BT 601: Analytical Biotechnology

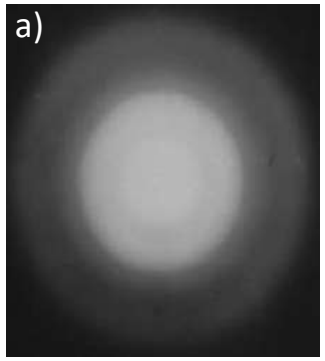
-Prof. Siddhartha Sankar Ghosh

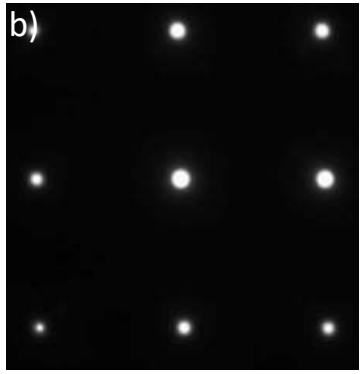
Lec-16

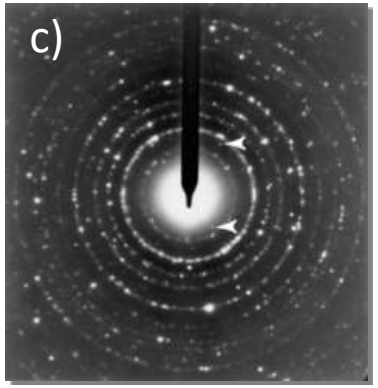
Electron diffraction

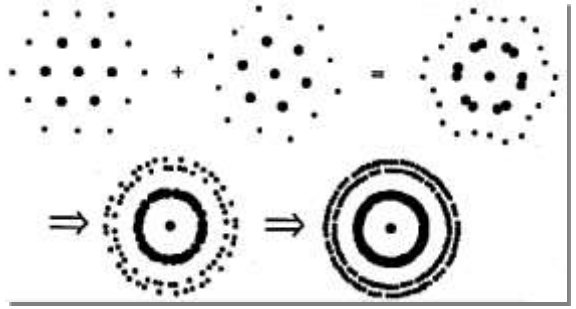
•Diffraction patterns give crystallographic information about a material from site specific small volumes

•Can determine if a material is a) amorphous, b) crystalline, c) poly crystalline quickly and effectively.









a)Amorphous material b) Crystalline material c) polycrystalline material

Detection of Glutathione by Glutathione-S-Transferase-Nanoconjugate Ensemble Electrochemical Device

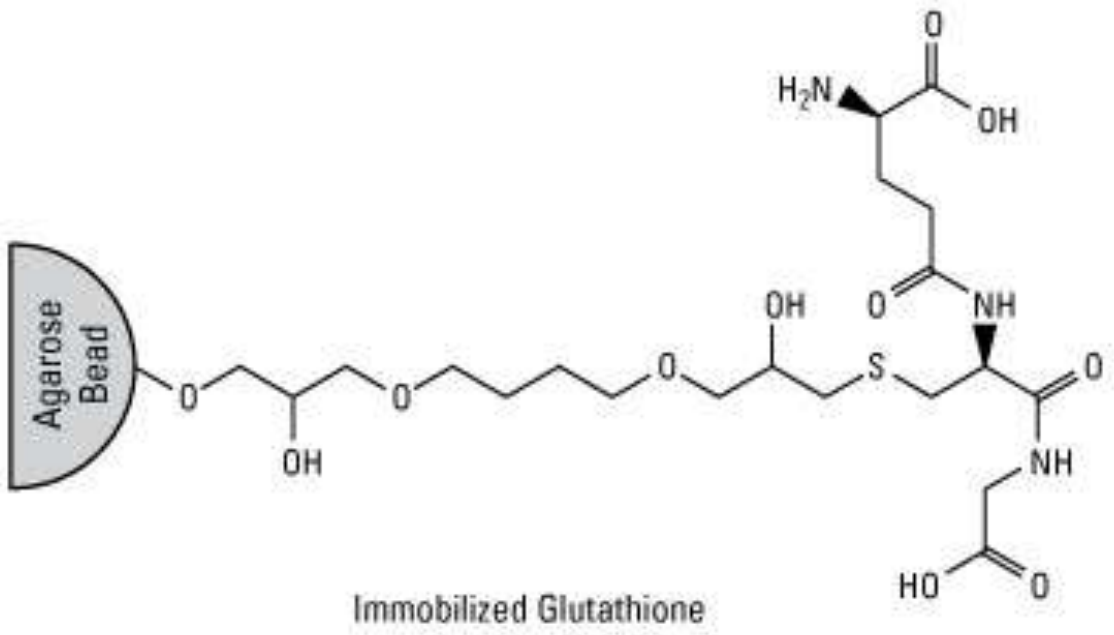
IEEE Trans Nanobioscience, 2017 Jun;16(4):271-279.

doi: 10.1109/TNB.2017.2698241

Glutathione and Glutathione-S-Transferase (GST)

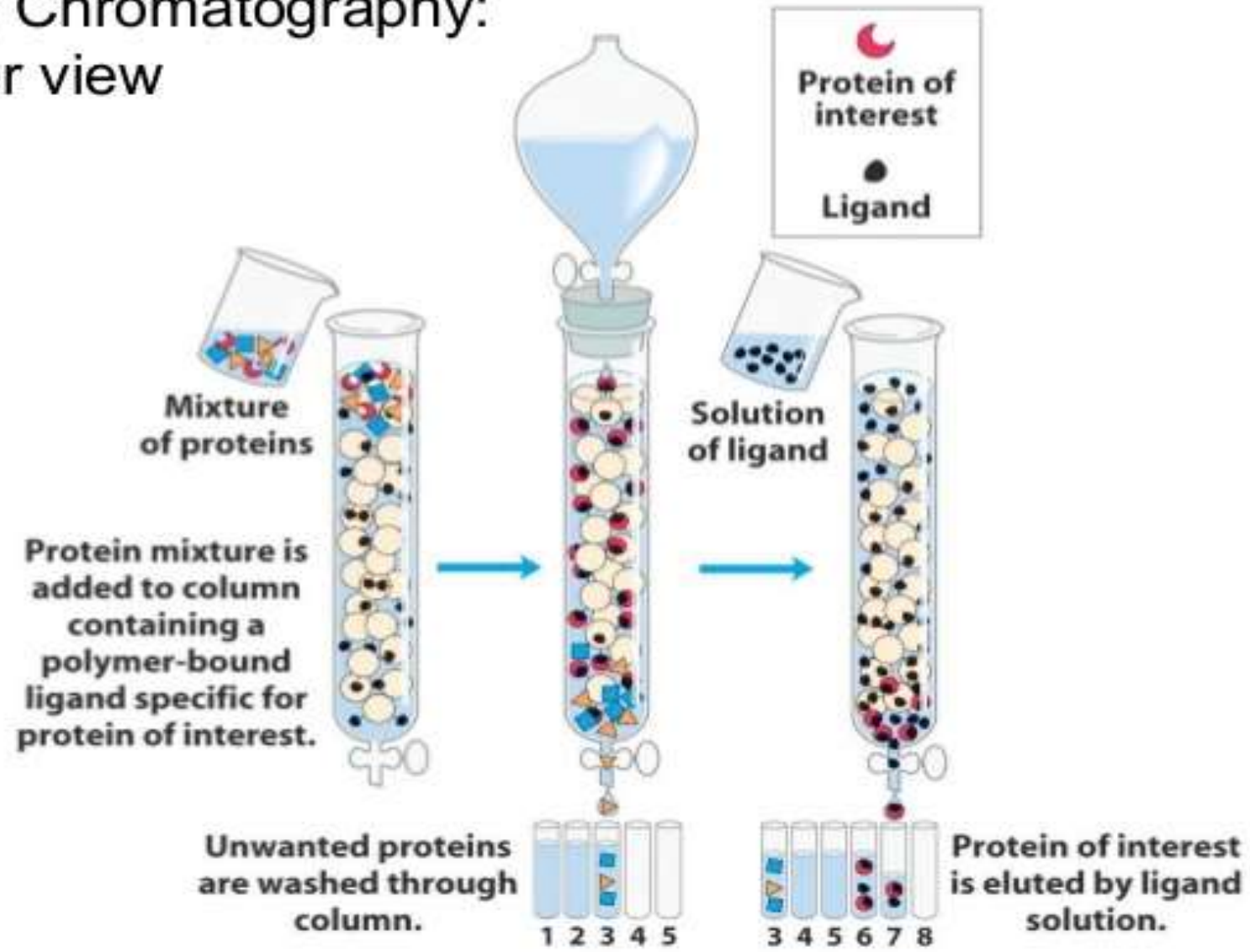
- Glutathione is a tripeptide (Glu-Cys-Gly) that is the specific substrate for glutathione S-transferase (GST).
- When reduced glutathione is immobilized through its sulfhydryl group to a solid support, such as cross-linked beaded agarose, it can be used to capture pure GST or GST-tagged proteins via the enzyme-substrate binding reaction.
- GST Purification is **an affinity chromatography**.
- GST is a 26 kDa protein
- Depending on the vector, many have a thrombin or other protease recognition site.
- Depending on the protein concentration (typically high levels) GST can dimerize.

Scheme for Glutathione-S-Transferase (GST) purification



Immobilized Glutathione

Affinity Chromatography: another view



Scheme for Glutathione-S-Transferase (GST) purification

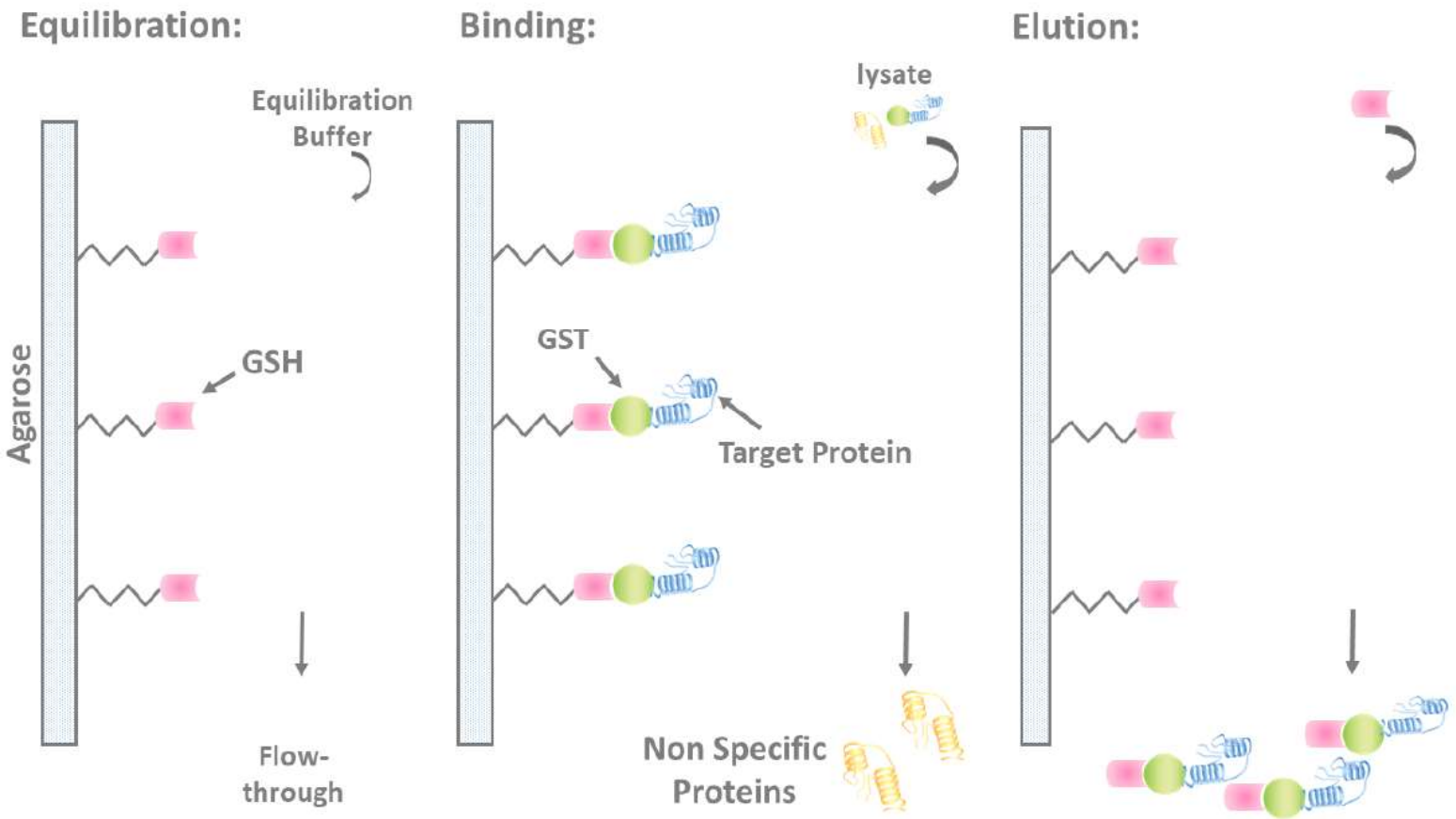
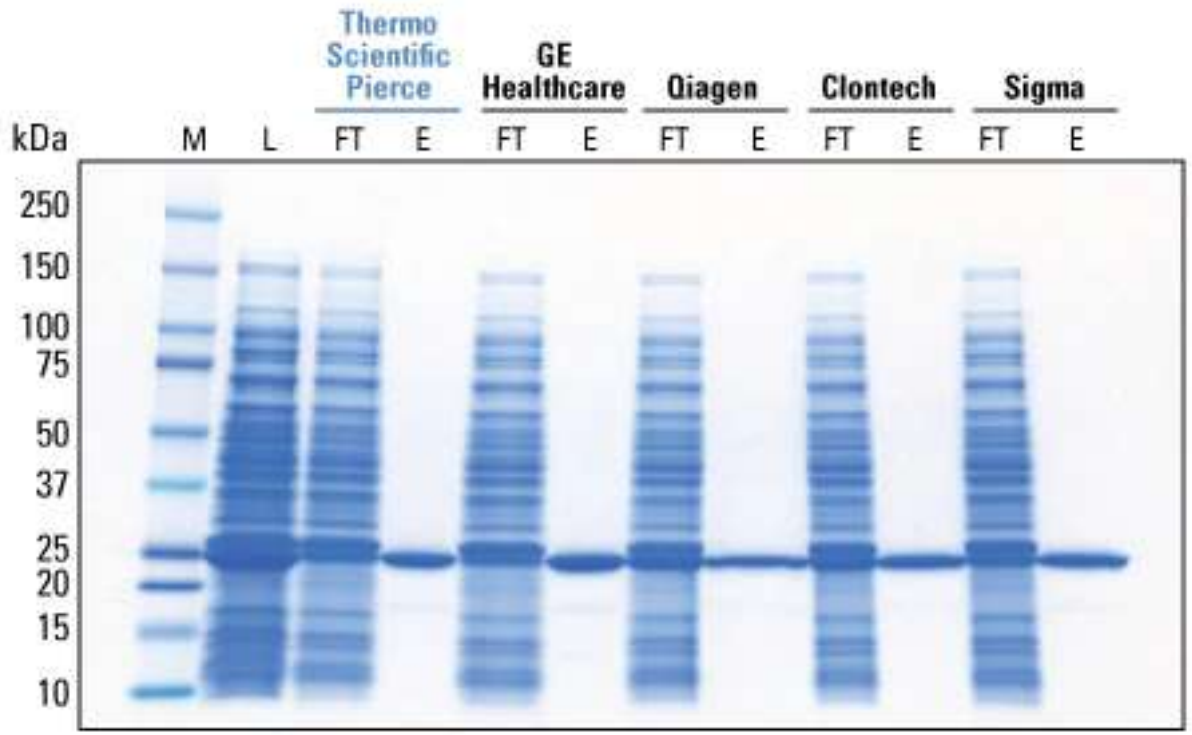


Figure 1. Purification of proteins fused to glutathione S-transferase (GST).

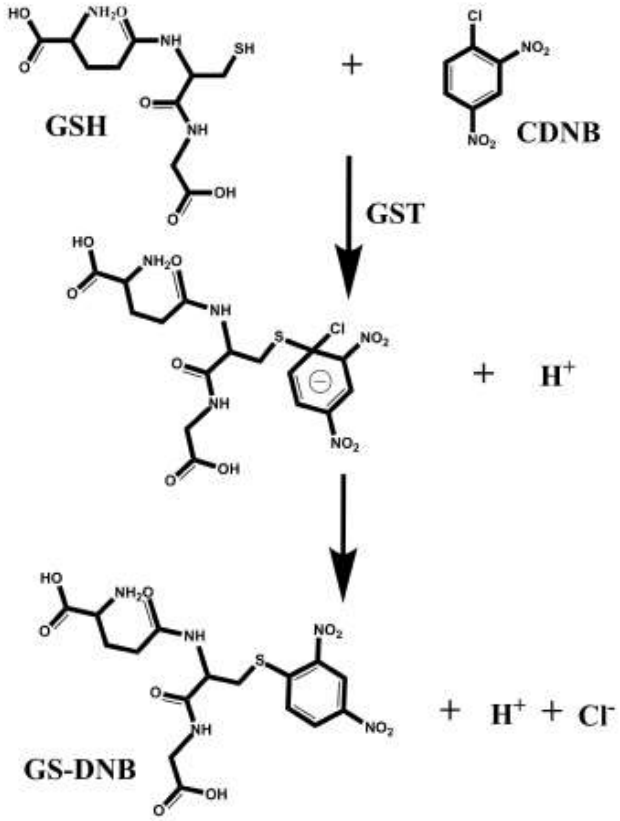
Gel electrophoresis to check GST



Vendor	Thermo Scientific Pierce	GE Healthcare	Qiagen	Clontech	Sigma
Yield	537µg	562µg	285µg	299µg	410µg
Purity	93%	93%	90%	91%	94%

Detection and Device

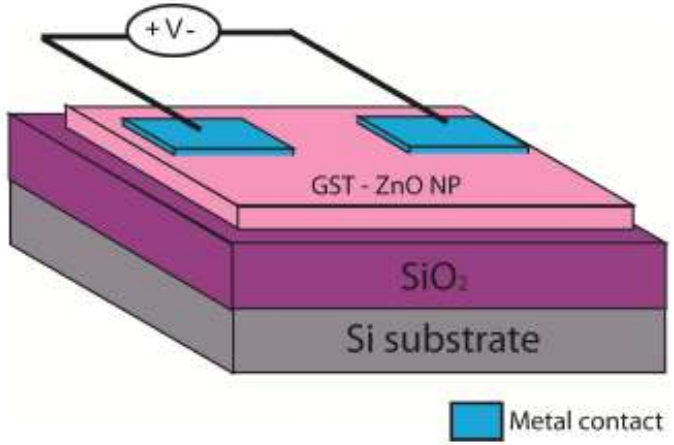
Detection process



Conjugation reaction of GSH and CDNB in presence of GST

- GST can be immobilized on the channel

Structure of the Device



Schematic of the structure

- Two terminal
- Resistive device
- Functionalized layer of nanoconjugate
- Change in resistivity

Highlights

- Fabrication of chemiresistive device
- Detection of glutathione using chemiresistive device
- Measurement with varying concentrations of glutathione
- Calculation of performance parameters

Abstract

- This paper reports a novel electrochemical method for detection of Glutathione (GSH) using Glutathione-S-Transferase (GST) - ZnO composite nanoparticles to investigate the prospects of the method for detection of cancer at an early stage.
- The purified GST enzyme was bound with ZnO nanoparticles by electrostatic interactions and the nanocomposite was dropcast on a silicon dioxide wafer.
- The GST functionalized deposited layer was then used as a chemiresistive channel to detect conjugation reaction between GSH and 1-Chloro-2, 4-Dinitrobenzene (CDNB).
- The zeta potential values of the ZnO nanoparticles and the GST were found to be 13.4 mV and-6.21 mV, respectively.
- Around 73.8% binding was observed between the enzyme and ZnO nanoparticles.
- I - V analysis of the chemiresistive channel showed an increase in conductivity of the channel due to conjugation reaction between GSH and CDNB as compared with that of GSH or CDNB alone.
- I - V characterization of the GST functionalized layer was performed at various concentrations of GSH and a sensitivity and limit of detection of 5.68 nA/ [Formula: see text] and 41.9 nM were obtained, respectively.
- Thus from I - V analysis of the chemiresistive channel, the detection and quantification of GSH could be obtained.
- The kinetic parameters of both GST and nanoconjugate of ZnO nanoparticles andGSTwere determinedwith respect to its substrates, GSH and CDNB, using Michaelis-Menten model.
- This novel approach of detection of GSH bymeans of ZnO nanoparticle and GST enzyme composite can be further analyzed for in vitro experiments, which will lead us to a new and efficient way of detecting certain types of cancers at an early stage.

