

Supplementary Figure 1

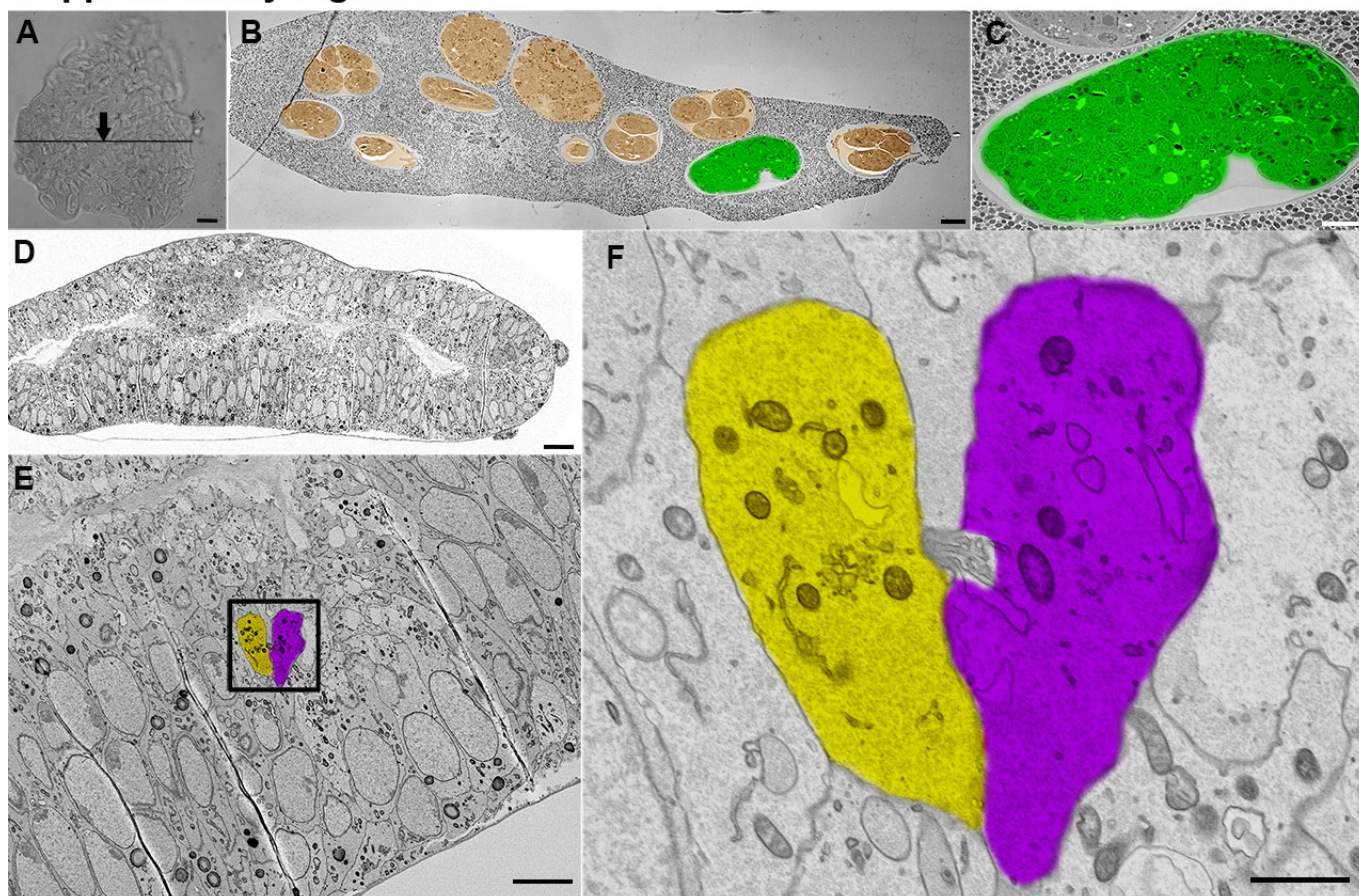


Figure S1. Examples of AT-assisted analysis of different complex samples

A. “Blind screening” of *C. elegans* embryos: A resin block containing a pellet of mixed stage embryos randomly sectioned through the area designated by the black line. Scale bar, 50 μm .

