



# Biomedical Imaging & Analysis

Lecture 6, Part 3. Fall 2014

*Basic Image Processing / Filtering (III)*

[Text:....]

*Prahlad G Menon, PhD*

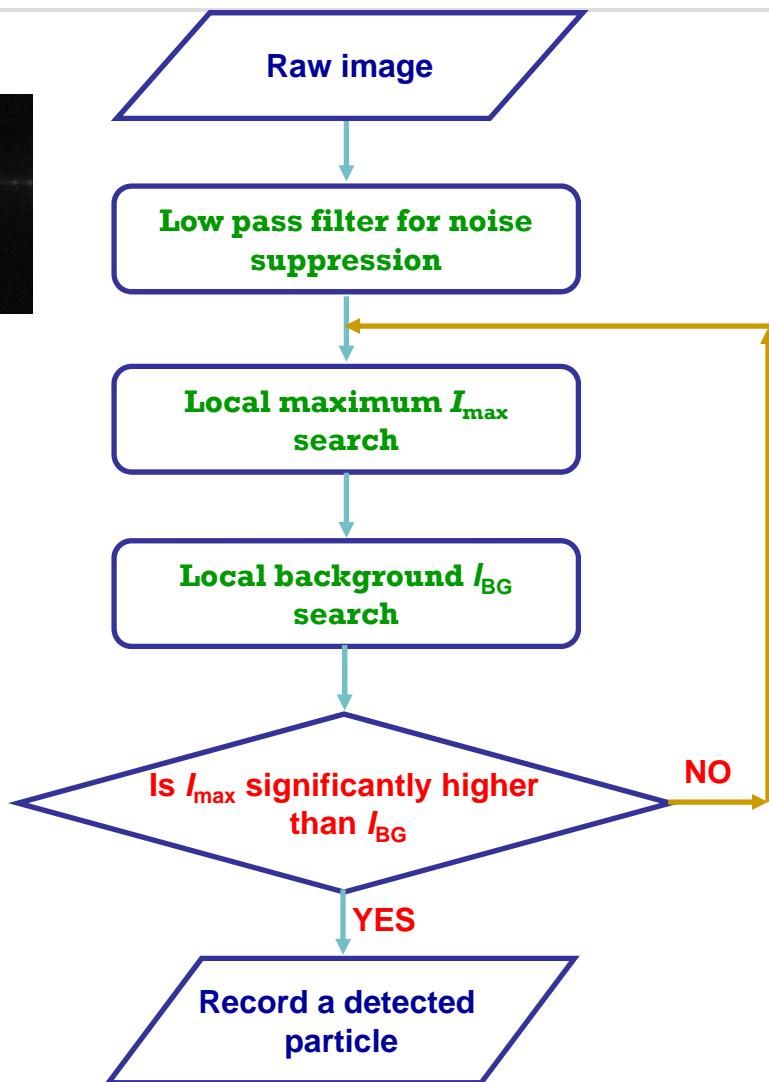
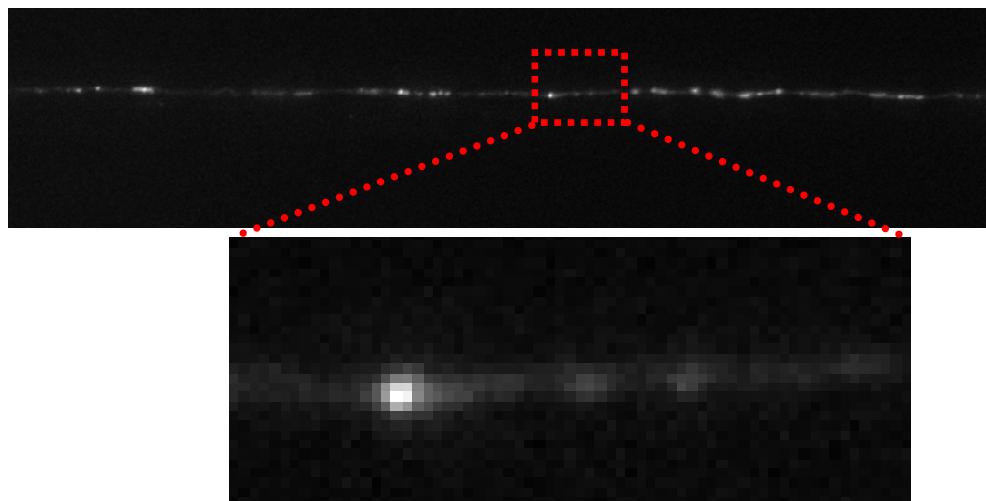
*Assistant Professor*

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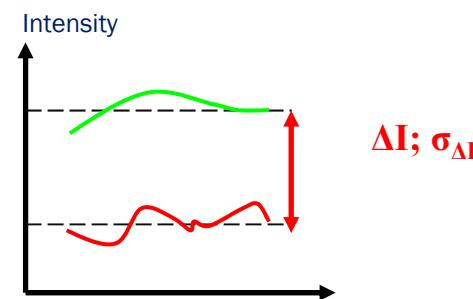
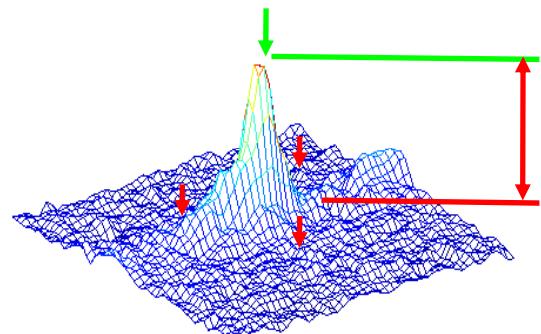
*Joint Institute of Engineering*



