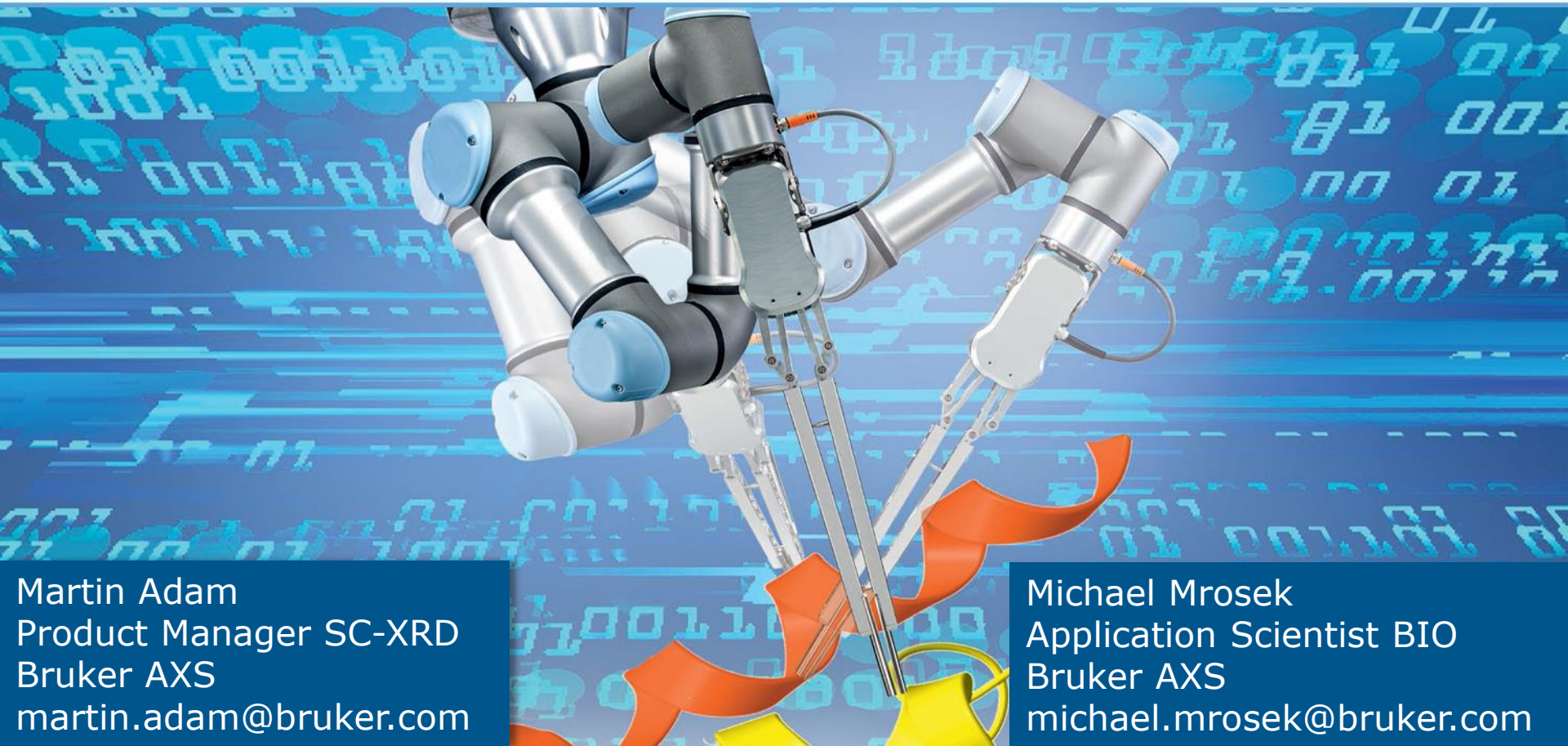
Increased productivity with automated crystal handling



Martin Adam
Product Manager SC-XRD
Bruker AXS
martin.adam@bruker.com

Michael Mrosek
Application Scientist BIO
Bruker AXS
michael.mrosek@bruker.com

SCOUT
Fast, reliable and safe sample changer

- SCOUT is an automated sample handler for the D8 VENTURE platform

Contents

- Project motivation
- SCOUT functionality
- Technical features of SCOUT
- Crystal centering with the AGH
- Software for Automation
- Benefits of SCOUT in your lab

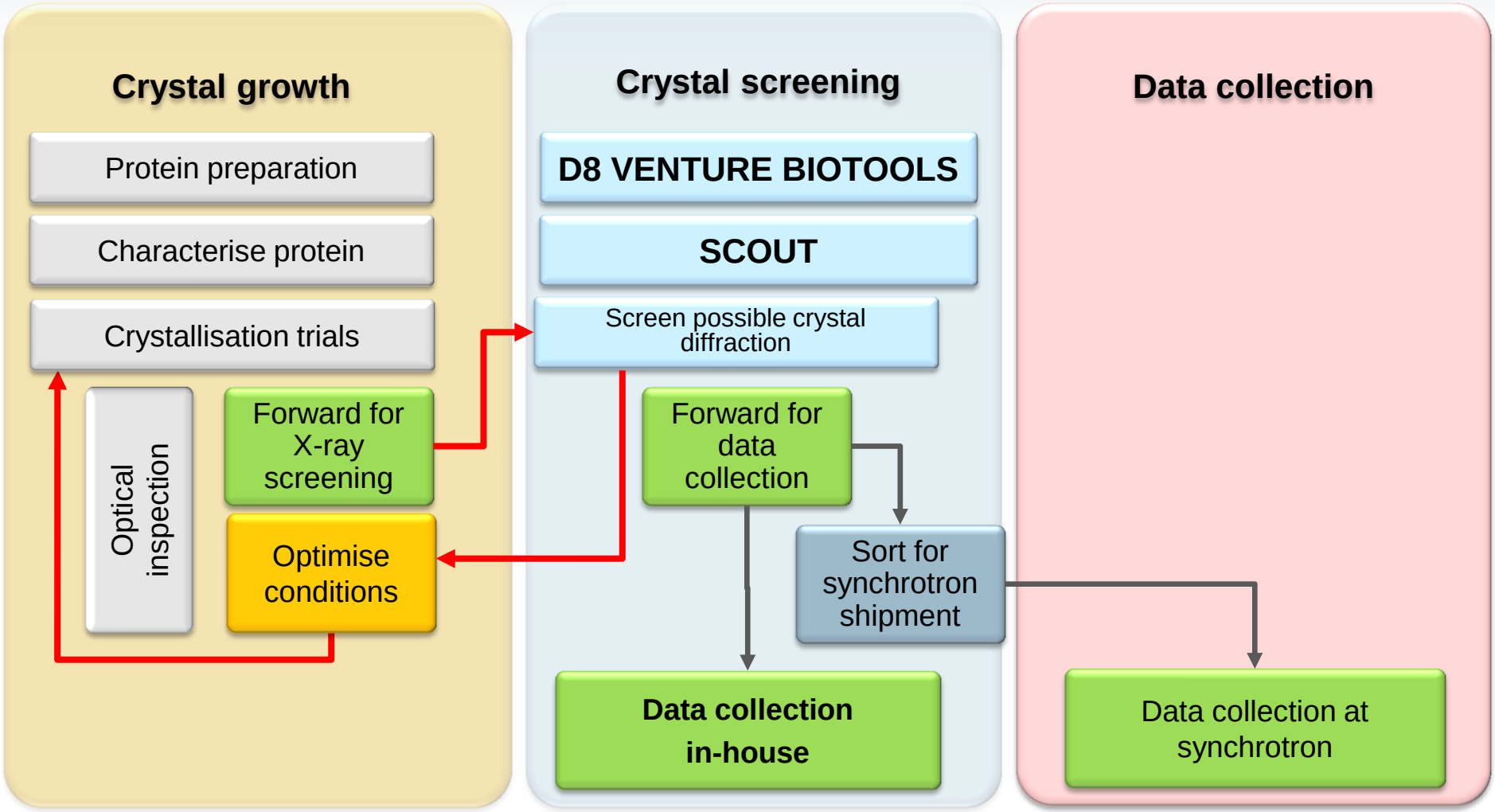
Protein Crystallography
The traditional way…

