



Mid-Infrared Transmission and Reflection Microspectroscopy: Analysis of a Novel Biological Imaging System: The Snake Infrared-Imaging Pit Organ

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Pit vipers (e.g., rattlesnakes), boas, and pythons possess facial pit organs that are part of unique infrared imaging sensors not found in any other living species. Pit organs allow these snakes to detect thermal contrast in the environment and to form spatial images of their thermal environments within infrared regions of the electromagnetic spectrum (1) (Figure 1). The infrared imaging system augments the snake's visual system by providing its brain with a spatial image of the thermal environment. Infrared-imaging snakes use the infrared system to effectively detect and target prey even at night and in very dark places, such as animal burrows. Infrared imaging may also allow these snakes to avoid potential predators and to locate suitable sites for thermoregulation (1–4). Snake infrared imaging systems are extraordinarily sensitive, providing greater absolute sensitivity than sophisticated manmade detection devices without supplemental cooling. Therefore, snake infrared imaging systems, and particularly the pit organs, are important natural models for the development of novel biomimetic infrared sensor technologies.

Because snakes detect potential thermal targets from some distance, two major windows of atmospheric infrared transmission are likely to be very important to snakes — the middle infrared 3–5 μm and the 8–12 μm wavelength regions. Biological tissues, such as those that make up the bodies of people and animals, contain high concentrations of water, which is highly absorptive in the mid-infrared region of the electromagnetic spectrum. It is therefore important to understand the optical properties of the pit organ epidermis that allows infrared photons to interact with neural receptors located just beneath the epidermal surface. Spectral investigation of the few millimeter size pit organs are facilitated by the use of mid-infrared microspectroscopy tools capable of focusing electromagnetic radiation onto a well-defined small area (Figure 2).

Prior research has shown that the epidermis of the pit organ in infrared imaging snakes is two orders of magnitude more reflective of visible light than that of non-pit organ epidermal regions, potentially to protect the infrared imaging sensor from the overwhelming high-energy photon flux from ambient light (5) (Grace, unpublished observations). The absorptive and/or reflective properties of pit organ epidermis may be due in part to



Figure 1: A pit viper (*Crotalus horridus*). Arrows indicate the locations of the infrared imaging pit organs. Photo by M.S. Grace.

unique nanopit arrays — regular arrays of microscopic surface invaginations — located on the epidermis covering the pit organs (5) (Figure 3). While nanopit arrays are found elsewhere on snake epidermal areas not associated with infrared sensing (such as interscale regions, and on the spectacle covering the eye), the architecture of nanopit arrays covering the pit organ epidermis is unique (5) (Safer and Grace, unpublished observations).

In a regular renewal process as their bodies grow, snakes periodically shed or slough off the outer layer of their epidermis, including the epidermis overlying the infrared-sensing pit organs. In the work reported here, samples of pit organ epidermis were used to investigate the spectral properties of the surface layer containing nanopit arrays, in isolation from the rest of the pit organ. Transmittance and reflectance of mid-infrared radiation were investigated because this region of the electromagnetic spectrum is believed to be important for infrared imaging in snakes (6).

Experimental

Shed epidermal samples from the pit viper *Crotalus adamanteus* (the eastern diamondback rattlesnake) were



Figure 2: μ MAX Sample Compartment Infrared Microscope.

mounted onto steel washers using black tape with a circular optical opening, approximately 1 mm in diameter. This mount was held without optical elements or windows; therefore, optical corrections were not necessary to establish the actual transmission and reflectance characteristics of samples. Samples from three distinct body regions were tested: from within the cavity of the infrared-sensing pit organ, from the epidermal spectacle that covers the eye, and from between scales of the dorsal body.

A Pike Technologies μ MAX sample compartment mounted microscope, capable of transmittance, reflectance, and attenuated total reflectance (ATR) measurement of samples smaller than 100 μm , was used (Figure 2). The microscope was placed in the sample compartment of an FT-IR spectrometer. Spectra were collected with DTGS detector using a 1-min scan time at 4 cm^{-1} resolution. Background for reflectance measurements was a gold-coated microscope slide, whereas for the transmission measurements an open beam spectrum with the same aperture was used. Samples were positioned using the visual image of the microscope to find the appropriate area of the epidermis, and measured using 100–300 μm illuminated apertures. The μ MAX microscope used in this work had a 7.5x infrared reflective objective and a 10x viewer.

Results

Samples of pit organ epidermis from the pit viper *Crotalus adamanteus* exhibited two major regions of strong infrared transmission, over approximately 1700–2900 cm^{-1} (3.4–6.0 μm) and 700–1200 cm^{-1} (8.3–14 μm) wavelength (Fig-

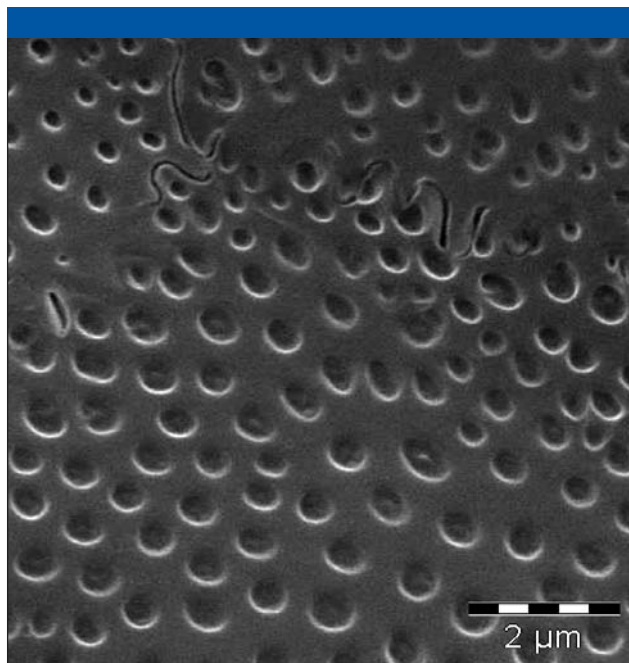


Figure 3: A scanning electron micrograph showing nanopits covering the epidermal surface of the pit organ of the pit viper *Crotalus horridus*. Photo by A.B. Safer.

