McGill UNIVERSITY

BIEN210
Coursepack

Electrical and Optical Properties of Biological Systems

Prepared by BUSS

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Preface

Welcome to the BIEN 210 — Electrical and Optical Properties of Biological Systems official course-pack! This document has been prepared by the Bioengineering Undergraduate Student Society (BUSS) to best assist you as you make your way through the course. The course-pack addresses the material according to an objective-based approach. This means that key questions from the slides, as well as other key concepts, are listed and expanded upon.

The course-pack is organized into four different parts:

- First, you will find course material information, such as explanations, notes, and figures organized to directly respond to each lecture's objectives.
- Then, an equations list has been compiled. It can serve as a quick way to find a particular equation you are looking for. For most of the equations you encounter in this course-pack, you can find variable definitions in this section.
- Afterwards, you will find an index of every italicized term in the course-pack. These are key concepts and ideas that you should be familiar with.
- Finally, you will find two final presentation examples from previous cohorts vetted and approved by the course's instructor.

The secret to best succeeding in this course is actually quite simple: attend the lectures and take notes on what is said in class! Do not rely solely on the contents of the PowerPoint slides, or even on the contents of this course-pack. While both can and should be used as key supplements to your learning, they cannot replace personalized notes taken in the moment which reflect your thoughts and ideas as they relate to the course material. It is also very important to ask questions as much as possible. The professor is there to help you!

Please note that this document was created using source material from the Winter 2019 and Winter 2020 semesters. Due to the ever-changing nature of courses offered by the Department of Bioengineering at McGill, it is possible that parts of this course-pack no longer accurately reflect the current contents or organization of the course. In case of doubt, refer directly to the course instructor, Professor Sebastian Wachsmann Hogiu.

This course-pack could not have been produced without the help of Professor Sebastian Wachsmann Hogiu, BUSS President Brenda Shen (2020 — 2021), and BUSS Vice-President of Academics Anna Ciprick (2020 — 2021).

Special thanks to Julie Chen who volunteered her impressive set course notes to help in the course-pack's production.

Written and designed by Alexander Becker
Reviewed by BUSS

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Course Material

Lecture #1: Introduction to Light

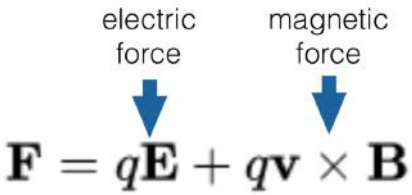
This lecture provides an introduction to optics and electromagnetism.

Lecture objectives:

1. Identify theories describing optical phenomena
2. Explain Maxwell's equations
3. Reproduce the wave equation
4. Describe the properties of light
5. Identify mechanisms of light generation
6. Describe lasers and laser properties

1. Identify theories describing optical phenomena

- *Classical electromagnetic theory* offers a global explanation for most phenomena.
  - The *Lorentz force* results from particle movement in an electromagnetic field:



- Simplified models, such as *geometric* and *physical optics* can also be used.
- *Quantum optics* describes these phenomena at the photon level, providing the best current model for optics.

2. Explain Maxwell's equations

Maxwell's equations answer the question: "How are electric and magnetic fields generated by charges, currents and field changes?"

- They describe the generation and propagation of light.
- There are four equations:
  - *Gauss's Law for Electric Charges*
    - Describes the generation of electric fields by single charges.

$$\nabla \cdot \mathbf{E} = \frac{\rho}{\varepsilon_0}$$

- *Gauss's Law for Magnetism*
  - Describes the generation of magnetic fields by *magnetic dipoles*. There are no single point "magnetic charges."

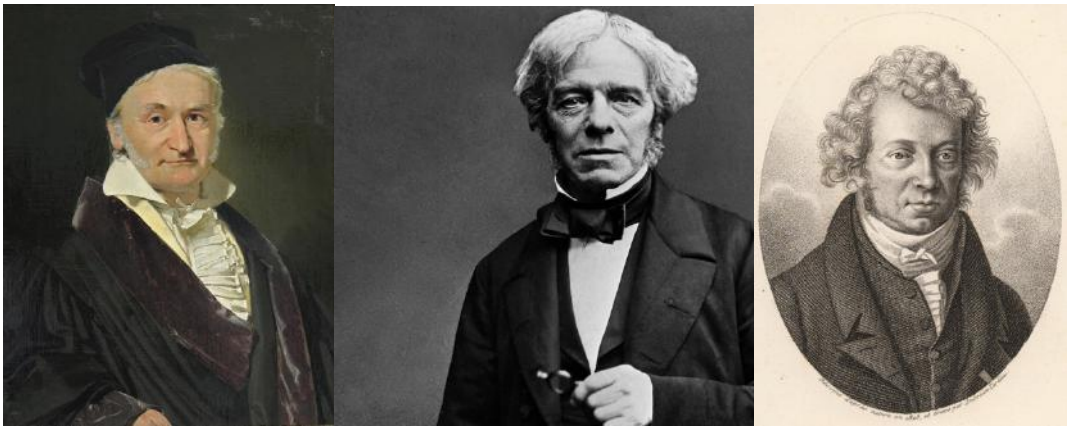
$$\nabla \cdot \mathbf{B} = 0$$

- *Faraday's Law*
  - Describes the creation of a magnetic field by a *moving electric field*.

$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t}$$

- *Ampere's Law*
  - Describes the creation of magnetic fields by *electric currents* and/or by moving *electric fields*.

$$\nabla \times \mathbf{B} = \mu_0\mathbf{J} + \mu_0\varepsilon_0\frac{\partial \mathbf{E}}{\partial t}$$



From left to right: Carl F. Gauss, Michael Faraday, and André-Marie Ampère

3. Reproduce the wave equation

- Maxwell's equations can then be used to derive the *electromagnetic wave equation*.

$$\left(v_{ph}^2 \nabla^2 - \frac{\partial^2}{\partial t^2}\right) \mathbf{E} = \mathbf{0}$$

$$\left(v_{ph}^2 \nabla^2 - \frac{\partial^2}{\partial t^2}\right) \mathbf{B} = \mathbf{0}$$

- The electromagnetic wave equation can then be solved, giving the following sinusoidal solutions:

$$\mathbf{E}(\mathbf{r}, t) = \mathbf{E}_0 \cos(\omega t - \mathbf{k} \cdot \mathbf{r} + \phi_0)$$

$$\mathbf{B}(\mathbf{r}, t) = \mathbf{B}_0 \cos(\omega t - \mathbf{k} \cdot \mathbf{r} + \phi_0)$$

- These equations also define two key values:
  - *Permeability*: the ability of a medium to support magnetic fields.
  - *Permittivity*: the capacitance encountered when forming an electric field in a medium. In other words, the energy-storage capacity of the field in the medium.

4. Describe the properties of light

- Light, an *electromagnetic wave*, carries electric and magnetic fields, allowing it to interact with electric charges.
- Light waves have a *frequency*, a *wavelength*, a certain *energy*, as well as a given *momentum*. These values can be computed using the following set of equations:

$$c = \lambda \nu$$

$$E = h\nu = \frac{hc}{\lambda}$$

$$p = \frac{E}{c}$$

- Light can also have a *polarization*, that is, the wave might oscillate in a specific plane of the electric field. If you have polarized sunglasses, you can try holding them up to your computer screen and then rotating them 90 degrees while keeping your head upright!
- As light is a wave, sets of these waves can be in or out of phase with each other. The relationship between these phases is called *coherence*.
- Light is also a particle! *Photons* have mass, and, as such, light will interact with gravity. Photons can also interact with each other.
- Photons are categorized as "*bosons*": more than one photon can occupy a given state. This means light obeys *Bose-Einstein statistics*.

5. Identify mechanisms of light generation

- While heat transfer can occur by conduction and convection, it can also occur by radiation. *Black body radiation*, otherwise known as thermal radiation, is emitted by all objects with temperatures above absolute zero (0K).
- This heat transfer can be calculated using the following equations:

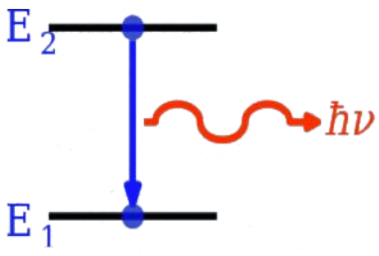
$$P_{\text{net}} = P_{\text{emit}} - P_{\text{absorb}}$$

$$P_{\text{net}} = A\sigma\varepsilon\left(T^4 - T_0^4\right)$$

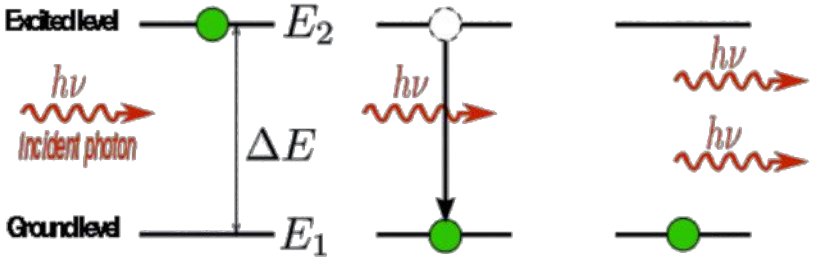
- Atoms emit light when their electrons are lowered to less energized states. The released energy takes the form of light. As such, the energy of the released photon can be found by using the following equation:

$$E_2 - E_1 = \Delta E = h\nu$$

- The decay described above can be *natural*:



- The decay could also be *stimulated*. This means a "catalyst" incident photon triggers the emission of a second photon from the atom:



6. Describe lasers and laser properties

- *Lasers* differ from other light sources for a few reasons:
  - They operate using a single wavelength, and, as such, are *monochromatic*.
  - They are coherent.
  - They are directional. This means the light is spatially targeted, the light barely diverges. This contrasts with flashlights, for example.
  - They are polarized.
- Lasers come in two varieties, *continuum wave* (CW) and *pulsed*.
  - Continuum wave lasers operate at a constant output power with no interruptions in the beam of light.
  - Pulsed lasers operate at similar average power outputs to the CW laser, but only emit light intermittently and for very short periods of time. As such, the power output per burst is much greater than that of CW lasers.
- Lasers have many practical applications:
  - Optical tweezers
  - Medical purposes (eg.: LASIK)
  - Motion detection
  - Mass spectroscopy
  - Scanning
  - DNA sequencing

Lecture #2: Light Sources

This lecture presents different ways in which light can be generated. It also introduces the concept of scattering.

Lecture objectives:

1. How is light generated?
2. Mechanisms by which light is generated by biological systems
3. What is fluorescence?
4. What is phosphorescence?
5. Understanding the different types of scattering

1. How is light generated?

- *Light* can be emitted by *heat sources*, such as through fusion and other chemical reactions.
- Light can be generated through *luminescence*. This is expanded upon below.
- Light can also be emitted through *radioactivity*.
- *Non-radiative decay* involves the release of energy through heat. In other words, instead of the energy being released in the form of a photon, the material simply heats up!

2. Mechanisms by which light is generated by biological systems

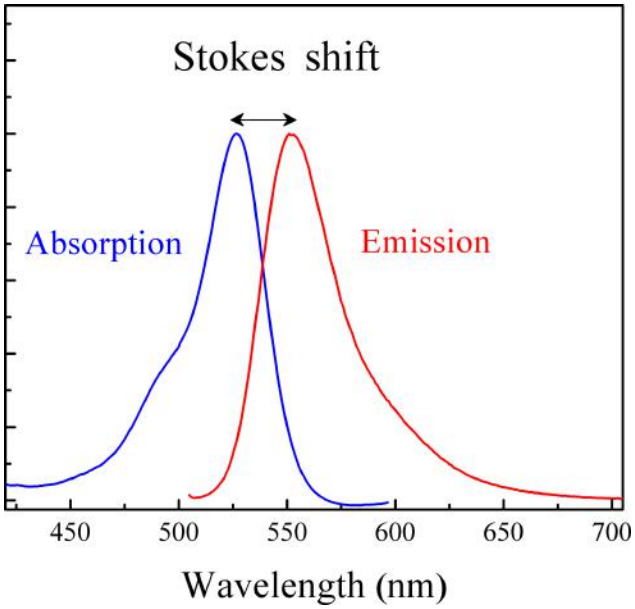
- Biological organisms generate light by luminescence.
- There are many types of luminescence, for instance:
    - *Chemiluminescence*: the emission of light by a chemical reaction.
    - *Electroluminescence*: the emission of light by an electric current.
    - *Mechanoluminescence*: the emission of light by a mechanical stress.
    - *Photoluminescence*: the emission of light by the absorption of another photon, recall stimulated emission from Lecture #1!
    - Other types of luminescence include *sonoluminescence* (sound!) and *electrical discharges*.
- *Bioluminescence*, a sub-type of chemiluminescence, is the emission of light by biological systems.
    - For example, fireflies use *adenosine triphosphate* (ATP) and an enzyme, luciferase, to oxidize a compound known as luciferin into oxyluciferin. Oxyluciferin, the excited state of luciferin, will then release a photon as it relaxes back to the ground state. That's why fireflies glow!

$$\text{Luciferin} + O_2 \xrightarrow{\text{Luciferase}} \text{Oxyluciferin} + \text{Light}$$

- Chemiluminescence is also used in many other cases, such as in glow-sticks and forensics.

3. What is fluorescence?

- *Fluorescence* is the absorption of light, quickly followed by its emission.
  - One property of compounds is the ability to absorb light within a particular wavelength range, and to then emit light at a different wavelength.
  - The difference between absorption wavelength and emission wavelength is referred to as the *Stokes shift*.



- The *quantum yield* of a compound is the ratio of the number of emitted photons over the number of absorbed photons. This value will always be less than one, as some energy is

always lost to heat.

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

- The fluorescence *lifetime* is the measure of how long the molecule in question will remain in the excited state. In other words, it is the amount of time the light emission will last. This is normally measured in nanoseconds.
- *Fluorophores* are molecules that fluoresce.
    - *Endogenous* fluorophores are naturally occurring within cells.
    - *Exogenous* fluorophores are introduced to cells, usually for experimental purposes. In fact, jellyfish-derived *Green Fluorescent Protein* (GFP) is often used in laboratory settings to tag other proteins, allowing for simplified visualization!

4. What is phosphorescence?

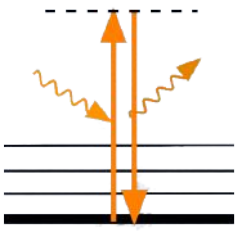
- Much like fluorescence, *phosphorescence* involves the absorption and subsequent emission of light. However, the emission takes far longer.
- While fluorescence lifetimes are on the order of nanoseconds, phosphorescence lifetimes are on the order of hours.
- Glow-sticks use phosphorescence to be able to glow for hours!

5. Understanding the different types of scattering

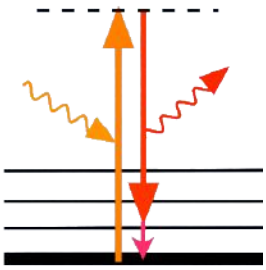
- *Scattering*, in general, is the deflection and/or diffusion of light. There are three main types of scattering: Rayleigh, Mie, and Raman.
- *Rayleigh scattering* is *elastic* scattering on objects smaller than the wavelength of light. Elastic scattering means that the interaction between the object and the light does not involve energy transfer. A popular example is the answer to the question "why is the sky blue?": atmospheric particles scatter the shortest wavelengths of light best, which corresponds to blue light with regards to the visible spectrum. This is a case of Rayleigh scattering as the particles are smaller than the wavelength of light being reflected!

- *Mie scattering* is similar to Rayleigh scattering in that it is *elastic*, but it applies to objects similar in size the light's wavelength instead. In both cases, the following formula and diagram apply:

$$x = \frac{2\pi r}{\lambda}$$



- *Raman scattering* is *inelastic* scattering, meaning an energy exchange occurs upon contact between the light and the object. This results in a *polarizability change* in the molecules struck by the light. As shown in the diagram below, the energy of the scattered photon does not match that of the incident photon! This contrasts with Rayleigh and Mie scattering (see the diagram above).



- Variants of Raman scattering can be used in the creation of biosensors. For example, many cell-based biosensors use Raman scattering to help researchers study cellular biochemistry in response to various toxins and conditions over time. Variants of Raman scattering include:
    - Spontaneous Raman scattering
    - Resonance Raman scattering
    - Coherent Raman scattering
    - Surface-Enhanced Raman scattering
    - Stimulated Raman scattering

Lecture #3: Manipulation of Light — Optical Components Fundamentals

This lecture covers how elementary principles regarding light help us design optical components we can use in all aspects of life, from consumer products to laboratory settings.

Lecture objectives:

1. How does light interact with matter?
2. Explain the manipulation of light from first principles
3. Identify the main optical components and describe how they work

1. How does light interact with matter?

Light interacts with matter through four main behaviours.

- *Reflection*
  - Reflection can be *specular*, meaning the angle of incident light is equal to the angle of reflected light. Think of a mirror!
  - Reflection can be *diffuse*, meaning light rays will be reflected in multiple different directions due to imperfections in the surface.
  - In general, the intensity of reflected light depends on the angle of incidence. Larger incident angles translate to lesser light intensities.
- *Absorption*
  - Many organic molecules are designed to absorb light. For example, chlorophyll in plants absorbs light, allowing plants to perform photosynthesis.
  - Absorption corresponds to a photon providing sufficient energy to a molecule for this molecule to be excited up from the ground state.
  - An absorption coefficient can be calculated as follows:

$$\mu_a = \rho_a \sigma_a$$

where: $\sigma_a = Q_a A$

  - Using the absorption, we can also compute the *transmission*, that is, the amount of light passing through a given material. As per the formula below, transmission follows exponential decay.

$$T = \exp(-\mu_a L)$$

- *Emission* is an encompassing term for phenomena such as fluorescence and phosphorescence. See lecture #2 for more information on these concepts!
- *Scattering*

- If a photon does not strike molecules in the "correct" way, it will be scattered instead of absorbed.
- If the light is scattered off a small molecule, Rayleigh scattering occurs.
- If the light is scattered off a larger molecule, or perhaps a cell or an organelle, Mie scattering occurs.
- Lipids are the main scatterers amongst the macromolecules.
- As with absorption, a scattering coefficient can be calculated.

$$\mu_s = \rho_S \sigma_S$$

where: $\sigma_s = Q_s A$

- There is also scattering transmission, computed as follows:

$$T = \exp(-\mu_s L)$$

The *mean free path* is the distance a photon travels without being absorbed or scattered. This value is different for different wavelengths of light, and will vary depending on the material.

A global interaction coefficient can be constructed from the individual interactions. Note that reflection does not feature in the equation as, by its nature, reflection does not involve the absorption or emission of light.

$$\mu_{total} = \mu_{absorption} + \mu_{fluorescence} + \mu_{scattering}$$

2. Explain the manipulation of light from first principles

Waves can be *spherical*, emanating from a point source (think of the splash after you throw a stone in a lake!), or *planar*, as is the case with most light waves. Many key principles explain waves and wave propagation.

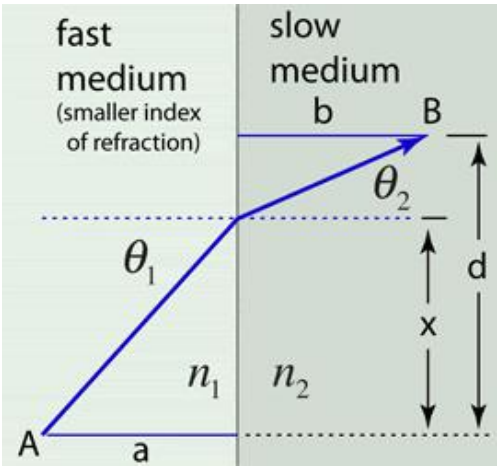
- *Hooke's Law*

$$F = -kX$$

- *Huygens-Fresnel Principle*
  - When light interacts with a material at an interface, points of interaction will be sources for new circular waves.
  - These waves will then interact with each other, creating a wave front.
- *Superposition*
  - Waves will combine, overlapping each other and propagating on top of each

other.

- *Fermat's Principle*
  - Light will always take the path requiring the shortest time when traveling between two points.
  - The shortest path distance-wise is not necessarily the shortest path time-wise!
  - In the figure below, notice that light spends less time in the slow medium, thus travelling as quickly as possible between points A and B.

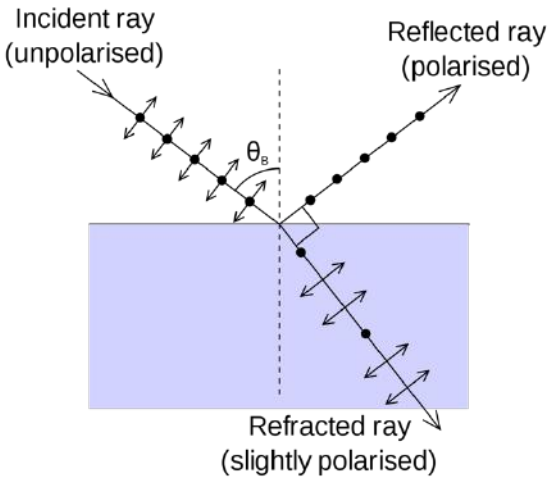


- *Snell's Law*
  - Snell's Law describes the relation between the angles and refractive indices from Fermat's Principle. Again, refer to the figure above to make sense of the following equation:

$$\frac{\sin\theta_1}{\sin\theta_2} = \frac{v_1}{v_2} = \frac{\lambda_1}{\lambda_2} = \frac{n_2}{n_1}$$

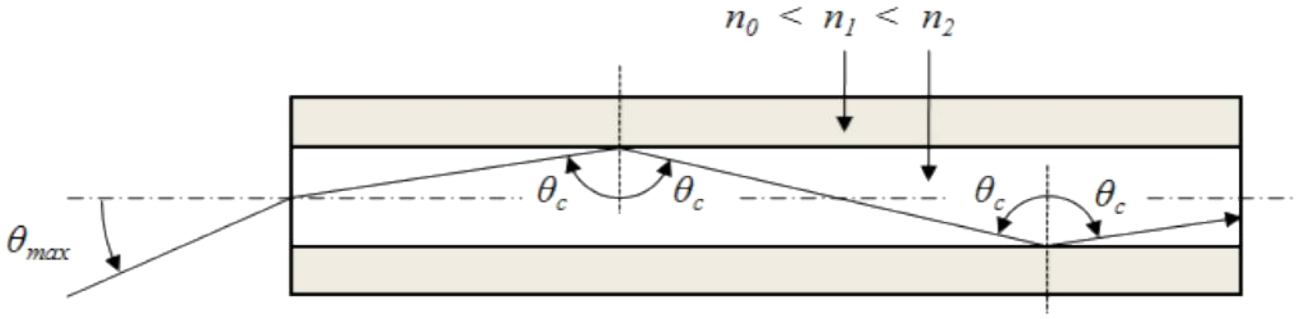
  - The *Brewster's angle* is the incident angle that causes the reflected and refracted rays to be exactly 90 degrees apart. At this angle, the reflected ray is completely polarized while the refracted ray is partially polarized.

$$\tan\theta_B = \frac{n_2}{n_1}$$



- *Total Internal Reflection* occurs when the incident angle equals the critical angle for the given pair of media. At this angle, the refracted ray is refracted entirely along the border. For greater angles, all the light is reflected. This can only occur when light travels from a medium with high refractive index to a medium with lower refractive index.

$$\sin \theta_c = \frac{n_2}{n_1}$$



3. Identify the main optical components and describe how they work

Optical components allow for the manipulation of light. These components operate thanks to the principles discussed above. Many such components exist:

- *Pinholes*
  - They control the amount and plane of light passing through to the system.
  - The iris is the human eye's equivalent of a pinhole!
- *Mirrors*
  - They (shockingly) reflect light.

- They control the polarization of light.
- Mirrors can be broadband or narrowband
- Mirrors can be flat or curved. The righthand mirror of a car, for example, is convex, hence the classic "objects in mirror are closer than they appear" warning!
- Mirrors can be metallic or dielectric.
    - Metallic mirrors are normally made of silver and aluminum. The metals making up the mirror determine the percentage of light of a given wavelength reflected by the mirror.
    - Dielectric mirrors are constructed by using dielectric materials to layer oil, water, and air such that light interferes with itself constructively and/or destructively.

◆ *Beam Splitters*

- Beam splitters are used to divide an incident beam into two separate beams: one reflected and one transmitted.
- They can be used in laboratory settings, for example, to perform flow cytometry.

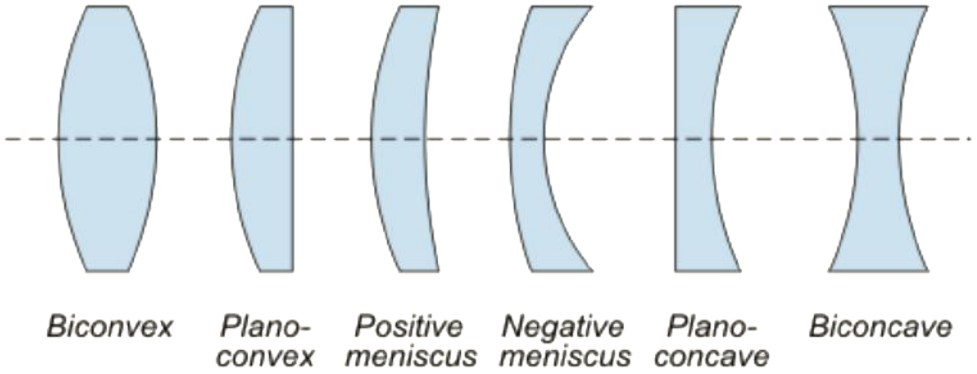
◆ *Filters*

- Filters control the intensity of light, as well as its spectral characteristics. This simply means that wavelengths can be filtered out as needed.
- Many different filter types exist:
    - *Neutral density* filters change the intensity of light, but not the wavelength.
    - *Bandpass* filters only allow a specific range of wavelengths through (they let a specific band pass).
    - *Edge filters* allow only long wavelength or short wavelength light to pass, depending on the type (long-pass or short-pass).
    - *Dichroic filters* only allow a very small range of wavelengths to pass. They are similar to bandpass filters.
    - *Notch filters* (also referred to as bandstop filters) let all wavelengths through except those within a specific range.
    - *Diffusers* make the incoming beam of light less abrasive, less intense and more ambient overall. These are often used in photography studios, for example, to properly light a subject!

◆ *Lenses*

- Lenses convert planar waves into circular waves to focus light on a specific point or scatter light away from a specific point.

- Lenses can be convex or concave, though many types exist overall.



- For double-convex lenses, also known as *converging lenses*, the image could be generated on either side of the lens, creating a real or virtual image. The image could also be upright or flipped, magnified or reduced in size.
- For double-concave lenses, also known as *diverging lenses*, the image will always form behind the lens (on the side of the incident light). This implies a virtual image. The image will also be upright and reduced in size.
- Unusual lenses are lenses that do not have the shapes shown above. The Fresnel lens, for example, operates like a convex lens, but uses less material.
- The lens equation which allows to pinpoint the focal point of a lens is as follows:

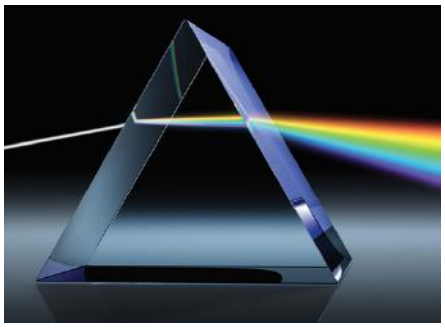
$$\frac{1}{d_0} + \frac{1}{d_i} = \frac{1}{f}$$

- The magnification of the image relative to the object is given by:

$$M = \frac{h_i}{h_0} = -\frac{d_i}{d_0}$$

◆ *Prisms*

- Prisms are constructed from at least two flat surfaces with an angle between them.
- They are used to disperse, split, or reflect light into various polarizations or wavelengths.
- They can be used in laboratory settings, for example, to allow a spectrophotometer to analyze the absorbance of materials at specific wavelengths of light.



- *Gratings*
  - A series of slits in a surface creating diffraction patterns. Maxima from constructive interference for specific wavelengths of light can be determined this way.
- *Polarizers*
  - Polarizers will polarize light depending on the orientation of the electrical field. The angle of incidence will determine the strength of polarization. At the Brewster's angle (see above), polarization is total.
- *Optical Fibers*
  - Optical fibers operate using total internal reflection, described above.
  - The incident ("acceptance") angle must obey the following expression:

$$\sin\theta \leq \sqrt{n_o^2 - n_c^2}$$

  - They are designed to transmit information. Transmission speeds using optical fibers are far superior to those of classic copper cables!
  - For the optical fiber to work correctly, the interior material (thin strands of glass) must have a larger index of refraction relative to the cladding (the material wrapped around the glass).
  - Many types of optical fibers exist, each with their particularities.
    - Step index fibers
    - Graded index fibers
    - Single mode fibers
    - Photonic crystal fibers

Lecture #4: Optical Components — How do biological systems manipulate light?

This lecture highlights how biological organisms have evolved to manipulate light.

Lecture objective:

1. How do biological systems manipulate light?

1. How do biological systems manipulate light?

- *Structural colouration* is the mechanism by which colour emerges from structure rather than as a result of absorption/emission. Colour is generated by precise constructive and destructive interference resulting from light reflecting off the object in question.
    - For example, butterflies' wings use structural colouration.
    - Diatoms, a type of algae, use structural features to in turn use reflected light for photosynthesis. A by-product of this process is structural colouration… and pretty images!
- *Metamaterials* are special materials that derive function from structure. While organisms do use structure to their advantage, we can engineer our own devices to mimic nature. Superhydrophobic surfaces can be designed by mimicking, for example, the lotus leaf.
- Certain organisms, such as pit viper snakes, giant clams, and nautili, use pinhole vision to their advantage. Some organisms also have bifocal eyes which allow for different focal planes.
- Some organisms use polarized light and optical-fiber-like mechanisms.

The butterfly's wings' colour is due to structural colouration.

