

# Tilt Geometry

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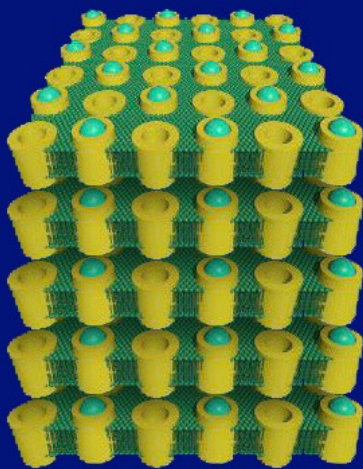
2dx Workshop  
Basel, August 23-26, 2016

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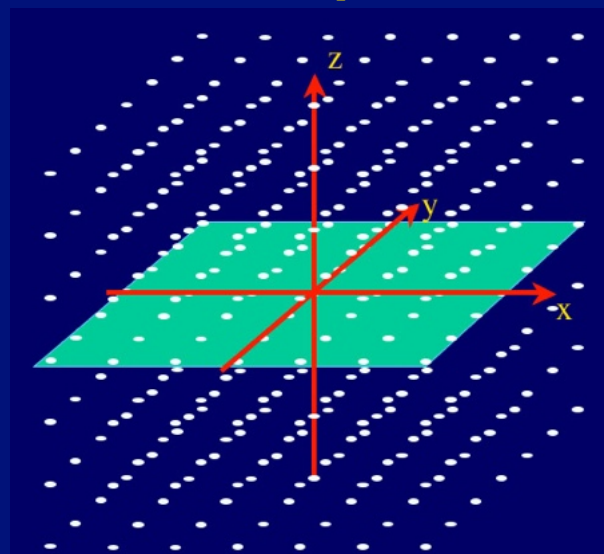
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## The Third Dimension

Real Space



Fourier Space



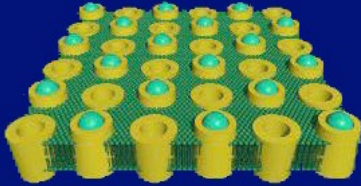
Diffraction Spots in 2D

2

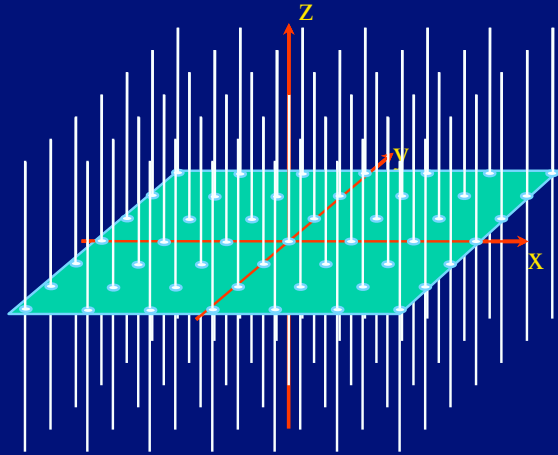
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# The Third Dimension

