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A New Method of Preparation of a Self-Perforated Micro Plastic Grid and its Application (I)*

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A new method of preparation of micro plastic nets for specimen supporting has been developed. A microscope slide glass is finished by a water-repellent. Next, the slide glass is cooled below the dew-point to form minute dew-droplets on the surface of it. This water-droplets are covered with dilute solution of plastic. After volatilization of the solvent, plastic nets with perforated holes can be made very simply. Within the extent of $0.2\sim10\,\mu$, a self-perforated plastic grid of a desired hole size can be obtained.

When a similar operation is carried out on a specially minute water-droplets, thin plastic film with holes of $0.05\sim0.15\,\mu$ can be obtained. This film is very useful for correction of astigmatism of an electron microscope as high as 100,000 times or more in direct magnification.

I. Introduction

ALONG with the development of the electron microscope and its application, the necessity of the specimen supporting method with combined use of a micro plastic net of small holes ($1\sim10\,\mu$) and a metal grid has recently been closed up. The pioneers in research of the micro plastic net are Sjöstrand[1] and Sakata[2]. Their preparation method for the micro plastic net consists of two stages, namely; in the first stage, minute water-droplets are brought in contact with the surface of the plastic film before volatilization of the solvent; in the next stage, after drying of the plastic film, the hollow parts made in the film by contact with the water-droplets are treated to change them into perforated holes. This method, especially the latter technique, is accompanied with technical difficulty and it is not easy to put into practice. The authors have completed study with an entirely different principles in the technique of making micro plastic net with various sizes of holes. Description of this new method will be given hereunder together with its applications.

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II. New Preparation Principle of Micro Plastic Grid

The new preparation principle of micro plastic grid is very simple. As shown in **Fig. 1**, numerous minute water-droplets are previously formed on a surface of such a substrate as a glass plate. This water-droplets are covered with very thin solution of plastic. After volatilization of the solvent, perforated micro holes can be made, in the plastic film, corresponding to those of the original water-droplets. The micro plastic grid made by this new principle is called "Self-perforated micro grid" (hereinafter simply called "micro grid").

With a view to putting this principle into practice, each of the undermentioned items was

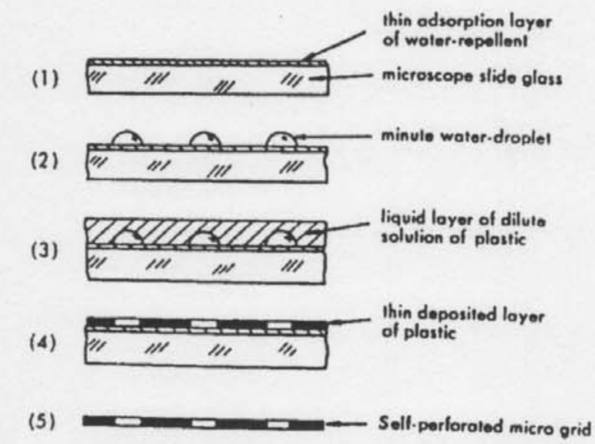


Fig. 1. Schematic diagram of the preparation principle of Self-perforated micro grid.

fully studied. (1) To form numerous minute water-droplets on the surface of the glass plate. (2) The size of these water-droplets are placed under control. (3) The plastic material is then selected. (4) Finally the solvent of the plastic material is selected.

(1) There are several techniques for formation of minute water-droplets on the surface of the glass plate. The authors have adopted undermentioned technique in consideration of the ease of control of the size of the minute water-droplets. A glass plate is cooled below the dew-point in a refrigerator, and then it is taken out into a room to form minute dew-droplets on the surface of it.