- Protein crystals are difficult to grow, small, fragile, weakly diffracting, require cryo-conditions
- Finding well diffracting crystals involves testing a large number of conditions
- Many crystals must be tested to assess diffraction quality and find suitable ones for complete data set collection
- Traditionally mounted by hand

Issues

- Slow, time consuming
- Laborious and delicate process
- Requires training and experience
- Crystal samples must be handled with great precision so they do not suffer any damage.

Protein crystallography workflow
Increasing degree of automation needed



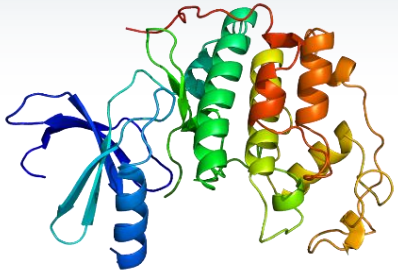
SCOUT
Compatibility with synchrotron workflow

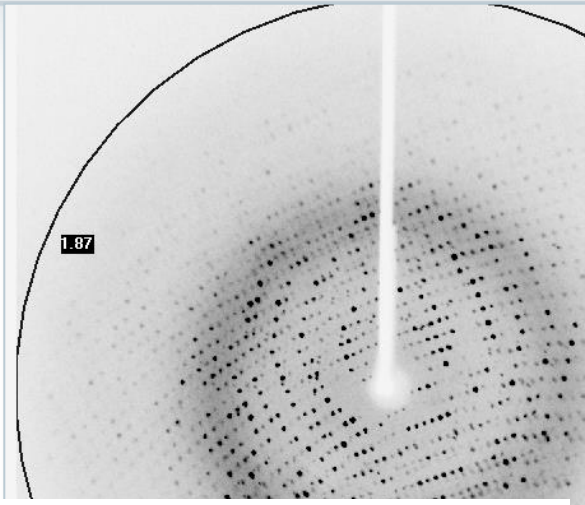
- SCOUT offers “Synchrotron-like” operation also for non-expert users
- Preparation for synchrotron trips very effective (Unipucks are compatible)
- Larger sample numbers can be screened prior to a visit
- Sensitive samples can be mounted reliably and safe
- Completes a state of the art automated in-house structure determination platform

http://www.diamond.ac.uk/Home/About.html

SCOUT - Rapid structure determination

High-throughput structure determination cyclin-dependent kinase (CDK2)





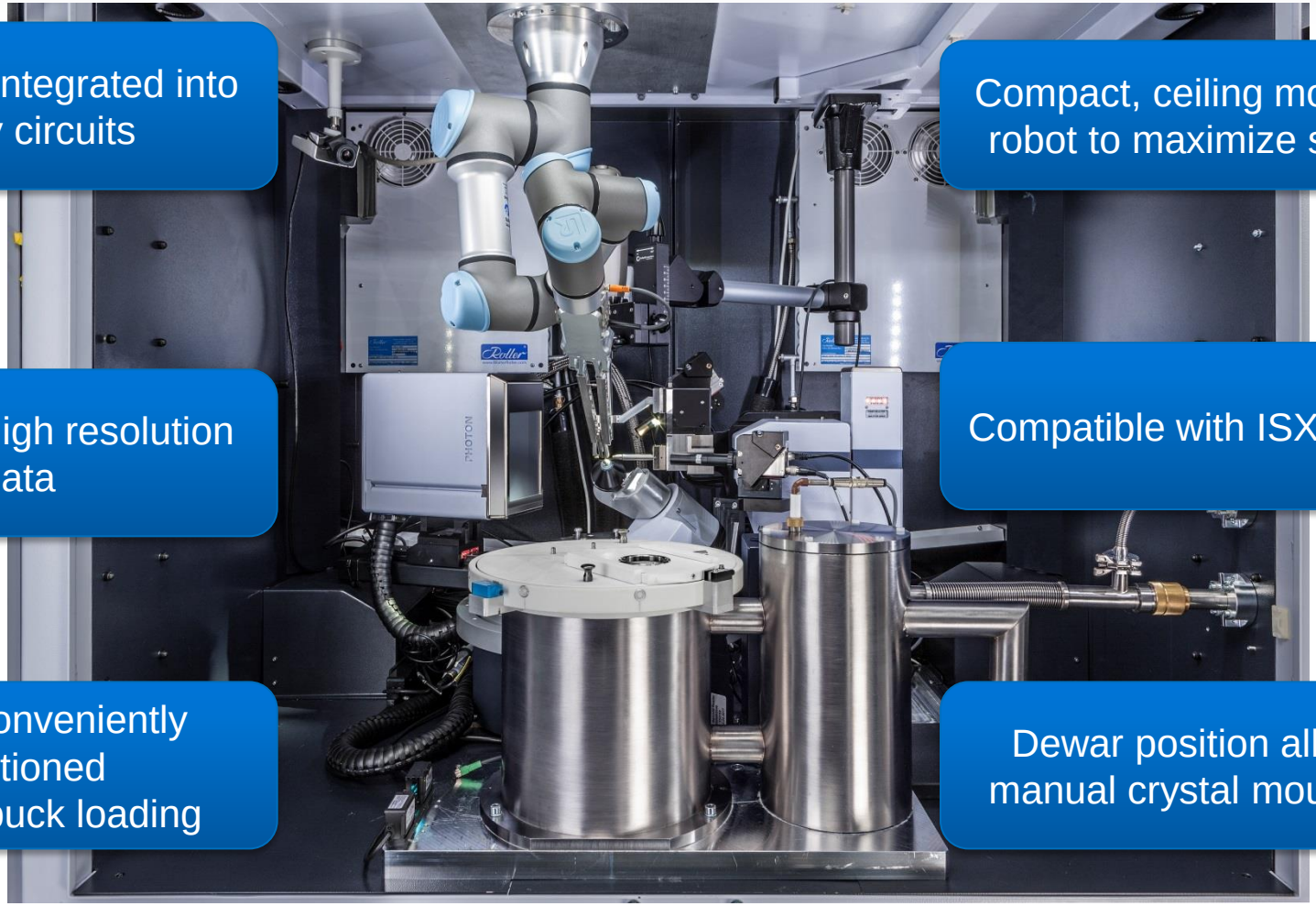
- CDK2 is involved in regulation of the cell cycle
- Diffraction of CDK2 is typical of the many kinases under investigation as anti cancer drug-targets
- Many thousands of crystal structures are solved to investigate binding of druggable lead compounds to the target proteins
- Chemists can make well-informed decisions (cost effective)
- **In house CDK2 data set in under 4 min**

