Bioeng 6460
Electrophysiology and Bioelectricity

Arrhythmias II (Arrhythmia Lab)

(Part 1)

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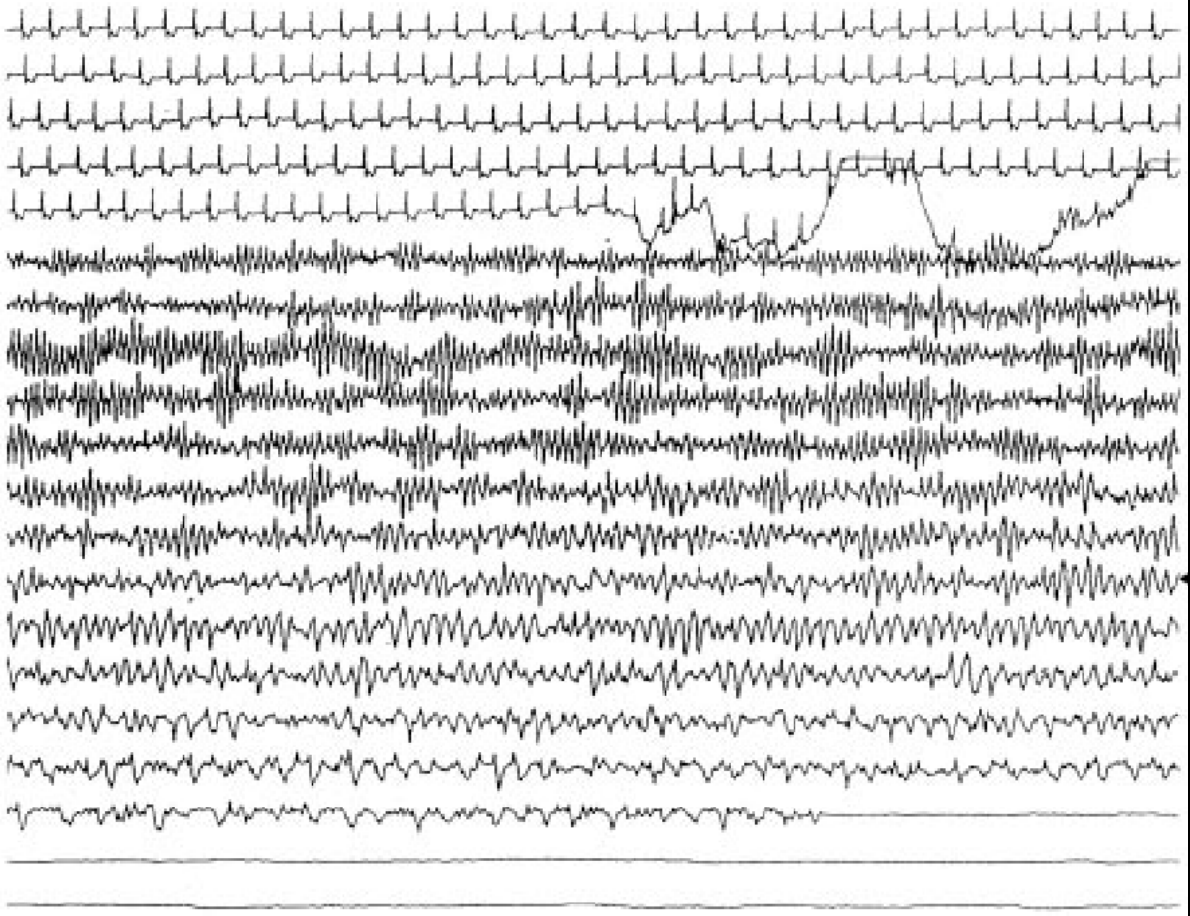
Overview

- Experimental preparations
  - In vivo heart
  - Langendorff perfused heart
  - Isolated right atrial and right ventricle preparation
  - Cell cultures
  - Isolated myocyes
  - Zebrafish heart
- Experimental techniques
  - ECG
  - Extracellular K^+ concentration
  - Myocardial tissue electrical impedance
  - Extracellular potentials
  - Fluorescent probes and their application to optical mapping of excitation and $[Ca^{++}]_i$

Ventricular fibrillation

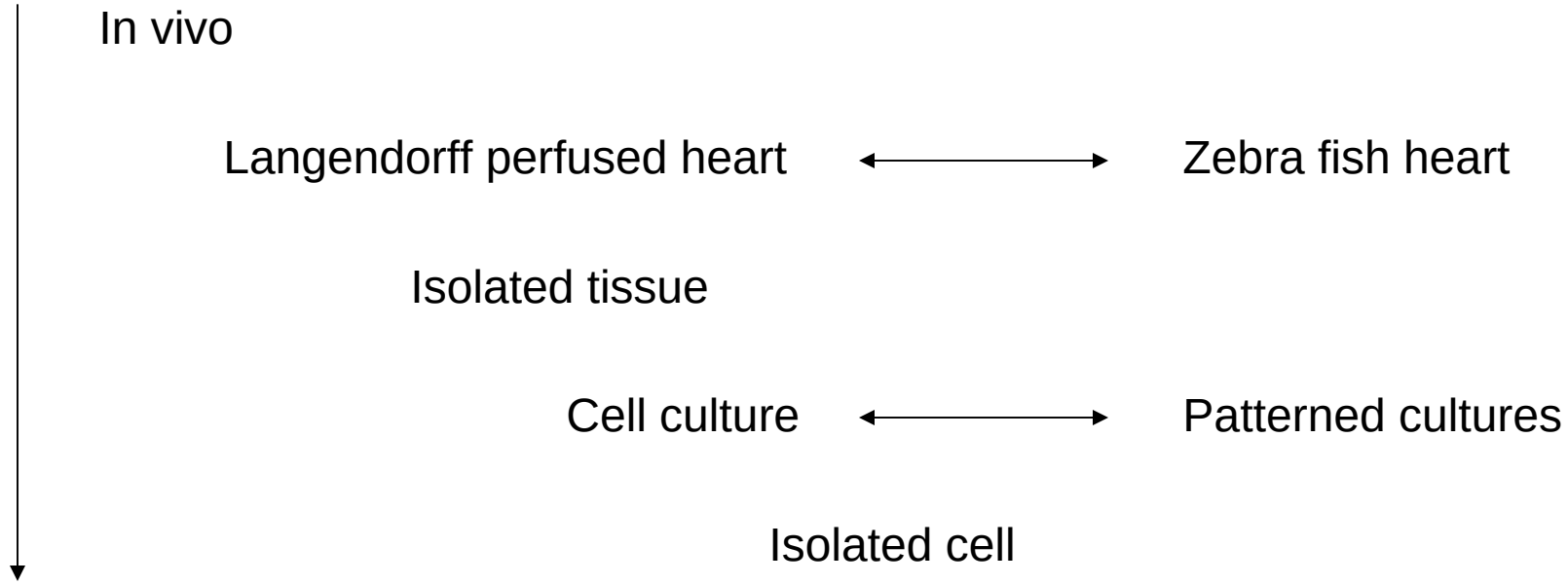
Evolution of Ventricular Fibrillation in a patient



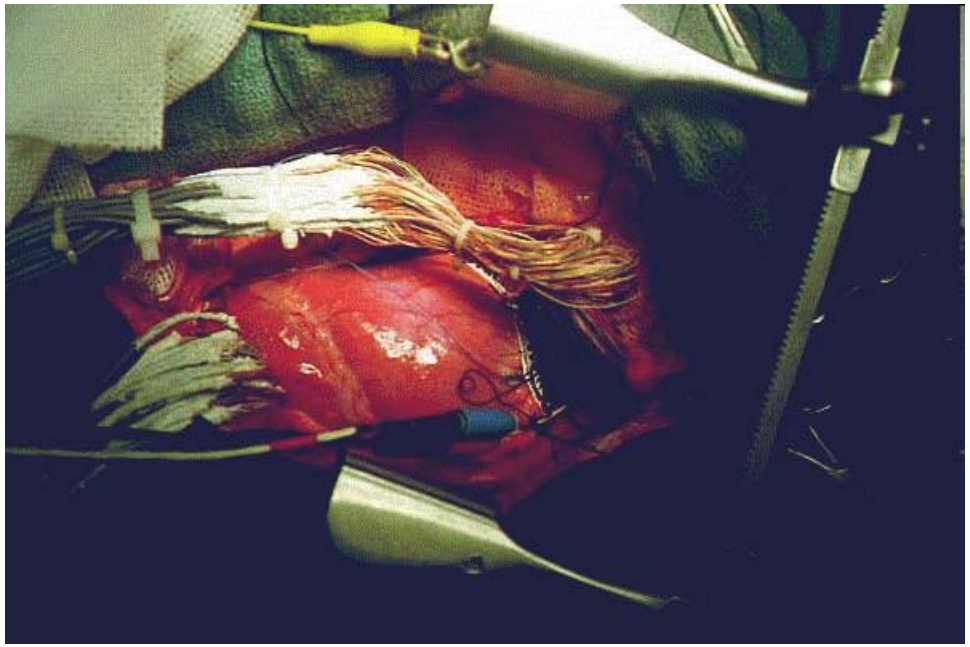
Ventricular fibrillation: Fast and rapidly changing electrical rhythm of the ventricles which precludes adequate pumping of the blood and thus causes an immediate systemic failure unless reverted. Success of defibrillation decreases markedly as the time since the onset of VF increases. Wiggers (Am Heart J 1930) was the first to describe the gross changes of rhythm that occur during the evolution of VF in the setting of ischemia and classified them into four stages: 1) undulatory or tachysystolic (1-2s); 2) convulsive incoordination (15-40s); 3) tremolous incoordination (2-3min); 4) atonic fibrillation (>3min).

How do we go about studying arrhythmia mechanisms?

Experimental preparations

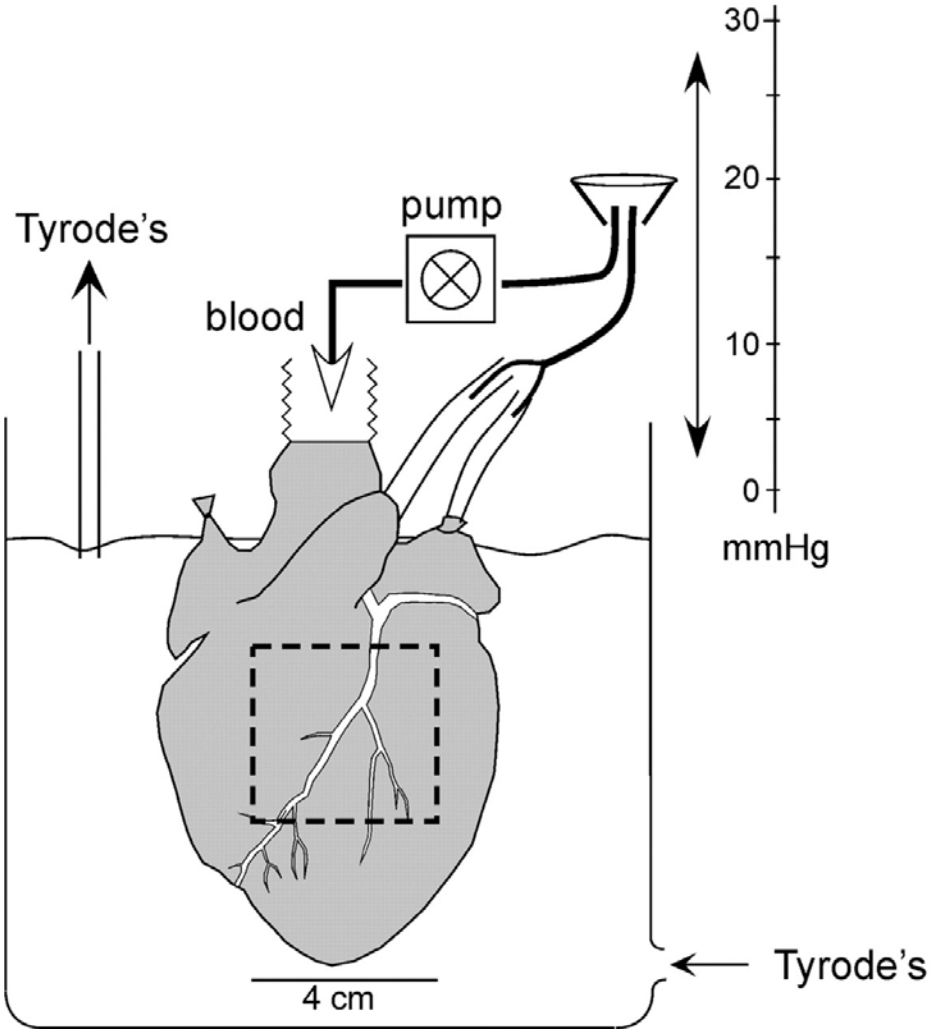


In vivo heart



There are various in vivo procedures. One common a approach is to access the heart via a thoracotomy performed once the animal is under the effect of anesthesia. The pericardium is incised and is sutured to the margins to form a cradle. The anterior wall of the ventricles can be easily accessed to insert electrodes or measuring devices.

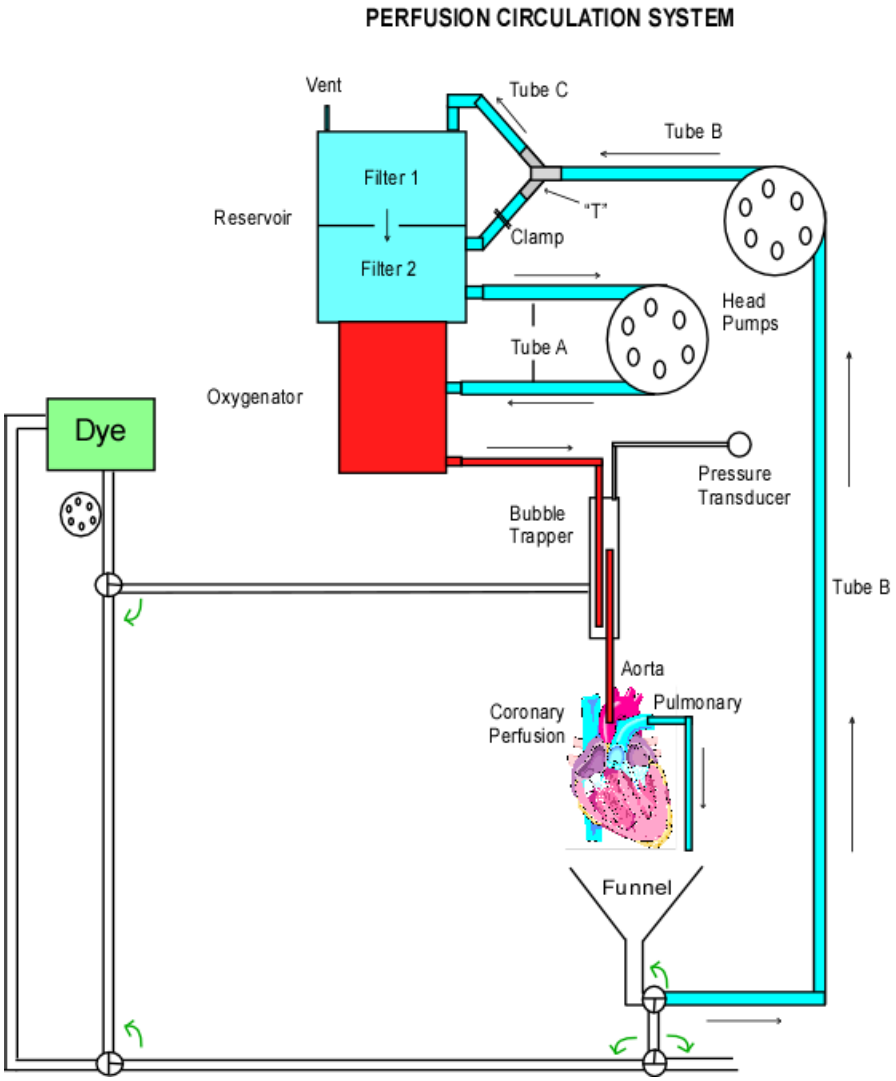
Langendorff perfused heart



Moreno J et al. Cardiovasc Res 2005;65:158-166

In the ***Langendorff*** heart preparation tissular perfusion is achived by retrograde circulation of the perfusion fluid. The two major modalities are a ***constant pressure*** or a ***constant flow*** Langendorff system. The perfusate is commonly a crystalline solution containing ions which closely mimic the physiological range. Alternatively, blood can be used for perfusion. In this preparation it is important to constantly monitor pressure and/or flow to ensure adequacy of perfusion. The heart is usually kept at a physiologocal temperature, and ideally it is submerged in fluid though this is not strictly necessary

Langendorff perfused heart II



Some common components which form part of the Langendorff system are a set of peristaltic pumps, oxygenators, fluid reservoirs, heat exchanger, bubble trap, and pressure and temperature transducers. Additionally, the heart is usually placed in a custom designed chamber which allows continuous perfusion of the heart and easy access in order to perform the various physiological measurements for the experiment.

Isolated right atria

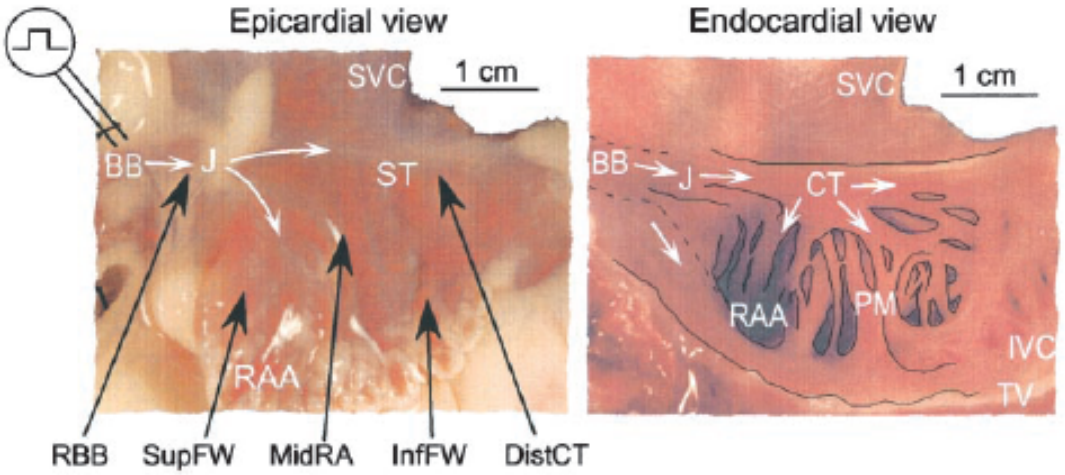


Figure 1. Isolated right atrium. Left, Epicardial view. RAA indicates right atrial appendage; SVC, superior vena cava; and ST, sulcus terminalis. Point J indicates the junction between BB and the ST. Right, Endocardial view. CT indicates crista terminalis; IVC, inferior vena cava. The network of pectinate muscles (PM) is outlined for clarity. Black arrows indicate 5 bipolar electrode recording sites (see Figure 4C below); RBB, right atrial end of BB; SupFW, superior aspect of the RA free wall (FW); MidRA, middle of the RA FW; InfFW, inferior FW; and DistCT, distal edge of CT.

Following excision of the heart, the right coronary artery is cannulated and the non-perfused areas of the heart are removed. The isolated preparation includes the right atria and the right ventricle, and can be placed in a frame or an appropriate fixture in order to perform experiment. In some cases, especially with smaller hearts, the atria can be maintained by superfusion of the tissue alone because the reduced thickness of the tissue wall allows diffusion of the perfusate through the tissue. RAA- right atrial appendage, SVC- superior vena cava, BB- bundle branch, CT- crista terminalis, ST- sulcus terminalis, TV- tricuspid valve, PM- pectinate muscle.

Berenfeld et al Circ Res 2002

Isolated right ventricle

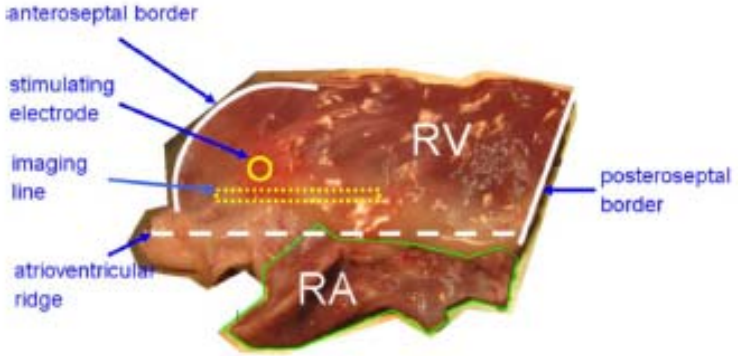


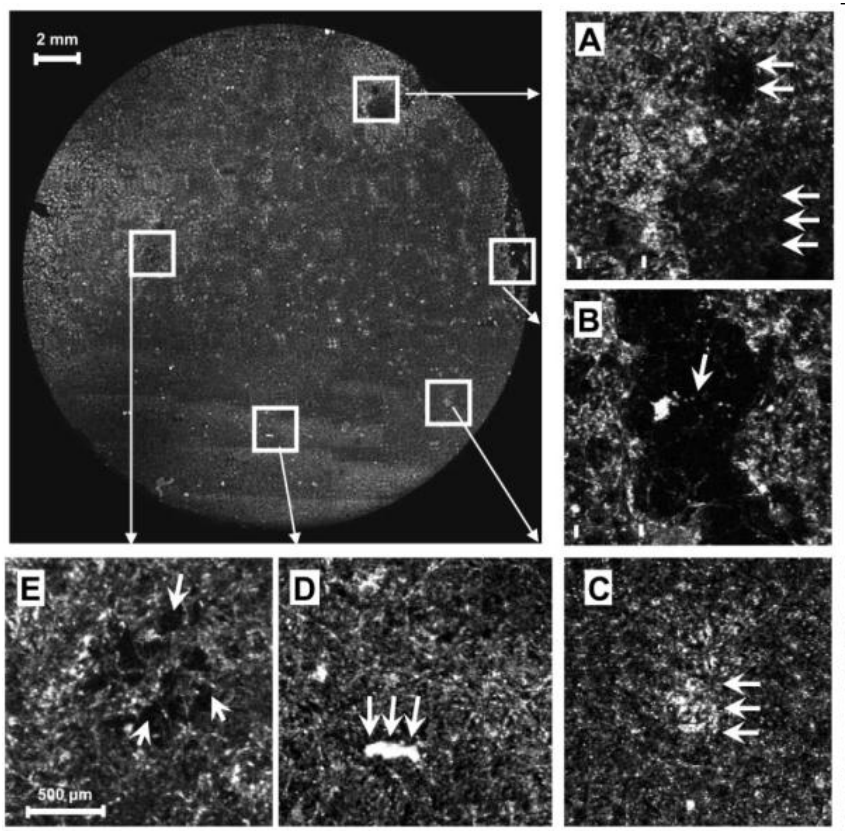
Fig. 5. Typical right ventricular preparation showing the epicardial surface of the right ventricular free wall. Dashed line demarcates the atrioventricular groove and the course of the right coronary artery. Right atria and appendix (RA) is below this line, whereas the right ventricle (RV) is above it. The imaging line (dotted yellow rectangle) is located 1–1.5 cm below and parallel to the atrioventricular groove. The stimulating electrode is placed close to the imaging line.

The right coronary is cannulated and the tissue dissected following a similar approach as that described for the isolated right atrial preparation. Tip: the use of food coloring or other types of colorants is helpful in determine the perfused and non-perfused areas.

Iravanian et al AJP 2007

Cell cultures

In this study the authors used neonatal rat ventricular myocytes obtained using a standard trypsin-collagenase digestion protocol. The cells were then plated on a 25 mm laminin coated coverslip and kept under standard culture conditions. By the third day the cells form interconnected confluent networks that exhibit rhythmic, spontaneous contractions. In particular this figure shows various heterogeneities occurring throughout the culture.



Agladze et al. AJP 2007

Patterned cell cultures

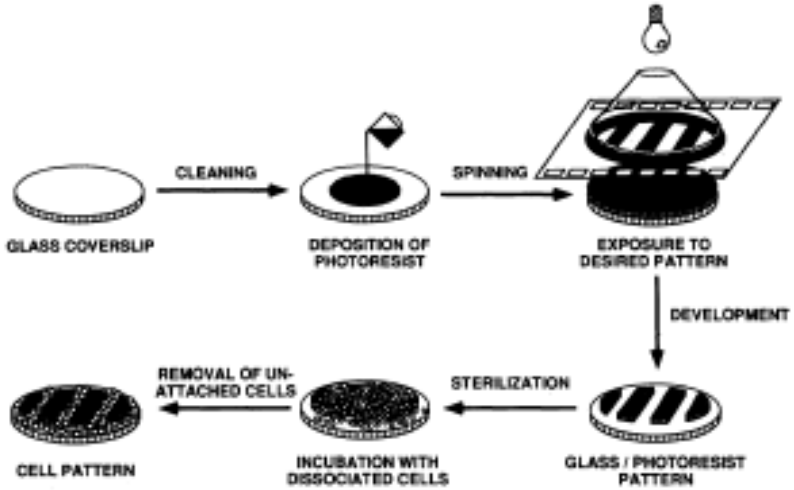


FIGURE 1. *Schematic presentation of main steps in the preparation of patterned cell cultures. Glass coverslips are cleaned, covered with photoresist, and spun and contact exposed to the desired pattern with UV light. After development, patterned coverslips are sterilized and incubated with freshly dissociated neonatal ventricular rat heart cells. Cells distribute themselves evenly over the whole coverslip but attach only to the exposed glass surfaces. The final pattern is obtained by the removal of nonadhering cells.*

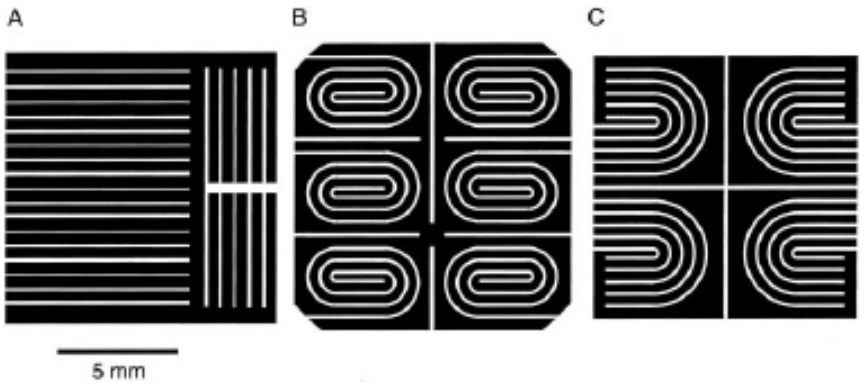
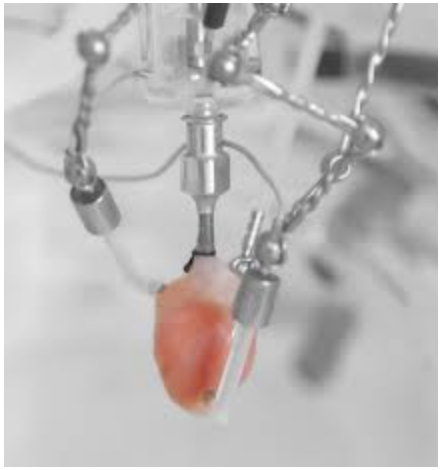
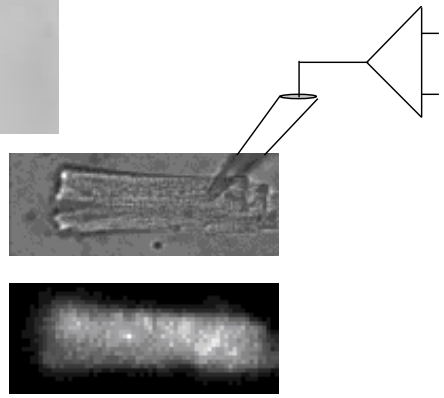


Fig. 1. Patterns used in the study. Three different patterns provided a variety of 100- to 150-μm-wide strands of cells in various combinations of straight line and semicircular segments.

Rohr et al Circ Res 1991 (upper) and Tung and Kleber AJP 2000 (lower)

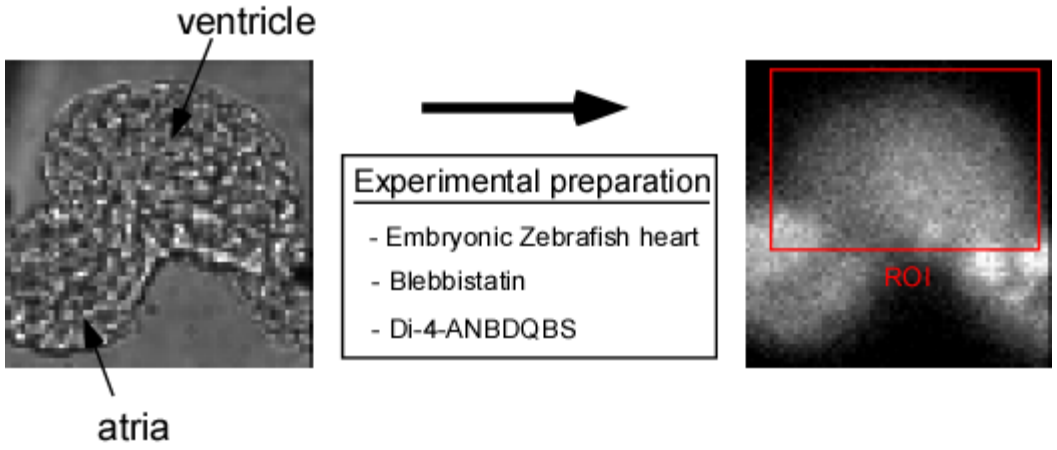
Isolated cells





To isolate the myocytes the excised heart is placed on a Langendorff perfusion system and a series of solutions containing zero calcium and enzymes such as collagenase a and protease are delivered to the heart in order to initiate digestion of the intracellular and collagen matrixes which hold the myocytes in the myocardium. Once isolated the cells may be used for diverse purposes ranging from electrical recordings using the patch clamp technique, or the measurement of various cellular parameters via the use of fluorescent probes.

