



# Odontoblast Apoptosis Contributes to Degraded Mechanical Properties in Root Dentin

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## Introduction

Vertical root fractures cannot be treated and result in tooth extraction. Recent studies have reported a significant reduction in fracture resistance of the root dentin with increasing dentin sclerosis and patient age, which appears to result from changes in the microstructure, chemical composition and increase in collagen crosslinking. Nevertheless, the role of odontoblast in the process of sclerosis and the degradation in strength of dentin is unknown. We hypothesize that odontoblast apoptosis may be responsible for the development of sclerotic dentin and degradation in mechanical properties of root dentin.



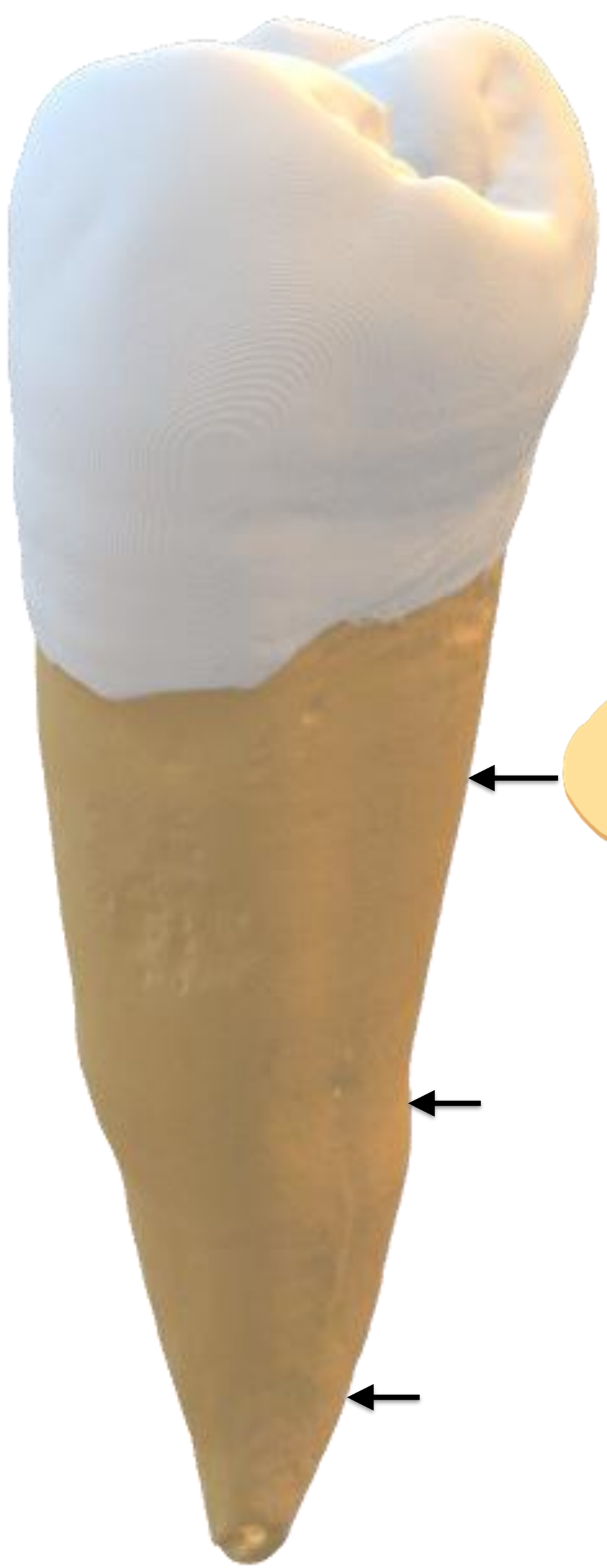
Figure 1. Vertical Root Fracture (Picture from Dr. Paul Caputo, D.D.S).

