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Title: High-Throughput Serial Tomography Using an Automated Sample-Changing Robot

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

If **Yes**, how far apart are the locations?

Filming will be in the same building (I13-2 beamline of Diamond Light Source), however we will shoot in the different rooms experiment control room, experiment hutch and I13 chemical lab all are bout 20m from each other.

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 15

Number of Shots: 48

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that the questions will not appear on screen. Please answer in stand-alone full sentence with sufficient context. The answers have been revised for length, clarity, and consistency with journal style guidelines.

INTRODUCTION:

~~**REQUIRED:** Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Christoph Rau:** This protocol describes automated sample exchange using a robot in I13-2, X-ray micro-tomography beamline of Diamond Light Source synchrotron facility.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer's NOTE: Christoph changed his lines from the introduction. He felt it was a bit too wordy and wanted the sentences to something he found more comfortable.

Shashidhara had a tough time remembering his lines for **Shot 1.2.1**, and avoiding eye contact of the camera when speaking. There are a handful of takes because of this. I would say **Takes 5 & 7 of Shot 1.2.1** are usable

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Shashidhara Marathe:** Manual sample exchange and alignment often exceed tomography scan time; this protocol automates them, improving beamtime efficiency and experimental throughput.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Christoph Rau:** This protocol enables preparation of robot-compatible samples, allowing high-throughput acquisition of statistically significant datasets for data-intensive scientific studies.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~**Describe the most important consideration or challenge when performing this protocol.**~~

- 1.4. **Shashidhara Marathe:** Sample preparation and sample alignment are two critical steps for the automatic sample changer robot to work correctly and effectively.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~**How can future studies build on this work? Describe the questions or research directions that can be investigated next.**~~

- 1.5. **Shashidhara Marathe:** Future studies can develop larger grippers for bigger samples and improve designs by incorporating side-approach mechanisms.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

1.6 Prof. Christoph Rau, Principal Beamline Scientist, Diamond Light Source Ltd.: (authors will present their testimonial statements live)

1.6.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What is the key takeaway you hope viewers remember after watching your video article?

1.7 Prof. Christoph Rau, Principal Beamline Scientist, Diamond Light Source Ltd.: (authors will present their testimonial statements live)

1.7.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Sample Preparation for Robot Experiments

Demonstrators: Shashidhara Marathe, Christoph Rau

2.1. To begin, ensure all the paperwork and safety training needed for the experiment are complete [1]. ~~Keep insect samples from the Natural History Museum mounted using CryoPin on SPINE (spine) compatible bases with Data Matrix barcodes, ready for the experiment [2-TXT]. Document the Data Matrix code associated with each sample [3]. Mount the CryoPin on the CryoCap [4]. Verify that the mounted sample assembly is properly secured [5].~~

2.1.1. WIDE: Talent checking the permissions and safety training certificates.

2.1.2. ~~CU: Shot of the insect samples mounted with a CryoPin on SPINE compatible sample bases equipped with a Data Matrix barcode. TXT: SPINE – Structural Proteomics in Europe~~ *Videographer: Please capture the mounted insect sample from different angles so that the pin, base, and barcode are seen clearly*

2.1.3. ~~SCREEN: Talent recording the Data Matrix code for a mounted sample.~~
Videographer's NOTE: This was a talent shot

2.1.4. ~~Talent mounting the CryoPin on the CryoCap~~ **NOTE: Don't use**

2.1.5. ~~Talent verifying that the assembly is properly positioned and secured.~~

NOTE: Move step 2.2 after step 2.4

2.2. Confirm that the mounted sample fits within one of the robot-compatible size envelopes [1]. Ensure that the mounted configuration is centered on the base [2] that the total height of the sample, mount, and base does not exceed 27 millimeters [3].

Videographer's NOTE: Shots 2.2.1, 2.2.2 & 2.2.3 were combined together. Take two of this series is the best.