Data collection	
X-ray source	METALJET (Ga)
Detector	PHOTON II
Type of scan	Omega, 100°
Exposure time (s/°)	**1 s / 0.5 °**
Measurement time (s)	**200**
Statistics	
Resolution (Å)	1.95
Completeness (%)	97.5 (92.2)
Multiplicity	3.68 (2.34)
<I/sigI>	10.75 (2.77)
$CC_{1/2}$ at 2.00 Å	86%
R_{pim} (%)	3.58 (17.95)

SCOUT - Functionality
Autonomous operation

- Reliable, safe mounting and retrieval of crystals
- Fully automated
  - Optical crystal centering
  - X-ray crystal centering
  - Crystal screening
  - Data collection
  - Crystal scoring
- Score crystals diffraction and select the best for data collection
- User defined or automated strategy determination
- Sample reorganization - prepare for synchrotron trips

SCOUT for D8 VENTURE
Features



Robot fully integrated into safety circuits

Compact, ceiling mounted robot to maximize space

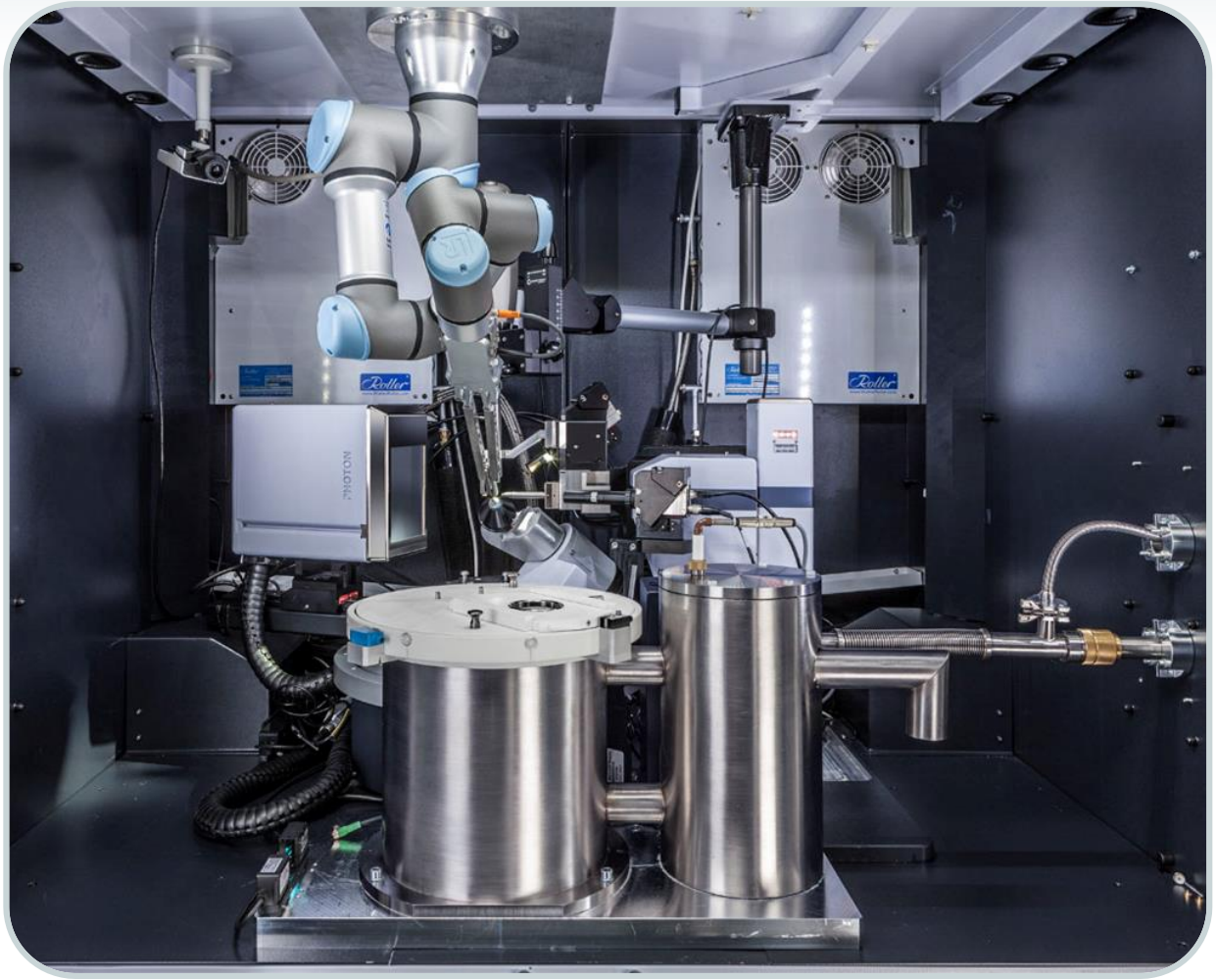
Access to high resolution data

Compatible with ISX Stage

Dewar conveniently positioned for easy puck loading

Dewar position allows manual crystal mounting

SCOUT – Compact, highly integrated
Components



- Cryogenic system
- AGH
- Air-conditioning

Optional:

- Bar-code reader
- Webcam
- Unipuck Starters Kit

SCOUT
Safety First

- SCOUT is the only sample automation system that meets the latest safety regulations:
  - Machinery Safety Directive 2006/42/EC
  - Pressure Equipment Directive 97/23/EC
  - Collaborative Robot Safety ISO/TS 15066:2016

The SCOUT system
Ultimate flexibility

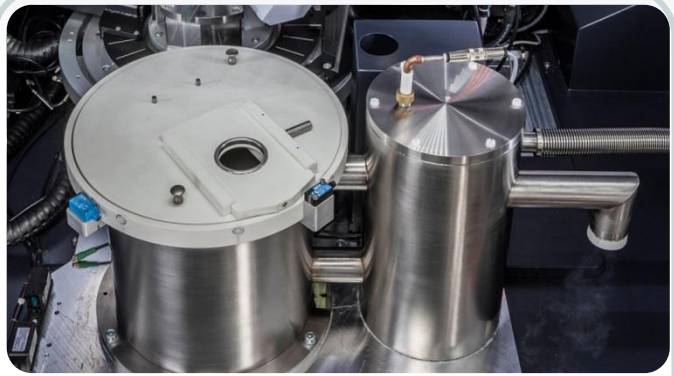
- Compatible with single source D8 VENTURE with KAPPA goniometer:
  - METALJET
  - IμS DIAMOND
  - TURBO X-RAY SOURCE (TXS)
  - IμS 3.0
- Compatible with all PHOTON II and PHOTON III detectors
- Compatible with ISX Stage
- Compatible with CRYOSTREAM 800 or COBRA low temperature devices
- Easy switch to manual mounting or ISX Stage (<5 minutes)
- Available with new system or as a later upgrade

