CPS
CPS Instruments Europe
P.O. Box 180, NL-4900 AD Oosterhout, The Netherlands
T: +31 (0)162 472478 F: +31 (0)162 421944 E: info@cpsinstruments.eu

Maximizing Baseline Stability in the CPS Disc Centrifuge

The CPS Disc Centrifuge is designed to maximize baseline stability. The light source/detector assembly uses internal feed-back control and temperature compensation. Time and modest ambient temperature changes do not cause significant changes in baseline signal. In many applications, baseline stability is seldom an issue. The recommendations in this document are for those applications where the most stable baseline possible is needed to give the best size distribution results.

Some type of samples yield strong light absorption/scattering signals in the CPS Disc Centrifuge. Materials that generally give strong signals are organic and inorganic pigments (or anything else that has a strong light absorption constant), amorphous carbon particles of all types, dispersed metals of all types (gold and silver sols, iron, nickel, aluminum, etc.), and non-absorbing materials with relatively high refractive index and diameters larger than about 40 nm and smaller than ~10 microns. These types of samples generally yield signals that are many times larger than any baseline drift, so baseline drift is generally not an important issue.

Some samples give weak signals in the CPS Disc Centrifuge. Materials that usually give relatively weak signals include virus particles, protein molecules (or clusters of molecules), non-absorbing particles of relatively high refractive index that are either very small (<40 nm) or very large (>10 microns), any nonabsorbing particle where the refractive index is close to the refractive index of the liquid medium in which they are measured, or any sample where the concentration of particles is extremely low.

Another important consideration in evaluating baseline drift is the time needed for an analysis. Any measurement that takes only a few minutes will normally not suffer loss of accuracy from baseline drift, while very long analyses (>30 minutes) will often present baseline drift problems unless the signal is strong.

Common Causes For Drift – Decreasing order of Importance

1. Intermittent Light Source Operation

   The light source intensity will be most stable if the source runs continuously. Any time that the instrument is turned off (unplugged from the AC power, or switched off with the manual switch on the back), will cause significant drift the next time the instrument is turned on. We recommend that the instrument be left on at all times to maximize baseline stability. The power used when the centrifuge is not turning is only about 35 watts, and the expected lifetime for the light source is more than 100,000 hours continuous – or more than 11 years. If the instrument must be turned off, then we suggest that the light source be allowed to “warm-up” for at least 1-2 hours before you attempt to run any critical analyses.

2. Oil and Fingerprints

   Any oil or fingerprints on the disc surfaces will cause baseline drift, because these tend to slowly “slide off” the disc surface under high g-force. As the oil disappears, the total light transmission falls (the signal voltage slowly goes down). This process can take remarkably long, even at high centrifuge speed (more than an hour!), and lead to very confusing results. It is recommended that you always wipe the outside disc surfaces with dry alcohol (methanol, ethanol, or propanol – and no water mixed in) before you start operation to remove all oil/fingerprints. Oil or oily materials on the inside surfaces can also cause drift, though the effect is normally less than the outside surfaces. If you think that the inside surfaces are contaminated, then it does not hurt to wipe these with dry alcohol as well.

3. Airborne Dust/Dirt/Oil

Very small particles floating in the air can cause mild to extreme baseline drift. Particles as small as smoke can deposit on the moving disc surface and gradually reduce light transmission. Oil mists generated by pumps that use an oil seal (like many laboratory vacuum pumps) are especially bad, since oil seems to allow other particles to cling to the disc surfaces (another reason to remove all oil/fingerprints, see 2 above). In extreme cases of dirty ambient air, it may never be possible to achieve a stable baseline; the instrument simply has to be moved to a cleaner environment.

Electrostatic charge buildup on the disc can also lead to rabid accumulation of dust particles from the air. Electrostatic charge is especially a problem when the ambient air is very low in relative humidity (winter months in colder climates). Static charge buildup can be eliminated by wiping the front and back disc surfaces with a very dilute solution (~50 PPM by weight) of an anionic emulsifier in alcohol. The recommended emulsifier is sodium dihexyl sulfosuccinate, but many others will also work to eliminate static charge build-up. Make sure that all outside surfaces are contacted with the emulsifier solution so that the static charge has a continuous path to ground.

4. Bacterial Growth in Aqueous Gradient Fluids

Aqueous gradient fluids are often based on materials (like sucrose) that provide a source of food to support bacterial growth. Experience shows that significant bacterial concentrations can be reached in less than 16 hours after a sucrose solution is formed, unless a preservative is used to inhibit this growth. When bacteria are present in the gradient, they can cause baseline drift for a long time, especially with more dense gradients. The bacteria can have net particle densities very close to the fluid density, so they may sediment very slowly even at high g-force. We recommend using a preservative (for example, ~20 PPM sodium hypo-chlorite) with any aqueous medium that may support bacterial growth, or making up fresh gradient solutions each time that you use the instrument.

5. Thermal Expansion of the Disc

At lower centrifuge speeds (<~10,000 RPM) total equilibrium temperature rise from air friction is only a degree or two, but as centrifuge speed increases, the temperature rise can reach 8 degrees C. The disc itself expands measurably due to the temperature rise. It is impossible to have absolutely constant optical transmission across the face of the disc, so the baseline may drift as the temperature rises and the disc expands... the light beams ends up passing through the disc at a slightly different place than when the disc was “cold”. This baseline effect is not normally large, but it can be important for critical applications. You can eliminate this effect entirely by allowing the centrifuge to warm up for ~40 minutes at the desired operating speed, so that the temperature is already approaching the equilibrium value before running any samples.

6. Refractive Index Effects

There are four disc interfaces that the detector beam passes through: two outside and two inside. Part of the beam is reflected and scattered by these surfaces, with the fraction of total intensity lost depending on the difference in refractive index for the materials at the interface. (If there is no difference in refractive index, then there is no reflection or scattering.) The outside surfaces are always in contact with air, so losses at these outside surfaces should be constant. The inside surfaces are in contact with the density gradient fluid. The gradient fluid generally is not constant in refractive index, because it is made of differing concentrations of two or more materials. As the density gradient degrades via molecular diffusion, the fraction of beam intensity lost at the inside interfaces changes, because the refractive index of the liquid at the point where the beam passes through gradually changes. The magnitude of this effect is usually not large, but if the inside surfaces have been damaged (scratched or rough) then the drift from changing refractive index can be very significant. The only way to completely eliminate this source of drift is to use a density gradient that is “constant” in refractive index. This type of gradient uses three or more components that are combined to give a constant refractive index. For example, a constant refractive index gradient can be formed from water, propylene glycol, and sucrose. (Please contact us by e-mail if you need some recommendations for forming a constant refractive index gradient.)