Standard Operating Procedure (SOP) #16

Benthic Image Analysis

Version 3.00 (August 22, 2019)

Change History

New Version #	Revision Date	Author	Changes Made	Reasons for Change	Previous Version #
2.00	6/30/2007	Eric Brown	See Track Changes	Response to reviewer's	1.00
2.01	7/20/2007	Larry Basch	See Track Changes	Corrections, additions	2.00
3.00	8/22/2019	Kelly Kozar	Substantial updates to the PhotoGrid© software instructions, benthic image analysis, and data certification process. Updated SOP #s	To accurately reflect the PhotoGrid© software instructions and benthic image analysis steps. Added a geospatial SOP and had to renumber subsequent SOPs.	2.01

Only changes in this specific SOP will be logged here. Version numbers increase incrementally by hundredths (e.g., version 1.01, version 1.02) for minor changes. Major revisions should be designated with the next whole number (e.g., version 2.0, 3.0, 4.0). Record the previous version number, date of revision, author of the revision, changes made, and reason for the change along with the new version number.

Purpose

This SOP documents the benthic image analysis protocol. The sections of this protocol are presented in order of execution. 1) Selecting the images for analysis, 2) PhotoGrid© analysis, 3) Identification standards, and 4) Exporting data from PhotoGrid©. Additionally, Appendix SOP 16.a shows various examples of substrate types from digital still images taken in different parks. The use of products identified in this protocol does not imply endorsement, effectiveness, or warranty by NPS.

A master equipment list for the entire Benthic Marine Community Vital Sign Monitoring Protocol can be found in Appendix SOP 1.a of SOP #1 Before the Field Season. The master equipment list should be updated simultaneously if any SOP requiring an equipment list is revised.

Benthic Image Analysis

Downloading Images for Analysis

Data will be downloaded from the camera as soon as possible following the dive. See SOP #15 Managing Photographic Images for instructions on downloading and storing photographs.

Setting up PhotoGrid©

1. Download the PhotoGrid© software from the PACN server, or the "I" drive. A zipped file of the software is located in the I:\data_mgmt\program_execution_files\PhotoGrid1b folder, file name PhotoGrid.zip.

2. Uncompress the zipped file. In Microsoft Windows® 10, right click on PhotoGrid.zip in and choose "Extract All". This will create the necessary files (e.g., setup.exe) used in the installation process. The location of the unzipped files can be specified for any folder on the hard drive.

3. Install PhotoGrid© on your hard drive in the folder of your choice by double clicking on the "setup.exe" file. The default folder of C:\Program Files (x86)\PhotoGrid 1.0 is adequate for this protocol. IT or someone with administrator rights will need to install the software. Contact the Data Manager or submit a Help Desk ticket for IT help.

4. After installation, navigate to the PhotoGrid© folder in the Program Files (x86) folder on your hard drive. Optional: create a shortcut icon for PhotoGrid© on computer desktop.

5. Select the "Species Buttons" folder
    a. Button files for each park are in the "I:\vital_signs\01_benthic_marine\Documents\Species Buttons" folder. Copy the button file for your park into the "C:\Program Files (x86)\PhotoGrid 1.0\Species Buttons" folder.
    b. If new species are detected during surveys, contact the Data Manager who will add the new species to the button file.

6. The Data Manager will edit the Button files using the following steps.
    a. Open one of the Button text files using a text editor such as Notepad®, Wordpad®, or Word®.
    b. Edit the text file to incorporate the species within the park (Figure SOP 16.1).
    c. Save the text file in the "C:\Program Files (x86)\PhotoGrid 1.0\Species Buttons" folder.
    d. Copy the file to the "I:\vital_signs\01_benthic_marine\Documents\Species Buttons" folder.

