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# Fiber-Optic Probes for Small-Scale Measurements of Scalar Irradiance

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## ABSTRACT

**A new method for producing fiber-optic microprobes for scalar irradiance (=fluence rate) measurements is described. Such fine-scale measurements are important in many photobiological disciplines. With the new method, it is possible to cast spherical 30–600 μm wide light integrating sensor tips onto tapered or untapered optical fibers. The sensor tip is constructed by first casting a clear polymethyl methacrylate (PMMA) sphere (~80% of the size of the final probe tip diameter) onto the optical fiber via dip-coating. Subsequently, the clear sphere is covered with light diffusing layers of PMMA mixed with $TiO_2$ until the fiber probe exhibits a satisfactory isotropic response (typically ±5–10%). We also present an experimental setup for measuring the isotropic response of fiber-optic scalar irradiance probes in air and water. The fiber probes can be mounted in a syringe equipped with a needle, facilitating retraction of the spherical fiber tip. This makes it, *e.g.* possible to cut a hole in cohesive tissue with the needle before inserting the probe. The light-collecting properties of differently sized scalar irradiance probes (30, 40, 100, 300 and 470 μm) produced by this new method were compared to probes produced with previously published methods. The new scalar irradiance probes showed both higher throughput of light, especially for blue light, as well as a better isotropic light collection over a wide spectral range. The new method also allowed manufacturing of significantly smaller scalar irradiance microprobes (down to 30 μm tip diameter) than hitherto possible, and such sensors allow minimally invasive high-resolution scalar irradiance measurements in thin biofilms, leaves and animal tissues.**

## INTRODUCTION

Light is essential for life on Earth and is an important environmental parameter in biology, but also in medicine, where light is used for diagnosis and treatment, *e.g.* in photodynamic therapy (PDT). It is a challenge to measure light in dense media such as sediments, biofilms and tissues, where intense scattering and absorption results in strong light attenuation and steep light gradients, and where phenomena such as photon trapping and path length enhancement come from multiple scattering and internal reflections at optical boundaries with variations in refractive indices (1–3). It is thus essential in both biomedical dosimetry and photosynthetic studies in plant physiology and microbiology to determine the total light the cells receive (1,4,5). This involves measuring the integral quantum flux from all directions about a point; this parameter ($E_0$) is often denoted as the photon scalar irradiance (μmol photons $m^{-2}$ $s^{-1}$) in environmental research or the radiant energy fluence rate (in W $m^{-2}$) in biomedical research.

The light field in a given medium can be described by detailed measurements of field radiance ($L$) with flat-cut optical fibers that have well-defined light acceptance characteristics (6, 7). The field radiance, $L(\theta, \varphi)$ from a given direction specified by the zenith and azimuth angles ($\theta$, $\varphi$) in a spherical coordinate system is defined as:

$$L(\theta, \varphi) = \frac{d^2\Phi}{dA d\omega}$$

where $\Phi$ is the radiant flux from that direction per unit solid angle, $d\omega$, per unit area perpendicular to the direction of light propagation, $dA$.

The scalar irradiance ($E_0$) or fluence rate at a given point can be expressed as the field radiance integrated over the whole sphere of $4\pi$ solid angle (1,4,5):

$$E_0 = \int_{4\pi} L(\theta, \varphi) d\omega$$

Scalar irradiance probes are usually built as spherical light collectors exhibiting an isotropic angular response to incident light, *i.e.* light from all directions is captured by the probe tip and channeled to the detector with equal probability. Scalar irradiance probes can be manufactured by fixing a light diffusing sphere with isotropic light collection properties at the end of an optical fiber. The size of the diffusing sphere, its angular isotropic response, the transmittance at different wavelengths, and the mechanical stability are important parameters for the choice of probe for a particular application. Ideal scalar irradiance probes should have a very good isotropic response, *i.e.* a standard deviation <10% of the mean detector response for different incident light angles as well as a small blind angle, where the optical fiber is in contact with the spherical tip. All wavelengths should be transmitted identically. Ultraviolet (UV) radiation is usually not transmitted well due to strong absorption in the probe material, and special optical fibers with high OH-content for transmission in the UV region are needed. To resolve the steep light gradients in scattering media, a small spherical tip is crucial for measurements in sediments, biofilms and tissues. A small tip diameter also minimizes local impact at the insertion point, especially in cohesive media where it can be necessary to precut a

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hole with a needle. However, untapered fibers with larger spheres are less fragile than tapered fibers with small spheres.

Several different methods for preparing fiber-optic scalar irradiance probes with spherical tip diameters <1000 μm have been described for application in biomedical and environmental sciences. Marijnissen and Star (8,9) developed scalar irradiance probes for applications in tissue optics and PDT. The sensors were constructed from a light diffusing plastic, Arnite® (polyethylene terephthalate), sphere with ~800 μm diameter that was machined on a lathe, and glued to a 200 μm wide flat-cut optical fiber. The probes exhibited a good isotropic response of ±10% and good mechanical strength; however, their size is quite large for most biological applications. They can be used in clinical medicine, and are commercially available (MedLight SA, Switzerland; PDT Systems, Buellton, CA, USA).

Henderson (9,10) developed an alternative manufacturing method based on a light-cured polymer probe using a white dental fissure sealant (Helioseal®; Ivoclar Vivadent) to form a <800 μm wide sphere on the tip of an untapered optical fiber. These sensors exhibited an angular light collection isotropy of ±5% at 488 nm, and ±7% at 632 nm. A similar method was used to cure a resin mixed with $TiO_2$ forming a 50 μm wide light-collecting sphere on a tapered fiber, albeit with a less ideal isotropic response (11).

Using a fundamentally different approach, Lilge *et al.* (12) developed two different types of scalar irradiance probes based on the use of a dye-doped measuring tip fixed at a distance from the light-collecting optical fiber by a transparent material. Type I was made from polymethyl methacrylate (PMMA) and different fluorescent dyes with measuring tip diameters of 265–615 μm and a light collection isotropy of ±10% in water. In type II, the fiber cladding was removed by HF etching, and inserted in a capillary tube with a "fluorescent dye-doped UV curing glue" at the end. The result was a cylindrical isotropic probe with diameter of 170 μm, and a sensing length of 200–400 μm showing an isotropy of ±20% in air. The responsivity of these designs was, however, two orders of magnitude lower than for probes made according to Marijnissen and Star (8,9,12). Their angular response was detected by rotating the light source around the probes. For measurements in water, the probes were held in a round container filled with water. The material of the container was not explained, and no comparison of probes measured in both air and water were given.

Dodds (13) fixed a drop of Titanium White acrylic paint at the end of a 125 μm step-index optical fiber that dried to a scattering sphere of ~250 μm in diameter. Such a probe was used to measure scalar irradiance in sediments and microbial mats. The angular response was less ideal and to make up for that, four measurements were done turning the sensor 90° along its axis and integrating the results, when a light profile was done.

Instead of paint, Lassen *et al.* (14) used polymethyl, and polybutyl methacrylate (PMMA and PBMA) dissolved in xylene (Plexisol, PM 560 and PM 709) and mixed with $TiO_2$ powder to cast a 70–100 μm wide scattering sphere on the end of a tapered fiber. These microprobes show a good isotropic angular response of ±10% at 450, 650 and 850 nm both in air and water and they have been widely used for visible and near-infrared light measurements in aquatic photosynthesis studies (6).

To alleviate the bad transmission in the UV region of the methacrylate-based scalar irradiance microprobes, Garcia-Pichel (15) produced an UV transmitting ~100 μm wide vitro-ceramic spherical tip by back melting of a long optical fiber taper coated with MgO crystals. Such sensors enable UV scalar irradiance measurements down to 250 nm with an isotropic angular response of ±15%.

The mentioned scalar irradiance probes have been applied in a variety of biomedical and ecological applications, where the small probe size has allowed new insights to the light microenvironment, and optical properties of biofilms, sediments and tissues (5,7,16–19). Some recent examples include the investigation on the effect of light on *in vitro* cultivated blastocysts in mouse embryos (20), detailed measurements of vertical, and lateral light gradients within and across coral tissues in different scleractinian species (21), characterization of the biophotonic properties of iridocytes in photosymbiotic giant clams (11), and measurements of radiative energy budgets in different photosynthetic microbial mats (22).

A comparison of different types of fiber-optic scalar irradiance probes in terms of their measuring characteristics and material properties has to our knowledge not been reported in the literature. In this study, we compare four different types of scalar irradiance probes, and present a further development of the type invented by Lassen *et al.* (14), along with details on a setup for measuring the isotropic performance of scalar irradiance probes in air and water. The new manufacturing method enables construction of ultrasmall scalar irradiance microprobes with 30–150 μm wide spherical light collectors cast onto tapered fibers, or 220–600 μm wide spherical collectors cast onto untapered fibers. These sensors show ~5 times less light attenuation, and good isotropic responses at different wavelengths. The optical fiber can be fixed within a syringe equipped with a needle, facilitating a retractable probe. This makes the microprobe easier to handle, which is crucial in various applications, such as for measuring in cohesive microbial mats, leaves and various tissues. The needle with the retracted probe can cut a hole in the object before the probe is inserted.

