



A smartphone integrated paper (SIP)-based platform for rapid and on-site screening of urinary tract infections

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ABSTRACT

We present a smartphone integrated paper (SIP)-based system that enables accurate screening of urinary tract infections (UTIs) by directly identifying white blood cells (WBCs) in human urine samples. The SIP platform mainly consists of a paper-based capillary filter and a smartphone-integrated fluorescence microscope. A paper filtration device allows a low-cost, simple method for selectively drying and isolating target WBCs in urine with more than 95% collection efficiency using capillary action through paper layers with several different pore sizes without extra pumps and tubes typically required for fluidic actuation in microfluidic systems. Furthermore, an integrated smartphone can be conveniently used for fluorescence imaging of the stained WBCs and microscopic analysis to rapidly provide UTI diagnostic results at the point of care without the need of bulky laboratory equipment and specialized training. The fabrication process for the SIP platform is simple and cost-effective, being beneficial for resource-limited field applications. We have experimentally demonstrated the capability of the SIP for isolation of target WBCs in a urine solution (more than 95% selectivity) using the paper capillary filter, the effectiveness of the smartphone microscope for fluorescence imaging of urine solutions with $14.4 \times$ magnification over a field of view of $\sim 12.25 \text{ mm}^2$, the reliability of the platform's cell counting with the 96.67% sensitivity and 100% specificity, and successful differentiation of the real urine samples between UTI patient and healthy person. The entire screening processes from filtration to quantification of WBCs in real human urine samples were rapidly completed within 10 min, showing its potential for point-of-care applications. The SIP technology provides a low-cost, rapid, and portable UTI diagnostic tool that can improve the access of accurate test results to developing areas and thus reduce suffering by speeding up therapy timelines.

1. Introduction

Urinary tract infections (UTIs) are the most common bacterial infections. They are responsible for about 150 ~ 250 million cases worldwide per year and account for significant healthcare expenditure with an annual cost of US\$3.5 billion in the USA [1–4]. Almost 50% of the global population experience UTIs at least once in their lifetime and those who suffer from recurrent UTIs have more than three episodes of infection per year [5]. Common symptoms of UTIs include painful urination, abdominal pain, and increased urination frequency, but more significant issues such as kidney damage and pregnancy complications for women can manifest if left untreated [1–3]. When complicated by factors like obstructing urinary stones, indwelling catheters, and urinary tract surgeries, UTIs carry the risk of urosepsis, which has an associated mortality of 20 ~ 40% [6].

The “Gold standard” method for UTI diagnosis is a urinalysis based

on typical laboratory analysis to see a urine condition called pyuria, in which the count of leukocyte cells, or white blood cells (WBCs), presents 10 cells/ μL or more in urine [7–11]. Once a urine sample is diagnosed as symptomatic of a UTI, biochemical and serological tests are subsequently required for determination of antibiotic susceptibility profile [12]. This standard method provides the most accurate result for screening of UTIs by directly counting the number of WBCs in urine [13, 14]. However, due to microbiological processes such as cell culturing, centrifugation, and identification typically conducted in centralized laboratories, a delay of two or three days is involved to get test results [15]. Furthermore, it also requires a great deal of labor, specialized training, and costly machines such as centrifuges and microscopes. To address these issues, the urine dipstick method has attracted great interest [7, 16–19]. It analyzes the color change of a chemical reagent strip to correlate with a measured amount of UTI biomarkers such as nitrite and leukocyte esterase, by-products of bacterial presence and WBC

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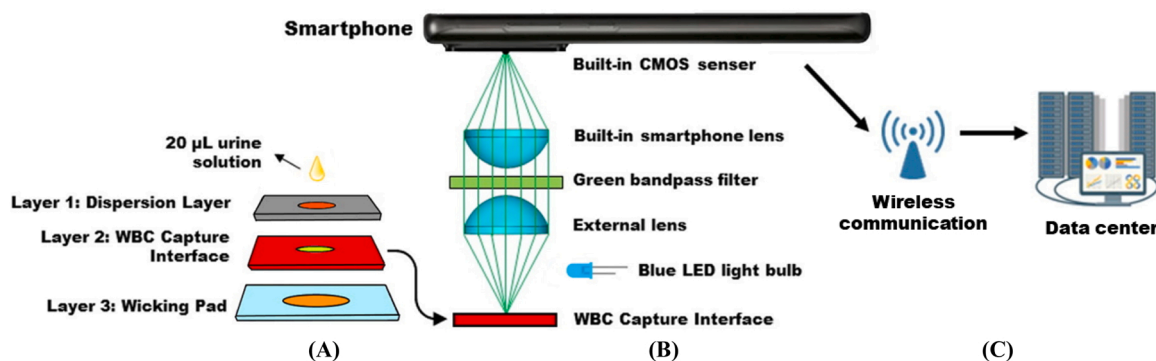


Fig. 1. A schematic of the smartphone integrated paper (SIP)-based platform as a simple, low-cost, portable tool for rapid, on-site screening of urinary tract infections (UTIs). (A) A paper-based filtration device consists of three layers of the papers with different pore sizes. 20 μL of a urine sample collected from a UTI patient is loaded onto the center of Layer 1, where sodium fluorescein dye is preloaded to selectively attach to target leukocyte cells (or WBCs). The stained WBCs are then collected in Layer 2, while smaller sizes of the cells are further filtered through to Layer 3. After completing the paper-based separation process of the target WBCs, the filter device is disassembled to remove the WBC capture interface of Layer 2. (B) The WBC capture interface is then placed below a smartphone-integrated fluorescence microscope for quantitative analysis of UTIs. The smartphone's rear camera is used to capture fluorescence images of the target WBCs isolated in Layer 2. Using an image processing software, the populations of the collected WBCs are quantified to diagnose the level of pyuria. (C) Additionally, a smartphone is equipped with various functions such as wireless communications, cloud storage, and global positioning system (GPS) capability. These capabilities allow the captured data (e. g., the number of the target WBCs, time of test conducted, etc.) to be shared instantly and wirelessly with a central host like a healthcare team for real-time monitoring and management of UTI patients.

