1 Introduction to biomedical Sensors

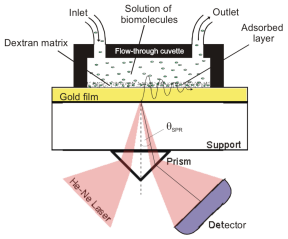
Biosensor:

- A device that detects, records and transmits information regarding a physiological change or process.
- A device that uses biological materials to monitor the presence of various chemicals in a substance

Bioelectronics:

- The application of the principles of electronics to biology and medicine.
- The study of the role of intermolecular electron transfer in physiological processes.

Surface Plasmon Resonance:
A resonant coupling between the light and the surface plasmons in the conducting film occurs at a specific angle.The reflected light will have a "shadow" at the resonance angle. This angle is *sensitive to the adsorption of proteins at the interface.*



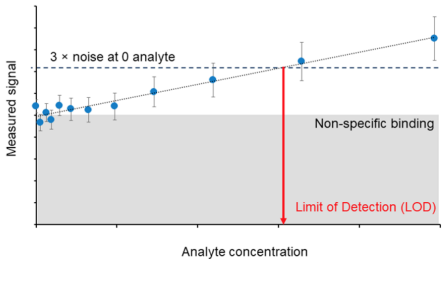
Biosensor Principle: Biosample → Element recognition → Transducer → Signal Processing → Signal
Can capture the wanted molecules with antibodies that were previously created by rats.

1.1 Current Problems:

Sample Size: Tumor is curable when it is really small, but hard to find without big Aufwand
Sensitivity: All biosensing techniques are limited by the non-specific interactions.
Specificity
Continous Monitoring: → need noninvasive sensors

1.2 Receptor binding:

$k_{on}[A][R] = k_{off}[AR] \rightarrow \boxed{K_a = \frac{k_{on}}{k_{off}} = \frac{[AR]}{[A][R]}}$ (equilibrium constant)
When half of the receptors are occupied, $[AR] = [R]$ → Good way to measure quality of sensor
Non-Specific Binding: other molecules that bind to the same receptor as the analyte. This is a fundamental limit related to the lack of receptors with high-affinity. (big net analogy when specific binding is small compared to non-specific: capture tons of fish, hard to find nemo)
The **limit of detection** (LOD) is defined as the concentration at which the signal $S_{LOD} = S_0 + 3 \cdot \ noise$, with S_0 the signal without analyte. For low analyte concentration the curve signal/(analyte concentration) is a straight line:



$\frac{dS}{d[A]} = \frac{3 \cdot noise}{LOD}$

2 Fundamentals of Microscopy

2.1 Maxwells equations

Continuity Equation: $\vec{\nabla} \cdot \vec{j}(\mathbf{r},t) + \frac{\partial \rho(\mathbf{r},t)}{\partial t}$
Gauss-Law: $\vec{\nabla} \cdot \vec{D}(\mathbf{r},t) = \rho(\mathbf{r},t)$
Farraday's Law: $\vec{\nabla} \times \vec{E}(\mathbf{r},t) = -\frac{\partial}{\partial t}\vec{B}(\mathbf{r},t)$
Ampere/Oersted $\vec{\nabla} \times \vec{H}(\mathbf{r},t) = \frac{\partial}{\partial t}\vec{D}(\mathbf{r},t) + \vec{j}(\mathbf{r},t)$
No magnetic Monopoles: $\vec{\nabla} \cdot \vec{B}(\mathbf{r},t) = 0$

2.1.1 Monochromatic fields: .

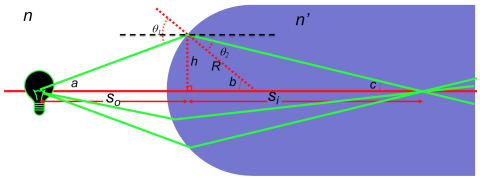
Gauss-Law: $\vec{\nabla} \cdot \vec{D}(\mathbf{r}) = \rho(\mathbf{r})$
Farraday's Law: $\vec{\nabla} \times \vec{E}(\mathbf{r}) = -i\omega\vec{B}(\mathbf{r})$
Ampere/Oersted: $\vec{\nabla} \times \vec{H}(\mathbf{r}) = i\omega\vec{D}(\mathbf{r}) + \vec{j}(\mathbf{r})$

2.1.2 The wave equation: .

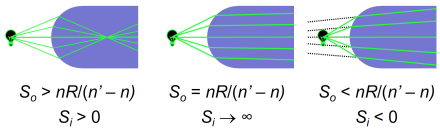
$\vec{\nabla} \times \vec{\nabla} \times \vec{E} + \frac{1}{c^2}\frac{\partial^2 \vec{E}}{\partial t^2} = -\mu_0 \frac{\partial}{\partial t}(\vec{j} + \frac{\partial P}{\partial t} + \vec{\nabla} \times M)$
$\vec{\nabla} \times \vec{\nabla} \times \vec{H} + \frac{1}{c^2}\frac{\partial^2 \vec{H}}{\partial t^2} = (\vec{\nabla} \times \vec{j} + \vec{\nabla} \times \frac{\partial P}{\partial t} + \frac{1}{c^2}\frac{\partial^2 M}{\partial t^2})$
Direction of propagation: $\vec{E} \times \vec{B}$
Velocity: $c \approx 3 \cdot 10^8 \ m/s$
$|\vec{E_0}| = c|\vec{B_0}|$; $ck = \omega$
At large distances from the source, a spherical wave may be approximated by a plane wave

