Leiden University
Medical Center

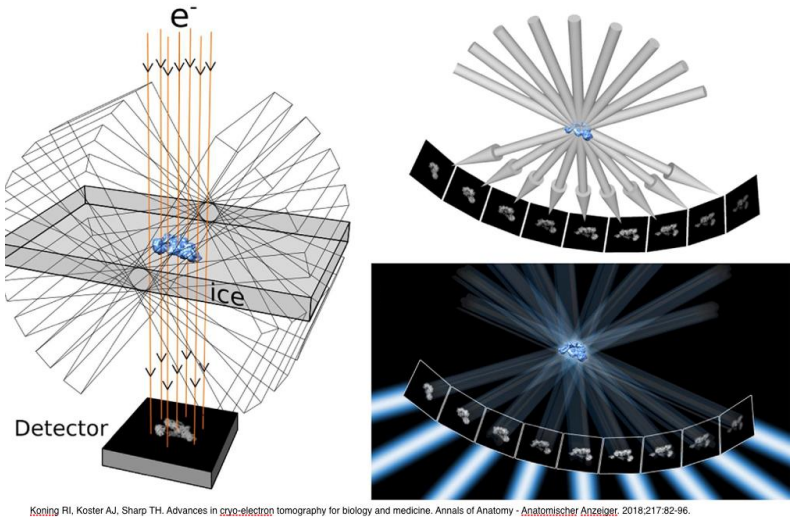
Challenges for High Speed Cryo Electron Tomography Introduction

28-29 November, 2018
NRAMM, NYSBC, New York
Clint Potter, Bridget Carragher, Bram Koster

Bram Koster

Cell and Chemical Biology

LEIDEN, THE NETHERLANDS



Koning RI, Koster AJ, Sharp TH. Advances in cryo-electron tomography for biology and medicine. Annals of Anatomy - Anatomischer Anzeiger. 2018;217:82-96.

Goals workshop

- Current performance of cryoET
  - speed & quality of data collection and reconstruction
- Common view on targeted performance of cryoET
  - Define the requirements for structural cell biology
- Identification workflow aspects to be improved
  - long-term & short term, technical & fundamental

Historical perspective

- Since the automation took hold during the 1990's cryoET matured into a widely used imaging technique
  - quality and speed of specimen preparation, reconstruction algorithms
  - advanced packages available both from scientific community as well as industry

- During the last five years, increased development of cryoET applications aiming for sub 0.5 nm resolution
  - more sensitive and faster detectors, better cryo TEMs, phase plates
  - algorithms, high performance computing

From my perspective early developments on automation

1985 Automated focusing – Delft University, Nethelands

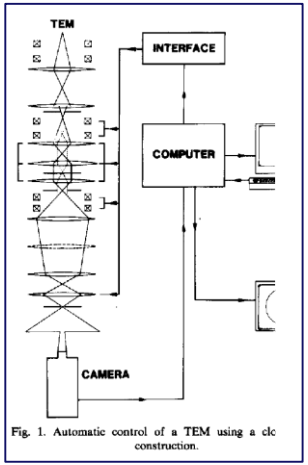


Fig. 1. Automatic control of a TEM using a clc construction.

Ultramicroscopy 27 (1989) 251–272
North-Holland, Amsterdam

Simulations & TEM control
Low dose, Autofocus

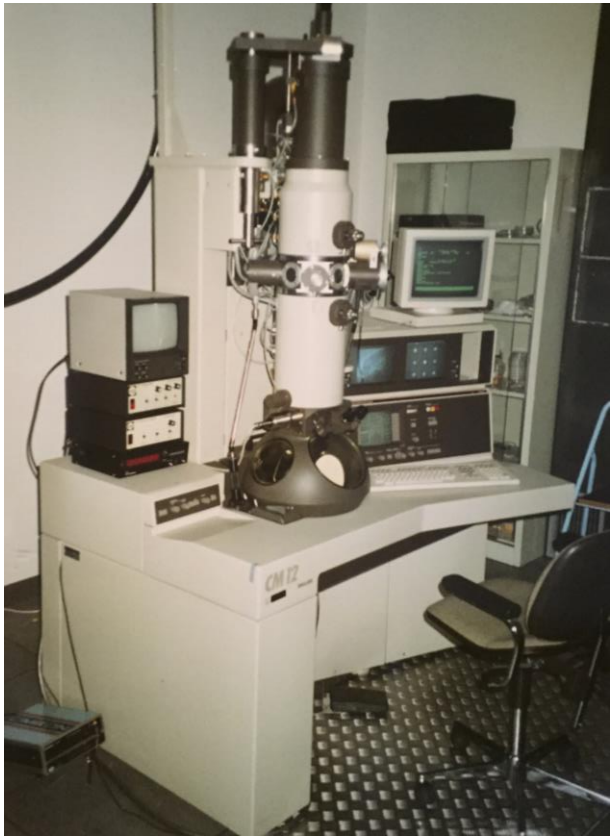
AUTOTUNING OF A TEM USING MINIMUM ELECTRON DOSE

A.J. KOSTER, W.J. de RUIJTER, A. VAN DEN BOS and K.D. VAN DER MAST

Research Group Particle Optics, Department of Applied Physics, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands

Received 26 August 1988; in revised form 27 December 1988

1989-91 Auto tomography – Tietz, Martinsried



Ultramicroscopy 40 (1992) 71–87
North-Holland

Automated tomography
Video camera, correlation

ultramicroscopy

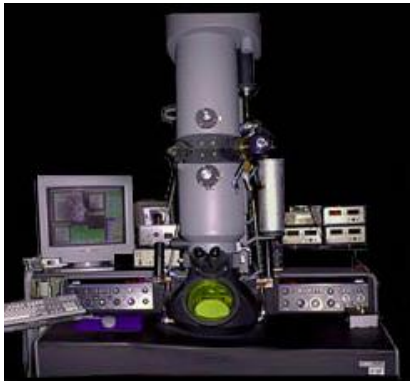
Cryo tomography
Handling drift, energy filter

Towards automatic electron to

K. Dierksen, D. Typke *, R. Hegerl, A.J. Koster [1] and W. Baumeister
Max-Planck-Institut für Biochemie, W-8033 Martinsried, Germany

Received 10 October 1991

1991-95 Auto tomography - UCSF



Ultramicroscopy 46 (1992) 207–227
North-Holland

Automated tomography
Slow Scan CCD
Metadata & Processing

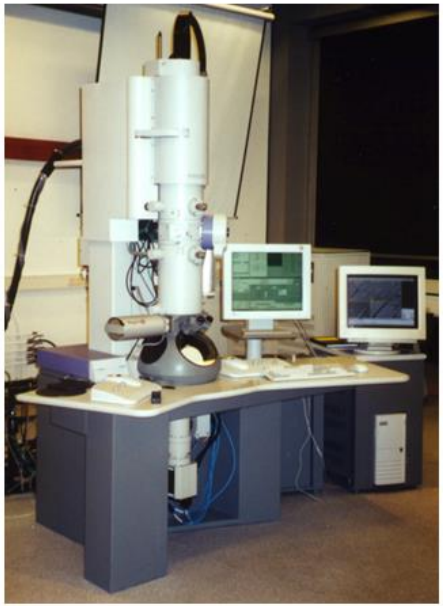
ultramicroscopy

Automated microscopy for electron tomography

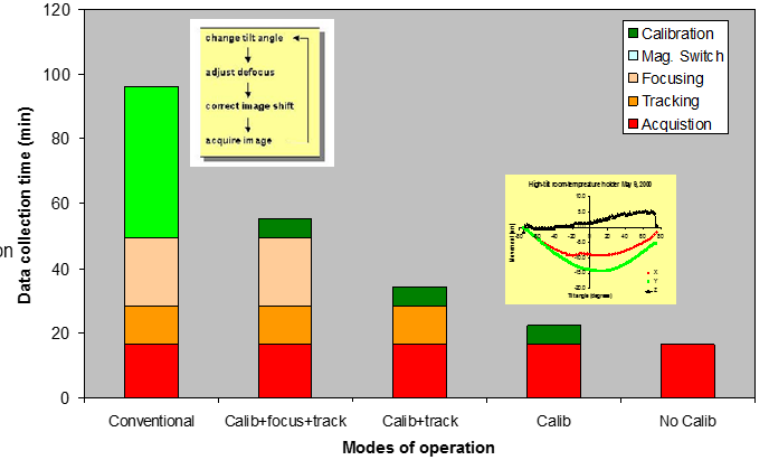
A.J. Koster, H. Chen, J.W. Sedat and D.A. Agard
Department of Biochemistry and Biophysics and the Howard Hughes Medical Institute, University of California at San Francisco, San Francisco, CA 94143-0448, USA

