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# BE-LIGHT

Improving biomedical diagnosis through light–based technologies and machine learning

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WP2: *Cardiac Arrhythmias*

**Deliverable D2.4**

**Fluorescence imaging system**

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| **Abstract (for dissemination)** | Deliverable D2.4 describes the fluorescence-based verification workflow and associated optical imaging and stimulation platform developed for cardiac optogenetic experiments.<br><br>Optogenetically modified HL-1 cardiac cell monolayers expressing Channelrhodopsin-2 fused to the fluorescent reporter mCherry (ChR2-mCherry) are used as the primary experimental preparation. Fluorescence imaging is performed before functional experiments to verify the expression of the optogenetic construct. Following expression verification, cardiac spiral-wave dynamics are investigated using high-speed MiCAM brightfield imaging combined with controlled blue-light stimulation at approximately 470 nm.<br><br>The experimental platform supports global, half-side, and gradient illumination patterns, enabling the investigation of illumination-dependent changes in cardiac wave dynamics. Quantitative analysis includes dominant frequency, apparent propagation velocity, apparent spiral-core size, and apparent core displacement.<br><br>The current implementation demonstrates fluorescence-based expression verification and functional optogenetic experiments in cardiac cell cultures. |
| **Keywords** | Optogenetics, HL-1 cells, Channelrhodopsin-2, cardiac arrhythmia, spiral waves, brightfield imaging, fluorescence imaging |



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# 1 Executive Summary

The primary objective is to establish an imaging workflow that enables the verification of optogenetic protein expression in cardiac preparations and supports subsequent investigations of cardiac excitation dynamics under controlled optical stimulation.

In the current cell-culture implementation, HL-1 cardiac cells are modified to express Channelrhodopsin-2 (ChR2), a light-sensitive ion channel, together with the fluorescent reporter mCherry. Fluorescence imaging is performed prior to optogenetic experiments to assess the presence of reporter expression in the cell preparation.

After fluorescence verification, the preparations are investigated using high-speed MiCAM brightfield imaging. The imaging system records contraction-related optical changes associated with propagating cardiac activity. Optical stimulation is delivered using blue light at approximately 470 nm.

Different illumination strategies have been investigated, including global illumination, half-side illumination, and spatial intensity gradients. The experimental observations demonstrate illumination-dependent changes in multiple parameters characterizing spiral-wave dynamics.

The developed workflow combines fluorescence-based expression verification, functional brightfield imaging, optical stimulation, and quantitative image analysis. The present document describes the experimental implementation, measurement principles, representative results, and current limitations.

# 2 Introduction

Cardiac arrhythmias are associated with abnormal electrical excitation patterns that disrupt the coordinated activity of cardiac tissue. Certain arrhythmias are maintained by re-entrant excitation waves, including spiral waves in two-dimensional preparations and scroll waves in three-dimensional cardiac tissue.

Understanding the initiation, propagation, and termination of these excitation patterns is important for developing more effective strategies for arrhythmia control. Conventional electrical stimulation methods can influence cardiac activity but offer limited spatial selectivity.

Optogenetics provides an alternative experimental approach in which cellular excitability can be modified using light-sensitive proteins. Channelrhodopsin-2 (ChR2) is a light-gated cation channel that can be activated by blue light. When expressed in cardiac cells, ChR2 enables optical perturbation of the cellular electrical state.

Successful optogenetic experiments require verification that the target cells express the introduced light-sensitive construct. Fluorescent reporter proteins provide a practical method for identifying expression in genetically modified cells.

In the present work, ChR2 is coupled to the fluorescent reporter mCherry. Fluorescence imaging is used to assess reporter expression before functional optogenetic experiments are performed.

Following expression verification, cardiac spiral-wave dynamics are investigated using high-speed brightfield imaging and controlled optical stimulation. Brightfield imaging detects contraction-related changes in transmitted light and therefore provides an indirect mechanical representation of propagating cardiac activity.

# 3 Methods and Materials

## 3.1 Experimental Model: HL-1 Cardiac Cell Monolayers

HL-1 cardiac cells are used as an in-vitro experimental model for investigating cardiac wave propagation and re-entrant excitation dynamics.HL-1 cells retain several characteristics of cardiomyocytes, including spontaneous contractile activity and the ability to form electrically coupled monolayers under suitable culture conditions. The two-dimensional geometry of the monolayer provides an accessible preparation for optical imaging and stimulation. Propagating

contraction waves can be observed across the field of view, allowing the investigation of complex spatio-temporal wave patterns.

