Physica Medica xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Physica Medica

journal homepage: http://www.physicamedica.com

Original paper

A multi-purpose imaging endstation for high-resolution micrometer-scaled sub-second tomography

Goran Lovric [a,b,*], Rajmund Mokso [c], Christian M. Schlepütz [a], Marco Stampanoni [a,d]

[a] Swiss Light Source, Paul Scherrer Institute, 5232 Villigen, Switzerland
[b] Centre d'Imagerie BioMédicale, École Polytechnique Fédérale de Lausanne, Lausanne 1015, Switzerland
[c] Max IV Laboratory, Lund University, SE-221 00 Lund, Sweden
[d] Institute for Biomedical Engineering, ETH Zurich, 8092 Zurich, Switzerland

ARTICLE INFO

Article history:
Received 9 May 2016
Received in Revised form 13 August 2016
Accepted 16 August 2016
Available online xxxx

MSC:
92C55
92C50

Keywords:
X-ray imaging
In vivo
Time-resolved tomography 2010

ABSTRACT

Time-resolved imaging of dynamic processes, ranging from biological *in vivo* studies to materials under *in situ* and *in operando* conditions, requires a flexible endstation capable of controlling complex components that interact in different configurations and at high speeds. At the X02DA TOMCAT beamline we have recently achieved *in situ* tomographic measurements at a rate of 20 Hz. Independently, we have shown the feasibility of *in vivo* lung imaging down to the micrometer scale. In the present paper, we discuss the latest developments in view of instrumentation and the accompanying components for achieving these two types of measurements. As the prime example, we focus on the technical requirements for *in vivo* tomographic microscopy of the lung at the micrometer scale in terms of acquisition schemes, triggering and radiation dose. We identify ultra-short single-projection exposures combined with accurate triggering capabilities as the main prerequisites to obtain high-quality reconstructions while limiting the X-ray dose imparted on the living sample. The presented endstation offers generic high-speed imaging capabilities, as it is compatible with a variety of experimental setups and suitable for a wide range of time-resolved studies.

1. Introduction

Capturing in three dimensions the time-resolved structural information of fast dynamic processes requires short exposure times, high frame rates and efficient tomographic acquisition protocols. At the tomography beamline [1] of the Swiss Light Source, we advanced high-resolution live animal imaging by correlating the individual frames with the motion of the living sample [2]. These correlative approaches coupled with prospective or retrospective gating perform particularly well in the case of essentially periodic processes such as those found in many biological systems. More recently, *in situ* crack propagation dynamics during tensile tests using synchrotron tomography were reported for the first time with a frequency of 20 tomograms per second [3]. In all cases, advanced triggering and synchronization is indispensable for capturing the dynamics at the desired state, which can either be externally induced, for example, by a compression rig or a high temperature furnace [4], or occur spontaneously and autonomously, as is the case for most *in vivo* samples [5]. Regardless of whether materials science or biological applications are concerned, the efficient integration of the sample controls and monitoring systems (furnace, small animal ventilator, compression rig, etc.) into the beamline infrastructure plays a central role in reducing the X-ray exposure, and hence the radiation dose received by the sample, while resolving the process under investigation in the greatest achievable detail.

Commercially available scanners are capable of routinely performing *in vivo* X-ray computed tomography of small animals down to a spatial resolution of about 10 μm [6]. At this level of detail, the synchronization of the image acquisition with the biological processes such as the heart beat is solved. New insights into the functional anatomy of various organs may, however, be gained by improving the spatial and temporal resolution further. As a result, the corresponding motion synchronization becomes very challenging. Likewise, in high-resolution X-ray imaging with dose-sensitive samples, the radiation dose is directly linked to the desired spatial resolution, where resolving more and finer details means also a higher X-ray dose. Due to these technical ;dif-

* Corresponding author at: Swiss Light Source, Paul Scherrer Institute, 5232 Villigen, Switzerland.
E-mail address: goran.lovric@psi.ch (G. Lovric).

http://dx.doi.org/10.1016/j.ejmp.2016.08.012
1120-1797/

ficulties in pushing the 3D spatial resolution to below about 10 μm, *in vivo* synchrotron-based lung imaging studies have been restricted until recently to mainly radiographic and particle tracking type experiments [7–10].

To achieve micrometer-scale resolution tomography at X-ray doses compatible with *in vivo* experiments, we need to be able to interpret the X-ray phase shifting properties of the sample (phase contrast imaging) in addition to the attenuation map used in all conventional medical CTs and small animal μCTs [11]. This implies the use of synchrotron radiation sources for their superior coherence properties and the much higher X-ray flux necessary to achieve sufficiently short exposure times. We have recently detailed this aspect in the case of tomographic lung imaging at the level of alveoli, where we have shown with fresh *ex vivo* mouse samples that micrometer spatial and sub-second temporal resolutions are feasible [12].

In this paper we present a route to implementing micrometer-resolution fast *in vivo* tomographic microscopy of rodent lungs. The main focus lies on the data acquisition, where we describe the technical solutions in view of instrumentation enabling a wide range of synchrotron-based time-resolved studies. These technical aspects are then discussed in light of *in vivo* measurements aimed at gaining a deeper understanding of lung physiology at the microscopic level.

