**Foraminifera cleaning procedures for laser ablation:**

Cleaning procedures vary by specimen type (e.g., cultured, robust fossil specimens, fragile fossil specimens, contaminated specimens, etc.). The recommended cleaning procedures, below, are only a guideline. Cleaning procedures vary considerably and depend on the specific scientific question(s) you are interested in, sediment type, and preservation state. In some cases, the initial cleaning may not be ideal, and you may learn, during the analysis, that the samples need an additional cleaning step or a modified cleaning protocol. If you are unsure of the necessary cleaning procedures for your samples, preliminary laser ablation data collection is highly recommended before you place all of your samples on double sided carbon tape for analysis.

General guidelines:

|  |  |
| --- | --- |
| **Specimen Type** | **Cleaning procedure** |
| Cultured/plankton tow | Oxidative step only and rinses |
| Fossil, robust, no evidence of metal oxide contamination\* | Rinses to remove clays, oxidative step to remove remnant organics |
| Fossil, fragile, no evidence of metal oxide contamination\* | Modified rinses to minimize sample loss, oxidative step to remove remnant organics (minimal or no sonication to reduce sample loss) |
| Fossil, robust, with evidence of metal oxide contamination\* | Rinses, oxidative step to remove remnant organics, reductive step |
| Fossil, fragile, with evidence of metal oxide contamination\* | Modified rinses to minimize sample loss, oxidative step to remove remnant organics, reductive step to remove metal oxides |

\*Surface contamination often can be excluded from the average TE/Ca ratio calculation by excluding the first few seconds of ablation during the data reduction step, using the contaminant phase as your guide. If you see evidence that contamination continues throughout the analysis (there may be contamination inside pores or on the surface of the calcite in the pores), reductive cleaning may be necessary. We rarely do the reductive step for shells prepared for laser ablation. The reductive step is HIGHLY corrosive, portions of your shells will dissolve. Utilize this step with caution.

**A few useful tips:**

If you are planning on doing paired isotope analyses with single specimens, we recommend measuring your samples on the longest dimension and weighing them prior to laser analysis. The weights are necessary to establish the minimum reference gas and to ensure the samples weigh enough for a single isotope measurement. The length/weight is useful for post analysis data interpretation, comparing the results to length/weight (which often covary) may be very useful. Photographs are also recommended if you plan to calculate area normalized weights.

Note: using a sieve is not the most accurate way to establish size because the samples can pass through the sieve on their shortest dimension. The laser has a measurement tool so you can get the size of your specimen using the laser, however, this adds time to your analysis, and it is probably worth it to measure the specimens beforehand. If you plan to calculate size normalized area data, take a photo of your specimens before analysis as well because some shells may break when they are removed from the carbon tape.

**Foraminifera Cleaning Protocols**

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# How to crack open single specimens of *O. universa*

Purpose: For fossil *O. universa*, the shell is cracked open to make it easier to remove finely adhering clays from the inner surface. For cultured *O. universa*, cracking the shells open exposes the inner surface, which may be covered with dried cytoplasm.

Note: For fossil specimens that are thinly calcified or appear poorly preserved, this step is done AFTER initial rinses. Keeping the specimens whole makes them stronger and less likely to shatter into tiny fragments (too small for LA) during the sonication steps (see instructions on the following page).

You will need:

1. DI water
2. Large black rubber stopper
3. Sable hair brush (5/0) (hair is better than synthetic bristles)
4. Surgical scalpel (note other labs use a needle – review literature...!)
5. Micropaleoslides for archiving the specimen OR microcentrifuge vials for cleaning

Instructions:

1. Place the black rubber stopper under the microscope and focus on the surface of the stopper
2. Place a single *very* small drop of DI onto the surface.
3. Dip the brush in DI, pick up the shell using a sable hair paintbrush (5/0), and transfer the specimen into the drop of water. Note that you can skip step 2 and transfer the specimen onto the rubber stopper using the DI that is in the brush. That is ‘usually’ enough water.
4. Using a surgical scalpel, gently crack the shell open. Practice this step on specimens that that you have that are in high abundances or from sediment intervals that are not being used for your study until you have mastered this step. Press the scalpel on the stopper just above the shell, then gently lower the scalpel onto the shell and press gently until the shell cracks. DI has a LOT of surface tension and the shell fragments often adhere to one another. To prevent this from happening, separate the fragments by separating them with a paintbrush BEFORE you remove the scalpel from the stopper.
5. After the samples are cracked open either transfer the cracked fragments back into a micropaleontology slide holder meant for single specimens OR transfer the specimens into a microcentrifuge vial in preparation for cleaning.

