Improved method of the MALDI-TOF analysis of DNA with nanodot sample target plate

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We report a new sample target plate for MALDI-TOF mass spectrometry made of SiO_2 with 30 nm Pt dots for which a highly reproducible and improved DNA analysis was achieved.

Matrix-assisted laser desorption/ionization (MALDI)[1,2] is a means of soft ionizing target samples *via* a matrix by laser beam irradiation. MALDI can ionize large-sized molecules without fragmentation. Time of flight (TOF) is a method to compute the molecular weight of an ionized molecule based on its flight time when accelerated by high voltage in a vacuum tube. MALDI TOF mass spectrometry, which is a combination of MALDI and TOF, is a powerful tool for analyzing biomolecules such as peptides and proteins. However, mass spectrometry is still used only in a limited fashion in the field of DNA research because DNA analysis by mass spectrometry lacks reproducibility, and the detectable range of the DNA size is more limited than other biomolecules.

For single nucleotide polymorphisms (SNPs) detection, mass spectrometry is sometimes used and several methods are known,[3–6] for example, PinPoint assay which detects the products of primers with a single extended base. However, these assays still need improvement to be practically used for genome-wide analysis. There are also some methods suggested for the sequencing of short DNAs using mass spectrometry,[7–9] but they are still not used as practical methods.

If the DNA analysis with mass spectrometry could achieve a high reproducibility by a simple method, it would be a powerful tool because an analysis based on its molecular size can distinguish adenine, guanine, cytosine and thymine without any labelling and also because measurement by mass spectrometry requires only a few seconds per sample, which is much shorter than other methods (for example, microchip electrophoresis requires several minutes). Since SNP analysis and genome sequencing are intensively performed, the rapid analysis of DNA with a higher reproducibility is significantly demanded. Therefore, improvement of the DNA analysis by mass spectrometry is an important issue.

In this report, we describe our improved mass spectrometry analysis of DNA by simply using our nanodot sample target without any modification of the sample preparation.

The newly developed nanodot sample target has an SiO_2 wafer which has 20 wells (rows 1–4, columns A–E) with nanosized Pt dots (60, 90 or 120 nm pitches) loaded in the adapter which is used to fit the wafer to the MALDI TOF-MS . The nanodots of Pt on the wafer were prepared by Nano F Consultant (Japan) using electron-beam lithography, followed by chemical etching to rim the wells. The diameter of each dot is 30 nm with pitches of 60 nm (row 1, columns A–E), 90 nm (row 2, columns A–E) or 120 nm (rows 3 and 4, columns A–E). The variation in the number of wells for the different pitch sizes is due to the manufacturing process when ordering the nanofabrication. The target sample plate is reusable by unloading the silicon wafer and washing the wafer with water in an ultrasonic bath. Representative SEM images of the nanodots on the sample target and the sample crystallization image of the nanodot sample target plate and the conventional target plate are shown in Fig. 1(a)–(c).

Reproducibility of the DNA mass spectrum using a conventional sample target (MTP 384 massive target, Bruker Daltonics) and the nanodot sample target plate are shown in Table 1. The DNA sample for this experiment was prepared by dissolving three different DNA molecules, 5′-ACT TCT GTG TTT AGG T-3′ (MW = 4893), 5′-ACT TCT GTG TTT AGG TGT C-3′ (MW = 5816) and 5′-ACT TCT GTG TTT AGG TGT CTC TCA-3 (MW = 6713) each at 5 pmol l^{-1} in H_2O as a mixture. 3-Hydroxypicolinic acid was dissolved at 10 mg ml^{-1} in acetonitrile–0.1% TFA (1 : 1). One μl of the sample solution containing three types of DNA molecules and 1 μl of the matrix solution were mixed on each well of the nanodot sample target or conventional sample target, then air dried. MALDI TOF mass spectrometry (Ultraflex, Bruker Daltonics) was loaded with either the nanodot sample target or the conventional sample target using a mass spectrometer equipped with a pulsed nitrogen laser and a delayed extraction ion source (positive mode, linear mode, ion source 1: 20.00 kV, ion source 2: 18.7 kV, lens: 5.6 kV). The mass spectra of 60 randomly chosen spots in the wells of A1–E4 were taken and the success rates for each well were calculated by dividing the number of successful spots by 60. The experiment was performed three times. In this experiment, a successful spot meant a spot which showed that the spectrum of all three DNA molecules were detected with $S/N > 5$ as the correct molecular weight, that the intensity for each molecule was greater than 300 and the resolution for each molecule was greater than 250 in one laser beamed spot (60–100 μm diameter), and it was counted as successful while the others were counted as failures.

