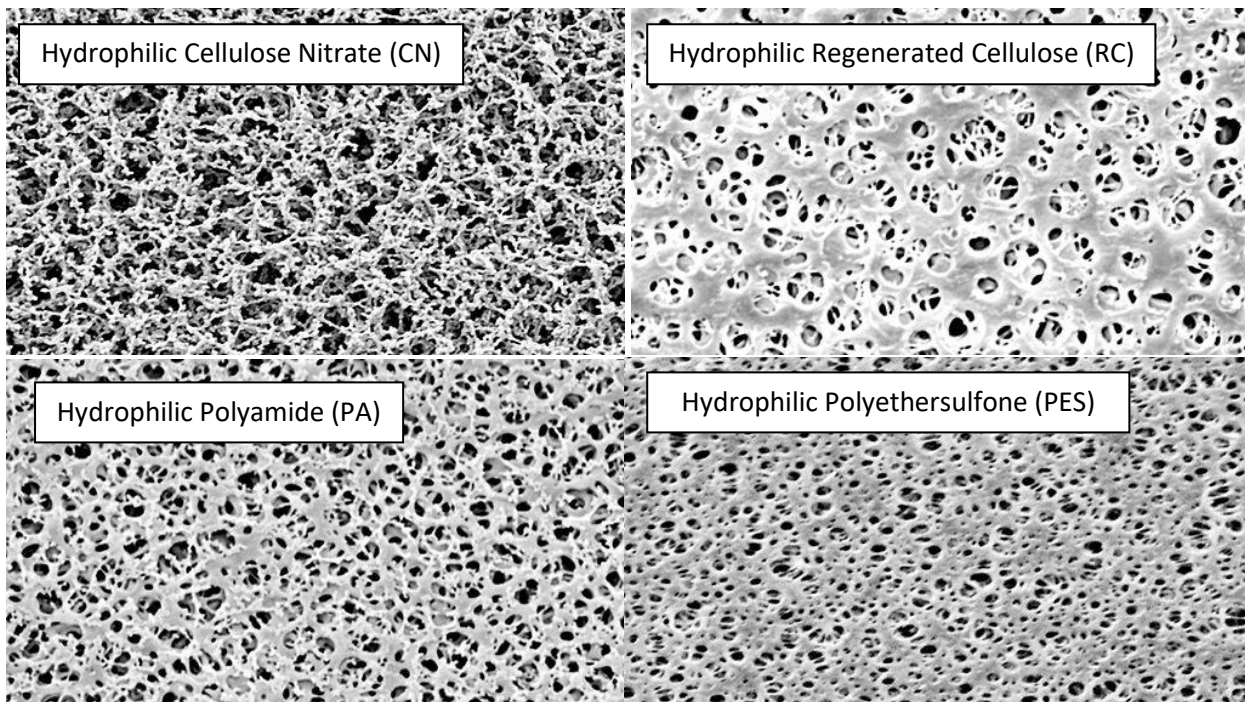


Why the CES filters are Special

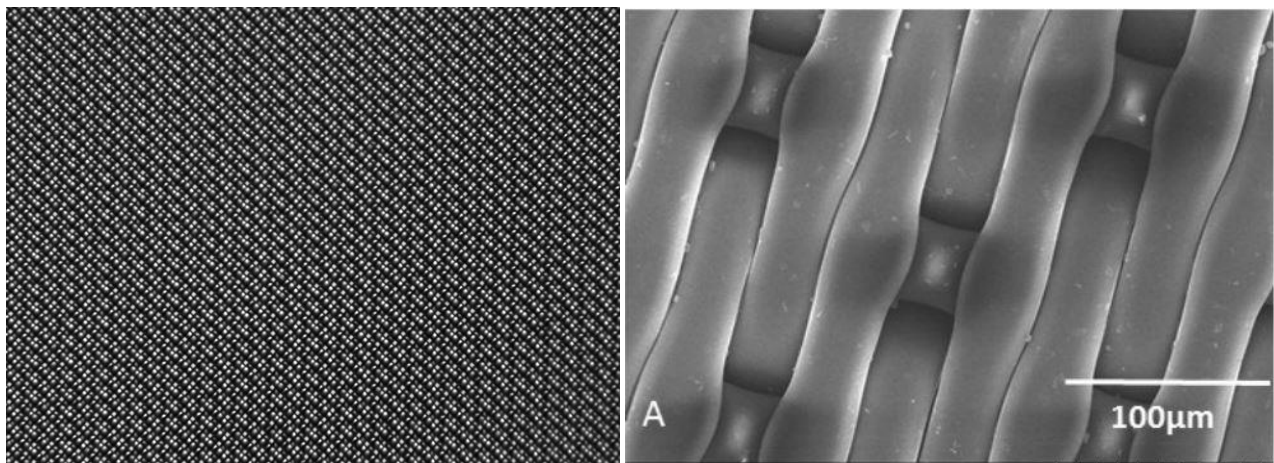
It took 4 years to develop the CES System and the special micro-filters it relies on. The initial intend was to use commercial available membrane filters, however by 'trial and error' we found that commercial available membranes do not work for a number of reasons:

Most filters are so called depth filters and do a great job at retaining particles throughout the filter medium, rather than just on the surface of the filter medium. Reality is also that they are not 'absolute', despite sometimes being advertised as such. Depth filters are problematic for the CES working principle, as it is difficult to recover the adults after the first step of the synchronization protocol without applying (back washing) pressure and thereby damaging the nematodes. Most disc filters are in many cases semi-depth filters, they just happen to be thin. Just take a look under the microscope or see some of the pictures we added below. All of them are 'membrane' filters of well-known brands, but none of them will accurately work in the CES protocol.

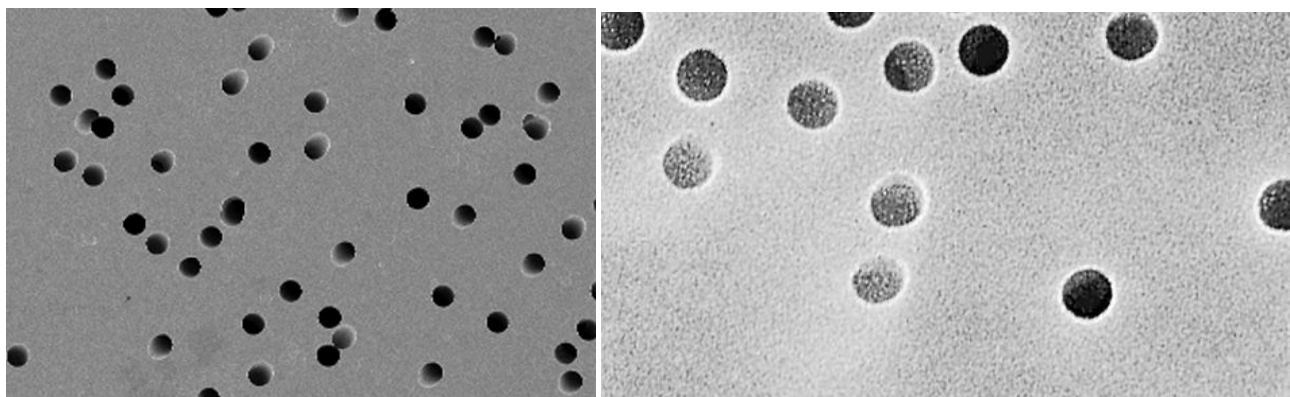


[images different type of Hydrophilic Sartorius Membrane filters]

The only type of filter that comes close to what we initially hoped could work are the so-called PET (membrane) filters, typically used in Cell Strainers.



[image PET – pluriStrainer® ‘netted’ filter]

