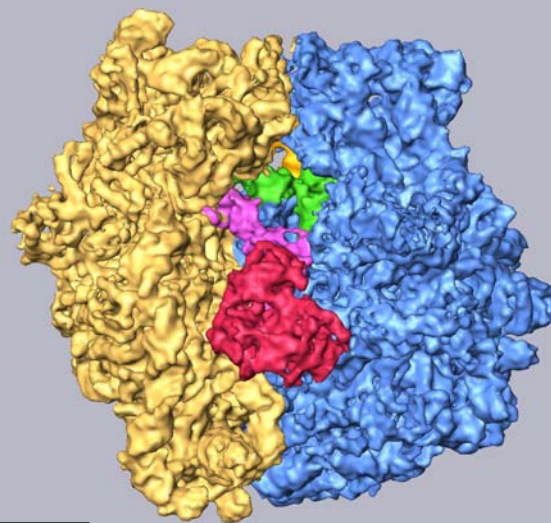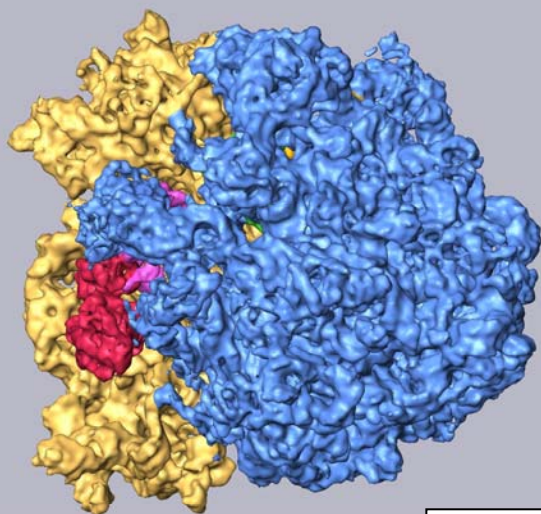# S P I D E R

Joachim Frank

SPIDER is a command-driven application written predominantly in FORTRAN 90.
Available for Linux, Irix, and AIX. More than 80 suites of operations (with a total of more than 450 operations)

- Deals with branching data flow typical for EM data processing
- Flexibility
- Modularity
- Hierarchical calling structure ("Procedures")
- Since 1978
- Associated tools: WEB, JWEB, SPIRE

OPERATIONS SUPPORTED

Overview of operations supported by SPIDER, broken into categories:

File handling and conversion
Copying, Converting, Stacking, and Montaging images, volumes, and stacks

Basic image processing
Contrast enhancement, Arithmetic operations, Rotating, Shifting, Scaling, Windowing, Masking, Thresholding, Filtering, Edge-detection, Histogram analysis, and Mirroring.

Fourier-space operations
Fourier transformation, Fourier filtering, Auto- and Cross-Correlation using Fourier theorem, Interpolation

Alignment
Rotational, Shift, Reference-based, and Reference-free alignments

Statistical analysis
Multivariate statistical analysis, Classification of image sets, Center of gravity, Variance map

Three-dimensional reconstruction
  Weighted back-projection, ART, SIRT

Resolution determination
  Fourier shell correlation, Differential phase residual, SSNR