2.2.1. Talent checking the mounted sample against the allowed size envelopes for robot handling.

2.2.2. Talent centering the mounted configuration on the sample base.

2.2.3. Talent checking the total height of the sample, mount, and base.

2.3. When mounting samples on SPINE sample pins, position the sample on top of the pin with no part obscured by the pin shaft [1-TXT]. Mount the sample vertically and centrally on the pin base [2].

NOTE: Shots 2.3.1 & 2.3.2. These two shots were combined together.

2.3.1. Talent positioning the sample on top of the sample pin. **TXT: SPINE - Structural Proteomics in Europe**

2.3.2. Talent adjusting the sample to a vertical and central position on the pin base.

2.4. Fix the pin securely and vertically onto the sample base [1]. Use a securing method or reliable adhesive to prevent detachment [2].

Videographer's NOTE: 2.4.1 and 2.4.2 were combined

2.4.1. Talent fixing the mounting element vertically onto the sample base.

2.4.2. Talent applying an adhesive or securing method to the mounting element.

NOTE: Show step 2.2 here after 2.4 and before 2.5

2.5. Check that all samples are positioned at a consistent height on the sample pin [1] and document the Data Matrix code associated with each sample [2].

2.5.1. Talent comparing sample heights across multiple mounted samples.

Videographer's NOTE: Shot 2.5.1 Take 2 is the best to use in my opinion. I advised the Author to adjust his head position after Take 1. I felt this was for the best for the shot

2.1.3,. Talent recording the Data Matrix code for a mounted sample. **NOTE: 2.1.3 is moved here**

2.6. Generate samples.csv (*samples c-s-v*) file that links each sample name to its corresponding sample-base Data Matrix code and includes the parameters required for running tomography scans [1].

2.6.1. SCREEN: 2026-05-27_2.5.2.mp4. 00:18-00:24 and 01:05-01:10

3. X-Ray System Setup and Automated Sample Alignment

3.1. Shift the full spectrum or filter the X-ray spectrum to achieve the required contrast [1]. Optimize the camera's sampling distance based on the X-ray spectrum and the required phase-contrast level for the sample [2]. Select the microscope magnification based on

the sample size and the required field of view [3]. Select the corresponding microscope objective [4] and align the camera's horizontal position along the x-axis of the laboratory with respect to the X-ray beam [5].

3.1.1. SCREEN: 2026-05-27_3.1.1.mp4 00:10-00:17 and 01:14-01:24

3.1.2. Shot of the stages moving in the experimental hutch the distance between the camera and the sample is being adjusted. **Videographer's NOTE: Shots 3.1.2 & 3.1.5 had variations of shots. Use the shots that had 3.1.2 & 3.1.5 combined together on the Clapperboard instead. The first take is a close up and the second take is a wide. Both of these takes can be cut together. The Author told me he preferred to have a sample on the robot**

3.1.3. SCREEN: 2026-05-27_3.1.3.mp4. 00:00-00:10

3.1.4. SCREEN: 2026-05-27_3.1.4.mp4.

3.1.5. Shot of the camera being aligned horizontally relative to the X-ray beam.

3.2. Check the samples and sample mounting thoroughly at the start of the experimental session for damage or loose mounting and inform the users [1]. Mount the samples on the respective puck and puck slots identified in the samples.csv file [2]. Perform a dry run of the robot sample exchange procedure on a few samples [3].

3.2.1. Talent inspecting multiple mounted samples at the start of the session and noting the details of any damage.

3.2.2. Talent placing samples into the specified puck and puck slots.

3.2.3. Talent initiating a dry run of the robot sample exchange procedure.

3.3. Use a sample base with a sample pin for aligning the axis of rotation of the rotation stage to the center of the detector in the horizontal direction [1]. Move the top of the pin to the center of the X-ray camera [2]. Align the telescope camera, set at 90 degrees to the beam, to the center of the field of view [3]. Determine the coordinates of this position in telescope camera pixel coordinates [4]. Update the sample target position in the script that handles sample alignment [5].