Characterizations

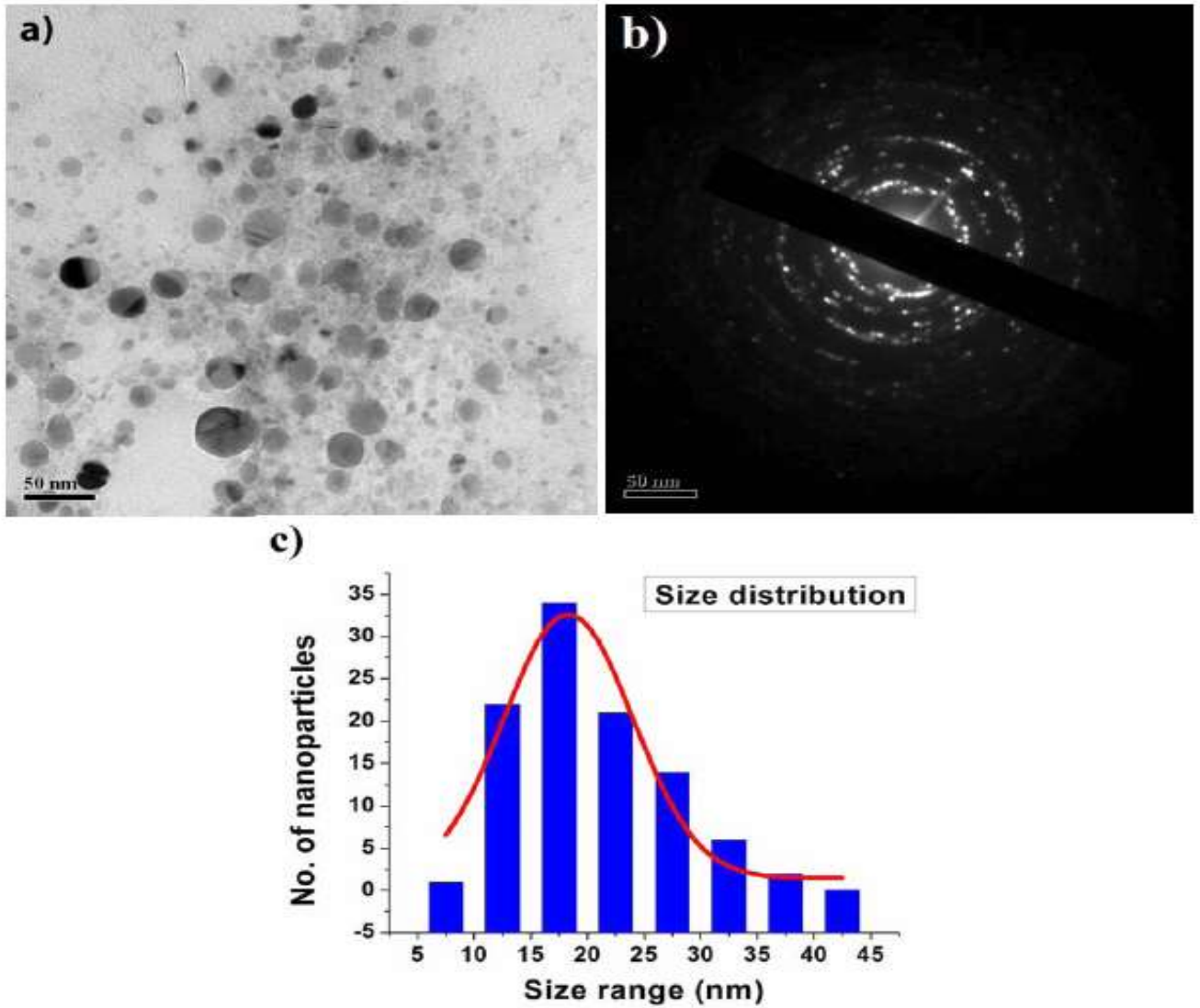


Fig. 1. a) TEM image b) SAED image c) Size distribution plot of synthesized ZnO nanoparticles.

Characterizations

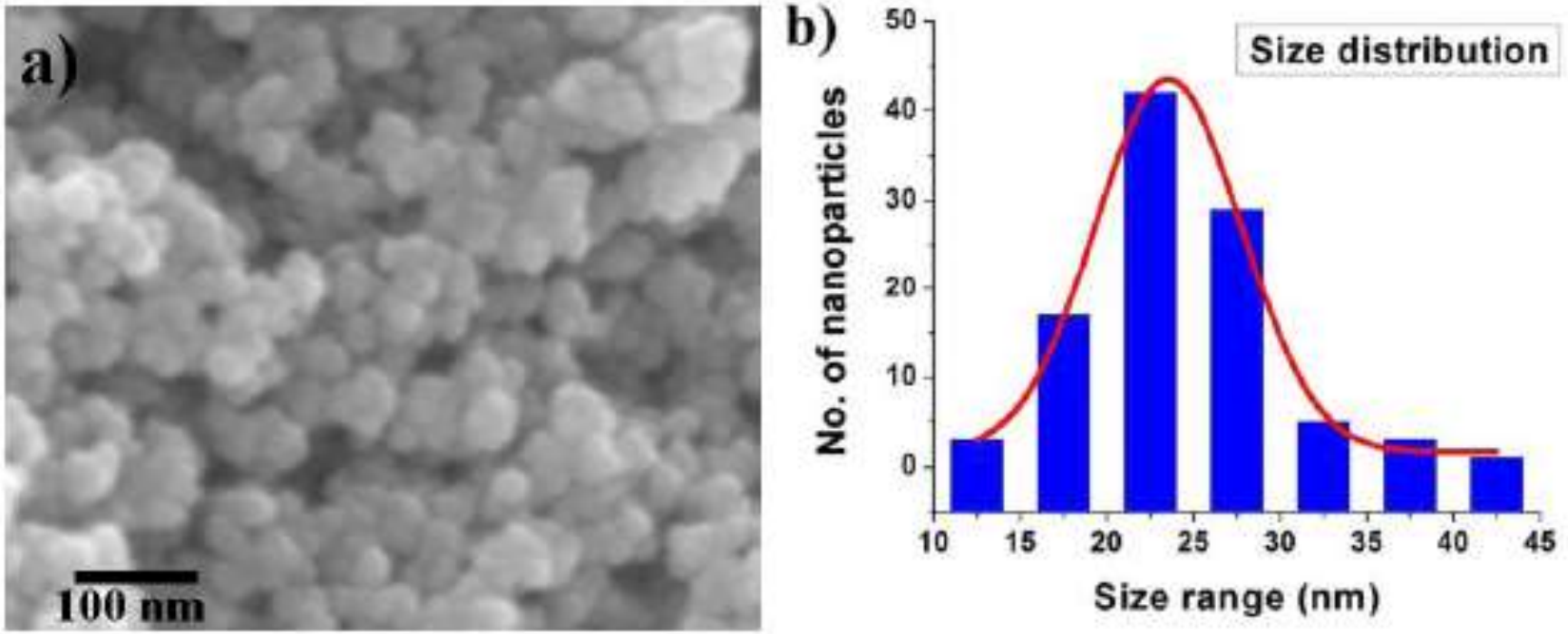


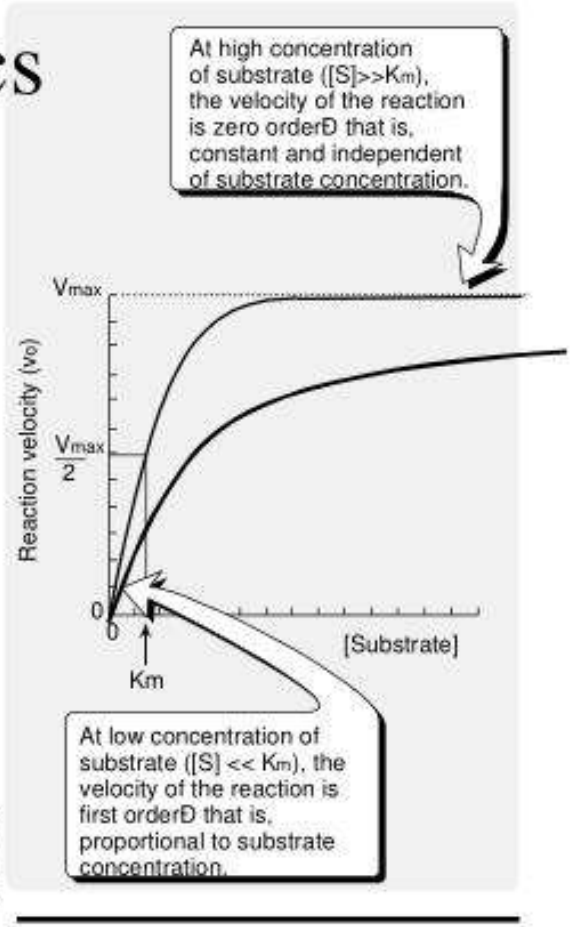
Fig. 2. a) FESEM image (with magnification 50000, acceleration voltage 5 kV and working distance 4.5 mm) b) Size distribution plot of the synthesized ZnO nanoparticles.

Michaelis-Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

K_m is the substrate concentration at which the rate of the reaction is half the maximum rate (V_{max})



Effect of substrate concentration on reaction velocity for an enzyme-catalyzed reaction.

Enzyme Kinetics

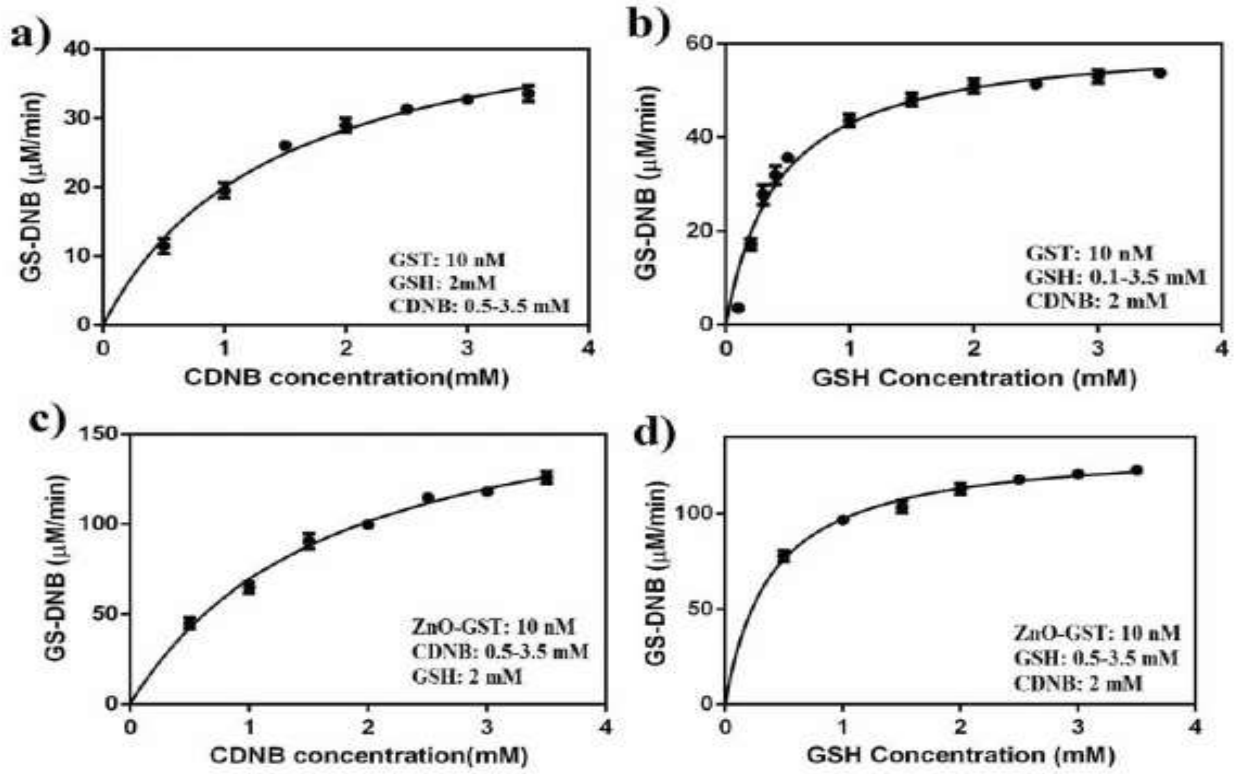


Fig. 5. Michaelis Menten plot with a) varying CDNB concentration b) varying GSH concentration using GST alone and c) varying CDNB concentration d) varying GSH concentration using GST-ZnO composite nanoparticles.

Enzyme Kinetics

TABLE I
KINETIC PARAMETERS

Parameters	GST	ZnO-GST	GST	ZnO-GST
	CDNB as substrate		GSH as substrate	
V_{max} (µmoles/ml/min)	48.67	188.2	61.32	135.3
K_m (mM)	1.431	1.713	0.427	0.3936
k_{cat} (s^{-1})	81.1	313	102.2	225
k_{cat}/K_m ($M^{-1}s^{-1}$)	$5.66x10^4$	$18.2x10^4$	$2.39x10^5$	$5.73x10^5$

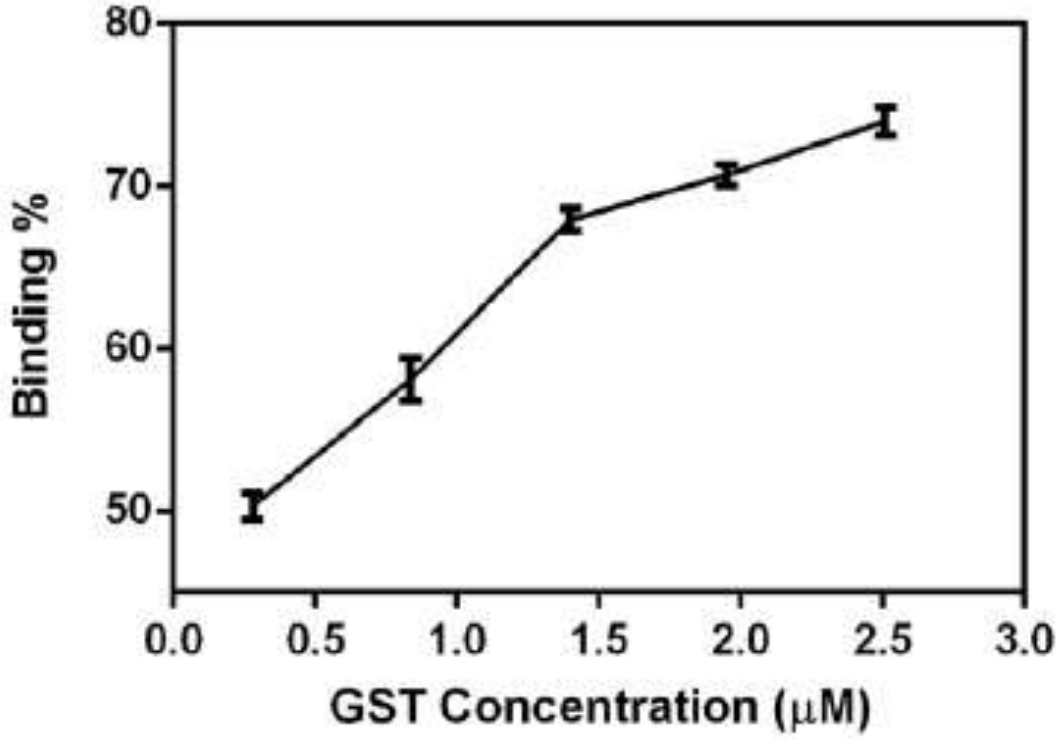
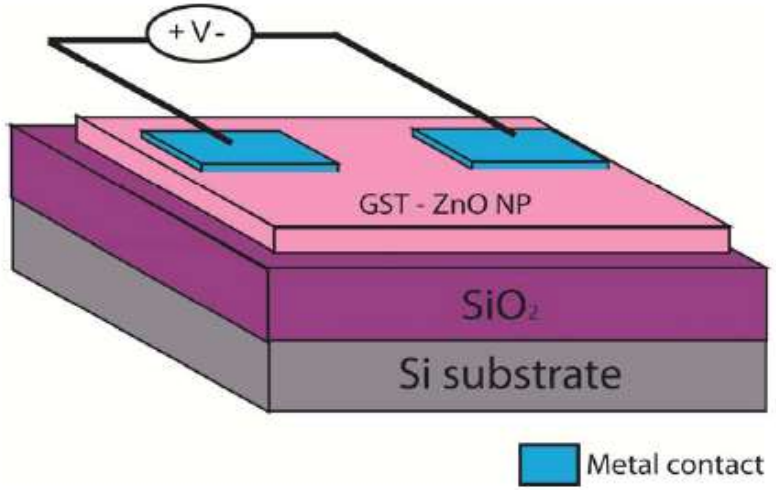


Fig. 7. Binding analysis of GST-ZnO nanocomposite.

Fabrication

TABLE II
CHARACTERIZATION OF DEPOSITED LAYERS

Method	Average Thickness	Average Roughness	Reusability
Dropcast	10.28 µm	3.627 µm	Good
Doctor Blade	1.35 µm	934.6 nm	Bad
Spin Coat	1.21 µm	61.16 nm	Bad



and

Fig. 8. Structural schematic of the experimental setup.