## 3.2 Optogenetic Modification and Fluorescence Expression Verification

The HL-1 cardiac cell monolayers are modified to express a Channelrhodopsin-2 construct coupled to the fluorescent reporter mCherry.ChR2 provides sensitivity to blue-light stimulation, while mCherry enables fluorescence-based assessment of construct expression.

Following transfection and before functional optogenetic experiments, the cell preparations are examined using fluorescence imaging. The purpose of this step is to verify the presence of the fluorescent reporter and assess whether expression is visually detectable in the preparation. Fluorescence images are acquired from the transfected HL-1 cells and inspected for reporter-associated fluorescence.

Preparations displaying suitable expression are subsequently used for functional optical stimulation experiments. The fluorescence measurement serves as an expression-verification step and is distinct from the high-speed functional imaging used to investigate cardiac wave dynamics.

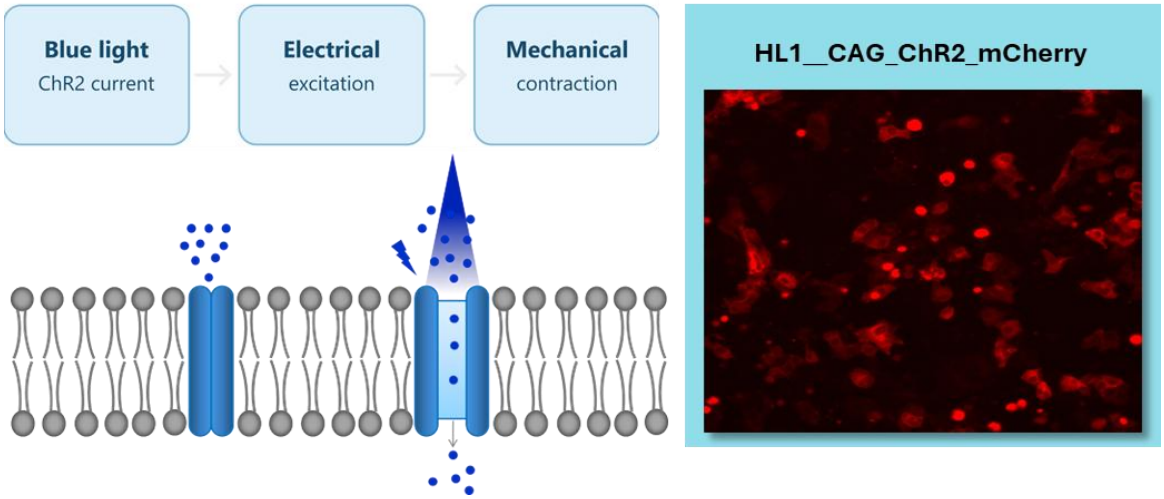


***Figure 1. Optogenetic activation mechanism and fluorescence-based verification of ChR2-mCherry expression in HL-1 cardiac cells. Left: Blue-light activation of ChR2 and its connection to electrical excitation and mechanical contraction. Right: Representative red mCherry fluorescence in transfected HL-1 cells, used to assess reporter expression before optogenetic experiments.***

## 3.3 Functional Brightfield Imaging

Following fluorescence-based verification of optogenetic expression, functional cardiac dynamics are investigated using a MiCAM high-speed brightfield imaging system.

The current acquisition configuration records images at a spatial resolution of 100 × 100 pixels and a sampling interval of 1 ms. Brightfield imaging measures variations in transmitted optical intensity associated with mechanical contraction of the cardiac monolayer.

Electrical excitation and mechanical contraction are coupled through the excitation-contraction process. Consequently, propagating contraction patterns can be used as an indirect indicator of cardiac activity. However, the brightfield measurements do not directly represent membrane voltage. Measurements derived from these recordings, including local propagation velocity and spiral-core position, are therefore interpreted as apparent mechanical quantities.

The imaging system is used to record cardiac wave dynamics before, during and after controlled optical illumination.

