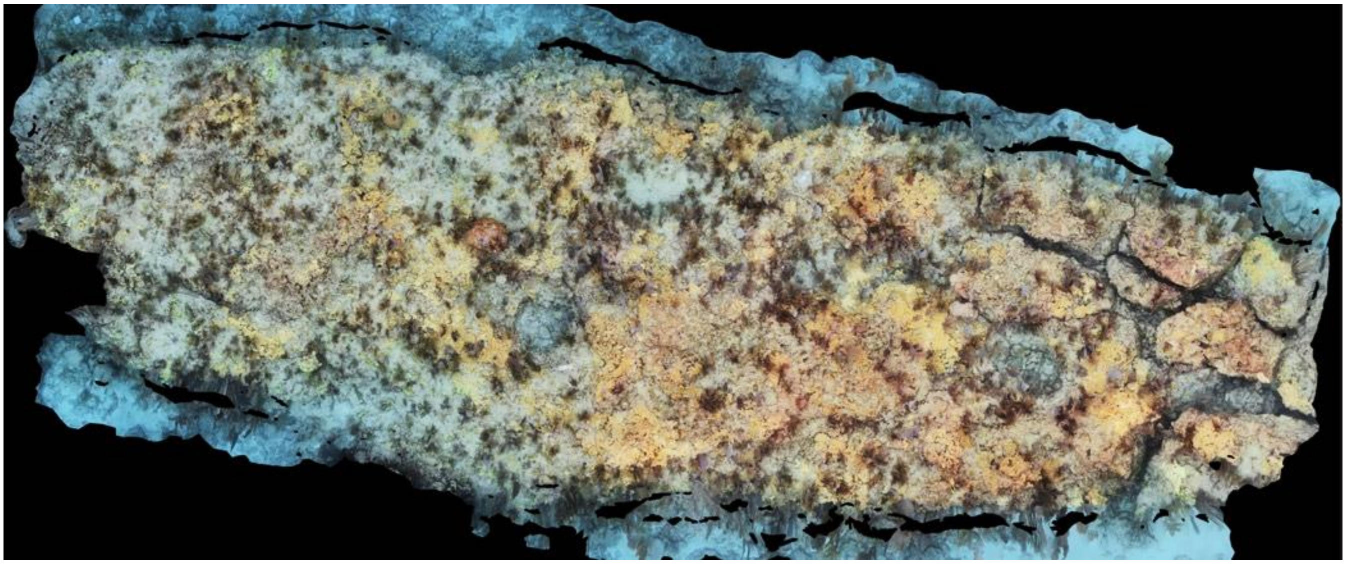
WHITE PAPER

OCTOBER 9, 2023

Coral Restoration Foundation™ Photomosaic Manual
Third Edition – October 2023



AUTHORS:
Alexander M. Neufeld, Garrett Fundakowski
Coral Restoration Foundation™, Tavernier, FL 33070

CORAL RESTORATION FOUNDATION™

Coral Restoration Foundation™ Photomosaic Manual

Alexander M. Neufeld
Garrett Fundakowski
Coral Restoration Foundation™, Tavernier, FL 33070

Suggested citation: Neufeld, A., Fundakowski G. (2023). Coral Restoration Foundation™ Photomosaic Manual: March 2023 Edition [White paper]. Retrieved [date], from Coral Restoration Foundation™

Table of Contents

Purpose

The purpose of this document is to (a) provide divers with the basic knowledge and skillset for creating 2-dimensional, underwater photomosaics- also referred to as orthomosaics, structure from motion (SfM), or several similar descriptors- and (b) explain one method for analyzing photomosaics to quantify coral reef restoration. It should be noted that while this document will describe several methods for acquiring photomosaics, many additional methods and techniques exist across the various underwater science communities. Additional sources of information on such techniques are listed on the final page of this manual. As photomosaics and their uses in underwater science are a continuously evolving field, the reader should carefully consider their needs and capacities, and choose techniques to suit their work. For readers interested in 3-dimensional modeling of reef restoration efforts and some of the measurements used therein, CRF™ has released an extensive 3-dimensional monitoring manual, available at www.coralrestoration.org/white-papers.

Background

A photomosaic is an image comprised of multiple individual and overlapping images that are digitally stitched together into a single image file. Thus, a photomosaic can depict a scene larger than can be encompassed by any individual photograph, without significant sacrifices in image resolution or vantage point.

In various disciplines of marine science, benthic photomosaics are a critical scientific tool in mapping submerged objects of interest, such as shipwrecks or topographic structure. Specific to reef restoration and conservation, photomosaics are invaluable in mapping large reef areas, assessing benthic coverage of different biota, and monitoring corals outplanted as a part of reef restoration efforts. Recent developments in underwater camera technology, as well as software and computing power, have allowed for the rapid creation of incredibly high-resolution photomosaics, without extensive financial investment or intensive training. Furthermore, these advances have granted the average diver the ability to produce quality photomosaics easily, provided the diver has been in trained in the appropriate techniques for image acquisition and digital photomosaic stitching.

Part I – Introduction to In-Water Image Acquisition

Theory

In order to consistently produce quality photomosaics, a method for the in-water acquisition of ”stitchable” individual images must be learned. Parts I and II of this document will detail a “Beginner/Easy” method for acquiring these images and an “Advanced/Hard” method for acquiring these images. Both methods are commonly used by Coral Restoration Foundation™ (CRF™) in day-to-day operations and differ only in the experience level of the dive team utilizing them. Part III of this document outlines several other methods for acquiring in-water imagery and while these methods are used by CRF, they require additional equipment or serve alternative purposes and therefore warrant separate explanation.

Regardless of the method(s) selected for use, the primary goal of the in-water photomosaic procedures is simple: obtain photos that can be stitched together to create the photomosaic. In order for a set of photos to be stitched together, they must meet several criteria. First, the photos must have sufficient overlap- at least 60% in each dimension. This overlap is ensured by placing the cameras in the appropriate locations on the camera rig, swimming with the cameras at a consistent pace, and setting the appropriate time lapse interval on the cameras. Second, the photos must be clear (i.e. without motion blur), a problem that can be avoided with clean/unscratched filters and housings, an appropriate time lapse interval, and by swimming smoothly and constantly. Finally, the photos should be organized and stored in a redundant and consistently labeled information architecture immediately after the in-water work is completed to streamline the stitching process and avoid confusion or the loss of files.

CRF’s Required Equipment

- Minimum (2) GoPro cameras (recommended Hero 5 or later model) with following accessories for each:
    - Dive housings
    - Red filters (if necessary for water conditions, though highly recommended)
    - GoPro attachment mounts
    - Batteries and microSD cards (recommended 32 GB or larger)
- PVC “hammerhead” rig for mounting cameras
- Scale bars
- Agisoft Metashape computer software
- Computer
    - Recommended computer requirements can be found at http://www.agisoft.com/downloads/system-requirements/
    - CRF™ uses a 2017 iMac Pro 27” Retina-Display CPU, with a 2.3 GHz 18-core processor and 128 GB of RAM, with a 12 TB RAID hard drive for file storage

Currently, Coral Restoration Foundation™ uses (2) GoPro Hero 9 Black cameras, each with a SuperSuit dive housing, and equipped with a red or magenta dive housing filter (dependent on water conditions). These cameras are waterproof to 9 meters without the SuperSuit housing but in our experience the cameras’ buttons often fail at depths below 3 meters, due to the pressure compressing them into a permanent “on” position. Dive filters are not required but CRF™ has had significantly more success utilizing the filters, even in shallow and very clear water. In addition to color correcting the blue-green tint of underwater light, the filters also aid in increasing the contrast of the individual GoPro photos, which in turn aids in creating an accurate stitch.

In initial phases of CRF™'s photomosaic program, we experimented with more than two GoPro cameras on a camera rig. While increasing the number of cameras used increases the diver's "field of view" as they swim, it was not found to lead to any significant increase in final photomosaic resolution. In fact, using more cameras increased the processing time necessary, since more GoPro cameras will result in more individual images that need to be stitched.

CRF™ has not found in-camera file storage to be a limiting factor on any photomosaic created to date. Each GoPro photo is saved to the internal storage card as a 1 to 3 megabyte JPG file. Thus, a microSD card of 32GB would hold over 10,000 images, equivalent to swimming for almost 3 hours on the 1-second interval timer mode.

To carry the GoPro cameras, CRF™ created a simple PVC rig, affectionately referred to as a "hammerhead" rig. T-connectors are used to attach handles to the main PVC pipe, where SuperSuit housings can be attached to mounts. The mounts shown in the below image are GoPro-brand handlebar/seat post/pole mounts.

The dual-camera "hammerhead" rig, with two GoPro mounts attached (clamps are positioned so that cameras are approximately 60cm apart).

Scale bars can be constructed from nearly any negatively buoyant material (CRF™ has used PVC, dive weights, and aluminum scale bars in the past). Our most commonly used scale bars are 50cm aluminum scale bars, with coded 12-bit targets etched into either end. These targets allow for rapid detection and auto-scaling during the stitching steps of Agisoft Metashape (described later in this manual). Larger, "square-end" PVC scale bars (~150cm) can also be beneficial, especially for very large areas or areas of poor visibility.

The placement of the scale bars within a photomosaic area is surprisingly important. In addition to providing a constant, known distance against which to scale the final photomosaic, scale bars can also provide direction to the divers acquiring the in-water imagery. Thus, this document recommends using scale bars that are large and/or brightly colored, especially if the area is unfamiliar to the divers or the visibility is poor. It should also be noted that small scale bars have a greater tendency to move in areas of high surge or territorial fish (i.e. damselfish). Scale bars that move over the course of the image acquisition process may not stitch properly in the final photomosaic and will be unreliable in scaling the photomosaic. Finally, be sure to place the scale bars level (i.e. horizontal or flat) across the seafloor to avoid warped perspectives from the cameras and to preserve the integrity of the orthorectification process, described below. This process is most accurate with 4-6 scale bars per mosaic, placed at a variety of depths and distributed non-linearly (i.e. not in a straight line) throughout the photomosaic area.

Agisoft Metashape has become the software of choice among the underwater photomosaic community and its ability to stitch large numbers of individual images quickly and accurately is impressive. So too, however, is the price tag and the computer system required to operate it. CRFTM has acquired a single Metashape license for processing its photomosaics on an iMac Pro computer (described above). Additional information on the software, as well as the pricing for commercial and educational licenses, can be found at the following link: www.agisoft.com.

Choosing Your Equipment (Cameras and Scale Bars)

Choosing the correct cameras for your photomosaics will largely come down to the balance of three things: cost, resolution, and ease of use/durability. Small action cameras like GoPros have relatively low associated costs, are easy to use, and are durable in underwater environments, but lag behind DSLR/mirrorless camera systems in photo quality and resolution (though the gap is rapidly closing). By contrast, DLSR/mirrorless cameras produce excellent images with sharp contrast and customizable white balance, which will in turn create consistent, comparable photomosaic images. However, these cameras are typically thousands of dollars, require 3rd party housings (Ikelite, Nauticam, etc.) to be used underwater, can be hard to transport and use underwater, and can become quickly ruined in the event of a housing leak or crack.

For the above reasons, CRFTM has opted to use GoPro cameras for its ongoing photomosaic monitoring program. We began the program in 2017 with GoPro Hero 5 Black model cameras and have since upgraded to Hero 9 Black model cameras. As expected, there are several differences between the cameras, two of which are highlighted here. First, the Hero 9 camera contains a much-improved, 20 megapixel camera sensor. When compared to the Hero 5 camera (12 megapixel sensor), photographs from the Hero 9 are clearer, higher contrast, and higher resolution than those of the Hero 5, resulting in substantial improvements in the quality and clarity of final photomosaic images.

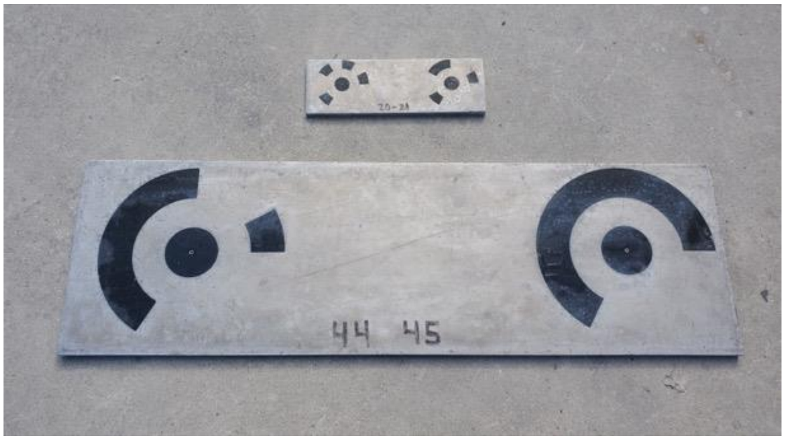
However, the second difference between the two cameras is the auto-white balance setting. Somewhere around the GoPro Hero 7 or Hero 8 models, GoPro significantly altered the way their auto-white balance setting reproduces underwater scenes. On the Hero 5, auto-white balance with a red dive filter produced consistent, properly balanced underwater photographs (albeit slightly purple-shifted). However, by the Hero 9's release, the underwater white balance had become noticeably yellow-shifted, producing "warm" tints on the images. Presumably, this change was made to more accurately depict yellow/brown skin tones underwater but the end result is much less accurate and constantly changing auto-white balance setting for benthic (coral reef) subjects.

In order to account for this poor auto-white balance performance, but continue using the incredible upgrades in image quality afforded by the Hero 9 model, CRFTM usually uses the "Native" white balance setting now found on GoPro 9 and 10 models, combined with a magenta color dive filter. We have found that the Native setting gives a consistent "faded green" color to underwater images taken throughout the acquisition process. Between the magenta filter and some light contrast increases via image editing software, a result acceptably close to the old Hero 5 auto-white balance colors can be achieved for clear water less than 10 feet in depth. For scenes taken between approximately 12 and 20 feet of depth, additional red-shifting must be applied in post-processing to generate a properly white balanced photo.

Note: Although GoPro Hero 10 and 11 cameras each have the capacity to shoot RAW+JPEG files in the Time Lapse mode, this feature is limited to intervals of 5 seconds or greater, rendering GoPro RAW file workflows impractical for large-scale photomosaic use.

As previously described, any negatively-buoyant object of known length can be used as a scale bar for the photomosaic image collection process. However, some items function better as scale bars than others. CRFTM currently uses 50cm aluminum bars, with 12-bit coded target etched into the aluminum. These coded targets are available from Agisoft via the "Tools->Print Markers" menu in Metashape. During the stitching process (described below), Agisoft will recognize these targets automatically, which helps to accelerate the scaling and orthorectification processes. Regardless of the material or style of scale bars used, CRFTM highly recommends incorporating the Agisoft coded targets in some way.

Our aluminum scale bars are high-contrast and heavy, which ensures that they show up well in both raw and final photomosaic imagery and can lay flat without moving when used in areas with strong current or surge. However, their weight does make them difficult to carry for long distances underwater; thus, CRFTM is currently experimenting with more lightweight scale bars constructed from durable underwater stickers, onto which targets are printed and which can then be adhered to thin weights. Targets may also be printed onto underwater paper and attached to some form of weight with good results, provided the paper does not bend, wrinkle, or tear over time.



Aluminum scale bars with etched Agisoft coded targets (50cm and 15cm lengths). Numbers correspond to Agisoft's numbering system for 12-bit coded targets.

Old square-end (above) and stick (below) scale bars, all made from PVC. Agisoft coded targets may be printed separately and affixed to the square-end bar with waterproof stickers or glue.

Part II – In-Water Procedures: Beginner/Easy Method

A diver mosaics an area of reef using the described two GoPro camera setup.

Pre-Dive Equipment Setup

Set up the GoPro cameras by checking for charged batteries, cleared microSD cards, and any scratches on the camera lenses. Place the cameras in the dive housings and attach the red dive filters. It is recommended that the camera's default mode be set to "Time Lapse" at 1-second intervals to minimize the risk of accidentally shooting in video mode or being unable to alter mode settings once in the water.

Attach the dive housings to the GoPro mounts at intervals of about one half meter. For very shallow areas where the diver will need to swim at or near the surface, mounting the cameras closer to each other will help ensure that there is sufficient overlap between the cameras, since the distance to target (reef) and field of view will be reduced. The cameras should typically be oriented to face straight down, though they may also be oriented at slight opposite angles (e.g. one camera ~5° forward and one camera ~5° backward) to aid in accurate stitching of very low rugosity or non-descript areas where more oblique views of the substrate can offer an increased number of points for alignment across photos.

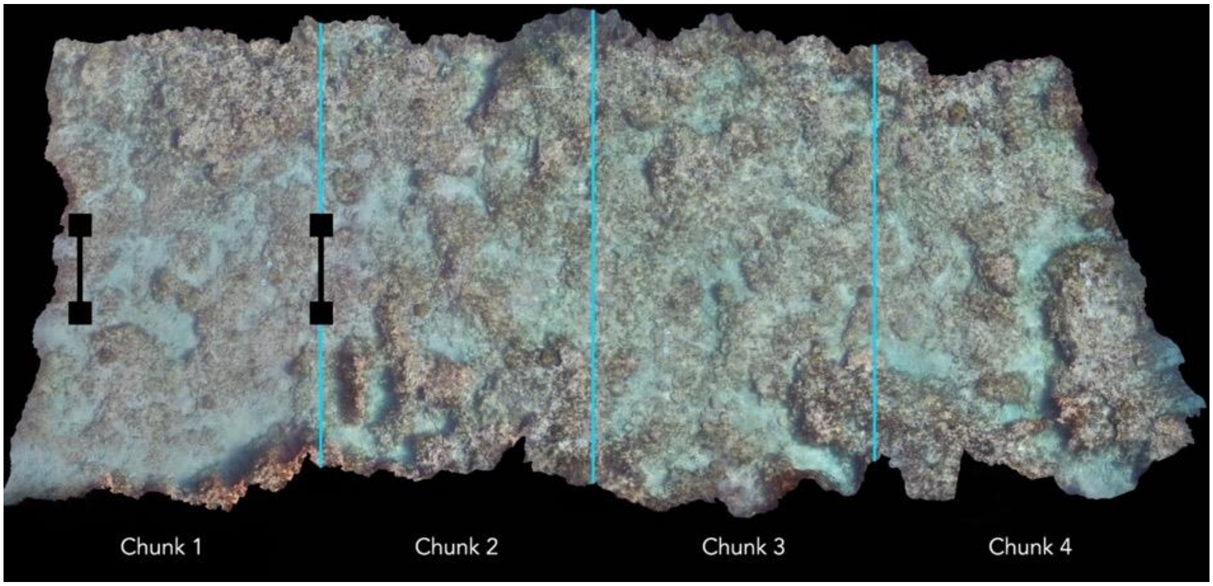
If necessary, assemble or prepare the scale bars.

Finally, the specific area of reef to be surveyed should be identified before entering the water, when possible. CRF™ uses Google Earth to assess mosaic areas of appropriate size, which are then confirmed as suitable for restoration upon arrival and mosaicked.

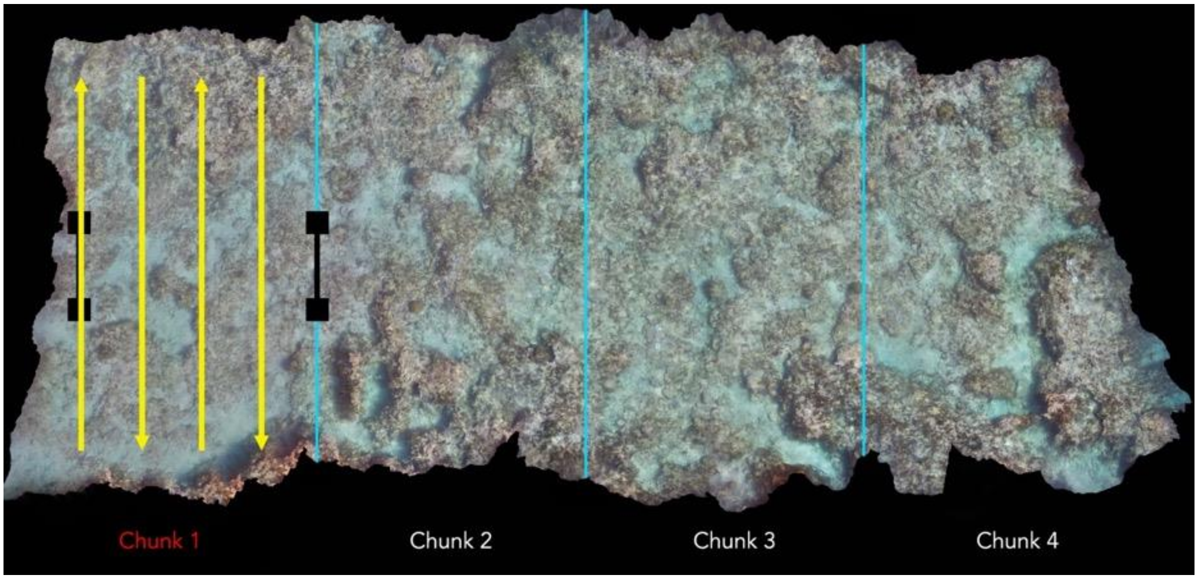
Image Capture and Diver Tasks

This method of image acquisition should be utilized if the diver team is inexperienced in the photomosaic process, water conditions (visibility, current, surge, etc.) limit the size of the area that can be efficiently mosaicked, or if the area to be mosaicked is larger than ~2,000 m². This manual recommends acquiring imagery for subsections of the full area to be mosaicked, referred to here as "chunks". The exact size of each chunk should be determined by water conditions, comfort and experience of both divers, and the presence or absence of significant navigational aids within the reef area. We recommend chunks of approximately 250 m² for new/inexperienced photomosaic divers and approximately 1000 m² for more experienced teams working to photomosaic much larger areas of reef. Diver 1 is the diver with the camera rig and Diver 2 is the support/scale bar diver.

