Laminated microfluidic paper-based analytical devices for clinical protein assays

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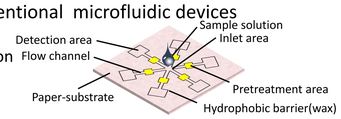
Background

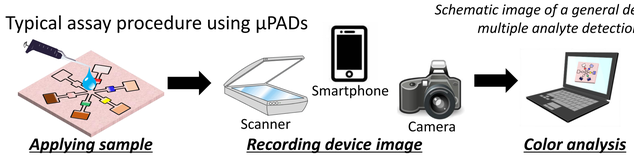
Microfluidic paper-based analytical devices (µPADs)

- General advantages compared to conventional microfluidic devices
  - Low cost and easy to dispose
  - On-device pretreatment of sample solution
  - Pump-free sample transport
  - Easy to fabricate



Schematic image of a general device for multiple analyte detection

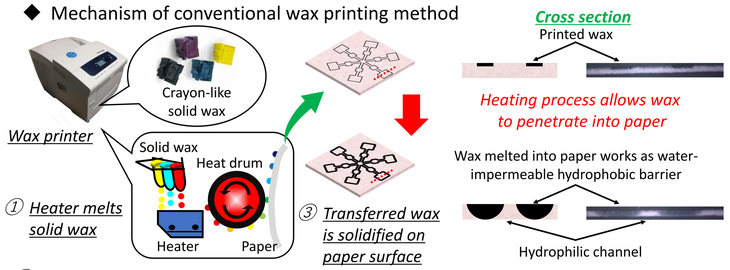
- Typical assay procedure using µPADs



µPADs have gained a lot of attention as new analytical devices

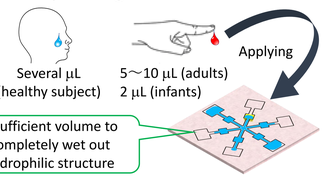
Patterning of microfluidic structure

- Wax printing technique evolved among a large number of patterning methods
  - ✓ ***Wax printing***
  - ✓ Photolithographic patterning
  - ✓ Knife cutting
  - ✓ Inkjet printing
  - ✓ Laser cutting
  - ✓ Flexographic printing
- ***Advantages of wax printing method***
  - ✓ Ease and flexibility of computer-based pattern design
  - ✓ Small number of process steps [printing followed by heating]
  - ✓ Suitability for mass production at low cost
- Mechanism of conventional wax printing method



General challenge of µPADs patterned by wax printing

- Relatively large sample volume required
  - Larger dimension of structure
  - Open system prone to evaporation
  - Slow sample liquid wicking velocity



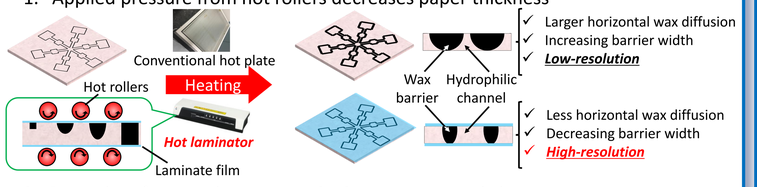
Sub-microliter sample assay by µPADs remains challenging

Research goal

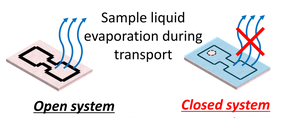
High-resolution µPADs for sub-microliter analysis fabricated by wax printing method

Using hot laminator as post-printing method for heating process

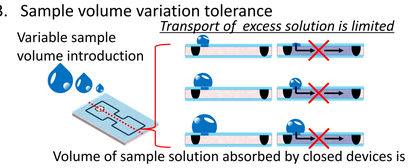
1. Applied pressure from hot rollers decreases paper thickness



2. Prevention of evaporation loss



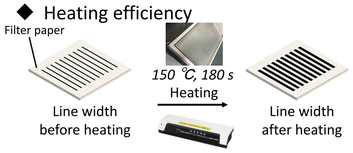
3. Sample volume variation tolerance

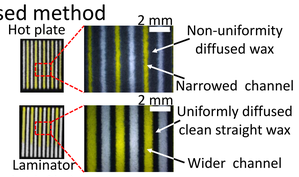


Design and evaluation of optimized µPADs

Comparison of conventional hot plate and laminator-based method

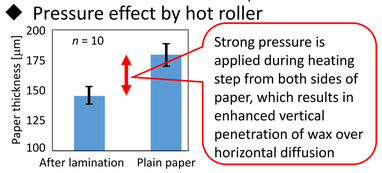
- Heating efficiency



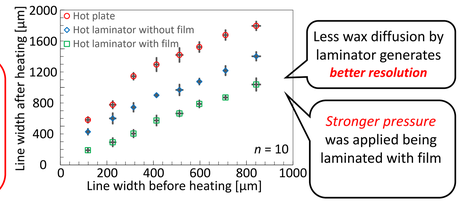


Line width before and after treatment by hot plate and hot laminator were measured and compared

