Detecting diabetic neuropathy in rodents by imaging corneal nerves

Nigel Calcutt
Department of Pathology
UCSD

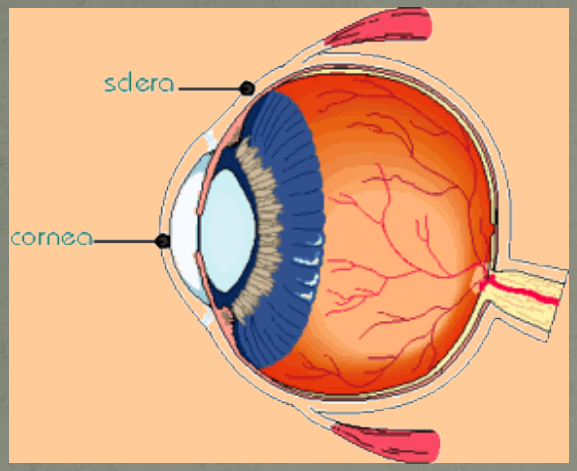
BACKGROUND AND RATIONALE

- Damage to small unmyelinated sensory nerve fibers is an early feature of human diabetic neuropathy
- The consequences are easily detected (sensory loss, pain) but difficult to quantify
- Skin biopsies allow visualization of small fiber damage and loss, but the technique remains invasive and slow
- Recent application of corneal confocal microscopy (CCM) shows loss of small sensory fibers in the cornea that is as sensitive as skin biopsy but entirely non-invasive
- CCM can be applied to both live animals and eyes stored in fixative/buffer
- **This project will establish whether CCM can detect neuropathy in eyes from mouse models of diabetes under development by the AMDCC and in eyes of living rodents, and will also compare CCM versus current functional (thermal tests) and structural (skin biopsies) assays of small fiber neuropathy**

Corneal Confocal Microscopy

- Rapid (2 min)
- Non-invasive
- Reiterative
- Images corneal nerve structure
- Shown to detect early neuropathy in diabetic patients
- Currently undergoing multi-site evaluation for potential use in clinical trials

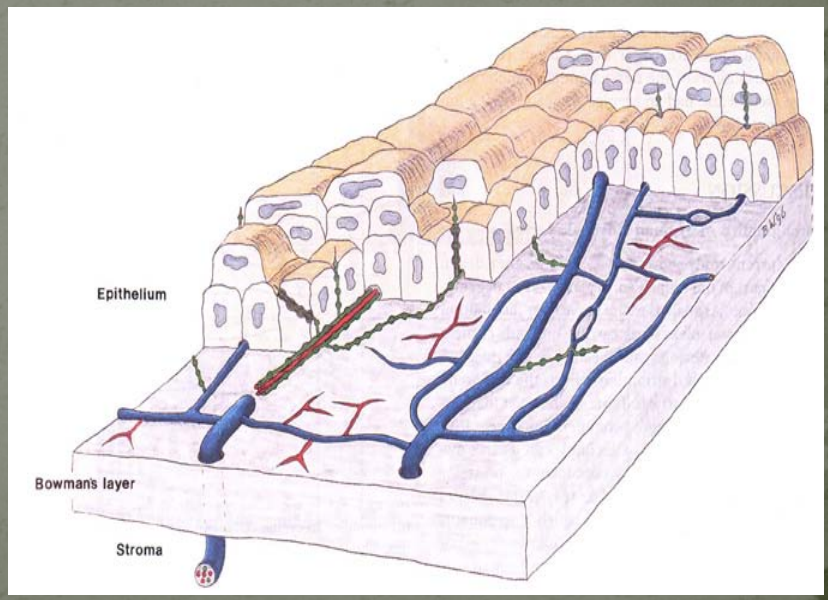
Corneal Anatomy



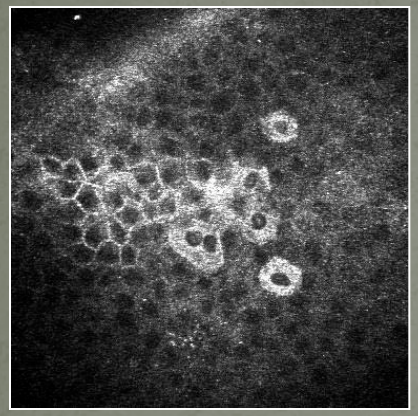
The cornea has the most dense innervation in the body (7000 nociceptors/mm^2)