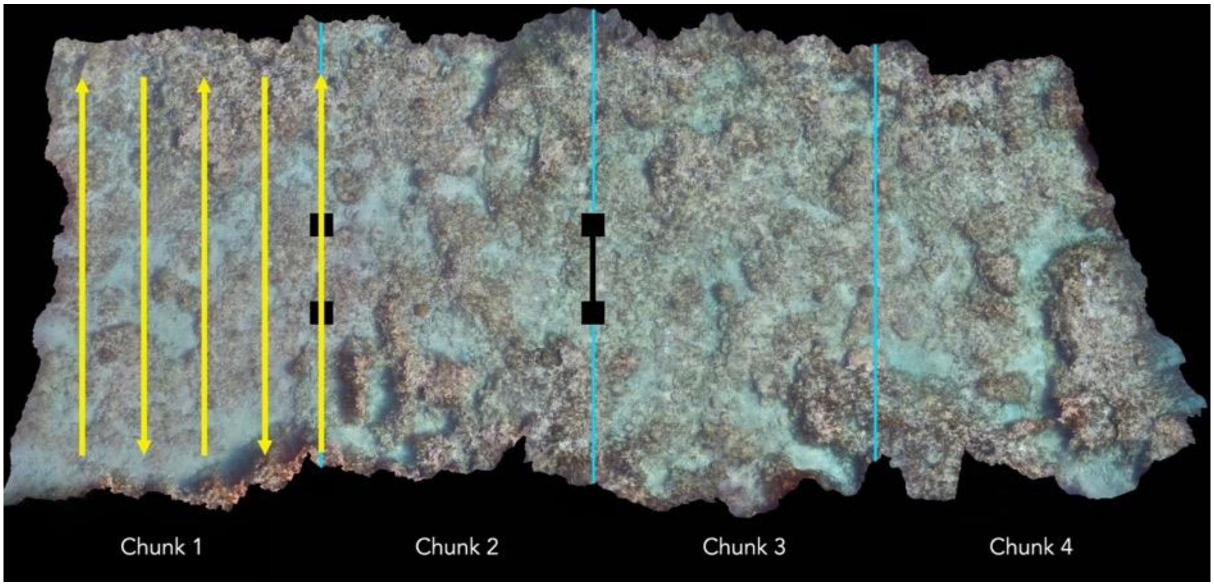
The following images explain the steps for photographing adjacent chunks of any size along an area of reef:



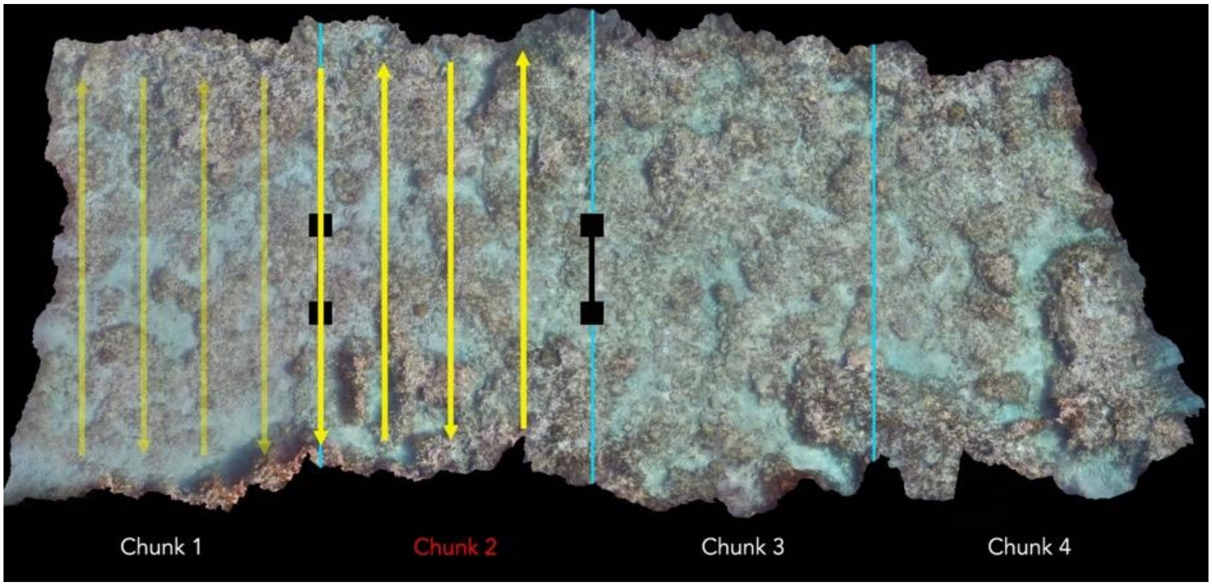
The above area of reef is to be photographed for a photomosaic. The area has been mentally divided into four chunks (blue lines) and Diver 2 has placed the scale bars (black) along the two borders of Chunk 1 and recorded the depths of the scale bars with their dive computer.



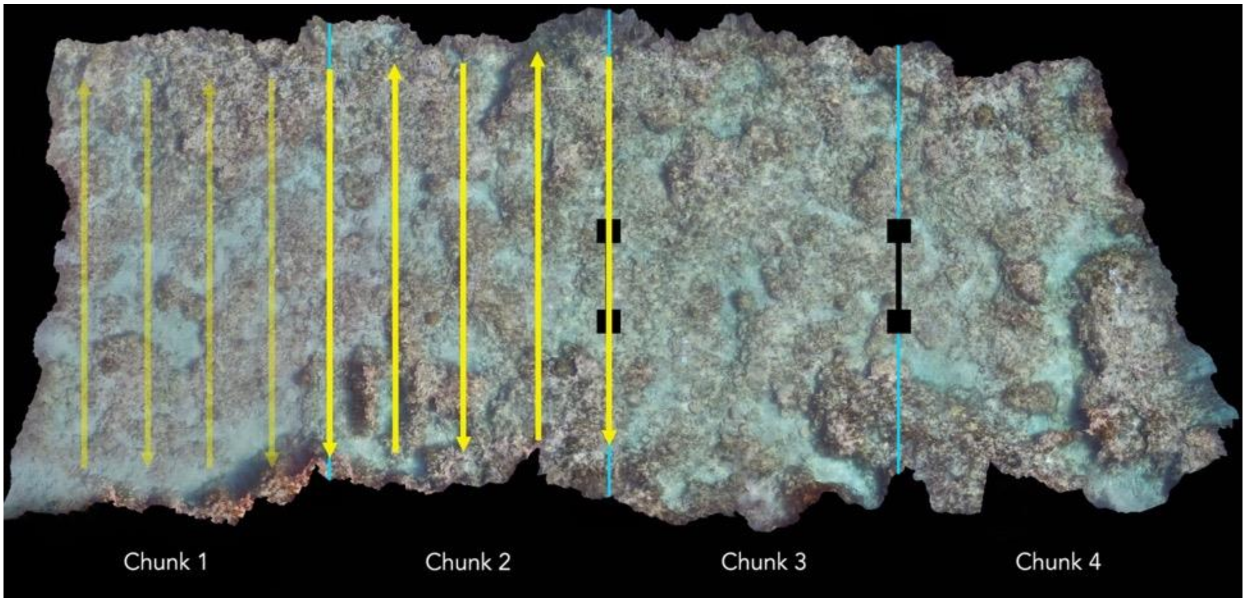
Diver 1 follows the pattern of the yellow arrows, with the cameras in Time Lapse Mode. The first pass should travel directly over the first scale bar and the second pass should put that scale bar in view of at least one camera. This will ensure sufficient overlap across passes.



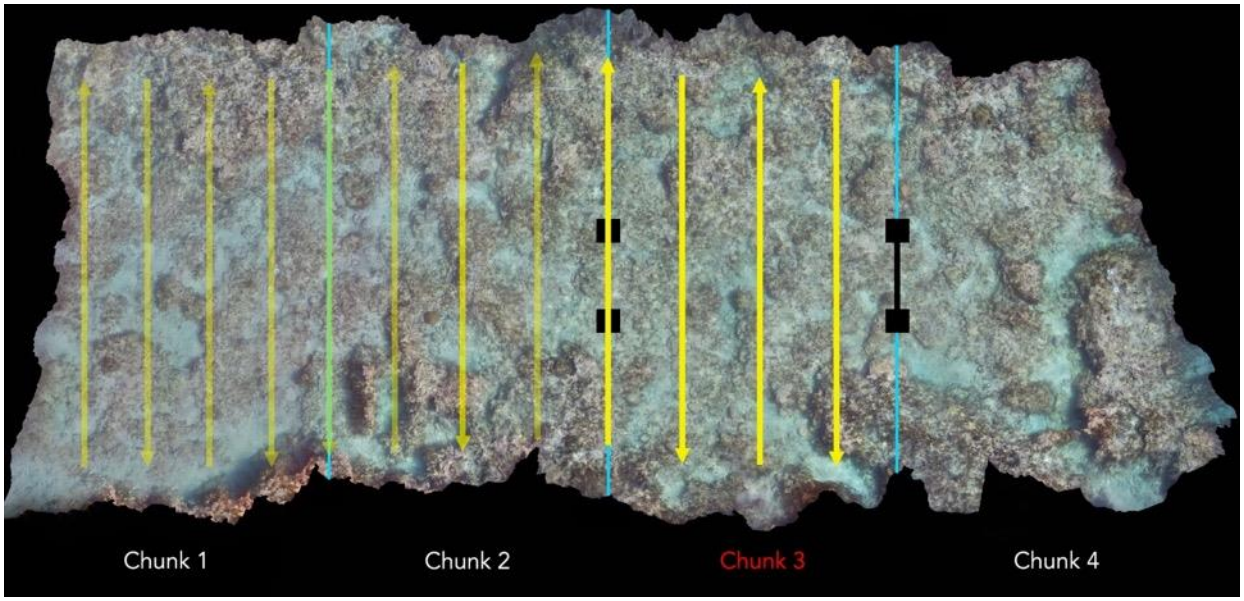
Diver 1 continues making passes until they pass over the second scale bar. This marks the completion of Chunk 1, and the cameras should be stopped/paused. Diver 2 now moves the first scale bar to the far side of Chunk 2, as shown above, and records the scale bar's depth. Chunk 2 is now prepped for photographing.



Diver 1 proceeds as before, with the first pass of Chunk 2 traveling directly over the scale bar on the border between Chunks 1 and 2. Again, the second pass of Chunk 2 should still include the scale bar on at least one camera.



When Chunk 2 is completed, the cameras are again stopped/paused, so that Diver 2 can leapfrog the scale bar and record a new depth measurement to prepare Chunk 3.



The photographing of chunks continues as explained until all chunks are photographed. Be sure to swim directly over scale bars TWICE- one time for each chunk that the scale bar borders- and that the depths of all scale bars used are recorded.

Additional Tips

- In ideal water conditions (>15 meters visibility, little to no current or surge), Diver 1 should attempt to swim ~2 meters above the reef at all times. This will allow for passes (yellow arrows in the diagrams above) that capture a 3-4 meter width of reefscape at a time, thus reducing the number of necessary passes and therefore, amount of swimming required. In shallower areas where this height above the reefscape cannot be maintained, note that the width of area captured by the images in a pass will decrease, and therefore, the distance between consecutive passes should also decrease.
- At the outset of each chunk, photograph Diver 1's hand holding up fingers that correspond to the chunk number. After each chunk is completed, turn off the cameras before photographing fingers for the following chunk. This will help with sorting the photos by chunk at the beginning of the stitching work.
- Passes should be swum continuously within a chunk. In other words, do not stop the camera in the middle of the chunk. Diver 1 can continue shooting on time lapse mode as they turn and proceed back and forth across the reef chunk.
- Diver 1 should hold the camera rig as far in front of their body as conditions (specifically, current and surge) allow. This will help remove the diver's shadow from the captured images and result in clearer imagery and a clearer photomosaic.
- Divers must communicate underwater to setup timing of moving of scale bars. If possible, plan for a set number of passes across each chunk before getting in the water.
- When in doubt, make extra passes. Additional images will slow the stitching process, but will still result in a completed image. There is no solution for images that do not overlap or that do not include a portion of the chunk.
- If desired, set the default mode of the GoPro cameras to time lapse mode at the correct interval. This will remove the chance of recording in a different mode when turning cameras on for the first time underwater.

- If the area to be mosaicked is shallow, be sure not to swim so fast that consecutive photos do not have sufficient overlap. Plan to swim more slowly in shallow areas (<2 meters depth) or set the GoPros to a faster time lapse interval (one picture per half second, for example) to help with picture-to-picture overlap. Decreasing the distance between cameras on the camera rig (i.e. moving the cameras closer together) may also help ensure overlap centrally between pairs of photographs.
- Be aware that water conditions that are "too" ideal may actually inhibit photomosaic imagery acquisition. When the water is clear and still (no surge or wave action) and the sun is bright overhead, light ripples appear across the seafloor. These light ripples, due to their constant motion, will confuse the stitching software as it attempts to reconcile the ripples as "points" that seem to change drastically from picture to picture. Some software settings can account for these caustics in the source imagery, but will drastically increase the time needed to generate a completed photomosaic stitch. Thus, the true "ideal conditions" for a mosaic are light surface wave chop, minimal current, clear water, and/or overcast, early morning skies.

Part III – In-Water Procedures: Advanced/Hard Method

Pre-Dive Equipment Setup

Set up the GoPro cameras by checking for charged batteries, cleared microSD cards, and any scratches on the camera lenses. Place the cameras in the dive housings and attach the red dive filters. It is recommended that the camera's default mode be set to Time Lapse at 1-second intervals to minimize the risk of accidentally shooting in video mode or being unable to alter mode settings once in the water.

Attach the dive housings to the GoPro mounts at intervals of about one half meter. For very shallow areas where the diver will need to swim at or near the surface, mounting the cameras closer to each other will help ensure that there is sufficient overlap between the cameras, since the distance to target (reef) will be reduced. The cameras should typically be oriented to face straight down, though they may also be oriented at slight opposite angles (e.g. one camera ~5° forward and one camera ~5° backward) to aid in accurate stitching of very low rugosity or non-descript areas where more oblique views of the substrate can offer an increased number of points for alignment across photos.

If necessary, assemble or prepare the scale bars.

Finally, the specific area of reef to be surveyed should be identified before entering the water, if possible. CRFTM uses Google Earth to assess mosaic areas of appropriate size, which are then confirmed as suitable for restoration upon arrival and mosaicked.

Image Capture and Diver Tasks

This method of image acquisition should be utilized if the diver team is experienced in the photomosaic process. Water conditions can vary from sub-optimal to ideal, depending on the skill and experience level of the diver team. This method is also suitable for very large areas of reef (between 1,500 m^2 and 3,000 m^2), dependent on the swimming abilities of the camera rig diver. In this method, Diver 1 is the diver with the camera rig and Diver 2 is the support diver.

Once the photomosaic area has been identified, Diver 1 places the scale bars throughout the area, remembering the notes on scale bar placement from this document's introductory section. For large, unfamiliar areas of reef, it may be helpful to place flagging tape, measuring tape transects, or subsurface floats around the perimeter or at the corners of the mosaic area.

Once the scale bars and navigation aids have been placed, Diver 1 begins swimming the lawnmower pattern described in the Beginner/Easy Method above. The difference with this method is that Diver 1 now continues swimming back and forth across the full width or length of the photomosaic area until the entire area has been photographed, utilizing their scale bars and the natural features of the reef to guide their passes and overlap.

This method eliminates "chunks" from the in-water acquisition and stitching processes, in favor of a more physically and mentally demanding swim by Diver 1. However, the result is a much more efficient method of in-water imagery acquisition and stitching. The total in-water swim is shorter because Diver 1 does not need to re-swim lines between chunks or wait for Diver 2 to maneuver the scale bars into place, image sorting post-dive is more straightforward since there are no chunks to organize, and the stitching speed improves dramatically because all photos are stitched as a single, massive "chunk" that does not need to be merged with its adjacent chunks.

Throughout this method, the most common role for Diver 2 is as a safety buddy diver for Diver 1, who can assist Diver 1 with any technical hiccups. However, this can change if the area to be mosaicked is quite large. The diver who is swimming the camera rig can easily become fatigued over the course of acquiring imagery for a large reef area and can consume their air much more quickly than their buddy.

To balance the fatigue and air consumption factors across the diver team, proceed with site setup as described above. Diver 1 begins to swim the lawnmower pattern across the area and when they have reached a pre-determined point within the area or a pre-determined air pressure in their scuba tank, they can stop the cameras and switch roles with Diver 2, who takes the cameras, turns them back on, and continues photographing from Diver 1's terminus. In this way, a well-coordinated team can photograph a large area of reef- neither diver becomes prohibitively fatigued and a situation where one diver is low on air and must surface while their dive buddy has air to continue the image acquisition work is avoided.

Additional Tips

- In ideal water conditions (>15 meters visibility, little to no current or surge), Diver 1 should attempt to swim ~2 meters above the reef at all times. This will allow for passes (yellow arrows in the diagrams above) that capture a 3-4 meter width of reefscape at a time, thus reducing the number of necessary passes and therefore, amount of swimming required. In shallower areas where this height above the reefscape cannot be maintained, note that the width of area captured by the images in a pass will decrease, and therefore, the distance between passes should also decrease.
- Diver 1 should hold the camera rig as far in front of their body as conditions (specifically, current and surge) allow. This will remove the diver's shadow from the captured images and result in clearer images and a clearer photomosaic.
- When in doubt, make extra passes. Additional images will slow the stitching process, but will still result in a completed image. There is no solution for images that do not overlap or that do not include a portion of the chunk.
- If desired, set the default mode of the GoPro cameras to time lapse mode at the correct interval. This will remove the chance of recording in a different mode when turning cameras on for the first time underwater.
- If the area to be mosaicked is shallow, be sure not to swim so fast that consecutive photos do not have sufficient overlap. Plan to swim more slowly in shallow areas (<2 meters depth) or set the GoPros to a faster time lapse interval (one picture per half second, for example) to help with picture-to-picture overlap.
- Be aware that water conditions that are "too" ideal may actually inhibit photomosaic imagery acquisition. When the water is clear and still (no surge or wave action) and the sun is bright overhead, light ripples appear across the seafloor. These light ripples, due to their constant motion, will confuse the stitching software as it attempts to reconcile the ripples as "points" that seem to change drastically from picture to picture. Some software settings can account for these caustics in the source imagery, but will drastically increase the time needed to generate a completed photomosaic stitch. Thus, the true "ideal conditions" for a mosaic are light surface wave chop, minimal current, clear water, and/or overcast, early morning skies.

Part IV – Additional In-Water Procedures

The following sections detail additional in-water procedures that may help with image acquisition for large reef areas (simultaneous diver teams, diver propulsion vehicles), areas where higher resolution photomosaics are needed (DSLR cameras), or small-scale areas depicting non-Acroporid coral restoration (massive/mounding corals). CRF™ has used each of these techniques to varying extents but their uses in day-to-day operations are too uncommon to warrant independent sections. Additionally, other organizations have developed more sophisticated procedures for utilizing DSLR cameras and 3-D modeling and should be consulted in conjunction with this document.

Simultaneous Diver Teams

A potentially useful amendment to the Advanced/Hard in-water image acquisition method described in the previous section is the use of two simultaneous diver teams. By utilizing two pairs of divers- each with their own set of scale bars, cameras, and camera rigs- massive areas of reef can be covered twice as quickly. With this technique diver teams ideally begin swimming their passes at opposite sides of a mosaic area and work towards each other until they meet in the middle. The photos from both teams are then combined during the stitching process to create the single, large photomosaic.

The most difficult aspect of this technique is confirming that the central region of the mosaic where the dive teams meet contains sufficient overlap between the teams, to ensure that all portions of the area have been imaged in the water. Coordination between the pair of “Diver 2”s may be necessary here.

Diver Propulsion Vehicles (Scooters)

Diver Propulsion Vehicles (DPVs), or “scooters”, also have the potential to make in-water imaging of larger reef areas more efficient. The largest hurdle to achieving results with this technique is simply finding the correct DPV model. Many models now come with GoPro attachment mounts but they are typically forward-facing and only offer space for a single camera. Thus, CRF™ has created a PVC bar system strapped to the underside of a Yamaha 220Li Seascooter with zip-ties, as shown below.

While DPVs can accelerate the process of in-water image acquisition for large areas, it is important to choose a model with appropriate speed settings. DPVs that move too quickly can create motion blur on photos or contribute a lack of front-to-back overlap between consecutive images. The recommended GoPro Time Lapse setting for most DPVs is therefore ½ second and DPVs should typically be run on their slowest speed setting.

DSLR and Mirrorless Cameras

If the final photomosaic resolution offered by the GoPro camera setup described above is not sufficient, either for monitoring or aesthetic purposes, DSLR or high-resolution mirrorless cameras can be used. These systems will offer much higher resolution of both in-water images and final photomosaics but that resolution will come with several associated costs.