2.2 Reflection & Refraction of plane waves

Law of reflection: $\theta_i = \theta_r$
Continuity of wavefronts across the boundary: $\lambda_{glass}/sin(\theta_i) = \lambda_{air}/sin(\theta_t)$ → Law of refraction (Snell's Law): $n_i sin(\theta_i) = n_t sin(\theta_t)$
total reflection for $\theta_i = \theta_c$, with $sin(\theta_c) = n_t/n_i$
$\lambda_{air} = \lambda_{vac}/n_{air}$

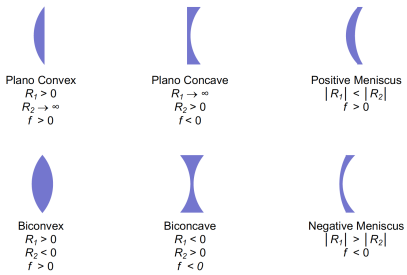


$n \ sin(\theta_1) = n' \ sin(\theta_2)$
$sin(\theta_1) \approx \sin\alpha + \sin b \approx \frac{h}{s_0} + \frac{h}{R}$
$sin(\theta_2) \approx \sin b + \sin c \approx \frac{h}{R} + \frac{h}{s_i}$
$\Rightarrow \boxed{\frac{n}{s_0} + \frac{n'}{s_i} = \frac{(n'-n)}{R}}$
- Paraxial approx. $\theta \approx \sin\theta \approx \tan\theta$
- Thin lens approx: $R << S_0, S_i$
- S_i does not depend on the angle



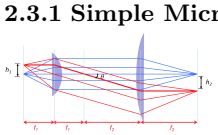
2.3 Lenses

Lens makers' fomrula: $\boxed{\frac{1}{S_0} + \frac{1}{S_i} = \frac{1}{f} = \frac{(n'-n)}{n}(\frac{1}{R_1} - \frac{1}{R_2})}$
f is the **focal length**

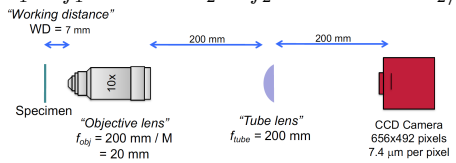


- Rays passing through the optical center of a lens continue in a straight line
- Rays traveling parallel to the optical axis pass through the focal point after refraction and vice versa
- Parallel rays pass through the same point in the focal plane after refraction and vice versa

2.3.1 Simple Microscope: .



Object is magnified by h_2/h_1
$h_1 = f_1 \sin\theta$ and $h_2 = f_2 \sin\theta \Rightarrow M = h_2/h_1 = f_2/f_1$



Preparing biological specimen for imaging: Refractive index matching liquid between the specimen and the lens determines the efficiency of light collection from the specimen, hence brightness and resolution.

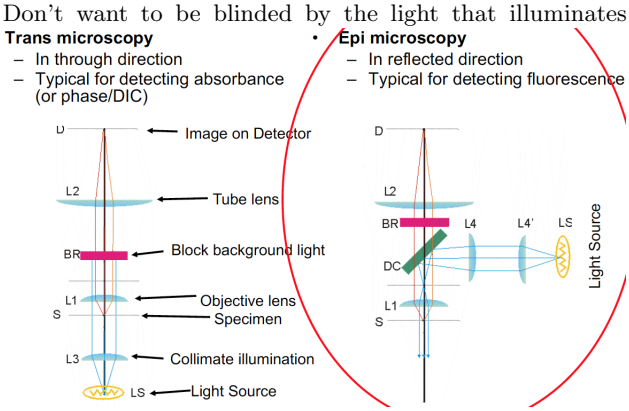
2.3.2 Lens limitations: .

Spherical aberration: lightrays will not focus on the same point along the horizontal axis
Coma: lightrays will not focus on the same point on the vertical axis
Chromatic aberration: Lens fails to focus all wavelengths on the same point can use different sheets of lenses to compensate for this)

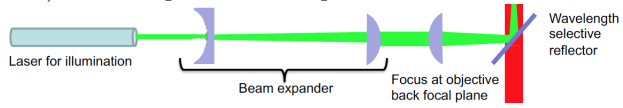
2.3.3 Resolution: .
When can two point sources imaged onto a screen be distinguished? Each point source gives an airy disk on the screen: $I(\theta) = I_0 \left(\frac{2J_1(kd\sin\theta)}{kd\sin\theta}\right)^2$, that can be distinguished when the maximum of one is not closer than the first minimum of the other. Where I_0 is the maximum intensity of the pattern at the Airy disc center, J_1 is the Bessel function of the first kind of order one, $k = 2\pi/\lambda$ is the wavenumber, d is the radius of the aperture, and θ is the angle of observation.
The first zero occurs at: $\sin\theta \approx 1.22\frac{\lambda_0}{d} \implies$ larger aperture, thinner PSF, better resolution.
Resolution: $R \approx f\sin\theta = 1.22\lambda_0 f/d = 0.61\lambda_0 n/NA$ (Rayleigh Criterion, works only when imaging process is only limited by diffraction)
Standart terminology: *Numerical Aperture (NA):* measure of light gathering capacity. $NA = n\sin\theta = nd/(2f)$ with n the index of refraction
can't use to small wavelengths to not damage the biological specimen

