

Evolution—the first single-molecule biophysicist

Phil Nelson

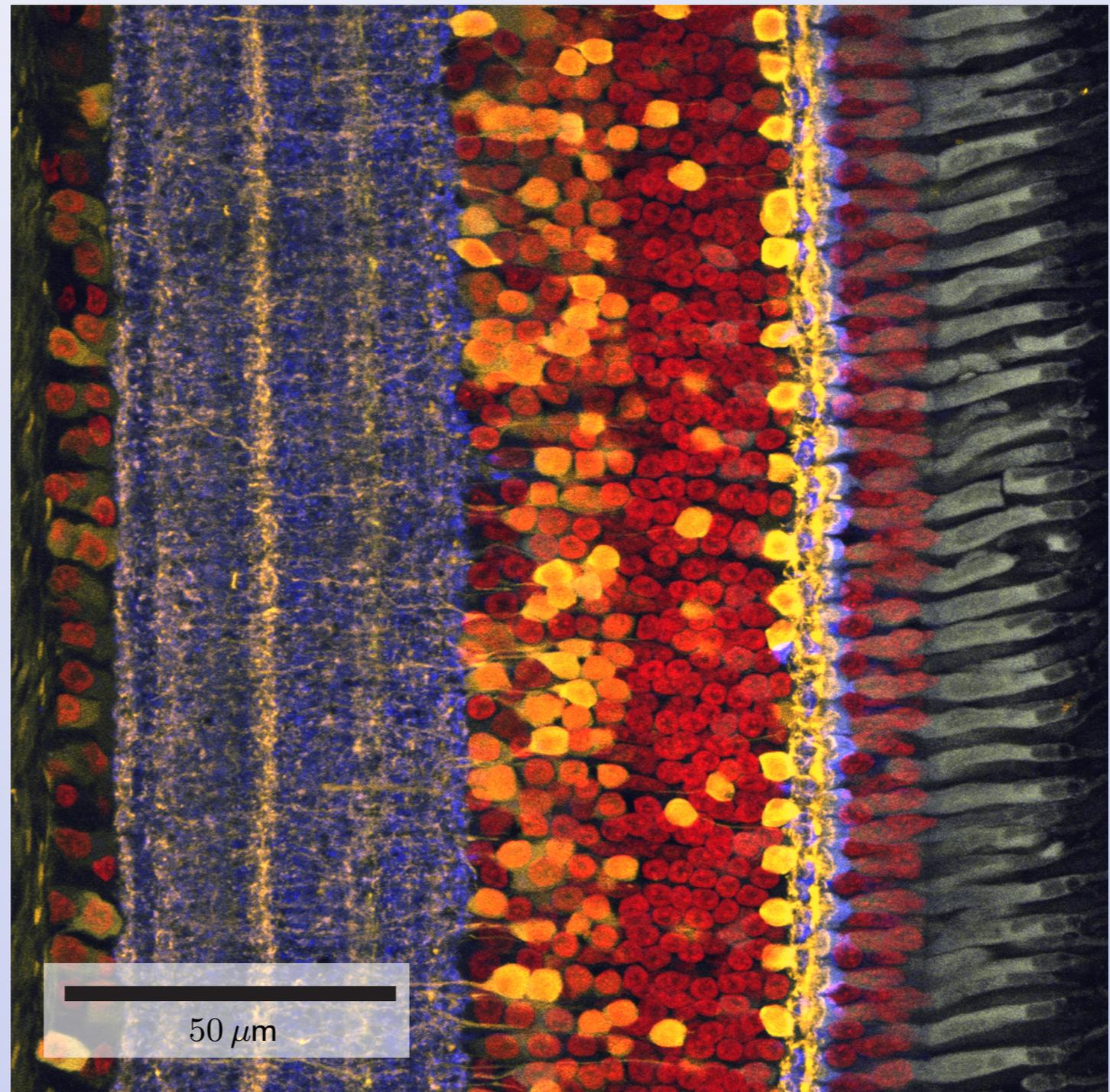
University of Pennsylvania

For these slides see:

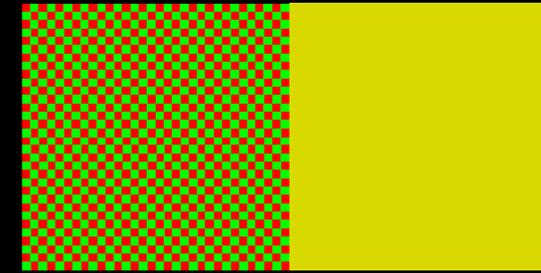
www.physics.upenn.edu/~pcn

Image by Andy J Fischer.

direction
of light



These two panels may look similar in color, particularly if you're sitting in back (or remove your glasses).

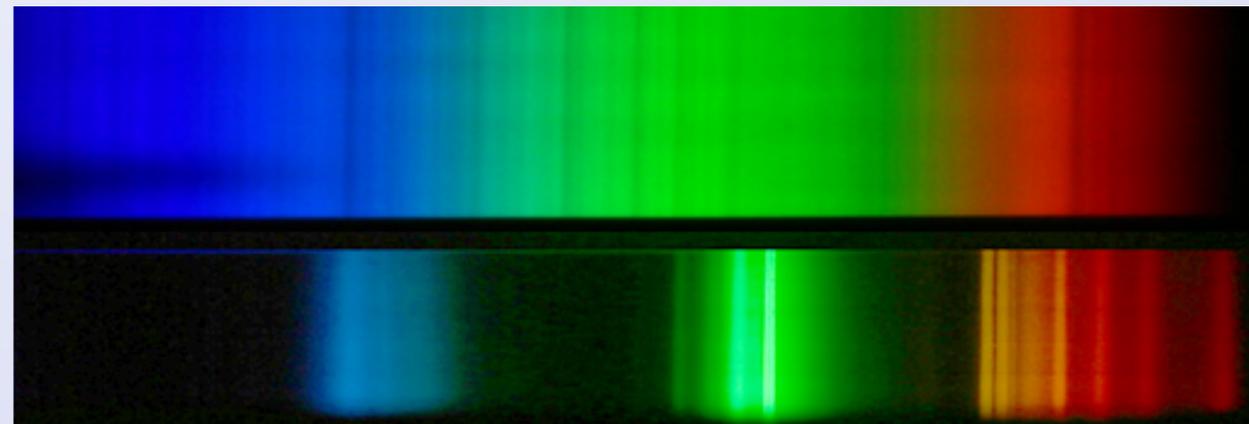


But when I blow them up you see that actually the left panel consists of vivid green and red!

How could anything like that possibly happen at all?
I.e., How could your eyes be so bad that they can't even tell spectral yellow from red+green?

Sunshine

Compact fluorescent
light bulb



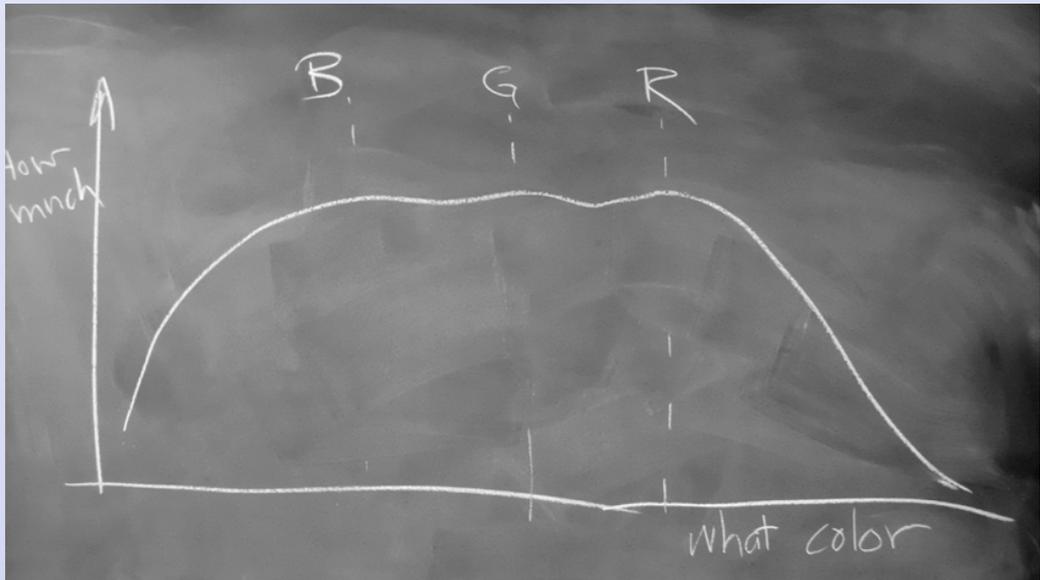
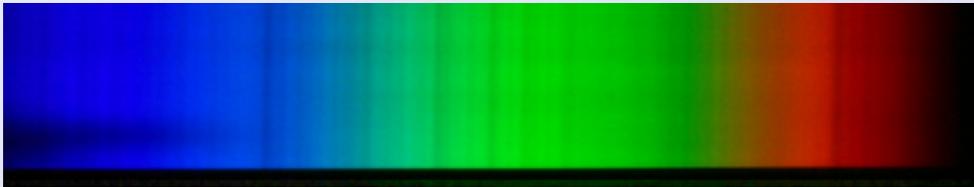
How can two such different kinds of light both look the same?

Just two topics

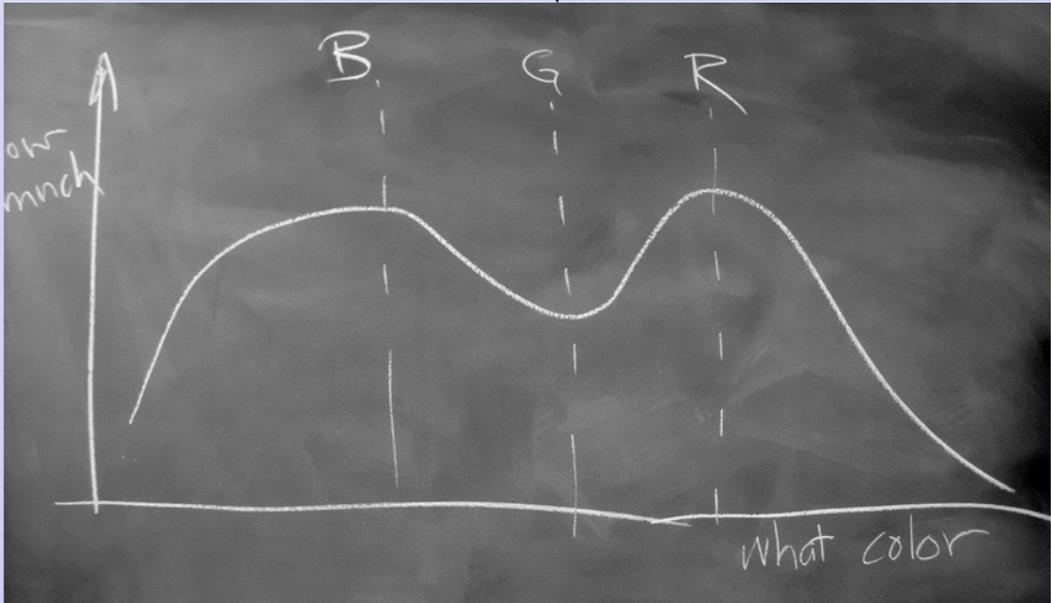
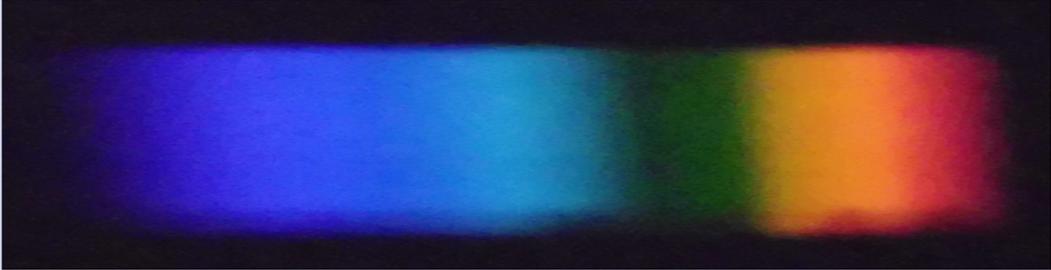
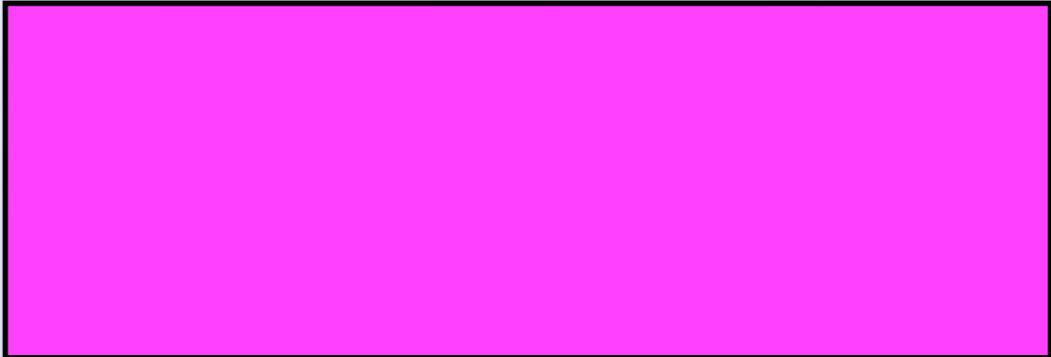
- What is color and how do we see it?
 - [Can we make a gadget that discriminates colors better than humans, and would that be useful?]
- What sets the ultimate limit on our visual sensitivity, and how close are we to that limit?
 - [And how do our eyes manage that?]

Light spectrum, or color content curve

Sunlight



Colored light



Thomas Young, 1802

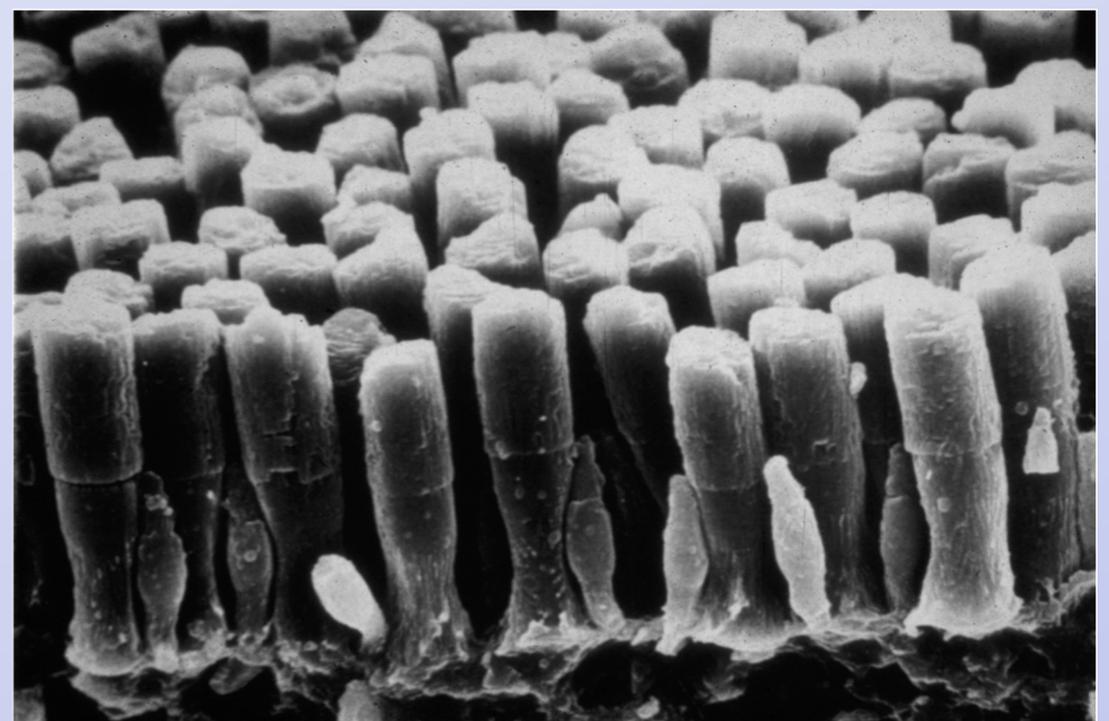
An astonishingly modern chain of hypotheses:

1. Light comes in different flavors (let's call them "spectral positions").
2. Even when mixed, those flavors retain their distinct character and can be re-separated.
3. "Color" involves the relative *amounts* of these flavors.
4. Our eyes contain a mosaic of "pixels" ("photoreceptor cells").
5. *All the brain can know* about color is what it hears these cells saying.

And the key point:

6. Each photoreceptor cell is *only sensitive to a particular range of color*: The cells are "tuned."

Rod cells and cone cells in the retina of the tiger salamander.
Image by Scott Mittman and Maria T. Maglio



Thomas Young (continued)

Continuing Young's chain of reasoning,

1. Light comes in different flavors (“spectral positions”).
2. Even when mixed, those flavors retain their distinct character and can be re-separated.
3. “Color” involves the relative *amounts* of these flavors.
4. Our eyes contain a mosaic of “pixels” (“photoreceptor cells”).
5. *All the brain can know* about color is what it hears these cells saying.
6. Each photoreceptor has a distinct sensitivity range.
7. They come in just 3 classes. Each cell has exactly the same sensitivity range as all the others in its class.

