Supporting Information

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1. Materials and methods

Compounds were visualized by UV light (254 and 365 nm) and all reactions were monitored by thin layer chromatography (TLC). Column chromatography was performed on silica gel (200-300 mesh). ¹H NMR and ¹³C NMR spectra were recorded on a Bruker instrument (400 MHz and 101 MHz, respectively). Data for ¹H NMR are reported as follows: chemical shift (δ , ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, q = quartet or unresolved, coupling constant (J) in Hz, integration). Data for ¹³C NMR were recorded in terms of chemical shift (δ , ppm) relative to the center of the triplet at 77.0 ppm for CDCl3. Mass spectra (MS) were measured with Bruker instrument. Fluorescence spectra were determined on a Multi-Mode Microplate Readers. Mass spectra were obtained on Shimadzu IT-TOF-MS or Shimadzu ESI-MS system. All analytical HPLC were carried out on Waters 2695 using reverse-phase Phenomenex Luna 5 μ m C₁₈₍₂₎ 100 Å 50 \times 3.0 mm columns. Water with 0.1% TFA and acetonitrile with 0.1% TFA were used as eluents and the flow rate was 0.6 mL/min. Imaging was done with the Leica TCS SP5X confocal microscope system equipped with Leica HCX PL APO 63×/1.20 W CORR CS, 405 nm diode laser, white laser (470-670 nm, with 1 nm increments, with eight channels AOTF for simultaneous control of eight laser lines, each excitation wavelength provides 1.5 mV), and a photomultipliertube (PMT) detector ranging from 410 to 700 nm for steady state fluorescence. Images were processed with Leica Application Suite Advanced Fluorescence (LAS AF). Human recombinant Monoamine Oxidase A (M7316) and B (M7441) (5 mg/ml) were purchased from Sigma Aldrich. MAO-B inhibitor, Pargyline hydrochloride (P8013) was purchased from Sigma.





Scheme S1: Syntheses of fluorogenic-Cy3 probes

Reagents and conditiongs (A): (a) NaOH/H₂O, 0°C; CH₃I/CH₂Cl₂, 4°C overnight, 73%. (b) (BOC)₂O/THF, 0°C \rightarrow r.t, 78%. (c) NaOH, (BOC)₂O, dioxane/water, 92%. (B): (d) MeSO₃H, r.f, 81%. (e) CH₃I, K₂CO₃/DMF, r.t, 91%; (f) NaBH₄/MeOH, 0°C, 88%; (C): (g) pyridine salt/MeCN–pyridine, rt, 73.5%; (h) NaBH₄/MeOH, 0°C, 91%; (i) NaOH/MeOH–H₂O, r.f, 67%; (j)*N*-Boc-3-Bromopropylamine, K₂CO₃/CH₃COCH₃, r.f., 77%; (k) NaOH/MeOH–H₂O, r.f, 61%; (D) *N*-Boc-ethylenediamine, HOBt, EDC, Et₃N/DMF, r.t, 72%; (m) TFA/CH₂Cl₂, r.t, 85%; (n) 3, HOBT, EDC, Et₃N /DMF, r.t, 16%; (o) 2, HOBt, EDC, Et₃N in DMF, r.t, 15%. (p) TFA/CH₂Cl₂, r.t, 55.6%.

1-Methyl-4-bromo-pyridyl iodized salt (S1). 4-Bromo-pyridine hydrochloride (4.86 g, 25.1mmol) was dissolved in 100 mL aqueous sodium hydroxide solution (0.008 g/ml), and the resulting solution was stirred for 10 min. The reaction mixture was extracted with CH_2Cl_2 (100mL × 3), and the organic layer was washed with water (100 mL × 2), dried over MgSO₄ and concentrated in vacuo to afford a pale yellow oil 4-bromo-pyridyl (4.374 g, 90 %). Next, 4-bromo-pyridyl all was dissolved in 20 mL CH₂Cl₂, followed by the addition of iodomethane (75 mmol). The resulting mixture was stirred overnight at 4 °C. The formed yellow precipitate was collected by filtration, washed with n-hexane affording yellow product **S1** (5.5 g, 73%). ¹H NMR (500 MHz, CDCl₃) δ 8.95 (d, 2H, *J* = 7.0 Hz), 8.51 (d, 2H, *J* = 7.0 Hz), 1.59-1.62 (m, 3H); ESI-MS *m/z* 173.3 (M+1)⁺.

