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╛ ⌐ ∆ ╢ ♃ fi Ʀ ▾ ─ ╩ ∫ √ ⁹ ≢ │ ⁸ SECM ─ ╛ ى ┼─
⁸ ─ ⌐ ≈ ™ ≡ ∆ ╢ ⁹

1.2 SECM, Scanning Electrochemical Microscopy

ķ ɔ Ķ SPM ≤ │ ⁸ ╩ ╟ ∑ √ ķ ɔ Ķ ╩ ⇔ ⁸ ─ ╩ ∆ ╢ ╩ ∆ ⁹ خ ⌂ ╙ ─ ≤ ⇔ ≡ ⁸ ﺭ fi Ⱡ ⁱ STM ╛ AFM ⅜ ╟ ╢ ≡ ⅔ ╡ ⁸ ═ ʐ ⁱ ╙ ⇔ ɔ │ ⁄ ╢ ﺨ ─ ʐ ⁱ ≢ ─ ╩ ∆ ╢ ↓ ≤ ⅜ ≢ ⅝ ╢ ⁹ ─ SPM │ ⁸ 1982 ⁱ ה ɔ ♬ ╟ ⌐ ╟ ∫ ≡ ↕ ╢ √ STM ≢ № ╢ [1] ⁹ ⁄ ─ ⁸ 1986 ⌐ ∂ ɔ ♬ ⌐ ╟ ∫ ≡ AFM ⅜ ↕ ╢ √ ث ⁸ ₁ ⌂ SPM ⅜ ╕ ≢ ⌐ ↕ ╢ ≡ ™ ╢ ⁹

SECM │ ⁸ ↓ ─ ╟ ℮ ⌂ SPM ─ پ ≢ № ╡ ⁸ ķ ɔ Ķ ≤ ⇔ ≡ nm ⅛ ╠ µm ╩ ⇔ ⁸ ⁄ ╢ ⌐ ╟ ╢ ≢ ─ ╩ ≤ ∆ ╢ ⁹ ⌐ ╟ ╢ ⅜ 1980 ⅛ ╠ ╥ ╢ ╢ ╟ ℮ ⌐ ⌂ ╡ ⁸ SPM ─ ⌐ ق ™ ⁸ SPM ─ ╩ ⇔ ⁸ ķ ɔ Ķ ≤ ⇔ ≡ ╩ ∆ ╢ SECM ⅜ Ᵽ ɔ ♪ ╟ ⌐ ╟ ∫ ≡ 1989 ⌐ ↕ ╢ √ [2, 3] ⁹ SECM ≢ │ ⁸ ≢ ─ ╩ ⌐ ╟ ╡ ∆ ╢ ↓ ≤ ⅜ ≢ ⅝ ╢ ⁹ SECM ─ ╩ ⁸ SPM ─ ≤ ⁸ ─ ⌐ ↑ ╢ ≤ ﺨ ─ ╟ ℮ ⌐ ⌂ ╢ ⁹ 1 ≈ │ ⁸ SPM ─ ≢ № ╢ ™ ╩ ∆ ↓ ≤ ≢ № ╡ ⁸ ث ─ ⌐ ═ ⁸ ─ ╛ ⅜ ≢ № ╢ ⁹ 2 ≈ ─ │ ⁸ ─ ⌐ ق ™ ⁸ ⌐ │ ⌂ ™ ⌂ ╩ ≢ ⅝ ╢ ≢ № ╢ ⁹ SECM ╩ ™ ╢ ↓ ≤ ≢ ⁸ ● ! ☻ ה ה w ⁱ ɔ ה ى ⌂ ≥ ─ ╛ ⁸ ה ⌂ ≥ ─ ≢ ⅝ ╢ ╩ ≢ ⅝ ⁸ ⁄ ╢ ╟ ─ ╩ ≢ № ╢ ⁹ ₁ ⌂ ى ╩ │ ∂ ╘ ⁸ ⁸ ⌂ ≥ ⁸ ™ ⌐ ╙ ↕ ╢ ≡ ™ ╢ [3] ⁹

─ SECM ≢ │ ⁸ ▬ ☻ ɔ ⁱ ─ ╩ ™ ≡ ɧ □ ! ♦ ɔ ╩ ∆ ╢ ɧ □ ! ♦ ɔ ≤ │ ⁸ ≢ ─ ⌐ ∆ ╢ ─ ↓ ≤ √ ╘ ⁸ │ STM ╛ AFM ≤ ⇔ ≡ ╢ ⅜ ⁸ ═ ʐ ⁱ ≢ ─ ╛ ⌐ ⇔ ⁸ ⁱ ▪ ⁱ ♃ ▬ ⊦ ⌂ ⅜ ≢ № ╢ √ ╘ ⅝ ⌂ ╩ ≢ ⅝ ╢ ⁹ ↕ ╟ ⌐ ≢ │ ⁸ ♫ ╪ ╩ ™ √ ⌂ ⅜ ≤ ⌂ ∫ ≡ ⅔ ╡ ⁸ ╕ √ ⁸ ╙ ɔ ╥ ╢ ≡ ™ ╢ ⁹ ≢ │ ⁸ SECM ⌐ ≈ ™ ≡ ⁸ ⁄ ─ ה ה ╩ ⇔ ⁸ ى ╩ ⌐ ₁ ⌂ ╩ ö ⇔ ≡ ™ ɔ ⁹

1.2.1 SECM ─

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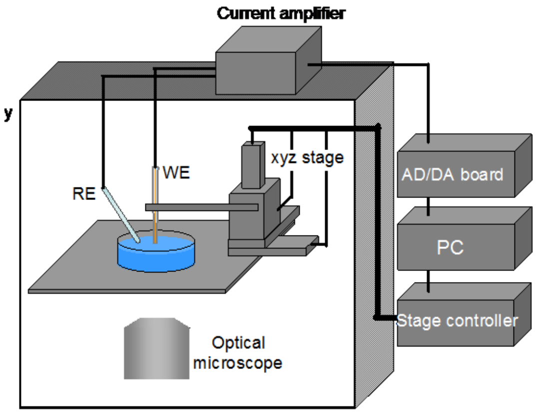

Figure 1.1 SECM system

1.2.2 ⌐ ╠ ╣

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╠ ╣ ╣ [4,5] ⁹
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⌐ ∆ ╣ ≤ ⁸ ≤ ⌂ ╡ $i_{tip,\ ä}$ Figure 1. 2 ⁸ 1 ⅜ ╠ ╣ ╣ [4-7] ⁹

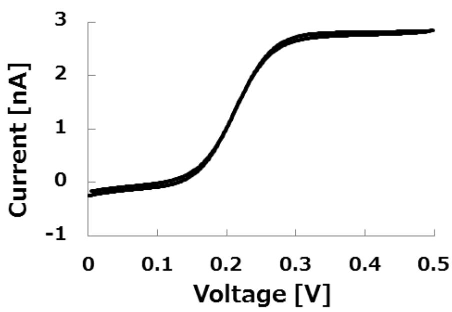

Figure 1.2 Cyclic voltammogram (CV) of microelectrode[8]. Pt microelectrode (radius = 10 îm), 1 mM $FcCH_2OH$ in PBS. Scan rate is 20 mV s^{-1}.

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(n: [-], F: ḩ □ ! ♦ ɔ =96500 [C/mol], D: [cm^2/s], C: [mol/cm^3], a: [cm])

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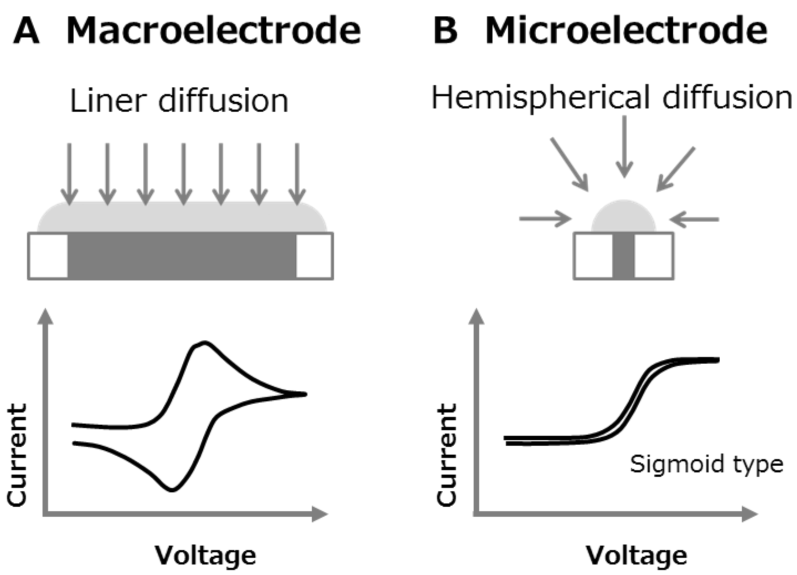

Figure 1.3 Diffusion layer of macroelectrode (A: liner diffusion) and microelectrode (B: hemispherical diffusion) [5].

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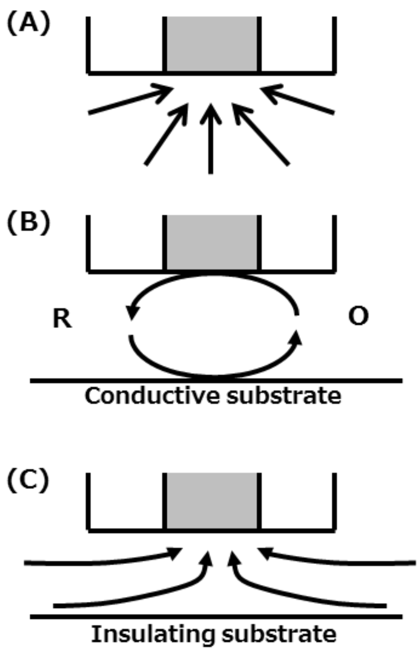

Figure 1.4 Basic principle of SECM[3].

A: hemispherical diffusion

B: positive feedback

C: negative feedback

1.2.4 Generation-Collection ≈ ɔ ♪

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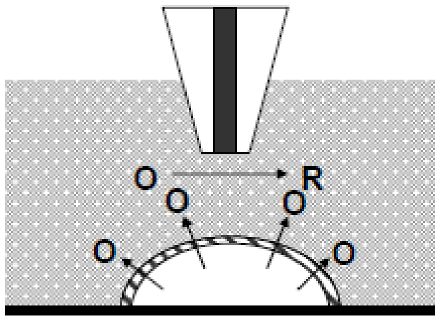

Figure 1.5 GC mode

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Figure 1. 6B ⌐ ↕ ╢ ╢ 9
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◦ ☻ ♥ ⊦ ⌐ ╟ ╡ — ⅜ ↕ ╢ ≡ ⅔ ╡ 8 ≢ ö ∆ ╢ 9

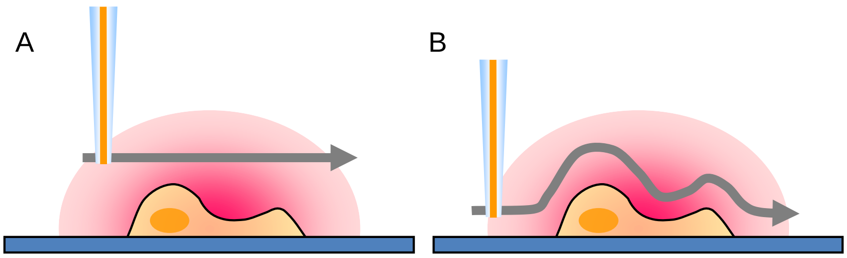

Figure 1.6 (A) constant height mode, (B) constant distance mode .

1.2.6 ╨ ≤ ∆ ╢ SECM

╨ پ ⌐ ʃ √ ╕ ╕ ﻸ ╨ ɐ پ ˵ ɔ ♪ ╨ ɐ √ ╘ ⌐ 8 ₁ ⌂
ɥ ▫ ɔ ♪ Ᵽ ɐ ◒ ◦ ◓ ♫ ᵢ ⅜ ↕ ╢ ≡ ⅔ ╡ 8 [11~15] 8 ▬ fi Ⱨ ɔ ♭ fi ☻ [9, 16-20] 8 ◦ ▪
ɥ ◊ ɔ ☻ [21-30] 8 ▬ ○ fi [31-33] ⌐ ╟ ʃ ≡ ⅜ ⌂ SECM ⅜ ↕ ╢ ≡ ™ ╢ 9
◦ ◓ ♫ ᵢ ╨ ™ √ ≤ ⇔ ≡ 8 Ł ● ♥ ▫ Ҝ ɥ ▫ ɔ ♪ Ᵽ ɐ ◒ 8 ▬ fi Ⱨ ɔ ♭
fi ☻ ⅔ ╟ ┘ ▬ ○ fi ╨ ™ √ ╙ — ⅜ № ╢ 9 Ł ● ♥ ▫ Ҝ ɥ ▫ ɔ ♪ Ᵽ ɐ ◒ ╨ ⇔ √
⌐ ≈ ™ ≡ │ ≢ ö ⇔ √ 9 ‗ ▬ fi Ⱨ ɔ ♭ fi ☻ ╨ ⇔ √ ≢ │ 8 ╨ ⌐ ↕ ∑
√ ⌐ ∂ ╢ ▬ fi Ⱨ ɔ ♭ fi ☻ — ╨ ɥ ▫ ɔ ♪ Ᵽ ɐ ◒ ◦ ◓ ♫ ᵢ ≤ ⇔ ≡ ⅔ ╡ 8 ⌐
پ ˵ ɔ ♪ ≤ ∂ ≤ ╨ ⇔ ≡ ™ ╢ 9 ▬ ○ fi ╨ ⇔ √ ≢ │ 8 ▬ ○ fi ◖

fi ♭ ◒ ♃ fi ☻ SICM — ╧ ™╢ [34, 35] ⁹ SICM ≢ │ ⁸ ķ ɔ Ķ ≤ ⇔ ≡ ⌐ 50
150 nm ╒ ≥ — — ↕ ╣ √ Ḩ Ɑ ɐ ╯ ╧ ∆ ╢ ⁹ Ḩ Ɑ ɐ ╯ ⌐ ≤ Ag/AgCl ╧
⇔ ⁸ ≤ ≤ — ⌐ ╧ ∆ ╢ ↓ ≤ ⌐ ╟ ╡ ⁸ ∂ ╢ ▬ ○ fi ╧ ∆ ╢ ⁹ ķ
ɔ Ķ ⅜ ⌐ ∆ ╢ ≤ ⁸ Ł ● ♥ ▫ Ķ ḩ ▫ ɔ ♪ Ᵽ ɐ ◒ ≤ ⌐ ▬ ○ fi — ⌐ ╟ ╡ ▬
○ fi ⅜ ∆ ╢ √ ╞ ⁸ ḩ ▫ ɔ ♪ Ᵽ ɐ ◒ ◦ ◓ ♫ ⁱ ≤ ⇔ ≡ ∆ ╢ ↓ ≤ ≢ ķ ɔ Ķ — ⅜
≤ ⌂ ╢ ⁹ SECM ⌐ ™╢ ╧ ∆ ╢ ↓ ≤ ≢ ⁸ ↓ — SICM — ╧ SECM ┼ ≤ ↕
∑ ╢ ↓ ≤ ⅜≢ ⅝ ⁸ ⌂ SECM ≤ ⌂ ╢ ⁹ ↓ ╣ ╕ ≢ ⌐ ⁱ fi ◓ [32] ╛ ♭ Ķ ⁱ Ᵽ ⁱ
[33] ⅜ ↕ ╣ ≡ ™╢ ⁹