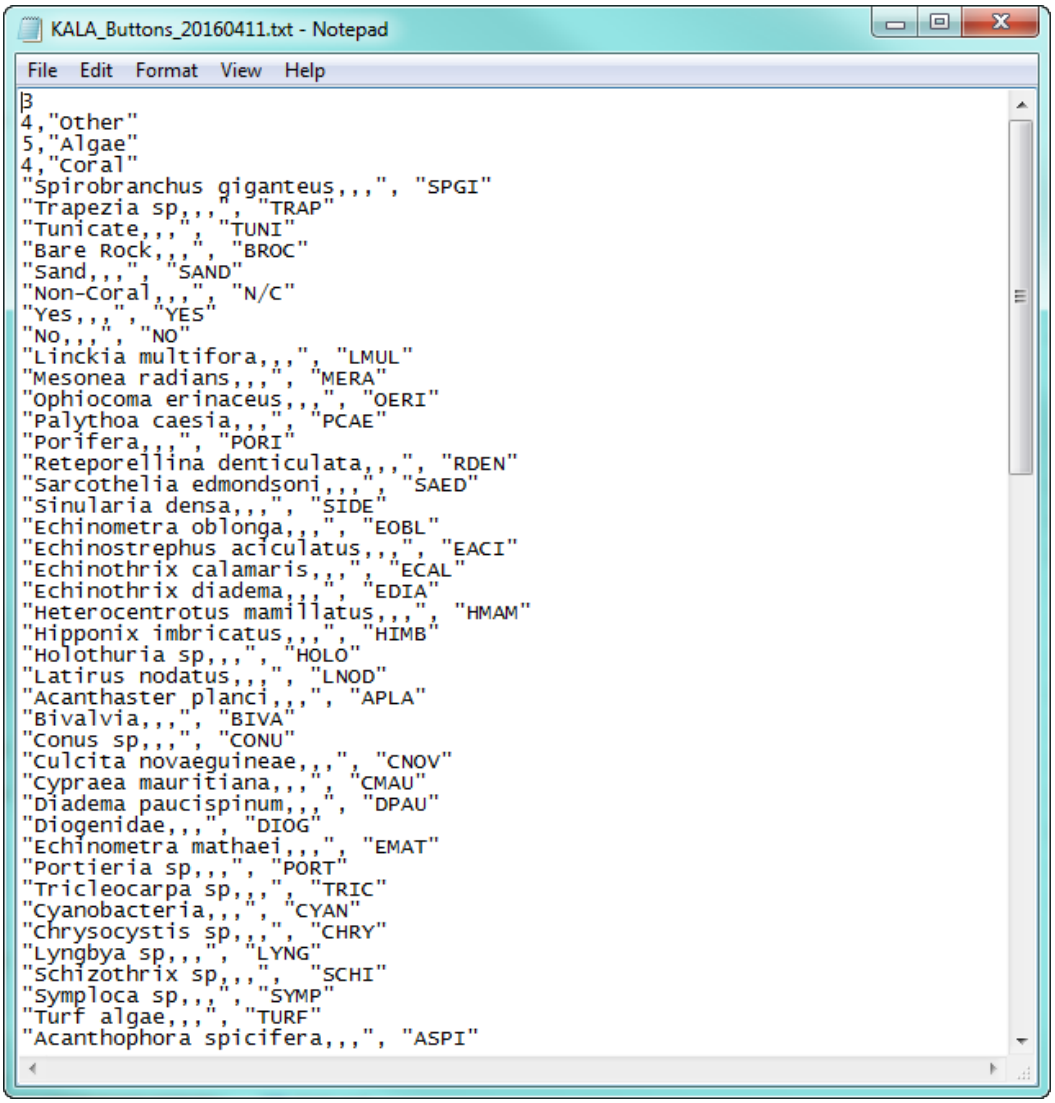

Figure SOP 16.1. Sample PhotoGrid© species buttons text file showing format for taxa entry.

7. Launch PhotoGrid© by clicking on the "Search" button on the bottom left of the task bar and typing in "PhotoGrid". The PhotoGrid© app will show in the search results, click on the PhotoGrid© app.

8. Once open, on the top left of the PhotoGrid© program, click on "Options". Click on "Preferences" under the "Options" menu. A dialog box will appear. The screen should look like Figure SOP 16.2.

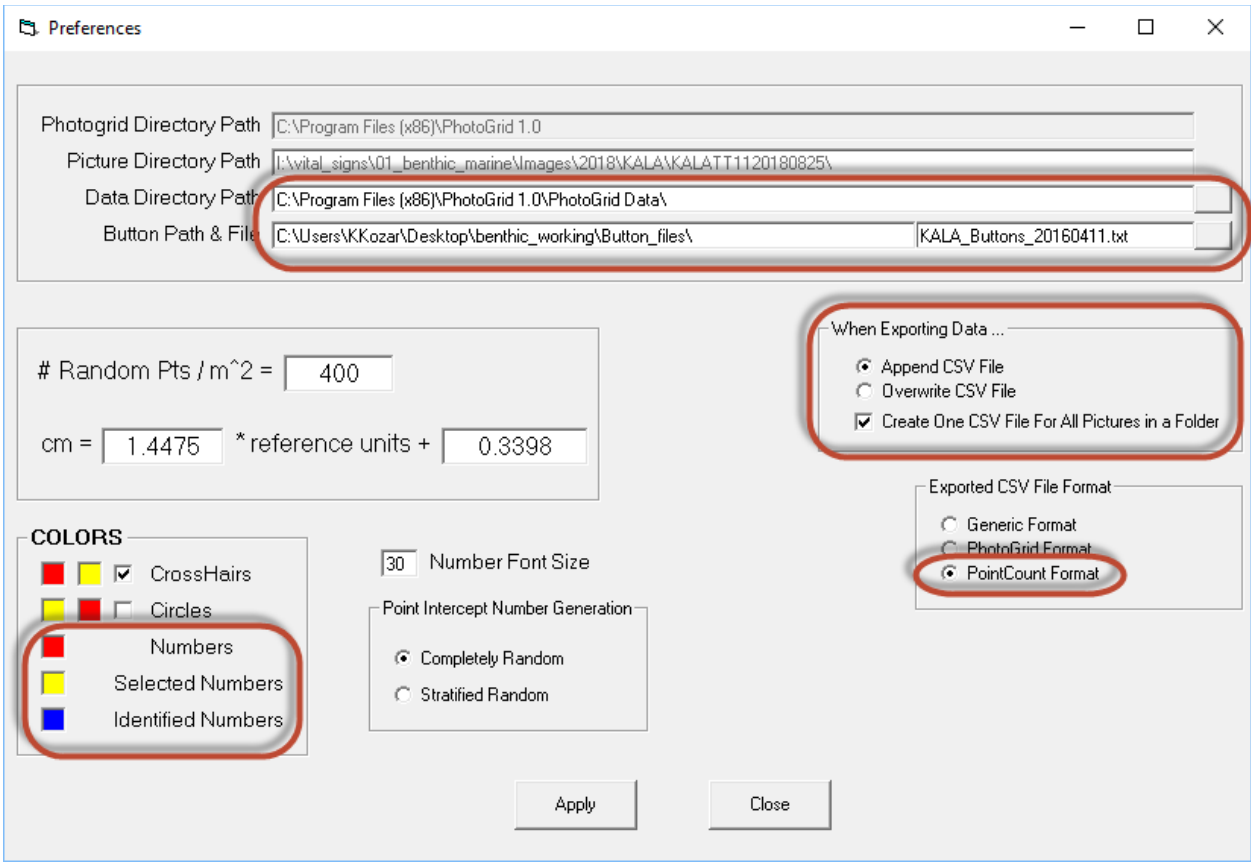

Figure SOP 16.2. PhotoGrid© preferences screen under the "Options" menu.

