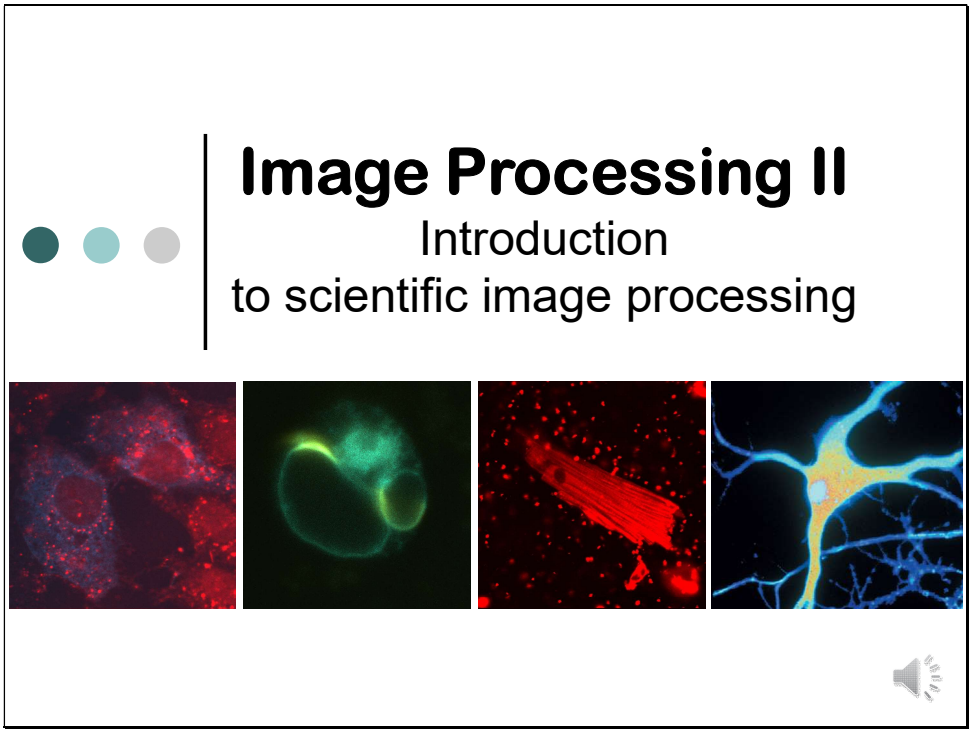
Christoph Biskup / Michael Habeck

Lecture notes

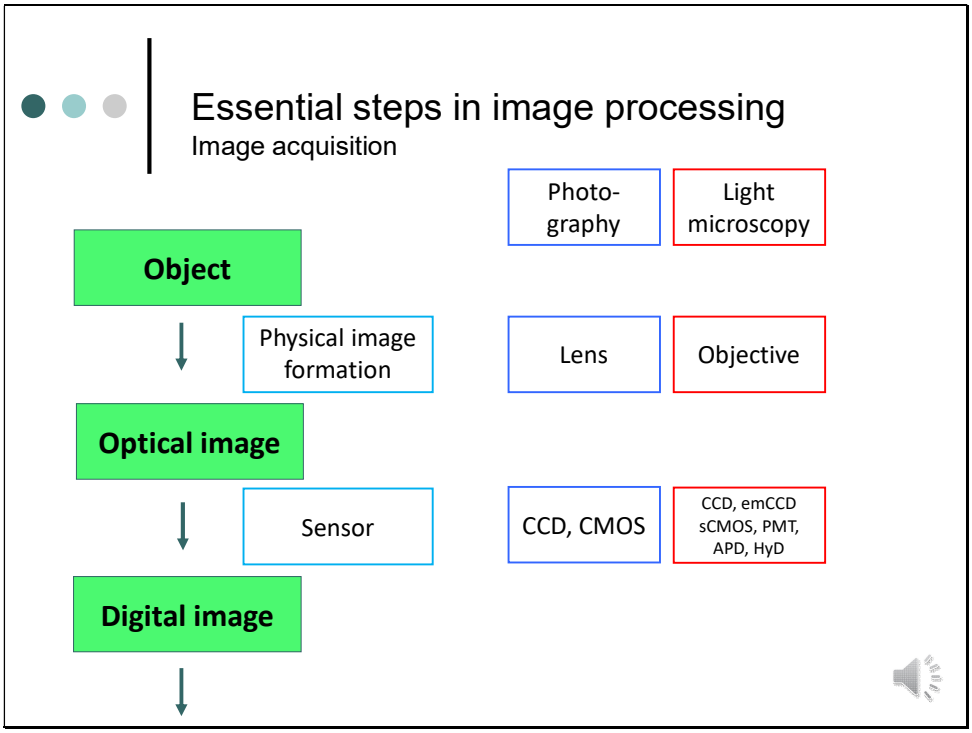


With this lecture we are opening a new chapter in our Image Processing course. So far, we processed only few images. But, by now you learned all the important basics, which we are now going to apply.

We will focus on processing microscopic images. At least most examples you see in this lecture will come from this application. We will not attempt to describe the workings of the microscope. This is subject of the courses in Physical Optics, Optical Engineering and Microscopy. Neither do we spend time on the proper use of the instrument for a given application. This will be subject of some of the courses (i.e. Biological microscopy) of the third semester. Of course, we will refer to knowledge taught in other modules to outline the reasons and limitations for certain image processing steps.

If you are not so much interested in microscopy, you might come to the conclusion that this course does not meet your needs. But this should not be true. Images play the most important part in our perception. Many algorithms discussed here can also be transferred to other medical and non-medical applications. Since there are many similarities to photography we will also discuss some examples from this field.

Slide #2



How are images processed?

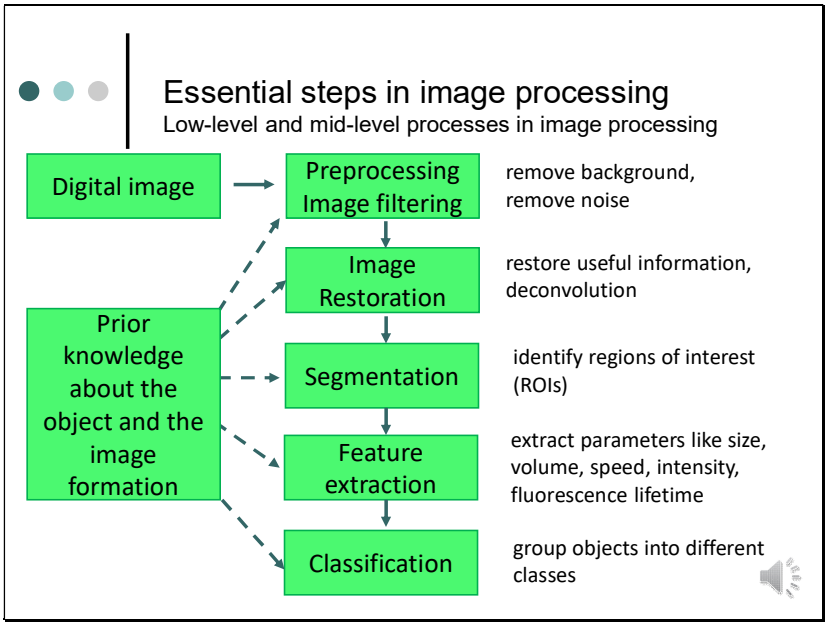
Before we can process digital images we need to get them. One might be inclined to not consider this process to be a part of image processing. But, at the end, as we will see in few minutes, it determines many properties of the image.

The first step is the physical image formation. In the case of a photographic camera this job will be done in the most simplest case by a pinhole or a lens. In the case of a microscope it's the objective.

To record the image we need a sensor. In the case of a photo or movie camera this is a CCD chip or a CMOS sensor, which are also used to acquire microscopic images. In the case of laser scanning microscopes, so called point detectors like a photomultiplier tube, an avalanche photodiode or some other devices might be used. In one of the lectures, which are going to come, we will discuss, how these sensors work.

But now, that the digital image is obtained, we can process it. The next slide shows which steps are involved in image processing.

Slide #3



The first steps in image processing are low-level processes: Input and output are images.

- The aim of **image preprocessing** is to remove background and noise. The quality of the image should be enhanced so that the result is more suitable than the original for a specific application. The word "specific" is important here, because it implies that the enhancement technique is problem oriented. A method used for microscopic images might be suitable for x-rays as well, but not for ultrasound images.
- **Image restoration** improves the appearance of an image. Unlike enhancement, which is subjective, image restoration is objective in the sense that restoration techniques tend to be based on mathematical, physical or probabilistic models of image degradation.

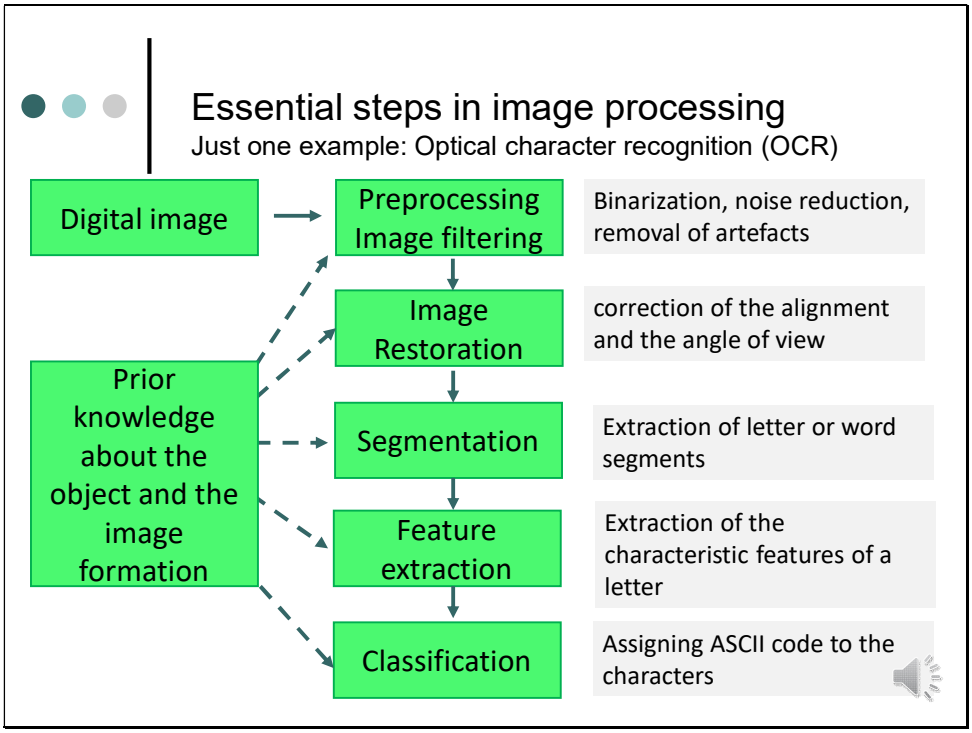
What follows are mid-level processes: Inputs are images, outputs are attributes extracted from the image like edges or contours.

- **Segmentation** means partitioning an image into regions or objects. The output are raw pixel data, constituting all the points of a region or the boundary of a region. Autonomous segmentation is one of the most difficult tasks in image processing. Its success determines the success of all the following steps.
- **Feature extraction** follows the output of the segmentation stage. It refers to finding the features of an image, region or boundary. Feature descriptors should be as insensitive as possible to a variation in parameters such as scale, translation, rotation, illumination and viewpoint.
- **Image pattern classification** assigns a label to an object based on its feature descriptors. Classification means recognition of individual objects.

What can follow are image analysis and higher-level processing steps involving cognitive functions which are usually associated with human vision. These steps are not part of this module.

In all steps of image processing knowledge of the object and the imaging process plays an important role. This might be simple knowledge as detailing regions of an image where the information of interest is located. But it might also involve knowledge about artefacts and the process of image formation.

Slide #4

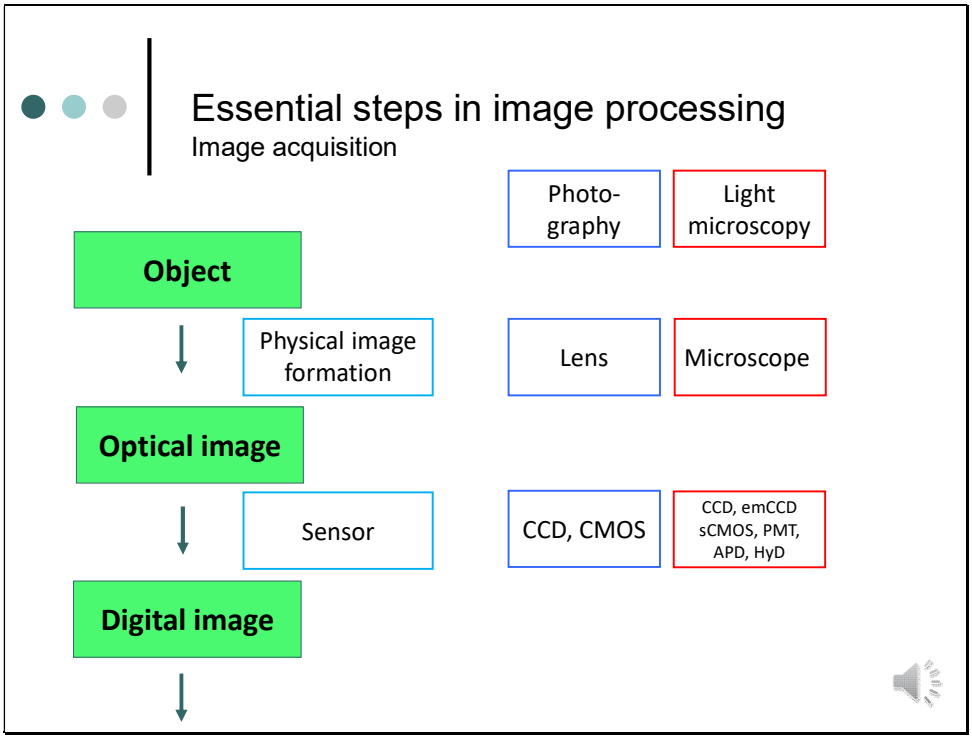


To elucidate this scheme we should compare this process with analyzing a piece of written text. This process is often called optical character recognition.

- **Image acquisition** is scanning the text. This yields the scanned text in a grayscale image format.
- **Preprocessing** aims to produce data that are easy for the OCR systems to operate accurately. The main objectives of pre-processing are removal of artefacts, binarization and noise reduction.
  - **Image binarization** refers to the conversion of a gray-scale image into a binary image. This can be done by applying a global threshold value to the entire document or by applying an adaptive (local) threshold for each pixel according to the local area information.
  - By applying **filters** noise can be reduced.
  - Also, artefacts such as dust in the scanner or coffee stains spilled on the paper can be removed at this stage.
- **Image restoration** might not be necessary. But at this stage the paper document might be aligned with the coordinate system of the scanner. Skew lines might be aligned.
- **Segmentation** means distinguishing letters or words from the background.
- **Feature extraction** means extracting characteristic features of a letter.
- **Classification** means describing the characters in a form suitable for computer processing, that is assigning ASCII code to them.
- Now we can include post-processing steps: The simplest way of incorporating the context information would be to make use of a dictionary for correcting minor mistakes.
- The last step would be making sense of the content of the text.

