A SiPM based novel approach to cytosolic calcium detection by bioluminescence

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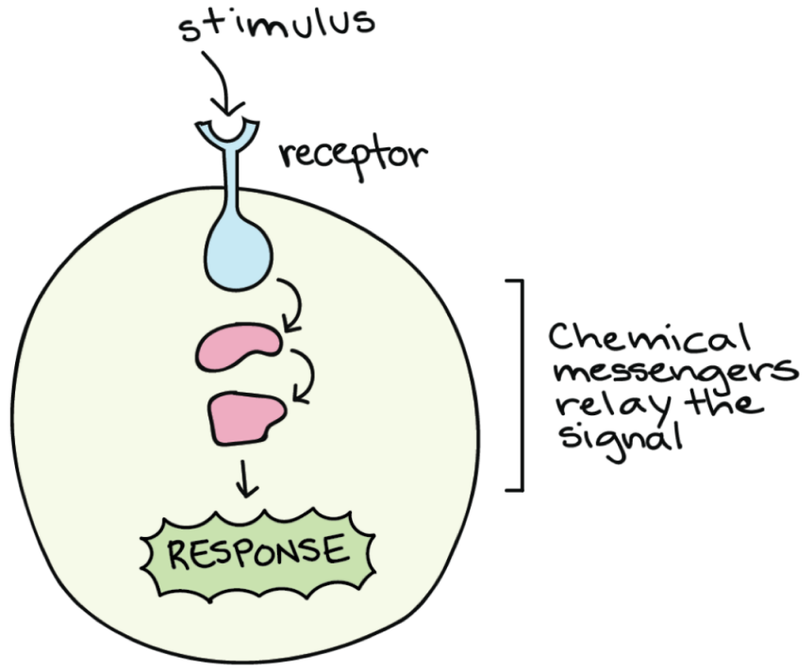
SiPM workshop: from fundamental research to industrial applications

4th October 2019 - Bari

Cell signaling

"The ability of cells to perceive and respond to the external stimuli"

- Signaling is the basis of cell growth and repair, immunity and tissue homeostasis.
- The stimulus is relayed through a chain of chemical messengers inside the cell.



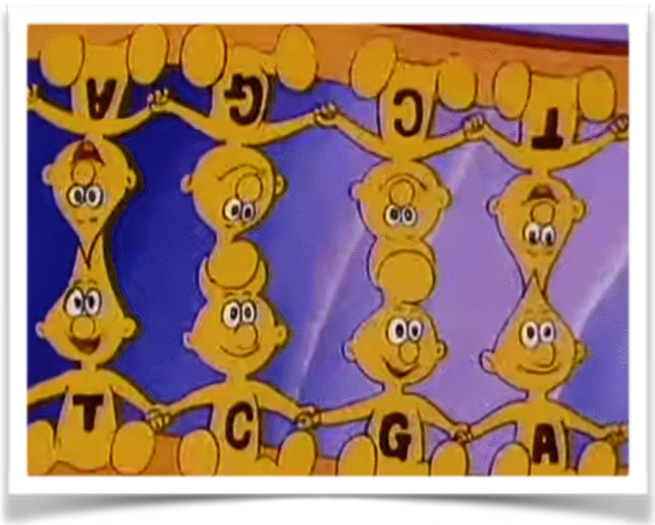
→ Ca^{2+} (the most abundant dication in human body) is an universal second messenger

Ca^{2+} signaling

"Second messengers are intracellular signaling molecules released by the cell in response to exposure to extracellular signaling molecules (first messengers)"

- Second messengers trigger physiological changes
- Many significant cellular processes are regulated by gradient Ca^{2+} concentration between the intracellular and extracellular enviroment and the cell itself

Neurotransmission

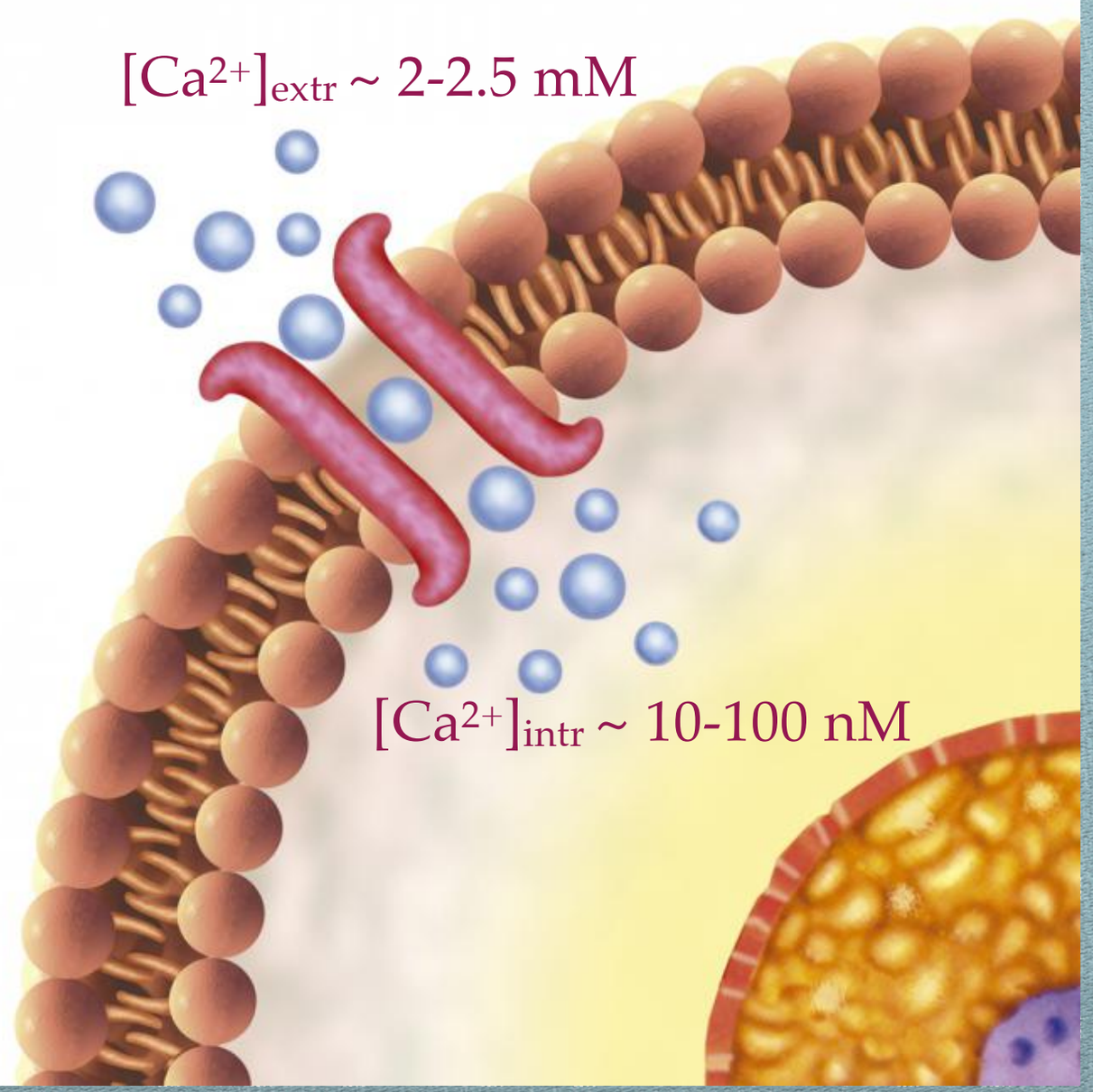


Gene Transcription

ATP production

Ca^{2+} signaling

- The intracellular concentration of Ca^{2+} in cytosol $[Ca^{2+}]_{intr}$ is very low in respect of extracellular concentration $[Ca^{2+}]_{extr}$
- Stimuli open channels for Ca^{2+} and allow Ca^{2+} extracellular to flow into the cytosol
- Ca^{2+} ions bind some proteins in the cell changing their activity providing a response to a stimulus

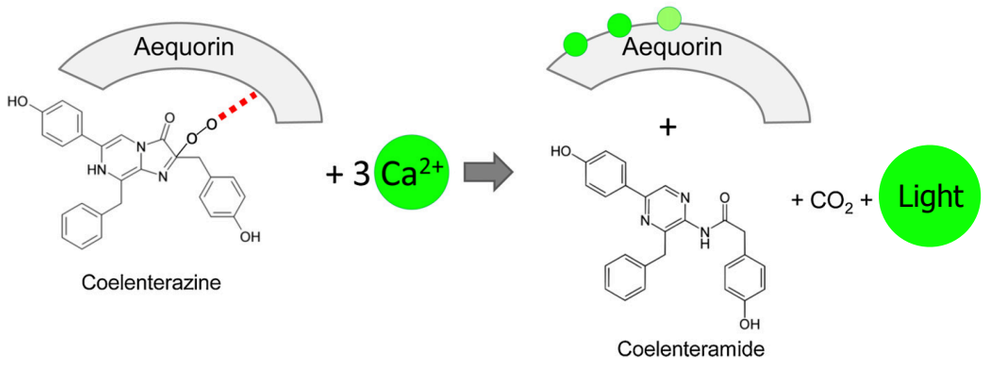


The Concept

- Reconstruct the shape of calcium spikes (gradients), which could be indication of diseases, by means of bioluminescent probes
- Develop a SiPM based instrument that could replace a custom made PMT based apparatus, offering at least comparable performance, cheaper, higher modularity, flexibility and portability

Aequorin (a protein) is a bioluminescent Ca^{2+} probe

A SiPM S13360-6050CS 6x6 mm^2 50µm pitch and 1 MHz of DCR



Low concentration = low light frequency

Biological Sample

- The linearity of the system response and the related dynamic range are assessed using a cell lysate obtained from cytosolic aequorin (cyt-AEQ)-transfected HeLa cells

