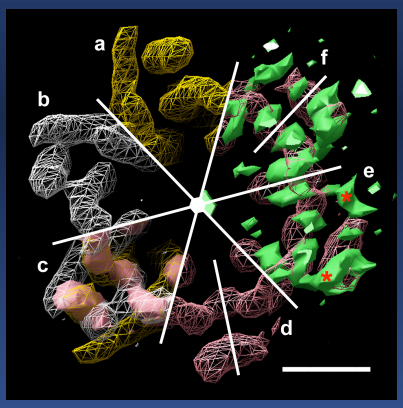
Bootstrap Resampling to Estimate the Error in 3D Difference Density Maps Derived by Electron Microscopic Image Analysis of 2D Crystals



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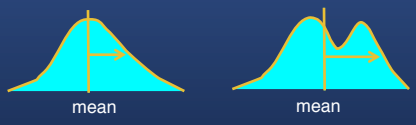
2dx workshop 09/08

Why and How to quantify "error" of a map

- Variance and Standard deviation of measurements of the same value ψ



$$\sqrt{v_\psi} = \sigma_\psi = \sqrt{\frac{\sum_{n=1}^{N}(\psi_n - \overline{\psi})^2}{N-1}}$$

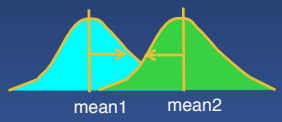
- Covariance-correlation between variates
- t-test: standard error weighted difference

$$\hat{t}_{\Delta\overline{\psi}} = \frac{\Delta\overline{\psi}}{\delta_{\Delta\overline{\psi}}}$$

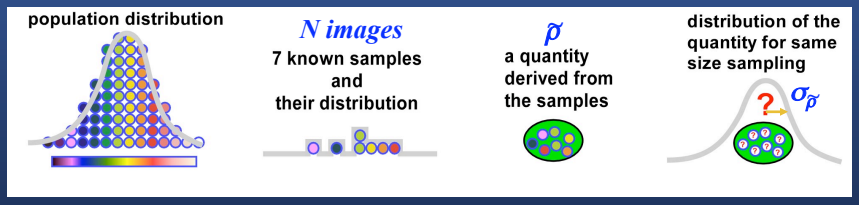
Difference

Standard error of the difference

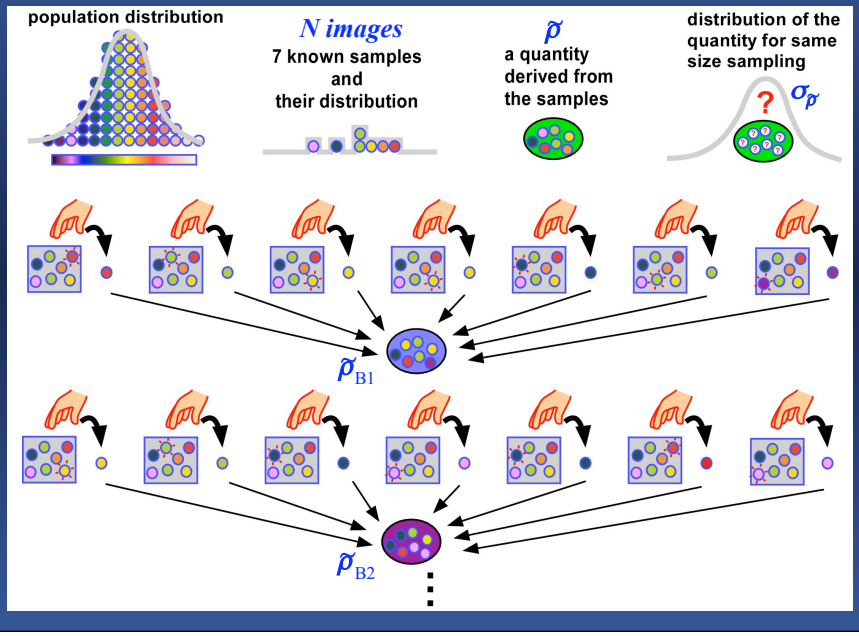
$$\delta_{\Delta\overline{\psi}} = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}$$



