

Verification, Validation and Electron Microscope Tomography

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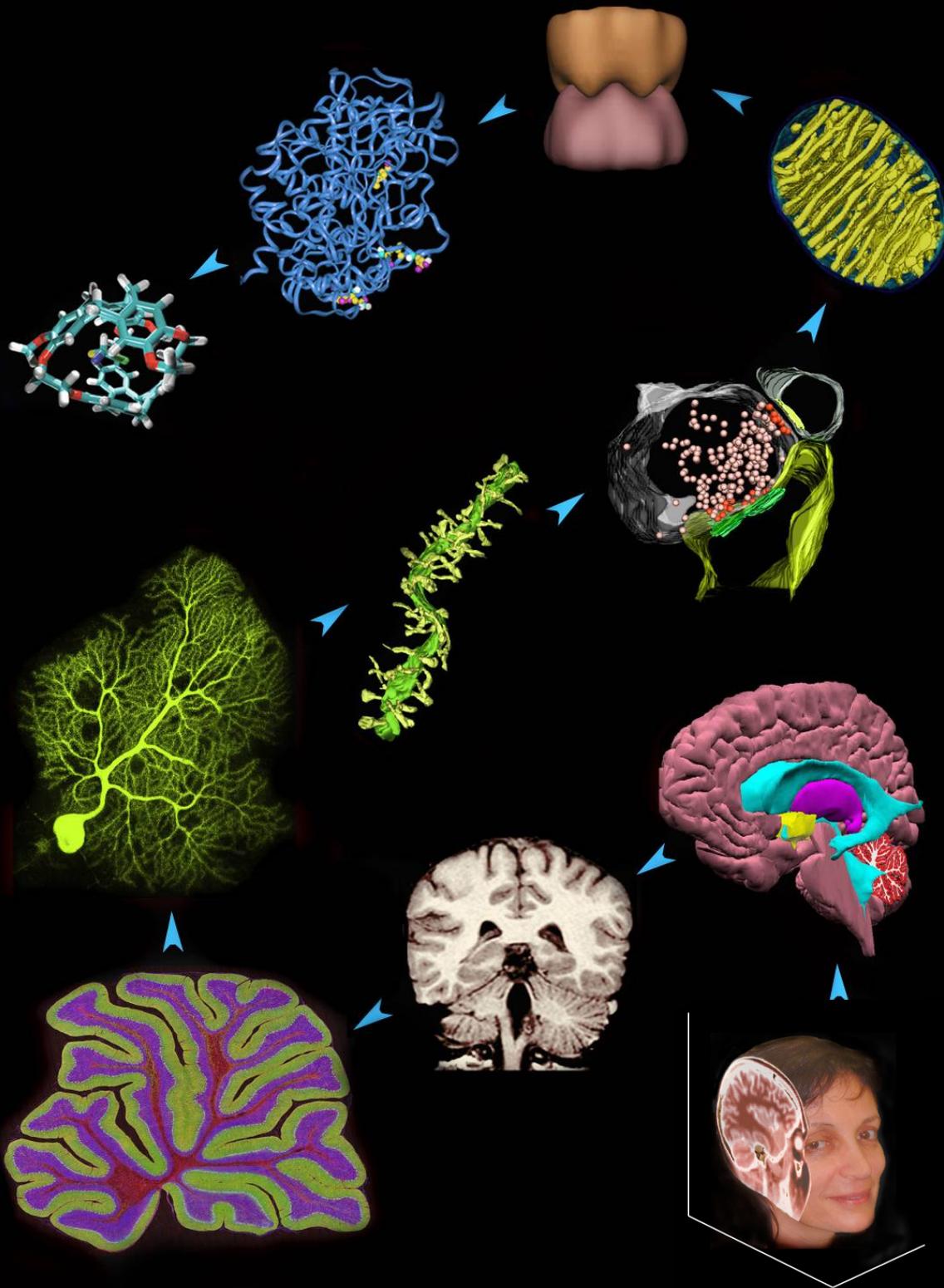
Purpose of Electron Microscope (EM) Tomography Development at CRBS

- Increase the range of spatial scales of 3D reconstructions from EM images
 - Connect with light microscopy at longer scales (10^{15} change in volume).
 - Improve resolution to level of molecular assemblies at shorter scales
- Integrate EM and fluorescence microscope data
 - Superresolution techniques localize emitting molecule to $\sim 1\text{nm}$
 - Fluorescence microscope techniques give functional and dynamical data, EM gives structural context
- Integration of fluorescence microscopy data and EM data will become an increasingly important (if not essential) technique for the validation of genetic and molecular biology data.
- Speed requirements are a constraint
 - Increase in data set size over past 10 years: 10^5 - 10^6
 - Improvement in processing times: 10^3 - 10^4 (algorithms + GPU)

The National Center for Microscopy and Imaging Research

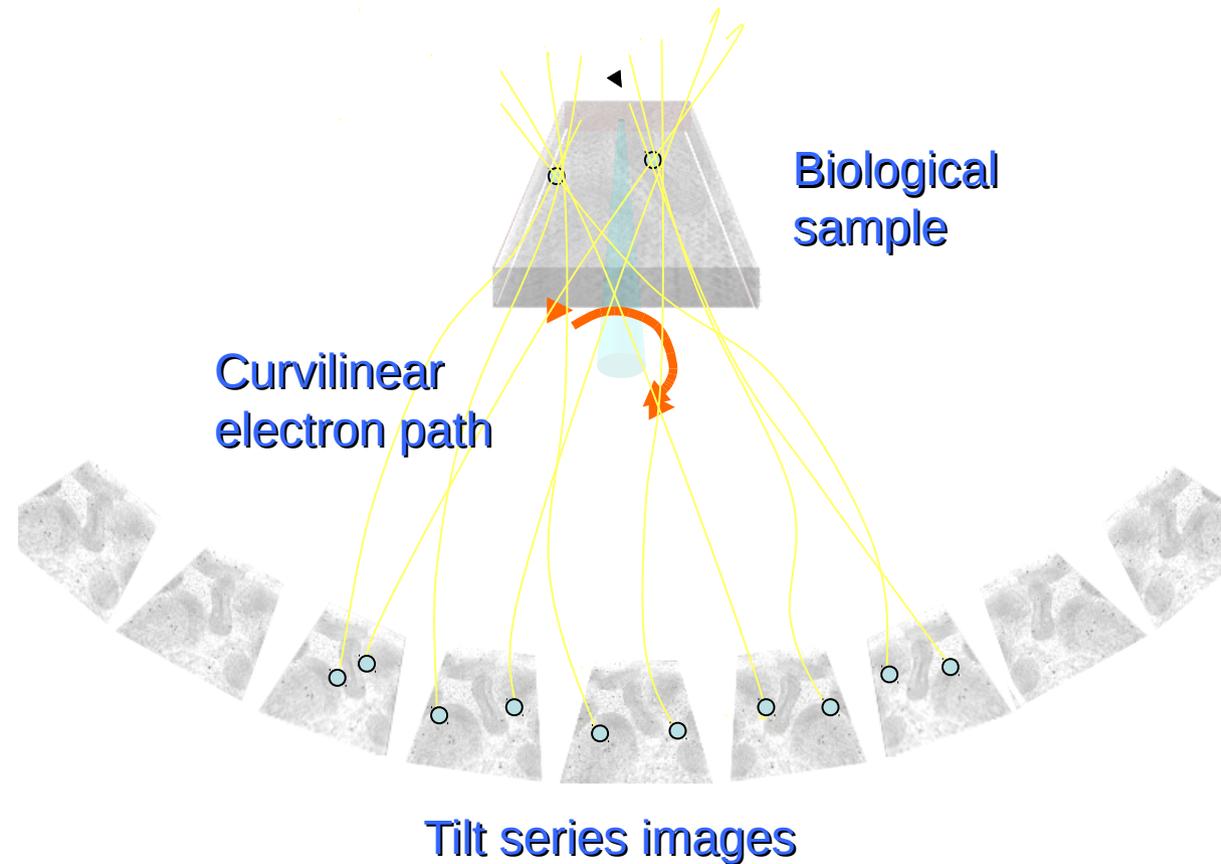
Mission:

- Develop and deploy technologies to determine and reveal supramolecular details in their cellular and tissue contexts.
- Focus on the 'Meso-scale' - ~0.5nm to > 100um

