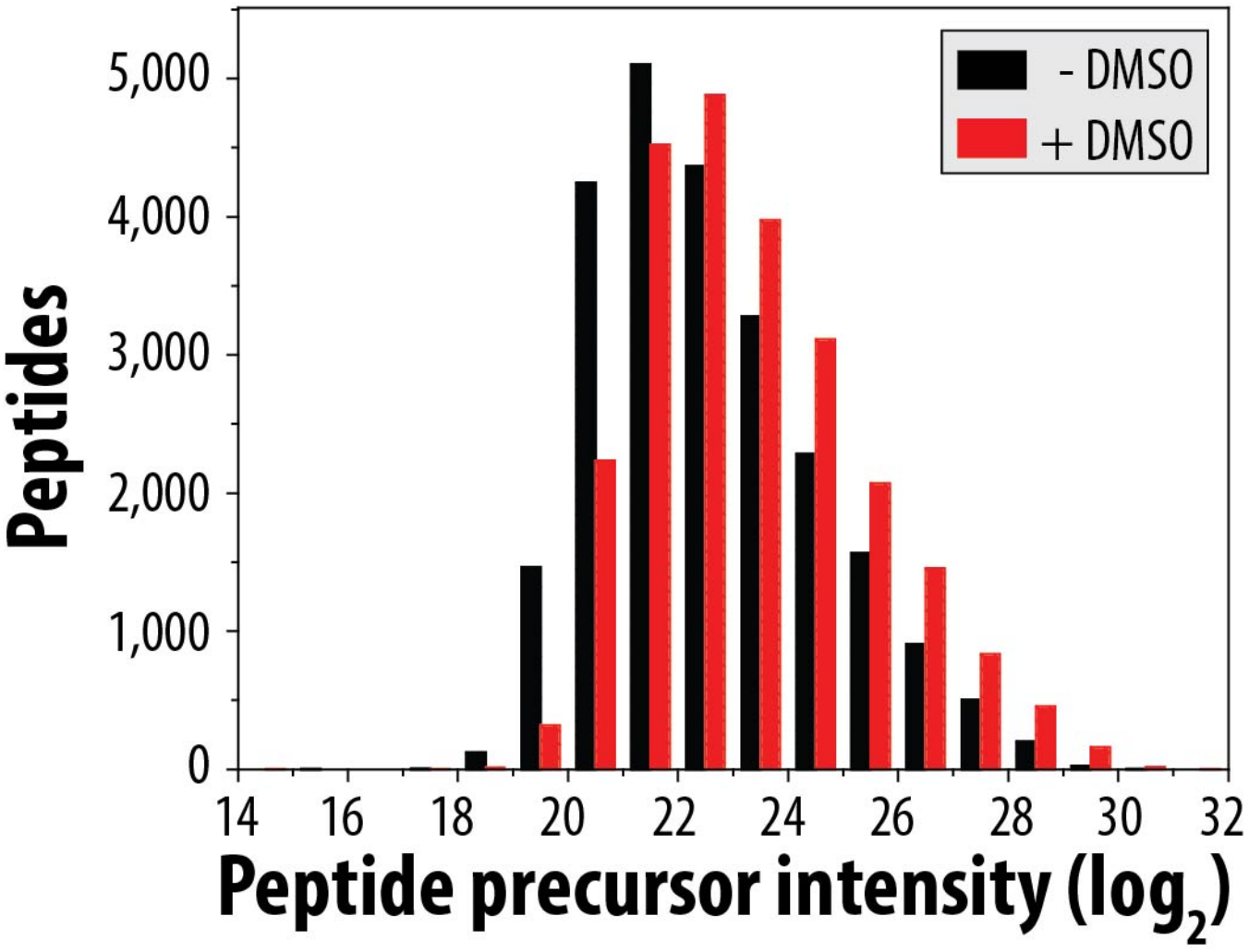
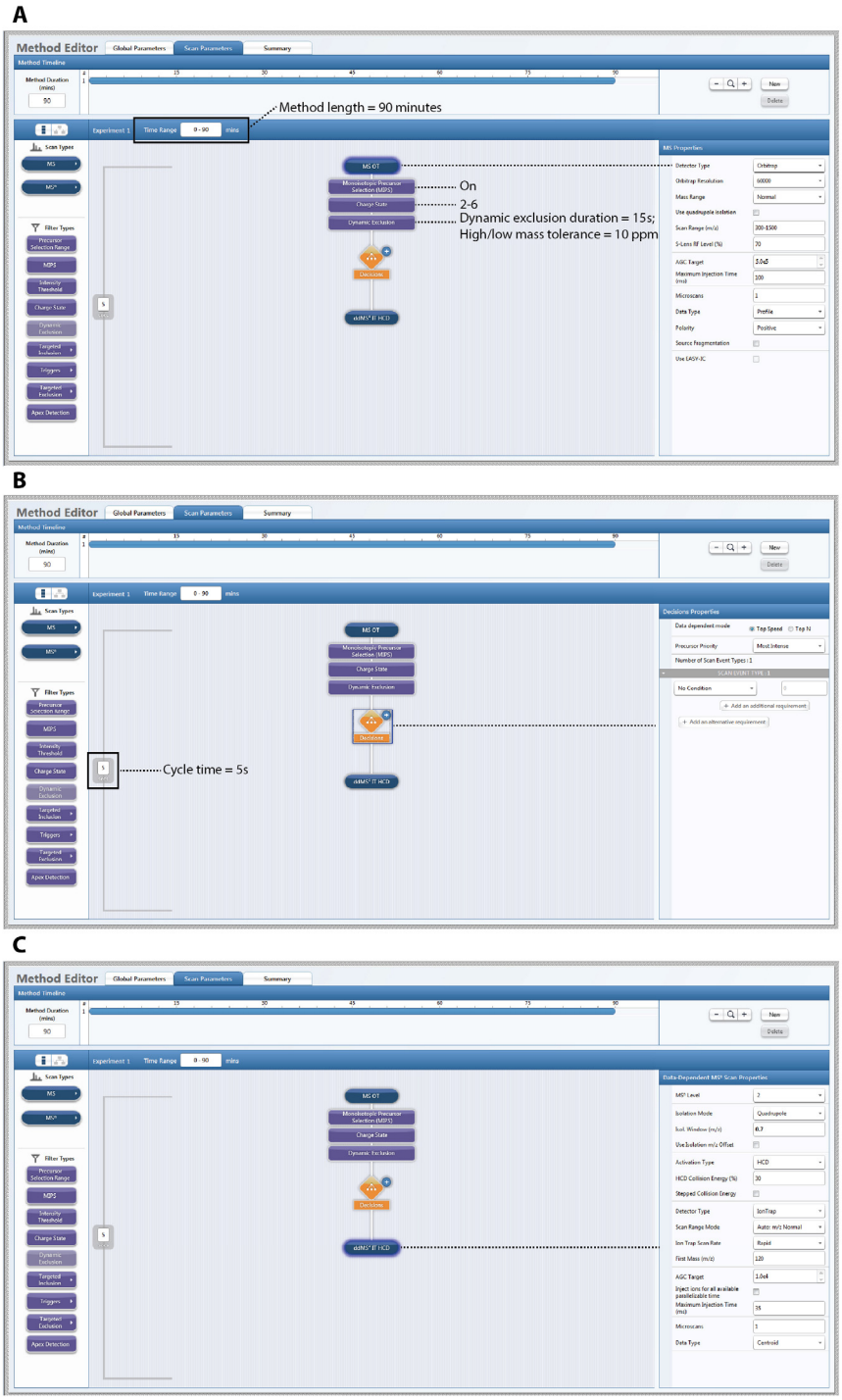


Supplementary Figure 1

Distribution of the intensities of peptide precursors in the survey scan.

