The study of anatomy is complex and difficult for students in both graduate and undergraduate education. Researchers have attempted to improve anatomical education with the inclusion of three-dimensional visualization, with the prevailing finding that 3D is beneficial to students. However, there is limited research on the relative efficacy of different 3D modalities, including monoscopic, stereoscopic, and autostereoscopic displays. This study analyzes educational performance, confidence, cognitive load, visual-spatial ability, and technology acceptance in participants using autostereoscopic 3D visualization (holograms), monoscopic 3D visualization (3DPDFs), and a control visualization (2D printed images). Participants were randomized into three treatment groups: holograms (n=60), 3DPDFs (n=60), and printed images (n=59). Participants completed a pre-test followed by a self-study period using the treatment visualization. Immediately following the study period, participants completed the NASA TLX cognitive load instrument, a technology acceptance instrument, visual-spatial ability instruments, a confidence instrument, and a post-test. Post-test results showed the hologram treatment group (Mdn=80.0) performed significantly better than both 3DPDF (Mdn=66.7, p=.008) and printed images (Mdn=66.7, p=.007). Participants in the hologram and 3DPDF treatment groups reported lower cognitive load compared to the printed image treatment (p < .01). Participants also responded more positively towards the holograms than printed images (p < .001). Overall, the holograms demonstrated significant learning improvement over printed images and monoscopic 3DPDF models. This finding suggests additional depth cues from holographic visualization, notably head-motion parallax and stereopsis, provide substantial benefit towards understanding spatial anatomy. The reduction in cognitive load suggests monoscopic and autostereoscopic 3D may utilize the visual system more efficiently than printed images, thereby reducing mental effort during the learning process. Finally, participants reported positive perceptions of holograms suggesting implementation of holographic displays would be met with enthusiasm from student populations. These findings highlight the need for additional studies regarding the effect of novel 3D technologies on learning performance.