2. Experimental methods and instrumentation

2.1. Rotation stage design

Performing micro-tomographic experiments to image processes with ideally (sub-) micrometer resolutions, requires high-precision angular and translational sample positioning, in conjunction with a sample rotation that occurs at rotation speeds that can vary by orders of magnitude, depending on the respective tomographic acquisition setting. Thus, the mechanical errors (radial, axial wobble and tilt) must be kept at a minimum to ensure motion-less and artifact-free CT reconstructions. In particular, the motion errors should be kept below 100 nm at the sample position (about 150 mm above the rotation table) to allow for nanoscopic imaging [13].

Here, we propose a combined semi-custom designed sample-manipulator and rotation axes system. The complete system is depicted in Fig. 1, where both the rotation axes unit and its implementation on the optical table of the X02DA TOMCAT beamline are visible. On top of the rotation axes, two linear translation stages are used for aligning the desired region-of-interest (ROI) when conducting tomography. For powering these stages (both the encoders as well as the electrical stepper motors), a slip-ring with 60 lead-throughs is used which further enables the transfer of digital and analog signals from the rotating top of the rotation stage to the fixed part below the axis. Two additional vacant D-Sub/DB-9 connectors are available for arbitrary user-specific connections. For decoupling the high-precision angular movement from any mechanical parts and vibrations, the complete rotation axes unit is implemented as a combined air bearing rotation axis (ABRT-200, Aerotech Inc.) synchronized with a mechanical rotation stage (ADRS-150, Aerotech Inc.) to drive the slip ring. This design assures that the high-precision positioning accuracy of the air bearing stage is not affected by the drag of the slip-ring during rotation. Additional mechanical locks are installed between the two rotation stages to prevent potential cables twisting in case the two stages lose their synchronization (e.g. if one fails).

From the controls point of view, the axes pair is operated in a master–slave mode and interacts with the Aerotech software running on a dedicated PC with a real-time extension. The in-house developed control software interfaces to the hardware via an EPICS driver implementation [14], by which the rotation stage can be powered in different flexible modes to synchronize with the detector and/or other external devices by use of standard transistor-transistor logic (TTL) triggering. These triggering modes are implemented through so-called "tasks" in the Aerobasic programming language (representing an integrative part of the Aerotech A3200 software controller) and are mainly based upon Aerotech's position-synchronized output (PSO) system, of which the following are currently available:

1. **Fixed distance trigger**: The user first specifies an arbitrary angular distance (e.g. 180°) and width of a pulse (e.g. 10 ms). When the stage starts rotating, it fires trigger signals (voltages) toggling between "HIGH" (+5 V) and "LOW" (0 V), each time it has rotated through the angular distance. Additionally, an offset for the starting point can be set, as well as the total number of trigger signals to be sent.
2. **Snap and step**: An external trigger signal initiates a move by a user-defined rotation angle. The stage sends back a trigger signal once it has completed the requested move, and then waits for the next input trigger to start the subsequent move. Used in conjunction with a camera's "exposure" trigger (from the rotation stage output) and the cameras "busy" signal (with its trailing edge triggering the next angular motion), one obtains perfect synchronization between the data acquisition and sample rotation processes. This mode is used for high-precision "slow" tomography.
3. **Sequence mode**: This is the most flexible mode, where several trigger sequences can be defined. Each sequence defines a number of repetitions and two angular ranges: the TTL signal is set to "LOW" for the first and to "HIGH" for the second range. Multiple such sequences can be executed sequentially. This is best illustrated through an example: Setting both the "LOW" and "HIGH" ranges to 180°, the rotation axis will output a continuous "LOW" TTL signal for the first 180 degrees followed by a "HIGH" signal for the next 180 degrees, repeating this same pattern for subsequent 360° turns. This way, one can obtain a specified number of tomographic scans with identical orientations during the continuous rotation of the sample. This is particularly useful in various time-resolved studies and can be modified to match the exact needs of the experiment.

In addition to these modes, the velocity and acceleration (in both negative and positive rotation direction) can be arbitrarily set/changed for each measurement as well as different offsets can be defined.

2.2. Sample alignment

Sample alignment is performed using two linear translation stages on top of the rotation axis that are used for both centering the sample on the axis as well as defining the field of view (FOV) in (local) region-of-interest tomography. For this purpose, we have developed a so-called "off-beam sample alignment" procedure where the sample and the respective region of interest (ROI) is aligned prior to exposing the sample with X-rays. An additional alignment camera is placed perpendicular to the X-ray beam and interfaces to the beamline controls system via the "areaDetector" EPICS application [15]. The detailed scheme is shown in Fig. 2 and can be explained as follows. Using an alignment pin in the X-ray beam, the ROI (red square in Fig. 2) on the alignment camera is adjusted in size and position to match the field of view of the X-ray detector. The centering and ROI selection on the real samples is then performed without exposure to X-rays by monitoring their position purely with the live preview video. Subsequently, the

Please cite this article in press as: Lovric G et al. A multi-purpose imaging endstation for high-resolution micrometer-scaled sub-second tomography. Phys. Med. (2016), http://dx.doi.org/10.1016/j.ejmp.2016.08.012