

Bioeng 6460  
Electrophysiology and Bioelectricity

Microstructural Basis of Conduction II  
Introduction to Experimental Studies

Frank B. Sachse  
fs@cvrti.utah.edu



# Overview

- Microstructural Basis of Conduction
  - Fibroblasts
    - Arrangement
    - Differentiation
    - Role in Electrophysiology
- Introduction to Experimental Studies
  - Whole Heart
  - Tissue Preparations
  - Cell Culture
- Summary



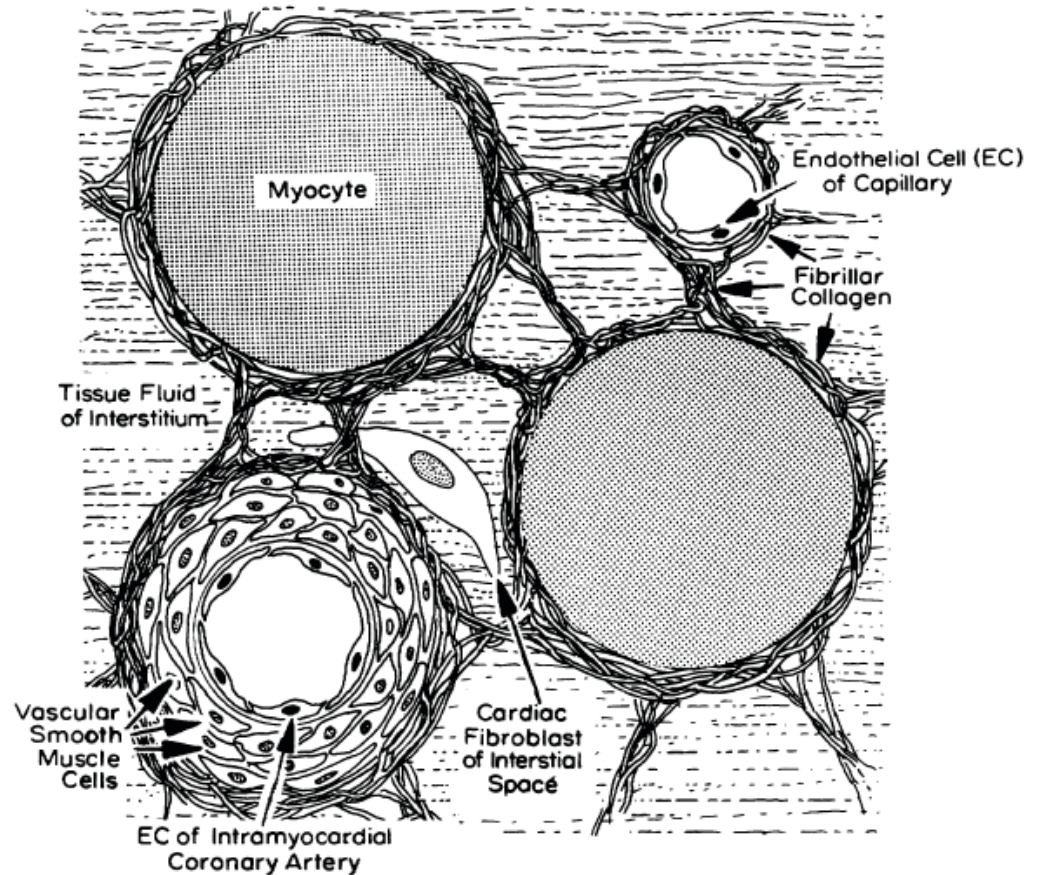
Group work



Group work

# Fibroblasts

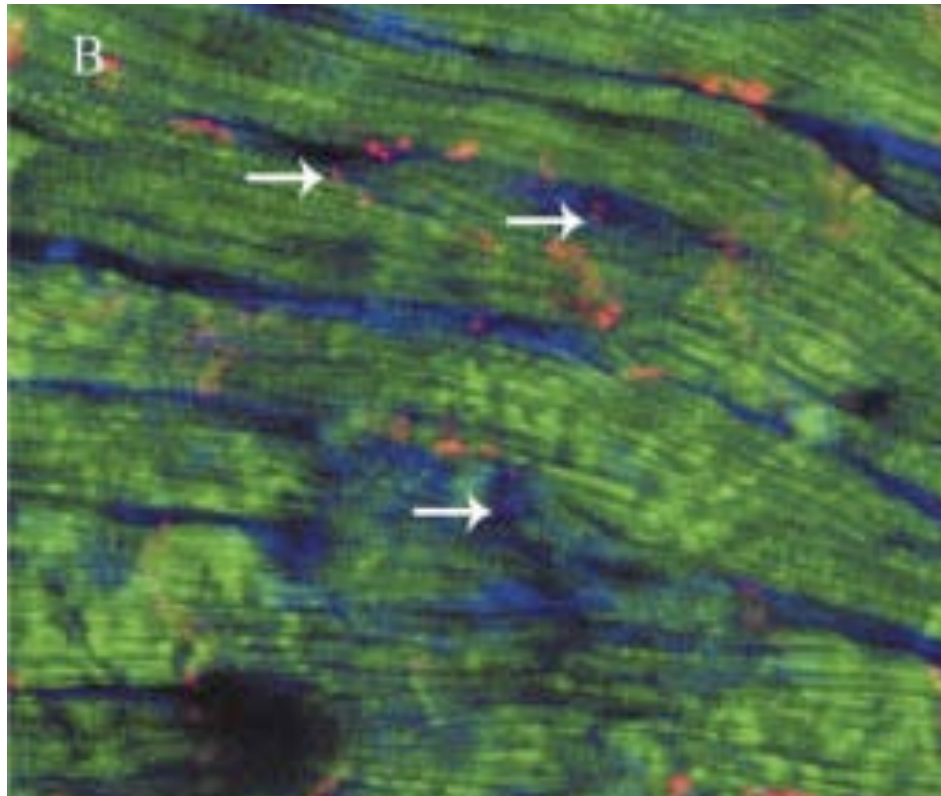
- most numerous cells in myocardium
- primary producer of extracellular matrix proteins
- change of phenotype in response to pathological conditions, eg infarction, inflammation and injury
- chronic activation can lead to fibrosis, hypertrophy and heart failure
- spatial organization in tissue still topic of research
- role in electrical conduction is still not completely understood



(K. T. Weber and C. G. Brilla, *Circ*, 1991)



# Fibroblast Organization in Rat Neonatal Myocardium



**Discoidin domain receptor (DDR) - Fibroblasts**

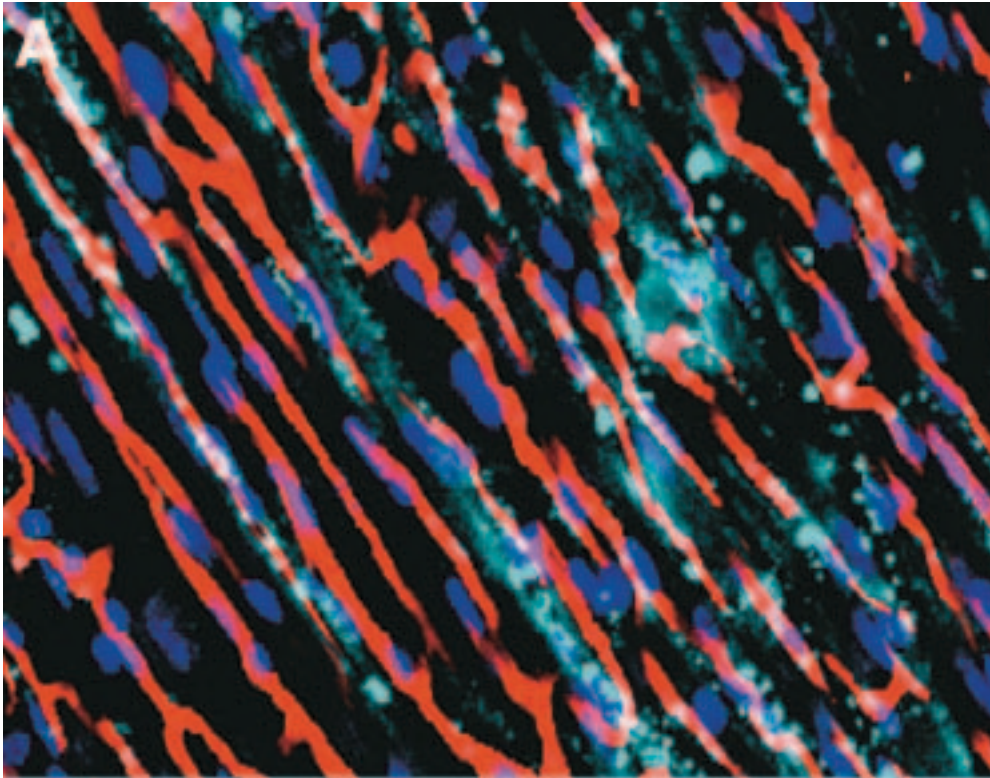
**Actin - Myocytes**

**Cx43 - Gap Junctions**

**Arrows indicate gap junctions of fibroblasts**

(E. C. Goldsmith et al, Develop Dyn 2004)

