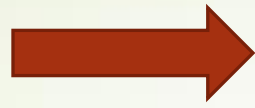


# Innovative Creation of a Laboratory Exercise

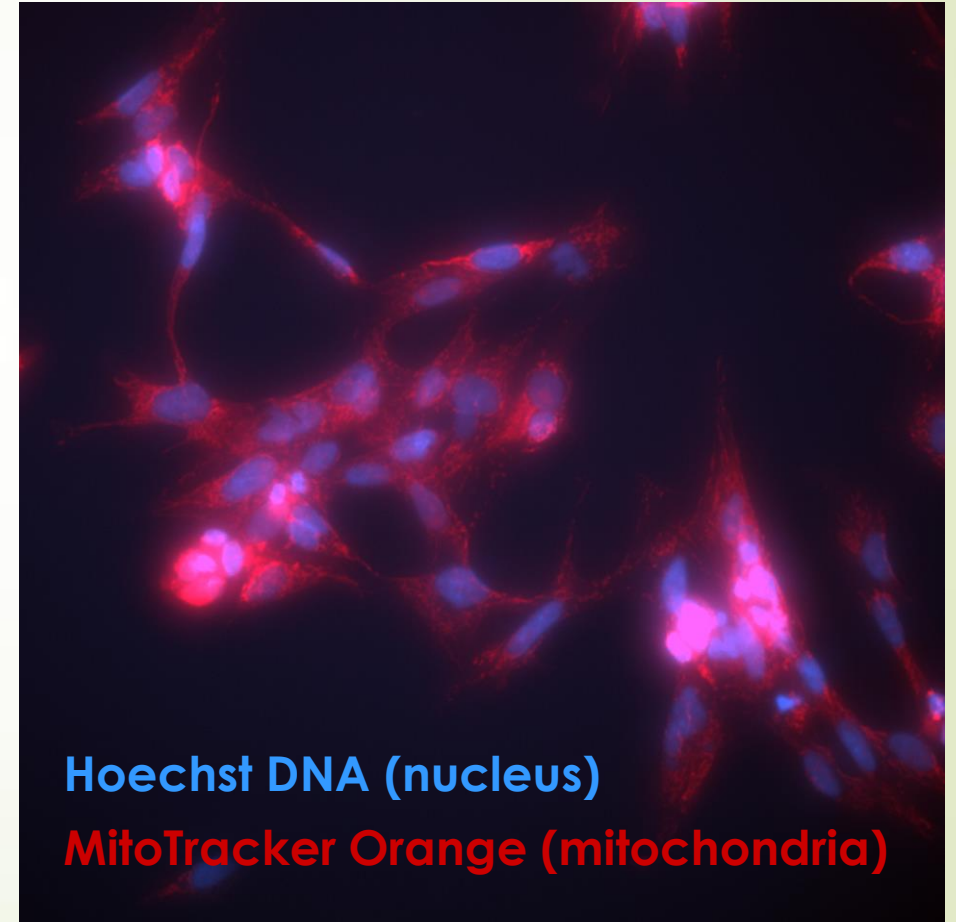
Fluorescent Staining of Primary Chicken Embryo Fibroblast Culture Using MitoTracher and Hoechst Stains

By  
Dwight Ginn, Ph.D.

Goal



Create a new exercise for my Developmental Biology lab using new fluorescent microscope



**Hoechst DNA (nucleus)**

**MitoTracker Orange (mitochondria)**

# New Exercise in Dev. Biology lab

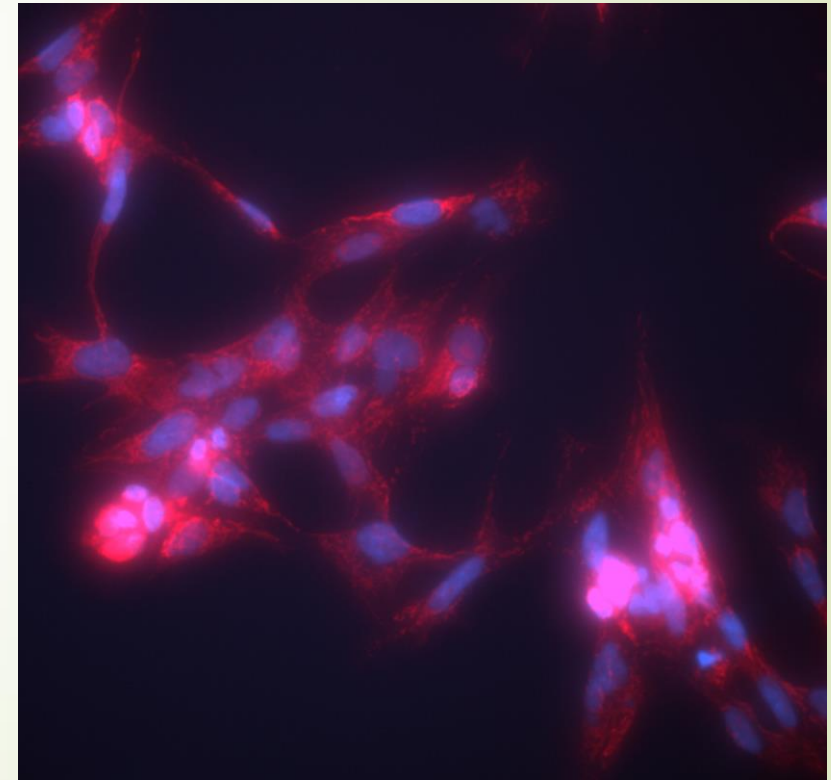
Create fibroblast cell cultures from chicken embryos  
(Existing exercise)



Fluorescent staining of cells  
(New exercise)



Fluorescence microscopy  
(New exercise)

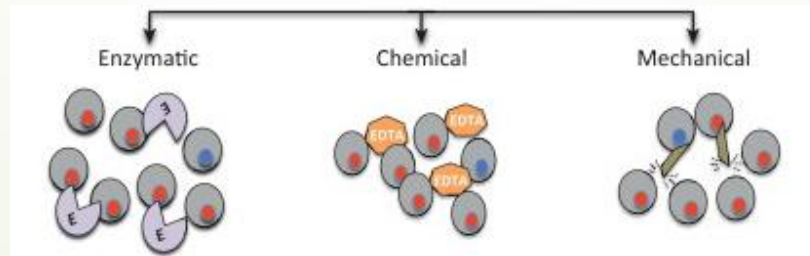
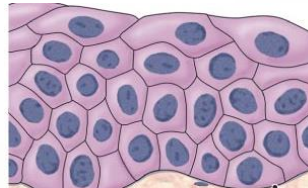




Grow fertilized chicken eggs  
in incubator for ~12 days



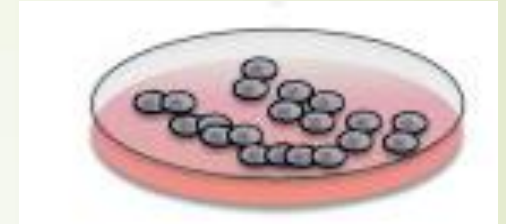
12 day chicken embryo



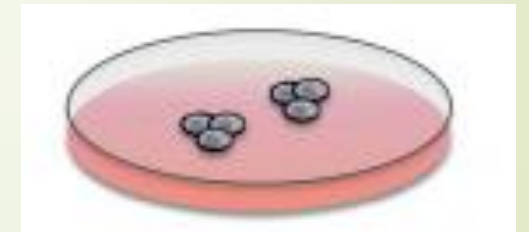
Tissue Disassociation with  
enzymatic, chemical &  
mechanical disruption