Real Space



Fourier Space

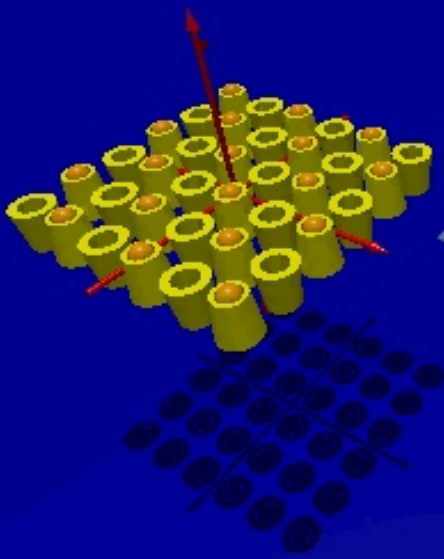


Diffraction Spots in 2D

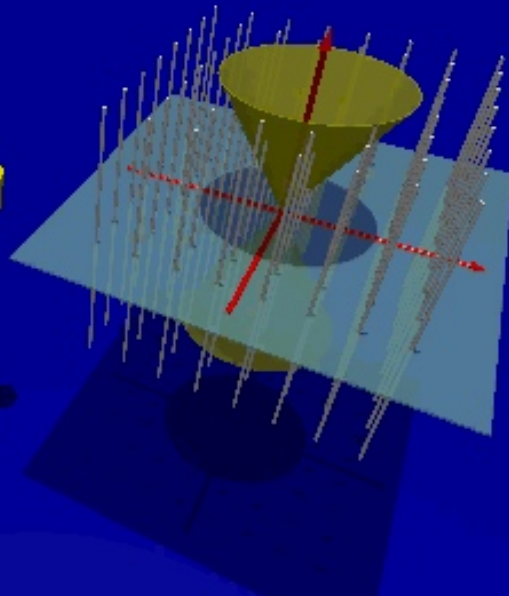
Lattice Lines in 3D

# The Missing Cone

Real Space



Fourier Space

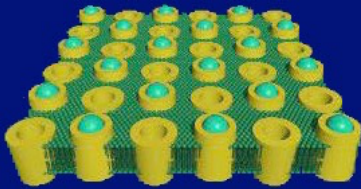


Diffraction Spots in 2D

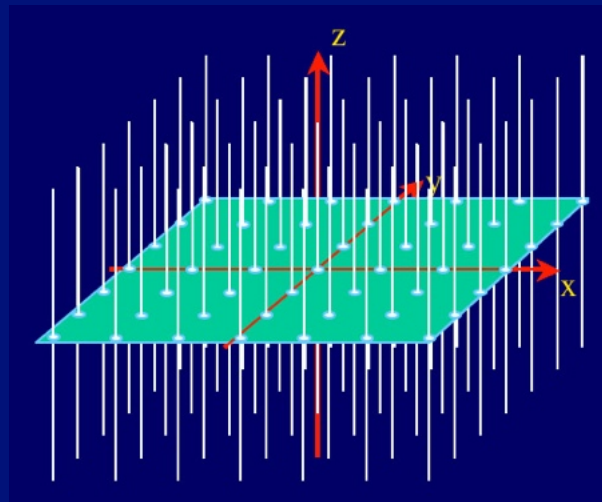
Lattice Lines in 3D

# The Third Dimension

Real Space



Fourier Space

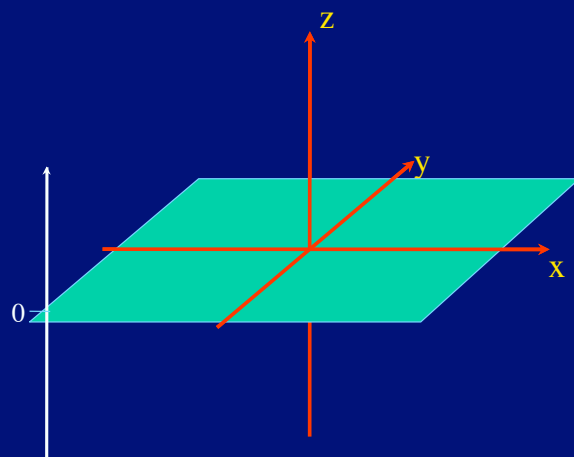
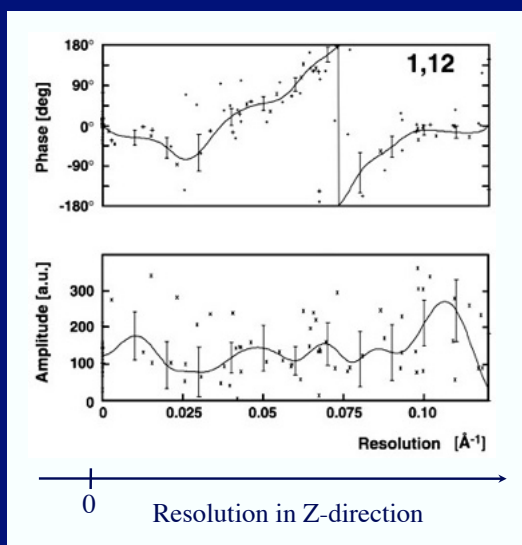


Diffraction Spots in 2D

Lattice Lines in 3D

# The Lattice Line

Fourier Space

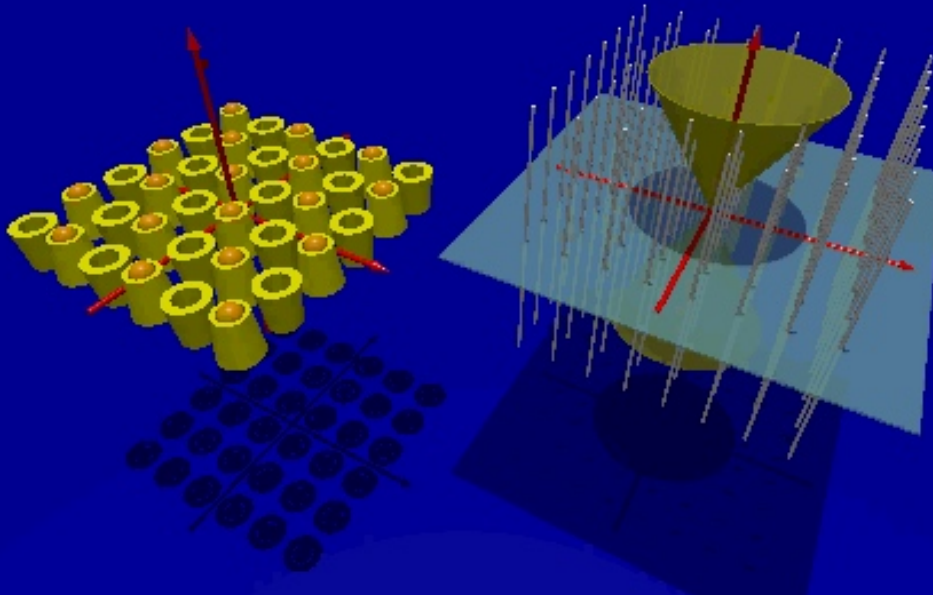


Complex Values for Amplitude and Phase in Z-direction

# The Missing Cone

Real Space

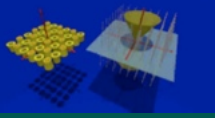
Fourier Space



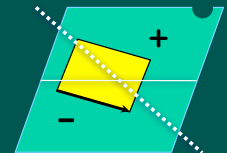
Diffraction Spots in 2D  
Lattice Lines in 3D

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## Tilt Geometry



### Coordinate System of the Recorded Image

1. Where is the tilt axis? **TLTAXIS:** angle from X-axis to tilt-axis
2. How much tilt was there? **TLTANG:** tilt angle of sample
3. How is the crystal oriented? **TLTAXA:** angle from tilt-axis to  $A^*$

### Coordinate System of the Sample

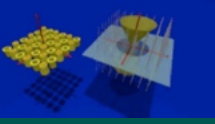
4. How much tilt was there? **TANGL:** tilt angle of sample  
(Same as **TLTANG**, but Sign dependent on:  
Is  $A^*$  above tilt axis? Sign of **TLTAXA**? Handedness of the lattice assignment?)
5. How is the crystal oriented? **TAXA:** angle from tilt-axis to  $A^*$  on sample  
(different than **TLTAXA**!)



