Bioeng 6460

Electrophysiology and Bioelectricity

Microstructural Basis of Conduction I

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Overview

- Basics and Concepts
- Imaging Approaches
- Microscopic Anatomy
  - Tissue
  - Cells
- Gap Junctions in Tissue
- Summary

Group work

Group work

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Basics

- Electrical signaling in cardiac tissue is a multiscale process
- Microscopic conduction is a function of the arrangement of cells and proteins
- Microscopic scale: from ~1 to ~100 µm
- Major cell types in cardiac tissue: myocytes and fibroblast
- Coupling through gap junctions channels

Objective of this lecture

- Insights into microstructure of cardiac tissue with perspective on electrical signaling
- Introduce research tools for studying tissue microstructure

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Conductive Tissue: Concepts

- Syncytium: Large cell-like structure
  - Structural/functional
  - Coupling: continuous/discrete
- Network
  - Topology: mesh, tree, ring, …
  - Coupling: gap junctions, synapses

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Approaches for Imaging of Tissue Microstructure

- Light Microscopy
  - Transmission / Reflection
  - Confocal Microscopy
- Electron Microscopy
- Thin Sectioning
  - Microtome / Ultramicrotome
  - Vibrotome
  - Cryosectioning
- Labeling
  - Antibody
  - Other

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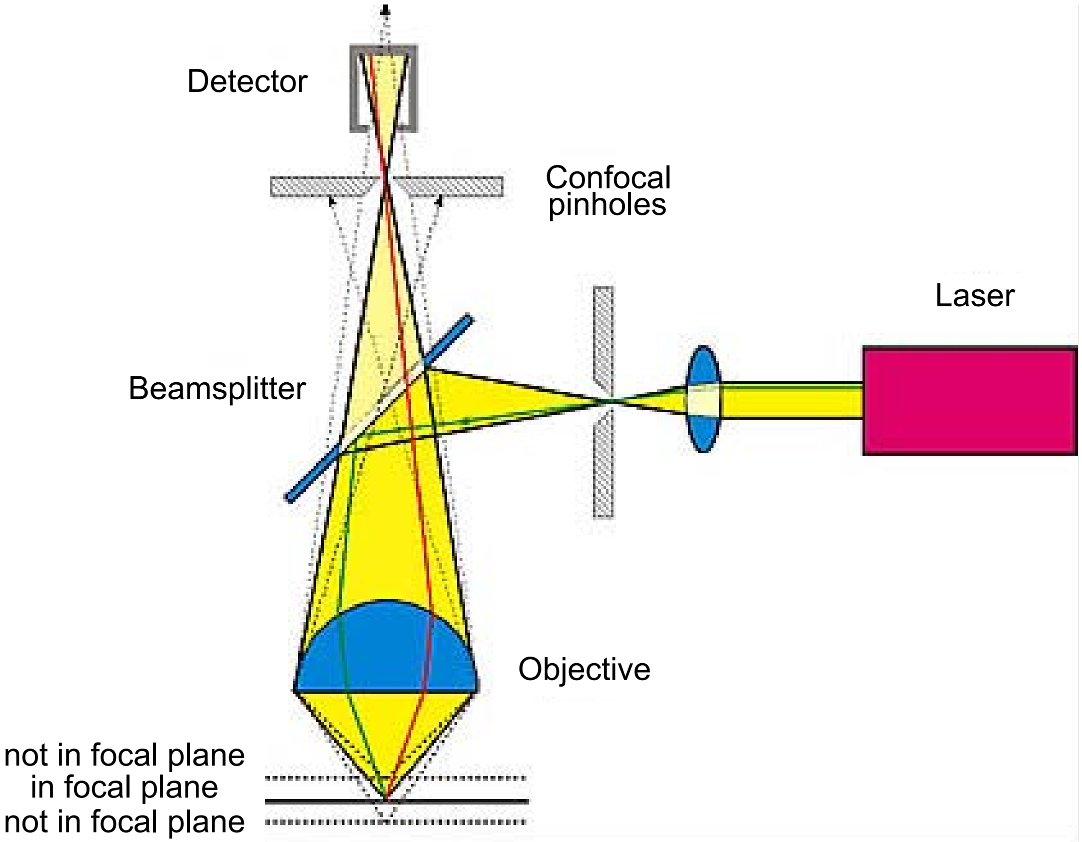
Principles of Confocal Microscopy



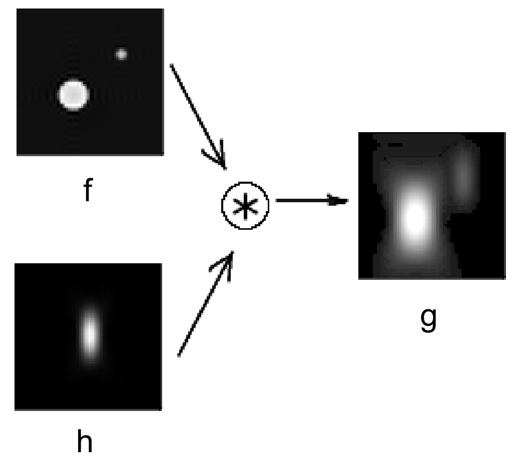
Mathematical description of imaging system:

$g = h * f$

g : Response of imaging system

h : Point spread function

f : Source image



(http://www.confocal-microscopy.com/)

(http://en.wikipedia.org/wiki/Point_spread_function)

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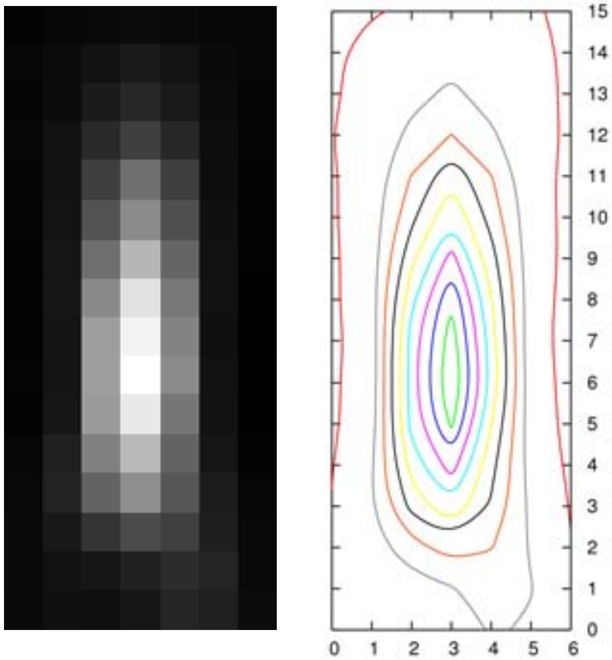
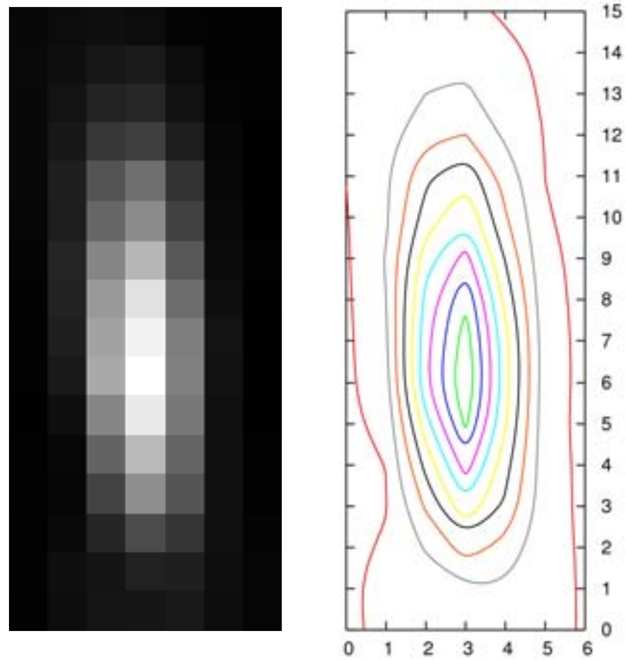
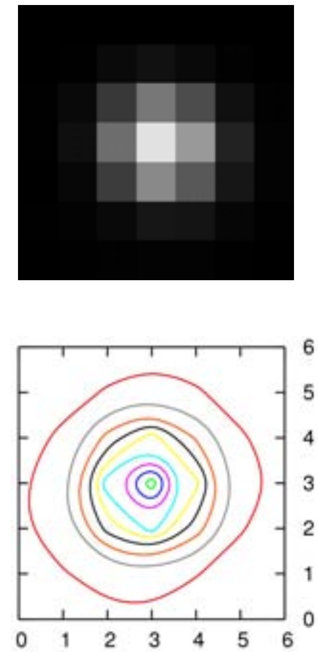
Point Spread Functions: BioRad, 60x Oil, NA 1.4