2.4 Illumination of sample

Don't want to be blinded by the light that illuminates the sample



blue lines = illumination light, orange lines = detected light
As we don't want our light to be focused on one point by the lens, we focus it at the objective back focal plane, so that it will then be evenly distributed on the sample (the objective lens will make the meams parallel). We also put a beam expamder first.

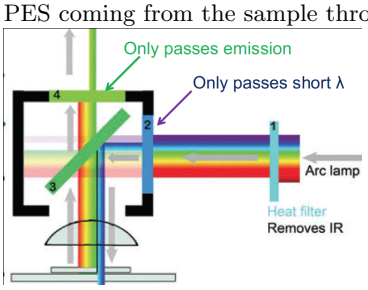


2.5 White-light Microscopy

Measures variations in refractive index, absorption, polarization and light-scattering. **Problems:** - Not specific enough to distinguish different types of molecules
- Not sensitive enough to detect single or even few molecules
- Cannot resolve objects much beyond the diffraction limit of light

2.6 Fluorescence Microscopy

Fluorescence: Photon absorption → electron excitation → electron loses some energy to nucleus vibrations → photon emission with less energy (gives a way to distinguish the illumination light to the reflected light)
It is best to illuminate the sample in it's peak absorption spectrum (PAS) and to capture in its peak emission spectrum (PES). The lamp light is first filtered by an "heat filter", which removes high λ. Above the sample is a filter which reflects PAS into the sample and let's the PES coming from the sample through.



The filters work with destructive interference and not with absorption.
Ideal lightsource: - Monochromatic (to excite the specimen at its peak absorption wavelength))
- High spectral radiance (for sufficient light intensity at particular wavelength)
- Low divergence - unidirectional (for tight focusing, or uniform illumination)
$\implies$ **Laser**

3 Mechanical Sensors

3.1 Surface Stress change detection

Molecules on top of a slice will apply mechanical stress to the surface and bend it. When shinig light on it, the change in angle of the reflected beam can be measured to measure the stress. Problem is non-specific binding. Could still be used for scent detection, as there is not much NSB in air.

3.1.1 Stress σ $[kg\cdot m^{-1}\cdot s^{-2}]$: .
Elastic media: $\sigma = \frac{F}{A} = \mu\frac{\partial u}{\partial z}$, μ being the elasticity and u the displacement
Viscous media: $\sigma = \frac{F}{A} = \eta\frac{\partial}{\partial z}(\frac{\partial u}{\partial t})$ η, being the viscosity
Viscoelastic media: $\sigma = \frac{F}{A} = \mu\frac{\partial u}{\partial z} + \eta\frac{\partial}{\partial z}(\frac{\partial u}{\partial t})$
3.1.2 Equation of motion: .
$u = u(z,t)$; $F = ma = m\cdot\frac{d^2u}{dt^2} \implies \rho\frac{d^2u}{dt^2} = \frac{\partial}{\partial z}(\mu\frac{\partial u}{\partial z} + \eta\frac{\partial}{\partial z}\frac{\partial u}{\partial t})$ (1)
with ρ being the density, $\tilde{\mu} = \mu + i\omega\eta$
harmonic solution: $u(z,t) = \tilde{u}(z)\cdot e^{i\omega t} \implies -\omega^2\rho = \tilde{\mu}k^2$
Elastic media: $\tilde{\mu} = \mu \implies k = \pm i\omega\sqrt{\frac{\rho}{\mu}}$
Viscous media: $\tilde{\mu} = i\omega\eta \implies k = \pm(i+1)\sqrt{\frac{\omega\rho}{2\eta}}$
Solution to (1): $\tilde{u}(z) = u_1e^{kz} + u_2e^{-kz}$
Crystal of thickness l in vacuum: $\omega_n = n\underbrace{\frac{\pi}{l}\sqrt{\frac{\mu}{\rho}}}_{\omega_0}$, $n = 0,1,2,..$
In water: $\omega_n = n\cdot\omega_0 + \Delta\omega_n$, $\Delta\omega_n = -\sqrt{n}\cdot\sqrt{\frac{\rho_w\cdot\eta_w\cdot\omega_0}{2}}\cdot\frac{1}{l\cdot\rho_q}$