MEASURED RESISTIVITY VALUES

Parameter	ZnO nanoparticles	ZnO nanoparticle bound GST
Resistivity (Ω.m)	21.8	5.61
Sheet Resistance (KΩ/sq)	-	545.72

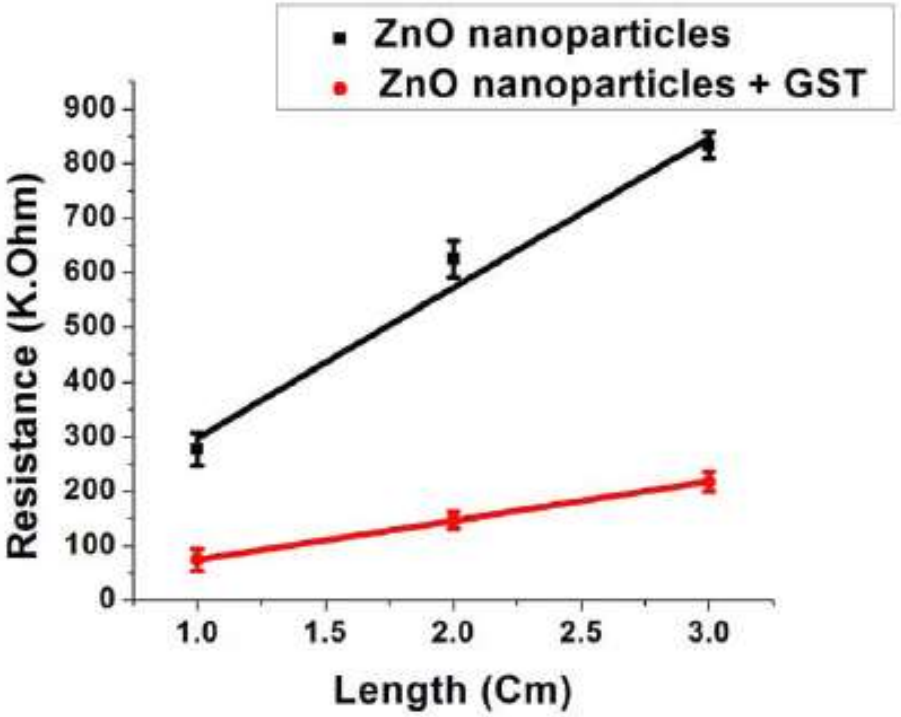


Fig. 9. Measurement of resistance values at different channel lengths.

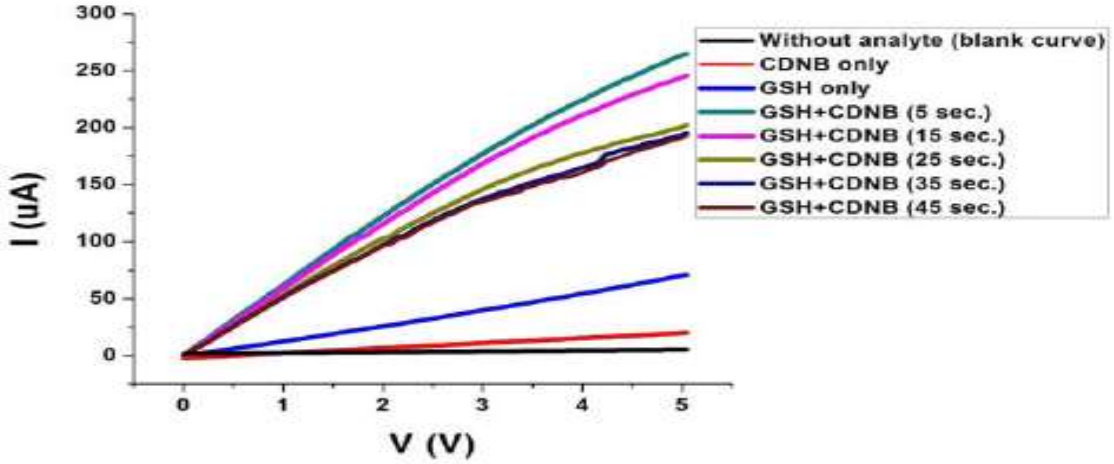


Fig. 10. I-V analysis and detection of GSH at different instants of time.

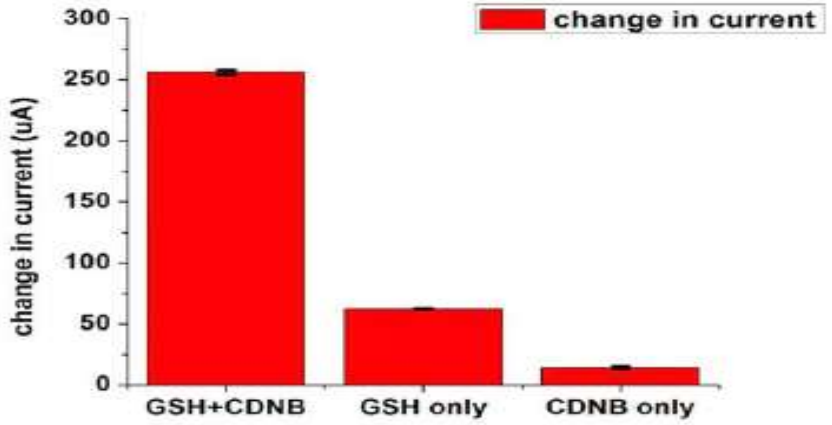
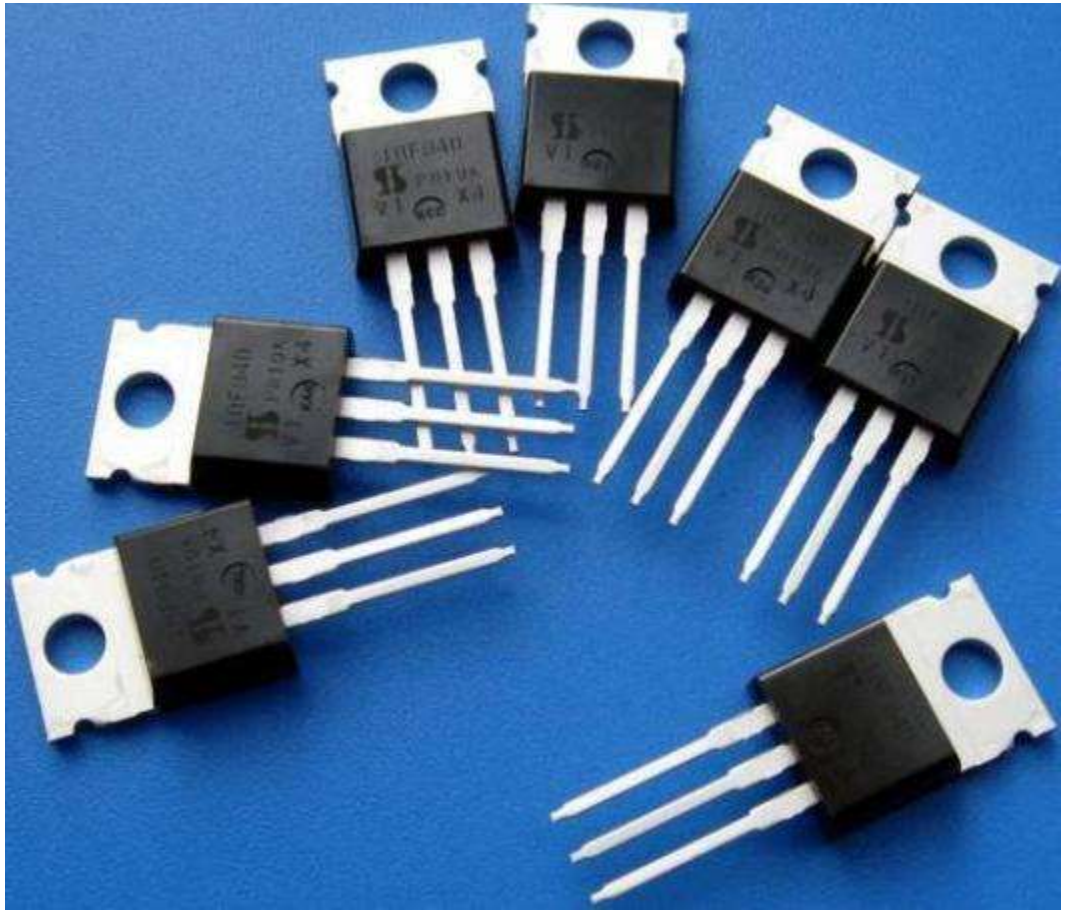


Fig. 11. Change in output current due to various analytes at V = 5V.

Fabrication of Glutathione-S-Transferase–ZnO Nanoconjugate Ensemble FET Device for Detection of Glutathione

Journal: IEEE Transactions on Electron Devices

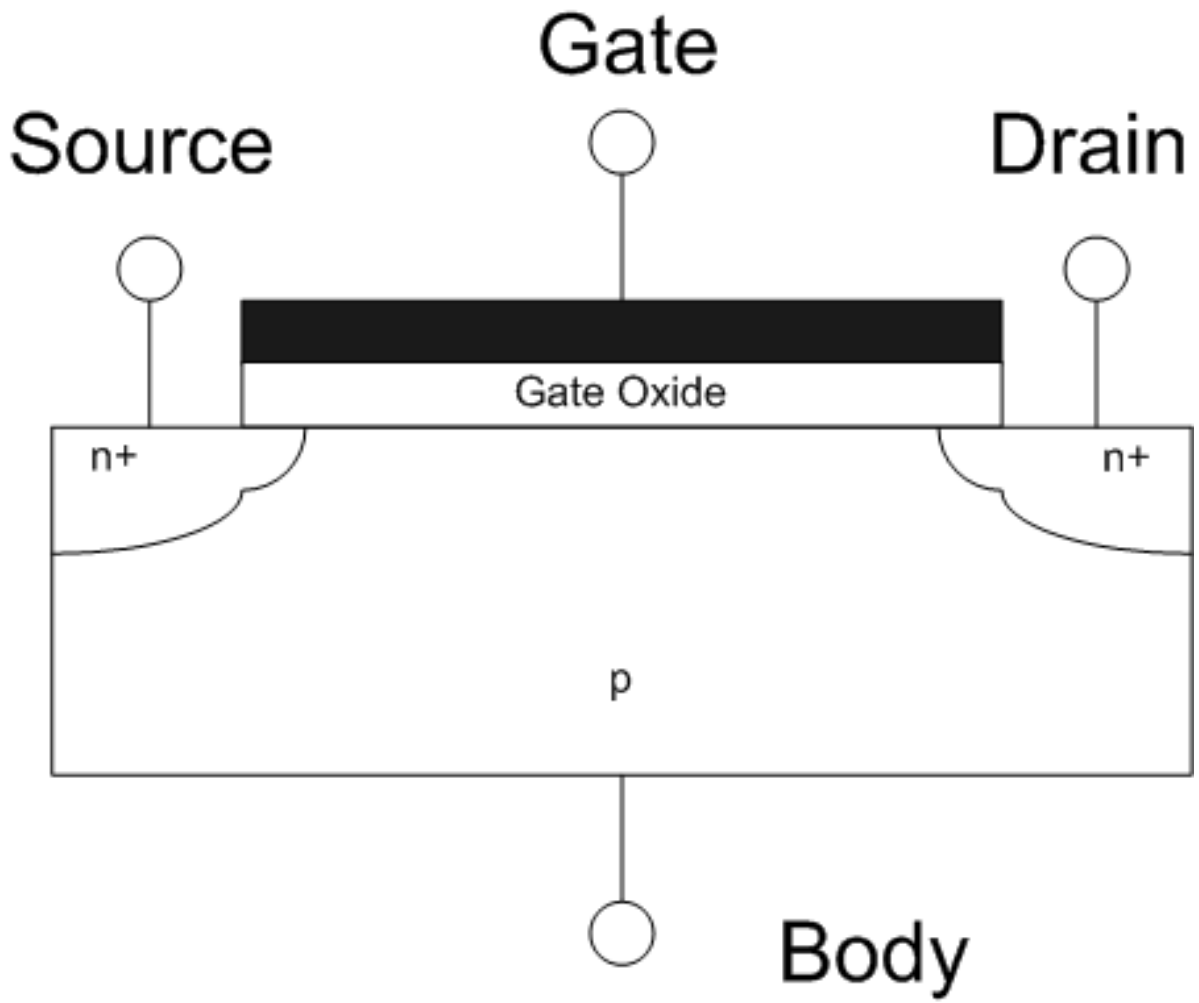
Commercial FET Transistor



FET Device

- Field-effect transistor (FET) is a type of transistor that uses an electric field to control the flow of current in a semiconductor.
- FETs are devices with three terminals: source, gate, and drain. FETs control the flow of current by the application of a voltage to the gate, which in turn alters the conductivity between the drain and source.
- FETs are also known as unipolar transistors since they involve single-carrier-type operation. That is, FETs use either electrons or holes as charge carriers in their operation, but not both.
- Many different types of field effect transistors exist. Field effect transistors generally display very high input impedance at low frequencies.

FET Device



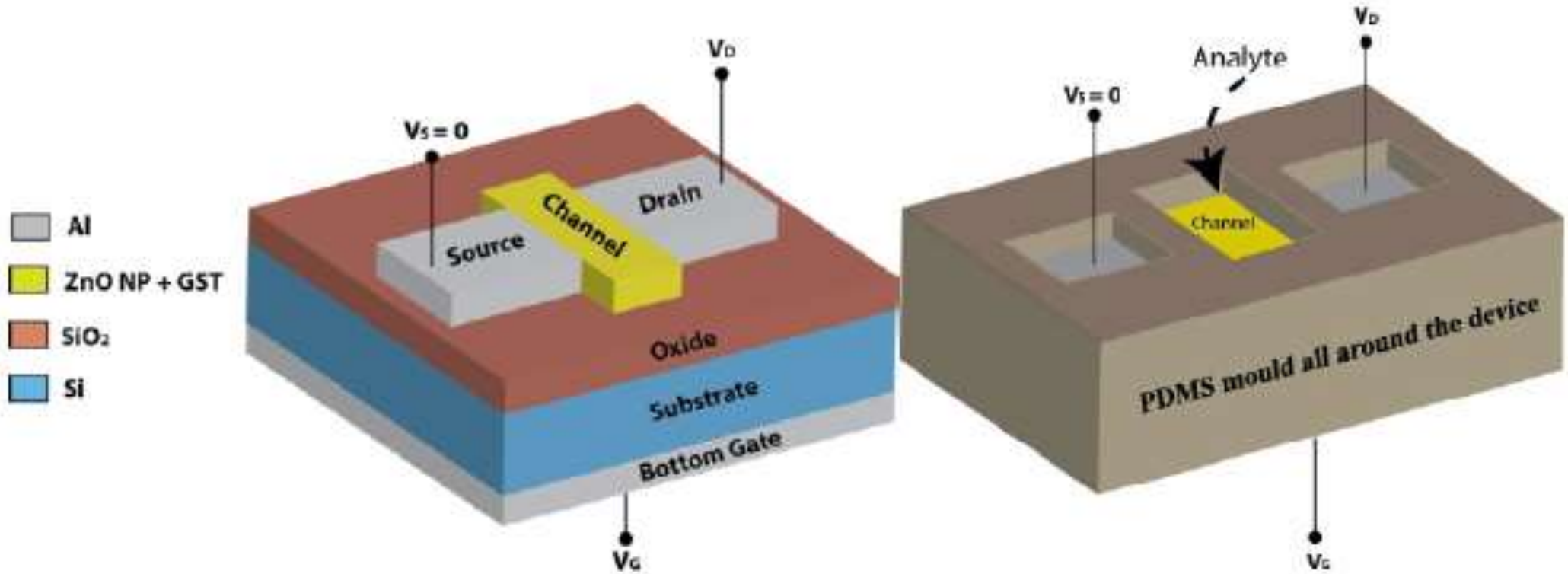


Fig. 2. Schematic of the fabricated device and PDMS mold around the fabricated device.

Challenges

- Semiconducting channel layer
- Immobilization of GST on the channel

Solutions

- Nanoparticles as channel
- GST binding with nanoparticles
- Bottom Gate structure

Bottom Gate produces good casting surfaces.

- Clean Room
- Lithography
- FET

Clean room

- A **cleanroom** is a facility utilized as a part of specialized industrial production or scientific research, including the manufacture of pharmaceutical items, integrated circuits, LCD, OLED and microLED displays.
- Cleanrooms are designed to maintain extremely low levels of particulates, such as dust, airborne organisms, or vaporised particles.
- Cleanrooms typically have a cleanliness level quantified by the number of particles per cubic meter at a predetermined molecule measure.
- The ambient outdoor air in a typical urban area contains 35,000,000 particles for each cubic meter in the size range 0.5 µm and bigger in measurement, equivalent to an ISO 9 cleanroom,
- while by comparison an ISO 1 cleanroom permits no particles in that size range and just 12 particles for each cubic meter of 0.3 µm and smaller.

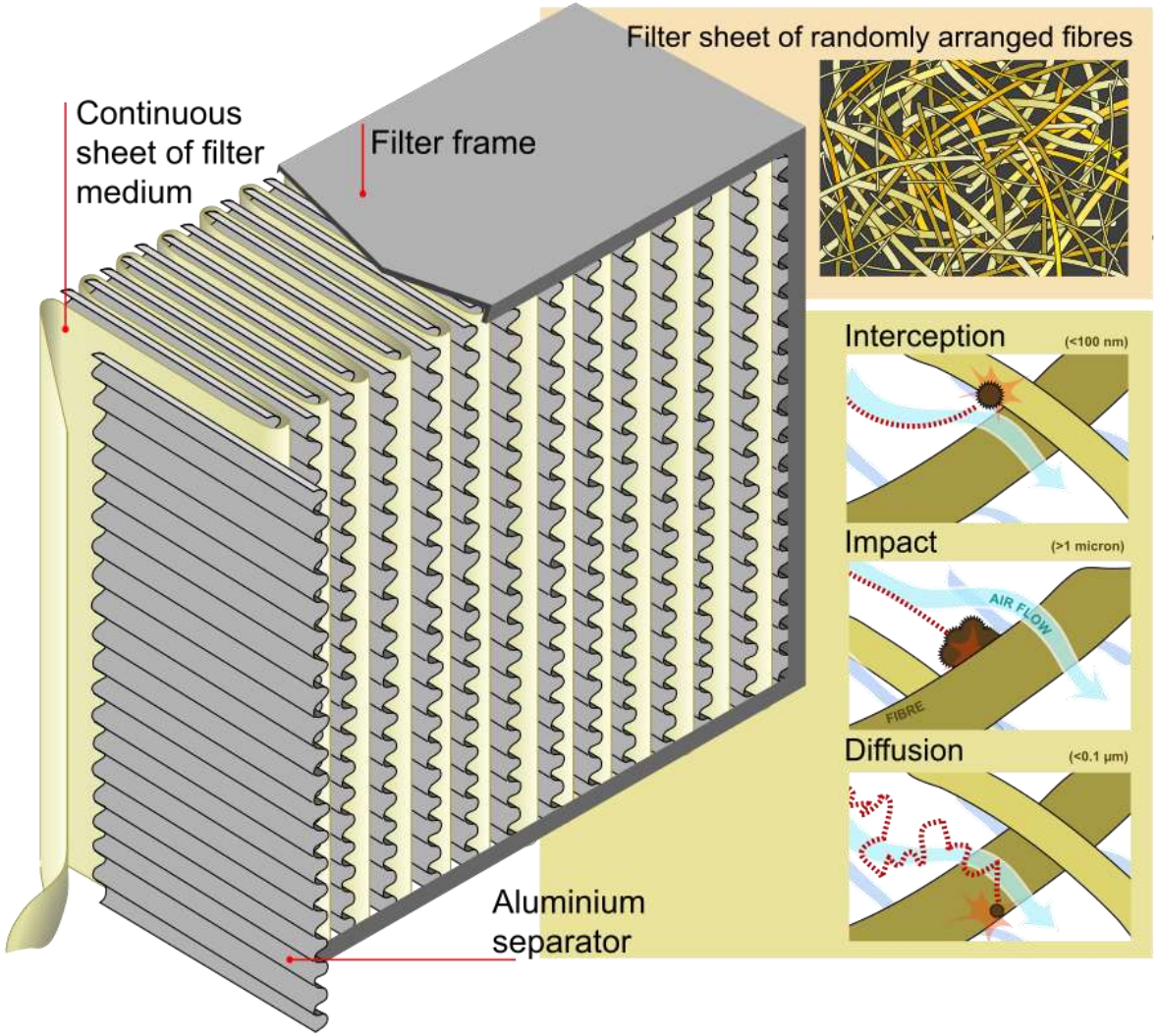
The yellow (red-green) lighting is necessary for photolithography, to prevent unwanted exposure of photoresist to light of shorter wavelengths.