B. Low-magnification SEM-BSE image of a section from Panel A (black line). Embryos at various stages are pseudo-colored in orange, and a 1.2-fold stage embryo in the desired orientation is colored in green. Scale bar, 10 μm .

C. High-magnification image of the embryo of interest (Panel B). Rapid AT screening will provide sufficient embryos at the desired stage for the operator to choose the desired orientation, based on previous knowledge of the anatomy of the specimen. Thus, sample orientation is unnecessary. Scale bar, 5 μm .

D. SEM section through a *Drosophila* larval wing disc. Scale bar, 10 μm .

E. High magnification of epithelial cells within the wing disc (Panel D). Cell division events are frequently studied in the *Drosophila* wing disc in fixed tissue processed for TEM. Locating the event of cell abscission and retrieving the complete volume of both dividing cells is technically challenging. Quick screening of multiple regions in numerous rows of sections facilitates this operation, allowing the timely location of the desired area. Scale bar, 2 μm .

F. High magnification view (Panel E): two cells (yellow and purple) at the end of cell division, in telophase, with the cellular bridge still connecting both cells. Scale bar, 1 μm .

Supplementary Figure 2

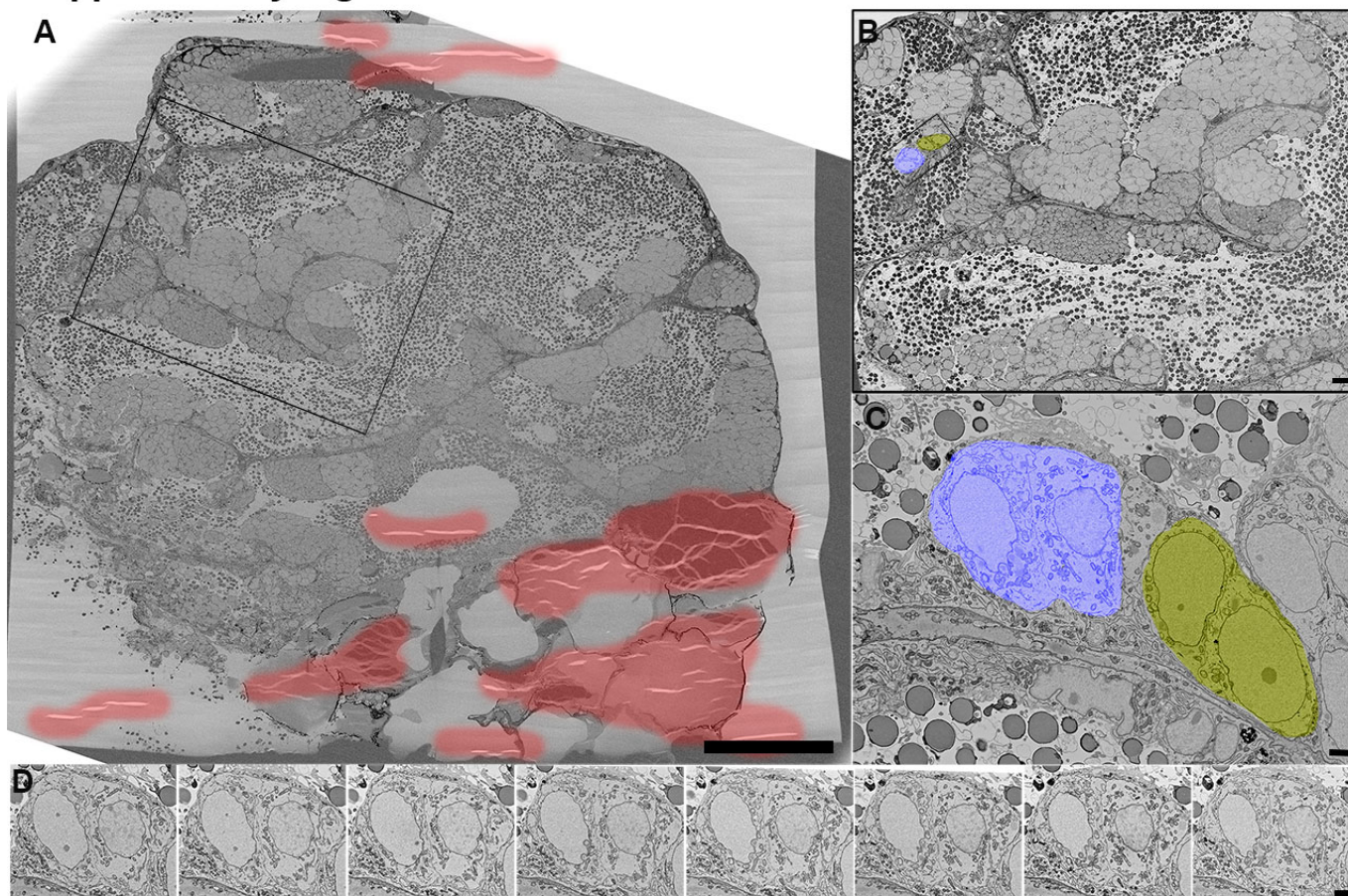


Figure S2. Examples of AT-assisted analysis of different complex samples

A. Low magnification view on a section an SEM image of a cross-section through a zebrafish testis. The regions containing the folds/microfolds are highlighted in red. Scale bar 100 μm .

B. Higher magnification on the global area of interest (black rectangle in Panel A) that contains no wrinkles. The structure is composed of cell clusters (cysts) at various stages of cell division. The mother cells that initiate such clusters by undergoing multiple rounds of cell division are randomly scattered throughout the spermatheca. Our AT approach with “blind” sectioning greatly facilitates locating the mother cells compared with standard sectional analysis. Black box encircles a potential ROI. Scale bar, 10 μm .

C. Close-up of two pairs of precursor cells highlighted in blue and in yellow (Box in Panel G). After a single section containing the ROI has been identified by “horizontal” screening (Figure 4C), the sections sequentially upstream and downstream of the initially identified section (i.e., “vertical”) within the same ribbon of consecutive sections can be analyzed. Scale bar, 2 μm .

D. A sequence of serial images obtained from the blue area (Panel G). None of the sections was affected either by the folds or the microfolds. Scale bar 2 μm .

