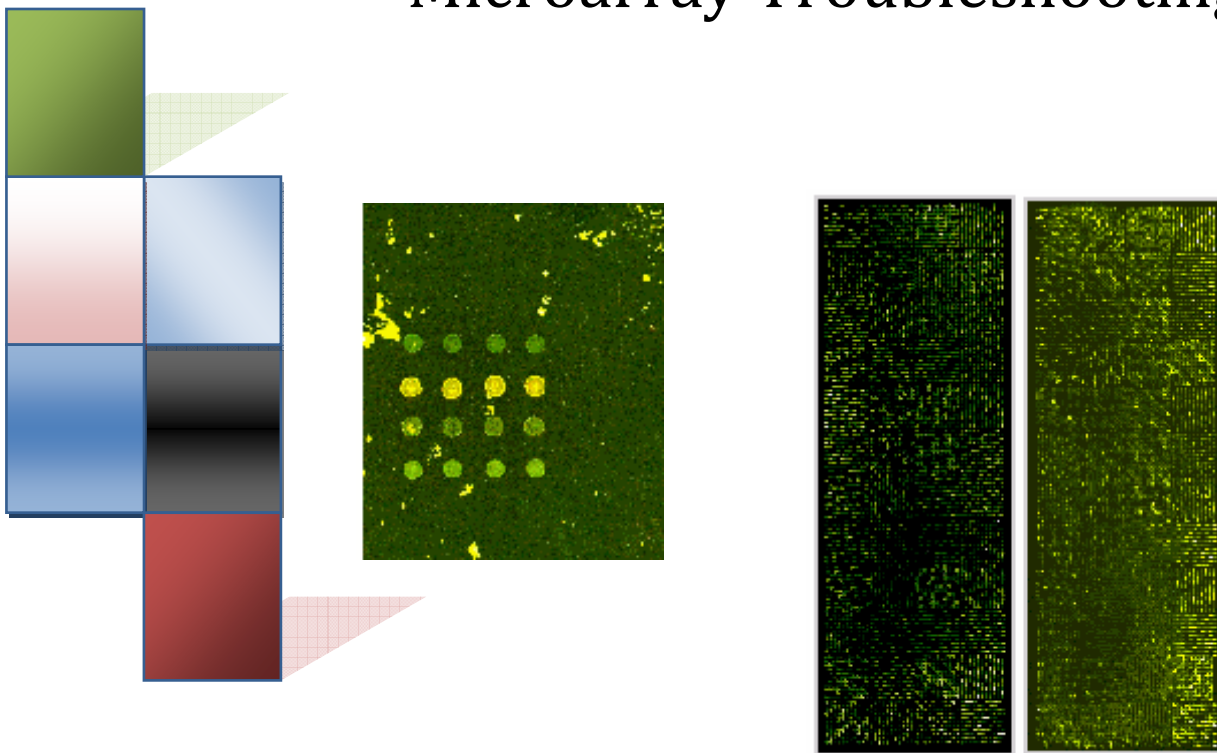


# NanoCinna

Pharmacogenomics Center

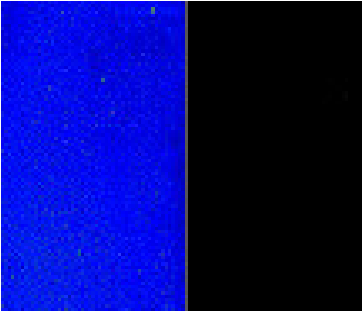
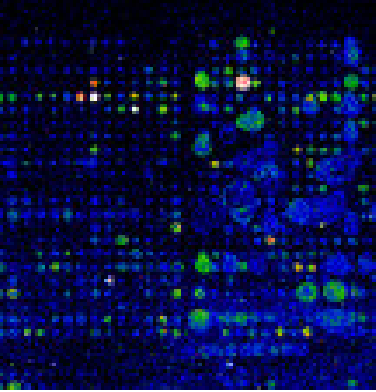
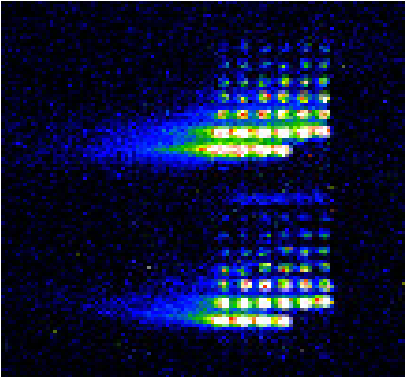
## Microarray Troubleshooting