(2) The control of the size of the minute water-droplets formed on the glass plate is the most important problem in making the micro grid of various hole sizes. The minute dew-droplets formed on the surface of the clean glass plate change into large flat droplets in combination with neighboring minute droplets by the phenomenon of spreading wetting. Such droplets do not suit the requirement of this study. To change the wetting phenomenon from a spreading type to an adhesional type, it is necessary that the surface of the glass plate is made with a water-repellent finish. As a result of experiments in various kinds of agents for water-repellent finish, it was found that the application of cation surface active agent was most effective. The control of the size of the dew-droplets thus formed was possible by next two factors, namely, (1) the grade of the hydrophobic characteristics of the surface active agents and (2) the cooling degree of the surface of the glass plate below the dew-point. On the other hand, the micro grid layer is difficult to separate from the surface of the glass plate as it is of a hydrophobic property. To solve this problem, the glass plate surface must be treated with anion surface active agent before separation, and the character of the glass plate surface be changed into the original hydrophilic nature.

(3) The plastic material must be easy to dissolve in the undermentioned solvents, its film-formation property must be excellent and it must easily be separated from the surface of the glass plate. Such plastic material can be found from cellulose derivatives.

(4) The organic solvent of the plastic must be selected as a good solvent of the plastic mentioned in the above (3), and of a large vaporization speed, not too high latent heat of vaporization and it must be difficult to dissolve in water but easy to get wet on the surface of the glass plate. The solvent suitable for this purpose can be selected from lower homologues of fatty acid ester.

III. Practical Data for Preparation

In this report, a description is given concerning the fundamental technique and technical data for making a self-perforated micro grid with desired hole size within the extent of $0.2\sim10\,\mu$. A glass plate was used as the substrate for making the micro grid. Several ways may be considered in the combinations of the solvent and the plastic material for the grid, but in this case the combination of cellulose acetobutyrate (Triafol) and ethyl acetate was adopted. As the water-repellent, quaternary ammonium salt and the derivative of polyoxyethylene were employed. These water-repellents were adopted as undermentioned, namely; in case of making a micro grid with the hole size under $1.5\,\mu$, the former was adopted for its strong hydrophobic property, while in case of the hole size $1.5\sim10\,\mu$, the latter was employed for its weak hydrophobic property.

As the agent for re-hydrophilic treatment, sodium dialkyl sulphosuccinate was selected from various kinds of anion surface active agents which possess strong penetrating power.

For cooling the glass plate below the dew-point, an electric refrigerator was employed. A metal block (1 cm in thickness) with a smooth flat surface is cooled in advance, being placed in a refrigerator. The pre-cooling temperature of the metal block was made at +5 °C∼−5 °C. When the dew-point (temperature: *d*) was above 10 °C, the glass plate was cooled by placing it on the metal block pre-cooled to 5 °C. When the temperature (*d*) is 5∼10 °C, it is cooled on the metal block of 0 °C, and when (*d*) is 0∼5 °C, it is cooled on the metal block of −5 °C.

Table 1. Plastic material and surface active agent.

▶Plastic material, Solvent and Concentration
Plastic material: Cellulose acetobutyrate (Triafol)
Solvent: Ethyl acetate (extra pure)
Concentration: 0.1∼0.5% (w/v)

▶Agent for water-repellent finish: Cation surface active agent
1) Quaternary ammonium salt
  i) Softex KWO (distearyl dimethyl ammonium chloride, Kao-Atlas Co.)
  ii) Osvan (benzalkonium chloride, Takeda Chemical Industries, Ltd.)
2) Derivatives of polyoxyethylene
  i) Amiet 105 (polyoxyethylene lauryl amine, Kao-Atlas Co.)
  ii) Diamiet 315 (polyoxyethylene stearyl propylene diamine, Kao-Atlas Co.)
  Concentration: 0.03% (w/v) aqueous solution

▶Agent for re-hydrophilic treatment: Anion surface active agent
Pelex OTP (sodium dialkyl sulphosuccinate, Kao-Atlas Co.)
Concentration: 0.5% (w/v) aqueous solution

