

## INSTRUMENTS AND METHODS

### PREPARATION OF SECTION PLANES IN SNOW SPECIMENS

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**ABSTRACT.** Diethyl phthalate, dimethyl phthalate, ethyl anisate, and  $\alpha$ -tetralone are examples of liquids that can be used to fill the pore space of snow specimens. After the pore space is filled, the specimen is frozen solid. A section plane is microtomed and polished. Photomicrographic contrast of the section plane is improved by gently rubbing on to the plane a water-insoluble powder, such as carbon powder, or by rubbing on a water-soluble stain such as powder of acid fuchsin. Photomicrographic contrast is also improved by adding a water-insoluble dye such as oil blue N or oil red O to the pore-space filler.

**RÉSUMÉ.** Préparation de sections planes dans des échantillons de neige. Le phthalate diéthylique, le phthalate diméthyle, l'anisate éthylique et le  $\alpha$ -tétralone sont des exemples de liquides qui peuvent être utilisés pour remplir les pores des échantillons de neige. Lorsque les pores sont remplis l'échantillon est congelé fortement. Une section plane est faite au microtome et polie. Le contraste des microphotographies de la section plane est augmenté en frottant doucement la surface avec une poudre insoluble dans l'eau telle que de la poudre de charbon de bois ou en appliquant un colorant soluble à l'eau tel que la poudre de fuschine acide. Le contraste des microphotographies est aussi amélioré en ajoutant au liquide remplisseur des pores un colorant insoluble dans l'eau tel que oil blue N ou oil red O.

**ZUSAMMENFASSUNG.** Vorbereitung von Dünnschliffen in Schneeproben. Diethyl-Phthalate, Dimethyl-Phthalate, Ethyl-Anisate und  $\alpha$ -Tetralon sind Beispiele für Flüssigkeiten, die zur Füllung der Porenräume von Schneeproben dienen können. Nach Auffüllung des Porenraumes wird die Probe eingefroren. Ein Dünnschliff wird geschnitten und poliert. Der photomikrographische Kontrast des Dünnschliffs wird durch leichtes Einstäuben der Oberfläche mit einem wasserunlöslichen Pulver wie Kohlenstaub, oder durch Einreiben einer wasserlöslichen Verunreinigung wie saures Fuchsin erhöht. Dieselbe Wirkung hat die Beifügung eines wasserunlöslichen Farbstoffes wie z.B. Ölblau N oder Ölrot O in das Porenfüllmittel.

#### INTRODUCTION

Bader and others (1939), Quervain (1948), Narita (1969, 1971), and Kry (1975) described techniques for preparing section planes in snow specimens for photomicrography and image analysis. Sample preparation includes: filling the pore space with a supercooled liquid, freezing the liquid so that the specimen becomes a rigid solid, cutting and microtoming the solid to obtain a microscopically plane surface, and treating this surface to enhance contrast for photomicrography.

#### PORE FILLER

The first task is to choose a water-insoluble liquid that can fill the pore space of the snow specimen without dissolving the ice skeleton. Just what constitutes acceptable "water insolubility" of snow grains has not been established. In this study, it is assumed that an "insoluble" pore filler would dissolve less than one part H<sub>2</sub>O for 100 parts of filler. For many families of organic liquids (alcohols, esters, ketones), 1:100 water insolubility is frequently observed if the number of carbon atoms exceeds about five. The pore filler should also perform as follows:

- (1) Melt above laboratory temperature, which is usually in the range of  $-20$  to  $0^{\circ}\text{C}$ .
- (2) Supercool 5 deg or more below its melt point, and remain in the liquid state when poured or gently agitated at laboratory temperature.

- (3) Have sufficiently low viscosity in the supercooled state to flow by capillary action into pore space.
- (4) Freeze into a rigid solid that can be microtomed at laboratory temperature; for convenience, freezing should initiate at dry-ice temperatures or higher.
- (5) Freeze into a solid that has a surface which can be polished and treated with dyes; that is, the surface must have a negligible liquid film at laboratory temperatures.

Virtually all liquids can be supercooled to some extent but, as a general rule, chemicals with symmetric structures, e.g. benzene, cannot be supercooled significantly below their melt point. By contrast, chemicals with asymmetric structures such as esters, ketones, aldehydes, and alcohols will normally supercool 5 deg or more, resist solidification when poured and agitated, and do not drastically increase in viscosity in the 5 deg supercooled region below the melt point.