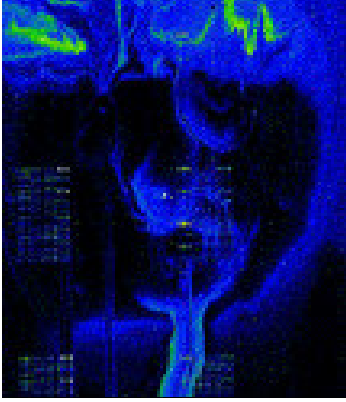
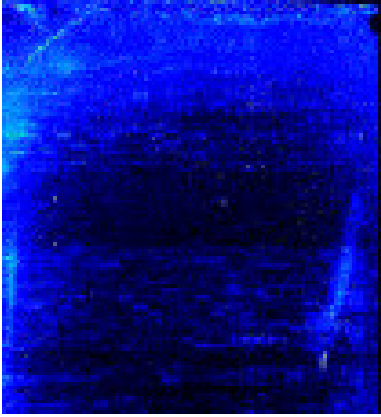


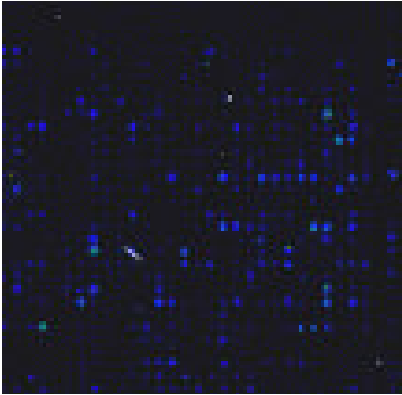
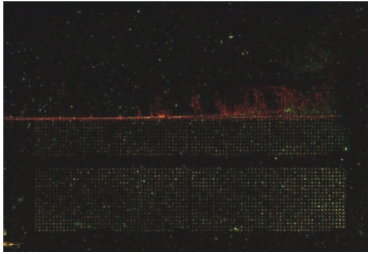
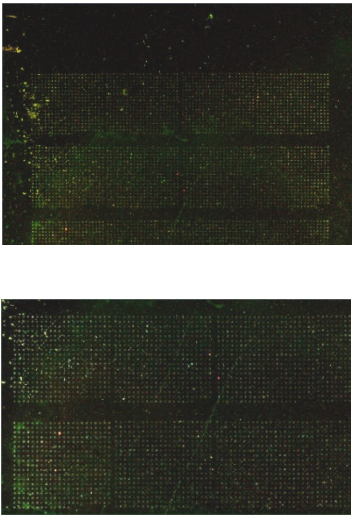
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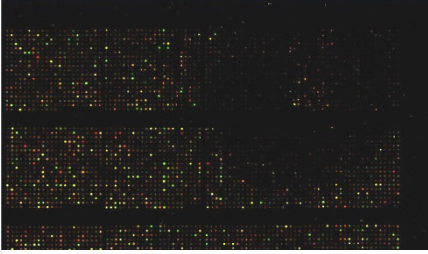
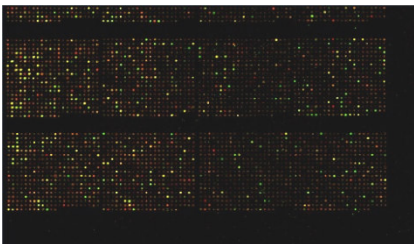
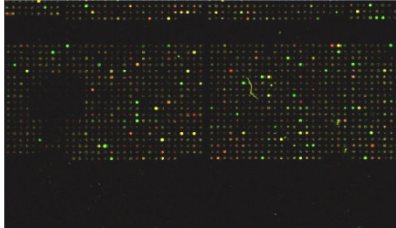
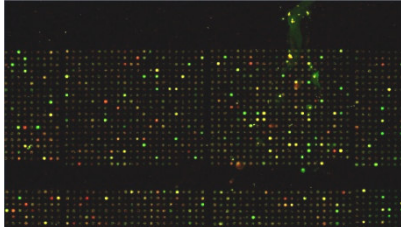
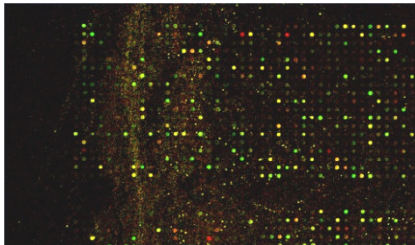
1. [http://www.stress-genomics.org/stress.flis/expression/array\\_tech/trouble\\_shooting/troubles\\_index.htm](http://www.stress-genomics.org/stress.flis/expression/array_tech/trouble_shooting/troubles_index.htm)
2. [http://www.bio.davidson.edu/projects/gcat/protocols/Troubleshooting\\_tiffs.html](http://www.bio.davidson.edu/projects/gcat/protocols/Troubleshooting_tiffs.html)
3. [http://www.corning.com/lifesciences/us\\_canada/en/technical\\_resources/doc\\_library/trouble\\_shooting\\_hybridization\\_kits\\_and\\_reagents.aspx](http://www.corning.com/lifesciences/us_canada/en/technical_resources/doc_library/trouble_shooting_hybridization_kits_and_reagents.aspx)
4. [http://www.corning.com/lifesciences/us\\_canada/en/technical\\_resources/doc\\_library/trouble\\_shooting\\_hybridization\\_kits\\_reagents.aspx](http://www.corning.com/lifesciences/us_canada/en/technical_resources/doc_library/trouble_shooting_hybridization_kits_reagents.aspx)