╧ ⇔ √ ≢ │ ⁸AFM — ○ fi Ᵽ ɔ ╧ ⇔ √ ≡ ↓ ≤ ⁸
◦ ▪ ḩ ◊ ɔ ☻ ∑ ╪ ⅜№╢ ⁹ ◦ ▪ ḩ ◊ ɔ ☻ ≢ │ ⁸ ◦ ☻ ♥ ⊦ ≤
╧ ™√ ⅜№╢ ⁹ ↓ ╣ ╠ │ ╧ ⌐ ∆ ╢ √ ╞ ⁸
╧ ™⌂ ⅜ ╠ — ╧ ɐ ↓ ≤ ⅜≢ ⅝╢ ⁹

⌂ SECM ╧ ∆ ╢ — │ ⁸ ◦ ☻ ♥ ⊦ — ≤ — ≢ №╢ ⁹
— ╧ ∆ ╢ √ ╞ ⌐ ⁸ ⌂ ◦ ☻ ♥ ⊦ ╧ ∆ ╢ ⅜№╢ ⁹ ╕ √ ⁸ ⌂ ╛
— ╧ ∆ ⅜ ≢ №╡ ⁸ ⁄ — ╟ ɐ ⌂ — ╛ │ ≢ │ ⌂™ ⁹ ╟ ╡
≢ — ™ — ⅜ ≢ №╢ ⁹

1.2.7 SECM ▪ ķ ⁱ ◔ ɔ ◦ ꜛ fi ⌐ ≈ ™ ≡ [3]

SECM — ▪ ķ ⁱ ◔ ɔ ◦ ꜛ fi │ ⌐ ╦ √ ╢ ⁹ SECM │ ₁ ⌂ ⌐ ≢ №╡ ⁸
— ╧ ⌐ ≠ ♫ ♃ ⁱ fi ◓ ≢ ⅝╢ √ ╞ ⁸ ▬ ɷ ɔ ☺ fi ◓ ╧ │ ∂ ╞ ⁸ — ⁸
╛ ⌐ ╟ ╢ ≤ ⁸ ≢ — ⌐ ∆ ╢ ⁸ —
⌂ ≥ ⌐ ╙ ↕ ╣ ≡ ™╢ ⁹ ╕ √ ⁸ ╛ ⁸ ⌂ ≥ ≢ — ╛
⌐ ╙ ↕ ╣ ╢ ⁹ ╧ ⇔ ≢ — ╧ ∆ ╢ ↓ ≤ ⅜ ≢ №╢ √ ╞ ⁸ ◄ ɐ
fi ◓ ╛ ⁸ w ⁱ ɐ ɔ ⌂ ≥ — ⌐ ╙ ↕ ╣ ≡ ™╢ ⁹ ╕ √ ⁸ — ⌐ ╙
↕ ╣ ╢ ⁹ ╛ ≢ │ ⁸ ⌂ ╛ ⁸ — ⌂ ≥ ⅜ ⇔ ™ ↓ ≤ ⅜
№╢ ⁹ ⌐ ⁸ ♫ ƚ ɷ ɔ ╯ ⁱ ◘ ▬ ☼ — ╛ ⁸ ⌂ — ≢ │ ⁸ ⌐ ╟ ╡ ╧ ɐ ⁹ W
ⁱ ♃ ≠ ◓ ! ⊦ ╛ ▪ ķ ɔ ○ ɔ Ķ ╧ ™╢ ↓ ≤ ≢ ↓ ╣ ╠ ╧ ≢ №╢ ⁹ ↕ ╠ ⌐ ⁸ ≢
│ ⁸ ⌐ ╟ ╢ ⅜ ™√ ╞ ⁸ ╧ ≢ №╢ ⁹

╙ ∫ ≤ ╙ ⌂ ⅜ SECM ▬ ɷ ɔ ☺ fi ◓ ≢ №╢ ⁹ ╧ ∆ ╢ ↓ ≤ ⌐ ╟ ╡ ⁸ 2
╙ ⇔ ɔ │ 3 — ▬ ɷ ɔ ☺ fi ◓ ⅜ ≢ №╡ ⁸ ◘ fi ķ ⁱ — ╛ ⁸ —
ɐ ɐ Ḩ fi ◓ ╧ ɐ ↓ ≤ ⅜≢ ⅝ ⁸ ≤ ╡ ╦ ↑ — ⌐ ↕ ╣ ≡ ™╢ ⁹
≢ │ ⁸ ♫ ƚ ≤ SECM — ⅜ ⌐ ╞ ╟ ╣ ≡ ⅔╡ ⁸
⅛ ╠ ⌂ ≥ ⌐ ╢ ╕ ≢ — ⅔ ╟ ┘ ⌐ ╦ ╢ ₁ ⌂ ⌐ ⅔
™ ≡ ⁸ ↕ ╠ ⌂ ╢ ⅜ ╦ ╢ ≡ ™╢ ⁹ ╕ √ ⁸ ╛ ◦ ☻ ♥ ⊦ — ≤ ≢ ⁸
⌂ — ╙ ≢ №╢ ⁹

ى ⌐⅔™≡│ ⁸ث— ⌐│⌂™ ה ה ﻪ ה ⌐
⌂ SECM — ⌐╟╡ ⁸ ى ╨◘Ķ⊦ ﻠ fi○Ͻ♭Ͻ⅛≈ ¡ ▪ ¡ ♃▬⊦≢ ≢
⅝╢√ ╘⁸ ┼— ⌐ ∆╢ ⌂ ⅜ ≢ ⅝╢ ⁹╕√⁸ ⅔╟┘
— — ⌐⅔™≡ ╙ SECM ⌐ ∆╢ ⅜ ↕╣≡⅔╡ ⁸♬ƚ ɷ Ͻ♩ ¡ ○
Ͻ♭Ͻ≢ — ⌐ ≢ №╢ ⁹SECM ╨│∂ ╘⁸ ⌐╟╢ — —
⌐│ ╨ق e ↓≤⅜ ™⅜ ⁸ث— ≤— ╛ ╙⇔Ͻ│ﻬ ∆╢↓≤⌐╟∫≡
/— ╨ e ↓≤⅜≢ ⅝╢ ⁹

1.3 SECM ⌐╟╢ ى

1.3.1

SECM ⌐╟∫≡ ⌂ ى ≤⇔≡ ╙ ⌂╙—— 1 ≈⅜ ≢ №╢ ⁹SECM │⁸ ⌐ ∆╢ ⌐ ╘≡ ⌂♠Ͻ ¡≢ №╢ ⁹ ₁⌂ — ⅜ SECM ⌐╟╡ ↕╣≡⅔╡ 2 ⁸ 3 [3] ⁸ ﺦ ⌂╙—≤⇔≡ ⁸ ◓ ¡ ◖Ͻ☻○◖ ◦♭Ͻ♀ GOx ⁸ ☺▪ħ◊ ! Ͻ♀ Dp ⁸ ▪ ¡ ◌ ¡ ѵ☻ħ□♃Ͻ♀ ALP ⌂≥⅜ →╠╣╢ ⁹ — ╨ Figure 1. 7 ⌐ ⇔√ ⁹

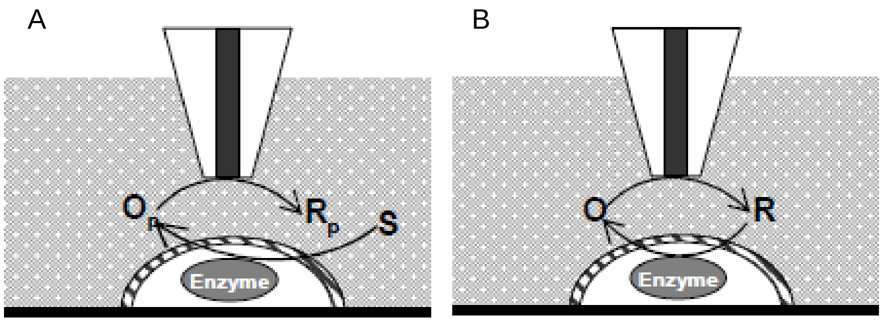

Figure 1.7 Detection principles of enzymes by GC mode (A) or FB mode (B).

╙ ⌂ ⅜ ⁸ — ╛ ╨ GC ⸗Ͻ♪⌐╟╡ ∆╢ Figure 1. 7 A ≢ №╢ ⁹ ╛┌ ⁸GOx ⅜◓ ¡ ◖Ͻ☻╨ ∆╢ ⁸ ﭽ— ╨ ⇔ ⅜ ∆╢ ⁹ ﭖ≢ ⁸ [52]╨ ∆╢↓≤≢ — ⅜ ≢ №╢ ⁹ALP — ⌐│ ≤⇔ ≡ *p*-▪⊦ƚħ▼♬ ¡ ѵ☻ħ▼▬♩ PAPP ⅜ ™╟╣╢ ⁹PAPP │ ALP ⌐╟╡ ↕╣⁸ *p*-▪⊦ƚħ▼ƚͻ ¡ PAP ≤⌂╢ ⁹ ≢ №╢ PAP ╨ ﭖ≢ ∆╢↓≤⌐╟╡ ⁸ALP ╨ ≢ ⅝╢ [56] ⁹ ╨ ﭖ≢ ┼≤ ≢ ⅝╢ ⁸ꟸ ♪ ᴘ ◡☻◘▬◡ ¡ fi◓⅜ ≢ №╡ ⁸ w☺♥▫Ķ FB ⸗Ͻ♪⌐╟╢ ⅜ ≤⌂╢ Figure 1.7 B ⁹

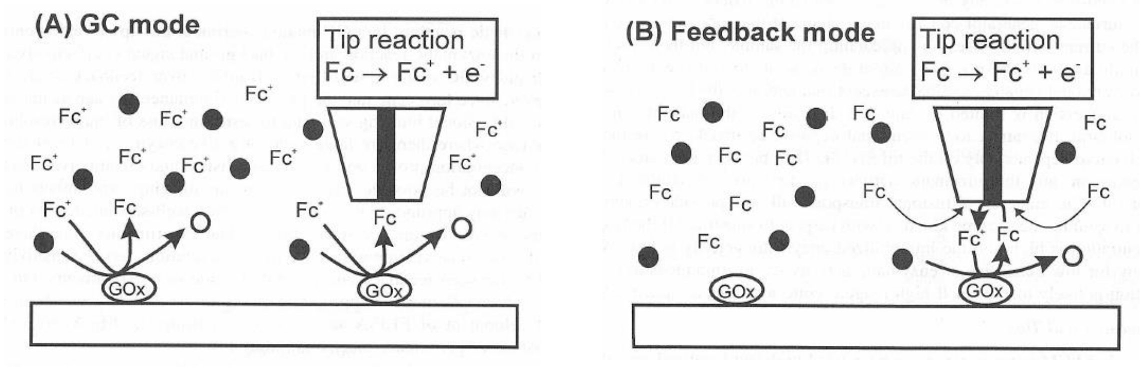

Figure 1.8 GOx detection methods by GC mode (A) and FB mode (B) [3].

