

The Staining of Polymers II

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Part I of this subject was presented earlier in *Microscopy Today* (September/October 2002, pp5-6). The objective of Part II is to cite additional contributions to staining technology that the authors feel are either seminal to, or graphically depict, staining processes that were previously referred to but not illustrated.

Osmium Tetroxide Staining to Differentiate Between Amorphous and Crystalline Regions:

E.H. Andrews [1] was the first to produce transmission electron micrographs that differentiated between crystalline and amorphous forms in long chain polymers. Working with thin cast films of cis-polyisoprene {natural rubber (NR)}; he examined them in the *unstrained* and *strained* states. Staining the *unstrained* thin films with osmium tetroxide vapors, he showed that spherulitic crystals were in light contrast whereas the darker contrasted matrix was the amorphous continuum (Figure 1). Working with *strained* thin films of natural rubber similarly stained; he showed light contrast shish-kebab crystalline regions embedded in the darker amorphous polymer (Figure 2). Quoting Andrews, "...since the crystalline regions are not readily penetrated by the (osmium) vapor these appear white against a dark background." The thin cast films of NR were cast from benzene solution onto water surface. The staining regimen was: vapor staining from dry OsO_4 crystals at temperatures of -50 to 10°C for periods of a few minutes to several days.

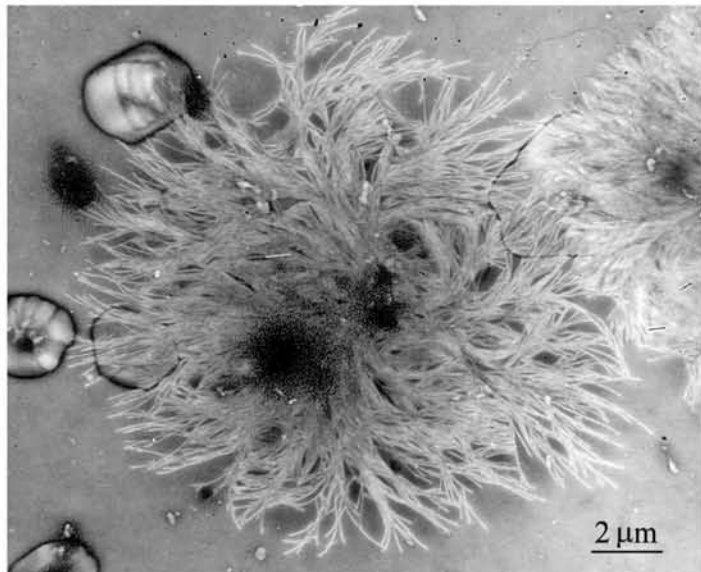


Figure 1: Spherulite in Unstrained NR Film, OsO_4 Stain

Understanding Ruthenium Tetroxide Staining:

Staining polymers with ruthenium tetroxide has become as popular as using osmium tetroxide. In addition to staining $>\text{C}=\text{C}<$ bonds, ruthenium provides contrast between aromatic and aliphatic moieties. Thus, ruthenium staining is particularly valuable for identifying polystyrene phases in polymer blends/alloys. An electron energy loss spectroscopy (EELS) study by Chou *et al.* [2] determined that π - π^* bonding was the mechanism for the attachment of ruthenium to the aromaticity in polystyrene (PS). The analysis magnifications required for these blend/alloy studies is not exceptionally great (1,000-40,000X) and completely satisfactory for the desired results. At nanometer resolu-