First and foremost, DSLR/mirrorless cameras are significantly more expensive than GoPros and may become prohibitively expensive when the costs associated with waterproof housings are factored into the equation. It should also be noted that these physically larger camera systems are much more difficult to handle underwater and can make the swimming of areas >1,000 m^2 incredibly physically demanding.

CRF™ has occasionally used a single DSLR camera and underwater housing to generate high resolution photomosaics for print and media/communications purposes. For these mosaics, a small (25-50 m^2) area of restored reef is photographed with the aforementioned lawnmower pattern with the single DSLR camera and 35mm lens facing straight down. In this scenario, the camera's built-in intervalometer is used to auto-focus and capture images at one second intervals. However, not all DSLR/mirrorless cameras come with built in intervalometers and after-market firmware upgrades may be necessary.

Multiple DSLR/mirrorless cameras may be used if an appropriate camera housing rig can be crafted. Be advised that while increasing the number of high-resolution images will create a more accurate and higher resolution photomosaic, the stitching process will require more time and/or computer power.

Massive/Mounding Corals – 3-D Acquisition

CRFTM has recently begun restoring mounding corals within the genus *Orbicella* by outplanting individual, half-dollar-sized plugs in groups of 10 to 70. Typically, these groupings are placed on existing *Orbicella* skeletons, which range in size from 25 centimeters to 2 meters in diameter. To gain a more accurate picture of these rounded structures, these skeletons are photographed before and after outplanting, with the intention of recreating a 3-dimensional skeleton and the outplanted plugs.

To accomplish this, a GoPro or Olympus Tough point-and-shoot camera is used to capture 10-15 images from directly above the skeleton (in the same was the lawnmower pattern captures the top-down imagery for the other photomosaic techniques) and 40-60 images are taken from vantage points surrounding and slightly above the skeleton, to ensure the sides of the skeleton are properly depicted by the software. To generate the 3-D model in the stitching software, the side-view images should minimize the amount of water shown in the background, hence the camera's orientation slightly above the skeleton.

Because of the relatively small size of these model areas, smaller scale bars, in the form of flattened circular disks or small aluminum bars are recommended. For the most accurate scaling measurements, several of these smaller markers should be placed on/around the skeleton substrate for models created both before and after outplanting.

Additional information on acquiring imagery of 3-D Orbicella spp. restoration and making relevant measurements in Agisoft Metashape can be found in the 3-dimensional monitoring manual, available at www.coralrestoration.org/white-papers.

Part V – Stitching and Computer Processing

Overall Procedures

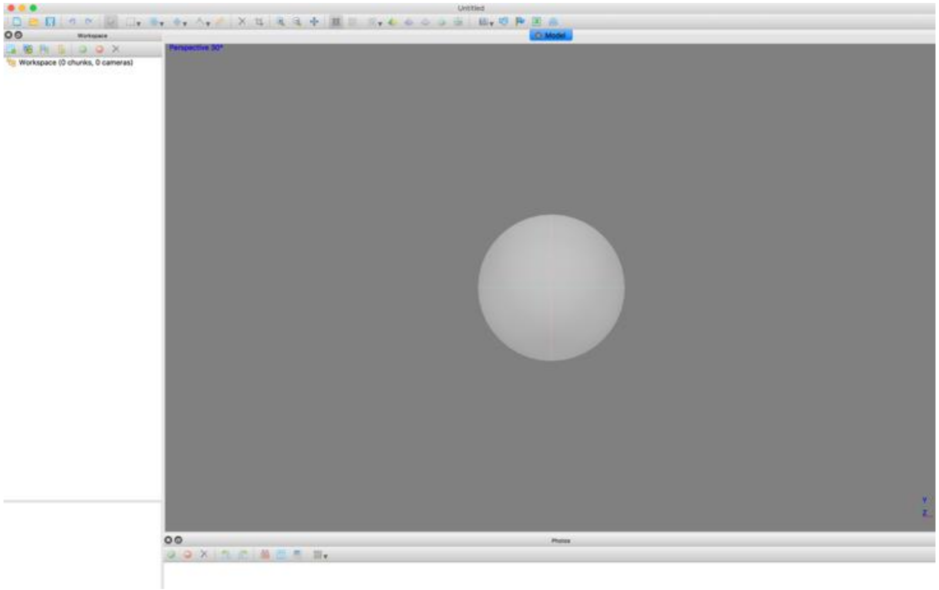
Stitching photographs into photomosaics requires two main steps. First, a photomosaic of each chunk must be built from that chunk's individual photos. Second, the chunk photomosaics must be aligned and merged to create the final photomosaic for the site. The first step is always accomplished with Agisoft Metashape while the second step can be accomplished in Metashape (recommended) or in Adobe Photoshop. (Note: if in-water image acquisition followed the Advanced/Hard method in Part II above, you will only have one chunk and therefore only need to complete the stitch for that chunk in Metashape).

Sorting Photos

Photos should be sorted immediately after the in-water work is completed to minimize the loss of memory or information from the day's work. Be aware that moving the location of photos after stitching has begun will break the project links in Metashape and will disrupt stitching. For this reason, it is recommended that a consistent, repeatable file naming and organization structure be created before mosaicking occurs and is followed over the life of photomosaic program or project.

Introduction to Agisoft Metashape

Agisoft Metashape is a powerful 3-D modeling software with the capacity to align and merge large numbers of photographs into a single mosaic or 3-D model. Upon opening Metashape for the first time, you will see a white Workspace on the left and grey model area in the center (note that all screenshots in this manual come from MacOS).



Immediately above the Workspace is a small menu bar with icons for adding chunks, photos, markers, and scale bars. Above those is another menu bar with options to open and save files, as well as change the appearance of the model displayed in the central gray area.

The standard order of operations for this manual's methodology is as follows:

1. Import Photos (into separate chunks)
2. Align Photos (for each chunk)
3. Detect/Assign Markers
4. Orthorectify and Scale

For alignment and merging of chunks in Agisoft Metashape, the steps continue as such:

6. Align Chunks
7. Merge Chunks

Once chunks have been aligned (or if the project only contained a single chunk and no chunk alignment is necessary):

8. Build Models
9. Build Orthomosaic
10. Export Orthomosaic

Importing Photos and Photo Alignment

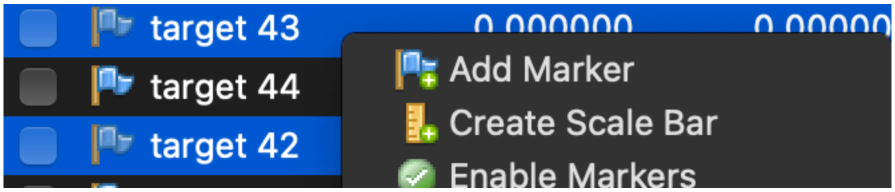
Open Agisoft Metashape and create new chunks as needed. Photos do not need to be sorted by camera in Metashape, so simply import all photos from a specific chunk into that Metashape chunk workspace. This can be accomplished either by clicking the icons immediately above the Workspace area or by selecting the "Import Photos" option under the "Workflow" menu. Be aware that a chunk must be selected in order to import photos and that selecting a different chunk requires double-clicking that chunk.

Note: If using a GoPro Hero camera or another camera with a rolling shutter, users should follow Tools -> Camera Calibration and select "Rolling Shutter Compensation -> Full (Txyz, Rxyz)" for all cameras used in the Chunk.

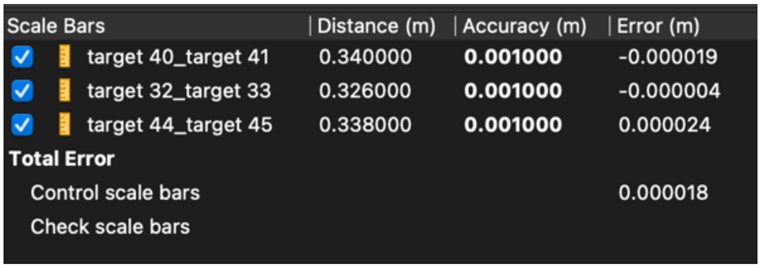
Once all photos are imported to their chunk workspaces, photos can be aligned. Follow Workflow -> Align Photos to align the photos in the selected chunk, or use Batch Processing, described below. In most cases, "High" accuracy is sufficient, along with default selections for the other settings. Click OK. However, for images with significant caustics (i.e. sunlight ripples, diver shadows in every photograph), users should switch the "Generic Preselection" setting from "On" to "Off". Note that this change will significantly increase the amount of time needed to align the photos but will allow Metashape to successfully stitch areas with caustics. At the completion of this step, each chunk should have a Sparse Point Cloud 3-D model in the project window.

Scaling and Orthorectifying the Model

If Agisoft's coded targets have been used during the in-water image acquisition process, begin the orthorectification and scaling steps by following Workflow -> Detect Markers. Once this is complete, create scale bars by selecting two targets, right clicking, and choosing "Create Scale Bar".



In the Scale Bars Panel in the lower left side of the screen, input the known distance between the center points of the coded targets (in meters) and the error of that measurement (usually 0.001m or 0.0005m depending on ruler used). Once all scale bars have been created and assigned their appropriate length, click the blue circular "Refresh" button at the top of this panel. Your model should re-size itself and a "[S]" will appear next to the chunk in the upper left corner panel. Your model is now scaled. Pressing "0" will automatically fit the model to the screen again.

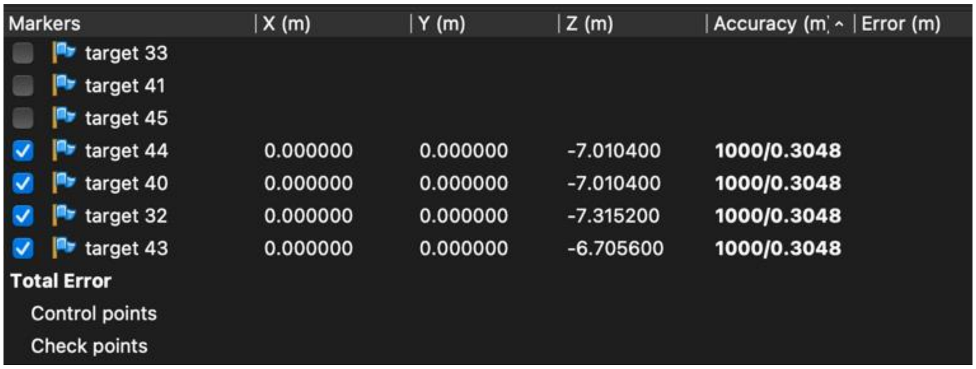


Scale Bars	Distance (m)	Accuracy (m)	Error (m)
☑ target 40_target 41	0.340000	**0.001000**	-0.000019
☑ target 32_target 33	0.326000	**0.001000**	-0.000004
☑ target 44_target 45	0.338000	**0.001000**	0.000024
Total Error			
Control scale bars			0.000018
Check scale bars			

After repeating the scaling process for each chunk, orthorectification can occur. Orthorectification is the process by which objects of varying distances from the camera can be "normalized" to the same 2-dimensional plane. In other words, objects at deeper depths are brought closer to the camera and objects at shallower depths are moved further away from the camera. Orthorectification is a critical step in the process as it allows for consistent measurements of objects throughout the image, regardless of those objects' depths or distances from the camera.

For each target or scale bar with a known depth, select one of the two targets and assign a depth measurement (in meters) for that target in the "Z" column of the target panel. Be sure to put a negative sign "-" before the depth measurement (e.g. "-4.0" for a target located at a depth of 4 meters). Repeat for at least three targets or scale bars found in a non-linear orientation throughout the mosaic model area, ignoring the X- and Y-coordinate (latitude and longitude) columns.

Next, assign an error value to each of these targets' depth measurements like so: "1000/0.1" for a dive computer or depth device set to metric units OR "1000/0.3048" for a dive computer or depth device set to imperial units. In Metashape's error column, the value before the "/" corresponds to the error of the X- and Y-coordinates, while a value after the "/" applies to the Z-coordinate. Since this technique does not utilize latitude and longitude values for the X- and Y-coordinates, assigning an arbitrarily high error value here (1000) relative to the error of the depth z-coordinate forces Metashape to accurately orient the model based on the z-plane only, without influence from the x- and y-planes, which exist in untethered space. The two z-coordinate error values (0.1m and 0.3048m) are simply taken from the common accuracy thresholds from metric and imperial (+/- 1ft is equal to +/- 0.3048m) dive computers. Click the blue circular Refresh button again, and now a “[R]” should be displayed next to the chunk. The model is now orthorectified.

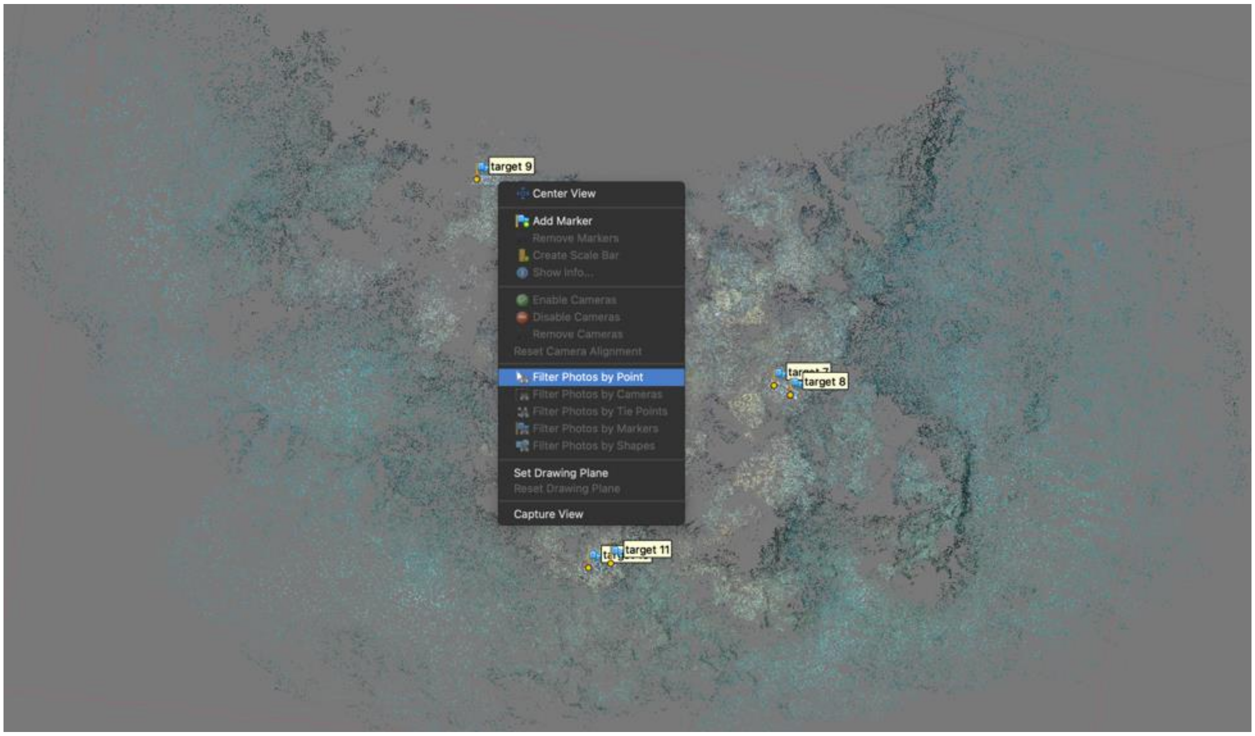


Markers	X (m)	Y (m)	Z (m)	Accuracy (m	Error (m)
☐ target 33					
☐ target 41					
☐ target 45					
☑ target 44	0.000000	0.000000	-7.010400	**1000/0.3048**	
☑ target 40	0.000000	0.000000	-7.010400	**1000/0.3048**	
☑ target 32	0.000000	0.000000	-7.315200	**1000/0.3048**	
☑ target 43	0.000000	0.000000	-6.705600	**1000/0.3048**	
Total Error					
Control points					
Check points					

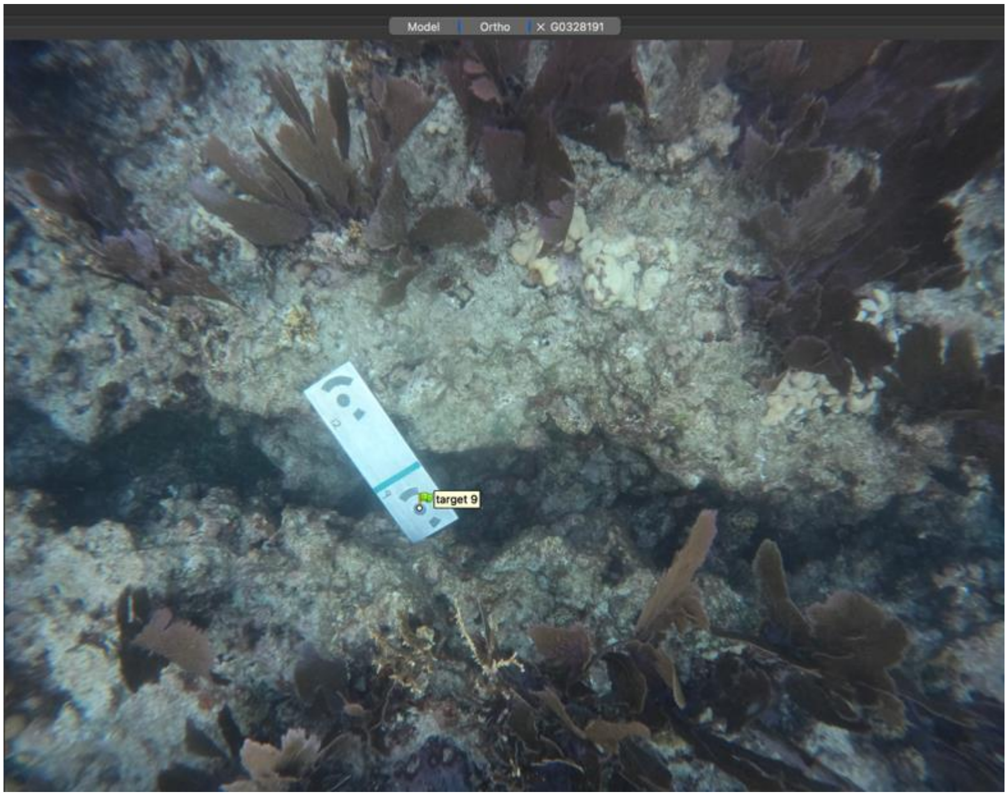
NOTE: In the above example, depths were taken in feet and then converted to meters for use in Agisoft (e.g. 23 feet = 7.0104 meters).

Manually Placing Points in Metashape

Occasionally, Metashape will fail to recognize a coded target (due to lack of clear imagery of the target or due to the size of the target relative to the size of the area). In these instances, it is necessary to manually place markers on the targets. To do this, locate a target in the model’s sparse point cloud. Once a target has been located, right click on this portion of the model and select “Filter Photos by Point”.



The selection of source images in the bottom panel of Metashape will filter to only show photos with the point that was clicked on in the model. Double click on a photo that shows the target clearly to bring the photo up in the main Metashape window. Then, right click on the center of the target and choose "Add Marker".



A new marker will be created, which can then be re-named in the lefthand panel. The marker will also appear in the other filtered photographs that show the target. Select 3-4 other photos and click-and-drag the newly created marker until it is accurately centered on the target in each. Note that markers that have been placed by the user will be green, while markers that have only been placed by Metashape's location estimator will remain blue until moved by the user.

Repeat these steps for any targets or scale bars that Metashape could not initially identify and then proceed to the scaling and orthorectification steps.