YZ



XZ



XY

Resolution: 130nm x 130nm x 130nm
Full width at half maximum Z: 1.04μm   XY: 0.260μm

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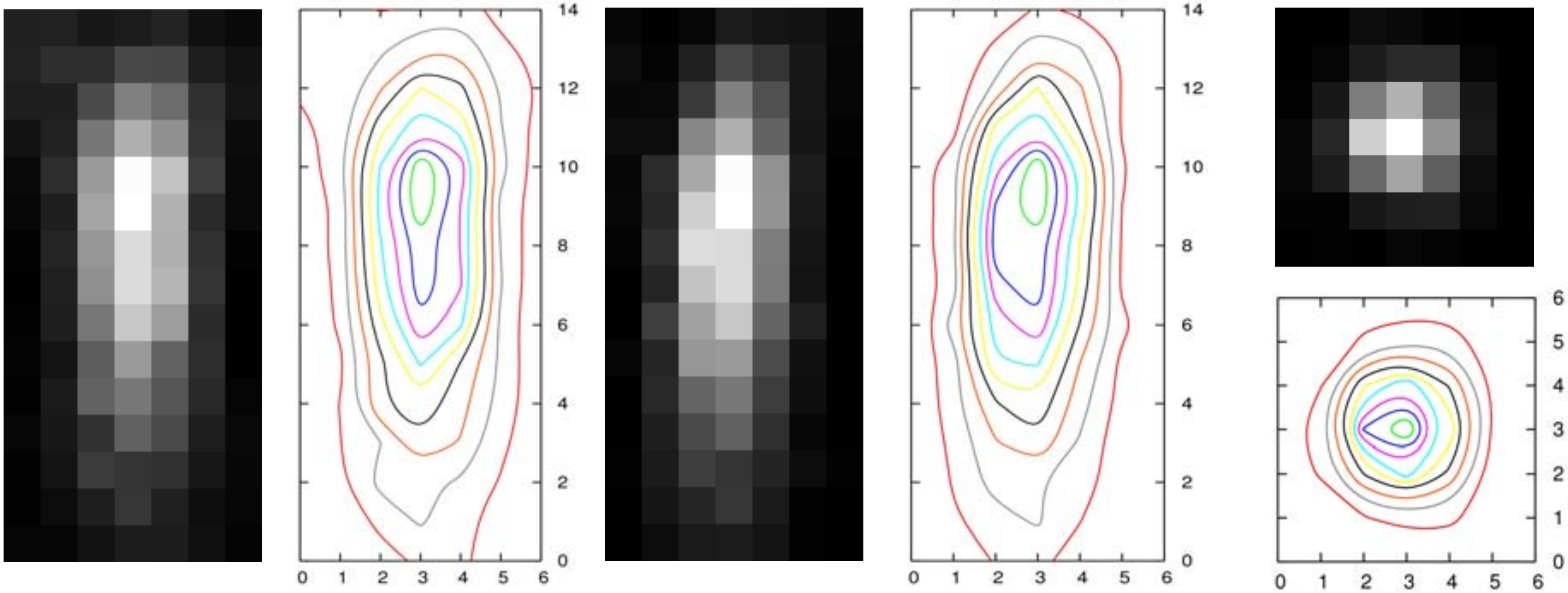
Point Spread Functions: Zeiss LSM5, 60x Oil, NA 1.4



YZ XZ XY

Resolution: 100nm x 100nm x 100nm
Full width at half maximum Z: 0.8μm XY: 0.2μm

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Image Deconvolution

Assumptions

Imaging system can be described by

$$g(\mathbf{x}) = f * h(\mathbf{x}) = \int\int_{-\infty}^{\infty}\int f(\mathbf{x})\, h(\mathbf{x}-\mathbf{x}')\, d\mathbf{x}'$$

g : Response of imaging system

h : Point spread function

f : Source image

- Linearity
- Translation independence

Deconvolution

Richardson-Lucy Algorithm

$$g_{n+1} = g_n\left(\frac{g_0}{g_n * h} \otimes h\right)$$

g_n : Solution for step n with $g_0 = g$

$\otimes$: Cross-correlation operator

- Sensitive to noise and imaging artefacts!
- Regularization

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Group Work

Imagine a horizontally and vertically oriented structure with a thickness of 1 voxel and length of 10 voxel.

Assume that the point spread function of an imaging system can be described with a Gaussian having the following properties:

- full width at half maximum XY: 1 voxel
- full width at half maximum Z: 3 voxel

Estimate the blurring of the vertical and horizontal structure by the point spread function.

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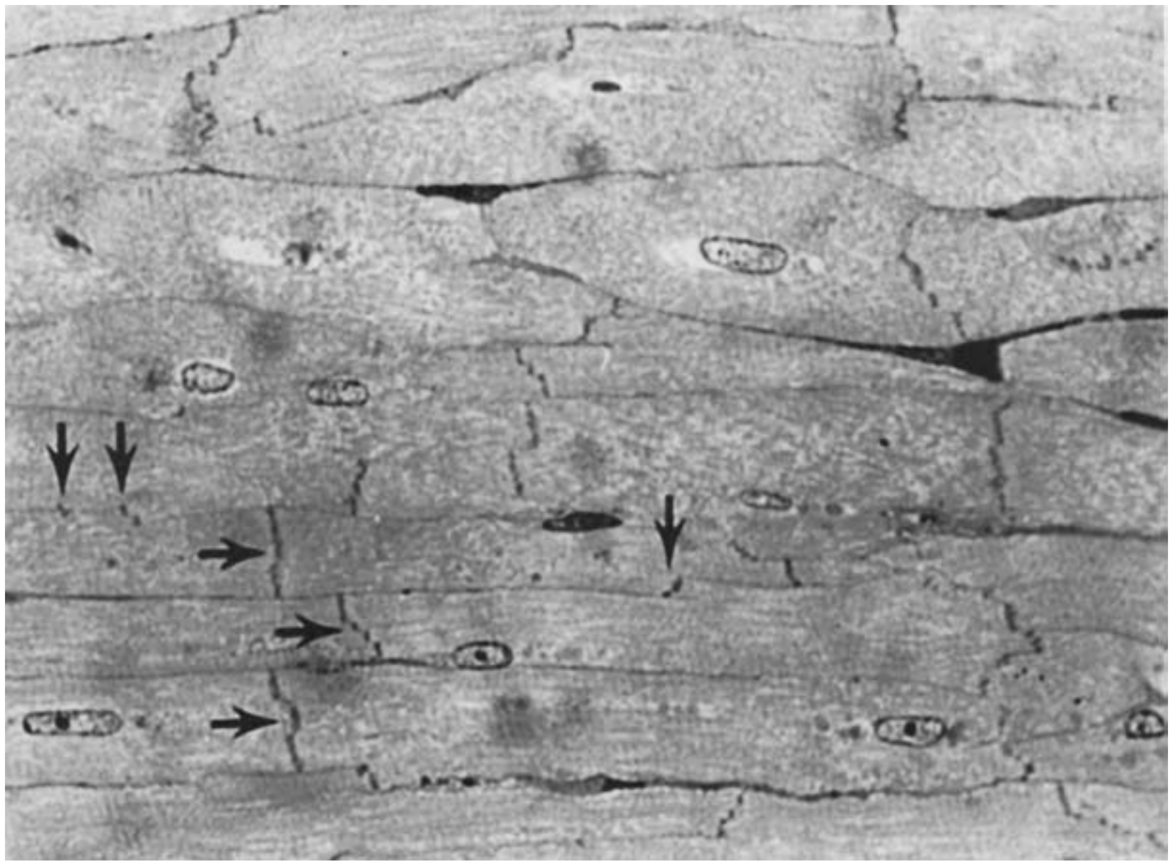
Microscopic Anatomy of Cardiac Tissue

