

Lecture 2:

Electromagnetic Spectrum and Biomedical Measurements

From Photons to Diagnostics

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Lecture Contents

Part 1: EM Spectrum Fundamentals

Part 2: Spectroscopy Methods

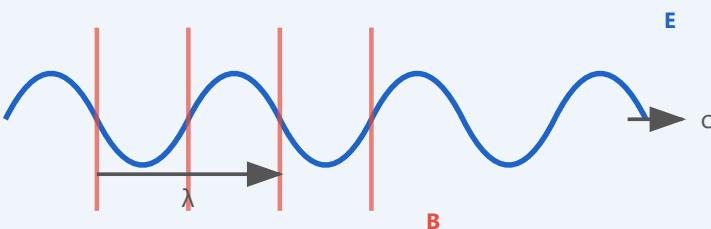
Part 3: Biological Applications

Part 1/3:

EM Spectrum Fundamentals

- 1.** Light as wave and particle
- 2.** Energy scales in biology
- 3.** Photon-matter interactions
- 4.** Absorption and emission principles
- 5.** Scattering phenomena
- 6.** Fluorescence foundations

Electromagnetic Wave Properties



Electric (E) and Magnetic (B) fields oscillate perpendicular to each other

$$E(x, t) = E_0 \cos(kx - \omega t + \varphi)$$

Wave equation describing electric field oscillation

$$k = 2\pi/\lambda \text{ (wave number)}$$

$$\omega = 2\pi\nu \text{ (angular frequency)}$$



Wavelength (λ)

Distance between wave crests

$$c = \lambda\nu$$



Frequency (ν)

Oscillations per second

Measured in Hertz (Hz)



Speed of Light (c)

3×10^8 m/s in vacuum

Reduced in media: c/n



Polarization

Direction of E-field oscillation

Linear, circular, elliptical



E and B Fields

Perpendicular oscillating fields

Energy transport mechanism



Coherence

Phase relationship maintenance

Critical for interferometry

Energy, Wavelength, Frequency Relationships

Planck-Einstein Relation

$$E = h\nu = hc/\lambda$$

$h = 6.626 \times 10^{-34} \text{ J}\cdot\text{s}$ (Planck constant)

Higher frequency → Higher energy
Shorter wavelength → Higher energy



💡 Energy in eV

$$E (\text{eV}) = 1240 / \lambda (\text{nm})$$

⚠️ Wavelength Conversion

$$\lambda (\text{nm}) = 10^7 / \nu (\text{cm}^{-1})$$

⌚ Frequency Relation

$$\nu (\text{Hz}) = c / \lambda (\text{m})$$

🌟 Photon Flux

$$\Phi = P / (h\nu)$$

photons per second

~2 eV

Visible light
photosynthesis

~0.1 eV

IR vibrations
molecular bonds

~4 eV

UV damage
DNA breaks

~25 meV

kT at 25°C
thermal energy

⚡ Biological Energy Scales

Electromagnetic Spectrum Overview

Full Electromagnetic Spectrum



Radio

Microwave

IR

Visible

UV

X-ray

Gamma

← Lower Energy

$\lambda:$ km → pm

Higher Energy →

Spectrum Range

Radio waves → Microwaves → IR → Visible → UV → X-rays → Gamma rays

Frequency: 10^3 Hz to 10^{20} Hz | Wavelength: km to pm



Biological Windows

Visible: 400-700 nm

Vision, photosynthesis

NIR: 700-1000 nm

Deep tissue penetration

UV-A: 320-400 nm

Minimal DNA damage



Atmospheric Transmission

Transparent: Visible light, radio waves

Absorbed: Most UV, IR, X-rays

Ozone layer: Blocks harmful UV-C radiation



Medical Imaging Regions

X-ray: 0.01-10 nm

Radiography, CT scanning

Gamma: <0.01 nm

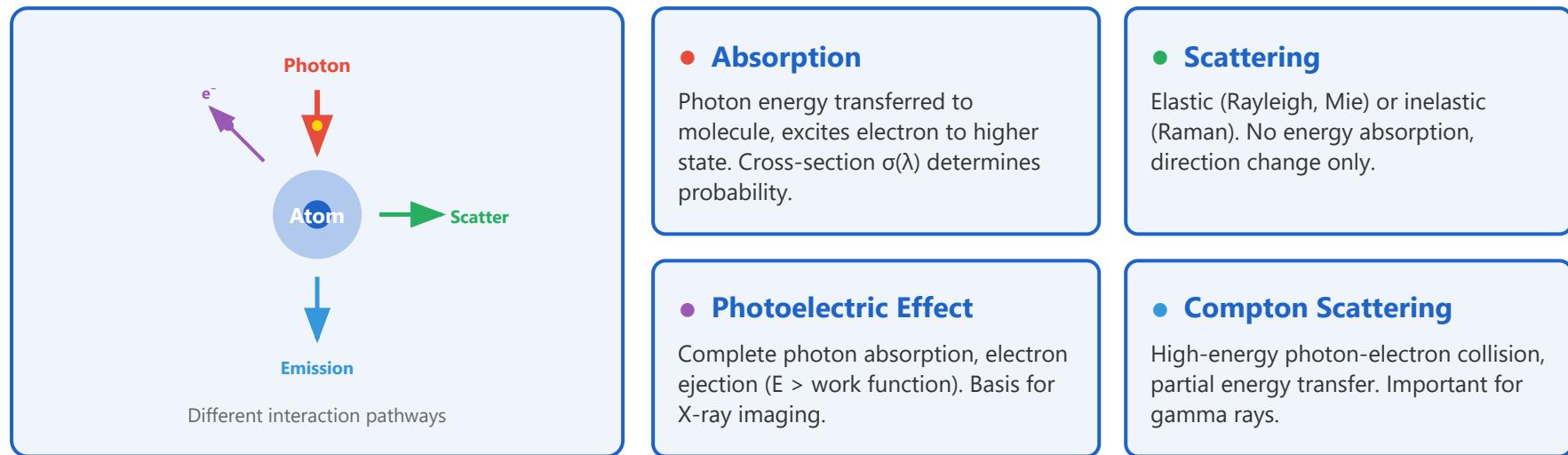
PET, SPECT imaging

Optical: Microscopy, endoscopy

Interactive Applications

- Spectral databases for reference
- Wavelength calculators
- Interactive spectrum explorers
- Energy conversion tools

Photon-Matter Interactions



⚠ Biological Damage Thresholds

UV: DNA damage, thymine dimers (<320 nm)

Visible/NIR: Generally safe, but high intensity causes thermal damage

Ionizing (X-ray, γ): Direct DNA breaks, ROS generation

Photobleaching: Fluorophore destruction limits imaging time

Absorption and Emission in Molecular Spectroscopy

Electronic Transitions

Ground state (S_0) → Excited states (S_1, S_2, \dots)

$$\Delta E = E_{\text{excited}} - E_{\text{ground}} = h\nu$$

Allowed transitions follow selection rules

Vibrational Modes

Molecular vibrations (stretching, bending)

IR absorption region

Fine structure in spectra

Characteristic frequencies for bonds

Selection Rules

Allowed: $\Delta l = \pm 1$ (dipole transitions)

Spin: $\Delta S = 0$ (singlet-singlet)

Symmetry: Determines intensity

Forbidden: Weak but observable

Stokes Shift

$\lambda_{\text{emission}} > \lambda_{\text{excitation}}$

Energy loss to vibrations

Typically 20-100 nm shift

Enables fluorescence detection

Quantum Yield (Φ)

$\Phi = \text{photons emitted} / \text{photons absorbed}$

Range: 0-1 (0-100%)

High $\Phi \rightarrow$ bright fluorophores

GFP: $\Phi \approx 0.79$

Detailed Explanations with Diagrams

1. Vibrational Modes

Molecules are not rigid structures but vibrate continuously at characteristic frequencies. These vibrations can be classified into different modes based on the atomic motion patterns.

Types of Vibrational Modes:

- **Symmetric Stretching:** Bonds extend and contract in phase
- **Asymmetric Stretching:** Bonds extend and contract out of phase
- **Bending (Scissoring):** Bond angles change periodically
- **Rocking, Wagging, Twisting:** More complex molecular deformations

Energy Levels:

Vibrational energy is quantized according to:

$$E_v = \hbar\nu(v + 1/2)$$

where $v = 0, 1, 2, \dots$ is the vibrational quantum number

Example: Water (H_2O) has three vibrational modes:

- Symmetric stretch: $\sim 3657 \text{ cm}^{-1}$
- Asymmetric stretch: $\sim 3756 \text{ cm}^{-1}$
- Bending: $\sim 1595 \text{ cm}^{-1}$

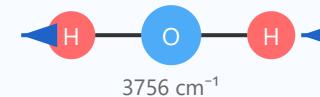
These frequencies are in the infrared region and are unique molecular fingerprints.

Vibrational Modes of H_2O

Symmetric Stretch



Asymmetric Stretch



Bending (Scissoring)



2. Selection Rules

Selection rules determine which electronic transitions are allowed or forbidden based on quantum mechanical principles. These rules govern the intensity and probability of spectroscopic transitions.

Key Selection Rules:

- **Orbital Angular Momentum ($\Delta l = \pm 1$):** For atomic transitions, the orbital quantum number must change by exactly one unit. This corresponds to electric dipole transitions.
- **Spin Selection Rule ($\Delta S = 0$):** The total spin must not change during the transition. This means singlet-to-singlet or triplet-to-triplet transitions are allowed, but singlet-to-triplet are forbidden.
- **Laporte Rule (Parity):** Transitions must involve a change in parity ($g \leftrightarrow u$ for centrosymmetric molecules).

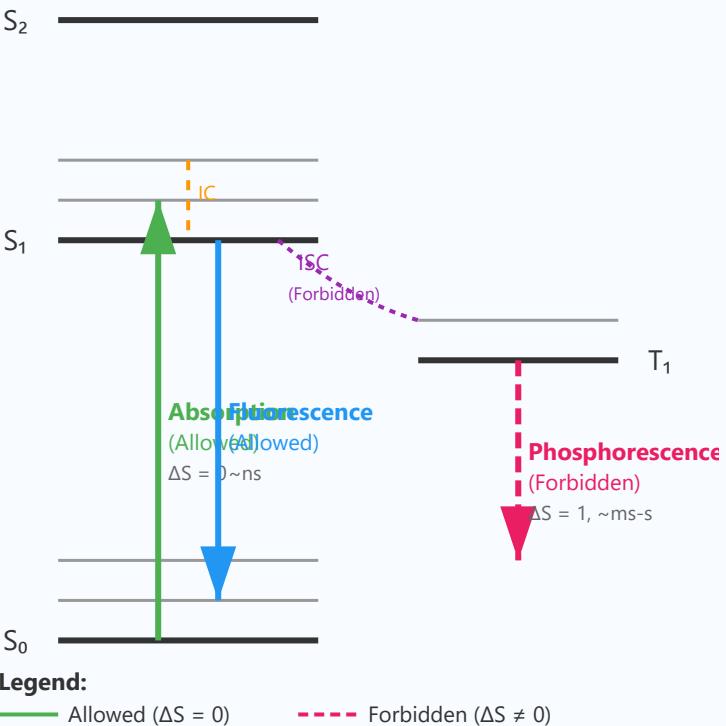
Forbidden Transitions:

"Forbidden" transitions can still occur but with much lower probability due to:

- Spin-orbit coupling (allows $S_0 \rightarrow T_1$ transitions)
- Vibronic coupling (mixing of electronic and vibrational states)
- Magnetic dipole or electric quadrupole mechanisms

Example: In fluorescence, the $S_1 \rightarrow S_0$ transition is spin-allowed ($\Delta S = 0$) and typically occurs in nanoseconds. In contrast, phosphorescence ($T_1 \rightarrow S_0$) is spin-forbidden ($\Delta S = 1$) and occurs over milliseconds to seconds, making it much weaker.

Jablonski Diagram



3. Stokes Shift

The Stokes shift is the difference between the wavelength positions of the absorption and emission maxima. This phenomenon is named after Irish physicist George Gabriel Stokes who first observed it in 1852.

