

## Electronic Supporting Information

Paper-Based Microfluidics for Experimental Design: Screening Masking Agents for Simultaneous Determination of Mn and Co

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### Overview of Included Data

ImageJ Analysis Procedure

Scheme S1: Image Analysis Flow Diagram

Table S1: Reagents

Table S2: Buffer Preparation

Table S3: Masking Agent Preparation

Figure S1: Buffer Screening Sample Image

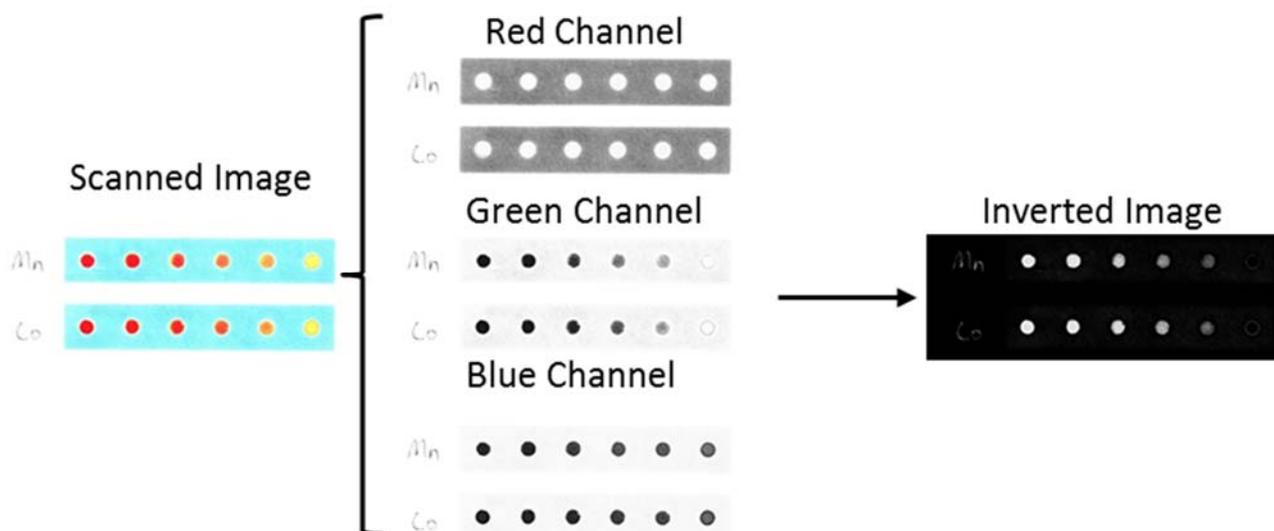
Figure S2: Masking Agent Screening Sample Images

Figure S3: Masking Efficiency Schematic and Sample Image

## Image J Analysis Procedure

1. Devices were scanned as .jpg images using a Xerox Documate 3220 scanner after all solutions were fully dried. Scanner settings were 600 dpi, 50% brightness, and 80% contrast.
  - Image → Color → Split Channels
2. The image files were cropped and opened in ImageJ, and the image was split into the red, green, and blue color channels
  - Image → Color → Split Channels
3. Using the green channel only, the image was inverted.
  - Edit → Invert
4. A 100 pixel by 100 pixel circle was drawn over the detection zone and the raw integrated density, which is the sum of the pixel intensities, was collected.
  - Analyze → Set Measurements → Integrated Density
5. The collected data was opened in Microsoft Excel 2010 for further analysis.
  - The two negative control spots of each device were averaged and used for a background subtraction.
  - The average intensity of the positive control spots, the average intensity of the control arms, and the intensities of the Mn and Co masking arms were plotted in Figure 4 of the main article text.
  - The masking efficiency was calculated as a percent change (Scheme 1 of the main article text) between the masking arms and the control arms as reported in Table 3 of the main article text.