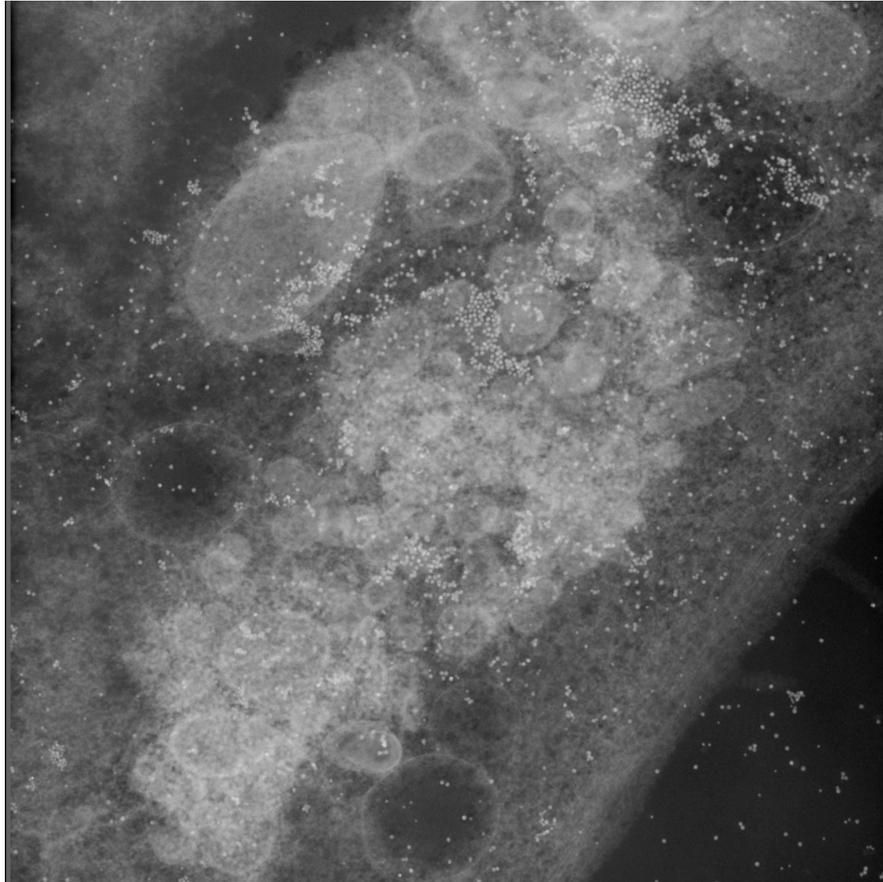


Electron Microscopic Tomography at a Glance

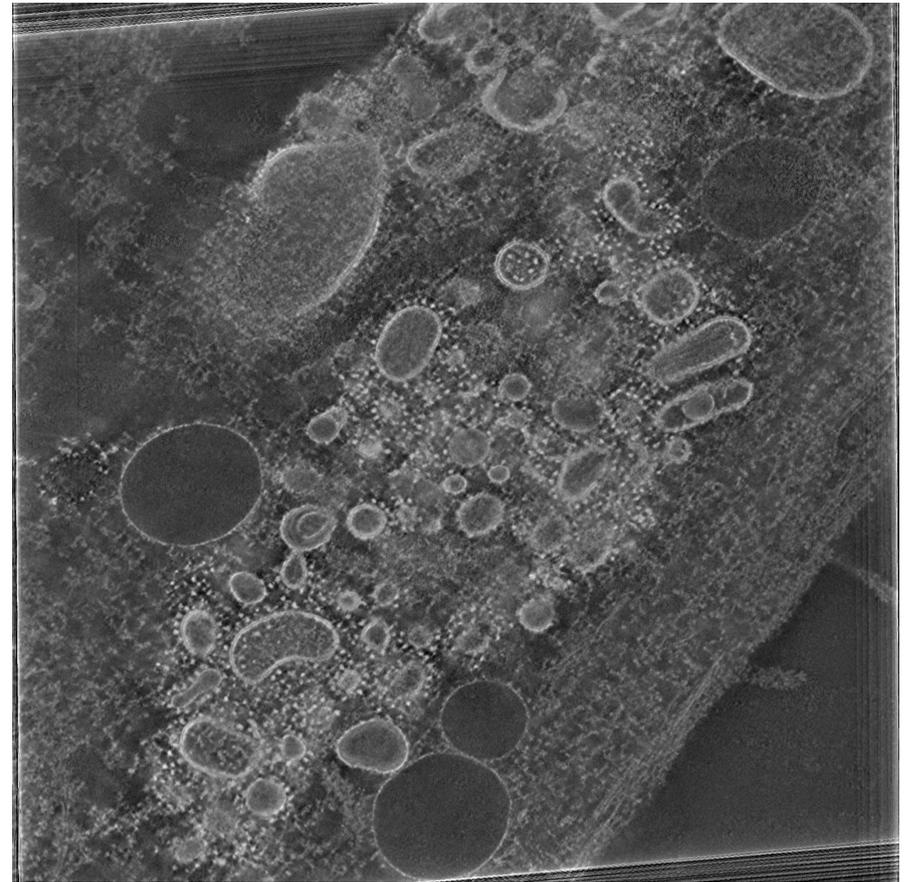
- Used for constructing 3D views of sectioned biological samples
- Sample is rotated around an axis and images are acquired for each 'tilt' angle
- Electron tomography enables high resolution views of cellular and neuronal structures.
- 3D reconstruction is a complex problem due to low signal-to-noise ratio, curvilinear electron path, sample deformation, scattering, magnetic lens aberrations...



High quality 3D reconstruction under these conditions is a difficult mathematical and computational problem...



Electron Microscope Image

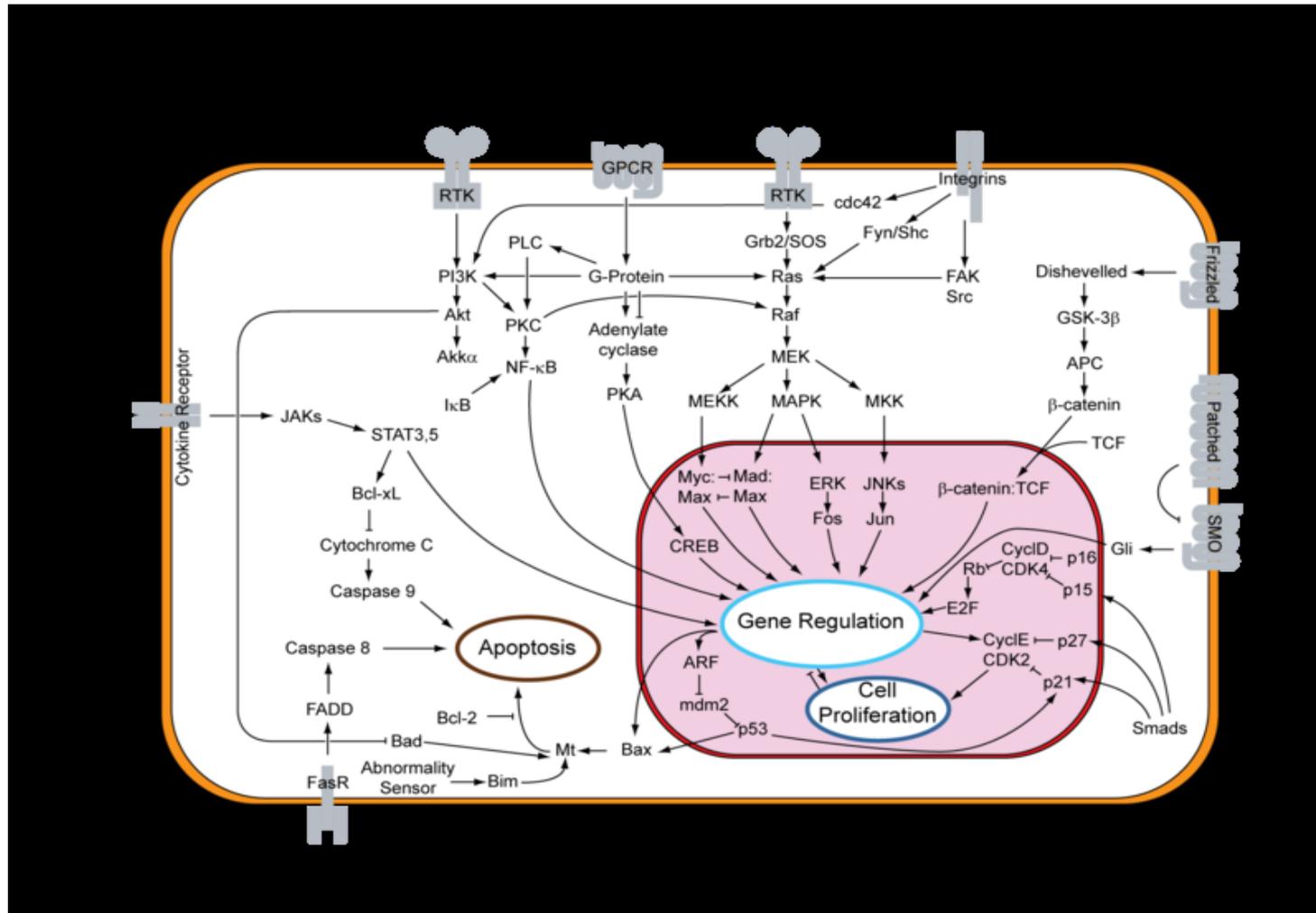


Section of Reconstruction

A Look at the Research Context

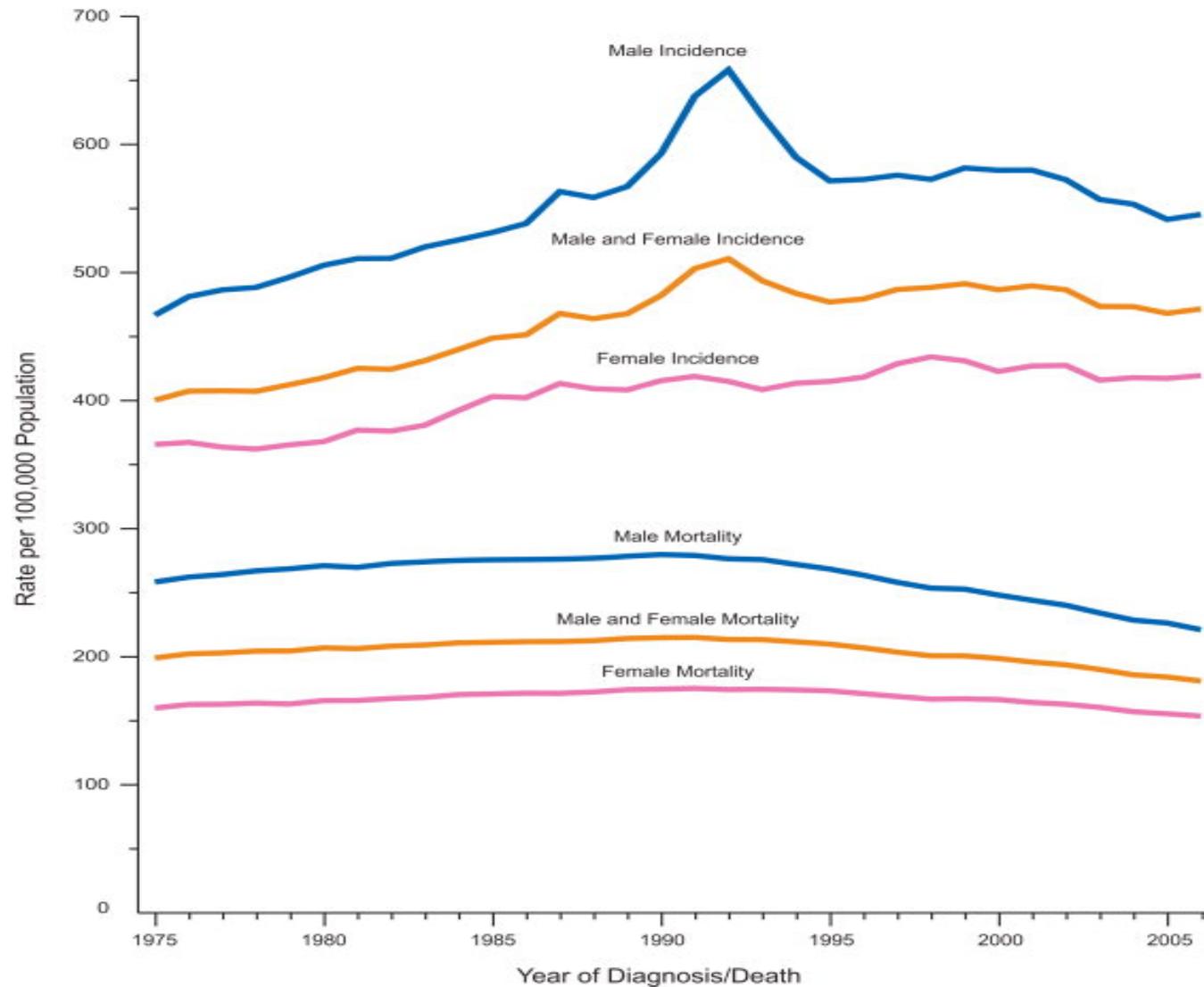
- Applications to
 - Engineered organisms
 - Defense (bio-terrorism)
 - Medicine
- Cancer

Cell death (Apoptosis) is an important component in the maintenance of the integrity of the organism