Primary chicken embryo  
fibroblast cell cultures  
(by Dr. Margaret Stevens, Ripon  
College)

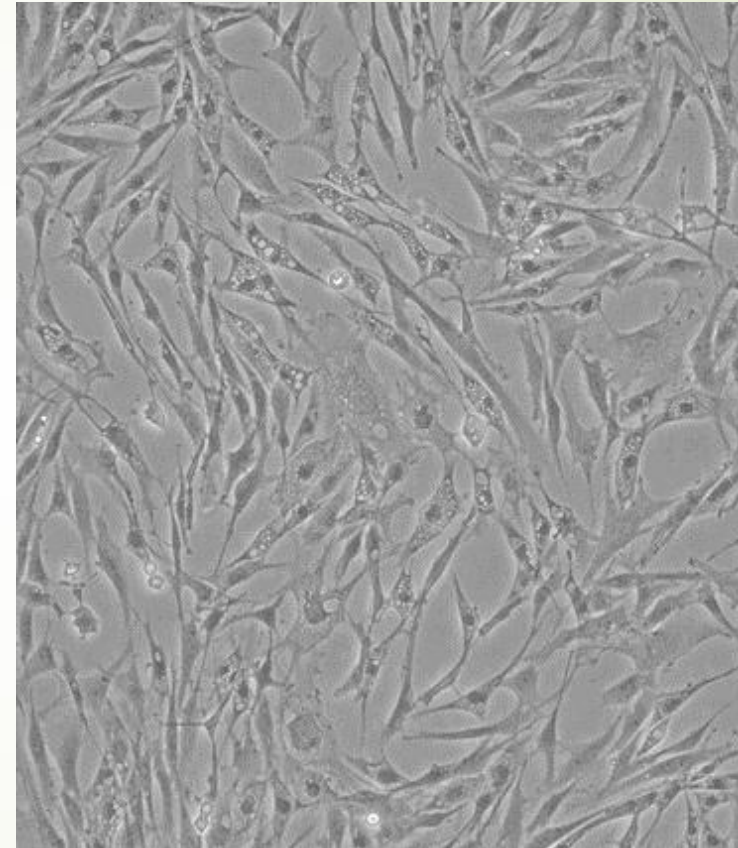
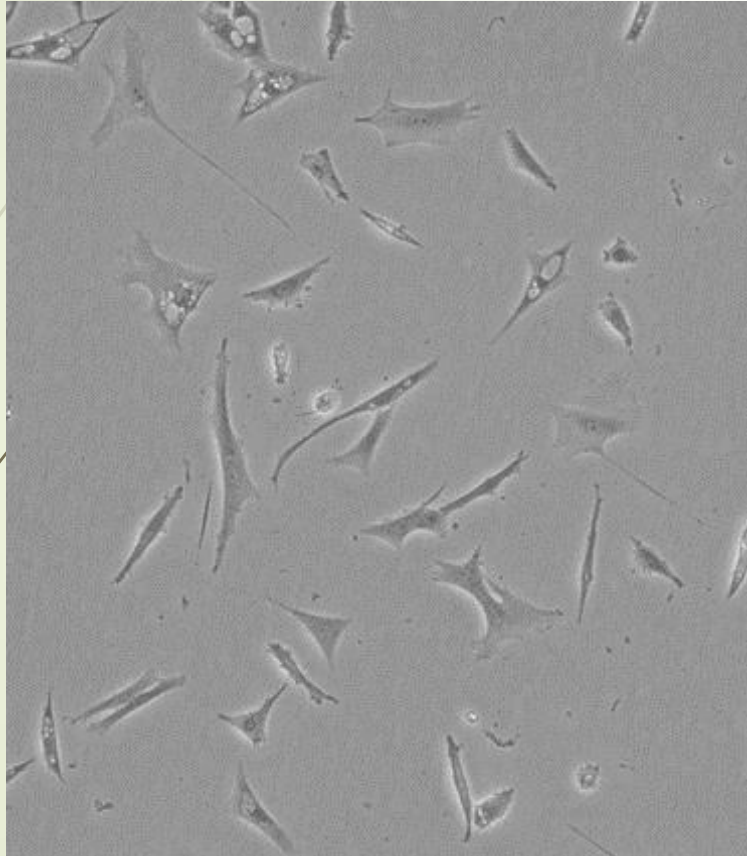


Grow in  
tissue culture

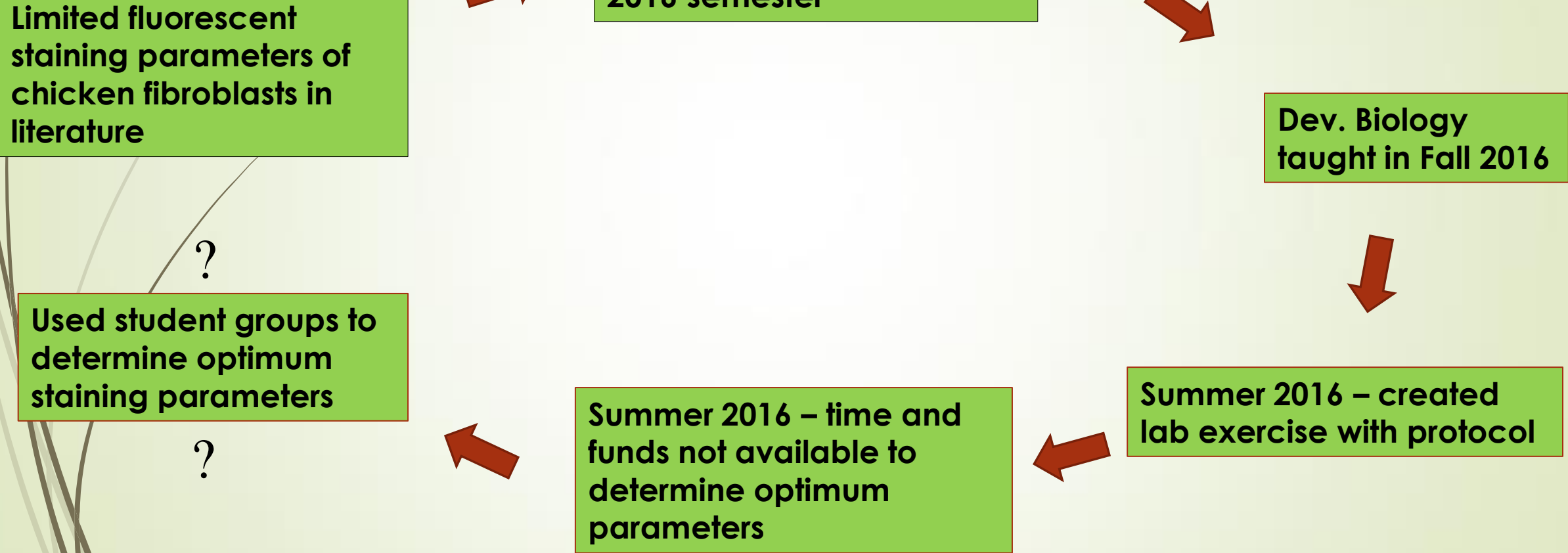




# Primary Chicken Embryo Fibroblast cell culture



# Lab Exercise Development



```
graph TD; A[Limited fluorescent staining parameters of chicken fibroblasts in literature] --> B[Fluorescent microscope purchased early spring 2016 semester]; B --> C[Dev. Biology taught in Fall 2016]; C --> D[Summer 2016 – created lab exercise with protocol]; D --> E[Summer 2016 – time and funds not available to determine optimum parameters]; E --> F[Used student groups to determine optimum staining parameters]; F --> A;
```

