

**PUNE DISTRICT EDUCATION ASSOCIATION'S**

**SETH GOVIND RAGHUNATH SABLE COLLEGE OF PHARMACY, SASWAD**

**DIVYANG CELL 2026-27**

A Divyang cell in a college aims to guarantee accessibility and equal opportunities for students with disabilities. Additionally, it promotes an atmosphere that is favourable to their general growth and motivates them to engage in various kinds of extracurricular and academic activities actively. The committee shall function from the Academic year 2026-27.

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**PRINCIPAL**  
PUNE DISTRICT EDUCATION ASSOCIATION'S  
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## 1. Cell Culture

### 1.1 Animal tissue culture / Cell culture

**Definition:** Cell culture is the technique of growing and maintaining animal cells under controlled, artificial conditions outside the body.

**Basic requirements:**

- Suitable culture medium
- Appropriate temperature, generally **37°C for mammalian cells**
- Controlled CO<sub>2</sub>, commonly **5% CO<sub>2</sub>**
- Sterile/aseptic conditions
- Appropriate culture vessel

**Interview answer:**

“Cell culture is an in-vitro technique used to maintain and grow animal cells under controlled environmental and nutritional conditions. It is widely used for drug screening, toxicity studies, cancer research, molecular biology and vaccine research.”

### 1.2 Aseptic culture handling

Aseptic technique prevents contamination of the culture by:

- Bacteria
- Fungi
- Mycoplasma
- Cross-contamination from other cell lines

**Important practices:**

- Work inside a biological safety cabinet.
- Disinfect the working surface before and after work.
- Use sterile media, pipettes and consumables.
- Minimize opening of culture vessels.
- Avoid unnecessary movement and talking over open cultures.

**Interview question:** Why is aseptic technique important?

“Because even a small microbial contamination can alter cell growth, metabolism and experimental results and may also contaminate other cultures.”

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### 1.3 Determination of cell count

Cell counting determines the number of cells present in a suspension.

A common method is **hemocytometer counting**.

**Formula:**

$$\text{Cells/mL} = \text{Average cells per square} \times \text{dilution factor} \times 10^4$$

**Interview point:** A hemocytometer is a specialized counting chamber with a known volume.

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### 1.4 Cell staining

Cell staining uses dyes to visualize cells or distinguish viable and non-viable cells.

Examples:

- Trypan blue – viability
  - Crystal violet – cell staining/biomass
  - DAPI – nuclear staining
  - Hematoxylin/eosin – tissue studies
- 

### 1.5 Cell viability assay

A viability assay determines the proportion of living cells in a population.

Common assays:

- Trypan blue exclusion
- MTT
- XTT
- Resazurin/Alamar Blue
- Live/dead fluorescence assays

**MTT principle:** Viable cells metabolically reduce MTT to purple formazan crystals. The intensity is related to metabolic activity/cell viability.

**Interview answer:**

“Cell viability assays are used to determine the effect of a treatment, such as a drug or nanoparticle, on cell survival.”

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## 1.6 Cell apoptosis assay

Apoptosis is **programmed cell death**.

Common methods:

- Annexin V/PI staining
- Caspase-3 assay
- TUNEL assay
- DNA fragmentation assay

**Annexin V principle:** During early apoptosis, phosphatidylserine becomes exposed on the outer surface of the cell membrane and binds Annexin V.

**PI** generally enters cells with compromised membranes and identifies late apoptotic/necrotic cells.

**Interview question:** Difference between apoptosis and necrosis?

| <b>Apoptosis</b>                        | <b>Necrosis</b>                    |
|-----------------------------------------|------------------------------------|
| Programmed cell death                   | Usually uncontrolled cell death    |
| Cell shrinkage                          | Cell swelling                      |
| Membrane generally maintained initially | Membrane damage                    |
| Minimal inflammation                    | Often associated with inflammation |

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## 1.7 Cell line revival and maintenance

You mentioned **SiHa, PA-1 and HEK**.