# Basic Principle of Particle Detection



## Step 4: Statistical Selection of Features

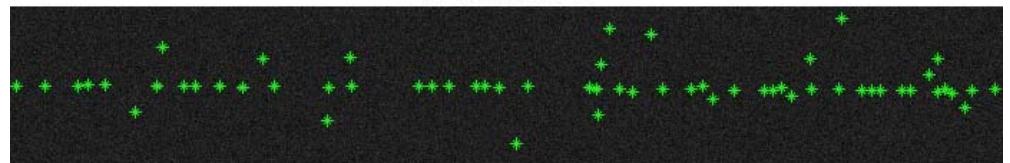


$$I_{max} - I_{BG} \geq Q \cdot \sigma_{\Delta I} ?$$

Q: selection quantile



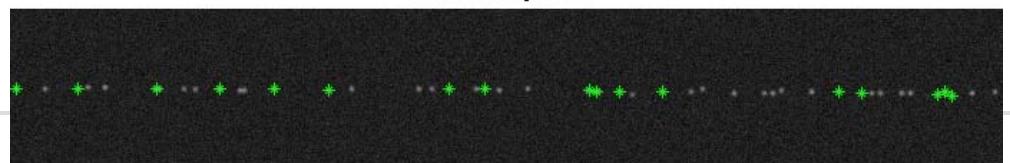
Q = 2.5, Sigma = 2



Q = 4.0, Sigma = 2



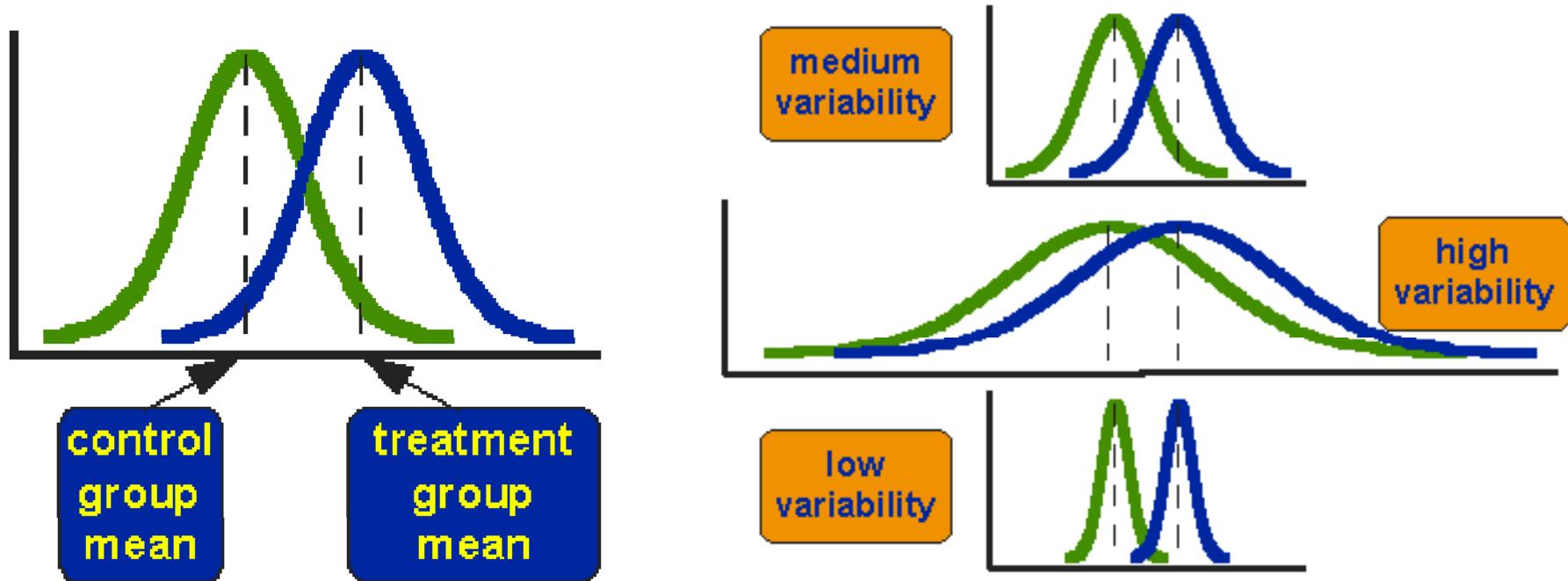
Q = 10.0, Sigma = 2



# Comparison between 2 groups: *t-test*

- **Basic idea:**

- When we are looking at the differences between scores for 2 groups, we have to judge the difference between their means relative to the spread or variability of their scores
  - Ex: comparison of 2 groups control and treatment



# Introduction to the t-distribution

- For a normally distributed variable  $x \sim N(\mu; \sigma)$ , the mean of  $n$  samples follows a normal distribution

$$\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i \square N\left(\mu, \frac{\sigma}{\sqrt{n}}\right)$$

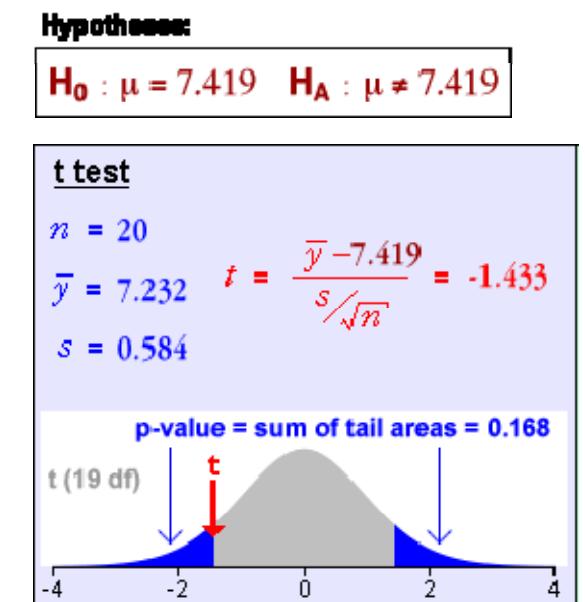
- The normalized  $\frac{x - \mu}{\sigma / \sqrt{n}} \square N(0,1)$

**Data:** The Food and Agriculture Organization of the United Nations reported that wheat yield in New Zealand was 7.419 tonnes per hectare in 2003. Wheat yield was found from a sample of 20 farms in 2004 before official figures were released.

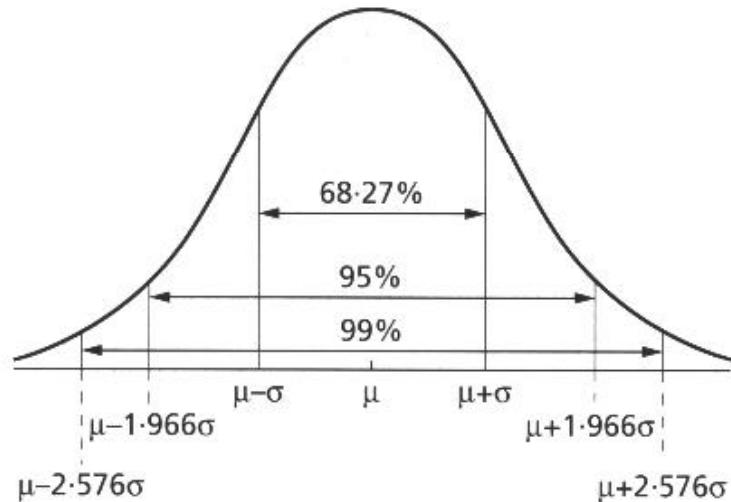
- The t statistic defined by

$$\frac{x - \mu}{s / \sqrt{n}} \text{ where } s = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2}$$

- One tail v/s Two tail P-Value for significance for alpha > 0.05 :



# Confidence interval (CI)



- 95% of observations in a normal distribution lie within  $\pm 1.96$  SEM
  - So limits of 95% CI:  
[Mean - 1.96 SEM; Mean + 1.96 SEM]
  - $\text{SEM} = \text{SD}/\sqrt{N}$

Error bars	Type	Description
<b>Standard deviation (SD)</b>	Descriptive	Typical or average difference between the data points and their mean.
<b>Standard error (SEM)</b>	Inferential	A measure of how variable the mean will be, if you repeat the whole study many times.
<b>Confidence interval (CI)</b> , usually 95% CI	Inferential	A range of values you can be 95% confident contains the true mean.

# A Review of Two Sample t-test

- Two-sample t significance test

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

**signal difference between group means**  
Web References → derivation: groups

<http://vassarstats.net/textbook/ch11pt1.html>  
 $\bar{x}_T - \bar{x}_C$   
 $SE(\bar{x}_T - \bar{x}_C)$

<http://vassarstats.net/textbook/ch9pt1.html>  
 $t$  value

<http://vassarstats.net/textbook/ch9pt2.html>

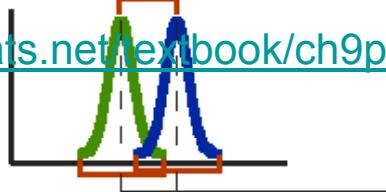
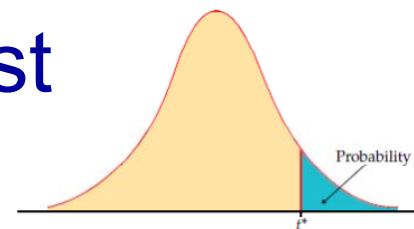


Table entry for  $p$  and  $C$  is  
 the critical value  $t^*$  with  
 probability  $p$  lying to its  
 right and probability  $C$  lying  
 between  $-t^*$  and  $t^*$ .