⌐ ⁸ ɧ ▼ ☿ fi ی ⌂ ≥ ─ ﻍ ꙍ ♦ ▫ ◄ ᴐ ♃ ᴐ ╩ ⇔ √ ⅜№╢ [45-47] ⁹ GOx ─ ⌐ ≈ ™ ≡ ∆ ╢ ⁹ ɧ ▼ ☿ fi ی Fc ╩ ™ √ GC ≠ ᴐ ♪ ≤ FB ≠ ᴐ ♪ ⌐ ╟ ╢ GOx ╩ Figure 1. 8 ⌐ ⁄ ╣ · ╢ ⇔ √ ⁹ ˗ ¡ ◖ ᴐ ☻ ⅜ ↕ ╢ ╢ ⁸ ─ ۼ ╦ ╡ ⌐ Fc ⅜ ↕ ╡ ╢ ↓ ≤ ╩ ∆ ╢ ⁹ GC ≠ ᴐ ♪ ≢ │ Fc+╩ ڇ ⌐ ⌐ ↕ ∑ ≡ ⅔ ᴐ ⁹ GOx ⌐ ╟ ╡ Fc+⅜ ↕ ╡ Fc ⅜ ∆ ╢ √ ╘ ⁸ ٿ≢ Fc ╩ ה ∆ ╢ ≤ ⁸ ≢ │ Fc ⌐ ╟ ╢ ⅜ ⅝ ᴐ ⌂ ╢ Figure 1. 8 A ⁹ FB ≠ ᴐ ♪ ≢ │ Fc ╩ ڇ ⌐ ↕ ∑ ≡ ⅔ ᴐ ⁹ ٿ≢ │ ⌐ Fc ╩ ה ∆ ╢ ⁹ GOx ≢ ─ ╖ ↕ ╡ √ Fc ⅜ ↕ ╡ √ ╘ ⁸ ٿ≢ ─ ה ⅜ ≤ ⌂ ╡ ⁸ ↓ ─ ╟ e ⌂ ♪ ꞓ ▾ ☻ ◘ ▬ ▾ ¡ fi ˗ ⌐ ╟ ╡ ⅜ ∆ ╢ Figure 1.8 B ⁹ GC ≠ ᴐ ♪ ≢ │ ⁸ Ᵽ ¡ ▾ ڇ ⌐ │ ─ Fc ⅜ ⇔ ⌂ ™ √ ╘ ⁸ Ᵽ ꞓ ▾ ˗ ! ► fi ♪ ╩ ҩ ᴐ ╛ ╢ ↓ ≤ ⅜≢ ⅝ ╢ ⅜ ⁸ GOx ⌐ ╟ ╡ ⇔ √ Fc ⅜ ∆ ╢ √ ╘ │ ᴐ ⌂ ™ ⁹ ٻ ≢ FB ≠ ᴐ ♪ ≢ │ ⁸ ≢ ─ ╖ ⅜ ↓ ╢ √ ╘ ⌐ ⌂ ⅜ ∞ ⅜ ⁸ ⅜ ҩ ™ ⌂ ≥ ─ │ ⇔ ᴐ ⌂ ╢ ⁹ ↓ ─ ╟ e ⌂ │ ⁸ ∆ ╢ √ ╪ ╕ ᴐ ╛ ELISA ⁸ꞈ w ᴐ ♃ ᴐ ﻍ ▪ ꞓ ☿ ▬ ⌐ ╙ ↕ ╡ ≡ ™ ╢ ⁹ NADH ─ ≢ №╢ ☺ ▪ ɧ ◊ ! ᴐ ♀ ─ ⅜ ⁸ w ☺ ♥ ▫ Ҟ FB ≠ ᴐ ♪ ╩ ™ ≡ ▬ ꙍ ᴐ ☺ fi ˗ ↕ ╡ ≡ ™ ╢ [49] ⁹ ⌂ ﻍ ꙍ ♦ ▫ ◄ ᴐ ♃ ᴐ ⅔ ╟ ┘ NADH ؽ ⌐ ⅔ ™ ≡ ⁸ ٿ≢ ꙍ ♦ ▫ ◄ ᴐ ♃ ᴐ ╩ ∆ ╢ ≤ ⁸ ☺ ▪ ɧ ◊ ! ᴐ ♀ ⌐ ╟ ╡ NADH ⅜ ↕ ╡ ╢ ≤ ≤ ╙ ⌐ ꙍ ♦ ▫ ◄ ᴐ ♃ ᴐ ⅜ ↕ ╡ ╢ ⁹ ☺ ▪ ɧ ◊ ! ᴐ ♀ ⌐ ╟ ╡ ↕ ╢ √ ꙍ ♦ ▫ ◄ ᴐ ♃ ᴐ │ ⌐ ╟ ╡ ↕ ╡ ╢ √ ╘ ⁸ ⌐ ⅔ ™ ≡ ꙍ ♦ ▫ ◄ ᴐ ♃ ᴐ ─ꞈ ♪ ꞓ ▾ ☻ ◘ ▬ ▾ ¡ fi ˗ ⅜ ∂ ⁸ │ ↕ ╡ ╢ ⁹ ╕ √ ⁸ w ☺ ♥ ▫ Ҟ FB ≠ ᴐ ♪ ⌐ ╟ ∫ ≡ ⁸ ٻ ٿ ⌐ Ɽ ♃ ᴐ fi ↕ ╡ √ ╦ ↕ ┘ ɑ ¡ ○ ◖ ◦ ♄ ᴐ ♀ HRP [50] ─ ╙ ╦ ╢ ≡ ™ ╢ ⁹ ʲ ● ! ▾ ♩ ◦ ♄ ᴐ ♀ ʲ Gal ╛ ▪ ¡ ○ ¡ v ☻ ɧ □ ♃ ᴐ ♀ ALP ─ │ ⁸ GC ≠ ᴐ ♪ ⌐ ╟ ╡ ≢ №╢ ⁹ ʲ Gal ─ ≢ №╢ PAPG │ ⁸ ⌐ ↕ ╡ √ ʲ Gal ⌐ ╟ ╡ ↕ ╡ ⁸ PAP ⅜ ↕ ╡ ╢ ⁹ ٿ≢ PAP ╩ ∆ ╢ ↓ ≤ ⌐ ╟ ╡ ʲ Gal ─ ⅜≢ ⅝ ╢ ⁹ GC ≠ ᴐ ♪ ≢ │ ⁸ ꙍ ♦ ▫ ◄ ᴐ ♃ ᴐ ─ꞈ ♪ ꞓ ▾ ☻ ◘ ▬ ▾ ¡ fi ˗ ⌐ ╟ ╢ ⅜ ⌐ ⇔ ⌂ ™ ⅜ ⁸ ∆ ╢ ⅜ ⅛ ╟ ⇔ ⌂ ™ √ ╘ ⌐ ⁸ Ᵽ ꞓ ▾ ˗ ! fi ♪ ╩ ↕ ᴐ ∆ ╢ ↓ ≤ ⅜≢ ⅝ ╢ ⁹

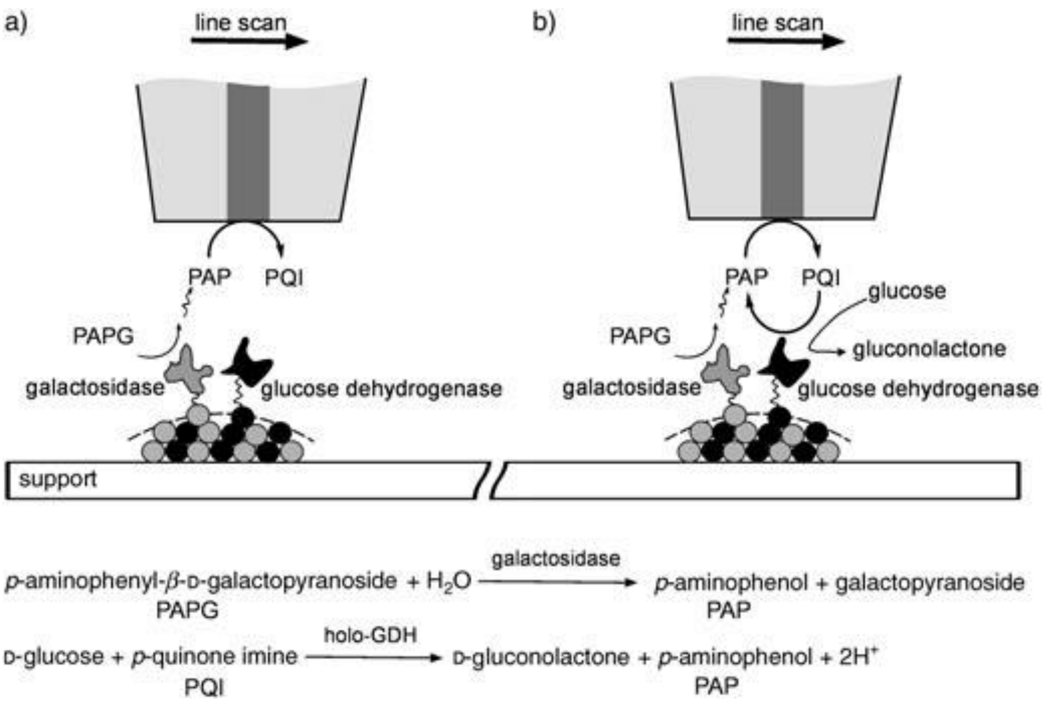

Figure 1.9 Detection by GC mode (A) or both GC mode and FB mode.

Wittstok ╠ │ ⁸ 2 — ⅜ ↕ ╣ √ ⅴ ▬ ▾ ⸗ ɟ ↄ ☼ ╩ ⇔ ≡ ⇔ ⁸ FB ⸗ ↄ ♪ ≤ GC ⸗ ↄ ♪ ╩ ↕ ∑ √ ◦ ☻ ♥ ⊦ ╩ ⇔ ≡ ™╢ Figure 1. 9 [59, 60] ⁹ ʲ ● ǃ ▾ ⅃ ◦ ♭ ↄ ♀ ⅔ ╟ ┘ ▴ ⁱ ◖ ↄ ☻ ♦ ₫ ♪ ◕ ♫ ↄ ♀ GDH ⇔ ≡ ™╢ ⁹ ⅜ ↕ ╣ √ ╩ ⁸ ▴ ⁱ ◖ ↄ ☻ ╩ ╗ ⌐ ⇔ PAPG ╩ ∆ ╢ ⁹ p- ▪ ⊦ ǂ ɦ ▼ ♬ ⁱ ʲ D ● ǃ ▾ ⅃ Ḩ ǃ ǂ ◦ ♪ PAPG │ ʲ Gal ⌐ ╟ ╡ ↕ ╣ PAP ⅜ ∆ ╢ ⁹ PAP │ ≢ ↕ ╣ p- ◖ ǂ fi ▬ ⊦ fi PQI ≤ ⌂ ╢ Figure 1.9 A ⁹PQI │ GDH ⌐ ╟ ╡ ↕ ╣ ↓ ≤ ≢ PAP ⌐ ↕ ╣ ⁸ PAP ╩ ≢ ה ∆ ╢ ↓ ≤ ≢ ⅝ ╢ Figure 1. 9 B ⁹ ↓ ─ ╟ e ⌂ ♪ ▾ ☻ ◘ ▬ ▾ ⁱ fi ▴ ⌐ ╟ ╡ ⁸ GC ⸗ ↄ ♪ ─ ╖ ─ ⌐ ═ ≡ 1.8 — ⅜ ↕ ╣ ≡ ™╢ ⁹ ≤ ╩ ⇔ √ ⅜ ╦ ╣ ≡ ™╢ [79-82] ⁹ ╩ ⌂ ꝍ ♦ ▫ ◄ ↄ ♃ ↄ ╩ ∆ ╢ ↓ ≤ ≢ ⁸ — ╙ ╦ ╣ ≡ ™╢ [61] ⁹ ╕ √ ⁸ ⌐ ∆ ╢ ALP ╩ ⇔ √ ⁸ ╙ ╦ ╣ ≡ ™╢ [53] ⁹ — ⌐ ⌐ ⌂ ▾ ♅ fi ⌐ ╟ ╢ ╙ ╦ ╣ ≡ ™╢ [83] ⁹ ʬ ↄ ♃ ↄ ╩ ™ ≡ ʝ-GAL ╛ ALP ╩ ↕ ∑ ⁸ ╩ ∆ ╢ ↓ ≤ ⌐ ╟ ╡ ╩ ≢ №╢ [84-86] ⁹

2 Measurements of enzymatic activities by FB mode.

		ꝍ ♦ ▫ ◄ ↄ ♃ ↄ	
▴ ⁱ ◖ ↄ ☻ ○ ◖ ◦ ♭ ↄ ♀	▴ ⁱ ◖ ↄ ☻	ה FcCOOH ה $(CH_3)_2NCH_2Fc$ ה $K_4[Fe(CN)_6]$ ה hydroquinone ה $[Os fpy(bpy)_2Cl]Cl$	45-47 45 45,48 48 46

NADPH ◦╛ ▾ ⊦ C‾ ♭ ▾ ♃ ↄ ♀	NADH	TMPD	48
☺ ▪ ɧ ◊ ! ↄ ♀	NADH	$FcCH_2OH$	49
: ◘ ɏ ᗡ ⁱ ○ ◖ ◦ ♭ ↄ ♀	H_2O_2	$(FcCH_2OH)^+$	50
	NO_3^-	MV^{2+}	51
Fc: ferrocene fpy: formylpyridine, bpy: bipyridine TMPD: N,N,N ǿN ǿtetramethyl -*p*-phenylenediamine MV^{2+}: methylviologen			

3 Measurements of enzymatic activities by GC mode.

	≠ ─	
◓ ⁱ ◖ ↄ ☻ ○ ◖ ◦ ♭ ↄ ♀	H_2O_2	18,52
▶‾ ▪ ↄ ♀	H+	54
▪ ⁱ ◌ ⁱ ⱱ ☻ ɧ □ ♃ ↄ ♀	PAPP	56
NAD^+ ▪ ⁱ ◖ ↄ ⁱ ♦ ₫ ♪ ◕ ♫ ↄ ♀	H^+	57
NADPH ○ ◖ ◦ ♭ ↄ ♀	O_2^-	58
PAPP: *p*-aminophenyl phosphate		

1.3.2 ≤ ─

─ ⅜ ⁸ ─ ─ ≤ ⌂ ╡ e ╢ √ ╘ ⁸ ⌐ ⅔ ↑ ╢ ⅜ ┿ ╕ ≢ ╪ ⌐ ╦ ╢ ≡ ⅝ ≡ ™ ╢ ⁹ ‾ ɀ ⁱ ≢ ─ ⅜ 1998 ⌐ ⌐ ↕ ╢ ≡ ⅔ ╡ ⁸ SECM ⌐ ╠ ╢ ─ ≤ ▬ ɷ ↄ ☺ ﬁ ◓ ╙ ╦ ╢ ≡ ™ ╢ [62 63] ⁹ ⌐ ⅔ ™ ≡ ⅛ ╠ ↕ ╢ ╢ ╩ ≢ ⇔ ⁸ ╩ ∆ ╢ ≤ ╩ ▬ ɷ ↄ ☺ ≤ ⇔ ≡ ≤ ╠ ╛ ╢ ↓ ≤ ⅜ ≤ ⌂ ╢ ⁹ ⅜ ⌂ ™ │ ⁸ ⌐ ╠ ╡ ⅜ ↕ ╡ ╢ √ ╘ ⌐ ⅔ ™ ≡ ⅜ ∆ ╢ ⁹ ─ ɦ ɧ □ ▬ ⁱ ╩ ∆ ╢ ↓ ≤ ⌐ ╠ ╡ ⁸ ─ ⌐ ╠ ╢ ⅔ ╠ ┘ ⌐ ╠ ╢ ╩ ⌐ ≢ ⅝ ╢ Figure 1. 11 ⁹ ╕ √ SECM ╩ ™ ╢ ⌐ │ ⁸ ⌐ ⅝ ⌂

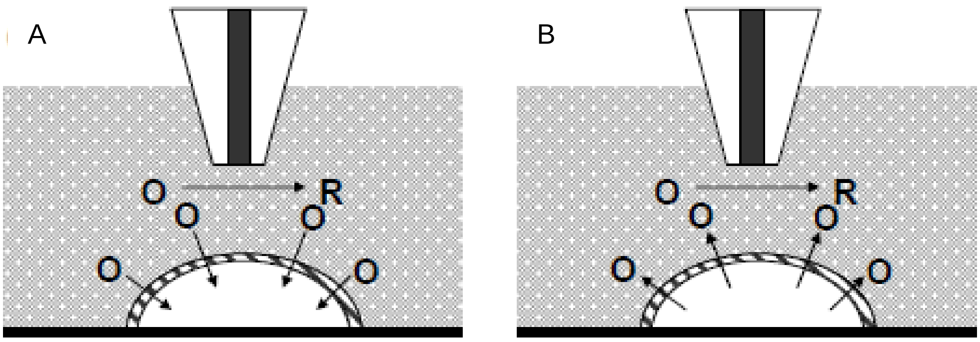

Figure 1.10 A: O_2 consumption by respiration , B: O_2 generation by photosynthesis