## Objectives

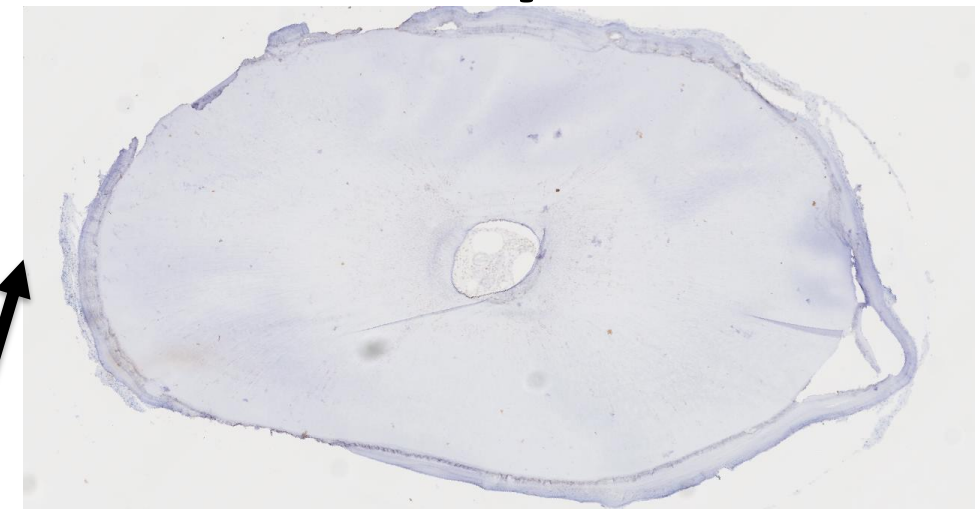
To evaluate the extent of odontoblast apoptosis along the radial direction of roots from teeth of different ages and assess the mechanical behavior of corresponding dentin.

## Methods

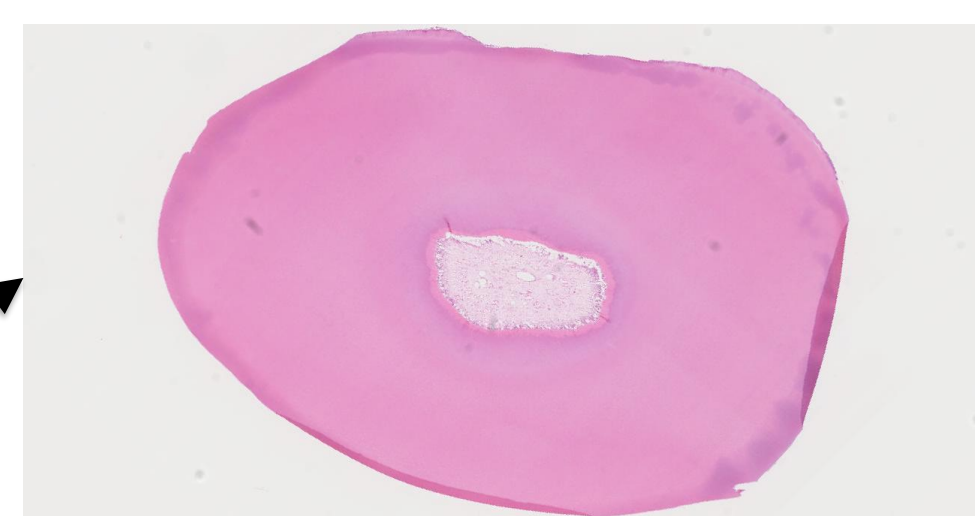
- 24 extracted human non-carious, vital teeth were obtained with approved protocol and divided into young (age ≤ 25, n=12) and old (age ≥ 60, n=12) groups.
- Immediately after extraction, half of the teeth were fixed in 10% formaldehyde solution, decalcified by Morse's solution, and processed for immunohistochemistry.
- Odontoblast apoptosis was determined by cleaved caspase-3 immunostaining and assessed using IMAGEJ software in the outer dentin, inner dentin, pulp chamber wall and pulp.
- Specimens prepared from the other half of the teeth were evaluated by nanoscopic Dynamic Mechanical Analysis (nanoDMA) in scanning mode.