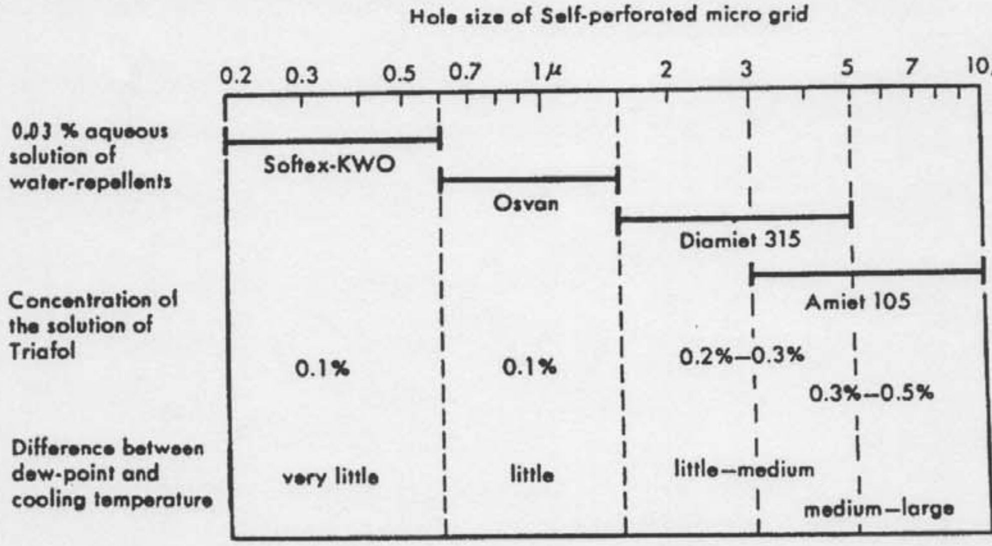
Data in detail concerning plastic materials and surface active agents will be described in **Table 1.** Technical data for the preparation will be given together in **Table 2.**

IV. Technique of Preparation

(1) *Cleaning the substrate.* A microscope slide glass of excellent quality (about 0.8 mm in thickness) is completely cleaned on the surface.

(2) *Water-repellent finish.* In the aqueous solution of the water-repellent (Refer to Table 1 and 2), a slide glass is immersed for 10∼30 minutes. Then it is taken out and is washed with water, eliminating excess water-repellent, and it is dried. By this treatment, a very thin adsorption layer of the water-repellent is formed on the glass

Table 2. Technical data of preparation for Self-perforated micro grid.



surface imparting a hydrophobic property to it.

(3) *Cooling the substrate.* A slide glass finished with the water-repellent is placed on a metal block pre-cooled in the refrigerator and is cooled below the dew-point. The extent of cooling varies according to the desired hole size of the micro grid as shown in Table 2. Generally speaking, when a micro grid of smaller hole size is intended, the size of the water-droplets formed on the glass surface must be made smaller, so the glass surface is cooled a little below the dew-point. Hence the time for cooling is also a little shorter. On the contrary, when a micro grid of the larger hole size is intended, the cooling time is made longer. Roughly the cooling time is generally 3~50 seconds. Concerning the cooling time, an optimum condition may be obtained after several trials. When the optimum cooling time becomes too short, the slide glass may be held in the air about 3 cm above the metal block instead of directly placing on it.

(4) *Making the micro grid layer.* After cooling, the slide glass is taken out of the refrigerator, and Triafol solution (Refer to Table 1 and 2) is quickly pored and spread on the surface of the slide glass. Then the slide glass is inclined to allow the excess solution to flow away, and it is left alone until the solvent volatilizes. The Triafol micro grid layer thus prepared is checked by an optical microscope as to the measurement of the hole size and the distribution of the holes. With a little skill, the micro grid layer with a uniform distribution of holes extending over a half the area of the slide glass can be made.

