Digital imaging systems

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Contents

1 Course concepts

The aim of the course is to give a unified perspective on the variety of digital imaging technologies that have been developed the last decades. Different aspects of the imaging technologies will be discussed such as:

- What they are imaging
- How
- With what quality
- For which applications

After this course you should be able to

- Describe the **physics** and **techniques** behind modern imaging techniques
- Describe the basic principles for sample preparation in relation to the imaging technique.
- Reason and analyze around possibilities and limitations in resolution with regards to:
  - density
  - space
  - time
  - spectrum
- Describe how the techniques affect the image and subsequent interpretation and analysis
- Reason about suitability of different imaging techniques in combination with image processing and machine learning for different applications.

The subject of imaging is interdisciplinary and cover a lot of subjects:

- Physics
  - Optics, wave propagation
  - Solid state sensing principles
- Electronics
  - Circuit designs
  - Sensor technology
  - Signal processing
- Mathematics
  - Geometry
  - Fourier analysis
- Computer science

No one is an expert on all imaging technologies and the course therefore consists of lectures of several guest lecturers. They will try to keep it in the common structure.

2 What is an image

An image is a multidimensional sample of the reality that consists of:

$$D = F(x, y, z, w, t) \tag{1}$$

- Densitometry **D**, signal intensity
- The spatial dimensions **x,y,z**
- Spectral dimension **w** – wavelength
- time **t** - the temporal dimension

We don't have any 5D sensors so we need to **multiplexing**. Since the subject is digital images, each dimension and the function value must be **quantized** into a limit range of discrete values.

The densiometric aspect **D** or intensity, what physical property is being imaged? is it a reflection, transmission of light, a density distribution of a molecule, a surface topology or an elastic property of an object? How well can we describe this property? and what physical effect do we use to measure: photo resistance, inducted charge or photon counting?

We create images from signals that can be of different types: electromagnetic waves (light, thermal, x-rays), pressure variations (sound) or contact forces (Braille).

We can use more than only the visible part of the electromagnetic spectrum, we can use all of it with different techniques.

Emission, excited emission, transmission or reflection

Imaged physical properties can be categories into : **Emission** (Astronomy, Autoradiography), **Exited emissions** (Flourescense), **transmission** (light microscopy, film scanning, classical x-rays) and **Reflections** (Normal photography, Document scanning, satellite sensors)

Consider where/what is the light source. In case of **emission** we have a well defined spectral properties, coming directly from the source. **Transmitted light** on the other hand is exponentially absorbed with a logarithmic intensity that is directly proportional to the absorbing matter. In the case of **reflection** is the surface orientation as well as the material properties and the direction and the spectral characteristics of the illumination that determines the signal. There is a need to differentiate between diffuse and specular reflection.

The illumination can be controlled in the case of a **Active** sensor system. This can be done either all at once for the whole scene or by scanning a pixel or a line at a time. An important example of a **Active** sensor system is: **LIDAR:** "laser imaging, detection and ranging".

Densitometric aspects: Resolution

It is important to consider what the densitometric resolution is and what is the signal to noise rate SNR. What pixel depth can we get or in other words how many greylevel do we get and are all of them meaningful. And what is the actual property that is measured?

- Material density
- Density
- Energy
- Photon count
- Topographic elevation

The contrast resolution is another aspect, the image should have a correct exposure time such that we use the most of the dynamic range of the sensor, not blowing up the brighter parts or under expose the image not showing any details in the darker parts of the image. Using most of the dynamic range of the sensor should result in white noise in the least significant bit. The optimal use of bits is therefore where we have about 1 bit of noise. If the conditions for imaging allows it, it can be possible to increase signal to noise ratio by taking the average of multiple exposure according to:

$$\sqrt{\text{number of exposures}} \tag{2}$$

We also need to have in mind the spatial consistency and ask: Will all positions in the image give the same density value for the same signal, there could be random or systematic variations where systematic variations

can be caused be for example defects or imperfections in the instrument/sensor that can be corrected for by calibration or other methods. One tool for correcting imperfections are: shading correction.

Imperfections is not only present considering spatial consistency where different part of the sensor register differently, we can have non linearity behavior of the registered sensor that must be corrected for with calibration. We need to know if the grey value is linearly or logarithmically related to the physical property we are interested in. In the field of normal photography is the intensity registered by our sensor linearly related to the reflected light and in transmission imaging is the light absorption logarithmically related to the amount of material the light passes through. If calibration is needed then it will be of importance to investigate of stable it is over time.

The spatial dimension (x,y,z)

Questions regarding the spatial dimensions to have in mind: How are the spatial dimensions **x,y,x** mapped into the image? Is the image a slice, a projection a depth map or something else? Are there any distortions that make the image not geometrically correct? What is the spatial resolution. Is it possible to get more than 2 dimensions with the available technology?

The spatial dimension can be mapped as **projection** that gives a 2D image of reflections from visible surfaces (in 3D) for the sensor or a transmission through the object. A **distance** image give explicit information as seen from a single point (2 1/2 D). With a **slice** we select a slice from a volume. **Tomographic reconstruction** is a method to compute information about the internal density structure using measurements of numerous line integrals.

The spatial resolution is limited in different ways in analogue and digital images. In Analogue there are constraints of the aperture of the lens and the wavelength of the light while in digital images the limiting factor is the sampling according to the sampling theorem. Under sampling can give problems such as aliasing and when it is caused be poor sampling the result is often worse then when it is because of limited resolution. It is therefore common to have low pass/blurring filters to prevent sharper images than can be digitized. There have been recent inventions that describes the ways of going beyond the resolution limits. example??

Distance images is a wat of representing 3d in 2D by making measurements of the distance to the surface of the object to the sensor for all points in the image. This can be done using either **passive** sensors with: Parallax camera or stereo images. With an **active** it can be done with the measurement of time of flight (lidar, radar, ultrasound, laser) or with triangulation, structured light.

Creating 3D images or image volumes is a rapid growing area in digital imaging mostly driven by medicine. In contrast to the 2D images these can not be viewed directly and it's necessary to use special visualization efforts to interpret the result. These types of imaging system generates very large data sets. Imaging volumes can also be done be psychically slicing (and destroying the sample in the process) and the result would be a 2D image of this thin slice. Another category of volume imaging is **Tomograpy** that includes: x-ray(CT scan, magnetic resonance (MRI), Emission (SPECT and PET), electron microscopic (EMT) and Optical coherence (OCT what is this?)). Confucal microscopy, ultrasound and holography are also volume imaging techniques.

Reconstructed images like tomographic reconstruction is a type of inverse problem with multiple dimensions that estimates a system from a finite number of projections. Examples of where this technique is used are:

- Transmitted X-rays, Computer Tomography (**CT**)
- Radioactive decay, Emission Tomography
  - PET
  - SPECT
- MRI, emitted excited radio frequency
- SAR (synthetic aperture radar)
  - CARABAS - long wave - incoherent

The spectral dimension w

In all imaging we need to limit the spectral range we are imaging by choosing a range in a wavelength interval. Different spectral ranges often give different image contrast. The signal in each of the pixels is the result of a convolution between:

- The spectral distribution of the illumination
- The spectral absorption/reflection properties of the object.
- The spectral sensitivity function(s) of the sensor.

The visual perception by the human eye is the result of the application of the three different spectral sensitivity ranges of the cones. Most imaging system are designed to be optimized for human color reproduction fidelity. There are many different illumination and reflection functions that can give the same color experience.

For capturing a color image we need three spectral samples which can be captured in different ways where the **Bayer filter** is the most common. This pattern leads to loss of resolution but is restored by interpolation of some sort. The alternative to having the **Bayer filter** is to have three sensor chips each color, splitting the image with a prism. This solution is expensive and requires also high mechanical precision. A third option is to stack the three sensing layers for the different color channels which have the benefit of not losing resolution/not need of interpolation.

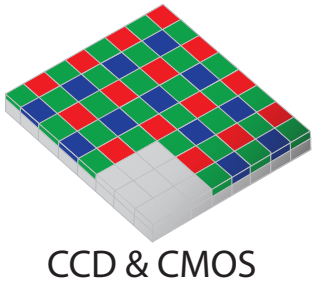

Figure 1: Bayer filter

What limit us to only use three spectral channels in image analysis is conventional thinking and that there are many cost effective camera in this range. When we register more than one spectral channel we need to use multiplexing, switching between channels either spatially or temporally. Ultimately it should be the application that decides how wide the spectral range should be and the number of channels. It is possible to achieve spectral imaging in a method similar to the Bayer filter, there are cameras with 9 (3 $\times$3) visible light channels and 16 (4 $\times$4) infrared channels that together can give us 25 channels simultaneously. As the number of channels increase to several hundred we call it **imaging spectroscopy** or hyperspectral imaging. This type of imaging creates a large amount of data and needs effective transmission and compression.

A solution to capture a large number of spectral channels is to have a rotating filter wheel in front of a wide band, single channel sensor/sensing chip. This requires the scene to be stationary since there will be several images registered.

Temporal aspects (t)

Each image will register something for defined time interval and if there is only one single temporal slice we have a still image, while if there are multiple exposures we get a film/movie. For each captured image or exposure we need to define the exposure time, the following questions should be considered:

- Does it give motion blur, how fast is the scene/object moving?
- Can it be varied freely?
- How will the quality of the image be affected by the exposure time? high ISO sensitivity more noise for example.

During the exposure is each pixel, line or image exposed for a certain time, so we need to consider the motion in the scene and of the camera relative to it and the light intensity can by limited. There are however ways

to freeze a moving object in the image by using a flash or follow the object in motion with the camera. There are even special solutions with sensors that have electronic object following.

Using sequences of images can be useful when measuring a motion. It is then crucial with timing:

- Repetition time
- Exposure time
- Data transfer and storage time
- Influenced by resolution in all five dimensions.

Sequences can also be used to detect changes in scenes by subtracting the previous stored reference image. For a sequence to be perceived as continuous by the human visual system it is necessary to have a repetition frequency of at least 25 Hz.

