Isolation, Calculation and Measurement of Healthy and Failing Cardiac Myocytes

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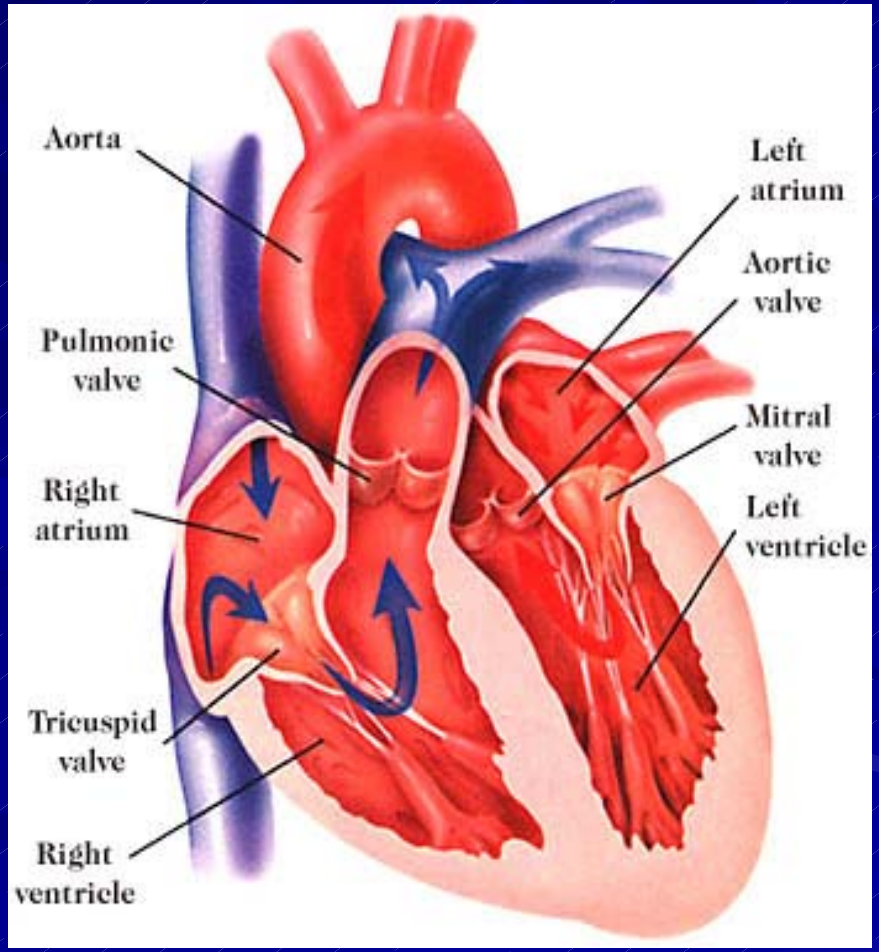
Background

The Heart

- Most vital organ in the body
- Job

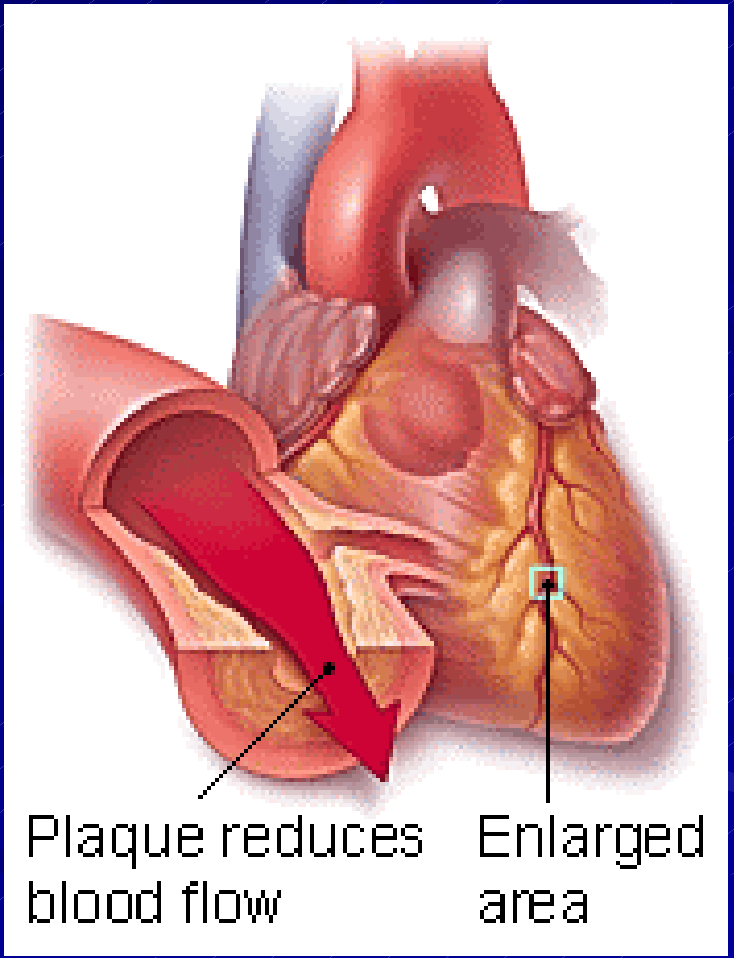
  - Pumps blood to the lungs to get O_2

  - Pumps oxygenated blood throughout the body



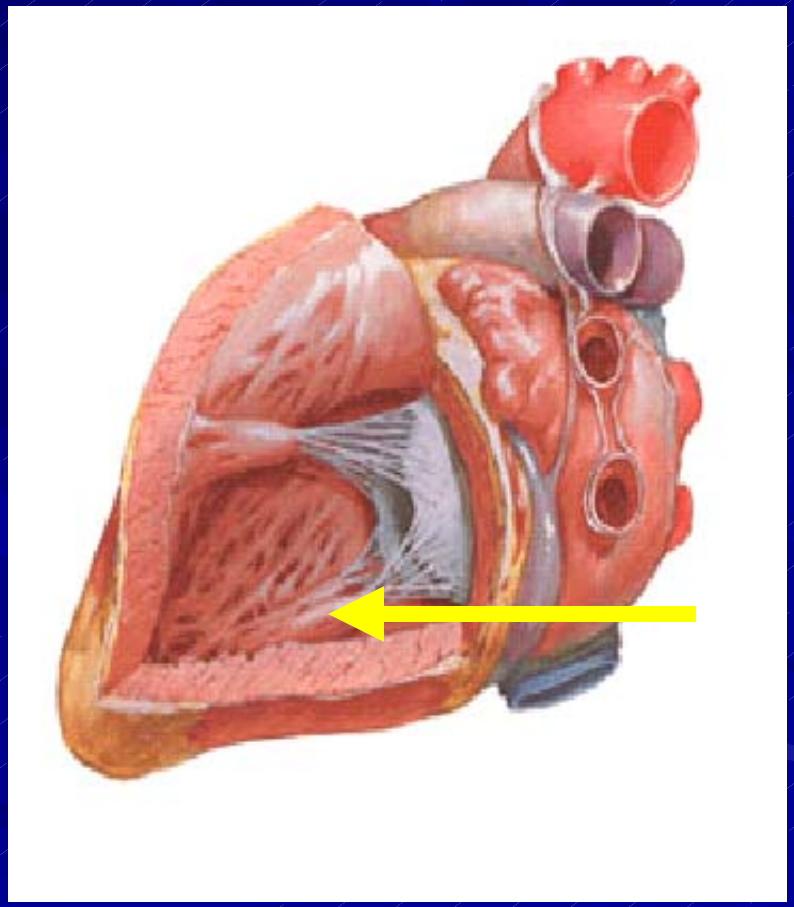
Myocardial Infarction

- Leading killer of Americans (1Million)
- Coronary Artery is blocked by plaque (arteriosclerosis)
- Blood cannot get through
- Heart begins to die