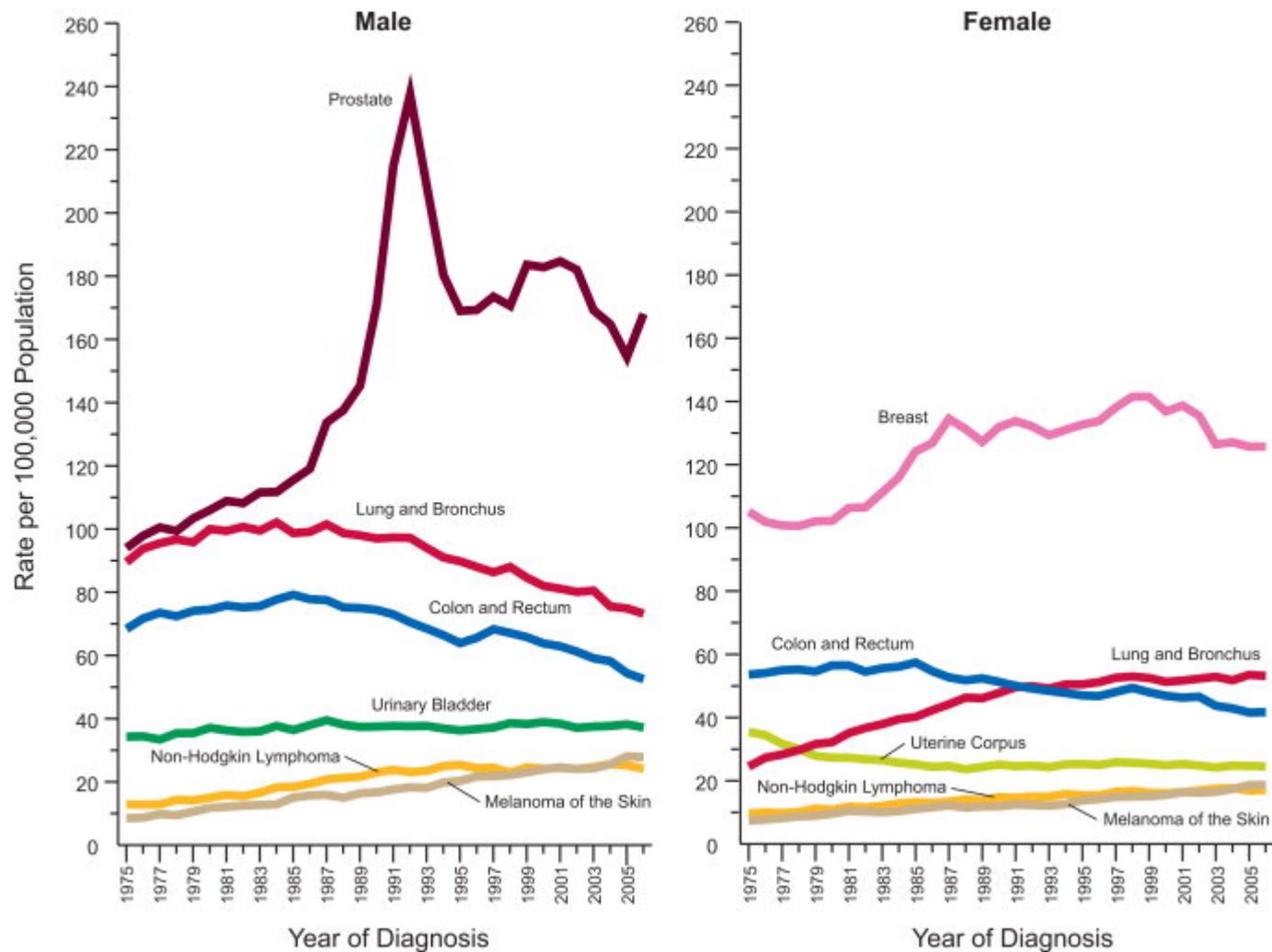


This fails in many cancers. Current non-surgical therapy techniques induce cell death in a highly non-selective way. More selective means of drug delivery are desirable.

Validation of Current Cancer Therapies?



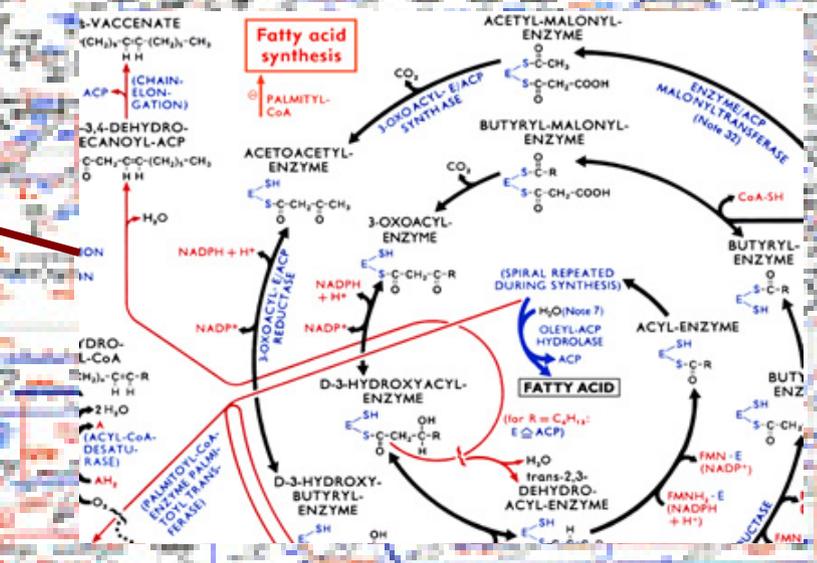
In Case You Were Curious About the Spike



Problem of Drug Delivery

- More selective drug delivery requires better knowledge of metabolic pathways and siting of critical reactions within the cell.
- Picture of cell ultrastructure may be incomplete. Current research tends to concentrate on specific organelles.
- Degree of organization of the cell nucleus and nucleolus is a subject of controversy.
- New techniques in microscopy can resolve these issues.
- Work on P53 gene may be a critical element of the research

As the network becomes larger intuition becomes more fuzzy...
And problems in validation become more complex.



As the size of the network increases, statistical modeling and inference can quickly become a problem requiring the introduction of new mathematical techniques.

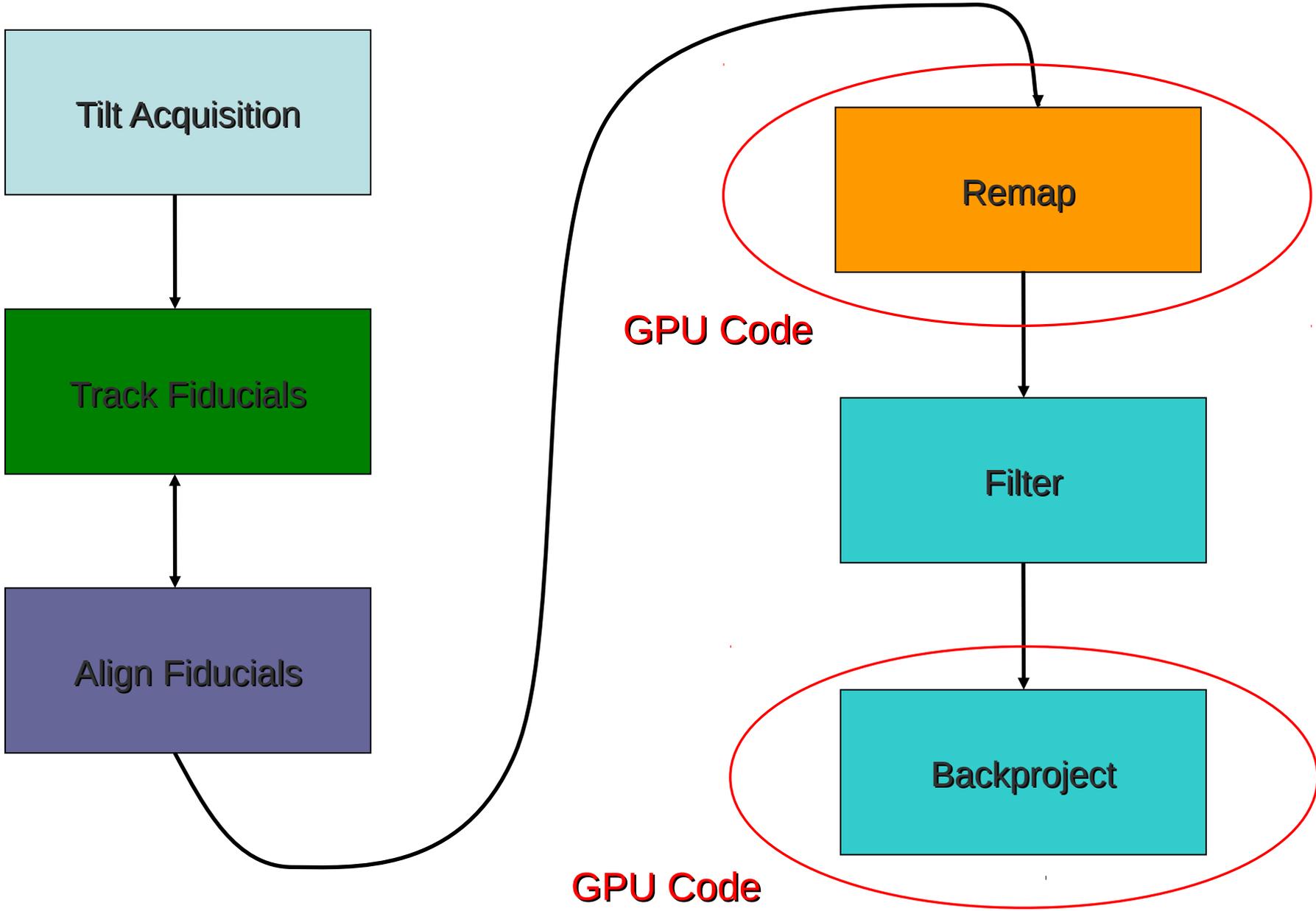
Inference can be computationally nontrivial for two reasons. In order to compute the partition function, the number of terms in the sum is equal to m which can be very large since many applications of graphical models require that the models have large numbers of random variables. One may easily encounter $n = 200$ binary random variables, in which case

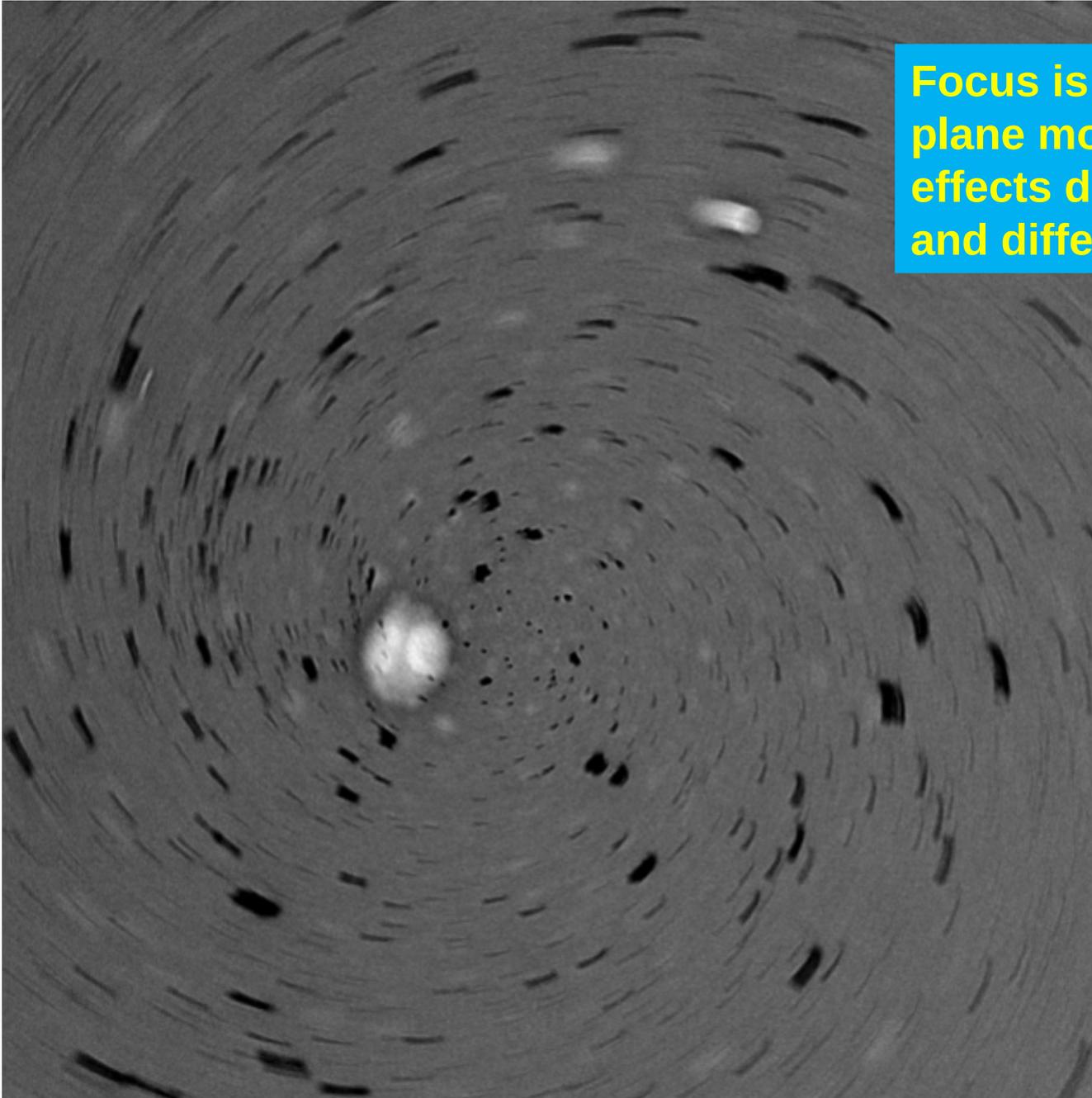
$m =$

1606938044258990275541962092341162602522202993782792835301376.