Embryonic zebrafish heart



Group work

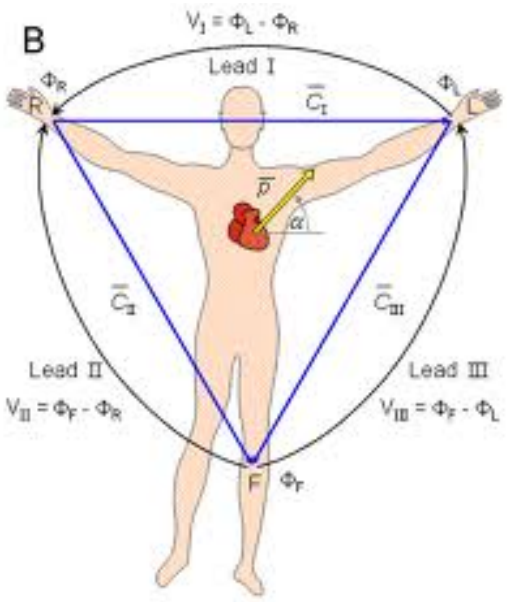
1- Identify advantages and disadvantages of the various experimental preparations that we have just reviewed.

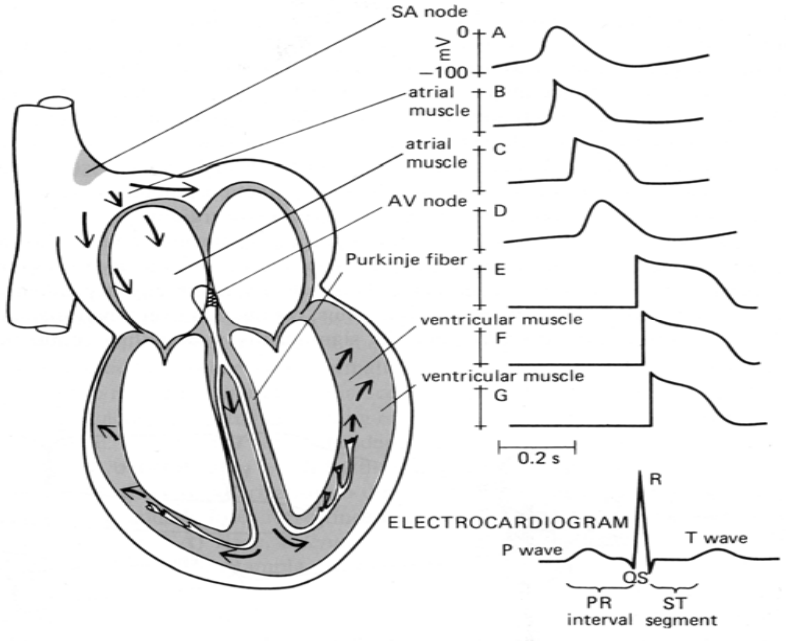
2- Can you think of any other preparation that was missed out?

Now that we have the cardiac preparation ready, lets measure something!!

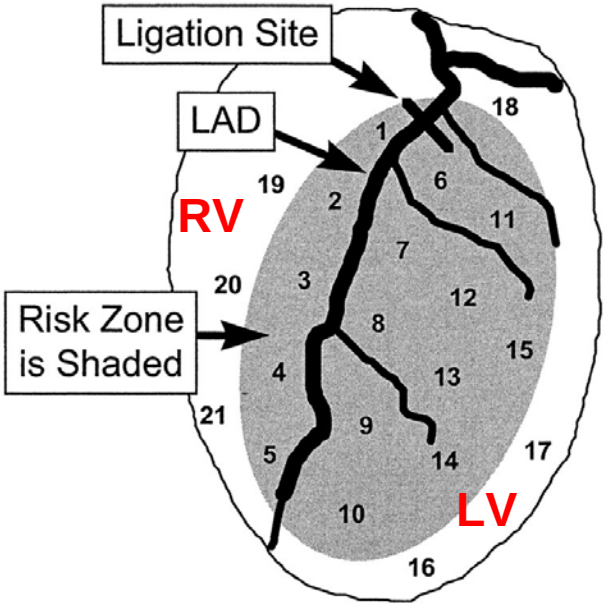
(Measurement of physiological data)

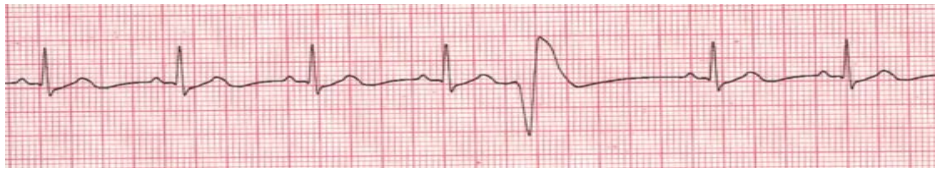
ECG

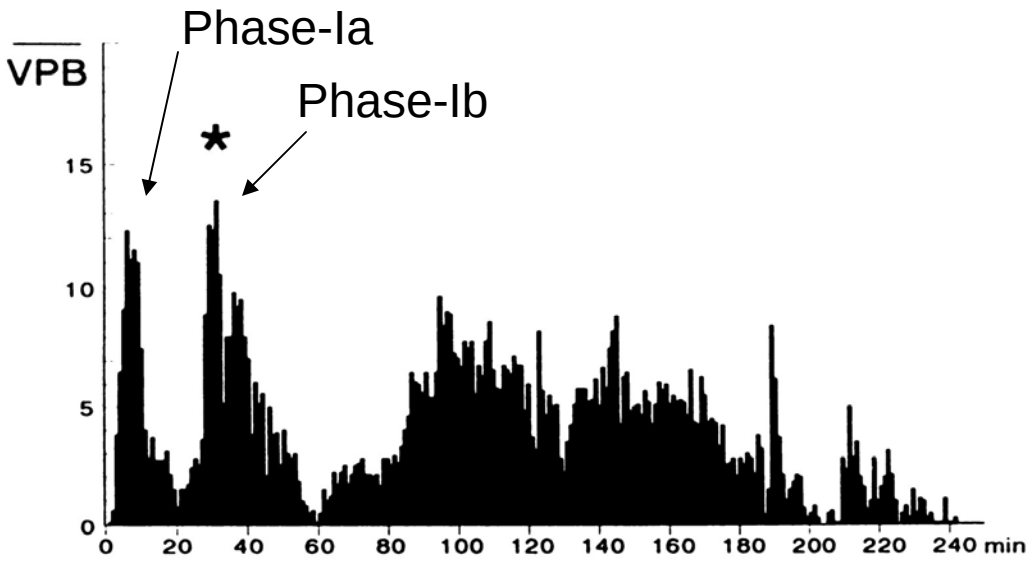

B
$V_I = \Phi_L - \Phi_R$
Lead I
Φ_R
R
Φ_L
L
$\vec{C}_I$
$\vec{p}$
α
$\vec{C}_{II}$
$\vec{C}_{III}$
Lead II
$V_{II} = \Phi_F - \Phi_R$
Lead III
$V_{III} = \Phi_F - \Phi_L$
F
Φ_F


SA node
mV
0
−100
A
atrial muscle
B
atrial muscle
C
AV node
D
Purkinje fiber
E
ventricular muscle
F
ventricular muscle
G
0.2 s
R
ELECTROCARDIOGRAM
T wave
P wave
QS
PR interval
ST segment

ECG (continued..)



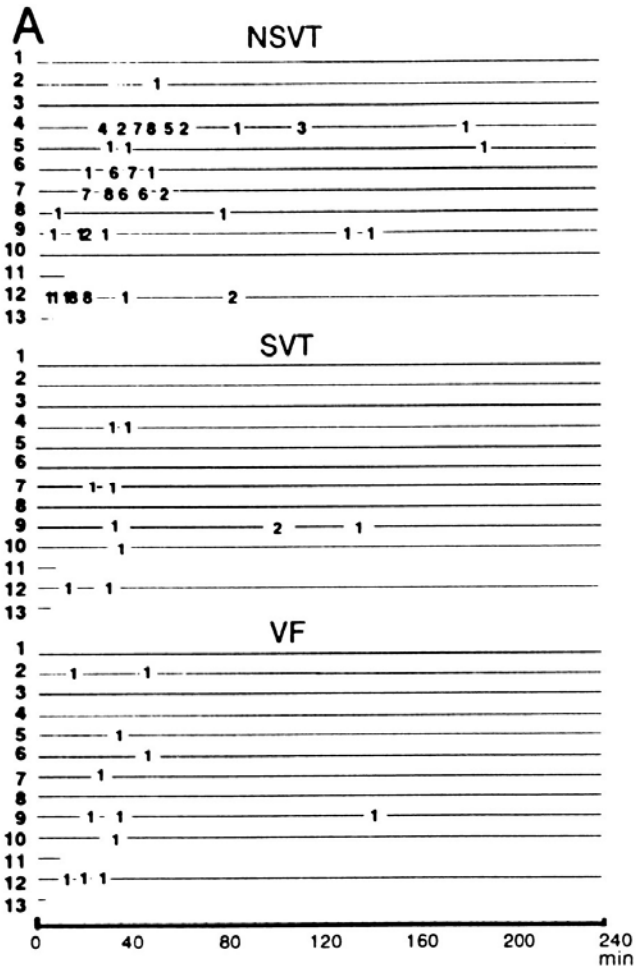




In an in vivo model of regional ischemia induced by ligation of the left anterior descending coronary artery (LAD), the incidence of ventricular premature beats following occlusion has a distinct and reproducible distribution which is characterized by two arrhythmic peaks named phase-Ia and phase-Ib. A number of studies have been directed to understanding the mechanisms underlying these arrhythmia given the potential clinical relevance of this experimental model.

Cinca et al. Circulation 1997

ECG (continued..)

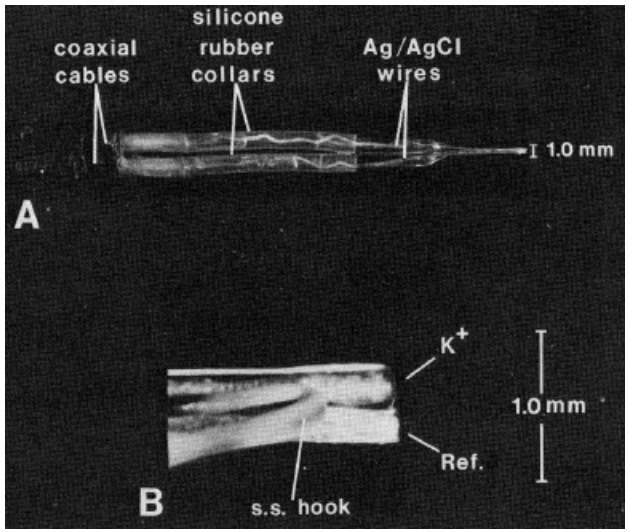


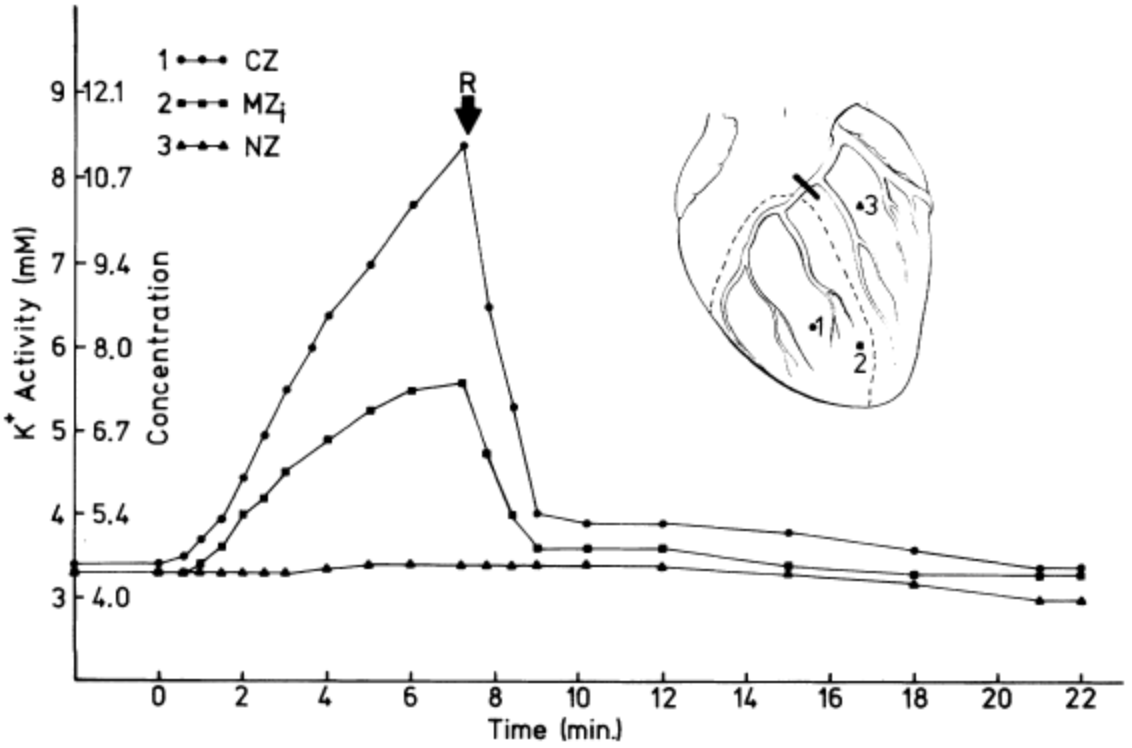
The clinical relevance of this model stems from the fact that in a significant number of experiment the arrhythmic events occurring during regional ischemia were ***ventricular fibrillation*** (VF) which leads to rapid death of the subject unless reverted back to sinus rhythm successfully. Other arrhythmia such as sustained and non-sustained ventricular tachycardia (SVT and NSVT, respectively) may also become life threatening since both arrhythmias can easily develop into VF. Note that actually two out of thirteen animals did NOT survive the ischemic insult.

Group Work: What arrhythmic phase is more vulnerable to the incidence of VF?

Cinca et al. Circulation 1997

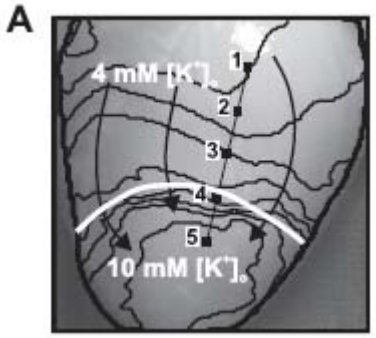
Extracellular potassium concentration



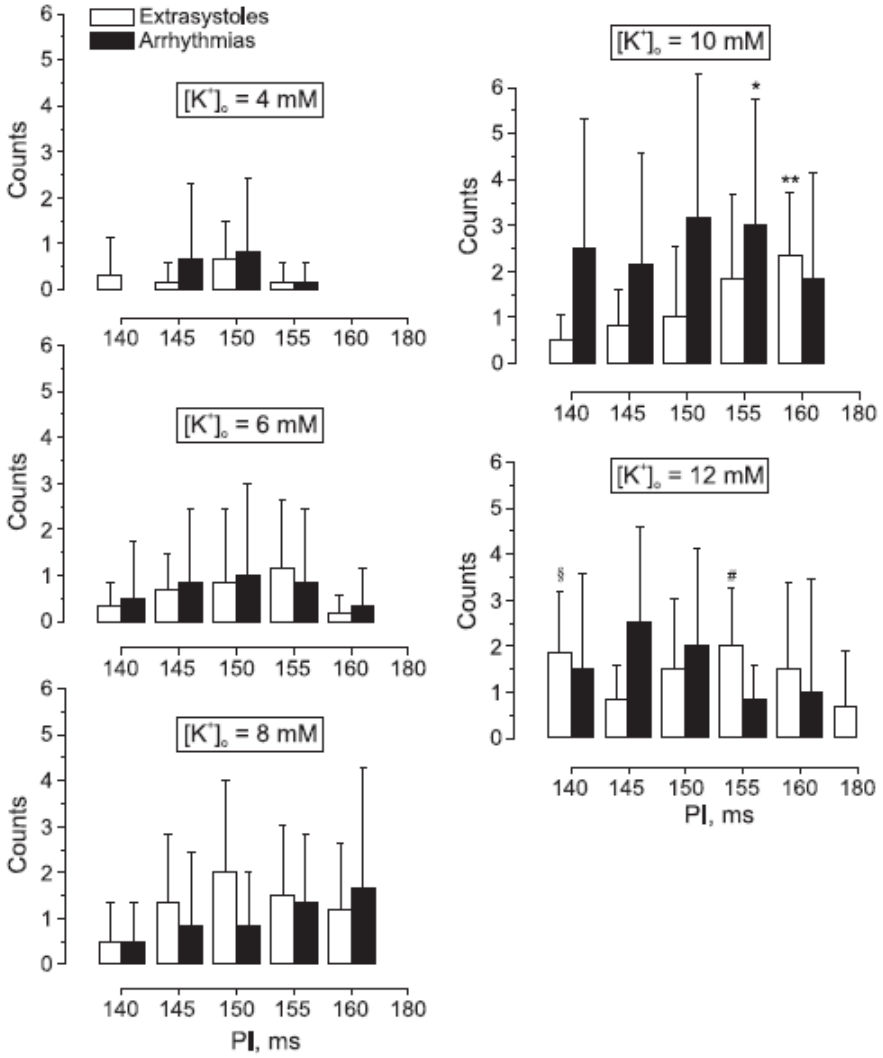


Hill and Gettes Circulation 1980

Extracellular potassium concentration (continued..)

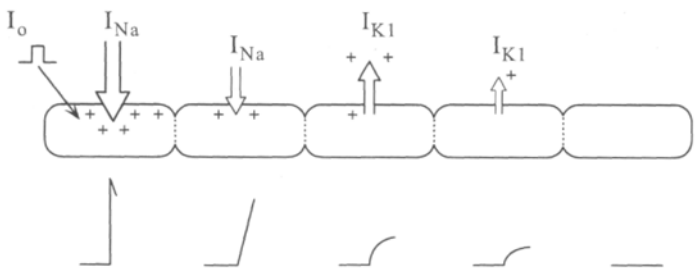


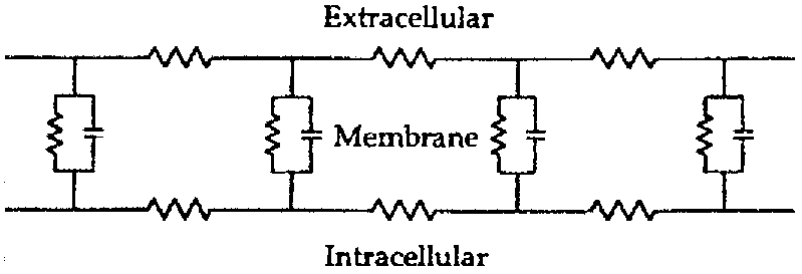
In this model the authors created a spatial gradient of extracellular K^+ by local cannulation of an artery which was infused with solutions of varying K^+ concentration. The relationship between the susceptibility to initiation of extrasystoles and arrhythmia and the K^+ concentration was biphasic, reaching a maximum susceptibility at 10 mM concentration. These results provide some mechanistic evidence regarding the role of K^+ gradients in the genesis of phase-Ia arrhythmias.

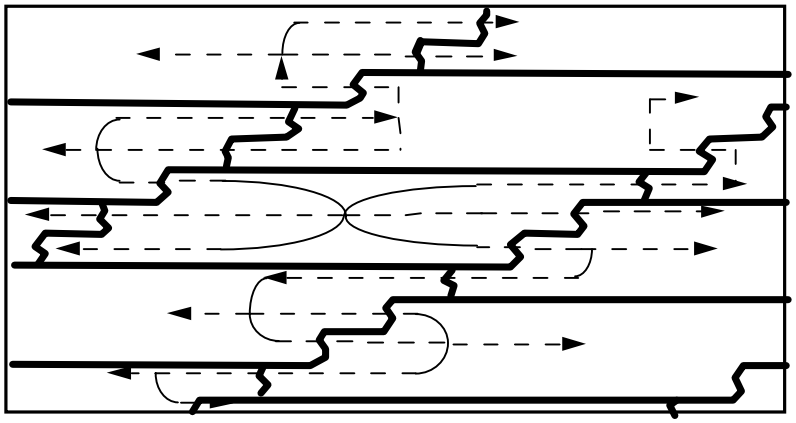


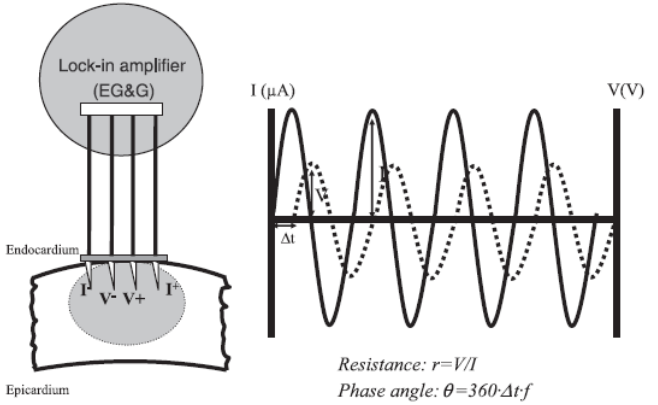
Sidorov et al. AJP 2011

Electrical impedance for measurement of the tissue passive electrical properties



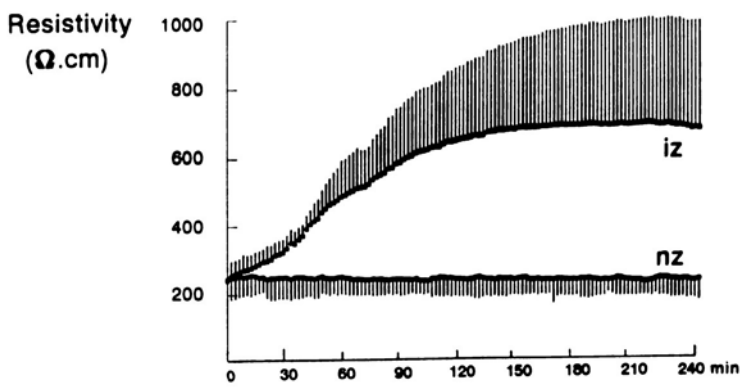


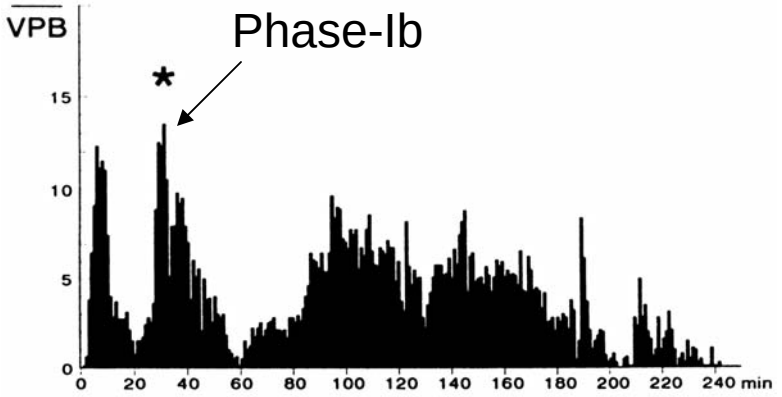


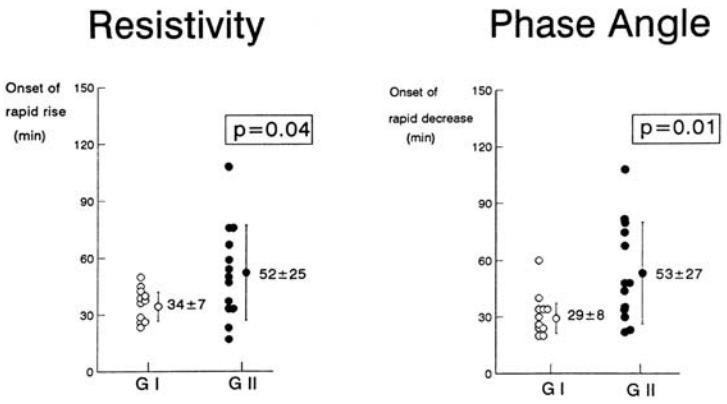


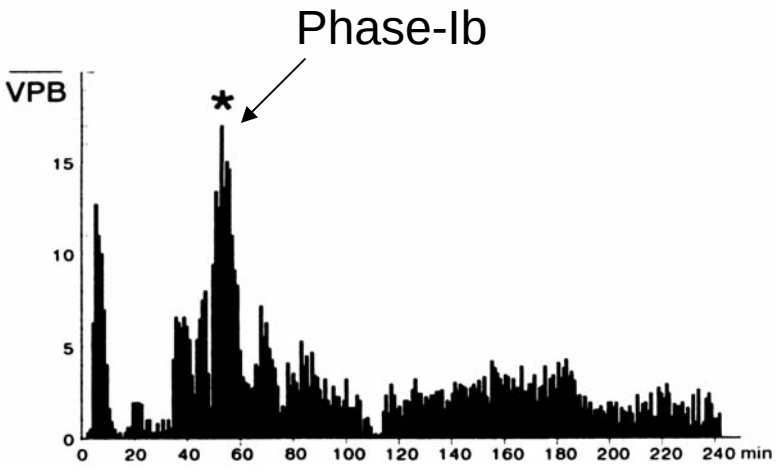
Injection of a sub-threshold current and measurement of the resulting voltage response can yield information regarding the passive electrical properties of the tissue. Given that cell membranes have capacitative and resistive properties, the tissue electrical impedance is a complex number and therefore an accurate description of the voltage response requires providing two parameters describing the change in magnitude and in phase angle.

Electrical impedance for measurement of the tissue passive electrical properties





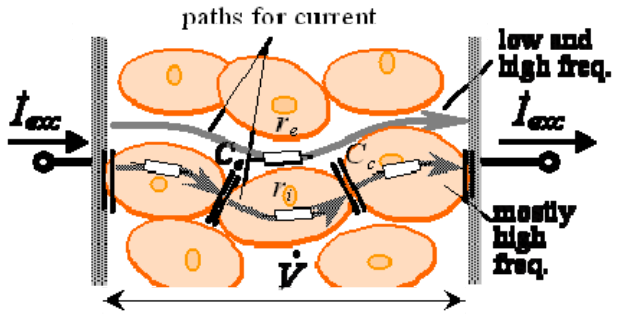


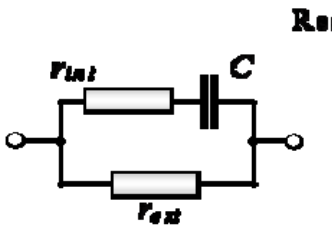


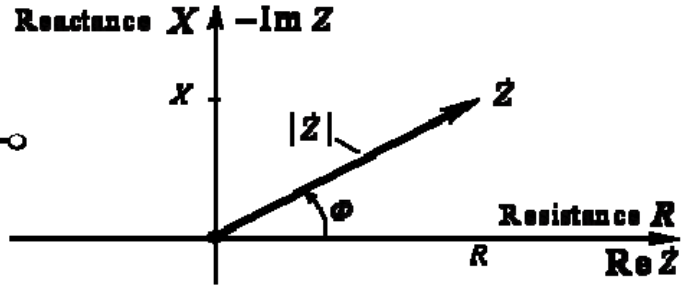
This study showed that changes in the myocardial passive electrical properties occurring during regional ischemia and ischemia-and-preconditioning correlate well with the peak incidence of ventricular premature beats (VPBs) occurring during the arrhythmic phase-Ib.