A large number of water-insoluble liquids which satisfy the above conditions (1) through (5) can be eliminated on the basis of cost, toxicity, corrosivity, and decomposition in storage. The cost factor can be offset somewhat if the chemical is re-usable (after use, the specimen is melted, then refrozen and the ice is easily separated from the water-insoluble chemical). Toxicity is a consideration because many refrigerated laboratories (typically, "walk-in" freezers) are poorly ventilated, confined work areas. The sawing and microtoming of specimens generates particulate wastes which accumulate and pose a health hazard despite precautions of wearing protective gloves, aprons, eyeshields, and respirators. Almost all water-insoluble inorganic liquids, aromatic amines, halogenated hydrocarbons, and aromatic nitro compounds can be excluded on the basis of toxicity. As a starting guideline, toxicity and corrosivity are usually minimized if the organic liquid has a formula restricted to  $C_xH_yO_z$ , but each possibility has to be studied individually, and moreover it can be assumed that there will be some risk even using the most innocuous of possibilities.

#### PORE FILLERS USED IN EARLIER STUDIES

Table I lists chemicals used earlier. Tetrabromoethane (Bader and others, 1939) is toxic (acutely and chronically), a potent mutagen, will corrode microtome knives, and cannot be recommended. Aniline (Kinosita and Wakahama, 1960) is toxic and water soluble. Before use as a pore filler it must be saturated with water; this lowers its melt point to about  $-12^{\circ}\text{C}$ , which seems unnecessarily low for most stereological studies. Ethyl laurate (Bader and others, 1939) freezes with a dendritic texture that is difficult to confine and too soft for microtoming. Diethyl phthalate (Quervain, 1950) is used by many investigators in Switzerland and North America. It is satisfactory if the laboratory temperature is colder than  $-10^{\circ}\text{C}$  during microtoming and surface preparation. A liquid surface film begins to appear at about  $-7^{\circ}\text{C}$ , depending on the particular brand, contamination, and decomposition in storage. The toxicity of diethyl phthalate has been discussed by Sax (1979) and many others (Lewis, 1979).

TABLE I. PORE FILLERS USED IN EARLIER STUDIES

Name	Formula	Formula weight	Approx.* melt point $^{\circ}\text{C}$	References			
				Weast, 1976	Windholz and others, 1976	Sax, 1979	Lewis, 1979
Tetrabromoethane	$\text{C}_2\text{H}_2\text{Br}_4$	345.67	0	C-293 e265	8902	338	K18225000
Aniline	$\text{C}_6\text{H}_7\text{N}$	93.13	$-12^{\dagger}$	C-112 a1002	692	379	BW6650000
Ethyl laurate	$\text{C}_{14}\text{H}_{28}\text{O}_2$	228.38	0	C-281 d301	3758	668	
Diethyl phthalate	$\text{C}_{12}\text{H}_{14}\text{O}_4$	222.24	-4	C-436 p821	3783	581	TI1050000

\* Depends on purity.

$\dagger$  Water-saturated mixture.

#### PORE FILLER AT WARMER TEMPERATURES

There are probably a large number of alternatives to diethyl phthalate that could be used at warmer temperatures ( $> -10^{\circ}\text{C}$ ). For example, several brands of dimethyl phthalate (see Appendix A) gave consistently good results with laboratory temperatures in the range of  $-10^{\circ} \leq T \leq -5^{\circ}\text{C}$ . A surface film appears on dimethyl phthalate at  $-3^{\circ}\text{C}$ ; this requires that the laboratory temperature remains below about  $-5^{\circ}\text{C}$  during microtoming, surface preparations, and photomicrography. Purified (99%) dimethyl phthalate supercools to below  $-15^{\circ}\text{C}$ , at least for short periods (c. 1 h), and particles of dry ice may be required to initiate freezing. After it has been re-used a few times, contaminated dimethyl phthalate may freeze spontaneously at  $T > -10^{\circ}\text{C}$ . Its toxicity has been discussed by Sax (1979) and others (Lewis, 1979).