9. Select which folder the saved data files will be generated in. The default folder is the C:\Program Files (x86)\PhotoGrid 1.0\PhotoGrid Data\ folder. To changes the folder, click on the box to the left of the "Data Directory Path" field and navigate to the preferred folder. These data files will have the extension ".PGC".

10. Select the buttons file that will be used for substrate identifications within the park. In this example, "KALA_Buttons_20160411.txt" is the file that has the species/substrate codes used for Kalaupapa NHP.

11. In the "When Exporting Data" box, select "Append CSV file" and "Create One CSV File for All Pictures in a Folder".

12. In the "Exported CSV File Format" box, select "PointCount Format".

13. In the "Color" settings note that the current point will be highlighted in yellow with completed points labeled blue and points yet to be identified still in red.

14. Disregard the "# Random Pts/m^2" box because the number of points projected on the screen will be entered on the main screen after an image has been loaded.

15. Apply any changes to the screen by clicking “Apply” and then close the window by clicking “Close”.

16. Under the “Options” menu, ensure that the Sampling Mode has been set to Point Intercept.

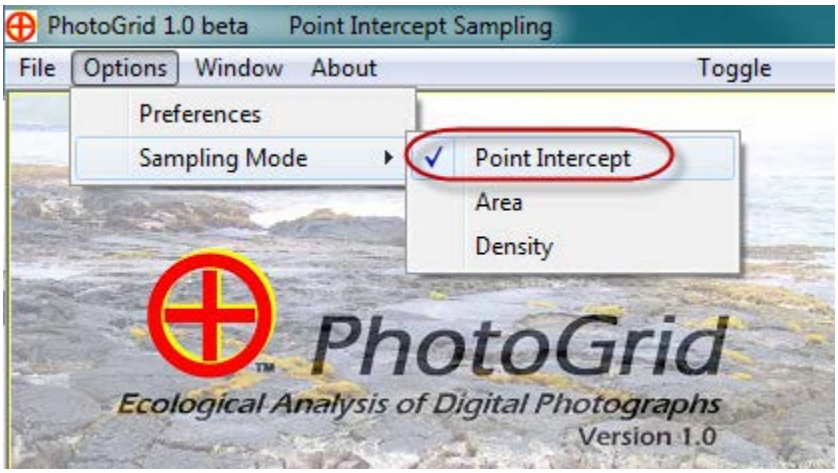

Figure SOP 16.3. PhotoGrid© sampling mode screen under the “options” menu.

PhotoGrid© Analysis

In 2017 it was decided that coral bleaching data would be collected in addition to species/substrate data. Directions below are broken out for the original species/substrate data being collected (“Original”) and the additional bleaching data being collected (“Bleaching”). Follow the instructions pertaining to what type of data is being analyzed.

1. On the menu select “File”, click “Open”, and navigate to the image being analyzed (Figure SOP 16.4).
    a. Benthic images should be in the I:\vital_signs\01_benthic_marine\Images\YYYY\PPPP folder on the I drive (refer to SOP #15 Managing Photographic Images for more information).
        i. If the network connection is too slow to analyze the images on the I drive, the images can be copied to a local folder on the user’s computer or external hard drive and analyzed from there. Make sure that the whole folder for a transect is copied over if doing so.
    b. Start with the first frame of the transect (000), identified by the last three digits of the file name, i.e. KAHO18FT01000.