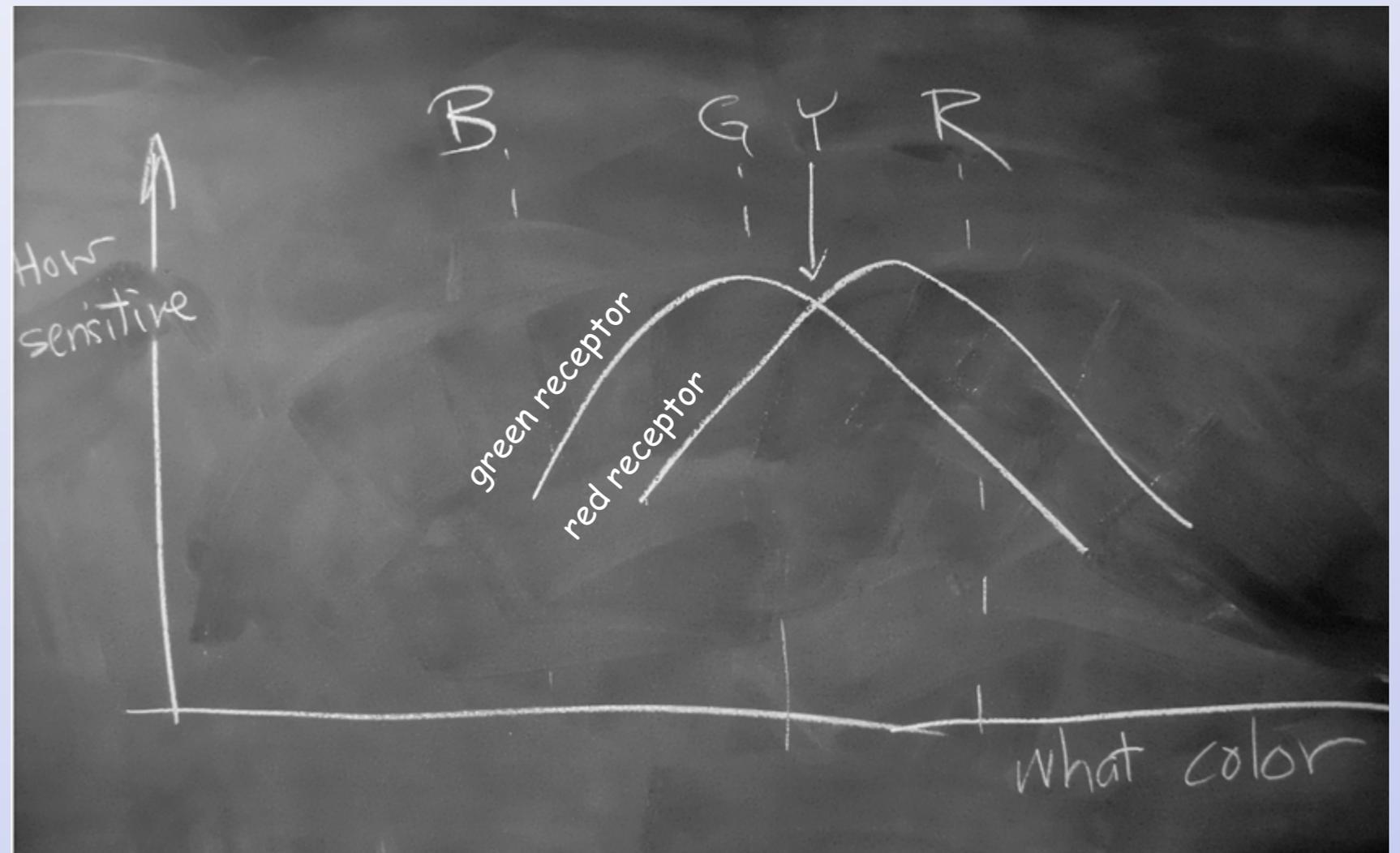
Resolution of the R+G=Y paradox

This list of the sensitivities of a photoreceptor cell to light of various spectral positions can also be drawn as a graph. Unlike the light spectrum, which tells “how much is present,” this **sensitivity curve** expresses “how much is *needed*” to get a response to each kind of light.

Forget about blue and consider only red- and green-sensitive cells:

If the sensitivity curves *overlap*, then sending in pure spectral yellow will excite both the green-sensitive and the red-sensitive cells equally.

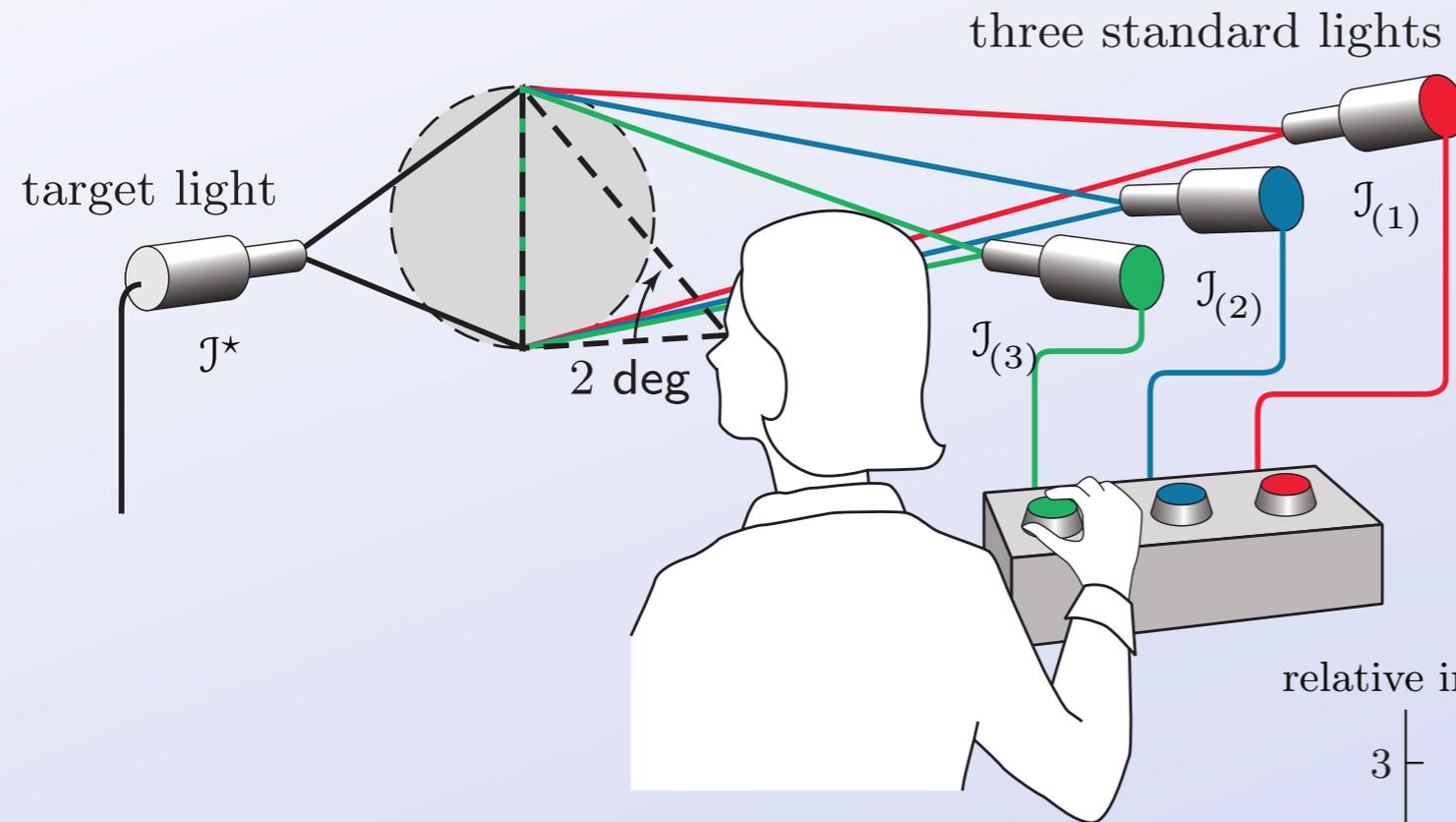
But the same result can be achieved by sending in equal amounts of pure green and pure red light!



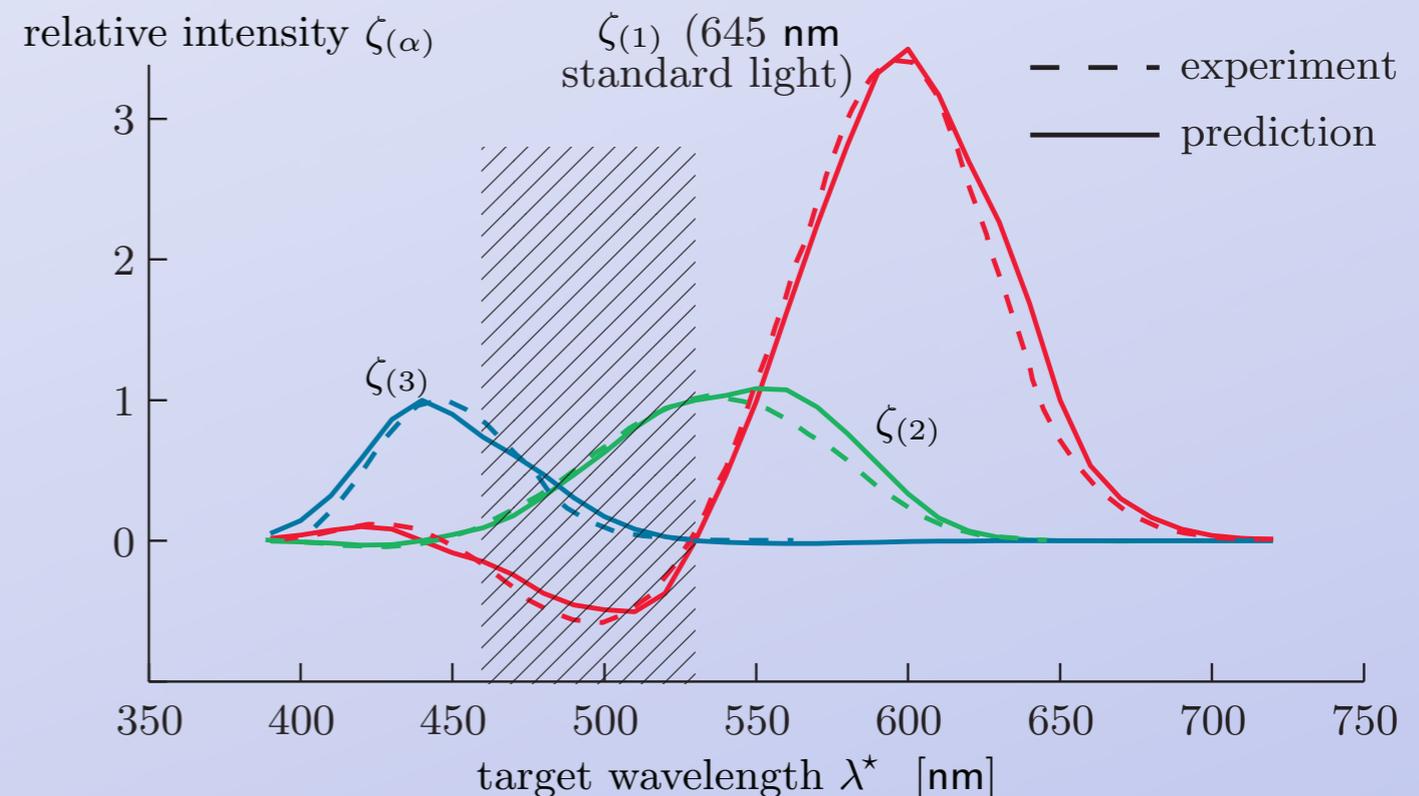
The brain can't tell the difference because *all it knows is what the receptor cells tell it.*

A quantitative test

Quantitative, detailed, testable prediction is crucial. Long before the underlying machinery was understood, people like Maxwell and Helmholtz were already testing Young's hypothesis:



Result: three “color matching curves”:

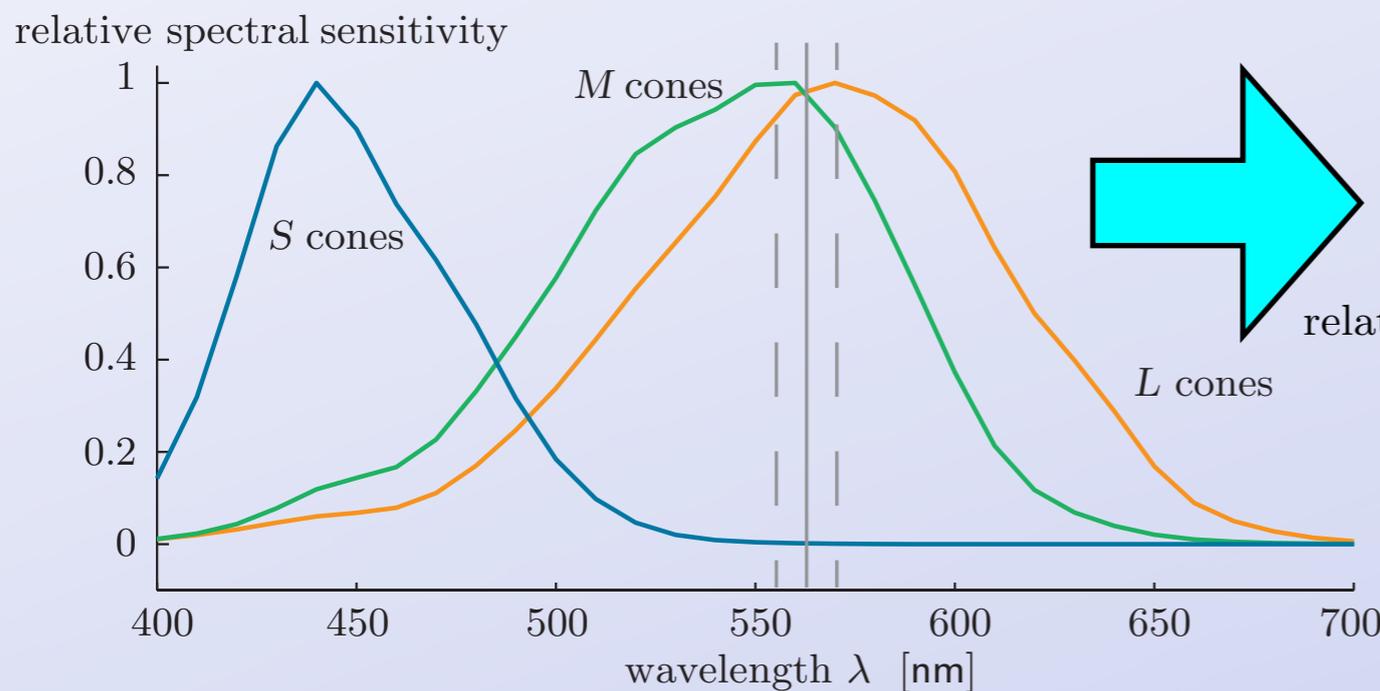


How the theory makes testable predictions

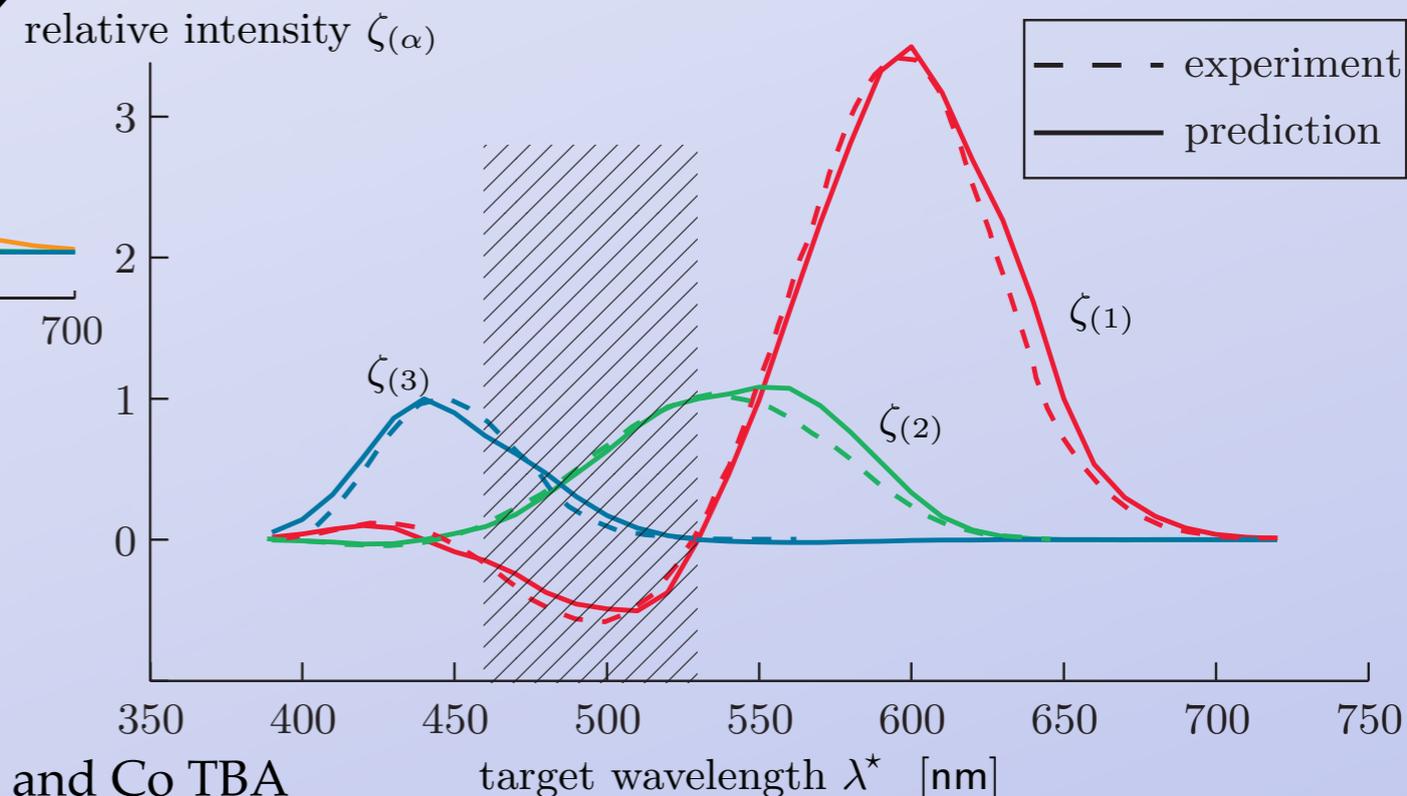
Once we measure the sensitivity curves, we can predict the response of each photoreceptor to any possible light spectrum. Then we can find out how much of each of the three standard lights is needed to *mimic* the response elicited by the target light, by solving *three linear equations*.

