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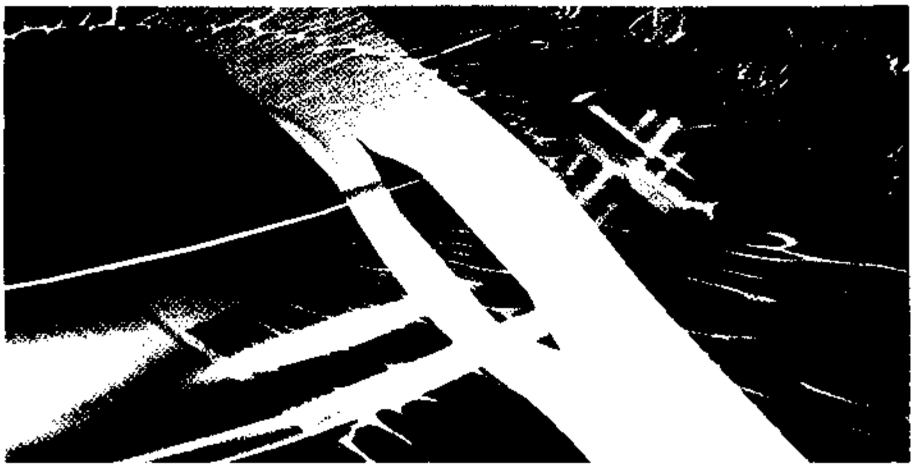


Photonics Unlocks Coral Reef's Beauty

MEASURING COLORS AROUND A CORAL REEF

by Justin Marshall

SUMMARY

Most creatures living in and around coral reefs are beautifully colored, sometimes reflecting wavelengths that are beyond the detection limits of the human eye. The first step in understanding the reasons behind the evolution of such a wide spectrum of body colors lies in simply identifying the colors that various species reflect. A versatile spectrometer developed in the United Kingdom facilitates this task.

Various technologies developed in recent years have made measuring the spectral reflectance of objects relatively simple. Small computers, sensitive CCD detectors and miniaturized spectrographs and gratings all make it possible to take a color reading in a few milliseconds using a small, easy-to-handle instrument.

The task is much more difficult, however, when the object is a swimming fish and the measurements must be made in situ at a depth of 150 ft. This is the problem encountered studying the extraordinarily colorful residents of coral reefs. In that environment, a spectrometer named Sub-Spec, the result of a collaboration between Sussex University in England and Andor Technology in Northern Ireland, is being used to quantify radiometric, color and polarization characteristics of the underwater world in a way not attempted before.

Humans and our close relatives use three color channels, or cone mechanisms. The mantis shrimp — a reclusive coral reef inhabitant — may have as many as 12 cones, several of which sample part of the ultraviolet region of the spectrum (300 to 400 nm). Human capabilities are also outdone by some reef fish that seem to have four cones for color vision, at least one of which is sensitive in the near-UV wavelengths to which we are blind.

In these circumstances, measuring or matching the colors of these creatures by human eyes is not accurate. Sub-Spec's empirical measurements can greatly aid us in understanding how the visual systems of reef creatures operate and why their colors evolved as they did.

The system's basics

Based on a highly modified version of Oriel Instruments' Instaspec IV system, Sub-Spec is controlled by a 486 microcomputer with a video graphics array (VGA) screen. The software makes it easily programmable to perform tasks with different parameters, and the computer is operable from outside the housing with a reduced numerical keypad.

The instrument, including the LCD monitor which faces out from one side, measures 35 × 25 × 24 cm and is powered by NiCad batteries (for up to 1.5 h) or a cable to the surface. For added durability, all circuit boards are plastic-coated, and the instrument is mounted on a relatively heavy aluminum chassis, so it should be able to withstand vibrations from boat transportation and a certain amount of sea spray.

The CCD is temperature-stabilized to reduce dark current and coated to enhance short-wavelength sensitivity. It is fitted with a grating that disperses light in the 300- to 800-nm spectral range and a spot-to-slit quartz optical fiber that yields 5-nm resolution. It can detect light levels as low as 10^{-12} W/cm^2/nm and where more light is available, can take rapid measurements (40 ms). Objects from the world outside are imaged onto the end of the quartz fiber while the operator of the Sub-Spec views them through a quartz Nikon 105-mm camera lens. Embedding the fiber end in a mirror behind the lens, similar to the configuration of an SLR camera, permits simultaneous detection and viewing and results in a black spot (the optical fiber end face) in the center of the operator's view.

