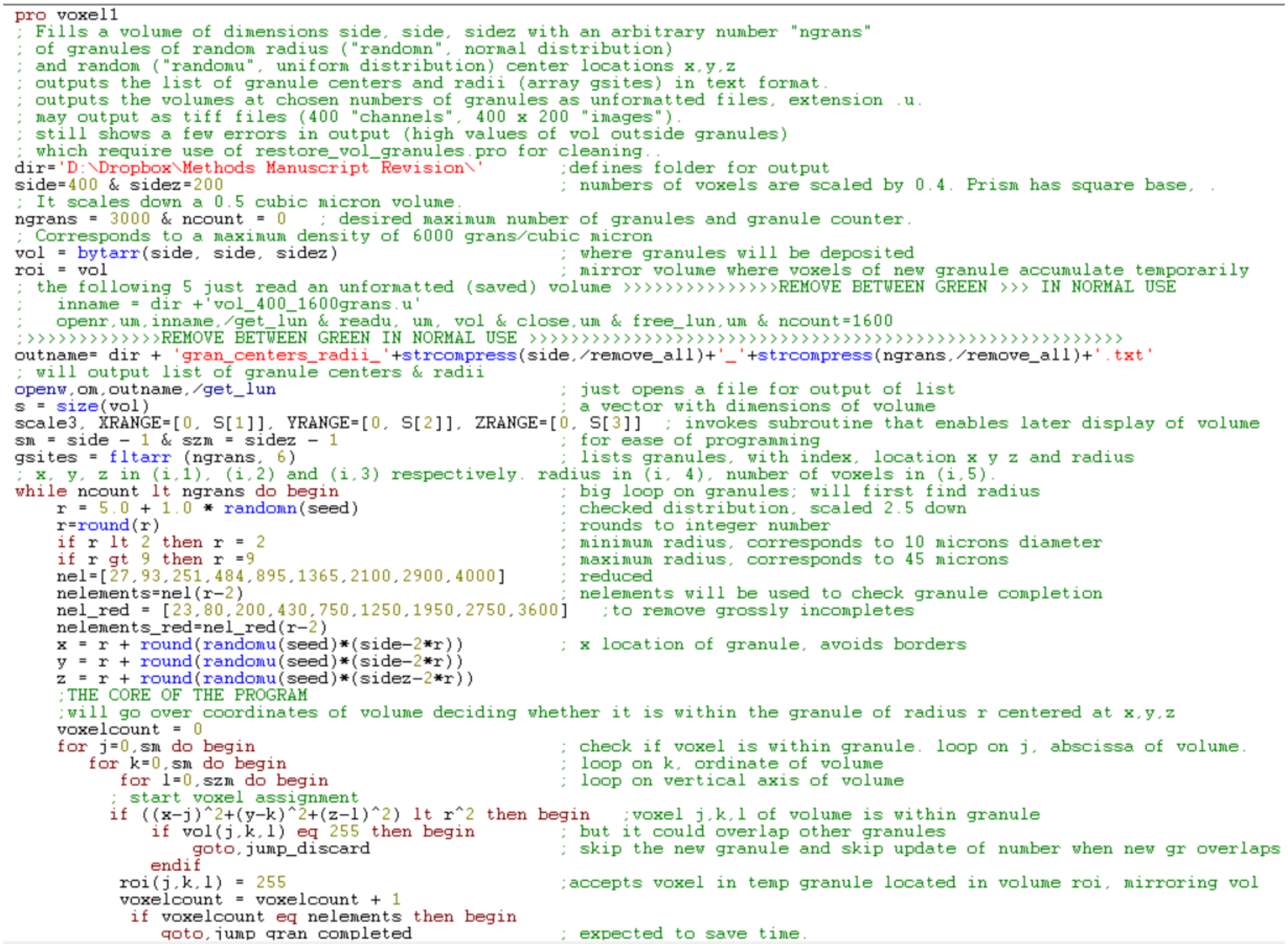
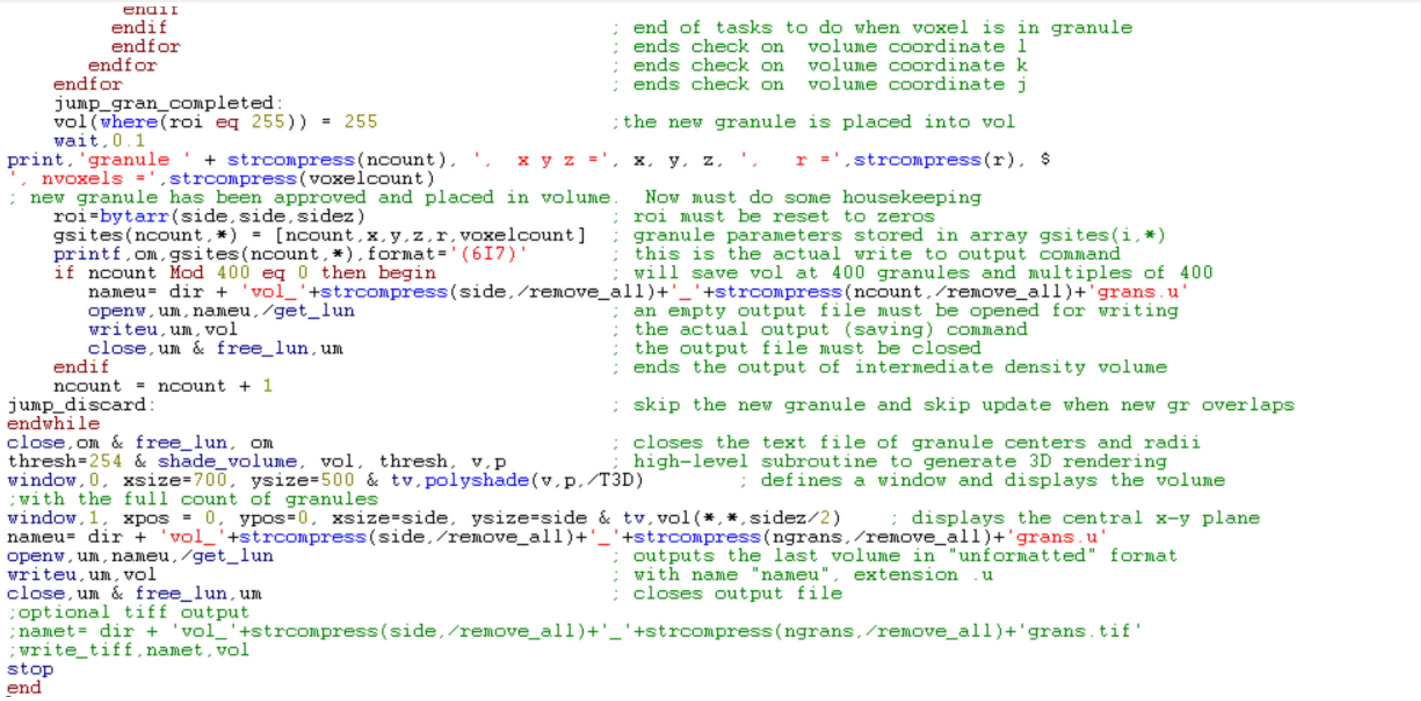
Supplement 3. Simulator of granules in a volume and detector and measurer of granules in label masks.

Programs written in IDL language (Harris Geospatiale, Paris, France).

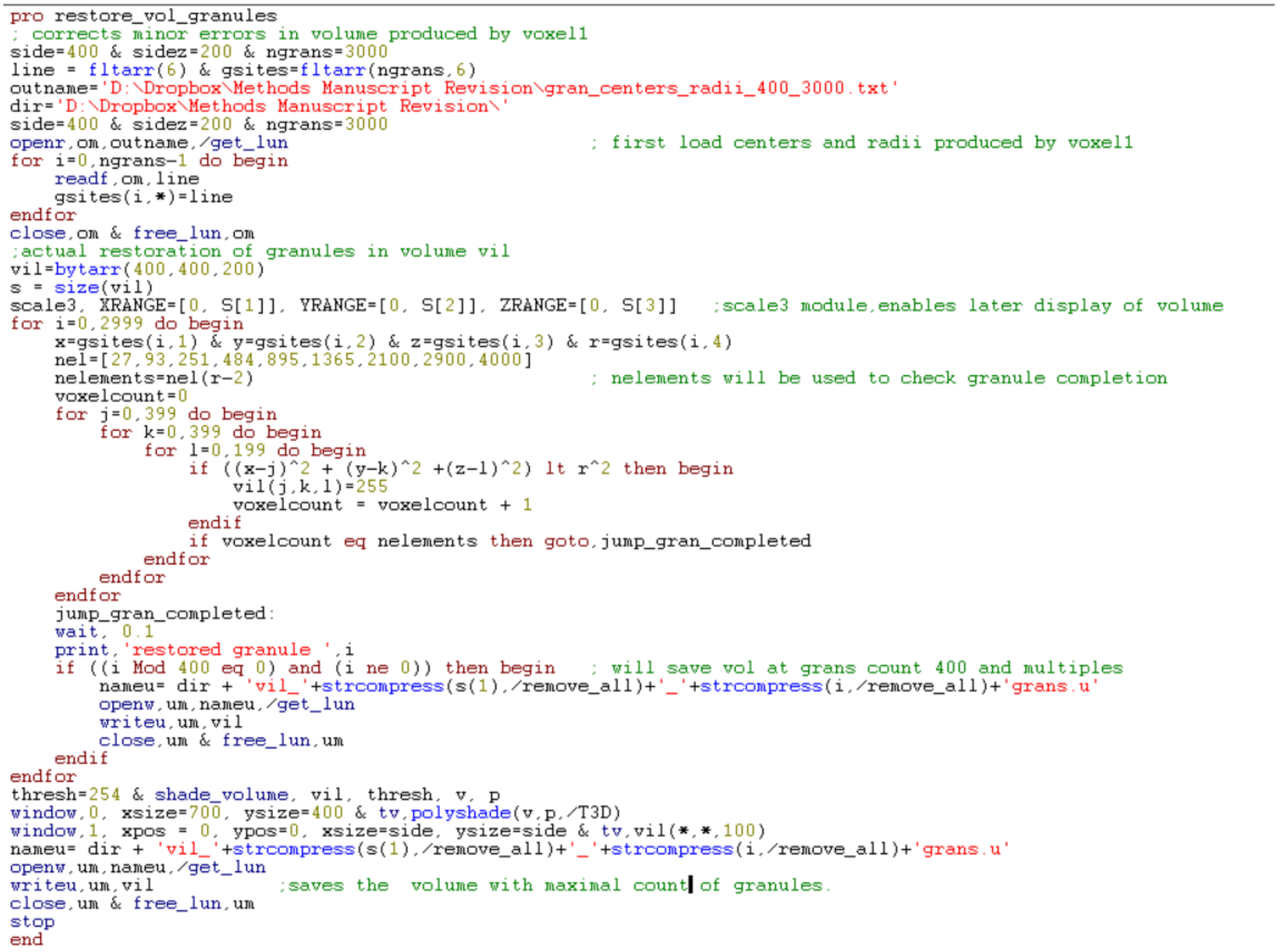


```
pro voxel1
; Fills a volume of dimensions side, side, sidez with an arbitrary number "ngrans"
; of granules of random radius ("randomn", normal distribution)
; and random ("randomu", uniform distribution) center locations x,y,z
; outputs the list of granule centers and radii (array gsites) in text format.