Left: The sensitivity curves of color photoreceptors indeed fall into three well-separated classes. Notice the big overlap between the “red” and “green” curves.

Right: Once those curves are known, the color-matching functions can be predicted, and they agree with psychophysical measurements.



Data from Julie Schnapf and Denis Baylor 1987.

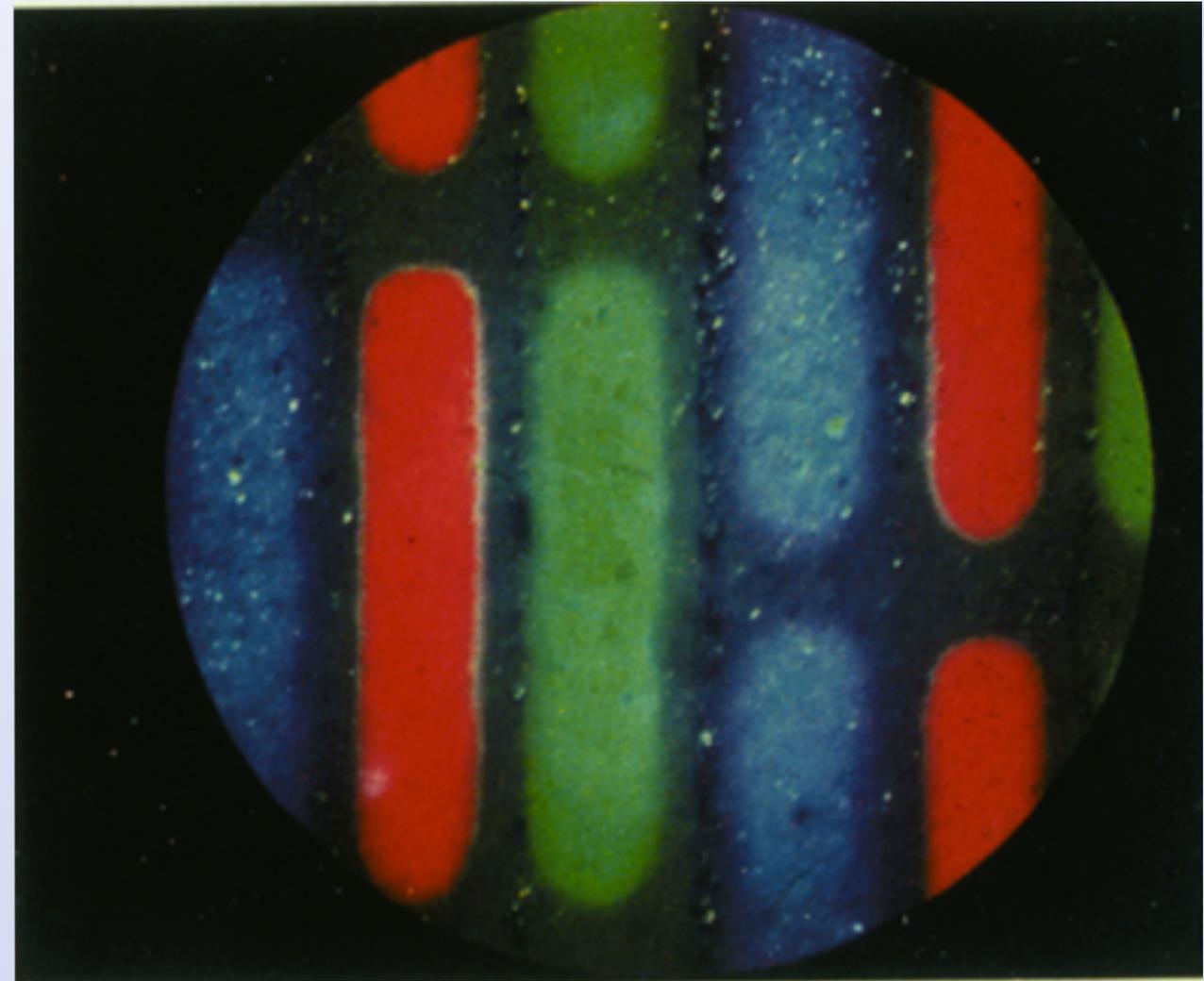


First tech payoff

You can fool the eye into thinking that a wide range of colors is present by using just three pixel types...

Mixing 3 colors is enough to match (almost) any color. That's good for making inexpensive computer displays.

But turning it around: Our eyes *discard* a lot of information about the spectrum of light entering any given visual field! **Can an artificial visual system discriminate *better* than that?**



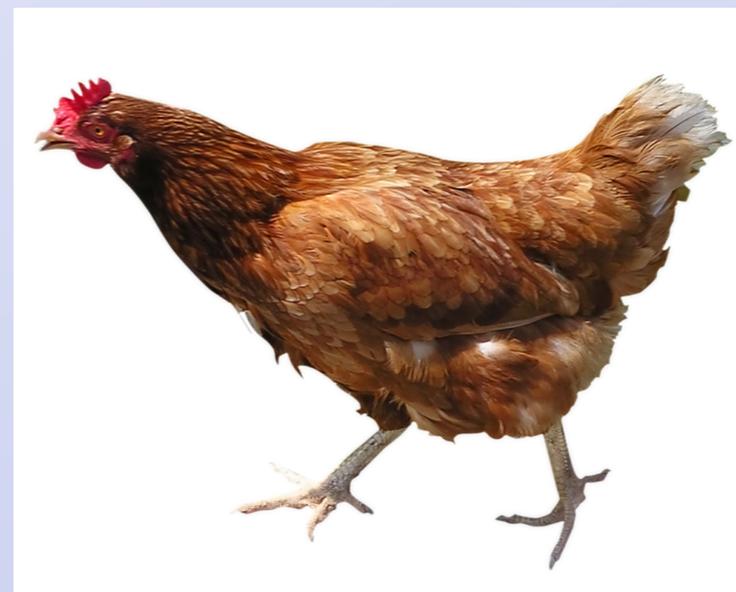
Superhuman color vision

OK, those were 19C experiments, confirmed in 1980s. Is that *all*?

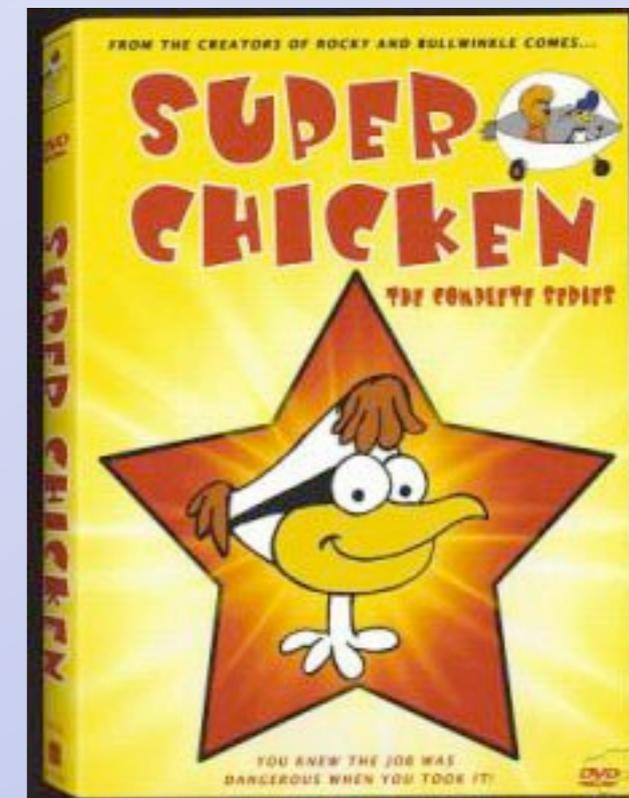
Subhuman ~~Human~~ color vision



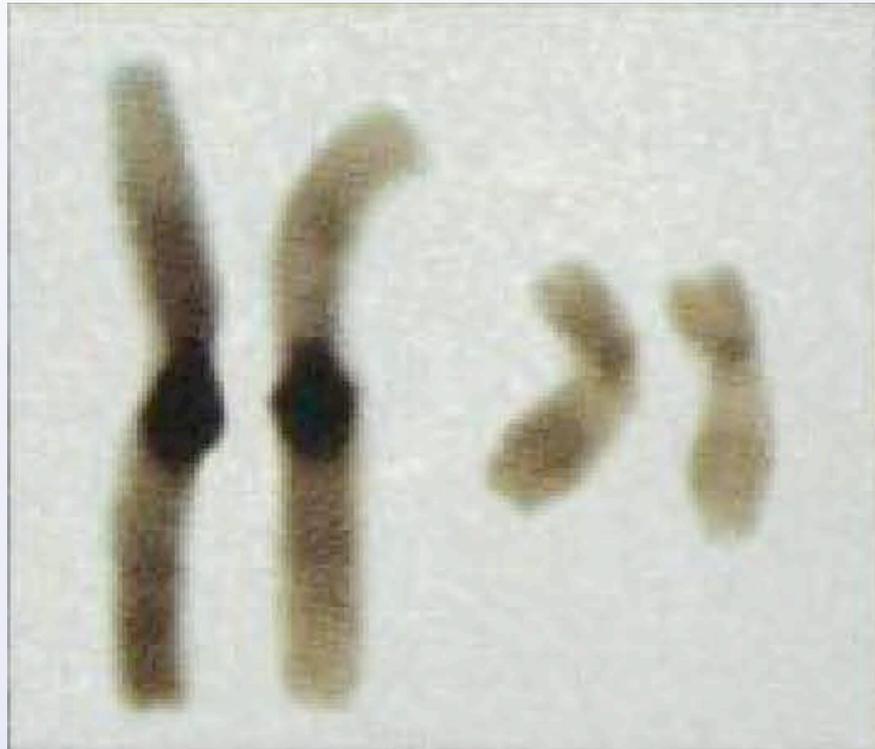
Superhuman color vision



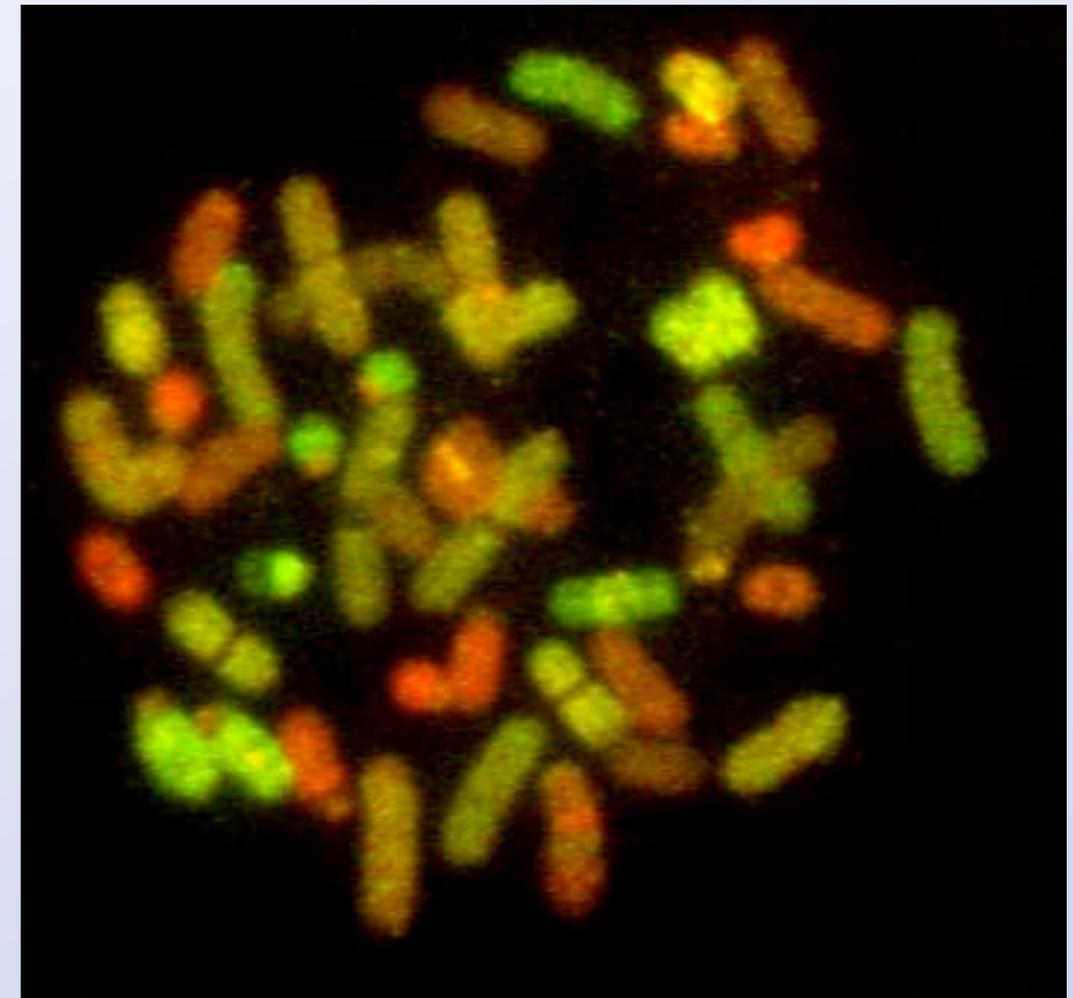
Superchicken?



Karyotyping



One-color (DAPI) staining can reveal, some, not all, chromosome abnormalities.

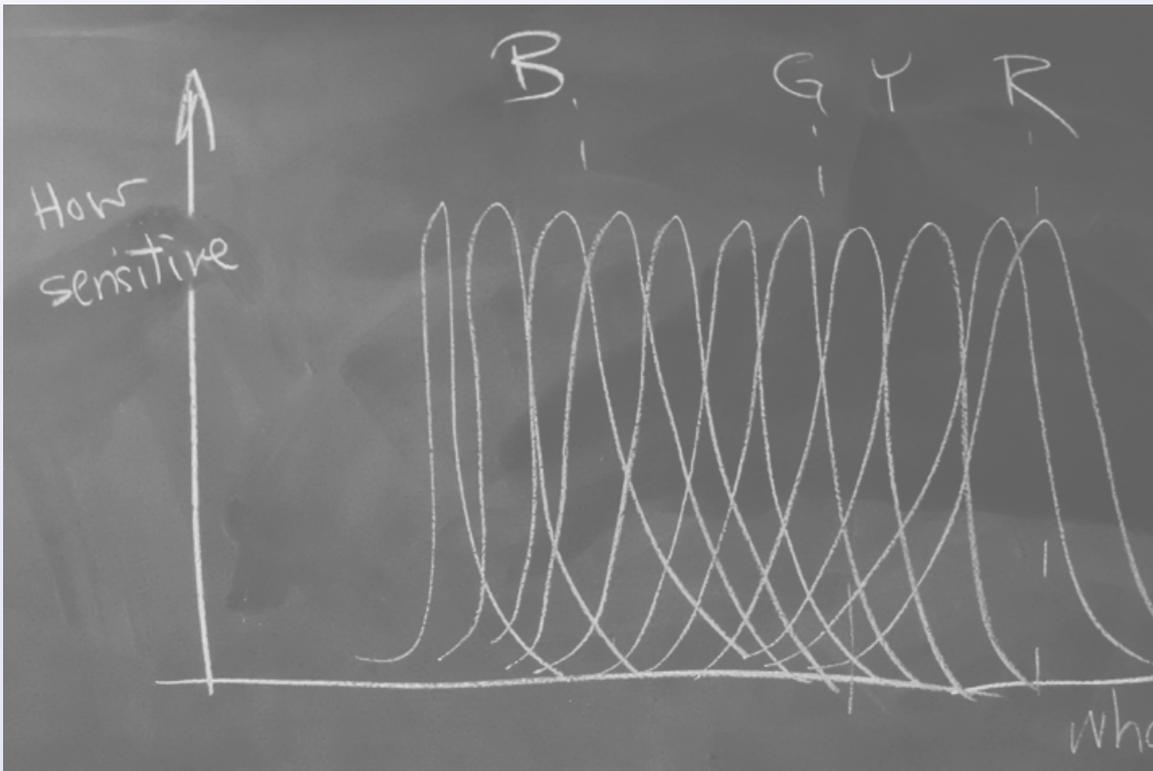


Multi-color (FISH) staining is hard to interpret when you go beyond two colors.