So much for an overview about image processing. In the following lectures we will focus on each of the steps in detail. But, for the remainder of this lecture we will stick to image acquisition.

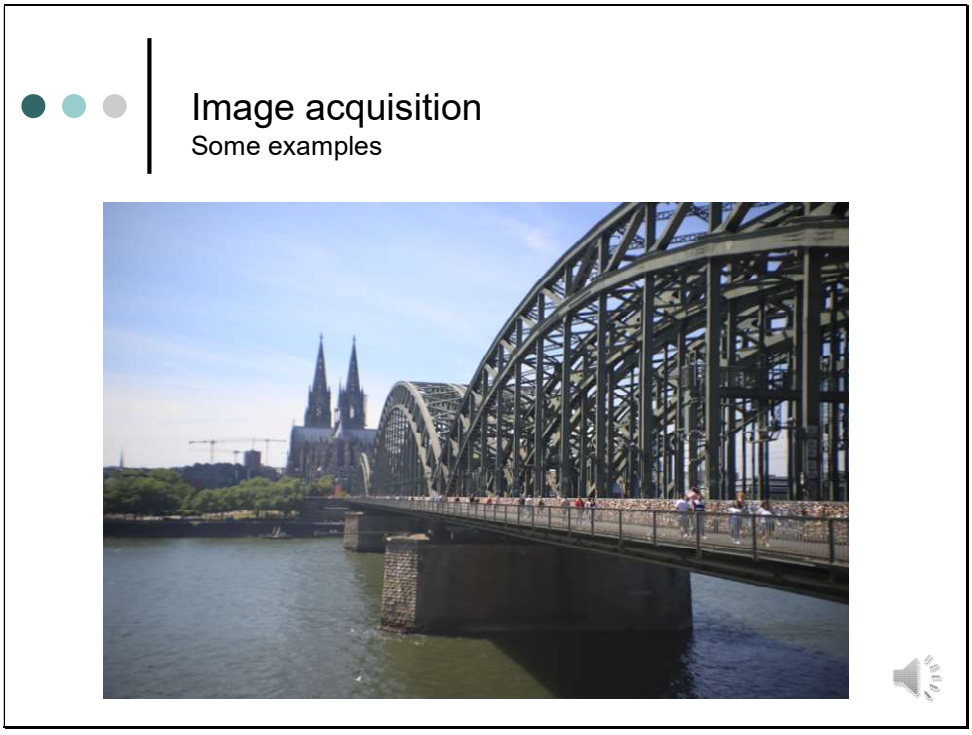
Slide #5



Why should we spend so much time on image acquisition?

The optics and technique we use to get the image determine the quality of an image. Some, but not all imperfections of the setup can be corrected by image processing. This, of course, applies to all experiments you do, the better your setup, the better the data, the more conclusions you can draw from your results. Just let me show with some simple examples taken from the field of photography how equipment and settings can affect the result.

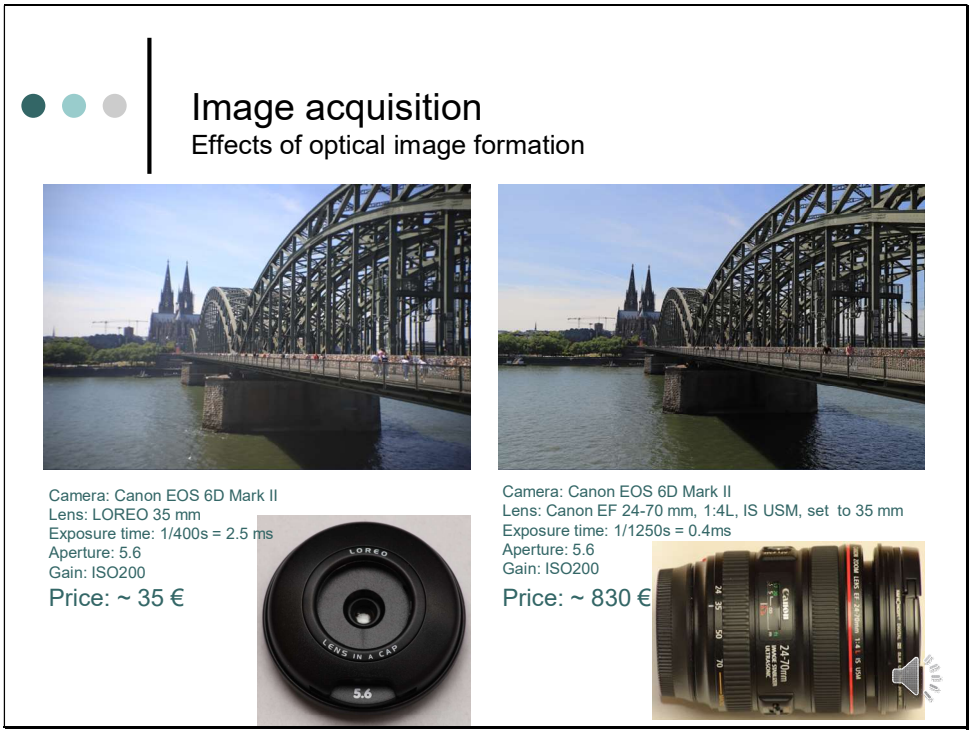
Slide #6



This is one example take from the skyline of Cologne. The image might seem to O.K. When you take a look at Cologne's cathedral you might come to the conclusion that the image was taken on a foggy day.

Equipment and settings used for the photograph: Canon EOS 6D Mark II, Lens LOREO 35 mm, A = 5.6, t = 1/400s = 2.5 ms, ISO200

Slide #7

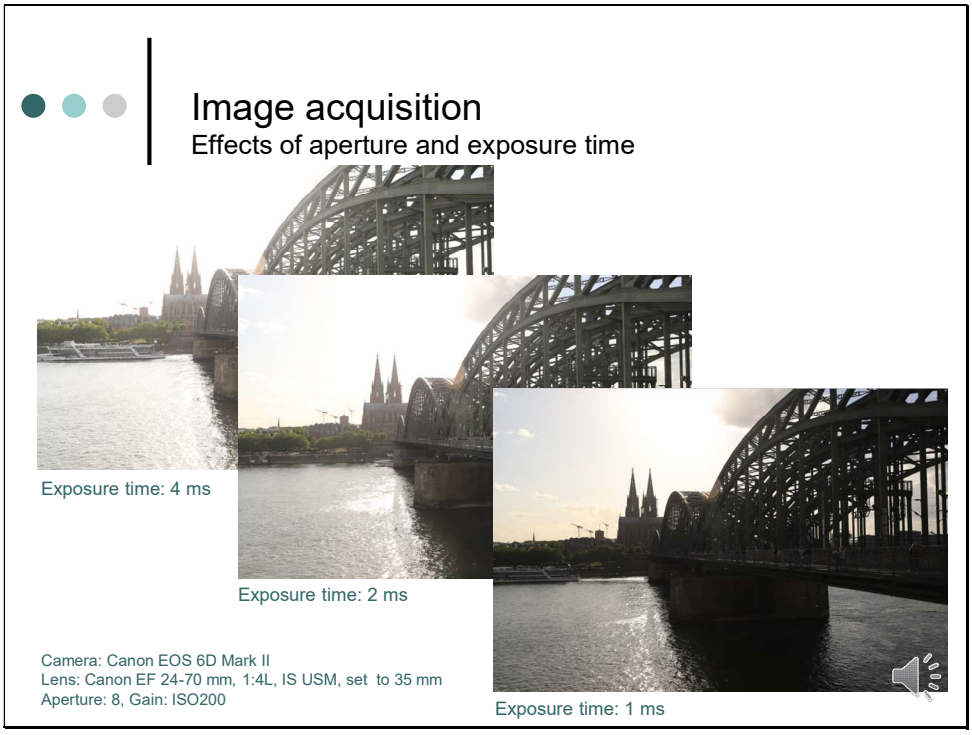


But if you compare the image to one taken with a different objective on the same day you will come to the conclusion that the blur is just due to the objective. The second image was taken with a state of the art objective, while the first image was taken with a simple lens.

Image processing might help to restore some imperfections in the image. But, good equipment can save a lot of work. On the other hand, if image restoration is able to correct for imperfections of the lens, image processing can save a lot of money. The same applies for microscopy. Researchers, however, tend not to make compromises, they need good equipment and good image processing algorithms to get most out of an experiment.

Equipment and settings used for the photographs:
Canon EOS 6D Mark II
Left photograph: Lens LOREO 35 mm, A = 5.6, t = 1/400s = 2.5 ms, ISO200
Right photograph: Lens Canon EF 24-70 mm, 1:4L, IS USM, set to 35 mm, A = 5.6, t = 1/1250 s = 0.4 ms, ISO200

Slide #8

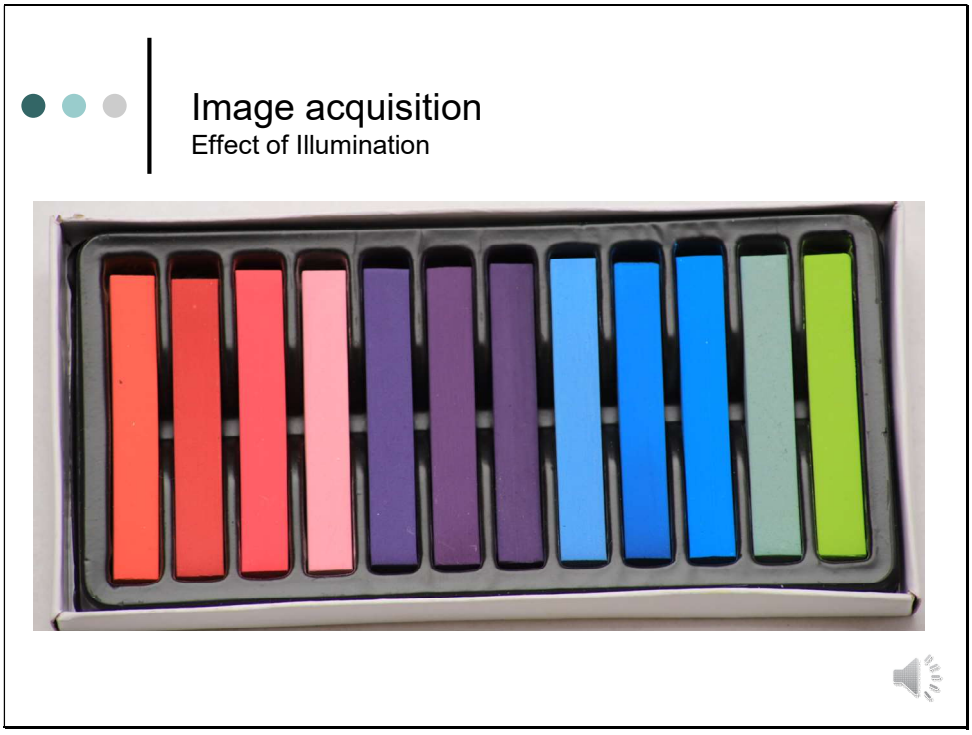


Several factors can affect the contrast and brightness of an image. Here you see the effect of exposure time. But, what is actually the right exposure time? How can you determine it? And if you do not know it in advance, is it better to get an underexposed or an overexposed image?

These are questions we will try to answer in one of the next lectures.

Equipment and settings used for the photographs:
Canon EOS 6D Mark II, Lens Canon EF 24-70 mm, 1:4L, IS USM, set to 35 mm, A = 8, ISO200, Upper image: t = 1/250 s, Middle: t = 1/500 s, Lower image: t =1000 s

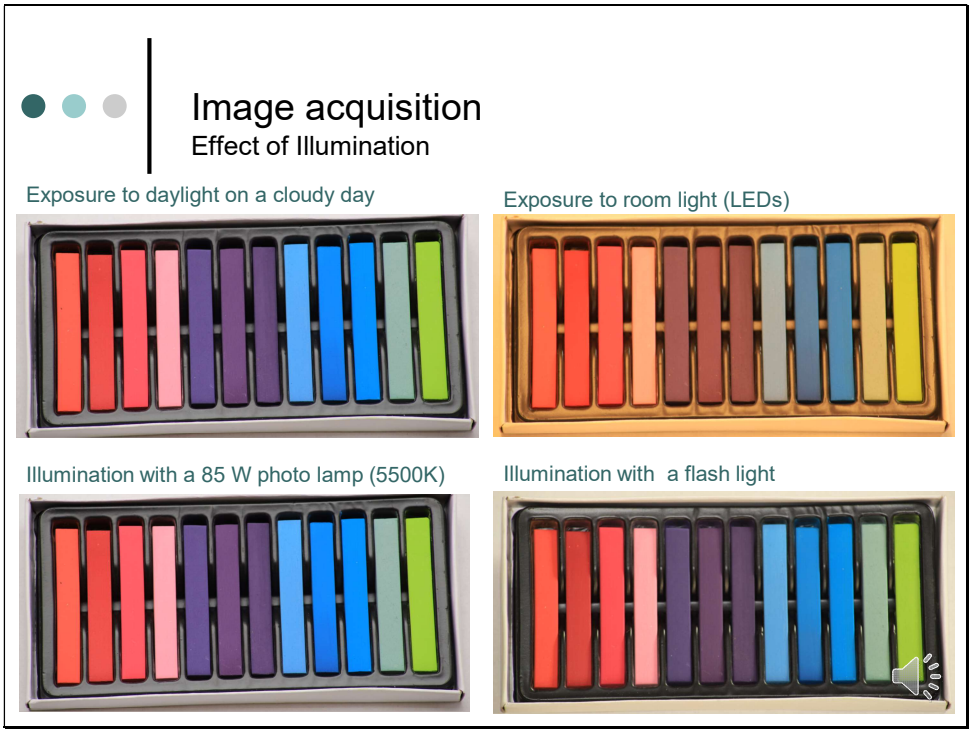
Slide #9



Something what is often underestimated is the effect of illumination. When we talk about colors we assume that they remain the same. Red appears always to be red, green appears always to be green.

Equipment and settings used for the photographs:
Camera: Canon EOS 6D Mark II, Lens Tamron 70-300mm, 1:4-5.6, set to Macro 180 mm, AV=8.0, ISO200
The photo was taken under illumination with daylight (on a cloudy day), t= 1/80 s.