Received at Editorial Office 20 May 1992

1999-2005 Auto tomography – Utrecht University



- **Data collection**
  - 141 images
  - Exposure (1 s) + Transfer (3 s)
  - Settling down tilt (3 s)
  - For focusing: 2 images
  - For tracking: 1 images
- **Conventional method**
  - 2 switches of magnification (10s)
- **Pre-calibration method**
  - Measurement at low magnification (25 img)
  - focusing, tracking



Pre-calibration

From manual to automated

Beam-exposure 10-100 times less

Acquisition time reduced from a hours to 20 minutes

Journal of Microscopy, Vol. 205, Pt 2 February 2002, pp. 187–200
Received 5 June 2001; accepted 11 October 2001

Automated high-throughput electron tomography by pre-calibration of image shifts

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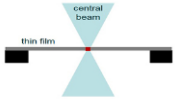
**Molecular Cell Biology, Utrecht University, 3584 CH Utrecht, the Netherlands*
†Inorganic Chemistry and Catalysis, Debye Institute, Utrecht University, 3584 CA Utrecht, the Netherlands
‡Laboratory for Cell Biology and Electron Microscopy, Faculty of Medicine, Utrecht University, 3584 CH Utrecht, the Netherlands

Technological developments revolutionized cryoET

- Direct electron detector
  - Increased sensitivity (5-10x): better resolution

- Dedicated cryo-TEM
  - Better mage quality, automation: higher throughput



- Phase Plate
  - Increased contrast (5x): better images

- GPU computing
  - Faster reconstructions (100x)
- Software
  - Better and smarter algorithms: faster and better reconstructions

Todays applications of cryo electron tomography can reveal structural information in the sub-nm range

Data acquisition time hardly improved

Current status

- Excellent data collection & processing available
  - High quality, user friendly, supported, flexible

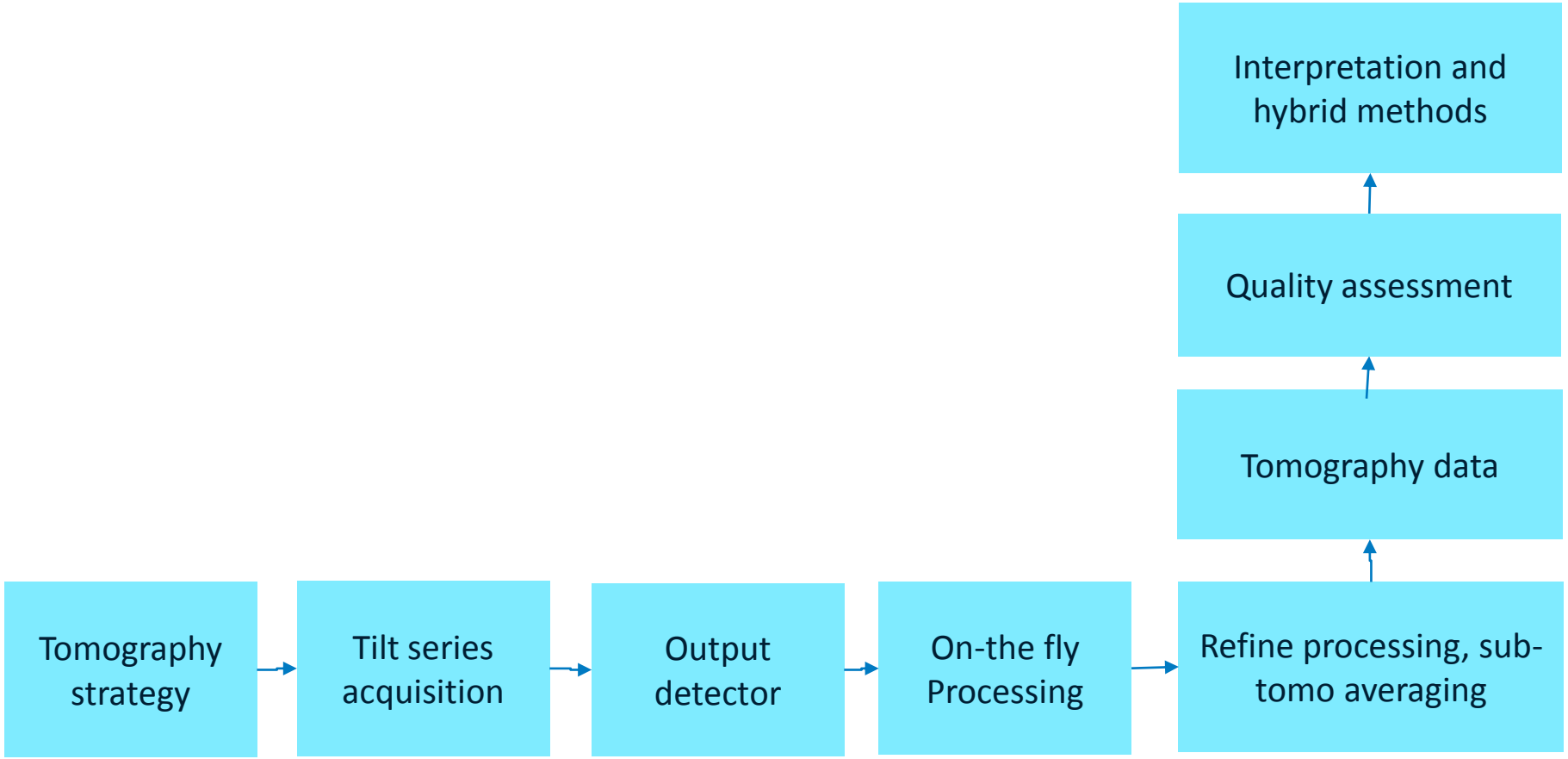
    Serial EM, UCSF Tomo, TOM, Leginon, Tomo4, EM-Menu, DMS

- Acquisition of a large number of high quality tomograms increasingly important important
  - increase resolution by sub-tomogram averaging
  - hybrid approach with single particle approaches
  - direct interpretation of densities in vivo and in situ

