Cell Biology

Unit 6 · Lesson 2 · Vision, insulin and microscopes

The notes for Lesson 2 of Unit 6: chapters 6.3 to 6.4 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 2 of 4 | 2 chapters | Chapters 6.3 to 6.4

About these notes

These are the notes for **Lesson 2 · Vision, insulin and microscopes** of the University free n easy video lessons on Cell Biology Unit 6, Specialized Cells and Cell Study Methods. They are chapters 6.3 to 6.4 of the unit book, unchanged, so a page here is the same page in the full book.

6.3 Cells of vision and pancreatic cells

Two more specialised cells show how the same signalling parts from Unit 5 are rewired for very different jobs. A rod or cone in the retina turns a photon into a change in membrane voltage. A pancreatic cell either secretes digestive enzymes into the gut or senses blood glucose and releases a hormone.

You need: G-protein coupled receptors and second messengers (Unit 5), the secretory pathway through ER and Golgi (Unit 3), and membrane potential (Chapter 6.1).

Rods and cones

Photoreceptors are neurons with a light-catching **outer segment**, a stack of membrane discs full of visual pigment, joined by a thin cilium to an **inner segment** rich in mitochondria, then a nucleus and a synaptic terminal. **Rods** (about 120 million per human eye) are very sensitive and work in dim light, but give no colour. **Cones** (about 6 million, packed in the fovea) need brighter light and give sharp, colour vision. Humans have three cone types, most sensitive to short (blue, about 420 nm), medium (green, about 530 nm) and long (red-yellow, about 560 nm) wavelengths.

Phototransduction: light closes channels

The rod pigment rhodopsin is a GPCR: the protein opsin with 11-cis-retinal (from vitamin A) bound inside. In the dark, cGMP holds cation channels open, so Na^+ and Ca^{2+} flow in (the "dark current"), the rod sits at about −40 mV and releases glutamate steadily.

1. A photon isomerises 11-cis-retinal to all-trans-retinal; rhodopsin changes shape (activated rhodopsin).
2. Activated rhodopsin activates many molecules of the G protein transducin.
3. Transducin's α subunit switches on phosphodiesterase (PDE6), which hydrolyses cGMP.
4. cGMP falls, the cGMP-gated channels close, and the rod hyperpolarises towards about −70 mV.
5. Glutamate release drops; the next neurons (bipolar cells) read this drop as "light".

Each step multiplies the signal, which is why a rod can respond to a single photon. Notice the twist: light makes a photoreceptor *hyperpolarise*, the opposite of most sensory cells.

Pancreatic cells: exocrine and endocrine

Nearly all of the pancreas is **exocrine** (the islets are only about 1–2% of its mass): acinar cells that make digestive enzymes (trypsinogen, amylase, lipase) on rough ER, pack them in the Golgi into zymogen granules at the cell apex, and release them by exocytosis into ducts. George Palade worked out the secretory pathway by following labelled protein through these very cells. Enzymes leave as inactive precursors so they do not digest the pancreas itself.

Scattered among them are the **islets of Langerhans**, the endocrine part: **β cells** make insulin, **α cells** glucagon, **δ cells** somatostatin and **PP cells** pancreatic polypeptide. β cells are the most numerous islet cell.

A β cell is a glucose sensor wired to a secretory machine:

1. Glucose enters through glucose transporters and is phosphorylated by glucokinase, which works in proportion to blood glucose.
2. Glycolysis and mitochondria raise the ATP/ADP ratio.
3. ATP closes ATP-sensitive K^+ channels (K_{ATP}); less K^+ leaves, so the cell depolarises.
4. Voltage-gated Ca^{2+} channels open; Ca^{2+} triggers exocytosis of insulin granules.