- **SiHa:** Human cervical cancer cell line, commonly used in HPV/cervical cancer research.
- **PA-1:** Human ovarian teratocarcinoma cell line.
- **HEK:** Human embryonic kidney-derived cells; HEK-293 is a commonly used line.

**Cell revival:** Recovery of cryopreserved cells from frozen storage and establishment of viable cultures.

**Basic sequence:**

Cryopreserved vial → thawing → transfer to suitable medium → centrifugation/washing where appropriate → culture → monitoring → subculture.

**Maintenance involves:**

- Monitoring morphology
  - Maintaining appropriate confluency
  - Regular medium replacement
  - Passaging
  - Checking contamination
  - Maintaining proper records
- 

### 1.8 Trypan blue dye exclusion

This is a simple method for determining cell viability.

**Principle:**

- Viable cells have an intact cell membrane and **exclude Trypan blue**.
- Non-viable cells have damaged membranes and **take up the blue dye**.

**Formula:**

$$\text{Viability (\%)} = \frac{\text{Number of viable cells}}{\text{Total number of cells}} \times 100$$

**Interview question:** Why are dead cells blue?

“Because membrane integrity is lost in dead cells, allowing Trypan blue to enter the cell.”

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## 2. Molecular Biology

### 2.1 DNA isolation from human blood and cell lines

**Purpose:** To extract genomic DNA for PCR, sequencing, genotyping and other molecular studies.

**General principle:**

Cell lysis → removal of proteins/contaminants → DNA purification → DNA elution.

Important components can include:

- Lysis buffer

- Detergent
- Proteinase K
- RNase
- Binding/purification system
- Elution buffer

**Interview question:** Why is DNA isolation performed?

“To obtain purified genomic DNA suitable for downstream applications such as PCR, restriction digestion, sequencing and molecular analysis.”

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## 2.2 Plasmid isolation from bacteria

Plasmids are **small, usually circular, extrachromosomal DNA molecules** found in many bacteria.

A common technique is **alkaline lysis**.

**Basic principle:**

1. Resuspend bacterial pellet.
2. Lyse cells using alkaline detergent.
3. Neutralize the lysate.
4. Chromosomal DNA and cellular debris precipitate.
5. Plasmid DNA remains in the soluble fraction.
6. Purify and elute plasmid DNA.

**Interview question:** Why does chromosomal DNA separate from plasmid DNA?

“Alkaline lysis followed by neutralization causes large, denatured chromosomal DNA and cellular debris to precipitate, while the smaller supercoiled plasmid DNA can renature and remain in solution.”

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## 3. Agarose Gel Electrophoresis

**Purpose:** Qualitative analysis and separation of DNA fragments according to size.

**Principle:**

DNA is negatively charged because of its phosphate backbone. When an electric field is applied, DNA migrates toward the **positive electrode**.

Smaller DNA fragments generally migrate faster through the agarose matrix than larger fragments.

**Important components:**

- Agarose gel
- DNA sample
- Loading dye
- Electrophoresis buffer
- DNA ladder
- DNA staining system

**Interview question:** Why do we use a DNA ladder?

“A DNA ladder contains DNA fragments of known sizes and is used to estimate the size of unknown DNA fragments.”

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#### **4. Quantitative Estimation of DNA and Protein by GeneQuant**

**GeneQuant** is a spectrophotometric instrument used for nucleic-acid and protein measurements.

##### **DNA**

DNA absorbs UV light strongly at approximately **260 nm**.

A commonly used relationship for double-stranded DNA is:

$$1 OD_{260} \approx 50 \mu g/mL$$

The exact calculation depends on the sample type and measurement conditions.

##### **Purity**

The **A260/A280 ratio** is commonly used as an indicator of nucleic-acid purity.

For relatively pure DNA, approximately **1.8** is commonly expected.

For RNA, approximately **2.0** is commonly expected.

**Interview question:** Why do we measure A260/A280?

“It provides an indication of nucleic-acid purity and helps identify contamination by proteins or other absorbing substances.”