**TABLE D**  
 $t$  distribution critical values

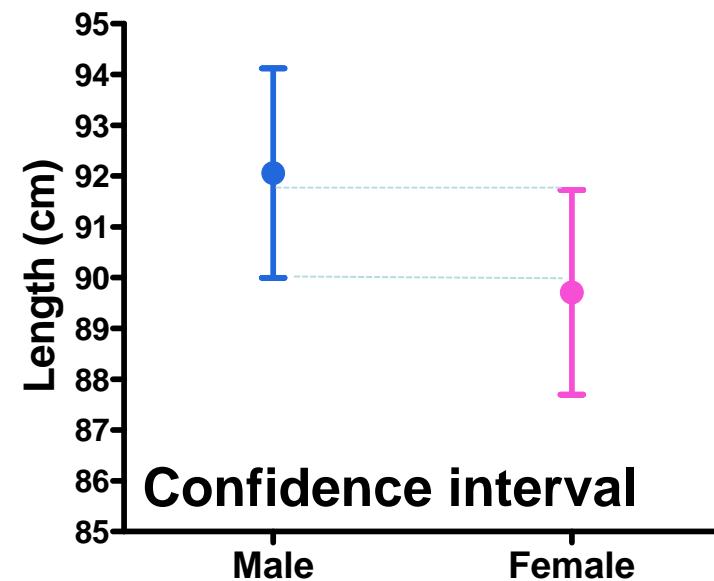
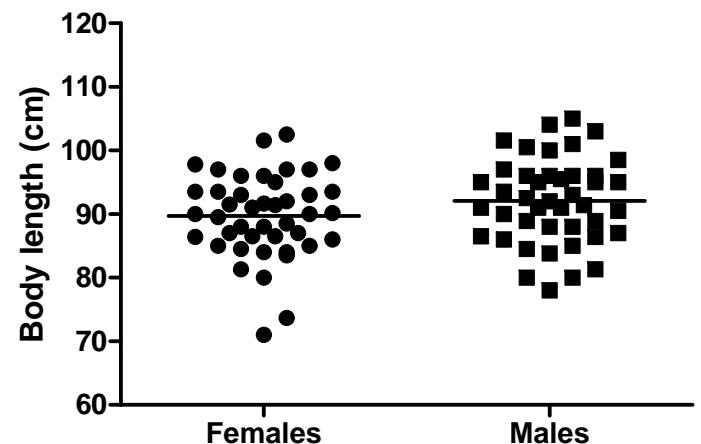
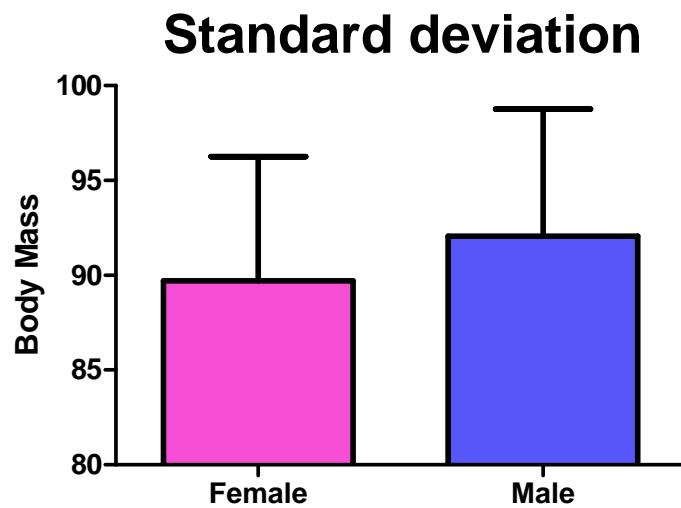
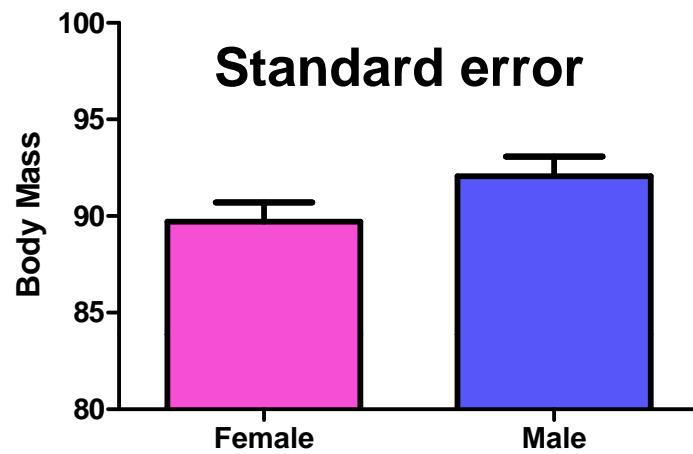
df	Upper-tail probability $p$											
	.25	.20	.15	.10	.05	.025	.02	.01	.005	.0025	.001	.0005
1	1.000	1.376	1.963	3.078	6.314	12.71	15.89	31.82	63.66	127.3	318.3	636.6
2	0.816	1.061	1.386	1.886	2.920	4.303	4.849	6.965	9.925	14.09	22.33	31.60
3	0.765	0.978	1.250	1.638	2.353	3.182	3.482	4.541	5.841	7.453	10.21	12.92
4	0.741	0.941	1.190	1.533	2.132	2.776	2.999	3.747	4.604	5.598	7.173	8.610
5	0.727	0.920	1.156	1.476	2.015	2.571	2.757	3.365	4.032	4.773	5.893	6.869
6	0.718	0.906	1.134	1.440	1.943	2.447	2.612	3.143	3.707	4.317	5.208	5.959
7	0.711	0.896	1.119	1.415	1.895	2.365	2.517	2.998	3.499	4.029	4.785	5.408
8	0.706	0.889	1.108	1.397	1.860	2.306	2.449	2.894	3.355	3.833	4.501	5.041
9	0.703	0.883	1.100	1.383	1.833	2.262	2.398	2.821	3.250	3.690	4.297	4.781
10	0.700	0.879	1.093	1.372	1.812	2.228	2.359	2.764	3.169	3.581	4.144	4.587
11	0.697	0.876	1.088	1.363	1.796	2.201	2.328	2.718	3.106	3.497	4.025	4.437
12	0.695	0.873	1.083	1.356	1.782	2.179	2.303	2.681	3.055	3.428	3.930	4.318
13	0.694	0.870	1.079	1.350	1.771	2.160	2.282	2.650	3.012	3.372	3.852	4.221
14	0.692	0.868	1.076	1.345	1.761	2.145	2.264	2.624	2.977	3.326	3.787	4.140
15	0.691	0.866	1.074	1.341	1.753	2.131	2.249	2.603	2.947	3.286	3.733	4.073
16	0.690	0.865	1.071	1.337	1.746	2.120	2.235	2.583	2.921	3.252	3.686	4.015
17	0.689	0.863	1.069	1.333	1.740	2.110	2.224	2.567	2.898	3.222	3.646	3.965
18	0.688	0.862	1.067	1.330	1.734	2.107	2.214	2.552	2.878	3.197	3.611	3.922
19	0.688	0.861	1.065	1.328	1.729	2.093	2.205	2.539	2.861	3.174	3.579	3.883
20	0.687	0.860	1.064	1.325	1.725	2.086	2.197	2.528	2.845	3.153	3.552	3.850
21	0.686	0.859	1.063	1.323	1.721	2.080	2.189	2.518	2.831	3.135	3.527	3.819
22	0.686	0.858	1.061	1.321	1.717	2.074	2.183	2.508	2.819	3.119	3.505	3.792
23	0.685	0.858	1.060	1.319	1.714	2.069	2.177	2.500	2.807	3.104	3.485	3.768
24	0.685	0.857	1.059	1.318	1.711	2.064	2.172	2.492	2.797	3.091	3.467	3.745
25	0.684	0.856	1.058	1.316	1.708	2.060	2.167	2.485	2.787	3.078	3.450	3.725
26	0.684	0.856	1.058	1.315	1.706	2.056	2.162	2.479	2.779	3.067	3.435	3.707
27	0.684	0.855	1.057	1.314	1.703	2.052	2.158	2.473	2.771	3.057	3.421	3.690
28	0.683	0.855	1.056	1.313	1.701	2.048	2.154	2.467	2.763	3.047	3.408	3.674
29	0.683	0.854	1.055	1.311	1.699	2.045	2.150	2.462	2.756	3.038	3.396	3.659
30	0.683	0.854	1.055	1.310	1.697	2.042	2.147	2.457	2.750	3.030	3.385	3.646
40	0.681	0.851	1.050	1.303	1.684	2.021	2.123	2.423	2.704	2.971	3.307	3.551
50	0.679	0.849	1.047	1.299	1.676	2.009	2.109	2.403	2.678	2.937	3.261	3.496
60	0.679	0.848	1.045	1.296	1.671	2.000	2.099	2.390	2.660	2.915	3.232	3.460
80	0.678	0.846	1.043	1.292	1.664	1.990	2.088	2.374	2.639	2.887	3.195	3.416
100	0.677	0.845	1.042	1.290	1.660	1.984	2.081	2.364	2.626	2.871	3.174	3.390
1000	0.675	0.842	1.037	1.282	1.646	1.962	2.056	2.330	2.581	2.813	3.098	3.300
$z^*$	0.674	0.841	1.036	1.282	1.645	1.960	2.054	2.326	2.576	2.807	3.091	3.291
	50%	60%	70%	80%	90%	95%	96%	98%	99%	99.5%	99.8%	99.9%
	Confidence level $C$											

# t-test Types

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- 3 types:
  - **Independent t-test**
    - it compares means for two independent groups of cases.
  - **Paired t-test**
    - it looks at the difference between two variables for a single group:
      - the second sample is the same as the first after some treatment has been applied
  - **One-Sample t-test**
    - it tests whether the mean of a single variable differs from a specified constant (often 0)

# Independent t-test: Example



# Analysis of quantitative data

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- Is there a difference between my groups regarding the variable I am measuring? P-values.
  - e.g.: are the mice in the group A heavier than the one in group B?
    - Tests with 2 groups:
      - Parametric: **t-test**
      - Non parametric: Mann-Whitney, **Wilcoxon rank sum test** (reference attached). Also results in p-values for significance estimation.
    - Tests with more than 2 groups:
      - Parametric: **Analysis of variance (one-way ANOVA)**
      - Non parametric: Kruskal Wallis
- Is there a relationship between my 2 (continuous) variables?
  - e.g.: is there a relationship between the daily intake in calories and an increase in body weight?
    - Test: **Correlation (parametric or non-parametric)**

# Analysis of quantitative data

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- Check for normality
- Choose the correct statistical test to answer your question:
  - They are 2 types of statistical tests:
    - Parametric tests with 4 assumptions to be met by the data,
    - Non-parametric tests with no or few assumptions (e.g. Mann-Whitney test) and/or for qualitative data (e.g.  $\chi^2$  test).