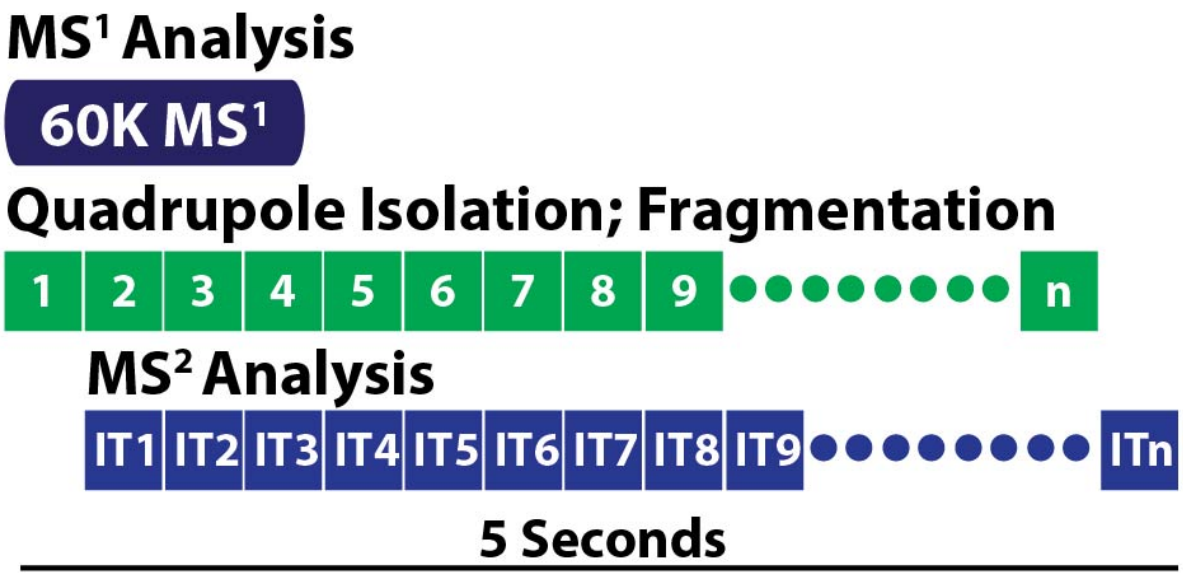
Red is with and black is without the addition of DMSO.



Supplementary Figure 2

Location of instrument parameters within Method Editor on the Orbitrap Fusion.

(A) MS^1 resolution, MS^1 detector type, and MS^1 AGC target are set within the MS OT section. (B) Top speed data dependent mode and precursor priority are selected within the Decisions section. (C) MS^2 detector type, MS^2 isolation window, MS^2 AGC target, MS^2 max injection time, activation type, collision energy, detector type and scan rate are set within the MS/MS IT section.#



Supplementary Figure 3

Orbitrap Fusion scan sequence.

#

Line 1 :	heat = 260	filament (fil) = 0	velocity (vel) = 10	delay (del) = 128	pull = 0
Line 2:	heat = 260	filament (fil) = 0	velocity (vel) = 20	delay (del) = 128	pull = 0
Line 3:	heat = 250	filament (fil) = 0	velocity (vel) = 15	delay (del) = 140	pull = 0
Line 4:	heat = 270	filament (fil) = 0	velocity (vel) = 15	delay (del) = 128	pull = 0

Supplementary Table 1

Recommended laser puller settings.

HEAT is the laser power output. FILAMENT is the scan length of the heat supplying laser; the manufacturer's protocol suggests a filament setting of 0 for fused silica. If tips are pulled too thin, the VELOCITY setting should be increased, while decreasing the HEAT setting. DELAY sets the time at which the fused silica is pulled relative to laser deactivation. PULL controls the force with which the fused silica is pulled. A PULL setting of 0 is recommended for fused silica.

Experiment	MS/MS scans	PSMs	Unique peptides
1	80,900	43,423	34,535
2	81,252	43,622	34,495
3	78,001	42,339	33,450
4	80,916	43,326	34,347
5	81,235	43,343	34,449