Myocytes are connected at intercalated discs: intracellular space via gap junctions; mechanical coupling



(Saffitz et al. 99)

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Microscopic Anatomy of Myocytes

Myocyte of ventricular myocardium

cylinder-shaped

length: 60-120 µm

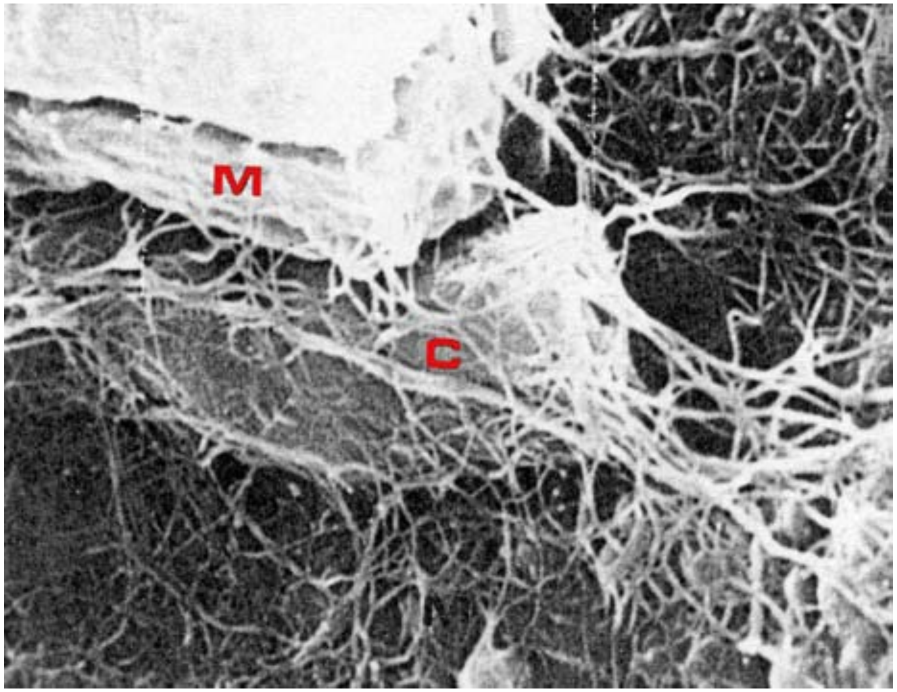
diameter: 8-15 µm

(Hoyt et al. 89)

Myocyte (M) and capillary (C)

mechanically coupled by fibers of connective tissue (collagen and elastin)

(Caulfield et al. 79)



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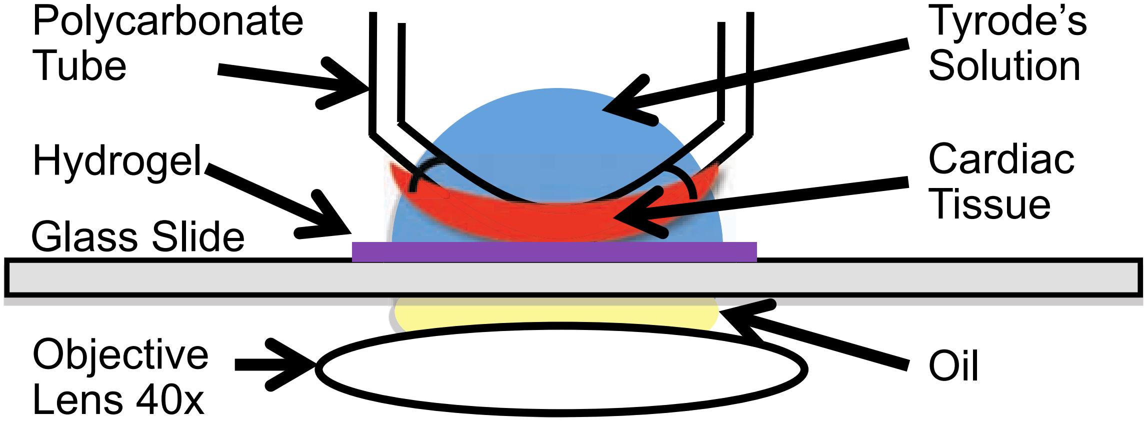
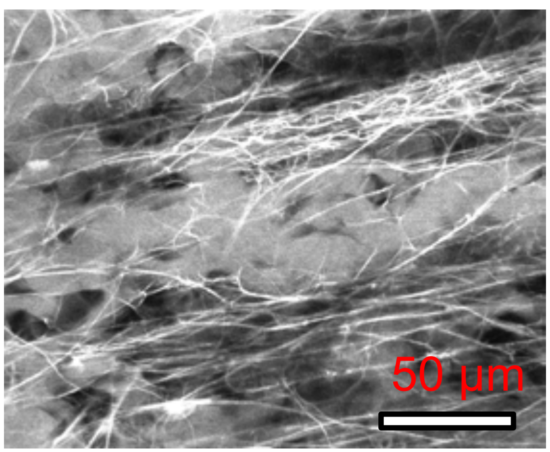
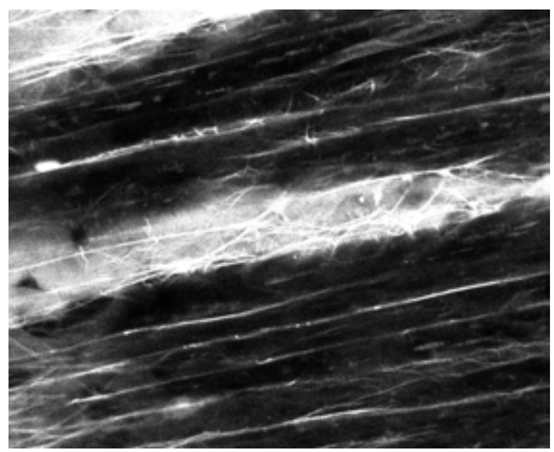
Microstructure of Living Tissue

