Supplemental Information for:

Development of an inexpensive Raman-compatible substrate for the construction of a microarray screening platform

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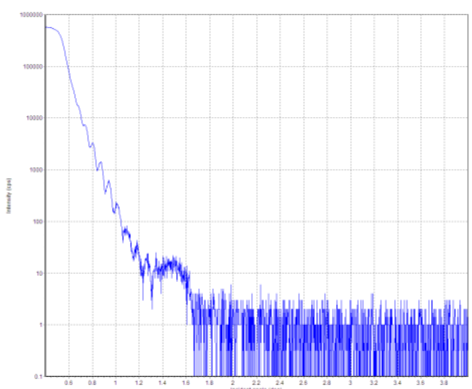
Supplemental Figures

(a)



(b)

Fit Settings

Sample Parameters

Layer	Layer Description	Density (g/cm3)	Thickness (nm)	Roughness (nm)
Substrate	DensityOnly, SiO2	2.904	1000000	0
1, 0	DensityOnly, Cr	6.471	6.005	1.34
2, 0	DensityOnly, Au	21.23	50.519	1.573

Convolution

Parameter	Value
BackGround	8
Divergence	0.008
Intensity	780407

Figure S1. (a) X-ray reflectivity plot of the thickness of the combined chromium adhesion layer (5 nm) and gold layer (50 nm) deposited on the glass substrates gives a combine value of 56.36 nm. (b) Fitting the curve using the Fourier method to calculate the thickness of the chromium adhesion layer and gold layer deposited on the glass substrate gives a combined value of 56.5 nm for the total thickness of the chromium and gold layers.

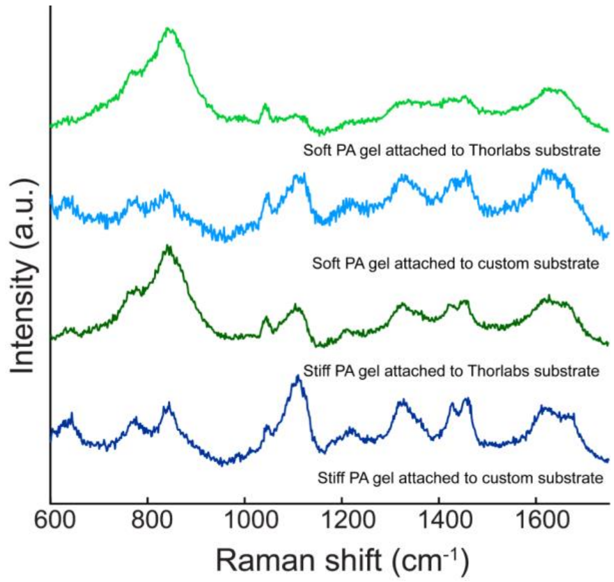


Figure S2. Spectra from soft and stiff fibronectin-functionalized PA gels covalently attached to either a custom Raman-compatible substrate or a Thorlabs substrate. Each spectrum was acquired by focusing on the surface of the gel.

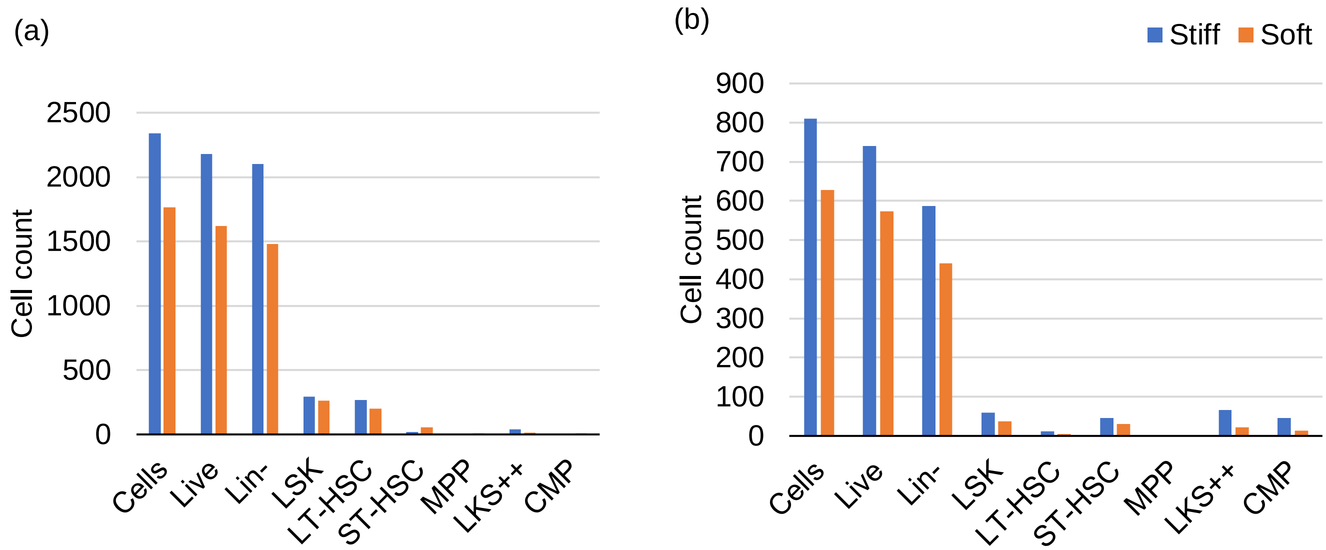


Figure S3. Flow cytometry cell counts of (a) LT-HSCs and (b) ST-HSCs, on custom Raman-compatible substrates covalently with an attached fibronectin coated soft and stiff PA gel, after Raman spectra acquisition shows maintained viability after exposure to 785 nm Raman laser.