Contrast transfer function
  CTF determination, CTF correction via Wiener filter
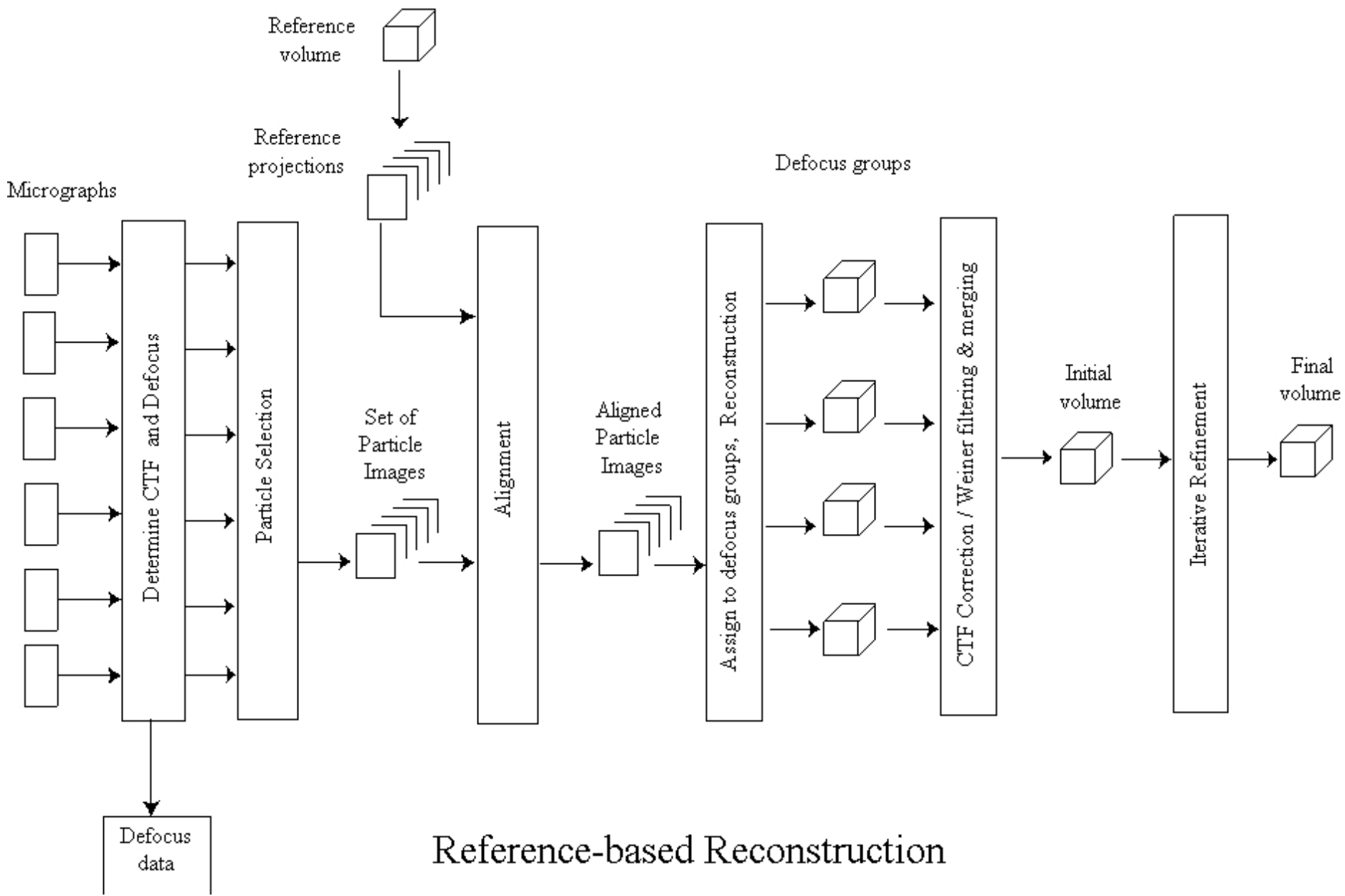
Graphical representations
  Contouring, Surface shading, Cluster analysis, and Graphing

Scripting and flow control
  DO-loops, IF statements, GO TO statements, Variable definition

Document file operations for handling numerical data
  Store, Retrieve, Sort, and Process numerical results in SPIDER
  document file format.

Reference volume

Reference projections

Micrographs

Defocus groups

Determine CTF and Defocus

Particle Selection

Set of Particle Images

Alignment

Aligned Particle Images

Assign to defocus groups, Reconstruction

CTF Correction / Weiner filtering & merging

Initial volume

Iterative Refinement

Final volume

Defocus data

Reference-based Reconstruction

NATURE PROTOCOLS (on-line Journal)

1) R.A. Grassucci, D. Taylor, J. Frank (2007) Preparation of macromolecular complexes for cryo-electron microscopy. *Nat. Prot.*, in press.

2) R.A. Grassucci, D. Taylor, J. Frank (2007) Visualization of macromoplecular complexes using cryo-electron microscopy with FEI Technai TEMs. *Nat. Prot.*, in press.

3) T. R. Shaikh, H. Gao, W. Baxter, F. Asturias, N. Boisset, A. Leith, J. Frank (2007) SPIDER image processing for single-particle reconstruction of biological macromolecules from electron micrographs. *Nat. Prot.*, in preparation.

