Sediment Particle Size Determination Using a Coulter LS230 Laser Diffraction Particle Analyzer and US Standard #10 Sieve

1. SCOPE AND APPLICATION

1.1. This method is used to measure the size of particles in sediment collected from freshwater and marine environments. Percentages of grain size are determined using laser diffraction within ranges set between 0.04 – 2000 μm based on two classifications: the Wentworth scale and the USDA sediment scale.

1.2. This procedure is used for samples with LIMS Test ID SED-PSZ-LS, SED-WWTH and SED-USDA.

1.3. Method Detection Limits (MDL)

1.3.1 No MDL has been established for sediment particle size. Particle range 0.04 – 0.4 μm is measured by Polarization Intensity Differential Scattering (PIDS). The reporting limit for laser particle size is 0.1%. The reporting limit for sieve particle size (>2 mm) is 1.0%.

2. SUMMARY OF METHOD

2.1. A small portion of sample is sieved and poured into the fluid module of the Coulter LS230 laser to measure grain sizes less than 2.0 mm using laser diffraction. A larger portion of sample is weighed and sieved over a pan to separate particles larger than 2.0 mm from those that are smaller than 2.0 mm. Particles from the pan and sieve are dried and weighed. Particles that are 2.0 mm and larger are analyzed as a percentage of the total dry weight.

2.2. Interferences

2.2.1. Samples must be stored at 4 °C until analyzed. The samples cannot be frozen, as the freezing and thawing cycle may cause permanent changes to the particle size distribution.

3. EQUIPMENT AND SUPPLIES

Record any equipment repairs or maintenance in LIMS Laboratory Logbook *Maintenance_Log*

3.1. Coulter LS230 Optical Bench with Variable Speed Liquid Module (S/N AA26207)

3.2. Computer with Coulter LS32 program

3.3. Mettler PB403-S balance (S/N 1128150043)

3.3.1. Check calibration each day before use. See SOP TA-06.38.

3.4. Drying oven, 105 ± 2°C (Model ERO 34000) Drying Oven, 105 ± 2°C (Isotemp 180 L Oven Gravity SN 42073712)

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

3.5. Sieve, US Standard #10 (2.00 mm)

3.6. Brass sieve pan

3.7. Beakers: Dry at 105 ± 2°C for 30 minutes prior to use. Cool completely before using to ensure beaker weight is constant.

3.7.1. 600 mL, 250 mL, 150 mL: One set for each sample to be analyzed

3.7.2. 250 mL: One used for all laser measurements

3.8. Metal spatula

3.9. Forceps

3.10. Rinse bottle for deionized water

3.11. Refrigerated storage

4. REAGENTS AND STANDARDS

4.1. Deionized (DI) water

4.2. Aqueous sodium hexametaphosphate

4.2.1. Add 6.2 g of $NaPO_3$ to 1 L of DI water.

4.3. Coulter LS Control G 15 garnet standard (15 μm)

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1. Samples must be stored at 4 °C until analyzed. Holding time is six months. For general sample collection and preservation, see SOP FS 1000.

6. SAMPLE PREPARATION

6.1. Refer to SOP BB-030 for sample custody and label instructions.

6.2. Coulter LS230 power up

6.2.1. Before power up, perform three instrument checks:

6.2.1.1. Check that the three Fluid Module switches (located on the back) are in the center position.

6.2.1.2. Check that there is suspension fluid in the sample vessel of the Fluid Module.

6.2.1.3. Check that the water source to the Fluid Module is turned on.

6.2.2. Turn on the power switch on the front of the Optical Module. After power up you must wait *at least two hours* for the instrument temperature to stabilize before running a control or sample.

6.2.3. Turn on the computer and open **COULTER LS** program (**LS32** icon).

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

6.2.4. Check **Use Optical Module** and click **OK**. This step establishes a communication link between the instrument and computer.