Slide #10

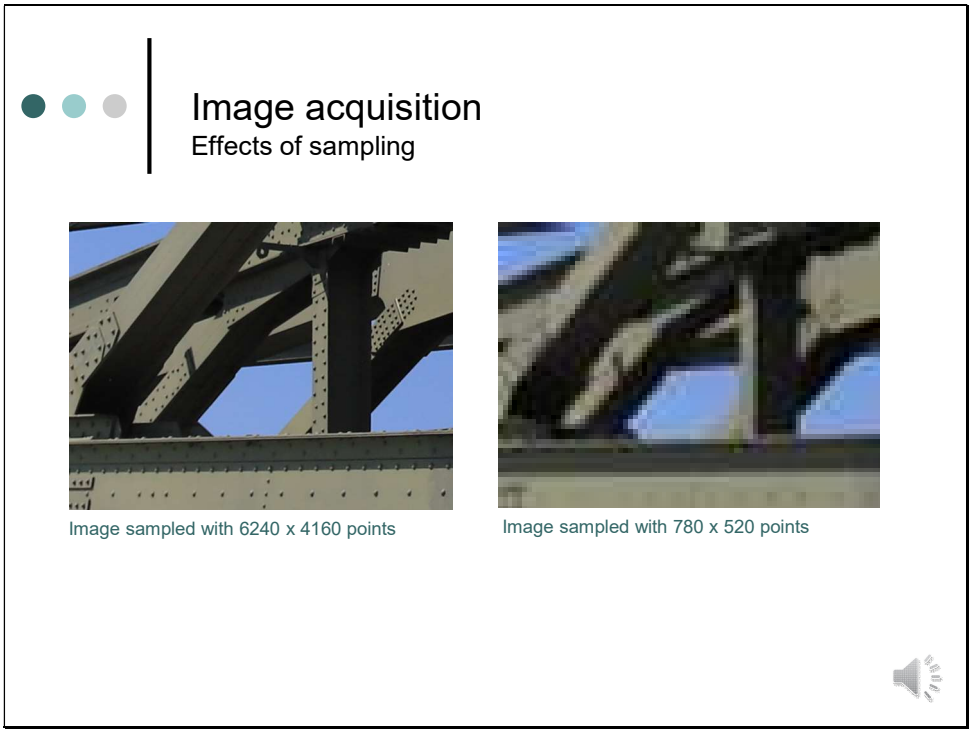


But this example shows that also the color we see with our eye differs largely with the illumination source we use. Just compare the blue and green pieces of chalk recorded at daylight and at room light. What applies to photography also applies to microscopy (at least to transmission microscopy).

We will focus on these and some more aspects during the next lecture.

Equipment and settings used for the photographs:
Camera: Canon EOS 6D Mark II, Lens Tamron 70-300mm, 1:4-5.6, set to Macro 180 mm, AV = 8.0, ISO200,
Upper row, left: daylight on a cloudy day, t= 1/80 s.
Upper row, right: exposure to room light (LED), t = 1 s.
Lower row, left: illumination with ESSDI photo lamp (energy saving lamp), t = 1/80 s.
Lower row, right: illumination with Canon Speedlite 430EXIII-RT, t = 2.5 s.

Slide #11

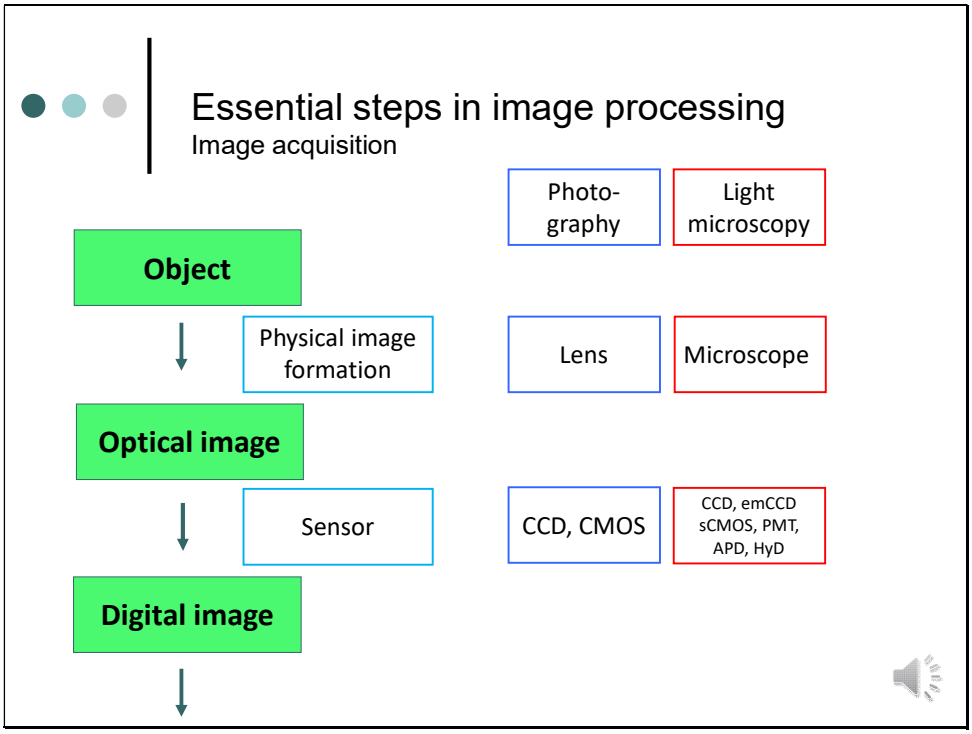


Last, but not least also sampling matters. What resolution is the right one to choose? Is a higher resolution always better?

We discussed sampling already when we talked about the Fourier transform. But this semester we will go more into details.

Equipment and settings used for the photographs:
Canon EOS 6D Mark II, Lens Canon EF 24-70 mm, 1:4L, IS USM, set to 35 mm, A = 5.6, t = 1/1250 s = 0.4 ms, ISO200
Left: Image sampled with 6240 x 4160 points
Right: Image sampled with 780 x 520 points

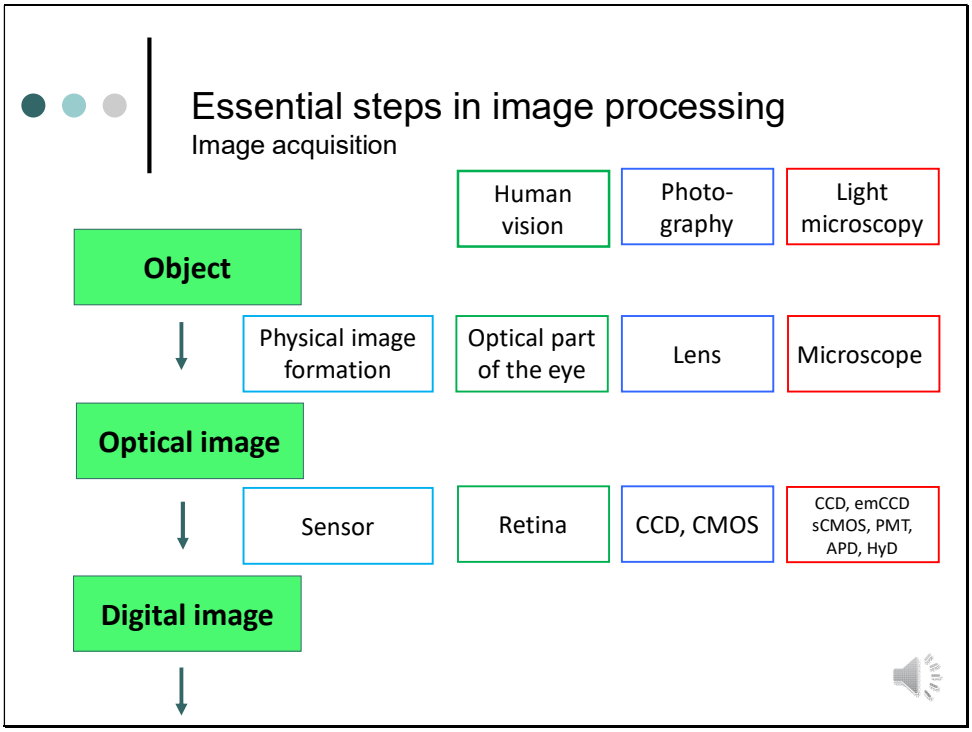
Slide #12



So much for some examples taken from photography. Later throughout this lecture we will cover similar examples from the range of microscopy.

But before we start discussing these systems in more detail, we should discuss another image acquisition system. It is the system you are using right now. Your eye!

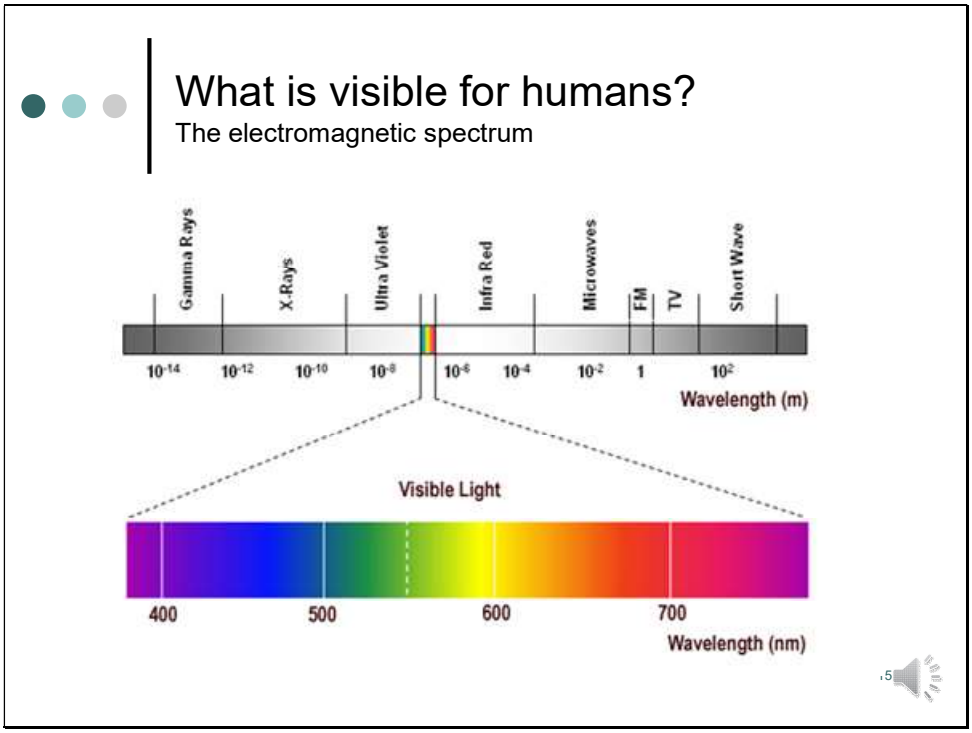
Slide #13



Although digital image processing is based on mathematical algorithms, human analysis (and perhaps intuition) often plays a role, when we judge the performance of image processing algorithms. Human vision is somehow a benchmark or perhaps the benchmark for technical systems. Factors such as how human and electronic imaging devices compare in terms of resolution and the ability to adapt to changes in illumination are not only interesting, they are also important from a practical point of view. Moreover, many imaging devices are made such that they produce images that are similar to what we perceive with our eye.

We will discuss the eye in detail in the Human Biology course of this semester. Here we will focus just on some basic features.

Slide #14

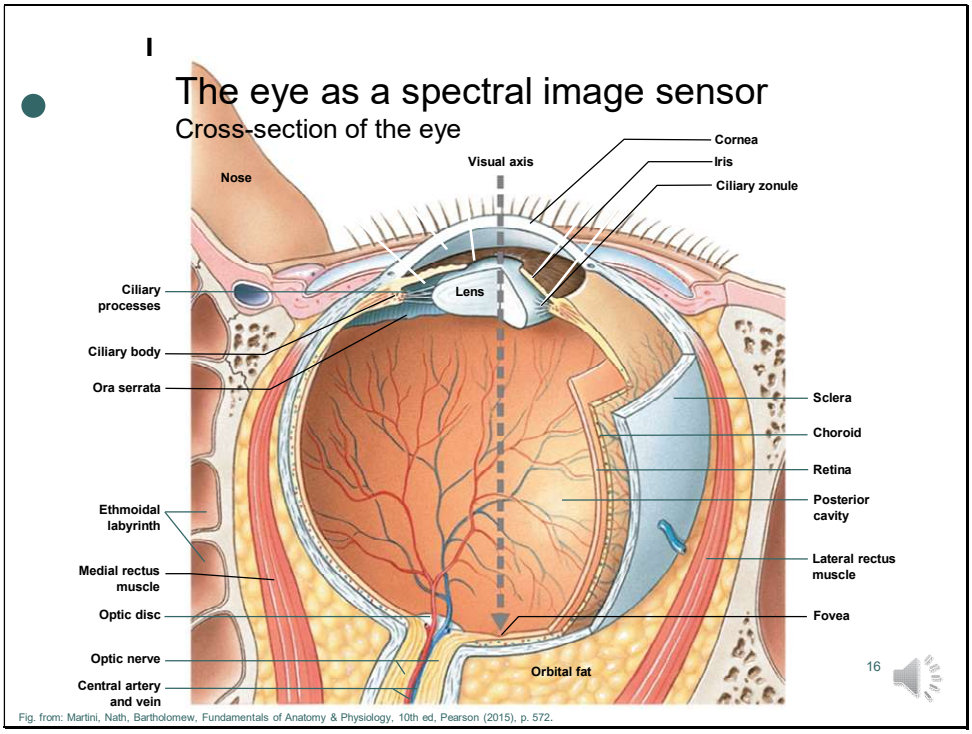


Humans are limited to the visual part of the spectrum, ranging from 380 to 780 nm. Wavelengths of electromagnetic spectrum shorter than 380 nm fall into the ultraviolet range, wavelengths of electromagnetic radiation longer than 780 nm fall into the infrared range. These are the mean values for many observers. There are observers, however, who can also see light in the near ultraviolet (UV) region down to 360 nm or in the infrared region (IR) of the spectrum up to 850 nm. Visibility, of course, depends on the intensity of the light. In the lab, laser light reflected by a sheet of white paper can be seen up to 850 nm by almost everyone.

Light in the short wavelength region from 380 nm to 430 nm appears violet, light with wavelengths up to 480 nm usually produces the sensation of blue light, radiation with wavelengths between 520 nm and 550 nm is seen as green light, light around 560 nm and 600 nm appears yellow, and above 650 nm we perceive the light as red color. These values however are not well defined. When you look at different textbooks you will find slightly different values. The actual perception depends on the adaption state of our eye and the light stimuli surrounding our test object. Also, the color scale which is reproduced to visualize the range of wavelengths we can see differs from textbook to textbook.

But how does our image sensor work? How is it possible that we can see colors?

Slide #15



This figure shows a cross section of the human eye. The eye is nearly a sphere (with a diameter of about 20 mm). It is enclosed by three layers of tissue.