> *"Lysate is a fluid containing lysed cell, i.e. suspend components of cells whose membranes are destroyed"*

- The Aequorin concentration is halved progressivly in order to explore 3 order of magnitude
- The administration of Ca^{2+} is the injected external stimulus

The apparatus

Bioluminescence is measured both by photon-counting and charge integration at the same time, splitting the SiPM output signal amplified (20 dB) by a custom and compact DC front-end with low noise

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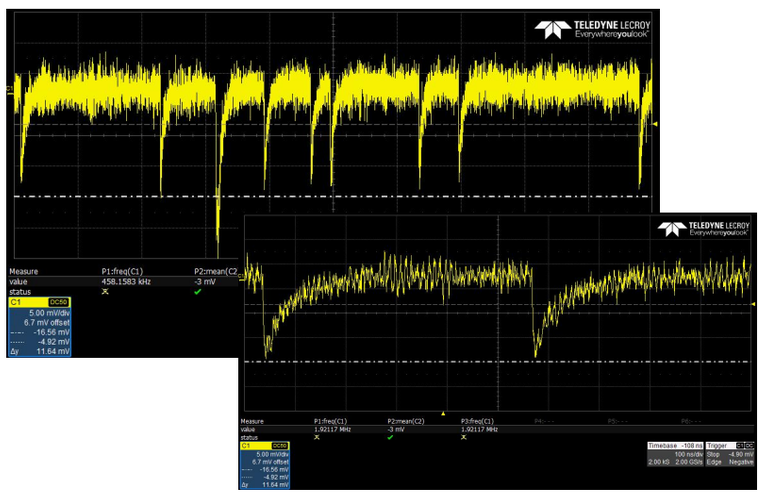
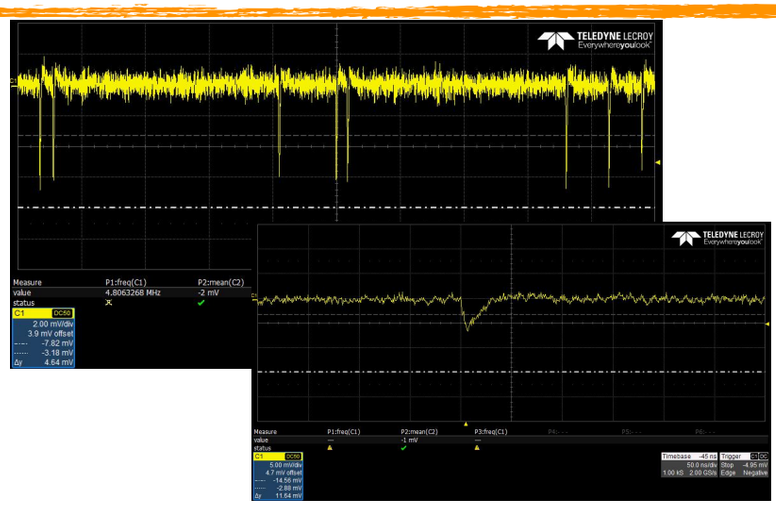
- polo-zero cancellation

Pile-up probability < 5% at 2MHz



before: time development 150 ns



After: time development 30 ns

The apparatus

Bioluminescence is measured both by photon-counting and charge integration at the same time, splitting the SiPM output signal amplified (20 dB) by a custom and compact DC front-end with low noise

- polo-zero cancellation
- leading-edge comparator (threshold ~0.5 photoelectron)
- 16-bit scaler, integrated in the PSAU SP5600 by CAEN S.p.A (time window = 100 ms)

The apparatus

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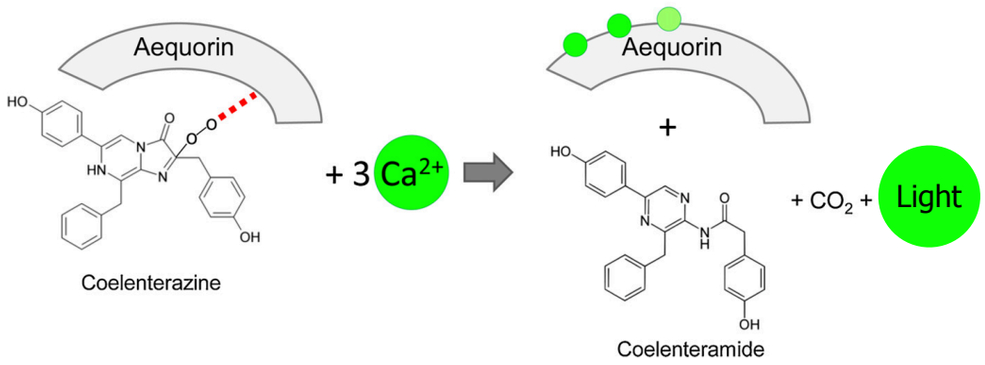
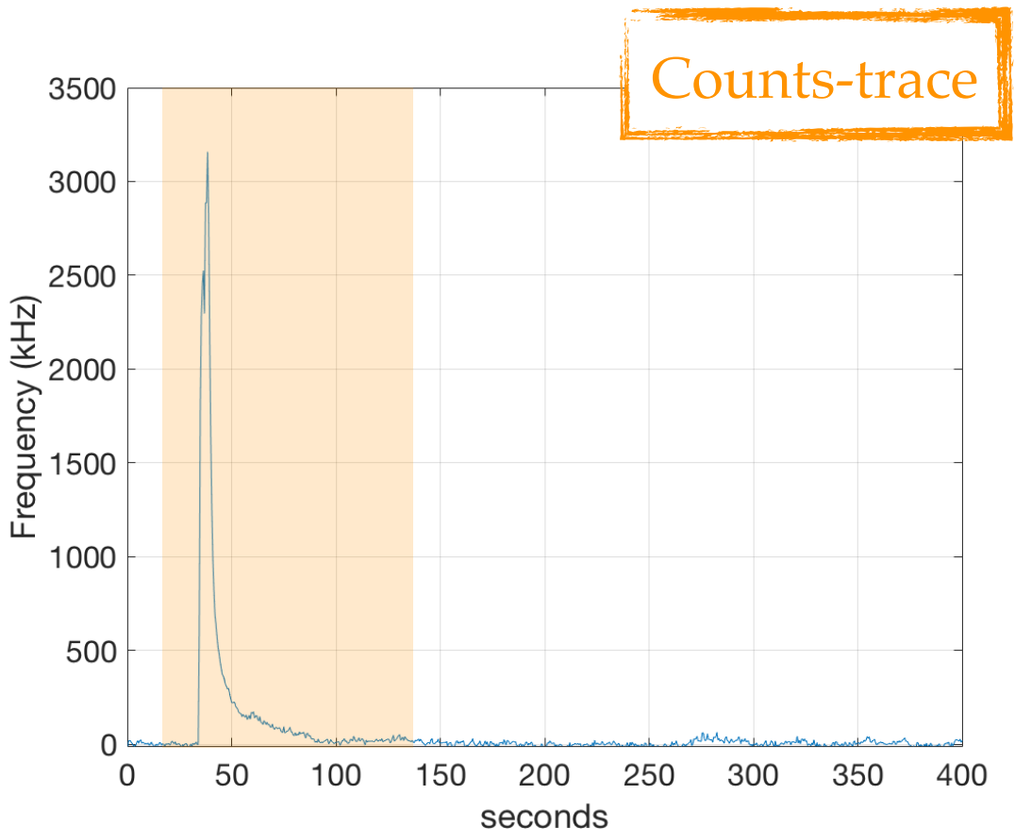
- Analog integrator V752N charge-to-digital converter (QDC) by CAEN S.p.A.
- External trigger at 11 kHz
- The amplitude of the signal and the integration gate were modified in order to cope with the dynamic range of the QDC

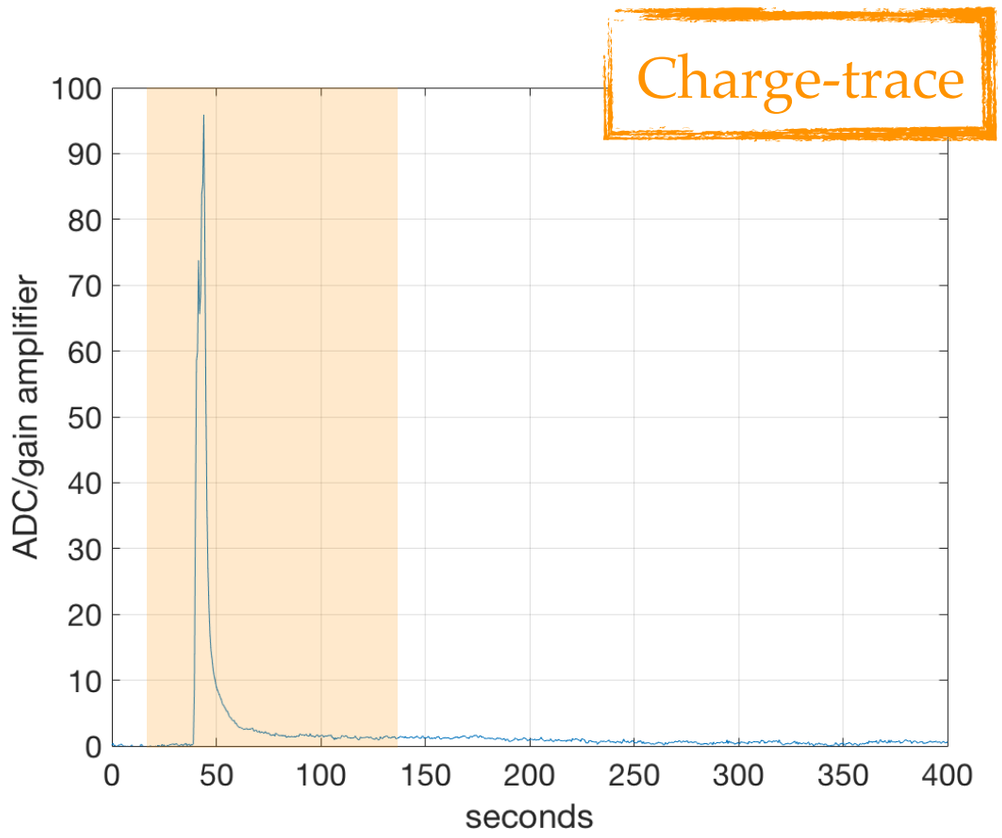
The Calcium Signal



A sequence of single photons. The frequency of the photons is strictly related to the Aequorin concentrations in the biological sample