Notes: Why do we crack the shells in water? It prevents sample loss. Without it, you risk losing fragments due to static or just simply the act of fracturing the shell causes a fragment to fly off the rubber stopper. The water keeps the fragments in place. The purpose of the rubber stopper: It provides a little ‘give’ and helps the shell fragment into large pieces. Without it, the shells may fracture into smaller pieces.

# Initial rinses for single specimens of ROBUST fossil *O. universa* or single specimens of other species

Purpose: Remove clays, loosen secondary precipitates

You will need:

1. Microcentrifuge vials (recommended size: 0.6 ml)
2. A cleaning rack for holding the vials
3. Milli-q
4. Methanol
5. Paintbrush
6. Pipette (<300 l) and pipette tips (300 l)

Instructions:

1. For *O. universa*: Place single shells **after** they have been cracked open into individual vials. For all other species: place shells into the bottom of the microcentrifuge vials. If you are tracking single specimens (i.e. you have weighed/measured/imaged the specimens) then you need to place the specimens into their *own vial*. I recommend doing the weighing/measuring/imaging AFTER cleaning so that you can clean more than one shell per vial. Also, weights are more accurate after cleaning. I don’t add more than 5 whole shells per vial because whole they can break apart during the ultrasonication step.
2. Add Milli-q to each vial (to about 3 mm from top of vial)
3. Rap the rack to remove air bubbles from the vials (sometimes you have to tap individual vials or flick them with your finger to remove stubborn air bubbles)
4. Ultrasonicate for 5-30 seconds (do this carefully until you know how robust your shells are). Very dirty shells, or ones that are very robust can be sonicated for up to 1 minute. Shorter sonication is needed for more fragile specimens to prevent fragmentation.
5. Rap the rack gently to ensure shells are at the bottom of the vial
6. Aspirate the water. You can aspirate using a pipette and manually remove the water (I do this for ‘precious’ samples – e.g. low abundances or cultured shells) or use a vacuum aspirator (which goes much faster)
7. For fossils: Are the fossils still dirty? Can you see sediment in the water? (Water might be slightly discolored.) Repeat steps 2-6 2-3x, the water should become clear and free of clay particles.
8. After initial Milli-q rinses, repeat rinse steps using methanol: Add methanol to each vial, rap to remove air bubbles
9. Ultrasonicate for 5-30 seconds
10. Rap the rack gently to ensure shell are at the bottom; then aspirate methanol
11. Repeat steps 8-11 2x.
12. Repeat steps 2-6 to rinse the samples. Rinse 1x if you are only doing the rinses, rinse 2x if you are also doing the oxidation step. If the oxidation step will be completed, aspirate the Milli-q so that very little water remains and continue onto the next step (pg 6).

Modifications: These steps are modified as needed. I skip the initial rinse in Milli-q for whole shells because the shells often float – the water doesn’t always go into the shells so they end up full of air. I start the cleaning with methanol instead – beginning at step 8. I do 1 methanol rinse followed by 1 milli-q rinse to check to see how dirty the shells are and if I don’t see a lot of clay in the water, I return to the methanol rinses and skip the repeat initial mill-q rinses.

# Modified initial rinses for single specimens of FRAGILE shells

Purpose: Removal of clays, loosen secondary precipitates. All shells remain whole and are cleaned individually to minimize sample loss.

You will need:

1. Microcentrifuge vials – one for each sample
2. A cleaning rack for holding the vials
3. Milli-q
4. Methanol
5. Paintbrush
6. Pipetter (<300 l) and pipette tip (300 l)

Instructions:

1. **KEEP SHELLS WHOLE!**
2. Place whole specimens in clean microcentrifuge vials, one vial per shell
3. Add methanol to each vial (to about 3 mm from top of vial).
4. Rap (sharply tap) the rack to remove air bubbles from the vials (sometimes you have to tap/flick individual vials to remove stubborn air bubbles).
5. Ultrasonicate for 5-20 seconds in 5 second intervals – observe the shell under the microscope or by eye to see if the shells are whole
   1. **STOP ultrasonicating if any specimens fragment and go to step 13**
6. Rap the rack gently to ensure shell is at the bottom of the vial, then remove methanol with aspirator or pipette.
7. Repeat steps 2-6 using Milli-q 2x
   1. **IF at any time the shells fragment during this process, go to step 13**
8. Add methanol to each vial, rap to remove air bubbles
9. Ultrasonicate for 5-20 seconds; Rap the rack gently to ensure shell is at the bottom of the vial, then aspirate methanol
10. Repeat steps 9-12 2x
11. Repeat steps 2-6 2x
12. If shell is still whole, break it open (see instructions on how to crack open the shells), and carefully clean out internal clays with a wet brush. Fragments can be analyzed easily by laser.
13. If clays are still present in the inside, additional rinses WITHOUT ultrasonication are necessary to remove clays.
    1. Add milli-q ‘energetically’ (i.e. squirt it into the bottom/down the side of the microcentrifuge tube) so it picks up and suspends all the loose clay particles), watch the shells settle to the bottom of the vials, and remove solution quickly.
    2. Repeat until the fragments appear clean and then do one final rinse with methanol, which evaporates quickly.

# Oxidative Step

Purpose: Remove remnant organic matter

You will need:

1. Reagents
   1. H2O2 (30%)
   2. 0.1N NaOH
2. Rack lid. If you are only cleaning single specimens or small samples, rack lid isn’t necessary. Do NOT use a rack lid if the specimens were cultured, recently alive (plankton tow) or have a lot of organics in them – too much pressure will build in the vials.
3. Milli-q
4. DI water and glass loaf pan (we use pyrex dishes) or crystallizing dish filled with DI
5. Beaker with extra DI water
6. Hotplate
7. Thermometer
8. Pipets (0-1000 l and/or 0-200 l) and pipette tips
9. Graduated cylinder for the NaOH

Instructions:

1. Make the solution (modify amount of reagent needed to clean the number of samples you are preparing for analysis. E.g. 10 samples will require MUCH less reagent):
   1. For **fossil** specimens (this recipe makes enough to clean 30 or more large fossil samples)
      1. 100 l of 30% H202 (for fossil specimens)
      2. 30 ml of 0.1N NaOH (trace metal clean grade)
   2. For **cultured** specimens:
      1. Equal parts H2O2 and 0.1N NaOH (e.g. 2 ml of each)
2. Set the pipet for oxidizing solution
   1. 250 l for samples large samples (multiple foraminifera or samples with many shells cracked open). This is the amount of reagent used to clean up to 500 g of shells.
   2. 100 l for samples that are small (e.g. single specimens)
3. Add solution to vial (250 l for large samples, 100 l for small samples)
4. Cap the samples (fossil specimens only; reminder: do NOT use a rack lid for organic rich samples)
5. Set each rack into an evaporating dish (large pyrex crystallizing dish or a loaf pan) filled with water and place it on a hot plate. The water should be ~65°C (warm it up before use)
6. Heat samples for about 5 minutes then flip rack (if cleaning fossil specimens) ultrasonicate briefly and return rack to the hot-water bath for another 5 minutes. Then repeat the ultrasonication step. For fragile samples, skip the sonication step, instead, gently rap rack on counter to remove bubbles and knock the samples to the bottom of the vials
7. After 10 minutes, remove samples from warm water bath. For fossil specimens, slowly open caps to release pressure
8. Add Milli-q to vial (fill to about 2 mm from top)
9. Rap the rack gently on the counter to remove bubbles and ensure that the specimens are at the bottom of the vial
10. Aspirate solution (clean caps too if you had the samples capped)
11. Repeat steps 7-9 3x to ensure complete removal of the oxidizing reagents
12. If skipping the reductive step, remove specimens from vials

# Reductive Step

Purpose: Removal of metal oxides/Mn-Mg carbonate overgrowths. This step is not recommend unless you are positive it is necessary because this step is very corrosive to the calcite.

Note: If your samples are very fragile or you are preparing the samples for laser ablation analyses, you might want to minimize ultrasonication time because it can cause the shells to break into very small fragments or shatter into a powder.

