Acoustic Cell Handling White Paper

A gentle, closed and scalable unit operation for cell therapy manufacturing

ænitis technologies

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Executive Summary

Cell and gene therapy manufacturing is entering a new industrial era, where fragile, high-value cellular products must be processed at scale under strict GMP constraints while preserving viability, functionality and safety. Legacy downstream operations such as centrifugation, filtration or magnetic sorting were not designed for these requirements. They introduce mechanical stress, rely on manual handling, increase contamination risks, and lack the modularity needed to adapt to rapidly evolving bioproduction workflows.

Acoustic Cell Handling is emerging as a new class of industrial unit operation, purpose-built for modern cell and gene therapy manufacturing. By using controlled acoustic forces to handle cells in continuous flow, it enables contact-free, low-stress processing without human intervention, while remaining fully compatible with closed, automated GMP environments.

This White Paper

This White Paper positions acoustic cell handling as a key enabling technology for next-generation bioproduction.
It reviews the state of the art in acoustic technologies, introduces Aenitis' proprietary acoustophoresis platform, and provides a theoretical framework allowing readers to assess whether acoustic processing may be compatible with their specific cell handling challenges.

Context:

Bioproduction Challenges in Cell and Gene Therapy

The cell therapy industry is at a critical inflection point. As the field matures and shifts from early-stage research to commercial-scale production, manufacturers face increasing regulatory scrutiny, higher expectations for process control, and the need for scalable, closed, and automated systems. Traditional equipment - centrifuges, filters, and bioreactors - originating from the blood industry or research labs, are often not designed for the unique requirements of cell therapy. These legacy systems are often manual, labor-intensive, and can compromise cell viability, yield, and process consistency - all of which are critical for regulatory compliance and commercial success. Shifting to more automated processing technologies, the industry faces its rapid evolution and requests more modular or agile technologies and devices to be able to adapt to new processes paradigm following major continuous improvement for safer and cost effective biotherapeutics production.

There is broad consensus in the field that advanced therapies are now moving into a space where long-standing GMP expectations fully apply. Regulatory agencies such as the FDA are not "tightening" rules as much as requiring manufacturers to align with existing guidance. A major strategic risk is relying on early-stage equipment that later proves unsuitable for commercialization, leading to costly comparability exercises and potential delays in market access.

Centrifugation remains the conventional technique used in downstream processing (DSP) for volume reduction and cell washing, often achieving a concentration factor greater than 40-fold. However, this method subjects sensitive therapeutic cells to high mechanical stress. The high G-forces required to pellet cells, followed by the necessary resuspension and supernatant exchange steps, introduce shear stress that can reduce cell viability and functionality. While centrifugation is effective for bulk separation, it is inherently aggressive compared to modern alternatives.

In contrast, acoustofluidic techniques have been shown to be exceptionally gentle. Studies demonstrate that acoustophoresis does not negatively affect cell viability or proliferation, even when processing sensitive cell lines[1]. This fundamental difference - avoiding the high shear forces and cell pelleting associated with centrifugation—presents a critical quality advantage for therapeutic products where cell viability, phenotype, and potency are paramount.

[1] Burguillos, M. A. et al. (2013). "Microchannel Acoustophoresis does not Impact Survival or Function of Microglia, Leukocytes or Tumor Cells." PLOS ONE, 8(5): e64233.

Zalis, M. C., Reyes, J. F., Augustsson, P., Holmqvist, S., Roybon, L., Laurell, T. & Deierborg, T. (2016). "Label-free concentration of viable neurons, hESCs and cancer cells by means of acoustophoresis." Integrative Biology, 8, 332–340. DOI: 10.1039/C5IB00288E.

Traditional flow filtration relies on mechanical barriers with defined pore sizes to separate cells from fluid media. While effective, these methods are highly susceptible to clogging and fouling caused by the accumulation of cell debris, proteins, or small particles. Clogging drastically reduces process consistency, necessitates operational pauses for cleaning or filter replacement, and introduces variability into continuous bioprocessing.

Acoustic filtration devices, such as those developed by AcouSort[2], Aenitis[3] or Getinge[4], overcome this limitation entirely by employing resonant ultrasonic waves to separate cells from the supernatant. This non-mechanical separation removes the inherent risk of clogging and fouling, offering all the benefits of filtration technologies without their limitations.

Industry guidance and regulatory authorities strongly advocate for replacing manual, open processing steps with fully closed and automated systems. This transition is essential for enhancing consistency, eliminating human error, and minimizing the risk of microbiological contamination, which is a major concern in high-throughput autologous manufacturing environments.

Automation offers a clear path toward industrialized production. AcouSort, for example, develops state-of-the-art components specifically to automate and integrate sample processing steps that traditionally required manual handling. By adopting automated acoustic devices, manufacturers fundamentally shift the source of process variability from human operators - a difficult-to-control factor - to machine precision. This shift creates a much clearer framework for establishing the process "design space" and providing robust evidence of consistency, thereby simplifying regulatory requirements for comparability protocols.

Finally, the scale-out model demanded by autologous therapies requires decentralized, modular, and often single-use facilities. Traditional blood processing equipment, often adapted from large clinical systems, represents an infrastructure mismatch. The small footprint and disposable nature of acoustic components are perfectly suited to support this decentralized, rapid-scale-out model, providing the necessary flexibility that legacy, large-footprint centrifuges and complex filtration rigs cannot offer.

Acoustic devices, such as those developed by Aenitis, Getinge and AcouSort, leverage acoustofluidics — a combination of sound waves (acoustics) and microfluidics. This technology enables the precise, gentle, and contact-free separation, washing, and concentration of cells based on their physical properties (size, density, compressibility). Unlike centrifugation or filtration, acoustic separation is continuous, biocompatible, and can be fully automated and integrated into closed systems.

[2] https://acousort.com/

[3] https://www.aenitis.fr/

[4] https://www.getinge.com/int/products/applikon-biosep/

Acoustic devices represent a **paradigm shift in cell therapy production**

Advantage	Acoustic Devices	Traditional Methods
Gentleness	Non-contact, preserves cell viability and function	High mechanical stress, risk of cell damage
Automation	Fully automatable, closed systems	Manual or semi-automated, open processes
Scalability	Continuous flow, modular, scalable	Batch processing, limited scalability
Yield & Purity	High recovery, high purity	Variable, often lower yield and purity
Regulatory Compliance	Designed for GMP, data-rich, transparent	Adapted from other industries, less documentation
Cost Efficiency	Reduces labor, consumables, and facility footprint	High labor needs, consumables, and infrastructure costs

Acoustic devices represent a paradigm shift in cell therapy production. By offering a gentle, scalable, automatable, and regulatory-friendly alternative to traditional methods, they address the most pressing challenges faced by manufacturers today. As the cell therapy market continues to grow - projected to exceed $40 billion by 2034[5] - adoption of innovative technologies like acoustofluidics will be essential for companies seeking to deliver safe, effective, and affordable therapies to patients worldwide. The importance of “phase-appropriate” equipment - using systems that can evolve with the product from early development to commercialization also needs to be emphasized. Thanks to their modularity and scalability, acoustic devices are particularly well suited to this requirement.

[5] https://www.grandviewresearch.com/industry-analysis/cell-therapy-market

https://www.transparencymarketresearch.com/cell-therapy-market.html

Acoustic devices: state of the art

Acoustic waves going through one medium to another, like two immiscible liquids, or a liquid and solid interface, will exert a force at that interface. That force is very small in most cases but can become significant if the wave intensity is high, or if the object is of similar size as the wavelength. This is called acoustic radiation force. A water/oil interface will deform if high intensity ultrasound is applied. In the case of particles and cells, free to move in a solvent, they will travel to specific areas. So would solid objects like plastic beads or rock particles. In the case of a standing wave in a resonator, they will travel to what are called pressure nodes and pressure antinodes. This mechanism is named acoustophoresis, or acoustic migration.

Since acoustic waves can displace any object, solid or liquid, and since the radiation force is directly proportionnal to the electric power applied to the acoustic source, it stemmed a large variety of applications and configurations. They can be categorized in high volume systems, like chambers or high flow rate channels, and in low volume systems, like microfluidic channels.

