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| **Issue** | **Probable cause** | **Proposed solution** |
| Very slow to no flow through one of the inlets. | An air bubble may be blocking tubings. | Set pressure setting to “change” and unplug tubbing of interest from the PDMS chamber. Set pressure to 300 mBar in this channel and let 3-5 droplets go to waste. Set pressure back to “change” and replug tubing into the PDMS chamber. Make sure that there is liquid coming out of the tubing tip and the PDMS inlet to avoid creating a new bubble. Make sure all buffers are well degased. |
| Very slow to no flow through all inlets. | The outlet tubing is most probably blocked. | Follow the same procedure with the outlet (unplugging, purging, plugging back). |
| One or several flows are unstable. | Bubble(s) inside chamber. | Locate the bubble(s) by moving within the chamber and observing with the microscope, using transmitted light. As PDMS is a porous medium, air bubbles can be evaded through PDMS. Apply equal high pressure in all inlets and the outlet. Monitor the disappearance of the bubble(s). |
| Larger flow rates than expected or rates do not add up. | Solutions leakage. | Check the PDMS-coverslip interface, PDMS-tube interface and any screwable part. |
| The measured pressure does not reach the desired pressure. | Pressured air leakage. | Make sure the reservoir tubes are well tightened. Check for any screwable part. |
| Inconsistent protein concentrations | When changing solution, the ‘dead’ volume has not been taken into account | Due to the tubing volume and flow profile (see figure 3), make sure that solutions have been injected with the appropriate pressure and duration. |
| Filaments fragment / Filament pause during depolymerization | Excessive light damages filaments. | Minimize the amount of light exposure : reduce light power, exposure duration or frame rate, or reduce actin labeling fraction. |
| Filaments stuck on the surface in unexpected directions | A large air bubble passed through the chamber | To prevent air bubbles, degas all solutions and make sure a droplet is coming out of the PEEK tubing when changing a reservoir tube (see 7.4). Surface might be bleached and passivated again. |