N-Boc-ethylenediamine (S2). Ethylenediamine (11.2 mL, 166.7 mmol) was dissolve in THF (200 mL), Di-tertbutyl dicarbonate (6.1g, 166.7 mmol) was added dropwise over a period of 6 hours at 0 °C dissolve in 100 mL THF. The mixture was allowed to warm up to room temperature and stirred overnight, the mixture was concentrated under reduced pressure, and saturated sodium carbonate solution (300 mL) was added. Aqueous phase extracted with EtOAC(300 mL×2),the organic layer was dried over MgSO₄. The solvent was removed by rotary evaporation and furnished **S2** as an oil liquid (78% yield).¹H NMR (DMSO, 400 MHz) δ 1.38 (s, 9H), 2.53 (m, 2H), 2.91 (m, 2H), 6.75 (bs, 1H); ESI-MS *m/z* 161.2 (M+1)⁺.

1-tert-Butoxycarbonylamino-3-bromopropane(S3), 1M NaOH aq. (30 mL) was added to a solution of 3bromopropylamine hydrobromide (3.0 g, 13.7 mmol) and di-*tert*-butyl dicarbonate (3.5 g, 16.4 mmol) in dioxane/water (40 mL/20 mL). The mixture was stirred at room temperature for 12 h, and then diluted with ethyl acetate and water. The organic layer was washed successively with 1N HCl aq., 5 wt % of NaHCO₃ aq., and brine, and then dried with MgSO₄. After filtration, the solvent was removed under reduced pressure. **S3** was obtained as colorless oil (3.0 g, 92% yield). ¹H NMR (500 MHz, CDCl₃) δ 4.31 (s, 1H), 3.46 (s, 2H), 3.18 (s, 2H), 2.11 (s, 2H), 1.42 (s, 9H); ESI-MS *m/z* 239.3 (M+1)⁺.

2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzene-3,5(6)-bicarboxylic acid (S4). 1,2,4-Benzenetri -carboxylic anhydride (2.5 g, 1.3 mmol) and resorcinol (2.86 g, 2.6 mmol) in methanesulfonic acid (15 mL) was heated with stirring at 80 °C. After complete consumption of the starting material (TLC monitoring), The dark tan solution was cooled and diluted with ice-water (60 mL) to precipitate the product. The solid was collected by filtration, washed with water, and dried. The crude residue was drided in. air oven. The resulting red solid was suspended in sodium hydroxide solution(4M), acidified with 1 N HCl to a pH of 2, filtered, and washed with water. Afforded dry product 4 g (81.8%) as red solid. ESI-MS m/z 377.3 (M+1)⁺.

Dimethyl 2-(6-methoxy-3-oxo-3H-xanthen-9-yl)benzene-3,5(6)-bicarboxylic acid methyl ester (1). MeI (2.3mL, 36.8 mmol) was added to the mixture of S4 (3.455 g, 9.2 mmol) and K₂CO₃ (5.71 g, 41.4 mmol) in 50 ml of DMF at room temperature. After stirring for 24 hours, the product was extracted with CH₂Cl₂ (100 mL× 3), and the organic layer was washed with water (100 mL×2) and brine (100mL), dried over Na2SO4 and concentrated under reduced pressure giving 3.5 g of 1 (91%).¹H NMR (500 MHz, CDCl₃) δ 8.30 (d, *J* = 0.9 Hz, 1H), 7.97 (s, 1H), 7.43 (d, *J* = 7.9 Hz, 1H), 6.97 (s, 1H), 6.89 – 6.69 (m, 3H), 6.55 (ddt, *J* = 9.7, 3.9, 1.8 Hz, 1H), 6.51 – 6.42 (m, 1H), 3.95 (dd, *J* = 35.3, 20.2 Hz, 6H), 3.67 (d, *J* = 11.9 Hz, 3H); ESI-MS *m/z* 419.2(M+1)⁺.

4-((9-(3,5(6)-bis(methoxycarbonyl)phenyl)-6-methoxy-9H-xanthen-3-yl)oxy)-1-methylpyridin (S6).

