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A methodology to separate single cells and single mitochondria to determine the location of heteroplasmy in mitochondrial DNA JOSEPH REINER, Physics Laboratory, NIST, Gaithersburg, RANI KISHORE, NIST, THOMAS ALBANETTI, SARAH PEERY, ASHLEY KNIPE, AMANDA SHEETS, NICHOLAS BOIRE, KOREN DECKMAN, Gettysburg College, BARBARA LEVIN, CSTL-NIST, KRISTIAN HELMERSON, NIST — A mixture of mutated and wild type mitochondrial DNA is referred to as a heteroplasmic population. Mitochondrial DNA heteroplasmies have been studied at the multi-cell level with some being linked to chronic symptoms of mitochondria-based diseases. However, the mechanism producing heteroplasmy is undetermined. One question is whether mitochondrial DNA heteroplasmies are present within single mitochondria. To address this issue we developed a protocol to isolate a single mitochondria from single human leukocyte cells. The cells from an HL-60 cell culture were labeled with Mitotracker Green FM and showed to contain a heteroplasmy at the cellular level (PCR and sequencing showed a 50/50 C/T heteroplasmy at nucleotide position 12071). In order to study heteroplasmy at the single mitochondria level a pulsed UV laser was used to lyse an individual cell. Mitochondria escaped from the cell and optical tweezers were used to transfer single mitochondria into a micropipette tip. Preliminary results suggest that single mitochondria also contain the heteroplasmy.

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