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Real and virtual image separation in digital in-line holography microscopy by recording two parallel holograms[1] HANGJIAN LING, JOSEPH KATZ, Johns Hopkins University — Maintaining high magnification and micron resolution in applications of digital in-line holography microscopy for 3D velocity measurements requires a hologram plane located very close or even within the sample volume. Separation between overlapping real and virtual images becomes a challenge in such cases. Here, we introduced a simple method based on recording two holograms through the same microscope objective that are separated by a short distance from each other. When the same particle fields are reconstructed from the two holograms, the real images overlap, whereas virtual images are separated by twice the distance between hologram planes. Thus, real and virtual images can be easily distinguished. Due to the elongation of the reconstructed particle in the axial direction, the distance between hologram planes is selected to exceed the elongated traces. This technique has been applied to record 3D traces of thousands of 2 um particles in a $0.5 \times 0.5 \times 0.5$ mm sample volume using hologram planes separated by 27 um. Experimental setup, alignment and data analysis procedures, including reconstruction, calibration, particles segmentation and precision particles positioning will be discussed.

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