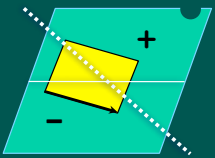
For all variables:  $[-90^\circ; 90^\circ]$

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# Tilt Geometry



**Coordinate System of the Recorded Image**

1. Where is the tilt axis? **TLTAXIS:** angle from X-axis to tilt-axis
2. How much tilt was there? **TLTANG:** tilt angle of sample
3. How is the crystal oriented? **TLTAXA:** angle from tilt-axis to A\*

**Coordinate System of the Sample**

4. How much tilt was there? **TANGL:** tilt angle of sample  
*(Same as TLTANG, but Sign dependent on:  
Is A\* above tilt axis? Sign of TLTAXA? Handedness of the lattice assignment?)*
5. How is the crystal oriented? **TAXA:** angle from tilt-axis to A\* on sample  
*(different than TLTAXA!)*

Four ways to determine/refine tilt geometry:

- From defocus of negative (ctfsearch3)

```

----- Defocus values in 49 positions on image ----
12780 11990 10960 9900 9510 8430 7500
12610 10920 10800 9480 9330 8070 7090
11770 11230 10190 8890 8460 7390 6890
11400 10190 9480 8685.35 7770 6790 6590
11090 9630 9360 8590 7570 6730 5930
10590 9540 9260 7850 7240 6060 5670
10580 9460 8330 7310 6640 5930 5200
          
```

# Determination and Refinement of the Tilt Geometry in 2dx

# Determination and Refinement of the Tilt Geometry in 2dx

## Defocus Gradient accross image

Rough Tilt Geometry, but correct sign of tilt angle (TLTAXIS, TLTANGL for grid)  
 No clue about crystal orientation (TAXA, TANGL for crystal)

## Lattice Distortion

Precise Tilt Geometry if tilt larger than 25°,  
 but no clue about sign of tilt angle (sign taken from above)

## SpotSplitting

Precise Tilt Geometry if tilt larger than 25°,  
 but no clue about sign of tilt angle (sign taken from above)

## 3D Merging

Precise Tilt Geometry for sample (TAXA, TANGL for crystal),  
 can calculate back to carbon film orientation (TLTAXIS, TLTANGL for grid)

**Get Defocus & Tilt**

Setup: Determine Tilt Geometry:  Yes  No  
 Which algorithm to use for Tilt Geometry from Defocus?  0=CFFIND on 7x7 locations  
 Grid Tilt Axis (TLTAXIS: X-axis -> Tilt axis, on image): 89.677

Results:

defocus	37741.16, 37573.21, 22.46
TLTAXIS	89.6767
TLTANG	-16.3541
TAXA	26.8226
TANGL	16.350
DEFOCUS_ACTIVE	1
DEFOCUS_TLTAXIS	89.677
DEFOCUS_TLTANG	-16.354
DEFOCUS_TLTAXA	26.823
DEFOCUS_TAXA	25.881

Output (Double click for logbrowser):

```

==== Mark Thon-ring fit panels
==== Merge Marked Thon-ring fit panels into one overview image
-----
37247.2  37267.2  37427.2  37507.2  37617.2  37987.2  37967.2
37307.2  37397.2  37437.2  37567.2  37737.2  37957.2  37947.2
37277.2  37337.2  37467.2  37457.2  0.0  0.0  0.0
37307.2  37327.2  37507.2  37657.2  37737.2  37657.2  0.0
37287.2  37297.2  37527.2  37617.2  37877.2  37697.2  37757.2
37307.2  37317.2  37507.2  37577.2  37777.2  37887.2  37937.2
37267.2  37337.2  37437.2  37617.2  37697.2  37837.2  37867.2
    
```

DEFOCUS = 37741.16, 37573.21, 22.46  
 TLTAXIS = 89.6767  
 TLTANG = -16.3541  
 ===== 2dx tiltgeom2: Determine other tilt angles with respect to lattice. =====  
 TLTAXA = 26.8226  
 TAXA = 25.8814

Calc. Mag=	22505.6						Last QVAL=	909.5	
Unbend	IQ1	IQ2	IQ3	IQ4	IQ5	IQ6	QVAL		
Unb. I	28	46	47	52	47	54	774.7		
Unb. II	29	60	51	59	63	49	909.5		
MovieA	-	-	-	-	-	-	---		
MovieB	-	-	-	-	-	-	---		
PowerBins (noise=7)			[A]	15	12	9	7	5	3
			#	808	81	56	14	8	7
Tilt Info	Defoc.	Latt.	SpSplit	Merge					
Grid TAxis	89.7	86.3	---	87.8					
Grid TAngle	-16.4	-23.3	---	-28.0					
Xst. TAxis	25.9	28.2	---	25.8					
Xst. TAngle	16.4	23.3	---	28.0					

MioK1-film | 2dx (4.0.0)

MioK1-K2-2014-11-17\_MioK1\_cAMP/2014-11-17\_12-05-02 MioK100/MioK10099001200

### Get Lattice & Tilt

Setup Level: Simplified

Crystal Tilt Angle (TANGL =TLTANG\*AxisAbove\*SignTLTAXA\*Hand) 16.350

**Lattice Determination**

Determine Lattice  Yes  No

Lattice Determination Algorithm to run

0=FindLattice (search for one or two expected lattice(s) based on real cell)

Real Unit Cell Length (for entire project) 131.0

Real Unit Cell Length (for project) 90.0

Regenerate Peak List  Yes  No

Exclusion radius for CSD peak list generation 15

17.205 -34.515 37.373 15.438

New 2nd lattice would have been 0.0,0.0,0.0,0.0 (Score = 0.0). Due to the bad score it will not be used.