Supplementary Table 2

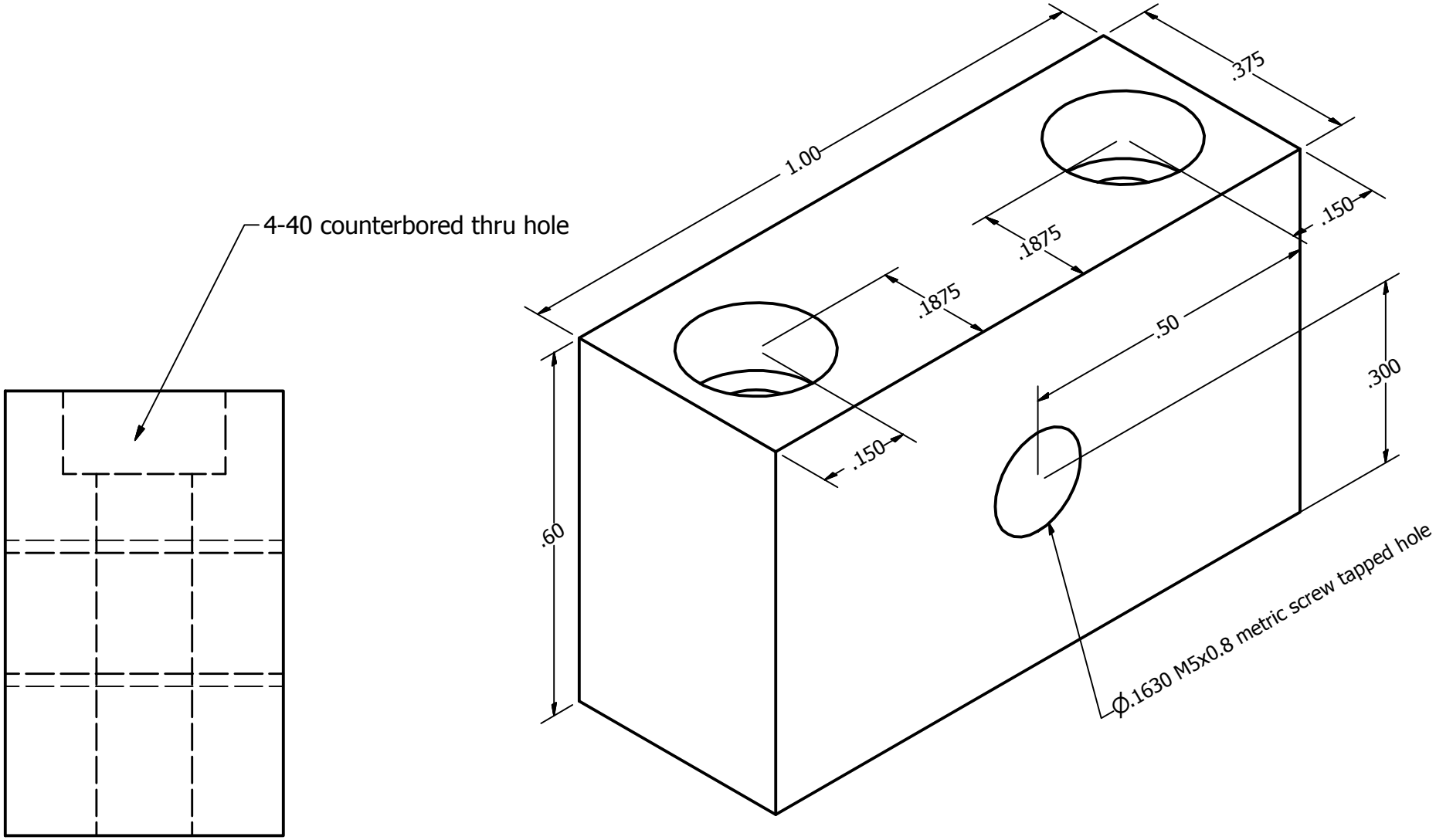
Number of MS/MS scans, PSMs and unique peptides for five replicate yeast experiments.

Gradient length(min)	MS/MS scans	PSMs	Unique peptides
30	45,632	27,700	18,152
45	60,656	35,421	26,931
70	86,267	52,872	36,900
120	124,014	74,813	44,743
180	169,181	96,121	48,970
240	212,088	115,077	51,211

Supplementary Table 3

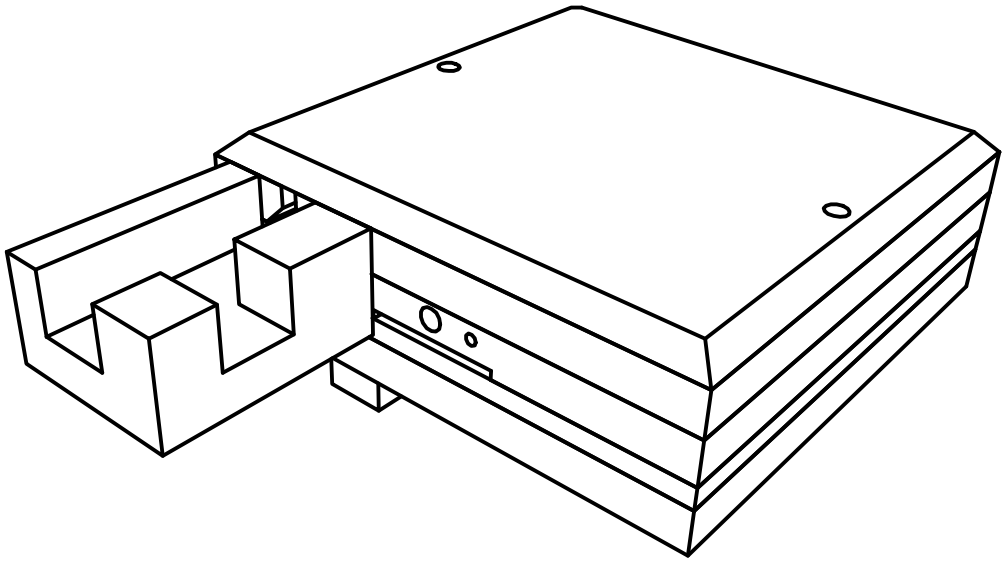
Number of MS/MS scans, PSMs and unique peptides for gradient length experiments (Figure 6).

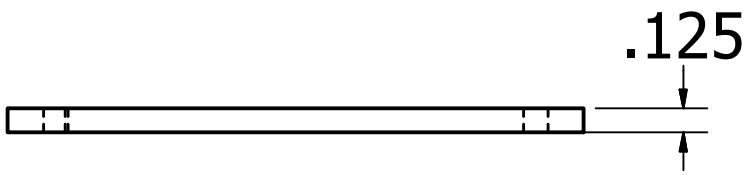
Attachment to Flex Ion Source (PC)

