Appendix 5. Annotated list of a new radiolaria- and diatom-slide preparation

Twenty-seven sub-samples from H.M.S. Challenger Collection deposited in the Natural History Museum in London were provided with the project. For the future studies on micofossils in the *Challenger Expedition* we made slides from the samples with the following procedure, and these should be deposited in the five institutions: NHM in London, MN in Berlin, Tohoku Univ., Utsunomiya Univ. and NMNS in Tokyo.

Additional **Nineteen sub-samples from H.M.S. Challenger Collection** housed in the NHM, London were provided to the project. Regarding these samples, only radiolarian slides were made and a 19 slide set was distributed and dposited in the same five institutions.

Methods for Preparation of Radiolarian Slides

Disaggregation	The sample was placed in a 300ml beaker with the boiled solution of hydrogen peroxide (H_2O_2, 10%, 150ml) and hydrochloric acid (HCl, 5%, 30ml).
Washing	After boiling for about 40-60 minutes, a suspension was added by water and poured into a 45μm-sieve and washed.
Cleaning	Washed residue was placed in a 300ml beaker with the warmed (60-80˚C) solution of sodium hexametaphosphate (1-2%, 200ml) to disperse clay grains within a shell for 40 to 60 minutes.
Drying	The cleaned residue was washed upon a 45μm-sieve and dried.

Preparation of Strewn Slides

Dispersion	The dried residue was placed on a glass slide to which was already applied a thin glue of a gum tragacanth. Then, the residue was moisted by a wet air and then fixed on the slide glass after it dried. Turning over the slide glass and an excess residue was freed.
Mounting	A single drop of xylen was poured on the center of the residue and then was mounted on cover glass with a Canada Balsam.

Methods for Preparation of Diatom Slides

Chemical & Physical Cleaning

Drying	sediment samples dried at 60°C for 24 hours
Weighing	about 0.5 - 1g were weighed at an accuracy of 0.001g
Disaggregation	the sample was placed in a 200ml beaker with the boiled solution of hydrogen peroxide (H_2O_2, 15%, 20ml) and hydrochloric acid (HCl, 5%, 1-5ml)
Centrifugation	after boiling for about 20 minutes, a suspension was poured into a centrifuge tube and filled with water the suspension was centrifuged for 2 minutes at a speed of 1200rpm fine material in suspension was carefully removed by decantation, and the tube was filled again with water this procedure was repeated 5 times
Suspension	the residue was diluted to 10ml with water and stored in a glass vial

Preparation of Slides

Dilution	the suspension was placed in a short test tube by the aid of an automatic micropipette, and diluted by water to obtain a suspension of proper density of diatom valves
Drying	the diluted suspension was placed on a square cover glass, 18 x 18mm, and dried at 45°C
Mounting	the sample was mounted on a slide glass with Styrax