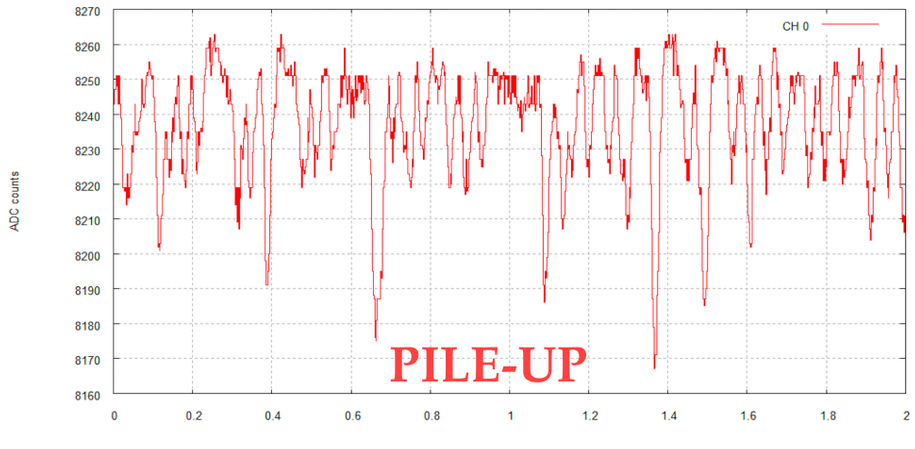
Low concentration = low light frequency





Counts vs Charge

HIGH aequorin concentration

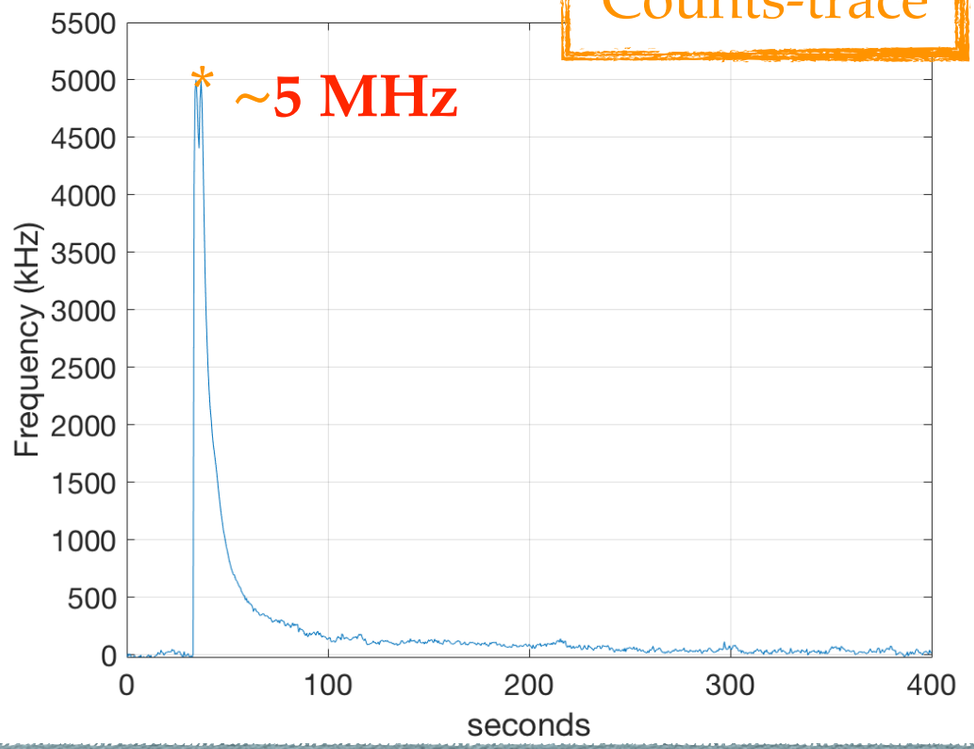


$$Freq = \frac{ADC}{gate_{int}} \cdot \frac{100\ fC}{272\ fC}$$

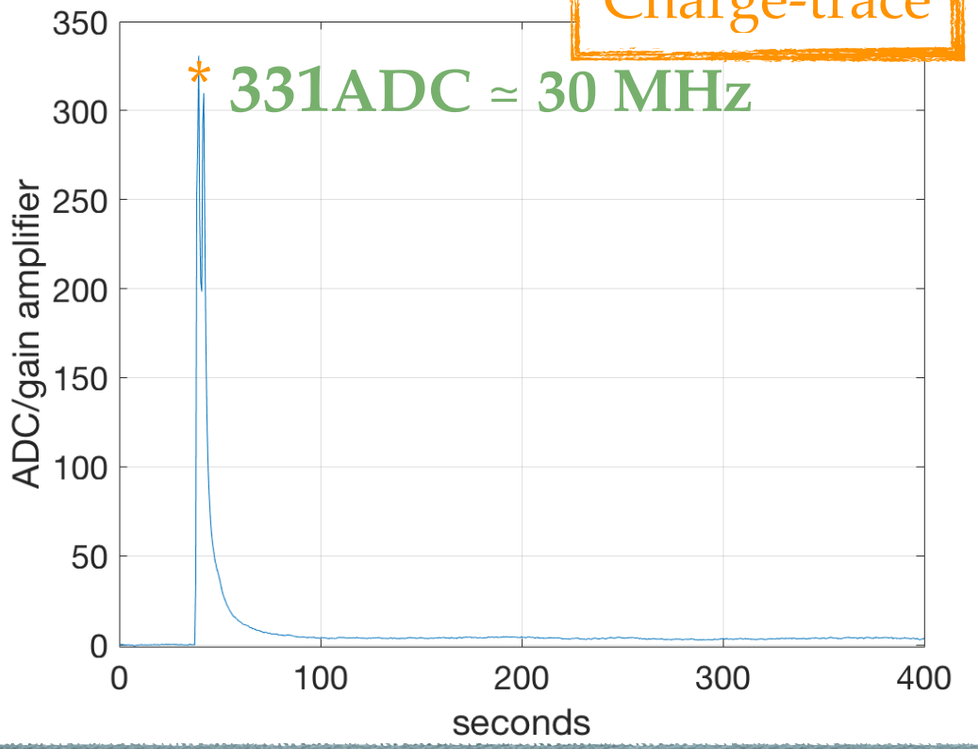
1 ADC = 100 fC from datasheet
1 phe = 272 fC (SiPM gain of $1.7 \cdot 10^6$)

Only as example we consider the maximum of the signal

Counts-trace



Charge-trace



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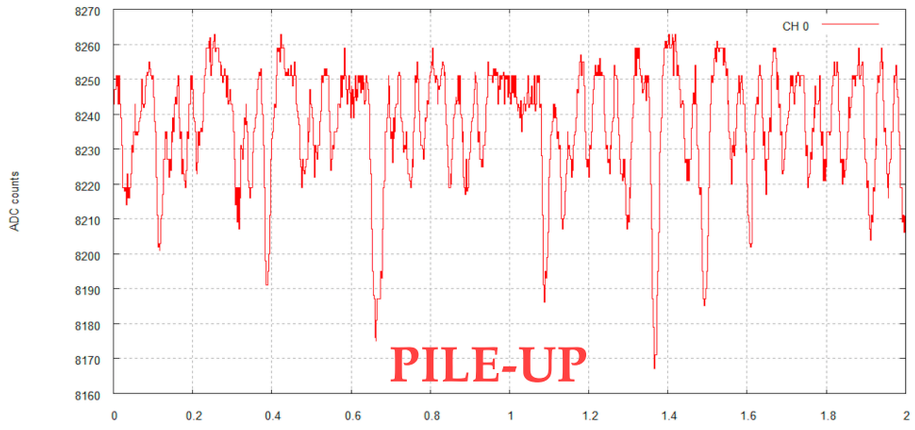