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#### **5. Protein Extraction**

Protein extraction involves breaking cells/tissues and releasing proteins into an appropriate buffer.

**General principle:**

Cell/tissue disruption → lysis → centrifugation → collection of protein-containing fraction.

**Important considerations:**

- Appropriate lysis buffer
- Protease inhibitors when required
- Keeping samples cold
- Avoiding repeated freeze-thaw cycles
- Proper centrifugation

**Applications:**

- Western blotting
  - ELISA
  - Protein quantification
  - Enzyme assays
- 

## **6. RNA Isolation**

RNA isolation is performed to obtain purified RNA for applications such as:

- RT-PCR
- qPCR
- RNA sequencing
- Gene-expression studies

**Basic principle:**

Cell lysis → disruption of RNases → separation of RNA → purification → elution.

**Important precaution:**

RNA is highly susceptible to **RNase degradation**.

Therefore:

- Use RNase-free materials.

- Wear gloves.
- Keep samples appropriately cold.
- Avoid contamination.

**Interview question:** Why is RNA more difficult to handle than DNA?

“RNA is more susceptible to degradation because RNases are widespread and can remain active under many laboratory conditions.”

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## 7. Western Blotting

Western blotting is used to **detect and analyze a specific protein** in a complex protein mixture.

**Major steps:**

**Protein extraction → protein quantification → SDS-PAGE → transfer to membrane → blocking → primary antibody → secondary antibody → detection**

**Principle:**

Proteins are first separated according to molecular size by SDS-PAGE. They are transferred onto a membrane and detected using specific antibodies.

**Primary antibody:** Recognizes the target protein.

**Secondary antibody:** Recognizes the primary antibody and carries a detectable label.

**Interview question:** Why do we perform blocking?

“Blocking reduces nonspecific binding of antibodies to the membrane, thereby reducing background signal.”

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## 8. Microbiology

### 8.1 Streak Plate Method

**Purpose:** To isolate individual bacterial colonies from a mixed or concentrated bacterial culture.

**Principle:** Progressive dilution of bacteria across the agar surface results in individual colonies.

**Applications:**

- Isolation of pure cultures
- Obtaining single colonies
- Identification and maintenance of microorganisms

**Interview question:** What is a colony?

“A colony is a visible mass of microorganisms that generally develops from a single cell or a group of closely associated cells.”

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## 9. Pour Plate Method

In the pour plate method, a measured volume of sample is mixed with molten agar and allowed to solidify.

Microorganisms grow:

- On the surface
- Within the agar

**Applications:**

- Viable microbial counting
- Determination of CFU/mL

**CFU:** Colony-forming unit.

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## 10. Bacterial Colony Picking

A single isolated colony is selected from an agar plate and transferred into fresh medium.

**Purpose:**

- Establish a pure bacterial culture
- Prepare cultures for further experiments
- Preserve/expand selected isolates

**Important point:** Select a well-isolated colony to minimize the possibility of mixed cultures.

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## 11. Antimicrobial Activity – Zone of Inhibition

A zone of inhibition assay determines the antimicrobial activity of a test substance.

In the **agar diffusion method**, the test compound diffuses through agar containing microorganisms.

If the compound inhibits microbial growth, a clear zone develops around the sample.

**Important term:**

**Zone of inhibition (ZOI):** Clear area around the antimicrobial agent where microbial growth is inhibited.

The diameter is generally measured in **mm**.

**Interview question:** Does a larger zone always mean a stronger antibiotic?

“Not necessarily. Zone size also depends on diffusion characteristics, concentration, agar conditions, organism susceptibility and other experimental factors. Therefore, zone diameter should be interpreted using standardized conditions.”

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## **12. Hands-on Instruments**

### **12.1 Biosafety Cabinet – Type I**

A **Class/Type I biological safety cabinet** provides personnel and environmental protection against aerosols generated during microbiological procedures.

**Important distinction:** A Class I cabinet does **not** provide product protection comparable to a Class II cabinet because room air is drawn over the work surface.

