

Extraocular Tissue Type Architecture

Joel M Miller ¹

Joseph L Demer ²

Vadims Poukens ²

Dmitri S Pavlovski ¹

Hien N Nguyen ¹

Ethan A Rossi ¹

¹ The Smith-Kettlewell Eye Research Institute, SF

² Depts of Ophthalmology and Neurology, UCLA

Supported by NEI EY-08313
and The Smith-Kettlewell Eye Research Institute

JM Miller Lab

Aim: Characterize Mechanical Properties of Distributed Extraocular Structures

EOM pulleys probably escaped earlier detection because they are **distributed condensations of smooth muscle, elastin and collagen** (and because biomechanical modeling of the orbit had not yet predicted their existence).

Few studies of distributed orbital structures have been able to determine **mechanical properties of tissues**. Characterization of distributed structures requires an **imaging approach**, which unfortunately cannot directly give mechanical properties. Imaging can, however, give strong hints about mechanical properties and direct subsequent mechanical measurements by showing the **types and distributions of constituent tissues**.

Approach: Reconstruction of Thin Histologic Sections

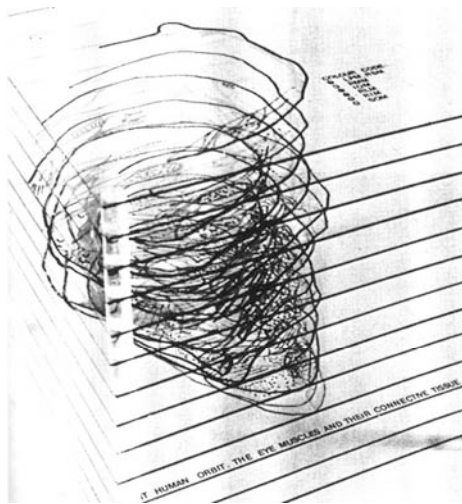
We have developed methods for producing 3D images of extraocular tissues, incorporating **histochemical and immunohistochemical processing** to distinguish

- **striated muscle**
- **smooth muscle**
- **collagen**
- **elastin**

Reconstruction from Thick Sections is Easy

3D reconstruction from slice data has become familiar in connection with tomography, confocal microscopy, and such projects as the Visible Human. It is easy to think that the problems of reconstruction have all been solved, but these applications require only relatively straightforward reconstruction methods:

- registration of each slice with the next is unambiguous.
- distortions in successive slices are similar.



The National Library of Medicine's

Visible Human Project (TM)

Human-Computer Interaction Lab
Univ. of Maryland at College Park

Reconstruction from Thin Sections is More Difficult

Histochemical and immunohistochemical processing, needed to reveal the fine structure and constituent tissues in a sample, requires **thin sections**.

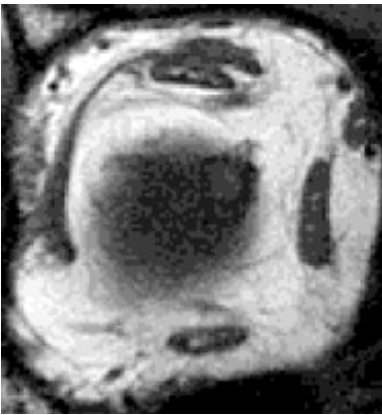
This leads to a much more difficult reconstruction problem:

- **bone must be removed** to avoid damage to the microtome knife, but this may cause soft, elastic tissues, to collapse, and spatial relationships to be lost.
- **imbedding compounds** used to support the sample during sectioning **distort the tissue as they harden**, especially when the tissue contains compartments such as the globe.
- **registration of sequential slices is lost** when they are cut.
- **each slice is uniquely and non-linearly distorted** by cutting and processing.

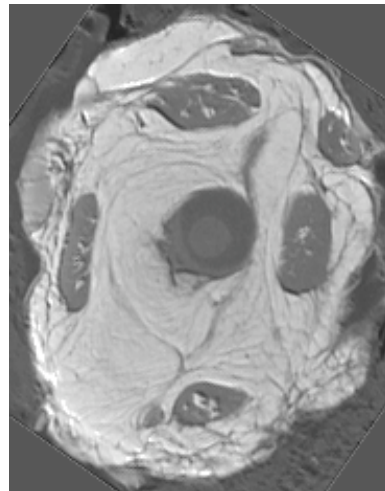
Here Today ... Histology Tomorrow

A tissue sample passes from life through the stages of histologic processing, **increasing resolution and tissue differentiation, at the cost of accumulating distortions.**

