- **Announcements**
- Pre-lab Lecture
  - ❖ Circuit analogy (enrichment)
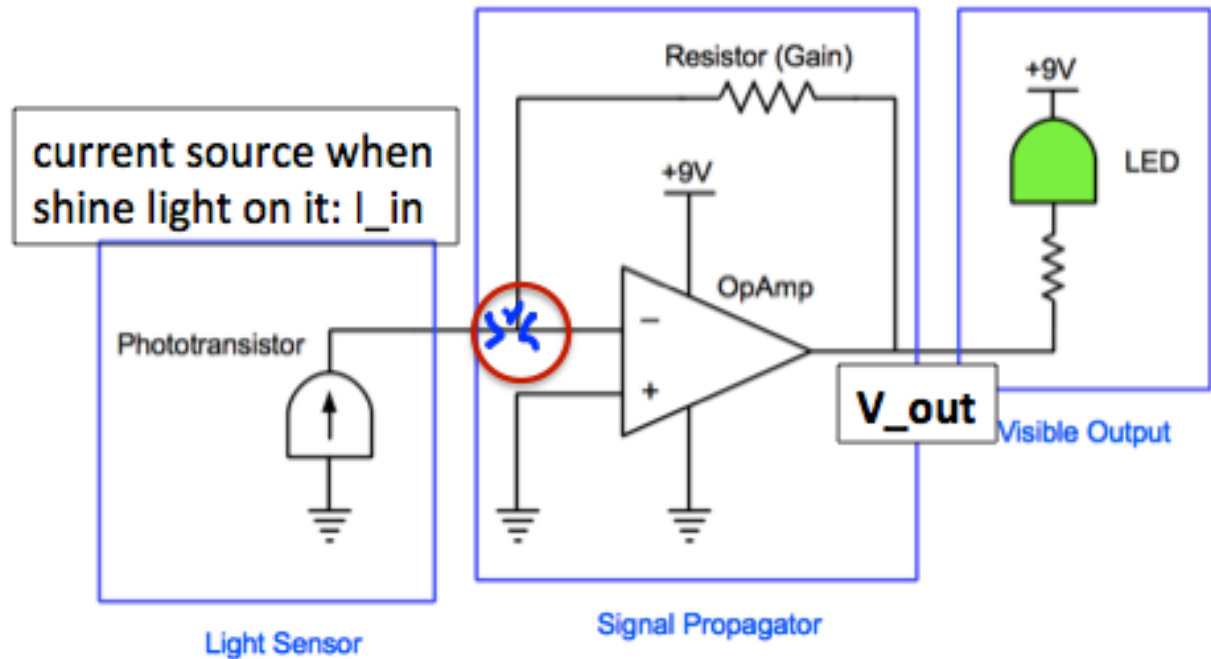 - ❖ Where we are/going
  - ❖ Today in Lab (M2D5)

Announcements

- FNW (and today's assay!) comments
  - when is time is an issue? → _short_ +/- 1 sec = larger % age
  - A420 vs. OD600 out of range. only _measurements_ matter
    - multiplying in-range OD600 by 10x does NOT make it "out of range" - not ever _measured_
- Journal club coming up
  - in one week
  - extra OH besides Tuesday? ⇒ cancel Tue.
  - poll by Doodle for one-on-one meetings? ✓
- Start thinking about best day for M2 extra OH

Revisiting circuit analogue: main equation

Combining information so far:
V_out/I_in = -R

V=IR
Vout = I_out * R
I_out = -I_in (red node balance)

current source when shine light on it: I_in

Resistor (Gain)

+9V

OpAmp

+9V

LED

Phototransistor

V_out

Visible Output
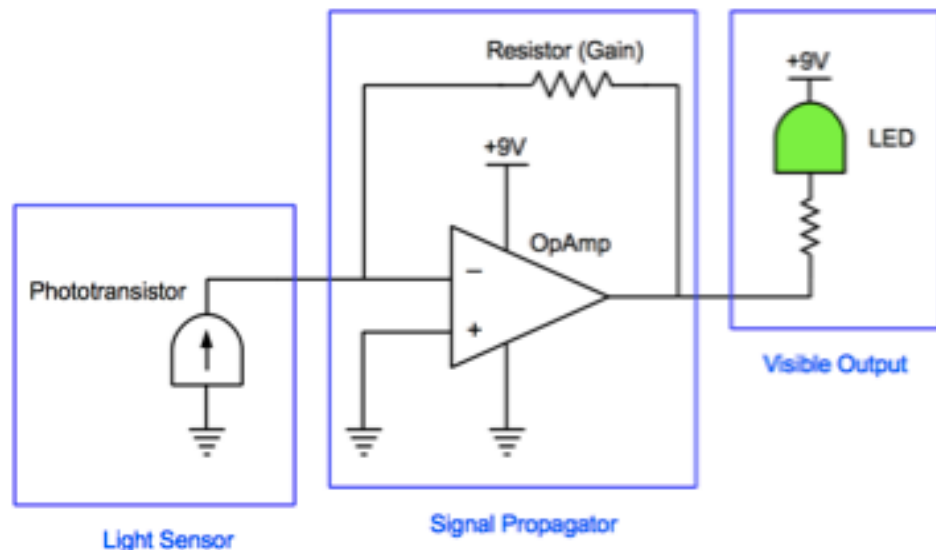
Light Sensor

Signal Propagator

current at (-) input of the op-amp = 0

Revisiting circuit analogue: changing R

$V\_out/I\_in = -R$

Shine light on photodiode →
I_in flows →
V_out decreases (- sign) →
Bigger voltage drop →
LED lights up!

As R increases →
V_out decreases faster →
takes less current for same brightness →
a more digital, less analog behavior

The extremes (R = 0 or R = inf) are tricky
because they vary with op-amp
characteristics. Theoretically, light always
on with wire (0), always off with air (inf).
Whether shine light or not!

Resistor (Gain)

+9V

LED

OpAmp

Phototransistor

Visible Output

Light Sensor

Signal Propagator

recall: voltage
difference lights LED

Where are we/going?

Discuss goal of each phase/experiment with a partner

- ## Construction phase:

    Make Cph8 library (NK) and put into cells (109)

    prepare to identify mutants, TS41X in Cph8 (K^+P^-)
    in the BP system, that might improve contrast

- ## Evaluation phase:

    DNA Sequencing   what is the mutant? # other mutations?

    protein {
    β-gal assay   does the mutation seem to affect relative
    [β-gal] in light and/or dark?

    Western   is [cph8] itself changed? # potential for artifacts #
    also: size dblchk   related to amount vs. K/P balance
    }

    Other ....   → D7

Chance for design/investigation

- Day 7 = Western = lots of long incubations
- Chance to design and carry out an additional experiment that could shed light on your results
- Start thinking about it now (or rather as soon as you have your first batch of mutant data)
- Also a day to share, collaborate with other groups

Today in Lab: M2D5

- Miniprep each mutant candidate (light *or* dark)
- Resuspend in sterile water, mix an aliquot with sequencing primer → ideally done by 3 pm!
- β-gal assay: WT, HK dead, and your mutants
  - choose *one* dilution that should give credible data
  - perform in triplicate, towards statistics ☆ team work! ✹
- Prepare culture for protein analysis
- Leslie will discuss elements of a successful research article (esp. Discussion) @ 4 pm