Multiplexing is needed since the intensity must be measured for all pixels to get a complete image matrix and we have multiple spectral channels (color RGB) to consider in the integration process. There are no effective sensors that can capture all dimension at once and we need to multiplex the light collection. The amount of parallelism in this light collection is an aspect imaging system and is strongly influenced by how the light is handled (economically) by the system.

Area integration

Area integration in imaging technology that uses a 2D image sensor to register the light for the whole image in parallel is by far the most common. It started of with image tubes and was later replaced with **CCD** (Charge Coupled Device) that are now being replaced with **CMOS** (Complementary Metal Oxide Semiconductor) that could be replaced by new technologies like QIS (photon counting devices) in the future.

Area integrations gives the best light collection efficiency since it collects "all the light". It also have a rigid geometry and gives stable and predictable imaging geometry that don't suffers from a lot of distortions. There is no need for mechanical motions and they can be mass-produced and therefore inexpensive. The drawbacks with area integration is that it need an even illumination of the whole image surface and there may be varying sensor sensitivities that need to be corrected for. It is also difficult to achieve high fill factor since there are other things that competes for space on the 2D area, multispectral scanning can therefore also be hard to achieve. The image size will be limited by the sensor size.

CMOS and CCD matrices/sensors are available in many different version with a variety of megapixel resolutions for special applications. In some applications they can be cooled to very low temperatures that allows for long integration times when the light levels are very low. In contrast they can also be used to capture fast events like laser flashes.

Linewise integration

This kind of sensors are often find in scanners and can be moved to capture an image of the object, or the object can be moved over the sensor. It is also common in remote sensing like satellites that move along the earth surface.

The technical advantages with line integrations are much better pointwise integration but worse economy compered to area. In motion the orthogonal line will be "frozen" which can be useful in some applications. It allows the use of the other dimension in a 2D sensor for the wavelength, for RGB and hyper spectral scanners ? read more. It possible to create whats called "intelligent sensors" by having a processor for each pixel. The disadvantages are similar to area integration, it needs an even illumination along the scan line, it needs corrections of sensor sensitivity along the line of pixels. The 1D fill factor is important but not often a problem meaning?. There is also a risk for x-y in-homogeneity because of the widely different methods of scanning.

Point-wise integration

With this methods we register light from one pixel at a time and it requires motion to create the image. Here we most distinguish between what is moving:

- The illumination

- The sensor
- The object

The method is mainly used in stationary conditions such as microscopy or scanning film or paper/document. Examples of applications are Drum scanner which produces very high quality scans of document or film. Other applications:

- Flying spot scanner
- Microscopy
  - Fluorescens
  - Confocal
  - Multi-photon
  - Moving stage

Some of the advantages are that it gives maximal possibilities for optimization of the measurement of each pixel and that i can have optimized optical path and sensor. No differences between the sensor properties of the different pixel sensors, i.e. only one pixel. It also have the advantage of not having a limit to the image size. The disadvantages are that it uses the incoming light poorly and need some sort of complex mechanical system for scanning which also results in it being very slow compared to the other methods.

Multiplexing for volume imaging

A few words about multiplexing and volume imaging; It is possible to register single voxels, a line, a plane at a time, the whole volume in parallel. Collecting data in the Fourier domain or through other transforms can be done. Today most tomographic systems collect data from the whole volume or from multiple planes simultaneously which is fast and therefore saves time and signal economy.

Wavefront imaging

Is new form of imaging that have high information density. The Wavefront sensing measure the amplitude and phase of the incoming optical field simultaneously. It is still under development but show promising results.

Stored intermediate analogue image

This category is mainly of historic interest and a few example are listed here:

- Photographic film
- Polaroids
- Magnetic tape
- Semiconductor materials (image plates)

3 Photography

The focus of this course are ways of creating images for scientific or medical applications but its worth noting that a majority of all digital images created are digital photography. Digital photography has grown tremendously the last 20 years and analog photography is now limited for some small applications or enthusiast.

Description of digital photography

Digital photography is an optical imaging technology and the aspects of it are the same as for analogue cameras: Lens quality, aperture, depth of focus and focal length.

The lenses come in large variety of sized and qualities and it should be chosen with the application in mind. Some basic guidelines are that: bigger is better and glass is better than plastic. Compact lenses has become better and there is a trend towards more compact lenses such as liquid based lenses that can change focal length and focus very rapidly.

The depth of focus depends on the lens focal length and aperture. A smaller aperture will result in a larger depth of focus but less light is reaching the sensor and therefore need longer exposure time.

Choosing between a fixed focal length or a zoom lens is depending on the application. For general hobby photography is a zoom lens often to prefer since it doesn't require the user to change lenses when the scene or application changes. Zoom lenses are in general not as good as a fixed lens but instead offer the flexibility. Some geometric distortions to the resulting image is caused by the lens and takes the form of **Pincushion** or **Barrel** distortion which can be corrected for in image processing.

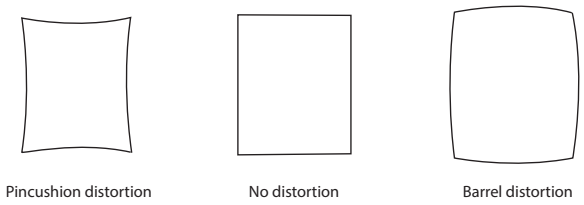

Figure 2: Example of caption

There are two types of sensors domination in the area of digital photography: **CCD** and **CMOS**. CCD sensors are matrices of photosites each comprised by a photodiode which converts light into a charge and region that can hold the charge. The charges are shifted/moved out of the sensor as a bucket brigade and converted to a digital signal at the end of the circuit. In CMOS sensors similar to the CCD are there photodiodes that converts the light into electrons/ a charge but there is also a reset and select transistor with an amplifier section. This mean that the amplification is done at each pixel.

In the **full frame** CCD sensor are the charge holding region integrated with the light sensing region. When the light is collected is happen over the entire imager but it needs to be shut off after that so the charge can be moved through a horizontal charge transfer register a charge voltage conversion, amplifier and analog to digital (A/D) converter. This results in a almost 100% fill factor of the sensor but there is need for an external shuttering. In the **interline** type of CCD sensor are the charge holding region shielded from the light meaning there is no need for a external shutter to keep light out. This type of sensor architecture results in lower fill factor but can be compensated with micro-lenses what is that?. A third type of CCD sensors are **frame transfer** sensors which have like the **full frame** photosites that cover almost all of the sensor/sensing area. The difference is that the charges are shifted quickly to a equally sized charge holding region that are shielded from the light. Thanks to the high speed is no shutter need and the readout can be done while a the light for a new image is captured.

The **CMOS** sensor is built with the same technique as processors and memory arrays. This architecture allow for readout of the entire array or only parts of it with a simple X-Y address. The fill factor is decreased compared to the full frame CCD since there is more electronic in each pixel. This can be compensated by either Micro-lenses or using thin sensors that can be exposed from the back side. CMOS sensors can ha

For a long time have megapixels been a selling point for sensor, the pitch has been: more is better. But this is not always true. A small sensor size with a high number of pixels can lead to decrease in sensitivity and worse signal/noise.

CCD	CMOS
Power consumption 2-5W	Power consumption 20-50mW
More light sensitive approx 1 lux	Less light sensitive approx 5 lux
Less digital noise	Uses same silicon design as other electronics
Better light sensitivity, up to 85% quantum efficiency	Any subset of pixels can be read out
More color depth, more dynamic range	Younger technology
Requires special chips with higher voltage, higher cost	CMOS pushing CCD:s out of the market

Table 1: Comparison CCD vs CMOS

Comparing priorities between consumer and scientific cameras we can see that consumer cameras generally focus on having good looking pictures while scientific camera priorities correct, quantitative pictures. The increasing use of digital photography has pushed on the development of cameras in the consumer segment, this has also resulted in much value for scientific needs also. Although the trend has now shifted towards smaller sensors since most of them are fitted to mobile phones. This creates a divergence between consumer cameras and scientific cameras that are not faced with the same constraints as mobile phone cameras. As today there is still a wide range of digital cameras for scientific applications. The sensors range from a couple of euros to 10 000 euros in price, but a typical camera can be bought for around 1000 euros.

A paradigm shift might be taking place with **Photon counting** sensors being developed. These sensors count each photon separately and is as sensitive as it is possible to be. This allow for new capabilities such as trade off in sensitivity and resolution that can be dependent on the scene. It will allow for motion blur compensation for multiple targets and high apparent SNT for a low photo flux.

Operational steps

We start of with the lens that most often includes a IR blocking filter and an optical anti-aliasing filter. The focal length is often adjustable (zoom lens) and the focus is controlled by the focus motor. Exposure and focus measuring is done by pressing the shutter button halfway. The lens focuses the light from the scene onto the sensor. The analog signal that is produced by the sensor is then converted to a digital signal (A/D converter). Then the shutter button is fully pressed down the the image is captured and stored on in DRAM. To long exposure times can lead to motion blur in the image which can be solved by active image stabilization, either by moving the lens or the sensor. Exposure control can be used to exposing different parts of the image differently to optimize quality of the image.

The final high-resolution image is processed by a digital image processor in the camera. The first step is to **de-mosaicing** since most cameras use the Bayer color filter array, here is interpolation used to fill in the missing color values for each pixel. An algorithm decides if the "missing" color values are in a smooth area or along an edge to determine the value for each missing color value. This process results in a full-color image but not a perfect one. To improve the result there is need for white balancing to compensate for spectral variations in the illumination of the scene. Both daylight from the sun and indoor lightning provides white light is the daylight more "high energy" in the blue portions while indoor lightning is more "high energy" in the red portion of the spectrum. An algorithm is used to analyze the scene and adjust the red and blue signal strength to match the green signal strength in white and neutral parts of the image. Continuing with color correction that is needed since the sensor has a different sensitivity than our eyes and the colors can for the human eye be perceived as unsaturated without correction. The color correction compensate for this and transform the output image to the output color space (often sRGB) that is ready to be displayed on a monitor.