## MATERIALS AND METHODS

*Fixation in syringe, cutting and fabrication of tapered fiber tips.* A 5 m long single strand fused-silica multimode optical fiber patch cord with standard SMA-connectors was used for manufacturing all scalar irradiance probes in this study. The optical fiber was a step-index fiber with 105/125 μm core/cladding diameter ratio, and a numerical aperture in air of NA = 0.22 (FG105LCA, Thorlabs, USA). The patch cord was cut in two, and the protective PVC coating and Kevlar fibers were removed over a length of ~15 cm from one end. The underlying Tefzel® polymer jacket enclosing the fiber was removed over ~7 cm with a mechanical fiber stripping tool (Micro-Strip®; Micro Electronics Inc., USA). For better handling, the fiber was fixed to the piston in a 1 mL syringe painted with an opaque black paint (Conductive Carbon Paint; SPI Supplies, West Chester, PA, USA); this also made the fiber retractable (see Fig. 1, where components are indicated with letters):

A 5 mm hole was drilled in the piston head (A). The black rubber gasket (B) was removed from the piston (C), and about a third of the gasket holder side was cut off with a Stanley knife. The gasket was put back, and a hypodermic needle with removed Luer connector (D) (Sterican 21G, 0.80 × 80 mm; B. Braun Melsungen AG, Germany) was pushed through the gasket. Then the fiber (E) was first put through the hole of the piston head (A), and then through the needle tube (D). The sharp end of the tube was pushed into the fiber protection, and the fiber protection was fixed to the piston (C) with black tape (Vinyl Electric Tape, Scotch® Super 33+; 3M Electrical Products Division, USA). The piston including the fixed fiber was put back into the black-painted syringe (F) with the fiber end (E) protruding from the opening. A hypodermic needle (G) (23G, 0.6 × 25 mm, Fine-Ject®; Henke-Sass Wolf

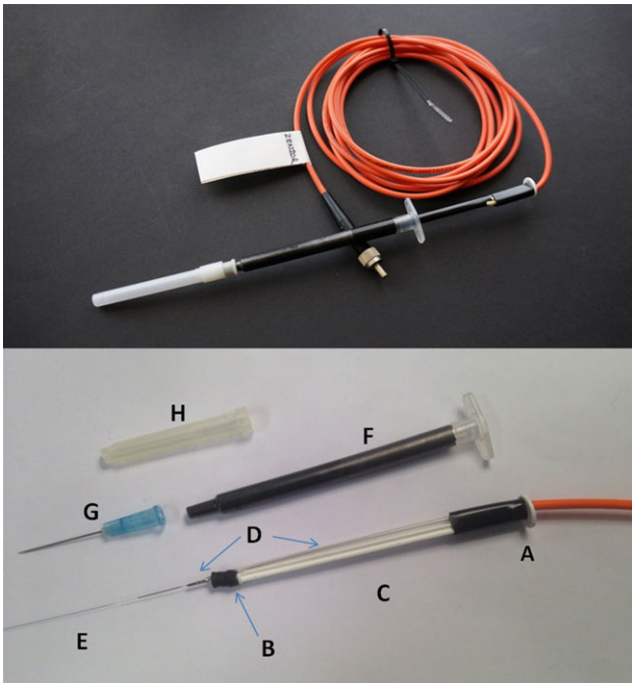


**Figure 1.** Overall design of the new scalar irradiance microprobe. The upper panel shows a complete probe with a protective cap on the needle and syringe containing the optical fiber. The lower panel shows a scalar irradiance microprobe before assembly comprised of the piston head (A), a rubber gasket (B) on the piston (C) with the protective metal tube (D) and the black-painted optical fiber (E) protruding out of the metal tube. The piston with the attached fiber is mounted inside a black-painted plastic syringe (F) and a hypodermic needle (G) equipped with a protective cap (H) when the light-collecting sensor tip is retracted into the needle.

GmbH, Germany) was attached to the syringe with the bare fiber pushed through the needle. The syringe was then mounted vertically in a micromanipulator (MM33; Märtzhäuser, Wetzlar, Germany) with a small weight (3.75 g) attached to the bare fiber end. A taper was made by heating the fiber with a small oxygen/propane flame from a miniature brazing and welding set (Roxy-Kit®; Rothenberger, Frankfurt a. M., Germany). Thereafter, the syringe was mounted horizontally in a micromanipulator with the fiber tip placed under a dissection microscope. The fiber outside the needle was painted with opaque black paint diluted 1:1 with isopropanol, and the taper was cut back manually with a sharpened forceps to the desired diameter (10–15 µm) of the tapered tip. Untapered fibers were cut with an optical fiber cleaving tool (Thomas & Betts, Raritan, New Jersey) to obtain a straight and flat-cut tip before it was put through a hypodermic needle (23G, 0.6 × 25 mm, Fine-Ject®). The outermost 2–3 cm of the fiber was coated with an opaque layer of the black paint diluted 1:1 with isopropanol. Finally, the plastic Luer connector of the needle was painted with undiluted black paint.

*Casting of light-collecting spheres onto fiber tips—PMMA/$TiO_2$—based probes.* For the construction of the light-collecting spheres, two stock solutions of 25% w/w of polymethyl methacrylate (PMMA) (Goodfellow Cambridge Ltd., UK; refractive index $n = 1.49$), were made in chlorobenzene: Solution A consisted of 5 g PMMA in 15 g chlorobenzene, and solution B consisted of 3 g PMMA 1 g $TiO_2$, 9 g chlorobenzene (25% w/w $TiO_2$ in the dry PMMA). From solutions A and B, three other solutions were made with a final content of $TiO_2$ in dry PMMA of 12.5% (solution C), 6.25% (solution D) and 3.15% (solution E), respectively. Chlorobenzene was chosen as the solvent, because it dissolves PMMA and evaporates with an adequate speed. It can be exchanged with xylene without problems.

The syringe with the fiber protruding out of the needle was mounted in a micromanipulator, and the tip was observed under a dissection microscope. Any black paint was carefully removed from the flat-cut fiber end, and a clear sphere was cast by dip coating the fiber tip in solution A aiming after placing the center of this sphere at the tip of the fiber. For this, a drop of the solution was placed on a small spatula, and was moved to the fiber until the drop on the spatula touched it. After retraction from the polymer droplet, the surface tension of the adhered material formed a small sphere on the fiber tip. After drying for 3–5 min, the process continued until the desired sphere size was obtained. The final sphere size usually was ~20–30% wider than the diameter of this clear sphere. A schematic drawing of the final result for a 470 µm probe with an inner 360 µm clear sphere is shown in Fig. 2. The casting process was then continued with mixtures containing different concentrations of $TiO_2$. The isotropic response was quickly checked after each adherence of a new layer (see *Measurement of isotropic response*) and the process was stopped when a sufficient isotropic response was obtained. If no satisfactory result could be obtained, the sphere was removed completely with $CHCl_3$, and the process repeated.

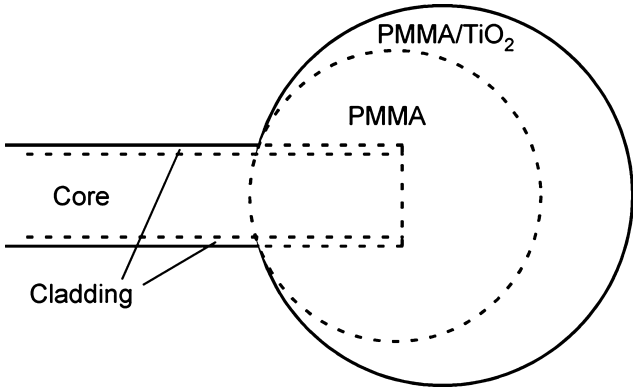


**Figure 2.** Schematic drawing of a scalar irradiance probe produced with the new method outlined in the text. The fiber is a multimode step-index fiber with a core/cladding ratio of 105/125 µm and painted with an opaque black paint. The inner sphere is made out of pure PMMA, whereas the outer sphere (470 µm) is made of PMMA with $TiO_2$; both materials are applied to the fiber tip by dip coating.

For spheres on both tapered and untapered fibers, the best result was obtained using mixture C for the first layers, and D or E for finer adjustments. Using this approach, scalar irradiance probes with five different sizes were used in this study: three supported by tapered fibers (30, 40 and 100) and two supported by untapered fibers (300 and 470 µm).

We also manufactured a large scalar irradiance probe (500 µm), with an untapered fiber and using only solution C for the sphere, following the casting method of Lassen *et al.* (14).