#### PORE FILLERS THAT ARE SOLID AT $0^{\circ}\text{C}$

In applications where it is necessary to transport rigid snow specimens from a field location to a laboratory, or where it is necessary to fill the pore space at  $0^{\circ}\text{C}$  (wet-snow studies), then the filler must melt above  $0^{\circ}\text{C}$ , and must supercool to  $0^{\circ}\text{C}$  or below. This added constraint greatly narrows the possibilities. Appendix A provides data on 20 water-insoluble chemicals with melting points in the range 0 to  $8^{\circ}\text{C}$ . These were chosen by first excluding all chemicals except those with the formula  $\text{C}_x\text{H}_y\text{O}_z$ , then eliminating chemicals of high cost ( $> \$100/\text{kg}$ , 1980 Canadian prices), and finally eliminating chemicals of high toxicity using guidelines from Sax (1979) and Lewis (1979). Best overall results were obtained using ethyl anisate and  $\alpha$ -tetralone.

#### DYEING THE FILLER

Diethyl phthalate, dimethyl phthalate, and ethyl anisate are almost colorless;  $\alpha$ -tetralone has a weak brownish red color. To enhance photomicrographic contrast, it is helpful to add a small quantity of water-insoluble dye to the above liquids. Two dyes which color all the chemicals shown in Table I and Appendix A are oil blue N (solvent blue 14) and oil red O (solvent red 27).

#### FILLING, MICROTOMING, AND POLISHING

An example of a quick and simple procedure is as follows. The snow specimen (approx. 30 mm by 30 mm) is placed in a small stainless steel tray. The supercooled liquid filler is poured into the tray. Capillary action carries the liquid up into the pore space to at least 20–30 mm above the liquid level in the tray. The capillary rise is easier to observe if the filler is dyed, as discussed above. If the snow specimen is relatively dense ( $> 600 \text{ kg/m}^3$ ) or relatively coarse-grained with large pores, then capillary action may not uniformly fill the specimen; total immersion may be necessary. Small chunks of dry ice are inserted in the corners of the tray to initiate freezing. (Frozen scrap particles of the filler can be used if dry ice is not available.) To accelerate freezing, the tray is then set in a small cold chamber ( $T < -20^{\circ}\text{C}$ ). After the filler completely solidifies (c. 2 h storage at  $-20^{\circ}\text{C}$ ), the tray is clamped in a microtome and shaved. Next, the surface of the specimen is polished gently with a high-quality lens-cleaning tissue and left undisturbed at laboratory temperature for about 5 to 10 min so that sublimation of the ice etches an observable boundary around the ice–filler interface. Exposed to incident light under the microscope, the ice appears darker since it is more transparent than the filler, an effect amplified considerably if the filler is dyed as discussed above. A ring illuminator connected to a fibre-optic light source will furnish relatively cool incident lighting with minimum disturbance to the surface of the specimen.

## CONTRAST ENHANCEMENT

Gentle polishing of the microtomed surface is an essential step for contrast enhancement. Presumably, removal of asperities minimizes surface scattering of incident light and accentuates the absorption difference between ice and filler. Contrast for photomicrography of the polished surface is further improved using either of two methods:

- (1) A very finely ground, water-insoluble powder (e.g. carbon powder) is gently rubbed on the surface with a cotton swab. To avoid accumulation of large particles on the surface, it is helpful to apply the powder through a lens tissue, or similar filter. The surface is then gently polished with lens tissue. The powder is rubbed off the surface, but remains in the microscale crevices at the ice–filler boundary, thus outlining the boundary and greatly improving photographic contrast. Various carbon powders were compared; best results were obtained using “fingerprint” powder and lampblack. Graphites gave poorer results.
- (2) The ice crystals are stained with a water-soluble compound. Good results were obtained by dipping a cotton swab into a finely ground powdered stain, picking up only a trace of the stain on the swab, which is then rubbed on the surface. It is sometimes helpful to clean the surface with lens tissue. Attempts to use liquid stains in place of powder stains gave poorer results.

Method (1) produces the sharpest outlines, whereas method (2) produces colourful photomicrographs (see Fig. 1).

A large number of water-soluble stains were compared in connection with method (2) (see Appendix B). Best results were obtained using acid fuchsin (Gurr, 1960, p. 202) which colors the ice violet-yellow and contrasts nicely against a filler dyed with oil blue N. The yellow is intensified by mixing eosin Y (Gurr, 1960, p. 173) with the acid fuchsin. Good results were also obtained using aniline blue water soluble (Gurr, 1960, p. 39), methyl blue (Gurr, 1960, p. 269), water blue (Gurr, 1960, p. 409), fast green FCF (Gurr, 1960, p. 195), fast sulphon black FCF, and sulfonazo III, all of which are acid stains with high water solubility, characterized by

