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COMMENTARY

Illumination geometry, detector position and the objective determination of animal signal colours in natural light

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The study of colour patterns used in animal signals often requires objective measurement of spectral quality and intensity under natural field conditions. In practice this can be difficult because animals are mobile and may display dynamically at particular locations or times of day (Endler 1992; Endler & Thery 1996). The most common solution is to measure the spectral reflectance of the coloured surface under controlled laboratory conditions, measure the light typically striking the surface in the field (habitat irradiance) and multiply the two values together to produce an estimate of the intensity and/or spectral quality (e.g. Andersson 2000; Macedonia 2001; Cummings et al. 2003; Stuart-Fox et al. 2003). In general, however, the geometrical arrangement of light striking the surface (i.e. the density distribution and directional characteristics of light rays) and the position of the viewer are not the same in laboratory and field (Endler 1990). If so, this procedure will produce an accurate estimate of signal appearance only if two critical assumptions are met. First, the measured surface must reflect light in a highly diffuse manner for all visible wavelengths (Fig. 1). Second, reflected light must dominate the spectrum, with little contribution from transmitted light.

In this paper, we illustrate a method for quantifying the effects of illumination and detection geometry on the spectrum and intensity of natural surfaces to test these critical assumptions. We use the body and dewlap (coloured throat fan) of the lizard *Anolis cristatellus* as examples. We then explore the consequences of failing to take deviations from these assumptions into account, by comparing direct measurements under natural light conditions to those obtained by calculating appearance.

Characterizing the Appearance of a Coloured Surface under Natural Light

Some specialized terms as used in this paper are described in Table 1. Light reflecting from or transmitting through a surface can be either specular or diffuse (Fig. 1). If rays striking a surface are equally likely to reflect (or transmit) at any angle, the surface is said to be an 'ideal' or a 'Lambertian' diffuser. Most natural surfaces have some combination of specular and diffuse reflection and/or transmission (Gates 1980; McCluney 1994; Palmer 1995).

If a surface with spectral reflectance (S) approximates a reflective Lambertian diffuser across all wavelengths of interest, and transmission is trivial, then its appearance, characterized by its radiance (R), can be calculated as

$$R = I \times S \times 1/\pi \qquad (1)$$

(Palmer 1995; Johnsen 2002). The radiance of a Lambertian surface does not change with distance or angle of view, making it possible to estimate radiance under a wide variety of field conditions for any viewing angle or distance from viewer to surface. Even more critically, the geometrical distribution of the light rays contributing to the irradiance (I) does not matter, as long as it is measured with a calibrated cosine-corrected probe oriented normal to the measured surface. Many published studies use equation (1), or a modification of it (e.g. if only spectral shape is important, the correction factor $1/\pi$, which converts the spectrum to units of absolute radiance, can be left out) to calculate signal appearance.

If the coloured surface deviates substantially from the assumption of Lambertian reflection, one cannot calculate radiance without a detailed knowledge of illumination

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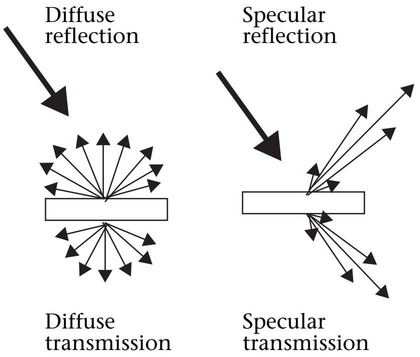

Figure 1. Light striking a surface can be absorbed, transmitted or reflected. The two major forms of reflection are specular and diffuse. In ideal diffuse reflection, the angle of reflection is independent of the angle at which the ray strikes the surface. In pure specular reflection, light rays reflect at an angle equal to the angle at which they strike the surface. Most real surfaces have some combination of these effects. Transmission can also be specular or diffuse.

Table 1. Definitions of important terms used

Term	Abbreviation	Definition
Steridians	sr	A measure of solid angle. A hemisphere covers a solid angle of 2π sr.
Radiance	*R*	Light emerging from a unit area of a flat surface measured over a small defined solid angle. It can be expressed as spectral radiance (measured as a function of wavelength) or as total radiance (integrated over a defined range of wavelengths). The units for spectral radiance are photons/m^2/s/sr/nm. It is the most useful descriptor of the appearance of the colour of a surface.
Irradiance	*I*	All of the light impinging on a unit area of flat surface measured over a hemisphere (2π sr) centred on and oriented normal to the surface. A given density of rays will spread out over a larger area if they arrive at an angle, which reduces their contribution to irradiance by the cosine of the angle of incidence. *I* is measured with a cosine-corrected irradiance probe, which corrects for the tendency of steeply angled rays to reflect off a detector surface and be undercounted. It can be expressed as spectral or total *I*. Spectral *I* has units of photons/m^2/s/nm.
Reflectance	*S*	The fraction of light striking a surface that is reflected from it. Spectral reflectance (S_λ) is measured as a function of wavelength. It is typically determined by comparing the light reflected from a surface of interest to the light reflected from a spectrally flat reflectance standard.
Transmittance	*T*	The fraction of light striking a surface that passes through. Spectral transmission (T_λ) is a function of wavelength.

geometry and detector position and their influence on radiance (Endler 1990), because radiance will depend on detector position relative to the directional distribution of the light rays impinging on the surface (Fig. 2). This difficulty has caused many studies to assume either explicitly (e.g. Johnsen 2002) or implicitly (e.g. Macedonia 2001; Cummings et al. 2003) that the surface has primarily diffuse reflectance. Although such an assumption may be reasonable for many surfaces (Johnsen 2002), if the assumption does not hold, the estimate of radiance may be inaccurate.

Many investigators are interested only in the spectral quality of a surface and not in total intensity. In such cases, it may not be critical that the surface be Lambertian with respect to intensity. However, all parts of the visible spectrum must interact with the surface in the same manner with respect to illumination geometry and viewer position. If not, the spectrum will change depending on the viewer's position and/or the geometry of illumination. This can occur for a variety of reasons. The most dramatic cases involve 'structural' colours, which often change hue or shimmer at certain viewing angles (reviews in Parker 1998; Osorio & Ham 2002). For pigment-based colours, like those we consider here, there are other, less dramatic ways that viewing and lighting geometry can alter spectral quality (Hailman 1986; Eckert & Carter 2000). For example, the surface may have more specular reflection for some wavelengths than for others, which would result in a shift in the overall spectrum with angle of view unless the illumination is highly diffuse.