ISO specification

Class	Maximum particles/ft^3					ISO equivalent
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥5 µm	
1	35	7.5	3	1	0.007	ISO 3
10	350	75	30	10	0.07	ISO 4
100	3,500	750	300	100	0.7	ISO 5
1,000	35,000	7,500	3000	1,000	7	ISO 6
10,000	350,000	75,000	30,000	10,000	70	ISO 7
100,000	3.5×10^6	750,000	300,000	100,000	830	ISO 8

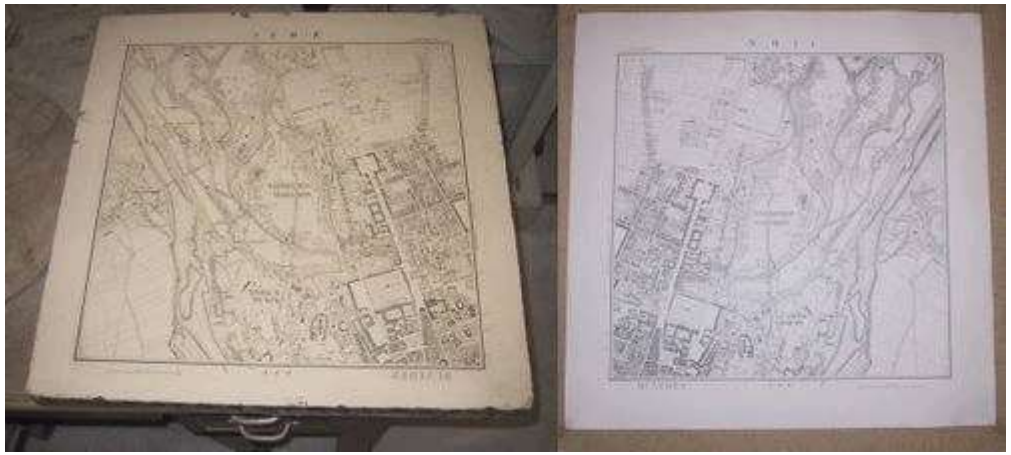
Cleanroom Air shower

HEPA filter (high-efficiency particulate air filter) corrugated internal structure and aluminum support along with the description of its functioning principle (interception, impact and diffusion of dust particles through a dense non-woven fiber material)



Lithography History

- Historically, lithography is a type of printing technology that is based on the chemical repellence of oil and water.
- Photo-litho-graphy: *latin*: light-stone-writing.
- In 1826, Joseph Nicephore Niepce in Chalon France takes the first photograph developed using oil of lavender and mineral spirits.
- In 1935 Louis Minsk of Eastman Kodak developed the first negative photoresist.
- In 1940 Otto Suess developed the first positive photoresist.
- In 1954, Louis Plambeck, Jr., of Du Pont, develops the Dycryl polymeric letterpress plate.



Lithography stone and mirror-image print of a map of Munich.

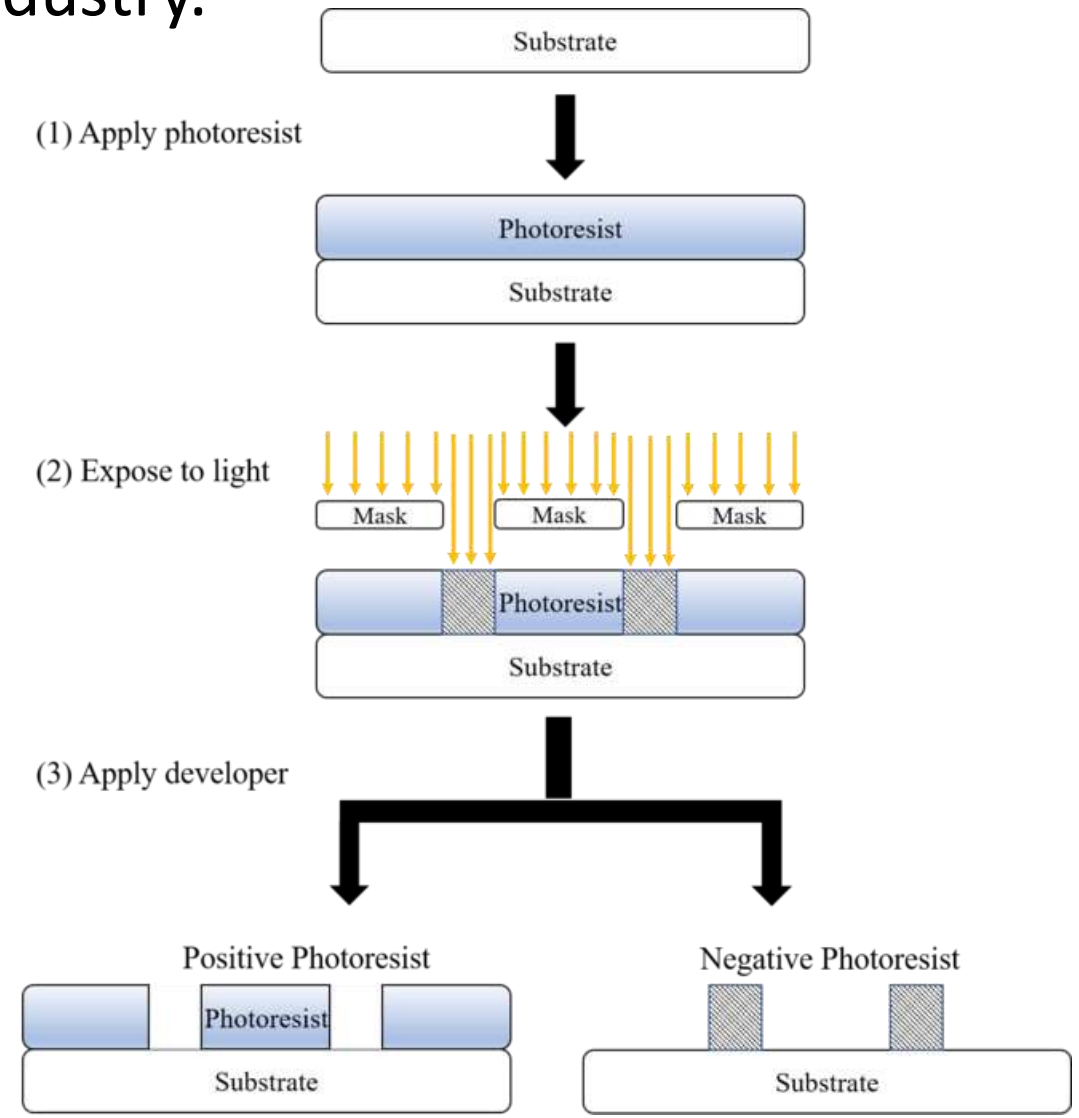
Lithography press for printing maps in Munich

Photoresist

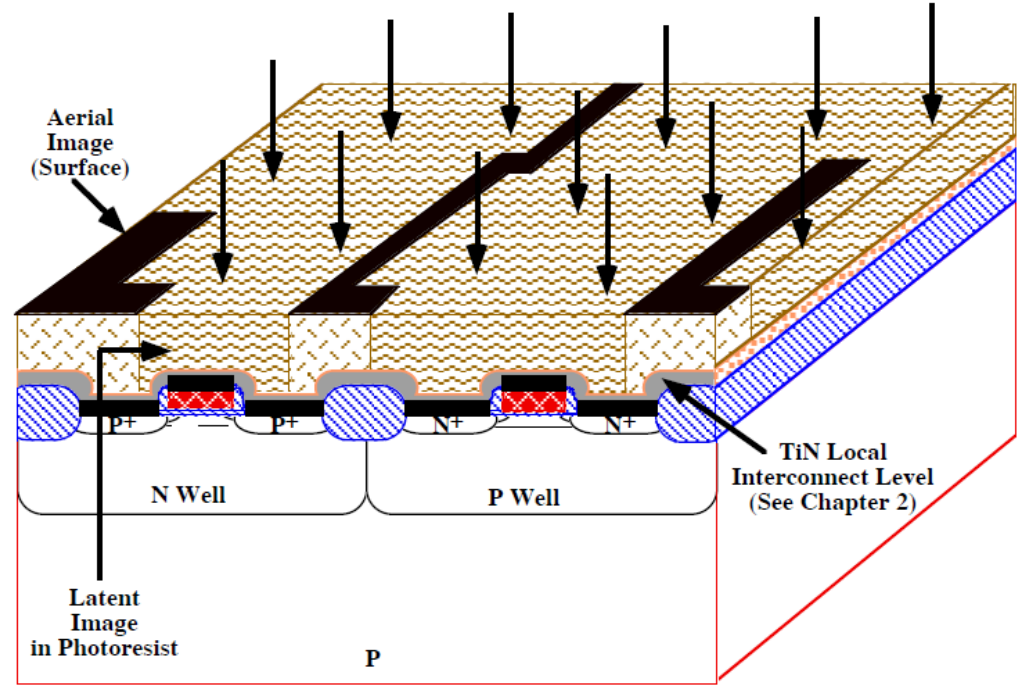
- A *positive photoresist* is a type of photoresist in which the portion of the photoresist that is exposed to light becomes soluble to the photoresist developer. The unexposed portion of the photoresist remains insoluble to the photoresist developer.

- A *negative photoresist* is a type of photoresist in which the portion of the photoresist that is exposed to light becomes insoluble to the photoresist developer. The unexposed portion of the photoresist is dissolved by the photoresist developer.

A photoresist is a light-sensitive material used in several processes, such as photolithography, to form a patterned coating on a surface. This process is crucial in the electronic industry.



Photolithography for IC manufacturing



- In IC manufacturing, lithography is the single most important technology.
- ~35% of wafer manufacturing costs comes from lithography.

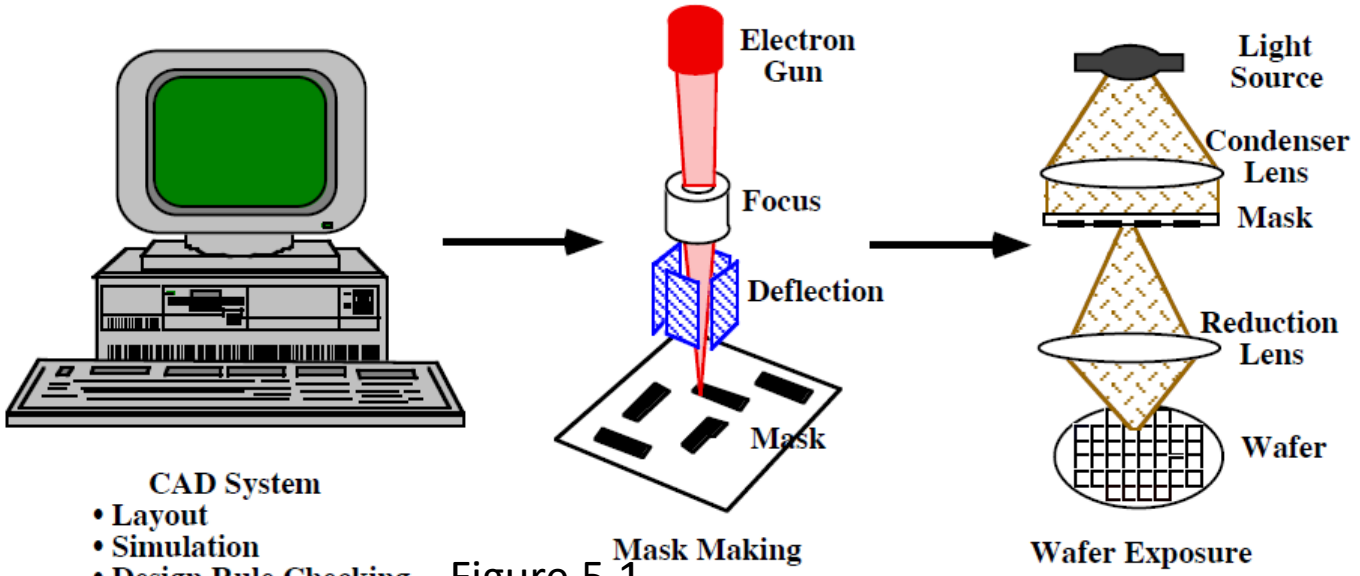


Figure 5.1

Patterning process consists of:

- Mask design
- Mask fabrication
- Wafer exposure

Lithography

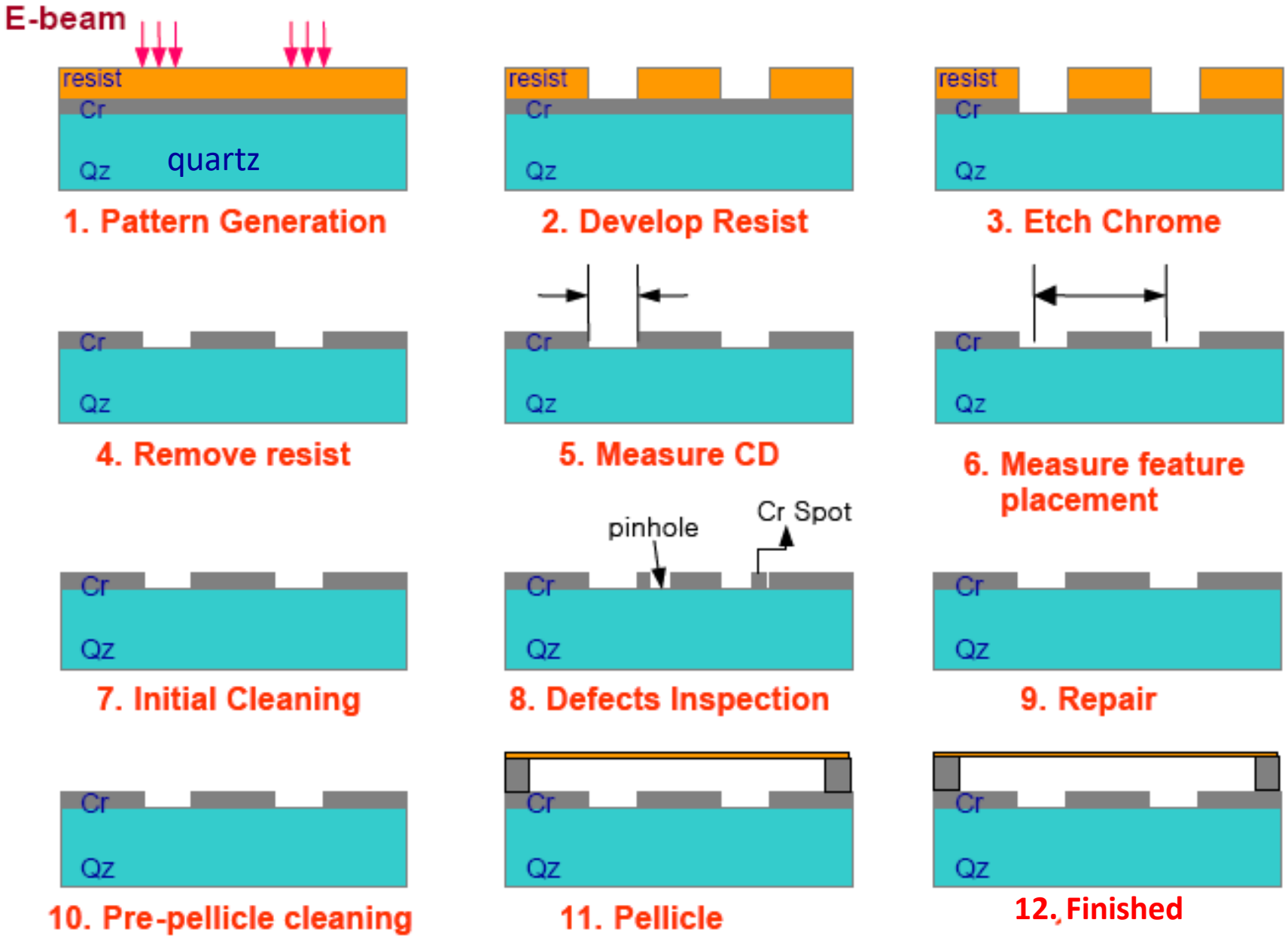
- It is printing technology.
- Microlithography and nanolithography refer specifically to lithographic patterning methods capable of structuring material on a fine scale.
- Typically, features smaller than 10 micrometers are considered microlithographic, and features smaller than 100 nanometers are considered nanolithographic.
- Photolithography is one of these methods, often applied to semiconductor device fabrication.
- Photolithography, or UV lithography, is a process used in microfabrication to pattern parts on a thin film or the bulk of a substrate (also called a wafer).
- It uses light to transfer a geometric pattern from a photomask (also called an optical mask) to a photosensitive (that is, light-sensitive) chemical photoresist on the substrate.
- A series of chemical treatments then either etches the exposure pattern into the material or enables deposition of a new material in the desired pattern upon the material underneath the photoresist.

Electron-beam lithography (EBL)

- Electron-beam lithography (EBL) is the practice of scanning a focused beam of electrons to draw custom shapes on a surface covered with **an electron-sensitive film called a resist (exposing)**.
- The electron beam changes the solubility of the resist, enabling selective removal of either the exposed or non-exposed regions of the resist by immersing it in a solvent (developing).
- The purpose, as with photolithography, is to create very small structures in the resist that can subsequently be transferred to the substrate material, often by etching.

- The primary advantage of electron-beam lithography is that it can draw custom patterns (direct-write) with sub-10 nm resolution. This form of maskless lithography has high resolution and low throughput, limiting its usage to photomask fabrication, low-volume production of semiconductor devices, and research and development.