Supplementary Figure 3

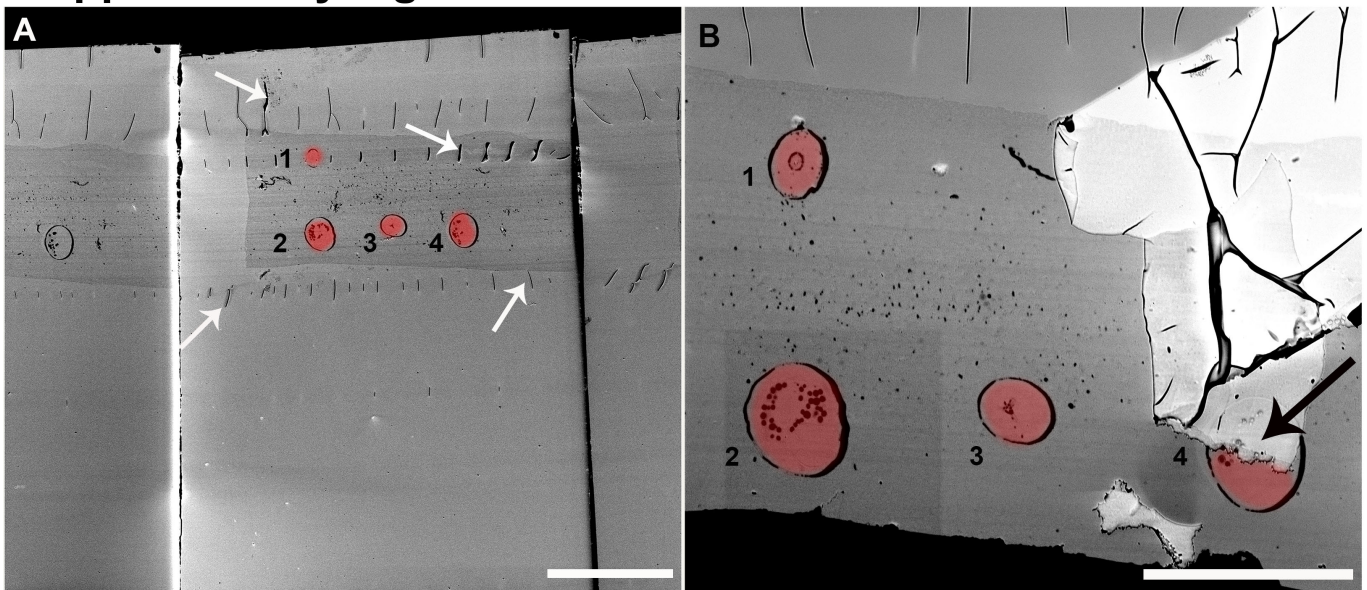


Figure S3. An example of the folds and micro folds on sections

A. An overview of several ribbons of consecutive sections on a wafer. Four *C. elegans* larvae, randomly cut through the body are shown. Arrows point to some microfolds. Scale bar 100 μm .

B. An example of a severe damage on section. Part of the resin from another section has landed on the adjacent section, partially ruining it (arrow). Scale bar 50 μm .