Physical Origin:

- **Vibrational Relaxation:** After photon absorption to S_1 , the molecule rapidly relaxes to the lowest vibrational level of S_1 through non-radiative processes (~ps timescale)
- **Energy Dissipation:** This energy is converted to heat in the surrounding medium
- **Lower Energy Emission:** Fluorescence occurs from this relaxed state, resulting in longer wavelength (lower energy) emission
- **Solvent Reorganization:** Excited state has different charge distribution, causing solvent molecules to reorient

Magnitude:

$$\text{Stokes Shift} = \lambda_{\text{em}} - \lambda_{\text{abs}}$$

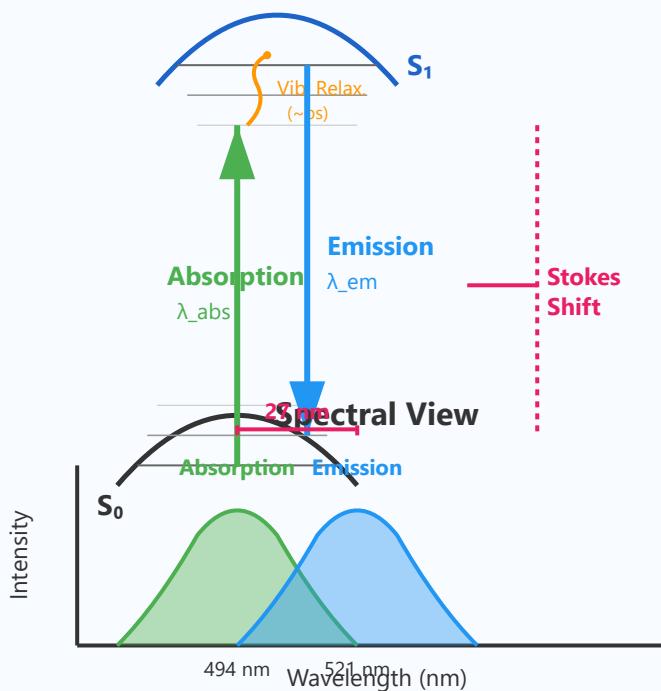
Typical values: 20-100 nm for organic fluorophores

Practical Importance:

- Enables spectral separation of excitation and emission
- Reduces self-absorption and inner filter effects
- Allows efficient fluorescence detection with minimal scattered light interference
- Critical for fluorescence microscopy and sensing applications

Example: Fluorescein has an absorption maximum at 494 nm and emission maximum at 521 nm, giving a Stokes shift of 27 nm. This shift allows use of dichroic mirrors to separate excitation and emission light in fluorescence microscopy.

Stokes Shift Visualization



4. Quantum Yield (Φ)

Quantum yield is the efficiency of the fluorescence process, representing the ratio of photons emitted to photons absorbed. It is a crucial parameter for characterizing fluorophores and determining their practical utility.

Definition:

$$\Phi = \frac{(\text{Number of photons emitted})}{(\text{Number of photons absorbed})}$$

Rate Constant Perspective:

Quantum yield can also be expressed in terms of rate constants:

$$\Phi = \frac{k_r}{(k_r + k_{nr})}$$

where k_r is the radiative rate constant and k_{nr} is the sum of all non-radiative rate constants

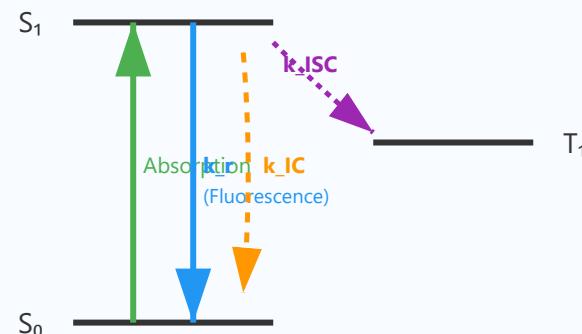
Non-radiative Deactivation Pathways:

- **Internal Conversion (IC):** $S_1 \rightarrow S_0$ without radiation
- **Intersystem Crossing (ISC):** $S_1 \rightarrow T_1$ (spin forbidden)
- **Collisional Quenching:** Energy transfer to other molecules
- **Photochemical Reactions:** Chemical bond breaking or formation

Factors Affecting Quantum Yield:

- Molecular rigidity (rigid molecules have higher Φ)
- Heavy atom effects (increase ISC, decrease Φ)

Quantum Yield Competition



Quantum Yield Formula

$$\Phi = \frac{k_r}{(k_r + k_{IC} + k_{ISC} + \dots)}$$

Examples of Different Quantum Yields

Fluorescein
 $\Phi = 0.92$

Very Bright
 $k_r \gg k_{nr}$

Tryptophan
 $\Phi = 0.14$

Moderate
 $k_r \approx k_{nr}$

Azulene
 $\Phi \approx 0$

Non-fluor.
 $k_r \ll k_{nr}$

- Temperature (higher T increases non-radiative decay)
- Solvent polarity and viscosity
- pH for molecules with ionizable groups

Examples of Quantum Yields:

- **Fluorescein (pH 9):** $\Phi \approx 0.92$ (very bright)
- **GFP:** $\Phi \approx 0.79$ (excellent for bioimaging)
- **Tryptophan:** $\Phi \approx 0.14$ (moderate)
- **Benzene:** $\Phi \approx 0.05$ (weak fluorophore)
- **Azulene:** $\Phi \approx 0.00001$ (essentially non-fluorescent)

High quantum yield fluorophores are preferred for imaging and sensing applications.

Scattering Phenomena

Rayleigh Scattering

Particles $<< \lambda$ (air molecules)

Intensity $\propto 1/\lambda^4$

Why sky is blue

Used in DLS for size measurement

Mie Scattering

Particles $\approx \lambda$ (cells, bacteria)

Complex angular distribution

Flow cytometry application

Forward/side scatter

Dynamic Light Scattering

Measures Brownian motion

Hydrodynamic radius determination

Protein aggregation studies

Nanoparticle characterization

Raman Scattering

Inelastic scattering

Molecular fingerprinting

Label-free chemical analysis

Surface enhancement (SERS)



Biological Applications

Cell sorting: Forward/side scatter in flow cytometry

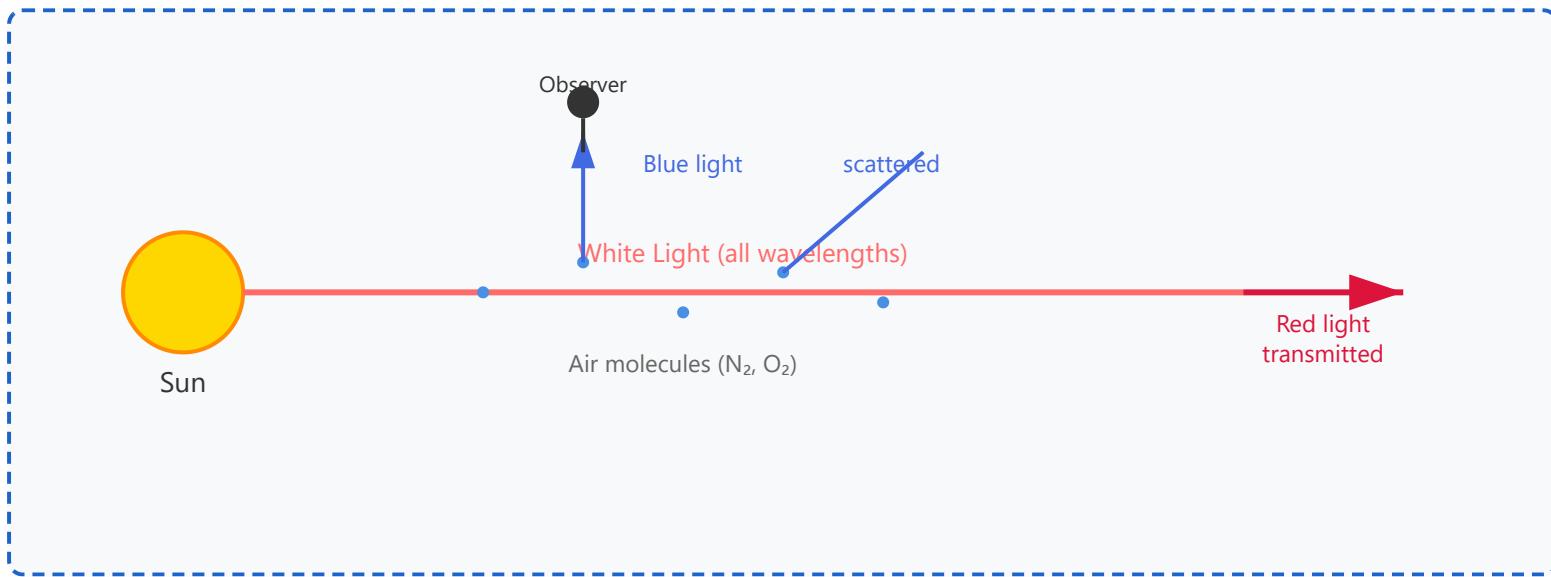
Protein analysis: DLS for aggregation and stability

Tissue imaging: Raman microscopy for cancer detection



Detailed Explanations and Visual Representations

► 1. Rayleigh Scattering



Physical Principle: Rayleigh scattering occurs when light interacts with particles much smaller than the wavelength of light (typically $< \lambda/10$). This elastic scattering phenomenon is responsible for many natural optical effects we observe daily.

$$I \propto 1/\lambda^4$$

Why the Sky is Blue

Sunlight entering Earth's atmosphere contains all visible wavelengths. Since scattering intensity is inversely proportional to the fourth power of wavelength, blue light ($\lambda \approx 450$ nm) is scattered approximately 10 times more than red light ($\lambda \approx 650$ nm). This scattered blue light reaches our eyes.

from all directions, making the sky appear blue. At sunset, light travels through more atmosphere, scattering away most blue light and leaving red-orange hues.

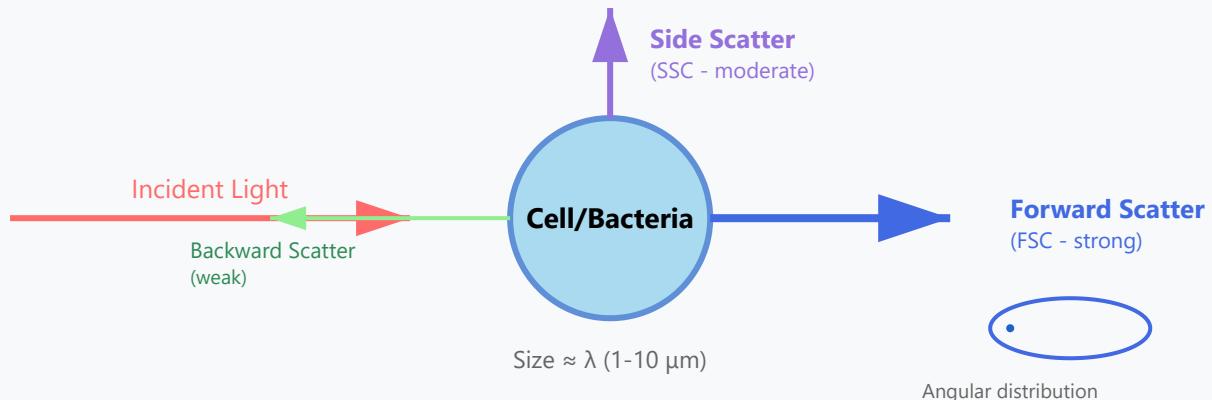
Application in Dynamic Light Scattering (DLS)

DLS exploits Rayleigh scattering to measure particle size in the nanometer range. By analyzing the time-dependent fluctuations in scattered light intensity caused by Brownian motion, we can determine the hydrodynamic radius of nanoparticles, proteins, and polymers. The technique is non-destructive and requires minimal sample preparation.