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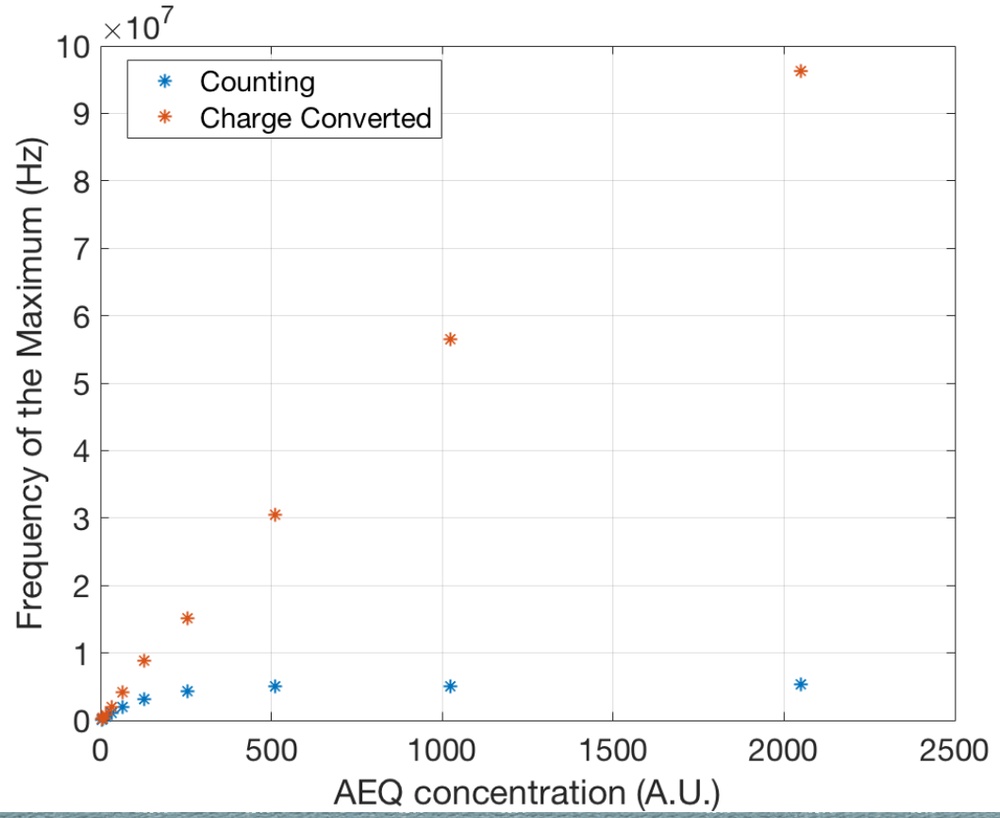
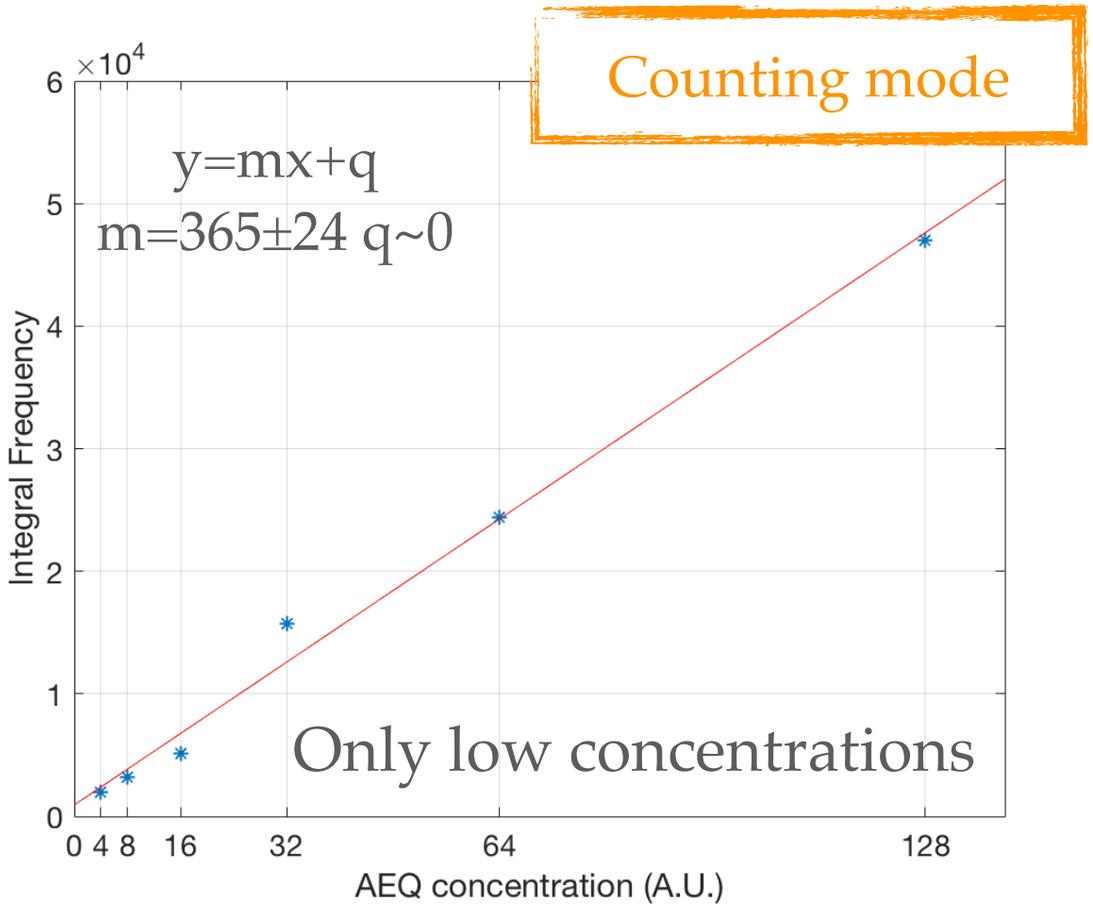
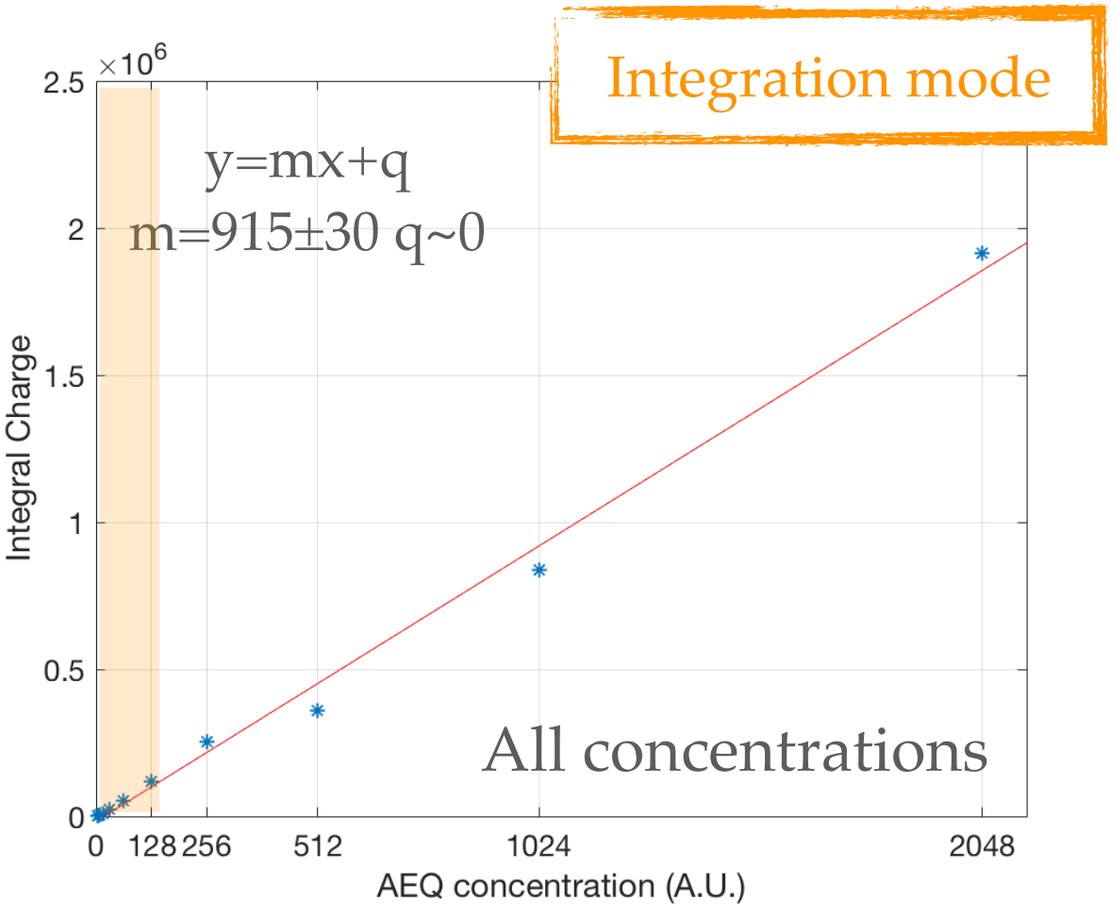


System Linearity

Aequorin concentration is scaled according to a geometric progression of common ratio 2 exploring a domain of ~3 orders of magnitude



