CRYO ELECTRON MICROSCOPY TOMOGRAPHY

ELECTRON MICROSCOPY

LONG USED FOR HIGH RESOLUTION IMAGING
ELECTRONS HAVE SHORT WAVELENGTHS (~0.01 nm)
VERY LITTLE CONTRAST WITH BIOLOGICAL OBJECTS
HISTORICALLY, COAT WITH METAL
DIFFICULT TO SEE INTERNAL STRUCTURES OF CELLS

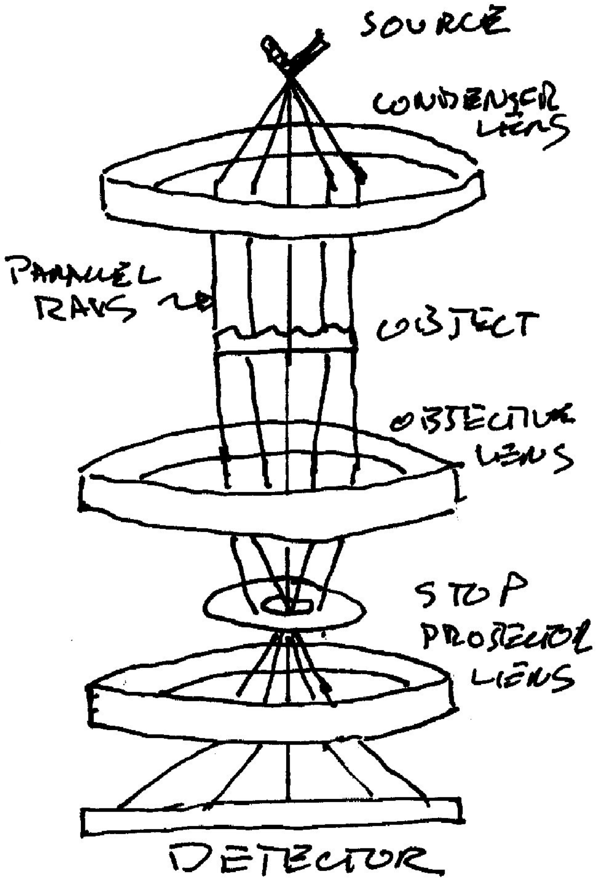
CRYO-EM

FLASH FREEZE THIN FILM OF CELLS IN WATER
  NORMAL HYDRATION STATE
WATER FORMS A GLASS, DOESN'T FORM CRYSTALS
  WHICH DESTROYS THE CELL
REDUCES DAMAGE FROM ELECTRON BEAM
STILL VERY LITTLE CONTRAST!

CRYO-EM TOMOGRAPHY

ACQUIRE A SERIES OF CRYO-EM IMAGES
  AT A RANGE OF TILT ANGLES
RECONSTRUCT 3D WITH FILTERED BACKPROJECTION
SEVERAL PRACTICAL ISSUES

ELECTRON MICROSCOPE (TRANSMISSION)

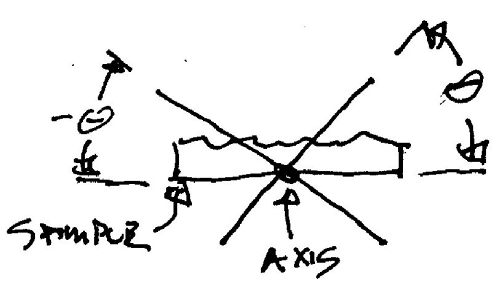


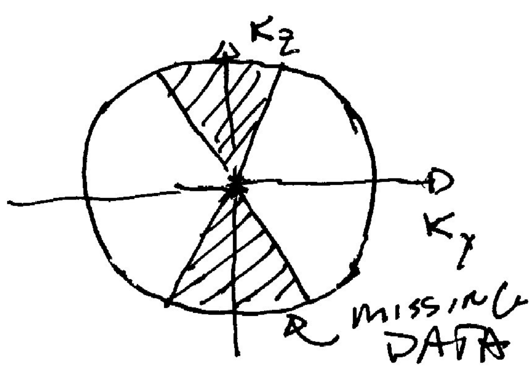
SOURCE ~25 nm TIP
100-300 kV ACCELERATION
LENS ARE ELECTROMAGNETS
OBJECT BOTH ATTENUATES AND DEFLECTS BEAMS
BOTH AMPLITUDE AND PHASE

