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13. ABSTRACT (Maximum 200 words) Scanning electron microscopy (SEM) was used to define the physical microscopic features of the pit viper IR detection system. Scanning electron microscopy was used to gain a familiarity with the structure and layout of the pit viper infrared receptor organ. The two pit organs were extracted from an in vitro Western Diamondback rattlesnake (Crotalus atrox) heads. This consisted of dissecting along the maxillary bone just under the back surface of the pit organ between the eye and nostril. Specimen preparation consisted of the following. The organs were cut into several pieces. Fixation took place in 20 ml of 2% glutaraldehyde aqueous solution. Forty-eight hours later the samples were put through a series of dehydration steps in ethyl alcohol.			
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Final Report

The Response of Specialized Biological Tissue to Infrared Radiation

F49620-98-1-0199 US Department of the Navy

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A one-year study was conducted to

1. Measure the physical, optical and thermal properties of pit vipers;
2. Create a system for measuring the electrical activity of the IR sense organs of beetles and pit vipers;
3. Develop an optical-thermal model;
4. Thermal lensing in tissue

Initial Progress

Task 1. Scanning electron microscopy (SEM) was used to define the physical microscopic features of the pit viper IR detection system.

Scanning electron microscopy was used to gain a familiarity with the structure and layout of the pit viper infrared receptor organ. The two pit organs were extracted from an *in vitro* Western Diamondback rattlesnake (*Crotalus atrox*) heads. This consisted of dissecting along the maxillary bone just under the back surface of the pit organ between the eye and nostril.

Specimen preparation consisted of the following. The organs were cut into several pieces. Fixation took place in 20 ml of 2% glutaraldehyde aqueous solution. Forty-eight hours later the samples were put through a series of dehydration steps in ethyl alcohol. The series were as follows: 15 to 30 minutes at 30, 50, 65, 75, 89, 95, 100, 100, and 100% solutions. The tissue samples were left in 100% ethyl alcohol overnight and introduced to a fresh vial of the alcohol before the critical point drying process. Critical point drying of the samples was performed on a Tousimis CPD. The dried samples were then mounted on aluminum specimen stubs and held in place with double-sided carbon tape. The samples were sputter coated with a gold palladium

mixture. This was done using a Pelco sputter coater. Parameters used were 20 mA and a cathode to anode potential of 2.5 kV, for sixty seconds.

The micrographs obtained of the specimen were taken using a Philips 515 scanning electron microscope and are shown in Figures 1-4. The integrity of the specimen seems to be well preserved. Obvious damage sites did occur along the caudal side of the supporting structure base tissue where the organ was dissected out of the pit.

Figure 1 shows the cross-section of a pit organ that was cut in half. One can see the soft tissue supporting unit of the organ that makes up the actual pit (arrow) and is adjacent to the maxillary bone. The pit membrane (which is thought to be the infrared receptor) is seen inside the supporting unit. An enlargement of the cross-section showing the various layers of the pit organ are shown on the right of Figure 2a. Only the top layer is believed to be the infrared receptor. It appears to have collapsed and has the appearance of soft folded cloth (Fig. 2b). the layers below it are believed to rest on the surface of the pit cavity along the maxillary bone. The thickness of the top membrane was measured to be 15 μm , which agrees with previous work¹.

Figure 2 is a micrograph of the surface of the pit membrane (x326 on the left), with an enlargement on the right side (x2600). Only an epithelial cell layer was seen on the surface, and inspection of a sample with the bottom pit membrane surface exposed (micrograph not taken) indicated an epithelial cell layer also lies on the bottom pit membrane surface.

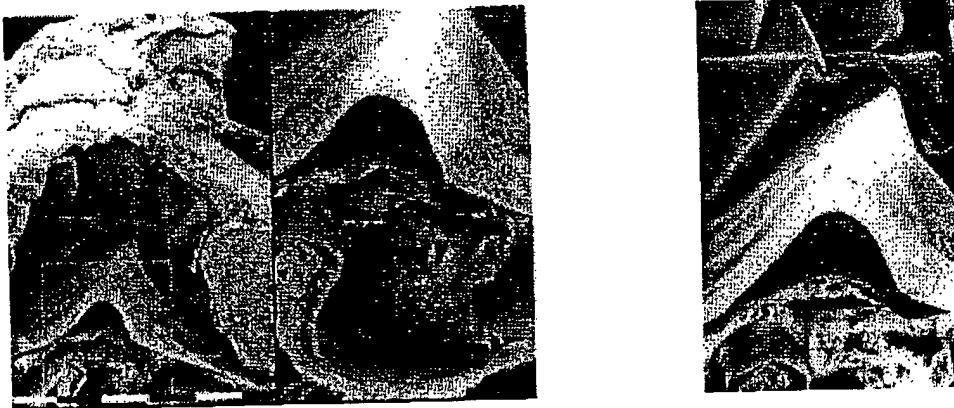


Figure 1. (a) Dual/split micrograph of pit organ cross-section. Left side: x20; Right side: x40. Left side shows the encasing supporting unit (arrow) of the pit and the collapsed receptor membrane inside. The enlargement shows the many layers of the organ. Only the top layer is believed to be suspended across the pit and have IT receptors. (b) Close-up of pit membrane.

