

AWARENESS OF CRYO ELECTRO TOMOGRAPHY AMONG DENTAL STUDENTS

Nithyanandham Masilamani¹, Dhanraj Ganapathy²

Abstract

CryoElectronomography (CryoET) is indeed an imaging method used to create high resolution (~1-4 nm) three-dimensional viewpoints of specimen, usually physiological macro molecules as well as cell lines. CryoET is really a highly specialised implementation of scanning electron microscopy cryomicroscopy whereby the specimen are scanned since they are tilted, triggering a series of Image data which can be processed to create a 3d image, analogous to 3D images, similar to a CT scan of the human body. This survey was done for assessing the awareness of Cryo electro tomography amongst dental students. This was a questionnaire oriented cross sectional type of survey comprising 100 dental college students in Chennai. A self designed questionnaire comprising ten questions based on the knowledge and awareness about Cryo-electron tomography amongst dental college students. Questionnaires were circulated through an online website survey planet. The questions explored the awareness on using Cryo-electron tomography as a tool to study various biological applications. After the responses were received from 100 participants, data was collected and analysed. 7% are aware about Cryo Electro tomography . 3% are aware of the mechanism of action of Cryo Electro tomography . 5% are aware of the diagnostic applications of Cryo Electro tomography . 3% are aware of the limitations Cryo Electro tomography. 91% are willing to learn about Cryo Electro tomography. This study concluded that dental students showed less knowledge and awareness toward Cryo electro tomography. There are large gaps in the knowledge and attitudes requiring strong remedial measures.

Keywords: Awareness, cryo electro tomography, dental students

Introduction

CryoElectronomography (CryoET) is indeed an imaging method used to create high resolution (~1-4 nm) three-dimensional viewpoints of specimen, usually physiological macro molecules as well as cell lines. CryoET is

¹ Tutor, Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India .

² Corresponding Author: Professor & Head of Department, Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India,
Email: dhanrajganapathy@yahoo.co.in

really a highly specialised implementation of scanning electron microscopy cryomicroscopy whereby the specimen are scanned since they are tilted, triggering a series of Image data which can be processed to create a 3d image, analogous to 3D images, similar to a CT scan of the human body (Dubochet et al., 1988).

In contrast to several other electron tomography methodologies, assessments are conjugated in non-crystalline ice or imaging under cryogenic environments ($< -150^{\circ}\text{C}$), allowing them to be imaging without shortage of hydration or substantiation fixation that can somehow or other disrupt or mutilate biodynamic structures (Dodonova et al., 2017; Gan & Jensen, 2012).

In a cryo-ET investigation, a physiological sample — cell, tissue, or living organism would be flash frozen, reduced to an appropriate thickness, and subsequently imaging using an electron magnifying device. The freezing process could save an exemplar in a hydrated, near-local state. Various images are taken as the specimen leans along a hub. The images are then modified and consolidated using computational strategies to recreate a three-dimensional image or tomogram (Dubochet et al., 1988; Oikonomou et al., 2016).

With all its ability to obtain nanometre-scale data on biological macromolecules in their local cell situation, cryo-ET produces scaffolding among light microscopy and in vitro structural assurance methodologies. This is important in view of the possibility that multiple structures can not be expunged, and understanding both the structure and the region of macromolecular structures is essential for understanding cell function (Lučić et al., 2013). Since use of Cryo electro tomography is helpful in the treatment of orofacial diseases, dental students should be aware of this. This survey was done for assessing the awareness of Cryo electro tomography amongst dental students.

Materials And Method:

This was a questionnaire oriented cross sectional type of survey comprising 100 dental college students in Chennai. A self designed questionnaire comprising ten questions based on the knowledge and awareness about Cryo-electron tomography amongst dental college students. Questionnaires were circulated through an online website survey planet. The questions explored the awareness on using Cryo-electron tomography as a tool to study membrane-associated complexes, image processing techniques for cryo-electron tomography, for analysis of glycoproteins in enveloped viruses, in the study of large molecular motors, Analyses of cellular vesicles and organelles associated proteins and study of Transmembrane proteins and pores. After the responses were received from 100 participants, data was collected and analysed.

