

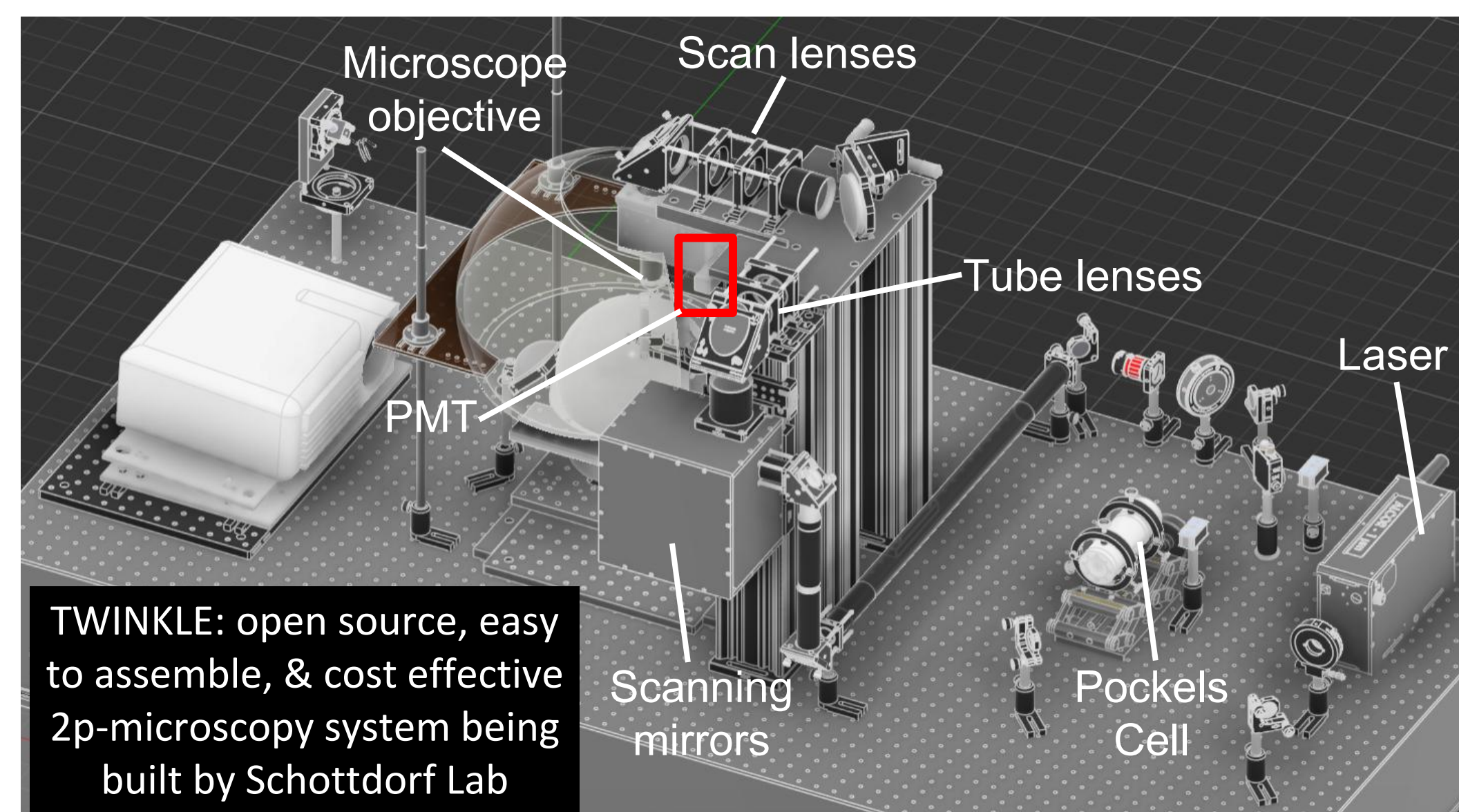
Overview

Photomultiplier tubes (PMTs) are photon-counting optoelectronic devices that play a critical role in multi-photon and confocal microscopy. Despite their sophisticated hardware, extreme sensitivity to light, and critical role in modern imaging, no open-source reliable measurement technique exists to characterize their performance and degradation. In this project, we begin to develop a system to analyze PMT health.