In case the room humidity is below 50 % (relative) the Triafol solution is previously cooled nearly to the dew-point. When it is above 70 %, the surface temperature of the Triafol layer goes down below the dew-point, due to latent heat of vaporization of the solvent. Other dew-droplets are formed again on the surface of the glass plate. Consequently there will be a different formation of the micro grid layer from that of the expected distribution of the size of holes. There are two methods to prevent the happening of this brushing phenomenon, namely to keep the surrounding humidity of Triafol layer under 70 % during the volatilization of the solvent, or to keep the surface temperature of Triafol layer above the dew-point by heating it. The simplest method is to carry on the operation under the lighting with an infrared lamp of about 250 W.

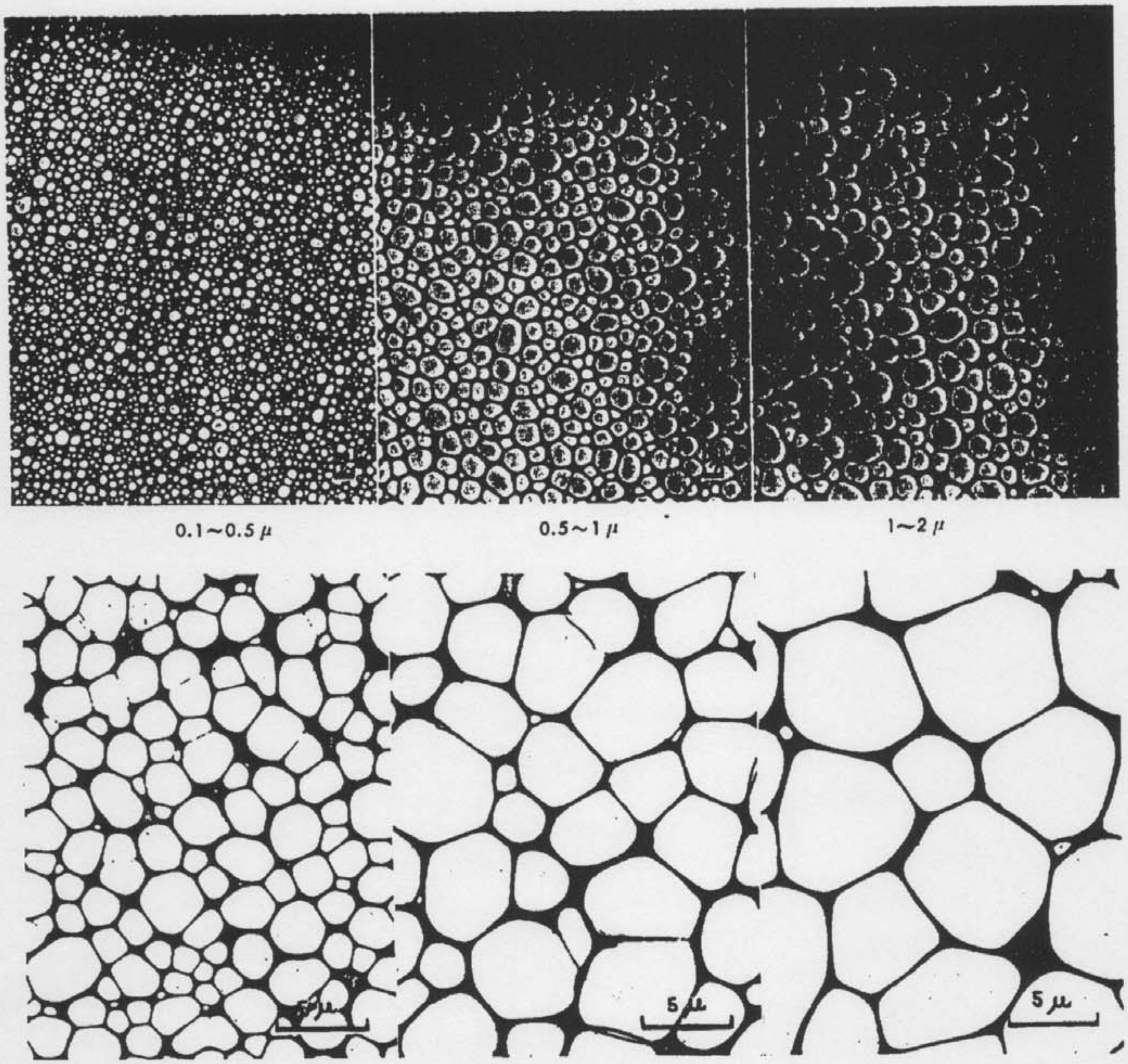
(5) *Re-hydrophilic treatment and separation of micro grid layer.* The slide glass, after finishing the operation mentioned in (4) is immersed in the re-hydrophilic agent (Refer to Table 1) for 3~10 minutes. Then taking it out, it is washed in water. The slide glass is then slowly put into the water at an angle under 10° with the water surface. Then Triafol micro grid layer is floated off onto the water surface. In case the Triafol layer cannot be easily floated off onto the water surface, it is again immersed in the re-hydrophilic agent for a much longer time and then the same process is carried out again.