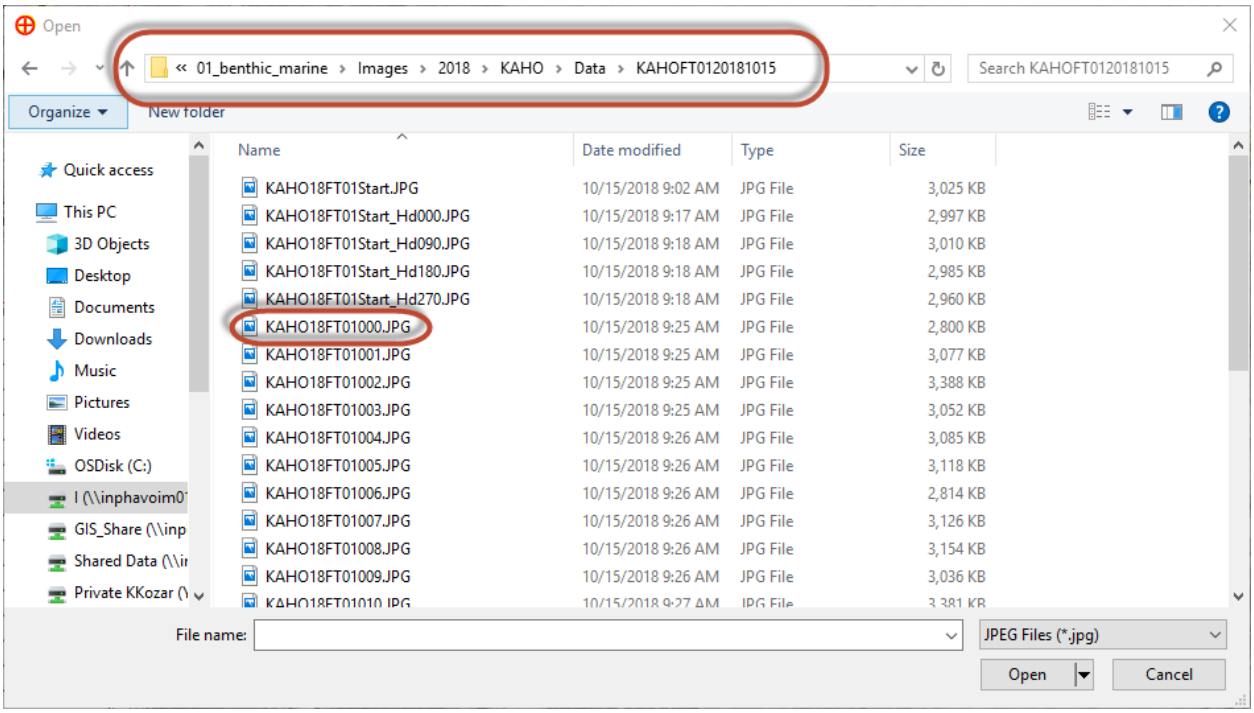

Figure SOP 16.4. Choosing an image to be analyzed in PhotoGrid©.

2. In the lower left-hand corner of the main screen set the number of random points (# Rnd Pts) to be generated depending on whether bleaching data is to be collected.

    a. Original – set to 51
    b. Bleaching – set to 52

3. Press the "MakeRandPts" button to generate the 51 (Original) or 52 (Bleaching) points on the image

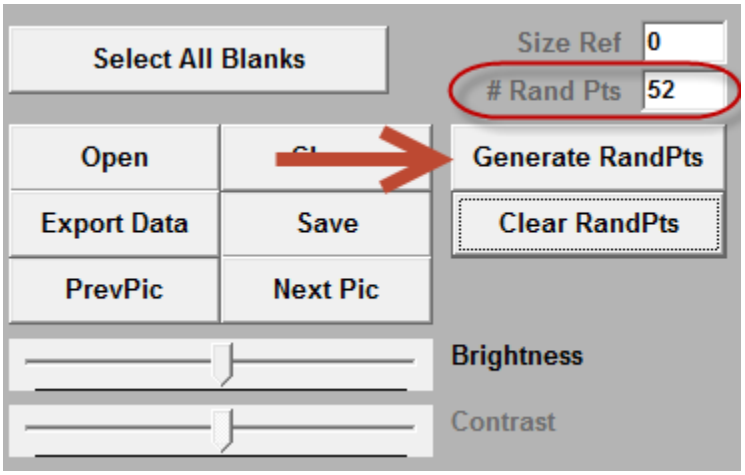

Figure SOP 16.5. Generate the random points to be analyzed.

4. The cursor appears in the blank space for point number one, and will advance automatically as subsequent identifications are made.

5. Fill in the PhotoGrid© table on the left side of the screen using the buttons corresponding to the substrate type at the bottom of the screen. The pixel used to make the identification is in the lower right-hand quadrant of the + (Figure SOP 16.6).

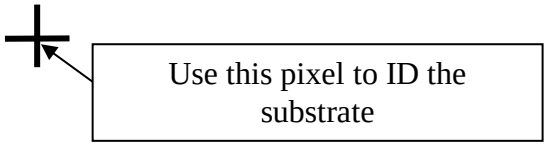

Figure SOP 16.6. Location of pixel used to make identification of substrate type.

   a. Bleaching - for coral species, bleaching data will need to be entered, which will go in the field with the coral species name on the left side of the screen (Figure SOP 16.7).
      i. Coral species all have a default value of "No" for coral bleaching, which is after the first comma after the species name.
      ii. If there is bleaching, replace "No" with "Yes", making sure it is between the commas. It is important that "Yes" goes between these two commas in order for the data to be exported to the correct fields.
      iii. If there is bleaching, add the bleaching category between the last two commas. Again, this is important for exporting the data to the correct fields.
         1. "BL" for bleached
         2. "BLA" for bleached with algal overgrowth

1	Turf algae,,,
2	Turf algae,,,
3	Turf algae,,,
4	Porites brighami,Yes,BL,
5	Turf algae,,,
6	Turf algae,,,
7	Porites brighami,Yes,BL,
8	Echinothrix calamaris,,,
9	Turf algae,,,
10	Turf algae,,,
11	Montipora patula,No,,
12	Amansia sp,,,
13	Porites brighami,Yes,BL,
14	Turf algae,,,
15	Turf algae,,,
16	Turf algae,,,
17	Turf algae,,,
18	Echinothrix calamaris,,,
19	Palythoa caesia,,,
20	Cyphastrea agassizi,No,,

Figure SOP 16.7. Populating species fields with bleaching data.

6. To magnify a part of the screen, point the cursor in the area and left-click (once) on the mouse. To reduce the image then right-click on the mouse.

7. Clicking on multiple point numbers and then assigning the appropriate identification to them with the corresponding substrate type button allows for multiple entries (of points on same substrate type) to be made with a single keystroke.

