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# Are Cell Death Proteins/Antigens Found on Interdigital Cells Dying During Limb Development Expressed in a Simple Organism Such as Tetrahymena?

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## Introduction

Apoptosis (cell death) occurs naturally and is a programmed event that is present during organ and tissue development such as the heart, synapses, and limbs. Apoptosis also is a common event that results from injury or disease, such as ischemia and cancer. Hallmarks of death include DNA fragmentation and cell surface expression of new protein molecules that participate in the removal of dying cells. The present poster describes a unique monoclonal antibody, B2AX4, that binds specifically to dying cells in the interdigital region of chick limbs during the programmed cell death on days 7-9.5. In order to characterize and isolate the unique cell death antigen we have selected a simple organism *Tetrahymena thermophila* that also dies by apoptosis under specific stimulation or stress. Various techniques including fluorescence microscopy, protein isolation and western blot analysis, indicates that B2AX4 appears to recognize a similar antigen in *Tetrahymena thermophila*.

Our aim is to verify the nature of the antigen through sequence, function, and timing of its expression. The *Tetrahymena thermophila* will serve as a model organism to further elucidate of the protein's role in apoptosis, is it a marker or does it initiate the process.

## Methods

### Western Blot Analysis:

Protein lysates were collected on day 7 of development, and prepared for standard electrophoresis and western blotting procedures. Tetrahymena lysates were collected under the similar conditions although specific time points are being established to enrich for apoptosis. Various gel electrophoresis experiments were conducted to verify size of protein, as initial experiments suggested a higher and lower molecular weight product.

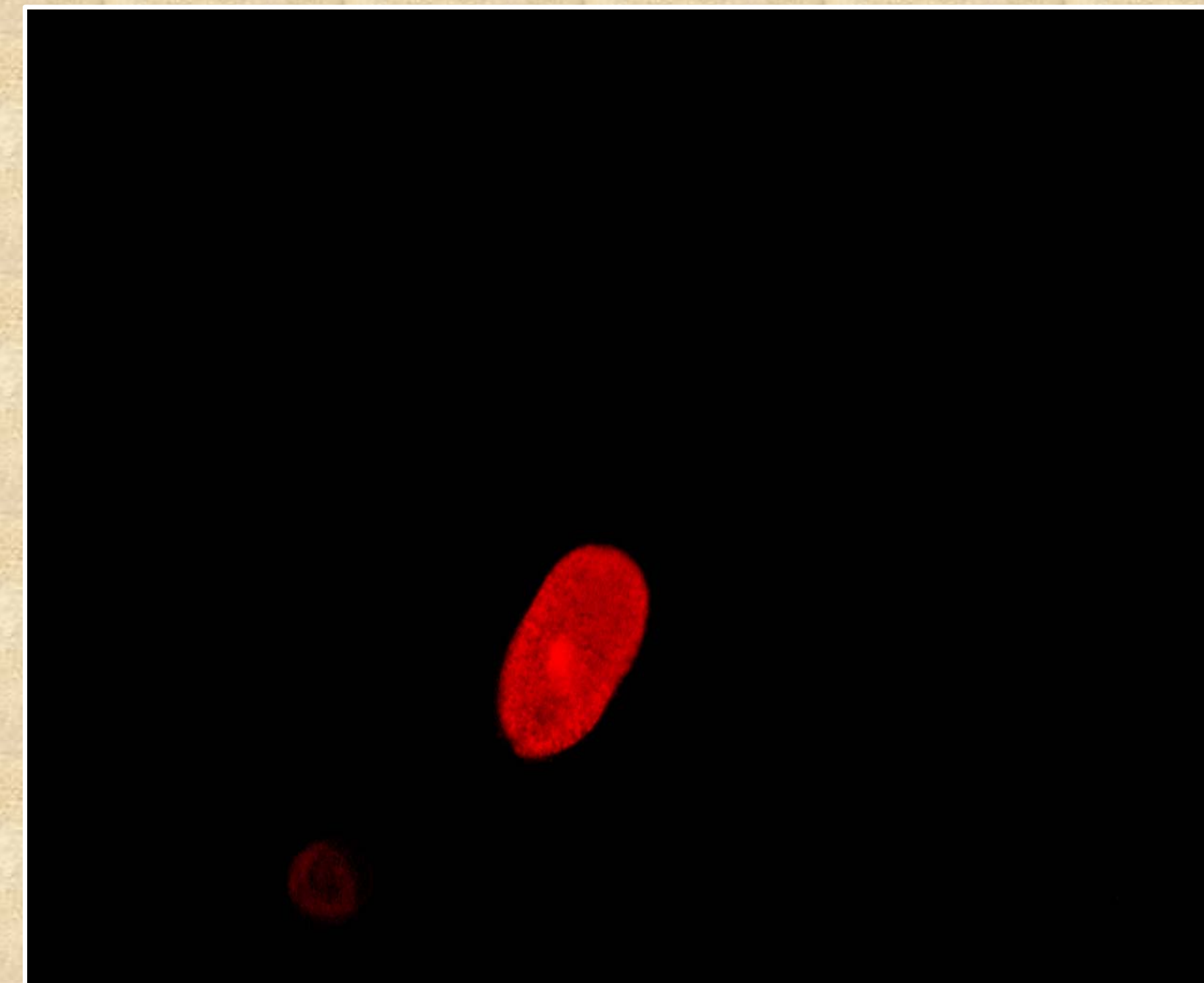
### Microscopy:

Tetrahymena apoptosis was determined using confocal microscopy. Healthy cells show a pear-like shape with intact cell membranes, while those undergoing apoptosis show blebbing, loss of membrane integrity, and, in later stages, cytosolic leakage.