Limited fluorescent staining parameters of chicken fibroblasts in literature

Fluorescent microscope purchased early spring 2016 semester

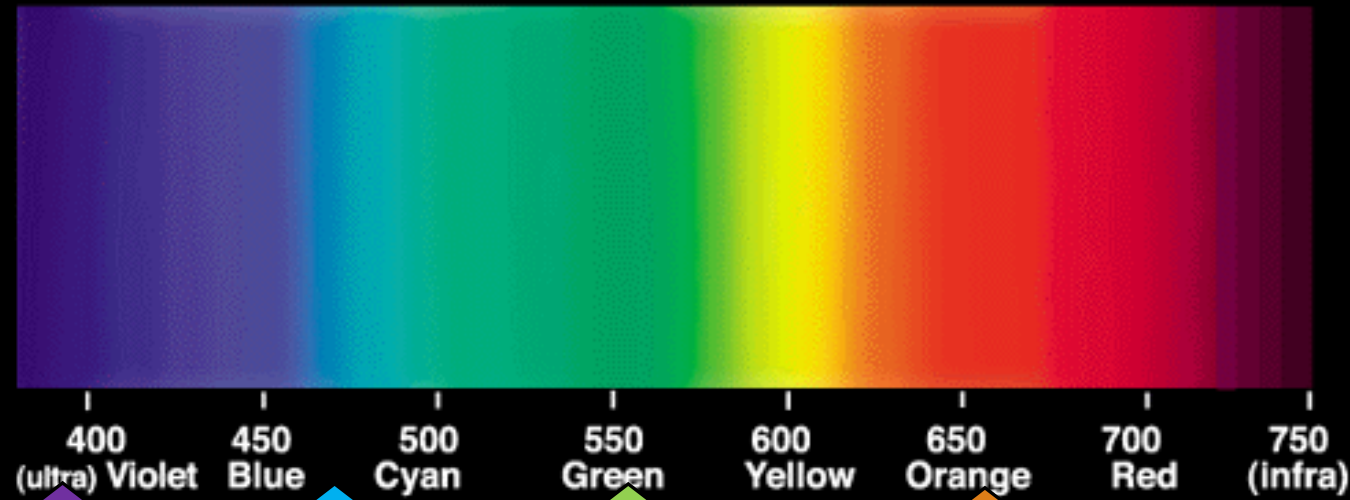
Dev. Biology taught in Fall 2016

Summer 2016 – created lab exercise with protocol

Summer 2016 – time and funds not available to determine optimum parameters

Used student groups to determine optimum staining parameters

## THE VISIBLE SPECTRUM • Wavelength in Nanometers



Exc.

Hoechst DNA  
(nucleus)

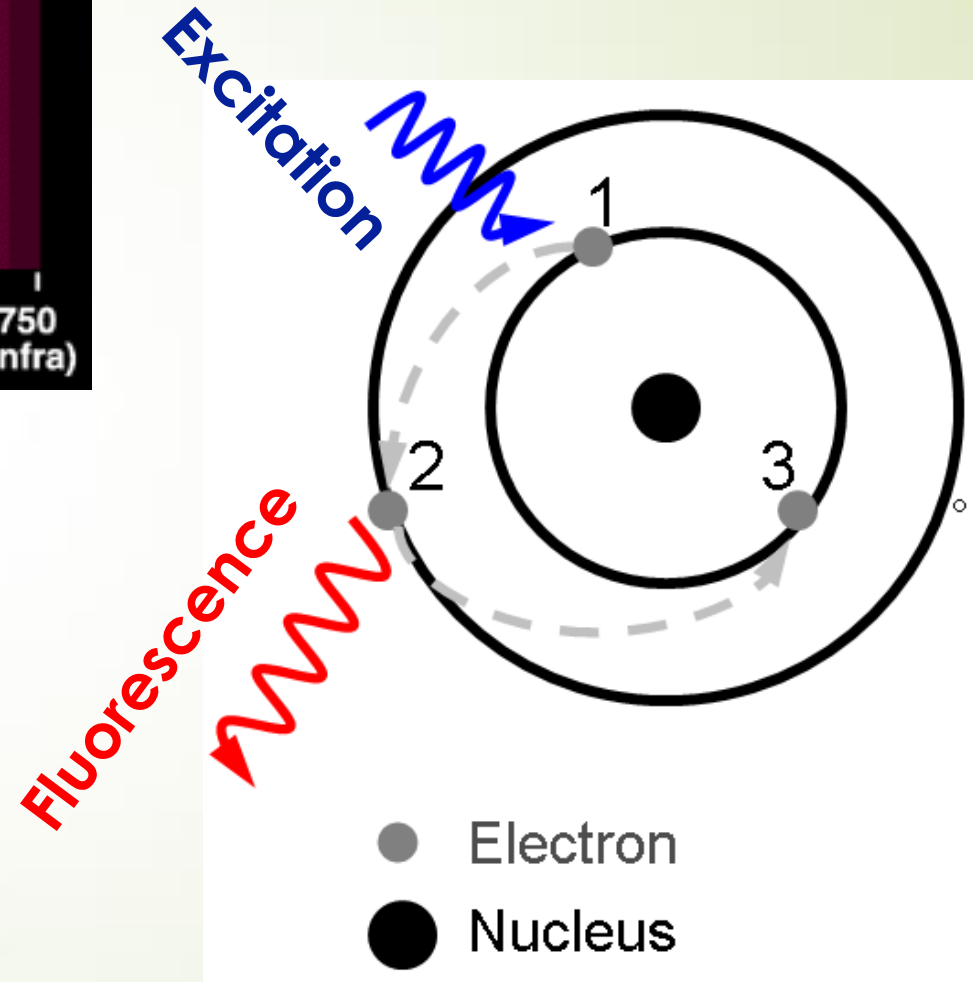
Fluor.

Exc.

MitoTracker Orange  
(mitochondrion)

Fluor.