You will need:

1. Reagents:
   1. Ammonium hydroxide
   2. Ammonium citrate
   3. Anhydrous hydrazine
2. Rack lid
3. DI water and glass loaf pan or crystallizing dish filled with DI
4. Beaker with extra DI water
5. Hotplate
6. Thermometer
7. Pipets (0-1000 l and/or 0-200 l) and pipette tips

Instructions

1. Make the solution used to remove metal oxides
   1. 10 ml ammonium hydroxide
   2. 10 ml ammonium citrate (citric acid/ammonia solution; you can make this solution by adding 25mg of citric acid to 500 ml of ammonium hydroxide – make in ice bath)
   3. 750 l of anhydrous hydrazine (very volatile, toxic, and explosive)
   4. Combine above in a small glass beaker, modify amount of reagent if less is needed (e.g. 5:5:375 or 2:2:150)
2. Add 50 l of solution to each vial for small samples (single specimens) 100 l to larger samples
3. Close caps very tightly
4. Ammonia has high vapor pressure so a rack lid is placed on top and screwed down
5. Place racks in hot water bath for 30 minutes (sub-boiling). Every 2-4 minutes, remove rack, flip it over one or two times, ultrasonicate for 5s, and place back into water bath. This agitates the reagents and discourages re-precipitation of oxides onto another surface.
6. Top off hot water in water bath as needed to ensure the samples are bathed in the hot water (can use two hotplates for this purpose or place a beaker of water on the hotplate with your samples so that hot water is readily available)
7. After the 30 minute hot water bath is complete, siphon off the reagent (make sure samples are at the bottom of the vials, rap vial rack on counter if necessary)
8. Add milli-Q to vial (fill to about 2mm top of vial)
9. Rap the rack, siphon water with aspirator, clean the caps too
10. Repeat steps 8-9 2x to ensure reagent is removed.

# 

# How to remove samples from microcentrifuge vials for laser ablation

Purpose: To remove the cleaned shells from the vials and transfer them to holders or glass slides prepared for LA. These instructions are for LA only. For solution analyses, samples have a final weak acid leach and are transferred into clean microcentrifuge vials. See page 8.

You will need:

1. Pipet tips
2. Scissors
3. Pipetter
4. Sable brush (5/0)
5. Milli-q or methanol
6. Archive slide or slide prepared for LA (glass slide with double-sided carbon tape)

Instructions:

1. Take a small pipet tip and cut off about ¼ inch of the tapered tip
2. Add 100 L of methanol or Milli-q to the vial and quickly remove the water by drawing it back into the pipet tip. The foraminifera ‘should’ be in the pipet tip with the water/methanol
3. Transfer the solution to a petri dish or welled slide.
4. Transfer specimen from the water/methanol using the paintbrush to one of the following:
   1. A micropaleoslide to archive the specimen until analysis. Specimen can be transferred using a sable hair brush **–OR–**
   2. Transfer the specimen onto a black piece of paper to remove some of the water/methanol then transfer specimen onto a carbon tape prepared sample slide. The sample can be moved around so that the aperture side faces down until the water/methanol dries.

Note: Methanol can be used to remove shells to adjust their placement or remove them from the tape after laser analyses are complete.

# How to amputate chambers for LA or single chamber isotope analyses

Purpose: To open the shell so the chamber can be analyzed from the inside using the laser or for single chamber isotope analyses. This step is typically done after the shells are clean. If the shells are dirty on the inside after the chambers are amputated, additional cleaning steps are necessary (repeating rinses, etc). We have not encountered this issue.

You will need:

1. DI water
2. Methonol
3. Large black rubber stopper
4. Sable hair brush (5/0) (hair is better than synthetic bristles)
5. Surgical scalpel
6. Micropaleoslides for archiving the specimen OR microcentrifuge vials for cleaning

Instructions:

1. Place a large (2-3 inch) black rubber stopper under the microscope and focus on the surface
2. Place a single *very* small drop of water onto the surface
3. Pick up the specimen using a sable hair paint brush (5/0) and transfer the specimen into the drop of water
4. Using a surgical scalpel, place the blade along chamber sutures and gently press down until the chamber cracks open. Practice this step on specimens that that you have that are in high abundances or from sediment intervals that are not being used for your study until you have mastered this step.
5. After the samples are cracked open either transfer the cracked fragments back into a micropaleontology slide labeled with the sample name/chamber number OR transfer the specimens onto a glass slide that has been prepared with double sided sticky tape and place specimen with the inner surface facing up. Use methanol to move the specimen or flip it over if needed.

## Removing shells for isotope ratio analyses after laser ablation:

You will need:

1. Methanol
2. Sable hair brush (5/0) (hair is better than synthetic bristles)
3. Microslides or the glass ampules for the kiel

Instructions:

1. Dip brush in methanol, wipe off excess on a Kim wipe
2. Place methanol on specimen and gently dislodge shell using the brush
3. Transfer shell to labeled microslide or glass ampule