

Electronic Supplementary Information (ESI)

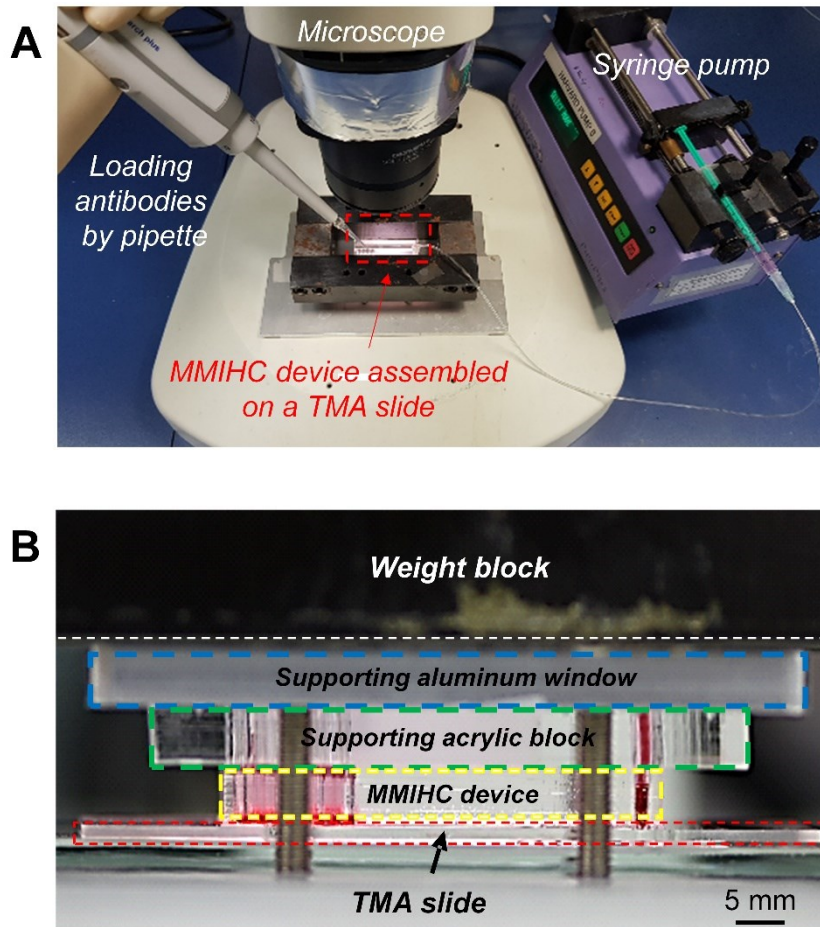
# **Biomarker barcodes: multiplexed microfluidic immunohistochemistry enables high-throughput analysis of tissue microarray**