High volumes
- Closed systems
- In-flow systems, high flow rate

Low volumes
- Surface acoustic waves (SAW)
- Transverse resonator

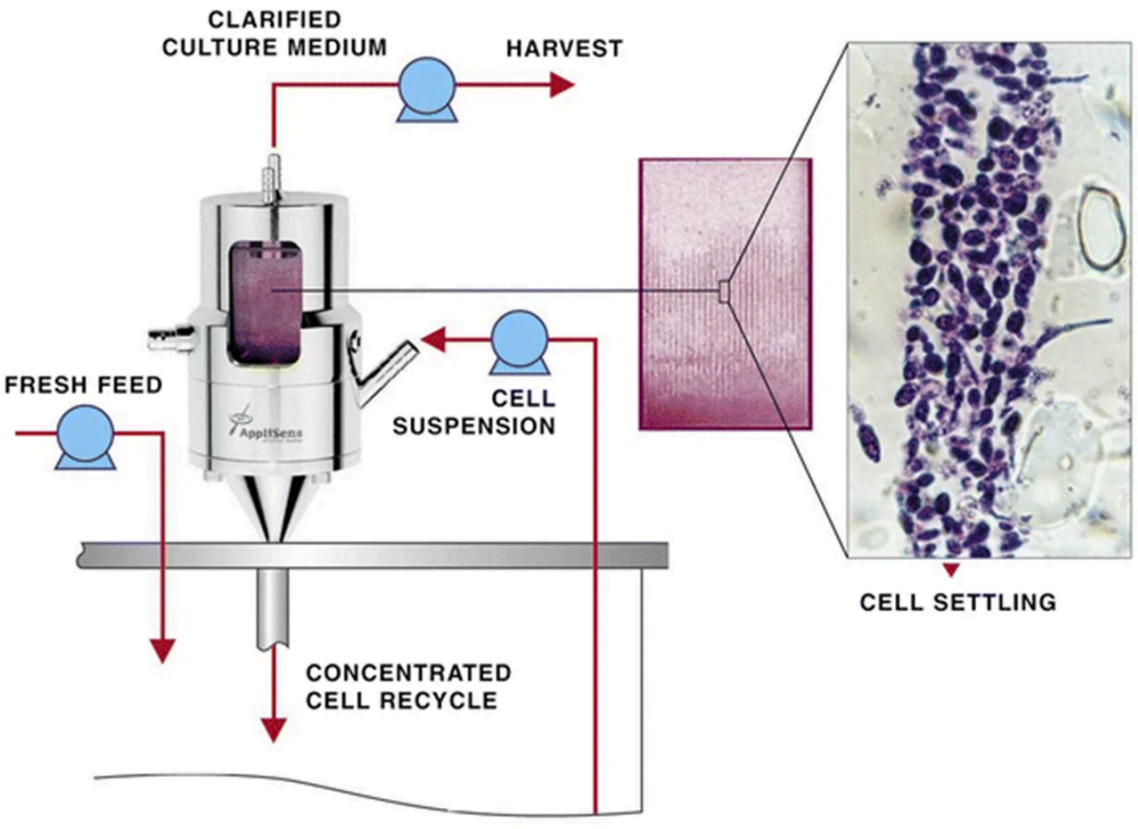
High volumes

Closed systems

Also called cell retention systems, this geometry uses multiple acoustic nodes to trap a cluster of cells in a chamber. That chamber can be temporarily closed, or a solvent can flow freely through it. This type of device allows for perfusion of cells that need constant fresh media and can also be used to aggregate cells that will naturally drop to the bottom of the tank by gravitation once reaching a critical mass. That aggregate is then sucked out of the chamber and collected.

Closed systems offer the possibility of high efficiency of transfusion, washing and concentration of cells. The chamber can be small, on the milliliter scale, or large, up to 10 liters. However, they're not good candidates for cell sorting, and they're not made to function with disposable chambers. The chambers need to be cleaned and reused. In some cases, the chamber is an expensive cartridge.

An example of this system is the Applikon BioSep from Getinge. It is sold in various chamber volumes, from 0.7mL to 1.45L. The cell suspension can be perfused, washed or concentrated.



Typical configuration of acoustic cell retention system.

Figure 1: BioSep system from Getinge. Source: https://www.getinge.com/dam/life-science/documents/english/biosep-flyer-82324-en.pdf

High volumes

In-flow systems, high flow rate

This geometry is basically a fluidic channel made from a type of plastic/resin, glass, or metal. The first two allow for visualization of the process, which is useful for cell sorting. A piezoelectric ceramic is usually glued or pressed against the channel, at different positions, allowing the formation of a fixed (or sometimes moving) pressure node/anti-node.

In microfluidic devices, syringe pumps are used (up to 0.5 ml/min) while in millifluidic channels, peristaltic pumps, which are contactless, are more common. (0.5 ml/min to 100 ml/min or more). Usually bulk acoustic waves (BAW) are used in high flow rate systems, with a type of resonator called a layered resonator..

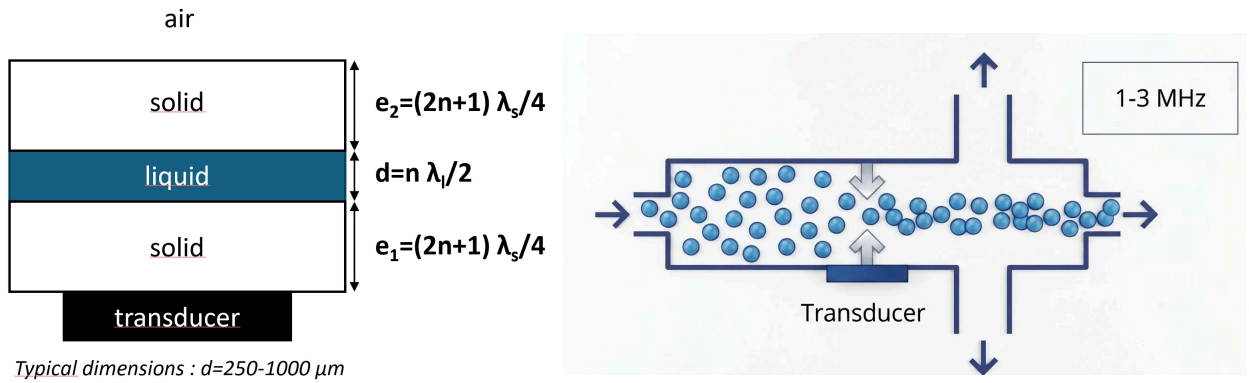


Figure 2: Principle of an acoustic layered resonator, and a 1 inlet 3 outlets concentration channel

Layered resonators use a geometry where the transducer, solid layers and liquid layers are all in line. The solid layers are usually plastic, glass or metal, and disposable. The channel is usually not glued to the transducer but temporarily fixed. This allows for the channel to be a disposable device. They're primarily used for washing and sorting cells. Moreover, since they are capable of high flow rates up to 100 ml/min, they can be used to quickly reconcentrate cells by removal of solvent through lateral outlets. For example, a solution of 1 million cells/ml can be reconcentrated to 3 million cells/ml if all the cells go out the central outlet, and the flow rates in all three outlets are equal. Experimentally, the yield varies between 95% and 99% depending on the flow rate and the type of cell.

Using plastic or metal channels makes this geometry highly versatile, in terms of flow rates, types of cells, sterilisation options etc. External tubing with peristaltic pumps is used, allowing it to be implemented into a cell production chain, or a cell therapy device. This high flow rate makes it a realistic alternative to mid sized to large cell centrifuges.

Low volumes

For precisely sorting small types of cells or biological objects (smaller than 1 µm), or for diagnostic purposes, microfluidics is preferred. The flow rate is inferior to 1 ml/min unless multiple channels are used. Two technologies dominate the field: surface acoustic waves (SAW), and transverse resonators.

Surface acoustic waves (SAW)

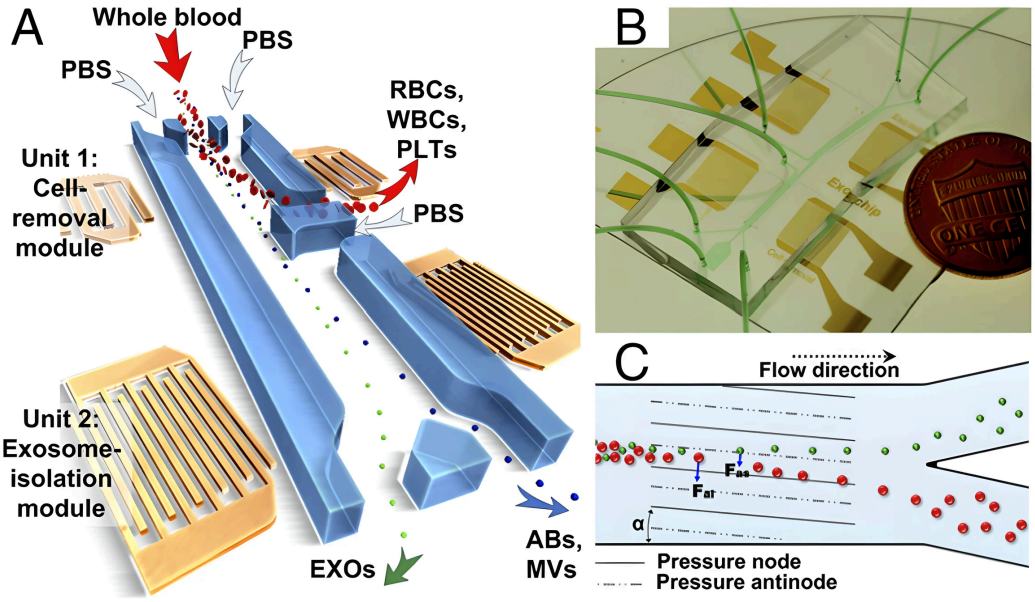


Figure 3: Isolation of exosomes from whole blood with 2 SAW sorting modules. Source: https://www.pnas.org/doi/10.1073/pnas.1709210114?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed

