Topics in Micro analytical Chemistry

- Inspiration for miniaturization.
- Micro analytical system building.
- Flow control in micro channels.
- Detection**.**
- Application.

❖ ***Micro analytical chemistry***

Micro analytical chemistry is a branch of science in which laboratory functions can be scaled down into a miniaturized system whilst maintaining the accuracy and precision observed in the larger scaled experiments. The development of micro analytical chemistry allows a new technology to be taken outside of the laboratory setting and away from the use of more costly conventional methods, it is time saving, more efficient and integrated several processes into one device.

As a result, there is a need for construction of a portable, disposable, automated and miniaturized systems, such as lab-on-a-chip (LOC) devices , point -of -care (POC) systems, micro total analysis system (μTAS), and drug delivery system (DDS), etc., which can provide valuable information. Due to the minimization of the device the in/out fluid must be minimized to microfluidic size. The microfluidic system should compile of generic components such as the introduction of a sample, movement of a sample, mixing/washing stages and a detection system. The design and approach for these components can vary greatly within research groups but with each sharing an end goal, to produce a device which can function easier and just as well as the conventional methods currently available.

Environmental sample Food sample Human sample Cell culture

Target analyte

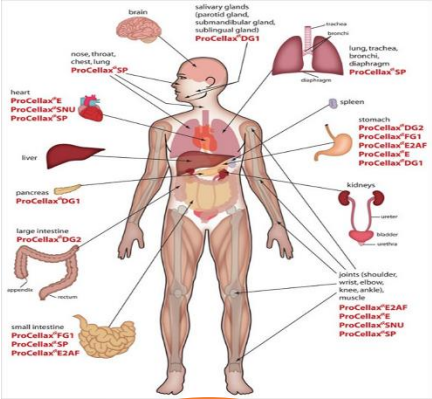
Chemical reaction

Transducers (electrical, mass based and optical)

Readout results

❖ ***History of micro analytical chemistry***

System miniaturization using Micro analytical chemistry include the use of microfluidics which can be best defined as "the science and technology of systems that process or manipulate small (10^{-9} to 10^{-18} liters) amount of fluid, using channels with dimensions of tens to hundreds of micrometers". This method can be used to control the concentration of molecules in space and time. Microfluidics concepts go back to 1950s, originating in the inkjet printer manufacturing. In 1970 a miniaturized gas chromatograph system was demonstrated and this has been followed by a huge rise in the number of publications in this field. Microfluidic systems have been successfully overcome the inherited problems related with traditional analysis as can be seen from below image. This encouraged researches to apply it in the diverse area including chemistry, medicine, engineering, forensics and bioanalytical researches, for example for DNA separation and analysis, cell separation and manipulation, enzyme kinetic studies, drug discovery, and immunoassay, etc.

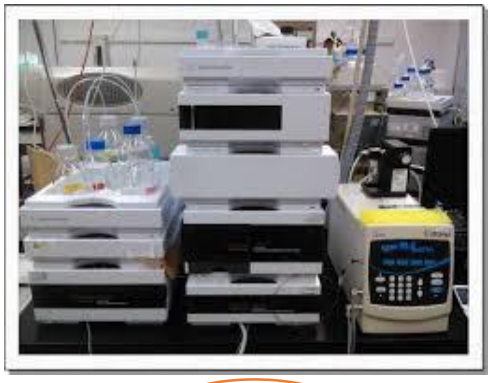


Sampling

Transport

Pretreatments

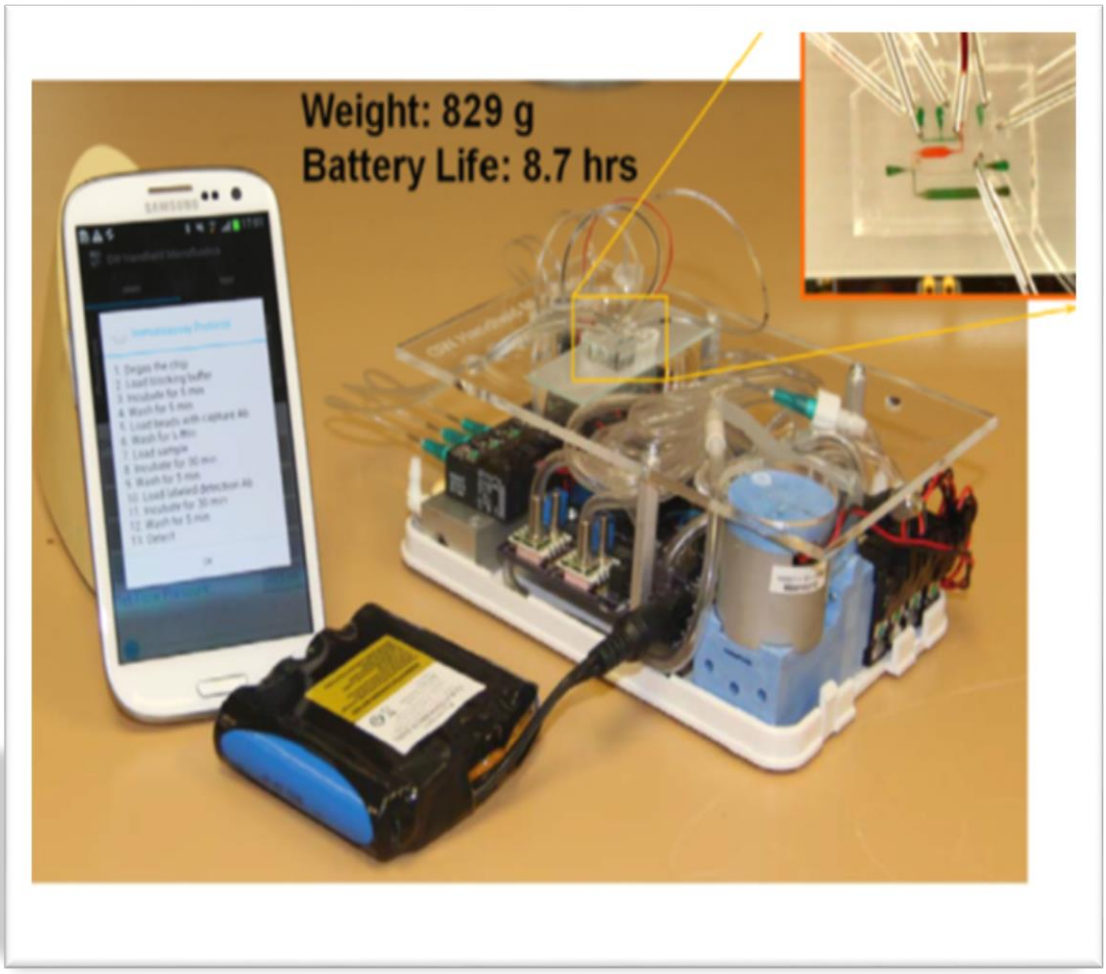
Chemical reaction

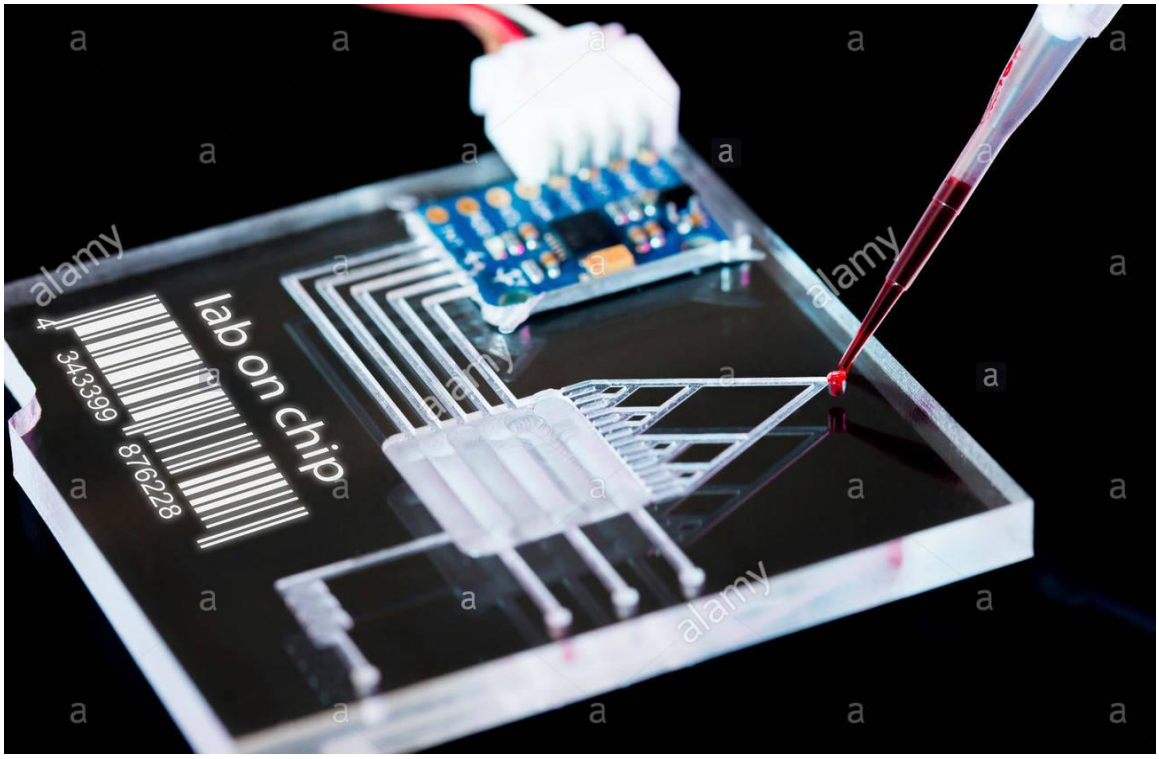


Detection

Data analysis

This diversity is related to major advantages of microfluidic systems: (i) the use of small sample and reagent volumes alongside the easier integration of multiple processes on a single device (ii) reduction of chemical waste and contamination (iii) construction of microfluidic device is relatively inexpensive and (v) improvement in sensitivity and resolution, such characteristics make this microfluidic system appropriate for dealing with limited or degraded samples. Finally, it can be said that due to its miniaturized design, microfluidic systems can be considered to be portable, compact and easy to use.