★ Key Characteristics

- Particle size: Much smaller than wavelength ($< 0.1\lambda$)
- Scattering pattern: Symmetric forward and backward scattering
- Wavelength dependence: Strong ($1/\lambda^4$)
- Examples: Air molecules (O_2, N_2), small nanoparticles (< 50 nm)
- Typical size range: 0.01 - 50 nm

► 2. Mie Scattering



Physical Principle: Mie scattering occurs when particles have dimensions comparable to the wavelength of incident light. Named after Gustav Mie, this theory provides complete solutions to Maxwell's equations for spherical particles. The scattering pattern is complex and strongly forward-directed.

Flow Cytometry Application

Flow cytometry exploits Mie scattering to analyze and sort cells. Forward scatter (FSC) intensity correlates with cell size, while side scatter (SSC) relates to internal complexity and granularity. By measuring both parameters simultaneously, different cell populations can be distinguished without fluorescent labels. This technique is essential in immunology, hematology, and cancer research.

Scattering Pattern Characteristics

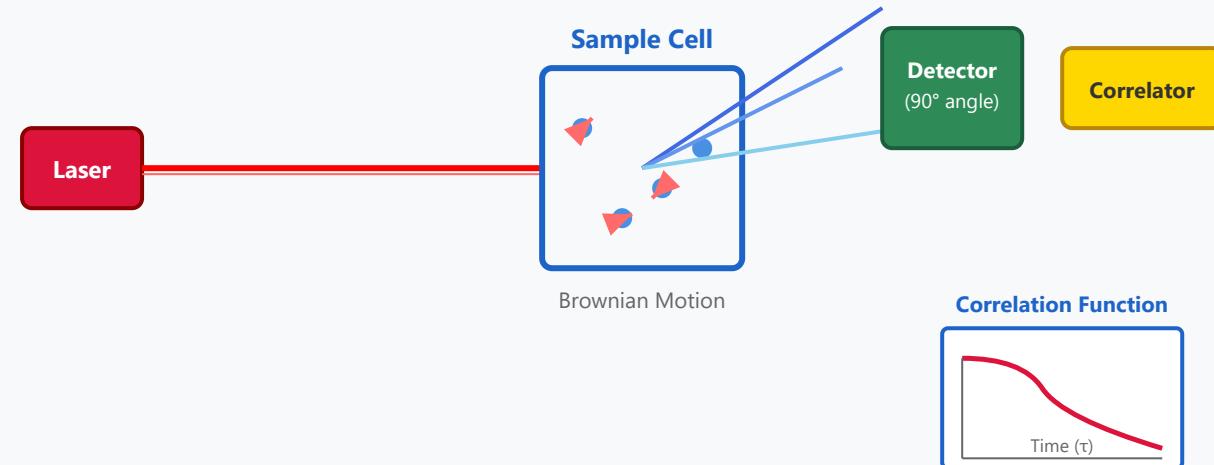
Unlike Rayleigh scattering's symmetric pattern, Mie scattering produces an asymmetric angular distribution with strong forward scattering. The exact pattern depends on the size parameter ($2\pi r/\lambda$)

and refractive index contrast. As particle size increases relative to wavelength, forward scattering becomes increasingly dominant, creating a narrow cone of scattered light.

★ Key Characteristics

- Particle size: Comparable to wavelength ($0.1\lambda - 10\lambda$)
- Scattering pattern: Asymmetric, strongly forward-directed
- Wavelength dependence: Complex, not simply $1/\lambda^4$
- Examples: Bacterial cells, yeast, blood cells, droplets in fog
- Typical size range: $0.5 - 10 \mu\text{m}$
- Applications: Flow cytometry, aerosol science, atmospheric physics

► 3. Dynamic Light Scattering (DLS)



Physical Principle: DLS, also known as Photon Correlation Spectroscopy (PCS) or Quasi-Elastic Light Scattering (QELS), measures the time-dependent fluctuations in scattered light intensity caused by Brownian motion of particles. The rate of intensity fluctuation is directly related to the diffusion coefficient, which can be used to calculate particle size.

$$D = kT / (6\pi\eta Rh) \rightarrow Rh = kT / (6\pi\eta D)$$

(Stokes-Einstein equation: D = diffusion coefficient, Rh = hydrodynamic radius)

Measurement Process

A monochromatic laser illuminates the sample, and scattered light is collected at a fixed angle (typically 90° or 173°). The detector measures intensity fluctuations over time. Fast-moving small particles cause rapid fluctuations, while slow-moving large particles produce slower fluctuations. An autocorrelation function is calculated to extract the decay time, which relates to particle size.

Protein Aggregation Studies

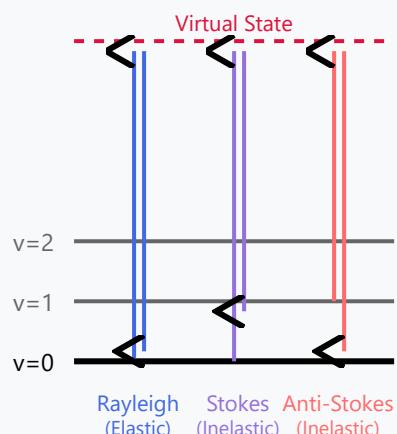
DLS is invaluable for monitoring protein stability and aggregation in pharmaceutical development. It can detect early-stage aggregates (oligomers) before they become visible precipitates. The technique tracks changes in hydrodynamic radius, polydispersity index, and size distribution during stress conditions (temperature, pH changes, freeze-thaw cycles), helping predict long-term protein stability and shelf life.

★ Key Characteristics

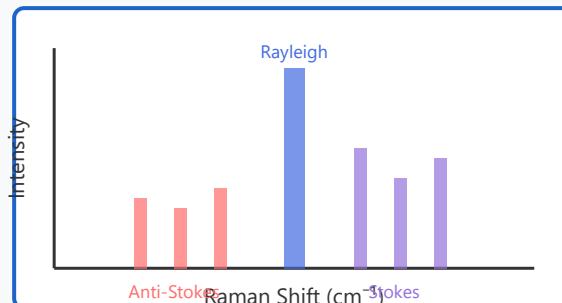
- Size range: 0.3 nm - 10 µm (optimally 1-1000 nm)
- Sample requirement: Low concentration, minimal preparation
- Measurement time: Seconds to minutes
- Non-destructive and non-invasive technique
- Applications: Nanoparticle characterization, protein stability, drug formulation, polymer science
- Limitations: Assumes spherical particles, sensitive to dust contamination

► 4. Raman Scattering

Energy Level Diagram



Raman Spectrum



Physical Principle: Raman scattering is an inelastic scattering process where incident photons interact with molecular vibrations, resulting in energy exchange. Unlike Rayleigh scattering (elastic), Raman scattering produces photons with shifted frequencies that correspond to specific molecular vibrational modes, creating a unique "molecular fingerprint."

Stokes vs. Anti-Stokes Raman

Stokes Raman: The molecule absorbs energy from the incident photon, moving from ground state ($v=0$) to an excited vibrational state ($v=1$). The scattered photon has lower energy (longer wavelength) than the incident photon. This is the dominant process at room temperature.

Anti-Stokes Raman: The molecule is already in an excited vibrational state and loses energy to the scattered photon, which has higher energy (shorter wavelength) than incident light. This is weaker because fewer molecules are in excited states at room temperature.

Molecular Fingerprinting and Chemical Analysis

Each molecule has a unique set of vibrational modes determined by its structure and chemical bonds. The Raman spectrum acts as a molecular fingerprint, allowing identification without labels or dyes. This label-free nature makes Raman spectroscopy ideal for studying biological samples in their native state, pharmaceutical quality control, forensic analysis, and material characterization.

Surface-Enhanced Raman Spectroscopy (SERS)

SERS amplifies Raman signals by factors of 10^6 - 10^{14} when molecules are adsorbed on rough metal surfaces (typically gold or silver nanoparticles). This enhancement enables single-molecule detection and trace analysis. SERS is revolutionary for biosensing, environmental monitoring (detecting pollutants at ultra-low concentrations), and cancer diagnostics through blood analysis.

★ Key Characteristics

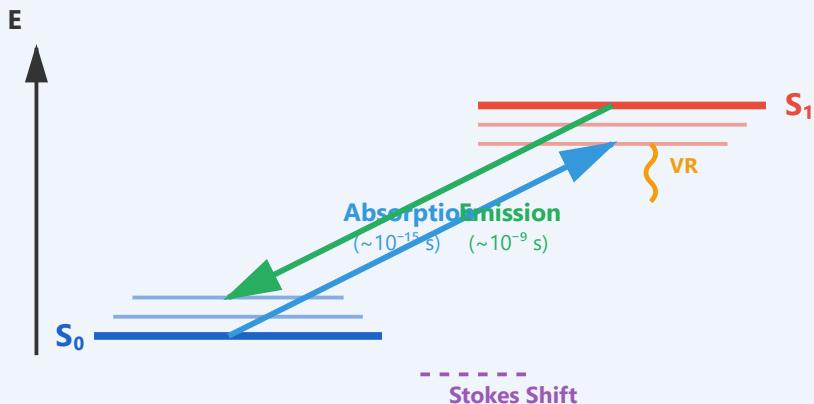
- Type: Inelastic scattering (energy exchange with molecules)
- Efficiency: Very weak (~ 1 in 10^7 photons undergo Raman scattering)
- Information: Molecular structure, chemical composition, crystallinity
- Advantages: Label-free, non-destructive, minimal sample preparation, works through glass/water
- Applications: Cancer detection, drug identification, quality control, geological analysis, art conservation
- Typical laser wavelengths: 532 nm, 633 nm, 785 nm, 1064 nm

►  **Comparative Summary**

Property	Rayleigh	Mie	DLS	Raman
Particle Size	$\ll \lambda$	$\approx \lambda$	0.3 nm - 10 μm	Molecular
Scattering Type	Elastic	Elastic	Elastic	Inelastic
Key Information	Size, concentration	Size, shape, structure	Size distribution, diffusion	Molecular structure, composition
Primary Application	Atmospheric optics	Flow cytometry, cell sorting	Nanoparticle sizing, protein stability	Chemical identification, imaging
Scattering Efficiency	Very weak ($\propto 1/\lambda^4$)	Strong	Weak-Moderate	Very weak ($\sim 10^{-7}$)

Fluorescence Principles

Jablonski Energy Diagram



Jablonski Diagram

$S_0 \rightarrow S_1$ (absorption)
↓ vibrational relaxation
 $S_1 \rightarrow S_0$ (emission)

Timescales:

Absorption: $\sim 10^{-15}$ s
VR: $\sim 10^{-12}$ s
Emission: $\sim 10^{-9}$ s

Stokes Shift:

$\lambda_{\text{emission}} > \lambda_{\text{excitation}}$

Excitation/Emission Spectra

Mirror image relationship due to vibrational structure
Stokes shift separation enables detection
Peak wavelengths for filter optimization
Spectral overlap considerations for multicolor imaging

Fluorophore Properties

Brightness: $\epsilon \times \Phi$ (extinction \times quantum yield)
Lifetime: τ (1-10 ns typical)
Stokes shift: 20-100 nm
Photostability: varies widely between fluorophores

Photobleaching

Irreversible fluorescence loss over time
Reactive oxygen species (ROS) mediated damage
Antifade reagents help preserve signal
Limits long-term imaging duration

FRET Basics

Förster Resonance Energy Transfer
Distance-dependent (2-10 nm range)
Requires donor-acceptor pair
Molecular ruler for protein interactions

PART 2/3

Spectroscopy Methods

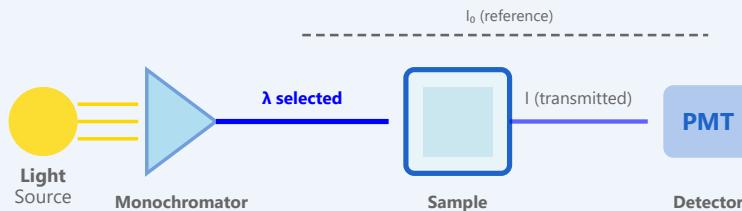
Instrumentation • Detection Principles • Quantitative Analysis