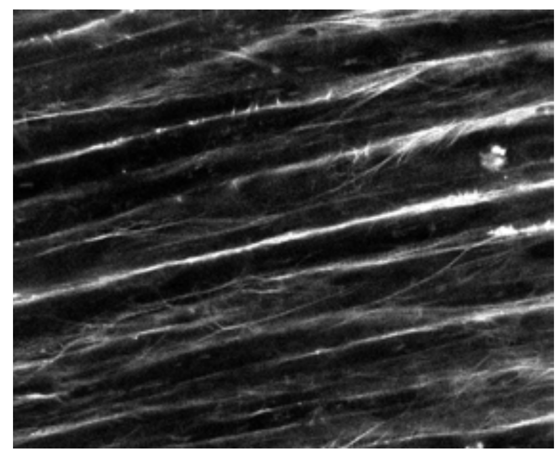




Epicardial surface



Depth: 5 μm



Depth: 15 μm

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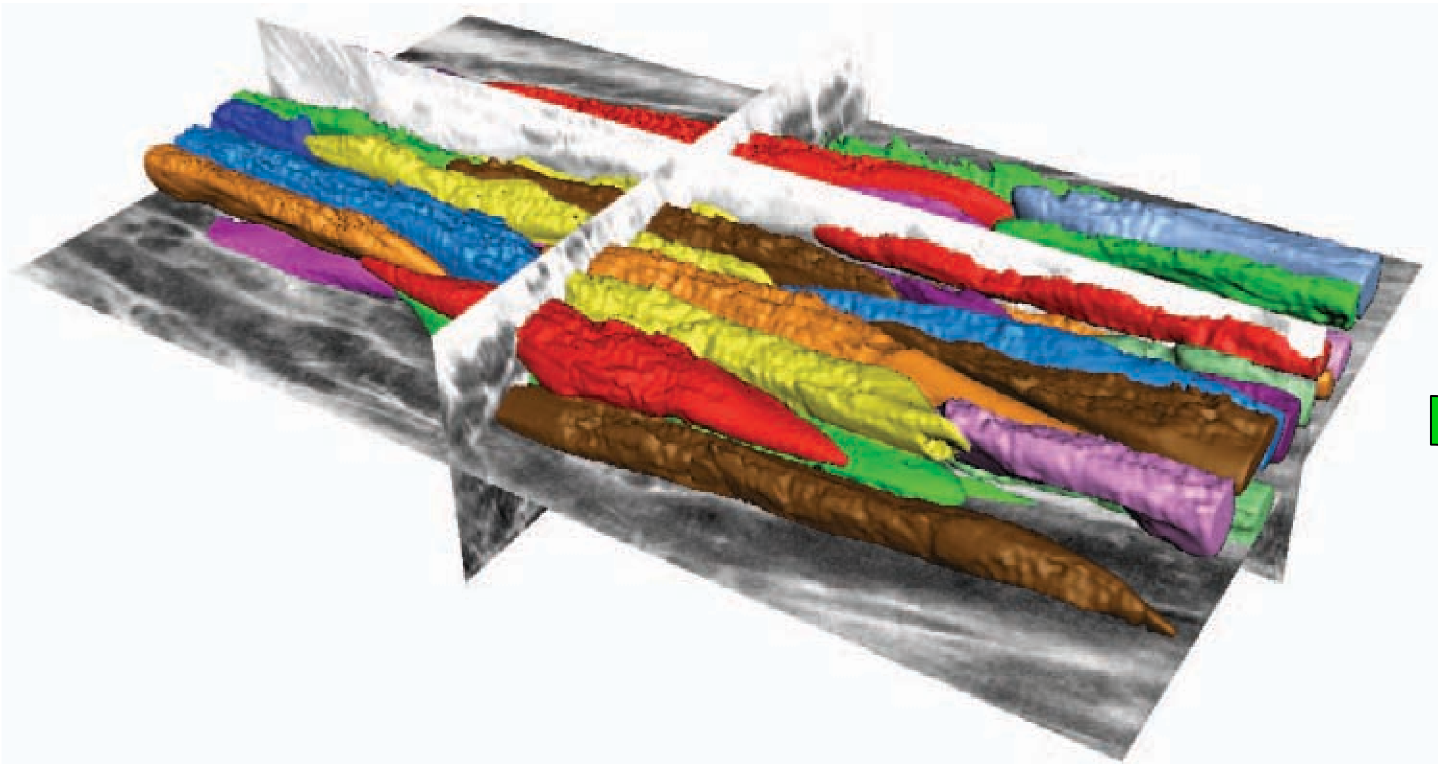
(Lasher et al, IEEE Trans Med Imaging, 2009)

Imaging-Based 3D Model of Cardiac Tissue



Tissue	Length (µm)	Width (µm)	Height (µm)	Volume (μm^3)
Atrial (n=28)	105.0±10.6	13.1±1.7	9.7±1.6	4901±1713
Vent. (n=20)	112.3±14.3	18.4±2.3	14.1±2.7	10,299±3598

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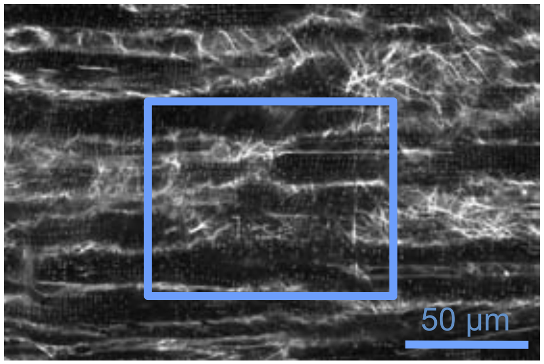
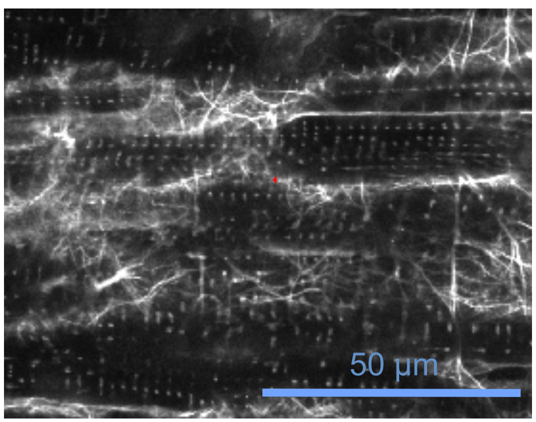
(Lasher et al, IEEE Trans Med Imaging, 2009)

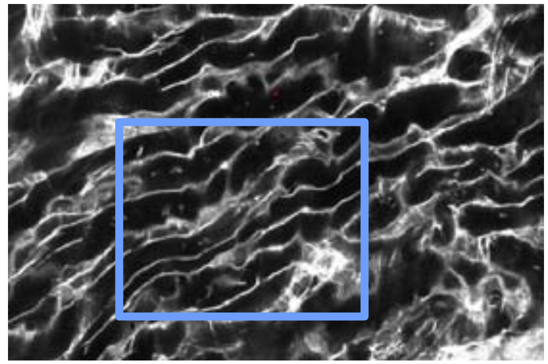
Microstructure of Fixed Rabbit Myocardium