Figure 3 shows the structure of the supporting unit cross-section corresponding to the area shown by the asterisk on Fig. 1a. We see it is mainly a fibrous structure.

Since the top and bottom surfaces are made up of an epithelial cell layer, it was not possible to see the structural layout of the area where the IR receptors are believed to be. A cross-section image of the membrane would be beneficial.



Figure 2. Dual/split micrograph of pit membrane surface showing an epithelial cell layer. Left side: x326; right side: x2600. Scale bar: 50 μ m.

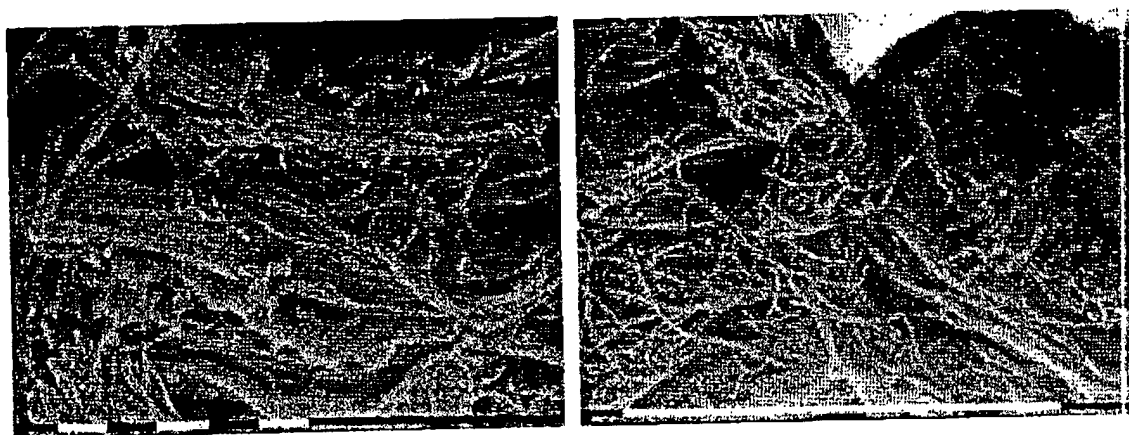


Figure 3. Micrographs showing the fibrous organization of the supporting unit of the pit organ. (a) High magnification micrograph (x10000). Specimen preparation: fixed in 2% glutaraldehyde, critical point dried, sputter coated with gold-palladium. Scale bar size 1 μ m. (b) Slightly lower magnification (x9150) in another area of the same structure. Scale bar size 10 μ m.

The purpose of this initial study was to become familiar with the pit organ and gain familiarity with its framework. The pit organ is about 5 mm in diameter. It consists of a 15 μm membrane exposed to the outside environment. Underneath the membrane is a cavity. At the bottom of this cavity is a fibrous supporting structure which cups up to allow the pit membrane to be suspended across it. The pit membrane is covered with an epithelial cell layer on both the top and bottom surfaces. Work continues to view the area between the epithelial cell layers where the actual innervation/receptor elements are believed to be.

A structural reconstruction of the pit will be incorporated in a Monte Carlo model to determine the actual distribution of IR irradiation upon the sense organ.

Task 2. A faraday cage was constructed on an optical table for measurement of sensory neuron response. A measurement system was assembled and micro-electrodes were pulled to make measurements in the jewel beetle. A series of electron microscope images were made to delineate the structure of the pit viper (rattlesnake) sensory pit and membrane. This system is being used in an AFOSR MURI project to investigate the electro-physiological response of the jewel beetle to IR stimulus.

Task 3. A Monte Carlo optical model was combined with a finite difference model of heat conduction with flexible geometry. This model will incorporate the unique shape of beetle and pit viper sensors. Structures are represented by approximately 10^6 volume elements. The code has been validated using analytic solutions for homogeneous semi-infinite geometry. Also, solutions for the model match published results of the rate of heat generation of laser irradiated blood vessels located in skin.

Task 4. Thermal lensing in tissue. The creation of thermal gradients in tissue produces gradients in density which in turn induces localized changes in index of refraction. We have demonstrated that thermal lensing occurs in continuous turbid media. Z-scan measurements in skin using a pulsed Nd:YAG laser ($\lambda = 1.06 \mu\text{m}$) illustrate the traditional thermal lensing signature. This signature is compared to transmission computed using a Monte Carlo model for light transmission in a scattering medium. Scattering is denoted in terms of albedo, a , which is

the scattering coefficient divided by the attenuation coefficient. We now have the capability of modeling and measuring effect of thermal lensing upon the retinal spot size of a laser beam directed into an eye phantom.