To a solution of 1(1.254 g, 3 mmol) in MeOH (100 mL) was added NaBH₄ (0.57 g, 15.0 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, and at room temperature for 2 h. After removal of the solvent, the product was extracted with CH₂Cl₂ (50 mL×3), and the organic layer was washed with brine (50 mL), dried over Na₂SO₄ and concentrated under reduced pressure to yield the air-sensitive product **S5** as a yellow powder (1.12 g, 88.9 %).

To a solution of S5 (1.12 g, 2.67 mmol) in anhydrous CH₃CN (60 mL) were added S1 (0.96 g, 3.2 mmol) and

Pyridine (5mL).The mixture was stirred at room temperature for 24 h. The remaining solid residue was removed by filtration. The filtrate was concentrated and purified by SiO₂ chromatography (CH₂Cl₂: MeOH = 50:1) to give compound **S6**. Yield: 73.5 %. ¹H NMR (400 MHz, CDCl₃) δ 7.88 (d, *J* = 8.2 Hz, 1H), 7.76 (s, 1H), 7.65 (s, 1H), 7.26 (d, *J* = 7.2 Hz, 2H), 7.17 – 6.96 (m, 1H), 6.83–6.69 (m, 1H), 6.62 (d, *J* = 10.8 Hz, 1H), 6.53 (s, 1H), 6.43 (d, *J* = 11.0 Hz, 1H), 6.31 (s, 1H), 6.14 (s, 1H), 4.42 (s, 3H), 3.92 – 3.61 (m, 9H); ESI-MS *m/z* 513.4(M+1)⁺.

Dimethyl-2-(3-methoxy-6-((1-methyl-1,2,3,6-tetrahydropyridin-4-yl)oxy)-9H-xanthen-9-yl)benzene-3,5(6)bicarboxylic acid methyl ester (S7). To a solution of **S6** (0.512 g, 1 mmol) in MeOH (30 mL) was added NaBH₄ (0.152 g, 4.0 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, after removal of the solvent, the residue was extracted with CH₂Cl₂ (30 mL× 3). The organic layer was then washed with brine (50 mL), dried over MgSO₄, and concentrated in vacuo. Yield the product **S7** as a yellow powder: 91 %. ¹H NMR (400 MHz, CDCl₃) δ 7.94 (d, *J* = 9.9 Hz, 1H), 7.88 – 7.76 (m, 1H), 7.19 (d, *J* = 8.3 Hz, 1H), 6.99 – 6.77 (m, 3H), 6.63 (d, *J* = 11.1 Hz, 2H), 6.51 (dt, *J* = 8.5, 2.6 Hz, 1H), 6.33 (s, 1H), 5.02 – 4.88 (m, 1H), 3.84 (dd, *J* = 48.1, 19.6 Hz, 9H), 2.99 (s, 2H), 2.65 (t, *J* = 5.3 Hz, 2H), 2.38 (s, 5H); ESI-MS *m/z* 516.4 (M+1)⁺.

2-(3-methoxy-6-((1-methyl-1,2,3,6-tetrahydropyridin-4-yl)oxy)-9H-xanthen-9-yl)benzene-3,5(6)-bicarboxylic acid (2). To a solution of **S7** (0.515 g, 1 mmol) in MeOH (30 mL) was added 2 M NaOH (7.5 mL), the mixture was stirred at r.f. After complete consumption of the starting material (TLC monitoring), the mixture was concentrated under reduced pressure and then acidified with 1M HCl (pH = 2). The product was extracted with EtOAc (30 mL×3), and the combined organic layer was dried over MgSO4 and concentrated. The crude residue was purified by SiO₂ chromatography to give the product **2** as a white solid 0.28g.Yield: 67 %. ¹H NMR (400 MHz, DMSO) δ 7.63 (d, *J* = 8.0 Hz, 1H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.19 (dd, *J* = 29.6, 8.4 Hz, 1H), 7.03 (t, *J* = 9.0 Hz, 1H), 6.75 (d, *J* = 8.1 Hz, 1H), 6.72 – 6.64 (m, 3H), 6.55 (dd, *J* = 13.1, 7.0 Hz, 2H), 5.08 (d, *J* = 8.0 Hz, 2H), 3.72 (d, *J* = 4.7 Hz, 3H), 3.17 (s, 2H), 2.84 (s, 2H), 2.51 – 2.40 (m, 3H), 2.32 (s, 2H); ESI-MS *m/z* 488.4 (M+1)⁺.