⅜№╢ ⁹ ķ ↄ Ķ ≤ ⇔ ≡ ⌐ ╛ ╢ │ ╘≡ ⌂™√ ╘ ─ ╨
≢ №╢ ⁹
╨ ⌐ SECM ╨ ™√⅜╪ ┘ ─ ▬ɷ Ͻ ☺ fi
⅜ ╥╣ ≡ ™╢ [64, 65] ⁹ ⌐ ↕╣ √ ─ ╨ Δ╢ ↓ ≤ ⌐ ╟╡ ⁸ ─ ɷ
○♬ ☼ ⊦ ⌐ Δ╢ ╨ ╢ ↓ ≤ ⅜≢ ⅝╢ ⁹ SECM ⌐ ╟╢ │ ⁸ ─ ◦ ☻
♥ ⊦ ┼ ╙ ↕╣ ≡ ™╢ [66, 67] ⁹ ─ ╨ ⇔ ≡ ה ⌂ ─ ⅜
╥╣ ≡ ⅔╡ ⁸ ⌐ ⅔ ↑ ╢ ─ ⅔ ╟┘ ♩ ─ ┼─ ⅜ ↕╣ ≡ ™╢ ⁹
SECM ╨ ™≡ ⅔ ╟┘ ⅛ ╠ ┼─ ⌐ ™≡ ↕
╣ ≡ ™╢ ⁹ ─ ™ ɷ ♦ ▫ ◄ Ͻ ♃ Ͻ ≢ №╢ fi ♂ fi │ ⁸ ⅔ ╟┘
⅛ ╠ ╨ ⇔ ♪ fi ┼ ≤ ↕╣╢ ↓ ≤ ⅜ ⅛ ∫ √ ⁹Mirkin ╟─ ⁱ Ͻ ķ
│ ⁸ ₁ ─ ─ ╨ ⇔ ⁸ ≤ ─ ⌐ ⇔ ≡ ™╢ [68] ⁹
⌐ TMPD ╨ ⇔ ⁸ ⌐ ↕ ∑ √ ╨ ™≡ TMPD ²⁺ ⌐ ⇔ √ ⁹
≢ ↕╣ √ TMPD ²⁺ │ ╨ ⇔ ⅛ ╠ ╨ ⇔ ≡ TMPD ⁺┼ ≤
↕╣╢ ⁹ ↓ ─ ↕╣ √ TMPD ⁺╨ ≢ Δ╢ ↓ ≤ ⌐ ╟╡ ₁ ─ ─ ╨
⇔ ≡ ™╢ ⁹ │ ⁸ ≤ ⇔ ≡ ⅜ ↄ ⁸ ɷ ♦ ▫ ◄ Ͻ ♃ Ͻ ─
⅜ ™√ ╘ ─ ⅜ ⅝™ ⁹ ╕ √ ⁸ ◦ ⊦ C ─ ─
⅜ ħ ▼ ☿ fi ɷ ♃ Ͻ ⁱ ╨ ɷ ♦ ▫ ◄ Ͻ ♃ Ͻ ⌐ ™≡ ╥╣ ≡ ™╢ [69] ⁹
Schuhmann ╟ │ ⁸ ◦ ▪ ħ ◊ Ͻ ☻ ╨ ⇔ √ ⌂ SECM ╨ ™ ⁸ ─
─ ╨ ∫ ≡ ™╢ [70] ⁹ │ ⅜ ↄ ⁸ ≢ ⅜ ⌐ ↕╣ ≡
⅜ ⅝ ↄ Δ╣ ┌ ⁸ ⌐ ∫ ≡ ─ ⅜ ™ ↕╣╢ ↓ ≤ ⌐ ⌂╢ ⁹
─ ⌐ ≢ ╙ ╨ Δ╢ √ ╘ ⁸ ≢ ─ ⅜ ⌐ ╟╢ ⌐ ═≡ ⅛ ⅝™
⁸ ╨ ™ ⇔ ≡ ⇔ ╕ ™ ⁸ ─ ╨ ⌐ ≢ ⅝ ⌂ ™ ⁹ ≢ ─
╨ ╟Δ │ 3 ≈ ╟╣ ⁸ ŋ ⌐ Δ╢ ╨ ↕ ↄ Δ╢ ⁸ ᵒ ◘▬☼╨ ↕ ↄ
Δ╢ ⁸ ↄ ╨ ↄ Δ╢ ↓ ≤ ≢ №╢ ⁹ Schuhmann ╟ │ ⁸ ╨ ⁱ ☻ ⌐ ⇔ ≡
⌐ ⇔ √ ⁹

1.3.3

≢ ─ ⅜ ⌐ ™ ⁸ ≤ ⌂╡ ─ ⌐ ╟╢
⅜ ╨ ╘╢ ⁹ ™ ╢ ┌ ⁸ ⅜ ⁸ ≢ ─ ⌂ ⌐
ↄ Δ╢ ↓ ≤ ╨ ⇔ ≡ ⅔╡ ⁸ SECM ╨ ™╢ ↓ ≤ ≢ ⁸ ⌂ ╨ Δ╢ ↓ ≤ ⅜≢ ⅝
╢ ⁹ ≢ │ ⌐ ╥╢ ╨ ⌐ ⌐ ═╢ ⁹

ɷ ♦ ▫ ◄ Ͻ ♃ Ͻ — ي

⅜ SECM ╩ ™≡ ↕ ╣ ≡ ™╢ [71] ⁹ Ru(NH $_3$)$_6^{3+}$ ≤ ђ ▼ ☿ fi ○ ⁱ Ẅ fi ╩ ╗ ╩ ™≡ ⁸ ⁄ ╢ · ╡ — ≤ — ╩ ℮ ≤ ⁸ ڊ ⌐ ⅜ ≠ Ͻ ⌐ ≈ ╣ ≡ Ru(NH $_3$)$_6^{3+}$ — ⅜ Δ ╢ Figure 1. 12 ⁹ Ru(NH $_3$)$_6^{3+}$ │ ⅜ ҩ Ͻ ⁸ ڊ ⌐ ╠ ╡ ⅜ ↓ ╢ √ ╘ ⁸ Ⱡ ● ♥ ▫ Ḳ ђ ▫ Ͻ ♪ Ᵽ ℮ ⅜ ╢ ╢ ⁹ پ ђ ▼ ☿ fi ○ ⁱ Ẅ fi — │ ⁸ pH ⅜ ™ ≤ ⅝ │ — ⅜ ╖ ╠ ╣ ╢ ⅜ ⁸ pH ╩ ҩ Ͻ Δ ╢ ≤ ╩ ⌐ ≠ ↑ ≡ ╙ ╒ ≤ ╪ ≥ ⇔ ⌂ Ͻ ⌂ ╢ ⁹ ↓ ╣ │ ⁸ pH ⌐ ╠ ʃ ≡ ђ ▼ ☿ fi ○ ⁱ Ẅ fi — ⅜ Δ ╢ ↓ ≤ ╩ Δ ╢ ⁹ ╕ √ — ķ ♩ ķ ! ☻ ♩ ╩ ™≡ ⁸ ₁ ⌂ — ɷ ♦ ▫ ◄ Ͻ ♃ Ͻ — ی ╙ ↕ ╣ ≡ ™╢ [72] ⁹ ↕ ╠ ⌐ Amemiya ╠ │ ⁸ ○ ◄ ⁱ — ⌐ ⅔ ↑ ╢ ╩ ⁸ SECM ⌐ ╠ ╡ ≤ ◦ ⊦ ⁼ Ͻ ◦ ꜛ fi ╩ ™╢ ↓ ≤ ≢ ⇔ ⁸ ⌐ ⅔ ↑ ╢ ɷ ○ ♬ ☼ ⊦ — ⌐ ⇔ ≡ ™╢ [73, 74] ⁹

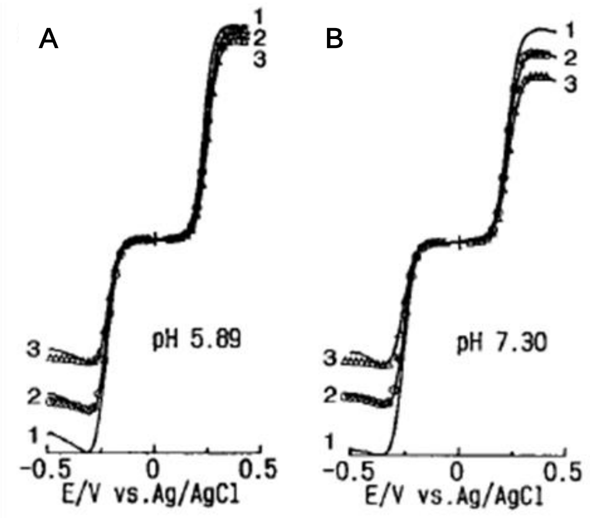

Figure 1.11 Relationship between CV s and membrane -electrode distance s. [71]

1: bulk, 2: 51 ᵞm, 3: 27 ᵞm, A: pH 5.89, B: pH 7.30

1.3.4 غ

SECM │ ⁸ ╩ ™≡ غ — ⌐ ↕ ╣ ≡ ™╢ [9, 22, 75-78] ⁹ ○ Ͻ Ẅ fi ђ □▬ Ᵽ Ͻ — ╩ ™√ ⁸ پ ⅛ ╠ — غ — ⁸ ⅜ ╦ ╣ ≡ ™╢ ⁹ ◄ ◖ ♁ ◘▬ ♩ Ͻ ◦ ☻ ⌐ ╠ ╡ ₁ — ⅛ ╠ ↕ ╣ ╢ ♪ Ͻ Ʀ ⊦ fi ⁸ ▪ ♪ ♬ ⁱ fi ⅔ ╠ ┘ ⱡ ⁱ ▪ ♪ ♬ ⁱ fi — ○ ♥ ◖ Ͻ ⁱ ▪ ⊦ fi — غ │ ⁸ ⌐ ≢ №╡ ⁸ ⌐ ↕ ╣ √ ╩ ™≡ ▪ fi Ꭰ ɷ ♩ ⁱ Ͻ ⅔ ╠ ┘ ◘▬ ◒ ⁱ ℮ ◒ Ẅ ⁱ ♃ fi ɷ ♩ ⁱ Ͻ FSCV ⌐ ╠ ╡ ⁱ ▪ ⁱ ♃▬ ⊦ ≢ ≢ ⅝ ╢ [9, 22] ⁹ ⅛ ╠ ↕ ╣ ╢ غ — │ ╘ ≡ ⌂ Ͻ ⁸ ⌐ ↕ ╣ ╢ ≤ ⁸ №╢ ™ │ ┘ ⌐ ╡ ╕ ╣ ╢ ⁹ ↓ — ⌐ │ ≢ ™ ╩ Δ ╢ ⅜ ≢ №╢ ⁹ ▪ fi Ꭰ ɷ ♩ ⁱ Ͻ │ ⊦ ⁱ ⁼ ʐ ⁱ — ™ ⅜№╢ ⅜ ⁸ ↕ ╣ ≡ ™╢ — ⌐ ⅜№╢ ⁹ پ ⁸ FSCV │ ↕ ╣ √ — ⅜ ∞ ⅜ ⁸ ⌐ ⅔ ™≡ ▪ fi Ꭰ ɷ ♩ ⁱ Ͻ ⌐ ╢ ⁹ ≢ │ ⁸ پ ≠ Ͻ ♪ SECM ╩ ⇔ ≡ ╩ — ⌐ ⇔ ╩ ⇔ ≡ ™╢ [9, 22, 76, 77] ⁹ ⇔ ⅛ ⇔ ⁸ ⌐ ⅜ ⌂ غ — ⌐ │ ⅜№╡ ⁸ ◓ ⁱ ♃ ⊦ fi ┛ ▪ ☿ ♅ ⁱ ◖ ⁱ fi — ⌐ │ Ꝟ▬ ◒ ╩ ™╢ ⅜№╢ ⁹

1.3.5

№╢ ™ │ ─ ⌐ ٿ∆ ╢ ─ ڿ≢ ⁸ ⌐ ⅜ ⌂
⌐ │ ╡ ⅜№╢ ⁹ ⁄ ↓≢ ─▬ ꙍ ↄ ☺ fi ◓ ⌐ │ ⁸ ڿ ⌐ ö ↕ ∑ √ ꙍ ♦ ▫ ◄ ↄ ♃ ↄ ╨
™╢ ⁹ ╙ ⇔ ↄ │ ⁸ ╛ ◦ ▪ ḩ ◊ ↄ ☻ ⌂ ≥ ─ ٿ─ ḩ ▫ ↄ ♪ Ᵽ ℮ ◒ ◦ ◓ ♫ ⁱ ╨ ™ √
⌐ ╟╡ ⅜ ≤ ⌂ ╢ ⁹

↕ پ ≠ ↄ ♪ ⌐ ╟╢ ≢ │ ⁸ Ł ● ♥ ▫ Ķ ḩ ▫ ↄ ♪ Ᵽ ℮ ◒ ╨ ⇔ ≡ ╤╢
╢ ⁹ ─ ๔ ™ ꙍ ♦ ▫ ◄ ↄ ♃ ↄ ╨ ™╢ ≤ ⁸ ─ ↕ ⌐ ⇔ ≡ Ł ● ♥ ▫ Ķ ḩ ▫ ↄ ♪
Ᵽ ℮ ◒ ⌐ ╟╡ ⅜ ∆ ╢ √ ╘ ▬ ꙍ ↄ ☺ fi ◓ ≢ №╢ ⁹ ꙍ ♦ ▫ ◄ ↄ ♃ ↄ
≢ №╢ $Ru(NH_3)_6^{3+}$ ╨ ™ ≡ PC12 ─ ▬ ꙍ ↄ ☺ fi ◓ ⅜ ╤╢ √ [78] ⁹