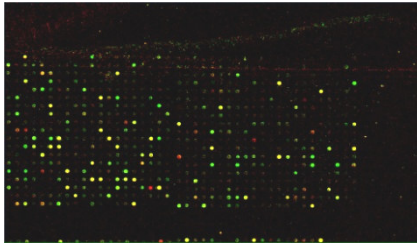
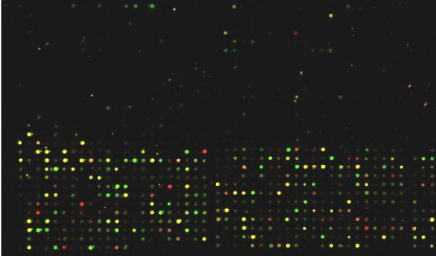
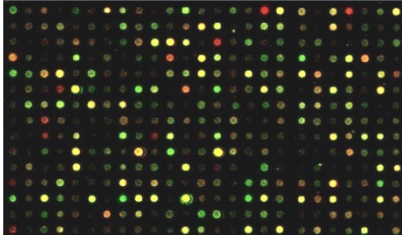
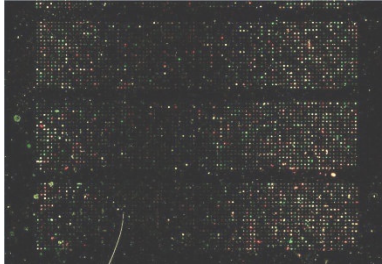
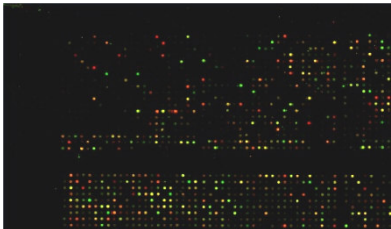
5<sup>th</sup> Floor, No.56, Azimi St. Phase 1, Shahrak Ekbatan. Tehran-Iran  
Tel: + 98-2144654896, +98-21-44654490  
Fax: +98-21-44654896  
[www.nanocinna.net](http://www.nanocinna.net)