Unmyelinated C fibres

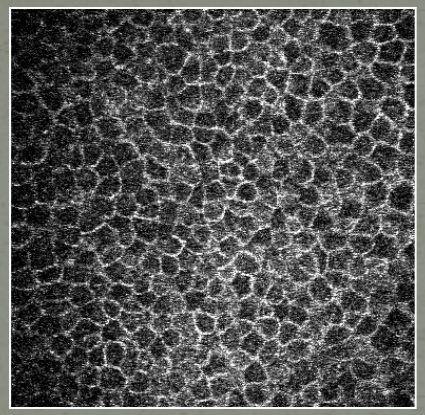
Can be viewed without stains by non-invasive confocal microscopy



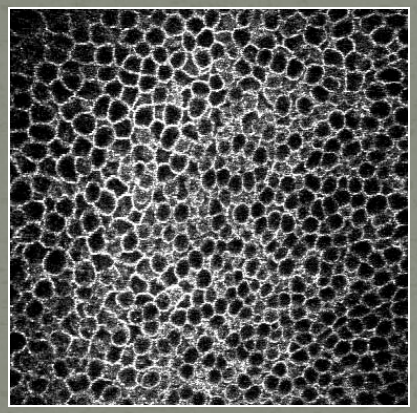
Corneal tomography



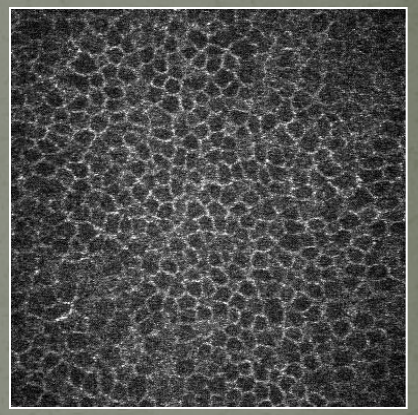
Depth: 0 µm



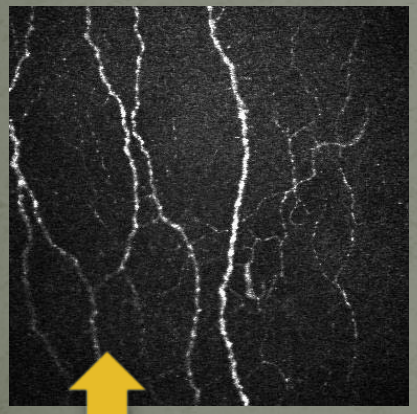
10 µm



30 µm

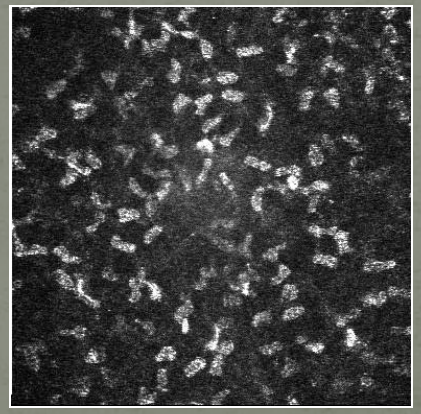


40 µm

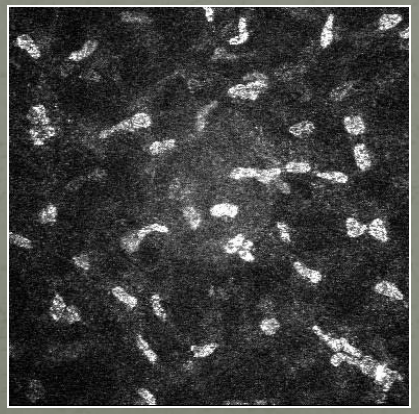


62 µm

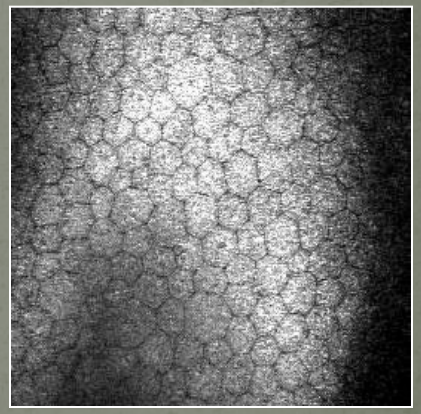
Corneal nerves



100 µm



300 µm



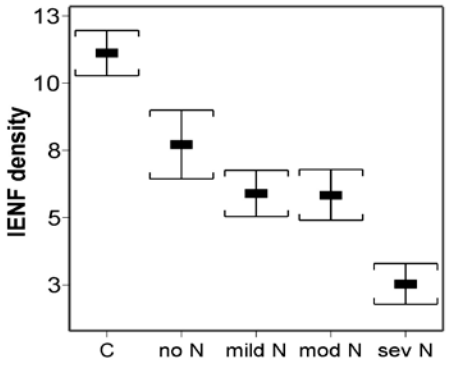
572 µm

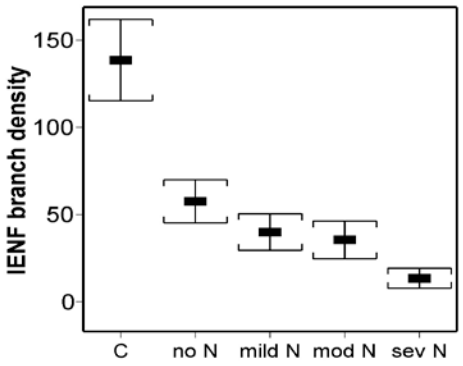
Skin biopsy vs CCM

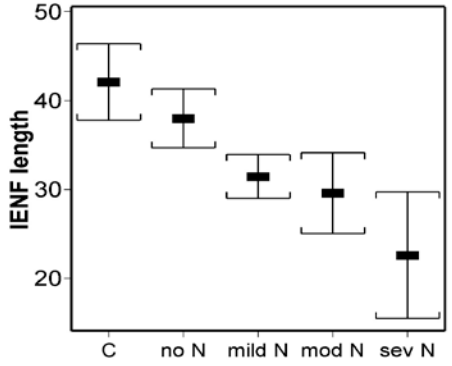
Group (Number)	Age *(yrs)*	Duration of diabetes *(yrs)*	HbA1c *(baseline)*	Neuropathy deficit score *(0-10)*
Control (15)	55 ± 4.8	–	–	0.0 ± 0.0
No neuropathy (10) *Diabetic*	54 ± 3.2	17 ± 4	7.2 ± 0.4	1.1 ± 1.6
Mild neuropathy (18) *Diabetic*	59 ± 3.0	18 ± 3	7.9 ± 0.4	4.5 ± 0.6
Moderate neuropathy (15) *Diabetic*	58 ± 2.7	25 ± 3	8.3 ± 0.4	6.6 ± 1.3
Severe neuropathy (11) *Diabetic*	62 ± 1.9	20 ± 3	7.9 ± 0.3	10.0 ± 0.0

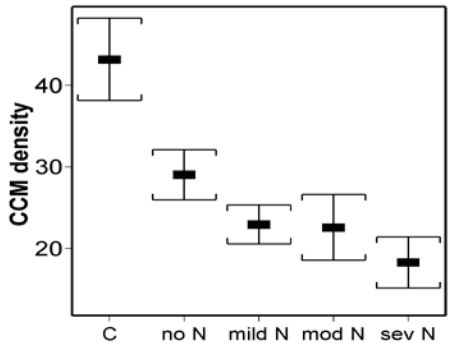