Lecture #5: Photon Diffusion, Biological Sensors, and Biological Membranes

This lecture reviews photons' interactions with matter, describes living organisms' biological sensors and laboratory-created artificial biosensors, and goes into detail regarding the plasma membrane and the ways it can be modelled.

Lecture objectives:

1. Understand how photons move and interact with matter
2. Understand the basics of biological sensors
3. Describe the plasma membrane from a chemical and structural point of view
4. Describe how membrane potentials are generated
5. Highlight similarities between the plasma membrane and known electrical components and draw simple electric circuits that mimic plasma membranes
6. Calculate electric potentials across plasma membranes

1. Understand how photons move and interact with matter

When light interacts with matter, it can be absorbed, scattered, or transmitted (in other words, it passes through without any interaction). The distance a photon travels on average through a given material is given by the mean free path. To learn more about these concepts, see lecture #3 in this course-pack!

The study of these interactions, however, can allow us to make clinical decisions in the real world.

2. Understand the basics of biological sensors

Biological organisms such as humans have "sensors" that pick up various environmental stimuli. The path of a signal from reception to reaction is as follows:

1) *Reception*: the receptor (for example, the eye) receives the stimulus.
2) *Transduction*: the stimulus is converted to an electrical impulse.
3) *Conduction*: the electrical impulse is carried to the brain through the nervous system through an action potential (more on that below!).
4) *Analysis and Interpretation*: the signal is processed by the brain.
5) *Feedback*: a reaction is triggered as a result of the stimulus.

There are three different types of biological sensors:

- *Light* (for example, the visual system)
- *Chemical* (for example, the olfactory system)
- *Mechanical* (for example, the auditory system where sound waves, in other words, pressure changes, make the ear drum vibrate)

By basing themselves on the above processes, researchers have created artificial biosensors. These biosensors operate similarly using a series of common devices:

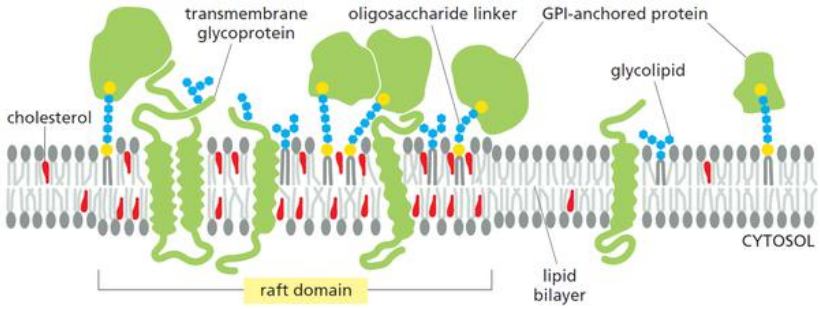
1) A *biorecognition element* which is designed to specifically detect a stimulus. This is the equivalent of the biological receptor.
2) A *transducer* which converts the stimulus into an electric signal.
3) An *amplifier* which boosts the voltage of the signal.
4) A *signal converter* which transforms the signal from analog to digital such that it can be processed by a computer.
5) A *recording device* which makes sense of the signal.

Refer to lecture #8 for more information on this topic!

3. Describe the plasma membrane from a chemical and structural point of view

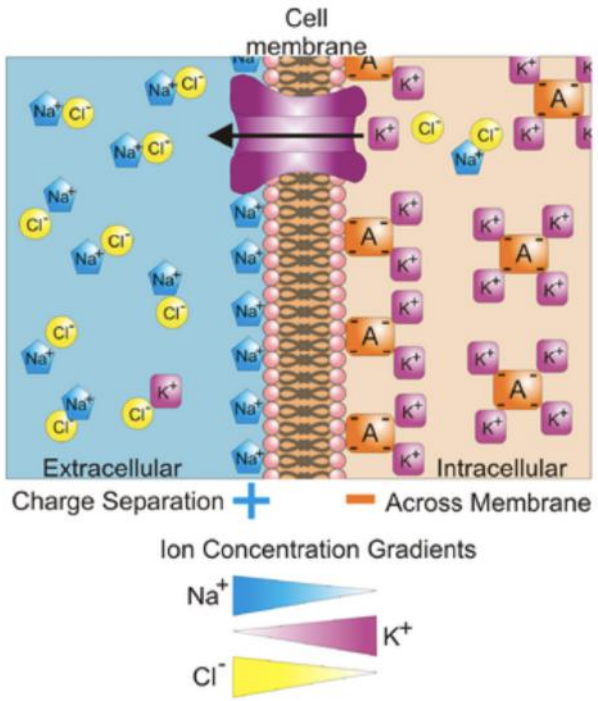
As you may have seen in too many biology courses to count, the *plasma membrane* is made up of *phospholipids* arranged in a bilayer. These macromolecules are in constant movement, frequently swapping places laterally and transversally. The phospholipids are made of a *hydrophilic head* (which is why the head points out towards the intracellular and extracellular regions) and *hydrophobic tails*.

- *Intercellular communication* occurs through this bilayer as well.
- *Lipid rafts* form occasionally due to the natural motion of the membrane. These rafts are simply little compartments of lipids within the greater membrane.



4. Describe how membrane potentials are generated

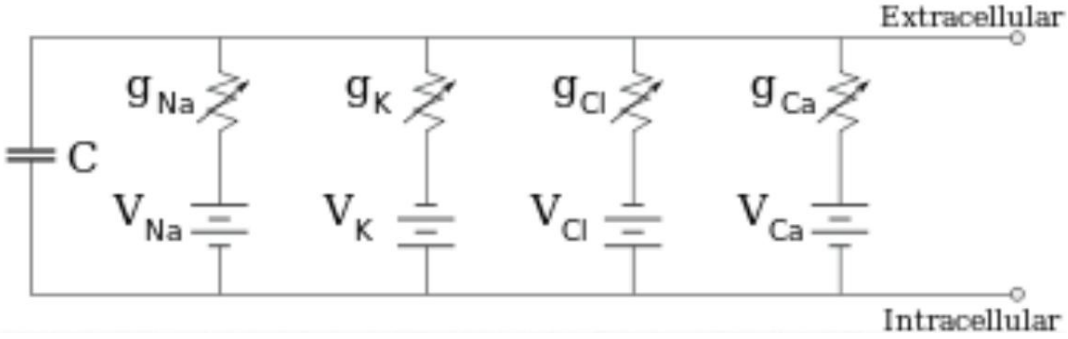
Throughout the membrane, *channel and pump proteins* allow for the passive and active displacement of key ions such as sodium, chlorine, and potassium. The chemical and electrical gradients created by these ions thanks to the channels and pumps generate the *membrane potential*: -73 millivolts at rest.



As a result of voltage changes, chemical ligand binding, and possibly other stimuli, channels will open and close, changing the potential of the membrane. This process can create or propagate an *action potential*, which is the way information travels through the nervous system, for example.

5. Highlight similarities between the plasma membrane and known electrical components, and draw simple electric circuits that mimic plasma membranes

The plasma membrane can be modelled as an electrical circuit by, for example, the *Hodgkin-Huxley Model*: the conductance is used to describe how action potentials are created and propagated.

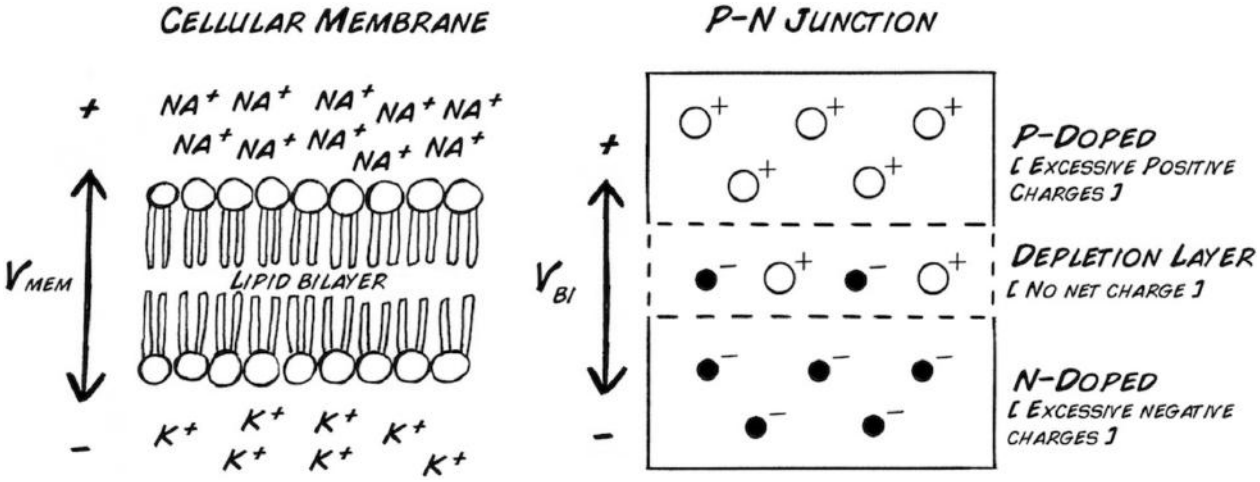


In this model:

- The lipid bilayer is represented by the capacitance (C)
- Voltage-gated ions channels are represented by the resistors (g)

- The electrochemical gradients driving ion flow are represented by the batteries (V)
- The active pumps are represented by the current source in the circuit (between "Extracellular" and "Intracellular" above)

The membrane can also be compared to the *P-N (or N-P) junction* in semiconductors.



In the "N-doped" area, group 5 elements are added in small quantities. The single fifth electron will be mobile in the system. In the "P-doped" area, group 3 elements are added in small quantities. The "gap" left by the mission valence electron will lead to current generation when free electrons from the N-doped region migrate to the P-doped region. Overall, a junction potential is created, much like the potential generated in the case of the plasma membrane!

6. Calculate electric potentials across plasma membranes

The potential of a membrane can be computed using the following equations. In the case of resting membrane potential, the *Nernst equation* is used:

$$E = \frac{RT}{zF} \ln \frac{[\text{ion outside cell}]}{[\text{ion inside cell}]} = 2.3026 \frac{RT}{zF} \log_{10} \frac{[\text{ion outside cell}]}{[\text{ion inside cell}]}$$

To include active ion pump contributions to the potential, the *Goldman equation* is used:

$$E_{\mathrm{m}} = \frac{RT}{F} \ln \left(\frac{\sum_i^N P_{\mathrm{M}_i^+} [\mathrm{M}_i^+]_{\mathrm{out}} + \sum_j^M P_{\mathrm{A}_j^-} [\mathrm{A}_j^-]_{\mathrm{in}}}{\sum_i^N P_{\mathrm{M}_i^+} [\mathrm{M}_i^+]_{\mathrm{in}} + \sum_j^M P_{\mathrm{A}_j^-} [\mathrm{A}_j^-]_{\mathrm{out}}} \right)$$

To study the dynamics of ion channels in the membrane, the *patch clamp technique* is used. This technique involves touching the membrane with a sharp pipette tip containing liquid. As ions move in and out of the pipette, a small current can be measured. This allows for the study of individual ion channels!

Lecture # 6: Photon Conversion into Electrical and Chemical Energy

This lecture covers ways in which light is processed by animals and plants. It also presents ways in which we can use light to our advantage for manipulating organisms and creating synthetic ones.

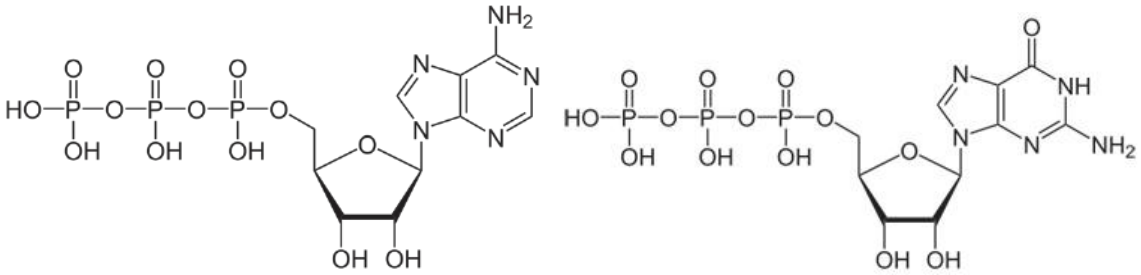
Lecture objectives:

1. Describe photoreceptors and phototransduction in the visual system
2. Understand the basics of optogenetics
3. Describe photosynthesis
4. Discuss artificial leaves

1. Describe photoreceptors and phototransduction in the visual system

The visual system is extremely complex.

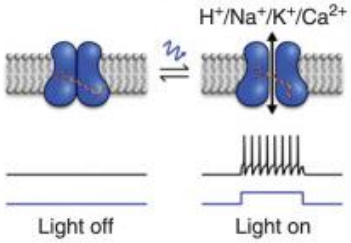
- Pigments in *photoreceptor cells* absorb light.
    - Light intensity detection is handled by the *rod* photoreceptor cells.
    - Colour vision is handled by the *cone* photoreceptor cells. They are unevenly distributed in the retina, with three different cone types handling different colour spectra.
    - If you are partially colourblind, you may be missing a cone type, or perhaps the spectrum of a particular cone type is shifted, creating a "colour blind spot" for you!
    - *Opsins* are light-sensitive proteins in the photoreceptor cells. *Retinal* is one such protein.
    - When light is absorbed by retinal it transforms into an activated version of itself.
    - This is called *photoisomerization*, as light ("photo") triggers a change in molecular configuration ("isomerization").
- *Phototransduction* is the process by which light detected by photoreceptor cells is converted to electrical impulses before these impulses are then sent to the brain.
    - Phototransduction is a result of membrane depolarization and multiple specialized proteins.
    - Guanosine Triphosphate (GTP) and Guanosine Diphosphate (GDP) are used in this process. They function similarly to Adenosine Triphosphate (ATP) and Adenosine Diphosphate (ADP) which you may be familiar with already.



2. Understand the basics of optogenetics

Optogenetics is a field that uses genetic engineering of ion channel proteins to trigger membrane potential changes (and, therefore, information transfer) from light input.

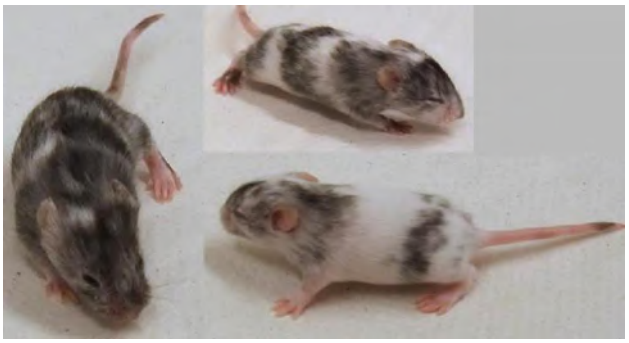
- *Channelrhodopsins* (a type of opsin) are light-gated ion channels, meaning they open and close in response to light. This is due to a chromophore located in retinal which responds to specific wavelengths. Blue light, for example, opens these channels.



- In order to control membrane potentials with light, opsins need to be expressed in neurons. This can be accomplished in three different ways:
    - *Virus transduction*: the gene coding for the opsin is introduced to a viral agent which is then inserted into the organism, for example, a laboratory mouse. It is very important to include the appropriate promoter along with the gene!
    - *Transfection*: new genetic material coding for the opsin is directly inserted into the organism.
    - *Transgenic animal lines*: techniques such as electroporation and knockout mice are used to grow genetically modified organisms from birth.
        - The *knockout mouse* is a mouse genetically engineered with a missing gene or set of genes (hence "knockout").
            - In the embryonic stage of growth, modified genetic material is introduced to embryonic stem cells. As a result, the knocked-out genetic material will be missing across all tissues.
            - *Electroporation* can be used to open up the cell membrane to allow for the insertion of genetic material. An electric field makes the membrane temporarily porous, hence the name.

- *Chimeric mice* can also be created for genetic experimentation. These mice carry two different genomes, depending on the tissue.
  - Again, electroporation is used to introduce new genetic material into an embryo.
  - The embryo is introduced to a foster mother, which will birth mice carrying both sets of genes.
  - By breeding these hybrids with each other, eventually, a mouse homozygous for the modified genetic material will be created naturally. These mice can be evaluated for viability, as well as other physical and behavioural changes.

- Once the organism (for example, the mouse) is genetically modified, light needs to reach the modified neurons. Small holes are drilled through the skull of the organism, and optical fibers carry light to the target region.



Phenotypic variations in chimeric mice.

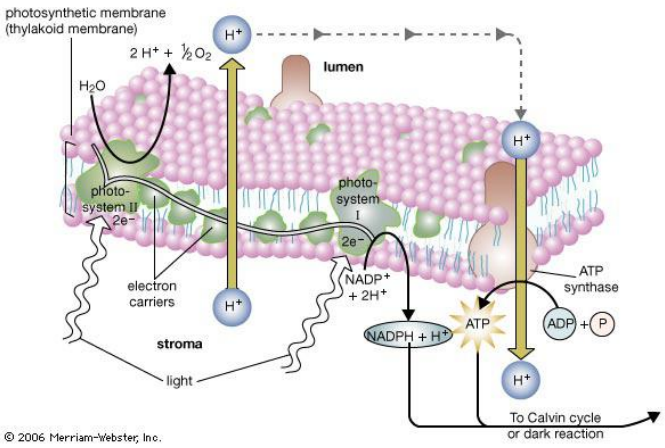
3. Describe photosynthesis

Plants are able to synthesize *glucose* (chemical energy stores) by harnessing light.

- Inside plant cells, there are *chloroplasts*, the organelles responsible for photosynthesis. Within those chloroplasts' stroma (the inner "liquid") are the *thylakoids*, stacked into grana. Finally, inside those thylakoids, there is *chlorophyll*, the pigment that absorbs light of specific spectra.
- Photosynthesis works by transferring energy from location to location, through ATP and NADPH energy carriers. Eventually, the *Calvin Cycle* uses this harnessed energy to create sugar molecules.
  - First, in *photosystem 2* (yes, 2, thank you science) chlorophyll molecules are excited by light, and transfer an electron to the reaction centre (P680, for 680 nanometre light). The electron is given away, passed down the electron transport chain (as it is progressively de-energized), giving P680 a positive charge. This

positive P680 steals an electron from water, breaking it down into molecule oxygen and a hydrogen ion. The hydrogen accumulates within the thylakoid, while the oxygen exits.

- The electron lost by P680 in the previous step will then trigger mechanisms that pull more hydrogen into the thylakoid. This electron then reaches photosystem 1.
- In the second photosystem, *photosystem 1* (again, round of applause for science) which contains P700, the electron is excited yet again by light, which allows for the creation of NADPH by combining NADP+ and hydrogen.
- Finally, the hydrogen accumulated within the thylakoid exits through *ATP synthase*, a "turbine" protein which uses kinetic energy from moving hydrogen ions to synthesize ATP from ADP and inorganic phosphate.
- In the Calvin Cycle, the ATP and NADPH created previously are used to create glucose, providing food for the plant.



4. Discuss artificial leaves

Artificial leaves are lightweight objects that operate by splitting water on a silicon platform using certain catalysts. This process generates molecular hydrogen, which could be used as an energy source.

Unfortunately, the drawbacks include high manufacturing costs and the unlikelihood that it could be used commercially.

Lecture #7: Artificial Photosynthesis and the Propagation of Electrical Signals

In this lecture synthetic biology is briefly introduced. The neuron and the action potential are then explained in greater detail.

Lecture objectives:

1. Further explore artificial photosynthesis
2. Describe some uses for synthetic biology
3. Understand the action potential in neurons
4. Describe the role of synapses and connexons
5. Name types of electrodiagnosis measurements

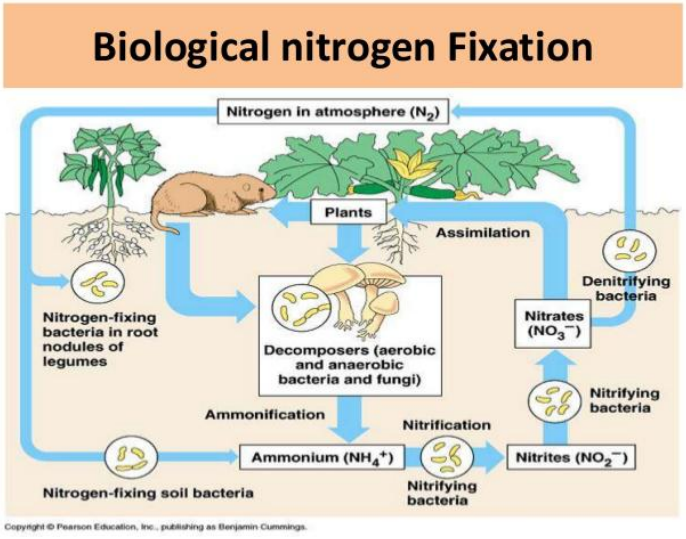
1. Further explore artificial photosynthesis

Artificial photosynthesis is a carbon-neutral, sustainable method for generating energy by splitting water to form molecular hydrogen. Water splitting like this can be achieved by a proton-coupled multi-electron transfer process, allowing for net energy to be produced.

This hydrogen can then be used as a clean fuel source for multiple industrial processes such as drug production, polymer synthesis, and more! Refer to the previous lecture for more information on the artificial leaf.

2. Describe some uses for synthetic biology

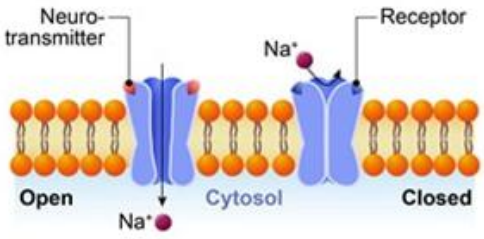
Nitrogen fixation is key to fertilization and therefore increasing crop yield. *Synthetic biology*, that is, the creation of new biological systems (or the modification of existing biological systems), can be used to engineer organisms designed to fix nitrogen. These organisms use molecules produced by artificial photosynthesis among others as food. As such, the overall process is more efficient and does not rely on traditional nutrition sources.



3. Describe how ion gradients generate electric potential

There are many different ion *channel proteins* in the membrane. Each channel has its own function:

- *Resting potassium channels* generate the resting potential across the membrane.
- *Voltage-gated channels* propagate the action potential. As their name suggests, a certain voltage is needed to activate them.
- *Ligand-gated* and *signal-gated channels*, present in dendrites and neuronal cell bodies generate electric signals in post-synaptic cells.
    - Ligand-gated channels have a site for a particular extracellular *neurotransmitter* to bind. This means they respond to external stimuli.
    - Signal-gated channels respond to intracellular signals resulting from a neurotransmitter binding to a distant receptor. Overall, a neurotransmitter binds to a receptor, which activates a *G protein* (a protein used in signal transduction within cells). This G protein travels to the signal-gated channel, activating it.



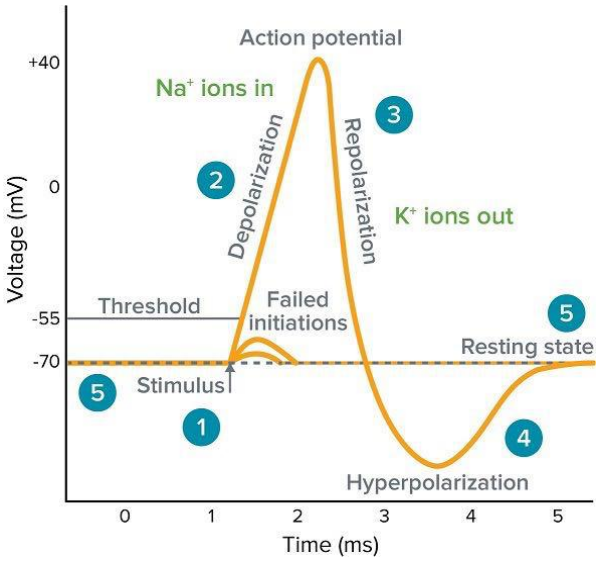
When a large enough signal is received by a neuron, the membrane begins to depolarize as a result of voltage-gated channels' activation. The resting potential is disrupted. As the depolarization grows, a point of no-return is reached, and an *action potential* becomes inevitable. The action potential leads to a *unidirectional propagation* of the signal. The action potential process is described here with numbered steps corresponding to those illustrated in the subsequent figure:

1) A sufficiently large stimulus is received, meaning a large number of voltage-gated channels have opened (protein channel conformation change). While resting potential is -70 millivolts, the membrane must depolarize to at least -55 millivolts for the action potential to be triggered.
2) A massive *depolarization* of the membrane occurs, spiking near +40 millivolts, transmitting the signal onward (unidirectionally).
3) The membrane will then polarize as the voltage-gated channels close, reversing the disruption to the membrane potential.
4) The membrane potential overshoots resting potential, dipping down near -80 millivolts.

This is called *hyperpolarization*. As the potential climbs back to -70 millivolts (resting potential), the neuron cannot fire another action potential. This period is known as the *refractory period*.

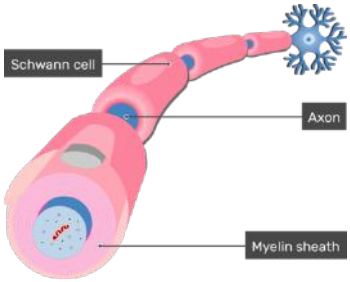
5) Finally, the membrane returns to its resting potential of -70 millivolts.

It is interesting to note that while the depolarization of the membrane is bidirectional, propagation of the action potential is unidirectional. In fact, the refractory period of the previous neuron prevents the action potential from going backwards: sodium channels cannot be activated during this period. As such, only subsequent sodium channels can be activated!



The speed of propagation of the signal along nerves depends heavily on *myelination* (the addition of myelin) of the neuron's axon. Myelinated neurons transmit information at least one order of magnitude faster than unmyelinated neurons.

Schwann cells are responsible for myelination. They add myelin at just the right locations along the axon to allow sodium channels to open at specific nodes, called *Nodes of Ranvier*. This allows for an action potential to move in a very targeted way, from node to node. Propagation of the signal like this is called *saltatory conduction* (meaning "jumping conduction") .

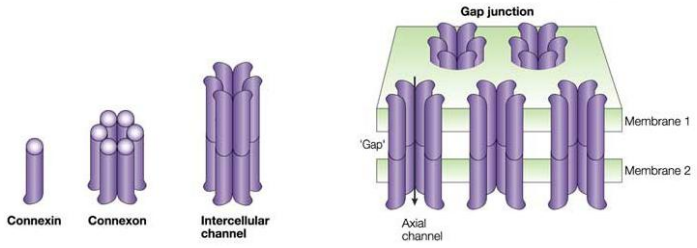


4. Describe the role of synapses and connexons

At the end of a neuron, the action potential must be communicated to the following neuron.

This is done through *synapses* and *connexons*.

- Synapses use neurotransmitters to transmit the action potential. This is a form of *chemical communication*. Synapses are useful because they prevent signals from traveling backwards (there are no receptors for the neurotransmitters at the end of the neuron) and because the signal is completely regenerated to full strength (*signal amplification*) after being passed on to the next neuron. The signal is transmitted as follows:
    1) The action potential arrives at the synapse and causes calcium-releasing voltage-gated channels to open. Calcium triggers neurotransmitter vesicle fusion with the membrane.
    2) Neurotransmitters are released into the synapse.
    3) Neurotransmitters diffuse across the synapse.
    4) Receptors on the accepting ("post-synaptic") neuron bind the neurotransmitters.
    5) The post-synaptic neuron's membrane depolarizes, propagating the action potential.
- Connexons use electric impulses to transmit information. This is instead a form of *electrical communication*. Connexons do not require the relatively slow diffusion of neurotransmitters, making them a faster method for communication. Connexons are made of *connexin* subunits. They make up *gap junctions* in many different types of cells. Chemical signals allow connexons to open or close, modulating signal transmission through gap junctions.



5. Name types of electrodiagnosis measurements

Electrodiagnosis is the use of electrical signals to obtain information about diseases. Various stimuli are used to trigger electrical signals in the body. These signals can then be measured. Three examples of electrodiagnosis are:

- *Electrocardiography* (ECG)
- *Electroencephalography* (EEG)
- *Electromyography* (EMG)

Lecture # 8: Electrical Phenomena

This lecture explains biosensors and biosensor components in more detail.

Lecture objective:

1. Understand biosensor components

1. Understand biosensor components

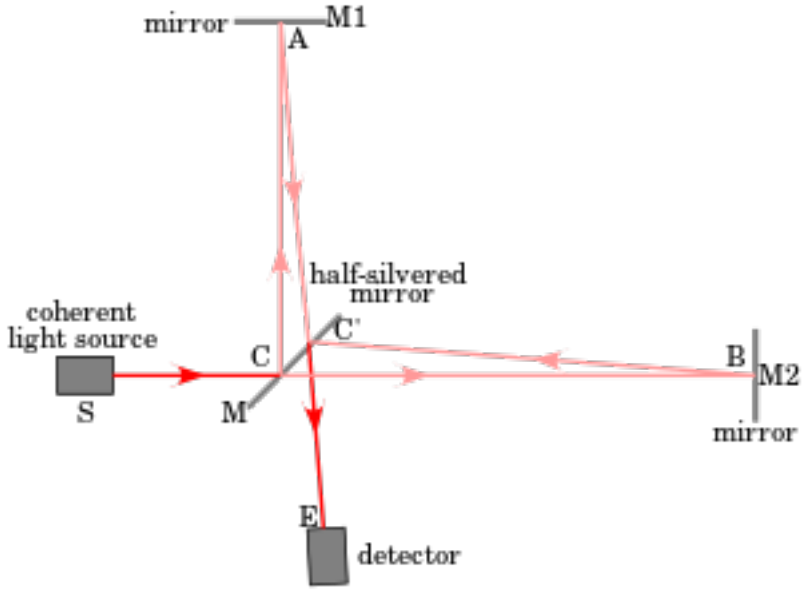
As briefly introduced back in lecture #5, biosensors are made up of five key elements:

1) The *biorecognition element*: this is the part of the biosensor that detects the stimulus. It is typically specific to that stimulus. For example, specific antibodies can be used to target particular proteins, and nucleic acid strands can be used to detect viruses.
2) The *transducer*: there are multiple different types of transducer available. Transducers react to the detection of the target stimulus.
    - *Mechanical transducers* (mass-based) operate by measuring vibration frequencies. An element will vibrate at a default frequency. When the biorecognition element binds the target, the mass increases, causing a change in the vibration frequency. This difference is measured electrically and can be used to determine the additional mass.
        - Devices such as *cantilever beams* are used for target binding. Techniques such as atomic force microscopy also use cantilever beams for ultra-precise terrain recognition, for example.
        - The *piezoelectric effect* is also popular. When a voltage is applied to a crystal, it will deform slightly.

