

## SUPPLEMENTARY INFORMATION

**Table S1.** The initial body weight as well as the absolute dose and dose rate received by each mouse.

**Fig. S1.** The specifically designed holder for mouse irradiation.

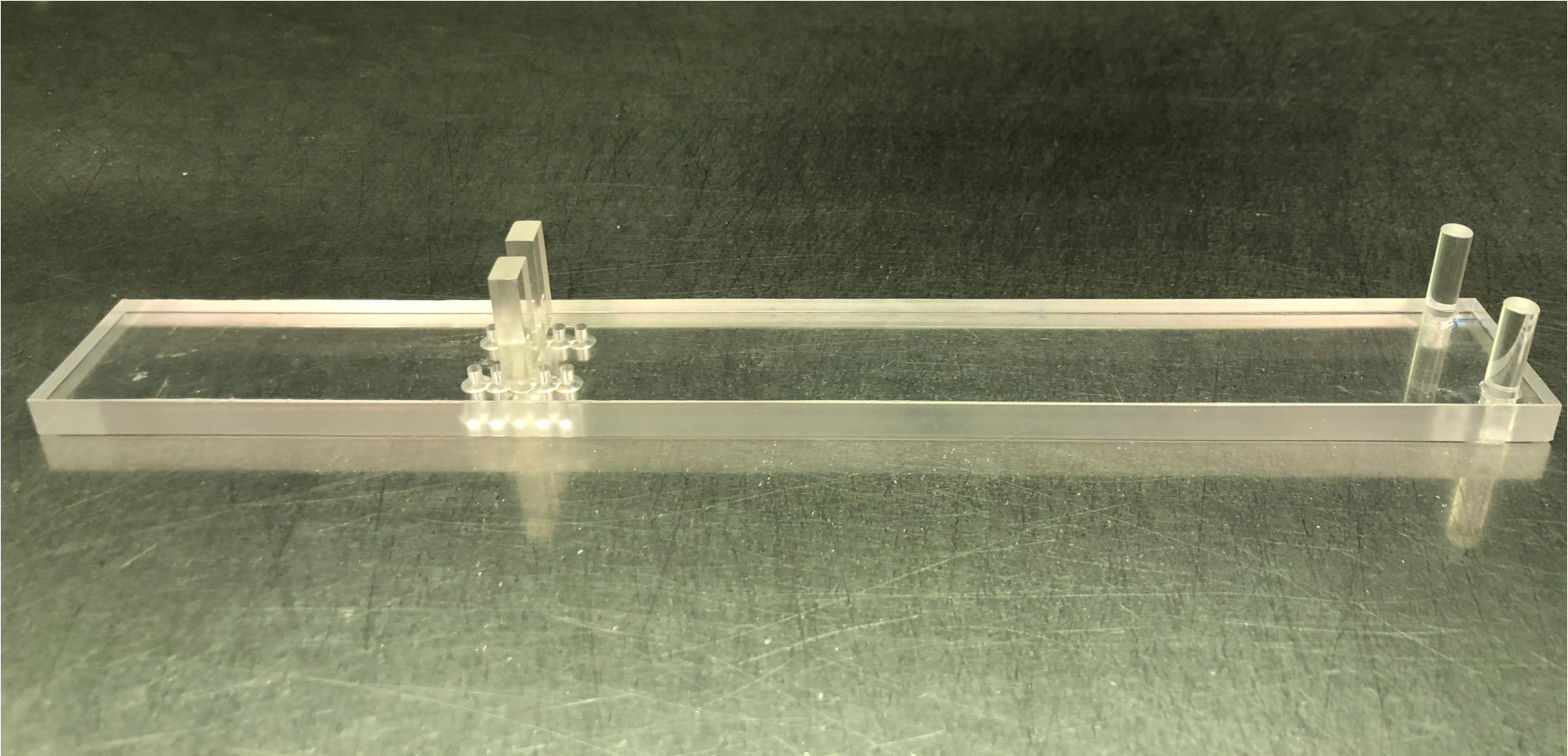
**Fig. S2.** The MC simulation result indicated ~16% dose difference between two mice.