Densitometric

Geometrical

Spectral

Temporal

History

4 SEM - Scanning Electron Microscopy

The electron microscopy is an instrument that allow us to create images/visualize organic and inorganic structures with impressive magnification.It is an invaluable instrument in the engineering and development of new materials where nano-meter sized imaging is very important. The SEM can also be used in chemical analysis and accessing the crystalline structures of materials. In comparison to light microscopy is the depth of field much larger in scanning electron microscopy thanks to the narrow electron beam. The resulting images get a 3D appearance due to this large field of depth that is very useful when examining the surface structures.

History

Operational steps

The idea behind the technique is to generate a beam of energetic electrons with emission from an electron source. This is typically done by heating up a metal (Tungsten for example) in vacuum and accelerate the electrons with a electric field. The electrons are stopped from leaving the atoms of the metal be an energy barrier between the emission tip and the surrounding vacuum. By adding an electric field in the vacuum the energy barrier is reduced to a "slope", but the electrons still need to perform the "work" W to pass this barrier. Luckily there is an quantum effect called tunneling allowing electrons to tunnel through this barrier out into to vacuum.

Illustration of tunneling

There are several types of electron emitters (cathodes) with different properties being used in electron microscopy today. One of the older and cheaper options are the so called **W Hairpin** made of Tungsten. These are widely used in older SEM and TEM microscopes. Tungsten is used because of its high melting point and low vapor pressure. This makes it possible to electrically heat to make electron emission possible. Another option is **Lanthanum Hexaboride $LaBa_6$** which has a low work functions, meaning the energy to remove an electron from the metal to a point in the vacuum outside the metal is low. This type of cathode is about 10 times "brighter" then Tungsten cathodes meaning?. A third alternative is FEG **Field Emission gun** which can create a beam that is smaller in diameter and more coherent and up to three times greater magnitude of "brightness/current density" The FEG is sharply pointed with an emitter that is held at several kilovolts of negative potential energy compared to a nearby electrode. This makes the potential gradient sufficient to cause electron field emission.

The energy of the electrons in this beam is measured in 1eV which is equivalent to what a electron in an electric field generated by 1 V would have. This energy is denoted E_0 and is often around 0.1 to 30keV. The electron beam is after acceleration modified/reshaped by lenses and apertures to reduce the diameter of the beam and to scan this beam into a raster of x-y coordinates which it sequentially is placed in. This coordinates are discrete but closely spaced. The lenses are no ordinary glass lenses since the electrons would pass through them or lose to much energy (wouldn't focus them either). Instead are they modified with electrostatic lenses and electromagnetic coils into desired shape and properties.

When the primary electrons in the electron beam hit the specimen it interacts with it forming a interaction volume. The electron penetrate the specimen material and looses energy by random scattering and absorption this process with all electrons in the beam form a volume of interaction that is teardrop shaped. The interaction results in different types of electrons being emitted/scattered from the specimen.

At each of the raster coordinates in the scan pattern are two types of outgoing electrons from the specimen created: **back-scattered electrons (BSE's)** and **secondary electrons (SE's)**. The BSE's are electrons that emerge from the specimen with a lot of the initial energy after interacting with the atoms electric fields of the atoms in the specimen, scattering and deflection. The SE's are electrons that emerge from the specimen surface after the electron beam have ejected them from the atoms in the sample. These electron escapes with very low energy compered to the typically high energy electron beam. They are in the range of 0-50eV with the majority below 5eV.

The secondary electrons are often measured with a Everhart-Thornley detector that is sensitive for both SE's and BSE's while the BSE's are measured with a dedicated BSE detector that is not sensitive for the SE's. The signal for each of the detectors are measured at each of the coordinates in the x-y raster. The location and the intensity is recorded and correspond to a gray value in the x-y coordinates. Other sensors

can also be used to capture for example X-ray signals that also can be emitted from the specimen due do the electron beam.

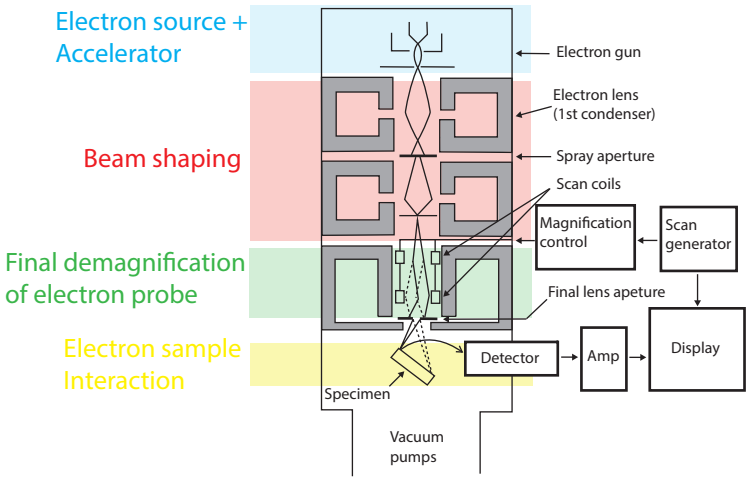

Figure 3: Example of caption

Densitometric

In order to create contrast between the different features of the sample there is need for a signal need to distinguish them. The BSE's and SE's give us different information about the specimen.

The physical property of being depicted in the imaging using BSE's are the mass of the atoms in the specimen/the chemical composition. Heavier atoms backscatter electron more strongly and they will therefore have more energy. Brighter spots on the image of BSE will therefore indicate areas with more heavier atoms. The contrast in the image is therefore used to identify areas with different chemical composition.

The secondary electron energy is linked to the incline of electron beam into the sample. If the beam enters the sample from an angle <90 degrees the interaction volume will be more exposed to the surface of the sample, making more SE's escape the specimen surface and being picked up by the detector. The SE's are counted and the increased number of them at inclines will give a brighter pixel in the digital image.

Sample preparation is important for non-conductive samples since it is otherwise a risk that the electron beam will build up a charge on the surface of the sample. This charge will start to deflect the incoming electron beam making it impossible to scan the sample properly. By sputter a thin layer of conductive metal onto the specimen this can be avoided. Typically is gold, platinum and iridium.

If an image is noisy or show low contrast then there have not been enough electron probe current to get a smooth noise-free image.

calibration, scanning same "sensor" for all x,y coordinates of the sample,

Geometrical

Scanning electron microscopy relates to the "real world" differently depending on if the information from BSE's or SE's are used. The energy of the BSE's are closely linked with the atomic number of the material the electron hits. The BSE's give an image of how the chemical composition is distributed over the specimen. Depending on the acceleration voltage used on the incoming primary electrons they penetrate differently deep before being backscattered. If the interest lies in the specimen surface it is important to use a acceleration voltage that make the primary electrons interact in the surface of the specimen.

In the case of SE's are the energy of the electrons dependent on the inclination of the point in the sample/specimen were the electron beam is hitting. The different intensities of the SE's give topographic contrast imaging. This is caused by the interaction volume having more escaping electrons in the inclined case than the normal case.

The resolution or the smallest observable feature in a SEM image is typically around 0.5 - 1.5 nanometers. This resolution depends on several things, including electron beam size and the interaction volume with the specimen. The beam size can be controlled with an aperture or with the condenser lenses but this has an negative effect on the beam current resulting in long exposure times. The resolution can be increased by reducing the wavelength by increasing the acceleration voltage. When increasing the acceleration voltage one must keep in mind that the electrons will penetrate deeper into the specimen before reflecting back as BSE's generating more SE's on the way. This will make it impossible to get a detailed image of the surface of the specimen. It is also an increased risk of surface charging if the specimen is not conducting. Building up charge on the surface will deflect the electron beam making it hard to get a clear image. Pre-treatment can be used to avoid this phenomenon. An image with BSE's has lower resolution since they originate from deeper within the sample.

The focus of a scanning electron microscope is intended to where the electron beam has the smallest diameter as it improves the resolution. When making the beam narrower it increases the angle of the cone of where the focal plane is. The increase in this angle makes the depth of field shorter. In SEM imaging the depth of field can be increased from a few microns to several millimeters.

Distortions such as astigmatism in the resulting image is the result of imperfections in the lens system. When increasing the magnification the effect get more apparent. The resulting image distorts round shapes so that they appear elliptical. This can be corrected for by manually (or use the automated correction) adjusting the stigmator control in order to get better results.

Spectral

SE's BSE's X-ray

Temporal

Exposure time, sample size, electron beam diameter,

5 Light microscopy

Introduction to light microscopy. This chapter will cover: simple and compound microscopes. The general concepts of

- resolution
- magnification
- staining

It will also cover: Darkfield, phase contrast, Fluorescence and confocal microscopy.

History

The father of microscopy: van Leeuwenhoek, he studied Hooke and refined his lenses to create a 1-lens microscope with approximately 270 times magnification. He used this to study insects, blood cells and bacteria.

Simple microscopes/magnifying glass

These types consist of one convex lens that enlarges the object. The magnification can be calculated with: $M = 1 + D/F$, where D is the least distance of distinct vision and F is the focal length of the convex lens. The maximum magnification that can be achieved this way is 10x.

Compound microscope

This type of microscopes are often referred to as **brightfield** microscopes. The concept: light from the light source is focused by a condenser onto the target/specimen. The transmitted light is then collected by the objective and forms a magnified, primary image. The primary image is magnified another time by the ocular lens. The total magnification of a compound microscope is given by the magnification of the ocular lens times the magnification of the objective lens.

Parts of a (typical) compound microscope

Light from the light source/illuminator goes through the condenser lens which focuses all the rays of light onto the specimen to maximize the illumination of it. By opening and closing the **diaphragm** between the condenser and the specimen it possible to adjust the amount of the light hitting the specimen. It is also possible to control the brightness of the light source with a **rheostat**. The light that hits the specimen is then differentially transmitted, absorbed, reflected or refracted by the different structures in the specimen. The **objective lens** collects the light and creates the magnified image which is magnified once again by the **ocular**.