Many biological structures are thin and transmit a great deal of light (e.g. insect wings, extended bird feathers, fish fins, thin-bodied animals, leaves, flower petals). In these cases, transmitted light must be taken into account to determine total or spectral radiance. If the transmission is Lambertian, its effects can be estimated in a manner similar to that for reflectance, and surface appearance can be estimated by adding reflected and transmitted radiance (Johnsen 2002; Leal & Fleishman 2004). However, as for reflected light, if transmission deviates substantially from Lambertian properties, there is no simple way other than direct measurement to determine appearance in the field without knowing details of the illumination geometry and viewer position.

For this paper, we illustrate a simple method for testing the critical assumptions required for estimation of signal colour under field conditions by precisely quantifying the relation between illumination and viewer geometry and the shape of the spectrum and the intensity of light for a few natural surfaces. We illuminated our test surfaces uniformly with a small beam of collimated light (all rays parallel) from different angles, and measured spectral radiance from a full range of angles around the surface. We started by measuring the properties of diffuse

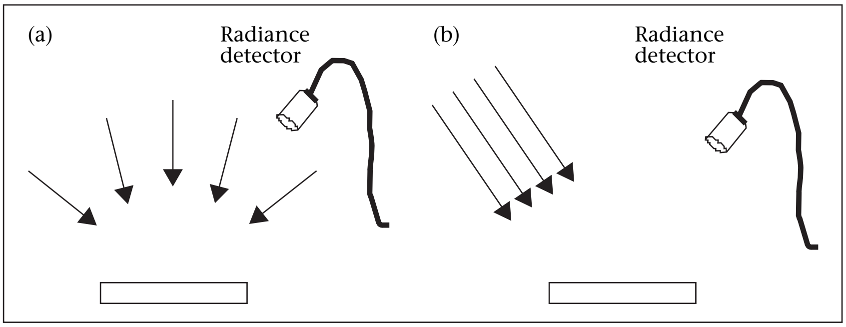

Figure 2. A hypothetical reflecting surface is illuminated with two different illumination geometries that are scaled to create identical irradiance (measured normal to the surface with a cosine-corrected irradiance probe). If the surface is a Lambertian reflector, the radiance recorded by the detector will be identical in (a) and (b). If the surface is not a Lambertian diffuser, the radiance values in (a) and (b) will differ substantially.

reflectance and transmission standards. We then measured the body and throat fan (dewlap) of the lizard *Anolis cristatellus*. For the lizard dewlap, we found that the assumptions of equation (1) were not satisfied. We tested the consequences by making direct measurements of spectral radiance under natural light conditions, and comparing these to radiance values calculated using two sets of assumptions: (1) that the dewlap acts as a diffuse Lambertian reflector, and (2) using a method that takes transmission and reflection into account (Leal & Fleishman 2004), assuming that both are Lambertian. The methods that we describe here are appropriate for testing any flat surface for the effects of illumination geometry on surface appearance, and our results illustrate the need to consider transmitted light, illumination geometry and viewer position when attempting to estimate the colour and brightness of animal signals under natural lighting conditions.

Methods

Laboratory measurements. The measurement apparatus is shown schematically in Fig. 3. For lizard measurements, the animal was gently held with tape to a small metal rod above and parallel to the surface of the platform. The leading edge of the dewlap was carefully grasped with a pair of fine forceps mounted on a two-axis micromanipulator whose opening was controlled by a small set screw. The dewlap was held in place in a naturally full open position. For measurements of the lizard's body, the position was shifted so that a relatively flat area of its flank, posterior to its front arm, was at the centre of the apparatus. We also measured a diffuse white reflectance standard and an opal glass diffuse transmission standard.

The measured surfaces were illuminated with a collimated beam of light produced by a 300-W xenon arc lamp passed through a small hole and a planoconvex lens positioned one focal length from the hole. This beam was deflected upwards off a front-surface mirror and then directed towards the coloured surface to be measured by a second front-surface mirror. This mirror was placed just above the platform to create a beam parallel to the platform even with the centre of the measured surface (defined as 'level'), or the mirror was elevated so that the beam was directed downward towards the measured surface, striking it at an angle of 45° ('elevated' illumination). The measured surface was mounted at the centre of a circular platform along the line connecting 0° and 180°. The platform was positioned so that the source beam was positioned at 90° or at 45° in the horizontal plane relative to the orientation of the measured surface.

Three illumination conditions were used: (1) light beam at the same vertical level as the measured surface, with the beam oriented 90° to the surface in the horizontal plane (Level-90°); (2) the light beam at the same vertical level, and the beam oriented at 45° to the surface in the horizontal plane (Level-45°); (3) the light beam elevated and striking the surface at 45° in the vertical plane and at 90° in the horizontal plane (Elevated-90°). In condition (3) the light path to the surface was longer than for the level conditions (Fig. 3). The beam was not perfectly collimated, so this resulted in a slight decrease in the intensity of the beam at the surface. We directly measured the effect of path length on beam intensity and included a correction for path length (independent of angle) where appropriate.

A radiance detector probe with a 4° acceptance angle was connected to a bifurcated, fused-silica fibre-optic cable. The single end consisted of two fibres mounted together in a single case, which was connected to the radiance probe. The fibre then divided into two separate, single fibres. One was connected to the input of an Ocean Optics USB2000 spectroradiometer, used to record spectral radiance. The second fibre was connected to a 50-W tungsten fibre-optic illuminator, which, when turned on, passed light out through the radiance probe, creating a small dot immediately adjacent to, and identical in size and shape to, the area from which the detector was sampling. We refer to this as the positioning light. To ensure that measurements were made repeatedly from the same location on the surface, a stand-mounted laser pointer was used to mark the measurement position. Before each measurement, the positioning light from the detector was matched to the position of the laser pointer. The laser pointer and positioning light were then turned off and the measurement was taken. The entire sampling area always fell within the area uniformly illuminated by the collimated light beam.