**Fig. S3.** Tissue necrosis was observed after conventional dose rate proton irradiation.

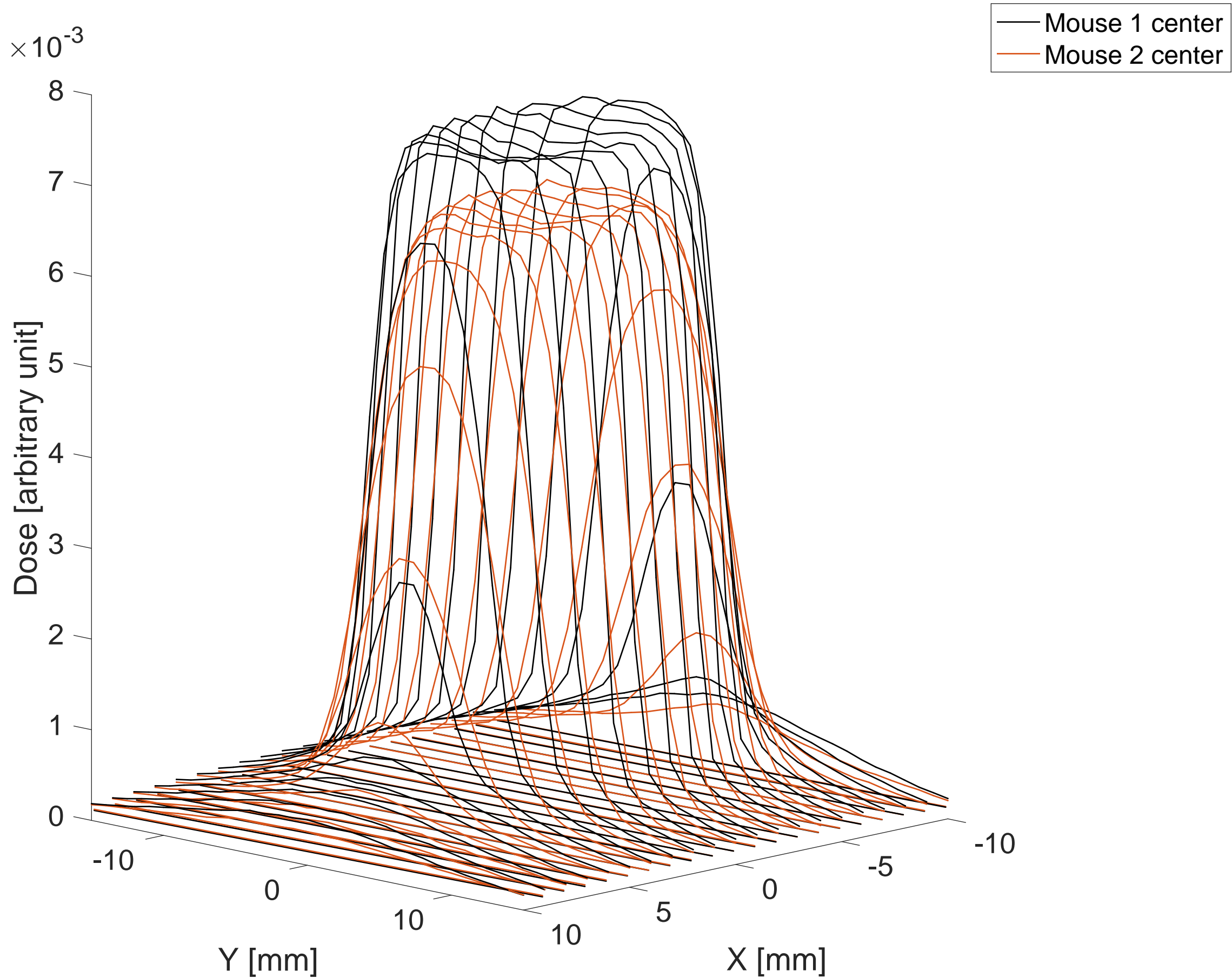
**Text S1.** The TOPAS parameter file that is used for the simulations.