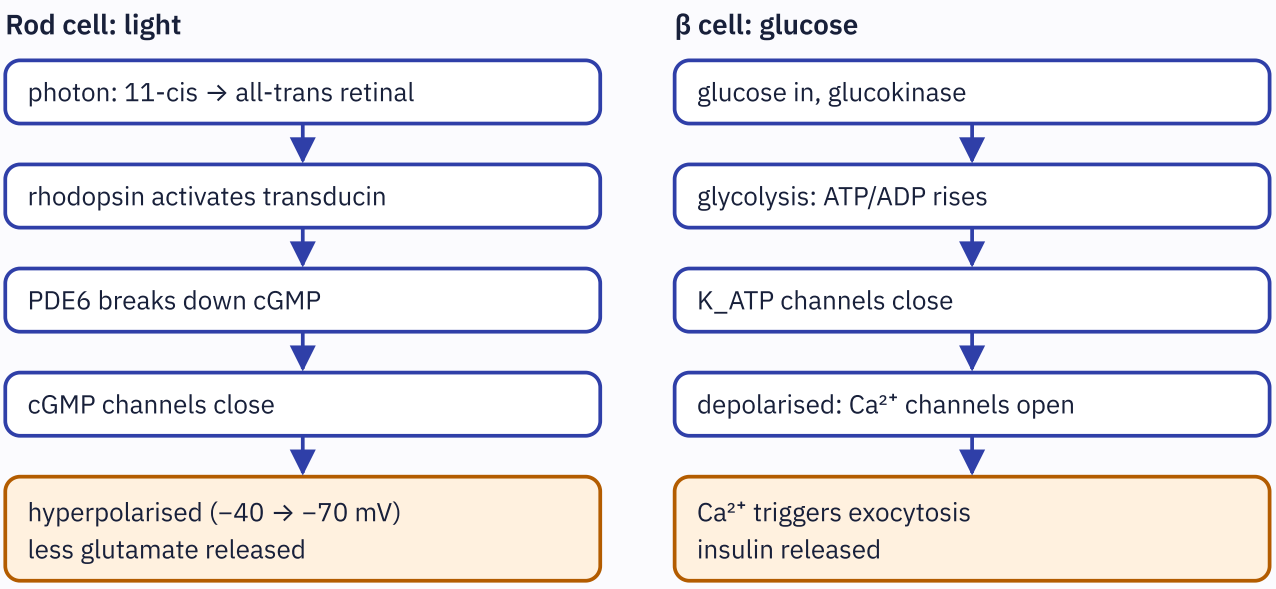


Figure 6.3.1 The two cascades side by side. In both, a chemical change closes a channel; in the rod the cell hyperpolarises and releases less transmitter, in the β cell it depolarises and releases more hormone.

WORKED EXAMPLE

Question (6 marks). (a) Sulfonylurea drugs, used in type 2 diabetes, bind and close the K_{ATP} channel. Predict their effect on insulin release. (b) Some babies are born with a K_{ATP} channel that stays open even when ATP is high. Predict the effect, and suggest a treatment.

1. (a) Closing K_{ATP} mimics high ATP: K^+ outflow drops, the β cell depolarises, Ca^{2+} channels open and insulin is released, even at modest glucose. So sulfonylureas **raise insulin secretion**; their risk is low blood sugar.
2. (b) If the channel cannot close, glucose metabolism raises ATP but the cell stays hyperpolarised. Ca^{2+} channels stay shut and **little insulin is released**: the baby has diabetes from birth (neonatal diabetes).
3. Treatment logic: a drug that closes the channel by another route can restore depolarisation. Many such patients are indeed treated with high-dose sulfonylureas instead of insulin injections.

EXAM TRAPS

- Light hyperpolarises rods and cones; it does not depolarise them. Glutamate release falls in light.
- cGMP, not cAMP, is the second messenger in photoreceptors; PDE breaks it down.
- β cells make insulin and α cells make glucagon; the letters are swapped often.
- Rods: dim light, no colour, one pigment. Cones: bright light, colour, three pigments.

CHECK YOURSELF

– REMEMBER **Name the four cell types of an islet and the hormone each makes.** 2 marks

β: insulin; α: glucagon; δ: somatostatin; PP: pancreatic polypeptide.

– REMEMBER **What are the two parts of rhodopsin?** 1 mark

The protein opsin and the chromophore 11-cis-retinal.

– UNDERSTAND **Why does vitamin A deficiency cause night blindness?** 3 marks

Retinal is made from vitamin A. Without it rods cannot rebuild rhodopsin, and rods are the cells that work in dim light, so vision fails first at night.

– APPLY **A drug blocks PDE6 in rods. What happens to the dark current when light falls on the rod?** 3 marks

cGMP cannot be broken down, so the cGMP-gated channels stay open; the dark current continues, the rod does not hyperpolarise and the light is not signalled.

– ANALYZE **Compare how an acinar cell and a β cell decide when to secrete.** 5 marks

Both store product in granules made through ER and Golgi and release by Ca^{2+}-triggered exocytosis. The acinar cell responds to hormones and nerves from the gut after a meal; the β cell reads blood glucose itself through its metabolism and the K_{ATP} channel. Acinar products go into ducts; insulin goes into blood.

BEYOND THE SYLLABUS

Not for the exam. Researchers now make insulin-secreting β-like cells from stem cells (see Unit 4, Stem cells) and transplant them, and some type 1 diabetes trials have reported patients producing their own insulin again. Gene therapy for an inherited retinal disease (RPE65 mutation) is an approved product. Search next: "stem cell derived beta cells" and "retinal gene therapy RPE65".