Results

7% are aware about Cryo Electro tomography (Fig.1) . 3% are aware of the mechanism of action of Cryo Electro tomography (Fig.2). 5% are aware of the diagnostic applications of Cryo Electro tomography (Fig.3) . 3% are aware of the limitations Cryo Electro tomography (Fig.4). 91% are willing to learn about Cryo Electro tomography (Fig.5) .

Fig 1: Awareness about Cryo Electro tomography

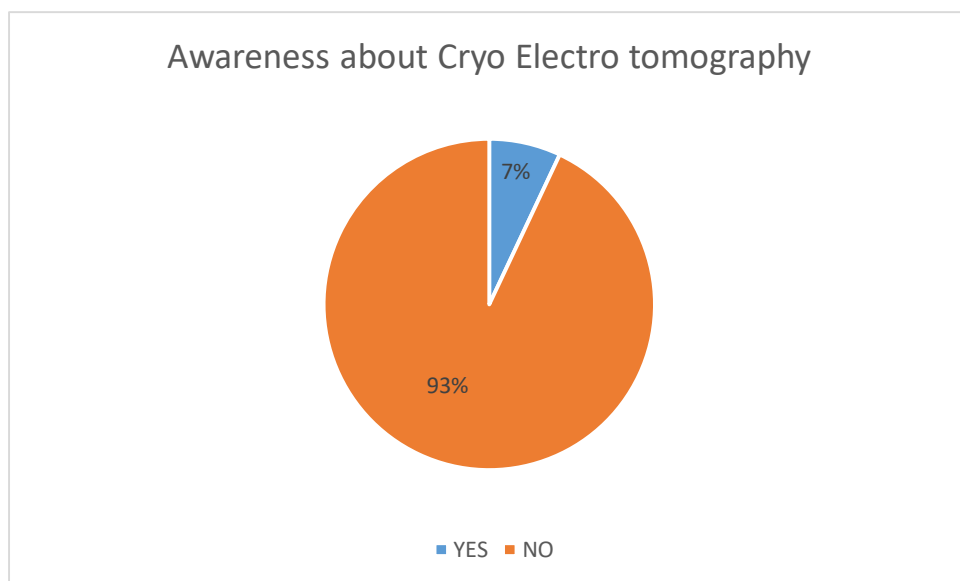


Fig 2: Awareness about mechanism of action of Cryo Electro tomography

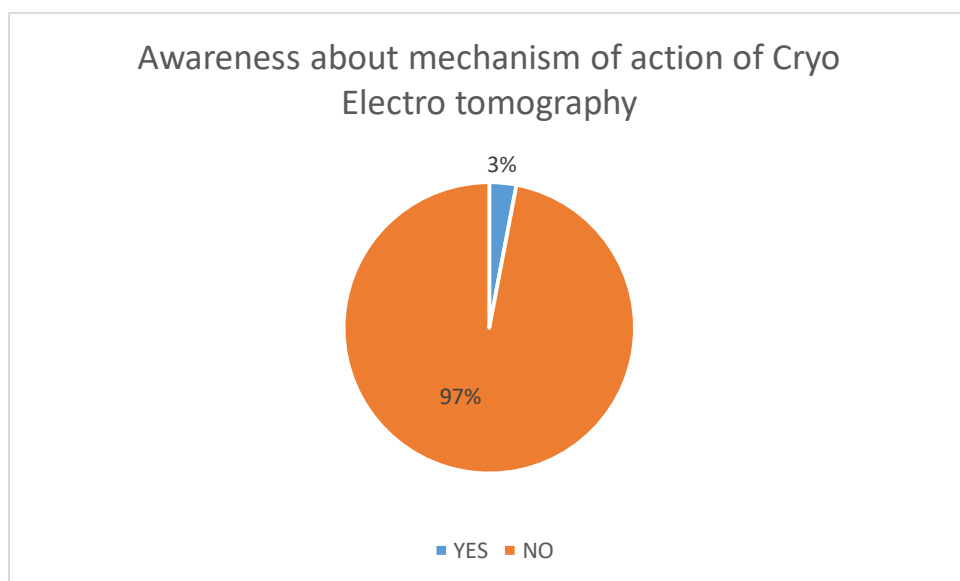


Fig 3: Awareness about diagnostic applications of Cryo Electro tomography

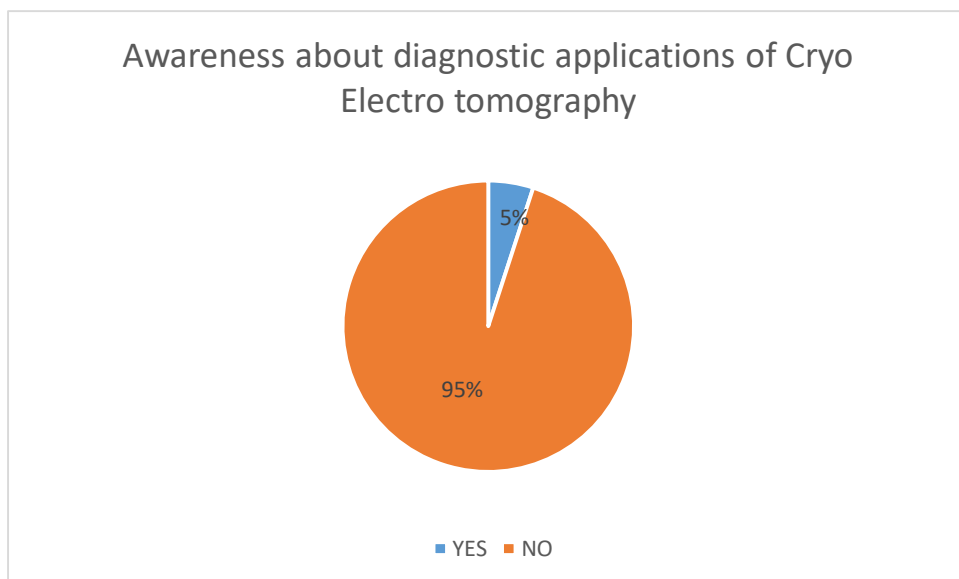


Fig 4: Awareness about limitations of Cryo Electro tomography

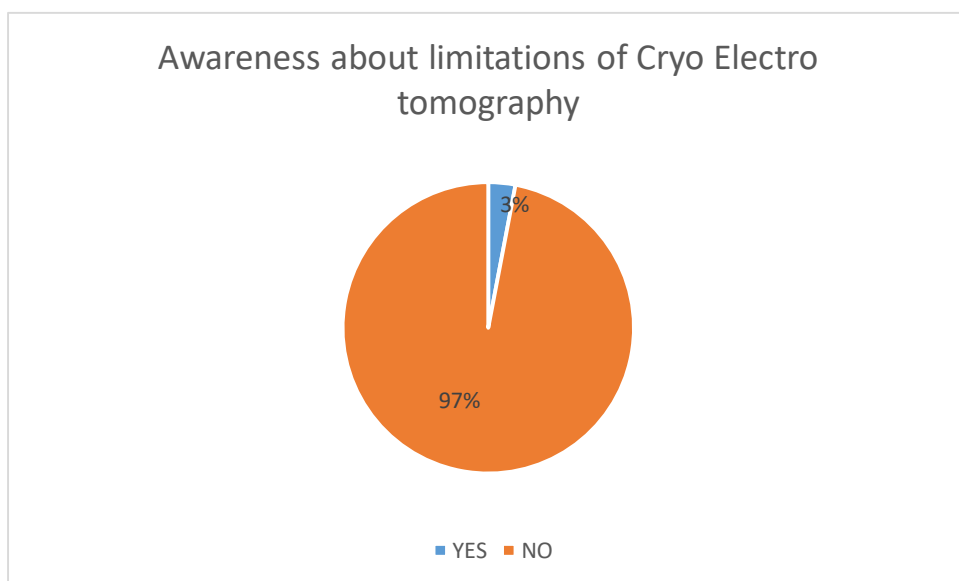
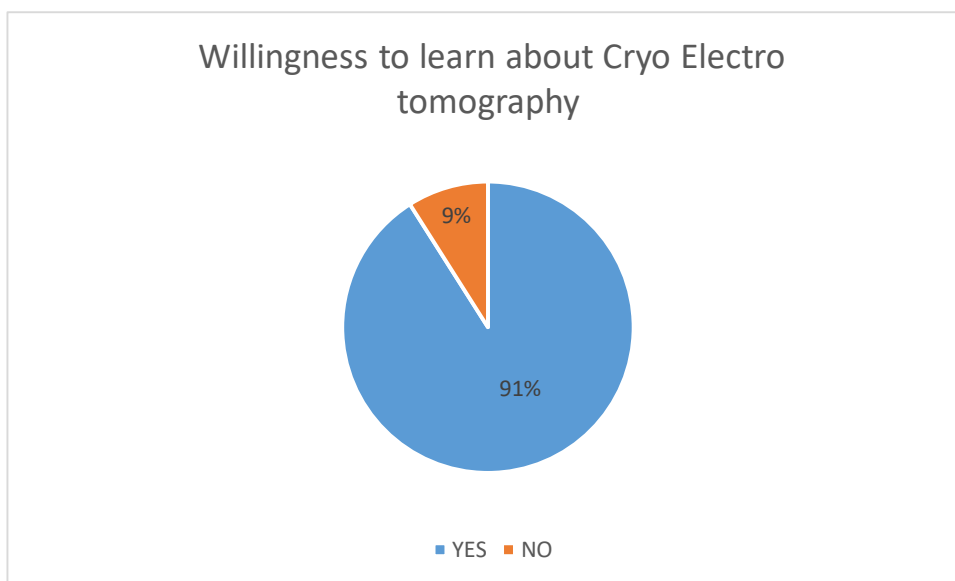


