53e High Speed and High Sensitivity Two Dimensional Capillary Electrophoresis with Laser Induced Fluorescence of Barrett's Esophageal Cells

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Protein expression fingerprints of Barrett's Esophageal biopsies and cultured cells are generated using a novel two dimensional capillary electrophoresis system. Proteins from cellular lysate are labeled with the fluorogenic reagent 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ), which reacts with lysine residues to produce a highly fluorescent product. Proteins are detected by laser-induced fluorescence inside a sheath flow cuvette using a fiber-coupled single photon counting module. The CE system requires only nL of sample, and has limits of detection in the zeptomole range (10^{-21}).

Protein separations are performed by capillary sieving electrophoresis (CSE) and micellar electrokinetic chromatography (MEKC). Field strengths in excess of 1000 V/cm produce CSE and MECC separation profiles in less than 3 minutes. Coupling the separation modes in two-dimensional capillary electrophoresis (2D-CE) increases the peak capacity. Proteins are separated according to their size by CSE on the first capillary. Fractions are then repeatedly transferred to the second capillary and subject to MEKC. 2D-CE analysis time has been reduced to less than 60 minutes. 2D-CE experiments are highly reproducible. Relative standard deviation in the CSE and MECC dimensions are less than 1% for the 50 most intense protein components.

Biopsies of esophageal epithelium are collected during endoscopic procedures and subject to 2D-CE analysis. Three tissue types (squamous, gastric, Barrett's) are compared from each of four patients. 2D-CE protein profiles show distinct differences between tissue types, as well as similarities between the same tissue type from different patients. In a second study, cultured cells are treated with acidic bile salts to simulate gastrointestinal reflux. 2D-CE analysis provides high sensitivity detection and rapid separation of complex protein mixtures.