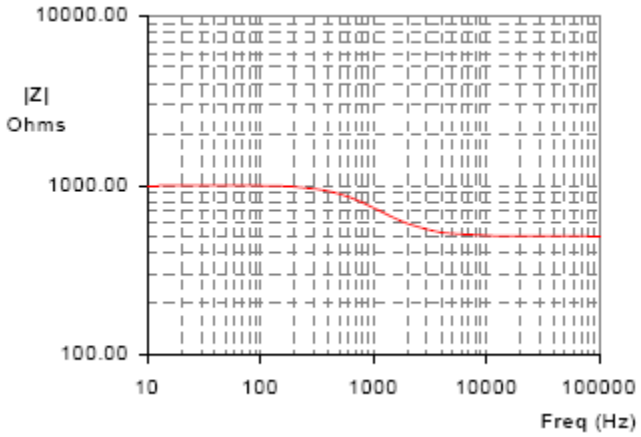
Cinca et al. Circulation 1997

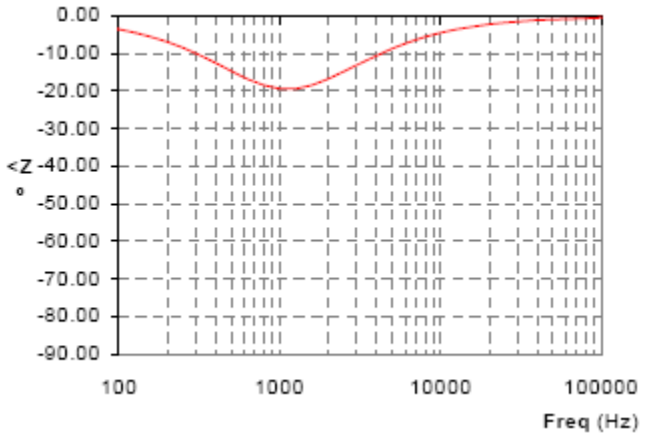
Frequency response of biological electrical impedance

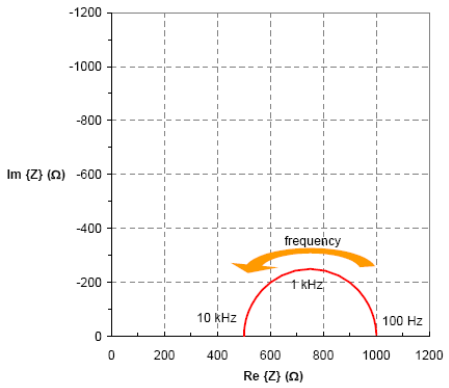






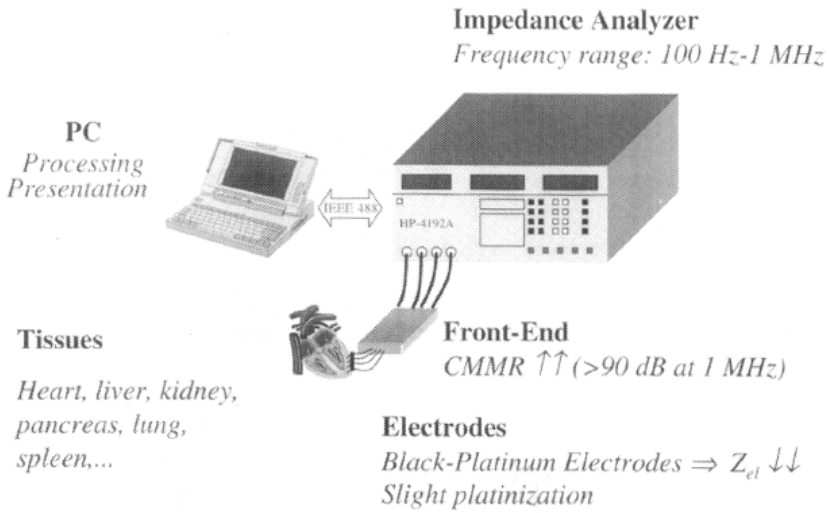


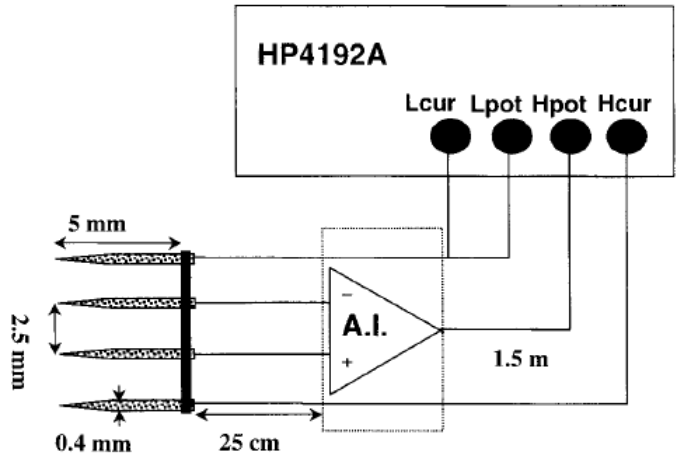




Ivorra, Centro Nacional Microelectronica 2003

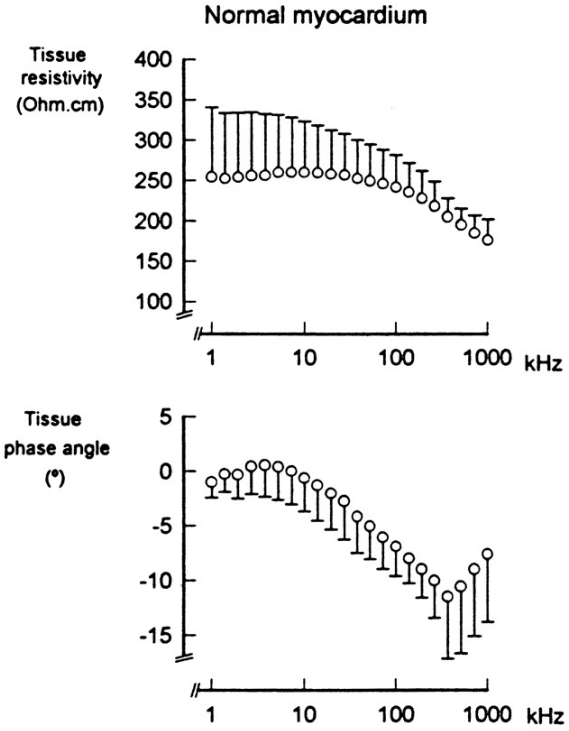
Frequency response of biological electrical impedance

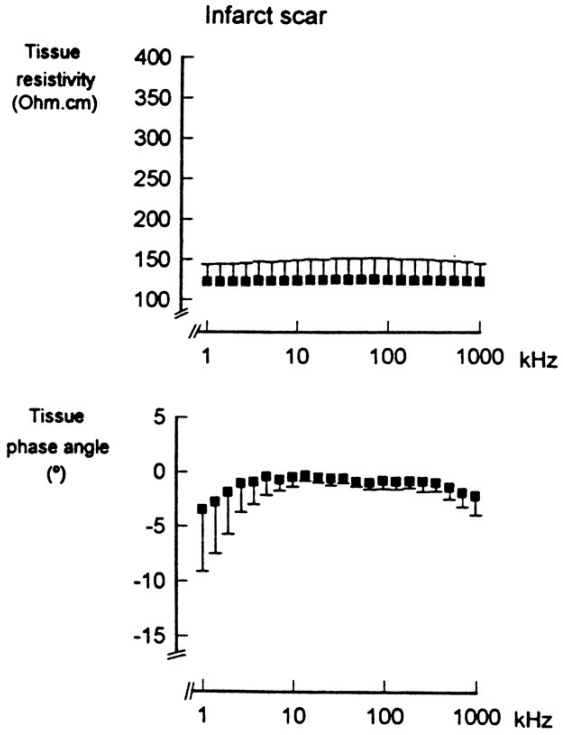


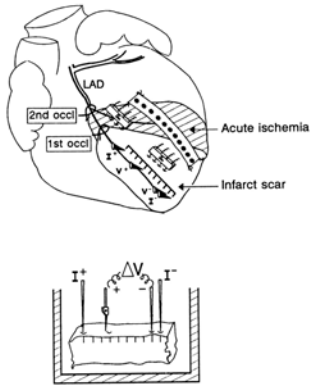


Casas et al. Ann NY Acad Sci 1999

Electrical impedance of the myocardial infarct scar



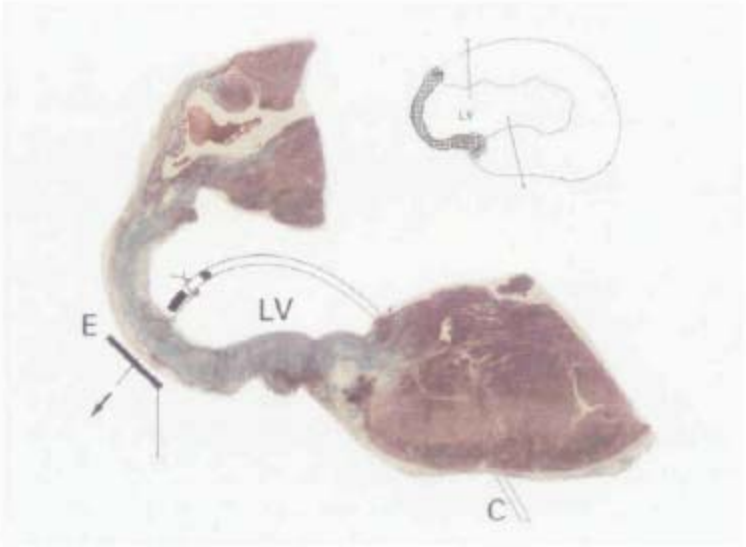


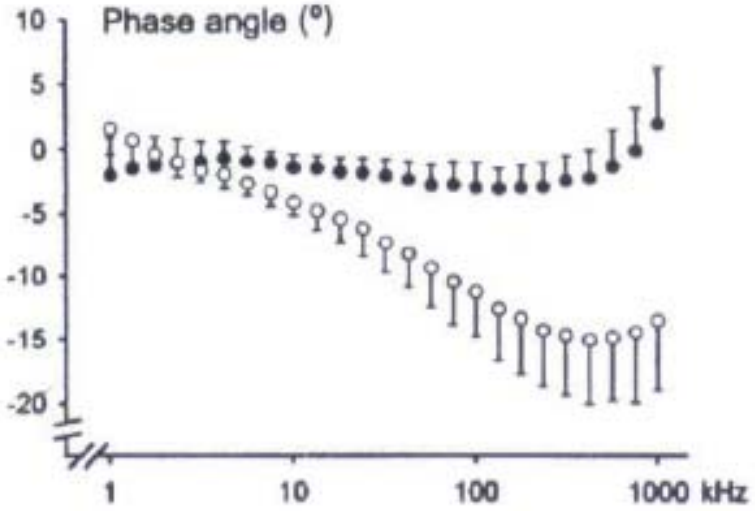


Normal tissue and 1-month old infarcted scar tissue have distinct electrical impedance frequency responses. The infarcted scar has low resistivity and does not exhibit a capacitative response in the 1-1000 Hz range.

Cinca et al. Cardiovasc Res 1998

Detection of the 1-month old myocardial infarct scar via electrical impedance measurements





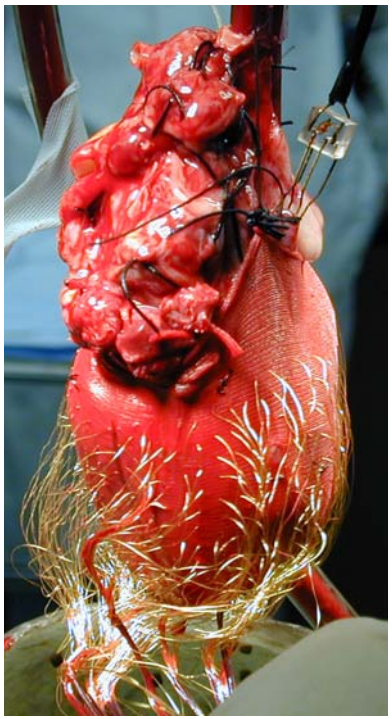
Warren et al. PACE 2000

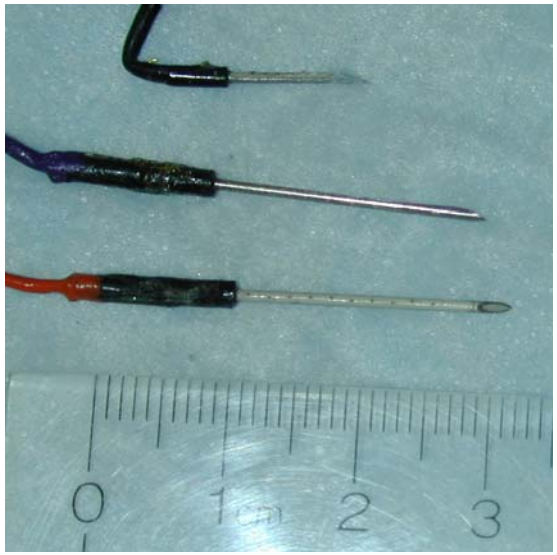
Group work

1- What role do you think the low resistivity of the myocardial infarct scar can have in the genesis of arrhythmias?

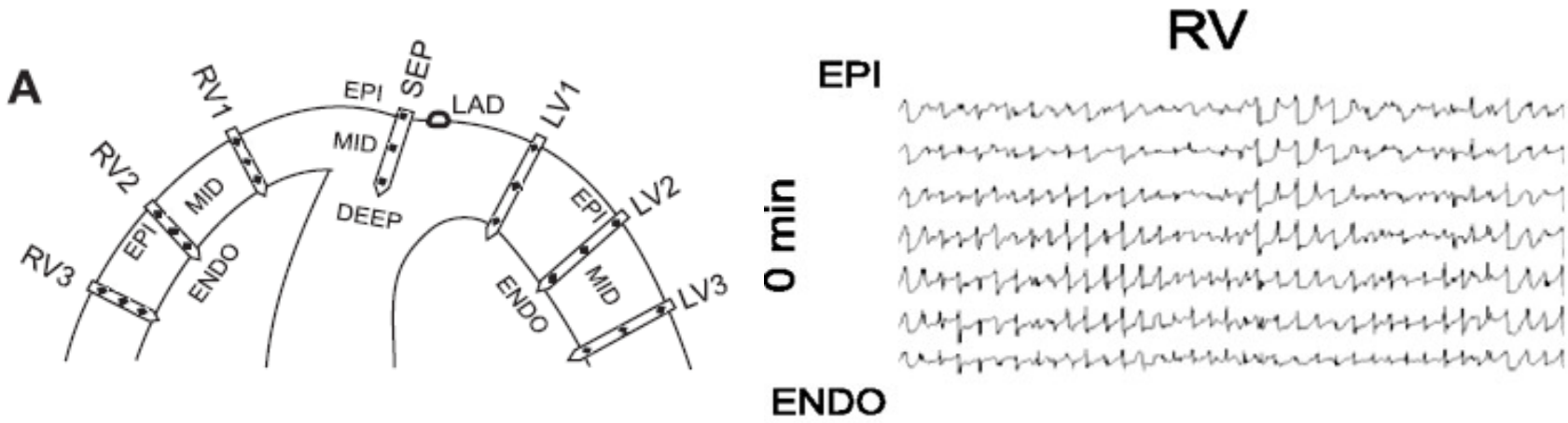
2- What is the benefit of being capable of detecting myocardial scar tissue by means of a catheter?

Extracellular potentials and cardiac mapping





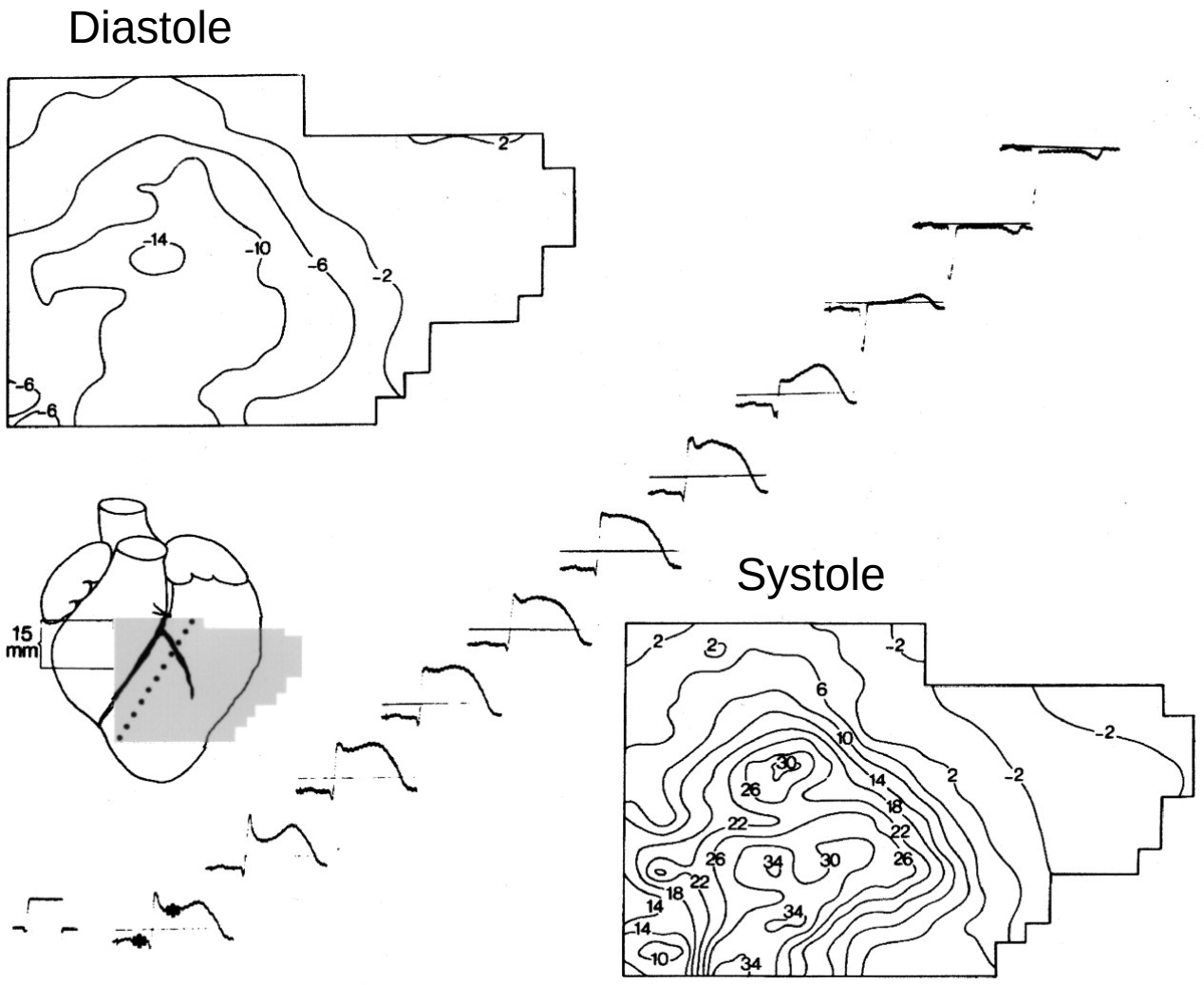
Extracellular potentials and cardiac mapping



Group Work: Can we learn anything regarding VF from these extracellular potential recordings obtained from a single transmural needle inserted in the RV?

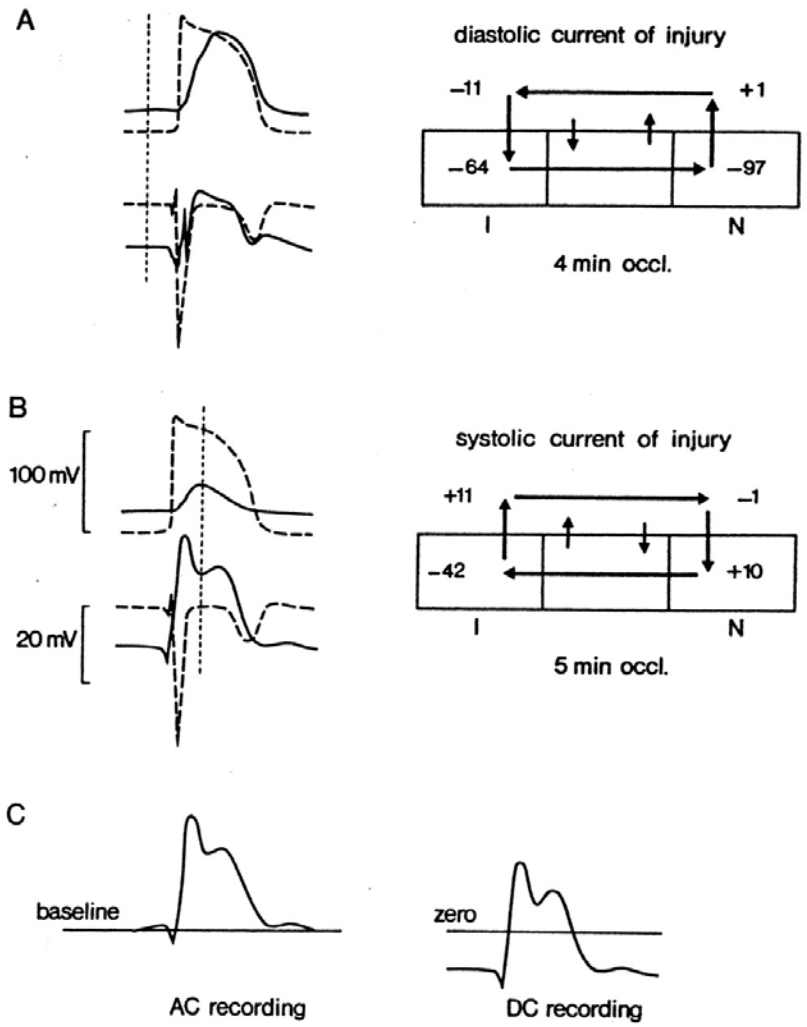
Venable et al. AJP 2010

Extracellular potentials and cardiac mapping



Kleber Cardiovasc Res 2000

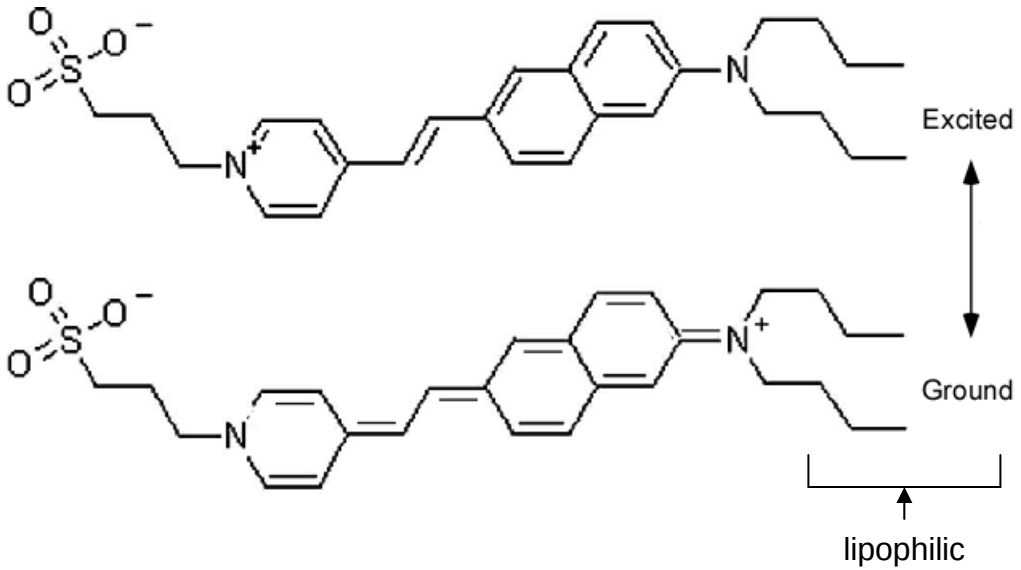
Extracellular potentials and cardiac mapping

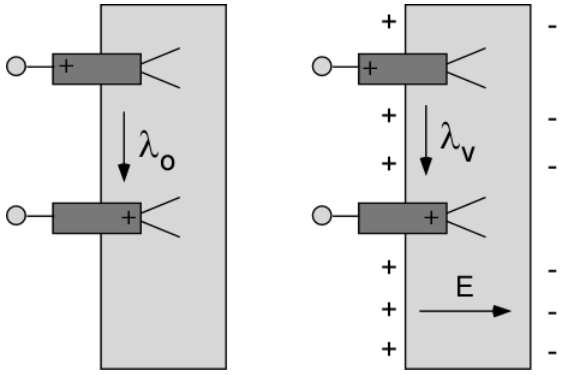


Janse Cardiovasc Res 2000

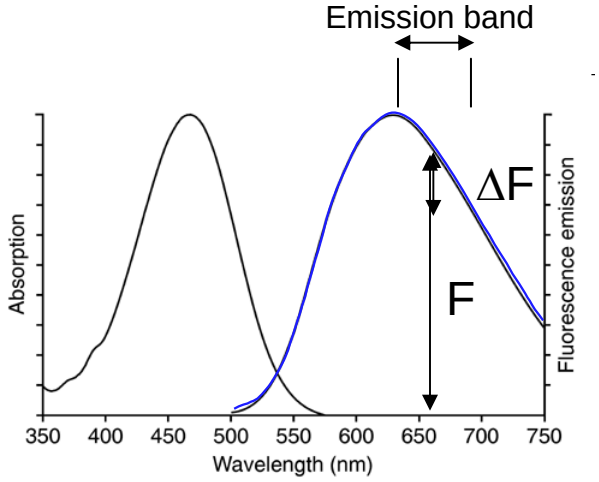
Fluorescent probes- voltage sensitive dyes

Di-4-ANEPPS

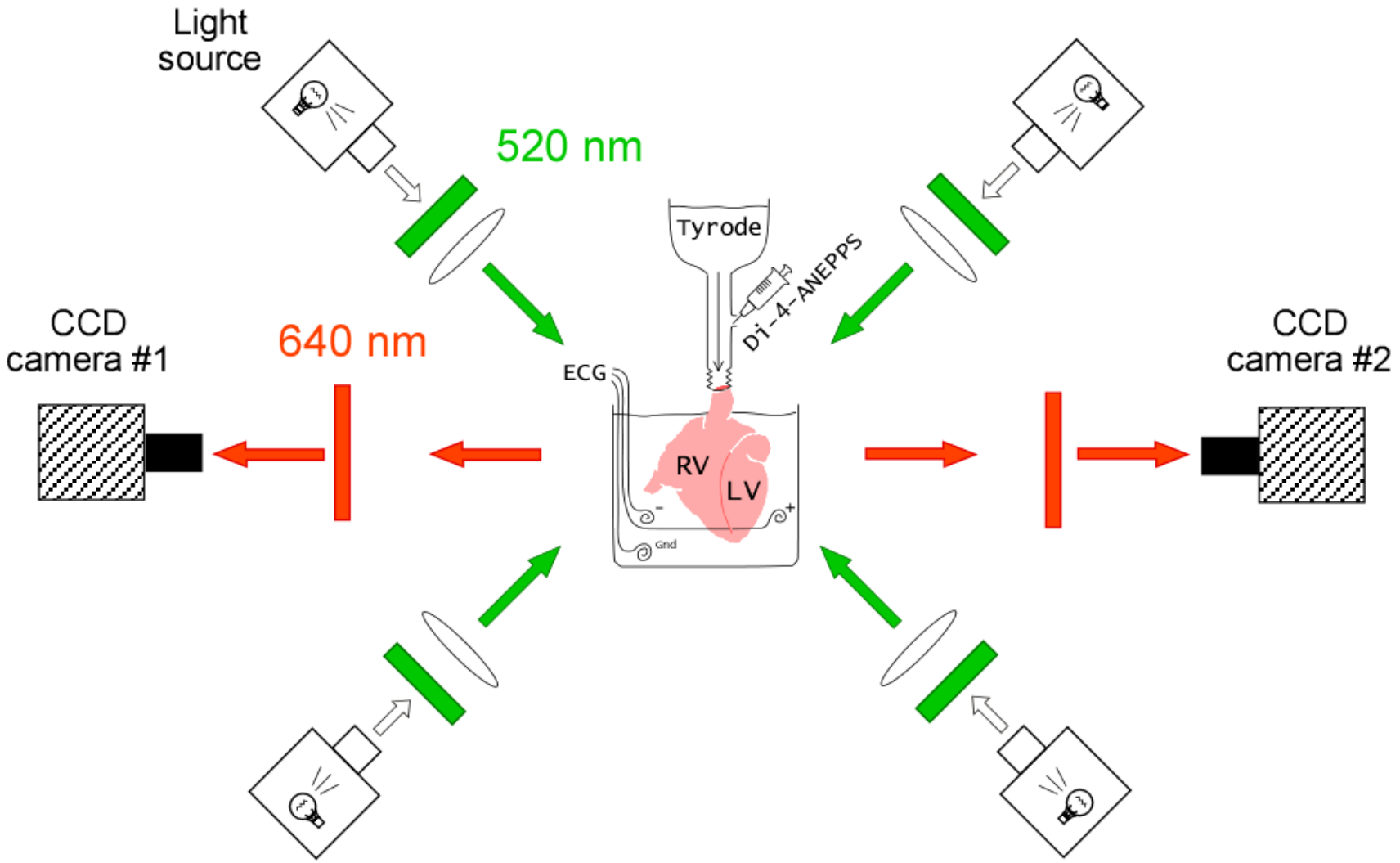




$\lambda_v - \lambda_o \sim E$

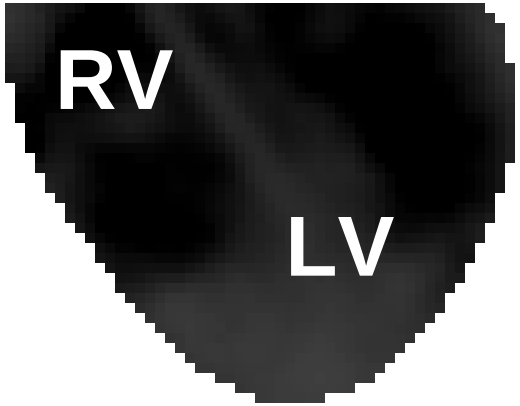


Optical mapping system



Optical mapping- imaging cardiac excitation

A



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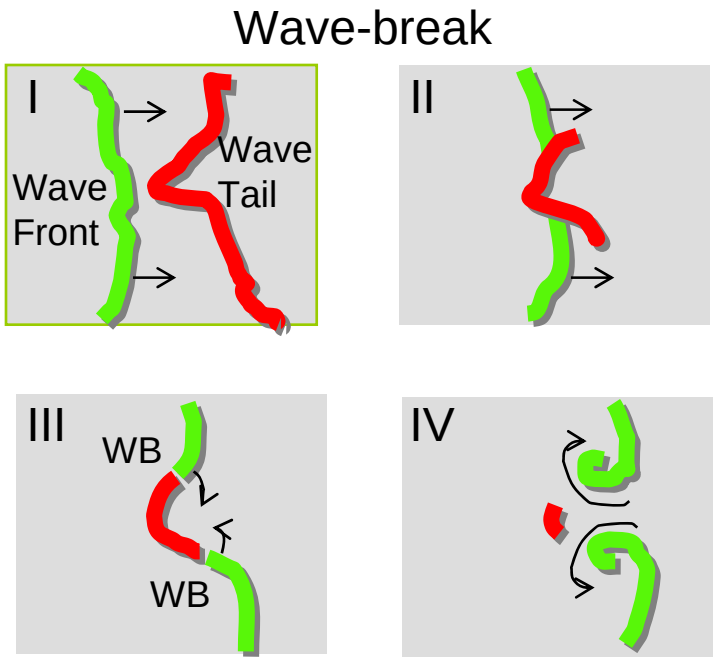