Devices using SAWs are made for very small volumes, and the use of high acoustic frequencies allows the manipulation of very small objects. They require special acoustic sources called Interdigitized Transducers (IDT). Connected to a glass or plastic microchannel, those devices allow for the visualization of the cells through a microscope. A notable result was the sorting of exosomes from whole blood by Tony Huang's team, that are on the 30-150 nm scale. With fluorescence, it was possible to observe the deviation of exosomes VS other objects in the biological solution and fine tune the acoustic power to achieve high yield and purity. This particular device contains two stages of acoustophoresis: the first 10MHz stage sends cells bigger than 2µm like platelets (PLTs), white blood cells (WBCs) and red blood cells (RBCs) into a first outlet. The second 100 MHz stage can affect smaller objects and aims at separating exosomes from other sub micrometer objects like apoptotic bodies (ABs) and microvesicles (MVs). This device potentially saves users the need to perform several steps of centrifugation, with trained personnel. It also increases the exosome isolation yield to 99% (against 5-40% with differential centrifugation methods) and the purity is 98.4%.

Low volumes

Transverse resonator

This type of device uses the same kind of wave as in the layered resonator, a bulk acoustic wave, but within a microfluidic channel and a different geometry. The channel is made of glass, metal, or a combination of the two. The transducer is fixed (often glued) on the channel, which allows for a strong acoustic transmission and also for a high yield and purity.

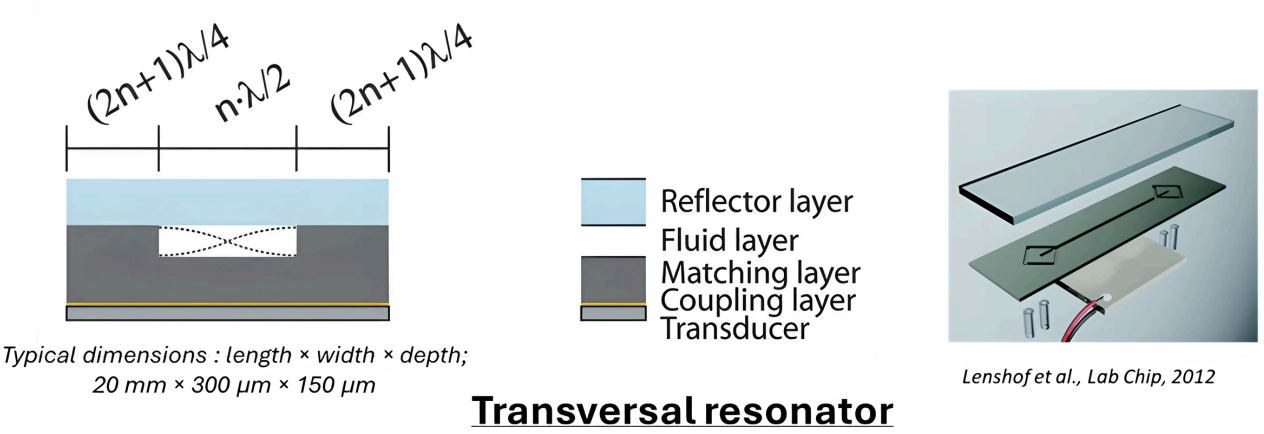


Figure 4: Principle of a transversal resonator for microfluidics

The acoustic wave is parallel to the piezo, meaning that cells are displaced in a plane parallel to the glass plate, allowing for the observation of the deviation of cells. Mostly developed by Acousort, different inlet/outlet geometries and multiple separation modules allow for a great variety of applications. Mostly, these devices are used as a single separation module for washing or sorting cells, for diagnostics purposes. They can also be used for production, if the volume required is small enough and the emphasis is on yield and purity.

Principle of acoustic cell manipulation

The patented technology used by Aenitis is based on the use of an acoustic force generated between the two walls of a fluidic channel to manipulate and/or separate, without contact, particles or biological objects (cells or cell clusters, bacteria, liposomes, solid particles, etc.) suspended in a fluid at rest or in flow (Fig. 5). Any wave that passes through two different media, whether optical or acoustic, generates a force at the interface between these two media. In the case of liquid media (cells or solid particles in an aqueous substrate, for example) and a stationary acoustic wave, a force is exerted on the object, and in return, the cell/particle moves: this is called acoustophoresis. No particular property of the object to be moved is required; acoustic waves can move solid, liquid, living objects, or objects of any geometric shape.

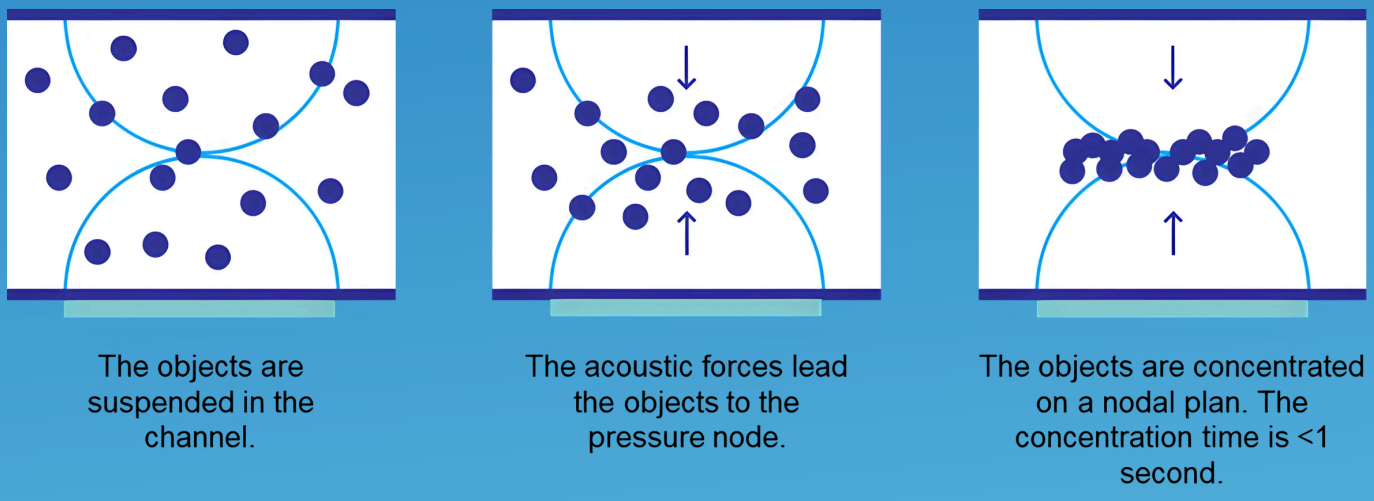


Figure 5 : Focusing of objects to a pressure node via acoustophoresis

The acoustic radiation force (ARF) pushes particles towards a pressure node or antinode located in the middle of the channel or at the walls in the geometry that Aenitis uses. The acoustic wave is traditionally represented by the crossing of the incident wave and reflected wave on fig. 5 in that X shape.