8. When you reach point number 51 on the left side of the screen then enter "Yes" if coral disease or bleaching is present anywhere in the frame or "No" if the corals appear to be disease free and unbleached.

    a. Disease symptoms include lesions and rings or bands that demarcate live healthy tissue from dead tissue. Bleaching is whitening of the tissue, which signifies a loss of the symbiotic zooxanthellae. This whitening can sometimes be confused with coral disease or Crown-of-Thorns (COTS) Sea Star predation, which may show a somewhat similar pattern. At present, treat any whitening as bleaching. However, when observed at field sites, record the following metadata: whether or not there are multiple bleached colonies, estimated number, species affected, approximate maximum diameter of patch(es) of bleached corals, presence-absence or (preferably) estimated number of COTS, and location(s) within the site relative to the transect start point (compass bearing, approximate distance in meters). LJ Raymundo et al. 2008. *Coral disease handbook: guidelines for assessment, monitoring and management* provides a good reference for coral diseases that may be encountered at PACN parks.

9. If bleaching data is being collected, point number 52 will be populated with the bleaching severity for the frame. This value is a visual estimate of how much of the coral in the photo is bleached. Make an estimate and choose the button with the corresponding severity range, i.e. 76-100%, 51-75%, etc. Severity buttons are on the lower right hand side under “Other”.

10. After completing the identification of all 51 or 52 points bring up the next image by clicking on the “Next Pic” button in the lower left of the screen. The data for each frame is being saved automatically in a PGC file for the transect in the folder “C:\Program Files\PhotoGrid 1.0\PhotoGrid Data”, or whichever folder was designated in Step 9 of the Setting up PhotoGrid© section.

Identification Standards

Identify the substrate under the lower right hand quadrant of the crosshair (“+”) using the following standards (Table SOP 16.1). See Appendix SOP 16.a for examples of different substrate types.

Table SOP 16.1. Substrate identification standards.

Substrate Type	Instructions
Unnatural objects	For unnatural objects such as pin, tape, reel, you can use the N/C (non-coral) category.
Fish	Since fish are not part of the benthos, treat these as unnatural objects and select the (non-coral) category.
Other invertebrates (e.g. urchins, sea cucumbers, mollusks)	Identify to the lowest possible taxon.
Dark areas	For dark areas (e.g., shadows obscuring objects) in the image use the Subs (substrate) category
Unknown substrates	For unknown substrate use the Subs (substrate) category
Other organisms	For organisms not listed in the button file, identify the organism to the lowest possible taxonomic group and notify the Data Manager of the new species. Notify the Marine Ecologist if necessary to verify the identification.
Snapping shrimp burrows	For snapping shrimp burrows in coral, use the TURF (Turf Algae) category since this category lines the burrow.
Recent broken areas	For areas of the reef that were recently broken, try to identify what it was prior to the damage. If this is not possible categorize as Unknown. In the next survey year, simply identify the new substrate (e.g., turf algae) based on whatever it now is.
Structures within algae or no structure	If structure within the algae is observable, select MALG (Macro algae) or lowest possible algal taxon. Typically this is evaluated as height (2-3 cm) above the substrate. Use the TURF (Turf Algae) category when the substrate has no observable structure or height, and it appears like a fuzzy growth.

Distinguishing between dead coral with turf algae and macroalgae in images captured from a still photograph may be difficult, but is worth taking the time to try to do accurately. The predominance of either category of substrate can be an important ecological indicator. Greater levels of macroalgae may reflect elevated nutrient levels, or lower levels of fish or urchin grazing (and possibly over-fishing). In comparison, increased dead coral with turf may indicate recent coral disease or high levels of grazing. Height (~ <2-3 cm) and the absence of an identifiable macroalgal structure can be considered in identifying dead coral with turf. Macroalgae generally attain a height/thickness >2-3 cm and may be fleshy, filamentous, or calcified, often with conspicuous branching, blades.

When deciding whether the photopoint is on dead coral with turf algae or macroalgae, you will need to evaluate other areas in the image for comparison. As with the hard corals, and other sessile benthic invertebrates, algae should be identified to the most descriptive level possible, i.e., lowest taxon possible, ideally genus or species. If this is not possible, but the alga appear fleshy and to have a height >2-3 cm, it should be identified as “macroalgae.” As the photograph provides only a two-dimensional view of the reef, the component’s “height" can be difficult to ascertain. This is where brief field notes of quadrat macrobiota composition are very important. Looking around the image, at substrates similar to and different from the one in question, will usually aid interpretation of the substrate features (colors, shading, textures, etc.). Also referring back to any field notes will help resolve the image in a third dimension resulting in more

accurate identifications. When in doubt, dead coral with turf algae may be the more conservative category to record.

Export Data from PhotoGrid©

1. After analyzing all the benthic images for a transect, the marine biological technician can now begin exporting the substrate identifications within each image along each transect.
2. Open PhotoGrid© if it's not already open and bring up the first image in the first transect for the year and park analyzed.

3. Press the "Export Data" button in the lower left corner of the screen. This procedure appends the information to the CSV file for that folder.
    a. Note - you will not receive any message that the data has been exported. Clicking the button twice will export the data again, causing duplicate records. While this will be error checked when importing into the Access® database, take care here not to export the same data more than once.

4. Click the "Next Pic" button to move to the second image. Click the "Export Data" button. This will export the data from the second image into the CSV file.

5. Advance to each successive image and export the data until you have exported data for the last image.

6. The CSV file is created in the "C:\Program Files\Photogrid 1.0\Photogrid Data" folder, or whichever folder was designated in Step 9 of the Setting up PhotoGrid© section.

