Serial Femtosecond Crystallography (SFX) has revolutionised macromolecular crystallography by enabling X-ray diffraction data to be collected sequentially from continuous stream of microcrystals at room-temperature. Serial crystallography approaches have been successfully applied to numerous time-resolved X-ray diffraction studies. Accurately assessing the quality and reliability of time-dependent changes in electron density remains a challenge. To address this challenge, we developed “Xshuffle”, a Python-based toolbox designed for systematic analysis of SFX diffraction patterns using resampling algorithms. Xshuffle exploits the high-multiplicity of SFX data to estimate data uncertainties, including coordinate errors after structural refinement and the identification of recurrent changes in electron density maps. Xshuffle’s python implementation enhances versatility and facilitates integration into environments such as Jupyter notebooks. Additionally, Xshuffle is designed to maintain the computational costs associated with analysing SFX data relatively low.