iii. Characterizations of nanoparticles, various microscopes...

iii. Characterizations of nanoparticles – transmission electron microscope (TEM), scanning electron microscope (SEM), fluroscence microscopy, atomic force microscope (AFM), Energy-dispersive X-ray spectroscopy (EDX), UV – visible absorption; photoluminescence; Fourier-transform infrared spectroscopy (FTIR), Atomic absorption spectroscopy (AAS) and dynamic light scattering spectroscopy (DLS)

Transmission Electron Microscope (TEM)

Principle and Instrumentation

1. **Electron Beam Transmission**
    - In **TEM**, a high-energy electron beam (typically 80–300 keV) passes *through* an ultrathin specimen (<100 nm thickness).
    - Interactions between the beam and the sample generate **contrast** (phase contrast, mass–thickness contrast), revealing internal structure at very high resolutions (<0.1 nm<0.1~\mathrm{nm}<0.1 nm under advanced instruments).
2. **Key Components**
    - **Electron Gun**: Often tungsten filament or field emission gun (FEG).
    - **Electromagnetic Lenses**: Condenser lenses focus the electron beam; objective lens forms the primary image.
    - **Projector Lenses and Screen/Detector**: Magnify and record the final image on a fluorescent screen or CCD/CMOS camera.
3. **Sample Preparation**
    - For powders (e.g., nanoparticles), typically dispersed in a solvent, then deposited on a **carbon-coated copper grid**.
    - Biological or sensitive samples may require cryogenic preparation (cryo-TEM) to preserve native structures.

Capabilities for Nanoparticle Characterization

1. **Size and Morphology**
    - Visualize individual particles and measure diameters, aspect ratios with near-atomic resolution.
    - Identify shape variations (spheres, rods, cubes, star-shaped, etc.).
2. **Lattice Resolved Imaging (HRTEM)**
    - **High-Resolution TEM (HRTEM)** reveals lattice fringes, crystallographic planes, defects, or grain boundaries.
    - Critical for analyzing crystalline materials (metals, metal oxides, semiconductors).
3. **Electron Diffraction and SAED**
    - **Selected Area Electron Diffraction (SAED)** patterns provide crystallographic information, confirming phase and identifying polycrystalline or single-crystal domains.
4. **Energy-Dispersive X-Ray Spectroscopy (EDS)**
    - Often integrated with TEM (STEM-EDS), enabling elemental composition mapping at the nanoscale.
    - Useful to confirm doping levels, alloy compositions, or core–shell distributions.

Scanning Electron Microscope (SEM)

Principle and Instrumentation

1. **Electron Beam Scanning**
    - **SEM** employs a focused electron beam (commonly 1–30 keV) that scans the sample's surface in a raster pattern.
    - Secondary electrons (SE) and backscattered electrons (BSE) ejected from the surface create topographical and compositional contrast.
2. **Key Components**
    - **Electron Column**: Includes electron gun, condenser lenses, objective lens to focus the beam onto the

sample surface.
- **Detectors**:
  - **SE Detector**: Captures low-energy secondary electrons from top surface layers, revealing fine surface details.
  - **BSE Detector**: More sensitive to atomic number contrast (Z-contrast).
- **Vacuum Chamber**: Minimizes electron scattering by air molecules.

3. **Sample Preparation**
   - **Nonconductive samples** (e.g., polymeric nanoparticles, biological specimens) often require conductive coating (Au/Pd sputtering).
   - For many inorganic nanoparticles (metals, metal oxides), minimal preparation is needed, though ensuring good adhesion to sample stubs is important.

Capabilities for Nanoparticle Characterization

1. **Surface Topography and Morphology**
   - SEM excels at imaging surface features (aggregates, interparticle spacing).
   - Typical resolution in high-end FE-SEM can approach ~1 nm (depending on instrument and sample).
2. **Particle Size Distribution**
   - By examining multiple fields of view, one can estimate size distributions, though resolution is lower than TEM for very small (<5 nm) particles.
3. **Elemental Analysis (EDS)**
   - As in TEM, **SEM-EDS** can map elemental composition across the sample's surface, distinguishing different nanoparticle types or confirming doping.
4. **3D-Like Imaging**
   - SEM images provide a pseudo-3D view of sample topography due to shadowing effects of secondary electron emission.