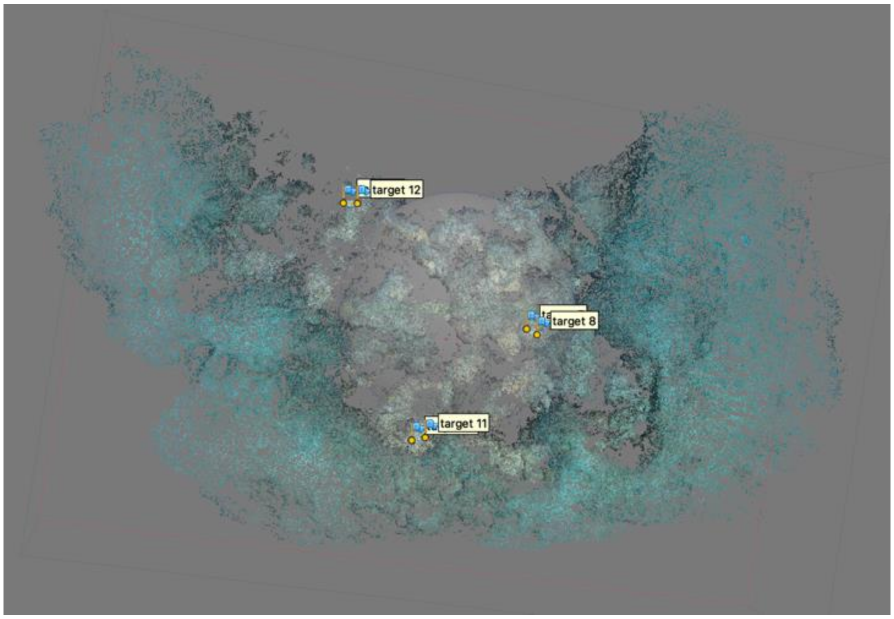
Aligning and Merging Chunks in Metashape

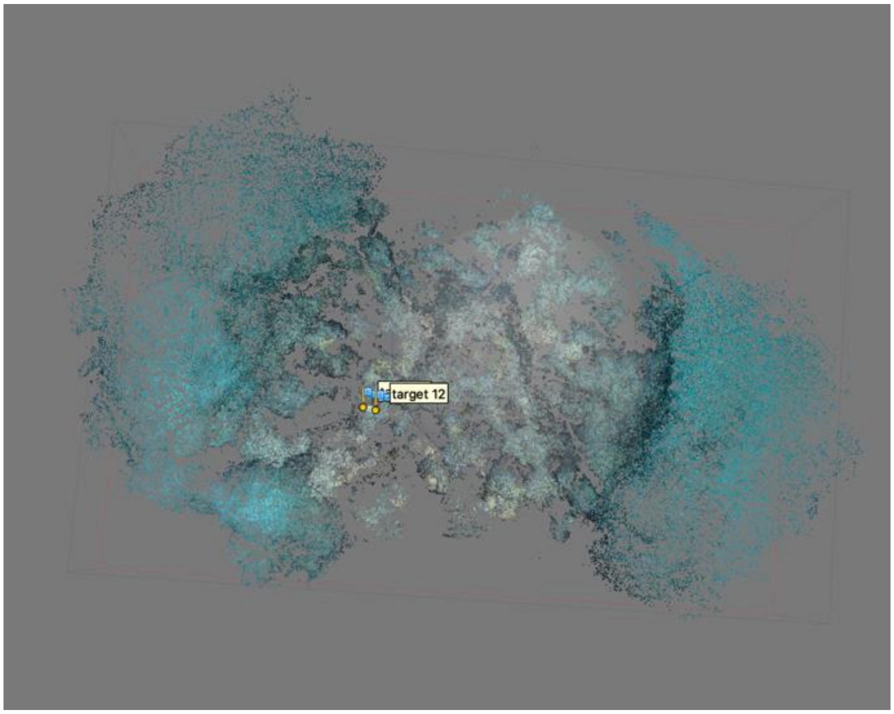
Once a series of chunks have each scaled and orthorectified, they can be aligned with adjacent chunks and then merged to create a larger photomosaic. This step has the potential to be the most time-consuming of the photomosaic process. Thus, any reduction in the number of photos or chunks that are used in the project is desirable.

Chunks should be merged two at a time and in a "cascading" fashion. For a series of chunks 1-8, with the reef section shown in Chunk 1 adjacent to the reef section shown in Chunk 2 and so on, the chunks should be aligned and merged like so:

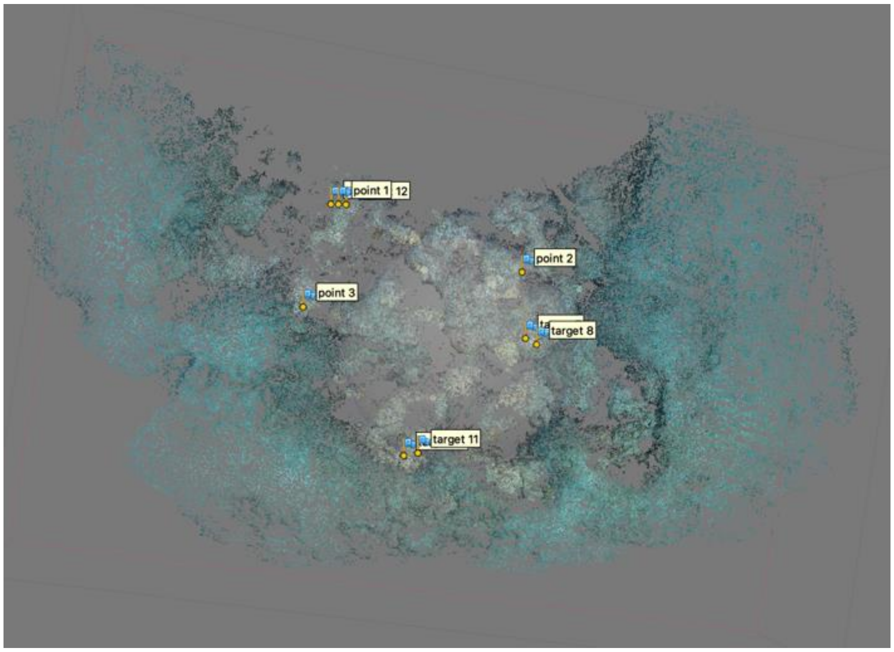
1. Align Chunks 1 and 2; Merge Chunks 1 and 2
2. Align Chunks 3 and 4; Merge Chunks 3 and 4
3. Align Chunks 5 and 6; Merge Chunks 5 and 6
4. Align Chunks 7 and 8; Merge Chunks 7 and 8
5. Align Chunk 1&2 with Chunk 3&4; Merge Chunk 1&2 with Chunk 3&4
6. Align Chunk 5&6 with Chunk 7&8; Merge Chunk 5&6 with Chunk 7&8
7. Align Chunk 1&2&3&4 with Chunk 5&6&7&8; Merge Chunk 1&2&3&4 with Chunk 5&6&7&8

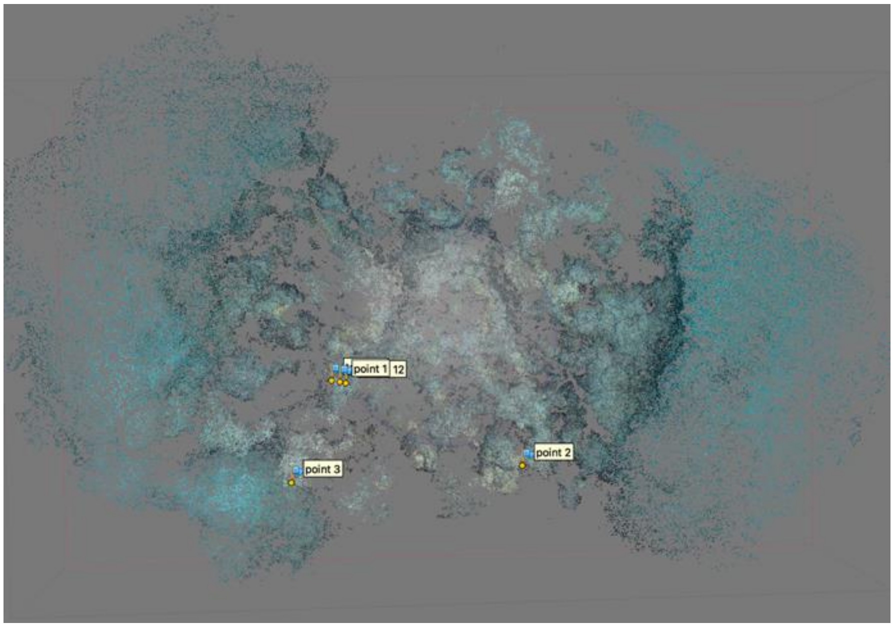
In the example images below, the photos of the two adjacent chunks have each been separately aligned and the resulting point clouds have been scaled. There is clear overlap between the two chunks around the scale bar labeled "Target 12".



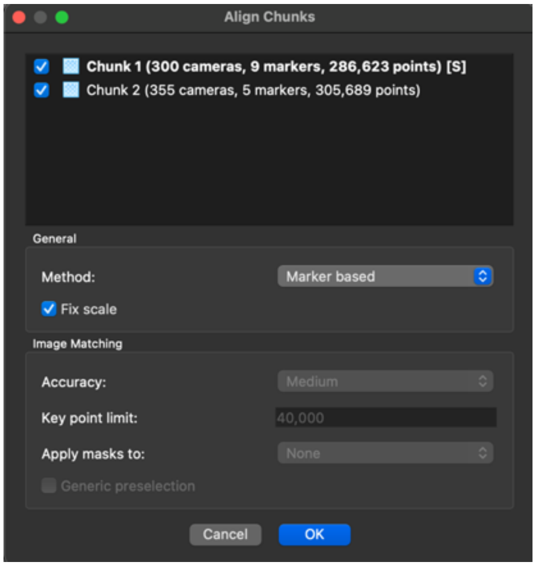


From here, the goal is to manually place 3-4 points in the exact same location on each chunk. In the images below, Points 1-3 have been added to the same parts of both chunks, using well-defined benthic features (a coral colony, a rock, etc.) to accurately place the points. Ensure that the points are labeled exactly the same between the overlapping chunks.





Follow Workflow -> Align Chunks and choose "Target Based" from the drop-down menu. Provided you have scaled your point clouds before this step, the chunks should align such that switching views between the two chunks displays the point clouds in relative orientation to each other. At this point, follow Workflow -> Merge Chunks and choose "Merge Markers" (and any other desired products that have been built to this point).



Align Chunks

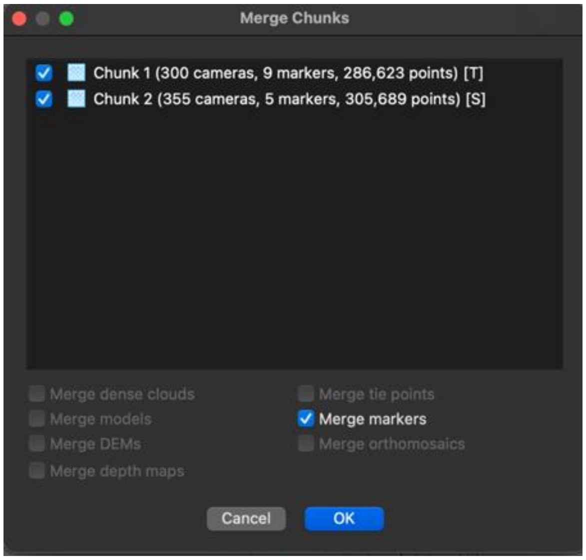
☑ **Chunk 1 (300 cameras, 9 markers, 286,623 points) [S]**
☑ Chunk 2 (355 cameras, 5 markers, 305,689 points)

General

Method: Marker based
☑ Fix scale

Image Matching

Accuracy: Medium
Key point limit: 40,000
Apply masks to: None
☐ Generic preselection

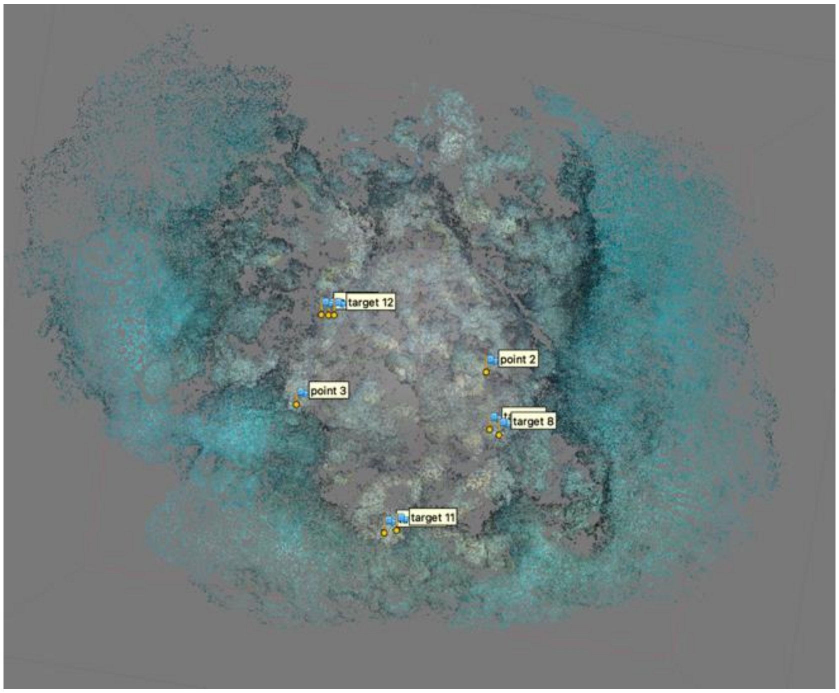


Merge Chunks

☑ Chunk 1 (300 cameras, 9 markers, 286,623 points) [T]
☑ Chunk 2 (355 cameras, 5 markers, 305,689 points) [S]

☐ Merge dense clouds
☐ Merge models
☐ Merge DEMs
☐ Merge depth maps
☐ Merge tie points
☑ Merge markers
☐ Merge orthomosaics

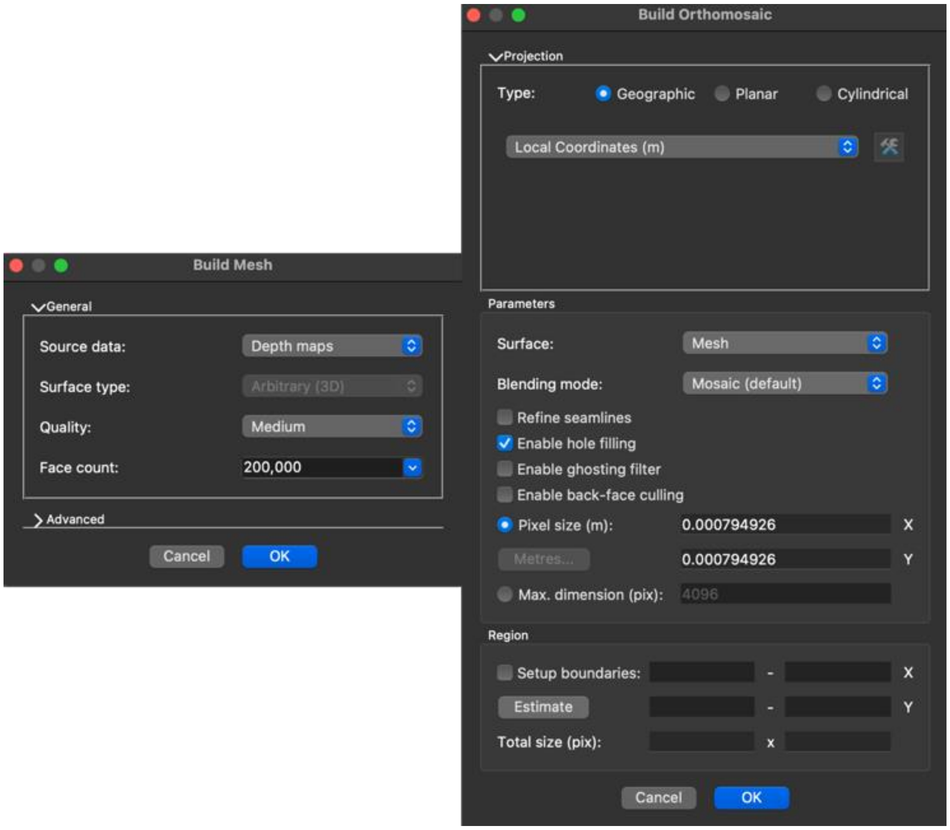
A new, merged chunk will be created. From here, this chunk can be scaled and orthorectified, and additional products such as models, orthomosaics, and DEMs can be built. Alternatively, this new merged chunk can also be used in additional Align/Merge steps if the project has additional chunks to be merged.



Once all chunks have been aligned and merged into a single, final chunk, a model can be built and used to create an exportable photomosaic.

Building Models, Building Orthomosaics, and Exporting Orthomosaics

To build the model (referred to as a "Mesh" in previous versions of Metashape) needed for creation of a photomosaic, follow Workflow -> Build Model (or Batch Process, as described below) and check that Source Data is set to "Depth Maps" and the face count is 200,000. Accept the default options for the remainder of the settings. Click OK.
Once this is completed, follow Workflow -> Build Photomosaic. Select Planar Type, Model Surface, and Mosaic Blending mode. Click OK.



Source data: Depth maps
Surface type: Arbitrary (3D)
Quality: Medium
Face count: 200,000

Type: ● Geographic ○ Planar ○ Cylindrical
Local Coordinates (m)
Surface: Mesh
Blending mode: Mosaic (default)
☐ Refine seamlines
☑ Enable hole filling
☐ Enable ghosting filter
☐ Enable back-face culling
● Pixel size (m): 0.000794926 X
0.000794926 Y
○ Max. dimension (pix): 4096
☐ Setup boundaries: - X
- Y
Total size (pix): x

To export a photomosaic, select the desired chunk. Right click and choose Export -> Export Orthomosaic -> Export JPEG/TIFF/PNG. Choose the file type, location, and any scaling desired (i.e. pixel dimensions set to 1mm x 1mm) for the photomosaic and click OK.

Aligning and Merging Chunks in Photoshop

With sufficient overlap between chunks, the final chunk photomosaics can be aligned and merged easily with Adobe Photoshop CC. For quickest results, this manual recommends exporting individual chunk photomosaics as JPEG files.

1. Open each chunk photomosaic as a "tab" in Photoshop CC.
2. Follow the path File→ Automate→Photomerge
3. Ensure "Layout" is set to "Auto" and click "Add Open Files"
4. Select each chunk photomosaic from the center list and then click "Ok"

With sufficient overlap between the Chunk files, this will generate a full site photomosaic that can be exported from the "File" menu and saved as a JPEG, TIFF, or Photoshop file format. However, this technique is not recommended, as it will not produce a truly ortho-rectified, scaled final photomosaic image and additional Metashape products such as 3-D models, DEMs, and Stitch Reports will be incomplete.

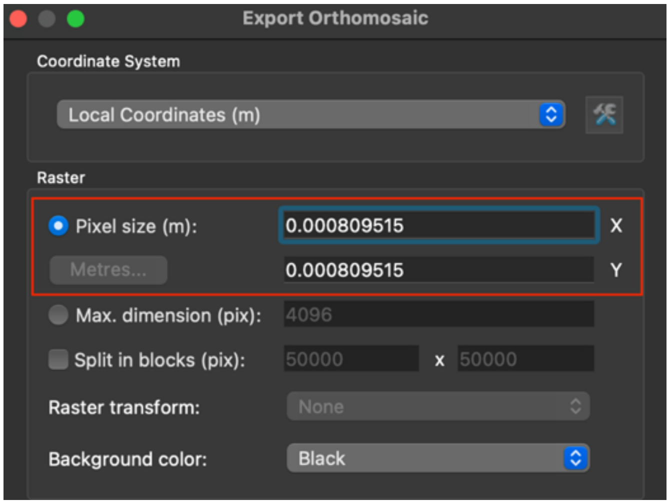
Batch Processing in Agisoft Metashape

Batch processing can automate the stitching process and save considerable time. However, batch processing should still be monitored to ensure that each step runs correctly and that the final products are complete and accurate.

Follow Workflow -> Batch Process. Click Add and define the job and parameters. Jobs can be added, edited, removed, and reorganized with the appropriate buttons. Taking advantage of the option to save the Metashape project file (.psx) after the completion of each job is highly recommended.

Exporting Large Files

Depending on the computer used and size of the orthomosaic being exported from either Metashape, exporting to a JPEG file format may fail. In this case, use the PNG image file format or, if a JPEG image is necessary, down sample the exported file through the Export Photomosaic dialog box by setting the pixel dimensions to larger values (e.g. 0.002m x 0.002m instead of a default value such as 0.00058m x 0.00058m).



Local Coordinates (m)

● Pixel size (m): 0.000809515 X

0.000809515 Y

○ Max. dimension (pix): 4096

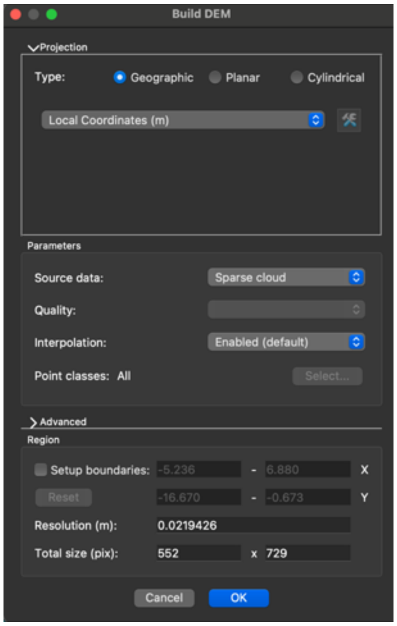
☐ Split in blocks (pix): 50000 x 50000

Raster transform: None

Background color: Black

Optional Steps – DEMs and Textured Models

Once models have been built for a scaled and orthorectified chunk, users may choose to build digital elevation models (DEMs) and model textures. To build a DEM, go to Workflow -> Build DEM and use the settings shown below. Then, the DEM may be viewed and exported from the list of options beneath the Chunk.



