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 microfossils 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 deposited 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\(_2\)O\(_2\), 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 45\(\mu\)-m 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 45\(\mu\)-m 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 moistened 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\(_2\)O\(_2\), 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