As shown in Table 1, samples on the nanodot sample target were all detected with a much higher reproducibility compared to the conventional sample target plate. No clear difference between the pitch sizes were seen among the pitches of 60, 120 and 180 nm. This result shows that good spectra can be obtained for more than 50% if the samples are measured using the nanodot target plate, while the success rate of the conventional plate is 13.9%. The slight dispersion of the data may be due to the preparation. The

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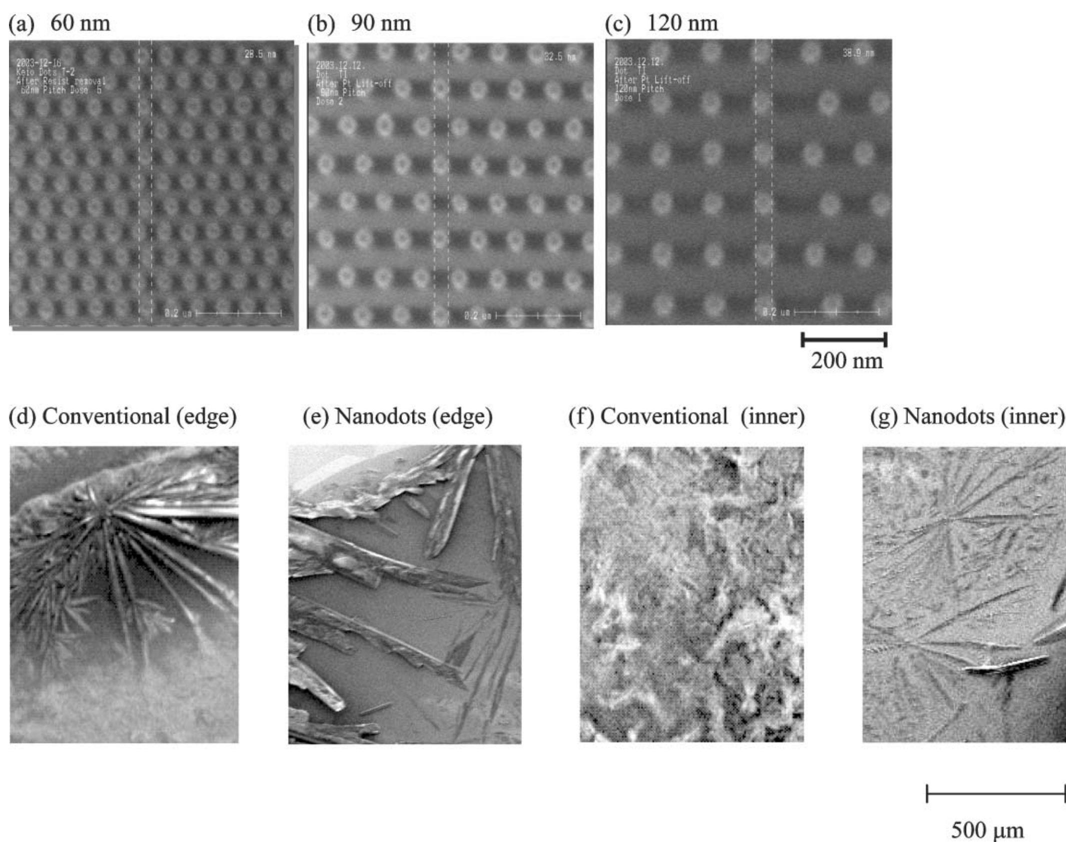

Fig. 1 Top: SEM images of the nanodots with pitches 60 nm (a), 90 nm (b) and 120 nm (c). Bottom: representative SEM images of crystals on the edge of the conventional target plate (d), the edge of the nanodot target plate (e), the inner area of conventional target plate (f) and the inner area of the nanodots target plate (g).