Measurements were taken from a full range of angles around the surface in increments of 22.5°, although for

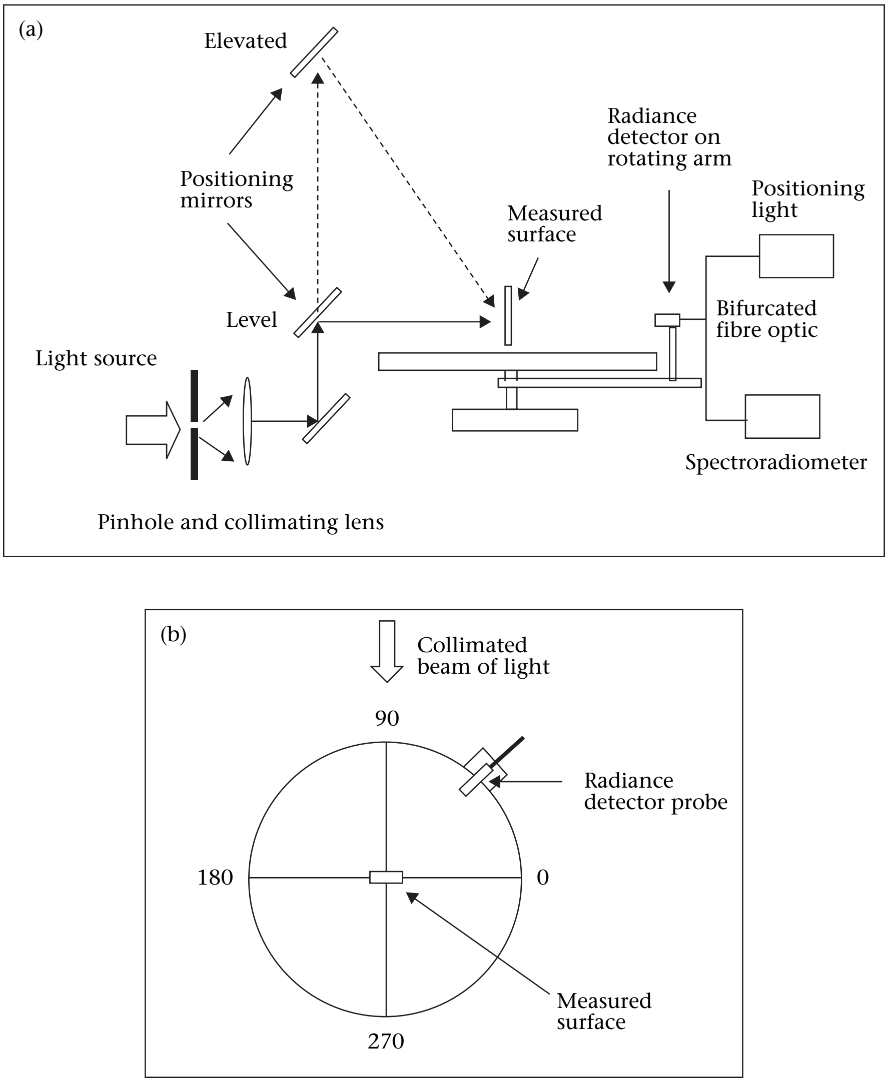

Figure 3. Schematics of the apparatus used to measure coloured surfaces as viewed (a) from the side and (b) from above.

some surfaces we were unable to record from the steepest angles. For the level illumination conditions, the detector blocked the light source when positioned at the same horizontal angle position as the collimated beam. In these cases, the measurement was taken 8° away from this angle, which was just sufficient to avoid blocking the light.

Immediately after each complete set of measurements for a surface, we placed the diffuse white reflectance standard at the centre of the apparatus. We positioned the light source at Level-90°, placed the detector at 45° and recorded spectral radiance. We used this value as a standard, and all other measurements are reported relative to this value. For each measurement, we collected light data in intervals of 1 nm. Each value was divided by the value at that wavelength recorded from the white standard in the calibration position. For intensity (i.e. total radiance), the area under the curve was summed from 350 to 700 nm. Thus, for example, the white standard intensity measured from the 45° position had a value of 350, because it had, by definition, a value of 1.0 at each wavelength and was then summed over 350 nm.

We used a single lizard, which was collected near the Cambalache State Forest in Puerto Rico (see Leal & Fleishman 2004 for details of collection and care). Variation in dewlap coloration is small within each population (Leal & Fleishman 2004), so this single sample is a reasonable representation of a dewlap of lizards from this population. The lizard was held in the measurement apparatus for a maximum of 1 h at a time and then returned to its cage. There were no observable adverse effects, and the lizard ate and drank normally the following day, which, in this species, is a good indication that they are not suffering from high stress.

For each sample, we made the complete set of measurements three times. Variation between measurements at the same angle and illumination condition was low, and we present only means of the three values measured.

Comparisons of field measurements and calculated radiance. In June 2003 we measured the radiance of the *A. cristatellus* dewlap directly under outdoor conditions near our laboratory in New York state, using the same individual from the laboratory measurements. The lizard was cooled to approximately 12°C (for stress reduction during handling) and then was gently taped, head upward, to a small wooden rod. We carefully opened the dewlap and attached a thin strip of clear plastic to the leading edge with a drop of cyanocryalate adhesive. The other end of the plastic strip was glued to a curved metal wire that extended from the top of the rod so that the dewlap was held fully open. The stick was placed in a holder mounted at the end of a metal rod so that the lizard was vertically oriented with its head upward and its dewlap fully extended. At the other end of the rod was mounted a small *X*–*Y*–*Z* stage, which held the 4° acceptance-angle radiance probe. The probe, attached to a bifurcated fibre-optic cable, was positioned so that it pointed at the dewlap, making an angle of 70° with the flat surface, 25 cm from the dewlap. A green laser pointer was used as the input to the positioning arm of the bifurcated fibre-optic cable to precisely locate the position from which the detector was sampling. This was always turned off during sampling. The second arm of the cable was input to the S2000 spectroradiometer. There was enough space between the probe and lizard and both were mounted well above the base so that most of the environmental light was allowed to strike the dewlap without obstruction. Laboratory tests showed that the amount of light obstructed by the apparatus resulted in less than 1.0% reduction in total radiance and no change in spectrum.

We measured radiance in an open field and in a heavily shaded forest on the same morning on a sunny day between 1000 and 1100 hours in Schenectady, New York, U.S.A. We measured dewlap radiance with the dewlap surface perpendicular to the ground and the line of sight between the detector and dewlap parallel to the ground. We made measurements with the detector pointed towards the sun (i.e. the sun on the opposite side of the dewlap from the radiance probe) and with the detector pointed away from the sun (dewlap and radiance probe on the same side of the sun). We also measured radiance reflected off a diffuse white standard in all relevant directions and used a modification of equation (1), ($I = \pi \times R$), to calculate irradiance striking the dewlap from each side at the position where it had been held. We measured spectral reflectance in the laboratory before and after the field measurements and determined that it did not change during the procedure.