Cardiac Hypertrophy

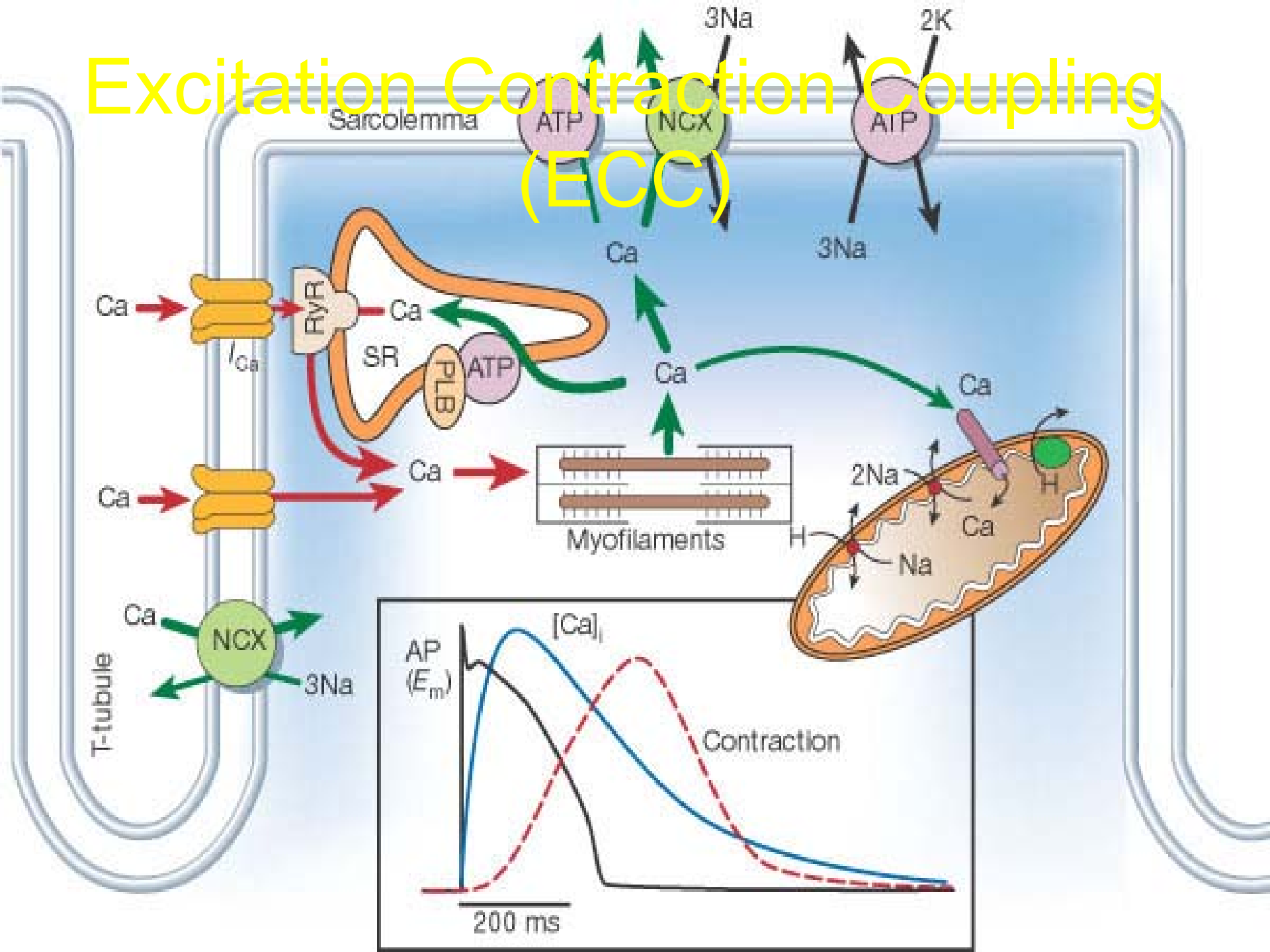
- Enlargement of the heart and it's walls
- An enlarged heart may be caused by a thickening of the heart muscle because of increased workload due to valve disease or high blood pressure



Myocytes

- Cardiac muscle cells
- Receive electrical impulses that causes a heartbeat
- Cells beat in unison
- If they don’t beat in unison arrhythmia occurs

Excitation Contraction Coupling (ECC)



Research Design

Purpose

- To see the effects of cardiac illnesses on myocytes
  - Counting cells
  - Measuring cells

Hypothesis

- There will be a difference in size of the myocytes.
- There will be a difference in the percent of mononucleated myocytes.

Materials for Myocyte Isolation

- Cat
- Langendorff apparatus
- Tray
- Cannula
- Funnels
- 5 Scissors
- Forceps
- 50mL conicals
- Petri dishes
- Transfer pipettes
- Surgical suture
- Vacuum
- Syringes
- Sodium Pentobarbital
- Heparin
- Ketamine
- Acepromazine
- Krebs Henseleit Buffer
- Bovine Serum Albumin
- .5M $CaCl_2$
- Collagenase
- Double Distilled Water
- Water bath
- Pressure Transducer

Materials

Cell Culture

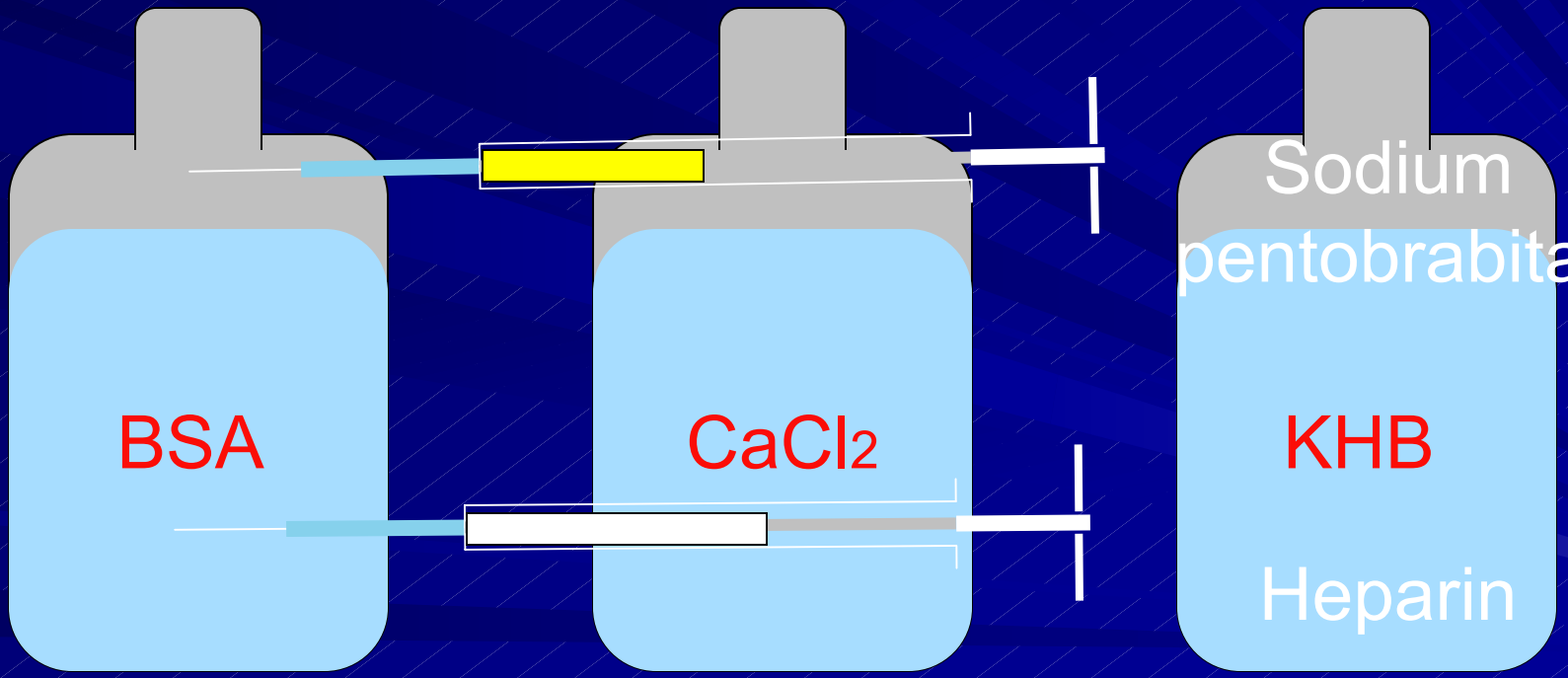
- Sterile Cell Culture Hood
- .5ul stock Penicillin
- .5ul stock Streptomycin
- .5µl stock Gentamycin
- 4 well rectangular 500µl/1000µl
- Laminin
- cell culture medium SigmaM-199
- L-glutamine
- Micro pipettes and tips
- Cells
- Incubator