## Fluorescent Stains



Grow chicken embryo  
fibroblast cell culture

## Fluorescent Staining Procedure

Add MitoTracker Orange  
stain to living cell culture

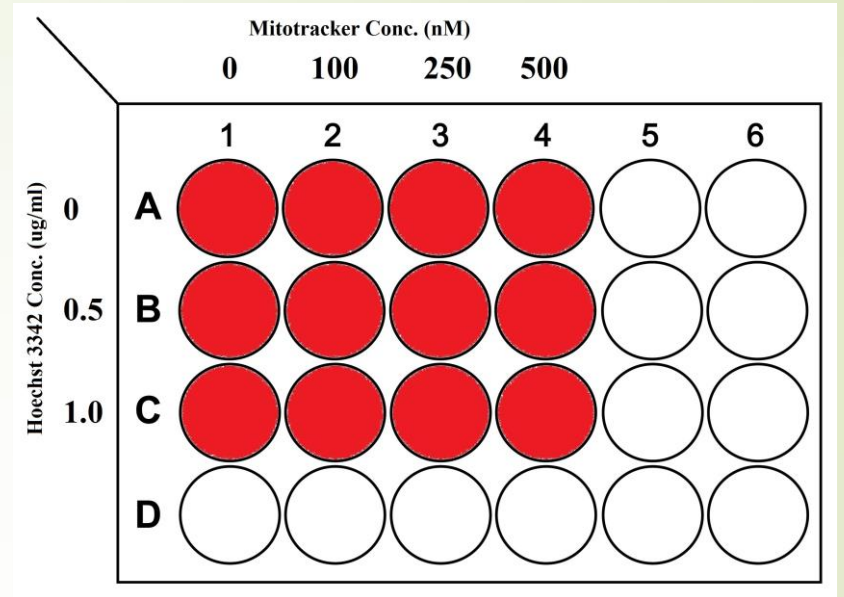
(100 nM, 250 nM, or 500 nM)  
(15, 30 or 45 min incubation)

Fixation with 3.7% formaldehyde

Permeabilization 0.2% Triton X-100

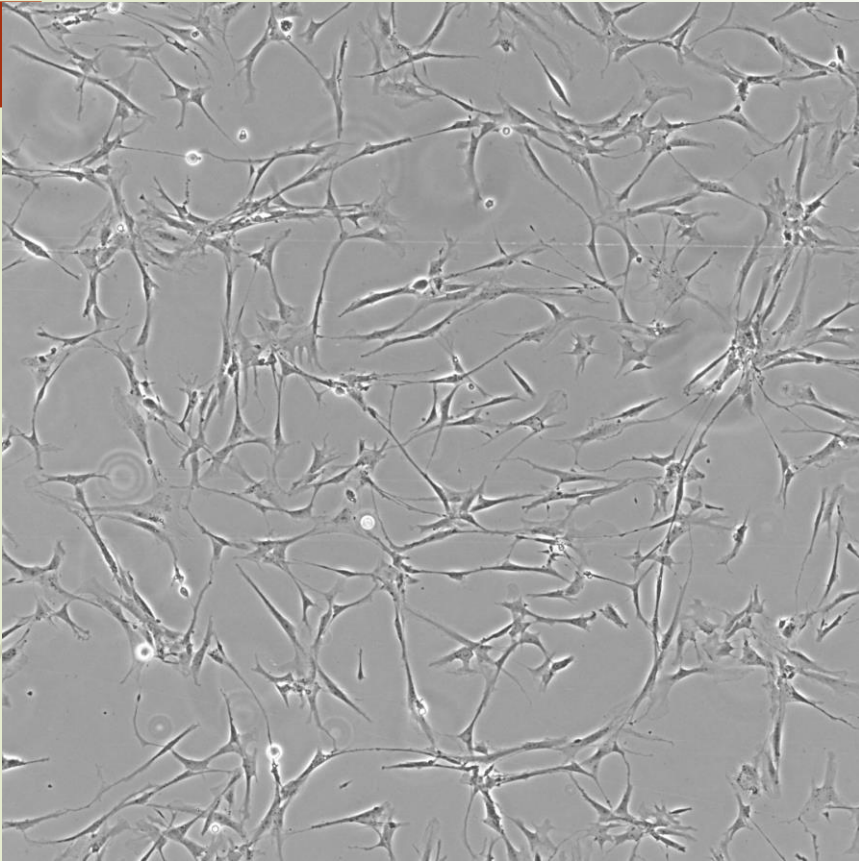
Counterstain with Hoechst DNA stain  
(0.5  $\mu\text{g/ml}$  or 1.0  $\mu\text{g/ml}$ )