For comparison, we calculated dewlap radiance in each locale using two sets of assumptions. First, we calculated dewlap spectral radiance using equation (1) (reflectance-only method). The dewlap reflectance was determined by measuring spectral radiance using the Level-90° illumination condition with the detector at 45°. We then measured the diffuse white reflectance standard under the same conditions, and divided the dewlap value by the reflectance standard value for each wavelength. Our second set of calculations used data and methods published by Leal & Fleishman (2004), for a lizard from the same population as that measured here. In the laboratory, Leal & Fleishman illuminated the open dewlap with a broad diffuse circle of white light 30 cm in diameter centred on and positioned 20 cm from the dewlap. They directly measured the spectral irradiance striking the front of the dewlap. They then measured dewlap spectral radiance on the same side as the source (reflected) and on the opposite side (transmitted). For each side, they calculated an *R*/*I* ratio for each wavelength. These ratios can then be multiplied by any set of field-measured spectral irradiances (one striking each side of the dewlap) to calculate dewlap radiance, which is the sum of the reflected and transmitted radiances. Thus, this method takes both reflected and transmitted light into account and allows for generalized calculation of dewlap radiance from field-measured irradiance based on the assumption that both reflection and transmission are Lambertian, because if they are not, the results will be valid only when the geometry of illumination in laboratory and field are the same.

The animal used in these experiments was treated in accordance with the guidelines for treatment of reptiles outlined in Pough (1991) and Union College IACUC Protocol No. 1056.

Results

Intensity effects. The results for total intensity ($R = 350$–700 nm) are reported in polar coordinates (Figs 4–6). Figure 4 shows the combined results for the diffuse white reflectance standard (reflection only) and the white opal glass diffuser (transmission only). These standards showed near-ideal Lambertian properties. The radiance was nearly equal when measured from different angles and deviated only at the most extreme angles. When the source struck the surface at 45°, either from the side or from above, the radiance was reduced by a factor of approximately 0.7 (equal to cosine 45°), because irradiance of the surface varies with the cosine of the angle with which light rays strike it. The intensity of the light transmitted through the diffuser was lower than that reflected from the white standard because it was reduced by reflection off the front surface (not shown) and absorption.

The results for the lizard body are shown in Fig. 5. The reflectance showed a reasonably good approximation of Lambertian reflection, similar to the white reflectance standard, with deviation only at the sharpest angles. In the Level-45° case the intensity was slightly elevated when measured from 45°. This property is known as retroflection (Palmer 1995), and probably results from some aspect of the fine-structure of the lizard scales. Transmission was approximately 100 times lower than reflectance, and was recorded only for the Level-90° light source position.

The results for the dewlap are shown in Fig. 6. For the Level-90° condition, the reflection showed reasonably good Lambertian properties, although some specular reflection was apparent. The transmission had a strong directional effect, with the greatest intensity occurring when the detector was directly opposite the source. When the source was positioned at Level-45°, the result was similar. Intensity was reduced by the expected amount, by a factor of approximately cos(45°), and the reflection and transmission properties were essentially

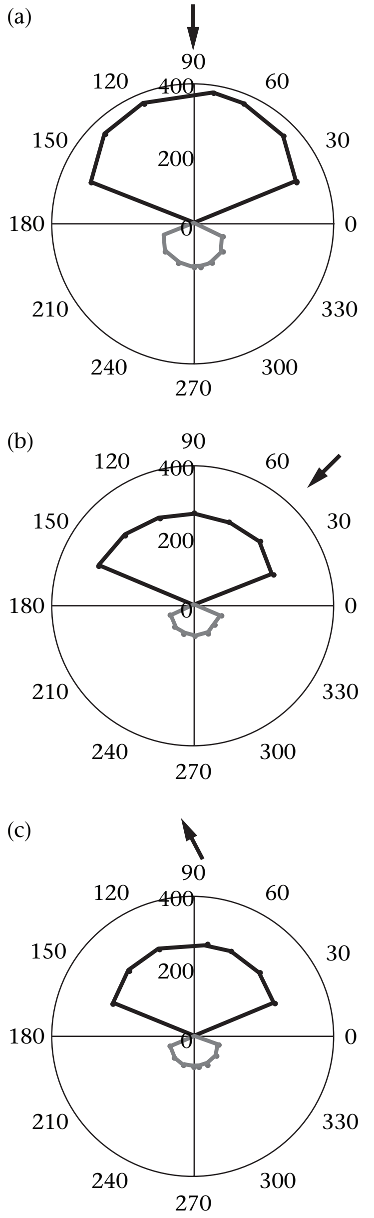

Figure 4. Radiance (summed from 350 to 700 nm) as a function of illumination condition and detector position for a diffuse white reflectance standard (black line) and a diffuse opal glass transmission standard (grey line). (a) Light source positioned at Level-90°. (b) Light source positioned at Level-45°. (c) Light source positioned at Elevated-90°. The dark arrows indicate the direction from which the collimated source beam was directed. In (c) the upward angled arrow indicates that the source beam was elevated so that it struck the surface at 45° from above in the vertical plane. Radiance was measured in 22.5° increments around the circular platform. The magnitude of radiance is indicated by numbers on the upper quadrant of the *Y* axis. Only transmission is shown for the diffuse transmission standard, and only reflectance is shown for the diffuse reflectance standard.

unchanged from the Level-90° case. For the Elevated-90° case, the directional transmission effects were reduced, and the intensity of transmitted light was much lower than for the Level-90° case. The specularly transmitted rays stay in the plane of the source beam and do not reach the detector, which is in a different plane. Only the diffuse rays are detected, so that the measured radiance is diffuse but also of lower intensity than would be expected if the surface were Lambertian.