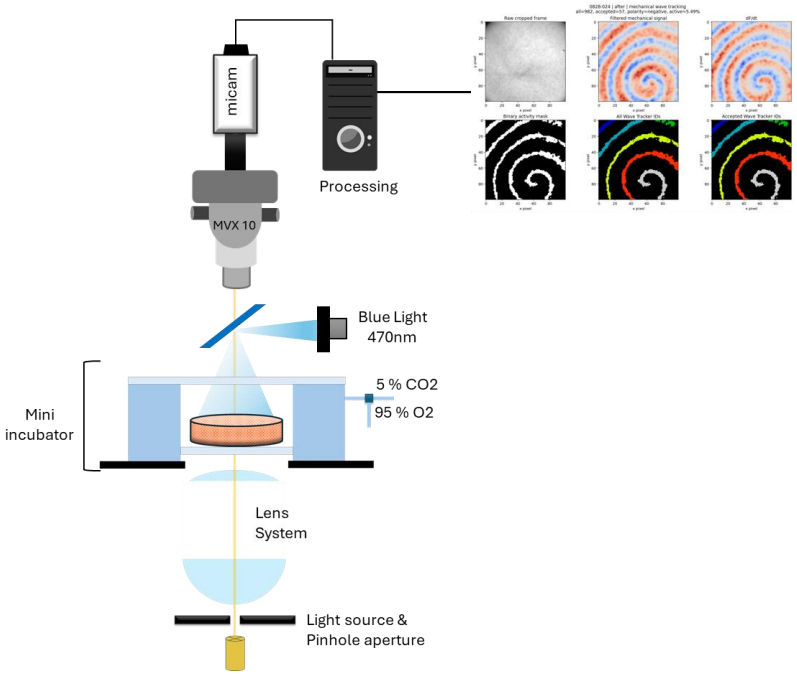


***Figure 2. Experimental optical stimulation and high-speed brightfield imaging setup for HL-1 cardiac monolayers. The MiCAM system records contraction-related wave dynamics, while fluorescence microscopy is used separately to verify ChR2-mCherry expression.***

## 3.4 Optogenetic Optical Stimulation

Optogenetic stimulation is delivered using blue illumination at approximately 470 nm to perturb cardiac activity in ChR2-expressing HL-1 monolayers. The illumination protocol is applied during recordings that contain baseline, light-on and recovery intervals. The functional imaging signal is acquired by MiCAM brightfield imaging and is not a direct measurement of membrane voltage.

Three spatial illumination strategies are investigated. Global illumination exposes the preparation broadly to blue light to assess overall modulation of existing wave dynamics. Half-side illumination produces an illuminated and a comparatively unilluminated region, allowing the response near a light–dark boundary to be studied. Gradient illumination provides a spatially varying light distribution for investigating apparent core displacement under nonuniform perturbation. Light-intensity calibration and optical power at the specimen should be included when verified measurement records become available.

# 4 Experimental Measurements and Results

## 4.1 Fluorescence-Based Expression Verification

Before functional optogenetic experiments, transfected HL-1 cardiac preparations are examined for mCherry-associated fluorescence. The representative fluorescence image in Figure 1 shows red reporter-associated signal in a subset of cells. This establishes a visual expression-check step in the experimental workflow. Fluorescence alone does not quantify transfection efficiency, functional ChR2 photocurrent, or spatially uniform expression. Instrument model, optical filter set, camera, objective, and exposure settings should be entered from the fluorescence-imaging laboratory records.

## 4.2 Global Illumination

Global illumination experiments were used to assess whether broad optical perturbation modifies established spiral-wave activity. The observed recordings indicate changes in several measured dynamical parameters during illumination, followed by a tendency toward baseline behavior after the light was switched off. These observations are consistent with reversible modulation of contraction-related wave dynamics.

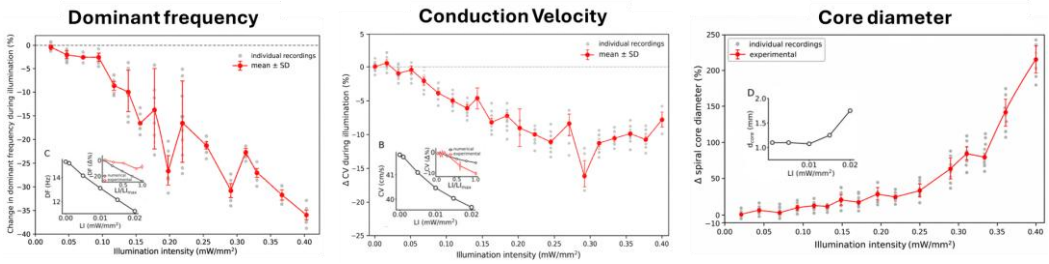


*Figure 3. Illumination-intensity-dependent changes in cardiac spiral-wave dynamics during global optical stimulation. Left: Relative change in dominant frequency; middle: relative change in apparent conduction velocity; right: relative change in apparent spiral-core diameter. Grey points indicate individual recordings and red markers indicate mean ± standard deviation.*

## 4.3 Half-Side Illumination

Half-side illumination was investigated to assess the influence of a spatially restricted light pattern. Preliminary analyses indicate changes in apparent spiral-core position and trajectory during the illumination period.