; outputs the volumes at chosen numbers of granules as unformatted files, extension .u.
; may output as tiff files (400 "channels", 400 x 200 "images").
; still shows a few errors in output (high values of vol outside granules)
; which require use of restore_vol_granules.pro for cleaning..
dir='D:\Dropbox\Methods Manuscript Revision\'       ;defines folder for output
side=400 & sidez=200                                ; numbers of voxels are scaled by 0.4. Prism has square base, .
; It scales down a 0.5 cubic micron volume.
ngrans = 3000 & ncount = 0   ; desired maximum number of granules and granule counter.
; Corresponds to a maximum density of 6000 grans/cubic micron
vol = bytarr(side, side, sidez)                     ; where granules will be deposited
roi = vol                                           ; mirror volume where voxels of new granule accumulate temporarily
; the following 5 just read an unformatted (saved) volume >>>>>>>>>>>>>>>REMOVE BETWEEN GREEN >>> IN NORMAL USE
;   inname = dir +'vol_400_1600grans.u'
;   openr,um,inname,/get_lun & readu, um, vol & close,um & free_lun,um & ncount=1600
;>>>>>>>>>>>>>REMOVE BETWEEN GREEN IN NORMAL USE >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
outname= dir + 'gran_centers_radii_'+strcompress(side,/remove_all)+'_'+strcompress(ngrans,/remove_all)+'.txt'
; will output list of granule centers & radii
openw,om,outname,/get_lun                           ; just opens a file for output of list
s = size(vol)                                       ; a vector with dimensions of volume
scale3, XRANGE=[0, S[1]], YRANGE=[0, S[2]], ZRANGE=[0, S[3]]  ; invokes subroutine that enables later display of volume
sm = side - 1 & szm = sidez - 1                     ; for ease of programming
gsites = fltarr (ngrans, 6)                         ; lists granules, with index, location x y z and radius
; x, y, z in (i,1), (i,2) and (i,3) respectively. radius in (i, 4), number of voxels in (i,5).
while ncount lt ngrans do begin                     ; big loop on granules; will first find radius
    r = 5.0 + 1.0 * randomn(seed)                   ; checked distribution, scaled 2.5 down
    r=round(r)                                      ; rounds to integer number
    if r lt 2 then r = 2                            ; minimum radius, corresponds to 10 microns diameter
    if r gt 9 then r =9                             ; maximum radius, corresponds to 45 microns
    nel=[27,93,251,484,895,1365,2100,2900,4000]     ; reduced
    nelements=nel(r-2)                              ; nelements will be used to check granule completion
    nel_red = [23,80,200,430,750,1250,1950,2750,3600]   ;to remove grossly incompletes
    nelements_red=nel_red(r-2)
    x = r + round(randomu(seed)*(side-2*r))         ; x location of granule, avoids borders
    y = r + round(randomu(seed)*(side-2*r))
    z = r + round(randomu(seed)*(sidez-2*r))
    ;THE CORE OF THE PROGRAM
    ;will go over coordinates of volume deciding whether it is within the granule of radius r centered at x,y,z
    voxelcount = 0
    for j=0,sm do begin                             ; check if voxel is within granule. loop on j, abscissa of volume.
       for k=0,sm do begin                          ; loop on k, ordinate of volume
          for l=0,szm do begin                      ; loop on vertical axis of volume
         ; start voxel assignment
         if ((x-j)^2+(y-k)^2+(z-l)^2) lt r^2 then begin   ;voxel j,k,l of volume is within granule
             if vol(j,k,l) eq 255 then begin        ; but it could overlap other granules
                 goto,jump_discard                  ; skip the new granule and skip update of number when new gr overlaps
             endif
          roi(j,k,l) = 255                          ;accepts voxel in temp granule located in volume roi, mirroring vol
          voxelcount = voxelcount + 1
           if voxelcount eq nelements then begin
              goto,jump_gran_completed              ; expected to save time.