**Alert Subject
Tomograph**



**Cadaveric
Tomograph**



**Thick
Slice**



**Thin
Slice**

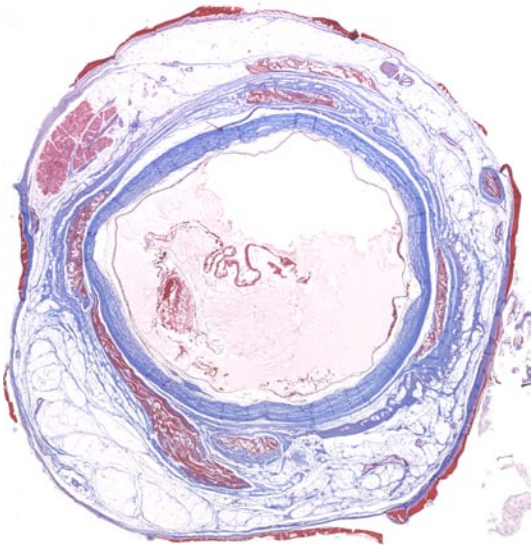


more resolution & tissue differentiation

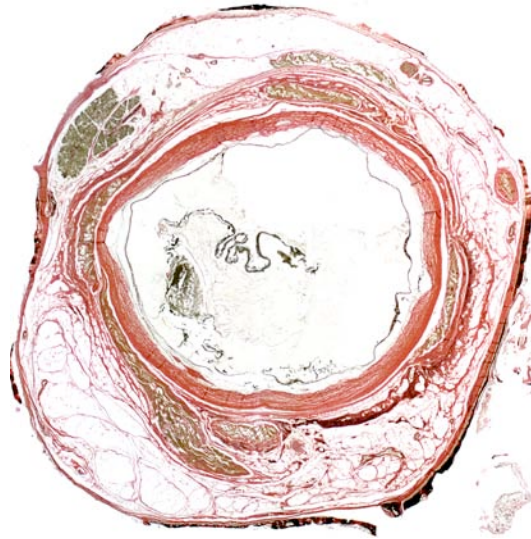
more realistic shape and topology

Thin Slices

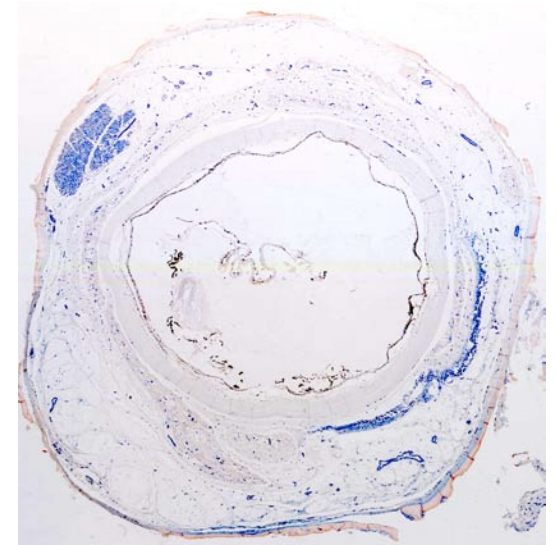
Our thin slices were 3 series of 10 μm thick sections, stained, stained for different tissues, mounted and digitally photographed.



Muscle (red) & collagen (blue)



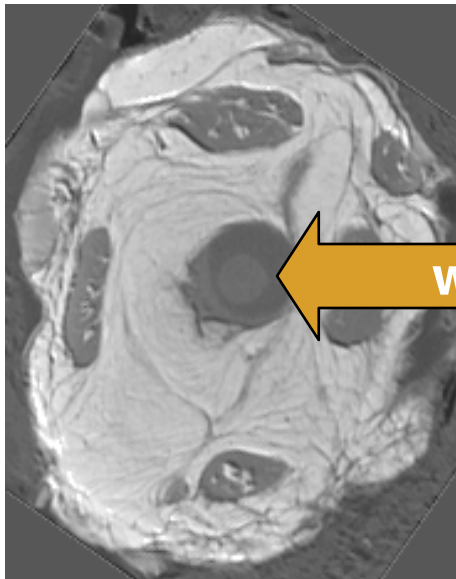
Elastin (brown-black)