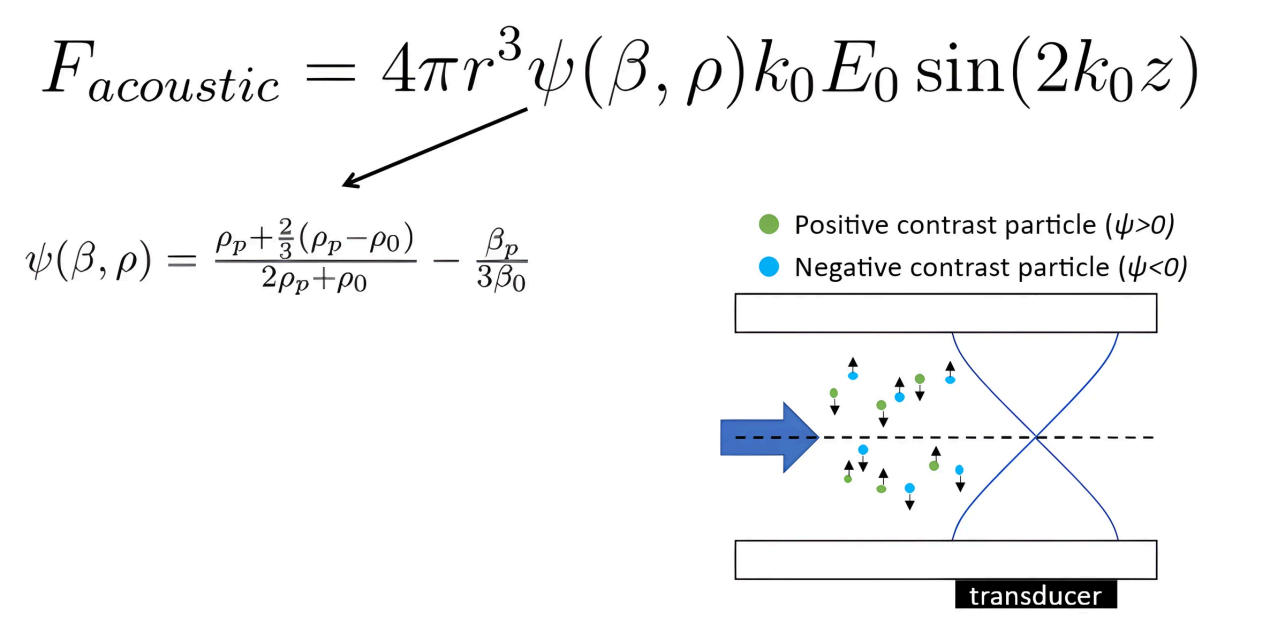


Figure 6 : Acoustic radiation force and direction of the displacement of objects

The acoustic force experienced by the particles in suspension depends on their physical properties (compressibility β, density ρ and speed of sound c) and geometric properties (radius r, for a spherical object). The larger the object, the easier it is to move. This force is also proportional to the frequency f (the wave number k0 is proportional to f) and to the electrical power (related to the acoustic energy E0). The parameter ψ is called the acoustic contrast factor, and the sign of this parameter determines the direction of particle migration. If ψ is positive, the particles migrate towards the acoustic pressure node at the center of the channel (the most common case). If ψ is negative, the particles migrate towards the two acoustic pressure antinodes at the ceiling and floor of the channel (see Figure 2). The absolute value of this parameter ψ will determine the effectiveness of acoustophoresis. To simplify, the greater the difference in density between the particle to be moved and the surrounding medium, the greater the force. Similarly, the greater the difference in sound velocity between the particle and the medium, the greater the force. Consequently, it is easy to move solid particles in a liquid (large contrast), and requires more input energy to move cells, whose density is closer to that of the medium. However, to date, we have never encountered cells (greater than 2 µm) that were impossible to move with our experimental setup.

As a result, objects denser than the media will exit the central outlet C, whereas objects lighter than the media will exit the lateral outlets O (opposite the piezo) and P (piezo side). This is the key to most acoustophoresis applications: sorting, washing, and isolating (see figure 3).

- Sorting can be achieved when one type of object to be recovered has different acoustic properties from the others, to be discarded. With normal cells, that are usually all denser than their surrounding media, this can be achieved through what we call acoustic beads. The beads will attach through antibodies to either the desirable cells (positive sorting) or the cells to be discarded (negative sorting). Beads need to be lighter than the media, and as a result are either lipid vesicles, or gas bubbles.

- Cell washing requires a cell population to change media. This can be achieved by having multiple inlets, the central being for example the fresh media, and the lateral inlets the solution with the wanted cells and the old media. If the solution has multiple cell species, this type of channel can also be used to achieve sorting at the same time.

- Cell concentration/isolation is useful when the user needs to change the concentration of a solution. In a 3 outlet channel, if all cells exit through the central outlet, and the flowrates and identical between the 3 outlets, then the concentration is tripled.

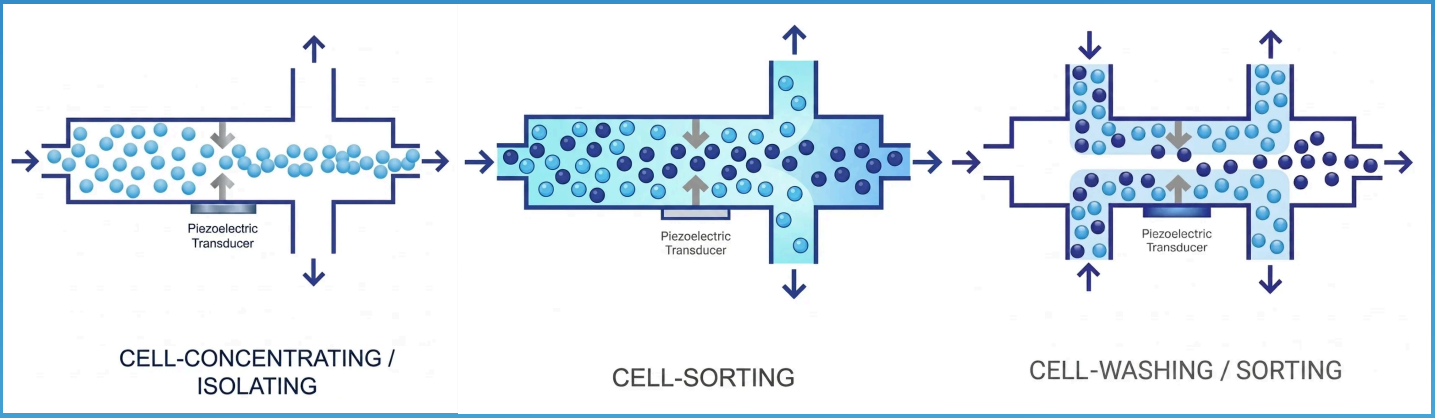


Figure 7: Different types of channels taking advantage of acoustophoresis properties.

On top of these three main applications, Aenitis has also explored two other applications using acoustophoresis to trigger specific cell properties: transduction, and vesicle productions. Both those applications result from the fact the pressure waves such as acoustic waves trigger a response in cells.

- In the case of a transduction protocol, it can improve the viral transduction efficiency by 100% by exciting the cell membranes and increasing the chances of transfer of DNA from the virus to the inside of the cells.
- In the case of vesicle production, cells will produce vesicles upon exposure to a type of stress, whether it's shear stress, heat, electrical or pressure waves. We successfully used acoustic waves in a concentration channel to make cells produce vesicles efficiently.

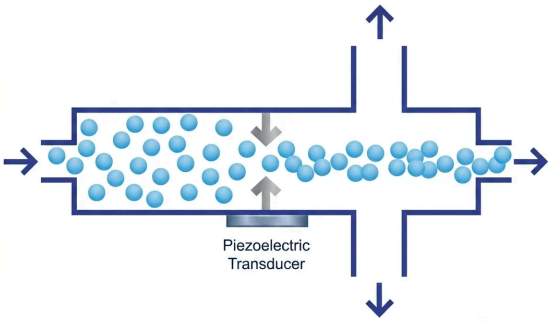
Advantages

The technology developed by Aenitis has several advantages compared to the existing acoustophoresis technologies:

- Flowrates: the channels are designed to be able to function in a continuous flow, in a flow rate range between 1 and 50 ml/min, allowing to treat several litres of solution per day.
- In-line flow: the entire system is open tubing and can be connected in line to any other cell production strategy
- Versatility: the sorting, washing and concentrating properties allow for easy tailoring of the technologies to one's needs. They can also be combined with other properties of acoustic waves. For example, making cells produce vesicles in a concentration channel, then collect the solution and sort cells from vesicles in a washing channel.

Types of applications

1 - Concentration



Concentration channel

This application aims at grouping objects into the center of the channel, and collecting them in a central outlet, to augment the concentration of this object, usually cells. A simple one inlet/three outlets channel allows to almost triple the concentration with a high recovery rate.

The first key step to building a reconcentration device is to accurately know the flow rates in each outlet. With 1/3 of the total flow rate in each of the outlets, and 100% the cells going in the central outlet, then the concentration factor is x3. If only 90% of the cells go in the same outlet, then the concentration factor is 0.9x3=2.7.

With most types of cells, around the 10 µm size, like Jurkat, Hela, or white blood cells, 95%/x2.85 is easily achieved and reproducible. The smaller the cell, the higher the required power to displace them. As a result, if we choose a typical flow rate of 5 ml/min, Jurkat cells (approx. 11 µm in diameter) require 2-3 W for 95% of them to migrate in the central outlet, against 25-30 W for platelet cells (approx. 2-3 µm in size).

