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Hybrid imaging - Novel Approaches and Recent Advances in Correlative Microscopy

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Hybrid imaging is the combined or correlative use of different imaging modalities. By using complementary approaches, structures can be studied on different length scales and with different ranges of resolution, dynamic and static aspects of the structure under scrutiny can be captured or purely structural information can be augmented by functional information. By choosing the right combination of methods, data are obtained which are much more informative than the data provided by one or the other method alone [1].
A combined approach can only be realized, if optimized preparation protocols, innovative hardware accessories and new methodologies are in place. The successful combination of formerly solitary methods into a robust and reliable workflow is a major challenge. To maintain the structural integrity of the sample, handling and transfer steps have to be robust and reliable and they have to be precisely controlled throughout the process to avoid adverse contamination effects. In addition suitable software tools have to be developed to provide a streamlined but transparent framework. Needless to say automation of repetitive tasks is instrumental to increase the throughput in hybrid imaging approaches.

The study of complex biological samples, from 'cellular landscapes to molecular territories [2]', is basically a threefold task: (i) identify features of interest, (ii) navigate these and (iii) finally target and capture high-resolution snapshots that represent *bona fide* cellular events. The first two tasks can be addressed by correlative cryo-fluorescence microscopy [3], which offers the possibility to navigate large cellular volumes and to localize specific cellular targets. These targets can then be accessed directly by focused ion beam milling (FIB) and further investigated by cryo-electron tomography [4]. We have assembled a 'pipeline' based on different technologies and tools to facilitate this strategy without compromising versatility and expandability (Fig. 1, A,B). Several key engineering issues have been addressed to optimize sample transfer and manipulation steps, including the design of suitable stages [5], transfer devices and alternatives for a more stable specimen support (Fig.1, C). The whole experimental setup has been streamlined to guarantee a reliable, reproducible and convenient workflow, which in turn could facilitate the routine application of cryo-electron tomography of larger cellular structures.

Here, we will discuss the current state of integrating optical (light) and electron microscopy with a view to performing cryo-electron tomography in an efficient and targeted manner. Moreover we will discuss novel and recent advancements in hybrid imaging, which could serve as alternative approaches to identify molecular inhabitants of particular intracellular neighborhoods (such as the combination of imaging with mass spectrometry [6,7]). Furthermore, we will give an outlook on developments regarding computational tools, which increasingly play a fundamental role not only in data acquisition and processing but also in integration and analysis [8].

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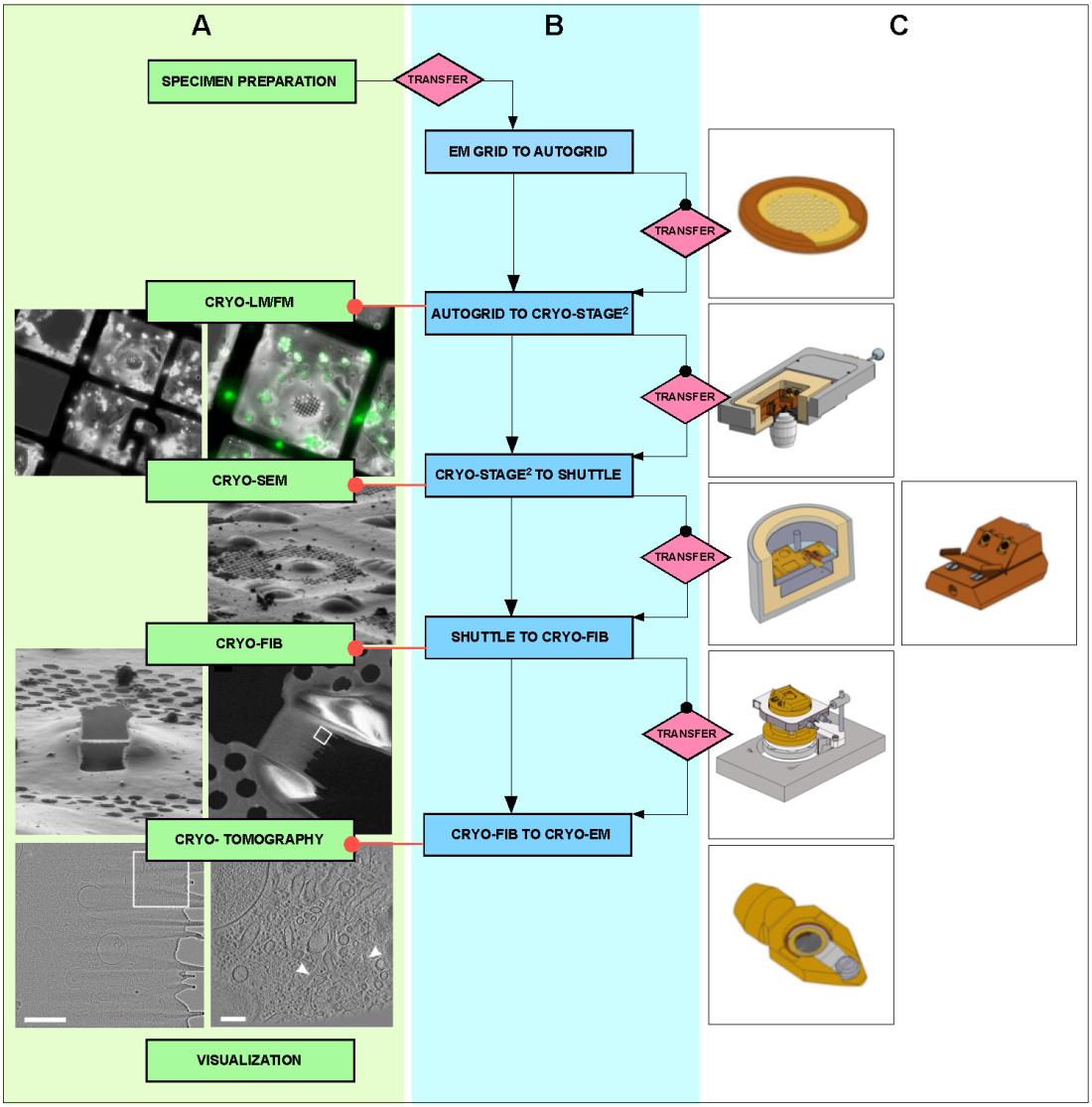

FIG. 1. Hybrid imaging pipeline. Schematic illustration depicting the different parts of the pipeline: transfer, identification, navigation and targeting steps of a frozen-hydrated specimen for tomographic analysis by electron microscopy. **A** experimental images from cryo-LM/FM (top), cryo-SEM/FIB (middle) and cryo-ET (bottom), **B** workflow and **C** necessary hardware accessories.

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