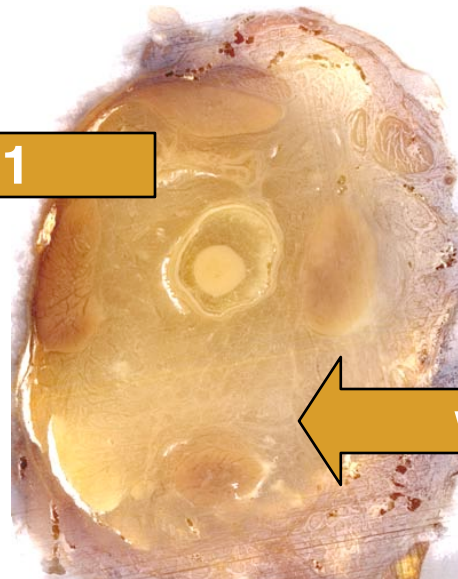
Smooth muscle (blue-black)

Multi-level Warping Restores Natural Shapes to High-resolution Thin Slices

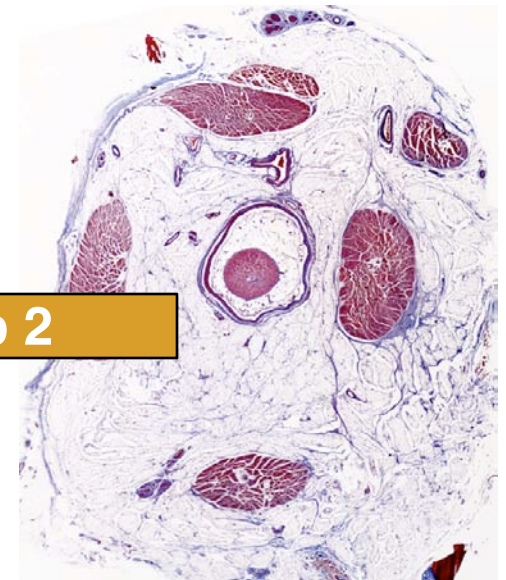
Cadaveric Tomograph



Thick Slice



Thin Slice



Strong Structures

- Nonlinear **warping** algorithms.
- Guided by **intrinsic fiducials**, ie, **strong structures**, which can be distinguished in every slice: the globe, optic nerve & EOMs.
- The remaining **weak structures**, the distributed collagen, elastin and smooth muscle, are thereby dragged into good alignment.

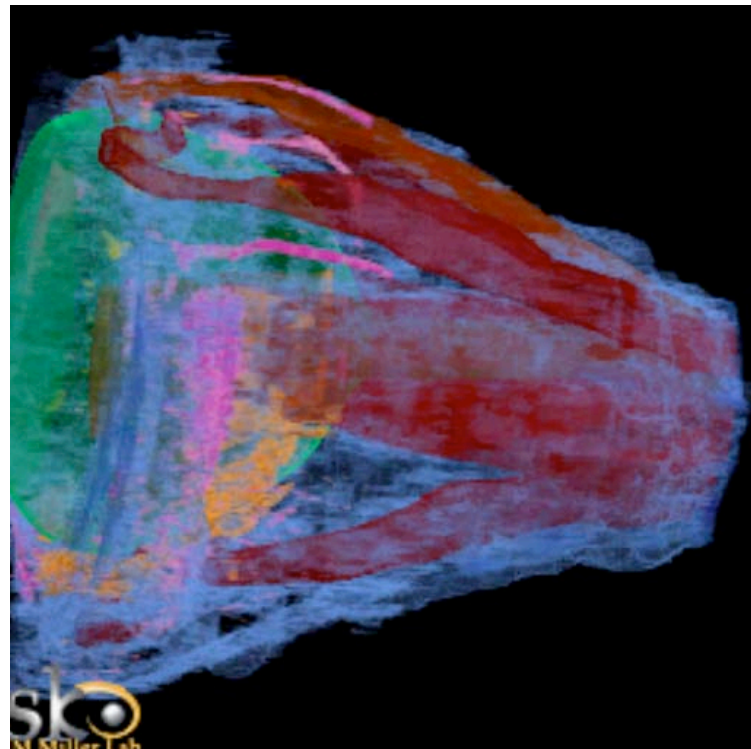


Alignment

Corrected slice images were then placed in register manually, or using an automatic method that maximized the cross-correlation of pixels in adjacent slices.