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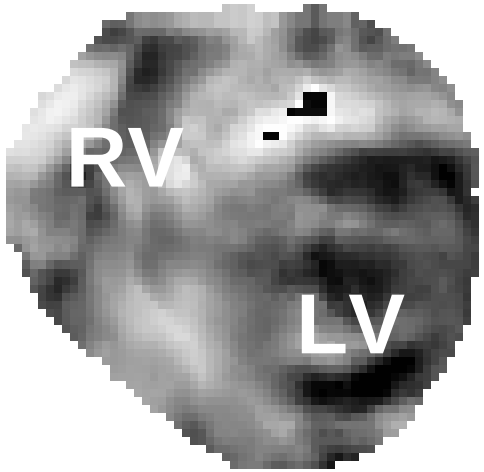
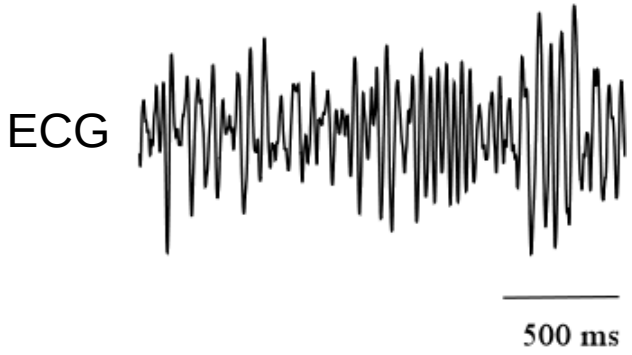
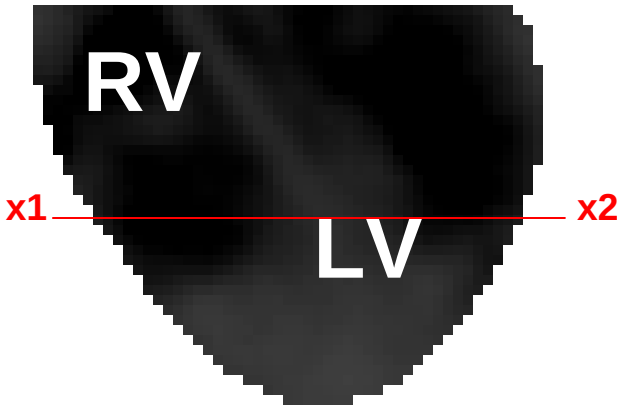




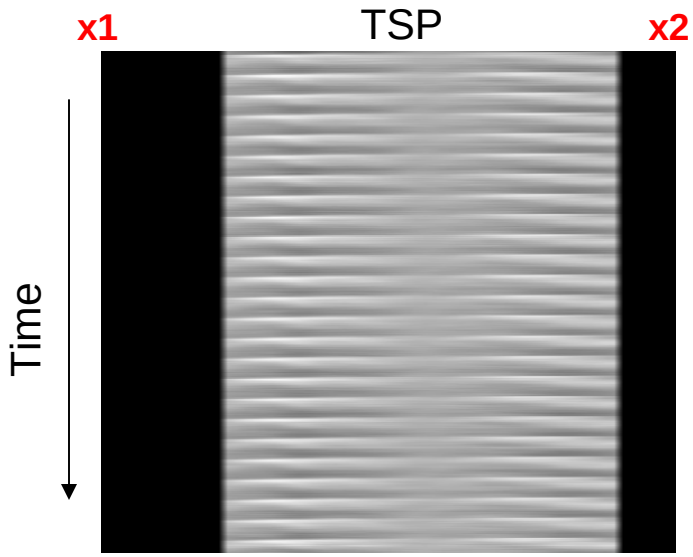
Warren et al JCE 2003

Optical mapping- Time-space-plots (TSP)

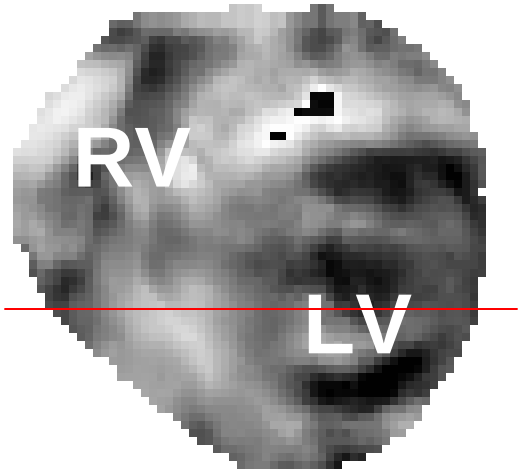
A



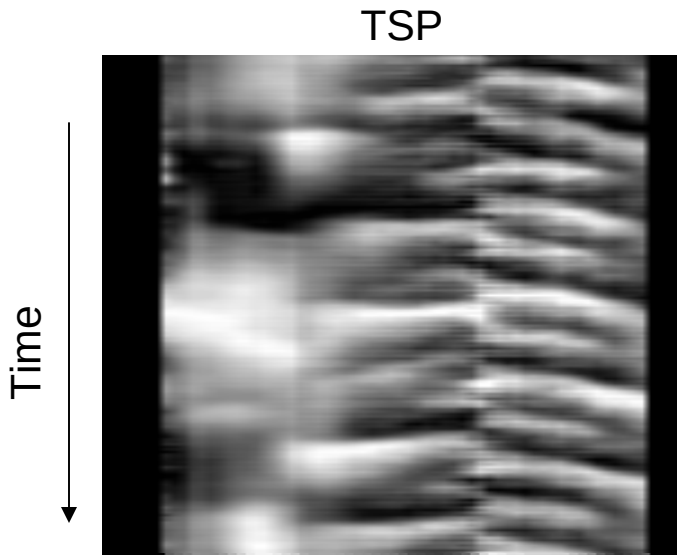
B



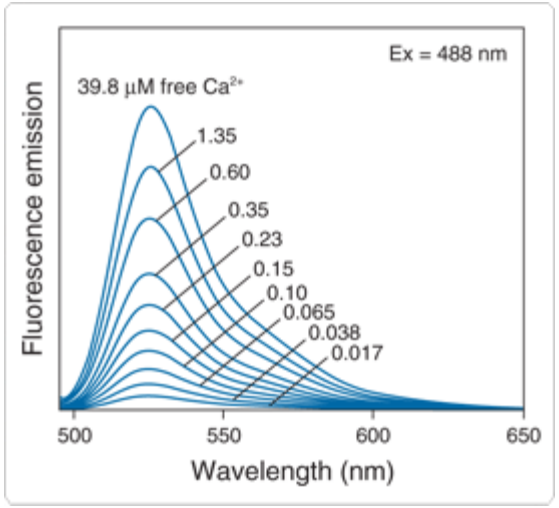
C

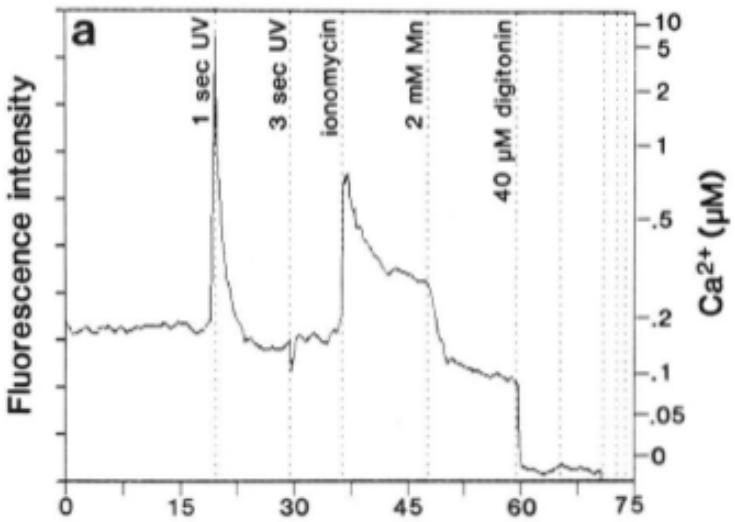


D



Fluorescent probes- calcium sensitive probes Fluo-3/Fluo-4

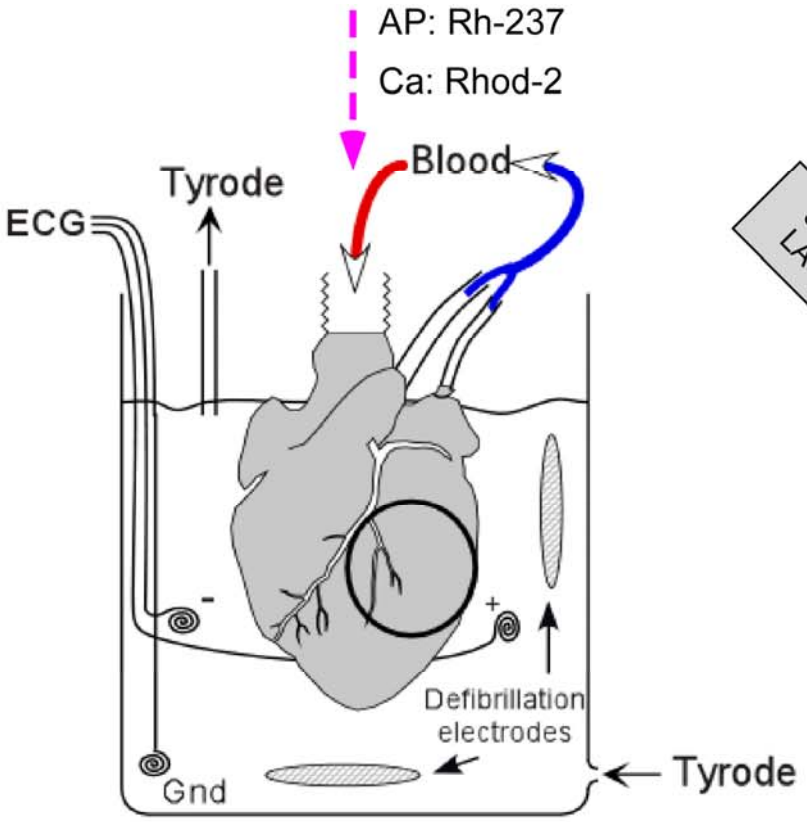




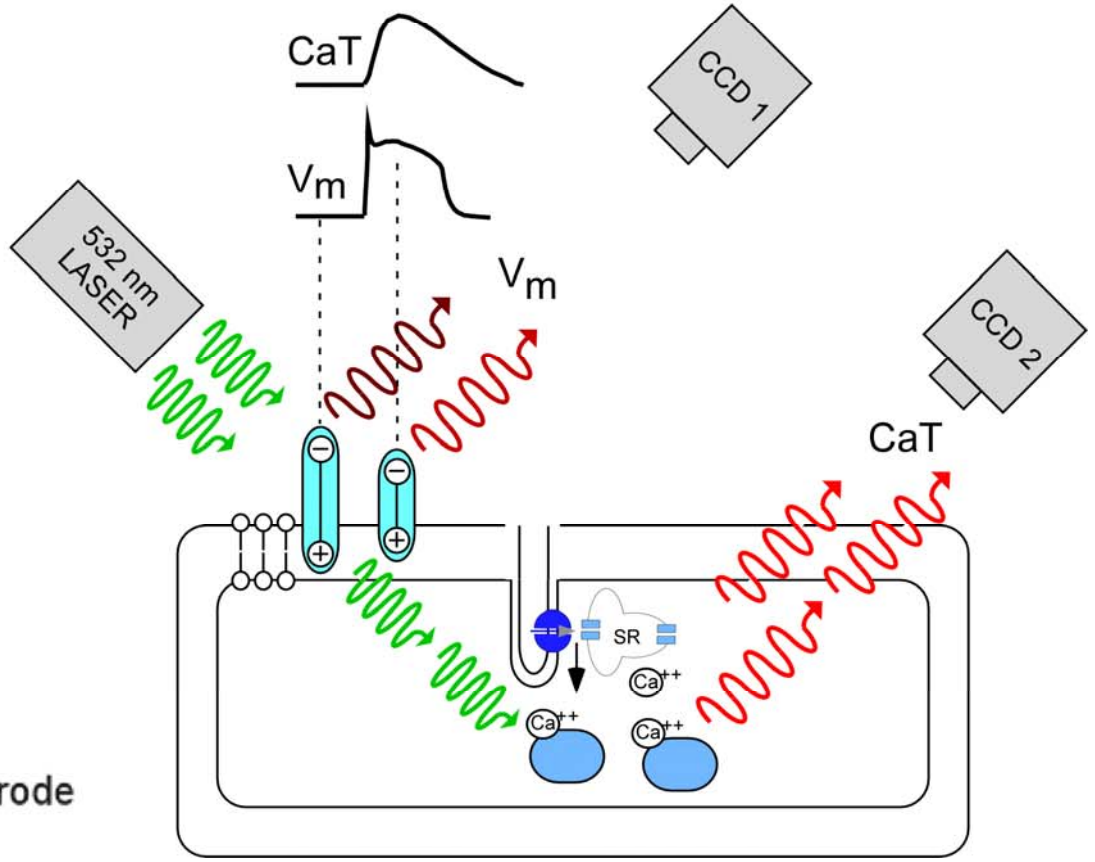
Kao et al J Biol Chem 1989

Optical mapping- dual imaging of excitation and $[Ca^{++}]_i$

Blood-perfused pig heart



Dual optical mapping

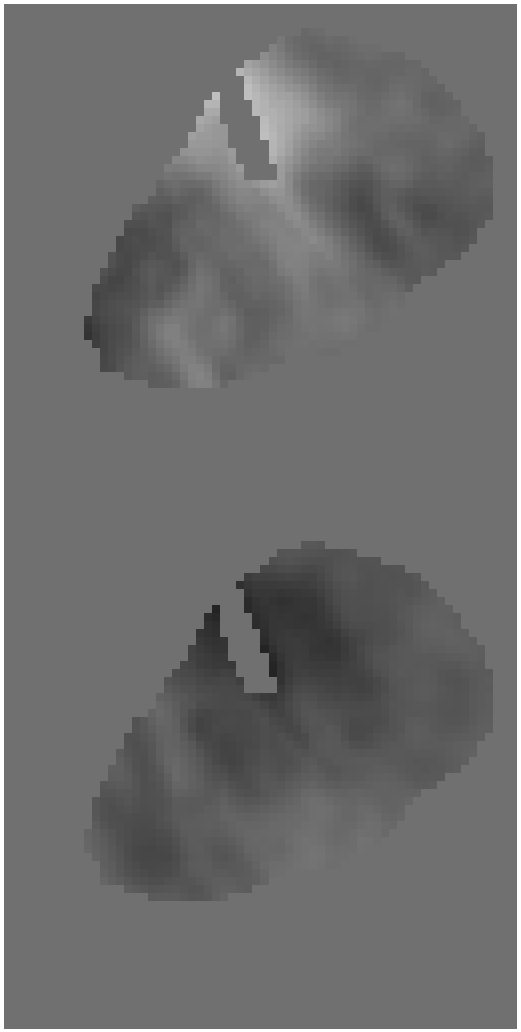


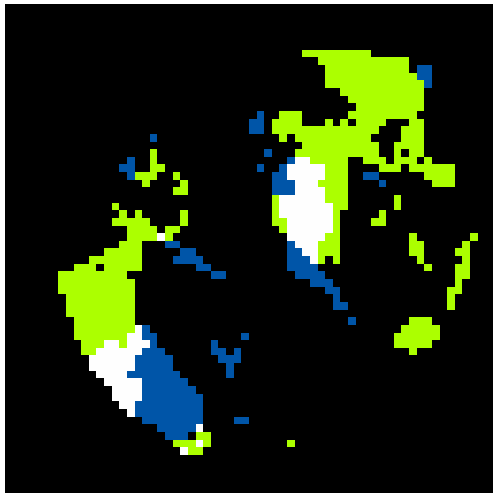
Optical mapping- dual imaging of excitation and $[Ca^{++}]_i$

RH-237 and Rhod-2 fluorescence

AP

Ca_iT





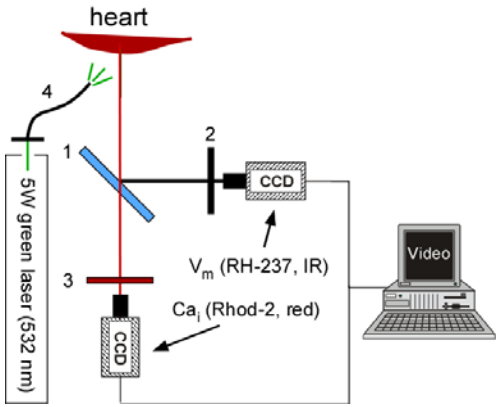


Figure 14. The optical setup for simultaneous imaging of V_m and Ca_i. ***(1)*** Dichroic mirror 634 nm; ***(2)*** longpass filter 700 nm; ***(3)*** bandpass filter 585±20 nm. See text for detail.

Warren et al Circ Res 2007

Imaging excitation and $[Ca^{++}]_i$ in isolated myocytes

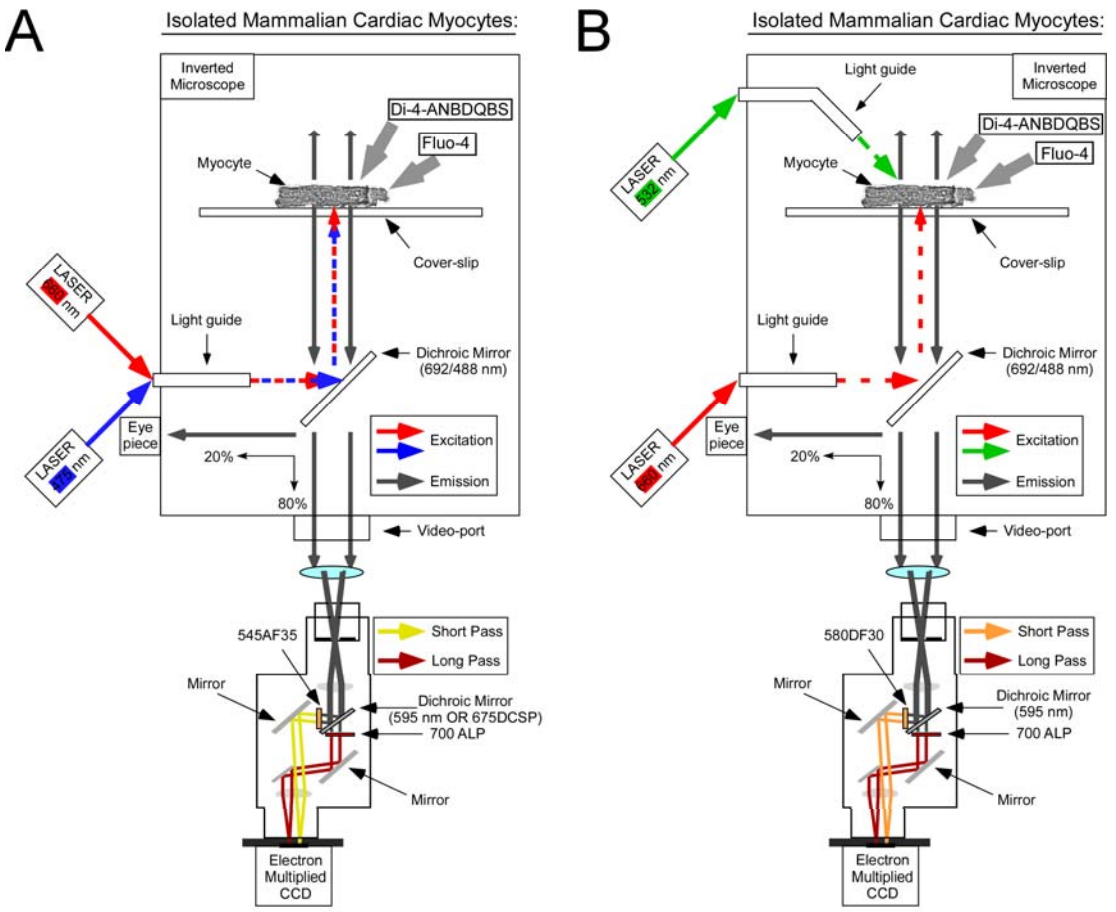
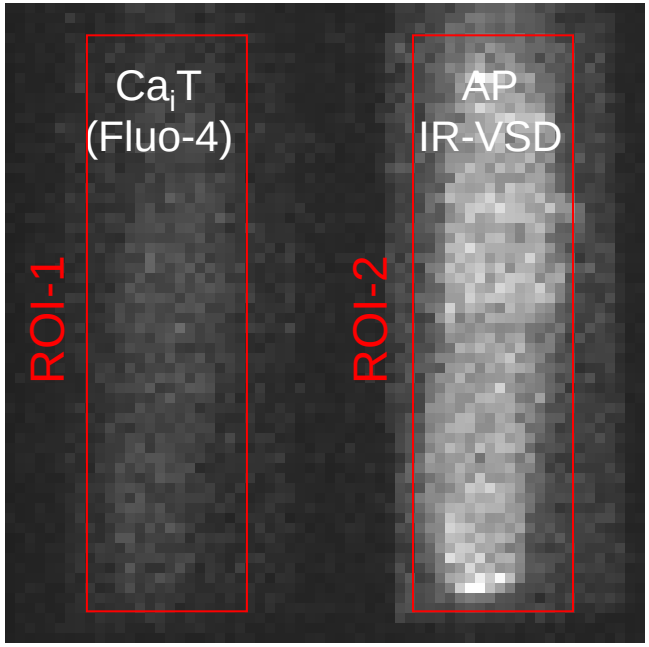
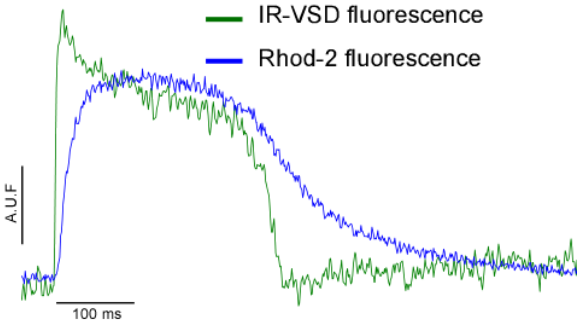


Figure 1





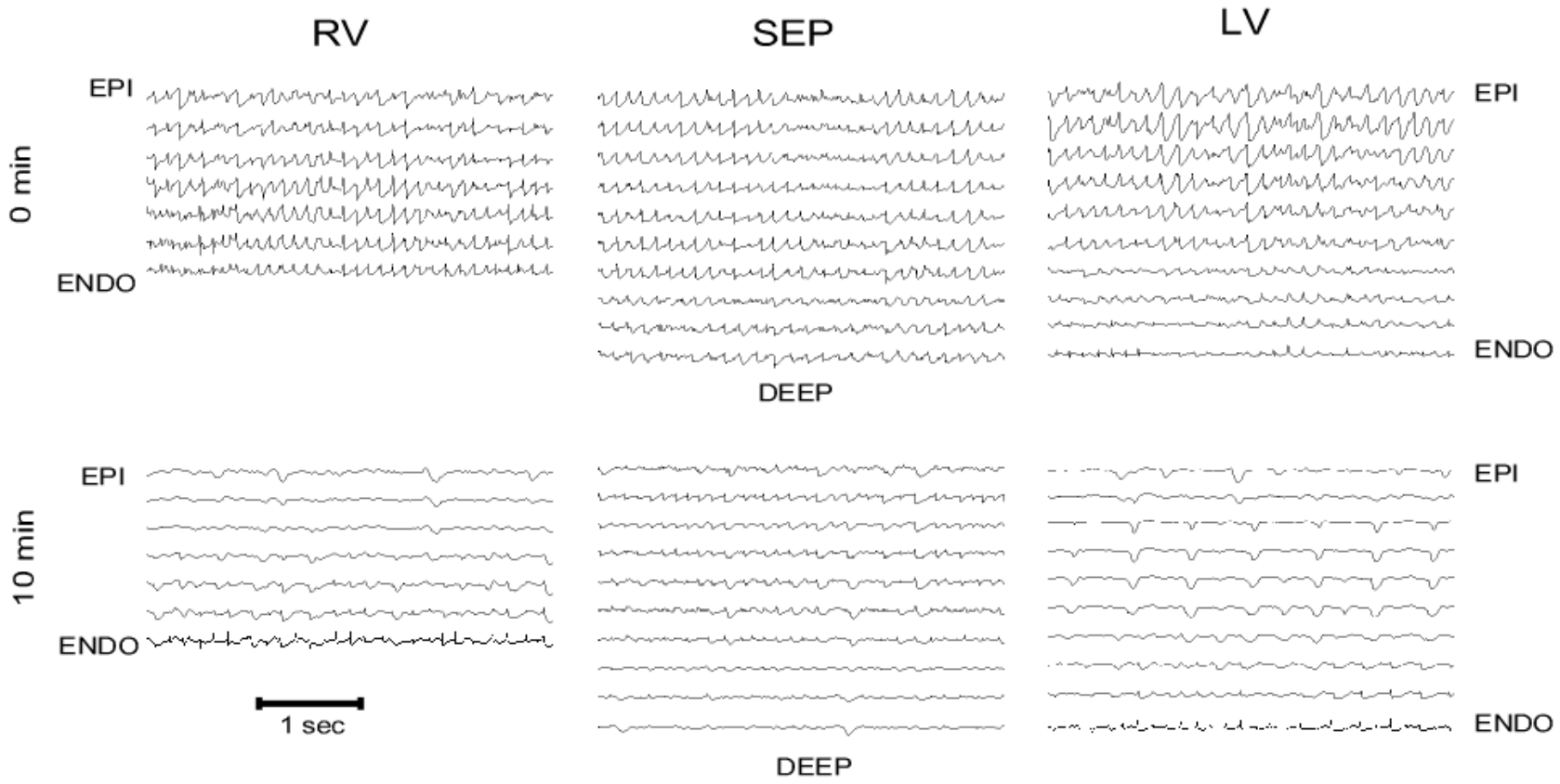
Bioeng 6460
Electrophysiology and Bioelectricity

Arrhythmias II (Arrhythmia Lab)

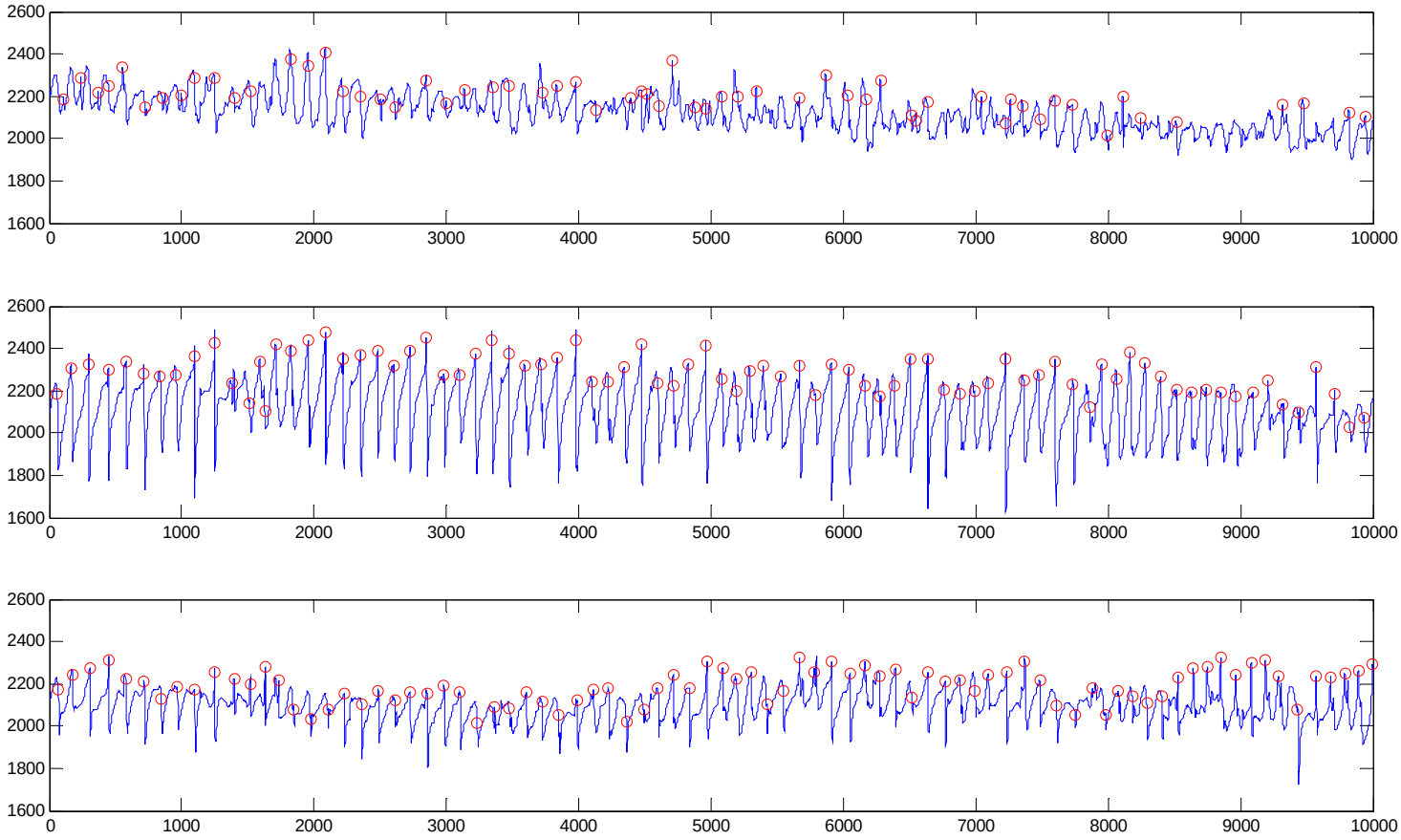
(Part 2 –Analysis Methods)