   76 pages, 60 figures

□

# SPIDER – new features since the last Scripps Workshop in 2005

- SPIDER and Web ported to Mac OSX for Intel
- Procedures for single-particle reconstruction now use image stacks for greater efficiency.
- New Operations: **RB 3F** and **RB 32F** speed up data flow and combine shift, rotate, and reconstruction from a data set.
- New syntax and added operations for **DO**, **ENDDO**, **CYCLE**, **EXIT**, **ELSEIF**.
- Explicit names for variables allowed, e.g., CCC or SIGMA instead of X11, X12, etc.
- Symbolic parameters are now true string variables
- Creation of angular grid: **VO NEA** replaces **VO EA** for more uniform coverage
- Operations for more convenient interaction with document files: **RR S**, **UD NEXT**, **FR N**, **RR G**
- SPIDER/Web released under GPL license. Free download.
- □

SPIDER Reconstruction Engine (SPIRE)

- Lets user run SPIDER batch files in a GUI.
- Keeps track of procedures and the output files they generate
- Provides interface to view all files related to the project
- Convenient environment for testing and debugging batch files
- Tools for handling file numbers and project-wide parameters
- Ability to save a project in html format
- A means for accessing external databases.
- http://www.wadsworth.org/spider_doc/spider/docs/SPIRE/index.html

SPIRE: SPIDER Reconstruction Engine V1.5.0
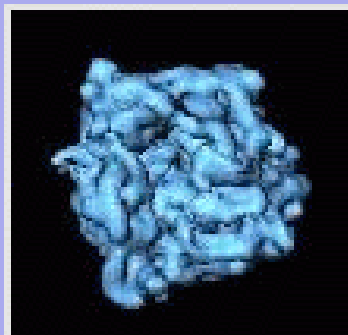
Single Particle Reconstruction with Reference Projections

Project: My Test Ribosome project

Directory: /home/bbaxter/projects/hcc

```
Sending command: /usr/local/bin/spider spi/hcc SPIRE@pnums &


 \__`0 0'__/          SPIDER  --   COPYRIGHT
,__xXXXx___          HEALTH RESEARCH INC., ALBANY, NY.
 __xXXXx__
 /  /xxx\  \          VERSION:  UNIX  14.16  ISSUED: 03/30/2006
 /       \           DATE:      28-NOV-2006    AT  14:39:27




Results file: results.spi.4
**** SPIDER NORMAL STOP ****
```

File numbers: 1-3

SPIRE basics

- SPIRE is organized around projects.

- A **Project** consists of an input data set of micrographs, the batch files that process the data to produce the result (usually a 3D density map) and numerous intermediate files.

- **Projects** are organized by project templates called "**configurations**" which indicate the location of the source batch file, the directory structure of the project, and how to present the project to the user.

- The graphical user interface is organized around dialogs, which allow the user to launch SPIDER batch files from buttons. With these dialogs, a large number of project batch files can be organized into a few conceptually related units such as "*particle picking*", "*alignment*", or "*power spectrum and defocus determination*".