UV-Vis Spectroscopy

$$A = \epsilon bc = -\log_{10}(I/I_0)$$

ϵ : molar absorptivity ($M^{-1}cm^{-1}$) | b: path length (cm) | c: concentration (M)

Spectrophotometer Design



Chromophores in Biology

Proteins: Trp, Tyr (280 nm)

DNA/RNA: 260 nm

Heme: Soret band (420 nm)

Cuvette Selection

Quartz: UV region

Glass/Plastic: Visible only

Standard: 1 cm path length

Applications

Protein quantification | DNA/RNA purity | Enzyme kinetics | Drug screening

Baseline Corrections

Buffer blank essential | Scatter correction for turbid samples | Temperature control

Linear Range

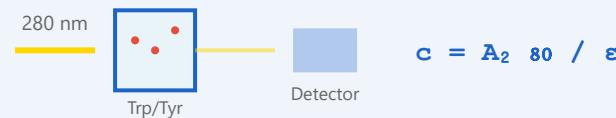
$A = 0.1-1.0$ optimal | Beyond $A=2$: non-linear | Dilute if necessary

Light Sources

Deuterium (UV) | Tungsten-halogen (Visible) | Xenon flash lamps

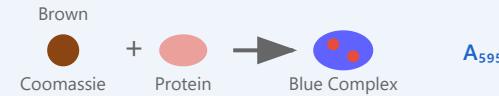
Protein Concentration Measurement

A₂₈₀ Method



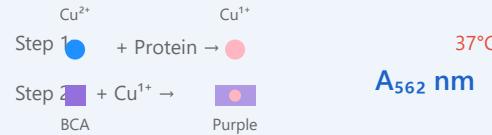
Direct, fast, needs pure protein. ϵ calculated from Trp/Tyr content.

Bradford Assay



Coomassie dye binding. Sensitive (1-100 µg/mL). Detergent interference.

BCA Assay



Cu²⁺ reduction. Compatible with detergents. 20-2000 µg/mL.

⚠ Interference Factors



Buffer composition critical for accurate measurements.

DNA/RNA Quantification

A260/A280 Purity Ratios

Pure DNA: ~1.8 | Pure RNA: ~2.0 | Protein contamination: <1.8

Absorbance (NanoDrop)

Fast, 1-2 μ L. A260/280, A260/230 ratios. Contamination detection.

Fluorometric (Qubit)

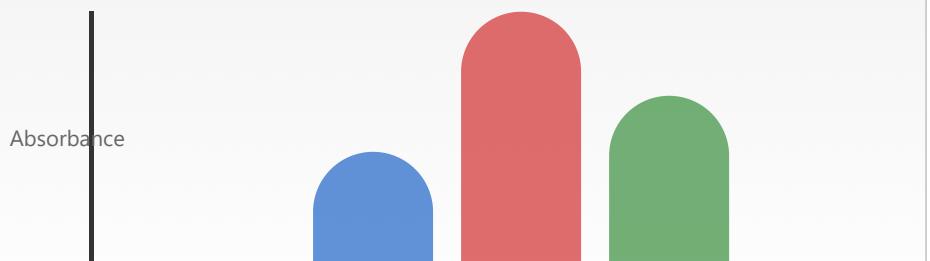
Selective dyes. More accurate, less contamination sensitivity.



Detailed Explanation and Principles

1. Absorbance Method (NanoDrop) - Spectrophotometry

Absorption Spectrum



NanoDrop Principle

Light Source (UV Light)



Sample (1-2 μ L)



230nm

260nm

280nm

Detector

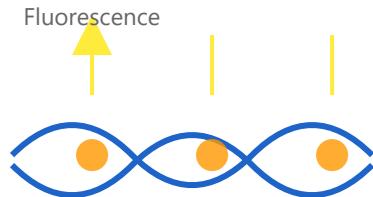
Absorbance Analysis

- **Measurement Principle:** Utilizes the property of nucleic acids absorbing UV light at 260nm wavelength
- **A260/A280 Ratio:**
 - Pure DNA: ~1.8 (no protein contamination)
 - Pure RNA: ~2.0 (RNA has higher ratio)
 - <1.8: Protein contamination suspected (proteins absorb at 280nm)
- **A260/A230 Ratio:** Ideal range 2.0-2.2 (low values indicate salt, phenol contamination)
- **Advantages:** Fast, minimal sample volume (1-2 μ L), simple operation
- **Disadvantages:** Susceptible to contaminants, relatively lower accuracy

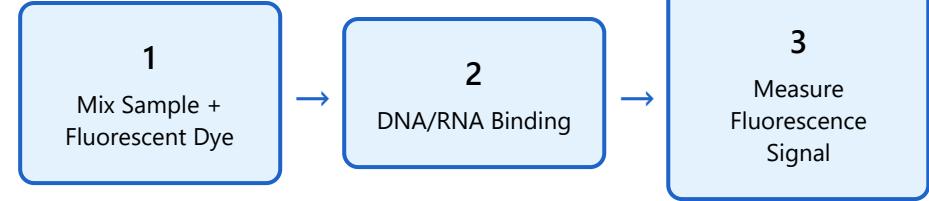
2. Fluorometric Method (Qubit) - Fluorescence-based Quantification

Fluorescence Principle

Measurement Process



DNA + Fluorescent Dye



- **Measurement Principle:** Uses fluorescent dyes that specifically bind to DNA/RNA
- **Selective Dyes:**
 - dsDNA-specific dye: Measures only double-stranded DNA
 - ssDNA dye: Measures single-stranded DNA
 - RNA-specific dye: Selectively measures RNA only
- **Advantages:** High accuracy, minimal contamination interference, high specificity
- **Disadvantages:** Relatively higher cost, requires dye reagents
- **Applications:** NGS, PCR, and experiments requiring precise quantification

3. Comparison of Two Methods

Comparison	NanoDrop (Absorbance)	Qubit (Fluorescence)
Measurement Principle	UV absorbance (260nm)	Fluorescent dye binding
Sample Volume	1-2 µL (minimal)	1-20 µL

Comparison	NanoDrop (Absorbance)	Qubit (Fluorescence)
Measurement Time	Very fast (seconds)	Fast (minutes)
Accuracy	Moderate (contamination-sensitive)	High
Purity Assessment	Possible (260/280, 260/230)	Not possible
Selectivity	Low (all nucleic acids)	High (specific nucleic acids)
Contamination Sensitivity	High (protein, salt effects)	Low
Cost	Low (no reagents needed)	High (dye reagents)
Primary Use	Quick screening, purity check	Precise quantification, NGS prep

4. Experimental Tips and Precautions

NanoDrop Usage

- Calibrate with blank before measurement
- Ensure no bubbles on sample surface
- Clean thoroughly after measurement
- Always check 260/280 ratio

Qubit Usage

- Always prepare standard curve
- Select appropriate dye
- Store in dark conditions
- Measure after fluorescence stabilization

Infrared Spectroscopy

Molecular Vibrations

Stretch, bend, rock, wag, twist modes. Each unique to molecular structure.

IR Regions

4000-2500: O-H, N-H | 2000-1500: C=O, C=C | 1500-400: Fingerprint

ATR-FTIR

Attenuated Total Reflectance. No sample prep required.

Water Interference

Strong O-H absorption. Use D₂O or dry samples.

Detailed Examples & Illustrations

1. Molecular Vibrations

Stretching Vibrations: Bond length changes periodically

- Symmetric stretch: Both bonds extend/contract together
- Asymmetric stretch: One extends while other contracts

Bending Vibrations: Bond angle changes

- In-plane bending: Scissoring, rocking
- Out-of-plane bending: Wagging, twisting

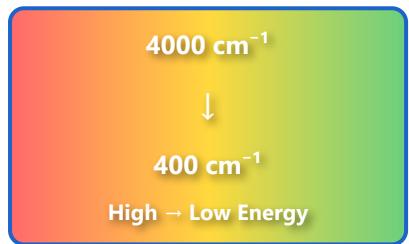
Typical Frequencies:



Vibration Modes

- C-H stretch: $\sim 3000 \text{ cm}^{-1}$
- C=O stretch: $\sim 1700 \text{ cm}^{-1}$
- C-C stretch: $\sim 1000 \text{ cm}^{-1}$

2. IR Spectral Regions



Functional Group Region ($4000\text{-}1500 \text{ cm}^{-1}$):

- 3600-3200: O-H stretch (alcohols, phenols)
- 3500-3300: N-H stretch (amines, amides)
- 3000-2850: C-H stretch (alkanes, alkenes)
- 1750-1650: C=O stretch (carbonyls)
- 1680-1600: C=C stretch (alkenes)

Fingerprint Region ($1500\text{-}400 \text{ cm}^{-1}$):

- Complex pattern unique to each molecule
- Used for definitive compound identification
- C-O, C-N, C-C stretches and various bending modes

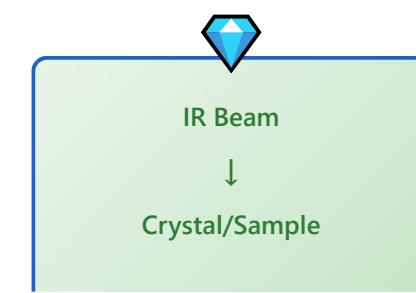
3. ATR-FTIR Technique

Working Principle:

- IR beam undergoes total internal reflection at crystal-sample interface
- Evanescent wave penetrates sample ($\sim 0.5\text{-}5 \mu\text{m}$ depth)
- Sample absorbs specific wavelengths
- Reflected beam carries absorption information

Key Advantages:

- No sample preparation needed
- Works with solids, liquids, powders, films
- Quick analysis (1-2 minutes)



- Non-destructive

Detector

Common Crystals:

- Diamond: Hardest, most durable, wide range
- ZnSe: Good for most organic samples
- Ge: Best for strongly absorbing samples

4. Water Interference Problem



The Problem:

- Water shows very strong O-H stretching (~3400 cm⁻¹)
- H-O-H bending band at ~1640 cm⁻¹
- Overlaps with important functional groups (O-H, N-H)
- Moisture in air can interfere with measurements

Solutions:

- **Use D₂O:** Deuterium shifts O-D stretch to ~2500 cm⁻¹
- **Dry samples:** Store in desiccator before measurement
- **Background subtraction:** Measure pure water spectrum first
- **Purge instrument:** Continuously flow dry N₂ or air
- **Use ATR-FTIR:** Less sensitive to atmospheric water

Best Practice:

Always record background in same conditions as sample measurement



Key Tips for IR Analysis

- Always run a blank/background spectrum first
- Clean ATR crystal thoroughly between samples

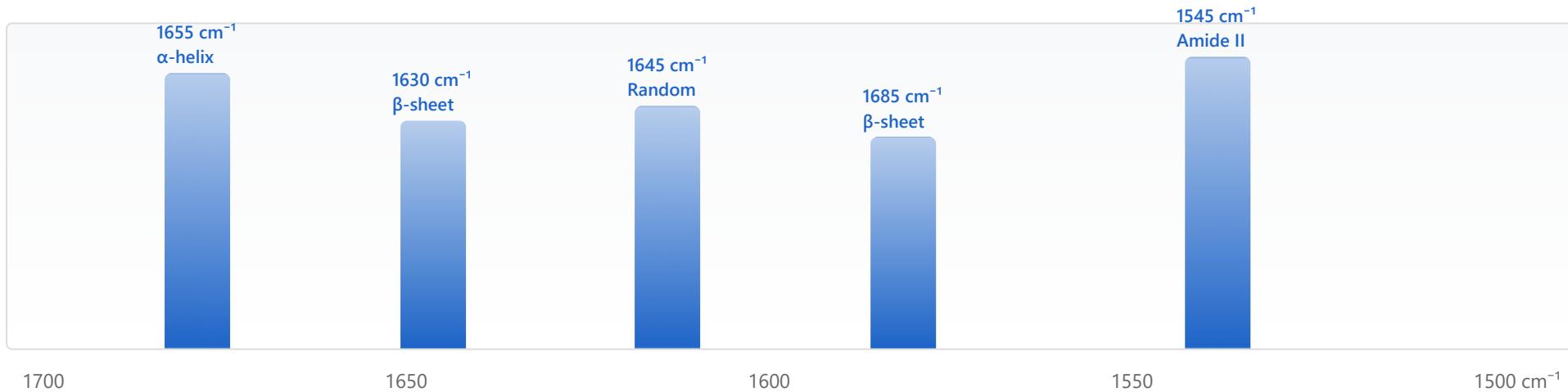
- Apply consistent pressure on ATR crystal
- Use correlation tables to identify unknown peaks
- Compare fingerprint region with reference spectra
- Consider both presence and absence of peaks
- Multiple complementary techniques (NMR, MS) confirm structure

FTIR for Biomolecules

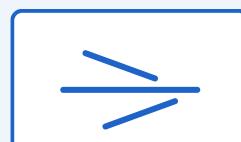
Amide Bands

- Amide I ($1600\text{-}1700\text{ cm}^{-1}$): C=O stretch, secondary structure sensitive
- Amide II ($1510\text{-}1580\text{ cm}^{-1}$): N-H bend, C-N stretch
- α -helix: 1650-1658 | β -sheet: 1620-1640, 1680-1690 | Random: 1640-1650

Representative Protein FTIR Spectrum



Secondary Structure Characteristics



α -helix

1650-1658 cm⁻¹

β -sheet

1620-1640, 1680-1690 cm⁻¹

Random coil

1640-1650 cm⁻¹

α -helix: Forms regular hydrogen bonding patterns due to helical structure, showing strong absorption at 1650-1658 cm⁻¹ from C=O stretching vibration.