If a concentration factor higher than x3 is required, then 2 channels connected back-to-back are used. The second channel inlet is then connected the first channel outlet. As a result, a x9 concentration factor is feasible.
A way to « boost » the concentration factor per channel is to use asymmetrical flow rates. If the inlet flow rate is kept the same, but the central outlet flow rate is lower, it is possible to reach x3.5-x4. This technique is useful when a high concentration is required, but the yield is not a priority.

Experimental protocol

Designing a reconcentration protocol starts with these 4 steps:

1. Determine the size of the cell/object to concentrate, whether the contrast factor is positive (which it usually is with most cells in media), the volume to process in a given time, and the final target concentration and yield.
2. Determine the variability of the samples. For example, density can vary a lot from donor to donor in the case of blood cells. This will affect the power required to concentrate the cells. A higher density will mean a lower power needed.
3. Conduct trials for the target flow rate at different powers, until finding the best yield (lowest cell loss). Check cell viability throughout the process.
4. Use the protocol for cell production.

Examples

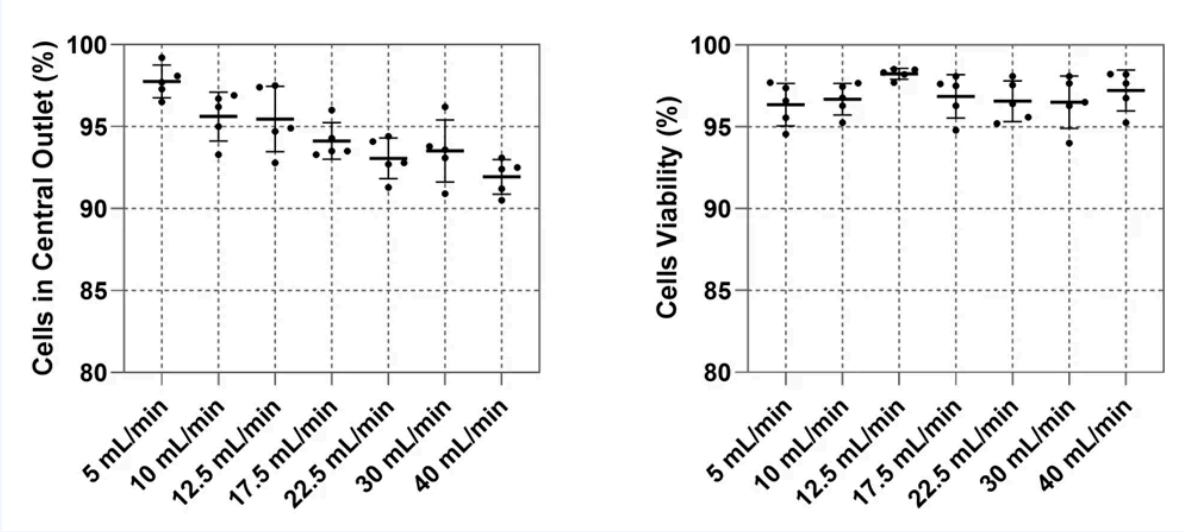


Figure 9 : Concentration of Jurkat cells at different flow rates.

An extensive study of Jurkat cells at different flow rates has been performed. From 5 ml/min to 40 ml/min in metal/PMMA channels, the reconcentration is between 92% and 98%, slightly going down with the flow rate. This particular study was done with 80 000 cells/ml, but more recent trials at 500 000 or 1 million cells/ml show identical results at 5 ml/min. Viability has never been affected in those experiments. More cells of similar sizes were trialed, like mesenchymal stem cell from bone marrow or fat donors (see fig. 10), HeLa cells, Hek cells, or red blood cells (7 µm in diameter, see fig. 11). It is important to note that the required power to reach more than 90% reconcentration varies a lot depending on the size of the cells. For Jurkat cells 5 ml/min is around 2 to 3 W. However, for platelets at 5 ml/min, the required power is higher than 25 W.

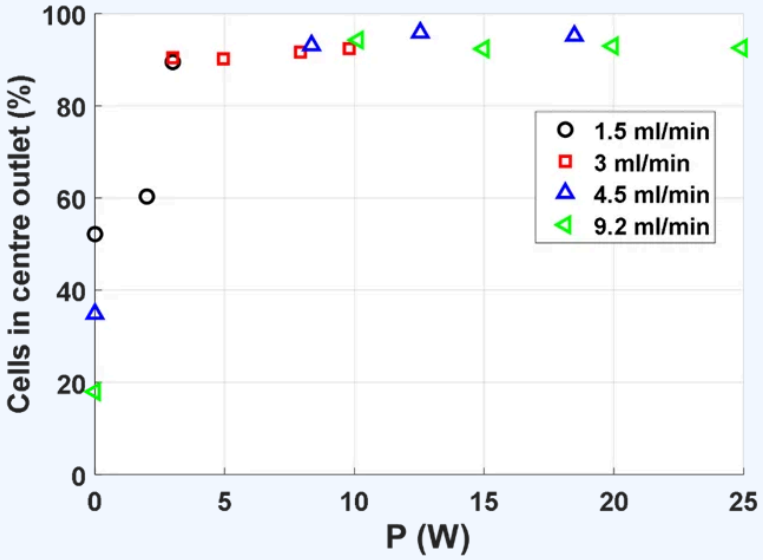


Figure 10: concentration experiments with MSCs (50-100 000 cells/ml)

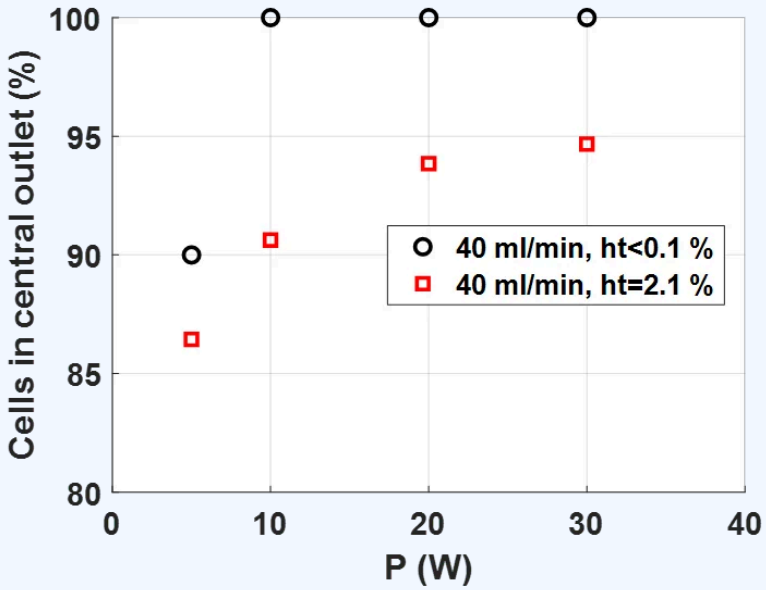


Figure 11: concentration experiments with diluted red blood cells (ht=hematocrit). Dilution media is Isoton.

Indeed, platelets are around 2 µm in size and can vary in shape. So, the required power is much higher than for white and red blood cells. The figure below shows the required power to reach 90% concentration at various flow rates specifically for platelet.

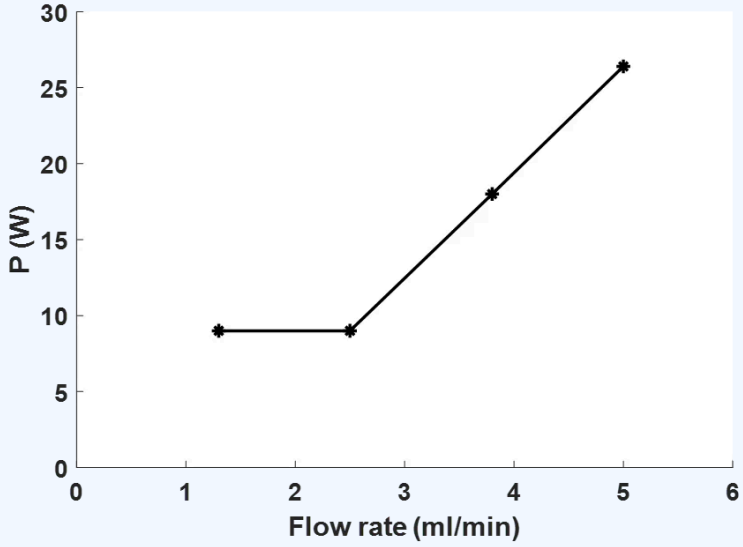


Figure 12: Required power (W) to reach more than 90% yield at various flow rates for platelets.

