機能化AFMプローブを応用した 生細胞のナノ加工・計測技術の開発

Functionalized AFM-Based Nanofabrication and Nanomeasurement Techniques for Living Cells

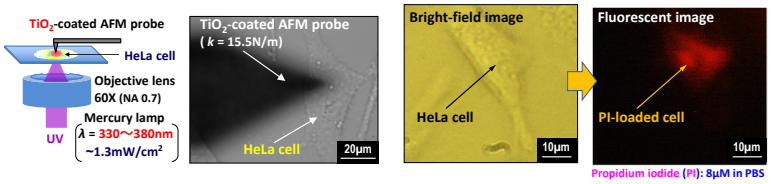
豊橋技術科学大学　機械工学系　　柴田 隆行，永井 萌土
Takayuki SHIBATA and Moeto NAGAI
Dept. of Mechanical Engineering, Toyohashi University of Technology

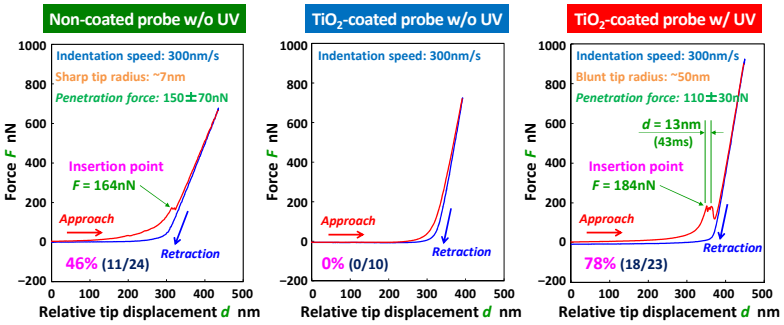
ABSTRACT

The atomic force microscope (AFM) provides an effective platform for *in vitro* manipulation and analysis of living cells in medicine and the biological sciences. In this study, we investigated the effect of indentation speed on the cell membrane perforation of living HeLa cells based on *highly localized photochemical oxidation* with a titanium dioxide (TiO_2)-coated AFM probe. Moreover, we also discussed the possibility of *intracellular tip-enhanced Raman spectroscopy (TERS) imaging* of molecular dynamics in living cells using an AFM probe coated with silver nanoparticles (AgNP) in the homemade Raman system integrated into an inverted microscope.

LOCALIZED PHOTOCATALYTIC OXIDATION

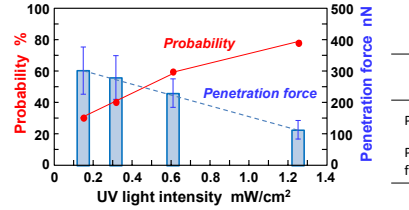
☞ *Cell membrane perforation for minimally invasive intracellular delivery*

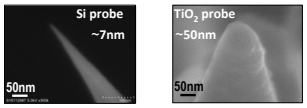




☞ *The probability of cell membrane perforation up to 78% compared with 46% with Si probe.*

EFFECT OF UV INTENSITY

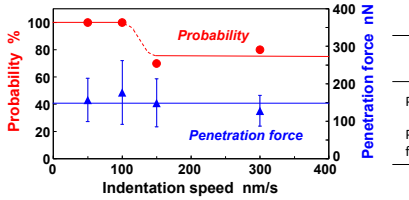




	UV light intensity (mW/cm²)			
	0.15	0.32	0.61	1.25
Probability (%)	30 (n = 10)	40 (n = 10)	60 (n = 10)	78 (n = 23)
Penetration force (nN)	300 ± 75 (n = 3)	280 ± 70 (n = 14)	230 ± 45 (n = 6)	110 ± 30 (n = 18)

☞ *The probability of cell membrane perforation increased with increasing UV light intensity, while the penetration force decreased.*

EFFECT OF INDENTATION SPEED

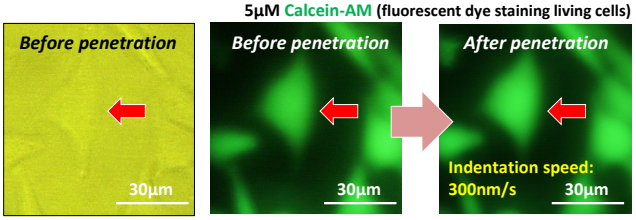


	Indentation speed (nm/s)			
	50	100	150	300
Probability (%)	100 (n = 20)	100 (n = 10)	70 (n = 10)	80 (n = 25)
Penetration force (nN)	157 ± 58 (n = 20)	177 ± 85 (n = 10)	149 ± 64 (n = 7)	128 ± 41 (n = 20)

☞ *The probability of cell membrane perforation reached 100% at indentation speeds less than 100 nm/s, while the penetration force was kept almost constant.*

