# Non-invasive detection of biomechanical and biochemical responses of human lung cells to short time chemotherapy exposure using AFM and Confocal Raman Spectroscopy

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## Electronic Supplementary Information

## Experimental

1. Cell viability test:

The cell viability was analyzed using LIVE/DEAD Viability/Cytotoxicity Assay Kit (Invitrogen) according to the manufacturer's instruction. Briefly, (1) Cells were cultured in poly-D-lysine coated glass-bottom dishes (MatTek Cop. USA) and MgF2 substrate which was put in Petri dishes for 24 hours; (2) cells were then washed with PBS twice; (3) 2 ml of mixed solution of 2 μM Calcein AM and 4 μM ethidium homodimer-1 (EthD-1) (both from Invitrogen) was added directly to cells, and incubated cells for 30 mins at room temperature; (5) cells were imaged using fluorescence microscope with DP30BW CCD camera (Olympus IX71) to analyze the relative proportion of live/dead cells. Here, a 10× objective was used to observe fluorescence. Calcein AM is well retained within live cells producing green fluorescence; however, EthD-1 enters cells with damaged membrane and binds to nucleic acids, thereby producing a red fluorescence in dead or membrane-damaged cells. Therefore, the live/dead cells were differentiated visually.

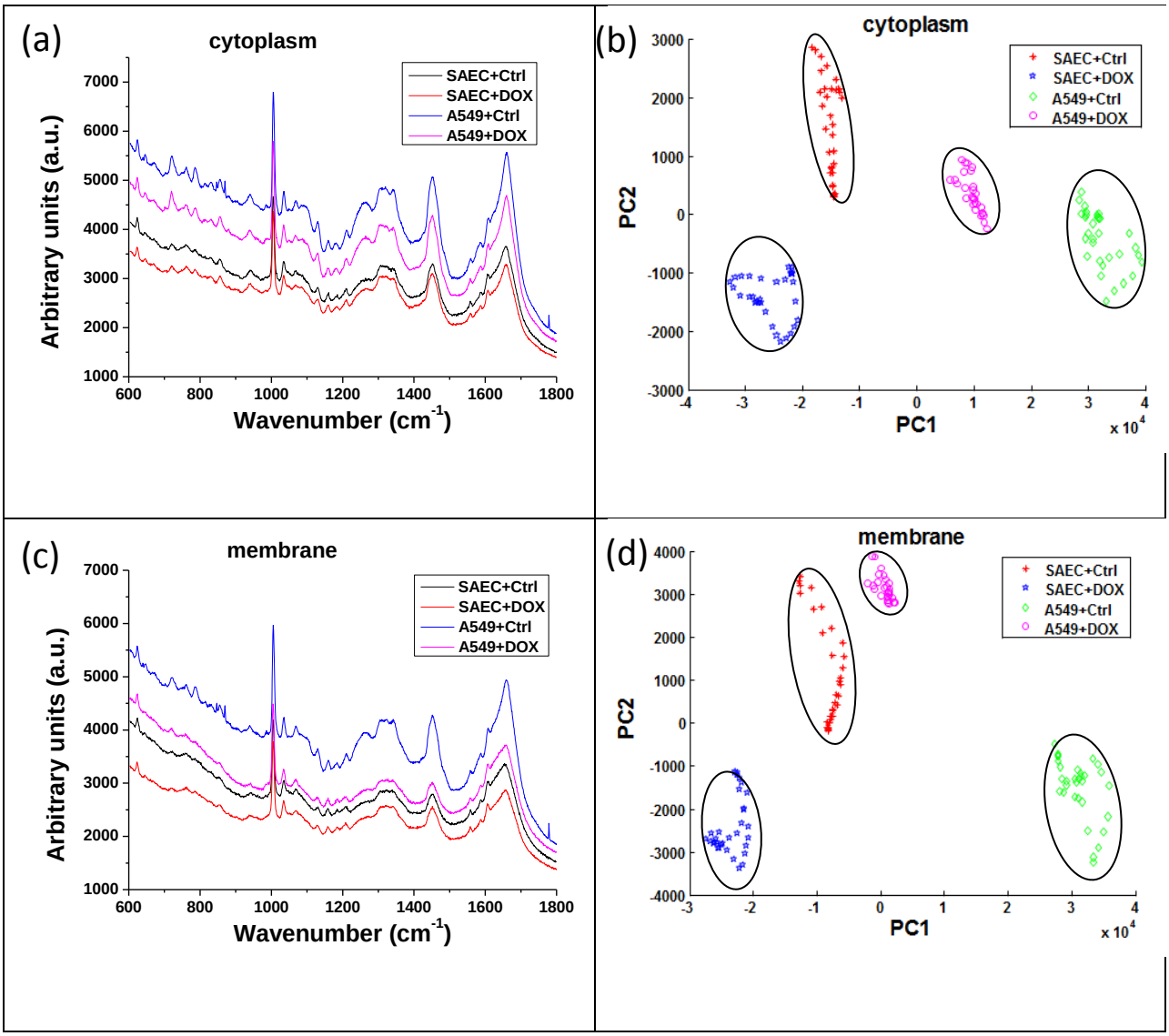


**Fig. S1.** Average Raman spectra and PCA analysis of A549 cells and SAECs for cytoplasm (a, b) and membrane (c, d) areas of control and DOX treatment (70nM, 4hr) experiment.