3.3.1. Talent placing an empty sample pin on the rotation stage for alignment.

3.3.2. Talent moving the top of the pin to the center of the X-ray camera.

3.3.3. Talent aligning the telescope camera to the center of the field of view. Videographer's NOTE: Shot 3.3.3 was broken up into two shots. The first part has the clapperboard showing 3.3.3 (clip C0088) and the second part has 3.3.3.B (clip C0096) showing on the board

3.3.4. SCREEN: 2026-05-27_3.3.4.mp4.

3.3.5. SCREEN: 2026-05-28_3.3.5_T1.mp4– 00:04 – 00:10 *Video editor: Highlight the “target : [355,260]” line where they are entering the number 355.*

3.4. After defining the target coordinates, mount a representative sample [1]. Run the automatic sample alignment script to place the sample in the center of rotation on the telescope camera [2]. Check whether the sample is centered in the X-ray data acquisition camera’s field of view [3].

3.4.1. Talent mounting a representative sample onto the stage.

3.4.2. SCREEN: 2026 05 27_3.4.2.mp4

3.4.3. SCREEN: 2026 05 27_3.4.2_T2.mp4 00:18 – 00:20 and 68273_3.4.3_t1.mp4 00:38-00:42

3.5. Repeat the previously demonstrated alignment procedure if the region of interest is not centered in the field of view [1]. Change the target position coordinates to refine the sample alignment [2]. Update the camera exposure time and the number of projections to acquire in the samples.csv file [3].

3.5.1. Representative shot of the talent repeating the sample alignment procedure.

Videographer’s NOTE: Shot 3.5.1 Take 2 is the take to use. Take 1 of this shot had the script in the shot

3.5.2. SCREEN: 2026-05-28_3.5.2_T1.mp4 00:03 - 00:09. *Video editor: Highlight the “target : [350,260]” line where they are entering the number 350*

3.5.3. SCREEN: 2026-05-28_3.5.3.mp4 00:05 to 00:20

4. Tomography Data Acquisition and Reconstruction

Demonstrator: Shashidhara Marathe

4.1. Mount all samples on the correct puck and pin positions as instructed in the samples.csv file [1]. Use the Generic Data Acquisition or GDA (*G-D-A*) workbench for data collection [2]. Issue the command tomomatic.load (*tomomatic dot load*) in the GDA terminal to load the file [3]. Load the input file [4] and , issue tomomatic.scan (*tomomatic dot scan*) to run the command [5].

4.1.1. Talent mounting all samples onto the designated puck and pin positions.

4.1.2. SCREEN: 2026-05-27_4.1.2_T2.mp4.

4.1.3. SCREEN: 2026-05-27_4.1.3_T1.mp4. *Video editor: Highlight the bottom of the screen where “tomomatic” is being typed*

4.1.4. SCREEN: 2026-05-27_4.1.4-4.1.5-4.3.2-4.4.2.mp4 00:27 – 00:38

4.1.5. SCREEN: 2026-05-27_4.1.4-4.1.5-4.3.2-4.4.2.mp4 00:42 – 00:51

- 4.2. Monitor the robot transferring the sample from the loading tray to the laser barcode reader [1]. Confirm that the barcode is read [2]. Ensure that the robot moves the sample to the tomography stage and mounts it correctly [3].

NOTE: Use 68273 4.2.2T1 LAB MEDIA for this step

4.2.1. Talent monitoring the robot as it transfers the sample from the loading tray to the barcode reader.

4.2.2. Shot of the sample barcode being read by the barcode reader.

4.2.3. Talent monitoring the robot as it mounts the sample onto the tomography stage.

- 4.3. After the tomomatic script starts, monitor the auto-center-pin function being executed to place the sample into the center of rotation [1]. Observe the stages as they move to the scanning position [2] before the tomo-fly-scan (*tomo-fly-scan*) function collects dark-field, flat images, and projection images [3].