Spectral effects. Direction of source and detector had no influence on the spectrum for the white standard, the opal diffuser or the lizard body. We limit our discussion here to the dewlap. In Fig. 7 the dewlap spectrum is plotted as a function of detector position for two illumination cases: Level-90° and Elevated-90° (the Level-45° results were similar to Level-90° and are not shown here). The reflected spectra were similar for elevated and level illumination, and these spectra did not change with detector angle. The spectra consisted of a UV portion, a low short-wavelength portion, a rapid increase at approximately 500 nm and a relatively flat long-wavelength portion. The transmitted spectra were similar for level and elevated illumination. For the level case, there was some change in spectral shape with detector position, but for the elevated case the spectrum did not change with detector position. The transmitted spectra differed from the reflected spectra: transmitted spectra showed less UV, a shallower shift from the short- to the long-wavelength portions of the spectrum near 500 nm and a continuous increase in intensity from 500 nm onward.

In Fig. 8 we present a plot of the relative stimulation of each of the four classes of cones found in the retina of *A. cristatellus*, for spectra recorded from three of the directions (Level-90° case) shown in Fig. 7. This plot illustrates that the changes in light direction result in modest changes in the ratios of stimulation of the cone classes. Table 2 shows the magnitude of the change in stimulation of each cone class when the detection angle was changed.

Field and calculated measurements for the dewlap. For the direct measurements of dewlap radiance under forest shade, the two estimation methods produced good approximations of the spectral shape (Fig. 9a, b). In both cases, the Leal & Fleishman method (reflectance plus transmittance) overestimated total radiance, whereas the reflectance-only method underestimated total radiance.

For measurements of direct radiance under full sun, with the sun and detector on the same side, spectral shape was well predicted by both estimation methods, although the reflectance-only procedure seriously underestimated total radiance (Fig. 9c). With the sun behind the dewlap, the reflectance procedure badly underestimated total radiance and yielded an incorrect spectrum shape (Fig. 9d). Here the Leal & Fleishman method gave a good approximation of intensity and spectral shape for both directions.

Discussion

The work described here illustrates that calculations of signal appearance made by multiplying spectral reflectance by habitat spectral irradiance are valid for general conditions only if the surface approximates a Lambertian reflector and if transmission is minimal. However, many surfaces of interest are thin enough to be transmissive,

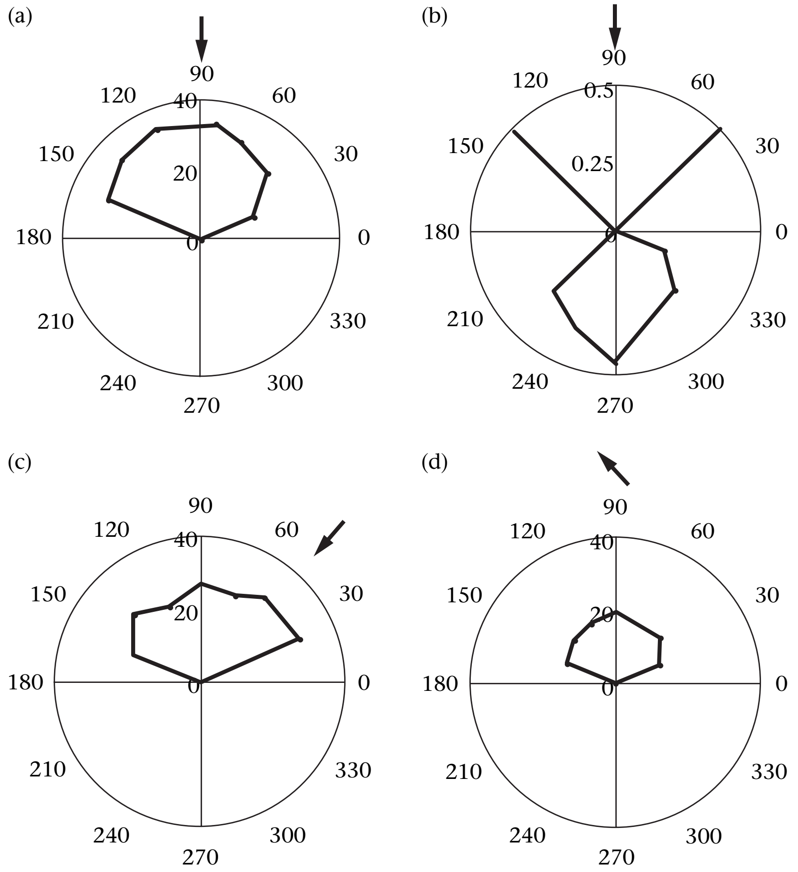

Figure 5. Radiance (summed from 350 to 700 nm) as a function of illumination condition and detector position for the flank of the body of a male *Anolis cristatellus*. Arrows indicate the position of the light source. Illumination conditions were (a) Level-90°. (b) Same as (a) with the scale expanded to show transmitted light. (c) Level-45° (reflection only). (d) Elevated-90° (reflection only). No attempt was made to measure transmission for the last two cases.

and a wide variety of natural surfaces show changes in radiance (spectral and/or total) with changes in illumination and viewing geometry (Hailman 1986). In general, if spectral quality or intensity change with the angular distribution of illuminating rays and/or with viewer position, estimates of appearance under field conditions that are based solely on laboratory spectral reflectance measurements may be inaccurate.

The plots of total radiance for the reflecting and transmitting white standards (Fig. 4) illustrate the properties of a Lambertian surface. The radiance is the same from all viewing angles, and intensity varies with the cosine of the angle that the rays make with the surface. The same result is observed whether the light comes from above at 45° or from the side. One can determine how far other surfaces deviate from being Lambertian by comparing to these standards.

The lizard body showed much greater reflection than transmission, and the reflection was approximately Lambertian in shape. Thus, the lizard body meets the assumptions underlying equation (1), and its spectral radiance could easily be calculated under field conditions. To the extent that this lizard body is typical of other animal body surfaces, the result supports the validity of using equation (1) to calculate radiance for signals on some animal body surfaces (Johnsen 2002). However, some animal bodies have subtle surface structure that results in complex relations between viewing geometry and radiance (e.g. Denton & Rowe 1998). Another caveat is that when using equation (1) to estimate radiance, the irradiance striking a surface must be measured with a cosine-corrected detector oriented normal to the surface. Investigators have often used values for downwelling irradiance (irradiance measured with a detector pointed straight up; e.g. spectra published in Endler 1993), even when calculating a colour on an animal's flank. There are substantial differences between irradiance spectra measured parallel to the ground and those measured with a detector pointed upward (Fleishman et al. 1997), and accurate estimates will depend on the correct irradiance orientation.