Rendering

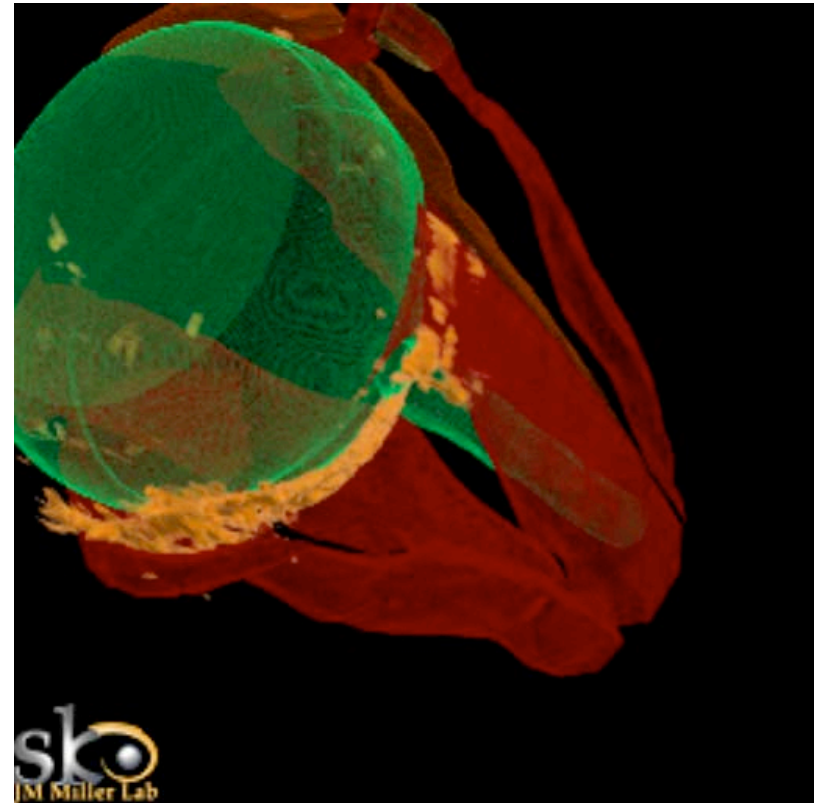
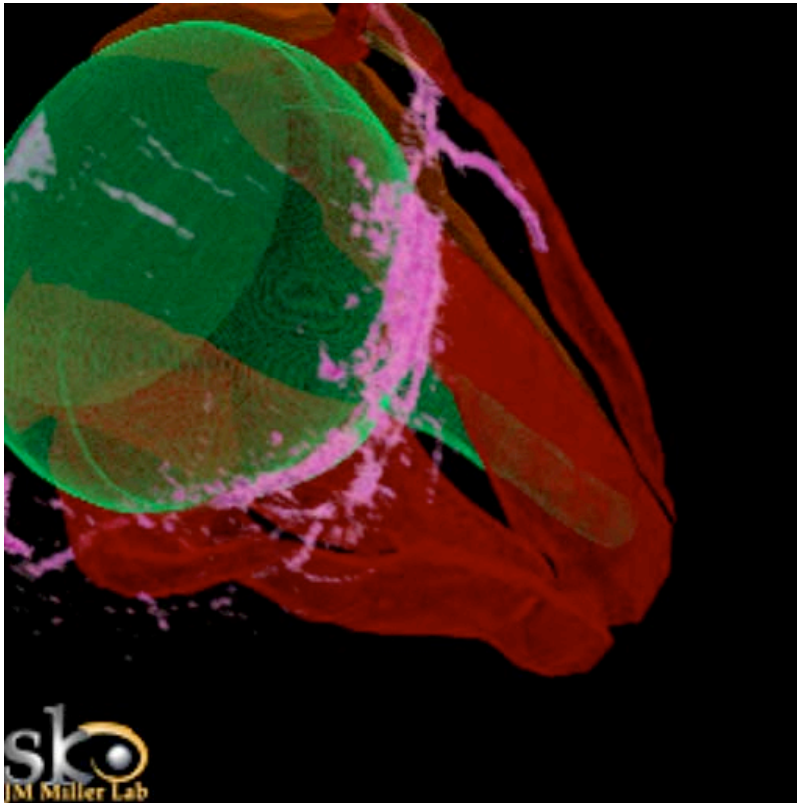
3D orbits were then reconstructed, fitting smooth surfaces to the strong structures, and using volumetric rendering for the weak structures, so as to visualize the distributions of collagen, smooth muscle and elastin.