7. The file naming convention for the PhotoGrid© format is: "c_TransectFolderName.CSV". The transect folder name contains the park code, transect number, and survey date.
    a. Data are now ready to import into the Benthic Marine and Marine Fish Monitoring Database. Refer to SOP #14 Data Entry, Verification, and Certification and Appendix I: Benthic Marine Database User Guide for instructions on importing the data into the database.

8. To close the PhotoGrid© program, select "File" at the top of the window, and scroll down and click on "Exit".

9. The marine biological technician uploads all of the PGC and CSV files for each transect that year to the "I:\vital_signs\01_benthic_marine\Data\Photogrid_data\YYYY\PPPP" folder, where YYYY is the current year and PPPP is the park unit code. These PGC and CSV files to be uploaded are located in the "C:\Program Files\PhotoGrid 1.0\PhotoGrid Data" folder, or whichever folder was designated in Step 9 of the Setting up PhotoGrid© section (Figure SOP 16.8).
    a. There will be one CSV file for each transect, and one PGC file for each frame analyzed.

10. The marine biological technician will inform the PACN Data Manager that the PhotoGrid© data is in the correct folder.

Data Certification

Certification is the process of checking substrate identifications for 10% of the images. The Marine Ecologist at each park or the PACN Marine Ecologist is responsible for completing this task (refer to SOP #14 Data Entry, Verification, and Certification).

The PACN Data Manager will randomly generate the transects to be checked by the Marine Ecologist after receiving notice from the marine biological technician that the annual CSV files are saved on the I drive.

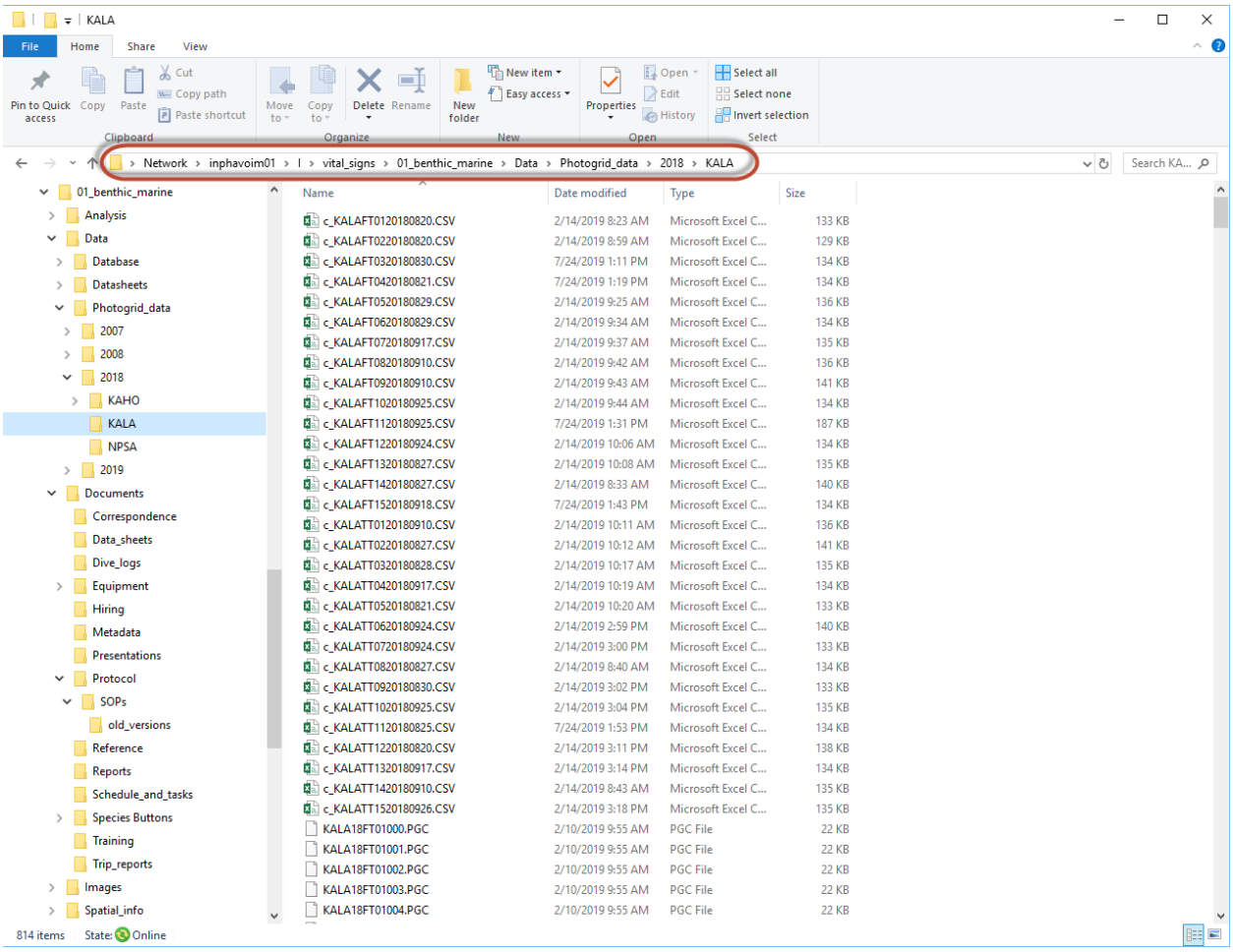

Figure SOP 16.8. Folder structure for PhotoGrid© data files.