# Fibroblasts in Mouse Ventricular Myocardium



Fluorescent microsphere in  
blood vessels

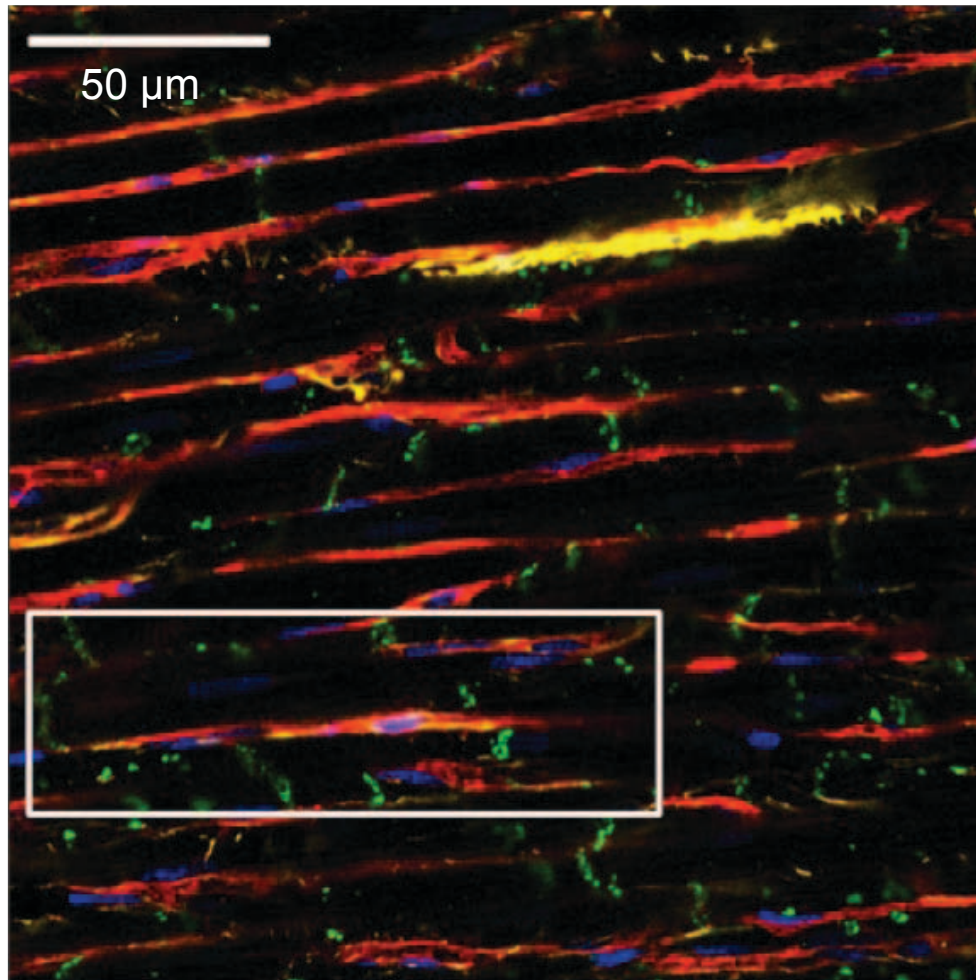
DAPI – Nuclei

DDR - Fibroblasts

(Sounders et al, Circ Res, 2009)



# Fibroblasts in Normal Rat Ventricular Tissue



WGA – Extracellular space

DAPI – Nuclei

Cx43

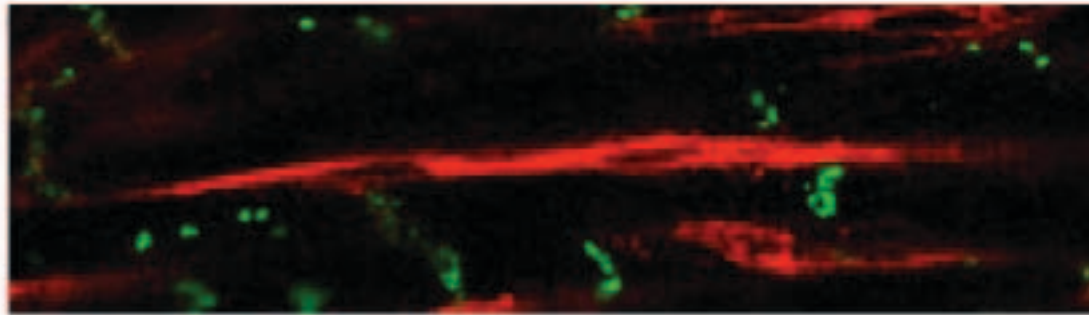
Vimentin - Fibroblasts

(M. Arp et al, Biomed Tech, 2011)



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## Fibroblasts in Rat Ventricular Tissue (Zoom)

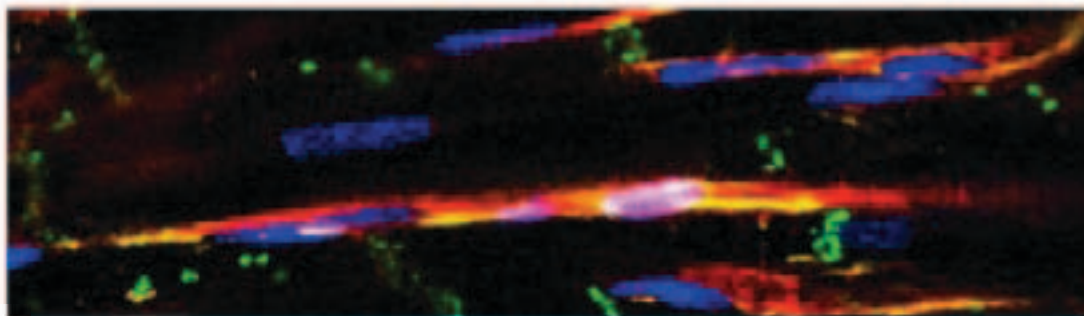
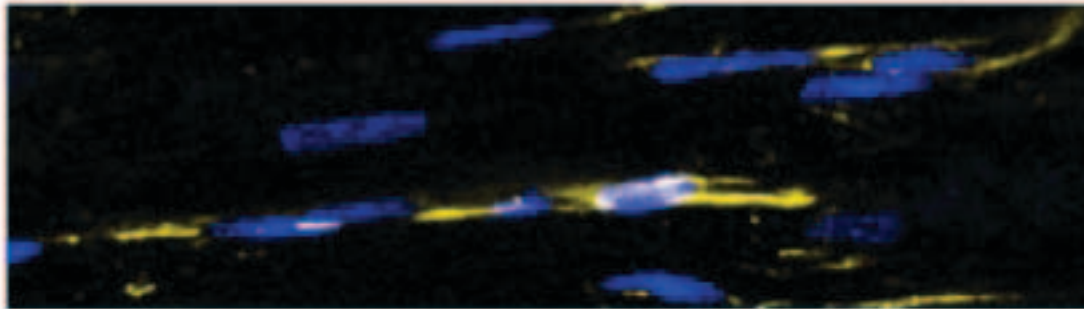