The critical angel for light reflected out of PMMA was calculated to 42.9° in air and 64.8° in water.

*Helioseal® curing-based scalar irradiance probes.* We manufactured scalar irradiance probes according to Henderson ([10] pp. 83–93, [9]) in the following way: After removal of the black paint at the fiber tip, the distal end of the fiber was connected to a UV light source (UV glue lamp, Dymax, Germany), and the fiber tip was placed into a small amount of an UV light curing white dental fissure sealant (Helioseal®, Ivoclar Vivadent, Liechtenstein; refractive index $n_0^{25} = 1.51$). The curing light was applied for 10 s; thereafter the fiber was pulled out of the sealant solution creating a 500 µm wide sphere of Helioseal® at the tip. The surface of the sphere was not completely cured, but was finished by turning the sphere directly in front of the UV source for some minutes. Helioseal® is cured with 400–500 nm light. The cured sphere was then washed in ethanol, and the fiber immediately behind the spherical tip was painted with diluted black paint. It was not possible to produce isotropic spheres on tapered fibers with this method. The critical angle for light reflected out of Helioseal® was calculated to 41.4° in air and 61.6° in water.

*Scalar irradiance probes based on gluing a diffusing sphere to fibers.* A scalar irradiance probe with a diffusing sphere glued to a step-index optical silica glass fiber was compared to the other types of probes. We used a commercially available version of a probe developed by Marijnissen and Star (8) (Medlight isotropic probe model IP850 with a gold radiomarker, tip diameter 850 µm, isotropy of ±10% in air 40–360°, wavelength range 480–800 nm; silica, low OH, 400 µm core, NA = 0.37; SMA-connector, Medlight S.A., Switzerland). After the

sphere was machined on a lathe out of Arnite™, a blind hole was drilled toward the sphere center and the fiber was glued into the hole with a transparent UV adhesive (9). The diameter of the fiber was measured between the sphere and the gold radiomarker to 450 μm. The refractive index of Arnite™ was 1.51 (23). The critical angel for light reflected out of Arnite™ was calculated to 41.4° in air, and 61.6° in water.

The probe was mounted within a hypodermic needle (17G, 1.5 × 50 mm) mounted on a 1 mL black-painted syringe for easier handling during measurements. It was also necessary to paint the fiber between the needle and the sphere including the part between the radiomarker and the sphere for correct measurements. Due to the good mechanical stability of the sphere, stains of paint on the probe could be removed by carefully scraping with a small dissection knife.

*Probe characterization—detectors and light sources.* A custom-built light meter, with a relatively flat spectral quantum responsivity for 400–700 nm light, developed for microscale measurements of photosynthetically active radiation (PAR, 400–700 nm) (24), was used for characterizing the fiber-optic scalar irradiance probes. The light meter signal was recorded on a strip-chart recorder (BD25; Kipp & Zonen, Netherlands).

A fiber-optic spectrometer (USB2000 operated with the Spectra Suite software; Ocean Optics, Dunedin, USA) was used to record the spectral response of the probes and bare fibers. All spectra were recorded as an average of 10 scans, using a boxcar smoothing width of 4, and with the spectrometers nonlinearity and stray light correction enabled. The integration time was set as high as possible, and the corresponding dark noise was automatically subtracted for each recorded spectrum. A light meter (Universal Light Meter, ULM-500; Walz, Germany) equipped with a calibrated photon irradiance sensor (LI-190; Li-Cor, USA) was used for measurements of the absolute photon irradiance of incident PAR (400–700 nm) from the collimated light source in units of μmol photons $m^{-2}$ $s^{-1}$.

Collimating optics was used for all sensor characterization measurements (Ovio Collimated Source; Ovio Optics, France) together with different light-emitting diodes (LED). The LED's were connected to a trigger box controlled by the software Look@RGB (both available from www.fish-n-chips.de), which enabled PC-controlled adjustment of the LED intensity (25). Three different LEDs were used in connection with the collimation optics: Two different white LEDs, one for low light intensities (Oslon 1 LED; ILH-OW01-STWH-SC211-WIR200; RS-Components, UK), and one for high light intensities (Oslon 4 LEDs; ILH-OO04-ULWH-SC211-WIR200; RS-Components, UK), and a 405 nm LED (Oslon 4 LEDs; ILH-OW04-UVBL-SC211; RS-Components, UK). The opening diameter of the Ovio optics was 32 mm, and at the backside of the box (at a distance of 54.5 cm) the beam diameter was 55 mm in air. The beam divergence for the collimated light was calculated to be <2.5° in air. The value was similar in water after a glass plate was placed in front of the collimation lens.

*Measurement of isotropic response.* For characterizing the angular light-collecting properties of fiber-optic scalar irradiance probes in air and water, a device was constructed from 1 cm thick black PVC (parts are identified by letters in Fig. 3): It consisted of a 60 × 60 × 10 cm box with a 10 cm disk (A) glued to the bottom in the middle of the box. A needle was put vertical through the center of the disk. Another 23.5 cm disk (B) with a 10 cm hole in the center exactly fitting the first disk was placed so it could be revolved freely. A holder (C) for the sensors was fixed on the outer disk with a metal rod so it could be rotated around its own axis. A plastic screw was used to lock the holder after centering of the probe sphere. A laminated print of a graduated circle was glued to the central disk (A). A pointer (D) was placed in front of the sensor holder for easy reading of the measurement angle.

The collimation optics was mounted water-tight on one side of the box in a holder (E) that was sealed with an O-ring (Simmerring®). A glass plate was placed in front of the collimator lens to prevent water from leaking, dew on the backside of the lens, and to keep the light collimation similar with air and water in the measuring chamber. The collimated light source and probe holders were constructed in a way, making the spherical probe tip position adjustable, as to be put in the center of the light beam precisely over the needle in the center of the disk. The chamber could be closed with a light-tight lid to prevent ambient light from disturbing the measurements.

For measurements, the scalar irradiance probe was attached to the holder on the revolving table with the sphere exactly over the guiding needle placed at the center of the disk (Fig. 3). The distal end of the fiber-optic scalar irradiance probe was connected to a light meter. The directional response of the probe was determined by rotating the revolving table from +160° to −160° in angular steps of 10° in a beam of collimated light. At 0° the probe fiber and the light source were aligned. To check for possible variations in the measuring setup such as light source fluctuations, fiber bending effects or air bubbles in the water, measurements at −90°, 0°, 90° and 160° were measured several times for each probe.

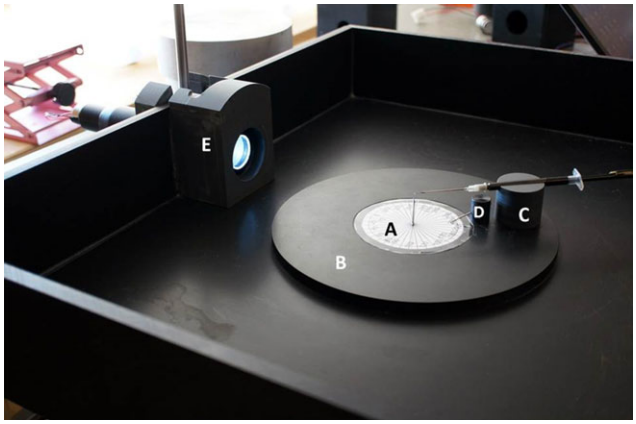


**Figure 3.** Setup for measuring the light-collecting isotropy of scalar irradiance probes comprised of a flat black box with a fixed disk (A) placed in the center with an angular scale and a needle, a rotational disk (B) on which a scalar irradiance probe is mounted in a holder (C) with the probe tip placed over the center of the fixed disk, a read out pointer (D), and the collimated light source mounted water-tight in a holder (E). During probe readout, the box is covered with a black lid, and the box can also be filled with water.

Four series of measurements were recorded with the PAR-meter, and two series with the scalar irradiance probes connected to the spectrometer. All probes were first measured in air, where after the chamber was filled with water and measurements continued. After a measurement series, the probe was turned 90° around its longitudinal axis in the sensor holder, and the angular light collection was measured again. The water was then drained from the chamber and the probe properties was measured once more in air.

*Calibration of scalar irradiance probes for photon irradiance measurements.* A 7 × 7 × 7 cm black PVC box with a lid and two aligned holes in opposite walls was used to calibrate the response of scalar irradiance sensors in absolute units of μmol photons $m^{-2}$ $s^{-1}$. The collimated light source and a photon irradiance sensor (LI190, LiCOR, USA; Universal Light Meter, ULM-500, Walz, Germany) were placed in opposite holes. The photon irradiance (μmol photons $s^{-1}$ $m^{-2}$) was measured at eight different current intensities (100–800 mA) for each of the white, blue and red LED's as adjusted by the LED power supply. Subsequently, the irradiance sensor was exchanged with a fiber-optic scalar irradiance probe with its measuring tip placed at exactly the same spot and distance in the light field relative to the collimator, and the sensor response was measured in mV with the PAR-meter. Measurements with the scalar irradiance probe could then be converted using the linear correlation between mV readings and μmol photons $m^{-2}$ $s^{-1}$.