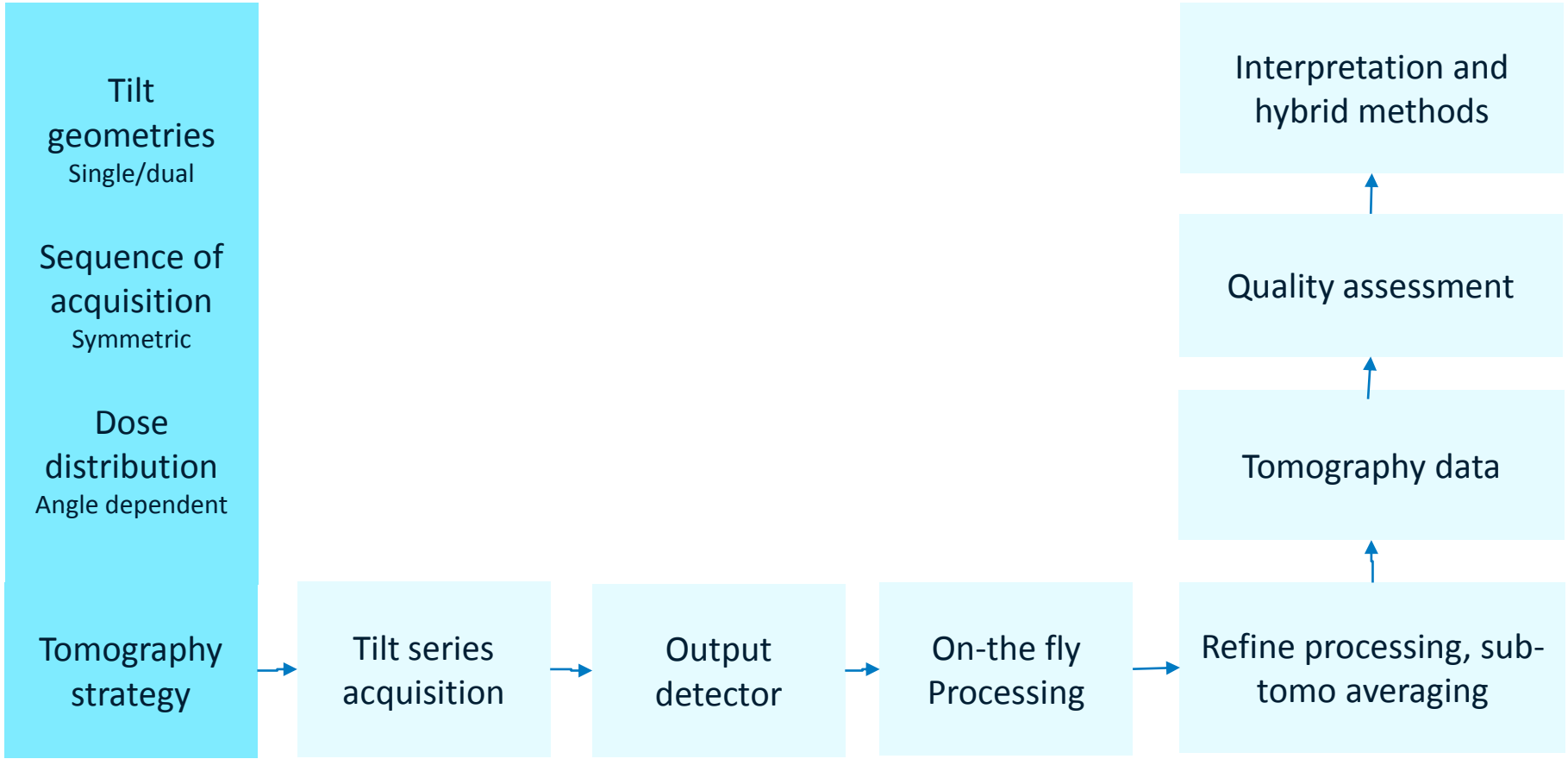
In many labs the time required for tilt series acquisition and processing becomes challenging

Volume of data, sparsity of data points on specimen, complex processing workflows

Automation approaches



Automation approaches



A view on automation

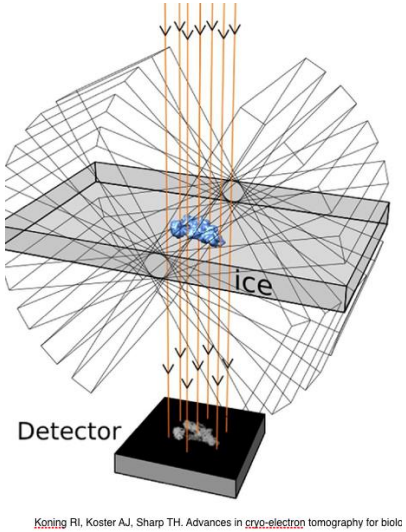
Tomography strategy

Tracking area of interest xyz
mechanical
electron optical

Detector and illumination
Single frame, movie, counting

Stability
various drifts, energy filter, Cs corr, phase plate

Tilt series acquisition



Koning RI, Koster AJ, Sharp TH. Advances in cryo-electron tomography for biolc

Output detector

On-the fly Processing

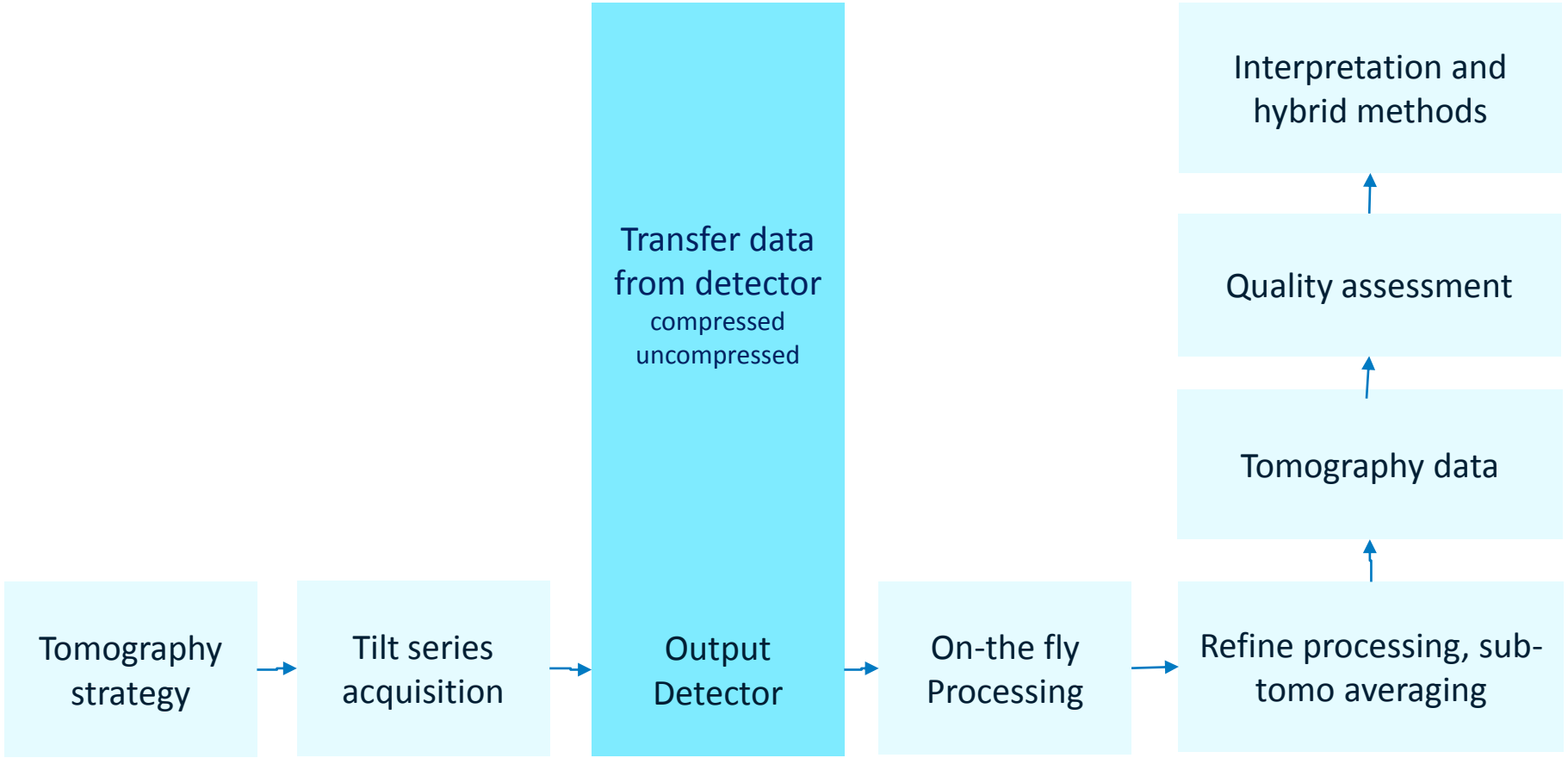
Refine processing, sub-tomo averaging

Tomography data

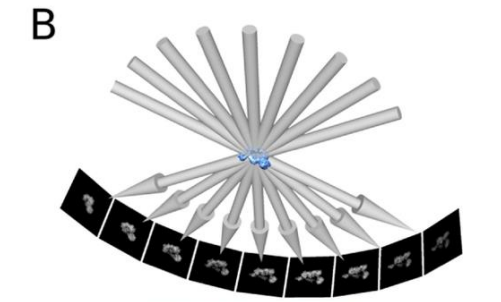
Quality assessment

Interpretation and hybrid methods

Automation approaches



Automation approaches



gy and medicine. Annals of Anatomy - Anatomischer Anzeiger. 2018;217:82-96.