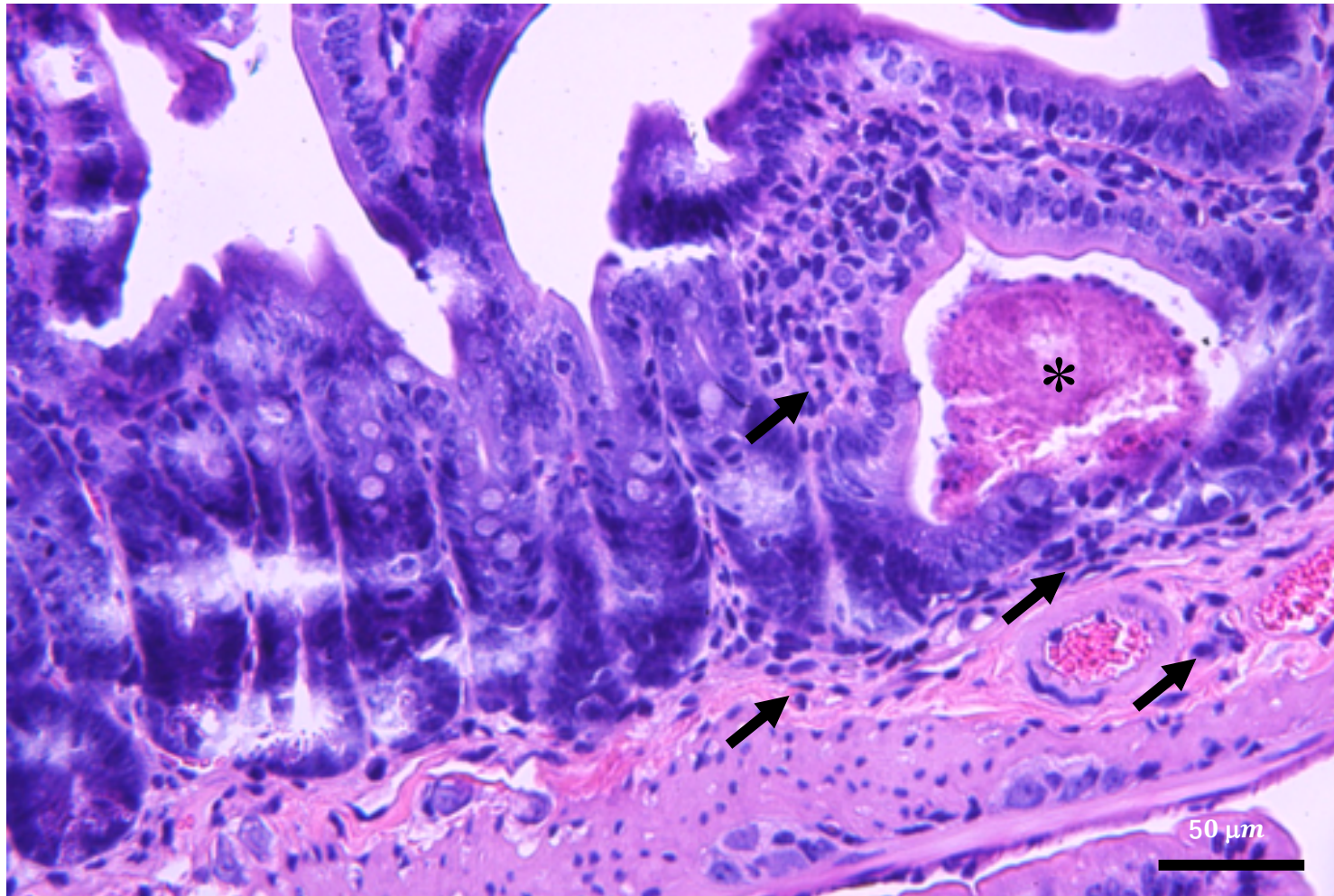
**Table S1**

Initial weight (g)	Dose (Gy)	Time (ms)	Dose rate (Gy/s)	Initial weight (g)	Dose (Gy)	Time (min)	Dose rate (Gy/s)
21	12.97	111.0	116.86	22	12.96	5.81	0.037
21	12.95	122.0	106.13	22	12.96	6.03	0.036
25	13.24	96.0	137.88	22	12.96	6.50	0.033
22	13.20	96.0	137.50	23	12.96	4.54	0.048
22	13.03	97.0	134.35	21	12.96	4.48	0.048
23	15.97	138.0	115.74	21	15.96	3.57	0.075
19	15.96	147.0	108.57	20	15.96	4.69	0.057
20	16.00	134.0	119.37	19	15.96	4.68	0.057
21	15.97	136.0	117.44	20	15.96	4.66	0.057
21	16.00	139.0	115.08	21	15.96	4.75	0.056
22	19.01	159.0	119.55	21	18.96	4.36	0.072
21	19.02	152.0	125.13	24	18.96	4.29	0.074
20	19.02	155.0	122.71	21	18.96	5.81	0.054
18	19.02	152.0	125.13	21	18.96	5.28	0.060
22	19.03	148.0	128.59	18	18.96	5.68	0.056
22	19.02	150.0	126.80	17	18.96	6.10	0.052
20	22.02	181.0	121.66	21	21.96	4.95	0.074
23	22.02	178.0	123.71	20	21.96	5.54	0.066
21	22.02	177.0	124.41	26	21.96	5.56	0.066
21	22.03	172.0	128.09	20	21.96	5.36	0.068
20	22.03	180.0	122.40	21	21.96	5.76	0.064