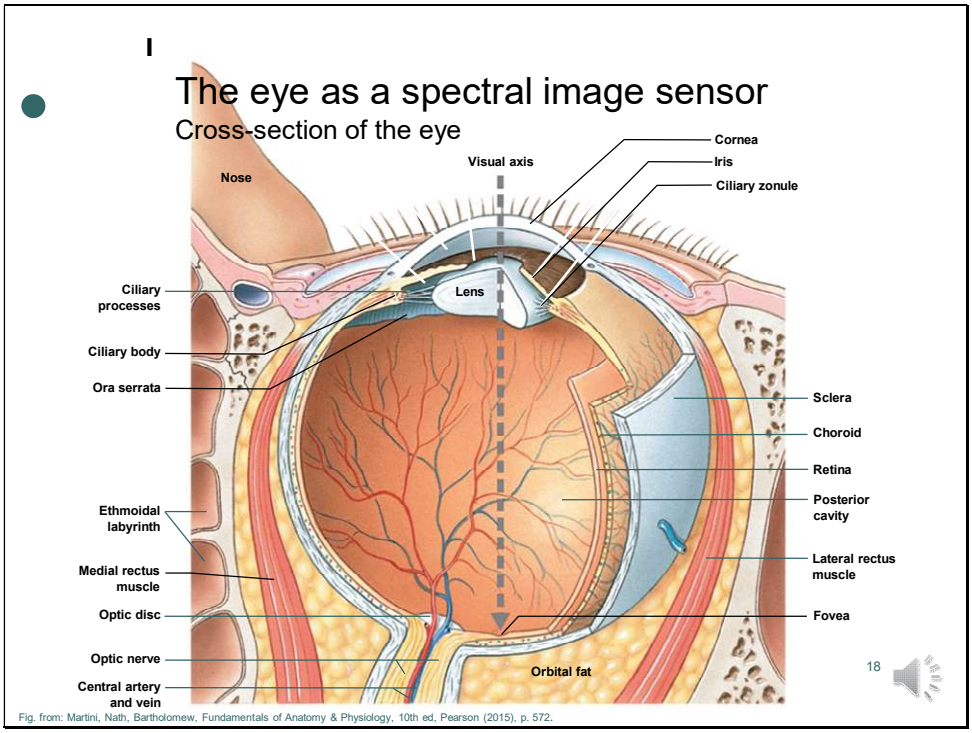
- The outermost layer is the **fibrous tunic**, which includes the white **sclera** and the clear **cornea**. Whereas the sclera accounts for five sixths of the surface of the eye, the transparent cornea covers the anterior tip of the eye and allows light to enter the eye.
- The intermediate layer of the eye is the **vascular tunic** (or uvea) which is mostly composed of the choroid, ciliary body, and iris. The **choroid** is a layer of highly vascularized connective tissue that provides blood supply to the eyeball.
- The innermost layer of the eye is the **neural tunic**, or **retina**, which contains the nervous tissue responsible for photodetection. Perception of light takes place by the excitation of light receptors, which encode the arrival of photons into an change of membrane potential. This changes the action potential frequency of subsequent neurons, which is ultimately decoded by the brain.

The eyeball itself is filled with fluid. Its interior can be divided into two cavities, the anterior cavity and the posterior cavity:

- The **anterior cavity** is the space between the cornea and lens including the iris and ciliary body. It is filled with a watery fluid called the **aqueous humor**. It has two chambers, the anterior chamber (between the cornea and the iris) and the posterior chamber (between the iris and the lens).
- The posterior cavity is the space behind the lens that extends to the posterior side of the eyeball, where the retina is located. The posterior cavity is filled with a more viscous substance called the **vitreous humor.**

The vitreous body and the aqueous humor help to stabilize the shape of the eye.

Slide #15 (Cont.)



Now, let's follow the path of light:

The light emitted by an object hits the surface of the cornea and is refracted here. In the human eye, the greatest amount of refraction occurs, when light passes from the air into the corneal tissue, which has a density close to that of water.

Additional refraction takes place when the light passes from the aqueous humor into the relatively dense lens. The lens provides the extra refraction needed to focus the light rays on the retina.

The lens can adapt its refractive power, so that images of nearby objects as well as images from distant objects can be focused on the retina. This process, which gives us (at least at young age) sharp vision over a broad range, is called accommodation.

The next slides shows how this works.

Slide #16

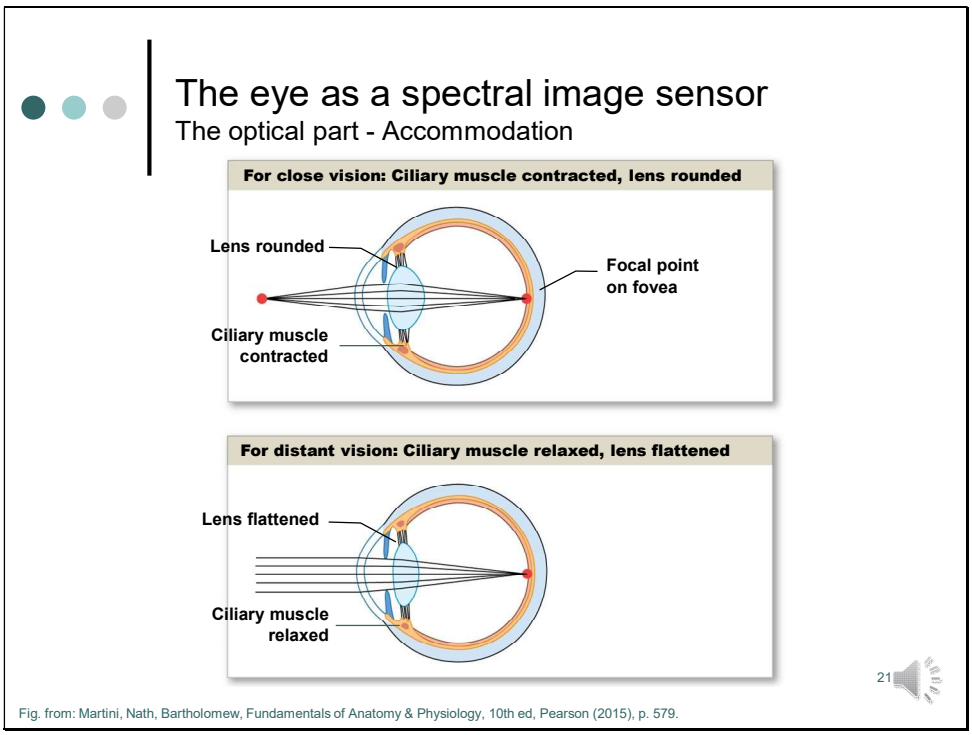


Fig. from: Martini, Nath, Bartholomew, Fundamentals of Anatomy & Physiology, 10th ed, Pearson (2015), p. 579.

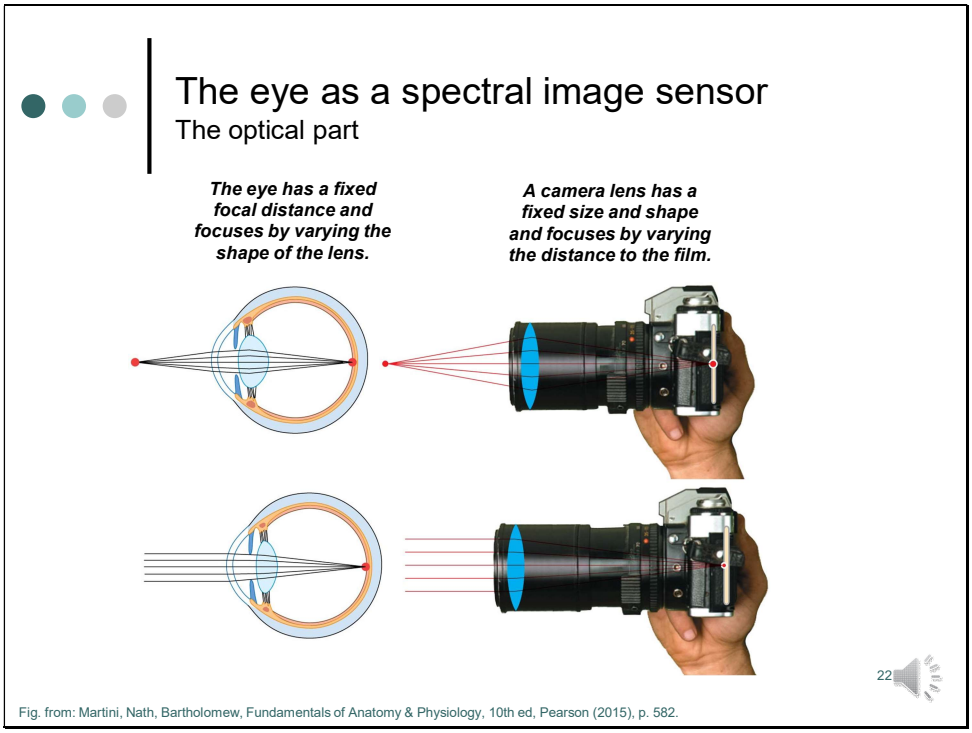
To focus on a nearby object the lens becomes rounder, to focus to a distant object the lens becomes flatter.

How does the lens change shape? The lens is held in place by the ciliary zonule that originates at the ciliary body. Smooth muscle fibers in the ciliary body act like sphincter muscles.

When the ciliary muscle contracts, the ciliary body moves toward the lens, thereby reducing the tension in the ciliary zonule. The elastic capsule then pulls the lens into a rounder shape, which increases the refractive (bending) power of the lens. This enables it to bring light from nearby objects into focus on the retina.

When the ciliary muscle relaxes, the ciliary zonule pulls at the circumference of the lens, making the lens flatter.

Slide #17



Hence, the optical system of the eye is very special.

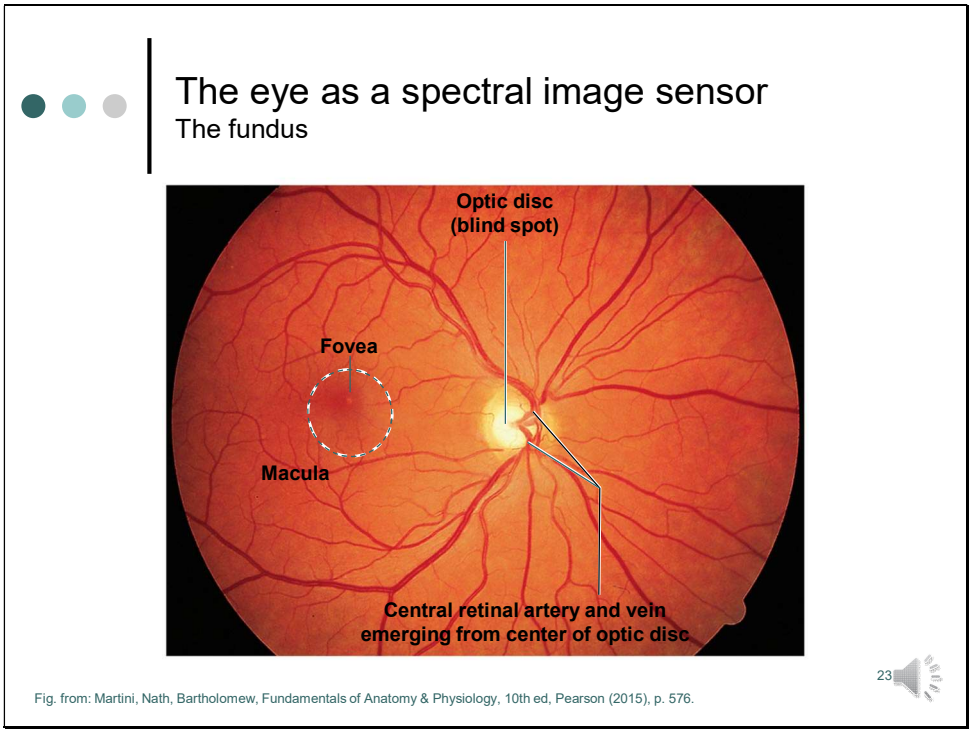
A camera focuses an image by moving the lens toward or away from the film or sensor. This method cannot work in our eyes, because the distance from the lens to the macula cannot change. We focus images on the retina by changing the shape of the lens to keep the focal distance constant.

Moreover, the spherical shape of the retina has the merit of reducing the complexity of the optical system by directly compensating the aberration from the curved plane.

Since microfabrication processes make hemispherical device fabrication almost impossible, most technical imaging systems use planar sensors. This requires complex methods to correct for spherical aberrations.

Now, let's have a closer look at the retina.

Slide #18



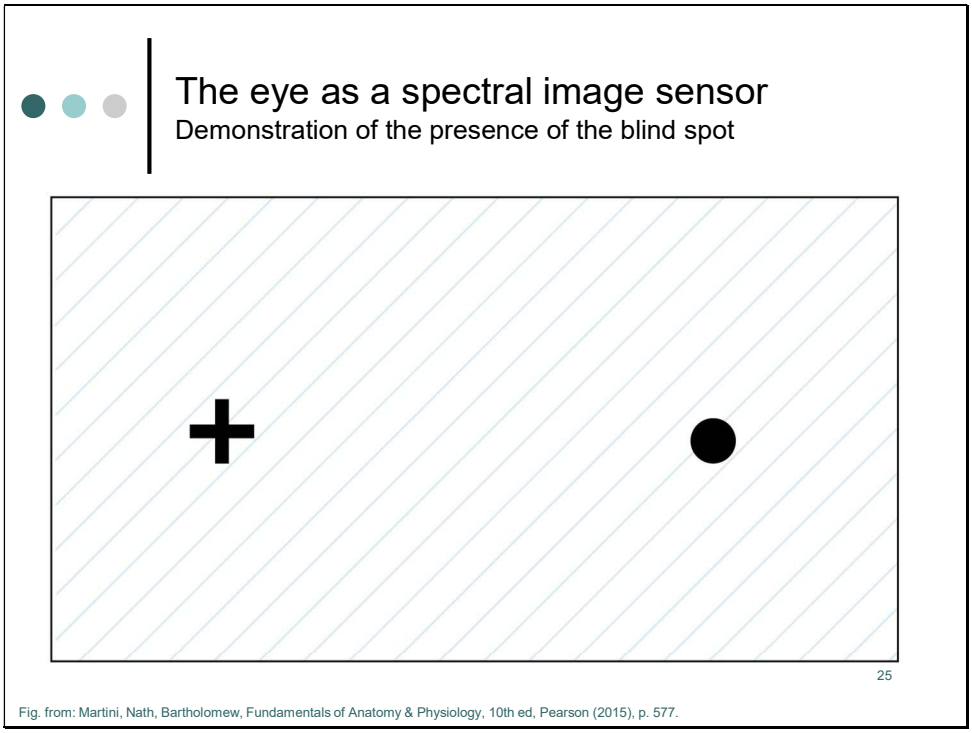
This slide shows a photograph of the retina as seen with an ophthalmoscope through the pupil.

Most prominent is the optic disc. At the optic disc the axons of retinal ganglion cells leave the eye. Also, arteries enter and veins leave the eye at this site. Because there are no photoreceptors, we cannot detect any light in this part of the retina. Therefore, the optic disc is also called blind spot.

Lateral (or temporal) to the optic disc is the **fovea**. At the fovea, the retina lacks the supporting cells and blood vessels, and only contains photoreceptors. Therefore, the sharpness of vision, the **visual acuity**, is greatest at this site. As one moves in either direction from this central point of the retina, visual acuity drops significantly.

In our daily life we move our eyeballs such that the object which attracts most of our attention is focused on the fovea. Therefore, we are not aware that acuity of vision is decreased in the periphery. We are neither aware that we have a blind spot. Our brain interpolates the lacking sensory input from information based on surroundings and information from the other eye, so that we do not perceive the blind spot.

