BP210: Biological Light Microscopy

Lab 7: Measuring Cellular Processes; Fluorescent Biosensors

Goal: The goal of this lab is to explore how different fluorescence-based biosensors and imaging techniques are used to measure dynamic cellular processes. We will compare methods for tracking electrical activity, calcium signaling, molecular interactions, and environmental responses across multiple biological systems. Through these experiments, we will evaluate how sensor design and imaging modality influence temporal resolution, spatial information, and quantitative accuracy, and will learn to select appropriate tools based on the biological question.

Location: GH S252

Samples provided by:

- Gladstone-CIRM Shared Resource Laboratory – Cardiomyocyte cultures
- Evan Miller (UC Berkeley) – BeRST voltage-sensitive dye
- Maria Ingaramo (Non-Fiction Labs) – Magnetofluorescent *E. coli*
- Bill Jia (UCSF) – Zebrafish embryos expressing GCaMP

1. Cardiomyocytes: BeRST (Voltage) + GCaMP (Calcium)

Objective: Compare membrane voltage and calcium dynamics.

1. While imaging, adjust the frame rate. What happens to your ability to resolve BeRST vs GCaMP signals?
2. From the recorded traces, which signal rises faster? Estimate the time difference.
3. What does each sensor measure? Which is direct vs indirect?
4. Do all voltage spikes correspond to calcium signals in your data? Why or why not?
5. What happens when you increase exposure time or illumination intensity? How does this affect signal quality and photobleaching?
6. Which sensor would better detect single action potentials based on your data?
7. Compare BeRST (dye) vs GCaMP (protein) based on your observations:
    a. Signal strength
    b. Variability
    c. Ease of use
8. If you wanted to measure fast neuronal activity in zebrafish, would GCaMP be sufficient based on what you observed?

2. Zebrafish Embryo: GCaMP (Light Sheet)

Objective: Whole-organism calcium imaging.

1. Adjust the light sheet thickness or focus. How does this affect image quality and background signal?
2. Compare different depths in the sample. How does signal quality change with depth?
3. Why is light sheet ideal for this sample based on what you observe?
4. What would happen if you used widefield instead? What differences would you expect in your images?
5. Do you observe photobleaching over time? How does it compare to widefield imaging?

3. Magnetic Sensor in E. coli

Objective: Observe magnetic response.

1. Record baseline fluorescence. Then introduce the magnet. What quantitative change (increase/decrease/localization) do you observe?
2. Move the magnet closer and farther. Does the response scale with distance?
3. Are all cells responding the same way? What variability do you observe?
4. How might differences in expression level affect what you see?
5. What control experiment would confirm the effect is due to the magnetic field?

4. FRET Imaging

Objective: Measure molecular interactions.

1. Acquire donor, acceptor, and FRET images. How do the raw intensities compare?
2. Calculate or estimate a FRET ratio. How does it change across cells or over time?
3. What happens to the FRET signal if expression levels vary between cells?
4. Adjust exposure or gain. How does this affect the apparent FRET ratio?
5. What sources of error (bleed-through, alignment) can you identify in your data?

5. Cross-Experiment Comparison

1. Based on your data, classify each sensor as Intensity-based, Ratiometric, Energy transfer-based, lifetime, or other.
2. Which dataset appears most quantitative? What evidence supports this?
3. What was the largest source of error you observed in any experiment? How would you fix it?
4. Choose one experiment and propose a modification (sensor or microscope). What improvement would it provide?