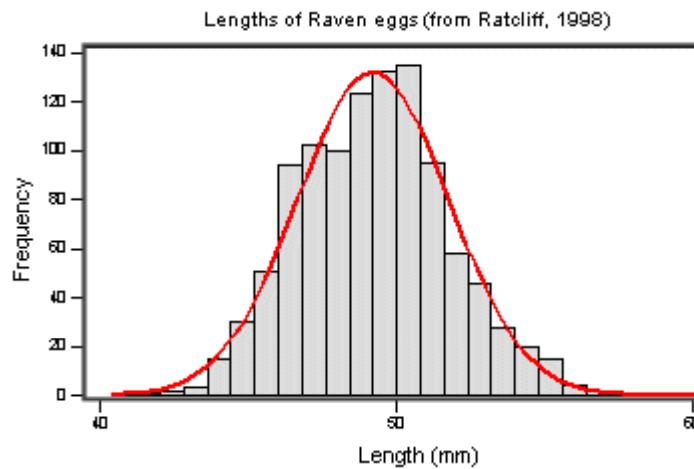
# Assumptions of Parametric Data

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- All parametric tests have 4 basic assumptions that must be met for the test to be accurate.

## 1) Normally distributed data

- Normal shape, bell shape, Gaussian shape



- Transformations can be made to make data suitable for parametric analysis
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# Assumptions of Parametric Data (2)

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## 2) Homogeneity in variance

- The variance should not change systematically throughout the data

## 3) Interval data

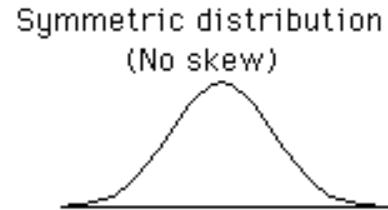
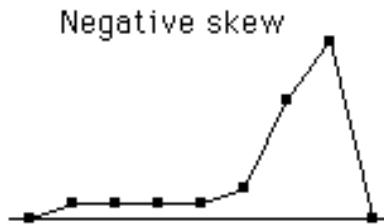
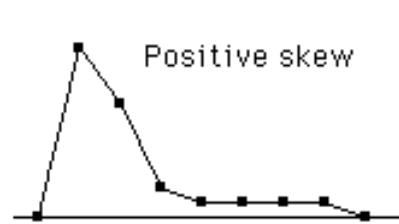
- The distance between points of the scale should be equal at all parts along the scale

## 4) Independence

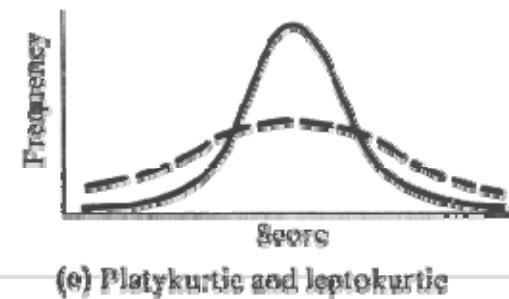
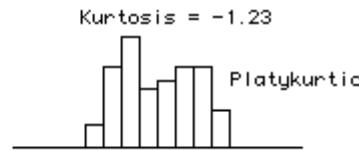
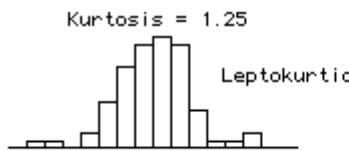
- Data from different subjects are independent
  - Values corresponding to one subject do not influence the values corresponding to another subject.
  - Important in repeated measures experiments

# Assumptions of Parametric Data (3)

- Frequent departure from normality:
  - Skewness: lack of symmetry of a distribution



- Kurtosis: measure of the degree of peakedness in the distribution
  - The two distributions below have the same variance approximately the same skew, but differ markedly in kurtosis.



# Analysis of variance – Normality check

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- The statistic for ANOVA is the **F ratio**. Useful for more than two group comparisons also!
  - $$F = \frac{\text{Variance between the groups}}{\text{Variance within the groups (individual variability)}}$$
  - $$F = \frac{\text{Variation explained by the model (= systematic)}}{\text{Variation explained by unsystematic factors (= random variation)}}$$
  - If the variance amongst sample means is greater than the error/random variance, then  $F > 1$ 
    - In an ANOVA, you test whether F is significantly higher than 1 or not. This results in a **P-value**.
    - If two groups are compared,  $t^2_{\text{crit}} = F_{\text{crit}}$
-

# Independent t-test: Example

1	Table Analyzed	Coyote
2	Column A	Female
3	vs	vs
4	Column B	Male
5		
6	Unpaired t test	
7	P value	0.1045
8	P value summary	ns
9	Are means signif. different? (P < 0.05)	No
10	One- or two-tailed P value?	Two-tailed
11	t, df	t=1.641 df=84
12		
13	How big is the difference?	
14	Mean ± SEM of column A	89.71 ± 0.9988 N=43
15	Mean ± SEM of column B	92.06 ± 1.021 N=43
16	Difference between means	-2.344 ± 1.428
17	95% confidence interval	-5.190 to 0.5012
18	R squared	0.03107
19		
20	F test to compare variances	
21	F, DFn, Dfd	1.045, 42, 42
22	P value	0.8870
23	P value summary	ns
24	Are variances significantly different?	No
25		
26		

Males tend to be longer than females  
but not significantly so (p=0.1045).

What about the Power of the analysis?

i.e. Power of the Statistical Test given a certain number of samples..? How many samples required for significance..?

Homogeneity in variance

# Power, $\pi$ / Sensitivity Analysis

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## What about the Power of a statistical comparison / test?

Power analysis is the name given to the process for determining the sample size for a research study. **It determines the probability of correctly accepting the alternative hypothesis when the alternative hypothesis is true** - that is, the ability of a test to detect an effect, if the effect exists.

Power,  $\pi=0.80$  ( $= 1-\beta$ ) is a standard for adequacy. This convention implies a four-to-one trade off between  $\beta$ -risk &  $\alpha$ -risk viz. trade off between false negatives v/s false positives.

Online Java applet for power analysis which can be used to ‘design’ a statistical test:  
<http://homepage.stat.uiowa.edu/~rlenth/Power/>

### There are a number of web resources related to statistical power analyses:

1. [StatPages.net](#) - This site provides links to a number of online power calculators.
2. [G-Power](#) - This site provides a downloadable power analysis program that runs under DOS. A Macintosh version is also available. The authors also provide online documentation and a brief tutorial on power analysis.
3. [Power analysis for ANOVA designs](#) - an interactive site that computes that calculates power or sample size needed to attain a given power for one effect in a factorial ANOVA design. This particular program can be found elsewhere on the web.
4. [PASS 2008](#) - a commercial site that allows you to download a 30 day trial version of their program. This is the software that I use. I don't think it's perfect, but I haven't come across anything that I think is better. Unlike many programs, PASS allows users to compute power for repeated measures designs.
5. SPSS makes a program called [SamplePower](#). I have only take a cursory look at it, and was disappointed that it didn't include repeated measures designs. However, one nice feature of the software is that it will output a complete report on your computer screen which you can then cut and paste into another document.

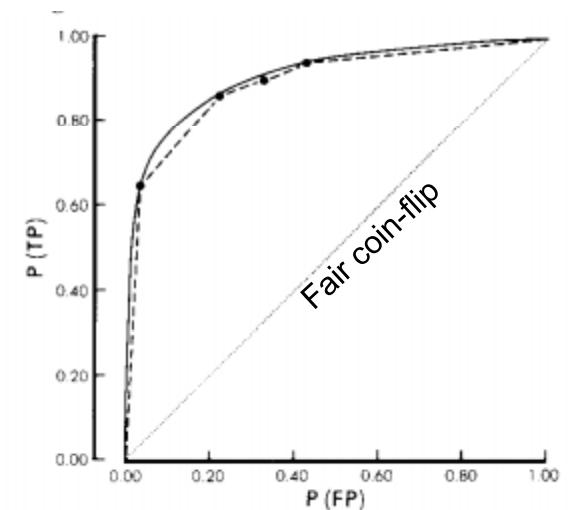
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**Assumptions:** Normality assumption for each group, same common variance.