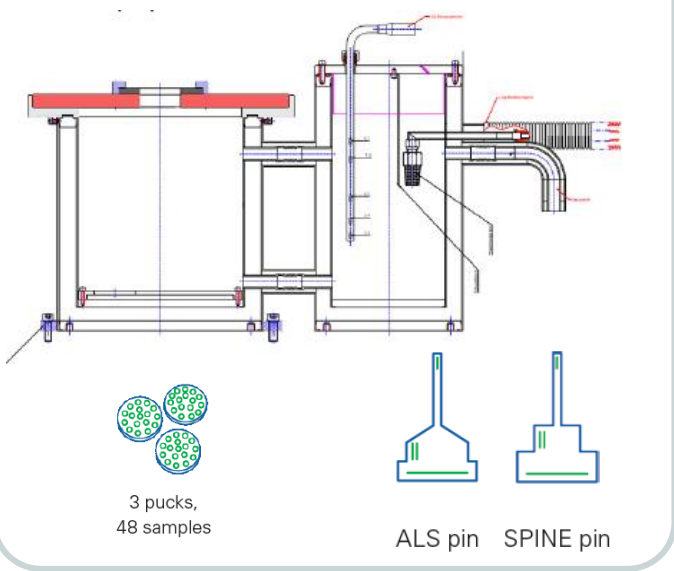
SCOUT
6-axis robot

- 6-axis robot ceiling mounted
- Lightweight “collaborative” robot
- High accuracy and reproducibility
- Easy teaching (~5 minutes)
- Meets CE safety standards
- No risk of damaging instrument
- Easy recovery when “stuck in action”
- Low footprint and easy access to cabinet
- Switch between automated and manual mode in seconds

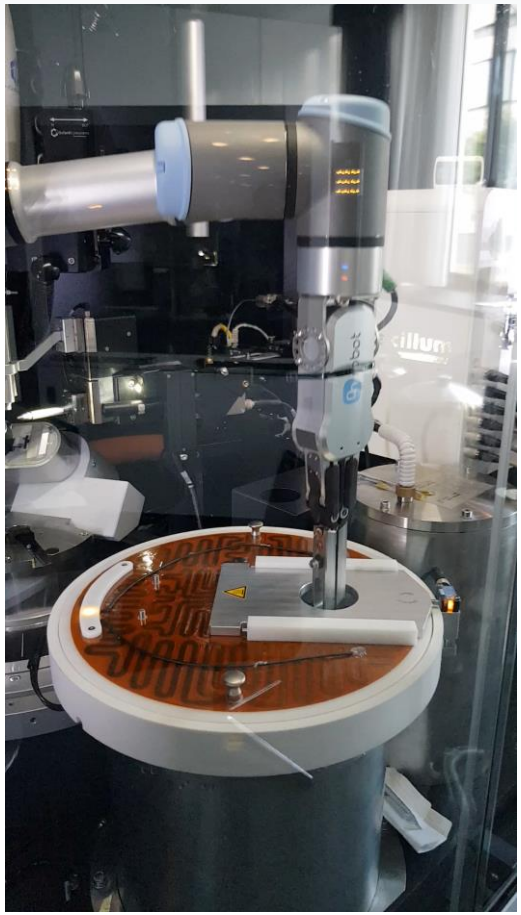
SCOUT
Cryogenic system

- Uses a unique Twin Dewar design to minimize icing:
  - Smooth refill without turbulence to minimize icing
  - Thick insulating air layer under the lid
  - Fully closed design with stable storage environment and minimized icing
- Fast and simple puck loading – tapered puck guidance pins
- Puck recognition in s/w
- 3 UNIPUCKS with up to 48 crystals
- UNIPUCKS and Starter Kit (optional)