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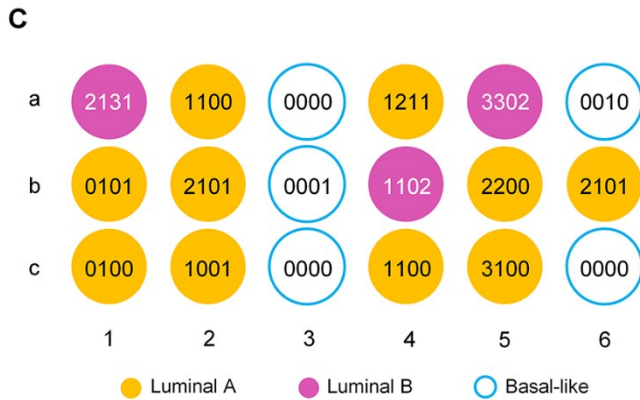
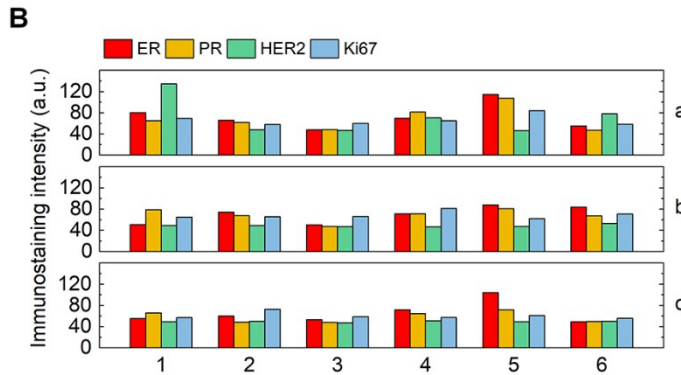
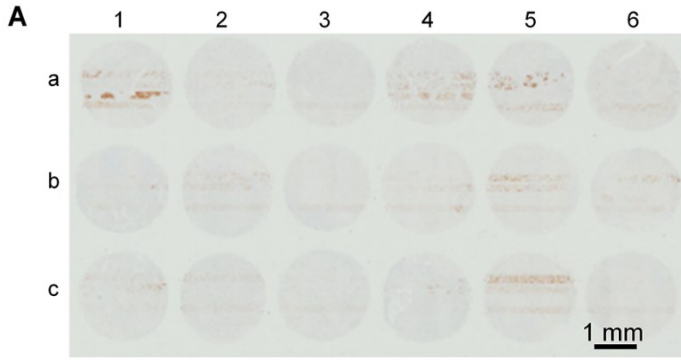
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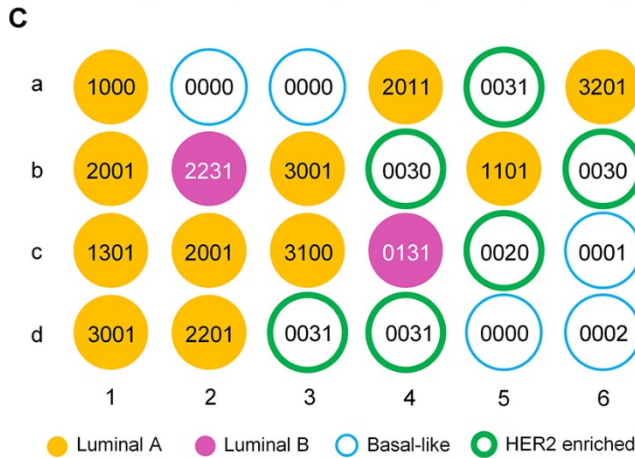
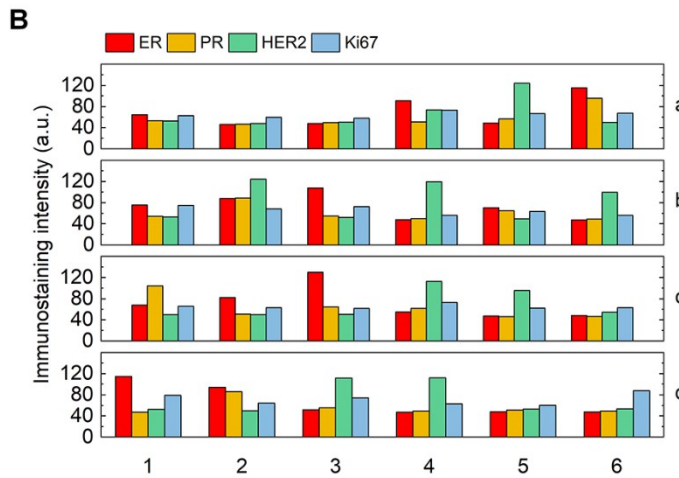
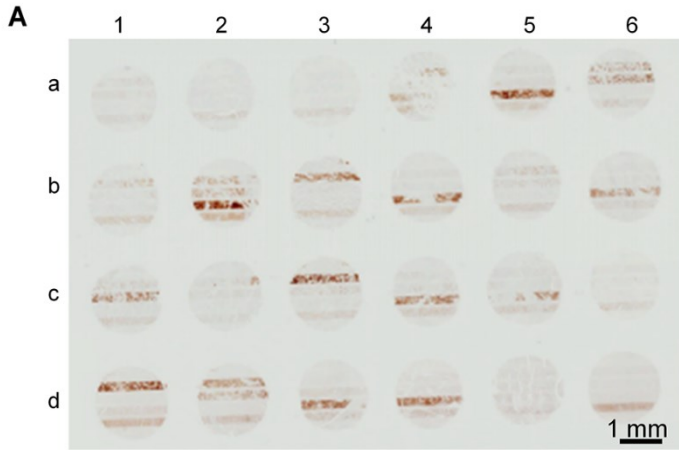
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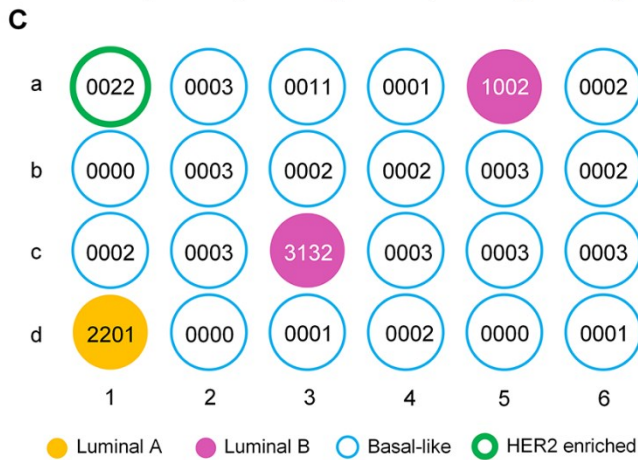
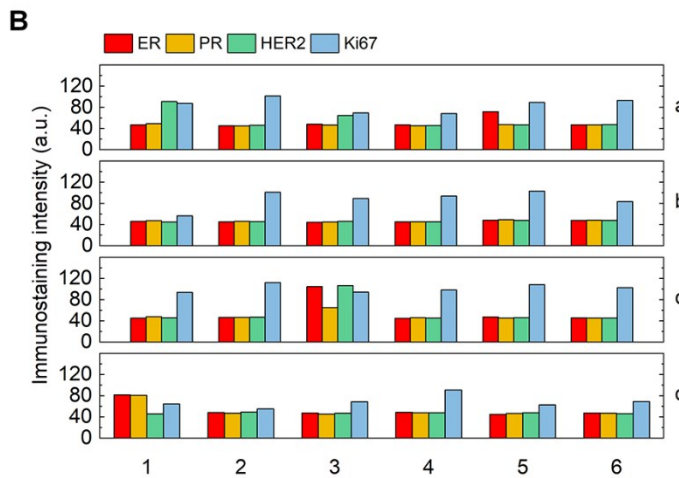
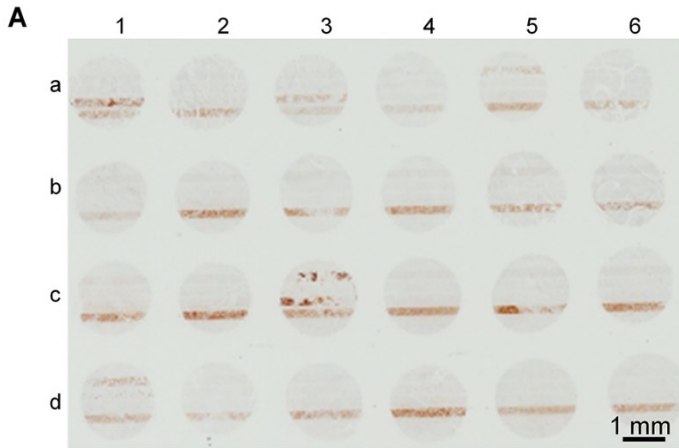
**Fig. S1** Experimental setup of multiplexed microfluidic immunohistochemistry (MMIHC). (A) Photo of the experimental setup of the MMIHC platform. The dashed box shows an MMIHC device assembled on a TMA slide. (B) The side view shows the stacking of components to aid in the reversible assembly of the MMIHC device with a TMA slide. A weight (1 kg), an aluminum window, and an acrylic block were placed in sequence on top of the device to provide constant pressure for the reversible setup of the assembly.