*Light attenuation of scalar irradiance sensors.* We compared the light attenuation in each of the four different types of scalar irradiance probes, based on different sphere types fixed on the same untapered fiber. The sensors were mounted in the light calibration box (see section above), and light spectra were recorded with the spectrometer system. The percentage of light passing from the light scattering sphere at the tip into the probe fiber was calculated by dividing the probe spectra with spectra recorded with bare fibers placed at similar distance and position in the light field (Fig. 4). A 400 μm core step-index fiber (Laser Components, Germany) was used corresponding to the Medlight scalar irradiance probe. All probes were supported by untapered fibers, and the results were corrected for different cross-sectional areas due to different sphere diameters. Besides measuring probe spectra with a white LED light source, additional spectra were recorded with a 405 nm LED to obtain sufficient signal in the 380–450 nm region.

*Photography of sensors.* The different types of scalar irradiance probes were photographed with a commercially available digital SLR camera (Canon EOS 7D MkII; Canon Europe Ltd., Middlesex, UK), connected via a 0.5× photoadapter (IMAG-AX; Heinz Walz GmbH, Effeltrich, Germany) to an epifluorescence microscope (Axiostar Plus FL; Carl Zeiss GmbH, Germany), fitted with either a 10× or 20× plan-Apochromate objective (Carl Zeiss GmbH, Germany). Illumination of the probes was achieved by means of the light source of the microscope and a fiber-optic halogen lamp (KL-1500; Schott AG, Mainz, Germany) fitted with a double-light guide for side illumination.

*Application of new scalar irradiance microsensors.* Spectral scalar irradiance was measured using a microprofiling setup with the sensor mounted in a motorized micromanipulator (MU-1; PyroScience GmbH, Germany) and controlled by PC-software (Profix; PyroScience GmbH, Germany); this allowed advances in 100 µm vertical steps through the sample. Spectra of the scalar irradiance were measured with the probe fiber connected to a fiber-optic spectrometer (USB2000+; Ocean Optics, FL, USA) that was interfaced to a PC running dedicated spectral acquisition software (SpectraSuite; Ocean Optics, FL, USA). Incident light was provided either with a fiber optic tungsten halogen lamp with a collimating lens (Schott KL2500LCD; Schott AG, Germany) or from a LED ring (Walz GmbH, Germany). Spectra of scalar irradiance can be integrated in the spectral region of interest, *e.g.* the PAR region (400–700 nm), and normalized to the incident downwelling irradiance measured over a black nonreflective light-well, to construct scalar irradiance profiles through tissues and characterize the amount of photosynthetically active radiation (PAR) in a certain tissue depth. For a detailed description of the collection and analysis of acquired spectra, see (26).

## RESULTS

### Light attenuation in the different scalar irradiance probes

A comparison of light attenuation in the four different probe materials showed that wavelengths <410 nm were strongly absorbed in probe tips made of PMMA or Helioseal, whereas the machined sphere probe made according to Marijnissen and Star (8) exhibited a much better performance in the UV region. At wavelengths >415 nm, probes manufactured according to Henderson (10) and Lassen *et al.* (14) exhibited similar light attenuation, whereas probes made according to Marijnissen and Star (8) and probes made with the new procedure described in this study showed about five times less light attenuation in the probe material (Fig. 4).

### Angular light collecting properties of scalar irradiance probes

We manufactured different types of scalar irradiance sensors with spherical tip diameters of 30–850 µm (Fig. 5). The isotropic response of the different scalar irradiance probes revealed relatively large differences in isotropy between different probes when measured in air and water and for different wavelength ranges (Table 1; Figs. 6–9). Sensors manufactured according to Marijnissen and Star (8) and Lassen *et al.* (14) showed similar isotropy for blue, green and red light, whereas the Helioseal-based sensor manufactured according to Henderson (10) showed a large color dependence, where the isotropy for green light was 3–5 times smaller than for blue and red light (Fig. 8C). Except for some minor peaks in water, for some sensors, in the region −120° to −160°, and 120° to 160°, the sensors manufactured with the new method presented here generally exhibited a good isotropic light collection in both air and water that was similar or better than the other types of scalar irradiance probes (Figs. 6 and 8) over a wide range of tip diameters (Figs. 7 and 9). The angular light response of most probes was symmetrical about 0°. The probe manufactured according to Marijnissen and Star (8) showed a characteristic parabolic shape, whereas the other types of scalar irradiance probes exhibited a more periodical variation in angular response.

### Application of scalar irradiance microprobes

The new manufacturing procedure presented in this study enabled production of small scalar irradiance microprobes with tip diameters <50 µm, which can be used for measuring scalar irradiance attenuation profiles in thin specimens. We successfully tested such probes in the tough thallus of the brown macroalga *Fucus serratus* and in the cohesive tissue of the reef-building coral *Montastrea curta* (Fig. 10).

In both specimens, it was possible to measure detailed light attenuation profiles of photon scalar irradiance of photosynthetically active radiation (PAR, 400–700 nm). The light microprofiles

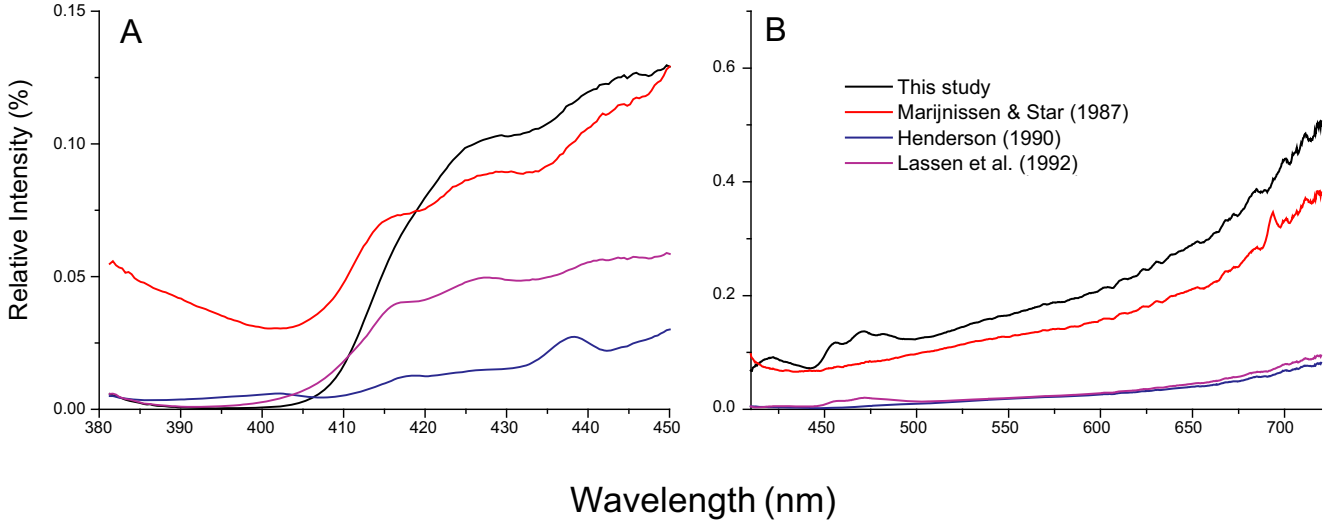


**Figure 4.** A comparison of spectral light attenuation in scalar irradiance probes made of four different types of light-collecting sphere materials, all supported by the same type of untapered fibers. (A) Measurements using a 405 nm LED as light source. (B) Measurements using a white LED light source. The light intensity is expressed relative to the measuring signal obtained with bare untapered fibers in the light path.