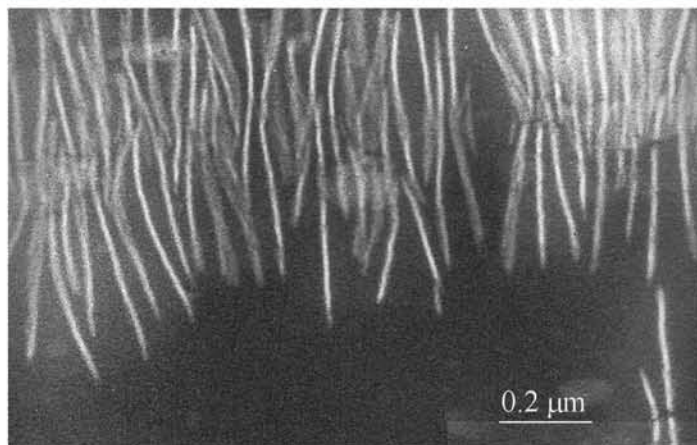


Figure 2: Shish-Kebab Crystalline Texture in Strained NR Film, OsO_4 Stain

tion studies, however, an anomaly appears that should be understood: the formation of RuO_2 nanocrystals on sample surfaces. Figure 3 shows a PS latex sphere supported on a holey carbon film that has a mottled appearance which, at higher magnification (Figure 4), shows that the mottled contrast is due to a distribution of randomly oriented 2-5 nm crystallites. Figure 5 is a cross section of a ruthenium stained surface of PS that shows a deposition of RuO_2 crystallites. The overall effect of the crystallite formation could be to obscure boundaries of polymer blends studied at lower magnifications. The ruthenium staining was performed by exposing dry on-grid specimens to vapors from a solution of 0.5 wt% RuO_4 in water.

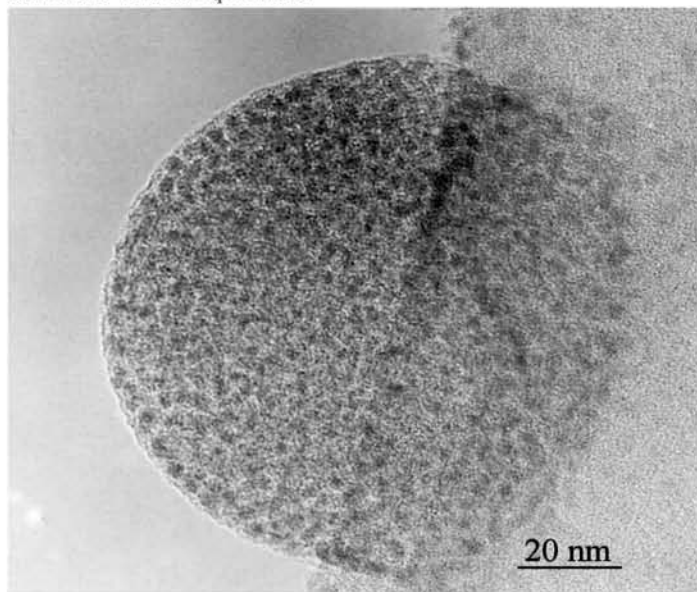


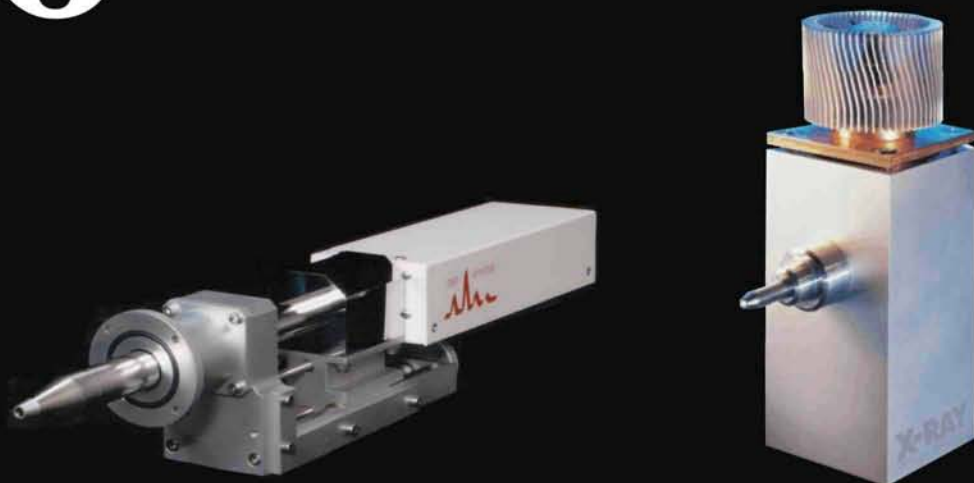
Figure 3: Mottled Appearance on the surface of a PS Sphere

This is an important point for those who desire in-depth information about ruthenium staining. For instance, it was suggested that problems associated with surface crystals can be avoided by using deep staining of bulk specimens and relying upon microtomy to shave off the questionable surface layers. However, under conditions of heavy staining, the electrically conductive RuO_2 might be desirable for reducing electron beam charging effects in both transmission and scanning imaging.