```



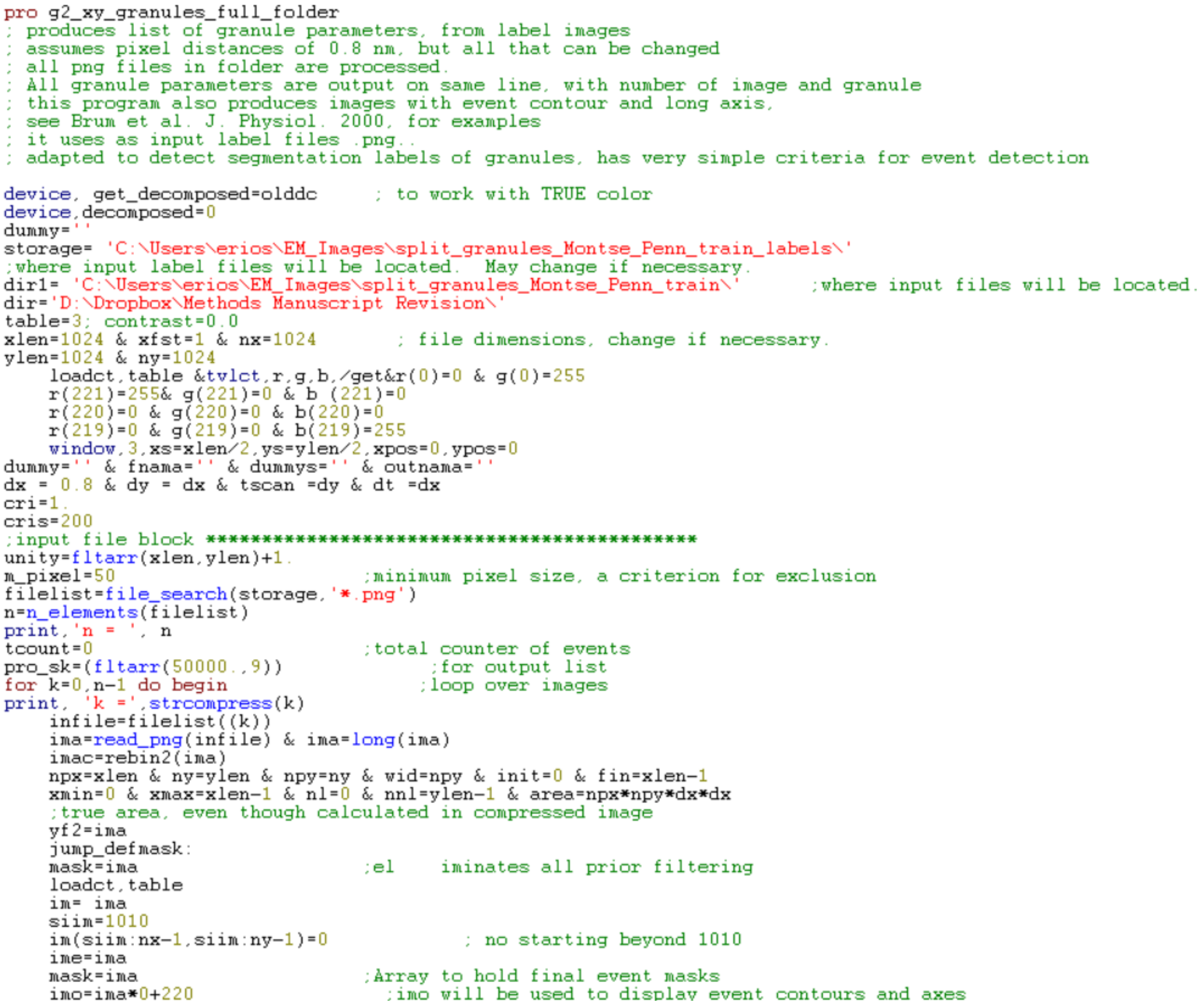
```
          endif
         endif                                      ; end of tasks to do when voxel is in granule
         endfor                                     ; ends check on  volume coordinate l
       endfor                                       ; ends check on  volume coordinate k
    endfor                                          ; ends check on  volume coordinate j
    jump_gran_completed:
    vol(where(roi eq 255)) = 255                    ;the new granule is placed into vol
    wait,0.1
print,'granule ' + strcompress(ncount), ',  x y z =', x, y, z, ',   r =',strcompress(r), $
', nvoxels =',strcompress(voxelcount)
; new granule has been approved and placed in volume.  Now must do some housekeeping
    roi=bytarr(side,side,sidez)                     ; roi must be reset to zeros
    gsites(ncount,*) = [ncount,x,y,z,r,voxelcount]  ; granule parameters stored in array gsites(i,*)
    printf,om,gsites(ncount,*),format='(6I7)'       ; this is the actual write to output command
    if ncount Mod 400 eq 0 then begin               ; will save vol at 400 granules and multiples of 400
       nameu= dir + 'vol_'+strcompress(side,/remove_all)+'_'+strcompress(ncount,/remove_all)+'grans.u'
       openw,um,nameu,/get_lun                      ; an empty output file must be opened for writing
       writeu,um,vol                                ; the actual output (saving) command
       close,um & free_lun,um                       ; the output file must be closed
    endif                                           ; ends the output of intermediate density volume
    ncount = ncount + 1
jump_discard:                                       ; skip the new granule and skip update when new gr overlaps
endwhile
close,om & free_lun, om                             ; closes the text file of granule centers and radii
thresh=254 & shade_volume, vol, thresh, v,p         ; high-level subroutine to generate 3D rendering
window,0, xsize=700, ysize=500 & tv,polyshade(v,p,/T3D)       ; defines a window and displays the volume
;with the full count of granules
window,1, xpos = 0, ypos=0, xsize=side, ysize=side & tv,vol(*,*,sidez/2)     ; displays the central x-y plane
nameu= dir + 'vol_'+strcompress(side,/remove_all)+'_'+strcompress(ngrans,/remove_all)+'grans.u'
openw,um,nameu,/get_lun                             ; outputs the last volume in "unformatted" format
writeu,um,vol                                       ; with name "nameu", extension .u
close,um & free_lun,um                              ; closes output file
;optional tiff output
;namet= dir + 'vol_'+strcompress(side,/remove_all)+'_'+strcompress(ngrans,/remove_all)+'grans.tif'
;write_tiff,namet,vol
stop
end
```

A module that corrects minor errors in the volumes generated by voxel1.pro.