6.4 Light and fluorescence microscopy

Most cells are 10 to 100 µm across, too small for the eye (which resolves about 0.1 mm), so cell biology began with the light microscope. A light microscope uses glass lenses to magnify an image formed by visible light; a fluorescence microscope lights the sample with one colour and collects the different colour that tagged molecules glow with, so you see only what you labelled.

You need: cell sizes and organelles (Unit 1, Overview of cell organelles and compartments).

Magnification is not resolution

In a compound microscope the **objective** lens near the sample forms a magnified image, and the **eyepiece** (ocular) magnifies it again. Total magnification = objective × eyepiece, so a 40× objective with a 10× eyepiece gives 400×. Below the stage, the **condenser** focuses light from the lamp onto the sample, and its diaphragm controls the cone of light.

Magnification only makes the picture bigger. **Resolution** is the smallest distance d at which two points still look like two. Because light is a wave, a point spreads into a small blur, and two blurs closer than about d merge. The Rayleigh criterion gives:

$$d = 0.61\,\lambda / NA \qquad NA = n \cdot \sin\theta$$

Here λ is the wavelength of light, NA the numerical aperture of the objective (engraved on its barrel), n the refractive index of the medium between lens and sample, and θ half the angle of the cone of light the objective accepts. A smaller d is better. Two ways to improve it: use shorter λ (blue rather than red) or larger NA. Air has n = 1.0, so a dry objective's NA stays below 1. Immersion oil has n ≈ 1.515, close to glass, so oil objectives reach NA ≈ 1.4. This sets the classic limit of about 0.2 µm: mitochondria and bacteria can be seen; ribosomes (about 25 nm)

and membranes cannot. Magnifying beyond about 1000 × NA adds size but no new detail ("empty magnification").

Getting contrast

Living cells are mostly water and nearly transparent, so in plain **bright-field** they show little detail. Four answers:

- **Staining** fixed cells with dyes: methylene blue or haematoxylin colour nuclei; eosin colours cytoplasm pink; Gram stain separates bacteria. Staining usually kills the cell.
- **Phase contrast** (Zernike, 1930s): converts the tiny delay that light suffers passing through denser parts (nucleus, organelles) into differences in brightness. Ideal for live, unstained cells in culture.
- **Differential interference contrast (DIC**, Nomarski): uses polarised light to show gradients of optical thickness, giving a shaded, 3D-looking image.
- **Dark-field**: blocks direct light, so only light scattered by the sample enters the objective; objects shine bright on black.

Sample preparation, as in a teaching lab: a **wet mount** is a drop of live sample under a coverslip, for motility and shape; a **dry, fixed smear** is spread thin, air-dried, heat- or chemically fixed, then stained. Thick tissues are fixed, embedded in paraffin wax and cut into sections a few micrometres thick with a microtome before staining.

Fluorescence microscopy

A **fluorophore** absorbs a photon of one wavelength (excitation) and, a few nanoseconds later, emits a photon of longer wavelength (emission), because some energy is lost as heat in between. This shift to longer wavelength is the **Stokes shift**, and it is the whole trick: if you block the excitation colour and pass only the emission colour, only labelled structures appear, glowing on a black background.

Most instruments are **epifluorescence** microscopes: light comes down through the objective and the glow goes back up through the same objective. The **filter cube** has three parts:

1. **Excitation filter**: passes only the band that excites the dye (blue for green-emitting dyes).
2. **Dichroic mirror** at 45°: reflects the short excitation wavelengths down to the sample but lets the longer emission wavelengths pass up.
3. **Emission (barrier) filter**: removes any leftover excitation light, passing only the emission band to the eye or camera.

Common labels: **DAPI** and Hoechst bind DNA and glow blue (excited by UV light near 360 nm); **FITC** and Alexa Fluor 488 glow green (excited near 490 nm); rhodamine-type dyes glow red.

Green fluorescent protein (GFP), from the jellyfish *Aequorea victoria*, can be fused to any protein by genetic engineering, so a living cell shows where that protein is (Chapter 6.8). Shimomura, Chalfie and Tsien shared the 2008 Nobel Prize in Chemistry for it. Two practical limits: **photobleaching** (the fluorophore is destroyed by strong light, so the signal fades) and out-of-focus glow from thick samples, which the confocal microscope removes (Chapter 6.5).