lab on chip
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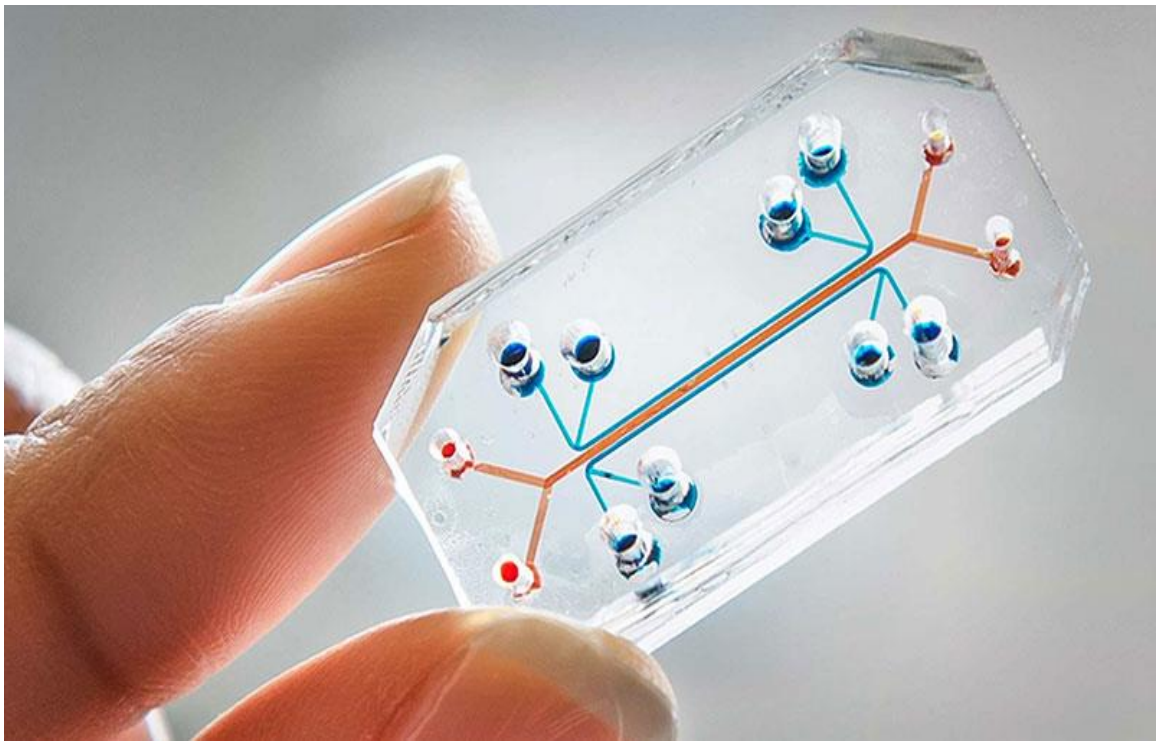

❖ ***Micro analytical systems:***

1. Lab-on-a-chip

A lab-on-a-chip (LOC) or so called "micro total analysis systems" (µTAS) is a device that integrates one or several laboratory functions on a single integrated circuit (commonly called a "chip") of only millimeters to a few square centimeters to achieve automation and high-throughput screening. LOCs can handle extremely small fluid volumes down to less than pico-liters. "lab-on-a-chip" indicates generally the scaling of single or multiple lab processes down to chip-format, whereas "µTAS" is dedicated to the integration of the total sequence of lab processes to perform chemical analysis.

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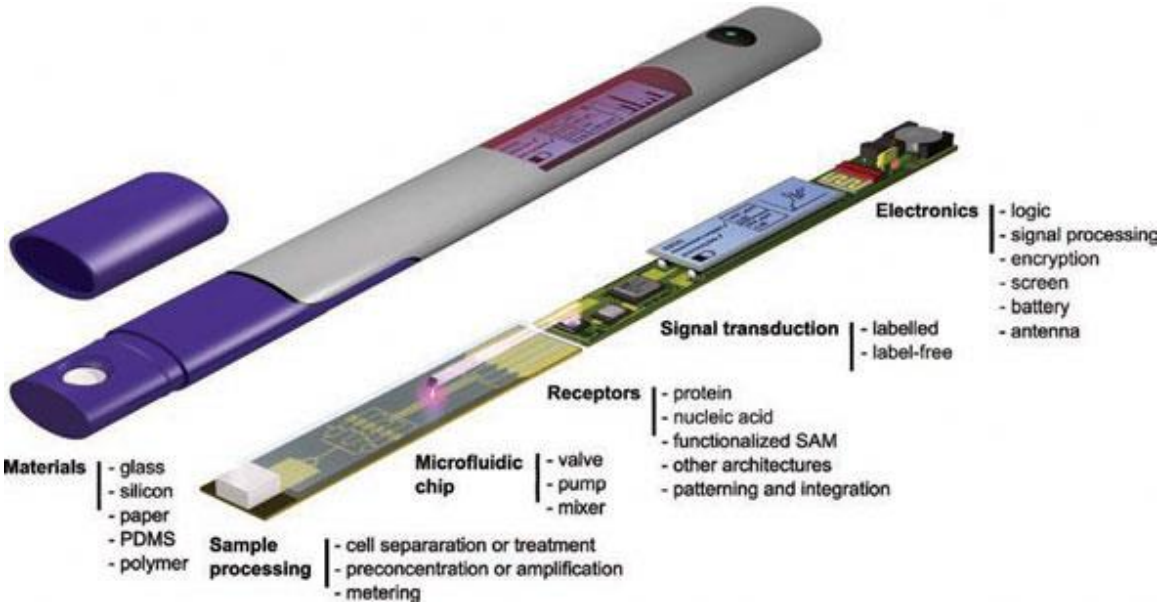
2. Point of care

Point of care testing is a medical device or system which can be brought to the patient's bedside or home rather than in a hospital or doctor's surgery and the results received by the patient either immediately after the test or a short time afterwards, as opposed to a few days. A POC device must also be small to allow for portability and in-expensive to manufacture. The features of microfluidics create a perfect platform for POC, the small consumption of reagents and sample allow for a fast turnaround in analysis, and the key idea is that rapid testing will lead to a rapid intervention improving the prognosis for the patient. One of the earliest POC system was used to detect glucose through a tablet, after which came the dipstick assays for pregnancy testing and human immunodeficiency virus (HIV) which only require the addition of a sample. Therefore most research has been aimed at producing a device for a POC system that is simple to use which in turn requires minimal equipment and an easy analysis.

A Famous aspect of POC devices currently undergoing development is a scheme referred to as "tele devices". This is aimed at who suffer from long term conditions such as diabetes or kidney failure or patients who have to continually travel to a hospital or general practitioners (GP) surgery for routine tests. It allows patients to live independently whilst maintaining stable health, a patient would be shown how to perform routine tests at home on a simple POC device and the results be transmitted automatically or emailed or telephoned by the user to their doctor. The doctor would monitor the results of

the patients and only contact the patient to visit the surgery or hospital if there was a change in status or unusual, thus reducing the burden on the health system.

