

Do you know which mounting medium you are using and why when mounting your sample?

You are all working hard in finding the right antibodies, probes etc and protocols for your immunostaining, but sometimes you are missing to pay attention to the last step in sample preparation, namely the **mounting medium to use when mounting your sample**.

If your sample is not close to the coverslip the choice of the mounting medium is crucial: choosing a mounting medium with a wrong RI will influence the quality of your images and ultimately your analysis due to refractive index mismatch between the sample and mounting medium. Choosing the correct mounting medium will improve your imaging by minimizing chromatic aberrations, reducing photobleaching and will increase your sample long term storage time.

Here are a few tips in what you should pay attention to when choosing mounting medium and how you should handle it:

1. Always chose your mounting medium with regard to your scientific question and what you need to analyze: if the scientific question requires measuring objects or distances use a non-hardening mounting medium, since the hardening mounting media dry and shrink the sample, thus your measurements will not be accurate.
2. Before ordering your mounting medium, read carefully on the data sheet if your mounting medium is a hardening or non-hardening mounting medium and the RI (refractive index).
 - a. If your mounting medium is a non-hardening mounting medium, seal the coverslip with nail polish and let the nail polish dry properly before you go to the microscope and start imaging.
 - b. If you chose a hardening mounting medium, let the mounting harden (cure) on a flat surface for 24 hrs before you start your imaging, thus to achieve the correct refractive index. The refractive index mentioned in the data sheet refers to the fully cured mounting medium!
 - c. Be aware of the RI (refractive index) of the mounting medium you are using. If the RI is not specified in the data sheet, write to the company and ask!
3. Always match the RI of your mounting medium with the RI of the immersion medium used by your objective: e.g., If you have a sample in PBS, always use a water immersion objective if high resolution is needed. If you have a sample in a mounting medium with high RI use an oil immersion objective
4. Always, remove all the PBS before adding the mounting medium to your sample so that you are not diluting it and thus change the RI.
5. Add as little as possible mounting medium to your sample, especially if the sample is placed on the slide and not on the coverslip.
6. Never use a mounting medium with DAPI in it since it will increase your background and thus lower your signal.