Dimethyl 2-(3-methoxy-6-(3-((tert-butoxycarbonyl)amino)propoxy)-9H-xanthen-9-yl)benzene-3,5(6)bicarboxylic acid methyl ester (S8). To a solution of **S5** (1g, 2.38 mmol) in anhydrous acetone (50 mL) were added K₂CO₃(0.493 g, 3.57 mmol) and **S3** (0.86 g, 2.86 mmol).The mixture was stirred at r.f overnight. The remaining solid residue was removed by filtration. The filtrate was concentrated and purified by SiO₂ chromatography (CH₂Cl₂:MeOH = 50:1) to give compound **S8** as a yellow solid 1.07g. Yield: 77.9 %. ¹H NMR (500 MHz, CDCl₃) δ 8.49 (s, 1H), 7.95 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.19 (d, *J* = 8.3 Hz, 1H), 6.93 (dd, *J* = 8.6, 3.1 Hz, 2H), 6.66 (dd, *J* = 4.2, 2.5 Hz, 2H), 6.53 (ddd, *J* = 10.3, 6.5, 3.0 Hz, 2H), 6.30 (s, 1H), 4.80 (s, 1H), 4.05 – 3.97 (m,6H), 3.90 (s, 3H), 3.82 – 3.79 (m, 5H), 3.32 (d, *J* = 5.9 Hz, 2H), 2.00 – 1.95 (m, 2H), 1.45 (s, 9H); ESI-MS *m*/z 578.4(M+1)⁺.

2-(3-methoxy-6-(3-((tert-butoxycarbonyl)amino)propoxy)-9H-xanthen-9-yl)benzene-3,5(6)-bicarboxylic acid (**3**). This compound was prepared according to the same process for the synthesis of **2** Yield: 61%; ¹H NMR (400 MHz, DMSO) δ 7.52 (d, *J* = 7.8 Hz, 1H), 7.48 (dd, *J* = 34.2, 7.7 Hz, 1H), 7.43 (d, *J* = 7.7 Hz, 1H), 7.26 (s, 2H), 7.06 (d, *J* = 5.6 Hz, 1H), 6.89 (t, *J* = 4.8 Hz, 1H), 6.63 (dd, *J* = 7.9, 2.4 Hz, 2H), 6.51 (dd, *J* = 5.3, 2.5 Hz, 2H), 6.32 (s, 1H), 3.93 (s, 2H), 3.72 (s, 3H), 3.05 (dd, *J* = 12.7, 6.5 Hz, 2H), 1.84 – 1.73 (m, 2H), 1.36 (s, 9H); ESI-MS m/z 572.4(M+Na)⁺.

2-((1E,3Z)-3-(1-(5-((2-aminoethyl)amino)-5-oxopentyl)-3,3-dimethylindolin-2-ylidene)prop-1-en-1-yl)-1,3,3trimethyl-3H-indol-1-ium (S10).The Cy3 (0.114 g, 0.2 mmol) was dissolved in DMF (5mL) and added with HOBt (0.033 g, 0.24 mmol), EDC (0.046 g, 0.24 mmol) and TEA (51 ul, 0.4 mmol) followed by stirring for 30 min. **S2** (0.0384 g,0.24 mmol) was added to the reaction mixture and stirred at room temperature for overnight. Water (10 mL) was added to the crude reaction mixture, the product was extracted with CH_2Cl_2 (50 mL× 3), dried over Na_2SO_4 and concentrated under reduced pressure giving 0.085 g of **S9** as red solid (72.6%). ESI-MS m/z 586.3(M+1)⁺.

