Microscopy Slide Preparation

A Few Tips for Various Approaches and Protocols

See the 0% waste idea

How Rethink your experiments

Note: Your experiment comes first. Do not impair the quality of your research! Go step by step and try out what works for you. You should think about controls and analyses to see whether your read-outs are affected– just like you do it in your research!

TAKE AWAY:

For every method there are green knacks

Hitting on some less obvious factors

A lovely poem for you at the very end :)

Think about Rethinking

Green Change Improves Your Science?

Intuitively, formaldehyde is more toxic than ethanol. This is not just important for your own health (given you inhale the gases). Additionally, it plays a role for its environmental impact. Yes, you should not pour PFA into the sink but even when you collect it, consider its environmental footprint downstream. What happens to this PFA? It ends up in a landfill…

Many labs have their fixation approach – one of the many habits. Make up your own mind to improve the quality of your samples! Regarding your fixative, consider further processing and tissue preservation. Different acids penetrate the tissue differentially fast, leading to better preservation. However, approaches also differ in their propensity to increase autofluorescence.

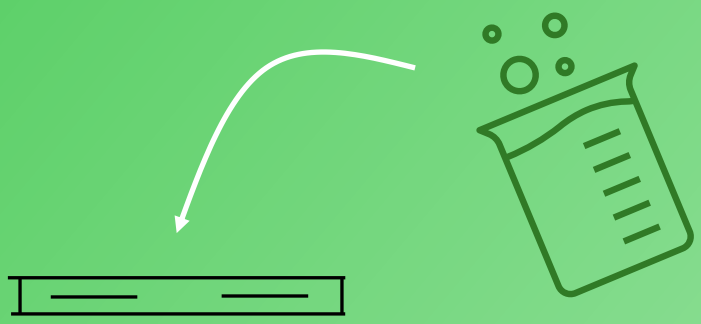
Rethink anything you do – don't just accept the status quo, try to understand it. For example, these days there is even xylene free media mounting availble that might be less toxic.

The 0% waste idea

Too Simple to Be True?

Short and Simple – pour instead of pipette.
That's all!

Alright, let's explain. To prepare your samples for cryosectioning (cutting a tissue block frozen in OCT) you fix your samples, dehydrate them and then freeze them.



It doesn't matter whether you work in a 12 well plate or in a tube. You can pour the PBS to wash as well as the sucrose to dehydrate from your bottle. Don't be afraid, just practice in an empty plate at first. And don't drink too much beer the night before for a steady hand ;) Whether dehydrating in 2.4mL instead of 2.5mL probably will not make a difference. And you do not use and tip…

Otherwise, if you do pipette, then start with PBS to wash, and then keep the tip to pipette the sucrose (there is no „bad" contamination or diluting effect given the minimal fluid retention in your tip).

For the ambitious

Right Is What Works

Fixation chains

Caution, for this one! However, we have experienced that some labs reuse their ethanol solutions which were less than 95% in concentration. Make up your mind if you establish something like a "chain" to reuse solutions as lower concentration of mix them with fresh ones 50/50.

Anti Wasting Antibodies

We all know this antibody fitting perfectly well for our experiment but … it simply does not work… First measure: varying the concentration. That also counts for sustainable practice. Titrating the antibody down and using just as few total liquid as possible on your slide to stain will 1) save lots of money and 2) save antibodies and solvent that otherwise goes to landfills or right into the nature.

Often, people will cover their slides with parafilm to avoid evaporation. Parafilm is a mix of wax and polyethylene, however it seldomly is re- or downcycled. If you have very sensitive samples e.g. ear skin from mice, not using parafilm can avoid dislodging your samples during removal of it. We, personally, left the parafilm out, always made sure that our sample is really covered in fluid and made use of a wet chamber (with a liquid reservoir) in the cold to incubate.

Cutting and assembling

Addressing Experimental Quality and Skill

Cutting - the time you need

It hurts but consider that every stop of your protocol produces waste in one other way. But that also means there is potential for optimization in each of them!

The more cuts of samples you can put on one slide, the less slides you need. And that not only saves solution and antibodies downstream but avoids your glass ending on a landfill (and being autoclaved beforehand). Continuously improving your performance will also make you faster = saving you a lot of time.

Think it green, think it through

Sustainability and improving research go hand in hand!

Good controls? Means you have a treated, a non-treated and a positive control sample right?

Yes, you would control for the treatment differences. Additionally, the sustainable scientist might control for staining differences among slides automatically.

why? Because he/she puts as many samples fit on one slide to reduce the number of slides. Thereby, he/she has multiple treatments on one slide and can assess how they stain.

Making it electric

Energy and Electricity

With the advent of advanced approaches such as scanning entire slides using confocal microscopy, running times increase up to 12h for a single session. Therefore, plan properly before scanning your sample. Run proper tests to assure that you only need to acquire once. Also, do not forget that the screen of your computer can be turned off during longer acquisitions.

Although it is hard to estimate, each terabyte (TB) of data stored might produce up to a kilogram of CO_2 per year. Therefore, be wary about where & what data is saved. Use a hard drive instead of cloud services if possible. Instead of saving copies/replicates after editing, consider to save the original picture and save the editing script as a separate protocol or text file.

Of course, which cloud-provider you use or how the electricity in your facility is procuced, plays into the equation as well.

The trivial and fun

Let’s Hit the “Extremes”

It might seem ridiculous but:
Please avoid using a stack of 20 towels to dry your slide, only to discard them all after using the first one!
Taking one or two is more than enough since they will soak up quite a bit of fluid and if some leaks, you can just wipe it off later.
Additionally, you can let these towels dry and use them again.

When you let picric acid or PFA drip off, do not throw the towel in the recycling waste, although it is tempting since it is paper. However, if it is just water or PBS, you can certainly do so.

We hope you got inspired to find our solutions although your protocol might look very different.

And now you also understand that:

Through lens and light, sustainability gleams,
Microscopy's beauty, in eco-friendly dreams.
Non-toxic dyes, mindful choices in play,
Green practice, preserving nature's array.

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