synthesis, respectively [16]. The darker the color, the greater degree of UTI infection. This dipstick test offers low-cost and rapid UTI detection at the point of care without the need of laboratory equipment and trained personnel [20]. However, it often displays false negative test results due to variability of urine samples associated with excessive specific gravity, protein concentration, or glucose concentration, leading to unreliable test results [3, 13–15]. Thus, numerous clinical studies of the dipstick tests for simultaneous leukocyte esterase and nitrite detection have measured the sensitivity as low as 25 ~ 65% for individuals tested at primary care settings [21–25]. This low sensitivity range leads to a high chance of a patient being tested as negative for UTI, although they are truly positive.

With recent technical advances, paper-based microfluidic methods have emerged as effective UTI detection tools [21–25]. By handling small volume of urine samples, paper-based systems allow less sample consumption, precise pathogen-specific detection, and amenability to automation, multiplexing, and high-throughput processing [26]. For example, Olanrewaju *et al.* presented a self-powered, PDMS-based capillary circuit where 100 μL of a synthetic urine sample was used to capture *E. coli* bacteria using antibody-functionalized microbeads [27]. Fluorescence images of the microbead column revealed the captured bacteria to measure bacterial concentrations up to 1.2×10^2 colony-forming-units per mL (CFU/mL). Cho *et al.* developed microfluidic paper analytic devices (μPADs) fabricated via wax printing for the detection of *E. coli* and *N. gonorrhoeae* from human urine samples [28]. 7 μL of spiked urine was input into a paper microfluidic chip pre-loaded with *E. coli* antibodies and underwent angle-specific Mie scatter analysis with a smartphone camera to quantify CFU with consistent UTI assay results. Later, origami immunosensors has been introduced using antibody-coated nanoparticles for *E. coli* detection combined with smartphone image pixel intensity analysis to quantify the presence of UTI [29]. These paper-based assay methods allowed for low-cost and rapid detection of specific UTI pathogens with both specificity and sensitivity at higher than 95%. However, they would be required to have separate inlets, valves, pumps, reservoirs, and affinity binders for specific pathogens. The addition of these microfluidic components consequently increases the complexity and cost of the detection devices above a reasonable level for bulk manufacturing and reliable use in resource-limited areas [1, 25, 30]. Hence, there is a critical need for simple, low-cost, and portable UTI detection tools that allow the benefits of diagnostic accuracy from conventional culture-based analysis and rapid detection from microfluidic-based methods.

In this article, we present a smartphone integrated paper (SIP)-based platform for rapid, on-site screening of UTIs with the advantages of cost effectiveness, device portability, and diagnostic accuracy. The SIP platform combines a multi-layer paper filter and a smartphone-integrated fluorescence microscope. A paper-based microfluidic approach allows pumpless and tubeless fluid handling for selective isolation of target WBCs in urine using low-cost paper layers with different pore sizes. Furthermore, an integrated smartphone works as a portable microscope to capture fluorescence images of the stained WBCs and count their populations for quantitative UTI analysis. By directly quantifying the concentrations of the WBCs in a urine sample, the SIP enables diagnostic accuracy provided by a conventional culture-based method, but without any bulky and pricey laboratory facilities such as centrifuges and microscopes. We have experimentally demonstrated the SIP's capabilities for (1) exclusive filtering and dying of target WBCs, (2) fluorescence imaging of the WBCs using an integrated smartphone, (3) quantification of the cell concentrations with the excellent sensitivity of 96.67% specificity of 100%, and (4) validation of test results with the hemocytometer. The entire screening processes from dye staining and isolation of target WBCs to quantitative UTI analysis were rapidly completed within 10 min, which was demonstrated with real human urine samples. The technology has a simpler fabrication method and higher sensitivity, compared to other point-of-care methods, allowing for greater accessibility. The SIP system truly offers a simple, low-cost, portable UTI screening tool to precisely identify UTI-positive individuals, being very beneficial for resource-limited field applications.

2. An overview of the SIP platform for rapid and on-site screening of UTIs

Fig. 1 shows a schematic of the smartphone integrated paper (SIP)-based platform for rapid screening of UTIs at the point of care. It includes a paper-based filtration device for separation of target cells in a urine solution and a smartphone-integrated fluorescence microscope for quantitative analysis of UTIs.

A urine solution collected from UTI patients typically includes a variety of different types of cells found in blood and urine such as platelets, erythrocytes, lymphocytes, bacteria, epithelial cells, and so on [31–33]. Hence, it is an essential process to selectively isolate target cells from heterogeneous components in a urine solution. However, a conventional urine culturing method typically requires bulky and expensive laboratory setup such as a centrifuge and a compound