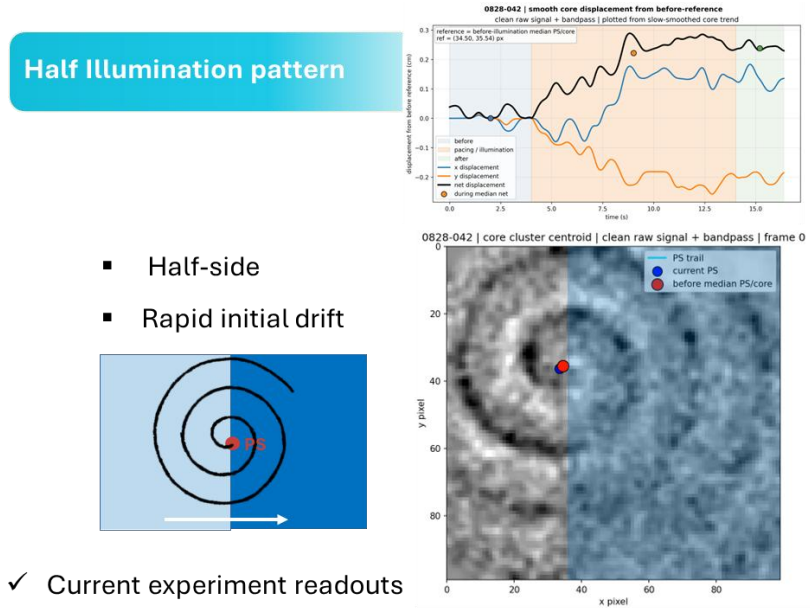


*Figure 4. Half-side illumination experiment. Spatially restricted blue-light illumination and representative recordings illustrate preliminary changes in apparent spiral-core position and trajectory near the illuminated region.*

## 4.4 Gradient Illumination

Spatial gradient illumination was explored to investigate the response of spiral-wave dynamics to nonuniform optical perturbation. Initial recordings suggest illumination-associated changes in apparent core displacement.

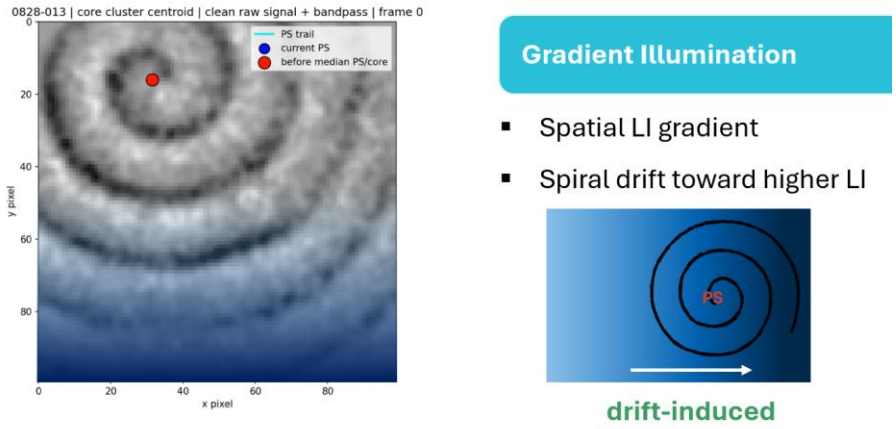


*Figure 5. Gradient illumination experiment. Spatially varying blue-light intensity and representative recordings illustrate preliminary illumination-associated apparent spiral-core displacement.*

## 5 SUMMARY

A fluorescence-based ChR2-mCherry expression-verification step has been incorporated into the HL-1 cardiac optogenetic workflow. Reporter fluorescence is checked before subsequent blue-light perturbation experiments. Functional wave dynamics are recorded separately with high-speed MiCAM brightfield imaging. Global, half-side and gradient illumination have been used to examine changes in contraction-related cardiac spiral-wave behavior. The observations indicate illumination-dependent changes in several dynamical parameters, with preliminary evidence of apparent spiral-core displacement under spatially patterned illumination.

The available results support a working cell-culture optogenetic measurement workflow.

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