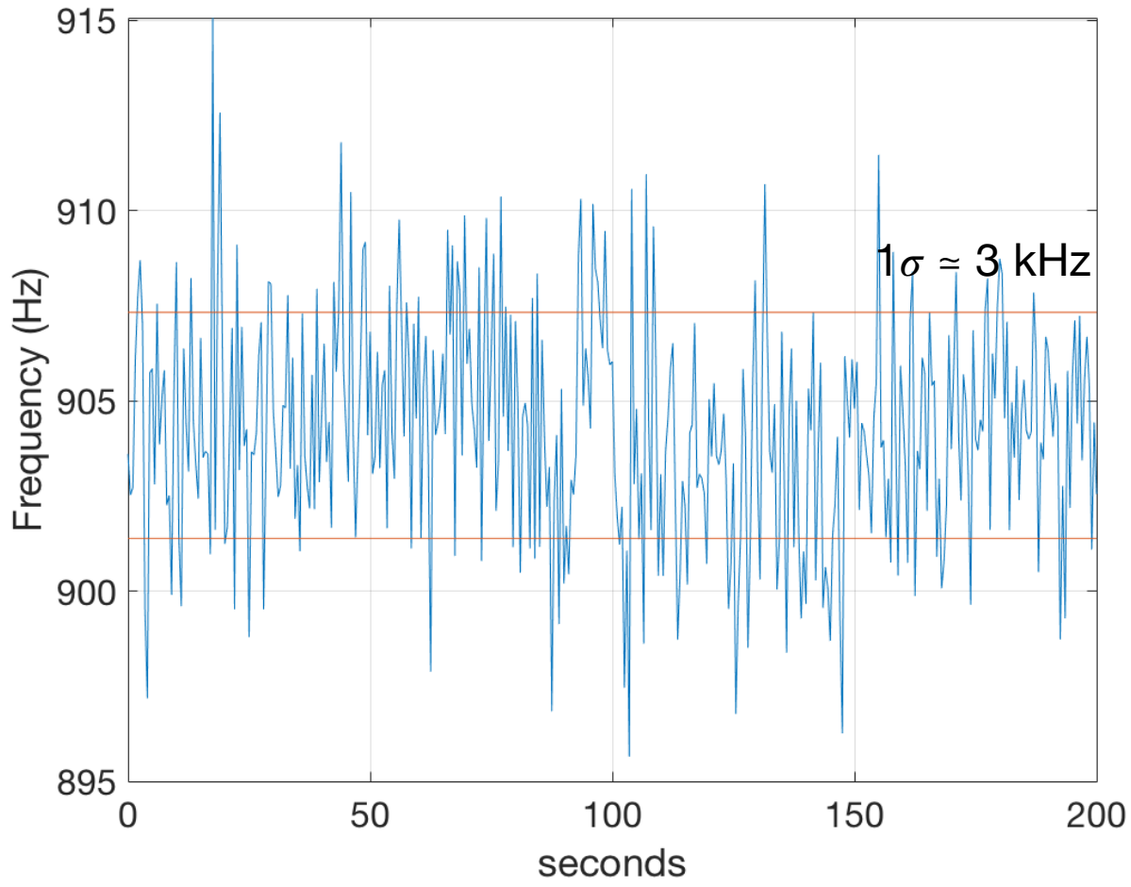


The two angular coefficient are compatible at 2σ if the conversione ADCvsFrequency is considered

The Sensitivity: counting

What happens at low light frequency?

- For the counting mode the standard deviation of the noise is about 3 kHz. Limit of detactable signal (LoD) a 3σ is ~10 kHz



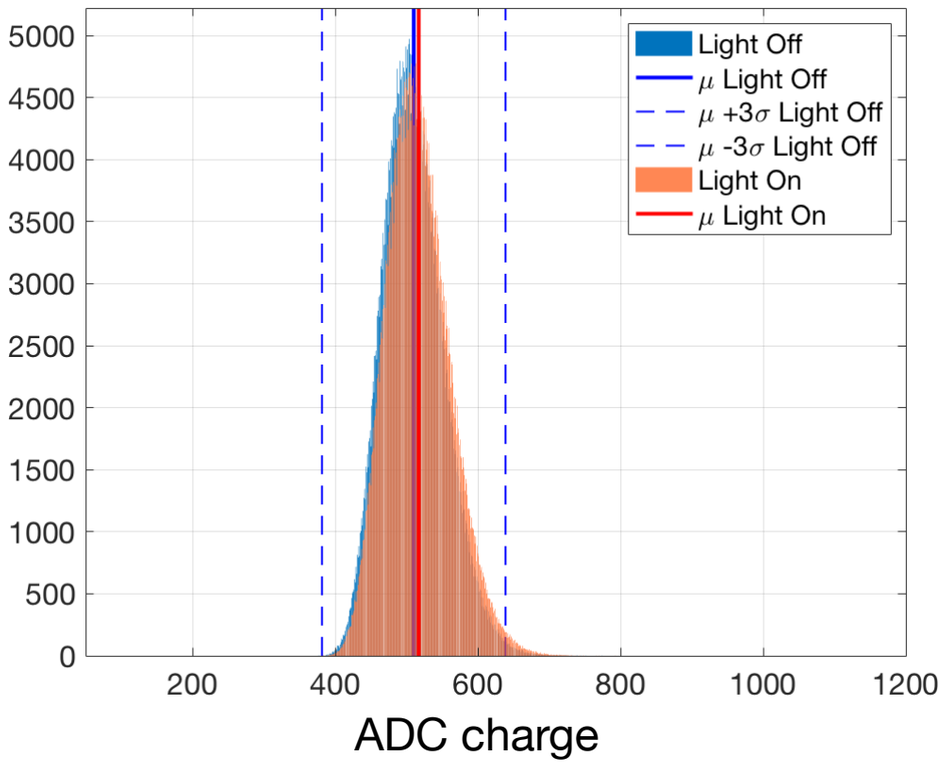
The Sensitivity: QDC

What happens at low light frequency?

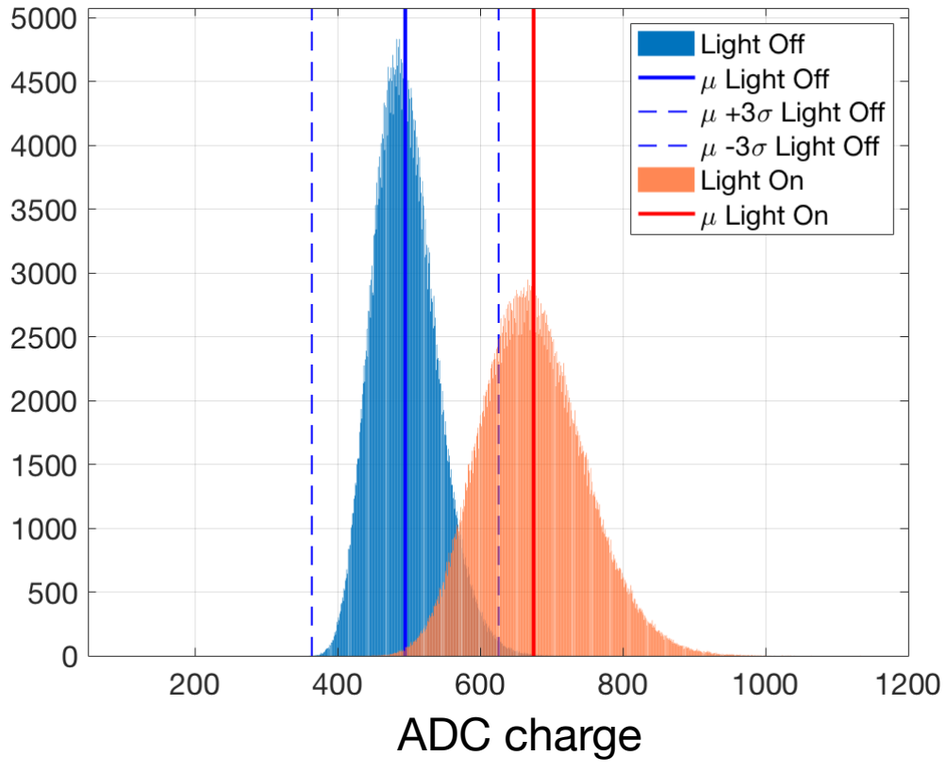
- Test in lab illuminating the SiPM with a led source, externally triggered at different frequencies, with mean valute of 1 photoelectrons
- Estimation of the mean difference between pedestal and light on

Charge distribution

55 kHz



2053 kHz



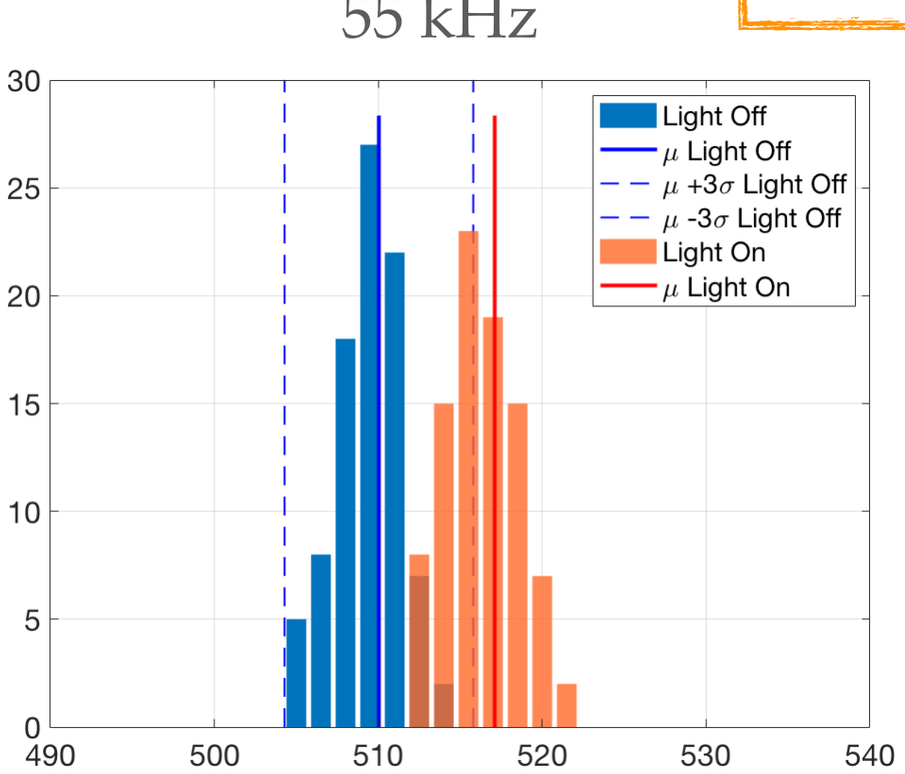
The Sensitivity: QDC

What happens at low light frequency?

- Thanks to the high event rate the averaging of events allows to increase the sensitivity at low frequencies.