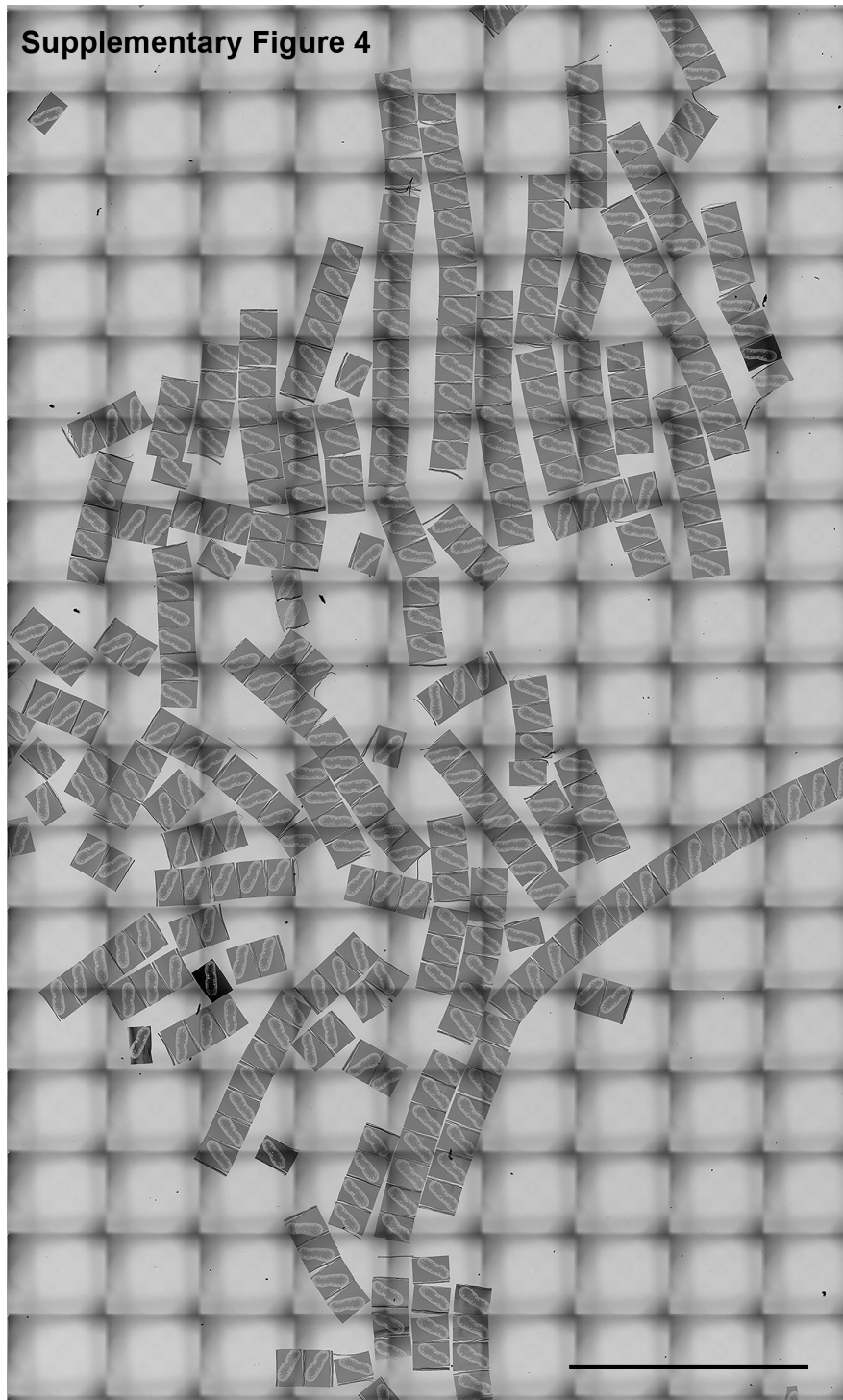


Figure S4. Scattered sections collected on wafer in a random orientation

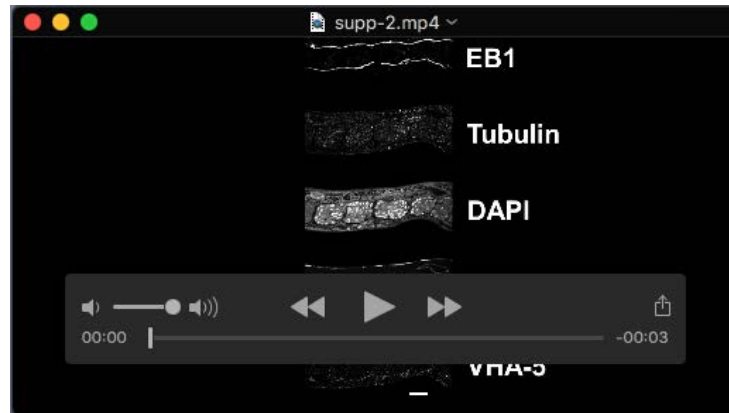
Drosophila gut sections transferred on the wafer. Multiple low magnification tiles stitched together. An example of particularly unsuccessful sectioning and arrays alignment. Sections are scattered in all directions and even in this severe case there are few to none folds on sections. The image can be zoomed in to see the details. Scale bar 500 μm .

Supplementary Movies

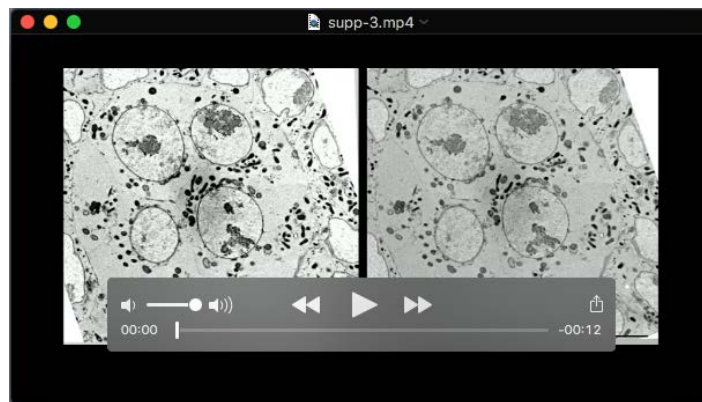


Movie 1. Sectioning and on-wafer transfer of the arrays using the modified diamond knife with the modified basin. There are several key steps in this procedure:

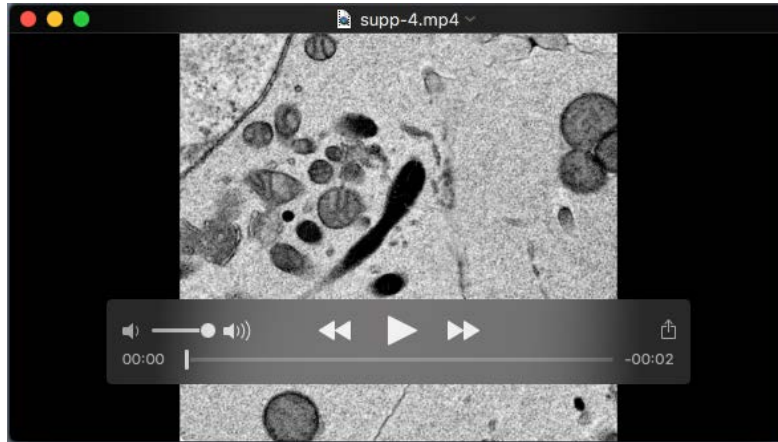
1. Sectioning. The time required for sectioning depends on the desired number of sections and the speed of sectioning. We used a rate of 0.8-1.0 $\mu\text{m}/\text{sec}$.
2. Alignment of ribbons of consecutive sections. During the first step, one or several ribbons can be generated. Multiple ribbons can be arranged side by side using gentle movements of an eyelash attached to a thin stick.
3. Water draining. Water retraction can be achieved by active aspiration through the syringe and by passive dripping through the plastic catheter. The entire procedure takes a couple of minutes at the most.
4. Readjustment of ribbons. While draining the water from the basin, the aligned ribbons often become misaligned. While water remains on the surface of the wafer, we can readjust the ribbons until the surface starts to dry.
5. Gradual evaporation of water from the surface of the wafer. We consider this step to be critical to avoid folds in the sections. The visual zoom-in on a drying portion of the ribbon illustrates this concept well. The length of time for drying will largely depend on the size of the surface area and the surrounding environmental conditions. In practice, drying takes 10 to 30 minutes for a wafer of 2x4 cm.



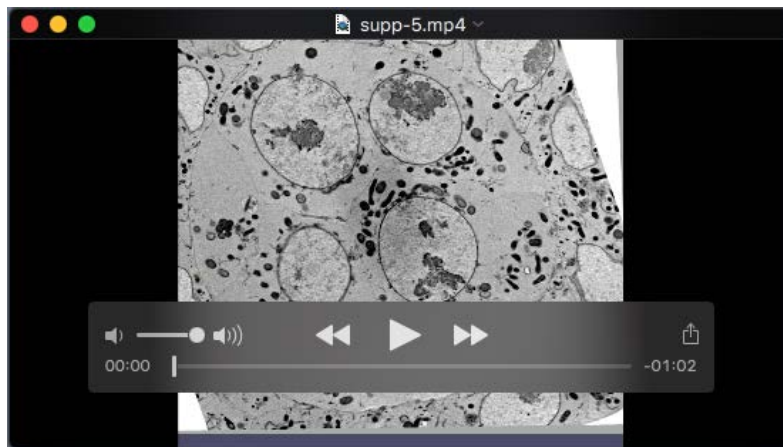
Movie 2. Alignment of the sequences of the labeled sections presented in Figure 2.



Movie 3. Alignment of multiple SEM-BSE images of sections through *Drosophila* ovarian chamber. All ring canals are pseudo-colored in orange with the selected ring in magenta. Side by side representation of the on-surface tracking (left) and a 3D ring canals rendering model (right).



Movie 4. Close-up of a ring canal from the dataset presented in the Movie 3, colored in magenta, captures with using higher-resolution SEM-BSE acquisition parameters.



Movie 5. Modeling and rendering data set of multiple SEM-BSE images of sections through a *Drosophila* ovarian chamber. An IMOD reconstruction of 80 sections featuring cells labeled in various colors as well as their interactions, and the connections through the ring canals (yellow).

