

"Basic Principles and Practical Aspects of CryoEM"			
Time	29.8.2022 - 9.9.2022		
Modul	CHE 485		
Speakers	Multi-User CryoEM Facility and Users		
Organizers	Carolin Seuring, Ulrike Laugks, Cornelia Cazey, Kay Grünewald		
Contact	carolin.seuring@cssb-hamburg.de		
Registration	https://lists.cssb-hamburg.de/sympa/info/cryoemcourse2022		
Rooms	CSSB Lecture hall, Harbor Lecture Hall, IT Rooms		
Format	Hybrid Talks, Live Demos (Microscope, Data Analysis)		
Zoom	Meeting ID: 687 5046 4138; pw: cryoem2022		
Practicals	12 pax only		
Week 1			
ID	CSSB Lecture Hall	Time Slot	Day 1, Monday, 29.8.2022
1.1	Set-up	8:30 - 9:00	Zoom Testing & Zoom Functionalities & Important Links
1.2	Welcome	9:00 - 9:15	About the Course & practical things you need to know
1.3	Welcome	9:15 - 9:30	Students introduce themselves
1.4	Facility Action	9:30 - 9:50	Live-Walkthrough: Multi-User CryoEM Facility at CSSB
	Break	9:50 - 10:10	Coffee Break
1.5	Talk + Interactive	10:10 - 11:00	Basic Introduction to TEM
1.6	Talk	11:00 - 12:00	From proteins to cells to cellular movies: Introduction to electron cryo-microscopy and modalities
	Break	12:00 - 13:15	Free Time
1.7	Credit Work	13:15 - 13:30	Paper Assignment
1.8	Talk	13:30 - 14:00	Talk Sample Prep Overview: neg. stain - vitrification
1.9	Groups 1-3	14:00 - 15:00	Hands on sample preparation: neg. stain, vitrobot, Leica GP2
1.10	Groups 1-3	15:00 - 16:00	Live Sample Imaging on Talos L120 + Arctica
ID	CSSB Lecture Hall	Day 2, Tuesday, 30.08.2022	
2.1	Q & A	8:30 - 9:00	Recap - Interactive Q&A
2.2	Talk + Interactive	9:00 - 9:45	Image Formation Basics 1: Phase Contrast, Amplitude Contrast, Plane Waves
2.3	Talk + Interactive	9:45 - 10:45	Image Formation Basics 2: Raydiagrams, CTF and Defocus
	Break	10:45 - 11:00	Coffee Break
2.4	Talk + Poll	11:00 - 11:30	Lens Aberrations
2.5	Talk	11:30 - 12:00	Tuning Imaging Parameters
	Break	12:00 - 13:30	Free Time
2.6	Talk	13:30 - 14:00	CryoEM SPA Workflow: Sample Screening to Data Collection (including movies, dose fractionation)
2.7	Live-Demo	14:00 - 16:00	Acquiring high-resolution images of a protein: High-throughput cryo-SPA data acquisition with EPU
2.8	Live-Demo	16:00 - 16:30	Monitoring incoming data with CryoSPARC live
ID	CSSB Lecture Hall	Day 3, Wednesday, 31.8.2022	
3.1	Q & A	8:30 - 9:00	Recap - Interactive Q&A
3.2	Talk	9:00 - 10:00	An overview to single particle analysis: principles for data analysis from 2D projection images to 3D model
	Break	10:00 - 10:45	Coffee Break
3.3	Talk	10:45 - 11:00	Explain Set-up cryoSPARC for student projects
3.4	Talk	11:00 - 11:30	Overview Webinterface cryoSPARC: from motion correction to 3D model
3.5	Demo	11:30 - 12:00	Theory Motion Correction + Theory CTF Correction
	Break	12:00 - 13:00	Free Time
3.6	Set-up	13:00 - 14:00	Split Students in Groups, Relocation to Seminar Room + Computer Room, Set-up Project in cryoSPARC: Import of MC+CTF corrected data
3.7	cryoSPARC Demo	14:00 - 15:00	Parameters to "Curate Exposures"
3.8	IT Room / Lecture Hall Action	15:00 - 16:00	Practice: Group 1 - 2 : Curate Exposures
ID	CSSB Lecture Hall	Day 4, Thursday, 1.9.2022	
4.1	Q & A	8:30 - 9:30	Q & A: Recap day 3, Plan for today, Making sure all students finished exposure curation
4.2	cryoSPARC Demo	9:30 - 10:30	Particle Picking Approaches Theory & Quality of picks
	Break	10:30 - 10:45	Coffee Break
4.3	Talk	10:45 - 12:15	The Theory behind 2D classification, 3D classification, Fourier Slice Theorem + Euler Angles
	Break	12:15 - 14:00	Free Time
4.4	IT Room / Lecture Hall Action	14:00 - 16:00	Practice: Group 1 - 2 : Particle Picking to 2D classification in cryoSPARC
	Socializing	16:00 - 18:00	Interactive
ID	CSSB Lecture Hall	Day 5, Friday, 2.9.2022	
