Electronic Supplementary Material (ESI) for Lab on a Chip.

Enhanced parylene-C fluorescence as a visual marker for neuronal electrophysiology applications

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SUPPLEMENTARY INFORMATION

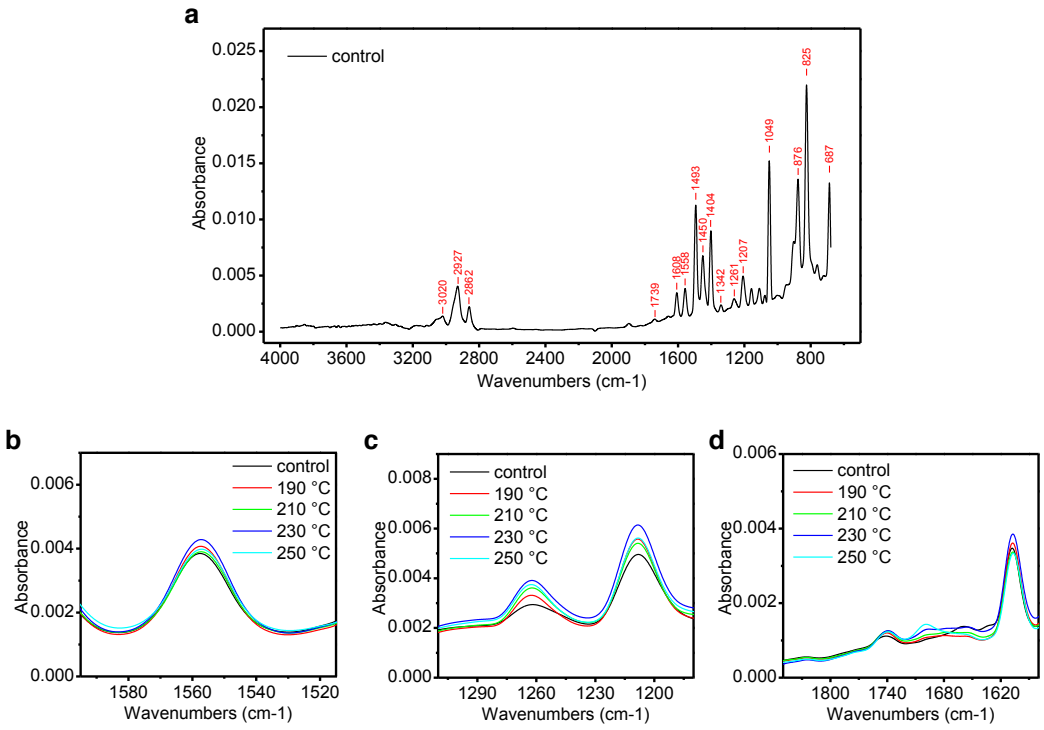

Fig. S1. Infrared spectra of parylene-C.

(a) Infrared spectra of as-deposited parylene-C. (b–d) Comparison of as-deposited and annealed parylene-C in the region of (b) C=C bond ~1560 cm^{-1}, (c) C–O bond ~1100–1300 cm^{-1} and (d) C=O bond ~1700 cm^{-1}.

In Lu et al. reported parylene-C fluorescence behaviors, the fluorescence spectra showed red shifting during short-time UV illumination and a reduction of emission-band magnitude during long-time UV illumination, through a possible mechanism of dehydrogenation and oxidation. The corresponding infrared spectra also showed significant variations in C=C (approximately 1560 cm^{-1}), C–O (1100–1300 cm^{-1}), and C=O (approximately 1700 cm^{-1}) peaks, suggesting the generation of new carbon bonds.

But in our work, the mechanism of fluorescence enhancement was different from previously reported light induced changes in chemical bonds. No noticeable shifts of the emission band in the fluorescence spectrum (Fig. 2a) were observed. Besides, no significant changes of the typical carbon-bond peaks in the infrared spectra (Fig. S1) were observed. The fluorescence enhancement was probably caused by physical factors