β -sheet: Shows two characteristic peaks depending on parallel or antiparallel structure. Absorbed at 1620-1640 cm⁻¹ (strong peak) and 1680-1690 cm⁻¹ (weak peak).

Random coil: Exhibits broad absorption band at 1640-1650 cm⁻¹ due to irregular structure.

Lipid Analysis

C-H stretch 2800-3000 cm⁻¹. Membrane fluidity studies.

Spectral Deconvolution

Fourier self-deconvolution resolves overlapping bands.

Lipid Analysis

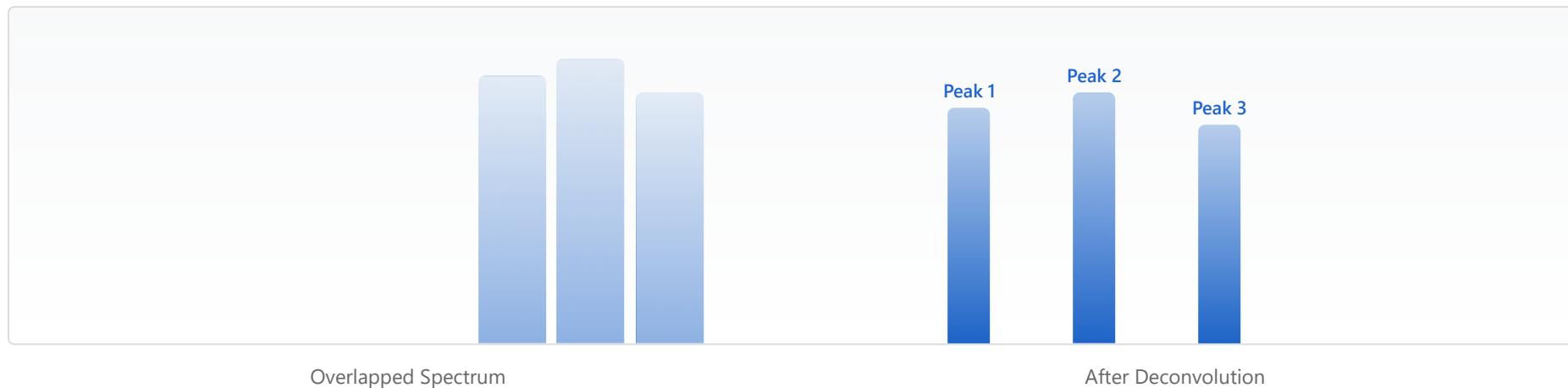


C-H Stretching Vibration (2800-3000 cm⁻¹):

- 2920 cm⁻¹: Asymmetric CH₂ stretch
- 2850 cm⁻¹: Symmetric CH₂ stretch
- Changes in position and intensity of these peaks can be used to analyze fluidity and phase transitions of lipid bilayers.
- Peak shift to higher wavenumbers with increasing temperature indicates increased membrane fluidity.
- Applied in biological membrane studies, drug-membrane interactions, and lipid oxidation research.

Spectral Deconvolution

Separation of Overlapped Peaks



Fourier Self-Deconvolution:

- Separates overlapped peaks into individual components using mathematical algorithms.
- Enables quantification of various secondary structure components in the complex Amide I region spectrum.
- Used in conjunction with second derivative to enhance resolution.
- Essential technique for protein aggregation, structural changes, and ligand binding studies.
- Caution: Excessive deconvolution can generate artificial peaks, so appropriate parameter selection is important.

Major Applications of Biomolecular FTIR

Protein Structure Analysis

Monitoring protein folding, denaturation, and aggregation states. Studying protein-ligand interactions in drug development.

Pharmaceutical Research

Drug crystalline forms, polymer formulations, stability assessment and quality control of protein therapeutics.

Cell Membrane Research

Phase transitions of lipid bilayers, membrane fluidity, and drug-membrane interaction analysis.

Biosensors

Label-free sensing for biomolecular recognition, immunoassays, and disease marker detection.

Experimental Tips & Precautions

Sample Preparation:

- Use D₂O solution or measure in dry film form to minimize water interference
- Appropriate concentration: Protein 10-50 mg/mL, Lipid 5-20 mg/mL

Spectrum Acquisition:

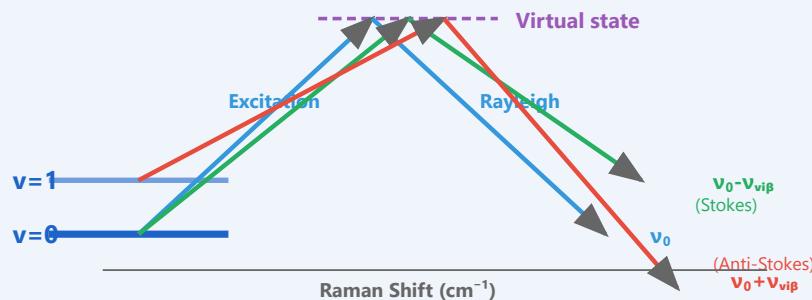
- Resolution: 2-4 cm⁻¹ (general), 1 cm⁻¹ (high-resolution analysis)
- Number of scans: Minimum 64 or more (improves signal-to-noise ratio)
- Acquire fresh background spectrum before each measurement

Data Processing:

- Baseline correction
- Atmospheric water vapor/CO₂ correction
- Appropriate smoothing (Savitzky-Golay filter recommended)

Raman Spectroscopy

Raman Scattering Energy Diagram



$$\Delta\nu = v_0 - v_s \text{scattered}$$

Raman Shift: Chemical fingerprint without labels

Stokes: Energy loss
(molecule gains vibrational energy)

Anti-Stokes: Energy gain
(less intense, temperature dependent)

Provides molecular vibrational information

Biological Applications

- Cell imaging:** Label-free analysis
- Drug distribution:** Tissue mapping
- Cancer diagnostics:** Tissue characterization
- Protein structure:** Secondary structure analysis

SERS (Surface-Enhanced)

- Enhancement:** 10^6 - 10^{14} fold
- Metal nanoparticles (Au, Ag)
- Single molecule detection possible
- Biosensing applications

Raman Imaging

- Spatial mapping of molecular composition
- Confocal Raman microscopy
- Chemical maps of cells/tissues
- Sub-micron resolution

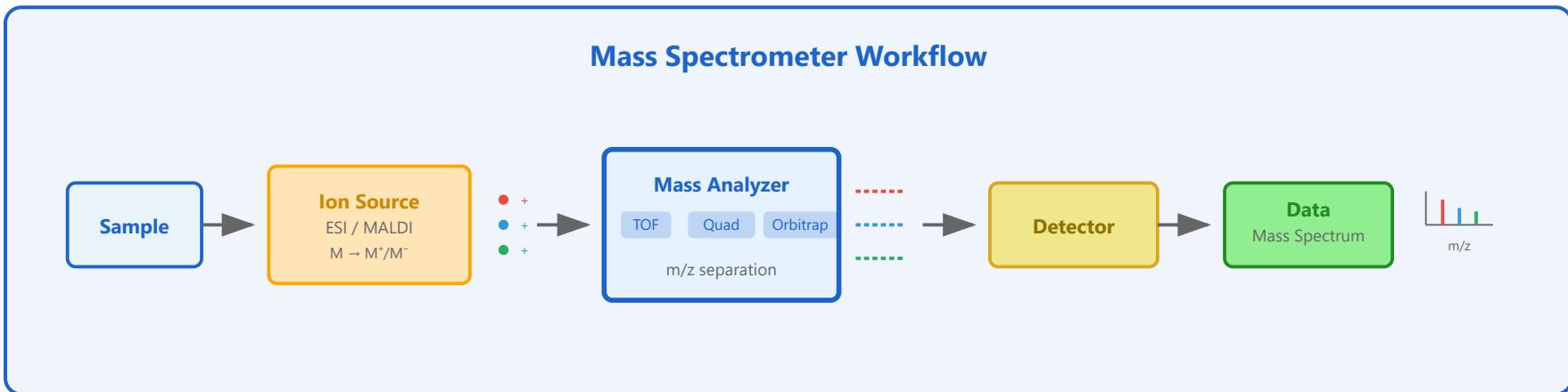
Label-free Analysis

- No fluorophores needed
- Native state biomolecules
- Non-destructive measurement
- Real-time monitoring possible

 **SERS Enhancement: 10^6 to 10^{14} Signal Amplification**

Enables ultra-sensitive detection for biosensing and single-molecule studies

Mass Spectrometry Basics



Ionization Methods



Mass Analyzers

TOF: Time-of-flight, fast
Quadrupole: Selective, robust
Orbitrap: High resolution
Ion Trap: MSⁿ capability

Tandem MS (MS/MS)



Proteomics workflow essential.

Resolution & Accuracy

Resolution: Distinguishes isotopes
Accuracy: <5 ppm for Orbitrap
Dynamic range: 10⁴-10⁶

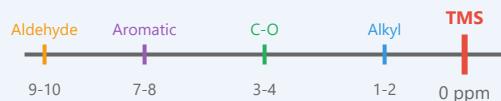
NMR Fundamentals

Nuclear Spin in Magnetic Field



^1H , ^{13}C , ^{15}N , ^{31}P nuclei have spin $1/2$

Chemical Shift (δ)



Electronic environment. ppm scale.

J-coupling



Spin-spin splitting. Connectivity information.

2D NMR



→ Structure determination

Biomolecular NMR



Solution state
Dynamics info

PART 3/3

Biological Applications

Translation to Diagnostics • Clinical Implementation • Point-of-Care
Devices

Fluorescent Proteins and Tags

GFP Family

GFP, YFP, CFP, RFP. Ex/Em: 395/509nm (GFP). Nobel Prize 2008.

Genetic Encoding

Fusion proteins. Minimal perturbation. Live cell compatible.

Multicolor Imaging

Simultaneous tracking. mCherry, mTurquoise, mVenus.

Photoswitchable

PALM, STORM superresolution. Dronpa, mEos.

1

GFP Family - The Foundation of Fluorescent Imaging

GFP Family Spectrum



Cyan
~475nm



Green
~509nm



Yellow
~527nm



Red
~583nm

The Green Fluorescent Protein (GFP) family originated from the jellyfish *Aequorea victoria* and revolutionized cell biology. These proteins spontaneously form a chromophore from their own amino acid residues, eliminating the need for external cofactors. The family has been engineered to span the visible spectrum, providing researchers with a palette of colors for cellular imaging.