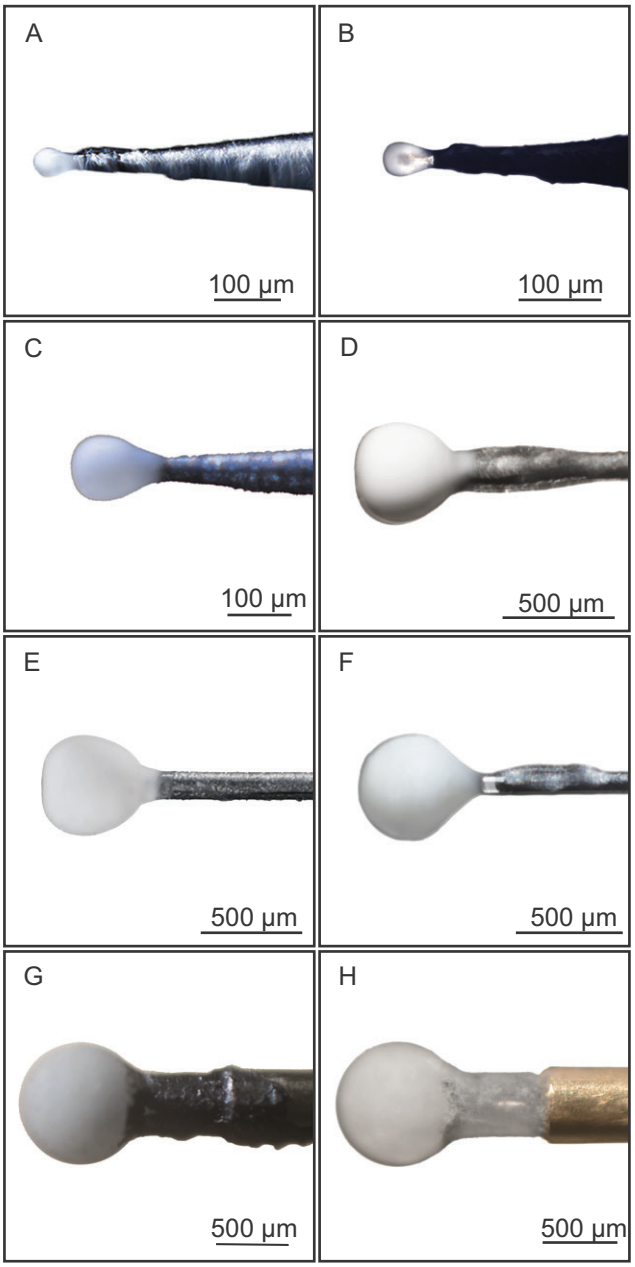


**Figure 5.** Photographs of different fiber-optic scalar irradiance probes. Sensors made with the new manufacturing procedure are shown in panels A, B (40 µm sphere diameter) at two different magnifications, where b was illuminated through the fiber, C (100 µm), and D (470 µm). Panel E shows a probe made according to Lassen *et al.* (1992) (500 µm). Panel F shows a probe (500 µm) made according to Henderson (1990). Panels G and H show a commercial probe (850 µm) made according to Marijnissen & Star (1987) with and without a black overcoat of the fiber, respectively.

showed local enhancement of scalar irradiance near the surface of the tissue due to photon trapping, created by multiple scattering and enhancement of the photon pathlength (6) followed by an exponential attenuation of light in deeper tissue layers.

## DISCUSSION

We present the first comparison of the light-collecting properties of different types of fiber-optic scalar irradiance probes along with a new manufacturing method that enables fabrication of scalar irradiance microprobes with a good isotropic response and significantly smaller tip diameters than previously realized. Furthermore, the new scalar irradiance probes exhibited a higher throughput of light in the visible to near-infrared spectral range, thus alleviating constraints on detector sensitivity. The detailed characterization of probe performance, using a new set-up for measuring the angular light collection of scalar irradiance probes, revealed distinct difference between the different probe types, as discussed below.

### Characterizing the angular light collection of scalar irradiance probes

To our knowledge, a thorough comparison of different types of scalar irradiance sensors has not previously been published. With the construction of a new device enabling angular response measurement in air and water (Fig. 3), it was possible to compare the performance of different scalar irradiance probes. If the probes and sensors are manufactured correctly, there is not a significant difference in the behavior of the sensors, whether they are measured in air or water, but such comparative measurements can effectively reveal any flaws in the production that can direct optimization of probe construction.

As an example, it was necessary to paint the fiber all the way to the sphere on the probe manufactured according to Marijnissen and Star (8), before a reasonable isotropic angular response was obtained; while the standard deviations in the isotropy plot where not improved by the painting, the shape of the plot became much different. Before painting, the plot in air was relatively flat from 90° to −90° with a decreasing angular response at the ends. In water, peaks appeared around 130° and −130°. After painting, the plots were similar in air and water (Fig. 6D; data before painting not shown). This underscores the importance of avoiding light entry or leakage via the fiber behind the spherical probe tip or via the optical fiber strand.

When the first recording of the "Marijnissen and Star" sensor was done, no protective painting was applied, not even on the syringe. This resulted in a large peak at −140° to −150° in both air and water, but only in one position of the syringe. This was not seen when the syringe was turned 90° in the holder. After painting of the syringe, this peak disappeared, but the overall plot still looked strange although the overall variability in angular response was ok. We concluded that the minor peaks in the angle response plots (Figs. 6 and 7) are artifacts due to flaws in the black painting of the fibers very close to the sphere where it is difficult to see (Fig. 5) and from which some light can leak out. The peaks arise because more light is apparently leaked out upon direct illumination of the front of the spherical tip and less when the light comes from the back. This may also explain the observed difference in angular response found in air and water, where in the latter case more light can be coupled out of the fiber more easily due to a lower difference in refractive index between fiber and water as compared to fiber and air. For a fiber with a 105/125 µm core/cladding ratio, 30% of the fiber cross section is cladding and the cladding refractive index is only a little smaller than the core refractive index. This indicates that a little <30% of the light from the sphere will enter the cladding. In air most of this light will rapidly be caught by the core, but in water the acceptance angle is ~20° wider and more of the light

**Table 1.** Light-collecting properties of different types of scalar irradiance probes.

| Probe no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Type | This study | This study | This study | This study | This study | Marijnissen and Star (1989) | Lassen *et al.* (1992) | Henderson (1990) |
| Diameter ($\mu$m) | n.d./30 | 35/40 | 80/100 | n.d./300 | 360/470 | 850 | 500 | 500 |
| Fiber tip geometry | | Tapered | | | | Untapered | | |
| Air | 9.3 | 4.6 | 7.2 | 5.5 | 6.8 | 17.2 | 8.9 | 7.3 |
| Air 90° | 6.3 | 5.4 | 7.3 | 4.5 | 9.3 | 17.8 | 12.3 | 5.1 |
| Water | 9.2 | 14.7 | 7.6 | 6.3 | 6.6 | 18.5 | 8.3 | 8.5 |
| Water 90° | 7.5 | 15.0 | 7.3 | 8.3 | 7.3 | 18.5 | 14.7 | 7.8 |
| Air blue (450 nm) | 13.2 | 9.2 | 7.8 | 6.9 | 8.0 | 18.7 | 13.1 | 27.4 |
| Air green (550 nm) | 7.5 | 5.6 | 6.6 | 6.8 | 7.0 | 19.5 | 13.5 | 5.5 |
| Air red (650 nm) | 7.6 | 7.2 | 8.7 | 7.7 | 9.3 | 20.2 | 13.8 | 23.5 |
| Water blue (450 nm) | 18.9 | 21.1 | 11.0 | 9.4 | 9.4 | 21.4 | 8.9 | 24.5 |
| Water green (550 nm) | 12.1 | 15.7 | 6.5 | 6.8 | 7.5 | 22.6 | 8.8 | 11.4 |
| Water red (650 nm) | 12.3 | 15.0 | 4.6 | 5.1 | 7.8 | 23.3 | 9.9 | 34.0 |

Probe isotropy was measured in the setup shown in Fig. 3 in air as well as in water with the sensor turned 90° around the longitudinal fiber axes between measurements. The isotropy was quantified as the standard deviation of probe signal normalized to the highest angular signal reading. The probes were manufactured with four different methods: Probe no. 1–5 (different diameters) were made with the new fabrication method based on casting first an inner sphere of PMMA and then a diffusing shell of PMMA doped with $TiO_2$ onto the fiber tip (the first number is the size of the clear sphere and the last number is the final probe diameter); Probe no. 6, was obtained with a 850 µm wide machined diffusing plastic sphere fixed to an optical fiber according to Marijnissen and Star (1987); Probe no. 7 was made by casting 500 µm wide sphere of PMMA doped with $TiO_2$ onto the fiber tip according to the method of Lassen *et al.* (1992); Probe no. 8 was made by curing a 500 µm wide sphere of Helioseal® on the fiber tip according to Henderson (1990).