The success of graphical models has been due to the possibility of efficient inference for many models of interest. The organizing principle is the generalized distributive law which gives a recursive decomposition according to the graph underlying the model.

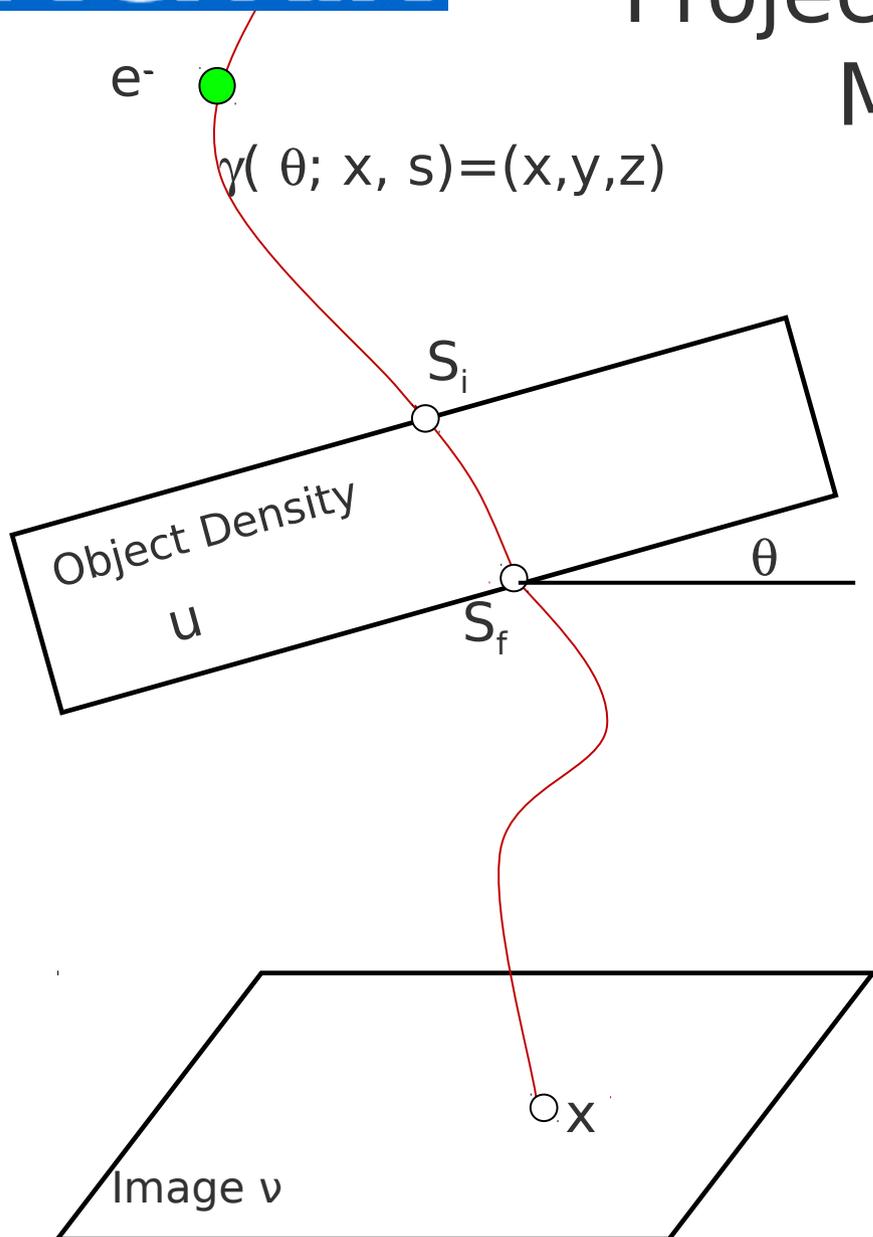
- Tomography, in practice, requires many steps
 - Sample preparation
 - Data collection
 - Feature isolation and tracking
 - Image alignment
 - Image filtering
 - Volume reconstruction
 - Object segmentation
- Each step carries it's own set of problems
- Choice of methods on one step affects subsequent steps
- **Lab Code:** Transform Based Tracking, Bundle Adjustment and Reconstruction
- In addition TxBR provides user choice of model, automated reconstruction, and statistical error reporting





Focus is changed in steps so focal plane moves through object. Note effects due to Helical trajectories and differential magnification.

Projection in Electron Microscope



- γ = electron path
- S_i = point of entrance
- S_f = point of exit
- θ = tilt angle
- $x = (x, y)_{\text{image}}$
- $X = (x, y, z)$
- u = object density
- v = image intensity

Linear “single scattering model”

$$\hat{v}(\theta; x) = I_0 \cdot e^{-\int_{S_i}^{S_f} u(\gamma(\theta; x, s)) ds}$$

$$v = \ln(\hat{v}(\theta; x)) = -\int_{S_i}^{S_f} u(\gamma(\theta; x, s)) ds$$

Mathematical Model

$$\Gamma = \{\gamma_{(\theta;x,y)}\}$$

Family of trajectories
Indexed by image point
and sample angle.

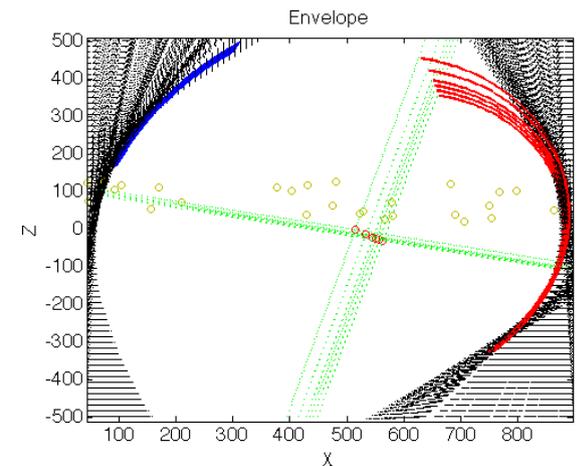
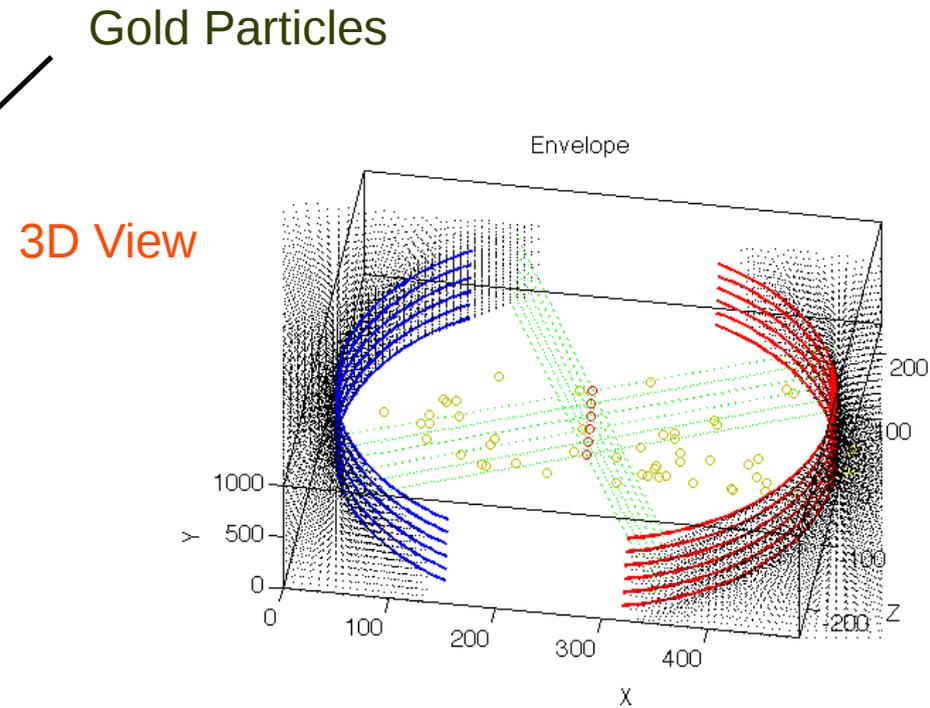
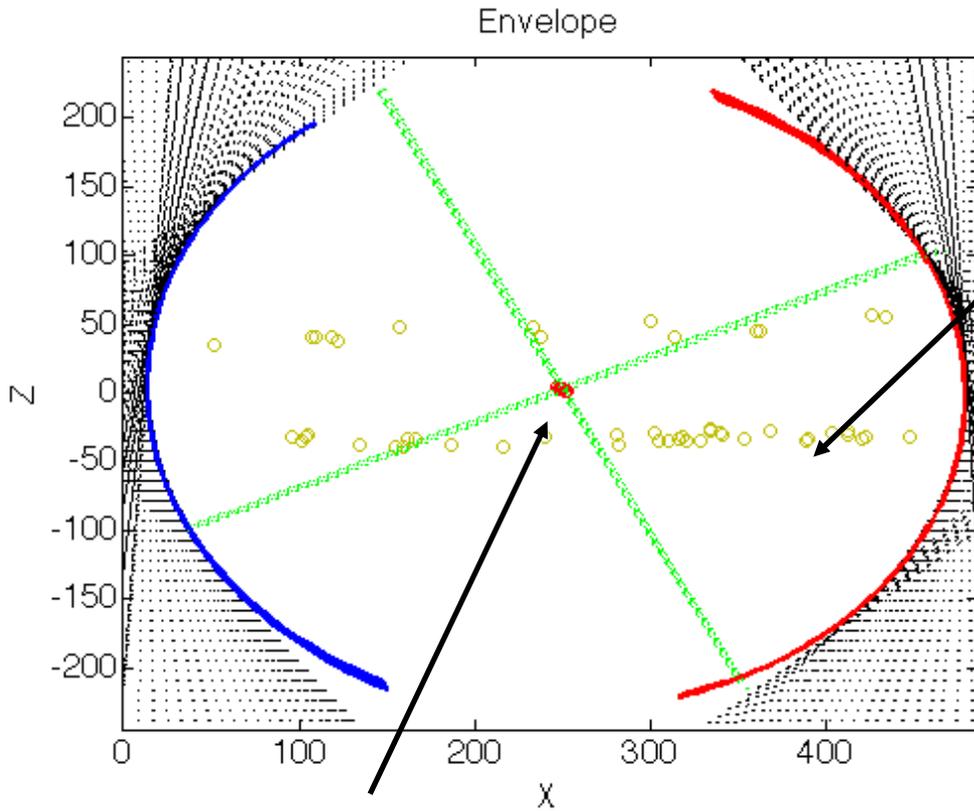
$$R_{\Gamma}(u)(\theta; x, y) = \int_{s=s_i}^{s=s_f} u(\gamma_{(\theta;x,y)}(s)) ds$$