پ ≠ ↄ ♪ ⌐ ╟╢ ╙ ╤╢ ≡ ™╢ ⁹ Wipf ╠ │ ⁸ ╨ ḩ ▫ ↄ ♪ Ᵽ ℮ ◒ ◦ ◓
♫ ⁱ ≤ ⇔ ≡ ⇔ ≡ ╨ پ ⌐ ≈ SECM ⅔ ╟ ┘ ⁸▬ fi Ḩ ↄ ҍ fi ☻ ╨ ḩ ▫ ↄ ♪ Ᵽ ℮ ◒ ◦ ◓ ♫ ⁱ
≤ ⇔ ≡ ⇔ ≡ ╨ پ ⌐ ≈ SECM ⌐ ╟╡ ⁸ PC12 ─ ▬ ꙍ ↄ ☺ fi ◓ ╨ ⇔ ≡
™╢ [9] ⁹ Takahashi ╠ │ ⁸ ◦ ▪ ḩ ◊ ↄ ☻ ╛ ▬ ○ fi ╨ ⇔ √ ḩ ▫ ↄ ♪ Ᵽ ℮ ◒ ⌐ ╟╢
پ ≠ ↄ ♪ ╨ ™ ≡ ⁸ PC12 ─▬ ꙍ ↄ ☺ fi ◓ ╨ ∫ ≡ ™╢ [21, 33] ⁹ ╕ √ Takahashi ╠ │ ⁸ Ł
● ♥ ▫ Ķ ḩ ▫ ↄ ♪ Ᵽ ℮ ◒ ╨ ⇔ √ ≤ ─ ╨ ⁸ ╨ ☻ ▬ ℮
♅ fi ◓ ⇔ ₁ ⌐ ∆ ╢ ↓ ≤≢ ≤ ⇔ √ [77] ⁹

1.3.6 ⁄ ─ ٿ ▬ ꙍ ↄ ☺ fi ◓ ⁸ ڂ ⁸ ⁸ Ɽ ♃ ↄ ♬ fi ◓

⁄ ─ ٿ ⌐ ╙ ⁸ ₁ ⌂ ⅜ ↕ ╢ ≡ ™╢ ⁹ ╨ ∆ ꙍ ♦ ▫ ◄ ↄ ♃ ↄ ─ ڂ [87, 88]
╛ ⁸ ROS ≤ RNS ─ پ ˘ ẓ ⁱ ≢ ─ [89-91] ⅜ ↕ ╢
≡ ™╢ ⁹ ╛ ى ⌂ ≥ ⁸ ₁ ⌂ ≢ ─ ─ ⌐ ╙ ↕ ╢ ≡ ™╢ [92-101] ⁹
پ ╨ ∆ ╢ ─ ⌂ ╨ ⌐ ≢ №╡ ⁸ ╕ √ ḩ ▫ ℮ ◒ ─ ⅛
╟ ─ ╨ ⇔ ⁸ ◘ Ķ ⊦ ◒ fi ○ ↄ ҍ ↄ ≢ ▬ ꙍ ↄ ☺ fi ◓ ∆ ╢ ↓ ≤ ⅜≢ ⅝╢ ⁹ ╨
™ ≡ ⌐ ╨ ↕ ∑ ╢ ↓ ≤ ⌐ ╟╡ ⁸ ٿ ⌐ ⌂ Ɽ ♃ ↄ fi ╨ ﻷ ∆ ╢ ↓ ≤ ╙ ≢ №
╢ ⁹ ₁ ⌂ ٿ┼ ─ ⁸ ⁸ ⁸ ⁸Ɽ ꜝ ☺ ► ⊦ [102-104, 111, 114-116] ⁸ [110, 115, 121, 122] ⁸
ى [49, 52, 123] ⁸ ʬ ⁱ ʬ ↄ [46, 106-108] ⁸ [109] ⌂ ≥ ─ Ɽ ♃ ↄ ♬ fi ◓ ⅜
↕ ╢ ≡ ™╢ ⁹ ה ה ⌂ ≥ ─ ◄ ℮ ♅ fi ◓ [103-105] ╛ ⁸ ى ─ ◄ ℮ ♅ fi ◓ [117-119] ⁸
─ ≤ ╨ ⇔ √ Ɽ ♃ ↄ ♬ fi ◓ ╙ ╤╢ ≡ ™╢ [116, 118, 120] ⁹ ⌐ ⅔ ↑ ╢
ķ ☿ ☻ ─ ⌐ ╙ SECM │ ≢ №╡ ⁸ ⇔ √ ─ [124] ⁸ ▬ ○ fi
─ [120, 125~130] ⁸ [113, 131-139] ⌂ ≥ ─ ⁸ ─ ⌐ ╤╢ ⌐ ↕
╢ ≡ ™╢ ⁹ ─(100) ┼─ Ag ▬ ○ fi ─ ⌂ ≥ ⁸ ٹ ⌂ ─ ⌐ ╙ ↕ ╢ ≡ ™╢
[140] ⁹

1.4 ─

≢ ═ √ ╟ e ⌐ 8 ⌐ ⌐ ╤ √ ╢ ⌐ SECM ⅜
™ ╠ ╣ ≡ ⅝ √ 9 ⌐ ى ⌐ ⅔ ™ ≡ │ 8 ٿ─ ⌐ │ ⌂ ™ پ ╒ ʑ ¡ ה ɑ ה
ה ⌐ ⌂ SECM ─ ⌐ ╟ ╡ 8 ى ╨ ◘ Ķ ⊦ ☁ fi ○ ɔ ♭ ɔ ⅛ ≈ ¡ ▪ ¡
♃ ▬ ⊦ ≢ ≢ ⅝ ╢ √ ╘ 8 ┼ ─ ⌐ Δ ╢ ⌂ ⅜ ≢ ⅝ ╢ 9
≢ │ 8 SECM ⌐ ╟ ╡ ⅔ ╟ ┘ ♃ fi Ʀ ☁ ─ پ ╒ ʑ ¡ ה ⌂ ╨
⇔ √ 9

3 ي ─ ϖ ♦ ▫ ◄ ɔ ♃ ɔ ╨ ™ √ پ ≠ ♬ ♃ ¡ fi ☁
─ ځ ─ ≤ ╡ ╢ ╨ 8 پ ╒ ʑ ¡ ה ה
⌐ Δ ╢ ↓ ≤ ╨ ≤ ⇔ √ 9 ╨ Δ ϖ ♦ ▫ ◄ ɔ ♃ ɔ ≤ 8 ╨ √ ⌂ ™ ϖ ♦
▫ ◄ ɔ ♃ ɔ ╨ ⇔ 8 ╨ ⇔ √ 9 ╠ ╣ √ ⅛ ╠ 8 ≢ ─ ځ ⌐ ╟ ʃ
≡ ┼ ≤ ↕ ╣ ╢ ϖ ♦ ▫ ◄ ɔ ♃ ɔ ╨ ⌐ ⇔ √ 9 ╕ √ ϖ ♦ ▫ ◄ ɔ ♃ ɔ ⌐ ╟ ╢
┼ ─ ╨ ⇔ 8 ۺ ╨ Δ ╢ ↓ ≤ ≢ ╨ ≤ ⇔ √ 9

4 SECM ⌐ ╟ ╢ پ ES ─
ES ─ │ ╛ ⅜ ≢ № ╡ 8 ╙ ╨ ⌂ ɑ
⌂ ⅜ ╘ ╠ ╣ ≡ ™ ╢ 9 ES ─ ɑ ⌂ ╨ ≤ ⇔ 8 ES ≢
⇔ ≤ ≤ ╙ ⌐ ⅜ Δ ╢ ─ ╨ SECM ⌐ ╟ ╡ پ ╒ ʑ ¡ ≢ ⇔ √ 9

5 SECM ⌐ ╟ ╢ پ ╒ ʑ ¡ ♃ fi Ʀ ☁
SECM ⌐ ╟ ╢ ─ ♃ fi Ʀ ☁ ─ ─ ╨ ≤ ⇔ √ 9 ─ ≤ ─
™ٿ ╒ ☿ ķ ♃ ɔ (EGFR) ╨ Δ ╢ ↓ ≤ ≢ 8 ╨ ʃ √ 9 ─ ى
─ ╛ 8 EGFR ─ ™ ╨ ⅛ ╠ ⇔ √ 9

6 ♃ fi Ʀ ☁ ─ ¡ ▪ ¡ ♃ ▬ ⊦ ≠ ♬ ♃ ¡ fi ☁
♃ fi Ʀ ☁ ─ پ ╒ ʑ ¡ ה ה ╨ ≤ ⇔ 8 5 ≢ ⇔
√ ⌐ ╟ ╡ EGFR ─ ╨ ʃ √ 9

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2

2.1 │ ∂ ╘ ⌐

≢ │ ⁸ ≢ ⇔ √ ─ ﻟٔ ⁸ ⁸ ≤ ⁄ ─ ﻟٔ
⌂ ≥ ⁸ ≡ ─ ≢ ⇔ √ ﻟٔ ≈ ™ ≡ ═ ╢ ⁹ ≢ ─ ╖ e │ ∆ ╢ ⁹

2.2 ─ ﻟٔ

ﭗ ﺋ ⌐ ⁸ ≢ ⇔ √ ∜ ▬ ◒ ≤ ─ ﻟٔ ⌐ ≈ ™ ≡ ═ ╢ ⁹

2.2.1 ∜ ▬ ◒ ─ ﻟٔ

≢ │ ⁸ 20 ㎜ ─ ♦ ▫ ☻ ◒ Pt ∜ ▬ ◒ RG = 2 ╩ ﻟٔ ⇔ ⁸ ⌐ ™ √ ⁹
╕ √ 6 ≢ │ ◌ ͻ W fi ђ □ ▬ Ᵽ ͻ ⌐ ╟ ╢ ﻟٔ ⇔ √ ⁹ ﭗ ﺋ ⌐ Pt ♦ ▫ ☻ ◒ ∜ ▬ ◒ ─ ﻟٔ
╩ ∆ Figure 2.1 ⁹

ŋ 1.65 mm 1.1 mm ─ ᶏ ● ! ☻ PG10165-4 World Precision Instrument
─ ╩ ⁸ ◖ Ḩ ! ¡ ͻ ķ ! ͻ PE-21 Narishige ≢ ∆ ╢ ⁹
º Pt 20 ㎜ ﭗ ╩ ↕ 10 mm ⌐ ╡ ⇔ ⁸ ↕ 10 cm ─ Cu ¡ ͻ
♪ ⌐ ☻ W ᴘ ♩ ME-35A Miyachi ≢ ∆ ╢ ⁹
ͻ ● ! ☻ ◖ Ḩ ! ¡ ͻ ⌐ Pt ╩ ͻ ⇔ ⁸ ◖ Ḩ ! ¡ ͻ ⌐ Pt ⅜ ⇔ ≡ ™
╢ ↓ ≤ ╩ № ╢ ™ │ ⌐ ╟ ╡ ∆ ╢ ⁹
ᵔ ◖ Ḩ ! ¡ ͻ ─ ● ! ☻ ⌐ ¡ ͻ ♪ ╩ ⱻ ͻ Ķ ⌐ ╟ ╡ ∆ ╢ ⁹
ᵕ ∜ ▬ ◒ ђ ◊ ͻ ☺ MF-77 Narishige ╩ ™ ≡ ⁸ ◖ Ḩ ! ¡ ͻ ╩ ─
ﭗ ⌐ ⇔ ⁸ Pt ≤ ◖ Ḩ ! ¡ ͻ ─ ─ ╩ e

p ◖ˈ Ң ! ¡ ɔ ╩ ♭ ▬ˇ ⸗ fi ♪ ◒ ! ▬ fi ♭ ɔ EG-6 number 5000 Narishige ≢ ╡ ⁸Pt ╩ ↕ ∑ ╢ ⁹ ● ! ☻ — ╡ ○ ☻ ⅜ ⌐ ⅈ ⇔ ≡ ™ ╢ │ 0.01 M — NaOH ڿ ⌐ ╝ ʃ ɔ ╡ ⇔ ⁸ ● ! ☻ ╡ ○ ☻ ╩ ⅛ ∆ ⁹ ⁄ — ڿ ≢ ⇔ ṧ ↕ ∑ ╢ ⁹

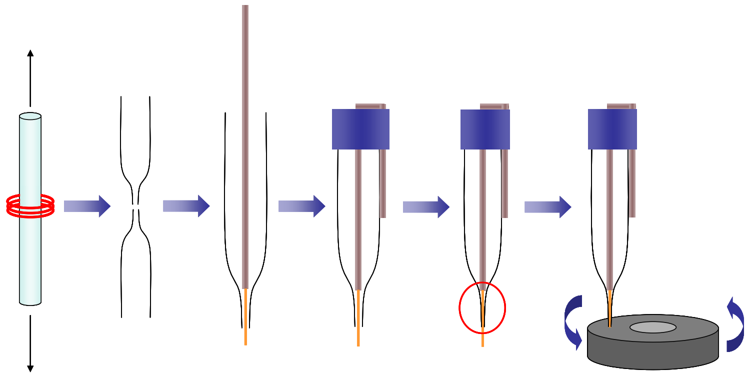

Figure 2.1 Fabrication procedure of Pt disk -type microelectrode.