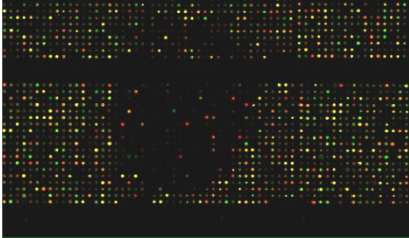
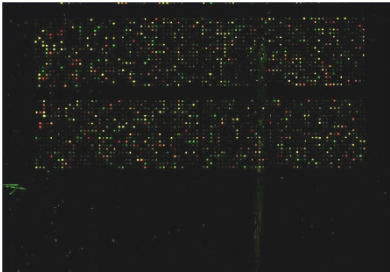
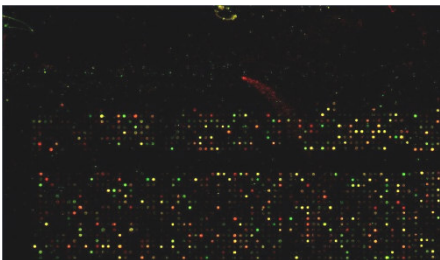
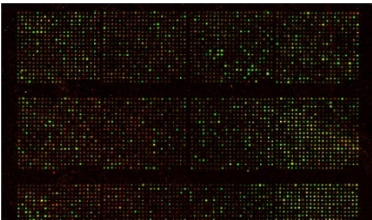
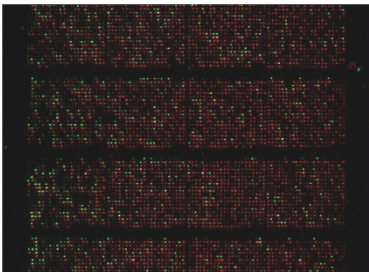
Problem	Image and Cause	Solution
<p><b>High background before hybridization</b></p> 		<p>a sample of slides from each production batch should be scanned prior to printing.</p>
<p><b>Irregular spot morphology</b></p> 	<p>When using quill-type printing pins, blotchy spots are not unusual among the first 1-5 slides of any print run. However, when these spots persist, they indicate a clogged or damaged pin. Even properly-printed spots may also run together if slides are rehydrated too long during immobilization.</p>	<p>Monitor spots and pins carefully, and clean the pins regularly with EDM wire. Some gridders allow the installation of a blotting pad, which increases the yield of useable arrays. Do not rehydrate slides for more than a few seconds.</p>
<p><b>Comet tails</b></p> 	<p>DNA that was printed on the slide, but which did not bind to the slide can produce “comet tails” following hybridization. This is common when DNA was printed at too-high of a concentration.</p>	<p>Remove unbound DNA by washing the printed slide with 1% SDS prior to hybridization (see our standard protocol)</p>

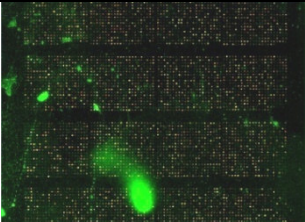
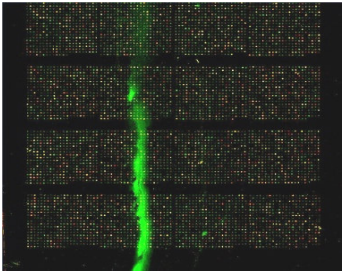
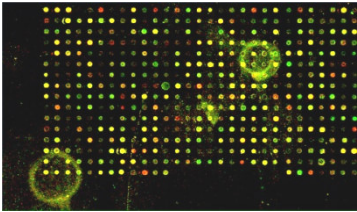
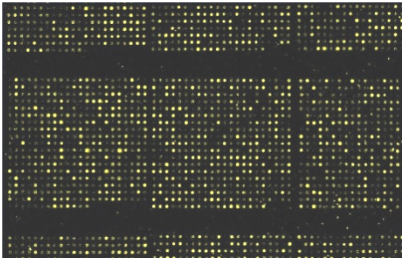
<p><b>Streaks</b></p> 	<p>The hybridization solution is intensely fluorescent and therefore must be fully removed prior to scanning. If any wash solution is allowed to dry on the slide, streaks will result. Slides are often centrifuged at low speed to hasten removal of residual liquid before drying.</p>	<p>Make sure the entire length of the slide is submerged in the wash solution, and do not allow the slides to sit in the centrifuge for more than a few seconds prior to spinning.</p>
<p><b>High background around the margins of the coverslip</b></p> 	<p>This is usually caused by dehydration of the hybridization solution.</p>	<p>Use a properly humidified hybridization chamber. We have found that a pre-warmed, 50mL screw-top tube containing a paper towel saturated with 2X SSC is effective. In some cases it may also be helpful to increase the volume of the hybridization solution, or to limit its evaporation during placement of the coverslip.</p>
<p><b>High background following hybridization</b></p>	<p>Unincorporated fluorochrome molecules are a common source of background signal.</p>	<p>We recommend using a Microcon YM-30 filter to purify the labeled target. The resulting solution should be almost colorless after purification. If it is not, it can be diluted and purified again through the YM-30 filter.</p>