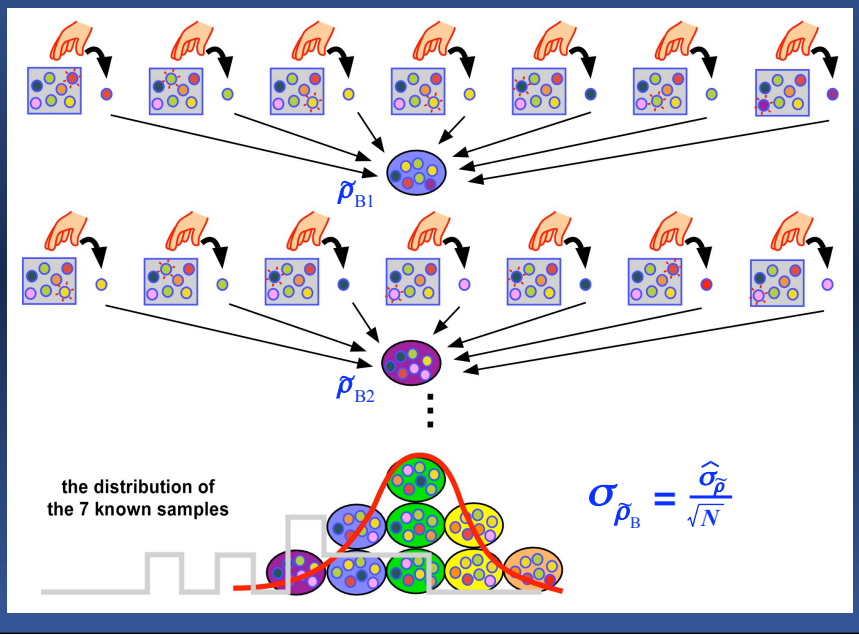
Bootstrap resampling-purpose



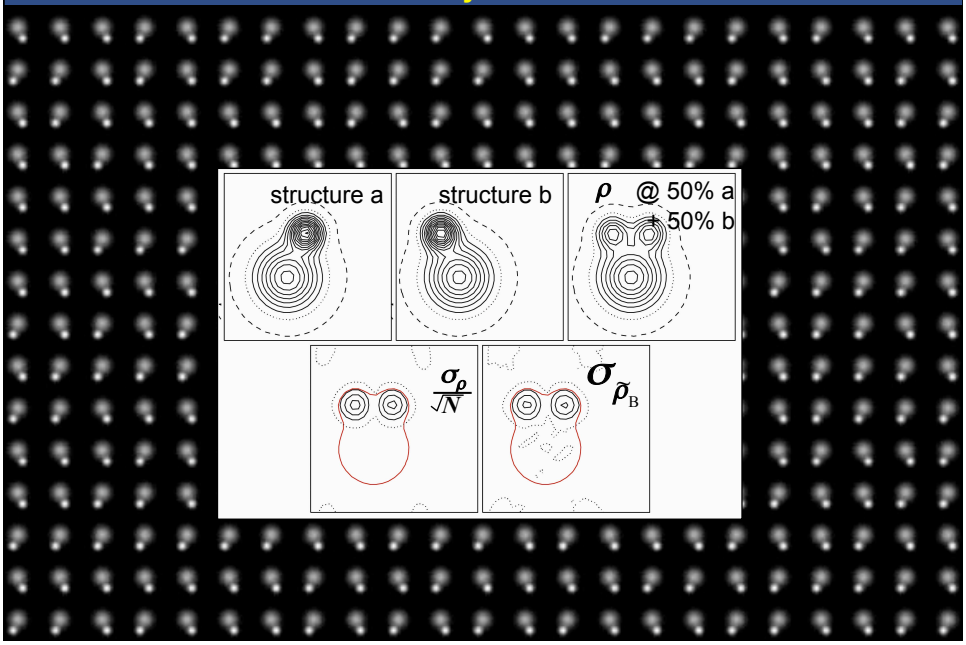
Bootstrap resampling-process



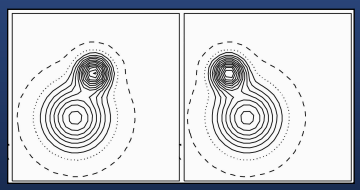
Bootstrap resampling-result

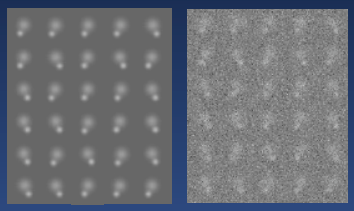


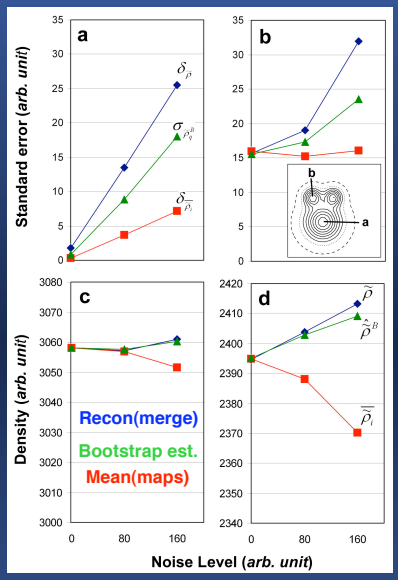
Test-simulated crystals without noise



Test-simulated crystals with noise

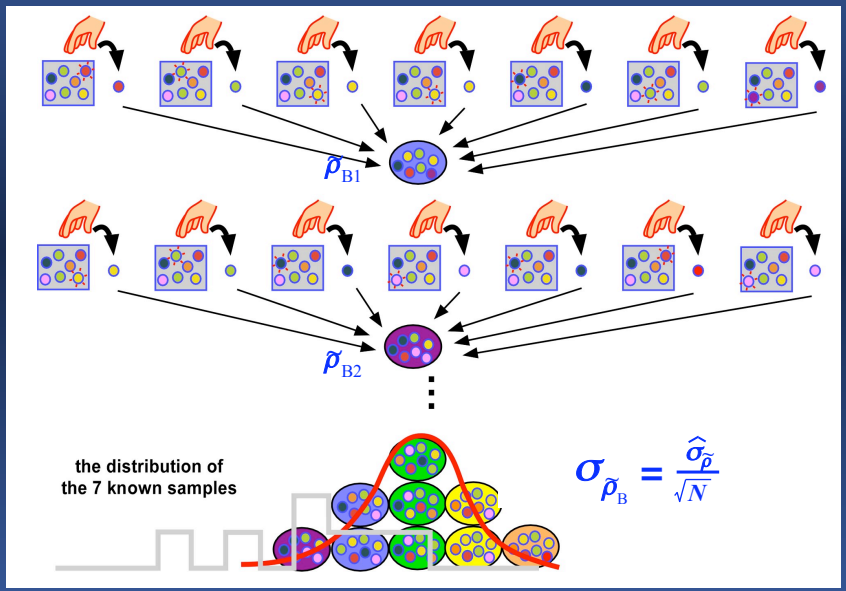