- Pressure effect by hot roller



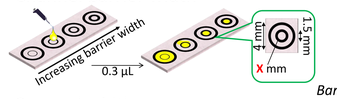
Preset design on software



Conventional heating method by hot plate cannot compete with hot laminator in terms of resolution

Optimization of wax dimensions

- Channel barrier width

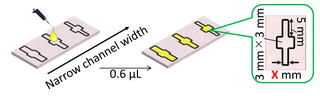


Contrast adjusted for improved visibility

Printed wax barrier width [µm]	200	250	300	350
Number of functional barriers (*n* = 20)	13/20	12/20	20/20	20/20
Actual pictures				

Barrier is regarded as being functional, if no leaking from the inner circle occurs

- Channel width

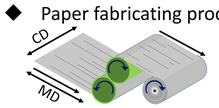


Printed channel width [µm]	300	400	500
Number of functional channels (n=20)	4/20	20/20	20/20
Actual pictures			

Channel is regarded as being functional,if liquid reached the terminal

Cellulose fiber direction effect

- Paper fabricating process generates non-uniform fiber orientation

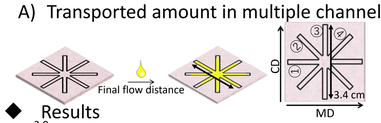


MD: Machine direction parallel to dominant fiber direction
CD: Cross direction perpendicular to dominant fiber direction

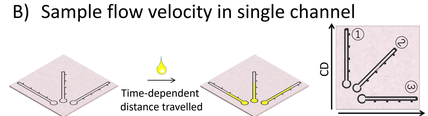
- ✓ *Liquid is easily transported along MD in a flow arrangement*
- ✓ *Inhomogeneous fiber direction affects the sample wicking process*

- Experimental procedure to evaluate fiber direction

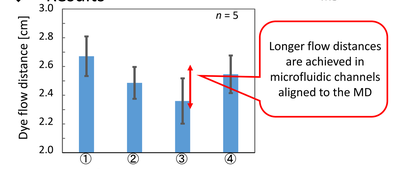
A) Transported amount in multiple channels

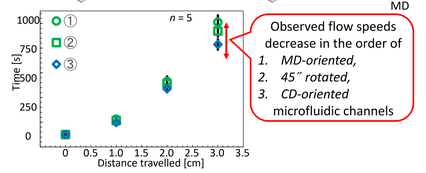


B) Sample flow velocity in single channel



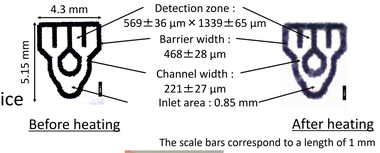
- Results





Optimized µPAD design

- Requirements
  - ✓ Double-sided wax printing of identical patterns
  - ✓ Symmetric pattern design
  - ✓ Equal flow distance to all detection zones
  - ✓ Channels aligned parallel to cellulose fiber direction
- Minimum sample volume required to fill the device



Sample volume [µL]	0.3	0.4	0.5	0.6
Success rate (*n*=5)	0/5	1/5	5/5	5/5
Actual pictures				

The scale bars correspond to a length of 1 mm, contrast adjusted for improved visibility

Actual pictures — Actual devices

0.5 µL liquid is sufficient to reliably fill all of the four detection zones

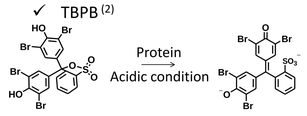
Analytical application

(1)HSA: human serum albumin
(2)TBPB: tetrabromophenol blue

[1] S. Mishima *et. al., Invest. Ophthalmol. Vis. Sci.*, **1966**, *5-3*, 264-276

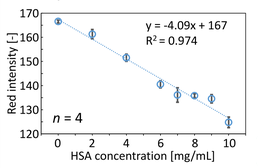
HSA[(1)] assay in human tear (6-10 mg/ml) as a model analysis on sub-microliter scale

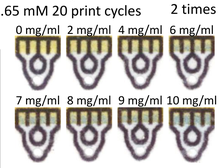
- Collectable tear volume[1]
  - ✓ 7.0 ± 2.0 µL (healthy subject)
  - ✓ 4.6 µL (palpebral conjunctiva)
  - ✓ 2.5 µL (dry eye)
- Diagnostic needs
  - ✓ Dry eye condition
  - ✓ Ocular injury
  - ✓ Ocular inflammation
- Detection mechanism
  - ✓ TBPB [(2)]