- For instance, the module "*Power Spectra*" provides access to all batch files related to computing power spectra, determining defocus, group data into defocus groups.
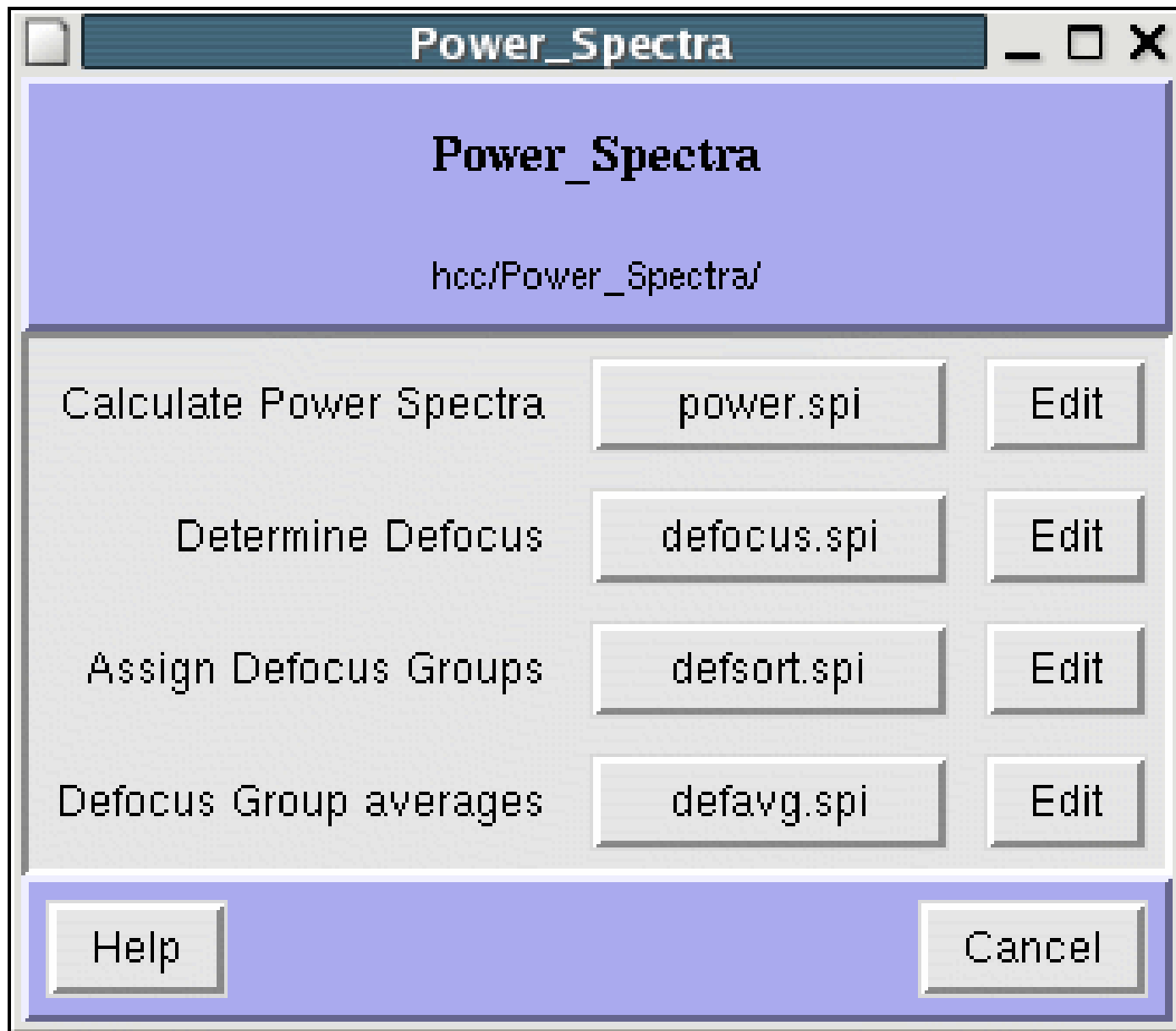
Module "Power Spectra". Start batch file by clicking button, or edit batch file first, then start.

- Contents and appearance of dialog are defined by the **Configuration File.**
- SPIRE configuration files are text files, using XML tags to organize their contents, that describe a set of frequently used batch files for various types of projects, e.g. single-particle reconstruction or electron tomography.
- Configuration tells SPIRE where to find the source batch files in the user's file system, and where they should be copied to in the working directory of the project.
- Also specifies how the GUI should present dialogs to the user.
- **Configuration Editor** allows one to change configurations or create new ones.

defsort.spi

defsort.spi

directory: /home/bbaxter/projects/hcc/Power_Spectra

Registers

max. defocus difference: `1000`

Input files

input defocus file : `defocus`

Output files

output defocus group file : `def_sort`

[Save] [Editor]   [Execute!] [Cancel]

Batch file form: shows default register values, input files and output files declared in the header. For programming changes outside header, use Editor and Save.

Project Viewer

ID	Directory	Procedure	Date	Time	File numbers
061128140811	Power_Spectra	defsort.spi	28-NOV-2006	14:08:11	
061128140818	Power_Spectra	defavg.spi	28-NOV-2006	14:08:18	
061128140842	Particles	noise.spi	28-NOV-2006	14:08:42	
061128141928	Particles	lfc_pick.spi	28-NOV-2006	14:19:28	
061128143927	Particles	pmums.spi	28-NOV-2006	14:39:27	1-3

Output files for 061128141928: lfc_pick.spi

coords/sndc0001.hcc	Document	1939	28-NOV-2006	14:23:49
coords/sndc0002.hcc	Document	1916	28-NOV-2006	14:31:39
coords/sndc0003.hcc	Document	1932	28-NOV-2006	14:35:43
win/ser000001.hcc	Image	75,75	28-NOV-2006	14:23:55
win/ser000002.hcc	Image	75,75	28-NOV-2006	14:23:55

Commands/View Projects in Main Menu opens the **Project Viewer,** which shows Current status of the project..