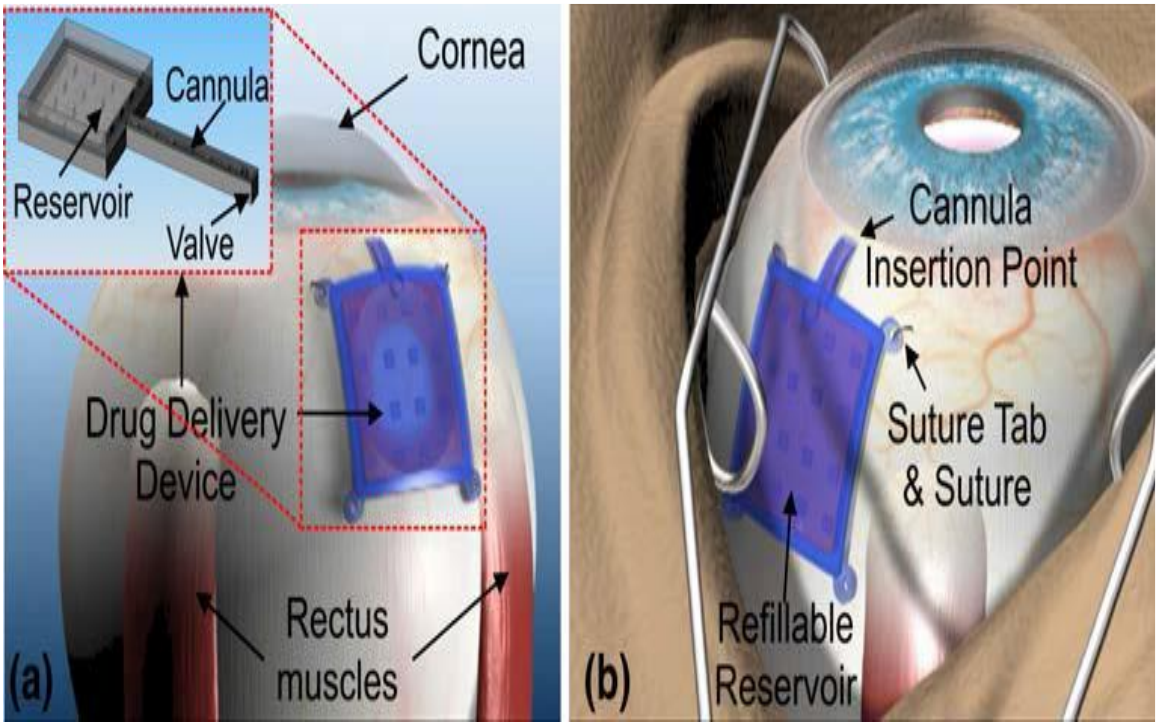


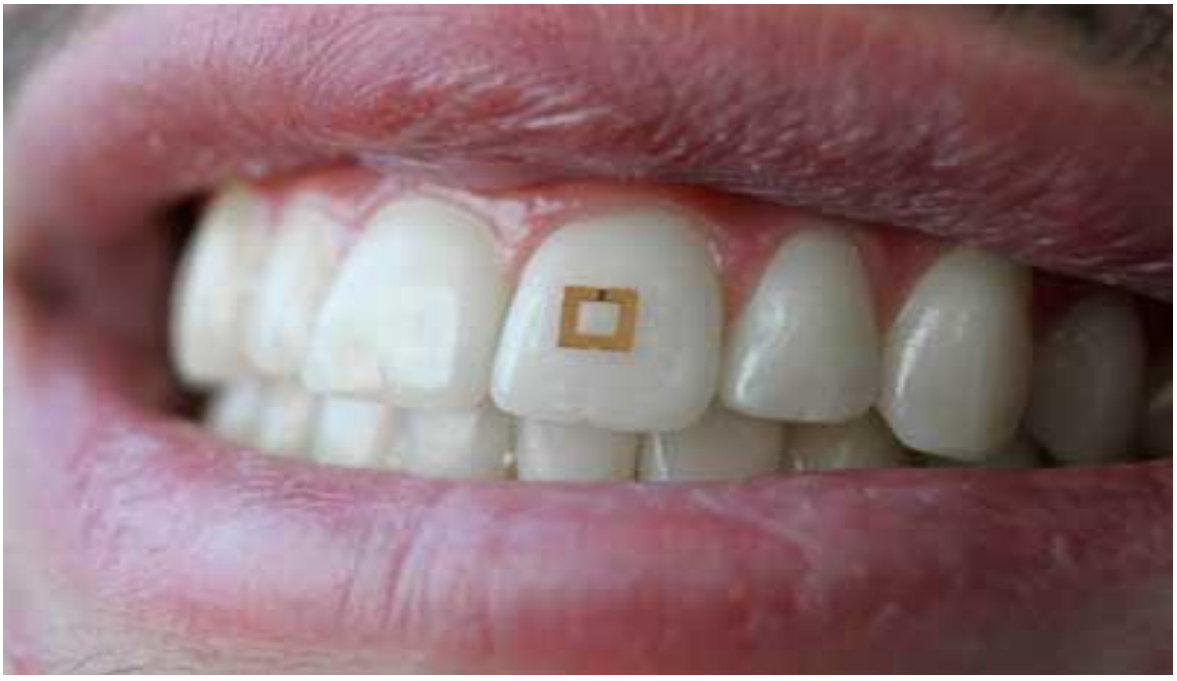
3. Drug delivery system

A drug delivery system is a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time and place of release of drugs in the body.

In the area of drug delivery science, microfluidics offers advantages, such as precise dosage, ideal delivery, target-precise delivery, sustainable and controlled release, multiple dosing, and slight side effects. These advantages bring significant assets to the drug delivery systems. Microfluidic technology has been progressively used for fabrication of drug carriers, direct drug

delivery systems, high-throughput screening, and formulation and immobilization of drugs.





❖ ***The prospects of microfluidic device***

To fulfil the important criteria of a simple, cheap, automated, and selective system; researchers seek for an analytical improvement, resulting in the automated and miniaturized microfluidic platform. Therefore, the basic requirements and principles of this promising technology was studied as follow:

1. Materials

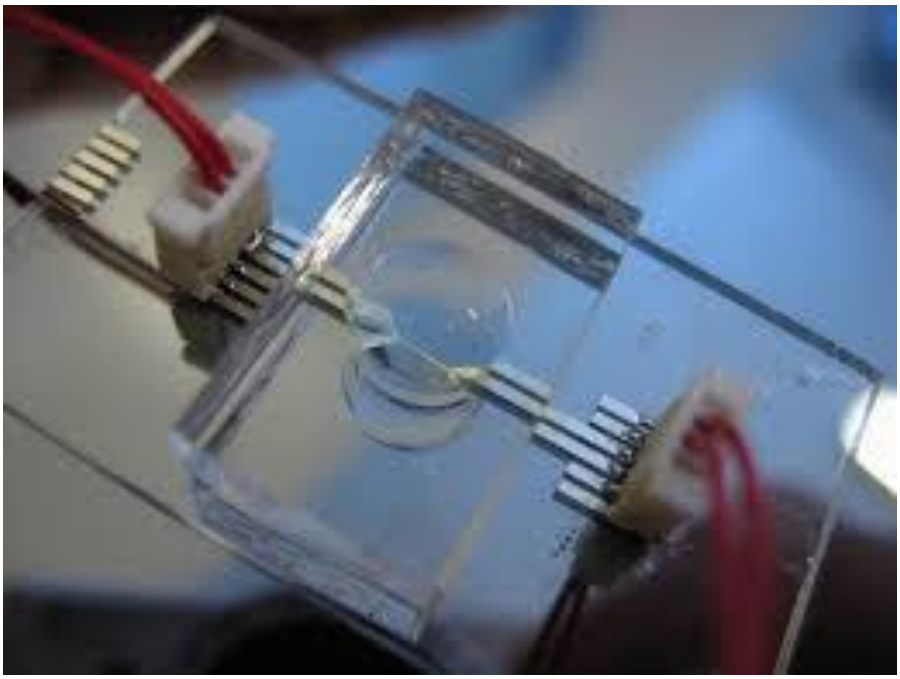
A key factor in microfluidic chip fabrication is the material substrate that is used. Various substrate materials have been reported, such as silicon, glass, and polymers. Silicon is the most common surface due to extensive surface chemistry studies plus its high-temperature resistance and good thermal conductivity. However, silicon suffers from some disadvantages such as it is not optically transparent, electrically conductive and it is expensive compared to other materials. On the other hand, glass has good optical transparency, and is not electrically conductive and is chemically resistant, which makes glass substrate good for practical application in a microfluidic chip construction. Nonetheless, handling with glass is slightly complicated due to the fact that glass is fragile which add extra attention and it is less expensive than silicon but more expensive than other materials. Polymers are popular for bioanalysis with desirable characteristics such as simple fabrication, biocompatibility and reduced cost. Suitable polymers include polymethylmethacrylate (PMMA), polydimethylsulfoxide (PDMS) and polystyrene(PS), etc. Nevertheless, polymers have some disadvantages such as limited temperature range, and lower optical transparency compared to glass.