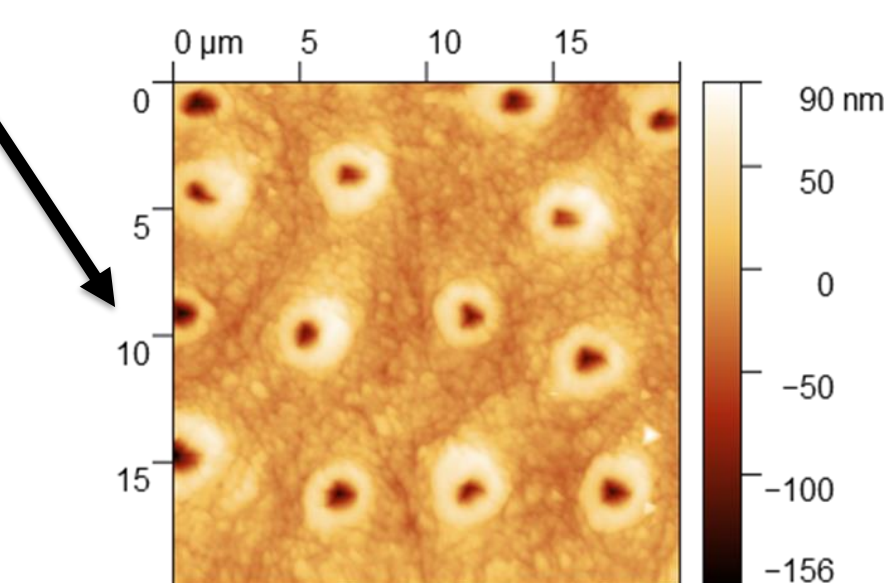
**Cellular Apoptosis:**  
**Cleaved Caspase-3 Staining**



**Cell Number:**  
**Haemotoxylin and Eosin**



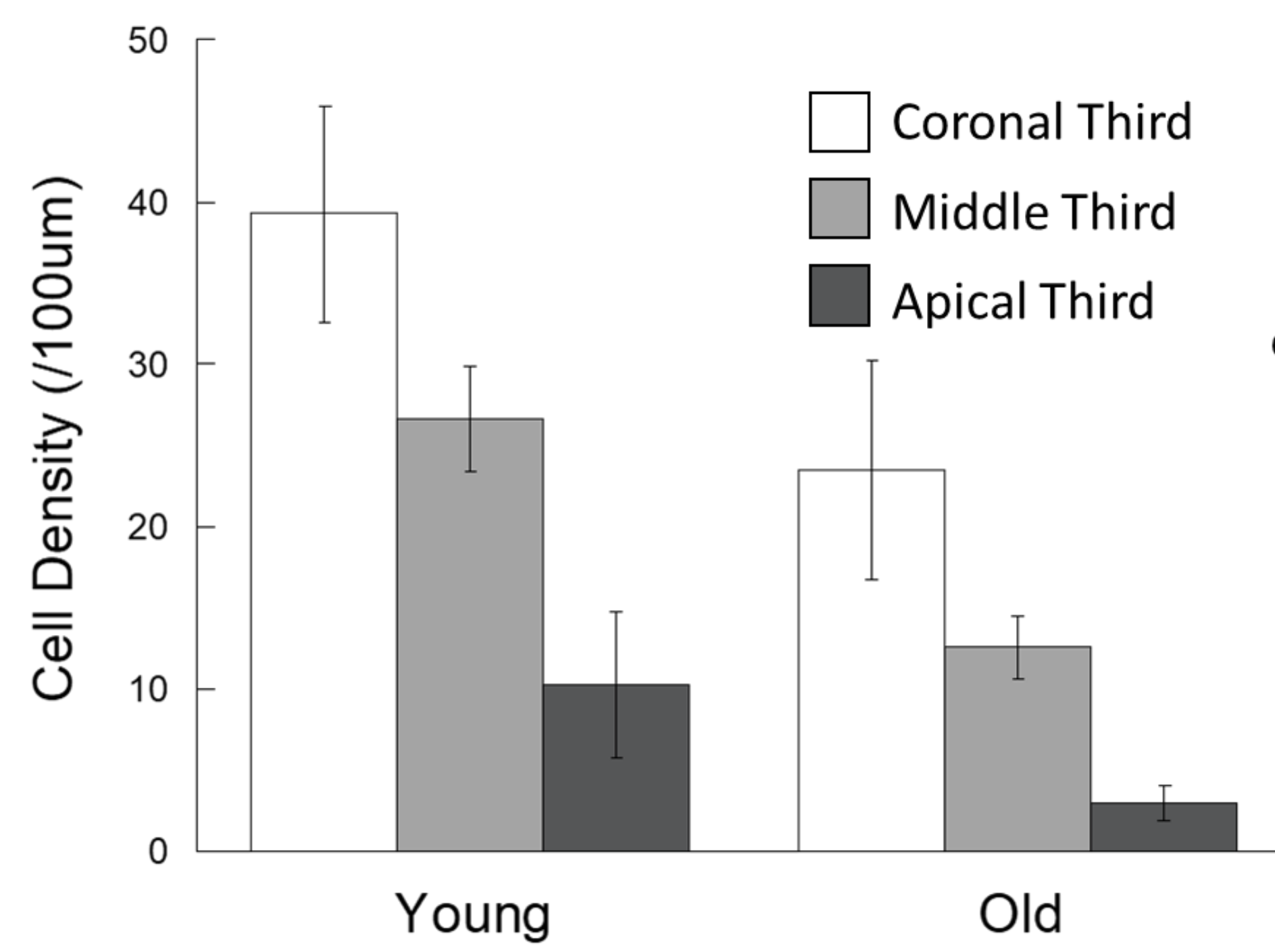
**Mechanical Properties:**  
**Scanning Probe Microscopy**