Charge distribution

55 kHz



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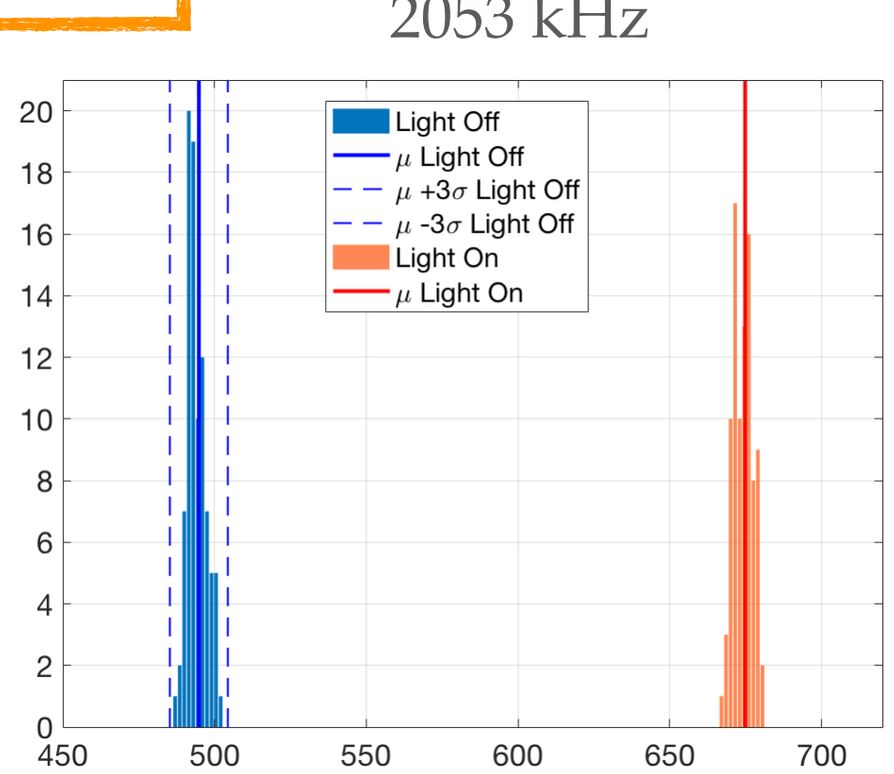
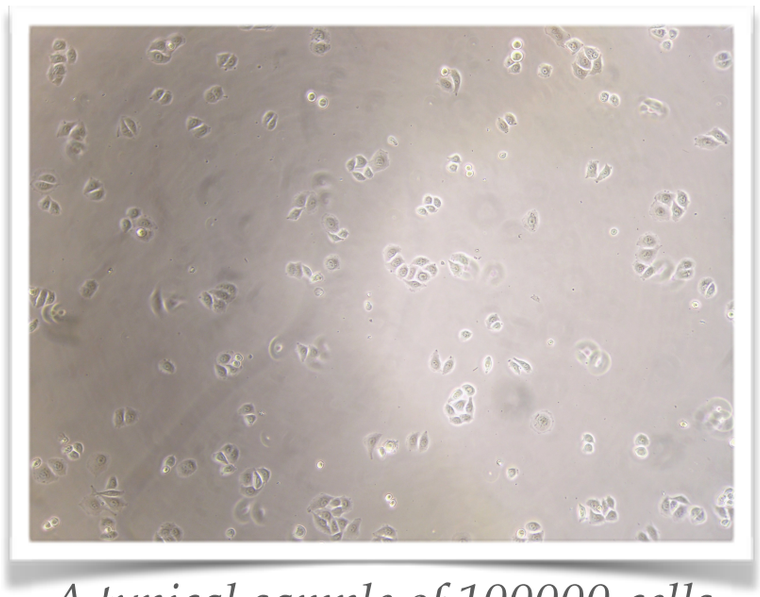


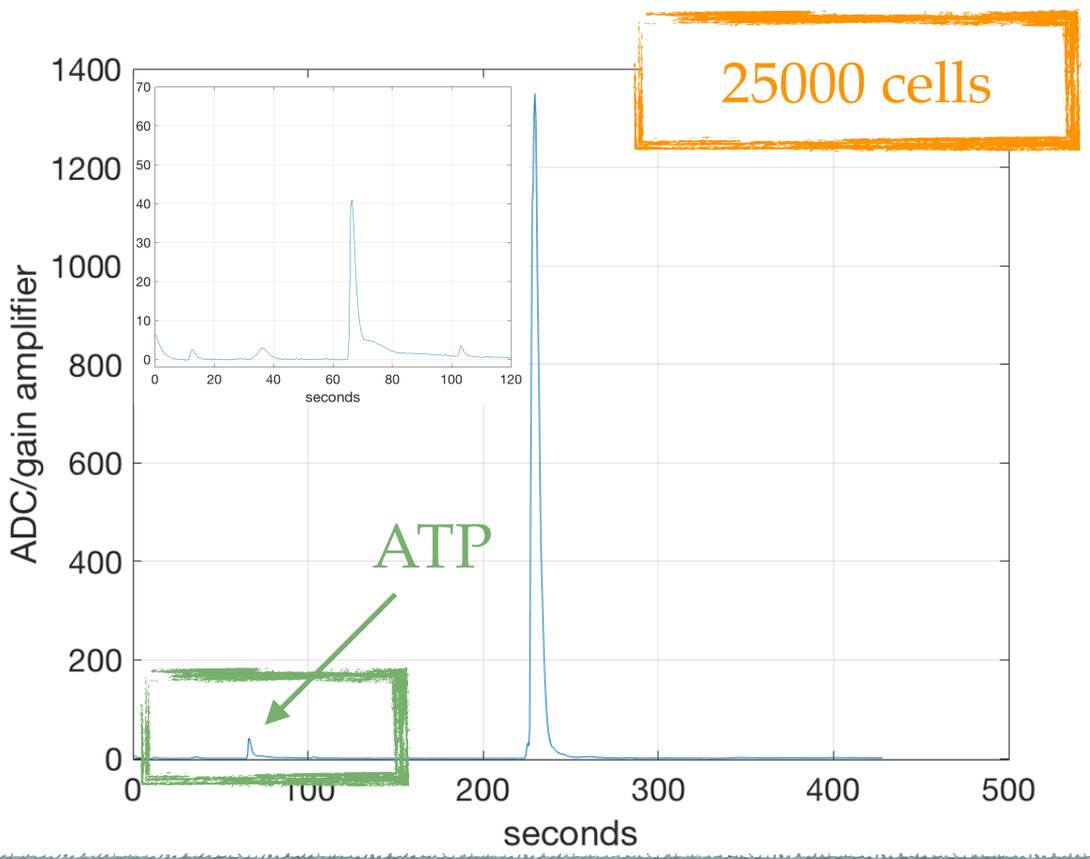
averiging on 5500 events in order to fit the trigger event of the counting branch

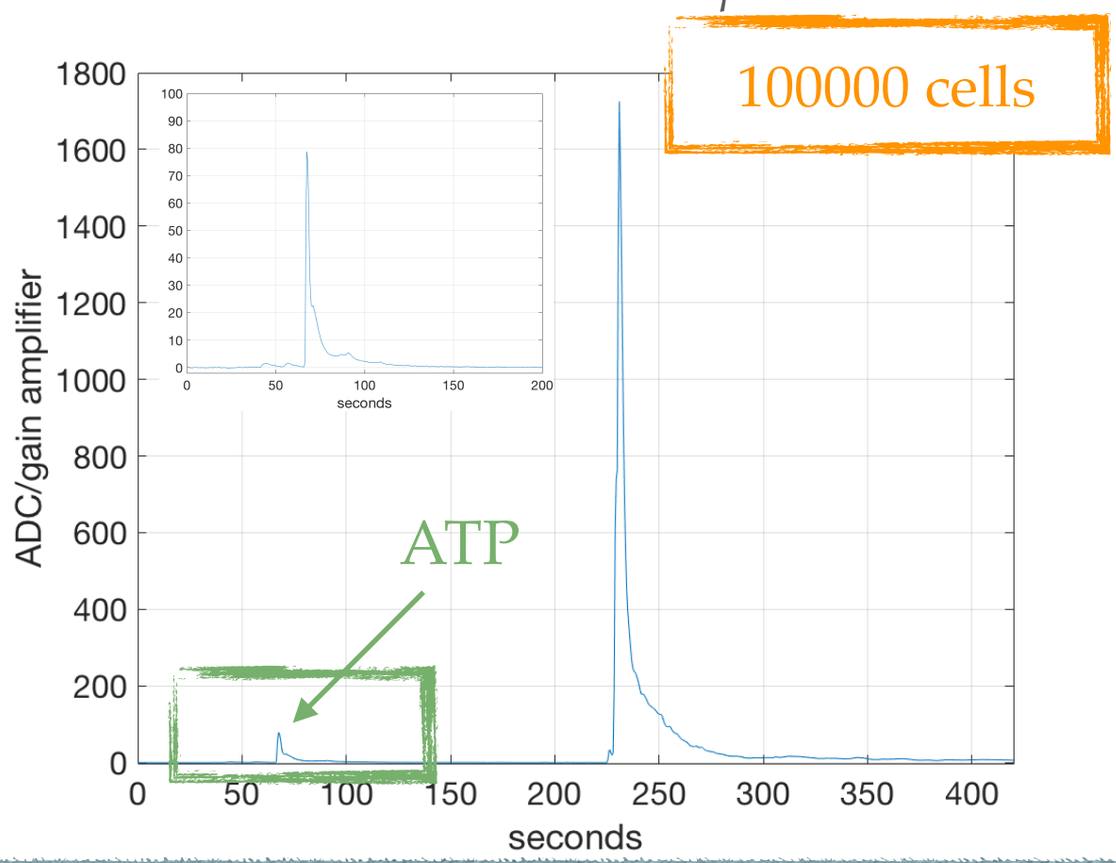
Live-cell Signaling

- HeLa cells (most common human cell line in research) are transfected in order to express aequorin in cytosol
- Administration of adenosine-triphosphate (ATP), as external stimulus, triggers intracellular Ca^{2+} release