E Schröck, S du Manoir, T Veldman, B Schoell, J Wienberg, M A Ferguson-Smith, Y Ning, D H Ledbetter, I Bar-Am, D Soenksen, Y Garini, T Ried.
Science 1996.

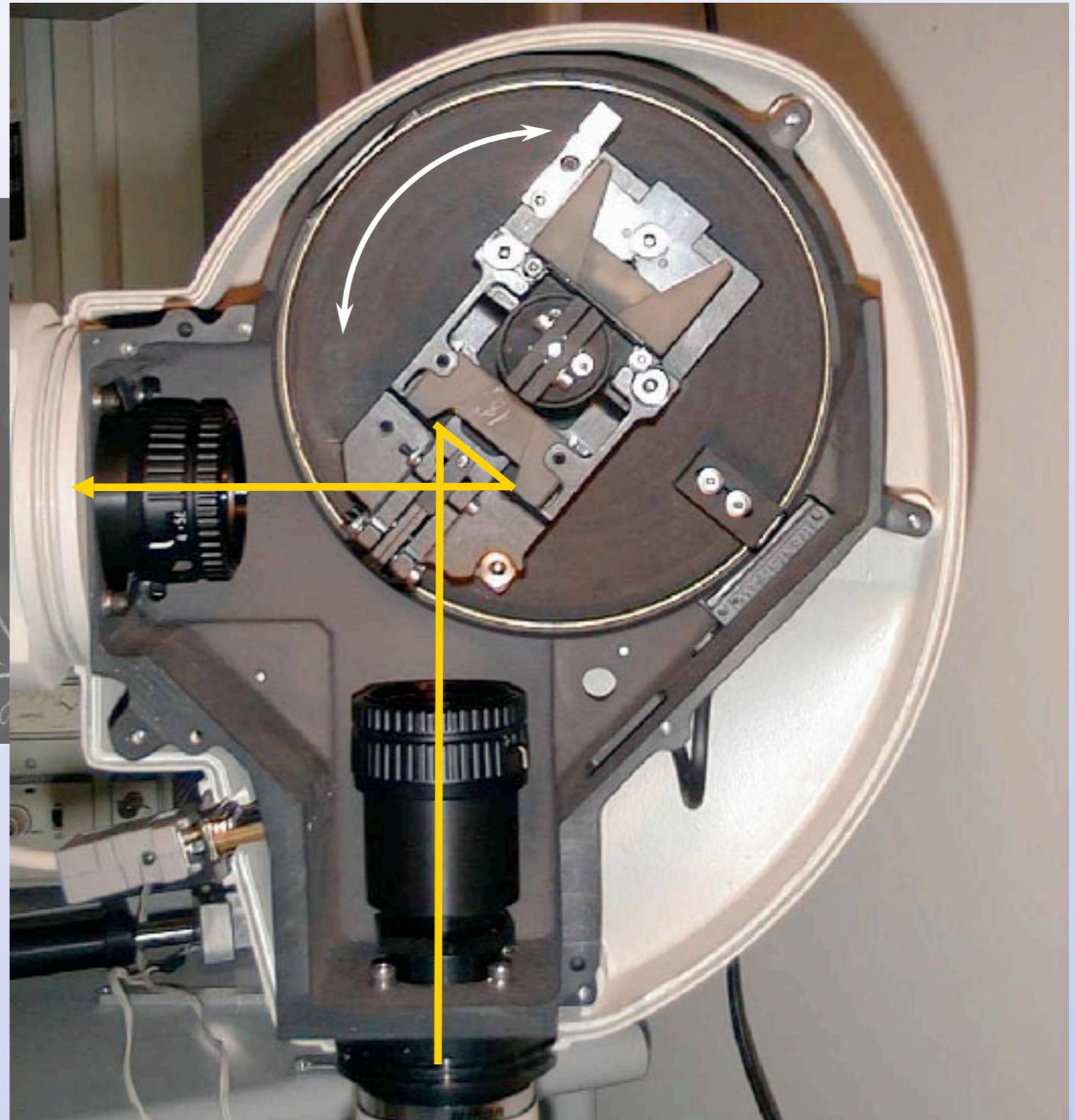
Spectral karyotyping, 1

Could we automatically sample each pixel with *many* sensitivity curves?



That would give us a detailed spectrum -- not just 3 numbers -- at every point in the image!

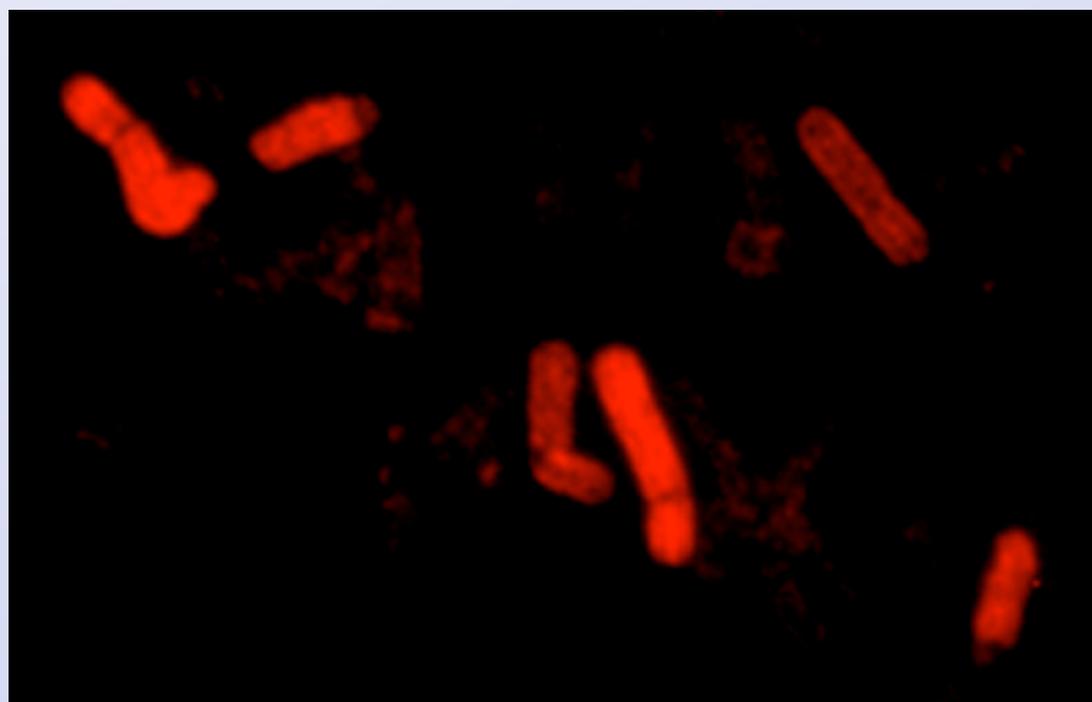
The Sagnac Interferometer is a gadget that mechanically scans through the full spectrum of every pixel in the image.



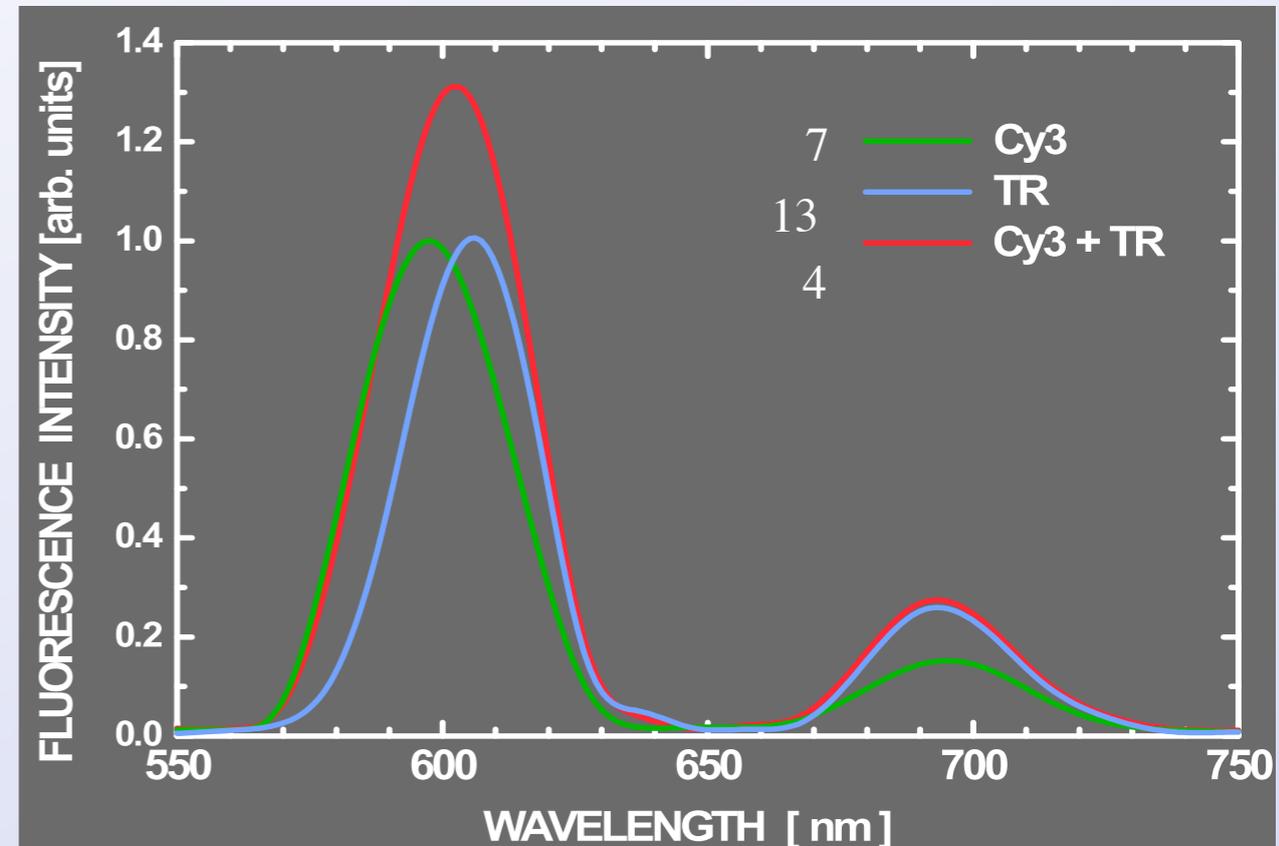
Spectral karyotyping, 2

1. Fluorescent dyes can be combined to give a lot of distinct spectra.
2. A lot of markers can be made that all bind to chromosome #1, and that all have the same combination of dyes.
3. Repeat with all the other chromosomes to visualize them all simultaneously.

Our unaided eyes are not so good at discriminating the resulting colors:



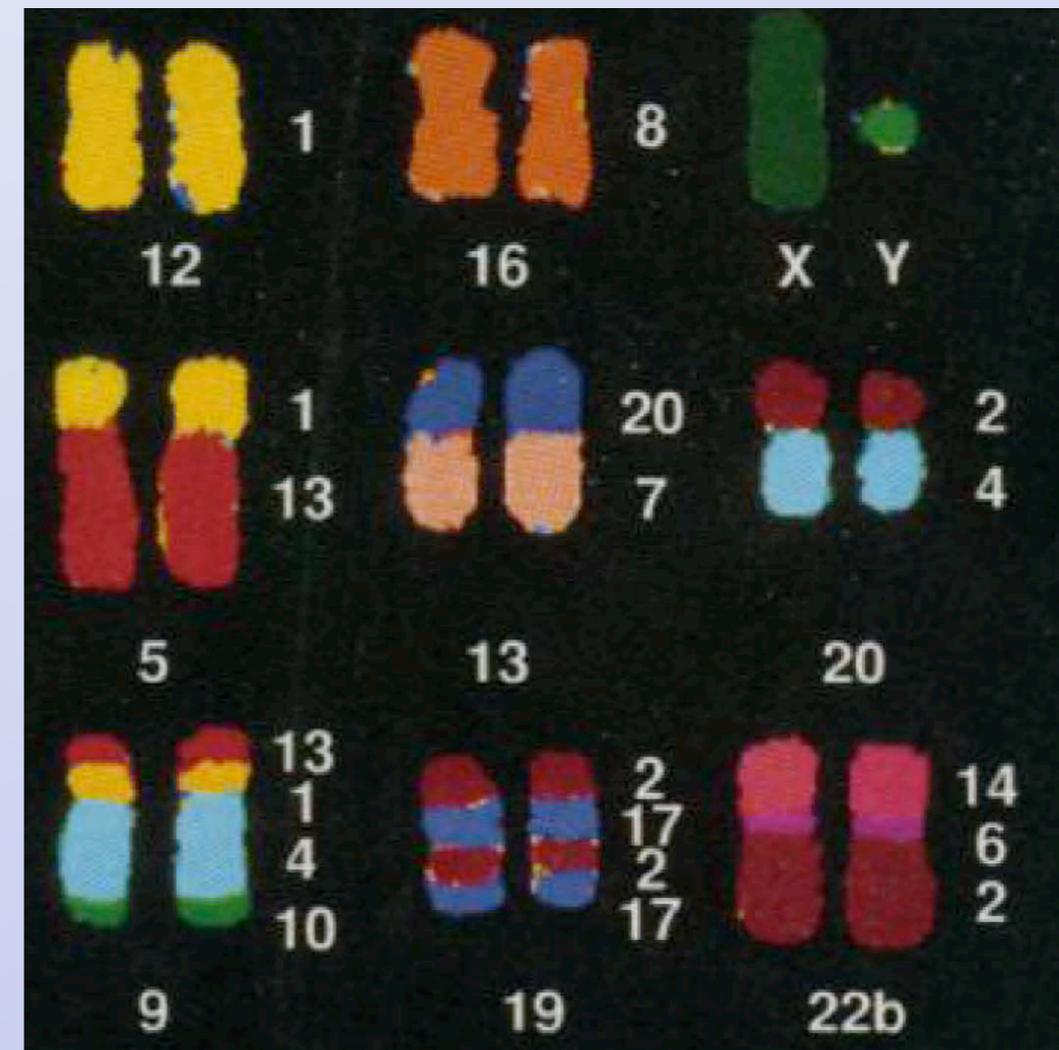
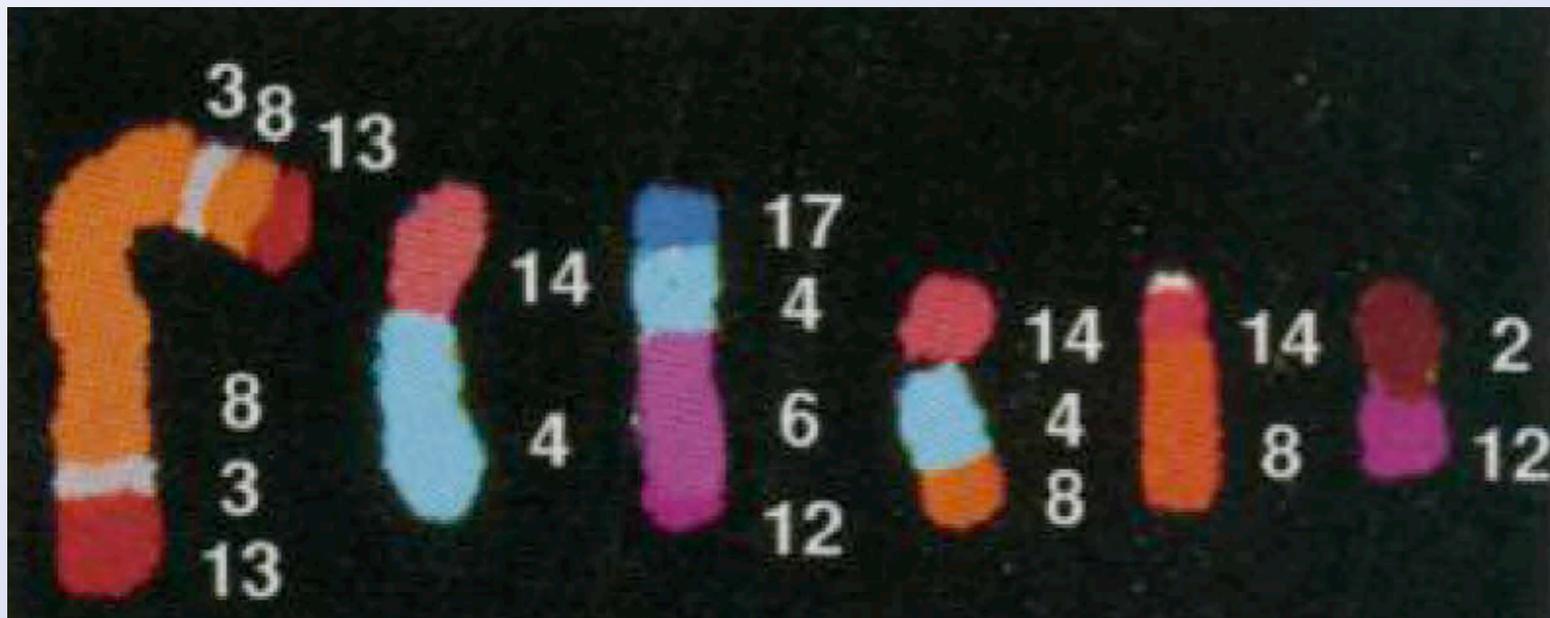
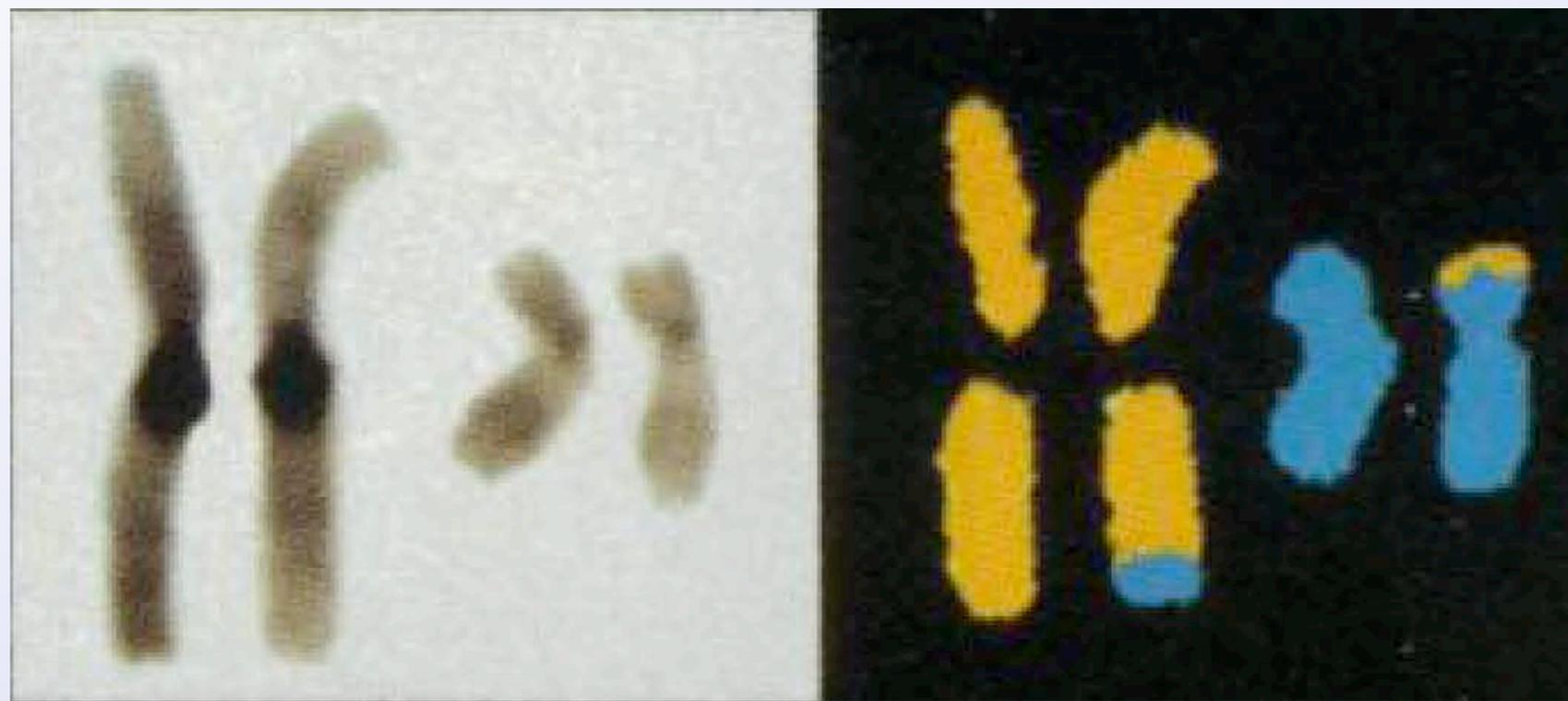
3/35



