# PrinCIPia

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#### Background

PrinCIPia is a Mac OS application that handles the calculation, display, analysis and manipulation of images representing the crystallographic orientation of grains in rock samples imaged by a polarising microscope. It is a customised edition of the freely available general purpose image analysis software Image SXM that was developed in collaboration with Professor Renée Heilbronner at the University of Basel. This document summarises the function of each menu item, shows the user input dialog boxes and describes how the calculations and analysis are carried out.

#### **More Information**

For more information on PrinCIPia see the web page For help using the other functions of Image SXM see If you have any problems using PrinCIPia email me http://www.liv.ac.uk/~sdb/PrinCIPia http://www.liv.ac.uk/~sdb/ImageSXM S.D.Barrett@liv.ac.uk

#### Contents

Introduction	3
Overview	4
Prepare Stack	6
Create Azi/Inc Stack	9
Recreate Azi/Inc Stack	
Masks	13
Mask Options	
Add Mask	
Load Mask	
Save Mask	
C-Axis Orientation Image	14
Make 24-Bit Image	16
Misorientation Image	17
North-South	
East-West	
Heaven	
Mean Az Inc	
Mode Az Inc	
Specify	
Orientation Gradients	18
Grain Analysis	19
Make Grain Map	
Flatten Grain Map	

Notes

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#### Introduction

PrinCIPia is an attempt to make crystallographic orientation analysis of polarisation microscope images of rock samples as user friendly as possible. Software already exists that can calculate azimuth and inclination angles of orientation from the variation in intensity of polarised light passing through rock samples that are rotated with respect to the light path. Computer–Integrated Polarisation (CIP) analysis software can do this but, with all due respect to the author of CIP, it is not the most user friendly program. PrinCIPia is the result of a physicist (SDB) with expertise in writing image analysis software collaborating with an earth scientist (RH) with expertise in applying image analysis techniques to rock samples. It is hoped that PrinCIPia will prove to be useful to earth scientists everywhere.

#### **Background Knowledge**

A detailed knowledge of image analysis techniques is not required to use PrinCIPia, but some familiarity with either Image SXM (or NIH Image, on which it is based) or other image analysis software would be beneficial.

#### **Acquiring Images**

It is assumed that the user has some polarisation microscope images to be analysed. Images from a firewire camera can be grabbed using the image capture routines in Image SXM. With a camera plugged in and the Video > Preview Window open, the keyboard shortcut  $\mathscr{K}$ -G will grab an image and add it to a stack of grabbed images. The stack can then be saved as a TIFF file. If you grab images with software provided by the manufacturer of your camera, then make sure that the images are saved as TIFF files rather than JPEG files to avoid unwanted artefacts in the images. If the images are saved as one image per file then load them into PrinCIPia, select Stack > Windows To Stack and then save the stack as a TIFF file.

#### **Document Layout**

The following pages are structured to mirror the PrinCIPia menu items. At the end of the document are some general comments and notes to guide the user, together with example images, screen dumps and output logs that illustrate PrinCIPia in use.





The first step is to prepare a stack from a set of raw microscope images – Circular Polariser image, No Polariser image, Rotation Images and Tilt Images. Each image is corrected with the camera calibration lookup table, flat fielded and brought into registry with the other images. Sets of tilt images are combined taking into account the limited depth of focus of each image. The resultant images are saved as a 'Pre Stack'.

Prepare Stack	
Create Azi/Inc Stack Recreate Azi/Inc Stack	
Masks C-Axis Orientation Image Make 24-bit Image	•
Misorientation Image Orientation Gradients	•
Grain Analysis	►



