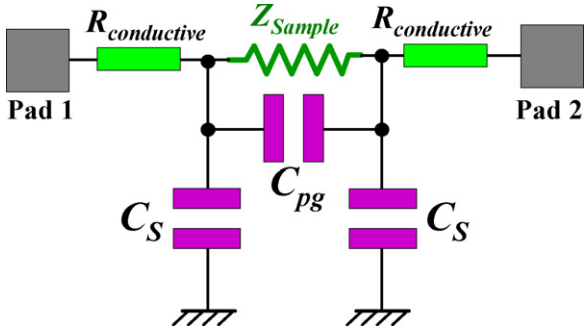

Fig. 2. Schematic equivalent circuit of the entire device.