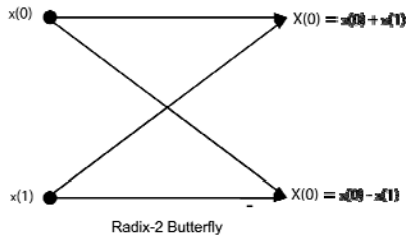
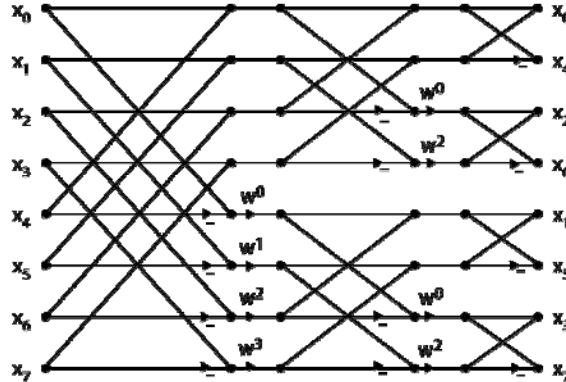


Fast Fourier Transform



- Cooley-Tukey FFT Algorithm
- Fast computation of Fourier xform
- Values combined in pairs
- Works with sizes of 2^n (powers of 2)
- Other algorithms for prime number sizes



2



FFTW – Fastest Fourier Transform in the West

<http://www.fftw.org/>

Dimension limits:

- Fastest for x, y, and z dimension of powers of 2
- Still fast if dimensions are small “prime factors”, some combination of multiple of 2, 3, 5, and 7
- Can handle arbitrary sizes, but switches to “Discrete Fourier Transform” – much slower than “Fast Fourier Transform”



----- Good x, y, and z dimensions -----

Powers of 2: 2 4 8 16 32 64 128 256 512 1024 ...

Prime factors of 2, 3, 5, and 7:

3	5	6	7	9	10	12	14	15	18	20	21	24	25	27	28	30	35	36
40	42	45	48	49	50	54	56	60	63	70	72	75	80	84	90	96	98	100
105	108	112	120	125	126	135	140	144	147	150	160	168	175	180	189	192	196	200
210	216	224	225	240	245	250	252	270	280	288	294	300	315	320	336	343	350	360
375	378	384	392	400	420	432	441	448	450	480	490	500	504	525	540	560	576	588
600	630	640	672	675	686	700	720	735	750	756	768	784	800	840	864	875	882	896
900	945	960	980	1000	1008	1029	1050	1080	1120	1125	1152	1176	1200	1225	1260	1280	1323	1344
1350	1372	1400	1440	1470	1500	1512	1536	1568	1575	1600	1680	1715	1728	1750	1764	1792	1800	1890
1920	1960	2000	2016	2058	2100	2160	2205	2240	2250	2304	2352	2400	2450	2520	2560	2625	2646	2688
2700	2744	2800	2880	2940	3000	3024	3087	3136	3150	3200	3360	3375	3430	3456	3500	3528	3584	3600
3675	3780	3840	3920	4000	4032	4116	4200	4320	4410	4480	4500	4608	4704	4725	4800	4900	5040	5120
5145	5250	...																

3



3D Deconvolution Software

Commercial Packages

- Autoquant
- Amira
- Huygens
- DeltaVision
- 3D Doctor
- Scanalytics
- VayTech

All are fairly expensive

Free Packages for iterative methods

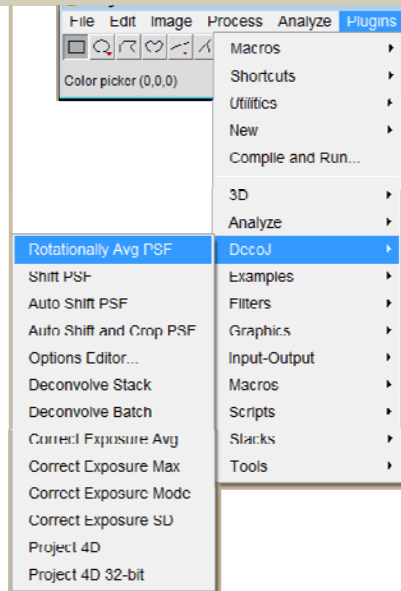
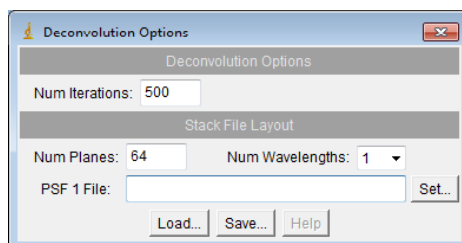
- XCosm
 - quite old (last update 2002)
 - Xwindows
 - linux only, can compile for OS-X
 - No batch processing
- DecoJ
 - Works with ImageJ,
 - batch processes files.
 - Developed by me.
 - Windows version of server

4



DecoJ

- Server runs in background
- Handles multiple timepoints
- Handles multiple wavelengths
- ImageJ plugin:
 - PSF creation
 - Batch file instructions (through options editor)
 - Processes all files in a subdirectory



5



Deconvolution Laboratory Exercises

- Make PSF slides
- Make PSF images for DecoJ
 - Capture PSF stacks
 - Crop and “shift” PSFs
 - Average several PSFs
- Capture image of large beads
 - Must capture extra slices
 - Necessary to compress Z information
 - Deconvolve
 - Compare # of iterations
 - Show “ringing” at edges
- Capture image of cells
 - Deconvolve single wavelength

6



Measuring the PSF

1. Use sub-resolution fluorescent beads (~200 nm diameter)
Tetraspeck PSF beads from Invitrogen
 2. Sonicate beads to disperse
 3. Dilute beads in solution
 4. Sonicate beads to disperse
 5. Spread drop of bead solution on slide
 6. Wait for water to dry
 7. Add drop of glycerol, water, immersion oil, or other mounting media – then coverslip
 8. Take image stacks of several beads
 9. Align and average bead images
- Take bead stack with SAME IMAGING PARAMETERS as image stack (same x-,y-,z-spacing)
 - Keep bead density low (prevents PSF overlap)
 - Stay near center of field of view



7

