

iii. Characterizations of nanoparticles, various microscopes...

iii. Characterizations of nanoparticles - transmission electron microscope (TEM), scanning electron microscope (SEM), fluorescence 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 $\sim 0.1\text{--}1\%$ by weight, less sensitive for lighter elements (e.g., H, He, Li).
- Spatial resolution depends on beam penetration and interaction volume; for SEM, $\sim 1\text{--}2\text{ }\mu\text{m}$ 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., $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ 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 ($400\text{--}4000\text{ cm}^{-1}$) 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.