WGA – Extracellular space

DAPI – Nuclei

Cx43

Vimentin - Fibroblasts

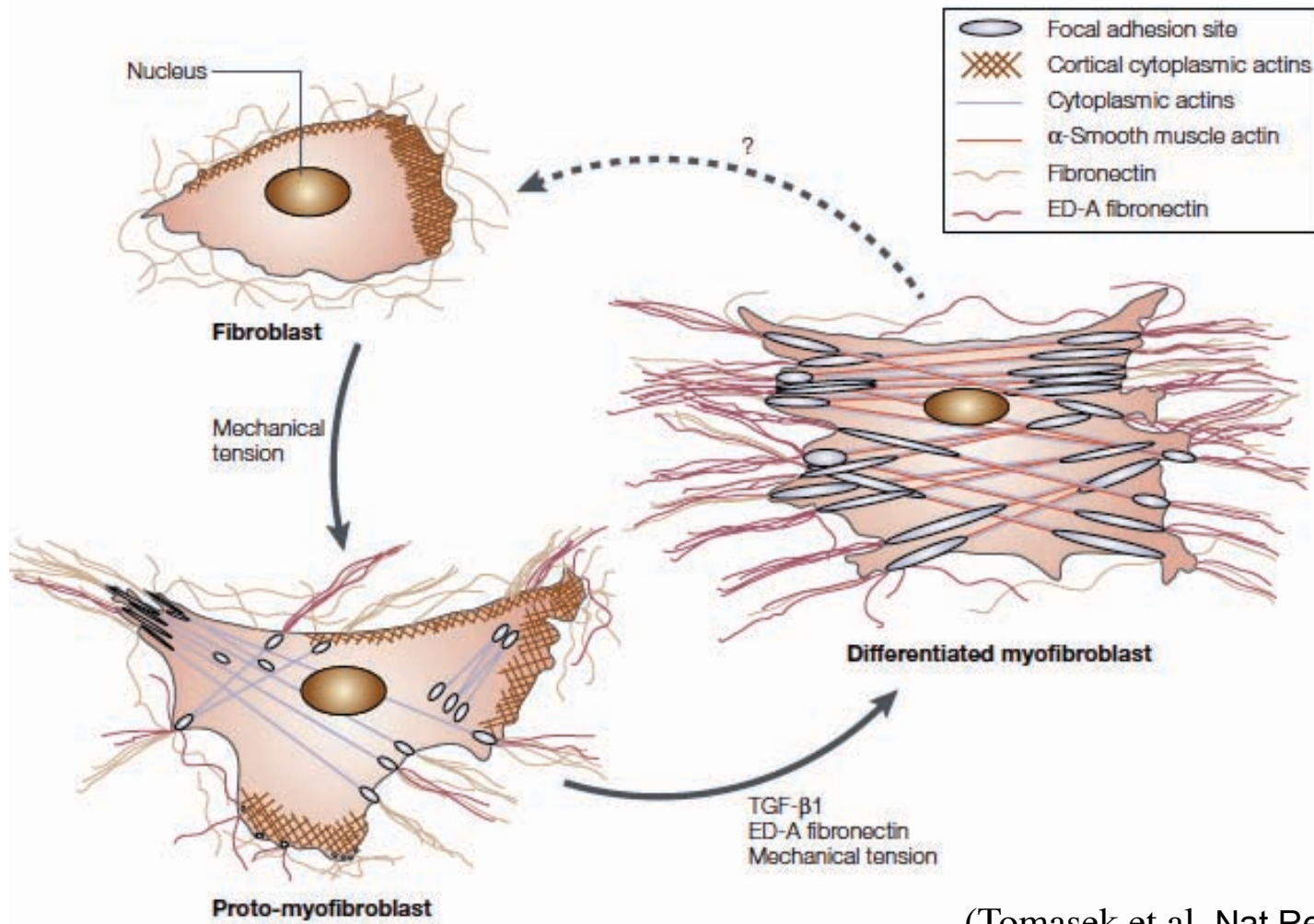


(M. Arp et al,  
Biomed Tech, 2011)



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# Fibroblast Differentiation



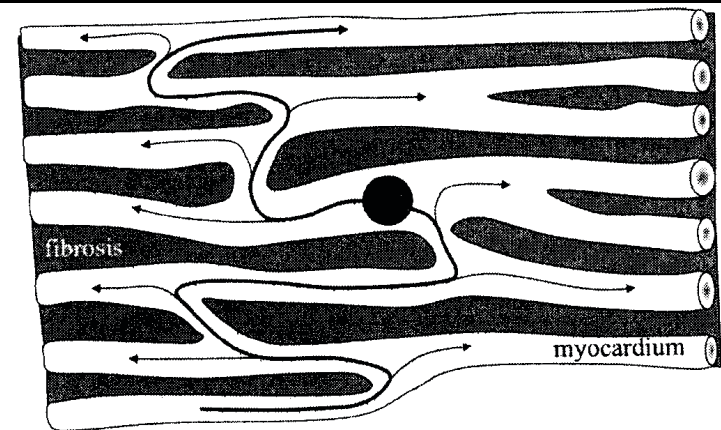
(Tomasek et al, Nat Rev, 2002)



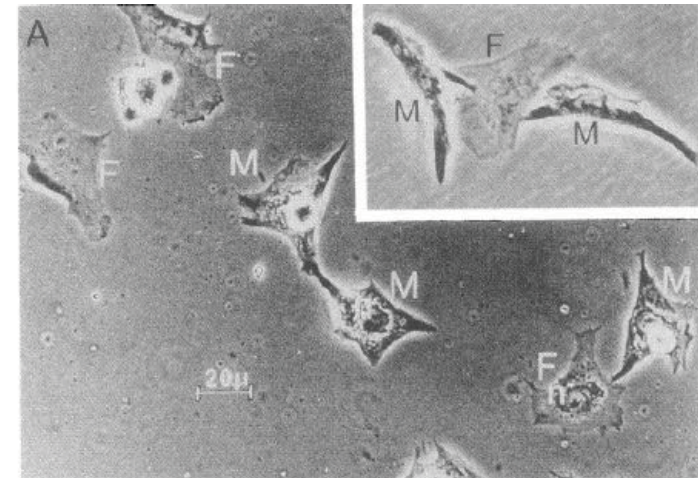


# Role of Fibroblasts in Electrophysiology

- electrically inexcitable
- passive role
  - septa due to fibrosis
  - reduced volume fraction of myocytes
  - reduced lateral coupling
- active role
  - electrical myocyte-fibroblast coupling via gap junction channels
  - electrical bridging of myocytes in culture: over distances up to 300 $\mu$ m (G. Gaudesius et al, Circ Res 2003)
  - additional sink or source for activation of myocytes
- role dependent on phenotype of fibroblast



(Jong et al, J Cardiovasc Pharm, 2011)



(Rook et al, Am J Physiol, 1992)

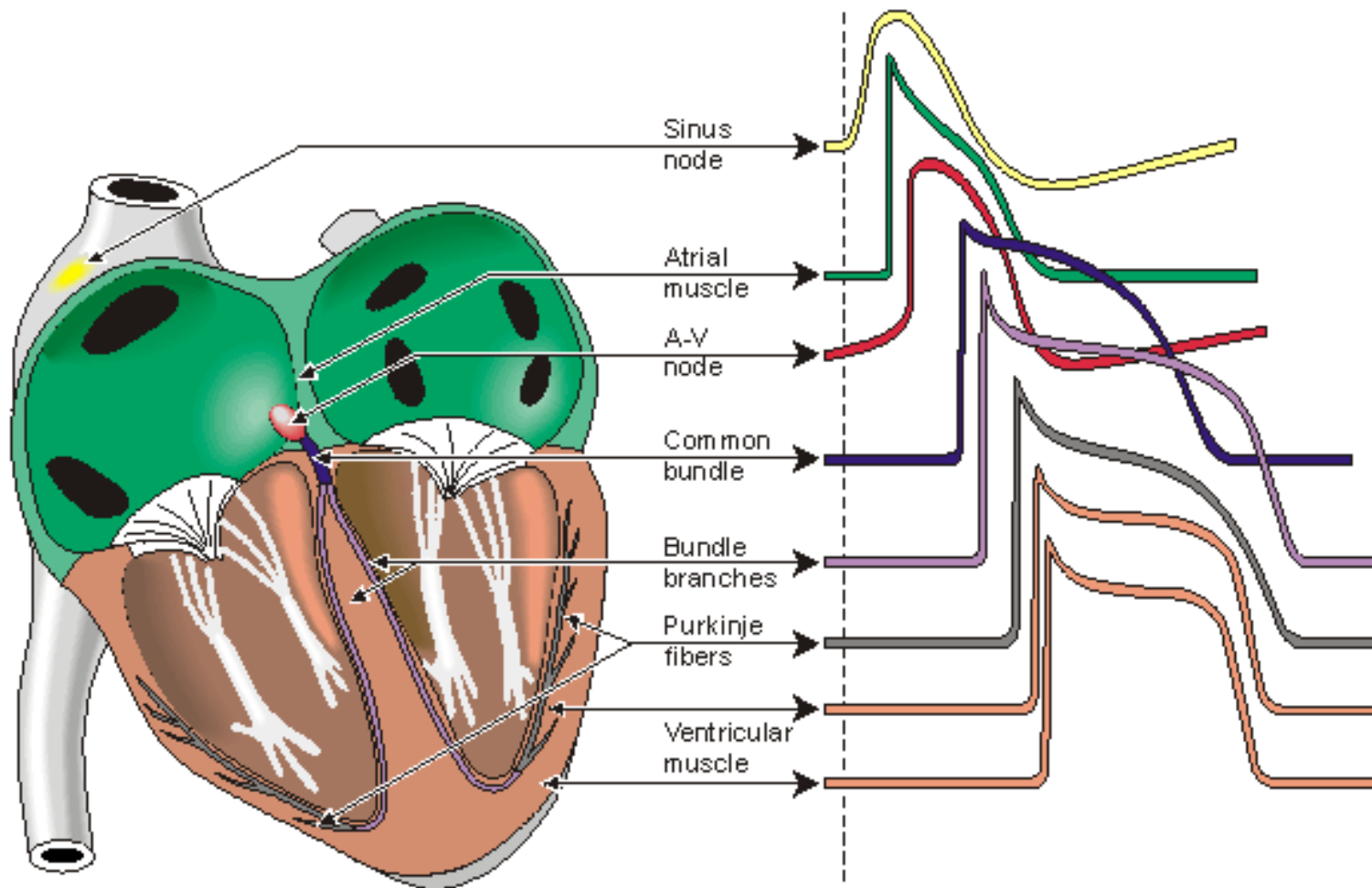
## Group Work

Discuss effects of fibrosis on extracellular electrograms.