Cantilever Beam

- *Electric transducers* operate by detecting changes in electrical parameters of the sample. An incomplete circuit may require the binding of specifically charged particles in order to complete the circuit. This completed circuit communicates the binding of the target.
- *Temperature-based transducers* operate through heat-generating reactions. When binding events occur, the heat generated by the reaction is detected. The exact change in temperature can then be used to determine the number of binding events.

- *Electrochemical transducers* operate on the principle that chemical reactions produce or consume ions or electrons. When this happens, solution properties change. Current, voltage, and impedance of the solution can then be measured.
- *Optical transducers* detect binding events through the optical phenomena associated with the captured target. Phenomena include fluorescence, luminescence, transmission, scattering, and more! A popular technique used here is interferometry. The *Michelson Interferometer* operates by comparing reflected wave coherence. A light source directs a beam toward a *half-silvered mirror*, which evenly splits the beam: half is transmitted (to M2 below) while the other half is reflected (to M1 below). The light then returns to the half-silvered mirror and recombines, continuing into the detector. If the mirrors are at exactly the same distance from the half-silvered mirror, the detector will register a coherent beam of light. Otherwise, the detector will notice a discrepancy. The mirrors can be moved as a result of a binding event, to name just one example.



- *Photodiodes* are a common type of transducer. They convert light into electrical signals. When light is detected, a small reverse current flows through the photodiode. This current is proportional to the incident light intensity. The sensitivity of the device is, however, proportional to the wavelength of the light. Photodiodes operate on a similar principle to semiconductors as they also make use of the P-N junction. To learn more about the P-N junction, return to lecture #5!

2) The *amplifier* boosts the signal for processing by amplifying voltages or currents. Differential or operational amplifiers are most commonly used.
3) The *signal converter* performs signal processing. Optical, mechanical, or direct electrical signals are converted to usable electrical signals. Field programmable gate arrays (FPGAs) contain logic gates and can be programmed for various processing tasks.
4) The *recorder* makes sense of the final signal and allows the data to be accessed at a later time.

Lecture # 9: Synthetic Biology

This lecture explores the fundamentals of synthetic biology.

Lecture objectives:

1. Define synthetic biology and relate it to the central dogma of biology and the discovery of DNA
2. Describe the role of restriction enzymes and ligation enzymes in synthetic biology
3. Explore inducible promoter systems as an alternate method for expression control
4. Understand how fluorescent proteins are used as laboratory tools
5. Describe genome sequencing methods
6. Understand the basics of CRISPR-Cas9

1. Define synthetic biology and relate it to the central dogma of biology and the discovery of DNA

Synthetic biology is the field of biology that works to design and create new biological systems from scratch. It also explores ways of reengineering existing biological systems to exploit various useful properties of biology.

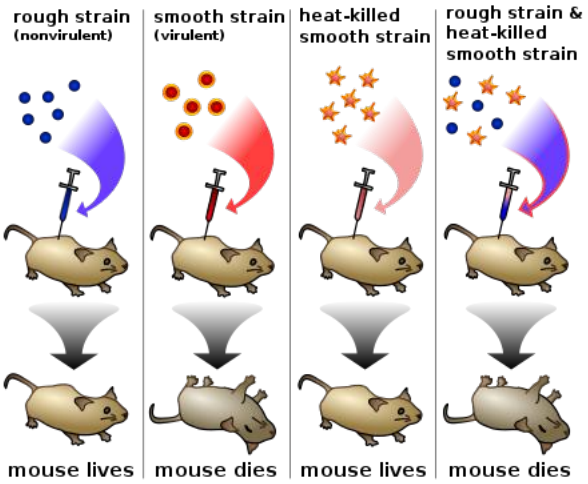
The central dogma of biology can be broken down into three steps:

1) *DNA Replication*: DNA will be duplicated continuously as cells divide and the organism grows and maintains itself.
2) *Transcription*: DNA will be read by RNA polymerase enzymes which produce messenger RNA strands.
3) *Translation*: RNA strands are processed by ribosomes which synthesize the proteins needed for the proper functioning of the organism.

With the exception of some RNA-based viruses, DNA is the molecule that encodes genetic information. Two famous experiments explored the nature of DNA and the ability of DNA to be transferred between organisms:

- *Griffith's experiment* involved two bacterial types. One bacterium was coated in a polysaccharide shell (the smooth strain) which prevented mice immune systems from detecting a threat. The presence of the shell essentially leads to the death of the infected mouse. The other bacterium, the rough strain, did not have this shell, therefore allowing immune system targeting, eliminating the threat and keeping the mouse alive.
    - Griffith heat-killed the smooth strain and injected it into a mouse: the mouse survived.
    - Griffith then injected the heat-killed smooth strain and living rough strain. While the rough strain should not be harmful, the mouse died. This was because the living rough-strain bacteria were transformed: the genes leading to the creation

of the protective shell were taken off the dead smooth-strain bacteria by the rough-strain bacteria and used to evade detection by the immune system.



- *Avery, McCarthy, and MacLeod's experiment* involved fluorescently tagging proteins and DNA to determine which was involved in storing genetic information.
    - One phage (a bacterium-targeting virus) had its outer protein shell tagged with sulphur-35 fluorescent dye.
    - Another phage had its viral DNA tagged with phosphorus-32 fluorescent dye.
    - Bacteria were infected with both phages. These cultures were then blended together. Using centrifugation, the phages and bacteria were separated. The sulfur-tagged protein was found in the supernatant (the liquid containing the phage) while the phosphorus-tagged DNA was found inside the bacteria. This revealed that it was in fact DNA that was transmitted and used to infect bacteria, indicating in turn that DNA carried genetic information.

2. Describe the role of restriction enzymes and ligation enzymes in synthetic biology

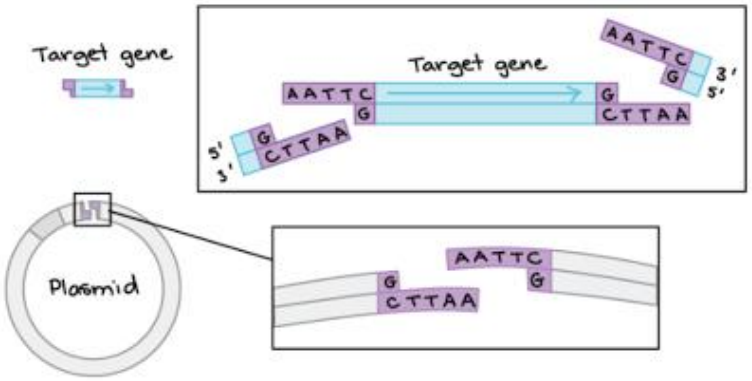
Synthetic biology involves the editing of genetic sequences. Nature has already provided molecular tools for basic genetic editing: restriction and ligation enzymes.

- *Restriction enzymes* will cut DNA at a specific recognition sequence, performing nuclease activity. In nature, restriction enzymes are used by bacteria to destroy foreign DNA. It is, essentially, a primitive form of an immune system! Restriction enzyme cuts can either give blunt or sticky ends:
    - A *blunt end* is the result of a cut straight down the double-stranded DNA: only phosphodiester bonds will be broken.
    - A *sticky end* is the result of a cut with overhang on each single DNA strand: both phosphodiester bonds and hydrogen bonds will be broken.

- *Ligation enzymes*, on the other hand, use ATP-catalyzed reactions to repair DNA and RNA damage, joining strands together.

These enzymes can be used as a pair to introduce genes of interest into other genomes. Typically, *plasmid DNA*, a circular chromosome found in bacteria, is used along with the enzymes. DNA recombination is then possible:

1) A plasmid is "opened" using a restriction enzyme. This plasmid can now accept DNA insertions.
2) A eukaryotic DNA fragment (typically a gene of interest) is added.
3) Ligation enzymes seal up the plasmid, locking the eukaryotic DNA into the circular chromosome. This creates recombinant plasmids: plasmids containing DNA from multiple different sources.
4) The plasmids are placed into bacteria (such as E. Coli). These bacteria are then allowed to multiply, creating an incredible number of copies of the target DNA in a short amount of time (you can create millions, if not billions of copies overnight by using E. Coli, which divides every 20 minutes!).
5) The bacteria will express the protein coded for by the inserted DNA.
6) Any number of tasks can then be performed: the protein could be harvested directly for experimental or commercial use, the plasmids could be stored in a DNA library, and more. One real life application of this technique: human insulin is, in fact, produced using this method!



In order to extract plasmid DNA from bacteria, *"prep" experiments* are performed. While there are many variants, they all follow similar core steps:

1) *Harvest*: the bacteria containing the plasmids are grown, typically overnight.
2) *Lysis*: lysis buffers are used to break apart the bacteria, releasing the plasmids into the surrounding solution.
3) *DNA binding*: a column filter is used to extract the plasmids from the solution. The filter in the column binds plasmids while allowing all other elements to pass through.

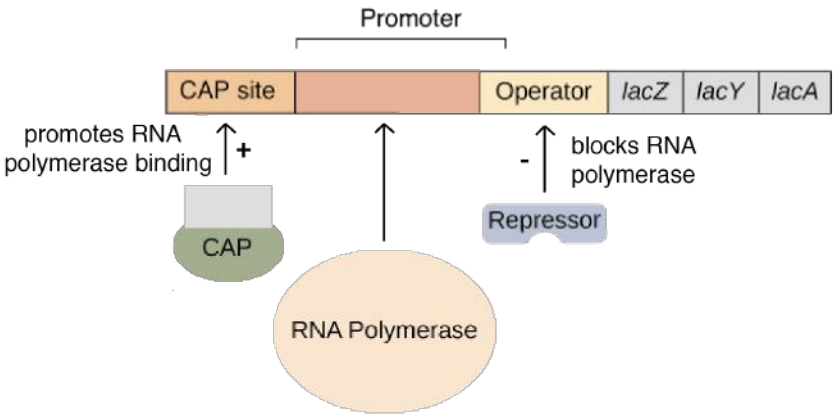
4) *Wash*: an elution buffer is passed through the column, forcing all bound plasmids to wash out of the column and into a separate flask. The plasmid has been isolated!

3. Explore inducible promoter systems as an alternate method for expression control

The *LAC* operon is an example of natural gene expression control in bacteria. An *operon* is a DNA fragment containing multiple closely linked genes controlled by a common upstream promoter. Operons are found exclusively in prokaryotic life. The *LAC* operon controls the expression of genes related to the breakdown of lactose into glucose such that the organism can create energy-carrying molecules: ATP. The control is in two parts:

- An *operator* region located just downstream of the promoter may bind an *inducible repressor protein*. This means that when lactose is <u>not</u> present, the repressor sits on the operator and prevents RNA polymerase from creating mRNA. When lactose is added to the system in excess, some lactose binds the repressor, releasing it from the operator and freeing up the operon for transcription.
- However, it is important to remember the goal of the *LAC* operon: the regeneration of the organism's energy supply. If energy supplies are already high, the organism should not waste resources on creating enzymes designed to break down lactose. This is where the *CAP protein* comes into play. Just upstream of the promoter is a CAP site for the CAP protein to bind. The CAP protein guides the RNA polymerase to the promoter region, allowing it to transcribe the DNA into mRNA. However, the CAP protein requires *cAMP* (cyclic adenosine monophosphate) to function. If energy reserves are high, all energy carrying molecules are in the ATP form. Slowly, as energy reserves are used up, ATP becomes ADP, and ADP becomes AMP. This AMP quickly becomes cAMP, activating CAP.
- In summary, the *LAC* operon only functions when there is low glucose (and therefore high cAMP) and lactose present. The lactose removes the repressor, opening up the operon for transcription, while the cAMP activates the CAP protein, which guides RNA polymerase to the promoter.

A system such as the *LAC* operon is called an *inducible promoter system* because certain key conditions must be met for the promoter to be accessible and used to produce mRNA transcripts. By using a similar system, genes embedded in a plasmid vector could be activated under specific conditions!

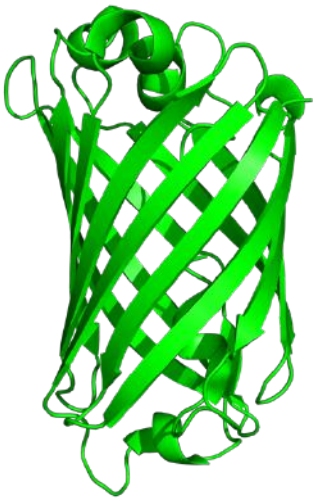


4. Understand how fluorescent proteins are used as laboratory tools

Green fluorescent protein (GFP) was discovered in jellyfish some time ago. GFP has a barrel-like structure, with this "barrel" containing a chromophore which gives it its characteristic fluorescent glow.

Since the discovery of GFP, experiments have been conducted to create differently coloured fluorescent proteins.

- *Undirected evolution* experiments place GFP into an organism and, as time passes, GFP accumulates mutations. Mutations leading to a colour change are then analyzed.
- *Directed evolution* experiments use genetic editing to directly modify GFP's gene's sequence in the hopes of changing the protein's fluorescence colour.

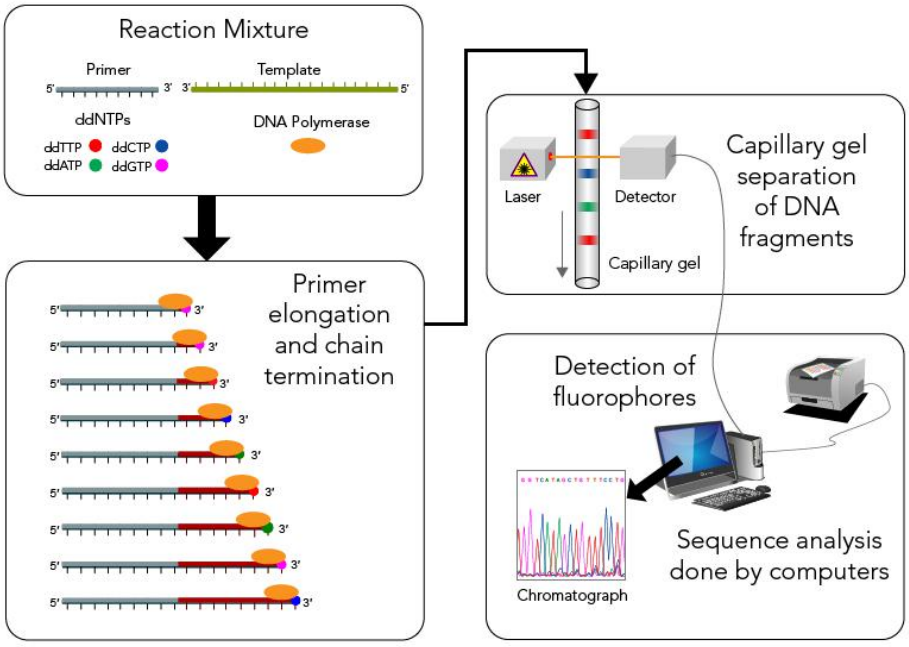


5. Describe genome sequencing methods

Gene sequencing is the process by which nucleic acid molecules' (most commonly, DNA) sequence is analyzed and recorded. With that kind of data, analysis can reveal the purpose of various genes and other DNA or RNA fragments. It can also be a first step towards gene

editing, for example. Due to the data-intensive nature of sequencing, *polymerase chain reaction* (PCR) was needed before sequencing techniques arose. As such, most sequencing experiments occurred as of the late 1980s. Today, multiple different sequencing techniques exist:

- *Sanger sequencing* is one of the oldest sequencing techniques. It is a time-consuming process relative to more modern alternatives. The technique proceeds as follows:
    1) A short DNA fragment is denatured such that the template strand can be isolated. Fragments are typically around 100 nucleotides in length.
    2) The fragment is extensively duplicated using PCR.
    3) Four different vials are then used: in each, some of the denatured DNA is placed along with DNA polymerase, regular nucleotides, and one of four *dideoxynucleotides* (ddNTPs). The ddNTPs lack an extra oxygen atom. As such, when one of them is added to a growing DNA strand, this caps off the strand for good. So, when these ddNTPs are placed in a vial with normal nucleotides, random chance will generate strands of varying lengths.
    4) The contents of each vial (one for each ddNTP) is then passed through a gel by gel electrophoresis. The resulting bands indicate the locations of the various nucleotides within the sequence. For example, if the vial containing ddCTP (dideoxycytidine) shows bands at a strand length of 56 nucleotides, the 56th nucleotide in the strand is likely cytosine! Note that computer software is typically needed to distinguish the bands.

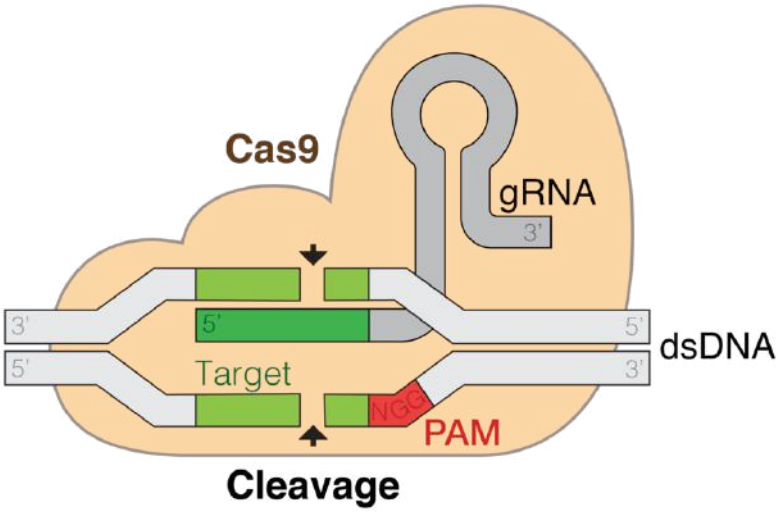


- *Shotgun sequencing* involves fragmenting much larger strands of DNA to allow for chunk-by-chunk sequencing. This technique proceeds as follows:
    1) Extracted DNA is broken into small fragments using restriction enzymes.
    2) The fragments are placed into plasmid vectors and replicated in bacteria.
    3) Each fragment is sequenced individually using Sanger sequencing.
    4) Computer algorithms will then look for sequence overlap in the multiple copies of each fragment and piece together the entire original strand.
- *Nanopore sequencing* is a far more advanced technique that is, in fact, portable! A strand of DNA is placed into a nanopore on the device. An electric current generated within the device will fluctuate slightly depending the nucleotide being monitored. These fluctuations can be decoded and tied back to the nucleotide in question.

6. Understand the basics of CRISPR-Cas9

CRISPR (Clustered Regularly-Interspaced Short Palindromic Repeats), short for *CRISPR-Cas9*, was originally found in bacteria which used CRISPR as a defence mechanism against viral infections:

1) A virus injects DNA into a bacterial cell.
2) The bacterial cell reacts by generating a new *spacer sequence* and incorporating it into the CRISPR DNA sequence. This adapts the CRISPR defence system to the particular viral DNA.
3) The DNA is transcribed into a special *crRNA* (CRISPR RNA). This special RNA will then guide the Cas9 complex (a *nuclease*, that is, it cuts DNA) to the viral DNA.
4) Cas9 uses the *PAM sequence* (a special targeting sequence) on the viral DNA to know where to make the cut in the strand. This ensures specificity in targeting.



Lecture #10: Long-Range and Short-Range Electrostatic Forces in Biology

In this lecture, various charge-based forces and phenomena are introduced and explained.

Lecture objectives:

1. Understand Coulomb's law
2. Know and explain the different types of intramolecular forces
3. Understand the Lennard-Jones potential
4. Know and explain the different types of intermolecular forces
5. Explain physical and biological phenomena using knowledge of electrostatic forces

1. Understand Coulomb's law

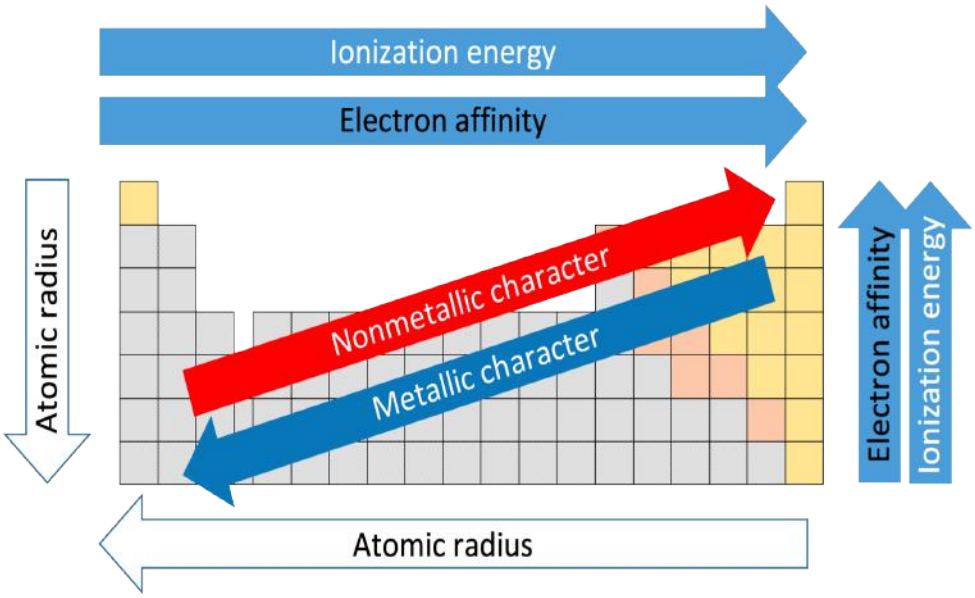
Coulomb's law, otherwise known as the *inverse-square law*, is used to quantify the electrostatic force between charged particles. The force is computed as follows:

$$F = k_e \frac{q_1 q_2}{r^2}$$

When the charges are of opposite signs (that is, the force is negative), the charges will attract each other. Conversely, when the charges are of the same sign (that is, the force is positive), the charges will repel each other.

2. Know and explain the different types of intramolecular forces

Intramolecular forces occur between the atoms of a same molecule. They are a type of short-range electrostatic force. Their formation often depends on one or more *electronegativity* trends, which describe atoms' tendency to attract electrons toward themselves:



Three types of intramolecular forces are discussed here:

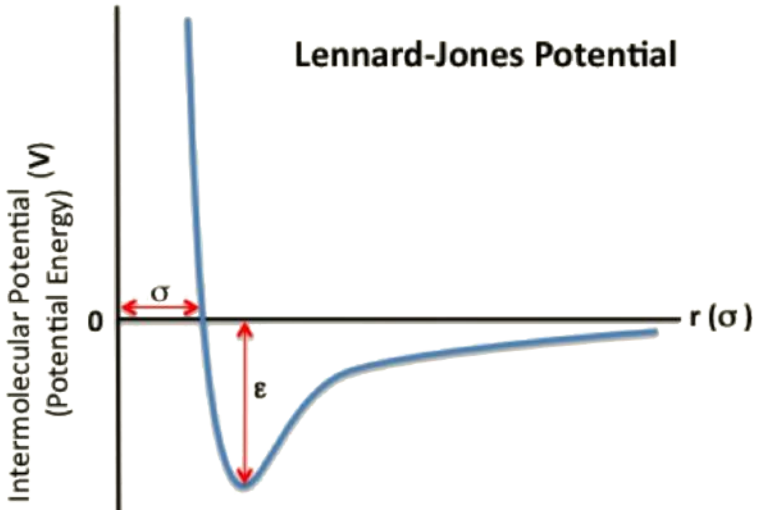
- *Ionic bonds* occur between metallic and non-metallic elements (this means that two metallic elements, or two non-metallic elements, could not form an ionic bond).
    - Valence electrons are completely transferred between atoms, converting each atom into charged ions which subsequently attract each other.
    - The metallic atom loses electrons, becoming a positively charged cation.
    - The non-metallic atom gains electrons, becoming a negatively charged anion.
    - Ionic bonds typically require a large difference in electronegativity between the involved atoms.
- *Covalent bonds* occur between non-metallic elements exclusively. Valence electrons are shared between atoms, completing the octets of both parties. The atoms involved in covalent bonds will have similar electronegativities. Covalent bonds can be non-polar or polar:
    - *Non-polar covalent bonds* require an electronegativity difference between the two atoms of less than 0.5. The resulting bond will not be polar.
    - *Polar covalent bonds*, on the other hand, require an electronegativity difference between the two atoms ranging from 0.5 to 1.9. The resulting bond will create a mild dipole by induction. The slight electronegativity difference pulls electrons more to one atom than the other, creating a charge imbalance in the bond.
- *Metallic bonds* are special covalent bonds that occur between metallic elements exclusively. Tightly packed metallic atoms create a lattice for electrons to move around freely. Metallic bonds occur in pure elemental metals (for example, gold) and alloys (for example, bronze).

3. Understand the Lennard-Jones potential

The *Lennard-Jones potential* describes the potential energy between non-bonding atoms given the distance between them. The potential itself (not the potential energy) computed as follows:

$$V(r) = 4\epsilon[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}]$$

It is also graphically represented as follows:



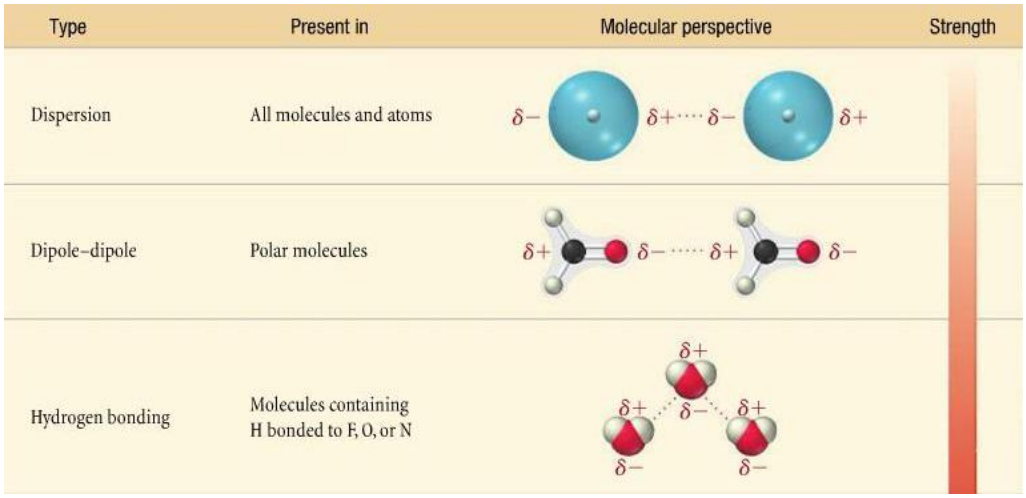
The Lennard-Jones potential is made of a large repulsive term (the first part of the plot above), followed by a smaller attractive term. This attractive term represents the London dispersion forces (described below in this lecture). The sigma value represents how close the two particles can get to each other. This value is known as the *Van der Waals radius*, and it is reached when potential is zero (in other words, the perfect distance between particles is reached to avoid attraction and repulsion).

4. Know and explain the different types of intermolecular forces

Intermolecular forces occur between atoms of different molecules. They are weaker than intramolecular forces, as they do not involve the sharing or exchanging of electrons. Intermolecular forces define matter's phase-related properties such as surface tension, viscosity, and vapour pressure. These forces can be short-range (when molecules' centres are separated by up to three angstroms) in which case they will usually be repulsive, or they can be long-range (when molecules' centres are separated by more than three angstroms) in which case they will normally be attractive. The long-range forces are known as *Van der Waals forces* which can be broken down into three categories:

- *Dipole-dipole* interactions occur when a partially positive atom from one molecule interacts with a partially negative atom from another molecule. These partial charges could, for example, be a result of polar covalent intramolecular bonding.
- *Hydrogen bonding* is a subtype of dipole-dipole interaction which is noticeably stronger due to the intensity of the partial charges involved. It typically occurs between hydrogen and either oxygen, fluorine, or nitrogen. The strength of these bonds explains, among other phenomena, the high boiling point of water!
- *London dispersion* forces are extremely short-lived and weak interactions between atoms

whose electrons shifted randomly. These shifts create (very) temporary dipoles within molecules that would normally not be considered polarized. Typically, more electrons in a molecule lead to stronger London dispersion forces, as more electrons are moving around, creating temporary dipoles.



5. Explain physical and biological phenomena using knowledge of electrostatic forces

The electrostatic interactions between atoms and molecules discussed above lead to a large variety of phenomena in chemistry and biology. Here are just a few:

- *Self-assembly* is the process by which disorganized particles will spontaneously form an organized structure as a result of multiple different forces and interactions (these are discussed in the next lecture!). This phenomenon is quite common in biology, from the folding of protein chains to the creation of lipid bilayers.
- *Disulfide bonding*, a type of intramolecular interaction, is an example of a stabilizing interaction within biomolecules. They occur between cysteine amino acids in protein chains and lead to stronger *tertiary structures*. Intermolecular forces are used to stabilize the *quaternary structure*, which holds together multiple protein complex subunits.
- *Capillary action* occurs due to *cohesion* and *adhesion* forces. Cohesion is the binding of liquid molecules to each other, while adhesion is the binding of liquid molecules to a surface. Capillary action can be observed in a straw for example.
- *Surface tension* is the result of cohesive forces between liquid molecules at an interface. It leads to a surface film: it is more difficult to break an object through this film than to move that object in the liquid once it is beneath the surface. *Surfactants* are compounds which lower surface tension by disrupting intermolecular forces at the interface.

Lecture #11: Self-Assembly and Networks

This lecture dives deeper into self-assembly and introduces the concept of networks.