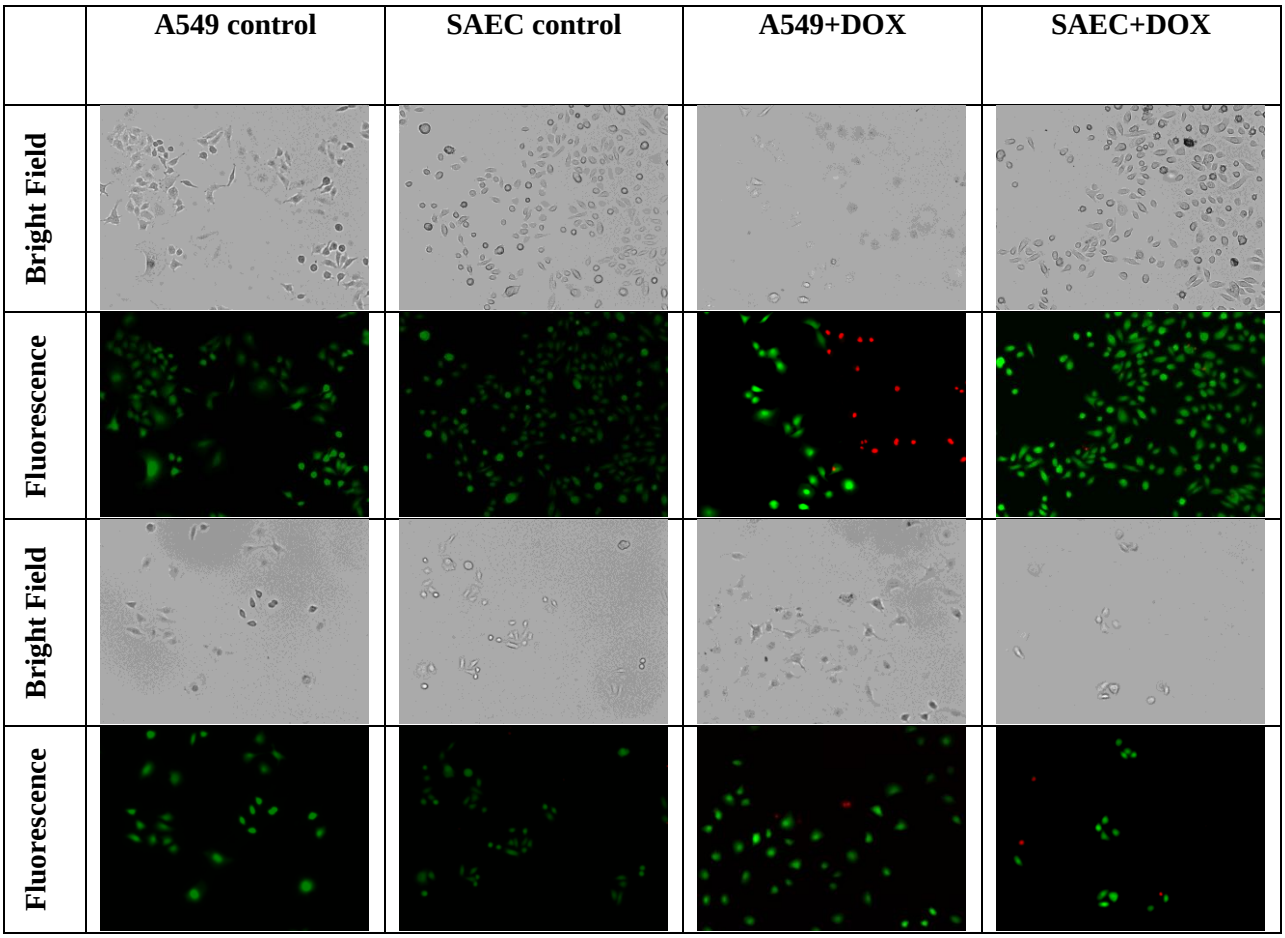


**Fig. S2.** Representative fluorescence images of cell viability test. Images of A549 cells and SAEC control (column 1, 2) and with DOX treatment (column 3, 4) were exhibited. Cells were stained with Invitrogen LIVE/DEAD Viability/ Cytotoxicity Assay Kit. Green fluorescence presented live cells, whereas red fluorescence showed dead or membrane-damaged cells. All images were obtained with 10× lens. These fluorescence images together revealed that A549 cells and SAECs which were used for AFM (row 1, 2) and Raman (row 3, 4) experiments were mostly alive.

**Table S1.** Tentative Raman band assignments of Small Airway Epithelial Cells (SAEC) and human lung adenocarcinoma epithelial cell (A549)

| **Raman shift ($cm^{-1}$)** | | |
|---|---|---|
| **SAEC** | **A549** | **Band assignment** |
| 624 | 624 | Phenylalanine |
| 643 | 643 | C-C twist Phenylalanine |
| 662 | 662 | C-S stretching mode of cystine (collagen type I) |
| 666 | 666 | G, T-tyrosine-G backbone in RNA |
| 672 | 669 | C-S stretching mode of cytosine |
| 719 | 719 | C-C-N+ symmetric stretching in phosphatidylcholine |
| 720 | 720 | DNA |
| 762 | 762 | Tryptophan |
| 786 | 785 | DNA & phosphdiester bands DNA |
| 813 | 813 | Phosphodiester bands RNA |
| 832 | 832 | $PO_2^-$ stretch nucleic acids |
| 854 | 853 | Tyrosine |
| 880 | 881 | Tryptophan |