Photomask fabrication by electron beam lithography



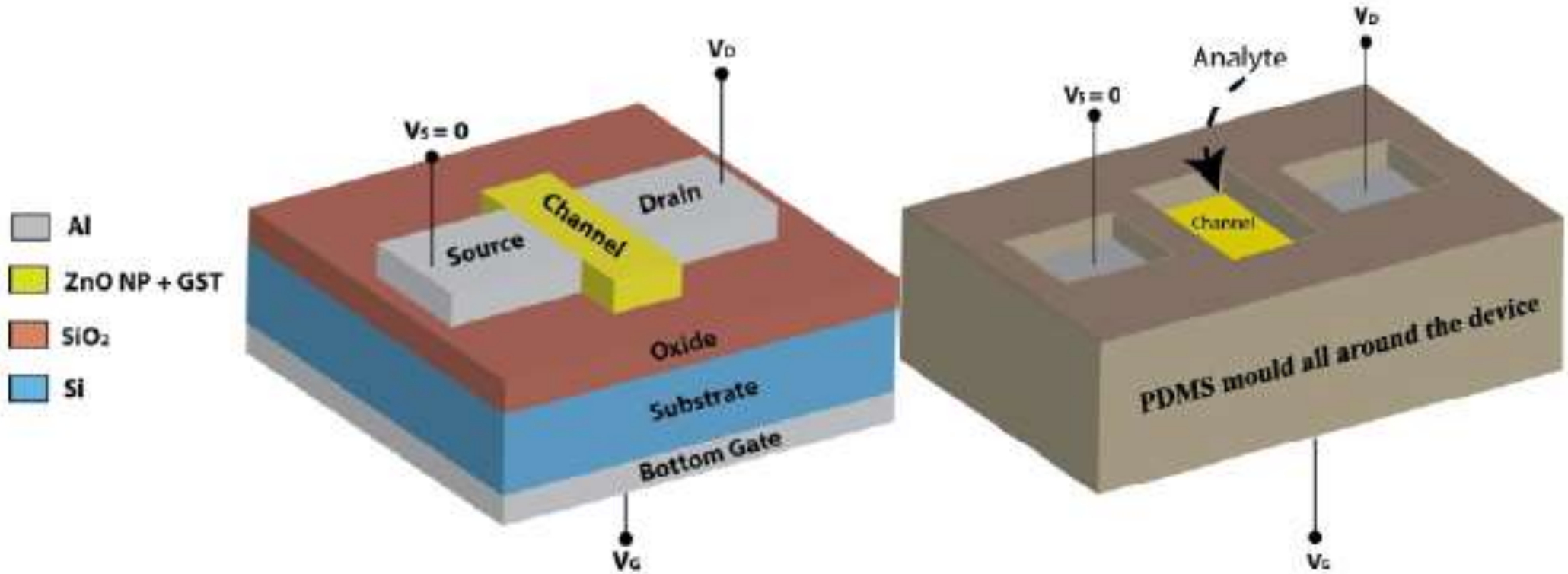
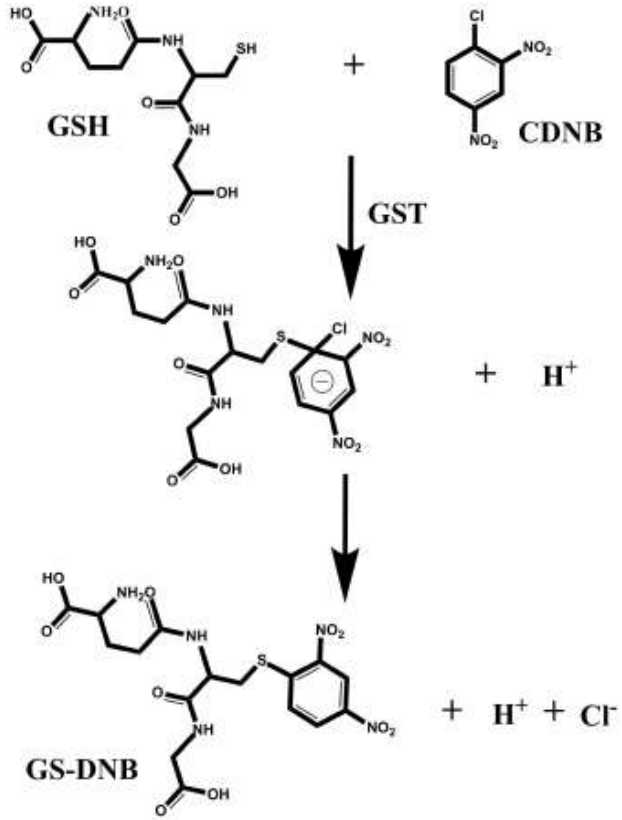


Fig. 2. Schematic of the fabricated device and PDMS mold around the fabricated device.

Detection process



Conjugation reaction of GSH and CDNB in presence of GST

- GST can be immobilized on the channel

Abstract:

- This article reports fabrication and characterization of field effect transistor (FET) device consisting of zinc oxide (ZnO) nanoparticle-glutathione-S-transferase (GST) composite channel for successful in vitro detection and quantification of glutathione (GSH) in solution and in cancerous cell.
- The functionalized channel layer is synthesized by means of electrostatic binding between **ZnO nanoparticles and GST.**
- Detection of glutathione can be confirmed by the change in output current characteristics of the device in real time whenever the conjugation reaction between GSH and 1-chloro-2, 4-dinitrobenzene (CDNB) in the presence of GST takes place on the channel of the device.
- The characterized device had a sensitivity of 60.22 µA/dec and limit of detection (LoD) of 43.96 nM.
- Thus, from the device characteristics, GSH could be quantified. Subsequently, laboratory grown HeLa, MCF-7 (both cancerous), and human embryonic kidney (HEK-noncancerous) cells were characterized with the fabricated device and both the cancerous cells resulted in significantly higher drain current compared to noncancerous HEK cells.
- The fabricated device resulted in a sensitivity of 210.86 nA/cell and LoD of 30 cells when characterized with HeLa cells. This detection paves way for a device-based early detection scheme for cancer by measuring the GSH concentration in human cell cytosol.

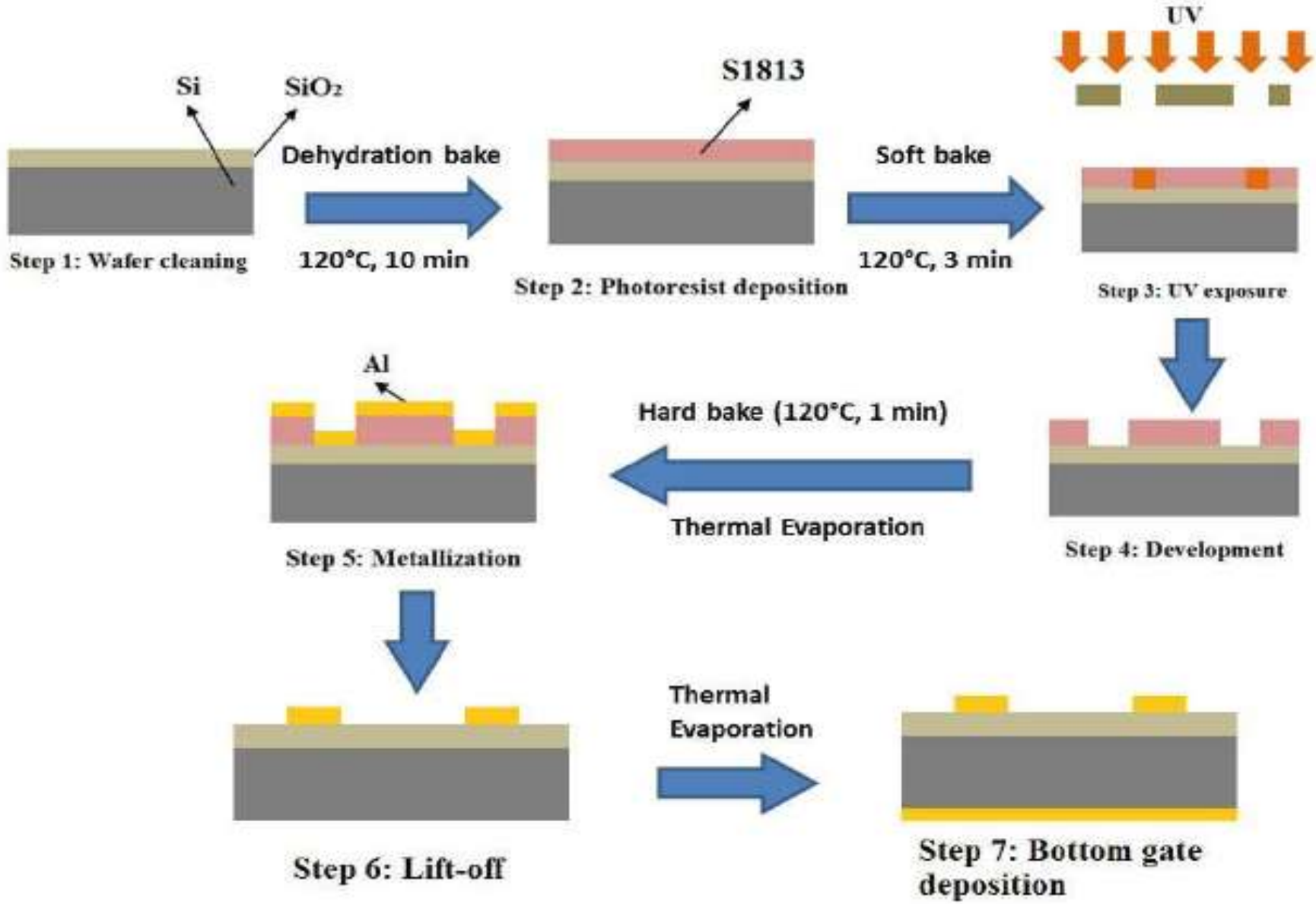


Fig. 1. Steps involved in the fabrication process.

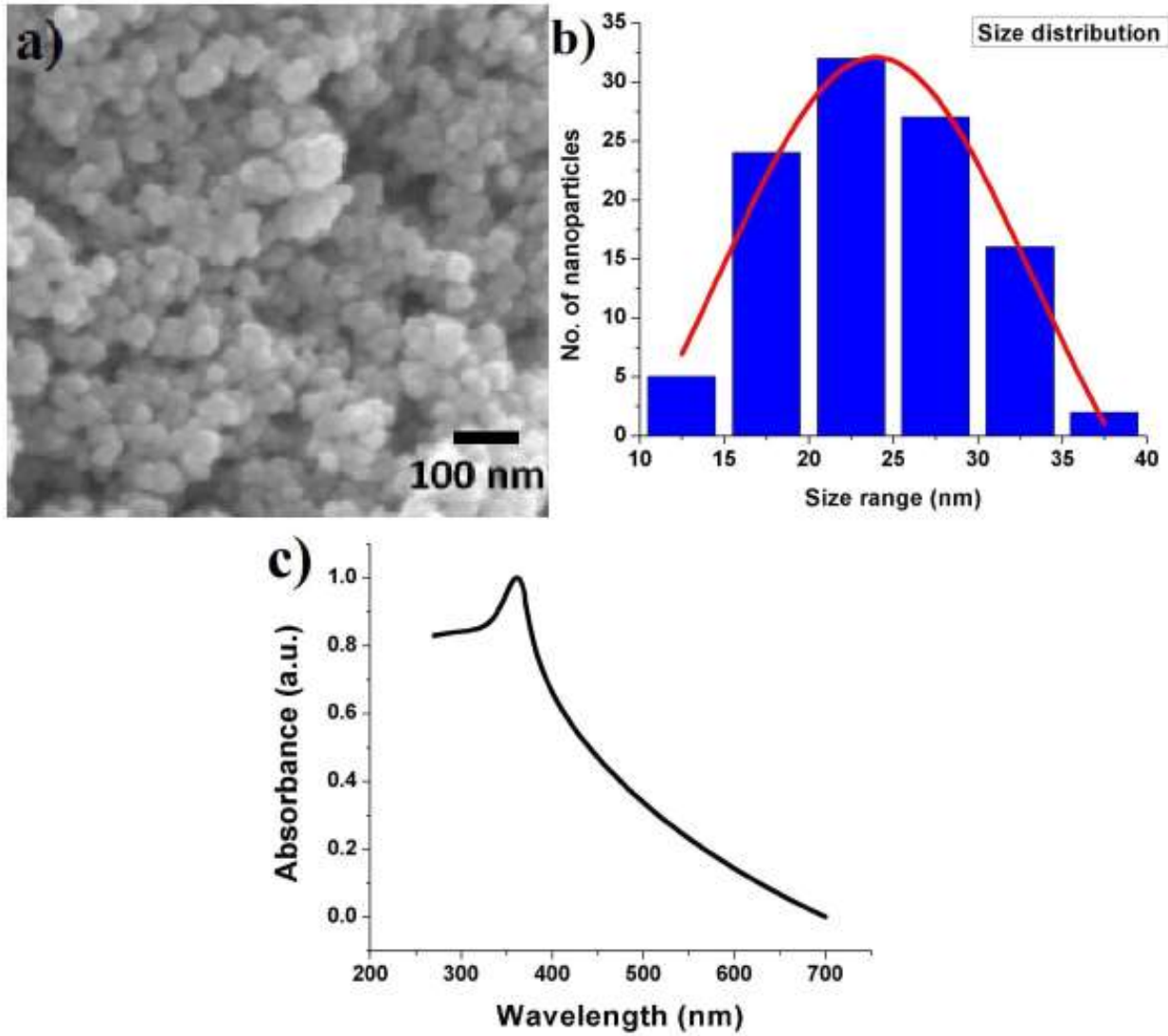


Fig. 3. (a) FESEM image (with magnification 50 000, acceleration voltage 5 kV, and working distance 4.5 mm). (b) Size distribution plot of the synthesized ZnO nanoparticles. (c) UV spectroscopic analysis of ZnO nanoparticles.

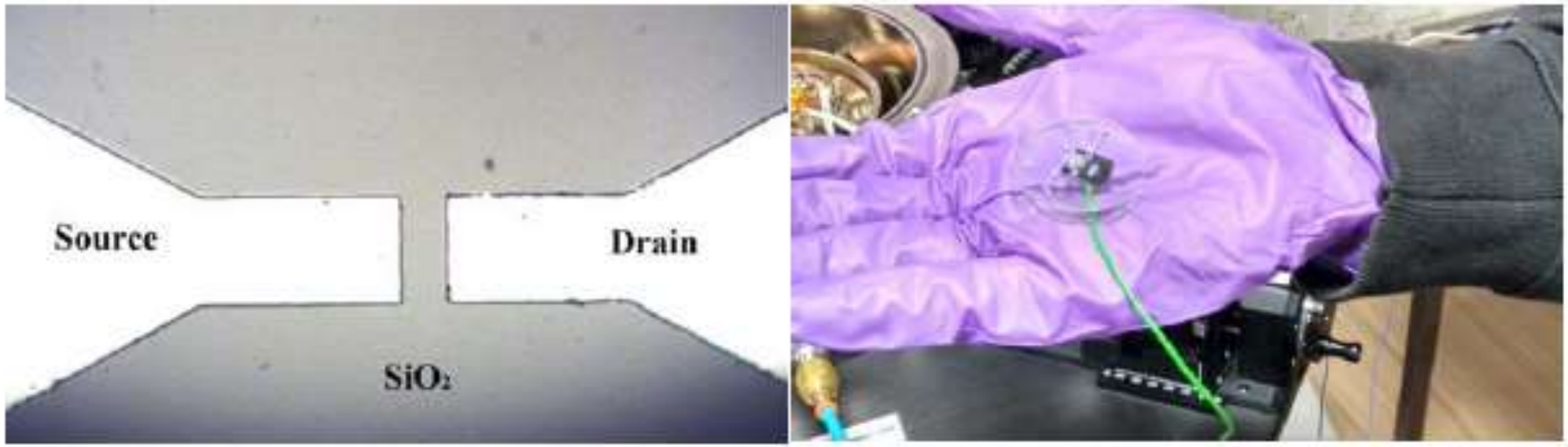


Fig. 4. Fabricated device under test.

TABLE II

FABRICATED DEVICE PARAMETERS

Parameter	Value
Channel length	5 μm
Channel width	10 μm
Oxide thickness	300 nm

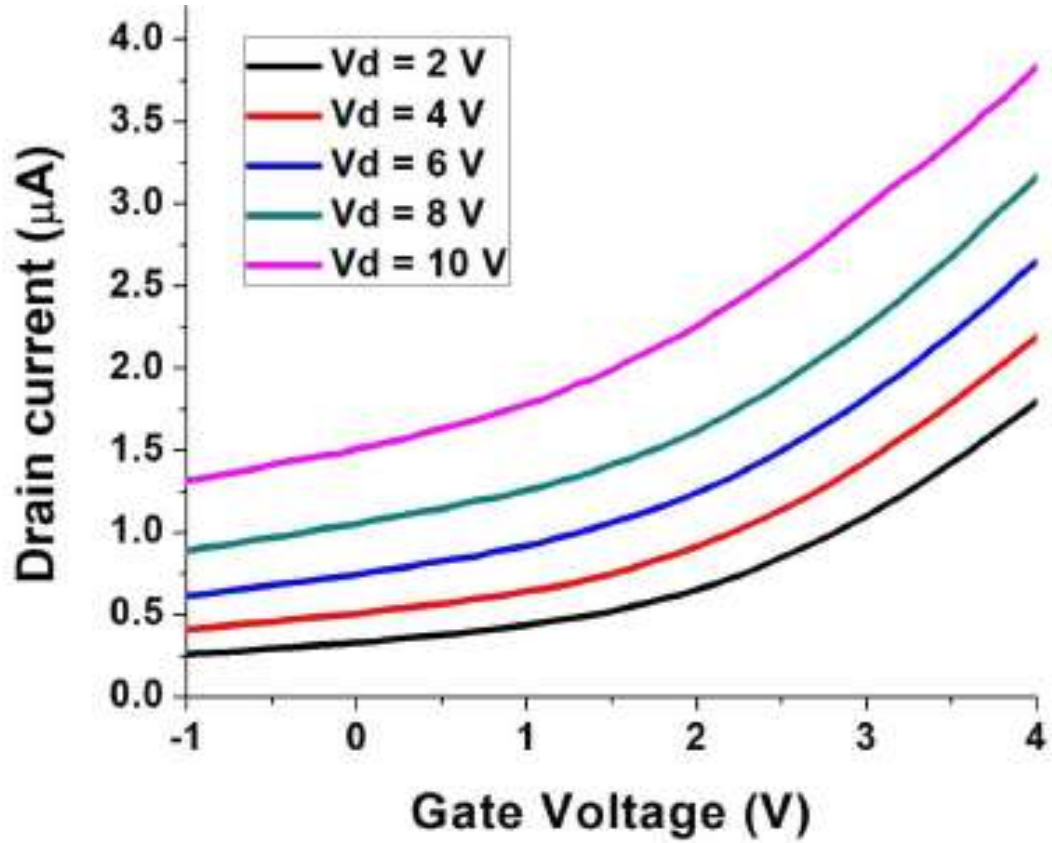


Fig. 5. Transfer characteristics of the fabricated devices without analyte.

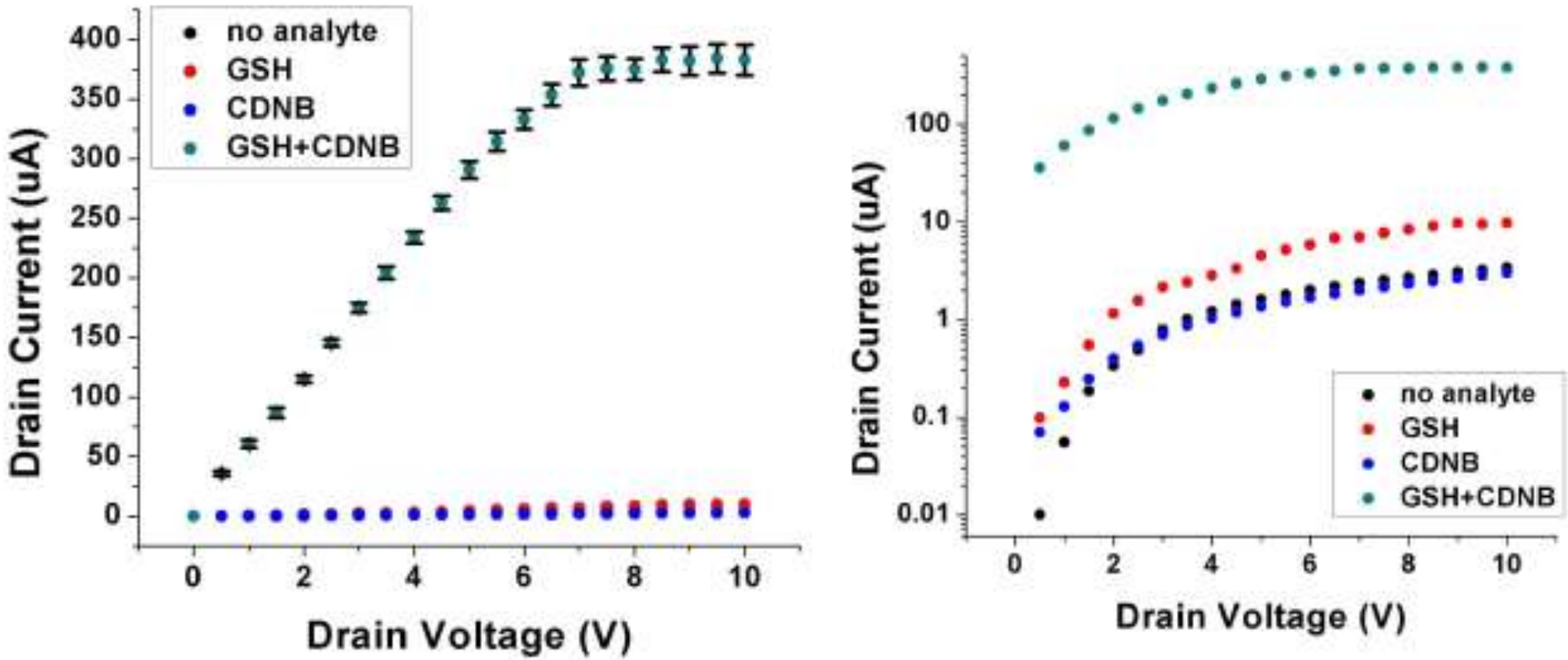


Fig. 6. Output characteristics of the fabricated devices ($V_g = 2$ V).

