

Aria Trouble Shooting Guide

Problem	Possible Cause	Solution	Notes
Stream Issues			
	Air Bubble	Restart the stream	Restarting the stream is the first step
	Nozzle clog	Sonicate nozzle	Place nozzle in water to cover the orifice
	Nozzle seated improperly	Remove and replace nozzle	Sometimes the O-ring will not create a complete seal
	Bad nozzle	Change to a different nozzle	Attempt using a different nozzle
	No stream	Turn blue knob on the fluidics cart towards the instrument	This is the house air pressure line
	Air in the system	Perform a "Fluidic Startup"	Brings sheath fluid into the system removing air
	Stream is crooked	Move the waste draw	Changing nozzles can change the seating of the nozzle
	Side streams are spraying during test sort	Adjust the frequency and amplitude	The stream needs to vibrate at certain times
	Side stream spraying during sort	Larger Nozzle	Stream will fan if cells too large
	Bar is jumping around	Change the drop 1 value	The sweet spot tries to adjust to input Drop 1 value
	No events seen	Filter sample and perform "sample line backflush"	This will decrease clumps and remove and line clogs







Problem	Possible Cause	Solution	Notes
Accudrop < 95%	Not enough beads	Increase the speed or add Accudrop beads	Event rate needs to be greater than 1000
	Stream is not bright enough	Tweak the micrometer to improve the brightness	Pause the tube and enable "test sort" and tweak the micrometer until dots are brightest
	Gap is too large	Change the gap value to a closer number	If the gap is too big then the sort will pause

	No side streams	Bring Left 1 slider bar to 30	Ensures the beads are in the box
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Problem	Possible Cause	Solution	Notes
Poor Sorting	Stream fanning	Use a large nozzle	stream will fan and be unstable if nozzle too small
	Pressure too high	Use a larger nozzle	Less pressure and a larger orifice will help viability
	Doublets	Create a tighter doublet gate	If two cells are sticking together they will end up in the collection tubes
	Sticky sample	Filter	Will create a better single cell suspension
	Dead Cells	Add a live/dead marker	The best combination is Calcein/PI to select live/dead
	Too concentrated	Dilute or lower the speed	The ideal events per second is Frequency *1000 / 4

Troubleshooting the Breakoff

Use the following examples to help troubleshoot problems with the breakoff image.

 <p>Ampl: 24.0 Freq: 87.0 Drop 1: 108 190 Gap: 6 8</p>	<p>Abnormal Stream Image</p>	 <p>Ampl: 8.0 Freq: 87.0 Drop 1: 306 71 Gap: 6 30</p>	 <p>Ampl: 8.0 Freq: 87.0 Drop 1: 306 163 Gap: 6 7</p>	 <p>Ampl: 1.0 Freq: 87.0 Drop 1: 306 499 Gap: 6 18</p>	 <p>Ampl: 44.0 Freq: 87.0 Drop 1: 306 163 Gap: 6 27</p>	 <p>Ampl: 80.0 Freq: 87.0 Drop 1: 306 163 Gap: 6 7</p>
<p>Normal stream image</p>	<p>Possible Causes</p>	<p>Nozzle inserted improperly</p>	<p>Nozzle inserted improperly or orifice is off center</p>	<p>Partial clog</p>	<p>Wet or dirty strobe lens</p>	<p>Attenuation is on at wrong pressure</p>
	<p>Recommended Solutions</p>	<p>Remove the nozzle and re-insert it.</p>	<p>Remove the nozzle and re-insert it.</p>	<p>Remove the nozzle, clean it, and then re-insert it.</p>	<p>Clean the lens as described in Cleaning the Camera Windows on page 239.</p>	<p>Turn off attenuation in the Side Stream window.</p>