Hold that pose

After background and reference readings have been taken to account for dark current and lighting conditions, the black spot is positioned on

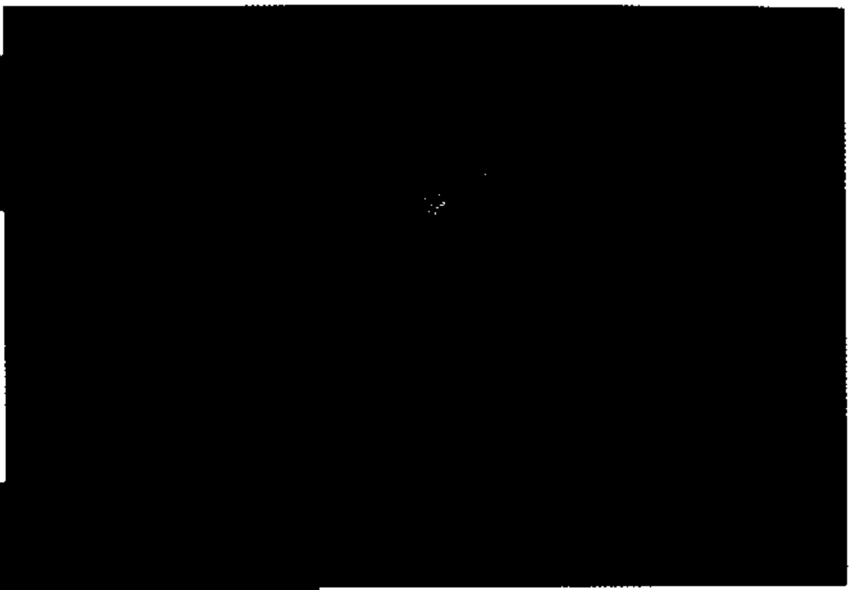
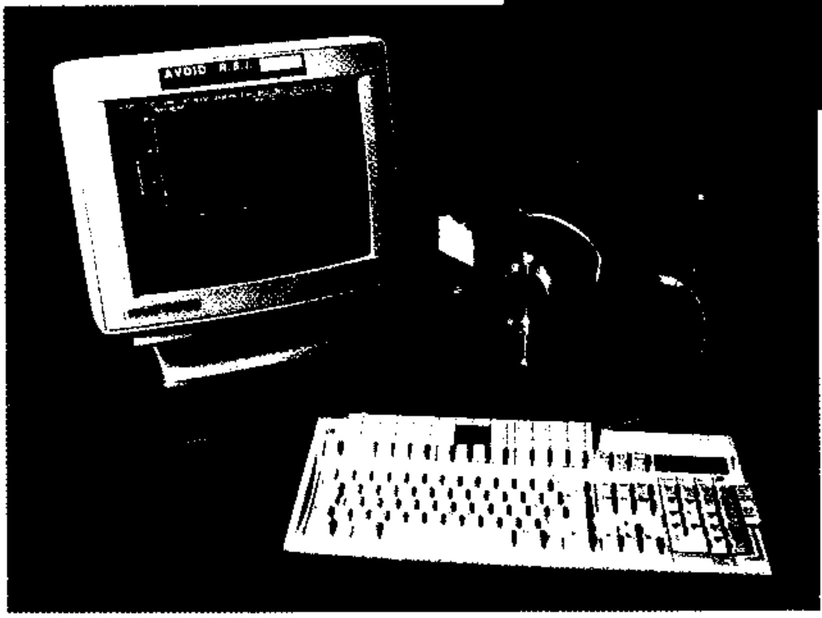


Figure 1. Sub-Spec operates at depths of 50 ft or more, as shown here near the Aquarius underwater habitat.

Figure 2. Sub-Spec can be used in or out of its housing in the laboratory.



the subject to be measured and a spectrum is recorded. To measure a moving fish this takes some practice. As is well known in the film industry, animals (and children!) rarely perform on the first take. Persuading a reef creature to swim in the same area where the reference was taken, hold still for a fraction of a second and stay in focus requires some patience.

Before presenting the tricks of using the instrument, consider the problem of fitting a computer-run optical bench into a one-user operable housing. Even with the instrument spread out on the bench, there are many problems associated with measuring such a broad spectral range of light. The light sources I used are natural sunlight or a cheap approximation to it: a xenon arc, usually in the form of an arc lamp (on land) or a camera flash unit (underwater), with the yellow filter removed to admit the UV.