Build DEM

Projection

Type: ● Geographic ○ Planar ○ Cylindrical

Local Coordinates (m)

Parameters

Source data: Sparse cloud

Quality:

Interpolation: Enabled (default)

Point classes: All

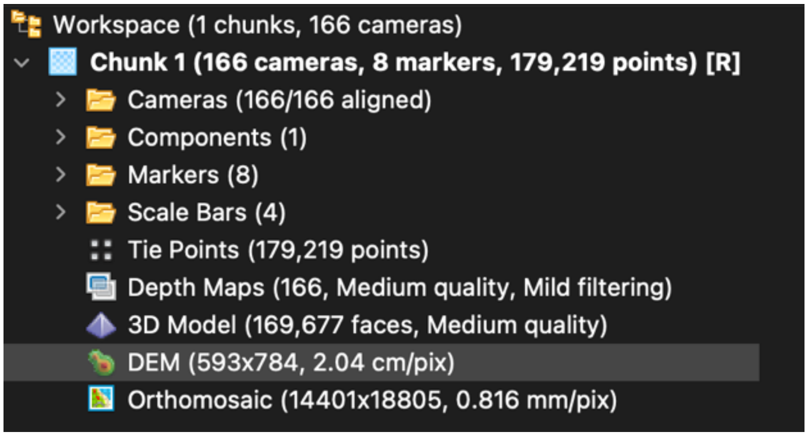
Region

☐ Setup boundaries: -5.236 - 6.880 X

-16.670 - -0.673 Y

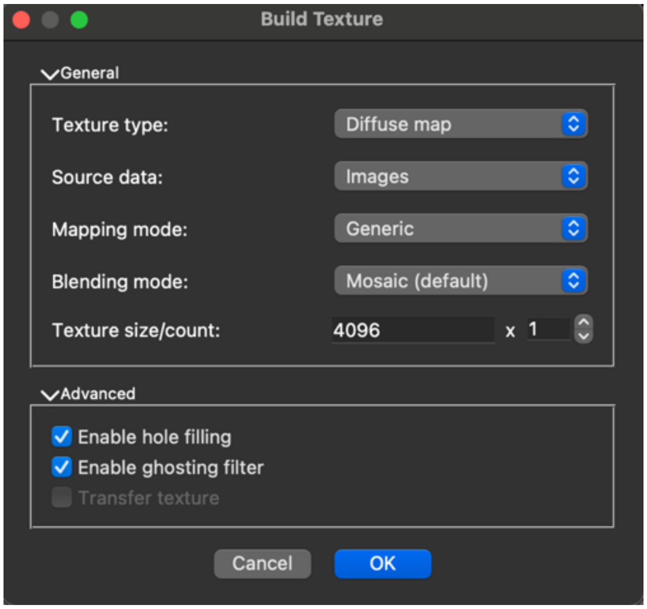
Resolution (m): 0.0219426

Total size (pix): 552 x 729



Workspace (1 chunks, 166 cameras)
- **Chunk 1 (166 cameras, 8 markers, 179,219 points) [R]**
  - Cameras (166/166 aligned)
  - Components (1)
  - Markers (8)
  - Scale Bars (4)
  - Tie Points (179,219 points)
  - Depth Maps (166, Medium quality, Mild filtering)
  - 3D Model (169,677 faces, Medium quality)
  - DEM (593x784, 2.04 cm/pix)
  - Orthomosaic (14401x18805, 0.816 mm/pix)

Building a texture of the model can be very useful for models/mosaics of individual coral colonies (particularly massives) and may also be done for large-scale mosaics, though the computer power and time needed may be prohibitive. Follow Workflow -> Build Texture and choose the settings shown below. The textured version of the model can then be seen in the model by choosing "Model Textured" from the Model icon's drop-down menu.



Build Texture

General

Texture type: Diffuse map

Source data: Images

Mapping mode: Generic

Blending mode: Mosaic (default)

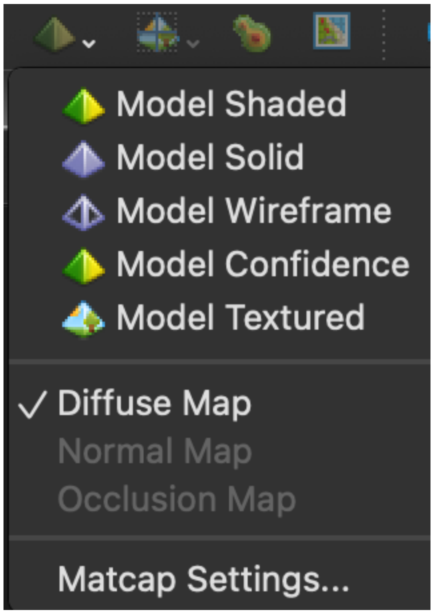
Texture size/count: 4096 x 1

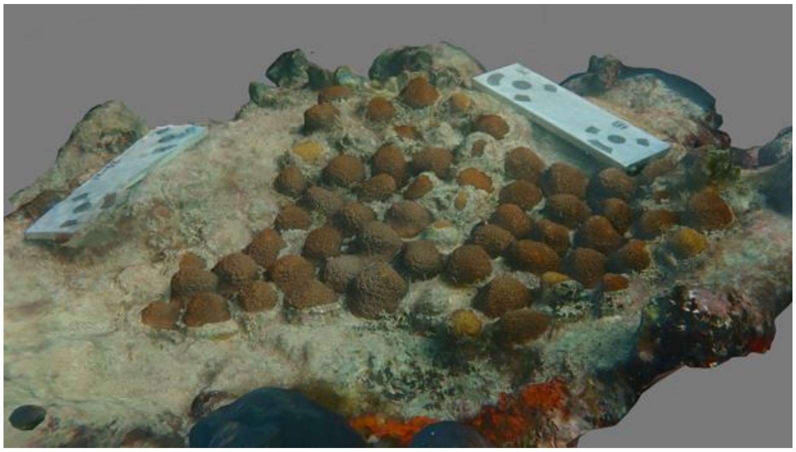
Advanced

☑ Enable hole filling

☑ Enable ghosting filter

☐ Transfer texture



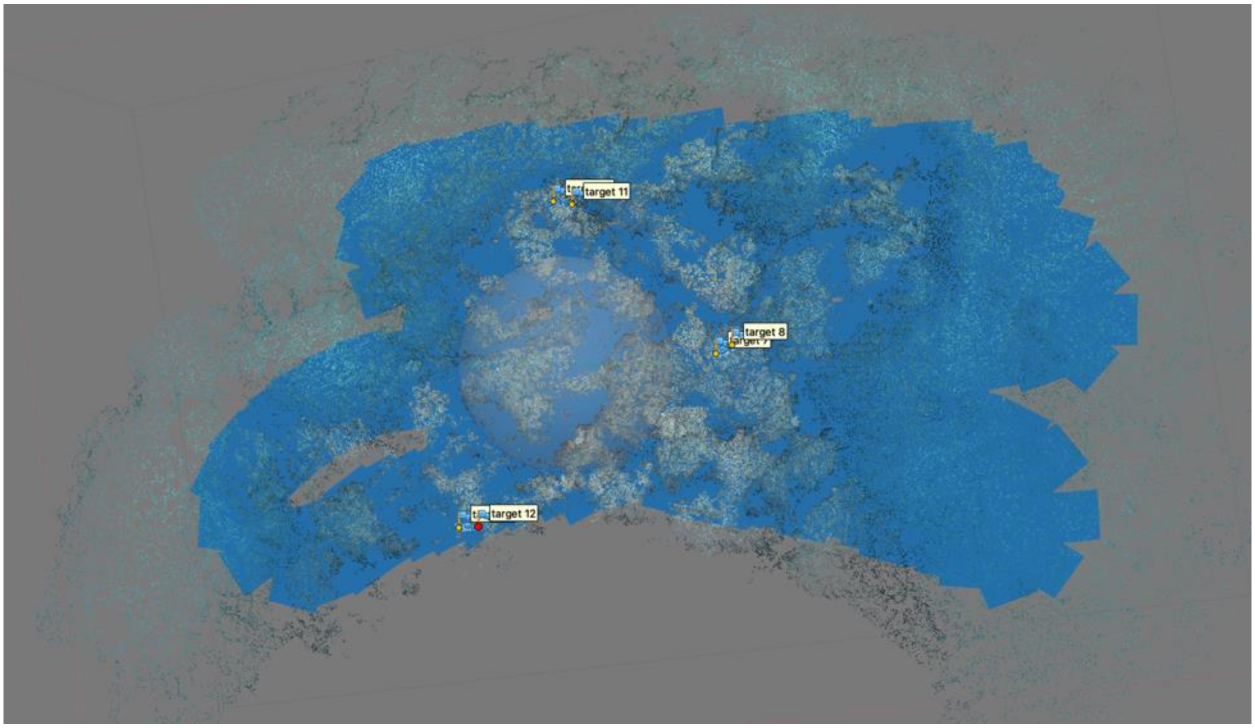


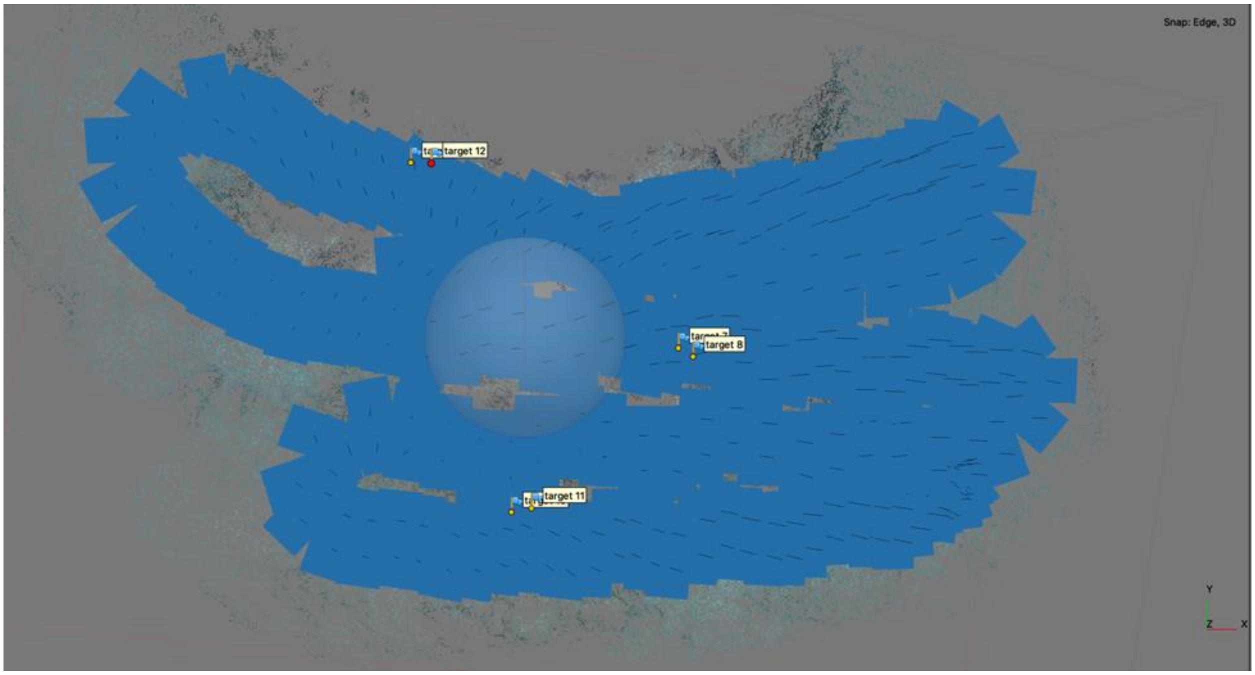
Tips for Orthorectification and Scaling

This manual recommends avoiding the use of the same target multiple times in a mosaic. Using unique targets for each scale bar and/or depth measurement will avoid confusion (both on the part of the user and on the part of Metashape) about which targets constitute which scale bars and at what depth or location they exist in a mosaic. The creation of accurate scale bars may fail if multiple instances of a target exist in a mosaic area, as Metashape will not know which instance of a target to use in creating a specific scale bar and assigning the known scale.

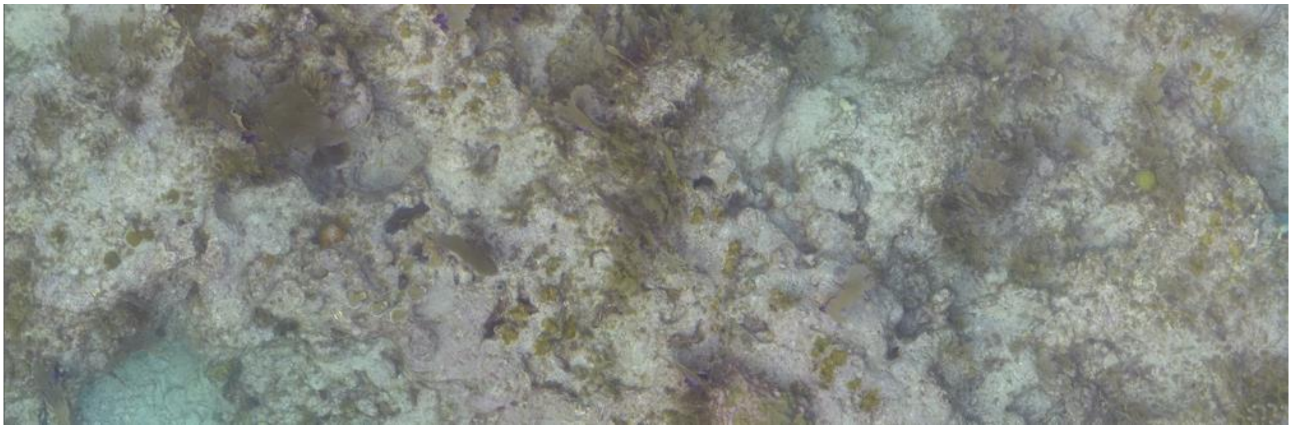
During the orthorectification of a model, Metashape will occasionally invert the orientation, so that the top-down view becomes defined as the "bottom-up" view. Thus, building an orthomosaic from this projection will result in an inverted image. To fix this, one or more defined targets with an assigned depth value (Z-coordinate) must be manually placed on the model and then given the known depth value. To do this, follow XXX to reset the default orientation of the model. Then, follow the steps described above for manual creation and placement of targets throughout the model. Note that these new targets will have arbitrary X- and Y-coordinate values assigned to them upon their creation. Assign the Z-coordinate values and errors as normal. Click the circular "refresh" icon above the targets panel and repeat this process until the model is properly orthorectified with the correct top-down view.

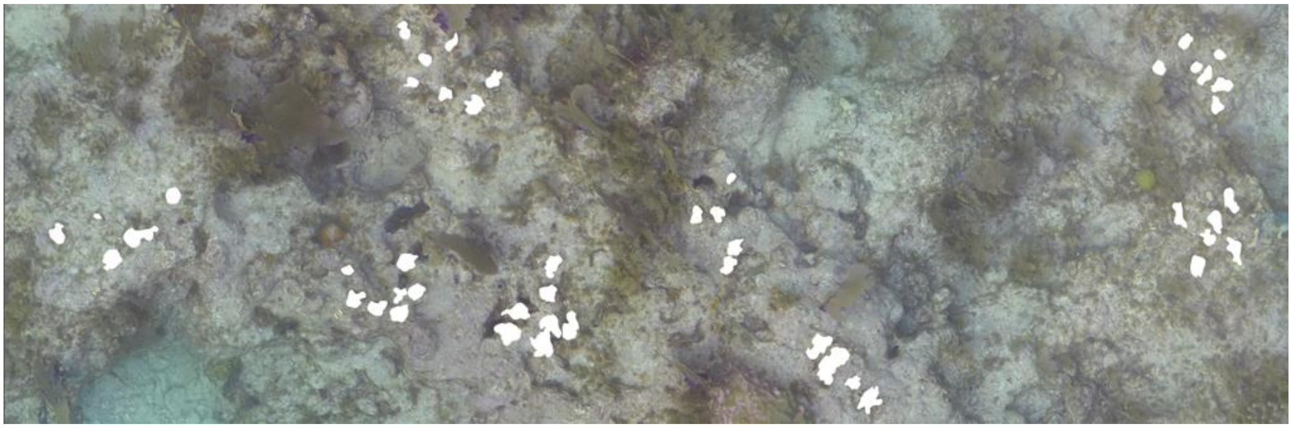
The quickest and easiest way to determine in a model or sparse point cloud is inverted is to turn on the camera locations by clicking the camera icon in the toolbar at the top of the main panel. If the model is inverted, the points of the point cloud will appear *in front of* the blue camera location markers (top photo, below). If the model is correctly oriented, the point cloud will appear *behind* the camera location markers (bottom photo, below).


target 11
target 8
target 7
target 12


Snap: Edge, 3D
target 12
target 7
target 8
target 11
Y
Z
X

Part VI – Final Photomosaic Analysis



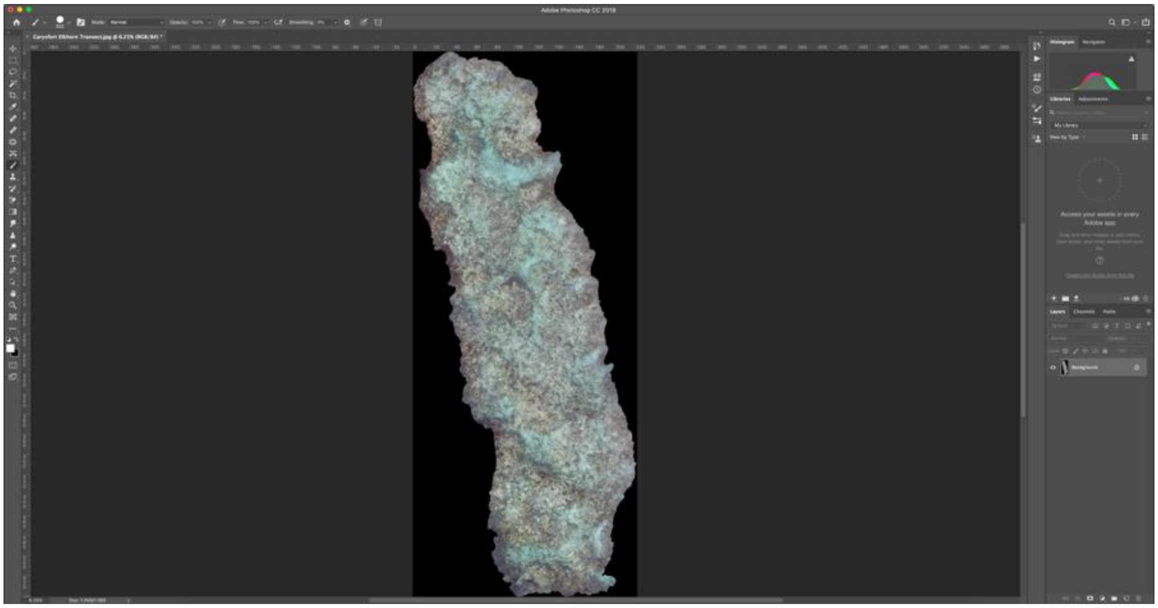


Goals

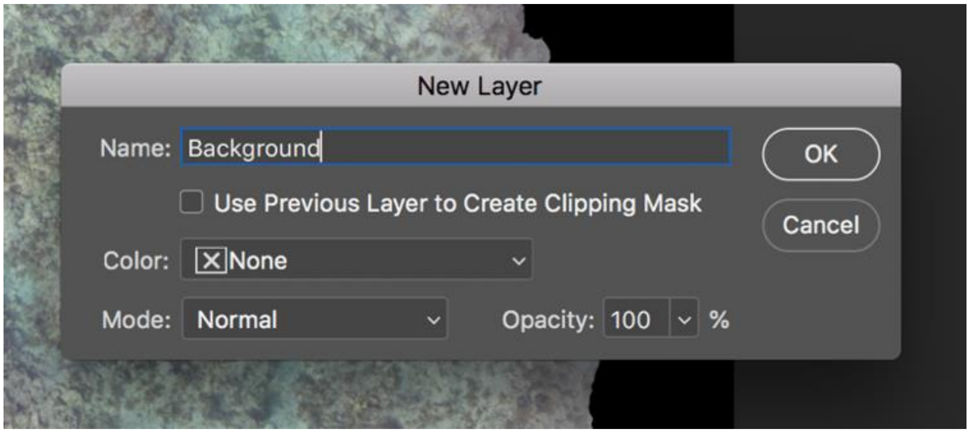
For CRF's purposes, basic ecological data is extracted from photomosaic stitches of a reef site. Images are layered in Adobe Photoshop CC by outlining all individual coral colonies and filling in each outlined area. These layered .psb files are then exported from Photoshop and analyzed in FIJI (formerly ImageJ) to extract a variety of metrics such as coral counts, size frequencies, and area cover. NOTE: the following steps reflect CRF's outplanting techniques and monitoring requirements; other methods or analyses may require additional or revised steps.

Photoshop Setup

1. Open the exported orthomosaic .jpg file in Adobe Photoshop CC. Right click the .jpg > open with > Adobe Photoshop CC.



2. Create a background fill layer. Go to Layer > New Fill Layer > Solid Color. Name it "Background." Click OK. Set the background to BLACK or #000000. Click OK.