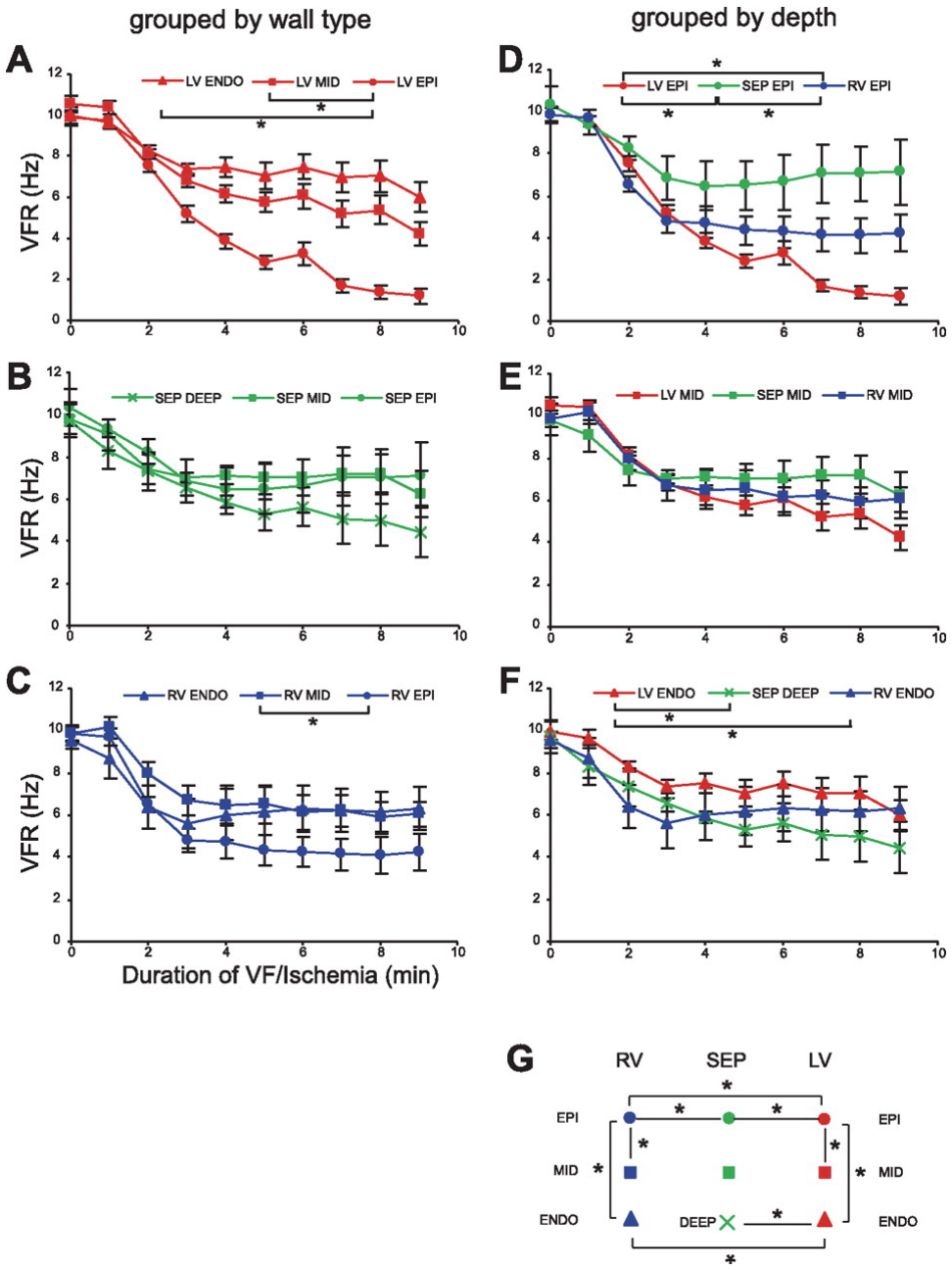
Mark Warren
warren@cvrti.utah.edu

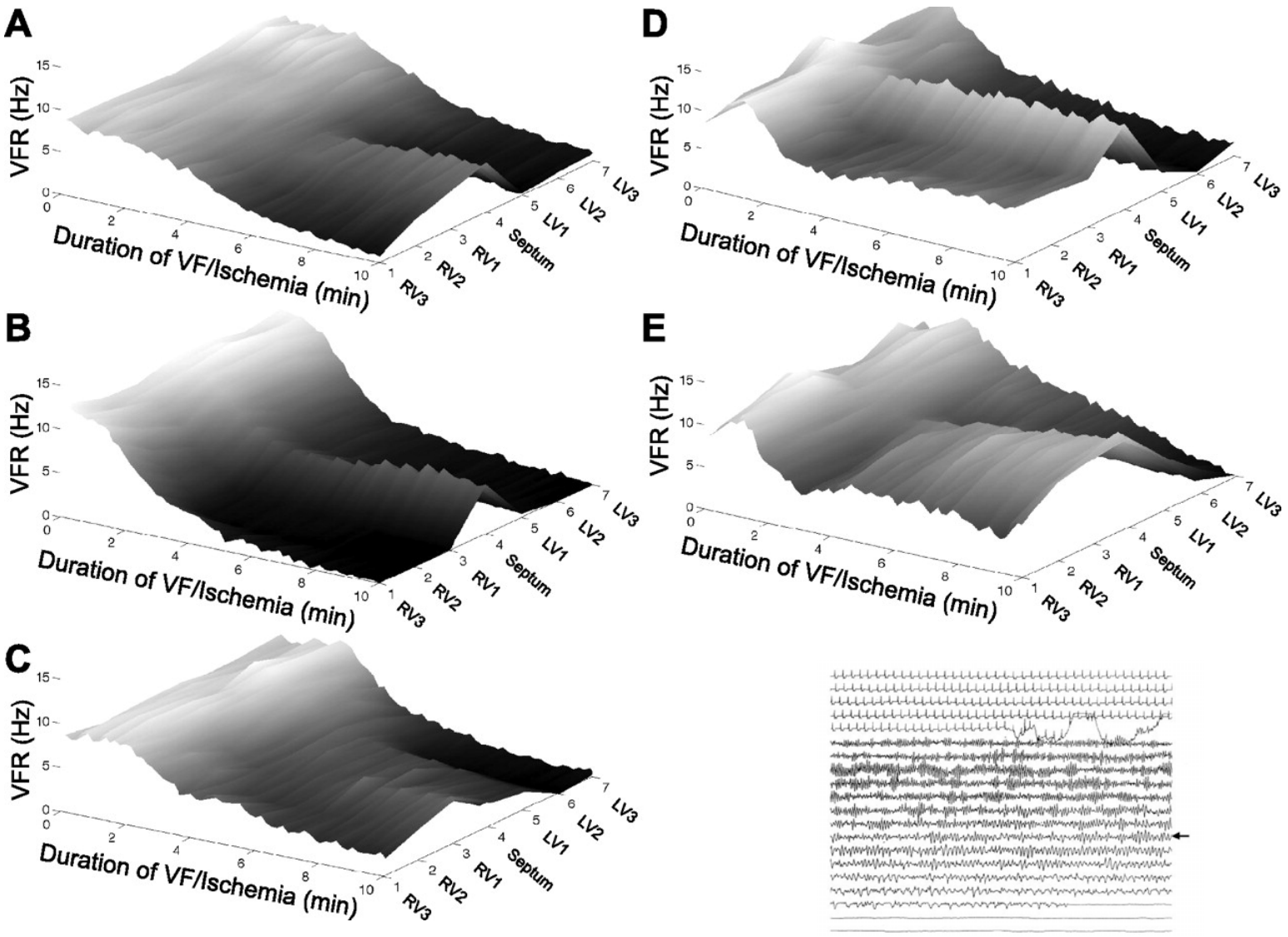
Exercise #1 (activation rate heterogeneity)



1- Determine the approximate excitation rate in the epicardium and the endocardium in each of the three regions. Choose one of the regions, and compare the rate in epi. and endo. in control and after 10 min of ischemia.


2600
2400
2200
2000
1800
1600
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000


grouped by wall type
grouped by depth
A
B
C
D
E
F
G
LV ENDO
LV MID
LV EPI
SEP DEEP
SEP MID
SEP EPI
RV ENDO
RV MID
RV EPI
LV EPI
SEP EPI
RV EPI
LV MID
SEP MID
RV MID
LV ENDO
SEP DEEP
RV ENDO
VFR (Hz)
Duration of VF/Ischemia (min)
RV
SEP
LV
EPI
MID
ENDO
DEEP

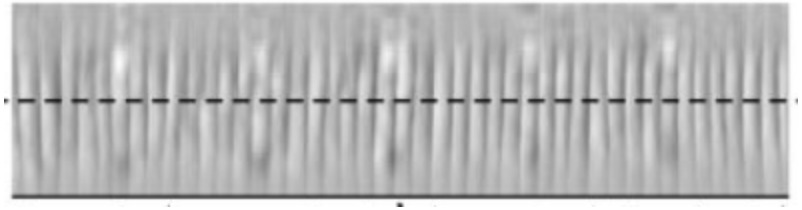

A
VFR (Hz)
Duration of VF/Ischemia (min)
RV3
RV2
RV1
Septum
LV1
LV2
LV3
B
C
D
E

Exercise #2 (temporal and spatial changes of AP)

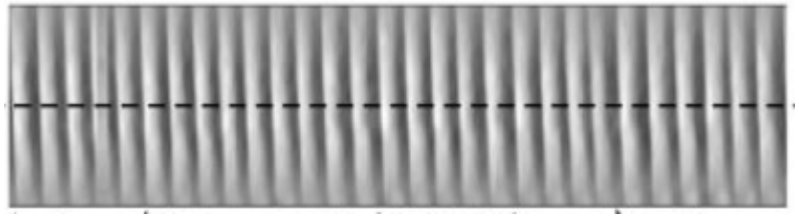
Time-space-plots (TSP)

Optical action potentials (OAP)

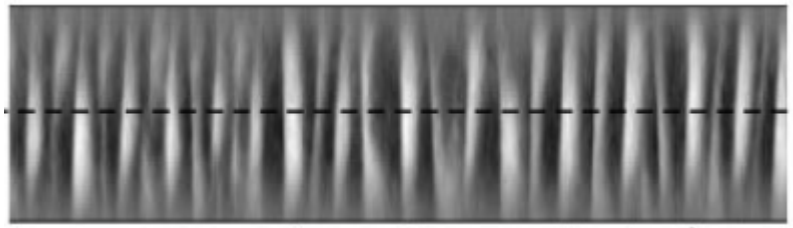
1)



2)



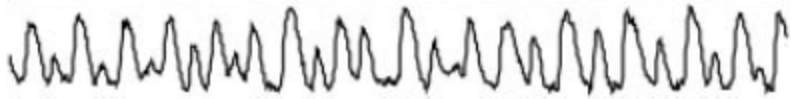
3)



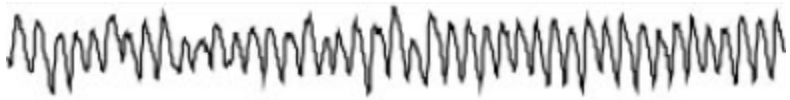
1 s

a)

b)



c)



1 s

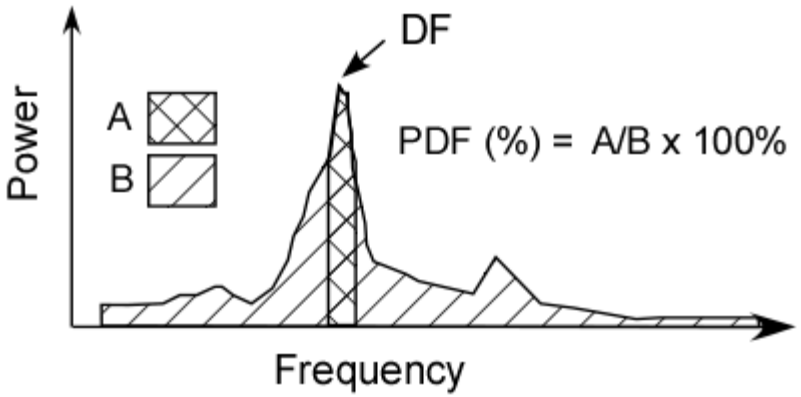
1- Determine the approximate excitation rate in each of the three experimental conditions.

2- Determine the approximate action potential duration (APD) and amplitude (APA) of four consecutive activations. What differences do you see between the three experimental conditions?

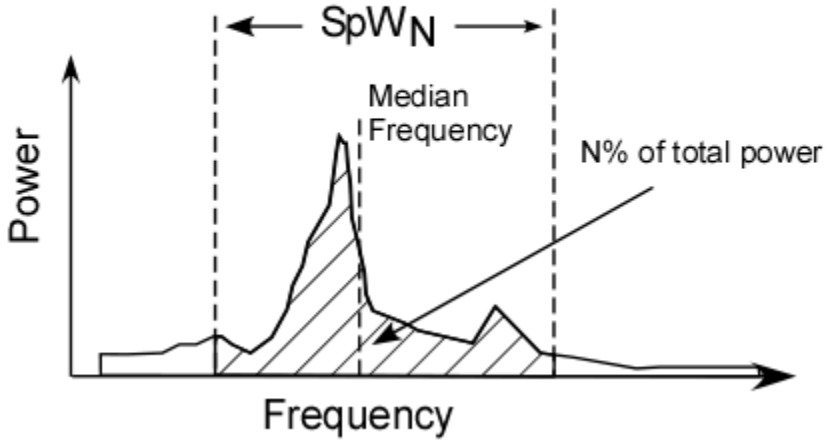
3- Match the TSP with its corresponding OAP

Fast Fourier Transform (FFT) ➔ Power Spectrum

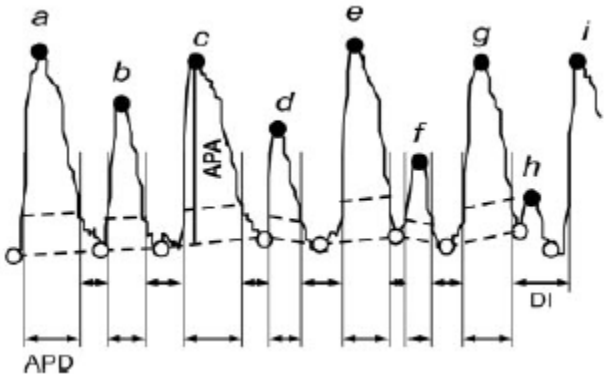
Power at the Dominant Frequency



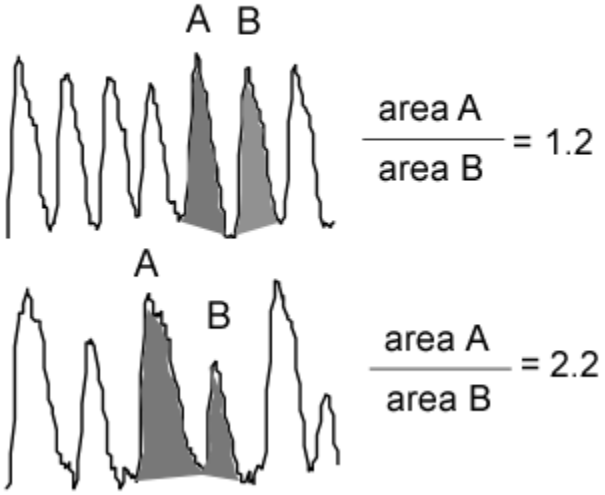
Spectral Width

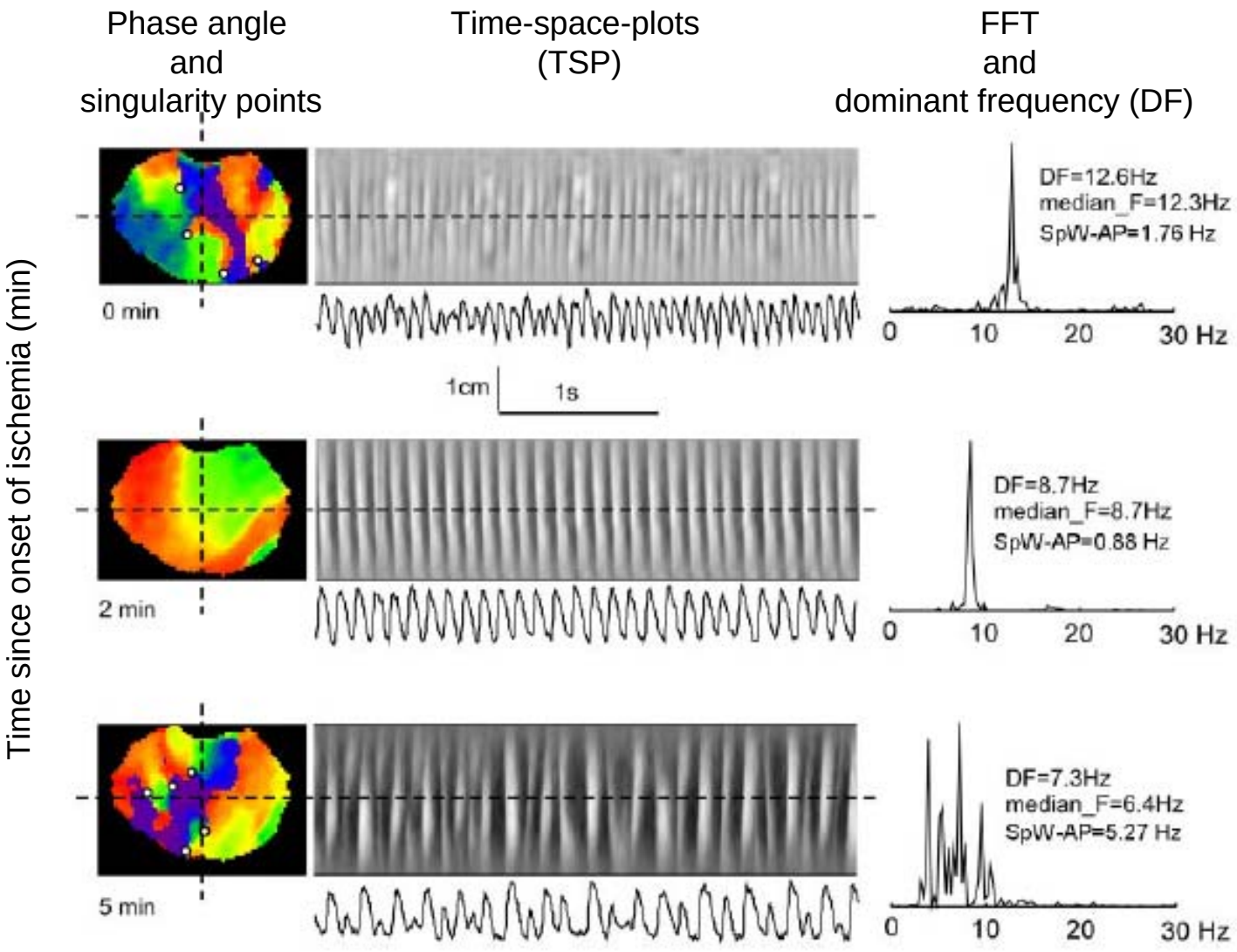


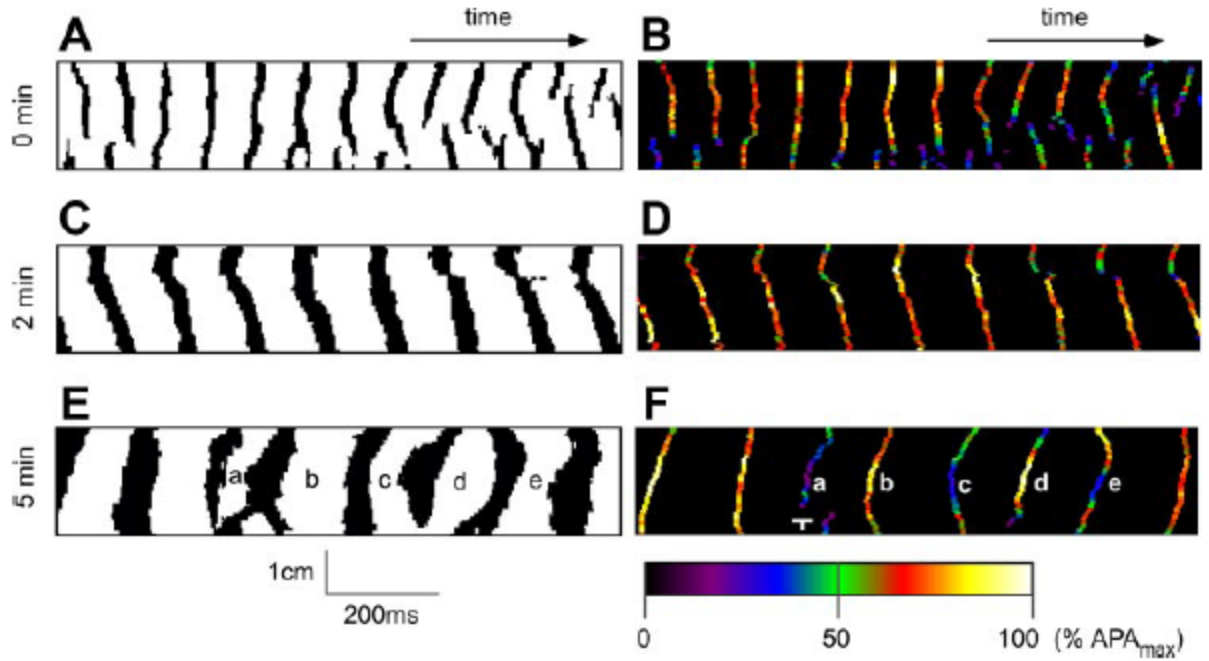
Individual AP Analysis

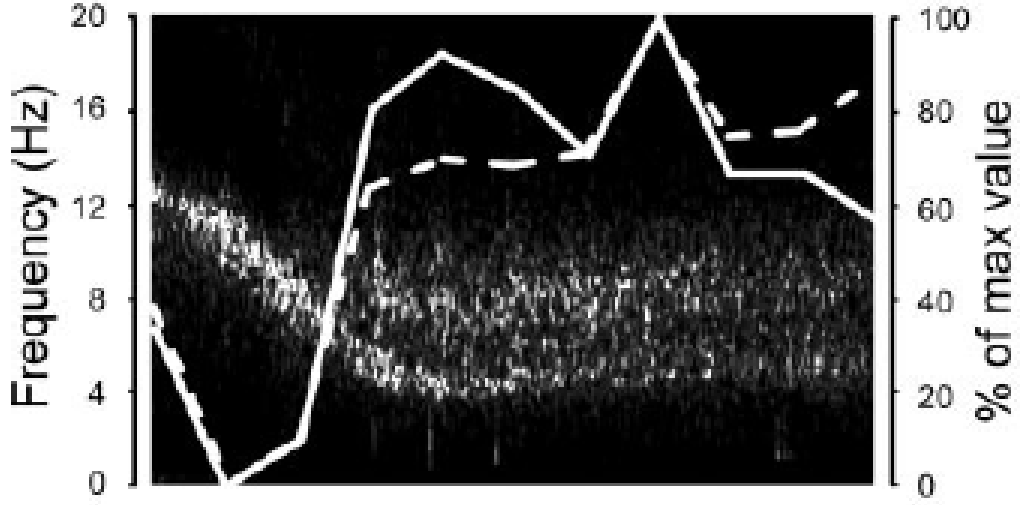


AP alternans index

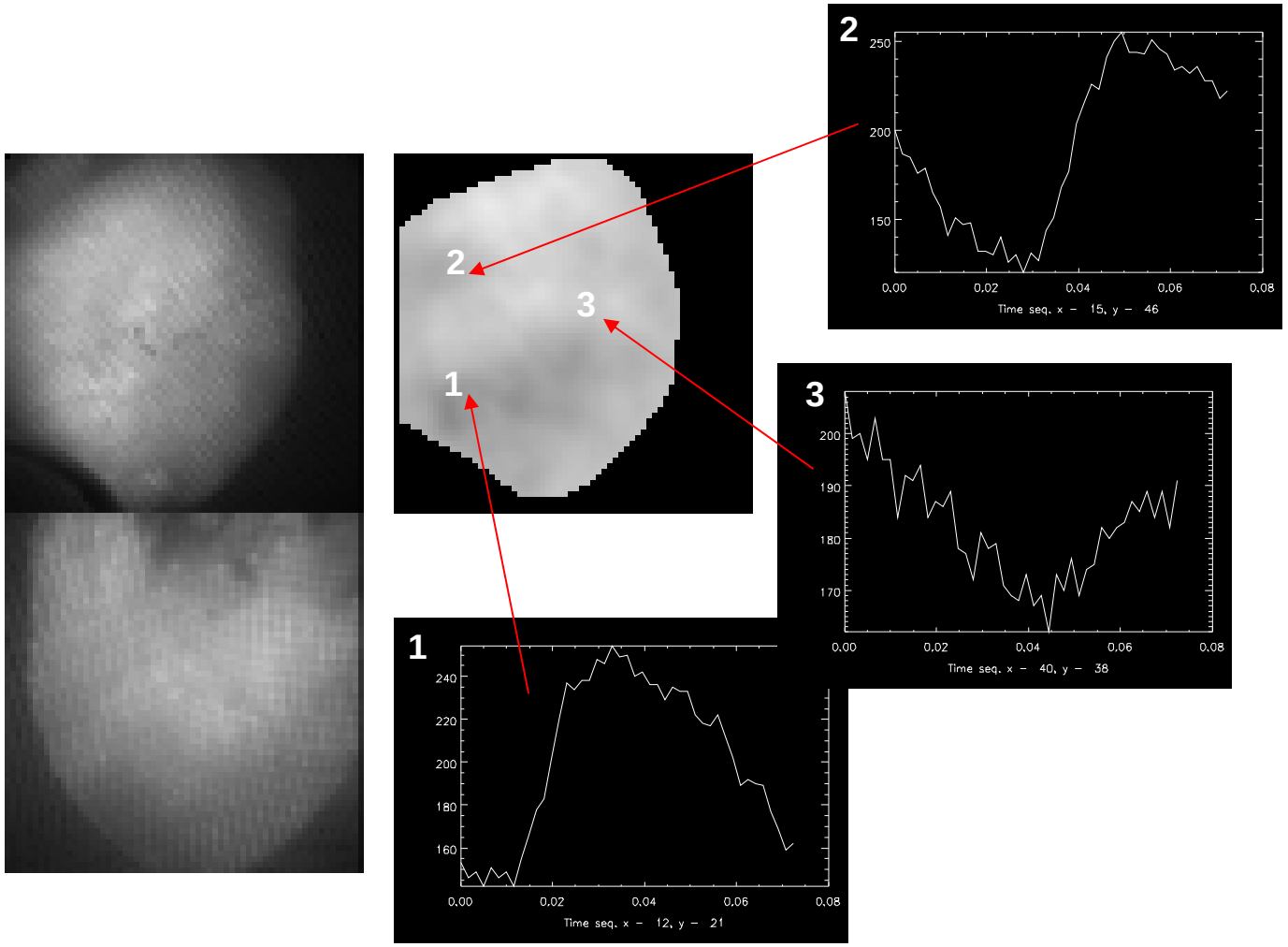



Phase angle
and
singularity points
Time-space-plots
(TSP)
FFT
and
dominant frequency (DF)
Time since onset of ischemia (min)
0 min
2 min
5 min
1cm
1s
DF=12.6Hz
median_F=12.3Hz
SpW-AP=1.76 Hz
DF=8.7Hz
median_F=8.7Hz
SpW-AP=0.88 Hz
DF=7.3Hz
median_F=6.4Hz
SpW-AP=5.27 Hz
0
10
20
30 Hz


A
B
C
D
E
F
time
time
0 min
2 min
5 min
a
b
c
d
e
1cm
200ms
0
50
100 (% APA$_{max}$)


Frequency (Hz)
20
16
12
8
4
0
100
80
60
40
20
0
% of max value

Exercise #3 (activation map -homework)



Algorithm

For each single pixel recording out of 64x64 pixels:

1- Determine the time at which the absolute maximum value of fluorescence occurs (t_{max}). If the maximum value is achieved at various instances of time, select the earliest.

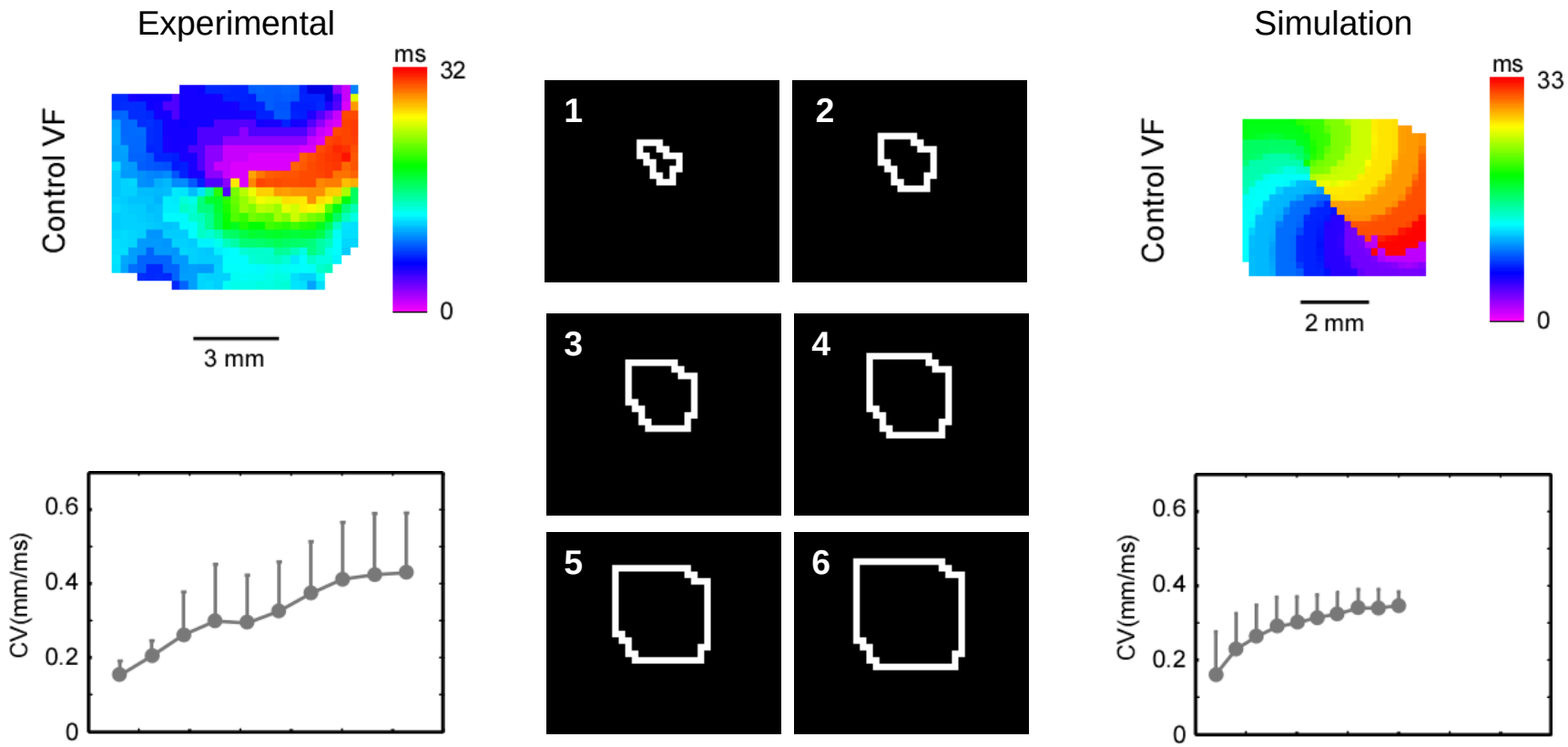
2- Determine the time at which the minimum value of fluorescence prior to the maximum value determined in 1) occurs (t_{min}).