Platelet Concentrate Conservation

Requirements:

- PC conservation 22°C,
- Tests day 1 to day 7, (day 1 = 3 days post donation),
- Small induced activation,
- No lack of activability up to 5 days post process.
- Slight change in morphology and metabolism and in diluted gas at day 5

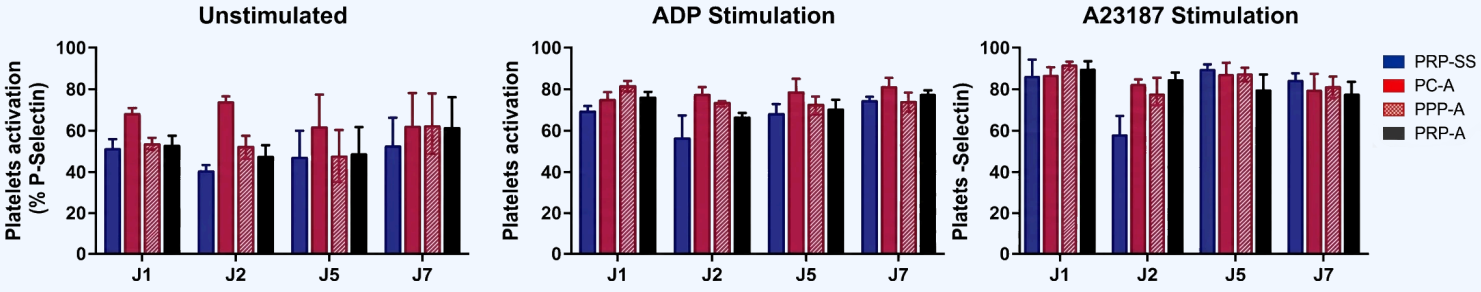


Figure 13: study on platelet conservation after acoustophoresis in 2 consecutive channels

A study of the storage quality of platelet concentrates (PC) maintained at 22°C from J1 to J7 has been performed (see fig. 13). Platelet integrity was evaluated using P-selectin expression, a well-established biomarker of activation that increases when platelets lose their resting state. Maintaining low baseline P-selectin levels is essential, as spontaneous activation compromises platelet survival and therapeutic efficiency.

The study includes a comparative analysis of four product types, each representing a distinct preparation method: PRP-SS (platelet-rich plasma obtained through soft-spin centrifugation), PC-A (apheresis-derived platelet concentrates), PRP-A (activated platelet-rich plasma), and PPP-A (activated platelet-poor plasma). This comparison aims to determine whether preparation techniques influence platelet stability and responsiveness during storage.

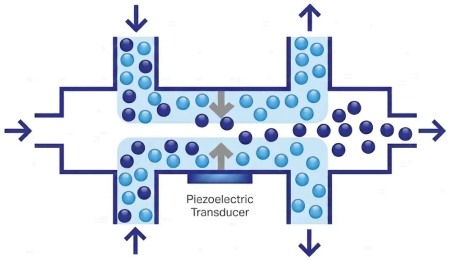
Three experimental conditions are analyzed: an unstimulated condition, which reflects intrinsic stability, and two stimulated conditions using ADP, a physiological agonist mimicking in-vivo platelet activation, and A23187, a calcium ionophore that induces maximal activation. The data show consistently low activation in the unstimulated condition for all product types, indicating preserved quiescence. In contrast, both ADP and A23187 show a robust activation for all days and products, demonstrating maintained functional reactivity.

Overall, the results indicate that all platelet preparations maintain stable morphology, adequate metabolic function, and sustained activation capacity during the storage period. The preservation of these parameters confirms that the different PC types remain of high quality and clinically suitable for at least seven days following processing.

Potential issues in concentration systems

- The system is limited by the maximum power of the amplifier used. Most of Aenitis' experiments were done with a limit of 30 W. But it is possible that a higher power will allow the use of very high flow rates (above 50 ml/min) or the manipulation of very small objects (below the size of platelets, 2 µm).
- The channel height for the 2.5 MHz system is 250-280 µm, although we have done prototypes with 500 µm. This means that the use of large cell clusters or tissues is not recommended. 50 µm objects maximum is the recommended limitation for 250 µm channels, double that for 500 µm channels.
- The volume fraction of cells is also a limitation of the system. In centrifuges, cells can be packed tighter together (leading to cell damage though) than in acoustic systems. We encountered this case with red blood cells especially: if the hematocrit is higher than 8%, then the concentration yield starts to drop. So, obtaining 24% hematocrit is possible. But starting at 10% and reaching 30% seems unattainable. A cell aggregate can then form in the middle of the channel, and potentially cells start to get damaged, just like in a high G centrifuge. With more commonly used cells like Jurkat cells, the upper limit for concentration is on the order of 250 million cells/ml. We have managed to concentrate jurkat cells from 81 million cells/ml to 243 million cells/ml.
- Cell loss needs to be checked, ideally the tubing used should be medical grade to reduce cell adhesion on the plastic tubing.

2 - Washing



Washing channel

In this document we call washing a change of media for one particular cell population. It can be useful to stop an enzyme digestion for example, or to give a fresh media to growing cells. This type of channel has 3 inlets and 3 outlets. Usually the « fresh » media is injected in the central inlet, and the cell mixture is in the lateral outlet. However, there is a very important property of acoustic systems that needs to be addressed here. If the liquid on the lateral inlets is denser than the liquid in the central inlet, then the acoustic radiation pressure will trigger what's called a phase shift, and the denser liquid layer will shift places with the less dense layer. That denser liquid is simply migrating towards the pressure node, just like cells would but in a more uniform manner. This can be prevented by adding a density gradient media to the liquid in the central inlet like Optiprep or Dextran. But this rarely is a practical option, and there is a simple alternative, similar to what a centrifuge does. The idea is to concentrate the cells into a small volume and then add the new media to that small volume. As we have seen, each channel divides the volume by 3 and ideally concentrate 95% or more of the cells in that volume. As a result, the protocol is similar to a concentration application but with the added media at the end.

Experimental protocol

Similar protocol to concentration:

- Determine the size of the cell/object to wash, whether the contrast factor is positive (which it usually is with most cells in media), the volume to process in a given time, and the final target concentration and yield.
- Determine the variability (density) of the samples, but this time, also the density of the clean media and the original cell media. The density of the cell media in the lateral inlets needs to be smaller or equal to the density of the clean media.
- Conduct trials for the target flow rate at different powers, until finding the best yield (lowest cell loss). Fine tune the inlet flow rates and outlet flow rates, again for best yield. Check cell viability throughout the process.
- Use the protocol for cell production.

Example with Jurkat cells

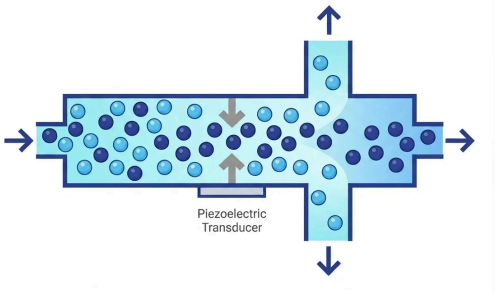
The key in this system is to achieve a high enough concentration at the end of the process that most of the medium has been washed away, while most of the cells are collected in small volume. Acoustophoresis has a lower concentration limit (i.e., the maximum number of cells/ml attainable) than a centrifuge. We experimentally estimated this limit for Jurkat cells. We have managed to concentrate Jurkat cells up to 250 million cells per ml. Above that we suspect that the packed cells disturbs the acoustic field, and that the cell layer becomes too big to all fit in the central outlet.

This means that for a 1 litre batch of 1 million jurkat cells/ml, we can use successive channels to concentrate the cells 3 times per channel. Two thirds of the media by volume is « washed away » in each channel. Consecutive channels, with the central outlet of the previous one plugged in the new one, will allow more media to be washed away. The process is worthwhile for the user as long as not too many cells are lost in the consecutive channels. If the yield is 95% in one channel, this means the cell loss stays at 5 % per channel. Indeed, although the volume is divided by 3 in each channel, the concentration is multiplied by 3. So, this means 5% loss per channel regardless. So extra care should be placed in achieving the highest yield possible for each individual flow rate. With 4 channels, the total theoretical cell loss is 20%, and 98.8 % of old media is washed away. With 3 channels, cell loss is 15% and 96.3 % of the media has been washed away. So, a compromise is possible between cell loss and fresh media percentage.