# Using Power Analysis & ROC to determine Sample Size for a Significant Test.

## Required input

1. **Type I error** - alpha: the probability of making a Type I error ( $\alpha$ -level, two-sided), i.e. the probability of rejecting the null hypothesis when in fact it is true.
2. **Type II error** - beta: the probability of making a Type II error ( $\beta$ -level), i.e. the probability of accepting the null hypothesis when in fact it is false.
3. Area under ROC curve: the hypothesized Area under the ROC curve (the AUC expected to be found in the study).
  - In a ROC curve the true positive rate (Sensitivity) is plotted in function of the false positive rate (100-Specificity) for different cut-off points of a parameter.
  - Null hypothesis value: the null hypothesis AUC viz. 0.5 or 50% .
4. Ratio of sample sizes in negative / positive groups: enter the desired ratio of negative and positive cases. If you desire both groups to have an equal number of cases you enter 1; when you desire twice as many cases in the negative than in the positive group, enter 2.



ROC curve for data in TABLE I. Dashed line = empirical curve; solid line = smoothed (Gaussian-based) curve; dotted diagonal line = no discrimination.

See:

[http://www.medcalc.org/manual/sampling\\_ROC1.php](http://www.medcalc.org/manual/sampling_ROC1.php)

Hanley JA, McNeil BJ (1982) The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology, 143:29-36. [[Abstract](#)]

# Correlation

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- Most widely-used correlation coefficient:
  - Pearson product-moment correlation coefficient “r”
$$r = \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2 \sum_{i=1}^n (y_i - \bar{y})^2}}$$
  - The 2 variables do not have to be measured in the same units but they have to be proportional (meaning linearly related)
  - Coefficient of determination:
    - r is the correlation between X and Y
    - $r^2$  is the coefficient of determination:
      - It gives you the proportion of variance in Y that can be explained by X, in percentage.
    - **P-value:** is the slope significantly non-zero..?

# Correlation: Example

	Linear reg. Tabular results		A	B
			Male	Female
	A	B	Y	Y
1	Best-fit values			
2	Slope	-4.621 ± 1.287	-1.888 ± 1.721	
3	Y-intercept when X=0.0	30.20 ± 3.025	25.04 ± 3.453	
4	X-intercept when Y=0.0	6.536	13.26	
5	1/slope	-0.2164	-0.5297	
6	95% Confidence Intervals			
7	Slope	-7.490 to -1.753	-5.637 to 1.861	
8	Y-intercept when X=0.0	23.46 to 36.94	17.51 to 32.56	
9	X-intercept when Y=0.0	4.902 to 13.47	5.738 to +infinity	
10	Goodness of Fit			
11	r <sup>2</sup>	0.5630	0.09119	
12	Sy.x	1.960	2.512	
13	Is slope significantly non-zero?			
14	F	12.89	1.204	
15	DFn, DFd	1,000, 10,00	1,000, 12,00	
16	P value	0.0049	0.2940	
17	Deviation from zero?	Significant	Not Significant	
18	Data			
19	Number of X values	12	14	
20	Maximum number of Y replicates	1	1	
21	Total number of values	12	14	
22	Number of missing values	14	12	

	Correlation		A	B
			Male	Female
	A	B	Y	Y
1	Number of XY Pairs		12	14
2	Pearson r		-0.7504	-0.3020
3	95% confidence interval		-0.5257 to -0.3098	-0.7177 to 0.2724
4	P value (two-tailed)		0.0049	0.2940
5	P value summary		**	ns
6	Is the correlation significant? (alpha=0.05)		Yes	No
7	R squared		0.5630	0.09119

There is a negative correlation between parasite load and fitness but this relationship is only significant for the males ( $p=0.0049$  vs. females:  $p=0.2940$ ).

# **Words of Advice – regarding publishing statistical results**

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- ✓ Every article that contains statistical testing should state the name of the statistical test, the n for each statistical analysis, the comparisons of interest, a justification for the use of that test (including, for example, a discussion of the normality of the data when the test is appropriate only for normal data), the alpha level for all tests, whether the tests were one-tailed or two-tailed, and the actual *P* value for each test (not merely "significant" or "*P* < .05").
  - ✓ Randomization procedures, or other ways to eliminate bias in sampling (in particular for experiments involving animals), should be clearly described.
  - ✓ It **should be clear what statistical test was used to generate every *P* value**. In the case of Brief Communications, these details should be reported in the text or the figure captions.
-

# Sub-pixel Particle / Point Detection

## *Gaussian fitting*

- Fit using a Gaussian kernel, which represents the ideal image of a point

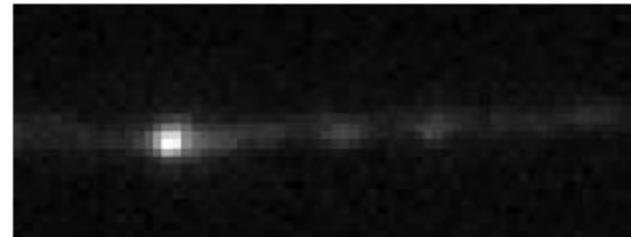
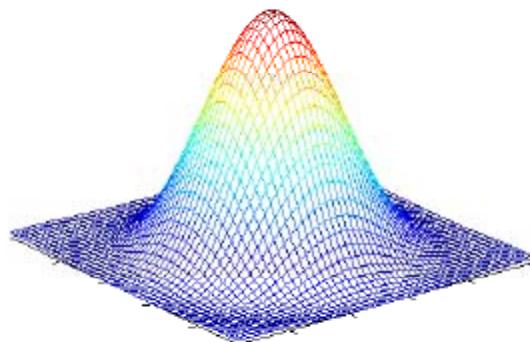
$$K(x, y; x_0, y_0) = K(x - x_0, y - y_0) = A \cdot \exp\left[-\frac{(x - x_0)^2 + (y - y_0)^2}{B}\right]$$

- Problem formulation: to minimize the difference between the translated kernel and the image

$$\min_{(x_0, y_0) \in \mathbb{R}^2} |I(x, y) - K(x, y; x_0, y_0)| \Rightarrow E(x_0, y_0) = \sum_{i=1}^M \sum_{j=1}^N |I(i, j) - K(i - x_0, j - y_0)|$$

May not be integer coordinates

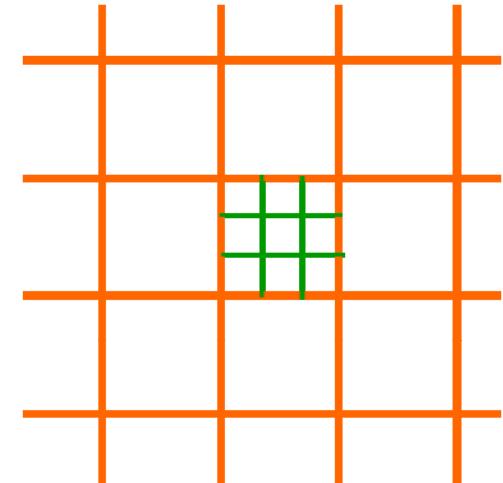
**In Matlab:**  
Intensity  
interpolation  
using interp2 .



# Sub-pixel Particle / Point Detection

## *Gaussian fitting*

- **Airy disc fitting to a Gaussian:**
  - $\sigma = \{0.61 \lambda / NA\} / 3$
  - **Spatial sampling:** three-times oversampling of Airy disk. 3 pixels at least across the airy-disc radius.
- **Implementation of exhaustive search:**
  - Oversampling the kernel and images. Use a small pixel size: e.g. 10nm
  - If multiple minima were identified, use their average position.

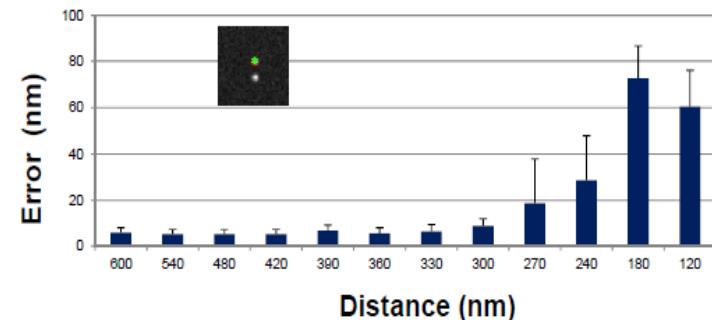


### Initialization:

- - Use detected local maxima to localize the search.

### Limitations:

When the distance between the two point features goes below the Rayleigh limit, they can no longer be resolved reliably unless under very high SNR.