STOP SUPPRESSES WIDELY SCATTERED ELECTRONS, REDUCE BACKGROUND
PROJECTOR MAGNIFIES IMAGE

DETECTOR, DIGITAL PHOTON COUNTING (WAS CCD, FILM)

TOMOGRAPHY





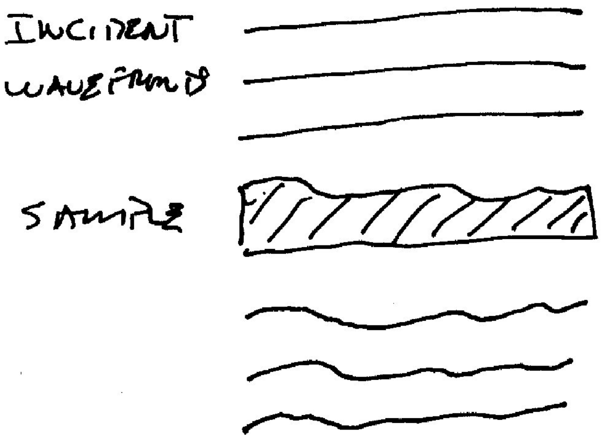
FOR TOMOGRAPHY TILT THE STAGE

ANGLES FROM ± 60° OR SO

MISSING 30° ON EACH END
"MISSING WEDGE"

IN K-SPACE, MISSING SPATIAL FREQUENCY DATA

SAMPLE CONTRAST



ψ_0

$$\psi_s(\underline{r}) = \psi_0 \, e^{(\underbrace{\sigma(\underline{r})}_{\text{ATTENUATION}} + i\underbrace{\phi(\underline{r})}_{\text{PHASE}})}$$

CONVENTIONALLY IN TRANSMISSION EM, SAMPLE IS COATED IN METAL TO MAKE $\sigma(\underline{r})$ LARGE (AND NEGATIVE)

IN BIOLOGICAL SAMPLES, $\sigma(\underline{r}) = 0$ AND $\phi(\underline{r})$ IS SMALL

SMALL ANGLE APPROXIMATION

$$\psi_s(\underline{r}) = \psi_0 \, e^{i\phi(\underline{r})}$$
$$\simeq \psi_0 (1 + i\phi(\underline{r}))$$

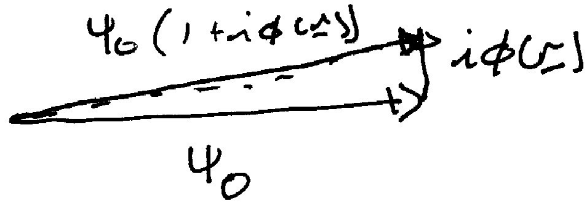
THE DETECTED INTENSITY IS

$$\psi_s(\underline{r})\psi_s^*(\underline{r}) = \psi_0^2 (1 + i\phi(\underline{r}))(1 - i\phi(\underline{r}))$$
$$= \psi_0^2 (1 + \phi^2(\underline{r}))$$

IF $\phi(\underline{r})$ IS SMALL, $\phi^2(\underline{r})$ IS <u>VERY</u> SMALL

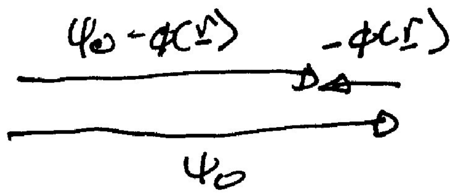
VERY LITTLE CONTRAST!

GRAPHICALLY



THE MAGNITUDE IS INSENSITIVE TO SMALL PHASE SHIFTS

WE WANT TO ADD $\pi/2$ PHASE SHIFT, SO THAT



THEN

$$\psi_s(\underline{r})\psi_s^*(\underline{r}) = \psi_0^2(1-\phi(\underline{r}))(1-\phi(\underline{r}))$$
$$\simeq \psi_0^2(1-2\phi(\underline{r}))$$

MUCH LARGER CONTRAST.

