Analysis of (cDNA) Microarray Data:

Part II. Intensities *versus* Intensity Ratios

Intensities vs Intensity Ratios

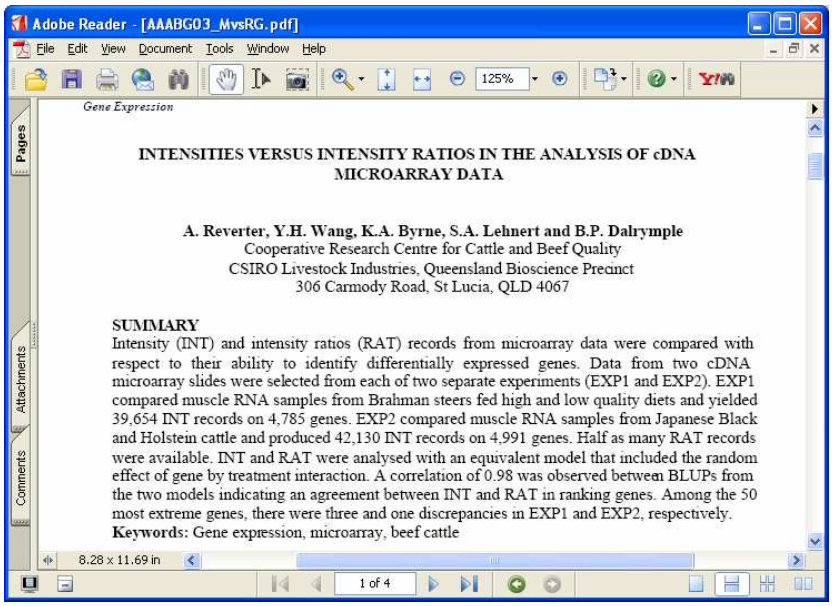
"It should be noted that there are disadvantages to using only expression ratios for data analysis. Although ratios can help to reveal some patterns in the data, they remove all information about the absolute gene expression levels. Various parameters depend on the measured intensity, including the confidence limits on any microarray measurement."

J Quackenbush, 2001
Nat Rev Gen, 2:418.

Objective:

- Compare RAT and INT in their ability to identify differentially expressed genes

Intensities vs Intensity Ratios



A Quantitative Overview to Gene Expression Profiling

Intensities vs Intensity Ratios

Introduction:

- Statistical challenges still evident at both level: design & analysis
  - RAT = Red to Green
  - INT = Red & Green
- Data quality controls performed at the INT level
- Analysis: Initial work developed for RAT but can also be accommodate to analyse INT

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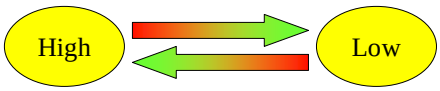
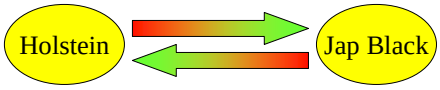
Intensities vs Intensity Ratios

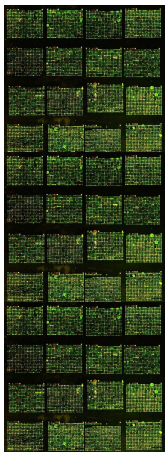
Materials & Methods:

EXP1: Diet



EXP2: Breed





Note: - Same microarray used across experiments
- Same (*basic*) criteria for data acquisition
- Equivalent models for data analysis across experiments and for both RAT and INT

A Quantitative Overview to Gene Expression Profiling

Materials & Methods:

Adobe Reader - [AAABG03_MvsRG.pdf]

Table 1. Summary statistics for intensities (INT) and red to green intensity ratios (RAT) for each experiment (EXP1 and EXP2) and by level of main effect

