Pathologists have long known that the functional state of a tissue is best understood from an overview of the different cells making up that tissue—cells that may differ radically in their lineage, their phase in the cell cycle, their responses to stimuli, and their clonal history of somatic mutation, e.g., in a tumor. With the radically expanding opportunities for the molecular analysis of nucleic acids, proteins, and combinations thereof, there is currently great interest in collecting information at cellular resolution, rather than recording population means, as has been common practice in molecular biology (1).

In this issue of *Clinical Chemistry*, Ståhlberg and coauthors (2) describe an interesting approach for jointly measuring DNA copy numbers and concentrations of mRNAs, microRNAs, and proteins in individual cells. These authors prepared single-cell lysates that were subsequently divided into separate reactions for each of the measured molecular species, and they recorded all of the results of the molecular-detection reactions via real-time quantitative PCR. This report represents an important first step in a new approach to detect multiple biological modalities in single cells with high analytical sensitivity and specificity.

Nothing is quite new under the sun, of course, and there are already a number of approaches for molecular analyses at the level of individual cells. Various in situ analyses that provide cellular-level resolution are routinely used for analyzing DNA, RNA, or protein in populations of cells and in tissue sections. The most commonly used method to analyze nucleic acids is fluorescence in situ hybridization, but the kind of information accessible for genomes and transcripts can be expanded with padlock probes (3), branched DNA probes (4), and techniques that make use of differentially labeled and designed probes, such as molecular beacons (5) or quantum dot–labeled probes (6).

For protein analyses in situ, immunohistochemistry and immunofluorescence have a long-established tradition. In these assays, antibodies are labeled with a substrate-converting enzyme such as peroxidase or a fluorophore. Methods such as the proximity ligation assay (7) or fluorescence resonance energy transfer (8) can be used with pairs of antibodies in situ to detect proteins, protein complexes, or posttranslationally modified proteins. These methods introduce an additional level of analytical specificity and reveal important functional properties of proteins.

In situ approaches preserve tissue architecture—and thereby spatial relations—between different cellular compartments, but the opportunities for parallel and multimodality analyses of different molecular species have been limited. The desire to study large numbers of cells for a greater number of markers has led to the development of methods that increase throughput but discard the architecture information of solid tissues. A pioneering report described the use of microarrays for single-cell measurements of both transcripts and copy number variation across the genome (9). At present, one can use highly parallel quantitative PCR (10), microarrays (11), and next-generation sequencing (12, 13) to measure large parts or all of a cell’s nucleic acid repertoire.

Proteomic measurements with cellular resolution are possible with flow cytometry. With this approach, up to 10–15 proteins in large populations of cells can be analyzed simultaneously with fluorescently labeled antibodies. Fluorescence-activated cell sorting, a form of flow cytometry, enables researchers to isolate cells on the basis of their protein expression profiles or physical characteristics (such as granularity or size) for subsequent molecular analyses. The opportunities for flow cytometry have recently been expanded further with a new process referred to as “CyTOF,” which replaces fluorescent tags on antibodies with stable isotopes that can be identified via TOF mass spectrometry (14). The use of stable isotopes overcomes problems related to background noise and spectral overlap between different fluorophores, and the approach has been used successfully to analyze up to 34 protein targets and protein modifications simultaneously, providing vast amounts of cellularly resolved information (15).

Despite the advances in protein measurements, analyses of proteins in single cells have been limited, both by the analytical sensitivity and specificity of the currently available methods and by difficulties in collecting comprehensive views of cell actions at the nucleic acid and protein levels.
The report by Ståhlberg et al. illustrates a means for specific analyses of different molecular modalities via the use of a common, convenient readout. This new process for codetecting various biological targets was established and characterized in a somewhat artificial system, which focused on a transfected plasmid and the transcript and fusion protein it encodes, as well as some genes regulated by that protein. Along with DNA copy number measurements, the authors made use of reverse transcription and the proximity ligation assay to access RNA and protein, respectively, for a common readout of DNA, RNA, and protein via TaqMan assays. The proximity ligation assay reports the presence of proteins via the ligation of DNA strands attached to pairs of antibodies that are brought into close proximity when they both bind to a target protein molecule. The fact that all measured molecular entities are represented by nucleic acids provides access to convenient, powerful technologies for detecting and quantifying nucleic acids. Besides real-time PCR, it is easy to see that the described approach can be generalized to obtain a higher throughput and a more complete understanding of processes under way in individual cells at the time of harvest, because it uses next-generation sequencing to read out DNA, RNA, and protein concentrations in parallel (16) from the same single cell.

With this report, the range of methods for molecular measurements of single cells—the smallest functional unit of biological organization—has been extended. Methods for single-cell analysis will have particular value in cancer research, where it is becoming increasingly clear that different cells have distinct histories, carry out separate roles in the biology of a tumor, and differ in their clinical importance for diagnosis and therapy. By their use of microscopy and flow cytometry, pathologists and cytologists have already adopted a single-cell perspective in evaluating diseases and responses to therapy. New approaches such as those Ståhlberg et al. have described illustrate the growing opportunity to characterize critical cellular functions more fully at the nucleic acid and protein levels in what might be referred to as “single-cell omniotics.”

References