Slide #19



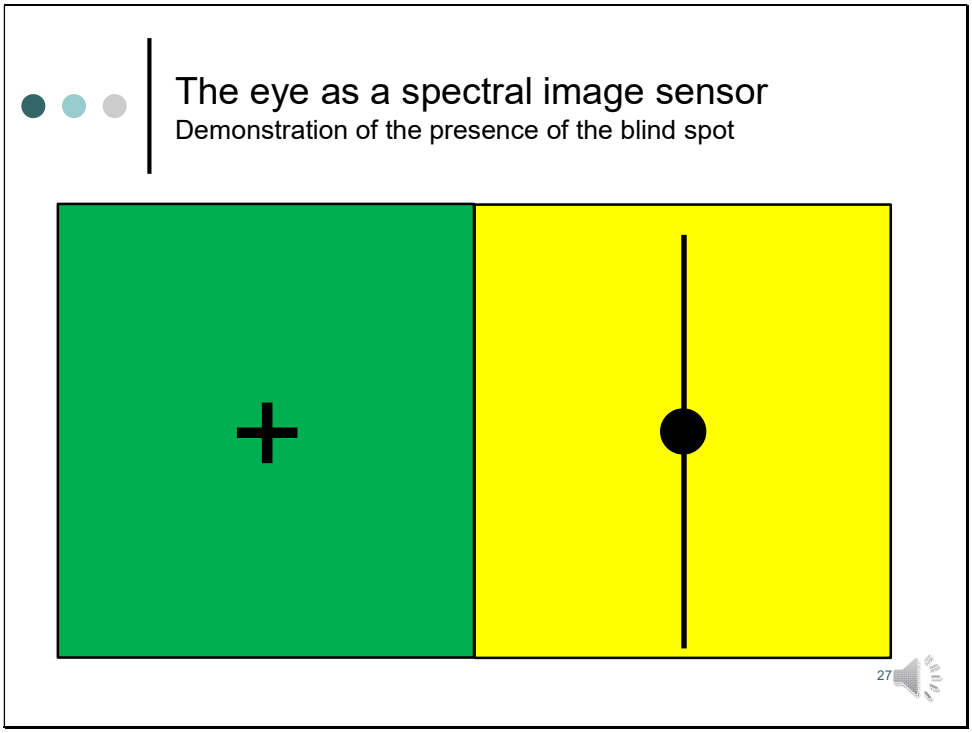
That we have a blind spot in our retina can be shown by a simple test.

Close your left eye and stare at the plus sign with your right eye, keeping the plus sign in the center of your field of vision. Begin with the screen few inches away from your eye, and gradually increase the distance. The dot will disappear when its image falls on the blind spot, at your optic disc.

To check the blind spot in your left eye, close your right eye and repeat the sequence while you stare at the dot.

The next test is even more fascinating. Try the same with the following figure.

Slide #20



This test appears to be similar to the previous one, but it is even more fascinating.

Now, just redo what you have done before. Close your left eye and stare at the plus sign with your right eye, keeping the plus sign in the center of your field of vision. Begin with the screen few inches away from your eye, and gradually increase the distance.

You should have seen a number of things: The dot went away, like in the previous figure. But it was replaced by a line! And a yellow image! Why a line? It's because our mind tries to fill in information from the context.

Slide #21

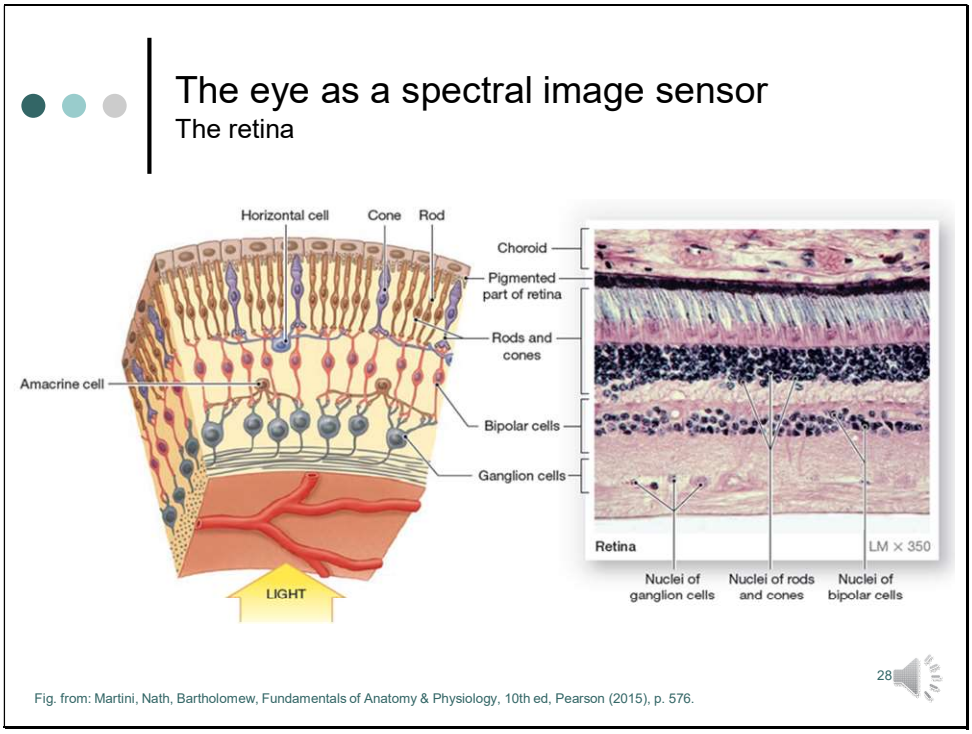


Fig. from: Martini, Nath, Bartholomew, Fundamentals of Anatomy & Physiology, 10th ed, Pearson (2015), p. 576.

Now, let's have a closer look at the retina. The retina is composed of several layers. It consists of a thin, outer layer called **pigmented epithelium**, and a thick, inner layer the **neural part**. The **pigmented part** of the retina absorbs light that passes through the neural part, preventing scattering. The pigment cells also have important biochemical interactions with the retina's light receptors.

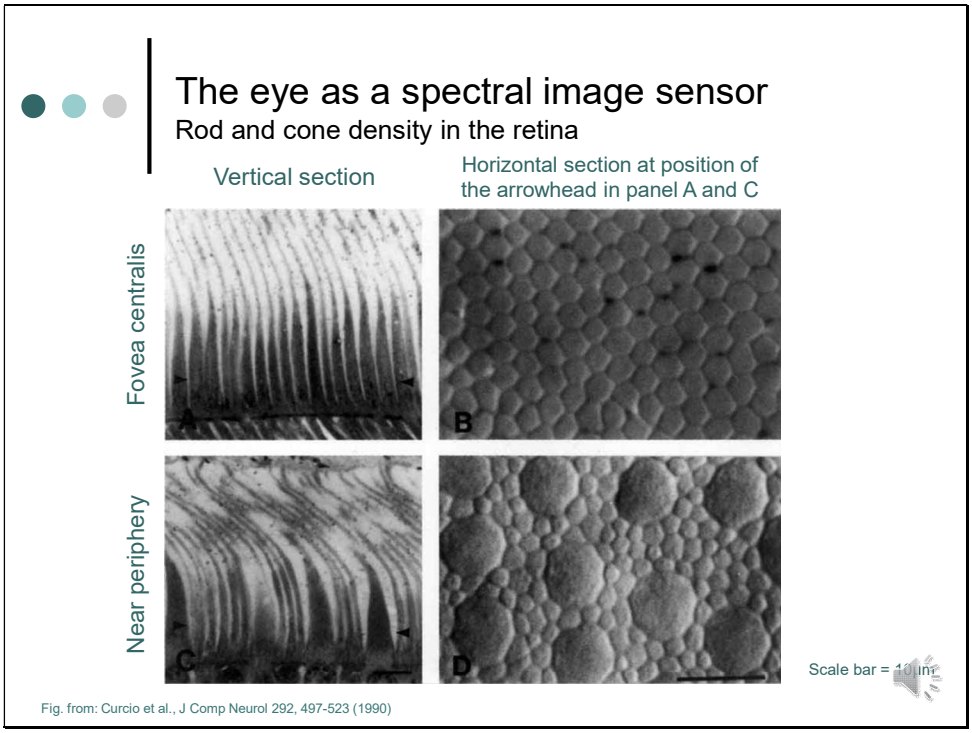
The **neural part** of the retina contains several layers of cells. Interestingly, the outermost layer, closest to the pigmented part of the retina, contains the cells that detect light, the photoreceptors. The human eye has two main types of photoreceptors: rods and cones, which differ in the shape of their outer segment. Rods are highly sensitive to light, but do not discriminate among colors. They are responsible for the **scotopic vision**. Cones give us color vision – or photopic vision. As we will discuss in more detail later, humans have (usually) three types of cones, and each type contains a different visual pigment which is sensitive for blue, green or red light. Rods and cones synapse with about 6 million bipolar cells. These cells in turn synapse with a layer of neurons called retinal ganglion cells (RGC). The axons of the ganglion cells, which constitute the innermost layer of the retina collect at the **optic disc** and leave the eye as the **optic nerve**.

A network of horizontal cells extends across the neural part of the retina at the level of the synapses between photoreceptors and bipolar cells. A similar layer of amacrine cells occurs, where bipolar cells synapse with ganglion cells. Horizontal and amacrine cells can facilitate or inhibit communication between photoreceptors and ganglion cells, altering the sensitivity of the retina. Details will be discussed in the lectures of Human Biology.

Note that the photoreceptors are located behind the axons, ganglion cells, bipolar cells, and retinal blood vessels. A significant amount of light is absorbed by these structures before the light reaches the photoreceptor cells.

This slide shows a stained cross-section of the retina. The next slide shows some more details.

Slide #22

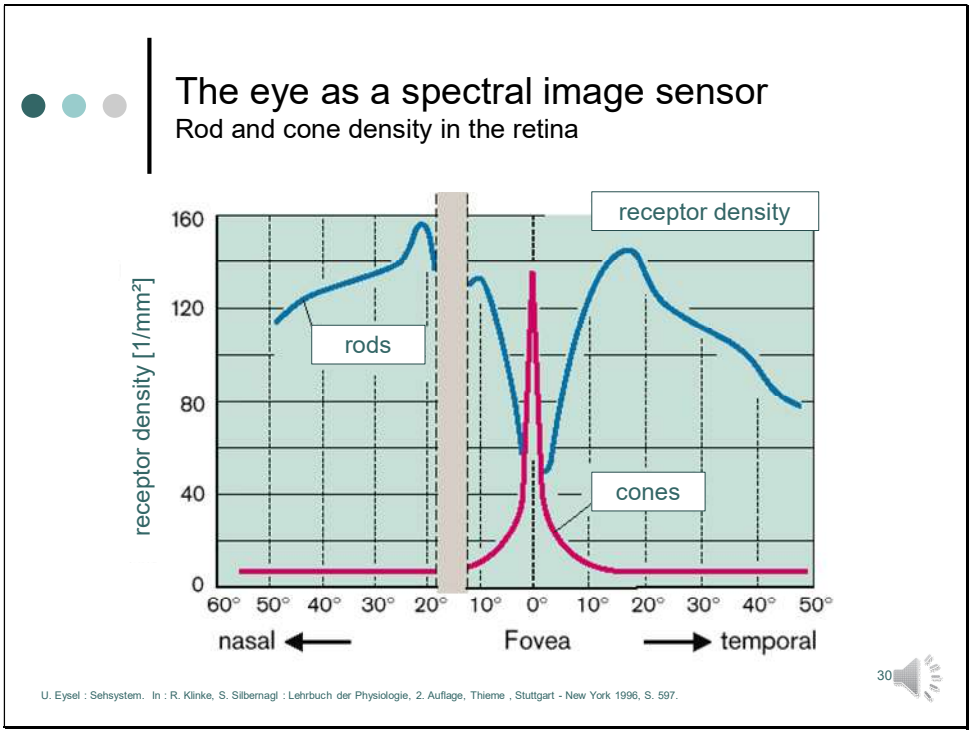


This slide shows vertical and horizontal cross sections, obtained at different sites of the retina. The arrowheads in the vertical cross sections indicate the approximate level where the horizontal photographs are taken. The scale bar has a length of 10 µm.

The first row is obtained at the fovea centralis. As you remember (hopefully), the fovea is the site of sharpest vision. When you look directly at an object, its image falls on this portion of the retina. At the fovea centralis all profiles are cones.

The second row shows micrographs taken at the near periphery. In the near periphery the large profiles are cones, and small intervening profiles are rods. Thus, rods and cones are not evenly distributed across the retina. The next slide shows you the numbers in more detail.

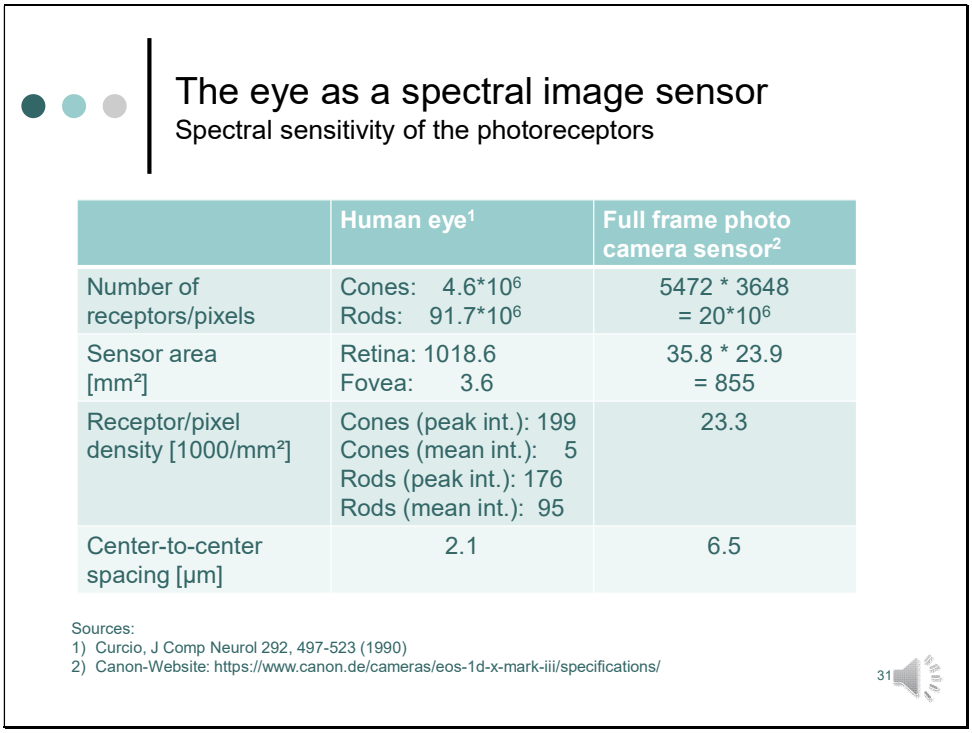
Slide #23



At the center of our field of vision about 5 million cones are located. The receptor density varies in between individuals. Peak intensities range in between 100000 and 300000 receptors per mm².

When we move away from the center toward the periphery of the retina, the density of cones gradually decreases, whereas the density of rods increases. Approximately 100 million rods form a broad band around the periphery of the retina. The peak density of rods in the retina is on the order of 100000 elements per mm².

Slide #24



Possibly, it is interesting to compare these numbers to some data of current imaging chips used in photography.