Macro

```
macro register_tomo_fluo{
    Dialog.create("Register tomo fluo");
    Dialog.addChoice("Macro", newArray("Split Stack", "Register Reference", "Apply
transformation"));
    Dialog.show();
    macroChoice = Dialog.getChoice;

    if (macroChoice == "Split Stack"){

        // params
        Dialog.create("Channel number");
        Dialog.addNumber("Channel number", 3);
        Dialog.show();
        channelNumber = Dialog.getNumber;

        // input image
        var inputImageID = getImageID();
        var inputImageTitle = getTitle();
        print("tomo");getDimensions(width, height, channels, slices, frames);print("tomo1");
        var slicesNb = slices;
        var imageMaxSize = maxOf(width, height);

        // parameters values
        var outputDir = getDirectory("Choose a Directory");

        // split the channels to individual files
        for(c=1 ; c <= channelNumber ; c++){
            File.makeDirectory(outputDir + "C" + c);
        }

        splitStackToImages(channelNumber);
    }
    else if(macroChoice == "Register Reference"){

        // get parameters
        Dialog.create("Register tomo fluo");
        Dialog.addChoice("Reference channel", newArray("C1", "C2", "C3", "C4", "C5", "C6"));
        Dialog.addNumber("Registered Iage Size", 1024);
        Dialog.show();
        var referenceChannel = Dialog.getChoice;
        var imageMaxSize = Dialog.getNumber;
        var outputDir = getDirectory("Select the Directory containing the Cx input images
folder");

        // register reference stack
        File.makeDirectory(outputDir + "reg_C1");
        File.makeDirectory(outputDir + "reg_C2");
        File.makeDirectory(outputDir + "reg_C3");
        File.makeDirectory(outputDir + "reg_C4");
        File.makeDirectory(outputDir + "reg_C5");
```



```

        File.makeDirectory(outputDir + "reg_C6");
        File.makeDirectory(outputDir + "reg_transform");
        register_reference();
    }
    else if(macroChoice == "Apply transformation"){

        var outputDir = getDirectory("Choose a Directory");
        applyTranformation();
    }
}

function splitStackToImages(channelNumber){

    //run("Stack to Hyperstack...", "order=xyczt(default) channels=3 slices="+slicesNb/3+" frames=1
display=Color");
    run("Split Channels");

    for (c = 1 ; c <=channelNumber ; c++){
        selectWindow("C"+c+"-" + inputImageTitle);
        run("Image Sequence... ", "format=TIFF save=["+outputDir + "C"+c+"\\"C"+c+"-
0000.tif]");
    }
}

function register_reference(){

    run("Register Virtual Stack Slices", "source="+outputDir+referenceChannel +
" output="+outputDir+"reg_"+referenceChannel +" feature=Rigid
registration=[Rigid -- translate + rotate ]" +
" advanced save initial_gaussian_blur=1.60 steps_per_scale_octave=3
minimum_image_size=64 maximum_image_size="+imageMaxSize+" "+
" feature_descriptor_size=8 feature_descriptor_orientation_bins=8
closest/next_closest_ratio=0.92 maximal_alignment_error=25 "+
" inlier_ratio=0.05 feature_extraction_model=Rigid registration_model=[Rigid --
translate + rotate ] interpolate");

}

function applyTranformation(){

    for(c = 1 ; c <=6 ; c++ ){
        channel = "C"+c;
        args = "source="+outputDir + channel+" output="+outputDir+"reg_"+channel+"
transforms="+outputDir+"reg_transform"+" interpolate";
        run("Transform Virtual Stack Slices", args);
    }
    run("Merge Channels...", "c1=[Registered C1] c2=[Registered C2] c3=[Registered C3]
c4=[Registered C4] c5=[Registered C5] c6=[Registered C6] create");
    Stack.setDisplayMode("color");
    saveAs("TIFF", outputDir + "reg_stack.tif");
}

```