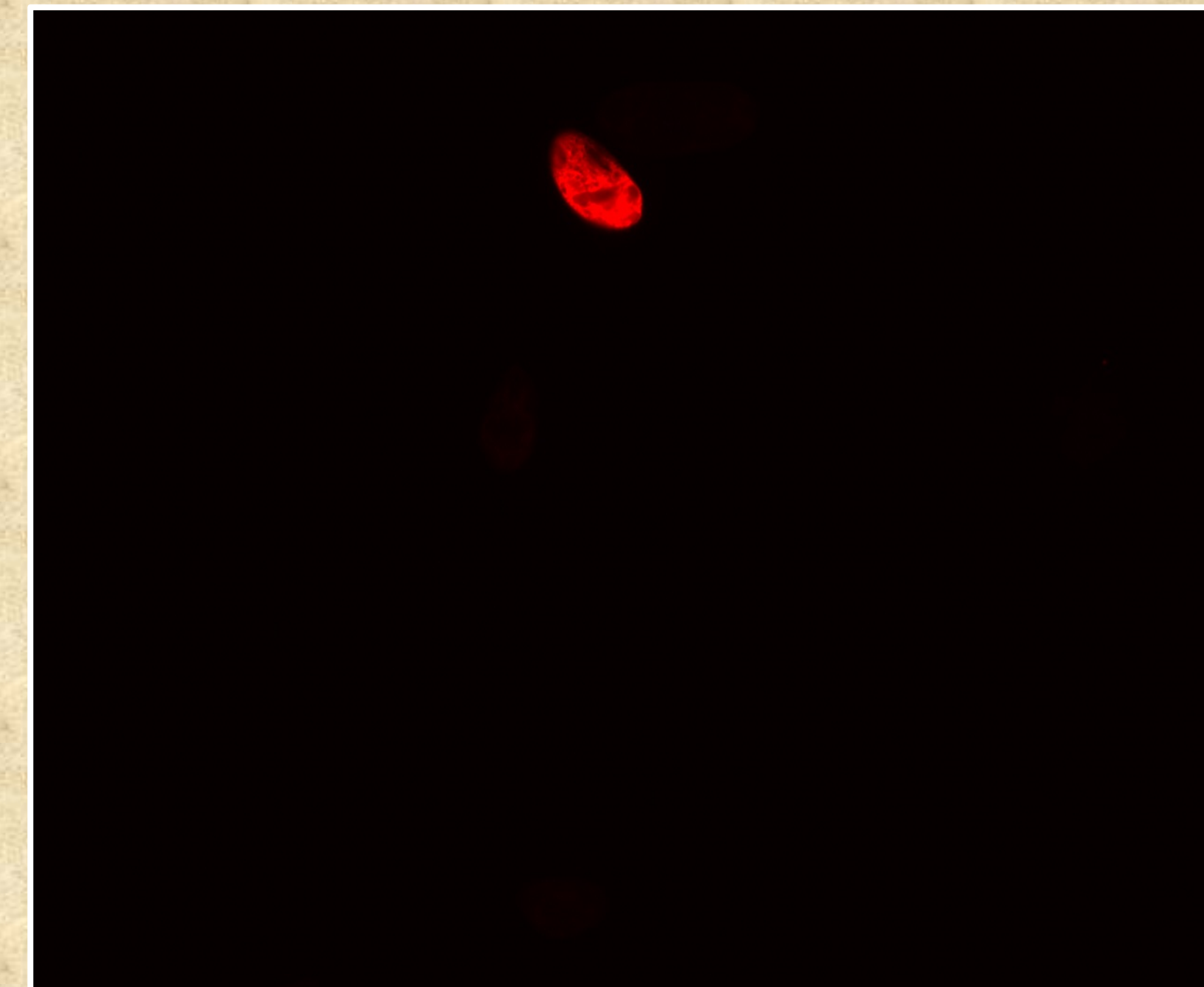
### Apoptosis Induction Methods:

All work is in progress. Cells are separately treated with 500 nM of staurosporine, a kinase inhibitor, and proprietary phosphatase inhibitor. At t=0 hours and 5 hours, two samples are taken: One is fixed to examine cell death morphologically, using confocal microscopy, and the other is processed using protein lysis methods for further analysis of protein size and charge. The western blot is used as an assay to compare the presence or absence of proteins expressed during apoptosis and control samples without cell death.