Lecture objectives:

1. Identify forces that are responsible for self-assembly
2. Explain hydrophobicity, superhydrophobicity, and the causes of these phenomena
3. Describe self-assembly with thermodynamics
4. Discuss the coffee ring as an example of self-assembly
5. Discuss biomimetics and its applications
6. Explore the basics of networks

1. Identify forces that are responsible for self-assembly

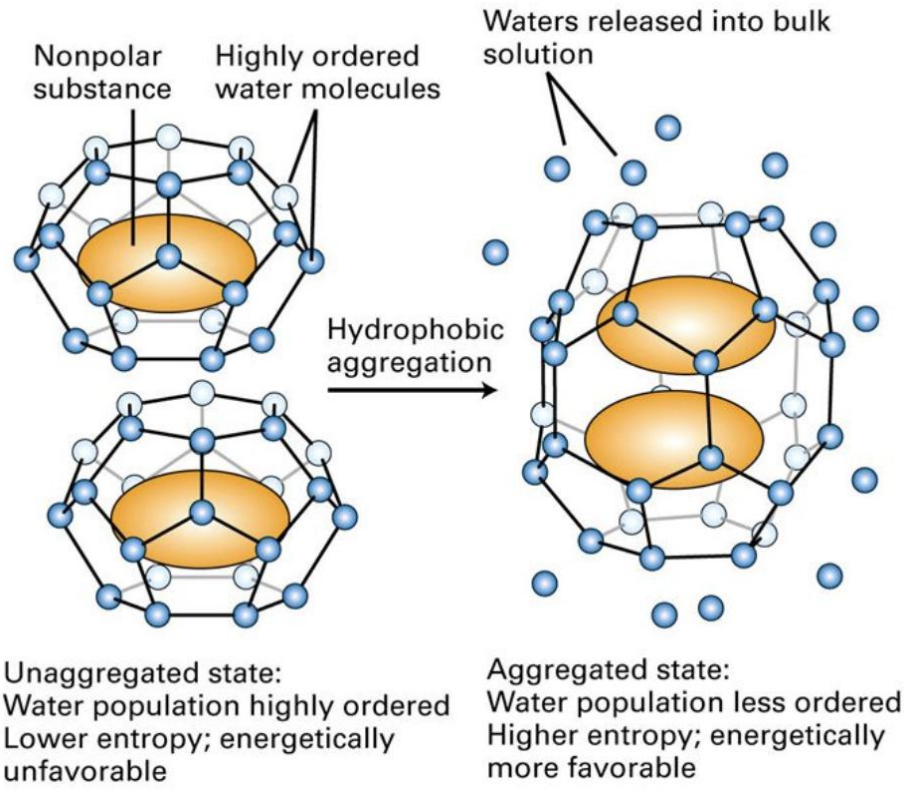
A number of forces, such as those discussed in the previous lecture, drive *self-assembly*. They include (though they are not limited to):

- *Electrostatic intramolecular* and *intermolecular* interactions, though mostly hydrogen bonding
- External forces such as *electric*, *magnetic*, *flow*, *osmotic* and *gravitational* forces
- *Interface forces*, both electrostatic and entropic

While biology is dominated by self-assembly, self-assembly occurs in all kinds of places! Snowflakes for example, are a case of self-assembly!

2. Explain hydrophobicity, superhydrophobicity, and the causes of these phenomena

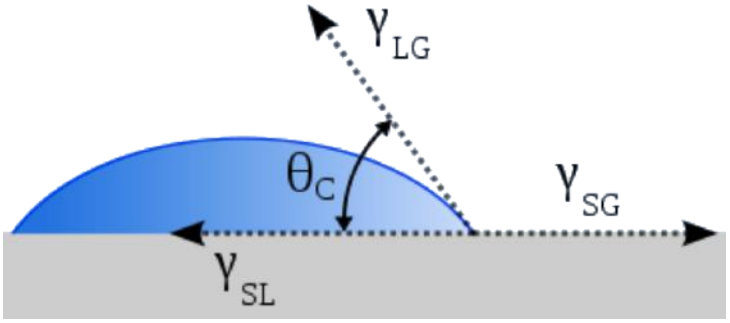
Hydrophobicity is a result of entropy and is often a major contributor to biological self-assembly. *Entropy* is the universal drive for the universe to be in a more random, disordered state. Hydrophobicity involves non-polar groups aggregating, which, at first, may seem counterintuitive. To see how this agrees with the theory of entropy, we need to look at the situation from another perspective: the perspective of the water molecules that envelop these non-polar groups! In fact, when non-polar molecules aggregate in water, fewer water molecules are needed overall to form the "cage" that surrounds the aggregates. As such, the water becomes more disorganized, increasing the overall entropy of the universe! This process is illustrated here:



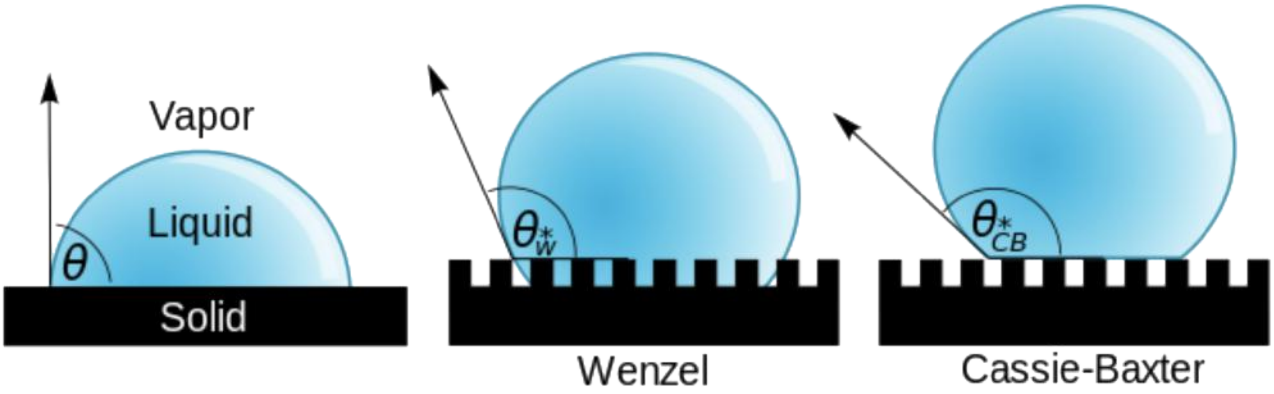
Superhydrophobicity is a result of micro- and even nano-structured patterns. Hierarchical structures increase hydrophobicity, in other words, making it easier for water to flow off of a material!

The difference between hydrophobicity and superhydrophobicity is mathematical: it comes down to the *contact angle* between the solid and the liquid. The more hydrophobic a surface is, the greater its contact angle will be. Generally, if the contact angle is greater than 90 degrees, the surface is considered superhydrophobic. Various regimes exist to describe the different degrees of hydrophobicity, including the *Wenzel regime* which is characterized by micro-structures into which the water droplet embeds itself, and the *Cassie-Baxter regime* which is characterized by hierarchical nano-structures that the water droplet "floats" above. The contact angle is calculated as follows, according to the accompanying illustration:

$$\gamma_{SG} = \gamma_{SL} + \gamma_{LG} \cos\theta$$



The superhydrophobic regimes can then be depicted as follows:



3. Describe self-assembly with thermodynamics

Self-assembly is a spontaneous process. In other words, the *Gibbs free energy* must be less than zero, and there must be a net gain in entropy. While you will learn more about this in future bioengineering courses, for now, note that the Gibbs free energy can be computed using the following set of equations for the Gibbs free energy, enthalpy, and internal energy, respectively (all terms are explained in the equations list later in this course-pack):

$$\Delta G_{SA} = \Delta H_{SA} - T\Delta S_{SA}$$

$$H(S,p) = U + pV$$

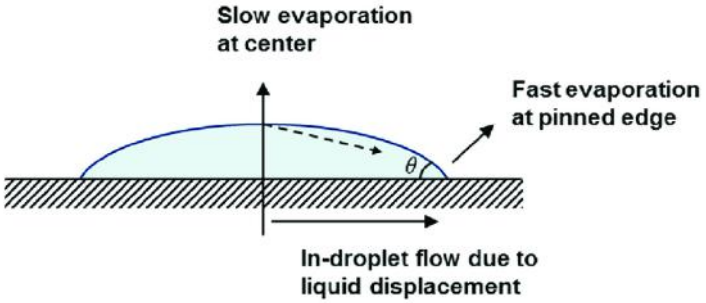
$$U = \sum_{i=1}^{N} p_i \, E_i$$

4. Discuss the coffee ring as an example of self-assembly

Have you ever noticed the ring that forms after you spill a bit of coffee around the base of

your cup? Believe it or not, that's an example of self-assembly! The *coffee ring effect* is driven by *surface tension* and *convective forces*. When the outer layer of the ring evaporates, it creates a slight temperature differential between the outside and inside of the ring. This then leads to convection within the ring, pushing smaller particles out towards to edge of the ring and keeping larger particles towards the middle. That's why the outside of the ring always looks darker: it is highly concentrated in small particles that were pushed to the edge of the ring!

This effect has potential for inspiring the development of methods for concentration and sorting of mixture components.

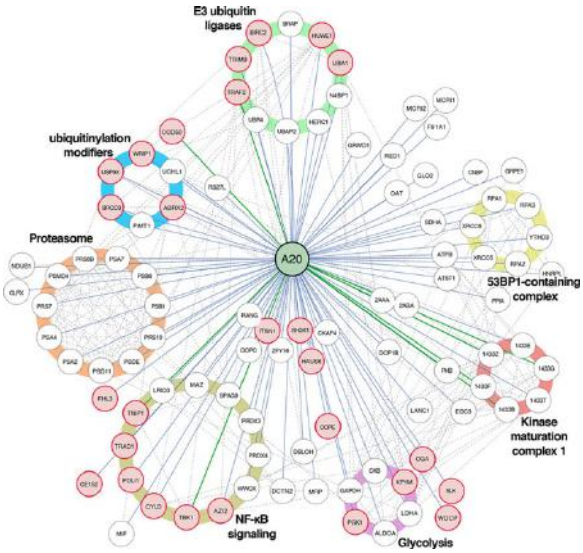


5. Discuss biomimetics and its applications

Biomimetics is the field that uses the structures and processes found in nature as inspiration for new synthetic structures and processes we can use in our lives. For example, when termites build their nests, they incorporate temperature-efficient design elements into their architecture. We can do the same in our buildings!

6. Explore the basics of networks

Networks are combinations of various *nodes* (elements) linked together through *edges* (the lines between elements). Many types of networks exist, such as *disease networks*.



Lecture #12: Microfluidics and Optofluidics

This lecture touches upon biomimetics, explains the principles behind microfluidics and introduces the concept of optofluidics.

Lecture objectives:

1. Give examples of biomimetics
2. Describe microfluidics and its fundamental principles
3. Describe applications of optofluidics technologies

1. Give examples of biomimetics

Biomimetics, as described in the previous lecture, is the field that uses the structures and processes found in nature as inspiration for new synthetic structures and processes we can use in our lives. There are examples of biomimetics all around us:

- *Velcro* is a biomimetic copy of the burdock plant. Hooks latch onto fibres, allowing for repetitive use.
- *Structural colouration*, first introduced back in lecture #4, can be mimicked to produce displays, detectors, and camouflage for example.
- *Compound eyes*, found, for example, in flies, can be mimicked to create detectors and displays as well.
- *Gecko's feet* are an example of *structure-induced functionality*. The feet are made of countless microscopic *spatulae* that maximize Van der Waals forces between the feet and the wall. This has led to the development of "gecko tape" and other nanotube-based adhesives.

2. Describe microfluidics and its fundamental principles

Microfluidics is the field that deals with manipulating microscopic volumes of fluids, typically by using micro-channels. In order to understand microfluidics, we first need to introduce *fluid dynamics*, the physics of flowing fluids. Fluids can move one of two ways:

- *Laminar flow* is extremely directional. There are no eddy currents, and all the fluid flows in one common direction. Have you ever seen a video of a garden hose stream where the water doesn't look like it is moving? That's laminar flow!
- *Turbulent flow* is what we observe most of the time when we see a fluid flowing. It is chaotic and unpredictable, in other words, completely random.

The flow type of a fluid can be determined by computing the *Reynolds number*, which is a ratio of inertial ("flowing") forces to viscous ("anti-flowing") forces. A large Reynolds number would indicate turbulent flow, while a smaller Reynolds number would indicate laminar flow. As such, an inviscid fluid (a fluid with zero viscosity) is typically prone to turbulent flow, while a highly viscous fluid is prone to laminar flow. Typically, a Reynolds number below 2300 is considered an indicator of laminar flow, while a number above 4000 is considered an indicator of turbulent flow. Between these values is a transitional flow region. The Reynolds number can be computed as follows:

$$\mathrm{Re} = \frac{\rho v L}{\mu}$$

Fluids can also be categorized according to whether they are Newtonian or Non-Newtonian:

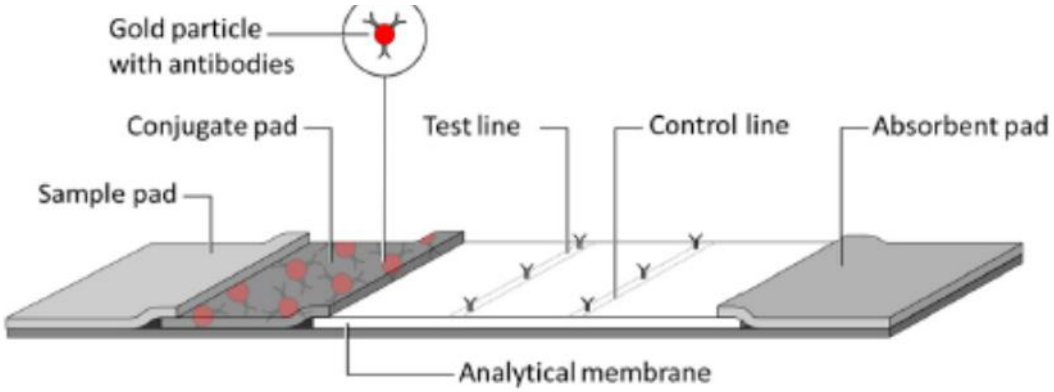
- A *Newtonian fluid* (such as water) will have a constant viscosity with respect to applied stress.
- A *Non-Newtonian fluid* (such as ketchup: brace yourself for BIEN314), on the other hand, does depend on applied stress. We say that the fluid is dependent on the shear rate. A *shear-thinning* fluid will see its viscosity decrease as applied stress grows. A *shear-thickening* fluid will see its viscosity increase as applied stress grows (to picture this, imagine silly putty: if you hit it really hard, in other words, with high applied stress, it behaves like a solid!). A Non-Newtonian fluid will also have its viscosity depend on time scale. A *rheopectic* fluid increases in viscosity with stress over time (such as cream), while a *thixotropic* fluid decreases in viscosity with stress over time (such as honey).

Surface tension and capillary action are also very important in microfluidics. Return to the previous lecture for a description of each! In order to compute the height of a fluid in a channel, the following equation can be used:

$$h = \frac{2\gamma \cos\theta}{\rho g r}$$

Lateral flow devices (such as a basic pregnancy test) are a simple type of microfluidic device that are driven by capillary action. The goal of such devices is the detection of an analyte in a sample solution. A lateral flow device will typically contain a series of elements:

- The *sample pad* accepts the sample solution.
- The *conjugate pad* contains antibodies for the analyte the test is looking to detect.
- The *test line* contains secondary antibodies that will bind only to a complex of the conjugate pad antibodies and the analyte. This line will only "light up" if the analyte is present in the sample solution.
- The *control line* contains antibodies that bind only to free conjugate pad antibodies. In other words, this line will "light up" every time the test is conducted. It acts as a way of ensuring the proper functioning of the test, as, whether or not the analyte is present, fluid will transport free antibodies to this line (not all antibodies will necessarily bind analyte).
- The *absorbent pad* will, as its name suggests, absorb the fluid at the end of the device.



In order to determine the smallest sample volume needed for a particular test, the following equation which factors in sensor efficiency is used:

$$V = \frac{1}{\eta_s N_A A_i}$$

In other words, lower analyte concentration in a solution implies a need for more of that solution for the test to detect the presence of the analyte.

The *volumetric flow rate* (the volume that flows per unit of time) of a fluid experiencing laminar flow in a tube is described by *Poiseuille's Law*:

$$Q = \frac{\pi D^4 \Delta p}{128 \mu \ell} = \frac{\pi R^4 \Delta p}{8 \mu \ell}$$

Furthermore, when two fluids flow in parallel under laminar flow, the only way they can mix is through diffusion (as velocity perpendicular to the direction of flow is zero). The average distance a particle will travel by diffusion under these conditions is given by the following equation:

$$\bar{x}^2 = 2Dt$$

Where the diffusion coefficient is given by:

$$D = \frac{RT}{6\pi r \eta N_A} \left[1 + C \left(\frac{\partial \ln y}{\partial C} \right)_{T,P} \right]$$

When designing a microfluidic device, the proper mixing of components must be considered. As such, the following guidelines are important:

- Optimal size is on the order of 10 to 100 microns. Any smaller and detection may be difficult. Any larger and the mixing may not be uniform and may be too slow.
- Typical channel cross sections are on the order of one thousandth of a square millimetre.
- Typical flow rates are on the order of 10 nanolitres per second.
- Uniform flow is necessary to achieve uniform mixing.

Mixing can be achieved through passive or active processes.

- *Passive mixers* use diffusion and channel geometry. T- and Y-mixing can use spatial (channel geometry) and temporal (opening and closing of various inlets) controls to mix components. Serpentine geometries are a popular channel geometry that allows for a more uniform and fast mixing process.
- *Active mixers* use piezoelectric, ultrasonic, and other types of active methods to increase the surface area of contact between fluids.

Fluids can be moved through microfluidic channels with mechanical or electrokinetic pumps.

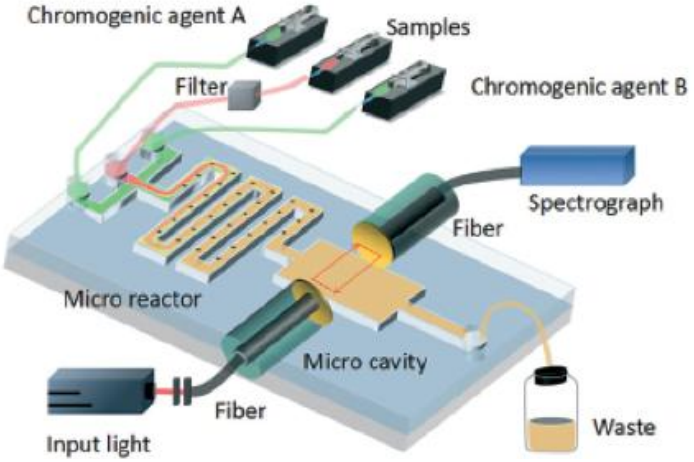
- *Mechanical pumps* use valves and other methods for pushing fluids along. Many types of mechanical pump exist, including *peristaltic*, *rotary*, *ultrasonic*, and *electromagnetic*.

- *Electrokinetic pumps* use electric fields and charges to move the fluid. There are many types of electrokinetic pump, such as those based on *electro-osmosis*, *electrophoresis*, *streaming potential*, and *dielectrophoresis*.

3. Describe applications of optofluidics technologies

Optofluidics combines microfluidics and optics into one field. The optical component can come in the form of light sources such as dye lasers, passive optics devices such as lenses, active optics devices such as modulators, and sensor devices such as microscopes.

Examples of optofluidic systems include photosynthesis, light-catalyzed reactions in microfluidic channels, certain water purification processes, and even PCR-based DNA testing!



Lecture # 13: M(O)EMS

This lecture introduces the small world of micro-(opto)-electromechanical systems.

Lecture objectives:

1. Identify major milestones in M(O)EMS manufacturing and technology development
2. Describe relevant M(O)EMS technologies

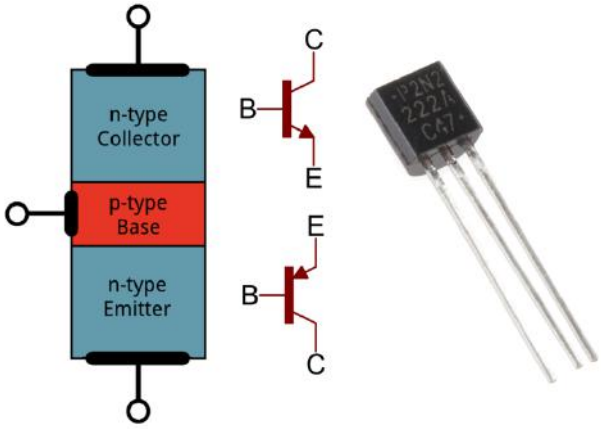
1. Identify major milestones in M(O)EMS manufacturing and technology development

Who knew it was possible to reach a level of interdisciplinary cooperation where you get a type of device so "feature-rich" that its acronym includes parentheses? *Micro-(opto)-electromechanical systems* (M(O)EMS) are micron-scale units designed to process data from one or multiple sensors. They can be incorporated into sensors, actuators, accelerometers, and light manipulation devices. They are typically built on semiconductor materials such as silicon and various metals. The development of M(O)EMS was a gradual process over decades. Here are a few development milestones (the underlying technologies will be covered in the following section!):

- 1947: The point transistor is developed in Bell Labs.
- 1954: The piezoresistive effect is discovered, paving the way for semiconductors.
- 1958: The first integrated circuit is constructed.
- 1959: Richard Feynman challenges the research and industrial communities to manipulate matter on the micro- and nano-scale.
- 1968: The resonant gate transistor, otherwise known as the *cantilever*, is developed. It was the very first MEMS, combining mechanical and electrical components. It was used as a frequency filter for integrated circuitry.
- Throughout the 1960s: Silicon was first processed using *chemical etching*.
- 1971: The first microprocessor is constructed by Intel.
- 1979: The first *thermal inkjet printer*, a MEMS, is developed by HP. The dense packing of nozzles in these printers was a big advancement in MEMS technology.
- 1982: *Lithography*, *electroplating*, and *molding* are developed. High-quality manufacturing of microstructures is henceforth possible.
- 1986: The *Atomic Force Microscope* (AFM) is built by IBM. It is based on cantilever technology, making it a very specialized MEMS designed to achieve sub-nanometre spatial resolution when analyzing surface topography of a sample.
- 1992: A *deformable grating light modulator* is developed at Stanford — the first ever M(O)EMS device.

- 1993: *Accelerometers* could be developed cheaply and quickly as a result of advanced micro-machining processes. Again, cantilevers are often a crucial component of these systems.
- 1994: The *Deep Reactive Ion Etching* (DRIE) manufacturing process is developed by Bosch. Even higher aspect-ratio manufacturing of MEMS devices was then possible. It has also allowed for the creation of high-density capacitors.
- In the late 1990s: Texas Instruments develops the *Digital Micromirror Device* (DMD), leading to more advanced projector technology.
- 1999: The *optical network switch* is developed by Lucent technologies; yet another example of a M(O)EMS device.

Now and in the future, M(O)EMS are and will be popular choices for implantable devices, lab-on-chip processes, point of care biosensing, and more!

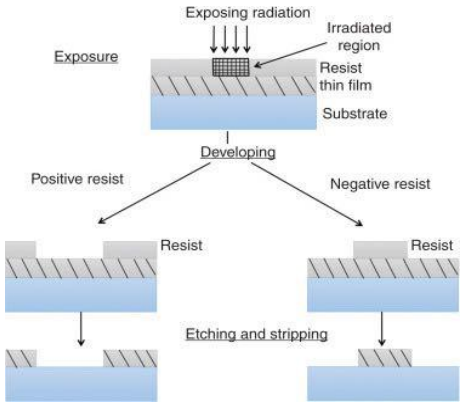


2. Describe relevant M(O)EMS technologies

- *Chemical etching* is a process whereby silicon is selectively chemically carved, usually by using hydrofluoric acid. This leaves behind specific patterns in the silicon.
- *Inkjet printing* is a common type of printing method used in many home printers nowadays. A large array of miniature nozzles is filled with ink. The surface tension within the ink prevents it from being deposited on the surface. The ink is heated until bubbles of ink form and burst, laying ink onto the surface. High-quality printing can be achieved by packing nozzles more densely. This process is not limited to ink, as it has been used with DNA printing!
- *Cantilevers* are a fundamental part of a large number of more modern MEMS and M(O)EMS. Cantilevers of different lengths will oscillate, resonating at different frequencies. If a force is applied in some way to the cantilever, the oscillation frequency

is altered slightly. This alteration is detectable and can produce images, topography, force maps, and more.

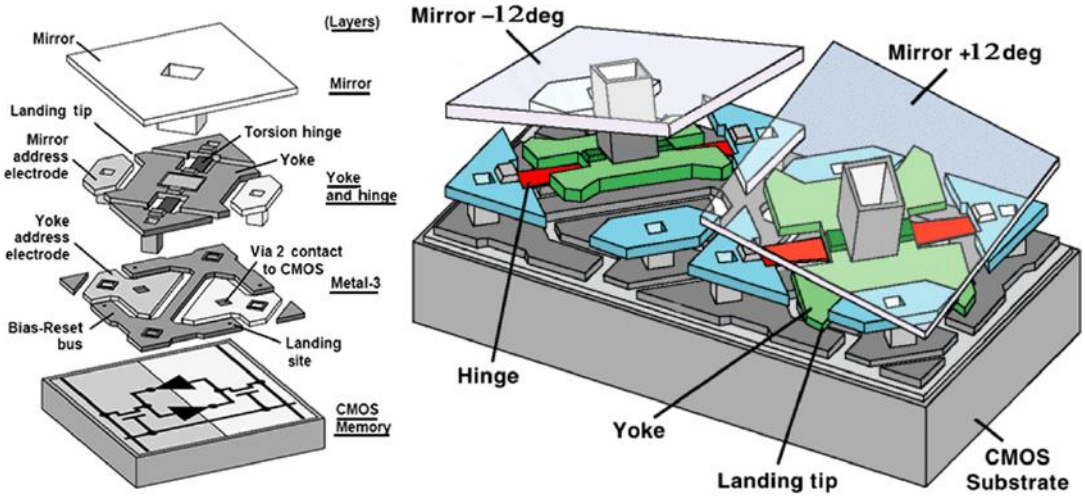
- *Atomic Force Microscopy* (AFM) is a widely used tool based on cantilever technology. It can be set to three configurations: contact mode which creates a direct map of the sample topography, tapping mode for liquid samples, and non-contact mode which keeps the cantilever tip at a constant height above the sample and uses laser deflection off the sample as a way to measure topography instead. The atomic force microscope is useful for obtaining high-resolution maps of a surface without interference and diffraction of light. There is also no need for staining or fluorescent labelling.
- *Accelerometers* come in two- or three-axis configurations that allow for the detection of movement and orientation along its configured axes. They are used in smartphones to determine orientation, for example! There are three types of accelerometers:
    - *Capacitance-based* accelerometers use silica capacitance layers that are attached to a mass of known mass (this is called the proof mass). Capacitance and voltage changes within the system allow the accelerometer to function properly.
    - *Piezoelectric-based* accelerometers rely on a single crystal such as quartz or ceramics. They are a very robust option when selecting an accelerometer.
    - *Heat-based* accelerometers use a miniature heater inside a small dome within the accelerometer, allowing the fluid within the device act as a proof mass. Small changes in temperature and pressure can be used to infer acceleration.
- There are many different ways M(O)EMS can be manufactured:
    - *Sputtering* is the process of coating surfaces in a thin film by spraying a gaseous plasma over the surface and accelerating the ions onto the surface.
    - *Deposition* is another process for coating surfaces in a thin film.
    - *Lithography* is the process by which a pattern is transferred to a photosensitive material with light. It can be either positive, where the pattern itself is transferred, or negative, where the opposite of the pattern is transferred.



- *Etching* can be wet or dry. Wet etching is a process whereby unmasked material is dissolved chemically, while dry etching is accomplished through sputtering, described above!
- *Deep Reactive Ion Etching* (DRIE) is a special type of etching that allows for the carving of 90-degree walls, which is not possible with classic etching techniques. This process can be used to create high density capacitors and allows for high aspect-ratio manufacturing of MEMS devices.

◆ *Digital Micromirror Devices* (DMDs) were the first ever M(O)EMS. An array of thousands of microscopic mirrors corresponding to pixels in the image to be projected can be rotated individually. This drastically improved projector technology.

BioM(O)EMS, the result of even more interdisciplinary cooperation, is the application of M(O)EMS devices to biology. These devices can be used for diagnostics, signal monitoring, drug delivery, and cell culture!



Lecture #14: Diagnostics, Part #1

This lecture introduces pathology as well as techniques commonly used in biology laboratories.

Lecture objectives:

1. Identify the main disciplines in pathology
2. Discuss infections and the nature of infectious disease
3. Describe optical and electrical technologies used in pathology
4. Describe nucleic acid amplification and DNA analysis techniques

1. Identify the main disciplines in pathology

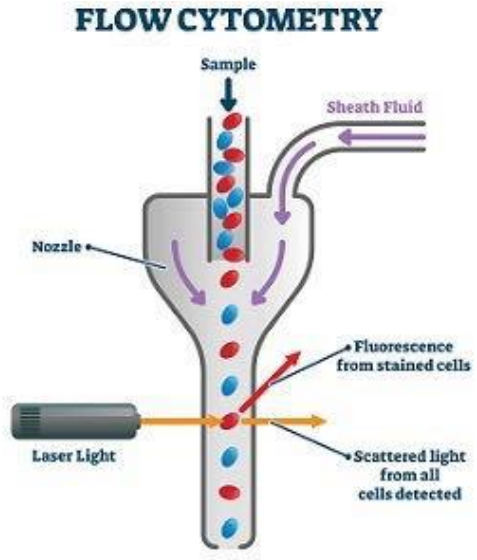
Clinical pathology is the field that searches for certain markers within a biological medium. Media include blood, saliva, urine, and tissues for example. On the other hand, markers include genetic anomalies, cell count, antibodies, and cell morphology for example.