The total magnification

The total magnification of the compound microscope can be expressed: $M_o \times M_e$, or alternatively :

$$m = \frac{D}{f_0} \times \frac{L}{f_e} \tag{3}$$

Where D is the least distance of distinct vision (25 cm), L the length of the microscope tube, f_o focal length of the objective lens and f_e the focal length of the eyepiece/ocular.

It is the light and optics that define the possible resolution, expressed:

$$d = \frac{\lambda}{2n \sin \theta} \tag{4}$$

Where d is the **point resolution** (the short the better for us), λ which is the **wavelength**, n the **refractive index** (1 for air, 1.3 for water or 1.4-1.5 for oil). $n\sin\theta$ is the **numerical aperture**.

Numerical aperture

The numerical aperture defined as: NA $= n \sin\theta$ where n is the refractive index for the medium between the specimen and the objective. The angle θ is the angle of the light cone. A high NA means that the objective collects light efficiently, means that θ is large. A high NA also means high resolution.

Resolution

Resolution, the smallest features that can be distinguished in the system. In a typical microcscope the resolution is decided by the wavelength of the light λ and the **resolving power** of the microscopes objective, which is defined by its numerical aperture NA. Somethings to consider: the maximum NA for an objective in air is 1. The resolution limit is given by the **Rayleight criterion**:

$$r = 0.61 \times \frac{\lambda}{NA} \tag{5}$$

An example using a wavelength of 550nm and objective with a NA of 0.7 gives a limit of 480nm. A cell is in the size of 10-30 μm and can therefore be observed in a light microscope. Viruses are on the other hand even smaller, $\approx$ 10-200nm and cannot be resolved by a ordinary light microscope.

With oil immersion its possible to increase the magnifications. This is possible thanks to the refractive index of oil is higher than air and similar to the glass that the specimen sits on. More light can be collected this way.

Remember

Magnification without resolution is useless: empty magnification

Airy disc

An Airy disc is the optimally focused point of light that can be determined by a circular aperture in the case of a perfectly aligned system limited by diffraction. If we view this from above it appears as a bright point with ripples around, also know as the airy pattern. The diffraction pattern is determined by the wavelength of light and the size of the aperture which the light passes through. The way a system images a point is called the **point spread function** or PSF for short.

Staining

Staining the sample is done to create contrast and or highlight details of interest. One very common stain is **H&E - Haematoxylin and eosin**

Immunostaining

With this type of staining are primary antibodies used that can bind to specific targets. There is also often a secondary antibodies that bind to the primary ones. The secondary antibodies have a dye or fluorescence attached to them or an enzyme that induces a coloring reaction.

DAB staining is a derivative of benzene and most often used in immunohistrochemical staining as a chromogen (a colorless chemical compound that can by reaction turned into colored). In DAB staining is DAB oxidized by hydrogen peroxide in a reaction typically catalyzed by HRP. The DAB forms a brown preciptate ? at the locations of the HRP, this can be visualized by a light microscope.

Typical immunohistochemistry staining procedure

Step by step:

1. Endogenous peroxidase are inactivated with hydrogen peroxide
2. Then are the antigen exposed with HIER (heat induced antigen retrieval)
3. Non-specific bindings sites and Fc repecptors get blocked by a normal serum (goat serum)

4. The primary antibodies are applied and bind to the exposed antigen in the cells and cannot bind to any of the blocked ones.
5. Then is the secondary antibody applied which got peroxide polymer attached bind to the primary antibodies.
6. In the last step are the peroxidase developed with DAB and hydrogen peroxide which gives dark brown deposits at the site of the bound antibody.

Possible issues - immunolabeling

- High background
  - Unspecific binding
  - Endogenous peroxidase
  - Excess peroxidase not washed away
- Weak signal
  - Not exposed antigen
  - Not good antibodies
  - Not enough time for reaction

Useful to compare the result with correct negatives, correct positive and false positive references. Other sources of errors can be that the sample is old, the chemical / solutions are old or wrong protocol is followed ?.

Dark field microscopy

Microscopy in which the central part of the light cone from the source is **blocked**, the only light that reaches the objective is light that has been refracted or reflected by the structures in the specimen (scattered light ?). This means that **no direct** light from the condenser enters the objective lens. The result has good contrast and resolution without any staining. This means that the technique is good for live specimens that otherwise would have been killed by the stains.

Phase contrast microscopy

Invented by Fritz Zernike in 1941. Uses the property that light phase shift when passing through a transparent specimen, different refractive index of different constituents turn into amplitude changes that creates the contrast in the image. This technique doesn't need any stain, no fixation, and is good for live cells. Good option for imaging objects with no color.

Differential interference contrast microscopy

Invented in the early 50's. It is a technique with good contrast without staining, for transparent objects for an example. It uses polarized light and interferometry. The contrast is created due to the differences in path length for two adjacent points in the sample. It can image small differences in the refractive index between neighboring points.

Reflected light microscopy

Also known as episcopic illumination metallography microscopy. Typically used for imaging the surface of metals, plastic, ores, ceramics, paper and so on. Same principle as but here are mirrors used to reflect the light onto the object.

Confocal microscopy

First patented in 1957. The method was improved with laser technology and became generally accepted and a very popular technique. A confocal microscope focus on one point at a time and can scan across the specimen in x,y **and** z direction. The results are 2D images at various depths which can be reconstructed

/ stacked to form a 3D image. Flourescent stain are often used to increase the contrast and resolution. The image clarity and resolution is improved by a narrow aperture that eliminates light that is not from the z-plane. This technique is useful for examining thick specimens and can also be used on live, unfixed samples.

Fluorescence microscopy

A technique that uses fluorescent molecules **fluorochromes** or **fluorophores** that absorb energy from a light source and emits the energy as light of a different wavelength. There are natural fluorecents (chlorophylls) and fluorescent stains that are added to the specimen to create contrast for the image.

The principle behind fluorescent microscopy:

- High energy light source emit light
- An excitation filter decides what wavelength to let through to illuminate specimen
- The fluorophores in the specimen absorb the energy from the light and emit energy with a longe wavelength.
- A dichroic mirror reflect the light of the excitation wavelength and tramsit light of the emission wavelength, so only the light of interest passes.
- This mirror is not perfect so not all light is blocked.

Fluorophores

In microscopy are most fluorophores excited near UV,blue or green light. A mercury arc lamp is an ideal light source that provide high intensity in the wavelength : 365, 405, 436 and 646 nm.

Stoke shift

The stoke shift is the energy difference between the peak of absorbance and the peak of the highest emission energy. Without the stoke shift it wouldn't be almost no way of distinguish between excitation and emitted light. Probes(?) with varying Stoke shift are useful for multicolor applications.

Autofluorescence

Autofluorescence is the natural emission of light by biological structures such as mitochondria and lysosomes after they absorbed light. This is then used to distinguish the light from the artificially added fluorescent markers (fluorophores). ?

green fluorescent protein

Green fluorescent protein also know as GFP exhibits green fluorescence when it is exposed to light in the UV range. GFP was first isolated from a jellyfish. This GFP has a major excitation peak at 395nm and a minor one at 475nm while the emission peaks at 509nm. This GFP gene is frequently used as a reporter of expression ?. The gene is inserted together with the gene of interest and if the GPF is expressed then we can assume the gene of interest is that also.

Immunofluorescence

Fluoochrome attached to primary or secondary antibodies can be used to visualize the location of targets. This is used a lot in cell biology.

2- or multi-photon microscopy

here the fluorophore is excited with 2 or more photons of lower energy (if two half excitation), hitting it simultaneously. One can use pulsed lasers for this and there is no need for pinhole since the emission will only happen from the focus spot. The advantage of this technique are the reduced phototoxicity and increased image depth.

6 Astronomical imaging: Telescopes and Doppler imaging

Astronomers need telescopes to collect photons from planets, stars and distant galaxies, track the objects on the sky and to create the image of a region on the sky. They strive to limit the FOV "Field of view" which can be done with large primary mirrors in the telescopes. Some examples of large telescopes:

- ELT, 39m
- LBT 2 × 11.8m
- Keck I & II 2 × 10m

All newer telescopes are tracking the objects on the sky with the Alt-Azimuth mount. Other mount are:

- German mount
- Fork mount
- English mount

Imaging: the points spread function

An image of a Point Spread Function, "PSF" created by a telescope is not a point due to diffraction. The intensity distribution in the focal plane produced by a point source located at infinity is the PSF. The ideal PSF for a circular mirror takes the shape of the Bessel function.

Image bessel

The resolution or the width of the peak is defined by the ratio of wavelength to the diameter of the telescope. So if we increase the diameter of the mirror it reduces the width of the peak. Using the sampling Theorem:

Whittaker-Nyquist-Kotelnikov-Shannon sampling theorem

The sampling frequency must be at least twice the highest frequency of the signal.

(Which has many names due to many worked with the problem in many different fields) gives us the minimum spacing between two Gaussians with a given width so we can tell them apart.

$$\text{PSF} \propto \frac{-x^2}{e^{2\sigma^2}} = \frac{1}{2} \Rightarrow x \equiv \delta = \sqrt{2\ln 2}\sigma \text{ and the separation } = 2\delta = 2\sqrt{2\ln 2}\sigma \approx 2.355 \cdot \sigma \tag{6}$$

The magical number in astronomy is therefore: 2.355 for sampling. This is important for making sure that we use a sensor that doesn't have to large pixels or smaller than necessary.

Resolution limit

Details that are smaller than the diffraction image of the telescope are smeared out, so after data reduction are anything smaller than $\frac{\lambda}{D}$ an artifact. So, is it possible to do better than the limit set by : $\frac{\lambda}{D}$, can we resolve features of distant objects smaller than the diffraction limit? YES!