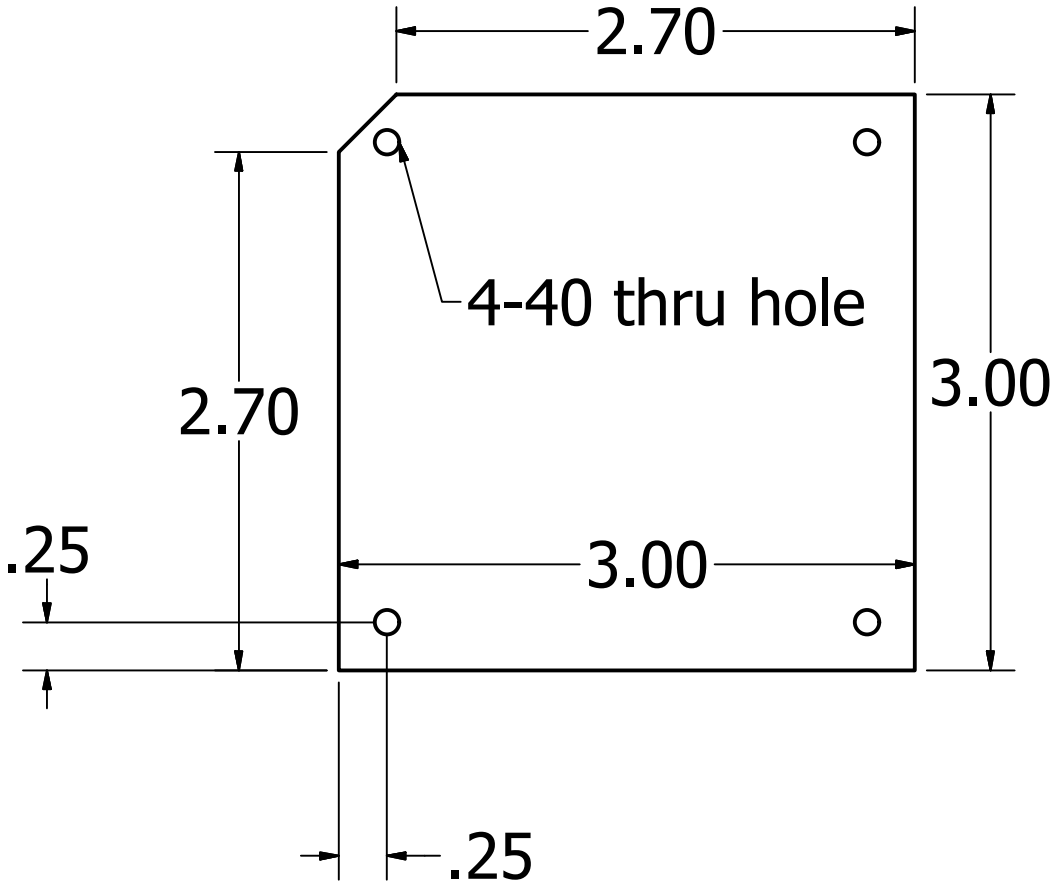


Nature Protocols: doi:10.1038/nprot.2015.040

Bottom Aluminum Layer

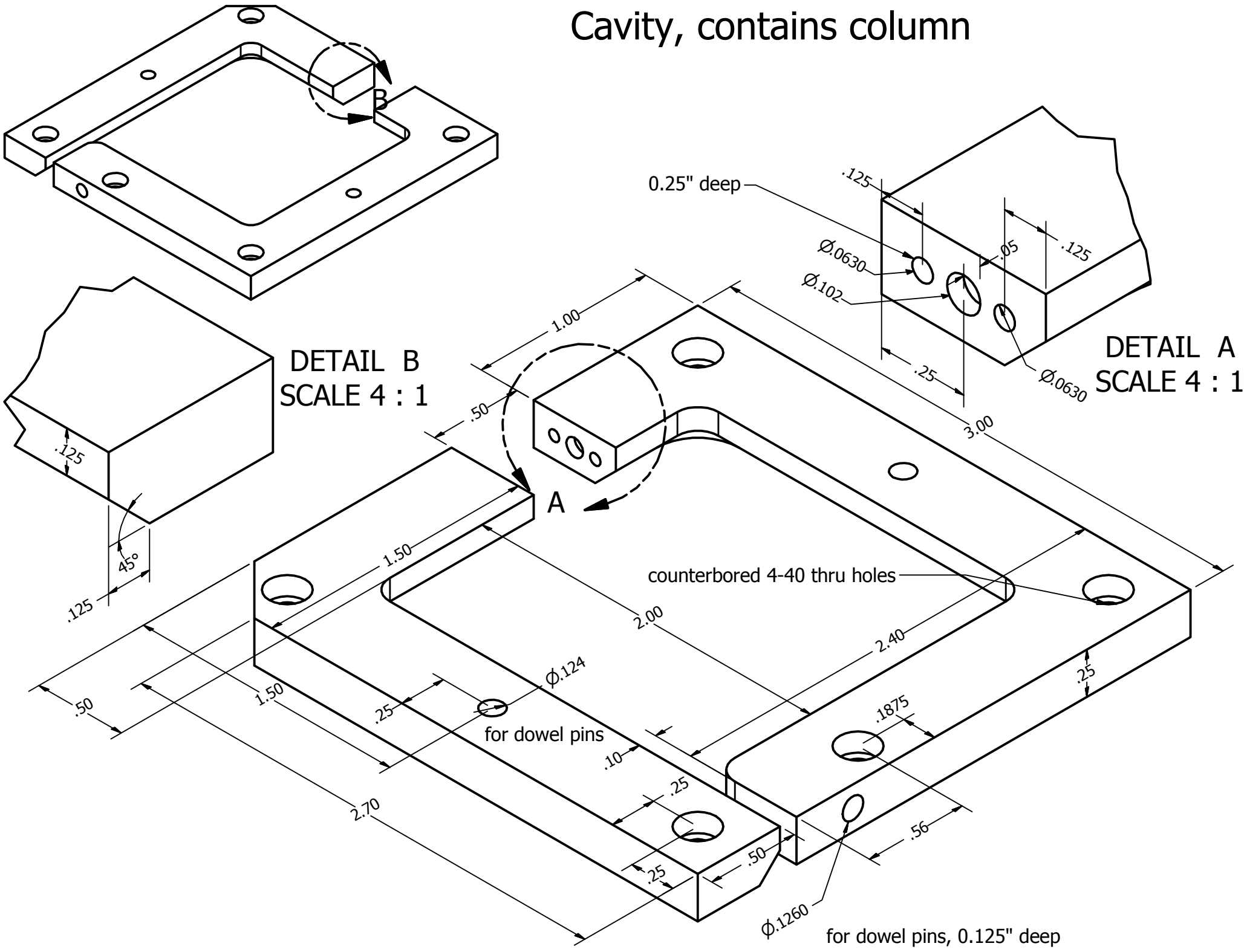






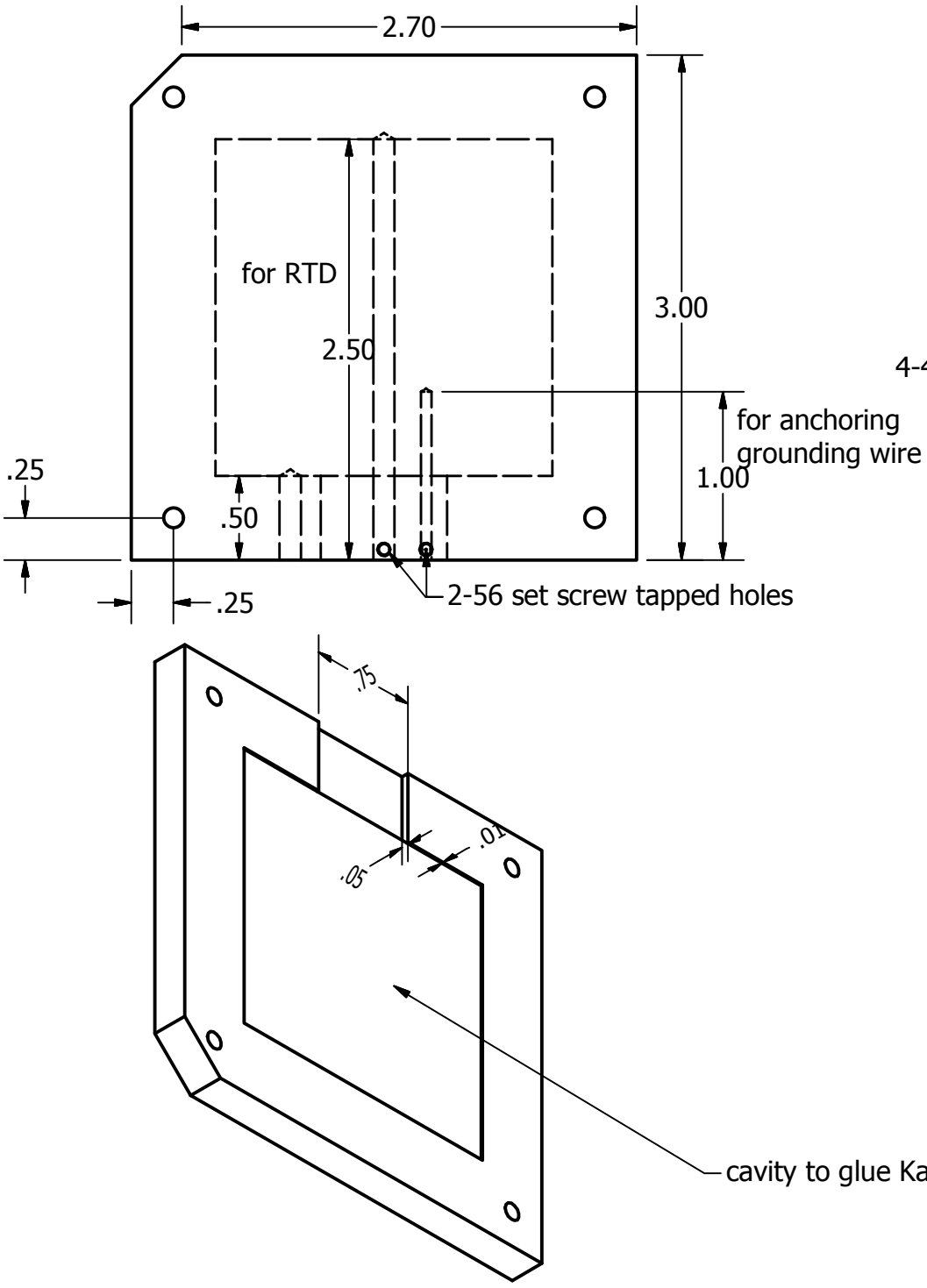
Nature Protocols: doi:10.1038/nprot.2015.040