**Interview question:** What is the purpose of a BSC?

“It provides containment and protects the operator and environment from potentially hazardous biological aerosols.”

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## **13. Simple Microscope**

Used for basic visualization of specimens using visible light and optical lenses.

**Applications:**

- Microorganism observation
  - Tissue/cell observation
  - Basic laboratory examination
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## **14. Inverted Microscope**

An inverted microscope has the **objective lenses below the specimen** and is particularly useful for observing cells growing in culture vessels.

**Why is it useful in cell culture?**

Because cells adhere to the bottom of flasks, plates or dishes, allowing observation without removing them from the culture vessel.

**Interview answer:**

“An inverted microscope is routinely used for cell culture because its objectives are positioned below the culture vessel, allowing direct observation of adherent cells.”

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## **15. Centrifuge**

A centrifuge separates components based on differences in **density** using centrifugal force.

Examples:

- Cell pellets
- DNA/RNA purification
- Protein extraction
- Blood component separation

**Important parameter:**

**RCF (×g)** describes relative centrifugal force.

Do not confuse **RPM** and **RCF**; they are not equivalent because RCF also depends on rotor radius.

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## **16. Autoclave**

An autoclave sterilizes materials using **pressurized saturated steam**.

A commonly used laboratory cycle is approximately **121°C at about 15 psi for around 15–20 minutes**, but the actual cycle depends on load, volume and validated equipment conditions.

**Applications:**

- Glassware
- Culture media
- Certain laboratory waste
- Sterilizable instruments

**Interview question:** Principle of autoclaving?

“Moist heat under pressure denatures and coagulates microbial proteins, resulting in sterilization.”

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## **17. Bacteriological Incubator**

An incubator provides controlled environmental conditions for microbial growth.

Commonly controlled parameters:

- Temperature
- Time
- Sometimes humidity/atmospheric conditions depending on application

**Application:** Culturing bacteria and other microorganisms under specified conditions.

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## 18. Soxhlet Apparatus

Soxhlet extraction is a **continuous solvent extraction technique** used mainly for extracting compounds from solid materials.

**Principle:**

Solvent is repeatedly evaporated, condensed and passed through the sample, allowing continuous extraction.

**Applications:**

- Extraction of plant constituents
- Lipids
- Bioactive compounds
- Natural products

**Interview question:** Why is Soxhlet useful?

“It allows repeated contact of the sample with fresh hot solvent, providing efficient extraction without manually replacing the solvent after every cycle.”

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## 19. PCR

**PCR = Polymerase Chain Reaction**

PCR is used to **amplify a specific DNA sequence exponentially**.

**Three fundamental steps:**

### 1. Denaturation

- Double-stranded DNA separates.

### 2. Annealing

- Primers bind to complementary target sequences.

### 3. Extension

- DNA polymerase synthesizes new DNA.

These steps are repeated for multiple cycles.

#### Requirements:

- Template DNA
- Forward and reverse primers
- DNA polymerase
- dNTPs
- $Mg^{2+}$
- Appropriate buffer

**Interview question:** What is the role of primers?

“Primers provide specific starting points for DNA synthesis and determine the region that will be amplified.”

#### PCR applications:

- Gene detection
- Mutation analysis
- Pathogen detection
- Cloning
- Molecular diagnostics

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## 20. GeneQuant

GeneQuant is a **UV-visible spectrophotometric instrument** commonly used for nucleic-acid and protein quantification.

It can provide measurements such as:

- Absorbance
- DNA/RNA concentration
- Purity ratios

**Key wavelengths:**

- Nucleic acids: ~260 nm
  - Protein: commonly ~280 nm for aromatic amino-acid absorbance, depending on the assay/sample
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**21. ELISA Plate Reader**

A microplate/ELISA reader measures the optical signal generated in assays performed in microplate wells.

Depending on the assay, it may measure:

- Absorbance
- Fluorescence
- Luminescence

**ELISA principle**

**Antigen–antibody interaction → enzyme-linked detection → substrate reaction → measurable signal**

The signal can be related to the amount of target analyte, depending on whether the assay is direct, indirect, sandwich or competitive.