#### **Operation Sequence**

- 1 The stack of raw microscope images specified in the **Prepare Stack Preferences** dialog box is opened. This stack must have the number of slices that matches the input stack structure specified in the **Input Preferences** dialog box.
- 2 If a camera calibration lookup table (LUT) has been specified in the **Calibration Preferences** dialog box then this is applied to every image in the stack. If the option is checked, the LUT is printed in the log.
- 3 If a flat field (background) image is included in the raw stack then this is used to flatten every image in the stack. A sample of pixel values is printed in the log.
- 4 The intensity of each pixel in the Rotation = 0° image is compared to the corresponding pixel in the Rotation = 180° image. The rms difference in intensity is printed in the log.
- 5 The Rotation =  $0^{\circ}$  image is compared to the Rotation =  $180^{\circ}$  image. If the images are out of registry with each other by more than one pixel then all rotation images are offset to bring them into registry with the Rotation =  $0^{\circ}$  image. A graphical indication of the offsets is printed in the log.
- 6 Every image in the stack has its spatial scale calibrated as specified in the **Calibration Preferences** dialog box.
- 7 A new stack is created with the slices in a standardised order (circular polariser, no polariser, flat field, rotation images, tilt images) regardless of the order in which they occurred in the raw stack.
- 8 A new folder is created in the folder specified in the **Prepare Stack Preferences** dialog box. This folder has a name formed from the prefix of the raw stack and a suffix of numbers in the format mmdd-hhmm (month-day-hour-minute). For the remainder of the analysis session, all files that are saved without prompting the user for a location are saved into this folder. If Image SXM is quit and subsequently relaunched, a new date/time-stamped folder is created.
- 9 The Pre Stack is saved with a file suffix as specified in the **Output Preferences** dialog box.
- 10 The log is saved.

### **Prepare Stack**

000	
PrinCIPia Prepare Stack Prefer	rences
Load Images From Stack:	Change File
Work/Image ark/PrinCIPia te	st/Raw-Stack-w920
Save Files To Folder:	Change Folder Same As Input Stack
Scratch/PrinCIPia	
Input Prefs Calib F	Prefs Output Prefs Cancel OK

#### **Prepare Stack Preferences**

Select the stack from which all raw microscope images are loaded and the folder into which the output files will be saved. The OK button is dimmed until a file and folder have been selected.



#### Input Preferences

Specify the order in which the raw images are located in the input stack; the incremental angle between rotation images, or the number of rotation images; the directions of the tilt images and the number of images for each tilt. The OK button is dimmed until a valid rotation increment is specified and 2 or 4 tilt images are selected.

000	
PrinCIPia Calibration Preferences	¥
Load Camera Calibration LUT	Select File Show LUT in Log
Image Scale	Cancel OK

#### **Calibration Preferences**

Select a camera calibration lookup table that will be applied to all image data loaded. Specify the image spatial calibration. The OK button is dimmed if a camera LUT is to be loaded but no file is specified.



#### **Output Preferences**

Specify the file suffixes used for the Pre Stack, CIP stack and log files; the labels used for the slices of the CIP stack; whether or not a comment is to be added to the stack(s) before they are saved.

The images in the Pre Stack are used to calculate the azimuth and inclination of the orientation of the crystallographic c-axis for every pixel in the image. The resultant images are saved in a CIP stack.

Prepare Stack	
Create Azi/Inc Stack	
Recreate Azi/Inc Stack	
Masks C-Axis Orientation Image Make 24-bit Image	•
Misorientation Image Orientation Gradients	•
Grain Analysis	►

Ontical Activity	- Tilt Images
<ul> <li>+ Quartz</li> <li>- Calcite</li> </ul>	Interpretation O Soft
Histograms Manual Limits 1% Auto 2% 5%	<ul> <li>Smooth Images Before Tilt Analysis</li> <li>Set Mean of Tilt = Mean of Rot 180°</li> <li>Process Tilt Index Image</li> <li>Median Filter</li> </ul>
Load Lookup Tables - ✓ Load Sin-Squared << No File Selected >	LUT Select File Show LUT in Log
🗹 Load Colour LUT	Select File

#### **Operation Sequence**

1 If the frontmost image is a Pre Stack then this is used to create an CIP stack comprising azimuth, inclination, error and mask images. If not, then the user is prompted to open an existing Pre Stack.

For every pixel in the image...