**Scheme S1:** Image Analysis Flow Diagram



**Table S1: Reagents**

Compound	Supplier	Compound	Supplier
<b>Metals</b>		<b>Masking Agents</b>	
Chromium(III) chloride, hexahydrate	Sigma	Sodium fluoride	Sigma
Potassium dichromate	Fisher	Potassium iodide	Fisher
Manganese chloride, tetrahydrate	Fisher	Sodium acetate	Fisher
Ferrous sulfate	Fisher	Sodium sulfate, anhydrous	Fisher
Ferric chloride	Mallinckrodt	Sodium persulfate	Kodak
Cobalt chloride, hexahydrate	Aldrich	Sodium phosphate tribasic, dodecahydrate	Fisher
Nickel sulfate, hexahydrate	Acros	Potassium cyanide	Sigma
Copper nitrate, hemipentahydrate	Sigma	Potassium thiocyanate	Sigma
Zinc nitrate, hexahydrate	Sigma	Sodium dithionite	Pfaltz & Bauer
Cadmium nitrate, tetrahydrate	Fisher	Potassium tetrathionate	Sigma
Lead nitrate	Fisher	Potassium pyrophosphate	Aldrich
Aluminum sulfate	Sigma	Sodium thiosulfate	Aldrich
Calcium chloride, dihydrate	Fisher	Ascorbic acid	Sigma
Magnesium chloride	Sigma	Malonic acid	Acros
Lead nitrate	Fisher	2-Picolinic acid	Fluka
Aluminum sulfate	Sigma	Sodium citrate tribasic, dihydrate	Fisher
Calcium chloride, dihydrate	Fisher	Sodium bitartrate, monohydrate	Sigma
Magnesium chloride	Sigma	Sodium oxalate	Aldrich
Sodium chloride	Macron	Urea	Sigma
Potassium chloride	Fisher	Thiourea	Acros
<b>Buffers</b>		L-Cysteine	Sigma
Sodium tetraborate, decahydrate	Fisher	Meso-2,3-dimercaptosuccinic acid	Acros
Sodium carbonate, anhydrous	Sigma	Acetylacetone	TCI
Sodium bicarbonate	Fisher	Ethylenediaminetetracetic acid, tetrasodium salt hydrate	Sigma
Sodium phosphate, dibasic	Sigma	Ethylenediamine	Fisher
Glycine	Fisher	Diethylenetriamine	Sigma
N-cyclohexyl-3-aminopropanesulfonic acid	Sigma	Triethylenetetramine hydrate	Aldrich
<b>Other</b>		Dimethyleglyoxime	Fluka
Hydrochloric acid	EMD	1,10-phenanthroline	Aldrich
Sodium hydroxide	Fisher	Hydroxylamine hydrochloride	Pierce
4-(2-pyridylazol)resorcinol	Fluka		