Ruthenium Tetroxide Staining to Differentiate Between Amorphous and Crystalline Regions:

A contribution to the subject of amorphous-crystalline staining was the successful use of ruthenium tetroxide for differentiating be-

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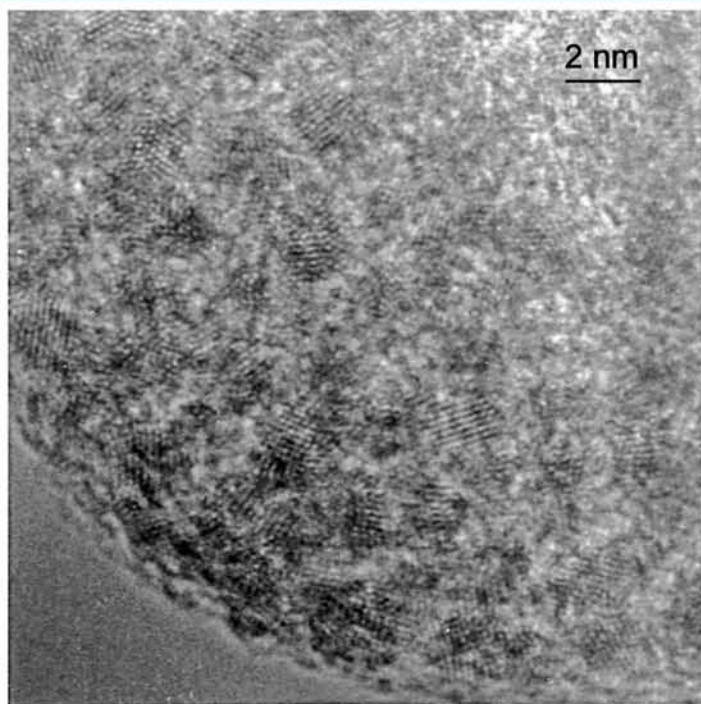


Figure 4: RuO₂ Crystallites Resolved on a PS Sphere

tween crystalline and amorphous phases of poly (ethylene terephthalate) (PET) by Haubruge [3]. Figure 6 shows side-by-side micrographs of PET stained at 80°C for 15 minutes (A) and for 15 minutes (B) at -15°C for two hours. The PET films were produced by spin coating and exposed to ruthenium tetroxide vapors from 5 min. to one hour, the vapors produced *in situ* by mixing 0.1 g ruthenium trichloride hydrate with 5 ml of 13% active chlorine aqueous sodium peroxide. Haubruge followed the chemistry of PET—RuO₄ vapor staining and corroborated Chou's conclusions (above) that it involved a complex solid-state reaction involving the oxidation of the amorphous phase of the polymer and the localized formation of RuO₂ crystallites. The chemical details of this paper should be considered before concluding precisely what moiety ruthenium tetroxide is staining in diverse polymer blends where chemical unsaturation may not be involved.

Sequential Staining:

Sequential staining can be used to advantage when applied to multicomponent blends when a third polymer (termed a compatibilizer) is added to a blend in order to enhance mixing properties. A typical system is a blend of a polyolefin (PO) and polystyrene (PS) where a