Speculate on effect in the near (interstitial space) and far field (ECG).



# Electrical Signaling in the Heart



(from Malmivuo and Plonsey)

# Experimental Studies of Cardiac Electrical Conduction

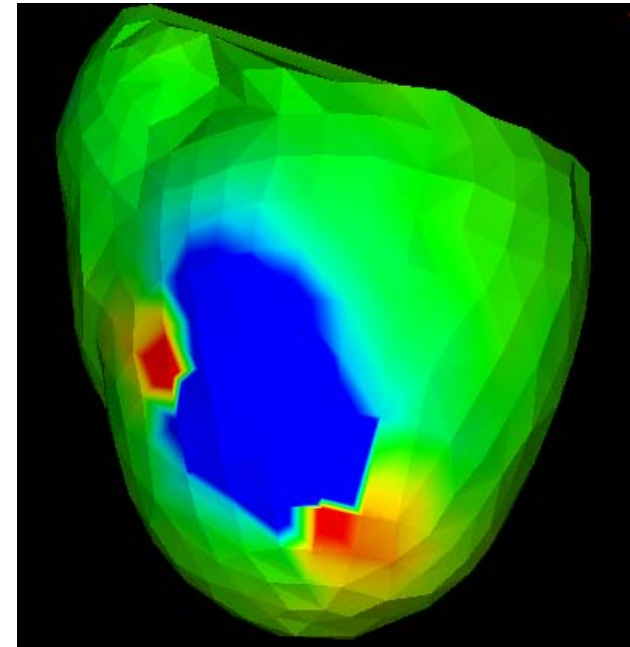
## Measurement methods

- Electrode arrays: Extracellular voltages (similar ECG measurements on body surface)  
Sampling rate up to several kHz  
Channels up to 2000
- Optical: Transmembrane voltages  
CCD-camera  
Photodiode array

## Preparations

- Cell strands - Purkinje fibers
- Small muscles - papillary muscle, trabeculae
- Sections - wedge preparations from ventricles
- Atria/ventricle
- Whole heart

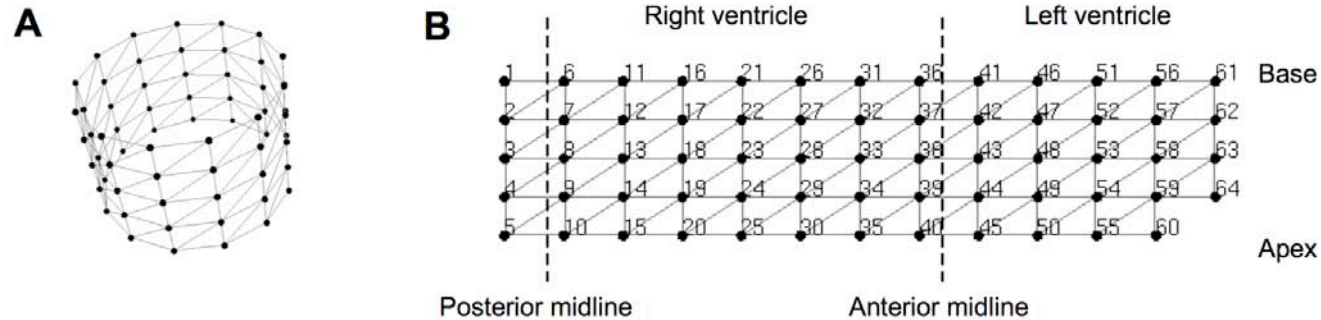
in vivo/in vitro



Color-coded visualization of extracellular voltages measured on surface of canine ventricles



# Epicardial Electrical Mapping System for Mouse Heart



**C**



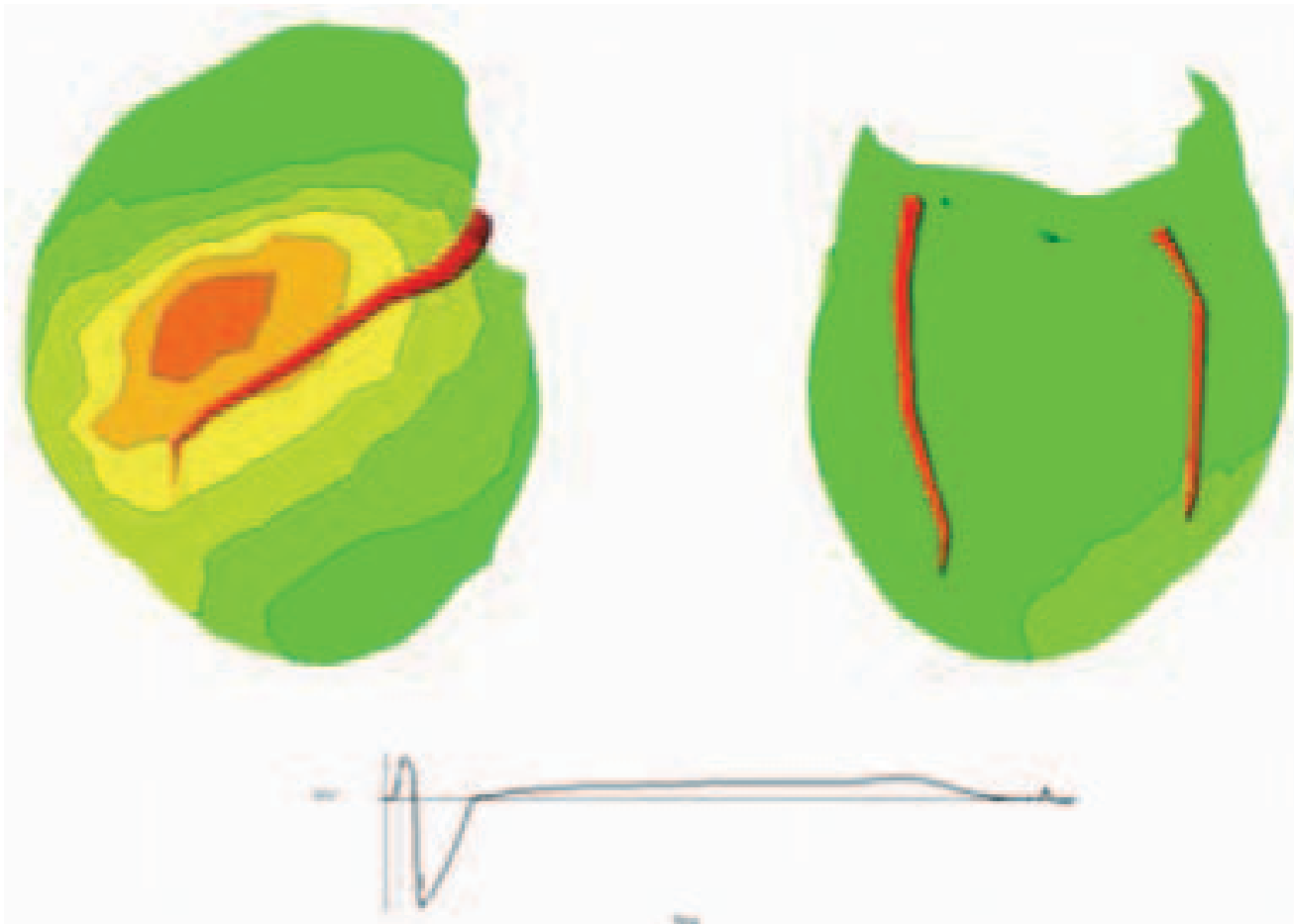
Perfusion line supplies modified Krebs solution through the coronary artery.