Table 1 The numbers of success spots (N) per 60 and the success rates (SR) of conventional target plates and wells A1–E4 of the nanodot target plate are shown. The measurement was repeated three times with different preparations. A successful spot means a spot which showed the spectrum of all three DNA molecules as main peaks with S/N $>$ 5, intensity $>$ 300, $m/\Delta m > 250$

			1st measurement		2nd measurement		3rd measurement		
		Pitch size/nm	N	SR (%)	N	SR (%)	N	SR (%)	Total SR (%)
Conventional target plate		—	5	8.3	7	11.7	13	21.7	13.9
Nanodot target plate	A1	60	56	93.3	42	70.0	54	90.0	84.4
	B1	60	60	100.0	31	51.7	47	78.3	76.7
	C1	60	60	100.0	60	100.0	47	78.3	92.8
	D1	60	46	76.7	55	91.7	50	83.3	83.9
	E1	60	58	96.7	49	81.7	42	70.0	82.8
	A2	90	58	96.7	46	76.7	43	71.7	81.7
	B2	90	45	75.0	47	78.3	52	86.7	80.0
	C2	90	59	98.3	41	68.3	58	96.7	87.8
	D2	90	51	85.0	57	95.0	49	81.7	87.2
	E2	90	22	36.7	29	48.3	37	61.7	48.9
	A3	120	60	100.0	58	96.7	30	50.0	82.2
	B3	120	33	55.0	39	65.0	45	75.0	65.0
	C3	120	57	95.0	52	86.7	47	78.3	86.7
	D3	120	24	40.0	38	63.3	52	86.7	63.3
	E3	120	27	45.0	24	40.0	21	35.0	40.0
	A4	120	30	50.0	31	51.7	16	26.7	42.8
	B4	120	43	71.7	46	76.7	45	75.0	74.4
	C4	120	33	55.0	29	48.3	48	80.0	61.1
	D4	120	54	90.0	52	86.7	51	85.0	87.2
	E4	120	59	98.3	44	73.3	33	55.0	75.6

reproducible effect of the nanodot target plate was confirmed by multiple experiments either with different samples or under different conditions.

When using the nanodot target plate, the S/N and resolution of the obtained spectrum were significantly improved, which resulted in the high success rate. On the conventional target plate, some of

the failure spots did not show any spectrum at all, but there were some spots showing an indefinite spectrum and were counted as failures. In these spots, the S/N was less than 5 and/or the resolution was less than 250.

An increased homogeneity of the matrix/analyte crystal distribution has been considered to be important for the high reproducibility of MALDI TOF-MS.[10] By taking SEM images, we found clear crystals of a matrix and DNA tend to localize at the edge of the sampling spots on the conventional sample target plate (Fig. 1(d)) and there was no clear crystal pattern in the inner area (Fig. 1(f)). On the other hand, when using the nanodot target plate, large crystals were formed on the edge of the sample spot (Fig. 1(e)), and fine needle crystals were formed in the inner area (Fig. 1(g)). Those fine crystals of the inner area contribute to the high reproducibility. The inner area of the conventional target plate hardly showed a successful spectrum, while most of those on the nanodot target plate were successful. On the nanodot target plate, the sample solution may be dried by forming fine crystals around the nanodots as an effect of the physiological structure of the nanosize asperity.

Recently, we prepared a new nanodot target plate with a wider range of dot pitches (60 to 1000 nm) and obtained a preliminary result showing that thin crystals are not formed if the dots pitches are 480 nm or more. The reproducibilities of the samples on the nanodots with pitches of 480 nm or more were not improved, which corresponded to the result of the crystallization. We also prepared a nanodot sample target which used a Si substrate instead of SiO_2, but it did not show any improvement in the mass spectral analysis of the DNA, thus we considered that the hydrophobic and hydrophilic contrast of the structure is also important.

There is a report describing a method of spotting a miniaturized DNA sample using a piezoelectric pipette, resulting in approximately the same crystal size as the irradiation area of the desorption laser, which allows detection of DNA by MALDI-TOF at the femtomol level.[11] This method may have a similar effect as our method. However, our method can be used more easily because the sample can be prepared using a normal pipette. A nanodot target plate costs approximately $5000, which is rather cheap considering the price of mass spectrometry equipment, and the target plate is reusable after washing.

In conclusion, we have reported the highly reproducible MALDI TOF mass spectrometry analysis of DNA using a newly developed nanodot sample target. The sample target also provided an improved analysis of larger oligo DNAs. We expect that our new method will contribute to the analysis of DNA sequences and SNPs which are now being examined in detail. It can be an especially powerful tool for automated detection. In addition, we would like to further investigate the role of the nano-sized structure for improving the mass spectrometry detection by preparing target plates with different materials or structures.

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