(6) *Mounting of the micro grid layer.* The Triafol micro grid layer floating on the water surface is picked up in the ordinary way and put on the metal grid. Then vacuum evaporation of carbon is taken on the micro grid layer to reinforce it. It is desirable to use an adhering method, as separately reported[8], on the metal grid. As a result of this method the micro grid layer and the metal grid adhere firmly together and an extremely stable micro grid will be made.

V. Discussion

Fig. 2 shows the examples of the micro grids with various hole sizes. When an observation of the specimen is carried out by utilizing a micro grid, it is necessary to select a micro grid which has the most appropriate hole size in proportion to the magnification of the observation. For instance, the micro grid of 0.2~0.5 μ class is suitable for observation of 100,000 times in direct magnification, because at 100,000 times, the area of the field is comparable to the area of a single hole.

It is one of the characteristics of this method that the open area of a self-perforated micro grid is made very wide. It is 40 % in 0.2~0.5 μ class of the micro grid, 65 % in 1~2 μ



0.1~0.5 μ 0.5~1 μ 1~2 μ

2~3 μ 3~6 μ 6~10 μ

Fig. 2. Self-perforated micro grids with various hole sizes.

class, and 75 % in 3~6 μ class.

The merit of the operation for making the micro grid described here is to cool the hydrophobic glass surface below the dew-point and to form minute dew-droplets on the surface of it. The progress of the occurrence and growth of the dew-droplets on the glass surface is as follows: On the surface of the glass plate treated with a strong hydrophobic water-repellent, a large number of specially minute dew-droplets are formed at the beginning. These droplets are formed comparatively in a uniform growth until they grow to a size of about 1.5 μ, however, they are subsequently able to form larger droplets in combination with neighboring droplets. Hence a micro grid with mixed sizes of holes of about 1~2 μ and considerably larger holes can be obtained. When treated by a water-repellent of this type, it is suitable to make the micro grid under the hole size of 1.5 μ with a wide open area by cooling the glass plate at a temperature slightly below the dew-point. When the glass plate surface is treated with a weak hydrophobic water-repllent, the number of dew-droplets forming at the beginning is smaller in comparison with that of the former case. Then afterwards they grow to a size of about 10 μ. On the stage of growth, the size of the dew-droplets nearly corresponds with the degree of cooling of the glass plate, and it is convenient to make the micro grid with a size of $2\sim10\ \mu$ hole size.

When making the micro grid with smaller holes, the concentration of Triafol solution is made dilute (Refer to Table 2). In case of larger holes of $5\sim10\ \mu$ class is required, a durable micro grid can be made with concentration of Triafol solution of $0.4\sim0.5$ %.