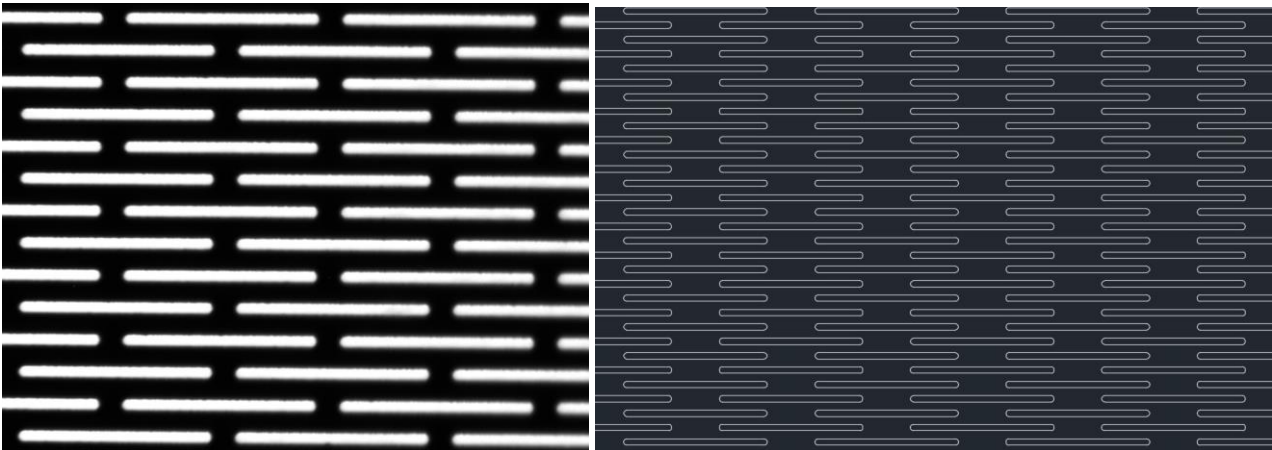


[image PET membrane - hydrophilic Polycarbonate Track-Etched (PC-TEM)]

Unfortunately it turns out the PET filters do not work for our CES application for the following reasons:

- a) Filters using a ‘netting’ made out of interlaced PET wires such as found in the pluriStrainers® didn’t work as the pores/apertures are not uniform when typically getting below 50µm as the PET wires are about 40 µm and pores/apertures are formed at the crossing point among the wires. Pores/apertures are not perfectly uniform and can vary up to 20-30 µm in lateral direction and as such not free from ‘defects’ in the definition of what the CES System requires.
- b) PET membrane (PC-TEM) pore size is typically limited to a pore size of max 8 µm. The pore/aperture is also a round capillary hole, great for holding back cells, however the typical L1 nematodes will not transfer through these filters.
- c) PET membranes (PC-TEM) typically have so called ‘doublets’ and even ‘triplets’ and even the best high quality filters still have a tolerance of +/- 10% . In our CES application we need an absolute (100%) guaranteed ZERO defect, i.e. no 'doublets' or 'triplets'.

Unlike traditional filters, the CES filters do not have 'round' or 'semi-round' pores/apertures however feature a special elongated design (slit) in order to prevent the filters from clogging while allowing L1's to pass through with ease. Below is a picture of the special metal alloy hydrophilic CES filter:



[image CES filter microscope and CAD image]

As mentioned before, a critical requirement for the CES to accurately work is an **Absolute Zero Defect for every single aperture** in order to get a perfect synchronization. Just imagine a single aperture (hole) to merge with an adjacent aperture (hole), and you have a so-called 'doublet' or 'triplet' and adult nematodes will also pass through and the system will not work as intended. For the CES application 99.999% is just not good enough!

It turns out there are NO commercial membranes available that meets the required specifications and therefore a different technology was needed. After several years of 'trial and error' prototyping, we were able to optimize the design and managed to manufacture a filter meeting all requirements guaranteeing a perfect (absolute) synchronization of nematodes.

Manufacturing the high precision CES micro-filters is done with a similar production process and clean room technology as found in the high-tech semi-conductor manufacturing. See the picture below of the actual manufacturing process of the CES filters.



[images CES filter manufacturing]

In addition, all CES glass parts are made from highest quality laboratory grade borosilicate glass and the high precision micro-filters are made from a very special high purity nickel alloy and are made to last. Did we mention that the CES system is developed and manufactured in the Netherlands, including the special micro-filters.

We hope this article explains 'Why the CES Filters are Special'. The CES System is sometimes perceived as expensive compared to high volume, low cost, commercial filters. However if used with care the CES filters will last 'forever' and the real cost of ownership is in fact low. Taking into account all the benefits* the CES provides including the long life span of the CES filters , the actual cost of ownership is lower compared to prior-art methods of synchronization.

(*) Healthy phenotype free worms synchronized without bleach, chemicals and starvation, improved level of synchronization with little variation, consistent results with little or no training required, less failed experiments!