Heart, isolated

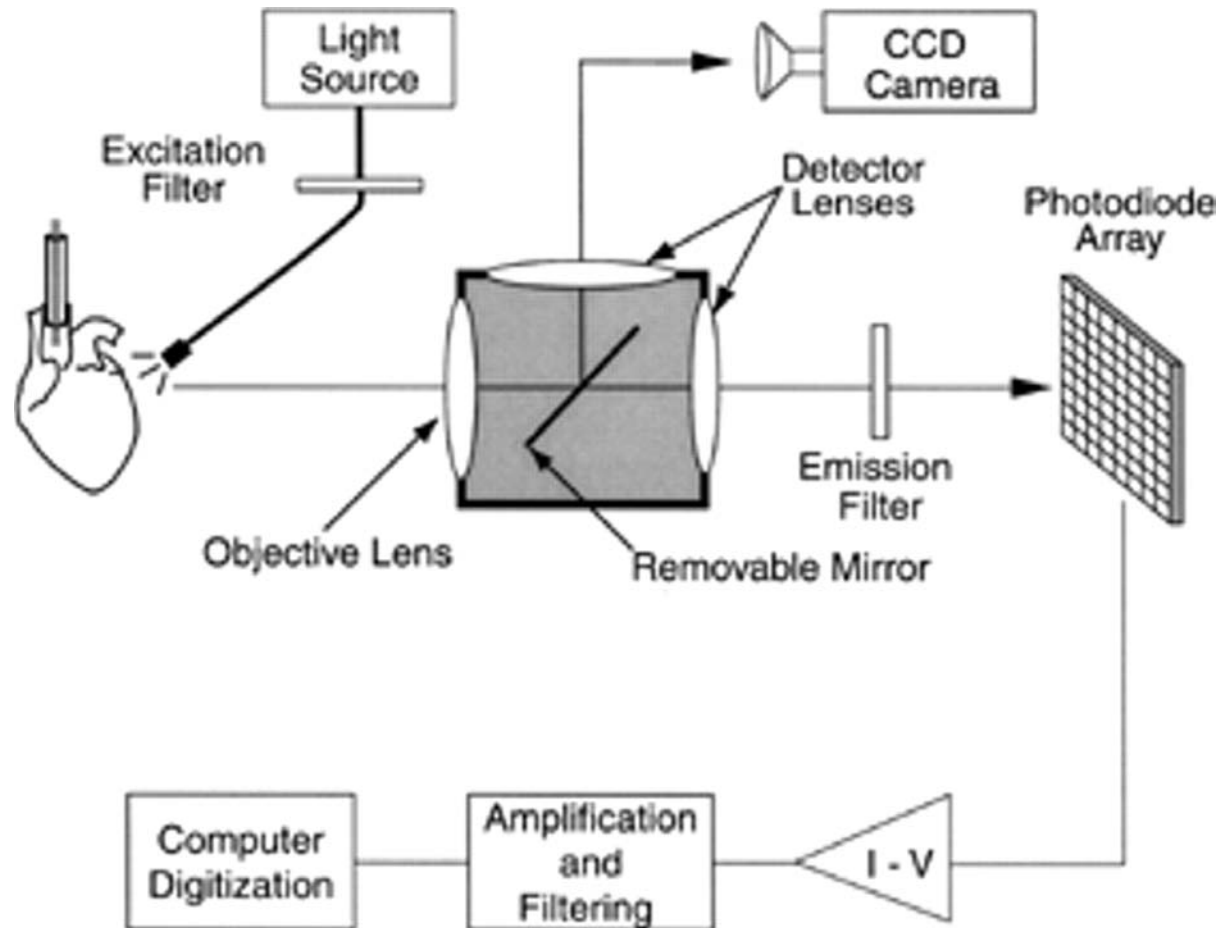
Cylindrical cage electrode array measures the extracellular potentials from the surface of the heart

Sohn et al, IEEE TBME, 2011

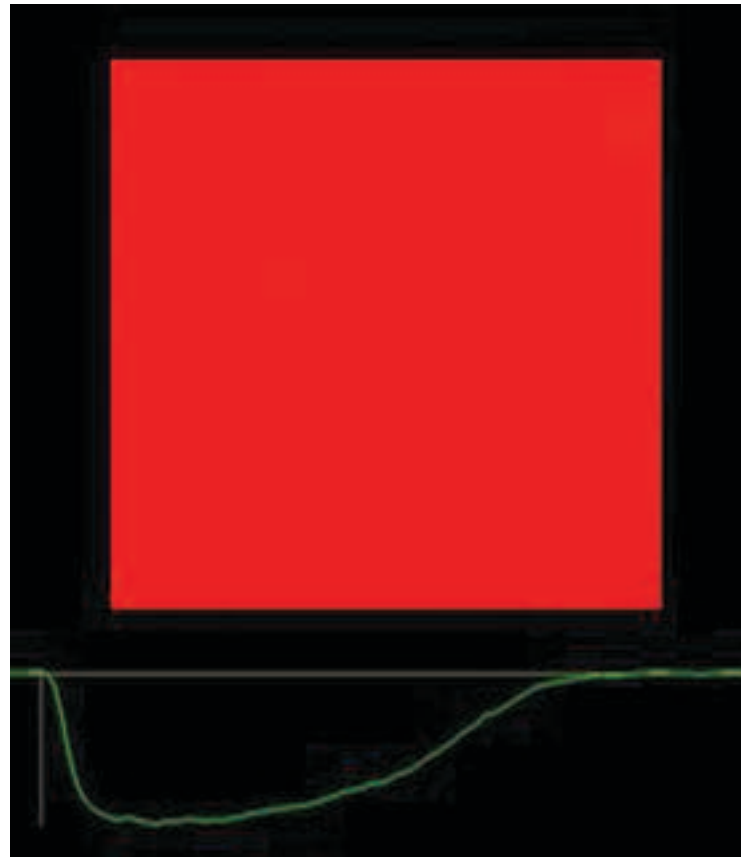
# Electrical Mapping of Canine Ventricles



# Optical Mapping System



# Optical Mapping of Canine Ventricular Area

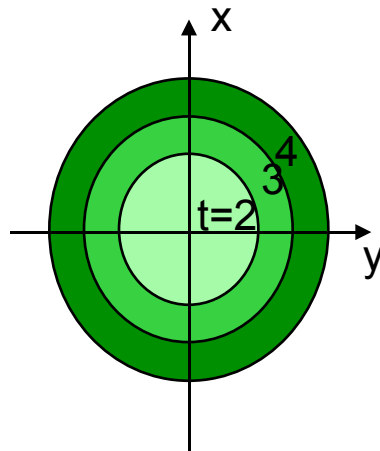




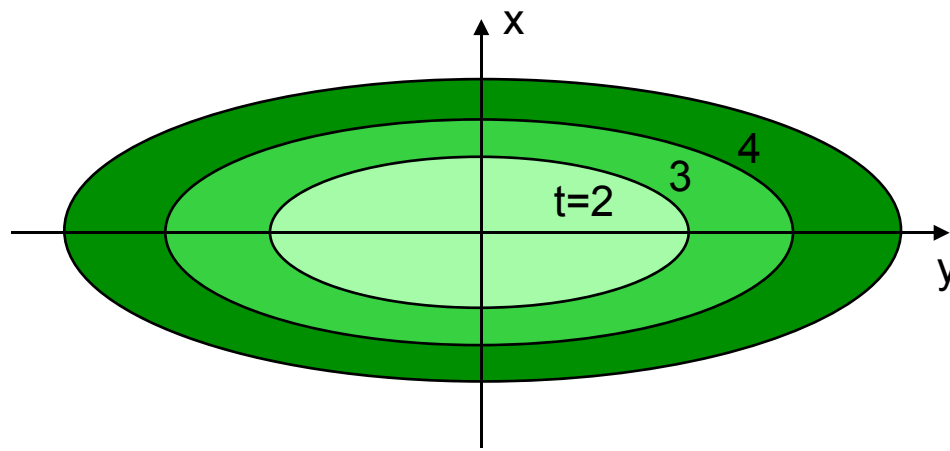
# Isotropic/Anisotropic Propagation of Excitation (2D)

- Long axis of myocytes parallel to y-axis
- Stimulus at point (0,0)

Isotropic x/y - 1/1  
Velocity  $v$ : 1 / s



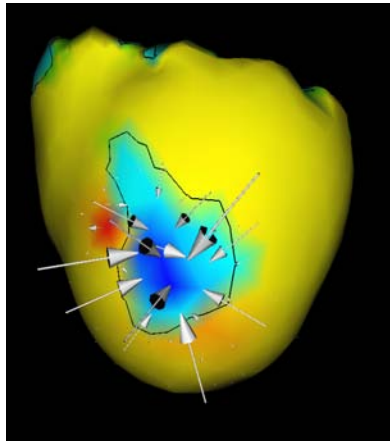
Anisotropic x/y - 1/3  
Velocity  $v_x$ : 1 / s,  $v_y$ : 3 / s