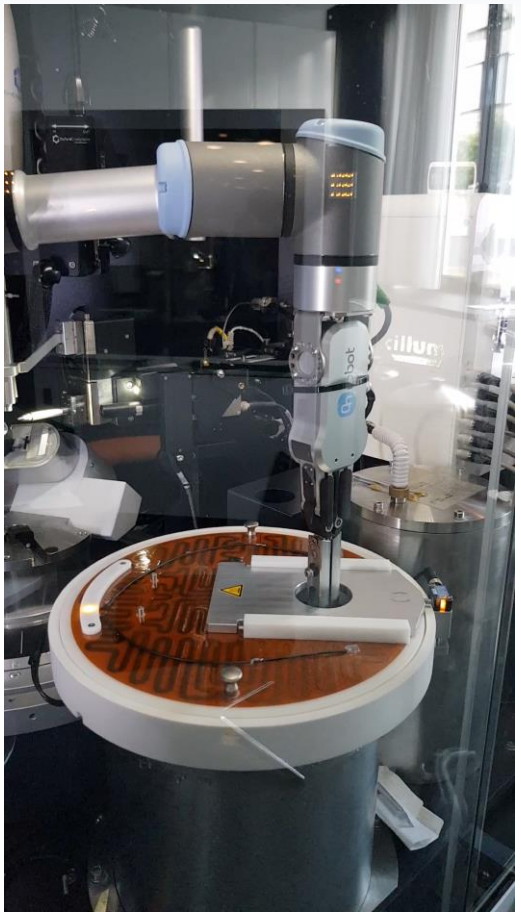




SCOUT sample changer
Very fast sample exchange times

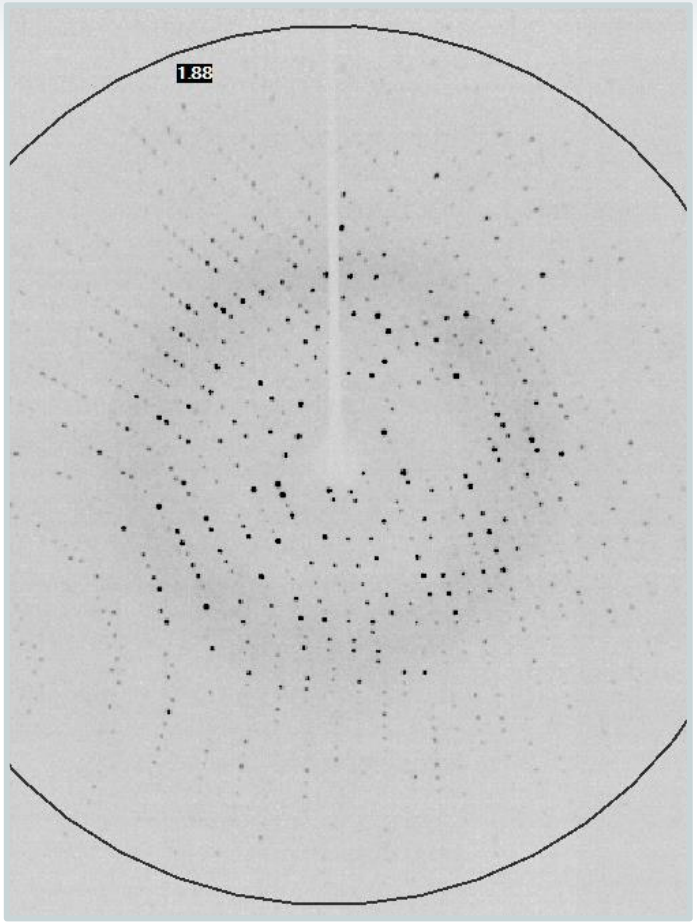


Sample load

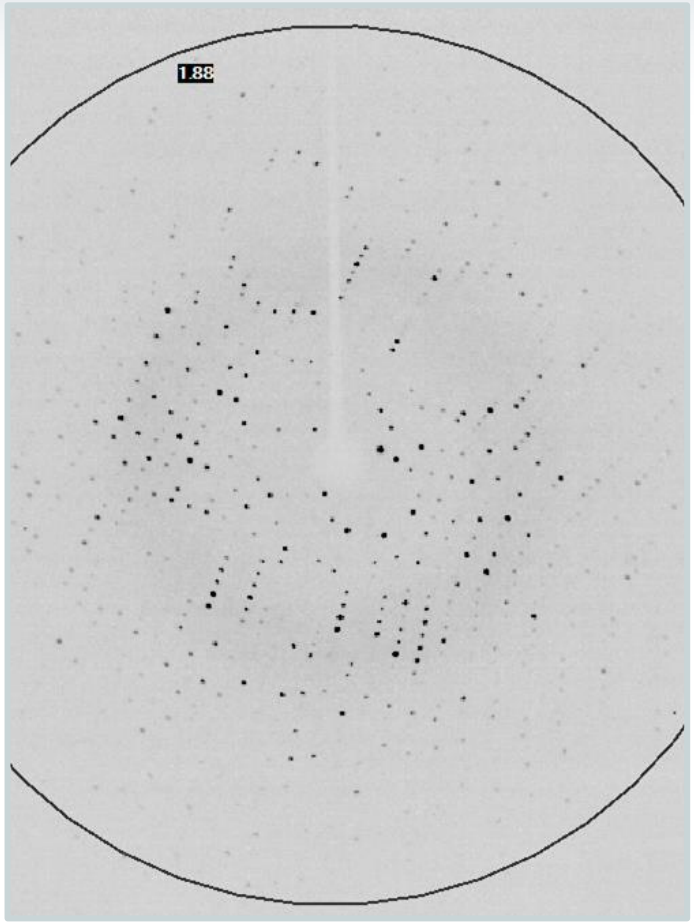


Sample unload

SCOUT Performance
Safe crystal transport and storage

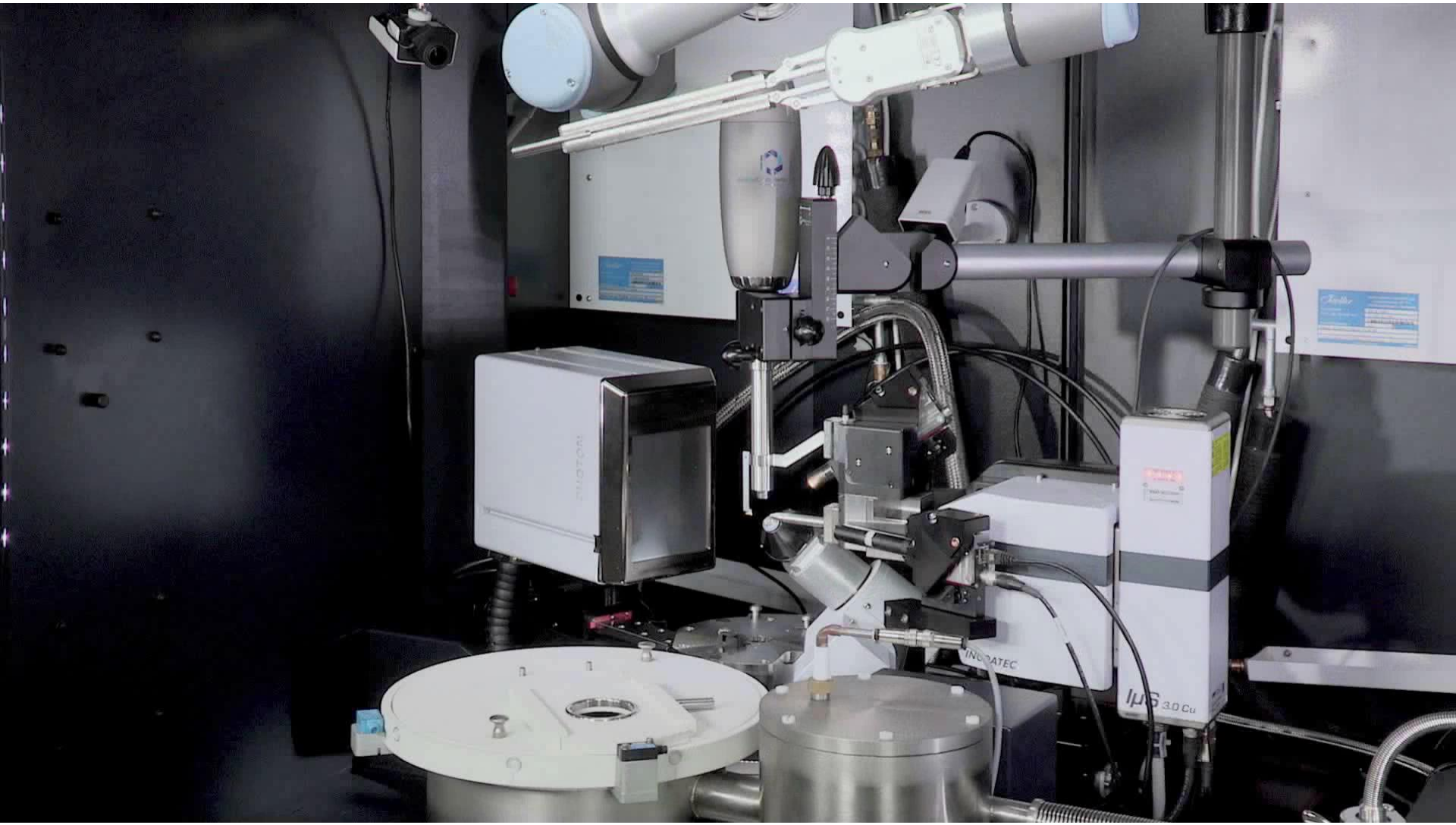


- Initial diffraction



- Same diffraction quality after five load/unload cycles of the same sample

SCOUT in action
Components and hardware features



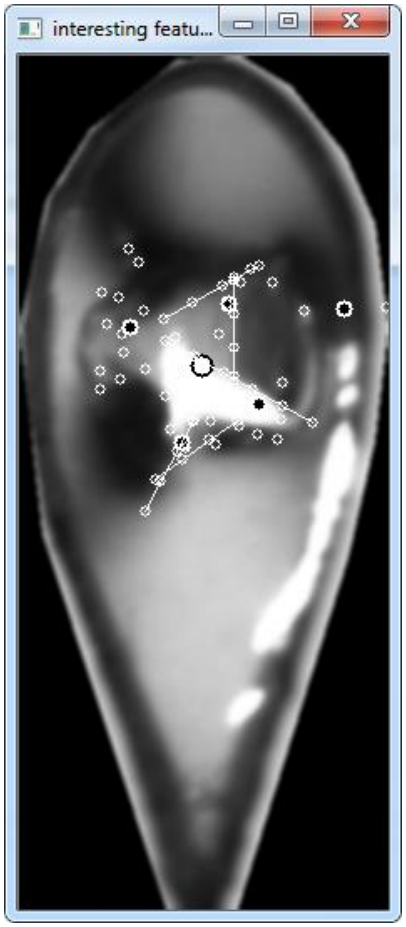
SCOUT
AGH - The Automated Goniometer Head

- **Compact Automated Goniometer Head (AGH)** for D8 QUEST and D8 VENTURE system with KAPPA goniometer (FIXED-CHI will follow)
- **Motorized sample alignment** for easy sample centering
- **Piezo-actuator** for high reliability and long life time
- **Pin-present sensor** eliminates sample crashes
- **Full software control** for fast two-click optical alignment
- **Automated X-ray centering** ensures that the best diffracting part of the sample is in the beam (finds crystals in LCP)
- **Centering time** less than 2min