The lizard dewlap is more complicated. As a reflector, it showed reasonably good Lambertian properties. The dewlap also transmitted light strongly. Transmission exhibited a mix of diffuse and specular properties. There was a very strong directional effect and a viewer directly opposite the light source sees a much brighter dewlap. For some detector positions, transmission was several times more intense than reflection. Under most natural conditions, both reflection and transmission contribute to dewlap radiance. The results suggest that for some viewer

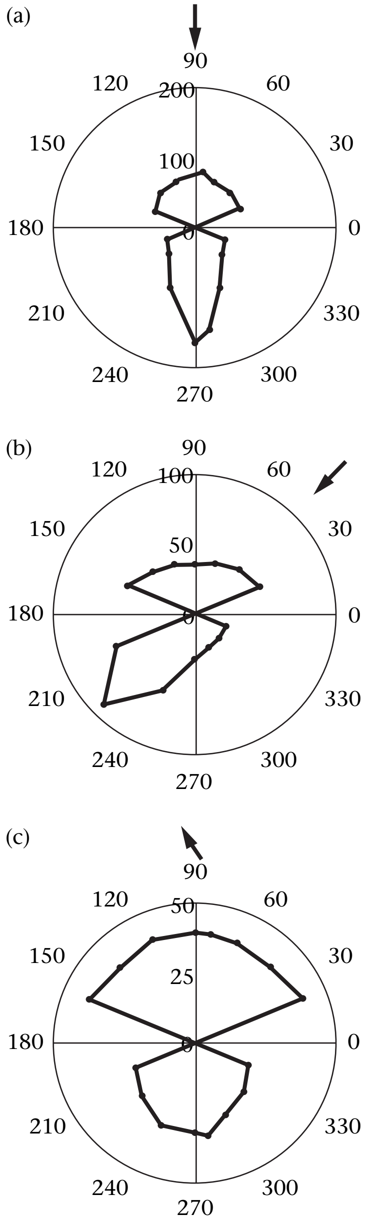

Figure 6. Radiance (summed from 350 to 700 nm) as a function of illumination condition and detector position for the extended dewlap (gular throat fan) of a male *A. cristatellus*. Both transmission and reflection are shown for each case. Arrows indicate the position of the light source. Illumination conditions were (a) Level-90°, (b) Level-45° and (c) Elevated-90°.

orientations, for example, with the sun on the opposite side of the dewlap from the viewer, transmission will dominate the radiance.

The spectral quality of the dewlap is also influenced by geometry. For reflection, the dewlap spectrum did not change with direction of viewer or source. For transmission, there was a directional effect. Moreover, the transmitted and reflected spectra differed. The natural radiance spectrum is a weighted sum of the transmitted and reflected spectra; therefore, it will change depending on the location of the strongest illumination (typically the sun) relative to the viewer.

The differences in spectral quality that result from changes in illumination and viewer geometry may be unimportant if they are too subtle for a viewer to detect. In Fig. 8, we examined how the changes in dewlap spectrum that resulted from changes in detection angle alter the stimulation of the four classes of cones that mediate colour vision in the retina of *A. cristatellus*. The cone spectral sensitivities were plotted relative to a maximum of 1.0 for each cone class. The change that occurs in the stimulation of each cone class from one detection angle to another is summarized in Table 2. The smallest change occurred between 270° and 315°, where the greatest difference was a 0.04-shift in L cone stimulation. Comparisons between the other detection angles produced a change of 0.06 in at least one cone class. The key question is whether changes of this magnitude in the stimulation of one or more cone classes are sufficient to produce a detectable change in colour appearance of the lizard by the viewer, but no such data exist for lizards. Detailed wavelength discrimination data are available for humans. If human cone sensitivities are plotted in the same manner (i.e. spectral sensitivity relative to a maximum value of 1.0), a change of this magnitude (0.04 or greater) in one or more cone classes is produced by a shift of 4–10 nm in a monochromatic stimulus, depending on the starting wavelength (Stockman & Sharpe 2000). A shift in wavelength of this size is well above the threshold for detection of colour difference by human viewers under daylight condition (Wyszecki & Stiles 1982), across most of the human-visible spectrum (450–650 nm). Thus, while we cannot say with certainty that lizards can detect the changes in the dewlap spectrum resulting from changes in viewing angle, changes in cone stimulation of the magnitude observed are sufficient to cause detectable changes in perceived colour by humans. There is no reason to expect lizards to be any less sensitive to colour differences. That being said, it is clear from Figs 6–8, that the most dramatic effects of illumination and viewing angle are changes in total radiance, and changes in colour are considerably more subtle.

Estimating radiance in field conditions. The optical analysis suggested that failure to include transmission in calculations of dewlap radiance should lead to an underestimate of radiance and fail to accurately reproduce spectral radiance, because the transmitted spectrum differed from the reflected spectrum. These predictions were supported for *A. cristatellus*. When radiance was calculated using equation (1) (reflectance-only method), the errors were quite large in the full sun condition with the sun on the opposite side of the dewlap. This result is predictable, because when the sun is opposite the viewer, transmission dominates. When the sun is on the same side of the dewlap as the detector, reflection dominates and, as expected, the reflectance-based estimate in our study was good. When the light conditions were more diffuse, as occurred in the forest, the use of equation (1) led to an underestimation of total radiance. The shape of the radiance spectrum, however, was fairly accurate. These results illustrate that failure to account for transmission can cause