Both natural and xenon illumination fluctuate over time. Intensity varies across the spectrum by a factor of 1000 in air. These imperfect aspects of the sources compromise the quality of the data. A tungsten source has a more constant emission, but such a lamp does not extend far into the near UV. Since reef fish and mantis shrimps in particular see and reflect light in the 300- to 370-nm range, it is important to use a source that provides near UV illumination. Unfortunately, this need makes the instrument about four times as expensive as it would be otherwise.

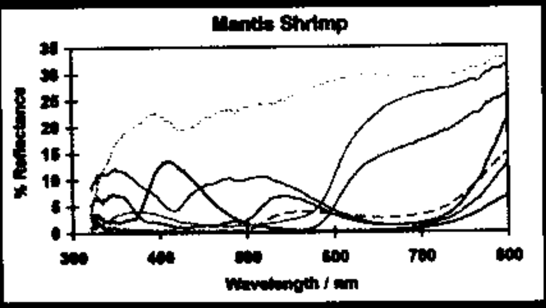
More problems with operating in the near UV come from the possibility of detecting the grating's second-order spectrum from 300 to 400 nm in the 600- to 800-nm region of the CCD. Sub-Spec solves this with a second-order filter placed over the long wavelength-region of the CCD, so the whole spectrum from 300 to 800 nm can be measured in one go.

Stray light

All spectrometers, no matter how carefully designed and how much baffling and black paint is used, suffer from the problem of stray light, especially when inputting broad-spectrum "white" light. In the laboratory, this problem is usually combated by using a double spectrograph. Because of the large size and the reduction in sensitivity of double systems, this solution could not be employed in Sub-Spec.

Both of Sub-Spec's sources (the sun or a xenon lamp) are relatively impoverished in the short wavelengths. This means that measurements in the UV region will be particularly susceptible to stray light problems. This can be demonstrated by examining the calculation for percentage of reflectance of an object, for example. The following equation is solved for each wavelength by the computer: % reflectance = 100 × (data − background)/reference. When the values

Figure 4. A mantis shrimp (stomatopod) looks out of its coral home and its reflectance spectra measured by Sub-Spec. Stomatopods use color for communication. Note several colors with significant reflection in the UV and far red, both not visible to humans. Colors here can be categorized as red, red/UV, yellow, green, green/UV, green/blue/UV (turquoise), blue/UV and white. The yellow-sided cube is a feeding cube used to train stomatopods to respond to color stimuli and thus demonstrate color vision. Color vision has yet to be shown for any other marine invertebrate.



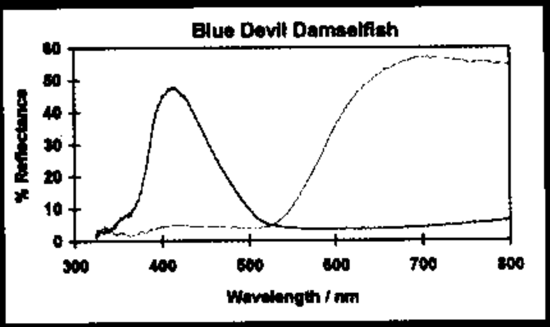


Figure 3. A blue devil damselfish and its reflectance spectra as measured by Sub-Spec. The sharply contrasting orange and blue can be used as an effective underwater signal. Data below 320 nm are not plotted because of the lack of light here and the resultant noisy spectrum. Notice that a substantial amount of the "blue" color lies in the UV region not easily visible to humans. This color is therefore perhaps better labeled "blue/UV."

in this equation are small (*i.e.*, there is very little light), a slight error in measurement produces a large change in the calculated reflectance percentage that is an artifact. In addition to stray light, artifactual numbers can also be caused by a change in the CCD dark current, which is affected by temperature.

Although Sub-Spec's CCD is temperature-stabilized with a Peltier cooler, confinement of the heat exchange mechanism in an underwater housing can cause the temperature to build up to well over 35 °C in a short time. Consequently the cooler has to work harder, draining battery power and eventually resulting in power failure or CCD instability. The water around the housing helps to dissipate the excess heat to some extent. The addition of a water circulation system in the future may further reduce heating. However, this would also increase the chance of water leakage into the housing.