AGH
Visual loop centering

- Advanced pattern-recognition algorythms
- Reliable, near 100% success rate
- Loop centering completed in 2 minutes

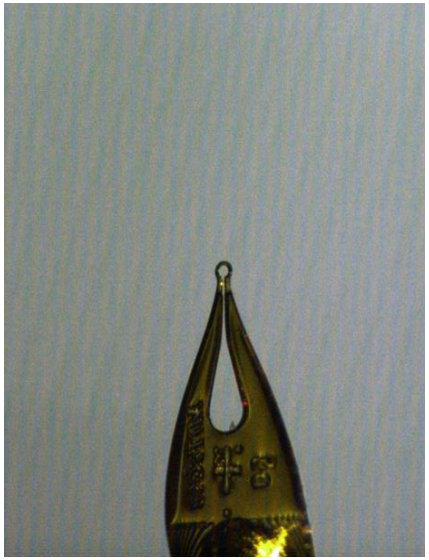
- Collect omega-phi scan: collect 60 images
- For these images:
  - Determine and subtract background to produce a binary b/w image
  - Find contour around foreground
  - Now only the loop remains
  - Determine center of the remaining contour
  - find area with most “features” inside above contour where features are defined as pieces of straight lines and edges and determine the center of these areas



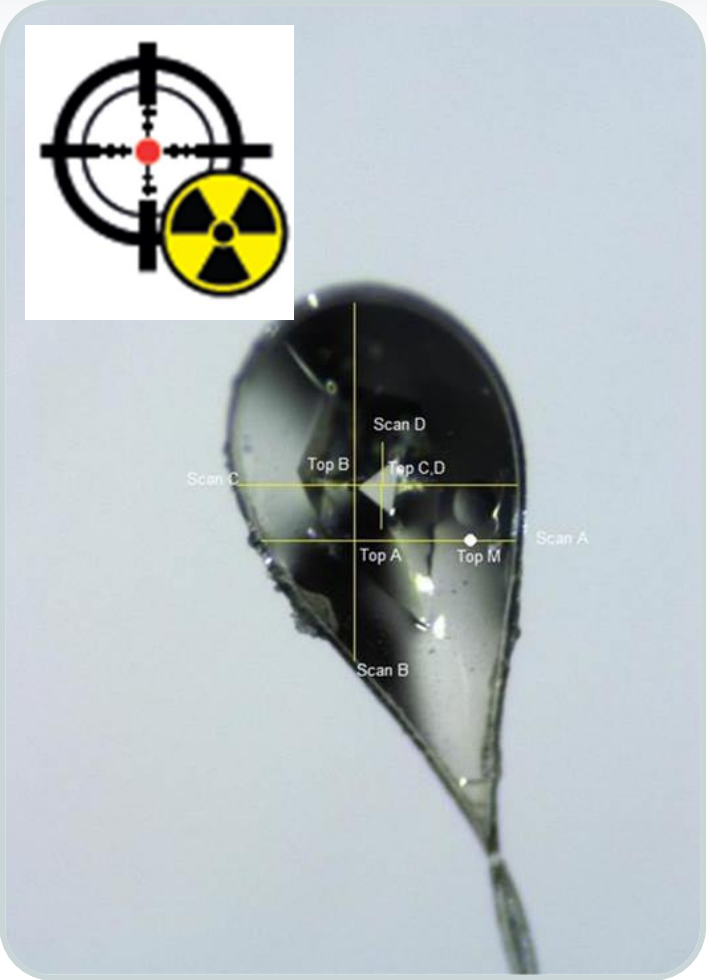
AGH

The AGH: compatible with all loop types

Experimenters basically use 2 type of mounts:
Loops and micromounts



AGH
X-ray crystal centering



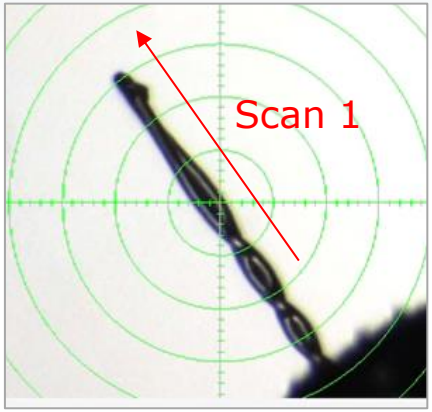
- Fully automated
- Improve precision after optical centering
- Get best diffracting part of the sample into the beam (based on overall intensity or resolution)
- Centers the sample and not the loop (USP)
- Takes a few minutes only
- Uses scanning feature of the AGH
- Ideal for centering very small crystals which are difficult to see in the microscope
- Ideal for centering crystals hidden in LCP (LCP is a method of growing crystals of membrane proteins)

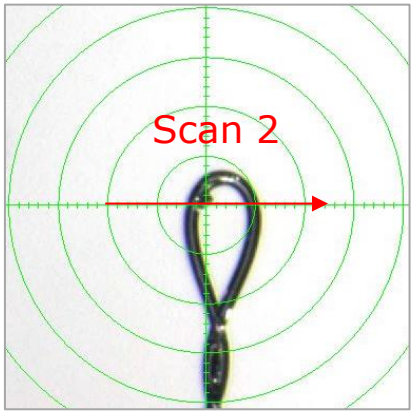
AGH
X-ray crystal centering

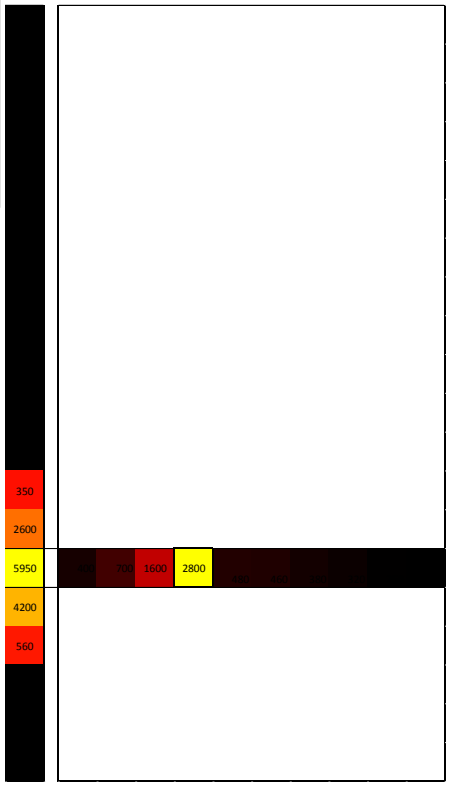
- Collects a sequence of quick X-ray images while performing a 2D scan of the loop
- Generates heat map to identify the strongest crystal diffraction
- Centres sample in at the intersection