6.2.5. From the menu bar, select **Control/Pump On**. Pump can remain on while completing sample preparation.

6.3. Network Drive Setup (If drives have already been set-up, skip to 6.4.)

6.3.1. First time users operating LS230 will map two network drives. Once established, the drives will remain assigned to the user's login on the sediment bench computer.

6.3.1.1. From the Start menu, open Computer. Click on Map Network Drive.

6.3.1.2. Choose Drive: "K:"

6.3.1.2.1. This will be where laser files are first saved (file extension.$01)

6.3.1.2.2. If K: drive is already in use, choose a drive that is available and substitute that drive for "K:" throughout this document.

6.3.1.2.3. Browse to find folder \\fldep1\DEAR\Labs\Lab5\Biology\Bench Common\Sediments

6.3.1.2.4. Click **OK**. Click **Finish**.

6.3.1.3. For second drive, Choose Drive: "N:"

6.3.1.3.1. This is where uploaded files will be saved (file extensions .txt, .raw, .rev)

6.3.1.3.2. If N: drive is already in use, choose a drive that is available and substitute that drive for "N:" throughout this document.

6.3.1.3.3. Browse to find folder: \\fldep1\DEAR\Labs\Lab3\Biology\Sediments. Click **OK**. Click **Finish**.

6.4. Create a Directory

6.4.1. Create a folder for raw data files at the beginning of each day that samples are analyzed.

6.4.1.1. In **COULTER LS**, go to **File/Open /K:** and open the yearly raw file folder. (ex: **SedRaw17)**

6.4.1.2. Create a new folder inside of **the yearly folder** and name it "**SEDMM-DD**" where MM-DD is the month and day of the analysis.

6.4.1.3. Record the new folder name as the Run ID in the *Sediment Run Log*. Close window.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

6.4.2. Go to **File/Change Directory**. Navigate to the daily folder just created. (ex: **K:\SedRaw17\SED07-13**)

6.4.3. Click **OK**. The directory appears along the bottom of the window.

7. SAMPLE ANALYSIS

7.1. <2.00 mm particle size

7.1.1. Refer to Section 9, **Quality Control**, to determine if a Garnet Control should be run. If so, run the control according to the directions that come with it.

7.1.2. Homogenize the sample by gently but thoroughly mixing with a metal spatula.

7.1.3. If a sample container is full, the sample can be added to a clean and dry beaker to avoid losing sample when mixed.

7.1.3.1. For sediment that is sticky or heavy with clay, add up to 10 mL of aqueous sodium hexametaphosphate. This surfactant aids in separating particles.

7.1.4. Stack a #10 sieve onto a 250 mL beaker. Using the spatula, place a small amount of sample (~1 g) onto the sieve. Rinse sample off the spatula and through the sieve into the beaker using DI water.

7.1.4.1. The portion of sample to be sieved should be representative of the entire sample.

7.1.4.2. Start with a very small amount of sediment. In general, the coarser the grain, the larger the amount needed. Finer silt sediment requires much less sample.

7.1.4.3. Any particles remaining on the sieve are greater than 2.0 mm and can be discarded.

7.1.5. In COULTER LS, select Run/Run Cycle from the menu.

7.1.5.1. Select New Sample.

7.1.5.1.1. Every sample needs **Auto Rinse**, **Measure Background**, and **Measure Loading**. The first control or sample of the day also needs **Measure Offsets** and **Auto Align**. After that the system puts an X next to **Measure Offsets** and **Auto Align** every hour to indicate when these functions need to be repeated.

7.1.5.1.2. Note: Often, the laser loses communication with the program. Auto Rinse can be deselected for each run. If the auto rinse is not selected, the vessel will have to be manually rinsed between each sample. Go to **Control**/**Rinse**.