==== 2dx\_getspot - To generate a first spotlist. =====

==== 2dx\_lencalc: To calculate some values for the tilt geometry determination. =====

==== emTilt: To calculate the tilt geometry from lattice distortions. =====

1. TILTAXIS = 86.34969 = ALPHA - PHITLT

2. TILTAXIS = -33.35969 = ALPHA + PHITLT

Taking first option.

==== NOT saving tilt geometry from lattice, because of low tilt. =====

==== 2dx\_calcmag - to calculate the theoretical magnification =====

==== Theoretical magnification is 22007.938, given magnification is 22500 =====

==== Angle in reciprocal lattice is 85.949. =====

==== Angle in real-space lattice is 94.051. =====

==== 2dx\_latorator to find fitness of current lattice. =====

Absolute Error: 51.6206

RMSDn ERROR: 1.27549

Peaks Used: 140 of 140

Nodes/Peak Density: 1.65014

==== 2dx\_getLattice - normal end. =====

#####2dx\_getLattice finished.#####

Status

Calc. Mag=	22007.9						Last QVAL=	909.5
Unbend	IQ1	IQ2	IQ3	IQ4	IQ5	IQ6	QVAL	
Unb.I	28	46	47	52	47	54	774.7	
Unb.II	29	60	51	59	63	49	909.5	
MovieA	-	-	-	-	-	-	---	
MovieB	-	-	-	-	-	-	---	
PowerBins (noise=7)	[A]		15	12	9	7	5	
			808	81	56	14	8	
Tilt Info	Defoc.	Latt.		SpSplit		Merge		
Grid Taxis	89.7	86.3		---		87.8		
Grid TAngle	-16.4	-23.3		---		-28.0		
Xst. Taxis	25.9	28.1		---		25.8		
Xst. TAngle	16.4	23.3		---		28.0		

Results

lattice "17.205,-34.515,..."

LATTICE\_TLTAXIS "86.34969"

LATTICE\_TLTANG "-23.31427"

LATTICE\_TLTAXA "30.14531"

LATTICE\_TAXA "28.07180"

LATTICE\_TANGL "23.31427"

CALCULATEDMAG 22007.938

latorator\_aberror 51.6206

latorator\_rmsderror 1.27549

latorator\_peakused 140 of 140

latorator\_nopeakdensity 1.65014

LATTICE\_done y

SPOTS\_done n

Preview

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MioK1-film | 2dx (4.0.0)

MioK1-K2-2014-11-17\_MioK1\_cAMP/2014-11-17\_12-05-02 MioK100/MioK10099001200

### Refine Tilt from SpotSplitting

Setup Level: Simplified

**Common Image Processing**

Lower Resolution Limit (RESMIN) 200

Upper Resolution Limit (RESMAX, in Angstroms) 5.0

ALAT (Z-dimension of unit cell to reconstruct) 200

**Switches For Algorithm Selection**

Mode of operation 0=nothing

Output (Double click for logbrowser) Verbosity Level: Low

#####2dx\_refinetilt#####

==== Anaconda Python executable not found. Is it installed ??? =====

==== /opt/anaconda/bin/python =====

==== Location of Anaconda Python is defined in Preferences. =====

==== 2dx\_refinetilt - To refine values. =====

==== 2dx\_refine.exe - To refine values. =====

TILTAXIS old 86.334, new 89.7

TILTAXA old 26.823, new 26.8183

TILTANG old 25.881, new 25.8773

==== 2dx\_refinetilt - normal end. =====

#####2dx\_refinetilt finished.#####

Note: The following floating-point exceptions are signalling: IEEE\_INVALID\_FLAG

Status

Calc. Mag=	22007.9						Last QVAL=	460.4
Unbend	IQ1	IQ2	IQ3	IQ4	IQ5	IQ6	QVAL	
Unb.I	35	74	66	96	168	219	415.3	
Unb.II	39	83	65	113	161	228	460.4	
MovieA	-	-	-	-	-	-	---	
MovieB	-	-	-	-	-	-	---	
PowerBins (noise=7)	[A]		15	12	9	7	5	
			818	77	59	15	7	
Tilt Info	Defoc.	Latt.		SpSplit		Merge		
Grid Taxis	89.7	86.3		89.7		87.8		
Grid TAngle	-16.4	-23.3		-16.4		-28.0		
Xst. Taxis	25.9	28.1		25.9		25.8		
Xst. TAngle	16.4	23.3		16.4		28.0		

Results

TTREFINE\_TLTAXIS "89.6770"

TTREFINE\_TLTANG "-16.3540"

TTREFINE\_TLTAXA "26.8183"

TTREFINE\_TAXA "25.8773"

TTREFINE\_TANGL "16.3540"

Preview

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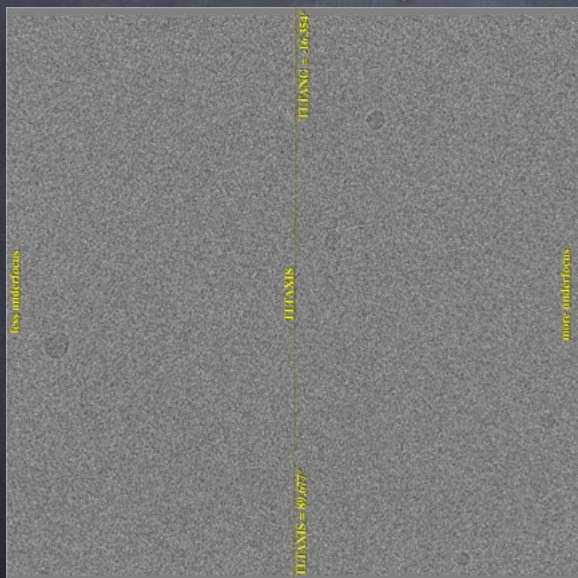
**3D Merging**  
 Precise Tilt Geometry for sample (TAXA, TANGL for crystal),  
 can calculate back to carbon film orientation (TLTAXIS, TLTANGL for grid)