Fluorescence microscopy

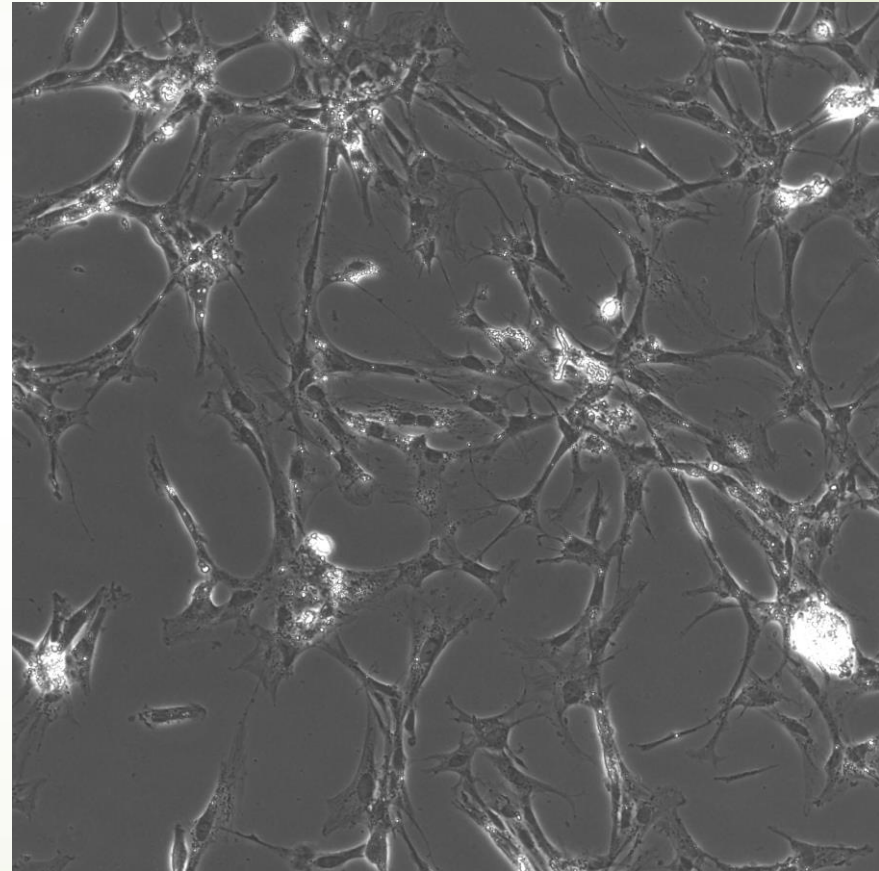




# Primary chicken embryo fibroblast cell cultures

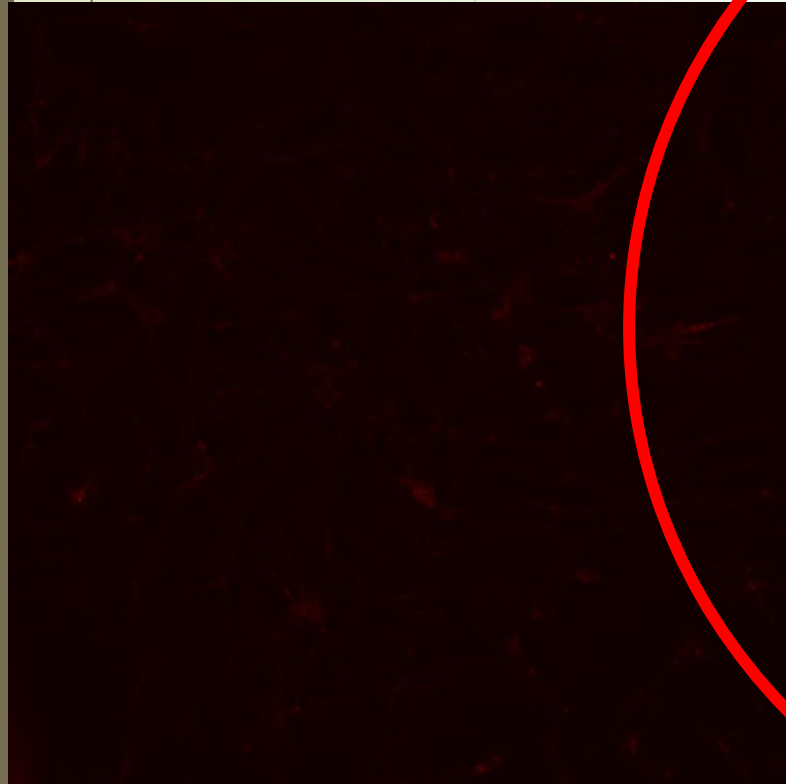


200x

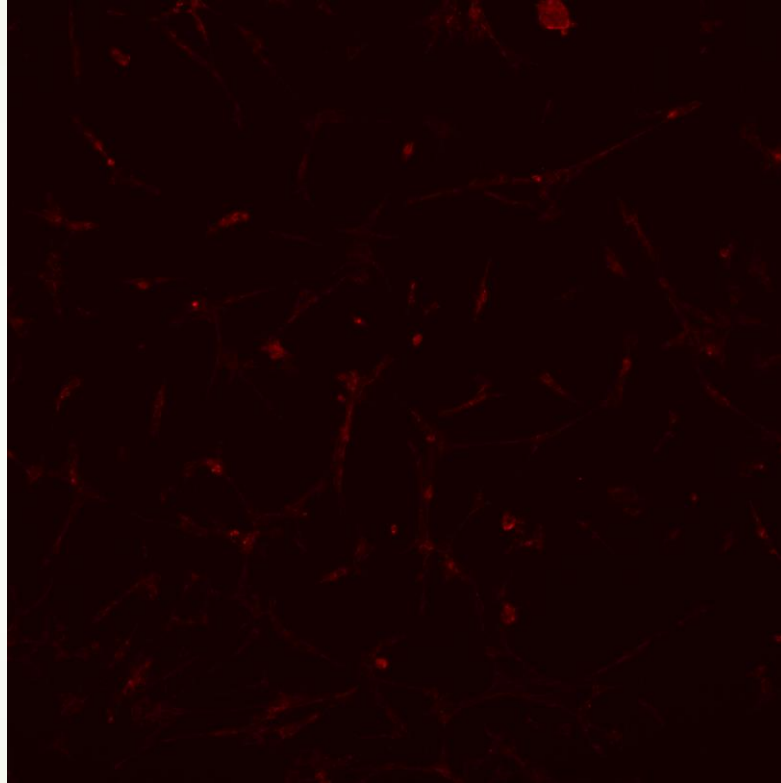


400x

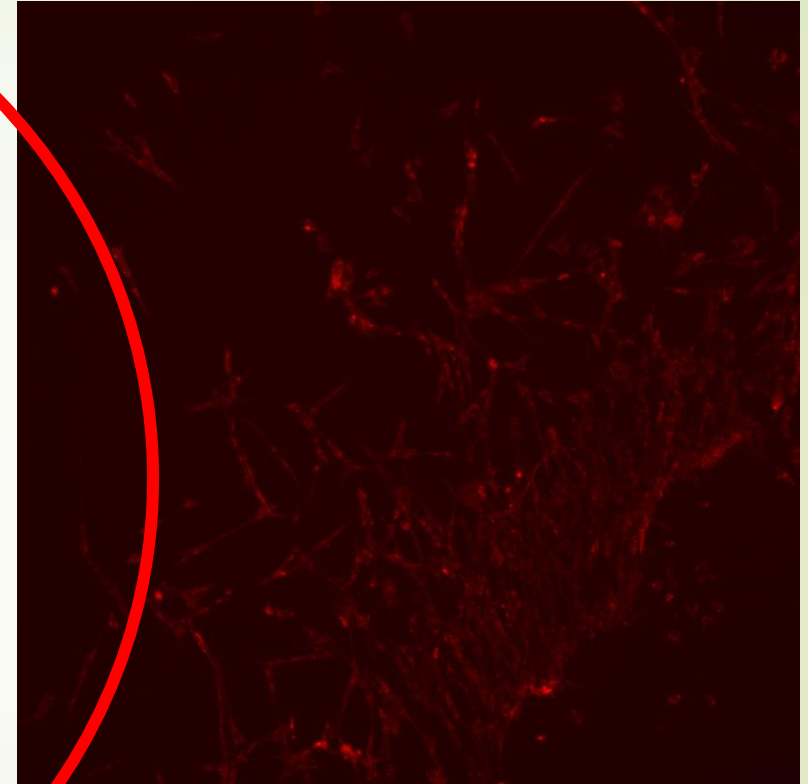
# Best MitoTracker Orange Concentration ?



100 nM



250 nM

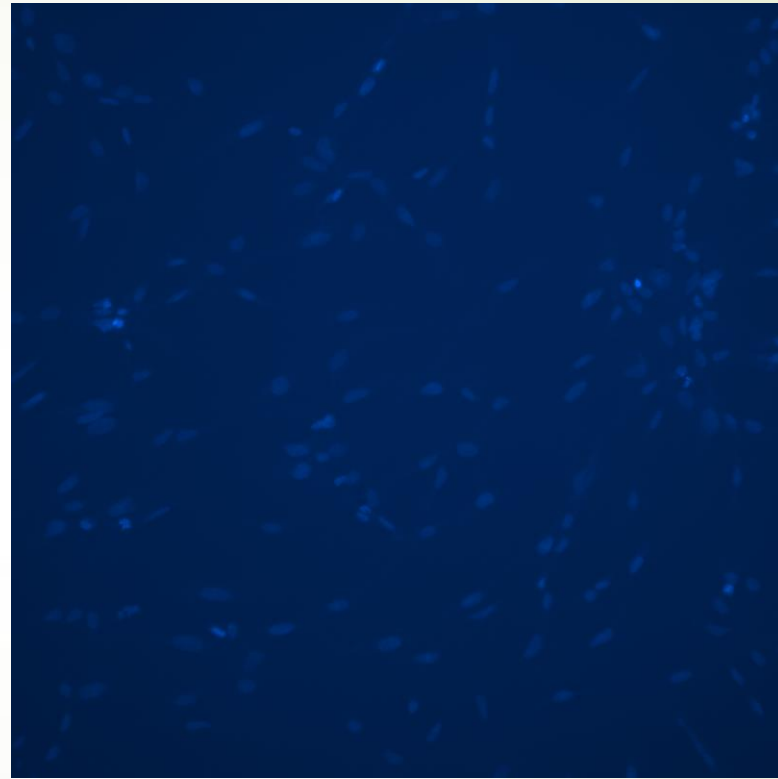


500 nM

# Best Hoescht DNA stain concentration ?



0.5 ug/ul



1.0 ug/ul

Best Mitotracker Orange incubation time ?