HOW DO WE INTRODUCE THIS PHASE SHIFT?

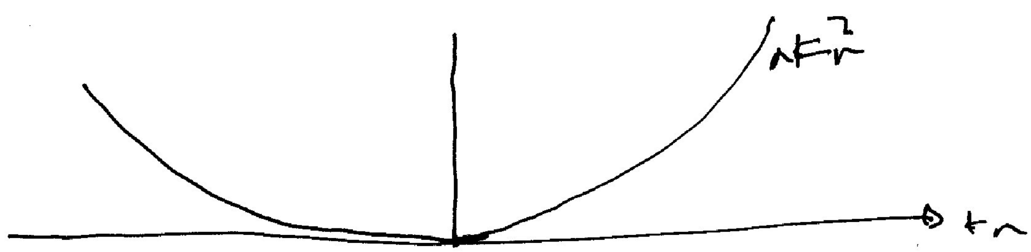
RECALL THAT BEING OUT OF FOCUS IN AN OPTICAL SYSTEM IS A SPHERICAL ABERRATION (MRI AUTOFOCUS LECTURE)

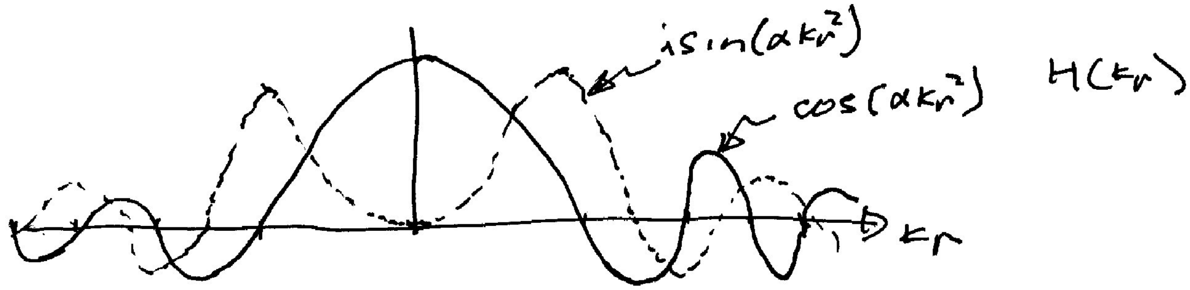
$$H(k_r) = e^{i \alpha k_r^2}$$

WHERE k_r IS THE RADIAL SPATIAL FREQUENCY

$$H(k_r) = \cos(\alpha k_r^2) + i \sin(\alpha k_r^2)$$

THIS LOOKS LIKE





<u>TWO TERMS</u>

$\cos(\alpha k_r^2)$ IS REAL, EVEN, AND LOWPASS. IT CORRESPONDS TO A REAL FILTER IN IMAGE SPACE. IT EFFECTS THE IN-PHASE COMPONENT.

$i\sin(\alpha k_r^2)$ IS IMAGINARY AND EVEN, AND BANDPASS. IT CORRESPONDS TO AN IMAGINARY FILTER IN IMAGE SPACE. THIS ROTATES THE PHASE SIGNAL TO THE REAL CHANNEL

IF WE INTENTIONALLY DEFOCUS, WE GET

LOWPASS REAL COMPONENT FOR REFERENCE

BANDPASS REAL COMPONENT

(IMAGINARY PHASE) · (IMAGINARY FILTER)