| 900 | 901 | Monosaccharides (b-glucose), (C-O-C) skeletal mode |
| 939 | 939 | Skeletal modes (polysaccharides) |

| | | |
|---|---|---|
| 961 | 961 | Phosphate of HA; Calcium-phosphate stretch band |
| 1006 | 1006 | Phenylalanine |
| 1034 | 1034 | Phenylalanine |
| 1066 | 1066 | $PO_2^-$ stretching; chain stretching; C-O, C-C stretching |
| 1070-90 | 1070-90 | Symmetric $PO_2^-$ stretching of DNA (represents more DNA in cell) |
| 1095 | 1095 | Phosphodioxy group ($PO_2^-$ in nucleic acids); Lipid |
| 1129 | 1129 | C-C skeletal stretch transconformation |
| 1158 | 1158 | Lipids and nucleic acids (C, G and A ) |
| 1179 | 1176 | Cytosine, guanine |
| 1213 | 1213 | Tyrosine, phenylalanine |
| 1254 | 1254 | Lipid; A,T breathing mode (DNA/RNA); Amide III (protein) |
| 1304 | 1304 | $CH_2$ deformation (lipid), adenine, cytosine |
| 1306 | 1306 | C-N stretching aromatic amines |
| 1317-9 | 1317-9 | Guanine (B,Z-marker) |
| 1343 | 1342 | G (DNA/RNA); CH deformation (proteins and carbohydrates) |
| 1400-30 | 1400-30 | $\gamma$(C=O) $O^-$ (amino acids aspartic & glutamic acid) |
| 1451 | 1450 | $CH_2$ deformation (nucleic acid, proteins, lipids) |
| 1579 | 1581 | Pyrimidine ring (nucleic acids) |

| | | |
|---|---|---|
| 1608 | 1608 | Phenylalanine, Tryptophan |
| 1660 | 1661 | Amide I |
| 1740 | 1740 | Collagen III |

Band assignment is based on[1-9].

## References


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