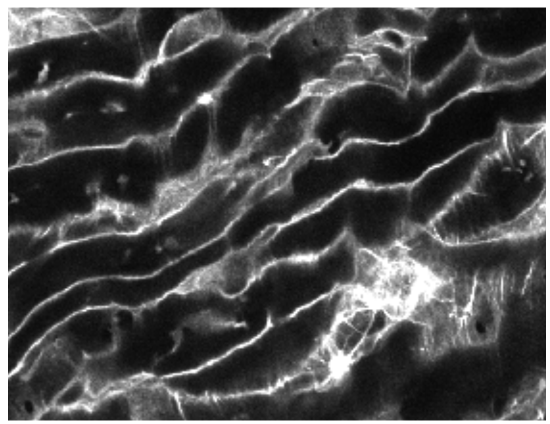
Ventricular myocardium



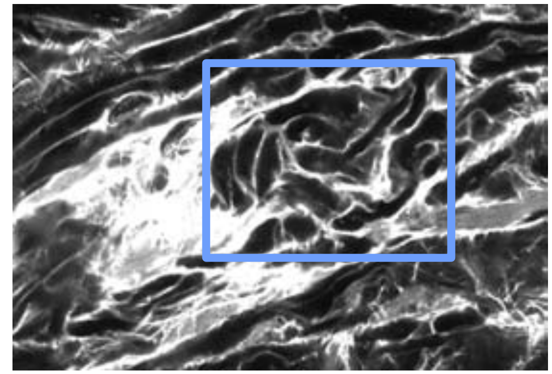


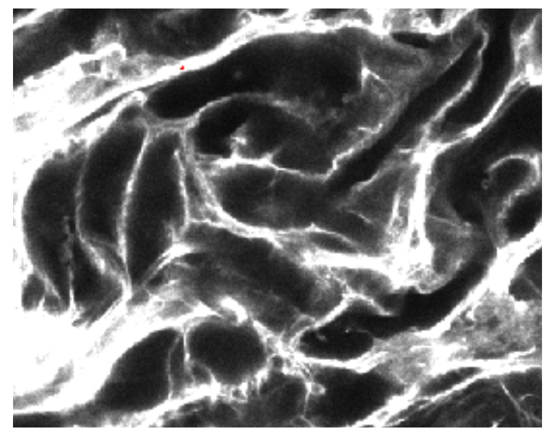
Atrial myocardium





Sinoatrial nodal tissue





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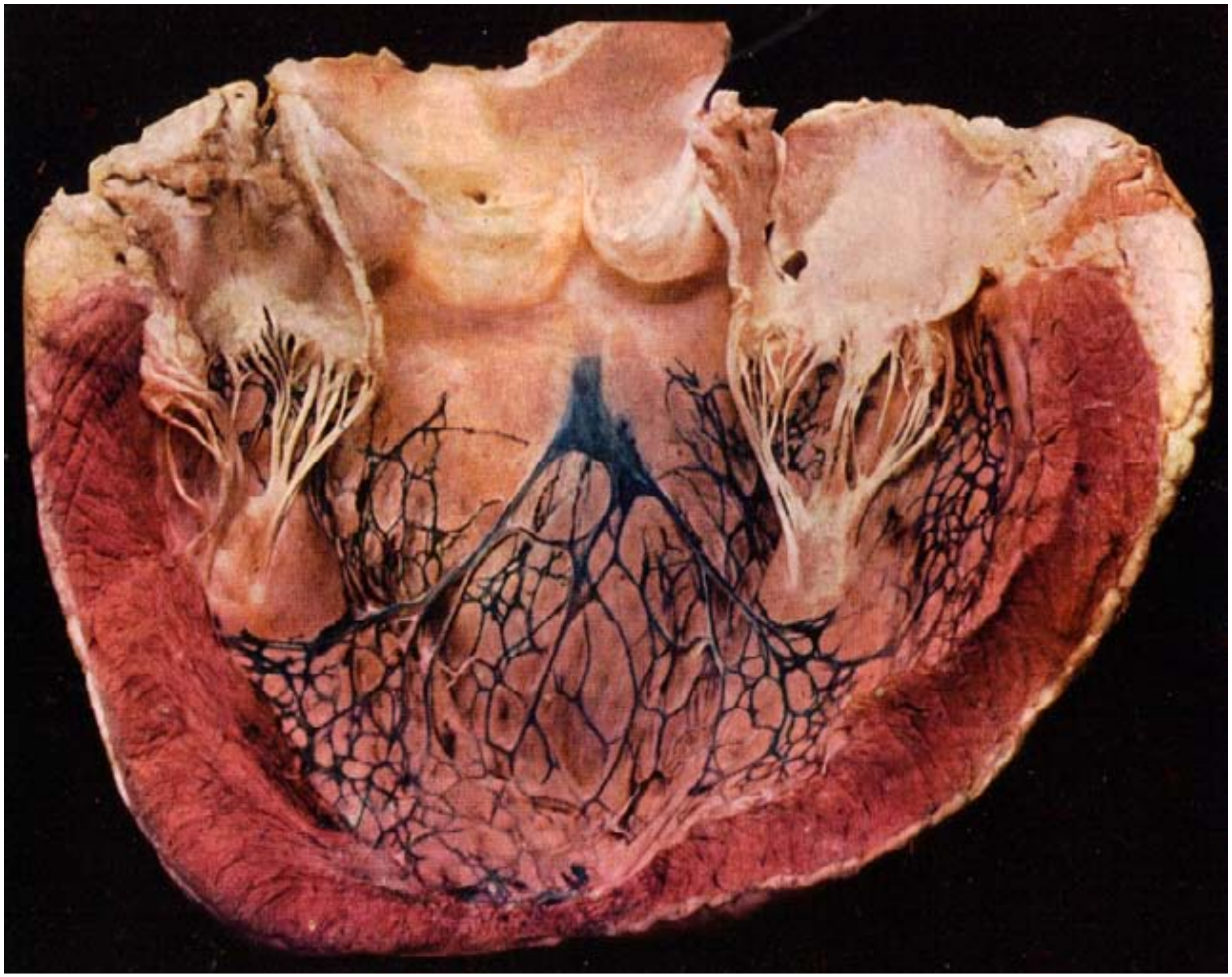
Conduction System in Cow Heart



(Lewis 1925)

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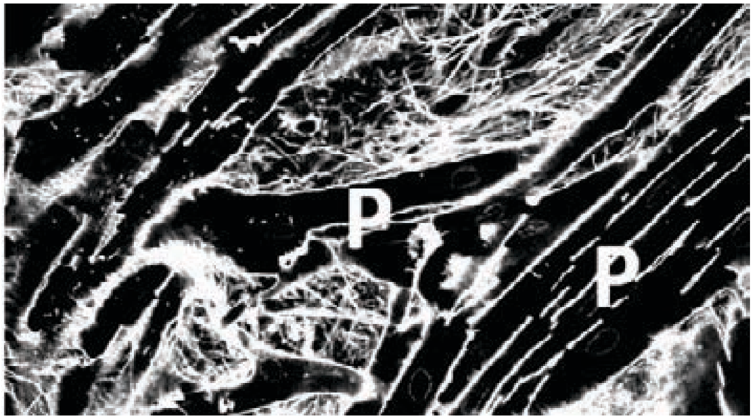
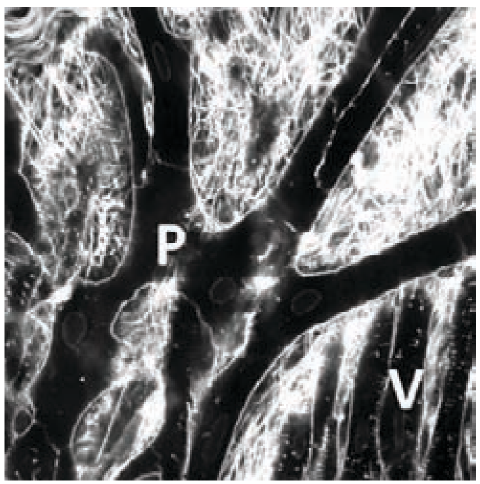
Microstructure of Rabbit Conduction System