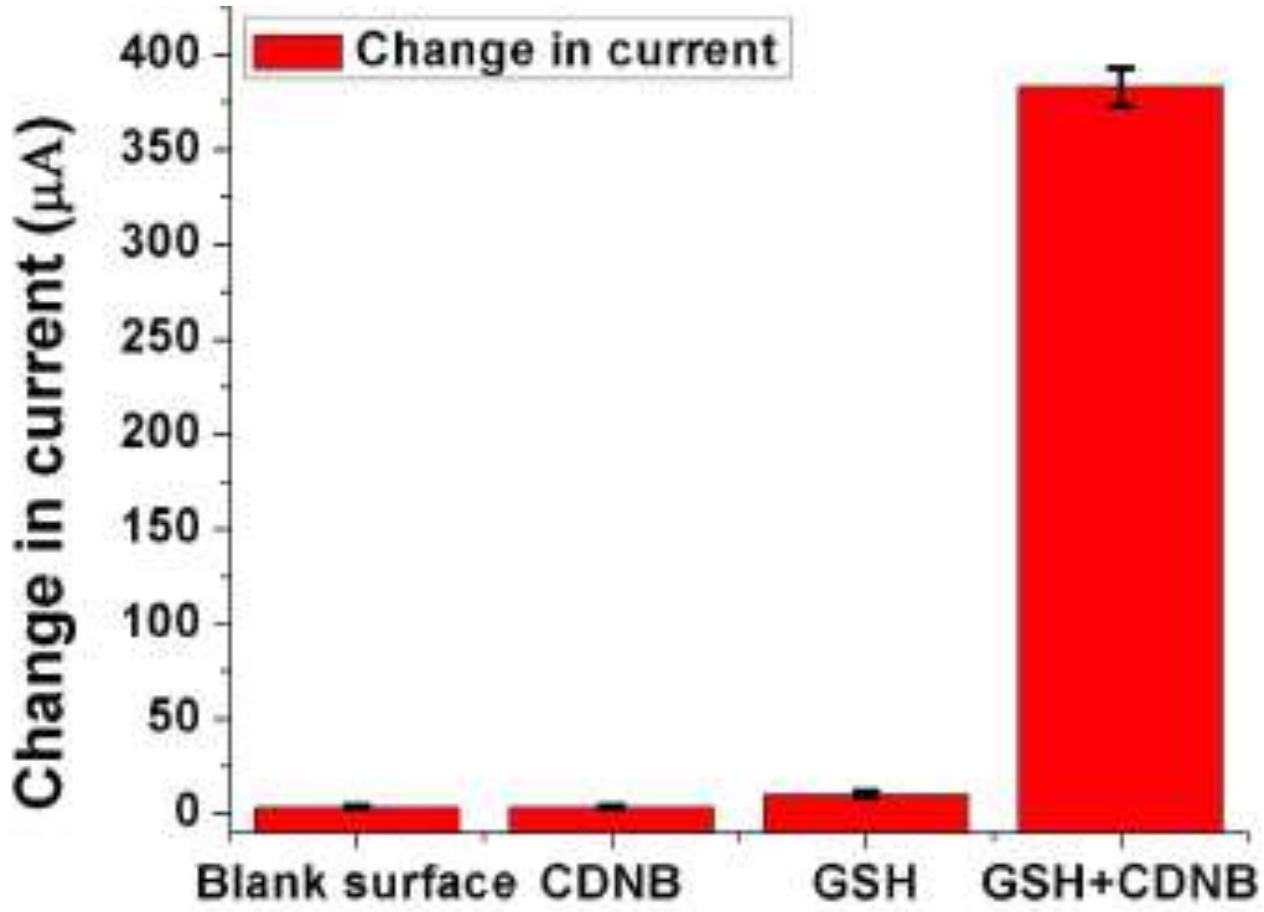


Fig. 7. Change in drain current ($V_d = 10$ V, $V_g = 2$ V).

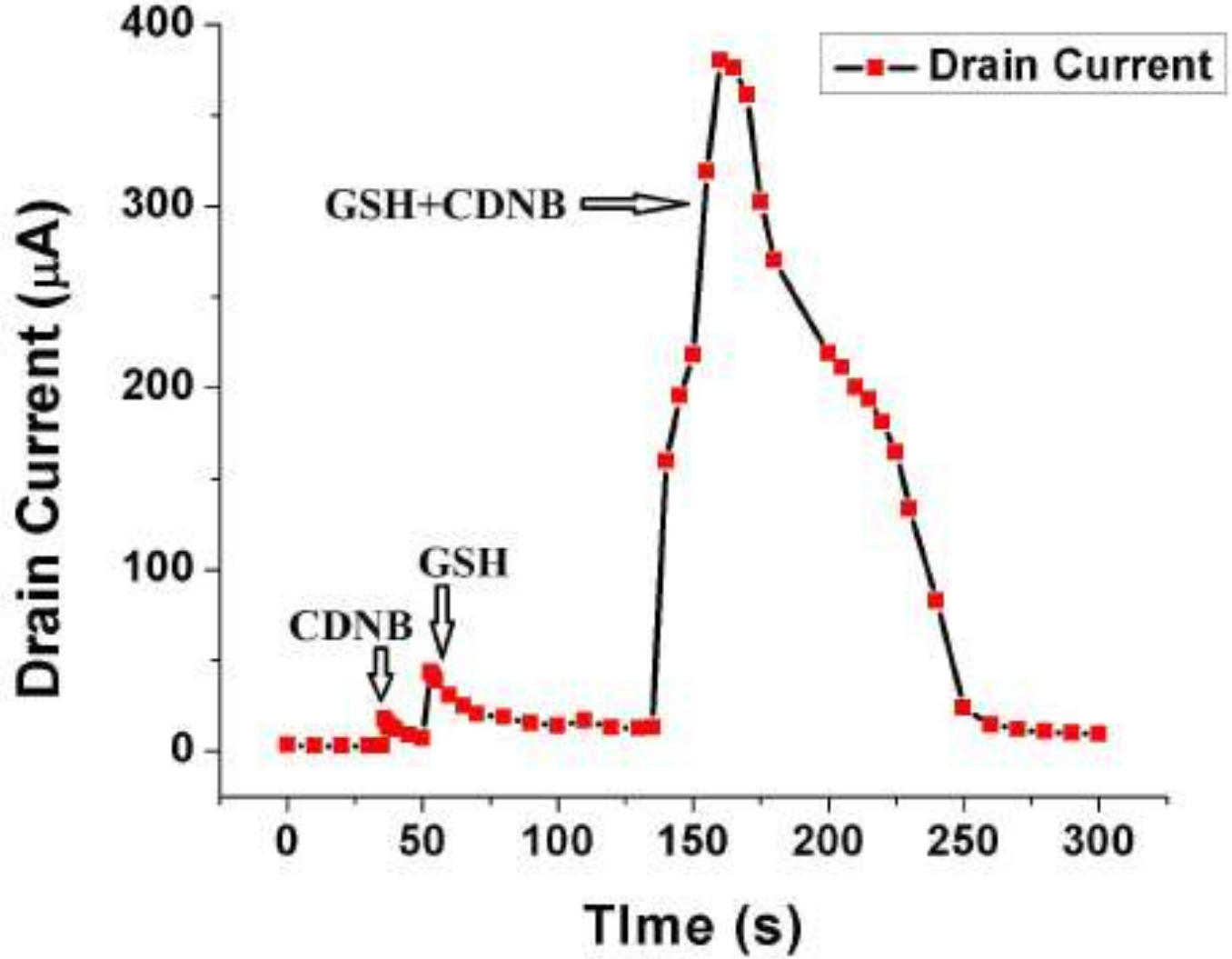


Fig. 8. Real-time analysis ($V_d = 10$ V, $V_g = 2$ V).

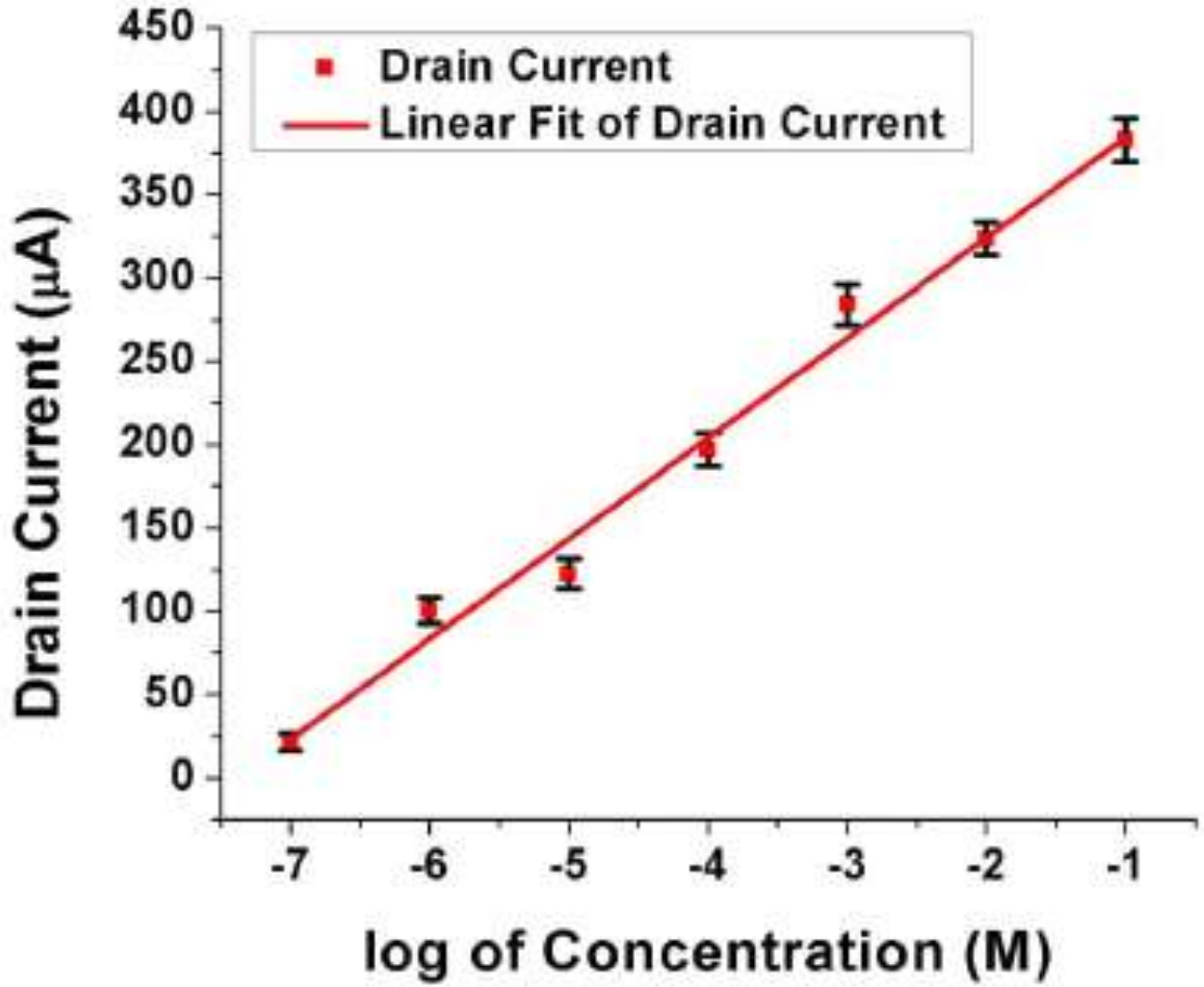


Fig. 9. Calibration curve (no. of trials = 10).

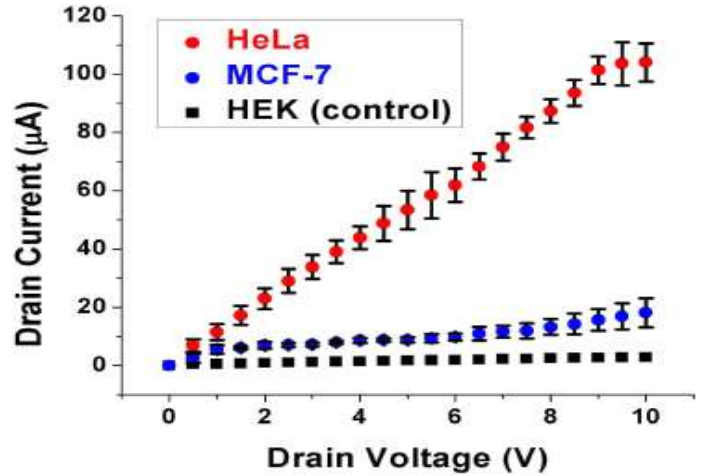


Fig. 10. Output characteristics for HeLa, MCF-7, and HEK cells (V_g = 2 V).

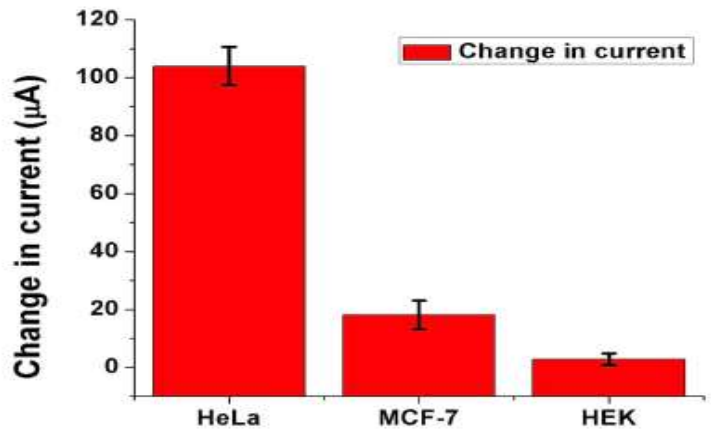


Fig. 11. Comparison of change in drain currents for HeLa, MCF-7, and HEK cells (V_d = 10 V, V_g = 2 V).

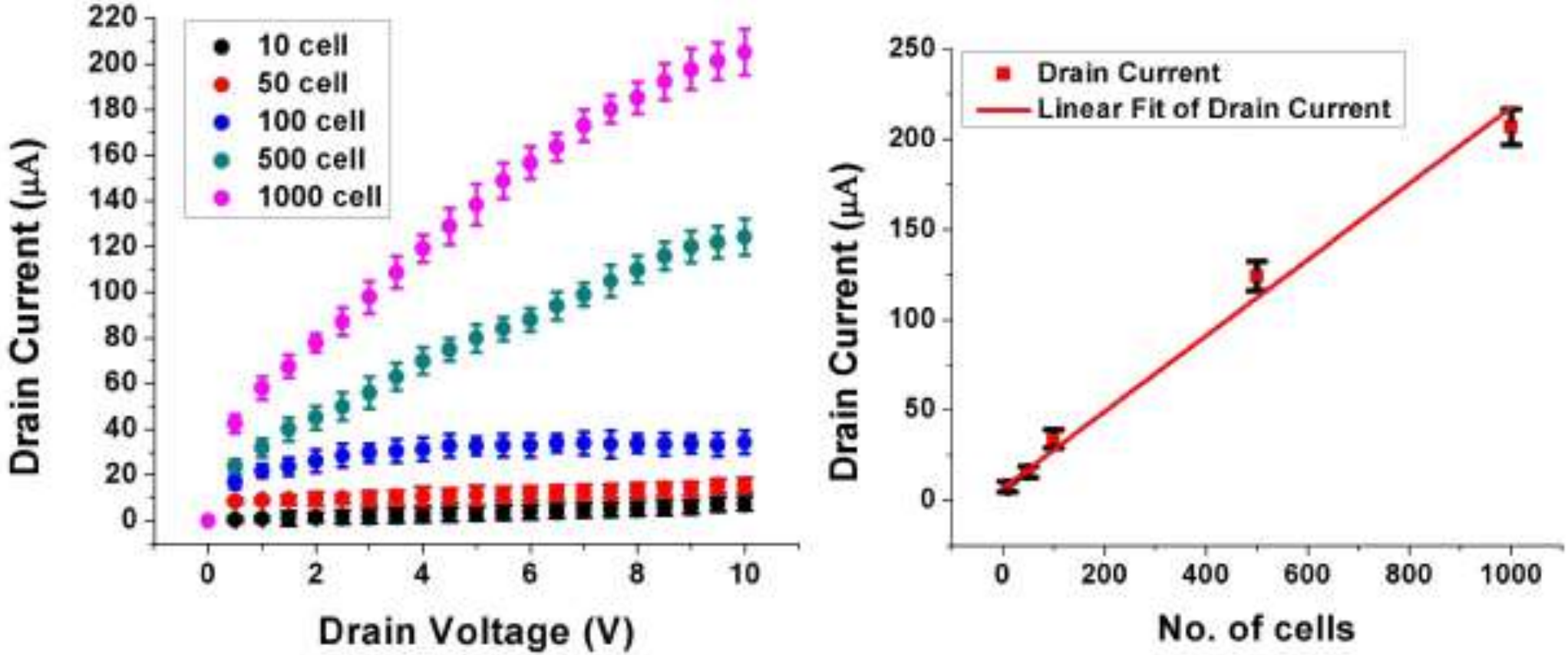


Fig. 12. Output characteristics of the different numbers of HeLa cells ($V_g = 2$ V) and calibration curve (no. of trials = 10).