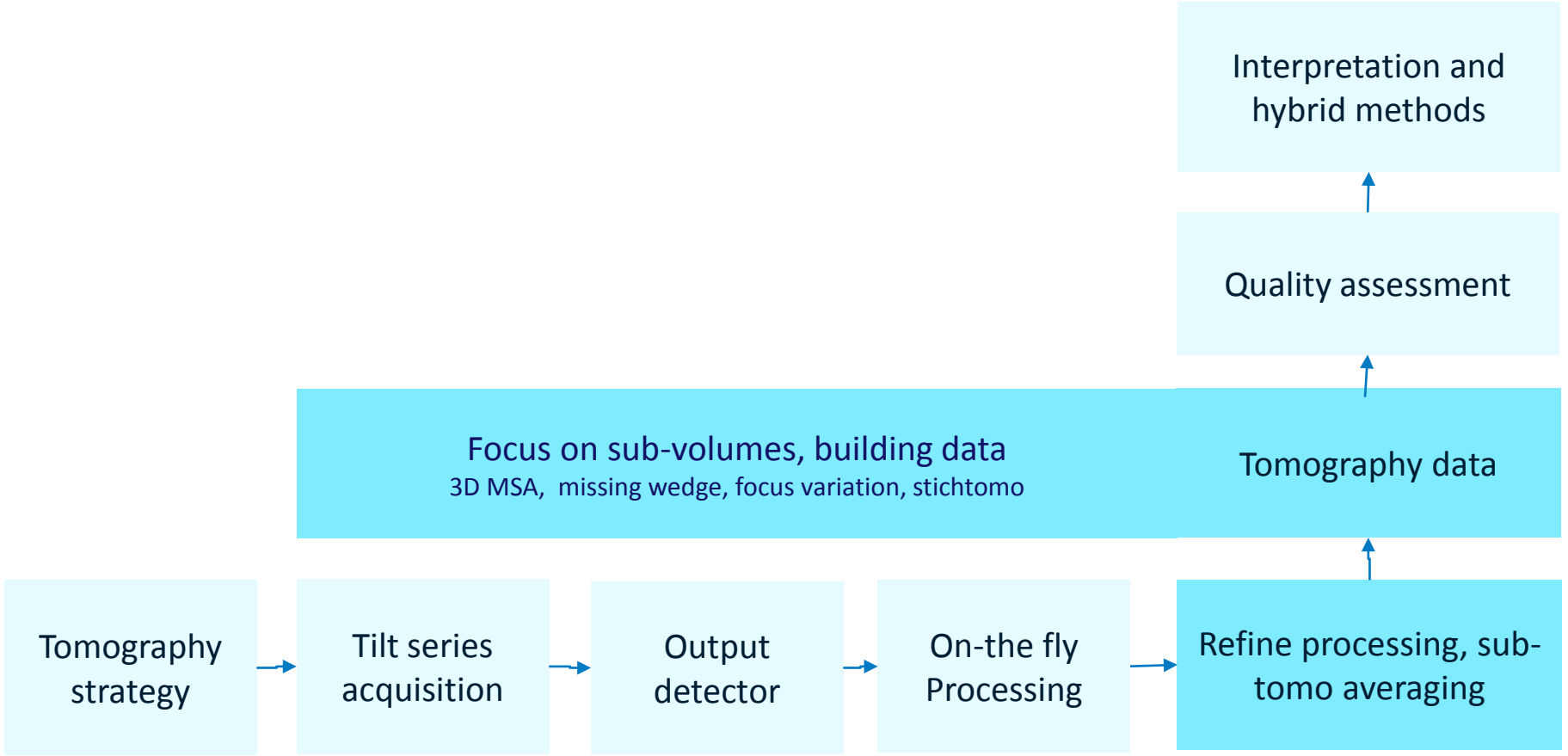
Tomography strategy → Tilt series acquisition → Output detector → On-the fly Processing → Refine processing, sub-tomo averaging → Tomogram data → Quality assessment → Interpretation and hybrid methods

Take into account image formation

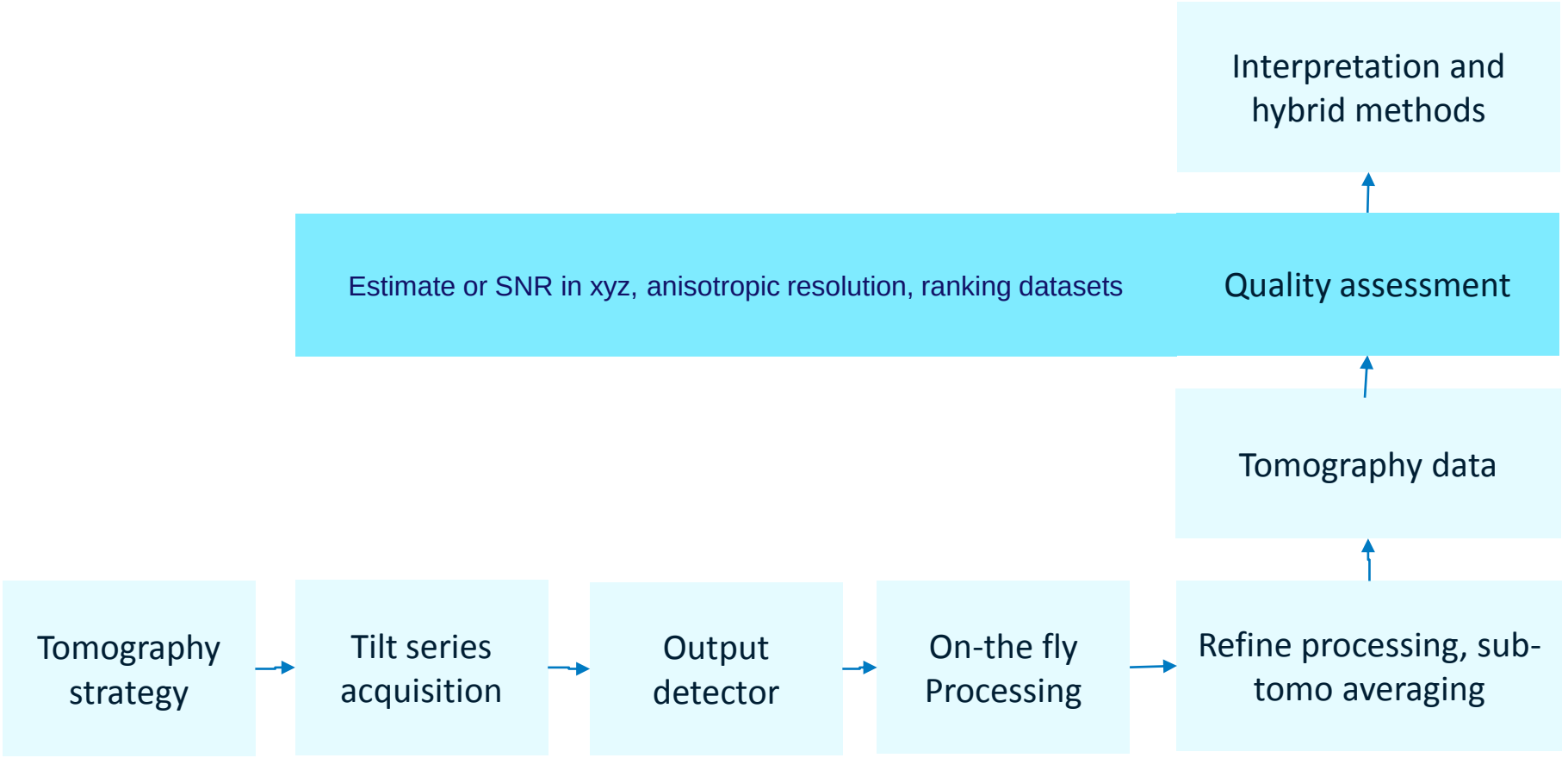
CTF, VPP
Dose-weighing
motioncorr2
alignframes
Cffind4, gctf,
gtfphaseflip