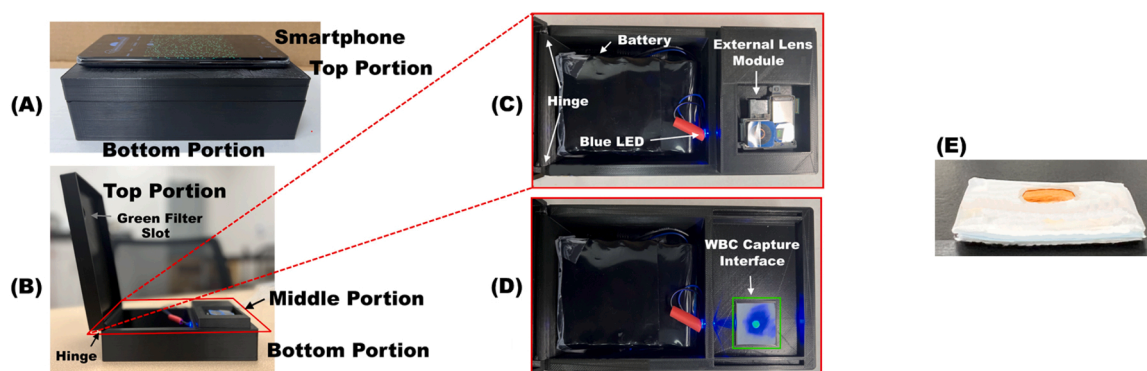


Fig. 2. (A) through (D) Photos of the 3D printed, hand-held SIP platform where all optical components, a WBC capture interface, and a smartphone are compactly assembled to serve as a portable diagnostic tool for rapid, on-site detection of UTIs. (E) An assembled paper-based filtration device with three layers meshed and a dye loaded onto the top layer.

microscope for a separation process [34–37]. This study utilizes a simple, low-cost, paper-based filtration device to achieve high-selectivity separation of target WBCs in a urine solution using three paper layers with different pore sizes. A patient's urine sample as little as 20 μL is loaded onto the paper-based filter device. While the urine solution flows downward through three layers of the papers by capillary action, target WBCs can be selectively stained and separated without additional laboratory facilities (see Fig. 1A).

For on-site screening and quantitative analysis of UTIs, a commercially available smartphone is used to capture fluorescence images of the stained WBCs and further quantify their populations to accurately diagnose UTIs on a smartphone (see Fig. 1B). Several optical components are assembled below a smartphone's rear camera to create a reversed smartphone lens microscope composed of two identical smartphone lens modules separated by a green bandpass filter [38,39]. An external smartphone lens module is reversely installed below a built-in rear camera of the phone. This reversed arrangement with the identical external lens module allows a full coverage of the smartphone's complementary metal-oxide-semiconductor (CMOS) sensor due to the perfectly matched angular field of view to the built-in camera lens module. With the presence of a blue LED light source for fluorescent excitation, the emission signals from the stained WBCs can be detected by a built-in CMOS sensor of the smartphone after passing through the reversed lens microscope that enables $14.4 \times$ magnification over a field of view of $\sim 12.25 \text{ mm}^2$. With a digital zoom-in function, up to $45 \times$ magnification can be achieved. Furthermore, a smartphone is equipped with various functions such as wireless communication, cloud data storage, and global positioning system (GPS). These capabilities allow the captured data (e.g., location tracking, time of test conducted) to be shared instantly and wirelessly with a central host like healthcare professionals for real-time monitoring and further health management of UTI patients (Fig. 1C).

Fig. 2 shows a three-dimensional (3D) printed housing platform where the optical components, battery, and WBC capture interface are all combined with a smartphone. The 3D printed housing platform is in a hand-held size of $25 \text{ (L)} \times 10 \text{ (W)} \times 4 \text{ (H)} \text{ cm}^3$ to serve as a portable diagnostic tool for rapid, on-site detection of UTIs. Fig. 2(A) shows a smartphone gently placed on the top portion of the 3D printed platform which works as a cover to block out all external light for fluorescent imaging of target WBCs taken by a rear camera of the smartphone. The captured fluorescent images of the WBCs can be displayed on a smartphone. The top and bottom portions of the 3D printed platform are connected by a hinge mechanism to allow for easy opening of the top and conveniently insert the internal components (Fig. 2B). A detachable middle portion holds an external smartphone lens module reversely installed, above which a green bandpass filter is positioned in a circular slot (Fig. 2B and C). Fig. 2(D) shows the bottom portion of the platform

where a 12 V battery (Tenergy) is placed in a hollow chamber to power a blue LED light source for fluorescent excitation of the stained target WBCs. Furthermore, a WBC capture interface of Layer 2 disassembled from the paper-based filter is inserted into a square slot of the bottom portion (indicated as green in Fig. 2D), where it can be well aligned with the external smartphone lens located 10.26 mm away from it. The stained WBCs collected in Layer 2 are excited by a blue LED at 455 nm and respond to emit the fluorescence signals at 515 nm to be detected by a built-in CMOS sensor of the smartphone via a green bandpass filter. Using various smartphone's features such as digital camera, zoom in-and-out function, high-resolution display, and image processing apps, not only can the 3D printed housing platform capture fluorescence images of target WBCs in a urine solution, but also quantify their populations to accurately determine whether a urine sample exhibits pyuria. Fig. 2(E) shows an assembled paper filter with three layers meshed and a dye loaded into the top layer.

3. Materials and methods

3.1. Urine sample preparations

For experimental demonstrations of the smartphone-based, on-site screening of UTIs, solutions of fresh human leukopak (PB001F-3, Charles River) and pooled healthy human urine (IRHUURE50ML, Innovative Research) were purchased, separately. A 1% leukopak-urine solution was prepared in volume of 900 μL by mixing 9 μL of leukopak with 891 μL of healthy urine. The concentration of leukocyte cells (or WBCs) in the solution was estimated at $8.35 \times 10^2 \text{ cells}/\mu\text{L}$. For measurement purposes, this original concentration is designated as the 100% reference solution and further sequentially diluted to provide a solution at various concentrations. For negative control, a pooled human urine solution with no leukocyte cells added was also prepared.