3- Determine the amplitude of the action potential (AP) by calculating the difference between the max and min values of fluorescence determined in 1) and 2).

4- For a given threshold value (expressed as a percentage) determine the value of fluorescence between the min and max values calculated in 1) and 2) which represents this number (F_{th}).

5- For a given threshold value, the activation time is the time between t_{min} and t_{max} at which the fluorescent signal first attains or surpasses F_{th}.

Activation maps



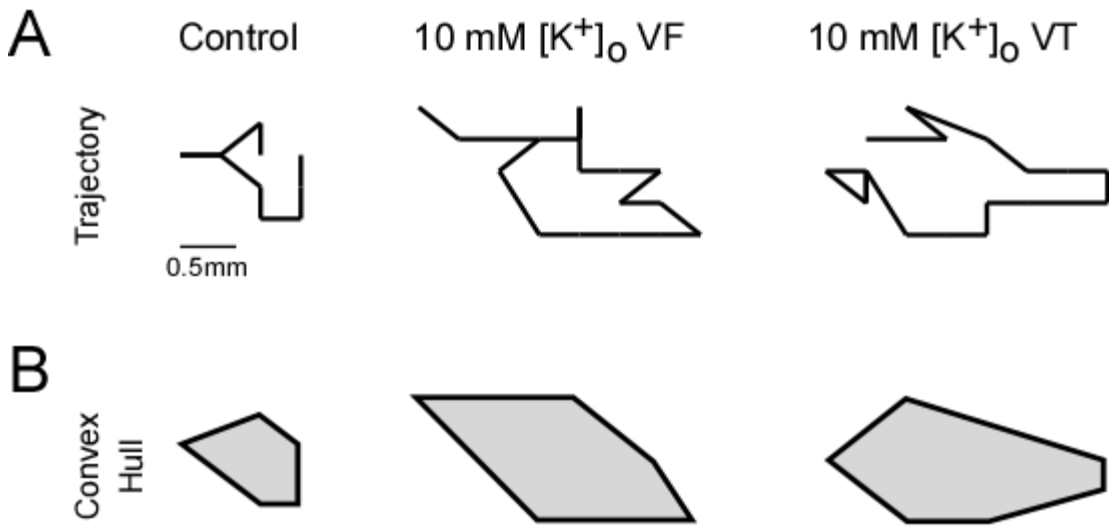
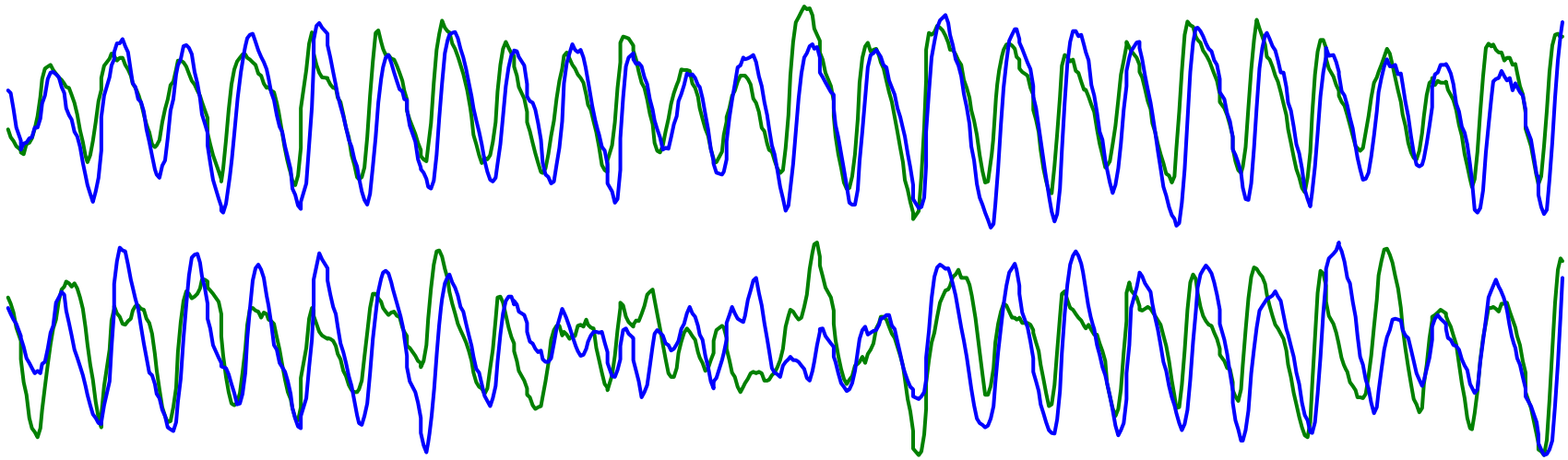
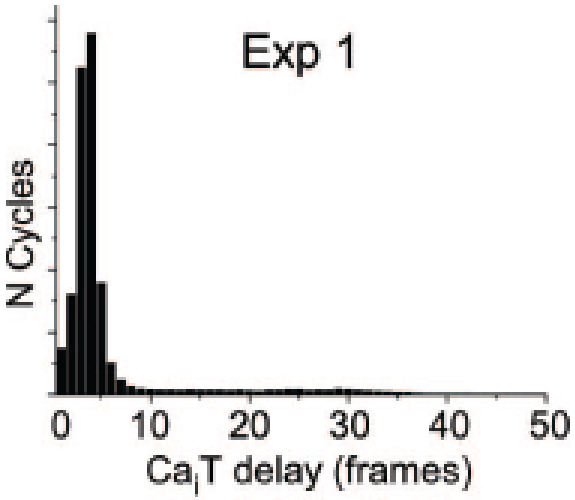
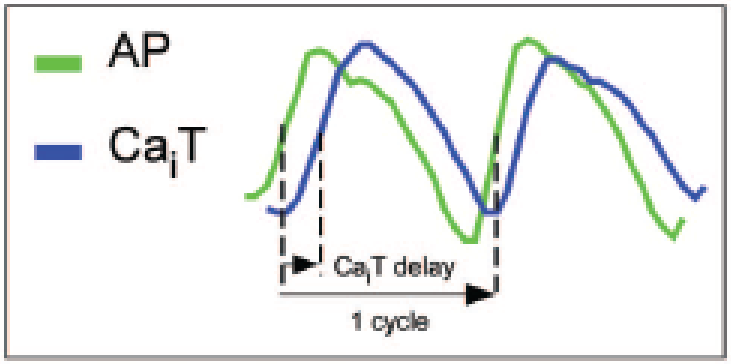


Figure 1S

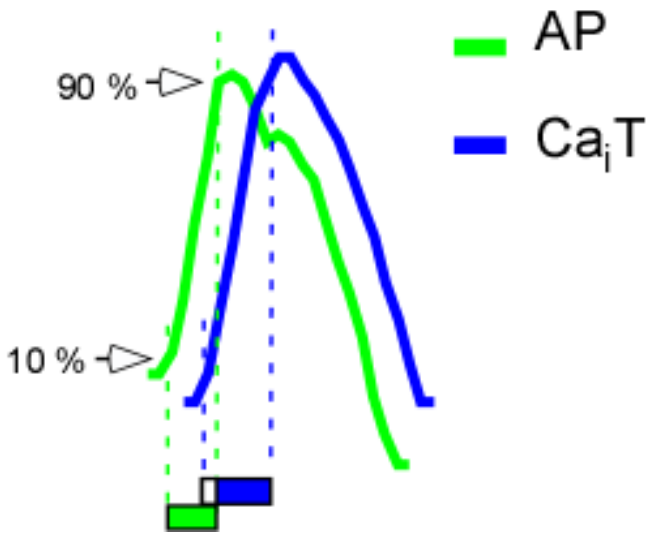
Abnormal calcium events

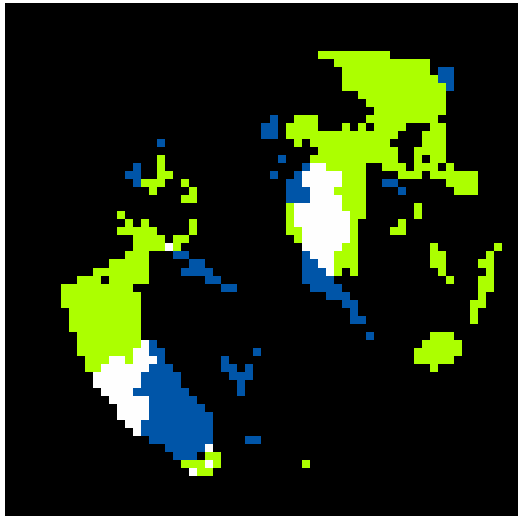




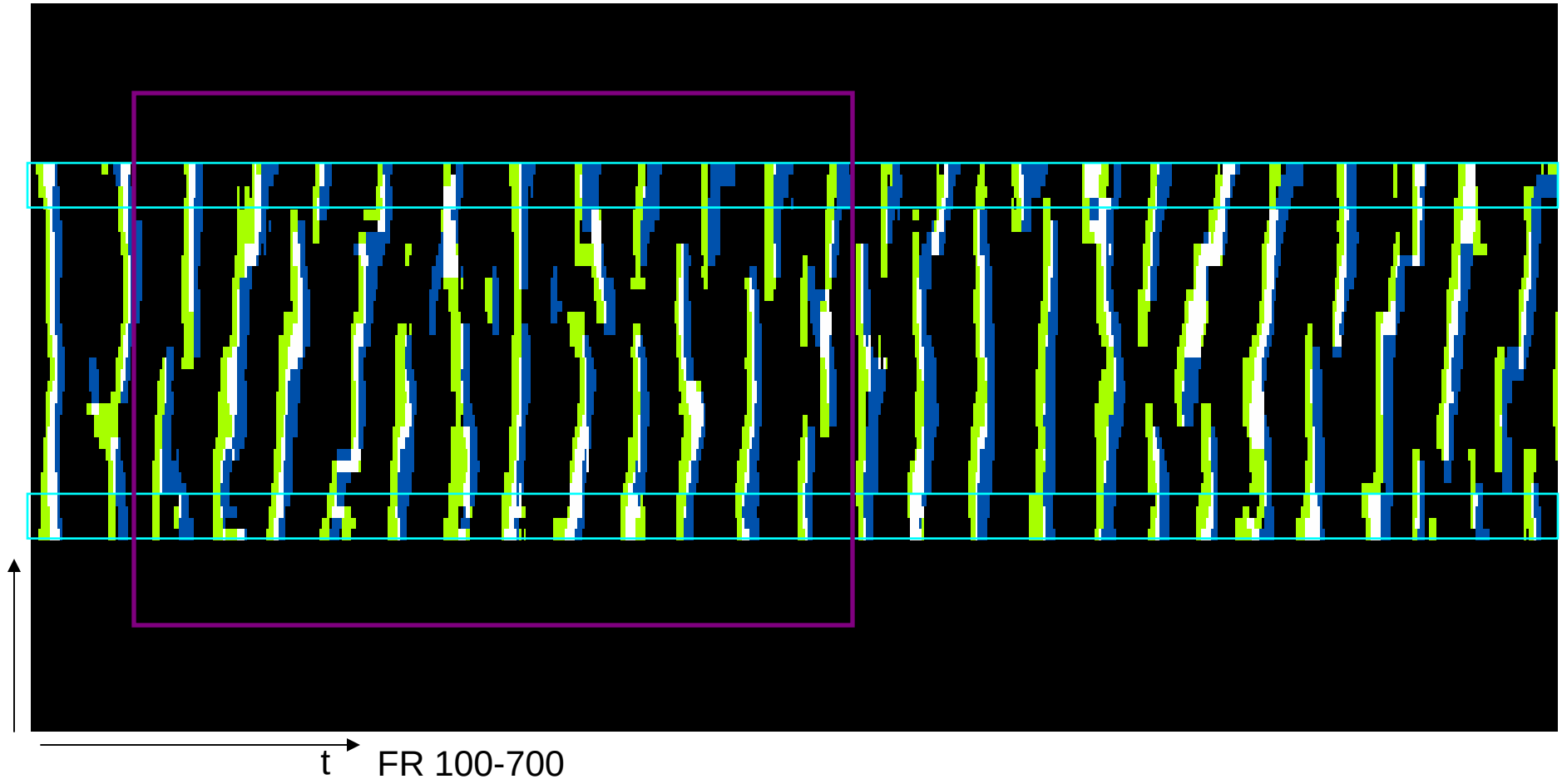


Abnormal calcium events





Exercise #4 (abnormal calcium events)

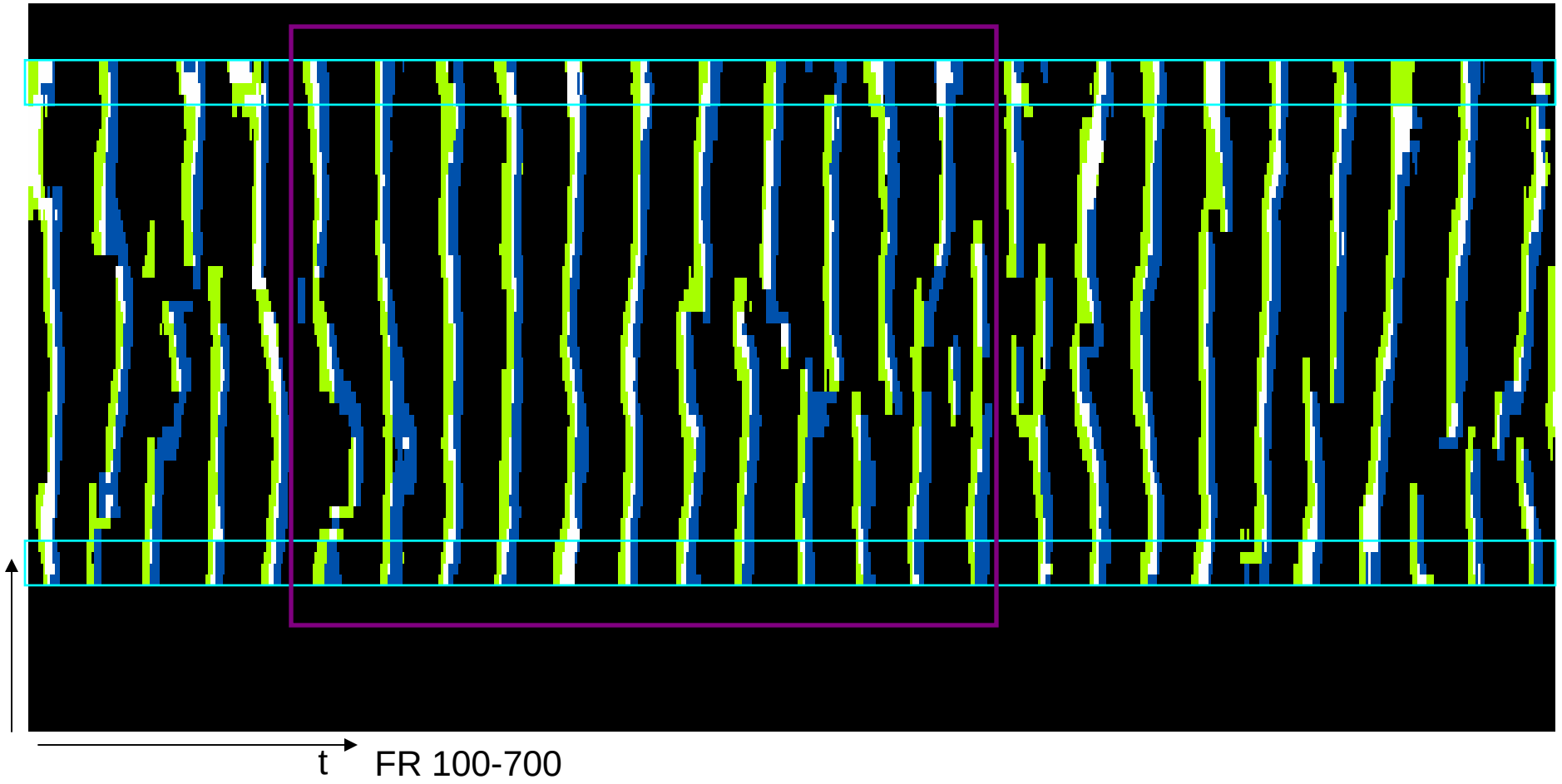


Lone Ca

Ca block

Ca breakthrough

Exercise #4 (abnormal calcium events)

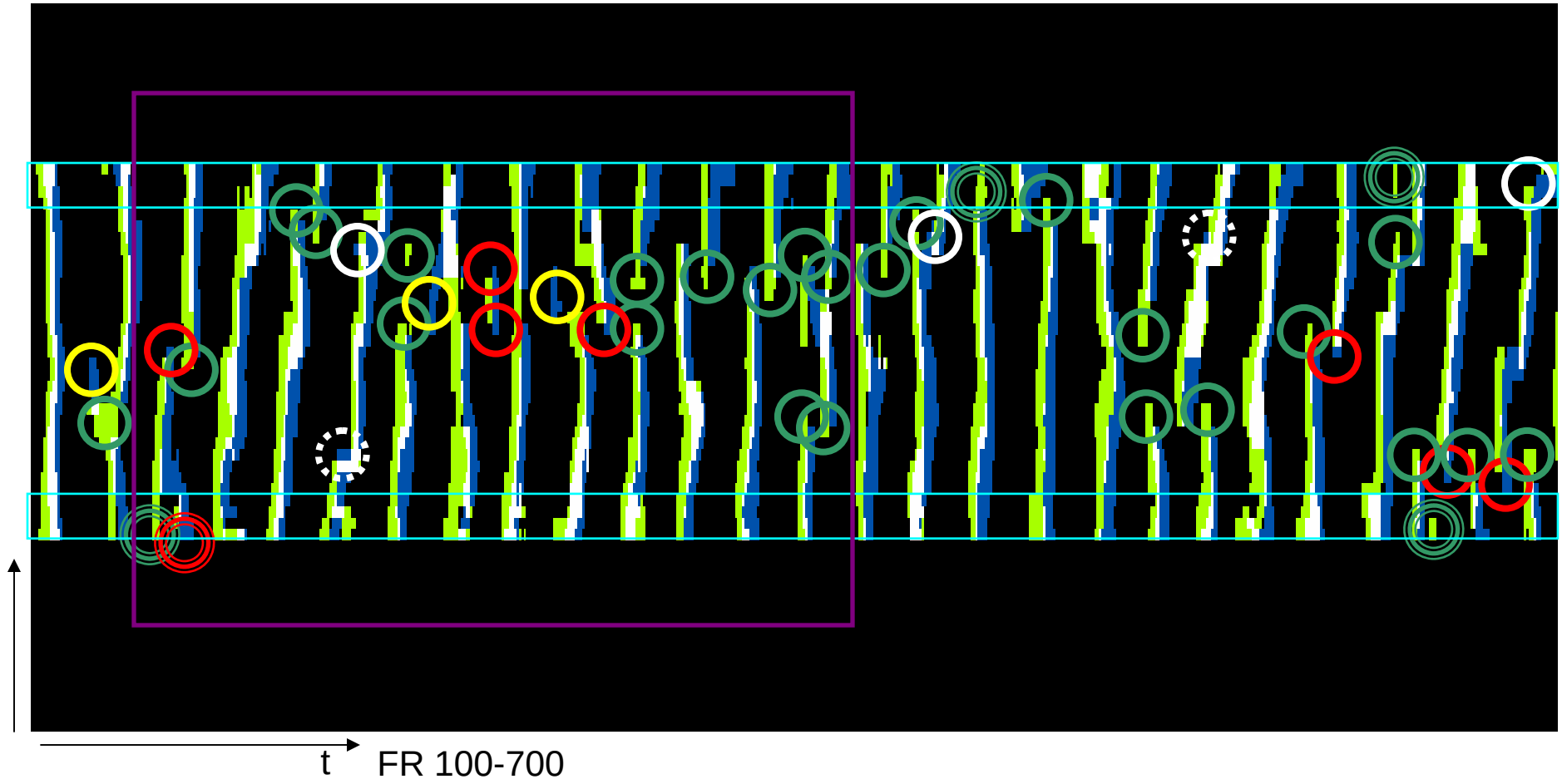


Lone Ca

Ca block

Ca breakthrough

Exercise #4 (abnormal calcium events)

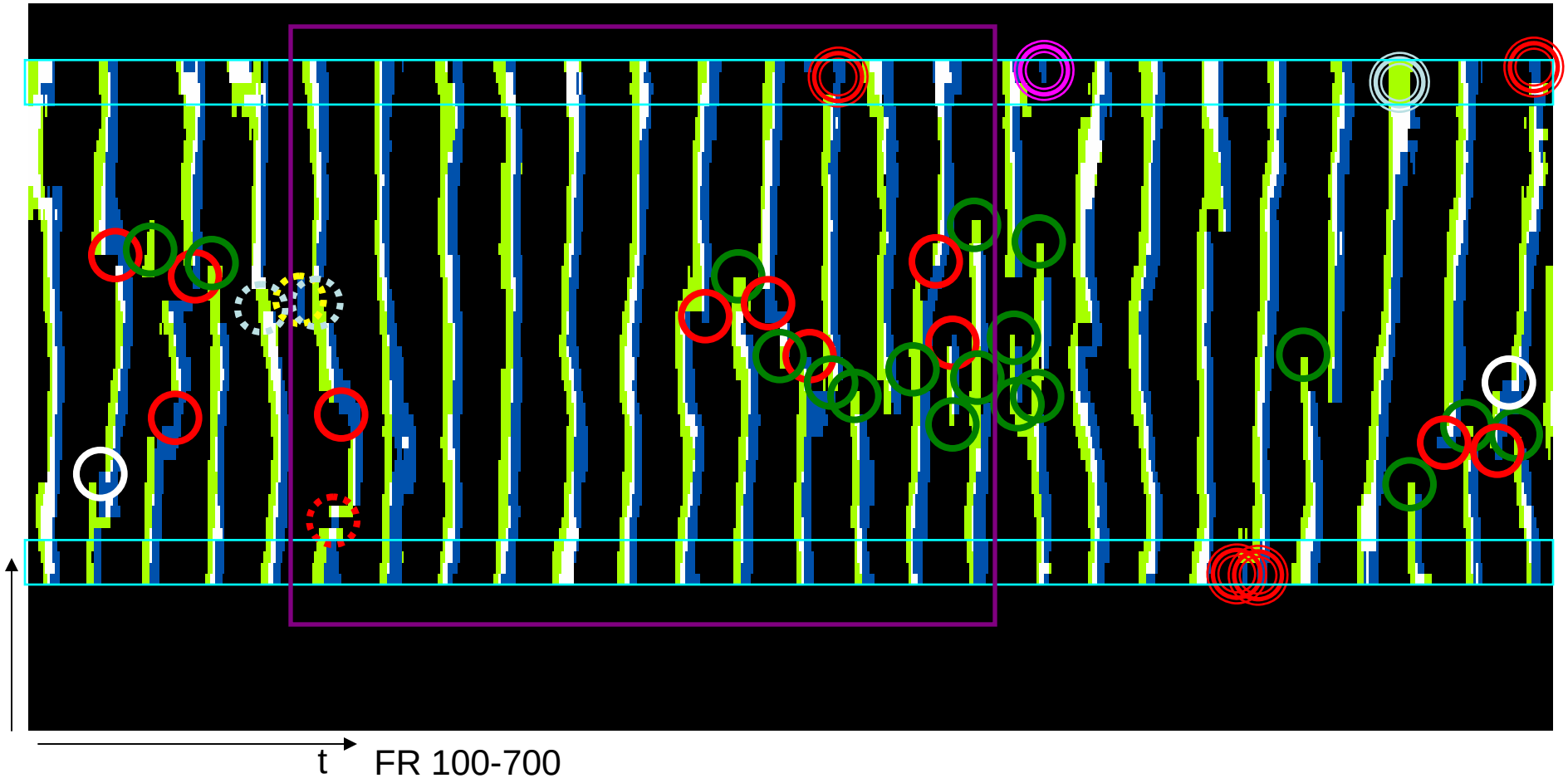


Lone Ca

Ca block

Ca breakthrough

Exercise #4 (abnormal calcium events)



Lone Ca

Ca block

Ca breakthrough

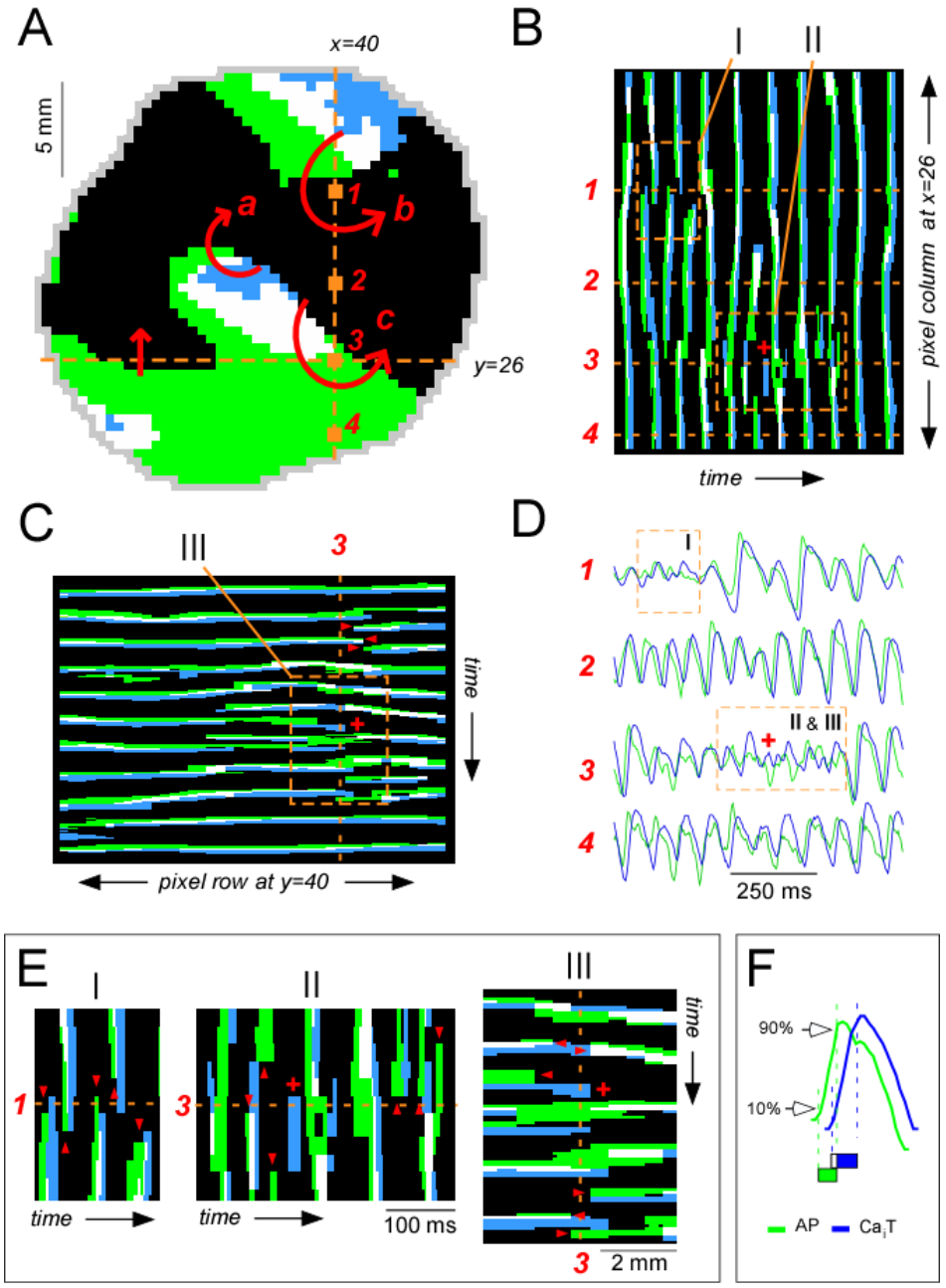
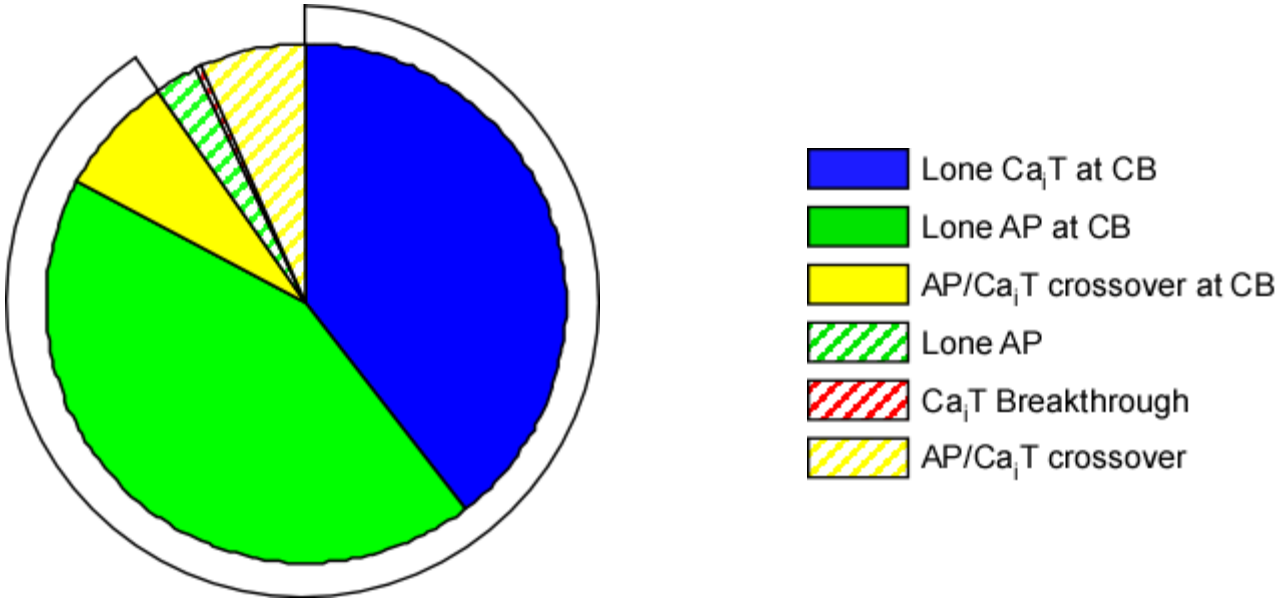