This principle is often applied to a large scale: analyses can be made in centralized facilities using high-throughput equipment. *Diagnostics medicine* can be split into two sub-disciplines, each with their own specializations:

- *Anatomical pathology*, including:
  - Surgical pathology
  - Cytopathology
  - Molecular pathology
  - Forensics pathology
  - Gross examination
  - Histopathology
    - Histopathology is a commonly used technique which involves the staining of very thin cross-sections of tissue. These slices are then imaged under a microscope.
  - Immunohistochemistry
  - Electron microscopy
  - Genomics
  - Proteomics
- *Diagnostic imaging*, including:
  - PET scans
  - CT scans
  - Acoustic imaging
  - Optical imaging
  - ECG and EEG scans (electrical imaging)
  - MRI scans (magnetic imaging)

- Thermography
- Ophthalmology

Flow cytometry is an extremely useful method used to count and sort stained cells. Using techniques from microfluidics, cells can be dripped through the flow cytometry device one at a time within fluid droplets. A laser will then identify cells based on fluorescence. An electric field generated at the end of the flow allows for the cells to be sorted based on the identification carried out by the laser.



Cells can also be counted (though not sorted) by impedance measurements. The impedance is essentially the ratio of voltage to current (or in other words, the resistance) in the system. A device called a *Coulter counter* is used: when a cell passes through the system, the impedance varies slightly. This variation is measured and counted as a cell.

2. Discuss infections and the nature of infectious disease

An *infection* is any entity (not necessarily living, think of viruses!) that can jump from one organism to another. Such infections normally create symptoms within the host. Certain viruses, bacteria, prions, parasites, fungi, and arthropods are known to be infectious.

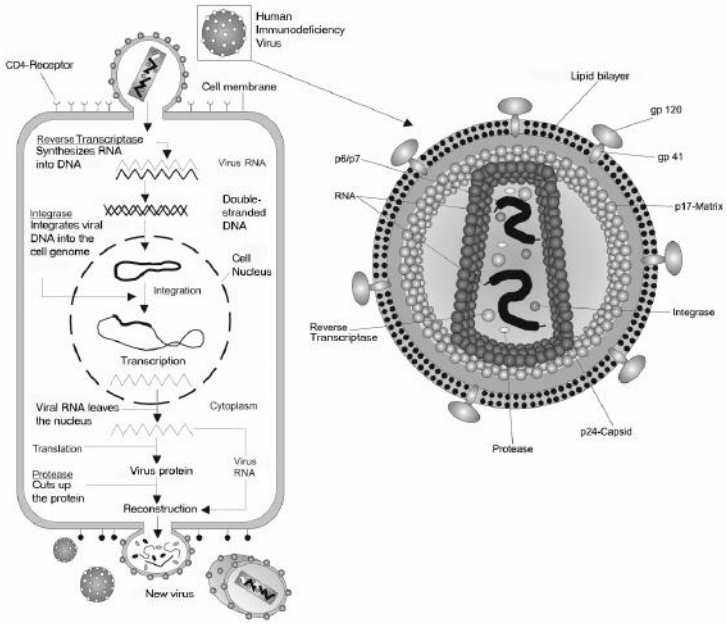
Viruses are extremely diverse due to three factors:

- *Mutations* may occur. RNA polymerase generates five times more errors in transcription when compared to DNA polymerase in DNA replication. This is mostly due to the fact that DNA polymerase proofreads its own work.
- *Reassortment* is the process whereby when two viruses infect a same cell, these two viruses recombine to form a new hybrid viral entity.
- *Recombination* is the process whereby DNA segments are swapped between

chromosomes. It has played a significant role in viral evolution.

Retroviruses are a specific type of virus that uses the host cell's own protein synthesis machinery to proliferate. HIV (the virus that causes AIDS) is a classic example of a retrovirus. The typical retrovirus life cycle is as follows:

1) The virus binds to the host cell.
2) Viral RNA is injected into the cell.
3) The cell's ribosomes synthesize reverse transcriptase, an enzyme that is used to create DNA from RNA.
4) Integrase integrates the viral DNA into the cell's genome.
5) The viral DNA is then used to create viral proteins, allowing the virus to proliferate.



Riboviruses are similar to retroviruses, but do not employ a DNA intermediate to proliferate. Any RNA virus that is not a retrovirus is a ribovirus. Their life cycle is as follows:

1) The virus binds to the host cell.
2) Viral RNA is injected into the cell.
3) The cell's ribosomes directly synthesize viral proteins.
4) Viral polymerases synthesize new negatively charged viral RNA strands by using positively charged RNA strands as templates.
5) RNA polymerase then uses the positively charged RNA strands to create new negatively charged RNA strands.
6) These negatively charged RNA strands are exported in new viruses, therefore allowing the virus to proliferate.

When pathologists take a look at infectious viral diseases, they could detect many different

signs of infection:

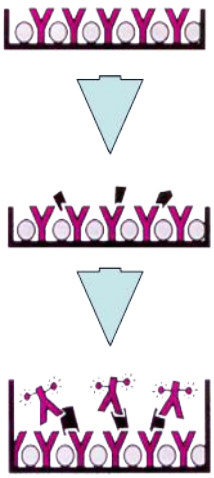
- The entire virus itself
- Specific proteins related to the virus
- The host's body's antibodies created to bind specific viral antigens
- Characteristic viral DNA sequences

Multiple methods exist to detect these indicators. These will be explored briefly in the next sections of this lecture.

3. Describe optical and electrical technologies used in pathology

Cells can be characterized using various techniques, including:

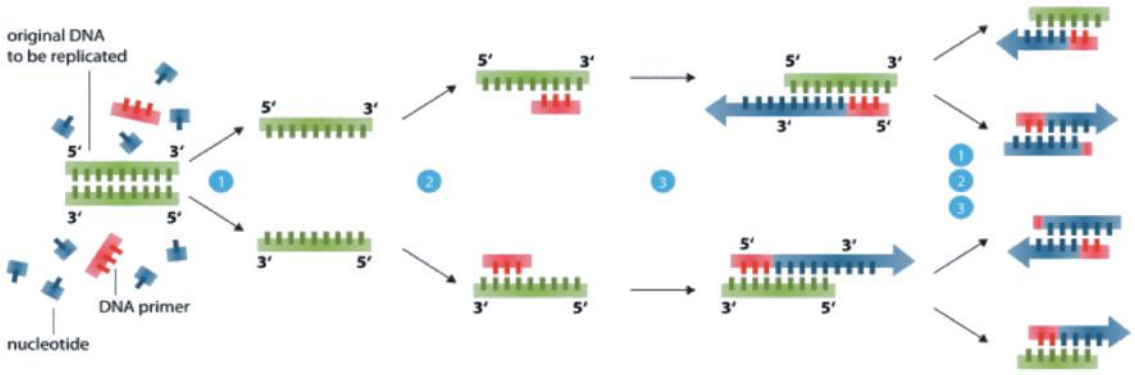
- Flow cytometry and Coulter counters (explained briefly above)
- Laboratory culture and microscopy
- *Immunoassays*
    - *ELISAs* (Enzyme-Linked Immunosorbent Assay), illustrated below, are a very popular example of an immunoassay. An ELISA detects and quantifies antigens present in a solution. The antigen to be detected is often fixed to the wells of a large well plate. Primary antibodies that target the antigen are then added to the wells, followed by secondary antibodies which target the primary antibodies. The secondary antibodies could be associated to a fluorescent tag, or perhaps a chromophore that can be activated when an enzyme is added to the solution. The intensity of the colour measured in a well is then proportional to the amount of antigen present in that well. Microbeads can be used to enhance the signal if too few antigens are present in all wells.



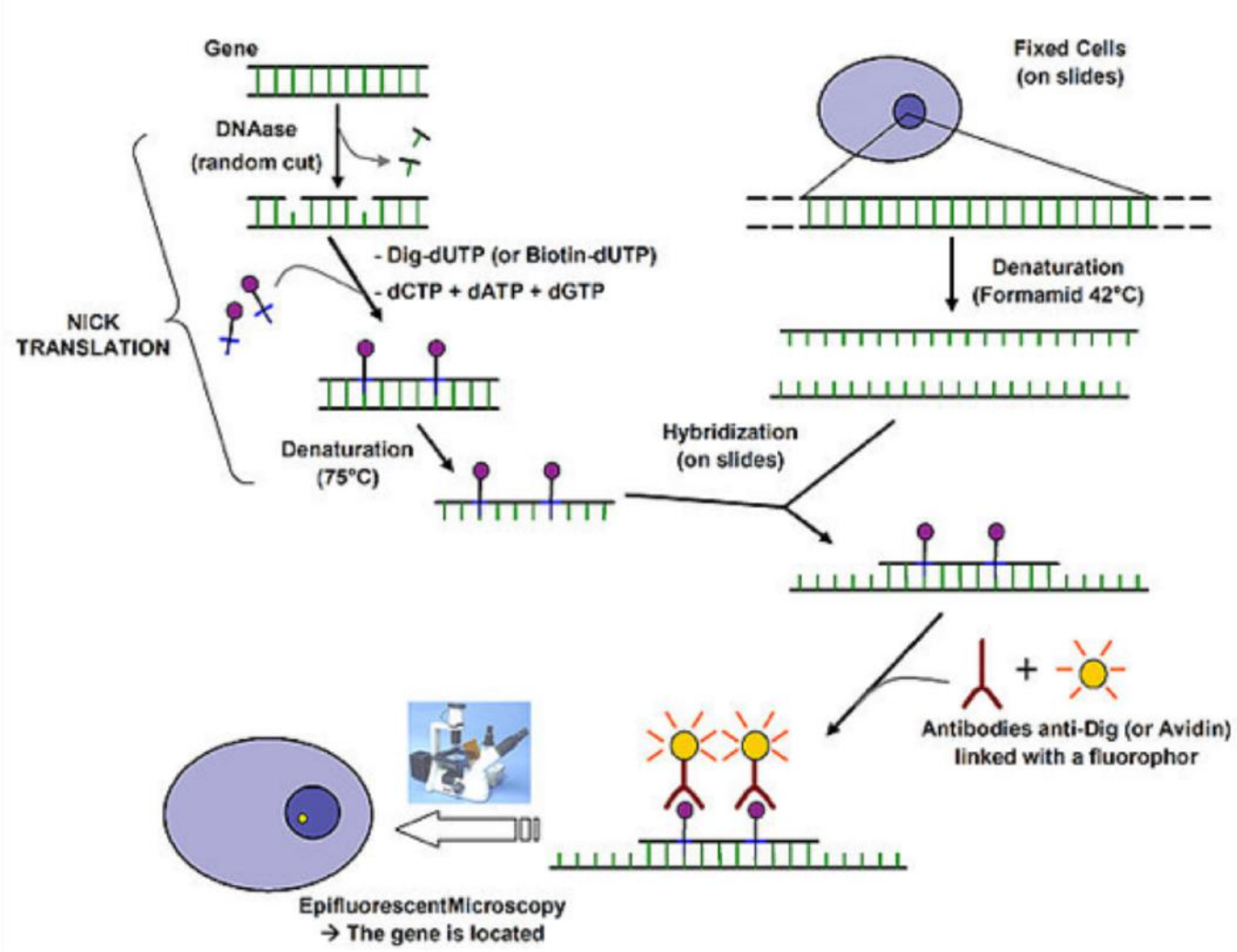
4. Describe nucleic acid amplification and DNA analysis techniques

When it comes to the analysis of nucleic acids, such as DNA, a huge variety of tests and techniques exist. Below are outlined some of the most notable ones:

- *PCR* (Polymerase Chain Reaction) is a process that amplifies nucleic acid sequences through thermal cycling. It leads to the exponential growth of chains assuming a sufficient number of polymerase enzymes. Variants of this technique exist such as qPCR (quantitative PCR), isothermal qPCR, and rtPCR (reverse transcriptase PCR).



- *Gel electrophoresis* involves the separation of nucleic acid fragments by molecular weight (size) in a gel by generating an electric field that acts on the innate charge of nucleic acids. By staining the gel after the experiment, bands allow for the identification of the fragment sizes. This is particularly useful in forensic analysis, as well as in sequencing.
- *Microarrays* allow for a bird's eye view of gene expression. Nucleic acid probes are introduced to wells containing complementary DNA sequences (cDNA). When a probe binds to a cDNA strand, fluorescence can be detected and even quantified by computer analysis. As such, it is a very sensitive procedure.
- *FISH* (Fluorescence In-Situ Hybridization) is a technique that allows for specific DNA sequences to be identified on particular chromosomes. This can be used to track a target DNA strand in space and time throughout an experiment, and to quantify specific gene expression. It can be particularly useful for identifying pathogenic DNA sequences within a sample. The steps to complete a FISH are as follows and are illustrated below:
    1) Clone the target DNA sequence and create complementary strands.
    2) Fluorescently tag these strands with biotin-dGTP.
    3) Denature the target DNA using heat.
    4) Allow the modified (tagged) strands to interact with the target DNA.
    5) Introduce antibodies into the solution.
    6) Fluorescence will then indicate the presence of the target DNA sequence.


Gene
DNAase
(random cut)
- Dig-dUTP (or Biotin-dUTP)
- dCTP + dATP + dGTP
NICK
TRANSLATION
Denaturation
(75°C)
Fixed Cells
(on slides)
Denaturation
(Formamid 42°C)
Hybridization
(on slides)
Antibodies anti-Dig (or Avidin)
linked with a fluorophor
EpifluorescentMicroscopy
→ The gene is located

Lecture # 15: Diagnostics, Part # 2

This lecture describes microscopy techniques used in pathology and biology laboratories.

Lecture objectives:

1. Understand the different microscopy techniques involved in biology laboratories
2. Outline cell staining and its use in biological microscopy

1. Understand the different microscopy techniques involved in biology laboratories

Countless microscopy techniques for imaging that which cannot be seen with the naked eye have been developed. Most often, *optical imaging* proceeds as follows:

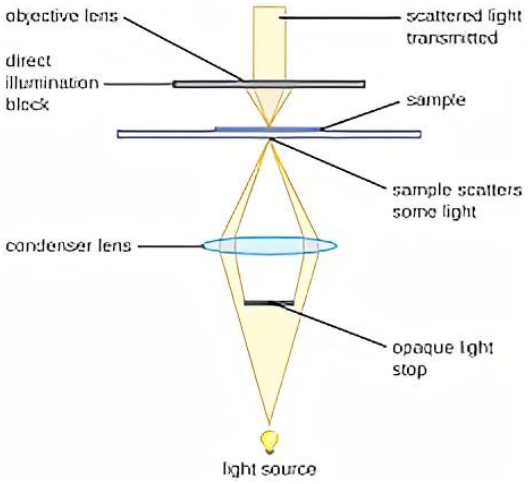
1. Excitation light strikes the object.
2. Collected light leaves the object and reaches a collecting lens.
3. The light then passes through a tuning element.
4. The light reaches a detector.
5. Image processing software can then assess and analyze the collected information.

Contrast is often a measure used in microscopy. The contrast for any given image is given by the following formula:

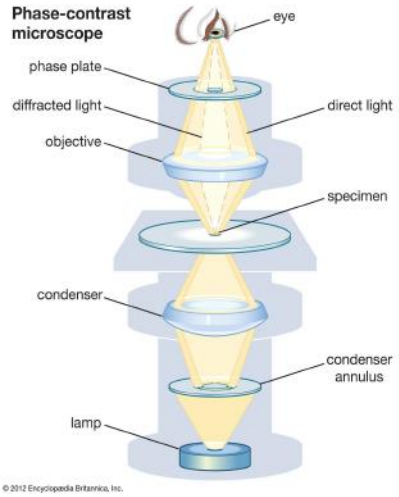
$$\frac{I - I_b}{I_b}$$

Here are a few commonly used microscopy techniques in laboratories:

- *Bright field microscope*: this is probably the microscope that comes to mind when you think of the word "microscope." When the light emitted by the microscope hits the sample, the light is scattered and absorbed, and, as a result, denser regions of the sample appear darker on the image. The sample can also be stained to improve the image quality.

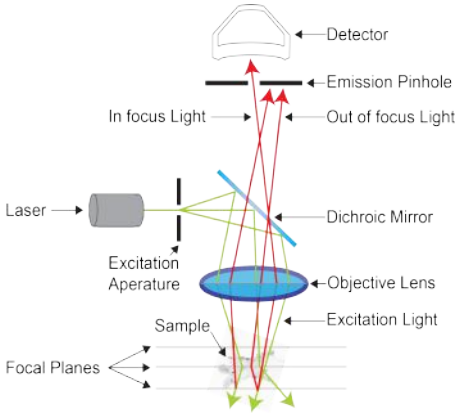


- *Phase contrast microscope*: this microscope uses light interference to construct a high-contrast image. Special rings, notably the *annular ring* and the *phase ring*, are used to convert constructive interference into bright regions and destructive interference into dark regions.



1) Light travels through the annular ring (condenser annulus) and hits the specimen.
2) The light may be either diffracted by the specimen, or it will pass right through undeflected. Diffracted rays will then be out of phase with those that are undeflected.
3) The phase ring (or phase plate) amplifies the contrast of the incoming light.

- *Confocal microscope*: this microscope is particularly useful for imaging and recording videos of live specimen. While other microscopes often require a dead, or fixed, specimen, the confocal microscope does not. The pinhole's height can be moved to take images in sequential planes. Together, these form a *Z-stack* (as they form a stack of XY-plane images into the Z-dimension). Subjects are often fluorescently labelled to aid in visualization.



1) A laser is directed through an excitation filter, bounced off a dichroic mirror, through an objective and finally onto the specimen. The dichroic mirror reflects the excitation wavelength.
2) Emission light from the specimen is passed through the dichroic mirror. The dichroic mirror transmits the emission wavelength.
3) This light is focused at another pinhole in front of the detector, which then leads to the creation of an image.

2. Outline cell staining and its use in biological microscopy

Cell staining is used to better visualize cell components. It is particularly useful for highlighting metabolic processes, varying morphologies, and cell component distributions. It can help answer questions such as "how large is this cell's nucleus?" and "how do mitochondria move in time?"

Cell staining is accomplished as follows:

1) *Permeabilization*: cells are treated with a mild surfactant to dissolve the cell membrane. The dye molecules can therefore be introduced into the cell. Note that electroporation is a valid alternative to this step.
2) *Fixation*: cell morphology is "frozen" in place. A chemical fixative, usually formaldehyde, is used to solidify bonds in proteins, increasing overall cell rigidity.
3) *Mounting*: cells are placed on a microscope slide.
4) *Staining*: cells are bathed in dyes and then rinsed. The cells can then be imaged under a microscope.

Lecture #16: Superresolution Microscopy

This lecture describes the limitations of traditional microscopy techniques and outlines more advanced alternatives to bypass those limitations.

Lecture objectives:

1. Describe microscopy techniques for different length scales
2. Identify and describe limitations of optical microscopy
3. Describe single-molecule-imaging superresolution microscopy techniques
4. Describe superresolution microscopy by spatially patterned excitation, photophysics, and photochemistry
5. Understand Moiré Fringes as a type of structured illumination superresolution microscopy technique

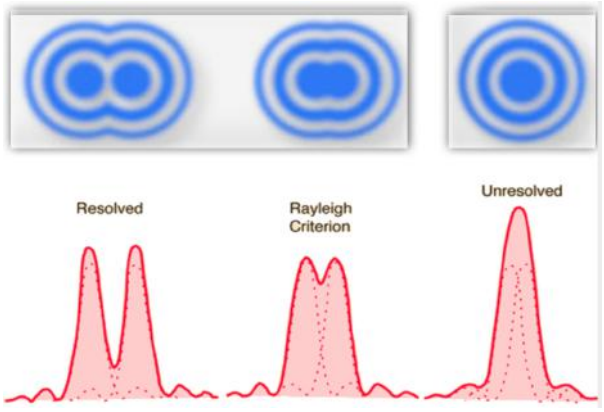
1. Describe microscopy techniques for different length scales

Different microscopy techniques are best suited for different length scales. For instance:

- Naked eye: down to about 100 micrometers.
- Light microscopy: one millimeter to 100 nanometers.
- Electron microscopy: one millimeter to one Angstrom (0.1 nanometers).
- Atomic force microscopy: 100 micrometers to one Angstrom.

2. Identify and describe limitations of optical microscopy

In 1873, Ernst Abbe concluded that for *far-field optics*, that is, optics in which the lens and the object are separated by a larger distance than the wavelength of the light used, the focal point will be imprecise. In other words, there is a limit to optical resolution. Such optics are *diffraction limited*, light cannot focus on an infinitely small spot. This is known as the *Rayleigh diffraction limit*: two images are just resolvable when the centre of the diffraction pattern of one image is perfectly superimposed with the minimum of the diffraction pattern of the other image. This is illustrated here:



The dimensions of a focal region can be computed using the follow equations for the radius (delta-r) and the height (delta-z) of the region:

$$\Delta r \approx \frac{\lambda}{2n \sin(\alpha)} \quad \Delta z \approx \frac{\lambda}{n \sin^2(\alpha)}$$

The *point spread function* is a three-dimensional intensity distribution of the image of a point object. It is essentially a measure of how a single point is "spread out" in an image. The best possible spatial resolution is given by:

$$Xmin = 0.61\ \lambda/NA$$
$$NA = n \sin(\alpha)$$

3. Describe single-molecule-imaging superresolution microscopy techniques

Superresolution microscopy techniques allow for imaging beyond the limits of diffraction-limited far-field optics by exploiting the properties of light and matter. There are many different types of superresolution microscopy, and their complexity is outdone only by the catchiness of their acronyms. First, here, we will be outlining superresolution microscopy techniques used to image individual molecules. Examples of such techniques include:

- *Stochastic Optical Reconstruction Microscopy* (STORM)
- *Photoactivated Localization Microscopy* (PALM): this method relies on point spread function fitting through the following equation, with the mean squared error of the position computed by the subsequent equation:

$$PSF = Ae^{-\frac{(x-x_0)^2+(y-y_0)^2}{2\omega^2}}$$

$$\left(\sigma_{x,y}^2\right) \approx \frac{s^2 + a^2/12}{N} + \frac{4\sqrt{\pi}s^3b^2}{aN^2}$$

- *Fluorescence Photoactivated Localization Microscopy* (FPALM)

These techniques are all based on the following concepts:

- Single fluorophores can be localized with a precision better than 1 nanometer. However, image deconvolution (a technique in image processing) may be required.
- If a large number of fluorophores overlap in the point spread function, photochemistry

methods may be used to darken and brighten various fluorophores to better isolate individual fluorophores.

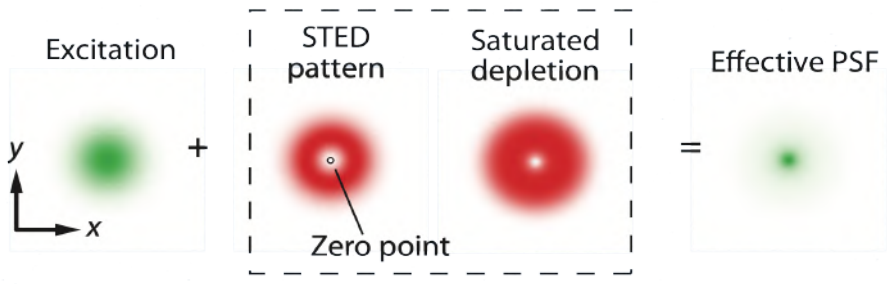
- Molecules within the point spread function are activated at different time points such that they can be individually localized. They are then deactivated.
- Extensive parallel localization is achieved through wide-field imaging such that the coordinates of multiple molecules may be determined. A high-resolution image can then be reconstructed.

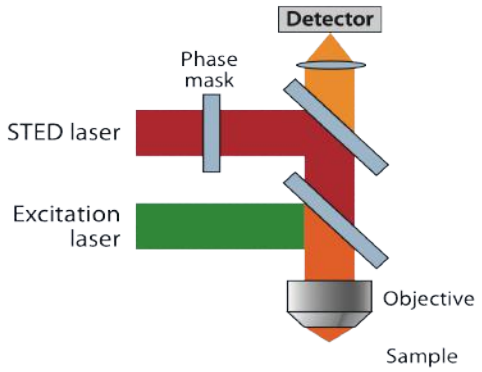
4. Describe superresolution microscopy by spatially patterned excitation, photophysics, and photochemistry

What follows are examples of superresolution microscopy techniques based in spatially patterned excitation, photophysics, and photochemistry:

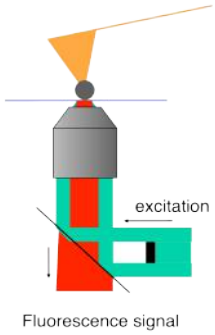
- *Stimulated Emission Depletion Microscopy* (STED): this technique creates images by the selective deactivation of fluorophores and minimizes the area of illumination at the focal point to enhance resolution.
  - Fluorescence in specific regions of the sample is depleted while leaving fluorescently-active focal points. This is accomplished with a STED laser.
  - The focal areas are engineered with extremely high precision by altering the properties of the pupil plane of the objective lens.
  - The effective point spread function is found with by following equation (note that, here, I_s is typically on the order of ten million watts per square centimetre, while I is on the order of one billion watts per square centimetre):

$$\Delta_{\text{eff}} \approx \frac{\Delta}{\sqrt{1 + I/I_S}}$$





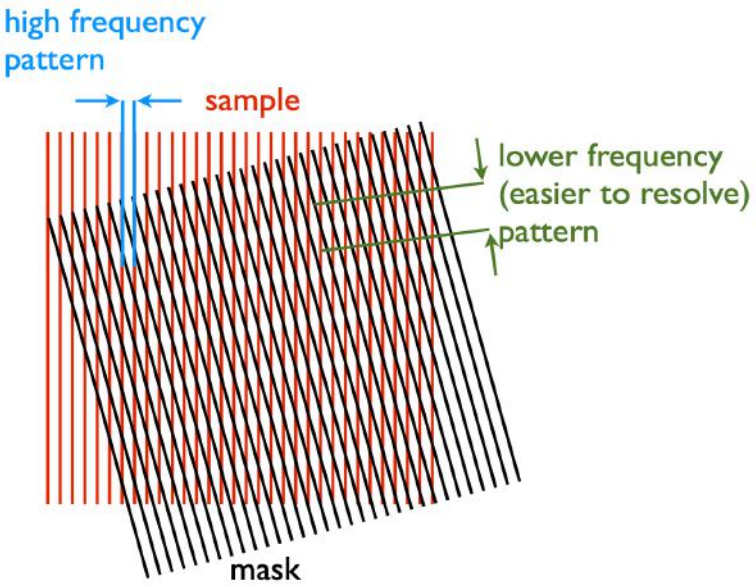
- *Reversible Saturable Optically Linear Fluorescent Transition* (RESOLFT)
- *Near-Field Scanning Optical Microscopy* (NSOM): this technique brute-forces a solution to diffraction-limited optics by simply focusing light through an aperture with a diameter smaller than that of the wavelength of excitation light. The optical resolution of the image is then limited only by the diameter of the aperture used.
- *Tip-Enhanced Near-Field Optical Microscopy* (TENOM): this technique provides an alternative to NSOM. A scanning probe is used to provide a broad range of spectroscopic information on single objects and structured surfaces at nanometer-resolution with extremely high detection sensitivity.



5. Understand Moiré Fringes as a type of structured illumination superresolution microscopy technique

In essence, *Moiré Fringes* down-convert non-resolvable high-frequency spatial information into resolvable low-frequency information. This is referred to as frequency mixing, or heterodyning. This mixing is computed as follows, and is illustrated below:

$$\sin\theta_1 \sin\theta_2 = \frac{1}{2}\cos(\theta_1 - \theta_2) - \frac{1}{2}\cos(\theta_1 + \theta_2)$$


high frequency
pattern
sample
lower frequency
(easier to resolve)
pattern
mask

Lecture #17: Diagnostics — Cellular and Tissue-Level Techniques

This lecture introduces nonlinear optics and the idea behind optical coherence tomography.

Lecture objectives:

- Describe nonlinear optical phenomena at a fundamental level and identify applications of nonlinear optics
- Explain optical coherence tomography

1. Describe nonlinear optical phenomena at a fundamental level and identify applications of nonlinear optics

When it comes to imaging within living organisms (such as with ultrasound or X-rays), results cover large areas but are in a low resolution. Imaging of this nature is dependent on light's penetration depth through tissue. The best results are typically obtained by using light with wavelengths around one micrometer, and by imagining at shallow depths. In other words, the mean free path of light must be maximized. The unfortunate truth is that it is difficult for light to penetrate deeply into tissue.

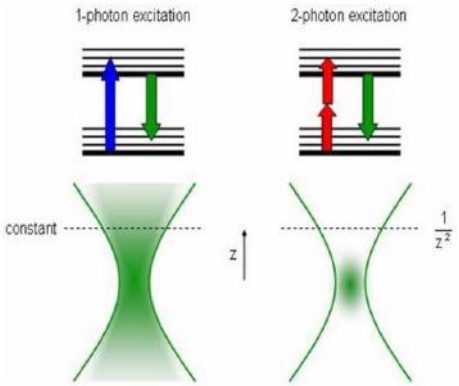
Nonlinear optical phenomena can be used, however, to improve the penetration depth of light into tissue. Light can interact nonlinearly with tissue molecules according to the following equations describing diffraction index and polarization, respectively:

$$N(\omega) = n(\omega) + i\kappa(\omega) = \sqrt{\varepsilon(\omega)\mu(\omega)} = \sqrt{[\varepsilon_1(\omega) + i\varepsilon_2(\omega)][\mu_1(\omega) + i\mu_2(\omega)]}$$

$$P(E) \approx \chi^{(1)}E + \chi^{(2)}EE + \chi^{(3)}EEE + \ldots$$

There are two types of nonlinear optics microscopy techniques:

- *Incoherent linear techniques*: one example of this type is two-photon microscopy. By using two photons instead of one to excite fluorescence in a sample, fluorescence is limited to the focal region of the microscope. This is particularly useful for imaging living cells. In essence, two lower-energy photons are used. As such, fluorescence could only be excited in a region where the energy of each photon sum to trigger the fluorescence.