Fluorescence Microscopy

Principle and Techniques

1. **Fluorophore Excitation and Emission**
   - **Fluorescence microscopy** uses light of a specific wavelength to excite fluorophores (intrinsic or extrinsic), which emit at longer wavelengths.
   - Nanoparticles can be intrinsically fluorescent (e.g., quantum dots) or labeled with dyes, facilitating visualization under an optical microscope.
2. **Instrumentation**
   - **Epifluorescence, Confocal, or Super-Resolution** microscopes commonly used.
   - **Confocal** systems employ a pinhole to eliminate out-of-focus light, yielding high-contrast optical sections.
3. **Quantum Dots**
   - Semiconductor nanocrystals (e.g., CdSe/ZnS) have size-dependent emission, high brightness, and photostability, well-suited for biological imaging.

Advantages for Nanoparticle Characterization

1. **Tracking and Localization in Biological Systems**
   - Fluorescently labeled nanoparticles enable real-time observation of cellular uptake, intracellular trafficking, and biodistribution.
   - Multi-color labeling reveals co-localization with organelles or other biomolecules.
2. **Structural Insights** at Subcellular Scales
   - Super-resolution methods (STORM, PALM) can push resolution below the diffraction limit (~20–50 nm), approaching the nanoscale domain.
3. **Limitation**: Optical diffraction limit typically ~200–300 nm for standard epifluorescence, insufficient for direct morphological analysis of single particles <20 nm (hence complementary to TEM/SEM).

Atomic Force Microscope (AFM)

Principle and Operation

1. **Atomic-Scale Interactions**
    - **AFM** uses a sharp tip (probe) attached to a cantilever to scan the sample surface.
    - Tip–sample interactions (van der Waals, electrostatic, etc.) cause the cantilever to deflect, measured by a laser–photodiode system.
2. **Imaging Modes**
    - **Contact Mode**: Tip remains in constant contact with the sample, and can damage soft samples.
    - **Tapping/Intermittent Mode**: The cantilever oscillates near its resonant frequency, gently touching the surface. Often preferred for delicate nano samples.
    - **Non-contact Mode**: Tip hovers above the surface at a short distance, reducing friction forces.

Advantages for Nanoparticle Characterization

1. **3D Surface Profiling**
    - Provides true topographical maps of individual nanoparticles or films with **vertical (z) resolution** down to sub-nanometer.
    - Useful for measuring surface roughness, thickness of nano-layers, or individual particle heights.
2. **Force Measurements**
    - **Force–Distance Curves**: Evaluate **adhesion**, **elastic modulus**, **mechanical properties** of single nanoparticles or thin films.
    - Enables study of interparticle interactions, doping effects on mechanical stability.
3. **Ambient or Liquid Environments**
    - AFM can operate in ambient, vacuum, or fluid cells, suitable for studying biological samples or colloidal dispersions in situ.
4. **Limitations**
    - Slower scanning speed than SEM; relatively smaller field of view.
    - Tip geometry can convolute measured size (tip–sample broadening effect).

Summary and Complementarity of Techniques

1. **TEM**: High-resolution internal structure and crystallography; ideal for sub-10 nm characterization.
2. **SEM**: Surface morphology and near-surface composition; simpler sample prep for many solids, 3D-like topographical views.
3. **Fluorescence Microscopy**: Optical imaging of labeled nanoparticles in biological contexts, real-time localization, and trafficking.
4. **AFM**: Nanoscale topography in 3D, surface forces, mechanical properties under ambient or liquid conditions.

Each approach addresses different aspects of **nanoparticle** structure, composition, and function. In research and industrial applications, **correlative microscopy**—combining two or more methods—often yields the most comprehensive picture, ensuring robust **size measurements**, **morphological details**, **chemical composition**, and **biological interactions** are accurately captured.

Concluding Remarks

Characterizing **nanoparticles** is a multidisciplinary endeavor, relying on **electron microscopy** (TEM, SEM) for direct morphological and compositional insights, **fluorescence microscopy** for functional/biological imaging, and **AFM** for high-resolution surface profiling and force measurement. A synergistic use of these techniques is critical to advance **nanotechnology** research—whether in designing next-generation drug delivery systems, constructing functional nanomaterials, or optimizing nano-enabled electronics and sensors.

Energy-Dispersive X-Ray Spectroscopy (EDS/EDX)

Principle and Instrumentation

1. **Electron-Sample Interaction**
    - Usually integrated with an electron microscope (SEM or TEM). An **electron beam** bombards the sample, ejecting inner-shell electrons from sample atoms.
    - **Characteristic X-Rays**: As electrons from higher shells fill these vacancies, element-specific X-ray photons are emitted.
2. **Detector and Signal Processing**
    - A **Si(Li)** or **silicon drift detector (SDD)** captures X-ray photons, measuring energy via generating electron–hole pairs.
    - The energy of these photons corresponds to specific electronic transitions, yielding characteristic peaks in the EDX spectrum.