**45 minutes**

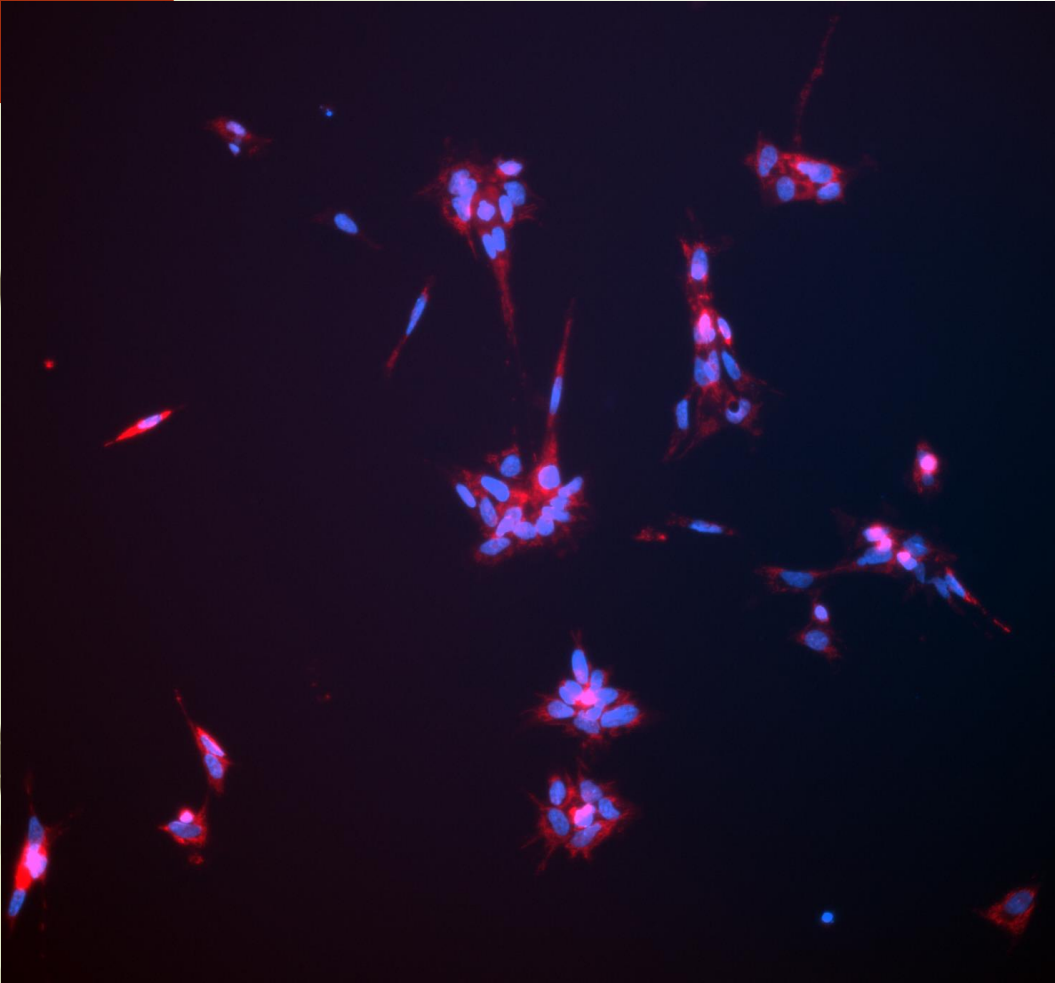
**30 minutes**

**15 minutes**

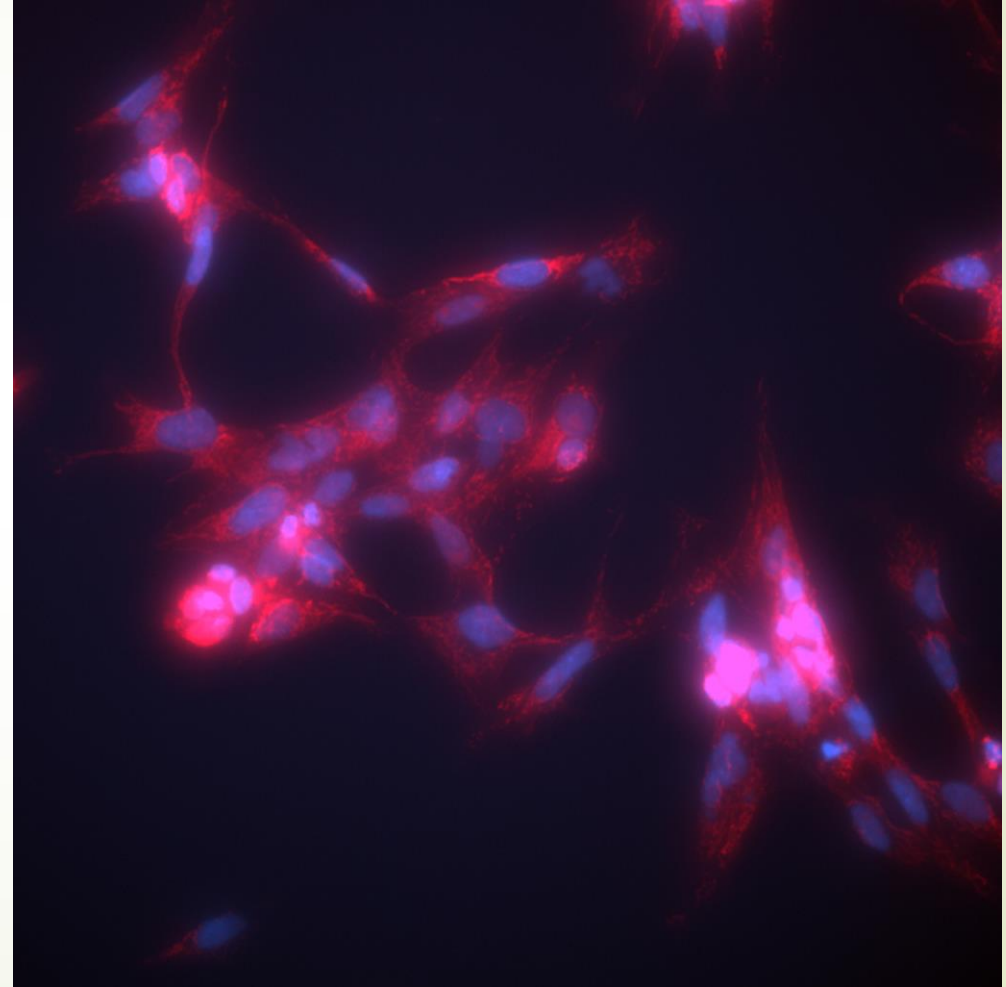


## Group 1 cells

## Fluorescent Staining of Primary