**Applications:**

- Hormone detection
  - Cytokine measurement
  - Antibody detection
  - Protein quantification
  - Diagnostic testing
- 

**22. Gel Electrophoresis Unit**

A gel electrophoresis unit is used to separate charged biomolecules through a gel under an electric field.

For **DNA agarose gel electrophoresis**:

- DNA is negatively charged.

- DNA migrates toward the positive electrode.
- Smaller fragments generally migrate faster.

For proteins, **SDS-PAGE** separates proteins primarily according to molecular size.

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## Most Important Interview Questions to Prepare

If this is for a **biotech/pharma research interview**, I would especially prepare these:

### Cell culture

1. What is cell culture?
2. What is aseptic technique?
3. Why is CO<sub>2</sub> used in mammalian cell culture?
4. What is confluency?
5. What is cell passaging?
6. What is Trypan blue?
7. How do you calculate cell viability?
8. Difference between apoptosis and necrosis?
9. What are SiHa, PA-1 and HEK cells?
10. Why do you use an inverted microscope?

### Molecular biology

11. Explain DNA isolation.
12. Explain plasmid isolation by alkaline lysis.
13. What is agarose gel electrophoresis?
14. Why does DNA migrate toward the positive electrode?
15. What is the purpose of a DNA ladder?
16. What is A260/A280?
17. Why is RNA more susceptible to degradation?
18. Explain Western blotting.
19. What is the role of primary and secondary antibodies?

20. What is PCR and what are its three steps?

### **Microbiology**

21. Explain streak plate technique.

22. Difference between streak plate and pour plate.

23. What is CFU?

24. What is zone of inhibition?

25. What factors affect zone of inhibition?

### **Instruments**

26. Difference between Class I and Class II biosafety cabinets.

27. Principle of centrifugation.

28. Difference between RPM and RCF.

29. Principle of autoclaving.

30. Principle and application of Soxhlet extraction.

31. Principle of ELISA.

32. What does an ELISA plate reader measure?

33. Principle of GeneQuant.

34. Difference between simple and inverted microscope.

35. Applications of gel electrophoresis.

### **A very useful interview strategy**

For **every technique you mention on your CV**, remember this 5-point format:

**Definition → Principle → Basic steps → Application → Precautions**

For example, if asked **“Explain PCR”**, don't only say *“PCR amplifies DNA.”* Say:

**“PCR is an in-vitro technique used to amplify a specific DNA sequence. It is based on repeated cycles of denaturation, primer annealing and extension by a thermostable DNA polymerase. The reaction requires template DNA, primers, dNTPs, polymerase, Mg<sup>2+</sup> and buffer. PCR is used in gene detection, molecular diagnostics, cloning and research. Proper controls and prevention of contamination are important for reliable results.”**