Background

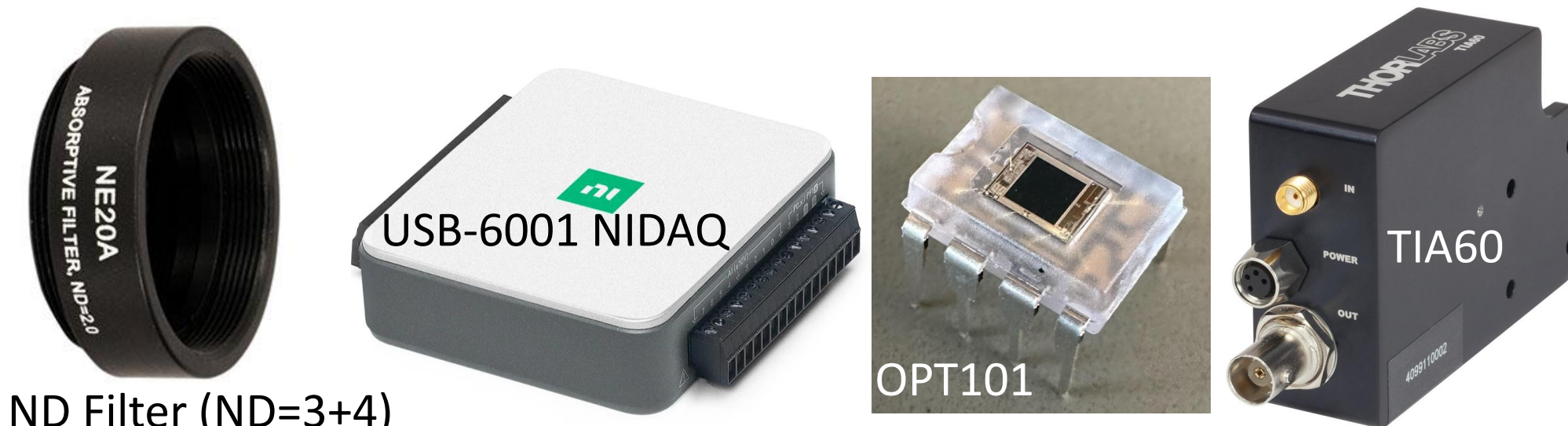
Two-photon (2p) Microscopy

- Fluorescent imaging technique of live specimens
- High resolution up to few hundred nanometers
- Multi-photon excitation of fluorescent molecule



Key Components of TWINKLE 2p-Microscopy System

- Laser: femtosecond pulsed laser
- Pockels Cell: electro-optical modulator for to control laser intensity
- Scanning mirrors: move laser beam to raster image
- Scan & tube lenses: magnify sample fluorescence to fill objective's back aperture (image collection)
- PMT: collect sample fluorescence**



Ancillary Equipment for PMT Analysis

- Data acquisition system (DAQ)
- LED, OPT101 (photodiode), neutral density filters
- Transimpedance amplifier (TIA), 1N4005 (diode)
- AD8047A (opamp), CD74HC390 (ripple counter)
- Oscilloscope, waveform generator