Key Features:

- **Nobel Prize 2008:** Awarded to Osamu Shimomura, Martin Chalfie, and Roger Tsien for the discovery and development of GFP
- **Autocatalytic:** Forms fluorescent chromophore without external enzymes or cofactors
- **Excitation/Emission:** Original GFP - 395nm (UV) & 475nm (blue) / 509nm (green)
- **Variants:** Enhanced GFP (EGFP), superfolder GFP (sfGFP) for improved stability and brightness
- **Beta-barrel structure:** 11-stranded β -barrel protects the chromophore from the environment

Excitation:

UV-Blue (395-488nm)

Emission:

Cyan to Red (475-630nm)

2

Genetic Encoding - Molecular Tagging Strategy

Fusion Protein Architecture



N-terminal or C-terminal fusion

Fluorescent protein is genetically fused to target protein via peptide linker

Genetic encoding allows fluorescent proteins to be fused directly to proteins of interest through molecular cloning. The DNA sequence encoding the fluorescent protein is inserted into the gene of the target protein, resulting in a single polypeptide chain that carries both functionalities. This approach enables real-time tracking of protein localization, dynamics, and interactions in living cells.

Advantages & Considerations:

- **No external addition required:** Once transfected, cells produce tagged proteins autonomously
- **Minimal perturbation:** Small size (27 kDa for GFP) and careful linker design reduce functional disruption
- **Live cell compatible:** Non-toxic, stable expression enables long-term imaging
- **Spatial precision:** Tags can be placed at N-terminus, C-terminus, or internal positions
- **Validation needed:** Must confirm that tagged protein retains normal function and localization
- **Linker design:** Flexible peptide linkers (e.g., GGGS repeats) prevent steric hindrance

Common Applications:

- Protein localization and trafficking studies
- Real-time monitoring of protein expression levels
- FRET-based biosensors for detecting protein-protein interactions
- Cell cycle and developmental biology visualization
- Organelle-specific markers (mitochondria, nucleus, ER, etc.)

Multicolor Imaging - Simultaneous Multi-target Tracking

Spectral Separation Strategy



mTurquoise

Ex: 434nm
Em: 474nm



mVenus

Ex: 515nm
Em: 528nm



mCherry

Ex: 587nm
Em: 610nm

Minimal Spectral Overlap = Clear Signal Separation

Multicolor imaging leverages the spectral diversity of fluorescent proteins to simultaneously visualize multiple cellular components in the same sample. By carefully selecting fluorescent proteins with distinct excitation and emission spectra, researchers can track multiple proteins, organelles, or cellular events in real-time without cross-contamination of signals. This powerful technique has become essential for understanding complex cellular processes involving multiple interacting components.

Technical Requirements & Benefits:

- **Spectral separation:** Proteins must have minimal overlap in emission spectra (typically $>30\text{nm}$ apart)
- **Modern variants:** Optimized proteins like mTurquoise2, mVenus, and mCherry offer superior brightness and photostability
- **Simultaneous acquisition:** Multiple fluorescence channels can be imaged concurrently or sequentially
- **Co-localization studies:** Determine if proteins occupy the same cellular location

- **Dynamic interactions:** Track how different proteins move and interact over time
- **3-4 color maximum:** Practical limit due to spectral overlap and detector limitations

Experimental Examples:

Organelle Interactions

Visualize mitochondria-ER contact sites using different colored markers for each organelle

Signal Transduction

Track receptor activation and downstream effector translocation simultaneously

Cell Cycle Markers

Monitor multiple cell cycle regulators in different colors throughout division

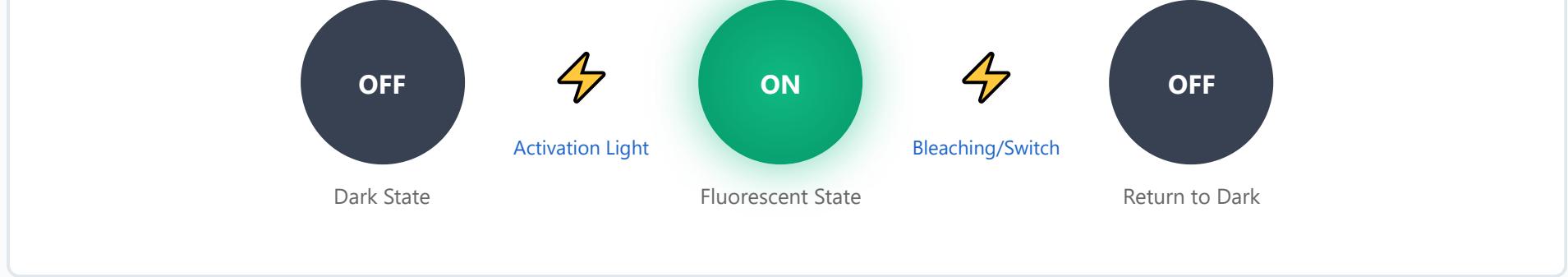
Multi-pathogen Studies

Distinguish different bacterial or viral strains within the same host cell

4

Photoswitchable Proteins - Superresolution Microscopy

Photoswitching Mechanism



Photoswitchable fluorescent proteins can be toggled between fluorescent and non-fluorescent states using specific wavelengths of light. This unique property forms the basis of superresolution microscopy techniques like PALM (Photoactivated Localization Microscopy) and STORM (Stochastic Optical Reconstruction Microscopy), which overcome the diffraction limit of conventional light microscopy (~200nm) to achieve resolutions of 20-50nm.

Key Photoswitchable Proteins & Mechanisms:

- **Dronpa:** Reversibly photoswitchable between fluorescent (488nm) and dark states (405nm activation)
- **mEos family:** Photoconvertible from green (516nm) to red (581nm) with 405nm light - irreversible switch
- **PA-GFP:** Photoactivatable - increases fluorescence 100-fold upon 405nm irradiation
- **Dendra2:** Green-to-red photoconversion with improved photostability and brightness
- **Single-molecule detection:** Sparse activation enables precise localization of individual molecules
- **Resolution breakthrough:** Achieves 10-20× better resolution than conventional microscopy

Superresolution Techniques:

PALM (Photoactivated Localization Microscopy)

Uses photoactivatable proteins to sequentially activate, localize, and bleach individual molecules

STORM (Stochastic Optical Reconstruction)

Employs photoswitchable probes to create stochastic blinking for superresolution imaging

Pulse-Chase Experiments

Photoconvert specific protein pools to track their fate separately from newly synthesized proteins

Subcellular ROI Marking

Selectively photoactivate proteins in specific cellular regions to study local dynamics



Impact on Cell Biology:

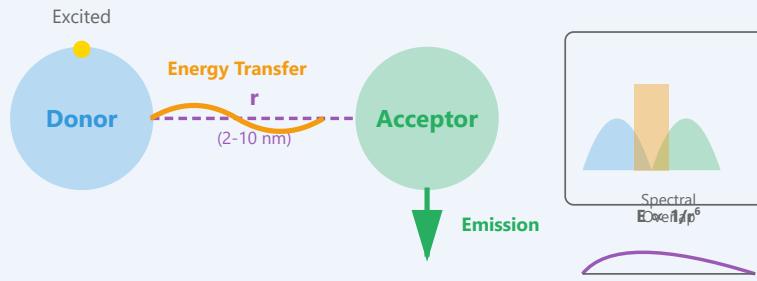
Photoswitchable proteins have revolutionized our ability to visualize cellular structures at the nanoscale level. They enable researchers to resolve previously invisible details such as synaptic vesicle organization, nuclear pore complex architecture, cytoskeletal ultrastructure, and the precise spatial organization of signaling complexes. The 2014 Nobel Prize in Chemistry was awarded to developers of superresolution microscopy techniques that rely on these remarkable proteins.

Fluorescent Proteins: Illuminating Life at the Molecular Level

From the discovery of GFP in jellyfish to the development of sophisticated photoswitchable variants, fluorescent proteins have transformed our ability to observe living cells. These genetically encoded tools continue to drive innovations in cell biology, neuroscience, developmental biology, and beyond.

FRET and Molecular Interactions

Förster Resonance Energy Transfer Mechanism



FRET Efficiency

$$E = R_0^6 / (R_0^6 + r^6)$$

R_0 : Förster radius

r : Donor-acceptor distance

When $r = R_0$, $E = 50\%$

Highly distance-dependent:

$1/r^6$ relationship makes FRET exquisitely sensitive to small distance changes

R_0 Calculations

Förster radius typically 2-10 nm
Depends on spectral overlap $J(\lambda)$
Quantum yield of donor
Orientation factor κ^2

FRET Pairs

Classic: CFP-YFP
Red-shifted: GFP-RFP
Organic dyes: Alexa Fluor, ATTO
Quantum dots: Semiconductor nanocrystals

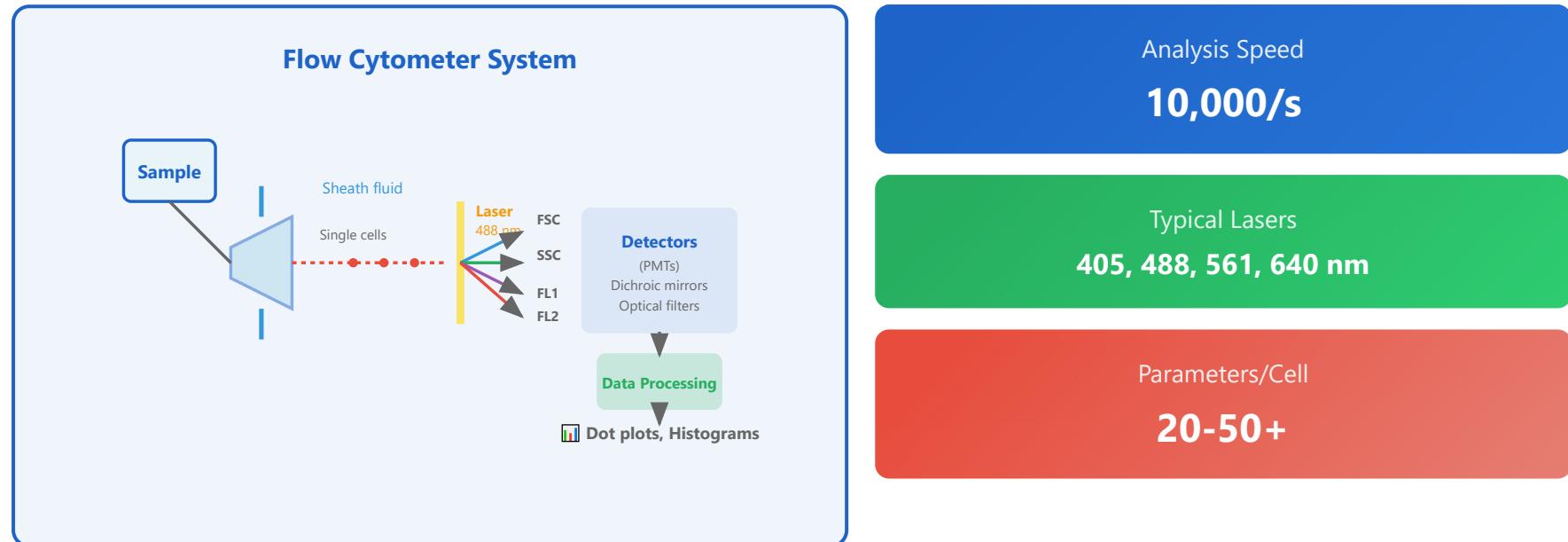
Biosensor Design

Conformational change sensors
Examples: Ca^{2+} , cAMP, kinases
Protein-protein interactions
Enzyme activity reporters

Live Cell Applications

Real-time protein interactions
Signaling pathway dynamics
Molecular proximity measurements
Drug screening assays

Flow Cytometry Principles



Fluidics System

Hydrodynamic focusing creates single-cell stream
Sheath fluid (PBS) surrounds sample
Laminar flow for precise alignment

Laser Excitation

Multiple lasers for multicolor detection
Common: 405, 488, 561, 640 nm
Each excites different fluorophores

Detection Channels

FSC: Forward scatter (cell size)
SSC: Side scatter (granularity)
FL1-FLn: Fluorescence PMTs

Compensation

Corrects spectral overlap between fluorophores
Single-color controls essential
Software or hardware compensation

 Applications:

Immunophenotyping • Cell cycle analysis • Apoptosis detection • Rare cell identification • Biomarker expression

FACS Sorting

Fluorescence-Activated Cell Sorting



Droplet Formation

High-frequency vibration creates uniform droplets. Poisson statistics ensure one cell per droplet.