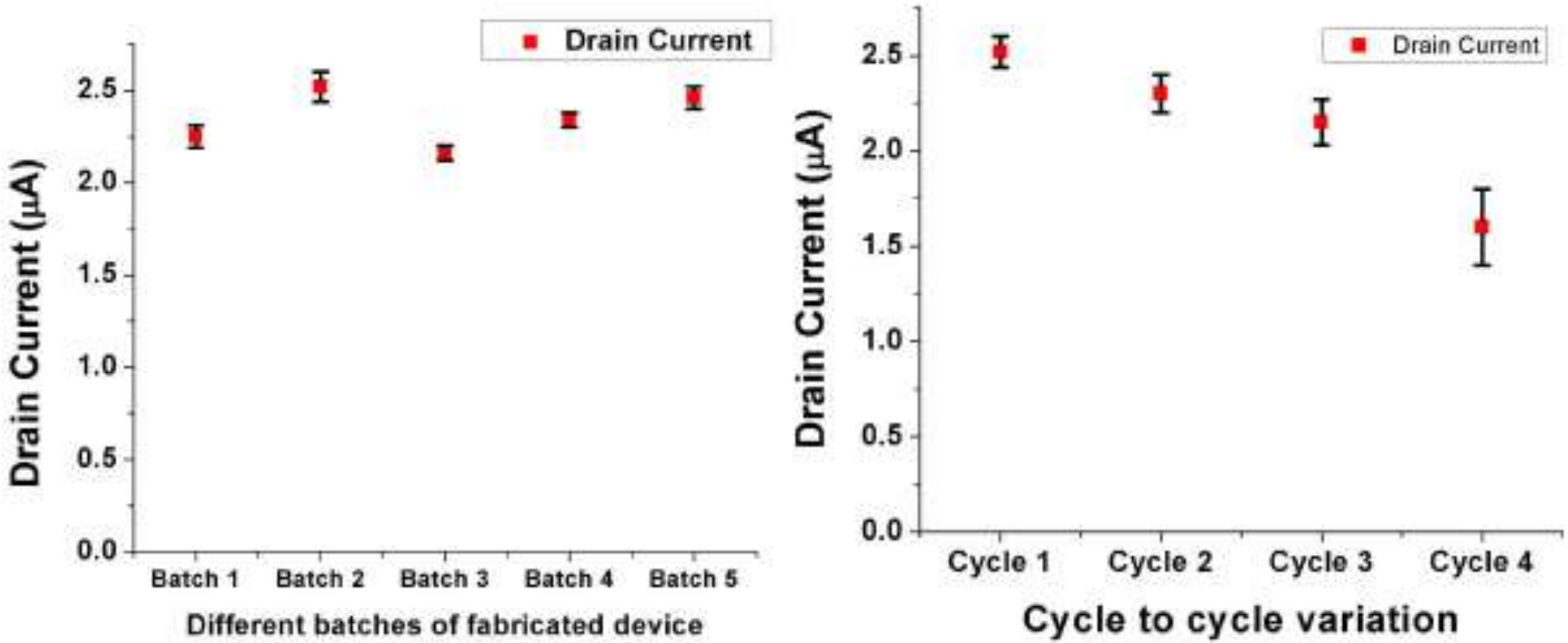


Fig. 13. Repeatability analysis ($V_d = 10$ V, $V_g = 2$ V).

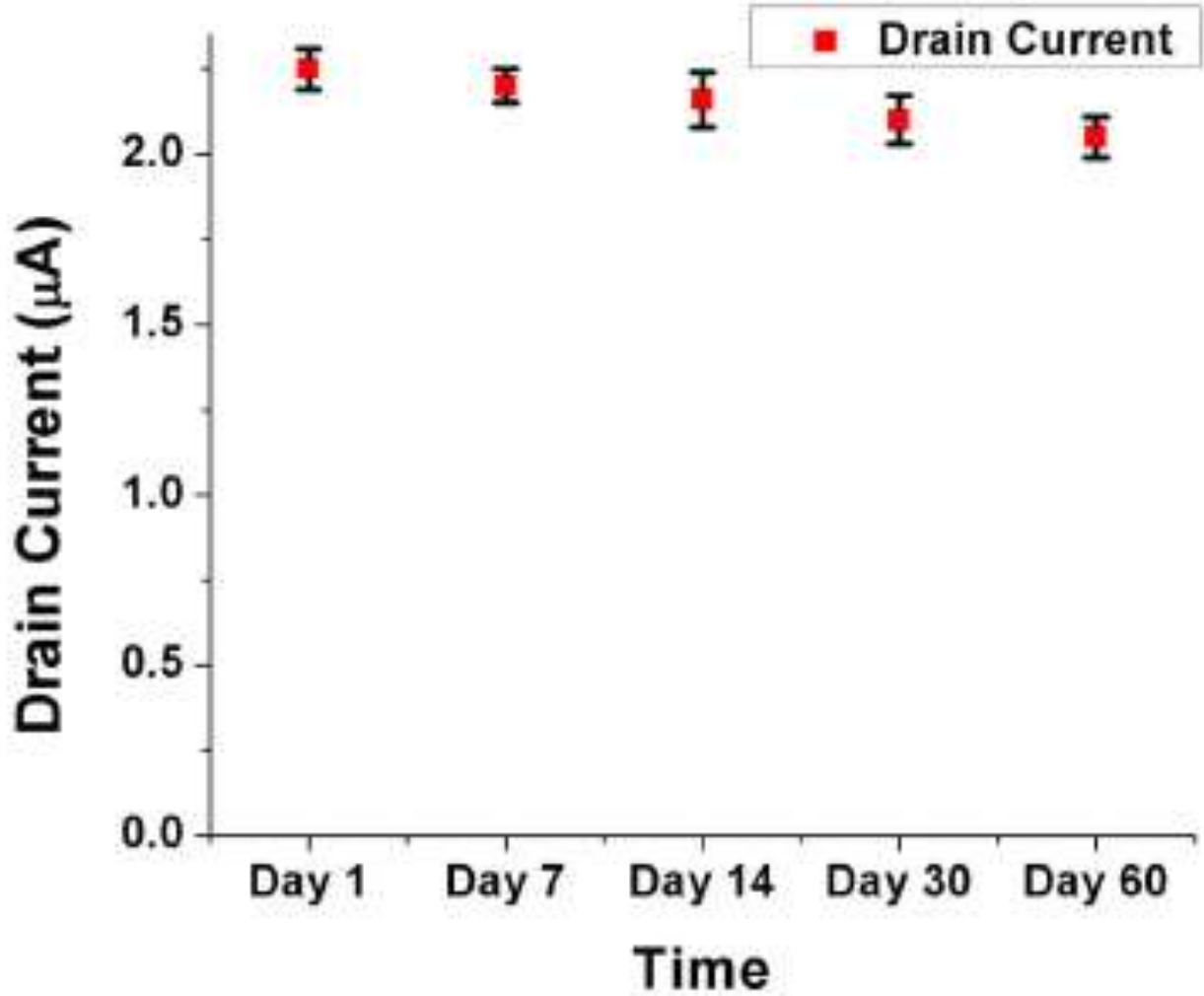


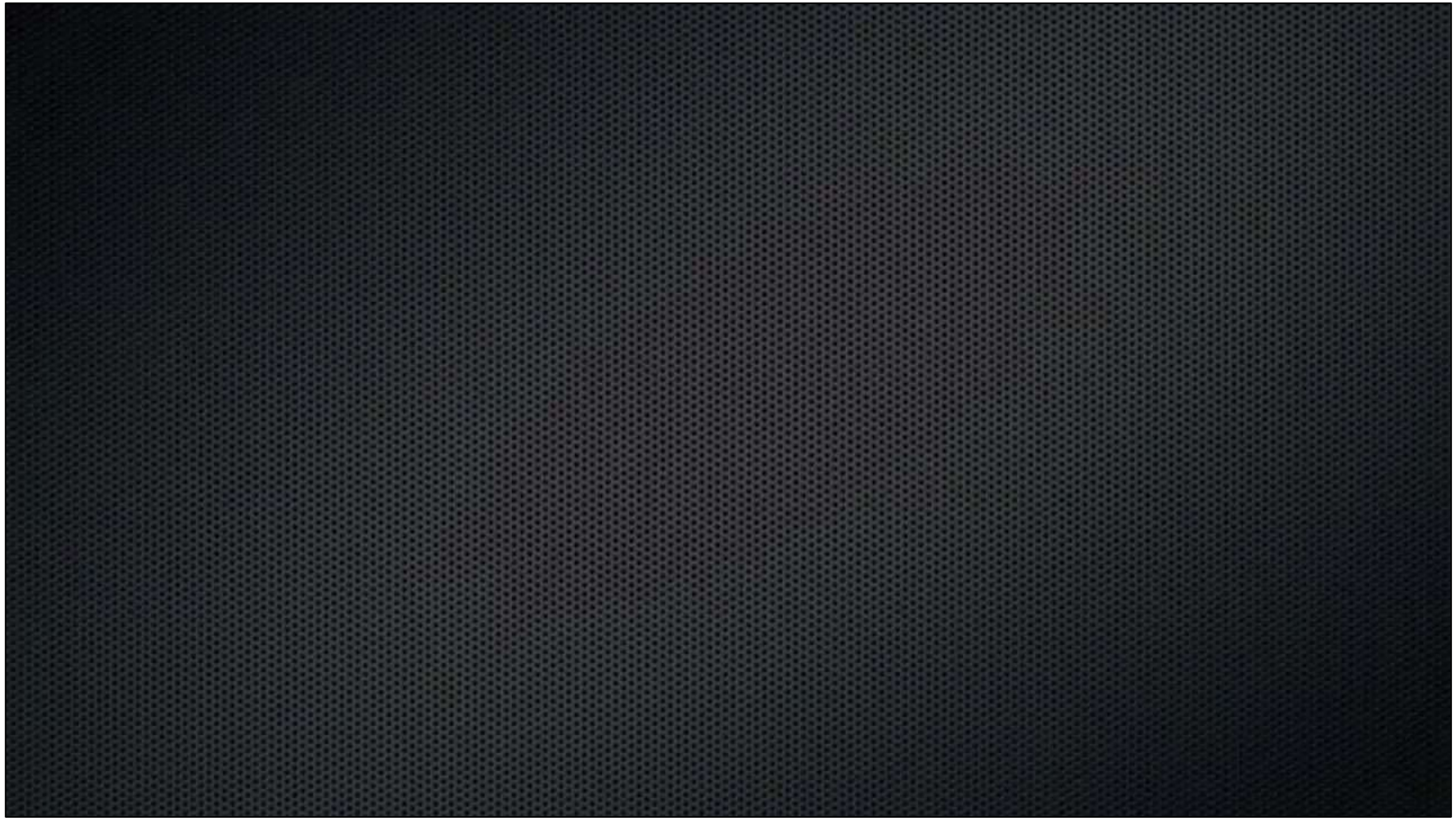
Fig. 14. Stability analysis ($V_d = 10$ V, $V_g = 2$ V).

COMPARISON WITH EXISTING TECHNIQUES

Detection Principle	Type	Assay Time	Sensitivity	Linear range of operation	Limit of Detection	Sample Type	Sample volume	Ref.
Colorimetric	-	10 min	0.06 U/mL	0.1 – 0.4 mM	10.5 nM	GST enzyme	200 µL	51
Liquid Chromatography	-	15 min	0.39 µA/µM	0.01 – 50 µM	0.6 nM	Human blood	20 µL	52
Chemiluminescence	-	8 s	0.8 au/µM	$1x10^{-2}$ – 10 µM	8 nM	Human blood	600 µL	53
Electrochemical	CV	-	$4.8x10^5$ cm^3/M	0.3 – 3350 µM	80 nM	GSH	-	44
Electrochemical	SWV	-	0.64 nA/µM	10 – 60 µM	3 µM	Human saliva	50 µL	54
Electrochemical	SWV	20 min	0.76 µA/µM	0.5 nM – 5 µM	0.14 nM	GSH	-	55
Electrochemical	DPV	20 min	96 nA/µM	1.0 nM – 1.0 µM	0.6 nM	GSH	50 µL	56
Electrochemical	CA	-	84.2 nA/µM	0.1 – 5.0 µM	0.095 µM	GSH	-	57
Electrochemical Device	Chemiresistive (ZnO)	30 s	5.68 nA/µM	100 nM – 10 mM	41.9 nM	GSH and CDNB	5 µL	30
Electrochemical Device	Chemiresistive (MoS_2)	30 s	0.7044 nA/µM	10 µM – 500 mM	703 nM	GSH and CDNB	1 µL	45
Electrochemical Device	FET	30 s	60.22 µA/dec	100 nM – 100 mM	43.96 nM	GSH and CDNB	1 µL	This work
Electrochemical Device	FET	30 s	210.86 nA/cell	10 – 1000 cells	30 cells	HeLa cells	1 µL	This work

Atomic Absorption Spectroscopy

ANIMATION



INTRODUCTION

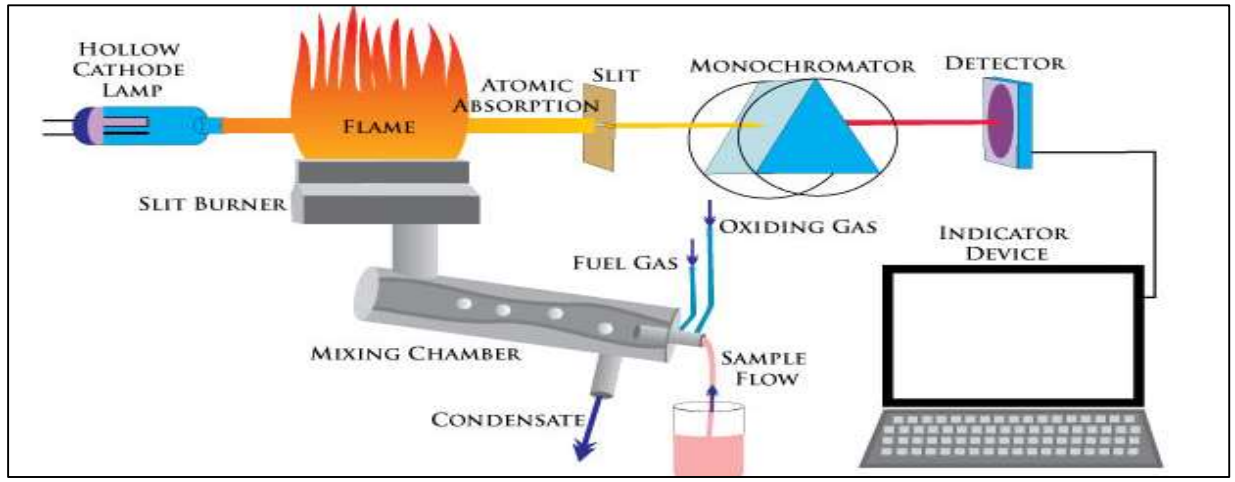
- Atomic absorption spectroscopy, or AAS, is a spectro-analyitcal technique for **measuring the concentrations of metallic elements** in different materials.

- **History:** Flame atomic absorption spectroscopy (Flame AAS or FAAS) was developed in 1952 and first commercially released as an analytical technique in the 1960s. Since then, the technique has remained popular for its reliability and simplicity.

- As an analytical technique, AAS uses **electromagnetic wavelengths**, coming from a light source. **Distinct elements will absorb these wavelengths** differently. It gives a picture of what concentrations of a specific element is present in the material, or liquid, is being tested.

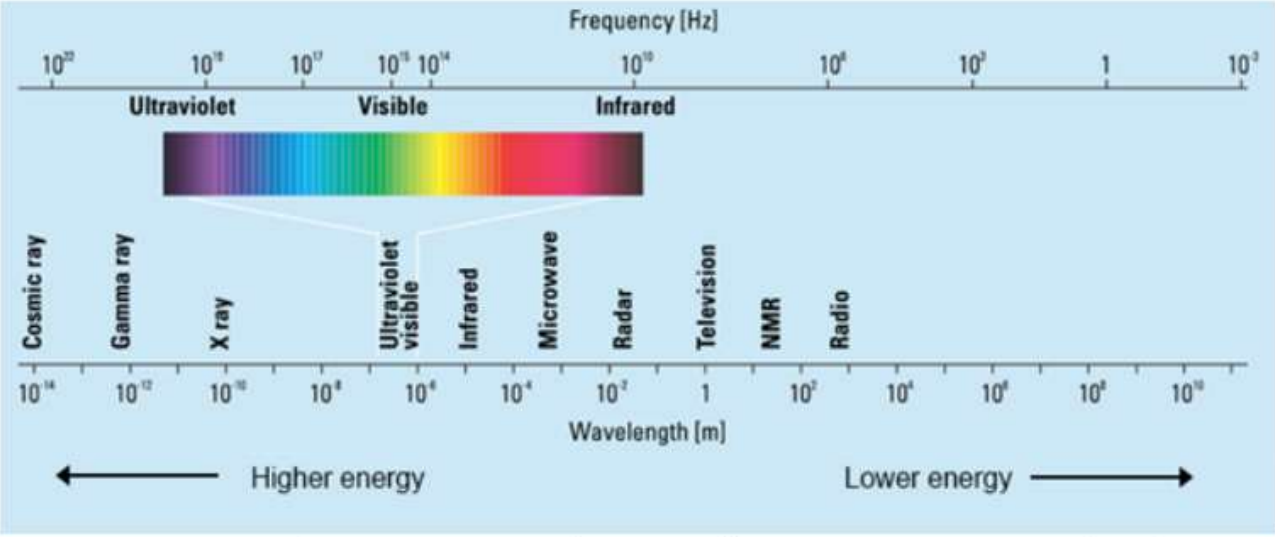
- **The amount of light absorbed at a defined wavelength corresponds to the known characteristics of the element being tested.**

- This techniques can analyze over 70 elements.

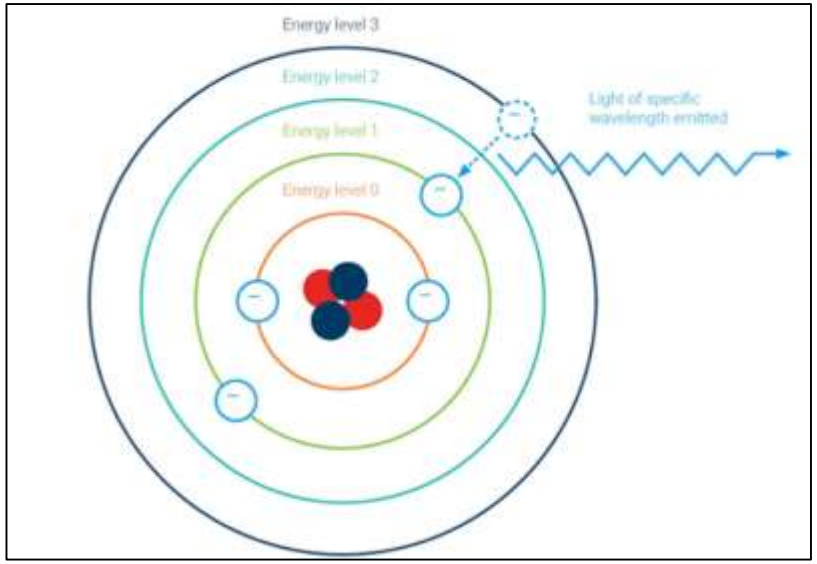


PRINCIPLE

- This technique is based on the principle that **free atoms (gas) generated in an atomizer** can absorb radiation at specific frequency.
- Atomic- Absorption spectroscopy **quantifies the atomic-absorption of ground state atoms in the gaseous state**. The atoms absorb the **ultraviolet or visible light** and make **transitions to higher electronic energy levels.**
- The **analyte concentration is determined from the amount of absorption.**
- Concentration measurements are determined from working curve after calibrating the instrument with standards of known conc.