Refine shifts, angles
WBP

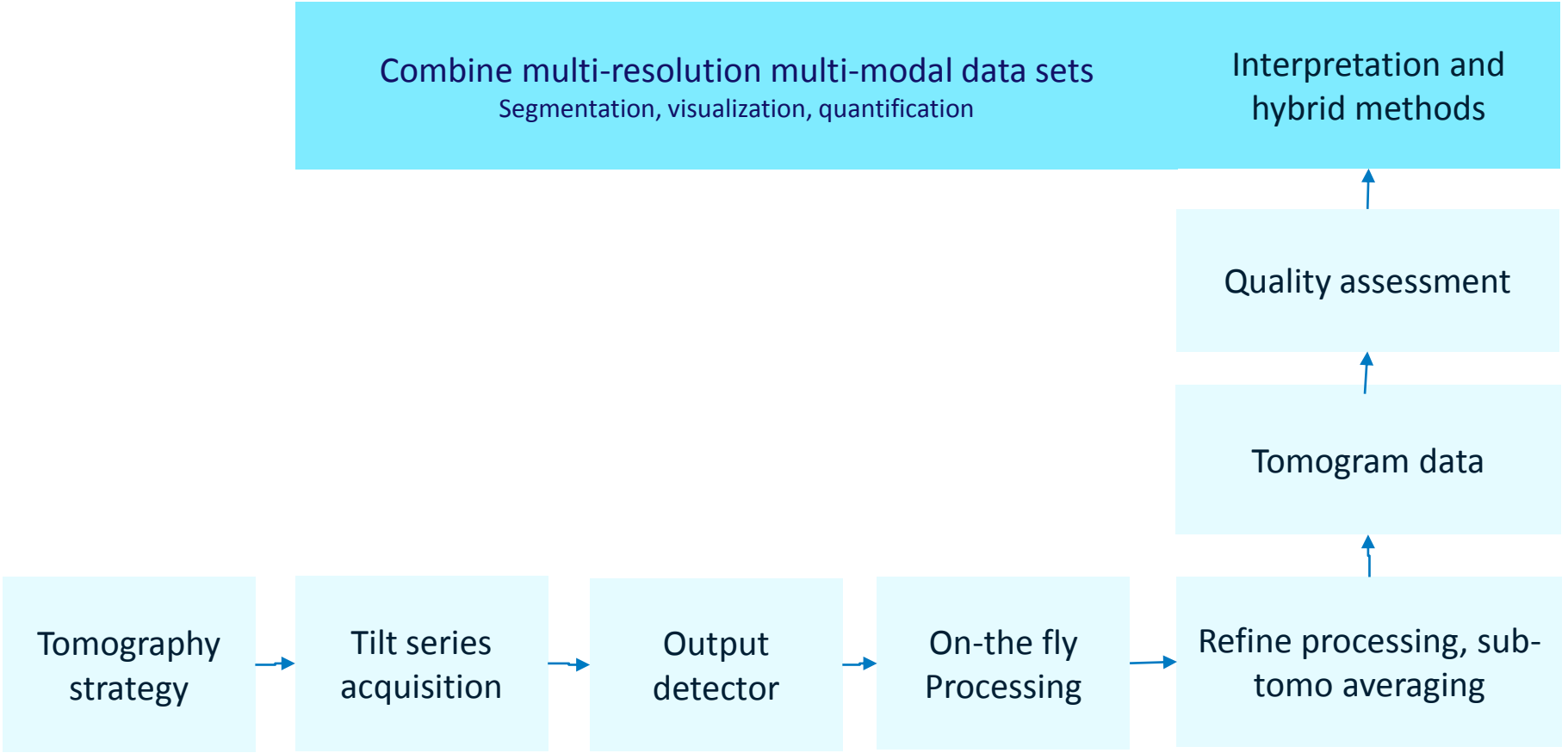
Automation approaches



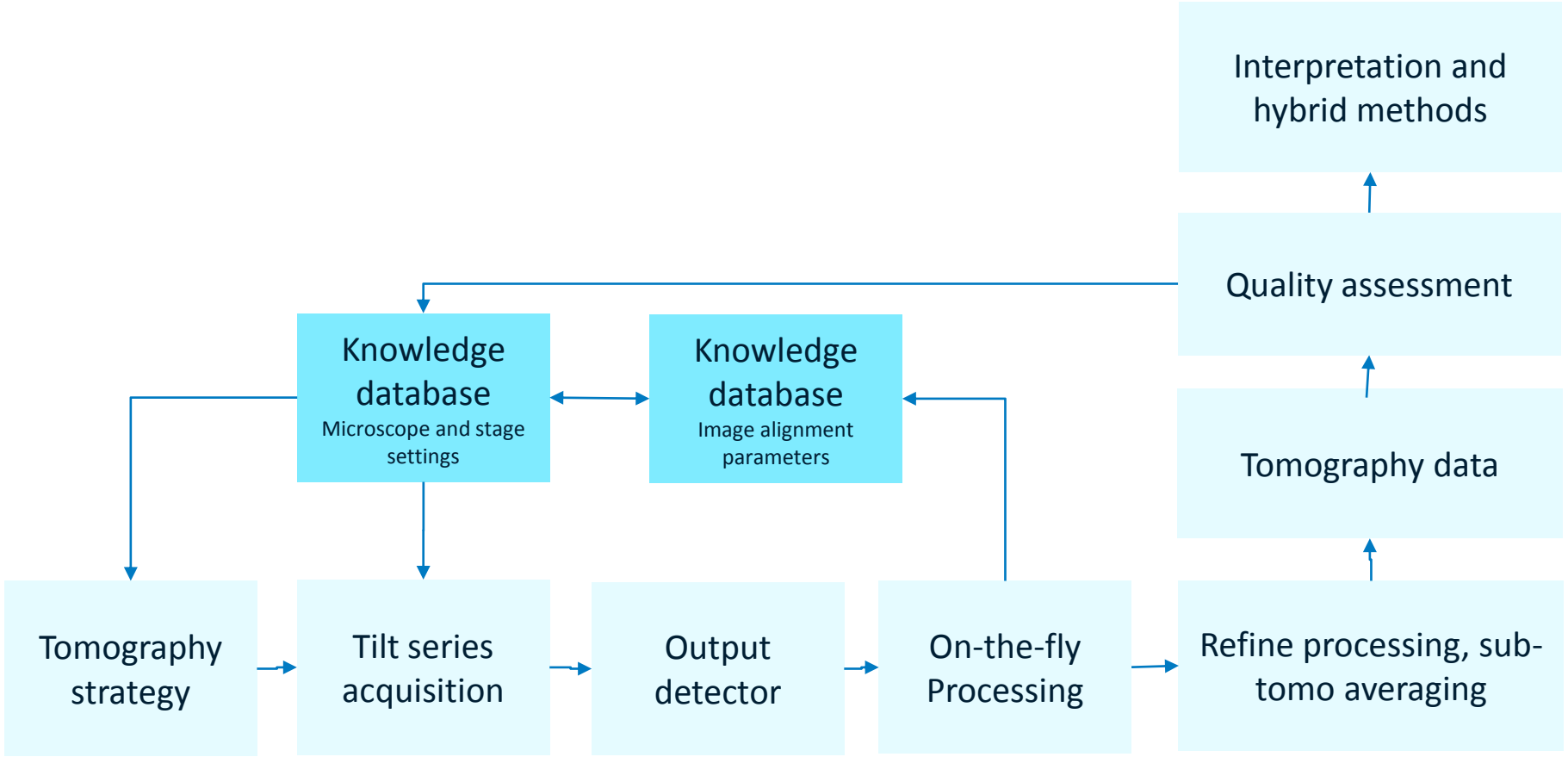
A view on automation



Automation approaches



Automation approaches



Presentations

Wednesday
9.00 Start

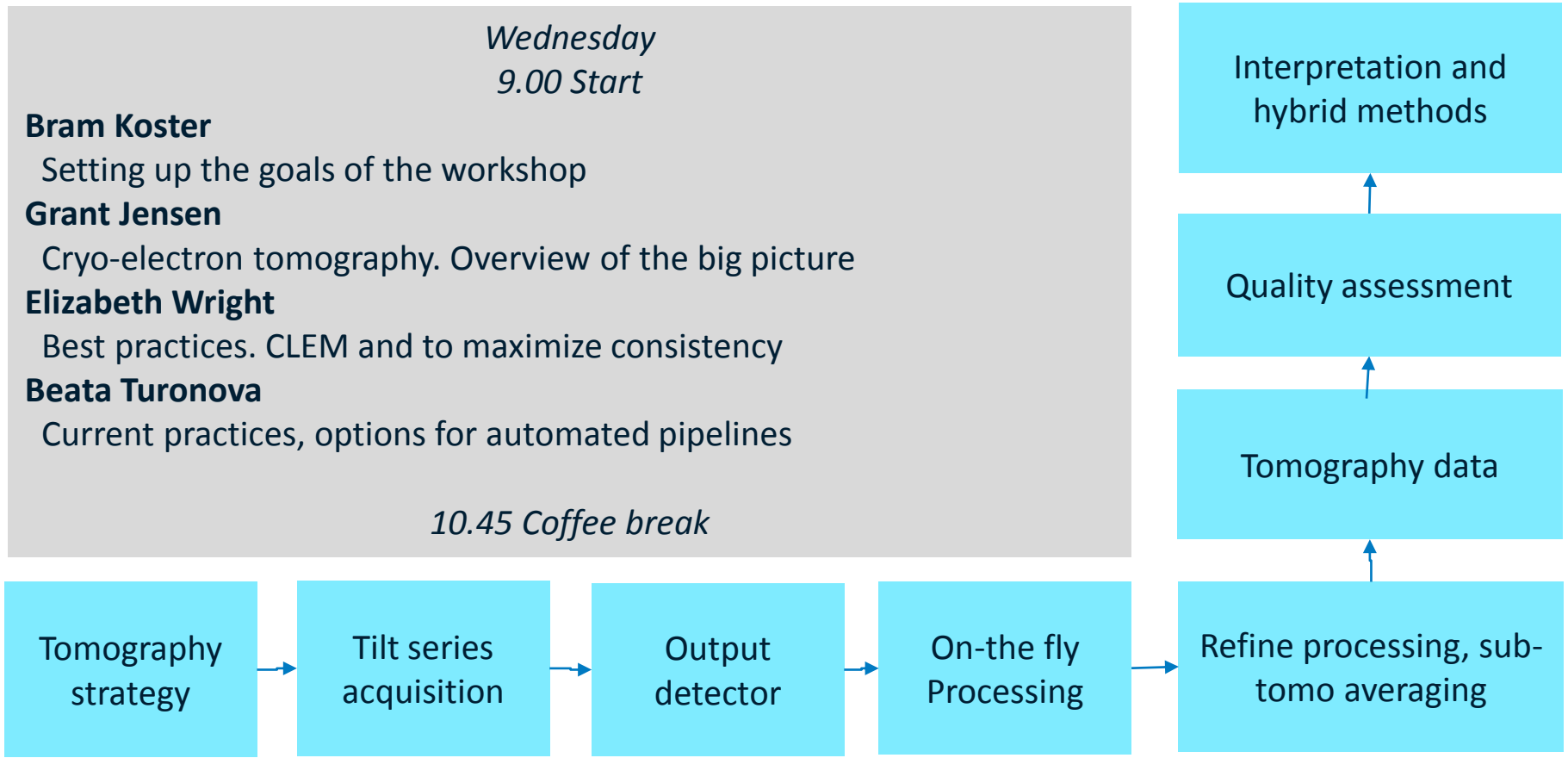
Bram Koster
Setting up the goals of the workshop

Grant Jensen