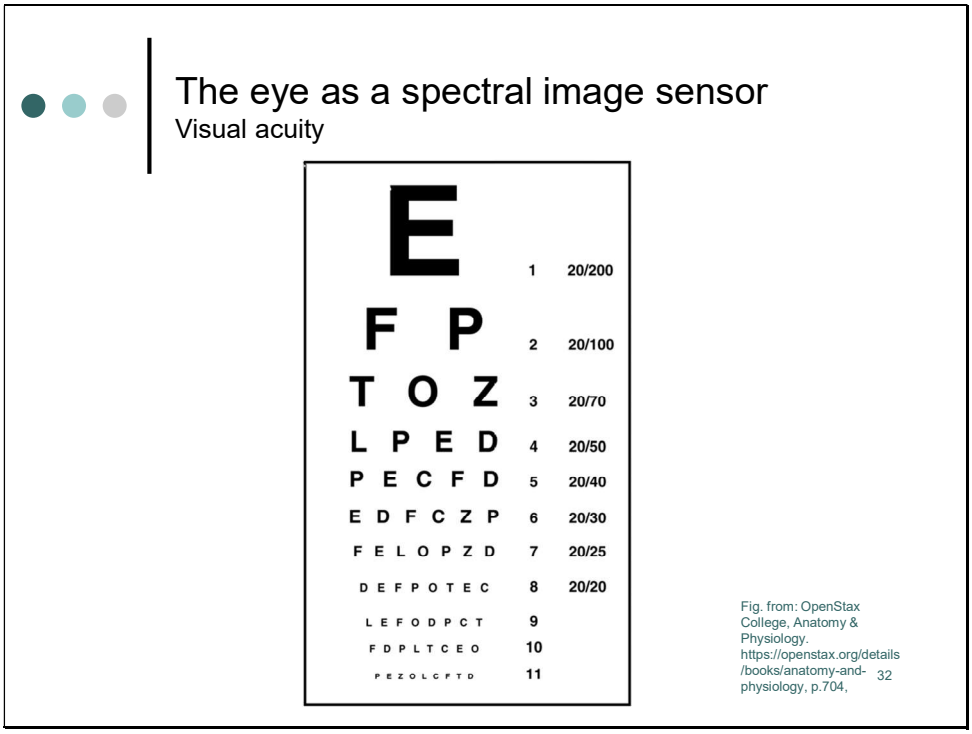
As you can see from this table, the number of sensor elements are in approximately the same order of magnitude. However, as discussed before the density of receptors varies throughout the retina, whereas pixels in imaging chips have equal size. Peak intensities in the retina are still almost an order of magnitude higher than in sensors used in professional photo cameras.

However, high-end imaging chips exceed this number by a large factor, so that electronic imaging sensors can easily exceed the capability of the eye in resolving image detail.

But, in terms of image processing and image understanding human vision is still superior (at least in some aspects) to machine vision. Keep in mind that the information collected by the photoreceptors is transferred via the bipolar cells to more than a million of retinal ganglion cells and that this information is processed in parallel.

But, now let's have a look, how incoming signals are transduced to an electrical signal.

Slide #25



The **Snellen chart** demonstrates visual acuity by presenting standard Roman letters in a variety of sizes. The result of this test is a rough generalization of the acuity of a person based on the normal accepted acuity, such that a letter that subtends a visual angle of 5 minutes of an arc at 20 feet can be seen. To have 20/60 vision, for example, means that the smallest letters that a person can see at a 20-foot distance could be seen by a person with normal acuity from 60 feet away. Testing the extent of the visual field means that the examiner can establish the boundaries of peripheral vision as simply as holding their hands out to either side and asking

the patient when the fingers are no longer visible without moving the eyes to track them. If it is necessary, further tests can establish the perceptions in the visual fields. Physical inspection of the optic disk, or where the optic nerve emerges from the eye, can be accomplished by looking through the pupil with an ophthalmoscope.

Slide #26

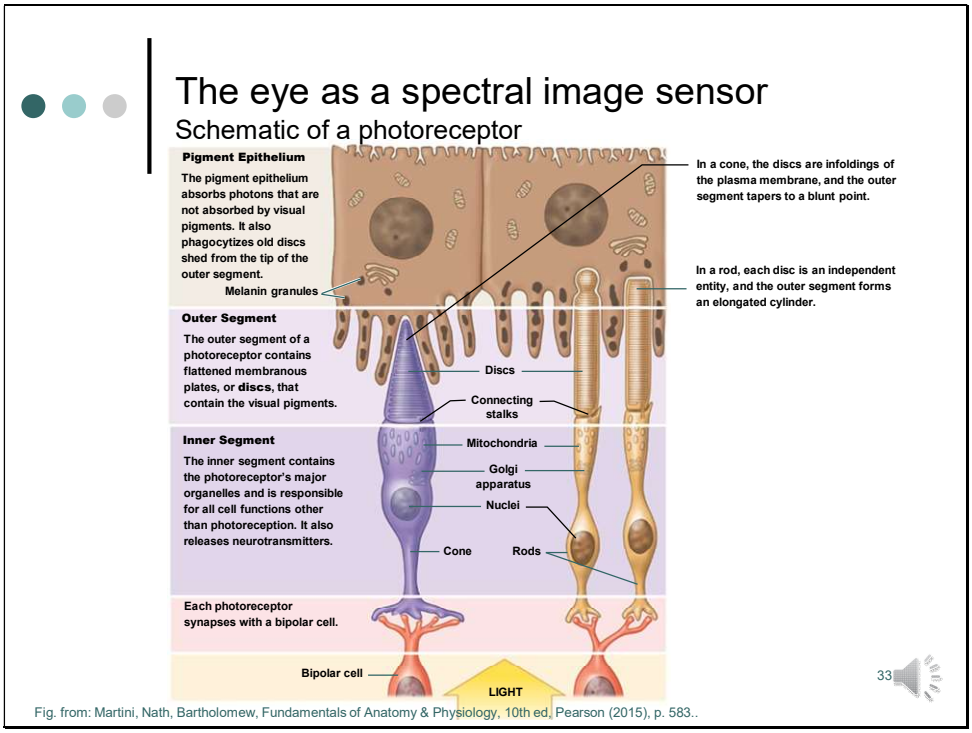
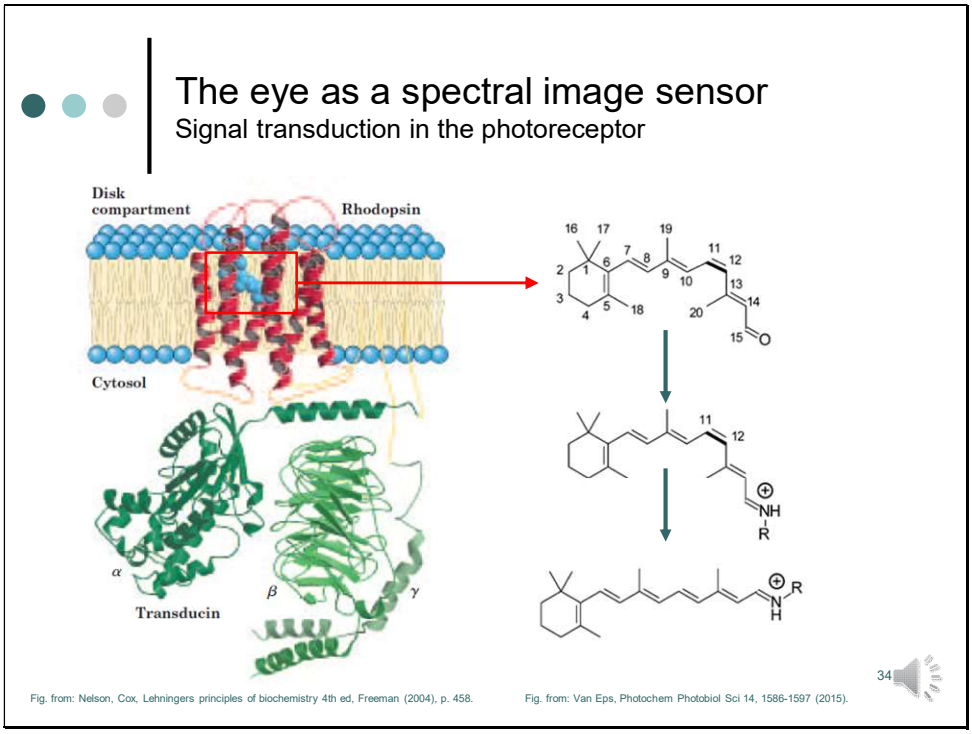


Fig. from: Martini, Nath, Bartholomew, Fundamentals of Anatomy & Physiology, 10th ed, Pearson (2015), p. 583..

This slide shows a magnified view of a photoreceptor.

Photoreceptor cells have two parts, the **inner segment** and the **outer segment:** The inner segment contains the nucleus and other common organelles of a cell, whereas the outer segment is a specialized region in which photoreception takes place. The rod-shaped outer segments of the **rod photoreceptor** contain a stack of membrane-bound discs that contain the photosensitive pigment **rhodopsin**. The cone-shaped outer segments of the **cone photoreceptor** contain their photosensitive pigments in infoldings of the cell membrane.

Slide #27



Rhodopsin consists of an apoprotein, called opsin, and a cofactor, retinal. Opsin is present at high concentrations in the disc membranes of photoreceptor rod cells. It belongs to the family of G-protein coupled receptors (GPCRs). As discussed in the Human Biology module of the 1st semester, GPCRs constitute a large family of seven-transmembrane proteins, that initiate signaling cascades [in eukaryotes]. Upon stimulation, conformational changes within GPCRs trigger the binding and activation of G proteins, which, in turn, amplifies and propagates the signal.

Light sensitivity is conveyed to opsin by retinal, which reacts with the amino group of a lysine residue in the 7th transmembrane domain to form an immine bond or a Schiff base, which is protonated at intracecellular pH.

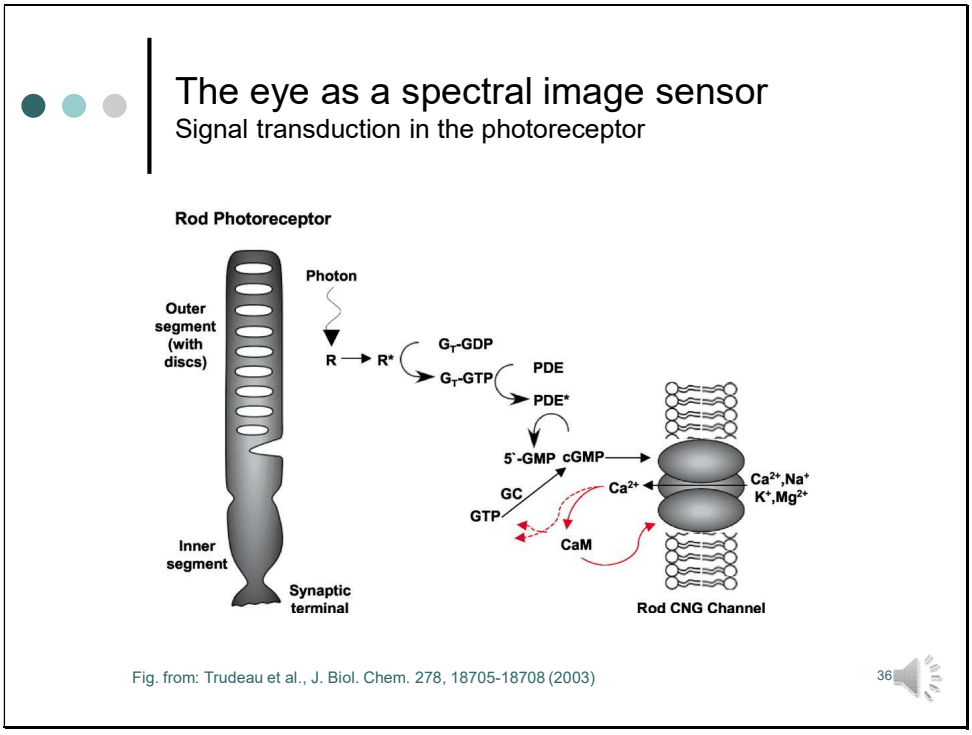
(Retinal is synthesized in the photoreceptors from vitamin A. Vitamin A is chemically all trans-retinol. This is converted to 11-cis-retinol, which then is oxidized to cis-retinal, the aldehyde. If you still remember the pre-course in chemistry, you might wonder about the numbering of the C-Atoms, which does not follow the general IUPAC rules. Since the alkane chain bears more C-atoms than the cyclohexene group, it should be parent compound. Numbering should start at the C-atom being part of the aldehyde group. According to the IUPAC rules, numbering should start from here. The systematic name of Vitamin A or retinol should be (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-l-yl)nona-2,4,6,8-tetraen-1-ol. Numbering in retinol and retinal, however, differs. It follows the system for carotenoids.)

All rods contain the same form of opsin. Cones contain the same retinal molecule that rods do, but the retinal is attached to different forms of opsin. Before interacting with a photon, retinal's double-bond at the 11-position is in the cis-(or Z-) configuration. Photoactivation of rhodopsin causes the 11-cis retinal to isomerize to an all-trans configuration, which triggers a sequence of conformational steps in the opsin molecule. In the activated state, rhodopsin, which then is

called, metarhodopsin, is capable of interacting with the G protein transducin, which in turn is activated.

The photoisomerization is reversed by a series of enzymatic changes so that the retinal responds to more light energy. Until the retinal molecule is changed back to the 11-*cis*-retinal shape, the opsin cannot respond to light energy. When a large group of photopigments is bleached, the retina will send information as if opposing visual information is being perceived. After a bright flash of light, afterimages are usually seen in negative.

Slide #28



The following events are shown in this slide. Transducin stimulates a phosphodiesterase (PDE), which degrades the second messenger 3′,5′-cGMP to 5′-GMP, lowering the concentration of cGMP. As consequence, cyclic nucleotide gated channels (CNG) in the plasma membrane close, because they are no longer activated by cGMP. Since the influx of sodium and calcium is blocked, the transmembrane potential hyperpolarizes. Thus, at the end of this long signaling cascade the arrival of a photon is transduced to a change in membrane potential. At the same time, this process also results in a substantial amplification of the original signal.

Here, we are not going to follow the series of events which transmit the signal to the brain. This will be covered in detail in the Human Biology II lectures.

This signal transduction cascade described here is similar for rods and cones. But, in rods and cones different isoforms of opsins, phosphodiesterase, and CNG channels are involved. Interestingly, in both, rods and cones photosensitivity is conveyed by the same retinal molecule. Accordingly, their distinct spectral properties must be related to the apoprotein opsin.

