

## NanoSuit® Solution II

For pathological specimens and CLEM (correlative light and electron microscope)

### Instruction manual for use

The NanoSuit solution Type II is developed for observing specimens such as tissue sections contact with glass slides and is useful for CLEM (Correlative light and electron microscopy).

NanoSuit membrane covers the sections and can keep the original structure of it.

After obtaining the color information by an optical microscope, it is possible to obtain high-resolution and high-magnification information as well as a three-dimensional structure with SEM. After SEM observation, it is possible to re-stain such as Hematoxylin and Eosin (H&E) staining, therefore, you can use this method for the specimens without any damage. It is possible to save again as it was. It is also possible to use the NanoSuit solution as a marker. When a very small amount of this solution were put at the part observed with an optical microscope, the tiny drop of the solution can see as a marker during SEM observation.

Since it can also be used for EDX (Energy Dispersive X-ray) analysis, it is possible to analyze the elements of biological samples as they are on a slide glass.

With these new observation methods, you can discover new findings in life science and new pathological findings by CLEM.

### Protocol

1. Make a paraffin section and attach it to a slide glass, and then remove the paraffin. Apply H&E stain, inspect the microscope, and take a picture of the necessary part. (If necessary, put a cover glass on it with a buffer solution and take a picture.)
2. In the case of old slide specimens that have already been observed and preserved, observe them as they are, and take a picture of the necessary parts by optical microscope. For slides in which the cover glass has adhered to the sliced sample with an embedding medium, soak the slide glass in a solution such as xylene and remove the cover glass (It may take some time to remove the cover glass depending on the type of product).
3. Marks (markers that are difficult to dissolve even in a solution such as xylene) should be pointed by hand on the opposite side of the section where you observed with the optical microscope (that is, the back surface of the slide glass) in 1 and 2 above.
4. Add 1 to 3 drops of NanoSuit Solution Type II onto the surface of the section and spread it thinly over the slide glass using a spin coater.
5. Put the slide glass in the SEM, and observe it as soon as the vacuum is closed, and take a picture where you are interested in by optical microscope.

6. If EDX (Energy Dispersive X-ray) analysis is required, the measurement is able to perform in the state of 5.
7. After the observation of described above (5 or 6 ), you can re-stain the specimen, and use cover glass with embedding medium, then you can store it again. If high energy is applied in the EDX analysis, the sample may be damaged, so be careful when you use a valuable sample.

### **Precautions**

■ NanoSuit Solution Type II is exclusively for observing specimens that have been fixed with paraffin. Since the base material contains an organic solvent, it may affect the sample when used for a fresh biological tissue sample. Please refrain from using it for other purposes.

For fresh biological tissue samples, please use NanoSuit Solution Type I (for micro-organisms, individuals, and living tissues).

■ When working, wear glasses and goggles to protect yourself from the scattering of glass. If NanoSuit Solution Type II gets on your skin during work, wash it with running water or detergent.

■ NanoSuit solution When a Type II solution is dropped on a sample, if the solution stays in one place, unevenness may occur or the sample may be adversely affected. After dripping, it is recommended to spread the solution thinly using a spin coater. If an electron beam is applied while the solution remains thick, the film formed may become too thick and the SEM image may be blurred.

■ The NanoSuit method is a technology that polymerizes by electron beam or plasma irradiation to form a film on the sample surface. Start electron microscope observation immediately after evacuation. You can use any light microscope and SEM models.

NanoSuit Inc.