Cryo-electron tomography. Overview of the big picture

Elizabeth Wright
Best practices. CLEM and to maximize consistency

Beata Turonova
Current practices, options for automated pipelines

10.45 Coffee break



Presentations

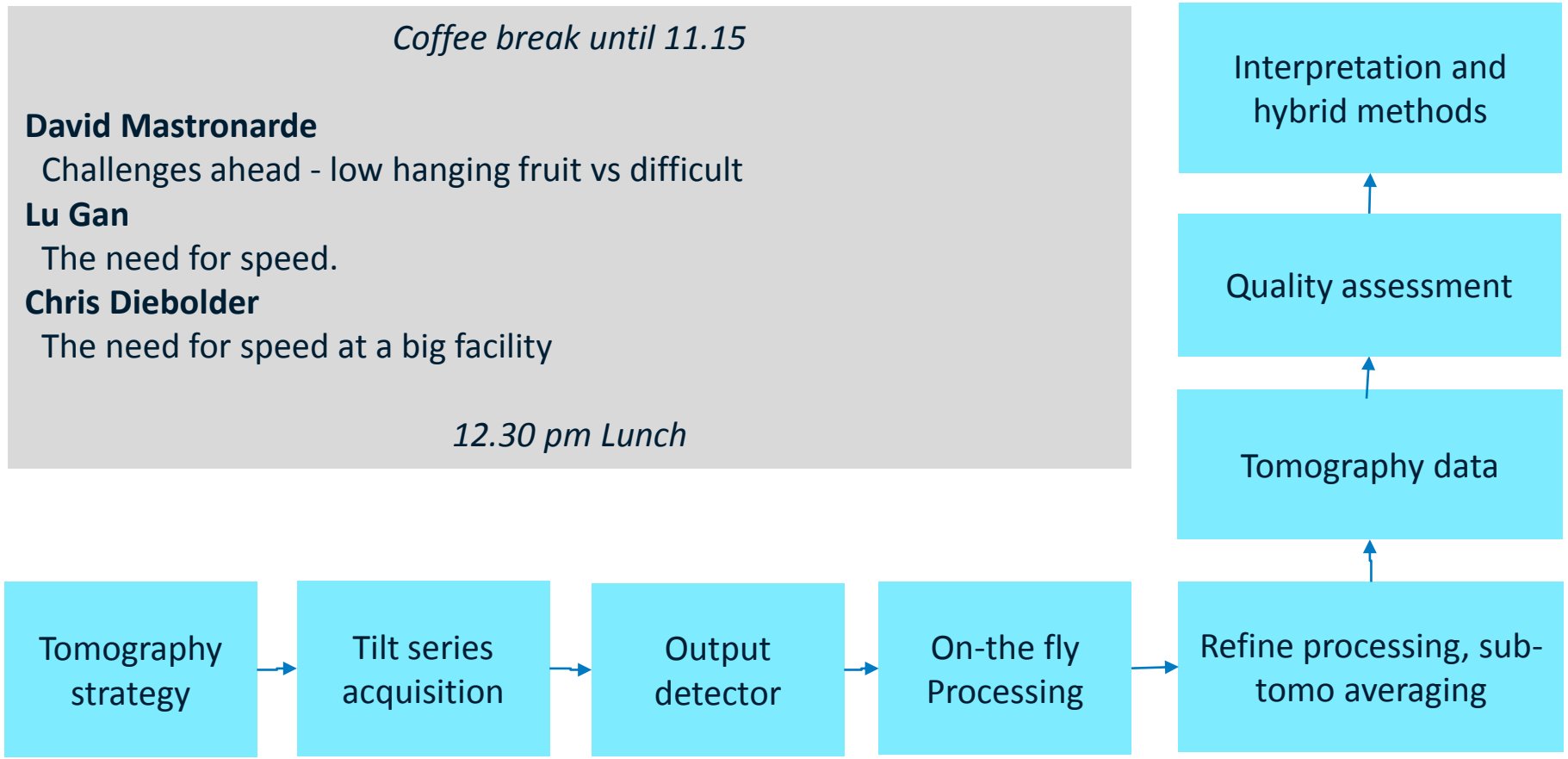
Coffee break until 11.15

David Mastronarde
Challenges ahead - low hanging fruit vs difficult

Lu Gan
The need for speed.