Calc. Mag#	22007.9						Last QVAL#	460.4
<b>Unbend</b>	IQ1	IQ2	IQ3	IQ4	IQ5	IQ6	OVAL	
Unb. I	35	74	66	96	168	219	415.3	
Unb. II	39	83	65	113	161	228	460.4	
MovieA	-	-	-	-	-	-	---	
MovieB	-	-	-	-	-	-	---	
<b>PowerBinn</b>	[Å]		15	12	9	7	5	3
(noise=7)	#		818	77	59	15	7	7
<b>Tilt info</b>	Defoc.		Latt.		Sp@plit		Merge	
Grid TAxis	89.7		86.3		89.7		87.8	
Grid TAngle	-16.4		-23.3		-16.4		-28.0	
Xst. TAxis	25.9		28.1		25.9		25.8	
Xst. TAngle	16.4		23.3		16.4		28.0	



# Display of Tilt Geometry in 2dx

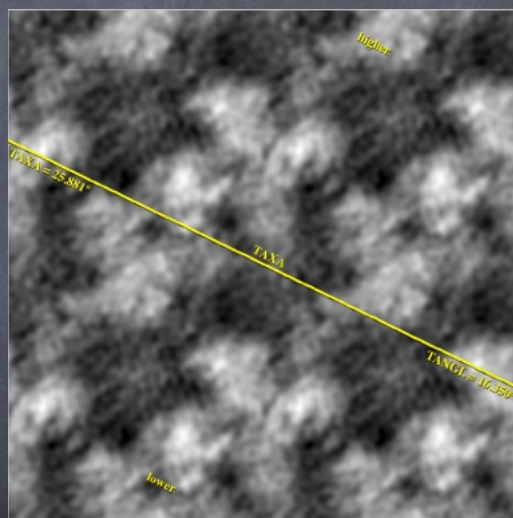
Sample Image



“T”

TLTAXIS, TLTANGL

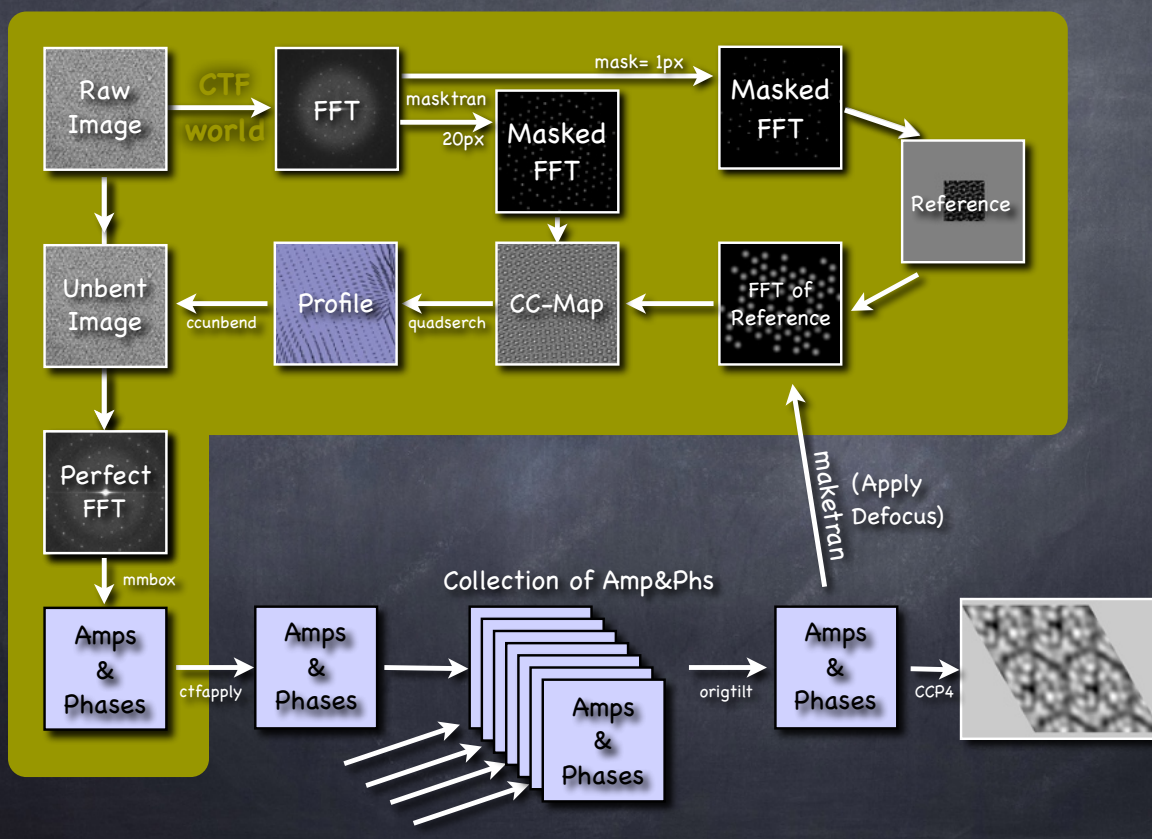
Final Map



“Shift-T”

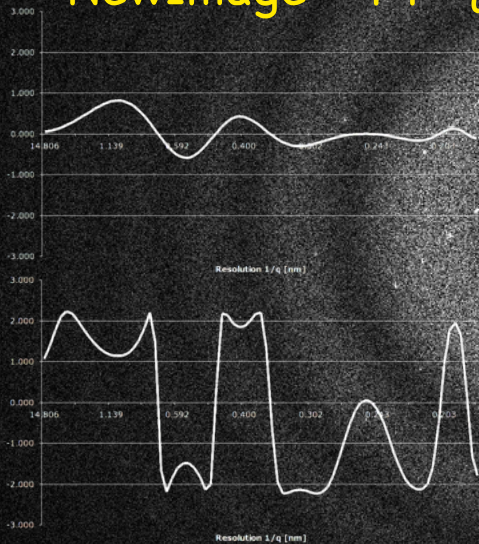
TAXA, TANGL

# Algorithm Non-tilted



## CTF correction: Wiener Filter

$$\text{NewImage} = \text{FT}^{-1} \left\{ \text{FT}(\text{Image}) \cdot \frac{\text{CTF}}{\text{CTF}^2 + N^2} \right\}$$



“Divide by the CTF (sort of...)”