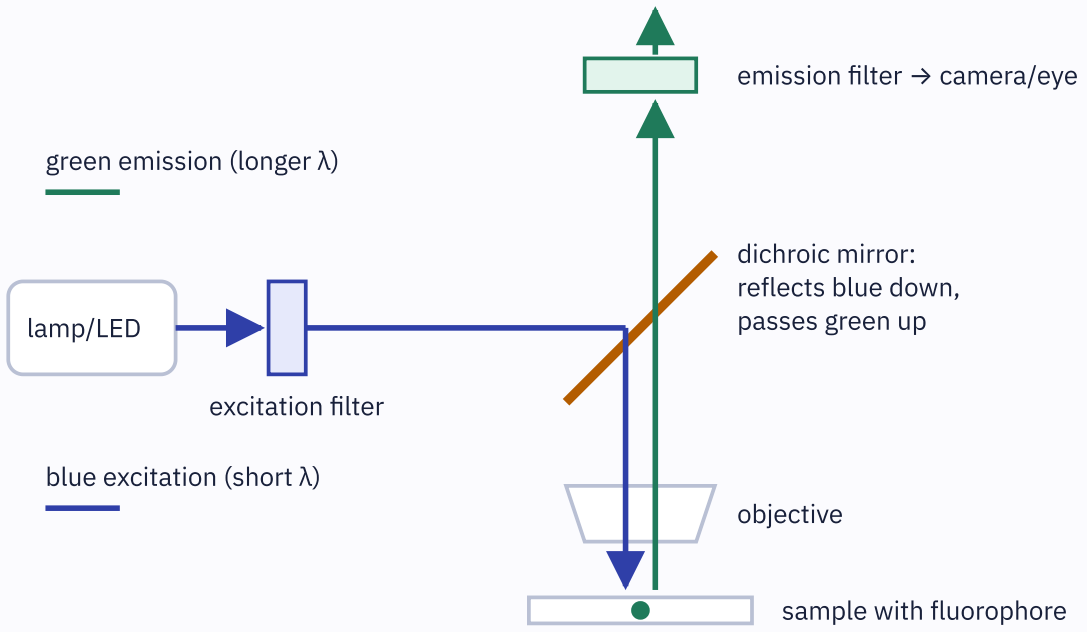


Figure 6.4.1 The filter cube of an epifluorescence microscope. Blue light (short wavelength) is reflected down by the dichroic mirror onto the sample; the green glow (longer wavelength, the Stokes shift) passes straight up through the mirror and the emission filter. Notice that one objective both lights and views the sample.

WORKED EXAMPLE 1: RESOLUTION

Question (4 marks). Find the resolution with green light (λ = 550 nm) for (a) a dry 40× objective, NA 0.65, and (b) an oil 100× objective, NA 1.40. Can either separate two vesicles 300 nm apart?

1. (a) d = 0.61 × 550 / 0.65 = 335.5 / 0.65 = **516 nm ≈ 0.52 µm**.
2. (b) d = 0.61 × 550 / 1.40 = 335.5 / 1.40 = **240 nm ≈ 0.24 µm**.
3. 300 nm is larger than 240 nm but smaller than 516 nm, so only the **oil objective** shows two vesicles; the dry lens shows one blur.

WORKED EXAMPLE 2: COUNTING CELLS WITH A HAEMOCYTOMETER

Question (4 marks). A yeast culture is diluted 1:10 and loaded on an improved Neubauer haemocytometer. Counts in the four large corner squares (each 1 mm × 1 mm, chamber depth 0.1 mm) are 52, 48, 55 and 45. Find the cells per mL in the original culture.

1. Mean count per large square = (52 + 48 + 55 + 45) / 4 = 200 / 4 = 50 cells.
2. Volume over one large square = 1 mm × 1 mm × 0.1 mm = 0.1 mm^3 = 0.1 µL = 1×10^{-4} mL.
3. Cells per mL in the diluted sample = 50 / 10^{-4} = 5.0×10^5.
4. Undo the dilution: $5.0 \times 10^5 \times 10$ = **5.0×10^6 cells/mL**.

EXAM TRAPS

- Higher magnification does not mean better resolution; resolution depends on λ and NA only.
- Smaller d means better resolving power. Students often say "resolution increased" when d grew.
- Emission is at a longer wavelength than excitation, never shorter.
- The dichroic mirror reflects excitation and transmits emission; it is not an ordinary mirror or a filter that blocks both.
- In the haemocytometer, one large square holds 10^{-4} mL; forgetting the depth (0.1 mm) gives an answer 10 times too small.

CHECK YOURSELF

– REMEMBER **Write the Rayleigh equation and define NA.** 2 marks

d = 0.61 λ / NA, with NA = n sin θ (n: refractive index of the medium; θ: half-angle of the light cone the objective accepts).