≢ │ ⁸˥ ♪ ᴘ ◒ ☻ — ⅜ ɔ ⁸ ⌐ ⌂ ₒ ╩ ⇔ √ ⁸˥ ♪ ᴘ ◒ ☻ — ⌐ ╟ ╢ ⅜ ≤ ⌂ ╢ ⁹ ↓ — — j_{bulk} │ ڂ ڗ — ≢ ∆ ↓ ≤ ⅜≢ ⅝ ╢ ⁹

ῼ ﭾ τὲ ῎ΟΌ ő ῶ 1

↓ ↓ ≢ j_{bulk} │ [A] ⁸ n │ ⁸ F │ ḩ □ ! ♦ ɔ [C/mol] ⁸ D │˥ ♪ ᴘ ◒ ☻ — [cm²/sec] ⁸ C │˥ ♪ ᴘ ◒ ☻ — [mol/cm³] ⁸ a │ [cm] ≢ № ╢ ⁹ ﻼ ⇔ √ — │ $FcCH_2OH$ ╛ $K_4Fe(CN)_6$ ڿ ≢ ʃ √ ⁹ ⁄ — — ╩ Figure 2.2 ⌐ ∆ ⁹ │ ∜ ▬ ◒ — ◦ ◓ ⸗ ▬ ♪ ≤ ⌂ ʃ √ ⁹ ≢ ﻼ ⇔ √ Pt │ RG ⅜⅔⅔ ╟ ⁄ 2 ≢ № ʃ √ ⁹ ⁄ ↓ ≢ ٽ — ╩ a 4.43 e ≤ ⇔ ⁸ ↕ ╡ √ ╩ ڂ ⇔ ╩ ⇔ √ ≤ ↓ ╤ ⁸ │ 10 ㎜ ≤ ≢ ⅝ √ ⁹ ⌂ ⅔ ⁸ $Fe(CN)_6^{4-}$ — │ 6.5 × 10^{-6} [cm² s⁻¹] ≤ ⇔ √ ⁹

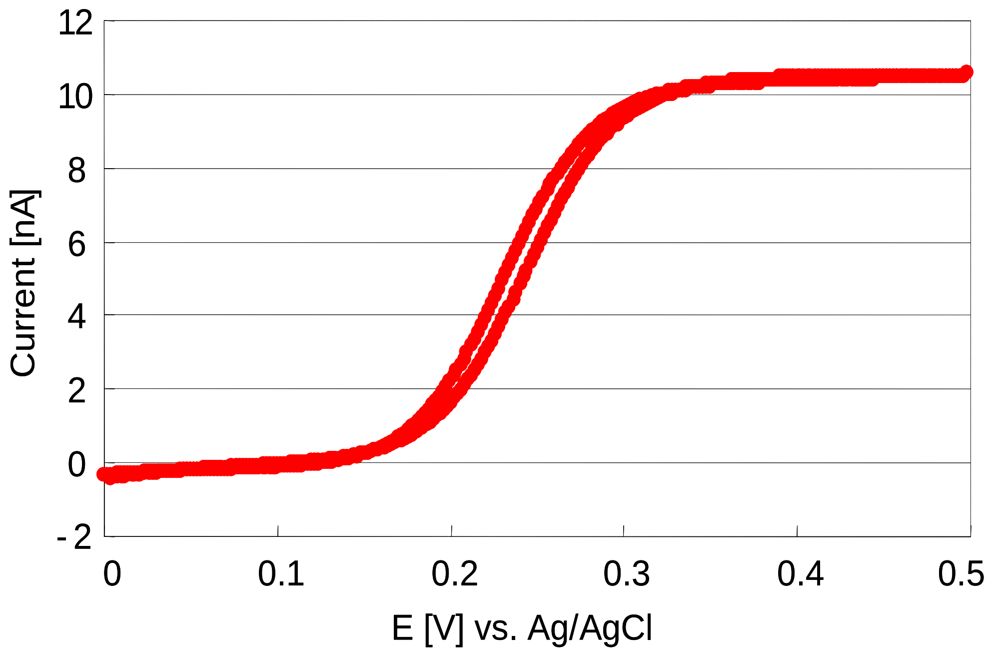

Figure 2.2 Cyclic voltammograms by a fabricated Pt disk -type microelectrode (radius = 10 µm). Measurement solution contains 4 mM $Fe(CN)_6^{4-}$. The scan rate was 20 mV vs. Ag/AgCl.

2.2.2 ─ ڵ

≢ │ ⁸ Ag/AgCl ╩ ڵ ⇔ ⌐ ™ √ ⁹ ϫ ; ⌐ ڵ ╩ ∆ ⁹

ŋ 300 mm ─ Ag ╩ ↕ 30 mm ⁸ 300 mm ─ Pt ╩ ↕ 10 mm ╡ ⇔ √ ⁹

o 3 mm ⁸ 2 mm ─ ● ! ☻ ╩ ↕ 10 cm ⌐ ⇔ ⁸ ╡ ⇔ √ Pt ╩ ⇔ √ ≢ Ᵽ ɔ ♫ ɔ ⌐ ╟ ╡ ⇔ √ ⁹

ɔ ⇔ √ ● ! ☻ ╩ KCl ≢ √ ⇔ ⁸ ● ! ☻ ⌐ KCl ⁸ AgCl ╩ ⌐ ╣ ⌐ ↕ ∑ √ ⁹

ᵔ ╡ ⇔ √ Ag ─ ╩ 0.1 M HCl ╙ ⇔ ɔ │ KCl ϫ ⌐ ╣ ⁸ ▪ ǂ ɔ ♪ ↕ ∑ ≡ AgCl ╩ ↕ ∑ √ Ag/AgCl ⁹

ᴗ Ag/AgCl ─ AgCl ╩ ● ! ☻ ⌐ ╣ ⁸ ╣ ⌂ ™ ╟ e ⌐ Ʀ ! ɧ ▫ i ⊦ ≢ ⇔ √ ⁹

p ڵ ⇔ √ Ag/AgCl │ ⁸ ╩ KCl ⌐ ⇔ √ ≢ ⇔ √ ⁹

2.3 SECM ─

≢ │ ⁸ Figure 2.3 ⌐ ∆ ╟ e ⌂ SECM ◦ ☻ ♥ ꜔ ╩ ™ ≡ ╩ ∫ √ ⁹ │ 2 ≢ ™ ⁸ ﻷ WE ⌐ │ ─ ≢ ﻷ ⇔ √ Pt ∜ ▬ ▾ ﹏ ╩ ⁸ RE ⌐ │ ─ ≢ ﻷ ⇔ √ Ag/AgCl ╩ ™ √ ⁹ ─ │ ⁸ ╕ ∏ PC ⅛ ╟ DA W ↄ ♪ ╩ ⇔ ⁸ ﻷ ≢ № ╢ ∜ ▬ ▾ ﹏ ≤ ⌐ ╩ ∆ ╢ ⁹ ∜ ▬ ▾ ﹏ ⅛ ╟ ⇔ √ │ Current amplifier ⁸ 427 current amplifier ⁸ KEITHLEY ⌐ ╟ ╡ ⇔ ⁸ AD W ↄ ♪ ╩ ⇔ ≡ PC ⌐ ⇔ √ ⁹ ꞵ ▬ ☼ ─ √ ╘ ⁸ SECM ◦ ☻ ♥ ꜔ │ ђ □ ! ♦ ↄ ◦ ↄ ☺ Faraday cage ⁸ HOKUTO DENKO ⌐ ⇔ √ ⁹ xyz ☻ ♥ ↄ ☺ ─ │ ☻ ♥ ↄ ☺ ◖ fi ♩ ﹏ ↄ ! ↄ ≢ ∫ √ ⁹ ﺵ ─ │ ⁸ ⌐ ≡ ∆ ╢ ⁹

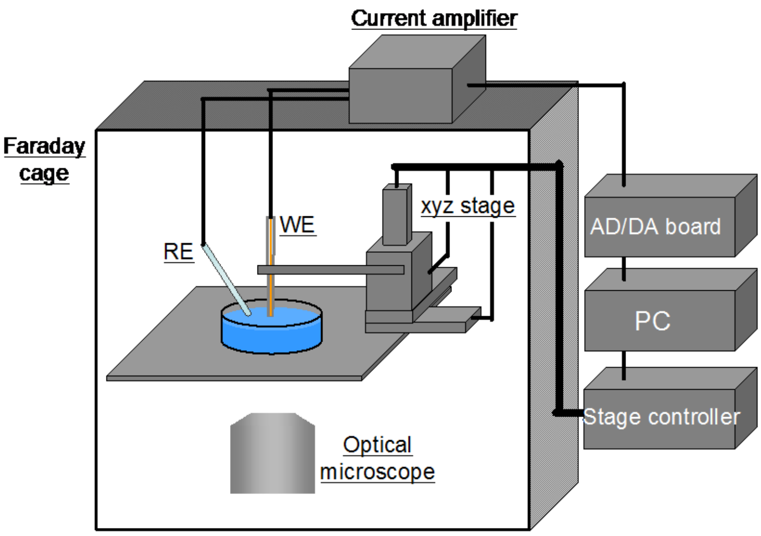

Figure 2.3 Schematic diagram of SECM system

2.4 ﻷ

ﻷ ⌐ │ ﺵ ⅜ ﭦ ⌂ √ ╘ ⁸ ∆ ╢ │ ∆ ═ ≡ ⇔ ⁸ ─ ﻷ │ ▾ ⁱ ↄ fi ʐ fi ╫ MCV-B131F ⁸ ﭖ ≢ ﻷ ╩ ∫ √ ⁹ ﺨ ﭲ ⌐ ⁸ ⇔ √ ≤ ⁄ ─ ﻷ ─ ╩ ∆ ⁹

2.4.1 ⇔ √

≢ │ ⁸ 4 — ╨ ⇔ √ ⁹

ŋ HeLa ∡ ♩ ⁸ ⅈ ☿ fi ♃ ɔ

º ∜ ▶ ☻ ES : Strain 129/ SVEV ⁸DS Pharma Biomedical Co. , Ltd ⅛ ╠

ɔ A431 ∡ ♩ ت ⁸ ⅈ ☿ fi ♃ ɔ

ᵔ MCF-7 ∡ ♩ ھ ⁸ ⅈ ☿ fi ♃ ɔ

2.4.2 ה

ŋ RPMI-1640 Gibco

500 mL — RPMI-1640 ⌐ 50 mL — ⇔ √ FBS ⁸ Gibco ⅔ ╠ ┘ 5 mL — Ɑ ♬ ◦ ⁱ fi / ☻ ♩ ‾ ķ ♩ ∜ ▬ ◦ fi 5000 U mL^{-1} ⁸5000 ㎍ mL^{-1} ╨ ⅎ ≡ ⇔ √ ⁹ │ ⁸ ╨ 37°C ≢ ⁸56°C ⁸30 min ⇔ ≡ ∫ √ ⁹ ⇔ √ RPMI-1640 │ ⌐ ≡ 4°C ≢ ⇔ √ ⁹

º 0.25 ♩ ⁱ ķ ◦ fi-EDTA Gibco

◒ ⁱ ɔ fi ʐ fi ♅ ≢ ⁸ ▪ fi ķ ⁱ ♅ ɔ Ķ ⌐ 1.0 mL ∏ ≈ ↑ ⌐ ⇔ ⁸ ≢ ⇔ √

ɔ PBS -

⁸ ⇔ √ ⌐ 8.00 g L^{-1} NaCl ⁸0.20 g L^{-1} KCl ⁸1.54 g L^{-1} $NaHPO_4$ה$12H_2O$ ⁸0.02 g L^{-1} KH_2PO_4 ╨ ⇔ ⁸ ○ ɔ ♩ ◒ ‾ ɔ Ķ KT-2346 ⁸ ▪ fi ķ ⌐ ≡ 121°C ⁸ 20 min ⇔ √ ⁹ ⌂ ⅔ - ≤ │ Ca^{2+} ⁸ Mg^{2+}⅜ ╕ ╣ ⌂ ™ ↓ ≤ ╨ ∆ ╢ ⁹

ᵔ ╖ — ķ ! ☻ ♅ ♇ ◒ Ḩ Ɑ ♇ ♩ 5 ⁸ 10 ⁸ 50 mL ⁸ Falcon

ᵕ ╖ — ķ ! ☻ ♅ ♇ ◒ ḩ ! ☻ ◖ 25 ⁸ 250 mL ⁸ Falcon

ᵖ ╖ — ķ ! ☻ ♅ ♇ ◒ ◦ ɔ ‾ 3.0 mL ⁸ Falcon

ᵗ ◖ ♬ ◌ ⁱ ♅ ɔ Ķ 15 ⁸ 50 mL ⁸ IWAKI

ᵘ C-Chip DHC-B02 ⁸ Digital Bio

— ◌ ▶ fi ♩ ⌐ ⇔ √ ⁹

ᵓ 70% ◀ ♅ ⁱ ▪ ⁱ ◖ ɔ ⁱ

⅝ ╨ ™ ≡ ◒ ⁱ ɔ fi ʐ fi ♅ ⌐ ╣ ╢ ⌐ ⅝ ≈ ↑ ⇔ √ ⁹

ɯ 70% ◀ ♅ ⁱ ▪ ⁱ ◖ ɔ ⁱ ≢ ╠ ∑ √ ◌ ♇ ♩

◒ ⁱ ɔ fi ʐ fi ♅ — ⁸ ⌐ ™ √ ⁹

ᵛ TE300 ⁸ Nikon

ᴥ

● ! ☻ ∤ ͻ ◌ ͻ ⌂ ≥ ╨ ⁸ ڔ ⌐ ≡ 85°C ≠ ڔ ↕ ∑ √

2.4.3 ─

ƞ ! ☻ ◖ ╨ ™ ⁸ 37°C ⁸ 5% CO_2 ─▬ fi ◖ ̂ ʐ ͻ ♃ APM-30D ⁸ ▪ ☻ ♥ ℮ ◒ ≢ ╨ ʃ √ ⁹ HeLa ⁸ A431 ⁸ MCF-7 │ ⁸ RPMI-1640 ڿ ≢ ⇔ √ ⁹ ES ⌐ │ ⁸ ♀ ! ♅ fi ◖ ͻ ♩ ⇔ √ ⅜ ≢ №╡ ⁸ ڂ ڗ ⌐ ڵ ╨ ═╢ ⁹ ♀ ! ♅ fi ◖ ͻ ♩ │ ⌐ ⅔ ↑ ╢ ─ ≤ ⌂ ╢ ⁹

ŋ Utlrapure water with 0.1 % Gelatin (Millipore, Cat. ES-006-B)╨ 25 cm^2 ƞ ! ☻ ◖ ⌐ 2 mL ╢ ⁸ ی ⌐ ⌂ ∂ ╕ ∑ ≡ ◒ ⁱ ͻ fi ʐ fi ♅ ≢ 4 ⇔ √ ⁹

º ♀ ! ♅ fi ╨ ≡ ⁸ 2 mL ─ PBS(-)╨ ╢ ≡ ⇔ ⁸ PBS ╨ ≡ ≡ ↕ ╠ ⌐ ◒ ⁱ ͻ fi ʐ fi ♅ ≢ ڔ ↕ ∑ √ ⁹

ͻ ڔ ⇔ √ ♀ ! ♅ fi ◖ ͻ ♩ ƞ ! ☻ ◖ │ ⁸ ♅ ℮ ◒ ʛ ─ ⌐ ڔ ƞ ! ☻ ◖ ≤ ⌐ ╢ ⁸ 4°C ≢ ⇔ √ ⁹

ES │ 1000 U mL^{-1} of mouse leukemia inhibitory factor (mLIF) ≤ 1 mM ȁmercaptoethanol ╨ ╖ Stem Medium DS Pharma Biomedical. Co., Ltd. , Tokyo, Japan ڿ ≢ ⇔ √ ⁹ ┼ ≤ │ ⁸ 1 µM ATRA ╨ ╖ ⁸ mLIF ╨ ╕ ⌂ ™ ڿ ≢ ES ╨ ∆ ╢ ↓ ≤ ≢ ʃ √ [1, 2] ⁹