New Layer

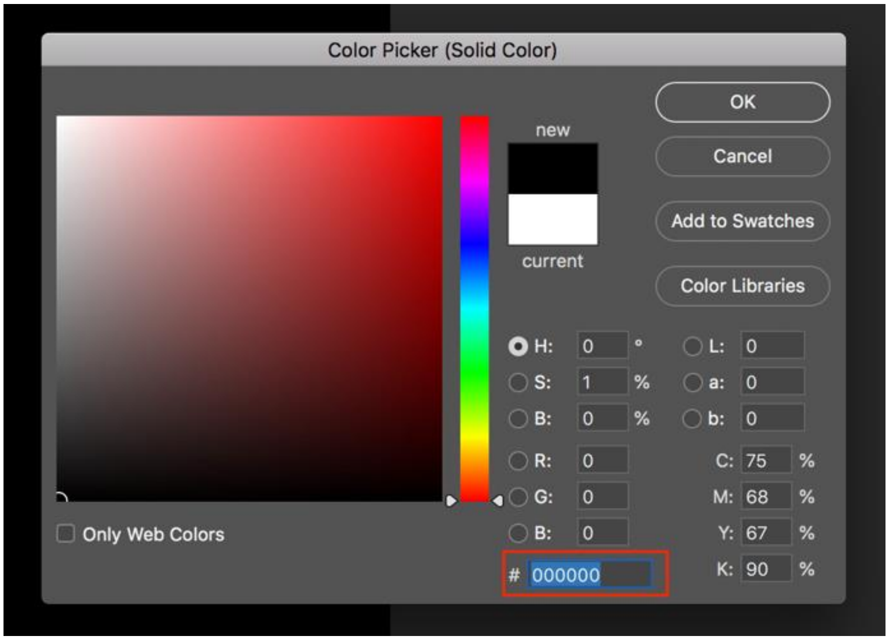
Name: Background

☐ Use Previous Layer to Create Clipping Mask

Color: ☒ None

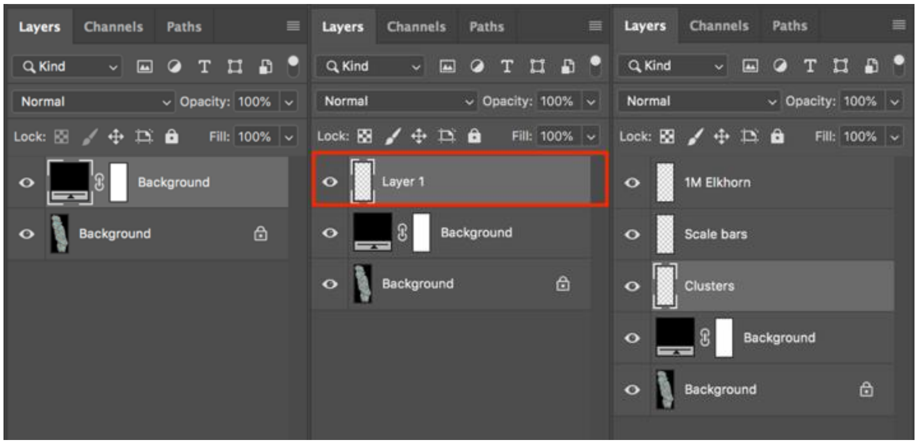
Mode: Normal

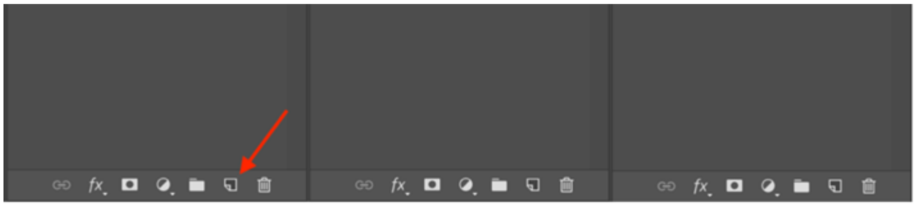
Opacity: 100 %



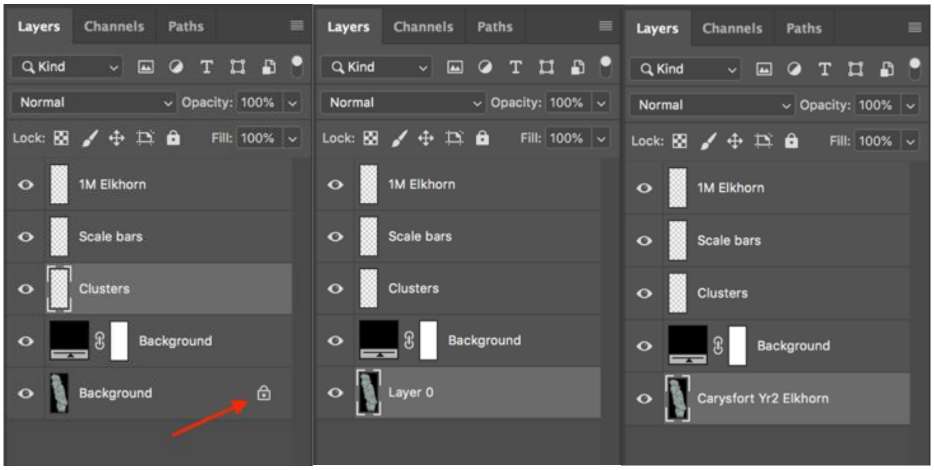
H: 0 °
S: 1 %
B: 0 %
R: 0
G: 0
B: 0
L: 0
a: 0
b: 0
C: 75 %
M: 68 %
Y: 67 %
K: 90 %
000000

a. Create new layers. In the lower right-hand corner of the Layers Panel, click the "Create a new layer" icon. (If the Layers Panel is absent, go to Windows > Layers.) A new layer, Layer 1, should appear in the Layers Panel. Create the following layers :
   a. "Timepoint CoralSpecies" This layer is for highlighting all the corals of a single coral species. Create an individual layer for each species of coral in the mosaic so that the species can be analyzed independently. Name the layer based on the monitoring timepoint (1M, 6M, 1Y, etc.) and the coral species being monitored (Staghorn, Elkhorn, etc.)
   b. "Scale bars" This layer is for tracing and highlighting the scale bars.
   c. "Clusters" This layer is for marking each outplanted "cluster" along the transect or within the mosaic area.

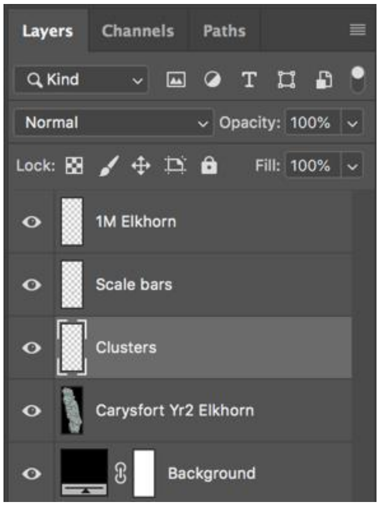




3. Unlock the orthomosaic layer. Click the lock. It will now become "Layer 0"; rename it based on the name of the area being monitored.



4. Rearrange the layers so that the Background layer is at the bottom. Click a layer and drag it to reorder the layers into the following order:

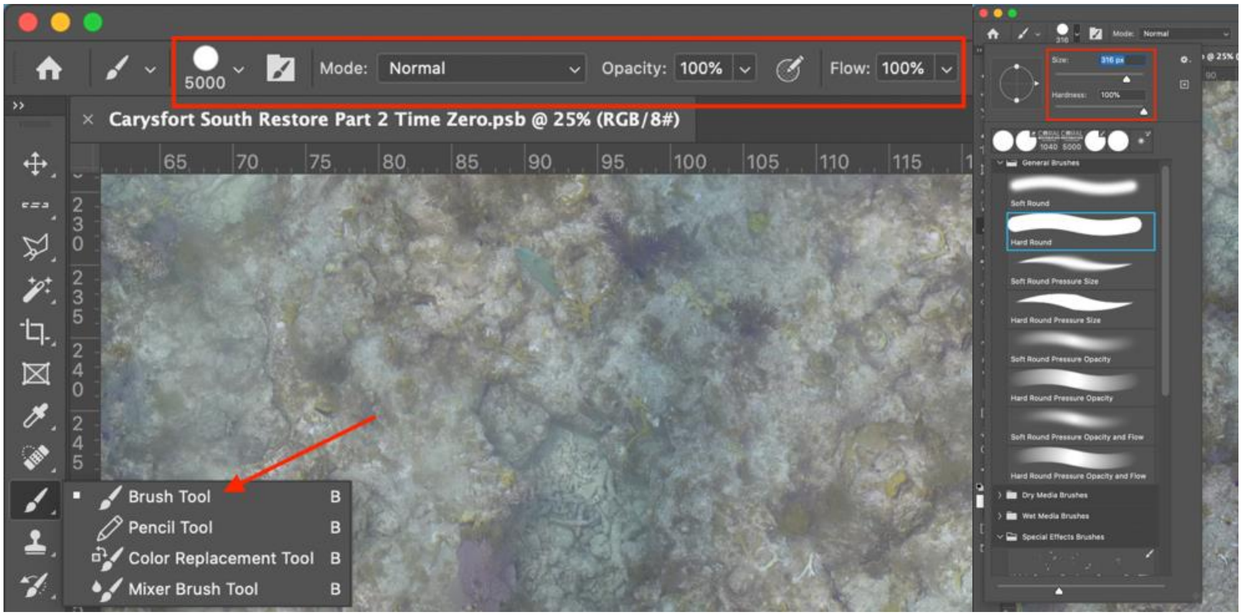


5. Zoom in (lower left corner) to get a better working view of the individual coral colonies in the photomosaic for labeling the clusters.

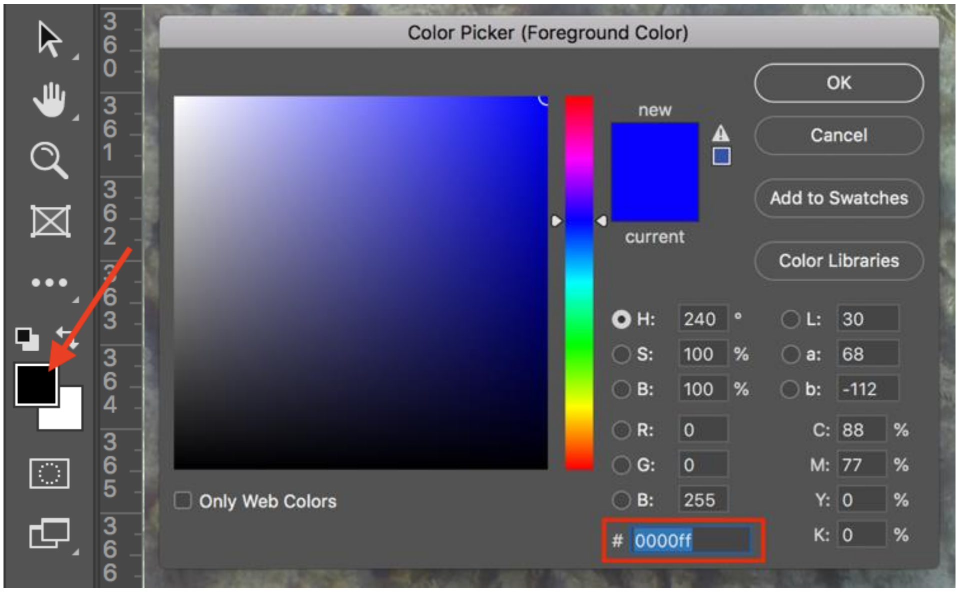

6.25%
348.556 in x 473.194 in (72 ppi)

6. Select the Clusters Layer in the Layers Panel.

7. Select the Brush Tool (B) from the Tools Panel. In the Brush panel (located at the top), make sure the opacity and flow are set 100%. Also make sure the brush is a decent size (~150 – 300 pixels, depending on the resolution of your photomosaic) and the hardness is set to the maximum. (Size and hardness are found in the first dropdown option in the Brush panel, boxed below).

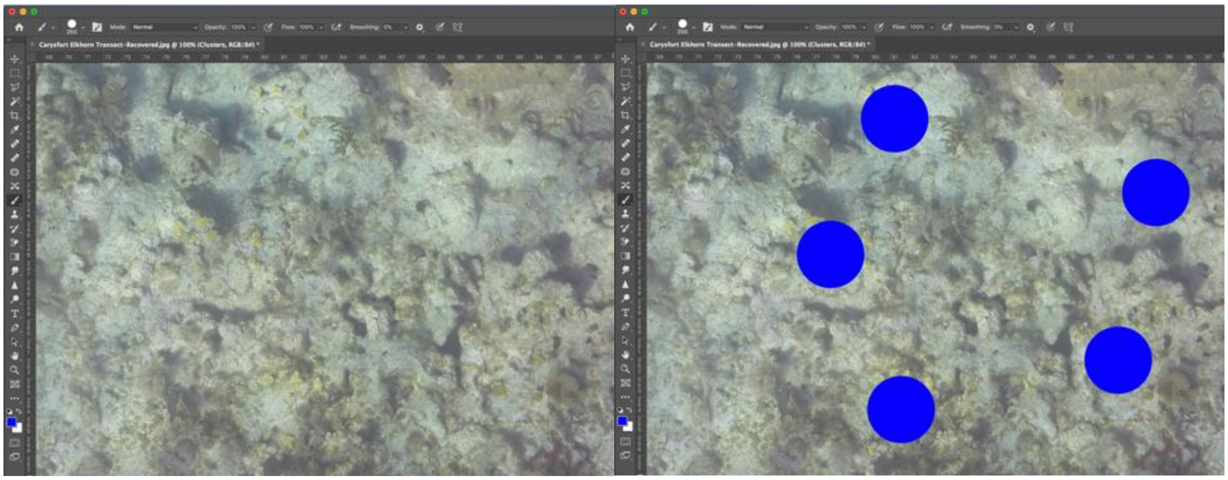

5000
Mode: Normal
Opacity: 100%
Flow: 100%
Carysfort South Restore Part 2 Time Zero.psb @ 25% (RGB/8#)
Size: 316 px
Hardness: 100%

8. Set the color using the Color Picker (located at the bottom of the left-hand Tools Panel) to BLUE or #0000ff.

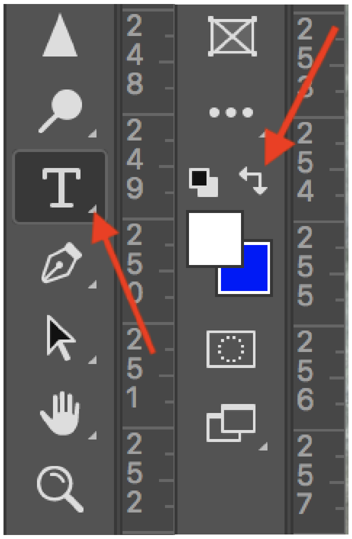


● H: 240 °
○ S: 100 %
○ B: 100 %
○ R: 0
○ G: 0
○ B: 255
0000ff
○ L: 30
○ a: 68
○ b: -112
C: 88 %
M: 77 %
Y: 0 %
K: 0 %

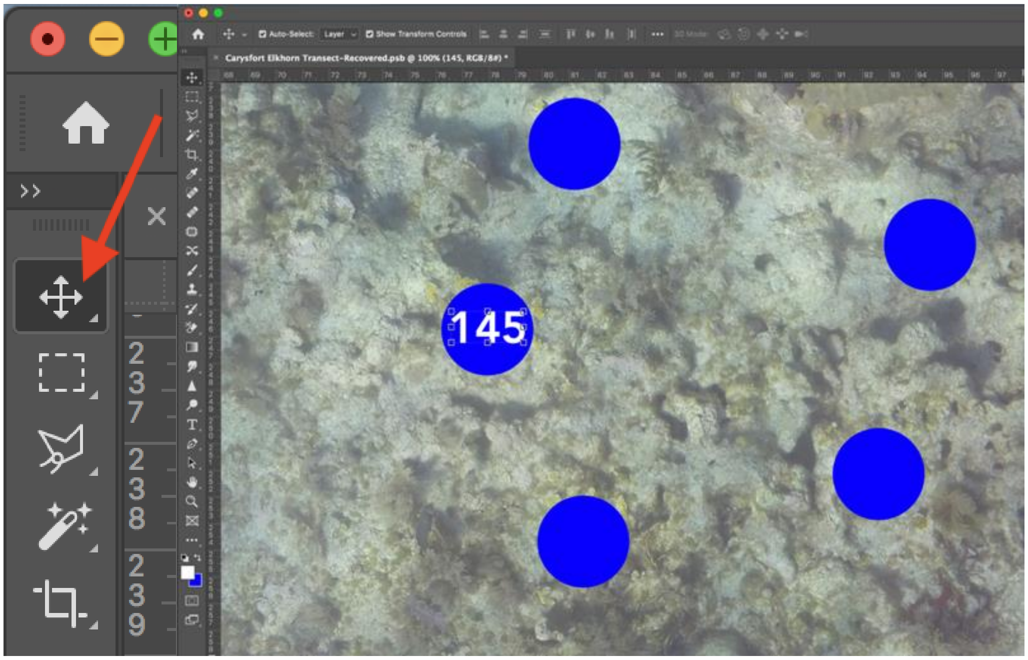
9. Paint one dot at the center of each cluster of corals. This will allow you to quickly locate and find the corals within the mosaic as you proceed through the tracing and highlighting steps below.



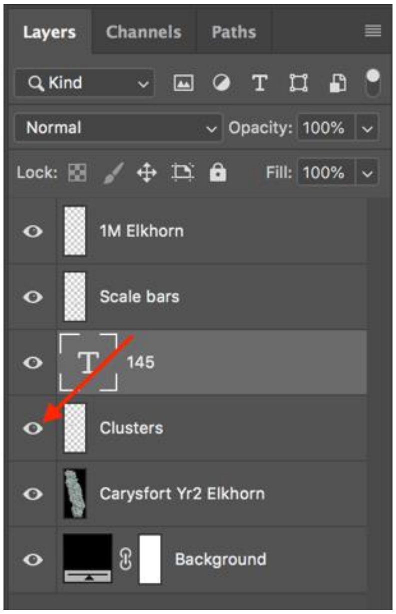
10. Select the Horizontal Type Tool in the left-hand Tools Panel. This tool is used to add text and will be used to mark the cowtag numbers for each cluster. Make sure to swap colors to white, using the Color Swatches (located at the bottom of the left-hand Tools Panel) so that the text can be seen over the blue dots.



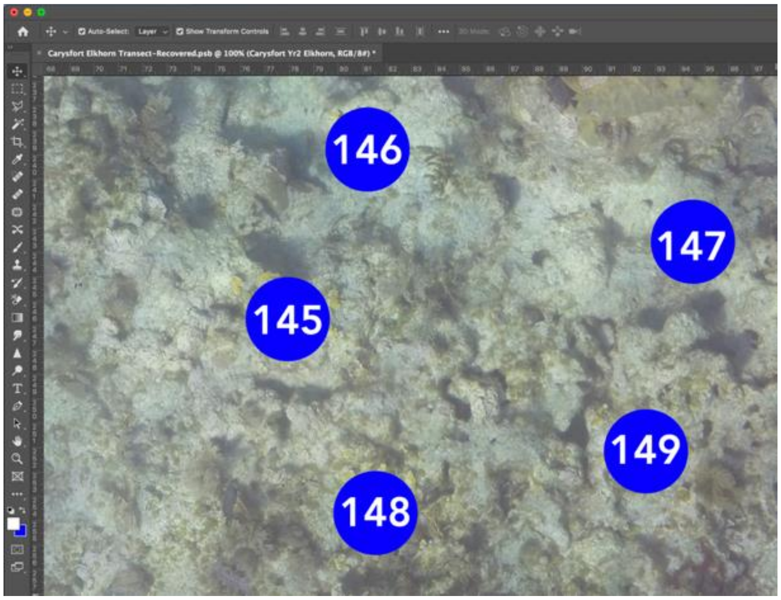
11. Add text. Click anywhere on the image to add a new text object and type in the correct cowtag number, genotypic information, or other identifier for a cluster of corals. Note: The text object will be added as a new Layer above the selected Layer in the Layer Panel, so be sure to have the Clusters Layer selected.
12. Select the Move Tool (V) in the left-hand Tools Panel. Hovering over a text object, click and drag to move. Place the text object to mark the correct cluster.



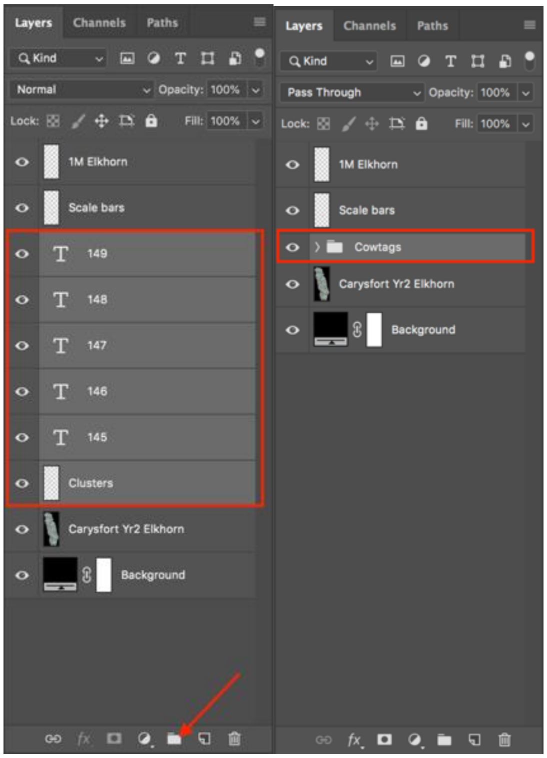
13. Hide/Unhide Clusters Layer as needed. In the Layers Panel, click the eye icon next to a Layer to make that layer invisible/visible again. This will help view the cluster tags, genetic tags, or other identifying markers.



14. Repeat until every cluster has been labeled appropriately. Note that tags with text or colors do not always render properly due to poor in-water conditions or stitching abberations. It is helpful to have in-water maps or notes to corroborate any coral identification information in the mosaic.

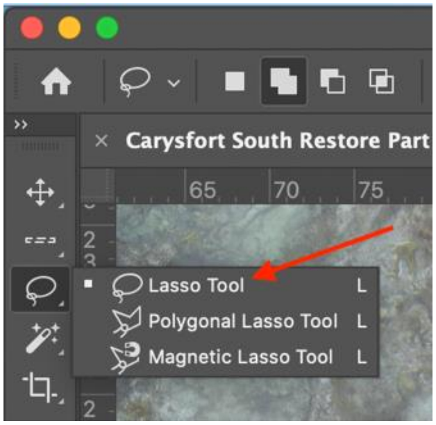


15. Group the label layers. In the Layers Panel, select all of the text layers and the Clusters Layer; click the topmost text layer and, while holding Shift, click the Clusters Layer (should be the bottom-most layer in the selection). Click the “Create a new group” icon and rename this group Cowtags/Genetic Markers/etc.. This will organize and declutter the Layers Panel, making it easier to work throughout the following steps.