P: Purkinje cell V: Ventricular myocyte

(Romero et al. 11)

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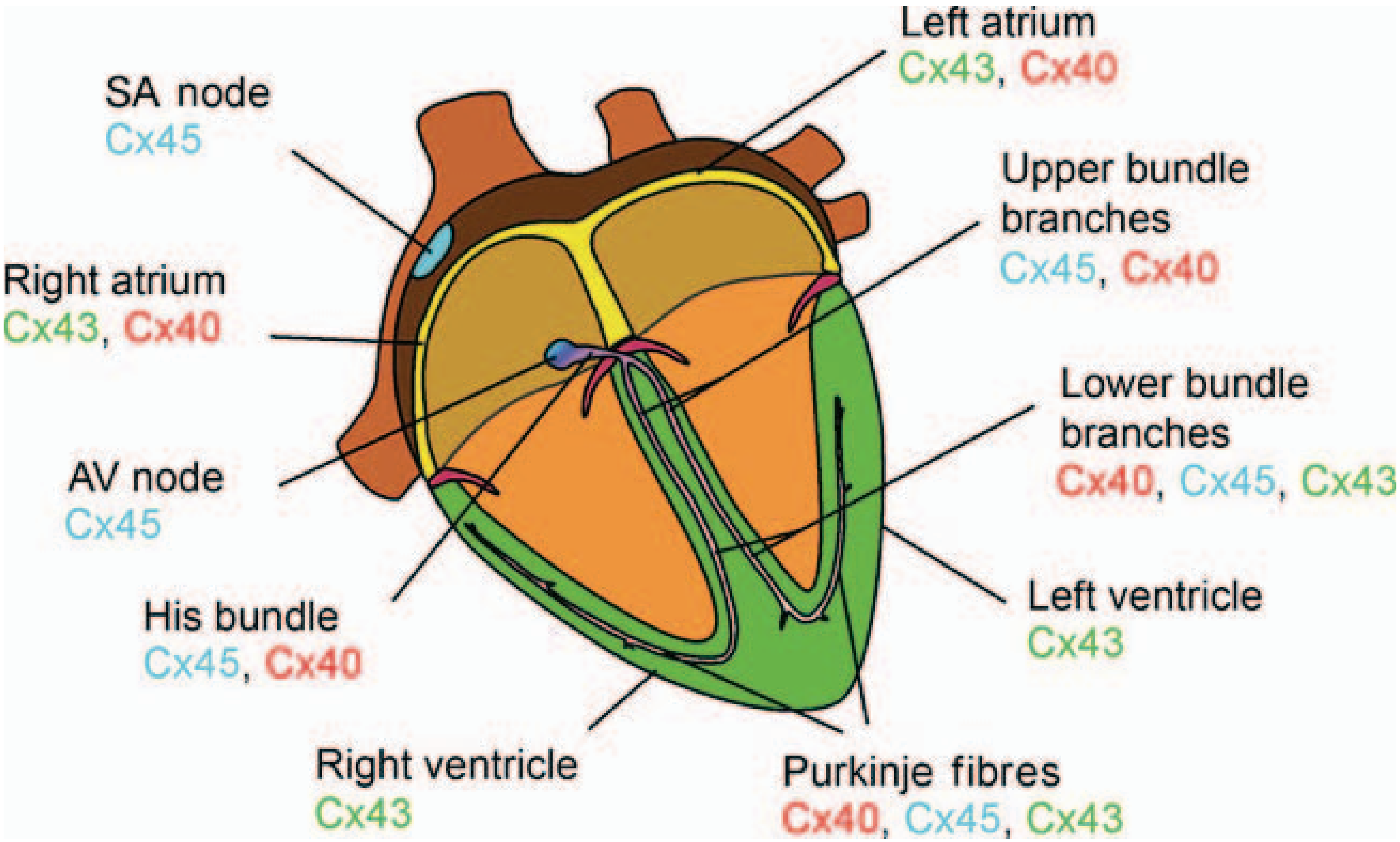
Gap Junctions in Mammalian Cardiac Tissue



(Severs et al. 08)

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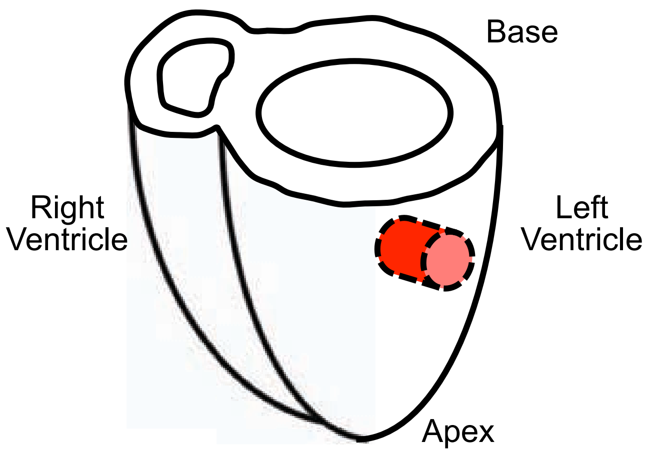
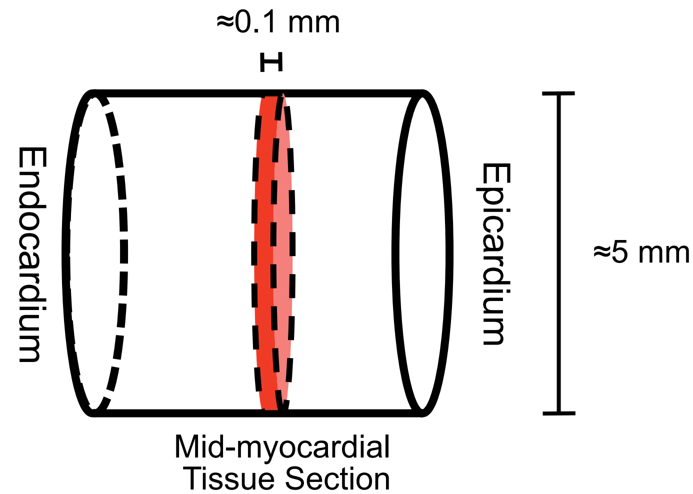
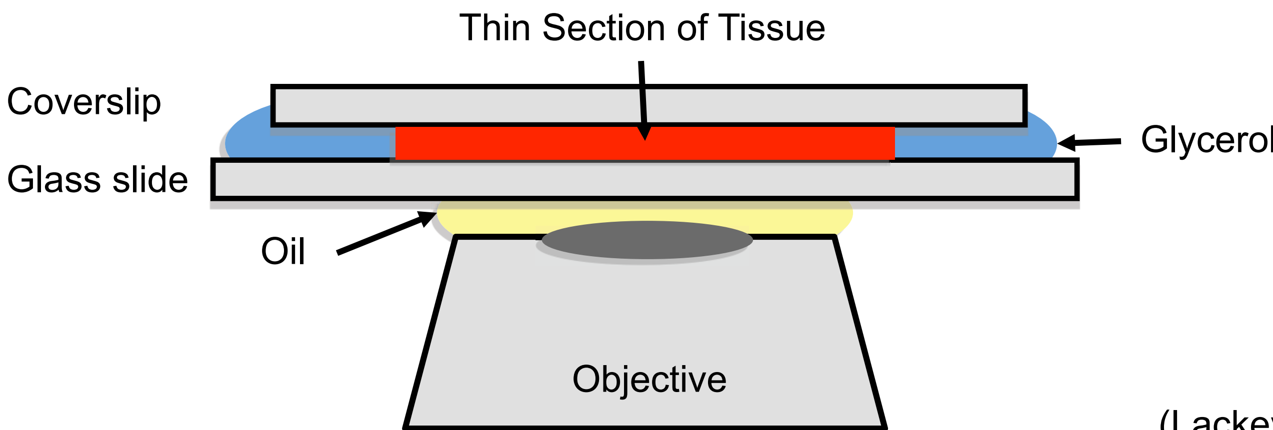
Imaging of Cx43 Distribution