Applications to Nanomaterials

1. **Elemental Composition**
    - Identifies and quantifies (semi-quantitative to quantitative) the elemental constituents of nanoparticles, thin films, or complex composites.
    - **Spatial Mapping**: When coupled with scanning electron microscopy (SEM-EDS mapping), reveals distribution of elements across a sample's surface or in specific regions.
2. **Advantages**
    - Rapid, direct elemental analysis, minimal additional sample prep when already using SEM/TEM.
    - Useful for verifying doping levels, detecting impurities, or analyzing core–shell structures.
3. **Limitations**
    - Detection limits typically ~0.1–1%\sim0.1–1\%~0.1–1% by weight, less sensitive for lighter elements (e.g., H, He, Li).
    - Spatial resolution depends on beam penetration and interaction volume; for SEM, ~1–2 µm typical (somewhat higher resolution in STEM mode).

UV–Visible (UV–Vis) Absorption Spectroscopy

Principle

1. **Electronic Transitions**
    - Molecules or nanomaterials absorb photons in the **UV–visible range** (200–800 nm) corresponding to **electronic excitations** (e.g., $\pi \to \pi^*$, $n \to \pi^*$ transitions in organic molecules, or band-to-band transitions in semiconductors).
    - **Metal Nanoparticles** (e.g., Au, Ag) exhibit **surface plasmon resonance (SPR)** absorption bands, highly dependent on particle size, shape, and dielectric environment.
2. **Setup**
    - **Light Source**: Typically a deuterium lamp (UV region) and tungsten/halogen lamp (visible region).
    - **Monochromator** selects specific wavelengths, passing through a sample in solution.
    - **Detector** measures intensity (I) relative to reference (I_0). Absorbance (A) = $\log(I_0/I)$.

Applications to Nanomaterials

1. **SPR Peak Analysis**
    - **Gold Nanoparticles**: Peak around 520–540 nm (depending on size). Shifts in SPR can indicate changes in particle size, aggregation state, or surface modifications.
2. **Band Gap Estimation**
    - For semiconductor nanoparticles (TiO_2, ZnO, CdS), Tauc plot or direct measurement of absorption edge to estimate band gap.
3. **Concentration Determination**
    - Beer–Lambert law can quantify nanoparticle or dye concentrations if molar extinction coefficient is known.

4. **Advantages and Limitations**
    - Rapid, non-destructive, widely available.
    - Does not directly provide morphological details or composition (complementary to microscopy, EDS).

Photoluminescence (PL)

Principle

1. **Emission After Excitation**
    - A sample is excited by a photon of suitable energy, promoting electrons to higher electronic states. Upon relaxation, photons are re-emitted at characteristic longer wavelengths (lower energy).
    - In solids (quantum dots, semiconductors), electron–hole recombination yields distinct PL signatures.
2. **Instrumentation**
    - **Excitation Source**: Laser or xenon lamp at a specific wavelength.
    - **Monochromator & Detector**: Collect emitted photons vs. wavelength; can measure intensity and spectral distribution.

Applications to Nanomaterials

1. **Band Gap and Electronic Structure**
    - Semiconductor quantum dots exhibit size-dependent PL peaks. Smaller dots → higher energy emission.
    - PL lifetime measurements elucidate carrier dynamics, defect states.
2. **Quality and Defect Analysis**
    - Changes in PL intensity or peak positions can indicate **surface passivation** quality, doping effects, or trap states.
    - For organic fluorophores or polymeric nanoparticles, reveals quantum yield and photostability.
3. **Biosensing and Imaging**
    - Fluorescent nanoprobes (e.g., carbon dots, upconversion nanoparticles) used in bioimaging or assays, harnessing stable photoluminescence under biological conditions.

Fourier-Transform Infrared Spectroscopy (FTIR)

Principle

1. **Molecular Vibrations**
    - IR radiation (400–4000 cm^{-1}) is absorbed by molecular bonds undergoing vibrational transitions (stretching, bending).
    - In **FTIR**, an interferometer (Michelson) collects an **interferogram**; Fourier transform yields the absorption spectrum in wavenumbers (cm^{-1}).
2. **Sample Forms**
    - **Solid Samples**: Often pressed into KBr pellets or measured in ATR (Attenuated Total Reflectance) mode.
    - **Solutions** or **Thin Films** can also be analyzed with appropriate sample holders or ATR crystals.