Charge Deflection

Electrostatic deflection: $\pm 3000\text{-}5000V$. Precise timing critical for accurate sorting.

Purity vs Yield

Index Sorting

Tradeoff in gating strategy. Purity mode: >99%. Yield mode: maximize recovery.

Link phenotype to well position. Enables single-cell sequencing correlation.

Spectroscopy in Diagnostics

Clinical Chemistry

Automated analyzers. Glucose, electrolytes, enzymes.

Immunoassays

ELISA, CLIA. Antibody-based detection. High sensitivity.

Molecular Diagnostics

PCR, qPCR, NGS. Pathogen detection, cancer markers.

Validation

Accuracy, precision, sensitivity, specificity. FDA/CLIA requirements.

1. Clinical Chemistry

Overview

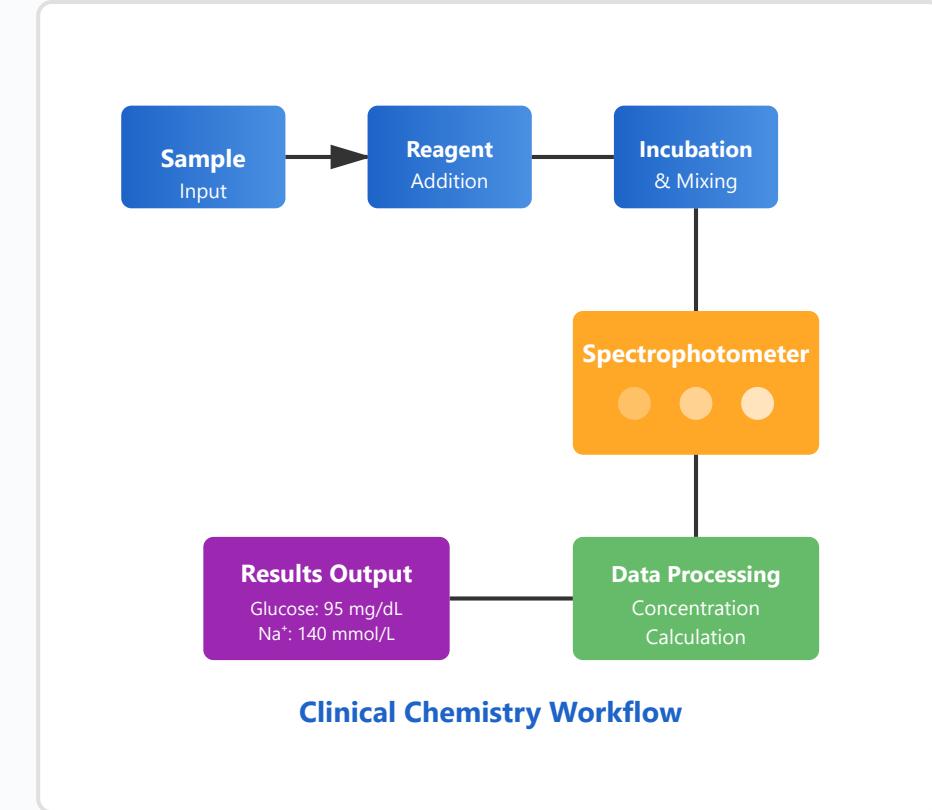
Clinical chemistry utilizes spectroscopic techniques to quantify biochemical substances in body fluids. Modern automated analyzers process hundreds of samples per hour using various spectroscopic methods.

Key Techniques

- **UV-Visible Spectroscopy:** Measures absorbance at specific wavelengths for colorimetric assays
- **Photometry:** Quantifies enzyme activities through substrate-product reactions
- **Ion-Selective Electrodes (ISE):** Measures electrolytes (Na^+ , K^+ , Cl^- , Ca^{2+})

Common Applications

- Blood glucose monitoring (diabetes management)
- Liver function tests (ALT, AST, bilirubin)
- Kidney function tests (creatinine, BUN)
- Lipid panels (cholesterol, triglycerides)
- Electrolyte balance assessment



2. Immunoassays

Overview

Immunoassays leverage antigen-antibody interactions combined with spectroscopic detection to identify and quantify specific analytes with exceptional sensitivity and specificity.

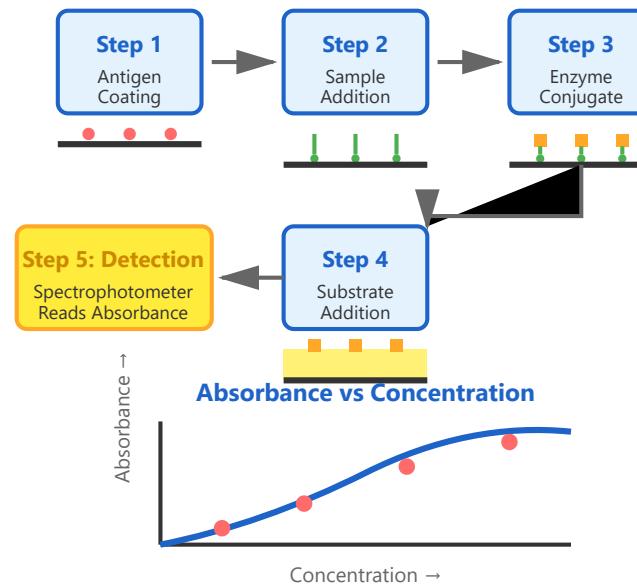
Major Techniques

- **ELISA (Enzyme-Linked Immunosorbent Assay):** Uses enzyme-substrate reactions producing colored products measured spectrophotometrically
- **CLIA (Chemiluminescence Immunoassay):** Measures light emission from chemical reactions, offering higher sensitivity
- **Fluorescence Immunoassays:** Utilize fluorescent labels for detection at very low concentrations

Clinical Applications

- Hormone level testing (thyroid, reproductive hormones)
- Tumor marker detection (PSA, CEA, CA-125)
- Infectious disease screening (HIV, hepatitis)
- Allergy testing (IgE antibodies)
- Autoimmune disease diagnosis
- Drug monitoring and toxicology

ELISA Process



3. Molecular Diagnostics

Overview

Molecular diagnostics analyzes biological markers in the genome and proteome using spectroscopic and fluorescence-based techniques to detect genetic variations, pathogens, and disease markers at the molecular level.

Core Technologies

- **PCR (Polymerase Chain Reaction):** Amplifies specific DNA sequences for detection
- **qPCR (Quantitative PCR):** Real-time fluorescence monitoring of DNA amplification
- **NGS (Next-Generation Sequencing):** Massive parallel sequencing with fluorescent detection
- **Fluorescence In Situ Hybridization (FISH):** Visualizes genetic material in cells

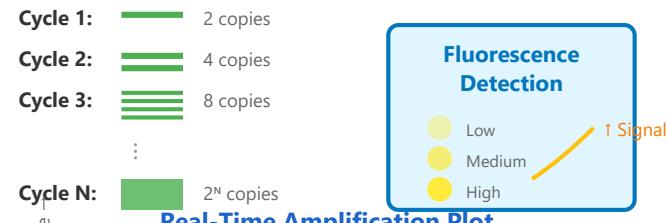
Clinical Applications

- Infectious disease identification (COVID-19, influenza, tuberculosis)
- Cancer genomics and personalized oncology
- Genetic disorder screening and prenatal testing
- Pharmacogenomics for drug response prediction
- Minimal residual disease monitoring
- Transplant compatibility testing

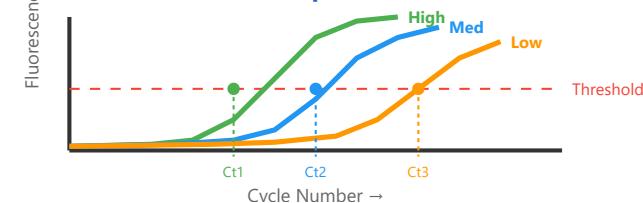
Real-Time PCR (qPCR) Process



Amplification Cycles



Real-Time Amplification Plot



4. Validation

Overview

Analytical validation ensures that diagnostic tests produce accurate, reliable, and reproducible results. Regulatory compliance with FDA and CLIA standards is essential for clinical implementation.

Key Performance Metrics

- **Accuracy:** Closeness of measured value to true value (assessed using reference materials)
- **Precision:** Reproducibility of results (intra-assay and inter-assay variability)
- **Sensitivity:** Ability to correctly identify positive cases (True Positive Rate)
- **Specificity:** Ability to correctly identify negative cases (True Negative Rate)
- **Limit of Detection (LOD):** Lowest detectable concentration
- **Limit of Quantification (LOQ):** Lowest accurately quantifiable concentration

Regulatory Requirements

- **FDA Approval:** Required for in vitro diagnostic devices
- **CLIA Certification:** Laboratory quality standards
- **Quality Control:** Daily calibration and control samples
- **Proficiency Testing:** External quality assessment programs

Validation Performance Metrics

Confusion Matrix

		Disease +	Disease -	Quality Control
		True Positive (TP) Correctly identified	False Positive (FP) Type I Error	✓ Daily calibration ✓ Control samples ✓ Proficiency testing ✓ Equipment maintenance ✓ Staff training
Test +	Disease +	True Positive (TP) Correctly identified	False Positive (FP) Type I Error	
	Disease -	False Negative (FN) Type II Error	True Negative (TN) Correctly rejected	

Performance Calculations

Sensitivity $TP / (TP + FN)$	Specificity $TN / (TN + FP)$	Regulatory FDA Approval CLIA Certification
Accuracy $(TP + TN) / Total$	Precision (PPV) $TP / (TP + FP)$	

Point-of-Care Devices: Comprehensive Overview

Lateral Flow Assays

Pregnancy tests, COVID-19, Rapid diagnostics. Gold nanoparticles.

Microfluidic Platforms

Lab-on-a-chip. Minimal sample volume. Integrated detection.

Smartphone Readers

Camera-based detection. AI image analysis. Telemedicine integration.

Colorimetric Tests

Visual readout. No instrumentation. Resource-limited settings.

1. Lateral Flow Assays (LFAs)

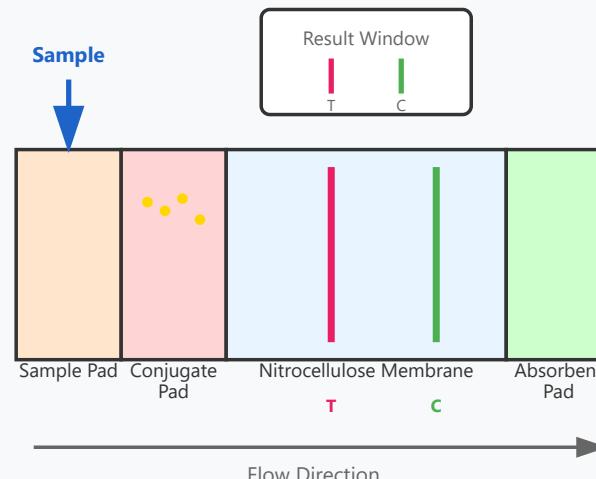
Lateral flow assays are paper-based diagnostic devices that enable rapid detection of target analytes in complex samples. They are among the most widely used point-of-care diagnostic tools globally.

