

### Three-dimensional paper-based digital measurements

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This study demonstrates a simple approach for fabricating a 3-dimensional microfluidic paper-based analytical device from a single sheet of paper by double-sided printing and lamination. First, a wax printer prints vertically symmetrical and asymmetrical wax patterns on double-sided surface of paper. Then, laminator melts the printed-wax-patterns to form microfluidic channel in a paper sheet. The vertically symmetrical and asymmetrical wax patterns form a 3D microfluidic network to move fluid laterally and vertically in a single sheet of paper. This method eliminates major technical hurdles related to the complicated and tedious alignment, assembly, bonding, and punching process. This 3-dimensional microfluidic paper-based analytical device can be used in a multiplex digital assay to measure the concentration of a target analyte in a sample solution simply by counting the number of colored bars at a fixed time. It does not require any external instruments to perform digital measurements. Therefore, we expect that this approach could be an instrument-free assay format for use in developing countries.