- Experimental procedure

- Experimental results





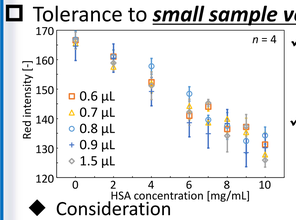
- ✓ Reagent composition

TBPB: citric acid buffer = 1:1
- TBPB 3.3 mM dissolved in 95 % (v/v) aqueous ethanol
- Citric acid buffer 0.1 M, pH 4.00

HSA standard solution (artificial tear matrix)
- 10 mM HEPES buffer pH7.4
- Na^+ : 150 mM
- K^+ : 20 mM
- Ca^{2+} : 1.0 mM
- Mg^{2+} : 0.6 mM
- Phospholipid : 379 mg/L
- HSA : X mg/ml

Single application of 0.6 µL sample liquid provides 4 data points

Tolerance to ***small sample volume variations***



- ✓ ***0.5 µL sample solution is not sufficient to completely fill device***
  - Hydrophobicity of TBPB
  - Adsorption of protein to paper
  - Increased viscosity of liquid
- ✓ ***More than 1.5 µL sample solution broke wax barrier***
  - Too narrow barrier
  - Thick barrier is required to achieve prevention of sample leakage

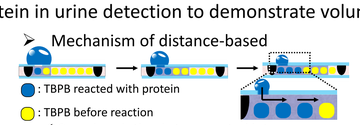
Sample [µL]	Slope	y-intercept	R^2 value	Average CV value [%]
0.6	-4.09	167	0.97	1.18
0.7	-4.21	166	0.98	2.08
0.8	-4.22	164	0.96	2.40
0.9	-4.01	163	0.93	1.78
1.5	-4.73	163	0.93	2.41

- Consideration
  - ✓ The signal is determined by the amount of analyte transported to detection zone which is limited to a constant value by wax barrier and lamination film on both sides.
  - ✓ Slopes and intercepts of the calibration curves are not significantly different.

The device has acceptable tolerance against minor variation in applied sample volume

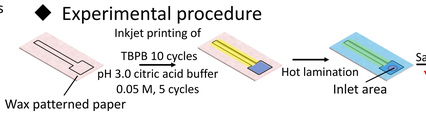
Tolerance to ***larger sample volume variations*** (not high-resolution)

- Distance-based protein in urine detection to demonstrate volume independent system
  - Diagnosis
    - ✓ Kidney disease
    - ✓ Cystitis
    - ✓ Urethritis
    - ✓ Prostatitis
  - Mechanism of distance-based



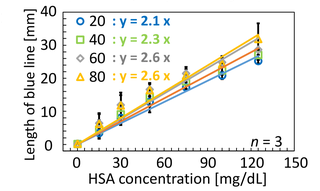
  - ✓ Sample solution is transported into paper substrate and reaction with TBPB takes place
  - ✓ After completely wetting out the closed hydrophilic area, sample wicking stops

- Experimental procedure

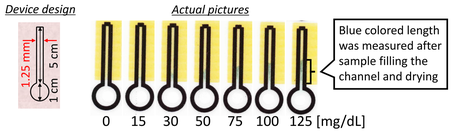


- TBPB 1.1 mM in (ethanol: pH 3.0 citric acid buffer = 1:2)
- Sample (albumin 0, 15, 30, 50, 75, 100, 125 mg/dL)
- • Y = 20, 40, 60, 80 µL

- Result



Device design — Actual pictures



- ✓ The slopes of each calibration curve depend on applied sample volume in the case of applying less than 60 µL (2.1, 2.3 and 2.6 in above figure)
- ✓ When more than 60 µL is applied, signal is identical independently from applied sample volume because excess sample solution cannot be absorbed by paper.

The device demonstrates volume-independent behavior by distance-based readout

Summary

- High-resolution patterning of µPADs for sub-microliter analysis
  - Barrier width: 850 µm → **374 µm**
  - Channel width: 561 µm → **268 µm**
  - The device is filled with as low as 0.5 µL liquid
  - 4 data points can be obtained by one sub-microliter pipetting
- Tolerance against small sample volume variation
  - Identical signal is obtained for 0.6-0.9 µL volume
  - When stronger and thicker wax barriers are used, identical signal over a wider volume range can be obtained
  - Direct sample application (pipetting-free) assay can be realized after establishment of completely volume independent system