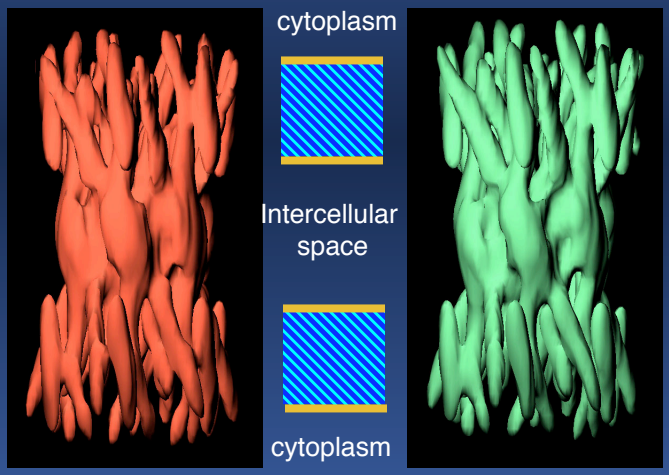
- standard error estimated by the bootstrap technique is closer to the true value calculated from the known population when the images are noisy

Why is bootstrap resampling better than direct calulation of map variation?

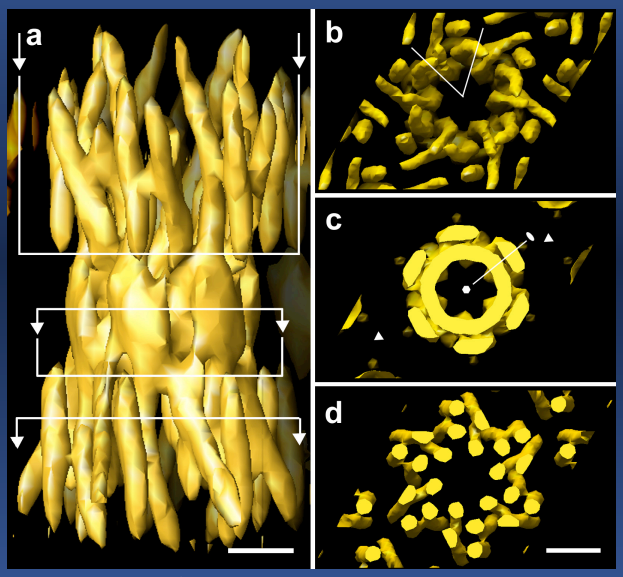


Gap junction channel structure at 7 Å

- Intercellular channel