3.2 Quartz Crystal Mycrobalance

Piezoelectric effect: Piezoelectricity is the electric charge that accumulates in certain solid materials (such as crystals, certain ceramics, and biological matter such as bone, DNA and various proteins) in response to applied mechanical stress.
Can use this effect to make the crystal resonate at resonance frequency $f = \frac{n\cdot v}{\lambda} = \frac{n\cdot v}{2t}$.
If a mass is added, the oscillation slows down: $\Delta m = Const\cdot\Delta f$ Measure the frequency decay of the oscillation and the dissipation: $D = \frac{1}{\pi f\tau}$ ceveral times to get viscosity, elasticity, density and thickness of adsorbed layer.
Adsorption of rigid film in vacuum: film: ρ_f, d_f; $\Delta f \propto \Delta m = \rho_f\cdot d_f$; $\Delta D = 0$
One side in an aqueous solution: Fluid: ρ_l, η_l; Δf, $\Delta D \propto \sqrt{\rho_l\cdot\eta_l}$
Sauerbrey Model: $\Delta m = \frac{C}{n}\cdot\Delta f$ with C: sensitivity factor, $n = 0,1,2,..$ and $\Delta m = \rho\cdot h$ (for C $[\frac{ng}{Hz\cdot cm^2}]$, might need to multiply both Δm and C by the area to get the right units/ the area should not appear in the end result)
Is an approx. for thin adlayers

3.3 Strain gauge transducers

Measure change in resistance to measure strain:
$\frac{\Delta R}{R} = K\frac{\Delta l}{l} = K\epsilon$; ϵ : strain, K: Gauge factor (≈ 2), R: resistance
Measure change in resistance on nanowires to measure strain: when nano-wire conductor gets elongated, some will lose touch and thus the resistance will go up, can attain extremely high gauge factors by controlling the contact resistance
3.3.1 Capacitive strain sensor : .
$C = \epsilon_0\cdot\epsilon_r\cdot\frac{A}{d}$ → change in capacitance will change the resonance frequency of RLC circuit $\omega_0 = \frac{1}{\sqrt{LC}}$.
Used to wirelessely control the bladder volume.
RLC circuit: $V_L = L\cdot\frac{dI_L}{dt}$ $I_C = C\cdot\frac{dV_C}{dt}$

4 Fluorescent Probes

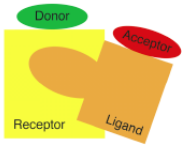
Use $\lambda = 510$nm for GFP markers. **Photobleaching:** the photochemical alteration of a fluorophore molecule such that it permanently is unable to fluoresce. Increasing light intensity increases photobleaching (molecules have a limit to how much they can emit).
Photon Absorption → Electron Excitation → Electron Relaxation by excitation of nuclear vibrations → Photon Emission OR Vibrational relaxation to ground state OR Chemical Reaction (Photobleaching)
Quantum Yield/efficiency: $\frac{\Gamma}{\Gamma + k + K_b} \sim 0 - 98\%$
Fluorescence lifetime: $\tau = \frac{1}{\Gamma + k + K_b} \sim 1ns$
with Γ: radiative decay rate, k: non-radiative decay rate, K_b: photobleaching rate

4.1 Fluorescence lifetime imaging

Decay of number of molecules in excited state: $\frac{dN_e}{dt} = -(k+\Gamma)N_e$
N_e: number of molecules in excited state
Decay in fluorescent emission intensity: $F = F_0 e^{-(k+\Gamma)t} = F_0 e^{-t/\tau}$
F and F_0 are the instantaneous and initial fluorescence intensity.
Fluorescence emission is characterized by exponential decay.

4.2 Förster resonance energy transfer (FRET)

Excited molecule can transfer its energy to nearby (few nanometers) molecules. The emission is then going to be at the emission wavelength of the neighbour. → permits to measure if there is receptor/ligand interaction.



FRET is a dipol-dipole interaction and is very sensitive to r (r^{-6})

Efficiency of FRET: $E = \frac{R_0^6}{R_0^6 + r^6} = 1 - \frac{\tau_{DA}}{\tau_D} = 1 - \frac{I_{DA}}{I_D}$

DA/D: Donor with/without acceptor A, $\frac{\tau_{DA}}{\tau_D}$: Lifetime/Intensity of donor emission with/without acceptor, **Förster ditance:** $R_0 = 40$ to 70 Å when efficiency of energy transfer is 50%
Smaller distance → Reduced donor lifetime and donor emission intensity but higher acceptor emission intensity
- Lightsource should excite donor but not acceptor -no overlap
- Need spectral overlap with donor emission and acceptor excitation spectrum for efficient energy transfer

4.3 Calcium Imaging

Calcium regulates cellular processes such as cell division, muscle contraction, fertilization, blood clotting, and synaptic transmission/plasticity → measure calcium to measure acitivity
Idea: Design molecules with optical properties that change upon calcium binding.
Single wavelength measurements:
$K_d = \frac{[Ca^{2+}]_i \cdot [Unbound\ dye \sim F_{max} - F]}{[Ca^{2+} - bound\ dye \sim F - F_{min}]}$
$\implies [Ca^{2+}]_i = K_d \frac{F - F_{min}}{F_{max} - F}$ with K_d: dissociation constant of the indicator, F_{min}: fluorescence at zero Ca^{2+} concentration, F_{max}: fluorescence at saturating Ca^{2+} levels. *Problem:* need to know the dye concentration
Dual-wavelength excitation measurements: Do two measurements of fluorescence F_1 and F_2 which are depending on excitation wavelength. $R = \frac{F_1}{F_2}$
$[Ca^{2+}] = K_{eff} \frac{R - R_{min}}{R_{max} - R}$ with R_{min}: ratio in Ca^{2+} free solution, R_{max}: ratio at Ca^{2+} saturating levels, $Keff$: effective binding constant