But we can compare the spectra to the known curves, make our assignments, and replace them by human-friendly false colors:



Spectral karyotyping, 3



E Schröck, S du Manoir, T Veldman, B Schoell, J Wienberg, M A Ferguson-Smith, Y Ning, D H Ledbetter, I Bar-Am, D Soenksen, Y Garini, T Ried. Science 1996.

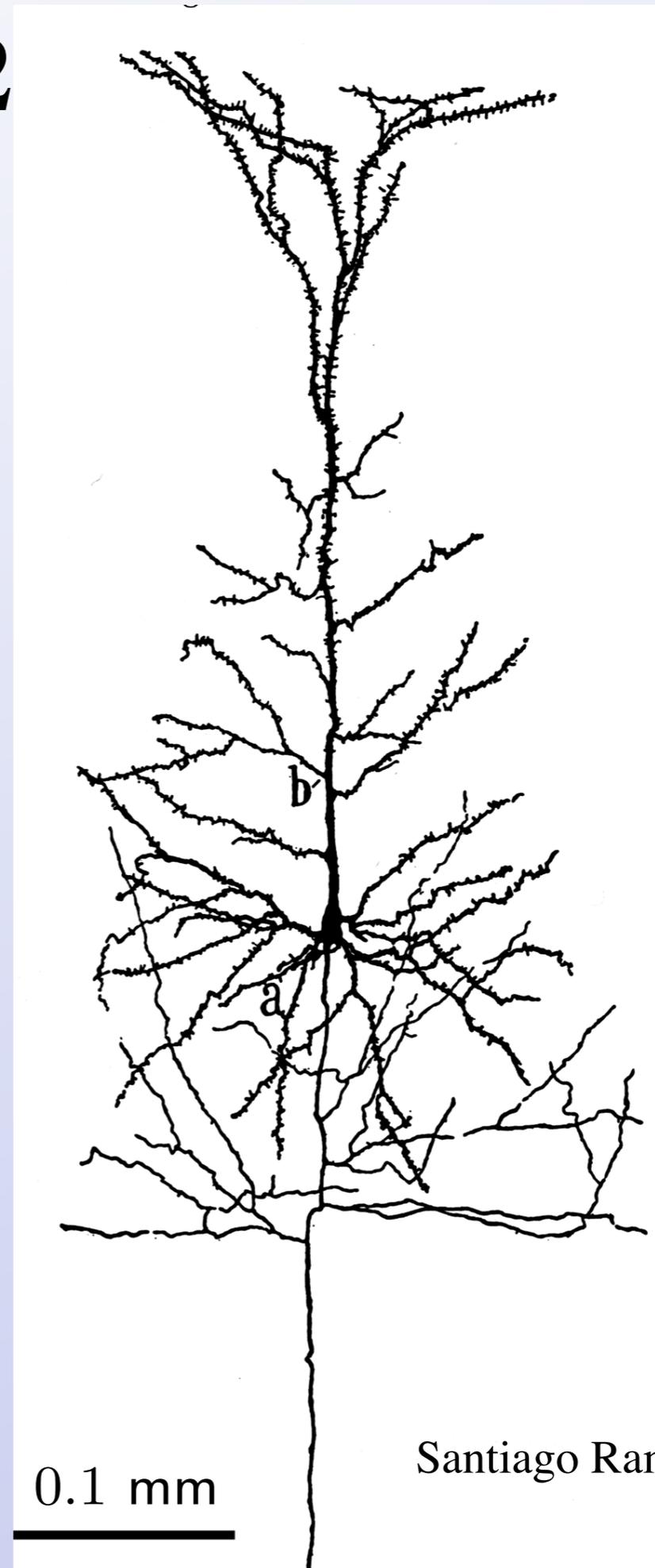
Superhuman vision, 2

If you look at a slice of brain in a microscope, all you see is a dense tangle.

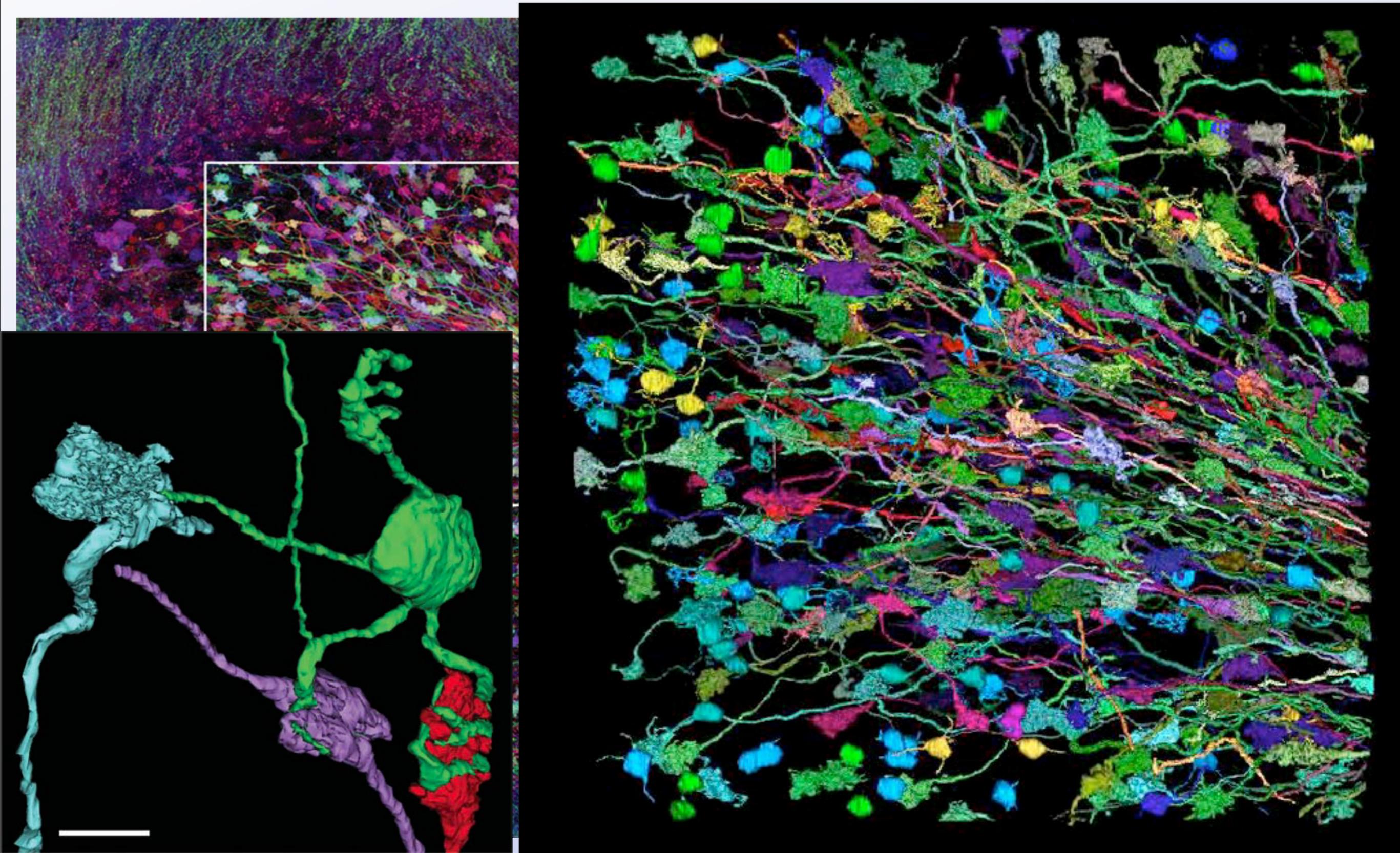
Neuroanatomy dates from Golgi's invention of a way to see a complete, single neuron amid the welter of its neighbors, and Cajal's breathtaking drawings of what it revealed.

Unfortunately, these images tell us nothing about the *connections* between all those neurons.

Also -- the method also kills the tissue.



Superhuman vision 2: “Brainbow” imaging



Jean Livet, Tamir A. Weissman¹, Hyuno Kang, Ryan W. Draft, Ju Lu, Robyn A. Bennis, Joshua R. Sanes & Jeff W. Lichtman. *Nature* 2007.

Part II: Quanta

OK, great! Fun demo, fun story, good applications -- let's quit.

No, wait. A few small matters remain, like:

- What sets the response curve of each cone cell? (Rod cells also have a sensitivity curve.)
- What actually happens when we transduce light into a neural signal?

Either way, an eye looks like a planar array of pixels-- superficially like a modern camera. About a hundred million photoreceptor cells in the human eye:

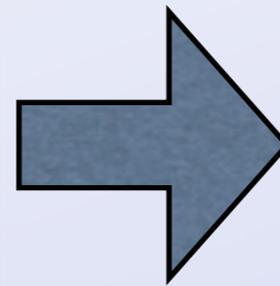
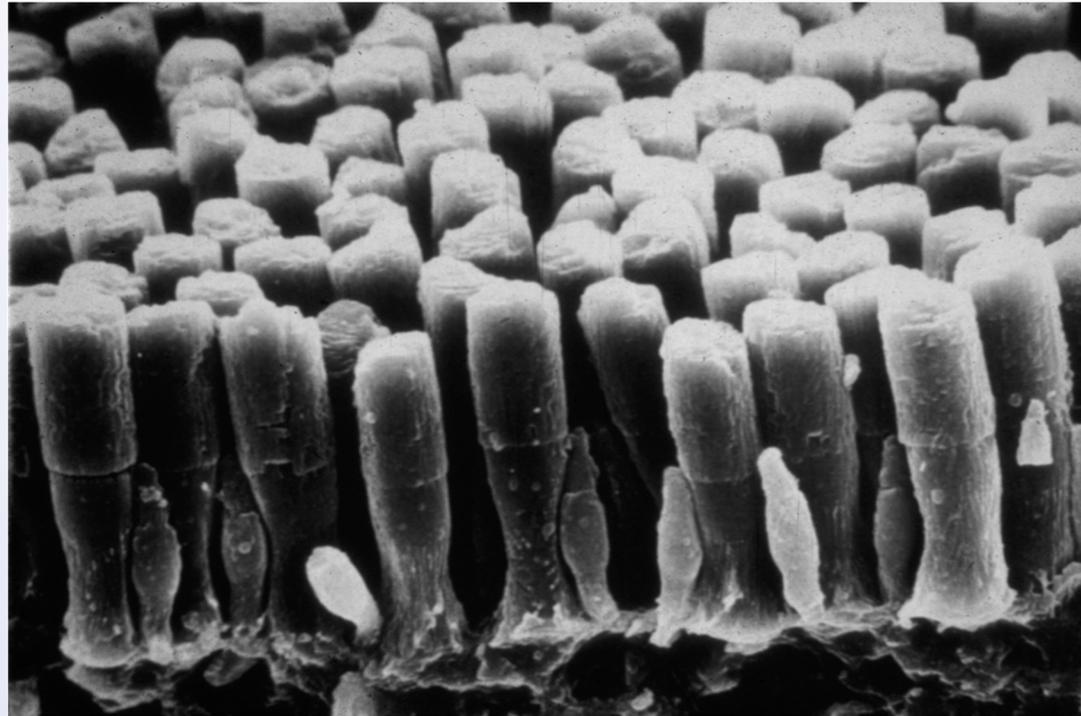


Image by Scott Mittman and Maria T. Maglio

Image by R. H. Masland.

When we get down to very few lumps, we see that individual arrivals are random in *space* as well as time:



Vision Hypothesis

- * Light comes in lumps (“photons”).
 - * Each lump has one distinguishing quality: its spectral position.
 - * These lumps arrive at random, no matter how hard we try to make a steady light. Their *average rate* (probability per second to arrive) corresponds to what we think of as “brightness.”
 - * The light spectrum/color content is the list of these average arrival rates, for each type lump.
- * Some *single molecule* in the photoreceptor cell can flip like a toggle when a photon comes by, absorbing it. Or, the photon can pass right by with no effect. The choice is random—a Bernoulli trial.
 - * The *probability* to be absorbed depends on the type of molecule and the spectral position of the photon. **That’s the meaning of the sensitivity curve** (tuning spectrum). It’s a property of that *single molecule*, like a fluorescence excitation spectrum.
 - * The three kinds of photoreceptors are each packed with just one of three kinds of sensitive molecule.
 - * Some cellular apparatus *counts* how many molecules flipped, and reports that to the brain. Each receptor type gets reported separately.

Sounds good, but what experiments could confirm (or demolish) such a story?

A crazy thought

The evolutionary payoff for good night vision is huge. Nature will certainly have tried very hard to get all the way to the absolute limit of sensitivity... which is *one isomerized molecule*, coming from *one absorbed photon*. (There's nothing between 0 and 1!)

This Astonishing Hypothesis was formulated by Lorentz very soon after Einstein's light-quantum paper.

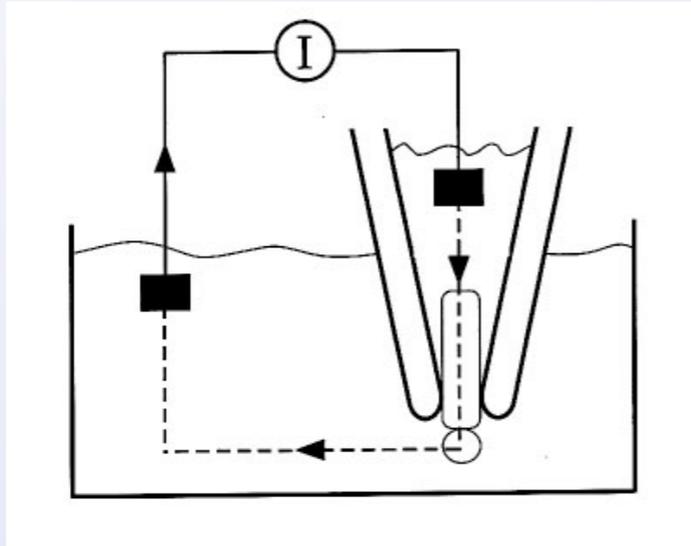
“But surely this photon stuff has got nothing to do with our eyes: Each photon carries an inconceivably small energy.”

