












# X-ray phase contrast imaging of endomyocardial biopsy samples preserved in formalin and embedded in paraffin – a comparison of tissue preparation methods

 Nikola Škreb<sup>1\*</sup>,  
 Filip Lončarić<sup>1</sup>,  
 Anne Bonnin<sup>2</sup>,  
 Hector Dejea<sup>3</sup>,  
 Ivana Ilić<sup>1</sup>,  
 Hrvoje Gašparović<sup>1</sup>,  
 Boško Skorić<sup>1</sup>,  
 Bart Bijmens<sup>4,5</sup>,  
 Davor Miličić<sup>1</sup>,  
 Ivo Planinc<sup>1</sup>,  
 Maja Čikeš<sup>1</sup>

<sup>1</sup>University Hospital Centre Zagreb, University of Zagreb School of Medicine, Zagreb, Croatia

<sup>2</sup>Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland

<sup>3</sup>European Synchrotron Radiation Facility, Grenoble, France

<sup>4</sup>Instituto de Investigaciones Biomédicas August Pi i Sunyer (IDIBAPS), Barcelona, Spain

<sup>5</sup>Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

**KEYWORDS:** heart transplantation, graft rejection, synchrotron imaging, histology.

**CITATION:** *Cardiol Croat.* 2023;18(11-12):314-5. | <https://doi.org/10.15836/ccar2023.314>

**\*ADDRESS FOR CORRESPONDENCE:** Nikola Škreb, Klinički bolnički centar Zagreb, Kišpatićeva 12, HR-10000 Zagreb, Croatia. / Phone: +385-92-380-4962 / E-mail: [nikola.skreb7@gmail.com](mailto:nikola.skreb7@gmail.com)

**ORCID:** Nikola Škreb, <https://orcid.org/0000-0003-1730-8768> • Filip Lončarić, <https://orcid.org/0000-0002-7865-1108> • Anne Bonnin, <https://orcid.org/0000-0001-5537-8682> • Hector Dejea, <https://orcid.org/0000-0003-2584-9812> • Ivana Ilić, <https://orcid.org/0000-0003-1988-6684> • Hrvoje Gašparović, <https://orcid.org/0000-0002-2492-3702> • Boško Skorić, <https://orcid.org/0000-0001-5979-2346> • Bart Bijmens, <https://orcid.org/0000-0003-3130-6937> • Davor Miličić, <https://orcid.org/0000-0001-9101-1570> • Ivo Planinc, <https://orcid.org/0000-0003-0561-6704> • Maja Čikeš, <https://orcid.org/0000-0002-4772-5549>

**Background:** Endomyocardial biopsy (EMB) is the gold standard in heart transplantation (HTx) follow-up, with samples commonly fixed with formalin, and then embedded in paraffin for histology analysis. Recently, EMB samples have been scanned with synchrotron X-ray phase-contrast imaging (X-PCI) to assess graft rejection.<sup>1</sup> We aim to compare imaging time efficiency and image quality between formalin-fixed and paraffin-embedded samples to determine the optimal scanning methodology.

**Methods:** Three adult patients undergoing EMB after HTx were included. EMB samples were initially stored in formalin and imaged by X-PCI at the Paul Scherrer Institute TOMCAT beamline (Villigen, Switzerland). On site samples were scanned in glass tubes in deionised, degassed water, and then embedded in paraffin, positioned on a holder, and scanned again using a multi-scale beamline set-up. Imaging time efficiency was measured by on-site sample preparation and scan time, and image quality was assessed with signal-to-noise ratio (SNR) and pixel resolution. Post-processing comparison included fibrosis quantification (using Ilastik for segmentation and Fiji for calculating the average percentage of collagen in 3 selected areas) and graft-rejection grading (assessed by two blinded observers based on the ISHLT 2004. criteria)<sup>2</sup>.

**Results:** Scanning F1-F3 and P1-P3 samples produced the same imaging resolution, while F1-F3 samples exhibited higher SNR values (clearer sample visibility) (**Table 1**). On site preparation and scan time were shorter with P1-P3 samples. Fibrosis quantification produced similar results in all samples, with

**TABLE 1. Imaging time (including preparation and scanning), technical parameters and imaging data analysis between the two sample preparation methodologies.**

Sample	Methodology	Imaging time efficiency		Technical image quality	Image post-processing analysis	
		On-site preparation time (min:sec)	Scan time (min:sec)	SNR (dB)	Average percentage of collagen in 3 selected areas (%)	Rejection grading (ISHLT 2004. criteria)
F1	Formalin	3:58	49:08	112,16	0.34	1R
P1	Paraffin	0:17	6:34	72,86	0.21	0R
F2	Formalin	4:13	37:24	119,39	0.16	1R
P2	Paraffin	0:20	12:03	54,65	0.11	0R
F3	Formalin	4:21	49:08	112,19	0.37	0R
P3	Paraffin	0:32	12:08	56,72	0.12	0R

RECEIVED:  
September 20, 2023

ACCEPTED:  
September 27, 2023



