THE "DRY" SILVER NITRATE METHOD

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INTRODUCTION

Because of the numerous problems with the basic dry Klein (3, 4) technique, Foissner (1) and others (e. g., 2, 5) introduced some improvements. The dry methods ("dry" because cells are air-dried and not chemically fixed before being treated with silver nitrate) provide preliminary information on the somatic and oral infraciliature (=ciliary pattern) and are often best for revealing the silverline system (= lines revealed by silver nitrate and connecting basal bodies and other cortical organelles such as extrusomes and the cytopyge). Although the results vary highly, the method is worthwhile because it is quick and often produces excellent preparations, which can be well documented since the cells are flattened during dehydration. Only cortical structures are revealed. Examples: Fig. 1 - 4.

PROTOCOL

1. Take 5-10 clean slides and spread a very thin layer of albumen over the middle third of each with a finger-tip. Dry for at least 1 minute.
   Remarks: The egg-albumen (remove germinal disk! do not add glycerol) must have kept open in a wide-necked flask for at least 20 hours; fresh albumen is often less satisfactory. It can be used for 2-3 days if the flask is subsequently sealed; do not, however, stir before use, but skim the albumen from the surface with a finger-tip. To facilitate spreading breathe on slide so that a film of condensation is produced on which the albumen can glide. The albumen layer must be very thin and uniform and should not cover cells.
2. Place a drop of fluid containing the ciliates on the albumized slide, spread with a needle (do not touch albumen layer!) and dry preparation at room temperature.
   Remarks: Even single specimens can be placed on the albumized slide with a micropipette. If necessary enrich ciliates by gentle centrifugation or by leaving sample to settle for a few hours, after which time oxygen depletion induces many ciliates to move to the water surface. The amount and chemical composition of the fluid with which the ciliates are air-dried as well as temperature and humidity greatly influence the results. Therefore, 5-10 slides should usually be prepared simultaneously to vary these parameters, e. g. by washing cells with different amounts of distilled water or fresh culture medium. Washing cells with distilled water or spreading the drop to a very thin film is especially recommended with saline fluids, e. g. seawater, sewage, and soils. Temperature and humidity are easily varied using an ordinary hair-dryer.
3. Apply some drops of silver nitrate to the dried material for about 1 minute.
   Remarks: Do not touch albumen layer with the pipette. The reaction time does not influence the results; a few seconds are adequate.
4. Wash slides for about 3 seconds under distilled water and re-dry.
   Remarks: Wash gently! Apply water current from the top third of the tilted slide so that water runs gently over the dried material. Leave slides tilted during drying.
5. Pre-develop dried slide by exposing it for 5-60 seconds to a 40-60 watt electric bulb at a distance of 3-10 cm.
   Remarks: Time and distance influence intensity of impregnation (see also next step).

6. Apply a few drops of developer to the dried preparation for about 30-60 seconds.
   Remarks: The pre-development (step 5), the composition of the developer, and the material itself influences impregnation intensity, and quality. The best ratio of these parameters must be evaluated in pilot experiments. If the developer is well adjusted, the albumen around the dried fluid turns brownblack; if the developer is too strong, the albumen appears black (add some component A [see Reagents] and/or reduce pre-developing time); if the developer is too weak, the albumen appears brownish (add some components B and/or C and/or increase pre-development time).
7. Pour developer off slide, rinse gently in tap water for 5-10 seconds and immerse in fixative (sodium thiosulfate).
8. Remove slide from fixative, rinse gently in tap water for 5-10 seconds and immerse in 96-100 % ethanol.
   Remarks: Fixative must be thoroughly removed, otherwise crystals are formed in the alcohol and remain on the slide, causing the impregnation to fade with time. Do not wash too long and do not use distilled water, otherwise cells swell and eventually detach from the slide! Use ethanol as denaturated alcohol may contain substances which cause fading of preparations. Preparations usually fade within a few weeks when the silver nitrate is reduced only by sunlight or a UV-lamp.
9. Transfer slides to fresh 100 % alcohol for 3 minutes and air-dry again. Mount in synthetic neutral mounting medium (e. g., Eukitt, Euparal).
   Remarks: Slides should be tilted during drying. Mounting medium should be of medium viscosity. The preparation is stable.

REAGENTS

a) Silver nitrate solution (long term stability in brown flask)
   1 g silver nitrate ($AgNO_3$)
   ad 100 ml distilled water

b) Developer (stable for about 1-3 days; must be renewed as soon as it turns dark brown or shows crystals; mix components in the sequence indicated)
   20 ml solution A
   1 ml solution B
   1 ml solution C

   Solution A (this is an ordinary developer for negatives; dissolve ingredients in the sequence indicated; stable for years in brown bottle)
   1000 ml hot tap water (about 40 °C)
   10 g boric acid (H_3BO_3)
   10 g borax ($Na_2B_4O_7$)
   5 g hydroquinone ($C_6H_6O_2$)
   100 g anhydrous sodium sulfite (Na_2SO_3)
   2.5 g metol = methylamino-phenol-sulfate = $(CH_3NHC_6H_4OH)_2 \cdot H_2SO_4$