Transform defined by
integration of density
along trajectories.

$$R_{\Gamma}^*(v)(x, y, z) = \int_{\gamma_{(\theta;x,y)}(s)=(x,y,z)} R_{\Gamma}(u)(\theta; x, y) d\theta$$

Adjoint transform
defined by integration
over sample orientations

Geometrical Characterization of the stage motion

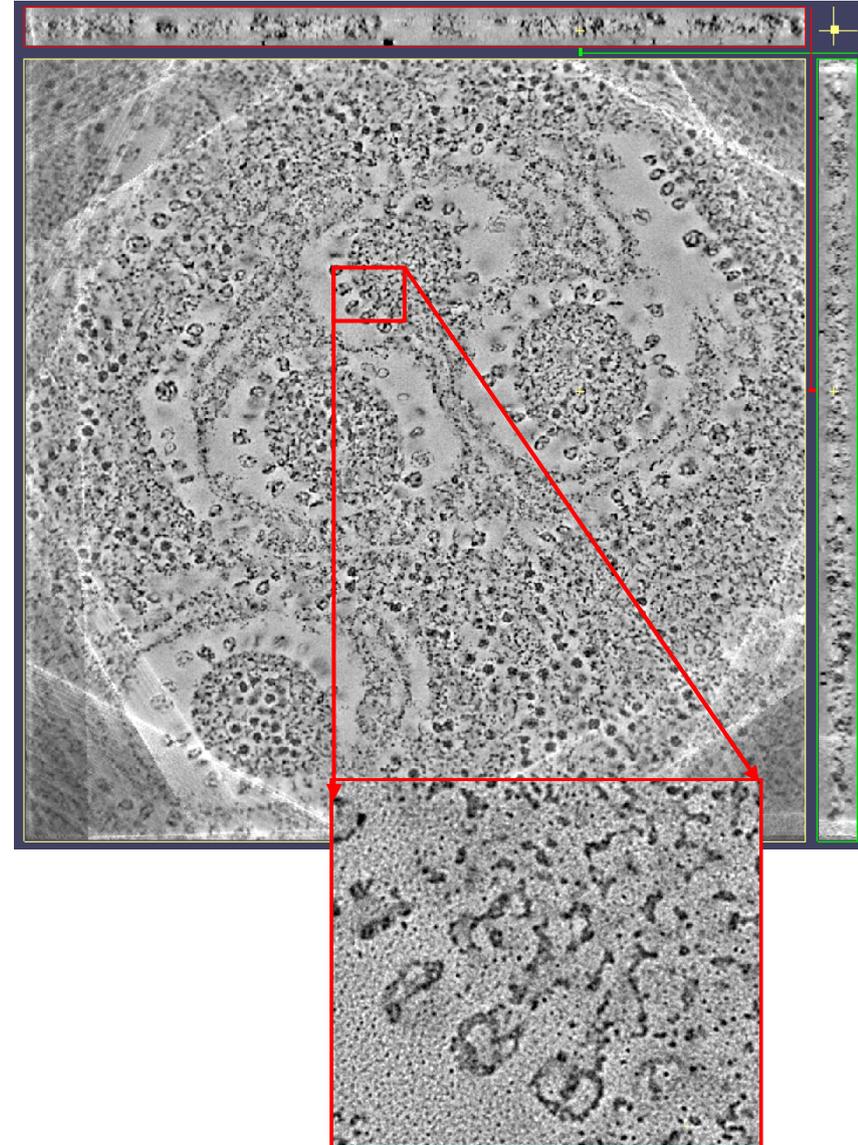


Verification of tracking and alignment

Calculation of tilt axis allows correction of filter

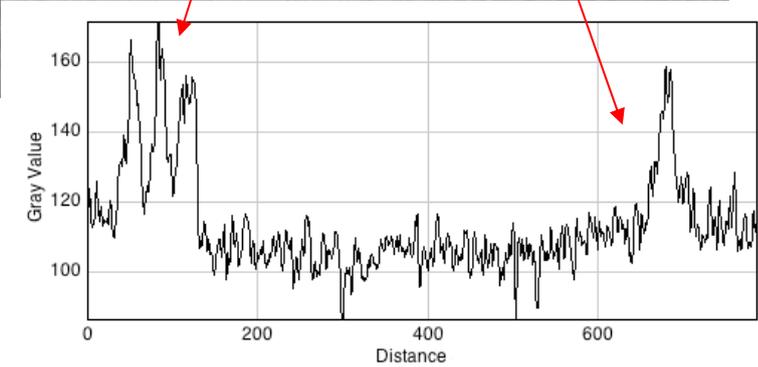
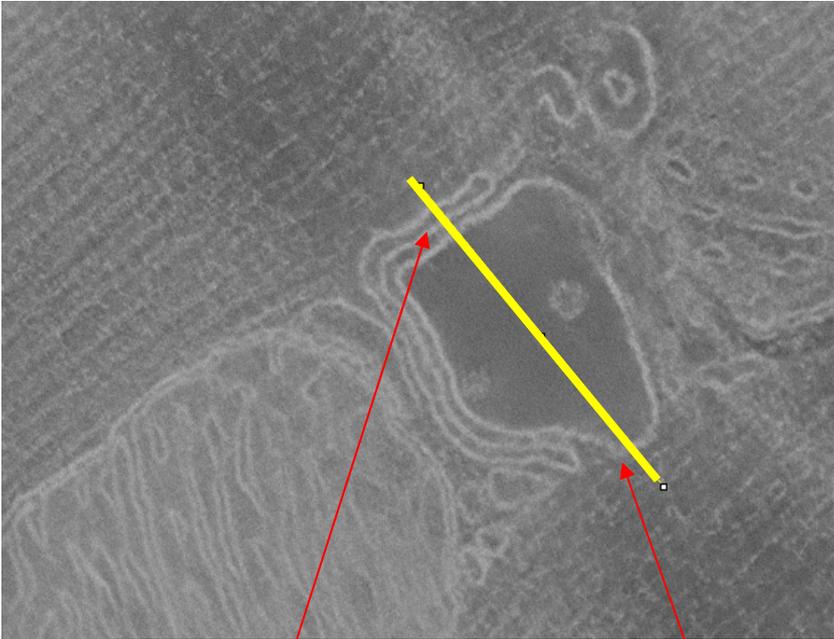
Automated estimation of object dimensions

- Use a Bundle Adjustment procedure to correct for curvilinear electron path and sample deformation
- Evaluation of electron micrographs correspondences needs to be done with double precision when using high-order polynomial mappings
- Non-linear electron projection makes reconstruction computationally intensive.
- TxBR works with wide field of view, large datasets
 - New cameras are now 24k x 24k

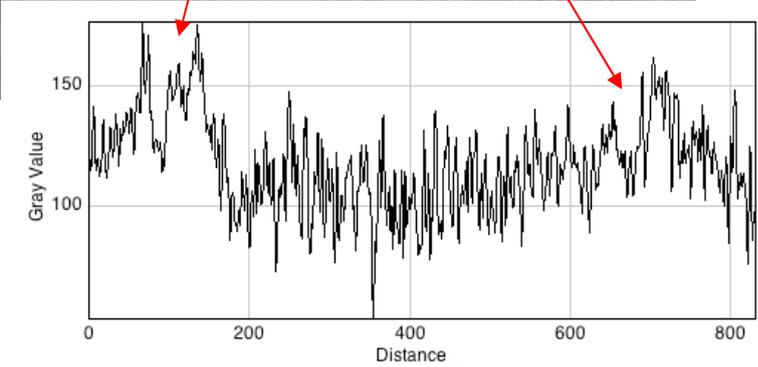
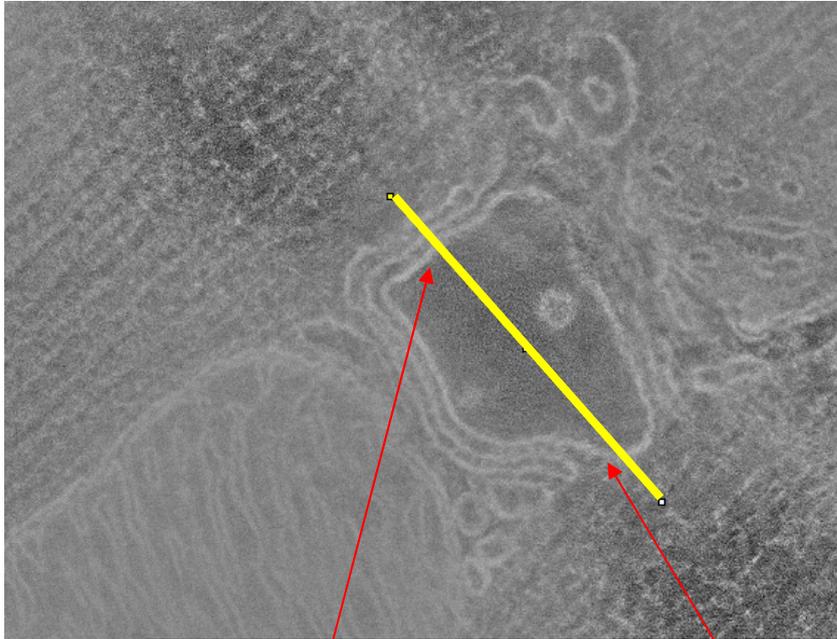