EVALUATION OF CELL VIABILITY

5μM Calcein-AM (fluorescent dye staining living cells)

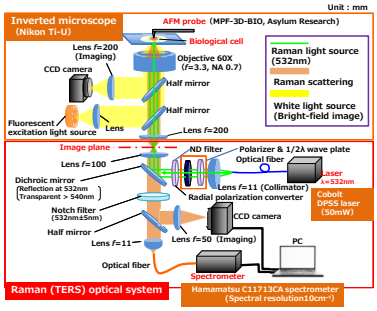


Cell viability

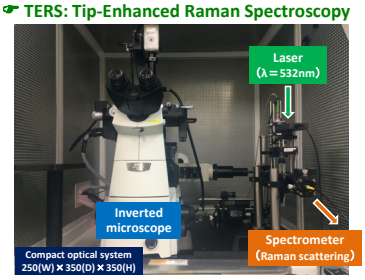
- 300nm/s 100% (8/8)
- 50nm/s 100% (10/10)

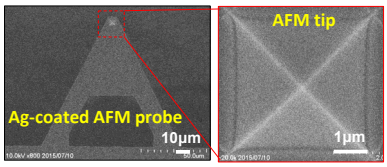
☞ *High probability of cell viability after cell membrane penetration for enabling minimally invasive intracellular delivery.*

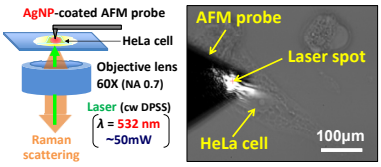
INTRACELLULAR TERS IMAGING

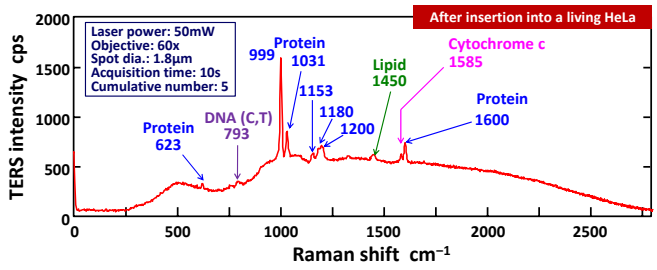


☞ TERS: Tip-Enhanced Raman Spectroscopy





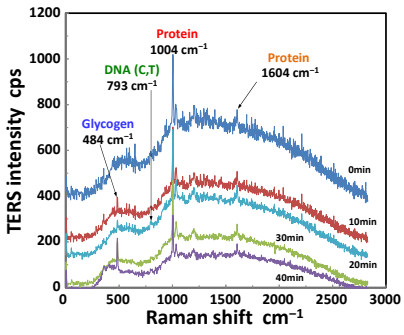


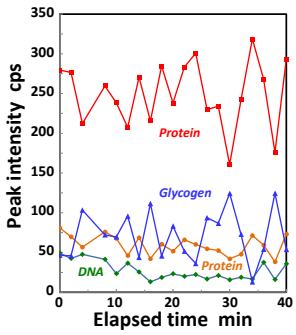


☞ *As expected, the peaks most likely associated with the protein, DNA and cell membrane lipids were clearly observed inside the living cell.*

☞ *This would allow us to quantitatively study dynamic processes inside living cells.*

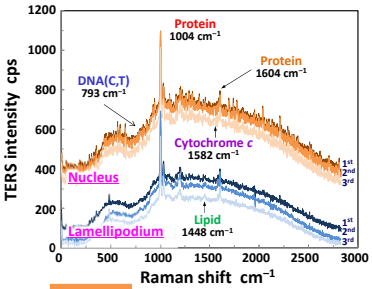
TIME-DEPENDENT TERS SPECTRA

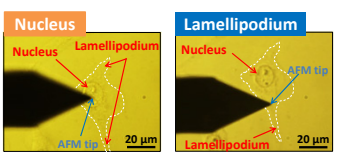




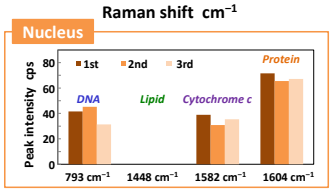
☞ *An increase in the peak intensity of protein may cause a decrease in that of glycogen, and vice versa.*

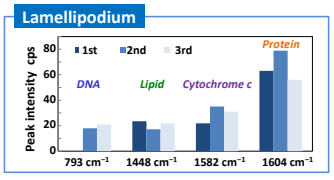
NUCLEUS vs LAMELLIPODIUM





DNA	Nucleus > Lamellipodium
Lipid	Nucleus < Lamellipodium
Protein & Cytochrome c	Nucleus ≈ Lamellipodium





The next step in this study is to combine both the above incomparable functions, photocatalytic nanofabrication and intracellular imaging of living cells, by using a functionalized Ag-TiO_2 coated AFM probe.