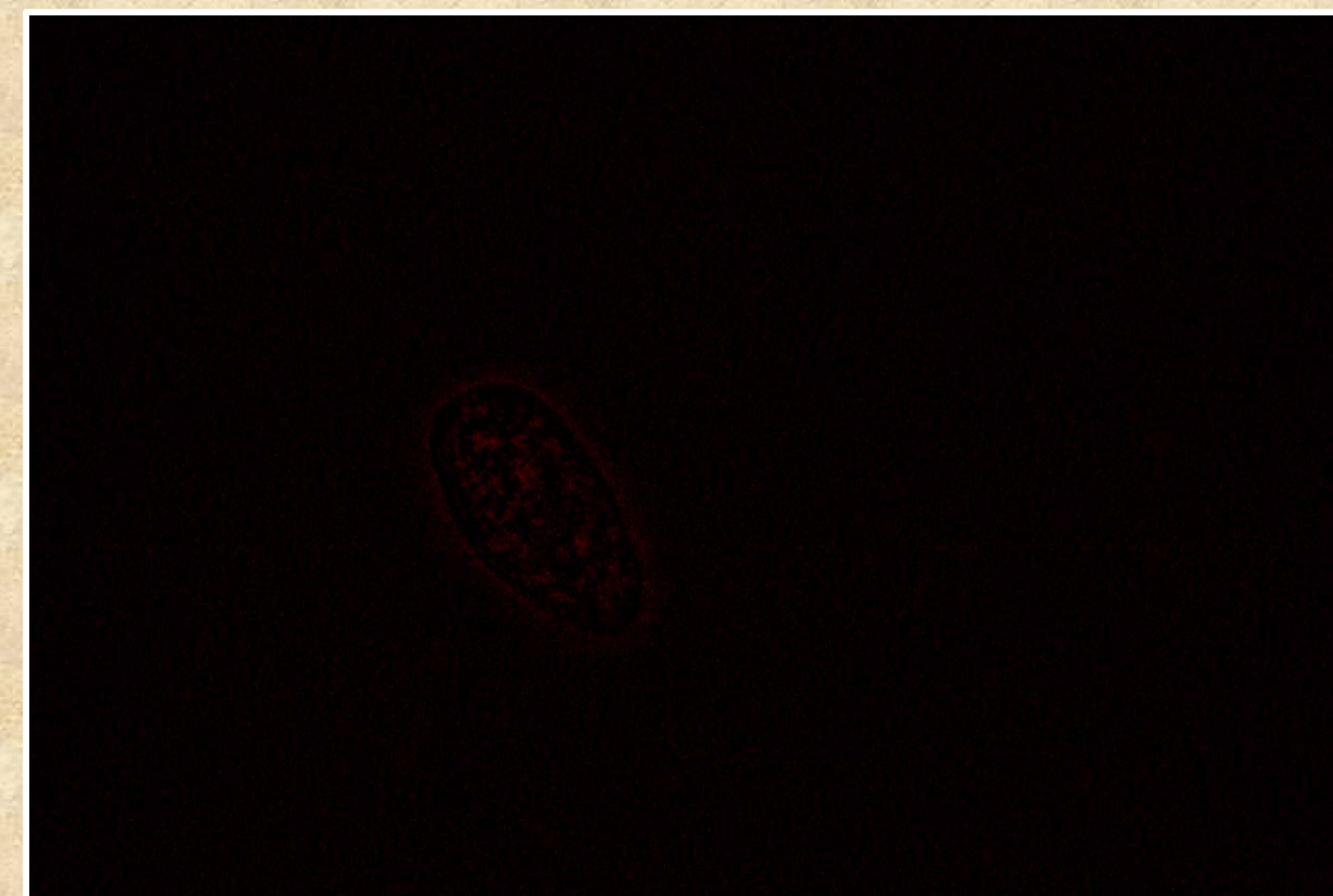
Recognition of Protein in Dying Tetrahymena with an Antibody that Stains Interdigit Dying cells in Limb. (J Lawhead)