- 10 μm step-size (100 μm beam)
- 1 s per exposure

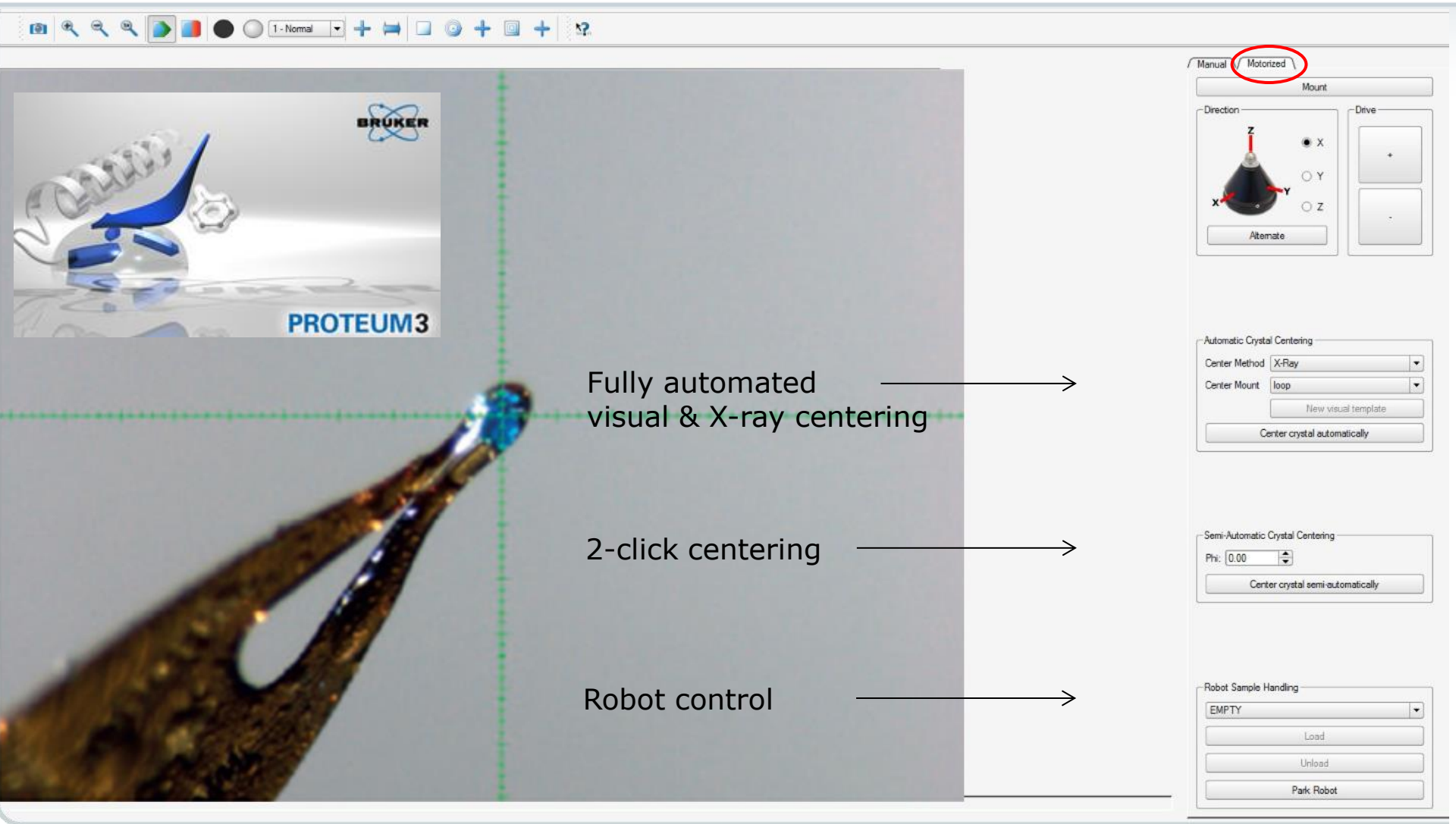
- Crystal 5 microns
- 30s automatic X-ray centering