Outlining and Shading

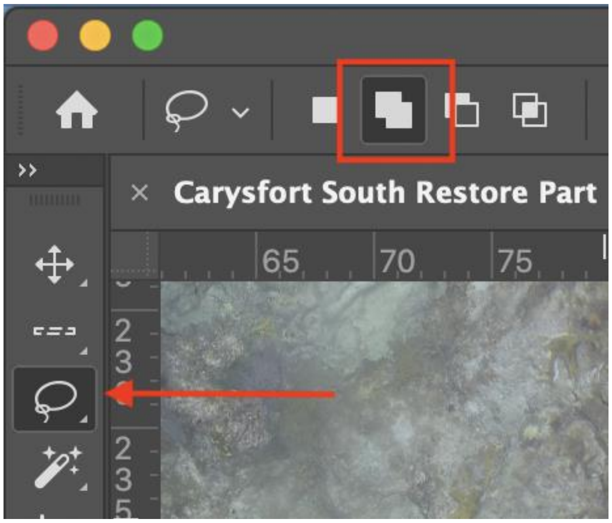
1. Select the Polygonal Lasso Tool (L) in the left-hand Tools Panel. Lasso Tools will be used throughout segmentation (tracing). Right click the icon to see the different options:
    a. Polygonal Lasso Tool: This tool is used to outline and select polygons (i.e. - the scale bars).
    b. Lasso Tool: This tool is used to outline and select freeform shapes (i.e. - the coral colonies).



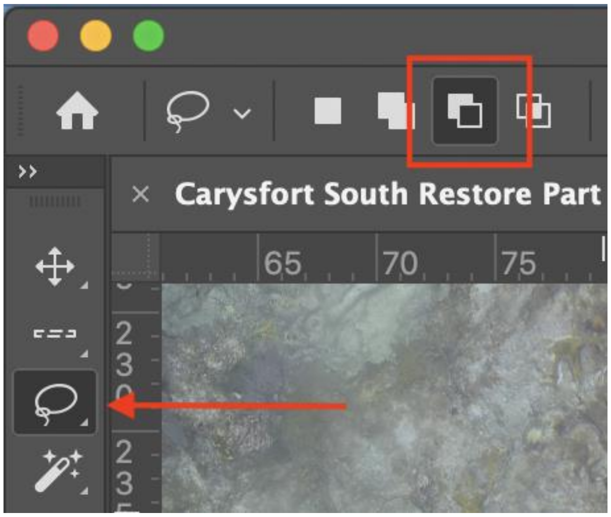
2. Outline the scale bars. The Polygonal Lasso Tool works by clicking to create corners while outlining an object. When finished outlining the object, double click to finish off the selection. A flashing

border will appear, surrounding your selection. There are two settings in the Lasso panel (located at the top) that are of note:

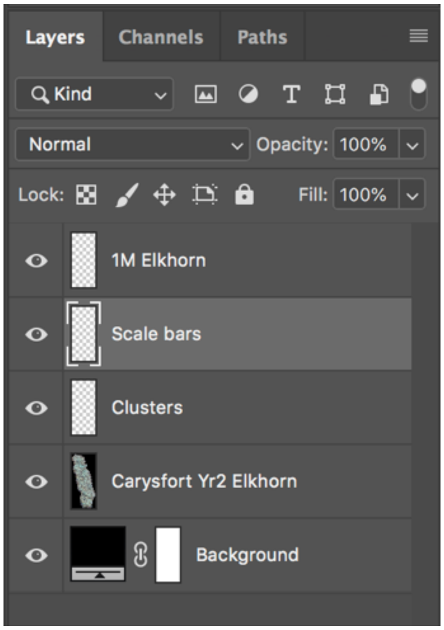
a. Add to selection: Use this tool to start a selection and continue adding to it.



b. Subtract from selection: Use this tool to remove areas from the current selection.



3. Select the Scale bars layer in the Layers Panel.



Normal
Opacity: 100%
Fill: 100%

4. Select the Brush Tool from the Tools Panel. In the Brush panel (located at the top), make sure the opacity and flow are set 100%. Also make sure the brush is large and the hardness is maximized.

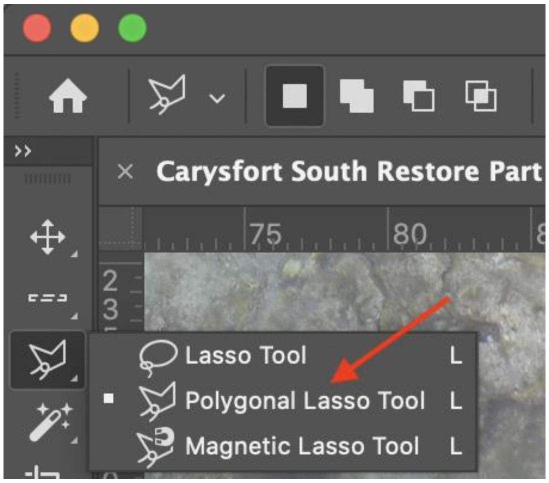
5. Set the color using the Color Picker (located at the bottom of the left-hand Tools Panel) to RED or #ff0000.

6. Paint over the entire scale bar selection. The selection ensures that only parts of the image bound by the flashing selection border are painted. Remember to paint all selected objects for the layer (i.e. all scale bars in the photomosaic).

7. Deselect the selection. Go to Select > Deselect.

8. Select the coral layer.

9. Select the Lasso Tool. Right click the Polygonal Lasso Tool in the left-hand Tools Panel to change Lasso Tools.

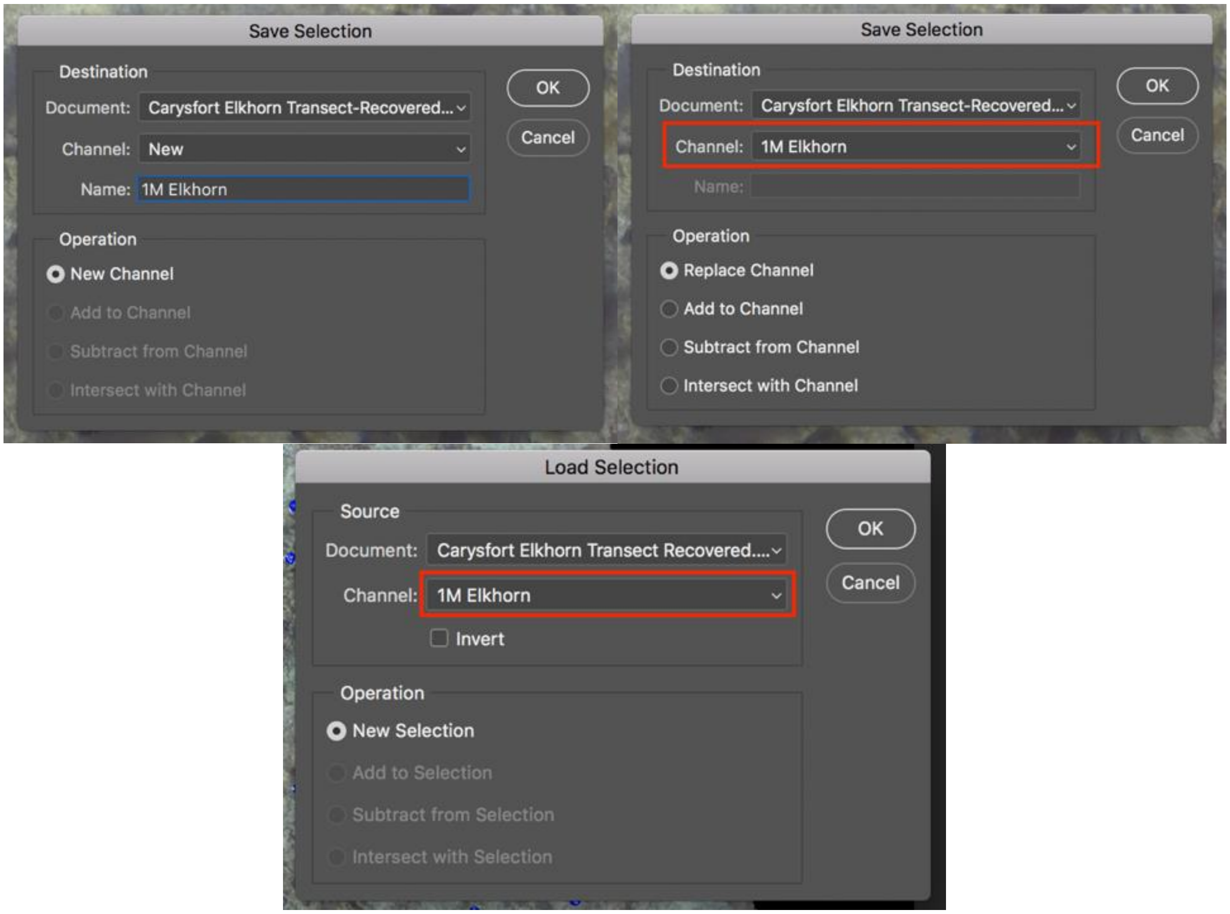


10. Select the “add to selection” option for the Lasso Tool.

11. Outline a live coral colony using the Lasso Tool. Recall the two settings in the Lasso panel (located at the top) that are of note – add to selection and subtract from selection – and use when appropriate. For this step, the use of a drawing tablet such as the Wacom Intuos Pro may be beneficial.

12. Repeat until every coral from a single species within the photomosaic is selected.

13. Save the Selection periodically to allow for re-selection (correction) and in case of a computer crash.
    a. Saving a new selection. Go to Select > Save Selection… and name the selection based on the species selected (below left). Do this the first time the selection is saved.
    b. Saving over a selection. Go to Select > Save Selection… When the Save Selection pop-up box appears, select the name of the previous selection from the Channel drop-down menu (below middle). Do this as more corals are added to the files selection and are saved.
    c. Loading a selection. Go to Select > Load Selection… When the Load Selection pop-up box appears, select the name of the previous selection from the Channel drop-down menu (below right). Do this when returning to the coral tracing and selection process after deselecting the selection or after closing out of Photoshop.



Save Selection

Destination
Document: Carysfort Elkhorn Transect-Recovered...
Channel: New
Name: 1M Elkhorn

Operation
● New Channel
○ Add to Channel
○ Subtract from Channel
○ Intersect with Channel

Save Selection

Destination
Document: Carysfort Elkhorn Transect-Recovered...
Channel: 1M Elkhorn
Name:

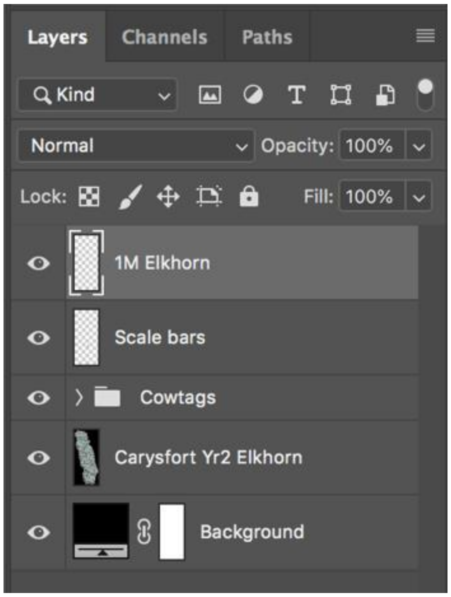
Operation
● Replace Channel
○ Add to Channel
○ Subtract from Channel
○ Intersect with Channel

Load Selection

Source
Document: Carysfort Elkhorn Transect Recovered....
Channel: 1M Elkhorn
☐ Invert

Operation
● New Selection
○ Add to Selection
○ Subtract from Selection
○ Intersect with Selection

14. After selecting all corals of a species in the photomosaic, click the coral Layer in the Layers Panel for the species of coral selected.



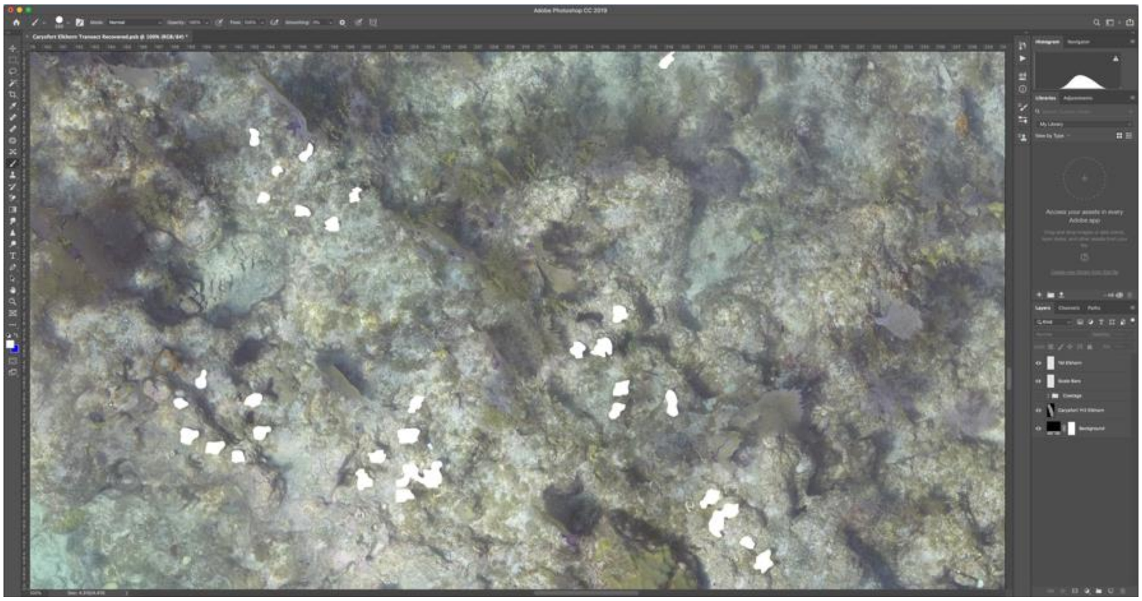
Normal
Opacity: 100%
Lock:
Fill: 100%

1M Elkhorn
Scale bars
Cowtags
Carysfort Yr2 Elkhorn
Background

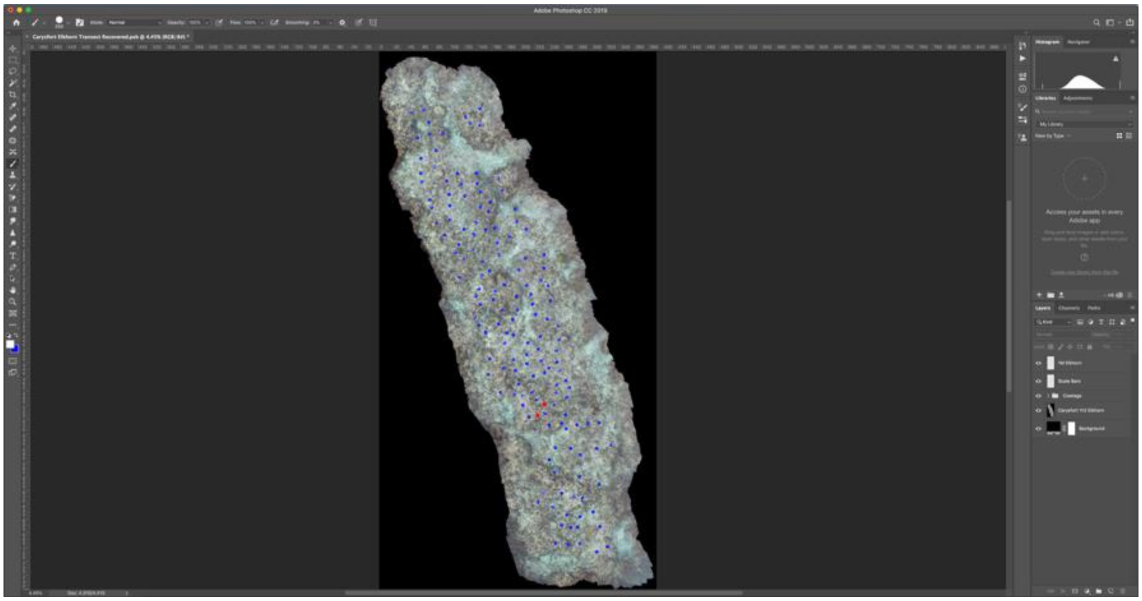
15. Select the Brush Tool from the Tools Panel. In the Brush panel (located at the top of the window), make sure the opacity and flow are set 100%. Also make sure the brush is large and the hardness is maximized.

16. Set the color using the Color Picker (located at the bottom of the left-hand Tools Panel) to WHITE or #ffffff.

17. Paint over the entire selection. Some tips:
    a. Paint as you go – Paint after every few clusters or after every cluster to ensure that you do not skip any corals.
    b. Make the brush size as big as possible – This will help cover more pixels with less effort.
    c. Go over everything a few times – This will ensure that no pixels representing live corals get left out of the highlighting.



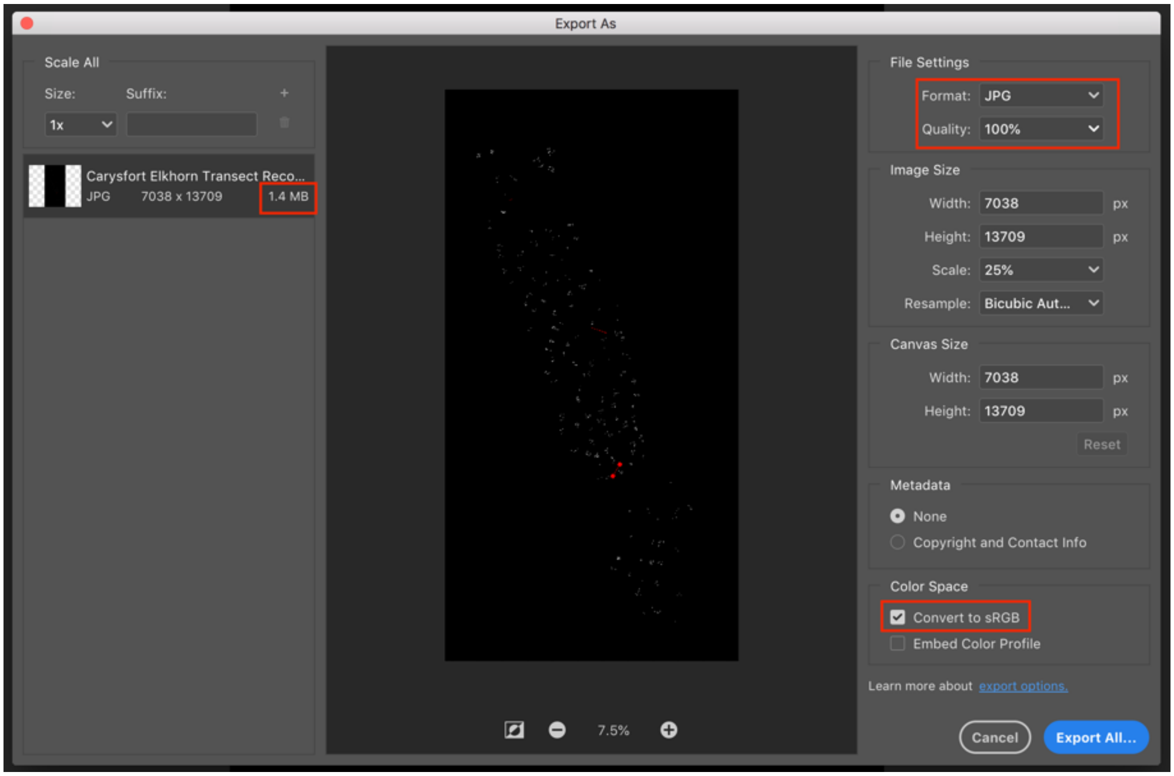
18. Save your final product! Go to File > Save As.



Exporting the Image

1. Hide the Cowtags Group and the orthomosaic/reef imagery Layer by clicking the eye icons in the Layers Panel.

2. Export the file. Go to File > Export > Export As… Export the file as a JPG. Some things to check before clicking Export All:
    a. Make sure the Quality is at 100%.
    b. Make sure the “Convert to sRGB” box is checked.
    c. Make sure the file size is not too large. It shouldn’t be above 5 MB.

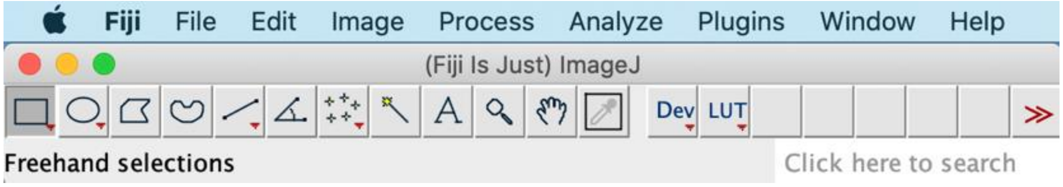


Size: 1x
JPG 7038 x 13709 1.4 MB
Format: JPG
Quality: 100%
Width: 7038 px
Height: 13709 px
Scale: 25%
Resample: Bicubic Aut...
Width: 7038 px
Height: 13709 px
● None
○ Copyright and Contact Info
☑ Convert to sRGB
☐ Embed Color Profile

3. Click Export All… and save the image to the desired location.
4. Now, hide the scale bars Layer by clicking the eye icon in the Layers Panel.
5. Export the file as before.