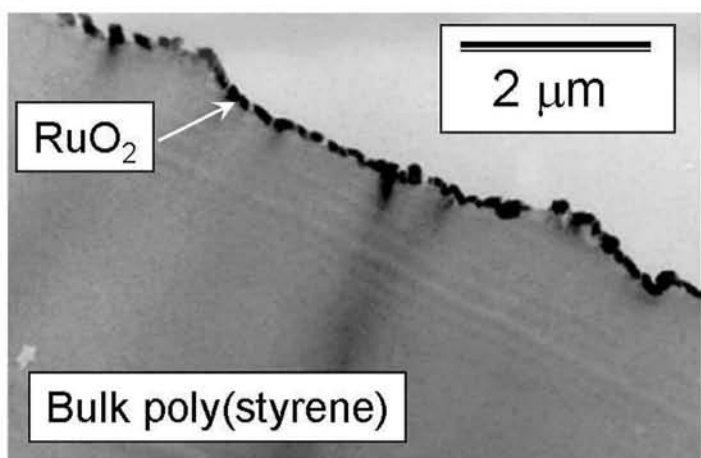


Figure 5: Microtomed Section Showing RuO₂ Crystallites on a PS Surface

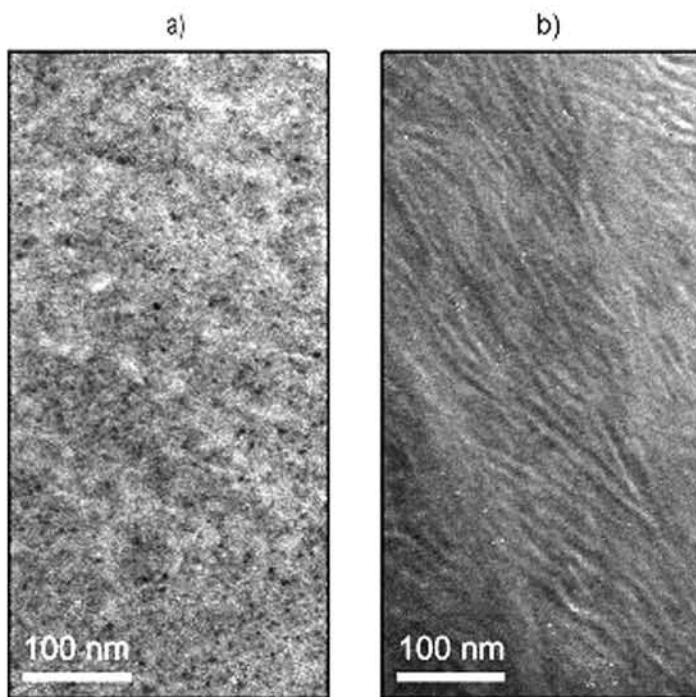


Figure 6: Ruthenium Tetroxide Stained PET: a) Amorphous Texture, b) Crystalline Texture

lesser amount of poly (styrene-butadiene) (SBR) has been added as a compatibilizer [4]. Figure 7 shows the unstained triblend which looks like a typical two component blend—a continuous phase and a dispersed phase. Osmium tetroxide was the first stain used and Figure 8 shows how the osmium, by reacting with residual >C=C< bonds in SBR, identifies the compatibilizer, which is found to envelope the dispersed phase globules. Subsequently, the system is stained with ruthenium tetroxide, which (Figure 9) identifies the disperse phase as PS by reacting with the aromaticity in PS. In all cases, the cryomicrotomed sections were vapor stained by vapors from their aqueous solutions.

Negative Staining:

The most prevalent negative stain for polymers is aqueous phosphotungstic acid (PTA). It has found widespread use in providing contrast for outlining latex particles where the resolution demands are not great. An example of a higher resolution application of PTA was demonstrated by Claussen, *et al.*, [5] where long chain polypeptide nanofibers, 6-10 nm in diameter with lengths in excess of one micron, are contrasted on a darker background of PTA (Figure 10).