## Results

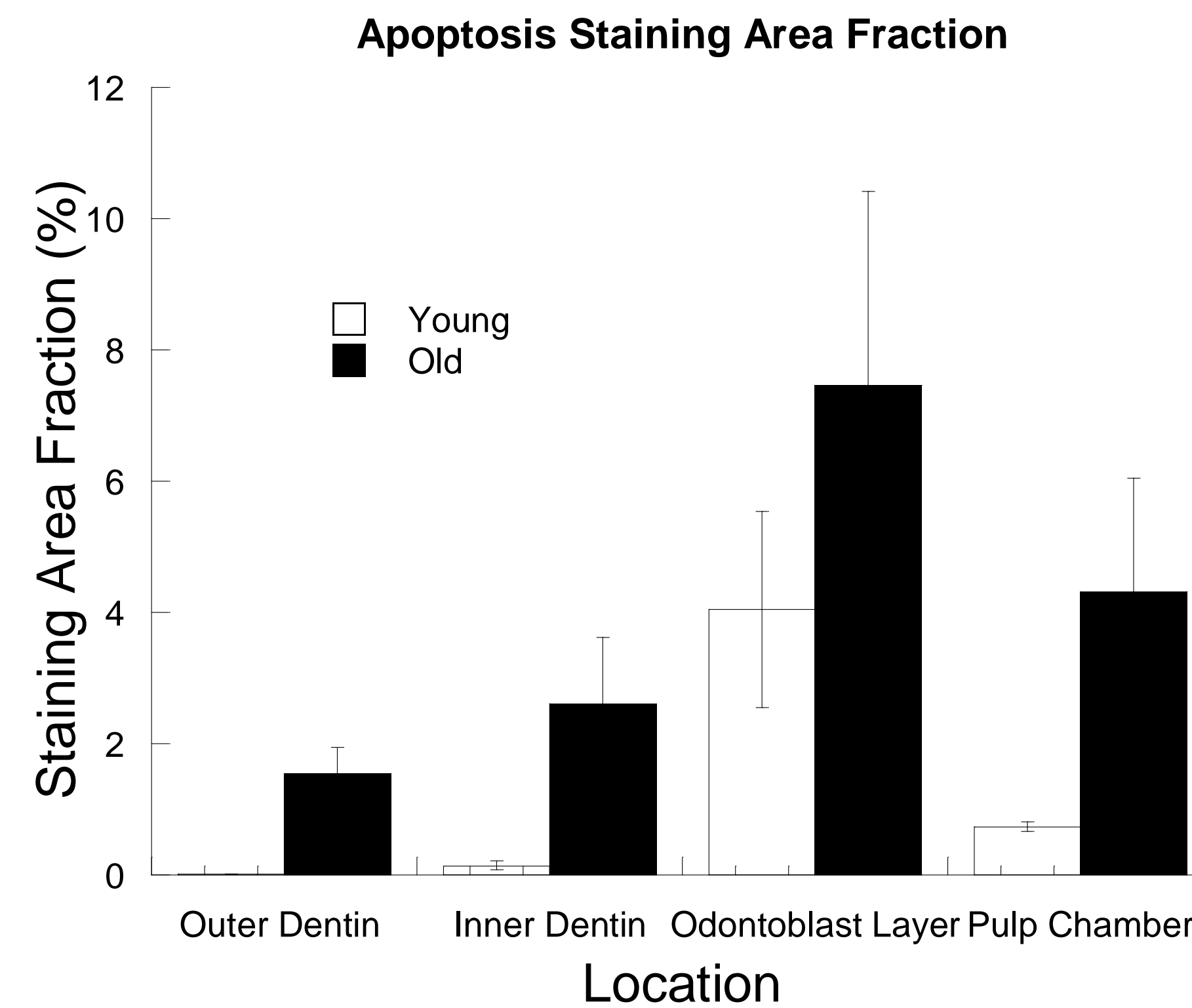
- There were no significant differences in odontoblast apoptosis staining in pulp chamber wall between the young and old teeth. However, the apoptosis staining was significantly higher in outer dentin, inner dentin and pulp in old teeth compared to young teeth ( $p < 0.05$ ).
- There are difference of Odontoblast density along the root canal. Numbers of odontoblasts decrease significantly with age. The most prominent change occur in apical third of root, which seems to be a result of cellular apoptosis with aging.
- Scanning based nanoDMA showed that the intertubular dentin of old teeth exhibited a significantly higher storage modulus and lower tan delta in comparison to that of the young teeth. The largest increase was observed in the outer dentin. However, the mechanical properties of peritubular dentin remain unchanged from young to old tissue.

