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Jun 20, 2022

In situ high-speed brightfield imaging for studies of aquatic organisms V.2

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dx.doi.org/10.17504/protocols.io.kxygxz4ykv8j/v2

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Behavioral measurements of fragile aquatic organisms require specialized in situ techniques. We developed an in situ brightfield camera set-up for use during SCUBA diving in aquatic ecosystems. The system uses brightfield illumination with collimated light and an underwater camera to highlight morphological details, body motion and interactions between organisms with high spatial (4K: 3840x2160 pixels) and temporal resolution (up to 120 fps). This technique is particularly useful for gelatinous organisms because of their large (centimeters in length), transparent bodies. Further, the measurements are not subject to experimental artifacts produced in laboratory studies. This method is useful for anyone seeking detailed brightfield images of organisms or nonliving material (e.g. marine snow) in the natural environment.

DOI

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Sean P. Colin, Brad J. Gemmell, John H. Costello, Kelly R R Sutherland 2022. In situ high-speed brightfield imaging for studies of aquatic organisms .

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<https://dx.doi.org/10.17504/protocols.io.kxygxz4ykv8j/v2>

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In situ, microscope, imaging, collimated light, SCUBA, marine, aquatic, plankton, morphology, camera

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Scientific SCUBA diving should be conducted by certified divers under the auspices of the American Academy of Underwater Sciences (AAUS) or another scientific diving organization.

- 1x Sony FDR-AX700 4K Camcorder
- 1x Memory card (e.g. SanDisk 128GB Extreme PRO UHS-I SDXC Memory Card)
- 1x Gates AX700/Z90 Underwater Housing for Sony AX700, HXR-NX80 and PXW-Z90 Video Cameras
- 3x Carbon fiber rods (15 mm diameter)
- SmallRig 15mm diameter carbon fiber rod clamps
- 1x 18" Aluminum 19 mm dovetail optical rail and 1/4 20 stainless hardware
- 1x Photographic scale (thin wire, 2-4 mm in diameter)
- 1x Suptig Underwater LED panel with 84 high-power, dimmable LEDs
- 1x Plastic light diffuser

Before diving, test camera and housing in a sink or pool to ensure that it is leak proof.

Select field site

- 1 This system is lightweight, compact and portable and can be used SCUBA diving from shore, docks or boats. The camera system can also potentially be towed vertically from a research vessel.

Assemble equipment

- 2 The brightfield camera system relies entirely on available off the shelf components and does not require custom fabrication of components, which keeps the overall cost reasonable. The camera/lens and underwater housing configuration are flexible in that there are numerous configurations of commercially available platforms that will work in a brightfield setup allowing users to choose a frame rate and image resolution that best suits their needs. The illumination source must be far enough away from the light diffusing plate to ensure an even light distribution onto the plate or else uneven brightness will occur in the resultant images. A variety of light sources can be used but the user must consider that the optimal distance from the light to the diffuser will likely change between the light sources.