4.3.1. SCREEN: 2026-05-27_4.1.4-4.1.5-4.3.2-4.4.2.mp4 02:22 -02:32 .

4.3.2. The stages are moving to the scanning position. **NOTE:** Use videographer's clip here

Videographer: Please capture the stages moving to the scanning position as a backup.

4.3.3. SCREEN: 2026-05-27_4.1.4-4.1.5-4.3.2-4.4.2.mp4 02:39 -02:56 and 07:20-07:21

- 4.4. Collect projection images with the sample in the beam at the sample rotation angle intervals indicated in the samples.csv file [1]. Acquire 3001 projection images [2].

4.4.1. SCREEN: 2026-05-27_4.4.1_T1.mp4 00:16-00:29

4.4.2. SCREEN: 2026-05-27_4.4.1_T1.mp4 01:24-01:36

- 4.5. Access the saved data by browsing to the appropriate file system and folder [1]. Use the Savu reconstruction pipeline to generate 2D slices from projection images [2]. Use 3-dimensional (*3-dimensional*) rendering software such as AVIZO (*avizo*) or Image-J to access and visualize the reconstructed images [3].

4.5.1. Talent browsing the file system to open the data folder.

4.5.2. SCREEN: 2026-05-28_4.5.2_T1.mp4 00:46- 00:52 and 2026-05-28_4.5.2_T2.mp4 00:20-00:34

4.5.3. SCREEN: 2026-05-27_4.5.3.2.mp4 00:40-01:00

Results

5. Results

5.1. Normalized projection images from a representative insect sample were obtained using 1.25x [1] and 4x magnification during the tomography experiment [2].

5.1.1. LAB MEDIA: Figure 4 *Video editor: Highlight the top row images*

5.1.2. LAB MEDIA: Figure 4. *Video editor: Highlight the bottom row images*

5.2. Projection images were acquired at 0 degrees [1], 90 degrees [2], and 180 degrees for both magnifications [3].

5.2.1. LAB MEDIA: Figure 4. *Video editor: Highlight the label 0° and the panels A and D*

5.2.2. LAB MEDIA: Figure 4. *Video editor: Highlight the label 90° and the panels B and E*

5.2.3. LAB MEDIA: Figure 4. *Video editor: Highlight the label 180° and the panels C and F*

5.3. Horizontal or transverse slices from the tomography reconstruction were well-centered within the field of view at both 1.25x [1] and 4x magnifications [2].