The electromagnetic spectrum. Atomic absorption spectroscopy uses light in the **ultraviolet-visible range.**



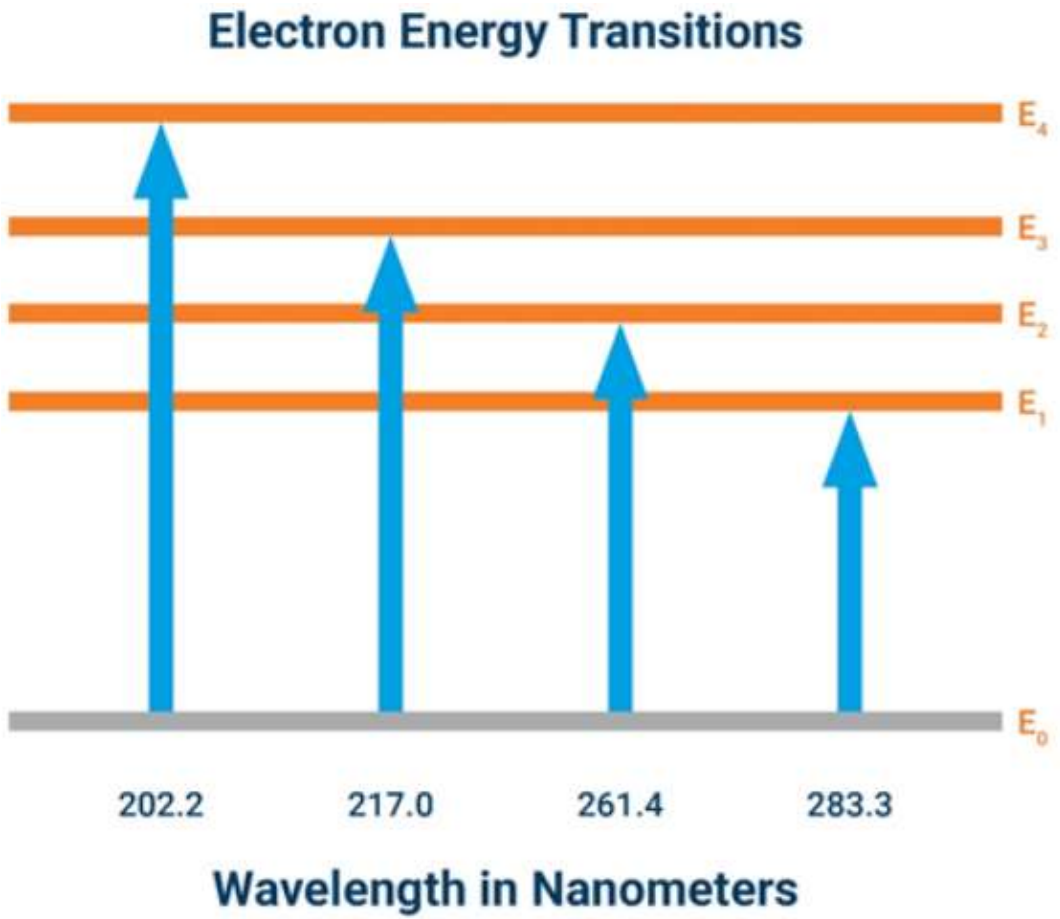
An electron is excited from the ground state to higher energy level by absorbing energy (light) at a specific wavelength. In atomic absorption spectroscopy, the wavelength of absorbed light is determined by the type of atom (which element it is) and the energy levels the electrons are moving to.

MECHANISM

The amount of energy required for the electrons to move between **energy levels corresponds to specific wavelengths of light**. As the diagram shows, moving an electron from the ground state of a Pb atom to the first energy level (E1) requires energy equivalent to light at 283.3 nm.

It requires more energy to move an electron from the ground state to the second energy level (which is further away from the nucleus).

For AAS analysis, the wavelength of the **ground state to the E1 level is frequently of most interest**, as it is **the most intense**. A strong absorbance band gives the best (lowest) detection limits.

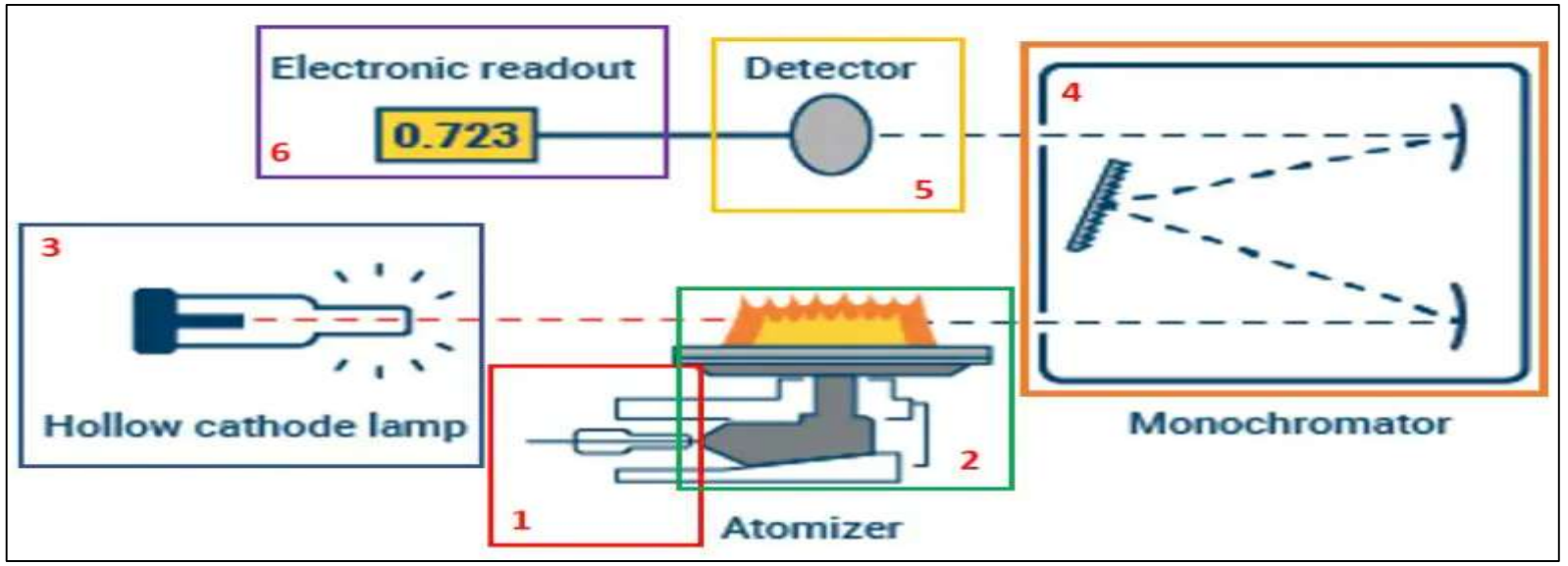


Energy level diagram for lead (Pb). **E_0 to E_1 is typically the transition of most interest for AAS analysis.**

Components of AAS Spectrometer (INSTRUMENTATION)

A simple flame atomic absorption spectrometer includes:

1. A sample introduction system
2. The burner (flame) and its associated gas supplies: air-acetylene or nitrous oxide-acetylene
3. A light source, the hollow cathode lamp (HCL)
4. A monochromator (the optical components inside the box in the diagram)
5. An optical detector (photomultiplier tube or PMT)
6. Computerized instrument control, data collection, and analysis.

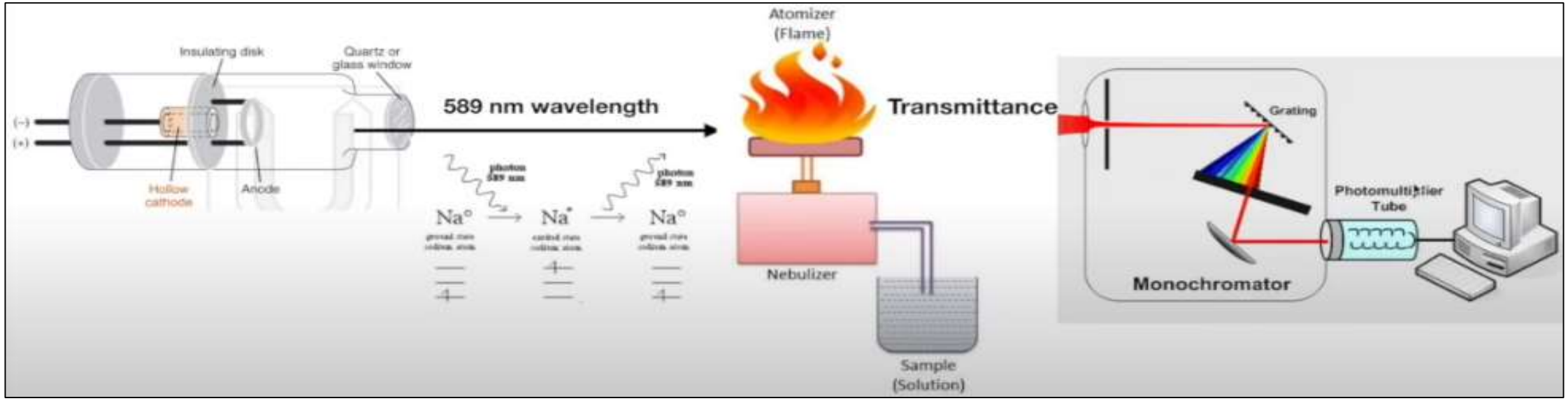


Components of AAS Spectrometer (INSTRUMENTATION)

1. In AAS, a solution containing the analyte is introduced into a flame.
2. The **flame** converts samples into free ground state atoms that can be excited.
3. **A lamp** emitting light at a wavelength specific to the atoms is passed through the flame, and as the light energy is absorbed, **the electrons in the atoms are elevated to an excited state.**

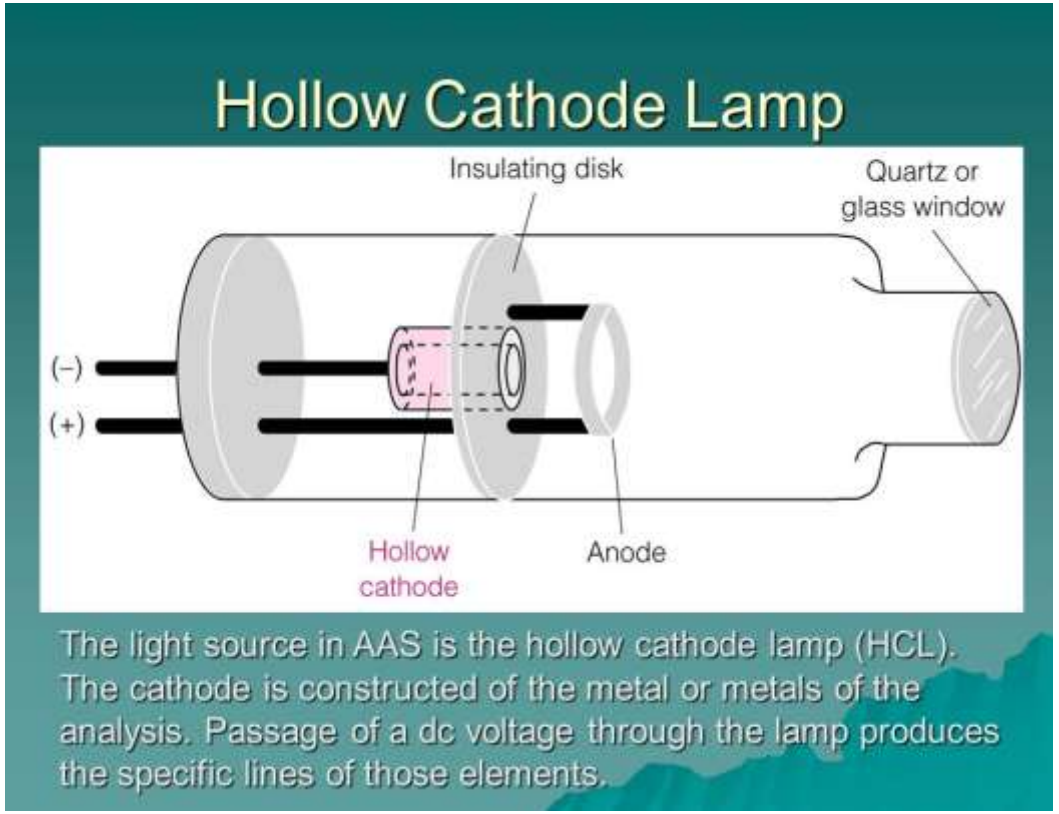
4. The **Beer Lambert law** describes the relationship between light absorption and concentration of the element.

5. According to law, **amount of light absorbed is proportional to the number of atoms excited from the ground state in the flame.**



1. LIGHT SOURCE

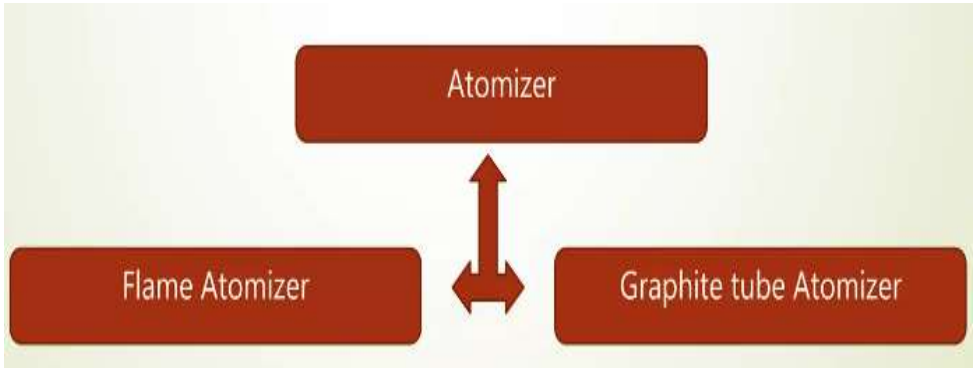
- The light source used in AAS is a **hollow cathode lamp**.
- This type of lamp contains a hollow **cathode** made of the element being analysed, and an **anode** electrode.
- Both these sealed in a hollow tube filled with a noble gas.
- Gaseous ions bombard the cathode, which ejects metal ions. The cathode concentrates most of these emitted ions into a beam that passes through a quartz window.
- Usually, atomic absorption spectrometers will have several different lamps for different elements.



2. ATOMIZER

There are two ways to atomise the sample:

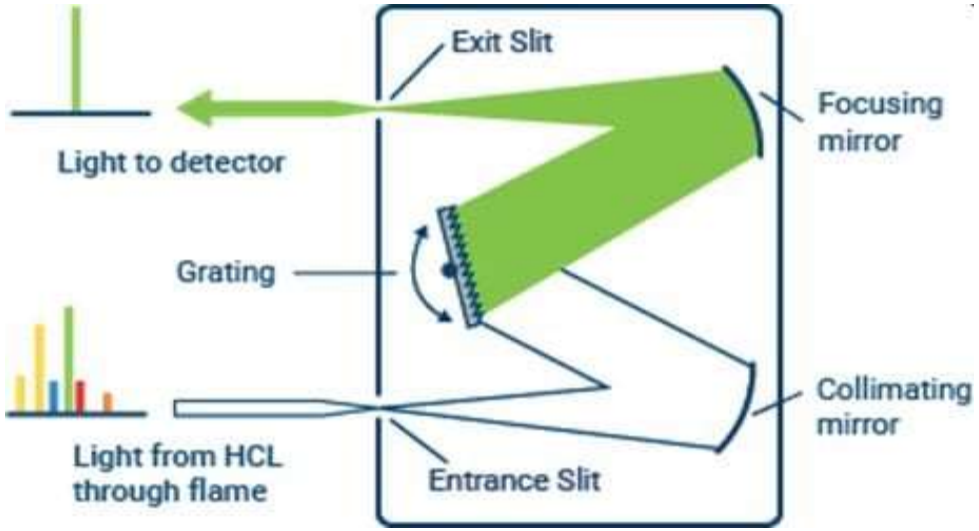
- **Flame aspiration** involves sucking a solution into a flame, where most of it is vapourised.
- **Electrothermal atomisation** where the sample is placed in a hollow tube, and, once heated, is vapourised completely.
- In most cases, vapourisation will have converted the sample into free atoms by providing a high temperature., regardless of its original chemical makeup.
- After vapourisation, the sample is ready for preparation for measurement.



3. MONOCHROMATORS

- Monochromators are used to **select the specific wavelength** which is absorbed by the sample and **exclude all other wavelengths**.
- The most common types of monochromators are,

1. Prisms
2. Gratings



4. DETECTORS

- Detectors are used to convert electromagnetic waves into electronic currents.
- For example, films and photomultiplier tubes are most commonly used as detectors.

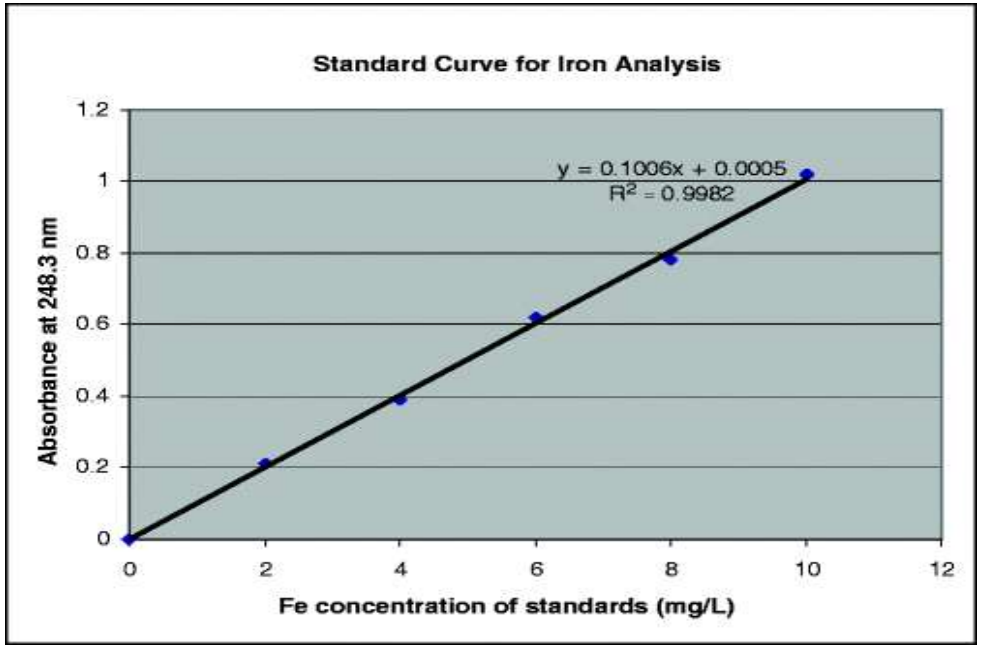
5. AMPLIFIER

- Electronic current from detectors is fed into an amplifier which amplifies the signal several times.

6. CALIBRATION OF AAS

As “AAS” is not an absolute method for quantification.

- A **calibration curve** is used for the quantification of analytes.
- Calibration curve provides comparison of signals of the **standard of known concentration and signals of unknown concentration.**
- Constructs a linear calibration curve of absorbance vs concentration of an analyte.



ADVANTAGES

As an analytical technique, AAS has several clear benefits:

- It is accurate, typically producing results within a 0.5 per cent to 5 per cent range
- As a method of detection, it is incredibly sensitive, measuring at parts per million (ppm)
- It can analyse specific elements because of the unique light-absorbent qualities of their atoms
- AAS can determine concentrations of over 65 elements
- It is a relatively simple process, drawing on well-documented protocols
- AAS allows for a high throughput of samples
- It is inexpensive in comparison to other analytical techniques.
- AAS supports a broad range of industries and sectors: environmental, chemical, petrochemical, food, drink and pharmaceutical.

LIMITATIONS

- It requires individual flame for every element
- It is used only for liquid samples.
- It can analyze only one atom at one time.
- It is a time-consuming technique.

APPLICATIONS

AAS has different laboratory and testing applications in industrial, clinical and research settings.

These processes include:

1. Quality control
2. Toxicology
3. Environmental testing

• Use in **environmental testing**, it can measure the concentration of various elements/metals/metalloids in rivers, drinking water and seawater.

• Use in the **food and drink industry**, it can measure various concentrations of elements in wine, beer and fruit drinks and test for types of contamination in food.

• For **pharmaceutical** companies, AAS can determine the minute quantities of catalyst materials used in the manufacture of drugs, and for other impurities.

• Use in **mining**, AAS can test for the concentration of valuable materials before excavation operations.

• AAS is used in **agriculture**, to analyse plants and soils for mineral content.

• Use in **petrochemical industry** to detect metals and other substances that could have a bad effect on oil and gas.

THANK YOU