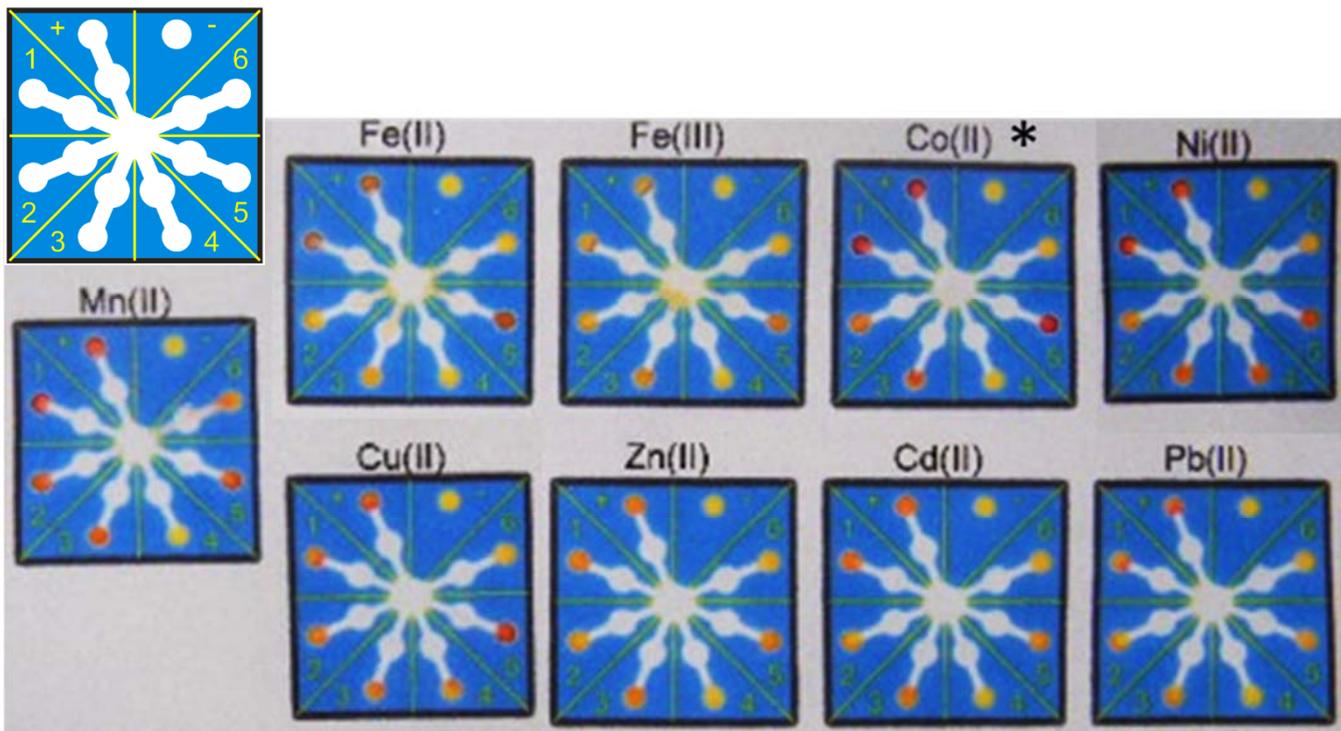
**Table S2: Buffer Preparation**

Buffer	Component 1	Component 2	Component 3	pH
NaOH/HCl	0.1 M NaOH	0.1 M HCl	-	9.921
0.125 M Borate	10 mL 0.25 M Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O	2.5 M 1 M NaOH	7.5 mL H <sub>2</sub> O	10.057
0.5 M Carbonate	6 mL 0.5 M Na <sub>2</sub> CO <sub>3</sub>	4 mL 0.5 M NaHCO <sub>3</sub>	-	9.933
0.1 M Phosphate	0.142 g Na <sub>2</sub> HPO <sub>4</sub>	9.98 mL H <sub>2</sub> O	0.02 mL 1 M NaOH	9.964
1 M Glycine	1.5 g glycine	0.4 g NaOH	20 mL H <sub>2</sub> O	9.935
0.25 M CAPS	9.5 mL 0.25 M CAPS	0.5 mL 1 M NaOH	-	9.946