2.4.4 ─ ڂ

HeLa ⁸ A431 ⁸ MCF-7 │ ڂ ڗ ─ ≢ ڂ ╨ ʃ √ ⁹ ڂ │ ⅜ ƞ ! ☻ ◖ ─ 80% ≤ ⌂ ʃ √ ≤ ↓ ╤ ≢ ʃ √ ⁹

ŋ ▬ fi ◖ ̂ ʐ ͻ ♃ ͻ ⅛ ╠ ƞ ! ☻ ◖ ╨ ╡ ⇔ ⁸ ◒ ⁱ ͻ fi ʐ fi ♅ ⌐ ∆ ╕ √ ⁸ ⇔ ™ ≤ ♩ ⁱ ƙ ◦ fi ╨ № ╠ ⅛ ∂ ╘ 37ϴ ⌐ ╘ ≡ ⅔ ͻ ⁹

º ƞ ! ☻ ◖ ─ ╨ ╡ ⅝ ⁸ PBS - ≢ ╨ 2 ∆ ╢ ⁹

ͻ ƞ ! ☻ ◖ ⌐ 0.25%♩ ⁱ ƙ ◦ fi -EDTA ╨ 1 mL ڗ ⇔ ⁸ 37°C ⁸ 5%CO_2 ≢ ▬ fi ◖ ̂ ʐ ͻ ♩ ⇔ ⁸ ≢ ─ ╨ ∆ ╢ ⁹

ᵔ ƞ ! ☻ ◖ ⌐ ⇔ ™ ╨ ⅎ ╢ ↓ ≤ ≢ ♩ ⁱ ƙ ◦ fi ─ ڵ ╨ ↕ ∑ ╢ ⁹

ᵕ ƞ ! ☻ ◖ ─ ╨ Ƕ ɑ ℮ ♥ ▫ fi ◓ ⇔ ⁸ ╨ ↕ ∑ ╢ ⁹

ᵖ ╨ ◖ ♬ ◌ ⁱ ♅ ̂ ͻ Ƙ ⌐ ⇔ ⁸ CT15D ⁸ ڵ ╨ ™ ≡ 1500

rpm ⁸ 5 min ≢ ╨ Δ ╢ ⁹

t ◖♬ ○ ¡ ♅ ̶ כ Ķ ─ ٿ ╨ ╡ ⅝ ⁸ ⇔ ™ ╨ ⅎ ≡ Ḩ Ɑ ℮ ♥ ▫ fi ◓ ⇔ ⁸ ╨ ╟ ɔ ↕ ∑ ╢ ⁹

u ⌐ ∂ ≡ C-Chip ╨ ™ ≡ ╨ Δ ╢ ⁹

ב ⇔ ™ ḩ ! ☻ ◖ ⌐ ⇔ ™ ╨ ╣ ⁸ ↕ ╠ ⌐ ╨ ڗ Δ ╢ ⁹

ɯ ḩ ! ☻ ◖ ╨ ▬ fi ◖ ̶ ʐ כ ♃ כ ⌐ ╣ ⁸ 37°C ⁸ 5%CO_2 ≢ Δ ╢ ⁹

ES ─ خ ╨ خ ڗ ⌐ ═ ╢ ⁹

ŋ ╨ ▶ ◊ כ ♃ כ Ᵽ ☻ 37°C ≢ ╘ ⁸ ╨ 4°C ⌐ ╛ ⇔ ≡ ⅔ ™ √ ⁹

o ╨ ⇔ PBS(-) ╨ 5 mL ⇔ ≡ ─ ╨ ∫ √ ⁹

ɔ PBS(-) ╨ ╡ ™ √ ⁸ Accutase Solution (Millipore, Cat. R-SCR005) ╨ 5 mL ⇔ ≡ 5min ▬ fi ◖ ̶ ʐ כ ♃ כ ⌐ ╣ ⁸ ╨ ⇔ √ ⁹

ᴖ ⇔ ≡ ™ ╢ ╨ ⌐ ⅜ Δ √ ╘ ⁸ ḩ ! ☻ ◖ ╨ ɐ ╟ ɐ ⌐ ⇔ ≡ 10 ⇔ √ . (ﻸ ٹ)

ᴗ 5 mL ╨ 15 mL ─ ⌐ ⇔ ⁸ Ḩ Ɑ ℮ ♥ ▫ fi ◓ ╨ 10 ™ ◦ fi ◓ ¡ ☿ ¡ ⌐ ⇔ √ ⁹

p 4 °C ⁸ 100 × g ≢ 3 min ╨ ∫ √ ⁹

t ٿ ╨ ⇔ √ ╨ 5 mL ⇔ ⁸ Ḩ Ɑ ℮ ♥ ▫ fi ◓ ⌐ ╟ ╡ Ɑ ℮ ♩ ─ ╨ ٻ ⌂ ⌐ ⇔ √ ⁹

u C-Chip ╨ ™ ≡ ○ ▶ fi ♩ ╨ ™ ⁸ ╨ ╘ √ ⁹

ב 1.0 × 10⁶ cells / 5 ml ≢ ♀ ! ♅ fi ◖ כ ♩ ─ 25 cm² ḩ ! ☻ ◖ ⌐ ™ √ ⁹

ɯ 37ϴ ⁸ ݜ 5% CO_2 ⁸ 95% Air ─ ▬ fi ◖ ̶ ʐ כ ♃ כ ≢ ╨ ∫ √ ⁹

ES │ خ ⅜ ™ √ ╘ ˍ │ ∫ √ ⁹

2.4.5 ─

ŋ ─ خ ≤ ─ ≢ ╨ ⇔ ⁸ ╨ ™ ⁸ ╨ Δ ╢ ⁹

o ٿ ╨ ⁸ ⅜ 1.0 × 10⁶ cells mL⁻¹ ⌐ ⌂ ╢ ╟ ɐ ⌐ ─ ╨ ⅎ ╢ ⁹

ɔ ⇔ √ ╨ ▪ fi ķ ¡ ♅ ̶ כ Ķ ⌐ ⇔ ⁸ -80°C ─ ﻫ ⌐ ╢ ₁ ⌐ ↕ ∑ ╢ ⁹

ᴖ ⌐ ⇔ √ ╠ ♅ ̶ כ Ķ ╨ ى ڃ ⌐ ⇔ ≡ Δ ╢ ⁹

2.4.6 ─

ŋ ◖♬◌ ꞁ ♅ ɔ Ḳ 2 ⌐ ╨ ╢ ⁸ ▶◊ɔ♃ɔ Ᵽ ☻≢ 37°C ⌐ ╘╢ ⁹ ╕√ ⁸ ḫ ꜝ ☻◖⌐ ╨ ╣ ⁸ ▬ fi ◖ ẓ ɔ♃ɔ ≢ 37Ɵ ⌐ ╘╢ ⁹

o ی ڿ ⅛ ╞ ⇔√ ─ ʃ ≡™╢ ▪ fi ķ ꞁ ♅ ɔ Ḳ╨ ╡ ⇔ ⁸ ▶◊ɔ♃ɔ Ᵽ ☻ ≢ ⅛Δ ⇔ ↑╡ ┌ ⁹

ɔ ⇔√ ≤ ↑⅝╣≡™⌂™ ╨Δ═≡ ╘√ ─ ʃ √ ◖♬◌ ꞁ ♅ ɔ Ḳ ⌐ ╣ ⁸ ⌐ ⅛Δ ⁹

ˆ 1500 rpm ⁸ 5 min ≢ ╨ Δ╢ ⁹

ˇ ٿ ╨ ╡ ⅝ ⁸ ⇔™ ╨ ɨ Ḩ ᗡ ꝑ ♥ ▫ fi ◓ ⇔ ⁸ ╨ ↕∑╢ ⁹

p ╘≡⅔™√ ḫ ꜝ ☻◖⌐ ╨ ɨ ⁸ ▬ fi ◖ ẓ ɔ♃ɔ ⌐ ╣ ⁸ 37°C ⁸ 5%CO_2≢ Δ╢ ⁹

t ⁸ ╨ ⇔ ⁸ ╨ ⇔ ⅜ ≢ ⅝╢ ┌ ╨ ↑╢ ⁹

2.5 ─ Ʀ♃ɔ♬ fi ◓

Ʀ♃ɔ♬ fi ◓ │ ⁸ ♃ fi Ʀ ⌐ ╨ ٿ⌐ش ⌐ Δ╢√╘⌐ ™╟╡ ⁸ ─ ⌐ق™ ⁸ ▬ ☻ ɔ ꞁ ─ Ʀ♃ɔ fi ⅜ ≤⌂ ʃ ≡™╢ ⁹ Ʀ♃ɔ♬ fi ◓ ─ پ≈≤⇔≡ ⁸ ⇔√ ╨ Δ╢ ☻♥ fi ◦ ꞁ ╨ ™√ ▬ ☻♥ fi ◦ ꞁ ⅜№╢ ⁹ ☻♥ fi ◦ ꞁ ≢ ╨ Δ╢ ↓≤≢ ⁸ ڂ ─ ┼─ ─ ⅜ ⌐ ↕╡╢ ⁹ ≢ │ ⁸ ی ⅜ ɔ ⁸ ♦▫ꝑ◦ ⌐ Δ╢ ≢ №╢ polydimethylsiloxane PDMS ╨ ™≡☻♥ fi ◦ ꞁ ╨ ⇔√ ⁹PDMS ☻♥ fi ◦ ꞁ ⌐ ╟╡ ⇔√ ♦▫ꝑ◦ ٿ≢ ╨ Δ╢ ≤ ⁸ ⇔√ ≤ ∂ ╨ Δ╢ Ʀ♃ɔ fi ⅜ ٿ⌐ ↕╡╢ ⁹ ↓ ─ PDMS ☻♥ fi ◦ ꞁ │ ⁸ Ḩ fi ☿ꝑ♩ ≢ ⌐ ╪≢ ⌐ ╡ ↑╢ ↓≤⅜≢ ⅝ ⁸ ⅜ ⇔√ ⁸ ⌐ ╨ ɨ ╢ ↓≤⌂ɔ ⅜Δ ↓≤⅜≢ ⅝╢ ⁹ ╕√ ⁸ ╨ ╥∏ ™╢ ↓≤⅜≢ ⅝╢ ⁹ Figure 2.5 ⌐ ≢ ™√ Ʀ♃ɔ fi ╨ Δ ⁹

ŋ ● ꜝ ☻ S1225 ⁸ MATSUNAMI ╨ 2 ⌐ ╡ ⁸ 3 ⁸ 3 ⁸ 4 ⁸ 4 ⁸ 5 ⁸ 5 ⁸ 6 ⁸ 6 ⁸ 6-Nonafluorohexyltrichorosilane 20 ㎕ ≤ ⌐ w fi ķ≢ ⅝Δ╢ ↓≤ ⌐ ╟ ʃ ≡ ⁸ ● ꜝ ☻ ╨ ◦ ꜝ fi Δ╢ ⁹

o ◦ ꜝ fi ⇔√ ● ꜝ ☻ ٿ⌐ PDMS (◦ ꞁ w ꝑ ♩ 184W/C ⁸ ƀ ▶◖ɔ♬ fi ◓)╨ ╟⇔≡ 1000 rpm ≢ 30 ☻ Ḩ fi ◖ɔ♩ ⇔ ⁸ ⁄ ─ 80 °C ⁸ 1 ≢ Δ╢ ↓≤⌐ ╟ ʃ ≡ ⁸ 100 ㎛ ─ PDMS ╨ Δ╢ ⁹

ɔ PDMS CO_2

PDMS 1

PDMS 70% ڿ ݽ

PDMS ڼ

1 0 10^6 cells mL^{-1} 100 ㎖ ٹ ڗ

37°C 5% CO_2 2

ٹ PDMS ڼ

ڿ 37°C 5% CO_2 1

80-90% PDMS

ݪ

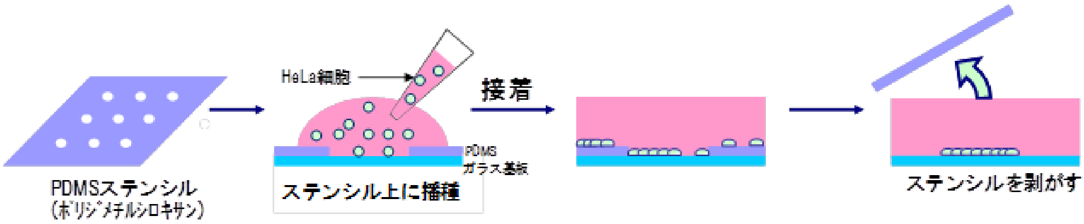

Figure 2.4 Manufacture method of patterned cells by PDMS stencil.

300 ㎜ ⅔ ╟ ┘ 100 ㎜ ─ ╩ ∆ ☻ ♥ fi ◦ i ╩ ™ ≡ ⌐ ݪ ⇔ √ Ʀ ൖ ͻ fi ─

╩ Figutr 2.6 ⇔ √ HeLa ⁹ Ʀ ൖ ͻ fi ─ ݪ ⌐ ⇔ √ ↓ ≤ ⅜ ≢ ⅝ √ ⁹

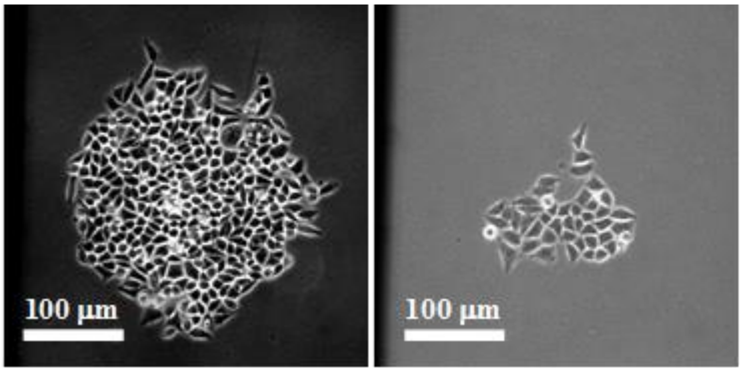

Figure 2. 5 Patterned HeLa cells by PDMS stencil.