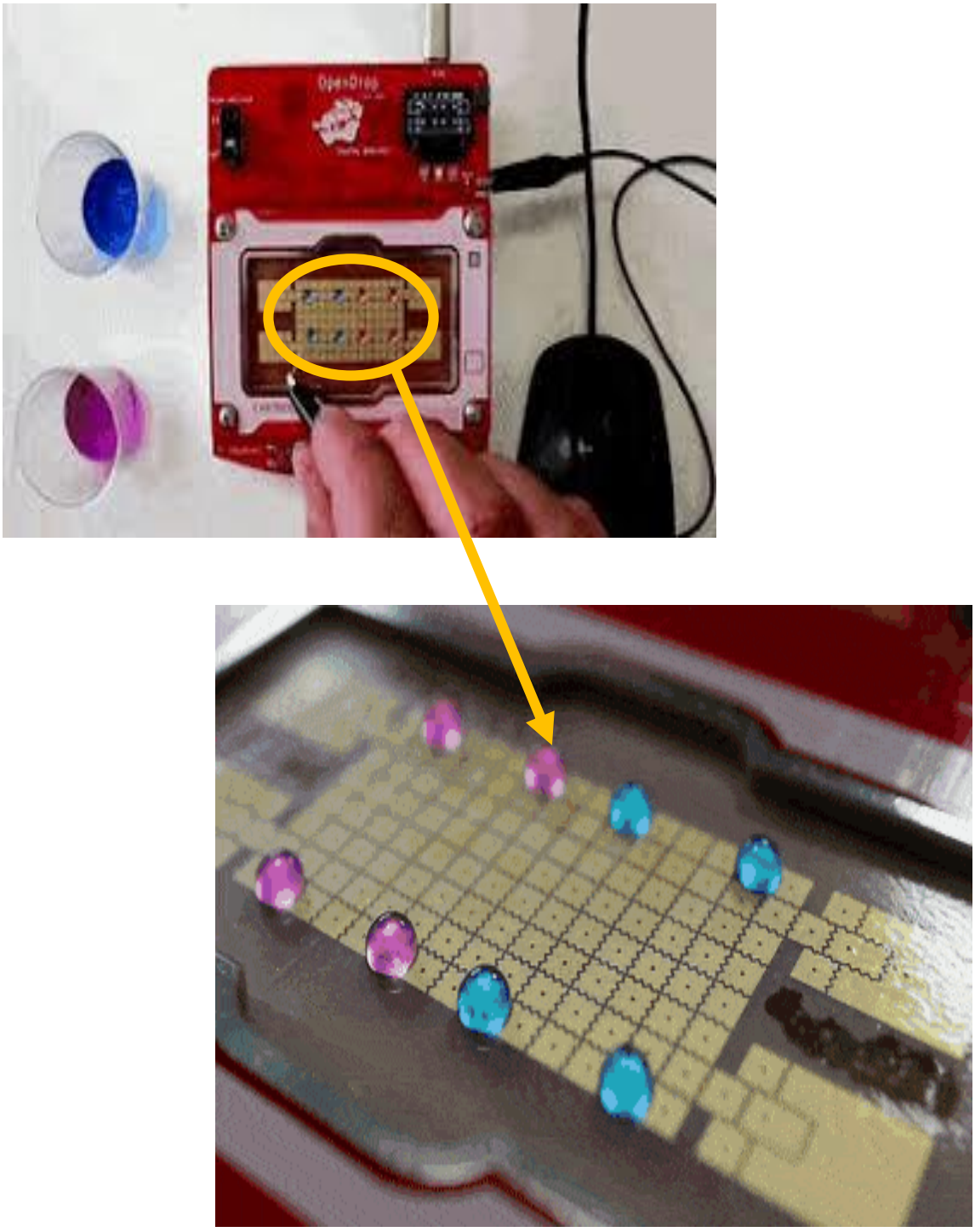
Recently different modified electrodes have beenused, including glassy carbon, gold (Au), carbon paste, screen-printed electrode and where they act as a support material for reagent immobilization, miniaturizing the microfluidic device components.

2. Sample/ Reagent transport

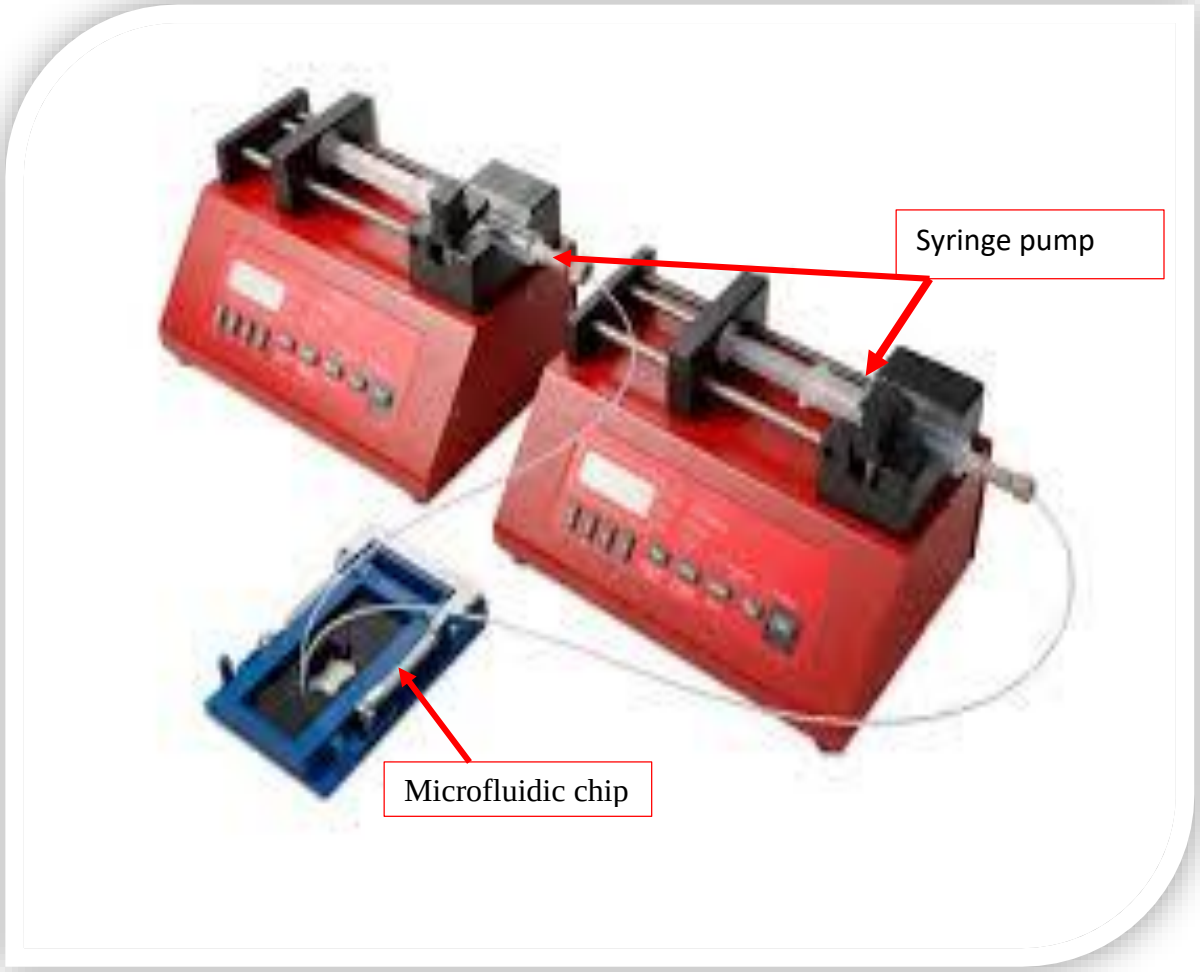
Microfluidic chips are directly affected by the performance of the fluid transport system. Therefore the fluid transfer plays an important role inside a microchannel. This can be categorized into three main fluid handling forces:

1. Electric forces where the fluid transport system depends on flow generated by the electrophoretic and electroosmotic interaction of ionic species with the applied electrical field, whereas electrowetting force depends on the manipulation of the fluid drop in the presence of program voltage applied to an electrode array.

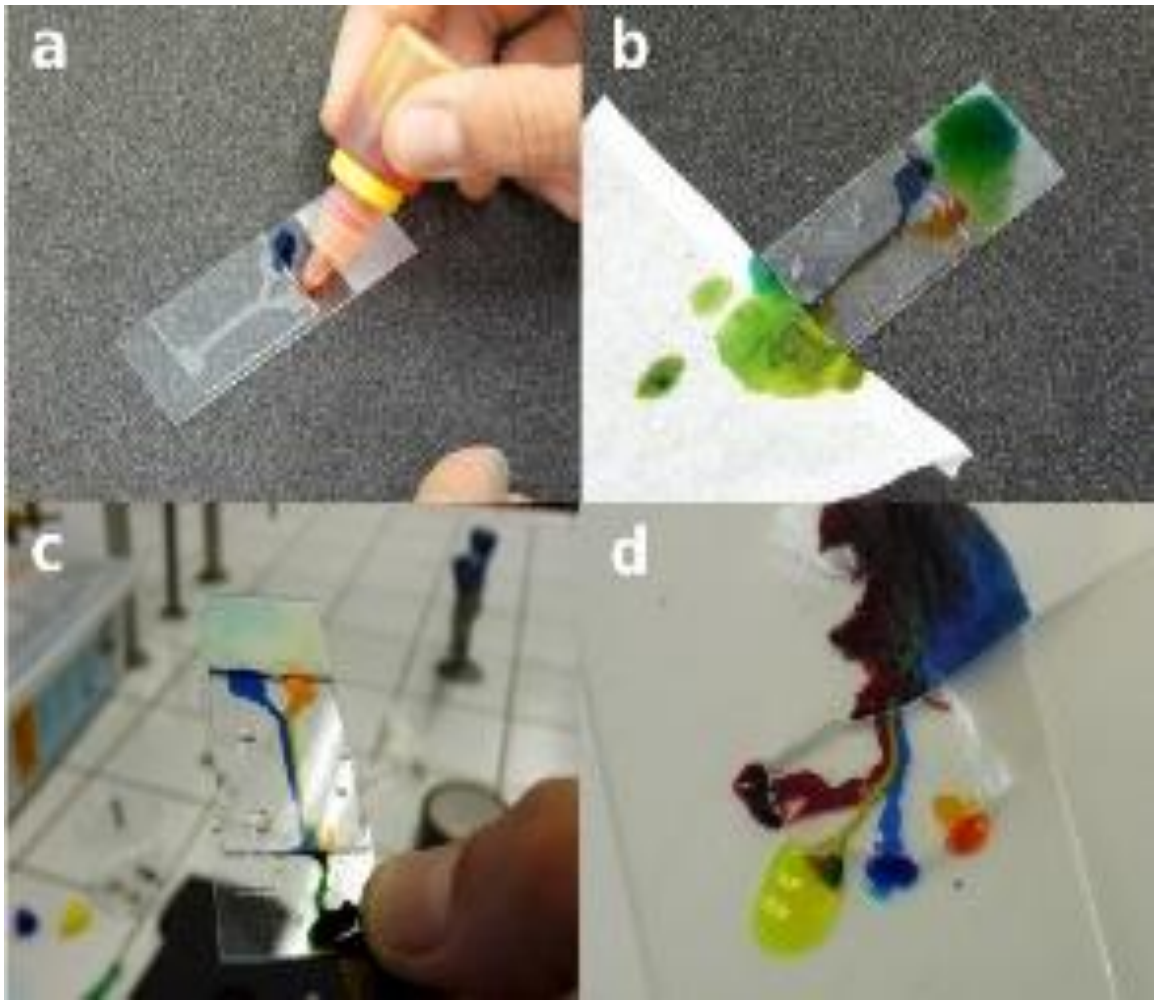




2. Pressure-driven flow, this can be achieved by the aid of a syringe pump or by applying vacuum at the outlet of the channel in order that the reagents can be delivered inside the microfluidic system.