7.1.5.2. Check box to Include PIDS data.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

7.1.5.3. Pump speed should be set at 51. Heavier particles may need a higher pump speed to keep sample suspended.

7.1.5.4. Run Info can be verified: 90 second Run Length, 0 second Wait Length, 1 Number of runs; Compute Sizes and Save File check marked; Model Fraunhofer.rfd selected. Select OK.

7.1.5.5. Click Start.

7.1.6. IMPORTANT: Manually open and close the valve on the front of the Fluid Module during the **Auto Rinse** phase of every sample. Continue flushing and filling until the water appears clean and the program initiates **Filling** in the communication bar at the top of the window and moves on to **Measure Background**. If the fluid module is not full or is not completely rinsed, cancel Run, go to **Control/Fill**, and then begin cycle again.

7.1.7. While the program executes diagnostics, review the following checklist to see if the parameters are within range. If out of range, run LS Control.

7.1.7.1. Offset is within +/-6 mV except for channel 127.

7.1.7.2. Alignment has similar pattern to reference file dfr*.$* in the calfile directory. (This file can be found on the computer at the Sediment station. It is located in **C:\Windows\LS\Calfiles**.)

7.1.7.3. Background is $\leq 2 \times 10^6$ for each channel.

7.1.8. When **Measure Loading** appears at the top of the screen, **make sure PIDS and Obscuration are both at 0%. If not, re-rinse the Fluid Module.** When **Measure Loading** reappears, go to **Control/Timed Sonication** and set for 90 seconds. Press **Start**. Continue sonication as needed until enough sample has been added (See 7.1.8.).

7.1.8.1. Add entire contents of sediment wash from the beaker to the Fluid Module. Rinse remaining particles from the beaker with DI water into the vessel.

7.1.9. Add sample until display values are within range and stable: Obscuration = 8 to 12% and PIDS = 45 to 55%. Click **Done** when sufficient amount of sample has been added.

7.1.10. The program displays the **Sample Info** box. Enter the six-digit Sample ID into the **Group ID** and **Sample ID** fields. Enter Run Number.

7.1.10.1. A duplicated sample is run as two separate samples. Add the suffix "_dup" to both runs with each duplicate having a different Run Number ([SampleID]_dup.$01 and [SampleID]_dup.$02).

7.1.11. Enter analyst's LIMS User Name in the **Operator** box. Select **OK**.

7.1.12. When done, go to **Run File/Get Info**. Verify that the Obscuration and PIDS ranges are acceptable and initial in the designated box in the *Sediment Run Log*.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

7.1.12.1. If more sample is needed, repeat step 7.1.3.

7.1.12.2. If too much sample was added, select *Cancel*. Go back to step 7.1.4 to rinse the vessel and start over.

7.1.12.3. If a sample is re-run, do not discard/delete file. Changing the Run Number in the Sample Info box will create a new file.

7.1.13. Record the date, time (from **Run File/Get Info**) and analyst in the *Sediment Run Log*. Record the name of the file that has the correct extension to be uploaded.

7.1.14. Repeat process starting with 7.1.2 for each sample, duplicate or control.

7.1.14.1. Rinse spatula, sieve and beaker thoroughly with DI water before starting next sample.

7.1.15. Turn water source off at shutdown.

7.2. <u>>2.00 mm particle size</u>

7.2.1. Homogenize the sample by gently but thoroughly mixing with a metal spatula.

7.2.1.1. For sediment that is sticky or heavy with clay, add up to 10 mL of aqueous sodium hexametaphosphate. This surfactant aids in separating particles.

7.2.2. Weigh out 50-70 grams of sediment into a tared 150 mL beaker. Record beaker ID and sediment weight in the comments section of the *Sediment Run Log*. The portion weighed should be representative of the entire sample.

7.2.3. Weigh one 250 mL and one 600 mL beaker, and record the Beaker #s and Beaker Weights in the *Sediment Run Log*. The 250 mL beaker is for >2mm and the 600 mL beaker is for Pan, or <2mm.