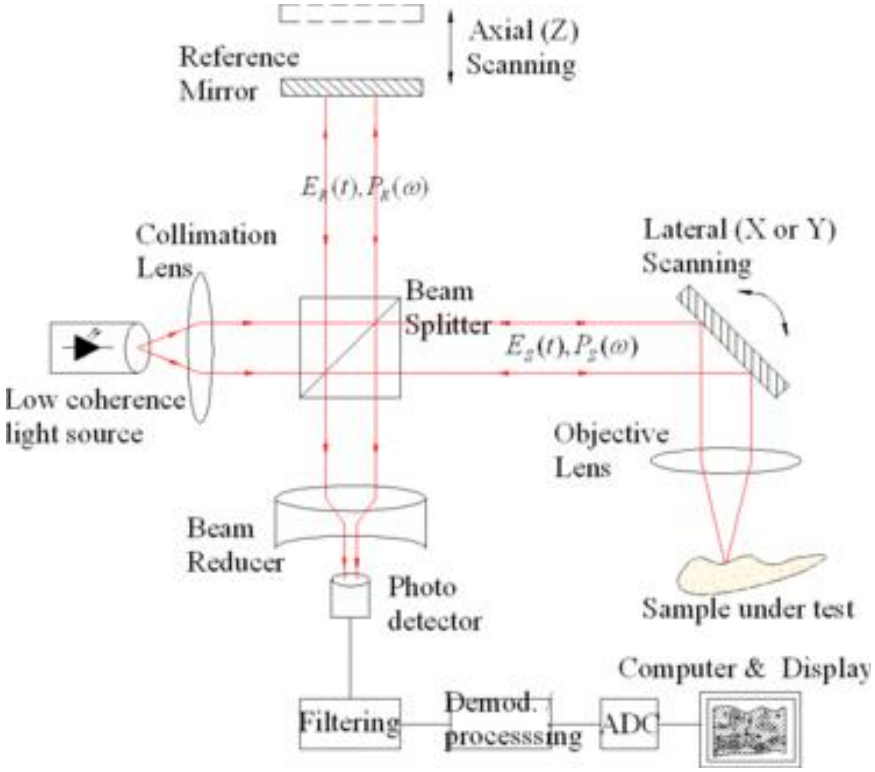


- Coherent linear techniques: one example of this type is *Second Harmonic Generation* (SHG). SHG is a nonlinear process by which incident light at a certain frequency is converted to light at double that input frequency. This occurs only at the focal point and it is the result of intense light interacting with matter that happens to be organized on the same scale of the light's wavelength without an inversion in symmetry. Imaging of this type does not require staining of the sample. Collagen structure, which satisfies the above requirement, is a perfect candidate for imaging using this technique.

2. Explain optical coherence tomography

Optical Coherence Tomography (OCT) uses interference to measure reflected light. This process is similar to that of an ultrasound, but OCT uses light instead. OCT uses a Michelson Interferometer configuration. If that sounds familiar, it's because the Michelson Interferometer was explained back in lecture 8!

A reference arm holds a mirror, while, in this case, a sample arm holds the item to be scanned. An interference pattern is created based on the coherence of the light that recombines at the beam splitter.



Time-domain OCT can be performed by changing the positioning of the reference mirror as well.

Frequency-domain OCT, on the other hand, can be performed by using spectrally separated detectors. The swept-source approach involves encoding optical frequencies in time with a

spectral scanning source, while the spectral-domain approach uses a dispersive detector to separate the different wavelengths involved. The depth scan be then be calculated by using the Fourier transform (brace yourself for BIEN350).

To scan a sample with OCT, the sample can be moved under a fixed beam, or the beam can be moved along a fixed sample:

- The *single point* approach uses a translation stage which allows the sample to move in two lateral dimensions as the beam of light scans the sample.
- The *parallel* approach uses a charge-coupled device camera in which the sample is fully illuminated. This eliminates the need for a lateral scan.

Lecture # 18: Biosensors

This lecture explains the theory behind biosensors in more detail.

Lecture objectives:

1. Describe the components of a biosensor
2. Understand different detection methods used by biosensors
3. Explain the basic characteristics of biosensors
4. Describe the ideal biosensor
5. Explain the advantages of IT-PCR over classic PCR

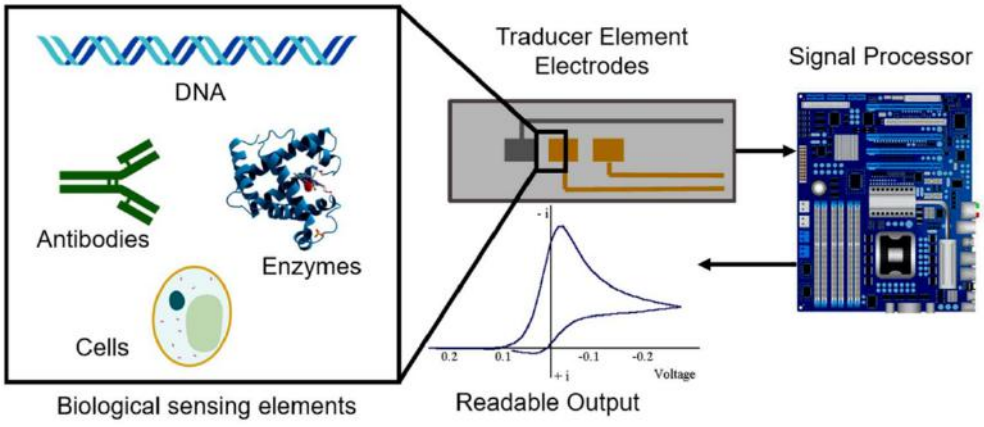
1. Describe the components of a biosensor

A *biosensor* is a device that uses bioactive materials along with a transfusing element to detect the activity of an analyte present in a sample. It could also detect that analyte's concentration in the sample.

A biosensor is crafted from four main components:

- The *bioreceptor* detects the analyte in the sample. A bioreceptor could be an enzyme, a cell, a nucleic acid strand, or a nanoparticle, for example.
- The *transducer* will then accept the signal from the bioreceptor. A transducer could be a photodiode (which accepts light), a thermistor (which accepts heat), a pH electrode (which accepts pH changes), or a quartz electrode (which accepts mass changes), for example.
- The *electronics* will then convert the signal from analog form to digital form. They will also process (or condition) the signal.
- The *display* outputs the collected data.

The transition from the bioreceptor to the transducer is called the *bio-recognition* step, the transition from the transducer to the electronics is the *signalization* step, and the transition from the electronics to the display is the *quantification* step.

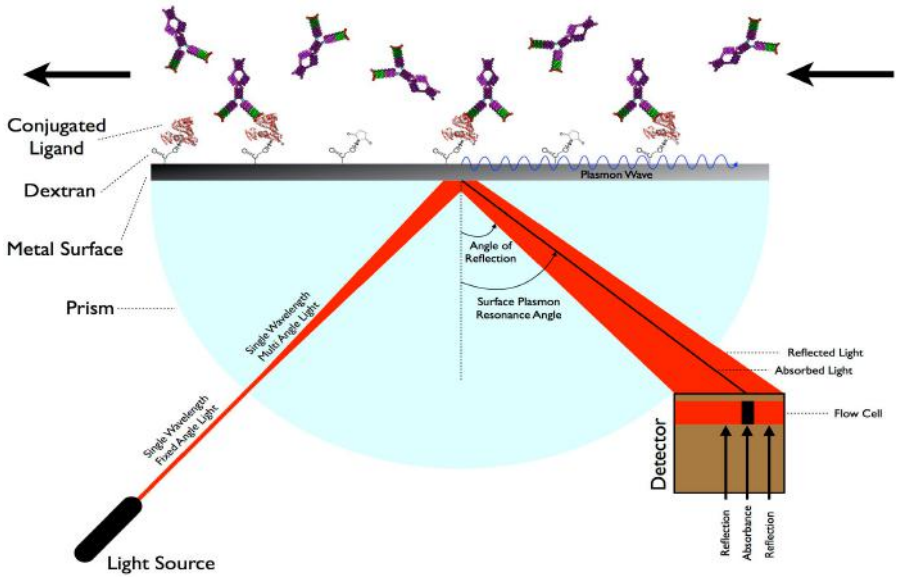


2. Understand different detection methods used by biosensors

Biosensors can detect analytes in three different ways:

- *Indirect detection* uses labels to highlight the analyte.
- *Direct detection* does not use labels. Instead, resonating photons take a path that will at least partially include the biomolecules used in the device. By using a biomolecule with a refractive index greater than that of the medium, the optical path can be deviated. The resonance of the photons is therefore altered. The wavelength shift can then be detected by the transducer elements as the change is proportional to the difference in effective refractive indexes.
- *Functional assay* detection uses the binding of proteins as a method of detection. An example of this is *Surface Plasmon Resonance* (SPR).

So, what exactly is SPR? In essence, light from the laser is reflected off the metallic plate and into a detector. At a certain angle dubbed the SPR angle, light is absorbed by the plate's electrons, making them resonate. These electrons are called surface plasmons. Since the light's energy is absorbed by the electrons, the intensity drops, leading to a darker band on the detector. When a binding event occurs, the dark band shifts, allowing for a localization of the binding event. Detection is therefore due to a change in reflectivity.



Biosensors could be referred to by their sub-type name:

- A *catalytic biosensor* uses enzymes.
- An *immunosensor* uses antibodies.
- A *genosensor* uses nucleic acids.
- An *aptasensor* uses *aptamers*. Aptamers can be a short sequence of oligonucleotides or a

small peptide sequence.

3. Explain the basic characteristics of biosensors

- *Linearity* is the accuracy of measured responses to a straight line. For a high substrate concentration, linearity should be quite high.
- *Sensitivity* is the minimum concentration of analyte that can be detected, or in other words, the probability of detecting the analyte (the *true positive rate*, or TPR). It measures how a signal changes in response to a varying analyte concentration.
- *Specificity* is the biosensor's ability to distinguish the analyte from other components in the sample (the *true negative rate*, or TNR).
- *Response time* is the time necessary for the biosensor to achieve 95% of the total response.

4. Describe the ideal biosensor

The ideal biosensor would feature all of the following characteristics:

- High specificity and sensitivity
- Multiplexing abilities, or, in other words, the ability to detect multiple different analytes
- Label-free (direct detection)
- Easy to use
- Portable
- Inexpensive

It is important to remember that for a device to be viable in the real world, it must not only be functional, but it must be affordable and convenient to use!

5. Explain the advantages of IT-PCR over classic PCR

Isothermal Polymerase Chain Reaction (IT-PCR) is not dependent on the rate at which thermal cycling can be performed. As such, it is a rapid, highly specific and sensitive molecular analysis tool. An IT-PCR device also consumes significantly less power than a classic PCR apparatus.

Equation Descriptions

In this section, you will find nearly every equation presented in the course; organized by the lecture in which it is first introduced. The equations are all named, and every variable is defined.

Lecture #1

The Lorentz Force

$$\mathbf{F} = q\mathbf{E} + q\mathbf{v} \times \mathbf{B}$$

- E: electric field
- B: magnetic field
- q: electric charge
- v: velocity of the electric charge

Gauss's Law for Electric Charges

$$\nabla \cdot \mathbf{E} = \frac{\rho}{\varepsilon_0}$$

- Del: vector gradient operator
- E: electric field
- Rho: volume charge density
- Epsilon naught: electric constant

Gauss's Law for Magnetic Fields

$$\nabla \cdot \mathbf{B} = 0$$

- Del: vector gradient operator
- B: magnetic field

Faraday's Law

$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t}$$

- Del: vector gradient operator
- E: electric field
- B: magnetic field
- t: time

Ampere's Law

$$\nabla \times \mathbf{B} = \mu_0\mathbf{J} + \mu_0\varepsilon_0\frac{\partial \mathbf{E}}{\partial t}$$

- Del: vector gradient operator
- B: magnetic field
- Mu naught: magnetic constant
- J: current density contribution
- Epsilon naught: electric constant
- E: electric field
- t: time

Maxwell's Equations

$$\left(v_{ph}^2\nabla^2 - \frac{\partial^2}{\partial t^2}\right)\mathbf{E} = \mathbf{0}$$

$$\left(v_{ph}^2\nabla^2 - \frac{\partial^2}{\partial t^2}\right)\mathbf{B} = \mathbf{0}$$

- v_{ph}: phase velocity of light
- Del: vector gradient operator
- t: time
- E: electric field
- B: magnetic field

Energy of a Photon

$$E = h\nu = \frac{hc}{\lambda}$$

$$E_2 - E_1 = \Delta E = h\nu$$

- E: energy of the photon
- h: Planck's constant
- v: frequency of the light wave
- c: speed of light
- Lambda: wavelength of the light wave

Momentum of Light

$$p = \frac{E}{c}$$

- p: momentum of the light
- E: energy
- c: speed of light

Stefan-Boltzmann Law

$$P_{\text{net}} = P_{\text{emit}} - P_{\text{absorb}}$$

$$P_{\text{net}} = A\sigma\varepsilon\left(T^4 - T_0^4\right)$$

- P: power
- A: surface area over which the radiative transfer occurs
- Sigma: Stefan-Boltzmann constant
- Epsilon: emissivity factor
- T: final temperature
- T naught: initial temperature

Lecture # 2

Quantum Yield

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

- Phi: quantum yield

Rayleigh and Mie Scattering Particle Size

$$x = \frac{2\pi r}{\lambda}$$

- x: dimensionless factor to characterize scattering
- r: particle radius
- Lambda: wavelength of the light

Lecture # 3

Light Absorption (a) and Scattering (s)

$$\mu_a = \rho_a \sigma_a$$
$$\sigma_a = Q_a A$$
$$T = \exp(-\mu_a L)$$
$$\mu_s = \rho_s \sigma_s$$
$$\sigma_s = Q_s A$$
$$T = \exp(-\mu_s L)$$

- Mu: absorption/scattering coefficient
- Rho: density
- Sigma: effective cross-sectional area
- Q: efficiency
- A: geometric cross-section
- T: transmission
- L: sample length

Hooke's Law

$$F = -kX$$

- F: force
- k: spring constant
- X: spring deformation

Snell's Law

$$\frac{\sin\theta_1}{\sin\theta_2} = \frac{v_1}{v_2} = \frac{\lambda_1}{\lambda_2} = \frac{n_2}{n_1}$$

- Theta 1: incident angle
- Theta 2: refraction angle
- v: velocity
- Lambda: wavelength of the light
- n: refractive index

Brewster's Angle

$$\tan \theta_B = \frac{n_2}{n_1}$$

- Theta B: Brewster's angle
- n: refractive index

Critical Angle (Total Internal Reflection)

$$\sin \theta_c = \frac{n_2}{n_1}$$

- Theta C: critical angle
- n: refractive index

Lens Equation

$$\frac{1}{d_0} + \frac{1}{d_i} = \frac{1}{f}$$

- d_o: distance from the lens to the object
- d_i: distance from the lens to the image
- f: focal length

Magnification

$$M = \frac{h_i}{h_0} = -\frac{d_i}{d_0}$$

- M: magnification factor
- h_o: height of the object
- h_i: height of the image
- d_o: distance from the lens to the object
- d_i: distance from the lens to the image

Acceptance Angle (Total Internal Reflection)

$$\sin\theta \leq \sqrt{n_o^2 - n_c^2}$$

- Theta: acceptance angle
- n_o: refractive index of the core of the fiber
- n_c: refractive index of the cladding of the fiber

Lecture # 5

Nernst Equation

$$E = \frac{RT}{zF} \ln \frac{[\text{ion outside cell}]}{[\text{ion inside cell}]} = 2.3026 \frac{RT}{zF} \log_{10} \frac{[\text{ion outside cell}]}{[\text{ion inside cell}]}$$

- E: membrane potential
- R: ideal gas constant
- T: temperature
- F: Faraday constant
- z: number of electrons

Goldman Equation

$$E_{\mathrm{m}} = \frac{RT}{F} \ln \left(\frac{\sum_i^N P_{\mathrm{M}_i^+} [\mathrm{M}_i^+]_{\mathrm{out}} + \sum_j^M P_{\mathrm{A}_j^-} [\mathrm{A}_j^-]_{\mathrm{in}}}{\sum_i^N P_{\mathrm{M}_i^+} [\mathrm{M}_i^+]_{\mathrm{in}} + \sum_j^M P_{\mathrm{A}_j^-} [\mathrm{A}_j^-]_{\mathrm{out}}} \right)$$

- E: membrane potential
- R: ideal gas constant
- T: temperature
- F: Faraday constant
- P: ion permeability

Lecture # 10

Coulomb's Law

$$F = k_e \frac{q_1 q_2}{r^2}$$

- F: electrostatic force
- k_e: Coulomb's constant
- q: charge
- r: distance between charges

Electrostatic Potential

$$V(r) = 4\epsilon[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}]$$

- V: potential
- Epsilon: well depth
- Sigma: distance for which r is zero
- r: separation distance

Lecture # 11

Superhydrophobic Effect

$$\gamma_{SG} = \gamma_{SL} + \gamma_{LG} \cos\theta$$

- Gamma$_{SG}$: solid-gas interface tension
- Gamma$_{SL}$: solid-liquid interface tension
- Gamma$_{LG}$: liquid-gas interface tension
- Theta: contact angle

Gibbs Free Energy Equation (for Self-Assembly)

$$\Delta G_{SA} = \Delta H_{SA} - T\Delta S_{SA}$$

- G: Gibbs free energy
- H: enthalpy
- T: temperature
- S: entropy

Enthalpy

$$H(S,p) = U + pV$$

- H: enthalpy
- U: internal energy
- p: pressure
- V: volume

Internal Energy

$$U = \sum_{i=1}^{N} p_i E_i$$

- U: internal energy
- E: microstate energy
- p: microstate probability

Lecture # 12

Reynolds Number

$$\mathrm{Re} = \frac{\rho v L}{\mu}$$

- Re: Reynolds number
- Rho: density
- v: velocity
- L: length
- Mu: viscosity

Capillary Action in a Vertical Capillary Tube

$$h = \frac{2\gamma \cos\theta}{\rho g r}$$

- h: height in the tube
- Gamma: medium-specific constant
- Theta: contact angle as measured from the side of the tube
- Rho: density
- g: gravitational acceleration
- r: tube radius

Smallest Sample Volume Needed for Testing

$$V = \frac{1}{\eta_s N_A A_i}$$

- V: smallest sample volume needed for testing
- Eta: sensor efficiency
- N_A: Avogadro's number
- A_i: analyte concentration

Poiseuille's Law

$$Q = \frac{\pi D^4 \Delta p}{128 \mu \ell} = \frac{\pi R^4 \Delta p}{8 \mu \ell}$$

- Q: volumetric flow rate
- D: tube diameter
- p: pressure
- Mu: viscosity
- l: tube length
- R: tube radius

Laminar Flow Mixing

$$\bar{x}^2 = 2Dt$$

$$D = \frac{RT}{6\pi r \eta N_A} \left[1 + C \left(\frac{\partial \ln y}{\partial C} \right)_{T,P} \right]$$

- x: distance the particle moves
- D: diffusion coefficient
- t: diffusion time
- R: gas constant
- T: temperature
- r: particle radius
- Eta: viscosity
- N_A: Avogadro's number
- C: concentration
- y: activity coefficient

Lecture # 15

Contrast

$$\frac{I - I_b}{I_b}$$

- I: specimen light intensity
- I_b: background light intensity

Lecture # 16

Focal Spot Diameter

$$\Delta r \approx \frac{\lambda}{2n \sin(\alpha)}$$

- Delta(r): focal spot diameter
- Lambda: wavelength
- n: refractive index
- Alpha: light cone angle

Focal Spot Length (Rayleigh Length)

$$\Delta z \approx \frac{\lambda}{n \sin^2(\alpha)}$$

- Delta(z): Rayleigh length
- Lambda: wavelength
- n: refractive index
- Alpha: light cone angle

Spatial Resolution

$$Xmin = 0.61\ \lambda/NA$$
$$NA = n \sin(\alpha)$$

- X: ideal distance (spatial resolution)
- Lambda: wavelength
- NA: numerical aperture
- n: refractive index
- Alpha: light cone angle

Point Spread Function

$$PSF = Ae^{-\frac{(x-x_0)^2+(y-y_0)^2}{2\omega^2}}$$

$$\left(\sigma_{x,y}^2\right) \approx \frac{s^2 + a^2/12}{N} + \frac{4\sqrt{\pi}s^3b^2}{aN^2}$$

- s: standard deviation
- a: pixel size
- b: number of background photons in the fitting window
- N: total number of signal photons from the molecule

Stimulated Emission Depletion Effective Point Spread Function

$$\Delta_{\text{eff}} \approx \frac{\Delta}{\sqrt{1 + I/I_S}}$$

- Delta: point spread function
- $\text{Delta}_{\text{eff}}$: effective point spread function
- I: intracavity intensity
- I_S: saturation intensity

Moiré Fringe Frequency Mixing

$$\sin\theta_1 \sin\theta_2 = \frac{1}{2}\cos(\theta_1 - \theta_2) - \frac{1}{2}\cos(\theta_1 + \theta_2)$$

- Theta: given angle *(this is a basic trigonometric identity)*

Index of Key Terms

In this section, you will find an alphabetized list of all the key (italicized) terms presented in this course-pack accompanied by the lectures in which they are highlighted as important.

C

D

- *Deep reaction ion etching*: Lecture #13
- *Deformable grating light modular*: Lecture #13
- *Depolarization*: Lecture #7
- *Deposition*: Lecture #13
- *Diagnostic imaging*: Lecture #14
- *Diagnostics medicine*: Lecture #14
- *Dichroic filter*: Lecture #3
- *Dideoxynucleotide*: Lecture #9
- *Dielectrophoresis electrokinetic pump*: Lecture #12
- *Diffraction limit*: Lecture #16
- *Diffuser*: Lecture #3
- *Diffuse reflection*: Lecture #3
- *Digital micromirror device*: Lecture #13
- *Dipole-dipole interaction*: Lecture #10
- *Direct detection*: Lecture #18
- *Directed evolution*: Lecture #9
- *Disease network*: Lecture #11
- *Display (biosensors)*: Lecture #18
- *Disulfide bond*: Lecture #10
- *Diverging lens*: Lecture #3
- *DNA binding*: Lecture #9
- *DNA replication*: Lecture #9

E

- *Edge (networks)*: Lecture #11
- *Edge filter*: Lecture #3
- *Elastic scattering*: Lecture #2
- *Electric current*: Lecture #1
- *Electric field*: Lecture #1
- *Electric force*: Lecture #11
- *Electric transducer*: Lecture #8
- *Electrical communication*: Lecture #7
- *Electrical discharge*: Lecture #2
- *Electrocardiography*: Lecture #7
- *Electrochemical transducer*: Lecture #8
- *Electrodiagnosis*: Lecture #7
- *Electroencephalography*: Lecture #7
- *Electrokinetic pump*: Lecture #12
- *Electroluminescence*: Lecture #2
- *Electromagnetic mechanical pump*: Lecture #12
- *Electromagnetic wave*: Lecture #1
- *Electromagnetic wave equation*: Lecture #1

F

G

H

I

K

L

M

N

O

P

S

T

U

V

- *Van der Waals force*: Lecture #10
- *Van der Waals radius*: Lecture #10
- *Velcro*: Lecture #12
- *Virus*: Lecture #14
- *Virus transduction*: Lecture #6
- *Voltage-gated channel*: Lecture #7
- *Volumetric flow rate*: Lecture #12

W

- *Wash (biology)*: Lecture #9
- *Wavelength*: Lecture #1
- *Wenzel regime*: Lecture #11

Z

- *Z-stack*: Lecture #15

Example Final Project PowerPoint Presentations

This final section includes two PowerPoint presentations used by teams in previous cohorts to present their final projects. Remember that these are simply examples, and you do not need to follow their format to the letter. Feel free to be creative and have fun!

As per the project's official instructions, every project should include the following elements unless marked otherwise:

- Problem statement
- Background and/or literature review
- Hypothesis (if applicable)
- Hypothesis testing procedure (if applicable)
- Presentation of the device, design, or system (if applicable)
- Conclusions
- Proposed future work (if applicable)

Project #1: Tahsin Abedi, Thomas Allen, Stefano Bambace, and Alexander Becker (2019)

The project is titled "Designing a Passive Cooling System for Vaccine Storage in Hot Climates" and responded to the following project prompt: "Design a passive cooling solution for vaccine storage in hot climates."

Project #2: Peter Chimienti, Anna Ciprick, and Griffin Copp (2020)

The project is titled "Electronic Nose" and responded to the following project prompt: "Review literature on artificial electronic nose [*sic.*] and describe 3 potential technologies currently explored. Highlight main limitations of current technologies."

https://docs.google.com/presentation/d/1-FP6-OlOeKfuEWWHC_Fvm0m9Bhh9ougcktFz00mY83k/edit?usp=sharing

Designing a Passive Cooling System for Vaccine Storage in Hot Climates

Tahsin Abedi, Thomas Allen, Stefano Bambace and Alexander Becker
McGill University, BIEN210, April 5th, 2019

Relevance to Bioengineering: stream #3, due to the use of techniques geared towards designing a device (and a system) with the goal of promoting human health.

WHAT IS PASSIVE COOLING?

- Design approach that focuses on heat gain control and heat dissipation with low or no energy consumption

Preventive techniques to provide protection and/or prevention of external and internal heat gains.

Modulation and **heat dissipation techniques** to dissipate heat gain through the transfer of heat from heat sinks to the climate.

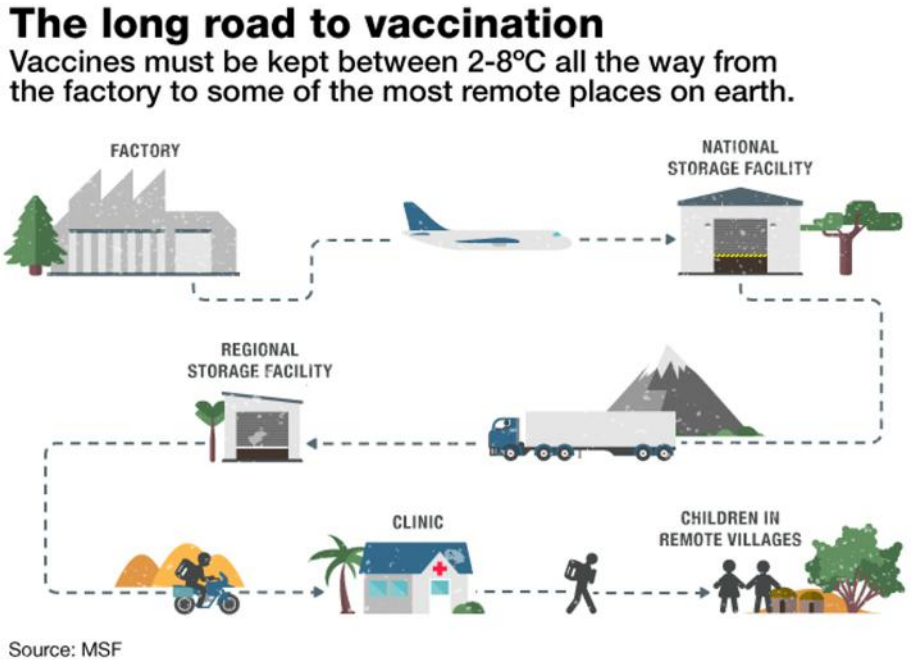
Why do vaccines need to be kept cold?

- To maintain potency. Once the potency is lost, it can never be restored
- Increased risk of vaccine-preventable diseases
- Some vaccines contain aluminum, which can precipitate at lower temperatures

Paho.org, 2019. [Online]. Available: http://www.paho.org/immunization/toolkit/resources/partner-pubs/ebook/Chapter5-Vaccine-Storage-and-Handling.pdf?ua=1. [Accessed: 31- Mar- 2019].

What is the Cold Chain?

- Process used to maintain optimal temperature conditions
- The recommended temperature for vaccine storage and handling is, at all times, at +2°C to +8°C.

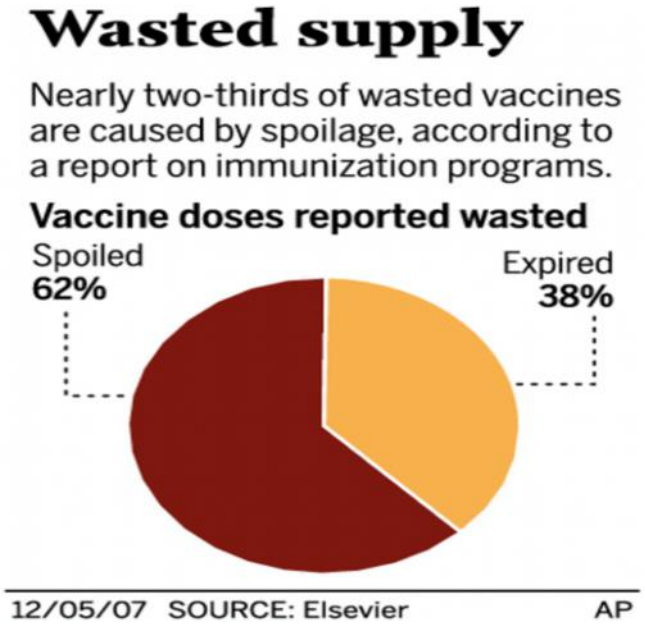


Bccdc.ca, 2019. [Online]. Available: http://www.bccdc.ca/resource-gallery/Documents/Guidelines%20and%20Forms/Guidelines%20and%20Manuals/Immunization/Cold%20Chain/bccdccoldchainresourcescreen.pdf. [Accessed: 31- Mar- 2019].