   Solution B (this is a concentrated photographic paper developer; dissolve ingredients in the sequence indicated; stable for about 6 months in brown bottle; soon turns brown [oxidizes], which, however, does not influence its activity)
   100 ml distilled water
   0.4 g metol = methylamino-phenol-sulfate = $(CH_3NHC_6H_4OH)_2 \cdot H_2SO_4$
   5.2 g anhydrous sodium sulfite (Na_2SO_3)
   1.2 g hydroquinone ($C_6H_6O_2$)
   10.4 g sodium carbonate (Na_2CO_3)
   10.4 g potassium carbonate (K_2CO_3)

0.4 g potassium bromide (KBr)

Solution C (stable for several years)
10 g sodium hydroxide (NaOH)
ad 100 ml distilled water

c) Fixative for impregnation (stable for several years)
25 g sodium thiosulfate ($Na_2S_2O_3 . 5H_2O$)
ad 1000 ml distilled water

LITERATURE CITED

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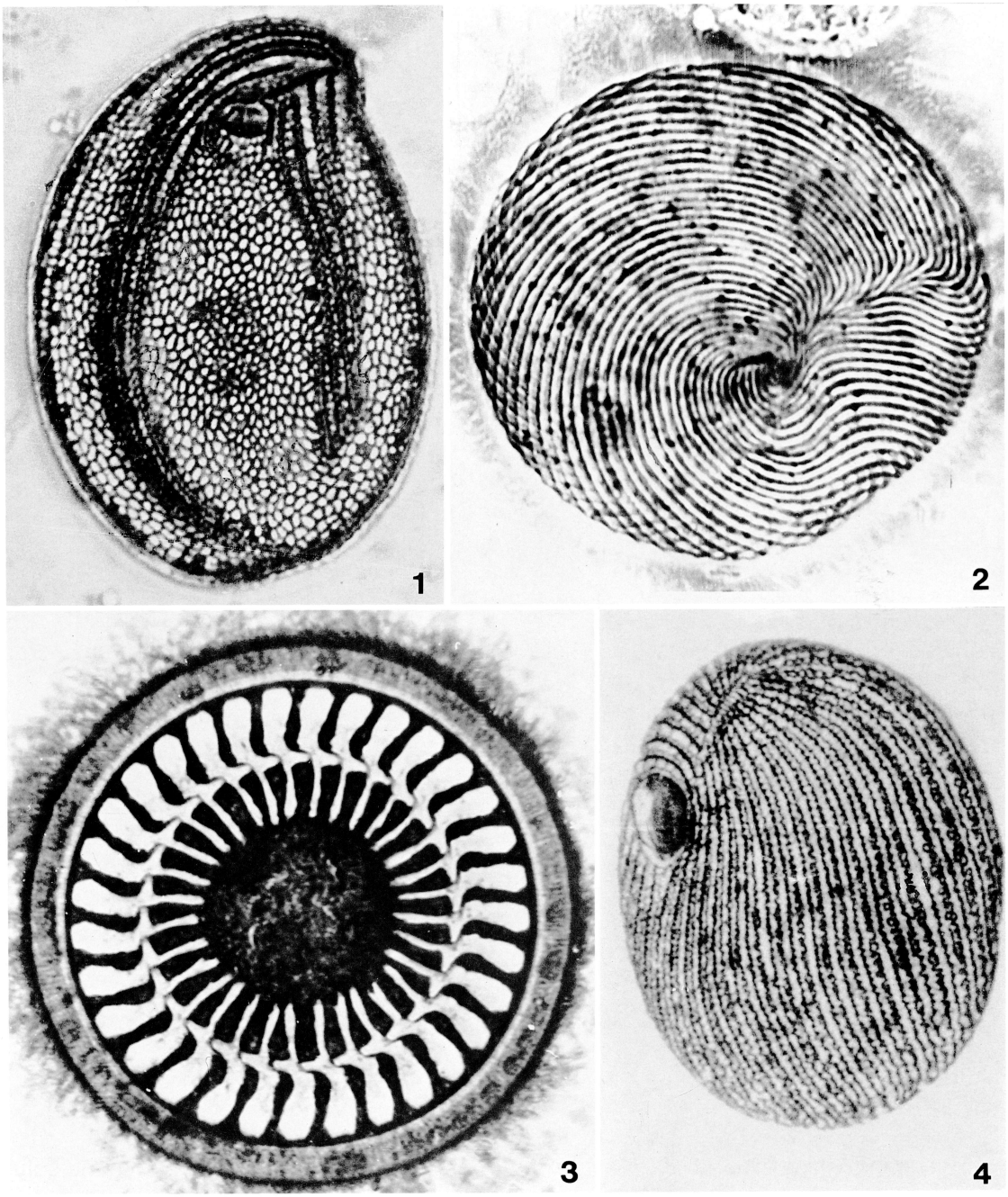

Fig. 1 - 4. Protists prepared with the dry silver nitrate protocol described. **1.** *Chilodonella uncinata*, a cyrtophorid ciliate; ventral view, length about 45 μm. **2.** *Peranema trichophorum*, a heterotrophic euglenoid flagellate; contracted specimen, diameter about 20 μm. **3.** *Trichodina mutabilis*, a mobiline peritrichous ciliate; adhesive disc, diameter about 50 μm. **4.** *Glaucoma scintillans*, a tetrahymenid ciliate; ventro-lateral view, length about 50 μm.