3. Passive forces, this force does not need an external power source that transport fluid. Mainly gravity and capillary forces have been employed to drive fluid within microchannel.

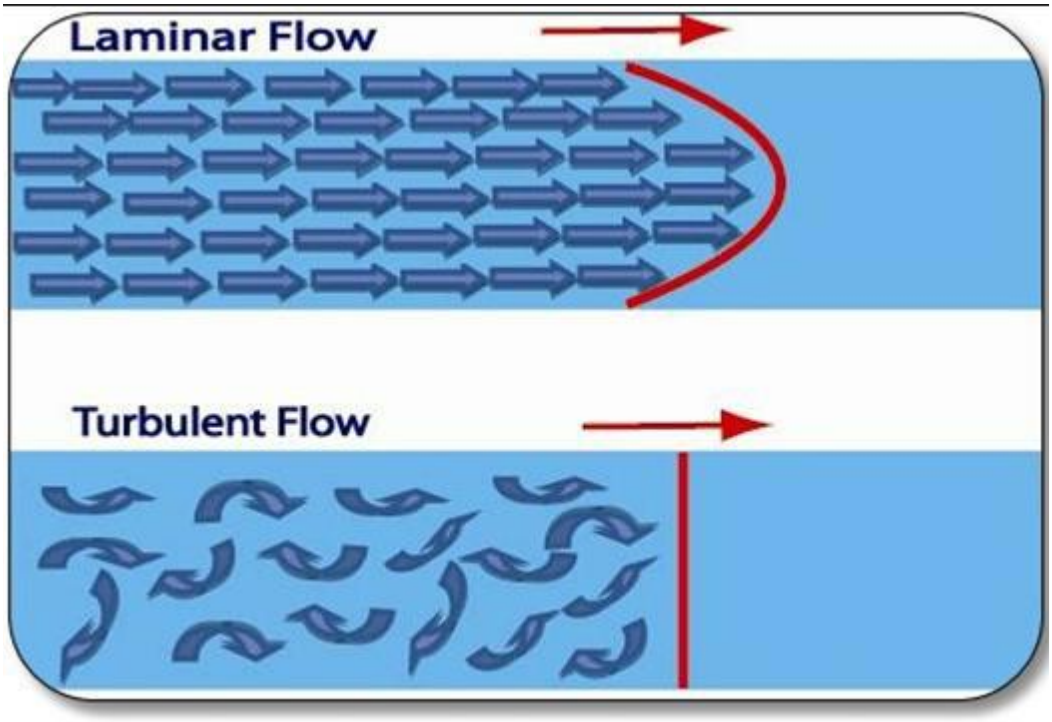


3. Reagents mixing

In microfluidic channels, the fluid flow through the channel must be considered. The flow of a fluid through a microfluidic channel is characterized by the Reynolds number (Re), defined as

$$Re=\rho U_0 L_0 \eta$$

Where ρ is the density of the fluid (kg m^{-3}), Uo is the velocity of the flow (m s^{-1}), Lo is the diameter of channel (m), and η is the viscosity (N S m^{-2}). Where there is a Reynolds number of < 2000 the flow is seen as laminar where mixing then only occurs via viscous forces of the two fluid, if the Reynolds number is greater than 3000 then the flow is seen as turbulent flow where mixing relies on diffusion of the reagent across the channel, if the Reynolds number is between 2000 and 3000 it is termed to be a transition from laminar to turbulent flow.



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3. Detection

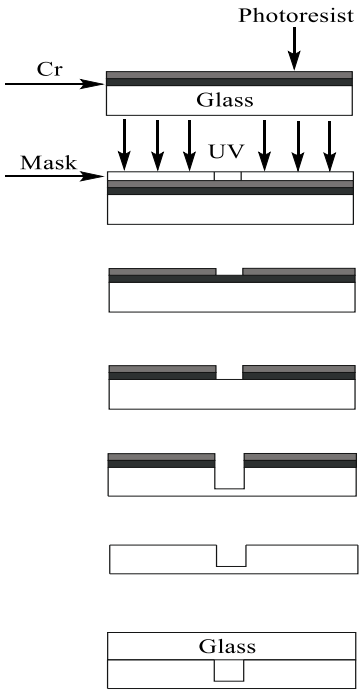
Coupling a detector to the microfluidic system is a crucial step, where most clinical diagnostic and environment monitoring require low power consumption, automation, high sensitivity and specificity of the target analyte. These include electrochemical detectors which include current (amperometric) and potential (potentiometric). The second category is optical detectors which consist of surface plasmon resonance (SPR), thermal lens microscope (TLM), wavelength-interrogated optical sensing (WIOS), absorbance, fluorescence, radioactive and chemiluminescence.

❖ ***Classification***

Microfluidic systems can be classified into:

1. Closed microfluidics

Over the past two-decades, the closed microfluidic concept has been growing due to minimization and integration of chemical and physical processes such as fluid transport, mixing, valving, separation, and detection. In order to perform the previous process, the closed microfluidic device consists of microfluidic unit operations including valves, pumps, actuators, switches, sensors, dispensers, mixers, filters, separators, heaters, etc. A closed microfluidic system composed mainly from glass and silicon which are the most substrate use for fabrication the microfluidic device, where the method based on the photolithography with wet etching is widely applied with this substrate. The procedure of this technique depends on using a chromium metallic layer that a photoresist is a spin coated to covering the glass or silicon with over, and final step put a mask which contains the design of the channels. The channel design firstly imprints onto the surface of the device rely on the UV light which penetrates the photoresist. After the channels are etched into the substrate surface, the chromium layer is then removed using a metal etch (typically hydrofluoric acid). The spin coating layer of sodium silicate used as adhesive to bond the cover plate onto the chip with heating the chip to complete the chip bonding. The fabrication process can be seen in figure below:



1. Chromium metal is deposited onto the glass surface followed by a photoresist.
2. A mask with the channel designed is placed on top of the glass surface; and is exposed to UV light to imprint the channel design.
3. The mask is then removed leaving the imprinted channel design.
4. The chromium metal is then chemically etched away.
5. The glass is etched with an etchant such as hydrofluoric acid to leave the channel design in the glass.
6. The metal and photoresist are removed leaving the glass with the channel present.
7. The top plate is bonded leaving the microfluidic device.

Despite the advantages offered by the closed microfluidic system, the device development is hindered by the need of mechanical components which add complexity to the device. In addition, the fabrication process using a material such as silicon and glass requires a complex process such as chemical etching and thermal bonding which takes time and cost, while the polymer fabrication using hot embossing suffer from the bubbles trapped between the stamp and the substrate which requires a specialized vacuum and this can reduce the reproducibility. Air bubble impacts significantly on the fluid flow behaviour within the microfluidic system which poses a challenge for the microfluidic platform operation. Handling biological samples such as blood through the microfluidic system adds more complexity to the system compared to pure water handling due to blockage issues. For these reasons closed microfluidic devices are not favoured from a commercial manufacturing perspective.

2. Open microfluidics

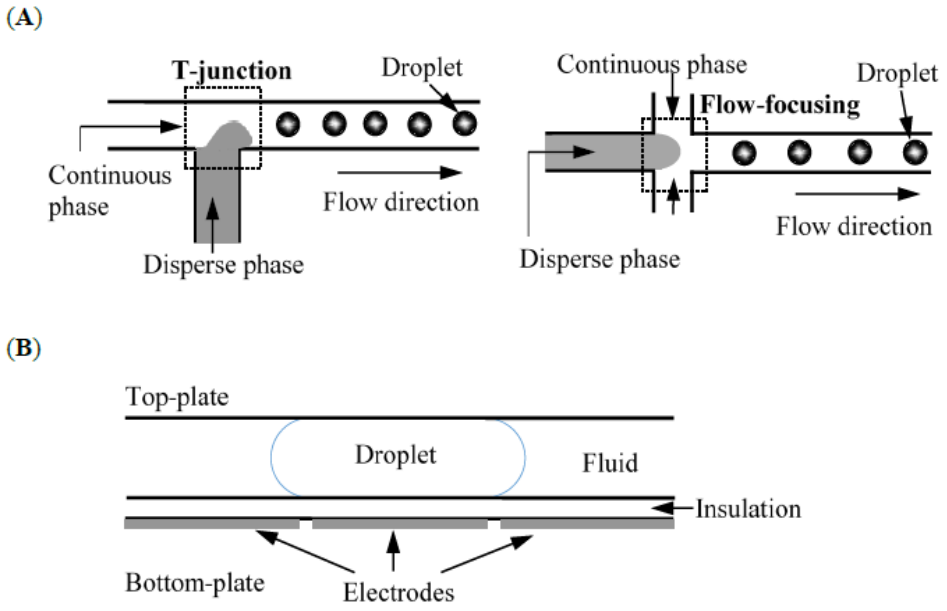
Since closed microfluidic systems were developed research into a related but distinct technology, open microfluidic systems have been carried out, primarily to overcome the inherited problems related to the closed microfluidic system. Open microfluidic system, have open boundaries where the biological, environmental, forensic samples, etc. should be able to interact with microfluidics in the open space. The fluid moves mainly due to capillary forces.