D. Matthias, J. Robertson, M. Garrison, S. Newland and C. Nelson, "Freezing temperatures in the vaccine cold chain: A systematic literature review", Vaccine, vol. 25, no. 20, pp. 3980-3986, 2007. Available: 10.1016/j.vaccine.2007.02.052

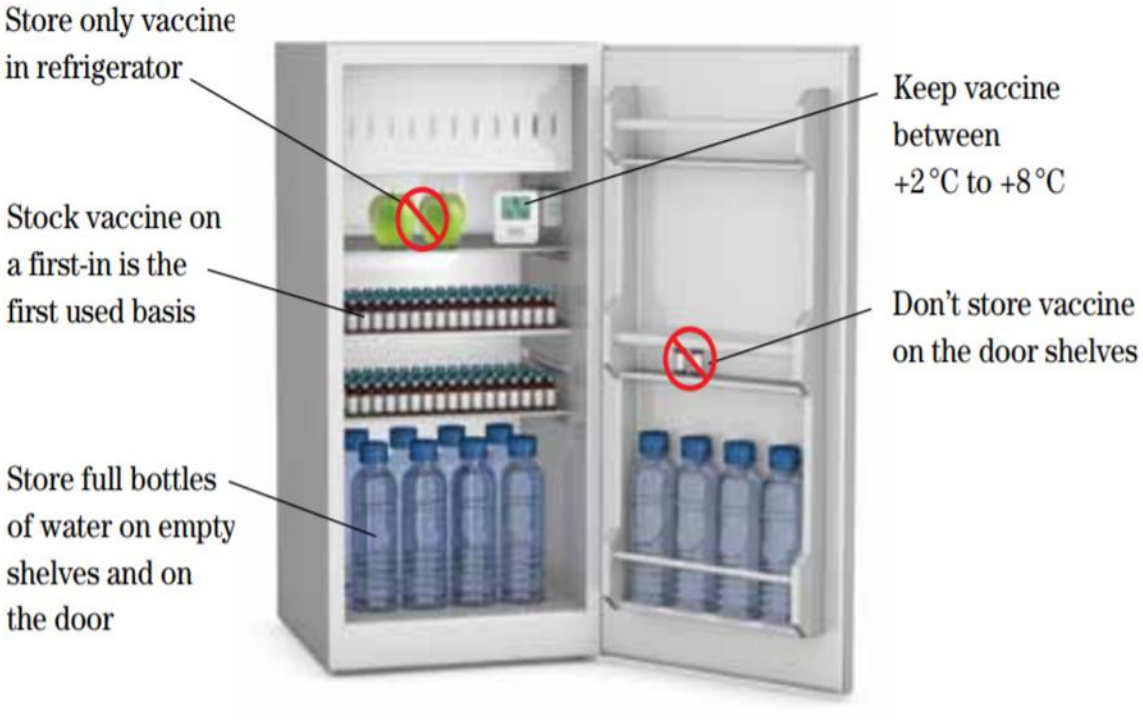
What happens if the Cold Chain breaks?

- Vaccine inactivation by exposure to excess **light**, **heat** or **freezing**
- Damage is **cumulative**
- Any loss of vaccine potency is **permanent** and **irreversible**



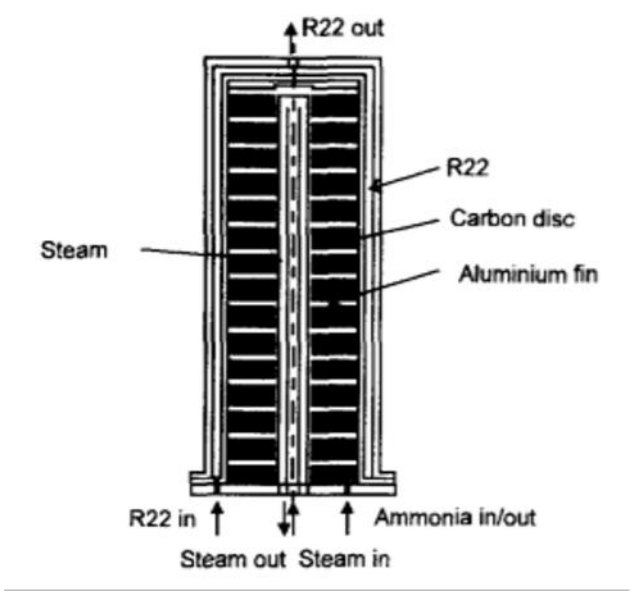
Government of Canada, 2019. [Online]. Available: http://www.health.gov.on.ca/en/pro/programs/publichealth/oph_standards/docs/reference/vaccine%20_storage_handling_guidelines_en.pdf. [Accessed: 31- Mar- 2019].

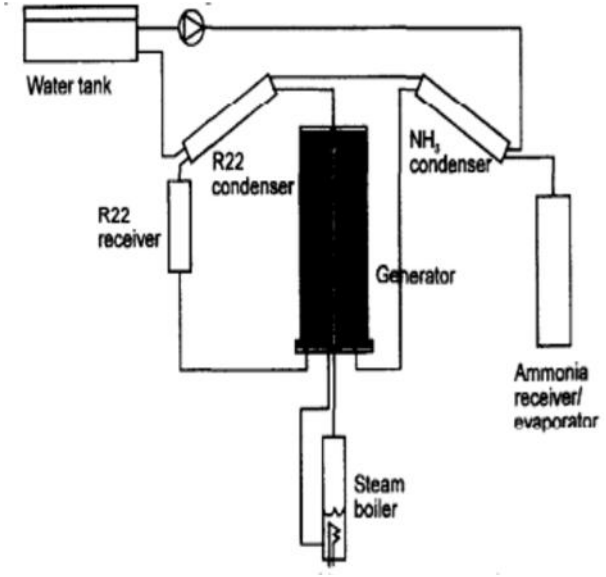
How are vaccines stored currently?



Government of Canada, 2019. [Online]. Available: http://www.health.gov.on.ca/en/pro/programs/publichealth/oph_standards/docs/reference/vaccine%20_storage_handling_guidelines_en.pdf. [Accessed: 31- Mar- 2019].

Alternate passive solution:





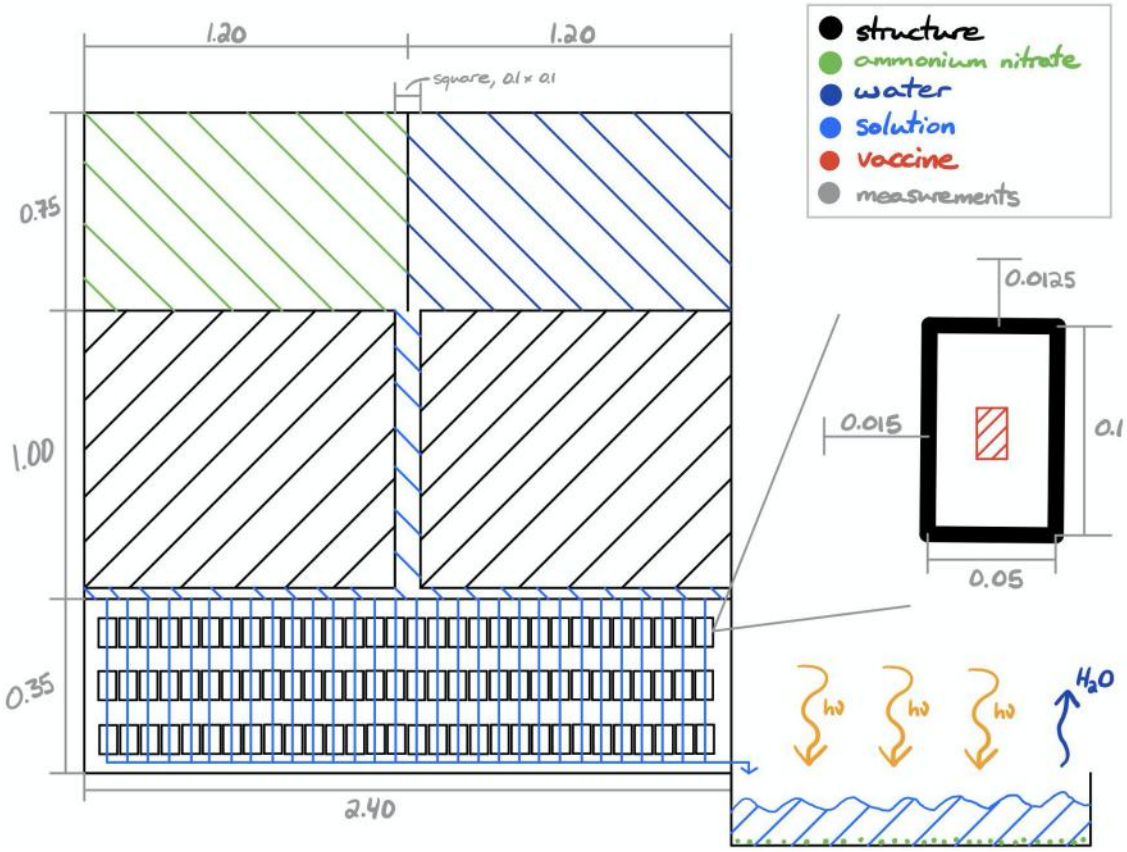
Research is underway for a passive vaccine storage solution that uses solar radiation to power a thermodynamic cycle using carbon - ammonia refrigerators driven by the heat of steam condensing in a heat pipe.

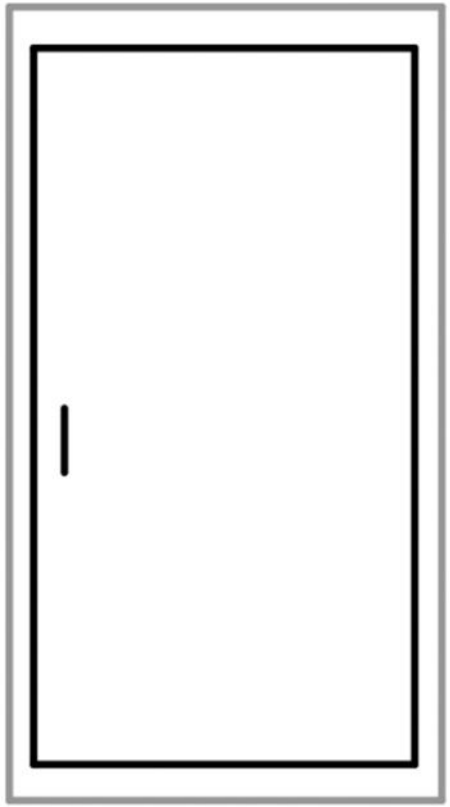
Critoph, R. E. (1996). Towards a one tonne per day solar ice maker. *Renewable Energy*, *9*(1-4), 626-631.

Hypothesis

Through the use of preventive and promotive strategies, we can design a passive cooling storage solution for vaccines in hot climates.

Keeping them between 2 — 8°C and ensuring minimal heat loss.





Design Sketch

Components

$$NH_4NO_{3(s)} \xrightarrow{H_2O_{(l)}} NH_4^+{}_{(aq)} + NO_3^-{}_{(aq)}$$

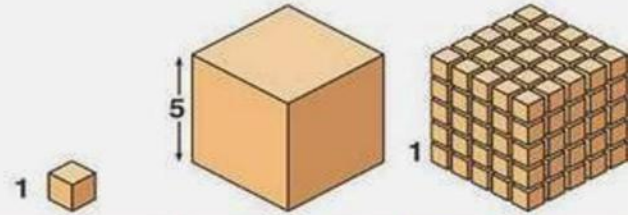
Means ions are surrounded by water molecules

Ammonium Nitrate Dissociation reaction

Components

Why Microfluidics?

Surface area increases while total volume remains constant



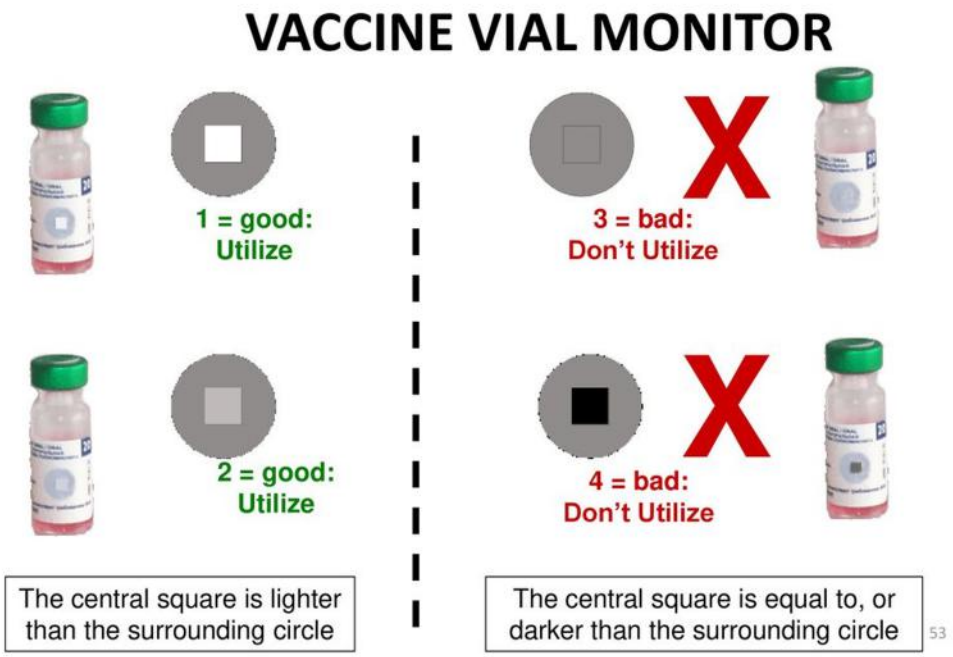
Total surface area (height × width × number of sides × number of boxes)	6	150	750
Total volume (height × width × length × number of boxes)	1	125	125
Surface-to-volume ratio (surface area / volume)	6	1.2	6

Components

Hyper-reflective surfaces and Insulation

Components

Vaccine vial monitors



Fundamental principles

Microfluidic Channel — slower volume flow and more surface area for heat transfer

Endothermic reactions — chemical reactions that use heat from the environment

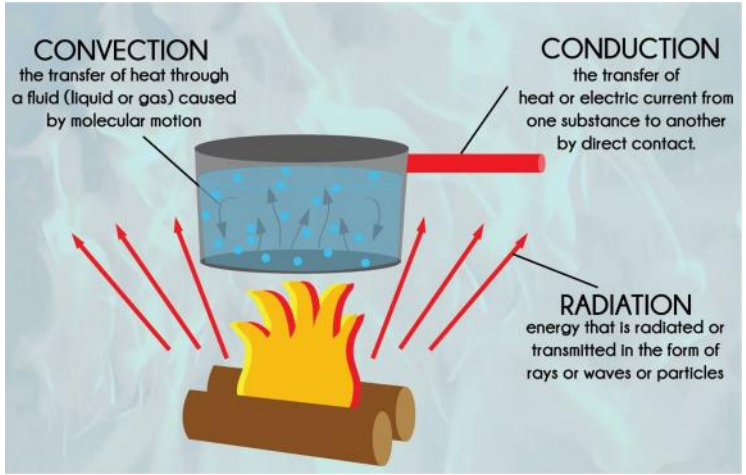
Evaporation — the process of turning from liquid into vapour

Insulator — a material with low thermal conductivity

Hyper Reflective Surfaces — metal alloy to reflect most incoming radiation

Thermochromic paper — changes colour in response to temperature

Methods - conductive and convective heat gain



$$U = \frac{1}{\frac{1}{h_1} + \frac{L_1}{k_1} + \frac{1}{h_2}}$$

$$U = \frac{1}{\frac{1}{5} + \frac{0.2}{0.04} + \frac{1}{5}} = \frac{5}{27}\frac{W}{m^2 * K}$$

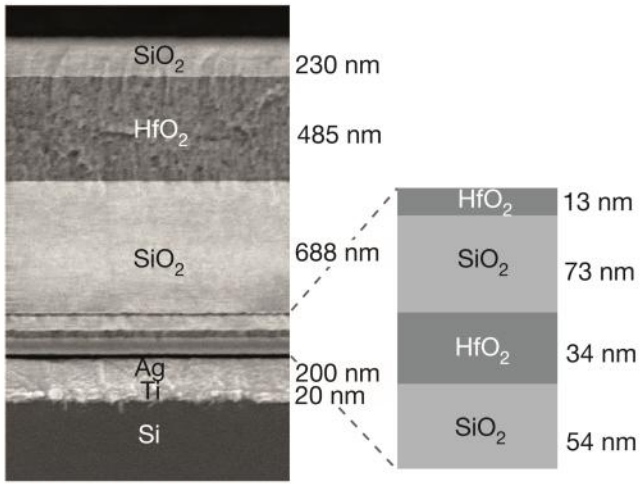
$$Q = U\Delta T = \frac{5}{27}\frac{W}{m^2 * K} * (27 - 5)\,K * 1.925\,m^2 = 7.843\,W$$

http://solarelectricityhandbook.com/solar-irradiance.html

Methods - radiative heat gain

$$5\frac{kWh}{m^2 * day} * \frac{1\ day}{24\ hours} = \frac{5}{24}\frac{W}{m^2} = 208.33\frac{W}{m^2}$$

$$208.33\frac{W}{m^2} * 0.03 = 6.25\frac{W}{m^2}$$

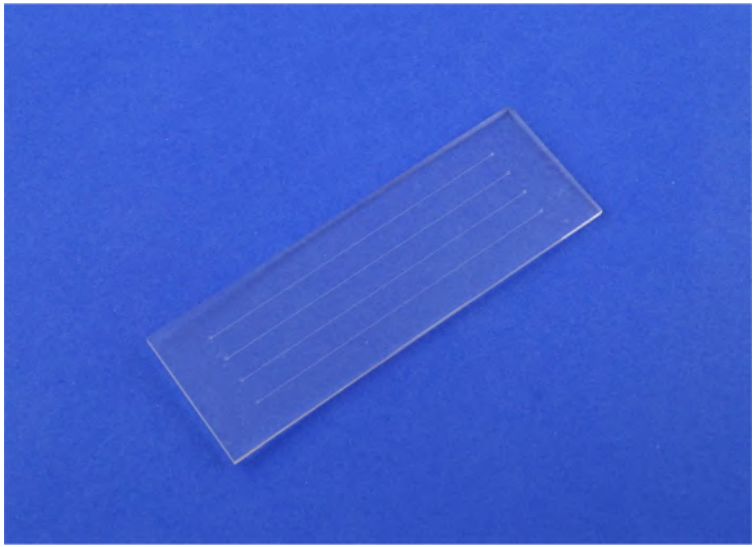


$$6.25\frac{W}{m^2} * 1.925\ m^2 = 12.031\ W$$

Methods - total theoretical heat gain

$$Q_{in} = 7.843\,W + 12.031\,W = 19.874\,W$$

Methods - effectiveness of one channel



$$Q_{conv} = A(k)(T_s - T_a)$$

$$A = 0.35\,m * 0.001\,m = 0.00035\,m^2$$

$$A = 0.00035\,m^2 * 2 = 0.0007\,m^2$$

$$Q_{conv} = 0.00035(5)(5-3) = 0.014\,\frac{W}{channel}$$

Design Component - putting it all together

$$\frac{19.874\ W}{0.014\ W} = 1{,}420 \text{ channels}$$

$$\frac{100\ W}{0.014\ W} = 7{,}143\ channels \approx 7{,}000\ channels$$

Design Component - putting it all together

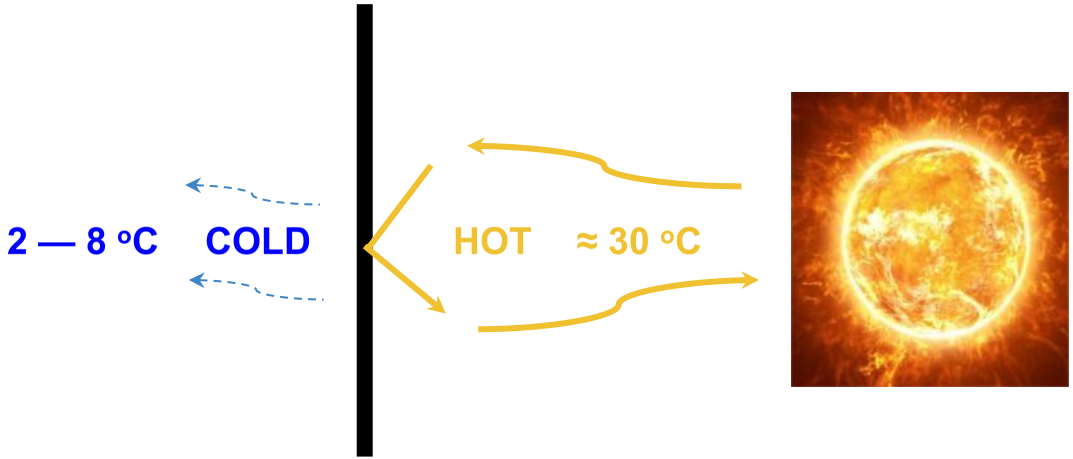
$$\dot{V} = \frac{A_c * l}{t}$$

$$\dot{V} = \frac{(1 * 10^{-6}) * (0.35)}{600} = 5.83 * 10^{-10}\,\frac{m^3}{s} = 5.83 * 10^{-7}\,\frac{L}{s}$$

$$7000 * 5.83 * 10^{-7}\,\frac{L}{s} = 4.08 * 10^{-3}\,\frac{L}{s} = 4.08\,\frac{mL}{s}$$

Expected Results: not feeling the burn

- Near-perfect **reusability** of ammonium nitrate
- Minimization of **heat gain**
- Massive **energy savings**
- Ideal temperature **achieved**, **maintained**



Potential Outcome: healthier vaccines, healthier humans

- **Decreased loss** of vaccines
- Applications in **developing countries** and areas lacking **consistent power access**
- Extensions into the **entire field of refrigeration**

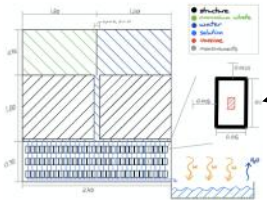
1/25 vaccines spoil in **developed countries** before being administered… with **millions more** spoiling in developing nations due to **cold chain breaks**

E. Weir, "Preventing cold chain failure: vaccine storage and handling", Canadian Medical Association Journal, vol. 171, no. 9, pp. 1050-1050, 2004. Available: 10.1503/cmaj.1041565 [Accessed 31 March 2019].

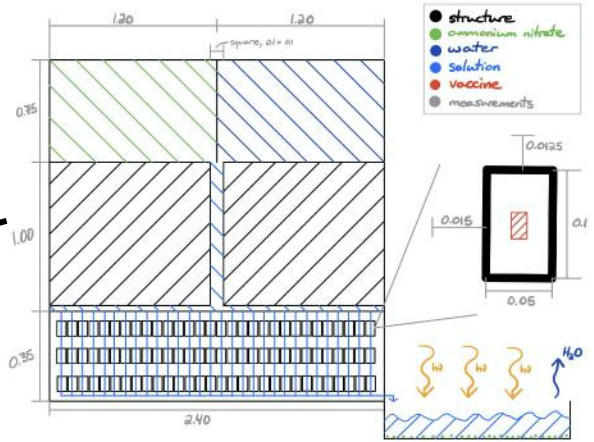
T. Comes, K. Bergtora Sandvik and B. Van de Walle, "Cold chains, interrupted", Journal of Humanitarian Logistics and Supply Chain Management, vol. 8, no. 1, pp. 49-69, 2018. Available: 10.1108/jhlscm-03-2017-0006 [Accessed 31 March 2019].

Future Work: pushing the science, and looking beyond it

- **Improve recycling system** (water recycling)
- Replace **ammonium nitrate**
- Development of methods for **further insulation**
- Improve **accessibility** and **economic viability**
- Adaptation for **more compact** or **portable equivalents**

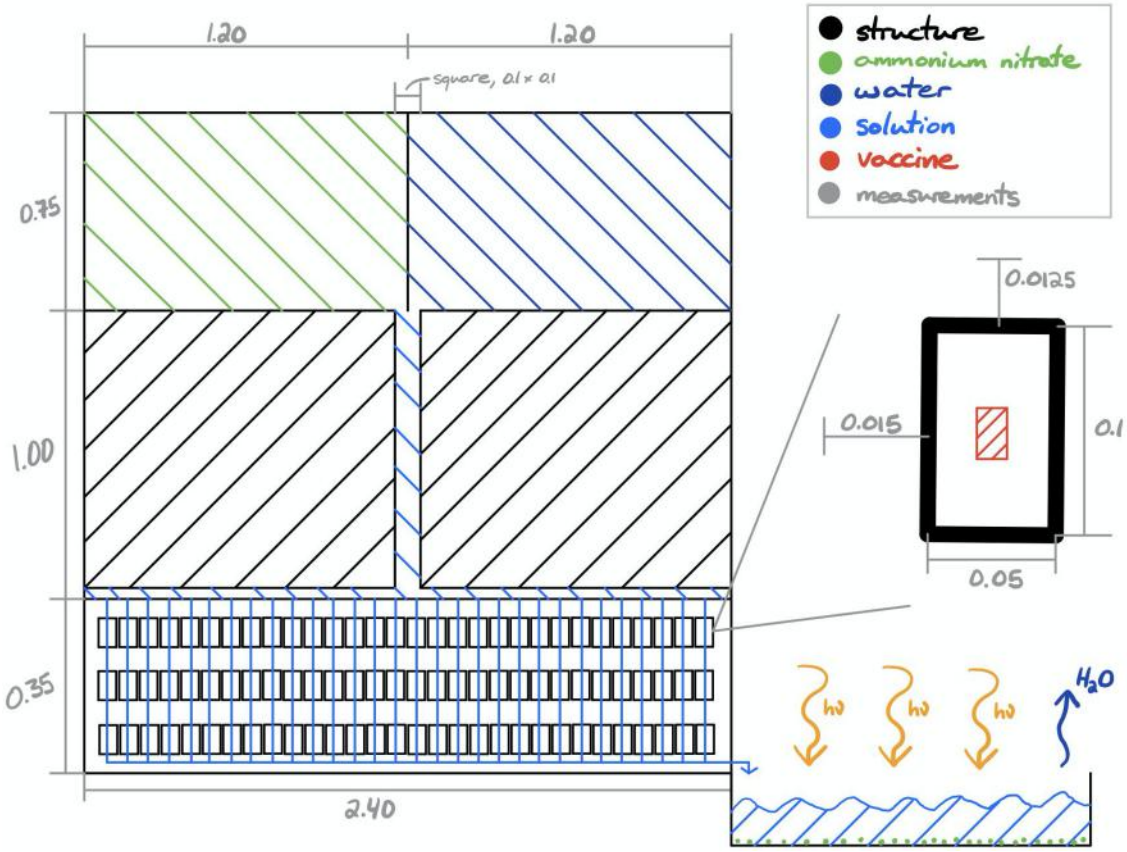


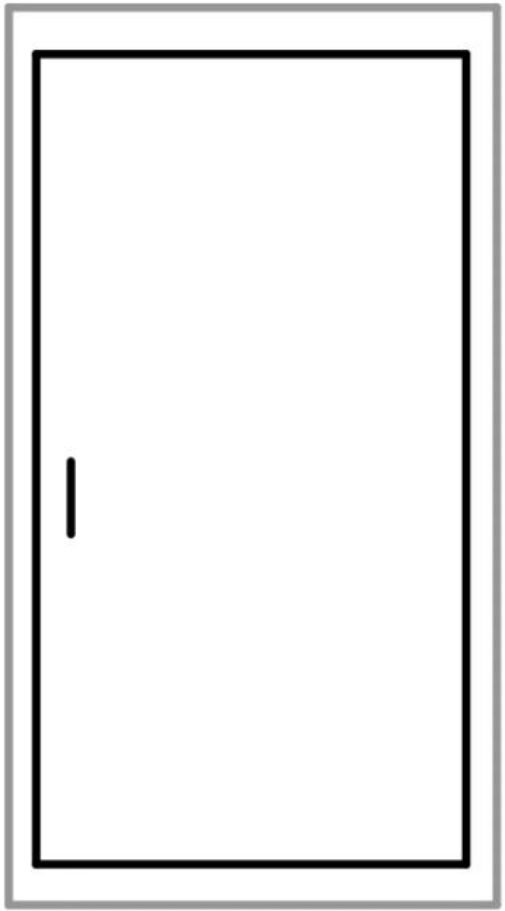
RESIZE



Conclusions: passive, but hardly inactive

- Entirely **passive** cooling system
- Use of **microfluidics** and **materials science**
- **Temperature** maintained in the required range
- High potential for **improvement** and **further innovation**
- **Applications** locally and abroad



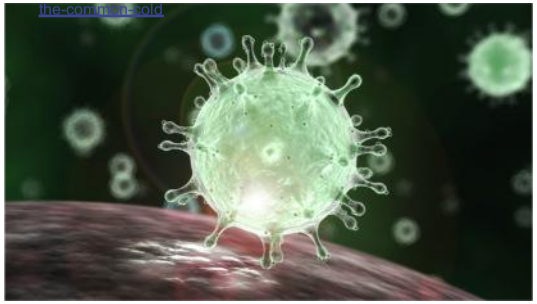


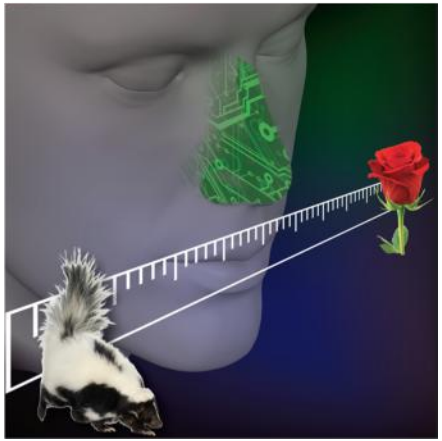
Design Sketch

Quick Demo of our Model

https://www.popsci.com/scitech/article/2009-09/state-art-electronic-noses/

https://www.npr.org/sections/goatsandsoda/2020/01/22/798277557/how-does-wuhan-coronavirus-compare-to-mers-sars-and-the-common-cold





https://en.wikipedia.org/wiki/Electronic_nose

Electronic Nose

By Anna Ciprick, Peter Chimienti, and Griffin Copp

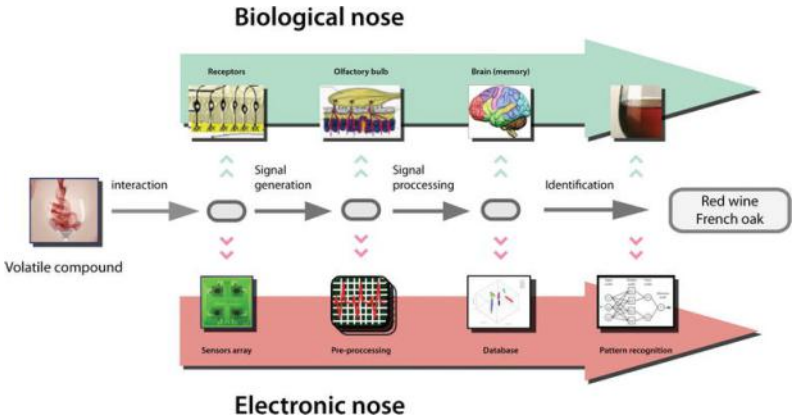
https://www.nanowerk.com/news/newsid=21758.php

Project Overview

Review literature on artificial electronic nose and describe potential technologies currently explored.