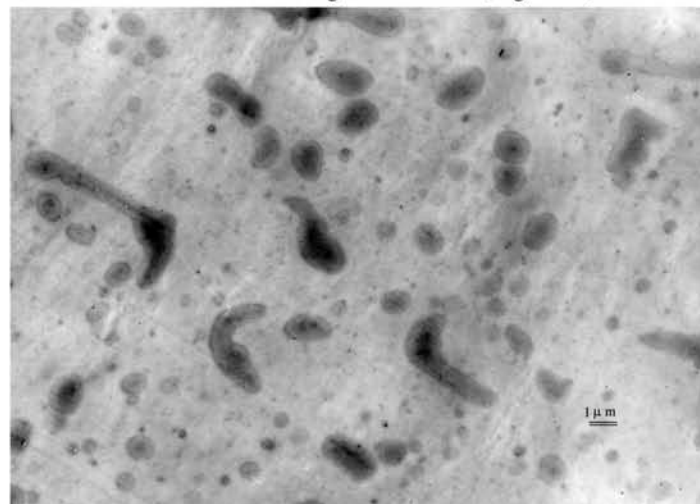


Figure 7: Triblend Ultrathin Section - Unstained

Eliminate the Element of Surprise

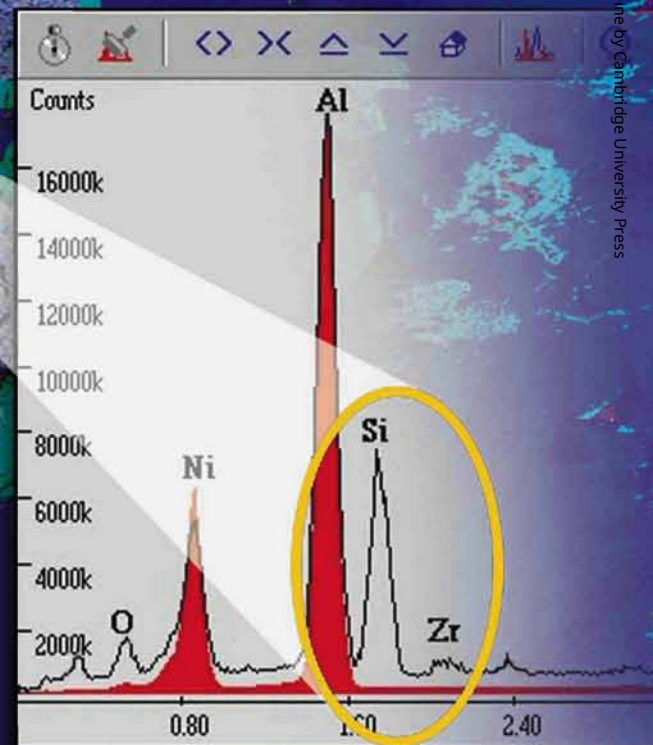
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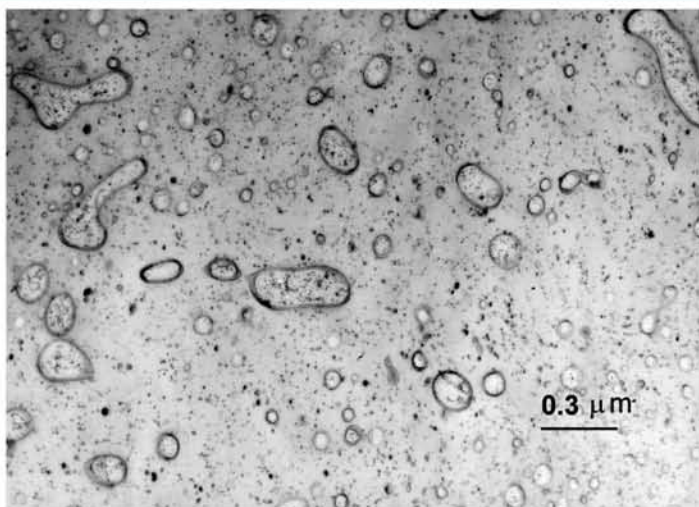


Figure 8: Triblend Ultrathin Section Stained with OsO_4

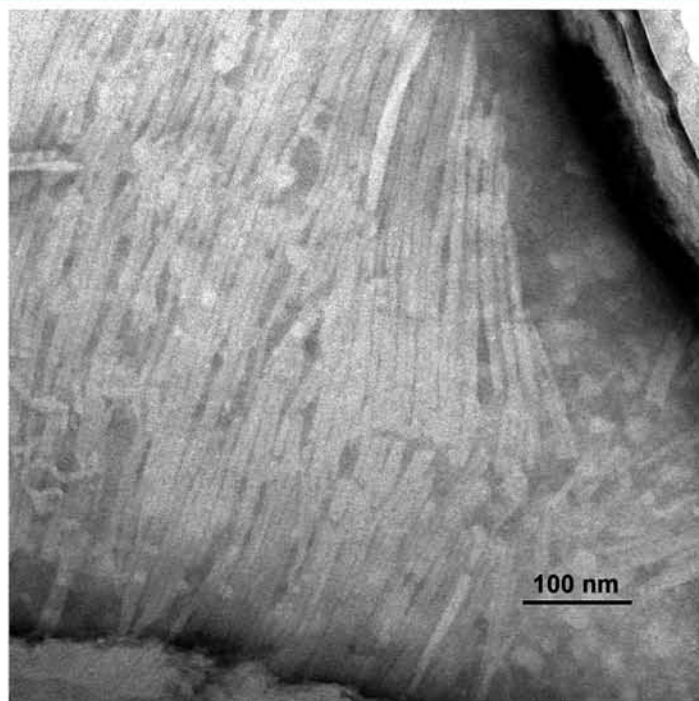


Figure 10: Polypeptide Nanofibers Negatively Stained with PTA

Staining Polymer Crazes (Microvoids):