To a solution of compound **S9** (84 mg, 0.14 mmol) in CH₂Cl₂ (5mL), was added TFA (2.5mL) at room temperature and the resulting mixture was stirred for 1.5 h. CH₂Cl₂ and TFA were removed under the reduced pressure by azeotrope with acetonitrile for several times. The resulting mixture was stirred at room temperature for 2 hours. Removal of the solvent and purified by SiO₂ chromatography produced **S10** as red solid (60 mg, 85.7% yield). 1H NMR (500 MHz, DMSO) δ 7.61 (d, J = 6.4 Hz, 2H), 7.43 (d, J = 7.3 Hz, 4H), 7.27 (d, J = 5.7 Hz, 2H), 6.49-6.38 (t, J = 13.1 Hz, 3H), 4.11 (s, 2H), 3.64 (s, 2H), 3.27 (d, J = 3.4 Hz, 2H), 3.08 (dd, J = 13.3, 6.3 Hz, 2H), 2.51 (m,2H), 2.22 (dd, J = 19.2, 13.2 Hz, 2H), 1.92-1.88(m,6H), 1.77-1.68(m,6H), 1.3-1.05 (m, 3H); ESI-MS *m/z* 486.4(M+1)⁺.

The synthesis of Probe 1. The 2 (58 mg, 0.12 mmol) was dissolved in DMF (5mL) and added with HOBt (0.020 g, 0.15 mmol), EDC (0.028g, 0.15 mmol) and TEA (34 uL, 0.24 mmol) followed by stirring for 1 h. **S10** (48.5 mg, 0. 1 mmol) was added to the reaction mixture and stirred at room temperature for overnight. Water (5 mL) was added to the crude reaction mixture, the product was extracted with CH_2Cl_2 (10 mL× 3), dried over Na₂SO₄ and concentrated under reduced pressure and purified by SiO₂ chromatography produced 15mg of **Probe 1** as red solid (15.7%). ¹H NMR (500 MHz, CDCl₃) δ 8.36 (dd, *J* = 37.1, 27.5 Hz, 1H), 7.88 – 7.80 (m, 1H), 7.74 (dd, *J* = 5.7, 3.3 Hz, 1H), 7.66 – 7.59 (m, 1H), 7.58 – 7.51 (m, 1H), 7.43 – 7.29 (m, 3H), 7.27 – 7.17 (m, 3H), 7.12 – 7.01 (m, 2H), 6.98 (dd, *J* = 10.9, 5.9 Hz, 2H), 6.75 – 6.61 (m, 2H), 6.51 (d, *J* = 13.2 Hz, 1H), 6.41 (ddd, *J* = 24.5, 12.0, 3.2 Hz, 3H), 4.84 – 4.59 (m, 1H), 4.10 (d, *J* = 6.7 Hz, 2H), 3.75 – 3.67 (m, 3H), 3.60 (dd, *J* = 17.0, 8.9 Hz, 2H), 3.54 – 3.49 (m, 2H), 3.18 (s, 2H), 2.75 (d, *J* = 13.3 Hz, 7H), 2.09 – 2.04 (m, 2H), 1.80 (s, 2H), 1.69 – 1.66 (m, 6H), 1.27 (s, 6H), 1.01 (s, 2H), 1.00 – 0.97 (m, 3H).

The synthesis of Probe 2. Compound **S11** was prepared according to the same process for the synthesis of **Probe 1** Yield: 61%. ESI-MS m/z 1017.2(M+1)⁺. To a solution of compound **S11** (10 mg, 0.01 mmol) in CH₂Cl₂ (2mL), was added TFA (0.2 mL) at room temperature and the resulting mixture was stirred for 1.5 h. crude reaction mixture was concentrated under reduced pressure and purified by SiO₂ chromatography produced 5 mg (55%). ¹H NMR (500 MHz, DMSO) 8.33 (dd, J = 68.4, 35.5 Hz, 1H), 8.24 – 8.10 (m, 3H), 8.01 – 7.83 (m, 1H), 7.68 (ddd, J = 30.2, 15.4, 4.9 Hz, 3H), 7.52 (dd, J = 6.3, 3.2 Hz, 1H), 7.45 (dd, J = 16.5, 8.7 Hz, 3H), 7.37 – 7.23 (m, 2H), 7.04 (d, J = 2.8 Hz, 1H), 6.94 (dd, J = 5.1, 2.3 Hz, 2H), 6.78 – 6.64 (m, 3H), 6.57 – 6.47 (m, 1H), 4.14 (d, J = 6.3 Hz, 2H), 3.84 – 3.76 (m, 3H), 3.65 (d, J = 6.9 Hz, 2H), 3.28 – 3.21 (m, 2H), 2.96 (s, 2H), 2.35 – 2.30 (m, 2H), 2.25 (s, 2H), 2.24 (s, 2H), 2.21 – 2.14 (m, 2H), 2.05 (dd, J = 11.4, 5.3 Hz, 3H), 1.77 (dd, J = 11.2, 5.6 Hz, 6H), 1.66 (d, J = 7.3 Hz, 6H), 1.41 – 1.32 (m, 3H);ESI-MS m/z 916.2(M-I)⁺.