5.1	Q & A	9:00 - 9:30	Q & A: Recap day 4, Making sure all students achieved 2D classes in cryoSPARC, plan for today
5.2	cryoSPARC Demo	9:30 - 10:00	Particle Cleaning From 2D classes to 3D
	Break	10:00 - 10:15	Break
5.3	cryoSPARC Demo	10:15 - 11:00	Ab initio Modelling (1 to 7 classes)
5.4	cryoSPARC Demo	11:00 - 12:00	3D refinement in cryoSPARC
	Interactive / Lunch	12:00 - 14:00	Free Time
5.5	IT Room / Lecture Hall Action	14:00 - 15:30	Practice: Group 1 - 2D class selection - Ab-initio modelling + homogeneous refinement in cryoSPARC

Week 2				
ID	HARBOR Seminar		Day 6, Monday, 05.09.2022	CryoET workflow part 1
6.1	Q & A	8:30 - 9:00	Recap: CryoSPARC results	CryoSPARC Team
6.2	Talk	9:00 - 10:00	Introduction to cryoET - workflow overview	Ulrike Laugks
	Break	10:00 - 10:15	Break	
6.3	Talk	10:15 - 10:30	Complexity of sample preparation: Overview and History	Christoph Hagen
6.4	Talk	10:30 - 11:00	Sample freezing expanded: plunge freezing of cells/high-pressure freezing	Christoph Hagen
6.5	Talk	11:00 - 11:15	Sample thinning 1 "CEMOVIS"	Christoph Hagen
6.6	Talk	11:15 - 11:45	Sample thinning 2 "Lamella": FIB-SEM Theory and CLEM	Josie Ferreira
	Break	11:45 - 13:00	Free Time	
	cryoEM Facility	13:00 - 13:15	Meeting at the facility and dividing the groups	Carolin Seuring
6.7	cryoEM Facility	13:15 - 14:45	Live Demo: CLEM + cryoFIB-SEM and cryoET data acquisition with Tomo (in rotation)	Tim Laugks, Ulrike Laugks, Carolin Seuring, Fereshta Hafizi
	Break	14:45 - 15:00	Break	
6.8	cryoEM Facility	15:00 - 16:30	Live Demo: CLEM + cryoFIB-SEM and cryoET data acquisition with Tomo (in rotation)	Tim Laugks, Ulrike Laugks, Carolin Seuring, Fereshta Hafizi
ID	HARBOR Seminar		Day 7, Tuesday, 06.09.2022	CryoET workflow part 2
7.1	Q & A	09:00 - 09:30	Re-cap, Q & A	Ulrike Laugks and Speakers
7.2	Talk	09:30 - 10:00	Tomogram reconstruction theory and software overview	Lindsay Baker
	Break	10:00 - 10:15	Break	
7.3	Talk	10:15 - 11:15	Sub-volume averaging	Daven
7.4	Discussion	11:15 - 12:00	Forum Discussion: Bright future of cellular cryoET	everyone from CSSB
	Break	12:00 - 13:00	Free Time	
7.5	Meeting	13:00 - 13:30	Walk over to computer room, get organized	all
7.6	IT Room / Harbor	13:30 - 13:45	Talk: Introduction to tomographic reconstruction software "IMOD"	Ulrike Laugks
7.7	IT Room / Harbor	13:45 - 16:00	Interactive tomographic reconstruction with IMOD	Ulrike Laugks, Emily Machala, Yuliia Mironova, Clara Feldmann
ID	HARBOR Seminar		Day 8, Wednesday, 07.09.2022:	Model Building
8.1	Q & A	09:00 - 09:30	Recap, Plan for today	Ulrike Laugks, Carolin Seuring
8.2	Talk	09:30 - 10:15	Introduction to cryoEM density fitting	Maya Topf
	Break	10:15 - 10:30	Coffee - Break	
8.3	Demo	10:30 - 11:15	Visualizing Spike protein in 3D - ChimeraX	Guendalina Marini
8.4	Demo	11:15 - 11:45	Introduction to "Interactive TEMPY" modelling software	Tom Mulvaney (recorded), Guendalina Marini
8.5	Follow-along	11:45 - 12:30	Flexible fitting of atomic models in cryoEM maps - RibFind and iTEMPY	Guendalina Marini
	Break	12:30 - 14:00	Free Time	
8.6	IT Room / Harbor	14:00 - 16:00	Interactive TEMPY in ChimeraX + Validation	Guendalina Marini, Carolin Seuring
ID	HARBOR Seminar		Day 9, Thursday, 08.09.2022	
9.1	Teaser Talk	9:00 - 09:20	Towards molecular mechanisms using cryoEM	Jiri Wald (Marlovits Group)
			Specimen Preparation	
9.2	Review Presentation	09:20 - 09:40	Russo et al. (2022). "Cryomicroscopy in situ: what is the smallest molecule that can be directly identified without labels in a cell?" Faraday Discuss.: 26.	http://dx.doi.org/10.1039/d2fd00076h
9.3	Paper Presentation	09:40 - 10:00	Danev et al. (2021) Routine sub-2.5 Å cryo-EM structure determination of GPCRs	https://www.nature.com/articles/s41467-021-24650-3