DAPI Staining

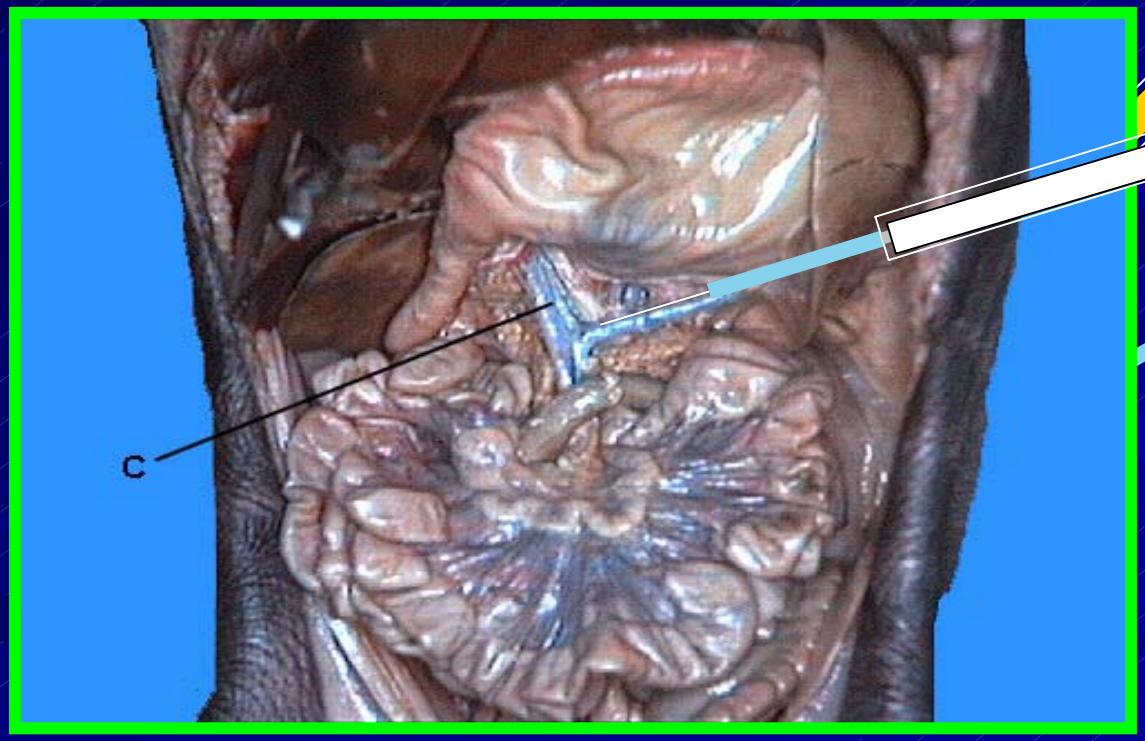
- Cultured cells
- 1X PBS
- 4% Paraformaldehyde
- Forceps
- Vacuum