Benchmarks for PMT Analysis

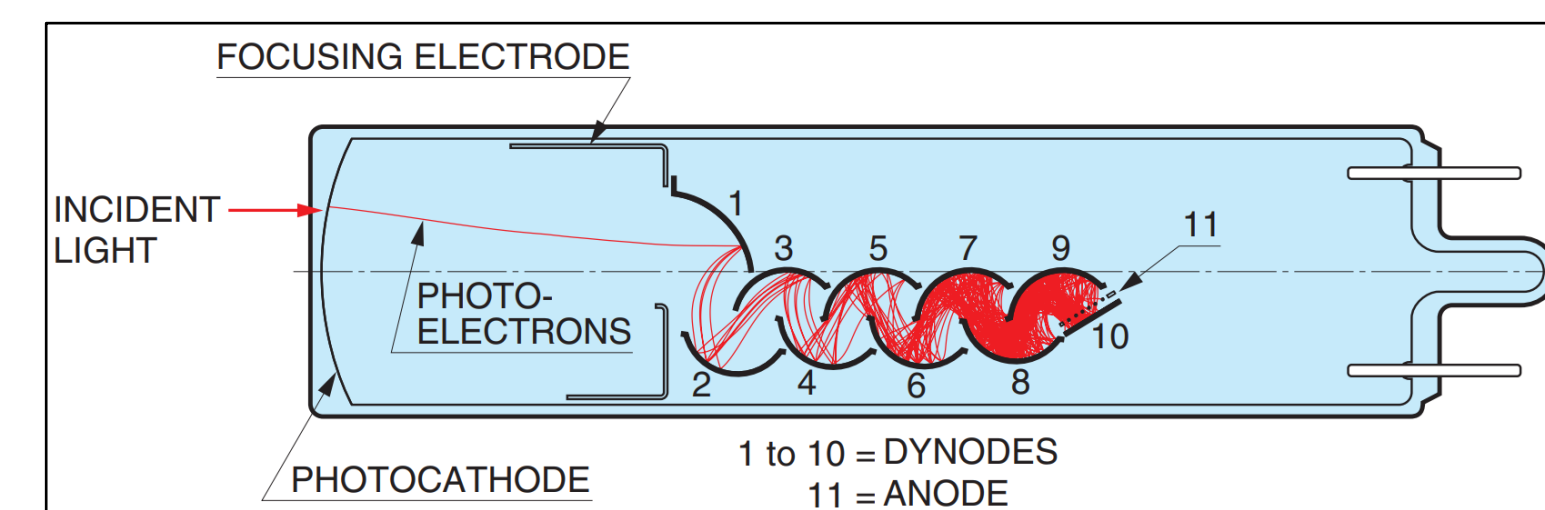
- Peak height: **amplitude** of photon events
- Peak count: **frequency** of photon events
- Peak width: **transit time** of photon events

Methodology

Photomultiplier (PMT) Design & Operation

PMT Design

- Light-sensitive devices in vacuum tube w/anodized aluminium light-tight housing
- Collection window (input light) → focusing electrodes → photocathode (photoelectric effect) → dynodes (electron cascade & amplification) → collection anode (current)