2 Human Orbits

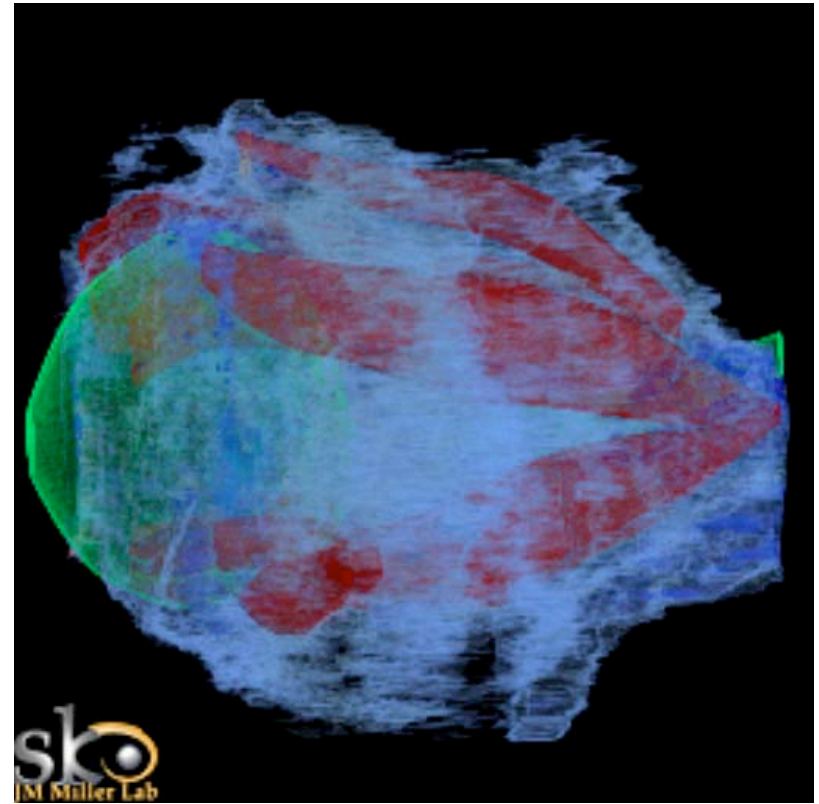
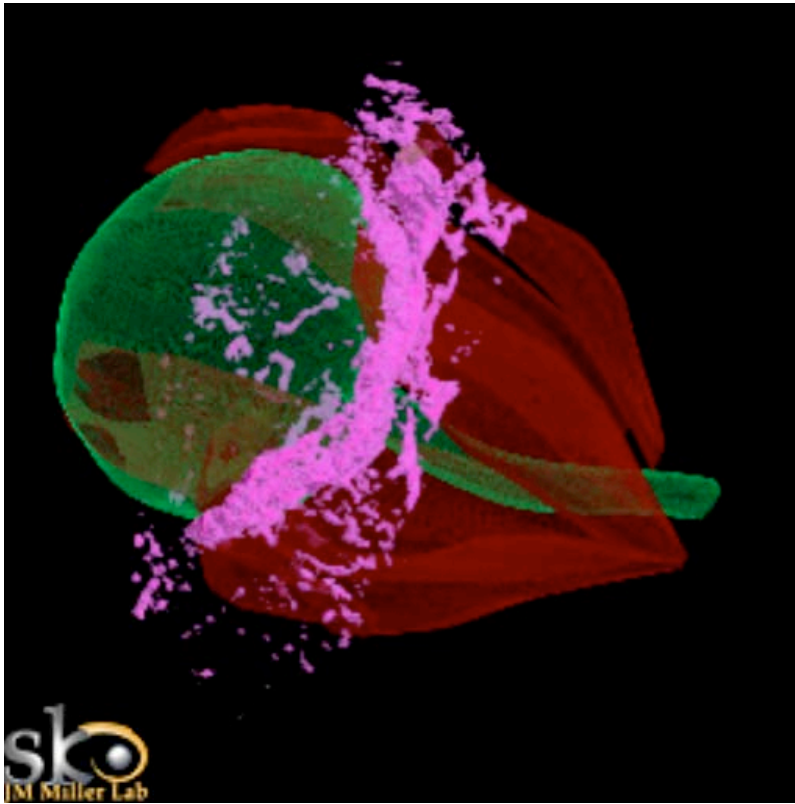
- “H5”, a 44 year old white male with Marfan syndrome.
- “H7”, a 17 month old male.