- Interferometry
- Doppler imaging

Using data from two telescopes we can try to overlap the images with an adjustable optical path way such the difference in optical path is zero (exact to the fraction of the wavelength). This result in the characteristic interference pattern: fringes which has amplitude and phase that we can analyze and use. By doing this its possible to create larger telescopes (but its not perfect). The fourier harmonics of the data gives us 6 points when using 4 telescopes. When the earth rotates it changes the projected baseline so it must be corrected during the night.

Using this technique made it possible to see the changes of the star Betelgeuse intensity change during 2019 in greater detail. With interferometry is the angular resolution limited by the ratio $\frac{\lambda}{B}$ where B is the the distance between the telescopes projected on the line of sight, see figure: fix ref to pic

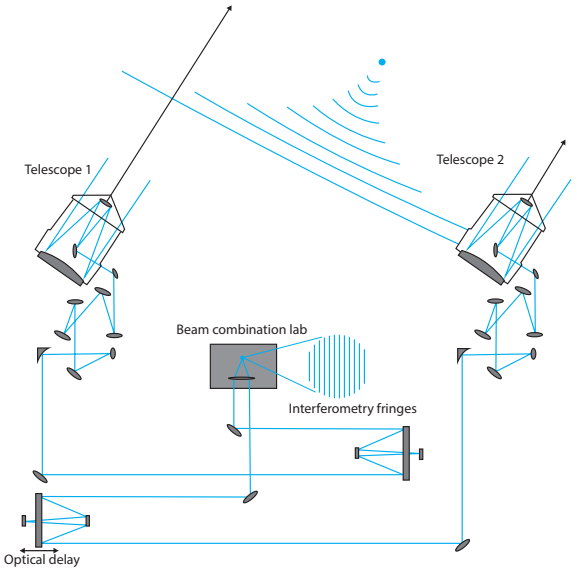

Figure 4: Example of caption

Radio interferometers are easier since the wavelength is longer making it cheaper to build and use. Here we can digitize the signal and transmit it with fiber cables instead of highly accurate mirrors and trolleys. The correlation between the telescopes can be done in supercomputers so its just to move the telescopes around and keep track of the position of them. This means that the array can be made more compact, not loosing a lot of flux.

Doppler imaging

During the 1900 century astronomers discovered a class of stars that we call "Peculiar". They have a chemical compositions of a lot of rare-earth metals which is unusual. The spectra of the star also showed a periodic variability. After systematic observations of these changes, it became clear that it was not only the strength of the spectral lines that changed but also the shape of them. It later became clear that these stars are fast rotating and have chemical spots on the surface.

These spots of chemicals gives a Doppler effect when rotating around the star, blue shift when the cloud moves towards the observer, the spectrum then appears to be broaden. Setting up the **forward problem** some assumptions were made:

- Each observed spectrum is taken during the time that is much shorter than the rotation period
- The surface structure doesn't change during the observing run
- The rotational broadening is resolved by our instrument: spectrometer.
- The star rotates as a solid body

Continuing with setting up the formulation of the forward problem:

- The telescope and spectrometer measure the flux coming from the star at a given wavelength and phase:

$$F(\lambda, \phi) = \oint I(\lambda + \Delta_m^{\mathrm{Dop}}, m) \cos\theta_m d\sigma_m \tag{7}$$

- Intensity I can be computed from a physical model of the stellar atmosphere locally parameterized by chemical composition
- Changes in composition along the stellar surface change the emerging I

The algorithm:

- For a given distribution of abundances, compute intensities for each surface element at each phase (non-linear)

- Integrate the intensities over the disk to obtain fluxes while taking into account Doppler shifts for all phases(convolution)
- Compare the synthetic spectra with observations
- Adjust the map(s) of abundance

This is an ill-posed problem and belongs to the class of integral equations: **Fredholm equations**. The properties of the DI kernel makes the problem ill-posed. Take as an example, if we would add a chess pattern to the surface with small/unresolved squares, it would not change the observed spectra? meaning?

Remote sensing problems

Doppler imaging is an example of remote sensing problems. These types of problems arrise when it is impossible or impractical to reach the actual target and measure it properly. What we do instead is to measure other properties that are related to what we want to measure with linear or non-linear transformations. Many of these problems are ill-posed (interferometric image reconstruction is an example). The solution to this is to introduce regularization.

Solving an inverse problem we need:

- Assume an initial guess for the abundance map
- Compute the local line profiles
- integrate over the disk for each of the rotations phases, taking Doppler shifts into account
- Find the correlations to local abundance that minimize the functional:

$$\sum_{\lambda,\phi} \left[F_\lambda^{\text{obs}}(Z,\phi - F_\lambda^{\text{calc}}(Z,\phi)^2\right] + \Lambda \cdot \mathrm{R}(Z) = \min \tag{8}$$

- Loop from the second point until convergence is reached. Example of $\mathbf{R}$ is Tikhonov regularization:

$$\mathbf{R}(Z)) \sum_{\text{surface}} (\overline{\nabla} Z)^2 \tag{9}$$

Extension of Doppler imaging

Doppler imaging is also used for monitoring active regions on solar-types starts (solar spots) where temperature variation influences line intensity differences. Can also be used for binary systems which is eclipsing (eclipsing binaries) were the geometry becomes more complex due to the eclipses. Can also be used for medical imaging such as computer aided tomography.

Conclusion

The inverse problems are used to infer information about an object that is not possible to measure directly. The solution in these cases are not often unique. For such cases we replace the problem with a regularized inverse problem. There exist various forms of regularization.

7 Transmission Electron Microscopy - TEM

The big data in TEM. A sample is only a couple of mm big and not the whole sample is examined, just tiny parts of it. It is therefore important to remember that what we are observing in TEM is only a small small part of the sample. If we were to examine the whole sample (3mm in diameter) it would generate approximately 29Tb of data.

What is it used for?

When we want to observe something at nanometer level the only way is to use electron microscopy. Other methods may offer some quantitative information but for looking directly we need electron microscopy. Some examples of applications are:

- Biology
  - Drug development and quality control
  - Diagnosis
  - Treatment planning
  - Understanding diseases
  - localization
  - Protein structures, create 3D maps
- Material science
  - Chemical compositions
  - Structures
  - Characterization
  - Quality control and material development

TEM systems are bulky

TEM systems are bulky and expensive systems that need expert skilled personnel to prepare the samples and operate the equipment. The facilities are adapted for the system and need many restrictions. Separate cooling and 380-400V 3 phase need for the system, makes it hard to place a system without careful planning. Sensitivity to vibration makes need for special flooring. The maintenance of the system of expensive requiring planned down time. Unplanned downtime increases the cost of the system. With the system comes also the need for careful waste handling since many samples can be toxic or covered with radioactive salts that are typically used to protect the samples during exposure.

The anatomy of a TEM system

Modern TEM system is looking more like a fridge/freezer combo than the previous generations that are illustrated below. Much similarity to an optical microscope, the lenses are replaced with magnetic lenses but the principle stays the same. Field emission is the most common electron source.

Types of TEM

Since the principle stays the same we have Brightfield and Darkfield imaging in TEM also. In Darkfield TEM we get enhancement of inorganic NPs that are easily hidden in Brightfield, Brightfield more used in biology. Mass contrast is another type of imaging from TEM that we dont see in optical microscopy. With mass contrast we can see contrast between regions consisting of different z-number. It is the objective aperture that creates the mass/z-contrast. Low mass gets more electrons through, more mass scatter more electrons which gives contrast.

Sample preparation

Sample preparation is very important since we are blasting electron through the sample to create an image. To to thick sample will now allow any electrons to pass through. Preparing a sample of a hard material often involves making a wedge shaped piece of the material where the thinnest part which is used need to be 5 to a few 100 nanometers thick. For **thin film** samples are the thin film of interest placed on top of a Ni/Ti film which sits on top of a Si substrate. Then is the process (similar with cross sections samples):

1. Grind away some of the substrate
2. Make a dimple in the substrate
3. Bombard the dimple with ions to make it thinner and brake the sample into two pieces
4. Image the sample

Stain such as heavy metals salts can be used to cover biological samples that otherwise would collapse of the electron beam. Gives the negative image of the object since we stain the background, improves contrast against the background to the biological sample that is otherwise poor. Unstained specimen is drying and suffers of deterioration which also affect the image. Embedding the specimen with the stain preserves the integrity of the sample.

nsTEM workflow

When working with liquid biological samples the workflow could look like this: Electron microscope grid is placed in a **Glow discharger** which makes the liquid sample able to cover the grid more evenly (reduces surface tension ?). The sample is placed on the grid with the excess blotted away, a wash of ultrapure water is applied with excess blotted away after which the stain is applied (excess blotted away). This process requires skill and patience, doing it once might not be that difficult but repeating it equally good each time is an art. Some problems that could arise are: too much or to little stain, dehydrated grid areas, stain deposits or stain crystals.

Ultrathin biological section preparation

When working with biological samples like cells it is important to work fast since it start to deteriorate as soon it comes contact with air. It is therefore fixed with **chemical fixation** after which a heavy metal **stain** is used to increase contrast, it is then **dehydrated** and **resin embedded**. A cell is typically to large to be observed in a TEM whole and must be sliced down using **ultramicrotomy**. When sliced it can be stained using **antibody** stain in combination with heating if needed. A secondary antibody stain connected with gold is then applied before putting it into the TEM system.

CryoTEM workflow

When using cryoTEM it is possible to prepare and observe the sample without using toxic heavy metal stains. The process of preparing the sample starts as the previous with placing the EM holder in a **glow discharger** to make the sample spread better on the grid. Instead of applying the stains it is plunged freezed (to avoid crystals forming). After it is stored in different ways until its observed in a cryoTEM.