7.2.3.1. There is room on the bench sheet for additional beakers if needed.

7.2.3.2. Add comments related to the sediment matrix. (Ex: shells, rocks, bottle caps)

7.2.4. Stack a #10 sieve on top of a brass sieve pan and pour sample onto the sieve. Use the rinse bottle of DI water to separate coarse and fine particles.

7.2.5. After separation is complete, use the DI rinse bottle and forceps to place the coarse fraction (>2mm) from the sieve into the 250 mL beaker. Be sure to flush all particles off the sieve.

7.2.6. Pour the fraction from the brass sieve pan into the 600 mL beaker. Rinse any remaining particles from the pan to the beaker with DI water. More than one beaker may be used, if needed. Record the Beaker # and Beaker Weight of all beakers used in the *Sediment Run-Log*.

7.2.7. Place all the beakers of sediment in the drying oven at 105 ± 2°C until completely dry. This may take anywhere from 24 hours to several days.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

7.2.8. Remove beakers from drying oven and allow them to cool completely. Weigh each beaker and record under "Beaker + Dry Sediment Weight" in the *Sediment Run Log.* Subtract the "Beaker Weight" from the "Beaker + Dry Sediment Weight" to record the actual "Dry Sediment Weight". Record total weights in the final column of the table.

7.2.9. A different analyst must check calculations and initial the "Calculations check by" box in the *Sediment Run Log* before uploading data.

8. DATA ARCHIVAL

8.1. Open **COULTER LS** and select **Preferences/Preference Files/Load Preferences.** Select appropriate file from \\fldep1\DEAR\Labs\Lab5\Biology\Bench Common\Sediments\Preferences backup.

8.2. Set preferences for sediment particle size tests as follows:

8.2.1. For Test ID SED-PSZ-LS, load preference file "ASTM12".

8.2.2. For Test ID SED-WWTH, load preference file "WWTH".

8.2.3. For Test ID SED-USDA, load preference file "USDA".

8.3. From menu, select **Preferences/Sieve/Listing**. Checkmark two boxes only: Mesh Size and Volume % Differential. Click **OK**.

8.3.1. Go to **Preferences/Data Export**. Export extension should be ".txt". Sample Info and Statistics boxes should be checked. Data format is Tab delimited and Numeric format is 123456.78. Click **OK**.

8.4. Select **File/Open** to open the first sample to be uploaded from the folder created during sample preparation and notated in the *Sediment Run Log.*

8.5. Select **RunFile/Get Info** and verify the date, time, and analyst in the *Sediment Run Log* against the info box. Also, verify that the Obscuration (8-12%) and PIDS (45-55%) are within range. If out of range, analyze sample again.

8.6. From menu, select **View/Listing**. Then select **View/Sieve**.

8.7. Select **RunFile/Export**. Click **Directory:** and select drive N:\SED Data 2012 created during user's initial setup. Click **OK**.

8.8. Click **Export**, then close file window.

8.9. Repeat steps 8.4-8.8 until all samples to be uploaded have been exported.

8.10. Refer to SOP BB-031 for QC Manager data uploading and verification instructions. Output data files are named by Sample ID and are located in the appropriate yearly folder in \\fldep1\DEAR\Labs\Lab3\Biology\Sediments.

8.11. After upload and verification, refer to SOP BB-032 for test level data authorization.

9. QUALITY CONTROL

9.1. Garnet Control

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

9.1.1. Coulter LS Control G 15 garnet grain is the control sample used to test the operation of the Coulter LS230 laser. Check values against the limits in the table on the control instructions at the bench.

9.1.2. Run a garnet control sample at least once a week. Save control files in the appropriate yearly folder in \\fldep1\DEAR\Labs\Lab5\Biology\Bench Common\Sediments\GARNET QC

9.1.3. Name file as: YYYY-MM-DD.

9.2. Sample Duplicates

9.2.1. Analyze a laboratory replicate for 5% of samples and a minimum of one replicate per batch. The acceptance limit is <25% RPD.