Maybe, like Calvin, we are taking a fundamental idea and naively shoehorning it onto an application for which it's irrelevant!

Actually, Calvin does have a key idea! Let's follow his lead.

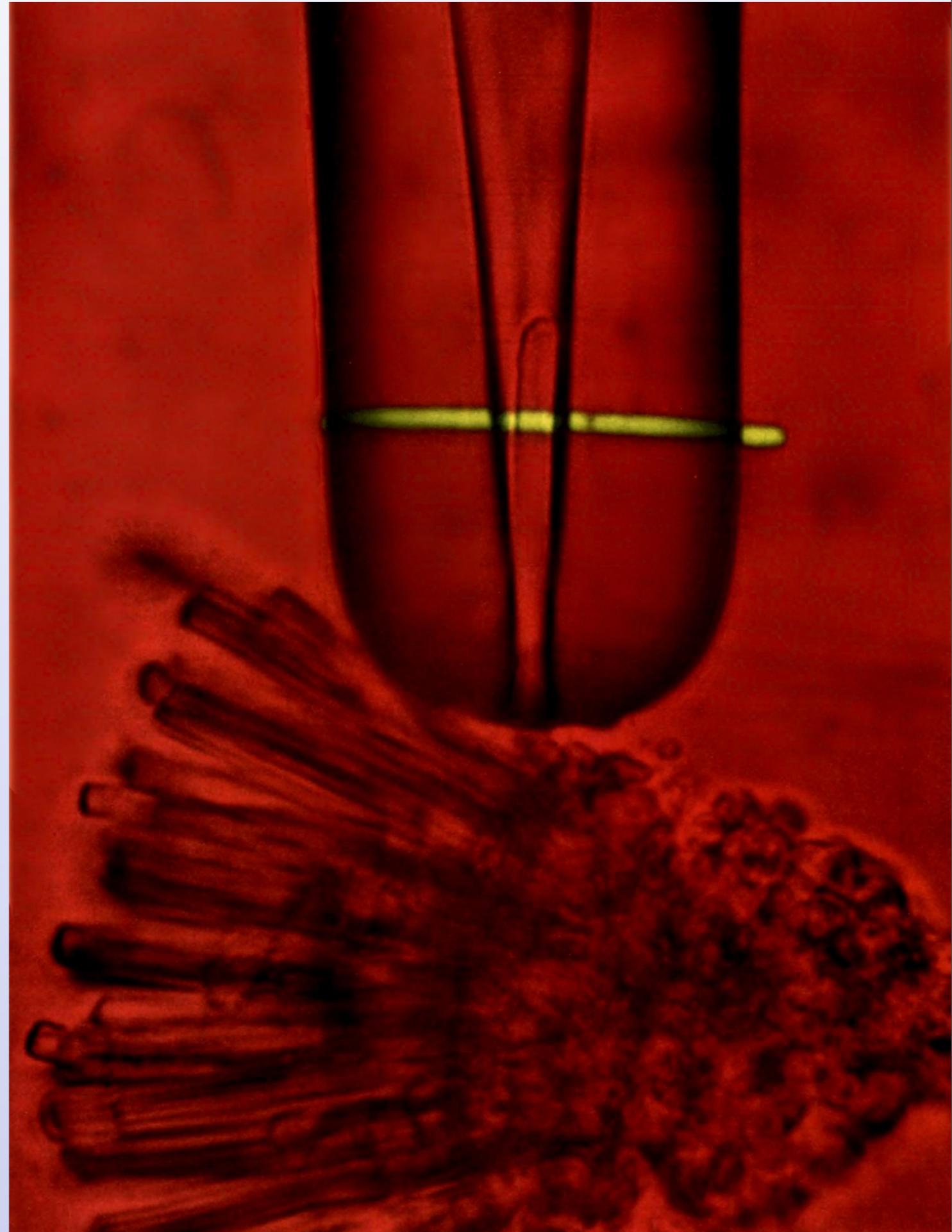


Receptors respond to single photons

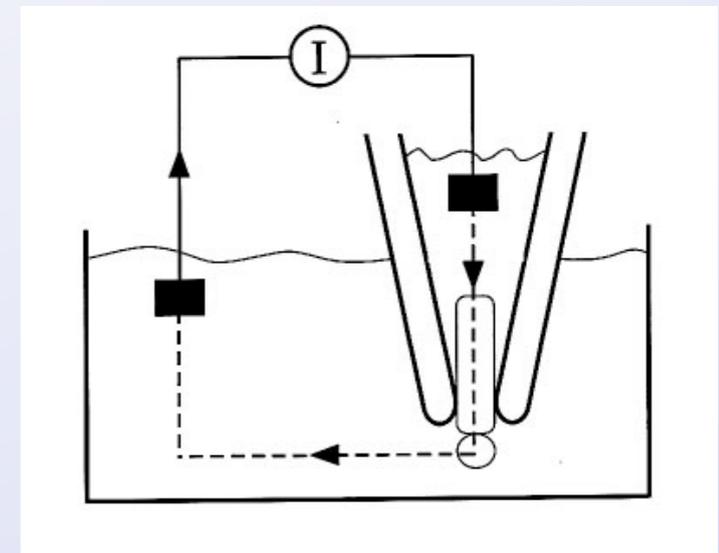
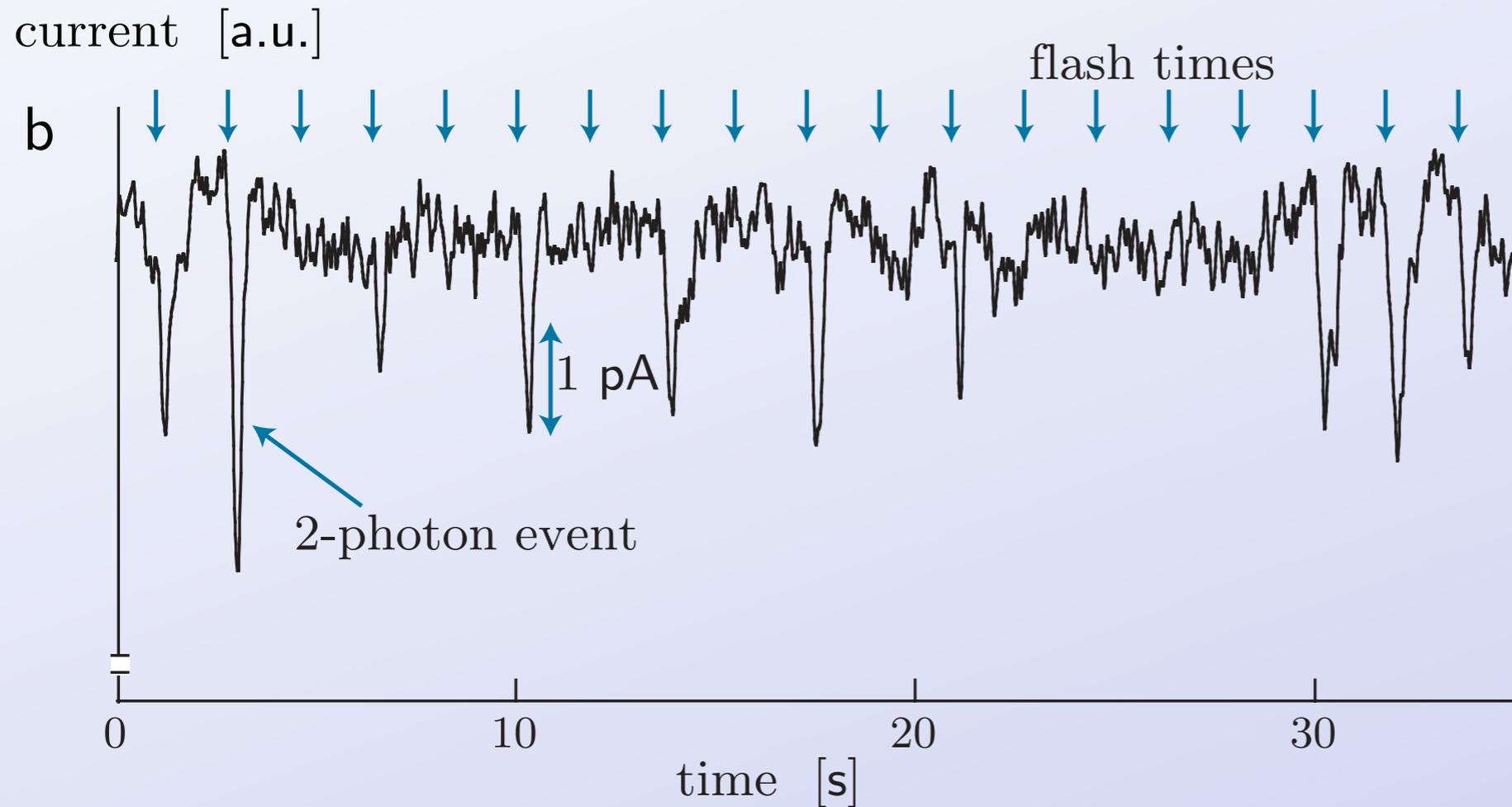


An individual rod or cone cell's response can be measured by gently sucking its outer segment into a pipette electrode and stimulating it with a flash of light (green).

P. Nelson, *Physical models of living systems*, WH Freeman and Co TBA



Receptors respond to single photons

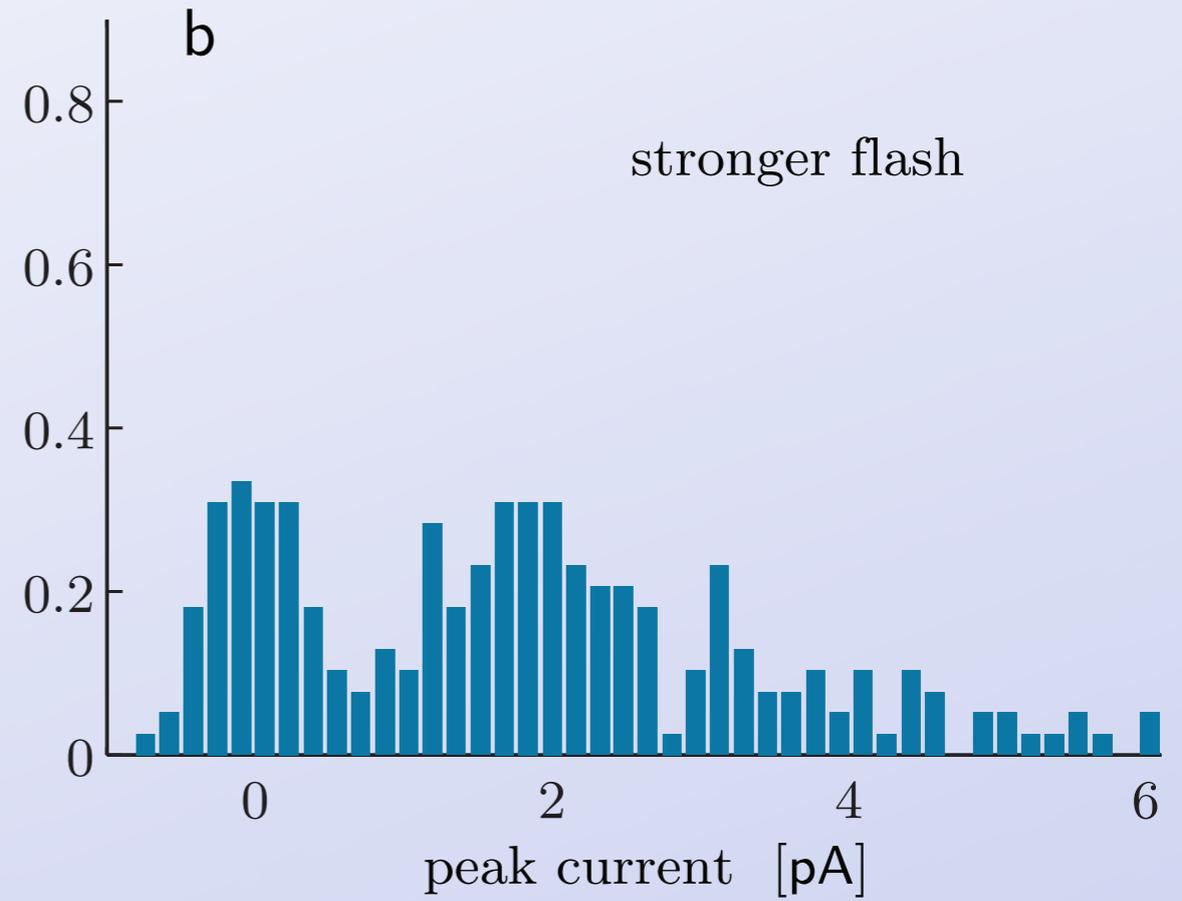
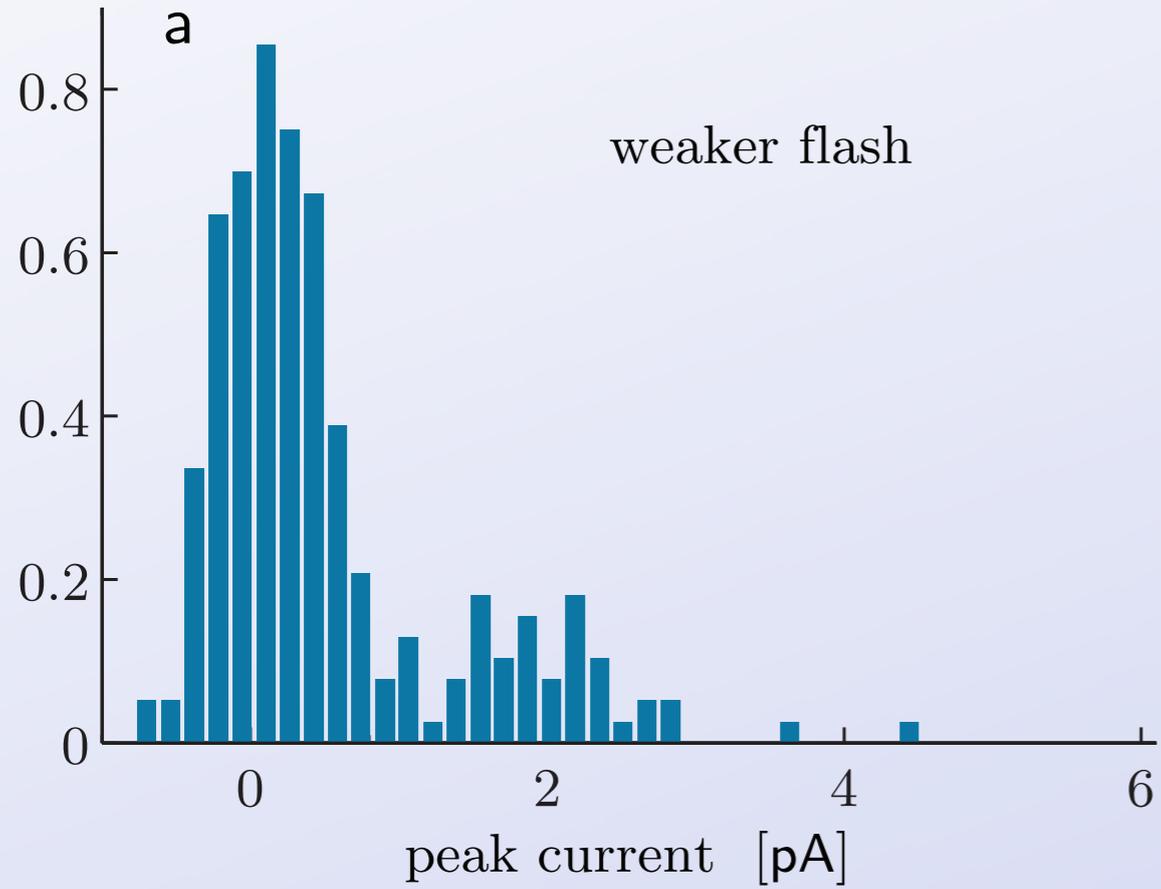


Data and sketch from Fred Rieke.

Flashes of light give rise to discrete current blips. Most observed blips fall into clearly separated categories: Here we see some 1-pA blips, and one with amplitude twice as great. Note that some photons are missed (pass through the rod cell without eliciting any response). Still, in the eye a pretty large fraction are productively absorbed. Also, there are occasional blips with *no* photon—false positive detections. But only one every few seconds.

Response probabilities

probability density [pA^{-1}]



Data from Greg Field.