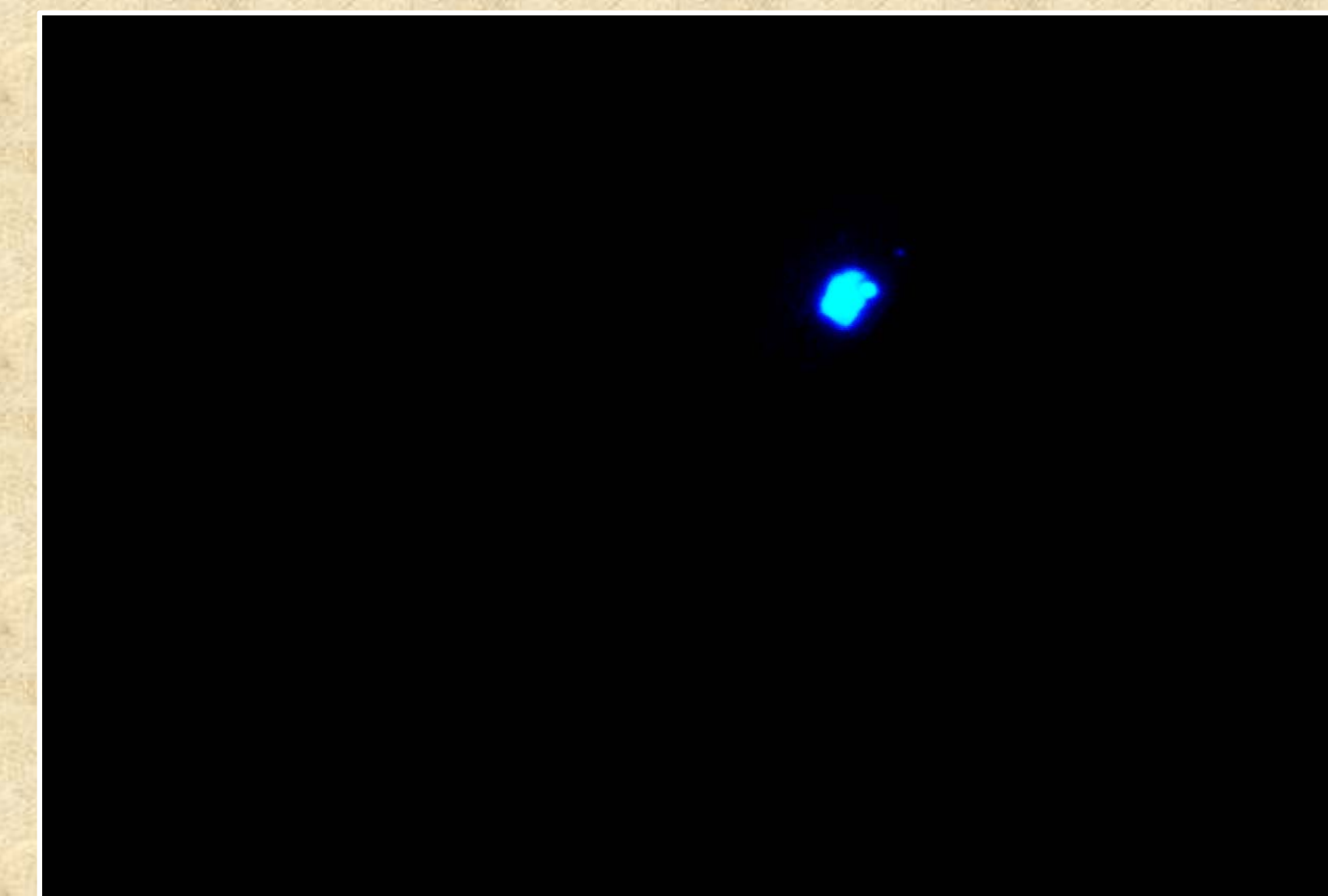
Positive B2 Antibody Staining



Positive B2 Antibody Staining

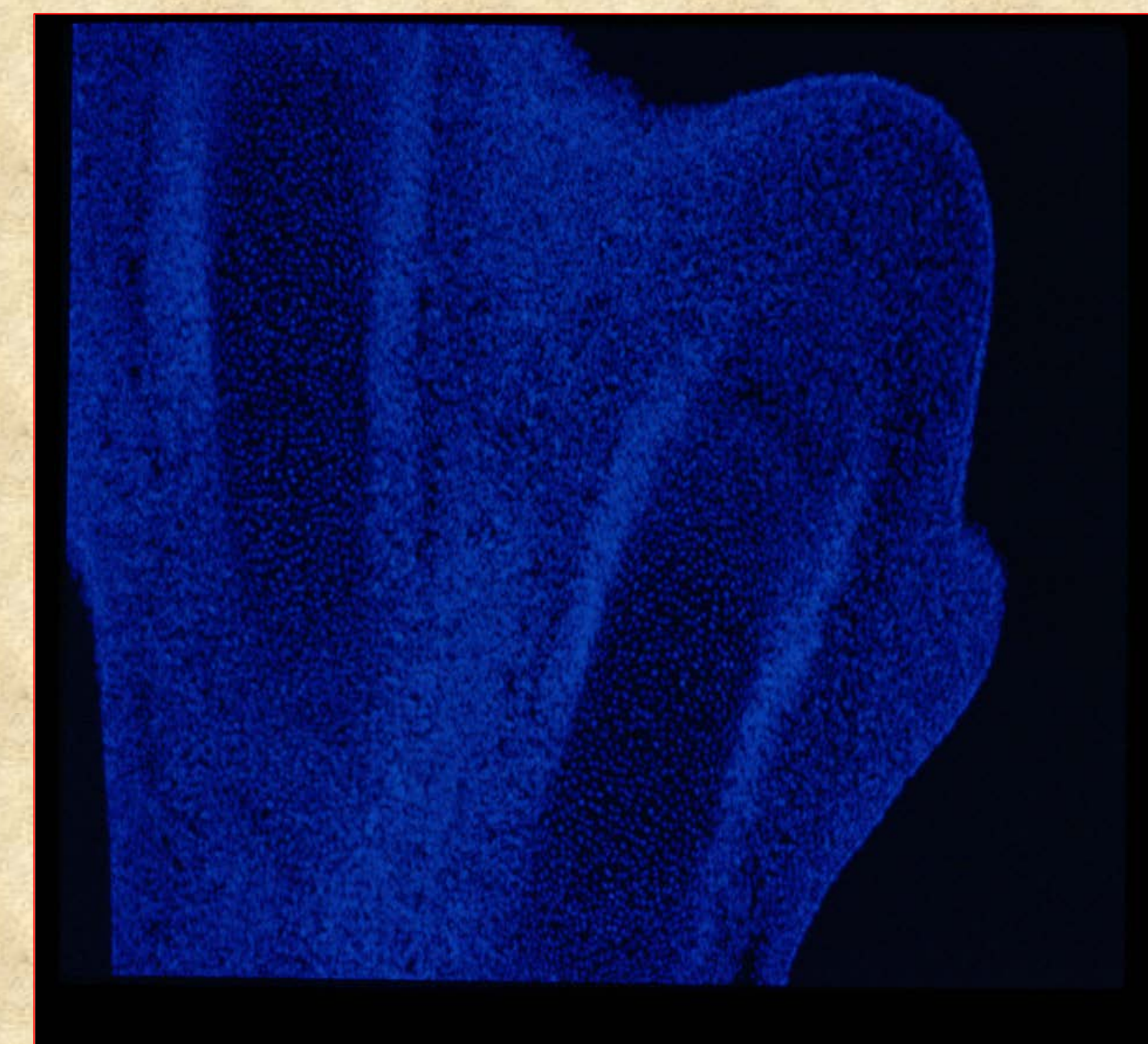