9.4	Paper Presentation	10:00 - 10:20	Schweighauser et al. (2022) Age-dependent formation of TMEM106B amyloid filaments in human brains. Nature: 22	http://dx.doi.org/10.1038/s41586-022-04650-z
9.5	Research Talk	10:20 - 10:40	CryoEM of membrane proteins	Katharina Jungnickel (L6w Group)
	Break	10:40 - 11:10	Break	
			Cryo electron tomography of infected cells	
9.6	Review Presentation	11:10 - 11:30	Saville et al. (2022). "Three-Dimensional Visualization of Viral Structure, Entry, and Replication Underlying the Spread of SARS-CoV-2."	http://dx.doi.org/10.1021/acs.chemrev.1c01062
9.7	Paper Presentation	11:30 - 11:50	Laughlin et al. (2022). "Architecture and self-assembly of the jumbo bacteriophage nuclear shell."	http://dx.doi.org/10.1038/s41586-022-05013-4
9.8	Paper Presentation	11:50 - 12:10	Chmielewski et al. (2022). Chikungunya virus assembly and budding visualized in situ using cryogenic electron tomography	https://www.nature.com/articles/s41564-022-01164-2
9.9	Research Talk	12:10 - 12:30	In situ biology: the rise of cryoET in virology and parasitology	Yuliia Mironova (Grünewald)
	Break	12:30 - 14:00	Break	
			Recent Advances of cryo tomography	
9.10	Review Presentation	14:00 - 14:20	Theveny et al. (2022). "Parasitology meets cryo-electron tomography-exciting prospects await." Trends Parasitol. 38(5): 365-378.	http://dx.doi.org/10.1016/j.pt.2022.01.006
9.11	Paper Presentation	14:20 - 14:40	Chua et al. (2022). "Better, Faster, Cheaper: Recent Advances in Cryo-Electron Microscopy."	http://dx.doi.org/10.1146/annurev-biochem-032620-110705
9.12	Research Talk	14:40 - 15:00	Form follows function: Variable microtubule architecture in the malaria parasite	Josie Ferreira (Grünewald)
ID	HARBOR Seminar		Day 10, Friday, 09.09.2022	
			Correlative light and electron microscopy	
10.1	Review Presentation	09:00 - 09:20	Lovatt et al. (2022). Bridging length scales from molecules to the whole organism by cryoCLEM and cryoET	https://pubs.rsc.org/en/content/articlepdf/2022/td/d2fd00081d?page=search http://dx.doi.org/10.3791/62992
10.2	Paper Presentation including Video	09:20 - 09:40	Sibert et al. (2021). "Micropatterning Transmission Electron Microscopy Grids to Direct Cell Positioning within Whole-Cell Cryo-Electron Tomography Workflows."	http://dx.doi.org/10.3791/62992
10.3	Research Talk	09:40 - 10:00	Structural insights into macromolecular self-assembly	Guendalina Marini (Topf)
	Break	10:00 - 10:30	Break	
			CryoEM data analysis and interpretation	
10.4	Paper Presentation	10:30 - 10:50	Edich et al. (2022). "The impact of AlphaFold2 on experimental structure solution." Faraday Discuss.	http://dx.doi.org/10.1039/D2FD00072E
10.5	Paper Presentation	10:50 - 11:10	Kinman et al. (2022). Uncovering structural ensembles from single particle cryo-EM data using cryoDRGN	http://dx.doi.org/10.1101/2022.08.09.503342
10.6	Research Talk	11:10 - 11:30	StarMap: a user-friendly workflow for Rosetta-driven molecular structure refinement	Vadim Kotov
	Feedback Form	11:30 - 12:30	Questionnaire / Feedback Online Form (mandatory)	Cornelia Cazey
	Break	12:30 - 13:30	Break	
			Challenges in cryoEM	
10.7	Research Talk	13:30 - 13:50	"Combining cryoEM and biophysics to study the membrane anchor of endocytic adaptors"	Javier Lizarrondo
10.8	Research Talk	13:50 - 14:10	AI-based structure prediction empowers integrative structural analysis of human nuclear pores	Jan Kosinski
10.9	Famous last words	14:10 - 14:30	"Ribosome-targeting antibiotics and bacterial resistance mechanisms"	Daniel Wilson
	Socializing	14:30 - 16:00	Cake and Coffee : Feedback Online Form / Interactive Discussion	