The advantage of this system lies in the simplicity of open microfluidic chip architecture design, resulting in lower fabrication costs, and this approach overcomes bubble trapping. Also, the easy accessibility of reagents and samples addition and optical observations are facilitated. All these aspects have emerged open microfluidic system as a versatile approach for the development of an integrated microfluidic device for the lab-on-chip application. Open microfluidic include droplet, and continuous open microfluidic systems that will be explained in the following section:

- ***Droplet microfluidics***

Droplet microfluidic system includes both electrowetting-based droplet (well known as a digital droplet or discrete droplet) and continuous-flow emulsion-based droplet microfluidics. In digital microfluidics, the discrete droplet for both reagents and sample are manipulated by applying an electrical potential to an array of the electrode on an open surface. This technique is driven by surface tension or so-called electrowetting or electrowetting-on-dielectric (EWOD). While for the other technique droplet is formed as a result of an emulsion created using two immiscible liquids including gas/liquid and

liquid/liquid systems. For the droplet generation control, various techniques are used such as dielectrophoresis and channel geometry (T-junction).



The figure above shows two droplet open microfluidic configurations (A) continuous flow emulsion-based droplet (from T-junction and flow focusing) (B) electrowetting-based droplet.

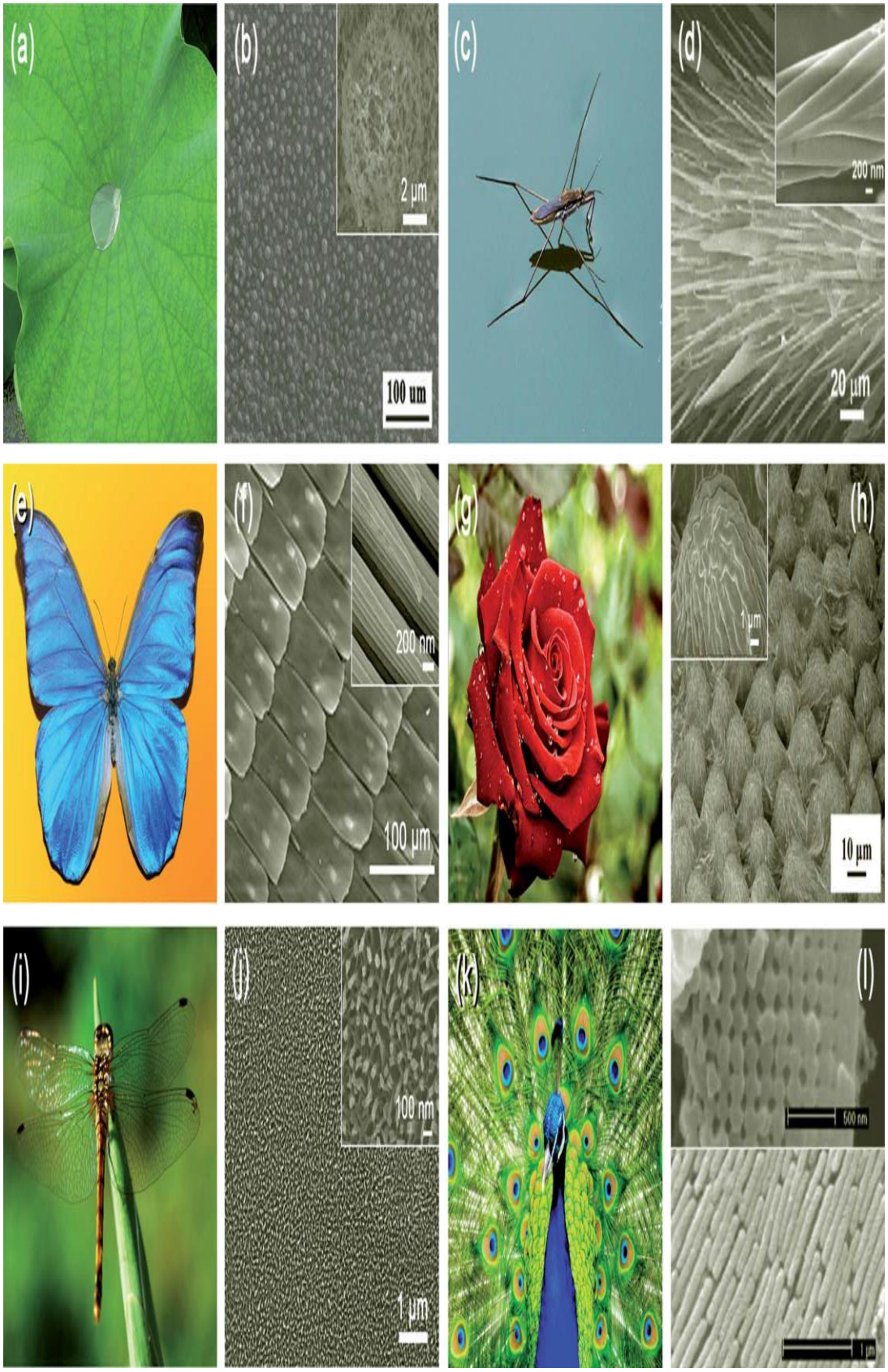
- ***Continuous flow open microfluidics***

A new promising generation of microfluidics has recently been investigated. The continuous open microfluidic technique includes devices that are designed using wettability pattern consisting of hydrophilic patterns on a hydrophobic substrate or conversely, by applying a hydrophobic pattern on a hydrophilic substrate. Once the surface is patterned, the aqueous sample flow will be along the hydrophilic channel fabricated on a hydrophobic substrate by capillary forces, provided the pressure is maintained below a critical value (thus this is called surface-directed fluid flow), since

the fluid (aqueous solution) will only wet the hydrophilic patterns as the hydrophobic region acts as “curbs” .

Hydrophobic surfaces have numerous profitable applications as these surfaces can be transferred to commercial products because of their self-cleaning, anti-sticking and anti-contamination properties. This remarkable phenomenon was first observed from natural plants self-cleaning after a simple rain shower as the natural environment get contaminated or polluted, also it is a self-defense against pathogenic organisms. A famous example of natural hydrophobic surfaces is lotus leaf which has a water contact angle of 161± 2.7°, this is attributed to epicuticular wax secreted by the lotus leaf itself forming a hierarchical micro- and nano-structure, the combination of roughness and wax contributed to the superhydrophobicity of this surface. In addition to a lotus leaf, there are various natural surfaces that present the hydrophobic surfaces from the plant kingdom including red rose petal, scallion, and garlic, etc.. Examples of hydrophobic surfaces are presented also in animals such as water strider legs, cicada orin’s wings and butterflies wings which are water repellent where water droplet roll-off their surfaces.

The knowledge from biomimetic (learning from the nature) inspired researchers to mimic hydrophobic surfaces by producing artificial surfaces taking in consideration the principle connections related to water repellency and surface roughness using different techniques including lithography, etching, plasma treatment, electro-spinning, templating, layer by layer deposition, chemical vapour deposition and use of nanoparticles.


(a)
(b)
2 μm
100 um
(c)
(d)
200 nm
20 μm
(e)
(f)
200 nm
100 μm
(g)
(h)
1 μm
10 μm
(i)
(j)
100 nm
1 μm
(k)
(l)
500 nm
1 μm

One of the most famous examples of hydrophobic/hydrophilic surfaces used for building the microfluidic device is paper as so-called “paper-based microfluidic” or “lab on paper”. The fundamental principle that paper microfluidics lies on is the paper basic material which is cellulose, it is considered naturally hydrophilic and allows penetration of fluid within its fiber matrix. Patterns are fabricated on the paper sheet aiming to create micron-scale capillary channels on paper which enable fluid to be confined inside the patterns and therefore fluid flow can be guided in a controlled manner.

The ubiquitous and cheap material of paper, comparability with a wide range of chemical/medical/biochemical applications and the omitting of external forces to enable fluid flow and depending on capillary forces, made paper becomes an attractive substrate for building microfluidic devices. Other surfaces include glass, polymer, electrodes, etc. which can transfer into hydrophobic surfaces after coating using dip coating or spin coating methods using for an example saline derivative.

❖ ***Applications***

One of the most important application of microfluidic systems is the integration of these systems with sensors. Sensors can be defined as devices that consist of an active detecting element incorporated with a signal transducer. These two components in the sensors can play a major role in transmitting either electrical, mass-based or optical signal to digital signal for selective compound analysis.

Sensors can be classified into two categories: chemical sensors and biosensors. According to the international union of pure and applied chemistry (IUPAC), “a chemical

sensor transforms chemical information ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal" . On the other hand, biosensors are composed of a biological sensing material well known as bioreceptor and a physical transducer.