**Table S3: Masking Agent Preparation (10 mL, 0.1 M in pH 10 glycine)**

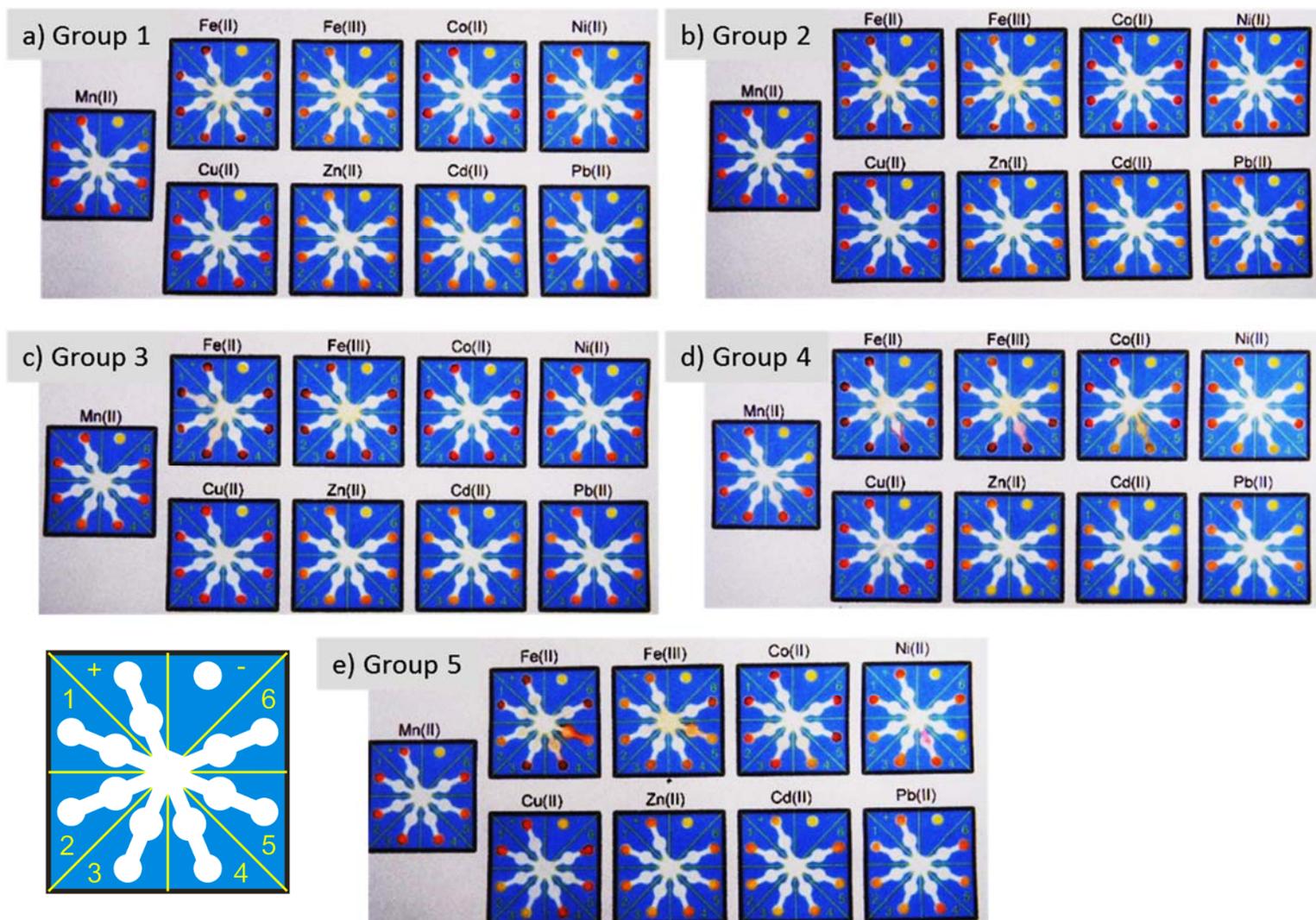
Group	Compound	Mass (g)	Initial pH	Adjusted pH
1	Sodium fluoride	0.0416	9.880	-
	Potassium iodide	0.1662	9.760	-
	Sodium acetate	0.0824	9.881	-
	Sodium sulfate	0.1471	9.905	-
	Sodium persulfate	0.2361	9.932	-
	Sodium phosphate, tribasic	0.3823	10.145	-
2	Potassium cyanide	0.0684	9.803	-
	Potassium thiocyanate	0.0965	9.816	-
	Sodium dithionate	0.2054	9.782	-
	Potassium tetrathionate	0.3003	9.821	-
	Potassium pyrophosphate	0.3333	10.013	-
	Sodium thiosulfate	0.2478	9.841	-
3	Ascorbic acid	0.1735	9.202	9.507
	Malonic acid	0.1041	9.381	9.560
	Picolinic acid	0.1238	9.664	9.735
	Sodium citrate	0.3082	9.939	-
	Sodium bitartrate	0.1955	9.689	9.760
	Sodium oxalate	0.1338	9.813	-
4	Urea	0.6105	9.892	-
	Thiourea	0.0760	9.645	-
	Cysteine	0.1336	9.292	9.699
	Dimercaptosuccinic acid	0.1827	9.161	9.566
	Acetylacetone	0.1040	9.616	9.763
	Ethylenediaminetetracetic acid, tetrasodium salt	0.3853	9.938	-
5	Ethylenediamine	0.0604	9.978	-
	Diethylenetriamine	0.1187	10.029	-
	Triethylenetetramine	0.1476	10.003	-
	Dimethyleglyoxime	0.1150	N/A – prepared in isopropanol	-
	1,10-phenanthroline	0.1812	N/A – prepared in isopropanol	-
	Hydroxylamine	0.0691	9.399	-

**Figure S1: Buffer Screening Sample Image**



**Figure S1:** Sample results from one run of the buffer testing experiment with H<sub>2</sub>O in the positive control arm, NaOH/HCl in arm 1, 0.125 M borate buffer in arm 2, 0.5 M carbonate in arm 3, 0.1 M phosphate in arm 4, 1 M glycine in arm 5, and 0.25 M CAPS in arm 6. PAR showed a color change with all of the metals in arm 5 indicated that glycine was the least interfering buffer solution. \*While Co appears to be masked by phosphate (arm 4), a subsequent test showed this was not the case.

**Figure S2: Masking Agent Screening Sample Images**



**Figure S2:** Sample results from one run of the masking agent screening experiment with masking agents as follows (numbers denote position on device):