# Dose profiles at center of mice





\* Focal necrosis; Arrows: Chronic inflammatory cells infiltration

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# Simulation of beam spread for two mice in a row
Ts/G4DataDirectory = "/Applications/geant4/G4Data"
Ts/UseQt = "False"
i:Ts/MaxInterruptedHistories = 1000
i:Ts/NumberOfThreads = 4
Ts/ShowHistoryCountAtInterval = 10000

#Source
s:So/ExpRoom/Type = "Emittance"
s:So/ExpRoom/Component = "SourceStart"
s:So/ExpRoom/BeamParticle = "proton"
d:So/ExpRoom/BeamEnergy = 228.5 MeV
s:So/ExpRoom/Distribution = "BiGaussian" # distribution name
d:So/ExpRoom/SigmaX = 5.0 mm # std of x positions
u:So/ExpRoom/SigmaXprime = 0.00433 # std of x', note that it's unitless. 1 equals to 1.0 rad.
u:So/ExpRoom/CorrelationX = 0.92101 # correlation of x and x'
d:So/ExpRoom/SigmaY = 7.5 mm # std of y positions
u:So/ExpRoom/SigmaYPrime = 0.00433 # std of y'
u:So/ExpRoom/CorrelationY = 0.777955 # correlation of y and y'
u:So/ExpRoom/BeamEnergySpread = 0
i:So/ExpRoom/NumberOfHistoriesInRun = 100000000

#Geometries
Ge/World/HLX = 1.5 m
Ge/World/HLY = 1.5 m
Ge/World/HLZ = 1.5 m

s:Ge/SourceStart/Type = "Group"
s:Ge/SourceStart/Parent = "World"
d:Ge/SourceStart/TransZ = -97. cm

#Scatterer 2:
s:Ge/Scatterer1/Type = "TsCylinder"
s:Ge/Scatterer1/Parent = "World"
s:Ge/Scatterer1/Material = "Lead"
d:Ge/Scatterer1/RMin = 0.0 cm
d:Ge/Scatterer1/RMax = 5.0 cm
d:Ge/Scatterer1/HL = 0.02032 cm
d:Ge/Scatterer1/TransZ = -96.49968 cm

#Scatterer 2:
s:Ge/Scatterer2/Type = "TsCylinder"
s:Ge/Scatterer2/Parent = "World"
s:Ge/Scatterer2/Material = "Mylar"
d:Ge/Scatterer2/RMin = 0.0 cm
d:Ge/Scatterer2/RMax = 5.0 cm
d:Ge/Scatterer2/HL = 0.01 cm
d:Ge/Scatterer2/TransZ = -60.97 cm

s:Ge/LeadScatterer2/Type = "TsCylinder"

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s:Ge/LeadScatterer2/Parent = "World"  
s:Ge/LeadScatterer2/Material = "Lead"  
d:Ge/LeadScatterer2/RMin = 0.0 cm  
d:Ge/LeadScatterer2/RMax = 0.165 cm  
d:Ge/LeadScatterer2/HL = 0.04064 cm  
d:Ge/LeadScatterer2/TransZ = -60.91936 cm

#### #Aperture

s:Ge/Aperture/Type = "TsCylinder"  
s:Ge/Aperture/Parent = "World"  
s:Ge/Aperture/Material = "Lead"  
d:Ge/Aperture/RMin = 0.0 cm  
d:Ge/Aperture/RMax = 5.0 cm  
d:Ge/Aperture/HL = 3.81 cm  
d:Ge/Aperture/TransZ = -17.24 cm

s:Ge/ApertureHole/Type = "G4EllipticalTube"  
s:Ge/ApertureHole/Parent = "Aperture"  
s:Ge/ApertureHole/Material = "Air"  
d:Ge/ApertureHole/HLX = 0.6 cm  
d:Ge/ApertureHole/HLY = 0.8 cm  
d:Ge/ApertureHole/HLZ = 3.81 cm  
d:Ge/ApertureHole/TransX = 0.0 cm  
d:Ge/ApertureHole/TransZ = 0.0 cm

#### #Scoring Plastic Block

s:Ge/Plastic/Type = "TsBox"  
s:Ge/Plastic/Parent = "World"  
s:Ge/Plastic/Material = "Lexan"  
d:Ge/Plastic/HLX = 2. cm  
d:Ge/Plastic/HLY = 2. cm  
d:Ge/Plastic/HLZ = 2. cm  
d:Ge/Plastic/TransZ = -11.43 cm  
i:Ge/Plastic/XBins = 40  
i:Ge/Plastic/YBins = 40  
i:Ge/Plastic/ZBins = 8

#### #Mouse position

d:Ge/Mouse/TransX = +0.1 cm  
d:Ge/Mouse1/TransZ = -7.43 cm  
d:Ge/Mouse2/TransZ = -3.43 cm