# Sub-pixel Particle / Point Detection

## Alternative: Correlation

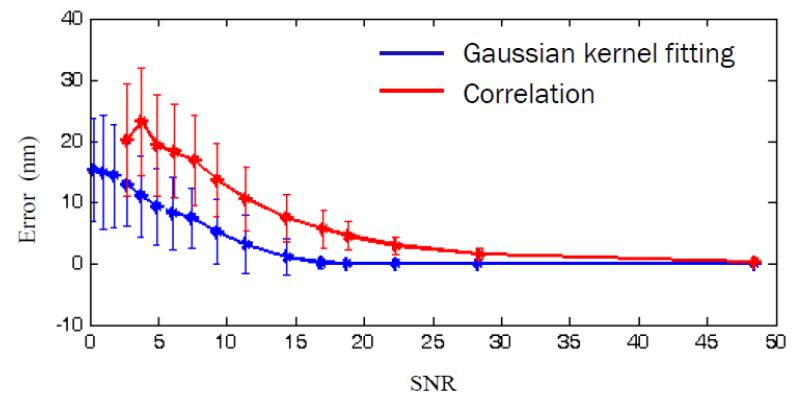
- **Detection by maximization correlation**
  - Same strategy as in Gaussian fitting as the only difference is the cost function:

$$C_{x_0, y_0} = \sum_{i=1}^M \sum_{j=1}^N I_{i,j} K(i - x_0, j - y_0)$$

- Often the correlation function is *normalized*:

$$C_{x_0, y_0} = \frac{\sum_{i=1}^M \sum_{j=1}^N I_{i,j} K(i - x_0, j - y_0)}{\sqrt{\sum_{i=1}^M \sum_{j=1}^N I_{i,j}^2} \cdot \sqrt{\sum_{i=1}^M \sum_{j=1}^N K^2(i - x_0, j - y_0)}}$$

- **Searching strategies:**
  - Strategy I: exhaustive search
  - Strategy II: optimization search

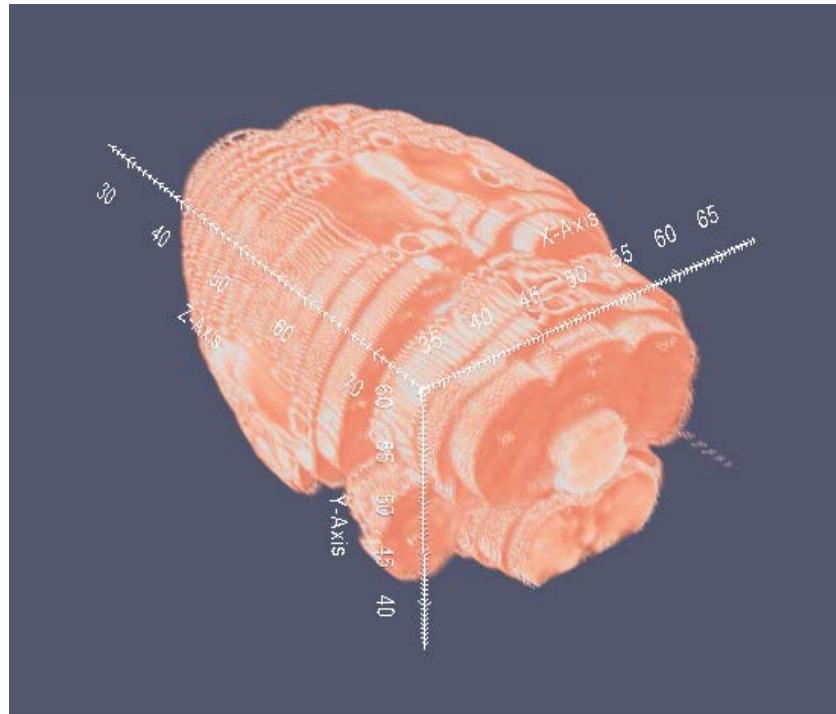


- ✓ For point features, Gaussian fitting is the best method overall.
- ✓ For larger non-diffraction limited features, correlation gives better resolution.

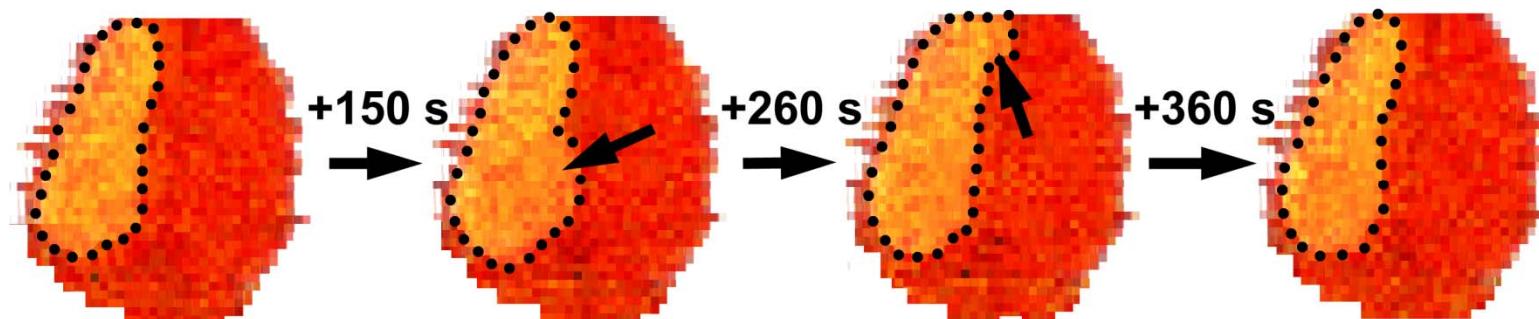
# After Point Detection – Perhaps Clustering.

**Brain MRI indicator of traumatic brain injury:**

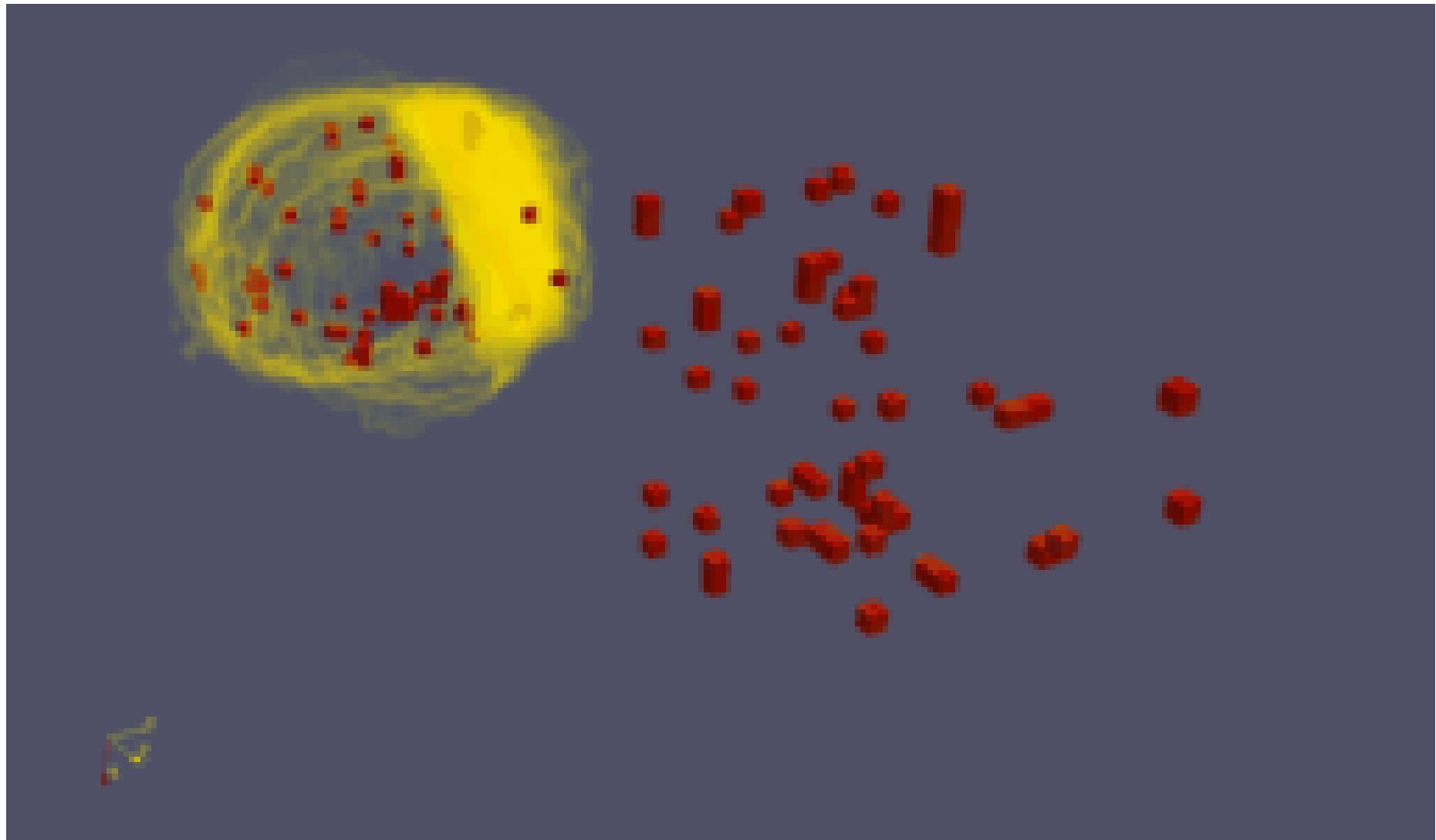
PIDs visualized as waves of ADC depression