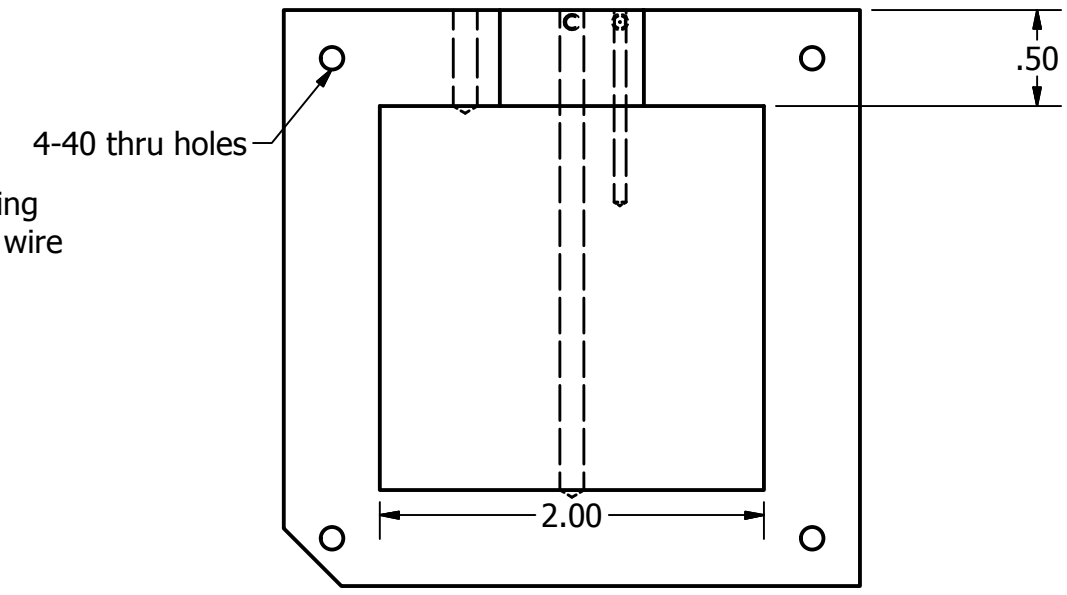
Cavity, contains column

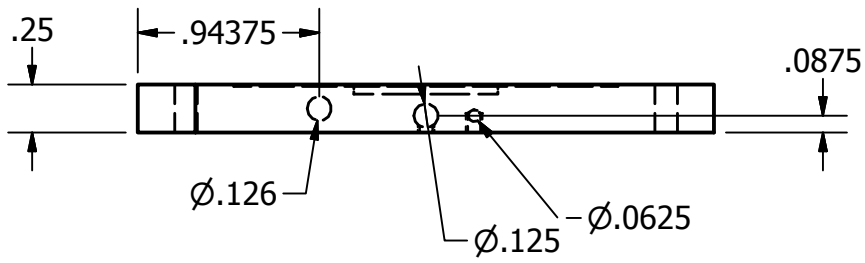


Nature Protocols: doi:10.1038/nprot.2015.040

Central Aluminum Plate with heater, RTD, grounding wire

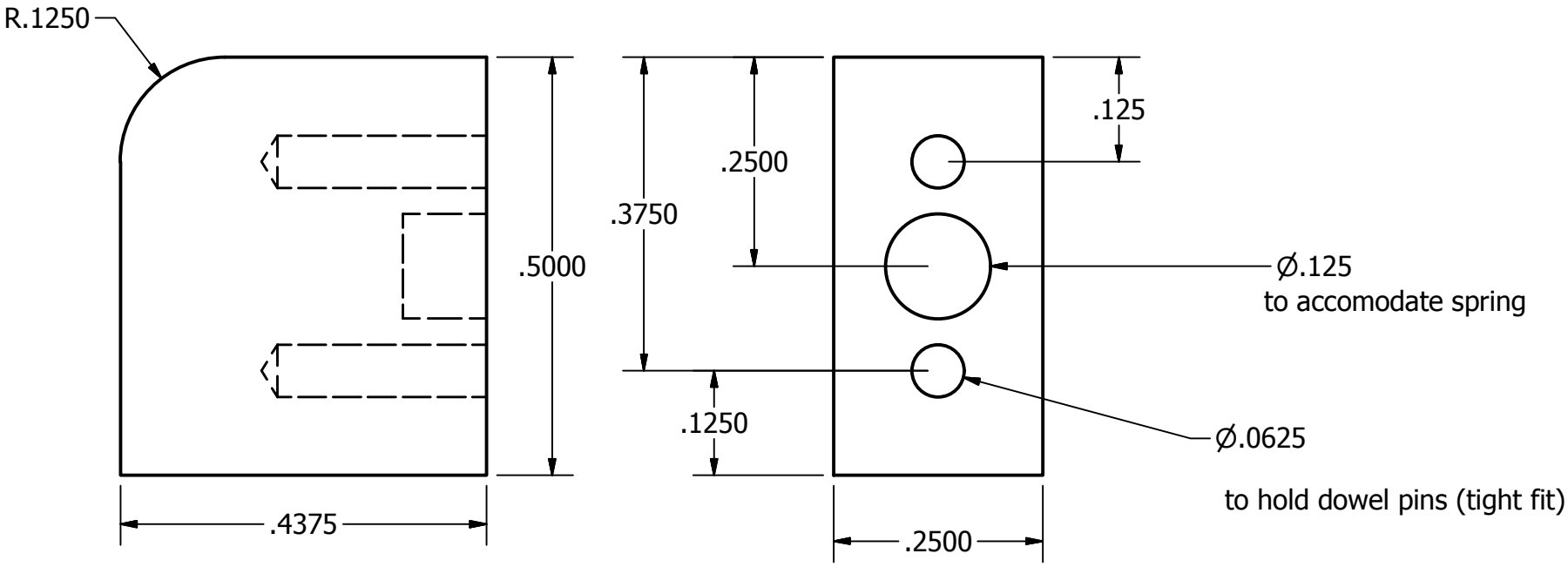