Pros and cons nsTEM and cryoTEM

nsTEM

- Straight forward method
- Allow to observe particles in diluted samples
- Debris and background easy to identified
- Grid/sample can be observed at multiple occasions
- Discrimination between filled and empty particles poorly reliable, "don't see inside"
- Preparation of specimen may alter particle morphology and integrity
- Artifacts from preparations common to observe

cryoTEM

- Specimen is observed close to its native state
- Possible to observe internal parts of specimen
- Discrimination between empty and filled particles good
- High resolution of structural information
- Tedious preparation
- The grid can only be imaged once
- Most work with low electron dosage to avoid damaging the specimen

8 Ultrasound

Ultrasound (and all other types of sounds) are acoustic waves, i.e. a mechanical perturbation traveling in a medium. The acoustic frequency range:

- Infrasound: < 20 Hz
- Audible sound: 20 Hz - 20 kHz
- Ultrasound: > 20 kHz
- (Medical ultrasound: 1 - 20 MHz)

Ultrasound is therefore mechanical oscillations with a frequency above 20 kHz that propagates through a medium that is elastic. Used for finding fungus in trees, used by bats to find their prey and in quality control of weld joint for finding cracks to give a few examples. The discovery of the piezoelectric property made this technique possible.

Ultrasound in medical imaging

Ultrasound is one of the most used imaging techniques in medicine and have an annual market growth of 3-4%.Pros for this technique are that is non ionizing, need minimal safety requirements, its real time, can be made portable and is quite low cost. The downside with real time is the need of training personnel to understand/interpret the image while making the examination. Signal to noise ratio is quite low compared to other techniques and it need a acoustic window to operate (can't "see through" bone).

Probe - probing mechanism

Ultrasound is a longitudinal wave, variation of pressure that propagates, this mean that it needs a medium to travel through. The speed of sound is a function of the medium:

$$c = \sqrt{\frac{K}{\rho}} \tag{10}$$

Where K is the stiffness, stiffer the faster and ρ is density: denser the slower. Like electrical circuits with resistance and current, the propagation of sound can be calculated with the Acoustic impedance:

$$Z = \rho c \tag{11}$$

Material	bf**Density (kgm^{-3})**	**Speed of sound**	**Acoustic impedance (kgm^{-2}s^{-1} $\times 10^6$)**
Air	1.3	330	0.000429
Water	1000	1450	1.50
Bone	1500	4000	6.0
Blood	1060	1570	1.59
Muscle (average)	1075	1590	1.70

Table 2: Example of a table with line breaks

The **wavelength** $[m]$ is determined by: **Frequency** $[Hz]$ (depends on the source) and **Sound-speed** $[m/s]$ (depends on medium).

Matter interaction

After the sound wave left the source (probe) it interact with the material with the tissue, being **back-scattered**, **reflected** and **refracted**. The sound wave is attenuated while moving through the tissue.

When the wave travels into a new medium the impedance of the medium and the incidence angle determines what happens to the wave (Huygens principle). If the wavefront is perpendicular to the medium the wave-length will change. If there is an oblique incidence angle it **refracts** giving a **reflection** against the new medium and **transmitted** wave through the new medium. The intensity of each part is dependent on: the **acoustic impedance difference** between the two materials and the **angle of incidence**.

Scattering is the directionless reemission of incident energy caused by local inhomogeneities. Inside tissue, this scattering is caused by "tiny" particles, cells, large proteins, calcifications and so on. The interaction between this scattered waves is what causes the noisy speckle texture pattern in ultrasound images.

Attenuation is the loss of energy of the wave in the material. This energy is lost over distance due to **absorption**, **scattering**, mode conversions ? and more. The amplitude decay increases with the depth (z) as a function of **some tissue properties** (a_0) and **acoustic frequency)**(f)

$$A(z) = A_0 e^{-a_0 f^n z} \tag{12}$$

The tradeoff

The higher the frequency, the better the imaging resolution
But, the worse the penetration depth

Piezo-electric effect

The purpose of the ultrasound transducer is to convert between electrical signal and acoustic energy. It act as both speaker and a microphone. By first emitting a very short sound pulse and then listen to the returning echoes (for a longer time then the emitting time). It is only possible to to one at a time.

The piezo-electric effect is a property that certain solids can have (some crystals and ceramics). Expose the material for electricity and there will respond with mechanical stress (like a speaker). Swapping places and expose it for mechanical stress will produce an electrical current (like a microphone)

Image production

Producing the image involves switching between Transmit (Tx) mode : active (speaker) and Receive (Rx) mode : passive (microphone). There are different modes for the imaging also:

- **A(mplitude)-mode:** one scan line
- **B(rightness)-mode:** 2D spatial images with multiple neighboring A-lines
- **M(otion)-mode:** sequences of A-modes in time, thus 2D (popular in cardiology)

Beamforming is the technique used to form sensor arrays for directional transmission and reception. By adjusting a time delay between element it is possible to mimic the spatially focus of a curved transducer.

insert US focus illustration here

Image quality

Contrast is what assesses visibility, so by comparing the image appearance between two distinct regions we want to be able to distinguish between object and background. The spatial resolution is the minimum distance at which is possible to distinguish two distinct point individually. The resolution of the ultrasound imaging system can be separated into **Axial resolution** and **Lateral resolution**. Axial resolution in which separating two reflectors or scatterers along the beams axis. This resolution depends on the length of the pulse, which need to be short for good axial resolution but needs to be long to deliver sufficient power for good signal to noise ratio. The lateral resolution separating reflectors side by side. This resolution depends on the focusing/beamforming.

Artifacts in ultrasound images are not uncommon and can even be used to interpret underlying causes some times. Examples of artifacts are: **Shadowing, speed-of-sound abberation, mirroring, reverberations and refraction**

Safety

In comparison with many other imaging systems in medicine, minimal. There are however four different metrics of safety that is regulated:

- **Thermal index** (TI): quantifies/limit the acoustic power to avoid overheating the tissue internally
- **Surface temperature**: limit how hot the probe can be, to avoid burn injuries
- **Spatial peak temporal average** (SPTA): limit the cumulative power at focus over time
- **Mechanical index** (MI): limit peak negative acoustic pressure to avoid cavitation and streaming

Other ultrasound imaging techniques

With **Harmonic (b-mode) imaging** method we transmit a signal at a frequency f and then listen at frequency 2 times f. Comparing this to the fundamental B-mode we here get better resolution and reduced noise. **Doppler** technique uses the shift in of frequency relative to the motion of the object, compare to red and blue light shift in optics. With this technique its possible to quantitatively predict the blood flow velocity for an example. **Functional ultrasound** (fUS) can be seen as an extension of Doppler imaging and is used to examine brain activity (blood flow in the brain) with high frame-rate. **Elastography**, due to pathological process there may be changes in stiffness of the tissue, this technique estimate the tissue biomechanical characteristics. **Strain imaging**, since a stiff tissue compresses less it possible to detect differences inside a tissue by compressing while imaging, then measure local displacement and look at relative values/axial derivative ?. **Acoustic Radiation Force** (ARF) uses focused acoustic energy to exert force in tissue by pushing it, remotely and locally, also known as "remote palpation". **Shear-wave Elastography** (SWE), here ultrasound is used to generate a **shear-wave** and then the temporal propagation is observed on consecutive ultrasound frames to infer the shear-wave speed. Stiffness difference in tissue can be observed that may indicate pathology. **Ultrasound computed tomography**. **Optoacoustic imaging** combines the use of the light pulses and ultrasound. Here is a light pulse transmitted and ultrasound received. The thermal expansion in the tissue creates the sound.

9 PET, CT and MRI

All of these techniques are so called tomography techniques (imaging slices of the sample). They are used in both clinical routine and in research. In common that they are: expensive, risks involved in using them (Radioactivity, Ionizing radiation and strong magnetic fields). The geometrical accuracy is good for all of them with some exceptions for MRI. Can perform both static and dynamic imaging (time series). They share the challenges of tunnel size (PET/MRI and MRI). Much development is ongoing, PET tracers, hardware and software.

PET - Positron Emission Tomography

Uses a **tracer** or radio pharmaceutical that is emitting positrons. This radionuclide isotope is attached on a molecule of interest, glucose for an example in Fluorodeoxyglucose ^{18}F. The doses are small (microdosing) and there is no expected pharmacological effect of the patient. The PET systems create an image of the concentration of tracer. There are many different types of tracers, and more under development. Depending on what is needed to be imaged or what is being looked for the tracer is chosen. The images are typically of quite low resolution and don't give much anatomical information, it is therefore most often combined with MRI or CT.

Workflow

The unstable isotope is created in a cyclotron often close to the PET facility, since the halftime is often short. It is then combined with the molecule of interest and we have the tracer that can be injected into or in some cases inhaled by the patient. The tracer emits positrons (beta particles) that can only travel short distances (mm) before it annihilates with an electron and create two 2 gamma rays shooting of approx 180 degrees apart. The detector rings picks up these gamma rays (Coincidence detection) and counts them, giving a line of response (LoR). This results in a**Sinogram** that is reconstructed with reconstruction algorithms including corrections for **random counts, attenuation and scatter**.

Use cases

PET scanning is used to diagnose cancer, dementia and cardiac problems. It is also used in drug development. Examples of some measurements are:

- Glucose metabolism
- Blood flow
- Heart function
- Bone formation
- Receptor expression ?

Tracers and isotopes

There are many different types of tracers for different kind of research and diagnosis purposes. Here are some to mention a few:

- ^{18}F-FDG used in oncology and neurology
- PSMA (prostate cancer)
- FLT (cancer)
- PiB (Alzheimer's)

The isotopes commonly used and their **half time (min)**:

- ^{82}Rb-Rubidium **1.3**
- ^{15}O-Oxygen **2**
- ^{13}N-Nitrogen **10**
- ^{11}C-Carbon **20**

Scanning

Three modes of PET scanning: Static scans with about 20cm of coverage, Dynamic scans: time series of scans and Whole-body scans: combination of multiple 20cm scans.

Reconstruction

Two types of image reconstruction: Filter back propagation which can be seen as fast and simple and Statistical likelihood-based which is iterative, uses more advanced modelling and corrections. The later one is therefore more computationally demanding. Corrections for scatter and random coincidences are typically included.

