



# Analysis of (cDNA) Microarray Data: Part II. Intensities *versus* Intensity Ratios



## Intensities vs Intensity Ratios

**INTENSITIES VERSUS INTENSITY RATIOS IN THE ANALYSIS OF cDNA MICROARRAY DATA**

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**SUMMARY**  
Intensity (INT) and intensity ratios (RAT) records from microarray data were compared with respect to their ability to identify differentially expressed genes. Data from two cDNA microarray slides were selected from each of two separate experiments (EXP1 and EXP2). EXP1 compared muscle RNA samples from Brahman steers fed high and low quality diets and yielded 39,654 INT records on 4,785 genes. EXP2 compared muscle RNA samples from Japanese Black and Holstein cattle and produced 42,130 INT records on 4,991 genes. Half as many RAT records were available. INT and RAT were analysed with an equivalent model that included the random effect of gene by treatment interaction. A correlation of 0.98 was observed between BLUPs from the two models indicating an agreement between INT and RAT in ranking genes. Among the 50 most extreme genes, there were three and one discrepancies in EXP1 and EXP2, respectively.  
**Keywords:** Gene expression, microarray, beef cattle



## 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



## 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.

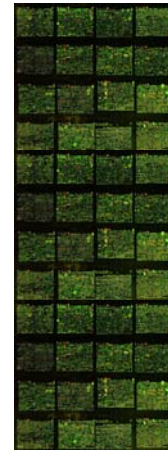
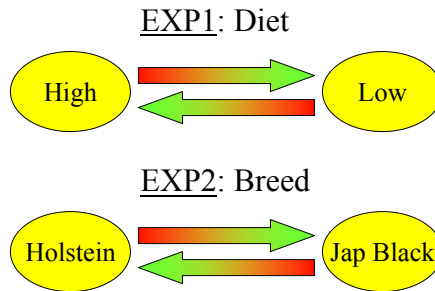
### Objective:

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



### Intensities vs Intensity Ratios

#### Materials & Methods:



- 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



#### Materials & Methods:

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 <sup>a</sup>	N	Mean	SD	Min.	Max.
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

<sup>a</sup>Each experiment (EXP1 and EXP2) contained two arrays, ARR1 and ARR2, and two treatments, TRT1 and TRT2.



Intensities vs Intensity Ratios

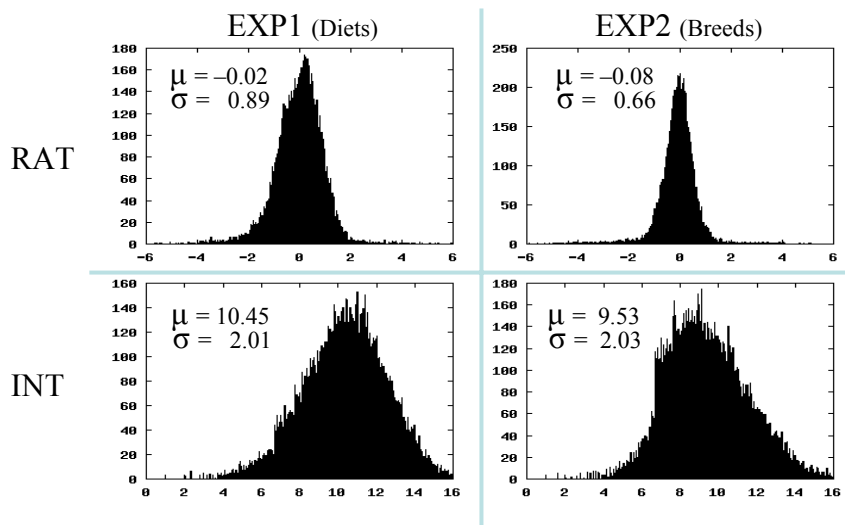
**Materials & Methods:**

	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



Intensities vs Intensity Ratios

**Materials & Methods:**





Intensities vs Intensity Ratios

Results & Discussions:

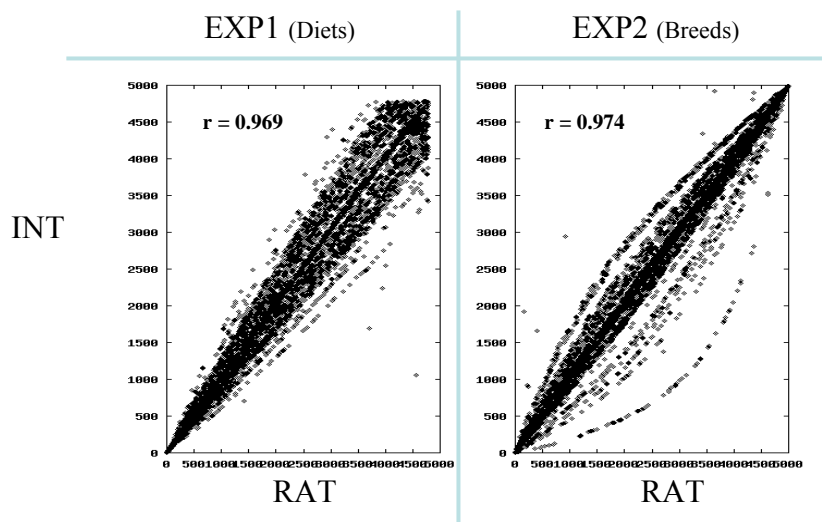
	EXP1 (Diets)	EXP2 (Breeds)
RAT	Array 1 = 0.17 (0.87) Array 2 = -0.20 (0.87)  Var(Tot) = 0.75 % GxT = 92	Array 1 = -0.08 (0.67) Array 2 = -0.09 (0.65)  Var(Tot) = 0.37 % GxT = 77
INT	Array 1 = 10.94 (1.64) Array 2 = 9.96 (2.21) Red = 10.45 (2.12) Green = 10.46 (1.89)  Var(Tot) = 3.73 % GxT = 76	Array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

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Intensities vs Intensity Ratios

Results & Discussions: Rank Comparison



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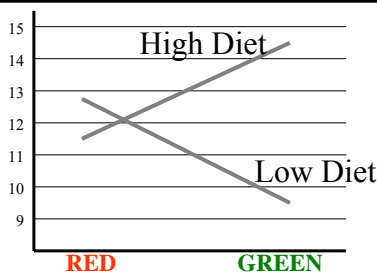


Intensities vs Intensity Ratios

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 ?



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