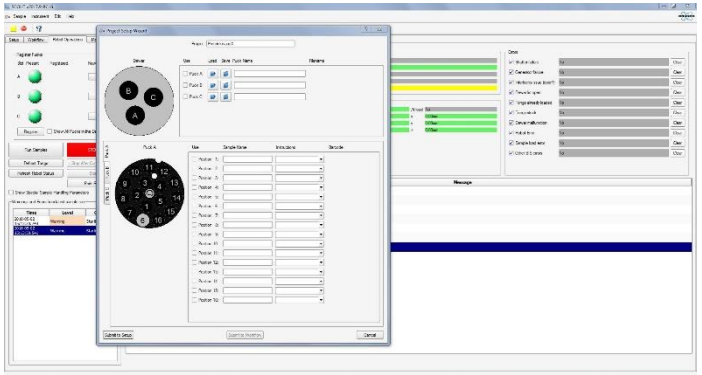


AGH standalone
Semi-automated operation using PROTEUM3

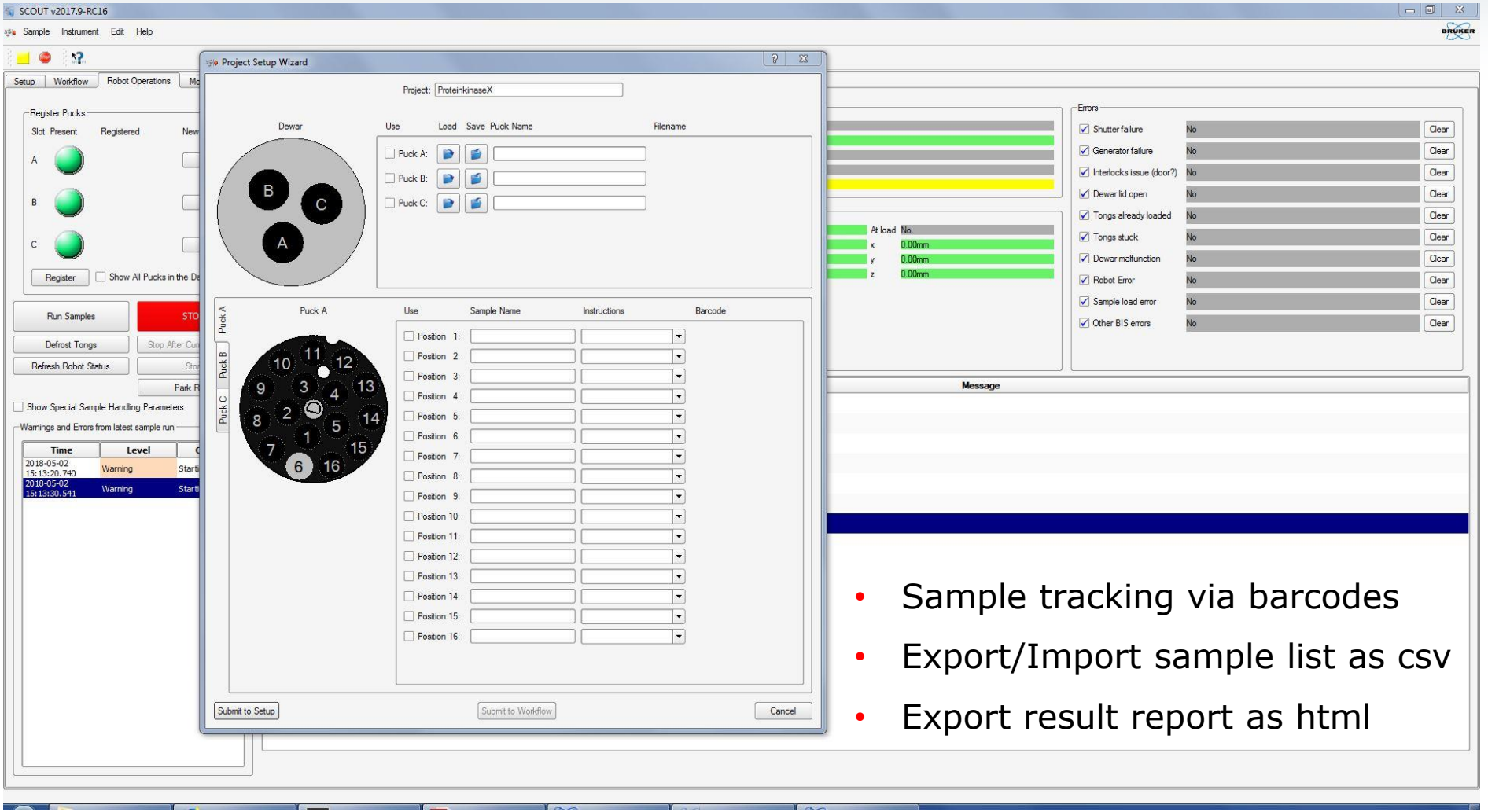


SCOUT Software
Software functionality

- Hardware management
- Sample tracking
- Automated sample mounting and centering
- Automated crystal screening
- Automated crystal scoring
  - Score and rank your crystals put forward the best for data collection
- Task management
- Automated data collection
  - User defined or automated strategy determination
- Sample reorganization
  - Prepare pucks for your synchrotron trip
- Remote data collection
  - Load your pucks and go home

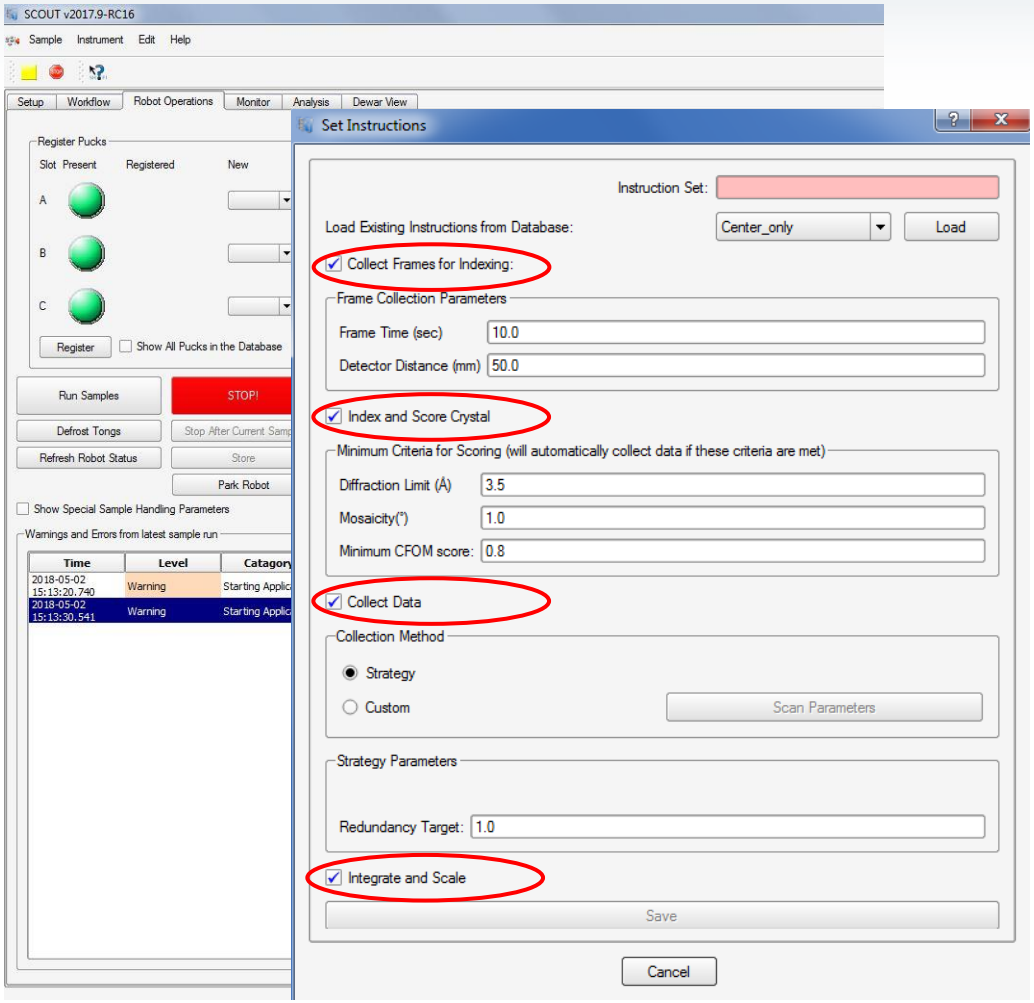


SCOUT
sample tracking for your convenience



- Sample tracking via barcodes
- Export/Import sample list as csv
- Export result report as html

SCOUT
Integration into the pipeline of your choice



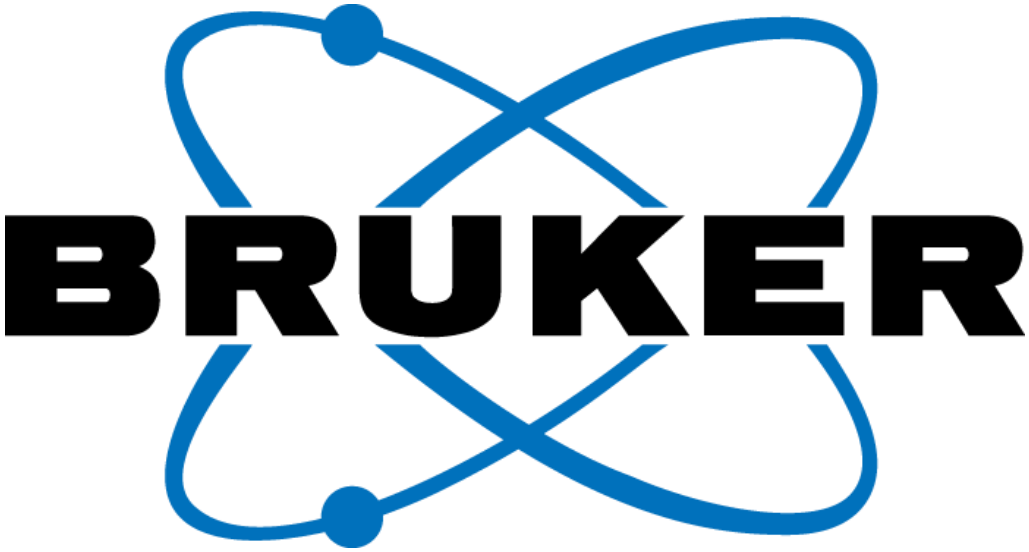
- Workflow includes: - indexing
  - strategy
  - data collection
  - integration
  - scaling
- Data export: hkl or mtz file

SCOUT
Summary

- Extend instrument operation to 24/7
- Protects precious crystals
- Fast, reliable
- Fully automated, prepare and monitor experiments remotely
- Find well-diffracting crystals faster
- Solve more structures

- Fully integrated into D8 VENTURE
- Full safety certification
- Synchrotron-like operation
- Compatible with all Bruker X-ray sources
- Compatible with KAPPA goniometer
- Compatible with ISX STAGE
- X-ray crystal centering
- Collect high-resolution data

- AGH available as standalone accessory



Innovation with Integrity