Key Components:

- **Sample pad:** Where the liquid sample is applied
- **Conjugate pad:** Contains labeled antibodies (often gold nanoparticles)
- **Nitrocellulose membrane:** Contains test and control lines
- **Absorbent pad:** Draws the sample through the device

Working Principle:

- Sample flows via capillary action through the device



Schematic diagram of a lateral flow assay showing sample application, flow path, and test/control lines

- Target molecules bind to labeled antibodies
- Complexes are captured at the test line
- Excess labeled antibodies bind at the control line

Common Applications:

- Pregnancy tests (hCG detection)
- COVID-19 rapid antigen tests
- Malaria detection
- Cardiac biomarkers (troponin)
- Drug screening

Advantages:

- Results in 5-30 minutes
- No specialized equipment required
- Low cost (~\$1-10 per test)
- Long shelf life at room temperature

2. Microfluidic Platforms (Lab-on-a-Chip)

Microfluidic platforms manipulate small volumes of fluids (microliters to picoliters) in microscale channels. These "lab-on-a-chip" devices integrate multiple laboratory functions on a single chip.

Key Features:

- **Channel dimensions:** 10-500 micrometers
- **Sample volume:** 1-100 microliters

- **Materials:** PDMS, glass, silicon, polymers
- **Fabrication:** Soft lithography, 3D printing, laser ablation

Integrated Functions:

- Sample preparation and purification
- Mixing and reaction chambers
- Separation (electrophoresis, chromatography)
- Detection (optical, electrochemical)
- Multiplexed analysis

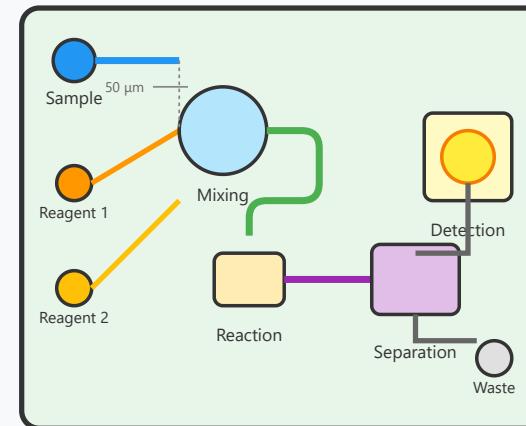
Applications:

- **Clinical diagnostics:** Blood analysis, immunoassays
- **Molecular biology:** PCR, DNA sequencing
- **Drug discovery:** Cell-based assays
- **Environmental:** Water quality monitoring

Advantages:

- Reduced sample and reagent consumption
- Faster analysis times
- Portable and automated systems
- High throughput and multiplexing
- Cost-effective per test

Microfluidic Lab-on-a-Chip



Microfluidic chip design showing sample/reagent inlets, mixing chambers, reaction zones, and integrated detection

3. Smartphone-Based Diagnostic Readers

Smartphone readers leverage the computational power, high-resolution cameras, and connectivity of mobile devices to transform them into portable diagnostic instruments.

System Components:

- **Hardware attachment:** Optical adapters, lenses, LED illumination
- **Mobile application:** Image capture, processing, analysis
- **Cloud connectivity:** Data storage, AI processing, telemedicine
- **Test cartridge:** Sample holder, assay platform

Detection Mechanisms:

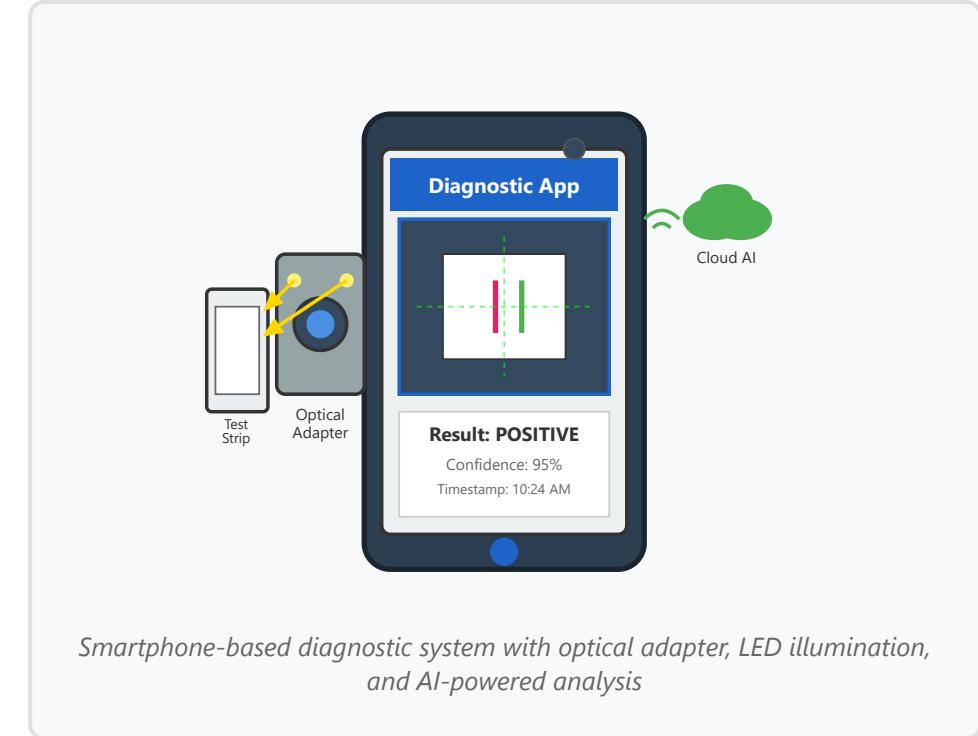
- **Colorimetric:** RGB analysis, color intensity quantification
- **Fluorescence:** LED excitation, filter-based emission detection
- **Microscopy:** High-resolution imaging of cells, pathogens
- **Chemiluminescence:** Light emission detection

AI Integration:

- Machine learning for image classification
- Automated result interpretation
- Quality control and error detection
- Personalized health recommendations

Applications:

- **Infectious diseases:** Malaria, tuberculosis detection
- **Chronic disease:** Diabetes monitoring, cholesterol
- **Water quality:** Contamination detection
- **Telemedicine:** Remote diagnosis and consultation



Advantages:

- Widespread device availability (global smartphone penetration)
- Built-in GPS for geotagging and disease tracking
- Real-time data transmission to healthcare providers
- Cost-effective infrastructure utilization
- Regular software updates and feature expansion

4. Colorimetric Tests

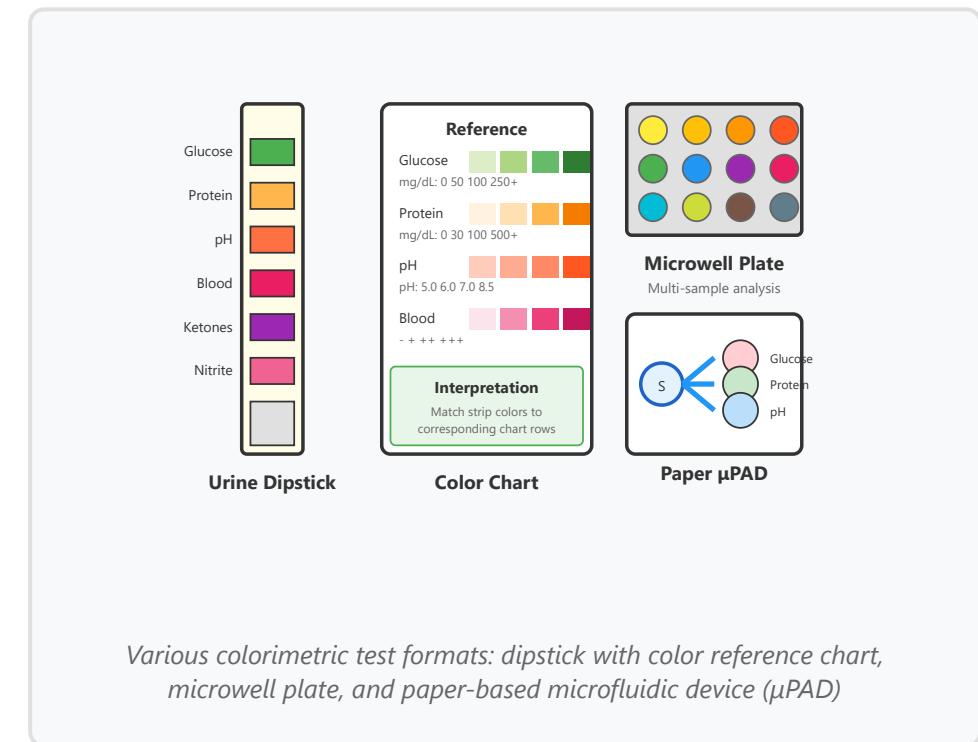
Colorimetric tests produce visible color changes that can be interpreted by the naked eye or simple optical devices. These tests are ideal for resource-limited settings where sophisticated instruments are unavailable.

Detection Principles:

- **pH indicators:** Color change based on acidity/alkalinity
- **Enzymatic reactions:** Chromogenic substrates
- **Metal complexation:** Colored metal-ligand complexes
- **Nanoparticle aggregation:** Gold/silver nanoparticle color shifts
- **Redox reactions:** Oxidation-reduction color changes

Test Formats:

- **Dipstick tests:** Paper or plastic strips with reagent zones
- **Microwell plates:** 96-well format for multiple samples
- **Microfluidic paper devices:** Patterned paper channels



- **Test tubes:** Simple solution-based assays

Common Applications:

- **Urinalysis:** Glucose, protein, pH, ketones, blood
- **Water quality:** pH, chlorine, nitrates, heavy metals
- **Food safety:** Contaminant detection, freshness indicators
- **Clinical chemistry:** Glucose, cholesterol monitoring
- **Agriculture:** Soil nutrient testing

Advantages:

- No instrument required for basic applications
- Immediate visual results
- Very low cost (\$0.10-\$2 per test)
- Minimal training needed
- Suitable for field deployment
- Long shelf life and stability

Quantification Methods:

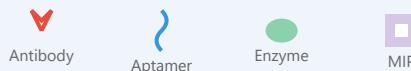
- Visual comparison to color charts
- Smartphone camera with color analysis apps
- Portable spectrophotometers
- Scanners with image processing software

Biosensor Technologies

Biosensor Architecture & Signal Transduction



Recognition Elements



High specificity biorecognition layers

Transduction Methods

- Optical: SPR, SERS, fluorescence
- Electrochemical: CV, DPV, EIS
- Piezoelectric: QCM, SAW
- Thermal: Calorimetric

Surface Chemistry

SAMs: Self-assembled monolayers
PEG: Anti-fouling coating
Blocking: BSA, casein
Minimize non-specific binding

Signal Amplification

Enzyme cascades: HRP, ALP
Nanoparticles: Au, Ag enhancement
SERS: 10^6 - 10^{14} fold
Improve LOD to fM-aM range

Hands-on: Spectral Data Analysis

Python/R for Spectral Analysis

- Libraries: NumPy, SciPy, Matplotlib, pandas
- Baseline correction: Polynomial, asymmetric least squares
- Peak fitting: Gaussian, Lorentzian, Voigt
- Multivariate: PCA, PLS-DA for classification
- Quality metrics: SNR, resolution, reproducibility

```
import scipy.signal as signal
peaks, _ = signal.find_peaks(spectrum, height=0.1)
```

Hands-on: Python for Signal Processing

Signal Processing Essentials

- FFT analysis: Frequency domain transformation
- Filtering: Low-pass, high-pass, band-pass, Savitzky-Golay
- Noise reduction: Moving average, Wiener filter
- Feature extraction: Peak detection, integration, moments
- Deconvolution: Separate overlapping signals

```
from scipy.signal import savgol_filter
smoothed = savgol_filter(data, window=11, polyorder=2)
```

Thank You & Resources

Thank You!

Questions?

Resources

- Lakowicz: Principles of Fluorescence Spectroscopy
- Skoog: Principles of Instrumental Analysis
- Online simulators: PhET, Fluorophores.org
- Software: ImageJ/Fiji, Python (SciPy, scikit-learn)
- Next lecture: Advanced Imaging Techniques

Thank You & Resources

Thank You!

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