```
pro restore_vol_granules
; corrects minor errors in volume produced by voxel1
side=400 & sidez=200 & ngrans=3000
line = fltarr(6) & gsites=fltarr(ngrans,6)
outname='D:\Dropbox\Methods Manuscript Revision\gran_centers_radii_400_3000.txt'
dir='D:\Dropbox\Methods Manuscript Revision\'
side=400 & sidez=200 & ngrans=3000
openr,om,outname,/get_lun                                       ; first load centers and radii produced by voxel1
for i=0,ngrans-1 do begin
     readf,om,line
     gsites(i,*)=line
endfor
close,om & free_lun,om
;actual restoration of granules in volume vil
vil=bytarr(400,400,200)
s = size(vil)
scale3, XRANGE=[0, S[1]], YRANGE=[0, S[2]], ZRANGE=[0, S[3]]   ;scale3 module,enables later display of volume
for i=0,2999 do begin
     x=gsites(i,1) & y=gsites(i,2) & z=gsites(i,3) & r=gsites(i,4)
     nel=[27,93,251,484,895,1365,2100,2900,4000]
     nelements=nel(r-2)                                         ; nelements will be used to check granule completion
     voxelcount=0
     for j=0,399 do begin
          for k=0,399 do begin
               for l=0,199 do begin
                    if ((x-j)^2 + (y-k)^2 +(z-l)^2) lt r^2 then begin
                         vil(j,k,l)=255
                         voxelcount = voxelcount + 1
                    endif
                    if voxelcount eq nelements then goto,jump_gran_completed
               endfor
          endfor
     endfor
     jump_gran_completed:
     wait, 0.1
     print,'restored granule ',i
     if ((i Mod 400 eq 0) and (i ne 0)) then begin              ; will save vol at grans count 400 and multiples
          nameu= dir + 'vil_'+strcompress(s(1),/remove_all)+'_'+strcompress(i,/remove_all)+'grans.u'
          openw,um,nameu,/get_lun
          writeu,um,vil
          close,um & free_lun,um
     endif
endfor
thresh=254 & shade_volume, vil, thresh, v, p
window,0, xsize=700, ysize=400 & tv,polyshade(v,p,/T3D)
window,1, xpos = 0, ypos=0, xsize=side, ysize=side & tv,vil(*,*,100)
nameu= dir + 'vil_'+strcompress(s(1),/remove_all)+'_'+strcompress(i,/remove_all)+'grans.u'
openw,um,nameu,/get_lun
writeu,um,vil                 ;saves the  volume with maximal count of granules.
close,um & free_lun,um
stop
end
```

Program to detect and measure granules in label masks. For convenience of presentation, the code is displayed as 3 separate images.



```
pro g2_xy_granules_full_folder
; produces list of granule parameters, from label images
; assumes pixel distances of 0.8 nm, but all that can be changed
; all png files in folder are processed.
; All granule parameters are output on same line, with number of image and granule
; this program also produces images with event contour and long axis,
; see Brum et al. J. Physiol. 2000, for examples
; it uses as input label files .png..