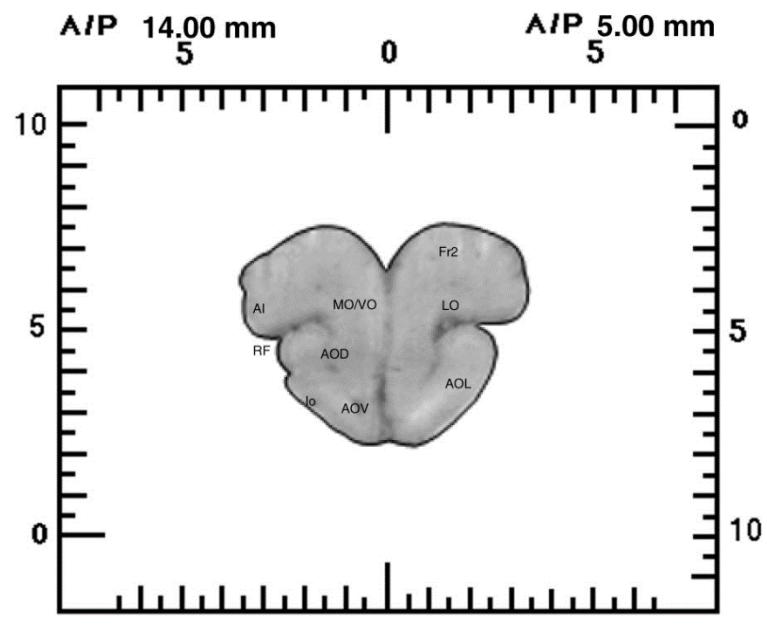
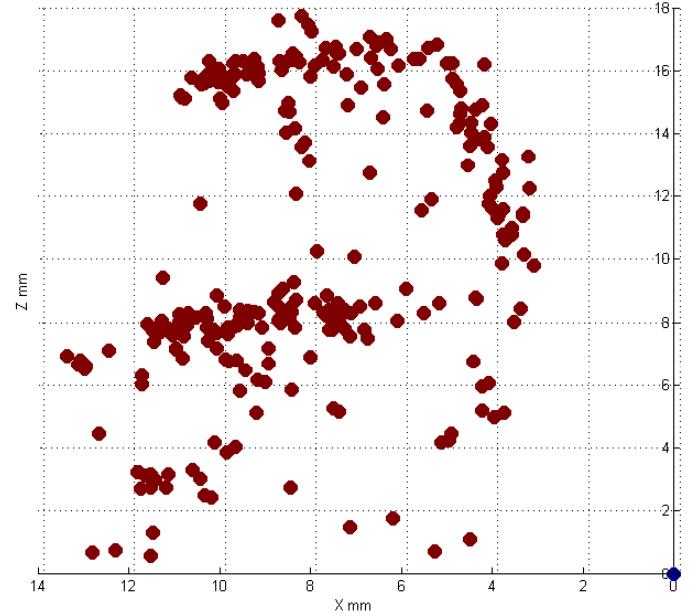
- Horizontal projections (i.e., sums of voxel intensities) of a 3D brain ADC map
- Yellow: ischemic lesion
- PID
  - originated at the edge of the ischemic lesion,
  - propagated in the caudal-rostral direction
  - eventually disappeared
  - 6 out of 8 PIDs in 80 mins originated in the dorso-caudal area of ischemic hemisphere and moved in the ventro-rostral direction



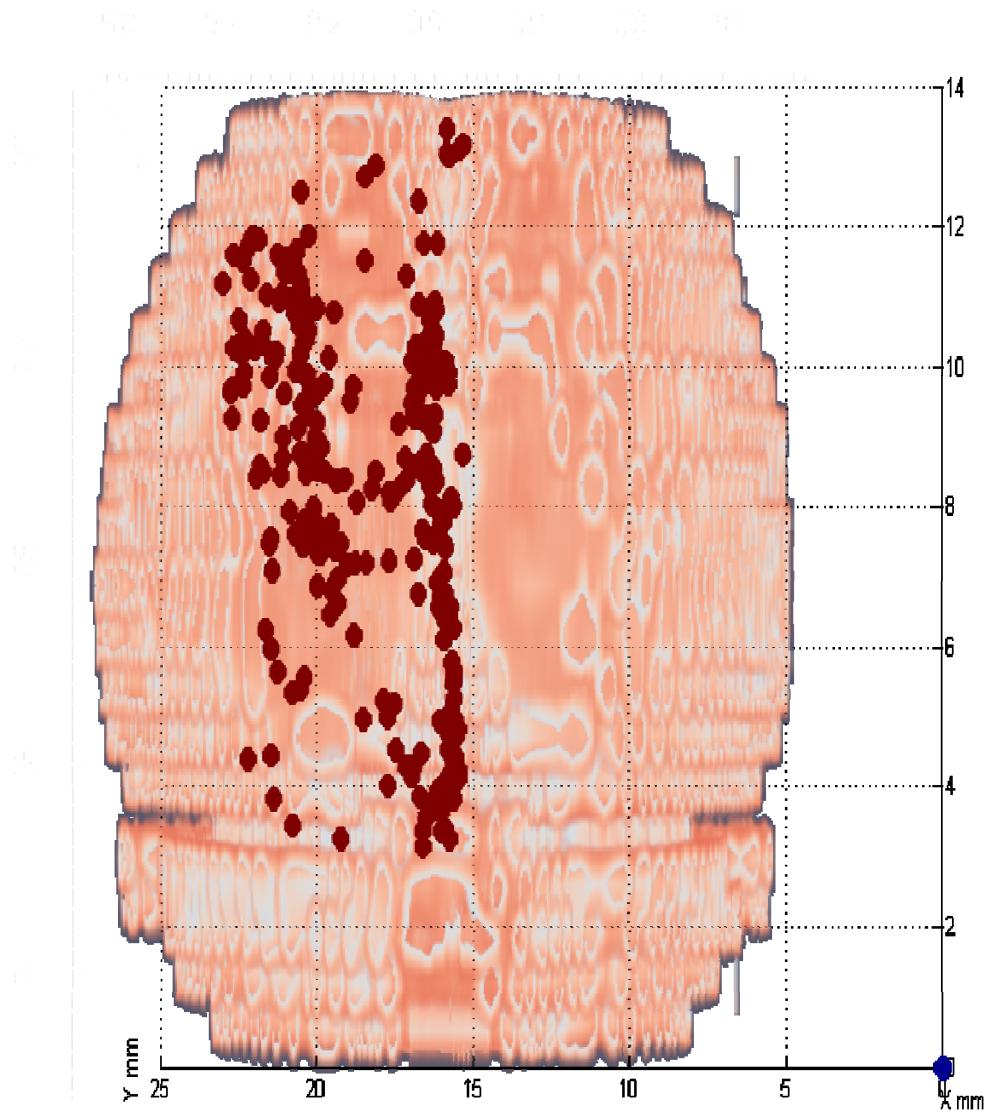
# Identifying the source of PIDs



The spark aligns with the curvature of the corona radiata →  
On the cortical side of the corona radiata i.e. layer 7 of the cortex