Quattrini et al. Diabetes, 2007

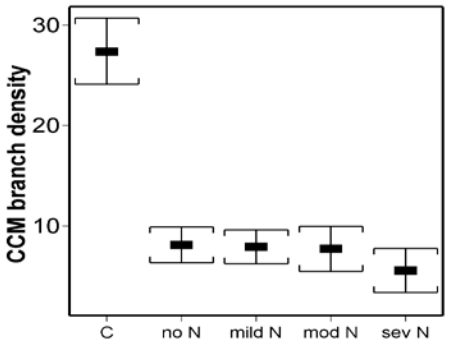
Skin Biopsy vs CCM

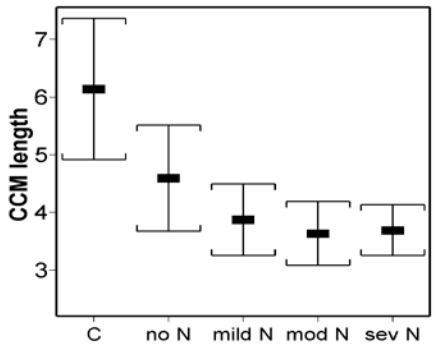












CCM has at least equivalent sensitivity as skin biopsy in detecting neuropathy in patients with no - mild clinical neuropathy

Quattrini et al. Diabetes, 2007

Plan

- **Study 1: Set-up (advised by Dr. Rayaz Malik)**
  - A) Image nerves in eyes removed from rodents and stored in assorted fixatives and buffers
  - B) Image nerves in live rats and mice
- **Study 2: AMDCC mice**
  - A) Have eyes sent from JAX using optimal conditions from Study 1
  - B) Compare with neuropathy status in same animals using sciatic nerve (large fiber) and paw skin (small fiber) sent at the same time
- **Study 3: Longitudinal study**
  - A) Longitudinal CCM on STZ-diabetic mice and compare with longitudinal NCV slowing and thermal hypoalgesia plus terminal axonal caliber and paw skin IENF measurements
  - B) As above, but using db/db mice
- Follow up: Extend longitudinal CCM validation to diabetic rats and open for business with anyone else who has models/eyes