; adapted to detect segmentation labels of granules, has very simple criteria for event detection

device, get_decomposed=olddc     ; to work with TRUE color
device,decomposed=0
dummy=''
storage= 'C:\Users\erios\EM_Images\split_granules_Montse_Penn_train_labels\'
;where input label files will be located.  May change if necessary.
dir1= 'C:\Users\erios\EM_Images\split_granules_Montse_Penn_train\'      ;where input files will be located.
dir='D:\Dropbox\Methods Manuscript Revision\'
table=3; contrast=0.0
xlen=1024 & xfst=1 & nx=1024       ; file dimensions, change if necessary.
ylen=1024 & ny=1024
    loadct,table &tvlct,r,g,b,/get&r(0)=0 & g(0)=255
    r(221)=255& g(221)=0 & b (221)=0
    r(220)=0 & g(220)=0 & b(220)=0
    r(219)=0 & g(219)=0 & b(219)=255
    window,3,xs=xlen/2,ys=ylen/2,xpos=0,ypos=0
dummy='' & fnama='' & dummys='' & outnama=''
dx = 0.8 & dy = dx & tscan =dy & dt =dx
cri=1.
cris=200
;input file block ********************************************
unity=fltarr(xlen,ylen)+1.
m_pixel=50                      ;minimum pixel size, a criterion for exclusion
filelist=file_search(storage,'*.png')
n=n_elements(filelist)
print,'n = ', n
tcount=0                        ;total counter of events
pro_sk=(fltarr(50000.,9))             ;for output list
for k=0,n-1 do begin                 ;loop over images
print, 'k =',strcompress(k)
    infile=filelist((k))
    ima=read_png(infile) & ima=long(ima)
    imac=rebin2(ima)
    npx=xlen & ny=ylen & npy=ny & wid=npy & init=0 & fin=xlen-1
    xmin=0 & xmax=xlen-1 & nl=0 & nnl=ylen-1 & area=npx*npy*dx*dx
    ;true area, even though calculated in compressed image
    yf2=ima
    jump_defmask:
    mask=ima                    ;el    iminates all prior filtering
    loadct,table
    im= ima
    siim=1010
    im(siim:nx-1,siim:ny-1)=0            ; no starting beyond 1010
    ime=ima
    mask=ima                    ;Array to hold final event masks
    imo=ima*0+220                 ;imo will be used to display event contours and axes
```

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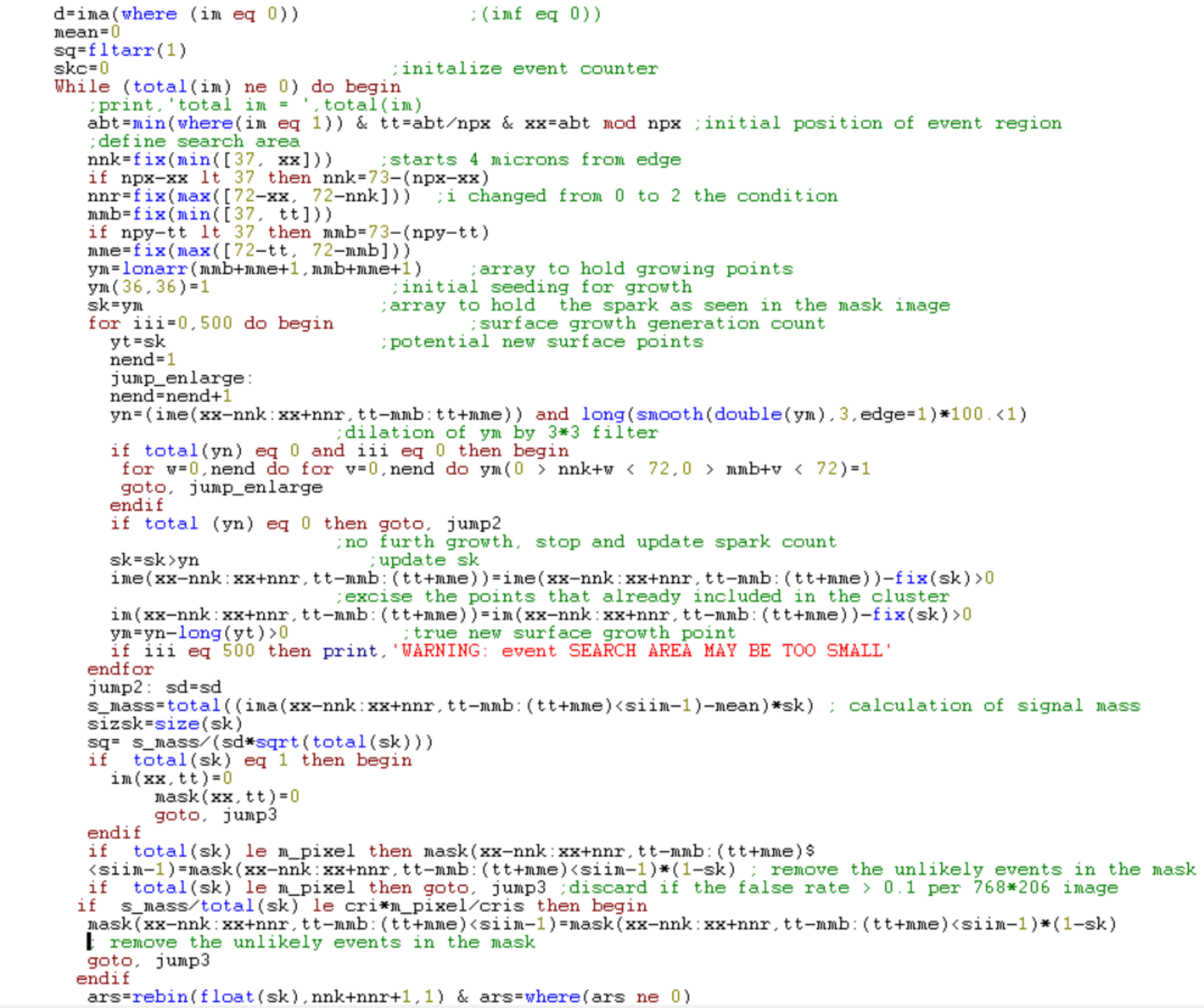

```
d=ima(where (im eq 0))               ;(imf eq 0))
mean=0
sq=fltarr(1)
skc=0                         ;initalize event counter
While (total(im) ne 0) do begin
   ;print,'total im = ',total(im)
   abt=min(where(im eq 1)) & tt=abt/npx & xx=abt mod npx ;initial position of event region
   ;define search area
   nnk=fix(min([37, xx]))    ;starts 4 microns from edge
   if npx-xx lt 37 then nnk=73-(npx-xx)
   nnr=fix(max([72-xx, 72-nnk]))  ;i changed from 0 to 2 the condition
   mmb=fix(min([37, tt]))
   if npy-tt lt 37 then mmb=73-(npy-tt)
   mme=fix(max([72-tt, 72-mmb]))
   ym=lonarr(mmb+mme+1,mmb+mme+1)     ;array to hold growing points
   ym(36,36)=1                 ;initial seeding for growth