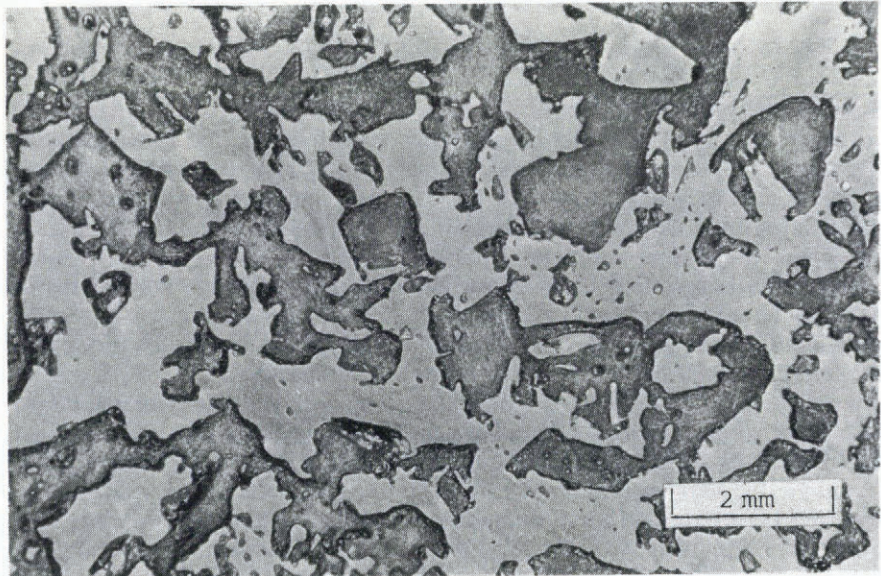


Fig. 1. Photomicrograph of a stereological plane cut through a coarse-grained snow specimen.

sulfonic acid groups ( $\text{SO}_3^-$ ). Narita (1969, 1971) also obtained good results with water blue. Less satisfactory or negative results were obtained using the remaining stains listed in Appendix B.

#### ACKNOWLEDGEMENT

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## APPENDIX

### A. PORE FILLERS TRIED IN THIS STUDY, AND RESULTS

Explanation of abbreviations used below:

FW	formula weight
d	density
mp	melt point, °C (purity dependent)
CRC b154	entry b154 in Part C of Weast (1976), CRC Press
Merck 696	entry 696 in Windholz and others (1976), Merck Inc.
Sax 802	see page 802 in Sax (1979)
NIOSH BZ2625000	entry BZ2625000 in Lewis (1979)

p-anisaldehyde  $\text{C}_8\text{H}_8\text{O}_2$  FW 136.15 d 1.119 mp 0° CRC b154 Merck 696 Sax 802 NIOSH BZ2625000. Supercooled below -5°C. Soft surface at -5°C. Could not prepare surface at -5°C.

Bicyclohexyl  $\text{C}_{12}\text{H}_{22}$  FW 166.31 d 0.864 mp 4° CRC b2186 Sax 416. Could not supercool below 0°C.

Cycloheptanol  $\text{C}_7\text{H}_{14}\text{O}$  FW 114.19 d 0.948 mp ? CRC c616. Supercooled below -10°C. Could not verify mp given in CRC c616.

Cyclohexane  $\text{C}_6\text{H}_{12}$  FW 84.16 d 0.779 mp 7° CRC c637 Merck 2728 Sax 529 NIOSH GU6300000. Could not supercool below 0°C.

Cyclohexylbenzene  $\text{C}_{12}\text{H}_{16}$  FW 160.26 d 0.950 mp 6° CRC b484 Sax 900 NIOSH CZ1330000. Supercooled below -5°C. Froze quickly when disturbed. Soft surface at -5°C.

n-decyl alcohol  $\text{C}_{10}\text{H}_{22}\text{O}$  FW 158.28 d 0.830 mp 7° CRC d57 Merck 2835 Sax 536 NIOSH HE4375000. Could not supercool below 0°C.

Didecyl phthalate  $\text{C}_{28}\text{H}_{46}\text{O}_4$  FW 446.67 d 0.960 mp 4° NIOSH TI0900000. Very viscous at 0°C. Difficult to freeze.

Diethyl fumarate  $\text{C}_8\text{H}_{12}\text{O}_4$  FW 172.18 d 1.053 mp 2° CRC f187 Sax 578 NIOSH EM5950000. Supercooled below -5°C. Hard surface at -5°C. Soft at 0°C. Vapour appears to irritate.