5.3.1. LAB MEDIA: Figure 5. *Video editor: Highlight panel A labeled 1.25x*

5.3.2. LAB MEDIA: Figure 5. *Video editor: Highlight panel B labeled 4x*

1. CryoPin

Pronunciation link: <https://www.howtopronounce.com/cryopin>

IPA: /'kraɪoʊ.pɪn/

Phonetic Spelling: kry·oh·pin

2. CryoCap

Pronunciation link: <https://www.howtopronounce.com/cryocap>

IPA: /'kraɪoʊ.kæp/

Phonetic Spelling: kry·oh·kap

3. Data Matrix
Pronunciation link: <https://www.merriam-webster.com/dictionary/matrix>
IPA: /'dɛrtə 'mɛtrɪks/, /'dætə 'mɛtrɪks/
Phonetic Spelling: day·tuh may·triks
4. Barcode
Pronunciation link: <https://www.merriam-webster.com/dictionary/barcode>
IPA: /'bɑːrˌkoʊd/
Phonetic Spelling: bar·kohd
5. Proteomics
Pronunciation link: <https://www.merriam-webster.com/dictionary/proteomics>
IPA: /ˌproʊtiˈɑːmɪks/
Phonetic Spelling: proh·tee·aa·miks
6. Tomography
Pronunciation link: <https://www.merriam-webster.com/dictionary/tomography>
IPA: /tə'mɑːɡrəfi/
Phonetic Spelling: tuh·maa·gruh·fee
7. Spectrum
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrum>
IPA: /'spɛktrəm/
Phonetic Spelling: spek·truhm
8. Phase Contrast
Pronunciation link: <https://www.merriam-webster.com/dictionary/phase>
IPA: /feɪz 'kənˌtræst/
Phonetic Spelling: fayz kon·trast
9. Magnification
Pronunciation link: <https://www.merriam-webster.com/dictionary/magnification>
IPA: /ˌmæɡnəfə'keɪʃən/
Phonetic Spelling: mag·nuh·fuh·kay·shuhn
10. Microscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscope>
IPA: /'maɪkrəˌskoʊp/
Phonetic Spelling: my·kruh·skohp
11. Objective
Pronunciation link: <https://www.merriam-webster.com/dictionary/objective>
IPA: /əb'dʒɛktɪv/
Phonetic Spelling: ub·jek·tiv
12. X-ray
Pronunciation link: <https://www.merriam-webster.com/dictionary/X-ray>
IPA: /'ɛksˌreɪ/
Phonetic Spelling: eks·ray
13. Detector
Pronunciation link: <https://www.merriam-webster.com/dictionary/detector>
IPA: /dɪ'tɛktər/
Phonetic Spelling: dih·tek·ter

14. Coordinates
Pronunciation link: <https://www.merriam-webster.com/dictionary/coordinate>
IPA: /koʊˈɔrdənəts/
Phonetic Spelling: koh·or·duh·nuhts
15. Acquisition
Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>
IPA: /ˌækwəˈzɪʃən/
Phonetic Spelling: ak·wuh·zish·uhn
16. Generic Data Acquisition
Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>
IPA: /dʒəˈnærɪk ˈdeɪtə ˌækwəˈzɪʃən/, /dʒəˈnærɪk ˈdætə ˌækwəˈzɪʃən/
Phonetic Spelling: juh·ner·ik day·tuh ak·wuh·zish·uhn
17. Projection
Pronunciation link: <https://www.merriam-webster.com/dictionary/projection>
IPA: /prəˈdʒɛkʃən/
Phonetic Spelling: pruh·jek·shuhn
18. Reconstruction
Pronunciation link: <https://www.merriam-webster.com/dictionary/reconstruction>
IPA: /ˌrɪkənˈstrʌkʃən/
Phonetic Spelling: ree·kuhn·struk·shuhn
19. AVIZO
Pronunciation link: <https://www.howtopronounce.com/avizo>
IPA: /əˈviːzoʊ/
Phonetic Spelling: uh·vee·zoh
20. ImageJ
Pronunciation link: <https://imagej.net/>
IPA: /ˈɪmɪdʒ dʒeɪ/
Phonetic Spelling: im·ij jay
21. Transverse
Pronunciation link: <https://www.merriam-webster.com/dictionary/transverse>
IPA: /trænzˈvɜːs/, /trænsˈvɜːs/
Phonetic Spelling: tranz·vurs
22. Rotation
Pronunciation link: <https://www.merriam-webster.com/dictionary/rotation>
IPA: /roʊˈteɪʃən/
Phonetic Spelling: roh·tay·shuhn
23. Alignment
Pronunciation link: <https://www.merriam-webster.com/dictionary/alignment>
IPA: /əˈlaɪnmənt/
Phonetic Spelling: uh·line·muhnt
24. Experimental
Pronunciation link: <https://www.merriam-webster.com/dictionary/experimental>
IPA: /ɪkˌspɛrəˈmentəl/
Phonetic Spelling: ik·sper·uh·men·tl

25. Representative

Pronunciation link: <https://www.merriam-webster.com/dictionary/representative>

IPA: /ˌrɛprɪˈzɛntətɪv/

Phonetic Spelling: rep·ruh·zen·tuh·tiv