$\pm 2\phi(r) * \mathcal{F}^{-1}\{\mathrm{Im}\{H(k_r)\}\}$ BANDPASS

$\psi_0 * \mathcal{F}^{-1}\{\mathrm{Re}\{H(k_r)\}\}$ LOWPASS

THE INFORMATION WE CARE ABOUT IS THE IMAGINARY PHASE, WHICH SEES THE FILTER

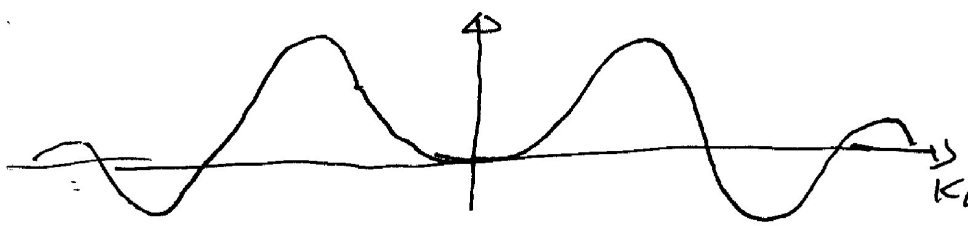
$$CTF(k_r) = i\sin(\alpha k_r^2)$$

THIS IS THE <u>CONTRAST</u> <u>TRANSFER</u> <u>FUNCTION</u>. THIS IS APPLIED <u>BEFORE</u> <u>INTENSITY</u> <u>DETECTION</u>.

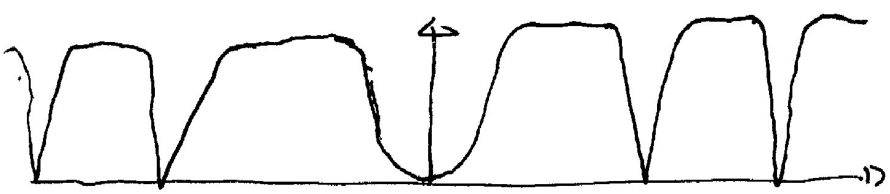
THE CTF IS BOTH POSITIVE AND NEGATIVE.