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[3] 8 ه 2008

[4] 8 ه 2008

[5] ث 8 ه 2009

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[4] ى 8 ه 20013

3 ىٍ — ϖ ♦ ▫ ◄ Ͻ ꟹ Ͻ ╨ ™ √ ۑ

≠ ♬ ꟹ ¡ fi ◓

≢ │ 8 ۑ — ⌐ ≈ ™ ≡ ═ ╢ 9 ϖ ♫ ☺ ○ fi ≤ ƾ ▼ ¡ ◦ ▪ fi ○ ¡ ► ⊦ $K_3Fe(CN)_6$ — 2 — ϖ ♦ ▫ ◄ Ͻ ꟹ Ͻ ╨ ╗ ╦ ∑ ╢ ↓ ≤ ≢ 8 ⌐ — ╨ ⇔ √ 9 SECM ╨ ™╢ ↓ ≤ ≢ 8 ۑ ⌐ ʐ ¡ ה ﻫ ה ⌂ ╨ ∫ √ 9

3.1 │ ∂ ╘ ⌐

│ 8 — خ ╛ — — ≈ ≤ ⌂ ╡ e ╢ √ ╘ 8 ﻫ ה ה ה ۑ ⌐ ʐ ¡ ≢ — ⅜ ╘ ╟ ╡ ≡ ™ ╢ 9 ≢ │ 8 غ ╨ ﺰ ة Δ ╢ ϖ ♦ ▫ ◄ Ͻ ꟹ Ͻ ╨ Δ ╢ ↓ ≤ ≢ 8 — ﻫ ה ⌂ ⅜ ≢ №╢ 9 ϖ ♦ ▫ ◄ Ͻ ꟹ Ͻ │ ╨ Δ ╢ ↓ ≤ ⅜ ≢ ⅝ 8 — ⌐ ≢ №╢ [1, 2] 9 SECM │ 8 ۑ ╙ ⇔ ɔ │ ⁄ ╣ ﺨ ﭫ — ◘ ▬ ☼ ≢ — ⌐ ⇔ ≡ ⅔ ╡ [3, 4] 8 ה — [5-12] — ⌐ ↕ ╣ ≡ ⅝ √ 9

ϖ ♫ ☺ ○ fi 2-methyl -1,4-naphthoquinone 8 ɏ ꟹ ⊦ fi K3 8 Figure 3.1 │ 8 ╨ Δ ◖ ƚ fi — ϖ ♦ ▫ ◄ Ͻ ꟹ Ͻ ≢ №╡ 8 — ⌐ ≢ ⅝ ╢ [5-7, 13, 14] 9 ⇔ ⅛ ⇔ 8 ϖ ♫ ☺ ○ fi │ H_2O_2 ╛ ☻ Ʀ Ͻ ○ ◖ ◘ ▬ ♪ ▪ ♬ ○ fi O_2 ⌂ ≥ — ROS — ≤ ⌂ ╡ 8 ⌐ ⇔ ≡ ╨ Δ ↓ ≤ ╙ ↕ ╣ ≡ ™ ╢ 9 ⌐ ⅔ ↑ ╢ ϖ ♫ ☺ ○ fi │ 8 NAD(P)H:quinone oxidoreductase NQO ⅔ ╟ ┘ ◒ ¡ ꟹ ♯ ○ fi GSH ⌐ ╟ ╡ ╦ ╣ ╢ [15] 9

NQO │ 8 ◖ ƚ fi — ╨ e ≢ №╢ 9 NQO ﺨ — ث — ⌐ ╟ ╡ ϖ ♫ ☺ ○ fi ⅜ ۑ ╨ ↑ ╢ ≤ 8 ☿ ⊦ ◖ ƚ fi ! ☺ ○ ¡ ⅜ ⇔ 8 ROS — ≤ ⌂ ╢ 9 ۑ 8 NQO │ 8 ◖ ƚ fi ╨ ىٍ ⇔ 8 ROS — ╨ ← Figure 3.1 9 ROS │ 8 ╛ ٹ 8 DNA — ╨ ⅝ 8 ╨ Δ 9 ╕ √ 8 ☿ ⊦ ◖ ƚ fi ! ☺ ○ ¡ ⁄ — ╙ — ⅜ 8 DNA ╨ ╗ ى ≤ ⇔ 8 ⅜ ╪ ╨ Δ [16] 9 ⅜ ╪ ≢ NQO ⅜ ټ ⇔ ≡ ™ ╢ ≤ ™ e ╙ ɔ №╡ 8 ⅜ ╪ — ≈ — ≤ ⇔ ≡ ≢ ⅝ ╢ ⅜ №╢ 9 NQO

╨ ⌐ ↕ ∑ ╢ ─ ⅜ ∆ ╢ ┌ 8
⅜ ╪ ┼ ─ ╙ ≢ ⅝ ╢ 9
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∄ ♩ ⅜ ╪ ⅛ ╠ ─ ꙍ ♫ ☺ ○ fi
GSH ى ─ ╨ ⇔ ≡ ™ ╢ [5, 6] 9
ꙍ ♫ ☺ ○ fi ≤ ɧ ▼ i ◦ ▪ fi ◌ i ► ⊦
$K_3Fe(CN)_6$ ╨ 8 ⇔ √ ♭ Ⱪ i ꙍ ♦ ▫ ◄ Ↄ ♃
Ↄ ◦ ☻ ♥ ⊦ ⅜ 8 NQO ─
⌐ ↕ ╣ ≡ ™ ╢ Figure 3.2 [13, 14, 17] 9 Pt
ﺖ≢ ─ ꙍ ♫ ☺ ○ fi ─ │ ™ ⅜ 8
⌐ ─ ™ $K_3Fe(CN)_6$ ╨ ∆ ╢ ↓ ≤ ≢ 8
↓ ─ ⌐ ∟ ∟ 8 ⌐ ⅜ ↕ ╢
╢ 9 ╕ √ 8 ﺖ≢ ↕ ╢ √ $Fe(CN)_6^{4-}$ │ 8
⅛ ╠ ↕ ╢ √ ꙍ ♫ ☺ ○ fi ى ⌐ ╠ ∫ ≡
∟ ⌐ ↕ ╣ ╢ √ ╘ 8 ≢ ─ ♪ ♇ ☁ ☻ ◘ ▬ ☁ i fi ☁ ⅜ ⅝ 8 ⅜
↕ ╣ ╢ 9Nagamine ╠ │ 8 ♭ Ⱪ i ꙍ ♦ ▫ ◄ Ↄ ♃ Ↄ ◦ ☻ ♥ ⊦ ≤ SECM ╨ ⇔ 8 ─ NQO
╨ ⇔ √ [7] 9 ╠ │ 8 ⅜ ≤ ⌂ ╢ ╠ e ⌐ ꙍ ♫ ☺ ○ fi ╨ ⇔ √ ⅜ 8 ↓
─ ≢ │ 8 ꙍ ♫ ☺ ○ fi ─ √ ╘ ⌐ ⌐ │ ↕ ⌂ ⅛ ∫ √ 9
≢ │ 8 SECM ≤ ♭ Ⱪ i ꙍ ♦ ▫ ◄ Ↄ ♃ Ↄ ◦ ☻ ♥ ⊦ ╨ ™ ≡ 8 NQO ─ ﭗ
ʐ i ה ה ≠ ♬ ♃ i fi ☁ ╨ ∫ √ 9 ꙍ ♫ ☺ ○ fi ≢ ─ ≤ SECM
⅛ ╠ 8 ⌐ ⇔ √ ꙍ ♫ ☺ ○ fi 8 $K_3Fe(CN)_6$ ╨ ⇔ √ 9 ⅛
╠ ﭗ ─ ꙍ ♫ ☺ ○ fi ى ╨ ⇔ 8 ⇔ √ 9 ꙍ ♫ ☺ ○ fi ⌐ ╠ ╢
─ ROS ≤ ☁ i ♃ ♅ ○ fi GSH ─ ╙ ⇔ √

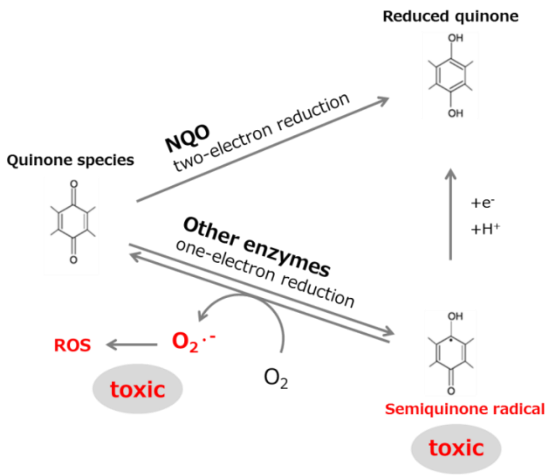

Figure 3.1 Schematic illustration of the reduction of quinone species within the cells. NAD(P)H:quinone oxidoreductase (NQO) can reduce quinone species by two-electron reduction without the generation of reactive oxygen species (ROS) and semiquinone radicals which lead to cytotoxicity.

3.2

3.2.1

≢ │ ﺨ ﺯ ─ ╨ ⇔ √ 9
D- ☁ i ◖ Ↄ ☻ 8 KCl 8 NaCl 8 Na_2HPO_4 $12H_2O$ 8 ɧ ▼ i ◦ ▪ fi ◌ i ► ⊦ $K_3Fe(CN)_6$ 8
2-methyl -1,4-naphthoquinon ꙍ ♫ ☺ ○ fi 8 $MgCl_2$ ה $6H_2O$ 8

N-2-hydroxyethylpiperazine -N ′-ethanesulfonic acid HEPES ﻑ ⁸ Cell Counting Kit -8 ﻑ ⁸ OxiSelectTM ROS Assay Kit CELL BIOLABS, INC. ⁸ GSH-GloTM Glutathione Assay Promega ⁸HEPES buffer 150 mM NaCl 4.2 mM K Cl 2.7 mM $MgCl_2$ 1.0 mM Na_2HPO_4 11.2 mM D -glucose 10 mM HEPES

3.2.2 SECM

ҍ Ҁ ⁱ ɷ ♦ ▫ ◄ Ↄ ҷ Ↄ ◦ ☻ ♥ ⊦ ⌐ ╟ ╢ NQO ╩ Figure 3.2 ⌐ ⇔ √ ⁹ ⌂ ╩ Δ ɷ ♬ ☺ ○ fi │ ⁸ ҷ fi Ʀ ◒ ⌂ ≥ ≤ ∑ ∏ ⌐ ⌂ ⌐ ╟ ∫ ≡ ╩ Δ ╢ ↓ ≤ ⅜≢ ⅝ ╢ [2, 5, 18] ⁹ ⌐ ╡ ┐ ╢ √ ɷ ♬ ☺ ○ fi │ ⁸ NQO ⌐ ╟ ∫ ≡ ↕ ╢ ╢ ⁹ ɷ ♬ ☺ ○ fi ی │ ≢ $Fe(CN)_6^{4-}$ ╩ ⇔ ⁸ $Fe(CN)_6^{3-}$ ⅜ Δ ╢ ⁹ $Fe(CN)_6^{3-/4-}$ │ ⁸ ɷ ♦ ▫ ◄ Ↄ ҷ Ↄ ≢ № ╡ │ √ ⌂ ™ ⁹ $Fe(CN)_6^{4-}$ ╩ ﺖ≢ Δ ╢ ↓ ≤ ≢ NQO ╩ ⌐ ≢ № ╢ ⁹ ≢ ↕ ╢ √ ɷ ♬ ☺ ○ fi │ NQO ≤ Δ ╢ ⁹ SECM ⌐ │ ⁸ 20 µm ─ Pt ♦ ▫ ☻ ◒ ∜ ▬ ◒ ﮪ ╩ ™ √ ⁹ ⌐ 0.5 V vs. Ag/AgCl ╩ ⇔ √ ⁹ 0.5 V vs. Ag/AgCl │ $Fe(CN)_6^{4-}$ ╩ ⌐ Δ ╢ ₒ ≢ № ╢ ⁹ ﻱ ╙ ⇔ Ↄ │ ⁸ ﺖ ╩ Δ ╢ ╟ ℮ ⌐ 1-line scan ╩ ∫ √ ⁹ 1-line scan ≤ │ ⁸ ﭗ ⌐ ╩ Δ ╢ ≢ № ╢ ⁹

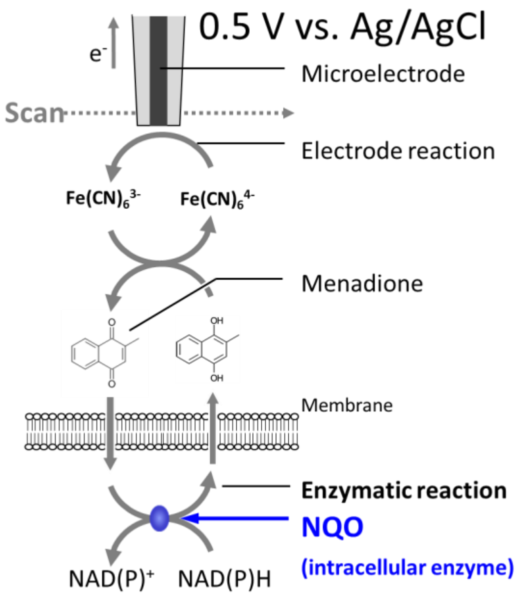

Figure 3.2 Principle of electrochemical detection of NAD(P)H:quinone oxidoreductase (NQO) activity in a HeLa cell. $Fe(CN)_6^{3-}$ and menadione were added in the measurement solution.

3.2.3 NQO ≤ NQO ≶ ♬ ҷ ⁱ fi ◓

SECM ⌐ ╟ ╡ ╟ ╢ ╢ ⅛ ╟ ⁸ ⌐ ╟ ╢ ҝ ɧ □ ▬ ⁱ ╩ ═ ╢ [19] ⁹ ≢ │ ⁸ ﭗ ⅛ ╟ ─ ɷ ♬ ☺ ○ fi ی ╩ ⇔ NQO ≤ ⇔ √ ⁹

ɷ ♬ ☺ ○ fi ی ─ ⅜ ∞ ≤ ﺫ Δ ╢ ≤ ⁸Fick ─ ﭗ ╟ ╡ *flux* │ ﺨ ﺓ ─ ╟ ℮ ⌐ ∑ ╢ ⁹