Background: Typically, optical microscopes for cytopathology use absorptive stain, while research microscopes for biomarker studies use fluorescent probes. Optical microscopes rely on stacks of 2D fluorescence images, but true 3D volumes cannot be measured accurately due to low resolution along the optical axis. By rotating the cell within a capillary tube in an optical microscope, the high 2D lateral resolution can be used to compensate for this shortcoming. The Cell-CT microscope utilizes a rotating cell mechanism with tomographic reconstruction to produce isotropic-resolution, true 3D images of cells for more accurate quantitative measurement of features. Imaging a cell in the Cell-CT equipped for dual labeling of both fluorescence and absorbance allows for exact registration of volumetric images of isometric resolution.

Methods: Chemically fixed and labeled cells are embedded in optical gel and flowed through a rotating capillary tube within the Cell-CT microscope. The same objective lens is used to image the cell in transmission-mode, using filtered white-light, followed by imaging in epi-illumination-mode using filtered arc-lamp light. Each image set consists of 500 image pseudo-projections, spaced evenly across a single 360-degree rotation (every 0.72 degrees). A pseudo-projection image is the result of rapidly scanning the sub-micron depth-of-focus of the objective lens through the cell and integrating the optical information at the CCD camera. To generate a 3D reconstruction of the cell, the 500 pseudo-projections are processed tomographically in the same manner as x-ray projections are used to generate X-ray CT images. To illustrate the Cell-CT instrument performance, fluorescent microspheres (100 nm diameter) and dual-labeled female muntjac cells (arrested in metaphase and labeled with hematoxylin and Sytox Green) were imaged.

Results: The 0.35 micron resolution of the Cell-CT was equal in lateral and axial directions when measuring the point-source of fluorescence in the 3D reconstructed images. The six large chromosomes of the female muntjac cell are clearly visible in the figure showing a series of absorbance and fluorescence views from the 3D reconstructions. Co-registration of images is within a rotational unit (0.72 degrees). Non-specific and sparse distribution of hematoxylin allows the cytoplasm boundary to be distinguished in the absorption images.

Conclusions: Isometric 3D resolution has been demonstrated in the Cell-CT instrument for fluorescence imaging which registers exactly with absorption imaging. Future work will provide 3D localization of biomarkers onto diagnostic cells with standard cytological classification. Within single cells, more quantitative protein expressions, Q-dot localizations, and 3D fluorescence in-situ hybridizations (3D-FISH) are now possible.