- 2 The intensity variation with rotation angle is extracted from the Pre Stack images.
- 3 The minimum and maximum intensities are noted.
- 4 Around these extremal intensity values, a quadratic fit of the intensity variation determines more accurate values for the rotation angles.
- 5 The rotation angles at which the extremal intensity values occur determine the azimuthal angle (Azi) taking into account the optical activity of the sample. The azimuthal angles are stored in the first slice of the CIP stack.
- 6 The difference between the maximum and minimum intensities determines the inclination angle (Inc). The inclination angles are stored in the second slice of the CIP stack.
- 7 The chi-squared error in the quadratic fits is stored in the third slice of the CIP stack. When thresholded this becomes the Error Mask (see page 13). This will be used when creating a C-Axis Orientation Image (COI) and subsequent images.
- 8 The number of pixels with chi-squared errors in the quadratic fits exceeding a few grey values are displayed in the log.
- 9 The number of pixels for which the extremal intensities do not occur at rotation angles differing by 90° are displayed in the log.
- 10 A histogram of inclination angles  $(0^{\circ}-90^{\circ})$  is displayed in the log.

For every pixel in the image...

11 The North (or South) and East (or West) Tilt Images are compared with the Rotation = 180° images to determine whether or not the inclination angle needs to be flipped from Inc to 180°–Inc.

#### **Operation Sequence (contd)**

- 12 A histogram of inclination angles  $(0^{\circ}-180^{\circ})$  is displayed in the log.
- 13 The number of pixels with inclination angles in the three ranges 0°-89°, 90° and 91°-180° are displayed in the log.
- 14 The No Polariser image of the Pre Stack is the fourth slice of the CIP stack. When thresholded this becomes the Defect Mask (see page 13). This will be used when creating a C-Axis Orientation Image (COI) and subsequent images.
- 15 The CIP stack is saved with a file suffix as specified in the **Output Preferences** dialog box.

## **Create Azi/Inc Stack**

		222
Optical Activity	Tilt Images	
● + Quartz ○ - Calcite	Interpretation	
	Smooth Images Before Tilt Analysis	
Histograms	☑ Set Mean of Tilt = Mean of Rot 180°	
Manual Limits	Process Tilt	
Auto 2%	Index Image Median Filter	
0 5%		
Load Lookup Tables – 🗹 Load Sin-Squared	LUT Select File Show LUT in L	og
<< No File Selected >	»	
🗹 Load Colour LUT	Select File	
	>>	
<< No File Selected >		

#### **Create Azi/Inc Stack Preferences**

Some explanation of...

Optical Activity Tilt Interpretation LUTs

Pixel values in the inclination image are mapped to actual inclination values in the range  $0^{\circ} - 90^{\circ}$  by analysis of the image histogram.

If the user selects Manual Limits then the upper and lower limits of inclination values can be set manually by adjusting the red (values < 0°) and green (values > 90°) ranges of pixel values using the mouse in either the LUT or the Histogram windows.

If the user selects Auto limits, then the red and green ranges are set such that a specified percentage of pixels lie within each range.



Two masks are generated automatically — the Error Mask and the Defect Mask. The error image and the defect image (stored in CIP stack slices 3 and 4) are thresholded at userspecified values to create these masks. The thresholds are set in the **Mask Options** dialog box. Optionally, additional User Masks can be added to the CIP stack or loaded/saved. When a mask is used to create an image, the superposition of all masks selected in the **Mask Options** dialog box is used. This is designated the Active Mask and is stored in CIP stack slice 5.

Prepare Stack Create Azi/Inc Stack Recreate Azi/Inc Stack	
Masks	
C-Axis Orientation Image	
Make 24-bit Image	
Misorientation Image	
Orientation Gradients	
Grain Analysis	►

		000
ck		PrinCIPia Mask Options
►	Mask Options	Error Mask Set Threshold 128 = 4.0 gv
mage e 🕨	Add Mask Load Mask Save Mask	<ul> <li>✓ Defect Mask</li> <li>✓ Set Threshold</li> <li>✓ User Mask</li> <li>&lt;&lt; none &gt;&gt;</li> </ul>
ents ►		Cancel OK

If a mask is selected to be used to create the Active Mask, then the CIP stack slice displaying that mask will have an asterisk appended to its title.