Chris Diebolder
The need for speed at a big facility

12.30 pm Lunch



Presentations

Lunch until 1.15 pm

Erik Franken
Image processing challenges for continuous tilt tomography

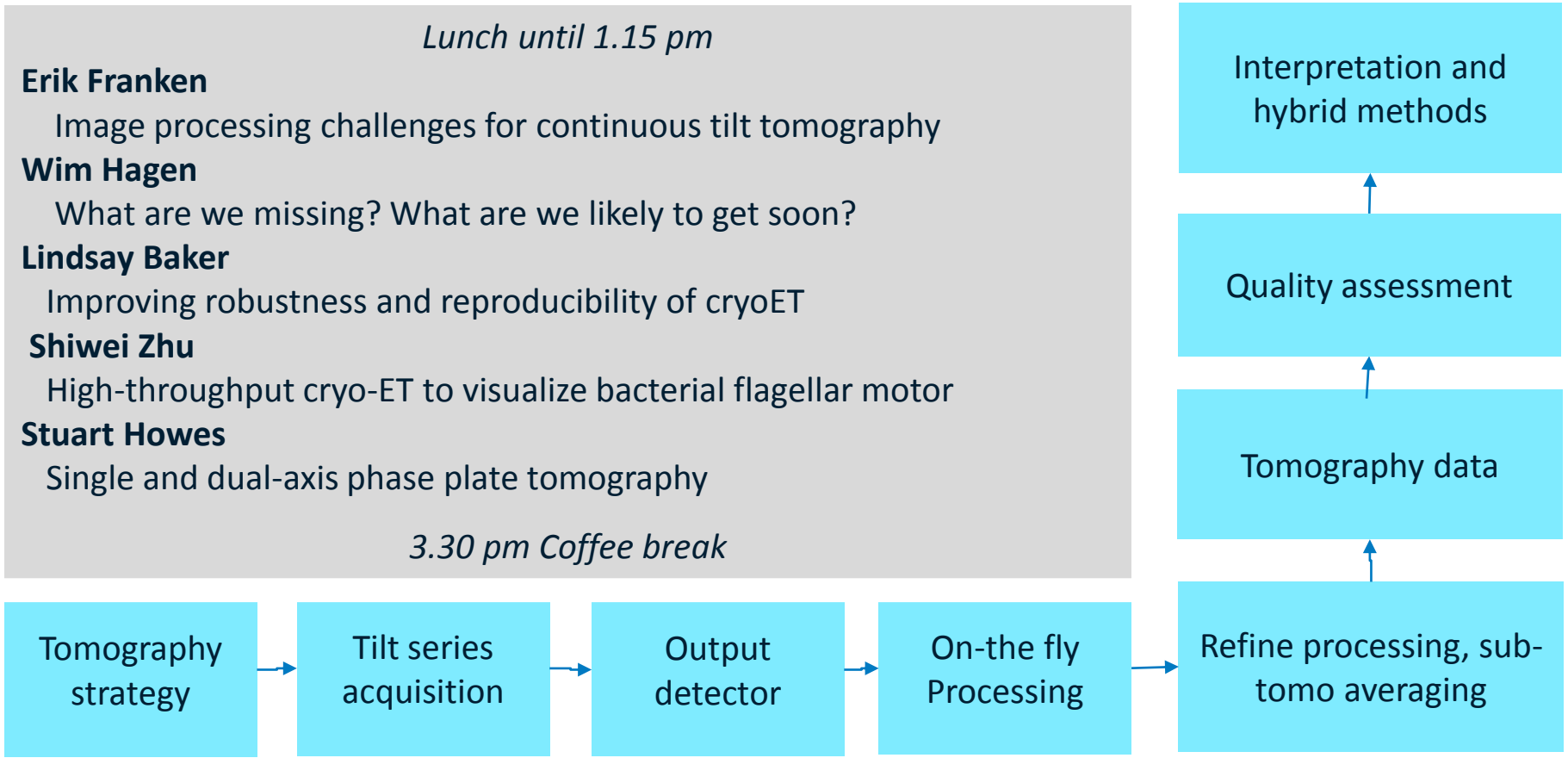
Wim Hagen
What are we missing? What are we likely to get soon?