The housing was designed with

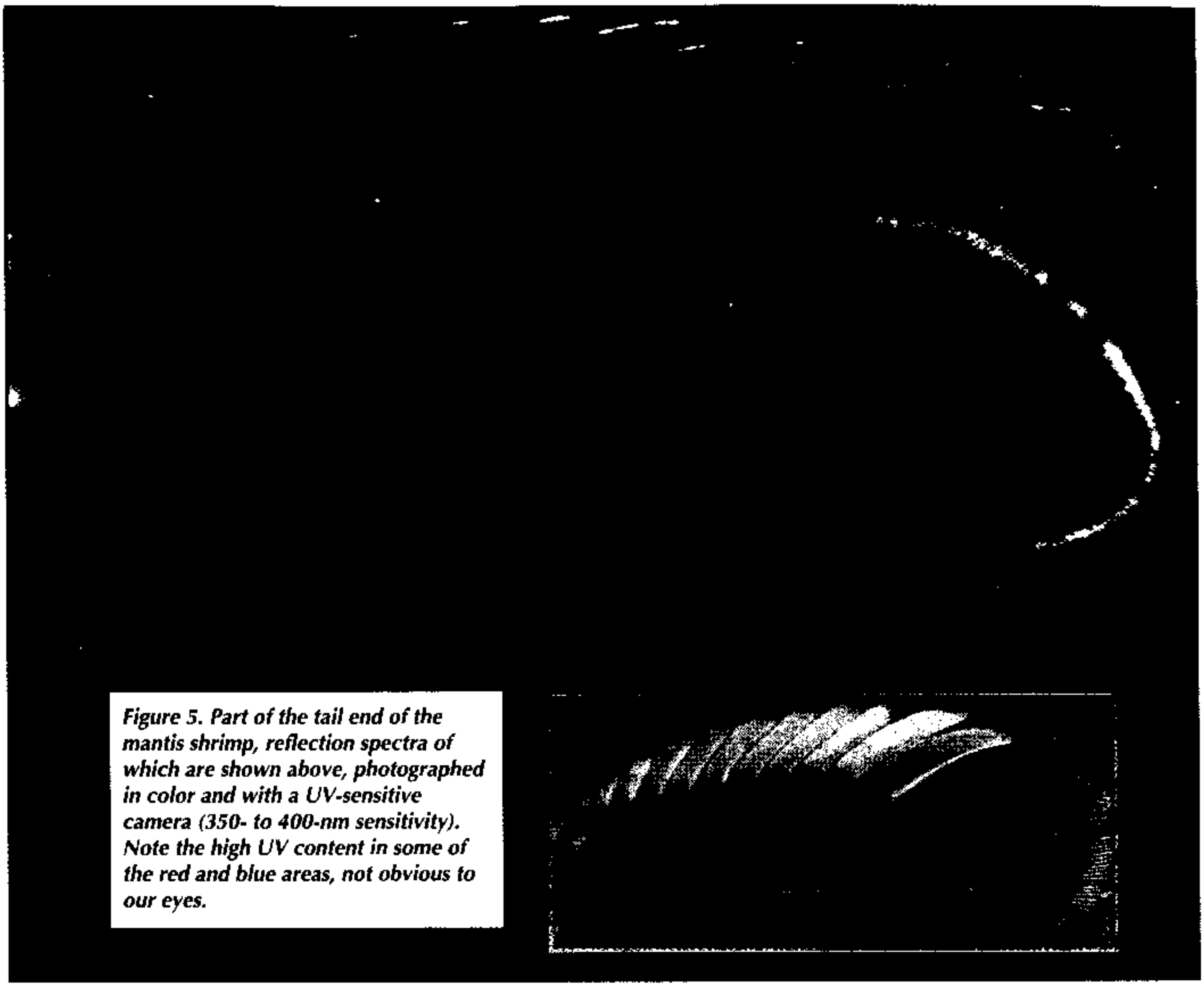


Figure 5. Part of the tail end of the mantis shrimp, reflection spectra of which are shown above, photographed in color and with a UV-sensitive camera (350- to 400-nm sensitivity). Note the high UV content in some of the red and blue areas, not obvious to our eyes.

three goals in mind: ease of operation for one diver both above and below the water, the need to communicate with the computer and instrument within and the need to view the VGA screen. The large size of the VGA screen, determined by the state of technology while we were developing our prototype, limited the housing design to a rectangular box. This design allowed us to see the monitor through a Plexiglas window in one side of the box while minimizing air space to keep buoyancy down.