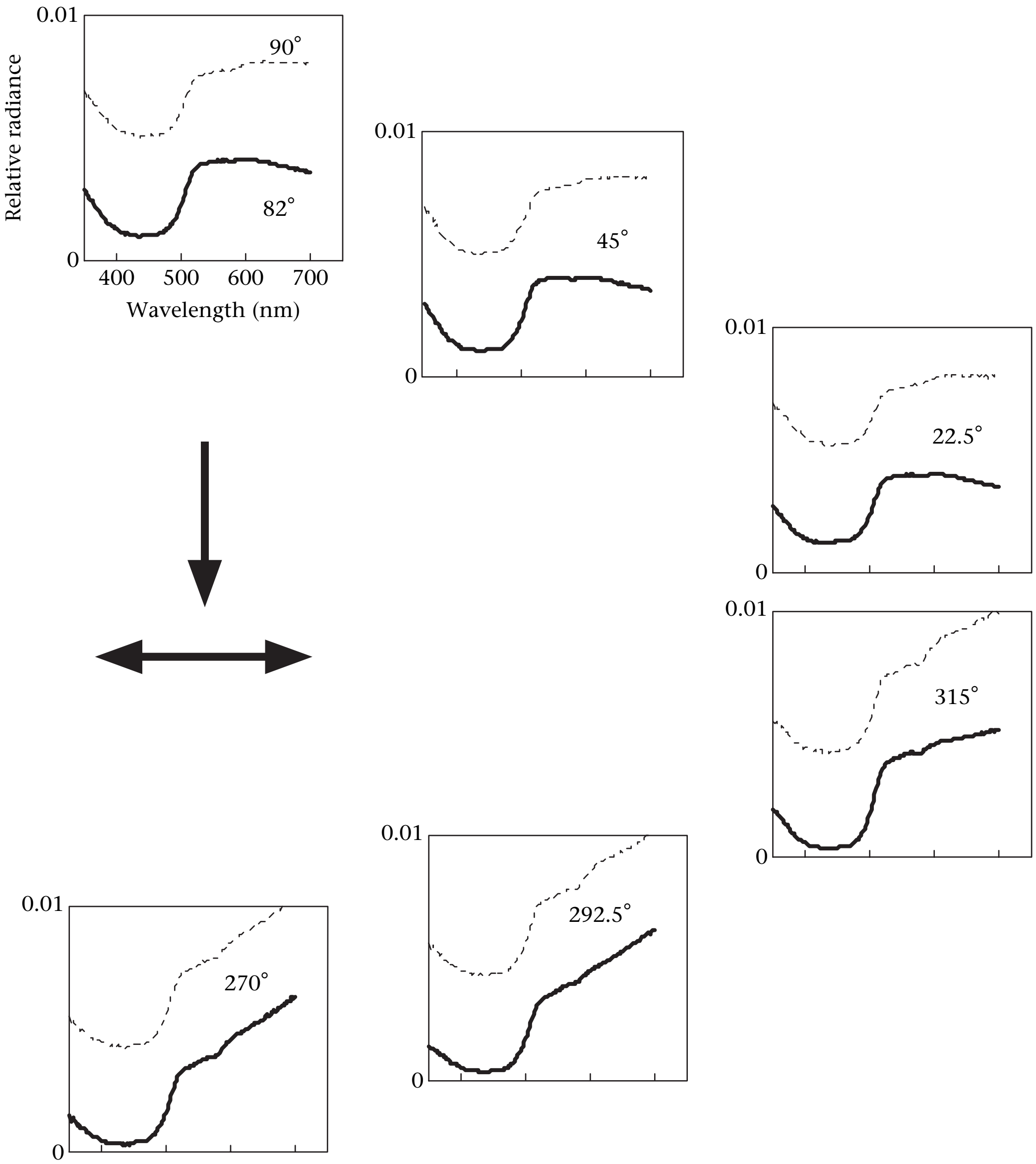

Figure 7. The radiance spectrum as a function of detector position for the same lizard dewlap shown in Fig. 6 for the Level-90° (—) and the Elevated-90° (- - - -) sampling condition. Each spectrum was normalized so that its area from 350 to 700 nm was equal to 1.0. The spectra for the two illumination conditions are displaced along the *Y* axis for clarity. For the level illumination, the detector could not be placed at 90° because it would block the source, so the measurement was made at 82°. The single arrowhead shows the light source position, and the double arrowhead shows the dewlap position. Spectra recorded from 22.5°, 45° and 82° are reflected light. Spectra recorded from 270°, 292.5° and 315° are transmitted light.

large errors in estimating both total intensity and spectral quality of objects that transmit light, for some viewing conditions.

The approach used by Leal & Fleishman (2004), which included an estimate of transmittance, considerably improved the estimate of radiance. Even here the assumption of Lambertian properties led to an inaccurate estimate for some lighting conditions. Not surprisingly, the estimate was best for the natural light conditions that were most similar to those used in the laboratory. Leal & Fleishman (2004) illuminated the dewlap with a broad, diffuse circle of light. This illumination geometry appears to have approximated direct illumination by the sun, and thus gave good estimates for radiance measured under direct sunlight. Forest shade presents a different illumination geometry, with much more diffuse illumination, and the estimates of intensity were not as good. These results illustrate the fundamental problem of estimating the colour of non-Lambertian surfaces based on laboratory measurements of reflectance and/or transmittance. They will be accurate only when the distribution of light contributing to the irradiance in the laboratory is similar to the natural distribution of light in the field (Endler 1990).

Conclusions

Animal colour patterns are typically measured with one of two aims in mind: (1) determination of the

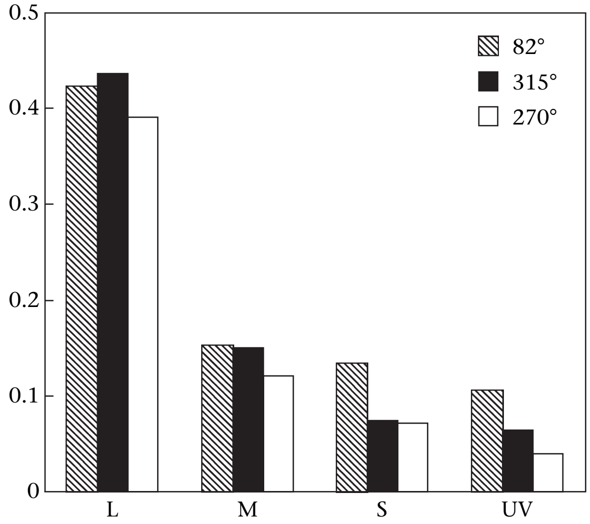

Figure 8. The relative stimulation of the four classes of *A. cristatellus* retinal cones produced by three normalized dewlap spectra from the Level-90° source position in Fig. 7. The dewlap spectra recorded from 82° (reflected), 270° (transmitted) and 315° (transmitted) were compared. The spectral sensitivity of each of the four cone classes (from Loew et al. 2002) was normalized to a maximum value of 1.0. Each of the three dewlap spectra was multiplied by the relative sensitivity of each cone class and the resulting value summed. The four cone classes are UV (ultraviolet), S (short wavelength), M (middle wavelength) and L (long wavelength). See Loew et al. (2002) for details.