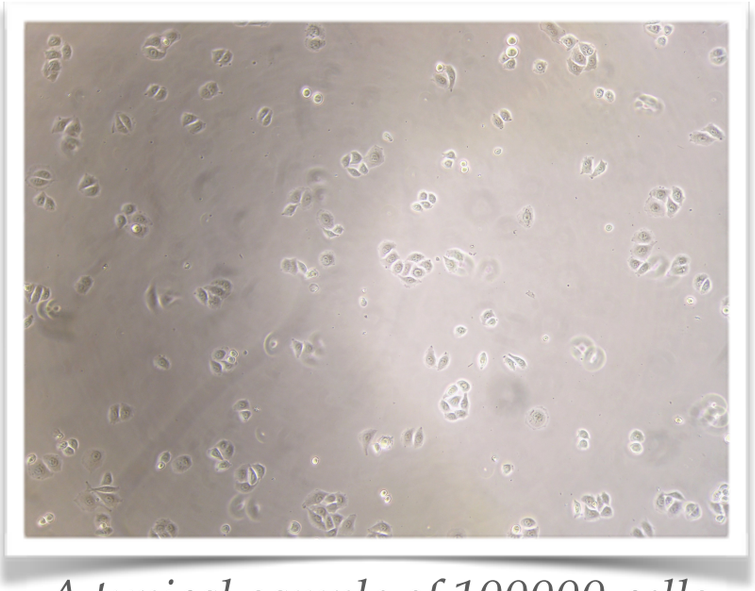
A typical sample of 100000 cells on plate





Live-cell Signaling

- To be quantitative, a normalisation of the ATP induced signal is obtained by a delayed disruption of all of the cells in the sample with a surfactant (TRITON)
- A factor 30 or more is expected between ATP and TRITON induced signals



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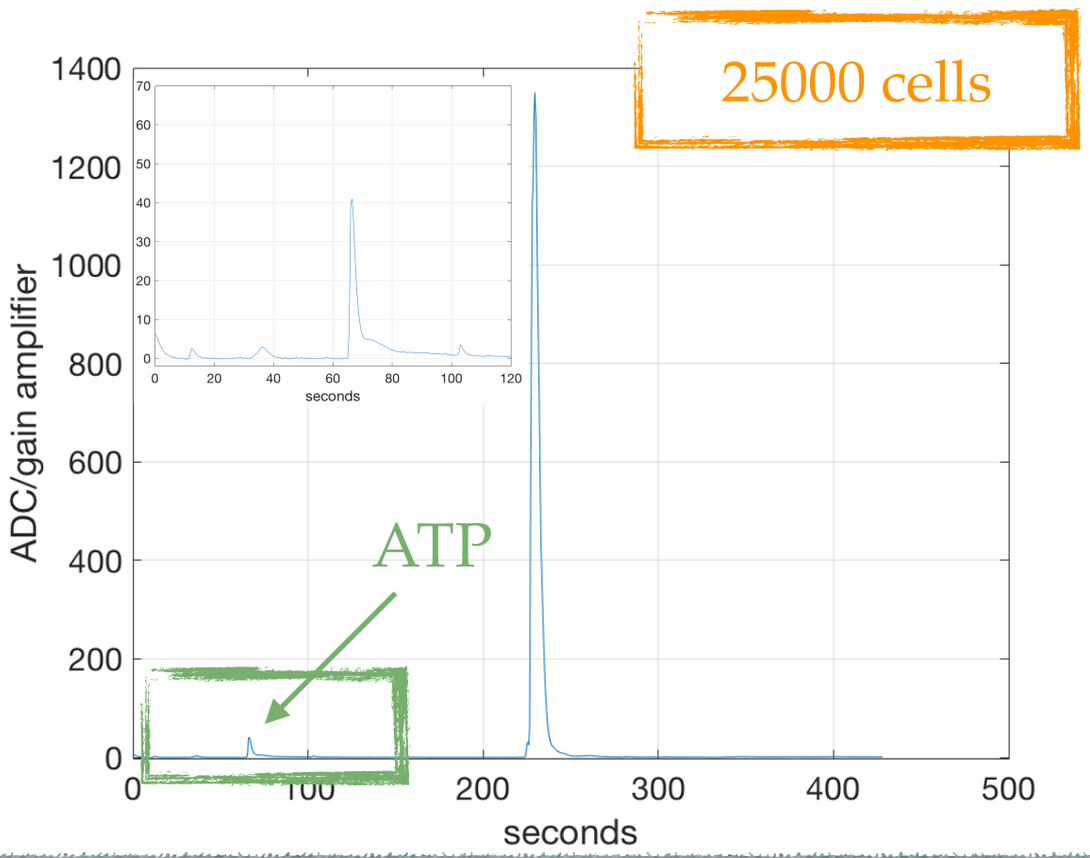
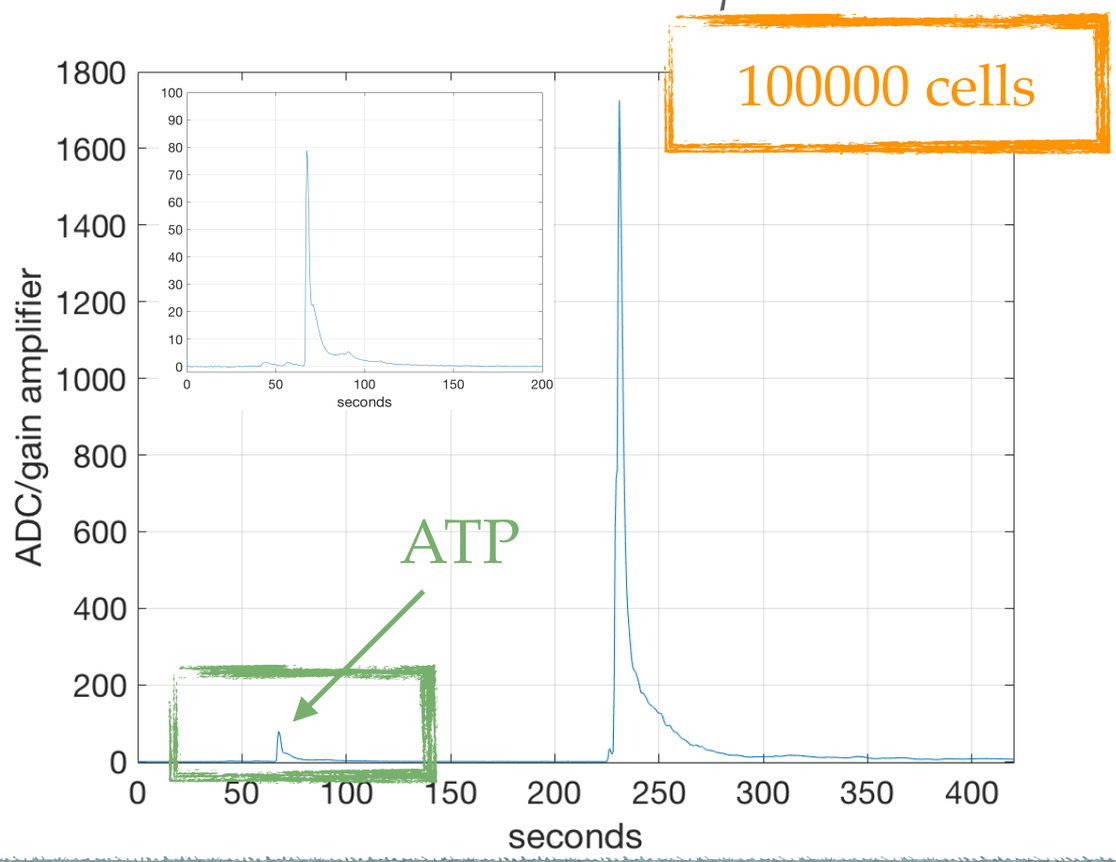




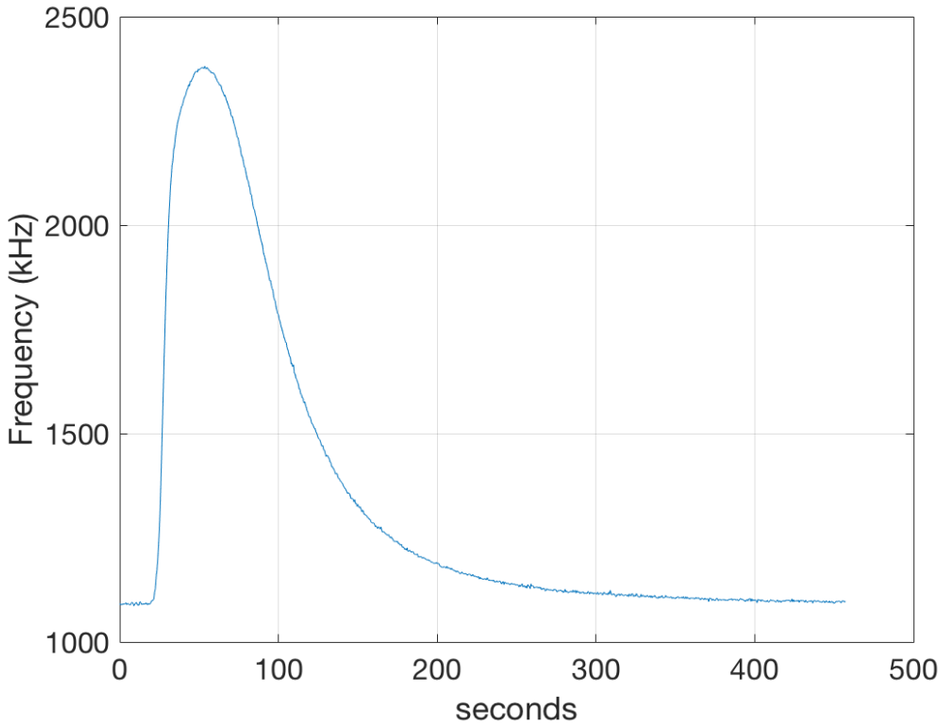
Conclusion & Outlooks

- Our experiments provide evidence of system linearity across three orders of magnitude of aequorin concentration.
- Charge integration and photon-counting prove to be two alternative and complementary modalities for handling the SiPM output signal (working with both strong and weak light signals)