Diethyl sebacate  $\text{C}_{14}\text{H}_{26}\text{O}_4$  FW 258.36 d 0.965 mp 1° CRC d35 Merck 3794 NIOSH VS1180000. Supercooled to -3°C. Surface softens < 0°C. Narrow range.

Diethyl sebacate  $\text{C}_{12}\text{H}_{22}\text{O}_4$  FW 230.31 d 0.981 mp 6°C CRC o144. Supercooled below 0°C. Hard surface at -5°C. Could not obtain outline of ice texture (decomposition and/or solubility problem?).

Dimethyl adipate  $\text{C}_8\text{H}_{14}\text{O}_4$  FW 174.20 d 1.063 mp 8° CRC h390 Merck 151. Could not supercool below 0°C.

o-dimethyl phthalate  $\text{C}_{10}\text{H}_{10}\text{O}_4$  FW 194.19 d 1.189 mp 2° CRC p824 Merck 3244 Sax 611 NIOSH TI1575000. Supercooled below -5°C. Hard at -5°C. Surface film > -5°C. Too soft at 0°C to support sample.

1-ethoxynaphthalene  $\text{C}_{12}\text{H}_{12}\text{O}$  FW 172.23 d 1.060 mp 6° CRC n179. Brown liquid. Supercooled below -10°C. Soft surface at -2°C. Expensive.

Ethyl anisate  $\text{C}_{10}\text{H}_{12}\text{O}_3$  FW 180.20 d 1.104 mp 7° CRC b1779 NIOSH BZ4697000. Supercooled below 0°C. Trace of surface film at 0°C. Rigid enough to support sample at 0°C.

Ethyl cinnamate  $\text{C}_{11}\text{H}_{11}\text{O}_2$  FW 176.22 d 1.049 mp 7° CRC c356 Merck 2288 NIOSH GD9010000. Supercooled to 0°C. Too soft at -5°C for surface preparation. Decomposes in storage.

Ethyl salicylate  $\text{C}_9\text{H}_{10}\text{O}_3$  FW 166.17 d 1.131 mp 3° CRC b1662 Merck 3793 NIOSH V03000000. Supercooled below 0°C. Hard and no surface film at 0°C. Inconsistent results due to water solubility and decomposition.

*l*-fenchone  $\text{C}_{10}\text{H}_{16}\text{O}$  FW 152.23 d 0.948 mp 5° CRC f12 Merck see 3896 NIOSH RB7875000. Supercooled below 0°C. Soft at 0°C. Could not obtain outline of ice texture at -5°C.

Isopropyl myristate  $\text{C}_{17}\text{H}_{34}\text{O}_2$  FW 270.46 d 0.853 mp 8° CRC t83 Merck 5075 NIOSH XB8600000. Supercooled below 0°C. Surface too soft at -5°C.

Methyl laurate  $\text{C}_{13}\text{H}_{26}\text{O}_2$  FW 214.35 d 0.870 mp 6° CRC d303. Could not supercool below 0°C.

$\alpha$ -tetralone  $\text{C}_{10}\text{H}_{10}\text{O}$  FW 146.19 d 1.099 mp 6° CRC t117 NIOSH QK4375000. Reddish brown. Supercooled below -10°C. Hard and no trace of film at 0°C.

## B. STAINS, DYES, AND INDICATORS TRIED IN THIS STUDY

Name	Color index*	Name	Color index*
Acid black 48	65005	Fluorescein WS	45350
Acid fuchsin	42865	Guinea green B	42085
Aniline blue WS	42755	Light green SF	42095
Bromophenol blue	—	Methyl blue	42780
Carminic acid	75470	Methyl red	13020
Congo red	22120	Nigrosin WS	50420
Copper sulphate	—	Orange G	16230
Direct red 75	25380	Phenol red WS	—
Direct yellow 50	29025	Phloxin	45405
Eosin B	45400	Ponceau S	27195
Eosin Y	45380	Potassium dichromate	—
Erioglaucine	42090	Potassium permanganate	—
Fast black K salt	37190	Rose bengal	45440
Fast green FCF	42053	Sodium dichromate	—
Fast red B salt	37125	Sulfonazo III	—
Fast red TR salt	37085	Trypan red	22850
Fast sulphon black F	26990	Water blue	42780

\* Based on Society of Dyers and Colourists (1971).

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