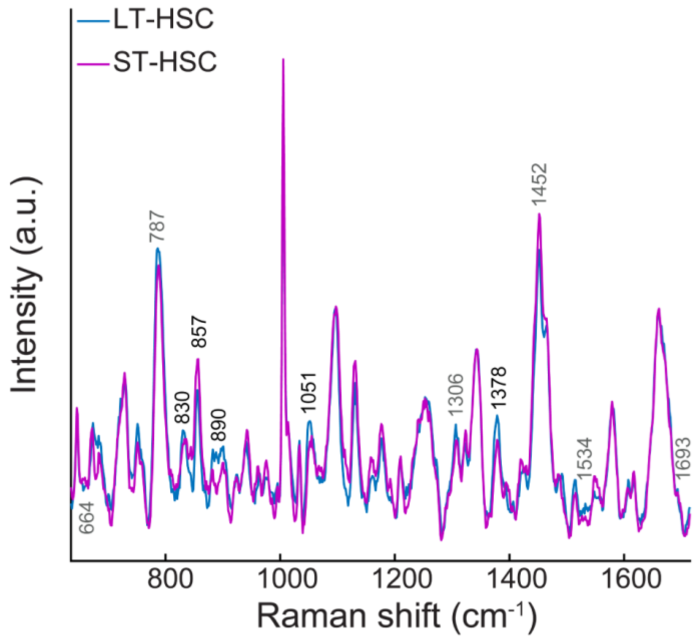


Figure S4. Average Raman spectra of individual living LT-HSCs (blue, *n = 143*) and ST-HSCs (purple, *n = 126*) on soft and stiff PA gels functionalized with fibronectin. Spectra were background subtracted, baseline corrected using automatic Whittaker filter and normalized for clarity. Peaks with VIP scores indicating importance for identification (**Figure 4.d**) are labeled black (>2, higher importance) and gray (>1.5, intermediate importance).

Supplemental Tables

Table S1. Cost assessment for the approximate cost of the presented custom Raman-compatible substrate fabricated with standard deposition techniques. Each batch consists of approximately 40 substrates and approximately one batch is made per month. Price of commercial Thorlabs substrates is $273.09 per 10 substrates. Fabrication facilities at UIUC serve the public (academic users) and the private (nonacademic) sector of the research community. Here we included the information of costs if a nonacademic user was interested in fabricating our presented custom Raman-compatible substrate within our academic fabrication facilities. If a nonacademic user used the same process and facilities to create a single batch of substrates, the cost of a single substrate would be approximately $12.13, 2.5 times lower than the cost of the commercially available (Thorlabs) protected gold mirror substrate. Also, the price of commercial Thorlabs substrates for a nonacademic purchase is $295.52 per 10 substrates.

Expense	**Cost (academic, nonacademic)**	**Cost/substrate (academic, nonacademic)**	**Reference**
Premium glass microscope slide	$45.04 / 144, $90 / 144	$0.30, $0.60	Fisherbrand™ Catalog No12-544-1
Gold and tungsten boat	$75 / g and $10 each, $77.08 / g and $10 each	$0.70, $0.78	Kurt J. Lesker, Part No EVMAUXX40G
Thermal evaporator use	$21 / h, $160 / h	$1.05, $8.00	MRL at UIUC
PECVD use	$17 / h and $35 / month, $75 / h and $35 / month	$1.30, $2.75	HMNTL at UIUC
Approximate total cost		$3.35, $12.13	

Table S2. Comparison of cross-validation (CV) errors for identification of CHO and CHO-T cells (**Figure 3.a** and **Figure 3.b**, *n=100*) as well as LT-HSCs and ST-HSCs (**Figure 4.b** and **Figure 4.c**, *n=269*). All spectra from calibration and test sets presented in PLS-DA models on **Figure 3** and **Figure 4** were combined for CV assessment. CV was performed on randomly distributed spectra using the venetian blinds method with splits of 2, 5 and 10.

PLS-DA model for identification of:		**CV with 2 splits**	**CV with 5 splits**	**CV with 10 splits**
CHO and CHO-T cells	sensitivity	0.840	0.880	0.860
	specificity	0.960	0.920	0.940
	error of CV	***10.0%***	***10.0%***	***10.0%***
LT-HSCs and ST-HSCs	sensitivity	0.951	0.958	0.958
	specificity	0.904	0.960	0.968
	error of CV	***7.2%***	***4.1%***	***3.7%***