For carrying out this operation, a room of $40\sim60$ % of relative humidity is most suitable. When the relative humidity is under 30 %, the difference between the room temperature and dew-point $(t-d)$ becomes too great, and it is difficult to make the micro grid with smaller holes. In case the humidity rises above 70 %, the difference $(t-d)$ becomes too small and not only does the control of the cooling degree become difficult but also brushing phenomenon is apt to arise.

VI. Application

On the surface of the glass plate treated with a strong hydrophobic water-repellent, considerable amount of specially minute dew-droplets appear at the beginning as described before. When the preparation process of the micro grid is given over the specially minute dew-droplets, Triafol film with very small holes can be obtained. **Fig. 3** shows one of the examples. As it will be observed from Fig. 3, a film with holes of minimum 0.01 μ or generally $0.05\sim0.15\ \mu$ can be obtained. This film is very effective when used as a specimen for correcting the astigmatism of an electron microscope. As shown in **Fig. 4**, it is quite easy to make an appearance of more than three holes within the field of 100,000 times in direct magnification. By the employment of this specimen, the trouble can be eliminated in correcting astigmatism that may occasionally arise due to defect of the individual hole. Even in the case of more than 200,000 times in direct magnification, it can be utilized as a specimen for correcting such aberration.

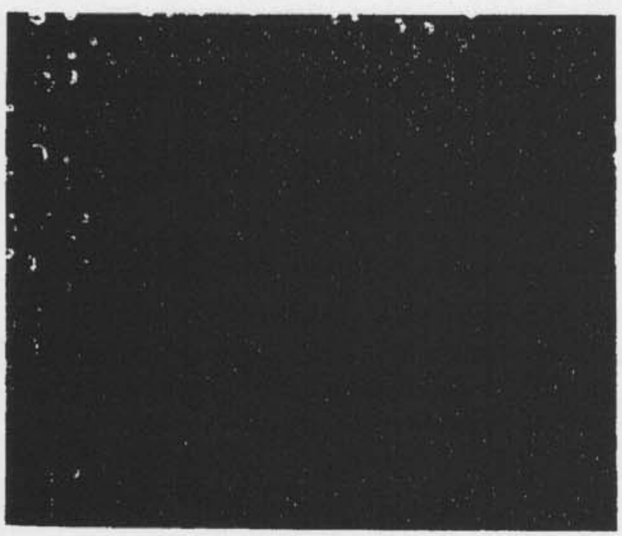


Fig. 3. Triafol film with very small perforated holes.

Fig. 4. Application for the correction of the astigmatism at high magnification.

VII. Conclusion

A new method of preparation of micro plastic nets for specimen supporting has been developed. At first, a microscope slide glass is finished by a water-repellent (cation surface active agent), and then the surface of glass is imparted hydrophobic property. Next, the slide glass is cooled below the dew-point in a refrigerator, and then it is taken out into a room to form minute dew-droplets on the surface of it. This water-droplets are covered with dilute solution of plastic. Actually 0.2~0.5 % ethyl acetate solution of cellulose acetobutyrate was employed. After volatilization of the solvent, plastic net with perforated holes can be made very simply. The size of the holes can be controlled by the size of the water-droplets. Within the extent of 0.2~10 μ the self-perforated micro plastic grid with holes of a desired size can be obtained. This micro plastic grid is mounted on an ordinally metal grid.

When a similar operation is carried out on a specially minute water-droplets, thin plastic film with holes of 0.05~0.15 μ can be obtained. This film is very useful as a specimen for correction of astigmatism of an electron microscope as high as 100,000 times or more in direct magnification.

In this report, descriptions are given on the fundamental technique for using the glass plate as the substrate for making the micro plastic grid. As it will be anticipated as a matter of course from the new principle of this method, this micro plastic grid can be made on various kinds of substrates. For instance, it may be made on either the cleavage surface of mica or vacuum evaporated film. It is expected that by the development of this method to a higher degree, extremely useful and new specimen supporting system may appear.

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