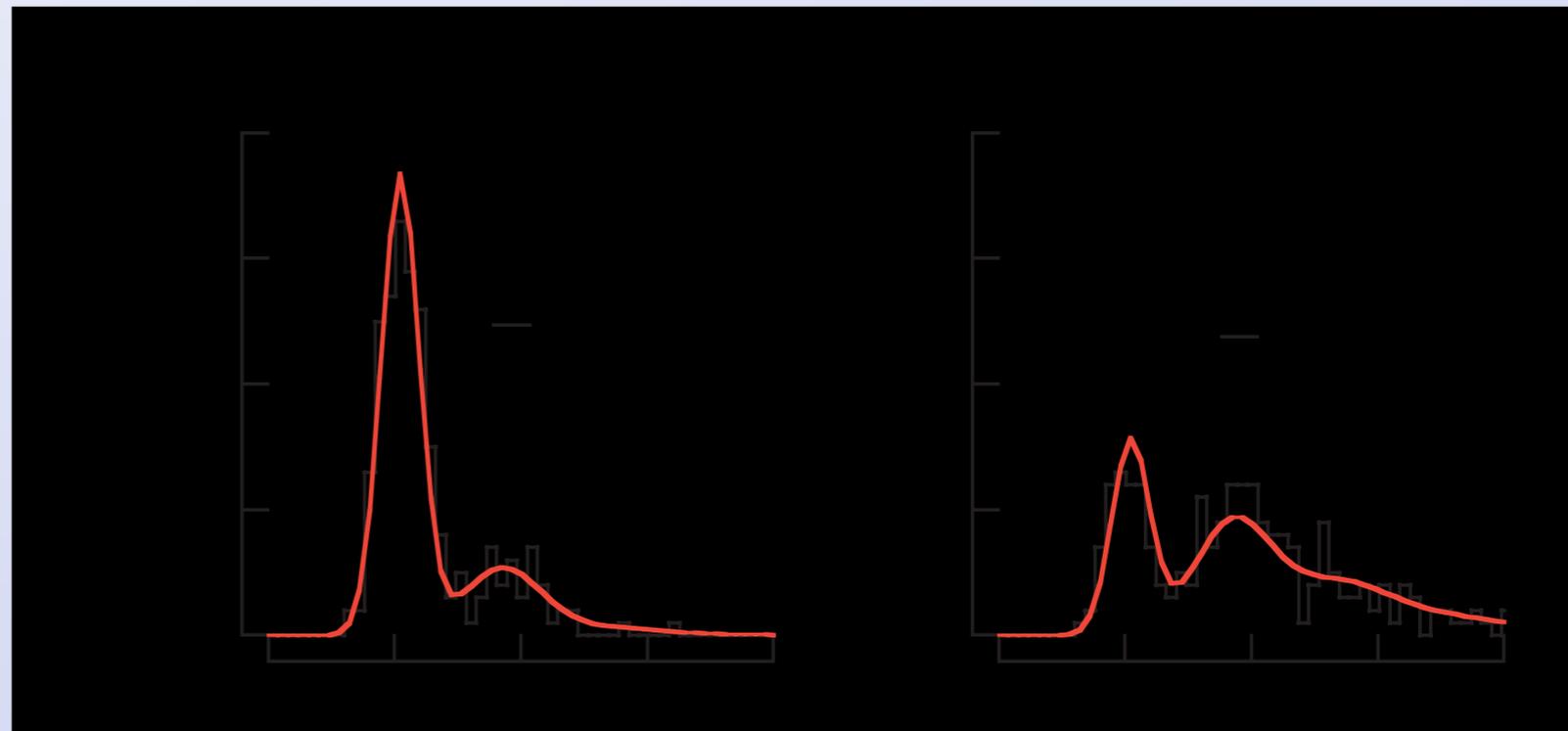
Test a quantitative prediction

It's tempting to imagine that each blip is the response to a single photon absorption. But we cannot relax till we've checked the detailed prediction of the theory: Identical flashes of light should make a *distribution* of blip types that agrees with the Vision Hypothesis.

We also get a hard prediction for how that distribution should *change* when we supply a stronger flash.

If those predictions pan out, then we'll have strong evidence not only that the Light Hypothesis is right, but also that all this matters for our vision.

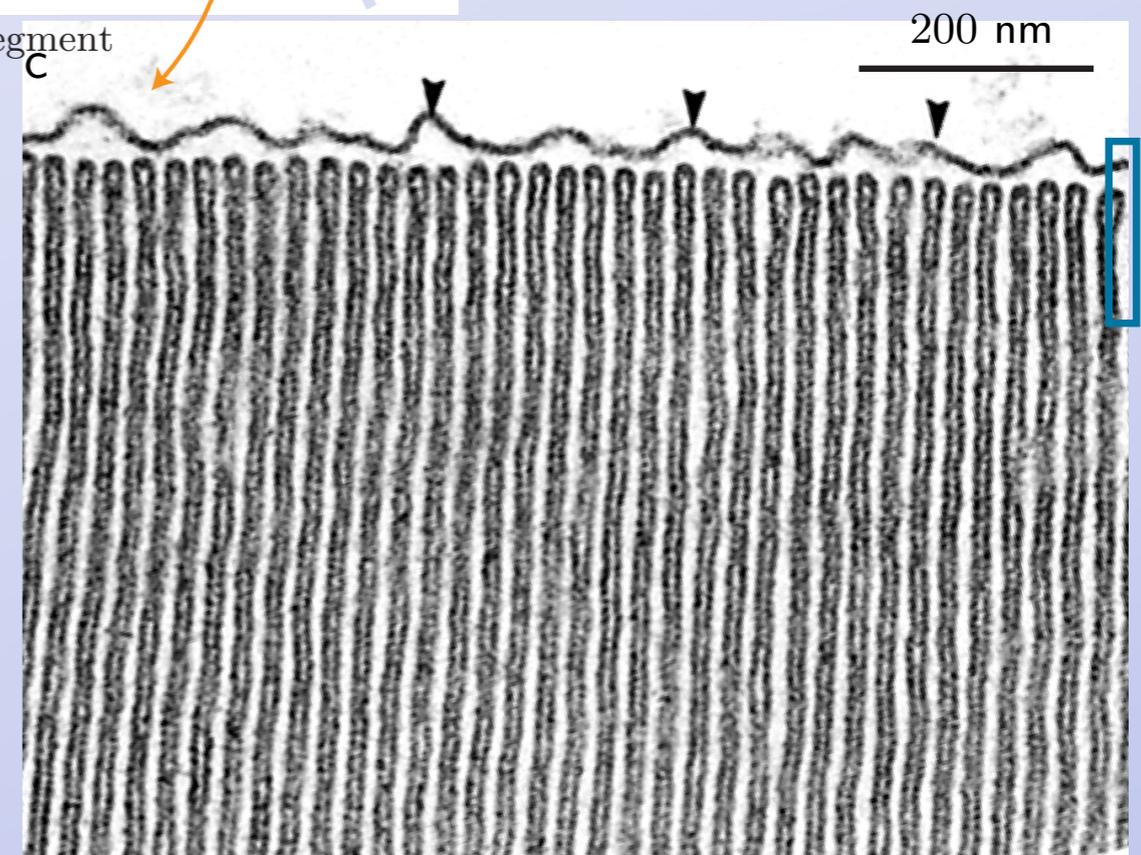
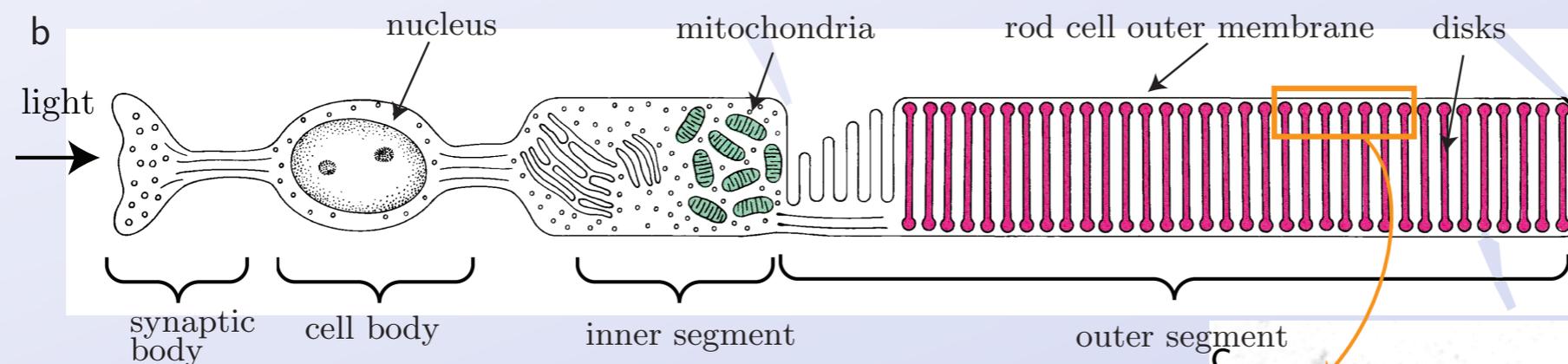
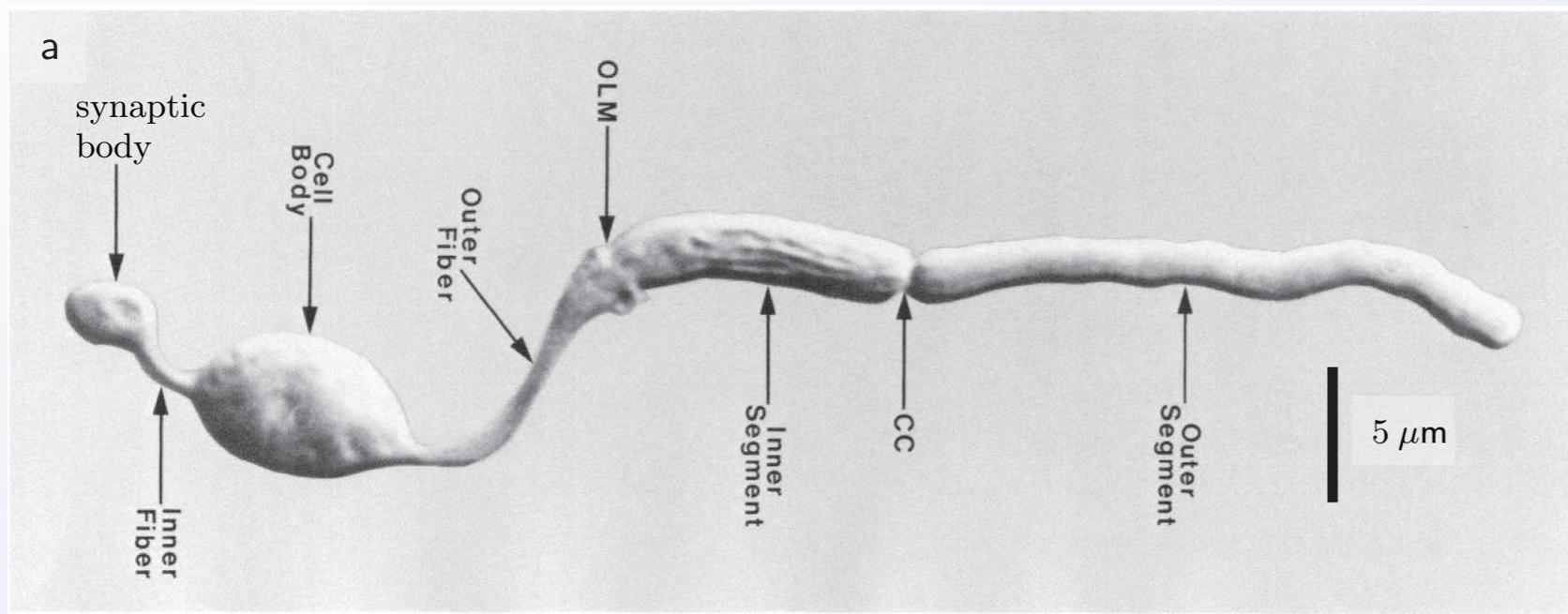
OK: looks promising. Baylor et al even confirmed that the average number of absorptions varies linearly with the flash strength.



From F. Rieke, in *The Senses: A Comprehensive Reference*; experimental data from G. Field.

“Ok, ok—somehow Nature invented single-molecule nanotech before we did. ***How does it work?***”

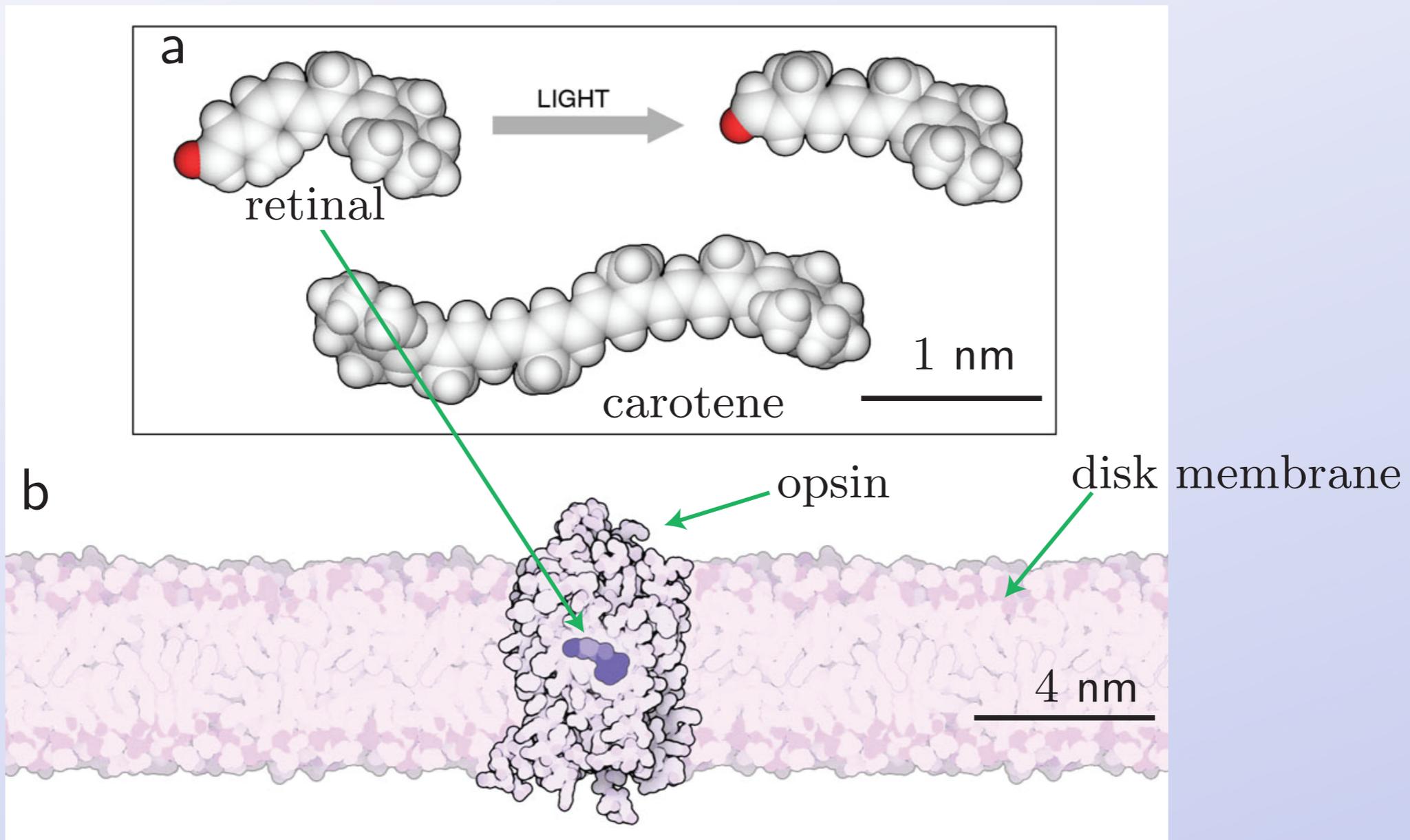
- How does a photon absorption (picosecond) turn into a nerve impulse (microsecond)?
- Most photons will cruise right past any normal chromophore without interacting at all. How can the rod cell’s capture cross-section be so high?
- How can the false-positive rate be so low?
- How can the *noise* be so low, with so much amplification?