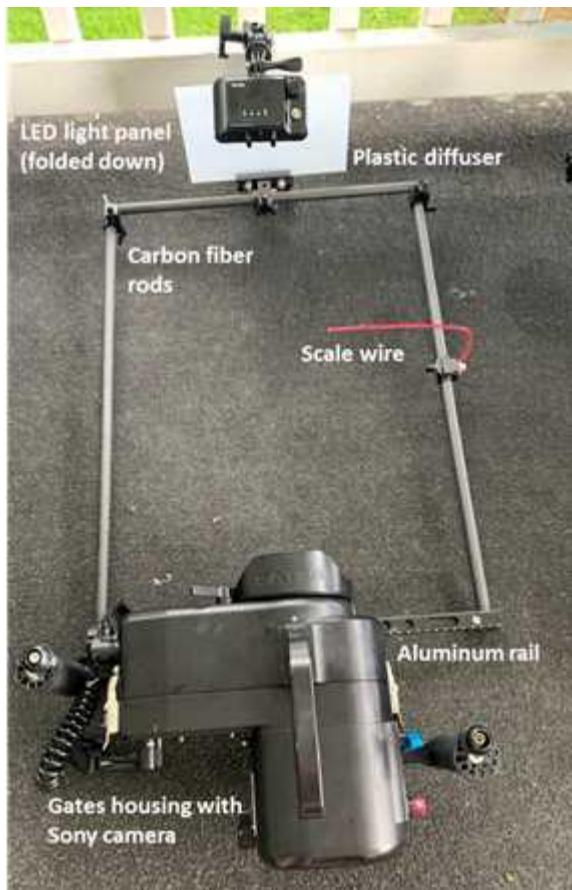


Image acquisition

- 3 The subject of study (animal or interaction) can be centered in the field of view using the viewfinder. The video camera can be used successfully in fully automatic mode whereby shutter speed, the International Organization for Standardization (ISO), f-stop and focus are continually monitored and altered by the camera. Alternatively, any or all of the camera settings can be manually controlled to produce a better image, such as the focal plane, which is beneficial when filming low contrast, mobile targets. A photographic scale can be placed on the edge of the field of view helps focus the camera and also provides the spatial scaling information for subsequent measurements. A thin wire of known dimensions provides a useful scale without creating hydrodynamic disturbance. The illumination source has several intensity modes which can be changed underwater for on-the-fly adjustments to best match environmental conditions.