4.3.1 Principles of different calcium indicators: .

- The binding of Ca^{2+} leads to a change in fluorescence intensity but not wavelength change (fluo-4, rhod-2, calcium green)
- The binding of Ca^{2+} results in a shift in excitation and sometimes emission peaks – ratiometric indicators (fura-2, quin-2, indo-1)
- The binding of Ca^{2+} results in changes in fluorescent resonance energy transfer (FRET e.g. chameleons) - The binding of Ca^{2+} leads to a change in fluorescence life time (FLIM, e.g. indo-1)

4.3.2 Delivery of Calcium Indicators to Cells: .

Loading cells using acetoxymethyl (AM) esters of Ca^{2+} indicators: AM will neutralise the charge of indicator, which will permit it to cross cell membrane. Inside the cell the indicator will get charged (thus becomes trapped in cell) and becomes fluorescent. Cautions: too high concentration of indicator can alter $[Ca^{2+}]$ and generate toxic byproducts.
A dye with too high Ca^{2+} affinity: very sensitive, but very slow recovery
A dye with too low Ca^{2+} affinity: very insensitive, but very fast recovery

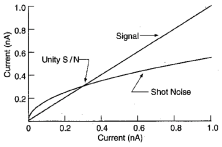
5 Signal and Noise

Technical noise: due to detector imperfections -can be avoided by good design

5.1 Shot Noise

Shot Noise (N_s): Shot noise exists because phenomena such as light and electric current consist of the movement of discrete 'packets'. It is is temperature and frequency independent. Statistical fluctuation of uncorrelated random events obey **Poisson statistics**: $P(n|\bar{n}) = e^{-n}\frac{\bar{n}^n}{n!}$,

$\sigma_n = \sqrt{\bar{n}} = \sqrt{< signal >}$



$< I^2_{noise} >= (\eta q/\Delta t)) < I_{signal} >$
$\implies < I_{noise} > \propto \sqrt{< I_{signal} >}$, with η: quantum efficiency of detector, $\bar{n}$: # photons, q: el. charge
Signal Power: $S =< I^2_{signal} > R$
Noise Power: $N =< I^2_{noise} > R$
Noise equivalent power (NEP): Signal power at which SNR =1

$N_s =< I^2_{noise} > R = 2R\eta q B < I_{signal} >$, with $2B \approx 1/\Delta t$: bw.

Responsivity: $< I > /P = \eta q \lambda/(hc)$

SNR: $\frac{P\lambda}{2hcB} = \bar{n}$

5.2 Dark current Noise

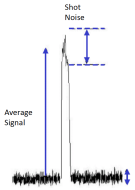
Thermal effect results in some probability of spontaneous production of free electrons. This effect is measured by the dark current amplitude of the device:$< I_d >$. N_d also obeys Poisson statistics: $N_d = 2R\eta q B < I_d >$

5.3 Jonson Noise

Johnson noise (N_j) originates from the temperature dependent fluctuation in the load resistance R of the detection circuit.

$I_J \propto \sqrt{\frac{kTB}{R}}$

Sum of all noises: $\langle I^2_{noise} \rangle = \langle I^2_s \rangle + \langle I^2_D \rangle + \langle I^2_J \rangle$
Total Noise: $N_{noise} = N_s + N_D + N_I$



shot noise dominates other noise at for large signal

6 Optical Detectors

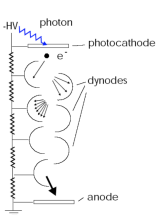
- **Quantum Efficiency:** The probability of generating a photoelectron from an incident photon
- **Internal Amplification:** The amplification ratio for converting a photoelectron into an output current
- **Dynamic Range:** What is the largest and the lowest signal that can be measured linearly
- **Response Speed:** The time difference and spread between an incoming photon and the output current burst
- **Geometric form factor:** Size and shape of the active area and the detector
- **Noise:** Shot noise vs. read-out noise dominated

$SNR = \frac{< signal >}{\sigma_{signal}} = \frac{QE \cdot N_\gamma}{\sqrt{\sigma_D^2 + \sigma_R^2 + \sigma_S^2}}$, QE: quantum efficiency, N_γ: # detected photons

6.1 Photoelectric effect

$hf = \phi + E_k$: incident photon energy = binding energy (ϕ is the work function) + kinetic energy of ejected electrons
- The kinetic energy of emitted electron depends only on the color (wavelength) of the photon but not light intensity (number of photons)
- The number of electrons ejected is proportional to the intensity (number of photons)