Fig 5: Willingness to learn about Cryo Electro tomography



Discussion

Biological material is quite susceptible to radioactive damage done by an electron beam, though, and researchers have to formulate a variety of ways to enhance sensitivity without damaging samples. Recent hardware innovations vital to strengthening the resolution of single-particle cryo-EMs, which include direct electron detectors as well as novel phase plates, also are trying to improve cryo-ET imaging. Computational manipulations specifically designed for cryo-EM, such as beam-induced implement appropriate algorithms and subtomogram averaging, often help to refine the essential characteristics interpreted by cryo-ET. These advancements are synergized with breakthroughs in the readiness of cryo-ET samples, in particular the implementation of concentrated ion beam filtration to thin samples with ideal imaging thicknesses (Al-Amoudi et al., 2004; Briggs, 2013).

At the basic level, cryo-ET can represent the entire proteome of a cell, but additional knowledge (Zhang, 2013) research programs are intended to parse those very thick images. With one successful methodology, known as format coordination, the design composition is used to find coordination structures in the tomogram. Although this strategy is already successful in mapping areas of relatively sized structures, for example, ribosomes, affectability and precision upgrades are needed to make it more relevant. We expect the progress of the new methodologies that will be implemented that will enable in situ structural science on a proteomic scale.

Cryo-ET is a rapidly increasing strategy that has recently started to demonstrate its innate capacity. The advancement of the equipment that has stated in the process 5 years, along with the recent advancement in picture handling processes, has made cryo-ET widely available to a continuously developing network. Cryo-ET gives an opportunity to carry out auxiliary scientific research investigations of cell structures in of their own

local needs. This trait tends to make cryo-ET the optimal device for the examination of movie-related proteins, especially those structures which are not stable when cleaned or cryo-stored.

It's been shown that it is possible to obtain near-atomic cryo-ET resolution when it is used in tandem with sub-tomogram quantization. A major limitation at this time is the large number of copies designed to accomplish such amendments and therefore it is unlikely that cryo-ET will be habitually used during high-resolution research. However, it also is reasonable to presume that use of cryo-ET will start increasing over the near future and be a method of choice for understanding the characteristics of complexes which can not be reconstructed in vitro or which can only be reconstructed in large liposomes. When used on cellular samples, this will enable an understanding of the determinants and components of macromolecular complexes within their natural environment (Zhang, 2013).