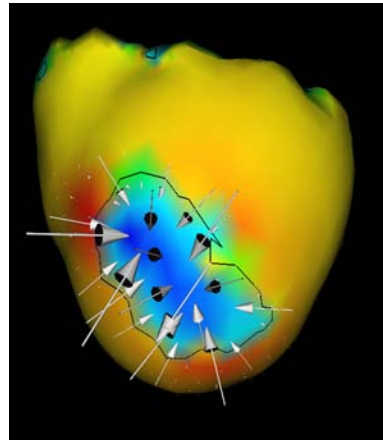
## Simplifications

- Homogeneous tissue
- Neglect of microstructure

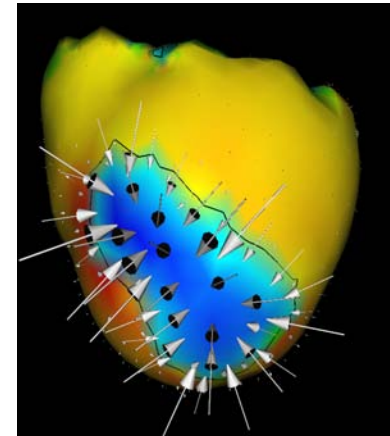
# In-/Outflow of Currents during Excitation



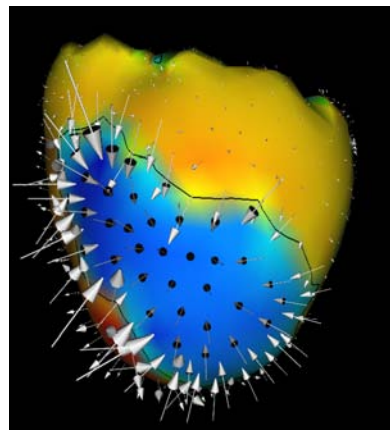
10 ms



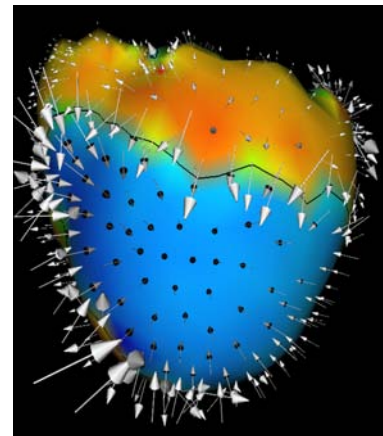
20 ms



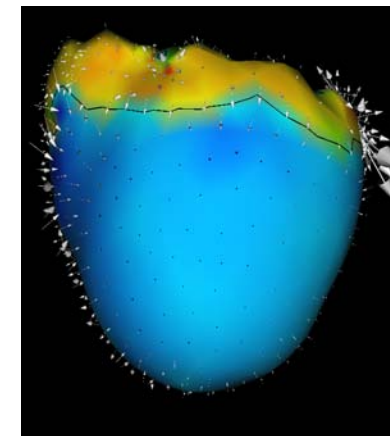
30 ms



40 ms

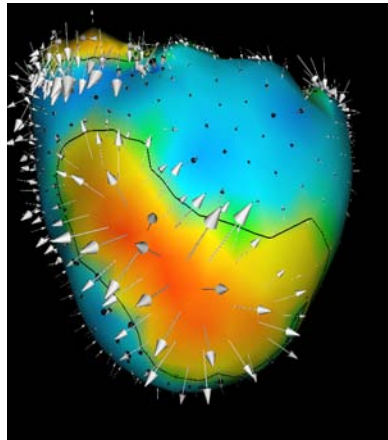


50 ms

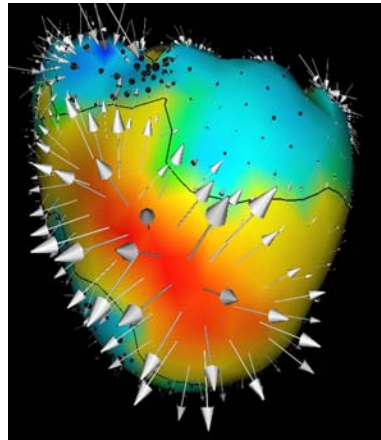


60 ms

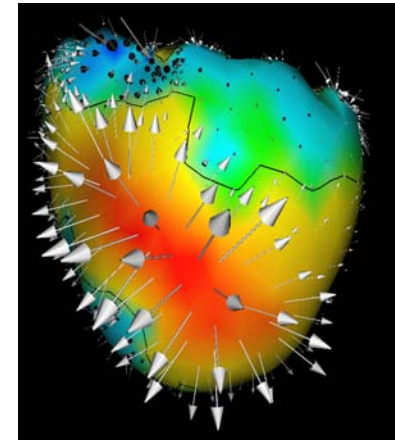
# In-/Outflow of Currents during Repolarization



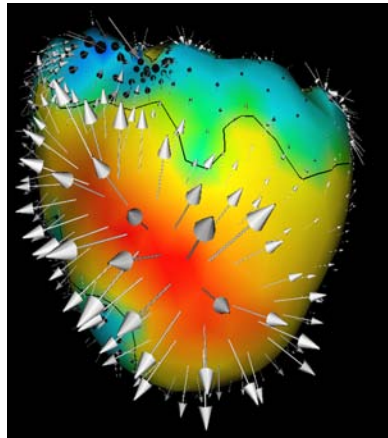
110 ms



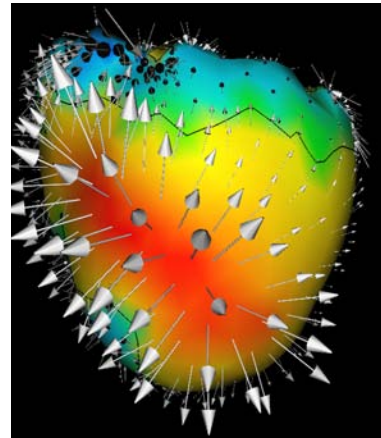
130 ms



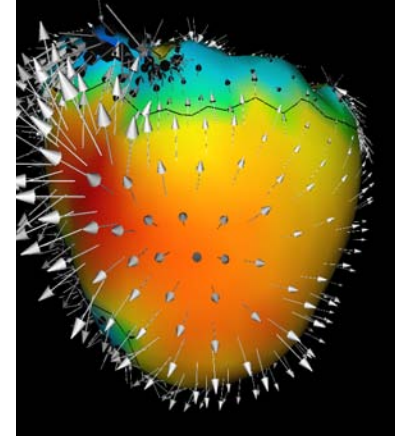
150 ms



170 ms



190 ms

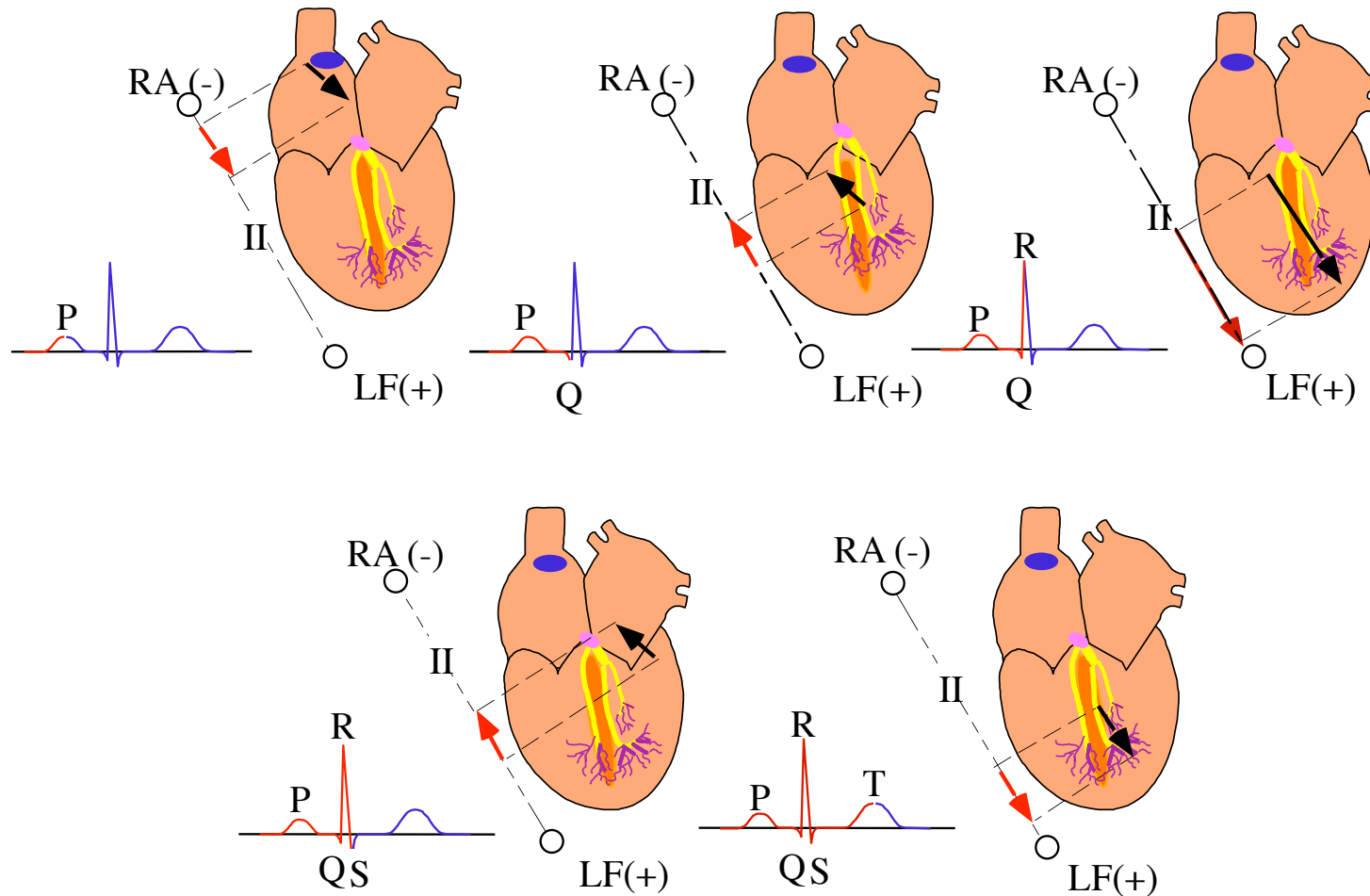


210 ms



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# Dipole Approximation and Surface ECG