<p><b>Low signal</b></p> 	<p>This is most often the result of problems of RNA quality or quantity, but can also be caused by problems generated during printing, hybridization or washing.</p>	<p>Increase the amount of RNA used in labeling. Use RNA that has been purified by binding to a column or a membrane, rather than by precipitation. Confirm that DNA was deposited on the slide during printing by staining a non-hybridized slide with propidium iodide.</p>
	<p>Cover slip not all the way up</p>	
	<p>Uneven mixing of hybe solution</p>	

	<p>bubble trapped under cover slip</p>	
<p><b>Dye gradient</b></p> 	<p>uneven mixing</p>	
<p><b>Dirt and grit</b></p> 	<p>poor mixing of probes prior to hybridization</p>	
	<p>Drops of liquid dried on slide</p>	
	<p>Drying of wash solution</p>	

	<p>Hybe did not cover all spots evenly</p>	
	<p>Top part of grid not covered by cover slip</p>	
	<p>Halo effect caused by printing, not your fault</p>	
	<p>Scratch and uneven distribution of hybe solution</p>	
	<p>Hybe solution did not reach top left corner.</p>	

	<p>Bubble covered a grid.</p>	
	<p>Drop of wash buffer dripped "up" from barcode at the bottom of the image.</p>	
	<p>Small chunk of <b>Cy5 dye</b> bled and the top half of grid not covered by cover slip</p>	
	<p>Hybe solution not evenly distributed with red dye biased in the middle.</p>	
<p><b>Overall red</b></p> 	<p>Overall red may be due to weak RT for green cDNA synthesis</p>	
	<p>SDS dried from wash solution</p>	

		
	<p>Drip of SDS from washing</p>	
	<p>Dried spot of wash solution</p>	
	<p>Loaded the same file for both colors</p>	

Reference:

1. [http://www.stress-genomics.org/stress.flx/expression/array\\_tech/trouble\\_shooting/troubles\\_index.htm](http://www.stress-genomics.org/stress.flx/expression/array_tech/trouble_shooting/troubles_index.htm)
2. [http://www.bio.davidson.edu/projects/gcat/protocols/Troubleshooting\\_tiffs.html](http://www.bio.davidson.edu/projects/gcat/protocols/Troubleshooting_tiffs.html)
3. [http://www.corning.com/lifesciences/us\\_canada/en/technical\\_resources/doc\\_library/trouble\\_shooting\\_hybridization\\_kits\\_and\\_reagents.aspx](http://www.corning.com/lifesciences/us_canada/en/technical_resources/doc_library/trouble_shooting_hybridization_kits_and_reagents.aspx)
4. [http://www.corning.com/lifesciences/us\\_canada/en/technical\\_resources/doc\\_library/trouble\\_shooting\\_hybridization\\_kits\\_reagents.aspx](http://www.corning.com/lifesciences/us_canada/en/technical_resources/doc_library/trouble_shooting_hybridization_kits_reagents.aspx)