## Odontoblast number decrease with increasing age

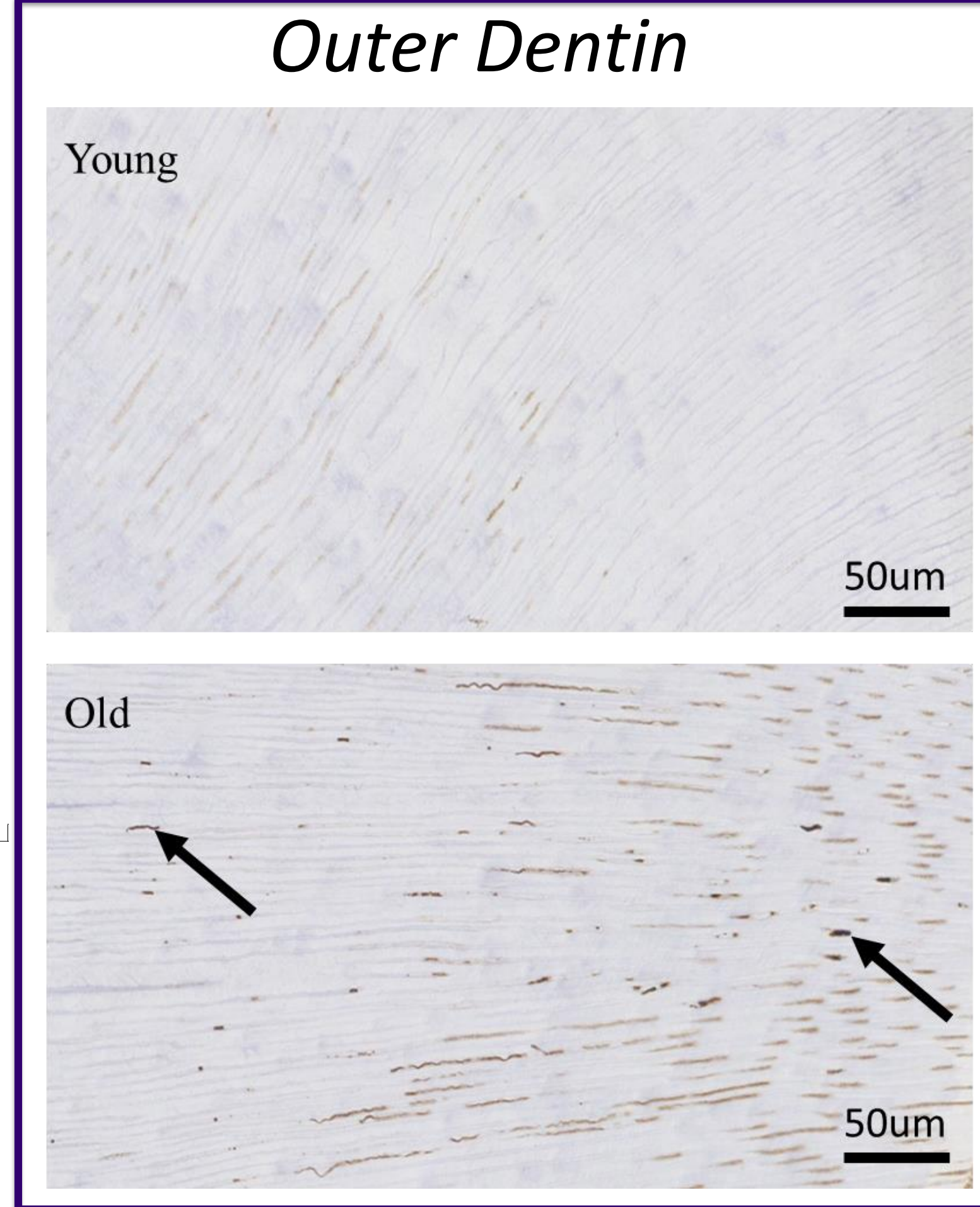


	Young	Old	Percentage Decrease
Coronal Third*	39.26±6.67	23.50±6.75	40.15%