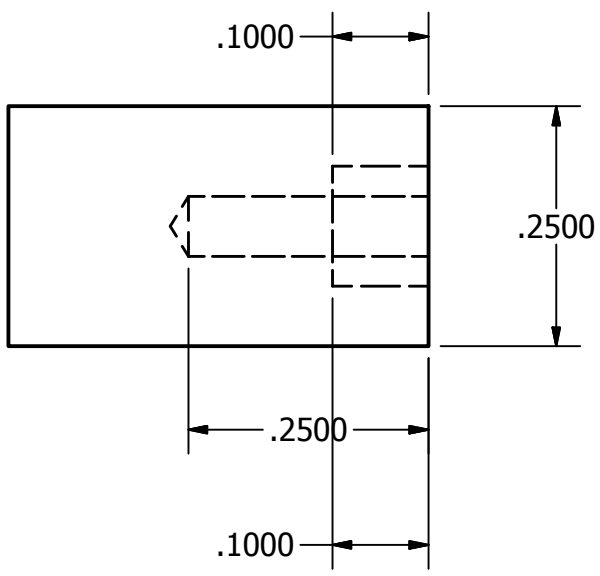


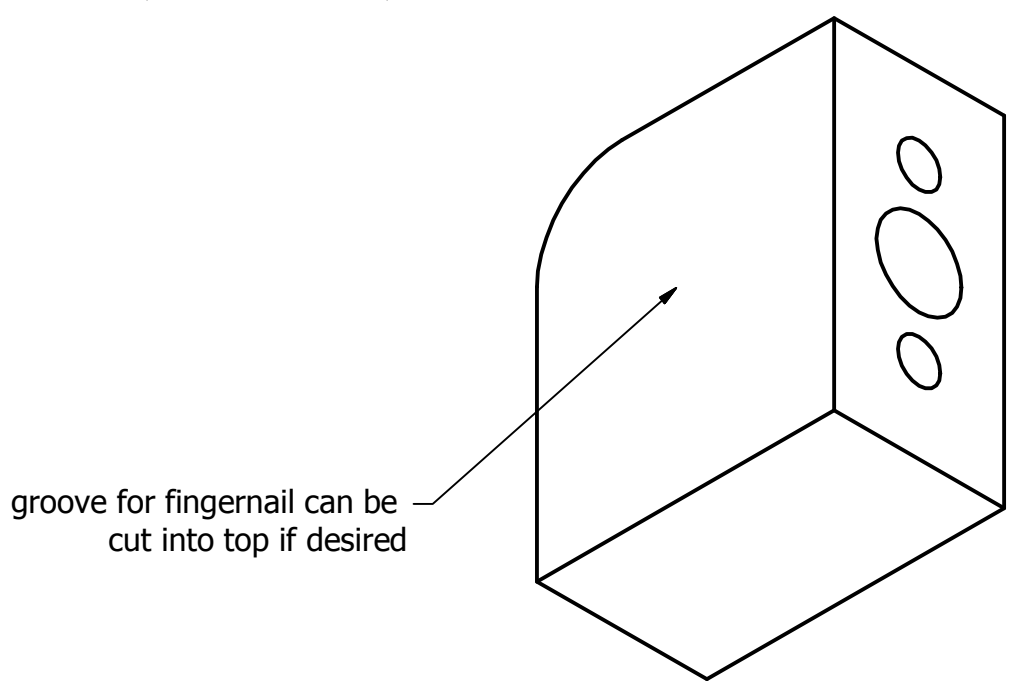


Nature Protocols: doi:10.1038/nprot.2015.040

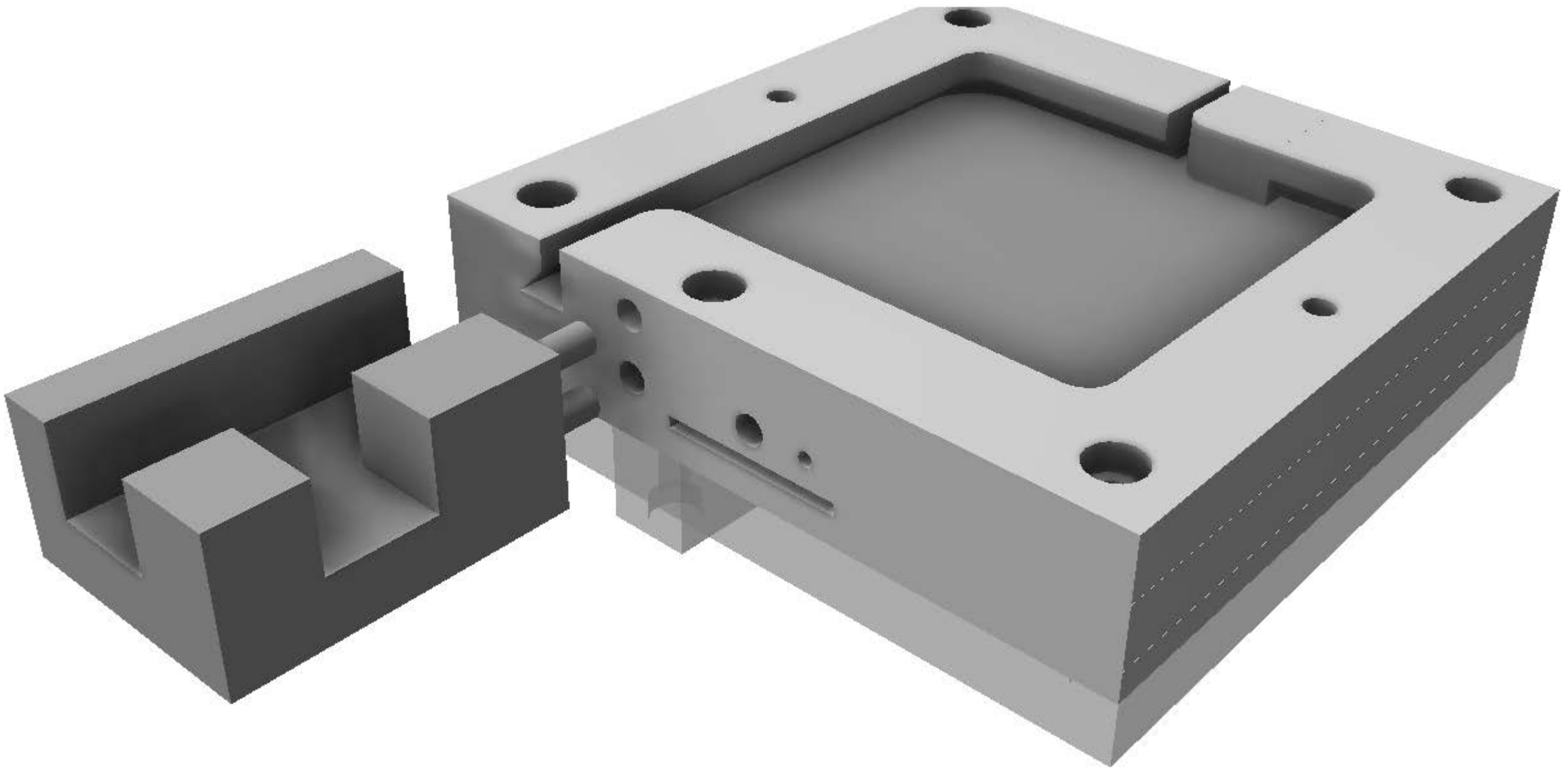
Column Retainer (spring loaded, with dowel pins)