- Cx43-major cardiac gap junction protein, m.w. 43 kD



General Features of Cx43 gap junction structure

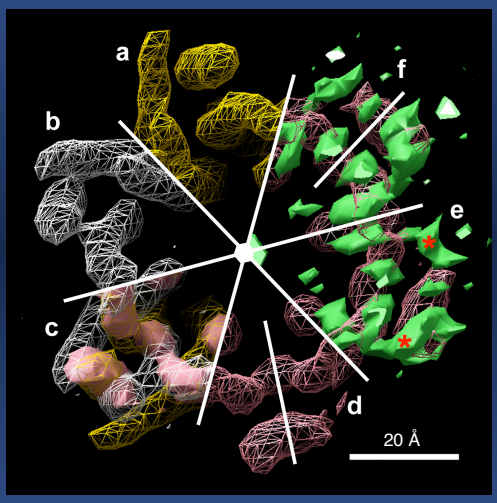


- 2 hexamer end -to-end docking
- helical in the membrane
- crystal-p6
- molecule-pseudo p622

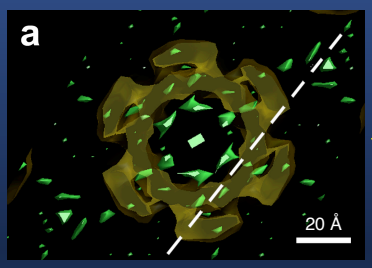
Unger et. al.,(1999) Science 283,1176-80

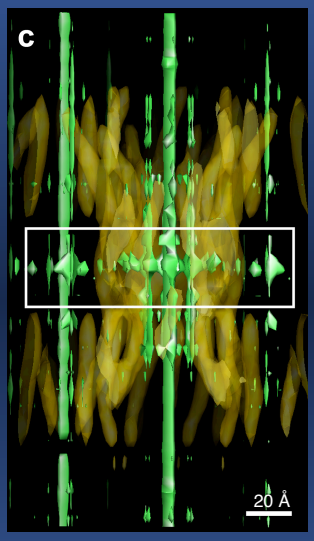
Test-Large conformation change in 3D map

