iFADO Best Practice:

Analysis of Faecal Pellets in Sediment Trap samples using the FlowCam Macro

Background

This method describes the analysis of Faecal Pellets in Sediment Trap samples using the FlowCam Macro to count and classify pellets using an image-based system.

The FlowCam macro uses dynamic imaging particle analysis (DIPA) to rapidly evaluate particulate matter in a moving fluid. Direct, image-based measurements of particle size and other parameters enable differentiation of particle types in a heterogeneous mixture. The 5% formalin preserved sample is gently split using a Folsom splitter. The Folsom splitter is used instead of the more usual (for sediment trap samples) rotary splitter to reduce breaking up particles. The fraction to be analysed is pumped through the FlowCam Macro so that each particle is photographed and measured. The sample is collected and placed into new, larger container to include additional preservative used to flush the sample through. Scientists using sediment traps and with an interest in carbon flux. Best practice is reliant on have flowcam instrumentation. Then the work is in sample flow through and in image analysis.

Procedure

Reagents

- Sediment trap preservative contains:
- Formaldehyde (technical grade, stabilised with methanol)
- Sodium tetraborate (borax)

Equipment

- Fume hood (496/05)
- Nitrile gloves (EN 374)
- Lab coat
- Safety glasses
- 1L plastic beakers
- Plastic bottles for Samples
- Squeezy bottles
- Folsom splitter
- FlowCam Macro and accessories
- Flow Cell (5mm)
- 5L buckets
- Tubing
- Funnel
- Self-adhesive (SA) tape
- Clamp stand
- Adjustable clamps
- Record book
- Paper towel
- External hard drive
- Hazardous waste chemical carboy labelled "formaldehyde waste"
- Marker pen

Set Up

- Fill a labelled squeezy bottle with Sediment Trap Preservative.
- Switch on the FlowCam Macro and pump. Attach the intake and outtake tubes and a clean flow cell. Set pump speed to 240 rpm.
- Fit funnel into intake tube, using SA tape to seal it in place. Clamp funnel to stand. See photo.
- On the FlowCam, open Visual Spreadsheet.
- Load the appropriate context file depending on samples being analysed.
- Fill a beaker with RO, place outtake tube in the sink; pour the RO into the funnel and pump through the FlowCam Macro to rinse the flow cell.
- Open 'Set up and Focus' mode to check that the flow cell is clean enough and make any necessary adjustments to the 'Acceptable region'.

- Place the outtake tube into a collection beaker in the fume hood,
- Prime the FlowCam Macro with STP. Fill the system up to just below the intake funnel.

Figure 1: Procedure picture

Sample Running

- Pour the sample into the funnel intake, and the outtake tube into a collection beaker.
- Open 'AutoImage' mode and save the sample as ..\Cruise_number\sample_type\sample_name_fraction_analysed.
- Start the pump once the software has taken the calibration image.
- Run the sample then rinse the sample through the funnel with STP.
- Stop the AutoImaging process when the whole sample has run through the flowcell and stop the pump after the samples all run through into the beaker.
- Flush the FlowCam Macro through with STP into the same bucket.
- Pour the collected sample into a new bottle and write the name and split onto the bottle.
- Re-prime the FlowCam Macro with STP.
- Run and preserve all samples.
- Flush the FlowCam Macro through with RO water. Remove the tubing and flow cell. Switch off the FlowCam Macro and pump.
- Back the data up on to an external hard drive.

Each sample is split into a fraction that can be diluted sufficiently for the FlowCam to image individual particles. This varies from ½ sample to 1/64 sample. The faecal pellets are vulnerable to being broken-up by rough handling. Fractions should not be obtained by rotary splitters. Each division has the possibility to break up/damage FPs, so should be minimised. **It is essential to keep a record of the fraction so the data can be related to concentration in the original sample.**

Gently pour the sample into the Folsom splitter. Rock the splitter gently to homogenise the sample and pour into the collecting bottles. Repeat if necessary. Pour the fractions that are not needed into separate bottles. Label all bottles, including the fraction. Make a note of the fraction of the sample that will be analysed.

Potential learning or transfer

The best practice is considered useful to provide additional information that may otherwise be missed. It is a new practice for NOC and was set up under 1 month of iFADO time.

The method allows for analysis of organic material that would otherwise be filtered away or lost as it characterises different types of organic material with visual analysis

Further information

Related practices (re: sediment traps) are found in the literature but this is considered a novel approach using Flowcam analysis of the sediment trap samples. It has not yet been published and would require further take up by the community.