Myocyte Isolation Prep

Ketamine-acepromazine



Isolation Procedure



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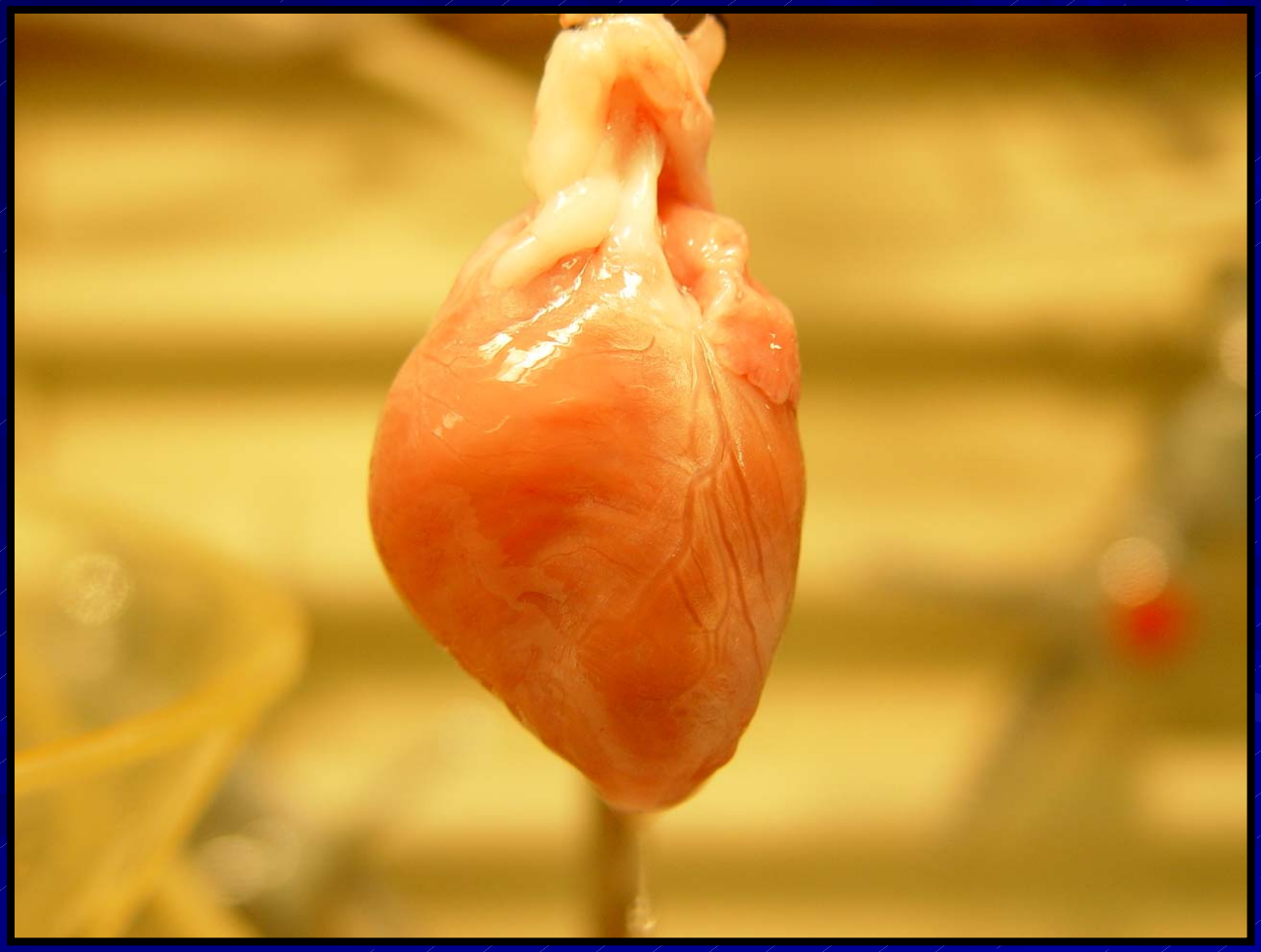
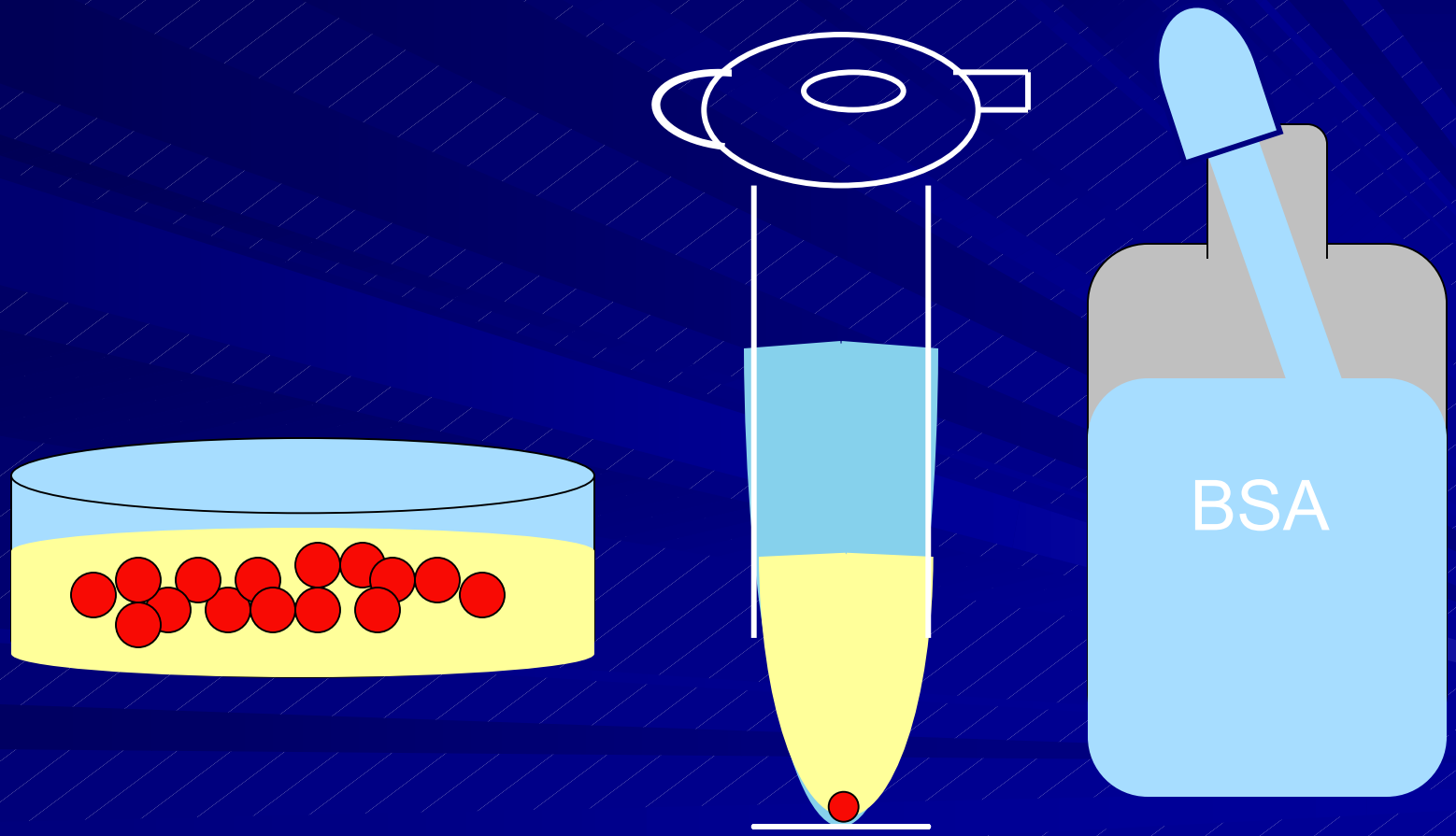
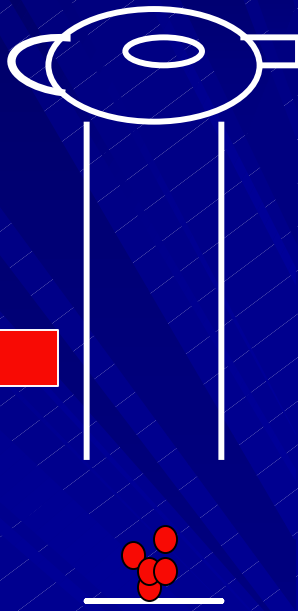