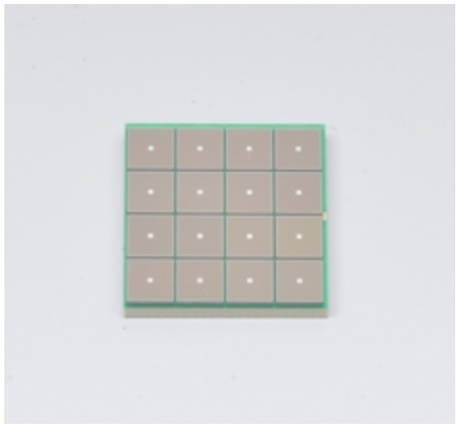
Conclusion & Outlooks

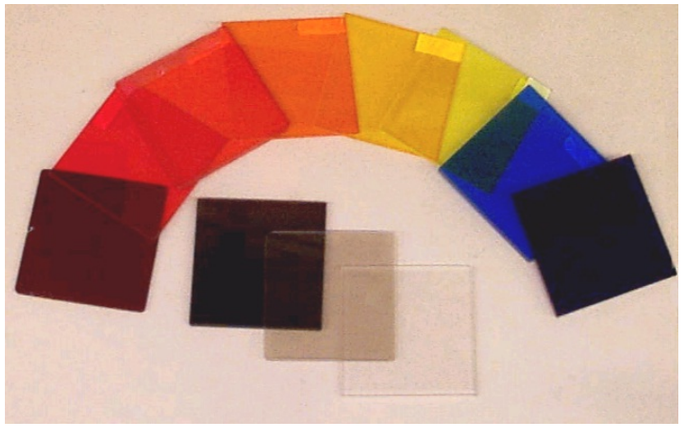
- This measurement can also be extended to a deeper study of Ca^{2+} pathways in cell signaling, reconstructing the Ca^{2+} spikes produced also in different organelles

- Target different organelles with different probes that emit light with different λ



An ATP induced signal in mitochondria





Thanks for the attention

ADDITIONAL SLIDES

Live-cell Signaling

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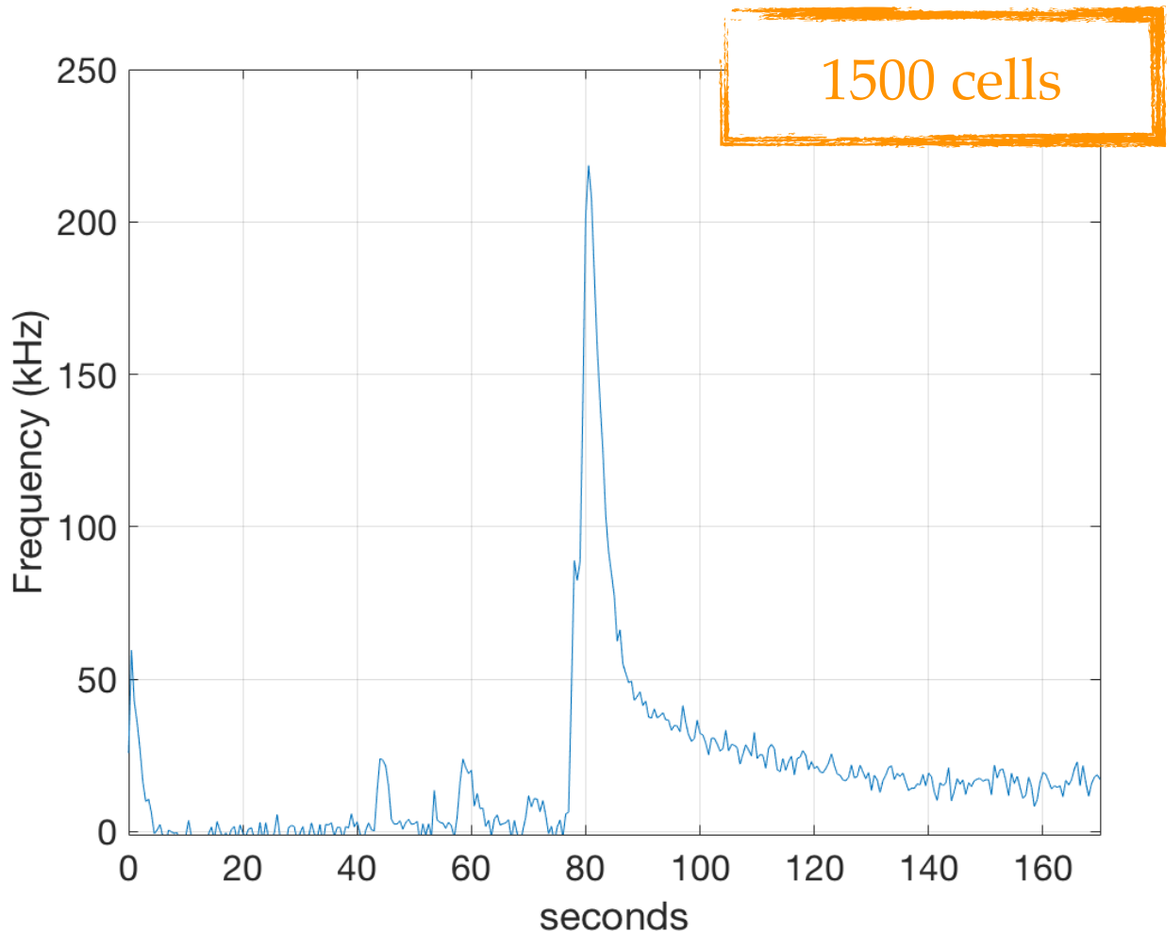
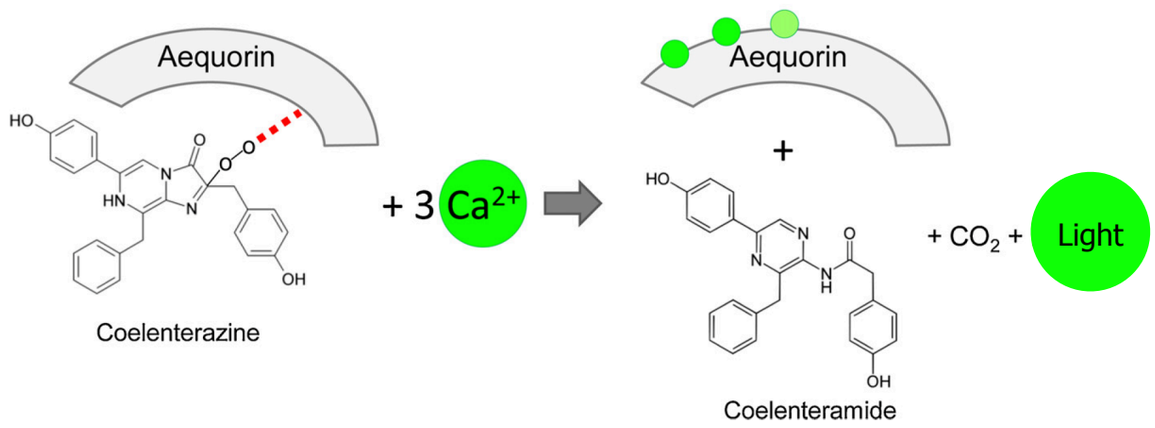


Aequorin

Aequorin features:

- measurement of a wide range of [Ca^{2+}] (μM-mM)
- response is directly proportional to the [Ca^{2+}]
- the cells are preserved by photodamage because external excitation is not needed
- low level of emitted light consisting in sequences of single photon pulses



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- The linearity of the system response and the related dynamic range are assessed using a cell lysate obtained from cytosolic aequorin (cyt-AEQ)-transfected HeLa cells

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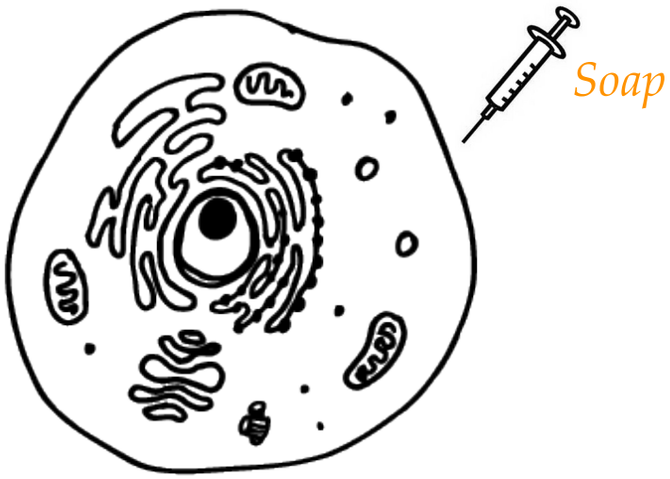
- The administration of Ca^{2+} is the injected external stimulus



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Lysate

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