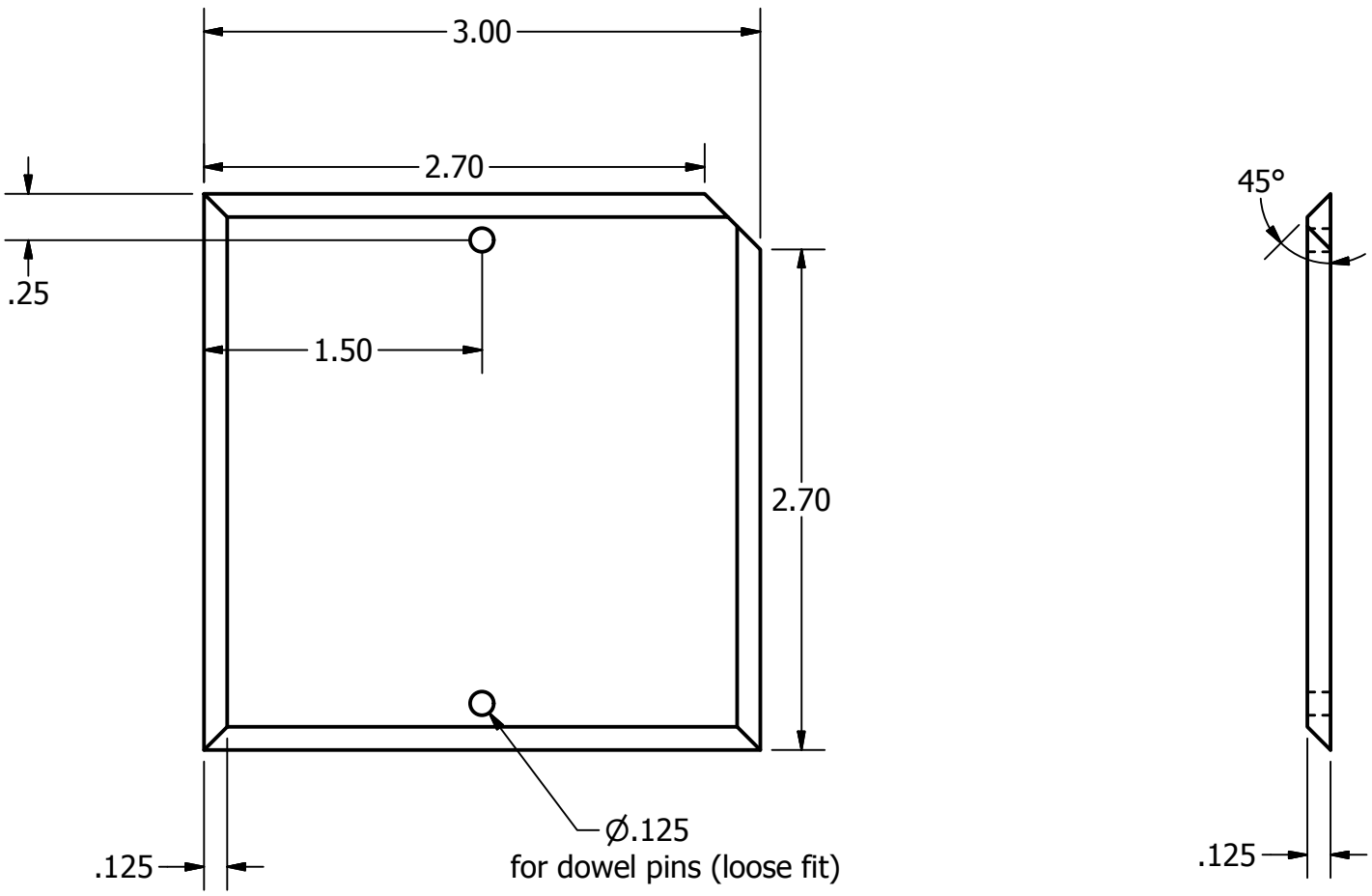


Nature Protocols: doi:10.1038/nprot.2015.040



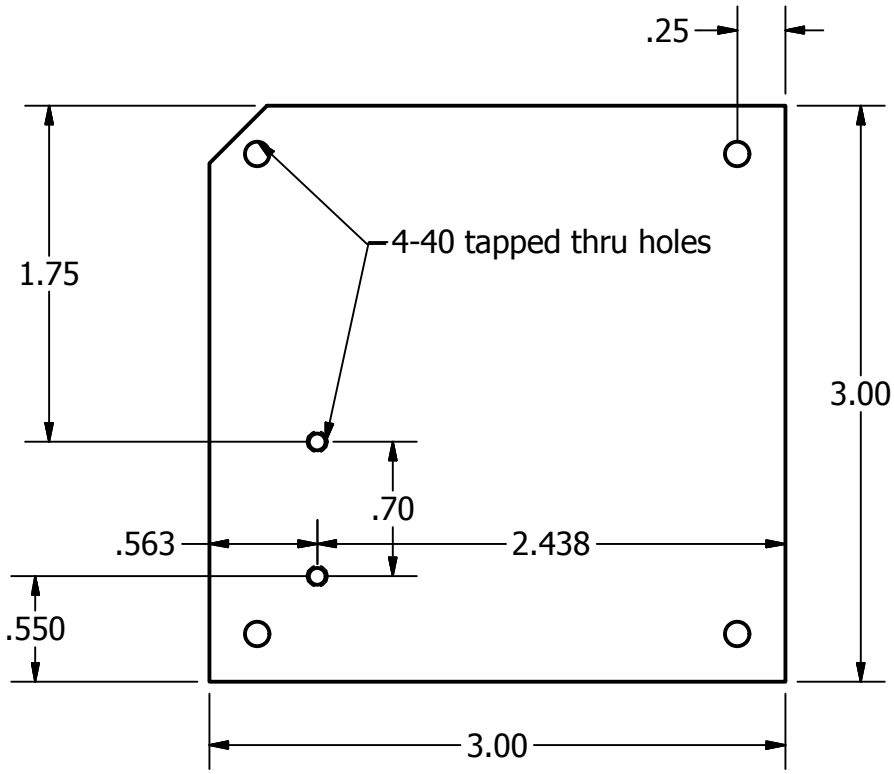
Nature Protocols: doi:10.1038/nprot.2015.040

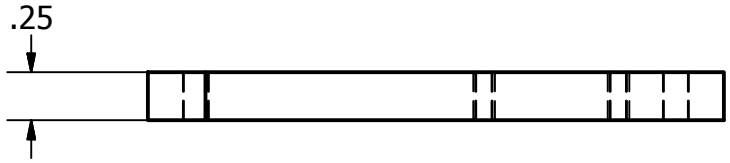
Heater Lid



Nature Protocols: doi:10.1038/nprot.2015.040

Polycarbonate Bottom Plate

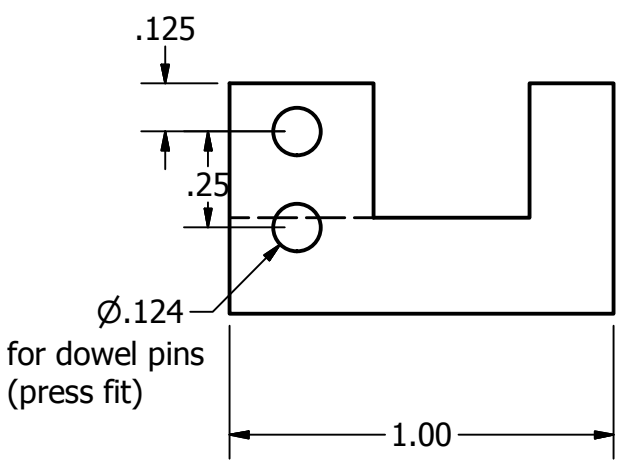