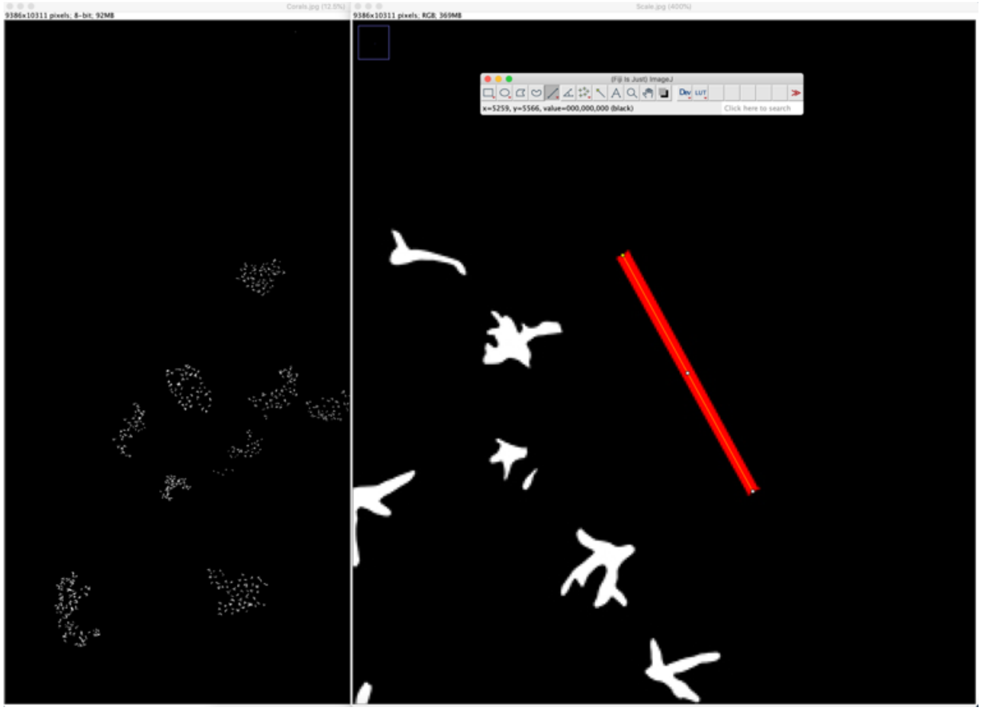
FIJI Setup

1. Open FIJI (previously, ImageJ). The FIJI Toolbar will appear.



2. Open both exported images in FIJI. Go to File > Open. Navigate to the .jpg images you exported from Photoshop. Alternatively, navigate to the .jpg images in a Finder window and then click and drag the images to the FIJI window. A new window should pop up for each image.

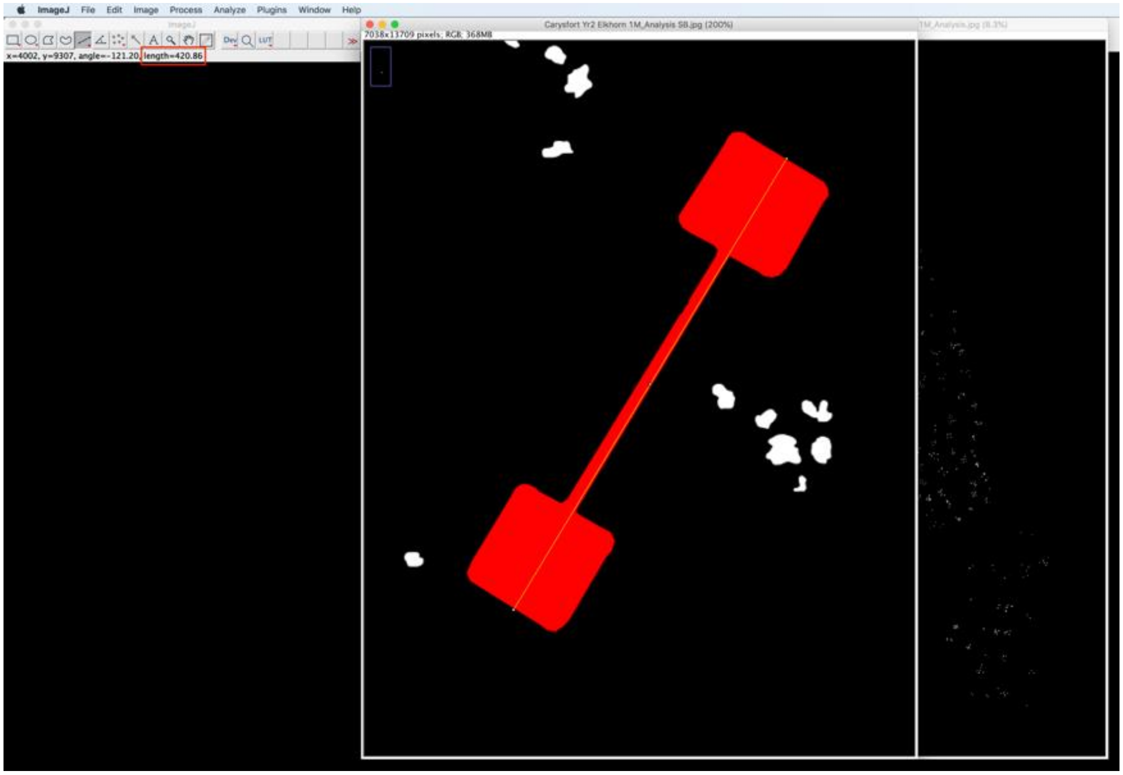
3. Select the FIJI window with the .jpg that has the scale bars.
4. Zoom in to the scale bar. Hover over the scale bar with the mouse cursor and press the "+" key to zoom in, readjusting after each keystroke.



5. Select the Straight tool from the FIJI Toolbar.

Straight, segmented or freehand lines, or arrows (right click to switch) Click here to search

6. Measure the length of the scale bar. Click on one end of the scale bar and drag the yellow line to the other end. Keep it parallel with the length of the scale bar.



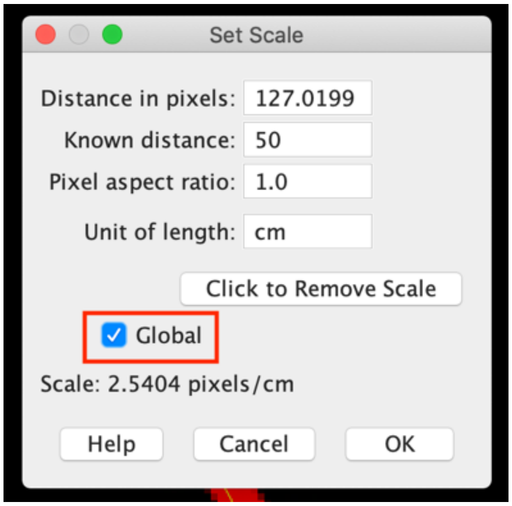
x=4002, y=9307, angle=-121.20, length=420.86

Carysfort Yr2 Elkhorn 1M_Analysis SB.jpg (200%)

7038x13709 pixels; RGB; 368MB

1M_Analysis.jpg (8.3%)

7. Set the scale. Go to Analyze > Set Scale… The "Distance in pixels" field should be filled out automatically from the last step. Enter the "known distance" and "unit of length" fields based on the scale bar. (The scale bar shown here is 50 cm.) NOTE: Make sure the "Global" box is checked. This should set the scale for any image you have opened in FIJI. Alternatively, you could set the same scale after selecting the other FIJI window, the exported image with only corals.

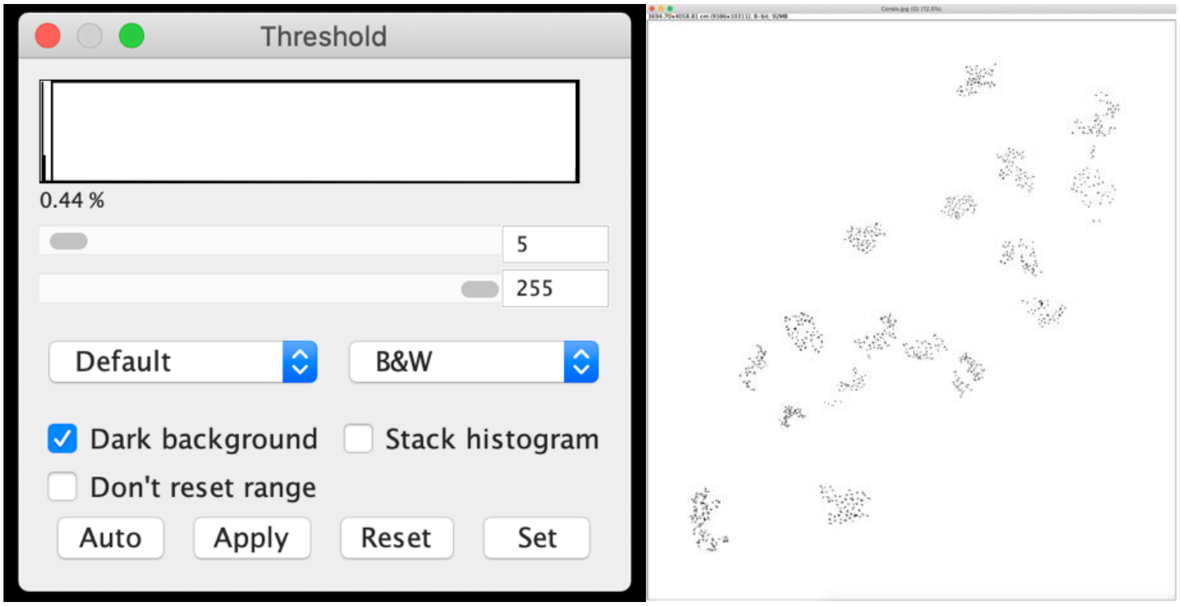


Distance in pixels: 127.0199
Known distance: 50
Pixel aspect ratio: 1.0
Unit of length: cm
☑ Global
Scale: 2.5404 pixels/cm

8. Select the other FIJI window, the .jpg with only corals.

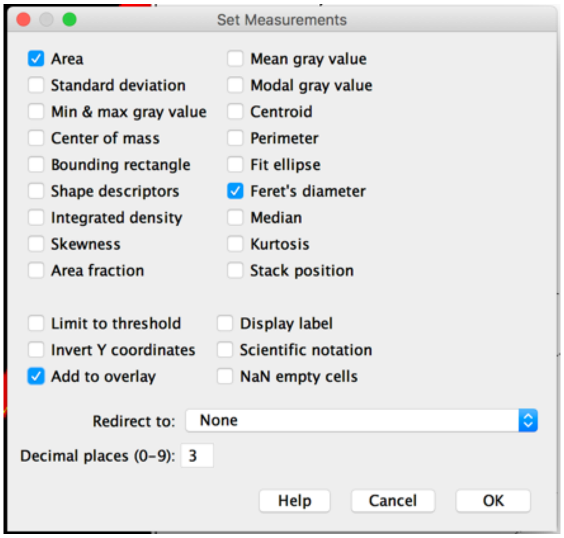
9. Convert the Image to 8-bit. Go to Image > Type > 8-bit. (It should already be selected.)

10. Adjust the Threshold. Go to Image > Adjust > Threshold. A dialogue box will appear to allow for customization. Set the values to run from 5 to 255. Notice the image will automatically change to B&W. This step allows FIJI to just analyze the black portions of the image, which are the corals, and exclude the background.



0.44 %
5
255
Default
B&W
☑ Dark background
☐ Stack histogram
☐ Don't reset range

11. Set Measurements. Go to Analyze > Set Measurements… A dialogue box will appear to allow for customization. Select the boxes next to Area, Feret's diameter, and Add to overlay. (Feret's diameter is defined in FIJI as the longest distance between any two points along the selection boundary, a.k.a. maximum caliper length.) Click OK.



Set Measurements

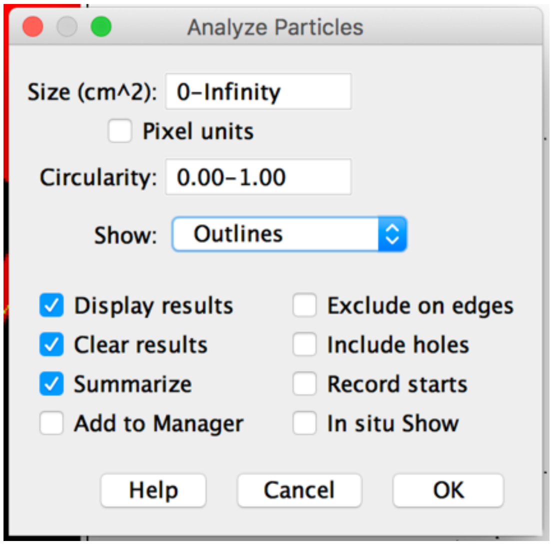
- ☑ Area
- ☐ Mean gray value
- ☐ Standard deviation
- ☐ Modal gray value
- ☐ Min & max gray value
- ☐ Centroid
- ☐ Center of mass
- ☐ Perimeter
- ☐ Bounding rectangle
- ☐ Fit ellipse
- ☐ Shape descriptors
- ☑ Feret's diameter
- ☐ Integrated density
- ☐ Median
- ☐ Skewness
- ☐ Kurtosis
- ☐ Area fraction
- ☐ Stack position
- ☐ Limit to threshold
- ☐ Display label
- ☐ Invert Y coordinates
- ☐ Scientific notation
- ☑ Add to overlay
- ☐ NaN empty cells

Redirect to: None

Decimal places (0-9): 3

Analyzing the Data

1. To analyze particles, go to Analyze > Analyze Particles… A dialogue box will appear to allow for customization. Select the boxes next to Display results, Clear results, and Summarize. Also select Outlines from the Show drop-down menu, then click OK.



Analyze Particles

Size (cm^2): 0-Infinity

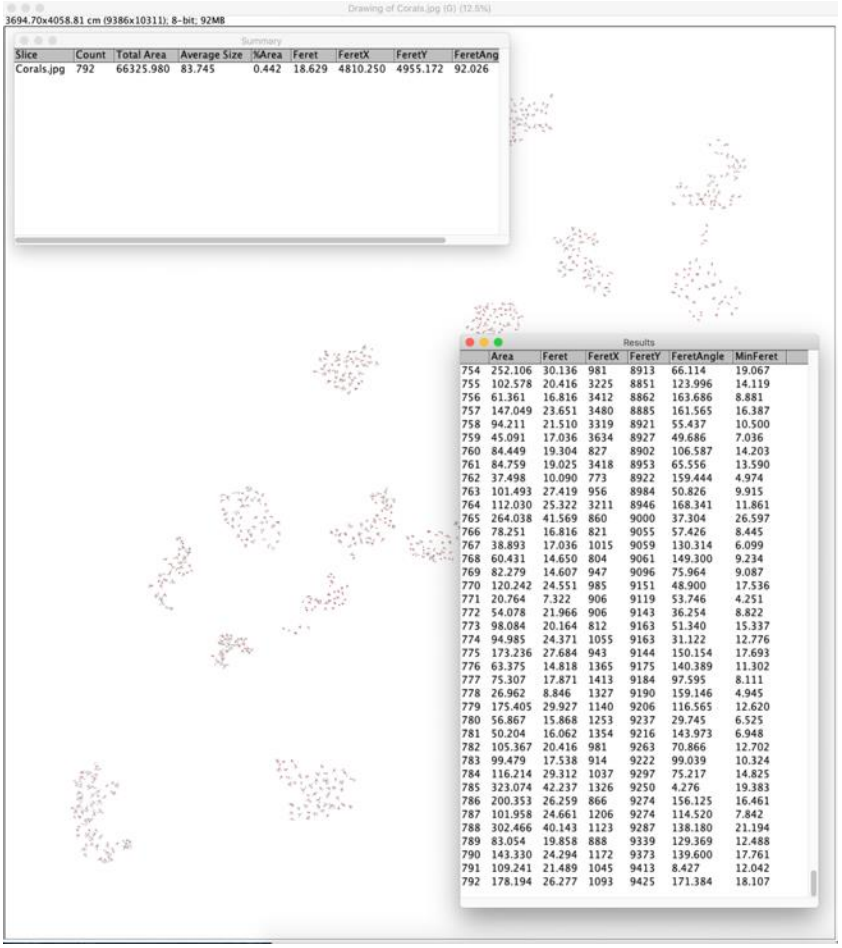
☐ Pixel units

Circularity: 0.00-1.00

Show: Outlines

- ☑ Display results
- ☐ Exclude on edges
- ☑ Clear results
- ☐ Include holes
- ☑ Summarize
- ☐ Record starts
- ☐ Add to Manager
- ☐ In situ Show

Multiple windows should appear. Two are data tables – a Summary table and a Results table with data from each "particle" (or coral). The last one is a drawing of the file with every "particle" outlined and numbered to correspond with the Results table.



3694.70x4058.81 cm (9386x10311); 8-bit; 92MB

Slice	Count	Total Area	Average Size	%Area	Feret	FeretX	FeretY	FeretAng
Corals.jpg	792	66325.980	83.745	0.442	18.629	4810.250	4955.172	92.026

	Area	Feret	FeretX	FeretY	FeretAngle	MinFeret
754	252.106	30.136	981	8913	66.114	19.067
755	102.578	20.416	3225	8851	123.996	14.119
756	61.361	16.816	3412	8862	163.686	8.881
757	147.049	23.651	3480	8885	161.565	16.387
758	94.211	21.510	3319	8921	55.437	10.500
759	45.091	17.036	3634	8927	49.686	7.036
760	84.449	19.304	827	8902	106.587	14.203
761	84.759	19.025	3418	8953	65.556	13.590
762	37.498	10.090	773	8922	159.444	4.974
763	101.493	27.419	956	8984	50.826	9.915
764	112.030	25.322	3211	8946	168.341	11.861
765	264.038	41.569	860	9000	37.304	26.597
766	78.251	16.816	821	9055	57.426	8.445
767	38.893	17.036	1015	9059	130.314	6.099
768	60.431	14.650	804	9061	149.300	9.234
769	82.279	14.607	947	9096	75.964	9.087
770	120.242	24.551	985	9151	48.900	17.536
771	20.764	7.322	906	9119	53.746	4.251
772	54.078	21.966	906	9143	36.254	8.822
773	98.084	20.164	812	9163	51.340	15.337
774	94.985	24.371	1055	9163	31.122	12.776
775	173.236	27.684	943	9144	150.154	17.693
776	63.375	14.818	1365	9175	140.389	11.302
777	75.307	17.871	1413	9184	97.595	8.111
778	26.962	8.846	1327	9190	159.146	4.945
779	175.405	29.927	1140	9206	116.565	12.620
780	56.867	15.868	1253	9237	29.745	6.525
781	50.204	16.062	1354	9216	143.973	6.948
782	105.367	20.416	981	9263	70.866	12.702
783	99.479	17.538	914	9222	99.039	10.324
784	116.214	29.312	1037	9297	75.217	14.825
785	323.074	42.237	1326	9250	4.276	19.383
786	200.353	26.259	866	9274	156.125	16.461
787	101.958	24.661	1206	9274	114.520	7.842
788	302.466	40.143	1123	9287	138.180	21.194
789	83.054	19.858	888	9339	129.369	12.488
790	143.330	24.294	1172	9373	139.600	17.761
791	109.241	21.489	1045	9413	8.427	12.042
792	178.194	26.277	1093	9425	171.384	18.107

It is important to notice how the particles are numbered on the Drawing. They are numbered in increasing order from top to bottom, left to right. Corals in a cluster will almost never be ordered consecutively. These numbers will also be altered if corals die before the next timepoint or if the next mosaic of the area has a different orientation, making colony-to-colony comparisons through time very difficult.

NOTE: At times, the .jpg will come with a white border surrounding the entire image or even some random white pixels that get measured as "particles". Take care to review the results FIJI gives you, so as not to include non-coral points and skew the results. Adjusting the threshold sliders in Step 10 above may also help avoid this problem.

If you notice any extremely large or small values, consult the Drawing and the original .psb file to verify if they are corals or not. If not, close out of the Results, Summary, and Drawing windows and redo the Analysis adjusting the "Size (cm^2):" value in the Analyze Particle window to exclude any particles that are above or below a certain size.

2. Copy the data into an Excel file for analysis. Some things to note:
    a. FeretX/FeretY. These are the (x,y) coordinates of the start of the Feret measurement.
    b. FeretAngle. This is the angle from the (x,y) coordinates that the Feret measurement runs.
    c. MinFeret. The minimum caliper distance of the particle.

3. Save the Drawing. Go to File > Save As > jpeg. This will allow you to cross-reference your results with the Drawing to determine which corals were which sizes, useful for comparisons across timepoints.

Additional resources can be found on the Coral Restoration Foundation™ website:
www.coralrestoration.org

The following persons have helped inform the CRF™ Photomosaic process throughout the years. For more information on different in-water image acquisition techniques, Agisoft processing advice, additional software such as TagLab or Viscore, and other general workflows, consider contacting them:

Art Gleason, University of Miami
Nicole Pederson, Scripps Institution of Oceanography
Clinton Edwards, Scripps Institution of Oceanography
Will Greene, Perry Institute of Marine Science