**Add Mask** adds another slice to the CIP stack that the user can edit. Images can be pasted into the mask and thresholded. The user can draw/paint/erase directly into the image.

**Load Mask** loads an image (of the correct height x width) as a new mask.

Save Mask saves the currently selected mask as an image.

The orientation information stored in the azimuth (Azi) and inclination (Inc) slices of the CIP stack are used to create a c-axis orientation image (COI) in which each 24-bit pixel colour encodes the Azi/Inc angles. In addition to the COI, two other windows are created:

(i) A colour lookup table (CLUT) shows how the pixel colours map to Azi/Inc values in a stereographic projection azimuthal angles determine the colour hue and inclination angles determine the colour saturation.

Prepare Stack Create Azi/Inc Stack Recreate Azi/Inc Stack	
Masks	►
C-Axis Orientation Image	
Make 24-bit Image	
Misorientation Image	►
Orientation Gradients	
Grain Analysis	►

(ii) A Pole Figure (PF) displays a stereographic projection in which the greyscale density at every point is proportional to the number of pixels in the image that have that Azi/Inc orientation.

#### **Colour Lookup Tables**



If a 'Standard' CLUT is selected, azimuth values of 0° – 360° map to a unique colour range: red-magenta-blue-cyan-green-yellow-red.

Pixels with a similar orientation in space may appear as quite different colours. For instance, the two orientations  $Azi/Inc = 0^{\circ}/89^{\circ}$  and  $180^{\circ}/89^{\circ}$  are actually only 2° apart, but would be represented as complementary colours red and cyan, respectively.



If a 'Spectrum' CLUT is selected, azimuth values of  $0^{\circ} - 360^{\circ}$  map to two repeating cycles of colour: blue-green-red-blue-green-red-blue. Using this CLUT, the two orientations Azi/Inc =  $0^{\circ}/89^{\circ}$  and  $180^{\circ}/89^{\circ}$  would both be represented as blue.

Thus, similar orientations are represented by similar colours, but the Azi/Inc values of any pixel cannot be determined unambiguously from the pixel colour.

A c-axis orientation image (COI) can be displayed using either CLUT by clicking in the corresponding corner of the CLUT window.

When the mouse is moved over the CIP stack or COI, the Azi/Inc values corresponding to the pixel under the mouse are shown by a cross in the CLUT.

#### **Pole Figure**



A Pole Figure (PF) is effectively a two-dimensional histogram of pixel values. The maximum in the PF is displayed in the top left corner of the PF window. The units are 'times uniform' — the value relative to that expected if all orientations were equally likely and so uniformly distributed over all orientation space. The Azi/Inc values at which the maximum in the PF occurs is displayed in the top right corner.



When the mouse is moved over the CIP stack or COI, the Azi/Inc values corresponding to the pixel under the mouse are shown by a cross in the PF.



If the user selects the Brush Tool from the Tools Window, then the cursor changes to a small circle and the Azi/Inc values corresponding to all the pixels inside the circle (radius = 8 pixels) are shown in the PF by a collection of red dots. As the user mouses over the CIP stack or COI, the red dots update.

If the user selects a rectangular, elliptical, polygonal or freehand region of interest (ROI) in the CIP stack or COI, then the red dots will correspond to all pixels within the ROI. If the ROI is large (> 1000 pixels) then it will take a finite time to update the PF window and the response to moving the ROI may appear sluggish.

Not yet implemented...

Prepare Stack Create Azi/Inc Stack Recreate Azi/Inc Stack	
Masks C-Axis Orientation Image	►
Make 24-bit Image	
Misorientation Image Orientation Gradients	•
Grain Analysis	►

In a misorientation image (MOI) each pixel represents the angle of the orientation with respect to a reference direction. The reference direction can be (i) one of the principal directions (North, East, Heaven), (ii) the mean orientation of a selection of pixels, (iii) the modal orientation of the all the pixels, or (iv) a user–specified orientation.

Prepare Stack Create Azi/Inc Stack Recreate Azi/Inc Stack	
Masks C-Axis Orientation Image Make 24-bit Image	•
Misorientation Image	١.
Orientation Gradients	
Grain Analysis	►



Any of the three principal directions can be selected directly from the menu.