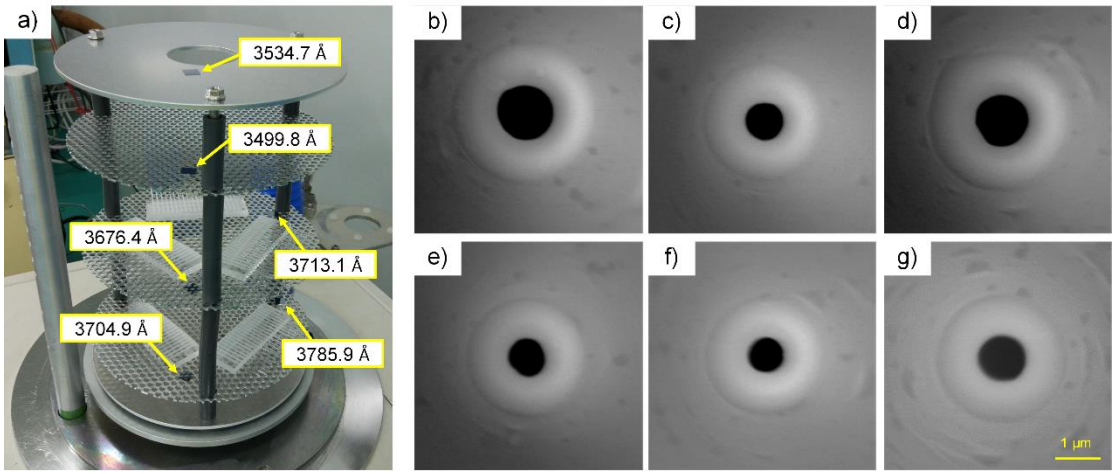

Fig. S2. The uniformity of parylene-C deposition.

(a) Parylene-C deposition thicknesses in the chamber. (b–g) SEM images showing the top view of parylene-C coated pipette tips.

Parylene-C was chemical vapor deposited (CVD) on the pipette. The CVD technique was a frequently used approach to achieve high-quality, conformal, thin films with precise thickness control. The deposition process was performed under under a low pressure (~ 15 mTorr) with a small sticking coefficient (~ 1×10^{-4} at 20 °C) for the monomers, so the deposition was slow and conformal. As shown in Fig. 3b, the grooved quartz plates was placed on a rotating stage (10 rpm during the deposition) which helped distribute any non-uniformity of the coating over the entire fixture.

For the uniformity of parylene-C deposition in the chamber, 6 simultaneously deposited silicon (100) substrates on different positions in the chamber were measured by a thin film-thickness measurement system (ST-2000, K-MAC, Korea), as shown in Fig. S2(a). The parylene-C deposition thickness was 3652 ± 111 Å, which indicated that the non-uniformity (standard deviateon divided by mean value) in the chamber was less than 5%.

For the uniformity of parylene-C deposition on the pipette tip, we performed SEM measurements as shown in Fig. S2(b-g). Although the pipettes showed different tip openings (mainly due to the opening variations after thermal pulling), the wall thicknesses were basically identical (910 ± 40 nm), demonstrating the good deposition uniformity of parylene-C.

Table S1. Numerical summary of values obtained in the electrophysiological experiments, as depicted in the figures.

Parameter	Conditions	Value (Mean)	Value (SEM.)	Value (S.D.)	n	Coefficient of Variation	p-value (t-test)	Fig.
Cm. (pF)	glass pipette	73.72	5.881	22.78	15	30.89%	0.8156	5b
Cm. (pF)	*f*-pipette	75.73	6.049	20.96	12	27.67%		
Rs. (MΩ)	glass pipette	14.26	0.8351	3.234	15	22.68%	0.8424	
Rs. (MΩ)	*f*-pipette	13.94	1.435	4.971	12	35.66%		
Rm. (MΩ)	glass pipette	57.78	5.185	20.08	15	34.76%	0.0547	
Rm. (MΩ)	*f*-pipette	41.26	6.483	22.46	12	54.43%		
mEPSC frequency (Hz)	glass pipette	0.1998	0.04217	0.1633	15	81.75%	0.4911	5d
mEPSC frequency (Hz)	*f*-pipette	0.2439	0.04693	0.1626	12	66.66%		
mEPSC amplitude (pA)	glass pipette	17.03	1.006	3.898	15	22.88%	0.1545	
mEPSC amplitude (pA)	*f*-pipette	20.04	1.918	6.643	12	33.15%		
Action potential half width (ms)	glass pipette	1.747	0.4833	0.8372	3	47.92%	0.4977	6c
Action potential half width (ms)	*f*-pipette	2.153	0.3291	0.7359	5	34.17%		
Action potential amplitude (pA)	glass pipette	86.28	9.73	16.85	3	19.53%	0.0801	
Action potential amplitude (pA)	*f*-pipette	66.78	4.464	9.983	5	14.95%		
Membrane resting potential (mV)	glass pipette	54.06	1.47	2.546	3	4.71%	0.5760	
Membrane resting potential (mV)	*f*-pipette	56.65	3.181	7.114	5	12.56%		
Action potential frequency (Hz)	glass pipette	0.3494	0.1735	0.3004	3	85.98%	0.8006	6e
Action potential frequency (Hz)	*f*-pipette	0.4238	0.1887	0.4219	5	99.54%		

Description of Additional Files and Videos

'**Video S1.mov**': This video shows targeted neuron patching by the *f*-pipette under two-photon microscope. The recording frame rate is 30 fps. Labelled by the parylene-C fluorescence, the pipette tip was easily observed during approaching the target neuron.

'**Cover.tif**': This figure is a 3D effect drawing, showing the concept of the fluorescently guided targeted neuronal electrophysiology by the parylene-C visual marker.