- **Biosensors**

Combining a biosensors ability to interact with an analyte at an extremely low concentration with extremely high selectivity, enables their application in a variety of applications using lab-on-a-chip (LOC), drug delivery system (DDS) and point-of-care systems (POC). Biosensors devices can be divided into bioreceptor that can be aptamers, protein receptors, DNA, enzyme, tissue, and antibodies; which transform biochemical information (substrate) into a form of energy to provide readable data.

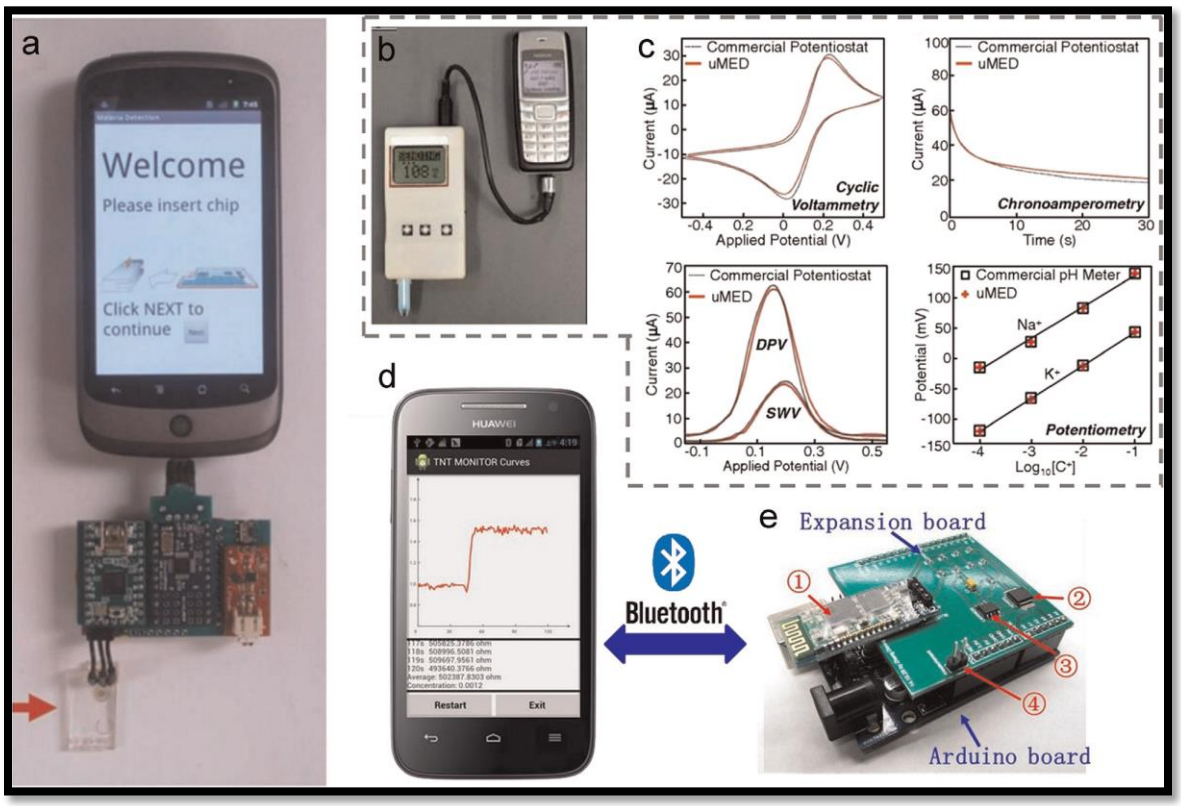
The second part is the transducers converts the biological response to a detectable analytical signal. Depending on the output signal, transducers can classify into electrochemical transducers (amperometric, potentiometric, impedimetric, and conductometric), mass-based transducers (piezoelectric, surface acoustic wave and magnetoelastic) and optical transducers (absorbance, luminescence, fluorescence, and reflective index).

The analogue signal is correlated to concentration unit and is transferred to a digital signal via a microprocessor and display on a computer or mobile phone screen for analysis. The combination of a biosensor, transducer, microprocessor and display screen

have given a rise to the construction of industrial, forensics, environmental, healthcare and medical biosensors platforms.

Traditional biosensor systems are relatively expensive, bulky and hard-to-handle, which largely limits their use in samples analysis. The problems mentioned above are successfully addressed with the popularization of smartphone and the development of microfluidic technology for their applications of biosensor, which integrates smartphones, microfluidic components and sensory elements together, paving the way for wide application of smartphone-based microfluidic biomedical sensory system.

Smartphones are now similar to miniature computers with operating systems, internal memory, and high-quality camera lenses. They are, however, potentially more accessible and cheaper than portable analytical laboratory devices. These portable, low cost devices could be used to run routine tests, which are currently performed by trained personnel using laboratory instrumentation such as microscopes and spectrophotometers. This would offer tremendous potential for improving the diagnosis and treatment of pathologies, particularly in low-resource countries. Below are examples of biosensor integrated with smartphone and microfluidic components.


a
Welcome
Please insert chip
Click NEXT to continue
b
c
Commercial Potentiostat
uMED
Current (μA)
Applied Potential (V)
Cyclic Voltammetry
Commercial Potentiostat
uMED
Current (μA)
Time (s)
Chronoamperometry
Commercial Potentiostat
uMED
DPV
SWV
Current (μA)
Applied Potential (V)
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uMED
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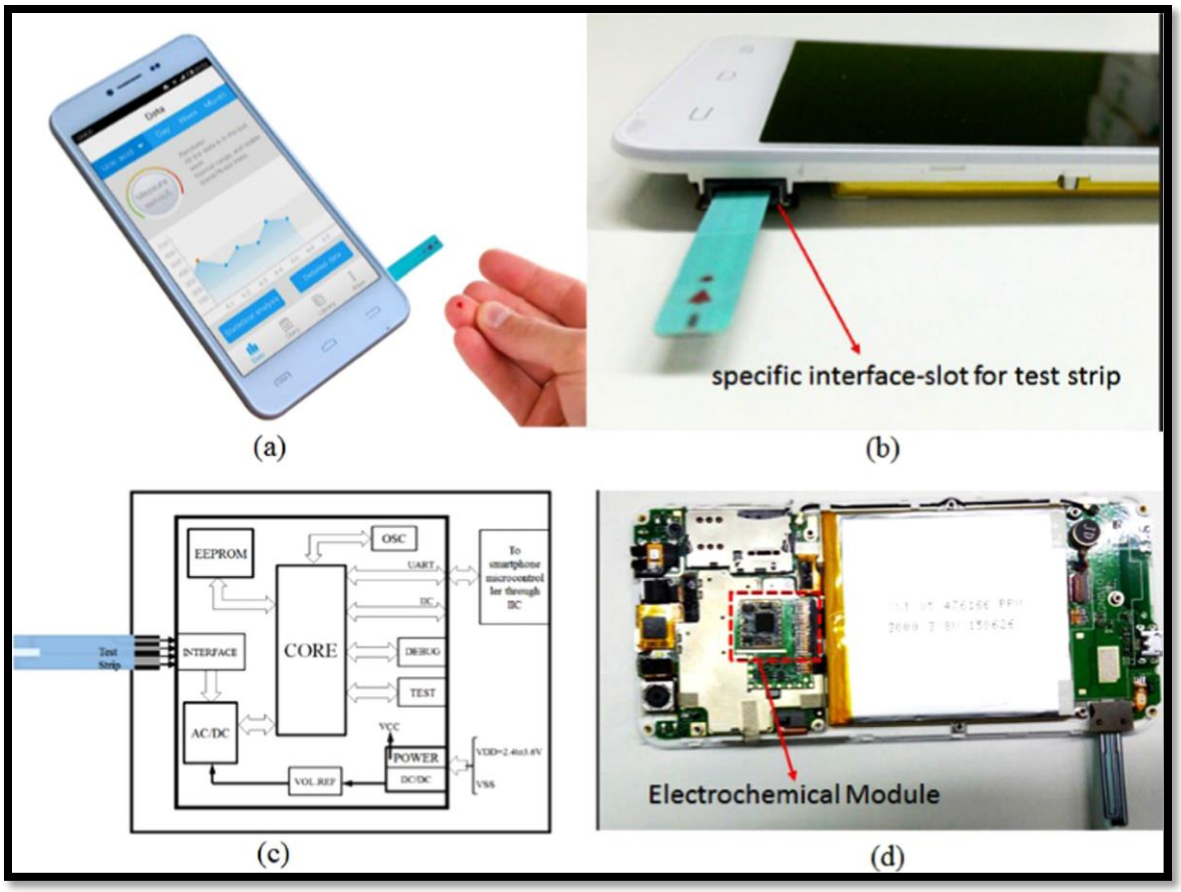
specific interface-slot for test strip
(a)
(b)
EEPROM
OSC
UART
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To smartphone microcontroller through I2C
Test Strip
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VSS
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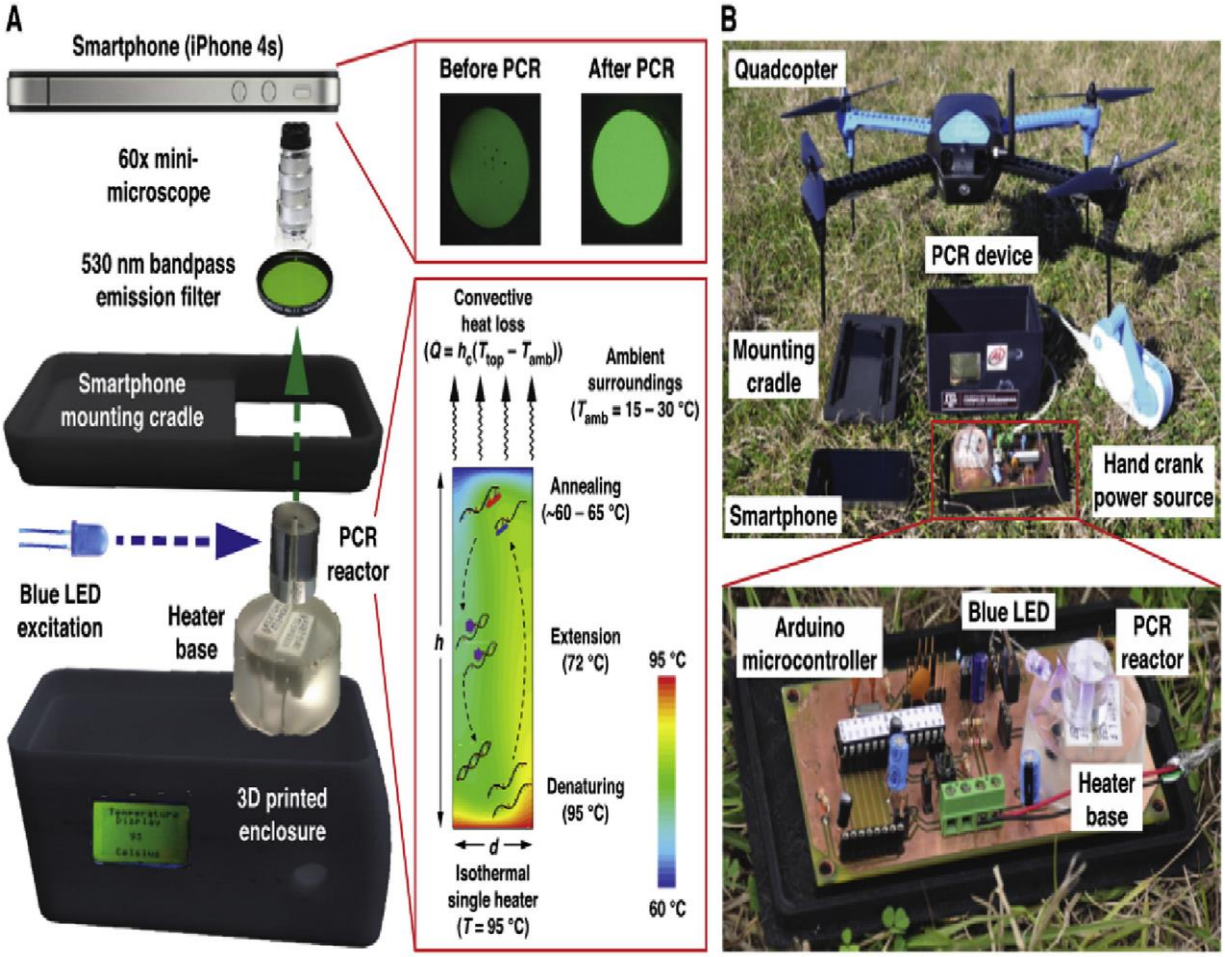
Electrochemical Module
(d)