A ton of water pressure

Disadvantages of the boxy shape include the difficulty of sealing square ends onto housing (this would be easier with round O-ring seals) and the tremendous pressure on the flat sides of the housing. Even at a depth of only 30 ft, each side receives around 1 ton of pressure, so the plastic used to build the housing had to be thick and heavy. This made the instrument difficult to handle by one person above water, but fortunately, the weight matched the buoyancy, making it maneuverable underwater.

Advances in technology since our original prototype construction would improve development of such an instrument today. Miniature spectrographs designed for installation into a computer and smaller VGA screens could compose a much smaller underwater spectrometer.

The fast speed of Sub-Spec's detection is invaluable for taking color reflectance measurements with natural or flash illumination. In addition, the instrument can record irradiance readings, radiance measurements and differences in polarized light content (using a rotatable UV-transmitting Polaroid sheet).

Calibration for these applications presents a whole new set of nightmares because each time the lens focus or *f*-stop is changed, recalibration is needed. And since the spectral variation of natural sunlight worsens with increasing ocean depth, it is very difficult to obtain a single accurate calibration across the entire spectrum in a single shot. In practice, we simply calibrate with the lens fixed at closest focus for three *f*-stops to allow extension of the dynamic range.

Silt and sand

Although environmental lighting conditions change rapidly when clouds cover the sun or silt and sand are stirred up by divers or currents, many of the unforeseen problems are biological; fish change color, sometimes in less than a second. Some of these colors are at least partially structural in nature, so the angle of measurement can be important.

Another problem is that animals tend to swim away or hide when approached by a diver. The relatively large size of the Sub-Spec housing can be an advantage here, because fish are often curious as to what might be in the box and hang around to have their spectra taken. On the other hand, I have found that it is difficult to measure "cleaner fish," (ones that clean parasites and dead skin off larger fish) because of their attempts to clean Sub-Spec.

The ideal solution to many of these practical problems would be to take a measurement in a small fraction of a second with equal sensitivity over the entire spectrum. Given the ten or more log units of lighting change seen during the diurnal cycle in the natural world, this is an impossibility. In practice, a shutter speed of 0.1 sec is fast enough to account for most changes in the animal itself and slow enough to smooth out the high-frequency variations in underwater lighting. Averaging, which improves signal strength and sensitivity, may obscure detail because of short-term color changes, but in some instances, can be useful for smoothing temporal noise. With some fish, anesthesia can permit slower, more accurate measurements, but other varieties respond to the stress with dramatic color changes, as does the damselfish, which turns black.

Underwater lab

Last year I spent a highly productive period measuring the reflectance of reef creatures both inside and outside the "Aquarius" habitat off Key Largo, Fla. This is an underwater laboratory at a 45-ft depth, where up to five scientists can work without returning to the surface for ten days. The purpose of our mission, led by Thomas W. Cronin [see *Biophotonics International*, March/April 1995, p. 38], was to measure color and polarization in this environment. With the expert help of Daryl Parkyn, I measured reflectance colors of over 45 fish species, two mantis shrimp species and a number of other crustaceans and corals.

Sub-Spec performed almost perfectly in this salty environment, both in and out of its housing. The only snag was caused by the ambient pressure of 45 ft. of water inside Aquarius, which prevented the hard disk from spinning. Therefore, the program had to be run and data gathered through the floppy drive. On return to the surface, the whole unit ran perfectly.

Although we can now measure the wide range of wavelengths reflected by the inhabitants of coral reefs, we have a long way to go in our understanding of why this coloration evolved. Camouflage, warning coloration and color communication in displays of sexual aggression or attraction are clearly important, but the subtleties behind these wonderful displays are lost to us, and we often misinterpret them.

It is presumed by many that octopuses, for example, with their near-perfect camouflage and flashes of colorful emotion — such as the vibrant blue of the blue-ringed octopus — must be able to see their own colors and those of their environment. However, these animals do not possess the complexity of eye design needed for color vision. Thus, the blue rings must have evolved for the benefit of other animals' visual systems, their message being, "Go away. I'm toxic and can deliver a lethal bite."☐

Meet the author

Currently a research fellow at the Sussex Centre for Neurosciences, Justin Marshall received his BSc in zoology from the University of St. Andrews in Scotland and earned his DPhil at the University of Sussex studying vision in mantis shrimp. He soon will start as a QEII research fellow at the Vision, Touch and Hearing Research Centre at the University of Brisbane in order to be closer to the Great Barrier Reef.