information content of signal colour or (2) attempts to quantify conspicuousness and/or crypsis. Many studies focus on the information contained in the colour pattern (e.g. condition, species identity, hormonal state, immunocompetency; reviewed in Grether et al. 2004). Visual systems are capable of correcting for habitat illumination (chromatic adaptation), so that the actual radiance of these signals is often not considered critical. In general, where the primary aim of a study is to objectively document differences between individuals or groups of individuals in signal colour, rather than to precisely estimate the appearance of these colours in nature, spectral reflectance measurements made under carefully controlled, constant illumination and detection conditions are often appropriate. However, if the spectral quality and/or intensity of the signal change with direction of view in a given habitat (e.g. Fig. 9), these changes cannot be completely discounted by a visual system, because it will be adapted to the overall habitat illumination spectrum, rather than to the particular location or direction where the signal occurs. The different radiance spectra that result purely from changes in illumination and viewer geometry in a given habitat represent the range of appearances that the surface can have under a given set of habitat illumination conditions. The extent to which the signal varies in this way sets limits on the precision of the information that it can broadcast.

Table 2. Changes in the relative stimulation of the different cone classes (Fig. 8) from the *A. cristatellus* retina that result from changes in the angle of the detector

Directions from which spectra were measured	UV cone	S cone	M cone	L cone
82° vs 315°	0.04	0.06	0.01	−0.01
82° vs 270°	0.06	0.06	0.03	0.03
270° vs 315°	0.02	0.01	0.03	0.04

Detection angles are from the Level-90° illumination condition (Fig. 7).

A second class of studies attempts to measure the extent of crypsis and/or conspicuousness of signals by comparing them to background elements. This is often done by measuring the spectral reflectance of signals and background elements in the laboratory and comparing them (Endler & Thery 1996; Macedonia et al. 2003; Stuart-Fox et al. 2003). However, these different elements will often be influenced in different ways by illumination geometry. Bark and soil, for example, will usually have diffuse reflection only. Other substrate objects, such as leaves and some types of rocks, may be sensitive to illumination geometry. Leaves, for example, are typically transmissive, their transmission and reflection spectra differ and reflection tends to be specular (Gates 1980). Thus, to accurately assess crypsis and conspicuousness, it is necessary to take into account how each of these background elements will change with the position of the sun relative to the position of the viewer.

While these difficulties need to be kept in mind, the present study also suggests that good first approximations of radiance for many signal surfaces can be successfully calculated. Many surfaces are, in fact, highly diffuse (Johnsen 2002). The results from Fig. 9 show that inclusion of transmission estimates, even using the inaccurate assumption of Lambertian diffusion, can yield reasonably good estimates of radiance for some habitat conditions. These estimates can be further improved by measuring reflectance in the laboratory, using an illumination geometry that is similar to that typical of the field (Endler 1990). For example, for forest understory animals, illumination with a highly diffuse source is appropriate, whereas illumination with a broad circle of light can approximate conditions under full sun.

Determining spectral radiance of natural objects under natural light can be complicated. However, it is relatively easy to determine whether one needs to be concerned with geometric effects. It is fairly simple to measure radiance from multiple angles in the laboratory and determine whether there are changes (Cuthill et al. 1999), and a simple observation can indicate whether transmission may be important. In this way, one can quickly determine whether a simple approach like that described in the introduction of this paper is sufficient for estimating signal appearance, or whether a more elaborate measurement strategy is required.

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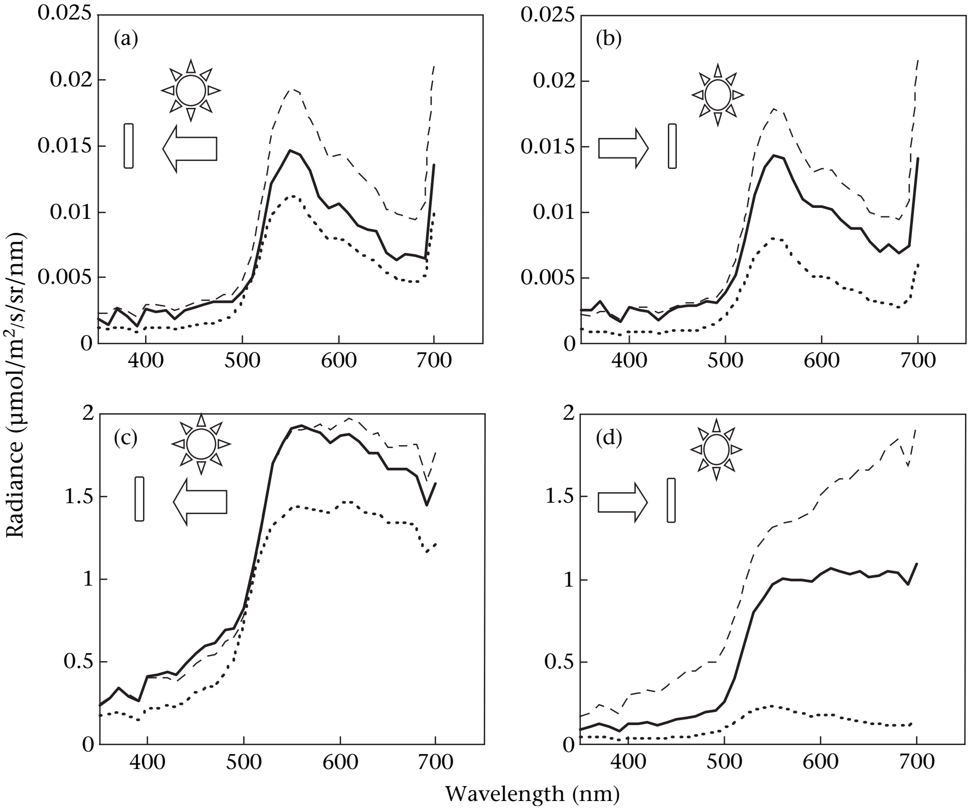

Figure 9. Spectral radiance of the dewlap of *A. cristatellus* in a heavily shaded forest (a, b) and in open sun (c, d) based on direct measures (—), and values calculated using the 'reflectance-only' method (······) and Leal & Fleishman's (2004) method (- - -), which includes transmission and reflection estimates (see Methods for details). In (a) and (c), the radiance detector pointed away from the sun; in (b) and (d), the radiance probe pointed towards the sun.

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