Middle Third*	26.62±3.26	12.56±1.91	52.81%
Apical Third*	10.24±4.50	2.95±1.05	71.19%

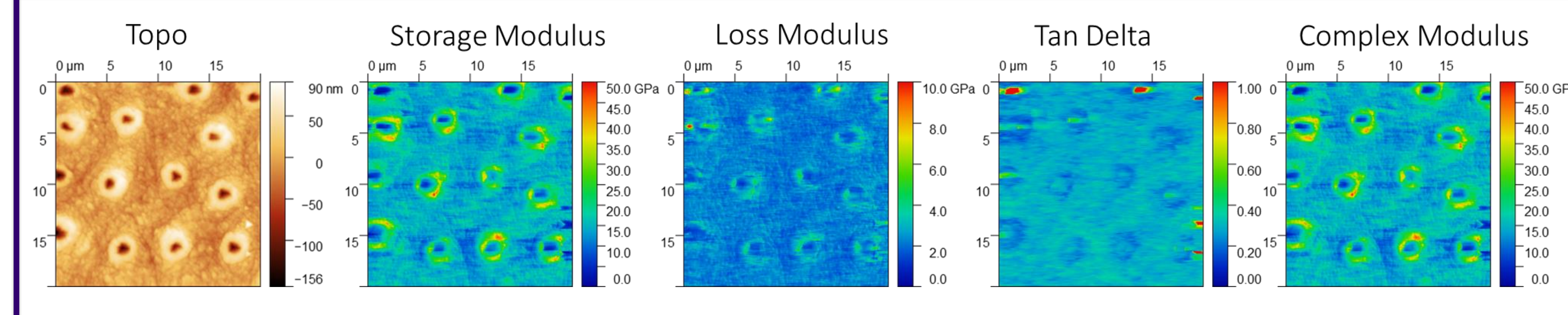
## Odontoblast apoptosis took place in aged dentinal lumen