Sodium fluorescein dye (F6377, Sigma-Aldrich) was selected for fluorescent dyeing of the target leukocyte cells in the urine solution. The dye is non-toxic, fluoresces green when excited by 460 nm light, and acts quickly on urine with a good retention rate [13,40]. It is also known as the dye commonly used for tracking leukocytes with high selectivity in binding with plasma proteins, such as albumin, which are found exclusively attached to leukocytes rather than urinary cells or other blood cells like erythrocytes, lymphocytes, and monocytes [13,41,42]. A 5% sodium fluorescein dye solution was prepared by dissolving 0.25 g of sodium fluorescein powder in 5 mL of distilled water. This dye solution was then preloaded in the middle of Layer 1.

For real human tests, two human urine samples were purchased, one from a healthy person (HMN0021695, BioIVT) and another from a patient diagnosed with UTI (HMN0027473, BioIVT). The healthy sample was pre-analyzed and confirmed to have a standard specific gravity and

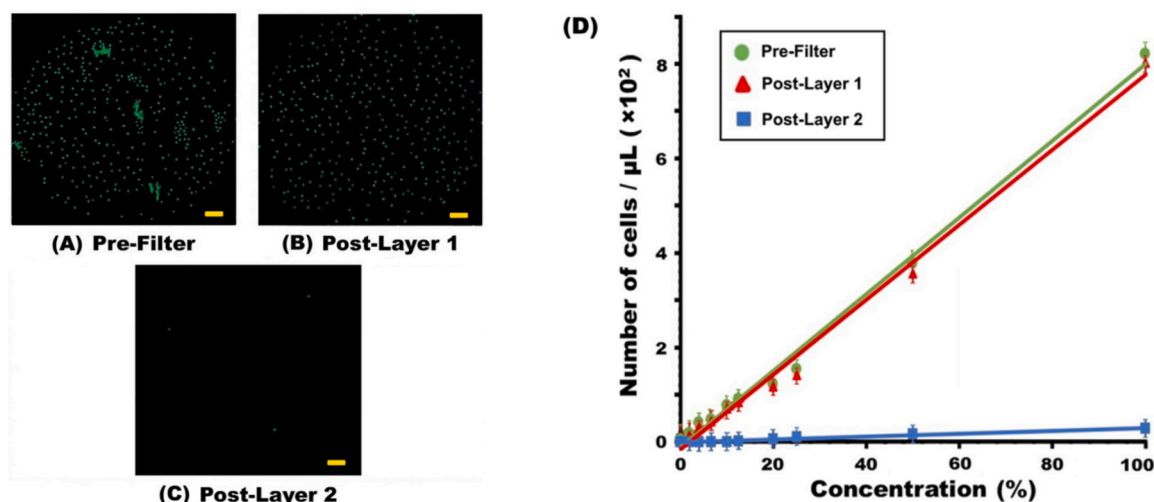


Fig. 3. Filtration capability of a paper-based device. Fluorescence images were taken on a microscope for the leukopak-urine solution (A) before the filtration process, (B) after passing through Layer 1 of the filter, and (C) after passing through both Layer 1 and Layer 2. The scale bars indicate 150 μm . (D) A graph shows the numbers of the leukocyte cells counted on a hemocytometer for ten different concentrations of the leukopak-urine solution. The concentrations of the leukocyte cells at the pre-Filter and post-Layer 1 are evaluated with no significant difference ($P < 0.05$, Student's t test). The measured data at the post-Layer 1 and post-Layer 2 show a significant difference in the concentrations of the leukocyte cells. For all ten concentrations of the urine solution, more than 95% of the total leukocyte cells can be collected in Layer 2, which will be used for fluorescence imaging and cell counting on a smartphone later. This experimental study confirms that the majority leukocytes (more than 95%) in urine solutions can be effectively collected by the paper-based filter device without any bulky and expensive laboratory setups.

test negative for blood cells and leukocyte esterase. The UTI sample tested highly for pathogen antibody bonding and provided the leukocyte concentration at 3.68×10^2 cells / μL from urinalysis. These human urine samples were directly loaded onto the SIP platform for selective filtration of target cells, fluorescence imaging, and cell counting for quantitative UTI analysis.

3.2. A simple, low-cost, paper-based filtration device

A paper-based filtration device was cost-effectively fabricated to selectively isolate the target leukocyte cells in a leukopak-urine solution. As presented in Fig. 1(A), it consists of the three layers of the paper sheets with several different pore sizes that allow two degrees of filtration to ensure high selectivity of the target cells from other urine components.

Layer 1 of the filter device works to selectively stain target leukocyte cells in a urine solution. It was fabricated by folding a 50 mm $\times$ 25 mm sheet of a 1 mm thick Paraffin wax film in half to make it in a 25 mm $\times$ 25 mm size. Then, it was punched to have a 6 mm diameter hole at the center before being wrapped by fiberglass (GFCP20300, Sigma-Aldrich) with 20 μm pores. The fiberglass and wax film were then compressed and put on a hot plate at 135 $^\circ\text{C}$ until evenly meshed. After cooling, the layer was preloaded with 10 μL of the sodium fluorescein dye coated at the center and then stored in a dark room. When a urine solution is loaded in Layer 1, the target leukocyte cells are selectively stained and the cells including the leukocytes smaller than the 20 μm pore size of the fiberglass can pass through it. It is noted that the size of leukocyte cells is around 10–15 μm in diameter [9]. Next, a borosilicate membrane (WHA70604714, Sigma-Aldrich) with 9 μm pores was used to capture the leukocyte cells in Layer 2. It was fabricated by first marking a 6 mm circle at the center of a 25 mm $\times$ 25 mm borosilicate membrane to align with the punched hole in Layer 1. A 0.7 mm high paste wax barrier was applied around the circular marking at the center using wooden sticks. This wax barrier ensures the urine sample flowing through only within the 6 mm circular area at the center without dispersion to outside, which helps for accurate quantification of the cells. Due to the pore size of the borosilicate membrane in 9 μm , the leukocyte cells around 10–15 μm in diameter can be effectively collected in Layer 2 without additional bulky and expensive laboratory setup like