Isolation Procedure



Cell Culture



Laminin

Slide

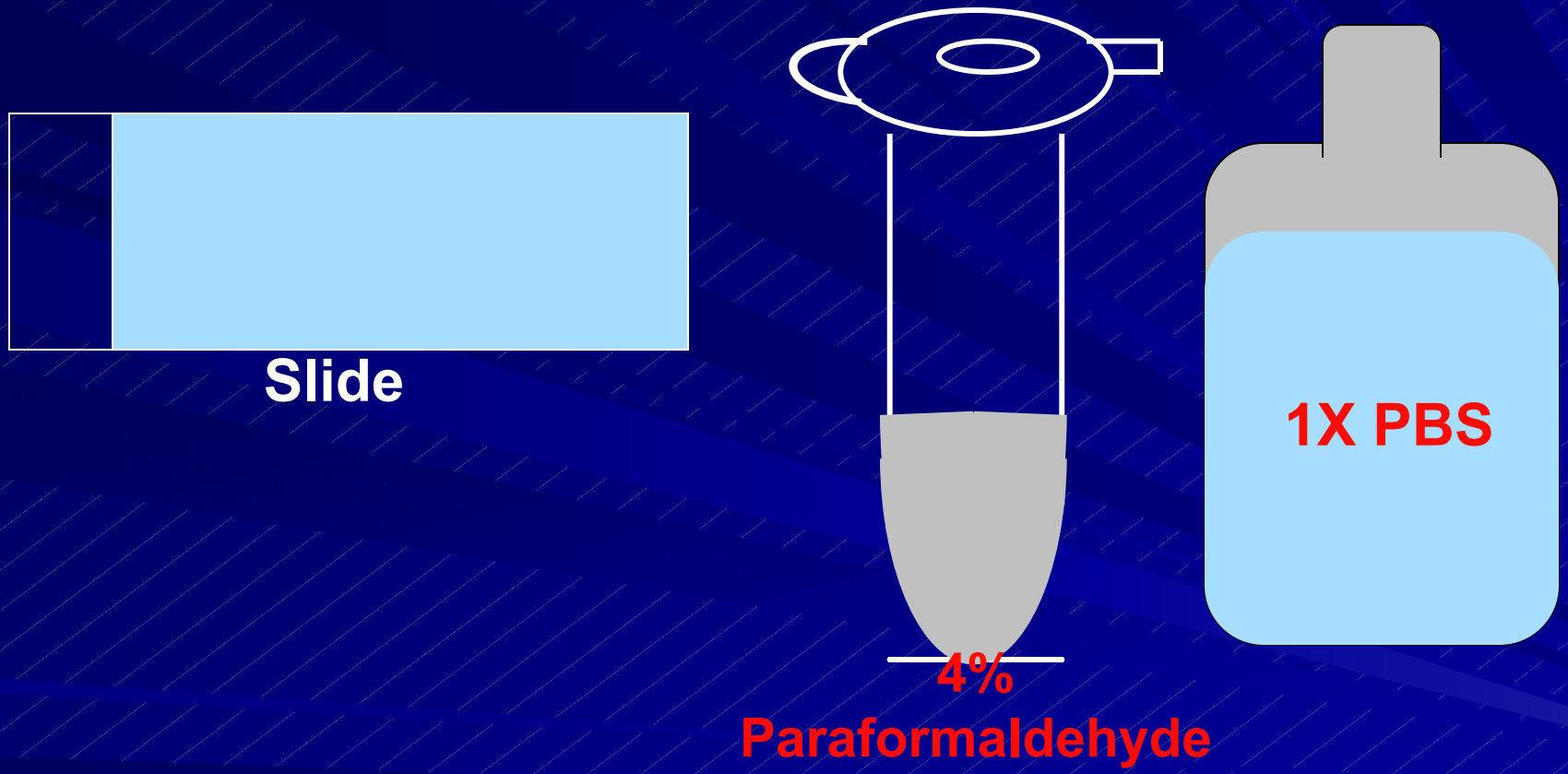
Cell culture medium
Sigma M199

Cell Culture

Wait 1 hour

36.7˚C Incubator

DAPI Staining

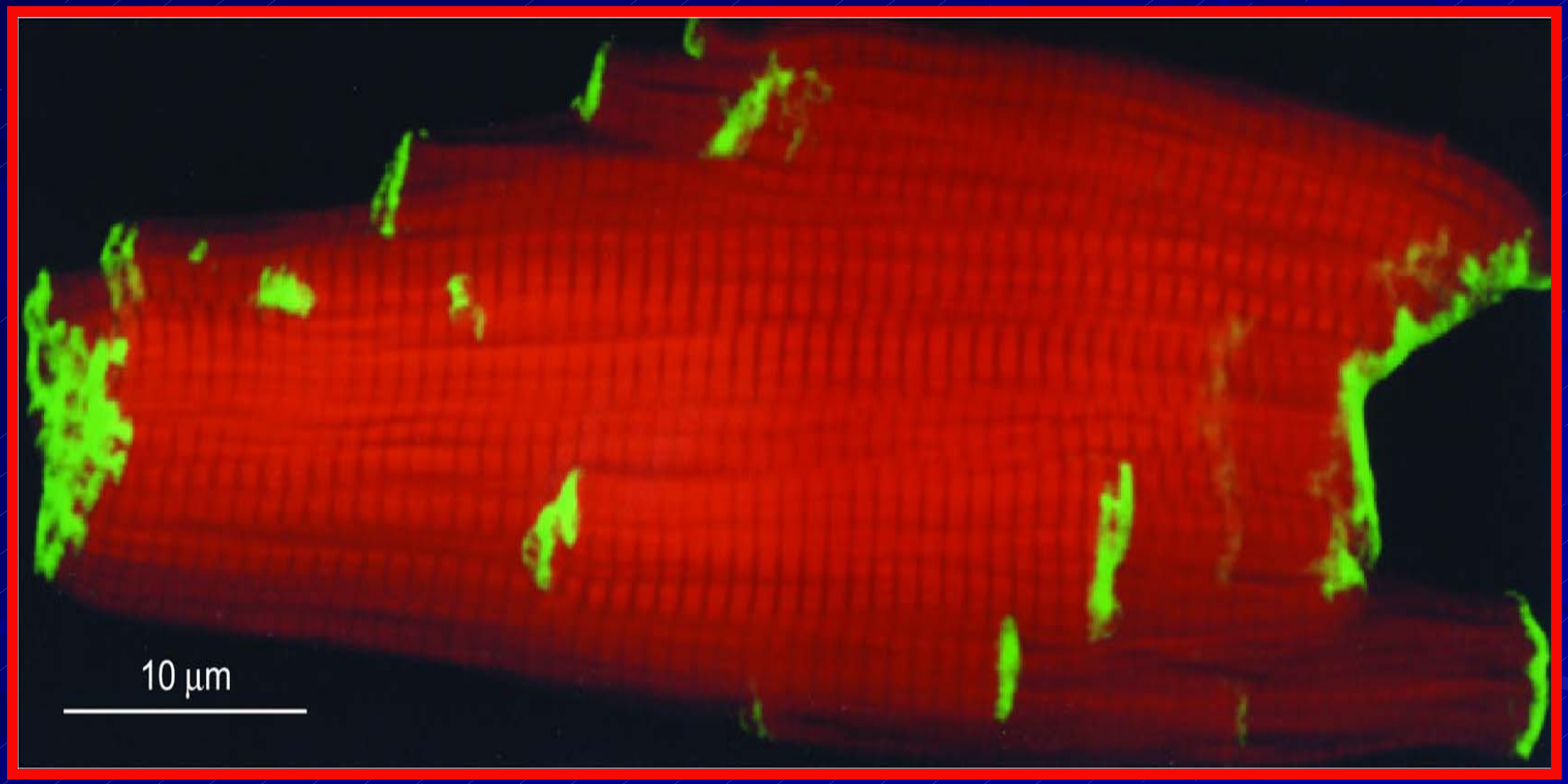


Photomicroscopy

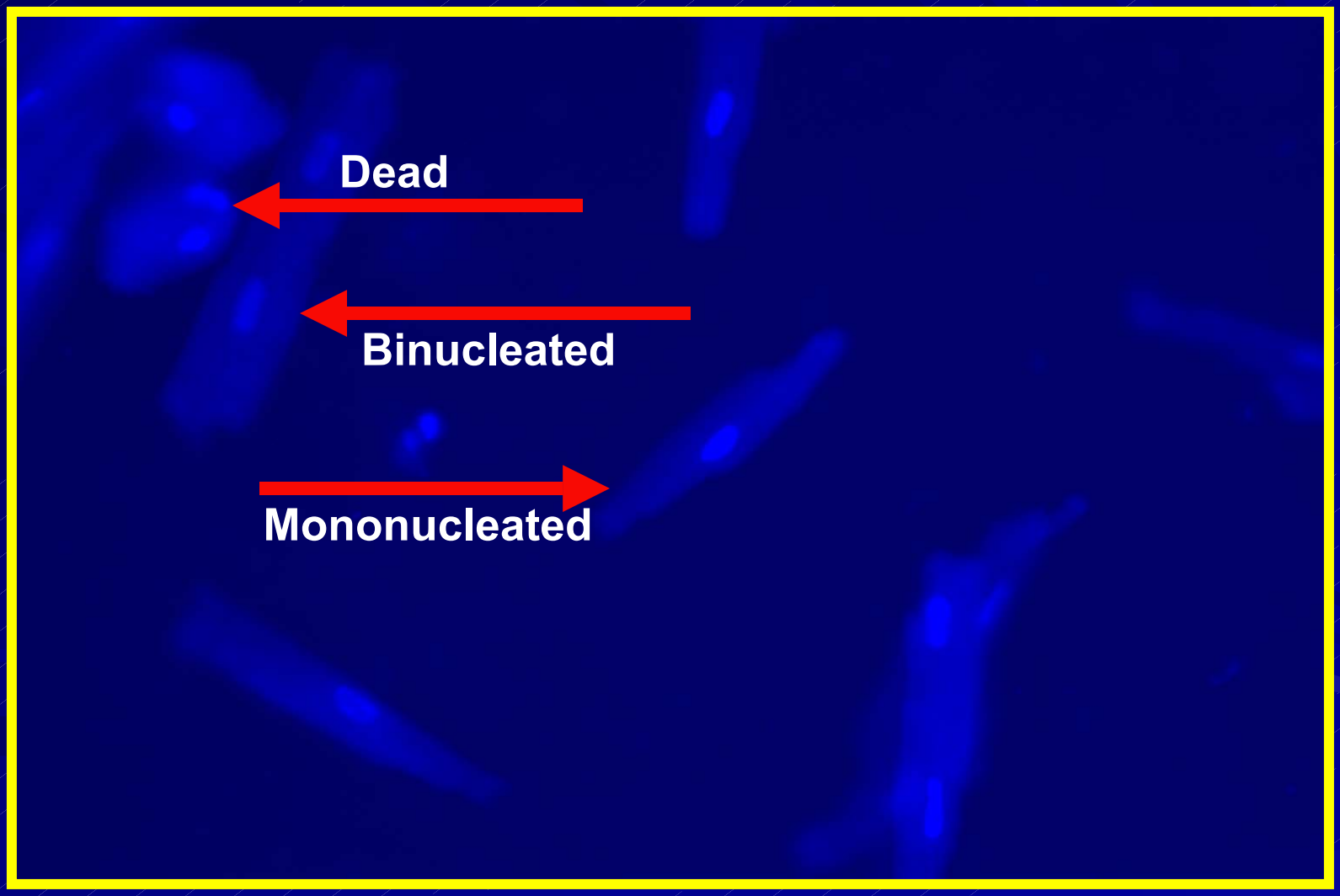
- Cells counted
- *Spot Advance* → cell pictures
- *Image Pro Plus* → cell measurements