Quality Comparison: EM Tomography

TxBR



IMOD

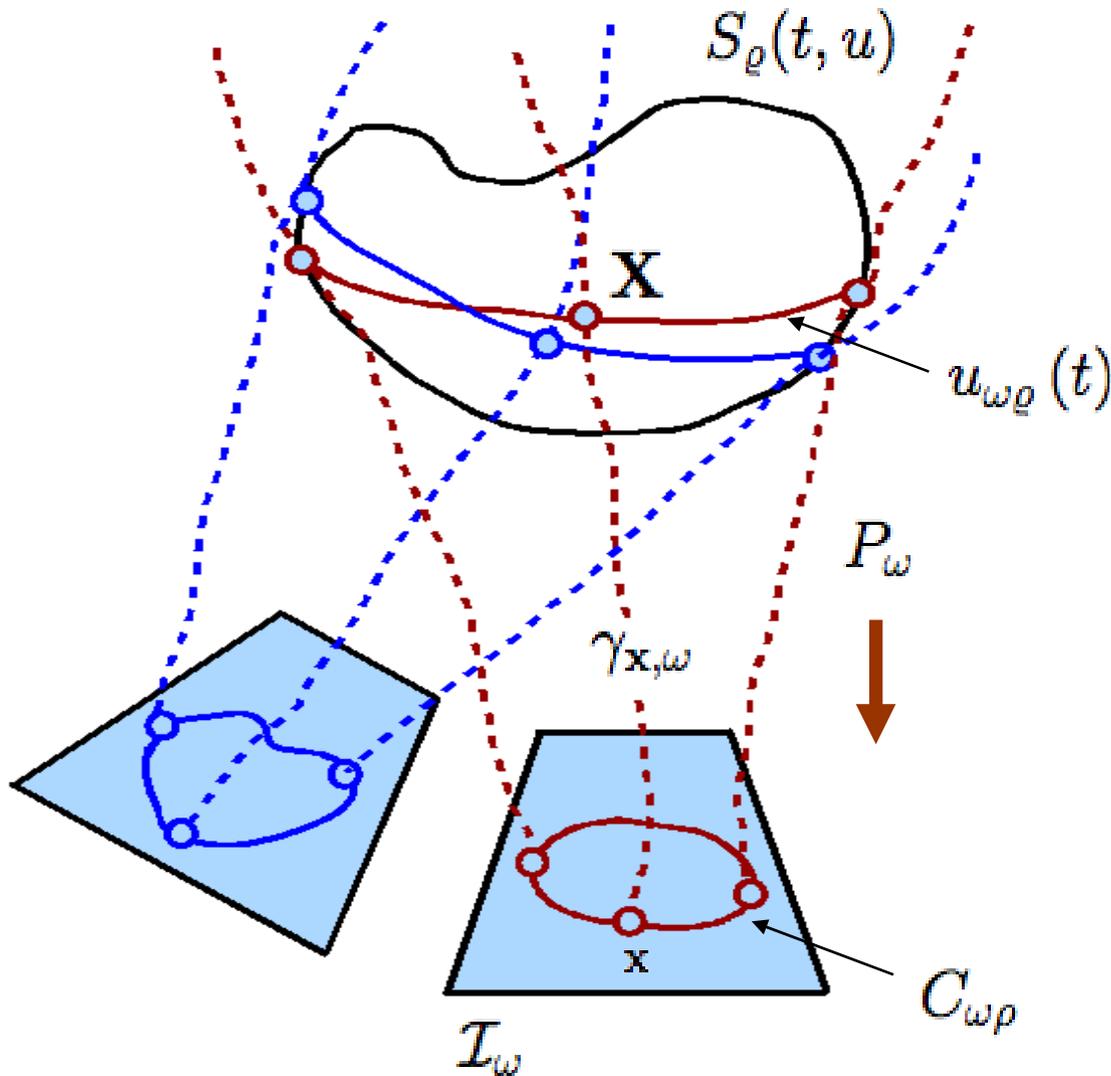


Cardiac tissue reconstruction sections

Validation and Verification of TxBR

- Comparison of multiple projection models: orthogonal, affine, projective, and polynomial
- Extensive checking of code evaluations of projection maps via MatLab
- Publication of 3D reconstructions and detailed reprojection error statistics for 5 datasets in the Journal of Structural Biology
- Mathematical discussion of failure modes of particular models
- Lawrence, et al., JSB 154:144-167

Projection Along Rays



- Surface S_ρ of a 3D object ρ is parameterized with (t, u) .
- We restrict S_ρ to be small patches. Use of polynomial expressions for $S_\rho(t, u)$.
- Curvilinear rays tangent to surface. Use of a polynomial expression for the projection map P_ω . Index ω represents a sample orientation.
- Contour in surface where $u_{\omega\rho}(t)$ projects to contour $C_{\omega\rho}$ in image \mathcal{I}_ω .

Contour Alignment Model

- Contour Tracks:

$$C_{\omega\rho} = (x_{\omega\rho 1}(t), x_{\omega\rho 2}(t)) \quad x_{\omega\rho i}(t) = \sum_{k=0, \dots, N_1} c_{\omega\rho ik} t^k$$

- Projection Map:

$$P_{\omega} (X_1, X_2, X_3) = (P_{\omega 1} (X_1, X_2, X_3), P_{\omega 2} (X_1, X_2, X_3))$$

$$P_{\omega i} (X_1, X_2, X_3) = \sum_{N_2 \geq j, k, l \geq 0} b_{\omega i j k l} X_1^j X_2^k X_3^l$$

- Structure Patches:

$$S_{\rho}(t, u) = (S_{\rho 1}(t, u), S_{\rho 2}(t, u), S_{\rho 3}(t, u))$$

$$S_{\rho i}(t, u) = \sum_{N_3 \geq k, l \geq 0} a_{\rho i k l} t^k u^l$$

- Surface Contours:

$$u_{\omega\rho}(t) \cong \sum_{k=0, \dots, N_4} d_{\omega\rho k} t^k$$

A Generalized Bundle Adjustment

Two Error Terms to minimize:

- A Projection Error: $P_{\omega} S_{\rho}(t, u_{\omega i}(t)) = C_{\omega \rho}^{(r)}(t)$

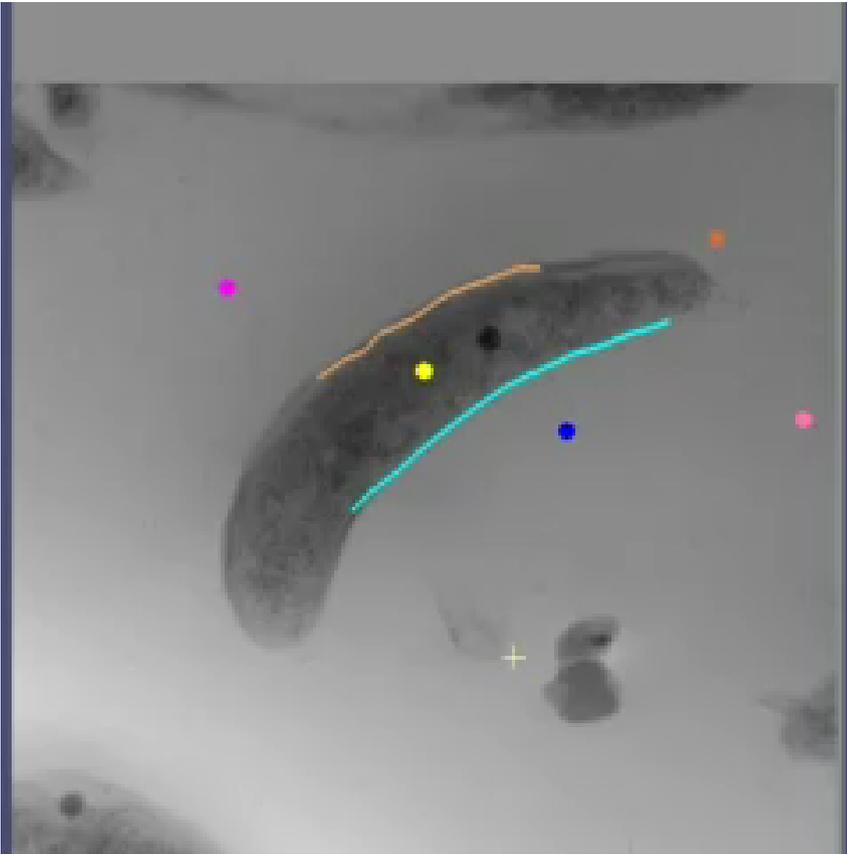
$$E_{\omega \rho}^P = \int_{t_0}^{t_1} \|P_{\omega} S_{\rho}(t, u_{\omega \rho}(t)) - C_{\omega \rho}(t)\|^2 dt$$

- A Tangency Error:

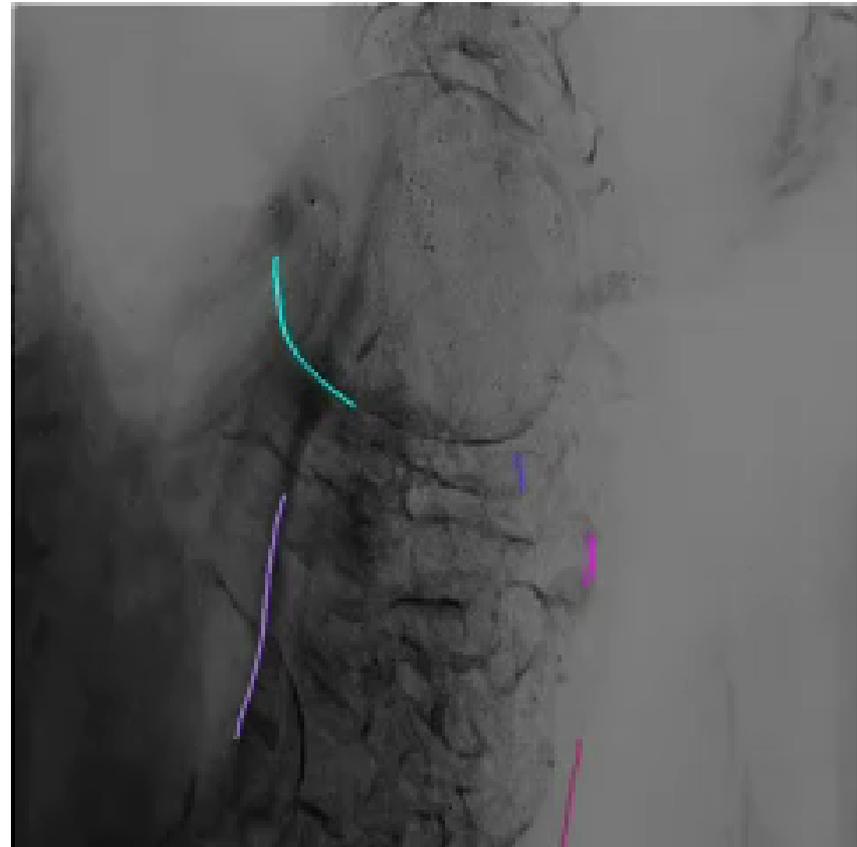
$$P_{\omega i}(\gamma_{\mathbf{x}, \omega}^1(t), \gamma_{\mathbf{x}, \omega}^2(t), \gamma_{\mathbf{x}, \omega}^3(t)) = x_i \quad \nabla P_{\omega i} \cdot \dot{\gamma}_{\mathbf{x}, \omega} = 0$$

$$E_{\omega \rho}^T = \int_{t_0}^{t_1} \left\| \nabla P_{\omega 1} \times \nabla P_{\omega 2} \cdot \left(\frac{\partial S_{\rho}}{\partial t} \times \frac{\partial S_{\rho}}{\partial u} \right) \right\|^2 dt$$