Negative Control Antibody Staining

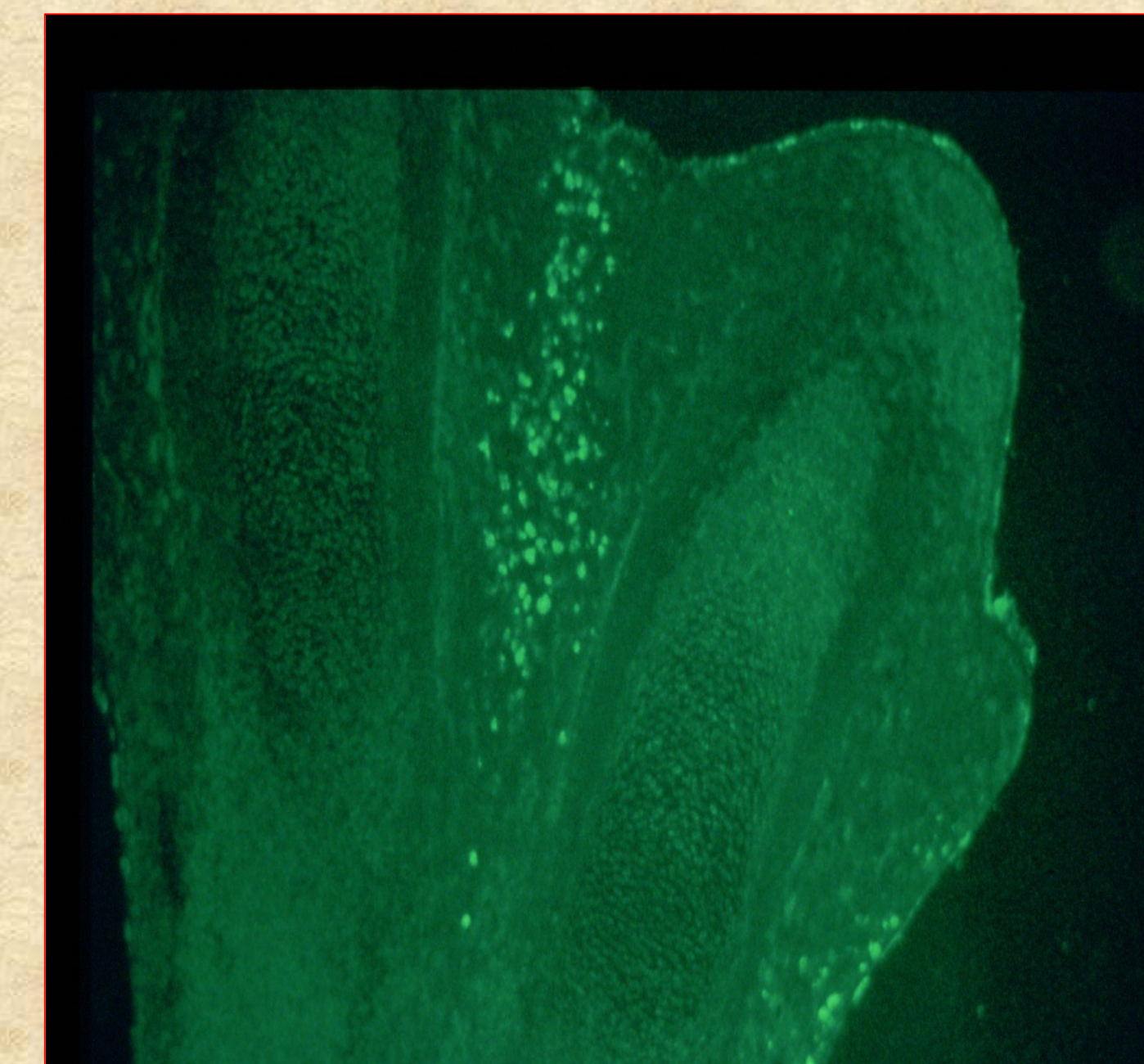


DNA Stain in Dying Organism

Localization of Cell Death (Apoptosis) in Day 8.0 Limb