- Gain & sensitivity (control voltage; 0.0-0.8V)



Innerworkings of linear-type PMT (from handbook)

Definitions

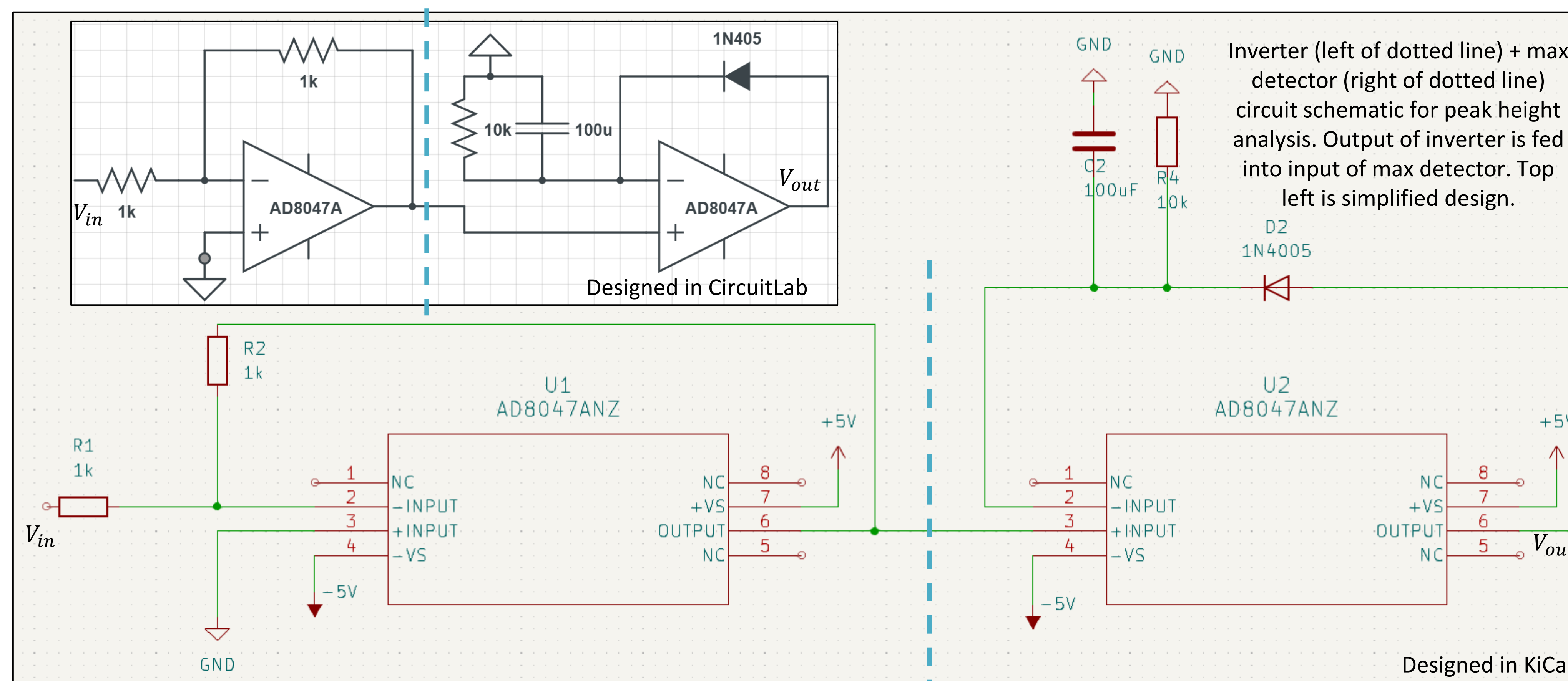
- Photon: fundamental particle of light
- Photoelectric effect: incident photon converted to electron when incident on surface
- Secondary emission: "secondary" electrons released from metal when bombarded by high-energy particles