IDEALLY WE'D LIKE IT TO BE ALL POSITIVE

WE CAN CORRECT FOR THIS IN <u>POST-PROCESSING</u>



$CTF(k_r)$



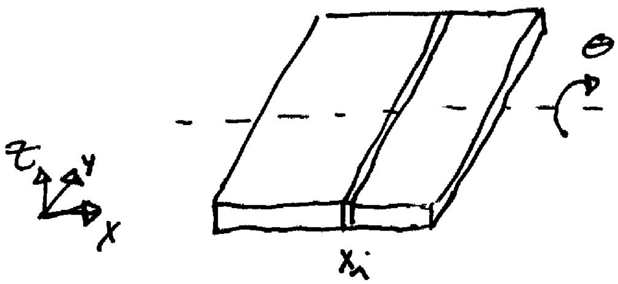
CORRECTED CTF

$$\frac{CTF(k_r)}{CTF(k_r)+\beta}$$

NOW ALL POSITIVE, NULLS AT ZEROS

THIS GIVES US MOST OF SPATIAL FREQUENCY EXCEPT DC, A FEW NULLS

TOMOGRAPHIC RECONSTRUCTION



THIN SLAB ROTATED ABOUT X AXIS

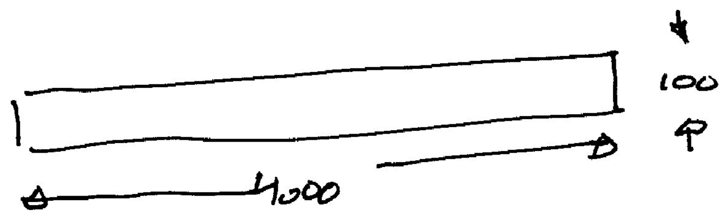
EACH PROJECTION IS ~4K X 4K

RECONSTRUCT ~100 SLICES

ANGLES FROM ±60 DEGREES AT 2° STEPS

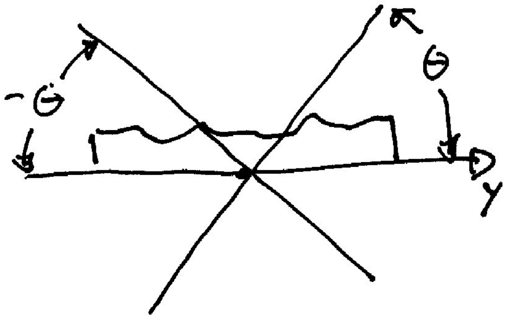
TOMOGRAPHY PROBLEM

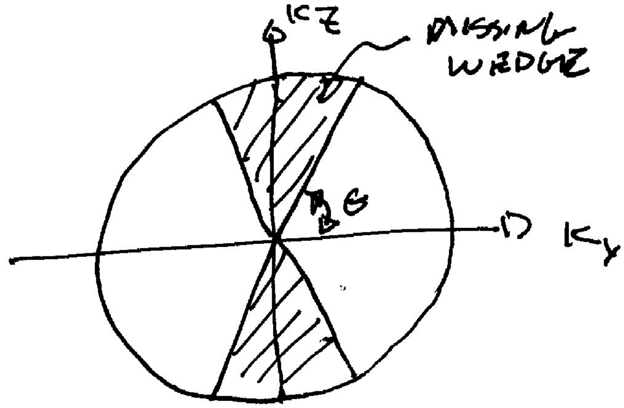
- RAYS ARE PARALLEL
- ROTATION ABOUT ONLY ONE AXIS
- EACH POSITION X IS A SEPARATE 2D RECONSTRUCTION IN Y AND Z
- GENERALLY SOLVED WITH FILTERED BACKPROJECTION
  - EFFICIENT FOR VERY ASSYMETRIC FOV



ONLY RECONSTRUCT THE VOXELS YOU WANT

LIMITED TILT ANGLES CAUSES ARTIFACTS

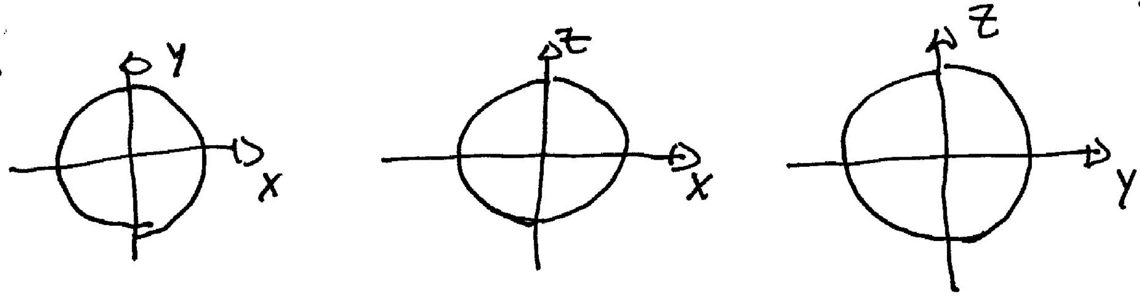




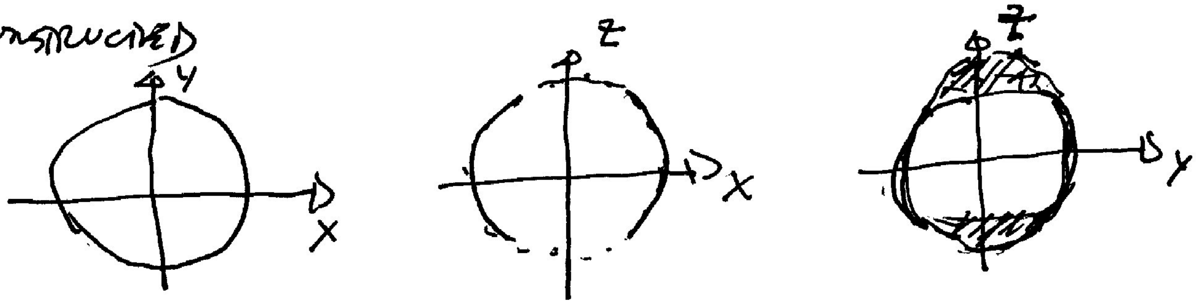
WE ARE MISSING HIGH SPATIAL FREQUENCIES IN Z

IF WE RECONSTRUCT SLICES THROUGH A SPHERICAL SHELL

IDEAL



RECONSTRUCTED



THE X-Y PLANE LOOKS VERY GOOD, X-Z NOT TOO BAD, THE Y-Z PLANE HAS THE WORST ARTIFACTS