Volume	Cell concentration	Cell recovery (%)	Washing efficiency
Initial volume of 1000 ml	1 million/ml	100%	0%
333 ml after channel 1	3 million/ml	95% after channel 1	66.7%
111 ml after channel 2	9 million/ml	90% after channel 2	88.9%
37 ml after channel 3	27 million/ml	85% after channel 3	96.3%
12.3 ml after channel 4	81 million/ml	80% after channel 4	98.8%
4.1 ml after channel 5	243 million/ml	75% after channel 5	99.6%

Table 1: Example of Jurkat cell washing after concentration in 5 consecutive channels with 95% concentration efficiency in each channel. Washing efficiency is defined as total % of media removed in the lateral outlets from the initial volume.

3 - Sorting



Sorting channel

Sorting two objects of very different densities and/or sizes

There are several ways of sorting cells/objects in an acoustophoresis system. The easiest one is similar to washing, but with two populations. The challenge is to find the power threshold at which one population will travel to the center channel, while the other population is little or not affected by the acoustic radiation force. This is the method used to sort cells in microfluidics, with transparent channels, as this tuning is more easily done with the help of a microscope and fluorescence. And just like with washing, playing with the solvents densities is also a way to help with sorting.

The protocol is then similar to a washing experiment and as follows:

1)Determine the size/density of the cells/objects in the solution and whether their contrast factor is positive (which it usually is with most cells in media), the volume to process in a given time, and the final target concentration and yield.

2)Determine the variability (density) of the samples, and the density of the clean media and the original cell media. The density of the cell media in the lateral inlets needs to be smaller or equal to the density of the clean media.

3)Conduct trials for the target flow rate at different powers, until finding the best yield. Some experiments might require purity to be the priority; some experiments might require a better yield instead. Check cell viability throughout the process.

4)Use the protocol for cell production.

Sorting two cell populations of similar size and density

This case is both more difficult and more common. For example, if one is trying to separate white blood cells from red blood cells. If the previous system was used, too many red blood cells would migrate to the center of the channel, alongside the white blood cells. However, red blood cells and white blood cells have different densities. It is then possible to tweak the density of the media with Optiprep or Dextran to have a positive contrast factor for one type of cell, and a negative one for another type of cell.

We measured the speed of sound and density of various ABS+Optiprep mixtures (see annex 2) in order to calculate the acoustic contrast factor ACF (see annex 1) for red blood cells and white blood cells. Then we plotted it against the optiprep percentage in the solution (see fig.14). For low concentrations of Optiprep, both ACFs are positive. But at 60% Optiprep, the red blood cell ACF is positive while the white blood cell ACF is negative (see dotted line). This means that RBCs will travel to the central outlet while the WBCs will travel to the lateral outlets. Then we repeat the protocol from the previous section to find the ideal power. In this particular example, however, the ACF of RBCs shows significant donor-to-donor variability, with values varying by up to ~20%. So, the Optiprep concentration that allows the ACFs to be positive and negative varies too. We've encountered one experiment where we managed to have a sorting effect, but then the following day, both cell populations would be focused to the center outlet with another donor. This was verified also with MSCs[1]. This system only works when the ACF doesn't change much from donor to donor.

[1] Characterization of mesenchymal stromal cells physical properties using acoustic radiation force, Bellebon et al., Front. Phys., 23 August 2022, Sec. Physical Acoustics and Ultrasonics, Volume 10 – 2022.

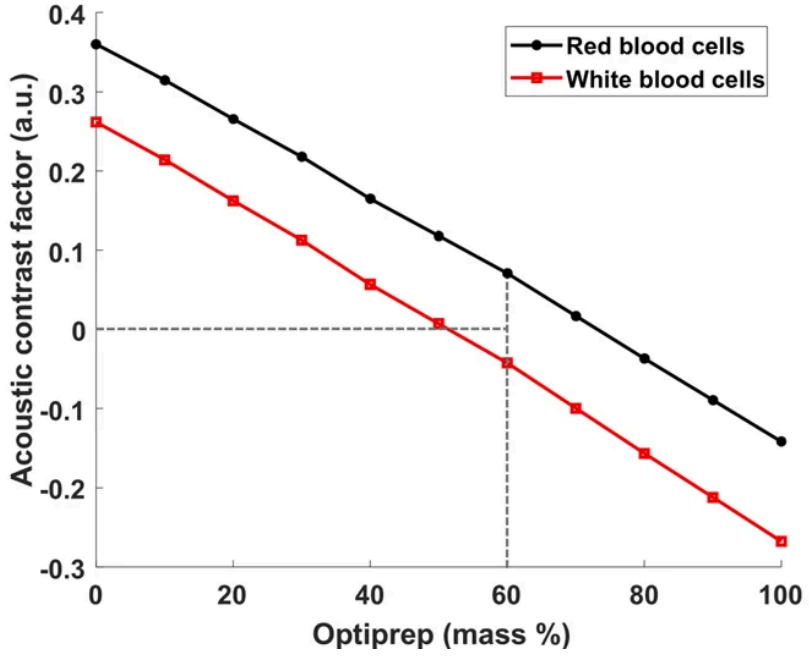


Figure 14: Acoustic Contrast Factor for RBCs and WBCs in Optiprep/ABS mixtures.

Sorting using negative contrast agents: acoustic bubbles and droplets

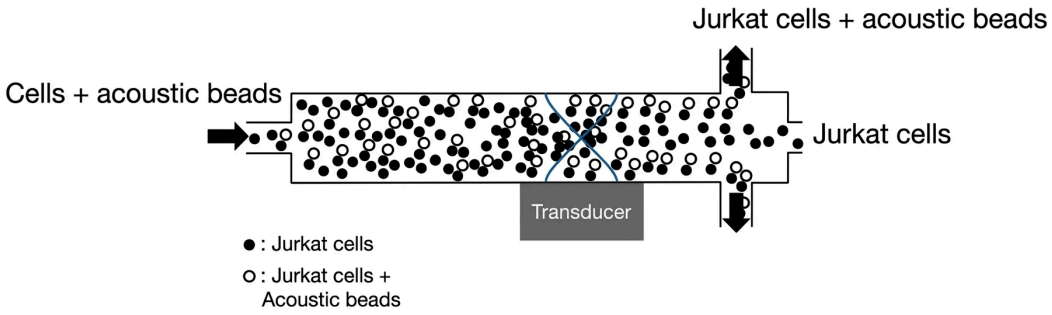


Figure 15: sorting channel with acoustic beads (bubbles or droplets)

When two or more cell populations have similar densities and sizes, and a high variation of these parameters depending on the donor or other parameters, there is still a way to achieve sorting. Using antibodies to attach objects to cells is a sorting technique that exists since the invention of fluorescence activated cell sorting (FACS) since the late 60s. But it required lasers and the process would sort cells one by one with that laser detection. Then magnetic-activated cell sorting was discovered in the late 80s, which allowed a magnetic field to sort all cells tagged with a magnetic bead. With the arrival of acoustophoresis to manipulate cells, it was then discovered that gas microbubbles could be attached to cells and used in the same way[1]. Oil droplets are also a possibility, and we will call both bubbles and oil droplets « acoustic beads » (see fig.15).

Bubbles are typically shorter lived, and many forms of contrast microbubbles are already biocompatible. The gas used in the bubble is usually a form of perfluorocarbon gas (low solubility in liquids, unlike air), like C4F10. One 3 µm diameter C4F10 bubble is enough to turn the acoustic contrast factor of a 10-12 µm cell negative. However, gas bubbles have a specific resonant frequency (Minnaert resonance) at which they vibrate violently. In the case of perfluorocarbon bubbles and a 2.6 MHz excitation frequency, we've identified that bubbles of sizes around 2-3 µm cause issues. They will vibrate, generate flows (acoustic streaming) and potentially harm cells. By using larger bubbles, around 5-6 µm, the effect is mitigated.