(Lackey et al. 11)

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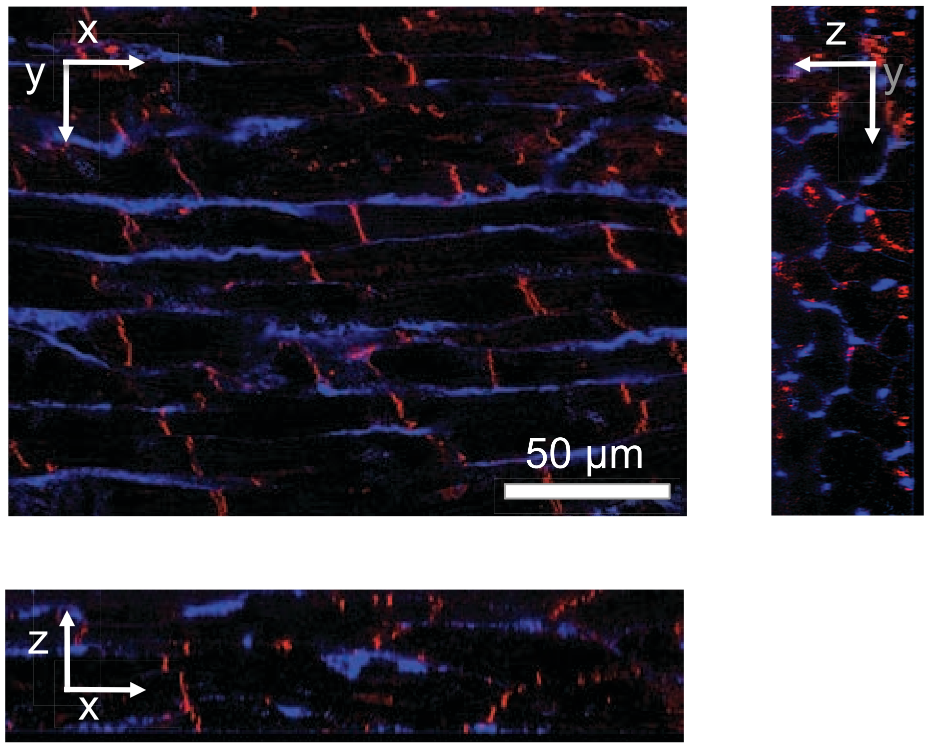
3D Cx43 Distribution in Rat Left Ventricular Myocardium



Wheat Germ Agglutinin - Extracellular Space

Cx43 - Gap Junctions

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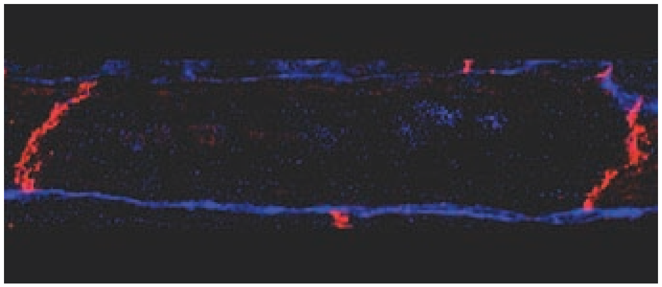
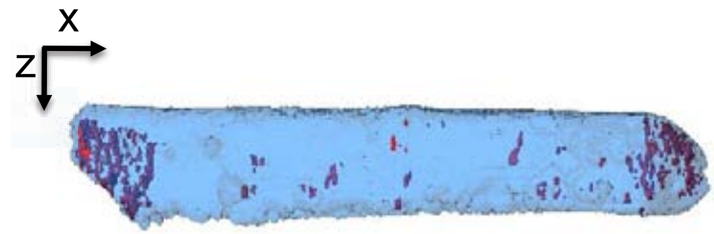
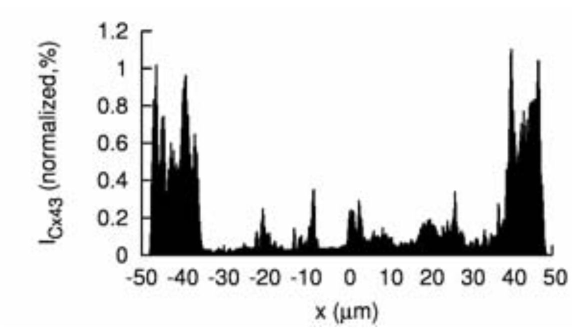
(Lackey et al. 11)

Analysis of Cx43 Distributions: Polarization



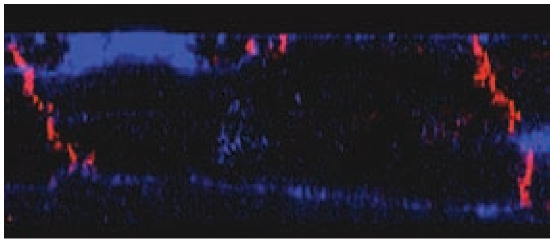


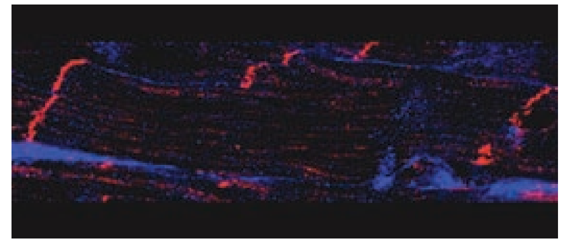


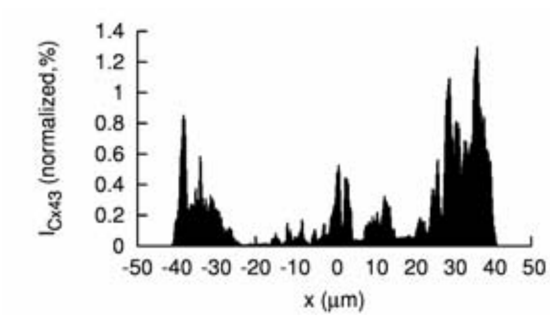
Measure	Value
Polarization $Pol_{10\%}$	59.2%
Polarization $Pol_{25\%}$	81.0%

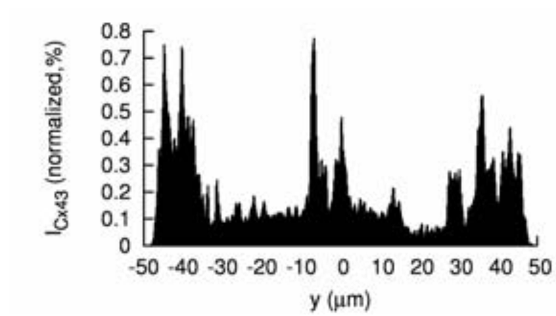
(Lackey et al. 11)

Analysis of Cx43 Distributions: Examples









Measure	Value	Value
Polarization $Pol_{10\%}$	40.1%	24.2%
Polarization $Pol_{25\%}$	77.6%	53.5%

(Lackey et al. 11)