Structure Segmentation



Caulobacter Crescentus



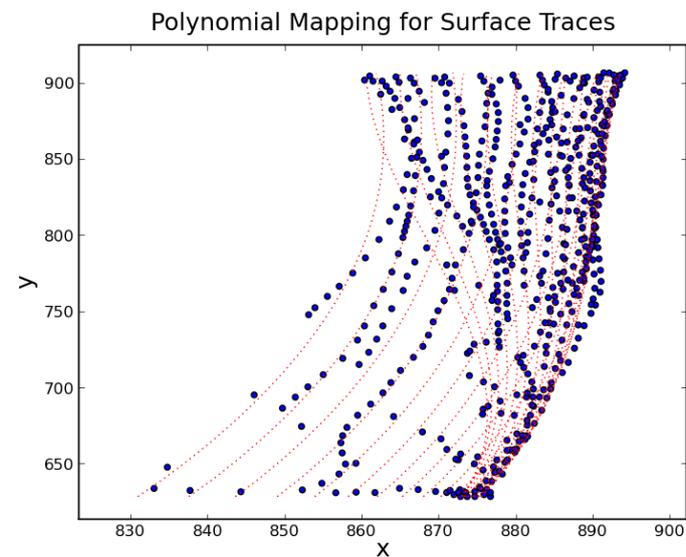
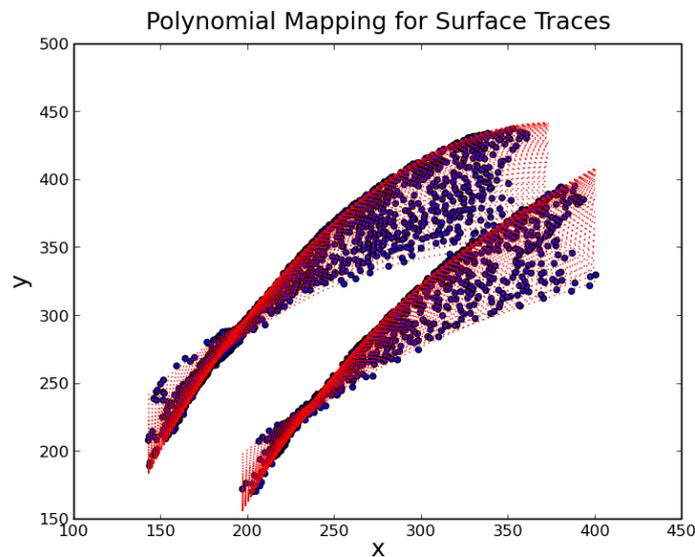
Glia

Parameterization of the contours

What choice? For a tilt series:

- t parameterizes the projection of a surface point onto the camera plane.
- u parameterizes the tilt index

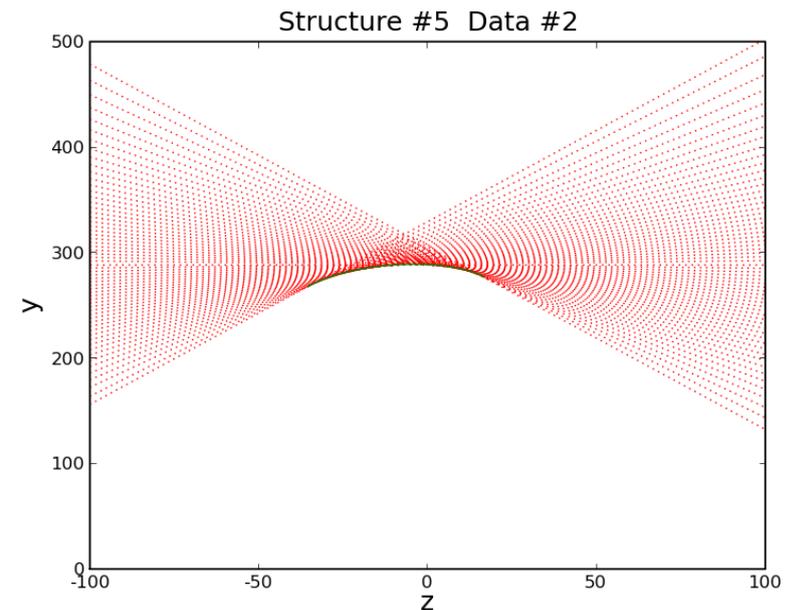
Simultaneous parameterization of tracks allows to assess optimal patch order to describe an object.



⇒ Minimization is then implemented with independent parameterization for each contours.

Initialization of the problem

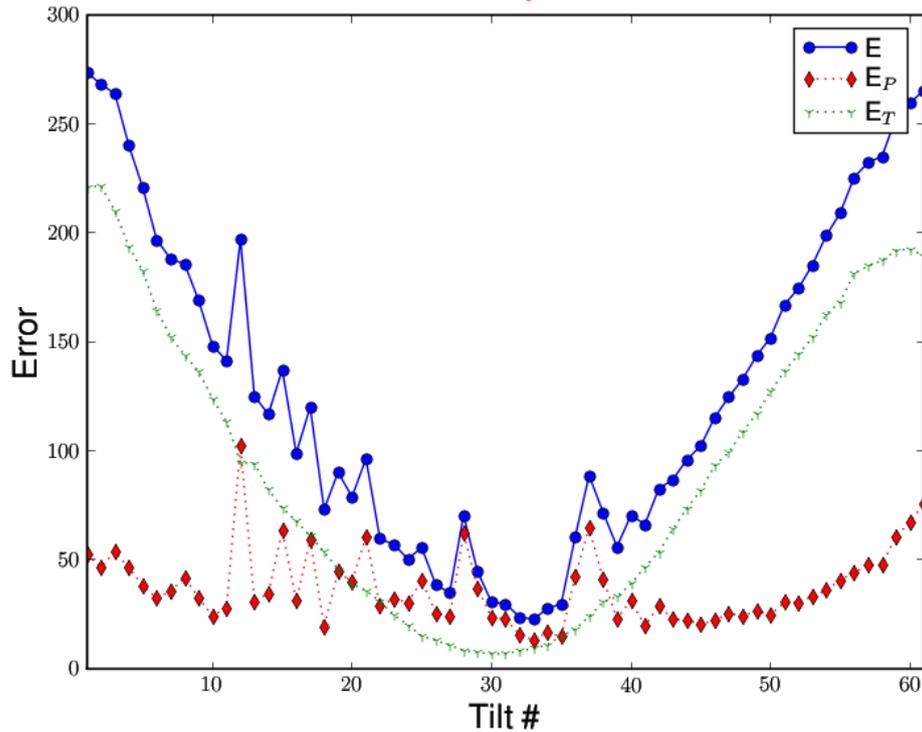
- Coefficients $a_{gikl}, b_{wijkl}, c_{wgik}, d_{wgk}$ need to be adequately evaluated at initialization to avoid sticking to a bad local minima during optimization.
- Projection Map b_{wijkl} is evaluated from experimental evaluation of sample orientation.
- c_{wgik} is evaluated from tracking contours on images
- Dual space method to evaluate a_{gikl} .
Dimension of dual space: 3d or 4d.
- Initialization of d_{wgk} tied to choice for Parameterization.



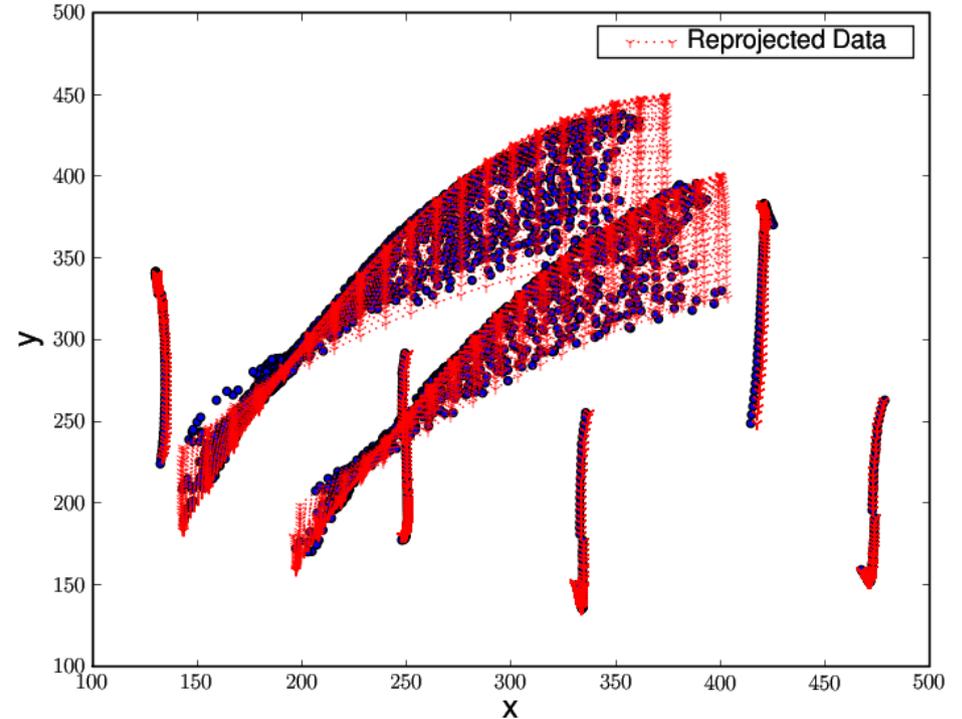
Re-projection Error at Minimum

Bundle adjustment applied on Caulobacter Crescentus dataset (500ptsx500pts) with parabolic patches

