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# CRYOGENIC CONTRAST-ENHANCED MICROCT ENABLES 3D HISTOPATHOLOGY OF SOFT BIOLOGICAL TISSUES

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## 1. Introduction

Visualization of the tissue's microstructure is crucial to fully understand their functioning, as well as the impact of diseases and treatments. However, classical 2D histology only partially reveals the complex 3D microstructure of tissues. In this regard, X-ray based 3D histology, using contrast-enhanced microCT (CECT), can provide important complementary information [1]. Here, we present an extension to conventional CECT, termed cryogenic contrast-enhanced microCT (cryo-CECT), which substantially improved the 3D visualization of individual tissue constituents, such as muscle and collagen fibers [2].

## 2. Materials and Methods

Cryo-CECT combines sample staining, using a contrast-enhancing staining agent (CESA), with subsequent freezing at an optimal freezing rate. Next, the sample is imaged by microCT in the frozen state, using an *in situ* cryo-stage.

## 3. Results

Cryo-CECT was first optimized in terms of CESA and freezing rate using bovine muscle tissue (Fig. 1 a-d). To demonstrate the added value of cryo-CECT to histopathology, we then applied cryo-CECT on murine hearts that underwent transverse aortic constriction (TAC) surgery, a well-established animal model for pressure overload-induced cardiac hypertrophy. Cryo-CECT allowed to analyze, in an unprecedented manner, the orientation and diameter of the individual muscle fibers in the entire heart (Fig. 1 e), as well as the 3D localization of fibrotic regions within the myocardial layers.

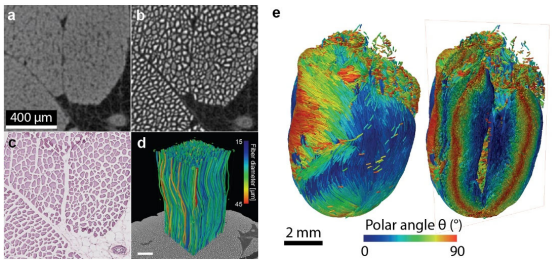


**Figure 1**: **Cryo-CECT allows histo(patho)logical assessment of skeletal and cardiac muscle tissues. a-b**, MicroCT images of the same bovine muscle sample stained with Hf-WD POM, acquired with conventional CECT (a) and with cryo-CECT (b). **c**, Matching 2D histological section (H&E staining) **d**, Fiber model based on (b). Color scale indicates fiber diameter. Scale bar = 500 µm. **e**, 3D spatial graph of the cardiac muscle fiber orientation of a TAC heart. Color scale indicates fiber orientation.

## 4. Discussion and Conclusions

In this study, we presented a novel approach to CECT, termed cryo-CECT, which enables nondestructive 3D histo(patho)logy of various individual soft tissue constituents by imaging the stained sample in its frozen state. The application of cryo-CECT to murine hearts that underwent TAC surgery demonstrated the histopathological potential and added value of this new technique.

## 5. References

1. De Bournonville S et al., Contrast Media and Molecular Imaging (2019).
2. Maes A et al., Nature Communications (2022).