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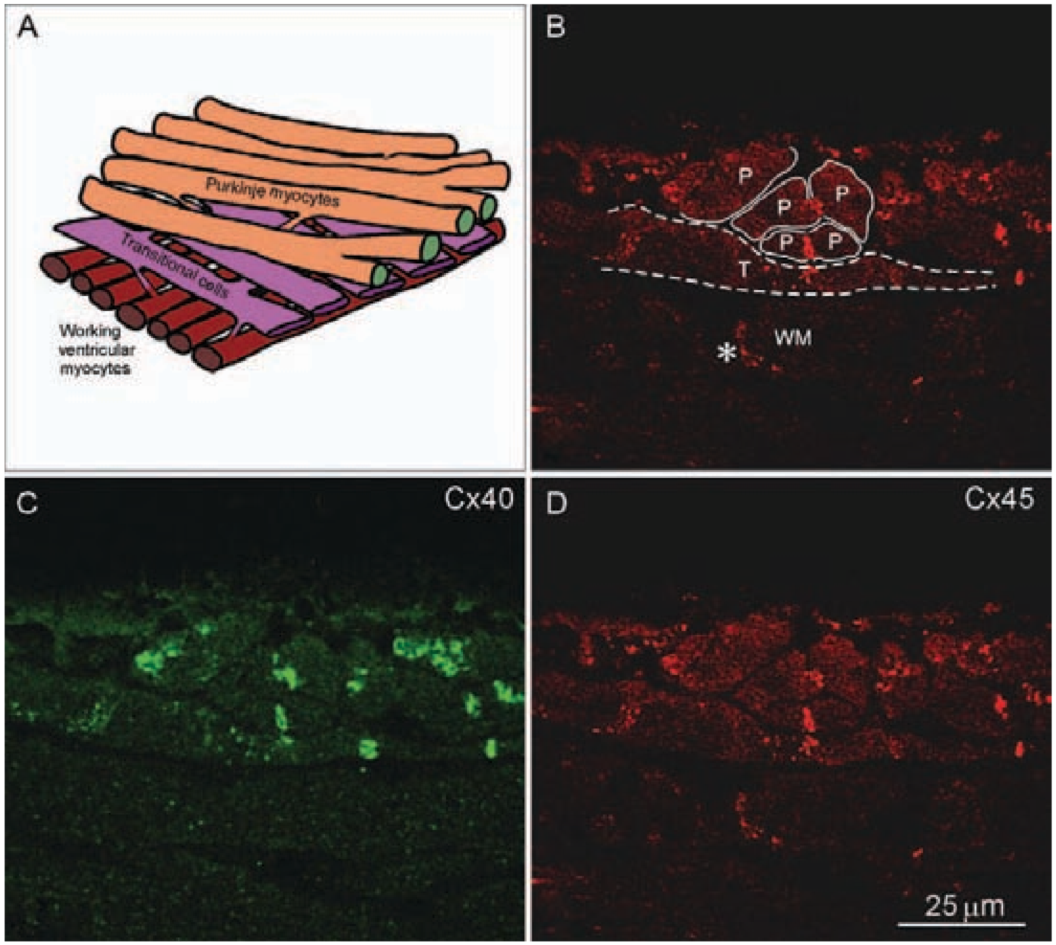
Cx40/45 Distribution in Mouse Conduction System



P Purkinje cell
T Transitional cell
WM Working myocardium
* Most superficial WM myocyte

(Severs et al. 08)

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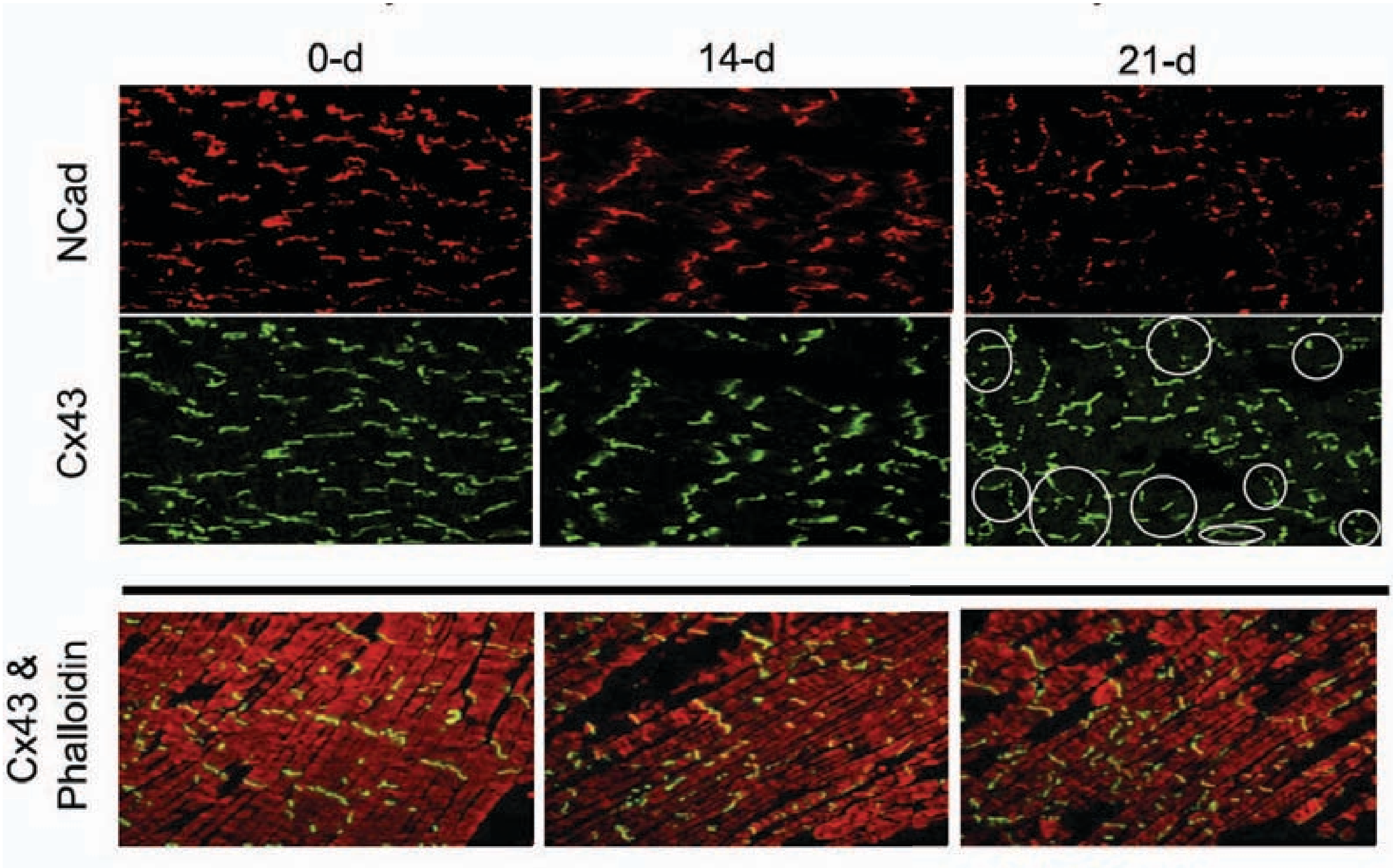
Cx43 Lateralization in Pacing Induced Heart Failure



(Akar et al. 07)

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Group Work

Compare coupling through gap junction channels with coupling through synapses. List 5 differences!

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Summary

- Concepts
- Imaging Approaches
- Microscopic Anatomy
  - Tissue
  - Cells
- Gap Junctions in Tissue

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