1. The PACN Data Manager will import the data in CSV files into the working copy of the database for the year (i.e. 2018 KAHO working database).

    a. To import the data, open the Benthic Marine and Marine Fish Monitoring database front-end database file and link to the working copy database back end file. Proceed to import the CSV files exported from PhotoGrid© (see Appendix I: Benthic Marine Database User Guide for instructions).

2. The PACN Data Manager will randomly select 10% of the images (~75 images per year) for re-examination and certification by the Marine Ecologist.

3. The Benthic Marine and Marine Fish Monitoring database has a proofing form that will randomly generate the transects to be re-examined.
    a. In the Benthic Marine and Marine Fish Monitoring database front-end database, click the Proof Benthic Data button on the database's main switchboard to run the proofing tool. See Appendix I: Benthic Marine Database User Guide for instructions for instructions on how to use the tool.

4. The PACN Data Manager will inform the Marine Ecologist which transects are to be re-examined for certification.

5. The PACN Data Manager will compile PGC and CSV files for the transects selected for certification. These files will be put in a "Pre-cert_review folder within the "I:\vital_signs\01_benthic_marine\Data\Photogrid_data\YYYY\PPPP" folder. These files will be re-examined by the Marine Ecologist.

    a. Keeping these files separate will keep a record of pre-certified data and certified data to archive.

6. The marine ecologist for each park should copy the PGC files for the randomly generated transects to be certified to the "C:\Program Files\PhotoGrid 1.0\PhotoGrid Data" folder on their PC.

7. The Marine Ecologist conducting the re-examination verifies that the correct substrate identifications have been recorded on the screen.

8. If corrections need to be made then the marine ecologist updates the PhotoGrid© screen and logs the transect and image number using the error data form in Appendix SOP 16.b. Logging the information will allow measurement error to be estimated.

9. Updating the screen identifications also updates the PGC files.

10. If the substrate identification error (overall measurement error for the 75 images) is lower than 10% (~375 total points) then the updated PGC and CSV files can now be sent to the PACN Data Manager for upload to the working copy database.

11. If the substrate identification error (measurement error) is higher than 10% then the errors need to be evaluated and corrected. For example, consistent misidentification of certain taxa would necessitate the marine biological technician going back and updating all of the images with the correct information.

    a. Once the errors have been resolved at the biological technician level, export the data using the previous steps for exporting the data, and upload updated PGC and CSV files to the

I:\vital_signs\01_benthic_marine\Data\Photogrid_data\YYYY\PPPP\Updated_files folder.

b. The marine ecologist will re-examine 10% of these updated records, using the same process as before.