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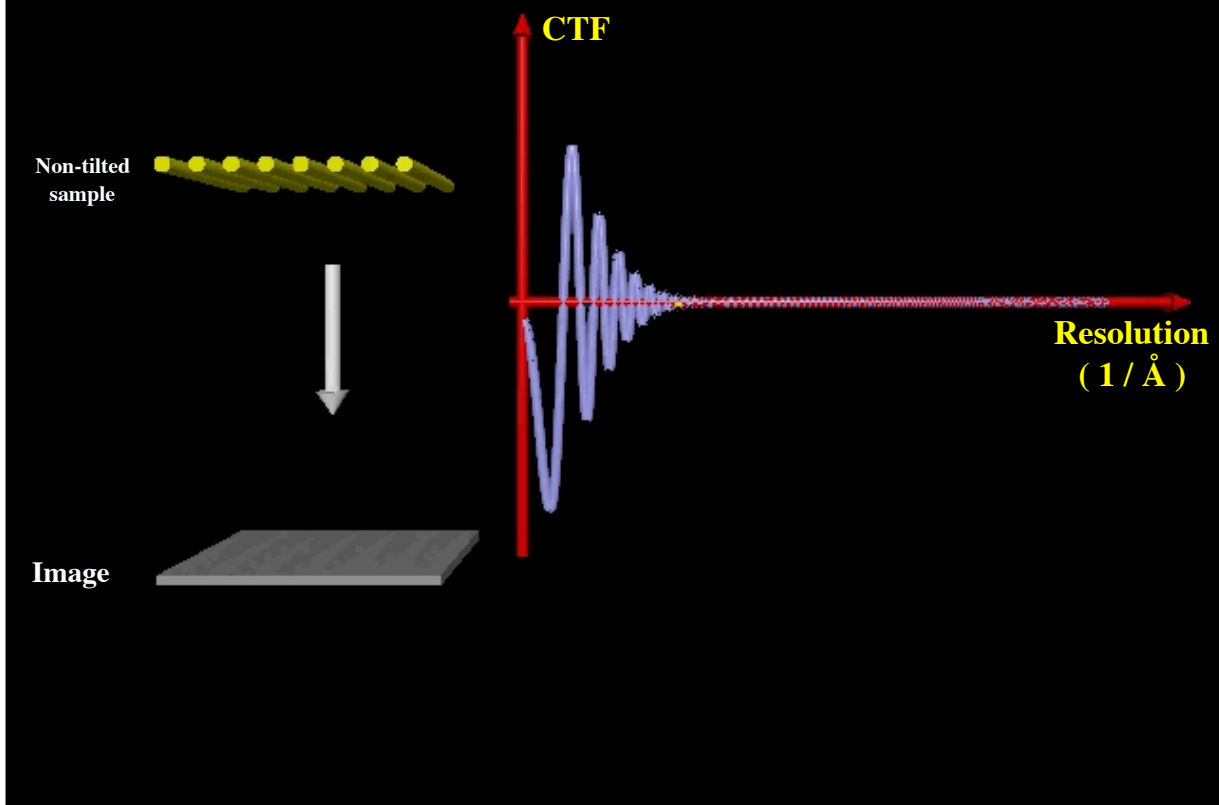
How does the CTF look like,  
if the sample is tilted?

This is called  
“Tilted Transfer Function”  
(TTF)