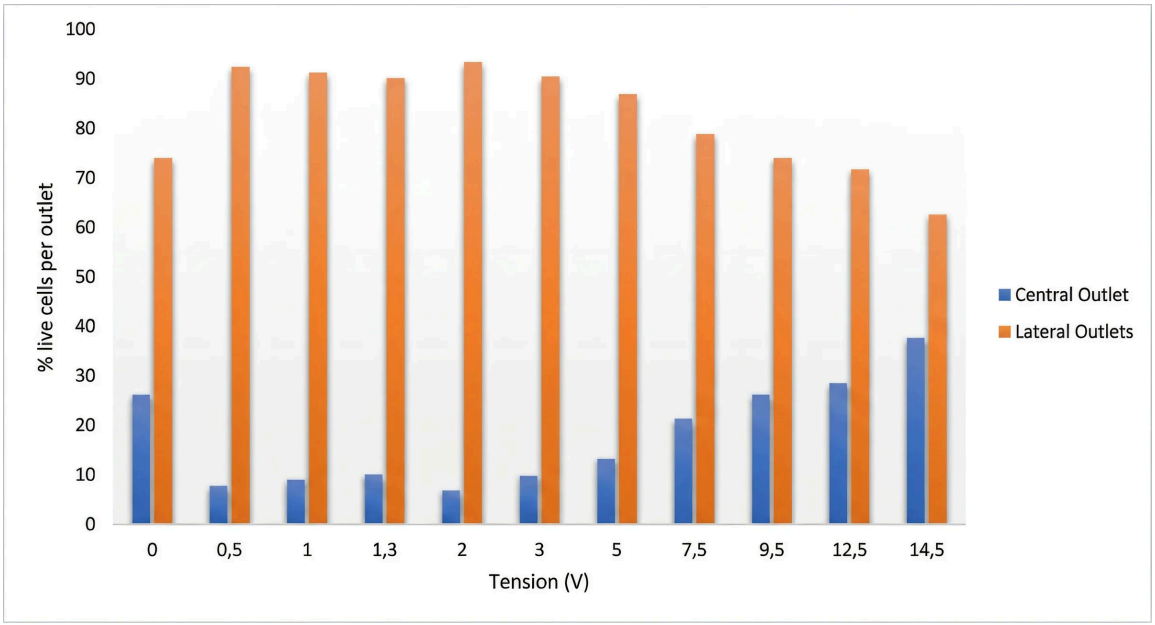


Figure 16: CCRF-CEM CCL119 cells, with decanted bubbles mostly centered around 5-6 µm diameter. 500 000 cells/ml, 5 bubbles/cell.

With these bubbles, it is then very easy to drive cells to the lateral outlets (see fig.16). Experiments are being conducted with 2 or more cell populations. The challenge is that the bubbles are so effective, they only require a very low power. That power is then not enough to make the untargetted cell migrate to the central outlet. And when the power is increased, we see that too many cells move to the central outlet. This is because aggregates form inside the channel, and expell cells from time to time in all directions. A number of bubbles per cell of 4 was tried, but that was too low, and the tagged cells did not migrate to the lateral sides anymore, meaning the system needs to be very finely tuned.

[1] Faridi, M.A., Ramachandraiah, H., Iranmanesh, I. et al. MicroBubble activated acoustic cell sorting. Biomed Microdevices 19, 23 (2017).

An alternative to gas bubbles is oil droplets. Most cells have a density between 1.05 and 1.15. An oil droplet with a density inferior to 0.9 is then potentially very useful to send cells to the lateral outlets, as the overall complex cell+droplet will be less dense than water, and the ACF becomes negative. The attachment system is the same, since the membrane of those droplets is similar to the gas bubbles (phospholipid layer). The benefit of droplets is that they're more stable and are mostly incompressible.

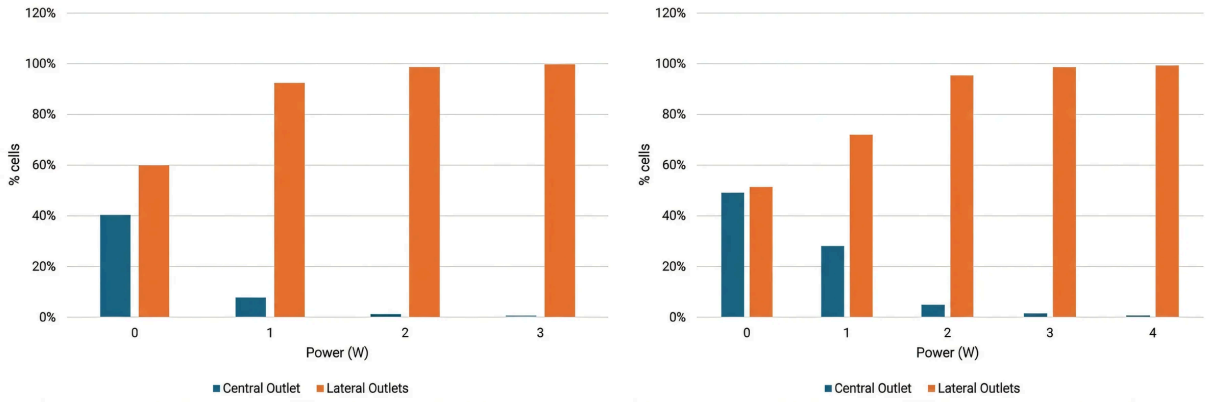


Figure 17: Acoustophoresis on 11.4 µm diameter oil droplet, density 0.84, 4 bubbles per Jurkat cell, 100 000 cells/ml, two identical experiments for repeatability.

Targeted cells migrate very easily to the lateral outlets (see fig.17), and experiments will be conducted with 2 or more cell populations. This time, the yield is excellent at 1-2 W, which is the power at which unmarked Jurkat cells would move to the central outlet. This means using droplet instead of bubbles would make fine tuning for the best yields/purity easier.

Flow rate optimisation

Increasing the flowrate is usually the last step of the creation of an acoustophoresis protocol. It is easier to first do low flow rate experiments both to save on cells, and to be able to try more power conditions. Once the ideal power and yield/purity is determined at 1 to 5 ml/min, then experiments with higher flow rates can be performed, and then experiments where only one power is used, and the whole cell batch is processed. The following table lists the usual required power depending on the cell size, as well as the maximum flow rate possible with 30 W as a maximum power.

Aenitis recommends using the current steel/PMMA channels up until 40 ml/min. Higher flow rates are possible, and the channels can handle them (tried up until 100 ml/min) but then cell aggregates start to form in the channel caps.

Cell size	Flow rate	Power	Example
1-5 µm	5 ml/min max	15-30 W	Platelets
6-9 µm	40 ml/min max	10-30 W	RBCs
10-50 µm	40 ml/min max	10-20 W	Jurkat, HeLa, Hek, CHO...

Advantages of the Aenitis Platform

Compared with existing acoustophoresis implementations, Aenitis' technology offers several distinctive advantages:

Relevant flow rates for manufacturing
Channels are designed for continuous operation in the 1–50 mL/min range, enabling the processing of several liters per day and making the technology compatible with clinical and commercial-scale workflows.

In-line integration
The entire system uses standard tubing and connectors, facilitating direct integration into upstream and downstream unit operations in a closed, GMP-compatible environment.

Versatility of operations
The same acoustic principles can support concentration, washing, and sorting, and can be combined with functional effects such as vesicle induction or transduction enhancement. Configurations can thus be tailored to specific unit operations or end-to-end process strategies.

Modularity and scalability
Channels can be operated in series (for multiple washing or concentration stages) or in parallel (for higher throughput), enabling phase-appropriate deployment from preclinical development through to commercial manufacturing.

Low mechanical stress
Preserving cell quality and functionality during processing

Biocompatible
Safe for delicate therapeutic cells

Continuous flow process
From mL to liters and exchanging media with no volume limit

Sterile conditions
No external contact, reducing contamination risk

GMP-compliant
Fully validated and audit-ready, 21 CFR Part 11, FDA-approved materials, CE-marked consumables

Flexible platform
Adapts to early research or full-scale production

Modular production units
Scale from lab to GMP manufacturing

4 months
to deliver custom single-use consumables

Conclusion

The transition of cell and gene therapies from the laboratory to the clinic exposes the limitations of hardware originally developed for blood processing or research use. High-g centrifugation, pore-based filtration, and manual handling steps introduce unnecessary stress and variability into processes in which every cell counts. At the same time, regulators expect fully controlled, well-characterized, and scalable manufacturing platforms from the earliest feasible stage.

Acoustofluidics offers a fundamentally different approach. By using acoustic radiation forces in well-defined micro- and millifluidic geometries, it becomes possible to concentrate, wash, and sort cells in a continuous, gentle, and contact-free manner. The result is a set of unit operations that preserves viability and functionality, is straightforward to automate, and naturally fits within closed, single-use architectures.

Within this landscape, Aenitis' acoustophoresis platform addresses both scientific and industrial requirements. The platform:

- Operates at flow rates and volumes that are relevant for real-world manufacturing.
- Supports a range of core functions—concentration, washing, sorting—within a single conceptual framework.
- Can be deployed in modular, phase-appropriate configurations, evolving with the product from discovery to commercialization.
- Opens the door to advanced applications, such as controlled vesicle production and improved viral transduction, which can further intensify and derisk manufacturing.

As cell therapy pipelines diversify and the number of clinical products grows, the pressure to standardize and industrialize processing will only increase. Acoustic technologies, and in particular the Aenitis platform, provide a robust physical basis on which to design the next generation of cell-handling equipment: gentle enough for fragile, high-value cells, powerful enough for industrial throughput, and flexible enough to keep pace with rapidly evolving therapeutic modalities.

In this context, acoustophoresis should no longer be viewed as an emerging niche technology, but as a core building block of future-proof cell therapy manufacturing.