6.2 Photomultiplier tube



$I = \alpha \cdot (S \cdot E_\gamma \cdot n/\Delta t)$ with S the **Cathode sensitivity** and α the **gain**. $E_\gamma = hc/\lambda = 4 \cdot 10^{-19} J$

6.3 Photovoltaic Effect

Electron-hole pair generation in semiconductor by incident light → the quantum efficiency of detector η is strongly wavelength dependent, because photons with energy less than Eg cannot generate conducting e-h pairs. The electrons are then moved via **CCD** (charge-coupled detectors -bucket arrays) or via CMOS to be analyzed. CMOS can be faster, but less uniform, noisier than CCDs.
Readout Noise: The noise that the camera's amplifier circuit introduces (specified in spec sheets). It is a combination of Johnson Noises + Amplifier Technical Noise. $N_{total} = N_{readout} + N_{dark} + N_{shot}$ → has high temperature dependency, can cool down to limit noise.
Can overcome readout noise with *Electron-multiplication CCDs (EM-CCDs)*: amplify electrons prior to amplifier circuit by impact ionization on the chip.

6.4 Pixel size

Separation of diffraction limited spots on CCD:
$\Delta x \cdot (f_t/f_o) = 0.61(\lambda/NA) \cdot M$ (magnification $M = f_t/f_o$) see 2.3.3
Maximum pixel size: $\boxed{0.5 \cdot \Delta x \cdot M = 0.3 \cdot (\lambda/NA) \cdot M}$ (Nyquist)
Too large pixels → cannot resolve the image
Too small pixels → too little signal/photon per pixel → readout circuit noise dominates
If CCD pixels are smaller than minimum pixel size OR if there is too little signal, then sum the neighboring pixels (*pixel binning*) to increase signal.

6.4.1 How to choose a camera with the right number of pixels?: .

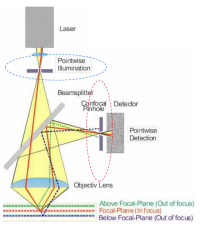
- First make sure FOV of objective (specified by manufacturer) is large enough for what you want to see
- Choose a region of interest (ROI) size within FOV of lens: Specimen within ROI is magnified M times: 250µm ROI w/ M=40 → you need 10 mm CCD size.
- Number of pixels: [CCD Area] / [Pixel size matching diffraction limit]

	PMT	Photodiode	APD (Photodiode with EM)	CCD/CMOS /EMCCD
QE	10-50%	80%	80%	60%-90%
Spectral Range	UV-Green	Blue-NIR	Blue-NIR	Blue-NIR
Internal Gain	10^6-10^8	1	100-1000	1 / 10^3 for EMCCD
Dark Noise (or Area)	e^-/sec	1000 e^-/sec	1 e^-/sec	e^-/sec
Multiplication Noise	Yes	No	Yes	No (Yes for EMCCD)
Electronic (Read) Noise	NA	1000 e^-	NA	3-1000 e^- <1 for EMCCD
Response Speed	+++	+++	+	-
Pixel Size	cm	mm	mm	3–30 µm

7 Scanning Microscopy

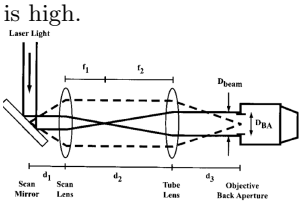
Problems: - out of focus absorption → bleaching
- scattering of light rays in sample → SNR↓

7.1 Confocal Microscope



- Constrain/focus the illumination to a small spot
- Rejects other fluorophores at different locations and scattered fluorescence
- Collect all the light onto a single detector i.e. photomultiplier tube (more efficient than CCD)
→ results in flattening of sinc ripples in PSF and narrows FWHM
Smaller pinhole: Higher xy and depth resolution BUT Less signal → Slower imaging

Smallest pinhole size should be proportional to the resolution of the rest of the optical system: $\boxed{\text{Pinhole size} \sim (0.61 \cdot \lambda/NA) \cdot M)}$
Faster scan: Less bleaching, observe fast physiology
Slower scan: Collect more photons per point (higher SNR, higher resolution/contrast) but increased bleaching
Image one point of sample → need to scan with the lasers to get full image. Direct laser with mirrors for x-y dimensions and move lens for $\vec{z}$
Upgrade: Confocal Nipkow spinning-disk: - Multiple laser points simultaneously illuminating the sample: Array of confocals.
- Spin the disc quickly to collect data from all the points on sample very fast - Limitation: needs CCD, which are less efficient than PMT's.
Problems: Still has out of focus absorption and scattering for illumination and emission + highly inefficient.
Solution: Two-photon excitation: if material interacts at freq. f, send light at f/2 that meets in focal point. For each excitation, two photons of f/2 are absorbed. As only light with freq. f interacts with the material, there is no scattering and no out of focus absorption.
→ less photobleaching
→ no need for pinhole for lightsource nor for detector (one knows exactly where the photons come from, so scattering is no issue)
Disadvantages: Need special lasers with ultrashort-light pulse s.t. likelihood of two photons simultaneously hitting/exciting fluorophore is high.