The **Mean Az Inc** item will be dimmed unless the mean values have been calculated by creating a C-Axis Orientation Image (COI) and either selecting a region of interest or selecting the paint brush tool to display (in red) orientations in the Pole Figure (PF).

The **Mode Az Inc** item will be dimmed unless a Pole Figure has been created. The modal values of Azi and Inc correspond to the maximum intensity in the Pole Figure.

Select the **Specify** item for a misorientation image with respect to an arbitrary direction.

In an orientation gradient image (OGI) each pixel represents the angle of the orientation with respect to those of neighbouring pixels. Prepare Stack... Create Azi/Inc Stack... Recreate Azi/Inc Stack... Masks C-Axis Orientation Image Make 24-bit Image Misorientation Image

Orientation Gradients

►

Grain Analysis

More about the algorithm to follow...

A map of the grain boundaries is created by applying an edge filter to a set of misorientation images. The orientations of all pixels within a grain are used to calculate the mean orientation for that grain. Setting the orientation of all pixels in a grain to the mean orientation produces a 'flat grain map'.





**Make Grain Map** uses misorientation images calculated for the principal directions (North, East, Heaven).

However, crystal grains aligned close to any of the <111> directions will have the same misorientation angles (~55°) with respect to all of the principal directions and so grain boundaries between such grains would be invisible. To reveal these grain boundaries, misorientation images are also calculated for each of four <111> directions.

If the option key is pressed when selecting the Grain Analysis menu, the **Make Grain Map** item changes to **Make Grain Map From OGIs**.

Instead of using a set of misorientation images (MOIs), the grain map is created from orientation gradient images (OGIs) in which the gradients are calculated in the neighbourhood of each pixel. Which approach produces the most accurate representation of the actual grain sizes and shapes will depend on the grain texture.

#### **Operation Sequence for Make Grain Map**

1 A misorientation image is calculated for each of the three principal directions plus the four <111> directions closest to Heaven.

For each misorientation image...

- 2 A median filter is applied.
- 3 The boundaries between areas with similar grey values are highlighted using a 5x5 Sobel (Find Edges) filter.
- 4 The image is thresholded at the mean pixel grey value.
- 5 A median filter is applied.
- 6 Each of the seven binary images is OR'd together to create a single binary image.
- 7 The image is skeletonised.
- 8 The skeleton is pruned to remove lines that are unterminated (ie, not grain boundaries).
- 9 The single-pixel grain boundaries are thickened to two pixels wide.
- 10 Steps 7, 8 and 9 are repeated three times to remove small artefacts.
- 11 The grain map is saved as a TIFF image.

#### **Operation Sequence for Make Grain Map From OGIs**

For each pixel in the image...

- 1 The orientation gradient is calculated in a 7x7 pixel neighbourhood for each of 8 directions ~ 22.5° apart.
- 2 The 8 values of orientation gradient are summed and divided by 4 to produce a pixel value in the range 0-255.
- 3 A median filter is applied.
- 4 A background is subtracted using a rolling ball algorithm with a radius of 10 pixels.
- 5 A median filter is applied.
- 6 The image is thresholded at the median pixel grey value.
- 7 The image is skeletonised.
- 8 The skeleton is pruned to remove lines that are unterminated (ie, not grain boundaries).
- 9 The single-pixel grain boundaries are thickened to two pixels wide.
- 10 Steps 7, 8 and 9 are repeated three times to remove small artefacts.
- 11 The grain map is saved as a TIFF image.



**Flatten Grain Map** uses the binary grain map to identify every grain large enough to analyse. The pixel colours within each grain are replaced with the colour corresponding to the mean Azi/Inc orientation within the grain.

The COI colours encode Azi/Inc values for every pixel in the image.



The Grain Map is a binary image showing the grain boundaries.

For each grain in the image the Azi/Inc values from the COI are used to calculate the mean orientation of all pixels in the grain. The mean Azi/Inc values are used to determine the colour to be used to display the entire (flattened) grain.