# All PID Cluster-Centroid tracks overlaid together

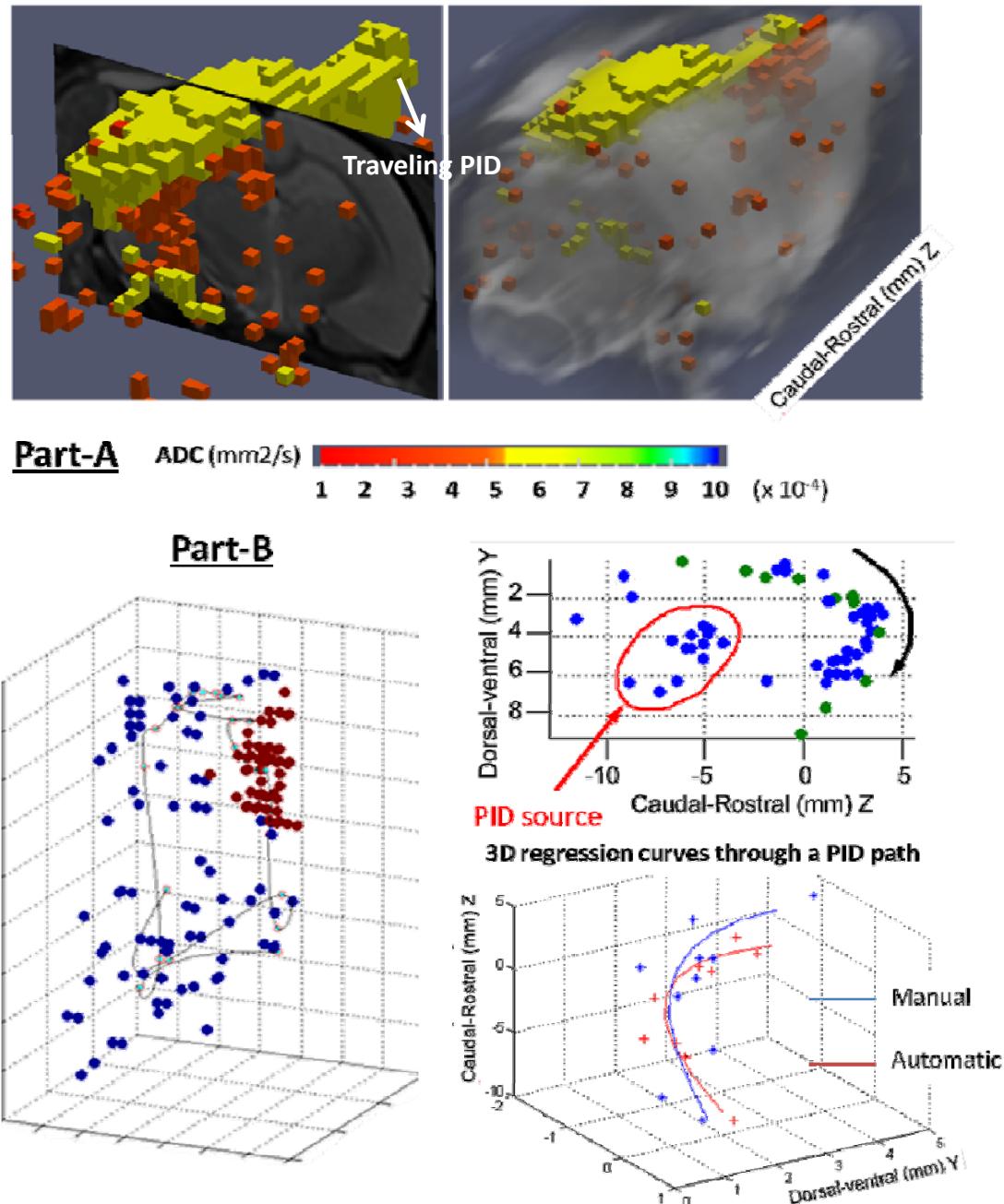




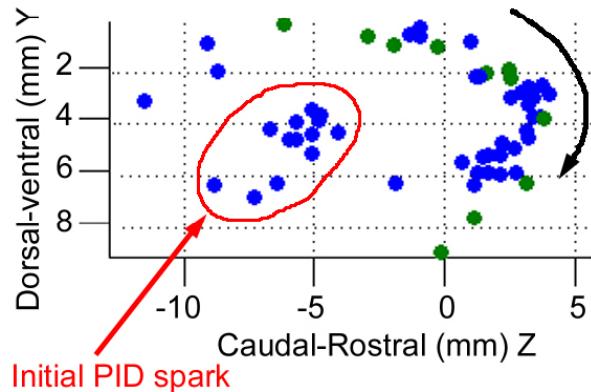
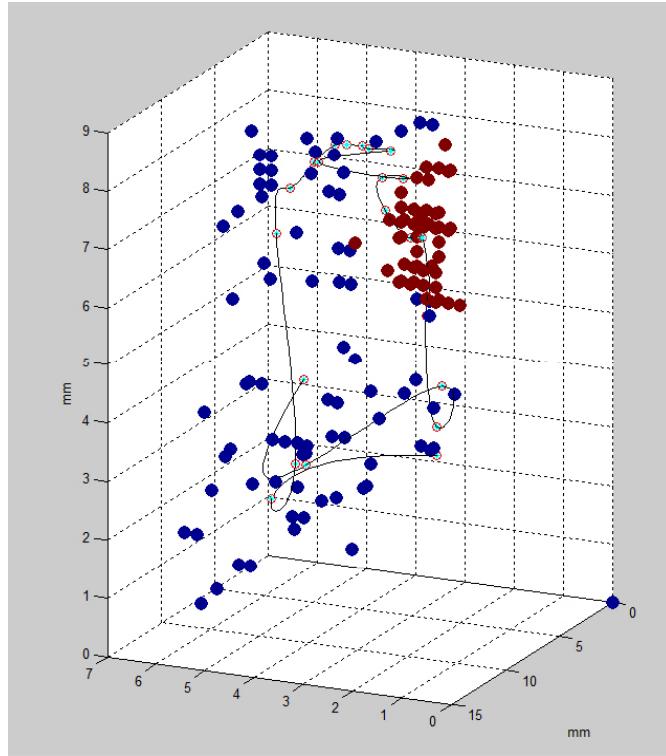
Melbourne  
AUSTRALIA



NEUROSCIENCE 2012  
NEW ORLEANS



# Automatic PID Tracking in stereotactic initial frame of reference

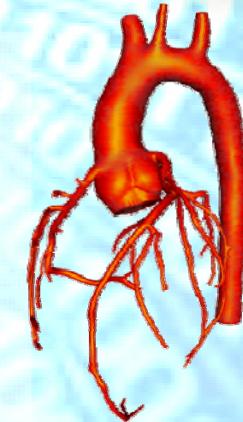
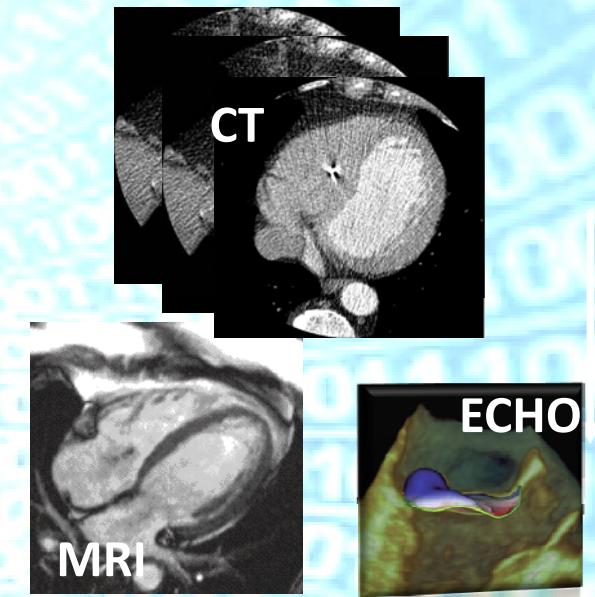
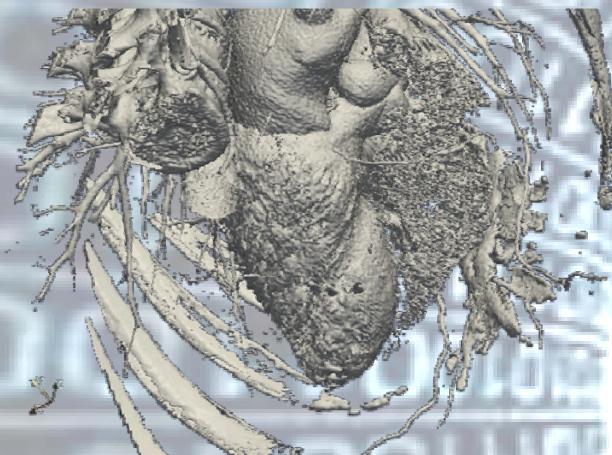


- PID identification
  - Removal of the 'static' lesion by 3D image subtraction
  - Cluster (brown) discrimination vs. noise (blue) using machine learning classification
- PID trajectories
  - Fully automated cluster tracking (blue) vs. observation-based tracking (green) detects an initial PID spark in the ventral part of the peri-infarct area (encircled)
  - Further PID propagation (in the arrow direction): good agreement of the two tracks



# BIA 2014

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