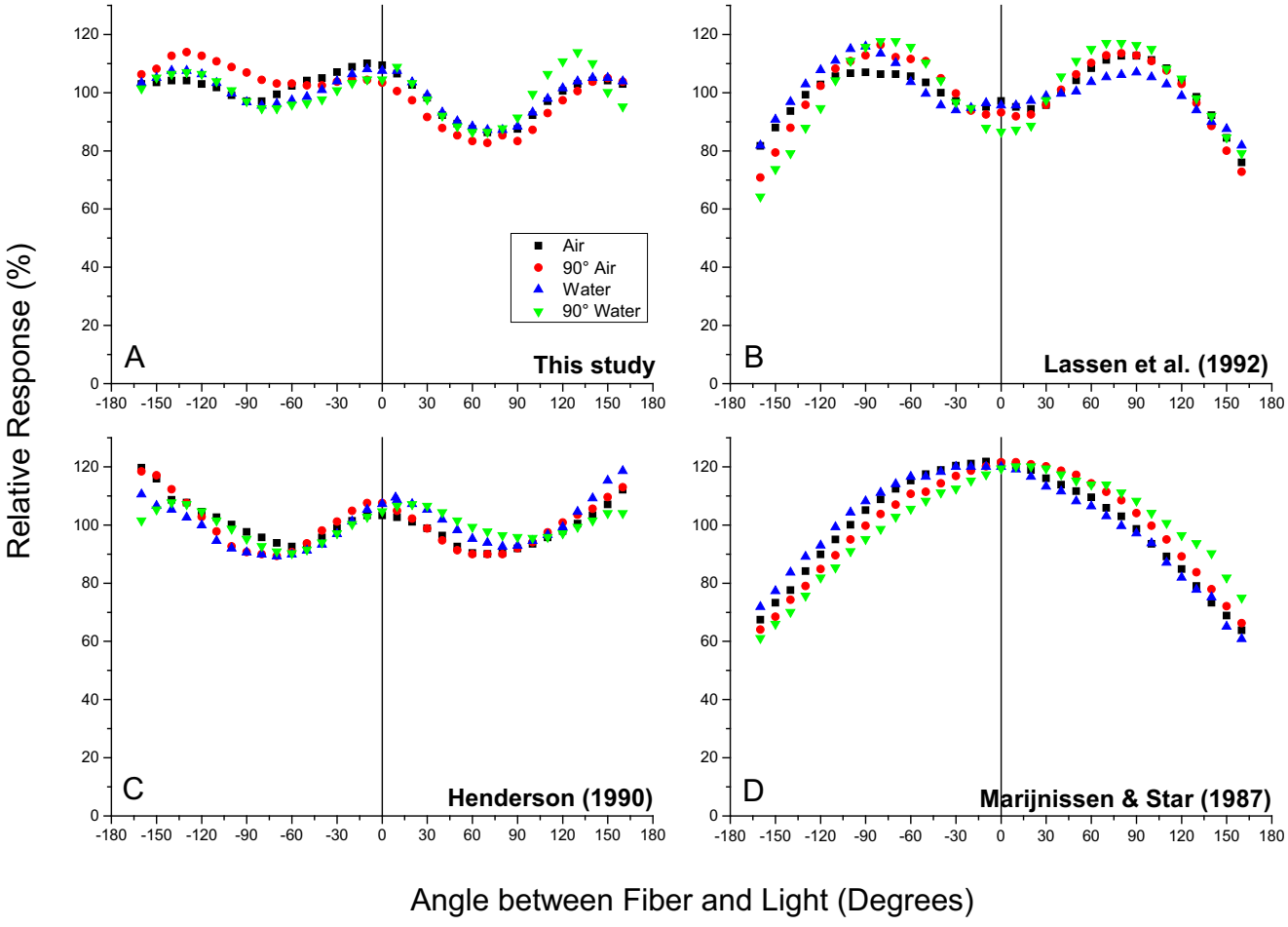


**Figure 6.** The isotropic light-collecting properties of four different types of scalar irradiance probes. The relative response was measured with the probes connected to a PMT light meter with a flat quantum response over 400–700 nm and illuminated with a collimated white LED light source. Each probe was mounted in the angular calibration setup shown in Fig. 3 and the probe response was measured as a function of incident light angle in 10° steps. Measurements were done in air and water. After each measurement, the sensor was turned 90° around its own axis and another set was recorded to check for spherical homogeneity in light collection.

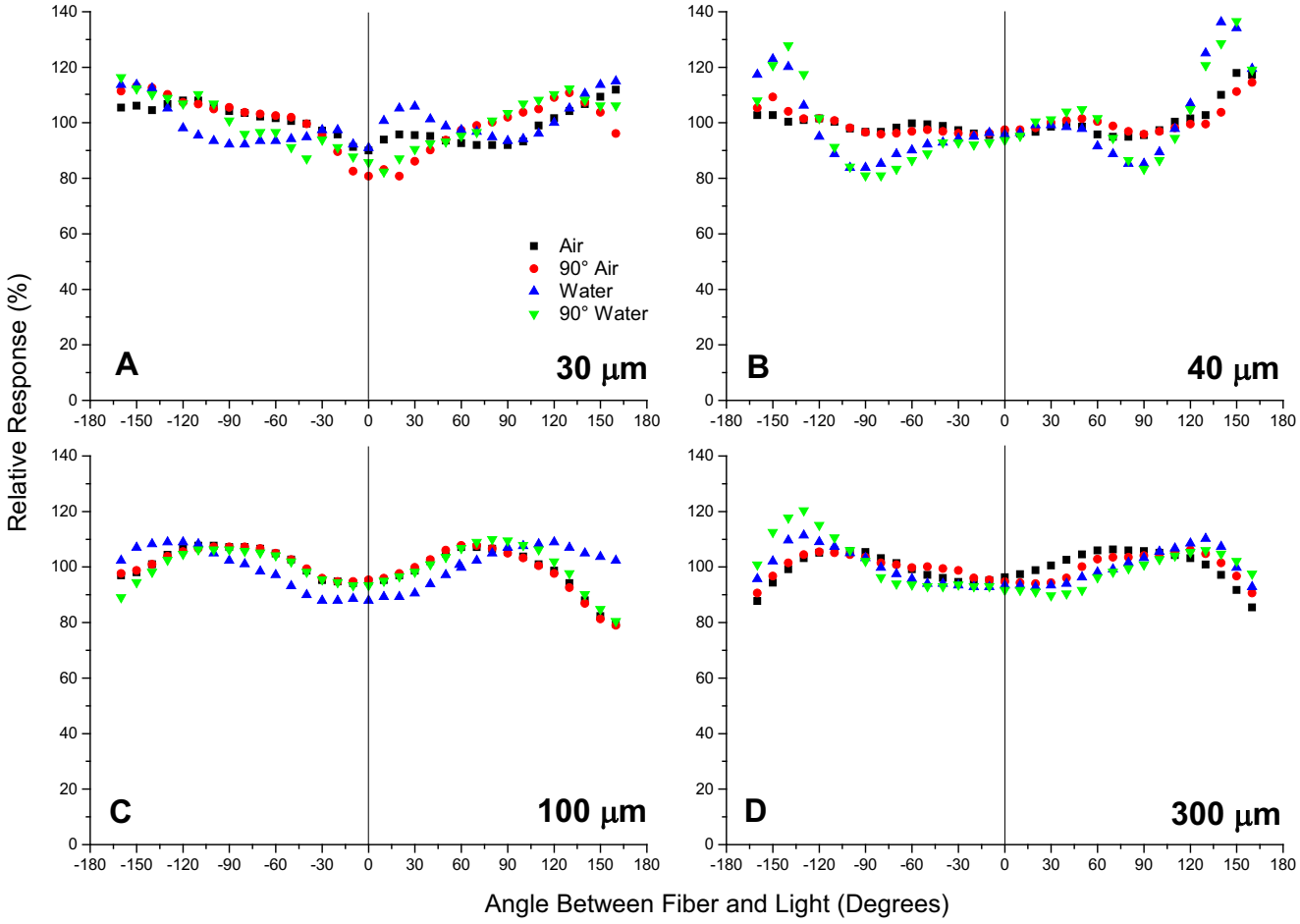


**Figure 7.** The isotropic light-collecting properties of four different sizes of scalar irradiance probes manufactured with the new method. The relative response was measured with the probes connected to a PMT light meter with a flat quantum response over 400-700 nm and with a collimated white LED light source. Probes were mounted in the setup shown in Fig. 3 and the angular response was measured as a function of incident light angle in 10° steps. Measurements were done in air and water. After each measurement, the probe was turned 90° around its own axis and another set was recorded to check for spherical homogeneity in light collection.

will escape out if the protective painting is insufficient. For tapered fibers, light coupling across the sides will be more pronounced than for untapered fibers. The general problem with light coupling out of the fibers was confirmed by redoing the 100 µm probe (3) (Fig. 7C), and the 500 µm Lassen probe (7) (Fig. 6B), where preliminary results showed large differences in isotropic response in air and water (data not shown). When the new probes were produced, great attention was put into ensuring a flawless painting of the fibers.