- Cx43 Crystal-P6
- Molecule-pseudo p622
- c,d: merged mirrored data set of a and b
- e,f: standard deviation map showing molecular pseudo p6 /mmm mirror symmetry



Cx43



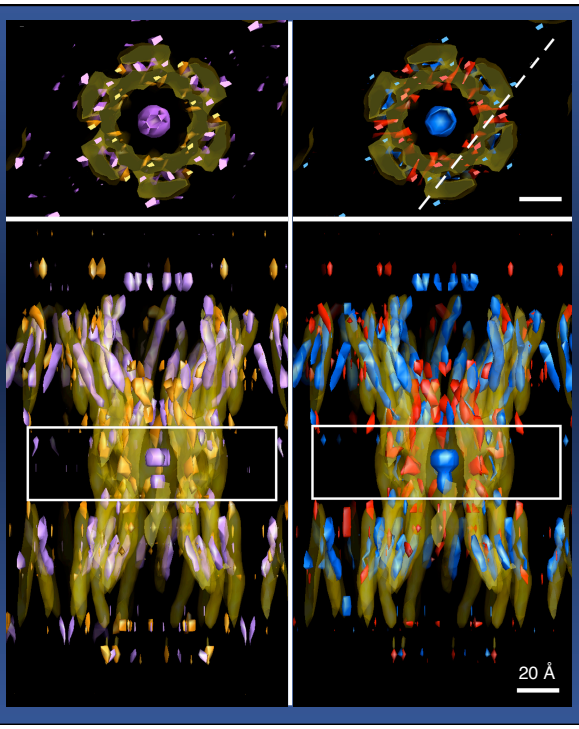


- Largest variance at symmetry axis
- Higher variance at the lumen wall in the extracellular domain.
- Cx43-TR variance > Cx43-WT
  - (87% of voxel α=0.05)

Contoured at 1.5 rms deviation above spatial mean(stdv)

t-map

- at 99% confidence level
- very similar to $\Delta\rho$ map since voxel-wise variation of the variance is small.



Comparison with single particle application

	2D crystallography	**Single particle averaging**
Sampling unit	Image	Particle
Number of samples	40-100's	1,000's to 1000,000's
Number of orientation	Same as # of images	Same as # of classes
Required resampling cycles for 99.5% confidence level of the error estimate	400	400
Required single processer time	< 3 hr for 400 cycles of 50 images	? Speed up by using only subset of the particles at uniform orientation distribution
Focused classification	Probably not applicable	applicable

Conclusions

- **The bootstrap resampling method yields a good quantitative estimate of the standard error of the reconstructed density.**
- **As expected, the error map is dominated by artifacts at the symmetry axes.**
- **The variance related to the Cx43 gap junction channel is largest at the boundary of the pore, with additional peaks within the helical transmembrane density.**
- **The 2D crystal reconstruction algorithm is not a simple average of maps from individual images.**

References

- A. Cheng, M. Yeager, "Bootstrap resampling for voxel-wise variance analysis of three-dimensional density maps derived by image analysis of two dimensional crystals" *J. Struct. Biol.* ***158, 19 (2007).***
- P. A. Penczek, C. Yang, J. Frank, C. M. T. Spahn, "Estimation of variance in single-particle reconstruction using the bootstrap technique" *J. Struct. Biol.* ***154, 168 (2006).***
- P. A. Penczek, J. Frank, C. M. T. Spahn, "A method of focused classification, based on the bootstrap 3D variance analysis, and its application to EF-G-dependent" *J. Struct. Biol.* ***154, 184 (2006).***

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http://nramm.scripps.edu

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