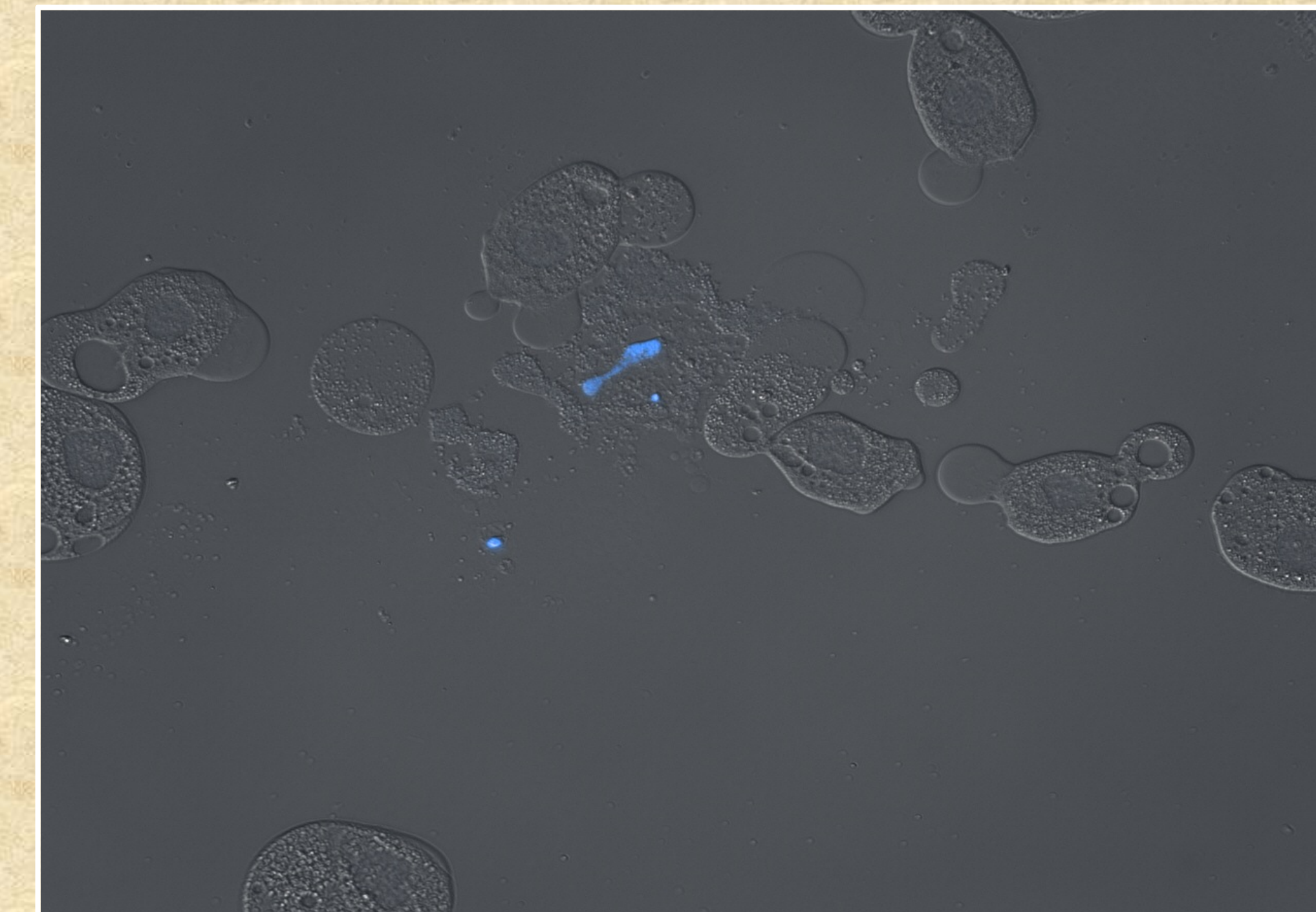


DNA Stain in Limb

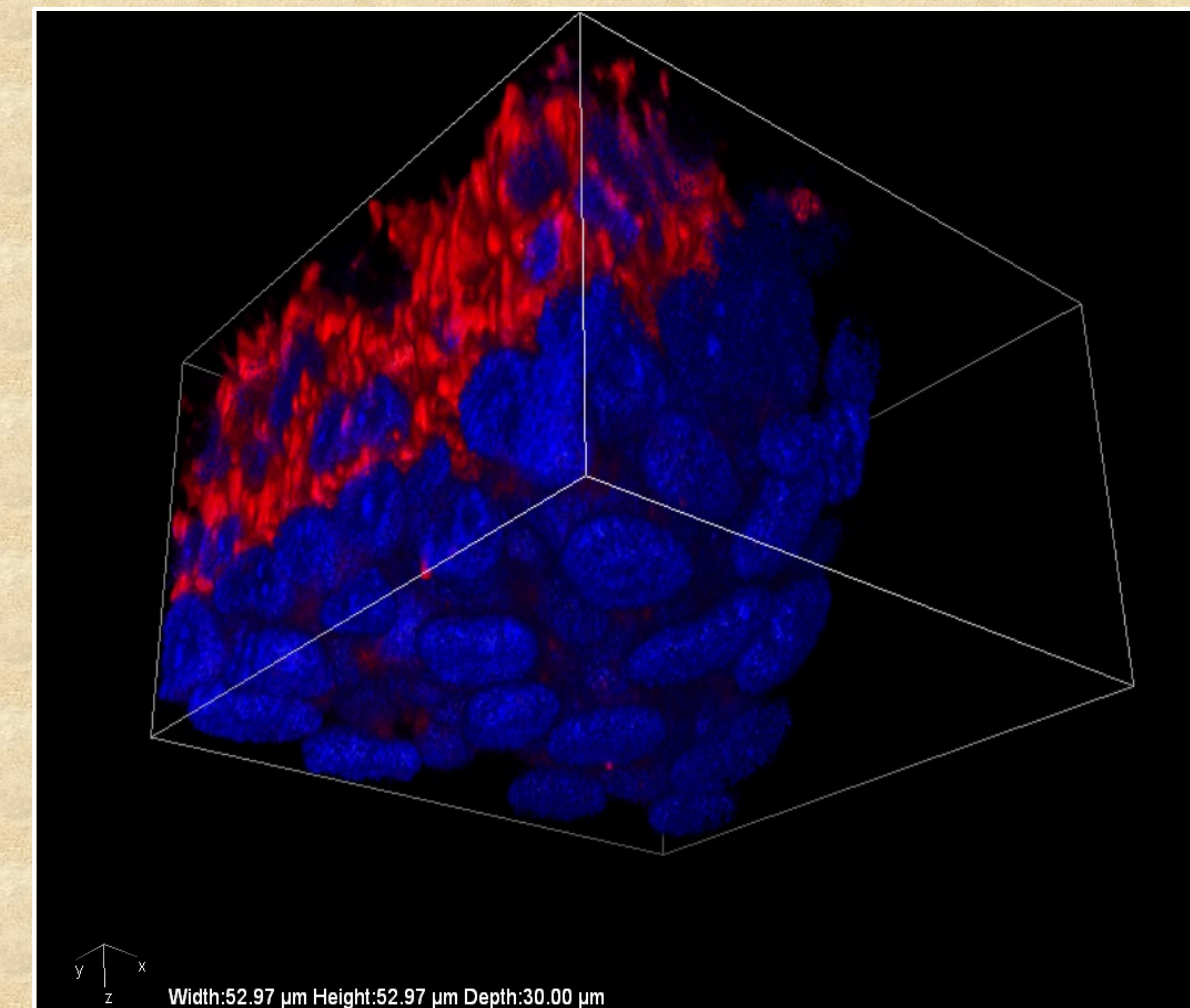


Cell Death Antigen B2 Antibody Staining

Confocal Image of DNA Staining in Dying Tetrahymena



Confocal Image of Chick Limb  
Blue represents Nuclei and Red represents Localization of Cell Death Antigen in Interdigit