9.2.2. After completing a run, overlay graphs to check similarity. Open the first run of the duplicated sample. Then, go to **RunFile/Open for overlay** and select the duplicate. If measurements for sample duplicates do not appear similar, run another replicate. Use "_dup" suffix for all replicate files. They will have different extensions based on the Run number (ex: .$02).

9.3. Refer to the Bureau Quality Manual for method performance, corrective actions and handling out-of-control data.

10. SAFETY/HAZARDOUS WASTE MANAGEMENT

10.1. Refer to the Laboratory Health and Safety Plan.

10.2. Read MSDS information for reagent handling and disposal.

11. REFERENCES

11.1. EPA Method 4805572010: Procedures for Handling and Chemical Analysis of Sediment and Water Samples

11.2. Coulter LS Series Control Program Version 2.11 Supplement

11.3. USDA Natural Resources Conservation Service Soil Survey Manual, Particle Size Distribution. https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/survey/

11.4. Wentworth Grain Size Classification Scheme. http://www-odp.tamu.edu/publications/197_IR/chap_02/c2_f6.htm

11.5. NELAC Quality Systems Manual

11.6. FDEP Laboratory Quality Manual http://www.dep.state.fl.us/labs/library/lab_sops.htm

11.7. FDEP Laboratory Health and Safety Plan http://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

11.8. DEP SOP-001/01, *FS 1000 General Sampling Procedures*

11.9. DEP SOP BB-030, *Sample Custody, Preparation Labels and Worksheet Instructions for Bench Biology Samples*

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

11.10. DEP SOP BB-031, *LIMS QC Manager Data Uploading Instructions for Bench Biology and Microbiology Samples*

11.11. DEP SOP BB-032, *Test Level Authorization of Uploaded Results for Bench Biology Samples*

11.12. DEP SOP TA-06.38, *Calibration and Use of Mettler® PB403-S Balance*

APPENDIX OF CHANGES

08/08/2008	Change SOP Format. Updated Data Archival methods.
08/07/2009	Added two new Test IDs. Included reporting limits in Section 1.3.1. Added balance calibration in Section 3.3.1. Changed Section 6.4 to reflect new folder name format. Added Section 7.1.1.1 for clay sediment. Updated preferences in Section 8.1.
08/13/2010	Updated references to new version of Health and Safety Plan.
07/29/2011	Updated reference to Bureau Quality Manual.
07/16/2012	Updated directory for preference files.
07/16/2013	The first step for <2.00 mm particle size was changed so that the garnet control should be run, if needed. Added the location of the dfr*.$* file mentioned in Step 7.1.6.2.
08/08/2014	Removed Network Drive Setup Section, updated Create a Directory, Data Archival and Uploading & Verification Sections to reflect current computer systems.
08/12/2014	Re-worded for clarification purposes in Sections 8 & 9. Added 9.15.2 to reflect new comments protocol, and added flow chart to 9.15.3
09/17/2014	Reverted to version 3.4 since instrument can now be connected to a Windows 7 computer and user does not have to move files via flash drive.
01/15/2015	Formatted for 508 compliance
02/24/2015	Updated to reflect new File location.
03/15/2017	Corrected format in Section 1; corrected balance SOP reference and added model number for drying oven in Section 3; added note and corrected format in Section 6.3; clarified language, explained how to name duplicates, and underlined the two different method headings in Section 7; corrected format in Section 8; corrected format and clarified duplicate check in Section 9; updated hyperlinks.
3/15/2018	Added new Drying oven to Section 3. Equipment and Supplies. Added a comment for mixing in a separate container in section 6. Sample Preparation. Added a note about troubleshooting with the auto rinse option in section 7. Sample Analysis. Updated hyperlink in Section 11. References.
3/15/2019	Updated balance SOP reference.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.