In particular, the data collected will be complemented by data from old-style supplementary science processes, such as X-beam crystallography, NMR or SP cryo-EM. In this scenario, all parts of the macromolecular complex would be determined exclusively at high targets, while the amass facility will be determined at moderate targets (1–2 nm) through cryo-ET and individual segments.

In view of the current state of cryo-EM and cryo-ET procedures, the objectives for which a facility can be established are not limited to the strategic plan being used and the ability to adapt of the objective complex or even the quantity of duplicates which can be imaged. Perception of different states or ability to adapt of a macromolecular complex is itself important information that can be used to inform crystallization methods (Chang et al., 2014; Kaufmann et al., 2014). Dental students have not been aware of the applications of cryo electro tomography. Exposure to this should be provided through a variety of awareness and professional development programmes.

Conclusion

This study revealed that whilst dental students had minimal knowledge and awareness of Cryo electro tomography and there seem to be large gaps in knowledge and attitudes that need enhancement. Large-scale health educational programmes on Cryo electro tomography must be launched by professional bodies to increase communication and to strengthen knowledge strongly.

Funding Support:

The authors declare that they have no funding support for this study.

Conflict of Interest:

The authors declare that they have no conflict of interest.

References

1. Al-Amoudi, A., Chang, J.-J., Leforestier, A., McDowall, A., Salamin, L. M., Norlén, L. P. O., Richter, K., Blanc, N. S., Studer, D., & Dubochet, J. (2004). Cryo-electron microscopy of vitreous sections. In *The EMBO Journal* (Vol. 23, Issue 18, pp. 3583–3588). <https://doi.org/10.1038/sj.emboj.7600366>
2. Briggs, J. A. G. (2013). Structural biology in situ—the potential of subtomogram averaging. In *Current Opinion in Structural Biology* (Vol. 23, Issue 2, pp. 261–267). <https://doi.org/10.1016/j.sbi.2013.02.003>
3. Chang, Y.-W., Chen, S., Tocheva, E. I., Treuner-Lange, A., Löbach, S., Sjøgaard-Andersen, L., & Jensen, G. J. (2014). Correlated cryogenic photoactivated localization microscopy and cryo-electron tomography. *Nature Methods*, 11(7), 737–739.
4. Dodonova, S. O., Aderhold, P., Kopp, J., Ganeva, I., Röhling, S., Hagen, W. J. H., Sinning, I., Wieland, F., & Briggs, J. A. G. (2017). 9 Å structure of the COPI coat reveals that the Arf1 GTPase occupies two contrasting molecular environments. In *eLife* (Vol. 6). <https://doi.org/10.7554/elife.26691>
5. Dubochet, J., Adrian, M., Chang, J. J., Homo, J. C., Lepault, J., McDowall, A. W., & Schultz, P. (1988). Cryo-electron microscopy of vitrified specimens. *Quarterly Reviews of Biophysics*, 21(2), 129–228.
6. Gan, L., & Jensen, G. J. (2012). Electron tomography of cells. *Quarterly Reviews of Biophysics*, 45(1), 27–56.
7. Kaufmann, R., Schellenberger, P., Seiradake, E., Dobbie, I. M., Yvonne Jones, E., Davis, I., Hagen, C., & Grünwald, K. (2014). Super-Resolution Microscopy Using Standard Fluorescent Proteins in Intact Cells under Cryo-Conditions. In *Nano Letters* (Vol. 14, Issue 7, pp. 4171–4175). <https://doi.org/10.1021/nl501870p>
8. Lučić, V., Rigort, A., & Baumeister, W. (2013). Cryo-electron tomography: The challenge of doing structural biology in situ. In *The Journal of Cell Biology* (Vol. 202, Issue 3, pp. 407–419). <https://doi.org/10.1083/jcb.201304193>
9. Oikonomou, C. M., Chang, Y.-W., & Jensen, G. J. (2016). A new view into prokaryotic cell biology from electron cryotomography. *Nature Reviews. Microbiology*, 14(4), 205–220.
10. Zhang, P. (2013). Correlative cryo-electron tomography and optical microscopy of cells. In *Current Opinion in Structural Biology* (Vol. 23, Issue 5, pp. 763–770). <https://doi.org/10.1016/j.sbi.2013.07.017>