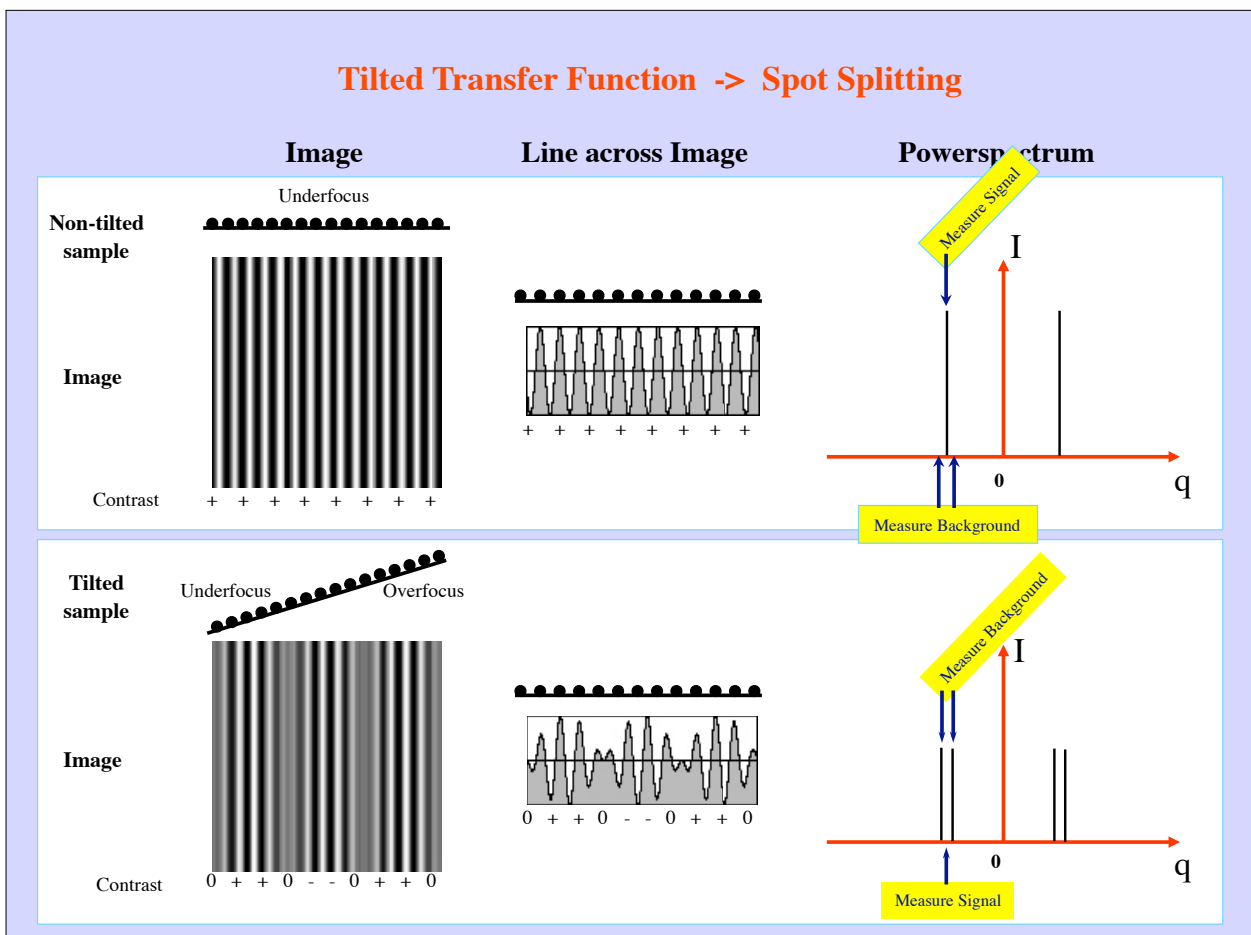
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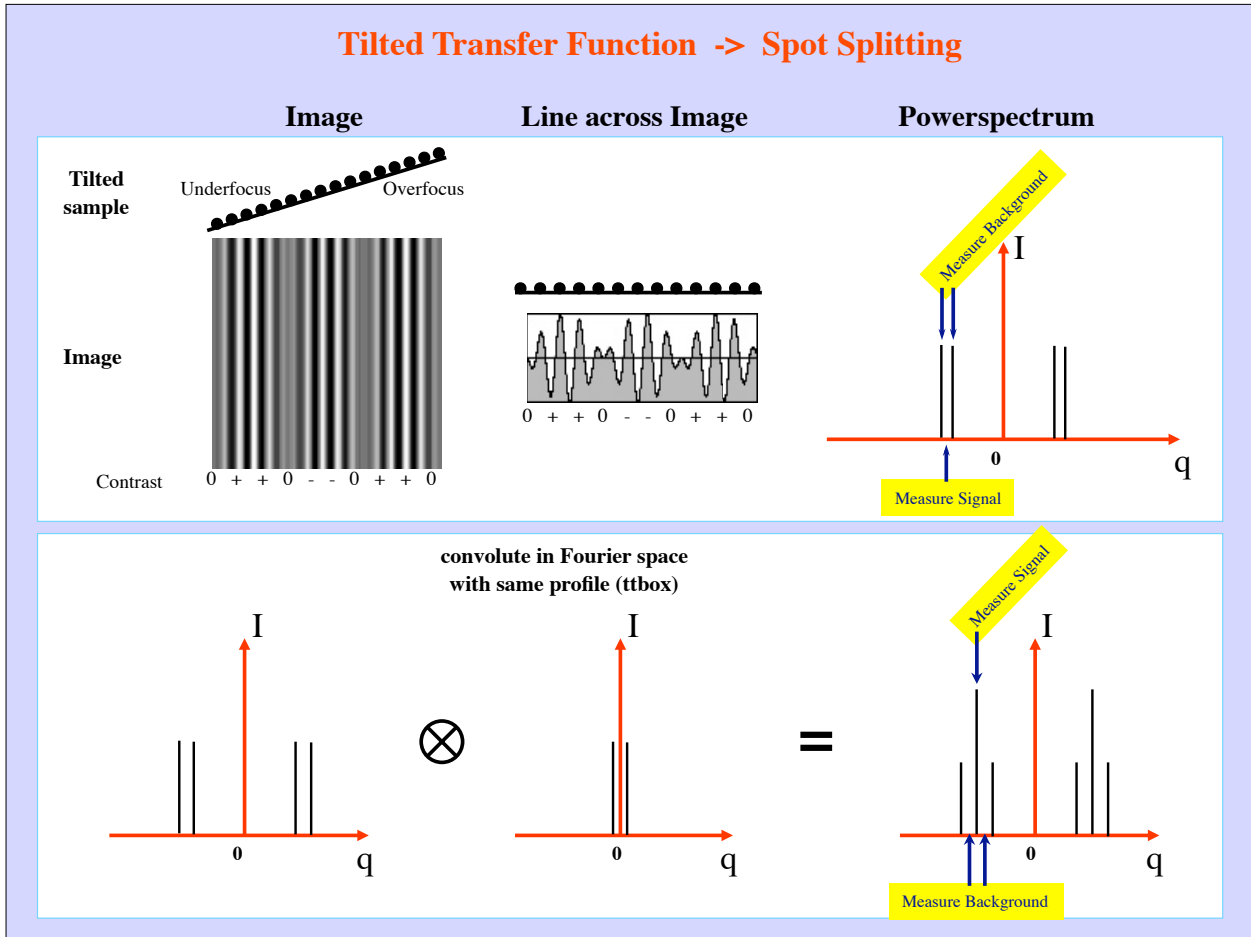
# Contrast Transfer Function