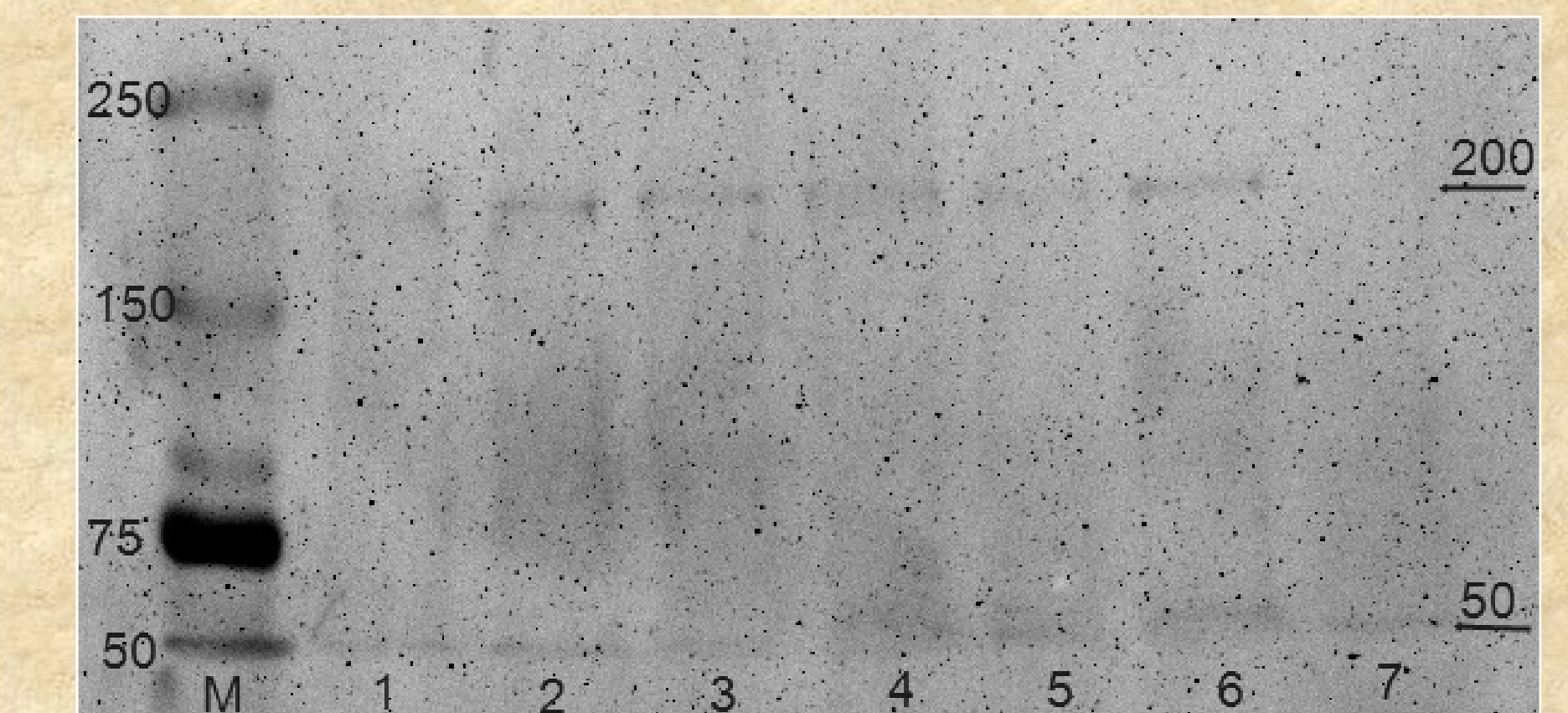


Width:52.97 μm Height:52.97 μm Depth:30.00 μm

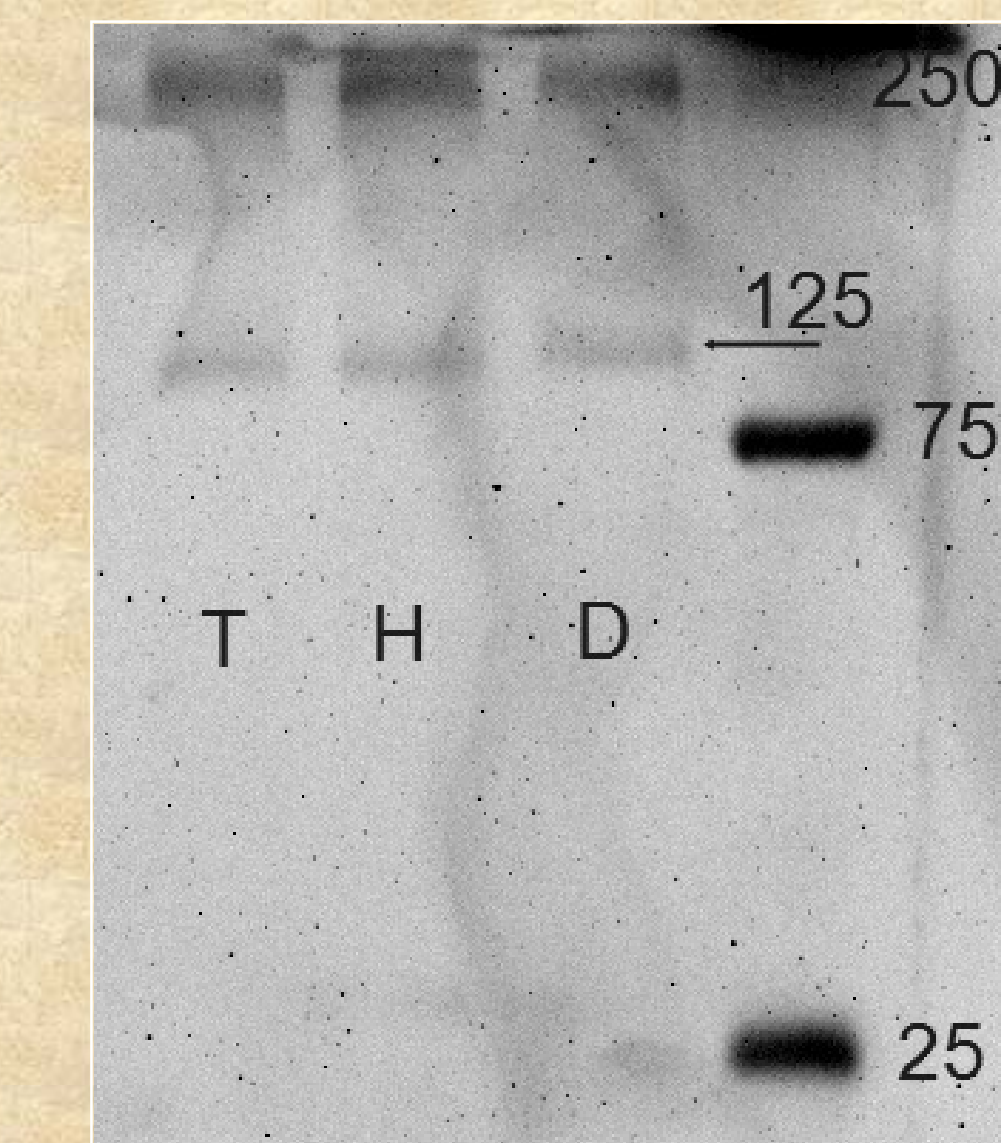
Protein Analysis Using Gel Electrophoresis



Medium, and Low Molecular Weight Protein Recognition in Tetrahymena (T) and Day 7 Limb (L) at -65 and -45 kD, respectively



Low Molecular Weight Protein Recognition in Tetrahymena (T) at -50 kD



High Molecular Weight Protein Recognition in Tetrahymena (T) and Chick Heart (H) and Digit (D) at -125kD

## Future Directions

### Future Directions:

Future work will utilize immunoprecipitation techniques at selected time points where *T. thermophila* death is prevalent, to enrich for the prospective cell death antigens. Ultimately, the goal is to obtain protein or peptide sequence from a isolated band(s) on a gel. The isolated band will be analyzed by mass spectrometry with an outside collaborator to obtain peptide sequence.

Confirmation of the antigen in *T. thermophila*, will be a useful in verifying other cell death related antigens and if there are potential interactions between cell death proteins. The main goal is to determine whether our described antigen is involved in the initiation or signaling of apoptosis, or if it is merely a result of apoptosis.