Left: H16200 Series, Center: H16201 Series, Right: H16204 Series

- TWINKLE uses H16201P-40 PMTs

- Red: power
- Black: ground
- White & blue: control voltage
- 15V operation
- GaAsP photocathode



Peak Height Analysis

- PMT (TIA) output = negative voltage (collection of electrons) → inverter + max detector circuit → DAQ for data collection & plotting
- For given PMT, systematically scan through light & gain settings to determine performance relationships

Peak Count Analysis

- PMT (TIA) output → inverter → Schmitt trigger → ripple counter
- Schmitt trigger: removes noise & digitizes analog signal (square waveform generator); comparator circuit w/positive feedback
- Ripple (asynchronous) counter: digital sequential circuit w/flip-flops; only first flip-flop receives external clock

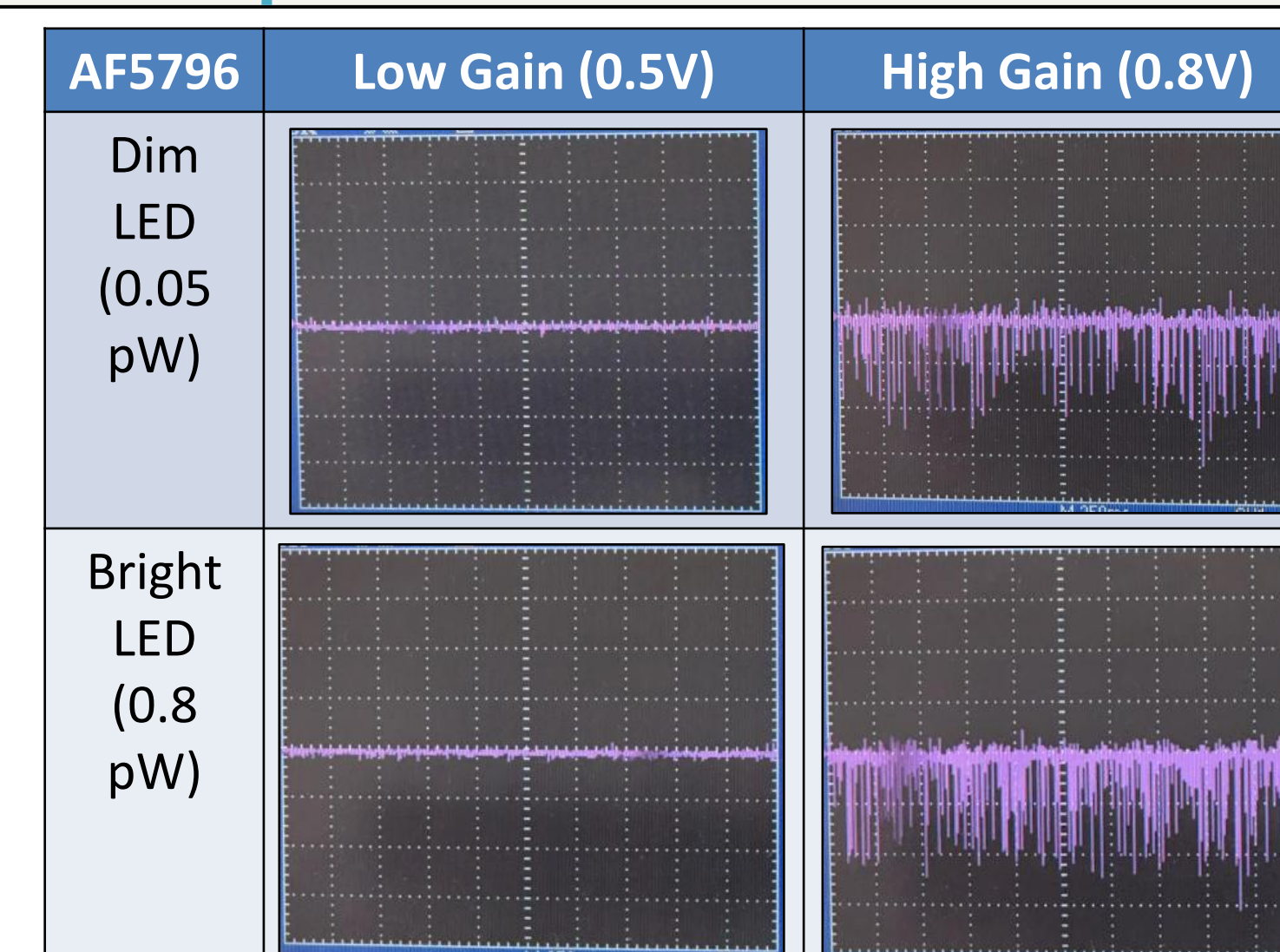
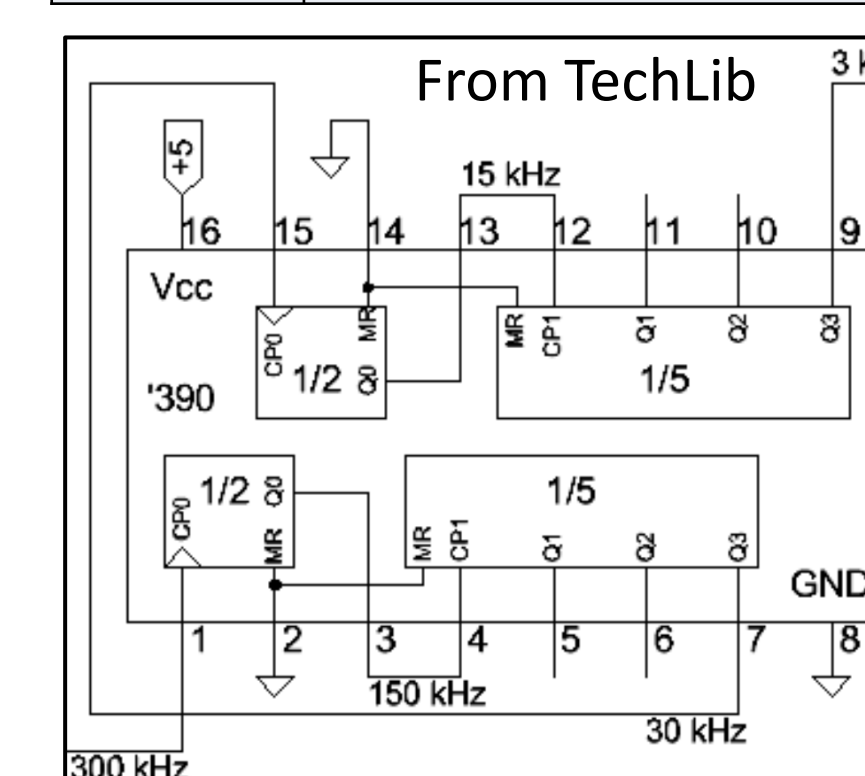