**Fig. S2** Biomarker barcodes on the BR248a (invasive ductal carcinoma) TMA consisting of 18 breast cancer tissue cores using four different antibodies. The fourth row of BR248a TMA was damaged during sample preparation and the corresponding six tissue cores were excluded from data processing. (A) Unique biomarker barcodes were observed on each TMA core representing the characteristics of biomarker expression of each patient. (B) Graph showing the immunostaining intensities of biomarkers on each TMA core. (C) The expression levels of four biomarkers and the molecular diagnosis result of each TMA core were represented as a four-digit number and color, respectively. The four digits indicate the expression level of ER, PR, HER2, and Ki67, respectively. The same colored codes represent the same molecular subtype obtained from the diagnosis results.



**Fig. S3** Biomarker barcodes on the BR249 (invasive ductal carcinoma) TMA consisting of 24 breast cancer tissue cores using four different antibodies. (A) Unique biomarker barcodes were observed on each TMA core representing the characteristics of biomarker expression of each patient. (B) Graph showing the immunostaining intensities of biomarkers on each TMA core. (C) The expression levels of four biomarkers and the molecular diagnosis result of each TMA core were represented as a four-digit number and color, respectively. The four digits indicate the expression level of ER, PR, HER2, and Ki67, respectively. The same colored codes represent the same molecular subtype obtained from the diagnosis results.



**Fig. S4** Biomarker barcodes on the BR2411 (medullary carcinoma) TMA consisting of 24 breast cancer tissue cores using four different antibodies. (A) Unique biomarker barcodes were observed on each TMA core representing the characteristics of biomarker expression of each patient. (B) Graph showing the immunostaining intensities of biomarkers on each TMA core. (C) The expression levels of four biomarkers and the molecular diagnosis result of each TMA core were represented as a four-digit number and color, respectively. The four digits indicate the expression level of ER, PR, HER2, and Ki67, respectively. The same colored codes represent the same molecular subtype obtained from the diagnosis results.