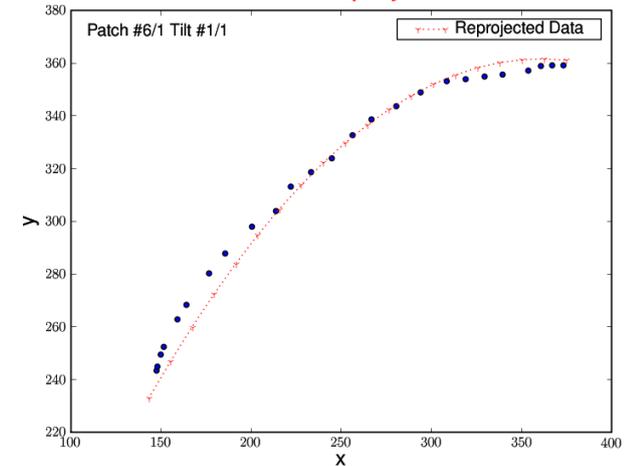
Error by Tilt



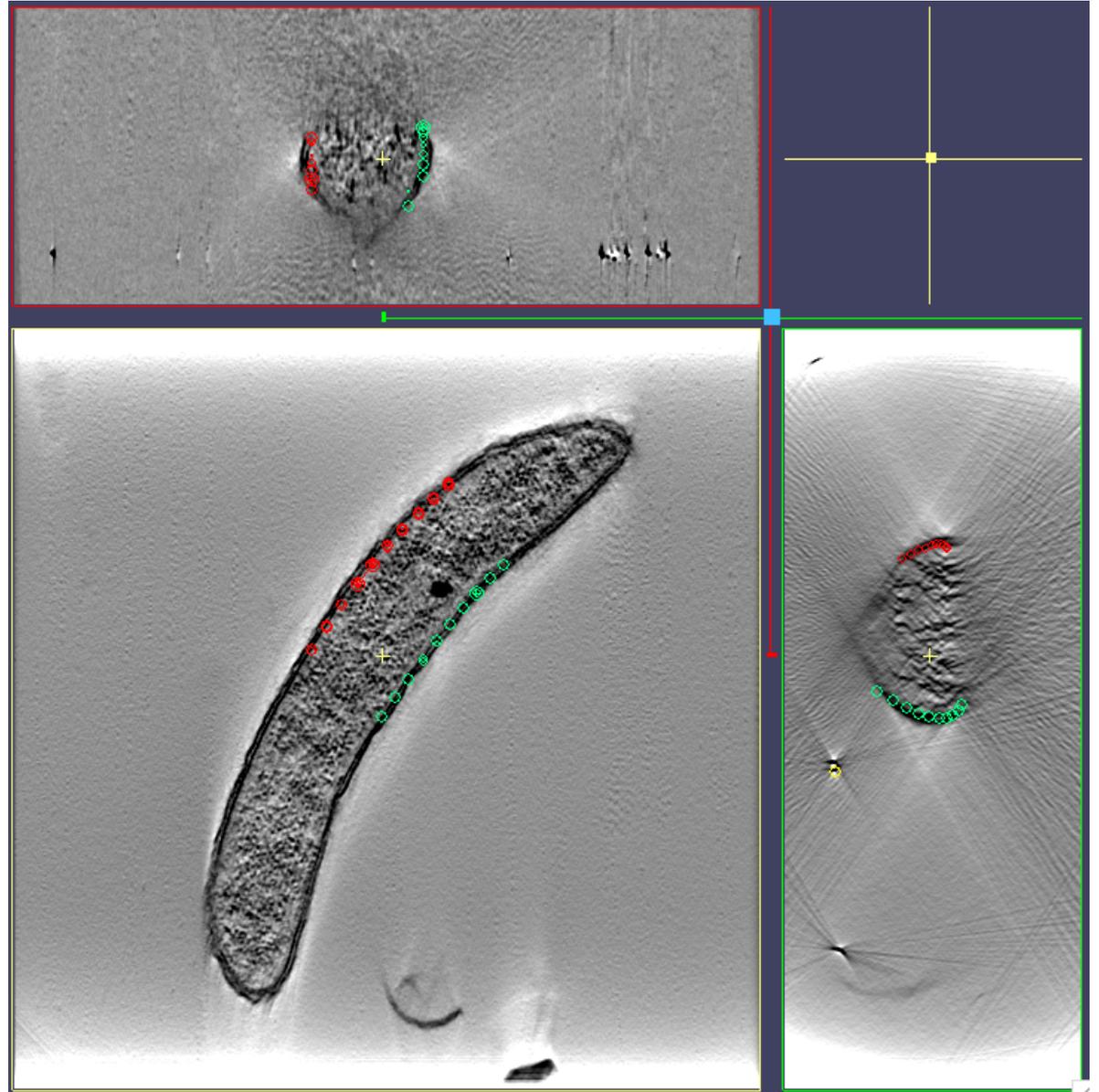
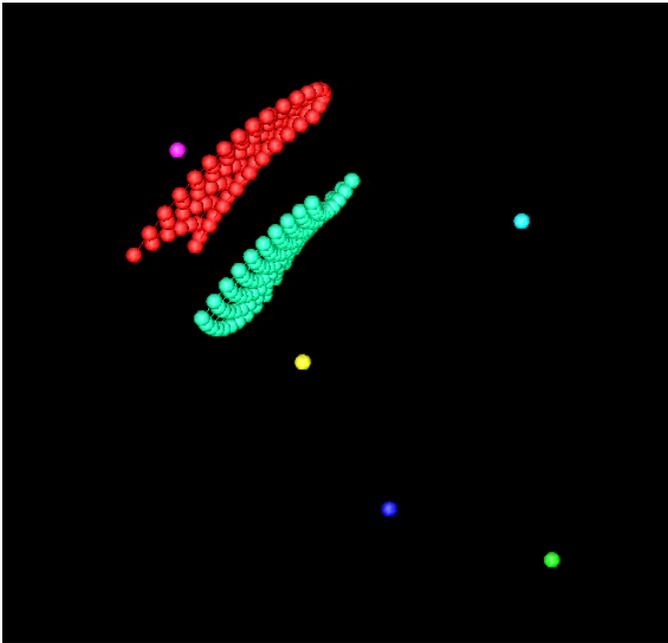
Contour Reprojection



Contour Reprojection

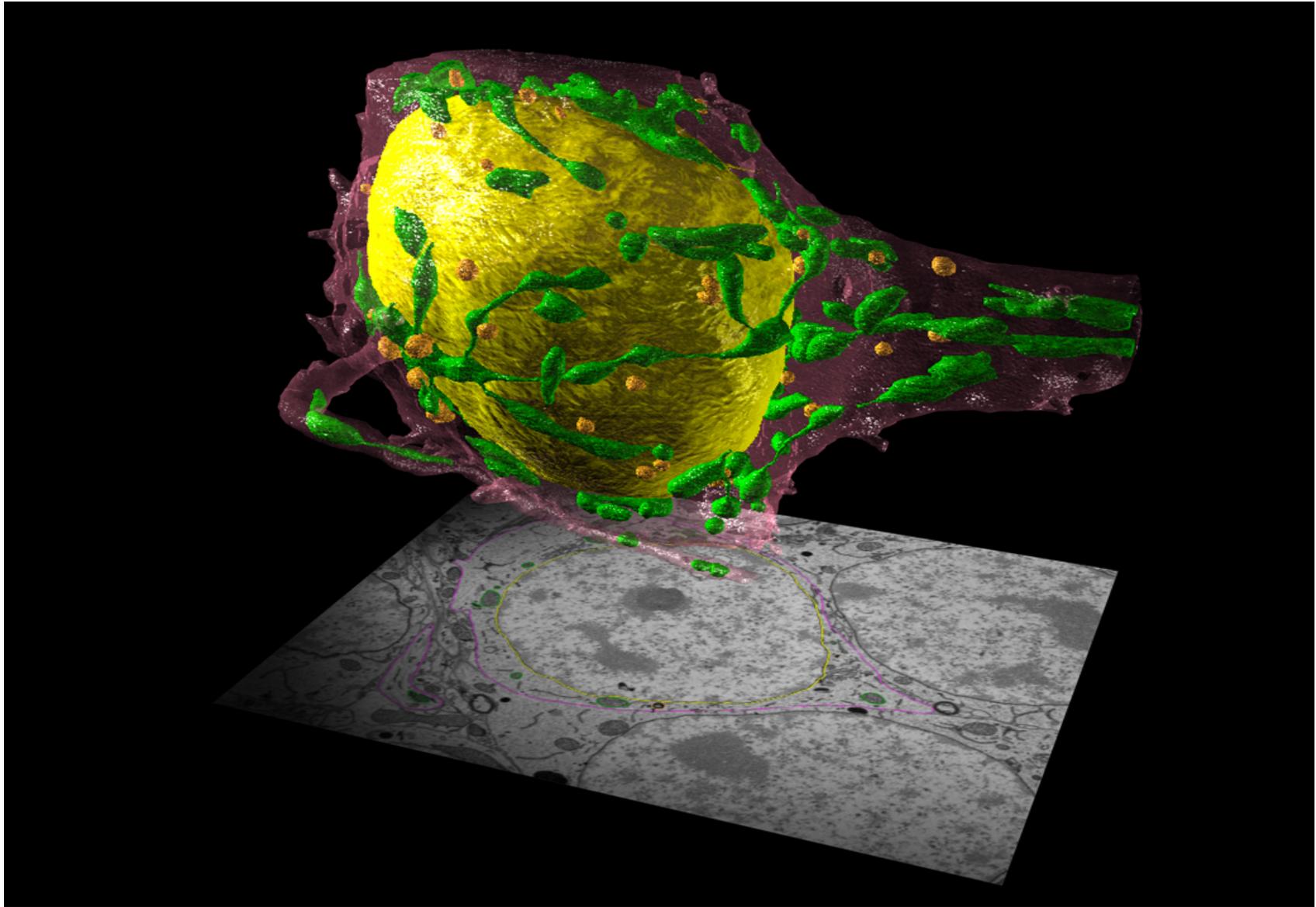


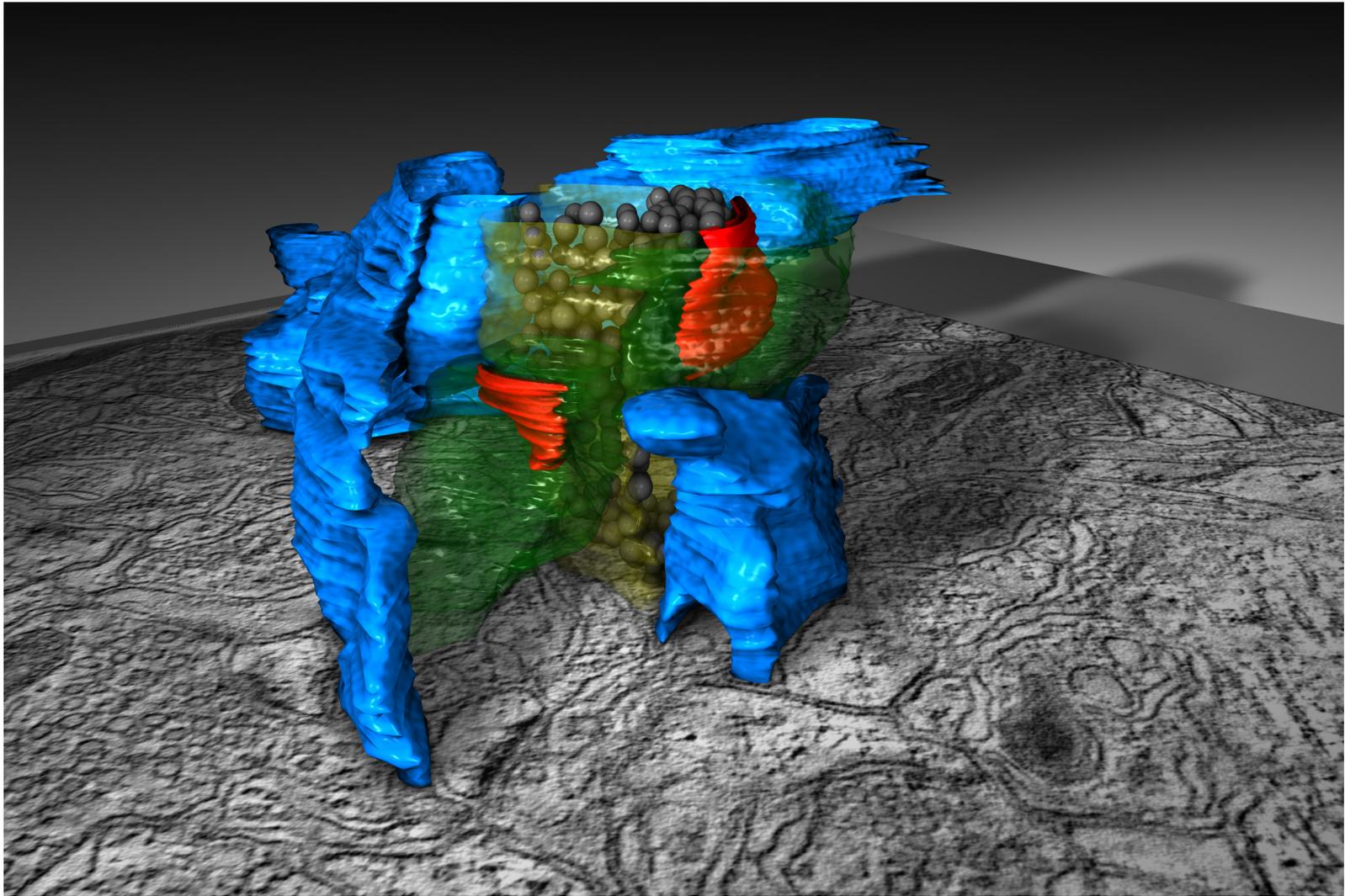
Volume and Patches Reconstruction



P53 Gene Experiments

- Adenovirus infection turns off P53 (cell death) gene
- High-resolution, large-scale 3D reconstructions via TxBR montages
- Extensive structural reorganization of cell nucleus shown in EM tomographic reconstructions
- Indications of cell nucleus structure correlated with cytoplasmic structure
- Further indications of spatial organization of metabolic pathways





Thank You for Your Attention

For further information:

<http://ncmir.ucsd.edu/>

<http://nbcrc.sdsc.edu/>

And thanks to Mark Ellisman, Director NCMIR; Peter Arzberger, Director NBCR; Michael Holst; Sebastien Phan; James Bower; Rajvikram Singh; Masako Terada and many others for the materials of this presentation.

https://www.nbcrc.net/pub/wiki/index.php?title=Tomography_Day_2008