$d_2 = f_1 + f_2$; $d_1 = \frac{f_1^2}{f_2} + f_1 - d_3(\frac{f_1}{f_2})^2$

8 Optical Biosensors

Ellipsometry: Change in the phase and amplitude of the polarized light provides information about the thickness and refractive index of the adsorbed protein layer.
Optical Immuno Assay: Optical film resonates with certain wavelengths, changes with thickness of film.

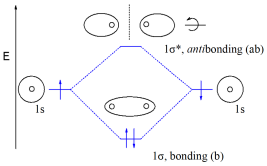
8.1 Surface Plasmon Resonance

Evanescent Field: $E(z) = E_0 e^{\frac{-z}{d_p}}$
Depth of penetration: $d_p = \frac{\lambda}{4\pi\sqrt{n_1^2 \sin^2\theta - n_2^2}}$

9 Molecular Adsorption and Electron Transfer

9.1 Schrödinger Equation

$\hbar = \frac{h}{2\pi} = 1.055 \cdot 10^{-34}\ Js$
$\Psi(r, \theta, \phi) = R(r)\gamma(\theta, \phi)$
Stationary: $\hat{H}|\psi\rangle = E|\psi\rangle$
time independent: $\hat{H}|\psi\rangle = i\hbar\frac{\partial|\psi\rangle}{\partial t}$
Hamiltonian H = kinetic energy + potential energy = $T + V = -\frac{\hbar^2}{2m}\Delta^2 + V$
One electron solution (Hydrogen Atom):
$H = T_n + T_e + Vn - e \implies -\frac{\hbar^2}{2m}\Delta^2\Psi - \frac{Ze^2}{4\pi\epsilon_0 r}\Psi = E\Psi$, $E = \frac{\hbar^2 k^2}{2m}$
Linear combination of atomic orbitals molecular orbital: $\Phi_i = \sum_k C_{ij}\Psi j$
A= # electrons in bonding MO
B= # electrons in anti-bonding MO



Bond Order: $\frac{A-B}{2} (\neq 0$ for stability)
HOMO: Highest Occupied MO
LUMO: Lowest Unoccupied MO

9.2 Formation of Crystal bands

For each atom, 1 energy level: infinite Atoms → infinite energy levels
Fermi-Dirac distribution: $f(E) = \frac{1}{e^{(E-E_F)/(k_B T)}+1}$

9.3 Adsorption

Physisorption: depends on van der Waals interaction, process in which the electronic structure of the atom or molecule is barely perturbed upon adsorption.
Chemisorption: depends on overlapping integral: $S_{ij} = \int \Psi_{mol,i}\Psi_{substr,j}dxdydz \implies$ charge transfer/sharing between surface and molecule
Electrostatic Interaction: $U_{coul} = \sum_{i\neq j}\frac{q_i q_j}{\epsilon_r\epsilon_0|\vec{r_i}-\vec{r_j}|}$

9.4 Electron transfer

Gibbs free energy: thermodynamic potential that can be used to calculate the maximum of reversible work that may be performed by a thermodynamic system at a constant temperature and pressure
$G = U + p \cdot V - T \cdot S$, U: internal energy; P: pressure; V: volume; S: entropy
$\boxed{\textbf{Reaction rate: } k \propto e^{-\frac{\Delta G}{RT}}}$; $R = \frac{k_B}{N_A} = 8.3\ J \cdot K^{-1} \cdot mol^{-1}$, with ΔG: acitvation energy
$\boxed{\textbf{Rate of electron transfer: } k_{et} = K_{P,O} \cdot \nu_n \cdot \kappa_{el} \cdot e^{-\frac{\Delta G}{RT}}}$
number of occupied states: $N_{occ}(E) = f(E) \cdot \rho(E)$, ρ: densitiy of states
In nanowires: $k_{el}(x) = k_{el}^0 \cdot e^{-\beta x}$ → exponential decay of the transmission coefficient with x

10 Potentiometric Biosensors

chemical potential: $\boxed{\mu_j = (\frac{\partial G}{\partial n_j})_{p,T,n'}}$, with G the Gibbs free energy
Equilibrium → $dG|_{p,T} = 0 \implies \mu_B = \mu_A$
electrochemical potential: $\bar{\mu_j} = \mu_0 + Z_j F\Delta\phi$ (for a metal, $\mu_0 = E_F$ at T=0K), $F = eN_a$ (Faraday constant)
$\mu_{redox} = E'$ where $D_0(\lambda, E') = D_R(\lambda, E')$
$\Delta\varphi = -\frac{\Delta_r G}{ZF} = C' + C_2 \cdot \ln[ion]$; C',$C_2$ constants
$\boxed{\textbf{Nernst Equation: } \Delta\varphi = \Delta\varphi_c^0 - \frac{RT}{nF} \cdot ln(Q)}$, with $R = N_A k_B$, $Q = \frac{[Red]}{[Ox]}$