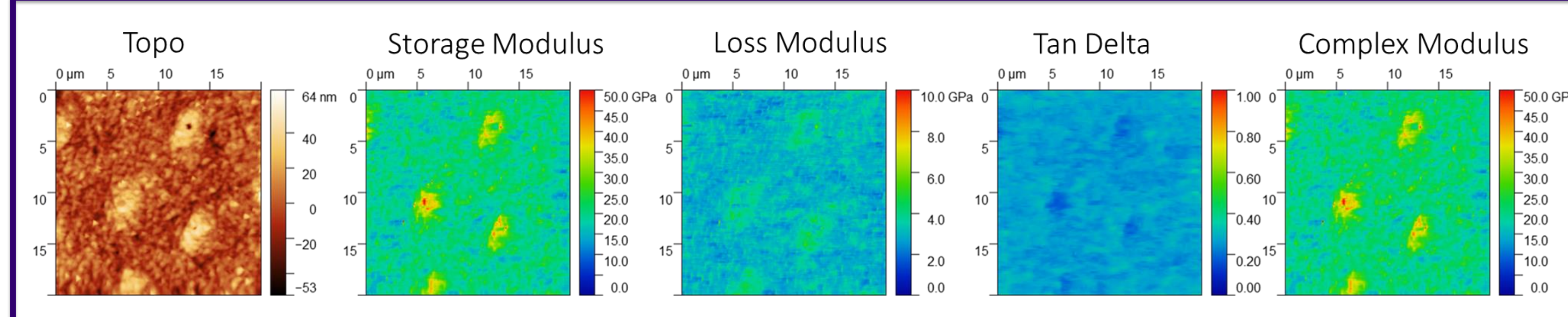
Apoptotic cell body not removed by autophagy can release cell content to cause change of properties in dentin