Lassen *et al.* (14) showed an isotropic plot of the same sensor in both air and water, but did not describe how it was carried out (14). Lilge *et al.* (12) measured some sensors in water and some in air, but did not present any comparison of the same sensor in both air and water. The set-up was described in some detail but it seems problematic as the probe was held in a cylindrical water bath and the collimated light was moved around it. It cannot be assumed that the light will still be collimated inside the water bath described due to focusing effects. The probes measured in air, was excited with a 351 nm laser, however, light attenuation in water is to strong (12). Van Staveren *et al.* (9) used a similar set-up as Lilge *et al.* (12) but placed a lens in front of the water bath to counteract the above mentioned focusing effects. They found differences in isotropy for probes inserted in different media and gave some correction factors for absolute calibration depending on the refractive index of the probe material and the surrounding medium. The probe fibers did not have any protection against light collection or leakage (3,9,17). They also compared isotropic properties for probes acting either as a light detector or as a light source, and measured large differences due to dissimilar light paths. A probe with an isotropic light emission (<±15%) could be anisotropic for light detection (>±35%), and vice versa (9). The isotropic response of a 50 µm scalar irradiance microprobe mentioned in the introduction produced by UV curing a resin mixed with $TiO_2$ on a tapered fiber was measured by emitting light out of the probe, and the result was plotted as logarithmic values (11). It is problematic to assess the isotropic response this way due to the different geometry for the light path as described by van Staveren *et al.* (9).

### Optical throughput and spectral differences in isotropic response

The difference in the angular response to blue, green, and red light of the various scalar irradiance probe types can largely be explained by absorption differences in the sphere materials. This

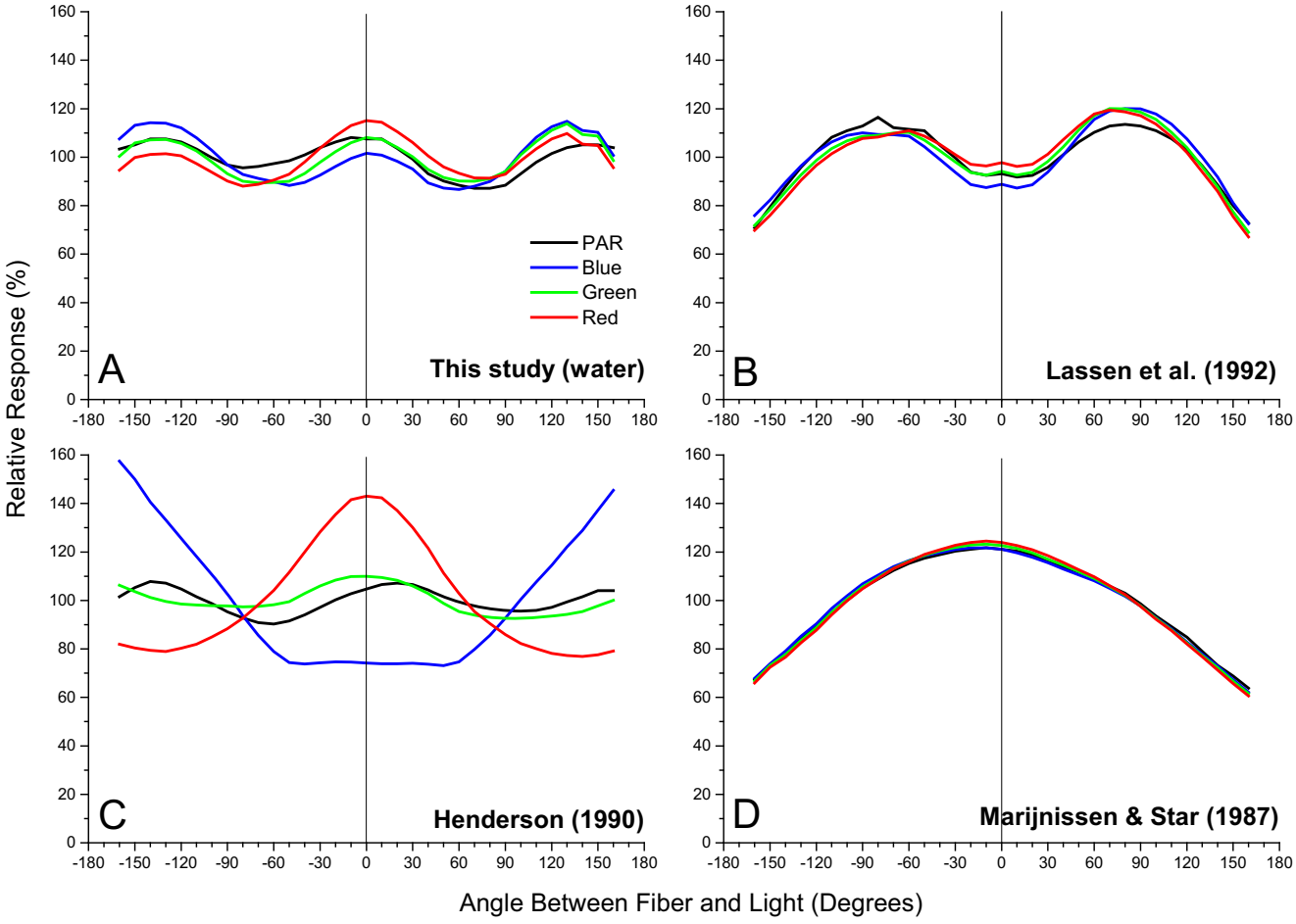


**Figure 8.** The spectral dependence of the isotropic light-collecting properties of four different types of scalar irradiance probes. The relative response was measured with the probes connected to a spectroradiometer and illuminated with a collimated white LED light source. Probes were mounted in the setup shown in Fig. 3 and the probe response was measured as a function of incident light angle in 10° steps. Measurements were done in air except for A.

affects the light attenuation in the light-collecting sphere due to scattering enhancing differences in the spectral absorption characteristics of the probe sphere material. That is, the path length enhancement for photons in the scattering sphere material increases the probability of photon absorption at wavelengths overlapping with the absorption maxima of the sphere material, while this effect is much smaller for photons in spectral ranges outside characteristic absorption maxima. In this way, both material properties, the homogeneity of the sphere in terms of material thickness, and the way the sphere is fixed onto the fiber will affect the probability of photons collected and channeled to the detector via the optical fiber.

The refractive index of the sphere material can also affect the light-collecting properties of scalar irradiance probes. The difference in refractive indices of air and water has no major significance for the light penetrating the probes but for the radiation reflected out of the probe there is a difference in the critical angel. For Helioseal® and Arnite™, the difference is ~20°, and for PMMA it is a little larger, ~22°. Photons collected in the sphere material are thus more efficiently retained in the sphere when measuring in air as compared to measurements in water. On the other side, backscatter of incident light in the sphere surface will be higher in air than in water. Such immersion effects are well known, also from other types of scalar irradiance probes, and for probes made according to Marijnissen and Star (8), such effects can lead to minor uncertainties in the range of <5% when measuring in turbid media (23,27).

Probes manufactured according to Marijnissen and Star generally exhibit a small uniform absorption of visible light (9), hence the observed difference in the three colors was very small (Fig. 8D). PMMA-based sensors (14) showed stronger absorption of blue light as compared to green and red light. The spectral variation in the isotropic response of such sensors could be related to the length of the path the light must travel inside the probe.

Such effect can be illustrated by looking at the construction of the new probes (Fig. 2), where the fiber tip is not placed in the center of the probe, but in the center of the clear sphere so the path through the $TiO_2$-doped PMMA is longer for light coming from the front than from the rear. This can give a somewhat stronger attenuation of the frontal light. In probes made according to Lassen *et al.* (14), the fiber tip is placed more central in the PMMA sphere, and such differences in path lengths are less pronounced. But as mentioned above, the solid PMMA + $TiO_2$ sphere of these probes causes a much higher overall attenuation