ure 4). Overall shapes of spectra were generally similar among pit organ, dorsal interscale, and spectacle epidermal samples, but epidermis covering the pit organ had the highest levels of transmission among the samples tested. Peak transmission through pit organ samples was approximately 85% in the 1700–2900 wavenumber range, whereas spectacle transmission was approximately 70%. Dorsal interscale skin exhibited much lower transmission (with a peak value of approximately 30%) in the same wavelength region. Pit organ also exhibited the highest transmission beyond 1700 cm^{-1} , but interscale samples were next highest and spectacle transmission was lowest of the three samples beyond 1700 cm^{-1} . The spectrum in all cases is dominated by keratin-type protein bands. On the other hand, other organ samples exhibited approximately the same low infrared reflectance as the epidermal samples from other body regions, up to about 500 cm^{-1} (Figure 5).

Conclusions

The results presented here show that pit organ epidermis from the rattlesnake *Crotalus adamanteus* provides substantially higher transmission of mid-infrared radiation than epidermis from other body regions. We also found that the pit organ epidermis exhibits very low reflectivity of mid-infrared radiation. Thus, the epidermis covering pit organs in infrared imaging snakes plays an important role by selectively allowing mid-infrared radiation to pass easily to the infrared sensitive receptors located just beneath the epidermis. The enhanced reflectance in the visible region apparently does not extend to the infrared; thus, in effect, the epidermis of the pit organ acts as an infrared

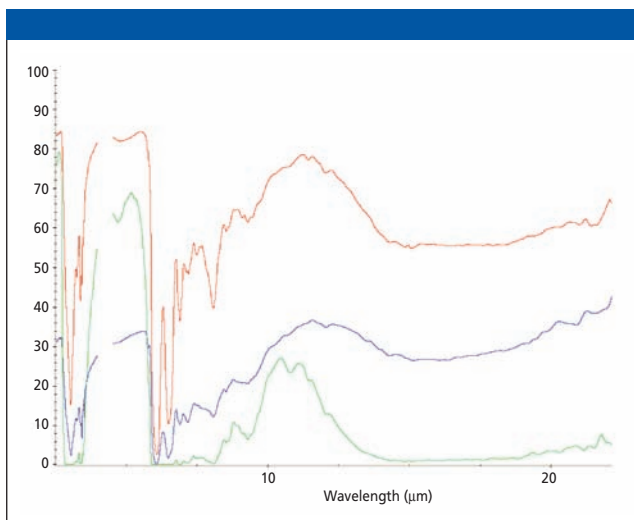


Figure 4: Percent transmission (2.5 μm –25 μm) of various regions of snake epidermis from the pit viper *Crotalus adamanteus*. (red = pit organ epidermis; blue = epidermis from between dorsal scales; green = spectacle).

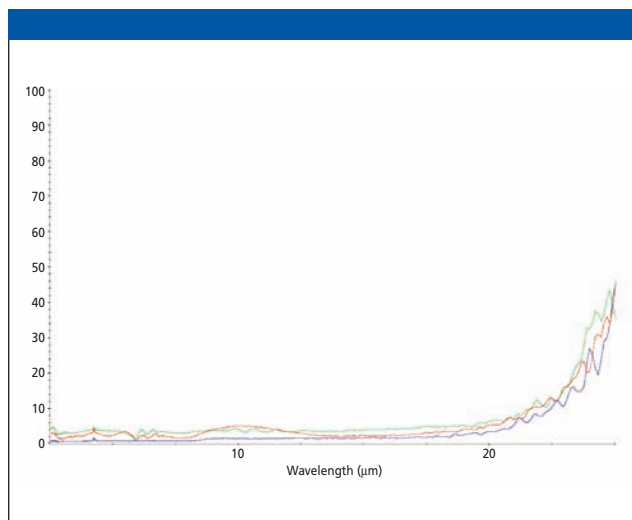


Figure 5: Percent reflectance (2.5 μm – 25 μm) of epidermis from the pit viper *Crotalus adamanteus*. (red = pit organ epidermis; blue = epidermis from between dorsal scales; green = spectacle).

selective low-pass filter. A detailed report of comparative results of different species and evolutionary significance of surface nanopit arrays to the infrared imaging system will be reported elsewhere.

Most of the samples used in this study were small, and nanopit arrays found on pit organ, spectacle, and interscale skin transition over very small distances to areas lacking nanopit arrays. Therefore, it was imperative to investigate the spectral properties of nanopit arrays using techniques that allow precise delivery of infrared radiation to well-defined spatial regions. The sample compartment mounted microscope used here, with its capability for transmittance and reflectance measurements in the mid-infrared, proved to be a powerful tool to perform precise and repeatable analyses on defined regions of a complex biological sensor, the pit viper infrared-imaging system.

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