Trait	Effect	Level[a]	N	Mean	SD	Min.	Max.
				EXP1			
INT	Total		39,654	10.45	2.01	0.00	15.99
	Array	ARR1	19,938	10.94	1.64	2.00	15.99
		ARR2	19,716	9.96	2.21	0.00	15.99
	Dye	Red	19,827	10.45	2.12	0.00	15.99
		Green	19,827	10.46	1.89	0.00	15.99
	Treatment	TRT1	19,827	10.55	2.01	0.00	15.99
		TRT2	19,827	10.36	2.00	0.00	15.99
RAT	Total		19,827	-0.02	0.89	-7.38	8.01
	Array	ARR1	9,969	0.17	0.87	-7.38	4.79
		ARR2	9,969	-0.20	0.87	-7.35	8.01
				EXP2			
INT	Total		42,130	9.53	2.03	0.00	15.99
	Array	ARR1	21,158	9.43	2.09	0.00	15.99
		ARR2	20,972	9.64	1.95	0.00	15.99
	Dye	Red	21,065	9.49	2.06	0.00	15.99
		Green	21,065	9.58	2.00	0.00	15.99
	Treatment	TRT1	21,065	9.54	1.96	2.32	15.99
		TRT2	21,065	9.53	2.09	0.00	15.99
RAT	Total		21,065	-0.09	0.66	-6.21	5.13
	Array	ARR1	10,579	-0.08	0.67	-5.58	5.13
		ARR2	10,486	-0.09	0.65	-6.21	5.04

[a]Each experiment (EXP1 and EXP2) contained two arrays, ARR1 and ARR2, and two treatments, TRT1 and TRT2.

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Intensities vs Intensity Ratios

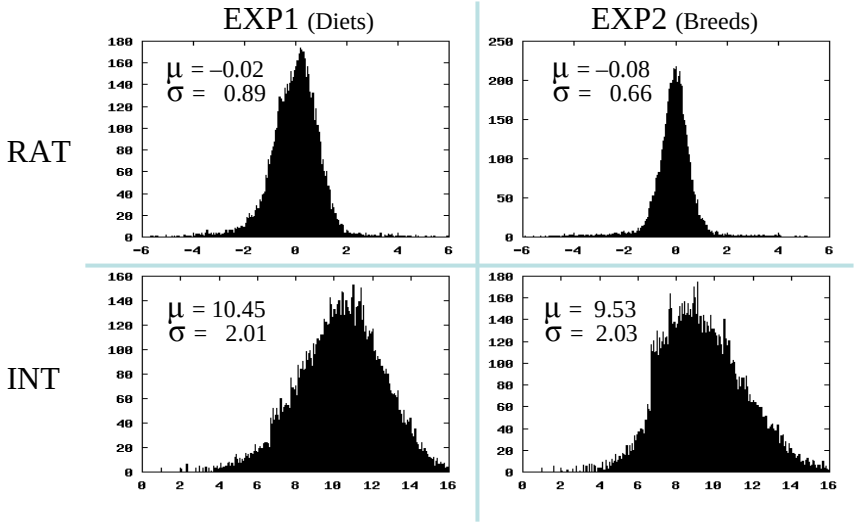
Materials & Methods:

		EXP1 (Diets)	EXP2 (Breeds)
INT =	Array|Block|Dye|Trt	192	192
+	(Gene)	4,785	4,991
+	Gene*Trt	9,570	9,982
+	Residual	39,654	42,130
RAT =	Array|Block|Trt_contrast	96	96
+	(Gene)	4,785	4,991
+	Gene*Trt_contrast	9,570	9,982
+	Residual	19,827	21,065

A Quantitative Overview to Gene Expression Profiling

Intensities vs Intensity Ratios

Materials & Methods:



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Intensities vs Intensity Ratios

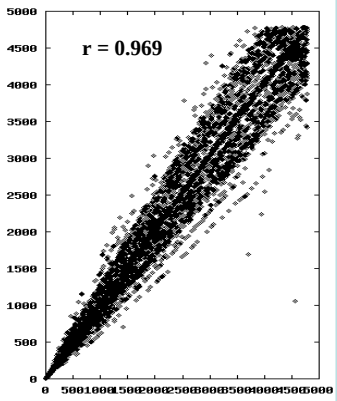
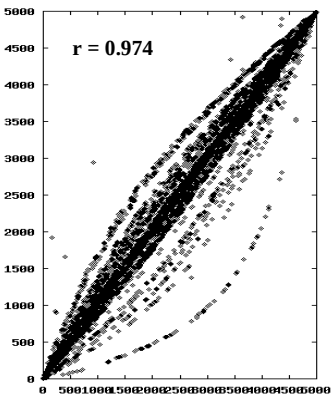
Results & Discussions:

	EXP1 (Diets)	EXP2 (Breeds)
RAT	Array 1 = 0.17 (0.87) Array 2 = -0.20 (0.87) Var(Tot) = 0.75 % GxT = 92	Array 1 = -0.08 (0.67) Array 2 = -0.09 (0.65) Var(Tot) = 0.37 % GxT = 77
INT	Array 1 = 10.94 (1.64) Array 2 = 9.96 (2.21) Red = 10.45 (2.12) Green = 10.46 (1.89) Var(Tot) = 3.73 % GxT = 76	Array 1 = 9.43 (2.09) Array 2 = 9.64 (1.95) Red = 9.49 (2.06) Green = 9.58 (2.00) Var(Tot) = 3.96 % GxT = 76

A Quantitative Overview to Gene Expression Profiling

Intensities vs Intensity Ratios

Results & Discussions: Rank Comparison

	EXP1 (Diets)	EXP2 (Breeds)
INT		

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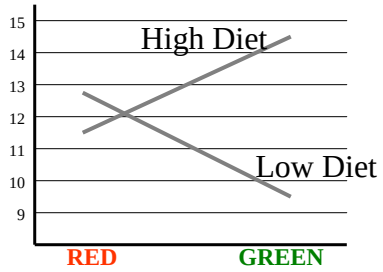
Intensities vs Intensity Ratios

Results & Discussions: Discrepancies

Discrepancies at the most extreme 50 elements (EXP1)

Gene in the top 50 with INT	Rank when analysing			Gene in the top 50 with RAT	Rank when analysing	
	INT	RAT			INT	RAT
CCL008103	10	56		CCL012284	57	41
CCL011618	49	53		CCL009178	67	49
CCL008010	50	72		CCL009304	69	50



Similar but non-significant trend for the other elements

INT is more Robust than RAT to Dye x Treatment ?

A Quantitative Overview to Gene Expression Profiling

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Intensities vs Intensity Ratios

Conclusions:

- Strong to very strong similarities between INT and RAT in their ability to ranking genes
- Possible evidence for better control of:
  - Overall Variation using RAT
  - Dye x Treatment using INT
- Further research is required (more arrays, samples, …)
- Initial concerns still hold:
  - RAT requires good signal on both channels
  - Not clear which RAT to use if > 2 Treatments

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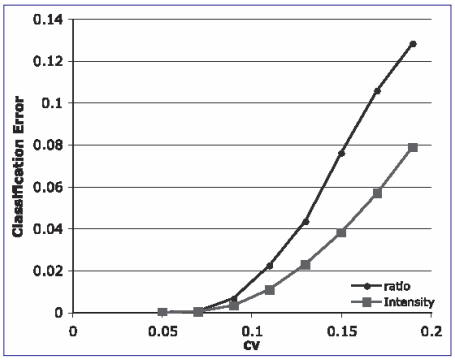
Intensities vs Intensity Ratios

BIOINFORMATICS
Vol. 20 no. 16 2004, pages 2513–2520
doi:10.1093/bioinformatics/bth272

Which is better for cDNA-microarray-based classification: ratios or direct intensities

Sanju Attoor[1], Edward R. Dougherty[1,2,], Yidong Chen[3], Michael L. Bittner[4] and Jeffrey M. Trent[4]*

Figure 2A shows that small and middling values of the true-signal coefficient of variation ($\alpha \leq 0.11$) have little effect on either method; however, beyond that both methods suffer, with the ratio method suffering significantly more. The greater effect on the ratio is likely due to the manner in which division accentuates the variability in the ratio for low signals.



My Conclusion:
Analyse intensities and, if needed, use ratios for reporting.