“Crazing” is a phenomenon of void formation caused by the application of a tensile strain to glassy polymers, causing the formation of microfibrils with microvoids interspersed between them. This phenomenon causes the formation of stress-whitening. Matsuo [6] exposed stress-whitened blocks of high impact polystyrene (HIPS) to vapors of osmium tetroxide for several days, holding the HIPS under strain. Ultrathin-sections were then cut parallel to the direction of strain. Figure 11 shows how the linear crazed formations have been

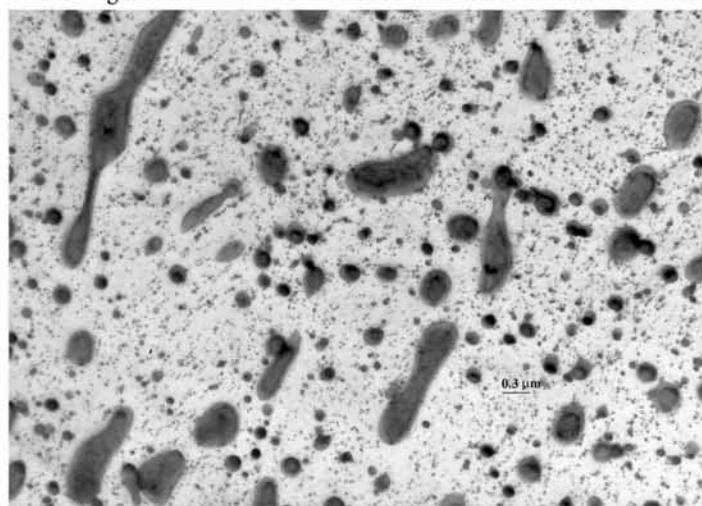


Figure 9: Triblend Ultrathin Section Stained with OsO_4 and RuO_4

stained (physically entrapped) by the osmium tetroxide. At the same time; osmium tetroxide has chemically stained the rubbery polybutadiene phase of HIPS. The arrow indicates the direction of applied strain. The crazes propagate from rubbery domain to rubbery domain – areas where the strain deformation is the greatest.

In Conclusion:

A survey of polymer-oriented journals from the 1950s to the present has indicated that over 700 articles have documented the reliance of polymer staining techniques on the experimental specimen preparation procedures. Polymer microscopists are successfully meeting the staining challenges presented by a plethora of new polymers, copolymers, blends, and alloys. Lastly, one must always be reminded that stains are chemically aggressive and environmentally sensitive. Proper safety and disposal procedures must be followed. ■

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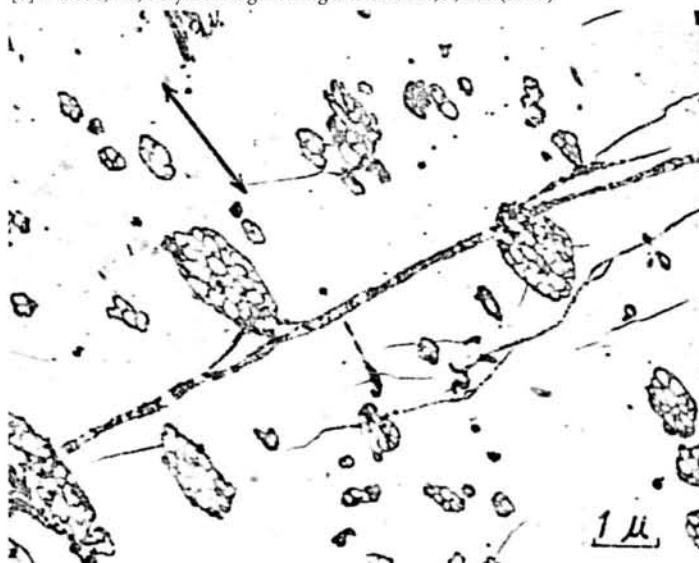


Figure 11: Stress-whitened PS Showing OsO_4 Stained Crazes

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