An Inferomedial Fibromuscular Band [H7]



A broad fibromuscular band was found to extend from the crossing point of the inferior rectus and inferior oblique to the medial orbit

An Inferomedial Fibromuscular Band [H5]



A similar smooth muscle band was found in sample H5. Thickened collagen was seen in the same region. (Elastin images were not available for H5).

Lockwood's Ligament is a Muscle!

“Lockwood's inferior ligament is a broad fascial sling...a connective tissue thickening in the inferior portion of Tenon's capsule, where the latter fuses with the conjoined sheaths of the inferior rectus tendon and the inferior oblique muscle.

The function of Lockwood's ligament has not yet been clearly defined, and its designation as the inferior suspensory ligament of the orbit may be misleading”

– JJ Dutton 1994

Let's call our new muscle the **infero-medial orbital muscle.**

Innervation of Extraocular Smooth Muscle

Innervation of Extraocular Pulley Smooth Muscle in Monkeys and Humans

Joseph L. Demer,† Vadims Poukens,‡ Joel M. Miller,§ and Paul Micevych*

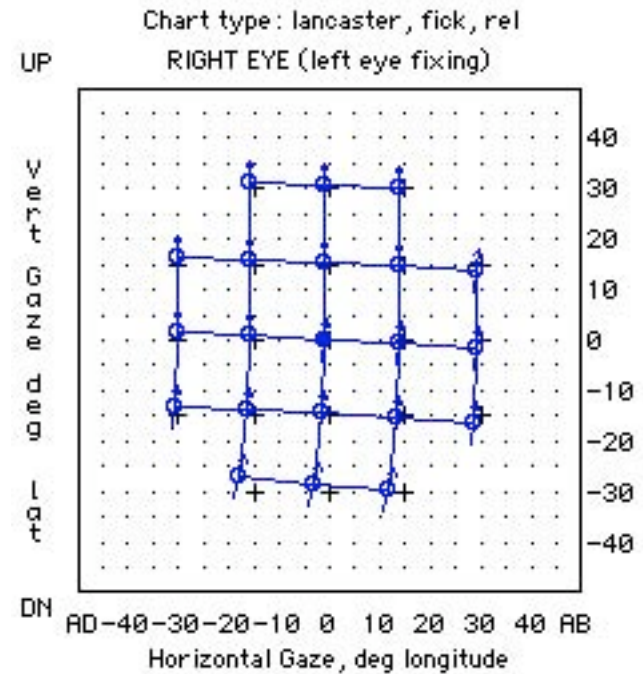
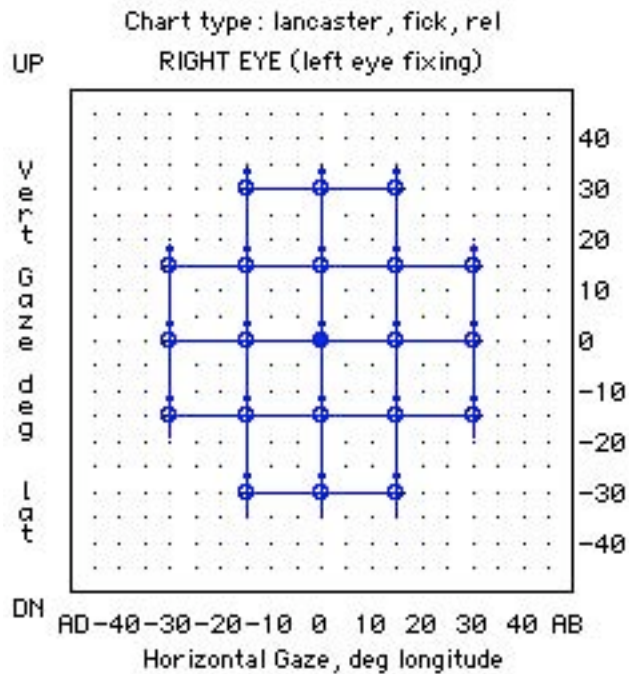
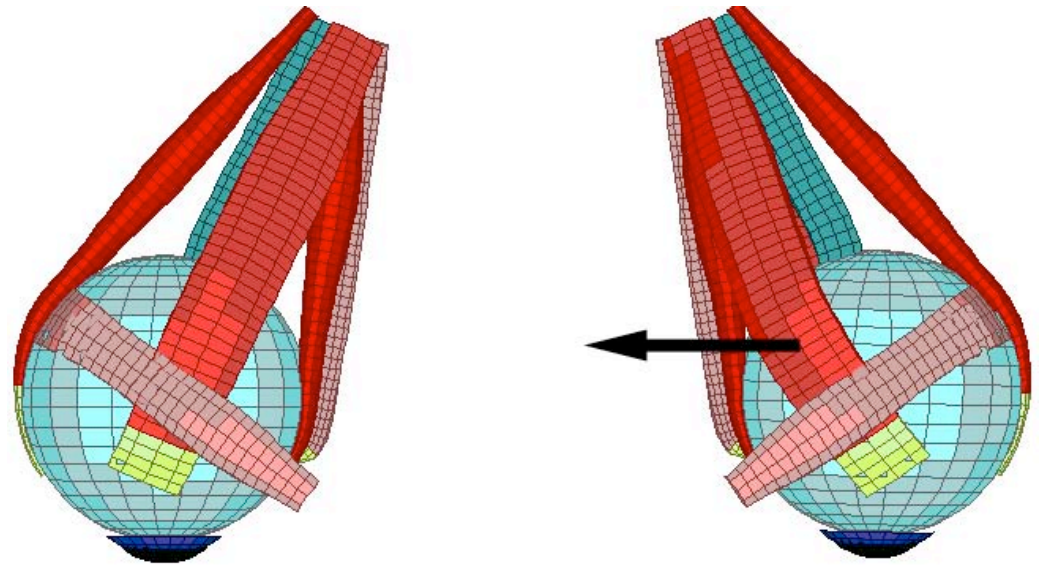
Purpose. Soft pulleys stabilize paths and determine pulling directions of the extraocular muscles (EOMs). This study was conducted to characterize innervation of smooth muscles (SMs) supporting these pulleys.