Lindsay Baker
Improving robustness and reproducibility of cryoET

Shiwei Zhu
High-throughput cryo-ET to visualize bacterial flagellar motor

Stuart Howes
Single and dual-axis phase plate tomography

3.30 pm Coffee break

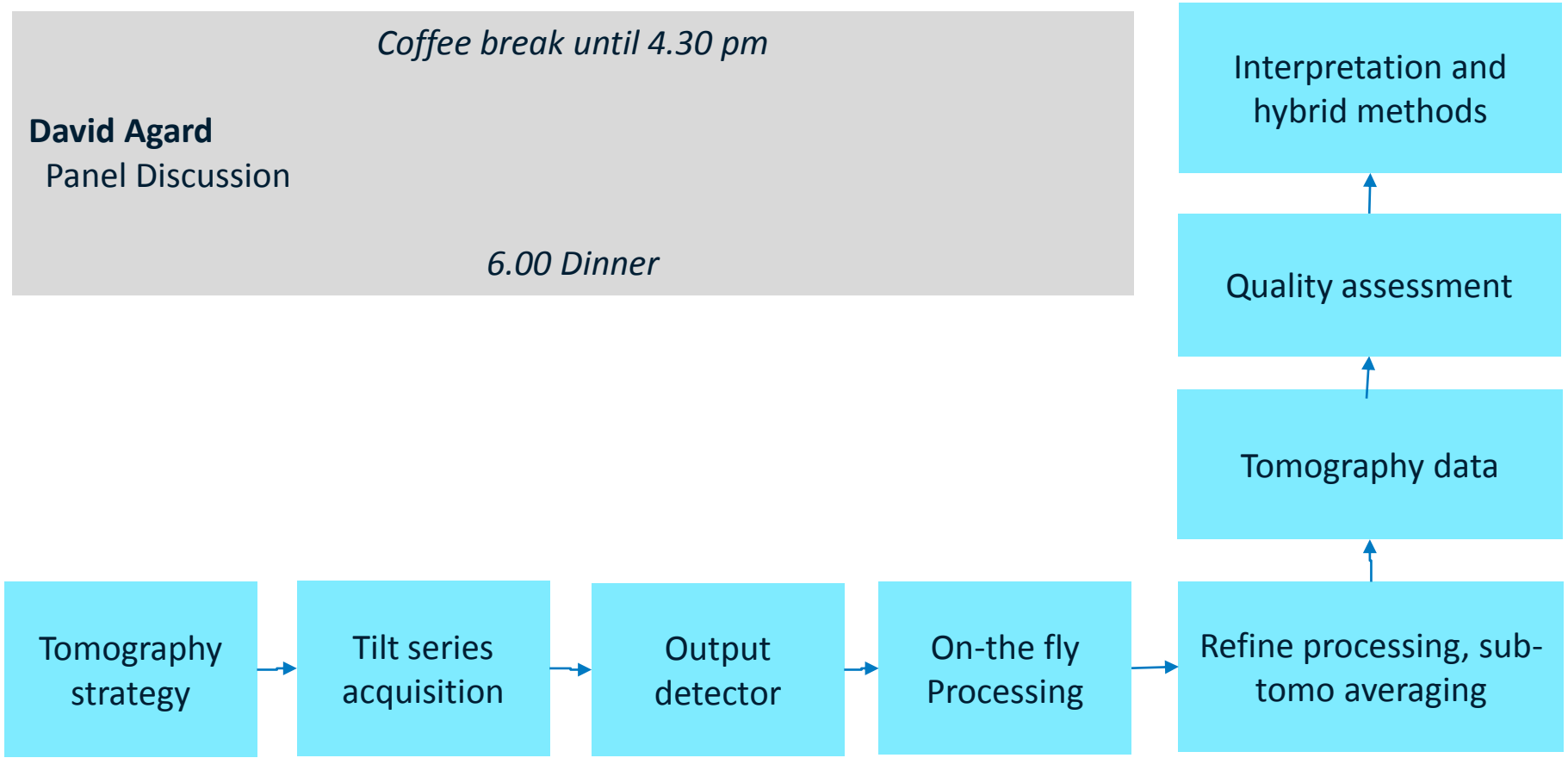


A view on automation approaches

Coffee break until 4.30 pm

David Agard
Panel Discussion

6.00 Dinner

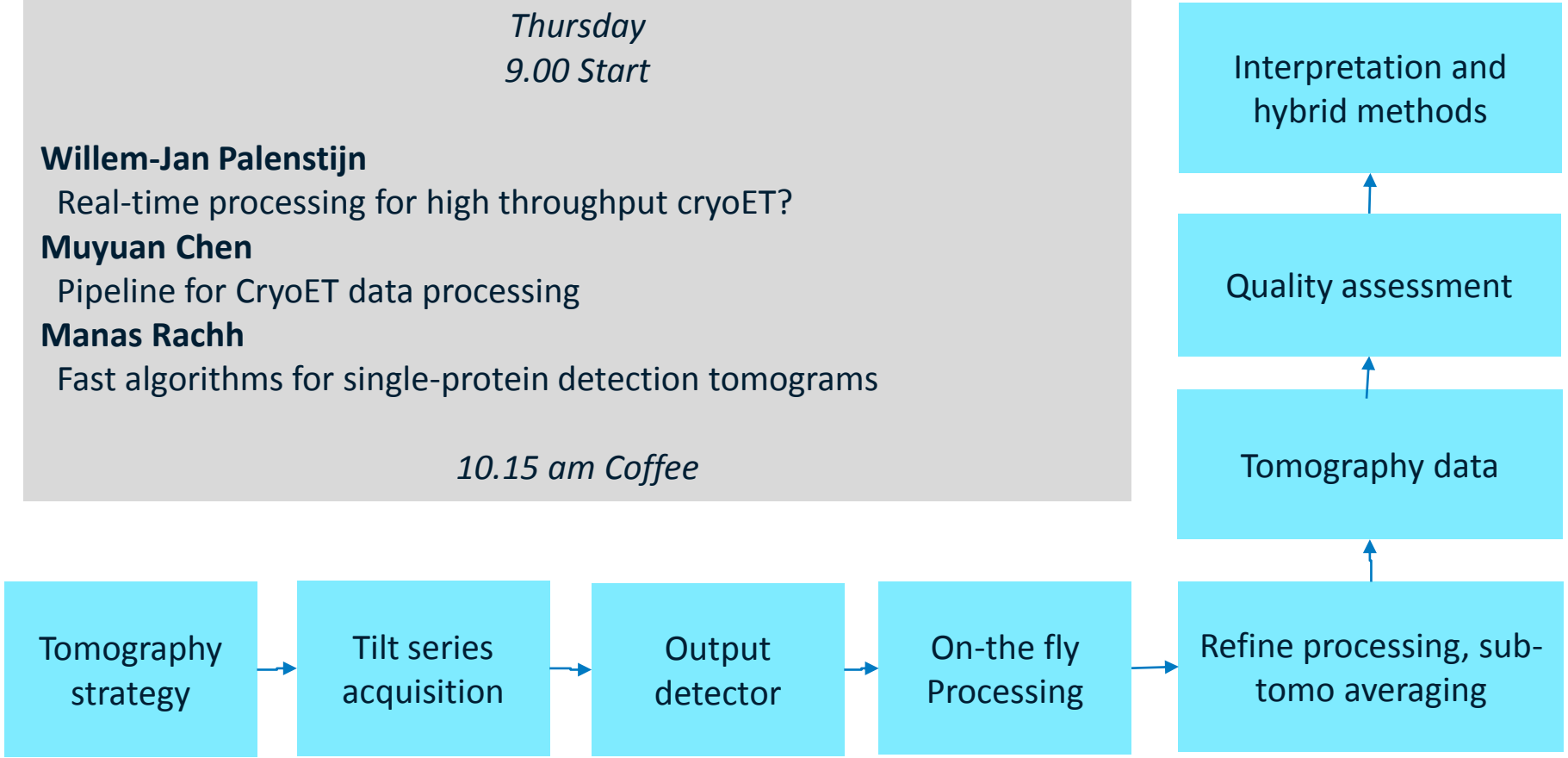


Presentations

Thursday
9.00 Start

Willem-Jan Palenstijn
Real-time processing for high throughput cryoET?
Muyuan Chen
Pipeline for CryoET data processing
Manas Rachh
Fast algorithms for single-protein detection tomograms

10.15 am Coffee



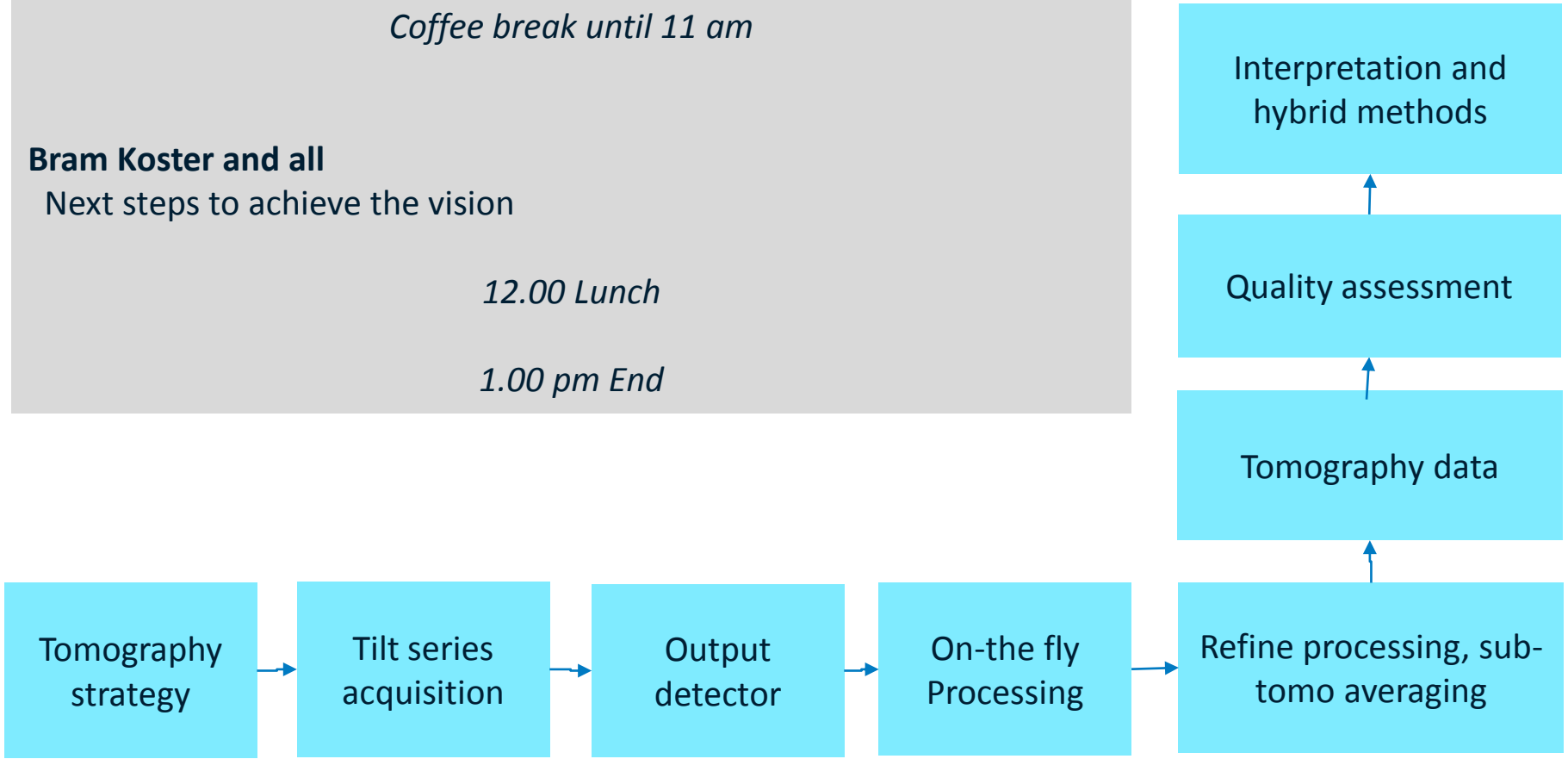
Presentations

Coffee break until 11 am

Bram Koster and all
Next steps to achieve the vision

12.00 Lunch

1.00 pm End



Presentations

Grant Jensen
Cryo Electron Tomography. Overview of the big picture