10.1 Nernst potential at Semipermeable Membranes

$j_{diffusion} = -|Z| \cdot D \cdot \frac{d[C]}{dt}$, C: concentration, Z: ion velence, D: diffusion constant $\implies$ can measure [ion] with voltage at electrode
$j_{drift} = -\mu \cdot |Z| \cdot F \cdot [C] \cdot \frac{dV}{dx}$
$\implies \boxed{\Delta\varphi = V_{in} - V_{out} = \frac{k_b T}{Q} \cdot \ln\frac{[C]_{out}}{[C]_{in}}}$

10.2 Ion-Selective Electrodes

$\Delta\varphi_m = \frac{RT}{ZF}\ln\left(\frac{[a_A]_1}{[a_A]_2}\right)$

10.3 Bioenzymatic Sensors

Enzymes: lower the activation energy (E_a or ΔG) $\rightarrow$ accelerate the rate of the reaction, are not consumed by the reaction and do not alter the equilibrium of these reactions.
Measure the amount of product after enzymatic reaction to know how much of the reactant was initially present.

11 Formulas

11.1 Mechanical waves

Speed: $c = 1/\sqrt{\kappa\rho}$; P: $p = \rho c u_z$; Impedance: $Z = p/u_z = \rho c = \sqrt{\rho/\kappa}$; Intensity: $I = p u_z/2$; WL: $\lambda = c/\nu = 1/(\nu\sqrt{\kappa\rho})$; Part.vel: $u_z = \sqrt{2I/Z}$

11.2 Basic Physics

photon energy $[J]$	E	hc/λ
arc length	s	$= \frac{\pi \cdot r \cdot \theta}{180}$
Force $[kg \cdot m \cdot s^{-1}]$	F	$= m \cdot a$
Work $[kg \cdot m^2 \cdot s^{-2}]$	W	$= F \cdot s = \int_{t_1}^{t_2} \mathbf{F} \cdot \mathbf{v} dt$
		$= \int_C \mathbf{F} \cdot d\mathbf{s}$
Power $[kg \cdot m^2 \cdot s^{-3}]$	P	$= \frac{\Delta W}{\Delta t} = F \cdot v$
		$= R \cdot I^2 = V \cdot I$
Momentum	p	$= mv = \sqrt{2Em}$
Drehmoment	L	$= I\omega$
Kinetic energy	E_{kin}	$= \frac{1}{2}mv^2$
Impulsenergie	E_{kin}	$= \frac{p^2}{2m}$
Potentielle Energie	E_{pot}	$= mgh$
Eletrische Energie	E_{el}	$= e \cdot V_0$
Rotationsenergie	E_{rot}	$= \frac{1}{2}I\omega^2$
Modell freies Elektron	E	$= \frac{\hbar^2}{2m^*}k^2$
Spannungsenergie	E_{pot}	$= \frac{1}{2}mx^2$
Federkraft	F	$= -kx$
Gravitationskraft	F_G	$= G\frac{m_1 m_2}{r^2}$
Gravitational Potential Energy	V_G	$= -G\frac{m_1 m_2}{r}$
Zentripetalkraft	F_Z	$= m\omega^2 r = ma$
		$(a = \frac{v^2}{r})$
Beschleunigung	a	$\frac{\vec{F}}{m}$
Glm.beschl. Bew	s	$= v_0 t + \frac{1}{2}at^2$

11.3 Conversions

Power in dB= $10 \cdot log(\frac{I}{I_0})$
gas constant R= $8.31 \frac{J}{mol \cdot K}$
Num Avogadro $N_A = 6.022 \cdot 10^{23}\ mol^{-1}$
Boltzmann constant $k_B = \ 1.380658 \cdot 10^{-23}\ J/K = 8.6 \cdot 10^{-5}\ eV/K$
Newton: $1\,\mathrm{N} = \mathrm{kg\,m\,s^{-2}}$
Pascal: $1\,\mathrm{Pa} = \mathrm{N\,m^{-1}}$
Joule: $1\,\mathrm{J} = \mathrm{N\,m} = \mathrm{kg\,m^2\,s^{-2}} = 6.2415 \times 10^{18}\,\mathrm{eV}$
Watt: $1\,\mathrm{W} = \mathrm{kg\,m^2\,s^{-3}}$
Angström: $1\,\text{Å} = 0.1\,\mathrm{nm}$

11.3.1 Trigonometric conversions:

$\sin(\alpha)\cos(\beta) = \frac{1}{2}(\sin(\alpha+\beta) + \sin(\alpha-\beta))$
$\cos(\alpha)\cos(\beta) = \frac{1}{2}(\cos(\alpha+\beta) + \cos(\alpha-\beta))$
$\sin(\alpha)\sin(\beta) = \frac{1}{2}(\cos(\alpha-\beta) - \cos(\alpha+\beta))$

11.4 Stuff

11.4.1 Harmonic Oscillator:

Average work done in one cycle ($T = \frac{2\pi}{\omega}$) $\bar{W} = \frac{1}{T}\int_0^T \vec{F} \cdot \vec{x}\ dt$
Average Power: $\bar{P} = \frac{\bar{W}}{T}$

11.4.2 Beer-Lambert:

$A = \epsilon \cdot l \cdot c = -log_{10}(I/I_0)$