Table of PMT AF5796 outputs for various gain & LED settings on oscilloscope (500mV/div). Sharp peaks represent single photon events. Higher gain → more frequent + higher amplitude photon events



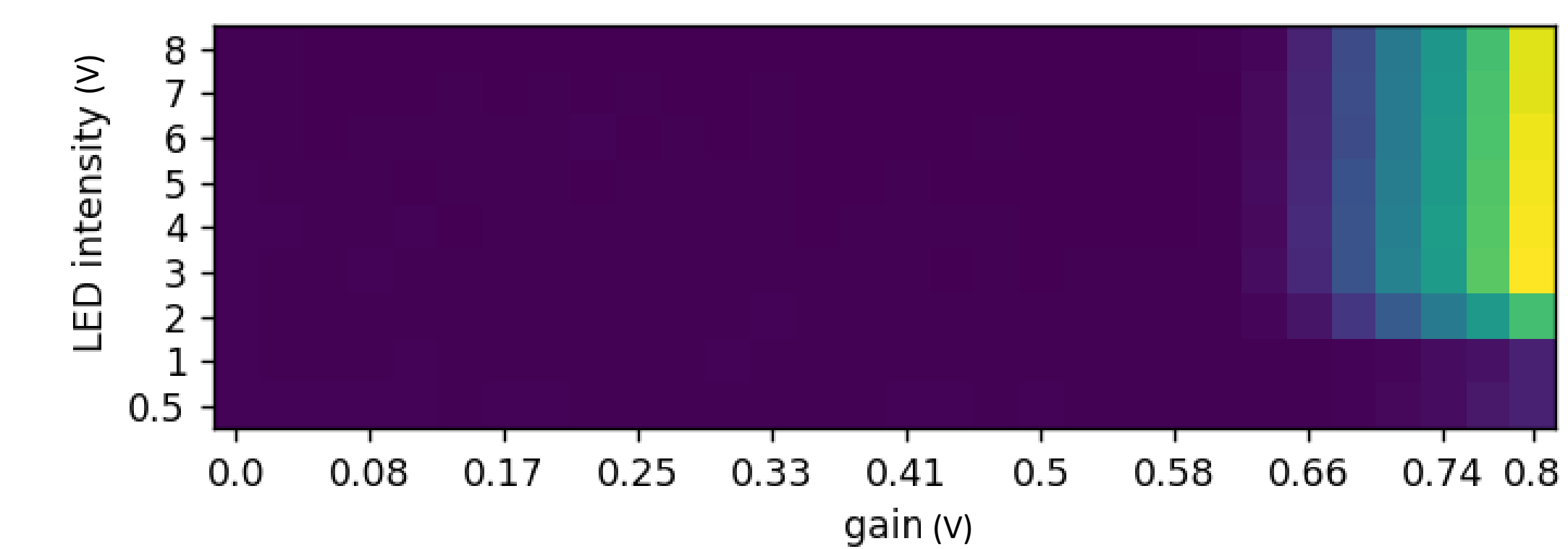
Left: Divide-by-100 ripple counter example