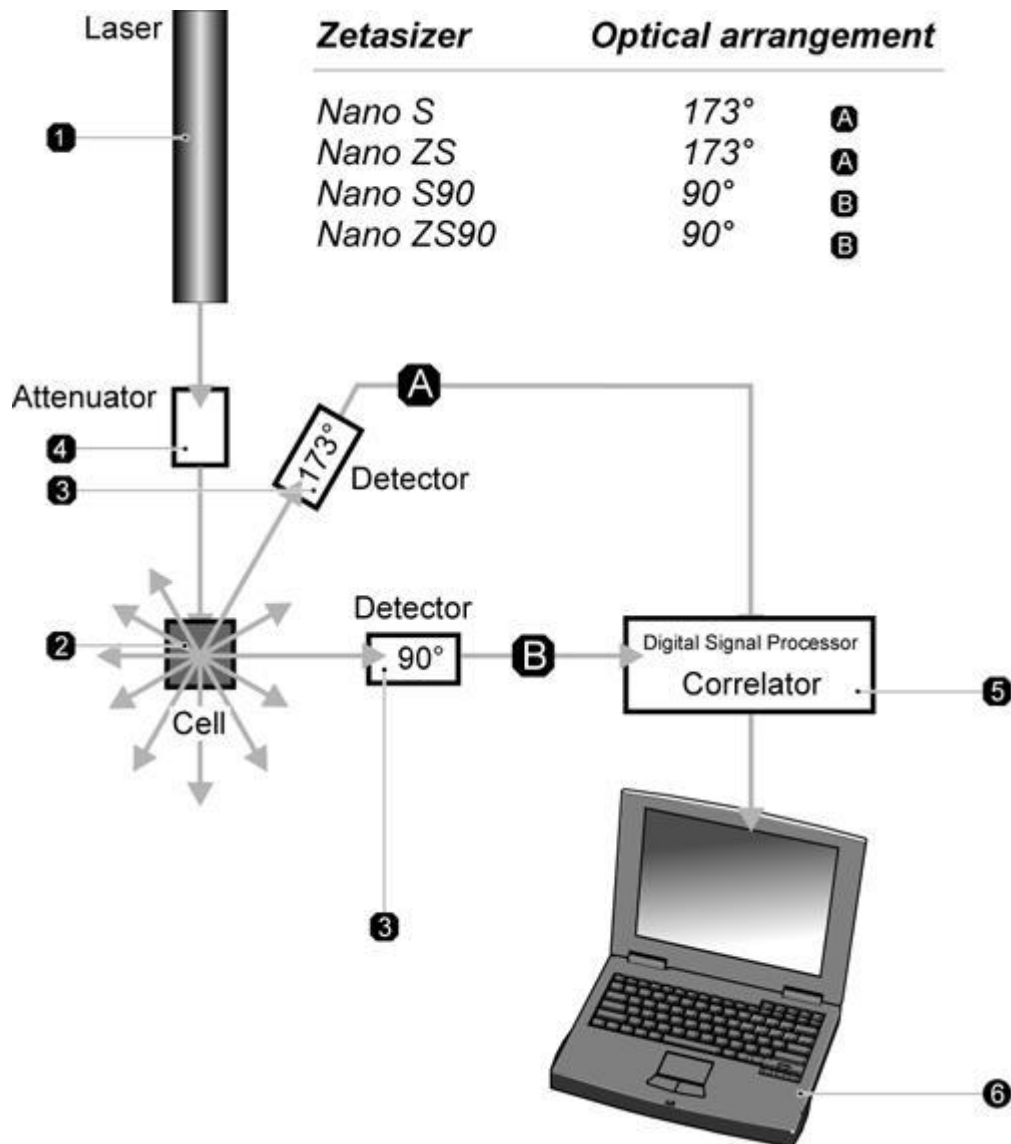
That style will make your answers sound much more **interview-ready and technically confident**.