A
x=40
5 mm
a
b
c
1
2
3
4
y=26
B
I
II
1
2
3
4
pixel column at x=26
time
C
III
3
time
pixel row at y=40
D
I
II & III
1
2
3
4
250 ms
E
I
II
III
1
3
time
time
100 ms
time
3
2 mm
F
90%
10%
AP
Ca$_i$T

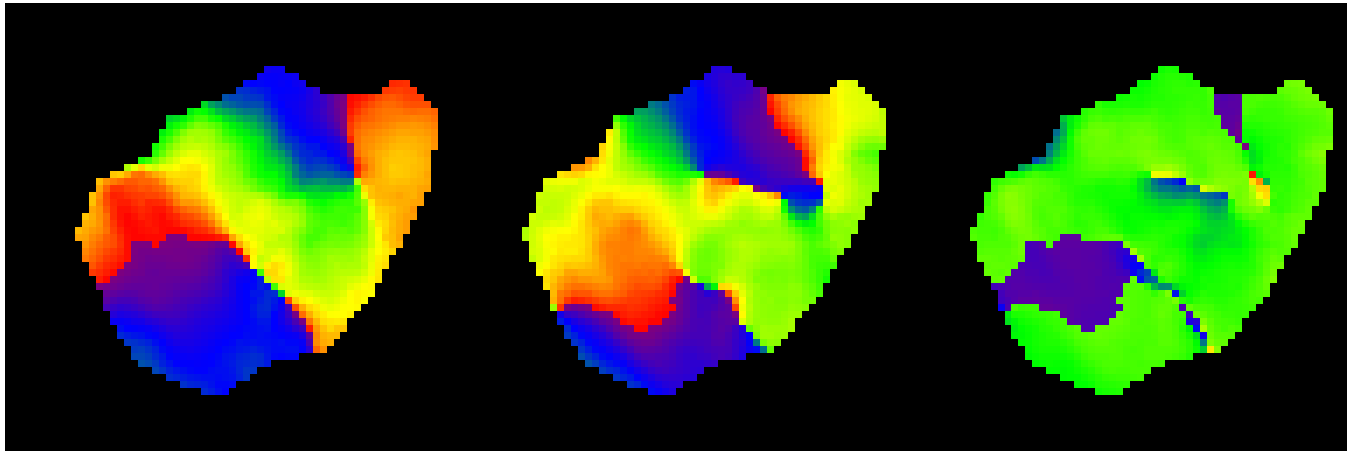
Figure 3

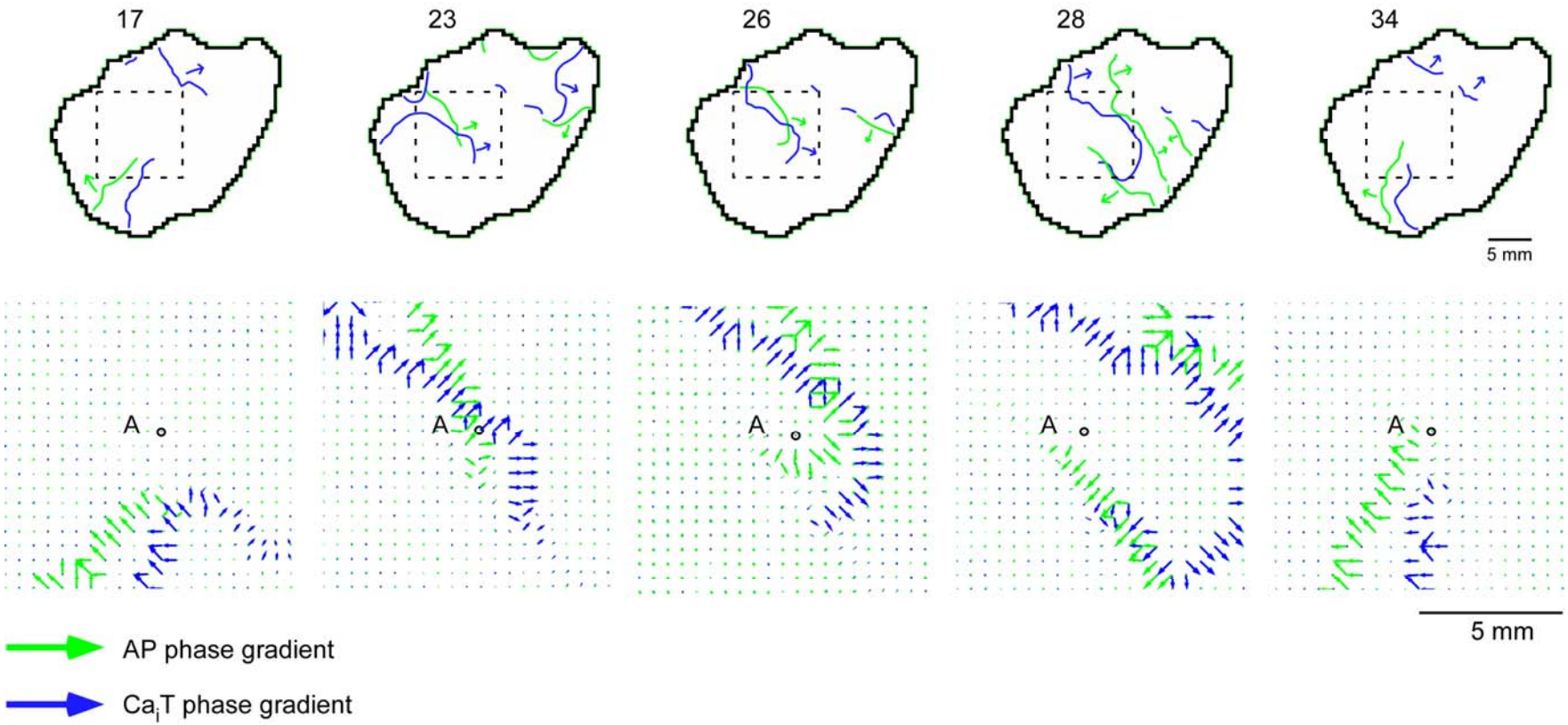


AP-phase

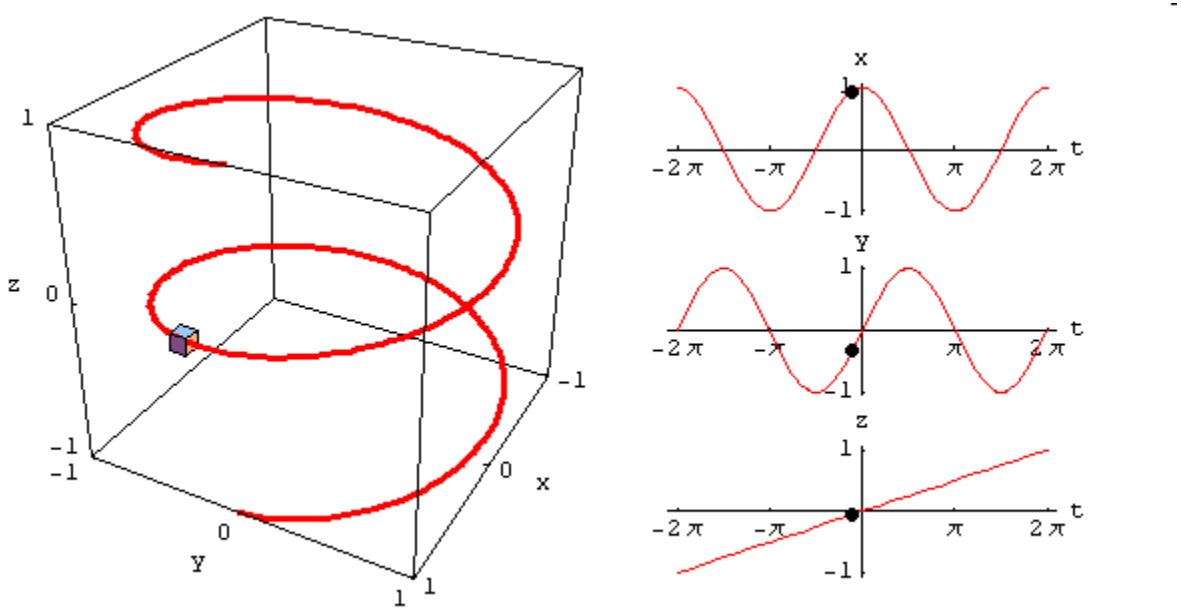
Ca_iT-phase

AP-Ca_iT
Phase difference

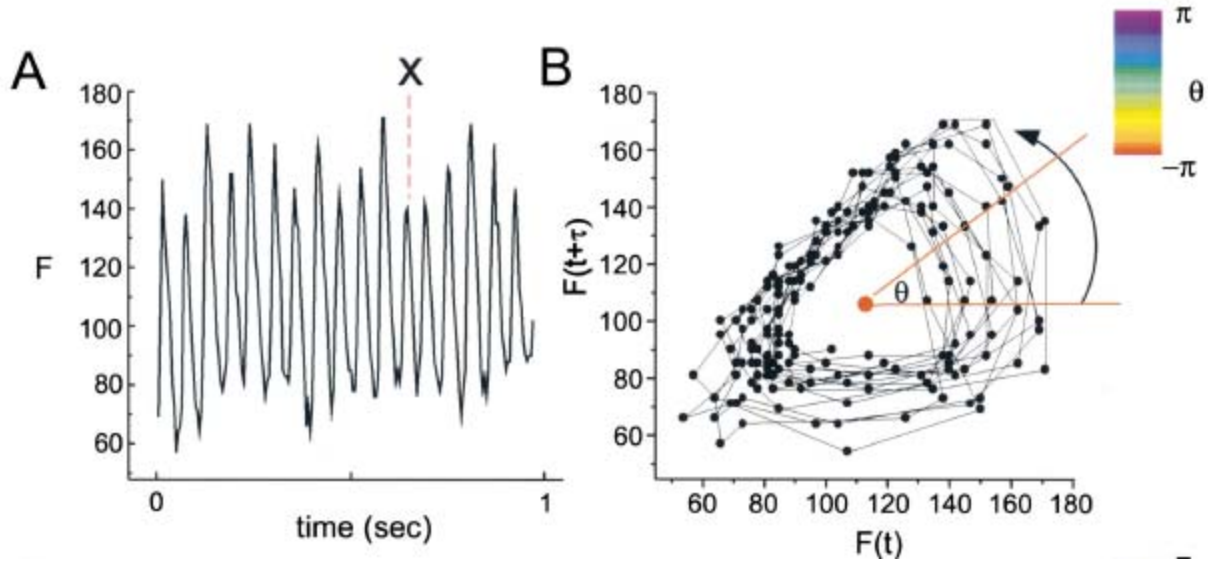



17
23
26
28
34
5 mm
A
A
A
A
A
5 mm
AP phase gradient
Ca$_i$T phase gradient

Phase analysis

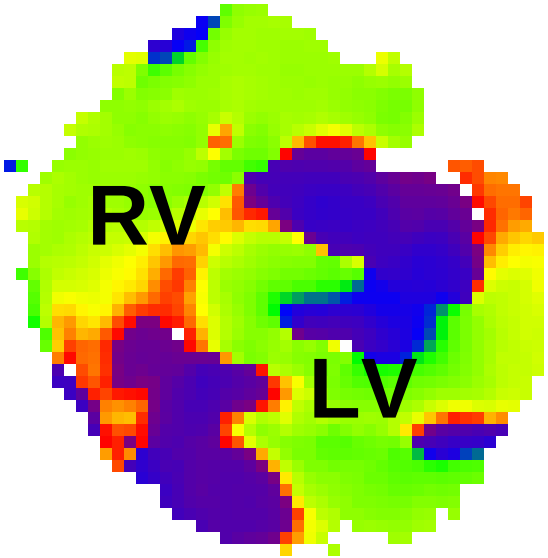


Phase analysis

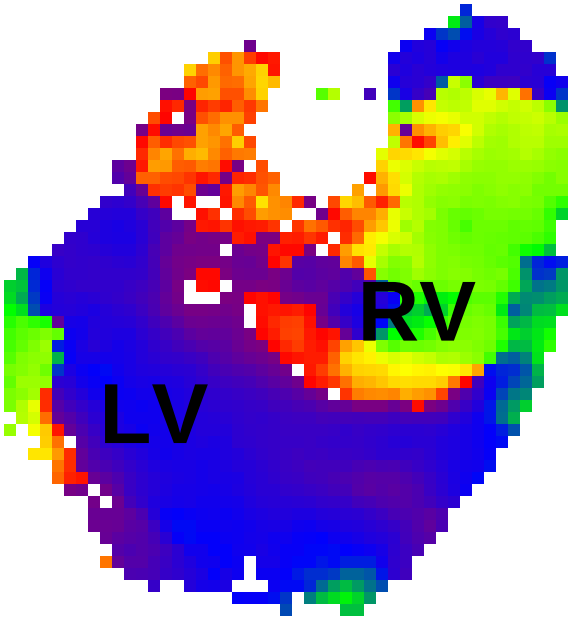


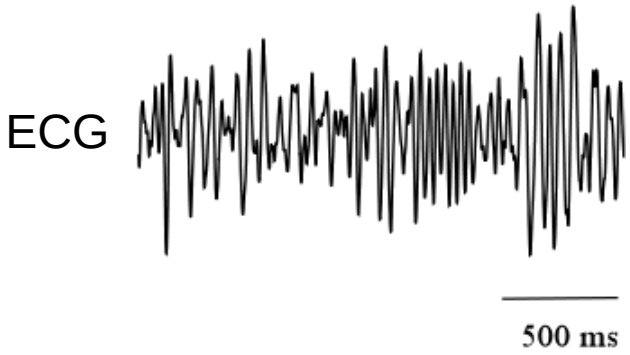
Phase analysis

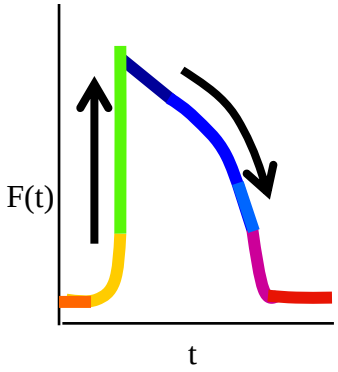
Anterior ventricular wall



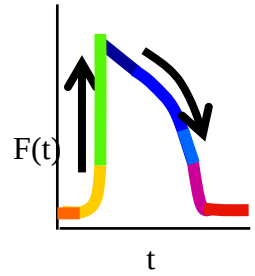
Posterior ventricular wall





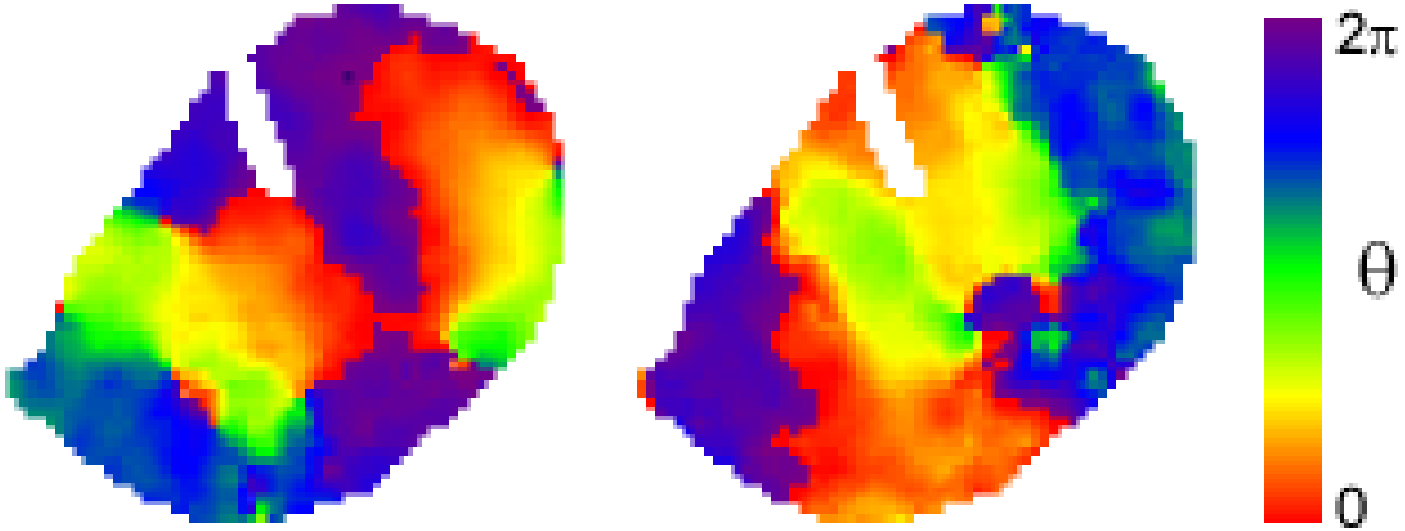


Exercise #5 (singularity points/wave-break)

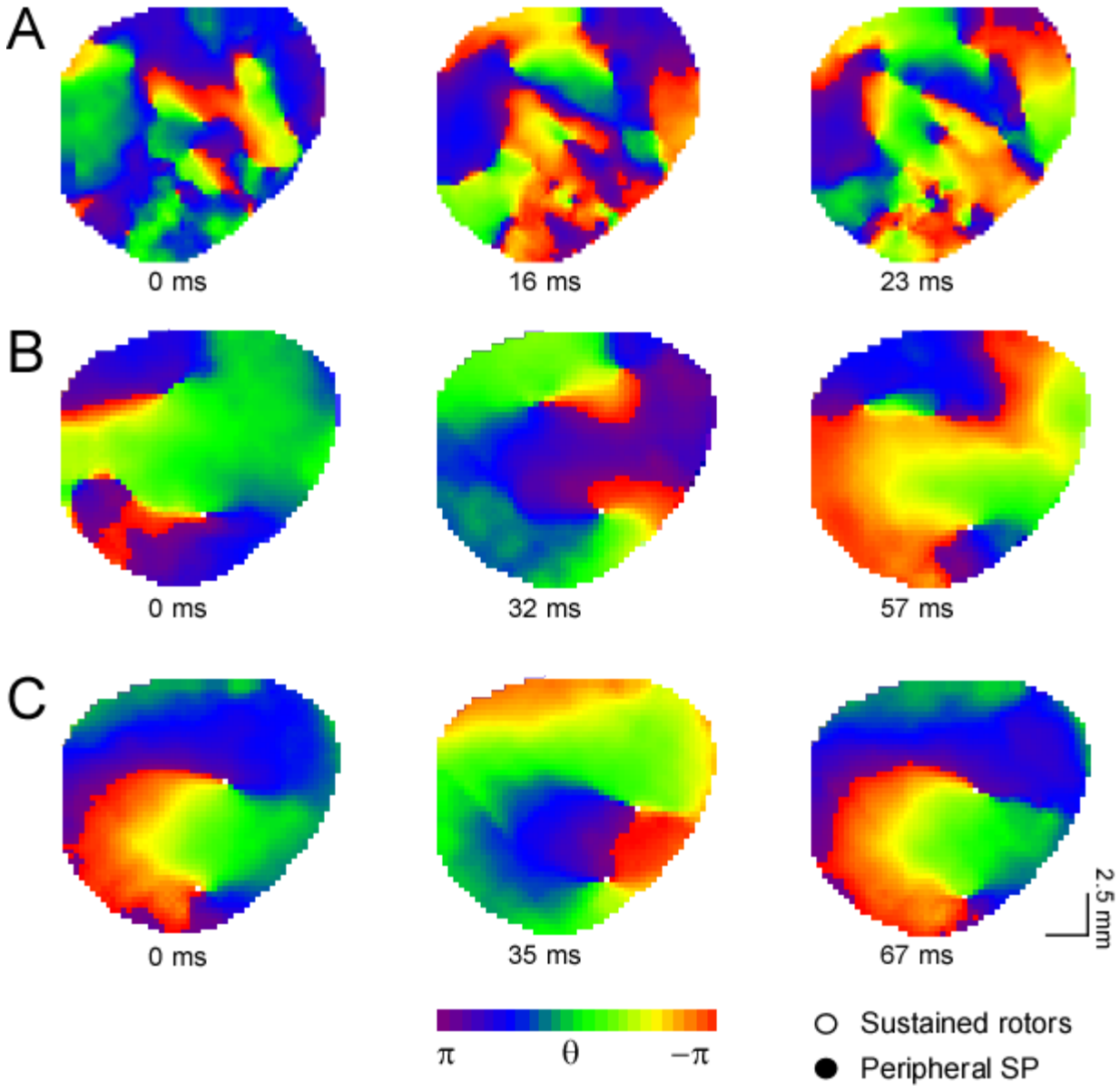


Experimental condition #1

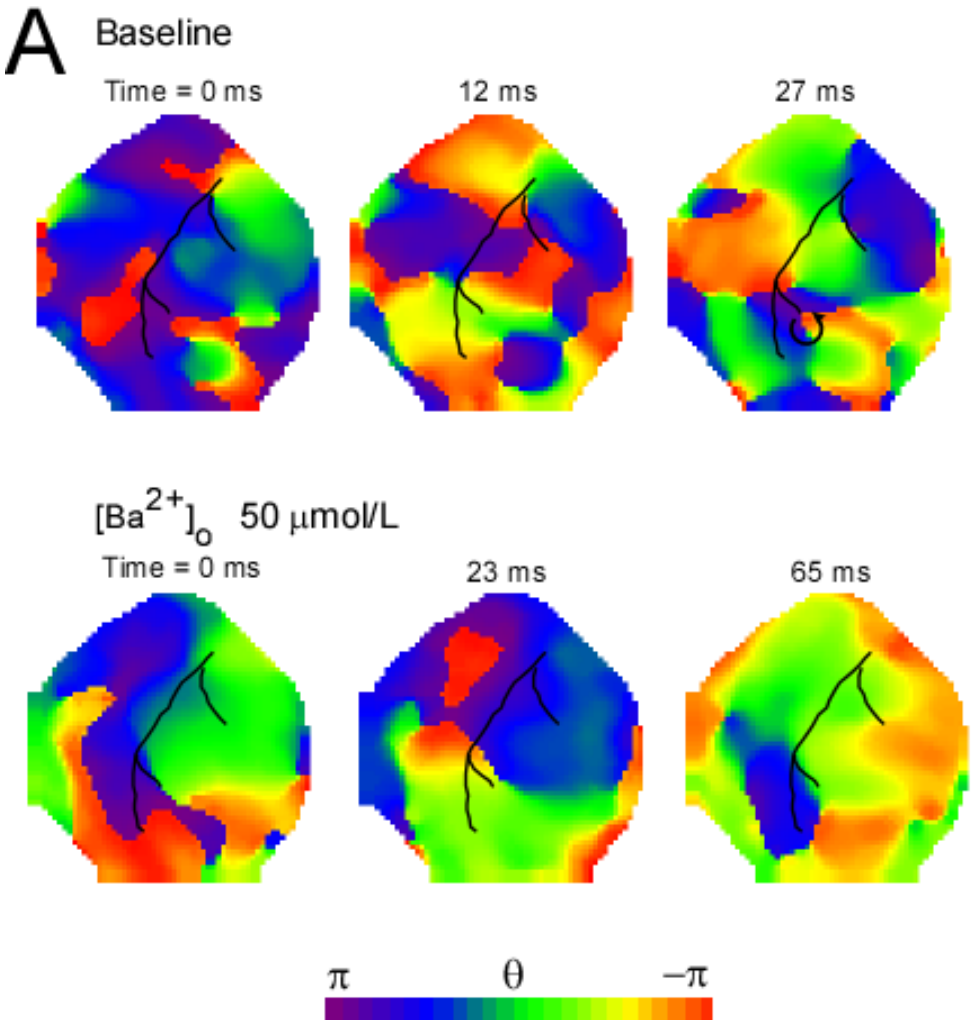
Experimental condition #2

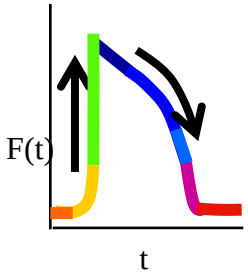


Exercise #5 (singularity points/wave-break)



Exercise #5 (singularity points/wave-break)

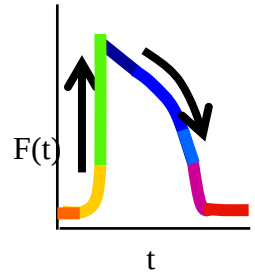


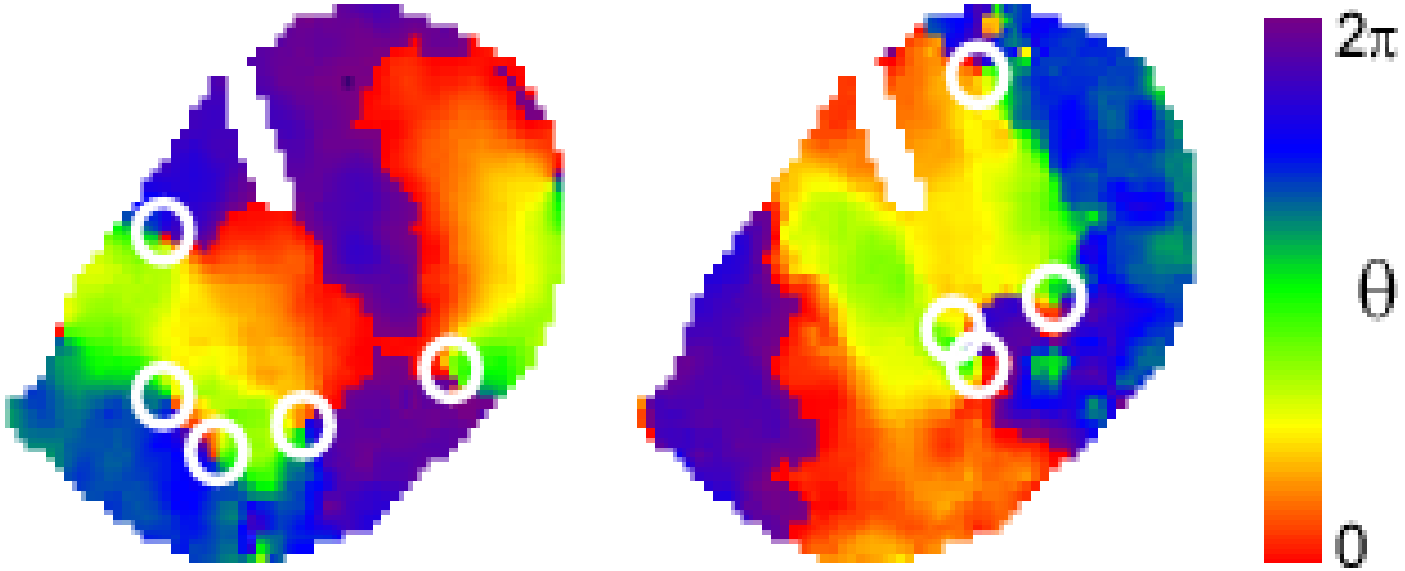


Exercise #5 (singularity points/wave-break)