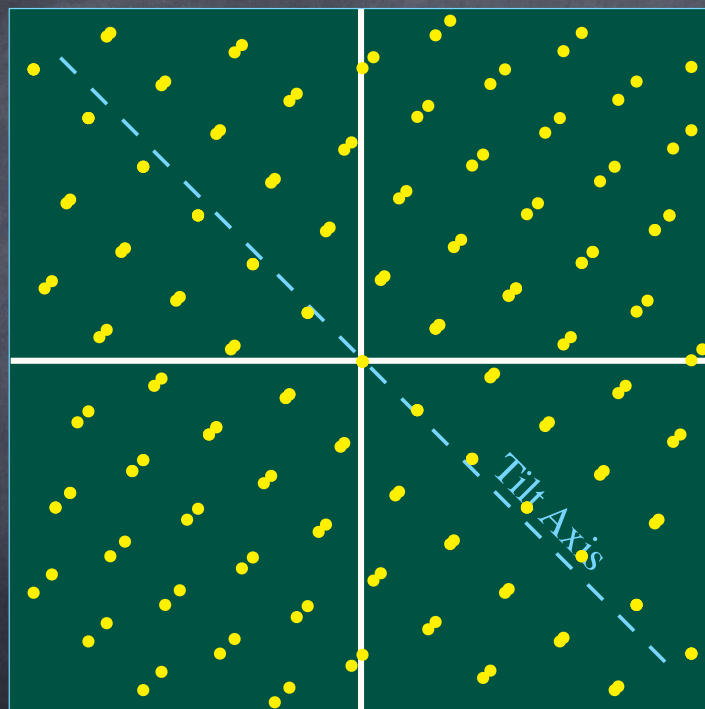
## Tilted Transfer Function -> Spot Splitting



## Tilted Transfer Function -> Spot Splitting



## Tilted Transfer Function -> Spot Splitting

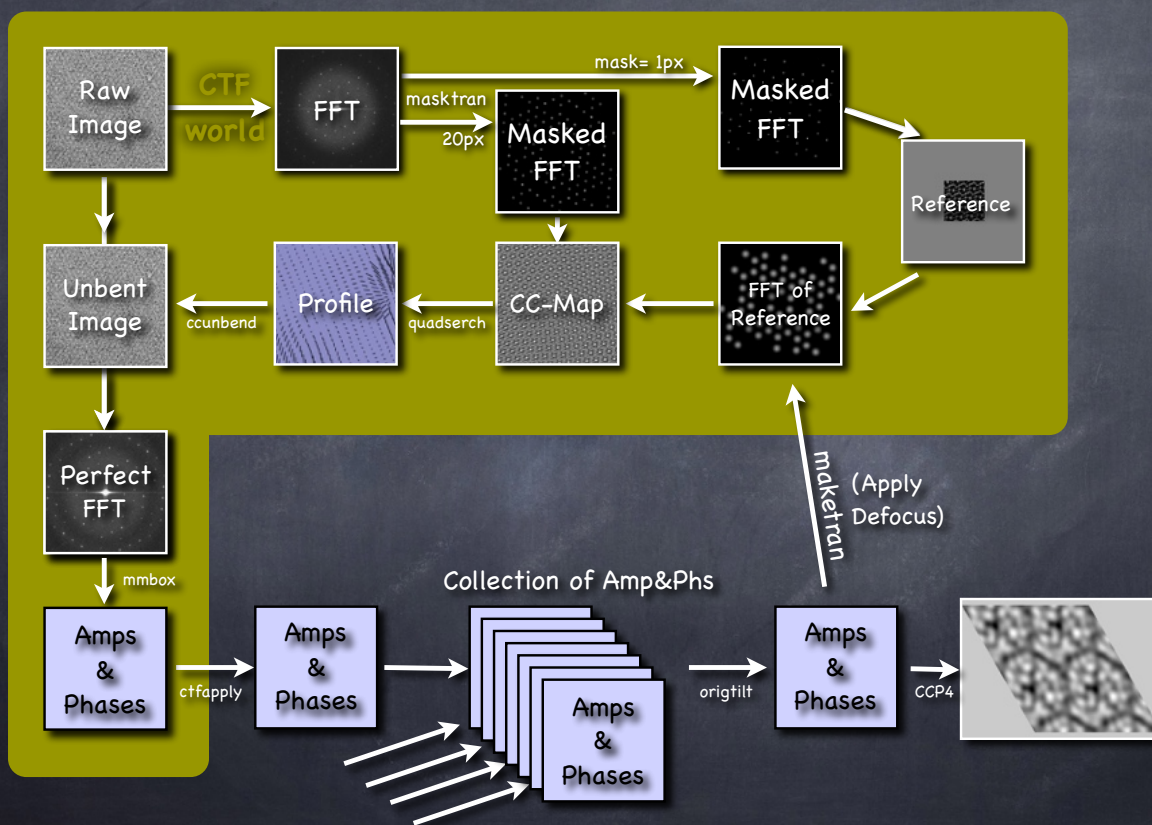


# Tilted Contrast Imaging Function (TCIF)

Philippsen et al., JSB 2006:  
 "The contrast imaging function of tilted specimen"

$$Q^{(2)}(\mathbf{p}) \approx 2\Phi[\mathbf{p}] - e^{-2iW_0(\mathbf{p})}\Phi[\mathbf{p} - d\mathbf{p}^2 \lambda \tan \alpha] + e^{2iW_0(\mathbf{p})}\Phi[\mathbf{p} + d\mathbf{p}^2 \lambda \tan \alpha].$$

## Algorithm Non-tilted



# Algorithm Tilted