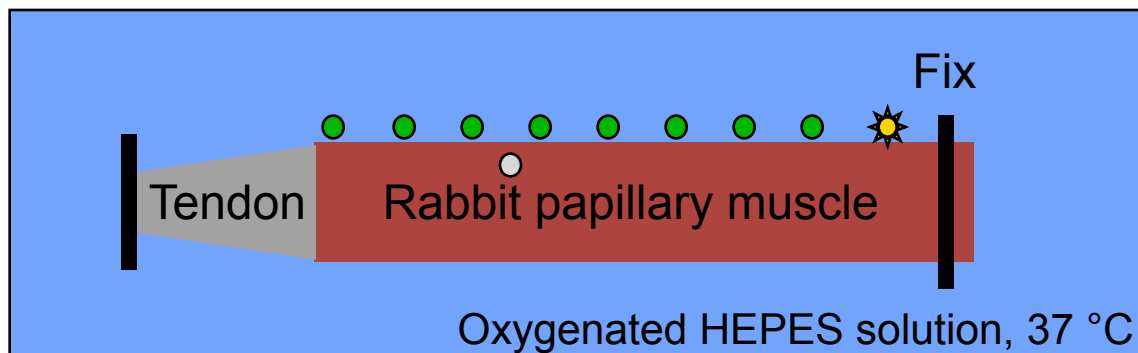
# One-Dimensional Cardiac Electrical Conduction

Species: Adult New Zealand White rabbits (1.5-3.0 kg)

1. Anti-coagulated with heparin and anesthetized with pentobarbital
2. Hearts are rapidly excised and moved to dissection tray
3. Retrograde perfusion via aorta with modified Tyrode solution
4. Opening of right ventricle
5. Selection and excision of papillary muscle including onset of chordae tendinae

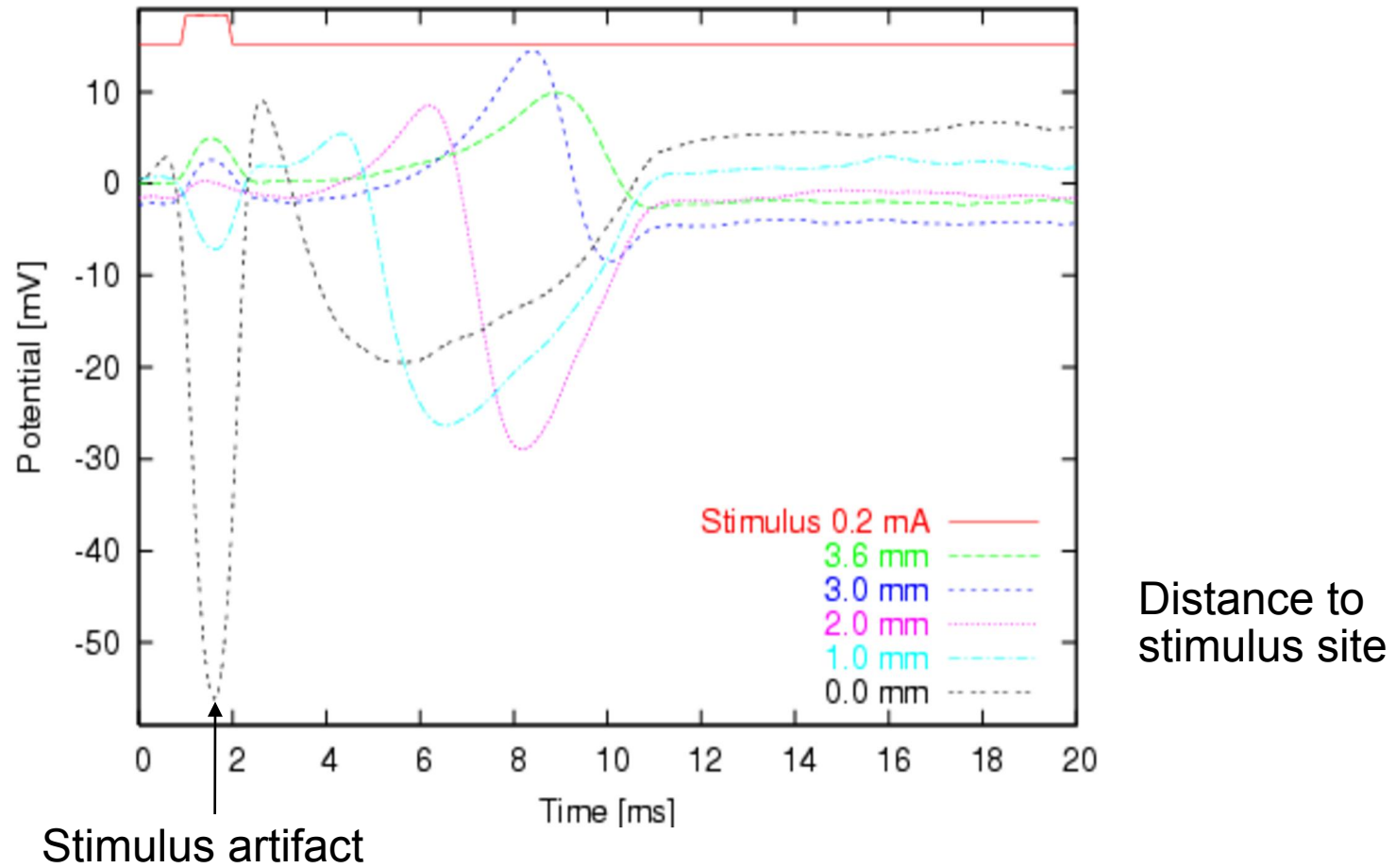
**Criteria:** Small diameter, large length, unramified