12. Substrate identification error must be lower than 10% in order to consider the data certified.

Appendix SOP 16.a. Substrate examples

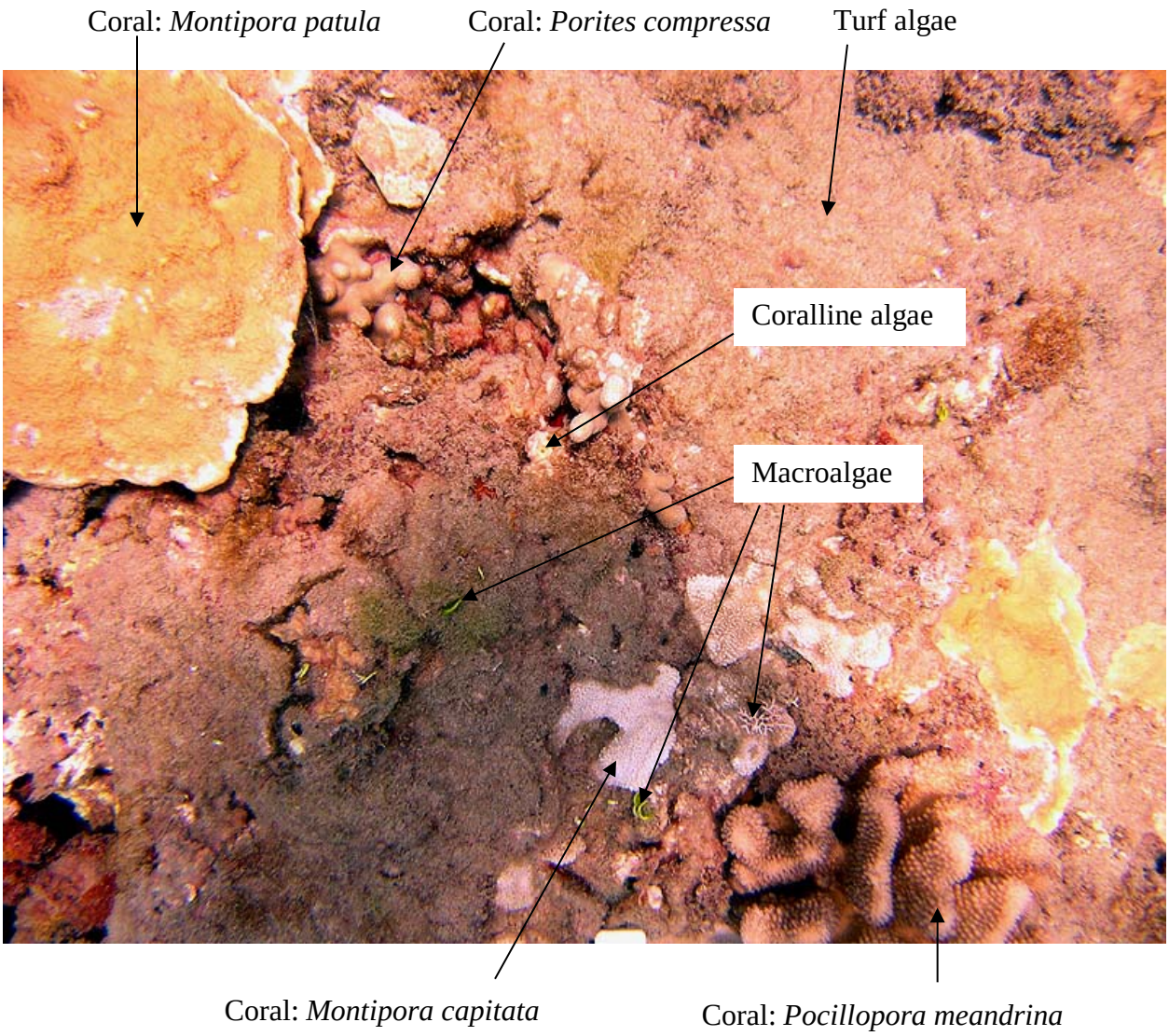

Figure SOP 16.9. Examples of substrate types from digital still images from Hawaii.

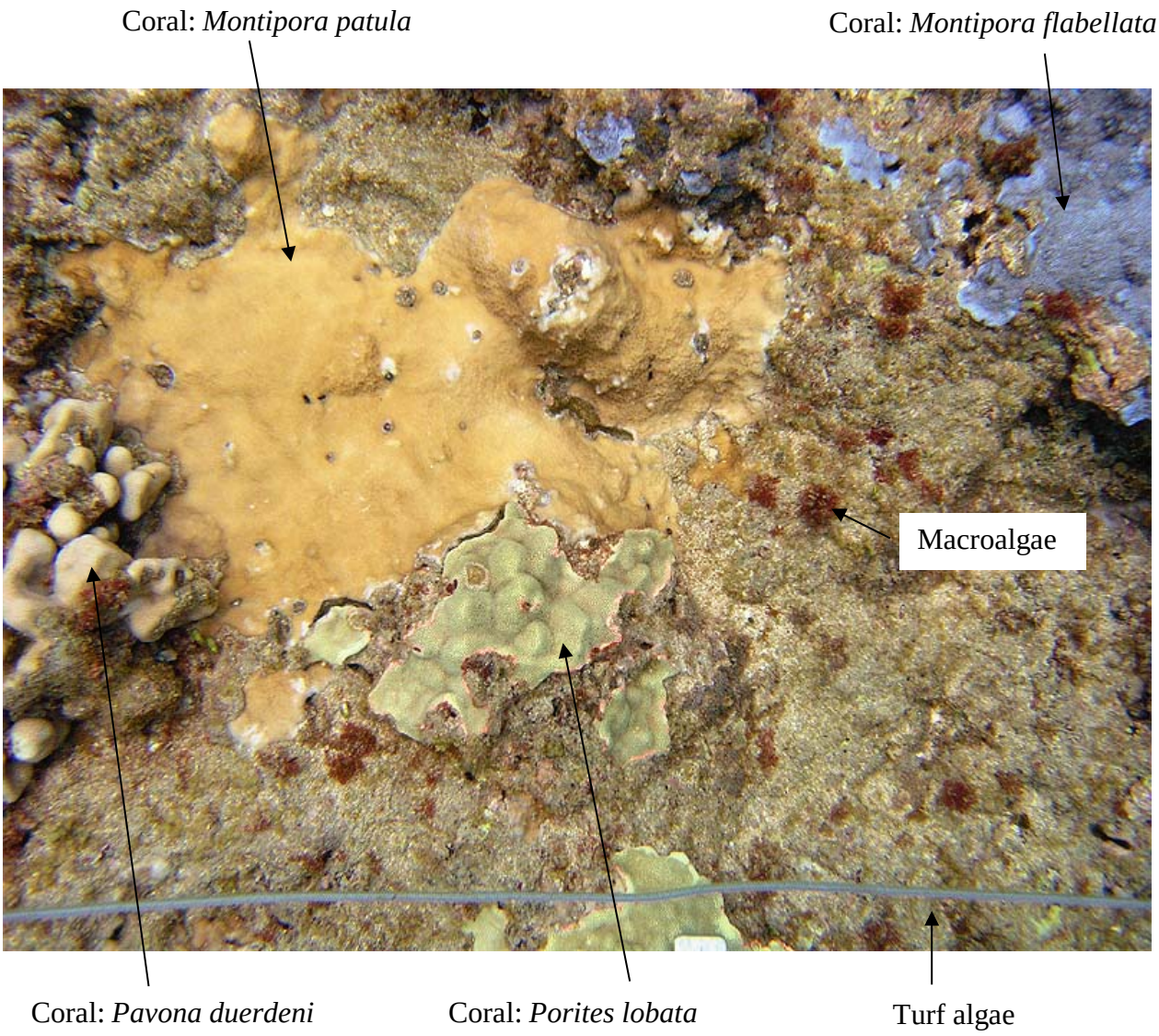

Figure SOP 16.10. Examples of substrate types from digital still images from Hawaii.

Appendix SOP 16.b. Error Checking Data Form

Data form for error checking of point identifications in photoquadrat images

Year: _____ PhotoGrid© IDer _____

Park: _____ Total Number of Images Checked: _____

Marine Ecologist: _____ Total Number of Points Checked: _____

Events	Transect	Image	Point #	Incorrect Substrate	Correct Substrate
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
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