- True Coincidence
  - One annihilation
  - Straight path of the photons in opposite direction
- Scatter coincidence
  - One annihilation
  - Photons scatter
  - Measured line of response places annihilation reaction along artifact projection
- Random coincidence
  - More than one annihilation
  - Photons from different annihilation detected at same time
  - Artifact line of response

CT - Computed Tomography

This technique uses X-rays that are sent through the body from different angles by rotating X-ray source and detector around the body. The X-rays are attenuated differently by different tissues and an image can be reconstructed from this "attenuation map". Arms up during the scan gives better image quality. It is a axial technique that is fast and high resolution. There are variations of it:

- High and low dose
- Contrast agents: intravenous or oral

Tissues have different attenuation, uses the Hounsfield scale. Calibrated with water = 0.

Tissue	**Hounsfield unit (HU)**
Bone	>400
Organs	-30 +150
Water	0
Fat	-190 -30
Aire	-1000

Table 3: example

Use cases

CT scanning is used for diagnosis and follow up in **Cancer**, Acute medicine such as **trauma**, **strokes** and **hemorrhage**, **Surgery** (planning: inflammatory disease) and **Cardiac** function (requires fast machines).

Scanning

The scanning is always done in axial plane. The result is of high resolution Sinogram that needs reformatting afterwards. Alternatives are :

- Axial (can be preferred for brain scan)

- Spiral (Helical scan)
- Single or multi slice (16/64/128), multiple detectors instead of 1 long.
- Dual-energy CT

Photon counting CT

There has recently been a major breakthrough in CT imaging thanks to photon counting sensors. These makes it possible to make smaller detector pixels that improves spatial resolution. Intrinsic spectral sensitivity: multi energy information, results in lower radiation exposure since there is lower electronic noise. Example of slice thickness: 0.2mm

MRI - Magnetic resonance imaging

Previously called: Nuclear Magnetic Resonance - NMR. The pros of MRI is the good soft tissue contrast, it uses no ionizing radiation and is very versatile: many different contrast mechanisms, used from morphology to physiology down to metabolism, can image angled slices. The Cons of the MRI systems are that they are: time consuming (examination time, patient motion problem), it is expensive, claustrophobia and competence need.

What is imaged

The hydrogen "protons" have a property called **spin** that is used for the creation if the MR images. Spin is a quantum mechanical property, something that spins have angular momentum. Isotopes that have an odd number of protons or neutrons have a spin that is non zero. These can be studied by MR, they are usually : ^{1}H or (^{13}C, ^{31}P, ^{3}H, ^{129}Xe). Hydrogen is the most common atom in the human body with spin.

Trade-off in MRI

To acquire an image with higher resolution means that it takes longer time if we want to maintain the same SNR, if we accept more noise we can do it with the same time. Since it can be required of the patient to hold their breath it might not be possible to change the time to much.

trade-off triangle image

General properties

The image elements are non-isotropic voxels and can be of the size: 1x1x6mm for an example. The intensities of the voxels are typically arbitrary units but can in some images be absolute. There are like in other imaging techniques artifacts. In MR there are often: intensity inhomogeneities, motion artifacts ans geometric distortions.

Spin, Precession, Larmor frequency

The hydrogen (proton) spin with a **Precession** (Like the earth has an angle in its rotation). This correspond to a frequency, the **Larmor frequency** which depends on the $\mathbf{B}_0$ field: 64MHz at 1.5T and 128MHz at 3T. We can think of this "spin" as small magnets. Even though we apply a strong magnetic field, the net magnetization $\mathbf{M}_0$ is very small, a few ppm compared to the unordered state without the magnetic field $\mathbf{B}_0$ applied. Increasing $\mathbf{B}_0$ increases the this magnetization $\mathbf{M}_0$.

Excitation and relaxation

Hitting the spins with a radio wave with the **Larmor frequency** changes the net magnetezation into a state of **Excitation**, the spins precess in phase. Enter a state with **Longitudinal** and **Longitudinal**-magnetization (can detected). After that comes **Relaxation** where the "echo" is picked up by RF-antenna.

Image contrast

"When and how the MR signal is measured determines image contrast "
"The whole process is repeated many times to collect the data needed"

T1 and T2 relaxation

The Longitudinal relaxation, when M_z increases is called **T1** relaxation. The transverse relaxation, when M_{XY} decreases is called **T2** relaxation.

- Difference in **T1** relaxation between tissues determine the contrast in **T1-weighted** imaging
- Difference in **T2** relaxation between tissues determine the contrast in **T2-weighted** imaging

Repetition time (TR), Echo time (TE)

By changing the scanning parameters TR and TE the weighting of the contrast for different tissues changes.

Weightings	**TE & TR**	
T2w	Long TE and long TR	Long T2 gives strong/bright signal
T1w	Short TE and short TR	Short T1 gives strong signal
PDw	Short TE and long TR	Many protons give strong signal

Table 4: example

INSERT TR TE IMAGE

T1 and T2 in different tissues

T1 and T1 can be seen as tissue properties, but they are dependent on: disease conditions (which is good for diagnostic), contrast agents (that are used for changing relaxation times), the field strength and temperature.

Image generation (2D)

Spatial encodingis used to determine what slice is being image and to determine what is up/down and right/left in that slice. The principle for spatial encoding is **frequency encoding** which can be described like choir that sing brighter the further away they stand from the observer, from this we can determine position from the notes (in one direction at least). This principle is used by adding a linear magnetic field gradient (**G**) to the static magnetic field ($\mathbf{B}_0$) in the scanner. By doing this, the total magnetic field and Larmor frequency will vary linearly with the position. **In-slice encoding** two directions are needed for encoding in the 2 dimensional slice, to do this simultaneously we use **phase encoding** also. The resulting signal readout repeated with different phase encoding results in a **k-space** image. Using the Fourier transform **FFT** we go from k-space to **image space**. To summarize: a combination of RF signals and a sequencing of linear gradients allow imaging.

Image generation (3D)

Comparing 2D and 3D imaging:

2D	**3D**
Many slices to be excited to cover volume	Two-phase encoding directions
K-space in 2D	takes time, many TR's
	Short TR (and TE) needed
	Mostly for T1w
	K-space in 3D
	Typically most time efficient for volume

Table 5: example

Multi-slicing, introducing the problem: A T2-weighted image used a long TR and TE, this means that there is a long waiting time to next excitation of the slice. This waiting time results in a lot of "deadtime" in the protocol. To be more effective was multi-slicing introduced. Additional slices are excited during the waiting time which means that the total scan time is reduced if TR increases (so that more slices fits under one TR).

Quantification of T1, T2 and PD

Instead of imaging with contrast weighting its possible to quantify the **relaxation parameters**. This is done by collecting data (multiple images) with different parameters, for example:

- For T2: different TEs
- For T1. different TRs

Then the signal **model** is fitted to the datapoints - voxel wise. This can be optimized in many ways, modelling and the data collection.

10 Thermal and multispectral imaging

Light and radiation

The light from the sun, created by the nuclear fusion in the core heating up the surfare that radiates towards earth. The radiation that we can see comes from light that is:

- Emitted
- Transmitted
- Reflected
- Absorbed

The thermal radiation for an object can be approximated as **black-body** radiation even though the thermodynamic system is not in equilibrium with its surrounding, this is done with Plack's law of black-body radiation:

$$B_v(T) = \frac{2v^2}{c^2} \frac{hv}{e^{\frac{hv}{kT}} - 1} \tag{13}$$

Where B(T) is the spectral radiance, h placks constant, k Boltzmanns constant, v is the frequency, T is the temperature of the body and c is the speed of light in vacuum.

Domain and wavelengths band

The bands are:

- ... X-ray
- Ultra violet
- Near infrared
- Shartwave infrared
- Thermal infrared
- Far infrared
- Microwave and radio...

Thermal infrared is divided into two bands: **Midwave infrared** and **Longwave infrared**, spanning from 3 μm to 12 μm. There is a gap in this division due to atmospheric transmission, between 5 and 8 μ. The division into these bands are because of the atmospheric transmission, behavior : reflective domain and missive domain and the type of sensors.

Radiation and matter

Incoming radiation interacts with matter in three ways: Absorbed, Reflected or transmitted. The matter can then emit radiation formed by processes of the absorption.

$$\begin{aligned} \alpha + \tau + r &= 1 \\ \alpha(\lambda) + \tau(\lambda) + r(\lambda) &= 1 \end{aligned} \tag{14}$$

Emissivity (ϵ), $\alpha = \epsilon$ for any object in thermal equilibrium with its environment.

- Absorptivity α
- Emissivity ϵ
- Transmittance τ
- Reflectance r

Dependent on wavelength and the angle. It is common that τ and r are close zero.

From object to sensor

The incoming energy is integrated over a certain bandwidth by the sensor. The radiance at the sensor is a sum of :

- Radiation **emitted** by the object and **transmitted** through the path to the sensor
- Radiation **reflected** by the object and **transmitted** through the path.
- Radiation **emitted** by the path to the sensor
- Radiation **scattered** by the path.

Summary: radiation & matter

- Radiators: Blackbodies, greybodies, general objects
- Properties: Emissivity, absorptivity, reflectance, transmittance
- Radiation: is often measured as flux, radiance and irradiance
- Domains: Reflective vs emissive
- Bands: UV, VIS, NIR, VNIR, SWIR, MWIR, LWIR, TIR, FIR

Thermal cameras

Thermal cameras are used for imaging (non radiometric) in the civilian and military. Radiometric thermal holds an actual temperature value for every pixel in the image. Non radiometric only contains a visual representation of the measured value of radiation for the specific time the image was captured.

Cooled and uncooled cameras

Depending on price and bands used for the camera can be either cooled or uncooled. The sensor material is different in uncooled and cooled cameras.