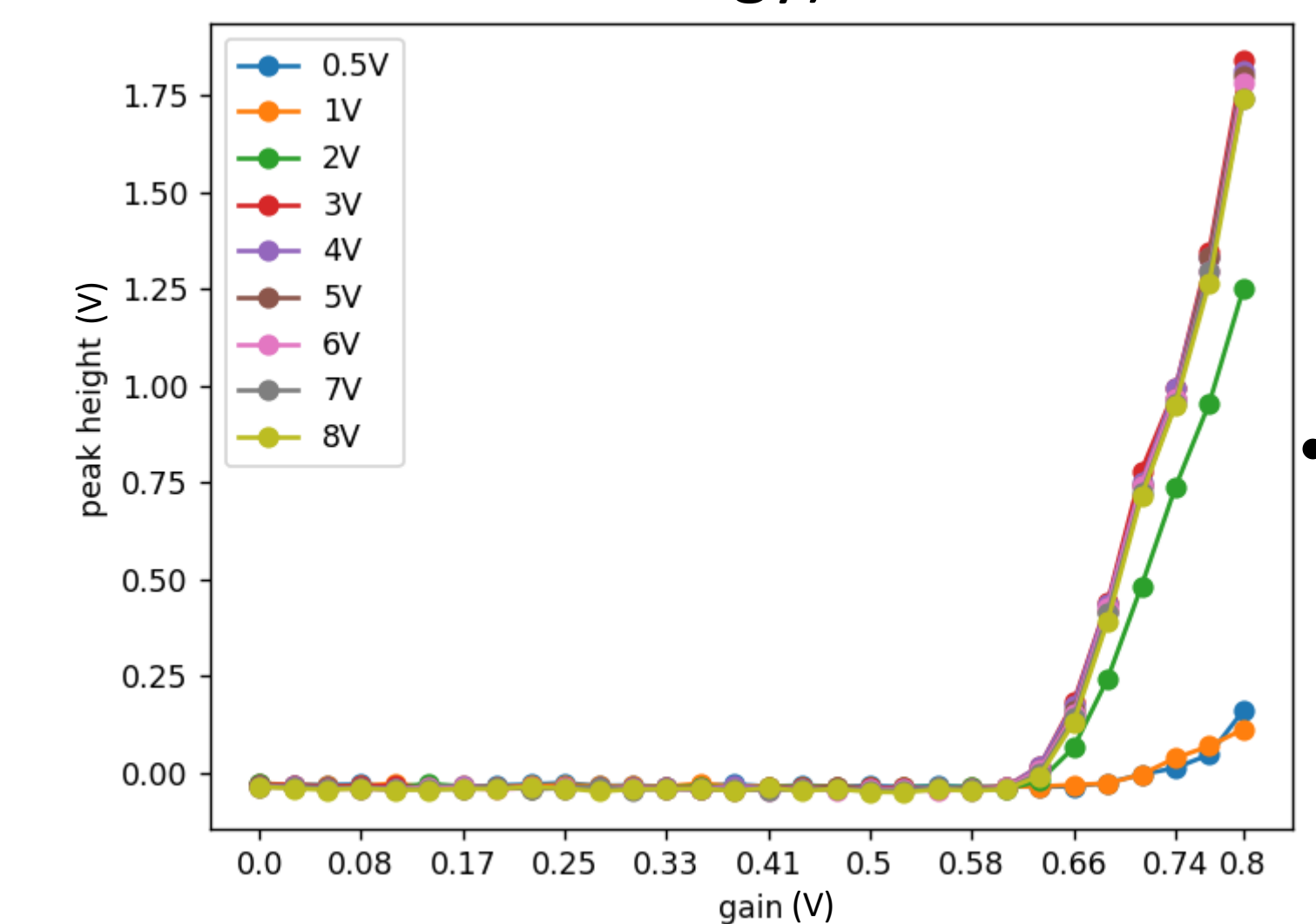
Above: Sine wave (yellow) digitized by Schmitt trigger (purple)

Results (Contributions)

Peak Height Analysis (PMT signals)



- Above: peak height plot across gain settings w/varying input light to determine sensitivity
- Peak height independent from light once adequate photon input reached (since photons all have same energy)



- Left: gain vs. peak height w/varying input light (in legend)
- Exponential growth from ~0.6V gain typical for working PMT

Peak Count Analysis (waveform generator)

- Left: sine wave (yellow) @ 1kHz → Schmitt trigger output (purple) → ripple counter output (green) @ 10Hz
- Half-wave rectified sine wave mimics PMT output after TIA & inverter

Future Work (2nd Semester of Thesis)

- Continue peak count analysis (w/ high-speed surface mount ripple counter)
- Analyze peak width to further characterize dynodes
- Design system & publish open-source documentation for community use for evaluating PMT health

Selected References

- Schottdorf M, Rich PD, Diamanti EM, Lin A, Tafazoli S, Nieh EH, et al. (2025) TWINKLE: An open-source two-photon microscope for teaching and research. PLoS ONE 20(2): e0318924.
- Hamamatsu. Photomultiplier Tubes Basics and Applications.
- NI-DAQmx Python Documentation. From <https://nidaqmx-python.readthedocs.io/en/stable/>