– REMEMBER **Name the three parts of a fluorescence filter cube.** 3 marks

Excitation filter, dichroic mirror, emission (barrier) filter.

– UNDERSTAND **Why does immersion oil improve resolution?** 3 marks

Oil (n ≈ 1.515) matches glass, so light bent at wide angles is not lost by refraction at an air gap; the objective collects a wider cone, NA rises to about 1.4 and d falls.

– UNDERSTAND **Which method would you use to watch unstained live cells divide, and why?** 2 marks

Phase contrast (or DIC): it turns differences in optical path into brightness, so transparent living cells show detail without stains that would kill them.

– APPLY **Find d for blue light (450 nm) with NA 1.40.** 2 marks

d = 0.61 × 450 / 1.40 = **196 nm ≈ 0.20 µm**; shorter λ gives finer resolution.

– APPLY **Haemocytometer counts of 30, 34, 28 and 28 cells in the four large squares, undiluted sample. Cells per mL?** 3 marks

Mean = 120 / 4 = 30; 30 / 10^{-4} = **3.0×10^5 cells/mL**.

– ANALYZE **A student sees a dim green signal everywhere in a thick tissue slice and it fades over a minute. Explain both observations and suggest fixes.** 5 marks

Everywhere-glow: fluorescence from planes above and below focus is also collected in a widefield microscope; use thinner sections or a confocal microscope. Fading: photobleaching; reduce light intensity or exposure, use an antifade mounting medium or a more stable dye.

BEYOND THE SYLLABUS

Not for the exam. Super-resolution methods such as STED, PALM and STORM beat the 0.2 µm limit by switching fluorophores on and off so that nearby molecules glow at different times; they won the 2014 Nobel Prize in Chemistry. Light-sheet microscopy lights only a thin plane, so whole embryos can be filmed for days with little bleaching. Expansion microscopy swells the sample itself in a gel. Search next: "STED microscopy", "light-sheet microscopy" and "expansion microscopy".

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
Phototransduction	Light → rhodopsin → transducin → PDE6 → cGMP falls → channels close → hyperpolarisation	6.3
β-cell coupling	Glucose → ATP/ADP up → K_ATP closes → depolarisation → Ca^{2+} in → insulin exocytosis	6.3
Resolution (Rayleigh)	$d = 0.61 \lambda / NA$; $NA = n \sin \theta$	6.4
Haemocytometer	cells/mL = mean count per large square × 10^4 × dilution factor	6.4

Glossary (terms used in this lesson)

cGMP Cyclic GMP; holds photoreceptor cation channels open in the dark.

Confocal microscope A point-scanning fluorescence microscope whose detector pinhole rejects out-of-focus light.

Dichroic mirror A mirror that reflects short wavelengths and transmits longer ones.

Haemocytometer A counting chamber of known depth and grid area used to count cells.

Islets of Langerhans Endocrine clusters in the pancreas containing α, β, δ and PP cells.

K_ATP channel A K^+ channel closed by ATP; it couples glucose metabolism to insulin release.

Photobleaching Loss of fluorescence as light destroys the fluorophore.

Resolution The smallest distance at which two points can be seen as separate.

Rhodopsin The rod visual pigment: opsin plus 11-cis-retinal.

Stokes shift The difference between excitation and longer emission wavelength of a fluorophore.

The full unit book

Every chapter of Unit 6, the unit summary, the exam question bank with model answers and the full glossary: Cell Biology · Unit 6 · Specialized Cells and Cell Study Methods (one file, works offline).

Sources and further reading

- Alberts B. et al., *Molecular Biology of the Cell*, 7th edition (2022), W. W. Norton: chapters on visualising cells, analysing cells and molecules, and the cytoskeleton (from memory; check).
- Lodish H. et al., *Molecular Cell Biology*, W. H. Freeman: chapters on culturing, visualising and fractionating cells, and on nerve cells (from memory; check).
- Karp G., *Cell and Molecular Biology: Concepts and Experiments*, Wiley: chapter on techniques in cell and molecular biology (from memory; check).
- Cooper G. M., *The Cell: A Molecular Approach*, Sinauer/Oxford University Press: chapter on tools of cell biology (from memory; check).
- Hall J. E., *Guyton and Hall Textbook of Medical Physiology*, Elsevier: chapters on membrane potentials, skeletal muscle contraction, the retina and insulin (from memory; check).
- Wikipedia, "Cell fractionation": en.wikipedia.org/wiki/Cell_fractionation (overview of homogenisation and isotonic, cold buffers).
- Abcam, "Cell fractionation: a complete overview": abcam.com knowledge center (differential and density-gradient centrifugation, purity checks by Western blot).

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