Applications to Nanomaterials

1. **Functional Group Identification**
    - Confirms presence of **organic ligands**, **surfactants**, or **biomolecules** capping nanoparticles. Characteristic peaks (C–H, N–H, C=O, O–H) reveal interactions.
    - In polymeric nanoparticles, FTIR identifies backbone vibrations (C–C, C–O, etc.) and crosslinking states.
2. **Surface Chemistry**
    - Monitoring **bond formation** or shifts (e.g., carboxylate binding to metal surfaces, or amine–metal interactions).
    - Study of **oxidation state** changes or doping effects in inorganic materials.
3. **Advantages**
    - Rapid, non-destructive method for chemical fingerprinting.
    - ATR-FTIR is particularly convenient for direct surface analysis with minimal prep.

Atomic Absorption Spectroscopy (AAS)

Principle

1. **Element-Specific Absorption**
    - **AAS** measures the absorption of characteristic wavelengths by vaporized atoms in a flame or graphite furnace.
    - A hollow cathode lamp (of the target metal) emits light at the element's resonance lines. The extent of absorption in the flame correlates to element concentration.
2. **Modes**
    - **Flame AAS**: Sample solution nebulized into an acetylene/air or nitrous oxide flame.
    - **Graphite Furnace AAS**: Higher sensitivity, smaller sample volumes, used for trace metal detection.

Applications to Nanomaterials

1. **Quantification of Metal Content**
    - For metallic or metal-oxide nanoparticles, AAS can measure total metal concentration after digestion in acid.
    - Tracking dissolution or release from nanoparticles (e.g., Ag^+ release from AgNPs) for toxicity or environmental impact studies.
2. **Advantages and Limitations**
    - Highly sensitive, element-specific.
    - Does not provide structural or morphological data—complements TEM, SEM, etc.
    - Requires sample digestion or dissolving NPs in a suitable solvent.

Dynamic Light Scattering (DLS)

Principle

1. **Brownian Motion and Light Scattering**
    - **DLS** measures the intensity fluctuations of **laser light** scattered by colloidal particles undergoing Brownian motion in a liquid.
    - The **autocorrelation function** of these intensity fluctuations is analyzed to extract the diffusion coefficient, translating to a **hydrodynamic diameter** via the Stokes–Einstein equation.
2. **Instrumentation**
    - **Laser source** (e.g., He–Ne, ~633 nm).
    - **Photodetector** collects scattered light at a set angle (commonly 90°, but also backscatter angles used).
    - **Correlator** calculates the time-dependent autocorrelation, yielding size distribution data.

Applications to Nanomaterials

1. **Particle Size Distribution in Liquids**
    - Commonly used in quality control of nanoparticle colloids, protein aggregates, polymer latexes.
    - Provides **z-average** diameter and polydispersity index (PDI).
2. **Stability and Aggregation Studies**
    - Repeated DLS measurements can track changes in size upon varying pH, ionic strength, or temperature.
    - Aggregation yields larger hydrodynamic radii, shifting the distribution.
3. **Advantages and Limitations**
    - Rapid, non-destructive, minimal sample volume, particularly for submicron range (~1 nm to 1 µm).
    - Cannot distinguish shape or complex multimodal distributions if peaks overlap.
    - Sensitive to refractive index assumptions and presence of large aggregates.

Concluding Remarks

Each of these techniques—**EDX**, **UV-Vis**, **Photoluminescence**, **FTIR**, **AAS**, and **DLS**—targets different facets of a material's **composition**, **structure**, **surface chemistry**, **optical properties**, and **size distribution**:

1. **EDX** (coupled with electron microscopy) probes **elemental composition** at submicron or nanoscale.
2. **UV–Vis** absorption reveals **electronic transitions**, plasmonic or band gap features crucial for optical properties and concentration estimates.
3. **Photoluminescence** highlights **emission** traits, enabling insights into electronic states, defects, or doping in semiconductors.
4. **FTIR** unravels **functional groups** and **bonding** on nanoparticle surfaces (organic or inorganic).
5. **AAS** quantifies **metal content** with high specificity—ideal for validating doping levels or measuring metal release.
6. **DLS** measures **hydrodynamic size** and **stability** in colloidal suspensions, indispensable for biomedical or environmental nanosafety studies.

By combining these complementary methods, researchers and engineers can obtain a holistic characterization of nanomaterials—ensuring robust design, functionality, and performance in applications spanning **drug delivery**, **catalysis**, **optical sensing**, and beyond.