Results

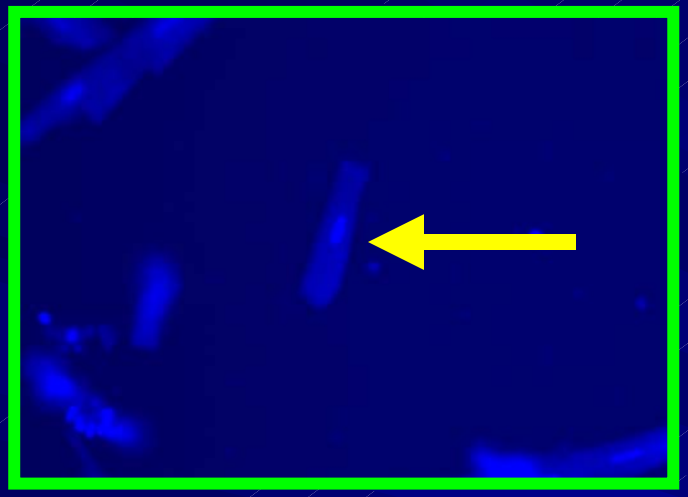
Myocytes Confocol



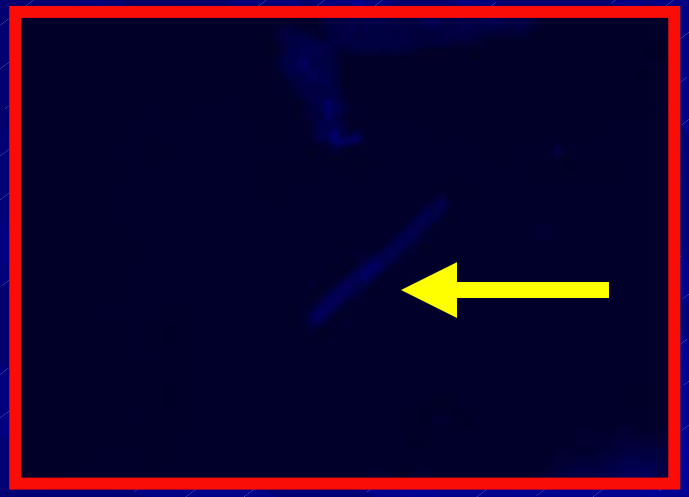
Myocytes DAPI



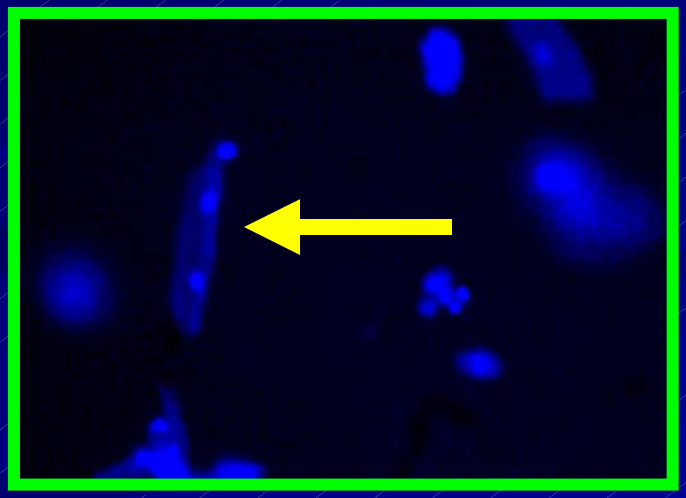
Myocytes

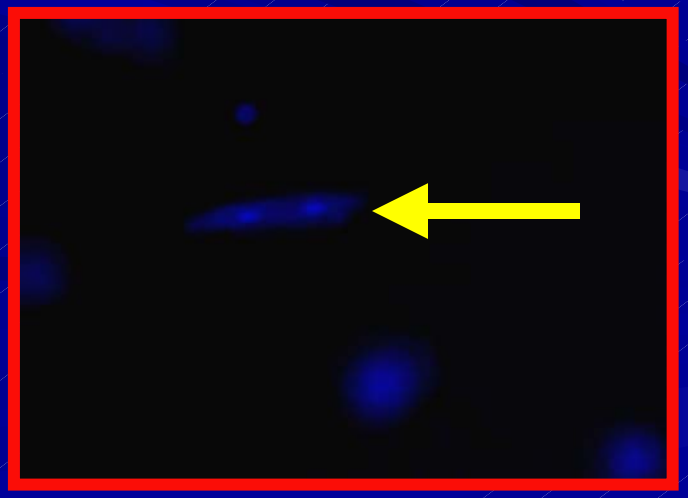


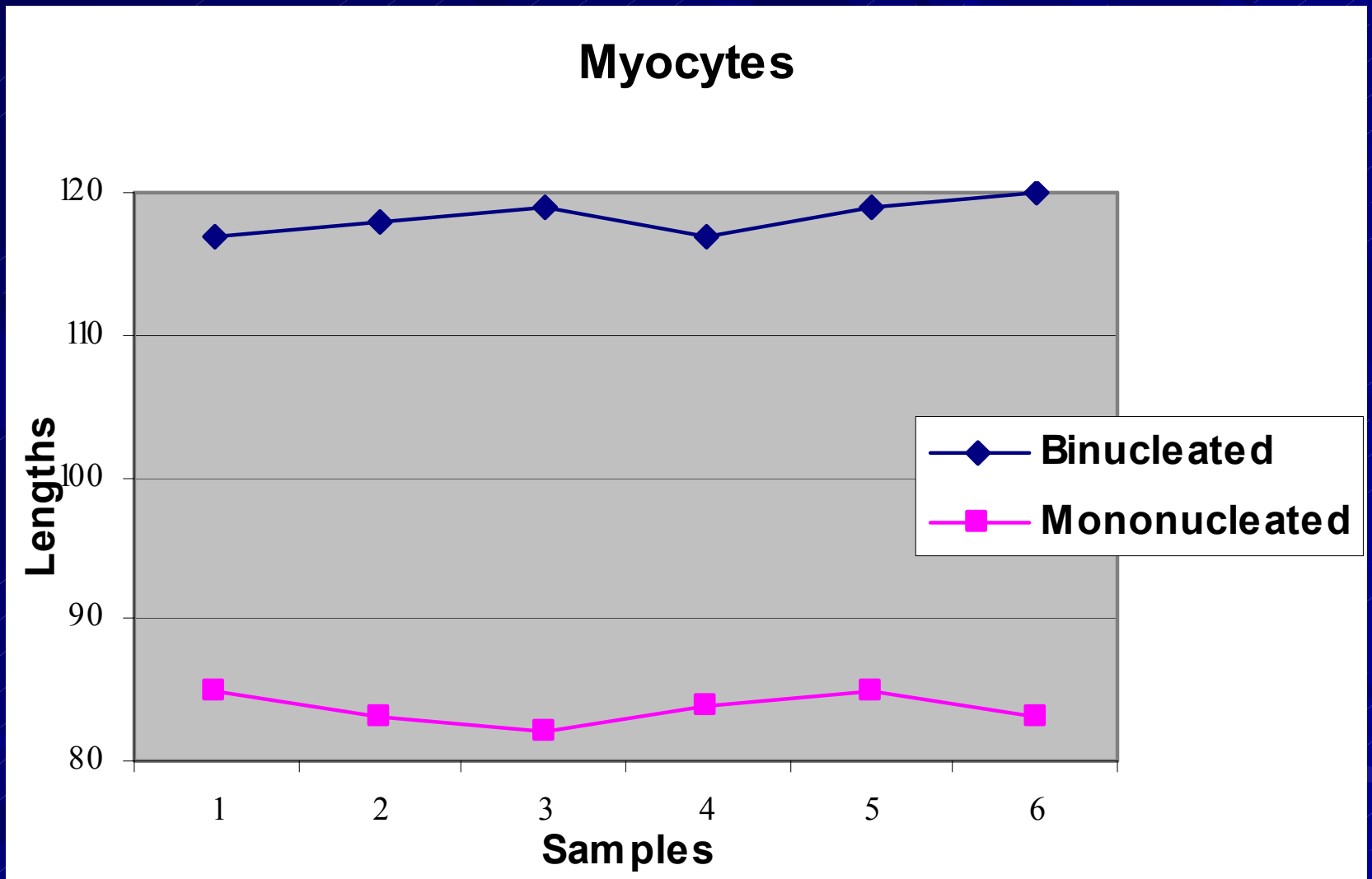
Healthy

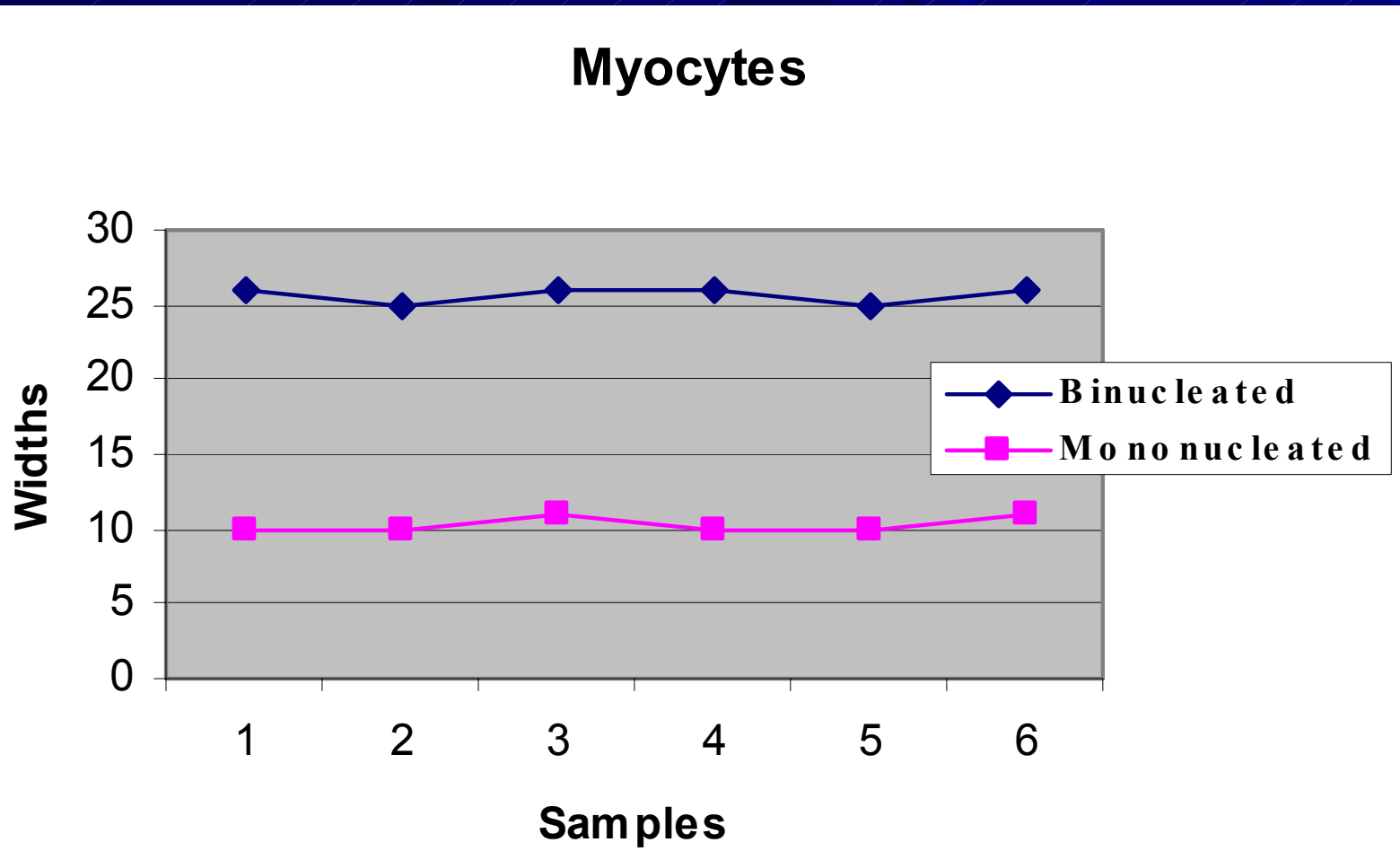


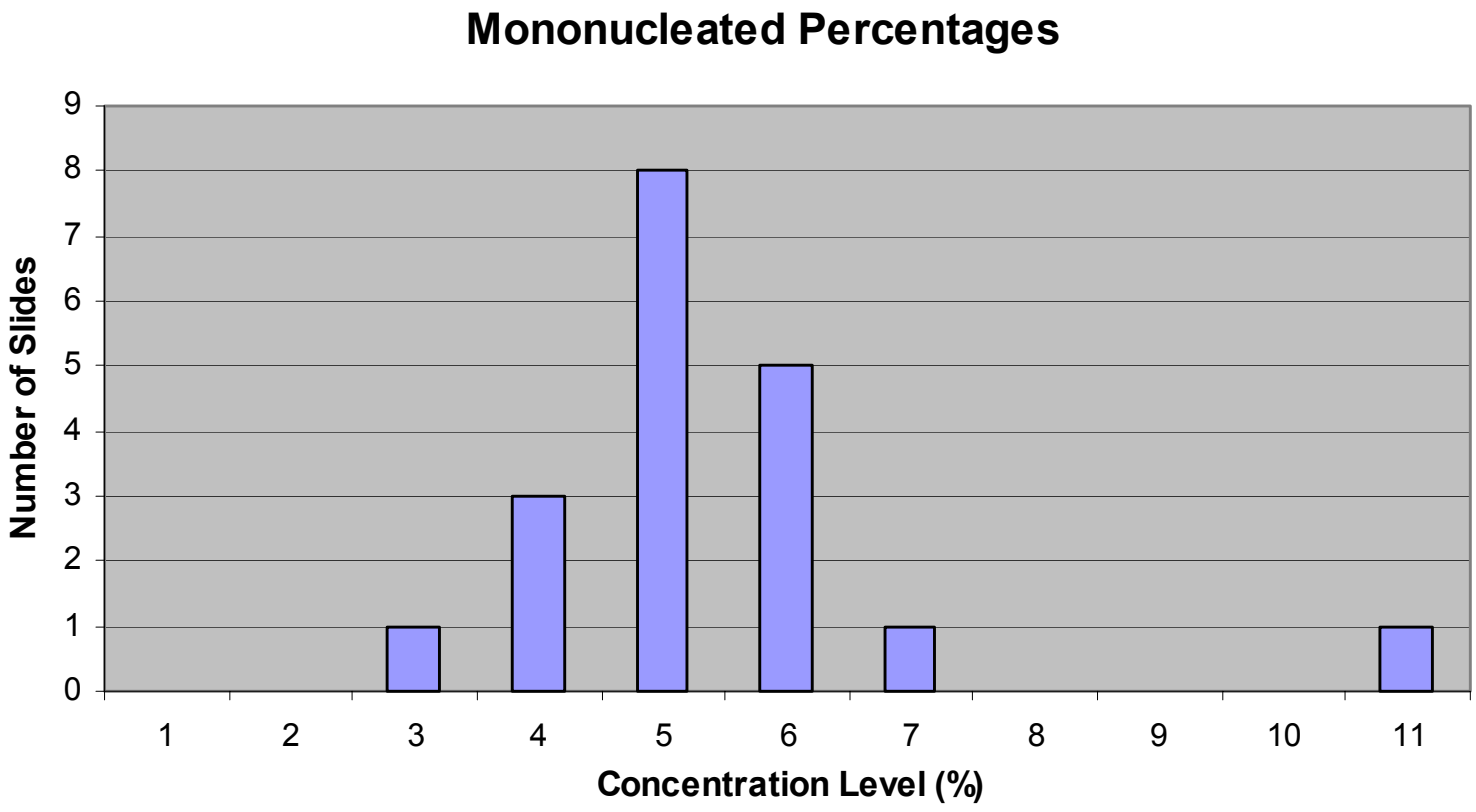
Failing






Myocytes
120
110
100
90
80
Lengths
1
2
3
4
5
6
Samples
Binucleated
Mononucleated


Myocytes
Widths
30
25
20
15
10
5
0
1
2
3
4
5
6
Samples
Binucleated