centrifuges. Whereas, other types of cells, including erythrocytes, lymphocytes, bacteria, and yeast, smaller than the 9 μm pore size keep passing through to a wicking pad of Layer 3 made of a 25 mm $\times$ 25 mm cellulose chromatography paper (WHA1001329, Sigma-Aldrich) with 3 μm pores. These three layers of the papers were then assembled using scotch tape and placing it hanging off one of the edges of Layer 1. Layer 2 was placed under Layer 1 without the overlap of the tape. Layer 3 was placed below Layer 2, and then the tape was wrapped around the bottom of Layer 3 to be adhered to the opposite edge of Layer 1. This keeps all three layers packed together and well aligned. An assembled paper filter with a dye loaded onto the top layer is shown in Fig. 2(E).

Once the fabrication of a paper-based filtration device is completed, 20 μL of the urine solutions at various concentrations was loaded at the center of the filter device. While it flows downward through three layers of the papers with different pore sizes, target leukocyte cells are selectively stained, collected, and evenly dispersed in Layer 2. Then, the tape is taken off to disassemble the Layer 2. This WBC capture interface is placed in a square slot of the bottom portion (indicated as green in Fig. 2D) to be imaged and analyzed for screening of UTIs.

3.3. Fluorescence imaging and counting of target cells on a smartphone

A commercially available smartphone (Samsung Galaxy S20) is used to capture fluorescence images of the leukocyte cells collected in Layer 2 of the paper device. The captured images of the cells are further analyzed to estimate their populations using an image processing software, ImageJ. For cell counting, the RGB-based fluorescent image is first converted in greyscale, and the background is subtracted with rolling ball to separate cells that are too close together. Then, the cells are analyzed by size and circularity to output a total image count. Using this image processing scheme, the target leukocyte cells are identified, and their populations are quantified based on the fluorescent images taken by a smartphone camera. After multiple repetitions over 3 times, an average cell count for each solution was calculated with standard deviations. This was used to estimate the measured cell concentrations in the urine solution. For validation purpose, the results measured by the SIP have been further compared to a conventional method based on a hemocytometer.

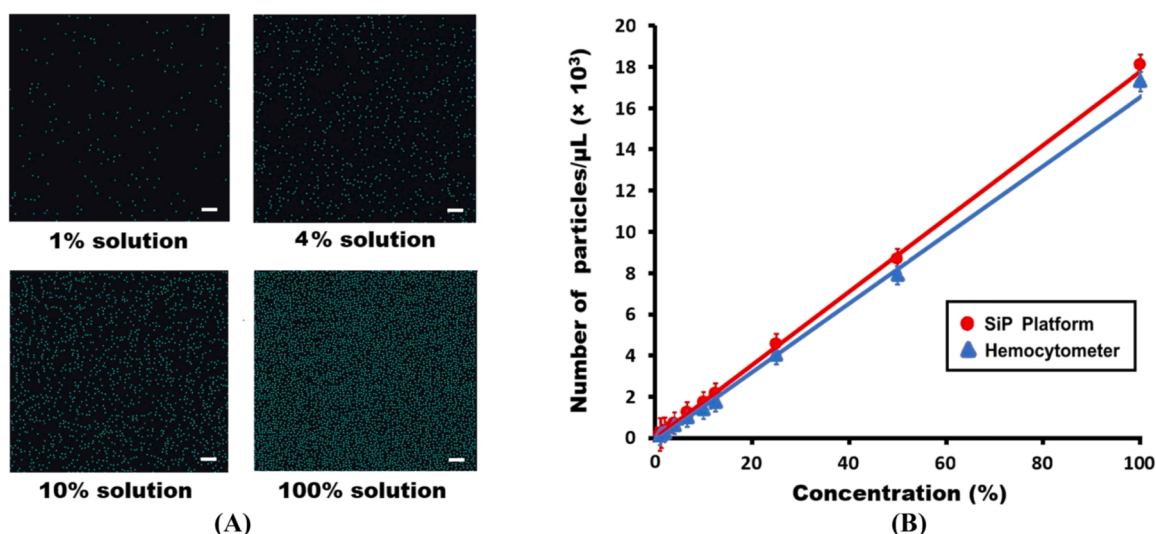


Fig. 4. Fluorescence imaging and counting of microparticles. (A) Fluorescent images of 10 μm particles at four different concentrations of the solution were taken by a smartphone's camera through a reversed lens microscope assembled in a 3D printed SIP platform. A scale bar indicates 250 μm. (B) Using the fluorescence images of the particles, their populations were quantified on the SIP and plotted at various concentrations. The particle counts show a linear increase with the particle concentrations of the reference solution. These cell population data measured by the SIP have been further compared to ones by a conventional hemocytometer method. Bland-Altman analysis reveals statistically no difference in the counts measured by both methods within a 95% confidence interval.