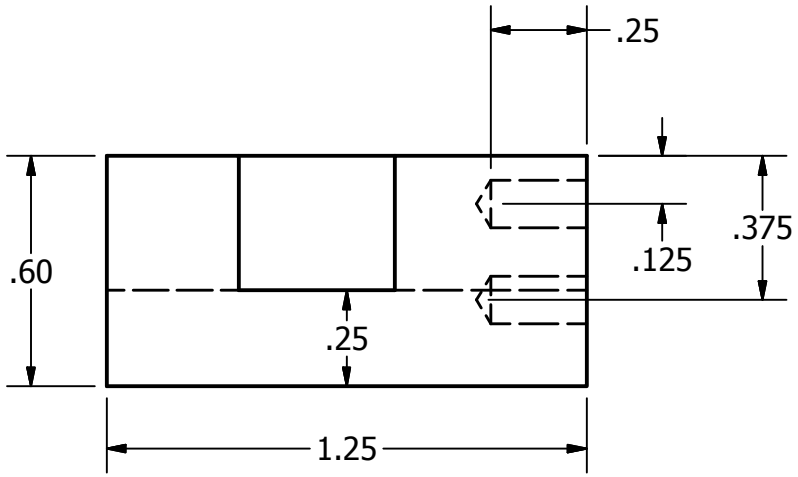


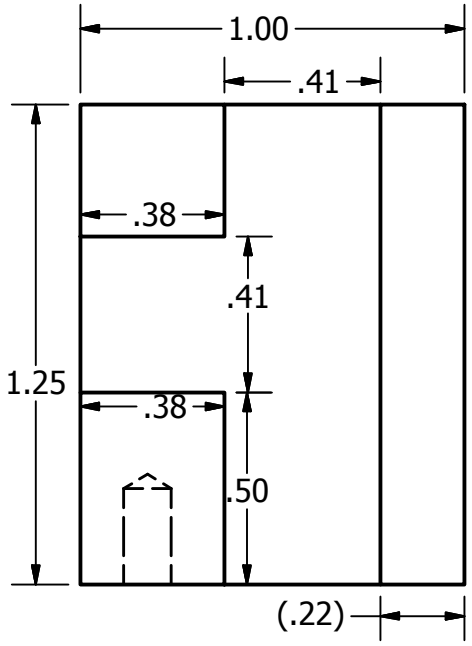
Nature Protocols: doi:10.1038/nprot.2015.040

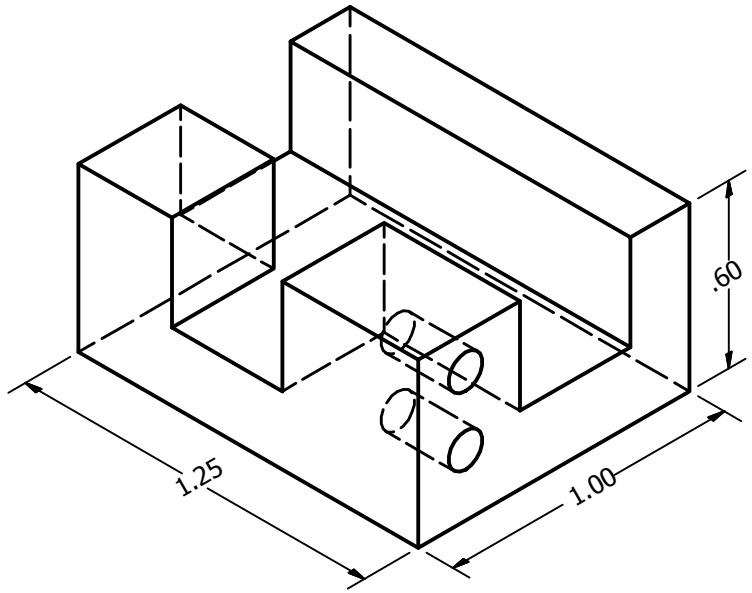
HPLC Union Bracket (PC)

for use with UH-750 microTee or UH-436 microUnion









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