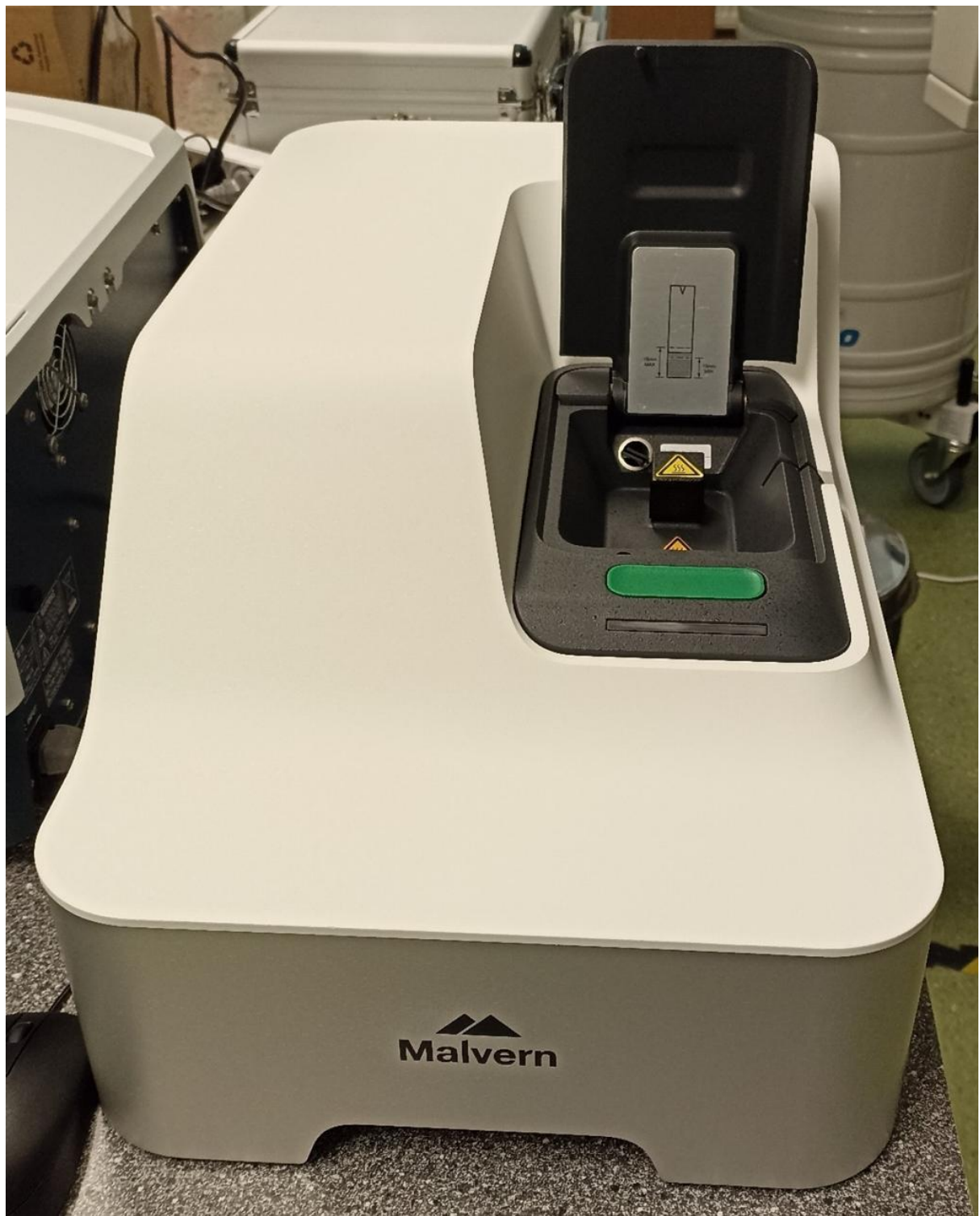
Today 10:35 PM

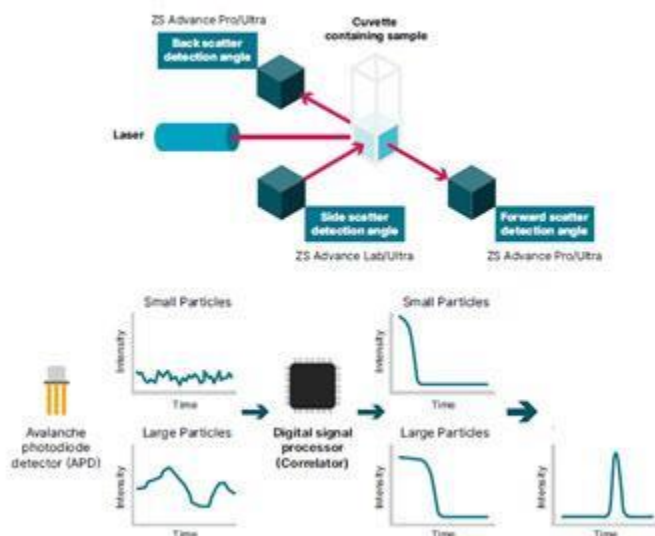
give parts of device for particle size and stability determination of nanoparticles

If you mean the **Dynamic Light Scattering (DLS) instrument / Zetasizer** used for **nanoparticle particle-size and stability determination**, these are the important parts to know for an interview.