3.4. Statistical analysis

A paired Student's *t*-test was performed to validate the paper filter's efficacy for isolation of target leukocyte cells from the leukopak-urine solution [43]. The analyses are statistically regarded as no significant difference when *p*-values are less than 0.05 ($P < 0.05$). Bland-Altman analysis is a more thorough method to determine the similarity between two instruments for measuring the same type of data. To compare SIP and hemocytometer measurement, Bland-Altman analysis was additionally conducted to determine their similarity for cell and particle counts [44]. The average measured values between the two measurement methods for each concentration value was plotted against the difference between the measured counts of the SIP and hemocytometer. The measurements between these two methods are regarded as statistically insignificant if all measurement values are within the 95% confidence interval range. A detailed description on Bland-Altman analysis is provided in a supplementary document. The error bars in the quantitative graphs indicate the standard deviations based on uncertainty in measurements [45].

4. Experimental results and discussion

4.1. Filtration capability of a paper-based device

A urine solution collected from UTI patients is typically heterogeneous, not only including leukocyte cells to be detected, but also other types of cells such as erythrocytes, lymphocytes, bacteria, and platelets. Hence, the first study conducted was to explore how effectively the paper-based filter device works for the isolation of target leukocyte cells from other types of cells in a urine solution. For this study, 1 mL of leukopak-urine solutions was prepared at ten different concentrations of leukocyte cells. Each solution was directly mixed with 0.5 mL of sodium fluorescein dye to selectively stain the leukocyte cells and then placed onto a hemocytometer to count the numbers of the cells before the filtration process. This was repeated five times for each concentration to get an average count of the leukocyte cells. The same cell counting processes continued for the urine solutions after being filtered in Layer 1 and Layer 2, respectively.

Fig. 3 (A) through (C) show the fluorescence images of the undiluted leukopak-urine solution, which were taken by a compound microscope

with 10 × magnification. Fluorescent signals indicate the stained leukocyte cells in the urine solution. Before filtration, most of the cells are well dispersed in the solution, but some of them are closely adjacent (Fig. 3A). When the solution passes through Layer 1, the cells, which might be aggregated to be larger than the 20 μm pore size, are collected in Layer 1. Majority of the cells can be filtered out through Layer 1 due to the cell size smaller than the 20 μm pore size. Fig. 3(B) shows the urine solution at post-Layer 1. The leukocyte cells are well dispersed in uniform size and no aggregated cells are observed. After being filtered by Layer 2 with 9 μm pore size, only a few numbers of the leukocyte cells are observed in Fig. 3(C).

To quantitatively evaluate filtration capability, the numbers of the leukocyte cells were further counted using a conventional cell counting method based on a hemocytometer under a microscope and the measured data are plotted in Fig. 3(D). An average count of the leukocyte cells for the 100% (i.e., undiluted) solution was estimated as 8.23×10^2 cells/μL before the filtration and 8.11×10^2 cells/μL after being filtered by Layer 1, respectively. These measurement data present no significant difference between them with less than 2% difference ($P < 0.05$, Student's *t* test). Such a negligible difference between pre-Filter and post-Layer 1 is consistently observed for all ten different concentrations of the urine solution, as presented in Fig. 3(D). This indicates that Layer 1 does not make any contribution to the separation process. It just works for staining of the leukocyte cells when being preloaded with the sodium fluorescein dye. However, after being filtered by Layer 2, negligible numbers of the cells were observed for each concentration of the urine solution (see a blue line in Fig. 3D). The test results indicate that more than 95% of the leukocyte cells can be effectively collected in Layer 2 without any bulky and expensive laboratory equipment like centrifuges, being very beneficial for low-cost, resource-limited field applications.

4.2. Fluorescence imaging and counting of microparticles on a smartphone

The next study conducted was to see the capability of the smartphone-integrated microscope as a portable tool for fluorescence imaging of target cells and quantitative analysis of their populations. Before doing experiment with urine samples, we have tested with fluorescent polystyrene particles in 10 μm diameter for this study. A particle