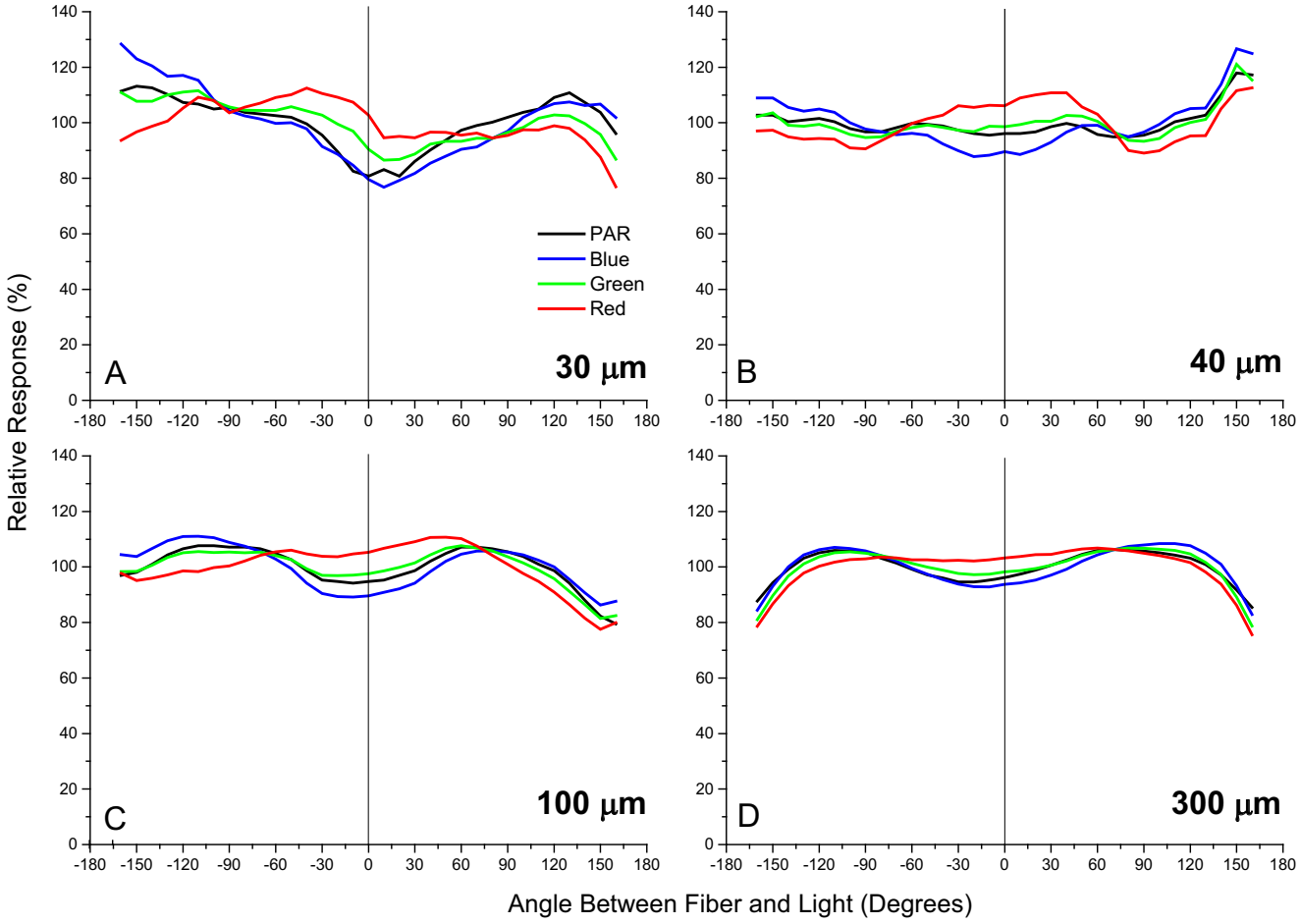


**Figure 9.** The spectral dependence of the isotropic light-collecting properties of four different sizes of scalar irradiance probes manufactured with the new method. The relative response was measured with the probes connected to a spectroradiometer and illuminated with a collimated white LED light source. Probes were mounted in the setup shown in Fig. 3 and the angular response was measured as a function of incident light angle in 10° steps. Measurements were done in air.

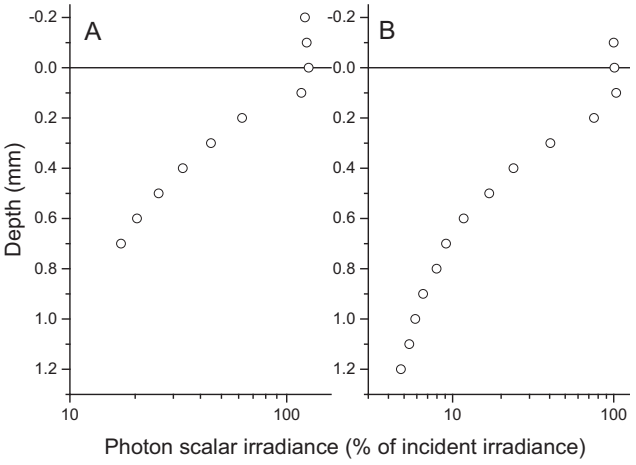


**Figure 10.** Two examples of scalar irradiance microsensor measurements in biological tissues. (A) A vertical profile of photon scalar irradiance (400–700 nm) in the living tissue of the scleractinian coral *Montastrea curta*. (B) Photon scalar irradiance measurements in a macroalgal thallus of the brown alga *Fucus serratus*. Values are expressed in % of the incident downwelling photon irradiance as measured with the fiber probe positioned over a black nonreflective light well.

of incident light as compared to probes made with the novel method presented here.

Scalar irradiance probes made according to Henderson (10), displayed a much stronger absorbance of blue light because the Helisoseal® matrix is produced to absorb in this wavelength range as it is used for curing of the matrix (9,10).

### Fabrication of scalar irradiance microprobes

The variance in isotropic light collection of the different scalar irradiance probes is strongly affected by the actual position of the fiber tip inside the spherical light collector casted on the fiber. This is also relevant for the Marijnissen-Star-type probes (9), where the parabolic shape of their angular light-collecting function is caused by the placement of the sphere on the fiber, as well as a small ratio between the sphere diameter (850 µm), and the fiber diameter (450 µm); in the original publications the ratio was recommended to be 4–5 (9).

The insertion point of the fiber itself creates a blind angle for light collection by the sphere and will in most cases set a limit for how efficient light is collected at incident angles >±120°. However, during the fabrication of scalar irradiance probes made according to the new method, one can partially compensate for

such effects. The construction based on a clear inner sphere coated with a scattering shell thus makes it possible to produce small probes down to 30 μm on tapered fibers with a tip diameter of ~15 μm, and on untapered fibers down to 240 μm with good isotropic response (Table 1).

It has not previously been possible to create probes made according to Lassen *et al.* (14) on untapered fibers. This is mainly due to the concentration of PMMA in xylene (40–45% w/w) of the used polymer solutions, which leads to a high surface tension in the material and larger spheres will not dry with a smooth surface (14). For larger spheres, the idea behind the Henderson method is elegant, but the optical properties of the probe material are not ideal for broad spectral light measurements as their angular light collection varies strongly with wavelength (10).

With the new method presented here, it is both faster to produce scalar irradiance probes with good isotropic performance and a higher optical throughput as compared to probes based on casting a sphere of only PMMA + $TiO_2$ (14). The new method makes it easier to vary the tip dimensions of the probe, and it is easier to predict the size of the final sphere during the fabrication process.

### Mechanical stability of scalar irradiance probes

It is difficult to give an exact evaluation of the mechanical stability of the probes without destroying them, but the material, and diameter of the employed optical fiber, and how well the sphere material sticks to the fiber are essential parameters. The fiber material is silica-glass for all probes compared in this study. Naturally, tapered fibers are thinner and more fragile than untapered fibers. Van Staveren *et al.* (9) found that the mechanical strength of sensors made according to Marijnissen and Star (8), and Henderson (10) was similar. Sensors made with PMMA spheres cast on the fiber tip are less sturdy, especially when measuring in very cohesive media, such as dense tissue, where there can be a substantial drag on the sphere when retracting the probe, with a potential loss of the spherical tip. Etching of the fiber tip (28) prior to casting the sphere results in better adhesion, and thus more robust scalar irradiance probes. Furthermore, the smaller probes made with the new method also appeared sturdier when used for measurements in plant and animal tissues (Fig. 10).

### Application of new scalar irradiance probes

Collection of scalar irradiance profiles in thin specimens, *e.g.* in the tough thallus of the brown macroalga *Fucus serratus*, and in the cohesive tissue of the reef-building coral *Montastrea curta* (Fig. 10) is only possible using very small sensors. This is due to (1) the thickness of the tissue under investigation, which is often <1 mm; and (2) the toughness of the outer tissue layers (epidermis in corals and outer cortex in algae), which makes it difficult for larger objects to penetrate. In addition, larger spheres are often actively pulled off the fiber tip by coral mesenterial filaments acting as a defense mechanism to fiber insertion, but apparently such filament attachment are unable to pull off the small spheres (<50 μm) (Daniel Wangpraseurt and Mads Lichtenberg, personal communication).

## CONCLUSION

The new fabrication method yields scalar irradiance probes with excellent isotropic light-collecting properties, being comparable or better than previously developed scalar irradiance probes, and spherical tip diameters ranging from as small as 30 μm on tapered fibers up to ~600 μm on untapered fibers. The presented experimental setup for measuring the isotropic light collection of the scalar irradiance probes in air and any relevant liquid is easy to build and operate. If probes are carefully produced, *e.g.* by an experienced technician, it is not necessary to test them both in air and water. However, testing can effectively reveal flaws in the probe performance due to imperfect coating/isolation of fiber tips or damage of the spherical tip. The possibility of making well-functioning scalar irradiance microprobes with tip diameters <50 μm now enables light measurements at higher spatial resolution in thin objects, such as terrestrial leaves and thin-tissue corals, and aquatic macrophytes. This new probe thus gives rise to many new fields of application in photobiology.

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