A
Smartphone (iPhone 4s)
60x mini-microscope
530 nm bandpass emission filter
Smartphone mounting cradle
Blue LED excitation
Heater base
PCR reactor
3D printed enclosure
Before PCR
After PCR
Convective heat loss
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Ambient surroundings
$(T_{amb} = 15 - 30\ °C)$
Annealing (~60 – 65 °C)
Extension (72 °C)
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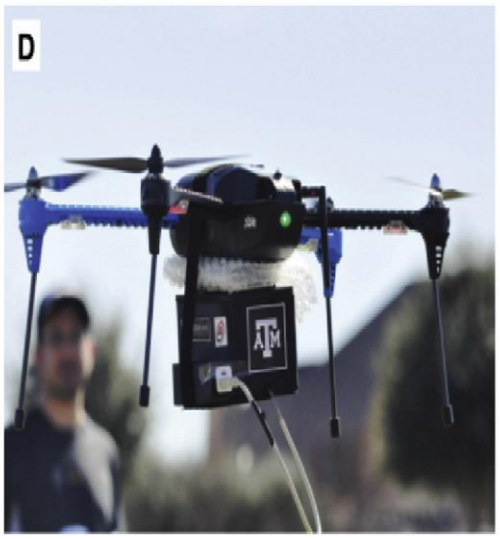
PCR device
Mounting cradle
Hand crank power source
Smartphone
Arduino microcontroller
Blue LED
PCR reactor
Heater base


C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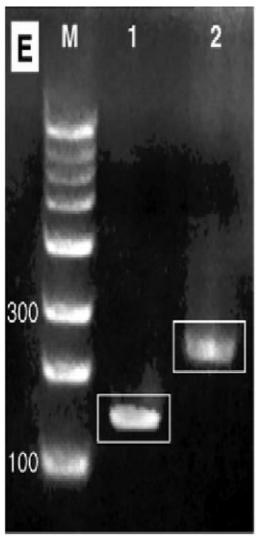
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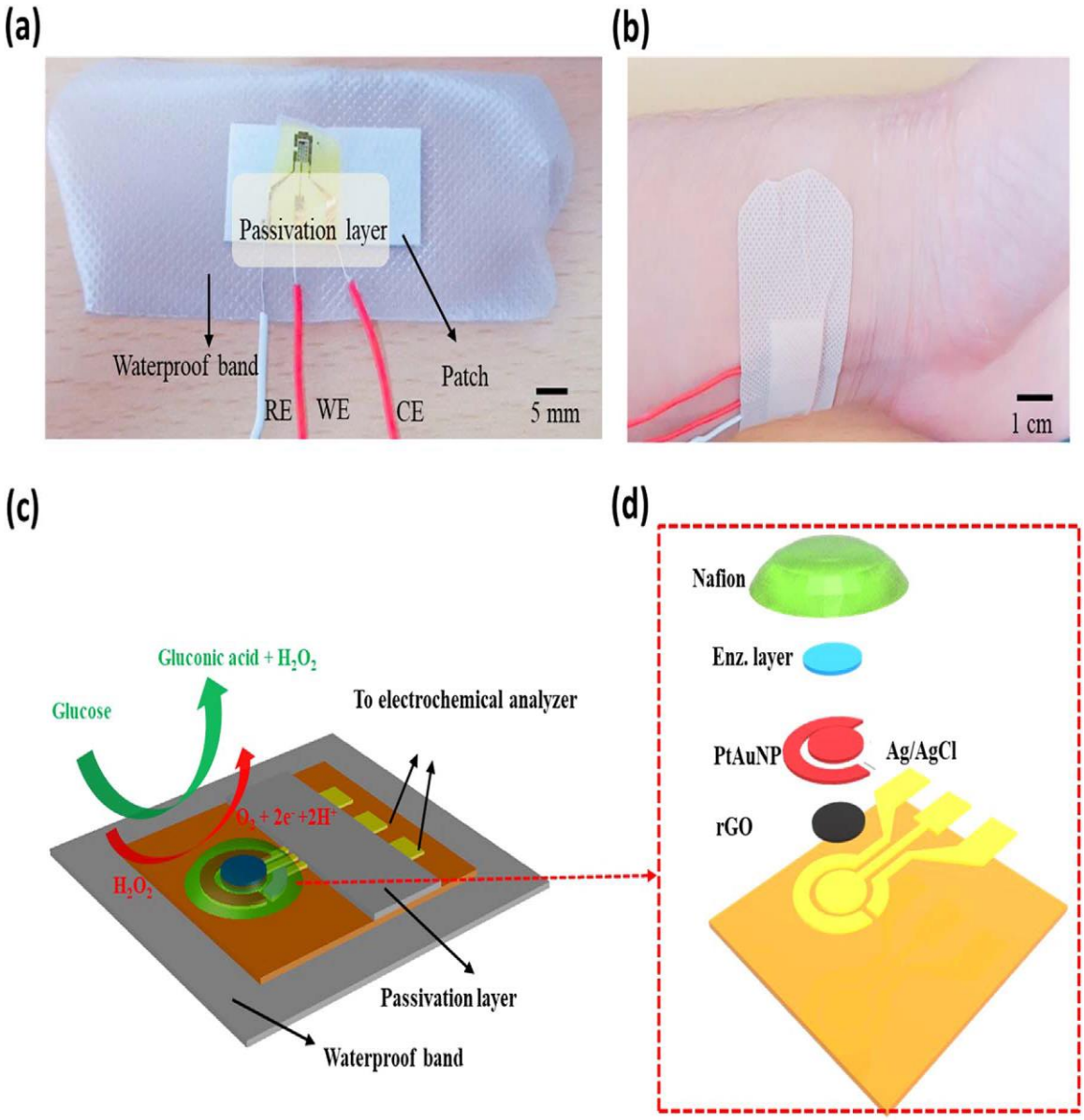

Photographs and schematics of the fabricated wearable sweat-based glucose biosensor. Optical camera images (a, b) of the fabricated biosensor.