- **Cooled**
  - Mercury cadmium telluride - MCT - **SWIR,LWIR**
  - Indium antimonide - InSb - **NIR,LWIR**
  - Strained layer superlattice - **MWIR,LWIR**
- **Un-cooled**
  - Charged-coupled device - CCD - **VNIR**
  - Indium gallium arsenide - InGaAs - **NIR,SWIR**
  - Microbolometer - - **LWIR**

Pyro-electric detectors commonly used in presence detector. Microbolometer is common in industrial hand-held IR cameras.

Internal radiation

Much of the radiation that hits the sensor of the camera is emitted by the camera itself. Somewhere around 90% of the radiation is a realistic value. It's therefore necessary to have one or more internal thermometers and onboard processing of the signal. Cooled cameras come with the benefits of having better spatial resolution, higher temperature resolution and faster. The downsides are that they are: louder, heavy, larger and much more expensive.

Performance measures

Comparing different camera can be done using these performance measures:

- NETD: noise equivalent temperature difference
- MRTF: Minimum resolvable temperature difference
- NEP: Noise equivalent power
- Normalized Detectivity D^*

Calibration and Narcissus

The pixel of the sensor needs to be calibrated to give a linear response to temperature. A dead pixel can be either completely dark or white. Narcissus is an unwanted effect where the detector images the reflection of itself. Caused by reflections from lens surfaces. The effect comes from the detector cold shield.

Optics for thermal cameras

Different parameters to consider when choosing optics for a thermal camera:

- Durability
- Refractive index
- Variability due to heat
- Cost
- Transmittance

One common lens material is: Germanium. It's good for MWIR and LWIR, durable and high refractive index. Have the downside of drop of transmittance with temperature.

Summary: thermal cameras

- Cooled vs uncooled
- Sensors: Thermal detectors vs photon detectors
- Optics: Transmitting in different bands
- Most common thermal camera: Uncooled bolometer camera LWIR with Germanium lens.

Hyperspectral imaging

Hyperspectral imaging compared to greyscale, color image and multispectral have many, many contiguous bands. One pixel of the image can be interpolated to a complete spectrum and is therefore very useful to identify different materials for an example.

Applications

- Defence
- Mineralogy
- Land-use classification
- Precision farming
- Food inspection
- Environmental monitoring
- and a lot more....

Imaging concepts

- Dispersive
  - Whiskbroom
  - Pushbroom
- Pushframe
  - Spatio spectral
- Snapshot
  - Mosaic
  - Tiled

Filter wheels can also be used.

Sensor types

- Diffraction grating
- Interferometer
- Prism
- Continuously variable optical band-pass filter

Summary: hyperspectral

- Uses multiple wavebands to see better
- Recognize materials by one pixel
- Many ways of making hyperspectral cameras
- Many applications.

11 Super resolution microscopy

This chapter covers super resolution fluorescence microscopy for enhancing spatiotemporal resolution.

The microscope - short summary

The numerical aperture NA = nsin(θ), where n is the refractive index of the medium between the specimen and the objective lens. The resolution limited by diffraction, Abbe diffraction limit for microscopy: $d = \frac{\lambda}{2NA}$. In the 4-F system the magnification M is equal to: f_1/f_2

Sampling in microscopy

The Nyquist Shannon sampling frequency tells us that a signal should be sampled at least at a factor 2 of the highest frequency component of the signal to avoid aliasing. Important to know what the dynamics of the sample is to avoid blur and aliasing.

Microscopy concepts

There are different types of concepts in microscopy, here are a few listed:

- white light transmission
  - Phase contrast
  - DIC
- Electron microscopy
  - SEM
  - TEM
- Fluorescence
  - Widefield
  - Confocal
  - Super resolution

The problem in white light transmission microscopy is that it is quite unspecific in its labeling. Different proteins and structures/substructures cannot be identified/differentiated.

Fluorescence

For identifying proteins can fluorescence be used. Electrons absorb high-energy photon and go to higher energy state, undergo vibrational relaxation which means a loss of energy, this is called **Stokes shift**. It then undergoes a shift to lower energy state and emits a photon when reaching ground state. This is happening during a short time period of 0.5-20ns. **Phosphorescence** on the other hand is a process that occurs up to hours.

Proteins vs Dyes

Proteins such as Green fluorescent protein **GFP** and **Dronpa** are good for live cell imaging, the downsides are that it's weak and there can be labeling errors due to the size. Dyes are on the other hand strong and tiny in size but can't be used in living cells.

Observing - dead tissue

When observing, taking snapshots of dead tissue involves the following steps. A fixation process with chemicals for preservation, the inactivation of proteolytic(avoid breaking down of the tissue) and strengthening of the tissue for the staining. Then the staining is applied, either direct or indirect immuno staining or biological reactive. The advantages with this process is that a sample will last for weeks/months or even years. Less issue with phototoxicity (sensitive to light, break down), longer exposure time can be used (since

dead tissue don't move around). The disadvantages are that the cell is dead and so is the the dynamics of it. The fixation process can be quite difficult to get right and the process is staining specific.

Live cell fluorescence imaging

In the process of live cell imaging are the incorporation of plasmid DNA into the cells of the target used, **transfection**. Upon protein synthesis are the plasmid DNA expressed. The fluorescent protein can then be detected with a microscope. The advantages of this is of course that the cell is alive and so the dynamics of it. The potential effects of fixations and the artifacts related to it are not there. A stable transfection is long lasting, "forever". The disadvantages with this technique is that it is sensitive to light, phototoxicity, weak compared to dye, the exposure time that is crucial since the cell is living and there can potentially be labeling errors.

Simple Widefield vs confocal microscopy

Widefield	**Confocal**
Axial out-of-focus light blur	Axial out-of-focus light blocked (pinhole)
Image seen by detector: superposition of all in and out of focus planes	Image is scanned latterly, one point/pixel at a time, yielding 3D volumetric data
The pixel i determined by the camera	Any pixel size since iteratively scanned
limited by diffraction	limited by diffraction

Table 6: example

Resolution problem and super resolution microscopy

The resolution of fluorescence microscopy is limited to 200nm XY and 500nm Z. If we want to observe the inside of a mitochondria at 80nm, that's not possible. For this we need super resolution microscopy or other methods to push the spatial resolution further.

Structured illumination microscopy - SIM

The methods basis consists of the theory around Moiré patterns and Fourier transform. Since periodic functions can be expressed as a sum of a series of sine/cosines with specific amplitude and phase coefficients. The optical transfer function specifies to what extent spatial frequencies are captured. With a well defined periodic illumination pattern, we get a moire image since these patterns are at a lower frequency and can therefore be resolved and captured by the microscope. Taking a series of these and with the known illumination pattern we can reconstruct the high frequency details that created the pattern, the sample!

The computational reconstruction separates the Fourier components from the raw images, then re-combining (?) them. Using Apodization and Wiener filtering (?) and then inverse fourier transform to get back to image domain.

SIM summary

The advantages with SIM: resolution extension: $d = 1 + \lambda/2NAr_{SIM}$, 2x extension max. There is no additional need for sample preparation. There are a lot of commercial microscopes available. It is compatible with live cell observations.

The disadvantages with SIM are the illumination pattern deteriorates deeper into samples. Instead of one image, it need 9 to 15. The computational reconstruction may introduce artifacts.

STimulated Emission Depletion - STED

STED is a point scanning microscopy technique (similar to confocal). The concept behind STEM is to make all other parts that is not illuminated of the fluorescence to light up creating contrast between the that way. The layout of a STED microscope involves a STED laser beam, an excitation laser beam, bandpass filter and detector. Polarization optics are used for shaping of the depletion beam, dynamic scanning mirrors (scanning technique), high NA objective is needed, dichroic filtes for combining the laser beams and a photo-multiplier tube for detector.

Shaping the STED beam

The beam is shaped using phase retardation. The lateral resolution improves with helical phase retardation 0-2$\pi$of STED beam. The axial resolution improves with phase retardation of inner circle area of π. This may be combined to obtain enhancement of the resolution in all three dimensions ???

STEM summary

Very high resolution in 3D, which theoretically is unlimited. The technique is confocal and have 3D capability. The RAW data is of super resolution. Can be possible to use 3-4 colors. The disadvantages of STEM is the need o the strong STEM laser which can be harmful for live cells, it is also quite slow since it is a scanning technique. The dyes needed are also strong which can be a disadvantage.

Single molecule localization microscopy - SMLM

A photo-activateable technique were typically a normal flourescent wide-field microscope can be used. THe fluorescent molecules are computationally localized from a diffraction limited sequence of images based on the concept of switching on and off. By activating the single dyes randomly it is possible, by combination off multiple frames to differentiate molecules that otherwise would have been to close to distinguish because of diffraction limit.

Some important acronyms:

- **PALM** - Photo-Activated-Localization Microscopy
- **STORM** - STochastic Optical Reconstruction Microscopy
- **DNA PAINT** - DNA-based point accumulation for imaging in nanoscale topography

Summary SMLM

The technique is 7-8 times better in resolution compared to widefield microscopy. It is a single molecule read out and multicolor is available are two other advantages. The disadvantages are the long light exposure, there is a limited number of on-off dyes, the number of raw images necessary, the process is slow and need data reconstruction.

Image processing

Common tools are: background correction, denoising, segmentation, labeling and tracking. Some of the challenges with these imaging techniques are: the size of the data sets, signal to noise ratios, object labeling the result can be tedious.

- Classic algorithms
  - Simple and computational expensive
- Deep /machine learning
  - black box, if trained it is easily applied

Denoising

Two more advanced techniques for improving image quality are **deconvolution** which is an iterative process that reconstructs which has been blurred by a **known** point spread function. Machine learning denoising by training a network with pair of high and low SNR. Both techniques are applicable to all imaging techniques.

Image segmentation

In microscopy we want to be able to label biological structures such as: Mitochondria, nuclei, specific cell types, cancer and more...

Tracking

Tracking is used for observe cellular behavior when drug is induced for example. Can be useful to track cells during development stages. Track Brownian motion of particles, diffusion coefficient.