Mononucleated


Mononucleated Percentages
Number of Slides
0
1
2
3
4
5
6
7
8
9
1
2
3
4
5
6
7
8
9
10
11
Concentration Level (%)

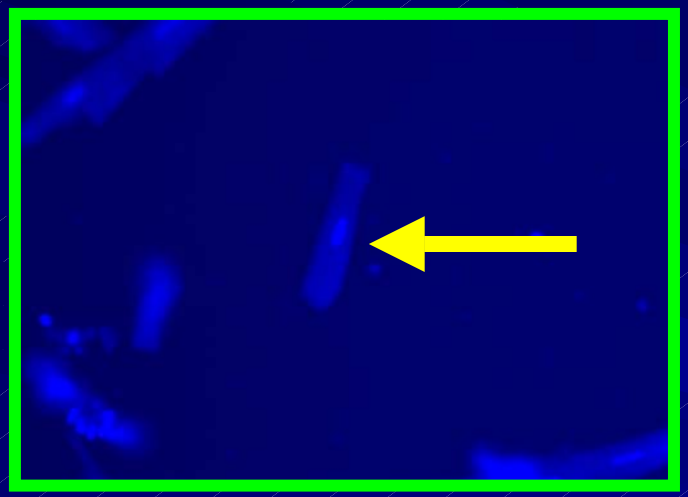
Cell Counts

- Percent mononucleated ranged from 3% - 11% (control)
- Average was between 5 and 6% mononucleated
- Average 2,000+ cells per slide

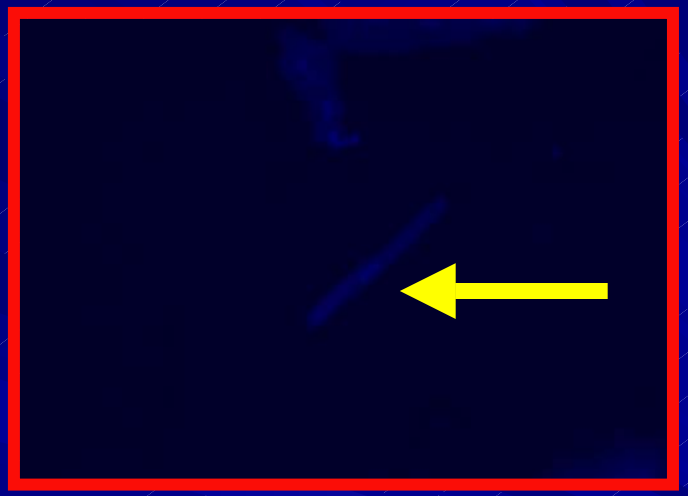
Conclusion

Conclusion

- There is a difference in cell size



Healthy



Failing

- Percent mononucleated was not found in failing myocytes due to time constraints

The Supporting Cast

God	The Creator
Family and Friends	As themselves
Dr. Steve Houser	Mentor
Dr. David Harris	Supporting mentor
Dr. Hsiang-wen Cheng	Supporting mentor
Dr. Hanine Kubba	Supporting mentor
James Moyer	Research supervisor
Remus Berretta	Research coordinator
Geoff Mills	Editor
Natalie Guerrero	Head supporting trainee
MoJaLeCaDiMaJaRi	Supporting trainees
Ben Wong <3	Technical advisor
TMARC peers	As themselves

THANK YOU ALL

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(Credits)

Dr. Adrian Widaya