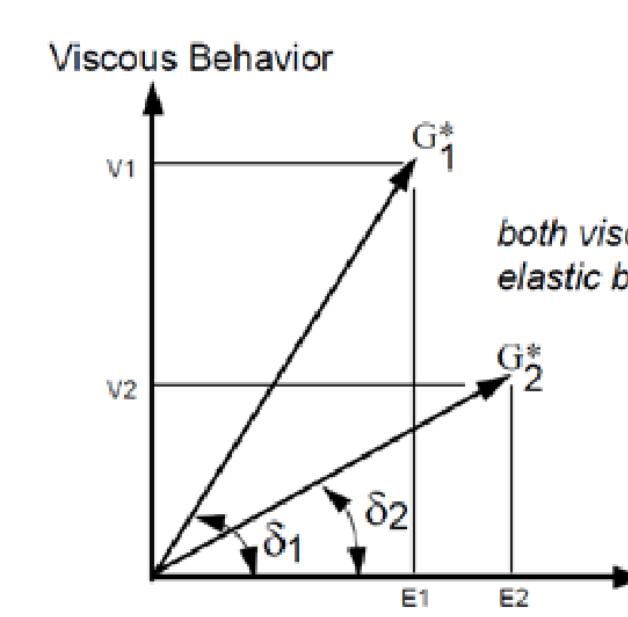
## Young: Lower Modulus, more viscous



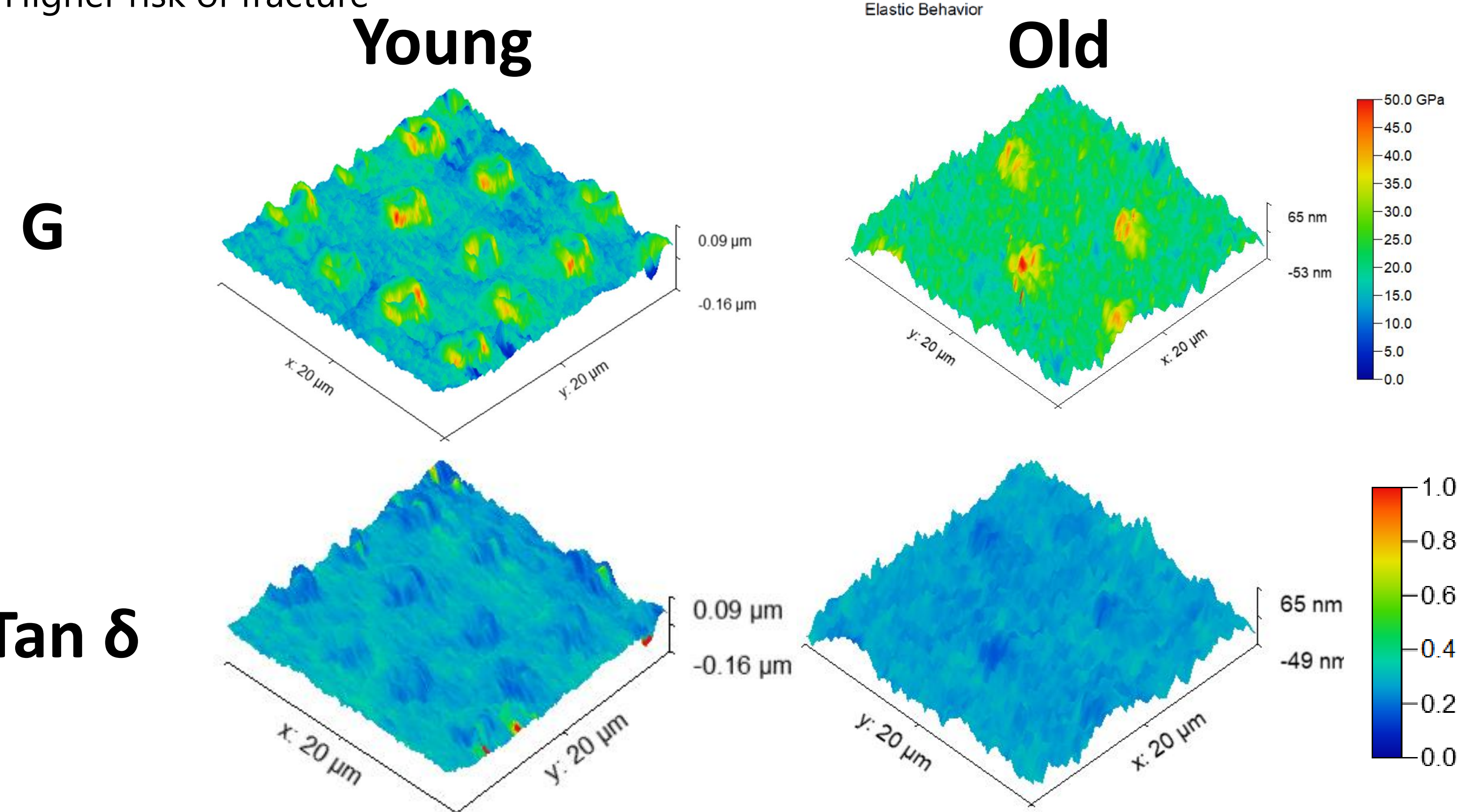
## Old: Higher Modulus, less viscous



- Intertubular outer dentin becomes stiffer with increasing age
- Tissue lose ability to behave plastically where more apoptosis signal is observed
- Higher risk of fracture



E: Storage Modulus  
V: Loss Modulus  
G: Complex modulus  
 $G = E + iV$   
 $\tan \delta = V/E$



## Conclusion

Odontoblast apoptosis appears to start at the cell extension in dentinal tubules, proceeds from outer to inner dentin, and contributes to a change in the complex modulus and tan delta in old root dentin.

## Acknowledgment

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