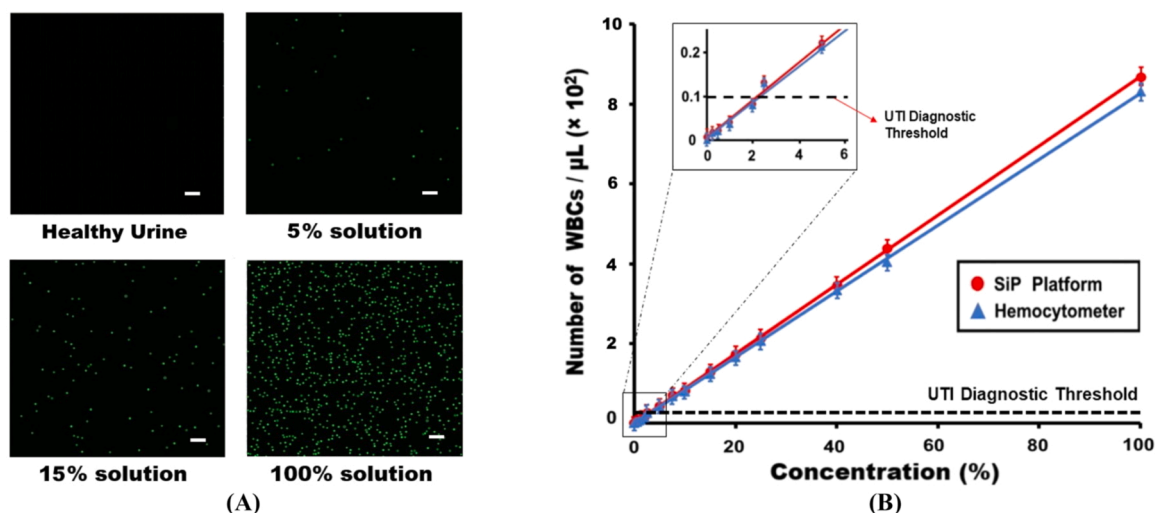


Fig. 5. SIP tests for on-chip filtration and counting of target WBCs in urine solutions. (A) Fluorescent images of the stained leukocyte cells at various concentrations including a pooled healthy human urine were taken by a rear camera of a smartphone through a reversed lens microscope assembled in a 3D printed SIP platform. The scale bars indicate 250 μm . (B) Using the fluorescence images of the cells at fifteen different concentrations, their populations were quantified on the SIP platform. The zoomed in black box displays the data at the concentrations below 5%. The numbers of the cells show a linear increase with the concentrations of the reference solution. These population data have been further compared to ones by a conventional hemocytometer method, showing a similarly increasing trend for both methods. Bland-Altman analysis shows a statistical similarity between the counts measured by both SIP and hemocytometer within a 95% confidence interval. A dotted line indicates the UTI threshold number of leukocytes at 10 WBCs/ μL , above which a patient's test sample is said to be diagnosed for UTI. Both counting methods have a similar positive linear correlation between expected and measured concentration, proving that the SIP technology accurately quantifies the numbers of target WBCs in urine solutions, comparable to the hemocytometer.

solution at the listed concentration of 1.82×10^4 particles/ μL was designated as the 100% solution for microparticle measurement experimentation and further sequentially diluted by adding water to provide ten different concentrations. 10 μL of each solution was pipetted onto a microscopic slide and then placed in a square slot of the bottom portion in the 3D-printed SIP platform. Fig. 4(A) shows the fluorescent images of the microparticles at four different concentrations, which were captured by a smartphone's rear camera through a reversed lens microscope installed in a 3D printed platform. The particle size in 10 μm can be clearly recognized. The captured fluorescence images were further analyzed to count their populations using an image processing software (ImageJ) and the measured data at various particle concentrations were plotted in Fig. 4(B). For validation purpose, the data measured by the SIP were further compared to the ones by a hemocytometer under a microscope. In Fig. 4(B), a linearly increasing trend in particle counts can be observed for both methods as increasing the solution concentrations. For example, an average count of the particles for the stock solution at 100% concentration was measured as 1.80×10^4 particles/ μL with the SIP and 1.74×10^4 particles/ μL with the hemocytometer. All counts measured by both SIP and hemocytometer were within a 95% confidence interval of each other. A supplementary document provides details on the Bland-Altman analysis conducted in this study. The statistical similarity proves that the cell population data measured on the SIP platform are comparable in accuracy to the conventional hemocytometer method. The positive linear correlation between expected and measured concentration confirms that the SIP can perform as a portable diagnostic tool for on-chip detection and quantification of urine samples.

4.3. Filtration and counting of target WBCs on the SIP

Previous works demonstrated the capabilities of the SIP for separation of target leukocyte cells using a paper-based filter in Section 4.1 as well as quantification of microparticles on a smartphone in Section 4.2, respectively. These two studies were combined and repeated for leukocyte cells in urine samples. To simulate the urine solutions with various levels of pyuria, leukopak-urine solutions were prepared at

fifteen different concentrations. 20 μL of each urine solution was first loaded onto the paper-based filtration device. When the solution flows through the three layers of the papers, the leukocyte cells exclusively bind with the dye preloaded in Layer 1, followed by selective isolation and even distribution in the WBC capture interface of Layer 2. Fig. 5(A) shows fluorescence images of the stained leukocytes (or WBCs) collected in Layer 2 at four different concentrations, including the one from the pooled healthy human urine sample (i.e., no WBCs expected). Similarly, the numbers of the WBCs were counted for the solution at various concentrations under the SIP, each averaged for three different measurement trials. These measured data were plotted in Fig. 5(B) and further compared with the ones by a hemocytometer for validation purpose. For the undiluted 100% solution, the concentration was measured at 8.46×10^2 cells/ μL by the SIP and 8.07×10^2 cells/ μL by a hemocytometer. The measurement data showed that the average cell counts measured by both SIP and hemocytometer for all fifteen different concentrations were within a 95% confidence interval of each other. A detailed information on the Bland-Altman analysis is provided in a supplementary document. The SIP method accurately measured cell concentrations as low as 1 cell/ μL , meaning there is no lower detection limit. The linear positive correlation between the expected and measured WBC concentrations verifies that the SIP technology can be used as an on-site detection tool to accurately screen for UTIs.

A dotted line in Fig. 5(B) indicates the diagnostic threshold for UTI at 10 WBCs/ μL , above which it can be said that the excessive level of WBCs indicates the presence of infectious foreign bodies in the urinary tract [7–11]. Of the fifteen different solution concentrations imaged and measured in the SIP, the five lowest concentrations at 0% (healthy urine), 0.25%, 0.5%, 1%, and 2% have average WBC counts below this diagnostic threshold for both methods (see a zoomed inset graph in Fig. 5B). All other concentrations had measured WBC counts characteristic of a UTI patient. If a positive result is similarly diagnosed for real patient samples, a patient would be directed to take medication or perform with subsequent tests for determination of antibiotic susceptibility profile [46–48]. A measurement capability of the SIP technology was evaluated using two performance parameters, the sensitivity of 96.67% and the specificity of 100%, with only one false negative and no