Methods. Cadaveric human and monkey orbits were step and serially sectioned for histochemical and immunohistochemical staining. Before perfusion, the superior cervical ganglia of one monkey had been injected with the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L). Immunoperoxidase staining to human SM α -actin confirmed pulley SM. Monoclonal and polyclonal antibodies were used to demonstrate PHA-L, tyrosine hydroxylase, dopamine β -hydroxylase, phenylethanolamine-N-methyltransferase, neuronal nitric oxide synthase (NOS), and synaptophysin. The NADPH–diaphorase reaction was also used as a marker for NOS and the acetylcholinesterase (AChE) reaction for acetylcholine.

Results. Pulleys, consisting of collagen and elastin sleeves supported by connective tissue containing SM, were observed around rectus muscles of humans and monkeys. The human and monkey SM was richly innervated. Axons terminating in motor end plates within SM bundles were immunoreactive to PHA-L, tyrosine hydroxylase, and dopamine β -hydroxylase, but not phenylethanolamine-N-methyltransferase, indicating **innervation of pulley SM from the superior cervical ganglion by projections using norepinephrine**. Smaller axons and motor end plates were also demonstrated in SM, using NADPH–diaphorase and NOS immunoreactivity, indicating nitroxidergic innervation, and using AChE, indicating cholinergic parasympathetic innervation. The pterygopalatine and, to a lesser extent, the ciliary ganglia, but not the Edinger–Westphal nucleus, contained cells immunoreactive to NOS, suggesting that **nitroxidergic innervation to pulley SM is mainly from the pterygopalatine ganglion**.

Conclusions. The SM suspensions of human and monkey EOM pulleys are similar and receive rich innervation involving multiple neurotransmitters. These complex projections **suggest excitatory and inhibitory control of EOM pulley SM**, and support their dynamic role in ocular motility. Invest Ophthalmol Vis Sci. 1997;38:1774–1785.

Medialization of IR Pulley



Conclusions

- We developed a (laborious, but effective) method for 3D reconstruction from thin histologic slices.
- We found a previously undescribed* elastic, smooth-muscle extraocular structure, which is related to the classically described ligament of Lockwood.
- The “infero-medial orbital muscle” might be involved in “A” and “V” strabismus patterns – specifically horizontal and torsional misalignments in downgaze
 - ◆ The infero-medial orbital muscle could be part of a normal binocular alignment corrective mechanism.
 - ◆ pathologic innervations could lead to “A” & “V” patterns.
 - ◆ this muscle could be target of surgical or pharmacologic therapy for strabismus.