Slide #29

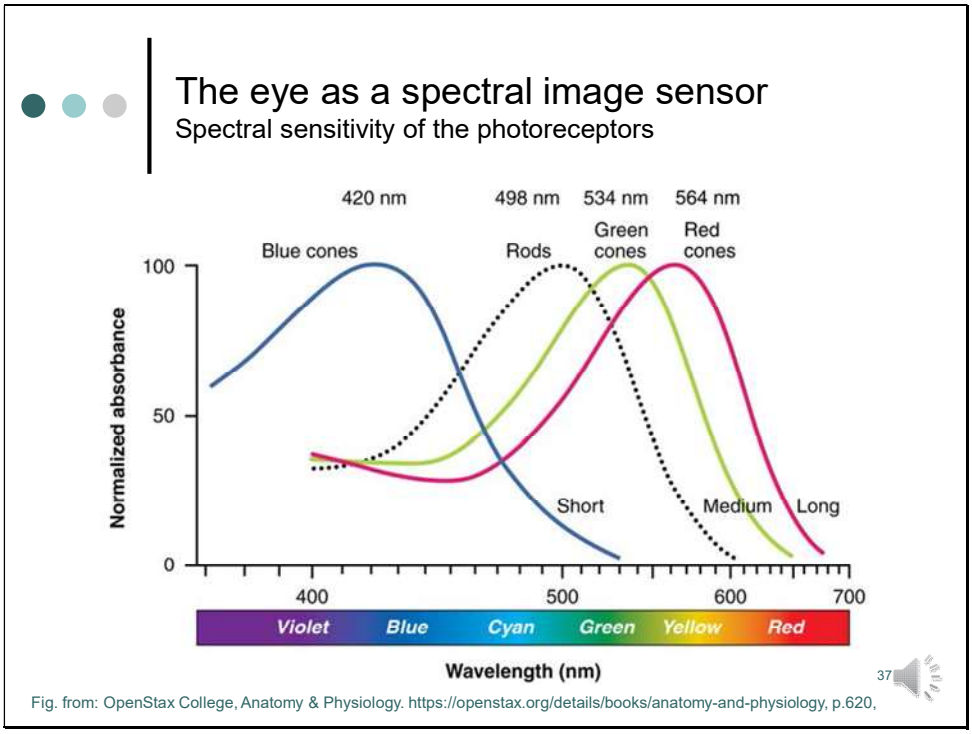


Fig. from: OpenStax College, Anatomy & Physiology. https://openstax.org/details/books/anatomy-and-physiology, p.620,

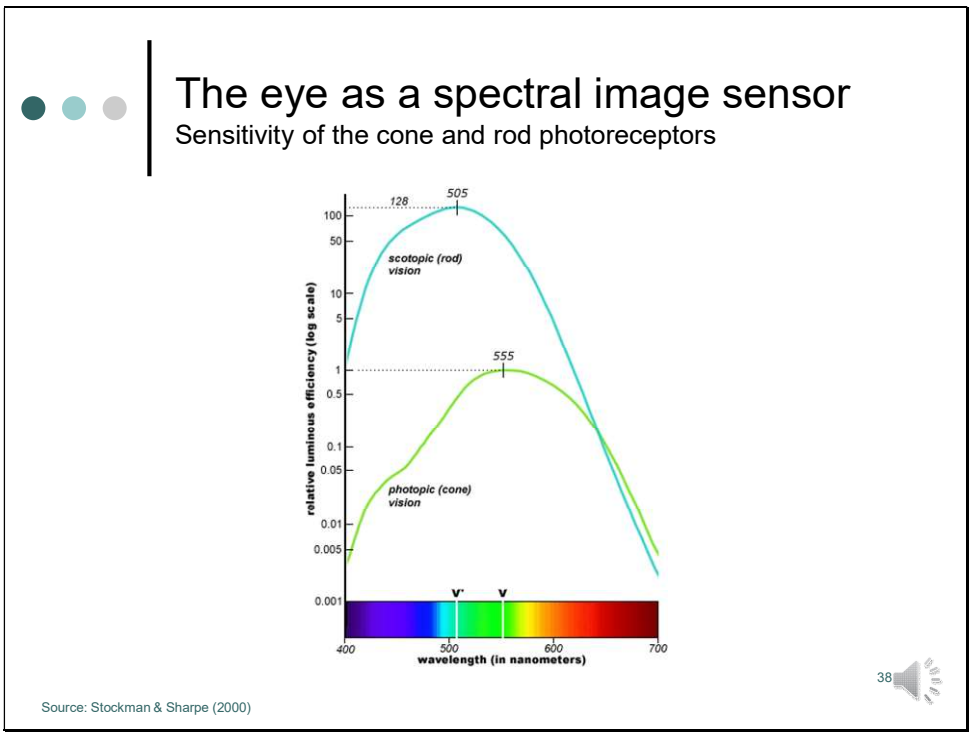
Rhodopsin, the photopigment in rods, is most sensitive to light at a wavelength of 498 nm. According to many medical textbooks, the three color opsins have peak sensitivities of 420 nm, 534 nm and 564 nm, corresponding to the primary colors of blue, green and red.

Interestingly, the absorption maximum of free retinal is located at 360–380 nm regardless of its configuration. Formation of a Schiff base with an amino group does not by itself shift the absorption maximum markedly, but the protonation causes a substantial red shift to 440 nm. Further red shift is explained by interaction between the protonated Schiff base and its counterion, distortion of the conjugated double bond system of the retinal chromophore, and electrostatic perturbation of the π-electron system by polar or charged residues. As it turns out, small variations in the amino-acid sequence of opsin yield pigments with peak sensitivity in different parts of the spectrum.

The three types of cone opsins, being sensitive to different wavelengths of light, provide us with color vision. By comparing the activity of the three different cones, the brain can extract color information from visual stimuli. For example, a bright blue light that has a wavelength of approximately 450 nm would activate the “blue” cones predominantly, the “green” cones marginally, and the “red” cones minimally. The relative activation of the three different cones elicits in the brain the sensation of a blue color.

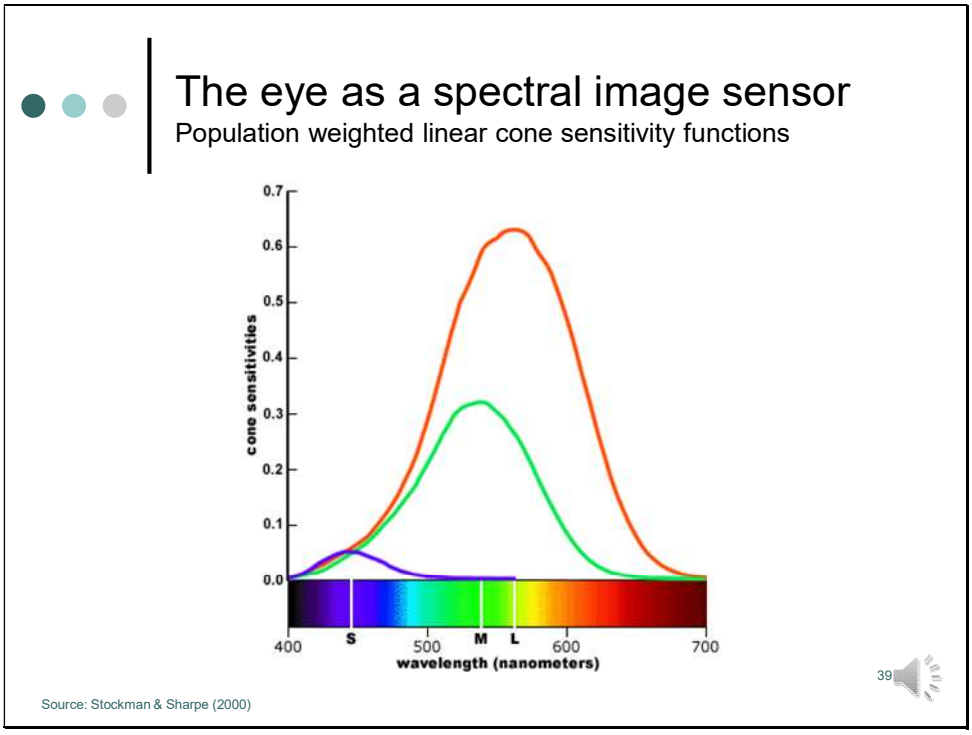
However, cones cannot react to low-intensity light, and rods do not sense the color of light. Therefore, our low-light vision is in essence in grayscale. In other words, in a dark room, everything appears as a shade of gray. If you think that you can see colors in the dark, it is most likely because your brain knows what color something is and is relying on that memory.

Slide #30



This slide compares the overall sensitivity of cone photoreceptors to the sensitivity of rods. As compared to cones the absorption maximum is shifted to shorter wavelengths and the overall sensitivity is more than two orders of magnitude higher.

Slide #31



Now, let's have a detailed look at the cone proportions in the retina.

Approximately 65% of all cones are sensitive to red light, 33% are sensitive to green light, and only about 2% are sensitive to blue light.

The diagram shows the population weighted linear cone sensitivity functions scaled to reflect the cone proportions in the retina. 1.0 is the total cumulative response by all three cones.

Slide #32

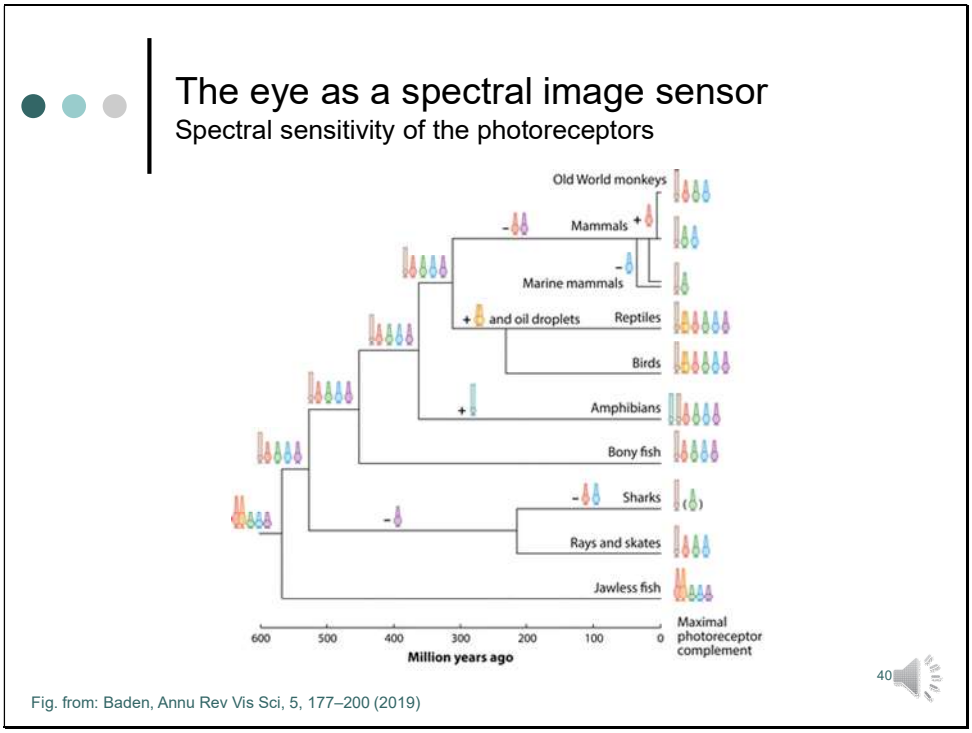
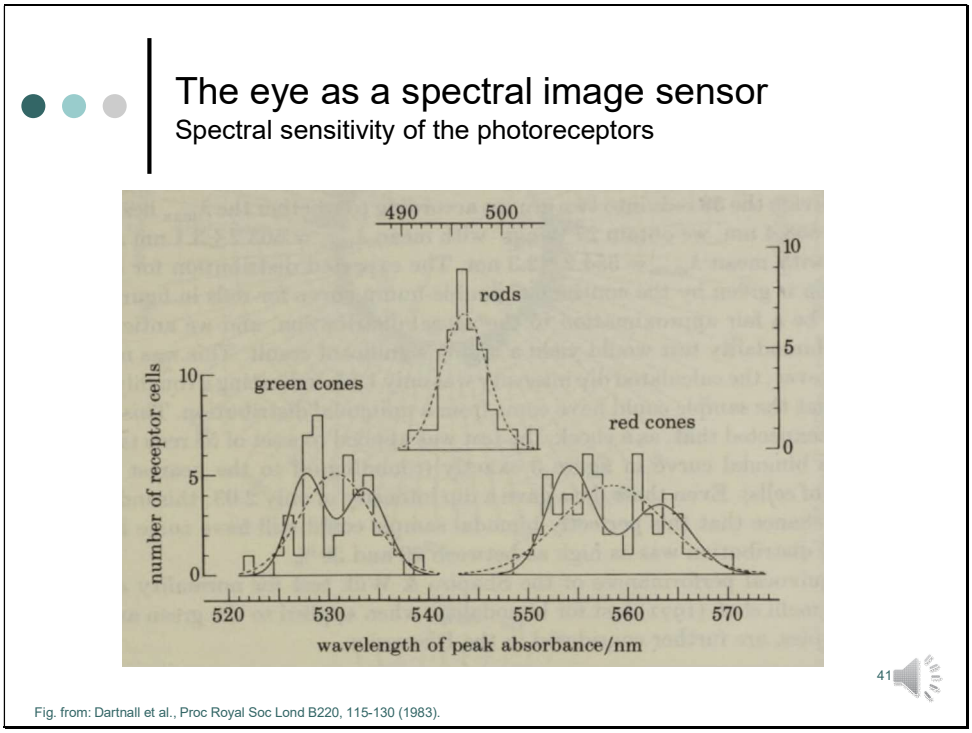


Fig. from: Baden, Annu Rev Vis Sci, 5, 177–200 (2019)

Interestingly, the number of photoreceptors varies in the animal kingdom. This slide shows some photoreceptor lineages. The ancient photoreceptor complement of jawless ancestral vertebrates (leftmost) gave rise to the photoreceptor complements present in jawed vertebrates today (right). At various time points along the way, different lineages added or lost particular photoreceptors. Amongst the mammals, only primates show true trichromatic color vision. The evolutionary drive behind the acquisition of trichromacy is thought to be improved color discrimination in the red/green region of the spectrum, so that ripe fruits and young nutritious leaves can be better detected against the green foliage of the rainforest.

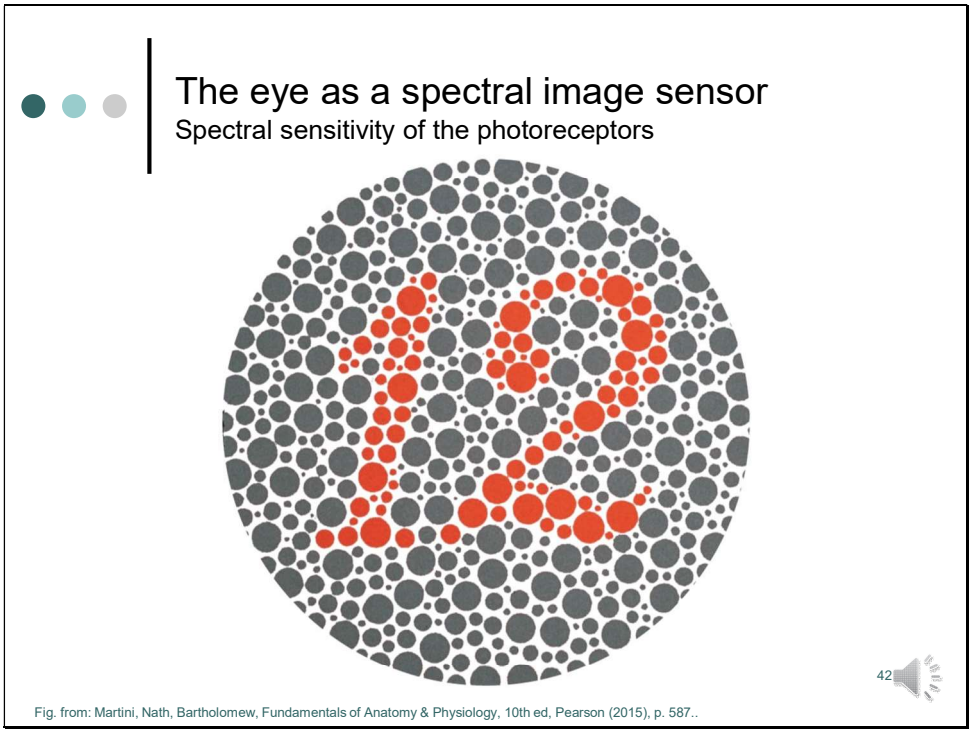
Slide #33



Just one little side remark: According to most medical textbooks there are usually taken to be three normal human cone pigments, with peak sensitivities in the blue, the green and the yellow-green regions of the spectrum. That's what I have shown in the slides before.