Highlight main limitations of current technologies.

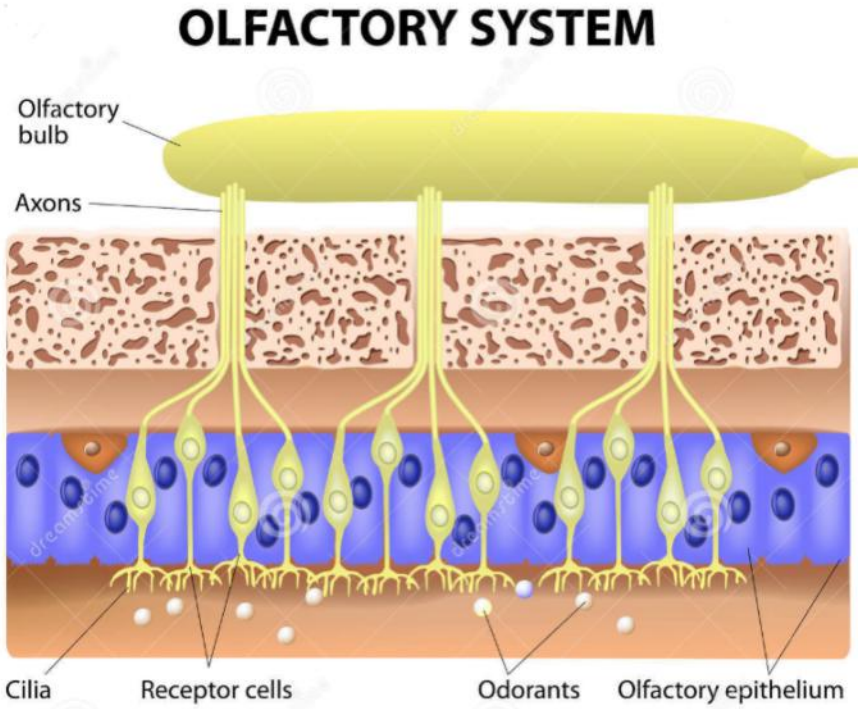
- Introduction to the olfactory system and the electronic nose concept
- General description of each sensory technique
  - Challenges & Limitations
  - Chemical, Optical, Piezoelectric
- Our design
  - Hypothesis
  - Approach
  - Expected Results
- Conclusion
  - Future directions



https://www.intechopen.com/books/brewing-technology/electronic-noses-applications-in-beer-technology

Human Olfactory System

1. Odorants bind to receptors on exterior of Olfactory epithelium (lining of the nose)
2. Olfactory receptor cells are activated and electric signal is sent
3. Signals are relayed in glomeruli (one for each molecule, located in olfactory bulb)
4. The signals are transmitted to higher regions of the brain via tufted/mitral nerves



https://www.khanacademy.org/science/health-and-medicine/nervous-system-and-sensory-infor/taste-gustation-and-smell-olfaction-topic/v/olfaction-structure-and-function

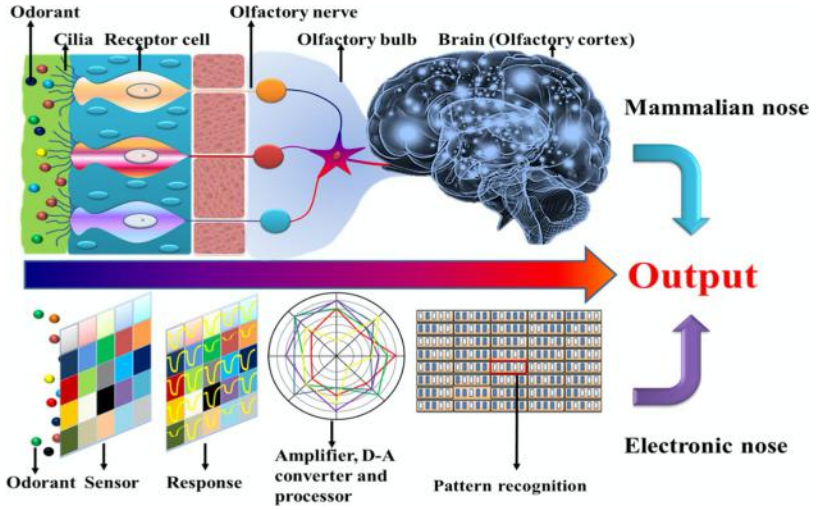
https://www.shutterstock.com/image-vector/olfactory-system-inside-human-head-bulb-192729188

The Electronic Nose (Stream 3)

- Biomimetic diagnostic device that detects and identifies specific molecules (VOCs) in gas odours [6].
- Compares known analytes from disease with test
- Provides qualitative & quantifiable data

https://spectrum.ieee.org/the-human-os/biomedical/devices/meet-the-enose-that-actually-sniffs

- Applications
  - Food & agriculture
  - Medicine
  - Quality control
  - Smoke detection

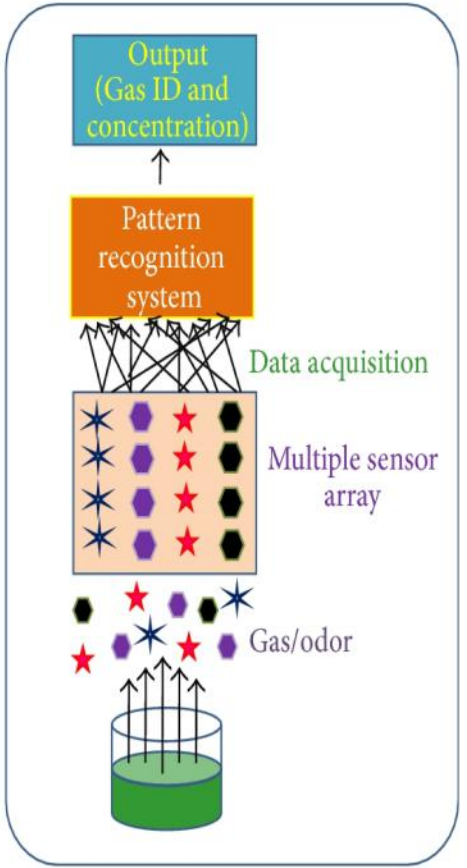


https://iopscience.iop.org/article/10.1088/1752-7163/aafc77

Electronic Nose Components & Mechanism

Components

1. **Multisensor array:** an array of sensors that change the acquired signal into a electrical signal [1,8].
2. **Data acquisition system:** Captures the signals and processes them [1].
3. **Pattern-recognition algorithm (PRA):** classifies, identifies and quantifies unknown gas analytes based on the data stored in the reference-library [1]. PRA techniques: Graphical analysis, Multivariate data analysis and Network analysis
    a. Reference-library database: Stores patterns or breath prints of know analytes.

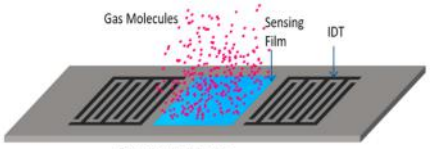


https://www.hindawi.com/journals/isrn/2013/941581/

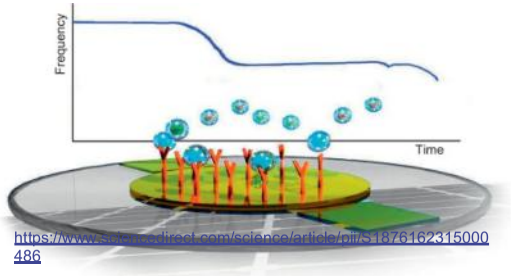
Technique #1: Mass-Based (Piezoelectric)

Mass-Based: Quartz-Based Microbalance and Surface Acoustic Waves: Piezoelectric effect

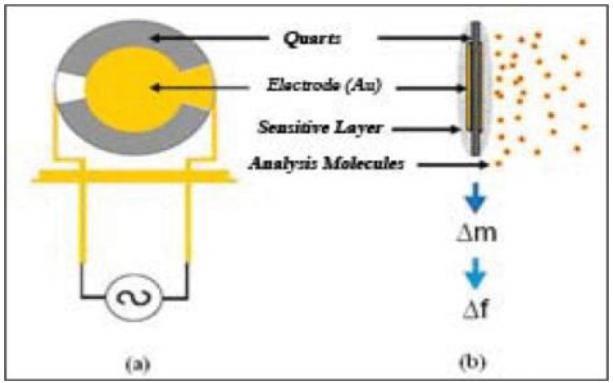
- Quartz-Crystal Microbalance (QCM): [3]
  - Thin plate of quartz crystal blanks, metal electrodes, electrical excitation, deformation and relaxation occurs, gives baseline natural resonant frequency
  - Utilizes mechanical resonance
  - Principle of QCM is based on changes in fundamental oscillating frequency with respect to adsorption of gas molecules, so a mass change
  - Advantages: high sensitivity and very high precision
- Surface Acoustic Waves (SAW):
  - Similar to QCM



https://www.mdpi.com/1424-8220/17/4/801/htm



https://www.sciencedirect.com/science/article/pii/S1876162315000486

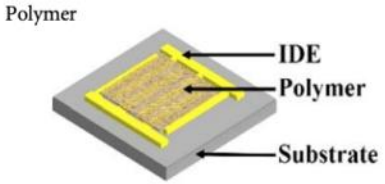


https://ieeexplore-ieee-org.proxy3.library.mcgill.ca/document/6531672

Technique #2: Chemical

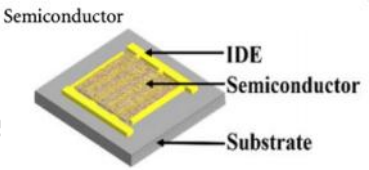
Chemical sensors rely on chemical interaction with gas or analyte to alter material properties

Conducting polymers [1]



- Polymer composites with different reversible physicochemical propertie
- high sensitivity, selectivity, and capability to operate at room temperatu
- The physisorption of VOCs is more efficient on a polymer's surface because of dipole/dipole interactions of polymers and VOCs

Metal-Oxide-Semiconductors (MOSFET) [11]

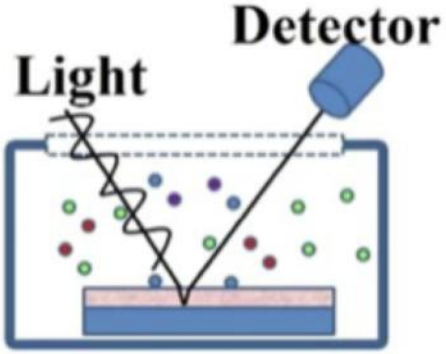


- Solid-state resistive metal oxide gas sensors are attractive because of thei simplicity, high sensitivity, low cost, stability, and compatibility with mode electronic devices
- The resistance of these semiconducting metal oxides changes during interaction with analytes

https://iopscience.iop.org/article/10.1088/1752-7163/aafc77/pdf

Technique #3: Optical

- Use light manipulation technology (optical fibers, photodiodes, and light-sensitive photodetectors) to measure changes in light properties (absorbance, fluorescence, polarization, scattering) [6,10,11]
- Excellent gas sensitivity and selectivity and simultaneous detection



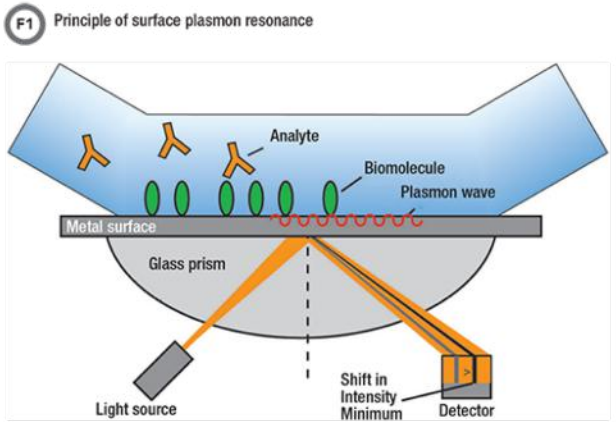
https://iopscience.iop.org/article/10.1088/1752-7163/aafc77/pdf

Types:

- Optical fibers [11]
- Surface Plasmon Resonance (SPR) [9]

Applications:

- Detection and quantification of bacteria in meat [11]
- Genomics



https://www.aacc.org/publications/cln/articles/2019/may/the-role-of-surface-plasmon-resonance-in-clinical-laboratories

Challenges of the Electronic Nose

https://spectrum.ieee.org/biomedical/devices/electronic-noses-sniff-success

General Challenges [1]

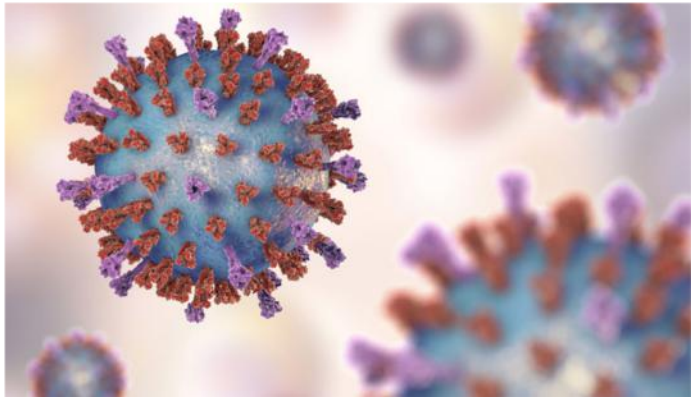
- Identifying odors that occur in small concentrations. There is a high probability of interference from other odors during signal processing when the odor of interest is found in small concentrations.
- Must be designed to fit its application, there is no “one size fits all” electronic nose available
- Loss of sensitivity in presence of water
- Inability to provide absolute calibration
- Some sensors have a short life

Specific Challenges [2]

- Optical: Difficulty in miniaturization making it not portable, high in cost, complex.
- Mass-based: QCM: complex, sensitive to humidity/temperature, poor SNR. SAW: temperature sensitive and coating on materials can affect certain compounds
- Chemical: Polymer: Highly sensitive to environmental changes, lower sensitivity than metal oxides.
    - Metal Oxide: high operating temperatures(300-500°C), limited range, greater operating cost

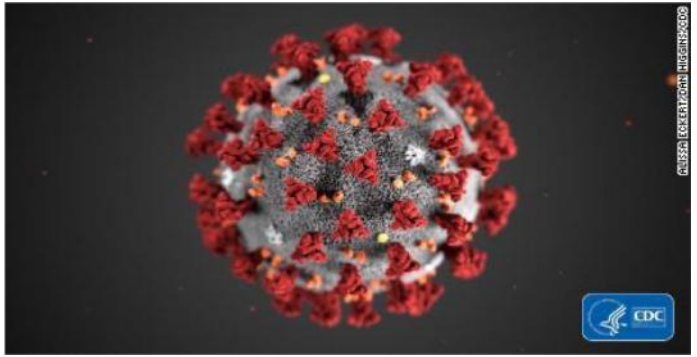
Hypothesis

- ➔ There is extensive research that detects and distinguishes respiratory diseases
- ➔ Data suggests the combination of quartz crystal microbalance and conducting nano-polymer sensors will increase capabilities
- ➔ The design will be easily adapted for the detection of COVID-19 VOCs once they are characterized



https://www.drugtargetreview.com/news/52992/structure-pneumonia-virus-enzyme-uncovered/

E-Nose for COVID-19 detection



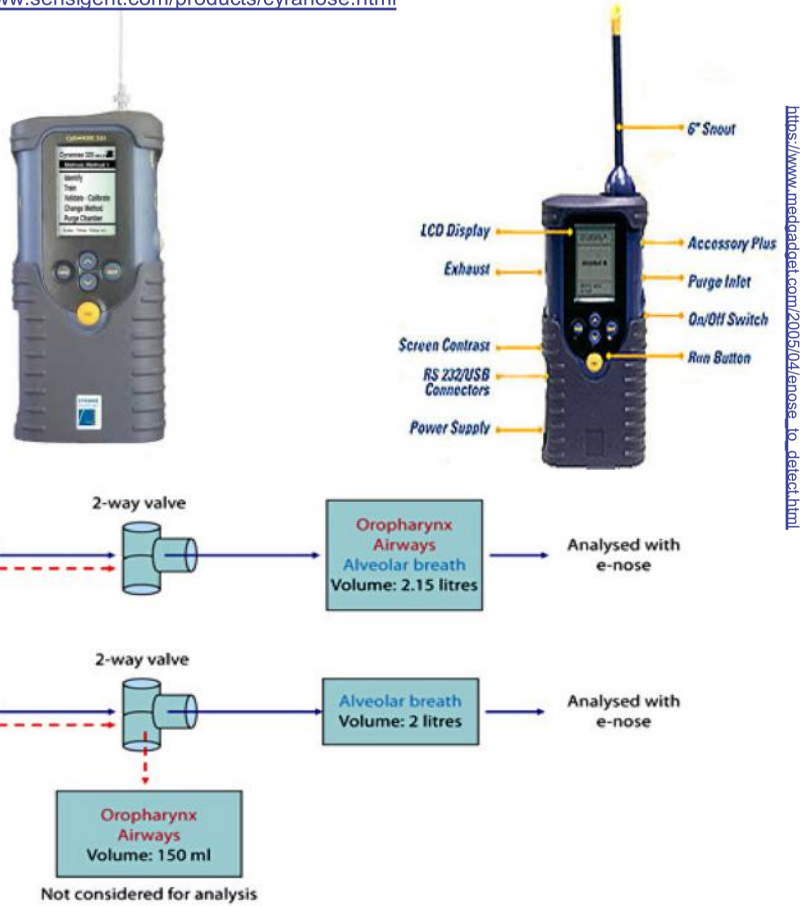
https://www.parsemus.org/breast_cancer/electronic-nose-sniffs-out-breast-cancer/

https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-at-higher-risk.html

Our Electronic Nose Design/Approach

- Miniature, portable device capable of diagnosing a wide range of respiratory diseases in a hospital setting.
- Coronavirus Approach
- Easy to use, filtered breath analysis [13]
- Sample Detection System [6]
- Artificial Neural Network [1]
- Aluminum sensor chamber [12]
- Gold Electrodes

https://www.sensigent.com/products/cyranose.html

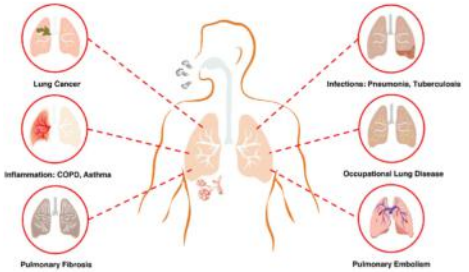


https://www.medgadget.com/2005/04/enose_to_detect.html

https://www.karger.com/Article/Fulltext/340044?fbclid=IwAR1JLLhDzt9fmA4GmvPkwG0-bo3w8W2fLUo_9LEILQOywDyIshQEM4V-7j4

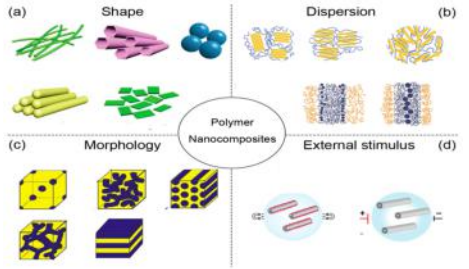
Approach for Choosing Sensor [1]

- The ideal sensor needed for an E-nose should have features such as affordable, sensitive, specific, user friendly, robust, equipment free, and deliverable to those in need (ASSURED)
- Sensor in electronic nose is MOST IMPORTANT COMPONENT
- VOC's of coronavirus are currently under investigation, we are predicting that the process of identifying VOC's linked to COVID-19 will be similar to other respiratory diseases such as lung cancer, asthma and COPD [4]
- Our sensor: 28 conducting polymer nanocomposite sensors (similar to sensors in Cyranose 320) and 4 quartz crystal microbalance sensors
- Why Nanomaterials? [7]



https://www.researchgate.net/figure/Common-respiratory-diseases-respiratory-diseases-are-among-the-leading-cause-of-deaths_fig1_349540845

https://www.rdsmed.com/news/respiratory-diseases-the-leading-cause-of-death-that-most-people-dont-know_446.html



https://www.mdpi.com/1099-4300/21/2/186

Expected Results

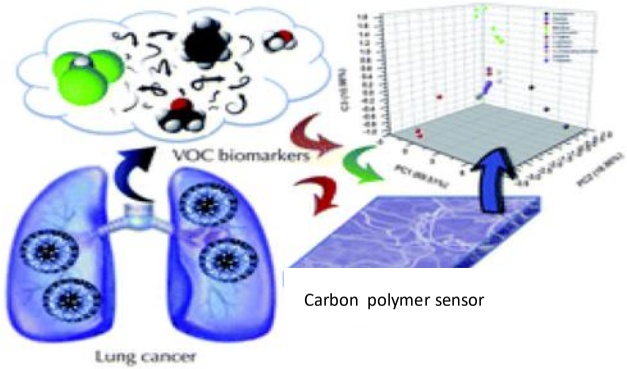
Lung Cancer: Quartz-Microbalance (QCM)

- 11 different VOCs identified [5,6]
- One study using 8 QCM sensor arrays coated with different metalloporphyrins to detect lung cancer
  - 100% sensitivity & 94% specificity [10]
- Distinction between LC and other diseases with a 93% sensitivity and 79% specificity [10]

Lung Cancer: Cyranose 320

- 71.4% sensitivity and 91.9% specificity [10]
- 66.6% positive and 93.4% negative prediction [10]

https://benthamopen.com/FULLTEXT/TOBEJ-9-228/FIGURE/F4/



https://pubs.rsc.org/en/content/articlelanding/2013/tb/c3tb20819b#!divAbstract

Expected Results (Cont)

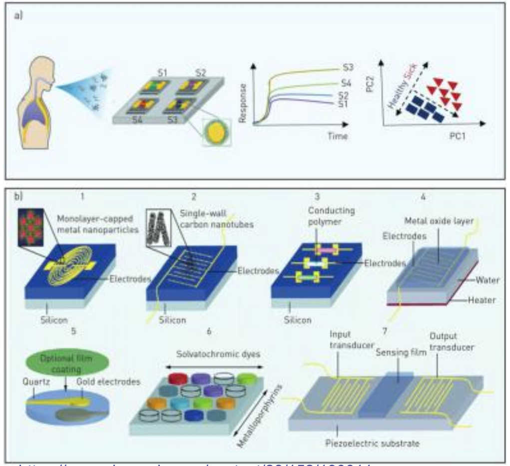
Asthma and COPD: Cyranose 320

- Netherlands 2011, study done to discriminate between patients having asthma or COPD
  - COPD vs classical asthma: sensitivity = 91% , specificity = 90% , accuracy = 96% [5,6]
- Netherlands 2016, study done to detect a viral or bacterial cause of acute exacerbations of COPD
  - Viral/non-viral: sensitivity = 83%, specificity = 72% [5,6]
  - Bacterial/non-bacterial: sensitivity = 73%, specificity = 76% [5,6]

Pneumonia: Cyranose 320

- Correctly discriminated between pneumonia-positive and pneumonia-negative patients. Good results were also obtained in a study of 38 people [8].

- E-Nose with polymer nanocomposites and gold electrodes was capable of detecting lung cancer with an accuracy of 94.2 %. [1]



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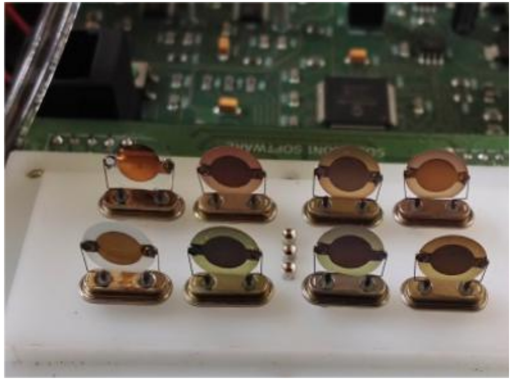


https://www.google.com/url?sa=i&url=http%3A%2F%2Feng.renluxcrystal.com%2Fsensor&psig=AOvVaw3IJO_x03eT1Mw5DVcNFssJ&ust=1586488725722000&source=images&cd=vfe&ved=0CA0QjhxqFwoTCIi_7aKx2ugCFQAAAAAdAAAAABAD

Table 3. (Continued.)

References	No. of Patients / Breath Samples	Diseases	Sensor Array Type	Dimensionality Reduction	Classifier	Validation	Results
							91.72% specificity, 95% CI
[82]	101 (58 healthy + 43 LC patients at different stages)	LC	MOS	NPLDA, PCA	Variants of k-NN, LD, QD, BPANN	Cross validation	92.6% accuracy,
							95.3% sensitivity, 90.5% specificity
[85]	335 (79 healthy + 165 LC + 91 other disease patients)	LC	Cyranose 320	Raw sensor values	SVM	Cross validation	77.3% for cancer versus non-cancer model,
							91.6% for cancer versus healthy volunteer's model
[86]	Not reported	LC ED	GC-MS + Cyranose360 + colorimetry + NanoNose (chemresistor with gold nanoparticles)	PCA	Fisher's LDA	Not reported	87% accuracy for GC-MS, 85% accuracy for Cyranose360, 73.3% sensitivity and 72.4% specificity for colorimetry, >90% reproducibility for NanoNose
[87]	30 (20 LC patients, 10 other lung disease patients)	LC	QCM	Raw sensor values	PLS-DA	Internal validation	>90% accuracy
[88]	89 (33 non-smokers, 11 ex-smokers, 18 smokers, 11 patients with respiratory disorders, 16 LC patients)	LC	Chemical (ENS Mk3 module)	PCA, analysis of variance	SPSS 12.0 software package	Not reported	p-values observed for different channels are 0.025, 0.045, and 0.001
[89]	32 (10 healthy + 15 LC patients + 7 CB patients)	LC ED	SAW	Raw sensor values	BPANN	Validated with 8 subjects	Accuracy not reported
[90]		LC	SAW	Raw sensor values	BPANN	Not Reported	

Table 3. (Continued.)

References	No. of Patients / Breath Samples	Diseases	Sensor Array Type	Dimensionality Reduction	Classifier	Validation	Results
	50 (18 healthy + 24 LC patients + 8 CB patients)						Sensitivity of decane is 2.12 ng Hz^{-1}, accuracy not reported
[91]	146 (76 healthy + 70 LC patients)	LC	Quartz microbalances	Raw sensor values	PLS-DA	LOOCV	81% sensitivity, 91% specificity for stage-I
[92]	475 (223 healthy + 252 LC patients)	LC	Cyranose 320	Raw sensor values	LRA	Not reported	95.8% sensitivity & 92.3% specificity for smokers, 96.2% sensitivity & 90.6% specificity for non-smokers

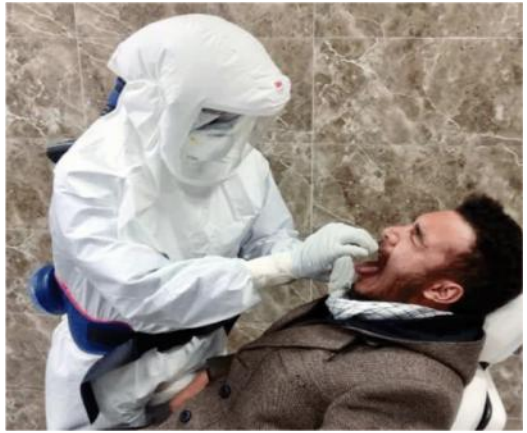
MOS: metal oxide semiconductor, SFS: sequential forward selection, PCA: principal component analysis, SVR: support vector regression, DWT: discrete wavelet transform, k-NN: k-nearest neighbor, ANN: artificial neural network, SVM: support vector machine, DFA: discriminant factor analysis, LOOCV: leave-one-out cross validation, CB: chronic bronchitis, LC ED: lung cancer early detection, BPANN: back propagation artificial neural network, , PLS-DA: partial least squares discriminant analysis, LDA: linear discriminant analysis, LE: Laplacian Eigenmap, LLE: local linear embedding, tSNE: t-stochastic neighbor embedding, CI: confidence interval, NPLDA: nonparametric linear discriminant analysis, LD: linear discriminant, QD: quadratic discriminant, QCM: quartz crystal microbalance, GC-MS: gas chromatography-mass spectroscopy, SAW: surface acoustic wave, LRA: logistic regression analysis.

Conclusion & Future Work

https://ca.proactiveinvestors.com/companies/news/182639/cyclopharm-begins-nuclear-imaging-trial-to-detect-respiratory-diseases-182639.html

- Advantages and disadvantages to sensor techniques
- Electronic noses can be applied to multiple applications
- Future work on identifying COVID-19 VOCs

https://www.google.com/search?q=covid+testing&sxsrf=ALeKk01YcsxqvKMvhK_RnoujrwyPf92iqg:1585787417081&source=lnms&tbm=isch&sa=X&ved=2ahUKEwj0uby9vsjoAhVTLs0KHX5FBQEQ_AUoAnoECA0QBA&biw=1062&bih=723#imgrc=6DpflTiivvww8M



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Questions?

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