   sk=ym                      ;array to hold  the spark as seen in the mask image
   for iii=0,500 do begin              ;surface growth generation count
     yt=sk                    ;potential new surface points
     nend=1
     jump_enlarge:
     nend=nend+1
     yn=(ime(xx-nnk:xx+nnr,tt-mmb:tt+mme)) and long(smooth(double(ym),3,edge=1)*100.<1)
                     ;dilation of ym by 3*3 filter
     if total(yn) eq 0 and iii eq 0 then begin
      for w=0,nend do for v=0,nend do ym(0 > nnk+w < 72,0 > mmb+v < 72)=1
      goto, jump_enlarge
     endif
     if total (yn) eq 0 then goto, jump2
                     ;no furth growth, stop and update spark count
     sk=sk>yn                ;update sk
     ime(xx-nnk:xx+nnr,tt-mmb:(tt+mme))=ime(xx-nnk:xx+nnr,tt-mmb:(tt+mme))-fix(sk)>0
                     ;excise the points that already included in the cluster
     im(xx-nnk:xx+nnr,tt-mmb:(tt+mme))=im(xx-nnk:xx+nnr,tt-mmb:(tt+mme))-fix(sk)>0
     ym=yn-long(yt)>0          ;true new surface growth point
     if iii eq 500 then print,'WARNING: event SEARCH AREA MAY BE TOO SMALL'
   endfor
   jump2: sd=sd
   s_mass=total((ima(xx-nnk:xx+nnr,tt-mmb:(tt+mme)<siim-1)-mean)*sk) ; calculation of signal mass
   sizsk=size(sk)
   sq= s_mass/(sd*sqrt(total(sk)))
   if  total(sk) eq 1 then begin
     im(xx,tt)=0
         mask(xx,tt)=0
         goto, jump3
   endif
   if  total(sk) le m_pixel then mask(xx-nnk:xx+nnr,tt-mmb:(tt+mme)$
   <siim-1)=mask(xx-nnk:xx+nnr,tt-mmb:(tt+mme)<siim-1)*(1-sk) ; remove the unlikely events in the mask
   if  total(sk) le m_pixel then goto, jump3 ;discard if the false rate > 0.1 per 768*206 image
  if  s_mass/total(sk) le cri*m_pixel/cris then begin
   mask(xx-nnk:xx+nnr,tt-mmb:(tt+mme)<siim-1)=mask(xx-nnk:xx+nnr,tt-mmb:(tt+mme)<siim-1)*(1-sk)
   ; remove the unlikely events in the mask
   goto, jump3
  endif
   ars=rebin(float(sk),nnk+nnr+1,1) & ars=where(ars ne 0)
```

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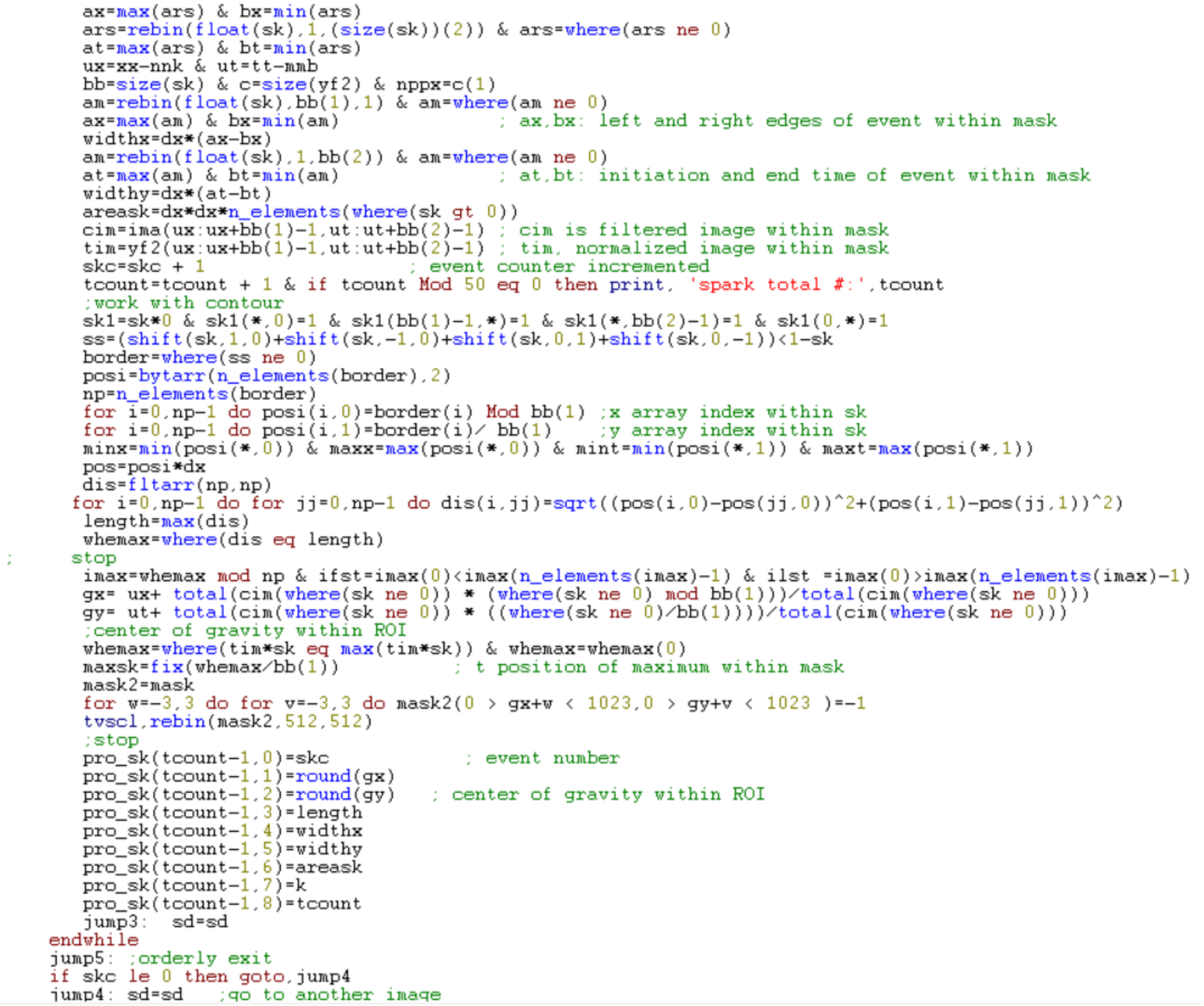
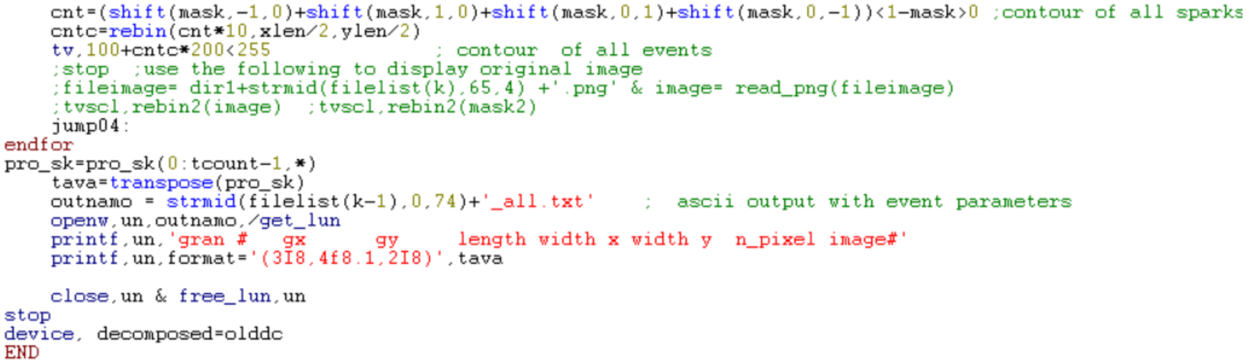


```
       ax=max(ars) & bx=min(ars)
       ars=rebin(float(sk),1,(size(sk))(2)) & ars=where(ars ne 0)
       at=max(ars) & bt=min(ars)
       ux=xx-nnk & ut=tt-mmb
       bb=size(sk) & c=size(yf2) & nppx=c(1)
       am=rebin(float(sk),bb(1),1) & am=where(am ne 0)
       ax=max(am) & bx=min(am)              ; ax,bx: left and right edges of event within mask
       widthx=dx*(ax-bx)
       am=rebin(float(sk),1,bb(2)) & am=where(am ne 0)
       at=max(am) & bt=min(am)              ; at,bt: initiation and end time of event within mask
       widthy=dx*(at-bt)
       areask=dx*dx*n_elements(where(sk gt 0))
       cim=ima(ux:ux+bb(1)-1,ut:ut+bb(2)-1) ; cim is filtered image within mask
       tim=yf2(ux:ux+bb(1)-1,ut:ut+bb(2)-1) ; tim, normalized image within mask