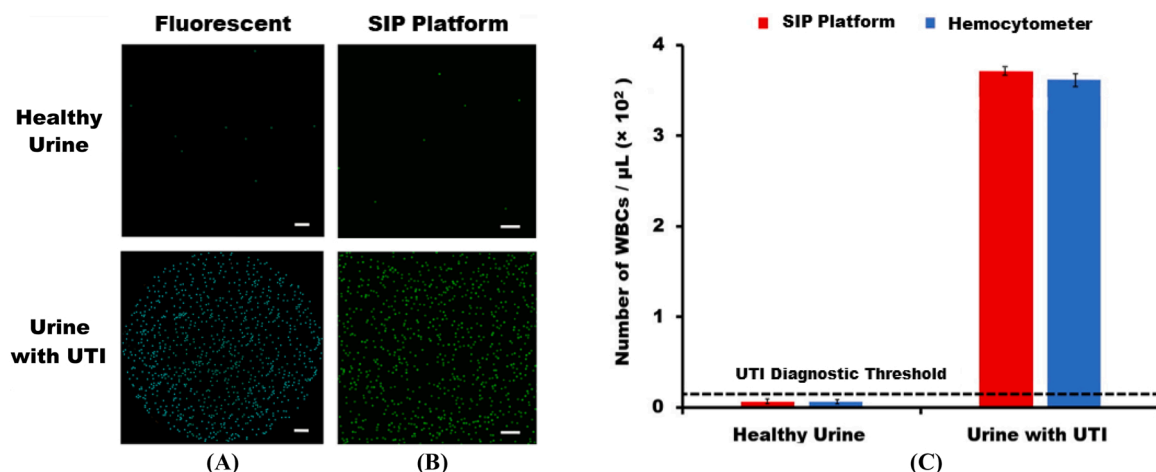


Fig. 6. Tests with real human urine samples. Fluorescence images of urine samples collected from a healthy person and a UTI-positive patient are taken (A) under a microscope and (B) on the SIP platform. Dramatically higher WBC counts are shown for the UTI urine sample. The scale bars indicate 150 μm for fluorescence microscope images, while indicating 250 μm for the SIP images. (C) A graph comparing the measured WBC concentrations for the healthy patient and UTI patient samples with the SIP device and using a hemocytometer under a microscope. Both counting methods measured similar concentrations with no significant difference ($P < 0.05$, Student's t test).

false positive results from total 45 measurement tests. These measurement performances indicate that the SIP method provides more accurate UTI screening than dipstick urinalysis, without bulky and complex microfluidic components typically required for microfluidic methods.

4.4. SIP tests with real human urine samples

The final experimental protocol was to test the SIP technology with real human urine samples. Two human urine samples were obtained, one from a healthy person and another from a patient diagnosed with UTI. Similarly, 20 μL of each urine sample was tested for cell counting on both the SIP and hemocytometer. Fig. 6(A) and (B) show fluorescence images of these two human urine samples taken on a regular microscope with a $10\times$ magnification and captured on the SIP. The numbers of WBCs were then counted, and the counting results were plotted in Fig. 6 (C). For the healthy urine sample, an average concentration of WBCs was estimated as 6.5 ± 0.23 cells/ μL by the SIP, while 6.2 ± 0.20 cells/ μL by the hemocytometer. For the UTI infected urine sample, $3.72 \pm 0.17 \times 10^2$ cells/ μL and $3.58 \pm 0.15 \times 10^2$ cells/ μL . These test results with real human urine samples clearly indicate that both healthy and UTI patient counts have no significant difference between the SIP and hemocytometer counts ($P < 0.05$, Student's t test). The urinalysis-tested leukocyte concentration in the UTI patient sample listed by the sample provider was 3.68×10^2 cells/ μL (BioIVT), and neither the SIP nor hemocytometer measured concentrations were significantly different from this value ($P < 0.05$, Student's t test). In relation to the UTI diagnostic threshold of more than 10 cells/ μL , the healthy patient sample tested below, while the UTI patient sample tested above.

Interestingly, on the SIP platform, the entire screening processes took no more than 10 min for each sample from filtration to final WBC count. Hence, the SIP technology successfully differentiated between real human urine samples that exhibit pyuria and do not exhibit pyuria, demonstrating the capability to diagnose UTIs dependably and quickly at the point of care with affordable materials ideal for resource-limited field applications.

5. Conclusion

A smartphone integrated paper (SIP)-based platform is presented for rapid and on-site screening of UTIs. It directly counts the numbers of target white blood cells (WBCs) in urine to accurately diagnose UTI-positive patients at the point of care without extra bulky and pricey

laboratory facilities. With a paper-based filter, the SIP enables to selectively dye target WBCs in a urine solution and isolate them with more than 95% collection efficiency using different pore sizes of low-cost paper layers. Furthermore, a reversed smartphone lens microscope captures fluorescence images of the target cells and quantifies their populations under the $14.4\times$ magnification over a field of view of ~ 12.25 mm². The capability of the SIP for accurate counting of target WBCs at various concentrations has been experimentally demonstrated with no significant difference, consistently within a 95% confidence interval from traditional hemocytometer measurements (Bland-Altman analysis). This statistical similarity proves that the cell population data measured by the SIP are comparable in accuracy to the hemocytometer count method. In addition, real human urine samples from healthy and UTI-positive persons were also tested on the SIP system, showing successful differentiation between infected and healthy person samples. The entire detection processes from isolation of WBCs to quantitative analysis can be rapidly completed within 10 min, allowing rapid and accurate screening of UTIs with 96.67% sensitivity and 100% specificity. The SIP technology offers a low-cost, portable UTI screening method to precisely identify UTI-positive individuals, being very beneficial for low-cost, resource-limited field applications. Using smartphone's various features, measured data can be wirelessly loaded to a central database monitored by medical professionals. Thus, UTI treatment can be prepared and dispatched earlier to drastically reduce patient morbidity.

CRediT authorship contribution statement

Janev: Methodology, Validation, Investigation, Formal analysis, Writing – original draft. **J. S. Kang:** Resources, Writing – review & editing. **S.-Y. Park:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sung-Yong Park reports financial support was provided by National Science Foundation (NSF).

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2023.133498.

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