chicken embryo fibroblast cell cultures

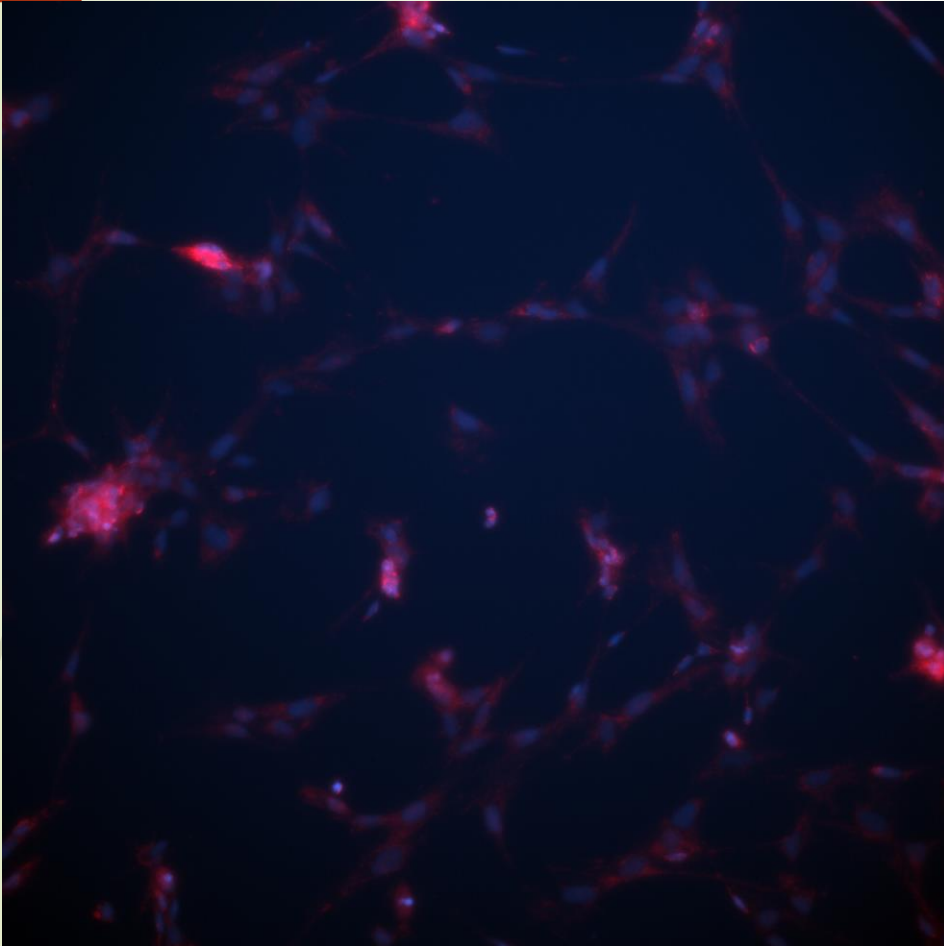


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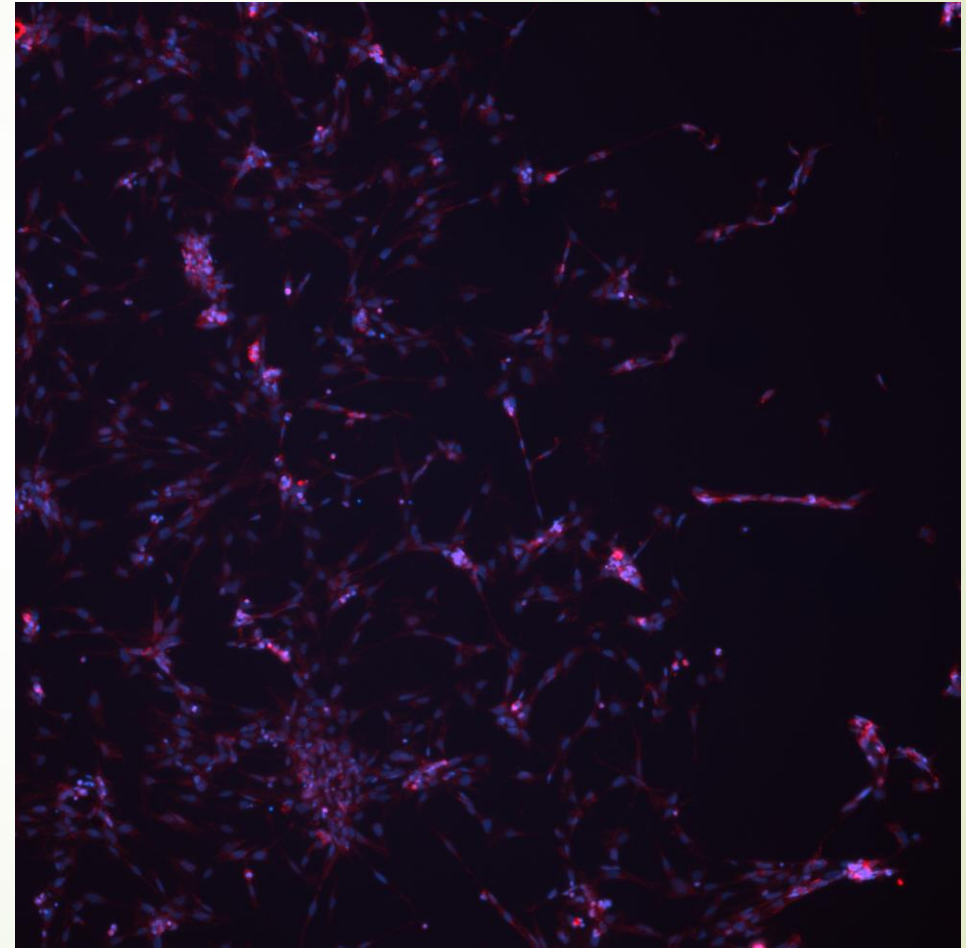