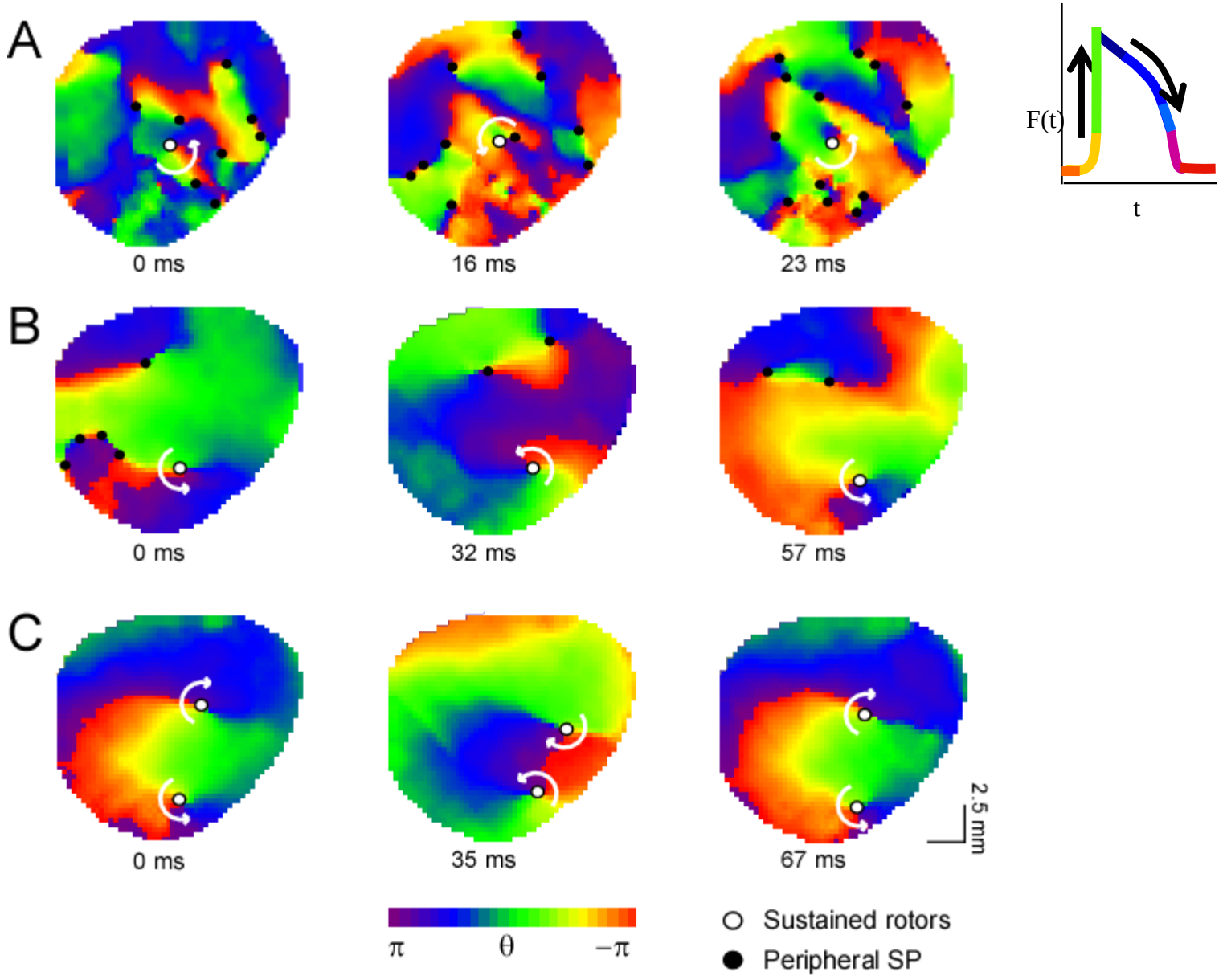
Experimental condition #1

Experimental condition #2

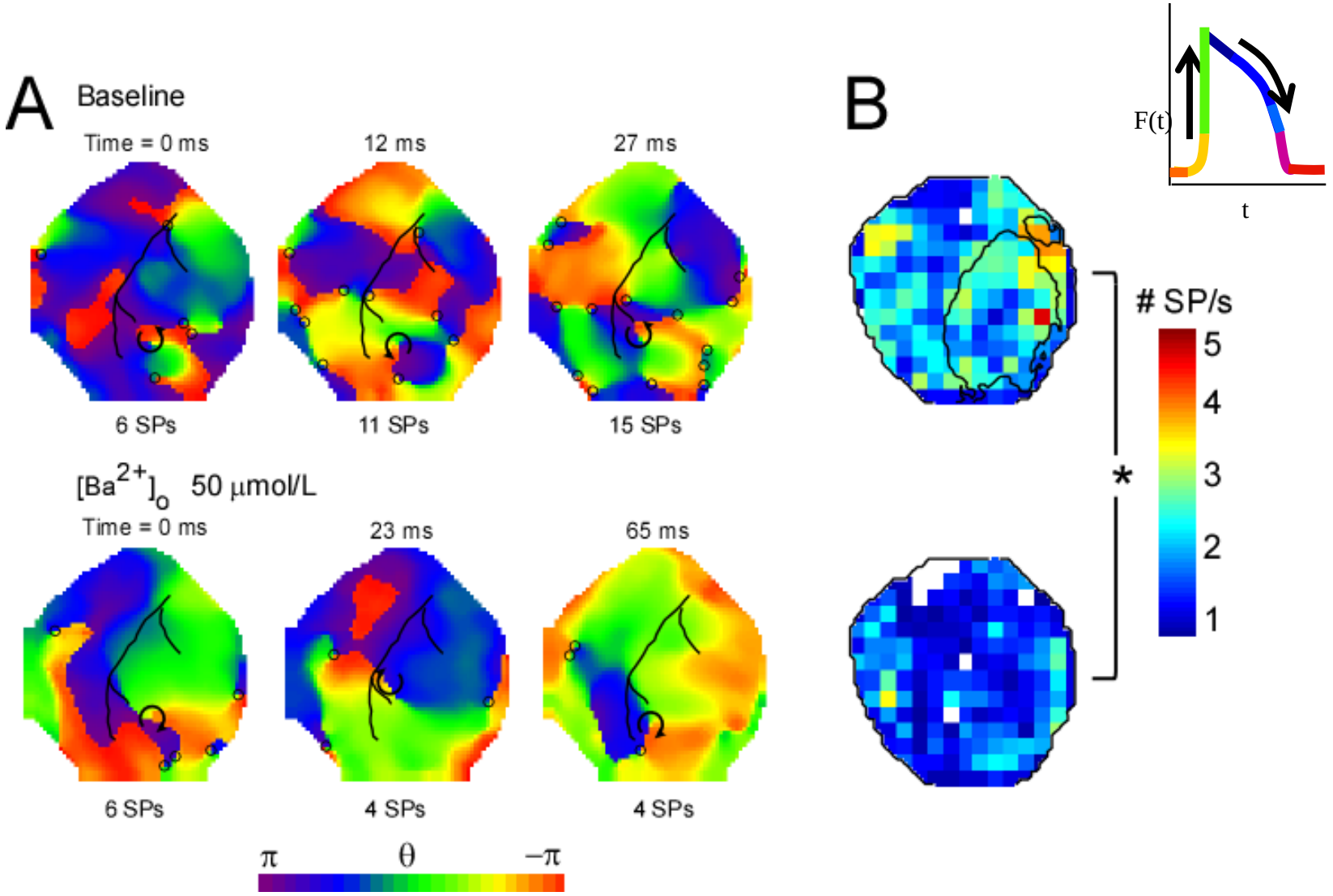




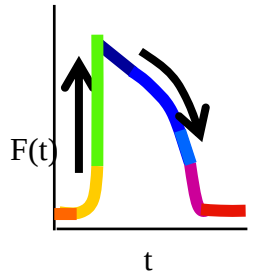
Exercise #5 (singularity points/wave-break)

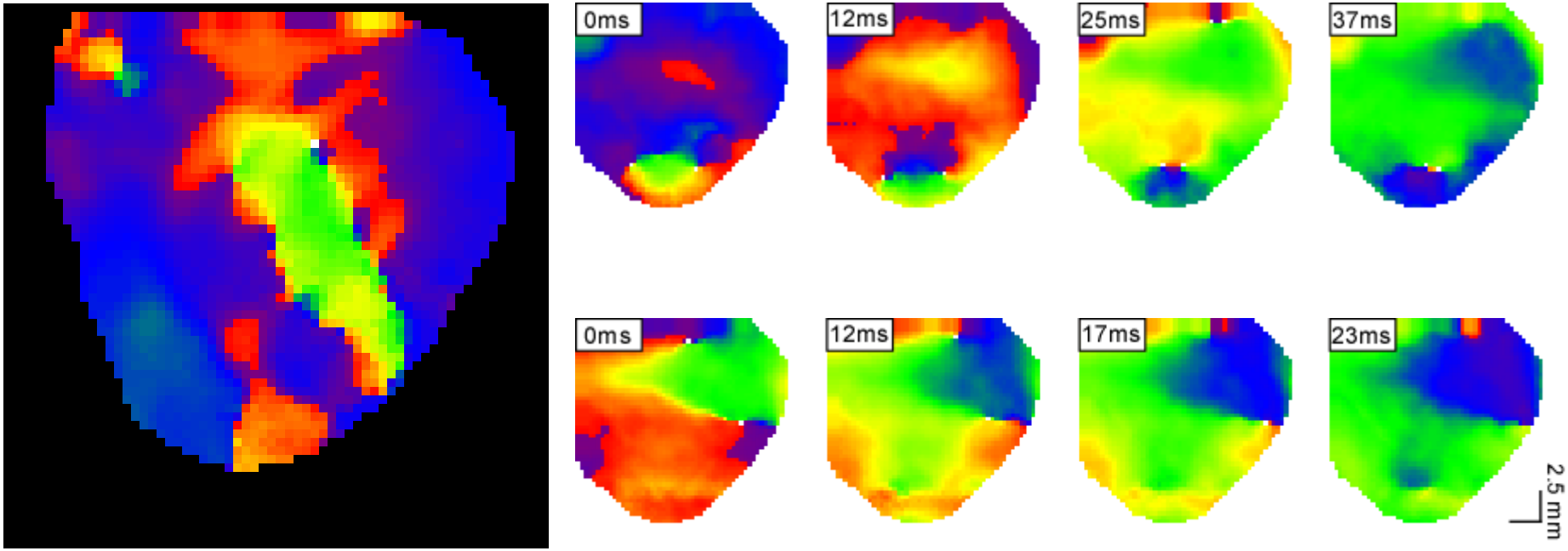


Exercise #5 (singularity points/wave-break)

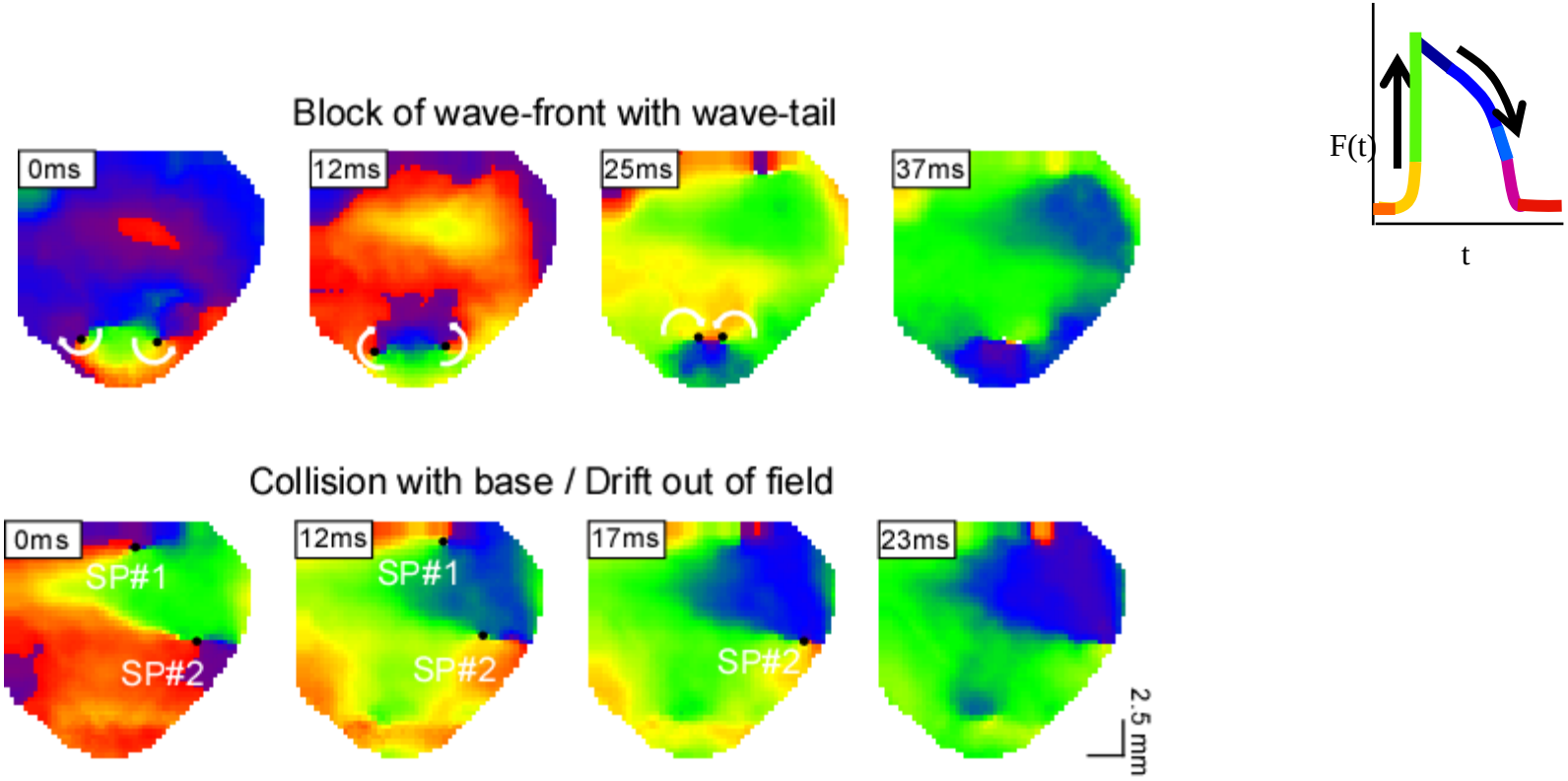


Exercise #6 (progression of phase)





Exercise #6 (progression of phase)



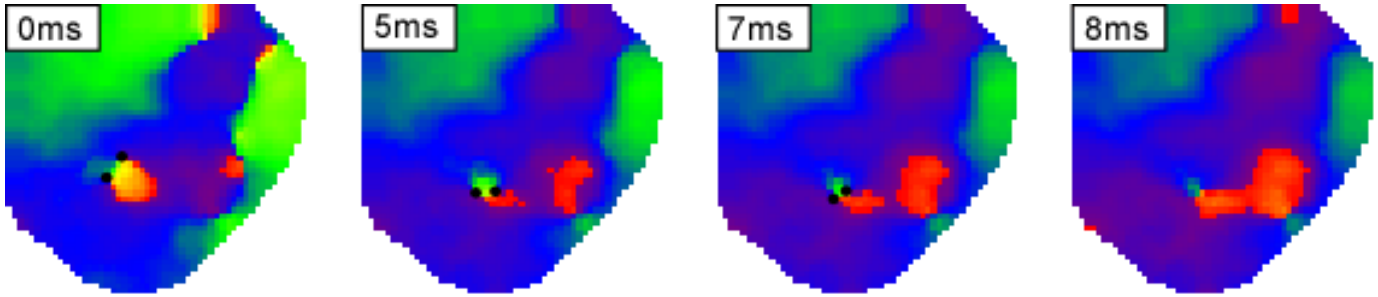
Some standard terminology: spiral wave; rotor; core; filament; wavelet; wave-break; singularity points; chirality of a spiral wave; pivoting point of reentry; counter-rotating spirals (also known as figure of 8); breakthrough; wave-front; wave-tail; conduction block; conduction failure.

Homework *

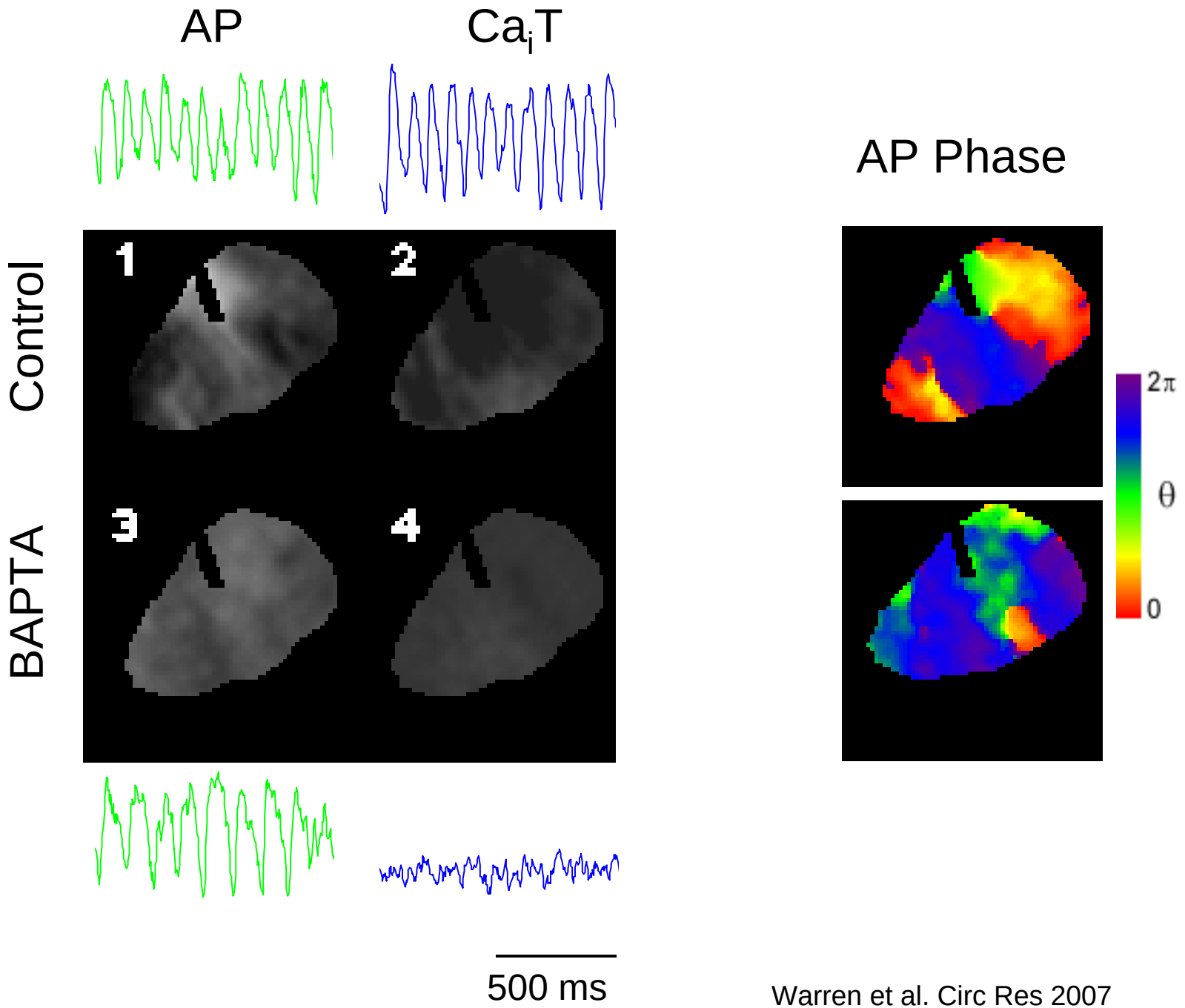
1- A) Perform a literature search to determine experimental values of conduction velocity (CV) and of action potential duration (APD) and/or effective refractory period (ERP) in the human and guinea pig myocardium. B) If possible find values of the aforementioned parameters for more than one cycle length. C) Calculate the wave wavelength associated to these values. D) Does the wavelength depend on the cycle length and how; on the species and how. E) Provide details regarding the type of tissue (i.e. atrial, ventricular, etc), regarding the types of heart (for ex. if they had disease, age, etc), as well as the experimental conditions under which measures were taken (for ex. cycle length or type of protocol used for the measure). F) Provide the reference/s and the keywords used for the search.

2- Construct an activation map from the movie of Di-4-ANEPPS fluorescence depicting one cycle of a reentrant excitation wave measured in the guinea pig anterior ventricular wall during perfusion with a high potassium solution. The 64x64 pixel and 45 frame long movie was recorded at a temporal resolution of 600 frames/s. Use a threshold of 50% as indicative of activation. Try one additional threshold value of your choice and observe the differences in the resulting activation map.

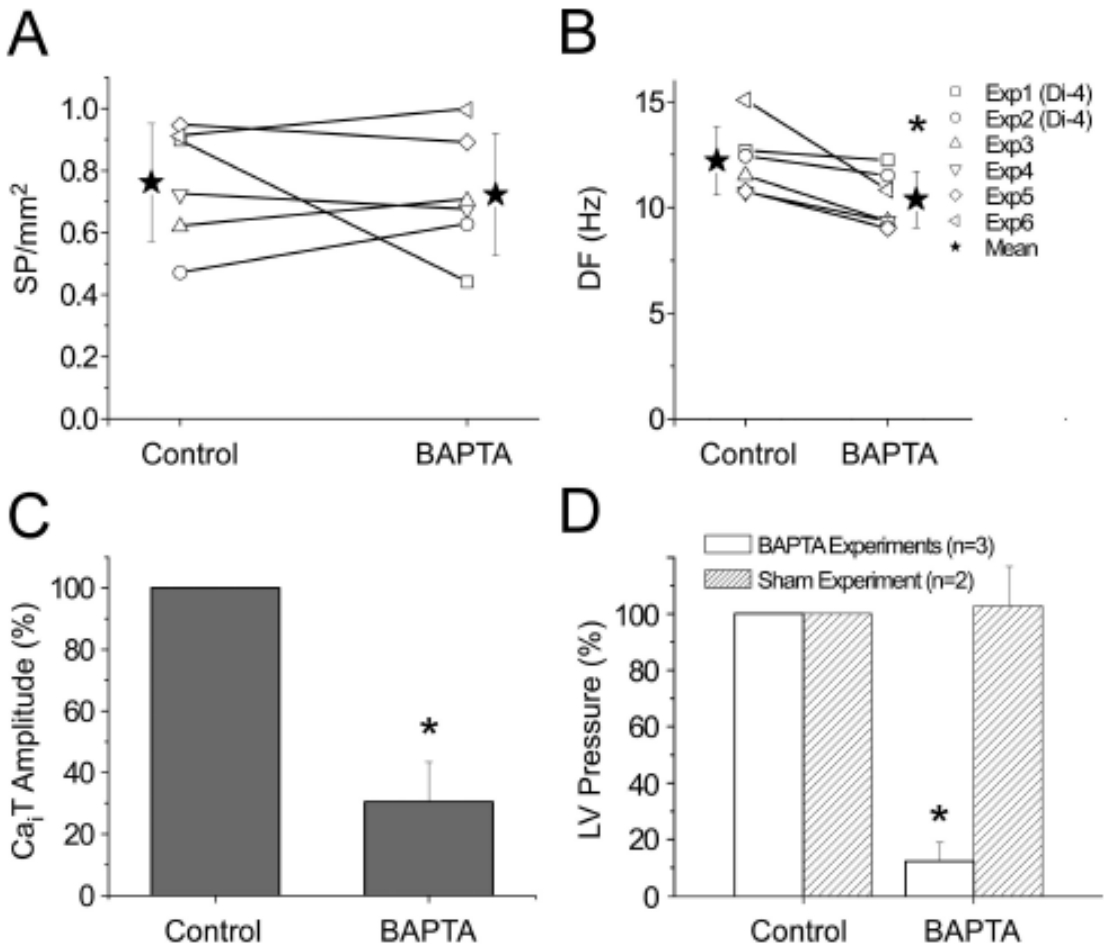
3- Describe as precisely as possible the sequence of events shown in the phase snapshots (see below). Suggest a possible mechanism by which the singularity points/wavebreaks were extinguished.



* Exercises 1 and 3 should be done individually. Exercise 2 can be optionally done in pairs.



Warren et al. Circ Res 2007



Warren et al. Circ Res 2007

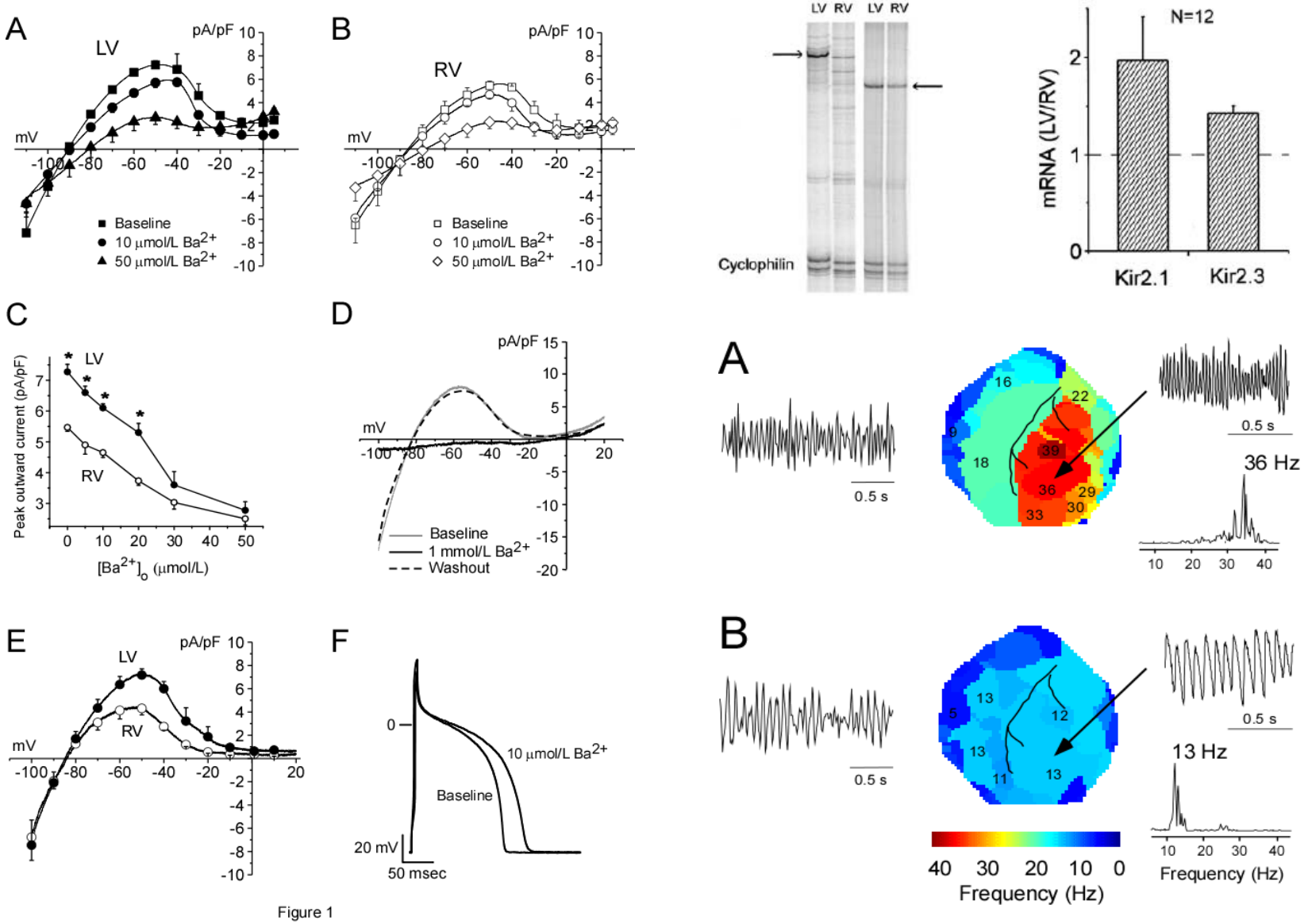


Figure 1

Warren et al. JCE 2003

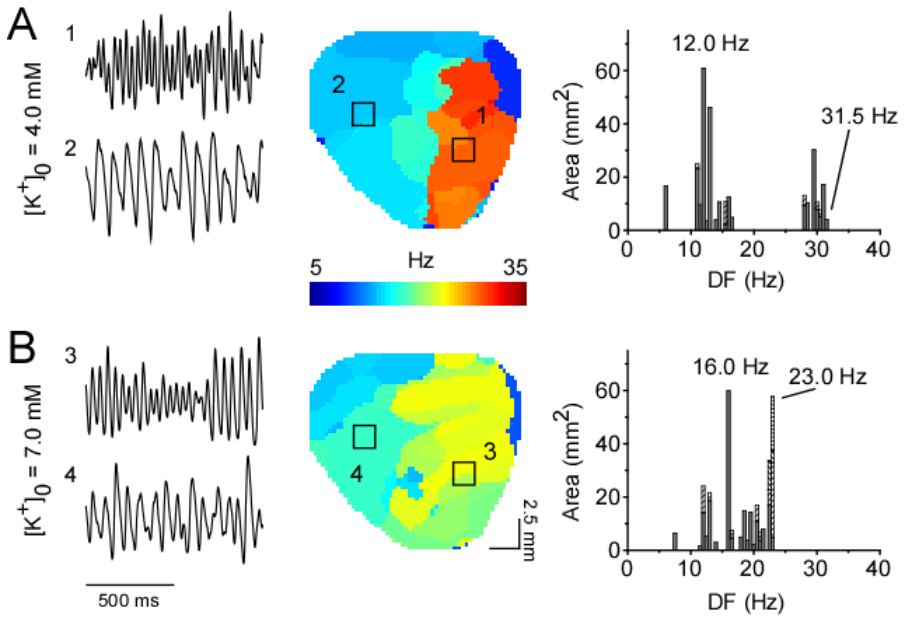


Figure 1

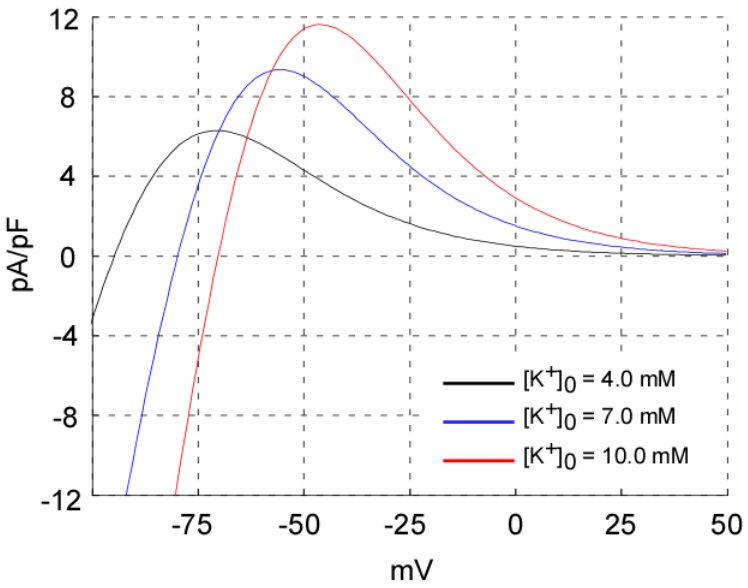
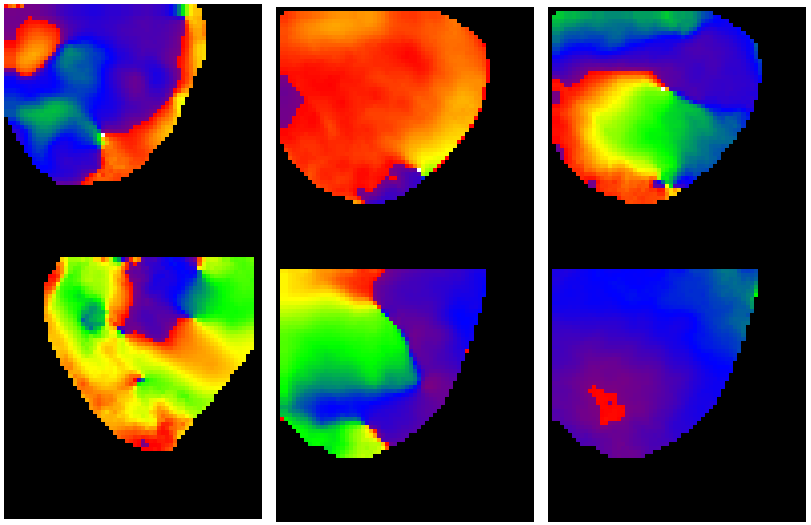


Figure 3S



Pandit et al. Biophys J 2010