Group 1: 1-fluoride, 2-iodide, 3-acetate, 4-sulfate, 5-persulfate, 6-phosphate

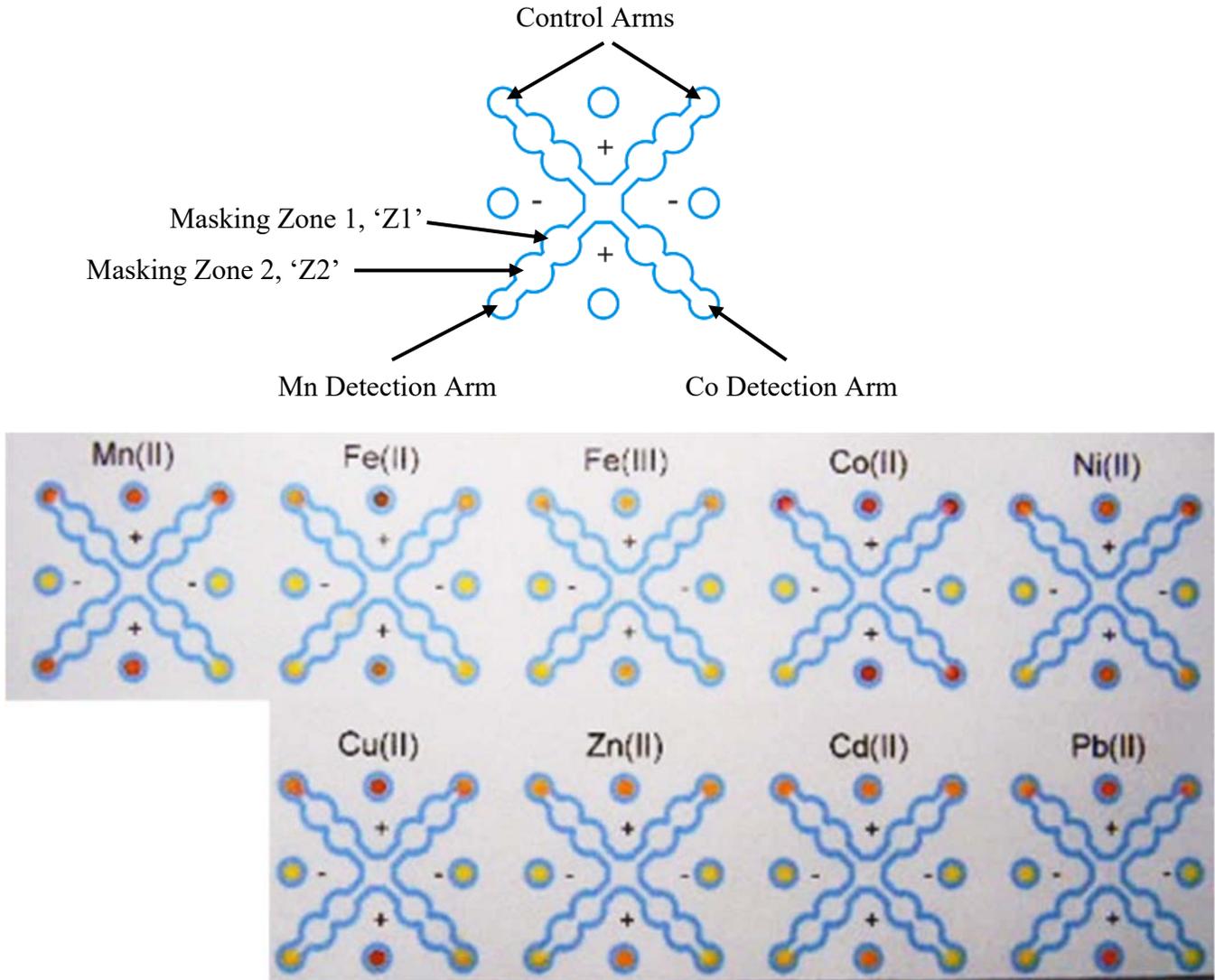
Group 2: 1-cyanide, 2-thiocyanate, 3-dithionate, 4-tetrathionate, 5-pyrophosphate, 6-thiosulfate

Group 3: 1-ascorbic acid, 2-malonic acid, 3-picolinic acid, 4-citrate, 5-bitartrate, 6-oxalate

Group 4: 1-urea, 2-thiourea, 3-cysteine, 4-dimercaptosuccinic acid, 5-acetylacetone, 6-ethylenediaminetetracetic acid

Group 5: 1-ethylenediamine, 2-diethylenetriamine, 3-triethylenetetramine, 4-dimethylglyoxime, 5-1,10-phenanthroline, 6-hydroxylamine

**Figure S3: Masking Efficiency Schematic and Sample Image**



**Figure S3:** Sample results from one run of the masking effectiveness study. Top Left (control): Z1 – H<sub>2</sub>O, Z2 – H<sub>2</sub>O; Top Right (control): Z1 – H<sub>2</sub>O, Z2 – H<sub>2</sub>O; Bottom Left (Mn procedure): Z1 – pH 10 borate buffer, Z2 – trien + DMSA in pH 10 borate buffer; Bottom Right (Co procedure): Z1 – EDTA in pH 10 phosphate buffer, Z2 – trien in pH 10 phosphate buffer.