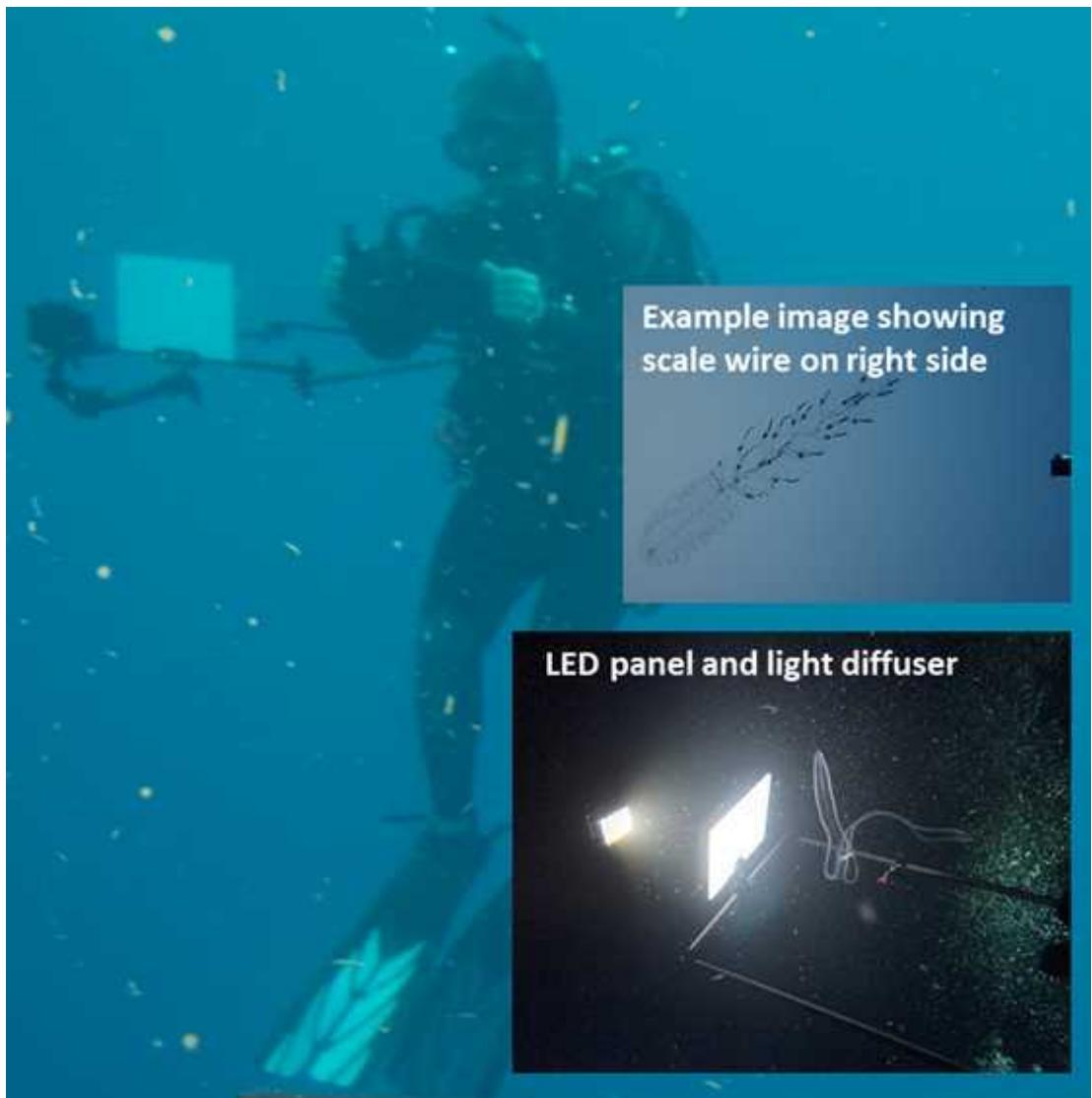


Figure 1. Brightfield system being operated by a SCUBA diver off the Kona coast of Hawaii, USA. Insets show 1) an example image of the siphonophore, *Forskalia* sp., with the scale wire and 2) the ctenophore *Cestum veneris*, being illuminated by the LED light panel with a diffuser.

Post-processing

- 4 Video files can be viewed and converted to image stacks in Adobe Premiere or a similar video editing software. Image stacks can then be imported to ImageJ or a similar image analysis software for obtaining measurements from still images or image sequences.

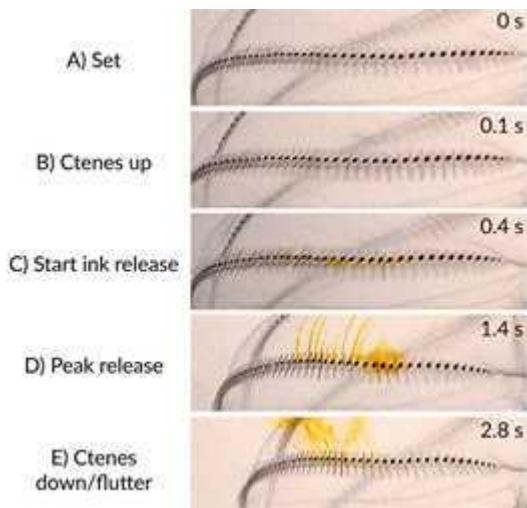


Figure 2. Time series of an ink release event in the ctenophore, *Eurhamphaea vexilligera* (Townsend et al. 2020).



Figure 3. Oral view of ctenophore, *Cestum veneris* with tentillae (fine hair-like structures important for feeding) draped over both sides of the body.

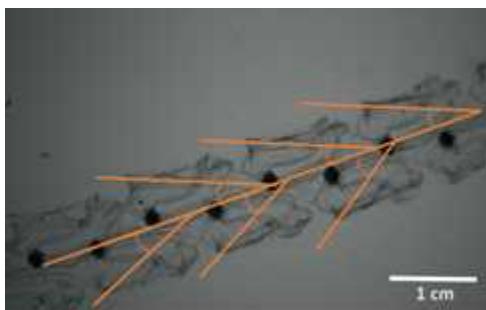


Figure 4. Siphon angles of the salp, *Iasis cylindrica* (aggregate stage), showing low jet angles which is important for swimming thrust.