OK, there's one answer—Each rod cell is stuffed with 10^8 rhodopsins. That's what gives it a high cross-section. But it only makes the other problem more urgent:

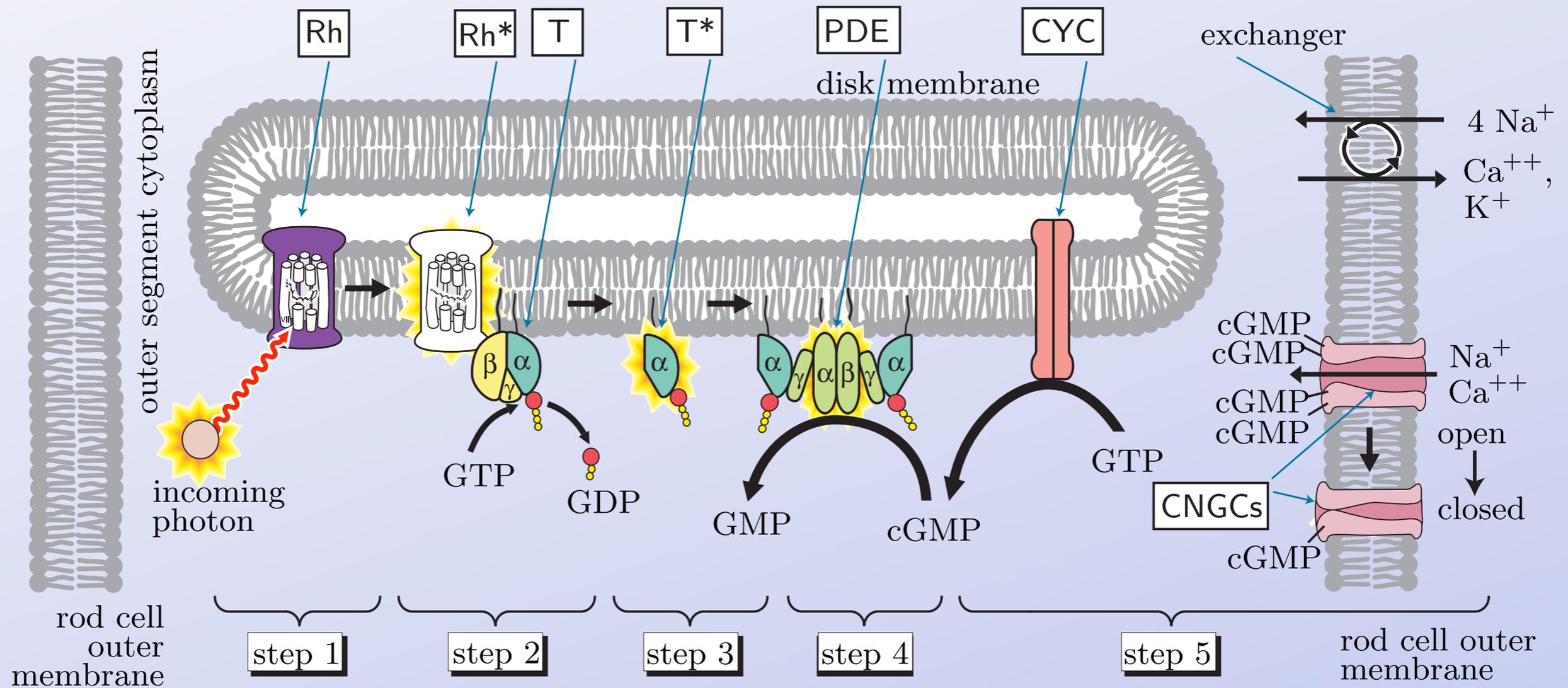
*How can the rod cell monitor 10^8 rhodopsins, respond when **any one of them** isomerizes, and do so with few false positives?*

A chromophore called retinal has a photoinduced conformational change. Retinal sits in a pocket of a protein, making a complex called rhodopsin. A change in the embedded retinal's shape gets transmitted allosterically to the surrounding rhodopsin. A high energy barrier to isomerization means very few spontaneous (no-photon) conversions. *That's step 1.*

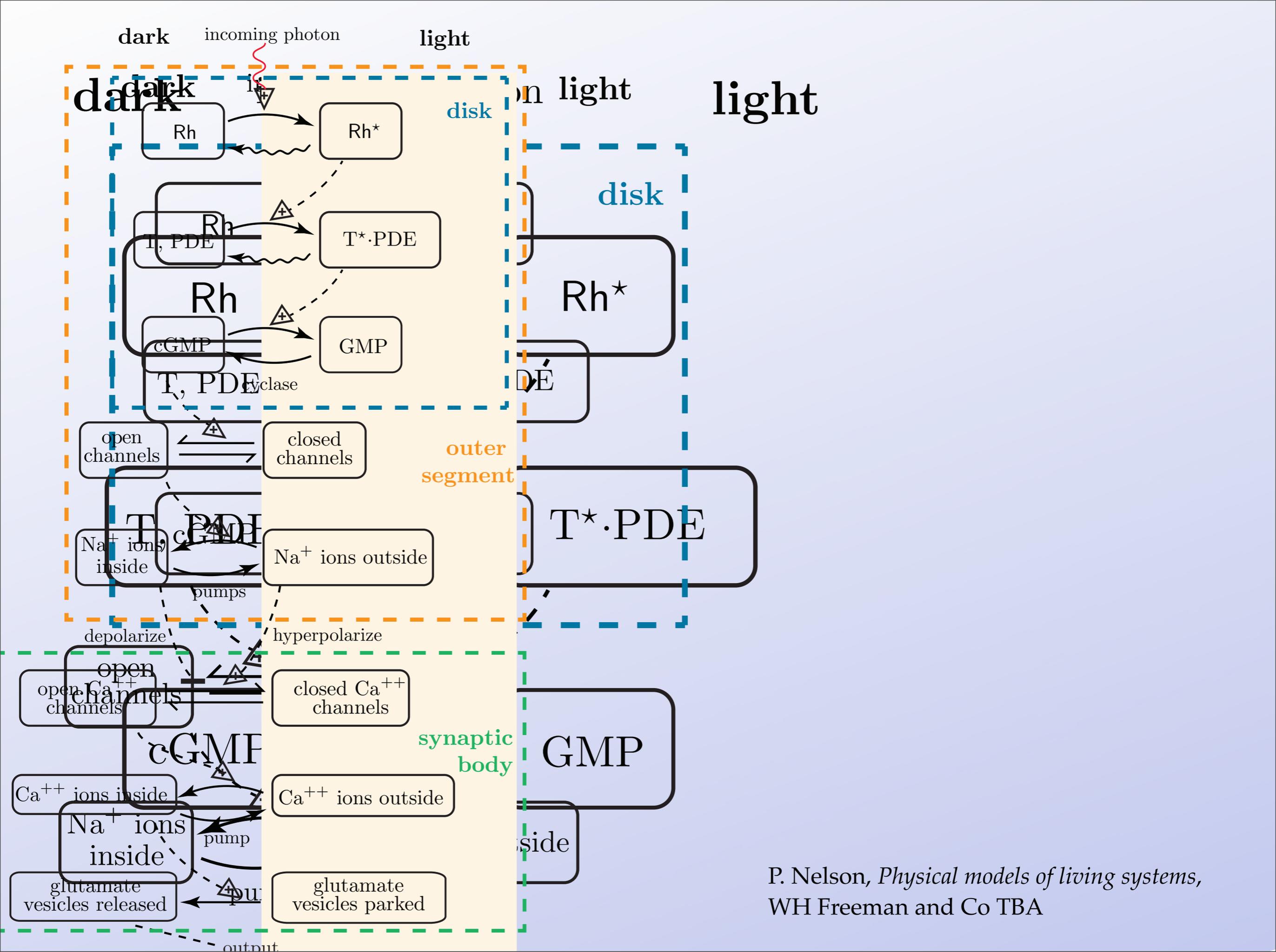


David Goodsell, 2009.

A G-protein cascade



From Trevor Lamb and Ed Pugh.

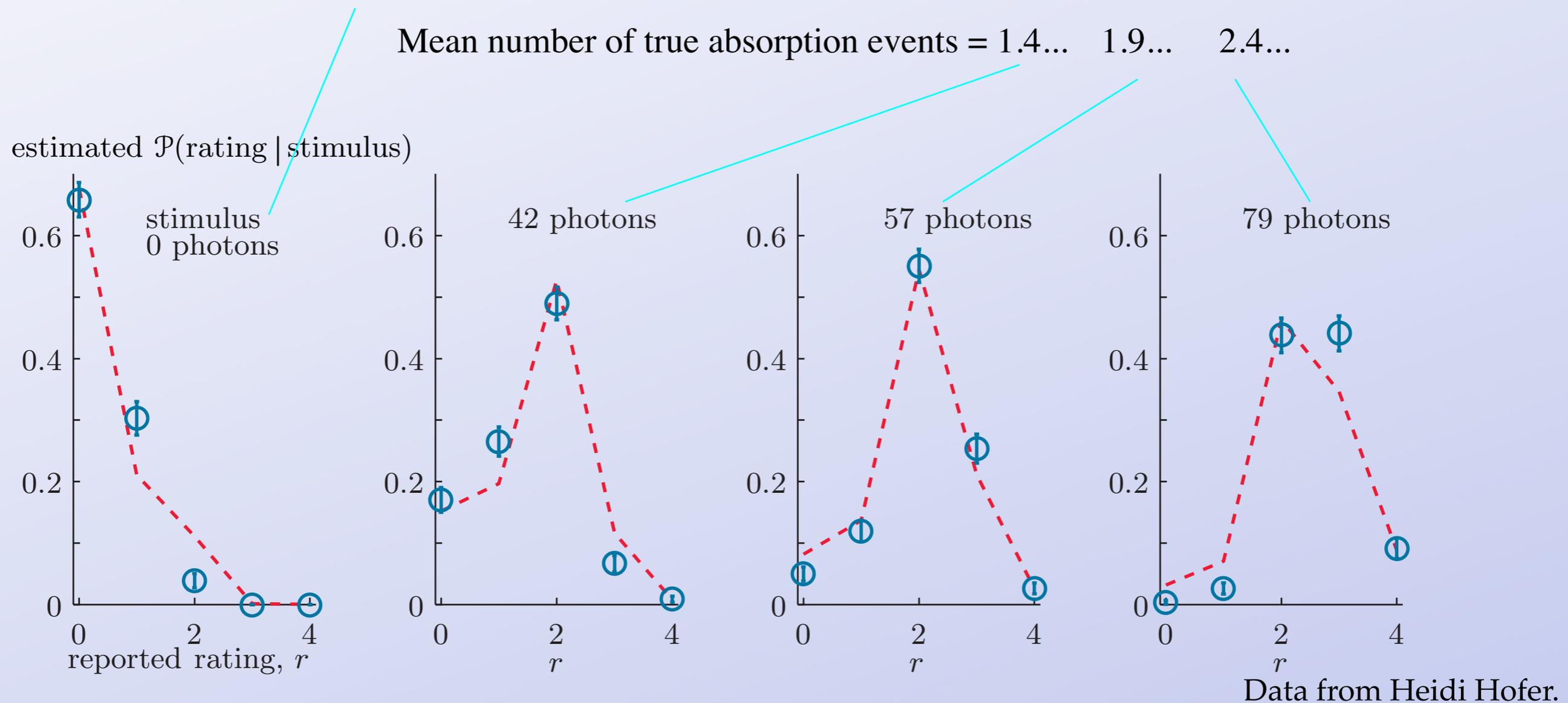


P. Nelson, *Physical models of living systems*,
 WH Freeman and Co TBA

Later visual processing can actually make use of single photon-absorption events

We now abruptly move from *single cells* all the way up to *whole organisms* (psychophysics).

Zero true absorption events.



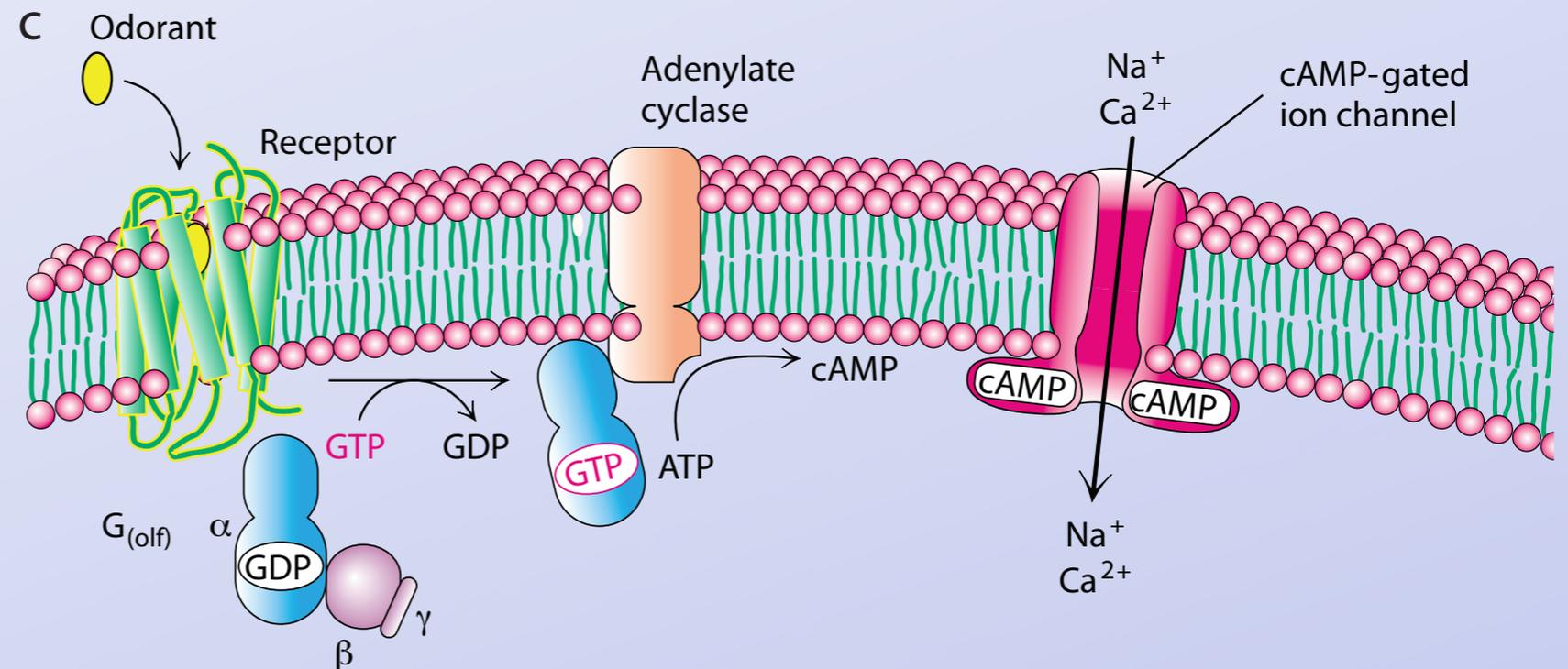
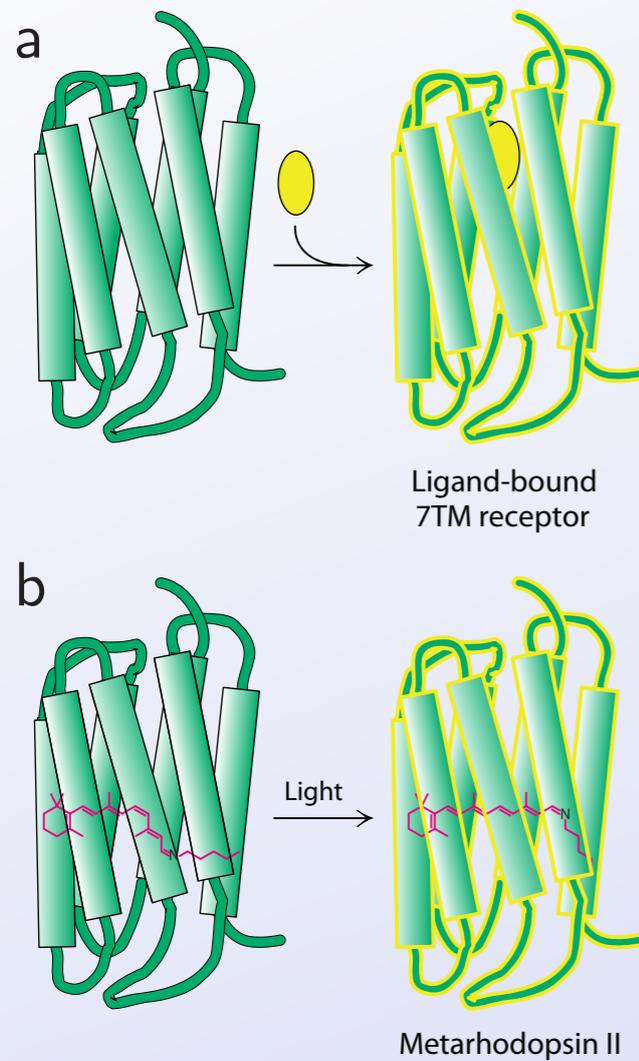
Each of these stimuli give rise to significantly different *behavior*. ***We are all single-molecule biophysicists*** (and so are insects, molluscs,...).

Why I like biophysics

These ideas are complicated, but they go way beyond vision. Nature has just *recycled* a much older mechanism, first invented for *olfaction* (chemoreception)!

Vision is ancient... but *smell* is even much more ancient.

(And the same mechanism is constantly looking for hormone molecules in your blood.)



Read More

I realize this was a whirlwind tour. You will enjoy reading...

Light:

RP Feynman, *QED: The strange theory of light and matter*.

Vision:

David Hubel, *Eye, brain, and vision*, also available free online:

<http://hubel.med.harvard.edu/index.html> .

Robert Rodieck, *First steps in seeing*.

Sean Carroll, *Making of the fittest*.

Jeremy Nathans lectures: <http://ibioseminars.org/nathans/nathans1a.shtml> .

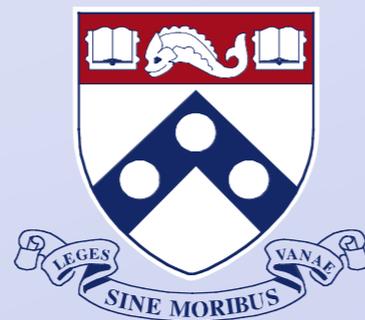
Everything else:

Phillips, Kondev, Theriot, Garcia, *Physical biology of the cell*.

Bill Bialek, *Biophysics: Searching for principles*.



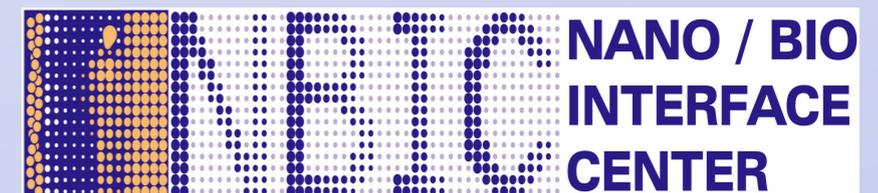
WH Freeman and Co



University of Pennsylvania



NSF BIO



NSF NSEC