3. Limit of detection

The limit of detection, expressed as the concentration, C_L , $C_L = 3\sigma/m$

$$y = \sqrt{\frac{\sum (\bar{x} - xi)^2}{n - 1}}$$

x is the mean of the blank measures (probe only), xi is the values of blank measures, n is the tested number of blank measure. m is the slope of the linear regression equation.

4. Enzymatic activity assays

The detection of activity of enzymes was implemented in 96-well fluorescence assay plates. The stock solutions of **Probe 1-2** were prepared in DMSO (10mM) and diluted in enzyme assay buffer (50 mM Borate buffer, pH=8.4) to a final concentration 100 μ m, [enzyme] =0-0.16 mg/mL(Figure S1).



Fig.S1 a-b: The emission spectrum Probe 1 reacted with different concentration of MAO-A and MAO-B separately; c-d: The emission spectrum Probe 2 reacted with different concentration of MAO-A and MAO-B separately. The concentrations of MAO-A and B were 0, 0.04, 0.08, 0.12, 0.16 mg/mL respectively.

5. LC-Mass characterization of the enzymatic reaction between Probe 1 with MAO-B.

Probe 1 (20 μ M) were added to a solution of MAO-B (50 μ g/mL) in 50 mM PBS buffer (pH 7.4). The mixture was kept at at 37 °C for 2 hrs and the mixture was subjected to LC-MS for analysis under same conditions.





Figure S2. HPLC spectra showing the reaction of MAO-B with Probe 1-2 with detection at 500 nm: (A) Probe 1 alone; (B) the products for the reaction after 2 h.



6. Enzymatic kinetics assays



Fig.S3 a-b: *Km* values of **Probe 1-2** with MAO-A and MAO-B. The *Km* and *Vmax* value of MAO-A or MAO-B was assessed by a series concentrations of **Probe 1-2**(0-200 μ M) reaction with MAO-A or MAO-B (0.16 mg/mL) at 37 °C in enzyme assay buffer(borate buffer, pH=8.4). The fluorescence intensity was collected at 570nm (λ ex=475nm).

7. Western blotting assays

The cells lysates for Western blotting assay were diluted (1 : 1000 for primary antibody if there is no specific highlight) in 5× SDS loading buffer and resolved on SDS-PAGE and transferred onto a nitrocellulose membrane (Hybond ECL, Amersham Pharmacia Biotech). The membrane were then blocked with 25 mM Tris-HCl, pH 7.4, 137 mM NaCl, 2.68 mM KCl, and 0.05% Tween 20 containing 3% BSA for 1 hr at room temperature. Membranes were incubated with primary anti-MAO-A, anti-MAO-B shaking 4 °C overnight. HRP conjugated secondary antibody (1: 5000) is added and incubated at room temperature for 1 hr. The antibody-reactive bands are visualized by chemiluminescent detection (ECL western detection kit; Amersham Pharmacia Biotech) in ImageQuant[™] LAS 500 (GE, GE Healthcare Life Sciences).





Fig.S3 Detection of MAOs activities in mammalian cells. MAO-B activities of eight different human cell lines were measured by enzymatic assay (a) and western blotting (b), respectively.

8. Cellular imaging

HepG2 cells were seeded in glass bottom dishes (Mattek) and grown until 70–80% confluency. Cells were then treated with **Probe 1** (1 μ M). After 2 h, the medium was removed, and cells were gently washed twice with PBS. The inhibition experiment was carried out with the same procedure described above except that the cells were incubated with Pargyline (50 μ M) in PBS for 2 h before the addition of the probes.