But, already in 1983 a microspectrophotometric study of retinae from human patients suggested that there were alternative types of cones. The distribution of the peaks was unimodal for the rods. For the green and red cones, however, there was evidence for bimodal distributions with sub-population maxima at 527.8 ± 1.8 nm (n = 22) and 533.7 ± 2.1 nm (n = 23) for the greens and 554.2 ± 2.3 nm (n = 31) and 563.2 ± 3.1 nm (n = 27) for the reds. Later it was found that the gene for opsin in the red cone was polymorphic.

Slide #34



Persons who are unable to distinguish certain colors have a form of color blindness. Color blindness occurs when one or more types of cones are nonfunctional. The cones may be absent, or they may be present but unable to synthesize the proper visual pigments. In the most common type of color blindness (red–green color blindness), the red cones are missing, so the individual cannot distinguish red light from green light.

The standard test for color vision involves picking numbers or letters out of a complex colored picture such as the one in this slide. People who lack one or more types of cones cannot see the number 12 in this pattern.

Slide #35

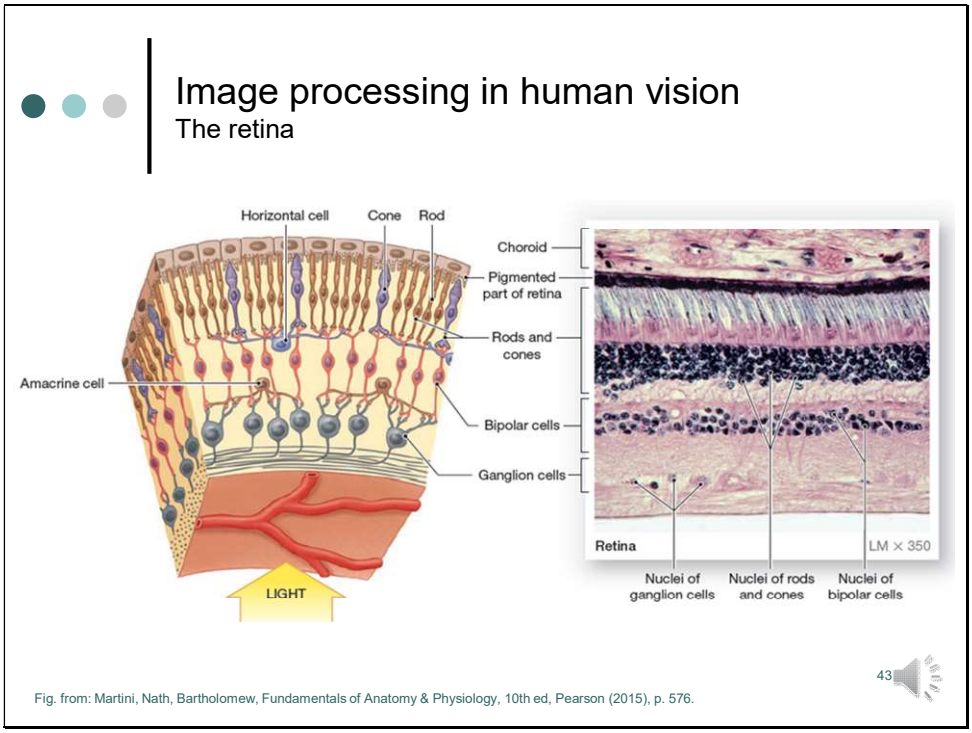


Fig. from: Martini, Nath, Bartholomew, Fundamentals of Anatomy & Physiology, 10th ed, Pearson (2015), p. 576.

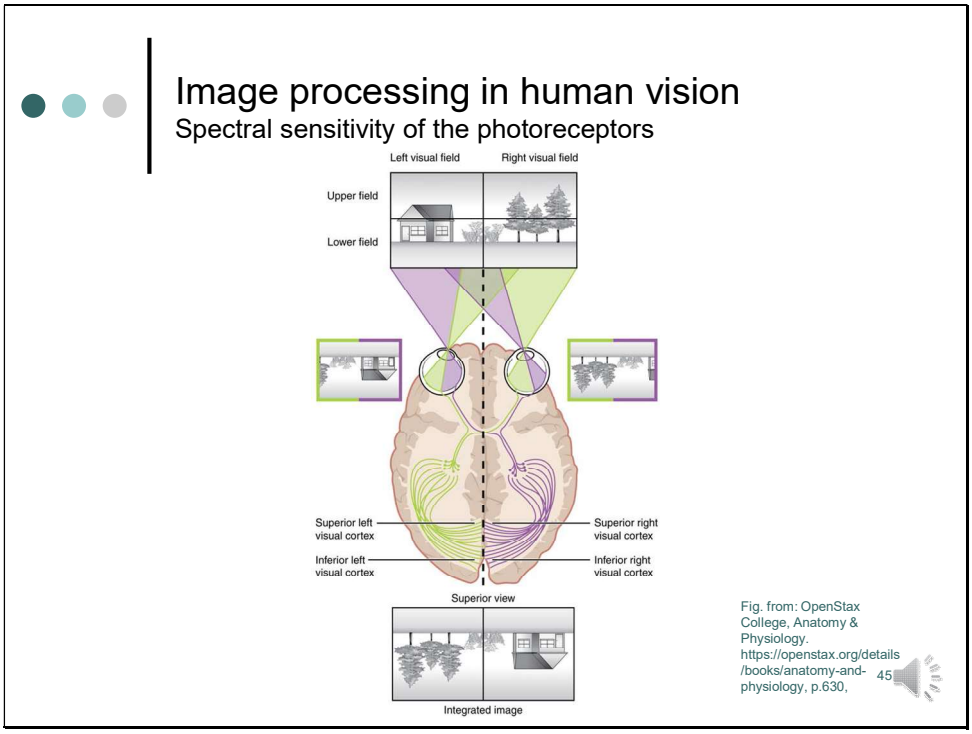
Now that we have discussed, how signal transduction works in the retina, we should briefly discuss how signals are processed.

As discussed before photoreceptors synapse to bipolar cells and bipolar cells synapse to retinal ganglionar cells. Some part of low level image processing is already done in the retina. Many ganglion cells respond strongly to edges in the image, which is the first important step in recognizing shapes. The response of many ganglion cells is also enhanced by moving objects. This makes sense, since objects that move or change suddenly are more worthy of immediate attention than those that do not. In brief, retinal processing extracts low-level features of the scene that are useful for guiding behavior and transmits those selectively to the brain.

Vision has also to operate under many different lighting conditions. The intensity of the light coming from an object depends on the intensity of the illuminating light. The range of intensities encountered in a day is enormous. Variations span 10 orders of magnitude.

Although all these details are fascinating, they are beyond the scope of this lecture. Some aspects will be discussed in the Human Biology lectures. Now, let's continue with our overview. The axons of the retinal ganglionar cells transmit the information to the brain.

Slide #36



A major part the axons projecting from the medial side of the retina decussate at the **optic chiasm**. The axons projecting from the lateral side of the retina do not decussate. Whereas both eyes get input from both fields of view, beyond the optic chiasm, the right field of view of each eye is processed on the left side of the brain, whereas the left field of view of each eye is processed on the right side of the brain.

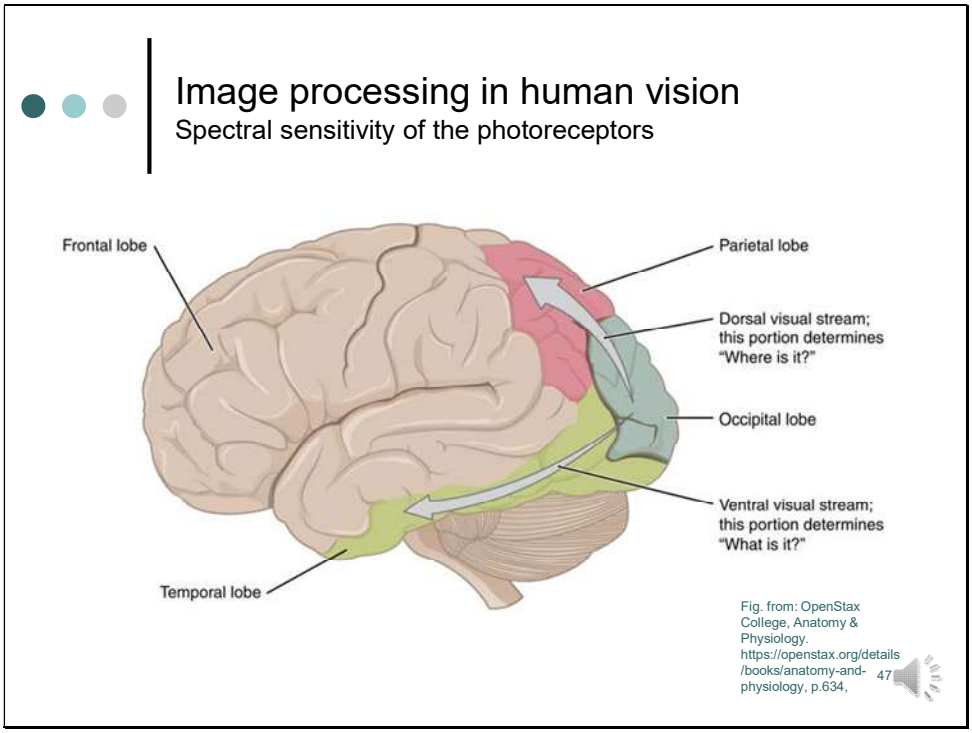
Extending from the optic chiasm, the axons of the visual system are referred to as the **optic tract** instead of the optic nerve. The optic tract has three major targets, one in the midbrain and two in the diencephalon.

The majority of the connections of the optic tract are to the thalamus - specifically, the **lateral geniculate nucleus**. Axons from this nucleus then project to the primary visual cortex, which is located in the occipital lobe.

Another target of the optic tract is the superior colliculus. The superior colliculi of the midbrain issue motor commands that control unconscious eye, head, or neck movements in response to visual stimuli.

In addition, a small number of RGC axons project from the optic chiasm to the **suprachiasmatic nucleus** of the hypothalamus. These RGCs are photosensitive, in that they respond to the presence or absence of light. Unlike the photoreceptors, however, these photosensitive RGCs cannot be used to perceive images. By simply responding to the absence or presence of light, these RGCs can send information about day length. The perceived proportion of sunlight to darkness establishes the **circadian rhythm** of our bodies, allowing certain physiological events to occur at approximately the same time every day.

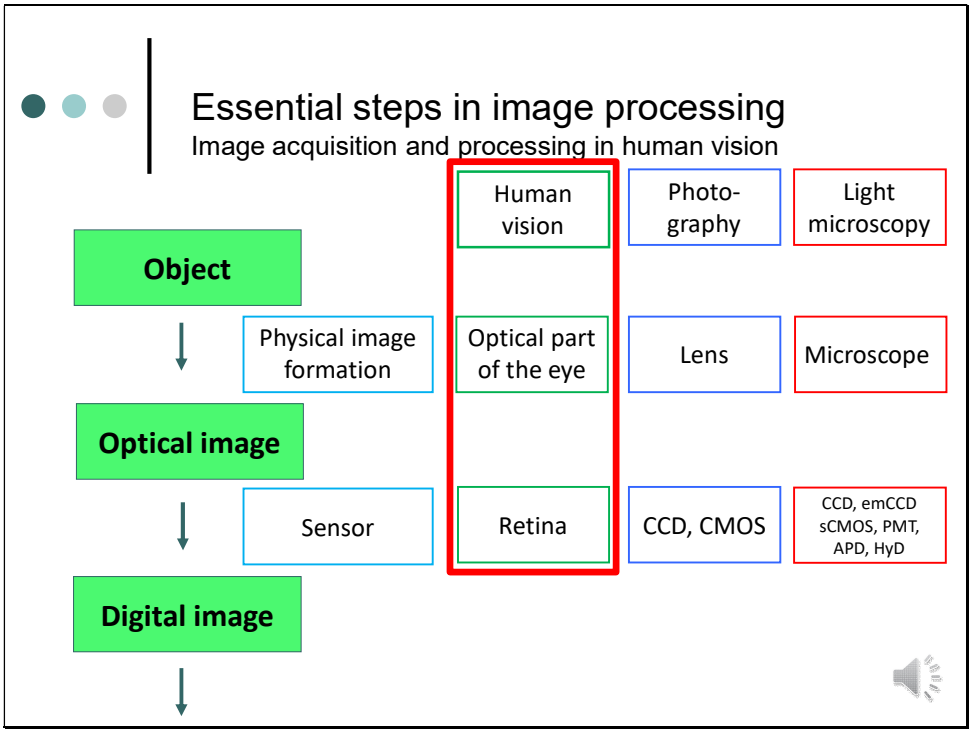
Slide #37



In the cerebral cortex, sensory processing begins at the **primary sensory cortex.** This area is primarily in the medial wall within the longitudinal fissure. Here, visual stimuli begin to be recognized as basic shapes. Edges of objects are recognized and built into more complex shapes. Also, inputs from both eyes are compared to extract depth information. Because of the overlapping field of view between the two eyes, the brain can begin to estimate the distance of stimuli based on **binocular depth cues.**

Sensory processing then proceeds to **association areas**, and finally, into a **multimodal integration areas**. But, details will be discussed in the lectures of Human Biology.

Slide #38



By now we have discussed how images are acquired by the human retina. Some part of low level image processing occurs already in the retina. Extended image processing occurs in the visual cortex. High-Level visual processing, involving object identification occurs in the associated brain regions.

Why is this knowledge so important? Many sensors are made to mimic visual vision. As we will see in future lectures digital cameras have pixels that are sensitive for blue, green and red light. Knowledge of how human vision works can also be an inspiration for technical development.

Moreover, photographic films or sensors are made such that they record what the human eye sees. Photographic color film has at least three sensitive layers, incorporating different combinations of sensitizing dyes, which are typically sensitive for the colors blue, green and red. These layers are often in combined with filter layer to stop light of shorter wavelengths affecting the layers below.