#### #Scoring Mouse 1

s:Ge/FluencePlane1/Type = "TsBox"  
s:Ge/FluencePlane1/Parent = "World"  
s:Ge/FluencePlane1/Material = "G4\_WATER"  
d:Ge/FluencePlane1/HLX = 2. cm  
d:Ge/FluencePlane1/HLY = 2. cm  
d:Ge/FluencePlane1/HLZ = 1.25 cm  
d:Ge/FluencePlane1/TransX = Ge/Mouse/TransX cm

d:Ge/FluencePlane1/TransZ = Ge/Mouse1/TransZ cm

s:Sc/Dose1/Quantity = "DoseToMedium"  
s:Sc/Dose1/Component = "FluencePlane1"  
s:Sc/Dose1/IfOutputFileAlreadyExists = "Overwrite"  
s:Sc/Dose1/OutputFile = "GMC177\_4cmBlock\_2.5cmMouse1"

s:Sc/Dose1\_binned/Quantity = "DoseToMedium"  
s:Sc/Dose1\_binned/Component = "FluencePlane1"  
s:Sc/Dose1\_binned/IfOutputFileAlreadyExists = "Overwrite"  
s:Sc/Dose1\_binned/OutputFile = "GMC177\_4cmBlock\_2.5cmMouse1\_binned"  
i:Sc/Dose1\_binned/XBins = 40  
i:Sc/Dose1\_binned/YBins = 40  
i:Sc/Dose1\_binned/ZBins = 3

#### #Scoring Mouse 2

s:Ge/FluencePlane2/Type = "TsBox"  
s:Ge/FluencePlane2/Parent = "World"  
s:Ge/FluencePlane2/Material = "G4\_WATER"  
d:Ge/FluencePlane2/HLX = 2. cm  
d:Ge/FluencePlane2/HLY = 2. cm  
d:Ge/FluencePlane2/HLZ = 1.25 cm  
d:Ge/FluencePlane2/TransX = Ge/Mouse/TransX cm  
d:Ge/FluencePlane2/TransZ = Ge/Mouse2/TransZ cm

s:Sc/Dose2/Quantity = "DoseToMedium"  
s:Sc/Dose2/Component = "FluencePlane2"  
s:Sc/Dose2/IfOutputFileAlreadyExists = "Overwrite"  
s:Sc/Dose2/OutputFile = "GMC177\_4cmBlock\_2.5cmMouse2"

s:Sc/Dose2\_binned/Quantity = "DoseToMedium"  
s:Sc/Dose2\_binned/Component = "FluencePlane2"  
s:Sc/Dose2\_binned/IfOutputFileAlreadyExists = "Overwrite"  
s:Sc/Dose2\_binned/OutputFile = "GMC177\_4cmBlock\_2.5cmMouse2\_binned"  
i:Sc/Dose2\_binned/XBins = 40  
i:Sc/Dose2\_binned/YBins = 40  
i:Sc/Dose2\_binned/ZBins = 3

#### # Dose along the beam line

s:Ge/BeamScorer/Type = "TsBox"  
s:Ge/BeamScorer/Parent = "World"  
d:Ge/BeamScorer/HLX = 0.5 cm  
d:Ge/BeamScorer/HLY = 0.5 cm  
d:Ge/BeamScorer/HLZ = 10. cm  
d:Ge/BeamScorer/TransZ = Ge/Plastic/TransZ + 8. cm  
b:Ge/BeamScorer/IsParallel = "T"  
i:Ge/BeamScorer/ZBins = 80

s:Sc/CenterDose/Quantity = "DoseToMedium"  
s:Sc/CenterDose/Component = "BeamScorer"  
s:Sc/CenterDose/IfOutputFileAlreadyExists = "Overwrite"



s:Sc/CenterDose/OutputFile = "GMC177\_4cmBlock\_2.5cmMouse2\_CenterDose"

s:Sc/CenterDoseW/Quantity = "DoseToWater"

s:Sc/CenterDoseW/Component = "BeamScorer"

s:Sc/CenterDoseW/IfOutputFileAlreadyExists = "Overwrite"

s:Sc/CenterDoseW/OutputFile = "GMC177\_4cmBlock\_2.5cmMouse2\_CenterDoseW"

s:Sc/CenterEdep/Quantity = "EnergyDeposit"

s:Sc/CenterEdep/Component = "BeamScorer"

s:Sc/CenterEdep/IfOutputFileAlreadyExists = "Overwrite"

s:Sc/CenterEdep/OutputFile = "GMC177\_4cmBlock\_2.5cmMouse2\_CenterEdep"