400x

Group 2 cells

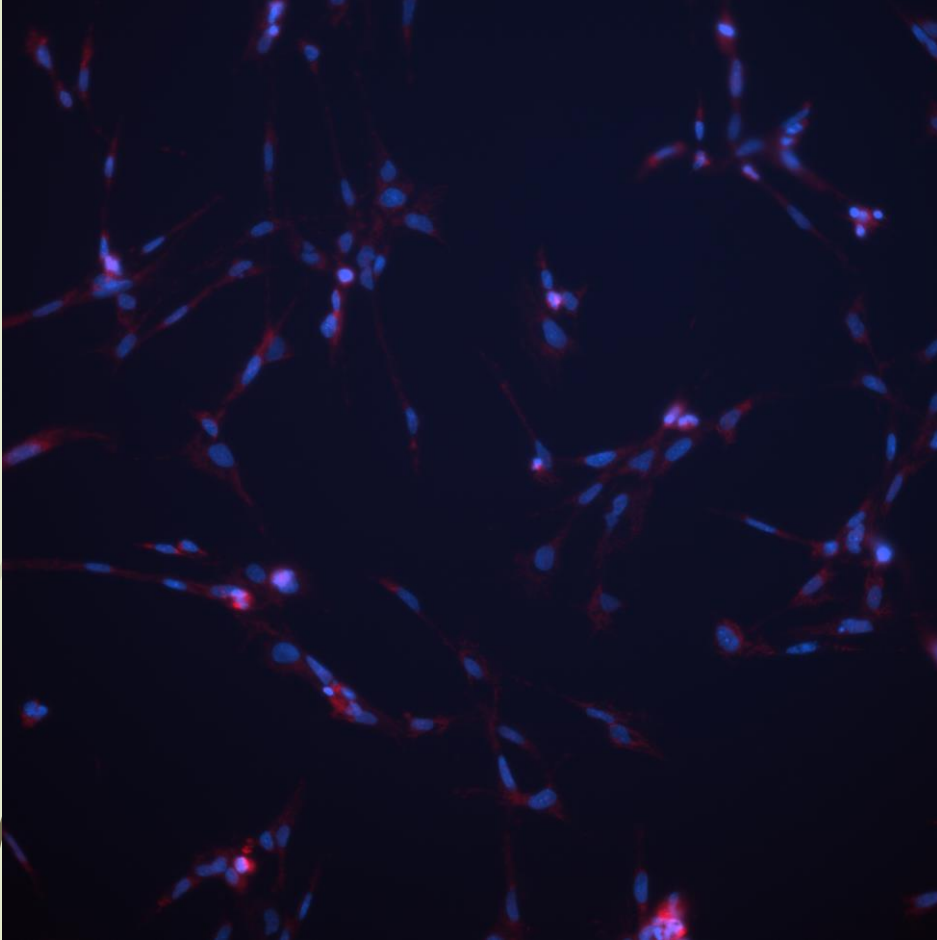


Fluorescent Staining of  
Primary chicken embryo  
fibroblast cell cultures

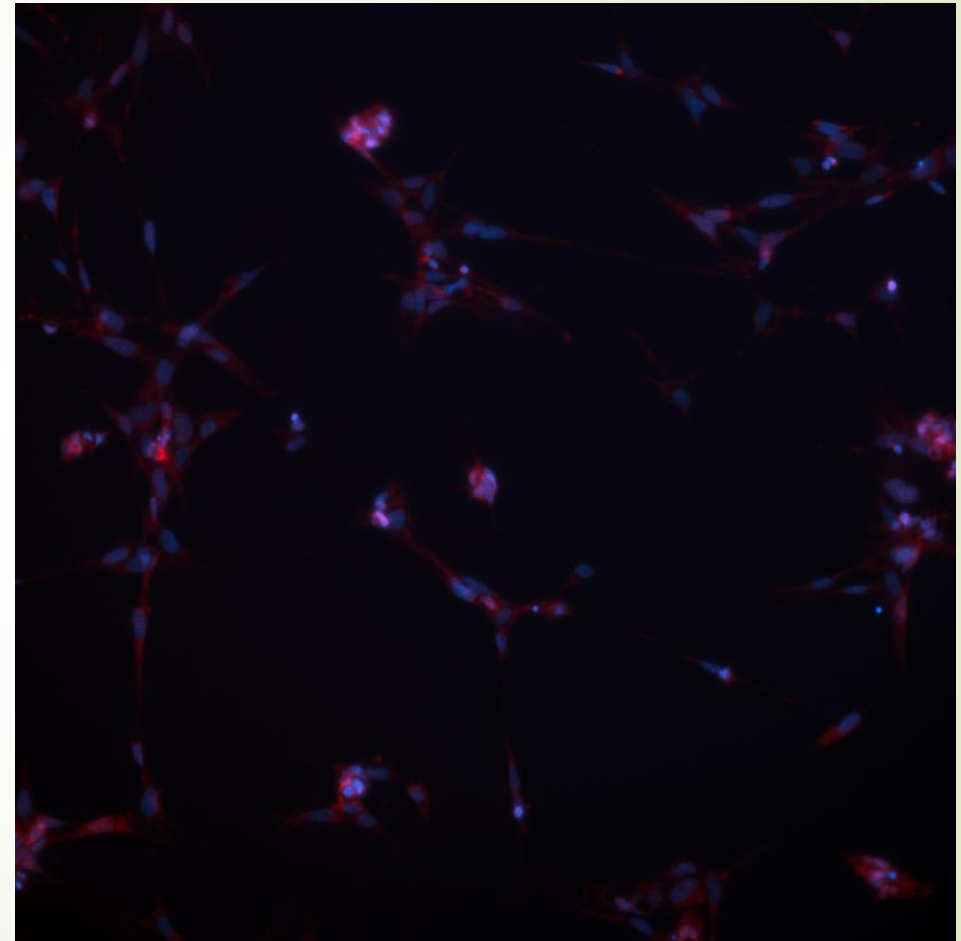


Group 3 cells

Group 4 cells



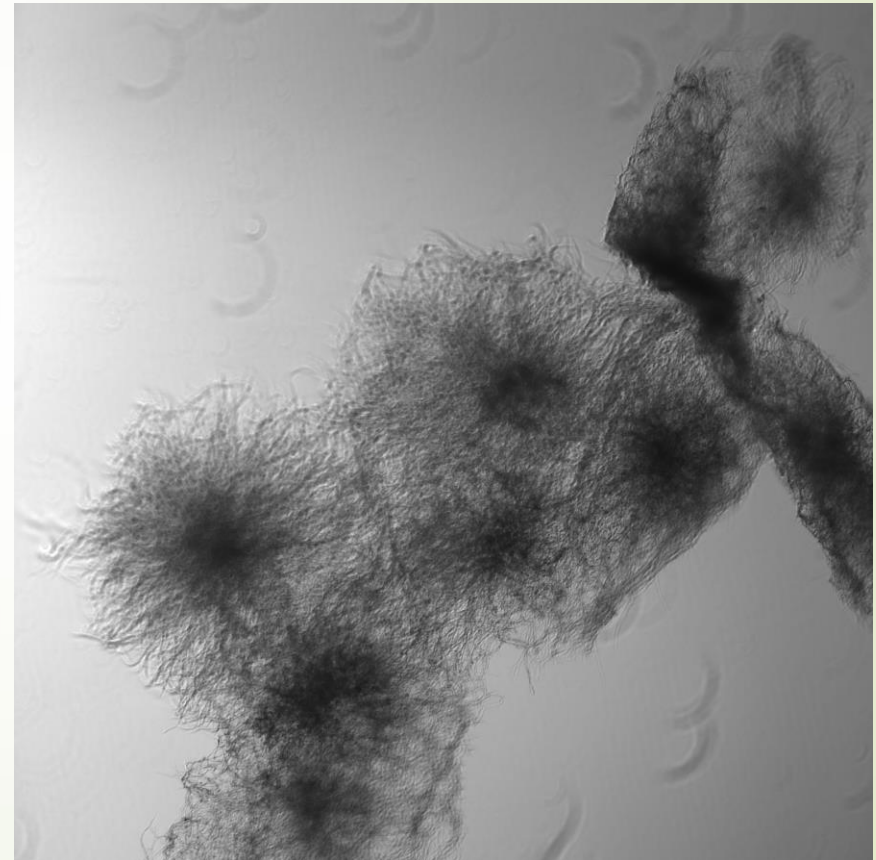
Fluorescent Staining of  
Primary chicken embryo  
fibroblast cell cultures



Group 5 cells

Group 6 cells

Fluorescent Staining of  
Primary chicken embryo  
fibroblast cell cultures







# Fluorescent Staining of Primary chicken embryo fibroblast cell cultures

- Conclusions:
  - Development Process worked quite well
  - Optimum staining parameters determined
    - 250 nM MitoTracker & ~20 minutes incubation, 1.0 ug/ml Hoechst
- Benefits for students:
  - Experience with New technique/instrument
  - Experience process of optimizing staining parameters
  - One of first course labs to use fluorescent microscope
- Additions/Improvements
  - Add immunostaining protocol
  - Use same development technique?



# Questions?