       skc=skc + 1                  ; event counter incremented
       tcount=tcount + 1 & if tcount Mod 50 eq 0 then print, 'spark total #:',tcount
       ;work with contour
       sk1=sk*0 & sk1(*,0)=1 & sk1(bb(1)-1,*)=1 & sk1(*,bb(2)-1)=1 & sk1(0,*)=1
       ss=(shift(sk,1,0)+shift(sk,-1,0)+shift(sk,0,1)+shift(sk,0,-1))<1-sk
       border=where(ss ne 0)
       posi=bytarr(n_elements(border),2)
       np=n_elements(border)
       for i=0,np-1 do posi(i,0)=border(i) Mod bb(1) ;x array index within sk
       for i=0,np-1 do posi(i,1)=border(i)/ bb(1)    ;y array index within sk
       minx=min(posi(*,0)) & maxx=max(posi(*,0)) & mint=min(posi(*,1)) & maxt=max(posi(*,1))
       pos=posi*dx
       dis=fltarr(np,np)
      for i=0,np-1 do for jj=0,np-1 do dis(i,jj)=sqrt((pos(i,0)-pos(jj,0))^2+(pos(i,1)-pos(jj,1))^2)
       length=max(dis)
       whemax=where(dis eq length)
;     stop
       imax=whemax mod np & ifst=imax(0)<imax(n_elements(imax)-1) & ilst =imax(0)>imax(n_elements(imax)-1)
       gx= ux+ total(cim(where(sk ne 0)) * (where(sk ne 0) mod bb(1)))/total(cim(where(sk ne 0)))
       gy= ut+ total(cim(where(sk ne 0)) * ((where(sk ne 0)/bb(1))))/total(cim(where(sk ne 0)))
       ;center of gravity within ROI
       whemax=where(tim*sk eq max(tim*sk)) & whemax=whemax(0)
       maxsk=fix(whemax/bb(1))          ; t position of maximum within mask
       mask2=mask
       for w=-3,3 do for v=-3,3 do mask2(0 > gx+w < 1023,0 > gy+v < 1023 )=-1
       tvscl,rebin(mask2,512,512)
       ;stop
       pro_sk(tcount-1,0)=skc            ; event number
       pro_sk(tcount-1,1)=round(gx)
       pro_sk(tcount-1,2)=round(gy)   ; center of gravity within ROI
       pro_sk(tcount-1,3)=length
       pro_sk(tcount-1,4)=widthx
       pro_sk(tcount-1,5)=widthy
       pro_sk(tcount-1,6)=areask
       pro_sk(tcount-1,7)=k
       pro_sk(tcount-1,8)=tcount
       jump3:  sd=sd
    endwhile
    jump5: ;orderly exit
    if skc le 0 then goto,jump4
    jump4: sd=sd   ;go to another image
```



```
    cnt=(shift(mask,-1,0)+shift(mask,1,0)+shift(mask,0,1)+shift(mask,0,-1))<1-mask>0 ;contour of all sparks
    cntc=rebin(cnt*10,xlen/2,ylen/2)
    tv,100+cntc*200<255              ; contour  of all events
    ;stop  ;use the following to display original image
    ;fileimage= dir1+strmid(filelist(k),65,4) +'.png' & image= read_png(fileimage)
    ;tvscl,rebin2(image)  ;tvscl,rebin2(mask2)
    jump04:
endfor
pro_sk=pro_sk(0:tcount-1,*)
    tava=transpose(pro_sk)
    outnamo = strmid(filelist(k-1),0,74)+'_all.txt'    ;  ascii output with event parameters
    openw,un,outnamo,/get_lun
    printf,un,'gran #   gx       gy      length width x width y  n_pixel image#'
    printf,un,format='(3I8,4f8.1,2I8)',tava

    close,un & free_lun,un
stop
device, decomposed=olddc
END
```