#### Parts of a DLS/Zetasizer







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| Part                                    | Function                                                                                            |
|-----------------------------------------|-----------------------------------------------------------------------------------------------------|
| 1. Laser source                         | Produces a laser beam that illuminates the nanoparticle sample.                                     |
| 2. Sample cell/cuvette                  | Holds the nanoparticle dispersion for measurement.                                                  |
| 3. Temperature control system           | Maintains the sample at a defined temperature during analysis.                                      |
| 4. Detector                             | Detects the intensity of light scattered by nanoparticles.                                          |
| 5. Correlator                           | Analyzes fluctuations in scattered-light intensity and calculates particle size-related parameters. |
| 6. Optical system/lenses                | Focuses and directs the laser and scattered light.                                                  |
| 7. Beam splitter/mirrors                | Directs the laser and scattered light through the optical pathway.                                  |
| 8. Measurement chamber                  | Holds and positions the cuvette correctly during measurement.                                       |
| 9. Autocorrelation electronics/software | Converts scattering fluctuations into hydrodynamic diameter and PDI.                                |
| 10. Computer/software interface         | Controls the instrument and displays particle size, PDI, intensity distribution and other results.  |

### Principle of particle-size measurement

DLS measures **Brownian motion** of nanoparticles by analyzing fluctuations in scattered laser light.

The instrument determines the **translational diffusion coefficient**, which is converted into **hydrodynamic diameter** using the Stokes–Einstein relationship:

$$D = \frac{k_B T}{3\pi\eta d_H}$$

where:

- **D** = diffusion coefficient
- **k<sub>B</sub>** = Boltzmann constant
- **T** = absolute temperature
- **η** = viscosity
- **d<sub>H</sub>** = hydrodynamic diameter

### Important parameters obtained

#### 1. Z-average particle size

- Average hydrodynamic diameter of the particles.

#### 2. PDI (Polydispersity Index)

- Indicates the width/heterogeneity of the particle-size distribution.
- Lower PDI generally indicates a more homogeneous dispersion.

#### 3. Intensity/volume/number distribution

- Different ways of representing the particle-size distribution.

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### For nanoparticle stability: Zeta Potential

Many Zetasizer instruments can also measure **zeta potential**, which is particularly useful for evaluating colloidal stability.

### Additional parts/functions involved

| Part                   | Function                                                          |
|------------------------|-------------------------------------------------------------------|
| Electrode cell/cuvette | Holds the nanoparticle dispersion for zeta-potential measurement. |
| Electrodes             | Apply an electric field to the sample.                            |

| Part                               | Function                                                       |
|------------------------------------|----------------------------------------------------------------|
| Laser                              | Detects movement/scattering of particles.                      |
| Detector                           | Detects the resulting scattered light.                         |
| Electrophoresis measurement system | Determines particle mobility under the applied electric field. |
| Software                           | Converts electrophoretic mobility into zeta potential.         |

### Principle of zeta-potential measurement

When an electric field is applied, charged nanoparticles move toward the oppositely charged electrode. The instrument measures their **electrophoretic mobility**, which is used to calculate zeta potential.

### Interview answer:

“Zeta potential is the electrical potential at the slipping plane of a particle in dispersion. It is commonly used as an indicator of electrostatic stability. A higher magnitude of zeta potential generally indicates stronger electrostatic repulsion and can reduce particle aggregation, although stability also depends on steric effects, ionic strength, polymer coating and other formulation factors.”

### Key interview distinction

**DLS → Particle size + PDI**

**Electrophoretic light scattering → Zeta potential**

**Particle size tells you:** *How large are my nanoparticles?*

**PDI tells you:** *How uniform is the particle-size distribution?*

**Zeta potential tells you:** *What is the surface electrokinetic potential and what does it suggest about colloidal stability?*

For a **nanomicelle/nanoparticle formulation interview**, you should especially know **DLS, Z-average size, PDI, zeta potential, hydrodynamic diameter, Brownian motion and Stokes–Einstein equation**.