6. Transfer to horizontal flow-through chamber
7. Fixation of muscle
8. Measurement



- ★ Stimulus position
- EG measurement

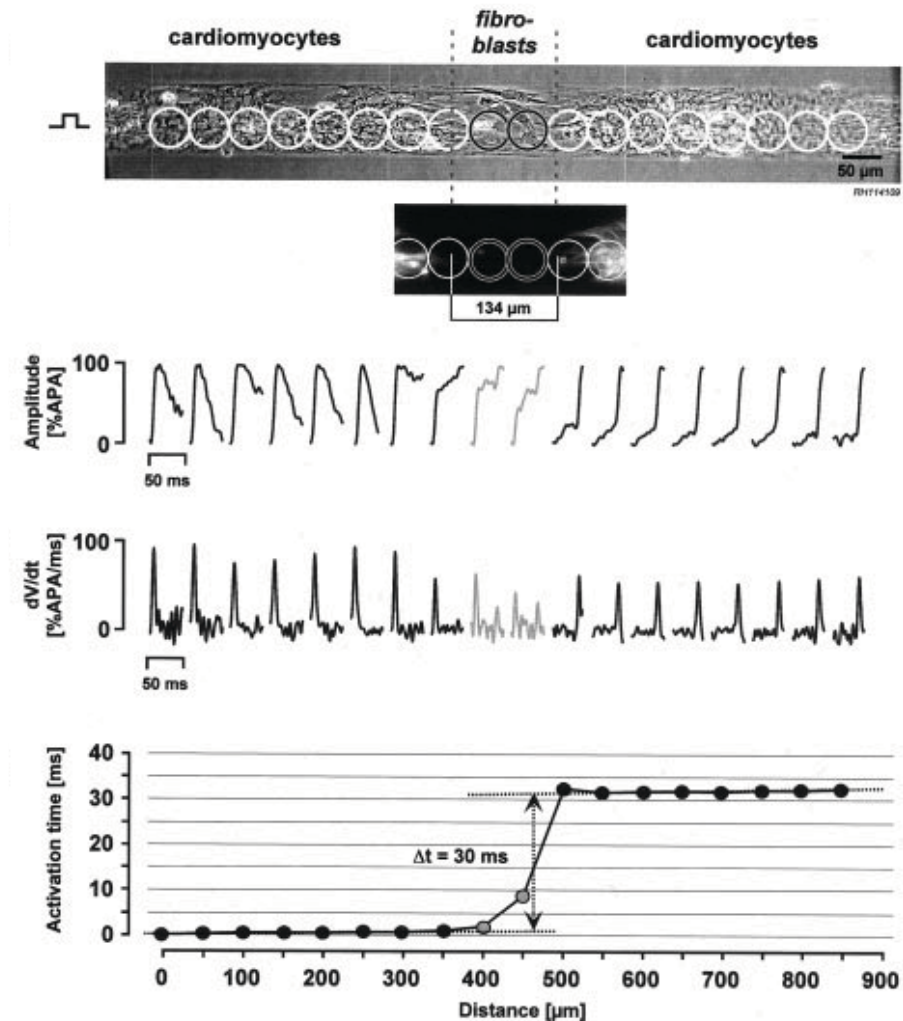
# Measurement Results: Electrograms



# Experimental Studies of Conduction in Cell Culture

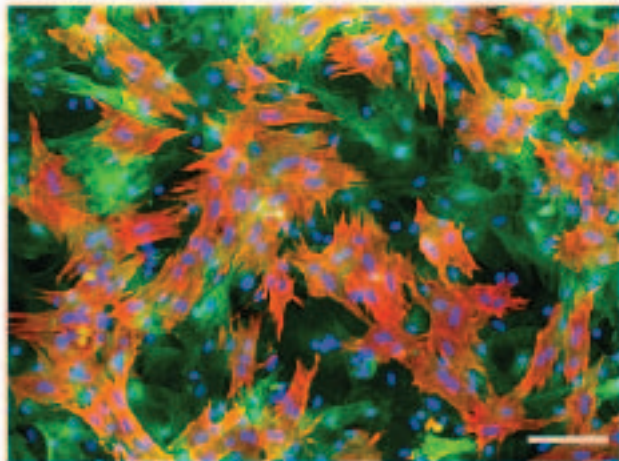
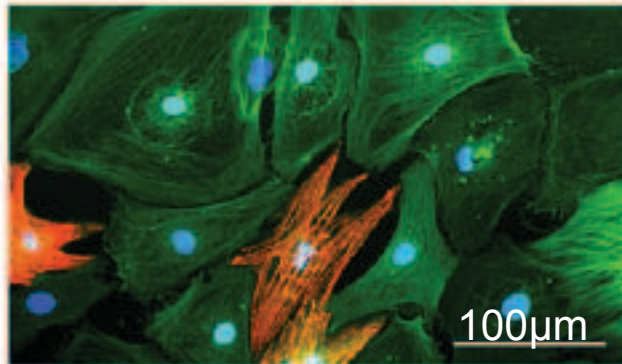
Myocyte strand with fibroblast insert

Optical mapping using voltage sensitive dyes

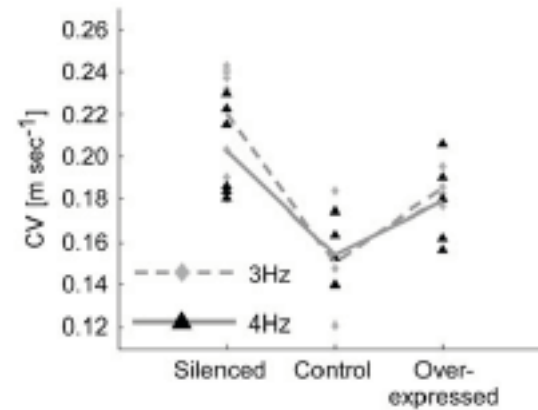
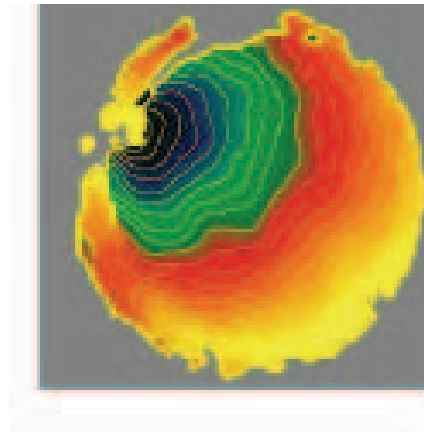


(Gaudesius et al, Circ Res, 2005)

# Optical Mapping of Co-Culture of Rat Myocyte/Myofibroblast

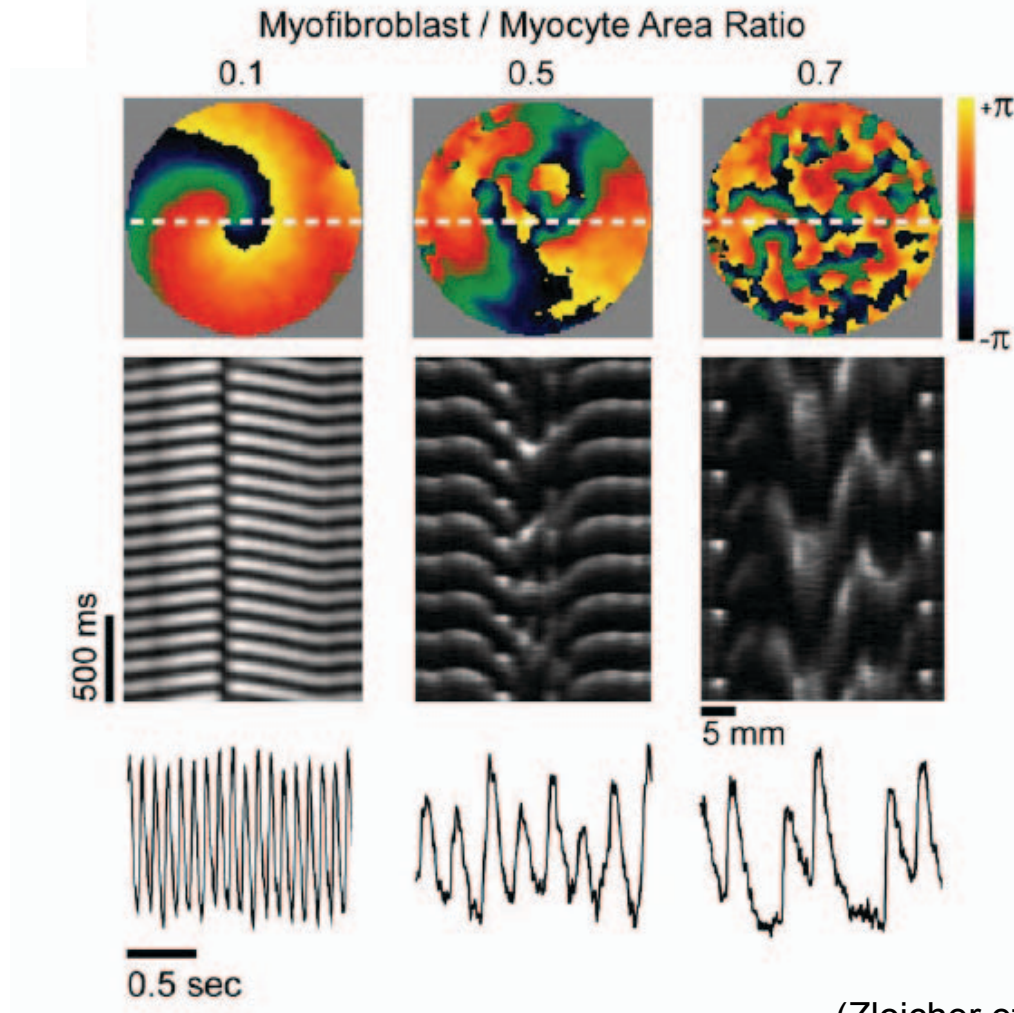


DAPI - nuclei  
Actin - myocytes  
 $\alpha$ -smooth muscle actin - myofibroblast



(Zloicher et al, Biophys J, 2008)

# Optical Mapping of Reentrant Arrhythmia



(Zloicher et al, Biophys J, 2008)



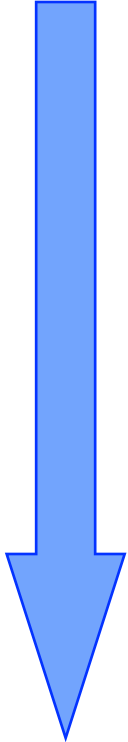
## Group Work

Identify the major mechanisms of cardiac conduction!  
What would be an electrical engineering description of those?

Which other systems exhibit similar phenomena? List at least 10.



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- 
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